CHARACTERIZATION OF THE PROTEIN LYSINE METHYLTRANSFERASE SMYD2

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ABSTRACT

Our understanding of protein lysine methyltransferases and their substrates remains limited despite their importance as regulators of the proteome. The SMYD (SET and MYND domain) methyltransferase family plays pivotal roles in various cellular processes, including transcriptional regulation and embryonic development. Among them, SMYD2 is associated with oesophageal squamous cell carcinoma, bladder cancer and leukemia as well as with embryonic development. Initially identified as a histone methyltransferase, SMYD2 was later reported to methylate p53, the retinoblastoma protein pRb and the estrogen receptor ERα and to regulate their activity. Our proteomic and biochemical analyses demonstrated that SMYD2 also methylates the molecular chaperone HSP90 on K209 and K615. We also showed that HSP90 methylation is regulated by HSP90 co-chaperones, pH, and the demethylase LSD1. Further methyltransferase assays demonstrated that SMYD2 methylates lysine K* in proteins which include the sequence $[LFM]_{-1}$-K*-$[AFYMSHRK]_{+1}$-$[LYK]_{+2}$. This motif allowed us to show that SMYD2 methylates the transcriptional co-repressor SIN3B, the RNA helicase DHX15 and the myogenic transcription factors SIX1 and SIX2. Finally, muscle cell models suggest that SMYD2 methyltransferase activity plays a role in preventing premature myogenic differentiation of proliferating myoblasts by repressing muscle-specific genes. Our work thus shows that SMYD2 methyltransferase activity targets a broad array of substrates *in vitro* and *in situ* and is regulated by intricate mechanisms.

”You turn the wheel and the mud whirls round, as if you were possessed while you stand over it and say "I'm going to make a jug, I'm going to make a plate, I'm going to make a lamp and the devil knows what more!"”

Nikos Kazantzakis, *Zorba the Greek*

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LIST OF ABBREVIATIONS

α-KEG ..... α keto-glutarate

AdoMet ..... S-Adenosyl-L-methionine

AHA1 ..... Activator of HSP90 ATPase 1

AR ..... Androgen receptor

ARID5B ..... AT-rich interaction domain 5B

ATM ..... Ataxia telangiectasia mutated protein kinase

BSA ..... Bovine Serum Albumin

C/EBPβ ..... CCAAT/enhancer-binding protein β

CDC5L ..... Cell Division Cycle 5-Like protein

CK2 ..... Casein Kinase II

CHD6 ..... Chromodomain Protein 6

CHIP ..... C-terminus of HSP70 Interacting Protein

Corto ..... Centrosomal and chromosomal factor

CPD ..... Computational Protein Design

Cren7 ..... Crenarcheal protein 7

DD ..... Dimerization Domain

DHX15 ..... DEAH box RNA helicase 15

DHX9 ..... DEAH box RNA Helicase 9

DNA ..... Desoxyribonucleic acid

DNMT1..... DNA Methyltransferase 1

Eaf3..... ESA1-associated factor 3

EF1..... Elongation Factor 1

EF-Tu..... Elongation Factor Tu

ERα..... Estrogen Receptor α

ESC..... Embryonic Stem Cells

ESI..... Electron Spray Ionisation

FBXL11 ..... F-box and leucine-rich repeat protein 11

FLAG..... DYKDDDDK octapeptide

FOXO3..... Forkhead Box O3

GAPDH..... Glyceraldehyde 3-Phosphate Dehydrogenase

GATA4 ..... GATA-targeting transcription factor 4

GNL3 ..... Guanine Nucleotide-binding Protein-Like 3

GREB1..... Growth Regulation By Estrogen In Breast Cancer 1

GST..... Glutathione S-transferase

HBHA ..... Heparin-Binding Haemagglutinin Adhesin

HIV ..... Human Immunodeficiency Virus

HOP..... HSP90/HSP70 Organizing Protein

HP1 ..... Heterochromatin Protein 1

HP1BP3..... Heterochromatin Protein 1 Binding Protein 3

HPLC ..... High Performance Liquid Chromatography

HSP70 ..... Heat Shock Protein 70

HSP90 ..... Heat Shock Protein 90

JARID1B..... Jumonji and ARID 1B

JHDM1..... Jumonji Human Demethylase 1

KMT..... Lysine Methyltransferase

L3MBTL1 ..... L3 Malignant Brain Tumor-like 1

LBP ..... Lamin Binding Protein

LC-MS ..... Liquid Chromatography coupled Mass Spectrometry

LSD1 ..... Lysine Specific Demethylase 1

LTR ..... Long terminal repeat

MBT ..... Malignant Brain Tumor domain

MC1-α ..... Methanococcus mazei protein 1α

MD ..... Middle Domain

MPP8..... M-Phase Phosphoprotein 8

MS..... Mass Spectrometry

MSD..... Multistate Design

MYND ..... Myeloid, Nervy, and DEAF-1 zinc finger

MyoD ..... Myoblast Determination Protein 1

MyoG ..... Myogenin

MYPT1 ..... Myosin Phosphatase Target Subunit 1

NBD ..... Nucleotide Binding Domain

NF-κB ..... Necrosis Factor κB

NPM1..... Nucleophosmin 1

OmpB..... Outer Membrane Protein B

PARP1..... Poly [ADP-ribose] Polymerase 1

PBS ..... Phosphate Buffer Saline

PCAF ..... p300/CBP-associated factor

PHD..... Plant Homeodomain

PHF2 ..... PHD finger protein 2

PKMT ..... Protein Lysine Methyltransferase

PR..... Progesterone Receptor

pRb..... Retinoblastoma protein

PTM ..... Post-translational modification

RelA ..... Reticuloendotheliosis-associated protein

RIF1 ..... RAP1 Interacting Factor

RNA ..... Ribonucleic acid

ROC ..... Receiver Operating Characteristic

ROR ..... RAR-related orphan receptor

Rubisco ..... Ribulose-1,5-bisphosphate carboxylase/oxygenase

RUVBL2 ..... Reptin (RuvB-like 2)

SAM..... S-Adenosyl-L-methionine

SDS-PAGE ..... Sodium dodevyl sufate – polyacrylamide gel electrophoresis

SET ..... SU(var), Enhancer of Zeste and Trithorax domain

SMYD..... SET and MYND

SILAC..... Stable Isotopic Labelling in Cell Culture

SIN3B ..... SIN3 homolog B

SIX1 ..... Sine Oculis-like Homeobox 1

SIX2 ..... Sine Oculis-like Homeobox 2

SPOT..... Synthetic Peptide on Membrane Support

SSD ..... Single-State Design

STAT3..... Signal transducer and activator of transcription 3

SUMO..... Small Ubiquitin-like Modifier

TNFα ..... Tumor Necrosis Factor α

Top1 ..... Topoisomerase 1

TPR ..... Tetratrico Peptide Repeat

tRNA..... transfer RNA

VCP..... Valosin-containing protein

WT ..... Wild-type

WDR5 ..... WD repeat domain 5

XRCC6..... X-Ray Repair Cross-Complementing Protein 6

1. INTRODUCTION

Sections 1.2 to 1.9 and Figures 1.2 to 1.5 of this chapter were published previously as:

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Author contributions:

S.L. researched the paper and data presented and prepared Figures 1.1, 1.2, 1.4 and 1.5. S.L. and J-F.C. wrote the paper. S.L. and V.M. prepared Figure 1.3. V.M. and D.F. provided insights on the paper

1.1. Modification of Lysine Residues

The combination of the 20 naturally occurring amino acids into polypeptides offers a remarkable number of possible conformational, structural and functional arrangements. The complexity of a set proteome is however still increased by several orders of magnitudes by specific modifications following translation and folding. Over 300 types of post-translational modifications (PTMs) have been reported from various organisms (1). Up to 5% of eukaryotic genomes encode enzymes responsible for post-translational modification of the proteome; it is estimated that the majority of proteins in eukaryotes and archae carry some type of PTM (2). Post-translational modifications direct processes as varied as chemotaxis, fluorescence, cellular signalling and localization, protein complex formation, turnover and polymerization.

Lysine presents one of the broadest arrays of post-translational modifications (Figure 1). Among those, acylation of the ε-amine is a common modification, of which the best studied is acetylation (3-5) but also include formylation (6), propionylation (7, 8), butyrylation (7), crotonylation (9), malonylation (10), succinylation (11-13) as well as acylation by fatty acids, such as palmitoylation & myristoylation (14) (Figure 1). Biotinylation of lysine by biotin ligases also form an acyl-lysine adduct (15).

Many other modifications of lysine in proteins are observed *in vivo*. Oxidation of the lysine sidechain leads to β-hydroxylation (16) or the production of allysine by deamination (17). Glycosylation of the lysine sidechain, either through N-glycation (18) or O-glycosylation following β-hydroxylation (19), is involved in protein stability (20) and ADP-ribosylation (21) or adenylylation (AMPylation) (22) are linked to the oxidative stress response. The modification of lysine can also be part of an enzyme catalytic cycle:

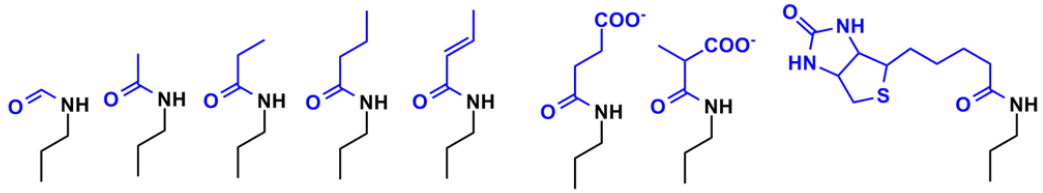
pyridoxal phosphate-dependent enzymes and rhodopsin form an imino-lysine adduct with their cofactor (23, 24). Protein degradation and cell signalling cascades lead to lysine modification by the covalent addition of ubiquitin or ubiquitinyl-like peptide chains (SUMO, NEDD8, FAT10, ISG15 and others.) (25-27). Lysine residues are also the target of non-enzymatic modification by urea, leading to its carbamylation (28).

Finally, the methylation of a lysine ε-amine is the only reported case of lysine alkylation (29-32) and involves the transfer of up to three methyl groups. To this day, lysine methylation has been observed in both nuclear and cytoplasmic proteins and is now considered a prevalent modification in eukaryotes, prokaryotes and archaea (33-36).

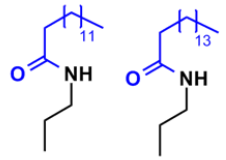
Figure 1.1: Post-translational modifications of lysine residues in proteins

Representation of known post-translational modifications of lysine residues; the lysine side chain is represented in black and added moieties are represented in blue.

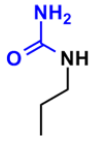
Acylation



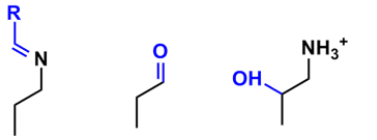
Fatty acylation



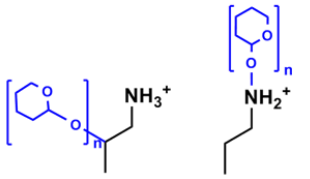
Acylamidation



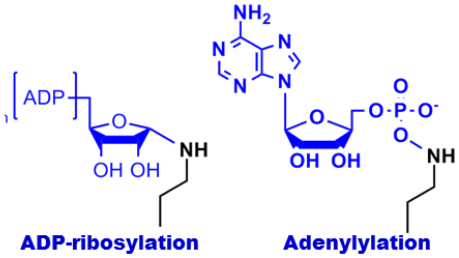
Oxidation



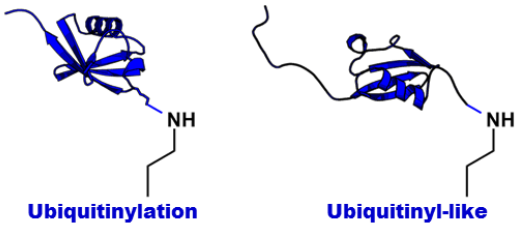
Glycosylation



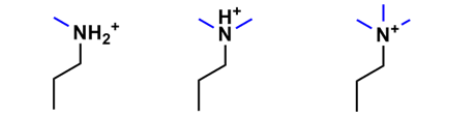
Ribosylation



Ubiquitinylation



Alkylation



1.2. Uncovering Lysine Methylation

Methylation of a lysine residue was first reported in 1959 by Ambler et al. (37) in the flagellin protein of *Salmonella typhimurium*. While the origin and the function of the methyllysine residue was a mystery at the time, the identification that histone proteins were also methylated suggested that this PTM is a prevalent modification (38). The subsequent discovery of the methylation of a wide range of proteins (39-47) confirmed the predominance of this PTM in both prokaryotes and eukaryotes.

In addition, the regulation of EF-Tu methylation by carbon, phosphorus or nitrogen availability (48) and the evolutionarily conserved character of multiple methylation sites identified in ribosomal proteins (49-52) hinted that lysine methylation could serve important biological functions. This was confirmed by the report that methylation of calmodulin K115 (53-55) lowers its capacity to stimulate NAD kinase activity (56). Methylation of calmodulin does not, however, prevent the activation of other calmodulin targets (56, 57). These findings showed that lysine methylation modulates the function of a protein and demonstrated that this PTM has the ability to affect only a subset of activity of the methylated substrate.

Interest in lysine methylation intensified following the observation that the methylation of lysine 9 on histone H3 leads to the recruitment of HP1 (Swi6 in *S. pombe*) to chromatin (58, 59), and consequently promotes heterochromatin formation. This effect suggested that the widespread modification of histone proteins by methylation could lead to dramatic effects on gene expression.

1.3. Protein Lysine Methyltransferases

Two groups of enzymes, both using S-adenosyl-L-methionine (SAM) as a methyl donor, catalyze the addition of a methyl group to the ε-amine group of a lysine side chain (60). The first type of protein lysine methyltransferase regroups the enzymes containing a catalytic SET domain (class V methyltransferases). The SET domain, named after SU(var), Enhancer of Zeste and Trithorax, the three first identified proteins harbouring this domain in *Drosophila* (61), is characterized by three regions folded into a mainly β-sheet knot-like structure that forms the active site consisting of the four conserved motifs GXG, YXG, NHXCXPN and ELXFDY (62-64). Binding of SAM and the substrate takes place on each side of a methyl-transfer channel formed by this knot-like structure. It is suggested that a catalytic tyrosine resting in this channel deprotonates the lysine's ε-amine group, triggering the methyl transfer from SAM to the lysine ε-amine (65-72). A network of aromatic residues and hydrogen bonds in this channel limits the possible orientations of the lysine substrate (73), controlling the SET domain proteins to transfer a specific number of methyl groups to a substrate.

Based on sequence similarities and domain organisation, the SET-domain containing proteins can be broadly divided in seven families (63): SUV3/9, SET1, SET2, SMYD, EZ, SUV4-20 and RIZ. Members of the SUV3/9 (G9a (74), GLP (75), SETDB1 (76)), SET1 (SET1 (77)), SET2 (NSD1 (78)), SMYD (SMYD2 (79), SMYD3 (80)) and EZ (EZH2 (81)) families methylate both histone and non-histone substrates (Appendix 1 & Figure 1.2) while substrates reported to this day for the SUV4-20 and RIZ families are limited to histone proteins (82, 83). Outside of these seven families, SET7/9 and SET8

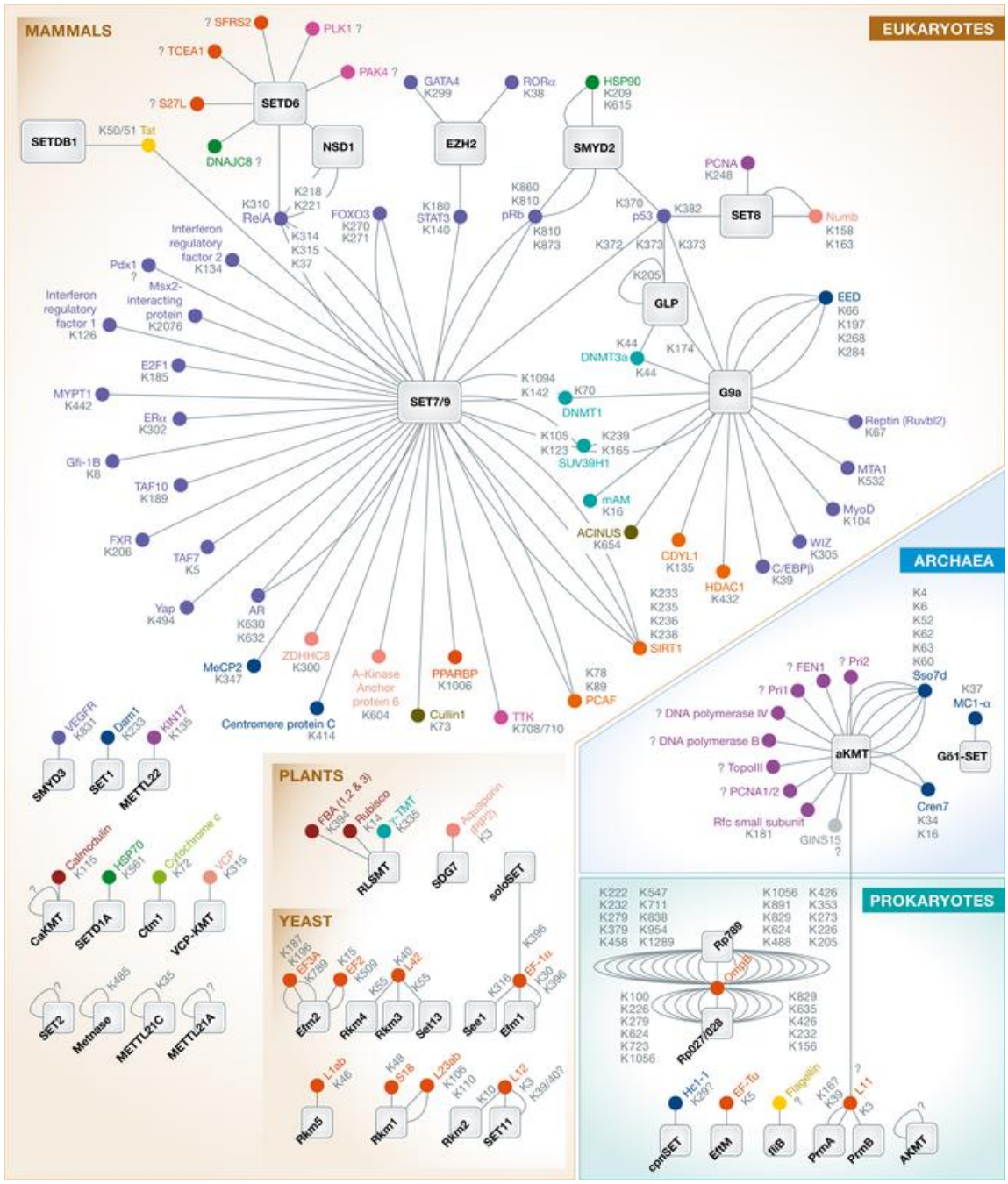
are also reported to methylate a substantial number of proteins (Appendix 1 & Figure 1.2).

The second class of PKMTs, the seven β-strand methyltransferases (class I methyltransferases), belongs to an extended superfamily of methyltransferases found throughout eukaryotes, prokaryotes and archaea. Members of this family methylate DNA, RNA or amino acids such as arginine, glutamine, aspartate and histidine (60, 84). They are named after its Rossman fold built around a central β-sheet structure, which includes the conserved, catalytic motifs hhXhD/E, XDAX and PXVN/DXXLIXL (h=hydrophobic residue) that allow the association of SAM and the protein substrate.

Across all three domains of life, a number of class I methyltransferases are reported to methylate lysine residues in proteins (Appendix 1). The bacterial methyltransferases PrmA and PrmB methylate the ribosomal units L11 (85) and L3 (86), respectively. In *S. cerevisiae*, Rkm5 methylates the ribosomal protein L1ab (87) and See1 methylates the elongation factor EF1-α on K316 (88). Recently, VCP-KMT, a newly identified class I methyltransferase, was shown to methylate the membrane protein VCP (89). Class I methyltransferases are also able to methylate histones, as Dot1 homologs trimethylate K79 of histone H3 (90). In crenarchaea, the methyltransferase aKMT, a broad specificity class I lysine methyltransferase, was shown to methylate the DNA-binding protein Cren7 (91).

Figure 1.2: Association of lysine methylation substrates with their PKMT suggests complex networks

Each PKMT or substrate node of the methylation networks is color-coded according to its functional classification. In Eukarya, 34 PKMTs methylate >65 substrates other than histones. SET7/9 is by far the most promiscuous PKMT, targeting close to half of eukaryotic substrates reported to this day. In contrast to eukaryotes, only 8 unique PKMTs have been identified in prokaryotes and 2 in Archaea. Those interactions, together with the 1018 methylation sites listed in Appendix 1, demonstrate the complexity of this modification and its regulatory potential for the proteome.


MAMMALS
EUKARYOTES
? SFRS2
PLK1 ?
? TCEA1
PAK4 ?
? S27L
SETD6
GATA4 K299
RORα K38
HSP90 K209 K615
SETDB1
K50/51 Tat
DNAJC8 ?
NSD1
EZH2
SMYD2
PCNA K248
K218 K221
K310 RelA
K314 K315 K37
FOXO3 K270 K271
K180 STAT3 K140
K860 K810 pRb
K810 K873
K370 p53
K382
SET8
Numb K158 K163
K372 K373
K373
Interferon regulatory factor 2 K134
K205
GLP
Pdx1 ?
Msx2-interacting protein K2076
Interferon regulatory factor 1 K126
EED K66 K197 K268 K284
K44
DNMT3a
K44
K174
E2F1 K185
K1094 K142
K70 DNMT1
G9a
MYPT1 K442
SET7/9
ERα K302
K105 K123
K239 K165
SUV39H1
Reptin (Ruvbl2) K67
Gfi-1B K8
MTA1 K532
TAF10 K189
mAM K16
MyoD K104
ACINUS K654
FXR K206
CDYL1 K135
WIZ K305
TAF7 K5
HDAC1 K432
C/EBPβ K39
K233 K235 K236 K238
SIRT1
Yap K494
AR K630 K632
ZDH4C8 K300
A-Kinase Anchor protein 6 K604
PPARBP K1006
K78 K89
PCAF
MeCP2 K347
Centromere protein C K414
Cullin1 K73
TTK K708/710
ARCHAEA
K4 K6 K52 K62 K63 K60
Sso7d
? FEN1
? Pri2
? Pri1
? DNA polymerase IV
? DNA polymerase B
? TopoIII
? PCNA1/2
Rfc small subunit K181
GINS15 ?
aKMT
K37
MC1-α
Gδ1-SET
Cren7 K34 K16
VEGFR K631
Dam1 K233
KIN17 K135
SMYD3
SET1
METTL22
PLANTS
FBA (1,2 & 3) K304
Rubisco K14
KTMT K335
RLSMT
Aquaporin (PIP2) K3
SDG7
soloSET
Calmodulin K115
HSP70 K561
Cytochrome c K72
VCP K315
CaKMT
SETD1A
Clm1
VCP-KMT
YEAST
K187 K196 EF3A K789
K15 EF2 K509
K49 L42
K55
K396
K316
EF-1α K30 K386
Efm2
Rkm4
Rkm3
Set13
See1
Efm1
K485
K35
SET2
Metnase
METTL21C
METTL21A
L1ab K46
K48 S18
L23ab K106 K110
K10
L12 K3 K39/407
Rkm5
Rkm1
Rkm2
SET11
PROKARYOTES
K222 K547 K232 K711 K279 K838 K379 K954 K458 K1289
K1056 K891 K829 K624 K488
K426 K353 K273 K226 K205
Rp709
OmpR
K100 K226 K279 K624 K723 K1056
K829 K635 K426 K232 K156
Rp027/028
Hc1-1 K39?
EF-Tu K5
Flagellin ?
K16? K39
L11 K3
cpnSET
EftM
fliB
PrmA
PrmB
AKMT

1.4. Detection of Lysine Methylation

Systematic high-throughput studies helped uncover the global implication of PTMs such as phosphorylation (92, 93) and acetylation (3-5) in different cellular processes. If the terms “phosphorylome” and “acetylome” can now properly be applied to our understanding of those modifications, an exhaustive description of the lysine methylome and the biological functions it regulates has yet to be produced. The challenges still associated with the detection of lysine methylation impede research on this PTM. The small molecular weight of a methyl group relative to other PTMs and the lack of a charge difference between methylated and unmethylated lysine residues leave few options for the detection of methylated lysine residues via direct physicochemical methods.

1.4.1 Targeted discovery of lysine methylation

Given the challenges associated with its detection, the identification of lysine methylation has long relied on the targeted identification of single sites by amino acid sequencing, radio-labelled assays or immunoblotting. Some of the earliest reports of lysine methylation were provided by Edman sequencing (46, 47, 94, 95). This method is reliable and precise enough to detect methyllysine (Figure 1.4A). However, Edman sequencing is time-consuming and necessitates large amounts of the target proteins, making it inapplicable to high-throughput approaches. Introduction of radioactively-labelled methyl donors either in culture media or lysate (Figure 1.4A-B) has also been used to detect methylated proteins in model systems, together with 2D SDS-PAGE or liquid chromatography (50, 96-98). The use of radioactive material on this scale is

however cumbersome and does not allow the identification of specific methylation sites. It also does not indicate what type of residue is labelled, as arginine, histidine, aspartate and glutamate residues as well as the amino terminus of proteins can be the targets of S-adenosyl-L-methionine-dependant methyltransferase (99-101). More recent studies have made use of immunoblotting to explore potential methylation sites on proteins (34). However, pan-methyllysine antibodies suffer from a low level of specificity, sensitivity and low reproducibility between suppliers and lots available. As for generic radioactive methylation assays, immunoblotting with pan-methyllysine antibodies does not allow the determination of the methylation site. Antibodies raised against a specific methylation site have however been invaluable in the identification and *in vivo* confirmation of methylated proteins (Figure 1.4A & Appendix 1).

1.4.2 High-throughput discovery of lysine methylation

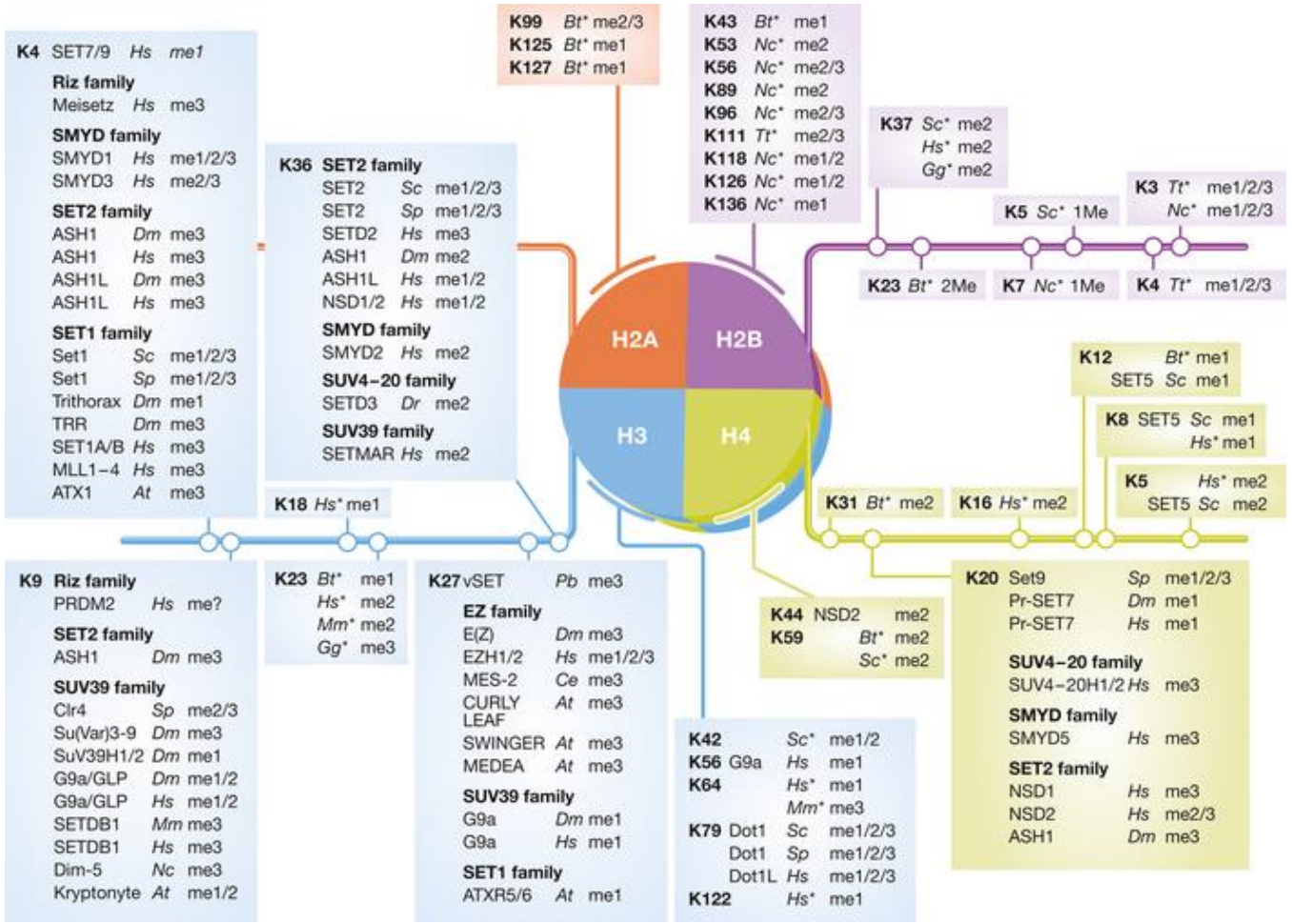
Mass spectrometry is the current method of choice to detect PTMs. This technique is sensitive and reproducible: it can detect the 14 Da shift in the mass of a given peptide corresponding to methyl group and is also capable to determine the residues being methylated (Figure 1.4B). Its use in recent years has nonetheless been impaired by the low abundance, *in vivo*, of methylated sites relatively to their non-methylated. In addition, the small mass difference between a trimethylated and an acetylated peptide (42.05 Da vs 42.01Da) cannot be separated using low resolution mass spectrometers. Fortunately, the precision of recent instruments such as Orbitrap and triple TOF makes easier their respective identification (91, 102). High resolution MS-MS is required to confirm that a lysine is methylated. Previous proteome-scale studies of

acetylation in human cells have used pan-acetyllysine antibodies to enrich acetylated proteins prior to mass spectrometry analysis (3). Low specificity and sensitivity of previously available pan-methyllysine antibodies have limited the use of this approach. Recently, a cocktail of antibodies was developed to enrich methylated peptides (103) and has successfully yielded a significant number of novel methylation sites. This novel approach identified 165 sites across a wide variety of sequences in histones, elongation factors and chaperone proteins in HCT116 cells. In addition, metabolic labelling methods, such as heavy methyl SILAC (104), are being developed and have been applied to the *de novo*, high-throughput discovery of chromatin-specific methylation sites (105).

Recently, a new approach for the detection of methylation was reported, based on known methyllysine-binding protein domains in lieu of a classic antibody fold (Figure 1.4B). Liu et al. used the HP1β chromodomain as bait against cell extracts and systematic peptide arrays to identify a methyllysine-dependant interactome for the protein (106). This led to the discovery of 29 new methylated proteins and demonstrated a role of HP1β in DNA-damage response, driven by its interaction with methylated DNA-PKc. Moore et al. (107) also made use of methyl-binding domains by engineering a generic methyl probe from the L3MBTL1 fold. This construct was then used to identify new targets for the PKMTs G9a and GLP directly from cell extracts, utilizing SILAC and specific PKMTs inhibitors.

Figure 1.3: Methyllysine residues on canonical histone H2A, H2B, H3 and H4.

Bold numbers indicate the methylated residue, italics indicate the organisms in which these modifications are found: At; *Arabidopsis thaliana*, Bt; *Bos taurus,* Ce; *Caenorhabditis elegans*, Dm; *Drosophila melanogaster*, Dr; *Danio rerio*, Gg; *Gallus gallus*, Hs; *Homo sapiens*, Mm; *Mus musculus*, Nc; *Neurospora crassa*, Pb; *Paramecium bursaria chlorella virus*, Sc; *Saccharomyces cerevisiae*, Sp; *Schizosaccharomyces pombe*, Tt; *Tetrahymena thermophila*. Known methylation states are indicated in parenthesis. An * indicates methyllysine residues modified by an unidentified enzyme.


K4 SET7/9 Hs me1
Riz family
Meisetz Hs me3
SMYD family
SMYD1 Hs me1/2/3
SMYD3 Hs me2/3
SET2 family
ASH1 Dm me3
ASH1 Hs me3
ASH1L Dm me3
ASH1L Hs me3
SET1 family
Set1 Sc me1/2/3
Set1 Sp me1/2/3
Trithorax Dm me1
TRR Dm me3
SET1A/B Hs me3
MLL1–4 Hs me3
ATX1 At me3
K36 SET2 family
SET2 Sc me1/2/3
SET2 Sp me1/2/3
SETD2 Hs me3
ASH1 Dm me2
ASH1L Hs me1/2
NSD1/2 Hs me1/2
SMYD family
SMYD2 Hs me2
SUV4–20 family
SETD3 Dr me2
SUV39 family
SETMAR Hs me2
K99 Bt* me2/3
K125 Bt* me1
K127 Bt* me1
K43 Bt* me1
K53 Nc* me2
K56 Nc* me2/3
K89 Nc* me2
K96 Nc* me2/3
K111 Tt* me2/3
K118 Nc* me1/2
K126 Nc* me1/2
K136 Nc* me1
K37 Sc* me2
Hs* me2
Gg* me2
K5 Sc* 1Me
K3 Tt* me1/2/3
Nc* me1/2/3
K23 Bt* 2Me
K7 Nc* 1Me
K4 Tt* me1/2/3
H2A
H2B
H3
H4
K12 Bt* me1
SET5 Sc me1
K8 SET5 Sc me1
Hs* me1
K5 Hs* me2
SET5 Sc me2
K31 Bt* me2
K16 Hs* me2
K18 Hs* me1
K9 Riz family
PRDM2 Hs me?
SET2 family
ASH1 Dm me3
SUV39 family
Clr4 Sp me2/3
Su(Var)3-9 Dm me3
SuV39H1/2 Dm me1
G9a/GLP Dm me1/2
G9a/GLP Hs me1/2
SETDB1 Mm me3
SETDB1 Hs me3
Dim-5 Nc me3
Kryptonyte At me1/2
K23 Bt* me1
Hs* me2
Mm* me2
Gg* me3
K27 vSET Pb me3
EZ family
E(Z) Dm me3
EZH1/2 Hs me1/2/3
MES-2 Ce me3
CURLY LEAF At me3
SWINGER At me3
MEDEA At me3
SUV39 family
G9a Dm me1
G9a Hs me1
SET1 family
ATXR5/6 At me1
K44 NSD2 me2
K59 Bt* me2
Sc* me2
K42 Sc* me1/2
K56 G9a Hs me1
K64 Hs* me1
Mm* me3
K79 Dot1 Sc me1/2/3
Dot1 Sp me1/2/3
Dot1L Hs me1/2/3
K122 Hs* me1
K20 Set9 Sp me1/2/3
Pr-SET7 Dm me1
Pr-SET7 Hs me1
SUV4–20 family
SUV4–20H1/2 Hs me3
SMYD family
SMYD5 Hs me3
SET2 family
NSD1 Hs me3
NSD2 Hs me2/3
ASH1 Dm me3

1.4.3 Prediction-based discovery of lysine methylation

As an alternative approach to high-throughput technologies, other research groups decided to focus on the determination of substrate recognition by PKMTs. A library of peptides spanning the sequence recognized by a PKMT and bearing targeted or systematic mutations is assayed for methylation optima. These, often together with structural studies, allow for the elucidation of the PKMT specificity and the prediction of new substrates. The approach has so far been applied to G9a (108), SETD6 (109), SET7/9 (73, 110) and SET8 (111). More specifically, the methyltransferase activity of SET7/9 toward TAF7 (73), TAF10 (112) and E2F1 (113, 114) was first predicted on the basis of methylation assays performed on a small library of peptides (73). To date, the majority of methylation sites reported for SET7/9 are included within the motif [R/K]-[S/T/A]-K*-[D/K/N/Q] inferred from these assays Appendix 1). However, a recent study expanded the range of SET7/9 putative substrates (110). The broader motif identified in this study [G/R/H/K/P/S/T]-[K/R]-[S/K/Y/A/R/T/P/N]-K*, suggests that SET7/9 may have a more relaxed specificity than previously assessed (110). An extensive peptide array based on a 21 residue peptide encompassing the N-terminus of histone H3 was also used to characterize the sequence recognized by the methyltransferase G9a (108). The team found that G9a recognizes the motif [N/T/GS]-[G/C/S]-[R]-K*-[T/G/Q/S/V/M/A]-[F/V/I/L/A], where K* is the methylated lysine (108). Among the candidates including this motif, CDYL, WIZ, ACINUS, DNMT1, HDAC1 and Kruppel were shown to be methylated both *in vitro* and *in vivo* by G9a. Furthermore, methylated peptides of the CDYL and WIZ target sequences were found to bind HP1β chromodomain, demonstrating that methyllysine effectors can recognize those sites. While peptide arrays

have proven useful in the identification of protein substrates, this approach may not be applicable to all PKMTs. For example, identification of a motif for SET8 based on a peptide array designed from the tail of histone H4 failed to provide new substrates for this PKMT, demonstrating that peptide substrates may lack important structural determinants required for substrate recognition and catalysis (111). In a variation on this approach, full-length protein arrays regrouping over 9000 candidate substrates were used to determine the motif recognized by the methyltransferase SETD6, only known at the time to methylate RelA. A total of 154 total putative targets were predicted. Of these, six substrates were confirmed *in vitro* and of these, PLK1 and PAK4 were found to be methylated in HEK293 cells overexpressing SETD6 (109). In summary, while the proteome-wide characterization of lysine methylation has recently progressed significantly, the success rates of linking a genuine methylation site to a proper biological cue have remained relatively low. However, even with the shortcomings of current methods, efforts from several groups have highlighted the roles played by lysine methylation in a myriad of cellular processes.

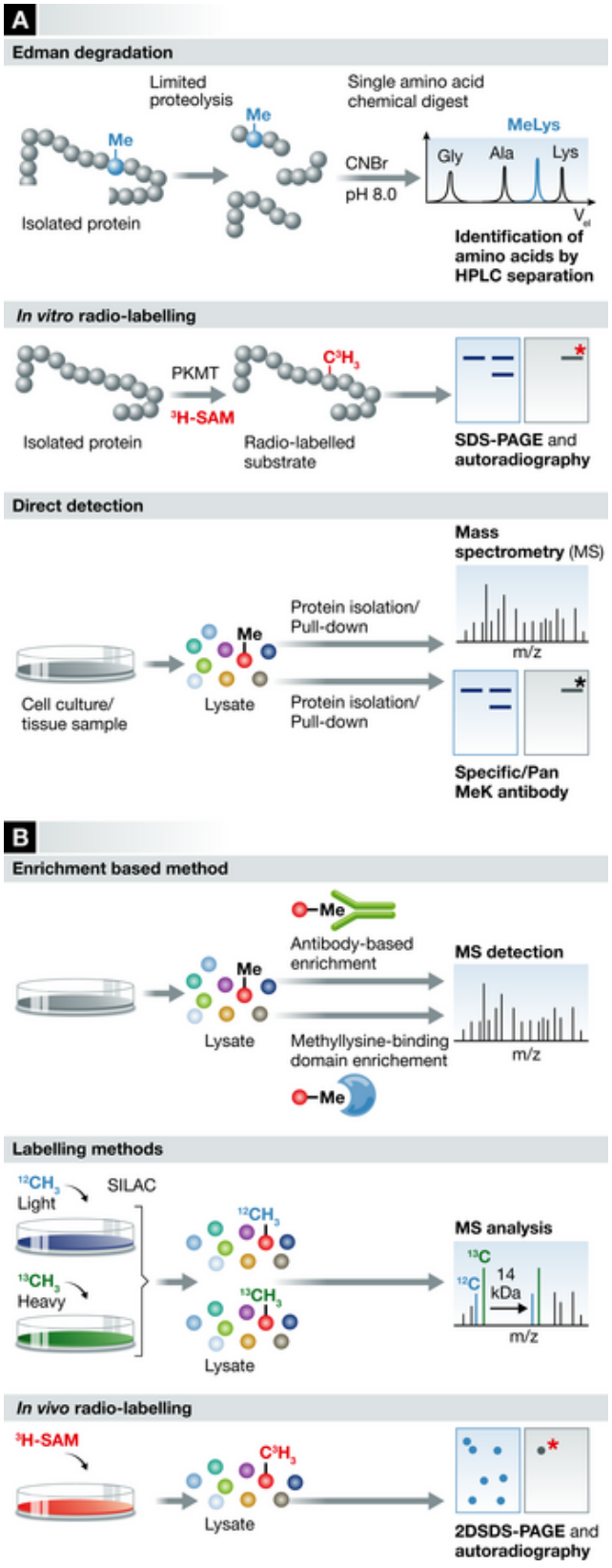
1.5. Functional Roles of Lysine Methylation

1.5.1 Methylation of Histone Proteins

Given their abundance and ease of preparation, histone proteins were one of the first characterized methyllysine proteins (38). Research efforts have subsequently mapped several methyllysine residues on histone proteins and related those modifications to specific biological cues (Figure 1.3) (comprehensively reviewed in (115-118). Among these biological roles, methylation of histones is associated with activity at transcription start sites (H3K4 (119)), heterochromatin formation (H3K9 (58, 59)), X chromosome silencing and transcriptional repression (H3K27 (120, 121)), transcriptional elongation and histone exchange in chromatin (H3K36 (122-126)) and DNA damage response (H4K20 (127, 128) and H3K79 (129)). Our view of this network of modifications increased in complexity with the recent discovery that the protomers of the same nucleosome can be methylated on different lysine residues (130). The combination of different PTMs forms patterns of modifications distributed throughout the genome and these configurations strongly correlate with the state, cell type and gene expression profile of the cell line studied (131-134).

Figure 1.4: Detection of lysine methylation

A) Most common experimental approaches in target-specific detection of lysine methylation. Edman degradation and direct detection either by mass spectrometry or immunoblotting allows for the analysis of in vivo samples. In vitro radio-labelling is most often used to confirm the PKMT associated to a given site. B) Recent approaches used to allow the high-throughput characterization of lysine methylation to overcome the limitations of direct detection approaches. Methylated peptides or proteins can be enriched, either by pan-methyllysine antibodies or methyl-binding protein domains. Alternately, proteins can be specifically labelled (isotopically, radioactively) to allow an easier identification of methylated peptides.


A
Edman degradation
Limited proteolysis
Single amino acid chemical digest
Me
Me
MeLys
Gly
Ala
Lys
CNBr
pH 8.0
Isolated protein
Identification of amino acids by HPLC separation
In vitro radio-labelling
PKMT
^{3}H-SAM
$C^{3}H_{3}$
Isolated protein
Radio-labelled substrate
SDS-PAGE and autoradiography
Direct detection
Mass spectrometry (MS)
Protein isolation/ Pull-down
Me
m/z
Cell culture/ tissue sample
Lysate
Protein isolation/ Pull-down
Specific/Pan MeK antibody
B
Enrichment based method
Me
Antibody-based enrichment
MS detection
Me
Lysate
Methyllysine-binding domain enrichement
m/z
Me
Labelling methods
$^{12}CH_{3}$ Light
SILAC
$^{13}CH_{3}$ Heavy
$^{12}CH_{3}$
$^{13}CH_{3}$
MS analysis
^{13}C
^{12}C
14 kDa
m/z
Lysate
In vivo radio-labelling
^{3}H-SAM
$C^{3}H_{3}$
Lysate
2DSDS-PAGE and autoradiography

1.5.2 Methylation of the transcription apparatus

The study of histone lysine methylation paved the way for the subsequent identification of an important number of sites on other proteins involved in the regulation of transcription and translation (Appendix 1). Among those, methylation of p53 by SET7/9 (135) was initially reported to promote the pro-apoptotic activity of the transcription factor in stimulating its acetylation by p300/CBP (136). Methylation of K370 by SMYD2 was later shown to prevent the methylation of K372 by SET7/9, thus keeping p53 in a "poised" state (79, 137). Methylation of K373 and K382, by G9a (138) and SET8 (139, 140) respectively, were also reported to regulate the function of p53. In the first case, the modification directly inhibits p53 pro-apoptotic activity (138). Methylation of K382 recruits the transcriptional suppressor L3MBTL1 to block the expression of p53 target genes such as *p21* and *PUMA* (140). Altogether, these findings suggest that lysine methylation tunes p53 activity in a variety of ways. Intriguingly, Lenhertz et al. (141) and Campaner et al. (142) reported recently that SET7/9 null mice do not show any defects in p53 acetylation or apoptotic activity. However, the authors did note the possibility that other compensatory mechanisms could exist – as p53 is regulated by redundant mechanisms (143-147). In addition, it remains to be investigated whether other p53 PTMs – such as the methylation of K370 by SMYD2 in control mice or redundant activating mechanisms such as the acetylation of K373 and 382 – buffer the impact of SET7/9 knock-out.

Besides p53, several transcription factors are methylated by SET7/9 and as a result their activity is modulated in different ways. Methylation of K185 inhibits E2F1 apoptotic activity by inducing its proteasomal degradation (113). TAF10 methylation

increases its affinity for RNA polymerase II thereby stimulating the transcription of specific target genes (112). Methylation of K630 on the androgen receptor (AR) stabilizes interaction of its N- and C- terminal domains, allowing transactivation of AR-responsive genes (148) while methylation of FOXO3 on K270 lowers the DNA binding affinity of the forkhead protein (149).

In apparently conflicting studies, SET7/9 was reported to methylate RelA (p65) on both K37 (150) and K314/315 (151). While Ea et al. (150) showed that methylation of K37 is required for NF-κB target gene expression in HEK293 cells following TNFα stimulation, Yang et al. (151) showed that, also in response to TNFα, methylation of K314 and K315 induces the proteasomal degradation of the protein in U2OS cells. It is possible that SET7/9 can methylate both residues and that another regulatory switch directs its activity specifically toward the activation or repression of RelA.

In addition to SET7/9, other methyltransferases also modulate RelA activity. Methylation of K310 on p65 by SETD6 tethers GLP through its ankyrin repeat domain, promoting the deposition of the repressive mark H3K9Me2 on inflammatory response NF-κB target genes (152). In contrast, cytokine stimulation induces methylation of RelA by NSD1, which promotes NF-κB activity through an unknown mechanism (78).

Similar to RelA, lysine methylation is a key PTM in the intricate regulatory network of the retinoblastoma protein (pRb) (153, 154). Methylation of K810 by SMYD2 enhances pRb phosphorylation and promotes cell cycle progression while methylation of K860 by the same PKMT stimulates the binding of the tumour suppressor to L3MBTL1 and induces cell cycle arrest (154). Interestingly, following DNA damage, pRb

methylation on K810 by SET7/9 leads to cell cycle arrest (155). Intriguingly, the same enzyme also methylates the tumour suppressor on residue K873, leading to the recruitment of HP1 to pRb target genes which also triggers cell cycle arrest (156).

Methyllysine residues have also been mapped on other pioneer transcription factors. Methylation of GATA4 by EZH2 regulates association of the activator to p300, regulating the expression of GATA4 target genes (81). Similarly, methylation of C/EBPβ (157) by G9a is important for the transactivation potential of the transcription factor. Conversely, methylation of Reptin by the same enzyme negatively regulates a subset of hypoxia responsive genes (158). Taken together, these studies suggest that lysine methylation of the same residue can lead to different outcomes depending on the cellular context. Overall, it is clear that different methylation sites on the same protein can lead to drastically different effects. These findings also suggest that additional mechanisms such as feedback loops, switches and even demethylation of methyllysine residues (*see below*) will mark which lysine will be methylated during a given cellular process.

1.5.3 Methylation of the translation apparatus

In contrast to the various effects reported for lysine methylation on gene transcription, investigation of the impacts of lysine methylation on translation has yielded far less details. Notably, although methylation of ribosomal proteins has been reported for three decades, the molecular and biological implications of these marks have remained elusive. Evidence that these PTMs are found in mammals, yeast, plants, bacteria and archaea, lends credence to the hypothesis that methylation of the ribosome is important

for its functions. However, systematic mutation of lysine residues known to be methylated failed to promote or impair either ribosomal assembly or cell survival, suggesting that methylation of ribosomal subunits plays a role in a novel, yet unexplored, biological pathway. It was recently suggested that methylation of K106 and K110 of L23ab could influence its precise positioning within the ribosome (159) while methylation of K55 on L42 might modulate association to tRNA (160). However, in both cases, further experimental evidence is needed to provide a definite answer. Interestingly, recent studies have shown that the *Drosophila* Polycomb interactor Corto (Centrosomal and chromosomal factor) recognizes trimethylated K3 of the ribosomal protein L12. This association, mediated by the chromodomain of Corto, recruits the RNA polymerase III and activates transcription of the heat shock responsive gene *hsp70* (161). The involvement of lysine methylation in the nuclear functions of ribosomal proteins (162) suggest that lysine methylation of ribosome components has the potential to modulate or elicit important functions beside its canonical functions. However, given the substantial number of methyllysine residues within the ribosomes (approximately 80), redundant mechanisms could mask the role of lysine methylation in the biology of the ribosome.

1.6. Functional diversity of lysine methylation beyond histones and transcription

In addition to transcription factors and the translation machinery, a wide variety of proteins are methylated by PKMTs, as demonstrated by both targeted and large-scale studies (34-36). Across all kingdoms of life, a critical set of functions is regulated by the methylation of lysine on proteins.

1.6.1 Lysine Methylation & Eukaryotes

Some chaperone proteins are regulated by lysine methylation in eukaryotes. For example, methylation of HSP90 by SMYD2 is involved in sarcomere assembly through titin stabilization (163, 164). Also, SETD1 methylation of HSP70 on K561 promotes the association of the chaperone to Aurora Kinase B and stimulates the proliferation of cancer cells (165). In the yeast kinetochore, methylation of Dam1 by SET1 at the yeast kinetochore is important for proper chromosome segregation during cell division (77, 166) while methylation of DNA methyltransferase DNMT1 by SET7/9 regulates global levels of DNA methylation (167-169). These examples further demonstrate that in eukaryotes lysine methylation is not limited to proteins of the transcriptional apparatus but it affects a wide variety of functions in the cell, many of them yet to be explored.

The role of lysine methylation in plants is even more elusive: the chloroplastic Rubisco large subunit (170) and Fructose 1,6-biphosphate aldolase (171, 172) are both methylated by RLSMT but their activity remains unaffected by the modification. Methylation of aquaporin PIP2 K3 is necessary for E6 methylation in *Arabidopsis thaliana*; yet the roles that these PTMs play remain unknown (173, 174).

1.6.2 Lysine Methylation & Prokaryotes

Similar to eukaryotes, lysine methylation modulates protein functions in bacteria. Methylation of pilin in *Synechocystis* sp. regulates cell motility (175) while methylation of EF-Tu's K56 lowers its GTPase activity and stimulates dissociation from the membrane (176). In the latter case, levels of methylated EF-Tu increase in response to deprivation in carbon, nitrogen or phosphate levels, suggesting that extracellular cues control the activity of lysine methyltransferases (48, 177). Other lines of evidence suggest that lysine methylation of surface proteins might play a role in optimizing bacterial adherence to their environment (see *Diseases and Lysine Methylation* and (178-182)). Recent large scale proteomic studies in *Desulfovibrans vulgaris* (183, 184) and *Leishmania interrogans* (185) reported a large number of methylation sites on a wide variety of proteins (Appendix 1), suggesting that lysine methylation is a prevalent and dynamic post-translational modification in bacteria.

1.6.3. Lysine Methylation & Archaea

Archaea are devoid of histone proteins capable of folding DNA into octameric nucleosomes reminiscent of those found in eukaryotes. Instead, DNA compaction is achieved by a family of small basic proteins (186). As an interesting parallel with lysine methylation in eukaryotes, several of these DNA-binding proteins are methylated on lysine residues. Among those, Sac7d from *S. acidocaldarius* was the first archaeal protein reported to be methylated (187). Other members of the archaeal histone-like DNA

binding proteins, such as CCI, Cren7, Sso7c, are methylated on multiple lysine residues (33, 188-190). However, no role has yet been ascribed to this modification in the context of archaeal chromatin (191). As a possible counterpart to eukaryotes, a SET protein able to methylate the DNA-associated protein MC1-α was identified in the crenarchaea *Methanococcus mazei* (192), illustrating that similar processes bring about lysine methylation across life's domains. Unique to an archaeal organism, the β-glycosidase of the hyperthermophile *Sulfolobus solfataricus* was reported to be methylated on up to five residues, a modification reported to protect the protein from thermal denaturation (193). Further proteomic studies uncovered a large number of proteins methylated in *S. solfataricus* (33). Interestingly, for a subset of these proteins such as the β-glycosidase, lysine methylation enhances the thermal stability of the modified protein (33, 189, 194). Altogether, these findings strongly suggest that lysine methylation in Archaea seems to be as critical to proper proteome function as in Eukarya.

1.6.4 Lysine Methylation of Viral Proteins

Viruses are able to use the arsenal of methyltransferases of their host cell. Burton et al. (195) were the first to report the methylation of the major capsid protein VP1 of the murine polyomavirus. Since then, other examples of methyllysine residue have been discovered in viral proteins. Methylation of the HIV-1 transcriptional activator Tat on K50 by SETDB1 inhibits LTR transactivation (76) while concurrent methylation of K51 by SET7/9 enhances HIV transcription (196, 197), demonstrating that, at least in a specific context, the virus uses the host's PKMTs to ensure proper viral propagation. Some viruses also have the ability to use their own methylation machinery: *Paramecium*

bursara chlorella virus 1 methyltransferase vSET site-specifically methylates histone H3 on K27 to trigger gene silencing (198, 199). Overall, viruses seem to take advantage of lysine methylation mechanisms in their invasion cycle as they do of other PTMs (200-203).

1.7. Lysine Demethylation

Evidence that purified cell extracts showed slow yet detectable activity toward methylated lysine suggested that the methyl moiety added to lysine residues could be removed by dedicated lysine demethylases (KDM) (204, 205). The discovery of the first KDM, LSD1, a flavine amine oxidase able to demethylate mono- and di-methylated histone H3K4, confirmed those initial reports and demonstrated that lysine methylation was part of a dynamic equilibrium. Jumonji-containing proteins, Fe(II)/α-KEG dependant dioxygenase, were subsequently shown to demethylate tri, di- and mono-methyllysine residues in histone proteins (206). In contrast to other KDMs, LSD1 shows a broad specificity and demethylates a large spectrum of methylated proteins. For example, demethylation of the poised K370-methylated pool of p53 by LSD1 is necessary for subsequent methylation and activation by SET7/9 (137). LSD1 also plays a role in the function of other transcription factors such as E2F1 (113), Sp1 (207), STAT3 (208) and MYPT1 (209). In addition to the demethylation of transcription factors, LSD1 also targets the DNA methyltransferases DNMT1 (210) and DNMT3 (75) and the molecular chaperone HSP90 (211). Notably, demethylation of DNMT1 by LSD1 enhances its stability and regulates global levels of DNA methylation in embryonic stem cells (210).

Only two jumonji proteins are reported to demethylate non-histone proteins. JHDM1 (FXBL11) demethylates RelA on K218Me and K221Me, opposing the activation of this transcription factor. Interestingly, given that RelA regulates *fxbl11* gene expression, the demethylase participates in a negative feedback loop that tightly controls the activity of FXBL11 (78). In another study, Baba et al. reported that the Jumonji demethylase PHF2, following activation by Protein Kinase A, demethylates the

transcription factor ARID5B. Demethylation of ARID5B stabilizes the PHF2/ARID5B complex and triggers the recruitment of PHF2's H3K9Me2 demethylase activity to, and regulates the expression of, ARID5B target genes (212). These examples demonstrate that demethylation is a key component of the signalization and modulation dynamics of the proteome.

1.8. Molecular functions of lysine methylation

In comparison to other post-translational modifications, methylation appears to present only limited ways to affect the chemistry of a residue. For example, acetylation of lysine ε-amine neutralizes its positive charge and the addition of a carbonyl's dipole makes possible new types of interactions. Phosphorylation drastically modifies the charge of a protein (-3 per phosphate group) and adds a relatively important mass to an amino acid side chain (95 Da; 80 Da for Ser and Thr phosphorylation). The addition of ubiquitin and ubiquitin-like molecules is linked to cell trafficking, transcriptional regulation and endocytosis (213, 214) and is coupled to a dedicated recognition pathway, leading to degradation by the proteasome (215, 216). Comparatively, methylation of a lysine residue does not modify the side chain's positive charge and causes only a small change in mass of a protein (14, 28 or 42 Da).

Following the large-scale identification of methylated lysine residues in *S. cerevisae*, Pang et al. (36) observed that 43% of these sites corresponded to potentially ubiquitinated residues, thus raising the possibility that methylation increases the stability of proteins by competing with ubiquitination (Figure 1.5A). Accordingly, pulse-chase experiments revealed an increase in the half-life of several proteins. Therefore, methylation can be considered as a regulator of ubiquitination. However, this means of regulating protein turnover rate cannot be applied to the entire proteome, as lysine methylation has been shown to increase global ubiquitination of E2F1, DNMT1, RORα and NF-κB (113, 168, 217, 218).

In the most direct case, methylation of a given lysine residue would preclude the addition of another modification on the same methylation site. However, "methyl

switches", in which methylation of one lysine residue stimulates or inhibits the modification of at least one neighbouring residue (Figure 1.5B) have been observed. For example, methylation of p53 K372 depends on the addition of an acetyl moiety on neighbouring lysine residues (219). Inhibition of cell cycle promoting activity of E2F1 is blocked by methylation of K185, thereby stimulating the ubiquitination of the transcription factor and preventing its phosphorylation by CK2 and ATM as well as its acetylation by PCAF (113). Another example is the methylation of K810 on pRb by SMYD2, which enhances phosphorylation of serine residues 807 and 811 by CDK4, inhibiting its cell cycle repressor activity (153). In *S. cerevisae*, methylation of Dam1 K233 prevents the phosphorylation of S232 and S234 by Ipl1, allowing its optimal phosphorylation at S235, which promotes efficient chromosome segregation (77). Overall, these observations support the fact that lysine methylation is connected to other networks of PTM and consequently to most signalling events.

In addition to controlling the deposition of neighbouring PTMs, lysine methylation creates a binding surface for the recruitment of other proteins (Figure 1.4C). Recognition of methylated lysine residues by chromodomain proteins - part of the Royal domain family - was first reported for histone proteins (58, 59, 220, 221). Members of the Royal domains family can specifically bind methylated lysine residues through an "aromatic cage" formed by combination of hydrophobic contacts and cation-π interactions (220, 222-225). Besides the Royal family, the Plant HomeoDomain (PHD) family also reads methyl-lysine residues. Despite structural divergence between chromodomain and PHD, the methyllysine engages in similar cation-π interactions (226-229). Interestingly, the presence of hydrogen bond networks in the aromatic cages allows

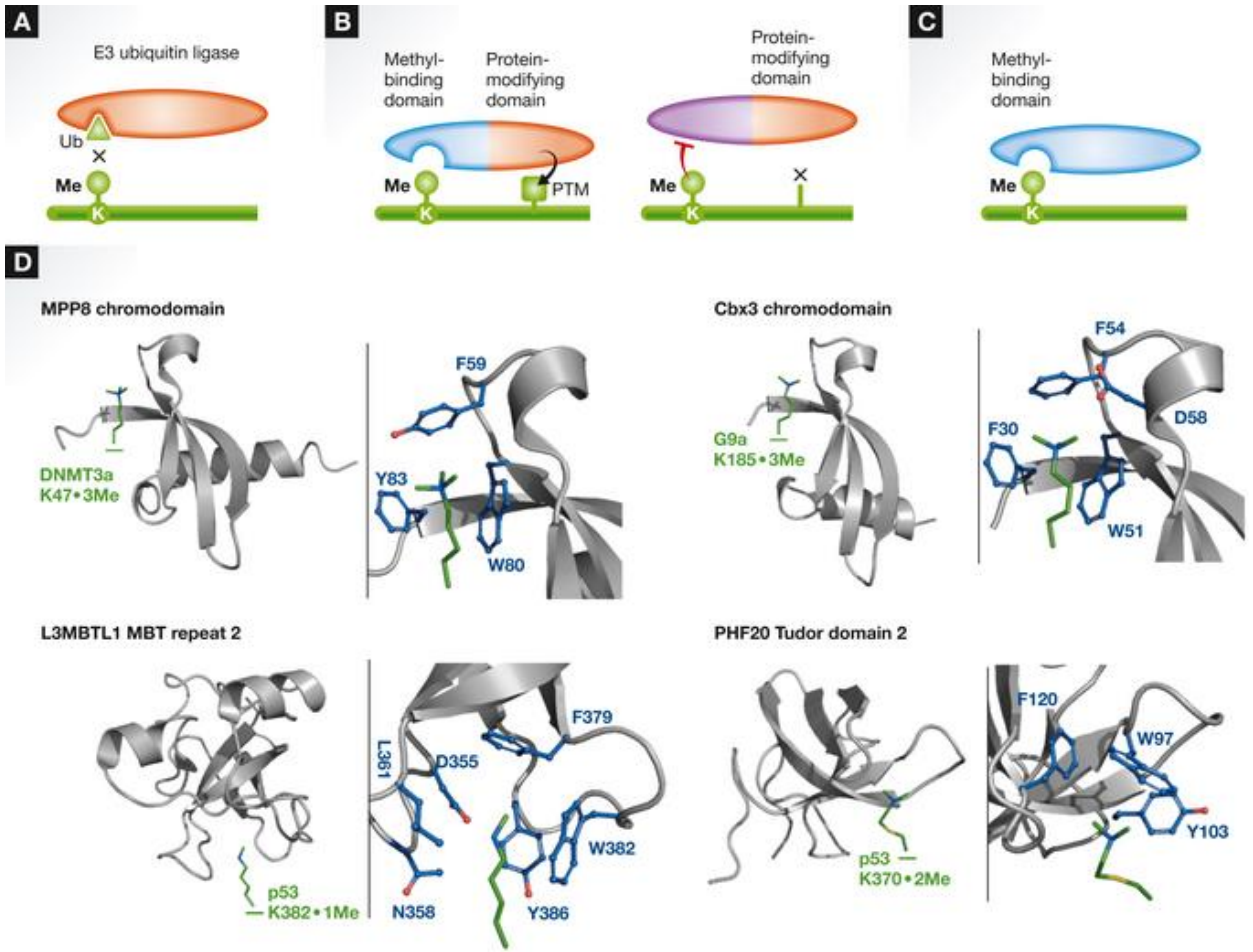
the specific recognition of either mono- or di- methylated over tri-methylated lysine (230) triggering a specific biological response.

For instance, in histone proteins, the Polycomb complex chromodomain recognizes di- or tri-methylated H3K27 (231) while the Eaf3 chromodomain protein recruits the Rpd3S deacetylase complex to regions enriched in H3K36 methylation (126). Among the numerous domains able to recognize methylated lysine residues on histone proteins (232), the Tudor (233) and MBT (230, 234) domains are also able to read specific methyl marks of both histone and non-histone proteins (Figure 1.4C). L3MBTL1 binds methyllysine residues on p53 (140) or pRb (154), and the MPP8 chromodomain associates with the methylated form of DNMT3 (75) (Figure 1.4D). Interestingly, ankyrin repeats also appear to recognize methyllysine residues, as illustrated in the recruitment of GLP to methylated RelA (152).

Lysine methylation can also affect biological outcomes through other mechanisms such as modulation of a protein's DNA affinity (114, 235, 236), resistance to tryptic cleavage (175, 181) and heat denaturation (193). Overall, despite its apparently simple character, lysine methylation regulates the proteome using a surprisingly wide range of mechanisms.

Figure 1.5: Molecular mechanisms of lysine methylation

A) Numerous instances of methylated lysines are able to regulate protein turnover by preventing the ubiquitination of their residue (see Molecular functions of lysine methylation). B) More indirectly than with ubiquitination, methyl "switches" can positively or negatively regulate the installation of other PTMs on neighbouring residues by recruiting other protein modifying enzymes or preventing their association with their substrate. C) "Readers" such as the chromo, PHD finger and MBT domains can specifically bind methylated lysine residues. In addition to numerous groups able to bind methylated lysine on histone tails, a few examples have been reported for non-histone substrates. In addition to HP1, the chromodomains of MPP8 and Cbx3 recognize (above) can bind methylated lysines (green) of non-histone proteins through residues forming an aromatic cage (blue) (PDB ID 3SVM and 3DM1). In addition, mono-methylated K382 and di-methylated K370 of p53 are bound respectively by the second MBT repeat of L3MBTL1 and the second Tudor domain of PHF20 (PDB ID 3OQ5 and 2LDM).


A
E3 ubiquitin ligase
Ub
Me
K
B
Methyl-binding domain
Protein-modifying domain
Me
PTM
K
Protein-modifying domain
Me
K
C
Methyl-binding domain
Me
K
D
MPP8 chromodomain
DNMT3a K47•3Me
F59
Y83
W80
Cbx3 chromodomain
G9a K185•3Me
F54
D58
F30
W51
L3MBTL1 MBT repeat 2
p53 K382•1Me
F379
L361
D355
W382
N358
Y386
PHF20 Tudor domain 2
p53 K370•2Me
F120
W97
Y103

1.9. Disease implications of lysine methylation

Several types of cancer involve the misregulation of PKMTs (237-244). For example, expression of SMYD2 is up-regulated in oesophageal squamous cell carcinoma (245) and bladder cancer cells (153). SMYD3 is overexpressed in breast carcinoma and correlates with tumour proliferation (246), while G9a is overexpressed in hepatocellular carcinoma and contributes to lung and prostate cancer invasiveness (138, 247-249). Accordingly, lysine methylation has been reported to influence processes directly linked to oncogenic pathways, providing a rationale for the involvement of PKMTs in cancer. For instance, methylation of pRb by SMYD2 promotes cell proliferation, possibly through E2F transcriptional activity (153). Similarly, SMYD2 methyltransferase activity prevents the activation of p53 pro-apoptotic function by the opposing modification of K372 by SET7/9 (79). Accordingly, these enzymes are currently explored as efficient cancer markers and potential anti-oncogenic drug targets (241, 242, 244, 250-254).

In addition to cancer, lysine methylation plays key roles in bacterial pathogenicity. Vaccination efforts against typhus' agent *Rickettsia typhi* are targeting the immunodominant antigen OmpB. Interestingly, a critical difference between OmpB from infectious and attenuated strains is the methylation of several lysine residues of the N-terminal region of the protein (255, 256). Chemical methylation of lysine residues on a recombinant peptide re-establishes serological reactivity of the OmpB fragment (255). In a similar fashion, *Mycobacterium tuberculosis* adhesins HBHA and LBP, important for adhesion to host cells, are also heavily methylated (178-182, 257). Similar to OmpB in *R. typhi*, immunological protection potential can be sustained by *Mycobacterium tuberculosis* HBHA only in its methylated form (182). Methylation of lysine residues in

HBHA or LBP *per se* does not appear to affect the adhesive potential of the pathogen but it instead protects the protein against proteolytic cleavage in mouse bronchoalveolar fluid, suggesting a possible role for methylation in the biology and pathogenicity of *Mycobacteria*. This hypothesis is further strengthened by the observations that the related species *Mycobacterium smegmatis* and *Mycobacterium leprae* possess methylated adhesins (181, 257). More recently, methylation of *P. aeruginosa* Ef-Tu K5 was shown to mimic the ChoP epitope of human platelet activating factor (PAF), allowing association to PAF receptor and strongly contributing to bacterial invasion and pneumonia onset (258). Given the increasing need for new and more efficient vaccines, understanding how lysine methylation impacts host-pathogen interaction will open exciting new avenues in understanding the mechanisms of pathogenicity.

