# Examining post-operative antigen specific $CD8^+$ T cell dysfunction

**Oladunni Olanubi**

Thesis submitted to the University of Ottawa
in partial Fulfillment of the requirements for the degree of Doctor of Philosophy
Department of Biochemistry, Microbiology and Immunology

Faculty of Medicine
University of Ottawa

# ABSTRACT

Surgery remains the most effective approach to cure patients with solid malignancies. However, surgical trauma results in significant cellular immunosuppression. $CD8^+$ T cell dysfunction post-operatively has been characterized, however the effects of surgery on antigen specific $CD8^+$ T cell suppression has not been fully explored. Here, we established that surgery-induces antigen specific $CD8^+$ T cell dysfunction, including impaired pro-inflammatory cytokine secretion and cytotoxicity in patients and mice. We developed a model to investigate surgical stress and cancer recurrence in C57Bl/6 mice, based on transfer of $CD8^+$ OT-I T cells, reactive to ovalbumin (OVA), followed by challenge with B16-OVA melanoma cells. This model demonstrates 100% protection against cancer in control animals but 0% protection following surgery. Using a COVID-19 immunological tool kit to characterize post-operative function, we observed a significant suppression in T cells from vaccinated cancer patients. The OT-I model was used to evaluate the mechanisms of post-operative T cell dysfunction. We first established that a population of surgery induce Myeloid Derived Suppressor Cells (sxMDSCs) also known as $GR\text{-}1^+$ cells in mice which expand after surgery in mice and cancer patients on post-operative day 1 (POD1), are suppressive of $CD8^+$T cells. We then performed single cell RNA sequencing, which identified the intracellular checkpoint CISH (Cytokine-inducible SH2-containing protein) as upregulated in antigen-stimulated human and murine $CD8^+$ T cells following surgical stress. Overall, this work highlights antigen specific $CD8^+$ T dysfunction post-operatively and uncovers a possible mechanism of dysfunction characterized by the expression of an intracellular checkpoint CISH.

# ACKNOWLEDGEMENTS

I would like to thank Dr. Rebecca Auer for the opportunity to work in her Lab. This project would not have been possible without her. Dr. Auer has provided me with invaluable support over the years. I have gained a lot from my time in working in her lab and I have developed into a stronger researcher because of her. To Dr. Rafeah Alam, I thank her for her assistance and support in completing this project, she has been a wonderful guide for me through the years. I would like to thank Christiano Tanese De Souza for his assistance with the experiments; this project would not have been possible without him. I would like to thank the entire Auer Lab; Marlena Scaffidi, Henna Mistry, Reagan MacDonald, Taylor Dion, Agrima Shrestha, Priyoshi Lahiri and past members for their amazing support. They provided me with an incredibly positive and encouraging atmosphere. I would like to acknowledge Dr. Michael Kennedy who was instrumental in my training and getting my project started. To my committee members, Dr. Harry Atkins, Dr. Angela Crawley, and Dr. Scott McComb, I thank them for their contribution and time during my project. A big thank you to Valerie Smaglinskie and Jennifer Valentino for all their amazing assistance through the years. To all the nurses and patients at the General Hospital, I thank them for their participation in the studies. A big thank you to the entire Cancer Therapeutics Department at the OHRI for their contribution to my research. I would also like to extend my appreciation to the funding sources, BioCanRx, The Terry-Fox Research Institute, Canadian Cancer Society, Cancer Research Grant, Translational Research Grant, Ontario Graduate Scholarship and the University of Ottawa.

I would like to dedicate this work to my family. Their unwavering and consistent support throughout the years has been nothing short of amazing. I could not have asked for a better support system. This journey would not have been possible without them. Thank you.

# Table of Contents

# LIST OF ABBREVIATIONS

ACK - Ammonia Chloride Potassium

Arg1 - Arginase-1

C/EBPβ - CCAAT/enhancer binding protein beta

CISH - Cytokine-Inducible SH2-Containing Protein

COX2 - Cyclooxygenase-2

CRC - Colorectal cancer

CRP - C-reactive protein

CTC - Circulating tumor cells

DAG - Diacylglycerol

DAMPs - Damage-associated molecular patterns

DTC- Disseminated Tumor Cells

ERK - Extracellular signal-regulated kinase

G-MDSC - Granulocytic MDSC

GM-CSF - Granulocyte-macrophage colony-stimulating factor

GSEA - Gene set enrichment analysis

HD - Healthy Donor

i.p. - Intraperitoneal injection

i.v. - Intravenous injection

IFN - Interferon

IL - Interleukin

iNOS - Inducer of Nitric Oxide Synthase

IP3 - Inositol 1,4,5-trisphosphate

JAK- Janus kinase

LGALS1- Galectin-1

M-MDSC - Monocytic MDSC

MDSC - Myeloid derived suppressor cell

MHC - Major histocompatibility

mTOR - Mammalian target of rapamycin

NES - Normalized enrichment scores

NFAT - Nuclear Factor of Activated T cells

NF-κB - Nuclear Factor kappa-light-chain-enhancer of activated B cells

NK - Natural Killer

NO - Nitric Oxide

PBMCs - Peripheral Blood Mononuclear Cells

PDE5 - Phosphodiesterase-5

PGE2 - Prostaglandin E2

PIP2 - Phosphatidylinositol 4,5-bisphosphate

PMN-MDSC - Polymorphonuclear MDSC

POD - Post-operative Day

PRR - Pattern recognition receptors

RBC - red blood cell

ROS - Reactive oxygen species

SBNO2 - Strawberry Notch Homolog 2

s.d. - standard deviation

scRNA-seq - single cell RNA sequencing

STAT- Signal Transducer and Activator of Transcription

sxMDSC - Surgery induced MDSC

TGF-β - Transforming growth factor beta

Th1 - T helper 1

Th2 - T-helper 2

TNF-α - Tumor necrosis factor alpha

# LIST OF FIGURES

# SUPPLEMENTAL FIGURES

# CHAPTER 1: GENERAL INTRODUCTION

## 1.0 RATIONALE

In this Chapter, I will provide an introduction into key topics that are related to my project. This chapter will provide background information necessary to understand the subsequent chapters. I will introduce topics such as Cancer, Cancer surgery, Immune suppression, $CD8^+$ T cells, and Myeloid derived suppressor cells. I will also establish the research objective and aims that will be explored.

## 1.1 CANCER AND METASTASES

Cancer can be defined as a pathological condition induced by the unchecked growth and division of abnormal cells, which can gradually acquire molecular and phenotypic changes that permit them to invade surrounding tissues and disseminate to distant organs(*1*). Healthy cells function by following gene instructions to carry out normal activities, such as cell division and regulated cell death. However, cancerous cells undergo specific genetic mutations brought on by different factors, including carcinogens, environmental influences, radiation, and genetic predisposition, all of which can alter their ability to carry out normal functions. These mutations can activate or dysregulate proto-oncogenes, converting them into oncogenes that drive uncontrolled cellular proliferation and promote cancer development.

Metastasis is the spread of cancer cells from the primary tumor to distant parts of the body(*2*). Before cancer cells start to spread, the cells have acquired the ability to break apart from the primary tumor and are able to invade surrounding tissues by travelling through the lymphatic system or the blood. Once cancer cells reach these sites, they can grow rapidly and populate the organ(*3–5*).

Cancer cells can spread to different parts of the body, in part because they evade the immune system. There are several ways cancer cells can combat the immune system. They alter the function of antigen-presenting cells (APC), which are generally meant to be responsible for sounding the alarm for the presence of cancer(*6*). Cancer cells secrete several growth factor signals such as transforming growth factor beta (TGFβ), vascular endothelial growth factor (VEGF) and IL-10, all of which act as tumor derived suppressors of APCs by inhibiting maturation, antigen presentation and T cell activation(*7–9*). For example, TGFβ can suppress

expression of the MHC-II (Major histocompatibility complex two) and other co-stimulatory molecules which results in immature APCs.

Cancer cells recruit several suppressor cells to the tumor microenvironment (TME), such as neutrophils, macrophages, and regulatory T cells (T-regs), by inducing the production of factors like colony-stimulating factor 1 (CSF-1), IFNγ and IL-8. These suppressive cells are responsible for the inactivity of other effector immune cells(*10*, *11*).

Additionally, cancer cells modify the proteins on their surface to inhibit the expression of co-stimulatory signals required for T cell activity. For instance, cancer cells express programmed cell death ligand 1 (PD-L1), which inhibits the activity of T cells and NK cells by binding to the checkpoint receptor Programmed Cell Death Protein 1 (PD-1) expressed on these immune cells(*12–14*).

Primary tumors are often localized to one area and are mostly curable, as there are several options including chemotherapy, surgery, and immunotherapy. However, metastasis is a systemic disease that can affect multiple organs, therefore making it a more complex process to cure.

### 1.1.1 Cancer surgery

Surgery is the oldest known method for treating cancer, and it involves physically removing the cancerous mass from the body. Surgical removal of tumors dates back over 4000 years ago(*15*). Over the years, surgical techniques have advanced significantly from basic procedures to highly complicated operations. Today, surgery remains an integral part of cancer therapy, particularly for tumors that haven't metastasized. Surgery is commonly used to remove localized primary tumors and, in some cases, surrounding tissue to ensure complete resection of cancerous cells. Although surgical resection offers the best chance of cure for many patients with solid malignancies, it can also trigger unintended adverse effects including bleeding, thrombosis, infections, and immune suppression that may ultimately worsen patient outcomes.

### 1.1.2 Cancer Surgery and Metastases

As a result of surgery, cancer cells can escape and enter the blood or lymphatic system and remain there; these are called circulating tumor cells (CTCs)(*16*). Due to the handling of tumors during surgery, various factors such as damaged blood vessels and inflammation can drive the escape of cancer cells from the tumor site (*17*, *18*)(Fig 1.1). This becomes a challenge because cancer cells can also travel to other sites, including the bone marrow, lymph nodes, and organs, by evading the immune system. Once these CTCs have migrated from the primary tumor site, they become disseminated tumor cells (DTCs)(*19*). The presence of DTCs indicates a primary step in metastasis because although these cells are dormant, they can colonize and repopulate(*20*). The effect of surgery consequently provides an ideal environment for tumor regrowth and metastasis due to multiple factors such as stress response, inflammation, and immune suppression. The detection of circulating and disseminated tumor cells following resection in several solid cancers, including gastric cancer, highlights the perioperative period as

a critical window for metastatic risk(*21–23*). These studies show that the presence of circulating and disseminated tumor cells is associated with increased cancer recurrence and poor prognosis. Notably, CTCs and DTCs can persist in a dormant state for years and may become reactivated by triggering events such as surgery. Surgery can trigger the awakening of these dormant cells by releasing growth factor signals such as VEGF, HIF-1α, and IL-6(*24*, *25*). These growth factors enable angiogenesis, which is a process that is necessary for cancer cells to grow and metastasize.

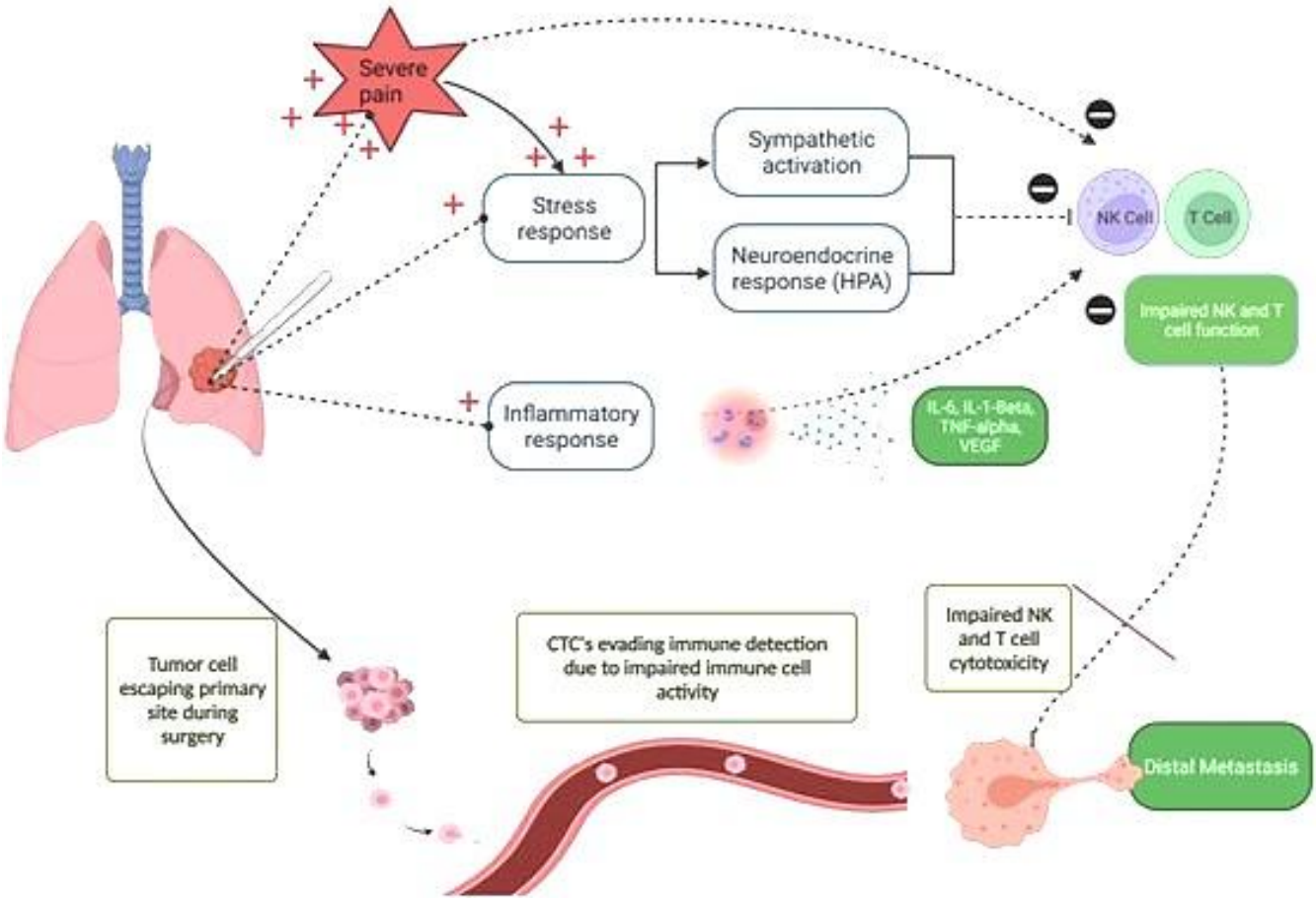

Severe pain
Stress response
Sympathetic activation
Neuroendocrine response (HPA)
NK Cell
T Cell
Impaired NK and T cell function
Inflammatory response
IL-6, IL-1-Beta, TNF-alpha, VEGF
Tumor cell escaping primary site during surgery
CTC's evading immune detection due to impaired immune cell activity
Impaired NK and T cell cytotoxicity
Distal Metastasis

**Figure 1.1: A model of Surgical stress**

Surgical resection is associated with a massive inflammatory and stress response. Surgery induces a stress response by initiating the sympathetic and neuroendocrine system, resulting in elevated levels of stress hormones such as cortisol that suppress NK and T cell activity. The inflammatory response is characterized by the recruitment of cells that secrete proinflammatory cytokines such as IL-6, IL-1β, TNFα, and VEGF. This enhanced postsurgical stress response and inflammation can result in immunosuppression of innate and adaptive immunity, including NK and T cells. This systemic stress response and inflammation provide an environment favorable for tumor metastasis, because disseminated cells (CTCs) from the surgical bed may form pre-metastatic niches because of immunosuppression. *Adapted with copyright permission from Moorthy et al., 2021.*

### 1.1.3 Cancer Surgery and the Surgical Stress Response

Surgery triggers a systemic stress response due to the stimulus released during the operation (Fig 1.1). The stress response to surgery often depends on the magnitude, complexity, invasiveness, and duration of the procedure. Major surgeries such as open-heart surgeries, abdominal surgeries, and joint replacement elicit a pronounced stress response(*26–28*) . The post-operative period is a critical time when the immune system is weakened, and the risk of cancer spread is higher because surgery induces a series of physiological changes in the body. These changes consist of two major categories, such as the neuroendocrine-metabolic axis and the inflammation-immune response axis.

**Neuroendocrine-metabolic axis**

The hypothalamus is the central neuroendocrine system that links the nervous system to the endocrine system. The hypothalamus regulates processes such as metabolism and stress response. The regulation of stress response occurs through the paraventricular nucleus (PVN), a central system within the hypothalamus(*29*, *30*). The paraventricular nucleus consists of neurons that can sense changes in the body such as inflammation and signals the pituitary gland of the endocrine system to release hormones thar regulate the stress response(*31*, *32*). The paraventricular nucleus also signals the sympathetic nervous system to release adrenaline, specifically epinephrine. The release of epinephrine induces energy production from storage sites like fats and sugars to elicit a stronger stress response. The paraventricular nucleus signals the endocrine system to release corticotrophin-releasing hormone (CRH), a hormone that activates the hypothalamic–pituitary–adrenal (HPA) axis. The activation of the HPA axis in turn secretes glucocorticoid (cortisol) often referred to as the stress hormone. Cortisol functions to meet the immediate energy demand of the body by triggering the release of glucose from the liver to the blood(*33*, *34*). Cortisol also plays a key role in metabolism and influences how the body

breakdown proteins and fats for energy production.

During surgery, there are molecules termed damage-associated molecular patterns (DAMPs) that are released due to tissue injury and non-programmed cell death into the extracellular environment(*35–37*). DAMPS also known as alarmins can be intracellular proteins or extracellular matrix that include high mobility group box protein (HMGB), heat shock proteins, reactive oxygen species, actin and mitochondrial DNA(*38–41*). HMGB1, a nonhistone chromatin nuclear peptide is a central DAMP that normally functions as a transcriptional factor but once released from a damage cell, it can act as a danger signal(*35*, *36*). Another well characterized DAMP is small, fragmented DNA such as mitochondrial DNA (mtDNA). Mitochondrial DNA are small DNA fragments that were released into the circulation because of tissue damage and cell death. Mitochondrial DNA can trigger robust inflammation responses and an increase in mtDNA after surgery has been linked to a high risk of post-operative complications(*42*, *43*). DAMPs function as chemoattracts for other cells such as macrophages, neutrophils, and dendritic cells to migrate to the site of tissue damage. These DAMPs bind to pattern recognition receptors (PRRs) such as membrane bound Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) found on immune cells such as monocytes, neutrophils and nonimmune cells (endothelial cells, fibroblasts)(*44*, *45*). The activation of the TLRs in turn leads to the large production of pro-inflammatory cytokines. DAMPs also bind to another set of receptors, NOD-like receptor (NLRs), which are mostly found in the cytoplasm of cells(*46*). The recognition of DAMPs by NLRs initiates the NLRP3 inflammasome, a large multiprotein complex that functions to drive the upregulation of proinflammatory cytokines that leads to an inflammatory state(*47–51*).

**Inflammation**

As stated above, DAMPs generated from surgery induce innate immune response as well as triggering both local and systemic inflammation during the process of wound healing which results in the recruitment of several immune cells and the production of inflammatory molecules(*52*, *53*). Immune cells such as neutrophils and macrophages are known as the first responders and are recruited to the site of tissue damage to regulate the inflammatory process. Both cells release various inflammatory factors such as IL-1(α and β), IL-6, IL-18, TNF-α, VEGF and cyclooxygenase (COX)(*52*, *54–56*).

The proinflammatory molecule IL-6 plays a major role in the post-operative period. Upon IL-6 secretion, it drives an acute phase response by initiating the JAK-STAT3 pathway in the liver(*57*, *58*). The acute phase response involves the release of inflammatory proteins including C-reactive protein (CRP), serum amyloid A and fibrinogen which are responsible initiating tissue healing, regulating inflammation and increased phagocytosis by innate immune cells. IL-6 also plays a role in the anti-inflammatory pathway by originating a negative feedback loop of TNF-α and IL-1 production. Some studies have shown a correlation between the level of immunosuppression and the inflammatory cytokines produced during surgery such as IL-6(*59*, *60*).

Surgery induces the expression of cyclooxygenase (COX) during inflammatory response to tissue injury(*61*, *62*). COX in turn induces the production of prostaglandin E2 (PGE2), which is one of the major inflammatory factors that plays a role in tumor progression(*63*, *64*). PGE2 increases the production of Matrix Metalloproteinase-9 (MMP9), which is an enzyme responsible for the breakdown of the extracellular matrix of tissues, a process that promotes tumor invasion and metastasis. PGE2 has also been implicated in the advancement of an

immunosuppressive environment by increasing the presence of regulatory T (Treg) cells and decreasing the population of cytotoxic $CD8^+$ T cells(*65*, *66*). The presence of Tregs have been linked to exacerbating immunosuppression, further worsening cancer prognosis(*67*, *68*). Similarly, the population of myeloid-derived suppressor cells (MDSCs) also increases after surgery and correlates with cancer recurrence and poor prognosis because they can inhibit both the innate and adaptive immune response(*69*, *70*).

## 1.2 MYELOID-DERIVED SUPPRESSOR CELLS (MDSCS)

Myeloid-derived suppressor cells were initially identified as suppressor cells that inhibited T cell differentiation(*71*). The identification of these myeloid suppressor cells in cancer came later, when MDSCs were discovered in patients with various forms of cancer. Prior studies referred to this population of cells as immature myeloid cells or "IMCs" and is now recognized as myeloid derived suppressor cells(*72–74*).

MDSCs are a heterogeneous population of myeloid progenitors. In healthy individuals, MDSCs are not largely present but exist as immature myeloid cells (IMCs) which originate from the bone marrow and differentiate into neutrophils, monocytes, and macrophages in response to a signal(*75–77*). The signal most often includes products of infection and trauma, such as DAMPs, which interact with pattern recognition receptors on these myeloid cells, resulting in a state of rapid differentiation and mobilization of the immature myeloid cells to mature cells in a process termed emergency myelopoiesis(*78–80*).

MDSCs are identified in mice by the expression of $CD11b^+$ and $Gr\text{-}1^+$ and in humans by $CD33^+$ cells. MDSCs consist of two subgroups, which are monocytic (M-MDSC) and polymorphonuclear (PMN-MDSC) cells also known as granulocytic MDSCs. M-MDSC are identified as $CD14^+CD15^-$ and PMN-MDSCs are $CD14^-CD15^+$ (*81*). MDSCs are characterized by their ability to suppress several key immune cell populations, including natural killer cells and T cells(*82*, *82*, *83*).

### 1.2.1 Myeloid Derived Suppressor Cells and Suppression of CD8⁺ T cells

MDSCs can facilitate immune suppression by utilizing numerous mechanisms, including the production of factors such as Reactive oxygen species (ROS), arginase (ARG1), inducible nitric oxide synthase (iNOS), Transforming growth factor beta (TGFβ), IL-6, IL-10, Cyclooxygenase-2 (COX2), indoleamine 2,3-dioxygenase (IDO), and many other factors(*69*, *84*, *85*).

The mechanism of immune suppression depends on the predominant MDSC subtype present. M-MDSCs primarily mediate suppression through iNOS and immunoregulatory cytokines, whereas PMN-MDSCs suppress target cells, particularly T cells through the production ROS and ARG1(*86*, *87*).

In murine cancer models, multiple studies have shown that MDSCs inhibit T-cell activity, and that depletion of these cells restores T-cell cytotoxic function(*88–90*). MDSCs can inhibit T cells by depleting important amino acids required for T cell function and activation, including arginine, tryptophan, and cysteine. For example, a study observed that when T cells were in the presence of MDSCs with high arginase activity, arginine levels decreased, leading to reduced CD3 expression, an important factor in T cell activation(*91*). MDSCs use molecules such as ROS and iNOS to inhibit the activation of the JAK-STAT axis, therefore inhibiting the function of T cells, including proliferation and cytotoxicity(*92*, *93*). MDSCs can also suppress T cells through the PD-L1/PD-1 checkpoint axis, a process that halts T cell effector function and drives T cells into an exhausted state(*94–96*) . The mechanism of MDSC suppression of T cells has been well characterized specifically in the context of cancer; however, the extent to which these mechanisms are responsible for surgery-induced T cell dysfunction has not been explored or understood.

## 1.3 $CD8^+$ CYTOTOXIC T CELLS

The importance of T cells was initially overlooked and misunderstood until studies from the 70s established that these cells were essential for immunity(*97*, *98*). $CD8^+$ T cells are the primary drivers of the adaptive immune system. They are generally identified as $CD3^+$ and $CD8^+$ T cells. $CD8^+$ T cells play an essential role in both infection and tumor surveillance. $CD8^+$ T cells become activated upon recognition of an antigen and undergoes certain mechanisms to carry out their effector functions. A major function of $CD8^+$ T cells is secreting cytokines such as IFNγ, TNFα, IL-2, which are cytokines that have anti-tumor effects and aid in effector immune responses(*99*). Another major function of $CD8^+$ T cells is the production of lysosomal cytotoxic granules composed of a lipid bilayer containing lysosomal-associated proteins (LAMPs), such as LAMP-1 or CD107a(*100*, *101*). These granules house perforin and granzyme, the two main cytotoxins required for lysis. The contents of granules are then deposited toward the target cell in a bid to kill the cell, which is a process termed degranulation. These functions are an integral part of $CD8^+$ T cell's ability to regulate primary tumor outgrowth.

When naive $CD8^+$ T cells encounter a pathogen, they begin to differentiate and divide into other subsets. In most infection and vaccination models, it has been shown that the majority of expanded $CD8^+$ T cells differentiate into short-lived effector T cells (TEC), characterized by $CD62L^{low}CD44^{high}$ phenotype peaks by 7 to 9 days post infection termed the expansion phase(*102*, *103*). These TECs rapidly disappear during the contraction phase, 12 days post infection. The second subset of $CD8^+$ T cells are Long-lived effector T cells or effector memory cells (TEM), which are smaller in number and are characterized by $CD62L^{high}CD44^{high}$ survive for longer periods of time.

### 1.3.1 $CD8^+$ Cytotoxic T cells and Metastases

Mouse models, such as colorectal cancer and mammary carcinomas, have been used to establish a critical role for $CD8^+$ T cells in preventing tumor outgrowth in mice with established micro-metastases. In these models, depleting the $CD8^+$ T cell population results in accelerated, progressive tumor outgrowth(*104*, *105*). In these depletion studies of tumor bearing mouse models, most CD8-depleted mice succumbed to metastatic disease, compared to CD8-sufficient mice. These data demonstrated that $CD8^+$ T cells delayed the spread of visceral metastases. Although they show that $CD8^+$ had no effect on established tumors, they can control disease spread and progression. $CD8^+$ T cells have been implicated in the ability to control the growth of disseminated tumor cells in visceral organs(*106–108*). $CD8^+$ cytotoxic T lymphocytes (CTLs) indirectly suppress metastatic dissemination by contributing to the adaptive immunological control of primary tumors and by preserving their normal vascularization. $CD8^+$ T cell depletion leads to increased intravasation due to enhanced vascularization and increased circulating tumor cell survival due to the reduction in the population of $CD8^+$ T cells(*109*, *110*). Furthermore, studies have shown that increased inflammation following surgery promotes the occurrence of tumor outgrowth which is attenuated by tumor specific T cell responses(*111*, *112*).

### 1.3.2 $CD8^+$ Cytotoxic T cells and Dysfunction after Surgery

While it is challenging to directly link post-operative $CD8^+$ T cell dysfunction with cancer recurrence there is accumulating data in multiple cancer types that has clearly established the presence of a profound defect in $CD8^+$ T cell function in the aftermath of cancer surgery(*113*–*117*). For example, in a clinical trial where patients had gastric bypass, significant decreases were found in effector $CD8^+$ T cell cytotoxicity compared with pre-operative levels(*117*). Furthermore, surgery decreases $CD8^+$ T cell numbers as well as their cytokine-secreting ability, elucidating that surgical stress causes T cell dysfunction post-operatively in most digestive surgeries(*113*, *114*, *116*). In a clinical study of 415 patients with colorectal cancer, high levels of infiltrating $CD45RO^+$ memory T cells were associated with improved survival and the absence of early metastatic invasion, reflected by a less advanced pathological stage defined by vascular emboli, lymphatic invasion, and perineural invasion (collectively referred to as VELIPI)(*118*). This study has led to the development of the immunoscore assay, which is a method that measures the infiltration of $CD3^+$ and $CD8^+$ T lymphocytes in the tumor site and is used to predict cancer recurrence(*119*).

In a previous study, our research group used mice vaccinated with a recombinant Adenovirus vector expressing human Dopachrome Tautomerase (hDCT) gene to characterize the effects of surgery on anti-tumor $CD8^+$ T cell responses(*120*). The study demonstrated that antigen-specific T cells are suppressed following surgical stress, which resulted in the recurrence of tumors and metastases. Mice that had been vaccinated with AdDCT and subjected to surgery developed more tumors and metastases than vaccinated mice that did not undergo surgery. Surgical stress also reduced $CD8^+$ T cell proliferation and recruitment to the tumor microenvironment(*120*). DCT specific $CD8^+$ T cells from vaccinated mice that had major surgery showed reduced ability to secrete IFNγ, TNFα and Granzyme B. Together, these findings suggest that surgery significantly attenuates $CD8^+$ T cell function and suppresses an anti-tumor T cell immunity. However, the mechanism(s) underlying the observed $CD8^+$ T cell dysfunction induced by surgery are not well understood and require a model that will permit further mechanistic analysis.

## 1.4 OBJECTIVE, RATIONALE, HYPOTHESIS, AND CHAPTER OVERVIEW

### 1.4.1 Overarching Objective

The overarching objective of this doctoral thesis is to identify the mechanisms that cause $CD8^+$ T-cell dysfunction during the post-operative period.

### 1.4.2 Aims

1. To establish an animal model to characterize post-operative, antigen-specific $CD8^+$ T cell dysfunction.

2. To use this model to investigate the mechanistic pathways involved in $CD8^+$ T cell suppression following surgery.

3. To validate the identified pathways that contribute to post-operative $CD8^+$ T cell dysfunction.

### 1.4.3 Hypothesis

This thesis hypothesizes that targeting the mechanisms underlying post-operative $CD8^+$ T cell dysfunction will improve cancer outcomes following surgery.

### 1.4.4 Chapter Overview

Chapter 2: I characterized $CD8^+$ T cell dysfunction in mice, focusing on antigen specificity, using an OT-I mouse model. I also characterized the impact of GR-$1^+$ cells (MDSCs) on $CD8^+$ T cell function.

Chapter 3: I investigated $CD8^+$ T cell dysfunction in patients undergoing cancer surgery to further establish its translational relevance.

Chapter 4: I sought to identify a possible mechanism of dysfunction by performing scRNA-seq on isolated $CD8^+$ T cell from the established animal model from chapter 2

In Chapter 5, I validated the results from Chapter 4 in mice and human $CD8^+$ T cells, as well as established a possible link between GR-$1^+$ cells (MDSCs) and the identified pathway from the scRNA seq data.

In Chapter 6, I discuss the findings from Chapters 2 to 5, while focusing on overarching concepts.

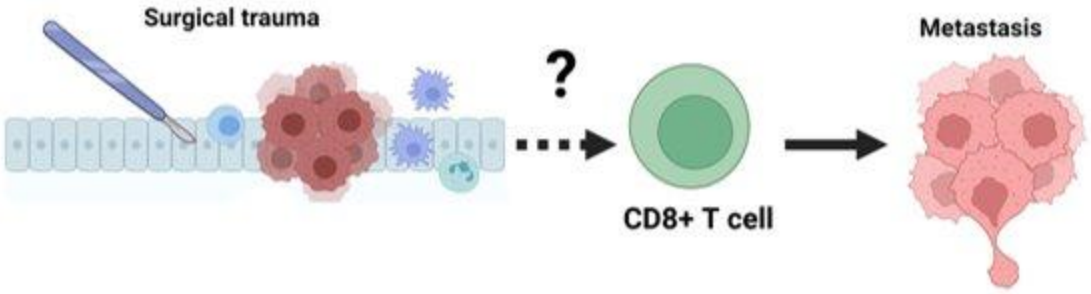

Surgical trauma
?
CD8+ T cell
Metastasis

**Figure 1.2: Schematic of proposed objective**

Surgical resection of tumors results in suppression of $CD8^+$ T cells, which can worsen cancer outcomes and increase the risks of metastasis. Therefore, identifying a mechanism of surgery-induced suppression is important for the development of immunotherapies that can alleviate immunosuppression. *Created with Biorender.com*

# Chapter 2: Surgical Stress Induces Post-operative murine $CD8^+$ T Cell suppression

## 2.0 RATIONALE

Surgical stress induces a significant decrease in $CD8^+$ T cell function in murine studies. However, the focus of these studies has been on non-antigen-specific dysfunction following surgery. Therefore, the objective of this chapter is to confirm non-antigen $CD8^+$ T cell dysfunction in mice and characterize antigen-specific dysfunction using an OT-I mouse model. I also characterized the impact of GR-$1^+$ cells (MDSCs) on $CD8^+$ T cell function by depleting these cells *in-vivo* and performing functional assays. Characterization of dysfunction in an animal model is important because it provides a foundation for future mechanistic studies.

## 2.1 INTRODUCTION

$CD8^+$ T cells play an essential role in tumor surveillance. $CD8^+$ T cells become activated upon antigen recognition and use specific mechanisms to carry out their effector functions. A key function of $CD8^+$ T cells is their ability to secrete effector cytokines such as IFNγ, TNFα, IL-2, which play an important role in effector immune response(*121*, *122*). IFNγ improves the cytotoxic function of $CD8^+$ by increasing antigen presentation of both target cells and antigen-presenting cells. TNFα helps induce cell death of target cells by binding to the TNF receptor and triggering apoptosis. $CD8^+$ T cells use IL-2 to promote their expansion as well as survival during immune response. These cytokines also act as proinflammatory signals to recruit additional immune cells, such as macrophages and additional $CD8^+$ T cells, to the appropriate site. Another major function of $CD8^+$ T cells is the production of lysosomal cytotoxic granules to kill target cells. These cytotoxic granules are composed of perforin and granzymes that are released from $CD8^+$ T cells. Perforin causes the production of pores on the target cell membrane, while granzymes act as a serine protease that cleaves the apoptosis related protein, caspase 3 to initiate cell death. During the release of cytotoxic granules, the membrane of the granules which are composed of a lipid bilayer containing lysosomal-associated proteins (LAMPs), such as LAMP-1 (CD107a) fuses with that of the target cell, a process known as degranulation. All together, these effector functions are integral to the ability of $CD8^+$ T cells to control primary tumor outgrowth.

As mentioned above, we first identified the effects of surgery on antigen-specific immunity using a murine model of anti-tumor vaccination with an adenovirus encoding the dopachrome tautomerase melanin antigen (AdDCT )(*120*) . The study demonstrated that tumor antigen-specific T cells are suppressed following surgical stress, which resulted in recurrence of tumors and metastases. Mice that had been vaccinated with AdDCT and subjected to surgery developed more tumors and metastases than vaccinated mice that did not undergo surgery. Surgical stress also reduced $CD8^+$ T cell proliferation and recruitment to the tumor microenvironment. We observed that surgery reduced the number of $CD8^+$ T cells, which was not due to increased apoptosis but rather to a significant decrease in T cell proliferation in response to the DCT antigen. This was done by using a tetramer specific to DCT, where the total number of DCT-specific $CD8^+$ T cells was similar in the non-surgically stressed mice and the surgically stressed mice, but IFNγ production by $CD8^+$ T cells in response to DCT antigen was reduced. DCT specific $CD8^+$ T cells from vaccinated mice that had major surgery showed reduced ability to secrete IFNγ, TNFα and Granzyme B on post-operative day (POD) 1, and recovered between POD7 and POD28. These findings suggest that surgery significantly attenuates $CD8^+$ T cell functionality and suppresses an anti-tumor T cell immunity.

However, the frequency of T cells that responded to tumor vaccine antigens are relatively low and did not allow for a comprehensive investigation of mechanistic questions. Therefore, this ultimately led us to generate a different model to characterize the antigen specific dysfunction which will be addressed in this chapter.

## 2.2 METHODS

### Mice

Six- to 8-week-old female C57BL/6 and inbred OT-I mice were purchased from The Jackson Laboratory. The OT-I mice background has been described previously(*123*). Animals were maintained in accordance with institutional guidelines at the Canadian Council on Animal Care and the Animal Care Committee of the University of Ottawa.

For adoptive transfer experiments, C57BL/6 mice received an adoptive transfer of (1 x $10^4$ OT-I cells/100 ul of PBS) intravenously (IV). Recipient mice (C57BL/6) were then immunized with *Listeria monocytogenes* expressing OVA (LM-OVA, $10^4$ CFU/injection/100 µl of PBS), strain 10403s. The formation of LM-OVA has been described previously(*124*). Next, recipient mice were challenged subcutaneously (sq) with B16-OVA (1 X $10^5$ cells/100 µl of PBS).

### Cell lines

B16-OVA cells were obtained from Dr. John Bell's laboratory at the Ottawa Hospital Research Institute. Cell lines are maintained in culture with Dulbecco's Modified Eagle's Media (DMEM) supplemented with 10% fetal bovine serum (FBS) and 400 µg/ml of geneticin (G418).

