The influence of diet-induced obesity and exercise on bone marrow extracellular vesicles in an irradiated mouse model

Matthew Ngu

Thesis submitted to the University of Ottawa
in partial fulfillment of the requirements for the
degree of Master of Science in Human Kinetics

School of Human Kinetics
Faculty of Health Sciences
University of Ottawa

Table of Contents

Acknowledgements

I would like to begin by thanking Dr. Michael De Lisio for accepting me to be his Masters student and providing me with guidance and creating a unique and enjoyable space for research and learning. I appreciate your style of mentoring and approach to science, it has changed how I research, and problem solve. I would like to thank you for always having an open door, listening to my concerns and assisting me throughout my time in your lab.

I would also like to express my sincere gratitude to my committee, Dr. Kristi Adamo, and Dr. François Haman, whose comments and feedback have helped me learn and increase the quality of my project.

Thank you to all the undergraduate, graduate and post-docs I have met in this lab and at the hospital. In no particular order thank you: PM, NC, EF, DD, SR, JL, JV, JL, JA, KH, FO, AA, PD, DB, NC. You were all accepting, kind and generous individuals and made the good days great and the tough days good.

Thank you, DD!!!!!!! For all your encouragement, wisdom and guidance. For listening and sitting through all my practice presentations. Your support helped me through my research, and I appreciate all you have done for me.

I would like to thank my family, for, without their support, none of this would have been possible. Dad, Mom, AN, RN.

Abstract

Background:

Between 2005 and 2015 the number of new cancer cases per year in Canada rose by 29% and this number is projected to increase to 277,000 cases per year. Ionizing radiation is used as therapy in the majority of cancer cases; however, it can have long-term detrimental effects on the hematopoietic system. Recent work from our lab, in a preclinical model of radiation damage, demonstrated that endurance exercise training can enhance hematopoietic recovery, while obesity can impair it. Extracellular vesicles (EVs) are a mode of cellular communication that has been implicated in regulating hematopoiesis acutely following radiation exposure. However, the long-term, radiation-induced changes to EVs, and the role of exercise and/or obesity at modulating marrow EVs remains unknown. Thus, the purpose of this project was to determine the extent to which obesity and exercise influence the regenerative potential of bone marrow-EVs following radiation.

Method:

Mice were randomly divided into control (n=20; CON) or high fat diet (n=20; HF) groups, then subdivided into exercise-trained (EX, n=10) or sedentary (SED, n=10). Mice underwent whole-body exposure to a 3 Gy dose of gamma-radiation at age 13 weeks of age followed by bone marrow collection at 20 weeks of age. EVs were then isolated from the bone marrow by ExoQuick and ultracentrifugation. A non-irradiated, sedentary, control diet group (n=10) was used to determine the effects of radiation alone. Data was evaluated using repeated-measures three-factor (diet, exercise, time) and two-factor ANOVA.

Results:

High fat diet-induced changes in body weight and composition and altered food consumption ($p<0.05$). Isolated EVs measured between 78 and 195 nm and western blot confirmed the presence of EV protein markers Alix, TSG101, and Flotillin. No size difference was observed between the groups. The concentration of EVs in irradiated mice was significantly lower compared to EVs from control mice ($p<0.01$). Radiation, obesity, exercise,

or their combination had no significant effect on hematopoietic stem progenitor cells (HSPC) content in co-culture assays. Conversely, EVs from irradiated mice significantly increased the number of CFU-GEMM, CFU-G, and the TOTAL number of colonies compared to EVs from non-irradiated mice ($p<0.01$). However, EVs from the CON+SED, CON+EX, HF+SED, and HF+EX groups did not have a significant effect on colony formation.

Conclusion:

Our findings demonstrate that ionizing radiation can diminish the concentration of bone marrow-EVs and that irradiated bone marrow-EVs can increase the total number of myeloid colonies formed *in vitro*. These results suggest that radiation induces myelopoiesis via a mechanism that includes EVs; however, exercise and obesity induce their effects via a different mechanism.

Chapter 1 - Literature Review

Introduction

According to the Canadian Cancer Society, there were an estimated 800,000 cancer survivors in Canada in 2009, with an additional 15.5 million in the United States ("Cancer Statistics," 2017). Also, in 2016 and 2017 6 in 10 Canadian adults aged 18 to 79 were classified as overweight or obese according to their (Government of Canada, 2018). Globally, it is estimated, that 1.9 billion adults, 18 years and older, are overweight, and over 650 million people are obese (World Health Organization, 2018). As such, a growing portion of the population is classified as both obese and cancer survivors (Courneya et al., 2008). The recent rise in cancer survivorship has increased the need to understand the late effects of cancer therapy. Radiation therapy is commonly used in cancer treatment, with 2/3 of patients receiving radiation therapy (Chen and Kuo, 2017). Late effects of radiation exposure can cause long-term immunosuppression, long-term damage to radio-sensitive tissues and secondary cancers after radiotherapy can develop. However these cancers can have a latency of 10 years or more after the initial treatment (Green and Rubin, 2014; Ng and Shuryak, 2014). The hematopoietic system is particularly susceptible to radiation damage (Shao et al., 2014). The hematopoietic stem and progenitor cells (HSPCs), the most primitive cells of the hematopoietic system from which all mature blood cells are derived (Metcalf, 2007) **(Figure 1.1)**, are particularly susceptible to radiation. Radiation induces DNA damage and senescence in hematopoietic stem cells (HSCs), and their progeny (Wang et al., 2006) as well as a prolonged inflammatory response within the bone marrow (Lange et al., 2011; Lorimore et al., 2013). These sides effects of radiation can lead to immunosuppression in the short-term, and long-term and can eventually progress to the development of hematological malignancies (Williams and McBride, 2011).

The increasing number of cancer survivors with obesity has created a critical need to develop a better understanding of how obesity influences the late-effects of radiation therapy

in the bone marrow, and to develop effective countermeasures. This information will be useful for informing oncologists, and patients of the risks of radiation therapy, and to design effective interventions to minimize the long-term effects of radiation exposure. Physiological and psychological benefits can result from an active lifestyle, and this lifestyle can be used as a preventative measure to disease. Heart disease and type II diabetes have been associated with limited physical activity. Physical activity and exercise interventions significantly reduce the risk of obesity-associated co-morbidities (Golbidi and Laher, 2012; Swift et al., 2014). Recent evidence from Emmons et al., 2019 suggests that exercise can reverse several of the detrimental effects of obesity on bone marrow stem/stromal cell populations following radiation exposure. However, the molecular mechanisms of these effects remain unknown.

Effects of radiation on the bone marrow

The bone marrow provides a specialized niche for HSCs to sustain hematopoiesis (Crane et al., 2017). Stromal cells present within the bone marrow direct HSC fate primarily through paracrine mechanisms (Schofield, 1978; Morrison and Spradling, 2008; Crane et al., 2017). Mesenchymal stem cells (MSCs) and their progeny are key stromal components of the bone marrow. MSCs differentiate into adipocytes, osteoblasts, and chondroblasts (Méndez-Ferrer et al., 2010; Frenette et al., 2013). They regulate HSC function directly through cell-cell contact, and indirectly through the release of cytokines, and growth factors (Méndez-Ferrer et al., 2010; Zhou et al., 2014; Crane et al., 2017). Shifts in MSC differentiation can result in adipogenic or osteoblastic cells to increase in the bone marrow (Hu et al., 2018). An increase in bone marrow adiposity impairs hematopoiesis and reduces HSPC number (Naveiras et al., 2009; Frenette et al., 2013).