1.10. Rationale and hypothesis

Although histone lysine methylation is held as a canonical example of the importance of lysine methylation, it still remains unclear whether it acts as a repository of epigenetic instructions or whether it is a consequence of transcriptional and replicative DNA-based processes. Importantly, an increasing number of methylation of lysine residues (see Appendix I) is now shown to influence cell function outside the context of chromatin. The difficulty to identify these sites however remains the major challenge to their study. The characterization of PKMTs presents one of the most promising avenues to study protein methylation as it can lead to the direct identification of methyltransferase/substrate pairs but also to the characterization of their roles *in vivo*. Among PKMTs, the SMYD, a family of SET proteins characterized by the insertion of a MYND zinc-finger within the conserved catalytic methyltransferase motifs, have been shown to be prolific PKMTs regulating protein function, transcription and developmental processes (section 2.1, 3.1 & 4.1). The range of their protein methyltransferase activity, together with their biological functions, makes the SMYD family interesting candidates to study the mechanisms of lysine methylation. As this project was undertaken, the SMYD protein SMYD2 was reported to methylate histone H3 and the apoptosis regulator p53 (79, 259) and to regulate the function of the latter. Moreover, SMYD2 levels were reported to be misregulated in various types of tumours (245). However, methylation of p53 was unable to explain the oncogenic phenotypes of all SMYD2-expressing tumours. Preliminary results from our laboratory and others (Chapter 2 and personal communications) also suggested that SMYD2 had additional substrates. The identity of those substrates was still unknown as well as the determinants of their recognition by

SMYD2, how the activity of SMDY2 was regulated and the normal biological functions of SMYD2. It was likely though that both the normal and oncogenic functions of SMYD2 depended on its methyltransferase activity. Therefore, we aimed to characterize the biochemical activity of SMYD2 in order to discover novel substrates and functions.

1.10.2 Hypothesises & Objectives

We hypothesized that SMYD2 possessed novel and yet unknown targets dictated by specific recognition mechanisms and that, in turn, those regulate biological processes associated with SMYD2 expression. Specifically, we aimed to:

1) Identify novel SMYD2 substrates
2) Use those substrates as models to understand SMDY2 activity
3) On the basis of these interactions, explore SMYD2 functions *in vivo*.

2. PROTEOMIC ANALYSES OF THE SMYD2 FAMILY INTERACTOMES IDENTIFY HSP90 AS A NOVEL TARGET FOR SMYD2

The work presented in this chapter was published as:

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* These authors contributed equally to this work

Author contributions:

S.L. performed the experiments and analyzed data related to figures 2.1, 2.3, 2.4, 2.5, 2.7, 2.8, 2.9 and 2.10. M.F. performed the experiments and analyzed data related to figures 2.2, 2.4B and 2.6 V.T and J.B. contributed to experiments F.E. and D.F. analyzed data and provided insights on the paper. S.L., D.F. and J-F.C. designed experiments and wrote the paper

2.1 Introduction

Lysine methylation by the SET domain protein lysine methyltransferases (PKMTs) has emerged as an important regulator of protein functions (260) and nuclear processes (261). Initially characterized as histone KMTs, SET domain enzymes have recently been shown to methylate a much broader repertoire of protein substrates. For example, VEGFR1 di-methylation by SMYD3 increases its kinase activity (80). The sensitivity of ER to estrogens is increased by its monomethylation on K302 and thereby stimulates the expression of estrogen-responsive genes (262). Methylation of the elongation factor E2F1 by SET7/9 accelerates its turnover rate whereas methylation of both p53 and pRb by the same enzyme protects their pro-apoptotic function (135, 156). Conversely, methylation of different target lysine residues on pRb and p53 by SMYD2, represses their apoptotic activity (79, 154).

Based on sequence homology, SET domain KMTs are divided in seven groups (263). Among these, the SMYD (Set and MYnD domain) group is characterized by the insertion of a MYND zinc finger (264) embedded within the SET domain (265). SMYD1, SMYD2 and SMYD3 share a high degree of sequence homology and, with the exception of SMYD5, human SMYD proteins harbor at least one C-terminal Tetratrico Peptide Repeat (TPR) domain. Both, TPR and MYND domains are important protein-protein interaction domains (266-268). In recent studies (266, 267, 269-271), interactions with different complexes were shown to modulate SMYD proteins localization and functions. Notably, HSP90 was shown to modulate the methyltransferase activity of SMYD1 (271), SMYD2 (266) and SMYD3 (270) toward lysine 4 of histone H3.

HSP90 is a homodimeric, ubiquitous and essential chaperone composed of three functional domains namely the nucleotide binding domain (NBD), the middle domain (MD) and the dimerization domain (DD). HSP90 is involved in a large variety of biological processes including, but not limited to, heat shock response, signal transduction, steroid signaling and tumorigenesis (272). These roles are finely regulated through the binding of co-chaperones and client proteins as well as post-translational modifications including phosphorylation (273), ubiquitylation (274), acetylation (275, 276) and nitrosylation (277, 278).

In this study, we generated the protein interaction network for SMYD2, SMYD3 and SMYD5. Characterization of each SMYD protein network revealed that they associate with both unique and shared subsets of proteins. Mass spectrometry and biochemical data also revealed that SMYD2 methylates HSP90α on lysine residues located on its NBD and DD, respectively. In addition, we found that HSP90 methylation by SMYD2 is partially reversed by LSD1 and inhibited by the presence of the co-chaperone HOP, suggesting that it can be dynamically regulated *in vivo*. Overall, our data provide insights into novel regulatory networks for three members of the SMYD family of KMTs and evidence that HSP90α is a novel substrate for SMYD2.

2.2 Results

2.2.1 SMYD proteins have non-overlapping protein networks

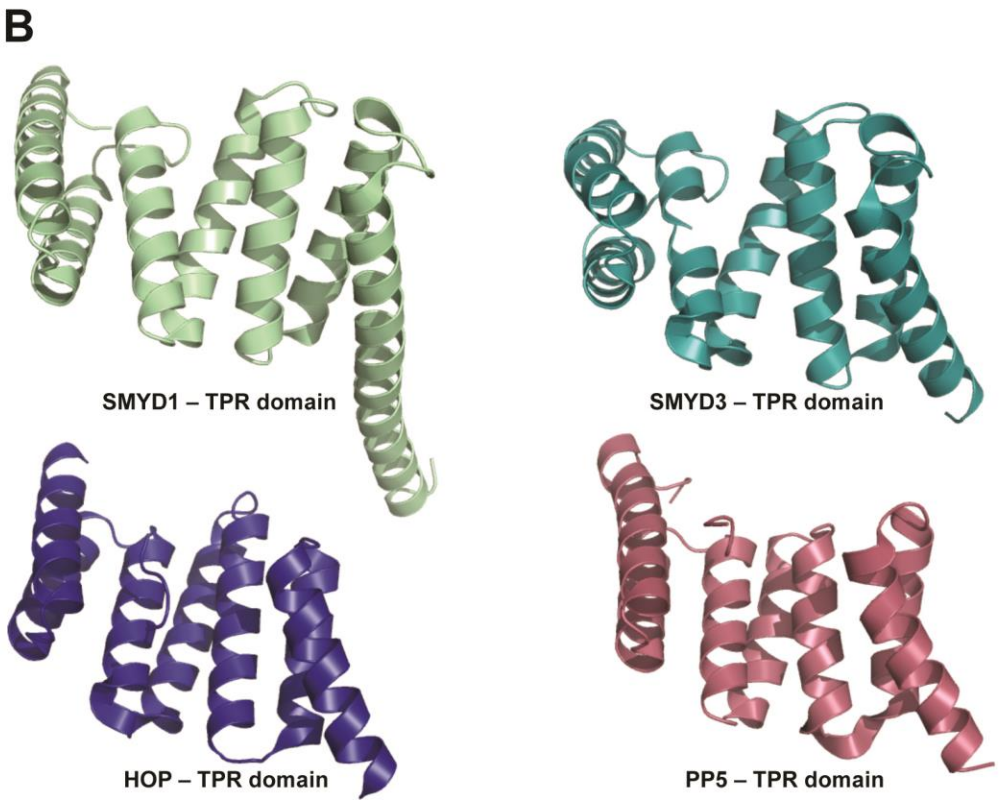
There is a growing interest in better understanding the roles and functions of the SMYD proteins as some have been linked to various forms of cancers (270, 279-283) and biological processes (259, 269, 271, 284-286). To date, only a handful of studies on SMYD interacting proteins have been reported (259, 266-268, 285). In addition, given that specific protein networks stimulate the enzymatic activity of other KMTs (287-293), we posited that SMYD proteins would be regulated by similar mechanisms. As shown in Figure 2.1A, all human SMYD proteins include the presence of the SET and MYND domain. In addition, the highly homologous SMYD1, SMYD2 and SMYD3 share a TPR-like domain found on their C-termini (Figure 2.1B). Comparatively, SMYD4 has a notable addition of a ~240 amino acids region on its N-terminus while SMYD5 lacks any clear domain other than the SET and MYND domain and a C-terminal glutamate-rich extension.

Figure 2.1: Organization of the five human SMYD proteins.

A) Domain organization of the five human SMYD proteins. Purple; SET domain, orange; MYND domain, green; TPR domain, Red; Glutamate-rich region. TPR domains of SMYD4 have been predicted based on sequence homology with SMYD1. B) The C-terminal domain of SMYDs (SMYD1; PDB ID 3N71) SMYD3; PDB ID 3PDN) folds as a 7-repeat TPR fold structurally homologous to HOP (PDB ID 1ELR) and PP5 (PDB ID 2BUG) TPR domains.


A
SMYD1
MYND
SET
TPR
SMYD2
MYND
SET
TPR
SMYD3
MYND
SET
TPR
SMYD4
TPR1
MYND
SET
TPR2
SMYD5
MYND
SET
Ex

FIG. 1

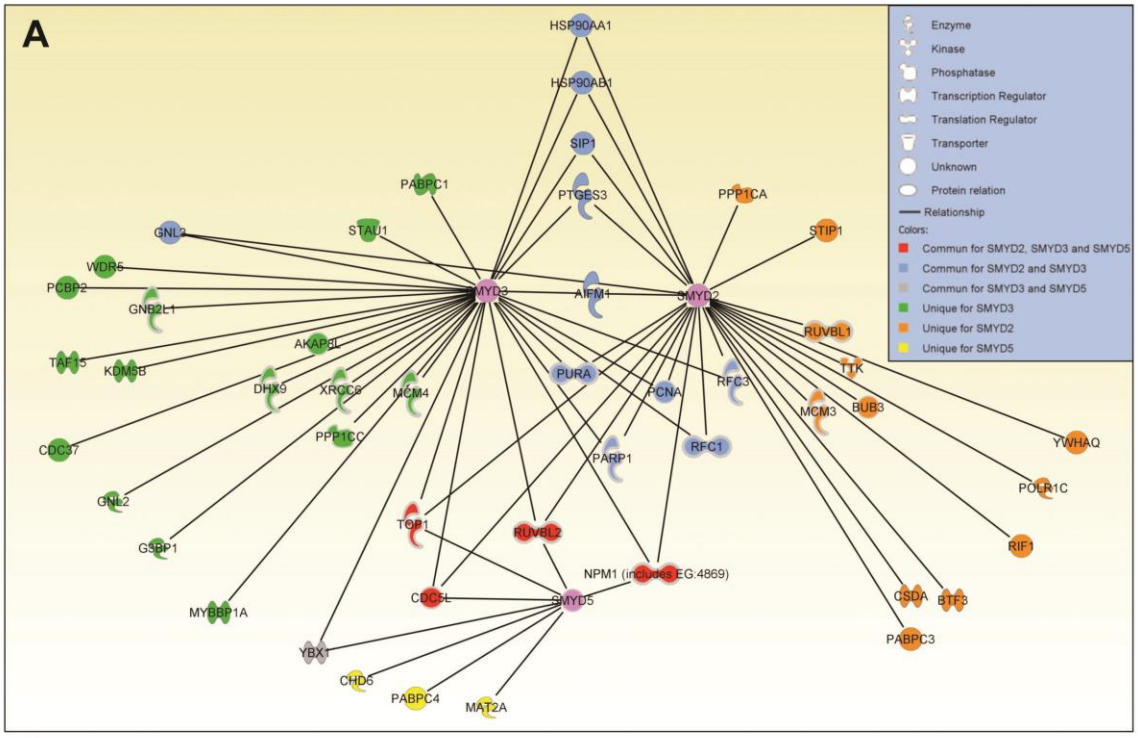

B
SMYD1 – TPR domain
SMYD3 – TPR domain
HOP – TPR domain
PP5 – TPR domain

To investigate whether the structural differences between SMYD2, SMYD3 and SMYD5 underlie the formation of alternative protein complexes, we have undertaken to establish the protein network specific for these SMYD proteins. Briefly, C-terminally FLAG-tagged SMYD2, SMYD3 and SMYD5 were ectopically expressed in HEK293T cells and subsequently immunoprecipitated using anti-FLAG antibodies. After visualization of the proteins by silver staining, SMYD-bound proteins were excised, trypsinized and analyzed by LC-MS/MS as previously described (n=3) (266). Overall, 28, 33 and 9 proteins co-purified with SMYD2, SMYD3 and SMYD5, respectively. Inspection of each SMYD protein network revealed only a limited number of shared protein interactions including nucleophosmin 1 (NPM1), guanine nucleotide-binding protein-like 3 (GNL3), cell division cycle 5-like protein (CDC5L) (Figure 2.2A), Topoisomerase 1 (Top1) and Reptin (RuvB-like 2; RUVBL2) (294-298). The interactions observed for the three SMYD proteins highlight the similarity between SMYD2 and SMYD3, as they share a high number of protein interactions (14 shared interactors) comparatively to SMYD5 (6 between SMYD2 and SMYD5 and between SMYD3 and SMYD5) (Figure 2.2A). Overall, SMYD bound proteins can be divided into three main functional groups. The first consists of proteins known to play a role in chromatin remodeling and histone modifications such as CHD6, WDR5, HP1BP3, TOP1 and JARID1B (299-303). The second group of SMYD interactors includes proteins that are involved in gene expression regulation and DNA damage repair, such as DHX9, PARP1, RIF1 and XRCC6 (304-307). Both groups include members either specific to each SMYD or shared between them. However, none of the two groups appear exclusive to one specific SMYD. The third group, unique to SMYD2 and SMYD3, includes the

molecular chaperone HSP90 (both isoforms α and β) and specific co-chaperones including p23, p50 and HOP. Overall, our data show that SMYD2, SMYD3 and SMYD5 share only a limited number of protein interactors. Consistent with recent studies, we have also found that SMYD2 associates with proteins involved in cell-cycle regulation, DNA damage response and HSP90 chaperone machinery (259, 266).

Figure 2.2: Interaction networks of SMYD2, SMYD3 and SMYD5.

A) Identification of SMYD2, SMYD3 and SMYD5 interaction partners following anti-FLAG immunoprecipitation coupled to MS. The protein networks highlight the common interactors of the three proteins. The combined interactors show enrichment of proteins related to DNA replication and repair, chromatin regulation and HSP90α chaperone machinery. The different classes of proteins are color-coded to reflect their binding to SMYD2, SMYD3 or SMYD5. Proteins are rendered in different shapes matching their specific biochemical functions (see legends in the inlet). This figure was generated using Ingenuity Pathway Analysis software. B) Reciprocal immunoprecipitation experiments using beads coupled with either anti-FLAG (left) or anti-HSP90 (right) antibodies. After extensive washes, proteins were separated on SDS-PAGE gel and detected by Western blot using specific antibodies. GAPDH was used as a loading control to normalize the amount of proteins added to the beads.


A
HSP90AA1
HSP90AB1
SIP1
PABPC1
PTGES3
PPP1CA
GNL2
STAU1
STIP1
WDR5
PCBP2
SMYD3
AIFM1
SMYD2
GNB2L1
RUVBL1
AKAP8L
TAF15
KDM5B
PURA
RFC3
TTK
DHX9
XRCC6
MCM4
PCNA
MCM3
BUB3
PPP1CC
CDC37
RFC1
YWHAQ
PARP1
GNL2
POLR1C
TOP1
RUVBL2
GSPP1
RIF1
NPM1 (includes EG:4869)
CDC5L
SMYD5
CSDA
BTF3
MYBBP1A
PABPC3
YBX1
CHD6
PABPC4
MAT2A
Enzyme
Kinase
Phosphatase
Transcription Regulator
Translation Regulator
Transporter
Unknown
Protein relation
Relationship
Colors:
Commun for SMYD2, SMYD3 and SMYD5
Commun for SMYD2 and SMYD3
Commun for SMYD3 and SMYD5
Unique for SMYD3
Unique for SMYD2
Unique for SMYD5
B
IP : α-FLAG
SMYD5
SMYD3
SMYD2
Mock
α-Hsp90α
α-FLAG
α-GAPDH
IP : α-HSP90
SMYD5
SMYD3
SMYD2
Mock
α-Flag
α-Hsp90α
α-GAPDH

To further confirm the binding of HSP90 to SMYD2 and SMYD3, we performed reciprocal co-immunoprecipitation experiments in HEK293 cells. Lysates were prepared in RIPA buffer and FLAG-tagged SMYD proteins were incubated with anti-FLAG M2-agarose beads. After washing, bound proteins were eluted with FLAG peptide and resolved on a 4–12% SDS-PAGE gel. Consistent with our mass spectrometry data, we detected a specific enrichment of HSP90α with SMYD2 and SMYD3 (Figure 2.2B). Similarly, reciprocal enrichment of HSP90-bound proteins revealed that both SMYD2 and SMYD3, co-eluted with the chaperone. However, similar to the immunopurified proteins from the control samples, we failed to detect an interaction between HSP90α and SMYD5, indicating that a shared feature between SMYD2 and SMYD3 determines their interaction with HSP90.

Concurrently, we sought to determine the region of HSP90 bound by SMYD proteins. HSP90 is composed of three functionally distinct domains: the nucleotide binding domain (NBD), middle domain (MD) and dimerization domain (DD). The full length HSP90 and the corresponding domains were homogeneously purified and added to bacterial lysates containing overexpressed GST or GST-SMYD2. After binding reactions, protein complexes were applied onto glutathione sepharose, washed extensively and eluted with reduced glutathione. Notable binding was observed for full-length HSP90 and its dimerization domain (Figure 2.7). However, similar to the binding reactions performed with the bacterial lysates containing GST alone, no enrichment of NBD or MD was detected. These results indicate that the NBD and MD of HSP90 are accessory to the binding of SMYD2, and likely SMYD3, to HSP90. Overall, our results demonstrate that HSP90 DD harbors the determinants controlling the recruitment of

SMYD2. Also, taken together with recent findings showing that HSP90 plays a role in the activity of both SMYD2 and SMYD3 (266, 270), our results suggest that these two PKMTs may play a role in the HSP90 chaperone functional network.

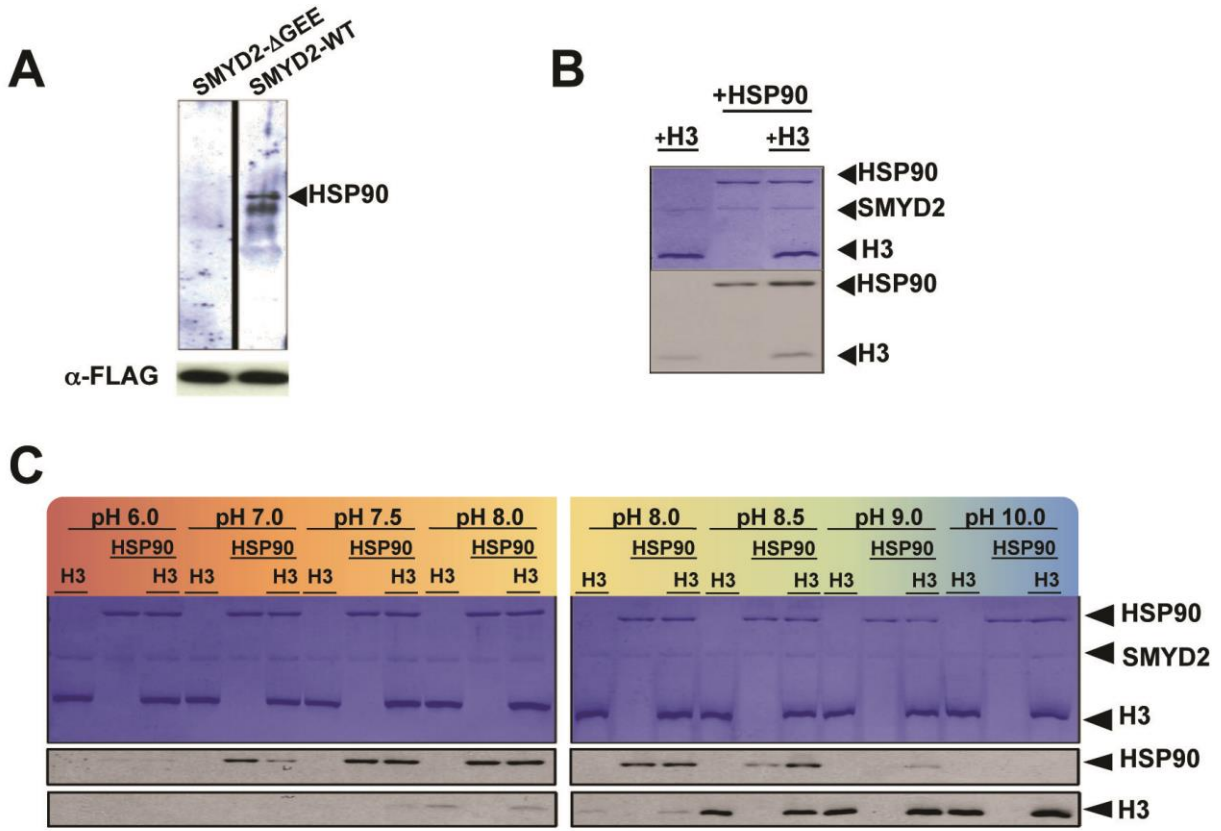
2.2.2 HSP90α is specifically methylated by SMYD2

Following the identification of the protein networks associating with different SMYD proteins, we surmised that specific interacting proteins could be genuine substrates for these enzymes. To examine this possibility, we probed the SMYD2-bound fraction with a pan methyl-lysine antibody and detected a prominent methylated protein migrating at approximately 90 kDa. Methylation of this protein was dependent on SMYD2 methyltransferase activity as no signal could be detected with a catalytically inactive SMYD2-ΔGEE (Figure 2.3A) (266). To confirm the methylation of HSP90α, we performed *in vitro* methyltransferase assays with recombinantly purified SMYD2 and HSP90α and observed that the chaperone is methylated by SMYD2 (Figure 2.3B). To investigate whether other SMYD proteins could mediate the methylation of HSP90, ectopically expressed FLAG-SMYD proteins were immunopurified from HEK293 cells. The enriched proteins were incubated with tritiated AdoMet, HSP90α and/or histone H3 (Figure 2.8A). Fluorography revealed that all human SMYD proteins catalyzed histone H3 methylation while methylation of HSP90α, in contrast to the other SMYD proteins, is specific to SMYD2. To verify whether HSP90α is a target for SET domain proteins known to share substrates with SMYD2 (308-310), methyltransferase assays were performed with SET7/9 and SET8. As shown in figure 2.8B, both SET domain KMTs

were unable to methylate HSP90, providing further evidence on the unique character of SMYD2 comparatively to other known non-histone KMTs. In addition, given that SMYD3 does not methylate HSP90, our results point to divergent roles of SMYD2 and SMYD3 in regards to specific biological processes regulated by HSP90.

Figure 2.3: SMYD2 methylates HSP90α.

(A) Evidence of HSP90α methylation by western blot from HEK293 cells transfected with either wild-type SMYD2 or a catalytically inactive mutant. (B) SMYD2, HSP90, and histone H3 were purified to homogeneity and incubated with 3H-AdoMet, separated on 15% (w/v) SDS–PAGE gel and Coomassie stained (upper panel). Methylated proteins were detected using autoradiography (lower panel). (C) Methylation of HSP90α and histone H3 was performed as in B with the exception that the pH of the buffer has been changed.


A
SMYD2-ΔGEE
SMYD2-WT
HSP90
α-FLAG
B
+HSP90
+H3
+H3
HSP90
SMYD2
H3
HSP90
H3
C
pH 6.0
pH 7.0
pH 7.5
pH 8.0
pH 8.0
pH 8.5
pH 9.0
pH 10.0
HSP90
H3
HSP90
SMYD2
H3
HSP90
H3

Methylation of histone proteins by SET domain KMTs is maximal at alkaline pH (311-315), which likely reflects on the basic environment of the nucleus. Given that HSP90α is predominantly found in the cytosol, we asked if the methylation of HSP90α by SMYD2 would diverge from other known histone KMTs. To determine the optimal pH for HSP90α methylation by SMYD2, we performed methyltransferase assays in conditions ranging from pH 6.0 to 10.0. Consistent with other histone KMTs, pH profile analysis of histone H3 methylation by SMYD2 revealed a sharp maximum of activity at alkaline pH values (pH 9.0-10). However, methylation of HSP90α by SMYD2, either in presence or absence of histone H3, peaked between pH 7.5-8.0 (Figure 2.3C). These results indicate that SMYD2 will preferentially methylate HSP90α and histone H3 at neutral and alkaline pH values, respectively. In addition, these results highlight a hitherto unknown mechanism for SET domain KMTs and, given that SMYD2 has been found in the cytoplasm and the nucleus, suggest that SMYD2 can methylate different proteins pending on its cellular environment.

2.2.3 SMYD2 methylates two residues in two domains of HSP90α

After establishing that HSP90α is a genuine substrate for SMYD2, we sought to identify the methylation sites on HSP90α (Figure 2.4). We first performed an *in vitro* methyltransferase assay with HSP90α constructs corresponding to the NBD, MD and DD. As shown in Figure 2.4A, we found that SMYD2 strongly methylates the dimerization domain of HSP90. In addition, in presence of histone H3, we detected a

noticeable band corresponding to the NBD of HSP90α. These results suggest that at least two lysine residues on HSP90α are methylated by SMYD2.

Figure 2.4: SMYD2 methylates two lysine residues on HSP90α.

A) Full-length HSP90α, NBD, MD and DD (wild-type and mutants) were homogeneously purified and incubated with tritiated AdoMet, recombinantly purified SMYD2 either in presence or absence of histone H3. Proteins were separated on a SDS-PAGE gel and stained by coomassie (upper panel). Methylated proteins were detected by autoradiography (lower panel). HSP90's NBD is methylated at pH ≥ 8.0 and only in presence of histone H3, while its DD is methylated at pH ≤ 8.5. B) MS/MS spectra showing the mono-methylation of HSP90α K615. C) Methyltransferase assays performed, using a gradient of pH ranging from pH6.0 to pH 10.0, as in A using the recombinantly purified wild-type, K209R, K615R or K209R/K615R mutant of HSP90. D) Methyltransferase assays were performed as in A using wild-type HSP90 NBD E), NBD K209R mutant F) or DD K615R mutant. G) Surface representation of yeast HSP90 crystal structure (PDB ID 3CG9) in which both methylation sites are depicted in red and found at the edge of α-helices.

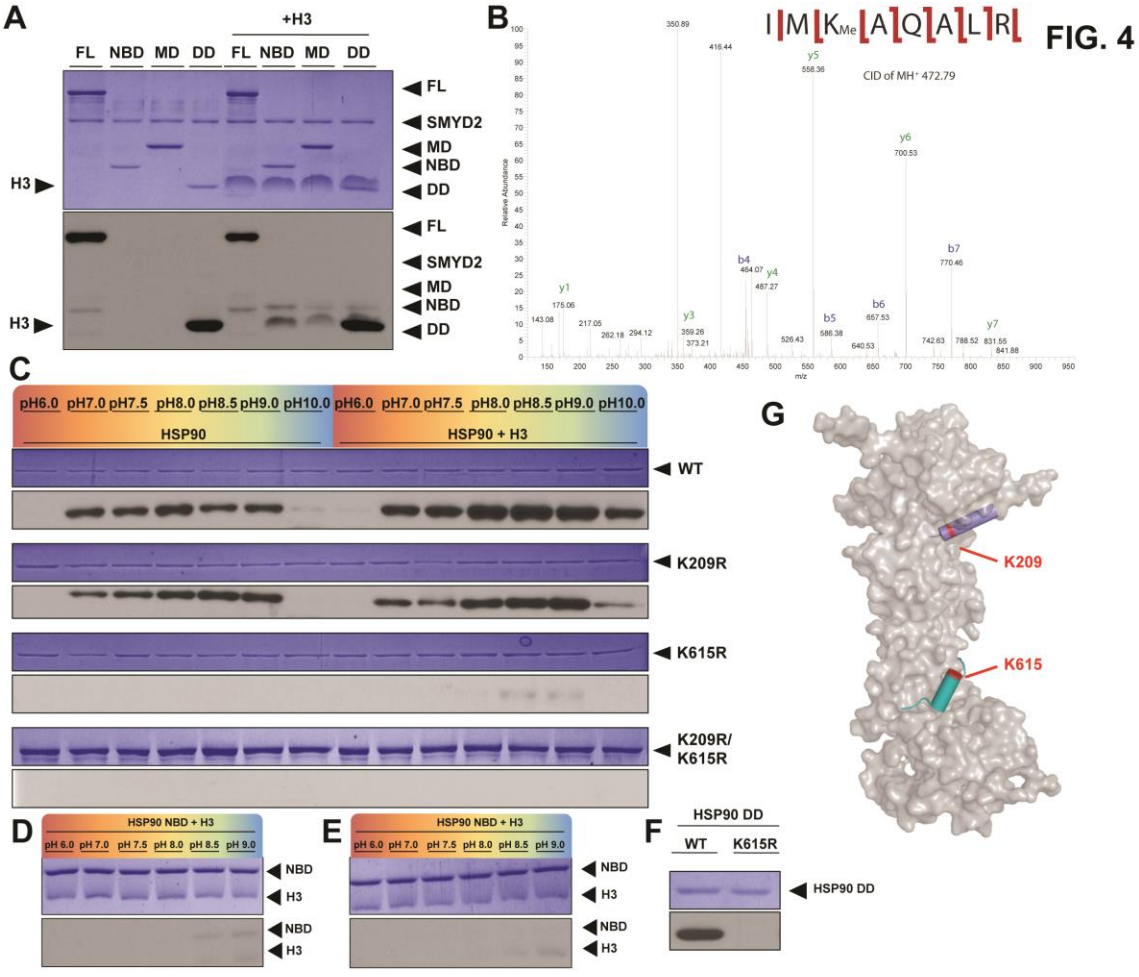

A
+H3
FL NBD MD DD FL NBD MD DD
FL
SMYD2
MD
NBD
DD
H3
B
I M K$_{Me}$ A Q A L R
CID of MH+ 472.79
C
pH6.0 pH7.0 pH7.5 pH8.0 pH8.5 pH9.0 pH10.0
HSP90
HSP90 + H3
WT
K209R
K615R
K209R/
K615R
D
HSP90 NBD + H3
pH 6.0 pH 7.0 pH 7.5 pH 8.0 pH 8.5 pH 9.0
NBD
H3
E
HSP90 NBD + H3
F
HSP90 DD
WT K615R
HSP90 DD
G
K209
K615

FIG. 4

Based on these results, we next sought to identify the lysine residues methylated by SMYD2. SMYD2-FLAG was transiently expressed in HEK293 cells and SMYD2 complexes were purified using anti-FLAG conjugated M2 agarose beads. The enriched HSP90α was detected by silver staining, trypsinized and the peptides were analyzed by HPLC-ESI-MS/MS. Two major methyllysine-containing peptides were identified corresponding to regions neighboring K209 and K615 residues (Figure 2.4B & Figure 2.6). To further confirm that these two amino acids were genuine SMYD2 methylation sites, we substituted K209 and K615 to arginine residues and homogeneously purified the full-length HSP90α mutants. As suggested by our MS/MS data, incubation of SMYD2 with HSP90 K615R mutant resulted in a severe loss of methylation. In addition, consistent with the stimulatory role of histone H3 on the SMYD2 mediated HSP90α K209 methylation, co-incubation of histone H3 with HSP90α K615R partially rescued the lost of methylation of HSP90α K615R mutant (Figure 2.4C). Given that K615 is the predominant methylation site on HSP90α, methylation reactions performed with the full-length HSP90α K209R mutant did not impact the overall level of methylation by SMYD2. However, reactions performed with HSP90α NBD K209R mutant fully abrogated the methylation by SMYD2 (Figure 2.4D-E). Accordingly, methylation reactions performed on HSP90α K209R/K615R double mutant completely impaired methylation by SMYD2. Overall, these results strongly suggest that SMYD2 methylates two lysine residues on HSP90α. Considering that methylation of K209 requires the presence of histone H3 or alkaline pH values and K615 is more readily methylated at neutral pH, our observations also suggest that, although catalyzed by the same enzyme, alternative mechanisms will control the methylation of these two lysine residues.

2.2.4 HSP90 methylation is modulated by its co-chaperones

After identifying K209 and K615 as genuine methylation sites, we sought to identify the regulatory mechanisms controlling the methylation of HSP90α by SMYD2. HSP90α is virtually always found in complex with co-chaperones and client proteins. For instance, the co-chaperone p50 associates to HSP90's NBD and is necessary for the regulation of numerous kinases (316). Upon binding to HSP90 middle domain, AHA1 increases HSP90 ATPase activity and stabilizes HSP90 N-terminal dimer (317). HOP recognizes HSP90 five C-terminal residues through its TPR domain and recruits HSP70 to the HSP90 "chaperone machinery" during certain chaperoning activities (318).

To determine the effects of these three canonical HSP90 co-chaperones, p50, AHA1 and HOP were homogeneously purified and increasing amount of each co-chaperone was incubated with HSP90. HSP90α/co-chaperone complexes were methylated by SMYD2, separated on a 15% SDS-PAGE gel and methylated proteins were detected by autoradiography. As shown in figure 2.5A, co-incubation of increasing amount of p50 and AHA1 did not result in a significant loss of methylation in all ratio assayed while the presence of HOP caused a drastic decrease in HSP90 methylation by SMYD2. Given that HOP mainly associates with HSP90 dimerization domain, the loss of methylation is consistent with our findings that this domain is the predominant site of methylation by SMYD2.

Figure 2.5: Regulation of HSP90α methylation by its co-chaperones and LSD1.

A) Methyltransferase assays were performed with homogeneously purified HSP90, SMYD2 and [^{3}H]-AdoMet. Increasing amounts of co-chaperones were added to HSP90 as indicated on the top of the gel. Proteins were separated, stained and detected as in Figure 2.4 B) Demethylase assays were performed using pre-methylated HSP90 (full-length or NBD) and increasing amount of LSD1 (Abcam). Proteins were separated by SDS-PAGE gel and either detected by Western Blot (full-length HSP90) or stained by coomassie (NBD). Methylated proteins (MeHSP90 or MeNBD) were detected by autoradiography.

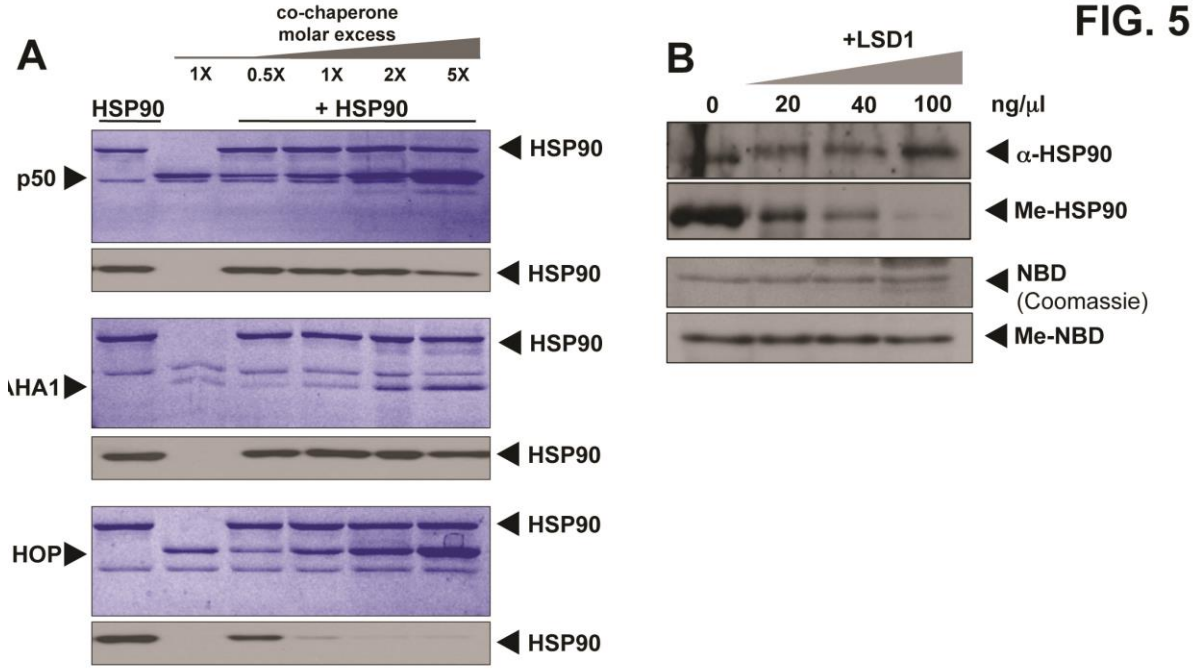

A
co-chaperone
molar excess
1X
0.5X
1X
2X
5X
HSP90
+ HSP90
p50
HSP90
HSP90
HA1
HSP90
HSP90
HOP
HSP90
HSP90
B
+LSD1
0
20
40
100
ng/μl
α-HSP90
Me-HSP90
NBD
(Coomassie)
Me-NBD

FIG. 5

2.2.5 Methylation of HSP90α K615 can be reversed by LSD1

LSD1 is a flavin dependent monoamine oxidase, which demethylates the methylated forms of p53 (319) and histone H3 (320). Given that LSD1 is the major enzyme reported to demethylate non-histone proteins and is also known to demethylate the SMYD2-methylated p53 (Figure 2.5B), we asked whether LSD1 would demethylate HSP90. Pre-methylated [^{3}H]-HSP90α (MeHSP90α) was incubated with increasing amount of LSD1 and MeHSP90α level was visualized by autoradiography. As shown in Figure 2.5B, we observed a noticeable decrease in the methylated form of full-length HSP90α, suggesting that LSD1 can demethylate, *in vitro*, HSP90α dimerization domain. Given that the modulation of K209 methylation by SMYD2 is challenging to monitor on full length HSP90, we repeated similar experiments with methylated HSP90α NBD. In clear contrast with full-length HSP90α, co-incubation of LSD1 with the methylated fragment of HSP90α NBD did not result in a significant loss of methylation, suggesting that the K209 methylation site is not a substrate for LSD1. In addition, our findings support the idea that methylation of the NBD and DD of HSP90α depend on different mechanisms, albeit elicited by the same methyltransferase.

2.3 Discussion

2.3.1 Interaction of SMYD2 with HSP90

Cancerous phenotypes associated with misregulated levels of SMYD proteins (279, 280, 283) and their underlying roles in gene expression regulation (259, 266, 270) were initially rationalized by their histone methyltransferase activity. Subsequently, their activities toward non-histone proteins were put in evidence, notably for SMYD2 and SMYD3 (267, 279, 286, 321). In an attempt to gain a global understanding of these functions, we determined their interaction network, along with their divergent family member, SMYD5.

The identification of SMYD2, SMYD3 and SMYD5 interaction networks suggests a set of similar roles for SMYD proteins involving chromatin modification, control of gene expression and DNA damage response. This is in agreement with previous studies linking SMYD family members with cell cycle progression and developmental processes (259, 266, 268-271, 283-285). Among the interactors we observed as shared between SMYD2 and SMYD3 include the DNA sliding clamp PCNA (Proliferating Cell Nuclear Antigen) together with its Replication Factors C RFC1 and RFC3 and Mini-Chromosome maintenance proteins MCM3 and MCM4. Interestingly, these proteins are known to interact with DNA polymerase δ during DNA damage response (322-328). It is thus possible that both SMYD proteins act in DNA polymerase δ dependent processes, possibly by methylating histone or non-histone proteins. In addition, the interactors shared between SMYD2, SMYD3 and SMYD5 (NPM1, TOP1, GNL3, RUVBL2) are associated with DNA repair and chromatin maintenance during cell cycle, notably through regulation of p53. This observation further link SMYD proteins

with the tumor suppressor, as SMYD2 was already shown to inhibit p53 apoptotic function (286).

The observation that the interaction with the HSP90 chaperone machinery group is restricted to SMYD2 and SMYD3 in our study suggests that it might be a feature of TPR-containing SMYD proteins. This hypothesis is supported by our pull-down assay showing that SMYD2 interacts physically and specifically with the C-terminal dimerization domain of HSP90, a mechanism likely applicable to SMYD1, SMYD3 and SMYD4. It is interesting to note that a TPR-like domain is a widespread feature among the SMYD family in higher eukaryotes and numerous SMYD proteins are known to interact with HSP90 (266, 270, 271). As phosphorylation and ubiquitylation signaling is already known to be regulated by HSP90 through its interaction with kinases (273) and E3 ubiquitin ligases (329), further investigation will determine the influence of HSP90 on the cell methylome.

2.3.2 Dual methylation of HSP90 by SMYD2

Our results illustrate that SMYD2 is unique among the SMYD proteins in its capacity to methylate HSP90α. This also represents the first direct report of lysine methylation of the chaperone, which can be added to a growing list of HSP90 PTMs along acetylation, phosphorylation, nitrosylation and ubiquitylation (273). Our results show that SMYD2 methylates two residues in two distinct regions of the protein, each under specific conditions *in vitro*. While K615 represents the major methylation site in

HSP90 C-terminal dimerization domain, its NBD is methylated at residue K209 at alkaline pH and in presence of histone H3.

Alignment of SMYD2 methylation sites reveals that both K209 and K615 neighboring sequences show only weak sequence homology with previously identified SMYD2 methylation sites (Figure 2.9). Moreover, crystal structure of HSP90 (PDB ID: 2CG9) shows that both methylation sites on HSP90 are located at the edge of α-helices (Figure 2.4G). These observations are in clear contrast with other SMYD2 methylation sites found on histone H3, p53 and pRb which are predominantly found in loops, raising the possibility that SMYD2 might recognize its substrates employing alternative binding modes.

2.3.3 Methylation of HSP90 complexes

Methylation of HSP90 appears to be modulated by different factors. As previously mentioned, methylation of K209 is dependent on the presence of histone H3. This infers that a tripartite complex between histone H3, HSP90 and SMYD2 elicits this additional activity. On the other hand, formation of the complex between HOP and HSP90, contrasting with other co-chaperones assayed, impairs the methylation of K615 by SMYD2, suggesting that the early phases of the HSP90 chaperoning cycle, as HOP mediates the recruitment of HSP70 (318, 330), would be impervious to lysine methylation. Other complexes might affect the installment of lysine methylation on HSP90. For example, K209 of human HSP90α is equivalent to R196 in HSP82 (homolog of HSP90 in budding yeast). Crystal structure of the full length HSP82 (PDBID: 2CG9)

reveals that R196 is located in α-helix 8, a hinge region between HSP90's NBD and MD (Figure 2.11). Analysis of this region of HSP90 shows that K209 might play a role in the stabilization of the closed conformation of the protein by engaging in polar contacts with the middle domain. Alternatively, given that R196 interacts with p50 (PDBID: 1US7), methylation of the structurally equivalent K209 would likely disrupt the formation of the HSP90-p50 complex. On the other hand, the same residue does not participate in the binding of the co-chaperone p23. Consequently, methylation of K209 could affect specific subsets of HSP90 co-chaperones and therefore client proteins, while leaving intact other HSP90 complexes.

2.3.4 Regulation of HSP90 methylation

Our findings that K209 and K615 are regulated, *in vitro*, by diverging mechanisms may be indicative of different roles in the cell. In fact, we have observed that the activity of SMYD2, at more alkaline pH, directs its activity toward the methylation of HSP90 K209 and histone H3 while pH closer to the neutral point are conducive for HSP90 K615 methylation. As a positively charged environment and the presence of histone proteins are hallmarks of the nuclear environment, it is likely that K209 will be methylated in the nucleus. In addition, our findings that LSD1, a nuclear lysine demethylase (320), specifically demethylates K615 raises the possibility that compartment-dependent subsets of complexes would direct the methylation of specific lysine residues on HSP90 by SMYD2. Interestingly, previous reports have shown that the cellular compartmentalization of various SMYD proteins changed during cell cycle

progression (269, 270, 285) and that such shuttling mechanism could modulate the activity of members of the SMYD family of PKMTs (267, 268).

Cross-talk between PTMs is a key mechanism controlling the addition or removal of other marks on proteins. As observed for pRb (331), RelA (332, 333) and DNMT1 (334), our data also point to an alternative model in which other PTMs affect the methylation of HSP90, or inversely, HSP90 methylation modulates other PTMs. HSP90 K615 was identified in a previous study to be ubiquitylated by the ubiquitin ligase CHIP. Based on our results, it is expected that both enzymes would compete for this residue. Accordingly, it is tempting to speculate that methylation of K615 would prevent HSP90α degradation and extend the half-life of HSP90 complexes, an hypothesis recently supported by Pang et al. (335).

2.4 Material and methods

2.4.1 Cell Culture and mammalian expression of SMYD-FLAG constructs and FLAG-Immunoprecipitation experiments

HEK293T cells were grown and transfected as previously described (266). Transfections and immunoprecipitation experiments (either using agarose beads or Dynabeads) are detailed in the Supplementary information.

2.4.2 Mass Spectrometry Analysis

Mass spectrometry experiments were performed and analyzed as previously described (266) with minor modifications (See supplementary Information for details).

2.4.3 Protein Purification, pull-down and *in vitro* enzymatic assays.

Protein purifications and pull-down experiments were performed as previously outlined (336, 337) (see Supplementary Information for details). Methyltransferase and demethylase assays are detailed in the supplementary materials section.

2.5 Supplementary material

2.5.1 Experimental procedures

2.5.1.1 Cell Culture

HEK293T cells were grown in high glucose Dulbecco's Modified Eagle medium (DMEM). The DMEM medium was supplemented with 10% fetal bovine serum and recommended antibiotics and antimycotics. Cells were maintained at 37ºC in a 5% CO_2 humidified incubator.

2.5.1.2 Mammalian Expression SMYD-FLAG constructs

Plasmids containing the open reading frames of SMYD2, SMYD3 and SMYD5 were obtained from Open Biosystems. Primers were designed with an extra 4 nucleotides (CACC) on the forward primer. After amplification of SMYD2, SMYD3 and SMYD5 cDNAs, the PCR products were directionally cloned into the Gateway Topo pENTR vector (Invitrogen). After bacterial transformation and plasmid purification, clones containing the correct DNA sequence were transferred into pCMV2 (Sigma). DNA sequence was verified by sequencing and protein expression was tested by transient transfection of HEK293T cells. C-terminally FLAG-tagged proteins were identified by western blotting using anti-FLAG antibodies (see below).

2.5.1.3 Agarose Beads FLAG-Immunoprecipitation experiments

FLAG-Immunoprecipitation experiments were performed as previously outlined (266). Briefly, plasmids expressing wild type SMYD2, SMYD3 and SMYD5 (Sigma) were transiently transfected into HEK293T using Lipofectamine 2000 as instructed by the manufacturer (Invitrogen). Cells were harvested after 48 hours and then stored at -80ºC. A total $2x10^7$ cells were used in each immunoprecipitation experiment. The cells

were lysed in 1 ml of modified RIPA lysis buffer (50 mM Tris HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.25% Na-deoxycholate, and 1% NP-40) containing the Mini Complete cocktail of protease inhibitors (Roche Diagnostics, Laval, Quebec) for 30 min at 4ºC. Lysates were incubated with pre-washed anti-FLAG M2 affinity gel and incubated in a shaker during 16 hours at 4ºC. The beads were washed five times with 50 mM Tris HCl pH 7.4 and 150 mM NaCl and bound proteins were eluted by incubation with 3xFLAG peptide (150ng/µl). Eluted proteins were then separated on an SDS-PAGE and visualized by silver staining or probed with specific antibodies.

2.5.1.4 Dynabeads Immunoprecipitation

Cells expressing SMYD2, SMYD3 and SMYD5 were harvested and lysed according to the previous protocol. The supernatant of these cells was then incubated with either an IgG control or anti-HSP90α at 4ºC during 16 hours. Protein G coupled Dynabeads (Invitrogen) were washed twice with PBS and added to the reaction mixture. Following an incubation of two hours at 4ºC, beads were washed 4 times with 50 mM Tris HCl pH 7.4 and 150 mM NaCl. Bound proteins were eluted by incubation with 50 µl of SDS-sample buffer without β-mercaptoethanol at 95ºC for 5 minutes. Eluted proteins were then analyzed by SDS-PAGE and visualized by silver staining or blotted on a nitrocellulose membrane and probed with anti-FLAG or anti-HSP90α antibodies.

2.5.1.5 Mass Spectrometry Analysis

After silver stain visualizations, gel bands were excised and processed as previously outlined (266). After tryptic digestion, peptides were extracted from the gel pieces with 5% formic acid (V/V) and 50% acetonitrile (V/V). The peptide solutions

were evaporated to dryness using a speed-vac and dissolved in 5% formic acid. Samples were then analyzed by LC-MS/MS as described previously (266). The peptides were then analyzed on an LTQ-Orbitrap XL (Thermo-Electron). MS/MS spectra were acquired in a data-dependent acquisition mode that automatically selected and fragmented the ten most intense peaks from each MS spectrum generated. The full MS spectra scan was performed on the LTQ-Orbitrap XL with a 60,000 resolution.

Peak lists were generated from the MS/MS .raw file using DTASupercharge (version 2.0a7, http://msquant.sourceforge.net/) to produce .mgf files. The .mgf files were then analyzed and matched to the 86845 protein sequences of the forward and reverse *Homo sapiens* International Protein Index (IPI) protein sequence database version 3.66 (European Bioinformatics Institute). The search was performed using the Mascot database search engine version 2.1.0.4 (Matrix Science) with Trypsin as digestion enzyme, carbamidomethyl of cysteine as a fixed modification and methionine oxidation, mono-methyl, di-methyl and tri-methyl lysine as a variable modification. Only the *Homo sapiens* protein sequences were selected since all experiments were performed on a human cell line. Peptides and MS/MS mass tolerances were set at ± 7 ppm and 0.5 Da, respectively, with 3 miss-cleavages allowed. An ion score cut-off of 30 was used for acceptance of individual MS/MS spectra. The protein significance threshold was set to 0.01 ($p < 0.01$) to ensure a false positive rate of less than 1%. Protein hit required at least one "bold red peptide", i.e. the most logical assignment of the peptide in the database selected to be considered. Finally, only peptides with a significance threshold of 0.01 ($p < 0.01$) were selected.

We developed an approach for the filtering of interaction datasets that takes into account multiple parameters. First, only the proteins that passed the MS/MS identification criteria described in the experimental section were considered. Then, we set-up a cutoff based on the number of peptide observed in the control versus experiment for every proteins. In particular, we used the number of peptide identified per protein as a filtering criterion to subtract proteins observed in the control experiments from the SMYDs immunoprecipitation experiments. The proteins that were present with approximately the same number of peptides in the controls and the immunoprecipitation of SMYDs were removed, which represent the majority of proteins observed in the control experiments. The proteins that had at least 3 times more peptides in the SMYDs immunoprecipitations than the control were kept. All of the SMYDs and corresponding immunoprecipitation experiments were repeated 3 times. Finally, protein had to be identified in at least 2 out of the 3 immunoprecipitation experiments to be kept in the list of interactors.

2.5.1.6 Cellular Protein Extraction and Western Blots

Cell extracts were prepared by lysing the cells in a modified RIPA buffer as outlined above. Proteins were separated on 4–12% SDS-PAGE gel (NuPAGE, NOVEX, San Diego) and then transferred to a nitrocellulose membrane. Western blot analyses for SMYD2, SMYD3, and SMYD5 expression were performed using monoclonal horseradish peroxidase-conjugated mouse anti-FLAG M2 antibodies (Sigma). GAPDH was used as a loading control and detected using a polyclonal rabbit anti-GAPDH antibody. Membranes were then probed with horseradish peroxidase-labeled goat anti-

rabbit IgG or goat anti-mouse secondary antibody (Dako Cytomation Inc., Mississauga, ON).

2.5.1.7. Protein Purification

The sequence encoding human HSP90α (residues 7-728), its NBD (residues 9-236), MD (residues 287-555) and DD (563-732) were PCR-amplified and cloned in a modified version of pET3d. Similarly, cDNAs corresponding to full-length AHA1, p50 and HOP were amplified by PCR and cloned in the same vector as for the HSP90α fragments (338). SMYD2 and p23 PCR fragments were inserted in a modified version of pET vector containing a sequence corresponding to a hexahistidine tag and the Small Ubiquitin-like Modifier (SUMO). Each protein was expressed in BL-21 Rosetta cells (Novagen) at 18ºC for 16 hours. For HSP90α, p50 and HOP, the cells were lysed by sonication in 50mM sodium phosphate (pH 7.0), 500 mM NaCl, 5mM β-mercaptoethanol and the soluble fraction was purified by metal affinity. After TEV cleavage during 16 hours at 4ºC, the protein solution was further purified by size exclusion chromatography (S200 Superdex for full-length HSP90 and S75 superdex for HSP90 NBD and DD and p23). AHA1 was purified as detailed before (339). Site-directed mutagenesis was performed using QuickChange site-directed mutagenesis kit (Stratagene) and mutations were confirmed by sequencing.

2.5.1.8 *In vitro* methyltransferase assay

Unless indicated, 60 ng of SMYD2 were incubated with 300 ng of HSP90α and 0.18 μCi of ^{3}H-labelled AdoMet for 2 hours at 30°C in 50mM Tris-HCl pH 8.0. For the pH profiling assays, 50mM sodium phosphate and CHES were used for pH 6.0 and 10.0

respectively. The reactions were stopped with the addition of loading sample buffer and the proteins were separated on a 15% SDS-PAGE. The methylated proteins were detected by fluorography as previously outlined (340). In order to prepare fully methylated HSP90, SMYD2 was incubated with HSP90 and a molar excess of AdoMet during 4 hours at 30ºC. Methylated HSP90 (HSP90me) was further purified by size exclusion chromatography on a Superose 6 pre-equilibrated in 20 mM Tris-Cl pH 8.0, 500 mM NaCl.

For the demethylase assay, increasing amounts of recombinant LSD1 (Abcam) were incubated with pre-methylated full-length HSP90 or its NBD during 1 hour at 37 °C in 50mM HEPES pH 8.0, 25% glycerol. The reactions were stopped with the addition of sample loading buffer and the proteins were separated on a 15% SDS-PAGE. Full-length HSP90 was normalized by Western blot (anti-HSP90 [S88] Abcam) while the NBD was normalized by Coomassie staining. As for methyltransferase assays, methylated proteins were detected by autoradiography.

2.5.1.9. Pull-down assays

SMYD2 was expressed in fusion with GST in conditions similar to the SUMO-SMYD2 fusion protein. Cells were harvested in PBS buffer and lysed by sonication. The supernatant was then incubated with 10 μg of purified HSP90 domains in PBS buffer during 2 hours at 4ºC. SMYD2 complexes were extracted from the mix by the addition of glutathione sepharose 4B beads (GE Healthcare) and incubated 1 hour at 4ºC. The beads were washed three times with PBS, and the bound fraction was eluted with 50 mM Tris 8.0 and 10 mM reduced glutathione. Eluted proteins were separated by SDS-PAGE gel (15%) and visualized by coomassie staining.

2.5.1.10 ATPase assays

HSP90 was incubated with 100 μM ATP in 100 mM Tris-HCl pH 7.5, 20mM KCl, 6 mM $MgCl_2$ at 37°C. The production of free phosphate was measured by adding 10 μl of the reaction mix to 10 μl of 8 M urea. The mix was then mixed with 70 μl of a 3:1 solution of 0.045% malachite green and 4.2% NH_4NO_3 in 4 M HCl previously incubated at room temperature for 30 minutes and filtered through a 0.22 μm filter. The mix was incubated for 10 minutes and the absorbance was measured at 620 nm.

Table 2.1: SMYD2 interacting proteins

IPI #	Protein Name	Repeat	Mascot Score	# Unique Peptide	% Coverage
IPI00220740	NPM1 Isoform 2 of Nucleophosmin	N1	964	8	43
		N2	229	3	24
		N3	268	2	22
IPI00024281	SIP1 Isoform 1 of Survival of motor neuron protein-interacting protein 1	N1	431	6	30
		N2	359	6	21
		N3	433	5	14
IPI00414676	HSP90AB1 Heat shock protein HSP 90-beta	N1	384	8	14
		N2	1351	2	44
		N3	660	13	22
IPI00221035	BTF3 Isoform 1 of Transcription factor BTF3	N1	292	4	31
		N2	372	5	36
		N3	365	5	36
IPI00784295	HSP90AA1 Isoform 1 of Heat shock protein HSP 90-alpha	N1	2709	39	48
		N2	3102	43	53
		N3	2505	35	42
IPI00003886	GNL3 Isoform 2 of Guanine nucleotide-binding protein-like 3	N1	204	4	7.4
		N2	93	3	18
		N3			
IPI00465294	CDC5L Cell division cycle 5-like protein	N1	172	4	6.2
		N2	185	5	7
		N3			
IPI00023591	PURA Transcriptional activator protein Pur-alpha	N1	142	3	14
		N2	160	4	15
		N3			
IPI00013468	BUB3 Mitotic checkpoint protein BUB3	N1	135	3	11
		N2			
		N3	120	3	11
IPI00021700	PCNA Proliferating cell nuclear antigen	N1	125	2	13
		N2	207	4	21
		N3			
IPI00301154	PABPC3 Polyadenylate-binding protein 3	N1	122	3	5.5
		N2	64	2	4.1
		N3			
IPI00018146	YWHAQ 14-3-3 protein theta	N1	115	2	5.7
		N2	332	7	29
		N3	97	2	5.7
IPI00031801	CSDA Isoform 1 of DNA-binding protein A	N1	107	2	9.7
		N2	98	2	9.5
		N3			
IPI00005179	POLR1C Isoform 1 of DNA-directed RNA polymerases I and III subunit RPAC1	N1	101	2	7.2
		N2	115	2	7.3
		N3			

Table 2.1: SMYD2 interacting proteins (cont.)

IPI00000690	AIFM1 Isoform 1 of Apoptosis-inducing factor 1, mitochondrial	N1	97	2	4.2
		N2			
		N3	84	2	3.4
IPI00375358	RFC1 Isoform 1 of Replication factor C subunit 1	N1	96	2	2.7
		N2	225	4	5
		N3			
IPI00413611	TOP1 DNA topoisomerase 1	N1	92	2	3.9
		N2	125	3	5
		N3			
IPI00015029	PTGES3 Prostaglandin E synthase 3	N1	82	2	8.1
		N2	149	3	19
		N3	133	2	19
IPI00449049	PARP1 Poly [ADP-ribose] polymerase 1	N1	76	2	2.1
		N2	83	3	5.1
		N3			
IPI00009104	RUVBL2 RuvB-like 2	N1	74	2	6.5
		N2	79	2	5.4
		N3			
IPI00031521	RFC3 Replication factor C subunit 3	N1	69	2	3.9
		N2	120	3	5.6
		N3			
IPI00013214	MCM3 cDNA FLJ55599, highly similar to DNA replication licensing factor MCM3	N1	68	2	2.3
		N2			
		N3	300	6	6.6
IPI00375454	RIF1 Isoform 2 of Telomere-associated protein RIF1	N1			
		N2	301	9	5.2
		N3	223	7	4.5
IPI00013894	STIP1 Stress-induced-phosphoprotein 1	N1	157	4	12
		N2	295	8	16
		N3			
IPI00027423	PPP1CA protein phosphatase 1, catalytic subunit, alpha isoform 3	N1			
		N2	228	5	19
		N3	225	5	18
IPI00796459	RUVBL1 20 kDa protein	N1	130	2	11
		N2	105	2	10
		N3			
IPI00151170	TTK Dual specificity protein kinase TTK	N1			
		N2	66	2	4.9
		N3	120	2	6.2
IPI00008524	PABPC1 Isoform 1 of Polyadenylate-binding protein 1	N1			
		N2	94	2	4.9
		N3	131	3	6.1

Table 2.2: SMYD3 interacting proteins

IPI #	Protein Name	Repeat	Mascot Score	# Unique Peptide	% Coverage
IPI00220740	NPM1 Isoform 2 of Nucleophosmin	N1	926	8	43
		N2	582	5	56.7
		N3	1437	14	53.2
IPI00414676	HSP90AB1 Heat shock protein HSP 90-beta	N1	705	11	15.9
		N2	889	17	47.7
		N3	2485	28	56.6
IPI00784295	HSP90AA1 Isoform 1 of Heat shock protein HSP 90-alpha	N1	2109	32	40
		N2	1950	37	48
		N3	2485	47	51
IPI00031812	YBX1 Nuclease-sensitive element-binding protein 1	N1	359	8	30.9
		N2	623	8	30
		N3	765	10	43.8
IPI00021700	PCNA Proliferating cell nuclear antigen	N1	265	4	20.7
		N2	285	5	21
		N3			
IPI00008524	PABPC1 Isoform 1 of Polyadenylate-binding protein 1	N1	250	4	8.2
		N2	325	5	8.5
		N3	337	4	9
IPI00644712	XRCC6 ATP-dependent DNA helicase 2 subunit 1	N1	241	4	7.7
		N2			
		N3	515	10	23
IPI00005705	PPP1CC Isoform Gamma-1 of Serine/threonine-protein phosphatase PP1-gamma catalytic subunit	N1	208	4	14.2
		N2			
		N3	147	2	9.9
IPI00012442	G3BP1 Ras GTPase-activating protein-binding protein 1	N1	186	2	7.3
		N2	78	3	4.6
		N3	409	7	23.6
IPI00023591	PURA Transcriptional activator protein Pur-alpha	N1	183	4	21.4
		N2	253	3	17.2
		N3	170	2	15.2
IPI00449049	PARP1 Poly [ADP-ribose] polymerase 1	N1	183	4	4.2
		N2			
		N3	318	6	6.2
IPI00015808	GNL2 Nucleolar GTP-binding protein 2	N1	177	4	6.6
		N2	133	3	5
		N3			
IPI00005024	MYBBP1A Isoform 1 of Myb-binding protein 1A	N1	169	4	3.2
		N2	510	10	7
		N3	409	9	6.5
IPI00012066	PCBP2 poly(rC) binding protein 2 isoform b	N1	164	2	8.6
		N2	120	4	17
		N3			

Table 2.2: SMYD3 interacting proteins (cont.)

IPI00465294	CDC5L Cell division cycle 5-like protein	N1	159	4	5.4
		N2			
		N3	406	9	14.6
IPI00000690	AIFM1 Isoform 1 of Apoptosis-inducing factor 1, mitochondrial	N1	148	3	6
		N2			
		N3	129	3	7.3
IPI00795318	MCM4 cDNA FLJ54365, highly similar to DNA replication licensing factor MCM4	N1	146	2	2.8
		N2	156	2	3
		N3			
IPI00003886	GNL3 Isoform 2 of Guanine nucleotide-binding protein-like 3	N1	141	2	4.7
		N2			
		N3	288	6	13.6
IPI00000001	STAU1 Isoform Long of Double-stranded RNA-binding protein Staufen homolog 1	N1	122	2	4.7
		N2	119	3	5
		N3	64	2	2.1
IPI00413611	TOP1 DNA topoisomerase 1	N1	122	3	6
		N2			
		N3	246	6	10.2
IPI00641950	GNB2L1 Lung cancer oncogene 7	N1	110	2	7.2
		N2	623	9	17
		N3	441	14	29.1
IPI00013122	CDC37 Hsp90 co-chaperone Cdc37	N1	100	2	7.9
		N2	125	3	8.5
		N3			
IPI00375358	RFC1 Isoform 1 of Replication factor C subunit 1	N1	94	2	2.2
		N2	185	4	4.3
		N3			
IPI00297455	AKAP8L A-kinase anchor protein 8-like	N1	92	2	4.5
		N2	102	2	5
		N3			
IPI00020194	TAF15 Isoform Short of TATA-binding protein-associated factor 2N	N1	90	2	3.4
		N2	125	3	5.2
		N3			
IPI00031521	RFC3 Replication factor C subunit 3	N1	76	2	3.9
		N2			
		N3	73	2	3.9
IPI00878947	HP1BP3 Heterochromatin protein 1, binding protein 3	N1	74	3	6.7
		N2	187	4	10
		N3			
IPI00024281	SIP1 Isoform 1 of Survival of motor neuron protein-interacting protein 1	N1	66	2	4.3
		N2	85	2	5.1
		N3			
IPI00009104	RUVBL2 RuvB-like 2	N1	84	2	2.2
		N2	100	2	4.5

Table 2.2: SMYD3 interacting proteins (cont.)

IPI00877939	JARID1B cDNA FLJ16281 fis, clone NT2RI3003104, highly similar to Homo sapiens Jumonji, AT rich	N1 N2 N3	51 52	2 2	2 2
IPI00844578	DHX9 ATP-dependent RNA helicase A	N1 N2 N3	 408 768	 7 15	 15 14.3
IPI00005492	WDR5 WD repeat-containing protein 5	N1 N2 N3	150 155	3 3	5 5.6
IPI00015029	PTGES3 Prostaglandin E synthase 3	N1 N2 N3	 66 95	 2 2	 17 18.8

Table 2.3: SMYD5 interacting proteins

IPI #	Protein Name	Repeat	Mascot Score	# Unique Peptide	% Coverage
IPI00220740	NPM1 Isoform 2 of Nucleophosmin	N1	274	5	18.1
		N2			
		N3	72	2	3.4
IPI00012726	PABPC4 Isoform 1 of Polyadenylate-binding protein 4	N1	165	5	9.3
		N2	150	4	4.3
		N3			
IPI00465294	CDC5L Cell division cycle 5-like protein	N1	117	2	3.2
		N2	121	2	3.7
		N3			
IPI00413611	TOP1 DNA topoisomerase 1	N1	100	2	3
		N2			
		N3	107	2	3.2
IPI00031812	YBX1 Nuclease-sensitive element-binding protein 1	N1	97	2	12
		N2	97	2	12
		N3	65	2	10.5
IPI00009104	RUVBL2 RuvB-like 2	N1	88	2	5.8
		N2	89	3	5.9
		N3			
IPI00168607	TTC21A tetratricopeptide repeat domain 21A isoform 1	N1	82	3	2.3
		N2	105	4	4.2
		N3			
IPI00639836	CHD6 17 kDa protein	N1	74	2	7.4
		N2	85	3	8.3
		N3			
IPI00010157	MAT2A S-adenosylmethionine synthetase isoform type-2	N1	69	2	2.5
		N2	106	3	3.1
		N3			

Figure 2.6: SMYD2 methylates K209 of HSP90 NBD

MS/MS spectra showing the methylation of K209 on HSP90.