Primary cells were harvested from the spleens manually. The cells were then treated with Ammonium Chloride Potassium (ACK) lysis buffer to remove red blood cells. For *ex vivo* studies, primary cells were cultured with Roswell Park Memorial Institute medium (RPMI) containing 10% FBS and 1% Non-Essential Amino Acids. All cells were cultured at 37ºC with 5% $CO_2$

**Ex-vivo Peptide Stimulation**

OT-I $CD8^+$ T cells were stimulated with H-2Kb OVA SIINFEKL (2 μg/mL) for 4 hours. For nonspecific antigen stimulation, anti CD3/CD28(2ug/ml/5ug/ml), PMA and Ionomycin(50ng/ml/1ug/ml), high dose IL-2 (1000U/ml) and phytohemagglutinin-P (5ug/ml) were used.

For intracellular cytokine staining, cells were first stimulated for 1 hour and were additionally stimulated for 4 hours with BD GolgiPlug transport or BD GolgiStop, with both inhibitors containing brefeldin A (10ug/ml) and monensin (10ug/ml), respectively.

**Immune Cell Profiling**

Following cell processing or stimulation, cells are first washed with FAC buffer (PBS with 1% BSA and 0.5mM EDTA). Subsequently, cells are labelled with Fixable Viability Stain 510 (BD, #564406) for 15 minutes at 4ºC. Primary cells are then treated with Purified Rat Anti-Mouse CD16/CD32 (BD, #553142) or Human Fc BD block (BD, #564219) for 5 minutes at 4ºC. Next, the cells are stained with an extracellular marker master mix at the appropriate dilutions for 20 min at 4ºC.

For intracellular staining, cells are fixed with BD Cytofix/Cytoperm™ (BD) following the manufacturer's protocol. A detailed list of antibodies can be found in appendix A. Following staining protocols, cells were acquired with flow cytometry acquired with a BD LSRFortessa using BD FACSDiva™ version 8.0.3 software. The flow cytometry data was analyzed with FlowJo, LLC version 10.8.2 software).

**Myeloid cell depletion**

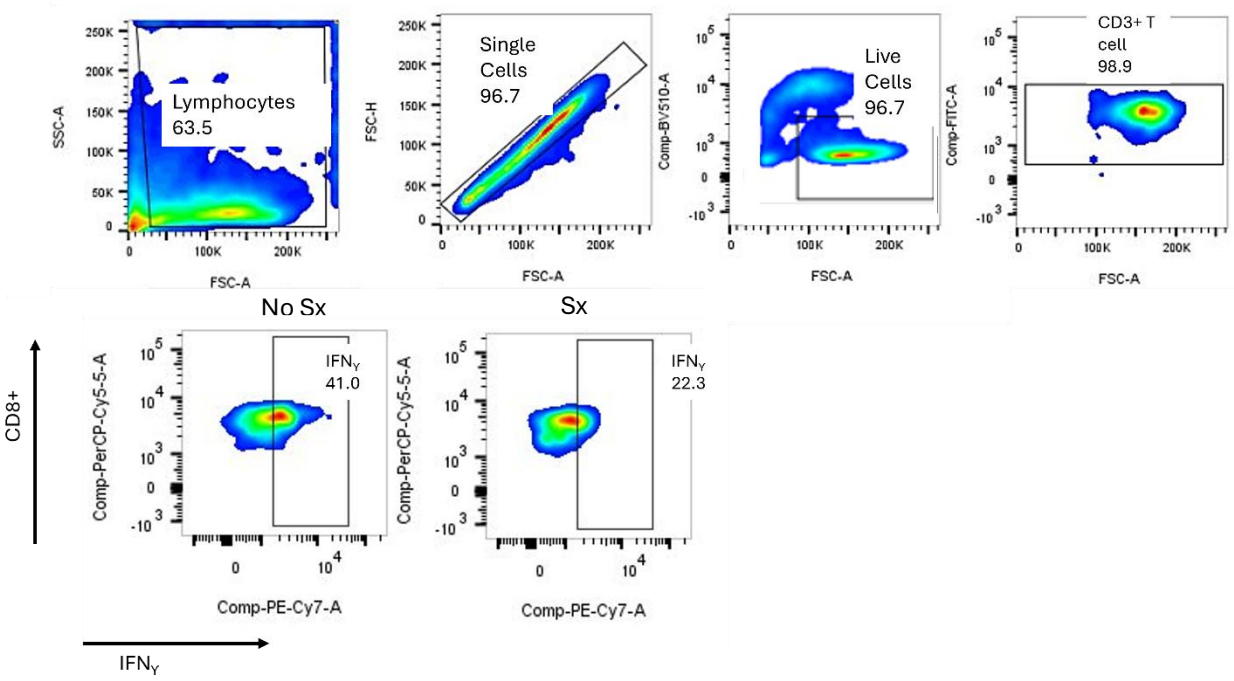
OT-I mice received a dose of intraperitoneal injections of anti-GR1 antibody (BioxCell InVivoMab anti-mouse Ly6G/Ly6C (Gr-1), Catalog # BE0075-5MG-A) prepared in PBS (100ug/dose) for 2 days prior to the day of the surgical procedure.

## 2.3 RESULTS

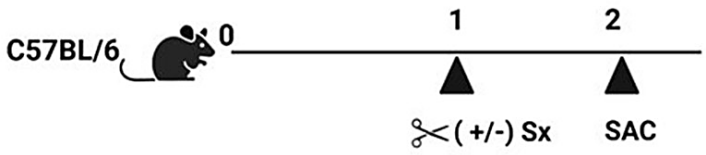
### Murine $CD8^+$ T cells are suppressed after surgery independent of stimuli

The first step was to establish that post-operative $CD8^+$ T cells are suppressed independent of the stimulation. Non antigen stimulation such as monoclonal anti CD3/CD28 antibodies which engages the TCR receptor results in the activation of $CD8^+$ T cells comparable to antigen specific stimulation. Another nonspecific stimulation is phytohemagglutinin-P (PHA-P). PHA-P is a plant derived lectin that can cross link the glycoproteins on the TCR of $CD8^+$ T cells which triggers a downstream cascade of intracellular signaling that ultimately results in activation(*125*). High dose IL-2 is another way to stimulate $CD8^+$ T cells, which bind to the IL-2 receptor on $CD8^+$ T cells, activating downstream signaling cascades that result in activation(*126*). Splenocytes isolated from the spleens of C57BL/6 mice of surgically stressed or control mice were stimulated with all three stimulation methods mentioned above for 4 hours ex vivo. Following stimulation, we assessed the effector function of surgically stressed $CD8^+$ T cells by measuring cytokine secretion and degranulation, a marker of cytotoxic function. According to the data, we observed an approximate 2-fold decrease in the production of IFNγ, TNFα, and CD107a in $CD8^+$ T cells after nonspecific stimulation 24 hours after surgery in the surgically stressed group (Fig 2.1).

Gating strategy



**A**


C57BL/6
0
1
2
(+/-) Sx
SAC

**B**

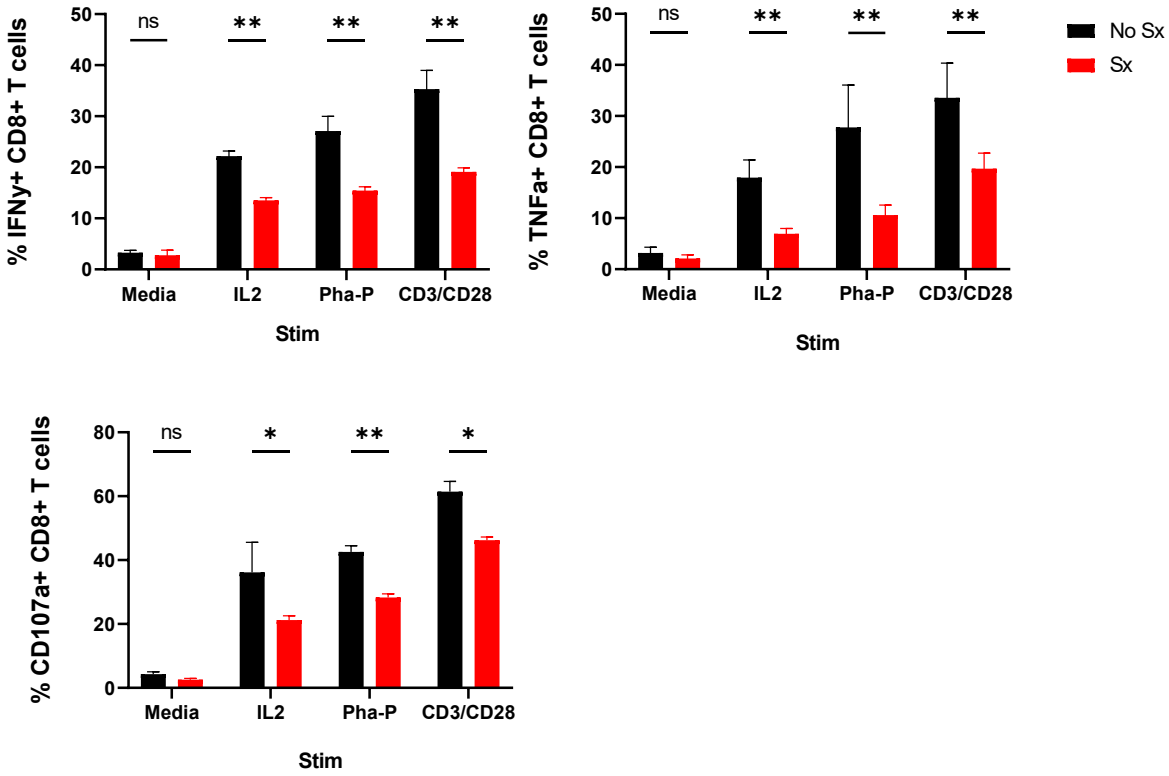

% IFNy+ CD8+ T cells
% TNFa+ CD8+ T cells
% CD107a+ CD8+ T cells
ns
**
*
Media
IL2
Pha-P
CD3/CD28
Stim
No Sx
Sx

**Figure 2.1: Murine $CD8^+$ T cells are suppressed after surgery independent of stimuli**

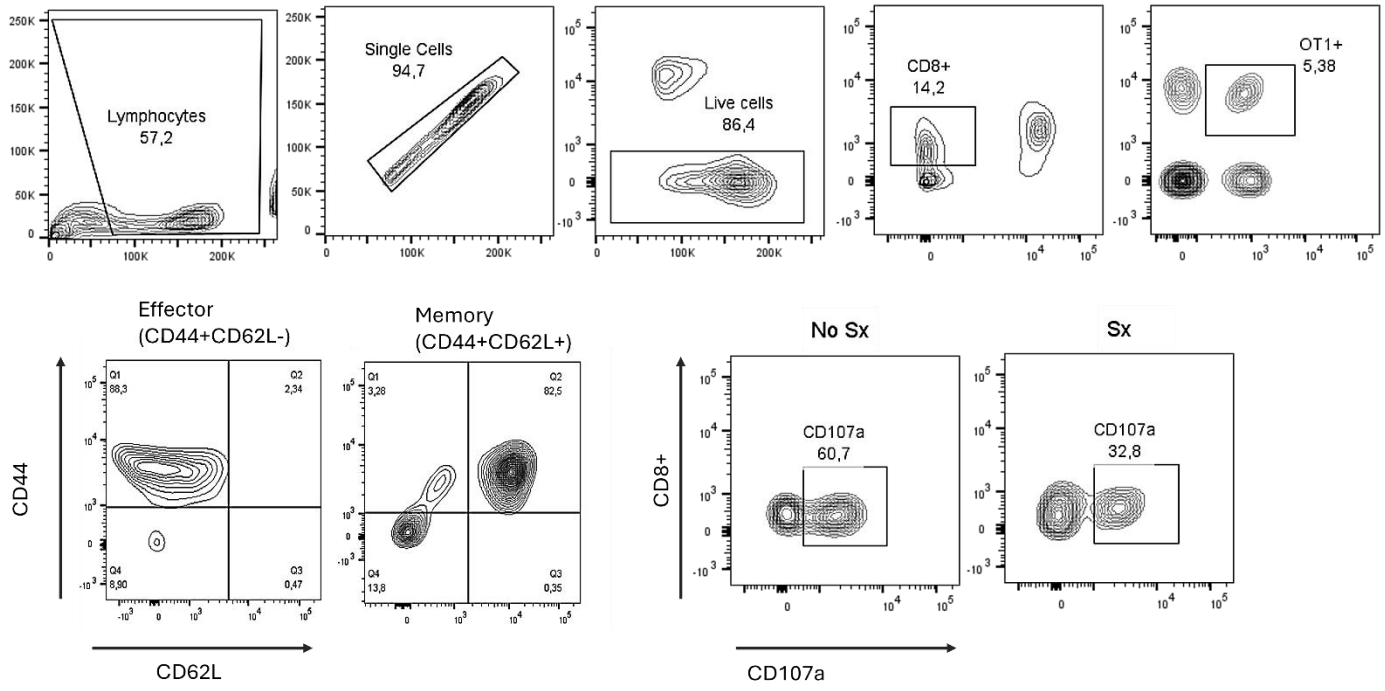
**(A)** Schematic diagram of the experimental plan. C57BL/6 mice underwent surgery (Nx-nephrectomy) +/- on day 1. On day 2, mice were sacrificed, and $CD8^+$ T cell characterization was performed. **(B-D)** Percentage of $IFN\gamma^+$, $TNF\alpha^+$, and $CD107a^+$ $CD8^+$ T cells reacting to IL-2 (1000U/Ml), Pha-P (5ug/ml), anti-CD3 and anti-CD28 (2ug/ml and 5ug/ml) stimulation. n =5. (*$p \leq 0.05$; **$p \leq 0.01$). Statistical analyses were analyzed by performing Mann-Whitney U test**.**

**Naïve and Antigen specific murine CD8$^+$ T cells are dysfunctional after surgery**

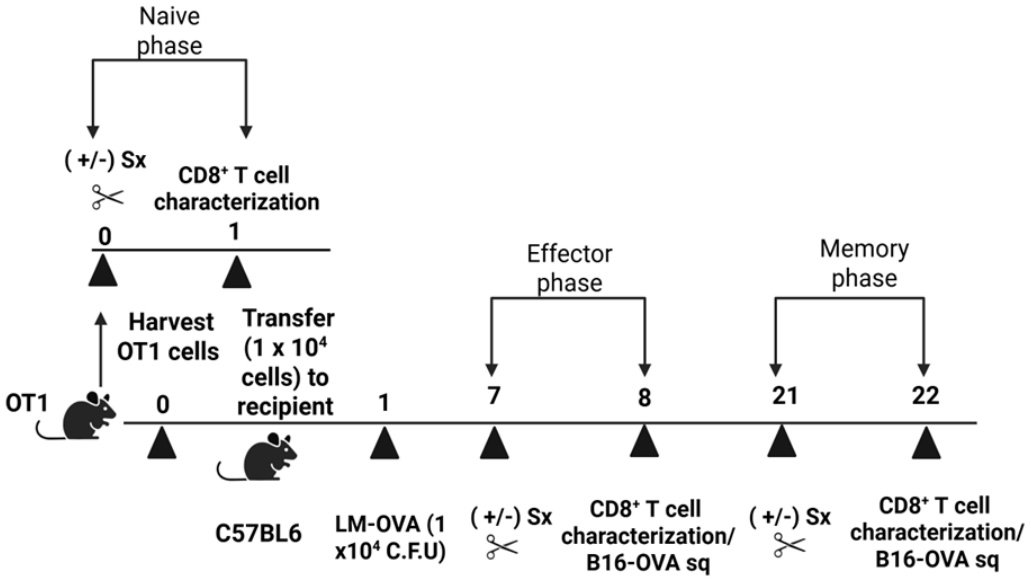
Subsequently after establishing that surgery suppresses CD8$^+$ T cells post-operatively independent of stimulation, the next step would be to characterize the effects of surgery in an antigen specific model. As stated above, we have previously characterized dysfunction in a mouse model of vaccination with AdDCT, however this model did not generate a large enough number of antigen specific CD8$^+$ T cell response to the DCT peptide. Therefore, to overcome this limitation, I utilized the well characterized OT-I mouse model as a quantitative tool to characterize anti-tumor specific T cell dysfunction post-operatively. OT-I is a transgenic strain of mice with CD8$^+$ T cells specific for the OVA peptide of amino acids 257-264 (SIINFEKL). The OT-I mouse model is a valuable tool because they allow for isolation and quantification of T cell responses to a specific antigen. We have developed a model where we transfer naïve OT-I CD8$^+$ T cells to a C57BL/6 mouse and generate effector and central memory T cells by vaccination with an OVA-expressing Listeria (Fig 2.2A).

We first characterized the dysfunction in an antigen specific naïve CD8$^+$ T cell population by sacrificing the mice on day 1 after surgery for CD8$^+$ T cell characterization (Fig 2.2). In this naive model, surgery causes significant CD8$^+$ T cell dysfunction in response to SINFEKL antigen on POD1 (Fig 2.2B), including a reduction in cytokine IFNγ (9.2% ± 1.3%), TNFα (22.0% ± 4.5%), and degranulation (CD107a; 19.2% ± 2.5) compared to non-surgically stressed CD8$^+$ T ( IFNγ; 24.0% ± 5.6%, TNFα; 41.6 % ± 5.9%, CD107a; 36.8% ± 7.4%).

## Gating strategy



**A**


Naive phase
( +/-) Sx
CD8+ T cell characterization
0
1
Effector phase
Memory phase
Harvest OT1 cells
Transfer (1 x 10^4 cells) to recipient
OT1
0
1
7
8
21
22
C57BL6
LM-OVA (1 x10^4 C.F.U)
( +/-) Sx
CD8+ T cell characterization/ B16-OVA sq
( +/-) Sx
CD8+ T cell characterization/ B16-OVA sq

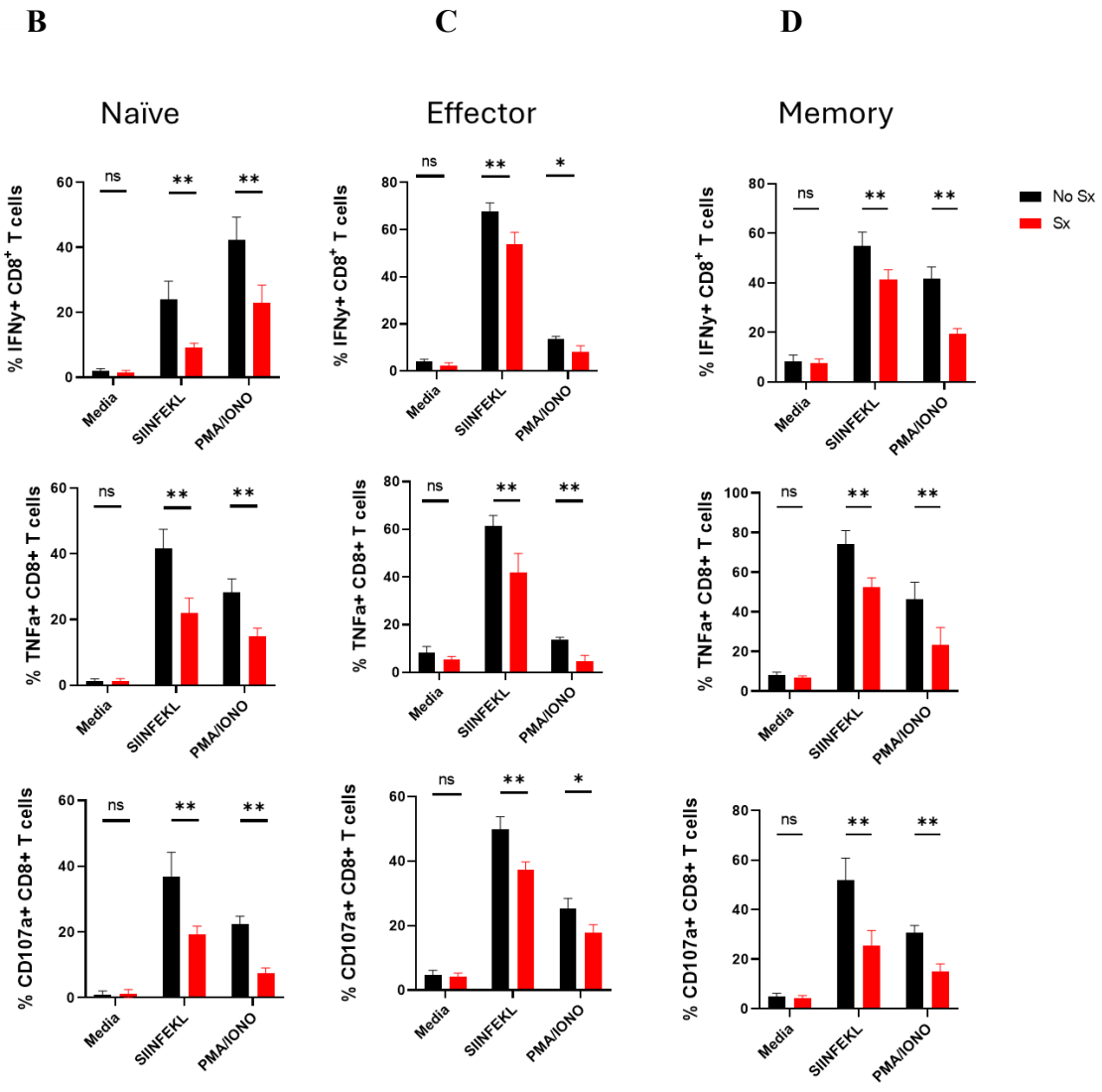

B
C
D
Naïve
Effector
Memory
No Sx
Sx
% IFNγ+ CD8+ T cells
% TNFa+ CD8+ T cells
% CD107a+ CD8+ T cells
Media
SIINFEKL
PMA/IONO
ns
**
*

**Figure 2.2: Naïve and antigen-specific murine $CD8^+$ T cells are dysfunctional after surgery**

**(A)** OT-I mice underwent abdominal laparotomy and nephrectomy (Sx) or no surgery (No Sx). At day 1, mice were sacrificed and underwent $CD8^+$ T cell characterization for naïve immune response. C57/BL6 mice received an adoptive transfer of 1 x 104 OT-I cells and 1 x 104 LM-OVA on days 0 and 1, respectively. At days 7 and 21, mice received Sx or No Sx. At days 8 and 22, mice were sacrificed and underwent $CD8^+$ T cell characterization for effector and memory immune responses. **(B)** Percentage of naive $IFN\gamma^+$, $TNF\alpha^+$, and $CD107a^+$ $CD8^+$ T cells reacting to SIINFEKL peptide stimulation**. (C)** Percentage of effector $IFN\gamma^+$, $TNF\alpha^+$, and $CD107a^+$ $CD8^+$ T cells ($CD44^{hi}$) reacting to SIINFEKL peptide stimulation**. (D)** Percentage of memory $IFN\gamma^+$, $TNF\alpha^+$, and $CD107a^+$ $CD8^+$ T cells ($CD44^{hi}$, $CD62L^{hi}$) reacting to SIINFEKL peptide stimulation**.** Percentage of $IFN\gamma^+$, $TNF\alpha^+$, and $CD107a^+$ $CD8^+$ T cells reacting to PMA and ionomycin (50ng/ml and 1ug/ml) stimulation for all CD8+ T cell populations was also included as a control. n = 5. (*$p \leq 0.05$; **$p \leq 0.01$). Statistical analyses were analyzed by Mann-Whitney U test**.**

Next, we generated a $CD8^+$ T cell response to ovalbumin peptide by first adoptively transferring OT-I cells to a C57BL6 mice, and then we administered an I.V dose of LM-OVA to elicit an immune response against the OVA antigen. Surgery was performed on the mice at the peak effector response which has been previously established in literature to occur on day 7. We performed functional assays on the effector population and observed that surgical stress reduces the ability of the $CD8^+$ T cells to secrete cytokines such as IFNγ (53.1% ± 5.1%), TNFα (42.1% ± 7.8%) in comparison to non-surgically stressed $CD8^+$ T cells (IFNγ; 70.0% ± 3.5%, TNFα; 61.4 % ± 4.9%). (Fig 2.2C). We detected a reduction in cytotoxicity by measuring degranulation; CD107a (37.2% ± 2.5%) in the surgically stressed $CD8^+$ T cells versus non-surgically stressed $CD8^+$ T cells (50.0% ± 3.9%). We also looked at the memory population which occurs at around day 20 where we performed functional assays and saw a similar result with surgically stressed $CD8^+$ T cells were unable to produce cytokines in comparison to non-surgically stressed cells (IFNγ; 40.1% ± 3.9% vs 55% ± 5.4, TNFα; 52.5% ± 4.6% vs 74.0% ± 7.0%) as well as degranulation (CD107a; 25.6% ± 6.0% vs 52.0% ± 8.8) (Fig 2.2D). We observed more suppression in the memory population than in the effector population, therefore the memory population was selected for downstream assays to further characterize dysfunction in $CD8^+$ T cells.

**Surgical stress impairs anti-tumor immunity and promotes subcutaneous tumor outgrowth**

Following the establishment of an impaired $CD8^+$ T cell effector function after surgery, we wanted to determine if surgical stress affects the ability of $CD8^+$ T cells to clear tumor cells in an *in vivo* model. Isolated $CD8^+$ T cells were adoptively transferred into a C57BL/6 naïve recipient mouse at a dose of 1 $x10^7$ cells. Surgery was then performed on some of the mice that received the adoptive transfer. A day after surgery, mice received a subcutaneous injection of melanoma tumor (B16F10) cells expressing ovalbumin. Following the tumor challenge, tumor growth was monitored, and a survival curve was created (Fig. 2.3A).

We observed that control groups all grew tumors post-tumor challenge within 14 days (Fig. 2.3 B). Subsequently, mice that received adoptive transfer and were subjected to surgical stress exhibited 100% tumor outgrowth within 20 days following tumor challenge, whereas no tumor outgrowth was observed in the non-surgically stressed group.

Analysis of tumor growth showed that both control groups exhibited the greatest tumor burden, followed by mice that received adoptive transfer and were subjected to surgical stress (Fig. 2.3 D). Mice that received adoptive transfer of $CD8^+$ T cells without surgical stress exhibited the least tumor growth. These findings suggest that surgical stress attenuates the ability of antigen-specific $CD8^+$ T cells to control tumor outgrowth.

In the survival analysis, both control groups that did not receive adoptive transfer succumbed by day 30 following tumor implantation (Fig. 2 .3C). Recipient mice that received OT-I T cells and were subjected to surgical stress had a median survival of 35 days, whereas all mice that received OT-I T cells without surgical stress survived to day 40 post-tumor implantation.

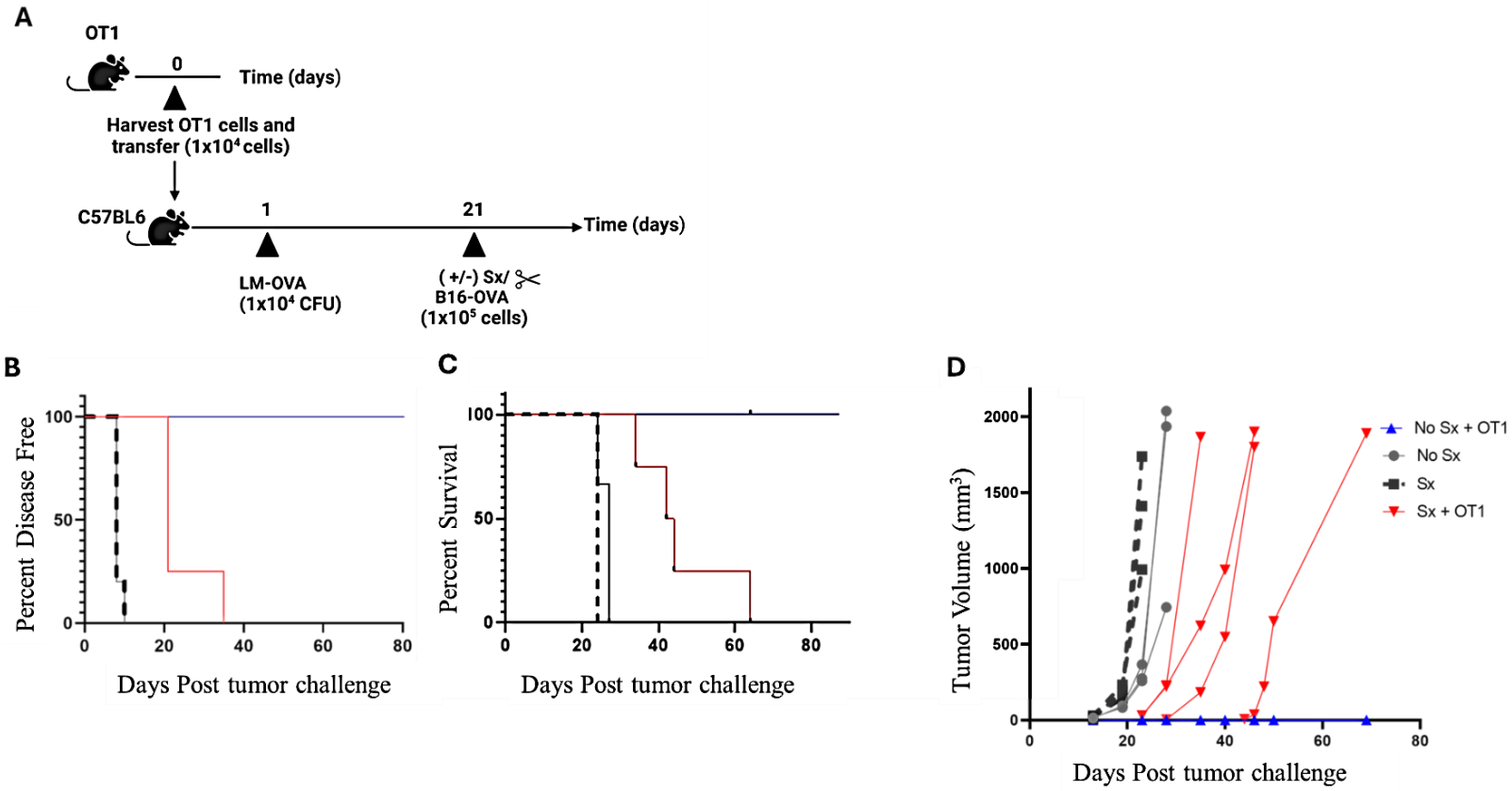

A
OT1
0
Time (days)
Harvest OT1 cells and transfer (1x10⁴ cells)
C57BL6
1
21
Time (days)
LM-OVA (1x10⁴ CFU)
(+/-) Sx/ B16-OVA (1x10⁵ cells)
B
Percent Disease Free
Days Post tumor challenge
C
Percent Survival
Days Post tumor challenge
D
Tumor Volume (mm³)
Days Post tumor challenge
No Sx + OT1
No Sx
Sx
Sx + OT1

**Figure 2.3: Surgical stress impairs anti-tumor immunity, resulting in subcutaneous tumor outgrowth**

**(A)** C57/BL6 mice received an adoptive transfer of 1 x $10^4$ OT-I cells and 1 x $10^4$ C.F.U of LM-OVA on day 0 and day 1, respectively. On day 21, recipient mice received surgery (+/). **(B)** On day 21, C57/BL6 recipient mice were challenged with 1 × $10^5$ B16-OVA cells, and the percentage of disease-free mice and **(C)** survival of treated B16-OVA tumor-bearing mice are shown (**D)** Corresponding **tumor** volumes of the mice were also plotted post tumor challenge. n = 5.

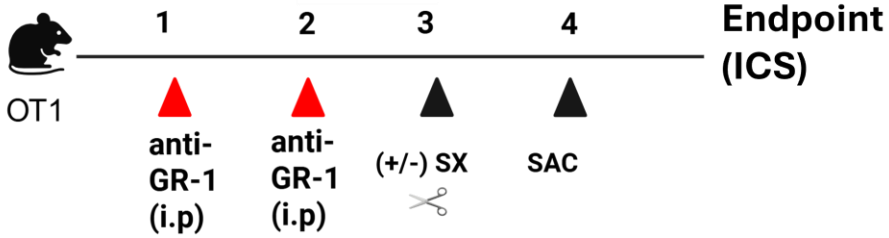
**Pre-operative depletion of myeloid cells enhances $CD8^+$ T Cell function in mice**

The suppressive effects of myeloid derived suppressor cells on immune cells such as NK and T cells have been well established in both preclinical and clinical studies, as stated above. In previous studies, our laboratory demonstrated that myeloid-derived suppressor cells (MDSCs/ GR-$1^+$ cells) expand markedly following surgery and are referred to as surgery-induced MDSCs (sx-MDSCs)(*120*, *127–129*). These sx-MDSCs cells have been shown to induce suppression of both NK(*127*, *130*) and $CD8^+$ T cells(*131*). To corroborate these findings in $CD8^+$ T cells, we assessed the suppressive capacity of these myeloid cells in mice. We hypothesized that depleting these myeloid cells can alleviate the dysfunction we previously observed to some extent. To determine whether myeloid cells play a role in post-operative immune suppression, OT-I mice received two intraperitoneal injections of anti-GR1 antibody. OT-I mice underwent surgery and were sacrificed on post-operative day 1. Splenocytes were isolated from both the surgery and no surgery groups of OT-I mice and stimulated with the SIINFEKL peptide as described previously. Cytokine production was measured by ICS staining and visualized by flow cytometry.

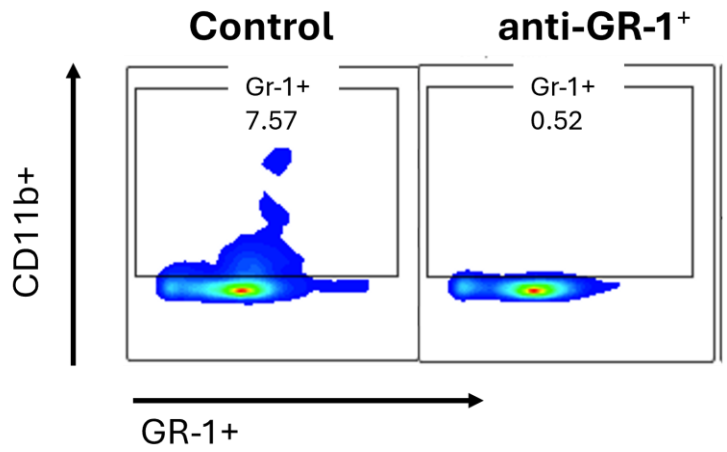
As expected, we observed a decrease in the production of IFNγ, TNFα and CD107a in the surgically stressed group (Fig 2.4C). However, there is an improved production of IFNγ (20.5%

± 2.0%), TNFα (51.7% ± 3.3%), CD107a (68.0% ± 8.4%) in the surgically stressed and GR-1$^{+}$ depleted treatment group compared with the non-treatment surgically stressed group, IFNγ (47.5% ± 1.7%), TNFα (34.5% ± 5.4%), CD107a (47.0% ± 9.8%).