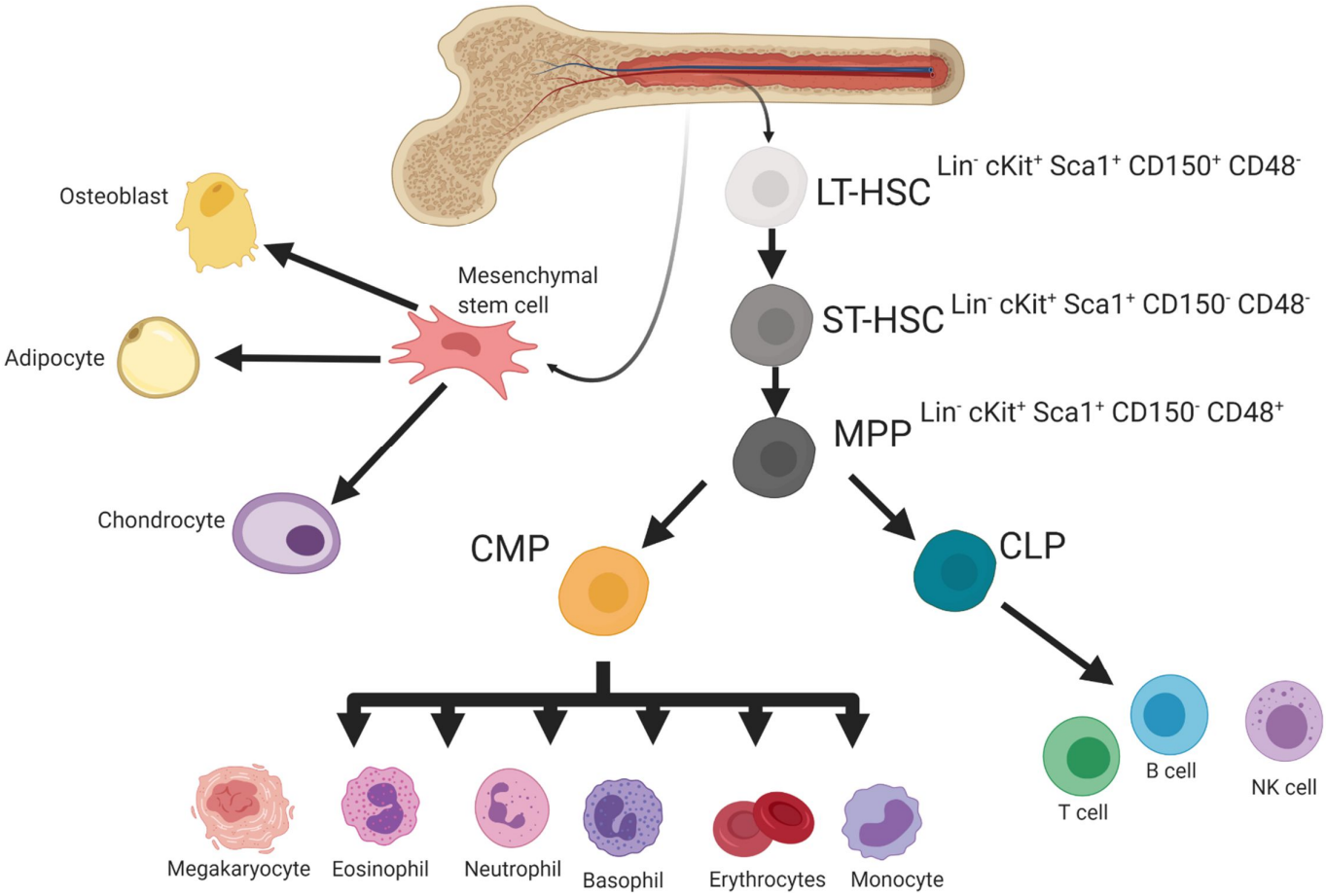


Figure 1.1. Hematopoietic and mesenchymal stem cell hierarchy in the bone marrow. Long-term hematopoietic stem cell (LT-HSC) differentiate into short-term hematopoietic stem cell (ST-HSC) which then differentiate into multipotent progenitor (MPP). MPP will then either differentiate into common myeloid progenitor (CMP) or common lymphoid progenitor (CLP). From the myeloid or lymphoid lineage mature blood and immune cells are produced. The protein markers used to identify LT-HSC, ST-HSC and MPP are listed beside. These markers include the "lineage" (Lin) antigens, which are specific to terminally differentiated blood cells. These antigens include but are not limited to: CD3, B220, Ly6G/Gr-1, CD11b/Mac-1, and TER-119. These lineage proteins are absent or only weakly expressed on HSPCs. Two commonly used markers, c-Kit and Sca1 are positively expressed on HSPCs. LT-HSC, ST-HSC and MPP are contained within a specific cell population in which the cells express Sca-1 and c-Kit, but lack the lineage (Lin) markers expressed on mature myeloid and lymphoid cells. This population is referred to as LSK or HSPC. Two other markers can be used to isolate HSCs and MPP and they are CD48 and CD150.

The bone marrow is a highly radiosensitive tissue (Ilnytskyy et al., 2009). Radiation can induce DNA damage, senescence in HSCs, HSC exhaustion, and apoptosis in HSCs (Wang et al., 2006; Shao et al., 2014). A moderate dose of irradiation will have the acute

effect of bone marrow suppression, with damage affecting HSPCs, and to a lesser degree HSCs (Dainiak, 2002). Under moderate doses of irradiation, HSCs undergo self-renewing proliferation, and differentiation to repopulate mature blood cells, thereby restoring homeostasis in the hematopoietic system. The application of hematopoietic growth factors, as well as stem cell transplants can promote the recovery of bone marrow hematopoietic function following radiation. As such, the majority of cancer patients can recover rapidly from acute bone marrow suppression after chemotherapy, and/or irradiation with or without hematopoietic growth factor treatment (Shao et al., 2013).

The rate and severity of damage following exposure to ionizing radiations depends on several radiation variables such as type of radiation (X- or γ-rays, α- or β-particles, and neutrons), dose, form (gas, particle, liquid), field (i.e. whole-, subtotal- or partial-body), dose-rate, route of exposure (external versus internal or combined), and fractionation. Also, an individual's ability to respond to irradiation is an important factor, this relates to age, sex and bone marrow before exposure. Acute single-fraction doses < 2 Gy can result in a modest decrease in blood cell concentrations. At intermediate doses (> 2- < 12 Gy) damage to the bone marrow is evident. In contrast, high doses > 12 Gy, especially at a high dose-rate, most individuals will die immediately (Gale and Armitage, 2020). High doses of radiation can severely damage HSCs, and impair their ability to self-renew, and differentiate (Macià i Garau et al., 2011; Singh and Seed, 2017). Patients and animals who have been exposed to high radiation doses, usually have normal blood cell counts, even though there is a decrease in HSC reserves, and impairment in HSC self-renewal (Mauch et al., 1995; Shao et al., 2013). Damage caused by persistent or high dose irradiation is long-lasting, shows little tendency to recover blood cell count, and can lead to the development of thrombocytopenia, agranulocytosis, lymphopenia, hypoplastic anemia or myelodysplastic syndrome (Fliedner et al., 2002; Shao et al., 2013). Since HSC injury is the primary cause of death after accidental or intentional exposure to a moderate or high dose of irradiation, protecting HSCs from the long-term effects of radiation should be a goal in the development of therapies which combat the side effects of radiation (Shao et al., 2014).