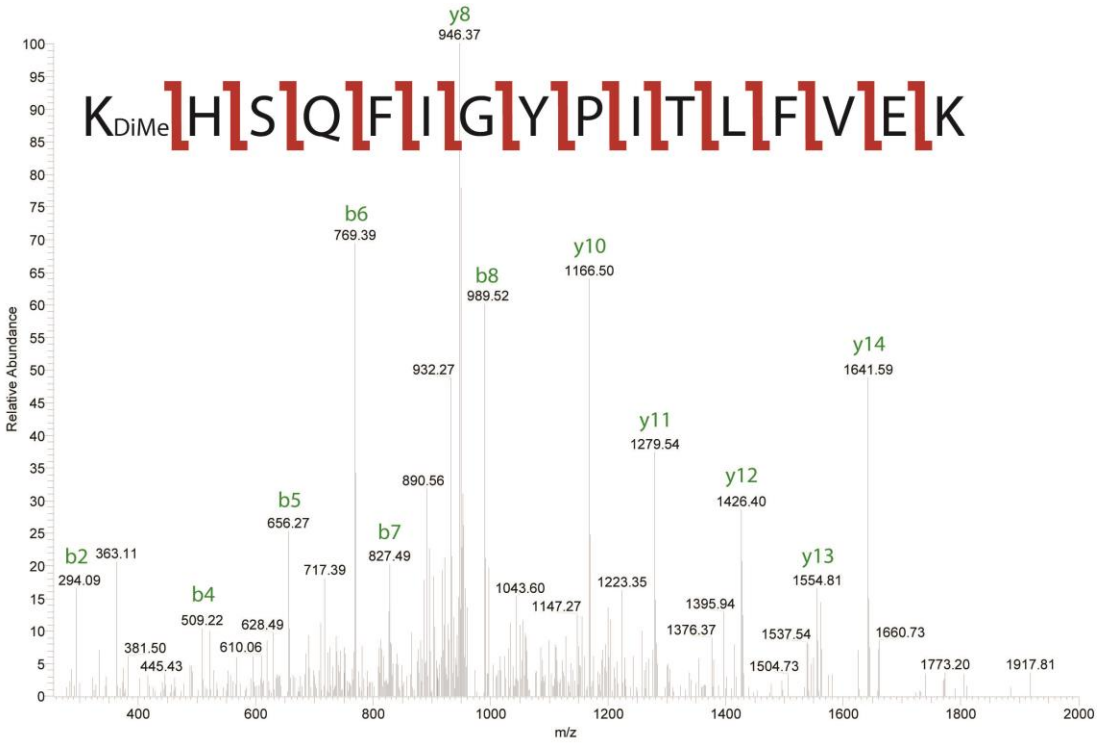

KDiMeHSQFIGYPITLFVEK
y8
946.37
b6
769.39
y10
1166.50
b8
989.52
y14
1641.59
932.27
y11
1279.54
890.56
y12
1426.40
b5
656.27
b7
827.49
b2
363.11
294.09
717.39
y13
1554.81
b4
509.22
1043.60
1147.27
1223.35
1395.94
628.49
1376.37
1537.54
610.06
381.50
445.43
1504.73
1660.73
1773.20
1917.81
Relative Abundance
m/z
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
400
600
800
1000
1200
1400
1600
1800
2000

Figure 2.7: SMYD2 binds the dimerization domain of HSP90

GST-tagged SMYD2 was incubated with and without equal amounts of full-length HSP90 or domains corresponding to NBD, MD or DD. Bound proteins were resolved on a 15% SDS-PAGE gel and stained by Coomassie. Loading controls for GST-SMYD2 fragments are shown on the left hand side of the gel.

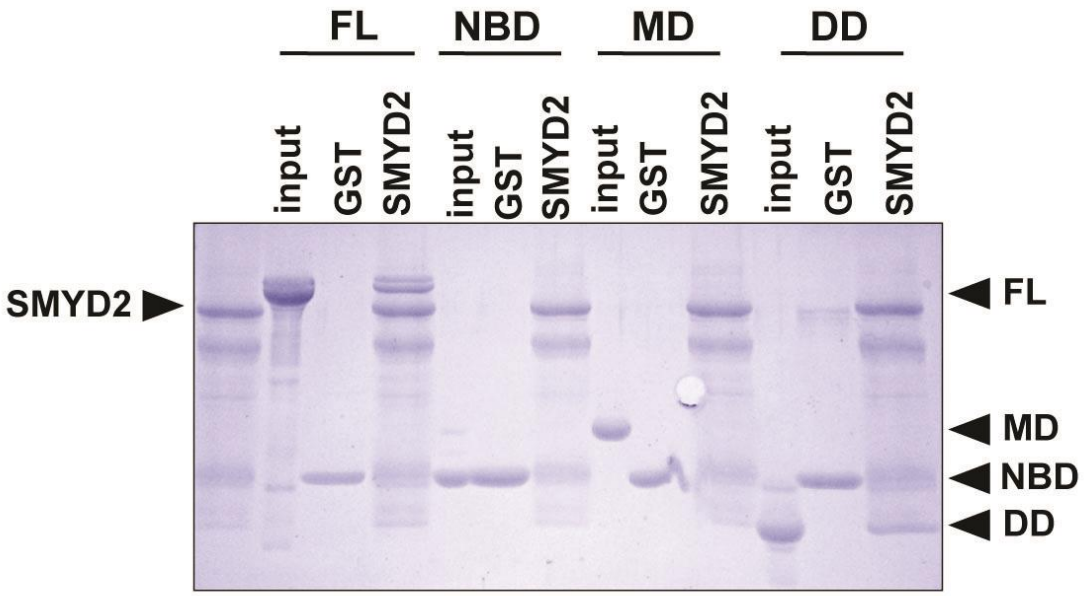

FL
NBD
MD
DD
input
GST
SMYD2
input
GST
SMYD2
input
GST
SMYD2
input
GST
SMYD2
SMYD2
FL
MD
NBD
DD

Figure 2.8 SMYD2 is the only member of the SMYD family to methylate HSP90α

A) Flag-tagged SMYD proteins were expressed in HEK293 cells and purified using Anti-Flag agarose beads. Eluates were incubated with or without purified HSP90α, histone H3 and [^{3}H]-AdoMet, separated on 15% (w/v) SDS-PAGE gel and Coomassie stained (upper panel). Methylated proteins were detected using autoradiography (lower panel). B) SET8, SET7/9, HSP90 and histone H3 were purified to homogeneity and incubated with ^{3}H-AdoMet Proteins were stained and detected as in (A).

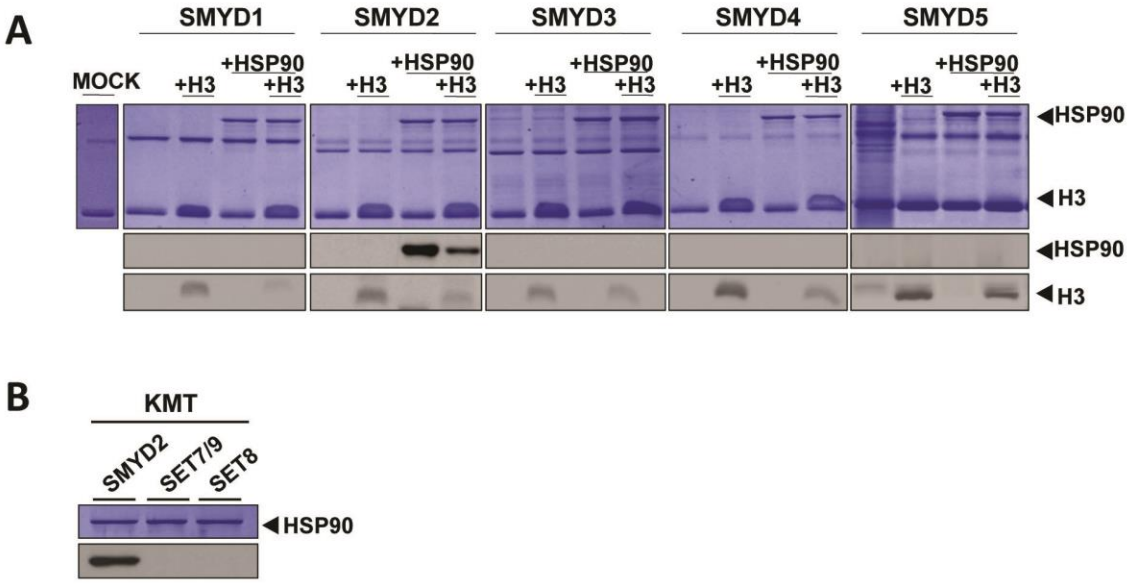

A
SMYD1
SMYD2
SMYD3
SMYD4
SMYD5
MOCK
+H3
+HSP90 +H3
HSP90
H3
HSP90
H3
B
KMT
SMYD2
SET7/9
SET8
HSP90

Figure 2.9: SMYD2 methylation sites do not converge toward a consensus motif

Sequence alignment of known SMYD2 methylation sites on proteins including Retinoblastoma (pRb), p53 and histone H3 (Lys-4 and Lys-36). Positions showing sequence homology are rendered in light blue.


HSP90 K615| WTANMERIMKAQALRDNST
HSP90 K209| ERRIKEIVKKHSQFIGYPI
pRb K860| MVCNSDRVLKRSAEGSNPP
p53 K370| GSRAHSSHLKSKKGQSTSR
H3 K4 | -----MARTKQTARKSTGG
H3 K36 | KSAPSTGGVKKPHRYRPGT

Figure 2.10 Methylation of HSP90 by SMYD2 does not impact its ATPase activity.

A) HSP90 was methylated and re-purified as stated in material and methods and incubated with ATP. Production of phosphate was detected using malachite green at 620nm. Results are shown as linear regression of the accumulation of phosphate during the assay. B) ATPase assays performed with HSP90 methylated in presence of histone H3. Results are depicted as in (A).

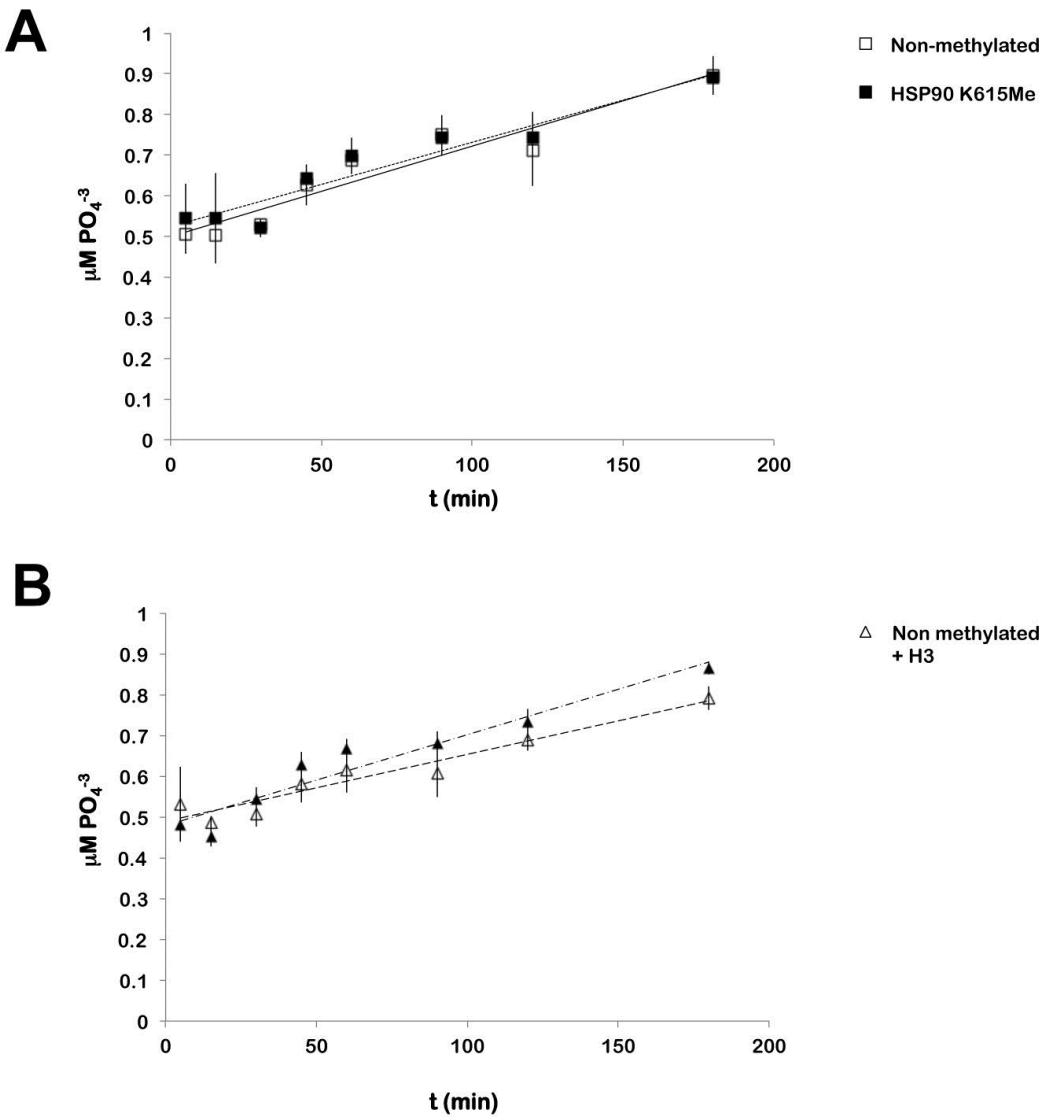
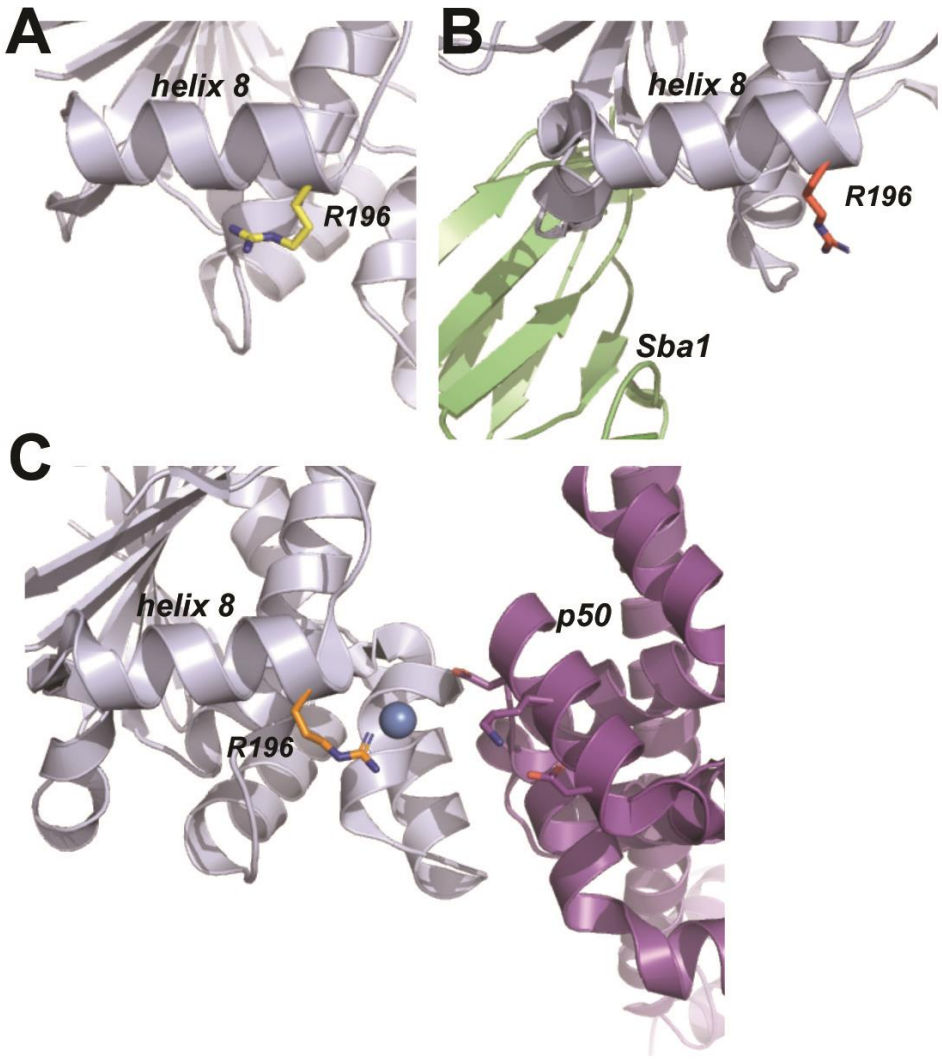

A
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
µM PO4-3
0
50
100
150
200
t (min)
Non-methylated
HSP90 K615Me
B
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
µM PO4-3
0
50
100
150
200
t (min)
Non methylated
+ H3

Figure 2.11 Residue K209/R196 mediates contacts with specific HSP90 co-chaperones

A) Secondary structure of HSP90 hinge region (white) in which the structurally equivalent K209, R196, is rendered as stick with carbon atoms highlighted in yellow. (PDB ID 1AM1). B) Secondary structure of HSP90 NBD domain (white) in complex with Sba1 (green) (PDB ID 1CG9). R196 is rendered as in (A) with carbon atoms are colored in red. C) Secondary structure showing the interaction of HSP90 NBD domain (White) with p50 (purple) (PDB ID 1US7). R196 is rendered as in A with carbon atoms highlighted in orange and the water is depicted as a blue sphere.


A
helix 8
R196
B
helix 8
R196
Sba1
C
helix 8
p50
R196

3. DISCOVERY OF SUBSTRATES FOR A SET DOMAN LYSINE METHYLTRANSFERASE PREDICTED BY MULTISTATE COMPUTATIONAL PROTEIN DESIGN

The work presented in this chapter was published as:

Sylvain Lanouette*, James A. Davey*, Fred Elisma, Zhibin Ning, Daniel Figeys, Roberto A. Chica, and Jean-François Couture: Discovery of Substrates for a SET Domain Lysine Methyltransferase Predicted by Multistate Computational Protein Design. Structure 23:206-15, 2015.

Author contributions:

* These authors contributed equally to this work

S.L. performed the experiments and analyzed data related to figures 3.1A, 3.1C, 3.4, 3.5, 3.6, 3.8 and table 3.3. J.A.D. performed the experiments and analyzed data related to figures 3.1B, 3.2, 3.7, table 3.1 and 3.2. F.E., N.Z. and D.F. analyzed data and provided insights on the paper. S.L., J.A.D., R.A.C and J.-F.C. designed experiments and wrote the paper.

3.1. Introduction

Post-translational modification (PTM) of proteins adds a layer of complexity to the proteome that is critical to biological regulation. Although proteomic studies have enabled the comprehensive study of PTMs such as phosphorylation and acetylation, the characterization of many other types of PTMs remains a significant challenge (3, 92). Among these, lysine methylation - the transfer by protein lysine methyltransferases (PKMTs) of one, two or three methyl groups to the ε-amine of a lysine side chain (60) - is a prevalent PTM associated with critical cellular processes including cell cycle progression, chromosome segregation, and pathogen infection (341). The relatively low abundance and chemically inert nature of lysine methylation hinder its detection by current proteomics methods. Moreover, the expensive and tedious protocols currently used to study lysine methylation limit the high-throughput biochemical characterization of PKMT substrates (342-344).

SMYD2 is a PKMT that plays critical roles in muscle development and myofibril formation (163, 164, 345) as well as proper endodermal development during embryonic stem cell differentiation (346). It is misregulated in oesophageal squamous cell carcinomas (245), bladder tumours (153), leukemia stem cells (347, 348) and doxorubicin-resistant breast cancer (349). The oncogenic phenotypes of SMYD2 depend on its methyltransferase activity (245, 348) which has also been shown to regulate gene transcription (methylation of histone H3 (266) and Estrogen Receptor α (350)), cell cycle progression (methylation of Retinoblastoma protein (351, 352)), apoptosis (methylation of p53 (286)) and oxidative stress (methylation of Poly (ADP-ribose) polymerase 1

(353)). The substrates currently known for SMYD2 are most likely only a subset of its full range of methylation targets.

The crystal structure of the SMYD2-p53 complex shows that substrate binding occurs in a narrow cleft between the catalytic SET domain and a C-terminal tetratrico peptide repeat domain (354-356). As SMYD2 substrates display little sequence or structural similarity, the mechanism by which this interface directs substrate specificity is currently unknown. A better understanding of the structural determinants of SMYD2 specificity would thereby allow for the identification of additional substrates, providing information on its underlying biological functions and disease associations.

We present here a computational protein design (CPD) approach to define a substrate recognition motif for SMYD2. This approach is based on multistate design (MSD), an emerging methodology in CPD that predicts stable protein sequences in the context of multiple backbones instead of a single fixed backbone template (357). We also appraised SMYD2 specificity using methyltransferase assays on both peptide and full-length protein substrates. The recognition motifs derived using both computational and experimental techniques were in remarkable agreement and established that SMYD2 recognizes the LFM-K*-AFYMSHRK-LYK sequence. In combination with bioinformatics analyses and methyltransferase assays, we discovered four previously unknown substrates of SMYD2: SIN3B, SIX1, SIX2 and DHX15, demonstrating the utility of MSD as a discovery tool for the study of PTM enzymes.

3.2. Results and Discussion

3.2.1. Prediction of SMYD2 substrate recognition motif by multistate design

Several CPD protocols have been developed to design the specificity and stability of protein-protein interactions (358, 359). These protocols are typically based on the single-state design (SSD) approach whereby protein sequences are optimized in the context of a single protein complex structure (360-362). In this approach, discrete amino acid side-chain rotamers are first threaded on the fixed polypeptide chain of each binding pair protein, followed by rotamer refinement to improve their packing interactions at the protein-protein interface. A list of ranked sequences is then returned based on their score value following sequence optimization. Additional procedures including protein docking (363, 364) and flexible backbone design (365) can also be incorporated in this process to improve predictions. Recently, MSD, a CPD methodology that utilizes conformational ensembles as inputs instead of a single backbone template, was developed as an alternate approach for the design of protein-protein interfaces (366). MSD improves the prediction of stable and functional protein sequences (367-369) through improved packing interactions resulting from small variations in backbone geometry. Although MSD has been used to recapitulate known binding interactions (370) and to engineer new ones (371, 372), it has not yet been applied towards the prediction and discovery of previously unknown substrates of PTM enzymes.

In this study, we developed a computational approach based on MSD to predict the substrate recognition space of SMYD2. We first generated *in silico* an ensemble of 180 unique backbone configurations of SMYD2 bound to a p53-derived peptide (PDB ID 3S7F, Figure 3.1A) to be used as our MSD input models. This ensemble was prepared

using the coordinate perturbation and energy minimization (PertMin) algorithm that we previously developed (367). PertMin involves the random perturbation of atomic coordinates followed by an energy minimization procedure that forces the calculation to adopt divergent descent trajectories resulting from the small coordinate changes to the input structure. Thus, PertMin results in an ensemble of similar protein structures found at alternate local minima that simulate conformational flexibility. As shown in Figure 3.1B, the resulting p53 peptide conformations in the PertMin ensemble adopt the same relative orientation in the SMYD2 binding cleft and occupy a tight conformational space. These conformations also preserve the interactions that the p53 sidechains (Figure 3.1C) and backbone (Figure 3.1D) make with the SMYD2 binding cleft. Inspection of the ensemble also shows that structural variation of the p53 peptide backbone is lower at positions -1, +1 and +2 relative to the K370 methylation site, suggesting that they dictate recognition through stronger interactions. We thus retained these positions for further design calculations.

Next, we used MSD to optimize each amino acid substitution with the exception of proline at positions -1, +1 and +2 of the p53 peptide in the context of the ensemble, evaluating a total of 57 substitutions. To predict the tolerated amino acid substitutions at each designed position, we calculated the fitness of each substitution by computing the Boltzmann weighted average of its interaction energy with the SMYD2-p53 complex (see Methods) in the context of each member of the ensemble. As a result, the fitness value does not directly reflect binding affinity since the change in free energy (bound *vs*. unbound) for each peptide is not computed. Instead, the fitness value reports on the

ability of each substitution to stabilize the SMYD2-p53 complex while preserving the binding mode represented in the ensemble.

Table 3.1: Fitness values calculated by MSD for amino acid substitutions found at three positions on the p53 peptide

Substitution	Fitness[a] (kcal/mol) at Position		
	-1	+1	+2
A	-62.39	-84.32	-65.32
C	-72.62	-86.53	-75.7
D	-64.14	-64.49	-52.54
E	-69.06	-72.43	-58.71
F	-92.88	-79.09	-61.56
G	-59.27	-73	-51.82
H	-79.14	-63.85	-72.19
I	-79.54	-16.6	-80.28
K	-91.28	-86.08	-84.64
L	-88.03	-75.05	-82.41
M	-90.02	-86.25	-82.63
N	-65.45	-65.05	-52.95
Q	-68.71	-75.06	-60.73
R	-71.98	-75.82	-36.08
S	-65.18	-81.27	-55.1
T	-75.2	-73.8	-61.75
V	-74.46	-51.4	-75.58
W	-78.52	-69	62.68
Y	-77.5	-76.63	140.53

a- Fitness values are the Boltzmann weighted average of interaction energies in the context of each member of the ensemble.

Table 3.2: k-Means cluster analysis for the calculated fitness of sequence substitutions

Position	Cluster (k) Substitutions[a] Fitness[b]: k1 < k2 < k3
-1	k1: F k2: K M L k3: C I H R T W V Y A E D G N Q S
+1	k1: A C F K M S k2: E D G H L N Q R T W Y k3: I V
+2	k1: I K M L k2: A C H V k3: E D G F N Q S R T

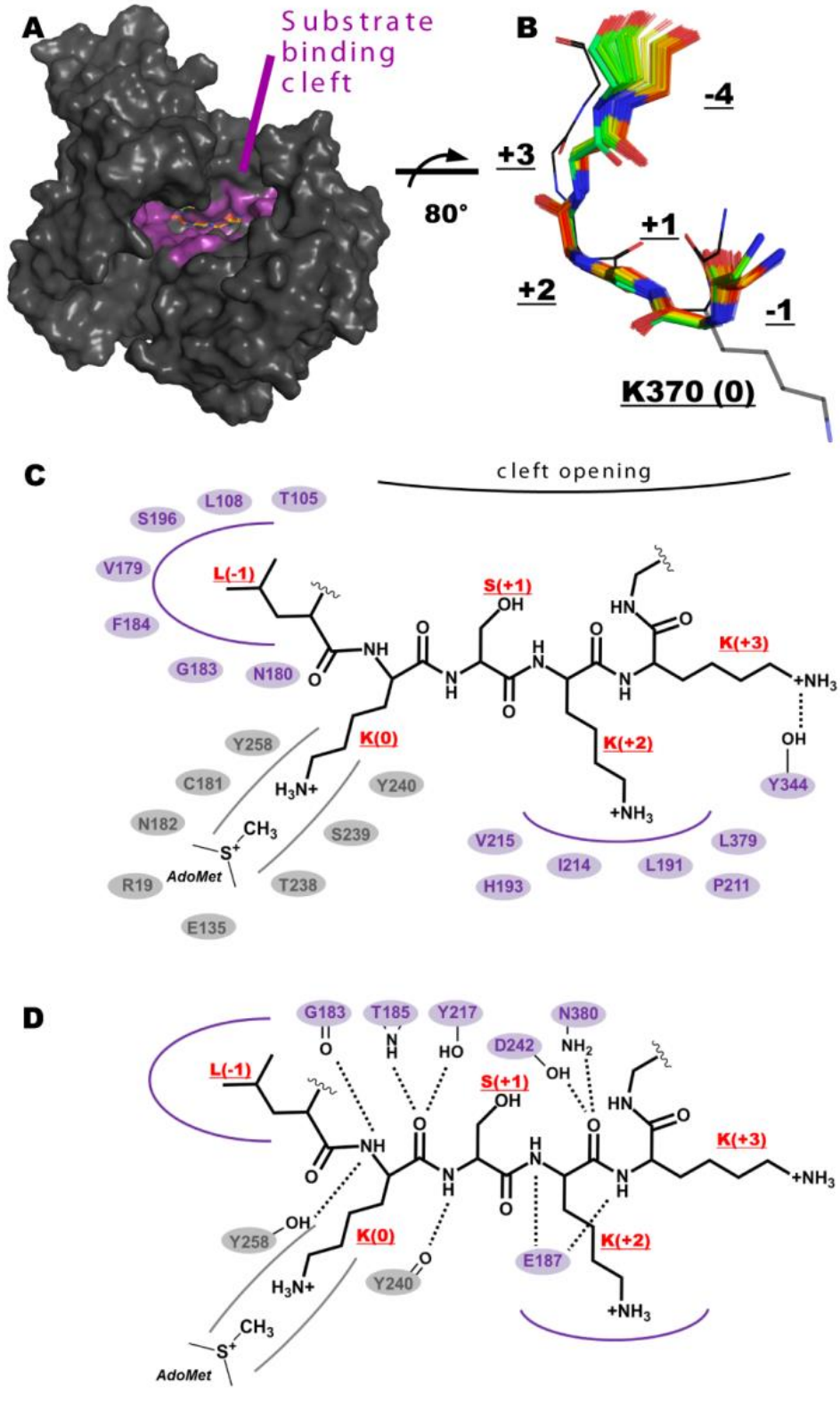
a- The wild-type p53 residue at each position is underlined and in bold.

b- Clusters ranked higher have more negative fitness values (kcal/mol)

As shown in Table 3.1, all substitutions with the exception of W and Y at position +2 result in negative and thus favourable fitness values, suggesting that they potentially stabilize the complex in the binding mode represented by the ensemble. To determine which residues to include in the predicted recognition motif, we clustered all substitutions at each designed position according to the similarity of their fitness values using the k-means cluster analysis algorithm (373). The k-means algorithm is an iterative method of clustering numerical values by partitioning them according to their Euclidian distance from each cluster centroid, which in this case is the average fitness value of each substitution belonging to the cluster. We partitioned each substitution with a negative fitness value into one of three clusters (Table 3.2 & Methods), with cluster k1 containing the most favourable substitutions. A substitution was predicted to be part of the SMYD2 substrate recognition motif if it is grouped in a cluster of equal or better fitness to the cluster containing the wild-type p53 amino acid ($L_{-1}/K_{0}/S_{+1}/K_{+2}$) at that position. Thus, residues found in the k1 cluster of positions +1 and +2 as well as those from the k1 and k2 clusters of position -1 were included in the recognition motif $[LFMK]_{-1}$-K*-$[AFMSKC]_{+1}$-$[KLIM]_{+2}$ (where K* is the methylated lysine) predicted by MSD. Interestingly, our k-means analysis showed that position +1 is more tolerant to substitution as more amino acids are included in the top cluster than that of positions -1 and +2. This result is in agreement with available crystal structures of SMYD2 in complex with Estrogen Receptor α and p53 which show that the side chain of the residue at position +1 is oriented outward of the binding cleft (354, 374), likely allowing for a broader range of substitutions by enabling a higher number of rotamers to be scored favourably at this position.

Figure 3.1: Peptide flexibility in SMYD2 substrate binding cleft

A) Structure of SMYD2 (PDB ID 3S7F) with bound p53 peptide (stick model). SMYD2 residues forming the substrate binding cleft are represented in purple. B) p53 substrate peptide backbones included in the 180-member PertMin ensemble. Amino acid positions relative to the methylated K370 residue are indicated. Crystallographic coordinates of the p53 peptide are shown in gray. C) Binding interactions between side chains of the p53 peptide and SMYD2 residues forming the binding cleft (purple) and the methyl transfer channel (grey). Two hydrophobic pockets (purple) stabilize the side chains of p53 residues at positions -1 and +2 while no interactions stabilize the side chain of the residue at position +1. D) Binding interactions of the p53 peptide backbone by SMYD2 residues forming the binding cleft (purple) and the methyl transfer channel (grey).


A
Substrate binding cleft
B
80°
-4
+3
+1
+2
-1
K370 (0)
C
cleft opening
L108
T105
S196
V179
L(-1)
S(+1)
F184
K(+3)
G183
N180
+NH3
K(0)
K(+2)
Y258
C181
Y240
Y344
N182
S239
V215
L379
AdoMet
I214
L191
R19
T238
H193
P211
E135
D
G183
T185
Y217
N380
D242
L(-1)
S(+1)
K(+3)
Y258
K(0)
E187
K(+2)
Y240
AdoMet

3.2.2. High-throughput methyltransferase assays reveals SMYD2 specificity

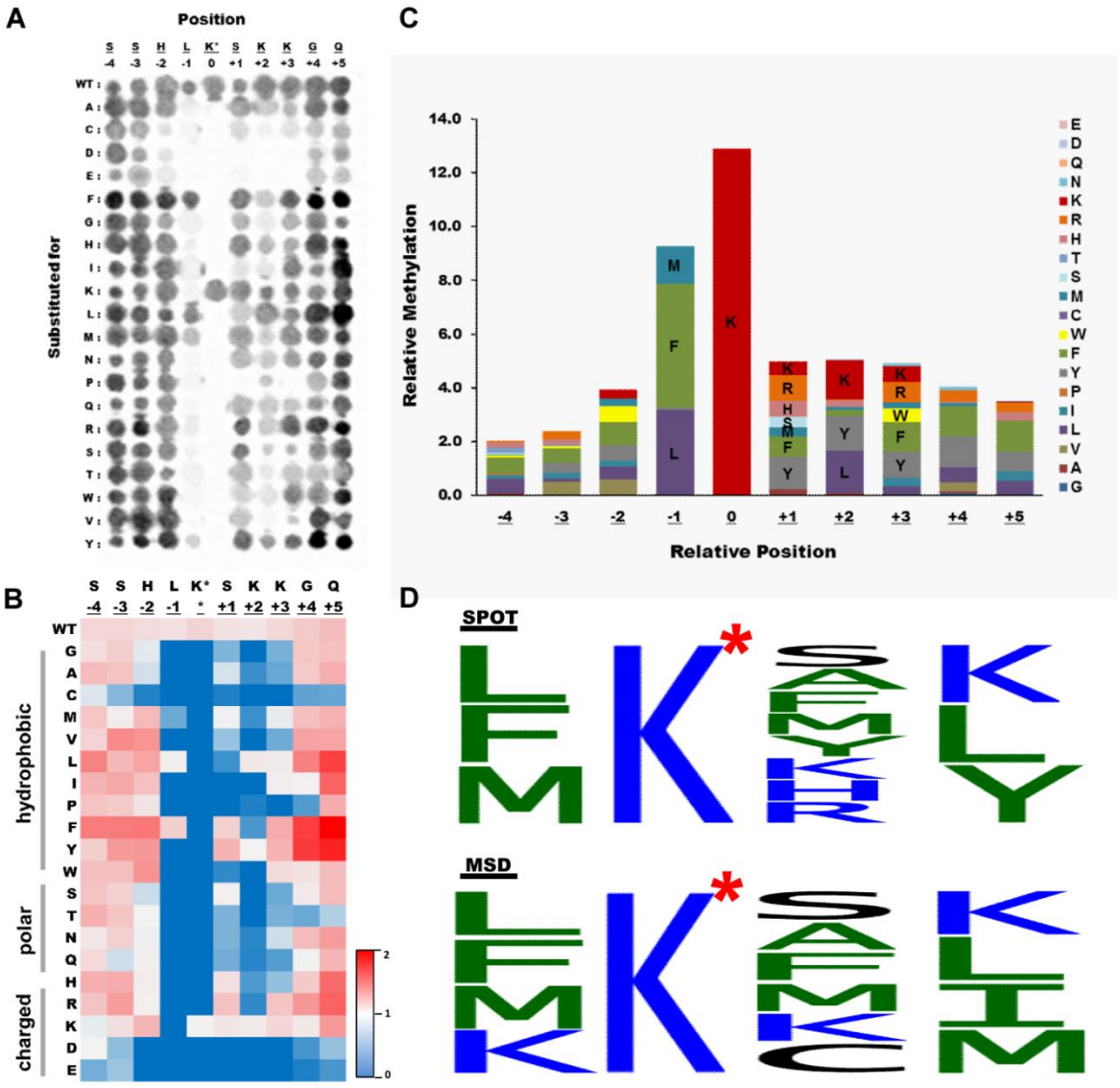
To validate the SMYD2 recognition motif predicted by MSD, we designed a SPOT peptide array (375, 376) based on the p53 sequence surrounding the SMYD2 target lysine K370 (SSHLK$_{370}$SKKGQ); each position of the peptide was substituted for every possible amino acid substitution. Three identical arrays were incubated with recombinant SMYD2 and ^{3}H-labeled AdoMet under conditions which reproduce SMYD2 specificity (Figure 3.6) and peptide methylation was quantified by phosphorimaging (Figure 3.2A). The normalized averaged intensities indicate that positions -1 to +2 surrounding K370 are the main determinants of SMYD2 activity (Figure 3.2B). In contrast, positions -4 to -2 and +3 to +5 show the highest tolerance to substitutions, validating our choice of positions designed by MSD.

To precisely define the SMYD2 substrate recognition motif, we calculated the relative methylation factor for each amino acid as the ratio of methylation for a given substitution relative to the difference of its methylation and the average methylation of all other substitutions at this position. This relative methylation factor thus reflects the preference of SMYD2 for a specific substitution at one position relative to all other substitutions. Consistent with MSD predictions, we observed that SMYD2 exhibits a strong preference for L, F and M residues at position -1 (Figure 3.2C). In contrast, position +1 has a broad tolerance with 8 out of 20 substitutions allowing preferential methylation. This result is consistent with the solvent exposed orientation of side chains at position +1 found in the PertMin ensemble. Similar to residues found at position -1, +2 residues remain in a deep pocket within the SMYD2 peptide binding cleft (Figure 3.1C). Accordingly, SMYD2 shows narrow specificity for peptides containing K, L or Y

substitutions at this position. Analysis of the relative methylation factors demonstrates that SMYD2 preferentially methylates the motif $[LFM]_{-1}$-K*-$[AFYMSHRK]_{+1}$-$[LYK]_{+2}$.

Figure 3.2: Substrate recognition motif of SMYD2

A) Methylation of a SPOT peptide array containing 200 peptides based on the p53 template sequence in which each position was systematically substituted for every amino acid. Labelling by tritiated methyl was detected by phosphorimaging after incubation with recombinant SMYD2 and 3H-AdoMet. B) Heat map representation of average methylation intensities measured by phosphorimaging. C) Analysis of the relative methylation factor for every amino acid at each position of the SPOT arrays (n=3). D) Comparison of SMYD2 substrate recognition motifs determined by methyltransferase high-throughput assays (SPOT) and MSD calculations. Green, blue, and black represent hydrophobic, charged, and polar residues, respectively. The methylated K370 residue is indicated with an asterisk.


A
Position
S S H L K* S K K G Q
-4 -3 -2 -1 0 +1 +2 +3 +4 +5
Substituted for
WT
A
C
D
E
F
G
H
I
K
L
M
N
P
Q
R
S
T
W
V
Y
B
S S H L K* S K K G Q
-4 -3 -2 -1 * +1 +2 +3 +4 +5
WT
hydrophobic
polar
charged
G
A
C
M
V
L
I
P
F
Y
W
S
T
N
Q
H
R
K
D
E
2
1
0
C
Relative Methylation
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
-4 -3 -2 -1 0 +1 +2 +3 +4 +5
Relative Position
E
D
Q
N
K
R
H
T
S
M
C
W
F
Y
P
I
L
V
A
G
D
SPOT
MSD

3.2.3. Accuracy of *in silico* predictions

The recognition motif predicted using MSD is in excellent agreement with results from our high-throughput methyltransferase assays (Figure 3.2D). Our *in silico* model accurately predicted that SMYD2 recognition is predominantly determined by amino acid identity at positions -1, +1 and +2 of the substrate peptide. The narrow specificity of SMYD2 for substitutions at position -1 observed in SPOT arrays is correctly identified by MSD, as position -1 includes only one false positive substitution (K) in the clusters considered (Table 3.2, k1 and k2 clusters). SMYD2 methyltransferase activity shows a strong bias toward long side-chain hydrophobic residues L, F and M at position -1, possibly due to the stabilization of these side chains in a hydrophobic pocket (Figure 3.1C) (354). MSD also correctly predicted a more relaxed specificity at position +1 (Table 3.1), with cluster k1 recapitulating five of the eight substitutions observed to be favourable *in vitro* (Figure 3.2D). Interestingly, Y at position +2 is one of the most unfavourable substitutions predicted by MSD whereas it is included in the recognition motif derived from the SPOT array experiments. This discrepancy may be due to the absence in our PertMin ensemble of an adequate p53 peptide backbone conformation required to favourably score this substitution. Available crystal structures of SMYD2 complexes (354, 374) show this residue embedded in a deep hydrophobic pocket (Figure 3.1C) that accommodates residues with long side chains, an observation consistent with the motif defined by MSD and the SPOT arrays. While our methyltransferase assays directly assessed the processivity of SMYD2 toward peptide substrates, MSD assessed the stabilization potential of its binding cleft. The agreement of both methods suggests that association may be the main driver of SMYD2 specificity.

While there is remarkable agreement between recognition motifs predicted by both MSD and peptide arrays, there are four false negative (H/R/Y and Y at positions +1 and +2, respectively) and four false positive (K, C and I/M at positions -1, +1 and +2, respectively) substitutions predicted by MSD. To evaluate whether an alternate binning method could have resulted in fewer false positives and negatives, we used the fitness value of the wild type at each designed position (Table 3.1) as a cut-off to include or exclude substitutions from the recognition motif predicted by MSD. As shown in Figure 3.6, use of the wild-type fitness value cut-off results in identical (position -1) or lower (positions +1 and +2) true positive ratios. In contrast, clustering substitutions with the k-means algorithm allowed an additional two substitutions to be correctly accepted in the recognition motif (one at position +1 and one at position +2) while incorrectly including only one false positive at position +2, demonstrating the benefit of using k-means clustering over binning with the wild-type fitness value.

To assess whether MSD provided improved prediction accuracy compared to SSD, we performed SSD on a single fixed backbone using identical parameters as those used for MSD except that single-state scores were used to rank sequences instead of the Boltzmann weighted average fitness value. Figure 3.3A shows a comparison of the SSD scores and MSD fitness values computed for all substitutions except for W and Y at position +2, which were omitted because both their MSD fitness values and SSD scores are > 0 kcal/mol. In all cases, regardless of substitution or position, MSD fitness values were more negative than SSD scores, indicating that substitutions are scored more favourably in the context of a backbone ensemble rather than a single structure. In the case of 10 substitutions (F/H/I/L/M/V/W/Y and F/I at positions +1 and +2, respectively),

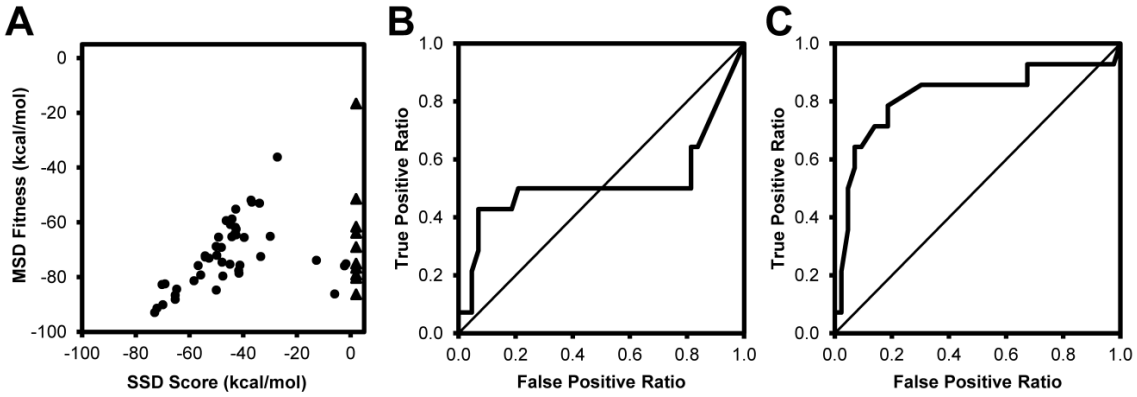
SSD scores fell above our accepted threshold of 0 kcal/mol. The high SSD scores for these 10 substitutions indicate unfavourable interactions likely resulting from steric clashes. This result is consistent with a known artefact of SSD arising from the combined use of a fixed protein backbone template with rigid rotamers, which can lead to the rejection of favourable amino acid sequences that would have been accepted on a slightly different backbone geometry or with a slightly different rotamer configuration (377).

To further compare the prediction accuracy obtained by SSD and MSD, we calculated the true positive (fraction of true positives out of the positives) and false positive (fraction of false positives out of the negatives) ratios for every possible cut-off value at 1 kcal/mol increments in the range of score/fitness values obtained for all substitutions, and plotted them to generate receiver operating characteristic (ROC) curves (Figure 3.7). ROC curves show that a significant improvement in prediction accuracy is obtained by using MSD as evidenced by the larger area under the curve that indicates a higher probability of ranking a randomly chosen positive higher than a randomly chosen negative. The lower prediction accuracy of SSD results from its poor scoring of substitutions to position +1, which results in four additional false negatives (F, H, M, and Y). This incorrect rejection of true positives decreases the true positive ratio to ≈0.5, a value that cannot be improved over a broad range of cut-offs that instead only increase the false positive ratio. In contrast, predictions made by MSD result in a large increase to the true positive ratio that is accompanied by a small increase to the false positive ratio. It is likely that similar improvements to prediction accuracy could also be achieved through the use of alternate CPD methodologies which incorporate backbone flexibility during calculation (365, 378). Although predictions could not be improved by using SSD or an

alternate binning method, MSD combined with k-means cluster analysis correctly binned substitutions as either being part or not of the recognition motif elucidated by SPOT array analysis with an overall accuracy of 86% (49/57), demonstrating the utility of this approach to the prediction of substrate recognition motifs for PKMTs.

Figure 3.3: Comparison of SSD and MSD predictions

A) MSD fitness values are plotted as a function of SSD scores for a total 55 substitutions found on the p53 peptide at positions -1, +1, and +2 relative to the methylated lysine. The 45 substitutions that resulted in negative (favourable) fitness and score values are shown as circles. The 10 substitutions that resulted in negative (favourable) MSD fitness values and positive (unfavourable) SSD scores are shown as triangles. To simplify, these 10 sequences are given identical arbitrary positive SSD scores on the plot. B) ROC curve for all substitutions at all positions obtained using SSD scores. C) ROC curve for all substitutions at all positions obtained using MSD fitness values. The diagonal line represents random binning.

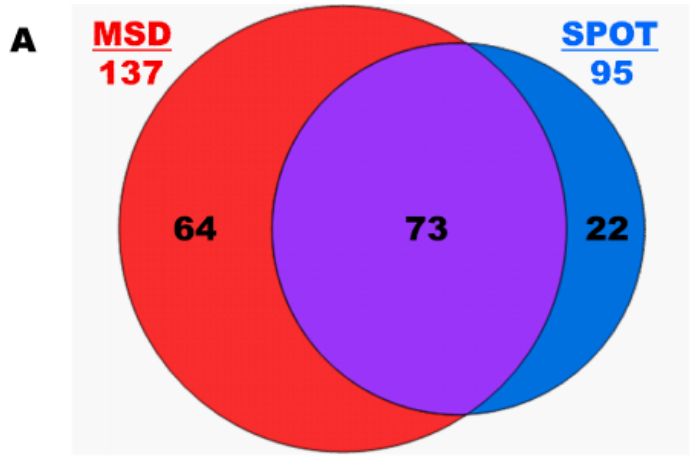

A
B
C
MSD Fitness (kcal/mol)
SSD Score (kcal/mol)
True Positive Ratio
False Positive Ratio
True Positive Ratio
False Positive Ratio

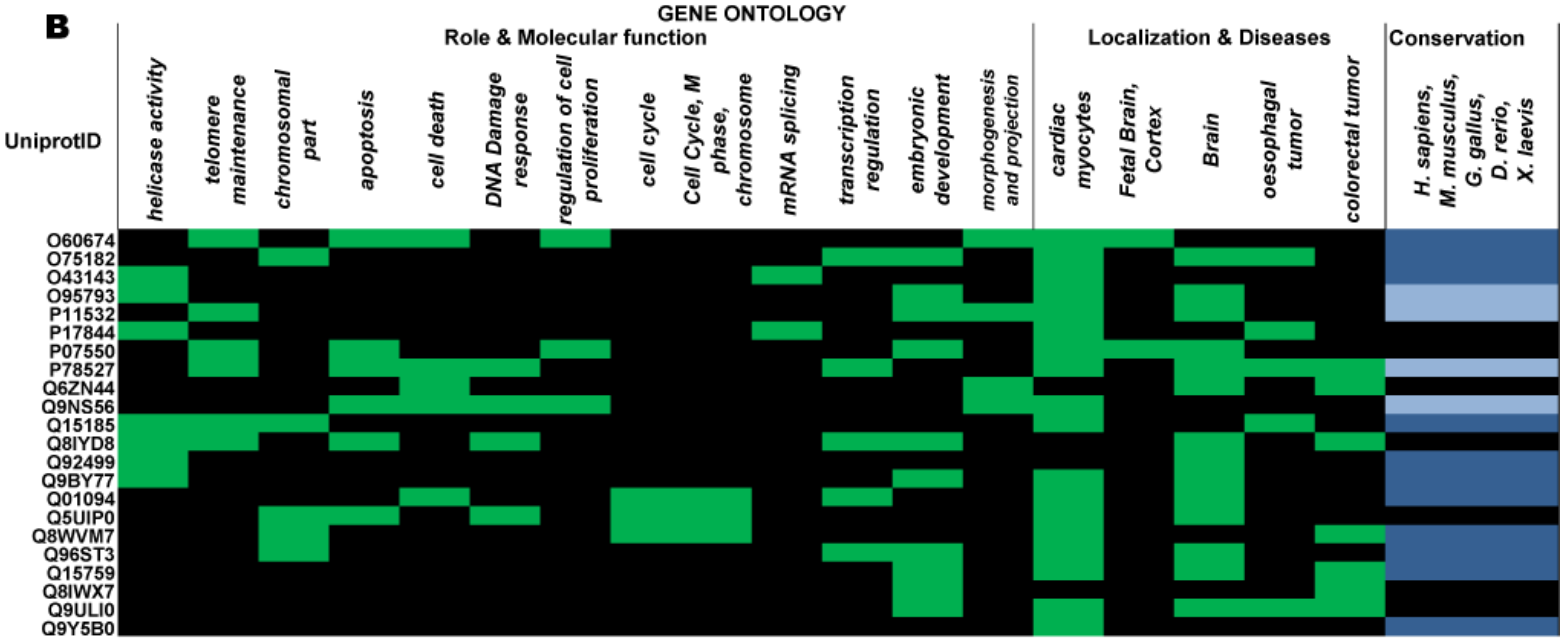
3.2.4. Novel SMYD2 substrates include the $[LFM]_{-1}$-K*-$[AFYMSHRK]_{+1}$-$[LYK]_{+2}$ motif

We next sought to determine whether the recognition motifs could be used to discover novel SMYD2 methylation targets. We probed a dataset of all reported genetic and physical SMYD2 protein interactors (259, 266, 286, 319, 351, 352, 379-381) using the ScanProsite motif search tool (382). Among our dataset, 95 SMYD2 interactors include the peptide array motif $[LFM]_{-1}$-K*-$[AFYMSHRK]_{+1}$-$[LYK]_{+2}$ and 135 include the MSD motif $[LFMK]_{-1}$-K*-$[AFMSKC]_{+1}$-$[KLIM]_{+2}$ (Table 3.3). As expected from their similarity, the recognition motif predicted by MSD identified a majority (77%) of the SMYD2 putative methylation targets identified using the motif determined by SPOT array (Figure 3.4A).

Figure 3.4: Prediction of SMYD2 substrates among its interactome

A) 95 proteins reported to interact with SMYD2 include the LFM-K*-AFYMSHRK-LKY motif determined from SPOT arrays while 137 include the LFMK-K*-AFMSKC-KLIM predicted by MSD. 73 of these proteins contain sequences common to both recognition motifs. B) Analysis of gene ontology terms associated to the 95 putative SMYD2 targets containing the LFM-K*-AFYMSHRK-LKY motif (green). Among the 22 hits corresponding to terms related to SMYD2 functions, localization and phenotypes, 11 include a lysine conserved in humans (*H. sapiens*), mice (*M. musculus*), frogs (*X. laevis*) and fish (*D. rerio*) (conservation of all sites across 2-3 species or across all four species are indicated in light or dark blue, respectively).

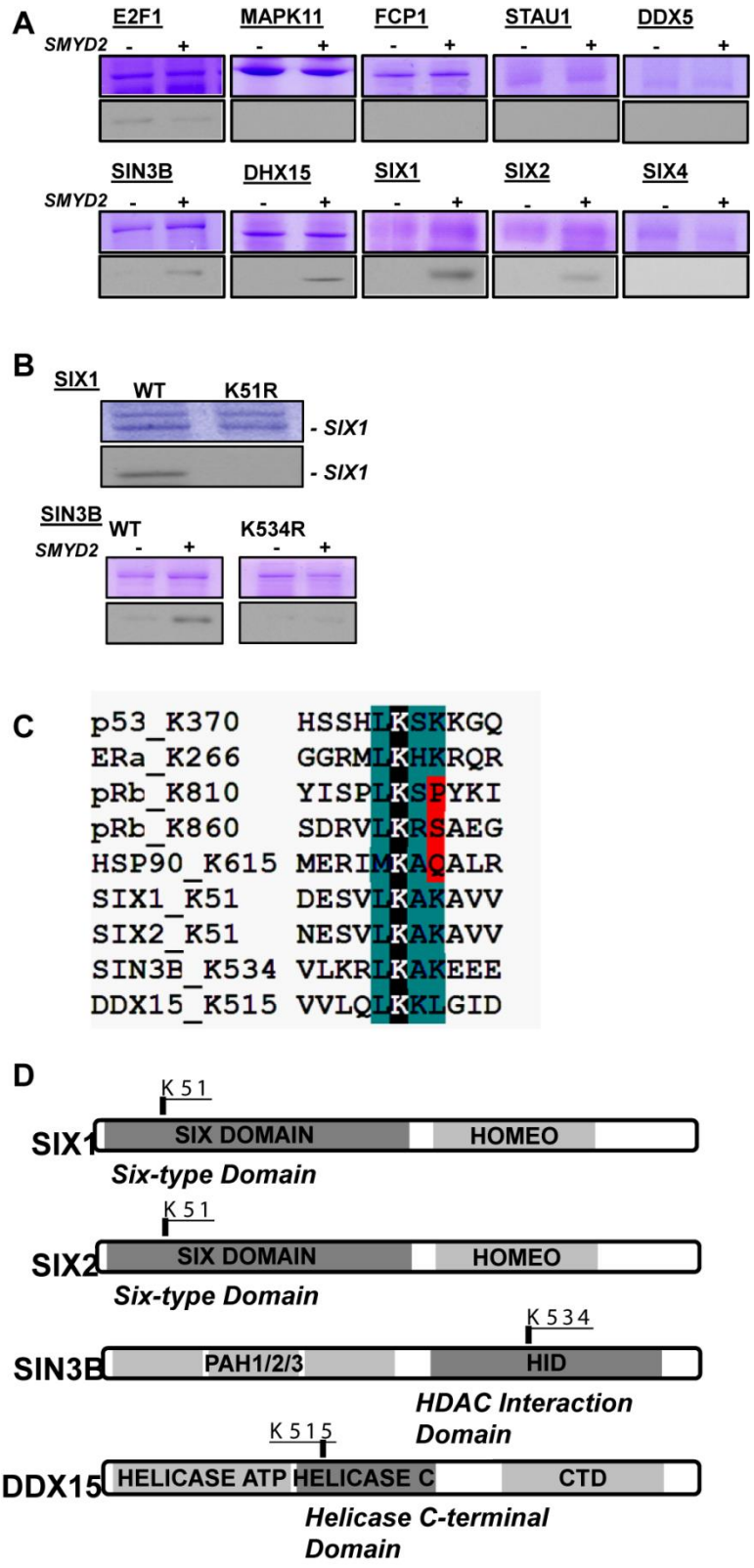

A
MSD
137
SPOT
95
64
73
22


B
GENE ONTOLOGY
Role & Molecular function
Localization & Diseases
Conservation
UniprotID
helicase activity
telomere maintenance
chromosomal part
apoptosis
cell death
DNA Damage response
regulation of cell proliferation
cell cycle
Cell Cycle, M phase, chromosome
mRNA splicing
transcription regulation
embryonic development
morphogenesis and projection
cardiac myocytes
Fetal Brain, Cortex
Brain
oesophagal tumor
colorectal tumor
H. sapiens, M. musculus, G. gallus, D. rerio, X. laevis
O60674
O75182
O43143
O95793
P11532
P17844
P07550
P78527
Q6ZN44
Q9NS56
Q15185
Q8IYD8
Q92499
Q9BY77
Q01094
Q5UIP0
Q8WVM7
Q96ST3
Q15759
Q8IWX7
Q9ULI0
Q9Y5B0

To refine our list of possible substrates, we cross-referenced the Gene Ontology (GO) terms of the 95 SMYD2 interactors with expression patterns and biological activities of this PKMT. Using the DAVID Bioinformatics Resources Functional Annotation Tool (383), we selected 22 proteins corresponding best to reported or proposed SMYD2 biological functions (Figure 3.4B & 3.7). We also assessed the conservation of the putative site in mammals, birds, and fish. Of the selected proteins, 10 were expressed as FLAG-tagged constructs and incubated with recombinant SMYD2 and ^{3}H-labeled AdoMet. Autoradiography confirmed that SMYD2 methylates the transcription factors SIX1 (384) and SIX2 (385), the transcriptional co-repressor SIN3B (386) and the RNA helicase DHX15 (387) (Figure 3.5A). Furthermore, SMYD2 cannot methylate single-point mutants of SIN3B and SIX1 in which the predicted target lysine is substituted for arginine, confirming that our motif correctly predicts novel protein methylation sites (Figure 3.5B). Notably, SMYD2 did not methylate six of the proteins identified by GO analysis. It is likely that the putative target lysine of these proteins is either not solvent exposed or engaged in intramolecular interactions with other residues of the motif, preventing methylation by SMYD2. All novel SMYD2 substrates identified in this study contain a recognition sequence (Figure 3.5C) that is included in both the SPOT array and MSD motifs, demonstrating the utility of MSD for the discovery of novel lysine methylation targets for a PKMT.

Figure 3.5: SIX1, SIX2, SIN3B and DHX15 are SMYD2 substrates

(A) Autoradiography of FLAG-tagged proteins incubated with recombinant SMYD2 and ^{3}H-AdoMet transcription factor E2F1, Mitogen-activated protein kinase 11 (MAPK11), RNA polymerase II subunit A C-terminal domain phosphatase (FCP1), Staufen 1 (STAU1), DEAD box RNA helicase 5 (DDX5), SIN3B, DEAH box RNA helicase 15 (DHX15), and Sine Oculis Homeobox homolog 1, 2, and 4 (SIX1, SIX2, SIX4). Upper panels show the proteins stained by Coomassie following SDS-PAGE and lower panels show the resulting autoradiography. Transcription factors SIX1 and SIX2, co-repressor SIN3B, and RNA helicase DHX15 are all methylated by SMYD2. (B) Autoradiography of wild type and mutant SIX1 and SIN3B incubated with recombinant SMYD2 and ^{3}H-AdoMet. Upper and lower panels are as described above. (C) Alignment of amino acid sequences surrounding all known SMYD2 lysine methylation sites (black). Residues included in the SMYD2 recognition motif are highlighted in green and those that are not are highlighted in red. (D) Localization of the methylation sites identified by our motif on SIX1, SIX2, SIN3B, and DHX15.


A
E2F1
MAPK11
FCP1
STAU1
DDX5
SMYD2 - + - + - + - + - +
SIN3B
DHX15
SIX1
SIX2
SIX4
SMYD2 - + - + - + - + - +
B
SIX1
WT
K51R
- SIX1
- SIX1
SIN3B
WT
K534R
SMYD2 - + - +
C
p53_K370 HSSHLKSKKGQ
ERa_K266 GGRMLKHKRQR
pRb_K810 YISPLKSPYKI
pRb_K860 SDRVLKRSAEG
HSP90_K615 MERIMKAQALR
SIX1_K51 DESVLKAKAVV
SIX2_K51 NESVLKAKAVV
SIN3B_K534 VLKRLKAKEEE
DDX15_K515 VVLQLKKLGID
D
K51
SIX1
SIX DOMAIN
HOMEO
Six-type Domain
K51
SIX2
SIX DOMAIN
HOMEO
Six-type Domain
K534
SIN3B
PAH1/2/3
HID
HDAC Interaction Domain
K515
DDX15
HELICASE ATP
HELICASE C
CTD
Helicase C-terminal Domain

The sequence surrounding the methylated K266 of the Estrogen Receptor α is included in our motif (350). Accordingly, the corresponding residues of the substrate peptide bind in a conformation nearly identical to the SMYD2-p53 complex (374). In contrast, our motif would not have predicted the methylation of HSP90 and pRb by SMYD2 (Figure 3.5C). Most likely, the recognition sites of these protein substrates adopt alternate peptide conformations not probed by our PertMin ensemble or consist of divergent sequences not covered in our SPOT arrays. MSD thus suggests that substrates included in our motif adopt a similar conformation as the SMYD2-p53 complex but that at least one alternative conformation or set of determinants is possible. This agrees with previous observations that SMYD2 is controlled by a biochemical "specificity-switch" in which reaction conditions direct its activity toward one substrate over another (379).

3.3. Conclusions

We showed that SMYD2 methylates the sequence motif $[LFM]_{-1}$-K*-$[AFYMSHRK]_{+1}$-$[LYK]_{+2}$ with position -1 and +2 exhibiting the highest degree of selectivity. Combining different bioinformatics and biochemical approaches with the motifs defined by MSD and SPOT arrays, a novel set of substrates for SMYD2 was discovered. Interestingly, the methylation sites in SIX1, SIX2, and SIN3B are located in the protein-protein interaction domain (Figure 3.5D). In SIX1 and SIX2, the methylation site K51 is located at the surface of the SIX1 domain of both transcription factors in a region known to interact with the EYA transcriptional activator (388). In SIN3B, the methylation site is located in the interaction domain that contacts the adaptor protein SUDS3 (389). Methylation of p53, HSP90, pRb and ERα by SMYD2 was already shown to control the association of their binding partners (79, 153, 164, 374); our findings suggest that this mechanism may be shared by numerous substrates of SMYD2. These four substrates also provide promising avenues to explore the critical role of SMYD2 in organ development (163, 390) and genetic regulation (391, 392).

High throughput methyltransferase assays showed that MSD correctly characterized the substrate sequence space recognized by SMYD2. In addition to biochemical characterization, CPD techniques can thus be used to study the spectrum of specificity of other post-translational modifying enzymes. In contrast with current methods, MSD provides a rapid and inexpensive mean to probe the specificity of a post-translational modifying enzyme on the basis of its enzyme-substrate interface. This novel method combining multistate computational protein design and k-means cluster analysis

is the first demonstration that CPD can be applied to the discovery of previously unknown substrates of PTM enzymes.

3.4. Methods

3.4.1. Multistate computational protein design

Atomic coordinates for the p53-bound structure of SMYD2 (354) were retrieved from the Protein Data Bank (PDB ID 3S7F). Hydrogens were added using the Protonate3D utility found in the Molecular Operating Environment (MOE) software suite (393). A 180-member PertMin ensemble (367) of the SMYD2-p53 complex was generated from the crystal structure with hydrogens added by randomly perturbing the coordinates of all atoms by ± 0.001 Å along each Cartesian coordinate axis. Because coordinate deviation from crystal structure increases proportionately with the number of minimization iterations, we energy minimized six sets of thirty perturbed structures using a truncated Newton minimization algorithm for 10, 25, 50, 100, 150 and 250 iterations, resulting in structures displaying a broad range of deviations from the crystal structure. The energy minimizations were performed using the AMBER99 force field (394) with a distance dependant dielectric of 80. The input structure used in SSD was prepared in a similar fashion from the initial SMYD2-p53 structure with added hydrogens by performing 50 iterations of conjugate gradient energy minimization.

MSD of the SMYD2-p53 complex was conducted using the fast and accurate side-chain topology and energy refinement (FASTER) algorithm for sequence optimization (395, 396). All amino acids with the exception of proline were introduced at p53 positions -1, +1 and +2 relative to the methylated lysine. Adjacent SMYD2 residues found in the binding cleft were allowed to sample alternate conformations during the design, but their identities were not modified (SMYD2 residues 19, 105, 108, 135, 179, 180, 181, 182, 184, 187, 191, 193, 196, 211, 215, 217, 238, 239, 240, 258, 344, 379 and

380). A backbone dependent Dunbrack rotamer library with expansions of ± 1 standard deviation around side chain χ_1 and χ_2 rotatable bonds was used (397). The interaction energy of amino acid substitutions were scored using a four-term potential energy function that includes a van der Waals term from the Dreiding II force field where atomic radii were scaled by 0.9 (398), a hydrogen-bond term with a well depth of 8 kcal/mol and direction specific variables (399), a Coulomb electrostatic term with a distance dependent dielectric of 40, and a surface area based solvation penalty term (400, 401). MSD was implemented using PHOENIX (402-404). Substitution fitness was computed as the Boltzmann weighted average of scores across the 180 members of the PertMin ensemble at 300 K and these fitness values were clustered using the k-means theorem (373).

3.4.2. Protein preparation and assays

Recombinant SMYD2 was expressed and purified as described previously (379). C-terminal bound peptides for high-throughput methyltransferase assays were synthesized by the SPOT method on a trioxatridecanediamine (TOTD) (Kinexus, Canada) (376). The methylation factor was calculated by using the formula $(I_i-I_j^*)/I_j^*$ were I_i is the intensity measured for peptide i and I_j^* is the average of intensities of all the peptides for this position, excluding i. For substrate validation assays, FLAG-tagged constructs of the putative substrates were transfected in HEK293T cells. Cells were lysed 24 hours after transfection in 50 mM Tris pH 7.5, 10 mM NaCl, 5 mM EDTA and 0.4% NP-40. FLAG-tagged proteins were immunopurified using anti-FLAG M2 magnetic beads (Life Technologies). Beads were adjusted to 50% v/v in 50 mM Tris pH 8.0 and incubated with 1.5 μCi of ^{3}H-AdoMet for 2 hours at 30°C either in the presence or

absence of recombinant SMYD2 (10 ng/μl). Proteins were separated on a SDS-PAGE gel and methylation was visualized by autoradiography (379). Additional methods are provided in SI text.