**A**


1
2
3
4
Endpoint
(ICS)
OT1
anti-
GR-1
(i.p)
anti-
GR-1
(i.p)
(+/-) SX
SAC

**B**


Control
anti-GR-1+
Gr-1+
7.57
Gr-1+
0.52
CD11b+
GR-1+

C

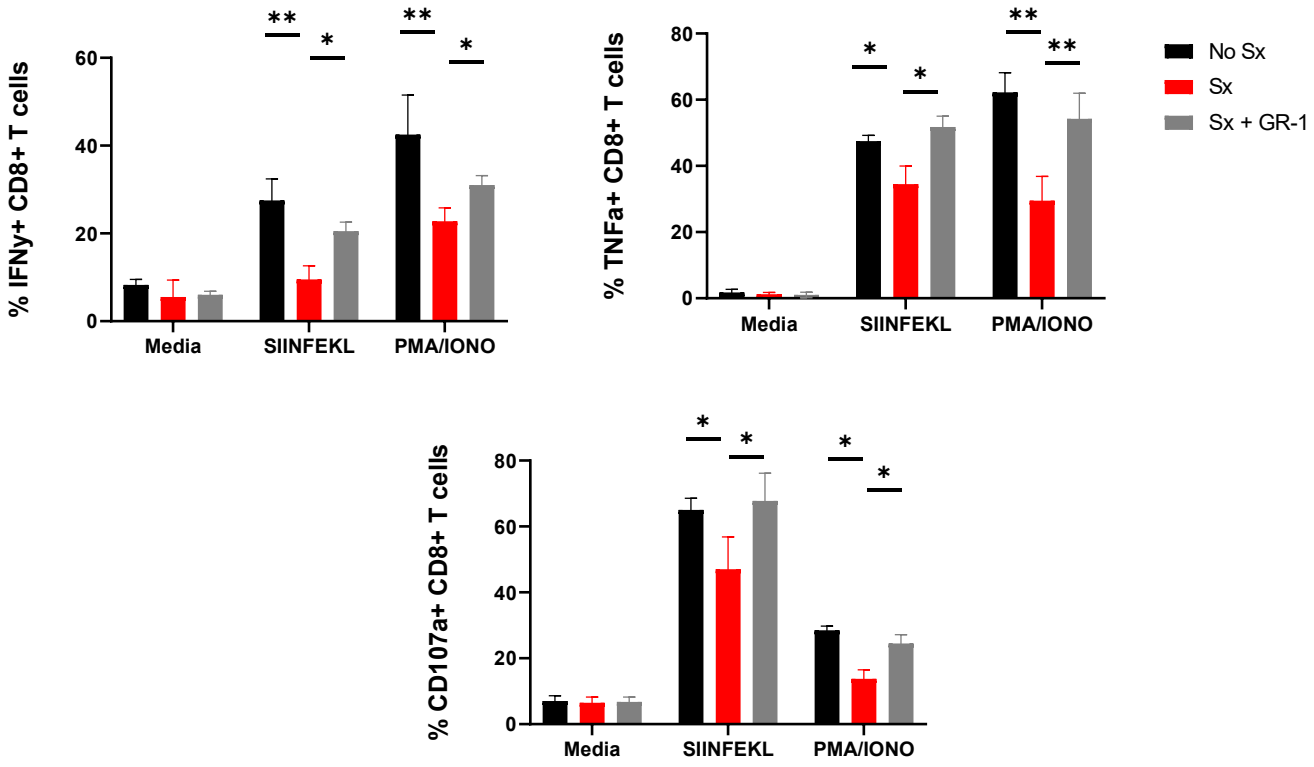

% IFNy+ CD8+ T cells
60
40
20
0
**
*
**
*
Media
SIINFEKL
PMA/IONO
% TNFa+ CD8+ T cells
80
60
40
20
0
*
*
**
**
Media
SIINFEKL
PMA/IONO
No Sx
Sx
Sx + GR-1
% CD107a+ CD8+ T cells
80
60
40
20
0
*
*
*
*
Media
SIINFEKL
PMA/IONO

**Figure 2.4: Depletion of murine MDSCs (GR-1$^+$) pre-operatively improves CD8$^+$ T cell function.**

**(A)** OT-I mice were treated with two doses of anti-GR-1 monoclonal antibody (200ug/ml) prior to surgery and underwent surgery (+/-). 24 hours post-surgery; mice were sacrificed and CD8$^+$ T cell immune characterization was performed on cells **(B)**Flow diagram illustrating GR-1$^+$/CD11b$^+$ depletion following anti-GR-1 monoclonal antibody. **(C)** Percentage of IFN$\gamma^+$, TNF$\alpha^+$, CD107a$^+$ CD8$^+$ T cells reacting to SIINFEKL n=5 (*$p \leq 0.05$; **$p \leq 0.01$) Statistical analyses were analyzed using Mann-Whitney U test**.**

## 2.4 DISCUSSION

In this study, we demonstrated that $CD8^+$ T cells are suppressed following surgery in both a non-antigen-specific and antigen-specific model that is independent of stimuli. We characterized the impact of surgery on both non antigen and SIINFEKL specific $CD8^+$ T cell responses and found a reduction in IFNγ, TNFα, and CD107a produced by $CD8^+$ T cells in the spleen 24 hours after surgery. Previous studies have also specified a similar decrease in cytokine secretion in response to stimulation following surgical stress(*113*, *115*, *131*). Although a lot of the previous studies reported this dysfunction in a non-antigen model, and only recently have studies started to focus on an antigen-specific $CD8^+$ T cell response. Here we demonstrate that surgical stress is not stimulation and antigen specific. There is an apparent reduction in $CD8^+$ T cell function regardless of stimulation, antigen, and subtype. To conclude that surgery impairs $CD8^+$ T cell mediated anti-tumor immunity, we adoptively transferred OT-I T cells into naïve recipient mice and subjected those mice to surgery and challenged the mice subcutaneously with B16-OVA tumor cells. Mice that received non surgically stressed OT-I cells exhibited 100% protection from tumor outgrowth, whereas recipient mice that received surgically stressed OT-I cells all developed tumors and succumbed to the tumor growth. These results indicate that the adoptively transferred OT-I cells are functional and able to prevent tumor outgrowth efficiently, but due to surgical stress, their cytotoxic functions are impaired enough to diminish their ability to clear tumor cells. We also confirmed and monitored the dysfunction of adoptively transferred OT-I cells after surgery in this tumor model by performing serial saph bleeds at day 1, 3, 5, and 20 days post-surgery. The data indicates that degranulation is reduced at 24hrs and 3 days after

surgery, with recovery by day 5 (Supplemental Fig 2.2). The OT-I cells are only suppressed for a few days and regain function during the tumor challenge. The effect of surgical stress to the OT-I cells is short lived, however, the impact of the suppression during this brief window of dysfunction is significant enough to impair the ability of OT-I cells to clear the tumor efficiently following tumor challenge. This highlights that a short time frame of surgical stress can still have significant consequences for an effective anti-tumor $CD8^+$ T response.

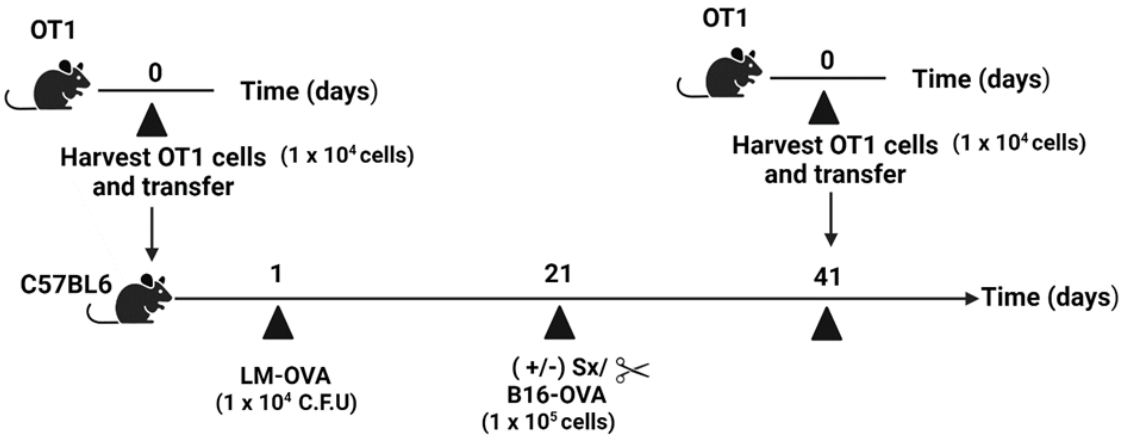
In a follow up study, we investigated that possibility of preventing tumor outgrowth in the surgically stressed mice by administering a second adoptive transfer of OT-I cells to inhibit tumor occurrence. The second adoptive transfer was able to delay tumor growth but ultimately these mice started to develop tumors (Supplemental Fig 2.1). These findings indicate that while the second adoptive transfer seemed to delay the tumor growth to some degree it wasn't enough to completely rescue the effect of the initial surgical stress on these mice. Similar results were obtained in the previous Ad-DCT $CD8^+$ T cell model, in which vaccinated mice demonstrated complete protection against subcutaneous tumor challenge and 100% survival(*131*). However, the vaccinated and surgically stressed mice displayed a loss of vaccine induced immunity, and 100% of these mice succumbed to tumor outgrowth. We have established that surgery suppresses $CD8^+$ T cells' function, which leads to tumor outgrowth. These observations provide some insight as to why cancer patients who undergo surgery with complete resection of tumors ultimately experience cancer recurrence and or metastatic disease. These results also suggest that perhaps a strong anti-tumor T cell immunity after surgery can potentially prevent tumor recurrence and reversing or inhibiting the effect of surgical stress can improve cancer prognosis. The immediate suppressive post-operative period is a critical window that allows the potential of cancer growth: therefore, the mechanism governing this suppression is necessary for the

development of therapies aimed at averting $CD8^+$ T cell dysfunction following surgery.

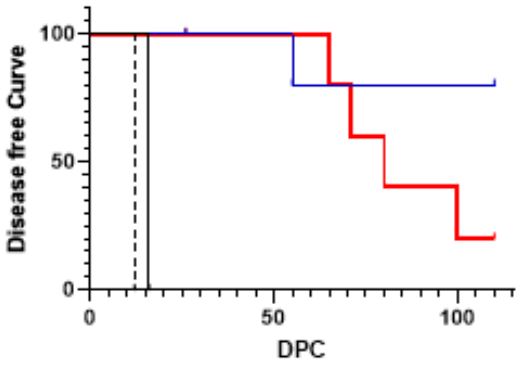
We also demonstrate that myeloid cells ($GR\text{-}1^+$) may impair $CD8^+$ T cell function, specifically secretion of cytokines post-surgery independent of stimulus type, following $GR\text{-}1^+$ depletion. Similar results have also been observed in NK cells, with depletion of sx-MDSCs preventing both post-operative immunosuppression of NK cells and the metastatic effects of surgical stress(*132*). Inhibiting the suppressive mechanisms of sx-MDSCs, including inhibiting arginase enzymatic activity and blocking TGFβ receptor signaling, prevented NK cell dysfunction(*132*). Although the precise mechanisms driving post-operative $CD8^+$ T cell dysfunction remain to be fully defined, our findings indicate that depletion of myeloid cells alleviates the effects of surgical stress on $CD8^+$ T cells, supporting a suppressive role for these cells.

## 2.5 Supplemental Figures

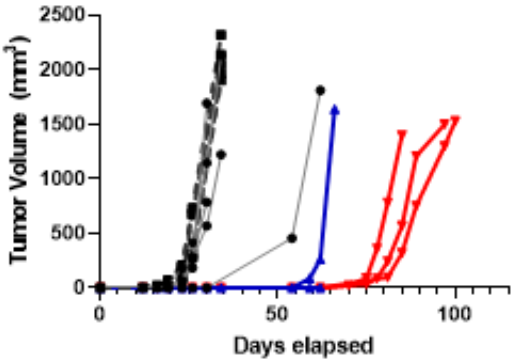
A



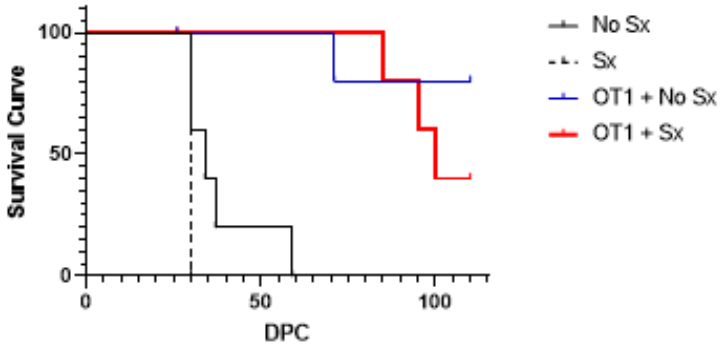
B


Disease free Curve
100
50
0
0
50
100
DPC

C


Tumor Volume (mm³)
2500
2000
1500
1000
500
0
0
50
100
Days elapsed

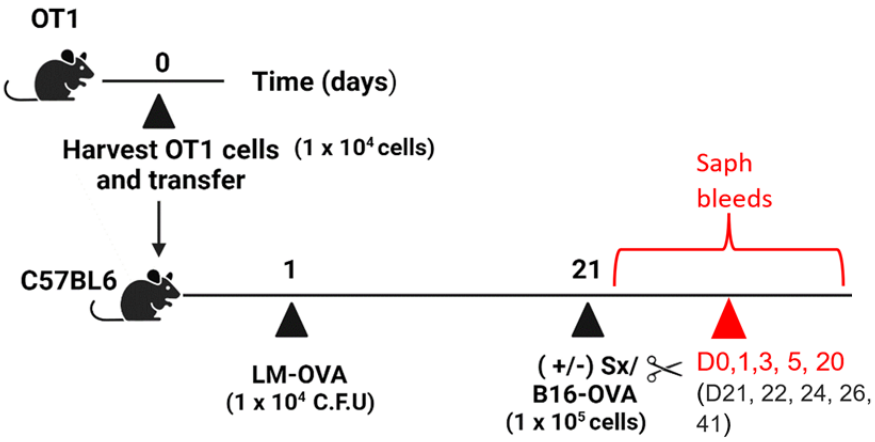
D


Survival Curve
100
50
0
0
50
100
DPC
No Sx
Sx
OT1 + No Sx
OT1 + Sx

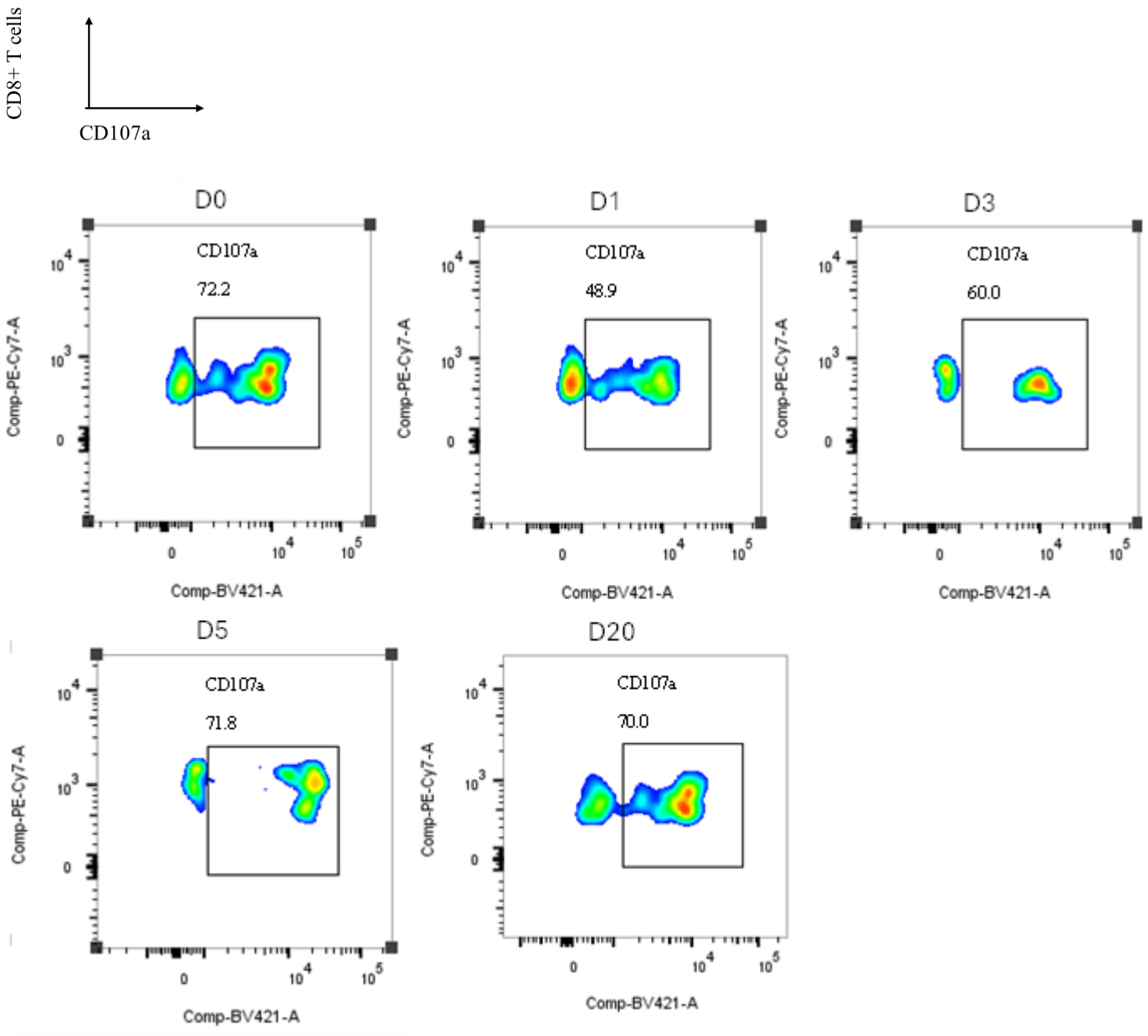
**Supplemental Figure 2.1: A second OT-I adoptive transfer delays tumor outgrowth**

**(A)** C57BL/6 recipient mice that received 1x $10^4$ OT-I cells by adoptive transfer and 1 x 104 C.F.U of LM-OVA. Following LM-OVA administration, recipient mice underwent surgery (+/-) on day 21. Recipient mice were challenged with 1x $10^5$ B16-OVA cells subcutaneously on day 21. Twenty days post tumor challenge, recipient mice received a second dose of 1x $10^4$ OT-I cells. **(B)** Percent disease-free curve is shown **(C)** Corresponding tumor volume over time **(D)** Corresponding survival curve of mice. n=5.

**A**


OT1
0
Time (days)
Harvest OT1 cells and transfer (1 x 10⁴ cells)
Saph bleeds
C57BL6
1
21
LM-OVA
(1 x 10⁴ C.F.U)
( +/-) Sx/
B16-OVA
(1 x 10⁵ cells)
D0,1,3, 5, 20
(D21, 22, 24, 26, 41)

B


CD8+ T cells
CD107a
D0
CD107a
72.2
D1
CD107a
48.9
D3
CD107a
60.0
D5
CD107a
71.8
D20
CD107a
70.0
Comp-PE-Cy7-A
Comp-BV421-A

**Supplemental Figure 2.2: Functional analysis of adoptively transferred $CD8^+$ T cells post tumor challenge in an LM-OVA model of surgical stress**

**(A)** C57BL/6 recipient mice received $1x10^4$ OT-I adoptive transfer and $1x\ 10^5$ C.F.U LM-OVA. Following LM-OVA administration, recipient mice underwent surgery (+/-) on day 21. Mice received $1x\ 10^5$ B16-OVA cells subcutaneously on day 21. Serial saph bleeds were performed on recipient mice on days 0, 1, 3, 5, and 20 for $CD8^+$ T cell assessment. **(B)** Flow plot illustrating $CD107a^+$ $CD8^+$ T cells at day 0, 1, 3, 5, and 20.

# Chapter 3: $CD8^+$ T cell is suppressed post operatively in vaccinated cancer patients

## 3.0 RATIONALE

In chapter 2, we established that surgical stress induces suppression in antigen-specific CD8+ T cells and results in this suppression, consequently impairing anti-tumor immunity in an established tumor mouse model. However, the effects of surgery on antigen specific $CD8^+$ T cells in cancer surgery patients have not yet been confirmed. In this study, we used a COVID-19 immunological toolkit to assess the duration and magnitude of antigen-specific T cell dysfunction following surgery by exploiting responses to the spike protein in vaccinated cancer patients. The goal of this study is to confirm the translational relevance of surgery-induced T cell dysfunction we observed in murine studies

The results in this chapter were published in the International Journal of Surgery on July 20th, 2025.

Olanubi, O., Dion, T., Macdonald, R., Alam, R., De Souza, C. T., Hu, R., Crawley, A. M., & Auer, R. C. (2025). Characterizing post-operative T and B cell dysfunction in cancer surgery patients, using COVID-19 as a model antigen. *International journal of surgery (London, England)*, *111*(7), 4785–4788. https://doi.org/10.1097/JS9.0000000000002429

## 3.1 INTRODUCTION

Our group has established that surgical stress suppresses $CD8^+$ T cell cytokine production and impairs $CD8^+$ T cell anti-tumor immunity in preclinical murine models(*131*). Additionally, the impact of surgery on natural killer cells of the innate immune system has also been characterized in cancer patients undergoing surgery(*133–135*). These studies have shown that NK cells are impaired in cancer patients and have demonstrated the consequences of a weakened NK cell response in metastatic disease. However, the unfavourable effects of surgery on the adaptive immune system, particularly $CD8^+$ T cells in cancer patients, have not been explored.

The widespread vaccination against COVID-19 and the accessibility of immunological assays to assess SARS-CoV-2-specific immune responses present an ideal opportunity to investigate the effect of surgery on antigen-specific $CD8^+$ T cell responses in a human model. The COVID-19 vaccine elicits a robust humoral and adaptive immune response that results in the production of a larger number of a SARS-CoV-2 specific T cell response. These T cells also recognize different epitopes of SARS-CoV-2 and, when exposed to these epitopes, elicit a robust response that can be measured.

We leveraged widespread COVID-19 vaccination and established SARS-CoV-2 immunological assays to investigate the magnitude and duration of post-operative dysfunction in pre-existing, vaccine-induced, antigen-specific $CD8^+$ T cell responses in cancer surgery patients.

## 3.2 METHODS

### Patients

A cohort of 30 patients undergoing major abdominal surgery for different types of malignancies and who had received at least two doses of the Pfizer BNT vaccine were included in this study. Blood was collected at baseline (POD0) and post-operative day (POD) 1, 3, and 28. This work was approved by the research ethics board for post-operative blood collection study at the Ottawa Hospital (OHSNREB# 2011884-01H).

### Blood Processing

Cancer patients' blood was collected into a BD Vacutainer Sodium-Heparin coated tubes. Blood was pooled together and diluted with equal volume of PBS. Blood was then carefully layered onto a falcon tube containing 15ml of Ficoll. Following the layering, PBMCs are isolated by Ficoll density centrifugation by spinning the tubes at 400g for 30 min at RT with the brakes off to maintain the gradient. PBMCs are cryopreserved at -80$^{o}$C and transferred to a nitrogen tank for longer preservation or used immediately for flow cytometry and functional assays.

## ELISpot

### T cell ELISpot

IFNγ secreting $CD8^+$ T cells were quantified by ELISpot (ImmunoSpot, Human IFN-γ Single-Color ELISPOT, #hIFNgp-2M) by stimulating thawed cryopreserved PBMCs with a peptide pool of SARS-CoV-2 spike (S1) protein (Miltenyi, #130-127-041) for 16 hours, following the ImmunoSpot ELISpot protocol.

### B cells ELISpot

IgG antibodies produced by $CD19^+$ memory B cells were quantified by ELISpot (Mabtech, Human IgG SARS-CoV-2 RBD ELISpot PLUS (HRP), #3850-4HPW-R1-1) by stimulating thawed cryopreserved PBMCs for 16 hours, following the Mabtech ELISpot protocol.

## Flow Cytometry

Following stimulation of thawed cryopreserved PBMCs with a peptide pool of SARS-CoV-2 spike (S1) protein (Miltenyi, #130-127-041), $1 \times 10^6$ cells were stained with Fixable Viability Stain 510 (BD, #564406) in the dark for 15 minutes and Human Fc BD block for 5 minutes (BD, #564219) at $4^o$ C prior to extracellular staining. Next, the cells are stained with an extracellular marker master mix at the appropriate dilutions for 20 min at 4ºC.For intracellular staining, cells are fixed with BD Cytofix/Cytoperm™ (BD) following the manufacturer's protocol. Following staining protocols, cells were acquired with flow cytometry acquired with a BD LSRFortessa using BD FACSDiva$^{TM}$ version 8.0.3 software. The flow cytometry data was analyzed with FlowJo, LLC version 10.8.2 software).

## 3.3 RESULTS

### Surgical stress reduces $CD8^+$ T-Cell cytokine production in response to SARS-CoV-2 spike protein and RBD in vaccinated patients

To quantify the degree of antigen-specific T cell dysfunction following surgery in humans, IFNγ production was measured using a T cell ELISpot kit (Mabtech) to determine the extent and duration of surgery-induced suppression of T cell responses to the RBD antigen. PBMCs from vaccinated cancer patients were isolated at POD 0, 1, 3, and 28 and cultured with the S protein peptide pool of SARS-CoV-2 (Miltenyi Biotech) for 16 hrs. This was followed by spot visualization and automated counting of cytokine-secreting cells. A total of 30 patients were included in the study. Surgical stress resulted in a significant reduction in the proportion of $CD3^+$ T cells that secreted IFNγ in response to the SARS-CoV-2 spike protein on POD1 by ELISpot (90.9±56.8 spots) as compared to POD0 (181.3±72.2, $p<0.0001$), which persisted on POD3 (128.5±49.2, $p<0.01$), but recovered by POD28 (163.2±51.9, $p=0.1$) (Fig3.1B). This result suggests that, in responsive patients, antigen-specific T cells suppress cytokine secretion post-operatively, a suppression that seems to persist up to day 3. Similarly, surgical stress also caused a decrease in the proportion of $CD3^+$ T cells that secreted IFNγ in response to anti-CD3 stimulation on POD1 by ELISpot (184.5±24.1 spots) as compared to POD0 (380.9±64.2, $p<0.0001$), which continued on POD3 (201.5±29.6, $p<0.01$), but recovered by POD28 (313.4±25.9, $p=0.1$)

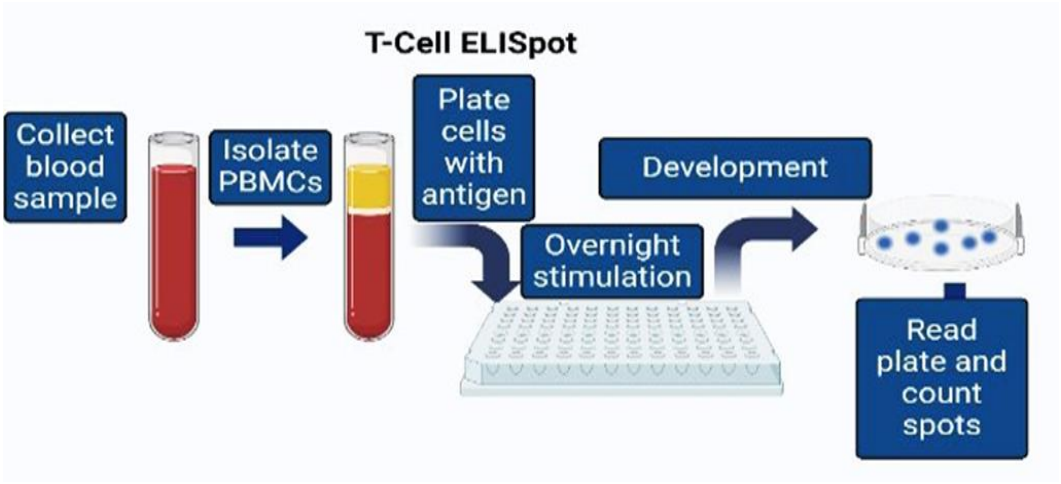
**Effect of surgical stress on the regulation of IFN$_\gamma$, TNFα, PD-1, TIGIT, TIM-3, T-bet in CD8$^+$ T cells.**

Given that objective 1 did not differentiate between CD4$^+$ and CD8$^+$ T cells or identify the characteristics of the target population in question, flow cytometry was used to identify the cell surface markers (CD45$^+$, CD4$^-$, CD8$^+$ TIGIT, TIM-3, LAG-3, and PD-1), transcriptional factors (T-bet, TOX, and GATA3) and cytokines (TNFα, and IFNγ) that may be altered on POD1 in CD8$^+$ T cells in response to the RBD antigen.

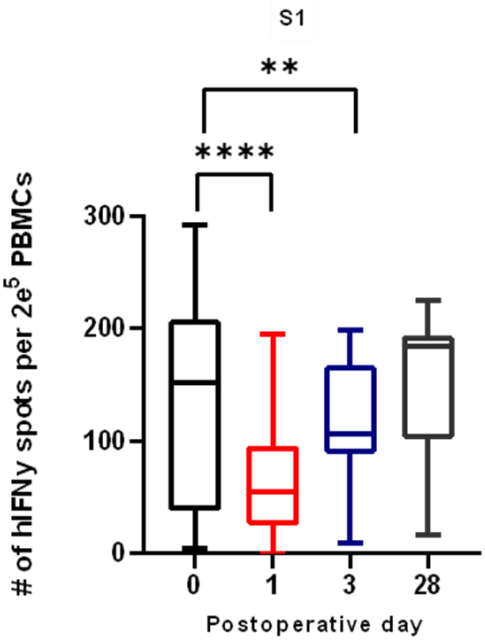
To characterize the phenotype of CD8$^+$ T cells, we further identified the top 8 patients who displayed the most significant T cell suppression from the T cell ELISpot data. Their PBMCs were stimulated with S1 peptide pool for 5 hrs. for cytokines and transcriptional factors and for 24 hrs. for cell surface markers.

We observe that surgery resulted in a decrease in intracellular IFNγ and TNFα expression ($p<0.05$) and an increase in cell surface expression of PD-1 with anti-CD3 monoclonal stim ($p<0.05$), but no changes in the expression of other exhaustion markers such as TIM-3, TIGIT, LAG-3, TOX nor of transcriptional factors such as T-bet, TOX, and GATA-3 (Fig 3.2).

**A**


T-Cell ELISpot
Collect blood sample
Isolate PBMCs
Plate cells with antigen
Development
Overnight stimulation
Read plate and count spots

**B**


S1
**
****
# of hIFNy spots per 2e5 PBMCs
300
200
100
0
0
1
3
28
Postoperative day

**C**

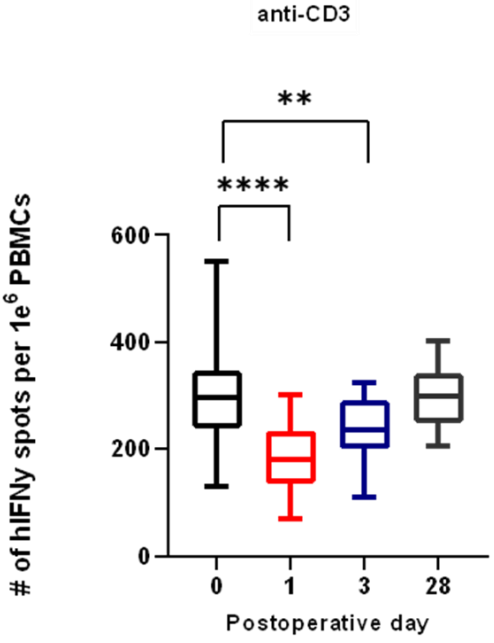
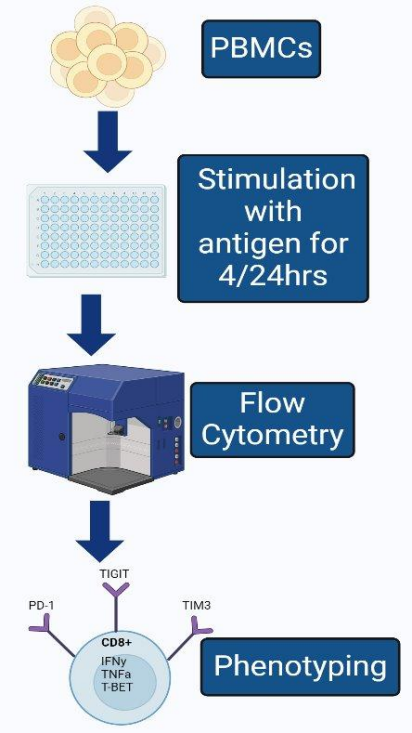

anti-CD3
**
****
# of hIFNy spots per 1e6 PBMCs
600
400
200
0
0
1
3
28
Postoperative day

**Figure 3.1: Surgical stress results in a decrease in CD8+ T cell cytokine production in response to the SARS-CoV-2 spike protein and RBD in vaccinated patients**

Adaptive immune responses by ELISpot against SARS-CoV-2 protein pool at pre-operative day 0 and post-operative day 1, 3, and 28 in Pfizer vaccinated cancer patients (n=30). (A) Proportion of $CD3^+$ T cells producing hIFNγ against the spike S1 protein of the SARS-CoV-2 peptide pool. (B)Proportion of CD3+ T cells producing hIFNγ with anti-CD3 stimulation as a positive control. Data are presented as a box plot, with a horizontal line at the median. Statistical differences were analysed by the paired T-test and the Wilcoxon Rank Test. P values $< 0.05$ were considered significant.


PBMCs
Stimulation with antigen for 4/24hrs
Flow Cytometry
TIGIT
PD-1
TIM3
CD8+
IFNγ
TNFa
T-BET
Phenotyping

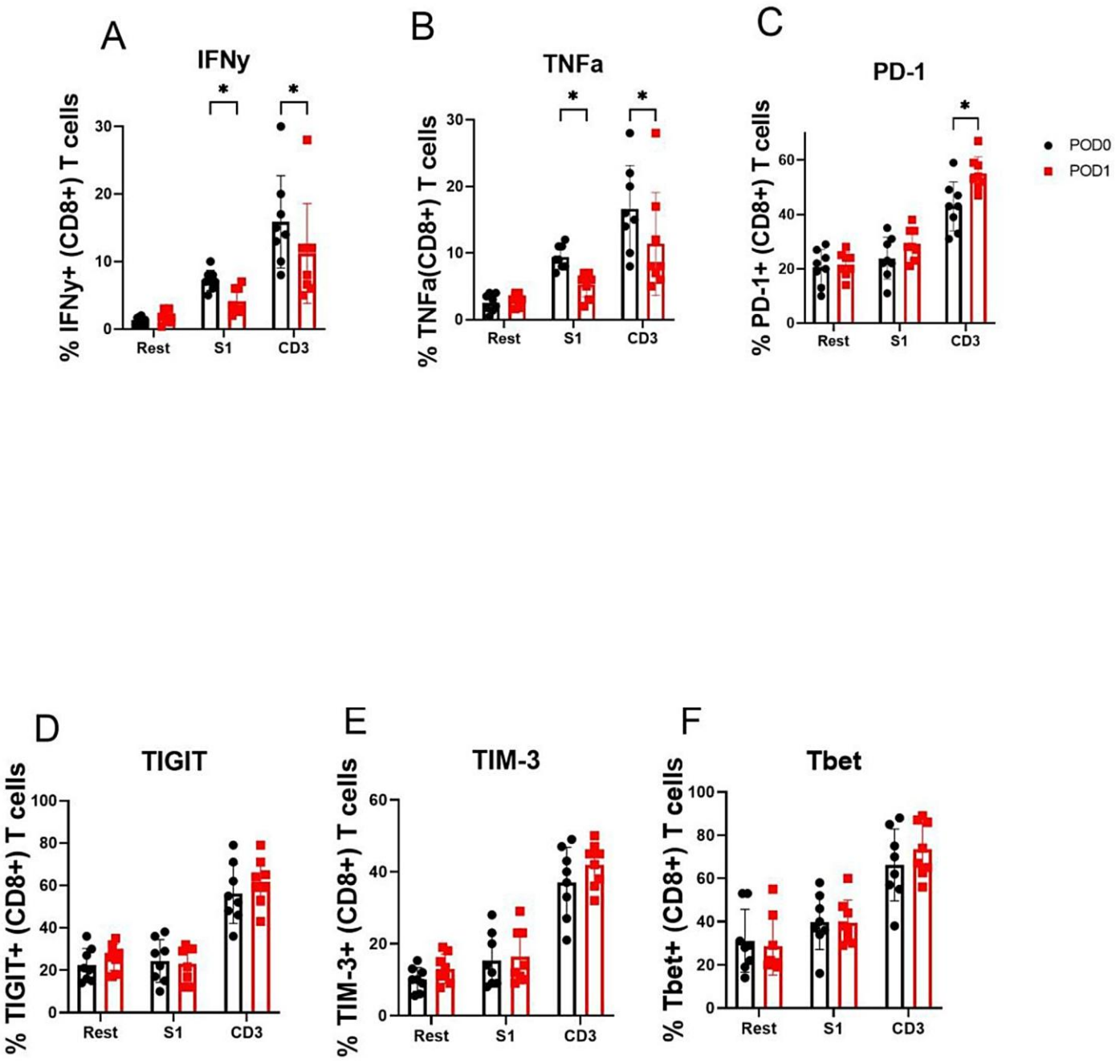

A
IFNy
% IFNy+ (CD8+) T cells
B
TNFa
% TNFa(CD8+) T cells
C
PD-1
% PD-1+ (CD8+) T cells
POD0
POD1
Rest
S1
CD3
D
TIGIT
% TIGIT+ (CD8+) T cells
E
TIM-3
% TIM-3+ (CD8+) T cells
F
Tbet
% Tbet+ (CD8+) T cells

**Figure 3.2: Effect of surgical stress on the regulation of IFNγ, TNFα, PD-1, TIGIT, TIM-3, T-bet in $CD8^+$ T cells of vaccinated patients**

$CD8^+$ T cells from patients with profound suppression in adaptive immune response post-operatively from ELISpot assay (n=8), were further analysed by flow cytometry for changes in intracellular cytokine markers IFNγ (A), TNFα (B), exhaustion markers PD-1 (C), TIGIT (D), TIM-3 (E), and transcription factor T-bet (F). Statistical differences were analysed by the paired T-test and the Wilcoxon Rank Test. P values $<0.05$ were considered significant.

## 3.4 DISCUSSION

In this study, we were able to use SARS-CoV-2 antigen as a tool kit to establish the suppression of antigen-specific $CD8^+$ T-cells following surgery in vaccinated cancer patients, with a 2-fold reduction in immune response in the early post-operative period. We also demonstrated that patients $CD8^+$ T-cell immune response had recovered by the time of their first post-operative visit, approximately 1 month after surgery. Unfortunately, we were unable to determine how long the dysfunction persisted, as patients were discharged before day 4. Although we could not directly investigate the duration of the dysfunction, prior studies have inferred that recovery of the immune response generally occurs within 7 days of surgery(*135*). This concept is also corroborated by a study indicating that vaccinated patients are not at risk of developing COVID-19 related infection following surgery, implying that the duration of post-operative dysfunction isn't prolonged(*136*).

In the murine models explored in chapter 2, we have demonstrated that surgery significantly suppressed an antigen-specific response and decreased an anti-tumor immune response. With this current study, we have also shown that a comparable degree of antigen-specific T cell immune suppression is observed in cancer surgery patients. We also quantified the degree of antigen-specific B cell dysfunction following surgery in humans to evaluate the humoral response (Supplemental Fig 3.1B). The production of IgG antibody measured by B cell ELISpot was used to determine the degree and duration of surgery-induced suppression of B cell responses to the RBD antigen. These findings parallel those of the T cell response and indicate that dysfunction not only occurs in the innate immune response but also in major players of the adaptive and humoral immune response.