In addition to the direct effects of radiation on the hematopoietic system and HSCs, radiation induces changes within the bone marrow microenvironment (Lange et al., 2011; Lorimore et al., 2013). The decline in bone marrow hematopoietic cells after radiation is accompanied by an increase in marrow adipose tissue, which can inhibit hematopoiesis (Green and Rubin, 2014). Research suggests obesity results in the systemic and bone marrow specific elevation of inflammatory cytokines. Given the role inflammatory cytokines have in directing MSC differentiation, it is likely these cytokines are contributing to the promotion of adipogenic differentiation (Emmons et al., 2017). Adipocytes have been shown to be negative regulators of hematopoietic progenitor cells (Naveiras et al., 2009). Naveiras *et al.* showed that adipocytes can suppress HSPC proliferation. With increased marrow adiposity, there is also chronic inflammation, late after radiation exposure. Increased marrow adiposity can induce increased inflammatory-related proteins, and cytokines, which can suppress HSC recovery (Lange et al., 2011). Therefore, regeneration therapies after radiation could potentially benefit from treatments aimed at reducing inflammation which would improve the weakened hematopoietic system.

Effects of obesity on the bone marrow

Interestingly, obesity and radiation have similar effects on the bone marrow. Obesity is characterized by an increase in adipose tissue, systemic inflammation, and modulations of the bone marrow microenvironment (Chan and Woo, 2010; Adler et al., 2014; Hardaway et al., 2014; Park et al., 2014). Obesity is associated with a low-grade chronic inflammatory state; resulting in an overly active immune system. Higher levels of circulating active immune cells have been observed in individuals with obesity (Schwartz and Weiss, 1991; Nanji and Freeman, 1985; Devêvre et al., 2015; Lancaster and Febbraio, 2014). The increased number of active immune cells, along with proteins, and cytokines released from adipose tissue creates a pro-inflammatory *milieu*, and positive feedback loop that perpetuates the inflammatory state (Vainio et al., 2002; Pellegrinelli et al., 2015). Obesity propagates detrimental changes within the bone marrow microenvironment by driving hematopoiesis, and

propels HSCs down the myeloid lineage (Styner et al., 2014; van den Berg et al., 2016). Obesity can increase HSC cycling, resulting in the pre-mature exhaustion of the HSC pool. Simply put, the chronic low-grade inflammation associated with obesity disrupts hematopoiesis.

Within the bone marrow microenvironment, obesity can increase marrow adipose tissue (MAT). This increase in MAT due to obesity is demonstrated in both individuals and mice with obesity (Emmons et al., 2017). The accumulation of MAT can negatively impact the bone marrow compartment as adipocytes physically occupy marrow space, and MAT secretes pro-inflammatory cytokines, as well as free fatty acids, affecting cells throughout the bone marrow including HSPCs (Halade et al., 2010; Emmons et al., 2017). MAT is also a negative regulator of hematopoiesis as the quantity of HSPCs collected from areas of high MAT is decreased compared to HSPCs collected from areas of low MAT (Naveiras et al., 2009). Given the similar effects of obesity, and radiation on the bone marrow, and hematopoiesis, the late effects of radiation therapy may be exacerbated in obese cancer survivors. It remains unknown; however, how obesity influences stromal control of hematopoiesis in patients who have received radiation therapy.

Effects of exercise on the bone marrow

An acute bout exercise has been demonstrated to increase the number of circulating monocytes and induce an acute inflammatory response which is dependent on the intensity of exercise (Emmons et al., 2017). While, exercise training can increase peripheral blood erythrocyte content, can enhance tissue regeneration, and is associated with an anti-inflammatory response, (Hu and Lin, 2012; Emmons et al., 2017). Exercise training induces numerous systemic benefits including increased cardiovascular function (Zheng and Liu, 2015), cognitive function (Taubert et al., 2015), bone health (Xu et al., 2016), immune function (Gleeson et al., 2011), and skeletal muscle remodelling (McGlory et al., 2017).

Exercise training is widely considered anti-inflammatory; thus, an inverse relationship exists between exercise, and circulating inflammatory markers (Lavie et al., 2011). The

alleviation of inflammation by exercise has been observed in pro-inflammatory conditions including obesity (Park et al., 2014; Dias et al., 2015), and cancer patients and survivors (Battaglini et al., 2009; Rogers et al., 2013; LaVoy et al., 2016). Although a large body of literature exists demonstrating the anti-inflammatory effects of exercise, relatively little attention has been paid to the effects of exercise on hematopoiesis, particularly the hematopoietic process of producing pro-inflammatory cells.

Work from our lab has shown that exercise training combats several of the negative effects of obesity on hematopoiesis, and the bone marrow. Exercise has been observed to increase the quantity of HSPCs within the bone marrow without compromising their self-renewal (Baker et al., 2011; De Lisio et al., 2013; De Lisio and Parise, 2012). Some studies have shown that exercise-trained individuals have increased amounts of HSPCs at rest in both the bone marrow, and peripheral blood (Baker et al., 2011; Braam et al., 2013). Baker and colleagues investigated HSPC content using colony-forming unit (CFU) assays following chronic exercise training and found that increased hematopoiesis was apparent in both the peripheral blood and the bone marrow (Baker et al., 2011). These data were confirmed, and extended by De Lisio, and colleagues who demonstrated that progressive treadmill exercise training increase HSPC content collected from the central bone marrow cavity of mice (De Lisio and Parise, 2012).

The effects of exercise on HSCs and hematopoiesis are likely mediated by changes in their niche. It was demonstrated, that mice that were on an exercise training program for 1 hour per day, 3 days a week for 8 weeks, had beneficial remodelling of the bone marrow microenvironment (De Lisio et al., 2013) even in obesity, and pro-adipogenic conditions in mice (Styner et al., 2014; Styner et al., 2015). Exercise skews MSC differentiation towards osteogenesis; as such, exercise is one potential tool for reversing adipogenesis, and increasing osteogenesis (Baker et al., 2011; Chen et al., 2016; Pagnotti and Styner, 2016). Adult rats that underwent a moderate intensity exercise program displayed enhanced osteogenesis compared to sedentary rats (David et al., 2007; Hell et al., 2012; Styner et al., 2014). In tandem with this “switch” in MSC differentiation, is the effect exercise has on

cytokines, and growth factors in the bone marrow (Zaldivar et al., 2006; Wardyn et al., 2008). Specifically, our group has demonstrated that an acute bout of exercise can stimulate the proliferation of HSPCs, and MSCs within the bone marrow (Emmons et al., 2016). HSPC proliferation is likely mediated by MSC expansion, and alterations in the MSC secretome (Emmons et al., 2016). These data demonstrated that MSCs isolated from mice 15 min after exercise, had increased secretion of granulocyte-colony stimulating factor (G-CSF), stem cell factor (SCF), and interleukin 3 (IL-3) compared with non-exercised mice (Emmons et al., 2016). G-CSF, SCF, and IL-3 are thought to be involved in controlling HSPC proliferation, and these factors are likely being upregulated to stimulate HSPC mobilization into the periphery. SCF is also involved in stimulating self-renewal, and proliferation of HSPCs (Li et al., 2015). One of the mechanisms through which progressive exercise training exerts its effect is through mechanical strain on bone marrow stromal cells (David et al., 2007). These mechanical forces are thought to improve the bone marrow microenvironment, through the regulation of transcription factors, and stromal cells (Pagnotti and Styner, 2016). Mechanical strain has been suggested to promote the reduction of the adipocyte related transcription factor peroxisome proliferator-activated receptor-γ (PPARγ), a regulator of adipocyte differentiation, and the increase in the osteoblast controlling protein Runx2 (Marie, 2008; Cook and Genever, 2013). Emmons and colleagues (2019) demonstrated that exercise training can differentially influence hematopoietic quantity within the bone marrow following sub-lethal radiation exposure. The exercise-trained mice had significantly higher quantities of several HSPC subpopulations, whereas HSPC subpopulations were significantly lower in obese mice after radiation. The full complement of factors secreted by MSCs following exercise training has not been evaluated.