3.5. Supplementary material

Discovery of substrates for a SET domain lysine methyltransferase predicted by multistate computational protein design

Sylvain Lanouette, James A. Davey, Fred Elisma, Zhibin Ning, Daniel Figeys, Roberto A. Chica, and Jean-François Couture

3.5.1. Supplemental methods

3.5.1.1. Preparation of SPOT peptide arrays

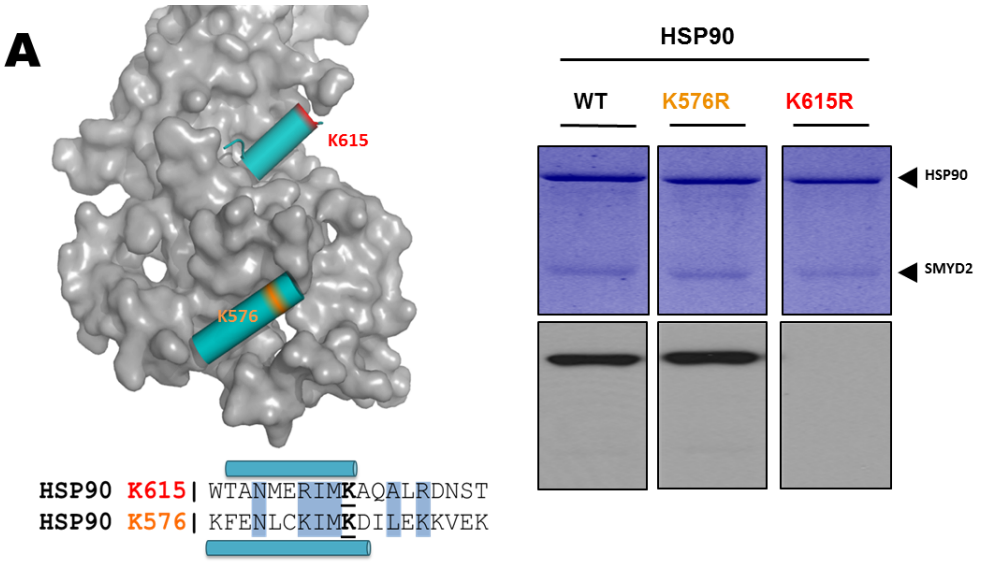
The specificity of SMYD2 against a selection of control peptides on SPOT membranes was evaluated against various buffer conditions to recapitulate its reported specificity (Figure 3.6) (79, 379). Before incubation with SMYD2, the SPOT arrays were primed three times with 95% ethanol and three times with Tris buffered saline-Tween 0.1%. The array was then incubated in reaction buffer (50 mM Tris pH 8.0, 50 mM NaCl, 2% Bovine Serum Albumin, 1 μg/ml recombinant SMYD2, 7.5 μM AdoMet and 2.5 μM 3H-AdoMet (10 μCi/ml)) for 1 hour at 30°C. The reaction was stopped by rinsing the membrane four times with a solution containing 8 M urea, 1% SDS, 0.5% β-mercaptoethanol. Then, the membrane was washed three times with 50% ethanol, three times with 10% acetic acid and three times with 95% ethanol. The array was then thoroughly dried before quantification by phosphorimaging (Typhoon FLA 7000 Phosphorimager (GE)). Spots in which K370 was mutated to arginine were used as negative controls.

3.5.1.2. Prediction of putative SMYD2 substrates

To increase the likelihood of identifying its genuine substrates, we first compiled all reported physical and genetic interactors of SMYD2 (259, 266, 286, 319, 351, 352, 379-381). We subsequently probed the dataset with the DAVID Bioinformatics Resources Functional Annotation Tool (http://david.abcc.ncifcrf.gov/) (383) to assess enrichment of GO terms. Putative targets sharing known roles with SMYD2 such as the regulation of cell cycle, as well as cell proliferation (153, 154, 392), gene transcription (153, 154, 391, 392), apoptosis and cell death (79) were selected for subsequent analysis. In addition, putative targets with biological activities related to mRNA splicing, helicase activity (211, 392) as well as chromosome segregation, chromosomal part, telomere maintenance and DNA damage response (211) were shortlisted for our comparative studies. Finally, we also selected potential substrates showing higher levels of expression in fetal brain and cortex, brain and cardiac myocytes (392, 405) and during embryonic development, morphogenesis and projection (346, 405, 406). Correlation of these GO terms across the 95 putative substrates (Figure 3.8) resulted in 22 candidates of interest (Figure 3.4B).

Figure 3.6: Recapitulation of SMYD2 specificity using SPOT arrays.

A) Autoradiography of wild-type (WT) and mutant Hsp90 incubated with recombinant SMYD2 and ^{3}H-AdoMet demonstrates that SMYD2 methylates K615 (red) but not K576 (orange), even though both lysines are found on α-helices (cyan) within similar sequences. Upper panels show the proteins stained by Coomassie following SDS-PAGE and lower panels show the resulting autoradiography. B) Sequences of peptides derived from p53 and Hsp90 used to probe reaction conditions required to recapitulate known SMYD2 specificity. C) Optimization of assay conditions enabling the recapitulation of SMYD2 specificity. Full, partial, or no methylation is expected for peptides 1/3/5, 6, and 2/4/7, respectively. Incubation of the array for 1 hour in 20 mM Tris•Cl pH 8.0, 50 mM NaCl, and 2% BSA accurately recapitulates SMYD2 specificity.



B

1. HSSHLKSKKGQ | p53 WT (365 - 375)
2. HSSHLRSRRGQ | p53 K370R/K372R/K373R
3. HSSHLKSRRGQ | p53 K372R/K373R
4. HSSHLRSKRGQ | p53 K370R/K373R
5. MERIMKAQALR | HSP90 WT (610 - 620)
6. MERIAKAQALR | HSP90 K614A
7. LCRIMKDILEK | HSP90 (571-81)

C

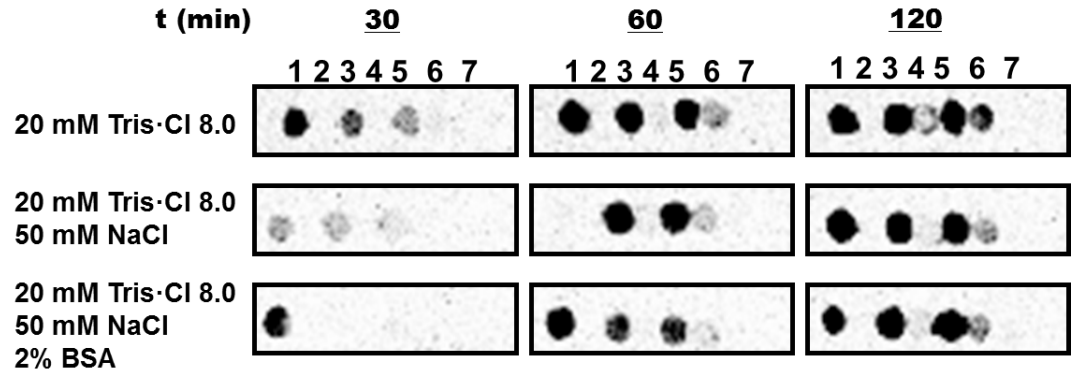


Figure 3.7 Receiver operating characteristic (ROC) curves for all substitutions to positions -1 (A), +1 (B), and +2 (C) relative to the methylated lysine.

The true and false positive ratios obtained by using the wild-type fitness value as a cut-off or by k-means cluster analysis are shown as white and black circles, respectively. For position -1, both binning methods yielded identical true and false positive ratios. The diagonal line represents random binning.

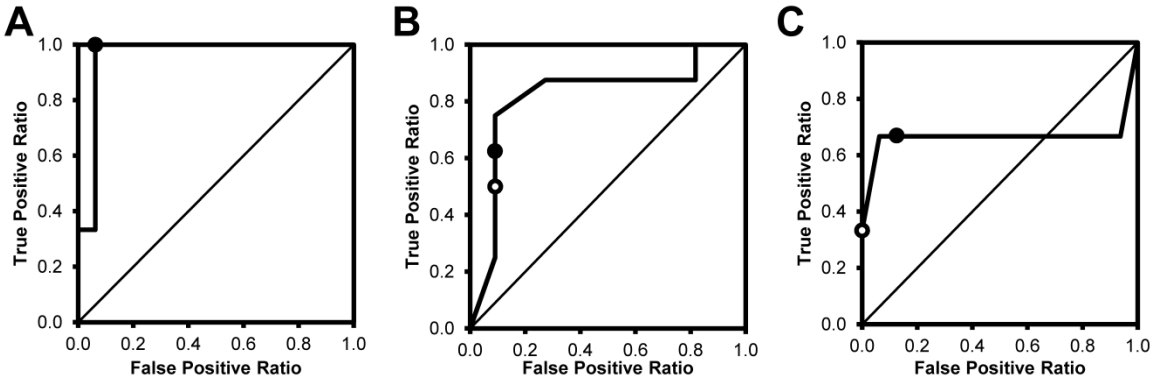

A
B
C
True Positive Ratio
False Positive Ratio
0.0
0.2
0.4
0.6
0.8
1.0

Figure 3.8: Correspondence of SMDYD2 interactors with selected gene ontology terms


GENE ONTOLOGY
Role & Molecular function
Localization & Diseases
UniprotID
helicase activity
telomere maintenance
chromosomal part
apoptosis
cell death
DNA Damage response
regulation of cell proliferation
cell cycle
Cell Cycle, M phase,
mRNA splicing
transcription regulation
embryonic development
morphogenesis and projection
cardiac myocytes
Fetal Brain, Cortex
Brain
oesophagal tumor
colorectal tumor
O60674
O95359
O75182
O43143
O75533
O95793
P07196
P11532
P23528
P27348
P40763
P42356
P14866
P17844
P33981
P50748
P07550
P09874
P78527
P80098
P11021
Q13402
Q15154
Q5TZA2
Q6ZN44
Q8TF72
Q96QS3
Q9NS56
Q00839
Q12830
Q15185
Q8IYD8
Q8NHQ9
Q92499
Q96IC2
Q9BY77
Q01094
Q5UIP0
Q8IZT6
Q8WVM7
Q93008
Q9NTJ3
Q96ST3
Q9H093
Q9NZC7
Q96JI7
Q9BVP2
P20290
Q9UQR0
Q9UQR1
Q86UP3
P11166
P15924
P31948
P39656
Q13393
Q01082
Q92783
Q15759
Q92817
Q16880
Q9H095
Q8IWX7
Q9H845
Q9NYQ8
Q9Y4I1
Q96PZ2
Q9Y4G6
Q9ULI0
O94915
P52569
P22003
Q03393
Q5JV73
Q5SRI9
Q8TBZ0
Q9UKF2
Q9Y2K3
O95944
P40227
Q86WR0
Q9BSJ5
Q9Y2B1
Q9Y5B0
O75335
Q8NDB2
Q01968
Q56UN5
Q9BRS2
Q8N157
Q01546

Table 3.3: SMYD2 interactors that contain the MSD and SPOT recognition motifs

OVERLAP		MSD		SPOT	
SWISSPROT ID	UNIPROT ID	SWISSPROT ID	UNIPROT ID	SWISSPROT ID	UNIPROT ID
1433T_HUMAN	P27348	ABCCB_HUMAN	Q96J66	ADRB2_HUMAN	P07550
AHI1_HUMAN	Q8N157	AKA11_HUMAN	Q9UKA4	ARX_HUMAN	Q96QS3
ACAD9_HUMAN	Q9H845	APC_HUMAN	P25054	BMP5_HUMAN	P22003
ADA30_HUMAN	Q9UKF2	ARSI_HUMAN	Q5FYB1	CC110_HUMAN	Q8TBZ0
ARX_HUMAN	Q96QS3	AT1A4_HUMAN	Q13733	CCD25_HUMAN	Q86WR0
ASPM_HUMAN	Q8IZT6	B4GN2_HUMAN	Q8NHY0	CCL7_HUMAN	P80098
ATD2B_HUMAN	Q9ULI0	CLAP2_HUMAN	O75122	CGT_HUMAN	Q16880
BANK1_HUMAN	Q8NDB2	CN166_HUMAN	Q9Y224	COF1_HUMAN	P23528
BPTF_HUMAN	Q12830	CO8A_HUMAN	P07357	DDX1_HUMAN	Q92499
BTF3_HUMAN	P20290	COBL_HUMAN	O75128	DDX5_HUMAN	P17844
CQ080_HUMAN	Q9BSJ5	CP3A4_HUMAN	P08684	DMD_HUMAN	P11532
CROCC_HUMAN	Q5TZA2	CP46A_HUMAN	Q9Y6A2	E2F1_HUMAN	Q01094
CTDP1_HUMAN	Q9Y5B0	DDX6_HUMAN	P26196	MK11_HUMAN	Q01094
CTR2_HUMAN	P52569	DLEC1_HUMAN	Q9Y238	NCTR2_HUMAN	O95944
DDX55_HUMAN	Q8NHQ9	DLGP1_HUMAN	O14490	PTPS_HUMAN	Q03393
DESP_HUMAN	P15924	DOT1L_HUMAN	Q8TEK3	SHRM3_HUMAN	Q8TF72
DHX15_HUMAN	O43143	DYH5_HUMAN	Q8TE73	SIX4_HUMAN	Q9UIU6
EVPL_HUMAN	Q92817	E2F5_HUMAN	Q15329	STIP1_HUMAN	P31948
F111A_HUMAN	Q96PZ2	E41L3_HUMAN	Q9Y2J2	UN45B_HUMAN	Q8IWX7
FANCM_HUMAN	Q8IYD8	E41LA_HUMAN	Q9HCS5	UNC5A_HUMAN	Q6ZN44
FAT2_HUMAN	Q9NYQ8	FER_HUMAN	P16591	YSK4_HUMAN	Q56UN5
FRPD3_HUMAN	Q5JV73	GLT13_HUMAN	Q8IUC8		
FRYL_HUMAN	O94915	HECW1_HUMAN	Q76N89		
GNL3_HUMAN	Q9BVP2	HS90A_HUMAN	P07900		
GRP78_HUMAN	P11021	HS90B_HUMAN	P08238		
GTR1_HUMAN	P11166	IF2B1_HUMAN	Q9NZI8		
HNRPL_HUMAN	P14866	IF2B3_HUMAN	O00425		
HNRPU_HUMAN	Q00839	IPO8_HUMAN	O15397		
IQCG_HUMAN	Q9H095	K1210_HUMAN	Q9ULL0		
JAK2_HUMAN	O60674	KIF11_HUMAN	P52732		
K22O_HUMAN	Q01546	LONF2_HUMAN	Q1L5Z9		
KNTC1_HUMAN	P50748	LSP1_HUMAN	P33241		
LIPA4_HUMAN	O75335	MACOI_HUMAN	Q8N5G2		
MANEA_HUMAN	Q5SRI9	MAMC2_HUMAN	Q7Z304		
MYO5A_HUMAN	Q9Y4I1	MCM5_HUMAN	P33992		
MYO7A_HUMAN	Q13402	MCM7_HUMAN	P33993		
NFL_HUMAN	P07196	MYH15_HUMAN	Q9Y2K3		
NUAK2_HUMAN	Q9H093	NAL12_HUMAN	P59046		
OCRL_HUMAN	Q01968	PAPOG_HUMAN	Q9BWT3		
OST48_HUMAN	P39656	PKP4_HUMAN	Q99569		
PARP1_HUMAN	P09874	PLXB2_HUMAN	O15031		
PCM1_HUMAN	Q15154	PTBP1_HUMAN	P26599		
PDIP3_HUMAN	Q9BY77	PTPRK_HUMAN	Q15262		
PI4KA_HUMAN	P42356	RBM34_HUMAN	P42696		
PLD1_HUMAN	Q13393	RHG28_HUMAN	Q9P2N2		
PRKDC_HUMAN	P78527	RL27_HUMAN	P61353		
REXON_HUMAN	Q96IC2	RPC6_HUMAN	Q9H1D9		
RIF1_HUMAN	Q5UIP0	RPP30_HUMAN	P78346		
RIOK1_HUMAN	Q9BRS2	RTF1_HUMAN	Q92541		
SCML2_HUMAN	Q9UQR0	RU2B_HUMAN	P08579		
SF3B1_HUMAN	O75533	S10A2_HUMAN	P29034		
SIN3A_HUMAN	Q96ST3	SHAN3_HUMAN	Q9BYB0		
SIN3B_HUMAN	O75182	SMC4_HUMAN	Q9NTJ3		
SIX1_HUMAN	Q15475	SMRCD_HUMAN	Q9H4L7		
SIX2_HUMAN	Q9NPC8	SPSY_HUMAN	P52788		
SPTB2_HUMAN	Q01082	SPTCS_HUMAN	Q96JI7		
STAG1_HUMAN	Q8WVM7	SRPK1_HUMAN	Q96SB4		
STAM1_HUMAN	Q92783	TAF2_HUMAN	Q6P1X5		
STAU1_HUMAN	O95793	TBA4A_HUMAN	P68366		
TACC2_HUMAN	O95359	THOC4_HUMAN	Q86V81		
TCPZ_HUMAN	P40227	TM110_HUMAN	Q86TL2		
TITIN_HUMAN	Q8WZ42	TNAP2_HUMAN	Q03169		
TLN2_HUMAN	Q9Y4G6	WDR7_HUMAN	Q9Y4E6		
TMEM5_HUMAN	Q9Y2B1	ZN148_HUMAN	Q9UQR1		
TOPRS_HUMAN	Q9NS56	ZN276_HUMAN	Q8N554		
TTK_HUMAN	P33981				
USP9X_HUMAN	Q93008				
WWOX_HUMAN	Q9NZC7				
ZFHX4_HUMAN	Q86UP3				

4. SMYD2 ANTAGONIZES MUSCLE GENE EXPRESSION AND SKELETAL MYOBLASTS DIFFERENTIATION

The work presented will be submitted as:

Sylvain Lanouette, Elias Horner, Yubing Liu, Myriam Cramet, Daniel Figeys, Alexandre Blais, and Jean-François Couture. Biochim. Biophys. Acta (To Be Submitted)

Author contributions:

S.L. performed the experiments and analyzed data related to figures related to figures 4.1 to 4.5 and tables 4.1 to 4.7. E.H. performed the experiments related to figure 4.4A and tables 4.5 and 4.6 Y.L and M.C. contributed to experiments. D.F. and A.B. provided insights on the paper. S.L., E.H. and A.B. designed experiments. S.L. and J.-F.C. wrote the paper.

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4.1. Introduction

The post-translational modification of proteins during tissue development is a tightly regulated mean of cell fate determination (407-411). In recent years, protein lysine methyltransferases were shown to regulate tissue development, regeneration, organogenesis and homeostasis (412-419). In particular, the SET-domain SMYD methyltransferase family has been associated with the formation of both cardiac and skeletal muscle tissues (163, 271, 420-423).

Both cardiac and skeletal muscle are characterized by the formation of sarcomeres - multimeric protein assemblages formed after myoblast fusion during the late stages of muscle development (424). During embryonic development, vertebrates SMYD1 and SMYD2 promote correct myofibril arrangement by associating with the molecular chaperone HSP90 at the sarcomere (163, 164, 425, 426). During embryonic development, cardiac tissue emerges from the lateral plate mesoderm and forms a chambered heart in a tightly regulated process driven in part by the transcription factors Nkx2.5, Hand1/2, Tbx2/3/5/20, Irx4/5, and GATA4/6 (427, 428). Both SMYD1 and SMYD3 regulate the expression of cardiac genes during cardiomyocytes differentiation and are important for correct cardiac morphogenesis (420, 423). In contrast, conditional knock-out of SMYD2 in *Xenopus* embryos does not significantly affect heart development (405) although studies in murine models showed that SMYD2 plays a role in cardiomyocytes by preventing p53-dependant apoptosis during cardiac injury (429).

The importance of SMYD proteins during the development of skeletal muscle precursors was also demonstrated in recent studies. Most skeletal muscle tissue derives from the paraxial mesoderm which eventually gives rise to myoblasts-containing

myotome. Myoblasts undergo terminal myogenic commitment under the control of several transcription factors, including members of the Pax, Mef2, Six and Myogenic regulatory factors families, and fuse into multinucleated myotubes to form the myofibers (430-433). Adult organisms can also undergo skeletal myogenesis following activation of progenitor myosatellite cells into proliferating myoblasts and their subsequent differentiation into new muscle fibers (432, 434, 435). In myoblasts, SMYD1 expression is controlled by the Serum Response Factor and myogenin (MyoG) and positively regulates myoblast differentiation to myotubes (284). SMYD3 regulates the late expression of the myogenic regulatory factors MyoG and MyoD in the later stages of muscle development (420) and positively regulates the expression of the muscle growth regulator myostatin and protect skeletal muscle from atrophy (421).

While the role of SMYD1 and SMYD3 in the regulation of myogenic differentiation has been explored, the role of SMYD2 in skeletal muscle development has remained elusive. In this study, we show that SMYD2 is expressed in proliferative myoblasts and negatively regulates their differentiation to myotubes. We also observe that the regulation of myogenic differentiation by SMYD2 depends on its methyltransferase activity. Finally, we demonstrate that SMYD2 controls myogenic differentiation by downregulating muscle-specific genes in myoblasts. Our results demonstrate a unique regulatory role for SMYD2 in skeletal muscle development and further highlight the role of SMYD proteins in myogenesis.

4.2. Results

4.2.1. SMYD2 is expressed in proliferating myoblasts

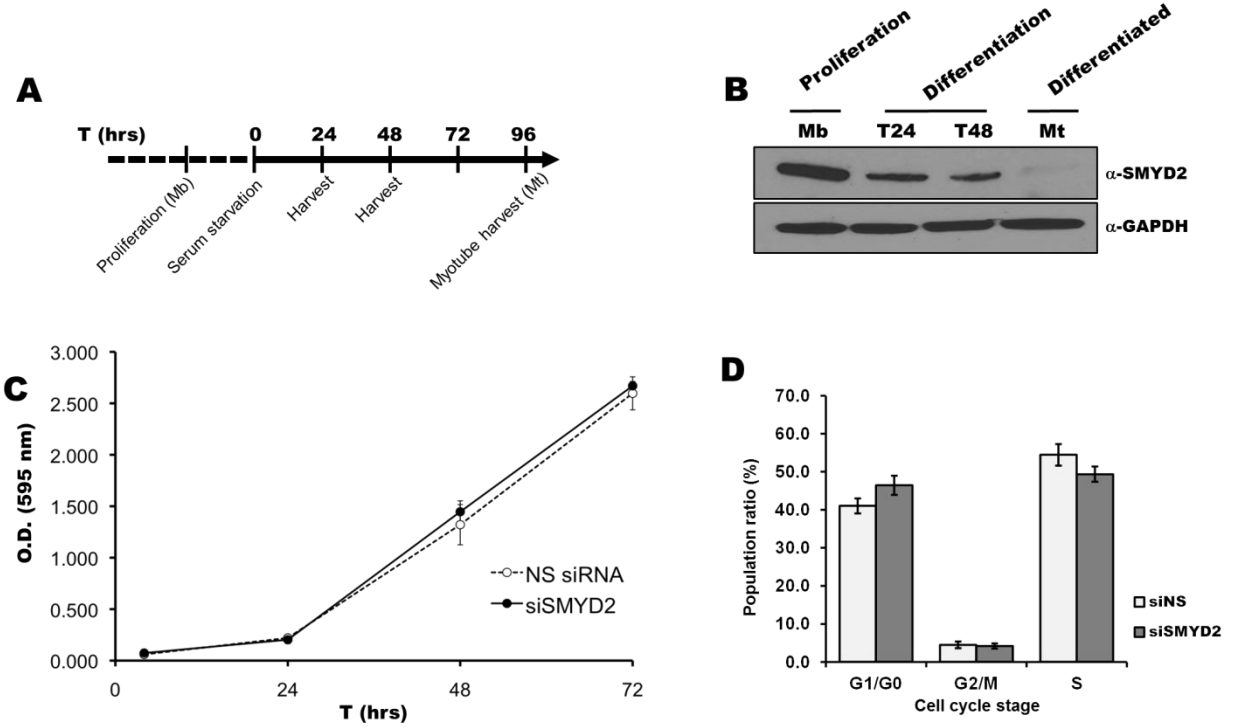
Functional studies of SMYD1 and SMYD3 have shown that these enzymes regulate myogenic differentiation of myoblasts. Given the high sequence homology within the SMYD family (379), we surmised that SMYD2 might play an important role not only in sarcomere stabilization formation but also during myogenic differentiation. First, we verified the expression of SMYD2 at different stages of myogenic differentiation using C2C12 myoblasts as a model (436). C2C12 myoblasts can be maintained in a strong proliferative state but undergo terminal myogenic commitment and fusion into myotubes upon serum starvation. To measure the levels of SMYD2 at different stages of differentiation, myoblasts were allowed to proliferate until confluence and myogenic differentiation was induced by serum starvation (Figure 4.1A). Detection of SMYD2 by western blotting shows that its expression is highest in proliferative myoblasts and decreases following induction of myogenic differentiation until it can barely be observed following fusion into myotubes (Figure 4.1B). This suggests that SMYD2 function in myoblasts is associated with the proliferative rather than the differentiated stages.

To determine the function of SMYD2 in the myoblast we first addressed whether it is involved in the maintenance of the proliferative stage. We determined the proliferation of sub confluent C2C12 myoblasts following treatment with either non-specific or SMYD2-targeted siRNA (Figure 4.1C). In contrast with previous results showing that SMYD2 regulates the proliferation of fibroblast cells (259), knockdown of SMYD2 did not impact the proliferation of myoblasts. As SMYD2 can also promote cell

cycle progression in other cell models (153, 154), we also assessed the impact of SMYD2 knockdown on cell cycle stages. Myoblasts treated with either non-specific or SMYD2-targeted siRNA were grown under proliferative conditions and treated with BrdU (*Material and methods*). Determination of the relative cell cycle stages populations by flow cytometry showed that SMYD2 knockdown did not markedly affect cell cycle progression in proliferating C2C12 myoblasts (Figure 4.1D).

Figure 4.1. SMYD2 is expressed in proliferating skeletal myoblasts

A) Experimental scheme for the treatment and harvest of differentiating myoblasts. B) Time course of SMYD2 expression in proliferating and differentiating myoblasts followed by western blotting. C) Crystal violet staining of proliferating C2C12 myoblasts shows that SMYD2 knock-down does not significantly affect the proliferation rate of C2C12 cells. D) Relative cell cycle stages population analysis of proliferating myoblasts by flow cytometry shows that SMYD2 knock-down does not impact cell cycle ratios during proliferation.


A
T (hrs)
0
24
48
72
96
Proliferation (Mb)
Serum starvation
Harvest
Harvest
Myotube harvest (Mt)
B
Proliferation
Differentiation
Differentiated
Mb
T24
T48
Mt
α-SMYD2
α-GAPDH
C
O.D. (595 nm)
3.000
2.500
2.000
1.500
1.000
0.500
0.000
0
24
48
72
T (hrs)
NS siRNA
siSMYD2
D
Population ratio (%)
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
G1/G0
G2/M
S
Cell cycle stage
siNS
siSMYD2

4.2.2. SMYD2 regulates myogenic differentiation

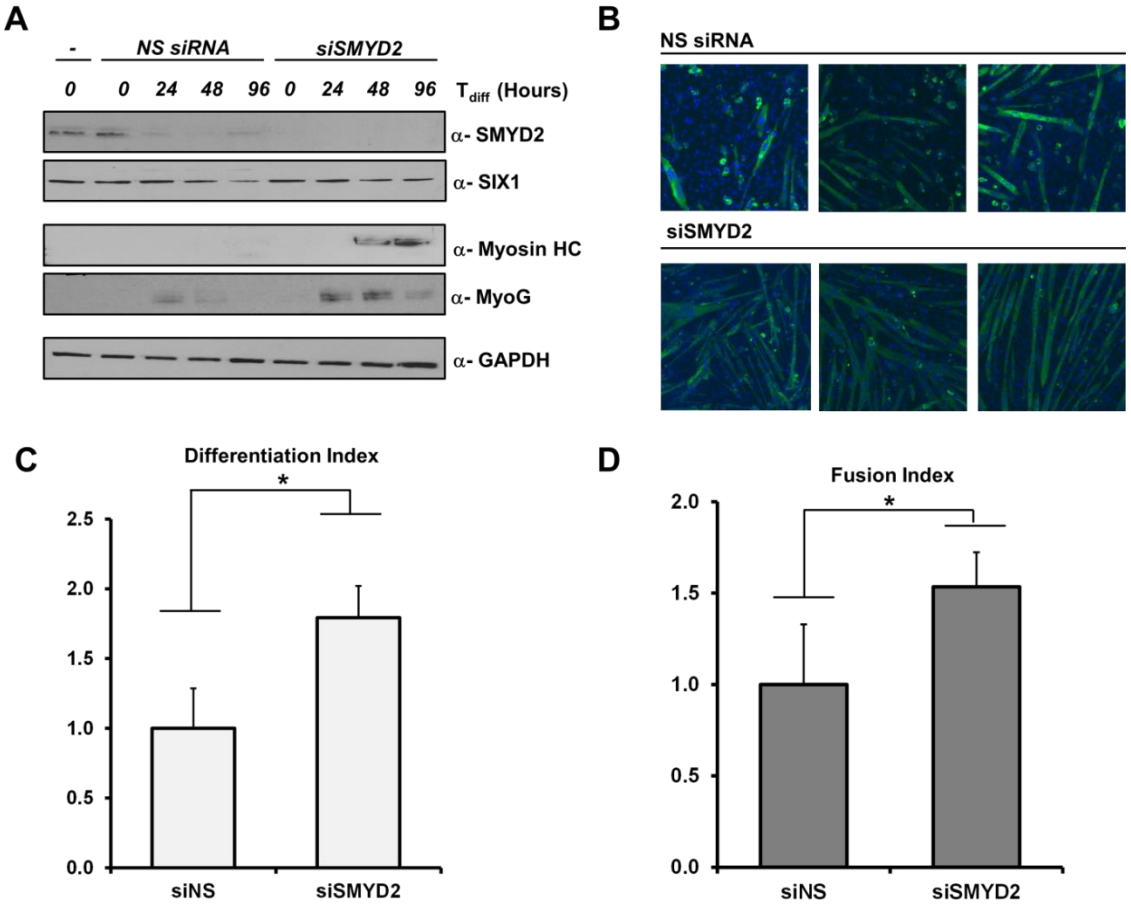
The decrease in SMYD2 levels during myoblast differentiation suggested that it may be linked with myogenic differentiation. We thus sought to verify whether SMYD2 repression affects the differentiation of myotubes from myoblasts. SMYD2 expression was repressed using siRNA as previously described and differentiation was initiated by serum starvation of fully confluent myoblasts. Early and late myogenic differentiation were followed by western blot, using antibodies directed against MyoG and Myosin Heavy Chain (MHC) (437) (Figure 4.2A). Strikingly, SMYD2 knockdown results in a significant increase in MyoG expression after 24 and 48 hours of differentiation (Figure 4.2A). In line with an increase in early myogenic differentiation, we observe higher levels of MHC during later differentiation stages (Figure 4.2A). These results suggest that myogenic differentiation is overall increased in response to SMYD2 repression.

Phenotypic differentiation of myoblasts into myocytes is rapidly followed by their fusion into multinucleated myotubes. To assess whether the differences in myogenic marker expression also reflected an increase in myocytes and myotube production, we followed myotube formation by immunofluorescence detection of MHC. C2C12 myoblasts were fixed after 72 hours of differentiation and expression of MHC was observed by immunostaining (Figure 4.2B). Consistent with the overall increase of MHC expression observed by western blotting, differentiated C2C12 cells show a higher proportion of MHC-positive cells following SMYD2 knockdown as calculated by their differentiation index (79% increase; Figure 4.2B-C). To assess whether this increase in myocyte production also results in increased myoblast fusion, we calculated the myoblasts relative fusion index. As shown in Figure 4.2D, SMYD2 knockdown causes a

53% increase in fusion relative to cells treated with non-specific siRNA. Together, these results strongly suggest that SMYD2 is a negative regulator of early myogenic differentiation.

Figure 4.2. SMYD2 limits skeletal myogenic differentiation

A) SMYD2 knock-down causes an increase in the expression levels of myogenic differentiation markers myogenin (MyoG) and myosin heavy chain (Myosin HC; MHC) in C2C12 differentiating cells. B) Immunofluorescence of C2C12 cells 72 hours after induction of myogenic differentiation shows that cells pre-treated with SMYD2-targeted siRNA (siSMYD2) have an increase in myotube formation (Green: MHC staining, Blue: DAPI staining; representative fields of three replicate experiments shown). Normalized differentiation (C) and fusion (D) indexes (*Material and methods*) of siSMYD2-treated C2C12 cells 72 hours after induction of myogenic differentiation relatively to cells treated with non-specific siRNA (siNS).


A
-
NS siRNA
siSMYD2
0 0 24 48 96 0 24 48 96 T diff (Hours)
α- SMYD2
α- SIX1
α- Myosin HC
α- MyoG
α- GAPDH
B
NS siRNA
siSMYD2
C
Differentiation Index
*
2.5
2.0
1.5
1.0
0.5
0.0
siNS
siSMYD2
D
Fusion Index
*
2.0
1.5
1.0
0.5
0.0
siNS
siSMYD2

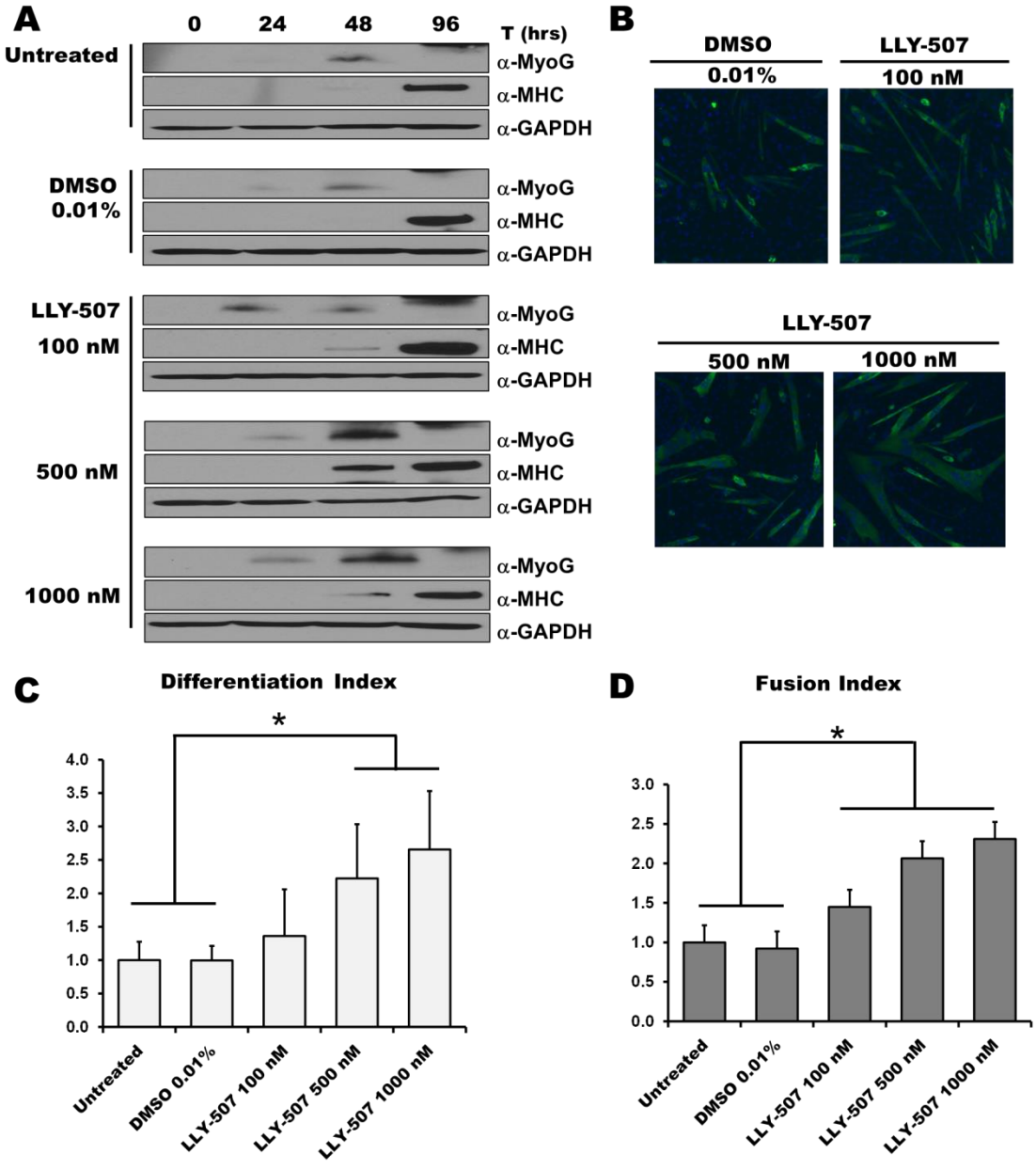
4.2.3. SMYD2 methyltransferase activity is important for myogenic differentiation

SMYD proteins regulate various aspects of muscle development but their methyltransferase activity is dispensable for certain aspect of muscle regulation. SMYD1 transcriptional role during muscle development can only be rescued by catalytically active SMYD1 (271) while its methyltransferase activity is not necessary for its association to the sarcomere and correct myofibril formation (438). To assess if the regulation of myogenic differentiation by SMYD2 depends on its methyltransferase activity, we treated C2C12 myoblasts with increasing concentration of the SMYD2 inhibitor LLY-507 (439). Consistent with SMYD2 knock-down, treatment with LLY-507 did not affect proliferation or cell cycle progression of myoblasts (Figure 4.5).

To determine whether inhibition of SMYD2 methyltransferase activity affects myogenic differentiation, we treated C2C12 myoblasts with LLY-507 and induced differentiation (Figure 4.3A). Similar to SMYD2 knock-down, levels of myogenic markers MyoG and MHC increases in response to SMYD2 inhibition (Figure 4.3). Moreover, immunostaining of differentiated cells treated with LLY-507 confirmed that inhibition of SMYD2 also results in an increase in MHC-positive myocytes and myotube fusion (Figure 4.3). Moreover, myogenic markers expression, myocytes population and myotube fusion increases concomitantly with inhibitor concentration (Figure 4.3). Together, those results indicate that the regulation of myogenic differentiation by SMYD2 is dependent on its methyltransferase activity.

Figure 4.3. SMYD2 methyltransferase activity is important for myogenic regulation

A) Treatment of C2C12 cells with the SMYD2 inhibitor LLY-507 promotes an increase in myogenic differentiation markers MyoG and MHC relatively to untreated cells and carrier-treated cells (DMSO 0.01%) B) Immunofluorescence of C2C12 cells 72 hours after induction of myogenic differentiation shows treatment with increasing amounts of LLY-507 increases myotube formation (Green: MHC staining, Blue: DAPI staining; representative field of each condition shown). Normalized differentiation (C) and fusion (D) indexes (*Material and methods*) of siSMYD2-treated C2C12 cells 72 hours after induction of myogenic differentiation relatively to cells treated with a non-specific siRNA (siNS).


A
0
24
48
96
T (hrs)
Untreated
α-MyoG
α-MHC
α-GAPDH
DMSO
0.01%
α-MyoG
α-MHC
α-GAPDH
LLY-507
100 nM
α-MyoG
α-MHC
α-GAPDH
500 nM
α-MyoG
α-MHC
α-GAPDH
1000 nM
α-MyoG
α-MHC
α-GAPDH
B
DMSO
0.01%
LLY-507
100 nM
LLY-507
500 nM
1000 nM
C
Differentiation Index
*
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Untreated
DMSO 0.01%
LLY-507 100 nM
LLY-507 500 nM
LLY-507 1000 nM
D
Fusion Index
*
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Untreated
DMSO 0.01%
LLY-507 100 nM
LLY-507 500 nM
LLY-507 1000 nM

4.2.4. SMYD2 regulates the transcription of muscle-specific genes in myoblasts

SMYD2 was shown previously to function as a transcriptional regulator to control apoptosis and macrophage activation (79, 440). To determine whether the regulation of myogenic differentiation by SMYD2 depends on the regulation of gene expression, we determined the transcriptional profile of proliferating C2C12 myoblasts following SMYD2 knockdown (*Materials and Methods*). A total of 186 genes showed at least a two-fold change of expression (Table 4.5 and 4.6). A majority (146; Table 4.5) shows increased expression following SMYD2 knock-down, which suggests that SMDY2 is predominantly a negative regulator of gene expression in myoblast.

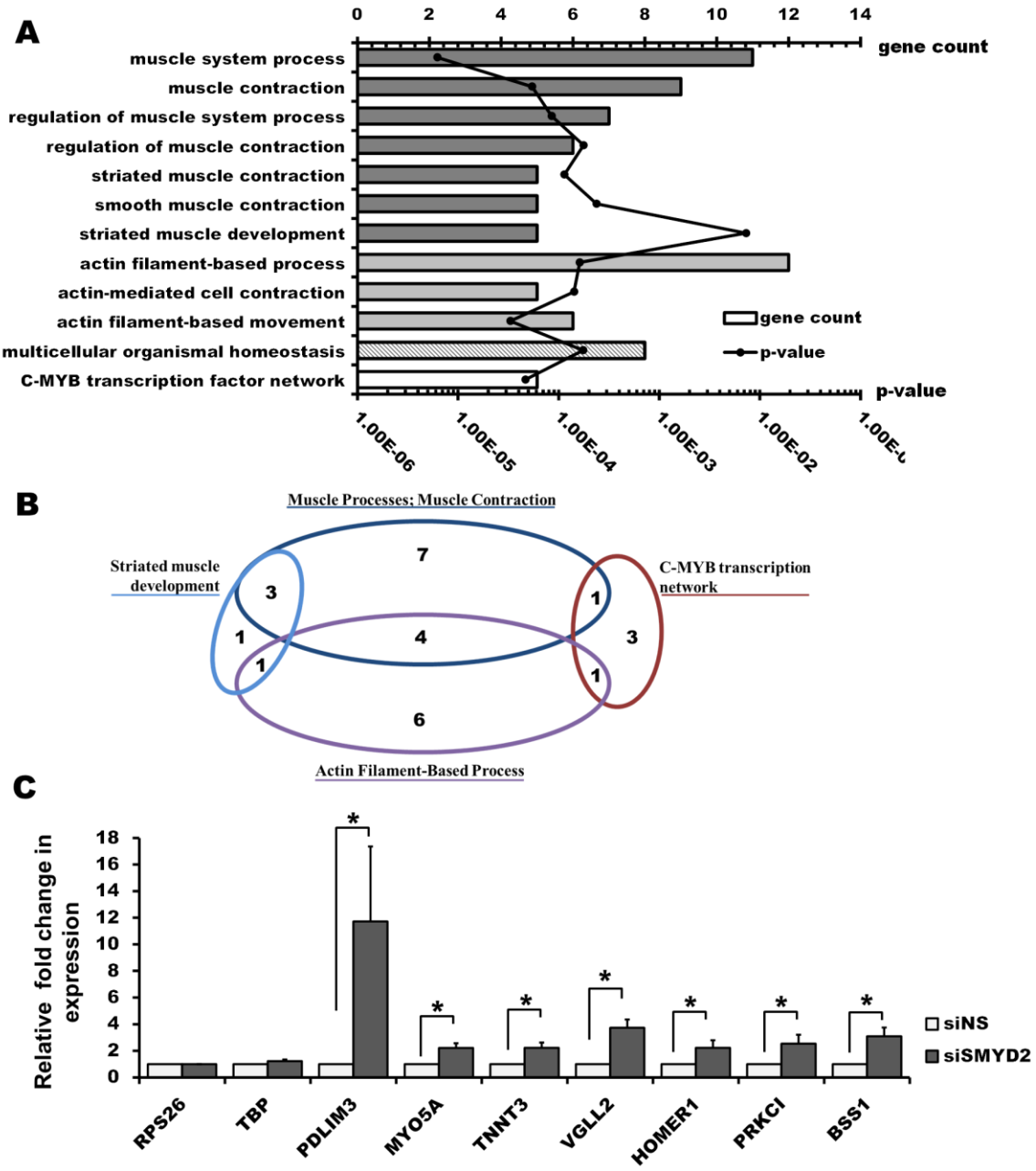
Surprisingly, we did do not observe changes in the expression of genes associated with SMYD2 activity in other models (79, 350). Previous work showed that methylation of p53 by SMYD2 in U2OS cells was shown to decrease *p21* expression (79). In addition, the methylation of ERα in MC7 breast cancer cells reduces the expression of *growth regulation by estrogen in breast cancer 1* (*greb1*) and *progesterone receptor* (*pr*) genes (350). However, repression of SMYD2 in myoblasts does not affect the expression of p21, *greb1* or *pr* (Table 4.5), which suggests that SMYD2 regulates unique pathways in myoblasts.

To confirm whether SMYD2 regulates specific pathways or functions in C2C12 myoblasts, we clustered our dataset using the DAVID and Toppgene gene ontology (GO) programs (383, 441, 442), and assessed the GO terms enrichment (Figure 4.4, Table 4.1-4.4 & 4.7). Genes upregulated in response to SMYD2 knockdown showed a significant enrichment in categories associated with muscle processes ($P=6.2x10^{-6}$), muscle

contraction ($P=5.4x10^{-5}$) and striated muscle development ($P=7.3x10^{-3}$) (Figure 4.4A, Table 4.1). We also observed that SMYD2 knockdown in myoblasts causes an increase in the expression of a set of genes involved in actin organization ($P=1.6x10^{-4}$) (Figure 4.4A & Table 4.2). Actin organization and contraction is tightly regulated during muscle development as it forms the basis of the muscle contractile apparatus (443). Overall, our results suggest that SMYD2 limits the expression in proliferating myoblasts of an overlapping set of genes related to muscle processes, development and actin organization (Figure 4.4B). We further confirmed, using RT-qPCR the upregulation of several genes associated with muscle and actin regulation (Figure 4.4C). *Pdlim3*, encoding the PDZ and LIM domain 3 Z-band associated protein (444, 445) and Vestigial-like like 2 (*Vgll2*) (446) are both early activators of the transcriptional network of myogenic differentiation and are upregulated in response to SMYD2 knockdown (Figure 4.4C). Homer 1 (*Homer1*) is also highly expressed in skeletal muscle and necessary for correct scaffolding of TRP channels in skeletal muscle (447, 448). Our data confirmed that it is aberrantly expressed following SMYD2 knockdown in proliferating myoblasts (Figure 4.4C). Moreover, expression of the sarcomeric protein troponin T3 (Tnnt3) (449) is also increased following SMYD2 knockdown.

Figure 4.4. SMYD2 controls the expression of muscle and actin – associated genes in C2C12 myoblasts

A) Statistical significance (p-value) calculated by DAVID and Toppgene analyses shows functional enrichment for muscle-associated genes (dark grey), actin-associated genes (pale grey), multicellular homeostasis (stripes) and the MYB-dependant transcriptional network (white)) among genes upregulated in response to SMYD2 knock-down. B) Venn representation of the overlap of upregulated genes associated with muscle processes, muscle development, actin regulation and MYB-dependant transcriptional network. C) RT-qPCR confirms that knock down of SMYD2 increases the expression of genes involved in muscle and actin regulation (* p-value ≤ 0.05).


A
0
2
4
6
8
10
12
14
gene count
muscle system process
muscle contraction
regulation of muscle system process
regulation of muscle contraction
striated muscle contraction
smooth muscle contraction
striated muscle development
actin filament-based process
actin-mediated cell contraction
actin filament-based movement
multicellular organismal homeostasis
C-MYB transcription factor network
gene count
p-value
p-value
1.00E-06
1.00E-05
1.00E-04
1.00E-03
1.00E-02
B
Muscle Processes; Muscle Contraction
Striated muscle development
C-MYB transcription network
Actin Filament-Based Process
7
3
1
4
1
3
1
1
6
C
Relative fold change in expression
18
16
14
12
10
8
6
4
2
0
RPS26
TBP
PDLIM3
MYO5A
TNNT3
VGLL2
HOMER1
PRKCI
BSS1
siNS
siSMYD2

Pdlim3 as well as the myosin VA isoform (*Myo5a*) and the protein kinase Cι (*Prkci*) regulate assembly of actinin during sarcomere and cytoskeletal formation (450-453). SMYD2 was previously shown to regulate myofibril assembly in developing muscle through direct association to the sarcomeric Z-discs (163, 164). Our data suggest that SMYD2 also regulates myofibril assembly in myoblasts by limiting the expression of actin assembly regulatory genes.

Table 4.1: Muscle processes genes upregulated in response to SMYD2 knock-down

Gene Symbol	Gene Name	Fold change (log 2)
Striated muscle tissue development		
Pdlim3	PDZ and LIM domain 3	5.38
Myog	myogenin	2.67
Homer1	homer homolog 1	2.52
Vgll2	vestigial like 2 homolog	1.48
Tnni1	troponin I, skeletal, slow 1	1.09
Muscle system processes		
Adrb1	adrenoceptor beta 1	3.11
Myl1	myosin, light chain 1, alkali; skeletal, fast	3.09
Myog	myogenin (myogenic factor 4)	2.67
Homer1	homer homolog 1 (Drosophila)	2.52
Pde4b	phosphodiesterase 4B, cAMP-specific	2.32
Lep	leptin	2.02
Tnnt3	troponin T type 3 (skeletal, fast)	1.59
Oxt	oxytocin/neurophysin I prepropeptide	1.52
Adora2b	adenosine A2b receptor	1.50
P2rx3	purinergic receptor P2X, ligand-gated ion channel, 3	1.28
Tnni1	troponin I type 1 (skeletal, slow)	1.09
Muscle contraction		
Adrb1	adrenoceptor beta 1	3.11
Myl1	myosin, light chain 1, alkali; skeletal, fast	3.09
Homer1	homer homolog 1 (Drosophila)	2.52
Pde4b	phosphodiesterase 4B, cAMP-specific	2.32
Tnnt3	troponin T type 3 (skeletal, fast)	1.59
Oxt	oxytocin/neurophysin I prepropeptide	1.52
Adora2b	adenosine A2b receptor	1.50
P2rx3	purinergic receptor P2X, ligand-gated ion channel, 3	1.28
Tnni1	troponin I type 1 (skeletal, slow)	1.09

Table 4.2: Actin – associated genes upregulated in response to SMYD2 knock-down

Gene Symbol	Gene Name	Fold change (log 2)
Actin Filament-based process		
Pdlim3	PDZ and LIM domain 3	5.38
Adrb1	adrenoceptor beta 1	3.11
Myl1	myosin, light chain 1, alkali; skeletal, fast	3.09
Pde4b	phosphodiesterase 4B, cAMP-specific	2.32
Tnnt3	troponin T type 3 (skeletal, fast)	1.59
Sdcbp	syndecan binding protein (syntenin)	1.34
Prkci	protein kinase C, iota	1.32
Myo5a	myosin VA (heavy chain 12, myoxin)	1.32
Rac2	ras-related C3 botulinum toxin substrate 2	1.30
Csf1r	colony stimulating factor 1 receptor	1.20
Kirrel	kin of IRRE like (Drosophila)	1.17
Tnni1	troponin I type 1 (skeletal, slow)	1.09

Analysis of the genes repressed in response to SMYD2 knockdown highlighted a small cluster of genes encoding neurofilament proteins (*Nefm, peripherin and keratin 8*; Table 4.3) normally expressed during the formation of the neuromuscular junction (454). Interestingly, expression of neurofilament proteins was observed to be inversely correlated myogenic potential of C2C12 cells (455). These results further suggest that repression of myogenic differentiation by SMYD2 is associated with transcriptional regulation. Interestingly, in addition to genes associated with muscle processes and actin organization, we observe an increase in the expression of genes associated with the MYB-dependant transcription network and multicellular homeostasis (Figure 4.4 & Table 4.4). This is consistent with previous observations that the MYB-dependant self-renewal of leukemic stem cells is associated with the misregulation of SMYD2 (347) and suggest that SMYD2 roles in those pathologies is associated with its normal function in muscle.

Together, these results suggest that SMYD2 negatively regulates myogenic differentiation in proliferating myoblasts by limiting the expression of positive regulators of myogenesis such as *Pdlim3* and *Vgll2*.

Table 4.3: Neurofilament genes downregulated in response to SMYD2 knock-down

Gene Symbol	Gene Name	Fold change (log 2)
Intermediate filament protein		
Nefm	neurofilament, medium polypeptide	-1.50
Krt8	keratin 8	-1.44
Prph	peripherin	-1.05

Table 4.4: Additional gene clusters upregulated in response to SMYD2 knock-down

Gene Symbol	Gene Name	Fold change (log 2)
C-MYB transcription factor network		
Tom1	target of myb1 (chicken)	2.56
Adora2b	adenosine A2b receptor	1.50
Casp6	caspase 6, apoptosis-related cysteine peptidase	1.50
Cebpd	CCAAT/enhancer binding protein (C/EBP), delta	1.29
Csf1r	colony stimulating factor 1 receptor	1.20
Multicellular organismal homeostasis		
Btbd9	BTB (POZ) domain containing 9	3.63
Adrb1	adrenoceptor beta 1	3.11
Homer1	homer homolog 1 (Drosophila)	2.52
Hoxa13	homeobox A13	1.60
Itgav	integrin, alpha V	1.49
Rac2	ras-related C3 botulinum toxin substrate 2	1.30
Csf1r	colony stimulating factor 1 receptor	1.20
Bbs1	Bardet-Biedl syndrome 1	1.01

4.3. Discussion

Our results show that SMYD2 is expressed in proliferating myoblasts and its methyltransferase activity negatively regulates myogenic differentiation. Moreover, we show that SMYD2 limits the expression of genes associated with muscle processes and development in proliferating myoblasts. The increase in transcription of muscle process genes observed before induction of myogenic differentiation in response to SMYD2 knockdown indicates that these regulatory effects occur in the proliferative stage of muscle development rather than after terminal phenotypic commitment.

Functional annotation of SMYD2-regulated genes in the myoblasts highlighted both new and previously reported roles for SMYD2. Previous work showed that SMYD2 is associated with the expression of cell cycle, transcription and chromatin remodelling genes in human embryonic kidney HEK293 cells and with the Nodal pathway in embryonic stem cells (ESCs). Our analysis of SMDY2-dependant gene expression in myoblasts did not show a significant enrichment of those pathways. In contrast, the increase in expression of genes involved in muscle-specific processes in response to SMYD2 repression suggests that the methyltransferase plays a unique role in transcriptional repression in myoblasts. Together with recent findings that SMYD2 negatively regulates the expression of endodermal genes in differentiating embryonic stem cells, our results suggest that SMYD may function predominantly as a repressor during transcriptional regulation of development.

Interestingly, we also observe an upregulation of a small MYB-dependant pathway gene cluster in response to SMYD2 knock-down. A recent study reported that SMYD2 is part of the MYB-dependant pathway in leukemic stem cells and that

repression of either SMYD2 or MYB-dependant genes in leukemic stem cells prevented their self-renewal (347). It is tempting to speculate that regulation of the MYB pathway by SMYD2 is part of a normal process in muscle that is misregulated in leukemia.

Our results also suggest that regulation of myogenic differentiation by SMYD2 depends on its methyltransferase activity. However, this regulatory function is difficult to explain by the substrates reported in previous studies (*see Results*). The lack of effect on *p21*, *greb1* and *pr* expression suggest that myogenic regulation of SMYD2 is also independent of p53 and ERα methylation. Moreover, the lack of effect of SMYD2 knockdown on myoblast proliferation, suggests that its role in myoblasts is independent of methylation of pRb K810 methylation as it was associated with cell cycle progression in other studies (153). Moreover, no clear association with specific transcription network could be established within the set of genes regulated by SMYD2 in myoblasts, which suggests that SMYD2 methyltransferase activity targets more than one transcription factor or regulatory complex. Also, previous studies showed the association of SMYD2 to its substrate HSP90 is important for proper myofibril formation during embryonic muscle development (163, 164). As sarcomere assembly occurs following the initiation of myogenic differentiation (456), this suggests that SMYD2 could play different roles as various stages of muscle formation as it regulates proper gene expression in proliferating myoblasts but localizes with HSP90 to the sarcomere in later stages of development.

Studies of SMYD1, SMYD2 and SMYD3 during cardiac morphogenesis have highlighted their functions during heart development (405, 420, 423) and injury (429). Consistent with their patterns of expression in muscle tissues (259, 270, 423), a role for SMYD1 and SMYD3 were shown to regulate myogenic differentiation in skeletal muscle

(420, 421, 425, 457, 458). Our results show that SMYD2 is also a regulator of myogenic differentiation and suggest that SMYD1, SMYD2 and SMDYD3 play unique roles during the myogenic differentiation of myoblasts. SMYD1 expression is upregulated following the initiation of myogenic differentiation and positively regulates the later stages of differentiation (271, 284, 457) while SMYD3 promotes the expression of myostatin (421) and the normal decrease in expression of myogenic regulatory factors in the later stages of myogenic differentiation (420). In contrast, SMYD2 limits the expression of muscle specific genes prior to the initiation of myogenic differentiation. As SMYD2 is expressed both in the somite (405, 406) and in adult skeletal muscle (163), it may, independently of its non-transcriptional role in sarcomere formation, regulate the premature differentiation of muscle progenitor cells during muscle formation.

4.4. Material and methods

4.4.1. Cell Culture

C2C12 myoblast cells (ATCC) were maintained at 37°C in 5% CO_2, below 70% confluence, in Dulbecco' Modified Eagle Media (DMEM; high glucose) containing Fetal Bovine Serum (10%), glutamine (1%) and gentamicin (28 μg/ml). To follow myogenic differentiation, C2C12 cells were allowed to reach confluence and the media was changed for DMEM containing Horse Serum (2%), glutamine (1%) and gentamicin (28 μg/ml).

4.4.2. Proliferation, cell cycle & apoptosis analysis

C2C12 myoblasts were seeded at low confluence and grown for 24 hours before siRNA transfection using RNAiMax (Life Technologies) as per manufacturer instructions and allowed to recover for 4 hours. Cells were fixed with 10% Formalin, washed with PBS 1X and stained with Crystal Violet (0.01%). Cells were then washed again and suspended in 1% SDS. The absorbance of the solution was measured at 595 nm. For cell cycle analysis, sub-confluent cells were treated as stated above, harvested and treated using the APC BrdU Flow Kit (Beckman Dickinson Bioscience) following the manufacturer's instructions. Dead cells population was assayed using the Vybrant® DyeCycle™ Violet/SYTOX® AADvanced™ Apoptosis Kit (Invitrogen).

4.4.3. Western blotting

Antibodies used in this study were as follow: anti-SMYD2 (#4251; Cell Signalling Technologies) anti-SIX1 (459), anti-myosin heavy chain (MF20,

Developmental Studies Hybridoma Bank, DSHB), anti-myogenin (F5D, DSHB), GAPDH (Immunetech).

4.4.4. Immunofluorescence

Cells were treated with siRNA as stated above, allowed to recover for 24 hours before inducing differentiation for 72 hours. Transfections were repeated after 48 hours of differentiation. After washing, cells were fixed with formalin (10%) and probed with anti-myosin heavy chain antibody. Myosin heavy chain was visualized with secondary antibody anti-mouse Alexa Fluor 488 (Life Technologies). For each condition, 9 fields were recorded at 10X magnification (800 ms) using an Axio Observer Z1 (AxioCamMR3, 1388 x 1040 pixels). The differentiation index was calculated by dividing the number of nuclei in myosin-positive cells by the total number of nuclei for each field. The fusion index was calculated by dividing the number of nuclei in myotubes containing $\geq$ 3 nuclei by the total number of nuclei for each field. Each index was averaged over 3 separate experiments.

4.4.5. Gene expression analysis & quantitative PCR

Subconfluent C2C12 cells were kept in a proliferative state, treated with either non-specific or SMYD2-targeted siRNA (*see above*), allowed to recover for 24 hours and harvested. Experiments were performed on independent biological duplicates. Gene expression profiling was done using the Agilent Technologies microarray gene expression platform Sureprint G3 Mouse (8x60k microarray kit). Labeled cDNA was prepared using a according to the manufacturer's instructions, using 200 ng of RNA per

microarray. Spot intensities were extracted using the Feature Extraction 1.1 software (Agilent) and gene expression data was analyzed using FlexArray (460). RT-PCR was performed using 250 ng of total RNA per reverse transcriptase reaction (Invitrogen). cDNA was diluted and quantified twice by real-time PCR using the SYBR green method.

4.5 Supplementary information

Supplementary information for:

Sylvain Lanouette, Elias Horner, Yubing Liu, Myriam Cramet, Daniel Figeys, Alexandre Blais, and Jean-François Couture. Biochim. Biophys. Acta (To Be Submitted)

Figure 4.5. LLY-507 and C2C12 myoblasts proliferation

A) Crystal violet staining of C2C12 myoblasts in proliferative conditions: treatment with increasing amounts of LLY-507 does not significantly affect proliferation of myoblasts up to 500 nM. Treatment with 1000 nM of LLY-507 shows an effect on myoblast proliferation after 72 hours of proliferation. B) C2C12 myoblasts were transfected with non-specific (siNS) or SMYD2 targeting (siSMYD2) siRNA and treated with LLY-507. Crystal violet staining shows that 1000 nM of LLY-507 decreases proliferation to a similar degree after SMYD2 knock-down as after treatment with non-specific siRNA. This suggests that inhibition of proliferation by 1000 nM of LLY-507 after 72 hours is independent of SMYD2. C) Relative cell cycle stages population of proliferating myoblasts following LLY-507.