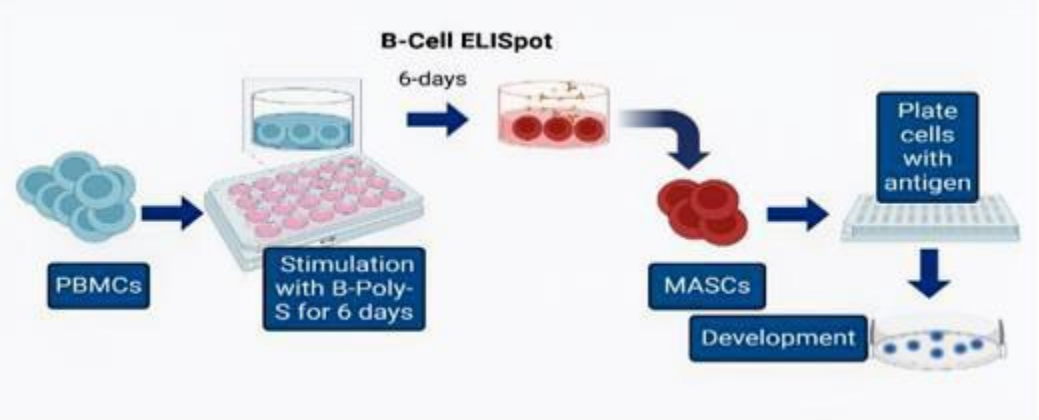
Furthermore, we investigated possible markers involved in exhaustion or checkpoint inhibition

of $CD8^+$ T cells and discovered that PD-1, a marker of T cell immune activation and exhaustion, was upregulated following surgery (Fig 3.2C). This result suggests a possible mechanism of immune dysfunction that could be targeted with approved therapeutic antibodies.

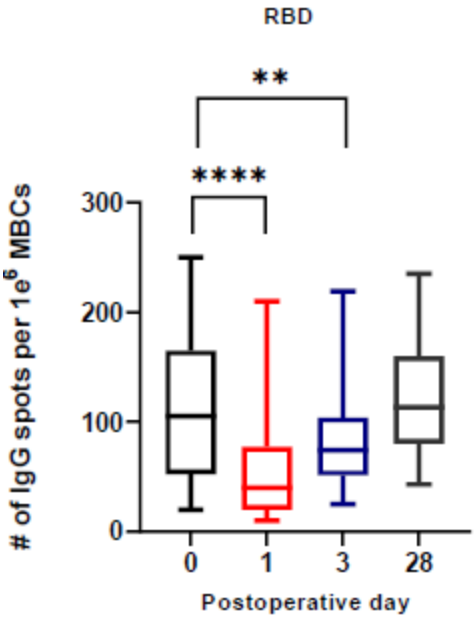
This study is the first to clearly show significant dysfunction of antigen-specific T and B cells in the early post-operative period. The findings emphasize the need for further exploration of the mechanisms underlying this dysfunction, which could inform strategies to enhance immune recovery in post-operative patients and ultimately improve oncological outcomes following cancer surgery.

## 3.5 SUPPLEMENTAL FIGURES

**A**



**B**


RBD
**
****
300
200
100
0
# of IgG spots per 1e6 MBCs
0
1
3
28
Postoperative day

**Supplemental Figure 3.1: Humoral immune responses by ELISpot against SARS-CoV-2 protein pool**

**(A)** Humoral immune responses by ELISpot in the serum of Pfizer vaccinated cancer patients at pre-operative day 0 and post-operative day 1, 3, and 28. IgG responses to RBD of SARS-CoV-2 spike protein are illustrated. **(B)** Data is presented as a box plot with a horizontal line in the middle representing the median. Statistical differences were analyzed using paired T-Tests and the Wilcoxon Rank-Sum Test. P values $< 0.05$ were considered significant.

# Chapter 4: Identifying a mechanism of post-operative $CD8^+$ T cell dysfunction

## 4.0 RATIONALE

In chapter 4, I focus on aim 2 of my project, which is to identify a mechanism of dysfunction by performing scRNA-seq on isolated $CD8^+$ T cells from the established animal model discussed in chapter 2. This chapter will address how surgical stress remodels the $CD8^+$ T cell transcriptional profile by performing clustering to identify cell states, differential expression analysis to identify gene signatures unique to surgically stressed $CD8^+$ T cells, and gene enrichment to determine gene signatures unique to surgically stressed $CD8^+$ T cells. The identification of a potential gene of interest mediating dysfunction in $CD8^+$ T cells will also be discussed in this chapter.

## 4.1 INTRODUCTION

Based on the findings from chapters 2 and 3, we observed that antigen-specific $CD8^+$ T cells are significantly suppressed following surgery in both murine and human models, as we examined using the OT-I transgenic model and the COVID-19 peptide toolkit. We observed the suppression of $CD8^+$ T cells across several analyses, such as in vitro experiments, which highlighted a substantial decrease in cytokine secretion, as well as in vivo studies that demonstrated that surgery attenuated $CD8^+$ T cell tumor killing ability. These results indicate that surgical stress dampens important and fundamental functions of $CD8^+$ T cells. The findings from both the murine and human models highlight the translational relevance of these results, indicating that surgery can disrupt the adaptive immune system, a concerning consequence of the procedure with implications for cancer patients. It is crucial to identify potential molecular pathways that suppress $CD8^+$ T cells after surgery. We opted to perform single-cell RNA sequencing because this would allow us to identify molecular pathways or genes altered by surgical stress, thereby highlighting possible targets for therapeutic intervention.

## 4.2 METHODS

### Study design

#### scRNA-seq

To analyze the transcriptional changes in the adoptively transferred OT-I cells after surgery, we conducted scRNA-seq on OT-I cells from 6 pooled surgically stressed mice and 6 pooled non-surgically stressed mice. Two duplicates from the following conditions were sent to STEMCORE:

1. No surgery (Unstimulated)
2. No surgery (Stimulated)
3. Surgery (Unstimulated)
4. Surgery (Stimulated)

Splenocytes were isolated from OT-I mice, and $CD8^+$ T cells were isolated. 1 x 104 OT-I T cells were then adoptively transferred to C57BL/6 recipient mice (Fig. 4.1A). Twenty-four hours post-adoptive transfer, mice received an IV administration of LM-OVA at a dose of $1 \times 10^4$ C.F.U. At day 21, surgery was then performed on the recipient mice. Twenty-four hours after surgery, the recipient mice were sacrificed, and splenocytes were isolated and divided into two groups: half underwent further ex vivo stimulation for 5 hours. Post stimulation, $CD8^+$ T cells were first isolated with beads and further isolated with MoFlo XDP sorter using an optimized T cell flow panel with antibodies against $CD45^+$, $CD3^+$, $CD8^+$, Vα2, Vβ5. The isolated cells were then sent to STEMCORE to be sequenced using Chromium Single Cell 3' Gene Expression.

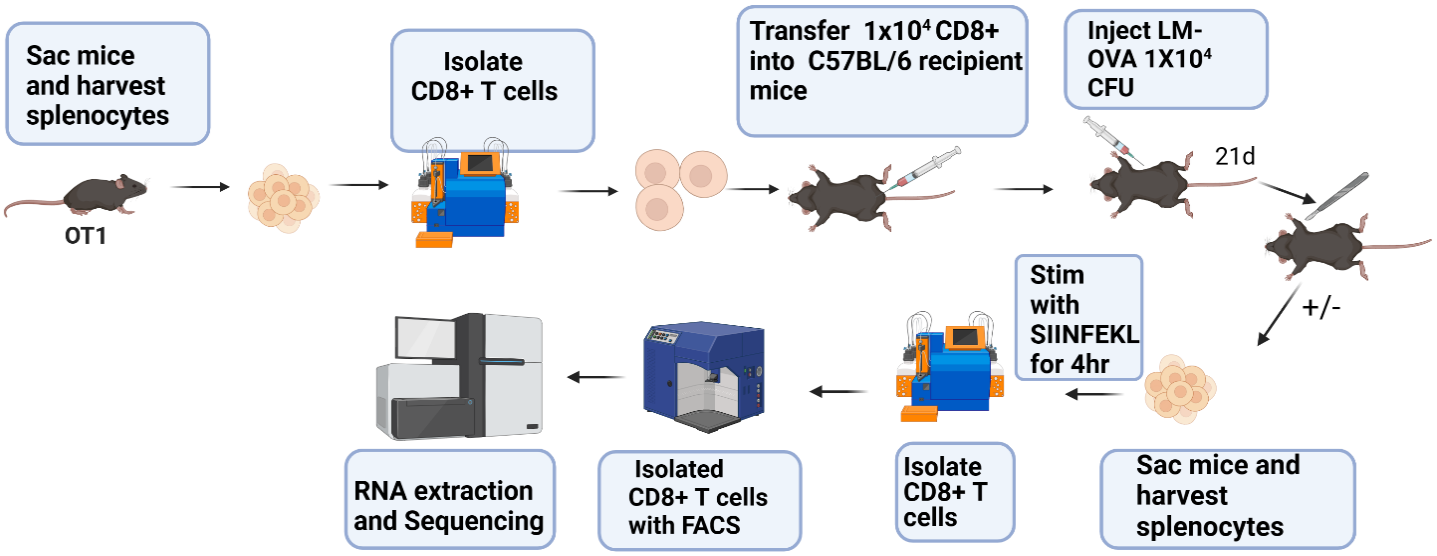
The Chromium Single Cell gene expression system uses a microfluidic program to assess over 200,000 cells. This technique enables the high-throughput single-cell sequencing of different cell types. This is achieved by capturing single cells in a droplet compartment using Gel Bead-In-Emulsion (GEM) technology, in which cells are barcoded for identification by integrating them with gel beads bearing a unique DNA barcode. After the cells have been barcoded, they are then pooled together. The single cells undergo lysis within each GEM, where reverse transcription of the mRNA takes place to create cDNA libraries. The GEM are then broken down, and the generated cDNA libraries are sequenced by using the barcodes to assign reads to individual cells. The libraries generated in this experiment were sequenced to a depth of close to 20,000 reads/cell. The cDNA Libraries were sequenced on the Illumina NextSeq 500.

Following sequencing, FASTQ files were generated, and gene expression libraries were processed and aligned to the mouse transcriptome (Fig 4.1B). Gene expression libraries were imported into R and processed with the Seurat package(*137*). We removed cells with low gene detection (<200 detected genes) and high mitochondrial gene content (>25%), normalized the data, and then integrated and clustered the remaining cells using principal component analysis (PCA) by treatment/condition, resulting in 7 clusters, 0-6. We also annotated the clusters based on the different subsets present using a tool for interpreting single cell transcriptomics based on their resemblance to reference TILS of $CD8^+$ T cell subtypes (Fig. 4). We next used the R package muscat to perform differential expression analysis between samples within each cluster.

**Gene set enrichment analysis**

We used the differential expression analysis data set and the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins database) to perform gene set enrichment analysis on genes ranked by their average log2 fold change and adjusted P values. The STRING database was used to acquire GO terms and to generate enrichment plots for biological pathways. All enrichment plots were adjusted by p-value.

**A**


Sac mice and harvest splenocytes
OT1
Isolate CD8+ T cells
Transfer 1x10⁴ CD8+ into C57BL/6 recipient mice
Inject LM-OVA 1X10⁴ CFU
21d
+/-
Stim with SIINFEKL for 4hr
Sac mice and harvest splenocytes
Isolate CD8+ T cells
Isolated CD8+ T cells with FACS
RNA extraction and Sequencing

**B**

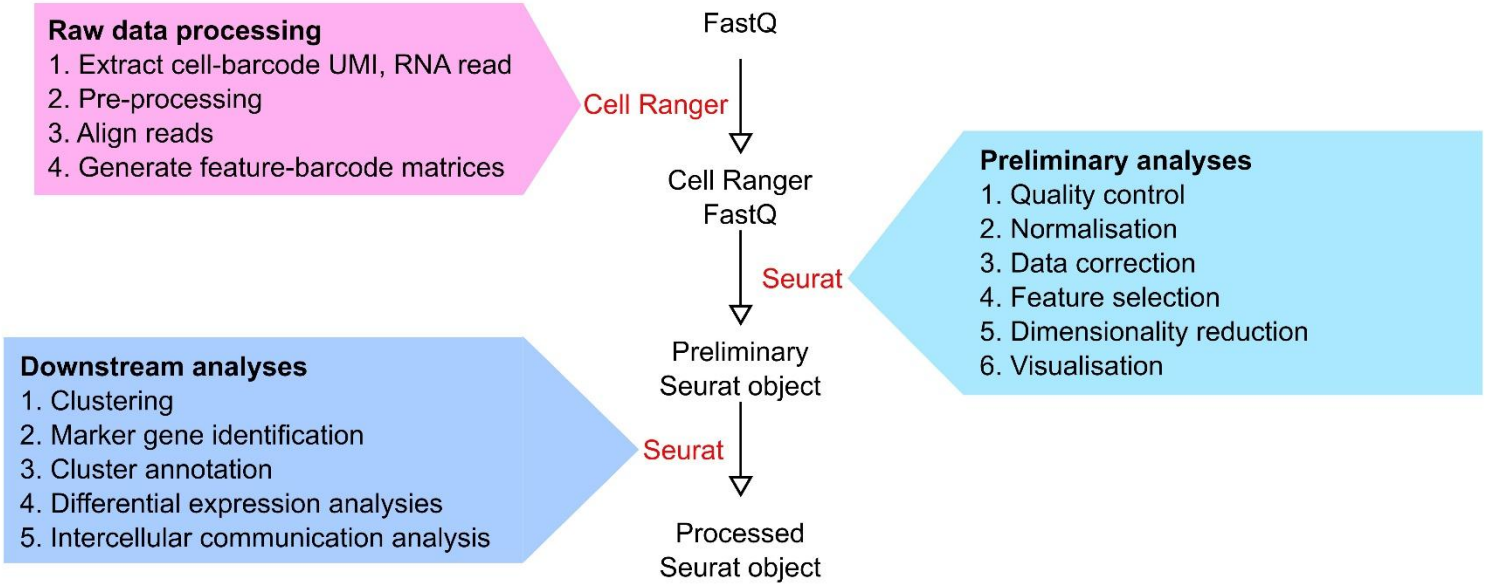

Raw data processing
1. Extract cell-barcode UMI, RNA read
2. Pre-processing
3. Align reads
4. Generate feature-barcode matrices
FastQ
Cell Ranger
Cell Ranger FastQ
Seurat
Preliminary Seurat object
Seurat
Processed Seurat object
Preliminary analyses
1. Quality control
2. Normalisation
3. Data correction
4. Feature selection
5. Dimensionality reduction
6. Visualisation
Downstream analyses
1. Clustering
2. Marker gene identification
3. Cluster annotation
4. Differential expression analysies
5. Intercellular communication analysis

**Figure 4.1: Schematic overview of the study design and analysis pipeline.**

**(A)** The experimental flowchart of this study. *Created with Biorender.com* **(B)** The bioinformatics pipeline used for data analysis. *Adapted with copyright permission from Baker Institute, Data Analysis Pipeline, https://clara.baker.edu.au/methods/data-analysis-pipeline/.*

## 4.3 RESULTS

We confirmed $CD8^+$ T cell dysfunction in isolated cells sent for scRNA-seq by performing degranulation assay (CD107a), and we observed a 2-fold decrease in surgically stressed stimulated $CD8^+$ T cell in comparison to non-surgically stressed cells (Supplemental Fig. 4.1B). As mentioned above, PCA and UMAP were used to capture the sequencing and provide a visual representation of the data, which resulted in 7 clusters (Fig 4.2). Cluster 0, 1, 2, 3 comprise the majority of $CD8^+$ T cells from all sample conditions. Cluster 4, 5 and 6 are smaller clusters that are represented by a naive $CD8^+$ T cell population, a dying population, and a cycling population, respectively. Subsequent downstream analysis was performed on the four major clusters.

**Clustering and identification of cell states**

Cluster 0 (the largest cluster) consists of all four conditions: No Sx, No Sx stim, Sx, and Sx stim. This cluster upregulates homing genes (CCR7, SELL), genes that regulate survival (BCL2 and IL7R), costimulatory genes for activation (CD27, CD28), and genes necessary for differentiation (EOMES, LEF1) in $CD8^+$ T cells (Supplemental Fig 4.2A)(*138*, *139*). These genes correspond to a central memory T cell (TCM) population (Fig 4.2B). The TCM subset largely consists of the No Sx stim, Sx, and Sx stim groups, which are the treatment group (Fig. 4.2A). The data suggests that stimulation induced the cells to undergo some reprogramming that enables them to upregulate early activation genes such as CD27 and CD69 which are markers that are typically present when naïve $CD8^+$ T cells encounter an antigen (Supplemental Fig 4.1A). Interestingly, the surgically stressed group that was not stimulated seems to follow a similar activation pattern.

Cluster 0 displays a significant interferon response which is typically marked by expression of cells that are activated in response to proinflammatory cytokines generated during surgery

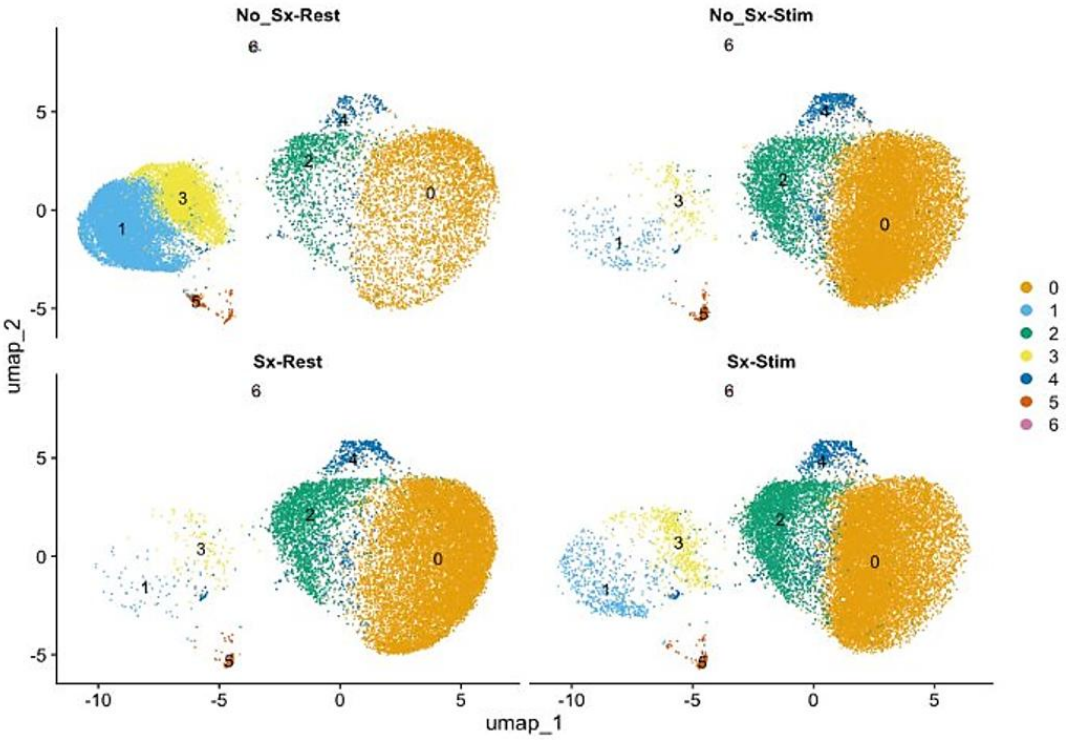
because of inflammation(*140*, *141*) . The expression of genes such as interferon induced guanylate binding protein 2 (GBP2) and signal transducer and activator of transcription 1 (STAT1) reflect a type 1 interferon response phenotype.

Cluster 2 consists of all four conditions; however, this cluster is mostly composed of the three treatment groups in comparison to the untreated group (No Sx) (Fig 4.2A). This cluster is referred to as central memory_2 or TCM 2, and it demonstrates an expression pattern similar to cluster 0 marked by the expression of canonical TCM genes such as CCR7, SELL, IL7R, TCF7, BCL2, LEF1, CD27, CD28, and EOMES(Supplemental Fig. 4.2A)(*139*). Cluster 2 differs from cluster 0 because it does not significantly express interferon response genes (Supplemental Fig 4.1A).

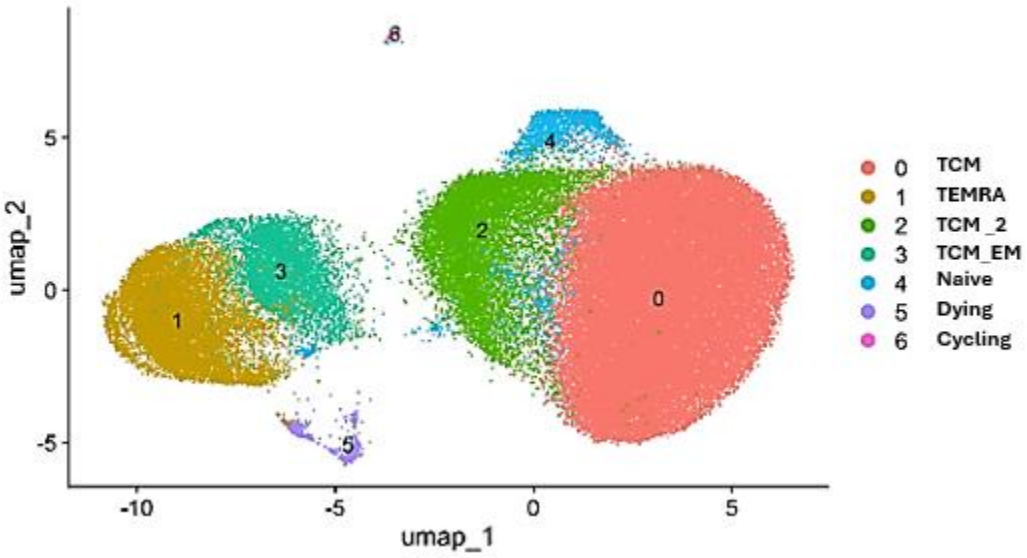
Cluster 3 primarily consists of cells from the No Sx group as well as a few cells from the No Sx stim, Sx, and Sx stim (Fig. 4.2A). This cluster demonstrates co-expression of most TCM canonical markers, such as CCR7, SELL, IL7R, and TCF7, along with a few effector memory markers, including CXCR6, ID2, and HOPX observed in $CD8^+$ T cells (Supplemental Fig. 4.2A, 4.2B)(*138*, *139*). This cluster represents a transitional state of $CD8^+$ T cells that are not fully committed to a terminally differentiated state or a memory state(*142*).

Cluster 1 consists of $CD8^+$ T cells predominantly from the No Sx group. Cluster 1 exhibits a terminally differentiated effector memory (TEMRA) phenotype described in Szabo et al., with upregulation of genes involved in senescence (KLRG1), effector genes (GZMB, PRF1), NK-associated genes such as KLRD1 and NKG7 (Supplemental Fig. 4.2C) (*143*). This state represents a cytotoxic but reduced proliferative potential, having previously experienced antigen stimulation and approaching terminal differentiation.

**A**



**B**


umap_2
umap_1
5
0
-5
-10
-5
0
5
0 TCM
1 TEMRA
2 TCM_2
3 TCM_EM
4 Naive
5 Dying
6 Cycling

**C**

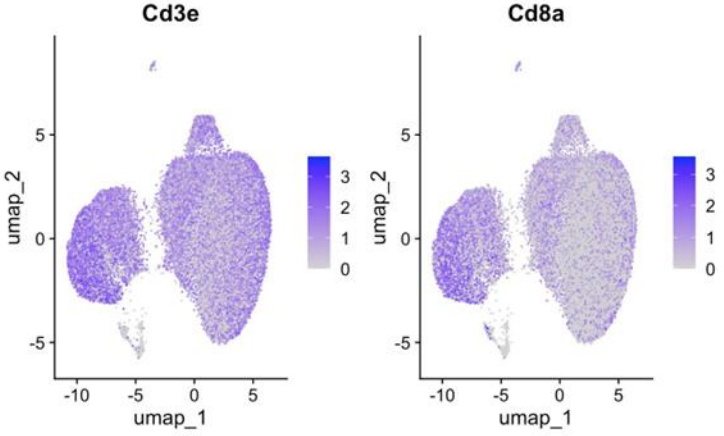

Cd3e
Cd8a
umap_2
umap_1
3
2
1
0

**Figure 4.2: Single-cell RNA-seq of surgically stressed vs. non-surgically stressed OT-I $CD8^+$ T cells indicates significantly altered expression profiles**

**(A)** UMAP plot of scRNA-seq data representing the four sample conditions (No Sx, No Sx Stim, Sx, and Sx Stim). Each point corresponds to a single cell and is coloured by cluster. **(B)** Matching UMAP displayed as in (A) with annotated $CD8^+$ T cell sub-types labelled according to Seurat cluster. **(C)** UMAP displaying expression of the canonical marker of lymphocyte cell type, $CD3^+$ and $CD8^+$ T cells.

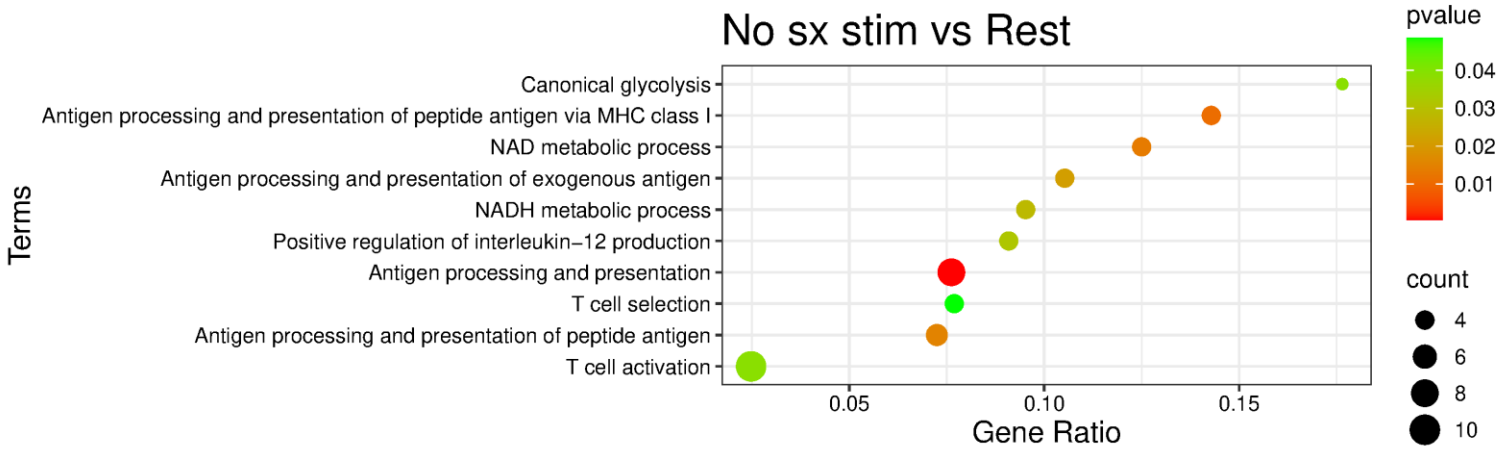
**Differential gene expression**

As stated previously, cluster 0 or the TCM subset consist of $CD8^+$ T cells that are considered activated central memory cells and are also composed of all four sample conditions (Fig.4.2A). Differential gene analysis of non-surgically stressed stimulated $CD8^+$ T cells versus resting cells (unstimulated) exhibited upregulation of genes such as RGCC (Regulator of Cell Cycle) and LAT (Linker For Activation of T Cells) which are markers indicating response to antigen (Supplemental Fig. 4.3A). However, stimulated $CD8^+$ T cells under surgical stress do not upregulate the same gene pattern as unstimulated surgically stressed cells within these three clusters. Surgically stressed stimulated $CD8^+$ T cells are upregulating genes such as ELOVL6 (Elongation of very long chain fatty acids protein 6) and MAP3K8 (Supplemental Fig. 4.3B) across all three clusters. Gene ontology enrichment analysis of stimulated non-surgically stressed $CD8^+$ T cells in comparison to resting non-surgically stressed $CD8^+$ T cells and this revealed an upregulation of expected biological pathways in response to antigen stimulation like glycolysis and antigen processing (Fig. 4.3A). Gene ontology enrichment analysis of stimulated surgically stressed $CD8^+$ T cells in comparison to resting surgically stressed $CD8^+$ T cells did not upregulate expected biological pathway but we see an upregulation in p38 MAPK signaling (Fig. 4.3B).

Next, we specifically wanted to identify markers that are differentially expressed in the surgically stressed stimulated group. We are interested in the surgically stressed and stimulated condition because this group should reveal transcriptional differences between how normal $CD8^+$ T cells respond to antigen in comparison to surgically stressed $CD8^+$ T cells. Therefore, we analysed potential genes that are differentially expressed in the surgically stressed group stimulated group in comparison to the non-surgically stressed stimulated group (Fig 4.4A-C).

Upon further analysis on the genes that may serves as a potential candidate gene for further mechanistic studies based on three criteria i) P-value which reveals the significance of the gene, ii) the log2 fold change (>1), which means the gene is expressed twice as much in the surgically stressed group vs control and iii) what is known about the genes in literature in terms of regulating $CD8^+$ T cell function. Considering these three criteria, we identified CISH as the primary gene of interest due to its significant differential expression across clusters in the surgically stressed and stimulated condition (Fig. 4.4A-C).

**A**


No sx stim vs Rest
Terms
Canonical glycolysis
Antigen processing and presentation of peptide antigen via MHC class I
NAD metabolic process
Antigen processing and presentation of exogenous antigen
NADH metabolic process
Positive regulation of interleukin−12 production
Antigen processing and presentation
T cell selection
Antigen processing and presentation of peptide antigen
T cell activation
0.05
0.10
0.15
Gene Ratio
pvalue
0.04
0.03
0.02
0.01
count
4
6
8
10

**B**

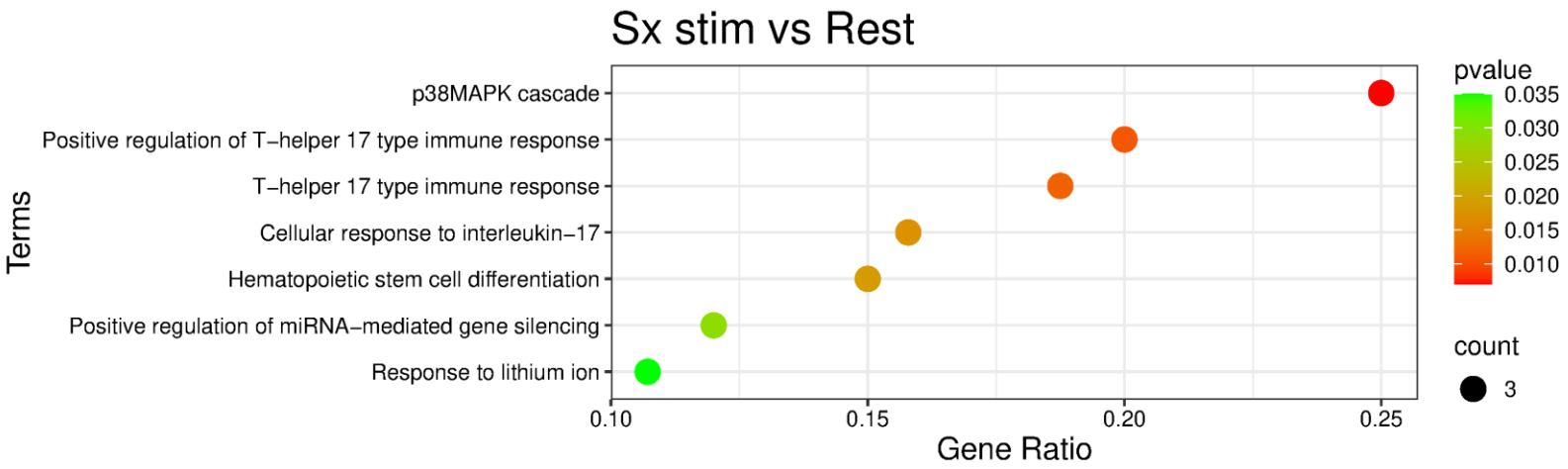
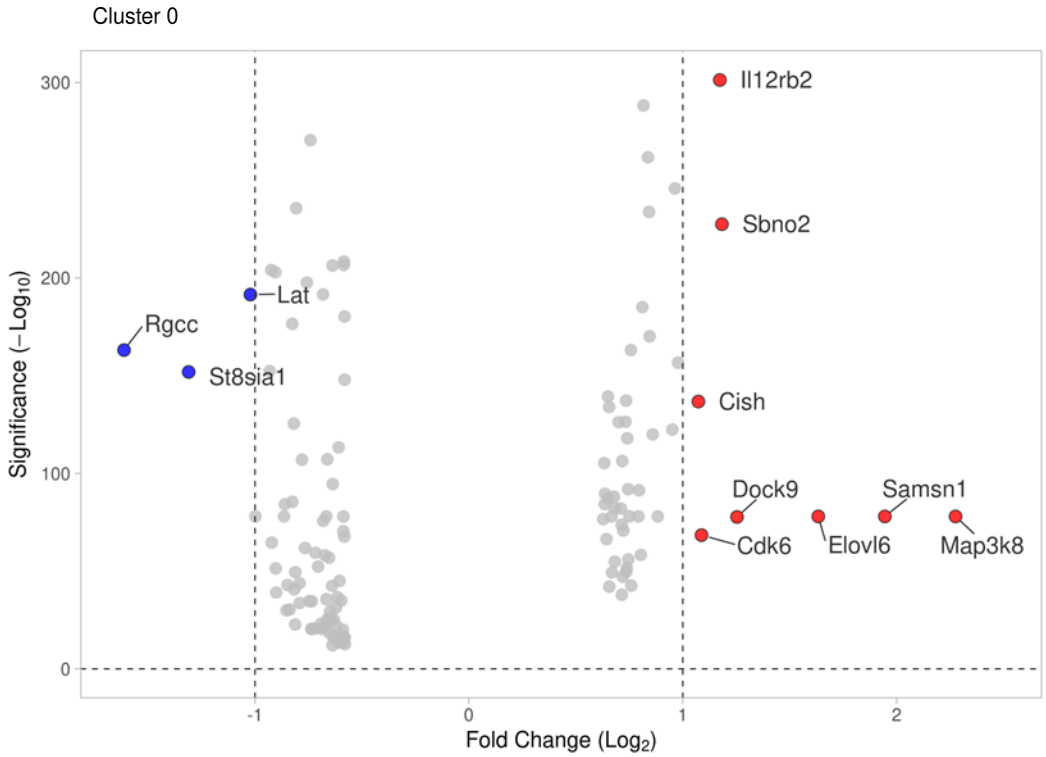

Sx stim vs Rest
Terms
p38MAPK cascade
Positive regulation of T−helper 17 type immune response
T−helper 17 type immune response
Cellular response to interleukin−17
Hematopoietic stem cell differentiation
Positive regulation of miRNA−mediated gene silencing
Response to lithium ion
0.10
0.15
0.20
0.25
Gene Ratio
pvalue
0.035
0.030
0.025
0.020
0.015
0.010
count
3

**Figure 4.3: Enrichment analysis of stimulated TCM CD8$^+$ T cells.** The STRING functional enrichment tool was used to perform Gene Ontology pathway enrichment analysis. **(A**) Enriched biological pathways in stimulated non-surgically stressed CD8$^+$ T cells in comparison to resting non-surgically stressed CD8$^+$ T cells. **(B)** Enriched biological pathways in stimulated surgically stressed CD8$^+$ T cells in comparison to resting surgically stressed CD8$^+$ T cells. $P < 0.05$ and gene count $\geq 2$.

**A**


Cluster 0
Significance (−Log10)
Fold Change (Log2)
Il12rb2
Sbno2
Lat
Rgcc
St8sia1
Cish
Dock9
Samsn1
Cdk6
Elovl6
Map3k8

**B**

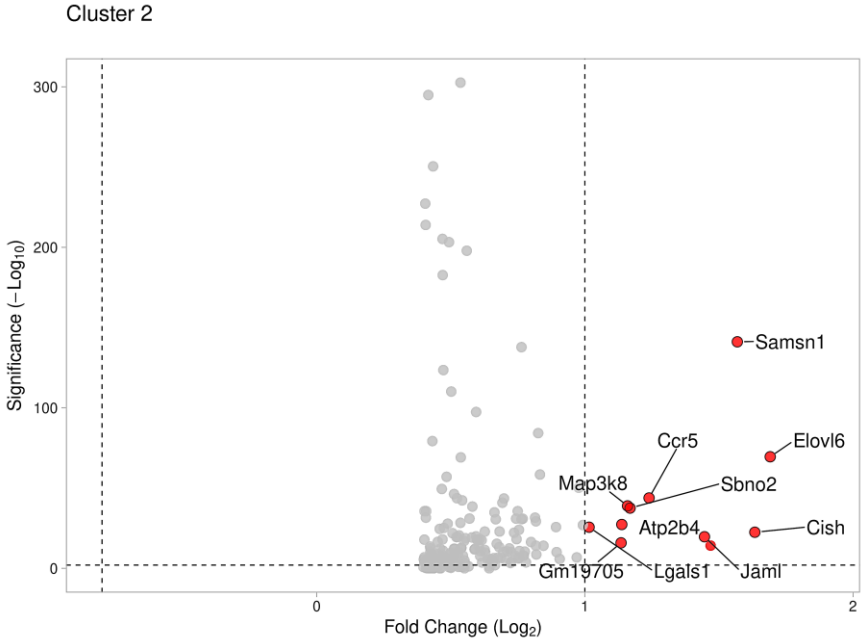

Cluster 2
Significance (−Log10)
Fold Change (Log2)
Samsn1
Ccr5
Elovl6
Map3k8
Sbno2
Atp2b4
Cish
Gm19705
Lgals1
Jaml

**Figure 4.4: Differential gene expression of surgically stressed $CD8^+$ T cells in response to antigen**

A volcano plot displays differential expression of genes in surgically stressed-stimulated $CD8^+$ T cells relative to their expression in non-surgically stressed-stimulated $CD8^+$ T cells. Red indicates upregulated genes with a positive Log2 fold change. **(A)** Volcano plot showing upregulated genes in surgically stressed-stimulated cells relative to their expression in non-surgically stressed-stimulated $CD8^+$ T cells in Cluster 0, and **(B)** Cluster 2. The volcano plots show -log10 FDR-adjusted p-value (y-axis). Genes that are not differentially expressed are coloured grey.