The literature presented above suggests that exercise training mitigates the negative effects of obesity on hematopoiesis, and the bone marrow, likely via altering paracrine factor secretion from bone marrow stromal cells. It remains unknown; however, if exercise can reduce the negative late effects of radiation therapy on the bone marrow in obesity, and subsequently, which paracrine factors are responsible for these exercise-induced effects.

Extracellular vesicles: characterization, cargo, and regulation of hematopoiesis

Recently, extracellular vesicles (EVs) have been identified as key regulators of HSC fate (Keating, 2006; Chou et al., 2013; Battiwalla and Barrett, 2014). EVs are membrane-bound particles, released by a variety of cell types, that are involved in local and distant intercellular communication (EL andaloussi et al., 2013; Aqmasheh et al., 2017; Butler et al., 2018). The generic term EV is used to describe various types of membrane-bound particles. These include exosomes, microvesicles (MVs) also sometimes referred to as microparticles, and apoptotic bodies. These groups of EVs can be classified based on size; with exosomes ranging in size from 40-100 nm, MVs having a size of 50-1000 nm, and apoptotic bodies having a size greater than 1000 nm (Yáñez-Mó et al., 2015; Butler et al., 2018). Exosomes are particles that are released by multivesicular bodies in the cell. Multivesicular bodies fuse with the plasma membrane, and the multivesicular bodies release smaller vesicles (exosomes) (Zaborowski et al., 2015). Conversely, MVs bud and cleave directly from the plasma membrane (Butler et al., 2018). Finally, apoptotic bodies are released during programmed cell death.

In addition to their classification by size, EVs can also be classified based on their protein expression. Tetraspanins, particularly proteins CD9, CD37, CD63, CD81, and CD82 are enriched in EV sub-populations, and are often used as markers for their identification (Raposo and Stoorvogel, 2013; Zaborowski et al., 2015). EVs are also enriched in proteins that associate with lipid rafts, including glycosylphosphatidylinositol-anchored proteins, and flotillin (Wubbolts et al., 2003; Raposo and Stoorvogel, 2013). MSC-derived EVs also maintain surface markers that are derived from their cell of origin, specifically CD29, CD73, CD44, and CD105 (Bruno et al., 2015; De Luca et al., 2016).

EVs specifically act as vehicles that transport proteins, lipids, and genetic material to recipient cells (Yeo et al., 2013). Lipid content can be important in maintaining immune proteins in an optimal and functional conformation (Laulagnier et al., 2004) as well as promoting lipogenesis (Subra et al., 2010; Sano et al., 2014). RNA is also transported via EVs; however, the RNA transported within EVs tends to be shorter (less than 200 nucleotides), and

includes, mRNA, microRNAs (miRNA), and transfer RNA (tRNAs) (Aqmasheh et al., 2017). It should also be noted, that EV cargo is dependent on their cellular origin. Therefore, the environment in which EV-producing cells originate, controls what effect that specific cell type's EVs may have.

New bodies of research are demonstrating how EVs and their cargo may play a role in controlling hematopoiesis, and HSC maintenance (Butler et al., 2018); however, it is still relatively unknown how EVs might contribute to controlling steady-state hematopoiesis. It has been suggested that EVs may be involved in the HSPC mobilization (Salvucci et al., 2012). Specifically, Salvucci *et al.*, 2012 demonstrated that G-CSF promotes the accumulation of microvesicles in the bone marrow, and that miR-126 delivered by microvesicles promotes the reduction of the surface protein vascular cell adhesion molecule 1 (VCAM1) in bone marrow HSPC, and other non-hematopoietic cells. Since VCAM1 is critical to the retention of HSPC in the bone marrow, this work illustrates that EVs can play a role in HSPC mobilization. This finding may also explain the results seen by our lab concerning HSC mobilization by exercise (Emmons et al., 2019). HSC-derived EVs have also been implicated in maintaining HSC stem cell properties such as self-renewal and the ability to differentiate into hematopoietic progeny (Gu et al., 2016). Specifically, HSC-derived EVs are abundant in angiopoietin-like protein 2 and 3, and these proteins have been reported to be critical for the regulation of HSC homeostatic features (Gu et al., 2016). This result demonstrated by Gu and colleagues (2016) could be associated to the increased content of primitive HSC populations observed in our previous work in exercise-trained mice (Emmons et al., 2019).

In terms of cargo carried by EVs, the content varies based on parent cell. However, EVs released by MSCs have been demonstrated to participate in maintaining cellular function within the bone marrow microenvironment (Yeo et al., 2013; De Luca et al., 2017). Specifically, EVs have a role in biological and pathological pathways. They have been demonstrated to participate in stem cell maintenance, tissue repair, immune surveillance, blood coagulation, and cancer proliferation (Ratajczak et al., 2006; Collino et al., 2010; Lai et al., 2010; EL Andaloussi et al., 2013). Further, EVs derived from human bone marrow-MSCs contain

intracellular proteins of the RAB family, which are involved in vesicle biogenesis, and trafficking as well as proteins associated with self-renewal, and differentiation (TGFβ, MAPK, PPAR) (Kim et al., 2012). RNA transcripts which have been found in MSC-EV have been associated with transcriptional control, cell proliferation, and immune regulation (Tomasoni et al., 2013; Abreu et al., 2016). MSCs support hematopoiesis through cytokines, chemokines, growth factors, and EVs that induce HSCs expansion, differentiation, and regeneration (Méndez-Ferrer et al., 2010; Butler et al., 2018). However, the molecular and cellular mechanisms that lead to the supportive effect of MSC EVs on hematopoiesis are still unknown. Several potential methods of control have been proposed, and they are linked to miRNA cargo found within EVs. Davis and colleagues (2017) conducted a miRNA profile of EVs isolated from bone marrow interstitial fluid, and they determined that transfection of bone marrow-MSCs with miR-183-5p suppresses osteogenic differentiation. miR-183-5p may not be restricted to its impact on bone formation as it also appears to stimulate bone resorption (Davis et al., 2017). Conversely, Shi and colleagues (2016) suggested that miR-486 can support erythroid differentiation. They observed that hypoxia-induced erythroid differentiation can be controlled via EV-bound miR-486. Lastly, an important study by Goloviznin and colleagues (2016) demonstrated that MSC-EVs can contribute to the paracrine crosstalk that is utilized in regulating hematopoiesis. Specifically, murine HSPCs exposed to MSC-EVs had increased activation, and differentiation, particularly down the myeloid lineage. The authors also demonstrated that EVs may act through toll-like receptor 4 (TLR4). When they incubated HSPCs with EVs in the presence of a TLR4 inhibitor, HSPC expansion and colony formation were inhibited. These data demonstrate that MSC-EVs are important regulators of hematopoiesis. Interestingly, work from our lab has shown that the obesity-induced increase in myeloid cells can be reduced through exercise training (Emmons et al., et al., 2019). With this in mind, exercise may reduce MSC-derived EV cargo that promotes myeloid differentiation of HSCs, while obesity may increase myelopoiesis through the same mechanism.