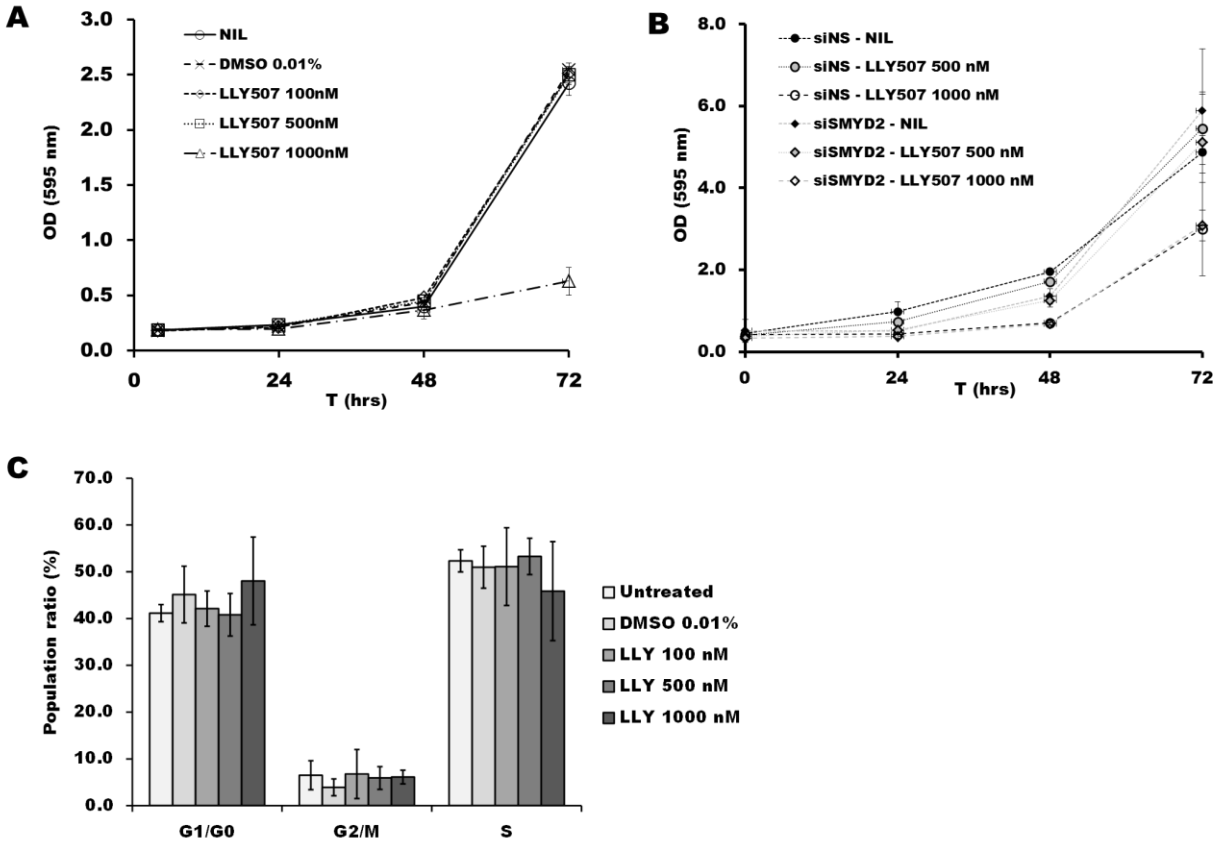

A
NIL
DMSO 0.01%
LLY507 100nM
LLY507 500nM
LLY507 1000nM
OD (595 nm)
T (hrs)
B
siNS - NIL
siNS - LLY507 500 nM
siNS - LLY507 1000 nM
siSMYD2 - NIL
siSMYD2 - LLY507 500 nM
siSMYD2 - LLY507 1000 nM
OD (595 nm)
T (hrs)
C
Population ratio (%)
G1/G0
G2/M
S
Untreated
DMSO 0.01%
LLY 100 nM
LLY 500 nM
LLY 1000 nM

Table 4.5: Genes upregulated by SMYD2 knock-down in proliferating C1C12 myoblasts

Gene Bank Accession Number	Gene Symbol	Gene Name	Chromosomal location	Fold change (log2)	P-value - SAM analysis
NM_145447	Mfsd7c	major facilitator superfamily domain containing 7C	chr12:87154338-87154397	7.385653	0.03967427
NM_001011803	Olfr1306	olfactory receptor 1306	chr2:111753043-111752984	5.857349	0.04301262
NM_001045484	Mef2b	myocyte enhancer factor 2B	chr8:72689021-72689080	5.83128	0.04186446
AK163379	Rad23b	RAD23b homolog (S. cerevisiae)	chr4:55364021-55364080	5.571117	0.04746054
XM_003945659	Gm2005	predicted pseudogene 2005	chrX:25941574-25941515	5.506342	0.04091892
NM_016798	Pdlim3	PDZ and LIM domain 3	chr8:47004658-47004717	5.375456	0.0468141
XR_168635	Gm7420	predicted gene 7420	chr5:29545623-29545682	4.932345	0.04184516
NM_013591	Madcam 1	mucosal vascular addressin cell adhesion molecule 1	chr10:79129132-79129191	4.888563	0.0452414
NM_009967	Crygs	crystallin, gamma S	chr16:22805336-22805277	4.751501	0.001958628
NM_026252	Cpeb4	cytoplasmic polyadenylation element binding protein 4	chr11:31831509-31831568	4.551496	0.01699085
NM_177719	Morc2b	microrchidia 2B	chr17:33272695-33272636	4.411819	0.04417043
NM_029494	Rab30	RAB30, member RAS oncogene family	chr7:99985127-99985186	4.116096	0.004110224
NM_181818	Olfr141	olfactory receptor 141	chr2:86646343-86646284	4.011475	0.007217012
	LOC100504608	protein FAM119B-like	chr10:126468304-126468245	3.702322	0.002277025
AK006275	Btbd9	BTB (POZ) domain containing 9	chr17:30551587-30551528	3.634075	0.01049747
NM_011600	Tle4	transducin-like enhancer of split 4, homolog of Drosophila E(spl)	chr19:14522621-14522562	3.47979	0.05066381
NM_008081	B4galnt2	beta-1,4-N-acetyl-galactosaminyl transferase 2	chr11:95725001-95724942	3.457546	0.01295782
NR_040491	1810053B23Rik	RIKEN cDNA 1810053B23 gene	chr16:93347193-93347134	3.422825	0.006223226
NM_017371	Hpx	hemopexin	chr7:112740363-112740304	3.400039	0.02265447
NM_029379	Tmem225	transmembrane protein 225	chr9:39958357-39958416	3.220993	0.002826985
NM_007419	Adrb1	adrenergic receptor, beta 1	chr19:56799215-56799274	3.11038	0.006059203
NM_021285	Myl1	myosin, light polypeptide 1	chr1:66971007-66970948	3.092081	0.01688472
NR_040409	Gm15441	predicted gene 15441	chr3:96359946-96359887	2.83489	0.04483617
NM_031189	Myog	myogenin	chr1:136188874-136188933	2.674512	0.005190846
NM_175434	Slc35f3	solute carrier family 35, member F3	chr8:128919283-128919342	2.583848	0.002055112
NM_001136259	Tom1	target of myb1 homolog (chicken)	chr8:77583100-77583159	2.557845	0.003666396
NM_011982	Homer1	homer homolog 1 (Drosophila)	chr13:94126009-94126068	2.520249	0.02970746

NM_147034	Olfr713	olfactory receptor 713	chr7:114180510-114180569	2.415719	0.0249315
AK051102	Pde4b	phosphodiesterase 4B, cAMP specific	chr4:102275988-102276046	2.321349	0.03174328
NM_016707	Bcl11a	B cell CLL/lymphoma 11A (zinc finger protein)	chr11:24073114-24073173	2.282814	0.001505152
NM_029993	Mlana	melan-A	chr19:29782737-29782796	2.264641	0.01936436
NM_027024	Cst13	cystatin 13	chr2:148656061-148656120	2.247539	0.04632203
NM_001161548	Tmem184a	transmembrane protein 184a	chr5:140280976-140280917	2.214702	0.00333835
NM_147034	Olfr713	olfactory receptor 713	chr7:114180510-114180569	2.171413	0.008413415
NM_001204916	H60c	histocompatibility 60c	chr10:7087176-7087235	2.16001	0.02198873
NR_027825	4930470P17Rik	RIKEN cDNA 4930470P17 gene	chr2:170427244-170427185	2.152447	0.02290533
NM_007887	Usp17la	ubiquitin specific peptidase 17-like A	chr7:112011106-112011165	2.137416	0.04222145
NM_177882	Zfp786	zinc finger protein 786	chr6:47769347-47769288	2.121874	0.02784532
NM_207683	Pik3c2g	phosphatidylinositol 3-kinase, C2 domain containing, gamma polypeptide	chr6:139570930-139570989	2.113606	0.02910926
NM_175271	Lpar4	lysophosphatidic acid receptor 4	chrX:104127529-104127588	2.081622	0.01588129
NM_028788	1300002K09Rik	RIKEN cDNA 1300002K09 gene	chr4:45899817-45899876	2.072829	0.01457875
NM_173379	Leprel1	leprecan-like 1	chr16:25968858-25965935	2.032228	0.04280036
NM_008493	Lep	leptin	chr6:29023234-29023293	2.024555	0.04824206
NM_146173	Tspan33	tetraspanin 33	chr6:29668498-29668557	2.021159	0.02273166
AK002412	Asb11	ankyrin repeat and SOCS box-containing 11	chrX:160897036-160897095	1.955232	0.02647524
NM_030229	Polr3h	polymerase (RNA) III (DNA directed) polypeptide H	chr15:81747646-81747053	1.931762	0.001234997
NM_133832	Rdh10	retinol dehydrogenase 10 (all-trans)	chr1:16122062-16122121	1.90006	0.02826985
NM_001277944	Apoc2	apolipoprotein C-II	chr7:20257086-20257027	1.899459	0.008702868
AK086119	Kcnt1	potassium channel, subfamily T, member 1	chr2:25750629-25750688	1.82118	0.01250434
NM_001177484	Gm11559	predicted gene 11559	chr11:99726135-99726194	1.820161	0.04720968
NM_053090	Fam126a	family with sequence similarity 126, member A	chr5:23467273-23467214	1.789267	0.002576126
NM_010218	Fjx1	four jointed box 1 (Drosophila)	chr2:102289748-102289689	1.781822	0.004968932
NM_053260	Prss29	protease, serine, 29	chr17:25459570-25459629	1.75488	0.00352167
AK151676	Fcnb	ficolin B	chr2:27933678-27933619	1.72016	0.02438154
NM_026887	Ap1s2	adaptor-related protein complex 1, sigma 2 subunit	chrX:160367276-160367335	1.714085	0.02300181
NM_011144	Ppara	peroxisome proliferator activated receptor alpha	chr15:85632110-85632169	1.684685	0.01509012
NM_139198	Plac8	placenta-specific 8	chr5:101001224-101001165	1.657432	0.02468064

NM_023423	Akirin1	akirin 1	chr4:123420761-123420702	1.653286	0.001756011
AK189299	Pcnp	PEST proteolytic signal containing nuclear protein	chr16:56024706-56024647	1.638147	0.01721277
NM_178257	Il22ra1	interleukin 22 receptor, alpha 1	chr4:135307221-135307280	1.634515	0.00856779
NM_053250	Crip3	cysteine-rich protein 3	chr17:46568642-46568701	1.625501	0.01869862
NM_008264	Hoxa13	homeobox A13	chr6:52209039-52208980	1.603029	0.0343387
AK041233	Tgfb1i1	transforming growth factor beta 1 induced transcript 1	chr7:135398222-135398281	1.597889	0.001997221
NM_011620	Tnnt3	troponin T3, skeletal, fast	chr7:149698500-149700262	1.589322	0.02743044
NM_147176	Homer1	homer homolog 1 (Drosophila)	chr13:94174815-94174874	1.586524	0.02047393
NM_026981	Dtwd1	DTW domain containing 1	chr2:125985757-125990514	1.574555	0.03526495
NM_030717	Lactb	lactamase, beta	chr9:66803425-66803366	1.520437	0.02481572
NM_011982	Homer1	homer homolog 1 (Drosophila)	chr13:94116595-94116654	1.518106	0.009098453
NM_011025	Oxt	oxytocin	chr2:130402665-130402724	1.516747	0.04278106
NM_007423	Afp	alpha fetoprotein	chr5:90937781-90937840	1.502404	0.005779399
NM_007413	Adora2b	adenosine A2b receptor	chr11:62079834-62079893	1.501357	0.03053722
NM_009811	Casp6	caspase 6	chr3:129616453-129616512	1.496034	0.01011154
NM_008402	Itgav	integrin alpha V	chr2:83643905-83643964	1.487457	0.02579021
NM_023423	Akirin1	akirin 1	chr4:123412443-123412425	1.48574	0.01470418
NM_153786	Vgll2	vestigial like 2 homolog (Drosophila)	chr10:51748095-51748154	1.48181	0.01144302
NM_001093749	Mpzl3	myelin protein zero-like 3	chr9:44882887-44882946	1.476019	0.02210451
NM_001163787	Ccdc151	coiled-coil domain containing 151	chr9:21794376-21794317	1.47194	0.02509552
NM_026484	Ccny	cyclin Y	chr18:9315372-9315313	1.469336	0.03399136
NR_002870	Dnm3os	dynamin 3, opposite strand	chr1:164150517-164150576	1.46078	0.02620509
NM_146660	Olfr1135	olfactory receptor 1135	chr2:87511649-87511590	1.455546	0.0276234
NM_030717	Lactb	lactamase, beta	chr9:66803463-66803404	1.453423	0.009976458
NM_010706	Lgals4	lectin, galactose binding, soluble 4	chr7:29626661-29626720	1.446812	0.00777662
NM_007423	Afp	alpha fetoprotein	chr5:90937781-90937840	1.441219	0.013035
NM_010090	Dusp2	dual specificity phosphatase 2	chr2:127163930-127163989	1.404283	0.01557254
NM_183296	Krtap16-3	keratin associated protein 16-3	chr16:88962965-88962906	1.382372	0.007091583
NM_134438	Gpr37l1	G protein-coupled receptor 37-like 1	chr1:137057805-137057746	1.375021	0.006966153
NM_145443	L2hgdh	L-2-hydroxyglutarate dehydrogenase	chr12:70791642-70791583	1.373678	0.01839952
AK048087	C130033p17rik	uncharacterized LOC402769	chr4:63689777-63689718	1.360711	0.02600247
NM_007423	Afp	alpha fetoprotein	chr5:90937781-90937840	1.358828	0.02471923
NM_010127	Pou6f1	POU domain, class 6, transcription factor 1	chr15:100405950-100405891	1.348308	0.01749257

DV059070	3010033K07Rik	RIKEN cDNA 3010033K07 gene	chr8:111107730-111107671	1.344146	0.04640886
NM_001098227	Sdcbp	syndecan binding protein	chr4:6323210-6323269	1.343012	0.0300934
NM_212450	Ctdspl2	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase like 2	chr2:121836400-121836459	1.342146	0.04876307
NM_011958	Orc4	origin recognition complex, subunit 4	chr2:48792375-48792316	1.340034	0.04898499
NM_008857	Prkci	protein kinase C, iota	chr3:30951503-30951562	1.324959	0.005142604
NM_153519	Txndc2	thioredoxin domain containing 2 (spermatozoa)	chr17:65987883-65987824	1.324647	0.02710239
NM_010864	Myo5a	myosin VA	chr9:75071413-75071472	1.323932	0.01041064
NM_001033192	C78339	expressed sequence C78339	chr13:46771083-46771142	1.320629	0.03322913
NM_007423	Afp	alpha fetoprotein	chr5:90937781-90937840	1.314332	0.03880591
NM_009008	Rac2	RAS-related C3 botulinum substrate 2	chr15:78391511-78391452	1.300706	0.00619428
NM_028834	Slx4ip	SLX4 interacting protein	chr2:136894055-136894114	1.300602	0.04565629
NM_021559	Zfp191	zinc finger protein 191	chr18:24170828-24170769	1.295304	0.03641311
NM_007679	Cebpd	CCAAT/enhancer binding protein (C/EBP), delta	chr16:15888459-15888518	1.291318	0.04258809
NM_001008499	Taar4	trace amine-associated receptor 4	chr10:23680515-23680574	1.291109	0.008587087
AK053021	P2rx3	purinergic receptor P2X, ligand-gated ion channel, 3	chr2:84836104-84836045	1.282715	0.005441704
NM_147176	Homer1	homer homolog 1 (Drosophila)	chr13:94157507-94157566	1.267202	0.01993362
NM_134172	Vmn1r26	vomeronasal 1 receptor 26	chr6:57958280-57958221	1.253759	0.003145382
NM_001166206	Erv3	endogenous retroviral sequence 3	chr2:131681477-131681418	1.251076	0.04635097
NM_144916	Tmem150a	transmembrane protein 150A	chr6:72309303-72309362	1.224763	0.04058122
NM_177597	March11	membrane-associated ring finger (C3HC4) 11	chr15:26339232-26339291	1.221473	0.006232874
NM_001037166	Gm4925	predicted gene 4925	chr10:88193122-88193063	1.201493	0.01246575
NM_001037859	Csf1r	colony stimulating factor 1 receptor	chr18:61289398-61289758	1.199124	0.04275211
NM_028264	Tmem55a	transmembrane protein 55A	chr4:14842049-14842108	1.187594	0.005567133
NM_146860	Olfr161	olfactory receptor 161	chr16:3593280-3593339	1.186445	0.04522211
NM_146957	Olfr170	olfactory receptor 170	chr16:19606000-19605941	1.186309	0.04582031
NM_130867	Kirrel	kin of IRRE like (Drosophila)	chr3:86893538-86893479	1.171123	0.01343059
NM_172253	Twistnb	TWIST neighbor	chr12:34124050-34124109	1.142874	0.0246324
NM_172882	Wdfy3	WD repeat and FYVE domain containing 3	chr5:102342192-102341192	1.141454	0.04137239
XM_887972	Gm6436	predicted gene 6436	chr13:61812762-61812703	1.123352	0.02985219
NM_001253916	Sez6l	seizure related 6 homolog like	chr5:112849205-112849146	1.121769	0.01982749
NM_027604	Usp15	ubiquitin specific peptidase 15	chr10:122550456-122550397	1.121741	0.03460885

NR_040298	4931408D14Rik	RIKEN cDNA 4931408D14 gene	chr19:37333005-37332946	1.094213	0.03329667
NM_021467	Tnni1	troponin I, skeletal, slow 1	chr1:137705328-137706203	1.090579	0.03484042
NM_010128	Emp1	epithelial membrane protein 1	chr6:135333061-135333120	1.090243	0.04782718
NM_146276	Olfr1394	olfactory receptor 1394	chr11:48974376-48974435	1.086487	0.007988885
NM_030687	Slco1a4	solute carrier organic anion transporter family, member 1a4	chr6:141754734-141754675	1.083976	0.01705839
NM_027819	Ggt6	gamma-glutamyltransferase 6	chr11:72251155-72251214	1.079988	0.02127475
NM_013885	Clic4	chloride intracellular channel 4 (mitochondrial)	chr4:134770475-134770416	1.076388	0.04931303
NM_001037098	Nacc2	nucleus accumbens associated 2, BEN and BTB (POZ) domain containing	chr2:25911126-25911067	1.069776	0.04324418
NM_001081234	Pigg	phosphatidylinositol glycan anchor biosynthesis, class G	chr5:108777113-108777172	1.069728	0.02765235
NM_026450	Zfp169	zinc finger protein 169	chr13:48585061-48585002	1.062832	0.02203697
NM_172614	Tmem44	transmembrane protein 44	chr16:30514162-30514103	1.053408	0.04745089
AK035805	A630026N12Rik	RIKEN cDNA A630026N12 gene	chr2:126495851-126495792	1.051973	0.03991548
NM_001001187	Zfp738	zinc finger protein 738	chr13:67784435-67784376	1.050695	0.04122766
NM_013885	Clic4	chloride intracellular channel 4 (mitochondrial)	chr4:134770475-134770416	1.047053	0.04682374
NM_010781	Tpsb2	tryptase beta 2	chr17:25504493-25504552	1.043373	0.004447918
NM_175937	Cpeb2	cytoplasmic polyadenylation element binding protein 2	chr5:43677337-43677396	1.034292	0.03126086
NM_001164320	Chadl	chondroadherin-like	chr15:81516695-81516636	1.034056	0.03494655
NM_001177841	Otub2	OTU domain, ubiquitin aldehyde binding 2	chr12:104644490-104644549	1.032193	0.01873722
NM_001013810	Gm5591	predicted gene 5591	chr7:39304732-39304673	1.031573	0.02909961
NM_001099299	Ajap1	adherens junction associated protein 1	chr4:152756433-152756374	1.027587	0.03546756
NM_177640	D030056L22Rik	RIKEN cDNA D030056L22 gene	chr19:18792806-18792865	1.014823	0.03582455
NM_001033128	Bbs1	Bardet-Biedl syndrome 1 (human)	chr19:4889219-4889160	1.005206	0.04300297
NM_026005	2610301B20Rik	RIKEN cDNA 2610301B20 gene	chr4:10809559-10809618	1.004829	0.03203273
NM_011146	Pparg	peroxisome proliferator activated receptor gamma	chr6:115440213-115440272	1.003551	0.003791826
NM_025294	Gm16515	predicted gene, Gm16515	chr11:60716627-60716568	0.9899526	0.04891745
NM_177687	Crebl2	cAMP responsive element binding protein-like 2	chr6:134806119-134806178	0.9862301	0.04516422

Table 4.6: Genes downregulated by SMYD2 knock-down in proliferating C1C12 myoblasts

Gene Bank Accession Number	Gene Symbol	Gene Name	Chromosomal location	Fold change (log)	P-value - SAM analysis
NM_177753	Sox21	SRY-box containing gene 21	chr14:118633410-118633351	-8.516808	0.04720003
NM_011481	Srms	src-related kinase lacking C-terminal regulatory tyrosine and N-terminal myristylation sites	chr2:180940666-180940607	-4.089402	0.04241442
NM_152220	Stx3	syntaxin 3	chr19:11859903-11857591	-3.824147	0.01496469
NM_001045527	Hsf5	heat shock transcription factor family member 5	chr11:87472974-87473028	-3.397849	0.01513836
AK033889	Mmd	monocyte to macrophage differentiation-associated	chr11:90129711-90129770	-3.031976	0.04456601
NM_011193	Pstpip1	proline-serine-threonine phosphatase-interacting protein 1	chr9:55975648-55976435	-2.607478	0.01188684
AK046043	Apol8	apolipoprotein L 8	chr15:77578413-77578354	-2.456539	0.04134344
AK046043	Apol8	apolipoprotein L 8	chr15:77578413-77578354	-2.418327	0.02379298
AK046043	Apol8	apolipoprotein L 8	chr15:77578413-77578354	-2.407273	0.02393771
NM_026796	Smyd2	SET and MYND domain containing 2	chr1:191704429-191704370	-2.378882	0.000627147
NM_176928	Brwd1	bromodomain and WD repeat domain containing 1	chr16:96288587-96288528	-2.102828	0.007149473
NM_001033632	Ifitm6	interferon induced transmembrane protein 6	chr7:148201773-148201714	-2.005123	0.03660607
NM_011474	Sprr2h	small proline-rich protein 2H	chr3:92191160-92191219	-1.891748	0.01836093
NM_031185	Akap12	A kinase (PRKA) anchor protein (gravin) 12	chr10:5991309-5991250	-1.857286	0.02956273
AK046043	Apol8	apolipoprotein L 8	chr15:77578413-77578354	-1.850582	0.0473737
NM_177793	Metl24	methyltransferase like 24	chr10:40530455-40530514	-1.841194	0.007438926
NM_178613	Gskip	GSK3B interacting protein	chr12:106941140-106941199	-1.701157	0.02088881
NM_133779	Pigt	phosphatidylinositol glycan anchor biosynthesis, class T	chr2:164333740-164333799	-1.658221	0.02317549
NM_025586	Rpl15	ribosomal protein L15	chr14:19100647-19100588	-1.57978	0.005036471
NM_008218	Hba-a1	hemoglobin alpha, adult chain 1	chr11:32183955-32184014	-1.537786	0.007853807
AK005901	1700012E03Rik	RIKEN cDNA 1700012E03 gene	chr1:122334975-122335034	-1.522503	0.03692447
AK043828	Cdhr3	cadherin-related family member 3	chr12:33735728-33735669	-1.503797	0.006638107
NM_008691	Nefm	neurofilament, medium polypeptide	chr14:68738252-68738193	-1.501013	0.04691058
NM_001177850	Asph	aspartate-beta-hydroxylase	chr4:9502652-9502593	-1.484183	0.01669175
NM_010937	Nras	neuroblastoma ras oncogene	chr3:102870149-102870208	-1.458166	0.007130176
AK146072	5830416P10Rik	RIKEN cDNA 5830416P10 gene	chr19:53518563-53518504	-1.45706	0.01575586
NM_152220	Stx3	syntaxin 3	chr19:11852097-11852038	-1.452097	0.0173382
AK166854	Krt8	keratin 8	chr15:101827197-101827138	-1.440781	0.0345992
NM_175105	Aqp11	aquaporin 11	chr7:104874948-104874889	-1.401373	0.04188376

NM_022417	Itm2c	integral membrane protein 2C	chr1:87804673-87804732	-1.3951	0.008529196
NM_001083955	Hba-a2	hemoglobin alpha, adult chain 2	chr11:32197245-32197304	-1.387386	0.002122651
NM_173414	Lancl3	LanC lantibiotic synthetase component C-like 3 (bacterial)	chrX:8835086-8835145	-1.338143	0.001254293
NM_178893	Coro2a	coronin, actin binding protein 2A	chr4:46556310-46556251	-1.276481	0.01119216
NM_009360	Tfam	transcription factor A, mitochondrial	chr10:70691162-70691103	-1.200964	0.02645595
NM_016866	Stk39	serine/threonine kinase 39	chr2:68048583-68048524	-1.147898	0.04643781
NM_001129803	Pcp2	Purkinje cell protein 2 (L7)	chr8:3623436-3623377	-1.131167	0.04489406
AF209907	Ramp3	receptor (calcitonin) activity modifying protein 3	chr11:6577424-6577483	-1.090826	0.02599282
NM_030175	Hhipl2	hedgehog interacting protein-like 2	chr1_random:409611-409670	-1.085523	0.04612906
NM_013639	Prph	peripherin	chr15:98889042-98889101	-1.048504	0.03902782
NM_178642	Ano1	anoctamin 1, calcium activated chloride channel	chr7:151774663-151774604	-1.037449	0.03194589

Table 4.7: Annotation of genes regulated by SMYD2 in proliferating C2C12 myoblasts

Type	ID	Annotation	Annotation tool	p-value	Genes	Regulation
Gene Ontology	GO:0014706	Striated muscle tissue development	DAVID	7.3E-03	*Pdlim3, Myog, Homer1, Vgll2, Tnni1*	Upregulated
Gene Ontology	GO:0003012	Muscle system process	TopGene	6.2E-06	*Homer1, Pde4b, Lep, Tnnt3, Oxt, Adora2b, P2rx3, Tnni1*	Upregulated
Gene Ontology	GO:0090257	Regulation of muscle system process	TopGene	8.5E-05	*Pde4b, Myog, Adora2b, Adrb1, Oxt, Tnni1, Tnnt3*	Upregulated
Gene Ontology	GO:0006936	Muscle contraction	TopGene	5.4E-05	*Adrb1, Myl1, Homer1, Pde4b, Tnnt3, Oxt, Adora2b, P2rx3, Tnni1*	Upregulated
Gene Ontology	GO:0006941	Striated muscle contraction	TopGene	1.1E-04	*Pde4b, Myl1, Adrb1, Tnni1, Tnnt3, Homer1*	Upregulated
Gene Ontology	GO:0006937	Regulation of muscle contraction	TopGene	1.8E-04	*Pde4b, Adora2b, Adrb1, Oxt, Tnni1, Tnnt*	Upregulated
Gene Ontology	GO:0006939	Smooth muscle contraction	TopGene	2.4E-04	*Pde4b, Adora2b, Adrb1, Oxt, P2rx3*	Upregulated
Gene Ontology	GO:0030029	Actin filament-based process	TopGene	1.6E-04	*Pde4b, Tnnt3, Sdcbp, Prkci, Myo5a, Rac2, Csf1r, Kirrel, Tnni1*	Upregulated
Gene Ontology	GO:0070252	Actin-mediated cell contraction	TopGene	1.4E-04	*Pde4b, Myl1, Adrb1, Tnni1, Tnnt3*	Upregulated
Gene Ontology	GO:0030048	Actin filament-based movement	TopGene	3.3E-05	*Pde4b, Myl1, Myo5a, Adrb1, Tnni1, Tnnt3*	Upregulated
Gene Ontology	GO:0048871	Multicellular organismal homeostasis	TopGene	1.8E-04	*Btbd9, Adrb1, Homer1, Hoxa13, Itgav, Rac2, Csf1r, Bbs1*	Upregulated
BioSystems pathway	BSID: 138073	C-MYB transcription factor network	TopGene	4.7E-05	*Tom1, Adora2b, Casp6, Cebpd, Csf1r*	Upregulated
Gene Ontology	GO:0001721	Intermediate filament protein	DAVID	6.5E-03	*Nefm, Krt8, Prph*	Downregulated

5. DISCUSSION

5.1 Summary

In the course of our characterization of SMYD2, I identified many novel substrates for this enzyme, delineated an amino acid motif driving its specificity and demonstrated the role of its methyltransferase activity during myogenic differentiation. I have first shown that SMYD2 methylates the molecular chaperone HSP90. I also showed that association to co-chaperones, reaction conditions and demethylation by LSD1 regulate the methylation of HSP90 by SMYD2. I have also shown, using high-throughput methyltransferase assays, that SMYD2 preferentially methylates lysine K* in the amino acid motif $[LFM]_{-1}$-K*-$[AFYMSHRK]_{+1}$-$[LYK]_{+2}$. This motif allowed me to identify the transcription factors SIX1 and SIX2, the co-repressor SIN3B and the RNA helicase DHX15 as novel substrates of SMYD2 *in vitro*. Moreover, my results confirmed that multistate computational protein design accurately recapitulates SMYD2 specificity. Finally, I determined that SMYD2 methyltransferase activity limits myogenic differentiation by regulating the expression of muscle-specific genes in myoblasts**.**

5.2 Extent of SMYD2 methyltransferase activity

At the start of this project, histone H3 and p53 were the only substrates reported for SMYD2 (79, 259). Since then, other groups have shown that SMYD2 methylates the retinoblastoma protein pRb (153, 154), the Poly [ADP-ribose] polymerase PARP1 (353) and the estrogen receptor ERα (350) (Table 5.1). The five novel substrates that I identified double this list of SMYD2 substrates. Together, they form one of the largest set

of substrates reported for a SET-domain methyltransferase (Appendix 1). Moreover, using the $[LFM]_{-1}$-K*-$[AFYMSHRK]_{+1}$-$[LYK]_{+2}$ motif, we identified the molecular basis underlying a majority of these substrates (SIX1, SIX2, DHX15 and SIN3B, p53, ERα). However, the sequences surrounding SMYD2 methylation sites on pRb and HSP90 diverge from this motif at position +2 and on PARP1 at positions +1 and +2. It is likely that these substrates are recognized through different motifs either determined by biochemical conditions, co-dependence of residue identity within our motif or relying on unique binding modes. Further methyltransferase assays would help infer the molecular basis and the specificity rules underlying these alternate motifs and predict new sets of SMYD2 substrates. Considering that we assayed only a limited number of proteins including the $[LFM]_{-1}$-K*-$[AFYMSHRK]_{+1}$-$[LYK]_{+2}$ motif and that more putative substrates would be predicted by alternate motifs, we postulate that SMYD2 methylates a much larger number of substrates.

5.3 Motif recognition by SET-domain methyltransferases

In addition to our characterization of the amino acid sequence recognized by SMYD2, amino acid motifs for the SET methyltransferases G9a (108), SETD6 (152), SET7/9 (110, 461), SET8 (111) and NSD1 (343) have been reported. SET7/9 recognizes the motif $[R/K]_{-2}$-$[S/T/A]_{-1}$-K*-$[D/K/N/Q]_{+1}$ (K* is the methylated lysine), or $[G/R/H/K/P/S/T]_{-3}$-$[K/R]_{-2}$-$[S/K/Y/A/R/T/P/N]_{-1}$-K* (110, 461), G9a recognizes the motif $[N/T/G/S]_{-2}$-$[G/C/S\text{-}R]_{-1}$-K*-$[T/G/Q/S/V/M/A]_{+1}$-$[F/V/I/L/A]_{+2}$ (108), NSD1 methylates the motif $[FY]_{-2}$-$[ILV]_{-1}$-K*-$[QKNM]_{+1}$-$[VI]_{+2}$ (343) and SET8 methylates the peptide motif R_{-3}-H_{-2}-$[RKY]_{-1}$-K*-$[VILFY]_{+1}$-$[LFY]_{+2}$-R_{+3} (111).

Alignment of these motifs (Figure 5.1) shows that these methyltransferases favour hydrophobic and positively charged residues, with a strong bias for K and R residues. SET-domain methyltransferases also select strongly against negatively charged residues, with only SET7/9 allowing for one acidic residue at a single position. However, despite the conservation of the SET-domain fold, each methyltransferase targets a unique set of sequences. In addition, the range and symmetry relatively to the substrate lysine of these sequences is not conserved between methyltransferases. For example, SMYD2 and SET7/9 recognize different asymmetric cassettes covering positions -1, +1 and +2 and -2, -1 and +1, respectively, while SET8 recognizes a symmetric cassette from positions -3 to +3. Moreover, there is no conservation in the degree of specificity for each position between SET domain methyltransferases.

Accordingly, inspection of the crystal structures of methyltransferase/substrate complexes shows few conserved characteristics (Figure 5.2). In each case, the target lysine is fully extended within the methyl-transfer channel and the Ψ_0 and Φ_0 dihedral angles orient the peptide backbone away from the catalytic site. However, the overall orientation of each backbone shows different binding conformations. For example, a histone H4 peptide bound to SET8 adopts an extended conformation while substrate peptides bound to SMYD2 or SET7/9 curve into sharp U-shapes.

The conformations of the peptide substrate backbones also show striking differences. The ψ dihedral angle Ψ_{-1} of the p53 peptide, when bound to SMYD2, is rotated by approximately 60° relatively to SET7/9, G9a and SET8 (Figure 5.2). The side chain of the residue in position -1 is stabilised by polar and water-mediated interactions in SET7/9 (461) and by two aspartic acid residues in GLP/G9a (75). In contrast, the side chain of the

corresponding residue of the SMYD2 substrate is bound within a tight hydrophobic pocket (354, 374). Position +1 of peptides bound to SMYD2, G9a and SET8 show similar relative Φ dihedral angle (Φ_{+1}) which orient the side chain of these residues away from the enzyme peptide binding clefts. Strikingly, in the peptide bound to SET7/9, the Φ_{+1} angle is rotated by approximately 180° (Figure 5.2) orienting the side chain of the residue in position +1 toward the peptide binding cleft. Accordingly, the SET7/9 motif shows a higher specificity (4 amino acids) at this position when compared to other SET domain lysine methyltransferases (Figure 5.1). Equivalent positions of each methyltransferase recognition motif are thus stabilized by unique mechanisms in each methyltransferase, independently of the conservation of the SET domain.

Comparison of crystal structures and motifs shows that the conformation of the lysine side chains within the methyl-transfer channel and a bias for positively charged residues are conserved across the SET-domain protein. However, the differences in binding conformations and recognition motifs suggest that SET-domain families have evolved divergent recognition mechanisms allowing them to recognize unique substrates. The SMYD2 recognition motif is thus representative of SET-domain methyltransferases as it shares the same amino acid bias but also presents unique amino acid combinations enabling the enzyme to methylate unique targets. However, in contrast with other post-translational modifying enzymes (462), SMYD2 lacks a stringent specificity, which strongly suggest that other factors can direct its methyltransferase activity (Chapter 3).

Figure 5.1: SET-domain methyltransferases recognition motifs

Comparison of all reported substrate recognition motifs for the SET-domain methyltransferases G9a, SETD6, SET7/9, SET8 and NSD1. Green, black, blue and red represent hydrophobic, polar, positively charged and negatively charged residues, respectively. The methylated lysine residue is indicated with an asterisk.

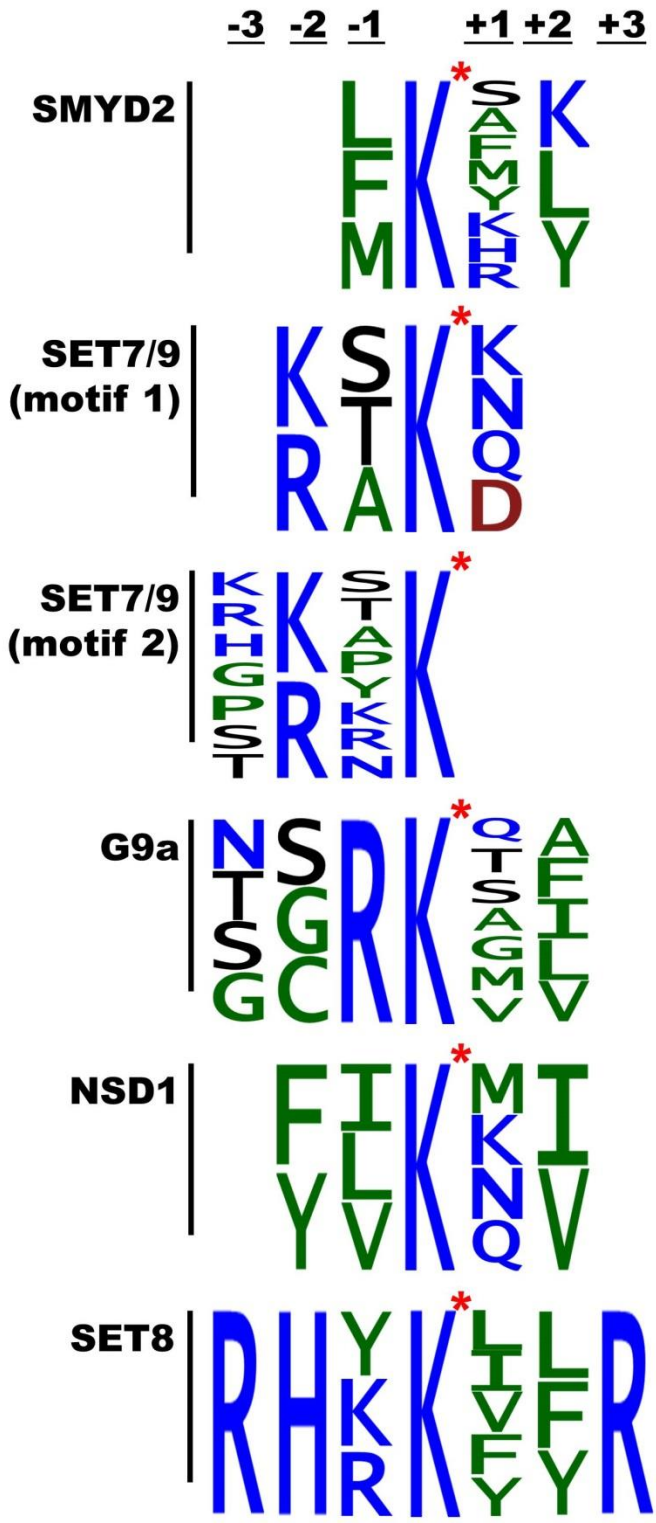
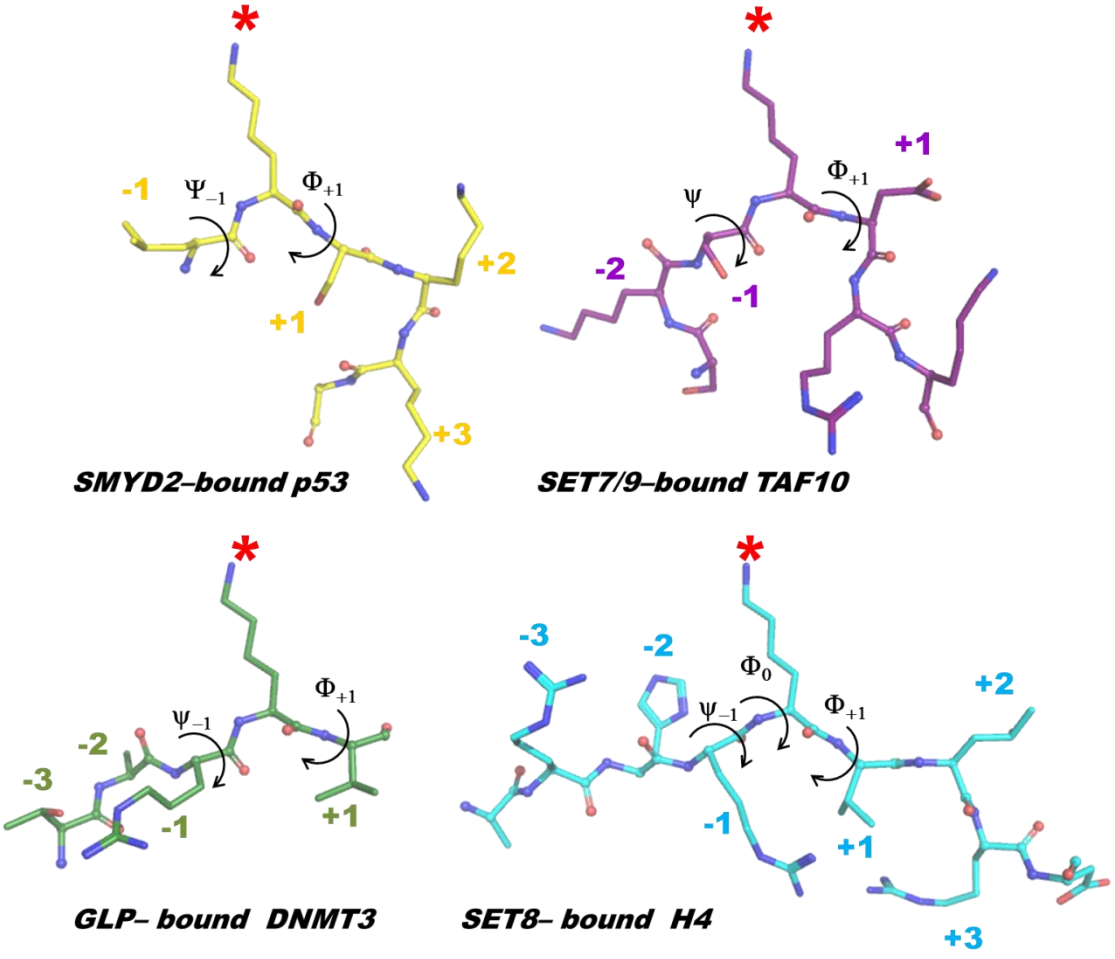

-3
-2
-1
+1
+2
+3
SMYD2
SET7/9
(motif 1)
SET7/9
(motif 2)
G9a
NSD1
SET8

Figure 5.2: SET domain – bound substrate peptides

Crystal structures of the p53 (*yellow*), TAF10 (*purple*), DNMT3 (*green*) and histone H4 (*blue*) peptides in complex with their respective methyltransferase SMYD2, SET7/9, G9a and SET8 (PDB ID 3S7F, 3M53, 3SW9, 1ZKK). The methylated lysine is indicated by an asterisk and positions within the methyltransferases are numbered relative to the central lysine, from the N-terminal (-X) to the C-terminal (+X) termini.


*
-1
Ψ−1
Φ+1
+2
+1
+3
SMYD2–bound p53
*
+1
Φ+1
ψ
-2
-1
SET7/9–bound TAF10
*
Φ+1
Ψ−1
-2
-3
-1
+1
GLP– bound DNMT3
*
-3
-2
Φ0
Ψ−1
Φ+1
+2
-1
+1
+3
SET8– bound H4

5.4 Regulation of SMYD2 methyltransferase activity

In addition to SMYD2 expression levels and recognition motif, several observations suggest that its activity is regulated by other factors. Methylation of pRB K810 increases following cell-cycle re-entry but the relative fraction of methylated pRb remains constant during myogenic differentiation (154) despite the decrease in SMYD2 expression (Chapter 4). Moreover, expression of SMYD2 in the breast cancer cell line model MCF7 is higher in the cytoplasm but methylation of ERα is detected mostly in the nuclear fraction (350). The activity of SMYD2 toward its substrates can thus be promoted or prevented by additional mechanisms.

Our biochemical characterization of SMYD2 provides several lines of evidence regarding those mechanisms. Demethylation by LSD1 limits the apparent levels of methylation of the SMYD2 substrates HSP90 (Chapter 2), p53 (137) and ERα (350); (Table 5.1). The inhibition or activation of LSD1 activity and expression could then regulate the methylation levels of SMYD2 substrates independently of SMYD2 itself.

The inclusion of SMYD2 substrates in protein complexes can also regulate their methylation, as in the inhibition of HSP90 methylation by its co-chaperone HOP (Chapter 2). Moreover, the stimulation of HSP90 K209 methylation in presence of histone H3 shows that the presence of other proteins can also enable methylation by SMYD2. Future studies of SMYD2, its substrates and their interactors might reveal activators and inhibitors of SMYD2 activity and illustrate the recruitment of SMYD2 to protein complexes.

In addition, the observations that pH can direct the activity of SMYD2 toward different substrates (Chapter 2) and that SMYD2 appears to recognize additional sequences (Chapter 3) suggest that its biochemical context (e.g. subcellular localization and protein interactors) could also direct SMYD2 activity toward alternate targets. Our results highlight that the broad specificity of SMYD2 is balanced by numerous factors directing its activity toward subsets of substrates depending on the biological context. New peptide arrays based on the HSP90 and pRb sequences, together with structural biology efforts toward the resolution of the structure of SMYD2 with either substrate would likely provide evidence of the determinants of SMYD2 specificity toward these substrates. Moreover, MSD simulations of these sequences with the recognition space of these conformations would provide a more complete assessment of the set of motifs recognized by SMYD2. It may be that SMYD2 inhibitors could be designed that would prevent the methylation of specific sets of under distinct conditions.

5.5 SMYD2 methyltransferase activity and myogenic differentiation

The methylation of numerous substrates by SMYD2 *in vivo* is evidenced by the impact on transcription following SMYD2 knock-down in myoblasts. I showed that SMYD2 methyltransferase activity negatively regulates myogenic differentiation by repressing a subset of muscle-specific genes in proliferating myoblasts. These genes however are not under the control of a specific muscle transcriptional network. This suggests that either SMYD2 methylates transcription factors such as SIX1, SIX2, SIN3B, pRB or ERα and modulates their transcriptional activity on a limited number of target genes or that SMYD2 modifies other proteins regulating myogenic differentiation.

It is also possible that methylation of HSP90, although mainly cytoplasmic (163), regulates indirectly the expression of myogenic genes. For example, deacetylation of HSP90 promotes the activation of glucocorticoid receptor signalling and downstream gene activation of its client genes (463, 464). By analogy, methylation of HSP90 could either activate or inhibit the function of client proteins playing a transcriptional function during myogenic differentiation. For instance, association to the HSP90/CDC37 complex regulates the activity of the c-Jun N-terminal and Akt kinases in a myoblast model and regulates myogenic differentiation (465, 466). Future research might determine whether SMYD2 affects the recruitment of HSP90 client proteins within the context of myogenic differentiation.

The identification of SMYD2 substrates in myoblasts remains however limited by the challenges associated with the discovery of lysine methylation. Characterization of histone methylation marks at the genes of interest would also bear little relevant information in this context, as it would remain ambiguous whether the changes observed derive directly from SMYD2 activity or a downstream consequence of other pathways controlled by SMYD2. The regulation of myogenic differentiation by SMYD2 whether it depends on substrates previously reported or yet to be identified most likely occurs through multiple targets.

5.6 SMYD proteins and muscle regulation

The role of SMYD2 in myoblasts demonstrates the importance of the SMYD family of lysine methyltransferases in muscle development. SMYD1 is essential for cardiac

morphogenesis by the regulation of *Hand2* expression - a positive regulator of myogenic differentiation (284) - and promotes correct myofibril organization by association with HSP90 and UNC45b at the sarcomeric M-line (438). Moreover, knock-down of SMYD3 causes the overexpression of late differentiation cardiac and myogenic differentiation markers in embryos (420) and causes the downregulation of myostatin both in proliferating and differentiating myoblasts (421). Muscle-specific knock-down of SMYD4 Drosophila homolog CG14122 in fly larvae leads to eclosion failure, indicative of muscle defects (422). While other SET-domain methyltransferases regulate muscle development (467-472), SMYD proteins in skeletal and cardiac muscle development suggest an evolutionary conserved function of this family.

The mechanisms enabling the regulation of muscle development by SMYD proteins are still elusive. Myofibril defects caused by the repression of SMYD1 during embryonic development can be rescued by a catalytically inactive protein (438). However, muscle defects resulting from the alteration of gene transcription can only be rescued by a catalytically active SMYD1 (271). Thus far, only the methylation of histone H3 by SMYD1 has been demonstrated (271) and no direct association has been made between the regulation of muscle development and the deposition of a histone mark by SMYD1. Also, it remains to be determined whether substrates reported for SMYD3 are important for the transcriptional regulation of cardiac and myogenic markers or the regulation of myostatin expression. Considering the high degree of sequence and structural homology between SMYD1, SMYD2 and SMYD3, it is likely that SMYD1 and SMYD3 also methylate a wide range of substrates which may underlie their functions during muscle development.

Our results indicate that SMYD2 methyltransferase activity regulates both the apoptotic potential in cardiomyocytes (429) and the differentiation of myoblasts. However, as for SMYD1 and SMYD3, the targets of SMYD2 methyltransferase activity in skeletal muscle remain unknown. This illustrates the challenges associated with the discovery of lysine methylation and makes the role of SMYD proteins during muscle development - more specifically the role of SMYD2 - a model of choice for the characterization of the cellular processes regulated by lysine methylation. A better understanding of SMYD substrates would also likely lead to the identification of numerous therapeutic targets for the treatment of SMYD-associated pathologies. Moreover, the identification of SMYD2 interacting proteins in myoblasts would provide potential mechanisms and substrates to explain its activity *in vivo*. These studies would also be facilitated by the recent developments in immunological, chemical and bio-orthogonal enrichment of methylated proteins (473-475). These techniques would enable us to catalogue SMYD2 substrates in the context of myogenic differentiation and assess the incorrect methylation state of these substrates in tumours overexpressing SMYD2.

Table 5.1 Roles of SMYD2 metyltransferase activity

Susbtrate	Site	Function	Regulation	References
Histone H3	K4	?	Optimal at pH ≥9	Abu-Fahra (2009) MCP, Lanouette & Abu Fahra (2011) JBMC
	K36	?	?	Brown (2006) Mol. Cancer
p53	K370	Inhibits p53 activation Prevention of apoptosis during cardiac injury	Inhibited by K370 methylation; Demethylated by LSD1; Within the SMYD2 recognition motif	Huang (2006) Nature Sajjad (2014) BBA
pRb	K810	Increases S807/811 phosphorylation, promotes cell cycle progression		Cho (2012) Neoplasia
	K860	Can recruit L3MBTL1 to pRb	Increases in proliferative cells and in reponse to DNA damage	Saddic (210) JBC
PARP1	K528	Enhances DNA damage signalling	Increases during oxidative stress	Piao (2014) Neoplasia
ERα	K266	Decreases activation of ERα transcriptional activity by p300	Limited to nuclear ERα; Decreases in response to estrogen stimulation; Demethylated by LSD1; Within the SMYD2 recognition motif	Zhang (2013) PNAS
HSP90	K209	No effect on ATPase activity	Optimal at pH ≥9, Necessitates presence of histone H3	Lanouette & Abu Fahra (2011) JBMC
	K615	No effect on ATPase activity; Correlates with localization at the sarcomere	Optimal at pH ≤8.5; Prevented by association with HOP; Demethylated by LSD1	Lanouette & Abu Fahra (2011) JBMC, Donlin (2012) Genes & Dev, Voelkel (2013) BBA
SIX1	K51		Within the SMYD2 recognition motif	Lanouette & Davey (2014) Structure
SIX2	K51		Within the SMYD2 recognition motif	Lanouette & Davey (2014) Structure
SIN3B	K534		Within the SMYD2 recognition motif	Lanouette & Davey (2014) Structure
DHX15	K515		Within the SMYD2 recognition motif	Lanouette & Davey (2014) Structure
?	?	Negative regulation of myogenic differentiation		Lanouette (2015) TBP

Note: Subtrates and functions reported in this work are represented in blue

5.7 Promotion of cell homeostasis by SMYD2

SMYD2 methyltransferase activity regulates myogenic differentiation by repressing the premature transcription of muscle genes. This may be analogous to the role of SMYD2 in mesoderm formation in embryonic stem cells not by directly promoting mesoderm-specific genes but rather by repressing the expression of ectoderm genes within the nodal pathway (346). In both cases, SMYD2 controls the transcriptional balance of a cell state by limiting the expression of genes perturbating this state rather than by promoting the progression of development. This is consistent with the prevention of a premature change of state – apoptosis - by the methylation of p53 by SMYD2 during cardiomyocyte injury (429). Similarly, the expression of SMYD2 in leukemic stem cells promotes the maintenance of their self-renewal (347, 348). In all these cases, SMYD2 activity promotes homeostasis of developmental stages in tissues in which it is expressed (mesoderm, myotome, cardiac and skeletal muscle, liver, bladder, etc.) by limiting the expression of destabilizing genes. Other groups have established a correlation between the recruitment of HSP90 to the sarcomere and its methylation by SMYD2, although a clear causal link remains to be demonstrated. It is tempting to speculate that the methylation could also affect the homeostatic functions of HSP90 (272). The promotion of homeostasis by SMYD2 suggests that it may have evolved as a buffering factor of vertebrate development.

5.8 Significance

This work presents evidence that SMYD2 methylates the five proteins HSP90, SIX1, SIX2, SIN3B and DHX15 within its interaction network *in vitro*. I have also shown that

SMYD2 recognizes a significant subset of substrates, including SIX1, SIX2, SIN3B, DHX15, p53, and ERα, through the motif [LFM]-$_1$-K*-[AFYMSHRK]+$_1$-[LYK]+$_2$. This motif provides an important tool for future investigations of SMYD2 targets *in vivo* and for the design of SMYD2 inhibitors. Moreover, this recognition motif, together with other regulatory mechanisms I identified – e.g. demethylation by LSD1 and a pH specificity-switch – can explain the regulation of SMYD2 activity *in vivo*. My work also shows the importance of this activity as a negative regulator of skeletal myogenic differentiation. This role of SMYD2 in myoblasts demonstrates the extensive regulation of muscle development by SMYD proteins and provides a model for future studies of SMYD2 targets *in vivo*.

REFERENCES

1. Walsh CT, Garneau-Tsodikova S and Gatto GJ, Jr.: Protein posttranslational modifications: the chemistry of proteome diversifications. Angew Chem Int Ed Engl 44: 7342-72, 2005.

2. Mann M and Jensen ON: Proteomic analysis of post-translational modifications. Nat Biotechnol 21: 255-61, 2003.

3. Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, Olsen JV and Mann M: Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science (New York, N.Y.) 325: 834-40, 2009.

4. Henriksen P, Wagner Sa, Weinert BT, Sharma S, Bacinskaja G, Rehman M, Juffer AH, Walther TC, Lisby M and Choudhary C: Proteome-wide analysis of lysine acetylation suggests its broad regulatory scope in Saccharomyces cerevisiae. Molecular & cellular proteomics : MCP 11: 1510-1522, 2012.

5. Weinert BT, Wagner Sa, Horn H, Henriksen P, Liu WR, Olsen JV, Jensen LJ and Choudhary C: Proteome-wide mapping of the Drosophila acetylome demonstrates a high degree of conservation of lysine acetylation. Science signaling 4: ra48, 2011.

6. Wisniewski JR, Zougman A and Mann M: N-epsilon-formylation of lysine is a widespread post-translational modification of nuclear proteins occurring at residues involved in regulation of chromatin function. Nucleic acids research 36: 570-7, 2008.

7. Chen Y, Sprung R, Tang Y, Ball H, Sangras B, Kim SC, Falck JR, Peng J, Gu W and Zhao Y: Lysine propionylation and butyrylation are novel post-translational modifications in histones. Molecular & cellular proteomics : MCP 6: 812-9, 2007.

8. Cheng Z, Tang Y, Chen Y, Kim S, Liu H, Li SSC, Gu W and Zhao Y: Molecular characterization of propionyllysines in non-histone proteins. Molecular & cellular proteomics : MCP 8: 45-52, 2009.

9. Tan M, Luo H, Lee S, Jin F, Yang JS, Montellier E, Buchou T, Cheng Z, Rousseaux S, Rajagopal N, Lu Z, Ye Z, Zhu Q, Wysocka J, Ye Y, Khochbin S, Ren B and Zhao Y: Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. Cell. 146: 1016-28, 2011.

10. Peng C, Lu Z, Xie Z, Cheng Z, Chen Y, Tan M, Luo H, Zhang Y, He W, Yang K, Zwaans BMM, Tishkoff D, Ho L, Lombard D, He T-C, Dai J, Verdin E, Ye Y and Zhao Y: The first identification of lysine malonylation substrates and its regulatory enzyme. Molecular & cellular proteomics : MCP 10: M111.012658, 2011.

11. Zhang Z, Tan M, Xie Z, Dai L, Chen Y and Zhao Y: Identification of lysine succinylation as a new post-translational modification. Nature chemical biology 7: 58-63, 2011.

12. Weinert BT, Scholz C, Wagner SA, Iesmantavicius V, Su D, Daniel JA and Choudhary C: Lysine succinylation is a frequently occurring modification in prokaryotes and eukaryotes and extensively overlaps with acetylation. Cell Rep 4: 842-51, 2013.

13. Park J, Chen Y, Tishkoff DX, Peng C, Tan M, Dai L, Xie Z, Zhang Y, Zwaans BM, Skinner ME, Lombard DB and Zhao Y: SIRT5-mediated lysine desuccinylation impacts diverse metabolic pathways. Mol Cell 50: 919-30, 2013.

14. Jiang H, Khan S, Wang Y, Charron G, He B, Sebastian C, Du J, Kim R, Ge E, Mostoslavsky R, Hang HC, Hao Q and Lin H: SIRT6 regulates TNF-alpha secretion through hydrolysis of long-chain fatty acyl lysine. Nature 496: 110-3, 2013.

15. Chapman-Smith A and Cronan JE, Jr.: The enzymatic biotinylation of proteins: a post-translational modification of exceptional specificity. Trends Biochem Sci 24: 359-63, 1999.

16. Van Slyke DD and Sinex FM: The course of hydroxylation of lysine to form hydroxylysine in collagen. The Journal of Biological Chemistry 232: 797-806, 1958.

17. Mirelman D and Siegel RC: Oxidative deamination of epsilon-aminolysine residues and formation of Schiff base cross-linkages in cell envelopes of Escherichia coli. J Biol Chem 254: 571-4, 1979.

18. Bunn HF, Shapiro R, McManus M, Garrick L, McDonald MJ, Gallop PM and Gabbay KH: Structural heterogeneity of human hemoglobin A due to nonenzymatic glycosylation. J Biol Chem 254: 3892-8, 1979.

19. Johansen MB, Kiemer L and Brunak S: Analysis and prediction of mammalian protein glycation. Glycobiology 16: 844-53, 2006.

20. Ling X, Sakashita N, Takeya M, Nagai R, Horiuchi S and Takahashi K: Immunohistochemical distribution and subcellular localization of three distinct specific molecular structures of advanced glycation end products in human tissues. Lab Invest 78: 1591-606, 1998.

21. Ziegler M: New functions of a long-known molecule. Emerging roles of NAD in cellular signaling. Eur J Biochem 267: 1550-64, 2000.

22. Itzen A, Blankenfeldt W and Goody RS: Adenylylation: renaissance of a forgotten post-translational modification. Trends Biochem Sci 36: 221-8, 2011.

23. Eliot AC and Kirsch JF: Pyridoxal phosphate enzymes: mechanistic, structural, and evolutionary considerations. Annu Rev Biochem 73: 383-415, 2004.

24. Bownds D and Wald G: Reaction of the Rhodopsin Chromophore with Sodium Borohydride. Nature 205: 254-7, 1965.

25. Kim W, Bennett EJ, Huttlin EL, Guo A, Li J, Possemato A, Sowa ME, Rad R, Rush J, Comb MJ, Harper JW and Gygi SP: Systematic and quantitative assessment of the ubiquitin-modified proteome. Molecular cell 44: 325-40, 2011.

26. Wagner Sa, Beli P, Weinert BT, Nielsen ML, Cox J, Mann M and Choudhary C: A proteome-wide, quantitative survey of in vivo ubiquitylation sites reveals widespread regulatory roles. Molecular & cellular proteomics : MCP 10: M111.013284, 2011.

27. Hochstrasser M: Origin and function of ubiquitin-like proteins. Nature 458: 422-9, 2009.

28. Golemi D, Maveyraud L, Vakulenko S, Samama JP and Mobashery S: Critical involvement of a carbamylated lysine in catalytic function of class D beta-lactamases. Proc Natl Acad Sci U S A 98: 14280-5, 2001.

29. Egorova KS, Olenkina OM and Olenina LV: Lysine methylation of nonhistone proteins is a way to regulate their stability and function. Biochemistry (Moscow) 75: 535-548, 2010.

30. Lan F and Shi Y: Epigenetic regulation: methylation of histone and non-histone proteins. Science in China. Series C, Life sciences / Chinese Academy of Sciences 52: 311-22, 2009.

31. Stark GR, Wang Y and Lu T: Lysine methylation of promoter-bound transcription factors and relevance to cancer. Cell Res. 21: 375-80, 2011.

32. Yang X-D, Lamb A and Chen L-F: Methylation, a new epigenetic mark for protein stability. Epigenetics : official journal of the DNA Methylation Society 4: 429-33, 2009.

33. Botting CH, Talbot P, Paytubi S and White MF: Extensive lysine methylation in hyperthermophilic crenarchaea: potential implications for protein stability and recombinant enzymes. Archaea 2010: ID106341, 2010.

34. Iwabata H, Yoshida M and Komatsu Y: Proteomic analysis of organ-specific post-translational lysine-acetylation and -methylation in mice by use of anti-acetyllysine and -methyllysine mouse monoclonal antibodies. Proteomics 5: 4653-64, 2005.

35. Jung SY, Li Y, Wang Y, Chen Y, Zhao Y and Qin J: Complications in the assignment of 14 and 28 Da mass shift detected by mass spectrometry as in vivo methylation from endogenous proteins. Analytical chemistry 80: 1721-9, 2008.

36. Pang CNI, Gasteiger E and Wilkins MR: Identification of arginine- and lysine-methylation in the proteome of Saccharomyces cerevisiae and its functional implications. BMC genomics 11: 92, 2010.

37. Ambler RP and Rees MW: Epsilon-N-Methyl-lysine in bacterial flagellar protein. Nature 184: 56-7, 1959.

38. Murray K: The occurence of epsilon-N-methyl lysine in histones. Biochemistry 3: 10-5, 1964.

39. DeLange RJ, Glazer AN and Smith EL: Presence and location of an unusual amino acid, epsilon-N-trimethyllysine, in cytochrome c of wheat germ and Neurospora. The Journal of biological chemistry 244: 1385-8, 1969.

40. DeLange RJ, Glazer AN and Smith EL: Identification and location of episilon-N-trimethyllysine in yeast cytochromes c. The Journal of biological chemistry 245: 3325-7, 1970.

41. Ames GF and Niakido K: In vivo methylation of prokaryotic elongation factor Tu. J Biol Chem. 254: 9947-50, 1979.

42. L'Italien JJ and Laursen RA: Location of the site of methylation in elongation factor Tu. FEBS letters 107: 359-62, 1979.

43. Motojima K and Sakaguchi K: Part of the Lysyl Residues in Wheat o-Amylase is Methylated as Af-e-Trimethyl Lysine. Plant Cell Physiol. 23: 709-712, 1982.

44. Hardy M, Harris I, Perry SV and Stone D: Epsilon-N-monomethyl-lysine and trimethyl-lysine in myosin. The Biochemical journal 117: 44P-45P, 1970.

45. Hardy MF and Perry SV: In vitro methylation of muscle proteins. Nature 223: 300-2, 1969.

46. Tong SW and Elzinga M: The sequence of the NH2-terminal 204-residue fragment of the heavy chain of rabbit skeletal muscle myosin. The Journal of biological chemistry 258: 13100-10, 1983.

47. Bloxham DP, Parmelee DC, Kumar S, Wade RD, Ericsson LH, Neurath H, Walsh Ka and Titani K: Primary structure of porcine heart citrate synthase. Proceedings of the National Academy of Sciences of the United States of America 78: 5381-5, 1981.

48. Young CC, Alvarez JD and Bernlohr RW: Nutrient-dependent methylation of a membrane-associated protein of Escherichia coli. Journal of bacteriology 172: 5147-53, 1990.

49. Amaro AM and Jerez CA: Methylation of ribosomal proteins in bacteria: evidence of conserved modification of the eubacterial 50S subunit. J. Bacteriol. 158: 84-93, 1984.

50. Dognin MJ and Wittmann-Liebold B: Purification and primary structure determination of the N-terminal blocked protein, L11, from Escherichia coli ribosomes. European journal of biochemistry / FEBS 112: 131-51, 1980.

51. Guérin MF, Hayes DH and Rodrigues-Pousada C: Methylated amino acids in the proteins of the cytoplasmic ribosome of Tetrahymena thermophila. Biochimie 71: 805-11, 1989.

52. Lhoest J, Costers E and Colson C: Methylated proteins and amino acids in the ribosomes of Saccharomyces cerevisiae. 590: 585-590, 1984.

53. Watterson DM, Sharief F and Vanaman TC: The complete amino acid sequence of the Ca2+-dependent modulator protein (calmodulin) of bovine brain. The Journal of biological chemistry 255: 962-75, 1980.

54. Lukas TJ, Wiggins ME and Watterson DM: Amino Acid sequence of a novel calmodulin from the unicellular alga chlamydomonas. Plant physiology 78: 477-83, 1985.

55. Marshak DR, Clarke M, Roberts DM and Watterson DM: Structural and functional properties of calmodulin from the eukaryotic microorganism Dictyostelium discoideum. Biochemistry 23: 2891-9, 1984.

56. Roberts DM, Rowe PM, Siegel FL, Lukas TJ and Watterson DM: Trimethyllysine and protein function. Effect of methylation and mutagenesis of lysine 115 of calmodulin on NAD kinase activation. The Journal of biological chemistry 261: 1491-4, 1986.

57. Molla A, Kilhoffer MC, Ferraz C, Audemard E, Walsh MP and Demaille JG: Octopus calmodulin. The trimethyllysyl residue is not required for myosin light chain kinase activation. The Journal of biological chemistry 256: 15-8, 1981.

58. Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, Allshire RC and Kouzarides T: Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature 410: 120-4, 2001.

59. Lachner M, O'Carroll D, Rea S, Mechtler K and Jenuwein T: Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 410: 116-20, 2001.

60. Schubert HL, Blumenthal RM and Cheng X: Many paths to methyltransfer: a chronicle of convergence. Trends in biochemical sciences 28: 329-35, 2003.

61. Tschiersch B, Hofmann A, Krauss V, Dorn R, Korge G and Reuter G: The protein encoded by the Drosophila position-effect variegation suppressor gene Su(var)3-9 combines domains of antagonistic regulators of homeotic gene complexes. The EMBO journal 13: 3822-31, 1994.

62. Cheng X and Zhang X: Structural dynamics of protein lysine methylation and demethylation. Mutation research 618: 102-15, 2007.

63. Dillon SC, Zhang X, Trievel RC and Cheng X: The SET-domain protein superfamily: protein lysine methyltransferases. Genome biology 6: 227, 2005.

64. Qian C and Zhou M-M: SET domain protein lysine methyltransferases: Structure, specificity and catalysis. Cellular and molecular life sciences : CMLS 63: 2755-63, 2006.

65. Couture J-F, Hauk G, Thompson MJ, Blackburn GM and Trievel RC: Catalytic roles for carbon-oxygen hydrogen bonding in SET domain lysine methyltransferases. The Journal of biological chemistry 281: 19280-7, 2006.

66. Kwon T, Chang JH, Kwak E, Lee CW, Joachimiak A, Kim YC, Lee J and Cho Y: Mechanism of histone lysine methyl transfer revealed by the structure of SET7/9-AdoMet. The EMBO journal 22: 292-303, 2003.

67. Manzur KL, Farooq A, Zeng L, Plotnikova O, Koch AW, Sachchidanand and Zhou M-M: A dimeric viral SET domain methyltransferase specific to Lys27 of histone H3. Nature structural biology 10: 187-96, 2003.

68. Min J, Zhang X, Cheng X, Grewal SIS and Xu R-M: Structure of the SET domain histone lysine methyltransferase Clr4. Nature structural biology 9: 828-32, 2002.

69. Trievel RC, Beach BM, Dirk LMA, Houtz RL and Hurley JH: Structure and catalytic mechanism of a SET domain protein methyltransferase. Cell 111: 91-103, 2002.

70. Trievel RC, Flynn EM, Houtz RL and Hurley JH: Mechanism of multiple lysine methylation by the SET domain enzyme Rubisco LSMT. Nature structural biology 10: 545-52, 2003.

71. Wilson JR, Jing C, Walker PA, Martin SR, Howell SA, Blackburn GM, Gamblin SJ and Xiao B: Crystal structure and functional analysis of the histone methyltransferase SET7/9. Cell 111: 105-15, 2002.

72. Xiao B, Jing C, Wilson JR, Walker PA, Vasisht N, Kelly G, Howell S, Taylor IA, Blackburn GM and Gamblin SJ: Structure and catalytic mechanism of the human histone methyltransferase SET7/9. Nature 421: 652-6, 2003.

73. Couture J-F, Dirk LMa, Brunzelle JS, Houtz RL and Trievel RC: Structural origins for the product specificity of SET domain protein methyltransferases. Proceedings of the National Academy of Sciences of the United States of America 105: 20659-64, 2008.

74. Rathert P, Zhang X, Freund C, Cheng X and Jeltsch A: Analysis of the substrate specificity of the Dim-5 histone lysine methyltransferase using peptide arrays. Chemistry & biology 15: 5-11, 2008.

75. Chang Y, Sun L, Kokura K, Horton JR, Fukuda M, Espejo A, Izumi V, Koomen JM, Bedford MT, Zhang X, Shinkai Y, Fang J and Cheng X: MPP8 mediates the interactions between DNA methyltransferase Dnmt3a and H3K9 methyltransferase GLP/G9a. Nature communications 2: 533, 2011.

76. Van Duyne R, Easley R, Wu W, Berro R, Pedati C, Klase Z, Kehn-Hall K, Flynn EK, Symer DE and Kashanchi F: Lysine methylation of HIV-1 Tat regulates transcriptional activity of the viral LTR. Retrovirology 5: 40, 2008.

77. Zhang K, Lin W, Latham Ja, Riefler GM, Schumacher JM, Chan C, Tatchell K, Hawke DH, Kobayashi R and Dent SYR: The Set1 methyltransferase opposes Ipl1 aurora kinase functions in chromosome segregation. Cell 122: 723-34, 2005.

78. Lu T, Jackson MW, Wang B, Yang M, Chance MR, Miyagi M, Gudkov AV and Stark GR: Regulation of NF-kappaB by NSD1/FBXL11-dependent reversible lysine methylation of p65. Proceedings of the National Academy of Sciences of the United States of America 107: 46-51, 2010.

79. Huang J, Perez-Burgos L, Placek BJ, Sengupta R, Richter M, Dorsey Ja, Kubicek S, Opravil S, Jenuwein T and Berger SL: Repression of p53 activity by Smyd2-mediated methylation. Nature 444: 629-32, 2006.

80. Kunizaki M, Hamamoto R and Silva FP: The Lysine 831 of Vascular Endothelial Growth Factor Receptor 1 Is a Novel Target of Methylation by SMYD3 Receptor. Cancer research 67: 10759-10765, 2007.

81. He A, Shen X, Ma Q, Cao J, von Gise A, Zhou P, Wang G, Marquez VE, Orkin SH and Pu WT: PRC2 directly methylates GATA4 and represses its transcriptional activity. Genes & development 26: 37-42, 2012.

82. Pinheiro I, Margueron R, Shukeir N, Eisold M, Fritzsch C, Richter FM, Mittler G, Genoud C, Goyama S, Kurokawa M, Son J, Reinberg D, Lachner M and Jenuwein T: Prdm3 and Prdm16 are H3K9me1 methyltransferases required for mammalian heterochromatin integrity. Cell 150: 948-60, 2012.

83. Yang H, Pesavento JJ, Starnes TW, Cryderman DE, Wallrath LL, Kelleher NL and Mizzen CA: Preferential dimethylation of histone H4 lysine 20 by Suv4-20. The Journal of biological chemistry 283: 12085-92, 2008.

84. Martin JL and McMillan FM: SAM (dependent) I AM: the S-adenosylmethionine-dependent methyltransferase fold. Current opinion in structural biology 12: 783-93, 2002.

85. Cameron DM, Gregory ST, Thompson J, Suh MJ, Limbach PA and Dahlberg AE: Thermus thermophilus L11 methyltransferase, PrmA, is dispensable for growth and preferentially modifies free ribosomal protein L11 prior to ribosome assembly. J Bacteriol 186: 5819-25, 2004.

86. Colson C, Lhoest J and Urlings C: Genetics of ribosomal protein methylation in Escherichia coli. III. Map position of two genes, prmA and prmB, governing methylation of proteins L11 and L3. Molecular & general genetics : MGG 169: 245-50, 1979.

87. Webb KJ, Al-hadid Q, Zurita-lopez CI, Young BD, Lipson RS and Clarke SG: The Ribosomal L1 Protuberance in Yeast Is Methylated on a Lysine Residue Catalyzed by a Seven- b -strand. J Biol Chem. 286: 18405-18413, 2011.

88. Lipson RS, Webb KJ and Clarke SG: Two novel methyltransferases acting upon eukaryotic elongation factor 1A in Saccharomyces cerevisiae. Arch Biochem Biophys. 500: 137-143, 2010.

89. Kernstock S, Davydova E, Jakobsson M, Moen A, Pettersen S, Mælandsmo GM, Egge-Jacobsen W and Falnes PØ: Lysine methylation of VCP by a member of a novel human protein methyltransferase family. Nature communications 3: 1038, 2012.

90. Nguyen AT and Zhang Y: The diverse functions of Dot1 and H3K79 methylation. Genes & development 25: 1345-58, 2011.

91. Chu Y, Zhang Z, Wang Q, Luo Y and Huang L: Identification and characterization of a highly conserved crenarchaeal protein lysine methyltransferase with broad substrate specificity. Journal of bacteriology 194: 6917-6926, 2012.