## 4.4 DISCUSSION

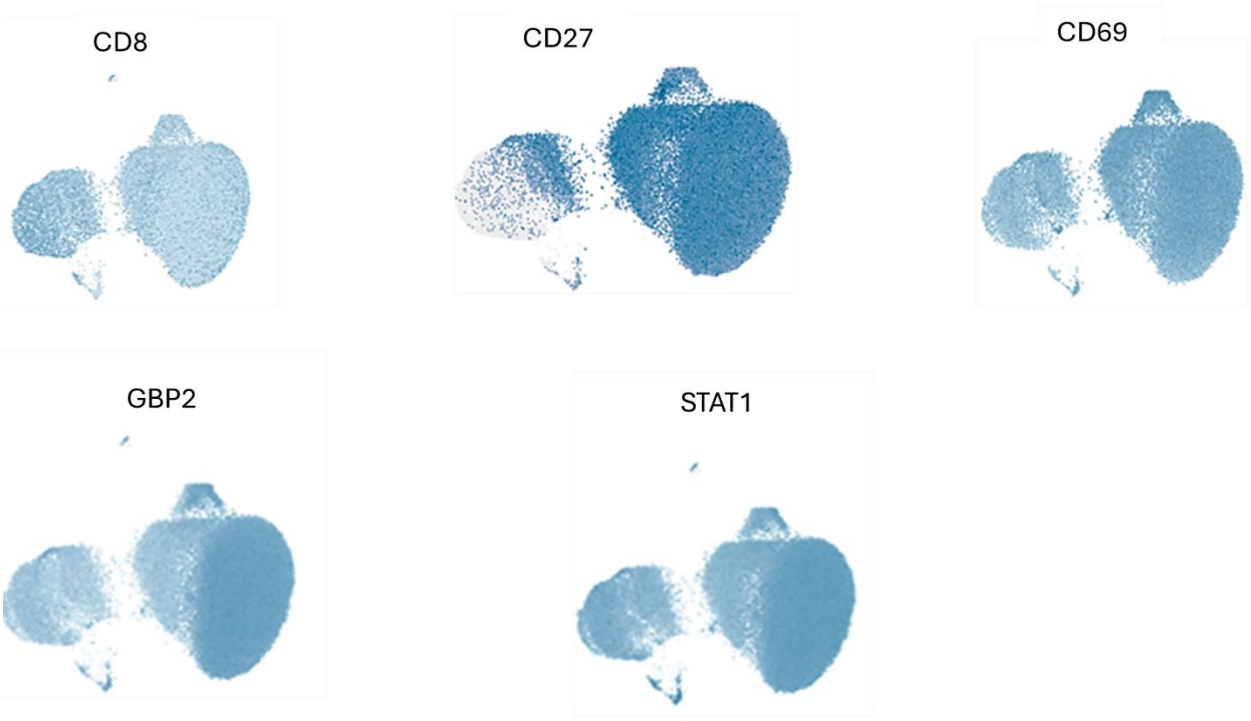
In the above experiment, scRNA-seq of surgically stressed and non-surgically stressed murine $CD8^+$ T cells was used to explore the implications of surgery on $CD8^+$ T cell's profile and activity. The analysis of the scRNA-seq data reveals differences in the differential expression analysis of surgically stressed versus non-surgically stressed stimulated $CD8^+$ T cells, compared to their unstimulated counterparts, yet they share a similar transcriptional profile, as highlighted by the clustering in the UMAP analysis. Interestingly, we observed a shift in the transcriptional profile of both surgically stressed and stimulated $CD8^+$ T cells (Fig. 4.2A). We observed that both stimulation and surgery influence the transcriptional profile of $CD8^+$ T cells. This reprogramming is defined by an increase in early activation genes CD27 and CD69 (Supplemental Fig.4.1A), as well as an active interferon response (GBP2 and STAT1), which is typically marked by cells that are activated in response to proinflammatory cytokines produced as a result of inflammation during surgery or in response to stimulus(*144*, *145*).

The data also demonstrated that surgically stressed $CD8^+$ T cells are unable to initiate standard signalling programs upon stimulation, defined by the lack of pathways related to canonical $CD8^+$ T cell function, such as glycolysis and antigen processing (Fig. 4.3B)(*146*, *147*). Instead, surgically stressed $CD8^+$ T cells appear to be engaged in stress response, as evidenced by upregulation of p38 MAPK signalling (Fig. 4.3B), which has been identified as one of the pathways upregulated in response to proinflammatory cytokines or environmental stressors(*148–150*). The inflammatory environment of surgery is characterized by the presence of pro-inflammatory cytokines such as IL-6, IL-1β, TNFα and oxidative stress that can induce the upregulation of p38 MAPK signalling pathway (*145*).

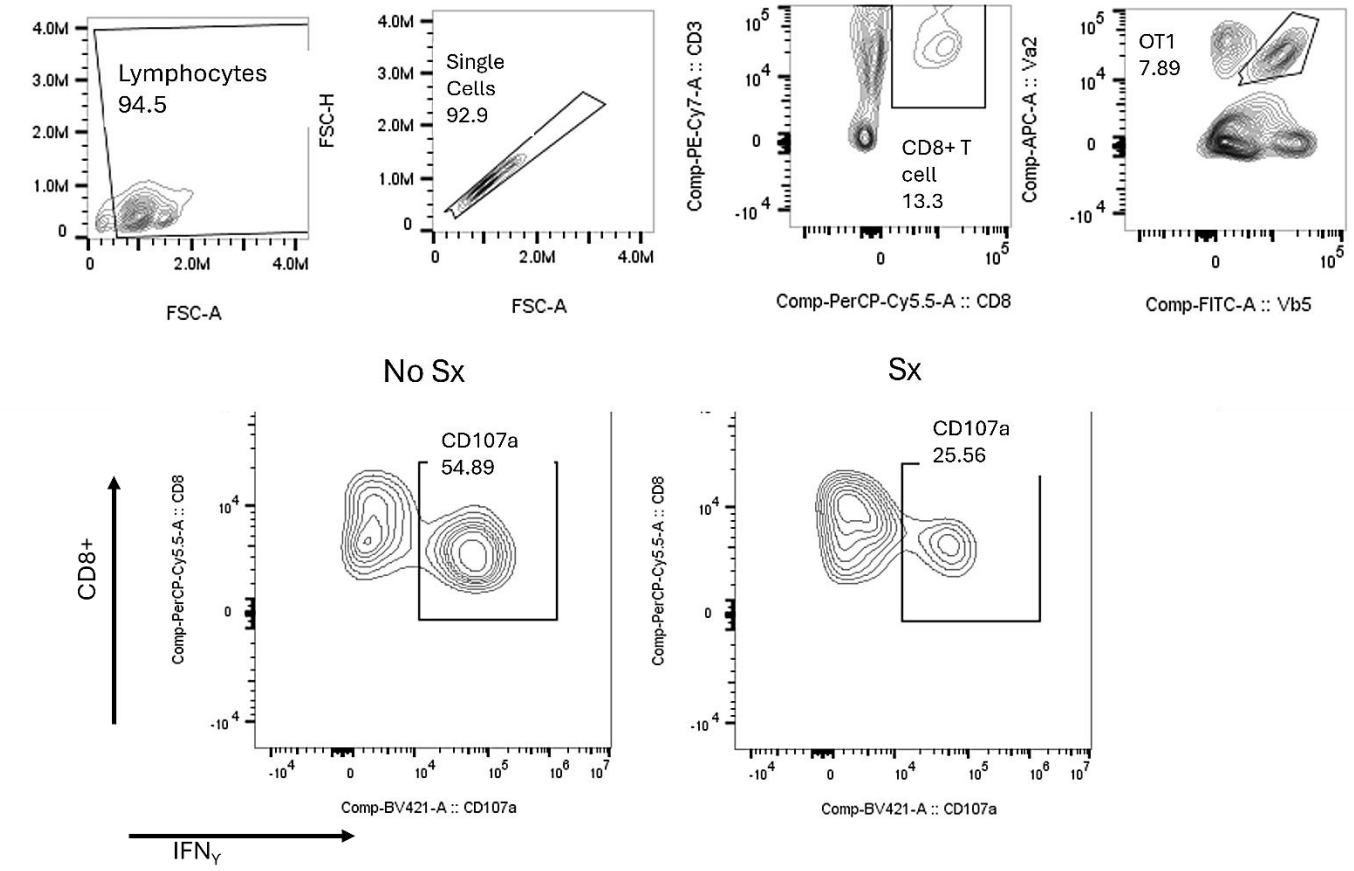
Upon further analysis we identified a potential gene that is differentially expressed in the surgically stressed group stimulated group in comparison to the non-surgically stressed stimulated group. The gene of interest CISH was identified as a potential candidate gene for further mechanistic studies because it was significantly expressed in surgically stressed stimulated group. Therefore, given the identification of CISH, the next chapter will focus on characterizing this gene in surgically stressed $CD8^+$ T cell.

## 4.5 SUPPLEMENTAL FIGURES

**A**



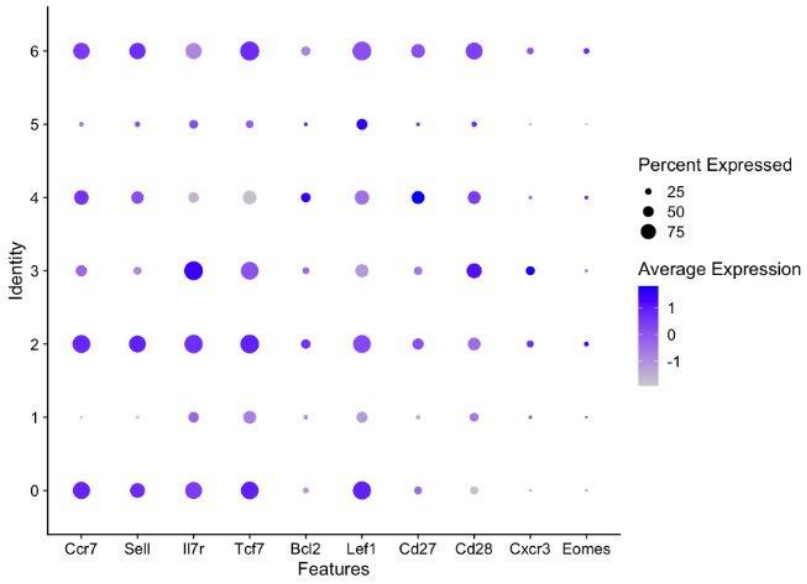
**B**


Lymphocytes
94.5
FSC-H
FSC-A
Single
Cells
92.9
FSC-A
Comp-PE-Cy7-A :: CD3
CD8+ T
cell
13.3
Comp-PerCP-Cy5.5-A :: CD8
Comp-APC-A :: Va2
OT1
7.89
Comp-FITC-A :: Vb5
No Sx
Sx
CD8+
Comp-PerCP-Cy5.5-A :: CD8
CD107a
54.89
Comp-BV421-A :: CD107a
CD107a
25.56
Comp-BV421-A :: CD107a
IFN$_\gamma$

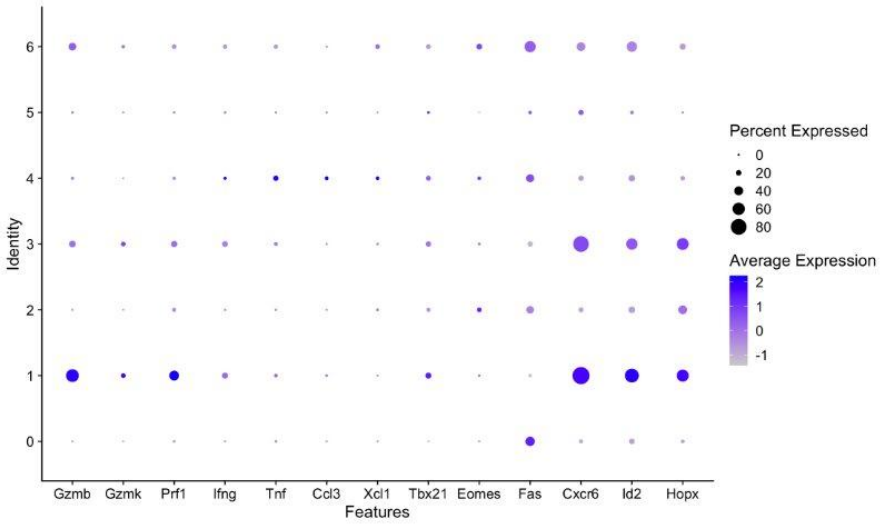
**Supplemental Figure 4.1: Expression of activation and interferon response genes**

**(A)**UMAP analysis of activation genes (CD69, CD27) and Type 1 interferon response-related genes, such as GBP2 and STAT1. **(B)** Representative flow plots showing surgically stressed $CD8^+$ T cells versus non-surgically stressed cells.

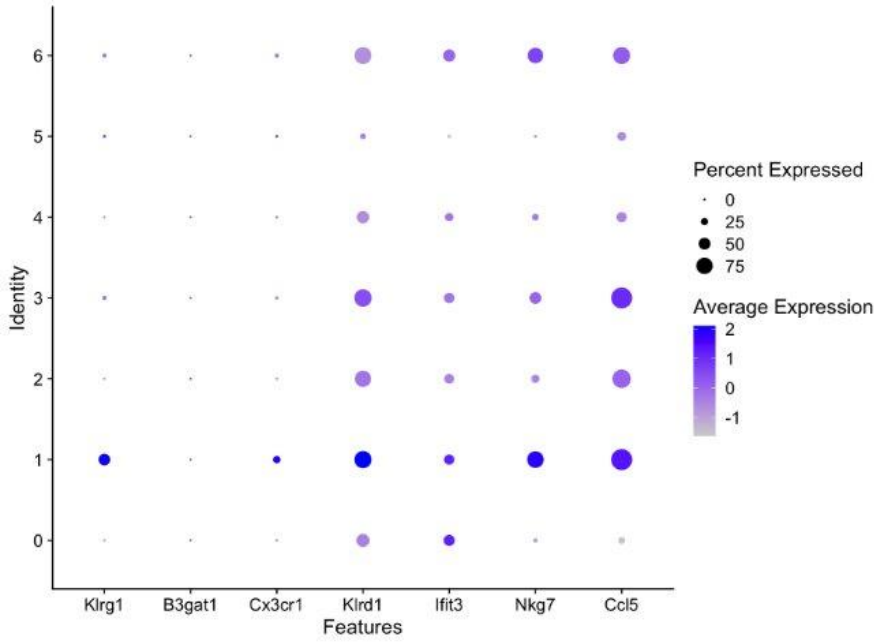
**A**


Identity
6
5
4
3
2
1
0
Ccr7
Sell
Il7r
Tcf7
Bcl2
Lef1
Cd27
Cd28
Cxcr3
Eomes
Features
Percent Expressed
25
50
75
Average Expression
1
0
-1

**B**


Identity
6
5
4
3
2
1
0
Gzmb
Gzmk
Prf1
Ifng
Tnf
Ccl3
Xcl1
Tbx21
Eomes
Fas
Cxcr6
Id2
Hopx
Features
Percent Expressed
0
20
40
60
80
Average Expression
2
1
0
-1

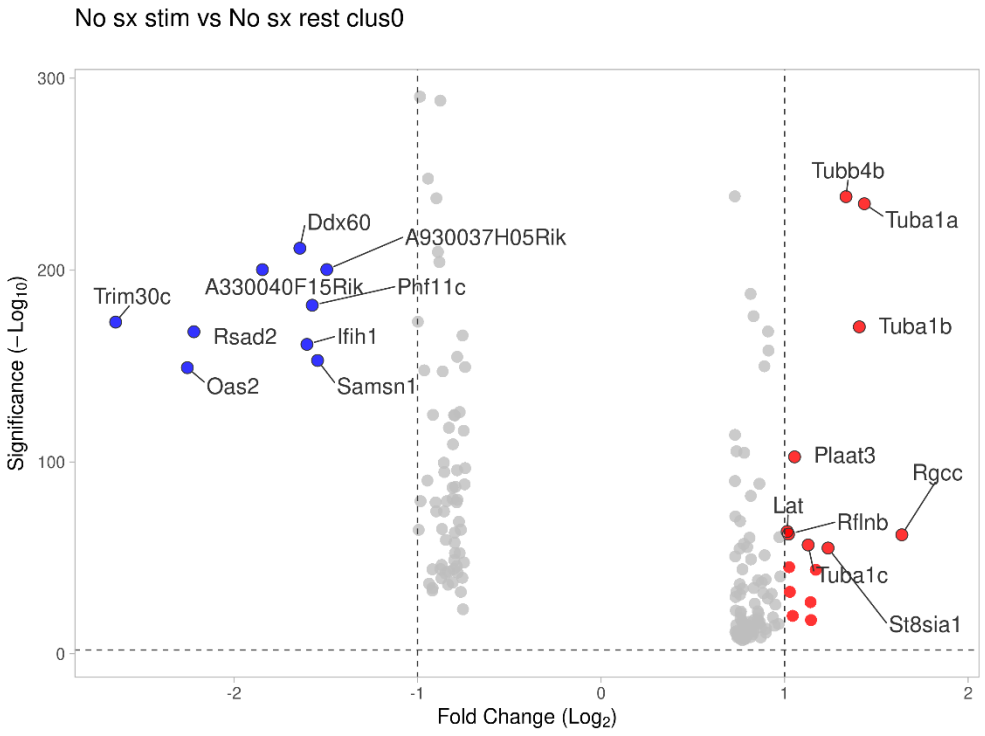
**C**


Identity
6
5
4
3
2
1
0
Klrg1
B3gat1
Cx3cr1
Klrd1
Ifit3
Nkg7
Ccl5
Features
Percent Expressed
0
25
50
75
Average Expression
2
1
0
-1

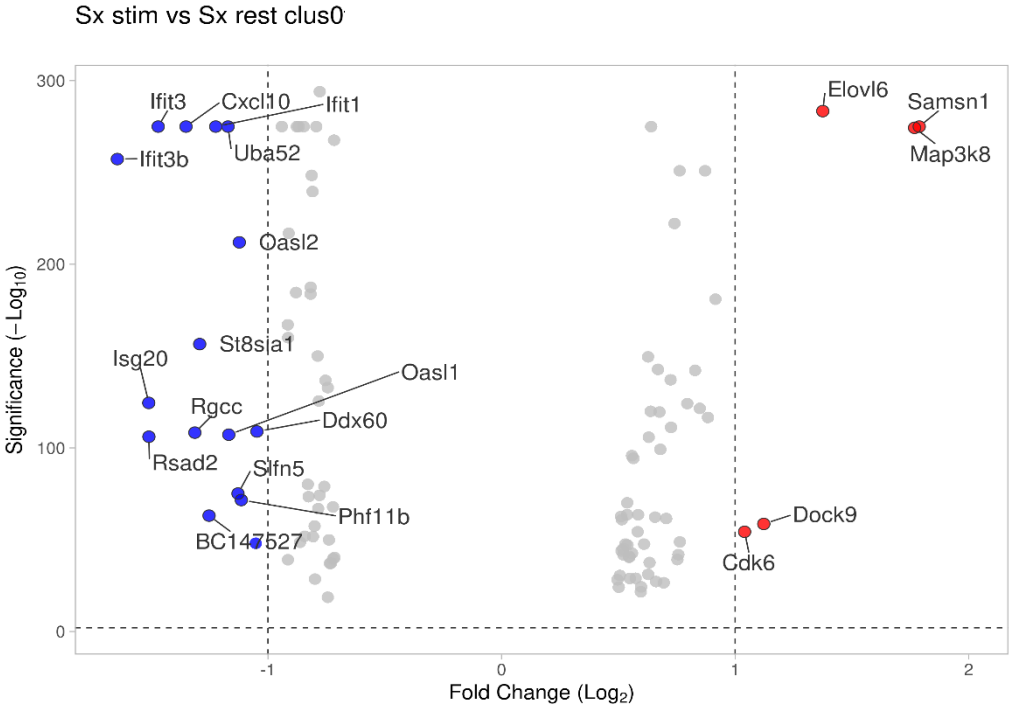
**Supplemental Figure 4.2: Identification of $CD8^+$ T cell subsets with signature markers**

**(A)**Expression of central memory $CD8^+$ T cell markers (TCM) on the x-axis and corresponding clusters on the Y-axis **(B)**Expression of $CD8^+$ T cell markers on the x-axis representing effector memory $CD8^+$ T cell population (TEM) and **(C)**Expression of $CD8^+$ T cell markers on the x-axis representing terminally differentiated effector cells (TEMRA).

**A**


No sx stim vs No sx rest clus0
Significance (−Log10)
Fold Change (Log2)
300
200
100
0
-2
-1
0
1
2
Ddx60
A930037H05Rik
A330040F15Rik
Phf11c
Trim30c
Rsad2
Ifih1
Oas2
Samsn1
Tubb4b
Tuba1a
Tuba1b
Plaat3
Rgcc
Lat
Rflnb
Tuba1c
St8sia1

**B**


Sx stim vs Sx rest clus0
Ifit3
Cxcl10
Ifit1
Uba52
Ifit3b
Oasl2
St8sia1
Isg20
Oasl1
Rgcc
Ddx60
Rsad2
Slfn5
Phf11b
BC147527
Elovl6
Samsn1
Map3k8
Dock9
Cdk6
Significance (−Log10)
Fold Change (Log2)
300
200
100
0
-1
0
1
2

**Supplemental Figure 4.3: Differential expression analysis of stimulated $CD8^+$ T cells relative to control cells**

**(A)** Volcano plot displays differential expression of genes in non-surgically stressed stimulated $CD8^+$ T cells relative to their expression in non-surgically stressed resting $CD8^+$ T cells in cluster 0, 2 and 3. Blue indicates upregulated genes with a positive Log2 fold change (x-axis). **(B)** Volcano plot showing upregulated genes in surgically stressed, stimulated cells relative to their expression in surgically stressed, resting $CD8^+$ T cells. The volcano plots show -log10 FDR-adjusted p-value (y-axis).

# Chapter 5: Identifying a potential mechanism inducing $CD8^+$ T cell suppression post-operatively

## 5.0 RATIONALE

In chapter 5, I address aim 3 of my project which is to validate the identified pathway that contribute to post-operative $CD8^+$ T cell dysfunction. I initially validate the results from chapter 4 in both murine and human $CD8^+$ T cells to confirm that the identified gene is demonstrating the same trend observed in the scRNA-seq data. I also propose a potential mechanism of post-operative $CD8^+$ T cell suppression from results observed in chapter 2 to 4.

## 5.1 INTRODUCTION

CISH is a cytokine-inducible SH2-containing protein and a member of the suppressor of cytokine signalling (SOCS) family. The SOCS family is expressed in a variety of non-immune and immune cells, such as macrophages, dendritic cells, NKs, and $CD8^+$ T cells. SOCS consist of 8 members, and they include SOCS 1-7 and CISH(*151*, *152*). CISH is primarily expressed in DCs, NK cells and $CD8^+$ T cells and function mainly as a negative regulator of cytokine signalling to keep immune responses in check. All 8 members of the SOCS family contain an N terminal that determines the specificity of cytokine receptor binding, a SH2 domain that binds to phosphorylated tyrosine residues on cytokine receptors and a conserved C terminal box that recruits E3 ubiquitin ligase complex to target ubiquitinated proteins for protein degradation(*153*). CISH was the first member of the SOCS family identified to be expressed during cytokine stimulation in hematopoietic cells. CISH is inducible by cytokines and growth hormones such as IL-1, IL-2, IL-3, IL-6, IL-7, IL-10, IL-13, IL-15, IL-21, TNFα, GM-CSF, prolactin and Erythropoietin(*154–156*). CISH is also typically induced in T lymphocytes after direct TCR stimulation(*157*, *158*). CISH is induced when cytokines or antigen bind to their associated receptor on $CD8^+$ T cell, which causes JAKs on the receptors to become phosphorylated and activated(*159*). The phosphorylation of JAK kinases recruits and activates STATs (STAT 5 or STAT 3), which results in the dimerization and translocation of STAT proteins to the nucleus. Once in the nucleus, STAT binds to CISH promoter regions to induce its expression (Fig. 5.1).

Upon T cell activation by antigen binding or TCR engagement, phosphatidylinositol 4,5 biphosphate (PIP2) which serves as a precursor for second messengers for downstream signalling molecules is hydrolysed into diacylglycerol and inositol triphosphate (IP3 & DAG) by

phospholipase C (PLCy1)(*159*, *160*). IP3 and DAG are secondary messengers essential for activating downstream signalling pathways that regulate activation, proliferation and survival, which are pathways that were shown to be downregulated according to the gene ontology analysis (Fig. 4.3). CISH functions by blocking PLCy; targeting it for degradation, thus blocking the conversion of PIP2 into IP3 and DAG(*161*) (Fig. 5.2).

In this chapter, we validated the contribution of CISH to post-operative $CD8^+$ T cell dysfunction, which was identified in Aim 2. Here I validated the expression of CISH in both murine and human $CD8^+$ T cells using RT-qPCR to verify that the results correspond to the scRNA seq data. I also propose a possible mechanism: CISH could act as an intracellular checkpoint that modulates $CD8^+$ T cell function in the post-operative period.

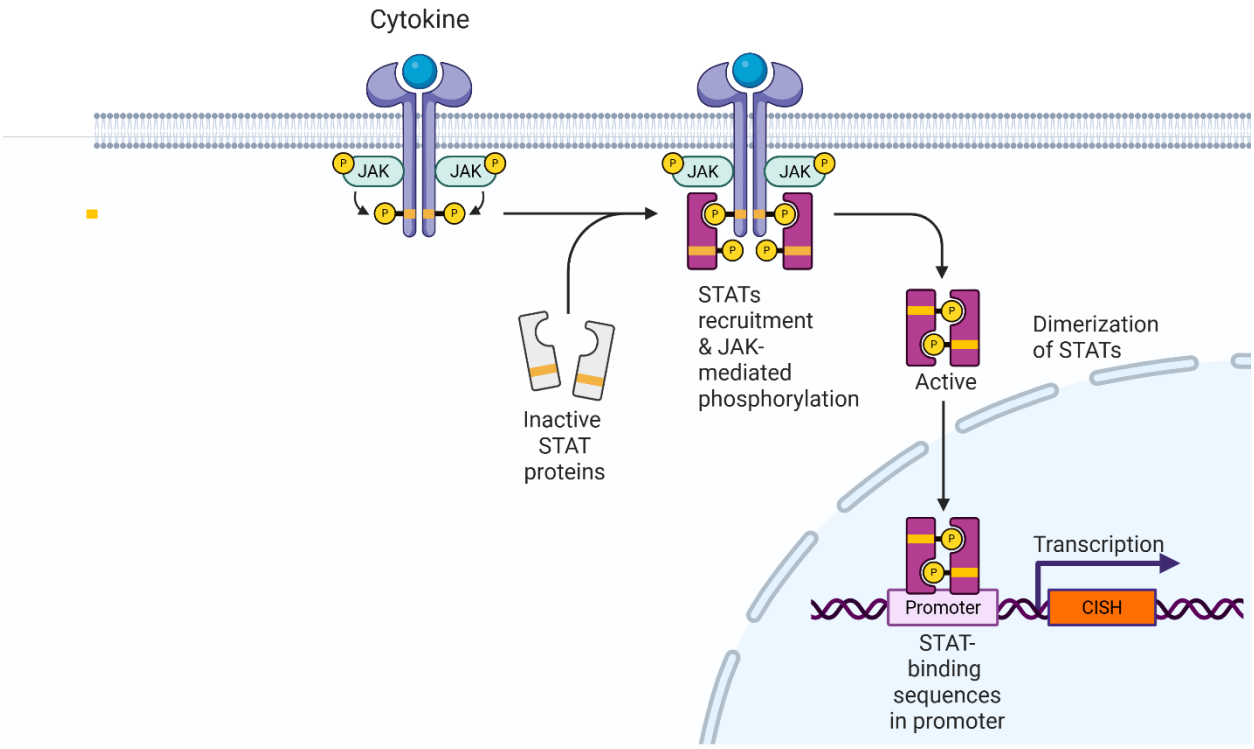

Cytokine
JAK
JAK
JAK
JAK
STATs recruitment & JAK-mediated phosphorylation
Inactive STAT proteins
Active
Dimerization of STATs
Transcription
Promoter
CISH
STAT-binding sequences in promoter

**Figure 5.1: Model of CISH expression by cytokine signaling through JAK-STAT pathway.** Stimulation of $CD8^+$ T cell results in the activation and phosphorylation of JAKs on intracellular receptors. The phosphorylation of JAK kinases recruits and activates STATs which results in the dimerization and translocation of STAT proteins to the nucleus. Once in the nucleus, STAT binds to CISH promoter regions to induce its expression. *Created in https://BioRender.com*

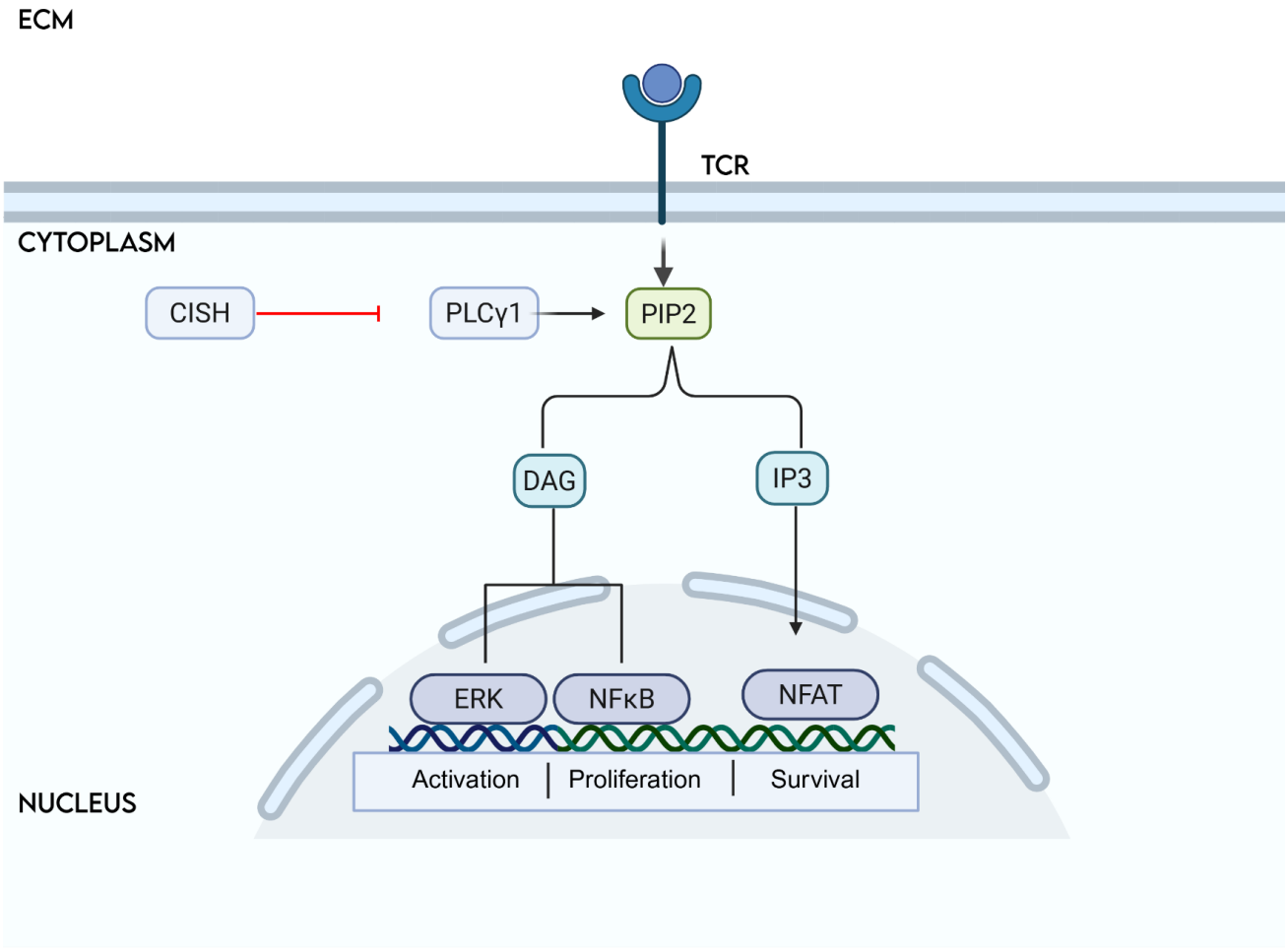

ECM
TCR
CYTOPLASM
CISH
PLCγ1
PIP2
DAG
IP3
ERK
NFκB
NFAT
Activation
Proliferation
Survival
NUCLEUS

**Figure 5.2: Model of CISH function in $CD8^+$ T cells.**

CISH inhibits $CD8^+$ T cell activity by interfering with PIP2- PLCγ1 signaling pathway. Upon $CD8^+$ T cell activation and engagement with TCR, PLCγ1 hydrolyzes PIP2 to generate DAG and IP3. DAG activates NF-Kb and ERK pathway, while IP3 facilitates the influx of calcium, which is essential for NFAT and subsequently $CD8^+$ T cell activation. CISH inhibits PLCγ1 and blocks $CD8^+$ T cell activation. *Created in https://BioRender.com*

## 5.2 METHOD

### RT-qPCR

Total cellular RNA was extracted using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Extracted RNA was used in reverse transcriptase reactions with high-capacity reverse transcriptase (Thermo Fisher) according to the manufacturer's guidelines, using random hexanucleotides as primers. The cDNA was used for real-time expression analysis using the Bio-Rad CFX96 real-time thermocycler. Fold changes in expression were determined by comparing expression levels with those of control knockdown cells or mock-infected cells and analyzing the data using the Pfaffl method.

### Myeloid cell depletion

OT-I mice received a dose of intraperitoneal injections of anti-GR1 antibody (BioxCell InVivoMab anti-mouse Ly6G/Ly6C (Gr-1), Catalog # BE0075-5MG-A) prepared in PBS (100ug/dose) for 2 days prior to the day of surgical procedure.

**Western Blot**

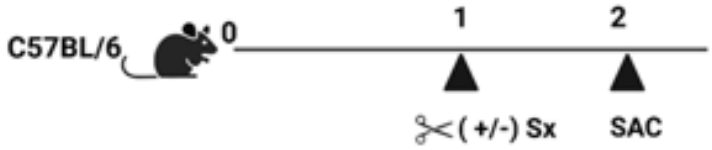
Stimulated $CD8^+$ T cells were lysed in NP-40 lysis buffer (0.5% NP-40, 50 mM Tris [pH 7.8], 150 mM NaCl) supplemented with a protease inhibitor cocktail (Sigma) at 4 °C for 10 min. Whole cell lysates were centrifuged at 12000 rpm for 5 min. Cell lysates containing 15 ug of total protein were used for the western blot. Samples lysates were resolved by 10% SDS–polyacrylamide gel electrophoresis and transferred onto PVDF membranes according to standard transfer conditions for 90 minutes at 4 °C. Membranes were blocked with 4% BSA in PBS and blots were incubated overnight at 4 °C with the corresponding primary antibody directed against, Vinculin (NEB #4650T), CISH D4D9 Rabbit monoclonal antibody (Cell Signaling Technology, #8731T ).

## 5.3 RESULTS

### Validating expression of CISH in murine CD8$^+$ T cells

The next step includes validating the upregulation of CISH in surgically stressed CD8$^+$ T cells according to the scRNA seq data. To validate the expression of CISH in mice, surgery (Nephrectomy and Laparotomy) was performed on C57BL6 mice (Fig. 5.3A). The mice were then sacrificed, and their spleens were processed. CD8$^+$ T cells were isolated using the EasySep Mouse CD8$^+$ T Cell Isolation kit and stimulated with plate-bound anti-CD3 and soluble anti-CD28 for 24 hours. Upon stimulation, the cells were counted and normalized to 1x $10^6$ cells and total RNA was extracted using a Qiagen RNA isolation kit. Using high-capacity reverse transcriptase, RNA (1.25µg) was converted to cDNA and used for real time expression analysis. Primers for target genes CISH and PLCγ1 (a downstream target for CISH) and IFNγ were included. IFNγ was included as a control to indicate the presence of suppression in surgically stressed CD8$^+$ T cells. Analysis of expression data was carried out using the Pfaffl method(*162*) and the results were normalized to housekeeping gene GUSB (beta-glucuronidase) mRNA levels and compared between non-surgically stressed and surgically stressed CD8$^+$ T cells. The expression of CISH was upregulated in three of the five mice that were suppressed after surgery (IFNγ was downregulated), with a fold change of ≥2 (Fig. 5.3B). This was correlated with the downregulation of PLCγ1 (fold change ≤ 0.5), the downstream target of CISH.