Effects of radiation on EVs – role in hematopoiesis

Radiation induces long-term HSC damage resulting in an increased risk of hematologic malignancies (Green and Rubin, 2014). In the disease state, bone marrow-EVs participate in the exacerbation and expansion of compromised bone marrow cells. An interesting study by Szatmári and colleagues (2017) investigated the effects of EVs that were directly isolated from irradiated mice (i.e., *in vivo*-derived) on hematopoiesis. When these EVs, were injected into non-irradiated mice, they induced radiation damage that was comparable to direct radiation and caused a significant reduction in HSC content. This group also evaluated the miRNA content of EVs, and they identified 90 miRNAs in the bone marrow-derived EVs of irradiated animals, which were differentially expressed compared to non-irradiated mice. Of the 90 miRNAs identified, miR-33, miR-140, miR-152, miR-199, miR-375, and miR-744 are implicated in regulating pathways responsible for hematopoiesis, and immune function (Ilnytskyy et al., 2008; Szatmári et al., 2017). With these results, Szatmári, and colleagues demonstrated that radiation induces alterations in bone marrow-EVs that negatively impact HSCs. It remains unknown; however, how relevant host factors (i.e., obesity and exercise) influence bone marrow-EVs following radiation exposure. Our work demonstrated that exercise training following sub-lethal radiation exposure, protected the bone marrow, increased bone marrow cellularity, and HSPC content (Emmons et al., 2019). Further, this increase in bone marrow cellularity and HSPC content was present in exercise-trained mice, regardless of obesity status. Whether EVs are involved in this response remains unknown.

Although EVs from irradiated mice exacerbates radiation damage, EVs from non-irradiated donors may be a potential strategy for mitigating the negative effects of radiation on hematopoiesis. Important work by Wen, and colleagues (2016) displayed that culture-derived MSC-EVs transplanted into irradiated mice can reverse the damaging radiation effects on bone marrow stem cells. Specifically, immature hematopoietic cells (lineage-negative; Lin^-) from irradiated mice were cultured with MSC-EVs before transplantation into irradiated mice. Lin^- cells cultured with MSC-EVs displayed a significant increase in engraftment compared to cells not cultured with EVs. Further, Wen and co-workers (2016) showed that human MSC-

EVs injected into mice that had been exposed to 0.5 Gy can reverse radiation damage in whole bone marrow cells (i.e., Lin+ depleted cells), lymphocyte, monocyte, and granulocyte counts. The findings of Wen and co-workers demonstrate that EVs may be used as a therapeutic tool to relieve acute radiation injury to the hematopoietic system, and enhance stress hematopoiesis. However, the effects of EVs on the long-term effects of radiation and recovery of steady-state hematopoiesis remain unknown.

The implication of obesity on the release and contents of EVs

In vivo, the effects of obesity on EVs released by tissues, and cell populations have been examined; however, this work is also limited. Togliatti and colleagues (2016) examined the therapeutic effect of EVs derived from adipose tissue-derived stem cells (ASCs) from individuals with and without obesity. They found that EVs from individuals with obesity had impaired pro-angiogenic potential, specifically through the reduced VEGF, MMP-2, and miR-126 content. The authors also observed that EVs from individuals with obesity were unable to induce endothelial cell (EC) migration, and tube-like structure formation (Togliatto et al., 2016). Lastly, ASC from lean individuals that were cultured under obesogenic conditions, had changes in EV cargo. These changes included an impaired EV-mediated pro-angiogenic signal activation as well as reduced VEGF, MMP-2, and miR-126 content (Togliatto et al., 2016). Exosomes from adipose tissue of obese mice have also been examined, and it was determined that the exosome can induce insulin resistance, and can act through bone marrow-derived TLR4 receptor (Deng et al., 2009). Macrophages exposed to obese-derived EVs had increased production of the pro-inflammatory cytokines IL-6, and TNF-α, and increased migration into adipose tissue, and the liver (Deng et al., 2009). The miRNA profiles of visceral adipose tissue-derived exosomes from individuals with obesity contain miRNAs with increased capacity to regulate TGF-β, and Wnt/β-catenin signalling pathways compared to lean (Ferrante et al., 2015). These pathways are implicated in several obesity-related comorbid conditions (Zhang et al., 2016). In a separate study, serum microvesicles were examined from a cohort of volunteers of which, ~18% had obesity. The study revealed that there was a

positive association with markers of obesity/adiposity, and increase in cystatin C, and CD14 in EVs (Kranendonk et al., 2014). These proteins have been linked to increasing the risk of myocardial infarction, vascular disease mortality, and future vascular events (Kanhai et al., 2013). So far, limited research suggests that diet/adiposity can have a role in manipulating EV function and cargo.

The effect of exercise on the release, and content of EVs

Exercise can stimulate the release of proteins, ions, nucleic acids, and polypeptides, which control pathways that ultimately result in metabolic, and morphological changes to tissue (Coffey ,and Hawley, 2007; Camera *et al*., 2016). Most of this work has focused on factors released from skeletal muscle, with relatively little attention paid to other cellular sources of paracrine factor release. Recently, our lab showed that a bout of acute exercise-induced the release of paracrine factors from MSCs into the HSC niche (Emmons et al., 2016). These paracrine factors included granulocyte-colony stimulating factor (G-CSF), and stem cell factor (SCF), which are implicated in HSPC maintenance and proliferation (Emmons et al., 2016). However, how MSC derived EVs are changing with exercise, and their effect in the HSC niche has not been examined (Wilhelm *et al*., 2018). Bei et al. (2017) investigated the effects of exercise stress tests on plasma EVs in human subjects as well as EVs from the serum from mice that were on a 3-week time progressive swimming protocol. In both humans and mice, exercise-induced an increase in the population of EVs compared to rest. Similarly, Frühbeis and colleagues (2015) demonstrated that acute exercise can increase the amount of circulating EVs retrieved from the plasma immediately after exercise and that the levels decrease during recovery. Similarly, Guescini and colleagues (2015) examined EVs from plasma after acute exercise in fit men. They profiled microRNAs from EVs which had been released by muscle. The authors proposed that the microRNA characterized in these EVs may act as a novel method of muscle communication. Interestingly, a study by Nielsen and colleagues (2014) demonstrated that miR-146 was down-regulated in the plasma of circulating blood after an acute exercise bout in humans. Aoi and co-workers (2013) showed that the

miR-486, which is enriched in EVs from muscle, was decreased in response to acute, and chronic aerobic exercise. As mentioned earlier, and proposed by the Shi group, miR-486 can support erythroid differentiation, indicating that exercise can manipulate EV cargo in a manner that is expected to impact haematopoiesis (Shi et al., 2017). Finally, Oliveira et al., 2018 showed that miR-103a-3p is down-regulated in EVs isolated from the serum of exercised rats and is a miRNA that has been linked to the inhibition of proliferation, and osteogenic differentiation in adipose-derived stromal cells (Sol Kim et al., 2015). This leads to the conclusion that the cargo, more specifically the microRNA of EVs from exercised tissue may be altered in a manner, which favour supporting the HSC niche. However, knowledge is limited in respect to how bone marrow-derived EVs are changing with exercise, and their effect in the HSC niche has not been examined (Wilhelm et al., 2018).