92. Ptacek J, Devgan G, Michaud G, Zhu H, Zhu X, Fasolo J, Guo H, Jona G, Breitkreutz A, Sopko R, McCartney RR, Schmidt MC, Rachidi N, Lee S-J, Mah AS, Meng L, Stark MJR, Stern DF, De Virgilio C, Tyers M, Andrews B, Gerstein M, Schweitzer B, Predki PF and Snyder M: Global analysis of protein phosphorylation in yeast. Nature 438: 679-84, 2005.

93. Sopko R and Andrews BJ: Linking the kinome and phosphorylome--a comprehensive review of approaches to find kinase targets. Mol Biosyst. 4: 920-33, 2008.

94. Ammendola S, Raia Ca, Caruso C, Camardella L, D'Auria S, De Rosa M and Rossi M: Thermostable NAD(+)-dependent alcohol dehydrogenase from Sulfolobus solfataricus: gene and protein sequence determination and relationship to other alcohol dehydrogenases. Biochemistry 31: 12514-23, 1992.

95. Schaefer WH, Lukas TJ, Blair Ia, Schultz JE and Watterson DM: Amino acid sequence of a novel calmodulin from Paramecium tetraurelia that contains dimethyllysine in the first domain. The Journal of biological chemistry 262: 1025-9, 1987.

96. Wang C and Lazarides E: Arsenite-induced changes in methylation of the 70,000 dalton heat shock proteins in chicken embryo fibroblasts. Biochemical and biophysical research communications 119: 735-43, 1984.

97. Wang C, Lazarides E, O'Connor CM and Clarke S: Methylation of chicken fibroblast heat shock proteins at lysyl and arginyl residues. The Journal of biological chemistry 257: 8356-62, 1982.

98. Wang C, Lin JM and Lazarides E: Methylations of 70,000-Da heat shock proteins in 3T3 cells: alterations by arsenite treatment, by different stages of growth and by virus transformation. Archives of biochemistry and biophysics 297: 169-75, 1992.

99. Petrossian TC and Clarke SG: Uncovering the human methyltransferasome. Mol Cell Proteomics 10: M110 000976, 2011.

100. Stock A, Clarke S, Clarke C and Stock J: N-terminal methylation of proteins: structure, function and specificity. FEBS Lett. 220: 8-14, 1987.

101. Webb KJ, Lipson RS, Al-Hadid Q, Whitelegge JP and Clarke SG: Identification of protein N-terminal methyltransferases in yeast and humans. Biochemistry 49: 5225-35, 2010.

102. Huq MDM, Ha SG, Barcelona H and Wei L-n: Lysine Methylation of Nuclear Co-Repressor Receptor Interacting Protein 140 research articles. J Proteome Res 8: 1156-1167, 2009.

103. Guo A, Gu H, Zhou J, Mulhern D, Wang Y, Lee KA, Yang V, Aguiar M, Kornhauser J, Jia X, Ren J, Beausoleil SA, Silva JC, Vemulapalli V, Bedford MT and Comb MJ: Immunoaffinity Enrichment and Mass Spectrometry Analysis of Protein Methylation. Mol Cell Proteomics epub ahead of print: 2013.

104. Ong S, Mittler G and Mann M: Identifying and quantifying in vivo methylation sites by heavy methyl SILAC. Nature Methods 1: 1-8, 2004.

105. Bremang M, Cuomo A, Agresta AM, Stugiewicz M, Spadotto V and Bonaldi T: Mass spectrometry-based identification and characterisation of lysine and arginine methylation in the human proteome. Mol Biosyst 9: 2231-2247, 2013.

106. Liu H, Galka M, Mori E, Liu X, Lin YF, Wei R, Pittock P, Voss C, Dhami G, Li X, Miyaji M, Lajoie G, Chen B and Li SS: A Method for Systematic Mapping of Protein Lysine Methylation Identifies Functions for HP1beta in DNA Damage Response. Mol Cell 50: 723-35, 2013.

107. Moore KE, Carlson SM, Camp ND, Cheung P, James RG, Chua KF, Wolf-Yadlin A and Gozani O: A general molecular affinity strategy for global detection and proteomic analysis of lysine methylation. Mol Cell 50: 444-56, 2013.

108. Rathert P, Dhayalan A, Murakami M, Zhang X, Tamas R, Jurkowska R, Komatsu Y, Shinkai Y, Cheng X and Jeltsch A: Protein lysine methyltransferase G9a acts on non-histone targets. Nature chemical biology 4: 344-6, 2008.

109. Levy D, Liu CL, Yang Z, Newman AM, Alizadeh Aa, Utz PJ and Gozani O: A proteomic approach for the identification of novel lysine methyltransferase substrates. Epigenetics & chromatin 4: 19, 2011.

110. Dhayalan A, Kudithipudi S, Rathert P and Jeltsch A: Specificity analysis-based identification of new methylation targets of the SET7/9 protein lysine methyltransferase. Chemistry & biology 18: 111-20, 2011.

111. Kudithipudi S, Dhayalan A, Kebede AF and Jeltsch A: The SET8 H4K20 protein lysine methyltransferase has a long recognition sequence covering seven amino acid residues. Biochimie 94: 2212-8, 2012.

112. Kouskouti A, Scheer E and Staub A: Gene-Specific Modulation of TAF10 Function by SET9-Mediated Methylation. Mol Cell 14: 175-182, 2004.

113. Kontaki H and Talianidis I: Lysine methylation regulates E2F1-induced cell death. Molecular cell 39: 152-60, 2010.

114. Xie Q, Bai Y, Wu J, Sun Y, Wang Y, Zhang Y, Mei P and Yuan Z: Methylation-mediated regulation of E2F1 in DNA damage-induced cell death. Journal of receptor and signal transduction research 31: 139-46, 2011.

115. Black JC, Van Rechem C and Whetstine JR: Histone lysine methylation dynamics: establishment, regulation, and biological impact. Molecular cell 48: 491-507, 2012.

116. Kouzarides T: Chromatin modifications and their function. Cell 128: 693-705, 2007.

117. Shilatifard A: Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. Annual review of biochemistry 75: 243-69, 2006.

118. Smith E and Shilatifard A: The chromatin signaling pathway: diverse mechanisms of recruitment of histone-modifying enzymes and varied biological outcomes. Molecular cell 40: 689-701, 2010.

119. Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NCT, Schreiber SL, Mellor J and Kouzarides T: Active genes are tri-methylated at K4 of histone H3. Nature 419: 407-11, 2002.

120. Cao R and Zhang Y: The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. Current opinion in genetics & development 14: 155-64, 2004.

121. Plath K: Role of Histone H3 Lysine 27 Methylation in X Inactivation. Science 300: 131-135, 2003.

122. Keogh M-C, Kurdistani SK, Morris SA, Ahn SH, Podolny V, Collins SR, Schuldiner M, Chin K, Punna T, Thompson NJ, Boone C, Emili A, Weissman JS, Hughes TR, Strahl BD, Grunstein M, Greenblatt JF, Buratowski S and Krogan NJ:

Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. Cell 123: 593-605, 2005.

123. Li B, Gogol M, Carey M, Lee D, Seidel C and Workman JL: Combined action of PHD and chromo domains directs the Rpd3S HDAC to transcribed chromatin. Science (New York, N.Y.) 316: 1050-4, 2007.

124. Venkatesh S, Smolle M, Li H, Gogol MM, Saint M, Kumar S, Natarajan K and Workman JL: Set2 methylation of histone H3 lysine 36 suppresses histone exchange on transcribed genes. Nature 489: 452-5, 2012.

125. Wagner EJ and Carpenter PB: Understanding the language of Lys36 methylation at histone H3. Nature reviews. Molecular cell biology 13: 115-26, 2012.

126. Carrozza MJ, Li B, Florens L, Suganuma T, Swanson SK, Lee KK, Shia WJ, Anderson S, Yates J, Washburn MP and Workman JL: Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. Cell 123: 581-92, 2005.

127. Greeson NT, Sengupta R, Arida AR, Jenuwein T and Sanders SL: Di-methyl H4 lysine 20 targets the checkpoint protein Crb2 to sites of DNA damage. The Journal of biological chemistry 283: 33168-74, 2008.

128. Sanders SL, Portoso M, Mata J, Bähler J, Allshire RC and Kouzarides T: Methylation of histone H4 lysine 20 controls recruitment of Crb2 to sites of DNA damage. Cell 119: 603-14, 2004.

129. Huyen Y, Zgheib O, Ditullio RA, Gorgoulis VG, Zacharatos P, Petty TJ, Sheston EA, Mellert HS, Stavridi ES and Halazonetis TD: Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. Nature 432: 406-11, 2004.

130. Latham JA and Dent SY: Cross-regulation of histone modifications. Nat Struct Mol Biol 14: 1017-24, 2007.

131. Ernst J, Kheradpour P, Mikkelsen TS, Shoresh N, Ward LD, Epstein CB, Zhang X, Wang L, Issner R, Coyne M, Ku M, Durham T, Kellis M and Bernstein BE: Mapping and analysis of chromatin state dynamics in nine human cell types. Nature 473: 43-9, 2011.

132. Heintzman ND, Hon GC, Hawkins RD, Kheradpour P, Stark A, Harp LF, Ye Z, Lee LK, Stuart RK, Ching CW, Ching KA, Antosiewicz-Bourget JE, Liu H, Zhang X, Green RD, Lobanenkov VV, Stewart R, Thomson JA, Crawford GE, Kellis M and Ren

B: Histone modifications at human enhancers reflect global cell-type-specific gene expression. Nature 459: 108-12, 2009.

133. Kharchenko PV, Alekseyenko AA, Schwartz YB, Minoda A, Riddle NC, Ernst J, Sabo PJ, Larschan E, Gorchakov AA, Gu T, Linder-Basso D, Plachetka A, Shanower G, Tolstorukov MY, Luquette LJ, Xi R, Jung YL, Park RW, Bishop EP, Canfield TK, Sandstrom R, Thurman RE, MacAlpine DM, Stamatoyannopoulos JA, Kellis M, Elgin SCR, Kuroda MI, Pirrotta V, Karpen GH and Park PJ: Comprehensive analysis of the chromatin landscape in Drosophila melanogaster. Nature 471: 480-5, 2011.

134. Yin H, Sweeney S, Raha D, Snyder M and Lin H: A high-resolution whole-genome map of key chromatin modifications in the adult Drosophila melanogaster. PLoS genetics 7: e1002380, 2011.

135. Chuikov S, Kurash JK, Wilson JR, Xiao B, Justin N, Ivanov GS, McKinney K, Tempst P, Prives C, Gamblin SJ, Barlev Na and Reinberg D: Regulation of p53 activity through lysine methylation. Nature 432: 353-60, 2004.

136. Ivanov GS, Ivanova T, Kurash J, Ivanov A, Chuikov S, Gizatullin F, Herrera-Medina EM, Rauscher F, Reinberg D and Barlev Na: Methylation-acetylation interplay activates p53 in response to DNA damage. Molecular and cellular biology 27: 6756-69, 2007.

137. Huang J, Sengupta R, Espejo AB, Lee MG, Dorsey Ja, Richter M, Opravil S, Shiekhattar R, Bedford MT, Jenuwein T and Berger SL: p53 is regulated by the lysine demethylase LSD1. Nature 449: 105-8, 2007.

138. Huang J, Dorsey J, Chuikov S, Pérez-Burgos L, Zhang X, Jenuwein T, Reinberg D and Berger SL: G9a and Glp methylate lysine 373 in the tumor suppressor p53. The Journal of biological chemistry 285: 9636-41, 2010.

139. Shi X, Kachirskaia I, Yamaguchi H, West LE, Wen H, Wang EW, Dutta S, Appella E and Gozani O: Modulation of p53 function by SET8-mediated methylation at lysine 382. Molecular cell 27: 636-46, 2007.

140. West LE, Roy S, Lachmi-Weiner K, Hayashi R, Shi X, Appella E, Kutateladze TG and Gozani O: The MBT repeats of L3MBTL1 link SET8-mediated p53 methylation at lysine 382 to target gene repression. The Journal of biological chemistry 285: 37725-32, 2010.

141. Lehnertz B, Rogalski JC, Schulze FM, Yi L, Lin S, Kast J and Rossi FMV: p53-dependent transcription and tumor suppression are not affected in Set7/9-deficient mice. Molecular cell 43: 673-680, 2011.

142. Campaner S, Spreafico F, Burgold T, Doni M, Rosato U, Amati B and Testa G: The methyltransferase Set7/9 (Setd7) is dispensable for the p53-mediated DNA damage response in vivo. Molecular cell 43: 681-8, 2011.

143. Cinelli M, Magnelli L and Chiarugi V: Redundant down-regulation pathways for p53. Pharmacological research : the official journal of the Italian Pharmacological Society 37: 83-5, 1998.

144. Gu B and Zhu W-G: Surf the post-translational modification network of p53 regulation. International journal of biological sciences 8: 672-84, 2012.

145. Kruse J-P and Gu W: Modes of p53 regulation. Cell 137: 609-22, 2009.

146. Ryan KM, Phillips AC and Vousden KH: Regulation and function of the p53 tumor suppressor protein. Current opinion in cell biology 13: 332-7, 2001.

147. Shadfan M, Lopez-Pajares V and Yuan Z-M: MDM2 and MDMX: Alone and together in regulation of p53. Transl Cancer Res 1: 88-99, 2012.

148. Gaughan L, Stockley J, Wang N, McCracken SRC, Treumann A, Armstrong K, Shaheen F, Watt K, McEwan IJ, Wang C, Pestell RG and Robson CN: Regulation of the androgen receptor by SET9-mediated methylation. Nucleic acids research 39: 1266-79, 2011.

149. Xie Q, Hao Y, Tao L, Peng S, Rao C, Chen H, You H, Dong M-q and Yuan Z: Lysine methylation of FOXO3 regulates oxidative stress-induced neuronal cell death. EMBO reports 13: 371-7, 2012.

150. Ea C-K and Baltimore D: Regulation of NF-kappaB activity through lysine monomethylation of p65. Proceedings of the National Academy of Sciences of the United States of America 106: 18972-7, 2009.

151. Yang X-D, Tajkhorshid E and Chen L-F: Functional interplay between acetylation and methylation of the RelA subunit of NF-kappaB. Molecular and cellular biology 30: 2170-80, 2010.

152. Levy D, Kuo AJ, Chang Y, Schaefer U, Kitson C, Cheung P, Espejo A, Zee BM, Liu CL, Tangsombatvisit S, Tennen RI, Kuo AY, Tanjing S, Cheung R, Chua KF, Utz PJ, Shi X, Prinjha RK, Lee K, Garcia Ba, Bedford MT, Tarakhovsky A, Cheng X and Gozani O: Lysine methylation of the NF-κB subunit RelA by SETD6 couples activity of the histone methyltransferase GLP at chromatin to tonic repression of NF-κB signaling. Nature immunology 12: 29-36, 2011.

153. Cho H-s, Hayami S, Kogure M, Kang D, Neal DE, Toyokawa G, Maejima K, Yamane Y, Suzuki T, Dohmae N, Ponder BAJ, Yamaue H, Nakamura Y and Hamamoto R: RB1 methylation by SMYD2 enhances cell cycle progression through an increase of RB1 phosphorylation. Neoplasia 14: 476-86, 2012.

154. Saddic La, West LE, Aslanian A, Yates JR, Rubin SM, Gozani O and Sage J: Methylation of the retinoblastoma tumor suppressor by SMYD2. The Journal of biological chemistry 285: 37733-40, 2010.

155. Carr SM, Munro S, Kessler B, Oppermann U and La Thangue NB: Interplay between lysine methylation and Cdk phosphorylation in growth control by the retinoblastoma protein. The EMBO journal 30: 317-27, 2011.

156. Munro S, Khaire N, Inche a, Carr S and La Thangue NB: Lysine methylation regulates the pRb tumour suppressor protein. Oncogene 29: 2357-67, 2010.

157. Pless O, Kowenz-Leutz E, Knoblich M, Lausen J, Beyermann M, Walsh MJ and Leutz A: G9a-mediated lysine methylation alters the function of CCAAT/enhancer-binding protein-beta. J Biol Chem. 283: 26357-63, 2008.

158. Lee JS, Kim Y, Kim IS, Kim B, Choi HJ, Lee JM, Shin H-JR, Kim JH, Kim J-Y, Seo S-B, Lee H, Binda O, Gozani O, Semenza GL, Kim M, Kim KI, Hwang D and Baek SH: Negative regulation of hypoxic responses via induced Reptin methylation. Molecular cell 39: 71-85, 2010.

159. Porras-Yakushi TR, Whitelegge JP and Clarke S: Yeast ribosomal/cytochrome c SET domain methyltransferase subfamily: identification of Rpl23ab methylation sites and recognition motifs. The Journal of biological chemistry 282: 12368-76, 2007.

160. Shirai A, Sadaie M, Shinmyozu K, Nakayama J-i, Unit PS, Way R and Kingdom U: Methylation of Ribosomal Protein L42 Regulates Ribosomal Function and Stress-adapted Cell Growth. J Biol Chem. 285: 22448-22460, 2010.

161. Coléno-Costes A, Jang SM, de Vanssay A, Rougeot J, Bouceba T, Randsholt NB, Gibert J-M, Le Crom S, Mouchel-Vielh E, Bloyer S and Peronnet F: New partners in regulation of gene expression: the enhancer of trithorax and polycomb corto interacts with methylated ribosomal protein l12 via its chromodomain. PLoS genetics 8: e1003006, 2012.

162. Bhavsar RB, Makley LN and Tsonis PA: The other lives of ribosomal proteins. Hum Genomics 4: 327-44, 2010.

163. Donlin LT, Andresen C, Just S, Rudensky E, Pappas CT, Kruger M, Jacobs EY, Unger A, Zieseniss A, Dobenecker M-W, Voelkel T, Chait BT, Gregorio CC, Rottbauer W, Tarakhovsky A and Linke Wa: Smyd2 controls cytoplasmic lysine methylation of Hsp90 and myofilament organization. Genes & development 26: 114-9, 2012.

164. Voelkel T, Andresen C, Unger A, Just S, Rottbauer W and Linke Wa: Lysine methyltransferase Smyd2 regulates Hsp90-mediated protection of the sarcomeric titin springs and cardiac function. Biochim Biophys Acta 1833: 812-822, 2013.

165. Cho H-S, Shimazu T, Toyokawa G, Daigo Y, Maehara Y, Hayami S, Ito A, Masuda K, Ikawa N, Field HI, Tsuchiya E, Ohnuma S-I, Ponder BaJ, Yoshida M, Nakamura Y and Hamamoto R: Enhanced HSP70 lysine methylation promotes proliferation of cancer cells through activation of Aurora kinase B. Nature communications 3: 1072, 2012.

166. Latham Ja, Chosed RJ, Wang S and Dent SYR: Chromatin signaling to kinetochores: transregulation of Dam1 methylation by histone H2B ubiquitination. Cell 146: 709-19, 2011.

167. Estève P-O, Chang Y, Samaranayake M, Upadhyay AK, Horton JR, Feehery GR, Cheng X and Pradhan S: A methylation and phosphorylation switch between an adjacent lysine and serine determines human DNMT1 stability. Nature structural & molecular biology 18: 42-8, 2011.

168. Estève P-O, Chin HG, Benner J, Feehery GR, Samaranayake M, Horwitz Ga, Jacobsen SE and Pradhan S: Regulation of DNMT1 stability through SET7-mediated lysine methylation in mammalian cells. Proceedings of the National Academy of Sciences of the United States of America 106: 5076-81, 2009.

169. Zhang J, Yuan B, Zhang F, Xiong L, Wu J, Pradhan S and Wang Y: Cyclophosphamide perturbs cytosine methylation in Jurkat-T cells through LSD1-mediated stabilization of DNMT1 protein. Chemical research in toxicology 24: 2040-3, 2011.

170. Houtz RL, Stults JT, Mulligan RM, Tolbert NE, Stultst JT and Mulligant RM: Post-translational modifications in the large subunit of ribulose bisphosphate carboxylase / oxygenase. Proceedings of the National Academy of Sciences of the United States of America 86: 1855-1859, 1989.

171. Magnani R, Nayak NR, Mazarei M, Dirk LMa and Houtz RL: Polypeptide substrate specificity of PsLSMT. A set domain protein methyltransferase. The Journal of biological chemistry 282: 27857-64, 2007.

172. Mininno M, Brugière S, Pautre V, Gilgen A, Ma S, Ferro M, Tardif M, Alban C and Ravanel S: Characterization of chloroplastic fructose 1,6-bisphosphate aldolases as lysine-methylated proteins in plants. The Journal of biological chemistry 287: 21034-44, 2012.

173. Sahr T, Adam T, Fizames C, Maurel C and Santoni V: O-carboxyl- and N-methyltransferases active on plant aquaporins. Plant & cell physiology 51: 2092-104, 2010.

174. Santoni V, Verdoucq L, Sommerer N, Vinh J, Pflieger D and Maurel C: Methylation of aquaporins in plant plasma membrane. Biochem J. 400: 189-97, 2006.

175. Kim YH, Park KH, Kim S-Y, Ji ES, Kim JY, Lee SK, Yoo JS, Kim HS and Park YM: Identification of trimethylation at C-terminal lysine of pilin in the cyanobacterium Synechocystis PCC 6803. Biochemical and biophysical research communications 404: 587-92, 2011.

176. Van Noort JM, Kraal B, Sinjorgo KM, Persoon NL, Johanns ES and Bosch L: Methylation in vivo of elongation factor EF-Tu at lysine-56 decreases the rate of tRNA-dependent GTP hydrolysis. European journal of biochemistry / FEBS 160: 557-61, 1986.

177. Young CC and Bernlohr RW: Elongation Factor Tu Is Methylated in Response to Nutrient Deprivation in Escherichia coli. Journal of bacteriology 173: 3096-100, 1991.

178. Biet F, Angela de Melo Marques M, Grayon M, Xavier da Silveira EK, Brennan PJ, Drobecq H, Raze D, Vidal Pessolani MC, Locht C and Menozzi FD: Mycobacterium smegmatis produces an HBHA homologue which is not involved in epithelial adherence. Microbes Infect 9: 175-82, 2007.

179. Delogu G, Chiacchio T, Vanini V, Butera O, Cuzzi G, Bua A, Molicotti P, Zanetti S, Lauria FN, Grisetti S, Magnavita N, Fadda G, Girardi E and Goletti D: Methylated HBHA produced in M. smegmatis discriminates between active and non-active tuberculosis disease among RD1-responders. PloS one 6: e18315, 2011.

180. Guerrero GG and Locht C: Recombinant HBHA boosting effect on BCG-induced immunity against Mycobacterium tuberculosis infection. Clinical & developmental immunology 2011: 730702, 2011.

181. Soares de Lima C, Zulianello L, Marques MADM, Kim H, Portugal MI, Antunes SL, Menozzi FD, Ottenhoff THM, Brennan PJ and Pessolani MCV: Mapping the laminin-binding and adhesive domain of the cell surface-associated Hlp/LBP protein from Mycobacterium leprae. Microbes Infect. 7: 1097-109, 2005.

182. Temmerman S, Pethe K, Parra M, Alonso S, Rouanet C, Pickett T, Drowart A, Debrie A-S, Delogu G, Menozzi FD, Sergheraert C, Brennan MJ, Mascart F and Locht C: Methylation-dependent T cell immunity to Mycobacterium tuberculosis heparin-binding hemagglutinin. Nature medicine 10: 935-41, 2004.

183. Chhabra SR, Joachimiak MP, Petzold CJ and Zane GM: Towards a Rigorous Network of Protein-Protein Interactions of the Model Sulfate Reducer Desulfovibrio vulgaris Hildenborough. PLoS One 6: e21470, 2011.

184. Gaucher SP, Redding AM, Mukhopadhyay A, Keasling JD and Singh AK: Post-Translational Modifications of Desulfovibrio vulgaris Hildenborough Sulfate Reduction Pathway Proteins research articles. J Proteome Res 7: 2320-2331, 2008.

185. Cao X-J, Dai J, Xu H, Nie S, Chang X, Hu B-Y, Sheng Q-H, Wang L-S, Ning Z-B, Li Y-X, Guo X-K, Zhao G-P and Zeng R: High-coverage proteome analysis reveals the first insight of protein modification systems in the pathogenic spirochete Leptospira interrogans. Cell research 20: 197-210, 2010.

186. Sandman K and Reeve JN: Archaeal chromatin proteins: different structures but common function? Curr Opin Microbiol. 8: 656-61, 2005.

187. Mcafee JG, Edmondson SP, Datta PK, Shriver JW and Gupta R: Gene cloning, expression, and characterization of the Sac7 proteins from the hyperthermophile Sulfolobus acidocaldarius. Biochemistry. 34: 10063-10077, 1995.

188. Guo L, Feng Y, Zhang Z, Yao H, Luo Y, Wang J and Huang L: Biochemical and structural characterization of Cren7, a novel chromatin protein conserved among Crenarchaea. Nucleic acids research 36: 1129-37, 2008.

189. Knapp S, Karshikoff A, Berndt KD, Christova P, Atanasov B, Ladenstein R and Huddinge S-: Thermal Unfolding of the DNA-binding Protein Sso7d from the Hyperthermophile Sulfolobus solfataricus. J Mol Biol 264: 1132-1144, 1996.

190. Oppermann UCT, Knapp S, Bonetto V and Ladenstein R: Isolation and structure of repressor-like proteins from the archaeon Sulfolobus solfataricus Co-purification of RNase A with Sso7c 1. FEBS Lett 432: 141-144, 1998.

191. Mcafee JG, Edmondson SP, Zegar I and Shriver JW: Equilibrium DNA Binding of Sac7d Protein from the Hyperthermophile Sulfolobus acidocaldarius : Fluorescence and Circular Dichroism Studies. Biochemistry 35: 4034-4045, 1996.

192. Manzur KL and Zhou M-M: An archaeal SET domain protein exhibits distinct lysine methyltransferase activity towards DNA-associated protein MC1-alpha. FEBS letters 579: 3859-65, 2005.

193. Febbraio F, Andolfo A, Tanfani F, Briante R, Gentile F, Formisano S, Vaccaro C, Scirè A, Bertoli E, Pucci P and Nucci R: Thermal stability and aggregation of sulfolobus solfataricus beta-glycosidase are dependent upon the N-epsilon-methylation of specific lysyl residues: critical role of in vivo post-translational modifications. The Journal of biological chemistry 279: 10185-94, 2004.

194. Fusi P, Grisa M, Mombelli E, Consonni R, Tortora P and Vanoni M: Expression of a synthetic gene encoding P2 ribonuclease from the extreme thermoacidophilic archaebacterium Sulfolobus solfataricus in mesophylic hosts. Gene 154: 99-103, 1995.

195. Burton KS and Consigli RA: Methylation of the polyomavirus major capsid protein VP11. Virus Res. 40: 141-147, 1996.

196. Pagans S, Sakane N, Schnölzer M and Ott M: Characterization of HIV Tat modifications using novel methyl-lysine-specific antibodies. 53: 91-96, 2011.

197. Sakane N, Kwon H-s, Pagans S, Kaehlcke K and Mizusawa Y: Activation of HIV transcription by the viral Tat protein requires a demethylation step mediated by lysine-specific demethylase 1 (LSD1/KDM1). PLoS pathogens 7: e1002184, 2011.

198. Mujtaba S, Manzur KL, Gurnon JR, Kang M, Van Etten JL and Zhou MM: Epigenetic transcriptional repression of cellular genes by a viral SET protein. Nat Cell Biol 10: 1114-22, 2008.

199. Manzur KL, Farooq A, Zeng L, Plotnikova O, Koch AW, Sachchidanand and Zhou MM: A dimeric viral SET domain methyltransferase specific to Lys27 of histone H3. Nat Struct Biol 10: 187-96, 2003.

200. Zheng Y and Yao X: Posttranslational modifications of HIV-1 integrase by various cellular proteins during viral replication. Viruses 5: 1787-801, 2013.

201. Keating JA and Striker R: Phosphorylation events during viral infections provide potential therapeutic targets. Rev Med Virol 22: 166-81, 2012.

202. Gustin JK, Moses AV, Fruh K and Douglas JL: Viral takeover of the host ubiquitin system. Front Microbiol 2: 161, 2011.

203. Van Opdenbosch N, Favoreel H and Van de Walle GR: Histone modifications in herpesvirus infections. Biol Cell 104: 139-64, 2012.

204. Paik WK and Kim S: Enzymatic demethylation of calf thymus histones. Biochemical and biophysical research communications 51: 781-8, 1973.

205. Paik WK and Kim S: Epsilon-alkyllysinase. New assay method, purification, and biological significance. Archives of biochemistry and biophysics 165: 369-78, 1974.

206. Tsukada Y-i, Fang J, Erdjument-Bromage H, Warren ME, Borchers CH, Tempst P and Zhang Y: Histone demethylation by a family of JmjC domain-containing proteins. Nature 439: 811-6, 2006.

207. Chuang J-Y, Chang W-c and Hung J-J: Hydrogen peroxide induces Sp1 methylation and thereby suppresses cyclin B1 via recruitment of Suv39H1 and HDAC1 in cancer cells. Free radical biology & medicine 51: 2309-2318, 2011.

208. Yang J, Huang J, Dasgupta M, Sears N, Miyagi M, Wang B, Chance MR, Chen X, Du Y, Wang Y, An L, Wang Q, Lu T, Zhang X, Wang Z and Stark GR: Reversible methylation of promoter-bound STAT3 by histone-modifying enzymes. Proceedings of the National Academy of Sciences of the United States of America 6: 21499-504, 2010.

209. Cho H-S, Suzuki T, Dohmae N, Hayami S, Unoki M, Yoshimatsu M, Toyokawa G, Takawa M, Chen T, Kurash JK, Field HI, Ponder BaJ, Nakamura Y and Hamamoto R: Demethylation of RB regulator MYPT1 by histone demethylase LSD1 promotes cell cycle progression in cancer cells. Cancer research 71: 655-60, 2011.

210. Wang J, Hevi S, Kurash JK, Lei H, Gay F, Bajko J, Su H, Sun W, Chang H, Xu G, Gaudet F, Li E and Chen T: The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. Nature genetics 41: 125-9, 2009.

211. Abu-Farha M, Lanouette S, Elisma F, Tremblay V, Butson J, Figeys D and Couture J-F: Proteomic analyses of the SMYD family interactomes identify HSP90 as a novel target for SMYD2. J Mol Cell Biol 3: 301-8, 2011.

212. Baba A, Ohtake F, Okuno Y, Yokota K, Okada M, Imai Y, Ni M, Meyer Ca, Igarashi K, Kanno J, Brown M and Kato S: PKA-dependent regulation of the histone lysine demethylase complex PHF2-ARID5B. Nat Cell Biol 13: 668-75, 2011.

213. Haglund K, Di Fiore PP and Dikic I: Distinct monoubiquitin signals in receptor endocytosis. Trends Biochem Sci 28: 598-603, 2003.

214. Hicke L: Protein regulation by monoubiquitin. Nat Rev Mol Cell Biol 2: 195-201, 2001.

215. Ciechanover A: Proteolysis: from the lysosome to ubiquitin and the proteasome. Nature reviews. Molecular cell biology 6: 79-87, 2005.

216. Glickman MH and Ciechanover A: The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiological reviews 82: 373-428, 2002.

217. Lee JM, Lee JS, Kim H, Kim K, Park H, Kim J-y, Lee SH, Kim IS, Kim J, Lee M, Chung CH, Seo S-b, Yoon J-b, Ko E, Noh D-y, Kim KI, Kim KK and Baek SH: EZH2 Generates a Methyl Degron that Is Recognized by the DCAF1 / DDB1 / CUL4 E3 Ubiquitin Ligase Complex. Mol Cell 48: 572-586, 2012.

218. Yang X-D, Huang B, Li M, Lamb A, Kelleher NL and Chen L-F: Negative regulation of NF-kappaB action by Set9-mediated lysine methylation of the RelA subunit. The EMBO journal 28: 1055-66, 2009.

219. Kurash JK, Lei H, Shen Q, Marston WL, Granda BW, Fan H, Wall D, Li E and Gaudet F: Methylation of p53 by Set7/9 mediates p53 acetylation and activity in vivo. Molecular cell 29: 392-400, 2008.

220. Jacobs SA and Khorasanizadeh S: Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. Science (New York, N.Y.) 295: 2080-3, 2002.

221. Jacobs SA, Taverna SD, Zhang Y, Briggs SD, Li J, Eissenberg JC, Allis CD and Khorasanizadeh S: Specificity of the HP1 chromo domain for the methylated N-terminus of histone H3. The EMBO journal 20: 5232-41, 2001.

222. Botuyan MV, Lee J, Ward IM, Kim J-E, Thompson JR, Chen J and Mer G: Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in DNA repair. Cell 127: 1361-73, 2006.

223. Hughes RM, Wiggins KR, Khorasanizadeh S and Waters ML: Recognition of trimethyllysine by a chromodomain is not driven by the hydrophobic effect. Proceedings of the National Academy of Sciences of the United States of America 104: 11184-8, 2007.

224. Ma JC and Dougherty DA: The Cation - pi Interaction. Chemical reviews 97: 1303-1324, 1997.

225. Taverna SD, Li H, Ruthenburg AJ, Allis CD and Patel DJ: How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. Nature structural & molecular biology 14: 1025-40, 2007.

226. Li H, Ilin S, Wang W, Duncan EM, Wysocka J, Allis CD and Patel DJ: Molecular basis for site-specific read-out of histone H3K4me3 by the BPTF PHD finger of NURF. Nature 442: 91-5, 2006.

227. Peña PV, Davrazou F, Shi X, Walter KL, Verkhusha VV, Gozani O, Zhao R and Kutateladze TG: Molecular mechanism of histone H3K4me3 recognition by plant homeodomain of ING2. Nature 442: 100-3, 2006.

228. Shi X, Hong T, Walter KL, Ewalt M, Michishita E, Hung T, Carney D, Peña P, Lan F, Kaadige MR, Lacoste N, Cayrou C, Davrazou F, Saha A, Cairns BR, Ayer DE, Kutateladze TG, Shi Y, Côté J, Chua KF and Gozani O: ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. Nature 442: 96-9, 2006.

229. Wysocka J, Swigut T, Xiao H, Milne TA, Kwon SY, Landry J, Kauer M, Tackett AJ, Chait BT, Badenhorst P, Wu C and Allis CD: A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. Nature 442: 86-90, 2006.

230. Li H, Fischle W, Wang W, Duncan EM, Liang L, Murakami-Ishibe S, Allis CD and Patel DJ: Structural basis for lower lysine methylation state-specific readout by MBT repeats of L3MBTL1 and an engineered PHD finger. Molecular cell 28: 677-91, 2007.

231. Min J, Zhang Y and Xu R-M: Structural basis for specific binding of Polycomb chromodomain to histone H3 methylated at Lys 27. Genes & development 17: 1823-8, 2003.

232. Musselman CA, Lalonde ME, Cote J and Kutateladze TG: Perceiving the epigenetic landscape through histone readers. Nat Struct Mol Biol 19: 1218-27, 2012.

233. Cui G, Park S, Badeaux AI, Kim D, Lee J, Thompson JR, Yan F, Kaneko S, Yuan Z, Botuyan MV, Bedford MT, Cheng JQ and Mer G: PHF20 is an effector protein of p53 double lysine methylation that stabilizes and activates p53. Nat Struct Mol Biol 19: 916-24, 2012.

234. Kim J, Daniel J, Espejo A, Lake A, Krishna M, Xia L, Zhang Y and Bedford MT: Tudor, MBT and chromo domains gauge the degree of lysine methylation. EMBO reports 7: 397-403, 2006.

235. Calnan DR, Webb AE, White JL, Stowe TR, Goswami T, Shi X, Espejo A, Bedford MT, Gozani O, Gygi SP and Brunet A: Methylation by Set9 modulates FoxO3 stability and transcriptional activity. Aging 4: 462-79, 2012.

236. Ito I, Fukazawa J and Yoshida M: Post-translational methylation of high mobility group box 1 (HMGB1) causes its cytoplasmic localization in neutrophils. The Journal of biological chemistry 282: 16336-44, 2007.

237. Black JC and Whetstine JR: Tipping the lysine methylation balance in disease. Biopolymers 99: 127-35, 2013.

238. Butler JS, Koutelou E, Schibler AC and Dent SYR: Histone-modifying enzymes: regulators of developmental decisions and drivers of human disease. Epigenomics 4: 163-77, 2012.

239. Campbell MJ and Turner BM: Altered histone modifications in cancer. Advances in experimental medicine and biology 754: 81-107, 2013.

240. Greer EL and Shi Y: Histone methylation: a dynamic mark in health, disease and inheritance. Nature reviews. Genetics 13: 343-57, 2012.

241. Hoffmann I, Roatsch M, Schmitt ML, Carlino L, Pippel M, Sippl W and Jung M: The role of histone demethylases in cancer therapy. Molecular oncology 6: 683-703, 2012.

242. Varier RA and Timmers HTM: Histone lysine methylation and demethylation pathways in cancer. Biochimica et biophysica acta 1815: 75-89, 2011.

243. Xhemalce B: From histones to RNA: role of methylation in cancer. Brief Funct Genomics. 12: 244-253, 2013.

244. Zagni C, Chiacchio U and Rescifina A: Histone Methyltransferase Inhibitors: Novel Epigenetic Agents for Cancer Treatment. Curr Med Chem. 20: 167-168, 2013.

245. Komatsu S, Imoto I, Tsuda H, Kozaki K-i, Muramatsu T, Shimada Y, Aiko S, Yoshizumi Y, Ichikawa D, Otsuji E and Inazawa J: Overexpression of SMYD2 relates to tumor cell proliferation and malignant outcome of esophageal squamous cell carcinoma. Carcinogenesis 30: 1139-46, 2009.

246. Luo X-G, Xi T, Guo S, Liu Z-P, Wang N, Jiang Y and Zhang T-C: Effects of SMYD3 overexpression on transformation, serum dependence, and apoptosis sensitivity in NIH3T3 cells. IUBMB life 61: 679-84, 2009.

247. Chen M-W, Hua K-T, Kao H-J, Chi C-C, Wei L-H, Johansson G, Shiah S-G, Chen P-S, Jeng Y-M, Cheng T-Y, Lai T-C, Chang J-S, Jan Y-H, Chien M-H, Yang C-J, Huang M-S, Hsiao M and Kuo M-L: H3K9 histone methyltransferase G9a promotes lung

cancer invasion and metastasis by silencing the cell adhesion molecule Ep-CAM. Cancer research 70: 7830-40, 2010.

248. Kondo Y, Shen L, Ahmed S, Boumber Y, Sekido Y, Haddad BR and Issa J-PJ: Downregulation of histone H3 lysine 9 methyltransferase G9a induces centrosome disruption and chromosome instability in cancer cells. PloS one 3: e2037, 2008.

249. Kondo Y, Shen L, Suzuki S, Kurokawa T, Masuko K, Tanaka Y, Kato H, Mizuno Y, Yokoe M, Sugauchi F, Hirashima N, Orito E, Osada H, Ueda R, Guo Y, Chen X, Issa J-PJ and Sekido Y: Alterations of DNA methylation and histone modifications contribute to gene silencing in hepatocellular carcinomas. Hepatology research : the official journal of the Japan Society of Hepatology 37: 974-83, 2007.

250. Cole PA: Chemical probes for histone-modifying enzymes. Nature chemical biology 4: 590-7, 2008.

251. He Y, Korboukh I, Jin J and Huang J: Targeting protein lysine methylation and demethylation in cancers Proteins that are Subject to Lysine Methylation Potential Biological Functions of PKMTs in Cancers. Acta biochimica et biophysica Sinica 44: 70-79, 2012.

252. Huang J, Plass C and Gerhauser C: Cancer chemoprevention by targeting the epigenome. Current drug targets 12: 1925-56, 2011.

253. Natoli G, Testa G and De Santa F: The future therapeutic potential of histone demethylases: A critical analysis. Current opinion in drug discovery & development 12: 607-15, 2009.

254. Poke FS, Qadi A and Holloway AF: Reversing aberrant methylation patterns in cancer. Current medicinal chemistry 17: 1246-54, 2010.

255. Chao C-C, Wu S-L and Ching W-M: Using LC-MS with de novo software to fully characterize the multiple methylations of lysine residues in a recombinant fragment of an outer membrane protein from a virulent strain of Rickettsia prowazekii. Biochimica et biophysica acta 1702: 145-52, 2004.

256. Chao C-C, Zhang Z, Wang H, Alkhalil A and Ching W-M: Serological reactivity and biochemical characterization of methylated and unmethylated forms of a recombinant protein fragment derived from outer membrane protein B of Rickettsia typhi. Clinical and vaccine immunology : CVI 15: 684-90, 2008.

257. Pethe K, Bifani P, Drobecq H, Sergheraert C, Debrie A-S, Locht C and Menozzi FD: Mycobacterial heparin-binding hemagglutinin and laminin-binding protein share

antigenic methyllysines that confer resistance to proteolysis. Proceedings of the National Academy of Sciences of the United States of America 99: 10759-64, 2002.

258. Barbier M, Owings JP, Martinez-Ramos I, Damron FH, Gomila R, Blazquez J, Goldberg JB and Alberti S: Lysine trimethylation of EF-Tu mimics platelet-activating factor to initiate Pseudomonas aeruginosa pneumonia. MBio 4: e00207-13, 2013.

259. Brown MA, Sims RJ, 3rd, Gottlieb PD and Tucker PW: Identification and characterization of Smyd2: a split SET/MYND domain-containing histone H3 lysine 36-specific methyltransferase that interacts with the Sin3 histone deacetylase complex. Mol Cancer 5: 26, 2006.

260. Huang J and Berger SL: The emerging field of dynamic lysine methylation of non-histone proteins. Current opinion in genetics & development 18: 152-8, 2008.

261. Martin C and Zhang Y: The diverse functions of histone lysine methylation. Nat Rev Mol Cell Biol 6: 838-49, 2005.

262. Subramanian K, Jia D, Kapoor-Vazirani P, Powell DR, Collins RE, Sharma D, Peng J, Cheng X and Vertino PM: Regulation of estrogen receptor alpha by the SET7 lysine methyltransferase. Molecular cell 30: 336-47, 2008.

263. Dillon SC, Zhang X, Trievel RC and Cheng X: The SET-domain protein superfamily: protein lysine methyltransferases. Genome Biol 6: 227, 2005.

264. Spadaccini R, Perrin H, Bottomley MJ, Ansieau S and Sattler M: Structure and functional analysis of the MYND domain. J Mol Biol 358: 498-508, 2006.

265. Xiao B, Wilson JR and Gamblin SJ: SET domains and histone methylation. Curr Opin Struct Biol. 13: 699-705, 2003

266. Abu-Farha M, Lambert JP, Al-Madhoun AS, Elisma F, Skerjanc IS and Figeys D: The tale of two domains: proteomics and genomics analysis of SMYD2, a new histone methyltransferase. Mol Cell Proteomics 7: 560-72, 2008.

267. Yamamoto K, Ishida T, Nakano K, Yamagishi M, Yamochi T, Tanaka Y, Furukawa Y, Nakamura Y and Watanabe T: SMYD3 interacts with HTLV-1 Tax and regulates subcellular localization of Tax. Cancer Sci 102: 260-6.

268. Sims RJ, 3rd, Weihe EK, Zhu L, O'Malley S, Harriss JV and Gottlieb PD: m-Bop, a repressor protein essential for cardiogenesis, interacts with skNAC, a heart- and muscle-specific transcription factor. J Biol Chem 277: 26524-9, 2002.

269. Gottlieb PD, Pierce SA, Sims RJ, Yamagishi H, Weihe EK, Harriss JV, Maika SD, Kuziel WA, King HL, Olson EN, Nakagawa O and Srivastava D: Bop encodes a muscle-restricted protein containing MYND and SET domains and is essential for cardiac differentiation and morphogenesis. Nat Genet 31: 25-32, 2002.

270. Hamamoto R, Furukawa Y, Morita M, Iimura Y, Silva FP, Li M, Yagyu R and Nakamura Y: SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cells. Nat Cell Biol 6: 731-40, 2004.

271. Tan X, Rotllant J, Li H, De Deyne P and Du SJ: SmyD1, a histone methyltransferase, is required for myofibril organization and muscle contraction in zebrafish embryos. Proc Natl Acad Sci U S A 103: 2713-8, 2006.

272. Taipale M, Jarosz DF and Lindquist S: HSP90 at the hub of protein homeostasis: emerging mechanistic insights. Nat Rev Mol Cell Biol 11: 515-28, 2010.

273. Scroggins BT and Neckers L: Post-translational modification of heat-shock protein 90: impact on chaperone function. Expert Opinion on Drug Discovery 2: 1403-1414, 2007.

274. Kundrat L and Regan L: Identification of residues on Hsp70 and Hsp90 ubiquitinated by the cochaperone CHIP. J Mol Biol 395: 587-94, 2010.

275. Scroggins BT, Robzyk K, Wang DX, Marcu MG, Tsutsumi S, Beebe K, Cotter RJ, Felts S, Toft D, Karnitz L, Rosen N and Neckers L: An acetylation site in the middle domain of Hsp90 regulates chaperone function. Molecular Cell 25: 151-159, 2007.

276. Yang Y, Rao R, Shen J, Tang Y, Fiskus W, Nechtman J, Atadja P and Bhalla K: Role of acetylation and extracellular location of heat shock protein 90alpha in tumor cell invasion. Cancer Res 68: 4833-42, 2008.

277. Martinez-Ruiz A, Villanueva L, Gonzalez de Orduna C, Lopez-Ferrer D, Higueras MA, Tarin C, Rodriguez-Crespo I, Vazquez J and Lamas S: S-nitrosylation of Hsp90 promotes the inhibition of its ATPase and endothelial nitric oxide synthase regulatory activities. Proc Natl Acad Sci U S A 102: 8525-30, 2005.

278. Retzlaff M, Stahl M, Eberl HC, Lagleder S, Beck J, Kessler H and Buchner J: Hsp90 is regulated by a switch point in the C-terminal domain. EMBO Rep 10: 1147-53, 2009.

279. Komatsu S, Imoto I, Tsuda H, Kozaki KI, Muramatsu T, Shimada Y, Aiko S, Yoshizumi Y, Ichikawa D, Otsuji E and Inazawa J: Overexpression of SMYD2 relates to

tumor cell proliferation and malignant outcome of esophageal squamous cell carcinoma. Carcinogenesis 30: 1139-46, 2009.

280. Hamamoto R, Silva FP, Tsuge M, Nishidate T, Katagiri T, Nakamura Y and Furukawa Y: Enhanced SMYD3 expression is essential for the growth of breast cancer cells. Cancer Sci 97: 113-8, 2006.

281. Wang SZ, Luo XG, Shen J, Zou JN, Lu YH and Xi T: Knockdown of SMYD3 by RNA interference inhibits cervical carcinoma cell growth and invasion in vitro. BMB Rep 41: 294-9, 2008.

282. Zou JN, Wang SZ, Yang JS, Luo XG, Xie JH and Xi T: Knockdown of SMYD3 by RNA interference down-regulates c-Met expression and inhibits cells migration and invasion induced by HGF. Cancer Lett 280: 78-85, 2009.

283. Hu L, Zhu YT, Qi C and Zhu YJ: Identification of Smyd4 as a potential tumor suppressor gene involved in breast cancer development. Cancer Res 69: 4067-72, 2009.

284. Li D, Niu Z, Yu W, Qian Y, Wang Q, Li Q, Yi Z, Luo J, Wu X, Wang Y, Schwartz RJ and Liu M: SMYD1, the myogenic activator, is a direct target of serum response factor and myogenin. Nucleic Acids Res 37: 7059-71, 2009.

285. Thompson EC and Travers AA: A Drosophila Smyd4 homologue is a muscle-specific transcriptional modulator involved in development. PLoS One 3: e3008, 2008.

286. Huang J, Perez-Burgos L, Placek BJ, Sengupta R, Richter M, Dorsey JA, Kubicek S, Opravil S, Jenuwein T and Berger SL: Repression of p53 activity by Smyd2-mediated methylation. Nature 444: 629-32, 2006.

287. Avdic V, Zhang P, Lanouette S, Groulx A, Tremblay V, Brunzelle J and Couture JF: Structural and biochemical insights into MLL1 core complex assembly. Structure 19: 101-8.

288. Xu C, Bian C, Yang W, Galka M, Ouyang H, Chen C, Qiu W, Liu H, Jones AE, MacKenzie F, Pan P, Li SS, Wang H and Min J: Binding of different histone marks differentially regulates the activity and specificity of polycomb repressive complex 2 (PRC2). Proc Natl Acad Sci U S A 107: 19266-71.

289. Dou Y, Milne TA, Ruthenburg AJ, Lee S, Lee JW, Verdine GL, Allis CD and Roeder RG: Regulation of MLL1 H3K4 methyltransferase activity by its core components. Nat Struct Mol Biol 13: 713-9, 2006.

290. Li Y, Trojer P, Xu CF, Cheung P, Kuo A, Drury WJ, 3rd, Qiao Q, Neubert TA, Xu RM, Gozani O and Reinberg D: The target of the NSD family of histone lysine methyltransferases depends on the nature of the substrate. J Biol Chem 284: 34283-95, 2009.

291. Pei H, Zhang L, Luo K, Qin Y, Chesi M, Fei F, Bergsagel PL, Wang L, You Z and Lou Z: MMSET regulates histone H4K20 methylation and 53BP1 accumulation at DNA damage sites. Nature 470: 124-8, 2011.

292. Cao R and Zhang Y: SUZ12 is required for both the histone methyltransferase activity and the silencing function of the EED-EZH2 complex. Mol Cell 15: 57-67, 2004.

293. Pasini D, Bracken AP, Jensen MR, Lazzerini Denchi E and Helin K: Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. EMBO J 23: 4061-71, 2004.

294. Pourquier P and Pommier Y: Topoisomerase I-mediated DNA damage. Adv Cancer Res 80: 189-216, 2001.

295. Maiguel DA, Jones L, Chakravarty D, Yang C and Carrier F: Nucleophosmin sets a threshold for p53 response to UV radiation. Mol Cell Biol 24: 3703-11, 2004.

296. Huber O, Menard L, Haurie V, Nicou A, Taras D and Rosenbaum J: Pontin and reptin, two related ATPases with multiple roles in cancer. Cancer Res 68: 6873-6, 2008.

297. Tsai RY: Nucleolar modulation of TRF1: a dynamic way to regulate telomere and cell cycle by nucleostemin and GNL3L. Cell Cycle 8: 2912-6, 2009.

298. Zhang N, Kaur R, Akhter S and Legerski RJ: Cdc5L interacts with ATR and is required for the S-phase cell-cycle checkpoint. EMBO Rep 10: 1029-35, 2009.

299. Lutz T, Stoger R and Nieto A: CHD6 is a DNA-dependent ATPase and localizes at nuclear sites of mRNA synthesis. FEBS Lett 580: 5851-7, 2006.

300. Scibetta AG, Santangelo S, Coleman J, Hall D, Chaplin T, Copier J, Catchpole S, Burchell J and Taylor-Papadimitriou J: Functional analysis of the transcription repressor PLU-1/JARID1B. Mol Cell Biol 27: 7220-35, 2007.

301. Kwon SH and Workman JL: The heterochromatin protein 1 (HP1) family: put away a bias toward HP1. Mol Cells 26: 217-27, 2008.

302. Trievel RC and Shilatifard A: WDR5, a complexed protein. Nat Struct Mol Biol 16: 678-80, 2009.

303. Tuduri S, Crabbe L, Conti C, Tourriere H, Holtgreve-Grez H, Jauch A, Pantesco V, De Vos J, Thomas A, Theillet C, Pommier Y, Tazi J, Coquelle A and Pasero P: Topoisomerase I suppresses genomic instability by preventing interference between replication and transcription. Nat Cell Biol 11: 1315-24, 2009.

304. Bouchard VJ, Rouleau M and Poirier GG: PARP-1, a determinant of cell survival in response to DNA damage. Exp Hematol 31: 446-54, 2003.

305. Schlegel BP, Starita LM and Parvin JD: Overexpression of a protein fragment of RNA helicase A causes inhibition of endogenous BRCA1 function and defects in ploidy and cytokinesis in mammary epithelial cells. Oncogene 22: 983-91, 2003.

306. Collis SJ, DeWeese TL, Jeggo PA and Parker AR: The life and death of DNA-PK. Oncogene 24: 949-61, 2005.

307. Wang H, Zhao A, Chen L, Zhong X, Liao J, Gao M, Cai M, Lee DH, Li J, Chowdhury D, Yang YG, Pfeifer GP, Yen Y and Xu X: Human RIF1 encodes an anti-apoptotic factor required for DNA repair. Carcinogenesis 30: 1314-9, 2009.

308. Huang J and Berger SL: The emerging field of dynamic lysine methylation of non-histone proteins. Curr Opin Genet Dev 18: 152-8, 2008.

309. Munro S, Khaire N, Inche A, Carr S and La Thangue NB: Lysine methylation regulates the pRb tumour suppressor protein. Oncogene 29: 2357-67, 2010.

310. Shi X, Kachirskaia I, Yamaguchi H, West LE, Wen H, Wang EW, Dutta S, Appella E and Gozani O: Modulation of p53 function by SET8-mediated methylation at lysine 382. Mol Cell 27: 636-46, 2007.

311. Zhang X, Tamaru H, Khan SI, Horton JR, Keefe LJ, Selker EU and Cheng X: Structure of the Neurospora SET domain protein DIM-5, a histone H3 lysine methyltransferase. Cell 111: 117-27, 2002.

312. Patnaik D, Chin HG, Esteve PO, Benner J, Jacobsen SE and Pradhan S: Substrate specificity and kinetic mechanism of mammalian G9a histone H3 methyltransferase. J Biol Chem 279: 53248-58, 2004.

313. Chin HG, Patnaik D, Esteve PO, Jacobsen SE and Pradhan S: Catalytic properties and kinetic mechanism of human recombinant Lys-9 histone H3 methyltransferase SUV39H1: participation of the chromodomain in enzymatic catalysis. Biochemistry 45: 3272-84, 2006.

314. Qian C, Wang X, Manzur K, Sachchidanand, Farooq A, Zeng L, Wang R and Zhou MM: Structural insights of the specificity and catalysis of a viral histone H3 lysine 27 methyltransferase. J Mol Biol 359: 86-96, 2006.

315. Dirk LM, Flynn EM, Dietzel K, Couture JF, Trievel RC and Houtz RL: Kinetic manifestation of processivity during multiple methylations catalyzed by SET domain protein methyltransferases. Biochemistry 46: 3905-15, 2007.

316. Roe SM, Ali MM, Meyer P, Vaughan CK, Panaretou B, Piper PW, Prodromou C and Pearl LH: The Mechanism of Hsp90 regulation by the protein kinase-specific cochaperone p50(cdc37). Cell 116: 87-98, 2004.

317. Lotz GP, Lin H, Harst A and Obermann WM: Aha1 binds to the middle domain of Hsp90, contributes to client protein activation, and stimulates the ATPase activity of the molecular chaperone. J Biol Chem 278: 17228-35, 2003.

318. Pratt WB and Toft DO: Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. Exp Biol Med (Maywood) 228: 111-33, 2003.

319. Huang J, Sengupta R, Espejo AB, Lee MG, Dorsey JA, Richter M, Opravil S, Shiekhattar R, Bedford MT, Jenuwein T and Berger SL: p53 is regulated by the lysine demethylase LSD1. Nature 449: 105-8, 2007.

320. Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA and Casero RA: Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell 119: 941-53, 2004.

321. Kunizaki M, Hamamoto R, Silva FP, Yamaguchi K, Nagayasu T, Shibuya M, Nakamura Y and Furukawa Y: The lysine 831 of vascular endothelial growth factor receptor 1 is a novel target of methylation by SMYD3. Cancer Res 67: 10759-65, 2007.

322. Overmeer RM, Gourdin AM, Giglia-Mari A, Kool H, Houtsmuller AB, Siegal G, Fousteri MI, Mullenders LH and Vermeulen W: Replication factor C recruits DNA polymerase delta to sites of nucleotide excision repair but is not required for PCNA recruitment. Mol Cell Biol 30: 4828-39.

323. Coue M, Kearsey SE and Mechali M: Chromotin binding, nuclear localization and phosphorylation of Xenopus cdc21 are cell-cycle dependent and associated with the control of initiation of DNA replication. EMBO J 15: 1085-97, 1996.

324. Cai J, Gibbs E, Uhlmann F, Phillips B, Yao N, O'Donnell M and Hurwitz J: A complex consisting of human replication factor C p40, p37, and p36 subunits is a DNA-

dependent ATPase and an intermediate in the assembly of the holoenzyme. J Biol Chem 272: 18974-81, 1997.

325. Uhlmann F, Cai J, Gibbs E, O'Donnell M and Hurwitz J: Deletion analysis of the large subunit p140 in human replication factor C reveals regions required for complex formation and replication activities. J Biol Chem 272: 10058-64, 1997.

326. Johnson A and O'Donnell M: Cellular DNA replicases: components and dynamics at the replication fork. Annu Rev Biochem 74: 283-315, 2005.

327. Li H, Xie B, Zhou Y, Rahmeh A, Trusa S, Zhang S, Gao Y, Lee EY and Lee MY: Functional roles of p12, the fourth subunit of human DNA polymerase delta. J Biol Chem 281: 14748-55, 2006.

328. Li X, Stith CM, Burgers PM and Heyer WD: PCNA is required for initiation of recombination-associated DNA synthesis by DNA polymerase delta. Mol Cell 36: 704-13, 2009.

329. Hatakeyama S, Matsumoto M, Yada M and Nakayama KI: Interaction of U-box-type ubiquitin-protein ligases (E3s) with molecular chaperones. Genes Cells 9: 533-48, 2004.

330. Chen S and Smith DF: Hop as an adaptor in the heat shock protein 70 (Hsp70) and hsp90 chaperone machinery. J Biol Chem 273: 35194-200, 1998.

331. Carr SM, Munro S, Kessler B, Oppermann U and La Thangue NB: Interplay between lysine methylation and Cdk phosphorylation in growth control by the retinoblastoma protein. EMBO J 30: 317-27.

332. Levy D, Kuo AJ, Chang Y, Schaefer U, Kitson C, Cheung P, Espejo A, Zee BM, Liu CL, Tangsombatvisit S, Tennen RI, Kuo AY, Tanjing S, Cheung R, Chua KF, Utz PJ, Shi X, Prinjha RK, Lee K, Garcia BA, Bedford MT, Tarakhovsky A, Cheng X and Gozani O: Lysine methylation of the NF-kappaB subunit RelA by SETD6 couples activity of the histone methyltransferase GLP at chromatin to tonic repression of NF-kappaB signaling. Nat Immunol 12: 29-36.

333. Yang XD, Tajkhorshid E and Chen LF: Functional interplay between acetylation and methylation of the RelA subunit of NF-kappaB. Mol Cell Biol 30: 2170-80.

334. Esteve PO, Chang Y, Samaranayake M, Upadhyay AK, Horton JR, Feehery GR, Cheng X and Pradhan S: A methylation and phosphorylation switch between an adjacent lysine and serine determines human DNMT1 stability. Nat Struct Mol Biol 18: 42-8.

335. Pang CN, Gasteiger E and Wilkins MR: Identification of arginine- and lysine-methylation in the proteome of Saccharomyces cerevisiae and its functional implications. BMC Genomics 11: 92, 2010.

336. Avdic V, Zhang P, Lanouette S, Groulx A, Tremblay V, Brunzelle J and Couture JF: Structural and biochemical insights into MLL1 core complex assembly. Structure 19: 101-8, 2011.

337. Avdic V, Zhang P, Lanouette S, Voronova A, Skerjanc I and Couture JF: Fine-tuning the stimulation of MLL1 methyltransferase activity by a histone H3-based peptide mimetic. FASEB J 25: 960-7, 2011.

338. Couture JF, Collazo E, Ortiz-Tello PA, Brunzelle JS and Trievel RC: Specificity and mechanism of JMJD2A, a trimethyllysine-specific histone demethylase. Nat Struct Mol Biol 14: 689-95, 2007.

339. Koulov AV, Lapointe P, Lu B, Razvi A, Coppinger J, Dong MQ, Matteson J, Laister R, Arrowsmith C, Yates JR, 3rd and Balch WE: Biological and structural basis for Aha1 regulation of Hsp90 ATPase activity in maintaining proteostasis in the human disease cystic fibrosis. Mol Biol Cell 21: 871-84, 2010.

340. Avdic V, Zhang P, Lanouette S, Voronova A, Skerjanc I and Couture JF: Fine-tuning the stimulation of MLL1 methyltransferase activity by a histone H3-based peptide mimetic. FASEB J: 2010.

341. Lanouette S, Mongeon V, Figeys D and Couture JF: The functional diversity of protein lysine methylation. Mol Syst Biol 10: 724, 2014.

342. Rathert P, Dhayalan A, Murakami M, Zhang X, Tamas R, Jurkowska R, Komatsu Y, Shinkai Y, Cheng X and Jeltsch A: Protein lysine methyltransferase G9a acts on non-histone targets. Nat Chem Biol 4: 344-6, 2008.

343. Kudithipudi S, Lungu C, Rathert P, Happel N and Jeltsch A: Substrate specificity analysis and novel substrates of the protein lysine methyltransferase NSD1. Chem Biol 21: 226-37, 2014.

344. Kudithipudi S, Dhayalan A, Kebede AF and Jeltsch A: The SET8 H4K20 protein lysine methyltransferase has a long recognition sequence covering seven amino acid residues. Biochimie 94: 2212-8, 2012.

345. Blais A, Tsikitis M, Acosta-Alvear D, Sharan R, Kluger Y and Dynlacht BD: An initial blueprint for myogenic differentiation. Genes Dev 19: 553-69, 2005.

346. Sese B, Barrero MJ, Fabregat MC, Sander V and Izpisua Belmonte JC: SMYD2 is induced during cell differentiation and participates in early development. Int J Dev Biol 57: 357-64, 2013.

347. Zuber J, Rappaport AR, Luo W, Wang E, Chen C, Vaseva AV, Shi J, Weissmueller S, Fellmann C, Taylor MJ, Weissenboeck M, Graeber TG, Kogan SC, Vakoc CR and Lowe SW: An integrated approach to dissecting oncogene addiction implicates a Myb-coordinated self-renewal program as essential for leukemia maintenance. Genes Dev 25: 1628-40, 2011.

348. Sakamoto LH, Andrade RV, Felipe MS, Motoyama AB and Pittella Silva F: SMYD2 is highly expressed in pediatric acute lymphoblastic leukemia and constitutes a bad prognostic factor. Leuk Res 38: 496-502, 2014.

349. Barros Filho MC, Katayama ML, Brentani H, Abreu AP, Barbosa EM, Oliveira CT, Goes JC, Brentani MM and Folgueira MA: Gene trio signatures as molecular markers to predict response to doxorubicin cyclophosphamide neoadjuvant chemotherapy in breast cancer patients. Braz J Med Biol Res 43: 1225-31, 2010.

350. Zhang X, Tanaka K, Yan J, Li J, Peng D, Jiang Y, Yang Z, Barton MC, Wen H and Shi X: Regulation of estrogen receptor alpha by histone methyltransferase SMYD2-mediated protein methylation. Proc Natl Acad Sci U S A 110: 17284-9, 2013.

351. Cho HS, Hayami S, Toyokawa G, Maejima K, Yamane Y, Suzuki T, Dohmae N, Kogure M, Kang D, Neal DE, Ponder BA, Yamaue H, Nakamura Y and Hamamoto R: RB1 methylation by SMYD2 enhances cell cycle progression through an increase of RB1 phosphorylation. Neoplasia 14: 476-86, 2012.

352. Saddic LA, West LE, Aslanian A, Yates JR, 3rd, Rubin SM, Gozani O and Sage J: Methylation of the retinoblastoma tumor suppressor by SMYD2. J Biol Chem 285: 37733-40, 2010.

353. Piao L, Kang D, Suzuki T, Masuda A, Dohmae N, Nakamura Y and Hamamoto R: The Histone Methyltransferase SMYD2 Methylates PARP1 and Promotes Poly(ADP-ribosyl)ation Activity in Cancer Cells. Neoplasia: 2014.

354. Ferguson AD, Larsen NA, Howard T, Pollard H, Green I, Grande C, Cheung T, Garcia-Arenas R, Cowen S, Wu J, Godin R, Chen H and Keen N: Structural basis of substrate methylation and inhibition of SMYD2. Structure 19: 1262-73, 2011.

355. Sirinupong N, Brunzelle J, Doko E and Yang Z: Structural insights into the autoinhibition and posttranslational activation of histone methyltransferase SmyD3. J Mol Biol 406: 149-59, 2011.

356. Sirinupong N, Brunzelle J, Ye J, Pirzada A, Nico L and Yang Z: Crystal structure of cardiac-specific histone methyltransferase SmyD1 reveals unusual active site architecture. J Biol Chem 285: 40635-44, 2010.

357. Davey JA and Chica RA: Multistate approaches in computational protein design. Protein Science 21: 1241-52, 2012.

358. Mandell DJ and Kortemme T: Computer-aided design of functional protein interactions. Nat Chem Biol 5: 797-807, 2009.

359. Karanicolas J and Kuhlman B: Computational design of affinity and specificity at protein-protein interfaces. Curr Opin Struct Biol 19: 458-63, 2009.

360. Alvizo O, Mittal S, Mayo SL and Schiffer CA: Structural, kinetic, and thermodynamic studies of specificity designed HIV-1 protease. Protein Science 21: 1029-41, 2012.

361. Sammond DW, Eletr ZM, Purbeck C, Kimple RJ, Siderovski DP and Kuhlman B: Structure-based protocol for identifying mutations that enhance protein-protein binding affinities. J Mol Biol 371: 1392-404, 2007.

362. Grigoryan G and Keating AE: Structure-based prediction of bZIP partnering specificity. J Mol Biol 355: 1125-42, 2006.

363. Jha RK, Leaver-Fay A, Yin S, Wu Y, Butterfoss GL, Szyperski T, Dokholyan NV and Kuhlman B: Computational design of a PAK1 binding protein. J Mol Biol 400: 257-70, 2010.

364. Huang PS, Love JJ and Mayo SL: A de novo designed protein protein interface. Protein Science 16: 2770-4, 2007.

365. Smith CA and Kortemme T: Backrub-like backbone simulation recapitulates natural protein conformational variability and improves mutant side-chain prediction. J Mol Biol 380: 742-56, 2008.

366. Humphris EL and Kortemme T: Prediction of protein-protein interface sequence diversity using flexible backbone computational protein design. Structure 16: 1777-88, 2008.

367. Davey JA and Chica RA: Improving the accuracy of protein stability predictions with multistate design using a variety of backbone ensembles. Proteins 82: 771-84, 2014.

368. Babor M, Mandell DJ and Kortemme T: Assessment of flexible backbone protein design methods for sequence library prediction in the therapeutic antibody Herceptin-HER2 interface. Protein Science 20: 1082-9, 2011.

369. Allen BD, Nisthal A and Mayo SL: Experimental library screening demonstrates the successful application of computational protein design to large structural ensembles. Proc Natl Acad Sci U S A 107: 19838-43, 2010.

370. Smith CA and Kortemme T: Structure-based prediction of the peptide sequence space recognized by natural and synthetic PDZ domains. J Mol Biol 402: 460-74, 2010.

371. Kapp GT, Liu S, Stein A, Wong DT, Remenyi A, Yeh BJ, Fraser JS, Taunton J, Lim WA and Kortemme T: Control of protein signaling using a computationally designed GTPase/GEF orthogonal pair. Proc Natl Acad Sci U S A 109: 5277-82, 2012.

372. Grigoryan G, Reinke AW and Keating AE: Design of protein-interaction specificity gives selective bZIP-binding peptides. Nature 458: 859-64, 2009.