**A**




**B**

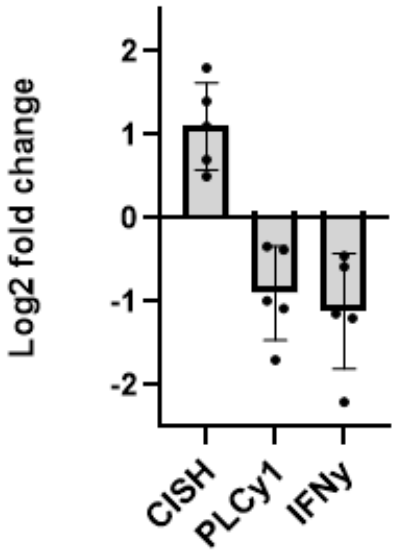


**Figure 5.3. Surgical stress induces CISH expression in murine $CD8^+$ T cells**

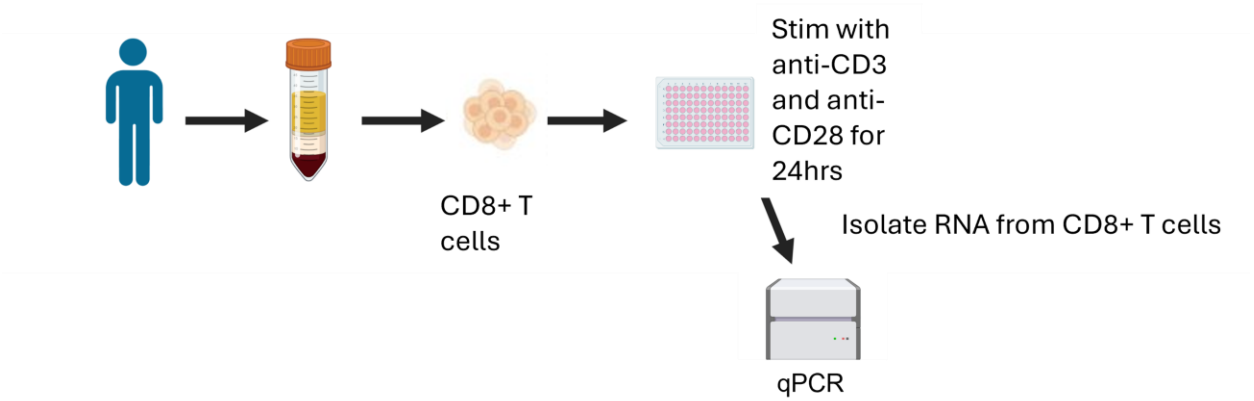
**(A)** C57BL/6 mice underwent surgery (Sx) or no surgery (No Sx) and were sacrificed on day 2 for $CD8^+$ T cell isolation. The cells were stimulated with plate bound anti-CD3 (5 μg/ml) and soluble anti-CD28 (2 μg/ml), followed by total RNA extraction and cDNA synthesis. **(B)** Gene expression was quantified, comparing log2 fold change in surgically stressed cells to non-surgically stressed $CD8^+$ T cells (n=5).

**Validating expression of CISH in human CD8$^+$ T cells**

To validate in human CD8$^+$ T cells, blood was collected from cancer patients undergoing abdominal surgeries at POD0 and POD1(Fig. 5.4A). CD8$^+$ T cells were isolated from the PBMCs and stimulated with plate-bound anti-CD3 and soluble anti-CD28 for 24 hours. Like the steps mentioned above, the cells were counted and normalized to 5 x $10^5$ and total RNA was extracted using a Qiagen RNA isolation kit. Using high-capacity reverse transcriptase, RNA (100 ng) was converted to $_C$DNA and used for real time expression analysis.

Similarly, we observe CISH upregulated in human CD8$^+$ T cells undergoing suppression, while PLCγ1 is downregulated (Fig. 5.4B). The expression of CISH was upregulated in six of the eight patients that were suppressed after surgery (IFNγ was downregulated), with a fold change of ≥2 (Fig. 5.4B). This was also correlated with the downregulation of PLCγ1 (fold change ≤ 0.5), the downstream target of CISH.

**A**


Stim with anti-CD3 and anti-CD28 for 24hrs
CD8+ T cells
Isolate RNA from CD8+ T cells
qPCR

**B**

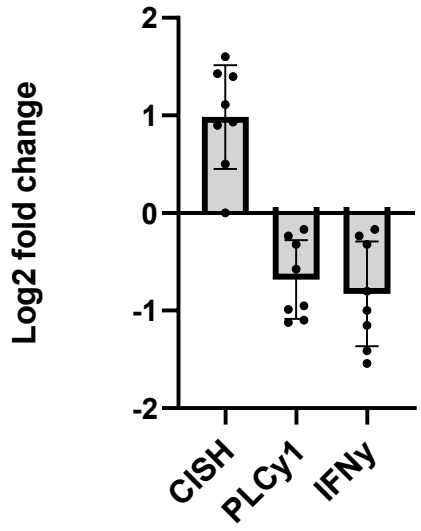

Log2 fold change
2
1
0
-1
-2
CISH
PLCγ1
IFNγ

**Figure 5.4: Surgical stress induces CISH expression in human $CD8^+$ T cells**

**(A)** $CD8^+$ T cells were isolated from cancer patients' PBMCs before and after abdominal surgery and stimulated with plate bound anti-CD3 (5 μg/ml) and soluble anti-CD28 (2 μg/ml), and total RNA was extracted to quantify gene expression. **(B)**The Log2 fold change in expression in surgically stressed cells was compared with that in non-surgically stressed human $CD8^+$ T cells (n = 8).

**Establishing a mechanism of dysfunction in post-operative CD8$^+$ T cells**

The role of CISH in cancer has attracted interest over the years due to its involvement in CD8+ T cell activation, exhaustion, and immunotherapy. CISH is induced in the tumor microenvironment (TME) and inhibits CD8$^+$ T cell proliferation and cytokine secretion (IFNγ, TNFα, IL-2) while knockdown of CISH in CD8$^+$ T cells resulted in a significant increase in cytokine secretion, antigen sensitivity, and enhanced anti-tumor immunity *in vitro* and *in vivo* (*163–165*). Currently, CISH silencing in adoptive T cells, both tumor-infiltrating lymphocytes (TIL) and CD19 chimeric-antigen receptor (CAR) T cells, is being investigated as a promising therapeutic strategy (*165*, *166*).

The role of CISH in suppressing CD8$^+$ T cells during surgery is unexplored. Our scRNA-seq and qPCR data indicate that CISH expression is upregulated in both murine and human CD8$^+$ T cells under surgical stress. As stated previously, CISH is induced by a broad spectrum of cytokines such as IL-1, IL-2, IL-3, IL-6, IL-7, IL-10, IL-13, IL-15, IL-21, TNFα, GM-CSF, prolactin, and Erythropoietin. Most of these cytokines are present in an inflammatory environment, such as cancer or, in this context, surgery. The cytokine rich environment present during surgery appears to create a favorable setting that drives the expression of CISH through the JAK-STAT axis. For example, IL-6 and IL-10 are two major cytokines abundantly expressed during surgery and are both strong inducers of CISH expression by activating the JAK1/STAT3 pathway(*167*). IL-6 can function as both a pro-inflammatory and an anti-inflammatory molecule depending on the context, while IL-10 mostly functions as an anti-inflammatory molecule that dampens the

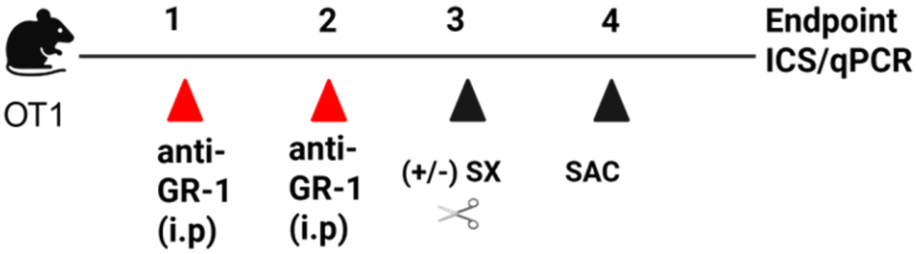
proinflammatory processes present during surgery. One of the primary producers of both IL-6 and IL-10 in the surgical environment are myeloid cells (GR-$1^+$) or MDSCs, which are known suppressors of $CD8^+$ T cells(*168–173*). In chapter two, we also established that depletion of GR-$1^+$ cells restored $CD8^+$ T cell function (Fig 2.4). Therefore, we hypothesized that increased expression of CISH in $CD8^+$ T cells causes post-operative dysfunction and MDSCs are one of the factors that modulate this effect.

To address this question, GR-$1^+$ cells were depleted by administering two doses of an anti-GR-1 antibody intraperitoneally in OT-I mice. Following the administration of the anti-GR-1 antibody, surgery was performed on the OT-I mice. The OT-I mice were sacrificed twenty-four hours later, followed by $CD8^+$ T cell isolation from the spleen, followed by RNA isolation. Real-time expression analysis was performed to detect CISH expression in $CD8^+$ T cells from GR-$1^+$ depleted and surgically stressed mice.

$CD8^+$ T cells that were surgically stressed displayed an increase in CISH expression compared to non-surgically stressed cells with a fold change >2 (Fig. 5.5B)

When GR-$1^+$ cells are depleted in the surgically stressed mice, mRNA levels of CISH are no longer elevated with a fold change of 0.57 (Fig.5.5B). This is correlated with an increase in IFNγ expression when GR-$1^+$ cells are absent.

**A**


1
2
3
4
Endpoint
ICS/qPCR
OT1
anti-
GR-1
(i.p)
anti-
GR-1
(i.p)
(+/-) SX
SAC

**B**

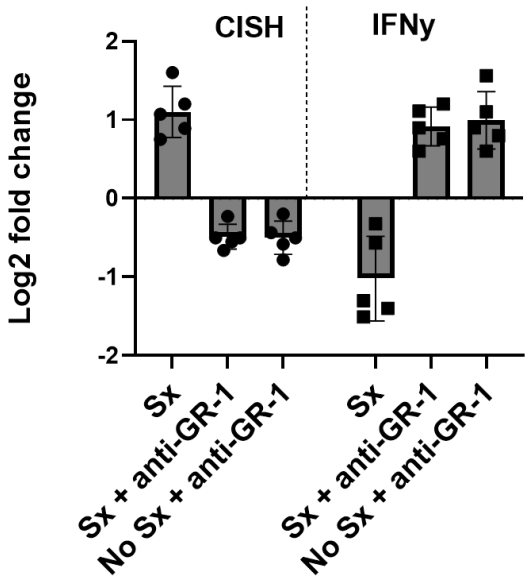

CISH
IFNy
Log2 fold change
2
1
0
-1
-2
Sx
Sx + anti-GR-1
No Sx + anti-GR-1
Sx
Sx + anti-GR-1
No Sx + anti-GR-1

**Figure 5.5: Surgery impairs $CD8^+$ T cell function and induces CISH expression that is reversed by the depletion of $GR\text{-}1^+$ cells**

**(A)**OT-I mice were treated with three doses of anti-GR-1 monoclonal antibody and either underwent surgery (Sx) or not (No Sx) **(B)** On day 1, mice were sacrificed, followed by isolation of $CD8^+$ T cells. $CD8^+$ T cells stimulated with plate bound anti-CD3 (5 μg/ml) and anti-CD28 (2 μg/ml). Total RNA was extracted, and gene expression was quantified using log2 fold change in surgically stressed cells relative to non-surgically stressed murine $CD8^+$ T cells (n = 5).

## 5.4 DISCUSSION

The results also indicate that the upregulation of CISH after surgery is not antigen specific, as the $CD8^+$ T cells were stimulated via TCR with anti-CD3. According to the data, surgery upregulates CISH expression upon stimulation, which correlates with $CD8^+$ T suppression, marked by reduced IFNγ levels in these cells. CISH functions by inducing the ubiquitination of PLCγ1, which results in the degradation and loss of the protein (*174*). The absence of PLCγ1 in $CD8^+$ T cells impairs the activation of the NFAT/NF-kB axis, leading to reduced $CD8^+$ T effector function(*175–177*).

CISH can also suppress $CD8^+$ T cells independently of TCR engagement via the JAK-STAT pathway. T cells are further activated through cytokine signaling, such as IL-2, IL-7, and IL-15, which bind to receptors associated with JAKs. This binding of cytokines to their respective receptors leads to the phosphorylation and dimerization of STAT proteins, which then translocate to the nucleus to regulate genes important for T cell effector function. CISH can inhibit the JAK-STAT axis by binding to JAK proteins on cytokine receptors and dampen their activity, thereby preventing STAT phosphorylation and subsequent gene regulation of T cell-related genes(*178*).

Here, we demonstrated that CISH, which is typically induced by TCR engagement, such as antigen stimulation, is upregulated at the mRNA level in both murine and human surgically stressed, antigen-stimulated $CD8^+$ T cells. We also examined CISH expression at the protein level using the same experimental procedure as for mRNA. We isolated lysates from stimulated $CD8^+$ T cells and performed a western blot to assess CISH protein levels. We detected a modest increase in CISH protein expression; however, this change did not reach statistical significance

in this cohort of mice (Supplemental Fig.5.1B). Similarly, the expression of CISH at protein level was investigated in surgically stressed human $CD8^+$ T cells. We did observe an increase in CISH expression at POD1; however, this was only observed in one patient (Supplemental Fig.5.1C). A limitation of this experiment is the low protein yield from primary $CD8^+$ T cells, which reduced assay sensitivity and prevented definitive conclusions regarding CISH protein expression.

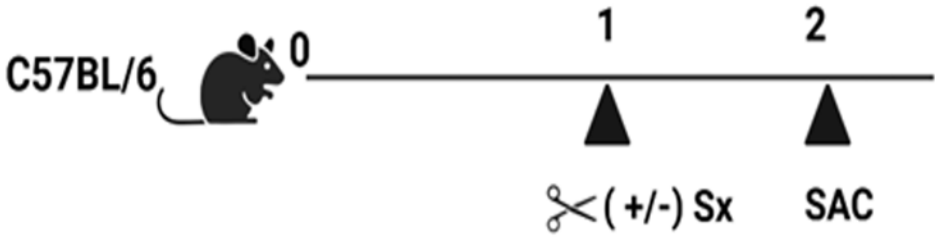
In literature, some studies have shown that CISH is upregulated in tumor-infiltrating $CD8^+$ T cells(*166*, *179*). The upregulation of CISH in tumor-infiltrating $CD8^+$ T cells has been observed to function as an intracellular immune checkpoint that regulates $CD8^+$ T cell function and prevents overactivation of T cells. Cancer cells can exploit these immune checkpoint molecules, such as CISH, to suppress $CD8^+$ T cell function. Factors in the tumor microenvironment, such as chronic antigen stimulation and inflammation that drive the expression of other external immune checkpoints, such as PD-1, also govern the expression of CISH in these cells(*165*, *180–182*). It is reasonable to propose that perhaps the upregulation of CISH in the context of surgery is also driven by similar factors, most notably inflammation and the surge of proinflammatory and anti-inflammatory cytokines.

Our findings suggest that regulation of CISH following surgery may be mediated by sxMDSCs, which have been shown to suppress $CD8^+$ T cell function in our model. Notably, depletion of $GR\text{-}1^+$ cells in the OT-I model of surgical stress prevented post-operative increase in CISH mRNA expression. It is important to note that $GR\text{-}1^+$ depletion targets multiple myeloid populations, and further work is needed to define the specific cells involved in mediating the observed suppression in $CD8^+$ T cells. Our previous AdDCT vaccinated mouse model did

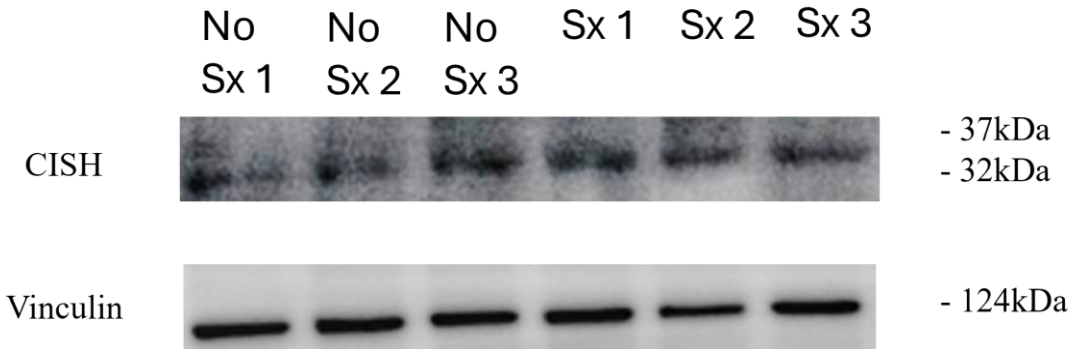
indicate that suppression was mediated by expanded granulocytic sxMDSCs and not by the monocytic population(*131*). Collectively, the data supports a model in which surgical stress promotes sxMDSC expansion, leading to CISH induction and downstream suppression of $CD8^+$ T cell effector function.

## 5.5 SUPPLEMENTAL FIGURES

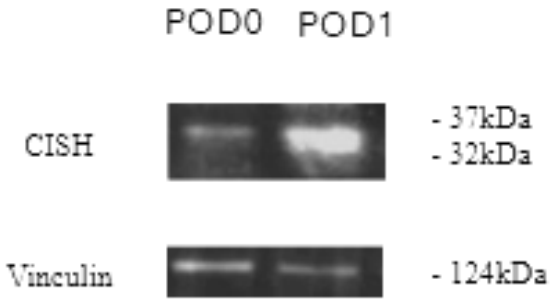
**A**




**B**

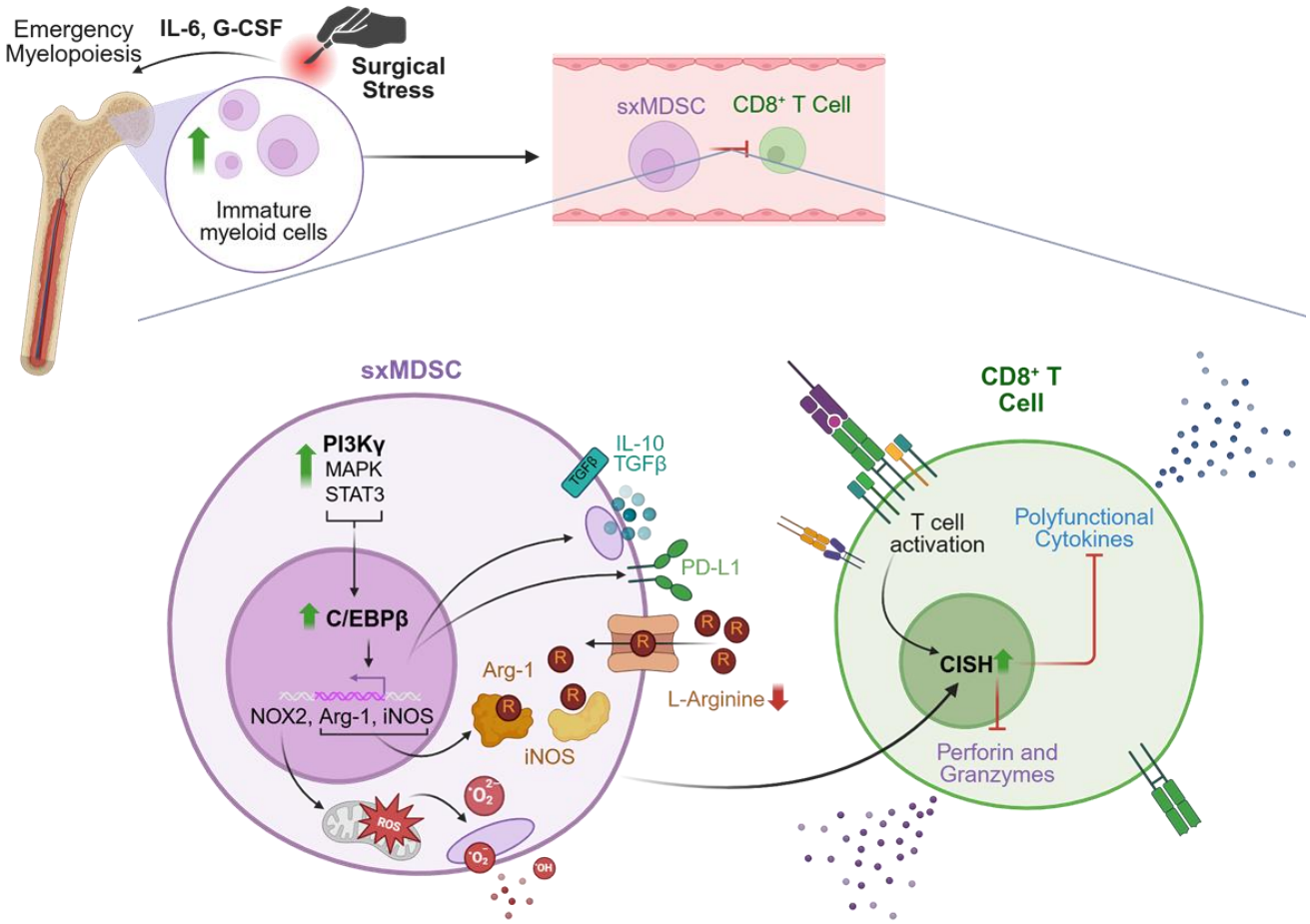



C



**Supplemental Figure 5.1: Surgical stress induces a modest upregulation in CISH protein expression in murine CD8$^+$ T cells**

**(A)** C57BL/6 mice underwent surgery (Sx) or no surgery (No Sx) and were sacrificed on day 2 for CD8$^+$ T cell isolation. The cells were stimulated with anti-CD3 (2 µg/ml) and anti-CD28 (1 µg/ml). **(B)** Western blot of whole cell lysate and 15 µg of total cell lysate from mice was loaded per lane, resolved by SDS-PAGE, and blotted for CISH and Vinculin. (n=3). (**C**) Western blot of whole cell lysate and 10 µg of total cell lysate from human CD8$^+$ T cells was loaded and blotted for CISH and Vinculin, n=1.


Emergency Myelopoiesis
IL-6, G-CSF
Surgical Stress
Immature myeloid cells
sxMDSC
CD8⁺ T Cell
sxMDSC
PI3Kγ
MAPK
STAT3
C/EBPβ
NOX2, Arg-1, iNOS
ROS
IL-10
TGFβ
PD-L1
Arg-1
iNOS
L-Arginine
CD8⁺ T Cell
T cell activation
Polyfunctional Cytokines
CISH
Perforin and Granzymes

**Supplemental Figure 5.2: Schematic demonstrating sxMDSCs impairs $CD8^+$T cell function through internal checkpoint CISH**

Surgical stress and inflammatory mediators (IL-6, G-CSF) promote emergency myelopoiesis and expansion of immature myeloid cells, which activate transcription factor C/EBPβ downstream of PI3Kγ, MAPK, and STAT3 signaling pathways, inducing the expression of immunosuppressive molecules including NOX2, Arg-1, and iNOS. sxMDSCs suppress $CD8^+$ T cell activity through depletion of L-arginine, release of reactive oxygen and nitrogen species, secretion of IL-10 and TGF-β, and engagement of PD-L1. These suppressive signals inhibit T cell activation, enhance expression of the checkpoint regulator CISH, and limit production of perforin, granzymes, and poly-cytokine secretion. The schematic is adapted from Dr. Rafeah Alam and Agrima Shrestha. *Created with Biorender.com.*

# CHAPTER 6: GENERAL DISCUSSION

## 6.0 RATIONALE

In this chapter, I discuss the findings from chapters 2 to 5, focusing on overarching concepts. I identify key results from each chapter, such as the characterization of $CD8^+$ T cell dysfunction in human and murine models and highlight their consequences for anti-tumor immunity. I discuss the suppression of $CD8^+$ T cell function by sxMDSCs observed in the OT-I model. I discuss key findings from the scRNA-seq dataset and summarize the roles of the identified factors that regulate post-operative $CD8^+$ T cell function. Lastly, I will discuss future studies related to my project.

## 6.1 INTRODUCTION

The studies in this thesis aimed to characterize antigen-specific $CD8^+$ T cell dysfunction after surgery. We used both murine (OT-I mouse) and human (COVID-19) immunological tool kits to define antigen-specific post-operative T-cell responses, an approach that has not been explored before in cancer patients undergoing surgery. In subsequent chapters, we investigated a possible mechanism mediating dysfunction using scRNA-seq and validated the results in mouse models. It's important to emphasize that $CD8^+$ T cells post-operative dysfunction is most likely caused by multiple mechanisms, and the mechanism examined in the thesis represents one of the possible pathways involved in post-operative dysfunction.

## 6.2 POST-OPERATIVE $CD8^+$ T CELL DYSFUNCTION

A good majority of the preclinical studies to date have established post-operative suppression of NK cells and non-antigen-specific $CD8^+$ T cells, leading to cancer regrowth and the formation of metastases(*130–132*, *183*). We have also previously observed post-operative dysfunction in murine antigen-specific $CD8^+$ T cells using a model of anti-tumor vaccination with an adenovirus encoding the AdDCT antigen(*183*). Although the AdDCT model was informative, we could not quantify a significant number of T cell responses as the vaccine model induced a modest immune response. In comparison to the AdDCT model, the OT-I and COVID-19 model enabled the quantification of a larger number of both murine and human antigen-specific $CD8^+$ T cell responses. We examined the function of antigen-specific $CD8^+$ T cells because this would enable the characterization of tumor-specific $CD8^+$ T cells. The investigation of antigen-specific $CD8^+$ T cells can offer valuable insights into how surgery impacts anti-tumor immunity, a question that non-antigen specific studies cannot fully address. Additionally, we confirmed and

demonstrated that dysfunction occurs independent of stimuli and $CD8^+$ T cell suppression is not solely dependent on TCR engagement but on the external factors induced by surgery. Total $CD8^+$ T cell dysfunction has been previously demonstrated; with cells exhibiting reduced cytokine secretion and impaired proliferation(*131*, *184–187*). We were able to confirm a similar reduction in cytokine secretion in both human and murine $CD8^+$ T cells in our models. The impact of surgery extends beyond cytokine secretion and degranulation. We detected a significant decrease in anti-tumor immunity of surgically stressed cells in the OT-I tumor model, signifying that these cells were unable to control tumor growth and the effect of surgery observed *ex-vivo* with cytokine secretion and degranulation was translated *in-vivo.* A similar observation was reported in the AdDCT vaccine model, in which surgically stressed $CD8^+$ T cells displayed a reduced ability to prevent tumor development in 100% of vaccinated mice(*120*). The suppression identified in mice studies was also confirmed in human $CD8^+$ T cells by using the COVID-19 model. These findings suggest that surgery and the resulting stress response significantly impair $CD8^+$ T cells, and this dysfunction has biologically relevant consequences.

## 6.3 IMPACT OF SXMDSCS ON THE FUNCTION OF CD8$^+$ T CELLS

Several preclinical studies have established that MDSCs suppress CD8$^+$ T cell function in different settings (*188–194*). In the OT-I model, we observed that depletion of the GR-1 population prior to surgery increased antigen-specific CD8$^+$ T cell cytokine secretion. Most studies to date have focused on the immunosuppressive effects of sxMDScs on NK cells rather than on CD8$^+$ T cells. Cancer, inflammation, trauma, and infection have all been shown to induce MDSC production and suppressive activity, but the mechanisms differ depending on the context. To intervene with a therapy targeting MDSCs, we must clearly understand these mechanisms and determine which upstream or downstream pathways are necessary to prevent post-operative immune suppression. Our data indicate that the PI3Kγ pathway is one of the pathways responsible for the suppressive phenotype of sxMDSCs following cancer surgery. Single-cell RNA sequencing was performed on MDSCs from cancer patients at baseline and POD1, and we detected an upregulation of the PI3K and MAPK pathway(*195*). This pathway was confirmed by performing a high-throughput screen of small molecules, and our lab identified a pan-PI3K inhibitor as the top hit molecule that prevented the suppression of NK cell cytotoxicity induced by sxMDSCs(*195*). Previous studies have also identified PI3Kγ as a key pathway that regulates immune suppression in macrophages(*196–198*). In macrophages, PI3Kγ functions as a molecular switch that controls immune suppression. By signaling through Akt and mTOR, PI3Kγ stimulates C/EBPβ expression, a critical transcriptional regulator of the immunosuppressive phenotype of these cells(*199*). C/EBPβ activates several immunosuppressive pathways, including increased expression of Arg1 (depletion of arginine), iNOS, NOX2 (generation of ROS), IL-10, PD-L1, and TGFβ, and is associated with reduced expression of proinflammatory cytokines, such as IL-12, IL1β, and TNFα(*200*)

Our group as well as others has established the immunosuppressive impact of sxMDSCs on NK cells(*130*, *195*, *201*). Depletion of sxMDSCs prevented suppression of NK cells post-operatively and reduced post-operative metastases(*202*). The effect of MDSCs was further confirmed in this study because the transfer of sxMDSCs into naïve mice recapitulated the effects of surgical stress on NK cell cytotoxicity. The mechanism of sxMDSCs suppression on NK cells has been demonstrated by our group by inhibiting arginase enzymatic activity and blocking TGFβ receptor signaling which prevented NK cell dysfunction(*133*, *202*). The mechanism of suppression by MDSCs in $CD8^+$ T cells in general has been explored. These mechanisms include the depletion of essential amino acids such as l-arginine, production of NOS, ROS, and PD-L1, all of which function by preventing proper T cell activation or function(*91*, *191*, *193*, *203*, *204*). However, the extent to which these mechanisms are responsible for post-operative $CD8^+$ T cell dysfunction has not been thoroughly explored. Therefore, future studies will focus on investigating the mechanisms responsible for sxMDSCs suppression to identify the most specific target that is both required and sufficient to induce post-operative T cell dysfunction.

## 6.4 MECHANISM OF POST-OPERATIVE $CD8^+$ T CELL DYSFUNCTION

The scRNA sequencing revealed an upregulation of genes in surgically stressed $CD8^+$ T cell that function as a negative regulator of T cell function, such as the gene of primary interest CISH, and SBNO2 (Fig. 4.4). SBNO2 (Strawberry Notch Homolog 2) is a gene that belongs to the ‘strawberry notch family’ of conserved, nuclear helicases. SBNO2 functions as a transcriptional factor that responds to IL-10 signaling and has been reported to inhibit NF-Kb transcription in a STAT3 (Signal Transducer and Activator of Transcription 3) dependent manner in macrophages (*216*, *217*). Although the role of SBNO2 in T cells is not well characterized, however we can

infer that it plays a possible role in suppressing $CD8^+$ T cell function through the NF-Kb pathway. Based on these observations, surgically stressed $CD8^+$ T cells may upregulate factors such as CISH, and potentially SBNO2, which are regulators that may diminish surgically stressed $CD8^+$ T cell function.

This raises the question of why surgically stressed $CD8^+$ T cells upregulate these negative modulators. A possible explanation is the pro-inflammatory and anti-inflammatory environment that surgery creates. As explained in chapter 1, surgery results in the generation of MDSCs, which are produced because of surgery induced DAMPs, such as HMGB1 which engage pattern recognition receptors on myeloid and stromal cells, triggering a pro-inflammatory state that leads to myelopoiesis and recruitment of these cells. We have also established that these cells suppress $CD8^+$ T cell function. MDSCs also produce suppressive factors such as TGFβ and IL-6, which are known suppressors of immune cells such as NK and T cells(*87*, *168*). Our data indicates that depletion of MDSCs results in reduced CISH expression and increased $CD8^+$ T cell function. Therefore, we infer that increased expression of the intracellular checkpoint CISH in $CD8^+$ T cells may be responsible for post-operative dysfunction and that MDSCs could potentially regulate this effect through suppressive mechanisms that are highlighted in Supplemental Fig. 5.2.

## 6.5 FUTURE STUDIES AND LIMITATIONS

Studies that will enable a comprehensive understanding of the main pathways from cytokines to signaling pathways, to immunosuppressive mechanisms responsible for post-operative T cell suppression should be investigated to provide insight into future therapeutic targets. To investigate the possible mechanism of suppression, we can use inhibitors to systematically evaluate the strength of downstream mechanisms of MDSC suppression, including inhibitors of arginase 1, iNOS, NOX2-dependent ROS production, TGFβ, IL-10, and monoclonal antibodies blocking PD-L1. The role that CISH plays in $CD8^+$ T cells following surgery as the basis for dysfunction needs to be explored further. For example, the role of CISH in causing post-operative $CD8^+$ T cell dysfunction in mice can be investigated in-vivo by knocking it out in $CD8^+$ T cells to determine if the effects of surgery on T cell function are attenuated. In tumor settings, several preclinical studies have demonstrated that genetic depletion of CISH with CRISPR-Cas9 technology improved $CD8^+$ T cell cytotoxicity, including a significant increase in cytokine secretion, antigen sensitivity and enhanced anti-tumor immunity *in vitro* and *in vivo*(*178–180*). A recent study is exploring CISH deletion in both tumor-infiltrating lymphocytes (TIL) and CD19 chimeric-antigen receptor (CAR) T cells, and this is being pursued as a promising therapeutic target to improve anti-tumor T cell function(*218*). Therefore, it is feasible to perform CRISPR–Cas9-mediated knockout of CISH in our OT-I tumor mouse model to confirm that CISH is responsible for $CD8^+$ T cell dysfunction during the post-operative period and determine whether the immunosuppressive effects of sxMDSCs are mediated through CISH upregulation.

This study has some limitations that should be considered when interpreting the results. Firstly, the depletion of GR-$1^+$ cells using the anti-GR-$1^+$ antibody does not selectively target only the MDSC population but also depletes other myeloid populations that express the GR-1 marker including neutrophils and some monocytic cells. Therefore, the result of the GR-1 depletion experiment cannot be attributed solely to the MDSCs but possibly the depletion of the other target cells that were also eliminated. Secondly, the LM-OVA infection model which was used to identify antigen specific responses following surgery does not fully represent the complex and chronic setting of a tumor microenvironment. Features of a tumor microenvironment include persistent cytokine signaling and $CD8^+$ T cell exhaustion, which are typically absent in an acute model such as LM-OVA infection. Therefore, the LM-OVA model may not have fully captured the effect of surgical stress in a chronic setting such as cancer.

## 6.6 CONCLUDING REMARKS

An effective anti-tumor immunity is one of the most important indicators of cancer outcomes, which is a notion that has enabled the development of several immunotherapeutics aimed at improving $CD8^+$ T cell function. While the mechanisms of cancer-induced immune suppression in the TME have been well studied, the impact of surgery on anti-tumor T cell function has not been fully explored. However, this phenomenon needs to be better understood for cancer patients undergoing surgery to have positive outcomes. The perioperative period represents a promising therapeutic window for immunomodulation, particularly for targeting factors that contribute to $CD8^+$ T cell dysfunction, such as sxMDSCs and potentially CISH. Therapeutic strategies aimed at reversing post-operative immunosuppression may therefore improve long-term cancer outcomes for the millions of patients who undergo cancer surgery each year.

## REFERENCES


1. H. Endo, M. Inoue, Dormancy in cancer. *Cancer Science* **110**, 474–480 (2019).

2. D. Hanahan, R. A. Weinberg, The Hallmarks of Cancer. *Cell* **100**, 57–70 (2000).

3. J. Massagué, K. Ganesh, Metastasis-Initiating Cells and Ecosystems. *Cancer Discovery* **11**, 971–994 (2021).

4. A. W. Lambert, D. R. Pattabiraman, R. A. Weinberg, Emerging Biological Principles of Metastasis. *Cell* **168**, 670–691 (2017).

5. J. E. Talmadge, I. J. Fidler, AACR Centennial Series: The Biology of Cancer Metastasis: Historical Perspective. *Cancer Research* **70**, 5649–5669 (2010).

6. D. Hanahan, R. A. Weinberg, Hallmarks of Cancer: The Next Generation. *Cell* **144**, 646–674 (2011).

7. R. Sabat, G. Grütz, K. Warszawska, S. Kirsch, E. Witte, K. Wolk, J. Geginat, Biology of interleukin-10. *Cytokine & Growth Factor Reviews* **21**, 331–344 (2010).

8. B. F. Johnson, T. M. Clay, A. C. Hobeika, H. K. Lyerly, M. A. Morse, Vascular endothelial growth factor and immunosuppression in cancer: current knowledge and potential for new therapy. *Expert Opinion on Biological Therapy* **7**, 449–460 (2007).

9. L. Yang, TGFß, a Potent Regulator of Tumor Microenvironment and Host Immune Response, Implication for Therapy. *CMM* **10**, 374–380 (2010).

10. J. David, C. Dominguez, D. Hamilton, C. Palena, The IL-8/IL-8R Axis: A Double Agent in Tumor Immune Resistance. *Vaccines* **4**, 22 (2016).

11. A. Teijeira, S. Garasa, M. C. Ochoa, M. Villalba, I. Olivera, A. Cirella, I. Eguren-Santamaria, P. Berraondo, K. A. Schalper, C. E. De Andrea, M. F. Sanmamed, I. Melero, IL8, Neutrophils, and NETs in a Collusion against Cancer Immunity and Immunotherapy. *Clinical Cancer Research* **27**, 2383–2393 (2021).

12. X. Lin, K. Kang, P. Chen, Z. Zeng, G. Li, W. Xiong, M. Yi, B. Xiang, Regulatory mechanisms of PD-1/PD-L1 in cancers. *Mol Cancer* **23**, 108 (2024).

13. C. Cantoni, H. Wurzer, C. Thomas, M. Vitale, Escape of tumor cells from the NK cell cytotoxic activity. *Journal of Leukocyte Biology* **108**, 1339–1360 (2020).

14. H. Raskov, A. Orhan, J. P. Christensen, I. Gögenur, Cytotoxic CD8+ T cells in cancer and cancer immunotherapy. *Br J Cancer* **124**, 359–367 (2021).

15. T. Tondini, A. Isidro, E. Camarós, Case report: Boundaries of oncological and traumatological medical care in ancient Egypt: new palaeopathological insights from two human skulls. *Front. Med.* **11**, 1371645 (2024).

16. X. Duan, Y. Zhu, Y. Cui, Z. Yang, S. Zhou, Y. Han, D. Yu, N. Xiao, X. Cao, Y. Li, S. Liu, Z. Wang, W. Zhang, L. Feng, K. Zhang, J. Shou, Z. Liu, S. Xu, Circulating tumor cells in the pulmonary vein

increase significantly after lobectomy: A prospective observational study. *Thoracic Cancer* **10**, 163–169 (2019).