Statement of Problem and Rationale

The late effects of radiation are well documented, and existing therapies are limited in their capacity to relieve symptoms. In parallel, the population of cancer survivors with obesity is also rising. These two factors can be detrimental to the bone marrow and can lead to damage to the hematopoietic system. Our work will investigate how exercise training can be used as a potential therapeutic option. As our lab has demonstrated, diet and exercise training has a significant effect on the paracrine factors produced by MSCs in the bone marrow. As described above, EVs participate in the crosstalk between HSCs and their niche, and they have been shown to regulate hematopoiesis. However, functional characterization of irradiated EVs after an exercise, and diet intervention has not been performed. EV research is limited in examining how EVs affect the bone marrow niche with most studies focused on EVs, derived *in vitro*. Our study will be examining EVs collected *in vivo* and how their cargo is changing in response to environmental factors. Since EV cargo has been implicated in HSC niche function and activity, it will be important to examine how physiologically relevant host factors (i.e., exercise and obesity) alter EVs and their effect on hematopoiesis (Salvucci et al., 2012; Shi et al., 2017; Davis et al., 2017) If EVs from the HSC niche are being altered in a

manner which promotes HSC maintenance, EV cargo may represent a novel therapeutic target for improving long-term hematopoiesis following radiation. Thus, the purpose of this thesis is to functionally characterize bone marrow-EVs from obese and exercise-trained mice following radiation. By identifying these EVs, we thus can develop a greater appreciation, for how exercise acts via novel mediators to improve hematopoietic recovery.

Aims and hypothesis

Our *overall objective* is to determine the extent to which obesity-induced alterations in bone marrow-EVs are responsible for reduced hematopoiesis in obese mice exposed to radiation, and the role of exercise in reversing these effects. We *hypothesize* that bone marrow-EVs from exercise-trained mice will increase HSPC expansion and inhibit obesity-induced myeloid skewing. We will test this hypothesis by completing the following three specific aims:

1. To compare bone marrow-EVs from irradiated and control mice alter hematopoiesis *in vitro*.
2. To determine if bone marrow-EV content or composition is altered by exercise, and/or obesity following radiation.
3. To determine if bone marrow-EVs from exercise-trained, and sedentary mice with or without obesity exposed to radiation influence HSPC expansion and colony formation *in vitro*.

References

1. Abreu, S.C., Weiss, D.J., Rocco, P.R.M., 2016. Extracellular vesicles derived from mesenchymal stromal cells: a therapeutic option in respiratory diseases? Stem Cell Res Ther 7. https://doi.org/10.1186/s13287-016-0317-0
2. Adler, B.J., Kaushansky, K., Rubin, C.T., 2014. Obesity-driven disruption of haematopoiesis and the bone marrow niche. Nat Rev Endocrinol 10, 737–748. https://doi.org/10.1038/nrendo.2014.169
3. Anno, G.H., Baum, S.J., Withers, H.R., Young, R.W., 1989. Symptomatology of acute radiation effects in humans after exposure to doses of 0.5-30 Gy. Health Phys 56, 821–838.
4. Aoi, W., Ichikawa, H., Mune, K., Tanimura, Y., Mizushima, K., Naito, Y., Yoshikawa, T., 2013. Muscle-enriched microRNA miR-486 decreases in circulation in response to exercise in young men. Front Physiol 4, 80. https://doi.org/10.3389/fphys.2013.00080
5. Aqmasheh, S., Shamsasanjan, karim, Akbarzadehlaleh, P., Pashoutan Sarvar, D., Timari, H., 2017. Effects of Mesenchymal Stem Cell Derivatives on Hematopoiesis and Hematopoietic Stem Cells. Adv Pharm Bull 7, 165–177. https://doi.org/10.15171/apb.2017.021
6. Baker, J.M., De Lisio, M., Parise, G., 2011. Endurance exercise training promotes medullary hematopoiesis. FASEB J. 25, 4348–4357. https://doi.org/10.1096/fj.11-189043
7. Battaglini, C.L., Hackney, A.C., Garcia, R., Groff, D., Evans, E., Shea, T., 2009. The effects of an exercise program in leukemia patients. Integr Cancer Ther 8, 130–138. https://doi.org/10.1177/1534735409334266
8. Battiwalla, M., Barrett, A.J., 2014. Bone marrow mesenchymal stromal cells to treat complications following allogeneic stem cell transplantation. Tissue Eng Part B Rev 20, 211–217. https://doi.org/10.1089/ten.TEB.2013.0566
9. Bei, Y., Xu, T., Lv, D., Yu, P., Xu, J., Che, L., Das, A., Tigges, J., Toxavidis, V., Ghiran, I., Shah, R., Li, Y., Zhang, Y., Das, S., Xiao, J., 2017. Exercise-induced circulating extracellular vesicles protect against cardiac ischemia-reperfusion injury. Basic Res Cardiol 112, 38. https://doi.org/10.1007/s00395-017-0628-z
10. Boppart, M.D., De Lisio, M., Witkowski, S., 2015. Exercise and Stem Cells. Prog Mol Biol Transl Sci 135, 423–456. https://doi.org/10.1016/bs.pmbts.2015.07.005
11. Braam, K.I., van der Torre, P., Takken, T., Veening, M.A., van Dulmen-den Broeder, E., Kaspers, G.J.L., 2013. Physical exercise training interventions for children and young adults during and after treatment for childhood cancer. Cochrane Database Syst Rev CD008796. https://doi.org/10.1002/14651858.CD008796.pub2
12. Bruno, S., Deregibus, M.C., Camussi, G., 2015. The secretome of mesenchymal stromal cells: Role of extracellular vesicles in immunomodulation. Immunol. Lett. 168, 154–158. https://doi.org/10.1016/j.imlet.2015.06.007
13. Butler, J.T., Abdelhamed, S., Kurre, P., 2018. Extracellular vesicles in the hematopoietic microenvironment. Haematologica 103, 382–394. https://doi.org/10.3324/haematol.2017.183335
14. Camera, D.M., Smiles, W.J., Hawley, J.A., 2016. Exercise-induced skeletal muscle signaling pathways and human athletic performance. Free Radical Biology and Medicine, Human Performance and Redox Signaling in Health and Disease 98, 131–143. https://doi.org/10.1016/j.freeradbiomed.2016.02.007
15. Cancer Facts & Figures 2013 | American Cancer Society [WWW Document], n.d. URL https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2013.html (accessed 7.27.18).