373. Kanungo T, Mount DM, Netanyahu NS, Piatko CD, Silverman R and Wu AY: An Efficient k-Means Clustering Algorithm: Analysis and Implementation. In: IEEE Trans. Pattern Anal. Mach. Intell. 2002, pp 881-892.

374. Jiang Y, Trescott L, Holcomb J, Zhang X, Brunzelle J, Sirinupong N, Shi X and Yang Z: Structural Insights into Estrogen Receptor alpha Methylation by Histone Methyltransferase SMYD2, a Cellular Event Implicated in Estrogen Signaling Regulation. J Mol Biol: 2014.

375. Winkler DF and Campbell WD: The spot technique: synthesis and screening of peptide macroarrays on cellulose membranes. Methods Mol Biol 494: 47-70, 2008.

376. Hilpert K, Winkler DF and Hancock RE: Peptide arrays on cellulose support: SPOT synthesis, a time and cost efficient method for synthesis of large numbers of peptides in a parallel and addressable fashion. Nat Protoc 2: 1333-49, 2007.

377. Choi E, Guntas G and Kuhlman B: Future challenges of computational protein design. . In: Park S and Cochran J (eds). Protein Engineering and Design. . Boca Raton: Talor and Francis Group, 2010, pp pp 367-370.

378. Murphy GS, Mills JL, Miley MJ, Machius M, Szyperski T and Kuhlman B: Increasing sequence diversity with flexible backbone protein design: the complete redesign of a protein hydrophobic core. Structure 20: 1086-96, 2012.

379. Abu-Farha M, Lanouette S, Elisma F, Tremblay V, Butson J, Figeys D and Couture JF: Proteomic analyses of the SMYD family interactomes identify HSP90 as a novel target for SMYD2. J Mol Cell Biol 3: 301-8, 2011.

380. Voelkel T, Andresen C, Unger A, Just S, Rottbauer W and Linke WA: Lysine methyltransferase Smyd2 regulates Hsp90-mediated protection of the sarcomeric titin springs and cardiac function. Biochim Biophys Acta 1833: 812-22, 2013.

381. Donlin LT, Andresen C, Just S, Rudensky E, Pappas CT, Kruger M, Jacobs EY, Unger A, Zieseniss A, Dobenecker MW, Voelkel T, Chait BT, Gregorio CC, Rottbauer W, Tarakhovsky A and Linke WA: Smyd2 controls cytoplasmic lysine methylation of Hsp90 and myofilament organization. Genes Dev 26: 114-9, 2012.

382. de Castro E, Sigrist CJ, Gattiker A, Bulliard V, Langendijk-Genevaux PS, Gasteiger E, Bairoch A and Hulo N: ScanProsite: detection of PROSITE signature matches and ProRule-associated functional and structural residues in proteins. Nucleic Acids Res 34: W362-5, 2006.

383. Huang da W, Sherman BT and Lempicki RA: Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 37: 1-13, 2009.

384. Heanue TA, Reshef R, Davis RJ, Mardon G, Oliver G, Tomarev S, Lassar AB and Tabin CJ: Synergistic regulation of vertebrate muscle development by Dach2, Eya2, and Six1, homologs of genes required for Drosophila eye formation. Genes Dev 13: 3231-43, 1999.

385. Boucher CA, Winchester CL, Hamilton GM, Winter AD, Johnson KJ and Bailey ME: Structure, mapping and expression of the human gene encoding the homeodomain protein, SIX2. Gene 247: 145-51, 2000.

386. Ayer DE, Lawrence QA and Eisenman RN: Mad-Max transcriptional repression is mediated by ternary complex formation with mammalian homologs of yeast repressor Sin3. Cell 80: 767-76, 1995.

387. Wen X, Tannukit S and Paine ML: TFIP11 interacts with mDEAH9, an RNA helicase involved in spliceosome disassembly. Int J Mol Sci 9: 2105-13, 2008.

388. Patrick AN, Cabrera JH, Smith AL, Chen XS, Ford HL and Zhao R: Structure-function analyses of the human SIX1-EYA2 complex reveal insights into metastasis and BOR syndrome. Nat Struct Mol Biol 20: 447-53, 2013.

389. Alland L, David G, Shen-Li H, Potes J, Muhle R, Lee HC, Hou H, Jr., Chen K and DePinho RA: Identification of mammalian Sds3 as an integral component of the Sin3/histone deacetylase corepressor complex. Mol Cell Biol 22: 2743-50, 2002.

390. Diehl F, Brown Ma, van Amerongen MJ, Novoyatleva T, Wietelmann A, Harriss J, Ferrazzi F, Böttger T, Harvey RP, Tucker PW and Engel FB: Cardiac deletion of Smyd2 is dispensable for mouse heart development. PloS one 5: e9748, 2010.

391. Abu-Farha M, Lambert J-P, Al-Madhoun AS, Elisma F, Skerjanc IS and Figeys D: The tale of two domains: proteomics and genomics analysis of SMYD2, a new histone methyltransferase. Molecular & cellular proteomics : MCP 7: 560-72, 2008.

392. Brown Ma, Sims RJ, Gottlieb PD and Tucker PW: Identification and characterization of Smyd2: a split SET/MYND domain-containing histone H3 lysine 36-specific methyltransferase that interacts with the Sin3 histone deacetylase complex. Molecular cancer 5: 26, 2006.

393. Chemical Computing Group I: Molecular Operating Enrivoment. In: Montreal, Quebec, Canada, 2012.

394. Wang JM, Cieplak P and Kollman PA: How well does a restrained electrostatic potential (RESP) model perform in calculating conformational energies of organic and biological molecules? Journal of Computational Chemistry 21: 1049-1074, 2000.

395. Allen BD and Mayo SL: An Efficient Algorithm for Multistate Protein Design Based on FASTER. Journal of Computational Chemistry 31: 904-916, 2010.

396. Allen BD and Mayo SL: Dramatic performance enhancements for the FASTER optimization algorithm. Journal of Computational Chemistry 27: 1071-1075, 2006.

397. Dunbrack RL and Cohen FE: Bayesian statistical analysis of protein side-chain rotamer preferences. Protein Science 6: 1661-1681, 1997.

398. Mayo SL, Olafson BD and Goddard WA: Dreiding - a Generic Force-Field for Molecular Simulations. Journal of Physical Chemistry 94: 8897-8909, 1990.

399. Dahiyat BI and Mayo SL: Probing the role of packing specificity in protein design. Proceedings of the National Academy of Sciences of the United States of America 94: 10172-10177, 1997.

400. Lazaridis T and Karplus M: Effective energy function for proteins in solution. Proteins-Structure Function and Genetics 35: 133-152, 1999.

401. Street AG and Mayo SL: Pairwise calculation of protein solvent-accessible surface areas. Folding & Design 3: 253-258, 1998.

402. Allen BD, Nisthal A and Mayo SL: Experimental library screening demonstrates the successful application of computational protein design to large structural ensembles. Proceedings of the National Academy of Sciences of the United States of America 107: 19838-19843, 2010.

403. Chica RA, Moore MM, Allen BD and Mayo SL: Generation of longer emission wavelength red fluorescent proteins using computationally designed libraries. Proceedings of the National Academy of Sciences of the United States of America 107: 20257-20262, 2010.

404. Privett HK, Kiss G, Lee TM, Blomberg R, Chica RA, Thomas LM, Hilvert D, Houk KN and Mayo SL: Iterative approach to computational enzyme design. Proceedings of the National Academy of Sciences of the United States of America 109: 3790-3795, 2012.

405. Diehl F, Brown MA, van Amerongen MJ, Novoyatleva T, Wietelmann A, Harriss J, Ferrazzi F, Bottger T, Harvey RP, Tucker PW and Engel FB: Cardiac deletion of Smyd2 is dispensable for mouse heart development. PloS one 5: e9748, 2010.

406. Kawamura S, Yoshigai E, Kuhara S and Tashiro K: smyd1 and smyd2 are expressed in muscle tissue in Xenopus laevis. Cytotechnology 57: 161-8, 2008.

407. Roidl D and Hacker C: Histone methylation during neural development. Cell Tissue Res 356: 539-52, 2014.

408. Strikoudis A, Guillamot M and Aifantis I: Regulation of stem cell function by protein ubiquitylation. EMBO Rep 15: 365-82, 2014.

409. Ramakrishna S, Kim KS and Baek KH: Posttranslational modifications of defined embryonic reprogramming transcription factors. Cell Reprogram 16: 108-20, 2014.

410. Gao C, Xiao G and Hu J: Regulation of Wnt/beta-catenin signaling by posttranslational modifications. Cell Biosci 4: 13, 2014.

411. Wang YC, Peterson SE and Loring JF: Protein post-translational modifications and regulation of pluripotency in human stem cells. Cell Res 24: 143-60, 2014.

412. Jacques-Fricke BT and Gammill LS: Neural crest specification and migration independently require NSD3-related lysine methyltransferase activity. Mol Biol Cell 25: 4174-86, 2014.

413. Schwarz D, Varum S, Zemke M, Scholer A, Baggiolini A, Draganova K, Koseki H, Schubeler D and Sommer L: Ezh2 is required for neural crest-derived cartilage and bone formation. Development 141: 867-77, 2014.

414. Aloia L, Di Stefano B and Di Croce L: Polycomb complexes in stem cells and embryonic development. Development 140: 2525-34, 2013.

415. Tan SL, Nishi M, Ohtsuka T, Matsui T, Takemoto K, Kamio-Miura A, Aburatani H, Shinkai Y and Kageyama R: Essential roles of the histone methyltransferase ESET in the epigenetic control of neural progenitor cells during development. Development 139: 3806-16, 2012.

416. Sun G and Shi YB: Thyroid hormone regulation of adult intestinal stem cell development: mechanisms and evolutionary conservations. Int J Biol Sci 8: 1217-24, 2012.

417. Delgado-Olguin P, Huang Y, Li X, Christodoulou D, Seidman CE, Seidman JG, Tarakhovsky A and Bruneau BG: Epigenetic repression of cardiac progenitor gene expression by Ezh2 is required for postnatal cardiac homeostasis. Nat Genet 44: 343-7, 2012.

418. Xiao Y, Bedet C, Robert VJ, Simonet T, Dunkelbarger S, Rakotomalala C, Soete G, Korswagen HC, Strome S and Palladino F: Caenorhabditis elegans chromatin-associated proteins SET-2 and ASH-2 are differentially required for histone H3 Lys 4 methylation in embryos and adult germ cells. Proc Natl Acad Sci U S A 108: 8305-10, 2011.

419. Chen X, Skutt-Kakaria K, Davison J, Ou YL, Choi E, Malik P, Loeb K, Wood B, Georges G, Torok-Storb B and Paddison PJ: G9a/GLP-dependent histone H3K9me2 patterning during human hematopoietic stem cell lineage commitment. Genes Dev 26: 2499-511, 2012.

420. Fujii T, Tsunesumi S-i, Yamaguchi K, Watanabe S and Furukawa Y: Smyd3 is required for the development of cardiac and skeletal muscle in zebrafish. PloS one 6: e23491, 2011.

421. Proserpio V, Fittipaldi R, Ryall JG, Sartorelli V and Caretti G: The methyltransferase SMYD3 mediates the recruitment of transcriptional cofactors at the myostatin and c-Met genes and regulates skeletal muscle atrophy. Genes Dev 27: 1299-312, 2013.

422. Thompson EC and Travers Aa: A Drosophila Smyd4 homologue is a muscle-specific transcriptional modulator involved in development. PloS one 3: e3008, 2008.

423. Gottlieb PD, Pierce Sa, Sims RJ, Yamagishi H, Weihe EK, Harriss JV, Maika SD, Kuziel Wa, King HL, Olson EN, Nakagawa O and Srivastava D: Bop encodes a muscle-restricted protein containing MYND and SET domains and is essential for cardiac differentiation and morphogenesis. Nature genetics 31: 25-32, 2002.

424. Granzier HL and Labeit S: The giant protein titin: a major player in myocardial mechanics, signaling, and disease. Circ Res 94: 284-95, 2004.

425. Li H, Xu J, Bian Y-H, Rotllant P, Shen T, Chu W, Zhang J, Schneider M and Du SJ: Smyd1b_tv1, a key regulator of sarcomere assembly, is localized on the M-line of skeletal muscle fibers. PloS one 6: e28524, 2011.

426. Gao J, Li J, Li BJ, Yagil E, Zhang J and Du SJ: Expression and functional characterization of Smyd1a in myofibril organization of skeletal muscles. PloS one 9: e86808, 2014.

427. Rana MS, Christoffels VM and Moorman AF: A molecular and genetic outline of cardiac morphogenesis. Acta Physiol (Oxf) 207: 588-615, 2013.

428. Snarr BS, Kern CB and Wessels A: Origin and fate of cardiac mesenchyme. Dev Dyn 237: 2804-19, 2008.

429. Sajjad A, Novoyatleva T, Vergarajauregui S, Troidl C, Schermuly RT, Tucker HO and Engel FB: Lysine methyltransferase Smyd2 suppresses p53-dependent cardiomyocyte apoptosis. Biochim Biophys Acta 1843: 2556-2562, 2014.

430. Buckingham M and Rigby PW: Gene regulatory networks and transcriptional mechanisms that control myogenesis. Dev Cell 28: 225-38, 2014.

431. Bentzinger CF, Wang YX and Rudnicki MA: Building muscle: molecular regulation of myogenesis. Cold Spring Harb Perspect Biol 4: 2012.

432. Tajbakhsh S: Skeletal muscle stem cells in developmental versus regenerative myogenesis. J Intern Med 266: 372-89, 2009.

433. Blais A: Myogenesis in the genomics era. J Mol Biol: 2015.

434. Chang NC and Rudnicki MA: Satellite cells: the architects of skeletal muscle. Curr Top Dev Biol 107: 161-81, 2014.

435. Le Grand F and Rudnicki MA: Skeletal muscle satellite cells and adult myogenesis. Curr Opin Cell Biol 19: 628-33, 2007.

436. Yaffe D and Saxel O: Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. Nature 270: 725-7, 1977.

437. Miller JB: Myogenic programs of mouse muscle cell lines: expression of myosin heavy chain isoforms, MyoD1, and myogenin. J Cell Biol 111: 1149-59, 1990.

438. Just S, Meder B, Berger IM, Etard C, Trano N, Patzel E, Hassel D, Marquart S, Dahme T, Vogel B, Fishman MC, Katus Ha, Strähle U and Rottbauer W: The myosin-interacting protein SMYD1 is essential for sarcomere organization. Journal of cell science 124: 3127-36, 2011.

439. Nguyen H, Allali-Hassani A, Antonysamy S, Chang S, Chen LH, Curtis C, Emtage S, Fan L, Gheyi T, Li F, Liu S, Martin JR, Mendel D, Olsen JB, Pelletier L, Shatseva T, Wu S, Zhang FF, Arrowsmith CH, Brown PJ, Campbell RM, Garcia BA, Barsyte-Lovejoy D, Mader M and Vedadi M: LLY-507, a Cell-active, Potent, and Selective Inhibitor of Protein-lysine Methyltransferase SMYD2. J Biol Chem 290: 13641-53, 2015.

440. Xu G, Liu G, Xiong S, Liu H, Chen X and Zheng B: Histone methyltransferase Smyd2 is a negative regulator of macrophage activation by suppressing IL-6 and TNF-alpha production. J Biol Chem: 2015.

441. Chen J, Bardes EE, Aronow BJ and Jegga AG: ToppGene Suite for gene list enrichment analysis and candidate gene prioritization. Nucleic Acids Res 37: W305-11, 2009.

442. Huang da W, Sherman BT and Lempicki RA: Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4: 44-57, 2009.

443. Epstein HF and Fischman DA: Molecular analysis of protein assembly in muscle development. Science 251: 1039-44, 1991.

444. Ohsawa N, Koebis M, Suo S, Nishino I and Ishiura S: Alternative splicing of PDLIM3/ALP, for alpha-actinin-associated LIM protein 3, is aberrant in persons with myotonic dystrophy. Biochem Biophys Res Commun 409: 64-9, 2011.

445. Pomies P, Pashmforoush M, Vegezzi C, Chien KR, Auffray C and Beckerle MC: The cytoskeleton-associated PDZ-LIM protein, ALP, acts on serum response factor activity to regulate muscle differentiation. Mol Biol Cell 18: 1723-33, 2007.

446. Chen HH, Maeda T, Mullett SJ and Stewart AF: Transcription cofactor Vgl-2 is required for skeletal muscle differentiation. Genesis 39: 273-9, 2004.

447. Salanova M, Volpe P and Blottner D: Homer protein family regulation in skeletal muscle and neuromuscular adaptation. IUBMB life 65: 769-76, 2013.

448. Stiber JA, Zhang ZS, Burch J, Eu JP, Zhang S, Truskey GA, Seth M, Yamaguchi N, Meissner G, Shah R, Worley PF, Williams RS and Rosenberg PB: Mice lacking Homer 1 exhibit a skeletal myopathy characterized by abnormal transient receptor potential channel activity. Mol Cell Biol 28: 2637-47, 2008.

449. Lee EJ, De Winter JM, Buck D, Jasper JR, Malik FI, Labeit S, Ottenheijm CA and Granzier H: Fast skeletal muscle troponin activation increases force of mouse fast skeletal muscle and ameliorates weakness due to nebulin-deficiency. PloS one 8: e55861, 2013.

450. Xing B, Wang L, Guo D, Huang J, Espenel C, Kreitzer G, Zhang JJ, Guo L and Huang XY: Atypical protein kinase Clambda is critical for growth factor receptor-induced dorsal ruffle turnover and cell migration. J Biol Chem 288: 32827-36, 2013.

451. Anderson BR, Bogomolovas J, Labeit S and Granzier H: The effects of PKCalpha phosphorylation on the extensibility of titin's PEVK element. J Struct Biol 170: 270-7, 2010.

452. Klaavuniemi T, Kelloniemi A and Ylanne J: The ZASP-like motif in actinin-associated LIM protein is required for interaction with the alpha-actinin rod and for targeting to the muscle Z-line. J Biol Chem 279: 26402-10, 2004.

453. Roder IV, Petersen Y, Choi KR, Witzemann V, Hammer JA, 3rd and Rudolf R: Role of Myosin Va in the plasticity of the vertebrate neuromuscular junction in vivo. PloS one 3: e3871, 2008.

454. Olson EN and Capetanaki YG: Developmental regulation of intermediate filament and actin mRNAs during myogenesis is disrupted by oncogenic ras genes. Oncogene 4: 907-13, 1989.

455. Abe M, Saitoh O, Nakata H, Yoda A and Matsuda R: Expression of neurofilament proteins in proliferating C2C12 mouse skeletal muscle cells. Exp Cell Res 229: 48-59, 1996.

456. Furst DO, Osborn M and Weber K: Myogenesis in the mouse embryo: differential onset of expression of myogenic proteins and the involvement of titin in myofibril assembly. J Cell Biol 109: 517-27, 1989.

457. Du SJ, Rotllant J and Tan X: Muscle-specific expression of the smyd1 gene is controlled by its 5.3-kb promoter and 5'-flanking sequence in zebrafish embryos. Dev Dyn 235: 3306-15, 2006.

458. Li H, Zhong Y, Wang Z, Gao J, Xu J, Chu W, Zhang J, Fang S and Du SJ: Smyd1b is required for skeletal and cardiac muscle function in zebrafish. Mol Biol Cell 24: 3511-21, 2013.

459. Liu Y, Chu A, Chakroun I, Islam U and Blais A: Cooperation between myogenic regulatory factors and SIX family transcription factors is important for myoblast differentiation. Nucleic Acids Res 38: 6857-71, 2010.

460. Blazejczyk M, Miron M and Nadon R: FlexArray: A statistical data analysis software for gene expression microarrays. . In: Genome Quebec, Montreal, 2007.

461. Couture JF, Collazo E, Hauk G and Trievel RC: Structural basis for the methylation site specificity of SET7/9. Nat Struct Mol Biol 13: 140-6, 2006.

462. Ubersax JA and Ferrell JE, Jr.: Mechanisms of specificity in protein phosphorylation. Nat Rev Mol Cell Biol 8: 530-41, 2007.

463. Echeverria PC and Picard D: Molecular chaperones, essential partners of steroid hormone receptors for activity and mobility. Biochim Biophys Acta 1803: 641-9, 2010.

464. Kovacs JJ, Murphy PJ, Gaillard S, Zhao X, Wu JT, Nicchitta CV, Yoshida M, Toft DO, Pratt WB and Yao TP: HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. Mol Cell 18: 601-7, 2005.

465. Wagatsuma A, Shiozuka M, Kotake N, Takayuki K, Yusuke H, Mabuchi K, Matsuda R and Yamada S: Pharmacological inhibition of HSP90 activity negatively modulates myogenic differentiation and cell survival in C2C12 cells. Mol Cell Biochem 358: 265-80, 2011.

466. Yun BG and Matts RL: Hsp90 functions to balance the phosphorylation state of Akt during C2C12 myoblast differentiation. Cell Signal 17: 1477-85, 2005.

467. Mal AK: Histone methyltransferase Suv39h1 represses MyoD-stimulated myogenic differentiation. EMBO J 25: 3323-34, 2006.

468. Tao Y, Neppl RL, Huang ZP, Chen J, Tang RH, Cao R, Zhang Y, Jin SW and Wang DZ: The histone methyltransferase Set7/9 promotes myoblast differentiation and myofibril assembly. J Cell Biol 194: 551-65, 2011.

469. Eom GH, Kim KB, Kim JH, Kim JY, Kim JR, Kee HJ, Kim DW, Choe N, Park HJ, Son HJ, Choi SY, Kook H and Seo SB: Histone methyltransferase SETD3 regulates muscle differentiation. J Biol Chem 286: 34733-42, 2011.

470. Ling BM, Bharathy N, Chung TK, Kok WK, Li S, Tan YH, Rao VK, Gopinadhan S, Sartorelli V, Walsh MJ and Taneja R: Lysine methyltransferase G9a methylates the transcription factor MyoD and regulates skeletal muscle differentiation. Proc Natl Acad Sci U S A 109: 841-6, 2012.

471. Sebastian S, Sreenivas P, Sambasivan R, Cheedipudi S, Kandalla P, Pavlath GK and Dhawan J: MLL5, a trithorax homolog, indirectly regulates H3K4 methylation, represses cyclin A2 expression, and promotes myogenic differentiation. Proc Natl Acad Sci U S A 106: 4719-24, 2009.

472. Tsang LW, Hu N and Underhill DA: Comparative analyses of SUV420H1 isoforms and SUV420H2 reveal differences in their cellular localization and effects on myogenic differentiation. PloS one 5: e14447, 2010.

473. Wang R and Luo M: A journey toward Bioorthogonal Profiling of Protein Methylation inside living cells. Curr Opin Chem Biol 17: 729-37, 2013.

474. Carlson SM, Moore KE, Green EM, Martin GM and Gozani O: Proteome-wide enrichment of proteins modified by lysine methylation. Nat Protoc 9: 37-50, 2014.

475. Cao XJ, Arnaudo AM and Garcia BA: Large-scale global identification of protein lysine methylation in vivo. Epigenetics 8: 477-85, 2013.

APPENDIX 1

This work was published as supplementary information in:

Sylvain Lanouette, Vanessa Mongeon, Daniel Figeys and Jean-François Couture: The functional diversity of protein lysine methylation. Molecular Systems Biology 8: 724, 2014.

This table presents all lysine methylation side identified in the available literature. The protein substrate common name and Uniprot ID are listed, along with the substrate lysine, methylation state (mono, di or tri-methylated; 1/2/3Me), organism in which it was observed, the method of identification*, the biological context and effects observed and the associated publication. The information has been updated since the initial publication.

Evidence code: viv: in vivo, vit: in vitro; AB: specific antibody, AB pan-methyllysine anitbody, MS: mass spectrometry, MS*: high-throughput mass spectometry, ED: Edman degradation, RD, radioactive assay

PROTEIN	UNIPROT ID	LYSINE	STATE	ORGANISM	KMT	KDM	EVIDENCE	EFFECTS	REFERENCES
HISTONES									
Histone H1.0		8	3Me?	Hs	BaSET	?	vit, viv; AB*	Reduces expression of NF-kB genes in macrophages during infection?	Mujtaba (2013) JBC
		12	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
	P07305	82	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
		102	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
		108	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
		155	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
Histone H1.1		22	1Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
	P43275	63	1Me, 2Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
		75	1Me, 2Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
		92	2Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
Histone H1.2		26	1Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
		34	1Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
		46	1Me, 2Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
		52	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
		63	1Me, 2Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
	P16403,	64	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
	P15864	75	1Me, 2Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
		97	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
		106	1Me	Hs, Mm	?	?	viv; MS	?	Lu (2009) J. Proteom. Res. (Mm: Wisniewski (2007) MCP)
		168	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
		187	1Me, 2Me	Hs	G9a, GLP1	?	viv, vit; MS, RD	?	Weiss (2010) Epigenetics & Chromatin
Histone H1.3		17	1Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
		52	1Me	Hs	?	?	viv; MS	?	Wisniewski (2007) MCP
		34	1Me	Hs, Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
		46	1Me, 2Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
	P16402,	63	1Me, 2Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
	P43277	64	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
		75	1Me, 2Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
		97	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
		106	1Me	Hs, Mm	?	?	viv; MS	?	Lu (2009) J. Proteom. Res. (Mm: Wisniewski (2007) MCP)
		169	1Me	Hs	?	?	viv; MS	?	Wisniewski (2007) MCP
Histone H1.4		17	2Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
		26					vit; RD	Important for transcriptionnal repression; requires isoform EED1	Kuzmichev (2004) Mol. Cell
				Hs, Mm E. gracilis	EZH2/PRC3	?		Recruits HP1 through its chromodomain; opposed by S27Pi	Pi: Garcia (2004) J. Proteom. Res. HP1: Daujat
			1Me				viv; MS, AB	(phosphorylation/methylation switch)	(2005) JBC
				Hs	G9a	JMJD2	viv, vit; AB, RD, MS	Promotes H1 deposition; required for retention on chromatin	Trojer (2009) JBC
			2Me	Hs	G9a	JMJD2	viv, vit; AB, RD, MS	Promotes H1 deposition; required for retention on chromatin; recruits Cbx3	Trojer (2009) JBC, Ruan (2012) PLoS One
		52	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
		63	1Me, 2Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
		64	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
	P10412	75	1Me, 2Me	Hs, Mm	?	?	viv; MS	?	Lu (2009) J. Proteom. Res. (Mm: Wisniewski (2007) MCP)
		97	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
		106	1Me	Hs, Mm	?	?	viv; MS	?	Lu (2009) J. Proteom. Res. (Mm: Wisniewski (2007) MCP)
		121	1Me	Hs	SET7/9	?	vit; RD	*In vitro* evidence only; necessitates ADP-ribosylation of H3	Kassner (2013) Epigenet. & Chromat.
		129	1Me	Hs	SET7/9	?	vit; RD	*In vitro* evidence only; necessitates ADP-ribosylation of H3	Kassner (2013) Epigenet. & Chromat.
		148	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
		159	1Me	Hs	SET7/9	?	vit; RD	*In vitro* evidence only; necessitates ADP-ribosylation of H3	Kassner (2013) Epigenet. & Chromat.
		171	1Me	Hs	SET7/9	?	vit; RD	*In vitro* evidence only; necessitates ADP-ribosylation of H3	Kassner (2013) Epigenet. & Chromat.
		177	1Me	Hs	SET7/9	?	vit; RD	*In vitro* evidence only; necessitates ADP-ribosylation of H3	Kassner (2013) Epigenet. & Chromat.
		192	1Me	Hs	SET7/9	?	vit; RD	*In vitro* evidence only; necessitates ADP-ribosylation of H3	Kassner (2013) Epigenet. & Chromat.
Histone H1.5		27	1Me	Hs	?	?	viv; MS	?	Lu (2009) J. Proteom. Res.
		34	1Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
	P16401,	75	1Me	Mm	?	?	viv; MS	?	Wisniewski (2007) MCP
	P43276	168	1Me	Hs	NSD1	?	vit, viv; RD, MS	?	Wisniewski (2007) MCP, Kuthidiputhi (2014) Chem. & Biol.
		169	1Me	Hs	?	?	viv; MS	?	Wisniewski (2007) MCP
Histone H1.11L	P08287	56	2Me	Gg	?	?	viv; MS	?	Snijders (2008) J. Proteome Res.
Histone H1t2	Q75WM6	183	2Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
Histone osH1	Q8IZA3	198	1Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
Histone H2A	P0C0S9	99	2/3Me	Bt	?	?	viv; MS	?	Zhang (2003) Chromosoma
		125/127?	1Me	Bt	?	?	viv; MS	?	Zhang (2003) Chromosoma
H2AX	P16104	134	1/2/3Me	Hs	SUV39H2	?	viv;MS	Necessary for H2AX Pi during DNA damage response	Sone (2014) Nat. Comm.
Histone H2A.Z	P0C0S5	6	1Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
			3Me?	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
		7	1Me	Hs	SETD6	?	viv, vit; AB, RD, MS*	Maintenance of ESC renewal	Binda (2013) Epigenetics, Jufvas (2011) PloS One

		8	1Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
		11	1Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
			3Me?	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
		13	1Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
			2Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
		15	1Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
			2Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
MacroH2A1.2		17	1Me	Hs	?	?	viv; MS	?	Chu (2006) MCP
	O75367	122	1Me	Hs	?	?	viv; MS	?	Chu (2006) MCP
		238	2Me	Hs	?	?	viv; MS	?	Chu (2006) MCP
Histone H2B									Medzihradszky (2004) MCP, Xiong (2010) Biochemistry
		3	1/2/3Me	Nc, Tt	?	?	viv; MS	?	
		4	1/2/3Me	Tt	?	?	viv; MS	?	Medzihradszky (2004) MCP
		5	1Me	Sc	?	?	viv; AB	Associated with active promoters downstream of transcription start sites	Barski (2007) Cell
		8	1Me	Nc	?	?	viv; MS	?	Anderson (2010) Biochemistry
		23	2Me	Bt	?	?	viv; MS	?	Zhang (2003) Chromosoma
		37	2Me	Sc (Hs,Gg)	?	?	viv; AB	?	Gardner (2011) PloS One
	P62807	43	1Me	Bt	?	?	viv; MS	?	Zhang (2003) Chromosoma
		54	2Me	Nc	?	?	viv; MS	?	Anderson (2010) Biochemistry
		57	2/3Me	Nc	?	?	viv; MS	?	Anderson (2010) Biochemistry
		90	2Me	Nc	?	?	viv; MS	?	Anderson (2010) Biochemistry
		97	2/3Me	Nc	?	?	viv; MS	?	Anderson (2010) Biochemistry
		111	2/3Me	Tt	?	?	viv; MS	?	Medzihradszky (2004) MCP
		119	1/2Me	Nc	?	?	viv; MS	?	Anderson (2010) Biochemistry
		127	1/2Me	Nc	?	?	viv; MS	?	Anderson (2010) Biochemistry
		137	1Me	Nc	?	?	viv; MS	?	Anderson (2010) Biochemistry
Histone H2B.d	P58876	125	3Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
		126	3Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
Histone H2B.e		11	1Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
	Q16778	12	1Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
		16	2Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
		29	1Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
Histone tsH2B	Q96A08	86	3Me?	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
		93	1Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
Histone H3		4	1Me						
			2Me				*See Ruthenburg (2007) Mol. Cell, Shilatifard (2008) Curr. Opin. Cell Biol., Eissenberg (2010) Dev. Biol., Malik (2010) FEBS J.*		
			3Me						
		9	1Me						
			2Me				*See Stancheva (2005) Biochem. Cell Biol., Shinkai (2007) Chromatin & Disease, Krishnan (2011) ChemBioChem*		
			3Me						
		18	1Me	Hs	?	?	viv; MS	?	Hake (2005) JBC
		23	1Me	Bt	?	?	viv, vit; MS, AB	Binds HP1β *in vivo* to dense heterochromatin	Zhang (2003) Chromosoma (HP1: Liu (2010) J. Proteome Res.)
			2Me	Hs, Mm	?	?	viv, vit; MS, AB	?	Liu (2010) J. Proteome Res.
			3Me	Gg	?	?	viv; MS*	Observed in H3.1 of avian eye	Thomas (2005) J. Proteome Res.
		27	1Me						
			2Me				*See Cao (2004) Curr. Opin. Genet. Dev. Jacob (2011) Epigenetics, Margueron (2011) Nature, Zheng (2011) Curr. Opin. Plant Biol., O'Meara (2012) Chromosoma, Yoo (2012) Int. J. Biol. Sci.*		
			3Me						
		36	1Me						
			2Me				*See Wagner (2012) Nature Rev. Mol. Cell Biol.*		
			3Me						
		42	1Me	Sc	?	?	viv, vit; MS, AB	Unique to Sc since not a K in other eukaryotes	Hyland (2011) Genes Dev.
							viv, vit; MS, AB	May prevent erroneous transcription	Hyland (2011) Genes Dev.
							viv, vit; MS, AB	Unique to Sc since not a K in other eukaryotes	Hyland (2011) Genes Dev.
							viv, vit; MS, AB	Enriched at 5' end of genes	Hyland (2011) Genes Dev.
			2Me	Sc	?	?	viv, vit; MS, AB	Higher levels dependant on Ctr9, Hpr1 & Rtf1 components of PAF1 cplx	Hyland (2011) Genes Dev.
							viv, vit; MS, AB	Deletion of PAF1 complex components Ccr4, Paf1 & Spt13 causes elevated levels	Hyland (2011) Genes Dev.
							viv, vit; MS, AB	May prevent erroneous transcription	Hyland (2011) Genes Dev.
		56	1Me	Hs	G9a	?	viv, vit; MS, AB, RD	:ruits PCNA in G1 at enriched spots different of transcribed regions or heterochroma	Yu (2012) Mol. Cell
		64	1Me	Hs	?	?	viv; MS	Enriched in H3.1 relatively to H3.2 and H3.3	Hake (2005) JBC
			3Me	Mm	?	?	viv; AB*	Enriched at pericentric heterochromatin, appears during oocyte growth until mitosis	Daujat (2009) NSMB
		79	1Me						
			2Me				*See Nguyen (2011) Gen. Dev.*		
			3Me						
		122	1Me	Hs	?	?	viv; MS	?	Hake (2005) JBC
Histone H4		5	2Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
				Sc	SET5	?	viv, vit; AB, RD		Green (2012) NSMB

		7	1Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
		8		Sc	SET5	?	viv, vit; AB, RD		Green (2012) NSMB
		12	1Me	Bt	?	?	viv; MS	?	Zhang (2003) Chromosoma
				Sc	SET5	?	viv, vit; AB, RD		
		15	2Me	Hs	?	?	viv; MS*	?	Jufvas (2011) PloS One
		20	1Me 2Me 3Me				*See Balakrishnan (2010) Crit. Rev. Biochem. Mol. Biol., Jorgensen (2013) Nucl. Ac. Res.*		
		31	2Me	Bt	?	?	viv; MS	?	Zhang (2003) Chromosoma
		44	?	?	NSD1, NSD2	?	vit; RD	*In vitro* evidence only and on octamer (NSD2 specificity changes for H3K36 in nucleosomes)	Li (2009) JBC, NSD1 : Kuthidiputhi (2014) Chem. & Biol.
		59	2Me	Bt/Sc?	?	?	viv; MS	Silent chromatin formation in yeast?	Zhang (2003) Chromosoma
Histone H5	P02259	12	1Me	Gg	?	?	viv; MS	?	Wisniewski (2007) MCP, Snijders (2008) J. Proteome Res.
TRANSCRIPTION									
p53		24	2Me	Hs	?	?	viv; MS	?	DeHart (2014) MCP
		101	1/2/3Me	Hs	?	?	viv; MS	?	DeHart (2014) MCP
		120	1/2/3Me	Hs	?	?	viv; MS	?	DeHart (2014) MCP
		132	1/2/3Me	Hs	?	?	viv; MS	?	DeHart (2014) MCP
		139	1/2/3Me	Hs	?	?	viv; MS	?	DeHart (2014) MCP
		164	2/3Me	Hs	?	?	viv; MS	?	DeHart (2014) MCP
		291	1/2/3Me	Hs	?	?	viv; MS	?	DeHart (2014) MCP
		292	1/2/3Me	Hs	?	?	viv; MS	?	DeHart (2014) MCP
		305	1/2/3Me	Hs	?	?	viv; MS	?	DeHart (2014) MCP
		321	1/2/3Me	Hs	?	?	viv; MS	?	DeHart (2014) MCP
		351	1/2/3Me	Hs	?	?	viv; MS	?	DeHart (2014) MCP
		357	1/2/3Me	Hs	?	?	viv; MS	?	DeHart (2014) MCP
			1Me	Hs	SMYD2	LSD1		Represses p53 activity; prevents methylation of K372	Huang (2007) Nature
		370	2Me	Hs	?	LSD1	viv, vit; AB, RD, MS	Prevents 53BP1 binding (represses p53 activity)	Huang (2006) Nature (LSD1 : Huang (2007) Nature)
	P04637							Recruits PHF20 with K382Me2 and inhibits p53Ub after DNA damage	Cui (2012) NSMB
								Stabilizes p53	Chuikov (2004) Nature (LSD1 : Huang (2007) Nature)
		372	1Me	Hs	SET7/9	LSD1	viv, vit; AB, RD	Inhibits methylation at K370 by SMYD2	Huang (2007) Nature
								Promotes acetylation of p53 (K373, K382)	Ivanov (2007) Mol Cell. Bio.
								Inhibited by HPV E6; protects p53 from E6-mediated degradation	Hsu (2011) Oncogene
								In contrast, deletion of SET7/9 in mice does not impairs p53 function, anti-oncogenic activity, transcriptional activity or its acetylation	Campaner (2011) Mol. Cell, Lehnertz (2011) Mol. Cell
								Inhibits apoptotic activity	Huang (2010) JBC
		373	2Me	Hs	G9a (Glp)	?	viv, vit; AB, RD	Stimulated by recruitment of MDM2; correlates with H3K9Me3 at p21 promoter	Chen (2010) EMBO J.
		381	1/2/3Me	Hs	?	?	viv; MS	?	DeHart (2014) MCP
		382	1Me	Hs	SET8	?	viv, vit; AB, MS	Suppresses transcription activation of highly responsive target genes	Shi (2007) Mol. Cell
								Recruits L3MBTL1 through MBT repeats	West (2010) JBC
		382	2Me	Hs	?	?	viv; AB, MS	Corelates with DNA damage, facilitate 53BP association	Kachirskaia (2008) JBC, DeHart (2014) MCP
								Recruits PHF20 with K382Me2 and inhibits p53Ub after DNA damage	Cui (2012) NSMB
		386	1/2Me	Hs	?	?	viv; MS	?	Kachirskaia (2008) JBC, DeHart (2014) MCP
53BP1		135	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
	Q12888	1175	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		1626	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
pRb		810	1Me	Hs	SET7/9	?	viv, vit; RD, MS	Elicited by DNA damage; cell cycle arrest, impairs Cdk binding and Pi of Rb; recruits 53BP1 Tudor domain to pRb in the context of E2 promoter genes during DNA damage response	Carr (2010) EMBO J, 53BP1: Carr (2014) PNAS
	P06400	810	1Me	Hs	SMYD2	?	viv, wit; AB, RD, MS	Enhances Pi of S807/S811; promotes cell cycle progression through E2F activity	Cho (2012) Neoplasia
		860	1Me	Hs	SMYD2	?	viv; AB, RD, MS	L3MBTL1 binding (repressor of target genes)	Saddic (2010) JBC
		873	1Me	Hs	SET7/9	?	viv; AB, RD	Required for cell cycle arrest and transcriptional repression; recruits HP1 to pRb	Munro (2010) Oncogene
E2F1								Decreases stability (increases ubiquitination) and impairs PCAF-Ac and CHK2/ATM-Pi (activating modifications)	Kontaki (2010) Mol. Cell
	Q01094	185	1Me	Hs	SET7/9	LSD1	viv, vit; RD	Prevents NEDDylation of E2F1, protecting its activity	Loftus (2012) EMBO rep.
								Inhibited by TMCG/DIPY which reduces RASSF1A expression	Montenegro (2012) PLoS One
								Levels correlate with DNA damage; increases DNA binding	Xie (2011) J. Recept. Signal Transduct. Res.
NF-kB (p65, RelA)	Q04206	37	1Me	Hs	SET7/9	?	viv, vit; AB, RD, MS	Regulates p65 promoter binding, necessary for certain target genes	Ea (2009) PNAS
		218/221	1Me/2Me	Hs	NSD1	FBXL11	viv; AB, MS	Activation of NF-kB; K221 recruits PHF20 which prevents PP2A recruitment and protects Pi and Ac of p65; Increases interaction with DNA	Lu (2010) PNAS, PHF20: Zhang (2013) Nature Comm.; Lu (2013) PNAS
		310	1Me	Hs	SETD6	?	viv, vit; AB, RD, MS	Recognized by ankyrin repeat domain of GLP which is recruited to RelA target genes and upregulates H3K9Me2 levels and downregulates their expression (GLP association inhibited in turn by S311Pi by PKC-ζ, which then allows expression of target genes)	Levy (2010) Nat. Immunol., Chang (2011) Nucl. Ac. Res.

		314/315	1Me	Hs	SET7/9	?	viv, vit; AB*, RD, MS	Stimulated by TNF-a; induces degradation of promoter-associated RelA (proteasome); stimulated by berberine and leads to ROS production	Yang (2009) EMBO J. (Berberine: Hu (2012) Acta Pharm. Sin.)
							viv, vit; AB, RD	Inhibited by K310Ac (which is opposed by SIRT1)	Yang (2010) Mol. Cell. Biol.
TAF6	P49848	537	1Me	Hs	?	?	viv; MS	?	Bremang (2013) Mol. BioSyst.
TAF7	Q15545	5	1Me	Hs	SET7/9	?	vit; RD	*In vitro* evidence only	Couture (2006) NSMB
TAF10	Q12962	189	1Me	Hs	SET7/9	?	viv, vit; AB*, RD	Potentiates transcription of certain TAF10-dependent genes	Kouskouti (2004) Mol. Cell
TAF15	Q86X94	192	1/3Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
		210	1/2Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
		576	1/3Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
Yap	P46938	494	1Me	Mm	SET7/9	?	viv; MS	Promotes cytoplasmic retention, allows Hippo pathway regulation	Oudhoff (2013) Dev. Cell
Sp1	P08047	?	?	Hs	?	LSD1?	viv; AB*	Levels upregulated by H2O2; lowers expression of Sp1 target genes; recruits Suv39H1 & HDAC1 to Sp1 and repress CyclinB1 expression	Chuang (2011) Free Rad. Biol. Med.
STAT3	P40763	140	2Me	Hs	SET7/9	LSD1	viv; MS, AB	Y705Pi dependent; activates or inhibits activation of certain target genes subsets	Yang (2010) PNAS
		180	?	Hs	EZH2	?	viv; MS, AB*	Stimulates EZH2 S21Pi by AKT signaling ; Increases STAT3 Y705Pi and its activation and signaling and tumor growth and glioblastoma	Kim (2013) Cancer Cell
FOXO3	O43524	270	1Me	Hs	SET7/9	?	viv, vit; MS, RD, AB	Inhibits FOXO3 transactivation and stress-induced neuronal apoptosis by lowering DNA binding	Xie (2012) EMBO rep.
		271	1Me	Hs	SET7/9	?	viv, vit; MS, RD	Lowers FOXO3 stability and activates transcriptional activity	Calnan (2012) Aging
MYPT1	O14974	442	1Me	Hs	SET7/9	LSD1	viv, vit; MS, RD	Cell cyle progression, protects E2F activity, RB1 PiS and MYPT1 levels	Cho (2010) Cancer Res.
MEF2D	Q63943	267	1/2Me	Mm	G9a	LSD1	viv; MS, AB	Disappeear during muscle cells differentiation; present on MEF2D at promoter sites; lowers association to chromatin and transcriptional activity on differentiation markers	Choi (2013) Nucl. Ac. Res.
GATA4	P43694, Q08369	299	1Me	Hs, Mm	EZH2 (PRC2)	?	viv, vit; MS, AB*, RD	Requires SUZ12 and EED, occurs in fetal hearts, prevents GATA4 C-terminal AcK by p300; limits GATA4 mediated recruitment of p300 to chromatin which represses these target genes expression	He (2012) Genes Dev.
MTA1	Q13330	532	2Me	Hs	G9a	LSD1	viv, vit; MS, AB*, RD	Necessary for NuRD complex assembly (through CHD4 binding), ATP binding and chromatin remodelling, inhibits binding of MTA1 to H3K9Me2 and H3K9Ac; demethylation by LSD1 necessary for association to the NURF complex and coactivator role	Nair (2013) Mol. Cell
Reptin (Ruvbl2)	Q9Y230	67	1Me	Hs	G9a	?	viv; MS, AB*	Negatively regulates hypoxia-responsive genes	Lee (2010) Mol. Cell
		412	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
C/EBPβ		20	2Me	Gg	?	?	viv; AB*, RD	?	Leutz (2011) Transcription
		39	?	Mm, Rn	G9a	?	viv, vit; RD	Inhibition of transactivation potential	Pless (2008) JBC
		99	1Me	Gg	?	?	viv; AB*, RD	?	Leutz (2011) Transcription
		102	1Me	Rn	?	?	viv; AB*, RD	?	Leutz (2011) Transcription
		134	1Me	Rn	?	?	viv; AB*, RD	?	Leutz (2011) Transcription
	Q05826, P28033, P21272	145	1Me	Rn	?	?	viv; AB*, RD	?	Leutz (2011) Transcription
		212	1Me	Rn	?	?	viv; AB*, RD	?	Leutz (2011) Transcription
		276	2Me/3Me	Gg	?	?	viv; AB*, RD	?	Leutz (2011) Transcription
		285	3Me	Gg	?	?	viv; AB*, RD	?	Leutz (2011) Transcription
		267	1Me/3Me	Rn, Gg	?	?	viv; AB*, RD	?	Leutz (2011) Transcription
		299	2Me	Gg	?	?	viv; AB*, RD	?	Leutz (2011) Transcription
		315	1Me/2Me	Rn, Gg	?	?	viv; AB*, RD	?	Leutz (2011) Transcription
ARID5B	Q14865	336	2Me	Hs	?	PHF2	viv; MS, AB	Demethylation of ARID5B necessary for binding to target promoters	Baba (2011) Nat. Cell. Biol.
MyoD	P15172	104	1Me/2Me	Hs	G9a	?	viv; AB*, MS	Lowers troponin levels; Inhibits muscle cell differentiation; increased by and recruits Sharp1 to MyoD, inhibiting myogenesis	Ling (2012) PNAS; Sharp1: Ling (2012) Mol. Biol. Cell
p63	Q9H3D4	?	?	Hs	?	JMJD3	viv; AB*	In TA domain; demethylation during neural differentiation stabilizes p63 and allows translocation to the nucleus	Fonseca (2012) PLoS One
NHP2L1	P55769	33	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
RBM25	P49756	97	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
GTF2i	P78347	219	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
DDX1	Q92499	234	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
DDX5	P17844	340	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
DHX15	O43143	18	2/3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
DDX46	Q7L014	113	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
		389	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
HNRPM	Q6P2D7	651	2/3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain; 2Me upregulated and 3Me downregulated in DNA damage response	Liu (2013) Mol. Cell
MAGOH	P61326	14	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
SAP155	O75533	290	2/3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain; upregulated in DNA damage response	Liu (2013) Mol. Cell
CBF-A	P67809	92	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
RHA	Q08211	120	2Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
SRp55	Q13247	242	3mE	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
SRm160	Q8IYB3	425	2Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
SRm300	Q9UQ35	239	2Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
Trap150	Q9Y2W1	756	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
NUMA1	Q3SYK8	1803	2/3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
G3BP1	Q13283	271	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
HCFC1	P51610	511	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
		558	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.

ILF3	Q12906	613	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
PABPC4	Q13310	524	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
PRPF8		1425	3Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
	Q6P2Q9	1516	3Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
		1517	3Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
RBM22	Q9NW64	185	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
RBM27	Q9P2N5	945	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
RBM39	Q14498	111	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
SMD3	P62318	99	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
SRP14	P37108	107	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
SRRM2	Q9UQ35	1588	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
U2AF1	Q8WU68	39	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
U2AF2	P26368	195	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
ZNF326	Q5BKZ1	238	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
		364	2Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
GTF2i	Q9UHL9	219	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
WIZ	O95785	305	3Me	Hs	G9a	?	vit; RD	Recruits HP1 to WIZ *in vitro*	Rathert (2008) Nat. Chem. Biol.
CyAbrB	Q1HGM1	17/19?	1Me	A. ovalisporum	?	?	viv; MS	?	Shalev-Malul (2007) Envir. Microbiol.
TFIID5	D3ZH66	285	1Me	Rn	?	?	viv; MS*	Affected in Experimental Autoimmune Encephalomyelitis?	Grant (2007) J Proteome Res.
Interferon regulatory factor 1	P10914	126/134	1Me/2Me?	Hs	SET7/9	?	viv, vit; RD, MS	?	Dhayalan (2011) Chem. & Biol.
Msx2-interacting protein	Q96T58	2076	2Me	Hs	SET7/9	?	vit; RD	?	Dhayalan (2011) Chem. & Biol.
Sin3b	Q62141	386	1Me	Mm	?	?	viv; MS	?	Le Guezennec (2006) Nucl. Acids Res.
AML1	Q01196	?	?	Hs	?	?	viv; AB*	Suggested by a band in a pan-MeK immunoblot	Chakraborty (2003) Oncogene
Pdx1	P52945	?	?	Hs	SET7/9	?	vit; RD	No obvious effect; interaction with Pdx recruits SET7/9 to H3K4 of target genes independantly	Francis (2005) JBC
Integration host factor β	Q726Y3	8	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
ERα	P03372	302	1Me	Hs	SET7/9	?	viv, vit; MS, RD, AB	Recruitment of ER to target genes and transactivation	Subramanian (2008) Mol. Cell
		472	3Me	Hs	?	?	viv; MS	Could be AcK	Atsriku (2009) MCP
AR	P10275	630	1Me	Hs	SET7/9	?	viv, vit; RD, AB*	Enhances AR transactivation through interdomain (N-C) interaction	Ko (2011) Mol. Endocr.
		632	1Me	Hs	SET7/9	?	viv, vit; RD, AB*	Enhances transcriptional activity and recruitment to target genes; site disputed	Gaughan (2010) Nucl. Acid Res. (disputed: Ko (2011) Mol. Endocr.)
PR	P06401	464	1Me	Hs	?	?	viv; MS	Might enhances activity in response to ligand activation; might affect the recruitment of co-activators SMRT, NCor1, SRC-1 ; established by A, Q and F mutants (doubtful method)	Chung (2014) JBC
RAR		109	1Me	Hs	?	?	viv; MS, RD, AB*	Positively modulates RA-dependant activation	Huq (2008) J. Proteome Res.
	P10276	171	1Me	Hs	?	?	viv; MS, RD, AB*	No significant effect observed	Huq (2008) J. Proteome Res.
		347	3Me	Hs	?	?	viv; MS, AB*	Enhances interactions with p300/CBP, RIP140, RXR (based on Phe mutants)	Huq (2007) MCP
RORα	P51448	38	1Me	Hs	EZH2	?	viv; MS, AB*	Recruits DCAF1/DDB1/CUL4, which induces Ub and proteasome degradation; represses transcription of target genes; might favor cancer cells proliferation	Lee (2012) Mol. Cell
FXR	P51114	206	?Me	Hs	SET7/9	?	viv, vit; MS, AB*	Synergistically promotes ligand-induced transactivation of target genes	Balasubramaniyan (2012) Am. J. Gastrointest. Liver Physiol.
		449	2Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
ATRX	P46100	1033	?	Hs	NSD1	?	vit; RD	?	Kuthidiputhi (2014) Chem. & Biol.
GluR-δ2	Q63226	38	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
		39	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
PPARδ	Q62879	152	1Me	Rn	?	?	viv; MS*	Affected in Experimental Autoimmune Encephalomyelitis?)	Grant (2007) J Proteome Res.
RIP140	P48552	591	1Me	Hs	?	?	viv; MS, AB*	Enhances gene repressive activity, inhibits AcK, necessary for interaction with HDAC3	Huq (2009) J. Proteome Res.
		653	1Me	Hs	?	?	viv; MS, AB*		Huq (2009) J. Proteome Res.
		757	1Me	Hs	?	?	viv; MS, AB*		Huq (2009) J. Proteome Res.
Gfi-1B	Q5VTD9	8	2Me	Hs	SET7/9?	LSD1	*indirect*	Promotes recruitment of LSD1 to Gfi-1B target genes promoters; promotes erythroid development	Laurent (2013) J. Cell Science
UBF1	P17480	232/254	3Me	Hs	ESET	?	viv; MS, AB*	Allows associaton of UBF to chromatin; in turn increases height and distance of nucleosomal particles; indirectly increases rDNA transcriptional activity	Hwang (2014) Nucl. Ac. Res.
SCML2	Q9UQR0	123	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
CDC5L	Q99459	165	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
NFIB	O00712	400	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
RFX5	P48382	321	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
SART3	Q15020	872	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
MKI67		263	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain; upregulated in DNA damage response	Liu (2013) Mol. Cell
	P46013	620	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
		1616	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
		2652	2Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell

MKI67IP	Q9BYG3	179	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain; upregulated in DNA damage response	Liu (2013) Mol. Cell
GON4L	Q3T8J9	1126	2Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
CHROMATIN/CHROMOSOMAL REGULATION									
Dam1								Tunes levels of Pi for S232, S234, S235 by Ipl1; important for proper chromosome segregation	Zhang (2005) Cell
	P53267	233	2Me	Sc	SET1	?	viv; AB	Occurs at kinetochore; Necessitates Paf1 independantly of transcriptional elongation	Latham (2011) Cell
								Requires H2BK123Ub; Requires Rad6 & Bre1;Ubp8 dowregulates levels	Latham (2011) Cell
Stellate	Q7KV12	92	3Me	Dm	?	?	viv; MS, AB	?	Egorova (2009) JMB
EED		66	2Me	Hs	G9a	?	vit; MS	?	Xu (2010) PNAS
	O75530	197	2Me	Hs	G9a	?	vit; MS	?	Xu (2010) PNAS
		268	2Me	Hs	G9a	?	vit; MS	?	Xu (2010) PNAS
		284	2Me	Hs	G9a	?	vit; MS	?	Xu (2010) PNAS
Centromere protein C	Q03188	414	1Me	Hs	SET7/9	?	viv, vit; RD, MS	?	Dhayalan (2011) Chem. & Biol.
HP1α		32	1Me	Hs	?	?	viv, MS	?	LeRoy (2009) MCP
	P45973	40	1Me	Hs	?	?	viv, MS	?	LeRoy (2009) MCP
		111	1Me	Hs	?	?	viv, MS	?	LeRoy (2009) MCP
		202	1Me	Hs	?	?	viv, MS	?	LeRoy (2009) MCP
HP1β	P83916	9	1Me	Hs	?	?	viv, MS	?	LeRoy (2009) MCP
		35	1Me	Hs	?	?	viv, MS	?	LeRoy (2009) MCP
HP1γ		21	1Me	Hs	?	?	viv, MS	?	LeRoy (2009) MCP
	Q13185	44	1Me	Hs	?	?	viv, MS	?	LeRoy (2009) MCP
		143	1Me	Hs	?	?	viv, MS	?	LeRoy (2009) MCP
SMC3		245	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		409	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		427	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
	Q9UQE7	429	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		445	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		486	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		644	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		997	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
MeCP2		119	2Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
	P51608	220	2Me	Hs	?	?	viv; MS*	viv; MS*	Jung (2008) Anal. Chem.
		347	1Me	Hs	SET7/9	?	viv, vit; RD, MS	?	Dhayalan (2011) Chem. & Biol.
HMGA1		6	1Me	Hs	?	?	viv; MS	?	Young (2010) J. Am. Soc. Mass. Spectrom.
		13	1Me	Hs	?	?	viv; MS	?	Edberg (2005) JBC
		22	1Me	Hs	?	?	viv; MS	?	Young (2010) J. Am. Soc. Mass. Spectrom.
		30	1Me	Hs	?	?	viv; MS	?	Zou (2007) J Proteome Res.
		45	2Me	Hs	?	?	viv; MS	Presence in tumor cells correlates with high metastatic potential	Edberg (2004) Biochem.
	P17096	54	1Me	Hs	?	?	viv; MS	?	Zou (2007) J Proteome Res.
		61	1Me	Hs	?	?	viv; MS	?	Young (2010) J. Am. Soc. Mass. Spectrom.
		64	1Me	Hs	?	?	viv; MS	?	Young (2010) J. Am. Soc. Mass. Spectrom.
		66	2Me	Hs	?	?	viv; MS	Specific to metastatic cells?	Edberg (2005) JBC
		70	1Me	Hs	?	?	viv; MS	?	Young (2010) J. Am. Soc. Mass. Spectrom.
		73	1Me	Hs	?	?	viv; MS	?	Young (2010) J. Am. Soc. Mass. Spectrom.
		80	1Me	Hs	?	?	viv; MS	Only in β isoform?	Edberg (2005) JBC
HMGB1	P09429, Q58EV5	42	1Me	Hs, Mm	?	?	viv; MS	Alters conformation; lower affinity for DNA; induces cytoplasmic localization	Ito (2007) JBC
		112	1Me	Hs	?	?	viv; MS	Possibly affects subcellular localization in clear cell renal carninoma?	Wu (2013) Asian Pacif. J. Canc. Prevent.
SSRP1	Q08945	143	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
NUCKS		9	?	Hs	?	?	viv; MS	?	Wisniewski (2008) Proteins
	Q9H1E3	64	?	Hs	?	?	viv; MS	?	Wisniewski (2008) Proteins
		175	?	Hs	?	?	viv; MS	?	Wisniewski (2008) Proteins
		218	?	Hs	?	?	viv; MS	?	Wisniewski (2008) Proteins
Cohesin subunit SMC1	Q14683	149	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		1133	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
Cohesin subunit SA-1	Q8WVM7	333	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		618	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
ARPP21	Q5FVI0	398	2Me	Rn	?	?	viv; MS*	Affected in Experimental Autoimmune Encephalomyelitis?	Grant (2007) J Proteome Res.
SNF2	P22082	1028	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
ISWI1	P43596	14	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
MED15A	F4I171	85	1Me	At	?	?	viv; MS*	?	Alban (2014) PLoS One
TFIIS	O49324	79	3Me	At	?	?	viv; MS*	?	Alban (2014) PLoS One
MC1-α	Q8PY15	37	?	M. mazei	Gö1-SET	?	vit; RD	?	Manzur (2005) FEBS letters
CC1	G4RKF6	12	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		21	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
Sac7d	P13123	4	1Me	S. acidocaldarius	?	?	viv; ED	? - No role in DNA binding)	McAfee (1995) Biochemistry (no role: McAfee (1996) Biochemistry
		6	1Me	S. acidocaldarius	?	?	viv; ED	? - No role in DNA binding)	McAfee (1995) Biochemistry (no role: McAfee (1996) Biochemistry
Sac7e	P13125	6	1Me	S. acidocaldarius	?	?	viv; ED	?	McAfee (1995) Biochemistry

		62	1Me	S. acidocaldarius	?	?	viv; ED	?	McAfee (1995) Biochemistry
		63	1Me	S. acidocaldarius	?	?	viv; ED	?	McAfee (1995) Biochemistry
Sso7c		4	1Me	S. solfataricus	?	?	viv; ED	?	Oppermann (1998) FEBS Lett.
		6	1Me	S. solfataricus	?	?	viv; ED	?	Oppermann (1998) FEBS Lett.
	P81552	60	1Me	S. solfataricus	?	?	viv; ED	?	Oppermann (1998) FEBS Lett.
		62	1Me	S. solfataricus	?	?	viv; ED	?	Oppermann (1998) FEBS Lett.
		63	1Me	S. solfataricus	?	?	viv; ED	?	Oppermann (1998) FEBS Lett.
Sso7d		4	1Me	S. solfataricus	aKMT?	?	viv, vit; ED, RD, MS	*Not* involved in thermostability, but correlated with temperature increase	Choli (1988) BBA, Snijders (2009) J. Am. Soc. Mass. Spectrom. (non-role: Knapp (1996) JMB); aKMT: Chu (2012) J. Bact.
		6	1Me	S. solfataricus	aKMT?	?	viv, vit; ED, RD, MS	*Not* involved in thermostability, but correlated with temperature increase	Choli (1988) BBA, Snijders (2009) J. Am. Soc. Mass. Spectrom. (non-role: Knapp (1996) JMB)
		52	1Me	S. solfataricus	aKMT?	?	viv; MS	*?*	Snijders (2009) J. Am. Soc. Mass. Spectrom. aKMT: Chu (2012) J. Bact.
	P39476	60	1Me	S. solfataricus	aKMT?	?	viv, vit; ED, RD, MS	*Not* involved in thermostability, but correlated with temperature increase	Choli (1988) BBA, Snijders (2009) J. Am. Soc. Mass. Spectrom. (non-role: Knapp (1996) JMB, increase with temp: Baumann (1994) NSB) aKMT: Chu (2012) J. Bact.
		62	1Me	S. solfataricus	aKMT?	?	viv, vit; ED, RD, MS	*Not* involved in thermostability, but correlated with temperature increase	Choli (1988) BBA, Snijders (2009) J. Am. Soc. Mass. Spectrom. (non-role: Knapp (1996) JMB, increase with temp: Baumann (1994) NSB) aKMT: Chu (2012) J. Bact.
		63	1Me	S. solfataricus	aKMT?	?	viv, vit; ED, RD, MS	*Not* involved in thermostability, but correlated with temperature increase	Choli (1988) BBA (non-role: Knapp (1996) JMB, increase with temp: Baumann (1994) NSB) aKMT: Chu (2012) J. Bact.
Ssh7	K1664	4	1Me	S. shibatae	?	?	viv; ED	?	Mai (1998) J. Bacteriol.
		6	1Me	S. shibatae	?	?	viv; ED	?	Mai (1998) J. Bacteriol.
Cren7		16	1Me/2Me	S. solfataricus S. islandicus	aKMT	?	viv, vit; RD, MS	?	Guo (2007) Nucl. Ac. Res.; aKMT: Chu (2012) J. Bact.
	Q97ZE3,C3N5A6	34	1Me	S. solfataricus S. islandicus	aKMT	?	viv, vit; RD, MS	?	Guo (2007) Nucl. Ac. Res.; aKMT: Chu (2012) J. Bact.
		31, 37, 42?	?Me	S. solfataricus	?	?	viv; MS	?	Guo (2007) Nucl. Ac. Res.
Hc1-1	Q9Z720	? (29?)	?Me	C. pneumoniae	cpnSET	?	vit; RD	*In vitro* evidence only	Murata (2007) Microbiology
DNA REPLICATION, SYNTHESIS & REPAIR									
PCNA	P12004	248	1Me	Hs	SET8	?	viv; RD, AB	Inhibits Ub and stabilizes PCNA, enhances interaction with FEN1, promotes maturation of Okazaki fragments	Takawa (2012) Cancer Res.
PARP1	P09874	508	1Me	Hs	SET7/9	?	viv, vit; RD, AB	Inihibited by auto-ADP-ribosylation; enhances catalytic activity in the context of DNA damage	Kassner (2013) Open Biol.
		528	1Me	Hs	SMYD2	?	viv, vit; AB, ED, MS	Increases DNA damage response	Piao (2014) Neoplasia
KIN17	O60870	135	3Me	Hs	METTL22	?	viv; MS*	Reduces association to chromatin, increases Kin17 concentration in the cytoplasm	Cloutier (2013) PloS Genet., Bremang (2013) Mol. BioSyst., Cloutier (2013) J. Proteomics
PCNA1/2	C3N5N1	?	?	S. islandicus	aKMT	?	vit; RD	?	Chu (2012) J. Bact.
TopoIII		?	?	S. islandicus	aKMT	?	vit; RD	?	Chu (2012) J. Bact.
BRCA1	P38398	?	?	Hs	?	?	viv; AB*	?	Guendel (2010) PLoS One
LIG1	P18858	795	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
MCM4	P33991	216	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
MCM7	P33993	449	1Me/2Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
MSH6	P52701	1126	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
TD-60	Q9P258	293	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
SAFB1/2	Q15424	226 (225)	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
YLPM1	P49750	643	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
DNA polymerase αB	P38121	84	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
DNA gyrase B	Q8FA30	98	1/2/3Me?	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
		101	1Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
CTP synthase 1	P28274	28	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
recA	G4RKE5	12	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		81	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		91	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		182	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
	A1VEQ5	330	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
recD/traA	A1VF89	716	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
MutL	A1VGA8	278	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
	Q8F6X4	546	3Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
DNApolI	A1VG95	420/423	1/2Me?	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
A/G-adenine glycosylase	A1VGZ3	180	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
OPRTase	A1VAI2	15	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
Phosphoribosylamine-glycine ligase	A1VGA3	221	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
ISlin1	Q8CM74	160	2Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
Ku70	P12956	92	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		114	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		166	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.