17. Z. Chen, P. Zhang, Y. Xu, J. Yan, Z. Liu, W. B. Lau, B. Lau, Y. Li, X. Zhao, Y. Wei, S. Zhou, Surgical stress and cancer progression: the twisted tango. *Mol Cancer* **18**, 132 (2019).

18. F. Tang, Y. Tie, C. Tu, X. Wei, Surgical trauma-induced immunosuppression in cancer: Recent advances and the potential therapies. *Clinical & Translational Med* **10**, 199–223 (2020).

19. A. Ring, M. Spataro, A. Wicki, N. Aceto, Clinical and Biological Aspects of Disseminated Tumor Cells and Dormancy in Breast Cancer. *Front. Cell Dev. Biol.* **10**, 929893 (2022).

20. M. Mohme, S. Riethdorf, K. Pantel, Circulating and disseminated tumor cells — mechanisms of immune surveillance and escape. *Nat Rev Clin Oncol* **14**, 155–167 (2017).

21. T. A. Allen, The Role of Circulating Tumor Cells as a Liquid Biopsy for Cancer: Advances, Biology, Technical Challenges, and Clinical Relevance. *Cancers* **16**, 1377 (2024).

22. H. Sakihama, N. Kobayashi, N. Minagawa, T. Shimokuni, S. Homma, N. Takahashi, T. Kamiyama, A. Taketomi, Comparison of circulating tumor cells (CTC) and disseminated tumor cells (DTC) in patients with colorectal carcinoma for a predictive marker for tumor recurrence. *JCO* **31**, e14548–e14548 (2013).

23. A. Dasgupta, A. R. Lim, C. M. Ghajar, Circulating and disseminated tumor cells: harbingers or initiators of metastasis? *Molecular Oncology* **11**, 40–61 (2017).

24. J. Hiller, G. Brodner, A. Gottschalk, Understanding clinical strategies that may impact tumor growth and metastatic spread at the time of cancer surgery. *Best Practice & Research Clinical Anaesthesiology* **27**, 427–439 (2013).

25. S. Tohme, R. L. Simmons, A. Tsung, Surgery for Cancer: A Trigger for Metastases. *Cancer Research* **77**, 1548–1552 (2017).

26. A. M. Binkowska, G. Michalak, R. Słotwiński, Current views on the mechanisms of immune responses to trauma and infection. *Cent Eur J Immunol* **40**, 206–216 (2015).

27. A. M. Dąbrowska, R. Słotwiński, The immune response to surgery and infection. *Cent Eur J Immunol* **39**, 532–537 (2014).

28. A. Lenz, G. A. Franklin, W. G. Cheadle, Systemic inflammation after trauma. *Injury* **38**, 1336–1345 (2007).

29. K. Pacak, M. Palkovits, I. J. Kopin, D. S. Goldstein, Stress-Induced Norepinephrine Release in the Hypothalamic Paraventricular Nucleus and Pituitary-Adrenocortical and Sympathoadrenal Activity: In Vivo Microdialysis Studies. *Frontiers in Neuroendocrinology* **16**, 89–150 (1995).

30. J. P. Herman, J. M. McKlveen, S. Ghosal, B. Kopp, A. Wulsin, R. Makinson, J. Scheimann, B. Myers, “Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response” in *Comprehensive Physiology*, Y. S. Prakash, Ed. (Wiley, ed. 1, 2016; https://onlinelibrary.wiley.com/doi/10.1002/cphy.c150015), pp. 603–621.

31. E. W. Salter, J. K. Sunstrum, S. Matovic, W. Inoue, Chronic stress dampens excitatory synaptic gain in the paraventricular nucleus of the hypothalamus. *The Journal of Physiology* **596**, 4157–4172 (2018).

32. J. P. Herman, J. G. Tasker, Paraventricular Hypothalamic Mechanisms of Chronic Stress Adaptation. *Front. Endocrinol.* **7** (2016).

33. J. A. Jesin, D. M. Walton, Cortisol as a Marker of Pain and Distress After Acute Musculoskeletal Trauma. *The Clinical Journal of Pain* **40**, 157–164 (2024).

34. M. M. Menger, T. Histing, M. W. Laschke, S. Ehnert, T. Viergutz, J. Fontana, Cortisol stress response after musculoskeletal surgery: a narrative review. *EFORT Open Reviews* **10**, 186–192 (2025).

35. D. Ojcius, N. Saïd-Sadier, Alarmins, inflammasomes and immunity. *Biomed J* **35**, 437 (2012).

36. H. E. Harris, A. Raucci, Alarmin(g) news about danger: Workshop on Innate Danger Signals and HMGB1. *EMBO Reports* **7**, 774–778 (2006).

37. S. J. Martin, Cell death and inflammation: the case for IL -1 family cytokines as the canonical DAMP s of the immune system. *The FEBS Journal* **283**, 2599–2615 (2016).

38. E. Lefrançais, S. Roga, V. Gautier, A. Gonzalez-de-Peredo, B. Monsarrat, J.-P. Girard, C. Cayrol, IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 1673–1678 (2012).

39. T. Kawasaki, T. Kawai, Toll-Like Receptor Signaling Pathways. *Front. Immunol.* **5** (2014).

40. C. Kawai, H. Kotani, M. Miyao, T. Ishida, L. Jemail, H. Abiru, K. Tamaki, Circulating Extracellular Histones Are Clinically Relevant Mediators of Multiple Organ Injury. *The American Journal of Pathology* **186**, 829–843 (2016).

41. T. Vogl, A. Stratis, V. Wixler, T. Völler, S. Thurainayagam, S. K. Jorch, S. Zenker, A. Dreiling, D. Chakraborty, M. Fröhling, P. Paruzel, C. Wehmeyer, S. Hermann, O. Papantonopoulou, C. Geyer, K. Loser, M. Schäfers, S. Ludwig, M. Stoll, T. Leanderson, J. L. Schultze, S. König, T. Pap, J. Roth, Autoinhibitory regulation of S100A8/S100A9 alarmin activity locally restricts sterile inflammation. *Journal of Clinical Investigation* **128**, 1852–1866 (2018).

42. A. De Gaetano, K. Solodka, G. Zanini, V. Selleri, A. V. Mattioli, M. Nasi, M. Pinti, Molecular Mechanisms of mtDNA-Mediated Inflammation. *Cells* **10**, 2898 (2021).

43. G. Zanini, V. Selleri, S. Lopez Domenech, M. Malerba, M. Nasi, A. V. Mattioli, M. Pinti, Mitochondrial DNA as inflammatory DAMP: a warning of an aging immune system? *Biochemical Society Transactions* **51**, 735–745 (2023).

44. T. Kawai, S. Akira, Toll-like Receptors and Their Crosstalk with Other Innate Receptors in Infection and Immunity. *Immunity* **34**, 637–650 (2011).

45. S. M. Schindler, M. G. Frank, J. L. Annis, S. F. Maier, A. Klegeris, Pattern recognition receptors mediate pro-inflammatory effects of extracellular mitochondrial transcription factor A (TFAM). *Molecular and Cellular Neuroscience* **89**, 71–79 (2018).

46. F. J. Velloso, M. Trombetta-Lima, V. Anschau, M. C. Sogayar, R. G. Correa, NOD-like receptors: major players (and targets) in the interface between innate immunity and cancer. *Bioscience Reports* **39**, BSR20181709 (2019).

47. D. M. Calcagno, A. Chu, S. Gaul, N. Taghdiri, A. Toomu, A. Leszczynska, B. Kaufmann, B. Papouchado, A. Wree, L. Geisler, H. M. Hoffman, A. E. Feldstein, K. R. King, NOD-like receptor protein 3 activation causes spontaneous inflammation and fibrosis that mimics human NASH. *Hepatology* **76**, 727–741 (2022).

48. V. Rai, G. Mathews, D. K Agrawal, Translational and Clinical Significance of DAMPs, PAMPs, and PRRs in Trauma-induced Inflammation. *Arch Clin Biomed Res* **06** (2022).

49. M. Ma, W. Jiang, R. Zhou, DAMPs and DAMP-sensing receptors in inflammation and diseases. *Immunity* **57**, 752–771 (2024).

50. J. Zindel, P. Kubes, DAMPs, PAMPs, and LAMPs in Immunity and Sterile Inflammation. *Annu. Rev. Pathol. Mech. Dis.* **15**, 493–518 (2020).

51. The PLOS ONE Staff, Correction: Evaluation of the Contribution of Multiple DAMPs and DAMP Receptors in Cell Death-Induced Sterile Inflammatory Responses. *PLoS ONE* **9**, e113029 (2014).

52. A. Elliot, H. Myllymäki, Y. Feng, Inflammatory Responses during Tumor Initiation: From Zebrafish Transgenic Models of Cancer to Evidence from Mouse and Man. *Cells* **9**, 1018 (2020).

53. N. Antonio, M. L. Bønnelykke-Behrndtz, L. C. Ward, J. Collin, I. J. Christensen, T. Steiniche, H. Schmidt, Y. Feng, P. Martin, The wound inflammatory response exacerbates growth of pre-neoplastic cells and progression to cancer. *The EMBO Journal* **34**, 2219–2236 (2015).

54. L. M. Coussens, Z. Werb, Inflammation and cancer. *Nature* **420**, 860–867 (2002).

55. W. Alazawi, N. Pirmadjid, R. Lahiri, S. Bhattacharya, Inflammatory and Immune Responses to Surgery and Their Clinical Impact. *Annals of Surgery* **264**, 73–80 (2016).

56. N. Ni Choileain, Cell Response to Surgery. *Arch Surg* **141**, 1132 (2006).

57. A. Murtha-Lemekhova, J. Fuchs, O. Ghamarnejad, M. Nikdad, P. Probst, K. Hoffmann, Influence of cytokines, circulating markers and growth factors on liver regeneration and post-hepatectomy liver failure: a systematic review and meta-analysis. *Sci Rep* **11**, 13739 (2021).

58. A. Kofler, M. Trattner, V. Mairinger, I. Urban, K. Søreide, S. Stättner, F. Primavesi, The Role of Perioperative Interleukin-6 Serum Levels on Liver Dysfunction and Infectious Complications After Hepatectomy—A Systematic Review. *Cancers* **17**, 3120 (2025).

59. R. M. Roumen, P. A. van Meurs, H. H. Kuypers, W. A. Kraak, R. W. Sauerwein, Serum interleukin-6 and C reactive protein responses in patients after laparoscopic or conventional cholecystectomy. *Eur J Surg* **158**, 541–544 (1992).

60. A. M. Cruickshank, W. D. Fraser, H. J. G. Burns, J. Van Damme, A. Shenkin, Response of Serum Interleukin-6 in Patients Undergoing Elective Surgery of Varying Severity. *Clinical Science* **79**, 161–165 (1990).

61. X. Zhu, D. Conklin, J. C. Eisenach, Cyclooxygenase-1 in the spinal cord plays an important role in postoperative pain. *Pain* **104**, 15–23 (2003).

62. J. S. Kroin, Z. D. Ling, A. Buvanendran, K. J. Tuman, Upregulation of Spinal Cyclooxygenase-2 in Rats after Surgical Incision. *Anesthesiology* **100**, 364–369 (2004).

63. S.-H. Chang, C. H. Liu, R. Conway, D. K. Han, K. Nithipatikom, O. C. Trifan, T. F. Lane, T. Hla, Role of prostaglandin $E_2$ -dependent angiogenic switch in cyclooxygenase 2-induced breast cancer progression. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 591–596 (2004).

64. A. Kulesza, L. Paczek, A. Burdzinska, The Role of COX-2 and PGE2 in the Regulation of Immunomodulation and Other Functions of Mesenchymal Stromal Cells. *Biomedicines* **11**, 445 (2023).

65. D. Wang, R. N. DuBois, Eicosanoids and cancer. *Nat Rev Cancer* **10**, 181–193 (2010).

66. D. Ruan, S.-P. So, Prostaglandin E2 produced by inducible COX-2 and mPGES-1 promoting cancer cell proliferation in vitro and in vivo. *Life Sciences* **116**, 43–50 (2014).

67. L.-S. Shen, J. Wang, D.-F. Shen, X.-L. Yuan, P. Dong, M.-X. Li, J. Xue, F.-M. Zhang, H.-L. Ge, D. Xu, CD4+CD25+CD127low/− regulatory T cells express Foxp3 and suppress effector T cell proliferation and contribute to gastric cancers progression. *Clinical Immunology* **131**, 109–118 (2009).

68. A. M. Wolf, D. Wolf, M. Steurer, G. Gastl, E. Gunsilius, B. Grubeck-Loebenstein, Increase of regulatory T cells in the peripheral blood of cancer patients. *Clin Cancer Res* **9**, 606–612 (2003).

69. F. Martin, L. Apetoh, F. Ghiringhelli, Role of Myeloid-Derived Suppressor Cells in Tumor Immunotherapy. *Immunotherapy* **4**, 43–57 (2012).

70. W. Li, K. Wu, E. Zhao, L. Shi, R. Li, P. Zhang, Y. Yin, X. Shuai, G. Wang, K. Tao, HMGB1 recruits myeloid derived suppressor cells to promote peritoneal dissemination of colon cancer after resection. *Biochemical and Biophysical Research Communications* **436**, 156–161 (2013).

71. A. Oseroff, S. Okada, S. Strober, Natural suppressor (NS) cells found in the spleen of neonatal mice and adult mice given total lymphoid irradiation (TLI) express the null surface phenotype. *J Immunol* **132**, 101–110 (1984).

72. D. M. R. Lathers, J. I. Clark, N. J. Achille, M. R. I. Young, Phase IB study of 25-hydroxyvitamin D3 treatment to diminish suppressor cells in head and neck cancer patients. *Human Immunology* **62**, 1282–1293 (2001).

73. J. E. Talmadge, E. C. Reed, A. Kessinger, C. A. Kuszynski, G. A. Perry, C. L. Gordy, K. C. Mills, M. L. Thomas, S. J. Pirruccello, B. A. Letheby, M. A. Arneson, J. D. Jackson, Immunologic attributes of cytokine mobilized peripheral blood stem cells and recovery following transplantation. *Bone Marrow Transplant* **17**, 101–109 (1996).

74. A. S. Pak, M. A. Wright, J. P. Matthews, S. L. Collins, G. J. Petruzzelli, M. R. Young, Mechanisms of immune suppression in patients with head and neck cancer: presence of CD34(+) cells which suppress immune functions within cancers that secrete granulocyte-macrophage colony-stimulating factor. *Clin Cancer Res* **1**, 95–103 (1995).

75. X. Zhu, G. Herrera, J. B. Ochoa, Immunosupression and Infection After Major Surgery: A Nutritional Deficiency. *Critical Care Clinics* **26**, 491–500 (2010).

76. A. G. Cuenca, M. J. Delano, K. M. Kelly-Scumpia, C. Moreno, P. O. Scumpia, D. M. LaFace, P. G. Heyworth, P. A. Efron, L. L. Moldawer, A Paradoxical Role for Myeloid-Derived Suppressor Cells in Sepsis and Trauma. *Mol Med* **17**, 281–292 (2011).

77. L. Akkari, I. Amit, V. Bronte, Z. G. Fridlender, D. I. Gabrilovich, F. Ginhoux, C. C. Hedrick, S. Ostrand-Rosenberg, Defining myeloid-derived suppressor cells. *Nat Rev Immunol* **24**, 850–857 (2024).

78. A. Sevko, V. Umansky, Myeloid-Derived Suppressor Cells Interact with Tumors in Terms of Myelopoiesis, Tumorigenesis and Immunosuppression: Thick as Thieves. *J. Cancer* **4**, 3–11 (2013).

79. K. Aliazis, S. Yenyuwadee, P. Phikulsod, V. A. Boussiotis, Emergency myelopoiesis in solid cancers. *Br J Haematol* **205**, 798–811 (2024).

80. T. J. Wildes, B. DiVita Dean, C. T. Flores, Myelopoiesis during Solid Cancers and Strategies for Immunotherapy. *Cells* **10**, 968 (2021).

81. V. Bronte, S. Brandau, S.-H. Chen, M. P. Colombo, A. B. Frey, T. F. Greten, S. Mandruzzato, P. J. Murray, A. Ochoa, S. Ostrand-Rosenberg, P. C. Rodriguez, A. Sica, V. Umansky, R. H. Vonderheide, D. I. Gabrilovich, Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun* **7**, 12150 (2016).

82. B. Hoechst, T. Voigtlaender, L. Ormandy, J. Gamrekelashvili, F. Zhao, H. Wedemeyer, F. Lehner, M. P. Manns, T. F. Greten, F. Korangy, Myeloid derived suppressor cells inhibit natural killer cells in patients with hepatocellular carcinoma via the NKp30 receptor #. *Hepatology* **50**, 799–807 (2009).

83. U. H. Mellqvist, M. Hansson, M. Brune, C. Dahlgren, S. Hermodsson, K. Hellstrand, Natural killer cell dysfunction and apoptosis induced by chronic myelogenous leukemia cells: role of reactive oxygen species and regulation by histamine. *Blood* **96**, 1961–1968 (2000).

84. E. D. Kramer, S. I. Abrams, Granulocytic Myeloid-Derived Suppressor Cells as Negative Regulators of Anticancer Immunity. *Front. Immunol.* **11**, 1963 (2020).

85. D. I. Gabrilovich, Myeloid-Derived Suppressor Cells. *Cancer Immunol Res* **5**, 3–8 (2017).

86. S. Ostrand-Rosenberg, C. Fenselau, Myeloid-Derived Suppressor Cells: Immune-Suppressive Cells That Impair Antitumor Immunity and Are Sculpted by Their Environment. *The Journal of Immunology* **200**, 422–431 (2018).

87. D. I. Gabrilovich, S. Nagaraj, Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* **9**, 162–174 (2009).

88. M. K. Srivastava, L. Zhu, M. Harris-White, U. Kar, M. Huang, M. F. Johnson, J. M. Lee, D. Elashoff, R. Strieter, S. Dubinett, S. Sharma, Myeloid Suppressor Cell Depletion Augments Antitumor Activity in Lung Cancer. *PLoS ONE* **7**, e40677 (2012).

89. B. Huang, P.-Y. Pan, Q. Li, A. I. Sato, D. E. Levy, J. Bromberg, C. M. Divino, S.-H. Chen, Gr-1+CD115+ Immature Myeloid Suppressor Cells Mediate the Development of Tumor-Induced T Regulatory Cells and T-Cell Anergy in Tumor-Bearing Host. *Cancer Research* **66**, 1123–1131 (2006).

90. K. Movahedi, M. Guilliams, J. Van Den Bossche, R. Van Den Bergh, C. Gysemans, A. Beschin, P. De Baetselier, J. A. Van Ginderachter, Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell–suppressive activity. *Blood* **111**, 4233–4244 (2008).

91. P. C. Rodriguez, A. H. Zea, K. S. Culotta, J. Zabaleta, J. B. Ochoa, A. C. Ochoa, Regulation of T Cell Receptor CD3ζ Chain Expression byl-Arginine. *Journal of Biological Chemistry* **277**, 21123–21129 (2002).

92. L. H. Wang, R. A. Kirken, R. A. Erwin, C. R. Yu, W. L. Farrar, JAK3, STAT, and MAPK signaling pathways as novel molecular targets for the tyrphostin AG-490 regulation of IL-2-mediated T cell response. *J Immunol* **162**, 3897–3904 (1999).

93. R. M. Bingisser, P. A. Tilbrook, P. G. Holt, U. R. Kees, Macrophage-derived nitric oxide regulates T cell activation via reversible disruption of the Jak3/STAT5 signaling pathway. *J Immunol* **160**, 5729–5734 (1998).

94. D. Basso, P. Fogar, M. Falconi, E. Fadi, C. Sperti, C. Frasson, E. Greco, D. Tamburrino, S. Teolato, S. Moz, D. Bozzato, M. Pelloso, A. Padoan, G. De Franchis, E. Gnatta, M. Facco, C.-F. Zambon, F. Navaglia, C. Pasquali, G. Basso, G. Semenzato, S. Pedrazzoli, P. Pederzoli, M. Plebani, Pancreatic Tumors and Immature Immunosuppressive Myeloid Cells in Blood and Spleen: Role of Inhibitory Co-Stimulatory Molecules PDL1 and CTLA4. An In Vivo and In Vitro Study. *PLoS ONE* **8**, e54824 (2013).

95. C. Lu, P. S. Redd, J. R. Lee, N. Savage, K. Liu, The expression profiles and regulation of PD-L1 in tumor-induced myeloid-derived suppressor cells. *OncoImmunology* **5**, e1247135 (2016).

96. M. Z. Noman, G. Desantis, B. Janji, M. Hasmim, S. Karray, P. Dessen, V. Bronte, S. Chouaib, PD-L1 is a novel direct target of HIF-1α, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. *Journal of Experimental Medicine* **211**, 781–790 (2014).

97. P. Golstein, H. Wigzell, H. Blomgren, E. A. J. Svedmyr, CELLS MEDIATING SPECIFIC IN VITRO CYTOTOXICITY. *The Journal of Experimental Medicine* **135**, 890–906 (1972).

98. J. C. Cerottini, A. A. Nordin, K. T. Brunner, Specific in vitro Cytotoxicity of Thymus-derived Lymphocytes sensitized to Alloantigens. *Nature* **228**, 1308–1309 (1970).

99. D. C. Tscharke, N. P. Croft, P. C. Doherty, N. L. La Gruta, Sizing up the key determinants of the CD8+ T cell response. *Nat Rev Immunol* **15**, 705–716 (2015).

100. J. M. McNally, H. A. Andersen, R. Chervenak, S. R. Jennings, Phenotypic Characteristics Associated with the Acquisition of HSV-Specific CD8 T-Lymphocyte-Mediated Cytolytic Function in Vitro. *Cellular Immunology* **194**, 103–111 (1999).

101. P. Wolint, M. R. Betts, R. A. Koup, A. Oxenius, Immediate Cytotoxicity But Not Degranulation Distinguishes Effector and Memory Subsets of CD8+ T Cells. *The Journal of Experimental Medicine* **199**, 925–936 (2004).

102. P. Wolint, M. R. Betts, R. A. Koup, A. Oxenius, Immediate Cytotoxicity But Not Degranulation Distinguishes Effector and Memory Subsets of CD8+ T Cells. *The Journal of Experimental Medicine* **199**, 925–936 (2004).

103. W. Cui, S. M. Kaech, Generation of effector $CD8^+$ T cells and their conversion to memory T cells. *Immunological Reviews* **236**, 151–166 (2010).

104. E. Beyranvand Nejad, R. B. Ratts, E. Panagioti, C. Meyer, J. D. Oduro, L. Cicin-Sain, K. Früh, S. H. Van Der Burg, R. Arens, Demarcated thresholds of tumor-specific CD8 T cells elicited by MCMV-based vaccine vectors provide robust correlates of protection. *j. immunotherapy cancer* **7**, 25 (2019).

105. S. Loeser, K. Loser, M. S. Bijker, M. Rangachari, S. H. Van Der Burg, T. Wada, S. Beissert, C. J. M. Melief, J. M. Penninger, Spontaneous tumor rejection by *cbl-b* –deficient CD8+ T cells. *The Journal of Experimental Medicine* **204**, 879–891 (2007).

106. T. Yamaguchi, J. Kinoshita, H. Saito, M. Shimada, S. Terai, H. Moriyama, K. Okamoto, I. Makino, K. Nakamura, H. Tajima, I. Ninomiya, S. Fushida, High CD8 / CD33 ratio in peritoneal metastatic lesions is associated with favorable prognosis in gastric cancer. *Cancer Reports* **4**, e1389 (2021).

107. P. Tallón De Lara, H. Castañón, M. Sterpi, M. Van Den Broek, Antimetastatic defense by CD8+ T cells. *Trends in Cancer* **8**, 145–157 (2022).

108. R. Joseph, R. Soundararajan, S. Vasaikar, F. Yang, K. L. Allton, L. Tian, P. Den Hollander, S. Isgandarova, M. Haemmerle, B. Mino, T. Zhou, C. Shin, M. Martinez-Paniagua, A. A. Sahin, J. Rodriguez-Canales, J. Gelovani, J. T. Chang, G. Acharya, A. K. Sood, I. I. Wistuba, D. L. Gibbons, L. M. Solis, M. C. Barton, N. Varadarajan, J. M. Rosen, X. H. Zhang, S. A. Mani, CD8+ T cells inhibit metastasis and CXCL4 regulates its function. *Br J Cancer* **125**, 176–189 (2021).

109. A. L. Magnussen, I. G. Mills, Vascular normalisation as the stepping stone into tumor microenvironment transformation. *Br J Cancer* **125**, 324–336 (2021).

110. L. Tian, A. Goldstein, H. Wang, H. Ching Lo, I. Sun Kim, T. Welte, K. Sheng, L. E. Dobrolecki, X. Zhang, N. Putluri, T. L. Phung, S. A. Mani, F. Stossi, A. Sreekumar, M. A. Mancini, W. K. Decker, C. Zong, M. T. Lewis, X. H.-F. Zhang, Mutual regulation of tumor vessel normalization and immunostimulatory reprogramming. *Nature* **544**, 250–254 (2017).

111. H. F. Aqbi, C. Coleman, M. Zarei, S. H. Manjili, L. Graham, J. Koblinski, C. Guo, Y. Xie, G. Guruli, H. D. Bear, M. O. Idowu, M. Habibi, X.-Y. Wang, M. H. Manjili, Local and distant tumor

dormancy during early stage breast cancer are associated with the predominance of infiltrating T effector subsets. *Breast Cancer Res* **22**, 116 (2020).

112. J. A. Krall, F. Reinhardt, O. A. Mercury, D. R. Pattabiraman, M. W. Brooks, M. Dougan, A. W. Lambert, B. Bierie, H. L. Ploegh, S. K. Dougan, R. A. Weinberg, The systemic response to surgery triggers the outgrowth of distant immune-controlled tumors in mouse models of dormancy. *Sci. Transl. Med.* **10**, eaan3464 (2018).

113. J. Shibata, S. Ishihara, N. Tada, K. Kawai, N. H. Tsuno, H. Yamaguchi, E. Sunami, J. Kitayama, T. Watanabe, Surgical stress response after colorectal resection: a comparison of robotic, laparoscopic, and open surgery. *Tech Coloproctol* **19**, 275–280 (2015).

114. C. Liu, J. Liu, S. Zhang, Laparoscopic versus conventional open surgery for immune function in patients with colorectal cancer. *Int J Colorectal Dis* **26**, 1375–1385 (2011).

115. H. Song, J. Song, Y. Liang, W. Fu, Y. Xu, J. Zheng, W. Xu, [Comparison of immune response after laparoscopic and open surgery for colorectal carcinoma: a meta-analysis]. *Zhonghua Wei Chang Wai Ke Za Zhi* **17**, 799–804 (2014).

116. M. Cristaldi, M. Rovati, M. Elli, S. Gerlinzani, A. Lesma, L. Balzarotti, A. M. Taschieri, Lymphocytic subpopulation changes after open and laparoscopic cholecystectomy: a prospective and comparative study on 38 patients. *Surg Laparosc Endosc* **7**, 255–261 (1997).

117. B. A. Whitson, J. D'Cunha, C. D. Hoang, B. Wu, S. Ikramuddin, H. Buchwald, A. Panoskaltsis-Mortari, R. A. Kratzke, J. S. Miller, M. A. Maddaus, Minimally invasive versus open Roux-en-Y gastric bypass: effect on immune effector cells. *Surgery for Obesity and Related Diseases* **5**, 181–193 (2009).

118. F. Pagès, A. Berger, M. Camus, F. Sanchez-Cabo, A. Costes, R. Molidor, B. Mlecnik, A. Kirilovsky, M. Nilsson, D. Damotte, T. Meatchi, P. Bruneval, P.-H. Cugnenc, Z. Trajanoski, W.-H. Fridman, J. Galon, Effector Memory T Cells, Early Metastasis, and Survival in Colorectal Cancer. *N Engl J Med* **353**, 2654–2666 (2005).

119. J. Galon, F. Pagès, F. M. Marincola, H. K. Angell, M. Thurin, A. Lugli, I. Zlobec, A. Berger, C. Bifulco, G. Botti, F. Tatangelo, C. M. Britten, S. Kreiter, L. Chouchane, P. Delrio, H. Arndt, M. Asslaber, M. Maio, G. V. Masucci, M. Mihm, F. Vidal-Vanaclocha, J. P. Allison, S. Gnjatic, L. Hakansson, C. Huber, H. Singh-Jasuja, C. Ottensmeier, H. Zwierzina, L. Laghi, F. Grizzi, P. S. Ohashi, P. A. Shaw, B. A. Clarke, B. G. Wouters, Y. Kawakami, S. Hazama, K. Okuno, E. Wang, J. O'Donnell-Tormey, C. Lagorce, G. Pawelec, M. I. Nishimura, R. Hawkins, R. Lapointe, A. Lundqvist, S. N. Khleif, S. Ogino, P. Gibbs, P. Waring, N. Sato, T. Torigoe, K. Itoh, P. S. Patel, S. N. Shukla, R. Palmqvist, I. D. Nagtegaal, Y. Wang, C. D'Arrigo, S. Kopetz, F. A. Sinicrope, G. Trinchieri, T. F. Gajewski, P. A. Ascierto, B. A. Fox, Cancer classification using the Immunoscore: a worldwide task force. *J Transl Med* **10**, 205 (2012).

120. A. A. Ananth, L.-H. Tai, C. Lansdell, A. A. Alkayyal, K. E. Baxter, L. Angka, J. Zhang, C. Tanese De Souza, K. B. Stephenson, K. Parato, J. L. Bramson, J. C. Bell, B. D. Lichty, R. C. Auer, Surgical Stress Abrogates Pre-Existing Protective T Cell Mediated Anti-Tumor Immunity Leading to Postoperative Cancer Recurrence. *PLoS ONE* **11**, e0155947 (2016).

121. W. Weninger, N. Manjunath, U. H. Von Andrian, Migration and differentiation of CD8$^+$ T cells. *Immunological Reviews* **186**, 221–233 (2002).

122. N. Zhang, M. J. Bevan, CD8+ T Cells: Foot Soldiers of the Immune System. *Immunity* **35**, 161–168 (2011).

123. K. A. Hogquist, S. C. Jameson, W. R. Heath, J. L. Howard, M. J. Bevan, F. R. Carbone, T cell receptor antagonist peptides induce positive selection. *Cell* **76**, 17–27 (1994).

124. L. Krishnan, K. Gurnani, C. J. Dicaire, H. Van Faassen, A. Zafer, C. J. Kirschning, S. Sad, G. D. Sprott, Rapid Clonal Expansion and Prolonged Maintenance of Memory CD8+ T Cells of the Effector (CD44highCD62Llow) and Central (CD44highCD62Lhigh) Phenotype by an Archaeosome Adjuvant Independent of TLR2. *The Journal of Immunology* **178**, 2396–2406 (2007).

125. G. Gulden, B. Sert, T. Teymur, Y. Ay, N. N. Tiryaki, A. K. Mishra, E. Ovali, N. Tarhan, C. Tastan, CAR-T Cells with Phytohemagglutinin (PHA) Provide Anti-Cancer Capacity with Better Proliferation, Rejuvenated Effector Memory, and Reduced Exhausted T Cell Frequencies. *Vaccines* **11**, 313 (2023).

126. V. Niederlova, O. Tsyklauri, M. Kovar, O. Stepanek, IL-2-driven CD8+ T cell phenotypes: implications for immunotherapy. *Trends in Immunology* **44**, 890–901 (2023).

127. L. Angka, C. Tanese De Souza, K. E. Baxter, S. T. Khan, M. Market, A. B. Martel, L.-H. Tai, M. A. Kennedy, J. C. Bell, R. C. Auer, Perioperative arginine prevents metastases by accelerating natural killer cell recovery after surgery. *Molecular Therapy* **30**, 3270–3283 (2022).

128. L.-H. Tai, A. A. Alkayyal, A. L. Leslie, S. Sahi, S. Bennett, C. Tanese De Souza, K. Baxter, L. Angka, R. Xu, M. A. Kennedy, R. C. Auer, Phosphodiesterase-5 inhibition reduces postoperative metastatic disease by targeting surgery-induced myeloid derived suppressor cell-dependent inhibition of Natural Killer cell cytotoxicity. *OncoImmunology* **7**, e1431082 (2018).

129. B. Gaudillière, G. K. Fragiadakis, R. V. Bruggner, M. Nicolau, R. Finck, M. Tingle, J. Silva, E. A. Ganio, C. G. Yeh, W. J. Maloney, J. I. Huddleston, S. B. Goodman, M. M. Davis, S. C. Bendall, W. J. Fantl, M. S. Angst, G. P. Nolan, Clinical recovery from surgery correlates with single-cell immune signatures. *Sci. Transl. Med.* **6** (2014).

130. L.-H. Tai, A. A. Alkayyal, A. L. Leslie, S. Sahi, S. Bennett, C. Tanese De Souza, K. Baxter, L. Angka, R. Xu, M. A. Kennedy, R. C. Auer, Phosphodiesterase-5 inhibition reduces postoperative metastatic disease by targeting surgery-induced myeloid derived suppressor cell-dependent inhibition of Natural Killer cell cytotoxicity. *OncoImmunology* **7**, e1431082 (2018).

131. A. A. Ananth, L.-H. Tai, C. Lansdell, A. A. Alkayyal, K. E. Baxter, L. Angka, J. Zhang, C. Tanese De Souza, K. B. Stephenson, K. Parato, J. L. Bramson, J. C. Bell, B. D. Lichty, R. C. Auer, Surgical Stress Abrogates Pre-Existing Protective T Cell Mediated Anti-Tumor Immunity Leading to Postoperative Cancer Recurrence. *PLoS ONE* **11**, e0155947 (2016).

132. L.-H. Tai, J. Zhang, K. J. Scott, C. T. De Souza, A. A. Alkayyal, A. A. Ananth, S. Sahi, R. A. Adair, A. B. Mahmoud, S. Sad, J. C. Bell, A. P. Makrigiannis, A. A. Melcher, R. C. Auer, Perioperative

Influenza Vaccination Reduces Postoperative Metastatic Disease by Reversing Surgery-Induced Dysfunction in Natural Killer Cells. *Clinical Cancer Research* **19**, 5104–5115 (2013).

133. M. Market, G. Tennakoon, M. Scaffidi, D. P. Cook, L. Angka, J. Ng, C. Tanese De Souza, M. A. Kennedy, B. C. Vanderhyden, R. C. Auer, Preventing Surgery-Induced NK Cell Dysfunction Using Anti-TGF-β Immunotherapeutics. *IJMS* **23**, 14608 (2022).

134. L. Angka, G. Tennakoon, D. P. Cook, A. B. Martel, M. Market, C. Tanese De Souza, E. Cummins, I. Samudio, N. Kekre, M. Ardolino, B. C. Vanderhyden, M. A. Kennedy, R. C. Auer, Preventing surgery-induced natural killer cell suppression and metastases by inhibiting PI3K-gamma signaling in myeloid-derived suppressor cells. *J Immunother Cancer* **14**, e013304 (2026).

135. L. Angka, A. B. Martel, M. Kilgour, A. Jeong, M. Sadiq, C. T. De Souza, L. Baker, M. A. Kennedy, N. Kekre, R. C. Auer, Natural Killer Cell IFNγ Secretion is Profoundly Suppressed Following Colorectal Cancer Surgery. *Ann Surg Oncol* **25**, 3747–3754 (2018).

136. S. T. Le, P. Kipnis, B. Cohn, V. X. Liu, COVID-19 Vaccination and the Timing of Surgery Following COVID-19 Infection. *Annals of Surgery* **276**, e265–e272 (2022).

137. T. Stuart, A. Butler, P. Hoffman, C. Hafemeister, E. Papalexi, W. M. Mauck, Y. Hao, M. Stoeckius, P. Smibert, R. Satija, Comprehensive Integration of Single-Cell Data. *Cell* **177**, 1888-1902.e21 (2019).

138. M. Andreatta, J. Corria-Osorio, S. Müller, R. Cubas, G. Coukos, S. J. Carmona, Interpretation of T cell states from single-cell transcriptomics data using reference atlases. *Nat Commun* **12**, 2965 (2021).

139. P. A. Szabo, H. M. Levitin, M. Miron, M. E. Snyder, T. Senda, J. Yuan, Y. L. Cheng, E. C. Bush, P. Dogra, P. Thapa, D. L. Farber, P. A. Sims, Single-cell transcriptomics of human T cells reveals tissue and activation signatures in health and disease. *Nat Commun* **10**, 4706 (2019).

140. M.-L. Eloranta, L. Rönnblom, Cause and consequences of the activated type I interferon system in SLE. *J Mol Med* **94**, 1103–1110 (2016).

141. H. Wang, J. Engesser, R. Khatri, D. P. Schaub, H.-J. Paust, Z. Sultana, S.-L. Jauch-Speer, A. Peters, A. Kaffke, S. Bonn, T. B. Huber, H.-W. Mittrücker, C. F. Krebs, U. Panzer, N. Asada, Type I interferon drives T cell cytotoxicity by upregulation of interferon regulatory factor 7 in autoimmune kidney diseases in mice. *Nat Commun* **16**, 4686 (2025).

142. E. Lugli, G. Galletti, S. K. Boi, B. A. Youngblood, Stem, Effector, and Hybrid States of Memory CD8+ T Cells. *Trends in Immunology* **41**, 17–28 (2020).

143. P. A. Szabo, H. M. Levitin, M. Miron, M. E. Snyder, T. Senda, J. Yuan, Y. L. Cheng, E. C. Bush, P. Dogra, P. Thapa, D. L. Farber, P. A. Sims, Single-cell transcriptomics of human T cells reveals tissue and activation signatures in health and disease. *Nat Commun* **10**, 4706 (2019).