16. Cancer Statistics [WWW Document], n.d. . National Cancer Institute. URL https://www.cancer.gov/about-cancer/understanding/statistics (accessed 8.10.18).
17. Chan, R.S.M., Woo, J., 2010. Prevention of Overweight and Obesity: How Effective is the Current Public Health Approach. Int J Environ Res Public Health 7, 765–783. https://doi.org/10.3390/ijerph7030765
18. Chen, Q., Shou, P., Zheng, C., Jiang, M., Cao, G., Yang, Q., Cao, J., Xie, N., Velletri, T., Zhang, X., Xu, C., Zhang, L., Yang, H., Hou, J., Wang, Y., Shi, Y., 2016. Fate decision of mesenchymal stem cells: adipocytes or osteoblasts? Cell Death Differ. 23, 1128–1139. https://doi.org/10.1038/cdd.2015.168
19. Chou, S.-H., Lin, S.-Z., Day, C.H., Kuo, W.-W., Shen, C.-Y., Hsieh, D.J.-Y., Lin, J.-Y., Tsai, F.-J., Tsai, C.-H., Huang, C.-Y., 2013. Mesenchymal stem cell insights: prospects in hematological transplantation. Cell Transplant 22, 711–721. https://doi.org/10.3727/096368912X655172
20. Coffey, V.G., Hawley, J.A., 2007. The Molecular Bases of Training Adaptation. Sports Med 37, 737–763. https://doi.org/10.2165/00007256-200737090-00001
21. Cogle, C.R., Wise, E., Meacham, A.M., Zierold, C., Traverse, J.H., Henry, T.D., Perin, E.C., Willerson, J.T., Ellis, S.G., Carlson, M., Zhao, D.X.M., Bolli, R., Cooke, J.P., Anwaruddin, S., Bhatnagar, A., da Graca Cabreira-Hansen, M., Grant, M.B., Lai, D., Moyé, L., Ebert, R.F., Olson, R.E., Sayre, S.L., Schulman, I.H., Bosse, R.C., Scott, E.W., Simari, R.D., Pepine, C.J., Taylor, D.A., Cardiovascular Cell Therapy Research Network (CCTRN), 2014. Detailed analysis of bone marrow from patients with ischemic heart disease and left ventricular dysfunction: BM CD34, CD11b, and clonogenic capacity as biomarkers for clinical outcomes. Circ. Res. 115, 867–874. https://doi.org/10.1161/CIRCRESAHA.115.304353
22. Collino, F., Deregibus, M.C., Bruno, S., Sterpone, L., Aghemo, G., Viltono, L., Tetta, C., Camussi, G., 2010. Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs. PLoS ONE 5, e11803. https://doi.org/10.1371/journal.pone.0011803
23. Cook, D., Genever, P., 2013. Regulation of Mesenchymal Stem Cell Differentiation, in: Transcriptional and Translational Regulation of Stem Cells, Advances in Experimental Medicine and Biology. Springer, Dordrecht, pp. 213–229. https://doi.org/10.1007/978-94-007-6621-1_12
24. Crane, G.M., Jeffery, E., Morrison, S.J., 2017. Adult haematopoietic stem cell niches. Nature Reviews Immunology 17, nri.2017.53. https://doi.org/10.1038/nri.2017.53
25. Dainiak, N., 2002. Hematologic consequences of exposure to ionizing radiation. Exp. Hematol. 30, 513–528.
26. David, V., Martin, A., Lafage-Proust, M.-H., Malaval, L., Peyroche, S., Jones, D.B., Vico, L., Guignandon, A., 2007. Mechanical loading down-regulates peroxisome proliferator-activated receptor gamma in bone marrow stromal cells and favors osteoblastogenesis at the expense of adipogenesis. Endocrinology 148, 2553–2562. https://doi.org/10.1210/en.2006-1704
27. Davis, C., Dukes, A., Drewry, M., Helwa, I., Johnson, M.H., Isales, C.M., Hill, W.D., Liu, Y., Shi, X., Fulzele, S., Hamrick, M.W., 2017. MicroRNA-183-5p Increases with Age in Bone-Derived Extracellular Vesicles, Suppresses Bone Marrow Stromal (Stem) Cell Proliferation, and Induces Stem Cell Senescence. Tissue Eng Part A 23, 1231–1240. https://doi.org/10.1089/ten.TEA.2016.0525
28. De Lisio, M., Baker, J.M., Parise, G., 2013. Exercise promotes bone marrow cell survival and recipient reconstitution post-bone marrow transplantation, which is associated

with increased survival. Exp. Hematol. 41, 143–154. https://doi.org/10.1016/j.exphem.2012.10.003
29. De Lisio, M., Parise, G., 2012. Characterization of the effects of exercise training on hematopoietic stem cell quantity and function. J. Appl. Physiol. 113, 1576–1584. https://doi.org/10.1152/japplphysiol.00717.2012
30. De Lisio, M., Phan, N., Boreham, D.R., Parise, G., 2011. Exercise-induced protection of bone marrow cells following exposure to radiation. Appl Physiol Nutr Metab 36, 80–87. https://doi.org/10.1139/H10-087
31. De Luca, L., Trino, S., Laurenzana, I., Simeon, V., Calice, G., Raimondo, S., Podestà, M., Santodirocco, M., Di Mauro, L., La Rocca, F., Caivano, A., Morano, A., Frassoni, F., Cilloni, D., Del Vecchio, L., Musto, P., 2016. MiRNAs and piRNAs from bone marrow mesenchymal stem cell extracellular vesicles induce cell survival and inhibit cell differentiation of cord blood hematopoietic stem cells: a new insight in transplantation. Oncotarget 7, 6676–6692. https://doi.org/10.18632/oncotarget.6791
32. Deng, Z., Poliakov, A., Hardy, R.W., Clements, R., Liu, C., Liu, Y., Wang, J., Xiang, X., Zhang, S., Zhuang, X., Shah, S.V., Sun, D., Michalek, S., Grizzle, W.E., Garvey, T., Mobley, J., Zhang, H.-G., 2009. Adipose Tissue Exosome-Like Vesicles Mediate Activation of Macrophage-Induced Insulin Resistance. Diabetes 58, 2498–2505. https://doi.org/10.2337/db09-0216
33. Devêvre, E.F., Renovato-Martins, M., Clément, K., Sautès-Fridman, C., Cremer, I., Poitou, C., 2015. Profiling of the three circulating monocyte subpopulations in human obesity. J. Immunol. 194, 3917–3923. https://doi.org/10.4049/jimmunol.1402655
34. Dias, K.A., Green, D.J., Ingul, C.B., Pavey, T.G., Coombes, J.S., 2015. Exercise and Vascular Function in Child Obesity: A Meta-Analysis. Pediatrics 136, e648-659. https://doi.org/10.1542/peds.2015-0616
35. EL Andaloussi, S., Mäger, I., Breakefield, X.O., Wood, M.J.A., 2013. Extracellular vesicles: biology and emerging therapeutic opportunities. Nat Rev Drug Discov 12, 347–357. https://doi.org/10.1038/nrd3978
36. Emmons, R., Ngu, M., Xu, G., Hernández-Saavedra, D., Chen, H., De Lisio, M., 2019. Effects of Obesity and Exercise on Bone Marrow Progenitor Cells following Radiation. Med Sci Sports Exerc. https://doi.org/10.1249/MSS.0000000000001894
37. Emmons, R., Niemiro, G.M., De Lisio, M., 2017. Hematopoiesis with Obesity and Exercise: Role of the Bone Marrow Niche. Exerc Immunol Rev 23, 82–95.
38. Emmons, R., Niemiro, G.M., Owolabi, O., De Lisio, M., 2016. Acute exercise mobilizes hematopoietic stem and progenitor cells and alters the mesenchymal stromal cell secretome. J. Appl. Physiol. 120, 624–632. https://doi.org/10.1152/japplphysiol.00925.2015
39. Ferrante, S.C., Nadler, E.P., Pillai, D.K., Hubal, M.J., Wang, Z., Wang, J.M., Gordish-Dressman, H., Koeck, E., Sevilla, S., Wiles, A.A., Freishtat, R.J., 2015. Adipocyte-derived Exosomal miRNAs: A Novel Mechanism for Obesity-Related Disease. Pediatr Res 77, 447–454. https://doi.org/10.1038/pr.2014.202
40. Fliedner, T. m., Graessle, D., Paulsen, C., Reimers, K., 2002. Structure and Function of Bone Marrow Hemopoiesis: Mechanisms of Response to Ionizing Radiation Exposure. Cancer Biotherapy and Radiopharmaceuticals 17, 405–426. https://doi.org/10.1089/108497802760363204
41. Frenette, P.S., Pinho, S., Lucas, D., Scheiermann, C., 2013. Mesenchymal stem cell: keystone of the hematopoietic stem cell niche and a stepping-stone for regenerative medicine. Annu. Rev. Immunol. 31, 285–316. https://doi.org/10.1146/annurev-immunol-032712-095919