Ku80	P13010	7	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain; downregulated in DNA damage response	Liu (2013) Mol. Cell
		598	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
HerA helicase	G4RJV2	169	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
DNA helicase TTX_0530	G4RNQ1	281	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		685	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
Rfc small subunit	C3N5N2, G4RLM4	181	?	S. islandicus, T. tenax	aKMT	?	viv, vit; MS, RD	?	Botting (2010) Archae; aKMT: Chu (2012) J. Bact.
Pri1	C3N549	?	?	S. islandicus	aKMT	?	vit; RD	?	Chu (2012) J. Bact.
Pri2	C3MQM0	?	?	S. islandicus	aKMT	?	vit; RD	?	Chu (2012) J. Bact.
DNA polymerase B	C3N3Z3	?	?	S. islandicus	aKMT	?	vit; RD	?	Chu (2012) J. Bact.
DNA polymerase IV	C4KH38	?	?	S. islandicus	aKMT	?	vit; RD	?	Chu (2012) J. Bact.
FEN1	C3NF65	?	?	S. islandicus	aKMT	?	vit; RD	?	Chu (2012) J. Bact.
PROTEIN SYNTHESIS									
EF-Tu	P09591	5	3Me	P. aeruginosa	EftM	?	viv; MS	Mimics platelet-activating factor to mediate interaction with PAF receptor; allows bacterial invasion in pneumonia	Barbier (2013) Pneumonia
	P02991, P0A1H5, P33166,	56	1Me/2Me	E. Coli, E. gracillus, S. typhimurium, B. subtilis , B. licheniformis	?	?	viv; RD	Affects bound tRNA conformation, lowers GTPase activity (2Me in stationnary phase), hypermetylation controlled by the availability of carbon, nitrogen and phosphate sources in external medium; induces dissociation of EF-Tu from membranes	L'Italien (1979) FEBS Lett. (role: Van Noort (1986) Eur. J. Biochem, Young (1991) J. Bacteriol., Toledo (1989))
EF-1α	P02994	30	1Me	Sc	Efm1	?	viv; ED, AB*	?	Cavallius (1993) BBA, Efm1: Couttas (2012) Proteomics
	P68104, P10126, Q41803	36	3Me	Hs, Mm, Oc, Zm	?	?	viv; ED, MS	?	Dever (1989) JBC (Zm: Lopez-Valenzuela (2003) Plant Physiol.)
	P0DH99	44	1/2Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
	P68104	55	1Me	As, Hs	?	?	viv; MS*	Necessary for neural crest migration	Dever (1989) JBC (Hs: Jung (2008) Anal. Chem., Moore (2013) Mol. Cell), Bremang (2013) Mol. BioSyst.; Neural Crest: Vermillion (2013) JCB
	P68104, P10126,		2Me	Hs, Mm, Oc, At	?	?	viv; ED, MS*	?	Dever (1989) JBC (At: Namukong (2011) NAR), Bremang (2013) Mol. BioSyst.
	P68104	79	1Me	Hs	?	?	viv; MS*	Necessary for neural crest migration	Bremang (2013) Mol. BioSyst.; Neural Crest: Vermillion (2013) JCB
	P68104, P10126, P02994, P0DH99		3Me	Hs, Mm, Oc, Sc, Zm, At	?	?	viv, ED: MS	?	Dever (1989) JBC, Couttas (2012) Proteomics (Zm: Lopez-Valenzuela (2003) Plant Physiol., At: Namukong (2011) NAR)
	P68104	84	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
	P68104	154	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
	P68104	165	1Me	Hs	?	?	viv; MS*	Necessary for neural crest migration	Bremang (2013) Mol. BioSyst.; Neural Crest: Vermillion (2013) JCB
	P68104, P10126		2Me	Hs, Mm, Oc	?	?	viv; ED, MS*	?	Dever (1989) JBC, Moore (2013) Mol. Cell, Bremang (2013) Mol. BioSyst.
	Q41803, P0DH99	187	3Me	Zm, At	?	?	viv; ED, MS*	?	Lopez-Valenzuela (2003) Plant Physiol. (At: Ndamukong (2011) NAR). Alban (2014) PLoS One
	P02994, Q9Y713	217	3Me	Sc, As	?	?	viv; ED	?	Cavallius (1993) BBA (As : Dever (1989) JBC)
	Q41803	227	3Me	Zm	?	?	viv; MS*	?	Lopez-Valenzuela (2003) Plant Physiol.
	P0DH99	261	1Me	At	?	?	viv; MS	?	Ndamukong (2011) NAR
	Q41803, P0DH99	306	3Me	Zm, At	?	?	viv; MS*	?	Lopez-Valenzuela (2003) Plant Physiol. (At: Namukong (2011) NAR)
	P02994	316	2Me	Sc	See1	?	viv; MS	Necessary for neural crest migration	Lipson (2010) Arch. Biochem. Biophys., Couttas (2012) Proteomics; Neural Crest: Vermillion (2013) JCB
	P68104, P02994, Q9Y713	318	3Me	Hs, Oc, Sc, As	?	?	viv; ED, MS*	?	Dever (1989) JBC, Bremang (2013) Mol. BioSyst.
	P68104	384	2Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
	P02994, Q41803, P0DH99	396	1Me	Sc, Zm, At	soloSET (At) (Sc: Efm1?)	?	viv; ED, MS	Important for correct cytoskeletal organization through actin bundling	Cavallius (1993) BBA, Couttas (2012) Proteomics (Zm: Lopez-Valenzuela (2003) Plant Physiol., At: Namukong (2011) NAR, Efm1 : Lipson (2010) Arch. Biochem. Biophys.
	P68104,	408	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
	P06805	?	?	M. racemosus	?	?	viv; ED, RD	19% of total K methylated in mycelia; correlated with protein synthesis; possible role in EF-1βγ association or activation during germination	Hiatt (1982) PNAS (role: Fonzi (1985) Mol. Cell. Biol., Sherman (1989) Arch Biochem Biophys)
EF2		15	3Me	Sc	Efm2?	?	viv; MS, AB*	?	Couttas (2012) Proteomics
	P02994	407	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
		509	2Me	Sc	Efm3	?	viv; MS, AB*	?	Couttas (2012) Proteomics; Efm3: Zhang (2014) BBRC
		594	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
EF3A	P02994	187	3Me	Sc	Efm2?	?	viv; MS, AB*	?	Couttas (2012) Proteomics
		196	3Me	Sc	Efm2?	?	viv; MS, AB*	?	Couttas (2012) Proteomics

		789	3Me	Sc	Efm2?	?	viv; MS, AB*	?	Couttas (2012) Proteomics
eIF4A3	P38919	70	2Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
		374	2Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
aEF-1α		273	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
	G4RQ54	385	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		440	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
Eif-1A	P47813	95	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
PABP1		299	1Me	Hs	?	?	viv; MS, AB	?	Brook (2011) Biochem J.
	P11940	312	2Me	Hs	?	?	viv; MS, AB	? - Might be opposed by K312Ac	Brook (2011) Biochem J.
		361	2Me	Hs	?	?	viv; MS, AB	?	Brook (2011) Biochem J.
		606	2Me	Hs	?	?	viv; MS, AB	? - Might be opposed by K606Ac	Brook (2011) Biochem J.
TCEA1	P23193	?	?	Hs	SETD6	?	viv, vit; RD, MS AB*	? - Detected *in vitro* and *in vivo*	Levy (2011) Epigenetics Chromatin
Elongation factor LepA	Q72E76	319	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
IF-2	Q8F500	375	1Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
	Q8F7K1	655	1Me	L. interrogans	?	?	viv; MS*	? - Could be E654Me1	Cao (2010) Cell. Res.
		660/663?	?Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
Gid-4		456	1Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
transcriptional regulator CarD	Q72VT2	85	2Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
transcriptional regulator cII	A1VCA4	33	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
PpiC DVU1901	Q72AT9	190	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
EF-G	A1VEB9	430	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
eIF3a	P38249	192	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
eIF3, p93 subunit	O14164	514	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
RNApolI subunit 2	P22138	513	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
rRNA biogenesis protein RRP5	Q05022	769	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
snRNP-B	P40018	138	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
		145	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
114kDa U5 snRNP	P36048	356	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
		935	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
FTSJ3	Q8IY81	749	2/3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
		763	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
LUC7L2	Q9Y383	166	2Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
PPARBP	Q15648	1006	?	Hs	SET7/9	?	vit; RD	*In vitro* evidence only	Dhayalan (2011) Chem. & Biol.
hnRNP D/A/B	Q14103	129	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
hnRNP K	P61978	139	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
RNApol σ45 factor	A1VAJ6	445	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
σ45 dependent regulator	E3IT45	206	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
RnaPol: Sso0225/RpoA'	Q980R2	395	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
		659	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
RnaPol: Sso0227/RpoB'-C	Q980R1	12	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
		311	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
		349	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
RnaPol: Sso0071/RpoD	P95989	115	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
RnaPol: Sso0415/RpoE'		20	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
	Q980A3	131	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
		133	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
		171	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
RnaPol: Sso0751/RpoF	Q7LXK4	54	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
		102	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
RnaPol: Sso5468/RpoH	Q980Q9	30	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
		68	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
RnaPol: Sso5577/RpoL	Q980K0	71	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
		88	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
RnaPol: Sso5865/RpoP	Q97ZX7	19	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
RnaPol: Sso0396/Rpo13	Q980B8	66	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
		98	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
		100	1Me	S. solfataricus	?	?	viv; MS*	?	Botting (2010) Archae
RpoA2	G4RL52	168	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		346	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
RpoD	G4RL48	277	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
Ribonuclease G/E	G4RME9	415	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
nusA	G4RPV8	52	?/2Me	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		97	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae

RL1ab	P0CX43, P0CX44	46	1Me	Sc	Rkm5	?	viv, vit; MS, RD	No effect versus protein synthesis inhibitors	Webb (2011) JBC
		207	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
		?	?	Sc	?	?	viv; RD	?	Lhoest (1984) Eur. J Biochem.
RL3	P14126	?	1Me	Sc	?	?	viv; RD	?	Lhoest (1984) Eur. J Biochem.
		384	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
RL4a	A6ZKX6	104	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
RL5	P26321	164	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
RL7a	P62424	101	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
RL8a	P17076	10	1Me	Sc	?	?	viv; MS*	?	Ladror (2014) BBRC
RL8b	A7A0L4	15	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
		43	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
		241	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
RL9a	A6ZU36	19	1Me	Sc	?	?	viv; MS*	?	Ladror (2014) BBRC
RL11ab	P0C0W9, Q3E757	75	3Me	Sc	?	?	viv; RD, MS	?	Lhoest (1984) Eur. J Biochem., Couttas (2012) Proteomics
Plastid RL11	Q9MAP3	109	Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
RL12	P0CX53, P0CX54,	3	3Me	Sp, Hs, Dm	SET11	?	viv, vit; MS, RD	"Growth defect" if SET11 overexpressed; recruits Corto chromodomain to Drosophila nucleus which recruits RNAPolIII to chromatin and activates transcription	Sadaie (2008) JBC; Corto: Coleno-Costes (2012) PLOS genet.
	P30050, O75000, Q9W1B9	10	2Me/3Me	Sc	Rkm2	?	viv; RD	?	Porras-Yakushi (2006) JBC
		39, 40?	3Me?	Sp	SET11	?	viv, vit; MS, RD	? - "Growth defect" if SET11 overexpressed	Sadaie (2008) JBC
RL15	P61313	54	2/3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
RL16A	P0C0W9	2	1Me	Sc	?	?	viv; MS*	?	Ladror (2014) BBRC
		148	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
RL17A	P05740	47	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
RL28	P02406	133	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
RL23ab	A6ZKL6	18	2Me	Sc	?	?	viv; MS*	?	Ladror (2014) BBRC
		106	2Me	Sc	Rkm1	?	viv; RD	No effect on RNA binding, may affect Rpl23ab position in the large subunit	Porras-Yakushi (2007) JBC
		110	2Me	Sc	Rkm1	?	viv; RD	No effect on RNA binding, may affect Rpl23ab position in the large subunit	Porras-Yakushi (2007) JBC
		223	2Me	Sc	?	?	viv; MS*	?	Ladror (2014) BBRC
RL24b	A6ZUG0	2	1Me	Sc	?	?	viv; MS*	?	Ladror (2014) BBRC
RL33a	A6ZP75	16	1Me	Sc	?	?	viv; MS*	?	Ladror (2014) BBRC
RL42		8	1Me	Sc	?	?	viv; MS*	?	Ladror (2014) BBRC
		9	1Me	Sc	?	?	viv; MS*	?	Ladror (2014) BBRC
	P0CX27, P0CX28, Q9UTI8	10	1Me	Sc	?	?	viv; MS*	?	Ladror (2014) BBRC
		40	1Me	Sc	Rkm3	?	viv; MS	?	Webb (2008) JBC, Couttas (2012) Proteomics
		55	1Me	Sc, Sp	SET13 (Sc:Rkm4)	?	viv; MS	Stress protection, survival in stationnary phase, cycloheximide protection	Shirai (2010) JBC (Sc: Webb (2008) JBC)
RL43	P0CX25, P0CX26	?	1Me	Sc	?	?	viv; MS, RD	?	Lee (2002) PNAS
RL17mt	P36528	70	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
RL20mt	P22354	104	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
RS1B	P2348	1	1Me	Sc	?	?	viv; MS*	?	Ladror (2014) BBRC
		5	1Me	Sc			viv; MS*	Decreases during stationnary phase	Ladror (2014) BBRC
RS6	Q72DH3	87	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
RS7	P21469	149	1Me	B. subtilis	?	?	viv; MS	?	Lauber (2008) J. Prot. Res.
RS11	P0A7R9	?	1Me	E. Coli	?	?	viv; MS	?	Arnold (2008) Methods Mol. Biol.
RS13	P05756	140	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
RS17A	A6ZM38	59	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
RS18	P0CX55, P0CX56	48	1Me	Sc	Rkm1	?	viv; MS	?	Couttas (2012) Proteomics
RS27A	P05759	?	3Me	Tt	?	?	viv; RD	?	Guerin (1989) Biochimie
RL1	Q727D0, Q8F0R9	88	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
		137	2/3Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
		230	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
RL3	P60438	?	1Me	E. coli	?	?	viv; MS	?	Arnold (2008) Methods Mol. Biol.
RL7ae		74	1Me	E. coli, B. subtilis, B. stearothermophilus, T. Thermophilus	?	?	viv; ED	?	Dognin (1980) Eur. J Biochem.
	P46350, Q9EV99, Q8ZTA5, Q4J8P1, P45955	102	1Me	S. acidocaldarius, P. aerophilum	?	?	viv; MS	?	Marquez (2011) JMB
		108	1Me	E. coli, B. subtilis, B. stearothermophilus, T. Thermophilus	?	?	viv; ED	?	Dognin (1980) Eur. J Biochem.
		116	1Me	S. acidocaldarius, P. aerophilum	?	?	viv; MS	?	Marquez (2011) JMB
RL10a	Q8VZB9, P62906	90	3Me	At	?	?	viv; MS	?	Carroll (2007) MCP
		106	2Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
RL11	P0A7J7, Q06796, P56210, Q5SLP6, P62433	3	3Me	E. coli, B. subtilis, B. stearothermophilus, T. Thermophilus	PrmB	?	viv; ED	? - Methylated before ribosome assembly	Dognin (1980) Eur. J Biochem.
		?	?	S. islandicus	aKMT	?	vit; RD	?	Chu (2012) J. Bact.
		17	1-3Me?	B. subtilis	?	?	viv; MS	?	Lauber (2008) J. Prot. Res.
		39	1-3Me?	B. subtilis	?	?	viv; MS	?	Lauber (2008) J. Prot. Res.

		39	3Me	E. coli, B. subtilis, B. stearothermophilus, T. Thermophilus	PrmA	?	viv; ED, RD	? - Methylated before ribosome assembly	Dognin (1980) Eur. J Biochem. (PrmA : Vanet (1994) Mol. Microbio.)
		50	3Me?	D. vulgaris	?	?	viv; MS*	? - Could be AcK	Gaucher (2008) J. Proteom. Res.
		? (16?)	3Me	T. thermophilus	PrmA	?	viv, vit; MS, RD	? - Methylated before ribosome assembly	Cameron (2004) J. Bacter.
		?	?	E. coli	?	?	viv; MS	9 total methyl groups reported	Arnold (2008) Methods Mol. Biol.
RL12		4	2Me	At	?	?	viv; MS	?	Caroll (2007) MCP
	P36210, P08055, Q8ZVA7, P0A7K2	39	1Me	S. acidocaldarius, P. aerophilum	?	?	viv; MS	?	Marquez (2011) JMB
		82	1Me	E. coli	?	?	viv; MS	?	Liu (2009) J Proteome Res.
		84	1Me	S. acidocaldarius, P. aerophilum	?	?	viv; MS	?	Marquez (2011) JMB
RL16	Q72CH3	107	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
RL18	P0CX49, P0CX50	50	3Me	Sc	?	?	viv; MS	?	Couttas (2012) Proteomics
RL21	P60492	?	3Me	Tt	?	?	viv; RD	?	Guerin (1989) Biochimie
RL27	Q8F7U1	11	3Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
RL29	P25886, P47914	4	1Me	Rn, Hs	?	?	viv; ED, MS	? - Specific to brain, liver and thymus in rat	Williamson (1997) Eur. J Biochem. (Hs : Odintsova (2003) J. Prot Chem.)
		?	1Me	Hs	?	?	viv; MS*	?	Tran (2011) Nature
RL29		15	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
	Q72CH2, Q4JB47, Q8ZW50	29	1Me	S. acidocaldarius, P. aerophilum	?	?	viv; MS	?	Marquez (2011) JMB
		39	?Me	S. acidocaldarius, P. aerophilum	?	?	viv; MS	?	Marquez (2011) JMB
		69	?Me	S. acidocaldarius, P. aerophilum	?	?	viv; MS	?	Marquez (2011) JMB
RL30/L7e	G4RKE6	101	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
RL32	P38061	?	1Me/2Me	Sc	?	?	viv; RD	?	Lobet (1989) BBA
RL33	P0A7N9	?	1Me	E. coli	?	?	viv; MS	?	Arnold (2008) Methods Mol. Biol.
RL36 (L44)	O80929	55	1Me	At	?	?	viv; MS	?	Caroll (2007) MCP
RL36AL		53	1Me	Hs	?	?	viv; MS	?	Hountondji (2012) Chembiochem, Bremang (2013) Mol. BioSyst.
	P0C6E6	89	1Me	Hs	?	?	viv, MS	?	Hountondji (2012) Chembiochem
		97	1Me	Hs	?	?	viv, MS	?	Hountondji (2012) Chembiochem
RL36L		?	1Me	Hs	?	?	viv; MS*	?	Tran (2011) Nature
RM40	P83565	22	3Me	Rn	?	?	viv; ED, MS	?	Williamson (1997) Eur. J Biochem.
RL18Ae	P38613	29	1Me	S. acidocaldarius, P. aerophilum	?	?	viv; MS	?	Marquez (2011) JMB
		66	1Me	S. acidocaldarius, P. aerophilum	?	?	viv; MS	?	Marquez (2011) JMB
RS27L	Q71UM5	?	?	Hs	SETD6	?		? - Detected *in vitro* and *in vivo*	Levy (2011) Epigenetics Chromatin
RpIL		77	1/2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res., Chahbra (2011) PloS One
	Q727C8	81	?	S typhimurium	?	?	viv; MS*	?	Ansong (2014) BMC Genomics
		88	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res., Chahbra (2011) PloS One
yfiA	Q72BK6	88	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
		17/18?	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
PheRS α	Q728R9	324	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
PheRS β	Q728S0	6	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
U3 snoRNA protein 2	P40362	189	?	Hs	NSD1	?	vit; RD	?	Kuthidiputhi (2014) Chem. & Biol.
U3 protein 12	Q12220	350	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
U3 protein 22	P53254	249	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
		1158	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
ERB1	Q04660	577	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
		581	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
tRNA(m1A58)MT subunit TRM6	P41814	436	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
GCN1	P33892	1446	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
Helicase HAS1	Q03532	444	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
Helicase JA1	P53131	662	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
Superkiller 3	P17883	1088	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
DDX3Y	O15523	546	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
FLJ20105	B5MDQ0	1156	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
SRA1	Q9H7N4	197	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
SFRS2	Q01130	?	?	Hs	SETD6	?	viv, vit; RD, MS AB*	? - Detected *in vitro* and *in vivo*	Levy (2011) Epigenetics Chromatin
NHP2	Q9NX24	56	2Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
Gln synthetase	Q8F6J3	434	3Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
Arginosuccinate lyase	Q8F4G5	33	3Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
L-lysine 2,3 aminomutase	Q8F2R8	146	1/2Me?	L. interrogans	?	?	viv; MS*	? - Could also be R145Me1/2	Cao (2010) Cell. Res.

Fumarate hydratase II	Q72FY3	292	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
Adenylosuccinate lyase	Q727B3	115	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
Aspartate aminotransferase	P14909	202	1Me	S. solfataricus	?	?	viv; ED	?	Zappacosta (1994) Eur. J. Biochem.
		384	1Me	S. solfataricus	?	?	viv; ED	?	Zappacosta (1994) Eur. J. Biochem.
P2 ribonuclease	Q9UXC7	4		S. solfataricus	?	?	viv; ED	Not involved in thermostability or ribonuclease activity *in vitro*	Fusi (1993) Eur. J. Biochem. (non-role: Fusi (1995) Genes)
		6		S. solfataricus	?	?	viv; ED	Not involved in thermostability or ribonuclease activity *in vitro*	Fusi (1993) Eur. J. Biochem. (non-role: Fusi (1995) Genes)
P3 ribonuclease		4		S. solfataricus	?	?	viv; ED	?	Fusi (1995) FEBS let.
		6		S. solfataricus	?	?	viv; ED	?	Fusi (1995) FEBS let.
METHYLTRANSFERASES/DEMETHYLASES									
DNMT1		70	2Me	Hs	G9a	?	vit; RD	?	Rathert (2008) Nat. Chem. Biol.
	P26358, P13864	142	1Me	Hs, Mm	SET7/9	LSD1	viv, vit; MS, AB, RD	Susceptibilize DNMT1 to proteasome degradation; inhibited by S143Pi by AKT1	Esteve (2009) PNAS (LSD1 : Wang (2009) Nat Genet.; PiS: Esteve (2011)NSMB))
		1094	?	Mm	SET7/9	LSD1	viv, vit; RD	Cyclophophamide increases levels of K142Me (increases LSD1) Reduces stability of DNMT1, decreases global levels of DNA methylation	Zhang (2011) Chem. Res. Toxicol. Wang (2009) Nature Genet.
DNMT3a	Q9Y6K1, O88508	44	2Me	Hs, Mm	G9a, GLP	?	viv,vit; MS, AB, RD	Recruits MPP8 chromodomain to DNMT3a; possible role in G9a/GLP/DNMT3a/ MPP8 complex formation	Chang (2011) Nature Comm.
SET2	Q9BYW2, E9Q5F9	?	?	Hs, Mm	SET2	?	vit; RD	?	Sun (2005) JBC
SUV39H1	O43463	105	1Me	Hs	SET7/9	?	vit; RD	Induced by DNA damage; lowers SUV39H1 HMT activity; promotes heterochromatin relaxation and genome instability	Wang (2013) PNAS
		123	1Me	Hs	SET7/9	?		Induced by DNA damage; lowers SUV39H1 HMT activity; promotes heterochromatin relaxation and genome instability	Wang (2013) PNAS
G9a		94	2/3Me	Hs	?	?	viv, vit; MS, AB	?	Sampath (2007) Mol. Cell
	Q96KQ7	114	3Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
		165	2/3Me	Hs	G9a	?	viv, vit; MS, AB	Recruits HP1 (reversed by T166Pi); recruits Cbx3	Sampath (2007) Mol. Cell, Ruan (2012) Mol. Cell
		239	3Me	Hs	G9a	?	vit; MS, RD	Colocalization of HP1 with G9a	Chin (2007) Nucl. Acids Res.
GLP		122	3Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
	Q9H9B1	174	?	Hs	G9a	?	vit; RD	*In vitro* evidence only	Chin (2007) Nucl. Acids Res.
		205	?	Hs	GLP	?	viv, vit; MS, RD, AB	Recruits MPP8 and GLP; possible role in G9a/GLP/DNMT3a/ MPP8 complex formation	Chang (2011) Nature Comm.
mAM	Q6VMQ6	16	?	Mm	G9a	?	vit; RD	*In vitro* evidence only	Chin (2007) Nucl. Acids Res.
Metnase	Q53H47	485	1Me	Hs	Metnase	?	viv, vit; MS	Blocks the enhancement of Topo IIα decatenation by Metnase	Williamson (2008) Nucl.Acids Res.
NUE	O84742	209/210	?	C. trachomatis	?	?	vit; RD	Enhances NUE HMT activity?	Pennini (2010) PLoS Pathogens
γ-TMT		335	3Me	Nt	RLSMT	?	vit; RD	Maintains stability? Mainly *in vitro* evidence	Magnani (2007) JBC
Nucleolar essential protein 1	Q06287	147	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
CARM1	Q86X55	276	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
METTL21C	Q5VZV1	35	1Me	Hs	METTL21C	?	viv; MS	?	Kernstock (2012) Nature Comm.
METTL21A	Q8WXB1	?	?	Hs	METTL21A	?	viv; MS	?	Kernstock (2012) Nature Comm.
CaKMT	Q7Z624	?	?	Hs	CaKMT	?	viv; RD	?	Magen (2012) PLoS One
SAM-dependent MT		5	1Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
		6	1Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
		######	1Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
MT-like LA1618	Q8F5P9	228	1Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
NOL1/NOP2/sun DVU3189	Q725K5	348	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
P-L-isoAsp O-MT	G4RPW3	202	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
AKMT	B9Q0K5	?	?	T. gondii	AKMT	?	vit; RD	? - Observed in *in vitro* assays	Heaslip (2011) PloS Pathog.
ACETYLTRANSFERASES/DEACETYLASES									
PCAF	Q92831	78/89	1Me	Hs	SET7/9	?	viv,vit; RD	? - Nuclei specific	Masatsugu (2009) BBRC
CDYL1	Q9Y232	135	3Me	Hs	G9a	?	vit; RD	Inhibits chromodomain binding to H3K9Me3	Rathert (2008) Nat. Chem. Biol.
SIRT1	Q96EB6	233, 235, 236, 238	1Me?	Hs	SET7/9	?	viv, vit; MS, RD	No obvious effect; SET7/9 association to SIRT1 activates p53 activity independantly	Liu (2011) PNAS
HDAC1	Q13547	432	?	Hs	G9a	?	vit; RD	Recruits HP1 to HDAC1 in vitro	Rathert (2008) Nat. Chem. Biol.
NCOA2	Q15596	705	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
NCOA3	Q9Y6Q9	840	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		1091	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
Formate AcyIT DVU2272	Q729S7	611	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
KINASES/PHOSPHATASES									
VEGFR1	P17948	831	2Me	Hs	SMYD3	?	viv, vit; AB, RD	Enhances kinase activity	Kunizaki (2007) Cancer Res.
VEGFR2	P35968	856	?	Hs	?	?	viv; MS, RD	Increases kinase activity, required for angiogenesis and tumor growth	Hartsough (2013) Sci. Signal.
		861	?	Hs	?	?	viv; MS, RD	Increases kinase activity, required for angiogenesis and tumor growth	Hartsough (2013) Sci. Signal.
		1041	?	Hs	?	?	viv; MS, RD	Increases kinase activity, required for angiogenesis and tumor growth	Hartsough (2013) Sci. Signal.

MAP3K2	Q9Y2U5	260	2Me	Hs	SMYD3	?	viv, vit; RD, MS	Promotes Ras-driven oncogenesis, activates Ras/Raf/MEK/ERK signalling	Mazur (2014) Nature
v-Ki-ras2	A0JN17	42	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
Creatine kinase	P07310	?	1/2 Me?	Mm	?	?	viv;AB*	?	Iwabata (2005) Proteomics
Cdk1	P04551	251	?	Sp	?	?	viv; MS*	?	MacCoss (2002) PNAS
		257	?	Sp	?	?	viv; MS*	?	MacCoss (2002) PNAS
TTK	P33981	369	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		708/710?	?	Hs	SET7/9	?	vit; RD	*In vitro e*vidence only	Dhayalan (2011) Chem. & Biol.
Rock1	Q63644	462	1Me	Rn	?	?	viv; MS*	Affected in Experimental Autoimmune Encephalomyelitis?	Grant (2007) J Proteome Res.
ATM	Q13315	1126	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		1656	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
PRKDC	P78527	1150	2/3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain; upregulated in DNA damage response; recruit to DNA double strand breaks	Liu (2013) Mol. Cell
		2746	2Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain; upregulated in DNA damage response; recruit to DNA double strand breaks	Liu (2013) Mol. Cell
		3248	2Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain; upregulated in DNA damage response; recruit to DNA double strand breaks	Liu (2013) Mol. Cell
		3372	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
NRK	Q9NWW6	818	2Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
S/T kinase LA1164	Q8F6Y5	903	3Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
		905	1Me	L. interrogans	?	?	viv; MS*	?- Could be E904Me1	Cao (2010) Cell. Res.
ppaC DVU1636	Q72BJ9	302	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
Sensor His kinase	A1V9G4	187	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
PAK4	O96013	?	1/3Me?	Hs	SETD6	?	viv; MS, AB*	? (Detected *in vitro* and *in vivo*)	Levy (2011) Epigenetics Chromatin
PLK1	P53350	?	1/3Me?	Hs	SETD6	?	viv; MS, AB*	? (Detected *in vitro* and *in vivo*)	Levy (2011) Epigenetics Chromatin
Yak1p	P14680	423	1Me	Sc	?	?	viv, MS*	?	Zhang (2010) Proteomics
Ypk2p	P14680	635	1Me	Hs	?	?	viv, MS*	?	Zhang (2010) Proteomics
PGK1	Q9LD57	424	1Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
PGK2	P50318	329	2Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
CHAPERONES									
HSP90	P07900	209	1Me	Hs	SMYD2	?	viv, vit; MS, RD	?	Abu-Fahra (2011) J. Mol. Cell Biol.
		615	1Me	Hs, Mm, Dr	SMYD2	LSD1	viv, vit; MS, RD, AB	Correlates with association of a SMYD2/HSP90 complex to titin and correct myofilament organization	Abu-Fahra (2011) J. Mol. Cell Biol., Donlin (2012) Genes & Dev, Voelkel (2013) BBA
HSP90β	P11499	355	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
HSP90AB3P	Q58FF7	496	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
HSP70		189	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		559	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
	P08107	561	2Me	Hs	SETD1A	?	viv, vit; MS, AB	Promotes association to AURKB which enhances its activity; enhances cancer cell growth	Cho (2012) Nature Comm., Cloutier (2013) PloS Genet.
			3Me	Hs, Mm	METTL21A	?	viv, vit; AB, MS, RD	Does not affect ATPase activity, reduces affinity for α-Syn and HSP70 ability to delay filament formation	Jakobsson (2013) JBC
		561	1/2/3Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
		567	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
		568	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
	Q61696	?	?	Mm, Gg	?	?	viv; RD	?	Wang (1981) PNAS
HSPA5	P11021	585	1/2/3Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
		591	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
	P20029, Q90593	?	?	Mm, Gg	?	?	viv; RD	?	Wang (1981) PNAS
HSPA8	P11142	538	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
		542	1/3Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
		548	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
		550	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
	P63017	?	?	Mm, Gg	?	?	viv; RD	?	Wang (1981) PNAS
dnaK	Q72DW8	183	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
		251	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
DNAJC8	O75937	?	?	Hs	SETD6	?	viv; MS*	? - Detected *in vitro* and *in vivo*	Levy (2011) Epigenetics Chromatin
GroEL	P61439	391/392?	1/2/3Me?	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
METABOLISM									
Calmodulin	P07463	13	2Me	P. tetraurelia	?	?	viv, ED	?	Schaefer (1987) JBC
	P62152	94	1Me/2Me	Dm	?	?	viv, MS	Eye-specific	Takemori (2007) Proteomics
	P62158, P62161, P06787, P07463	115	3Me	Hs, Rn, Oa, Nt, Ps, Sc, Sp, P. tetraurelia	CaKMT	?	viv, vit; ED, RD, MS	Reduces NAD kinase activation; reduces *in vitro* T_m of linker region, not required for myosin light chain activation; role in stem internode growth, seed production and seed and pollen viability; for mammals, no effect on cell growth, proliferation or calmudulin stability; necessitate chaperoning of CAKMT by middle domain of HSP90; limits root length and CaM protein complex formation in *A. thaliana*	Watterson 1980 (JBC) (role: Roberts (1986) JBC; myosin: Molla (1981) JBC, Roberts (1992) PNAS CAKMT: Sitaramayya (1980) JBC, Oh (1990) Plant Physiol., Han (1993) Biochemistry, Pech1994 BBA, Magani (2010) Nat. Comm.; Tm: Magnani (2012) Protein; Expr. and Pur. Mammals; Panina (2012) JBC ; HSP90 : Magen (2012) PLoS One), Bremang (2013) Mol. BioSyst.; Roots: Banerjee (2013) Plant Cell
Calmodulin-like 3	P27482	115	1Me	Hs	?	?	viv, MS*	Observed in hair shaft	Jin Lee (2006) MCP
CaVP	P04573	95	3Me	B. lanceolatum	?	?	viv; ED	?	Kobayashi (1987) JBC

		116	3Me	B. lanceolatum	?	?	viv; ED	?	Kobayashi (1987) JBC
Rubisco large subunit	P11383, P00876, P04717, P27064	14	3Me	T. aestivum, Nt, P. sativum, Solanaceae, Cucurbitaceae	RLSMT	?	viv, vit; ED, RD, MS	? - Not methylated in Arabidopsis	Houtz (1989) PNAS, Houtz (1992) Plant Physiol. (RLSMT: Houtz (1991) Plant Physiol. At: Mininno (2012) JBC)
		32	2/3Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
		79	2Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
	O03042	201	1/2/3Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
		236	1/3Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
		356	2Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
Rubisco small subunit		66	1/2Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
	P10795	140	2Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
		147	2Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
Rubisco activase	P10896	204	1/3Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
PORC	F4I2F8	87	2Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
FBA (1,2 & 3)	Q9SJU4, Q944G9, Q9ZU52, F2VJ75	394	3Me	At, Nt	RLSMT	?	viv, vit; RD, MS, MS*	No effect on kinetics *in vitro* but increases aldolase activity in Nicotinia	Mininno (2012) JBC, Alban (2014) PloS One
Ribose 5-Pi isomerase	Q9C998	200	1Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
PPA1	Q9LXC9	118	1Me	At	?	?	viv; MS*	?	Alban (2014) PloS One
CMAS	Q8NFW8	399	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
β-glycosidase		116/135?	1Me/2Me?	S. solfataricus	?	?	viv; MS	Enhances thermal stability	Febbraio (2004) JBC
	P22498	272	2Me	S. solfataricus	?	?	viv; MS	Enhances thermal stability	Febbraio (2004) JBC
		311/322?	1Me/2Me?	S. solfataricus	?	?	viv; MS	Enhances thermal stability	Febbraio (2004) JBC
ADH	P39462	11	1Me	S. solfataricus	?	?	viv; ED	?	Ammendola (1992) Biochemistry
		213	1Me	S. solfataricus	?	?	viv; ED	?	Ammendola (1992) Biochemistry
NDUFS8	O00217	31	2Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
GluDH		254	1Me	S. solfataricus	?	?	viv; ED	?	Maras (1992) Eur. J Biochem
		260	1Me	S. solfataricus	?	?	viv; ED	?	Maras (1992) Eur. J Biochem
	O93715	372	1Me	S. solfataricus	?	?	viv; ED	?	Maras (1992) Eur. J Biochem
		391	1Me	S. solfataricus	?	?	viv; ED	?	Maras (1992) Eur. J Biochem
		392	1Me	S. solfataricus	?	?	viv; ED	?	Maras (1992) Eur. J Biochem
		393	1Me	S. solfataricus	?	?	viv; ED	?	Maras (1992) Eur. J Biochem
MDH	Q704B2	34	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		298	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
Lys synthethase	G4RM74	196	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
GAPDH		5	2Me	Hs	?	?	viv; MS	?	Seo (2008) J. Proteom. Res.
		194	2Me	Hs	?	?	viv; MS	?	Seo (2008) J. Proteom. Res.
		214	2Me	Hs	?	?	viv; MS	?	Seo (2008) J. Proteom. Res.
	P04406	227	2Me	Hs	?	?	viv; MS	?	Seo (2008) J. Proteom. Res.
		260	2Me	Hs	?	?	viv; MS	?	Seo (2008) J. Proteom. Res.
		263	2Me	Hs	?	?	viv; MS	?	Seo (2008) J. Proteom. Res.
		334	2Me	Hs	?	?	viv; MS	?	Seo (2008) J. Proteom. Res.
GDH	P10860	346	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
		352	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
LDHA		42	1Me/2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
	P04642	57	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
		59	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
LDHB	P42123	58	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
		60	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
α-amylase	P08117	?	3Me	T. aestivum	?	?	viv, ED	?	Motojima (1982) Plant & Cell Physiol.
Citrate synthase	P00889	368	3Me	Ss	?	?	viv; ED	No effect on catalysis	Bloxham (1981) PNAS (no effect: Evans (1988) BBRC)
IPMS/HCS DVU1914	Q72AS7	471	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
17-βHSD-3	P26439	155	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
		156	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
CNP	P13233	K27	2Me/3Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
		K29	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
Phosphoglycerate mutase 2	P16290	173	2Me	Rn	?	?	viv; MS*	? - Affected in Experimental Autoimmune Encephalomyelitis?)	Grant (2007) J Proteome Res.
GlmM	Q72CK1	256	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
ribAB	Q72CT4	5	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
Mo-Fe Nitrogenase α	A1VI38	484	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
Thr synthase	Q728R5	464/470?	1/2Me?	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
NDP-sugar epimerase LA1644	Q8F5M6	66/67	1/2Me?	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
Citrate lyase LA2841	Q8F2D0	80	2Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
dUTP diphosphatase	Q8F729	117	1Me	L. interrogans	?	?	viv; MS*	? - Could be R116Me1	Cao (2010) Cell. Res.
MhpC-like LA2857	Q8F2B7	63	1/2Me?	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
		2547	2Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.

ELECTRON TRANSFER & OXIDATIVE STRESS

ETFβ	P38117	199, 202	3Me	Hs, Mm	METTL20	?	viv,vit; MS,ED	Decreases oxidation of fatty acids; reduces reductase potential from dehydrogenase reactions dehydrogenase	Rhein (2014) JBC, Malecki (2015) JBC
Cytochrome c	P00042	55	1Me/2Me	H. anomala	?	?	viv; ED	?	Becam (1981) Eur. J. Biochem
	P00047	72	2Me	H. lanuginosa	?	?	viv; ED	?	Morgan (1972) JBC
	P00044, P00068, P62898, P00048, P00043, P00041		3Me	Rn, Sc, Nc, T. aestivum, N. crassa, H. anomala, D. kloeckeri, C. krusei	Ctm1	?	viv, vit; ED, RD	Blocks cytochrome c apoptotic activity; minor role in transfer to mitochondria in yeast; absent from most higher mammals, vertebrates	DeLange (1969) JBC, DeLange (1970) JBC, Sugeno (1971) J. Biochem. Brown (1973) Biochem. J. (Ctm1p: Polevoda (2000) JBC; roles: Kluck (2000) JBC)
	P00042	73	3Me	H. anomala	?	?	viv; ED	?	Becam (1981) Eur. J. Biochem
	P00068, P00047, P00066, P62773, P00064	86	3Me	T. aestivum, E. Gracillis, N. damascena, S. oleracea, A. porrum, H. languinosa	?	?	viv, vit; ED, RD	?	DeLange (1969) JBC, Morgan (1972) JBC, Lin (1973) Nature, Brown (1973) Biochem. J.
Ferredoxin	P00219, P49949, P00218, P81542, P81543, P81541	29	1Me	S. acidocaldarius, S. solfataricus, A. ambivalens, T. acidophilum, M. prunae, S. metallicus, A. infernus	?	?	viv; MS	?	Minami (1985) J. Biochem, (other than acido.: Gomes (1998) JBIC)
Ferredoxin-NADP reductase	P53991	102	1Me	T. tenax, A. ambivalens	?	?	viv; MS*	?	Botting (2010) Archae
		83	3Me	C. reinhardtii	?	?	viv; MS	?	Decottignies (1995) Arch Biochem Biophys. (ambivalens: Gomes (1998) BBRC)
		89	3Me	C. reinhardtii	?	?	viv; MS	Role in cytochrome c reductase activity?	Decottignies (1995) Arch Biochem Biophys (Role:Aliverti (1994) FEBS Lett.)
		135	2Me	C. reinhardtii	?	?	viv; MS	?	Decottignies (1995) Arch Biochem Biophys.
2-Cys Peroxiredoxin	Q96291	202	1/2/3Me	At	?	?	viv; MS*	?	Alban (2014) PLoS One
Photosystem I subunit E-2	Q9S714	145	1Me	At	?	?	viv; MS*	?	Alban (2014) PLoS One
Photosystem II subunit O-2	Q9S841	292	1Me	At	?	?	viv; MS*	?	Alban (2014) PLoS One
Chlorophyll A-B binding family protein	Q9SYX1	101	1Me	At	?	?	viv; MS*	?	Alban (2014) PLoS One
SDH	Q8LGI2	6	3Me	At	?	?	viv; MS*	?	Alban (2014) PLoS One
iNOS	P35228	422	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
sdhB	G4RPM3	38	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
SucDH/ fumarate reductase	Q8F4Z2	253	3Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
etfB	Q8F8Y6	188	3Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
thioredoxin reductase	Q72F38	293	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res., Chahbra (2011) PloS One
ApsB		18	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res., Chahbra (2011) PloS One
	Q72DT3	91	2/3Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res., Chahbra (2011) PloS One
		101	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res., Chahbra (2011) PloS One
ApsA		83	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res., Chahbra (2011) PloS One
	Q72DT2	298	3Me?	D. vulgaris	?	?	viv; MS*	? - Could be AcK	Gaucher (2008) J. Proteom. Res., Chahbra (2011) PloS One
		538	2Me	D. vulgaris	?	?	viv; MS*	? - Could be AcK	Gaucher (2008) J. Proteom. Res., Chahbra (2011) PloS One
DsrC		48	2Me/3Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res., Chahbra (2011) PloS One
	P45573	58	1Me/2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res., Chahbra (2011) PloS One
		78	3Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res., Chahbra (2011) PloS One
		90	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res., Chahbra (2011) PloS One

PROTEASES, PROTEASOME									
ACINUS	Q9UKV3	654	3Me	Hs	G9a	?	vit; RD	Weakly recruits HP1 to ACINUS in vitro	Rathert (2008) Nat. Chem. Biol.
RPN2	P32565	376	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
RPN3	P14685	92	2Me	Mm	?	?	viv; MS	?	Gomes (2009) MCP
UBA3	Q8TBC4	9	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
		10	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
Complement C1s	Q6P6T1	592	1Me	Rn	?	?	viv; MS*	? - Affected in Experimental Autoimmune Encephalomyelitis	Grant (2007) J Proteome Res.
Cullin1	Q13616	73	?	Hs	SET7/9	?	vit; RD	*in vitro* evidence only	Dhayalan (2011) Chem. & Biol.
Proteasome activator 28$_\gamma$	P61289	132	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		225	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
Sjogren syndrome antigen A1	P19474	138	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
ClpA	Q72BN3	718	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
ClpB	Q72AW6	265/299?	1/2Me?	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
U32 peptidase	Q72EC4	404/413?	1/2Me?	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
U62 peptidase TTX_0298	G4RN30	7	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
VIRAL PROTEINS									
Tat	P04610	50/51	3Me?	HIV-1	SETDB1	?	vit; RD	Inhibits LTR transactivation, does not modify affinity for RNA	Van Duyne (2008) Retrovirology, RNA binding: Kumar (2013) PloS One
		51	1Me	HIV-1	SET7/9	LSD1	viv, vit; RD, AB, MS	Enhances HIV transcription, inhibited by K50Ac by p300 but demethylation LSD1 independent of K50Ac; LTR transactivation by LSD1 demethylation; directly increases affinity for HIV RNA (increases Kon)	Pagans (2010) Cell Host Microbe (LSD1 & Ac interaction : Sakane (2011) PloS Pathog.), RNA binding: Kumar (2013) PloS One
VP1	A8Y983	5?	3Me	polyomavirus	?	?	viv; RD	?	Burton (1995) Virus Res.
S-HDAg	P0C6L3	?	?	HDV	?	?	viv; RD	?	Li (2004) J. Virol.
Virion morphogenesis protein	Q72D17	329	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
P4 phage primase	Q72FM4	141	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
LIPOPROTEINS									
Ape2211 (putative lipoprotein)	Q9Y9S7	7	1/2Me?	A. pernix	?	?	viv; ED	Methylated pept EKFKIV copurifies along endogenous OppA	Balestrieri (2011) J. Bacteriol.
		9	1/2Me?	A. pernix	?	?	viv; ED	Methylated pept EKFKIV copurifies along endogenous OppA	Balestrieri (2011) J. Bacteriol.
Lipoate-protein ligase A	Q8F699	248	1Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
nlpC precursor	K6F5K9	46	2Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
MEMBRANE PROTEINS									
ATP-synthase c	P05496	43	3Me	Hs, Bt, Cf	?	?	viv; ED, MS	Related to Ceroid lipfuscinose?	Katz (1994) JBC, Chen (2004) JBC (Hs: Katz (1996) BBA, Katz (1997) BBA, Katz (1995) Biochem. J.)
ATP synthase beta chain	Q42139	129	1/3Me	At	?	?	viv; MS*	?	Alban (2014) PLoS One
ATP synthase subunit beta	P19366	447	1/2Me	At	?	?	viv; MS*	?	Alban (2014) PLoS One
VCP	P55072	315	3Me	Hs	VCP-KMT	?	viv, vit; RD, MS	Methylated prior to hexamer assembly, Does not affect ATPase activity (contested: also observed to lower VCP ATPase activity)	Kernstock (2012) Nature Comm., Lower ATPase activity: Cloutier (2013) PloS Genet., Bremang (2013) Mol. BioSyst.
ADP/ATP translocase 1	P12235	52	3Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
Numb	P49757	158	2Me	Hs	SET8	?	viv; MS, AB*	Prevents association to p53 and promotion of apoptosis; prevented by doxorubicin	Dhami (2013) Mol. Cell
		163	2Me	Hs	SET8	?	viv; MS, AB*	Prevents association to p53 and promotion of apoptosis; prevented by doxorubicin	Dhami (2013) Mol. Cell
IFITM3	Q01628	88	1Me	Hs	SET7/9	?	viv; MS, AB	Increases during viral infection; Reduces interferon antiviral activity	Shan (2013) JBC
Aquaporin (PIP1)	Q9XHG7	155	1Me	Brassica	?	?	viv; MS, AB	? - Observed in roots samples)	Casado-Vela (2010) J. Proteom. Res.
Aquaporin (PIP2)	P43286	3	2Me	At	SDG7	?	viv, vit; MS, RD	? - Necessary for E6 1Me)	Santoni et al.(2006) Biochem. J (SDG7:Sahr (2010) Plant & Cell Physiol.)
OmpA	Q8F012	516	3Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
		526/527	1Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
OmpB	Q53020, P96989	100	3Me	R. prowazekii, R. typhi	Rp027/028	?	viv, MS	Virulence factor	Ching (1993) Techniques in Protein Chemistry IV/Chao (2004) BBA
		156	3Me	R. prowazekii, R. typhi	Rp027/028	?	viv, MS	Virulence factor	Ching (1993) Techniques in Protein Chemistry IV/Chao (2004) BBA
		205	1/3Me	R. prowazekii, R. typhi	Rp789	?	viv, vit; RD, MS, AB*	Virulence factor	Ching (1993) Techniques in Protein Chemistry IV/Chao (2004) BBA, Abeykoon (2012) J. Bact.
		222	1Me	R. prowazekii, R. typhi	Rp789	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		226	1/3Me	R. prowazekii, R. typhi	Rp789, Rp027/028	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.

		232	1/2/3Me	R. prowazekii, R. typhi	Rp789, Rp027/028	?	viv, vit; RD, MS, AB*	Virulence factor	Ching (1993) Techniques in Protein Chemistry IV/Chao (2004) BBA, Abeykoon (2012) J. Bact.
		273	1Me	R. prowazekii, R. typhi	Rp789	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		279	1/2/3Me	R. prowazekii, R. typhi	Rp789, Rp027/028	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		353	1Me	R. prowazekii, R. typhi	Rp789	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		379	1/2Me	R. prowazekii, R. typhi	Rp789	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		426	1/2Me	R. prowazekii, R. typhi	Rp789, Rp027/028	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		458	1/2Me	R. prowazekii, R. typhi	Rp789	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		488	1/2Me	R. prowazekii, R. typhi	Rp789	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		547	1/2/3Me	R. prowazekii, R. typhi	Rp789	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		624	1/2/3Me	R. prowazekii, R. typhi	Rp789, Rp027/028	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		635	3Me	R. prowazekii, R. typhi	Rp027/028	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		711	2Me	R. prowazekii, R. typhi	Rp789	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		723	3Me	R. prowazekii, R. typhi	Rp027/028	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		829	1/3Me	R. prowazekii, R. typhi	Rp789, Rp027/028	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		838	1/2Me	R. prowazekii, R. typhi	Rp789	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		891	1Me	R. prowazekii, R. typhi	Rp789	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		954	1Me	R. prowazekii, R. typhi	Rp789	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		K1056	1/3Me	R. prowazekii, R. typhi	Rp789, Rp027/028	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
		K1289	1Me	R. prowazekii, R. typhi	Rp789	?	viv, vit; RD, MS, AB*	Virulence factor	Abeykoon (2012) J. Bact.
HBHA	A1KFU9, P0A5P6, Q3I5Q7	162-195	1Me/2Me	M. bovis, Mtb, M. smegmatis	?	?	viv, vit; RD, MS	Possible role in resistance to proteolysis; important for T cell antigenicity and protective immunity to Mtb infection (only for aerosol infection), non-active TB patient have a stronger response to Me form; does not affect heparin-binding	Pethe (2002) PNAS, Biet (2007) Micr. & Infect., (antigenicity: Temmerman (2004) Nat. Med., aerosol: Guerrero (2011) Clin. Dev. Immunol., immun response: Delogu (2011) PLoS One)
LBP		?	?	M. smegmatis, M. leprae	?	?	viv; MS	Possible role in resistance to proteolysis; does not affect heparin/laminin binding	Pethe (2002) PNAS, (leprae: Soares de Lima (2005) Microbes & Infect.)
CFTR	P13569	K698	1Me	Hs	?	?	viv; MS	?	McClure (2012) Protein Eng. Des. Sel.
Connexin26	P29033	61	?	Hs	?	?	viv; MS	?	Locke (2009) Biochem. J
		221/223?	?	Hs	?	?	viv; MS	?	Locke (2009) Biochem. J
ATPase 4A	P09626	31	1Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
ATPase A1 α3	P06687	717	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
		718	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
		763	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
		764	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
FOF1 ATP synthase β	Q8F2J0	293	3Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
F1F0 ATP synthase	A6ZYP6	196	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
ATPase DNF1	P32660	541	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
Neuroplastin	P97546	147	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
		149	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
Clathrin Hc	P11442	245	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
		260	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
RAB2	P05712	8	3Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
Myelin	P02688	103	1Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
RhoGDI		50	2Me	Rn	?	?	viv; MS	?	Guerrera (2007) J. Proteome Res.
		52	2Me	Rn	?	?	viv; MS	?	Guerrera (2007) J. Proteome Res.
	Q5XI73	210	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
		264	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
		269	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
GABA-AT	P50554	142	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
EEA1	F1LUA1	178	2Me	Rn	?	?	viv; MS*	? - Affected in Experimental Autoimmune Encephalomyelitis	Grant (2007) J Proteome Res.
DLGAP5	B1WC75	706	1Me	Rn	?	?	viv; MS*	? - Affected in Experimental Autoimmune Encephalomyelitis	Grant (2007) J Proteome Res.
		708	2Me	Rn	?	?	viv; MS*	? - Affected in Experimental Autoimmune Encephalomyelitis	Grant (2007) J Proteome Res.
GCN20	P43535	656	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
MPG1	P41940	299	2Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
ZDHHC8	Q9ULC8	300	1Me	Hs	SET7/9	?	viv, vit; RD, MS	?	Dhayalan (2011) Chem. & Biol.

AKAP6	Q13023	604	?	Hs	SET7/9	?	vit; RD	*In vitro* evidence only	Dhayalan (2011) Chem. & Biol.
Silaffin-1 A1	Q9SE35	4	2Me	C. fusiformis	?	?	viv; MS	In conjunction with δ-hydroxylated, required for silica-precipitating activity	Kroger (1999) Science, Kroger (2001) JBC
		12	3Me	C. fusiformis	?	?	viv; MS	In conjunction with δ-hydroxylated, required for silica-precipitating activity	Kroger (1999) Science, Kroger (2001) JBC
Silaffin-1 A2	Q9SE35	4	2Me	C. fusiformis	?	?	viv; MS	?	Kroger (2001) JBC
		15	2Me	C. fusiformis	?	?	viv; MS	?	Kroger (2001) JBC
Silaffin-1/2L	Q5Y2C2	412	2Me	T. pseudonana	?	?	viv; MS	?	Poulsen (2004) JBC
Silaffin-3		20	2Me	T. pseudonana	?	?	viv; MS	On the C-ter K of repeated K-(A/S/Q)-X-K motifs if apart by 6 a.a from others	Sumper (2007) Angew. Chem. Int.
		61	2Me	T. pseudonana	?	?	viv; MS	On the C-ter K of repeated K-(A/S/Q)-X-K motifs if apart by 6 a.a from others	Sumper (2007) Angew. Chem. Int.
		83	2Me	T. pseudonana	?	?	viv; MS	On the C-ter K of repeated K-(A/S/Q)-X-K motifs if apart by 6 a.a from others	Sumper (2007) Angew. Chem. Int.
		100	2Me	T. pseudonana	?	?	viv; MS	On the C-ter K of repeated K-(A/S/Q)-X-K motifs if apart by 6 a.a from others	Sumper (2007) Angew. Chem. Int.
	Q5Y2C0	133	2Me	T. pseudonana	?	?	viv; MS	On the C-ter K of repeated K-(A/S/Q)-X-K motifs if apart by 6 a.a from others	Sumper (2007) Angew. Chem. Int.
		155	2Me	T. pseudonana	?	?	viv; MS	On the C-ter K of repeated K-(A/S/Q)-X-K motifs if apart by 6 a.a from others	Sumper (2007) Angew. Chem. Int.
		169	2Me	T. pseudonana	?	?	viv; MS	On the C-ter K of repeated K-(A/S/Q)-X-K motifs if apart by 6 a.a from others	Sumper (2007) Angew. Chem. Int.
		185	2Me	T. pseudonana	?	?	viv; MS	On the C-ter K of repeated K-(A/S/Q)-X-K motifs if apart by 6 a.a from others	Sumper (2007) Angew. Chem. Int.
		199	2Me	T. pseudonana	?	?	viv; MS	On the C-ter K of repeated K-(A/S/Q)-X-K motifs if apart by 6 a.a from others	Sumper (2007) Angew. Chem. Int.
LipL32	O34094	171	1/2/3Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
LipL45-like LA4267	Q8EYE9	198	2Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
		199	2Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
Cation/anion pump LA0550	Q8F8K5	171?	2Me?	L. interrogans	?	?	viv; MS*	? - Could be R169Me2	Cao (2010) Cell. Res.
Membrane protein LA2244	Q8F404	105	1Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
ABC transporter LA3694	Q8F003	44	1Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
Membrane protein LA3725	Q8EZX6	2536/ 2541?	1Me	L. interrogans	?	?	viv; MS*	? - Could be E2542Me1	Cao (2010) Cell. Res.
Dipeptide ABC transporter	Q8EYI5	123	1/2Me?	L. interrogans	?	?	viv; MS*	? - Could be R131Me2	Cao (2010) Cell. Res.
Heavy metal pump LA2439	Q8F3G5	272	3Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
TolC-like LA0957	Q8F7I8	357	1Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
ABC transporter DVU3161	Q725I8	8	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
Fe-compound ABC transporter		211	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
ABC transporter DVU1017	Q72DB2	748	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
Pi ABC transporter DVU2477	Q728X5	91	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
Dipept. ABC transp. DVU0169	Q72FP4	206	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
		500/503?	1/2Me?	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
K+ transporter β		389/396?	1Me/2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
DVU0149 (putative membrane protein)	Q72FR4	115	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
outer efflux pump DVU3097	Q726L0	266	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
MCP DVU1962	Q72AN0	351	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
MCP DVU3035	Q726S1	310	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
MCP DVU1975	Q72AL7	602	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
MCP DVU0018	Q72G42	563	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
MCP DVU0591	Q72EI7	165	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
cheA	Q72AN2	542	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
YajC	Q72B19	118/121?	1/2Me?	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
tonB dependent receptor	Q72FW3	112	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
Hemolysin CaBD	Q72BT9	766	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
SBP bac 3	A1VC88	242	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
vWF protein DVU2127	Q72A70	257	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
VirB11/GspE ATPase	A1VEE3	469	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
porA	G4RP74	249	?	T. tenax	?	?	viv; MS*	Proteolytic substructure of brain myosin.	Botting (2010) Archae
		373	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
CYTOSKELETON/CELL WALL/STRUCTURAL/TRAFICKING									
Myosin	P02604	34	1Me	Oc, Gg	?	?	viv; ED	?	Tong (1983) JBC
		129	3Me	Oc, Gg	?	?	viv; ED	Lowers ATP affinity	Tong (1983) JBC (effect: Bivin (1994) PNAS)
		549	3Me	Gg	?	?	viv; ED	?	Maita (1987) PNAS

		?	1/3Me	Gg	?	?	viv; RD	?	Krzysik (1971) Arch. Biochem. Biophysics.
Actin	P60709	84	1Me	Hs	?	ALKBH4	viv; MS, AB	Promotes actin/actomyosin interaction	Li (2013) Nat. Comm.
		86	1Me	Hs	?	?	viv; MS*	?	Bremang (2013) Mol. BioSyst.
	P02578	326	2Me	A. castellanii	?	?	viv; RD	?	Weihin (1970) Nature
	P60706	?	1/2Me	Gg	?	?	viv; RD	?	Krzysik (1971) Arch. Biochem. Biophysics.
Flagellin	P06179	?	2Me	S. typhimurium	*fliB*	?	viv; ED	Resistance against tryptic cleavage?	Ambler (1959) Nature (fliB: Burnens (1997) Microbiology; poss. Role: Joys (1979) BBA)
ERC1/2		?	?	S. Serpens	?	?	viv; ED	?	Glazer (1969) BBA
Flagellin A	Q8ECA5	159	1Me	S. oneidensis	?	?	viv; MS	?	Budendorfer (2013) PLoS One
Flagellin B		159	1Me	S. oneidensis	SO4160?	?	viv; MS	? (not required for motility of flagellar assembly)	Sun (2013) J. Bact., Budendorfer (2013) PLoS One
		163	1Me	S. oneidensis	SO4160?	?	viv; MS	? (not required for motility of flagellar assembly)	Sun (2013) J. Bact., Budendorfer (2013) PLoS One
	Q8ECA6	167	1Me	S. oneidensis	SO4160?	?	viv; MS	? (not required for motility of flagellar assembly)	Sun (2013) J. Bact., Budendorfer (2013) PLoS One
		188	1Me	S. oneidensis	?	?	viv; MS	?	Budendorfer (2013) PLoS One
		189	1Me	S. oneidensis	SO4160?	?	viv; MS	? (not required for motility of flagellar assembly)	Sun (2013) J. Bact., Budendorfer (2013) PLoS One
		190	1Me	S. oneidensis	SO4160?	?	viv; MS	? (not required for motility of flagellar assembly)	Sun (2013) J. Bact.
DMP1	Q13316	?	1Me	Hs	?	?	viv; ED	? - In resting state?	Kalasz (1978) J Dent. Res.
profilin	P68696	103	3Me	A. castellanii	?	?	viv; ED	?	Ampe (1985) JBC
actobindin	P18281	35	3Me	A. castellanii	?	?	viv; ED	?	Vandekerckhove (1990) JBC
		72	3Me	A. castellanii	?	?	viv; ED	?	Vandekerckhove (1990) JBC
pilin	P74465	168	3Me	Synechocystis	?	?	viv; MS	Pili elongation and cell motility	Kim (2011) BBRC
troponin C	P90687	20	1Me/2Me?	B. floridae	?	?	viv; MS	?	Takagi (1994) Eur. J Biochem.
troponin I	P05547	142	3Me	A. Pallipes	?	?	viv; ED	?	Kobayashi (1989) JBC
		146	3Me	A. Pallipes	?	?	viv; ED	?	Kobayashi (1989) JBC
ERC1/2	Q8IUD2	319/315	2Me	Hs	?	?	viv; MS	?	Moore (2103) Mol. Cell
Nopp140	Q14978	80	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
XIRP2	A4UGR9	1877	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain; downregulated in DNA damage response	Liu (2013) Mol. Cell
		1944	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
LAP2A	P42166	94	3Me	Hs	?	?	viv; MS	Interacts with HP1β chromodomain	Liu (2013) Mol. Cell
Tau		24	2Me	Hs	?	?	viv; MS	Observed in normal brain, reduces Tau aggregation and limits filaments extension	Funk (2014) Biochem J.
		44	1Me, 2Me	Hs	?	?	viv; MS	? - Enriched in neurofibrillary tangles?, Observed in normal brain, reduces Tau aggregation and limits filaments extension	Thomas (2011) Acta Neuropathol., Funk (2014) Biochem J.
		67	2ME	Hs	?	?	viv; MS	Observed in normal brain, reduces Tau aggregation and limits filaments extension	Funk (2014) Biochem J.
		163	1Me	Hs	?	?	viv; MS	? - Enriched in neurofibrillary tangles?	Thomas (2011) Acta Neuropathol.
		174	1Me	Hs	?	?	viv; MS	? - Enriched in neurofibrillary tangles?	Thomas (2011) Acta Neuropathol.
		180	1Me	Hs	?	?	viv; MS	? - Enriched in neurofibrillary tangles?	Thomas (2011) Acta Neuropathol.
		190	1Me	Hs	?	?	viv; MS	Observed in normal brain, reduces Tau aggregation and limits filaments extension	Funk (2014) Biochem J.
	P10636	254	1Me	Hs	?	?	viv; MS	? - *In vivo* , might compete with Ub at this residue; enriched in neurofibrillary tangles?	Thomas (2011) Acta Neuropathol.
		259	1Me	Hs	?	?	viv; MS	Observed in normal brain, reduces Tau aggregation and limits filaments extension	Funk (2014) Biochem J.
		267	1Me, 2Me	Hs	?	?	viv; MS	? - Enriched in neurofibrillary tangles?, Observed in normal brain, reduces Tau aggregation and limits filaments extension	Thomas (2011) Acta Neuropathol., Funk (2014) Biochem J.
		290	1Me, 2Me	Hs	?	?	viv; MS	? - Enriched in neurofibrillary tangles?, Observed in normal brain, reduces Tau aggregation and limits filaments extension	Thomas (2011) Acta Neuropathol., Funk (2014) Biochem J.
		311	2Me	Hs	?	?	viv; MS	Observed in normal brain, reduces Tau aggregation and limits filaments extension	Funk (2014) Biochem J.
		317	2Me	Hs	?	?	viv; MS	Observed in normal brain, reduces Tau aggregation and limits filaments extension	Funk (2014) Biochem J.
		363	2Me	Hs	?	?	viv; MS	Observed in normal brain, reduces Tau aggregation and limits filaments extension	Funk (2014) Biochem J.
		385	1Me	Hs	?	?	viv; MS	Observed in normal brain, reduces Tau aggregation and limits filaments extension	Funk (2014) Biochem J.
Neurofilament triplet-I	P08553	?	1/2 Me?	Mm	?	?	viv; AB*	?	Iwabata (2005) Proteomics
α-actin	P68134	?	1/2 Me?	Mm	?	?	viv; AB*	?	Iwabata (2005) Proteomics
β-actin	P60710	?	1/2 Me?	Mm	?	?	viv; AB*	?	Iwabata (2005) Proteomics
γ-actin	P63260	?	1/2 Me?	Mm	?	?	viv; AB*	?	Iwabata (2005) Proteomics
α-tubulin	P68369	?	1/2 Me?	Mm	?	?	viv; AB*	?	Iwabata (2005) Proteomics
		60	2Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
tubulin-α4	Q5XIF6	40	3Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
tubulin-β5	P69897	103	1Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
Vimentin	P31000	143	Me	Rn	?	?	viv; MS*	?	Wu (2003) Nature Biotech.
Keratin II HB2		137	2Me	Hs	?	?	viv; MS*	?	Jin Lee (2006) MCP
	Q9NSB4	207	2Me	Hs	?	?	viv; MS*	?	Jin Lee (2006) MCP
		354	2/3Me	Hs	?	?	viv; MS*	?	Jin Lee (2006) MCP
Keratin II HB6		193	2Me	Hs	?	?	viv; MS*	?	Jin Lee (2006) MCP
	O43790	222	2Me	Hs	?	?	viv; MS*	?	Jin Lee (2006) MCP
		377	2Me	Hs	?	?	viv; MS*	?	Jin Lee (2006) MCP

Desmoplakin	P15924	1802	2Me	Hs	?	?	viv; MS*	?	Jin Lee (2006) MCP
Crystallin α A	P02489	88	?	Hs	?	?	viv; MS*	?	MacCoss (2002) PNAS
Crystallin β B1	P53674	235	?	Hs	?	?	viv; MS*	?	MacCoss (2002) PNAS
Crystallin β B2	P43320	42	?	Hs	?	?	viv; MS*	?	MacCoss (2002) PNAS
		68	?	Hs	?	?	viv; MS*	?	MacCoss (2002) PNAS
		121	?	Hs	?	?	viv; MS*	?	MacCoss (2002) PNAS
Crystallinβ B3	P26998	128	?	Hs	?	?	viv; MS*	?	MacCoss (2002) PNAS
Crystallinγ S	P22914	6	?	Hs	?	?	viv; MS*	?	MacCoss (2002) PNAS
Plastin-3	Q63598	444	2Me	Rn	?	?	viv; MS*	? - Affected in Experimental Autoimmune Encephalomyelitis?	Grant (2007) J Proteome Res.
ARp2/3 Complex subunit 1	P38328	121	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
NOC2	P39744	384	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
transport protein USO1	P25386	119	1Me	Sc	?	?	viv; MS*	?	Pang (2010) BMC Genomics
TUBB2C	P68371	19	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
Actin	P60709	18	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
DAB2	P98082	163	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
		173	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
BICD2	Q8TD16	54	1Me	Hs	?	?	viv; MS*	?	Jung (2008) Anal. Chem.
TIC110	Q8LPR9	946	2Me	At	?	?	viv; MS*	?	Alban (2014) PLoS One
TIC62	Q8H0U5	79	3Me	At	?	?	viv; MS*	?	Alban (2014) PLoS One
TPT	F4KG20	96	1Me	At	?	?	viv; MS*	?	Alban (2014) PLoS One
Rho	Q8F7C5	247	2Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
murF	Q8F648	124	1Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
FtsA	Q728V2	113	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
UNKNOWN FUNCTION/ACTIVITY									
FAM50A	Q14320	5	1Me	Hs	?	?	viv; MS	?	Moore (2013) Mol. Cell
BIR6	Q9STK5	394	1Me	At	?	?	viv; MS*	?	Alban (2014) PLoS One
MJ0556	Q57976	3/6/15/21/25?	1Me	M. jannaschii	?	?	viv; MS	?	Forbes (2004) PNAS
LA0471	Q8CXU3	136?	1/2Me?	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
LA0940	Q8F7K4	231	1Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
LA1017 (TPR repeats)	Q8F7C9	99	2Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
		102	2Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
LA1037		36	2Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
LA1883	Q8F505	242	3Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
LA2208	Q8F439	261/264	1Me	L. interrogans	?	?	viv; MS*	? - Could be R262Me1	Cao (2010) Cell. Res.
LA2490	Q8F3B7	176	2Me	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
LA3378	Q8F0W3	739	1/2Me?	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
LA3818	Q8EZN4	103/105/106/107?	1/2Me?	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
LA4032	Q8EZ29	162	1/2/3Me?	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
		183	1/2/3Me?	L. interrogans	?	?	viv; MS*	?	Cao (2010) Cell. Res.
DVU1005	Q72DC4	152	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
DVU2025	Q72AH0	1040	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
DVU0618	Q72EG0	16	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
DVU0843	Q72DT6	144	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
DVU1121	Q72D10	70	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
DVU1247	Q72CN6	144	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
DVU1265	Q72CL8	199/201?	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
DVU1826	Q72B13	76	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
DVU1747	Q72B89	839	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
DVU2649	Q728F4	8	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
DVU0337	Q72F77	188	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
TPR protein DVUA0036	Q72WQ3	189	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
DVUA0098	Q72WJ1	182	1Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
Radical SAM protein DVU0182	Q72FN1	213	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
Decarboxylase DVU1529	Q72BV5	15	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
Rhodanese-like DVU1368	Q72DW8	374	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
AMP-binding DVU2250	Q729U8	73	2Me	D. vulgaris	?	?	viv; MS*	?	Gaucher (2008) J. Proteom. Res.
TTX_0535	G4RNQ6	128	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		172	1/2Me	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
TTX_1155	G4RJQ0	149	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
TTX_1565	G4RKU7	20	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
TTX_1576	G4RKV8	20	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		98	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		165	1/2Me	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		187	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
TTX_1840	G4RLL3	69	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		138	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae

TTX_1876	G4RLP7	28	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		39	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
TTX_1885	G4RLQ7	53	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
TTX_2059	G4RM77	33	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
TTX_2090	G4RMM6	41	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
		113	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
NAD(FAD)-DH	G4RNE7	221	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
DMI6/NTAB-like	G4RLA0	106	?	T. tenax	?	?	viv; MS*	?	Botting (2010) Archae
GINS15	C3NHF9	?	?	S. islandicus	aKMT	?	viv; MS	?	Chu (2012) J. Bact.

Evidence: viv: in vivo, vit: in vitro; AB: specific antibody, AB* pan-methyllysine anitbody, MS: mass spectrometry, MS*: high-throughput mass spectometry, ED: Edman degradation, RD, radioactive assay