144. N. Kopitar-Jerala, The Role of Interferons in Inflammation and Inflammasome Activation. *Front. Immunol.* **8**, 873 (2017).

145. L. I. Labzin, M. A. R. Lauterbach, E. Latz, Interferons and inflammasomes: Cooperation and counterregulation in disease. *Journal of Allergy and Clinical Immunology* **138**, 37–46 (2016).

146. H. Zhang, J. Liu, Z. Yang, L. Zeng, K. Wei, L. Zhu, L. Tang, D. Wang, Y. Zhou, J. Lv, N. Zhou, K. Tang, J. Ma, B. Huang, TCR activation directly stimulates PYGB-dependent glycogenolysis to fuel the early recall response in CD8+ memory T cells. *Molecular Cell* **82**, 3077-3088.e6 (2022).

147. Q. Shan, S. S. Hu, S. Zhu, X. Chen, V. P. Badovinac, W. Peng, C. Zang, H.-H. Xue, Tcf1 preprograms the mobilization of glycolysis in central memory CD8+ T cells during recall responses. *Nat Immunol* **23**, 386–398 (2022).

148. N. W. Freshney, L. Rawlinson, F. Guesdon, E. Jones, S. Cowley, J. Hsuan, J. Saklatvala, Interleukin-1 activates a novel protein kinase cascade that results in the phosphorylation of hsp27. *Cell* **78**, 1039–1049 (1994).

149. C. Merritt, H. Enslen, N. Diehl, D. Conze, R. J. Davis, M. Rincón, Activation of p38 Mitogen-Activated Protein Kinase In Vivo Selectively Induces Apoptosis of $CD8^+$ but Not $CD4^+$ T Cells. *Molecular and Cellular Biology* **20**, 936–946 (2000).

150. I. N. Foltz, J. C. Lee, P. R. Young, J. W. Schrader, Hemopoietic Growth Factors with the Exception of Interleukin-4 Activate the p38 Mitogen-activated Protein Kinase Pathway. *Journal of Biological Chemistry* **272**, 3296–3301 (1997).

151. J. U. Kazi, N. N. Kabir, A. Flores-Morales, L. Rönnstrand, SOCS proteins in regulation of receptor tyrosine kinase signaling. *Cell. Mol. Life Sci.* **71**, 3297–3310 (2014).

152. W. S. Alexander, R. Starr, D. Metcalf, S. E. Nicholson, A. Farley, A. G. Elefanty, M. Brysha, B. T. Kile, R. Richardson, M. Baca, J.-G. Zhang, T. A. Willson, E. M. Viney, N. S. Sprigg, S. Rakar, J. Corbin, S. Mifsud, L. DiRago, D. Cary, N. A. Nicola, D. J. Hilton, Suppressors of cytokine signaling (SOCS): negative regulators of signal transduction. *Journal of Leukocyte Biology* **66**, 588–592 (1999).

153. L. Larsen, C. Röpke, Suppressors of cytokine signalling: SOCS: Review article. *APMIS* **110**, 833–844 (2002).

154. M. J. Aman, W. J. Leonard, Cytokine signaling: Cytokine-inducible signaling inhibitors. *Current Biology* **7**, R784–R788 (1997).

155. A. Yoshimura, T. Ohkubo, T. Kiguchi, N. A. Jenkins, D. J. Gilbert, N. G. Copeland, T. Hara, A. Miyajima, A novel cytokine-inducible gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin 3 and erythropoietin receptors. *The EMBO Journal* **14**, 2816–2826 (1995).

156. R. Starr, T. A. Willson, E. M. Viney, L. J. L. Murray, J. R. Rayner, B. J. Jenkins, T. J. Gonda, W. S. Alexander, D. Metcalf, N. A. Nicola, D. J. Hilton, A family of cytokine-inducible inhibitors of signalling. *Nature* **387**, 917–921 (1997).

157. V. Laletin, P.-L. Bernard, C. Costa Da Silva, G. Guittard, J. A. Nunes, Negative intracellular regulators of T-cell receptor (TCR) signaling as potential antitumor immunotherapy targets. *J Immunother Cancer* **11**, e005845 (2023).

158. T. S. Abraham, A. E. Snook, A novel role for Cish in the inhibition of TCR signaling. *Transl. Cancer Res* **5**, S142–S145 (2016).

159. H. Sudholz, R. B. Delconte, N. D. Huntington, Interleukin-15 cytokine checkpoints in natural killer cell anti-tumor immunity. *Current Opinion in Immunology* **84**, 102364 (2023).

160. A. P. Menon, B. Moreno, D. Meraviglia-Crivelli, F. Nonatelli, H. Villanueva, M. Barainka, A. Zheleva, H. M. Van Santen, F. Pastor, Modulating T Cell Responses by Targeting CD3. *Cancers* **15**, 1189 (2023).

161. S. Mahmoudi, D. McCulloch, A. Ward, 123. *Cytokine* **70**, 57 (2014).

162. M. W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research* **29**, 45e–445 (2001).

163. D. C. Palmer, B. R. Webber, Y. Patel, M. J. Johnson, C. M. Kariya, W. S. Lahr, M. R. Parkhurst, J. J. Gartner, T. D. Prickett, F. J. Lowery, R. J. Kishton, D. Gurusamy, Z. Franco, S. K. Vodnala, M. D. Diers, N. K. Wolf, N. J. Slipek, D. H. McKenna, D. Sumstad, L. Viney, T. Henley, T. Bürckstümmer, O. Baker, Y. Hu, C. Yan, D. Meerzaman, K. Padhan, W. Lo, P. Malekzadeh, L. Jia, D. C. Deniger, S. J. Patel, P. F. Robbins, R. S. McIvor, M. Choudhry, S. A. Rosenberg, B. S. Moriarity, N. P. Restifo, Internal checkpoint regulates T cell neoantigen reactivity and susceptibility to PD1 blockade. *Med* **3**, 682-704.e8 (2022).

164. D. C. Palmer, G. C. Guittard, Z. Franco, J. G. Crompton, R. L. Eil, S. J. Patel, Y. Ji, N. Van Panhuys, C. A. Klebanoff, M. Sukumar, D. Clever, A. Chichura, R. Roychoudhuri, R. Varma, E. Wang, L. Gattinoni, F. M. Marincola, L. Balagopalan, L. E. Samelson, N. P. Restifo, Cish actively silences TCR signaling in CD8+ T cells to maintain tumor tolerance. *Journal of Experimental Medicine* **212**, 2095–2113 (2015).

165. J. Lv, L. Qin, R. Zhao, D. Wu, Z. Wu, D. Zheng, S. Li, M. Luo, Q. Wu, Y. Long, Z. Tang, Y.-L. Tang, X. Luo, Y. Yao, L.-H. Yang, P. Li, Disruption of CISH promotes the antitumor activity of human T cells and decreases PD-1 expression levels. *Molecular Therapy - Oncolytics* **28**, 46–58 (2023).

166. E. Lou, M. S. Choudhry, T. K. Starr, T. D. Folsom, J. Bell, B. Rathmann, A. P. DeFeo, J. Kim, N. Slipek, Z. Jin, D. Sumstad, C. A. Klebanoff, K. Ladner, A. Sarkari, R. S. McIvor, T. A. Murray, J. S. Miller, M. Rao, E. Jensen, J. Ankeny, M. A. Khalifa, A. Chauhan, B. Spilseth, A. Dixit, P. P. Provenzano, W. Pan, D. Weber, M. Byrne-Steele, T. Henley, D. H. McKenna, M. J. Johnson, B. R. Webber, B. S. Moriarity, Targeting the intracellular immune checkpoint CISH with CRISPR-Cas9-edited T cells in patients with metastatic colorectal cancer: a first-in-human, single-centre, phase 1 trial. *The Lancet Oncology* **26**, 559–570 (2025).

167. A. Matsumoto, M. Masuhara, K. Mitsui, M. Yokouchi, M. Ohtsubo, H. Misawa, A. Miyajima, A. Yoshimura, CIS, a Cytokine Inducible SH2 Protein, Is a Target of the JAK-STAT5 Pathway and Modulates STAT5 Activation. *Blood* **89**, 3148–3154 (1997).

168. Y. Yang, C. Li, T. Liu, X. Dai, A. V. Bazhin, Myeloid-Derived Suppressor Cells in Tumors: From Mechanisms to Antigen Specificity and Microenvironmental Regulation. *Front. Immunol.* **11**, 1371 (2020).

169. M.-J. Park, S.-H. Lee, E.-K. Kim, E.-J. Lee, J.-A. Baek, S.-H. Park, S.-K. Kwok, M.-L. Cho, Interleukin-10 produced by myeloid-derived suppressor cells is critical for the induction of Tregs and attenuation of rheumatoid inflammation in mice. *Sci Rep* **8**, 3753 (2018).

170. H. Tsukamoto, R. Nishikata, S. Senju, Y. Nishimura, Myeloid-Derived Suppressor Cells Attenuate TH1 Development through IL-6 Production to Promote Tumor Progression. *Cancer Immunology Research* **1**, 64–76 (2013).

171. M. M. Yaseen, N. M. Abuharfeil, H. Darmani, A. Daoud, Mechanisms of immune suppression by myeloid-derived suppressor cells: the role of interleukin-10 as a key immunoregulatory cytokine. *Open Biol.* **10**, 200111 (2020).

172. Y. Ge, D. Cheng, Q. Jia, H. Xiong, J. Zhang, Mechanisms Underlying the Role of Myeloid-Derived Suppressor Cells in Clinical Diseases: Good or Bad. *Immune Netw* **21**, e21 (2021).

173. R. Ren, C. Xiong, R. Ma, Y. Wang, T. Yue, J. Yu, B. Shao, The recent progress of myeloid-derived suppressor cell and its targeted therapies in cancers. *MedComm* **4**, e323 (2023).

174. D. C. Palmer, G. C. Guittard, Z. Franco, J. G. Crompton, R. L. Eil, S. J. Patel, Y. Ji, N. Van Panhuys, C. A. Klebanoff, M. Sukumar, D. Clever, A. Chichura, R. Roychoudhuri, R. Varma, E. Wang, L. Gattinoni, F. M. Marincola, L. Balagopalan, L. E. Samelson, N. P. Restifo, Cish actively silences TCR signaling in CD8+ T cells to maintain tumor tolerance. *Journal of Experimental Medicine* **212**, 2095–2113 (2015).

175. C. H. June, M. C. Fletcher, J. A. Ledbetter, G. L. Schieven, J. N. Siegel, A. F. Phillips, L. E. Samelson, Inhibition of tyrosine phosphorylation prevents T-cell receptor-mediated signal transduction. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7722–7726 (1990).

176. L. J. Berg, L. D. Finkelstein, J. A. Lucas, P. L. Schwartzberg, TEC FAMILY KINASES IN T LYMPHOCYTE DEVELOPMENT AND FUNCTION. *Annu. Rev. Immunol.* **23**, 549–600 (2005).

177. C. L. Sommers, J. Lee, K. L. Steiner, J. M. Gurson, C. L. DePersis, D. El-Khoury, C. L. Fuller, E. W. Shores, P. E. Love, L. E. Samelson, Mutation of the phospholipase C-γ1–binding site of LAT affects both positive and negative thymocyte selection. *The Journal of Experimental Medicine* **201**, 1125–1134 (2005).

178. J. Lv, L. Qin, R. Zhao, D. Wu, Z. Wu, D. Zheng, S. Li, M. Luo, Q. Wu, Y. Long, Z. Tang, Y.-L. Tang, X. Luo, Y. Yao, L.-H. Yang, P. Li, Disruption of CISH promotes the antitumor activity of human T cells and decreases PD-1 expression levels. *Molecular Therapy - Oncolytics* **28**, 46–58 (2023).

179. D. C. Palmer, G. C. Guittard, Z. Franco, J. G. Crompton, R. L. Eil, S. J. Patel, Y. Ji, N. Van Panhuys, C. A. Klebanoff, M. Sukumar, D. Clever, A. Chichura, R. Roychoudhuri, R. Varma, E. Wang, L. Gattinoni, F. M. Marincola, L. Balagopalan, L. E. Samelson, N. P. Restifo, Cish actively silences TCR signaling in CD8+ T cells to maintain tumor tolerance. *Journal of Experimental Medicine* **212**, 2095–2113 (2015).

180. F. Cano, A. Bravo-Blas, M. Colombe, C. Cerrato, R. K. C. Venigalla, O. Preham, E. Burns, P. Mortimer, A. Choudhry, N. Slipek, M. J. Johnson, B. R. Webber, B. S. Moriarity, E. Lou, M. Choudhry, C. A. Klebanoff, T. Henley, CISH, a key intracellular checkpoint, in comparison and combination to

existing and emerging cancer immune checkpoints. *Commun Biol*, doi: 10.1038/s42003-026-09579-x (2026).

181. D. C. Palmer, B. R. Webber, Y. Patel, M. J. Johnson, C. M. Kariya, W. S. Lahr, M. R. Parkhurst, J. J. Gartner, T. D. Prickett, F. J. Lowery, R. J. Kishton, D. Gurusamy, Z. Franco, S. K. Vodnala, M. D. Diers, N. K. Wolf, N. J. Slipek, D. H. McKenna, D. Sumstad, L. Viney, T. Henley, T. Bürckstümmer, O. Baker, Y. Hu, C. Yan, D. Meerzaman, K. Padhan, W. Lo, P. Malekzadeh, L. Jia, D. C. Deniger, S. J. Patel, P. F. Robbins, R. S. McIvor, M. Choudhry, S. A. Rosenberg, B. S. Moriarity, N. P. Restifo, Internal checkpoint regulates T cell neoantigen reactivity and susceptibility to PD1 blockade. *Med* **3**, 682-704.e8 (2022).

182. V. Laletin, P.-L. Bernard, C. Costa Da Silva, G. Guittard, J. A. Nunes, Negative intracellular regulators of T-cell receptor (TCR) signaling as potential antitumor immunotherapy targets. *J Immunother Cancer* **11**, e005845 (2023).

183. R. Seth, L.-H. Tai, T. Falls, C. T. De Souza, J. C. Bell, M. Carrier, H. Atkins, R. Boushey, R. A. Auer, Surgical Stress Promotes the Development of Cancer Metastases by a Coagulation-Dependent Mechanism Involving Natural Killer Cells in a Murine Model. *Annals of Surgery* **258**, 158–168 (2013).

184. M. Albertsmeier, D. Quaiser, V. Von Dossow-Hanfstingl, H. Winter, E. Faist, M. K. Angele, Major surgical trauma differentially affects T-cells and APC. *Innate Immun* **21**, 55–64 (2015).

185. W.-H. Wang, H.-Y. Xu, Z.-M. Zhao, G.-M. Zhang, F.-W. Lin, Dynamic and significant changes of T-cell subgroups in breast cancer patients during surgery and chemotherapy. *International Immunopharmacology* **65**, 279–283 (2018).

186. J. J. Carter, R. L. Whelan, The immunologic consequences of laparoscopy in oncology. *Surg Oncol Clin N Am* **10**, 655–677 (2001).

187. P. Xu, P. Zhang, Z. Sun, Y. Wang, J. Chen, C. Miao, Surgical trauma induces postoperative T-cell dysfunction in lung cancer patients through the programmed death-1 pathway. *Cancer Immunol Immunother* **64**, 1383–1392 (2015).

188. V. P. Makarenkova, V. Bansal, B. M. Matta, L. A. Perez, J. B. Ochoa, CD11b+/Gr-1+ Myeloid Suppressor Cells Cause T Cell Dysfunction after Traumatic Stress. *The Journal of Immunology* **176**, 2085–2094 (2006).

189. L. Cheng, J. Wang, X. Li, Q. Xing, P. Du, L. Su, S. Wang, Interleukin-6 Induces Gr-1+CD11b+ Myeloid Cells to Suppress CD8+ T Cell-Mediated Liver Injury in Mice. *PLoS ONE* **6**, e17631 (2011).

190. R. P. Ranocchia, C. V. Gorlino, M. I. Crespo, M. F. Harman, M. V. Liscovsky, G. Morón, B. A. Maletto, M. C. Pistoresi-Palencia, Arginase-dependent suppression by CpG-ODN plus IFA-induced splenic myeloid $CD11b^+$ $Gr1^+$ cells. *Immunol Cell Biol* **90**, 710–721 (2012).

191. V. Bronte, P. Serafini, A. Mazzoni, D. M. Segal, P. Zanovello, L-arginine metabolism in myeloid cells controls T-lymphocyte functions. *Trends in Immunology* **24**, 301–305 (2003).

192. V. Bronte, P. Serafini, C. De Santo, I. Marigo, V. Tosello, A. Mazzoni, D. M. Segal, C. Staib, M. Lowel, G. Sutter, M. P. Colombo, P. Zanovello, IL-4-Induced Arginase 1 Suppresses Alloreactive T Cells in Tumor-Bearing Mice. *The Journal of Immunology* **170**, 270–278 (2003).

193. A. Mazzoni, V. Bronte, A. Visintin, J. H. Spitzer, E. Apolloni, P. Serafini, P. Zanovello, D. M. Segal, Myeloid Suppressor Lines Inhibit T Cell Responses by an NO-Dependent Mechanism. *The Journal of Immunology* **168**, 689–695 (2002).

194. S. A. Kusmartsev, Y. Li, S.-H. Chen, Gr-1+ Myeloid Cells Derived from Tumor-Bearing Mice Inhibit Primary T Cell Activation Induced Through CD3/CD28 Costimulation. *The Journal of Immunology* **165**, 779–785 (2000).

195. L. Angka, G. Tennakoon, D. P. Cook, A. B. Martel, M. Market, C. Tanese De Souza, E. Cummins, I. Samudio, N. Kekre, M. Ardolino, B. C. Vanderhyden, M. A. Kennedy, R. C. Auer, Preventing surgery-induced natural killer cell suppression and metastases by inhibiting PI3K-gamma signaling in myeloid-derived suppressor cells. *J Immunother Cancer* **14**, e013304 (2026).

196. M. C. Schmid, C. J. Avraamides, H. C. Dippold, I. Franco, P. Foubert, L. G. Ellies, L. M. Acevedo, J. R. E. Manglicmot, X. Song, W. Wrasidlo, S. L. Blair, M. H. Ginsberg, D. A. Cheresh, E. Hirsch, S. J. Field, J. A. Varner, Receptor Tyrosine Kinases and TLR/IL1Rs Unexpectedly Activate Myeloid Cell PI3Kγ, A Single Convergent Point Promoting Tumor Inflammation and Progression. *Cancer Cell* **19**, 715–727 (2011).

197. M. M. Kaneda, K. S. Messer, N. Ralainirina, H. Li, C. J. Leem, S. Gorjestani, G. Woo, A. V. Nguyen, C. C. Figueiredo, P. Foubert, M. C. Schmid, M. Pink, D. G. Winkler, M. Rausch, V. J. Palombella, J. Kutok, K. McGovern, K. A. Frazer, X. Wu, M. Karin, R. Sasik, E. E. W. Cohen, J. A. Varner, PI3Kγ is a molecular switch that controls immune suppression. *Nature* **539**, 437–442 (2016).

198. P. Foubert, M. M. Kaneda, J. A. Varner, PI3Kγ Activates Integrin α4 and Promotes Immune Suppressive Myeloid Cell Polarization during Tumor Progression. *Cancer Immunology Research* **5**, 957–968 (2017).

199. I. Marigo, E. Bosio, S. Solito, C. Mesa, A. Fernandez, L. Dolcetti, S. Ugel, N. Sonda, S. Bicciato, E. Falisi, F. Calabrese, G. Basso, P. Zanovello, E. Cozzi, S. Mandruzzato, V. Bronte, Tumor-Induced Tolerance and Immune Suppression Depend on the C/EBPβ Transcription Factor. *Immunity* **32**, 790–802 (2010).

200. M. M. Kaneda, K. S. Messer, N. Ralainirina, H. Li, C. J. Leem, S. Gorjestani, G. Woo, A. V. Nguyen, C. C. Figueiredo, P. Foubert, M. C. Schmid, M. Pink, D. G. Winkler, M. Rausch, V. J. Palombella, J. Kutok, K. McGovern, K. A. Frazer, X. Wu, M. Karin, R. Sasik, E. E. W. Cohen, J. A. Varner, PI3Kγ is a molecular switch that controls immune suppression. *Nature* **539**, 437–442 (2016).

201. H. Grauers Wiktorin, E. Aydin, R. Kiffin, C. Vilhav, J. Bourghardt Fagman, M. Kaya, S. Paul, B. Westman, S. O. Bratlie, P. Naredi, K. Hellstrand, A. Martner, Impact of Surgery-Induced Myeloid-derived Suppressor Cells and the NOX2/ROS Axis on Postoperative Survival in Human Pancreatic Cancer. *Cancer Research Communications* **4**, 1135–1149 (2024).

202. L.-H. Tai, A. A. Alkayyal, A. L. Leslie, S. Sahi, S. Bennett, C. Tanese De Souza, K. Baxter, L. Angka, R. Xu, M. A. Kennedy, R. C. Auer, Phosphodiesterase-5 inhibition reduces postoperative

metastatic disease by targeting surgery-induced myeloid derived suppressor cell-dependent inhibition of Natural Killer cell cytotoxicity. *OncoImmunology* **7**, e1431082 (2018).

203. L. Wang, E. W. Y. Chang, S. C. Wong, S.-M. Ong, D. Q. Y. Chong, K. L. Ling, Increased Myeloid-Derived Suppressor Cells in Gastric Cancer Correlate with Cancer Stage and Plasma S100A8/A9 Proinflammatory Proteins. *The Journal of Immunology* **190**, 794–804 (2013).

204. J. Schmielau, O. J. Finn, Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of t-cell function in advanced cancer patients. *Cancer Res* **61**, 4756–4760 (2001).

205. B. Huang, H. Li, Q. Jiang, Y. Li, Z. Jiang, H. Cao, S. Wang, X. Wang, J. Li, G. Li, Elevated type I IFN signalling directly affects CD8+ T-cell distribution and autoantigen recognition of the skeletal muscles in active JDM patients. *Journal of Autoimmunity* **146**, 103232 (2024).

206. R. M. Welsh, K. Bahl, H. D. Marshall, S. L. Urban, Type 1 Interferons and Antiviral CD8 T-Cell Responses. *PLoS Pathog* **8**, e1002352 (2012).

207. B. N. Newby, T. M. Brusko, B. Zou, M. A. Atkinson, M. Clare-Salzler, C. E. Mathews, Type 1 Interferons Potentiate Human CD8+ T-Cell Cytotoxicity Through a STAT4- and Granzyme B–Dependent Pathway. *Diabetes* **66**, 3061–3071 (2017).

208. A. K. Pinto, S. Daffis, J. D. Brien, M. D. Gainey, W. M. Yokoyama, K. C. F. Sheehan, K. M. Murphy, R. D. Schreiber, M. S. Diamond, A Temporal Role Of Type I Interferon Signaling in CD8+ T Cell Maturation during Acute West Nile Virus Infection. *PLoS Pathog* **7**, e1002407 (2011).

209. M. Sade-Feldman, K. Yizhak, S. L. Bjorgaard, J. P. Ray, C. G. De Boer, R. W. Jenkins, D. J. Lieb, J. H. Chen, D. T. Frederick, M. Barzily-Rokni, S. S. Freeman, A. Reuben, P. J. Hoover, A.-C. Villani, E. Ivanova, A. Portell, P. H. Lizotte, A. R. Aref, J.-P. Eliane, M. R. Hammond, H. Vitzthum, S. M. Blackmon, B. Li, V. Gopalakrishnan, S. M. Reddy, Z. A. Cooper, C. P. Paweletz, D. A. Barbie, A. Stemmer-Rachamimov, K. T. Flaherty, J. A. Wargo, G. M. Boland, R. J. Sullivan, G. Getz, N. Hacohen, Defining T Cell States Associated with Response to Checkpoint Immunotherapy in Melanoma. *Cell* **175**, 998-1013.e20 (2018).

210. L. Zheng, S. Qin, W. Si, A. Wang, B. Xing, R. Gao, X. Ren, L. Wang, X. Wu, J. Zhang, N. Wu, N. Zhang, H. Zheng, H. Ouyang, K. Chen, Z. Bu, X. Hu, J. Ji, Z. Zhang, Pan-cancer single-cell landscape of tumor-infiltrating T cells. *Science* **374**, abe6474 (2021).

211. Y. Zhang, H. Chen, H. Mo, X. Hu, R. Gao, Y. Zhao, B. Liu, L. Niu, X. Sun, X. Yu, Y. Wang, Q. Chang, T. Gong, X. Guan, T. Hu, T. Qian, B. Xu, F. Ma, Z. Zhang, Z. Liu, Single-cell analyses reveal key immune cell subsets associated with response to PD-L1 blockade in triple-negative breast cancer. *Cancer Cell* **39**, 1578-1593.e8 (2021).

212. W. Chen, J. M. N. Teo, S. W. Yau, M. Y.-M. Wong, C.-N. Lok, C.-M. Che, A. Javed, Y. Huang, S. Ma, G. S. Ling, Chronic type I interferon signaling promotes lipid-peroxidation-driven terminal CD8+ T cell exhaustion and curtails anti-PD-1 efficacy. *Cell Reports* **41**, 111647 (2022).

213. S. D. Liu, T. Tomassian, K. W. Bruhn, J. F. Miller, F. Poirier, M. C. Miceli, Galectin-1 Tunes TCR Binding and Signal Transduction to Regulate CD8 Burst Size. *The Journal of Immunology* **182**, 5283–5295 (2009).

214. E. Corapi, G. Carrizo, D. Compagno, D. Laderach, Endogenous Galectin-1 in T Lymphocytes Regulates Anti-prostate Cancer Immunity. *Front. Immunol.* **9**, 2190 (2018).

215. T. Clemente, N. J. Vieira, J. P. Cerliani, C. Adrain, A. Luthi, M. R. Dominguez, M. Yon, F. C. Barrence, T. B. Riul, R. D. Cummings, T. M. Zorn, S. Amigorena, M. Dias-Baruffi, M. M. Rodrigues, S. J. Martin, G. A. Rabinovich, G. P. Amarante-Mendes, Proteomic and functional analysis identifies galectin-1 as a novel regulatory component of the cytotoxic granule machinery. *Cell Death Dis* **8**, e3176–e3176 (2017).

216. T. Brandstoetter, J. Schmoellerl, R. Grausenburger, S. Kollmann, E. Doma, J. Huuhtanen, T. Klampfl, T. Eder, F. Grebien, G. Hoermann, J. Zuber, S. Mustjoki, B. Maurer, V. Sexl, SBNO2 is a critical mediator of STAT3-driven hematological malignancies. *Blood* **141**, 1831–1845 (2023).

217. K. C. El Kasmi, A. M. Smith, L. Williams, G. Neale, A. Panopolous, S. S. Watowich, H. Häcker, B. M. J. Foxwell, P. J. Murray, Cutting Edge: A Transcriptional Repressor and Corepressor Induced by the STAT3-Regulated Anti-Inflammatory Signaling Pathway. *The Journal of Immunology* **179**, 7215–7219 (2007).

218. E. Lou, M. S. Choudhry, T. K. Starr, T. D. Folsom, J. Bell, B. Rathmann, A. P. DeFeo, J. Kim, N. Slipek, Z. Jin, D. Sumstad, C. A. Klebanoff, K. Ladner, A. Sarkari, R. S. McIvor, T. A. Murray, J. S. Miller, M. Rao, E. Jensen, J. Ankeny, M. A. Khalifa, A. Chauhan, B. Spilseth, A. Dixit, P. P. Provenzano, W. Pan, D. Weber, M. Byrne-Steele, T. Henley, D. H. McKenna, M. J. Johnson, B. R. Webber, B. S. Moriarity, Targeting the intracellular immune checkpoint CISH with CRISPR-Cas9-edited T cells in patients with metastatic colorectal cancer: a first-in-human, single-centre, phase 1 trial. *The Lancet Oncology* **26**, 559–570 (2025).

# APPENDIX

| **Appendix A: List of antibodies (Murine)** | **Company** |
|---|---|
| APC CD11c Clone N418 | BioLegend |
| APC IFN gamma Clone XMG1.2 | eBioscience |
| APC Vα2 Clone B20.1 | BioLegend |
| BV421 CD107a Clone ID4B | BD Biosciences |
| BV421 CD62L Clone MEL 14 | BD Biosciences |
| BV421 CD69 Clone H1.2F3 | BD Biosciences |
| BV786 CD11b Clone M1/70 | BD Biosciences |
| BV786 CD4 Clone RM4-5 | BD Biosciences |
| BV786 CD44 Clone IM7 | BD Biosciences |
| BV786 CD45 Clone 564225 | BD Biosciences |
| BV786 CD45.2 Clone 563686 | BD Biosciences |
| FITC Vβ5.1 Clone MR9-4 | BD Biosciences |
| PE CD4 Clone RM4-5 | BD Biosciences |
| PE CD40 Clone 3123 | BD Biosciences |
| Pe-Cy5 Ly-6G (Gr-1) Clone RB6-8C5 | eBioscience |
| Pe-Cy7 CD3 Clone 17A2 | BioLegend |
| PE-Cy7 CD3e Clone 145-2C11 | eBioscience |
| PerCP-Cy5.5 CD8 Clone 53-6.7 (RUO) | BD Biosciences |
| PerCP-Cy5.5 TNFa Clone MP6-XT22 | BioLegend |

| **Appendix B: List of antibodies (Human)** | **Company** |
|---|---|
| APC CD8 Clone SK1 | BD Biosciences |
| APC-Cy7 TIGIT Clone A15153G | BioLegend |
| BV421 GATA-3 Clone 16E10A23 | BioLegend |
| BV650 CD45 Clone HI30 | BD Biosciences |
| BV786 TIM-3 Clone CD366 | BD Biosciences |
| FITC IFN gamma | BioLegend |
| FITC T-bet Clone 4B10 | BioLegend |
| PE CD40 Clone 3123 | BD Biosciences |
| Pe-Cy7 CD3 | BioLegend |
| Pe-Cy7 CD33 | BioLegend |
| PerCPCy5.5 PD-1 Clone NAT105 | BioLegend |
| PerCP-Cy5.5 TNFa Clone MAb11 | BioLegend |

## Appendix C

Murine qPCR Primers

| Primer | Forward Primer | Reverse Primer |
|---|---|---|
| GUSB | CTCATTTGGAATTTTGCCGATT | CCGAGTGAAGATCCCCTTTTTA |
| CISH | GTACAACAGCTACGCCTGGA | GAGTCCGCCTCTGATGCTTA |
| PLCγ | GAGACGCGCCAGATCACAT | AAAGTCCCGAGAAGTCTTCCC |
| IFNy | AGTGATGGCTGAAACTGTCGC | TCTTCGACCTCGAAACAGCA |
| SBNO2 | CATCCAGCTACAGAACCGACT | GAACCCACGAACTGTTGGTTT |

## Appendix D

Human qPCR Primers

| Primer | Forward Primer | Reverse Primer |
|---|---|---|
| GUSB | GTCTGCGGCATTTTGTCGG | CACACGATGGCATAGGAATGG |
| CISH | GAACTGCCCAAGCCAGTCAT | GCTATGCACAGCAGATCCTCC |
| PLCγ | GGAAGACCTCACGGGACTTTG | GCGTTTTCAGGCGAAATTCCA |
| IFNy | TCGGTAACTGACTTGAATGTCCA | TCGCTTCCCTGTTTTAGCTGC |
| SBNO2 | ACTCCCTGTCGGACATCGT | GAACAGCTTATCGTGGGTGGA |

# CURRICULUM VITAE

**Oladunni Olanubi**

DOCTOR OF PHILOSOPHY [MICROBIOLOGY AND IMMUNOLOGY)-UNIVERSITY OF OTTAWA | 2020|
. **Thesis Topic**: Investigating the mechanism of post-operative CD8+ T cell dysfunction in cancer surgery patients.
. Research Area Focus: Characterizing CD8+ T cell dysfunction post-operatively induced by surgical stress in both murine and human model.

MASTER OF SCIENCE (MICROBIOLOGY)-UNIVERSITY OF MANITOBA | 2015-2017 |
. **Thesis Topic:** Exploitation of human RuvBL1 by HAdV E1A to inhibit interferon response for efficient viral growth.
. **Research Area Focus**: Interaction of Eukaryotic Human Protein RuvBL1 AAA+ ATPase with Human Adenovirus E1A protein.

**Coursework**: Graduate Seminar in Microbiology, Graduate Microbiology

BACHELOR OF SCIENCE (BIOTECHNOLOGY-): -YORK UNIVERSITY | 2008-2012 |
. **Thesis Topic:** Autophagy: a comparative review.

**Research Area Focus**: molecular biology, more specifically autophagy in yeast, mammalian, and ciliate cells.

**Coursework**: Lab courses such as biotechnology, microbiology, advanced biochemistry & molecular genetics Lab, organic chemistry, analytical chemistry, Honors thesis program.

# WORK EXPERIENCE

RESEARCH STUDENT- UNIVERSITY OF MANITOBA | 2015-2017|

- Conducted various molecular techniques such as Western Blot, Immunoprecipitation, Co-Immunoprecipitation, cloning, PCR, real time PCR, ChIP, FISH, Viral growth assay, Transformations, Transfections, Infections, Immunofluorescence, Pull-down Assays, Electrophoresis, Luciferase Assay etc
- Preparing and maintaining tissue culture such as HT1080, IMR90, U20S, 293 cell lines, as well as bacteria culture BL21's and DH5 alpha.

- Conducted basic and applied research on Adenovirus and immunity.
- Interpreted research findings and summarized data into reports.
- Managed overall laboratory functions.
- Designed and optimized assays.
- Ordered laboratory equipment and supplies.

RESEARCH STUDENT- OTTAWA HOSPITAL RESEARCH INSTITUTE | 2020-2025|

- Performed various experiments with techniques such as Flow Cytometry, ELISpots, ELISA, scaRNA sequencing. Clinical trials etc.
- Completed Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans Course on Research Ethics course (TCPS2 Certified)
- Carried out Patient screening for studies including using EPIC, calling patients, acquiring consent from patients (collecting signatures), recording patients' information in confidential database
- Planning for various study (contacting various hospital resources for blood collecting program - OHSNREB)
- Collecting Blood, Plasma (storing all samples appropriately and recording data).
- Processing Blood using Ficoll centrifugation, isolating PBMCs and carrying out endpoint assays

# COURSES

- MIC8166 – Professionalism and Professional Skills
- MIC 8120 – Advanced Topics in immunometabolism
- MIC9998 – Comprehensive examination April 2023
- BCH8109 – Advanced Topics in Cell Death

# PERSONAL INFORMATION

VOLUNTEER WORK

**Children's hospital of Eastern Ontario telethon march (CHEO):** | 2007|

Accept pledges and donations on behalf of the hospital to help provide research, equipment,

pediatric program etc.

**March of dimes**: | 2009|

Campaigned door to door fundraising for the Aphasia Communication Disabilities Program.

**Information days (University of Manitoba)**: | 2015|

Meet with potential students to discuss programs and research topics that departments offer.

# ASSOCIATIONS

. 1. **UMGSA (Graduate student association) (University of Manitoba) (2016-2018)**

- Council members meet monthly to discuss issues related to different departments and graduate student life.

**2. BIOCANRX HQP Working Group (2021)**

- The BIOCANRX HQP provides trainees with the opportunity to stay current in the field of cancer immunotherapies, laboratory techniques as well as the latest technological advances. Working group members get together to discuss and plan the details of the Immunotherapy Cancer summit conference held annually. Here we present ideas on how to enhance the trainees experience during the summit.

# SCHOLORASHIPS

**University of Manitoba Admission Scholarship** | 2020-2024|

**Ontario Graduate Scholarship (OGS)** | 2021-2024|

# CONFERENCES

**DNA Tumor Virus Conference (Montreal)** | 2016|

**Canadian Student Health Research Forum (Manitoba)** | 2016|

**Faculty of medicine Research day (Ottawa)** | 2021-2025|

**The Ottawa Hospital General Surgery Day (Ottawa)** | 2021|

**Canadian society for Immunology conference (Halifax)** | 2022|

**Summit for cancer Immunotherapy** | 2022-2025|

# PUBLICATIONS


1. Olanubi, O., Frost, J. R., Radko, S., & Pelka, P. (2017). Suppression of Type I Interferon Signaling by E1A via RuvBL1/Pontin. *Journal of virology*, *91*(8)

2. Radko S, Jung R, Olanubi O, Pelka P (2015) Effects of Adenovirus Type 5 E1A Isoforms on Viral Replication in Arrested Human Cells. PLoS ONE 10(10)

3. Frost, J. R., Olanubi, O., Cheng, S. K., Soriano, A., Crisostomo, L., Lopez, A., & Pelka, P. (2017). The interaction of adenovirus E1A with the mammalian protein Ku70/XRCC6. *Virology*, *500*, 11–21.

4. Market, M., Angka, L., Martel, A. B., Bastin, D., Olanubi, O., Tennakoon, G., Boucher, D. M., Ng, J., Ardolino, M., & Auer, R. C. (2020). Flattening the COVID-19 Curve with Natural Killer Cell Based Immunotherapies. *Frontiers in immunology*, *11*, 1512.

5. Olanubi, O., Dion, T., Macdonald, R., Alam, R., De Souza, C. T., Hu, R., Crawley, A. M., & Auer, R. C. (2025). Characterizing post-operative T and B cell dysfunction in cancer surgery patients, using COVID-19 as a model antigen. *International journal of surgery (London, England)*, *111*(7), 4785–4788.