42. Frühbeis, C., Helmig, S., Tug, S., Simon, P., Krämer-Albers, E.-M., 2015. Physical exercise induces rapid release of small extracellular vesicles into the circulation. J Extracell Vesicles 4. https://doi.org/10.3402/jev.v4.28239
43. Gale, R.P., Armitage, J.O., 2020. Use of molecularly-cloned haematopoietic growth factors in persons exposed to acute high-dose, high-dose rate whole-body ionizing radiations. Blood Rev. 100690. https://doi.org/10.1016/j.blre.2020.100690
44. Gleeson, M., Bishop, N.C., Stensel, D.J., Lindley, M.R., Mastana, S.S., Nimmo, M.A., 2011. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. Nat. Rev. Immunol. 11, 607–615. https://doi.org/10.1038/nri3041
45. Golbidi, S., Laher, I., 2012. Exercise and the Cardiovascular System. Cardiol Res Pract 2012. https://doi.org/10.1155/2012/210852
46. Goloviznina, N.A., Verghese, S.C., Yoon, Y.M., Taratula, O., Marks, D.L., Kurre, P., 2016. Mesenchymal Stromal Cell-derived Extracellular Vesicles Promote Myeloid-biased Multipotent Hematopoietic Progenitor Expansion via Toll-Like Receptor Engagement. J. Biol. Chem. 291, 24607–24617. https://doi.org/10.1074/jbc.M116.745653
47. Government of Canada, S.C., 2015. Overweight and obese adults (self-reported), 2014 [WWW Document]. URL https://www150.statcan.gc.ca/n1/pub/82-625-x/2015001/article/14185-eng.htm (accessed 8.13.18).
48. Grammaticos, P., Giannoula, E., Fountos, G.P., 2013. Acute radiation syndrome and chronic radiation syndrome. Hell J Nucl Med 16, 56–59.
49. Green, D.E., Rubin, C.T., 2014. Consequences of irradiation on bone and marrow phenotypes, and its relation to disruption of hematopoietic precursors. Bone 0, 87–94. https://doi.org/10.1016/j.bone.2014.02.018
50. Gu, H., Chen, C., Hao, X., Wang, Conghui, Zhang, X., Li, Z., Shao, H., Zeng, H., Yu, Z., Xie, L., Xia, F., Zhang, F., Liu, X., Zhang, Y., Jiang, H., Zhu, J., Wan, J., Wang, Chun, Weng, W., Xie, J., Tao, M., Zhang, C.C., Liu, J., Chen, G.-Q., Zheng, J., 2016. Sorting protein VPS33B regulates exosomal autocrine signaling to mediate hematopoiesis and leukemogenesis. J. Clin. Invest. 126, 4537–4553. https://doi.org/10.1172/JCI87105
51. Guescini, M., Canonico, B., Lucertini, F., Maggio, S., Annibalini, G., Barbieri, E., Luchetti, F., Papa, S., Stocchi, V., 2015. Muscle Releases Alpha-Sarcoglycan Positive Extracellular Vesicles Carrying miRNAs in the Bloodstream. PLOS ONE 10, e0125094. https://doi.org/10.1371/journal.pone.0125094
52. Halade, G.V., Rahman, M.M., Williams, P.J., Fernandes, G., 2010. High fat diet-induced animal model of age-associated obesity and osteoporosis. J. Nutr. Biochem. 21, 1162–1169. https://doi.org/10.1016/j.jnutbio.2009.10.002
53. Hardaway, A.L., Herroon, M.K., Rajagurubandara, E., Podgorski, I., 2014. Bone marrow fat: linking adipocyte-induced inflammation with skeletal metastases. Cancer Metastasis Rev. 33, 527–543. https://doi.org/10.1007/s10555-013-9484-y
54. Hell, R.C.R., Ocarino, N.M., Boeloni, J.N., Silva, J.F., Goes, A.M., Santos, R.L., Serakides, R., 2012. Physical activity improves age-related decline in the osteogenic potential of rats' bone marrow-derived mesenchymal stem cells. Acta Physiol (Oxf) 205, 292–301. https://doi.org/10.1111/j.1748-1716.2011.02397.x
55. Hu, L., Yin, C., Zhao, F., Ali, A., Ma, J., Qian, A., 2018. Mesenchymal Stem Cells: Cell Fate Decision to Osteoblast or Adipocyte and Application in Osteoporosis Treatment. Int J Mol Sci 19. https://doi.org/10.3390/ijms19020360
56. Hu, M., Lin, W., 2012. Effects of exercise training on red blood cell production: implications for anemia. Acta Haematol. 127, 156–164. https://doi.org/10.1159/000335620

57. Ilnytskyy, Y., Koturbash, I., Kovalchuk, O., 2009. Radiation-induced bystander effects in vivo are epigenetically regulated in a tissue-specific manner. Environ. Mol. Mutagen. 50, 105–113. https://doi.org/10.1002/em.20440
58. Ilnytskyy, Y., Zemp, F.J., Koturbash, I., Kovalchuk, O., 2008. Altered microRNA expression patterns in irradiated hematopoietic tissues suggest a sex-specific protective mechanism. Biochem. Biophys. Res. Commun. 377, 41–45. https://doi.org/10.1016/j.bbrc.2008.09.080
59. Kanhai, D.A., Visseren, F.L.J., van der Graaf, Y., Schoneveld, A.H., Catanzariti, L.M., Timmers, L., Kappelle, L.J., Uiterwaal, C.S.P.M., Lim, S.K., Sze, S.K., Pasterkamp, G., de Kleijn, D.P.V., 2013. Microvesicle protein levels are associated with increased risk for future vascular events and mortality in patients with clinically manifest vascular disease. International Journal of Cardiology 168, 2358–2363. https://doi.org/10.1016/j.ijcard.2013.01.231
60. Keating, A., 2006. Mesenchymal stromal cells. Curr. Opin. Hematol. 13, 419–425. https://doi.org/10.1097/01.moh.0000245697.54887.6f
61. Kim, H.-S., Choi, D.-Y., Yun, S.J., Choi, S.-M., Kang, J.W., Jung, J.W., Hwang, D., Kim, K.P., Kim, D.-W., 2012. Proteomic Analysis of Microvesicles Derived from Human Mesenchymal Stem Cells. J. Proteome Res. 11, 839–849. https://doi.org/10.1021/pr200682z
62. Kordelas, L., Rebmann, V., Ludwig, A.-K., Radtke, S., Ruesing, J., Doeppner, T.R., Epple, M., Horn, P.A., Beelen, D.W., Giebel, B., 2014. MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease. Leukemia 28, 970–973. https://doi.org/10.1038/leu.2014.41
63. Kranendonk, M.E., de Kleijn, D.P., Kalkhoven, E., Kanhai, D.A., Uiterwaal, C.S., van der Graaf, Y., Pasterkamp, G., Visseren, F.L., 2014. Extracellular vesicle markers in relation to obesity and metabolic complications in patients with manifest cardiovascular disease. Cardiovasc Diabetol 13, 37. https://doi.org/10.1186/1475-2840-13-37
64. Lai, R.C., Arslan, F., Lee, M.M., Sze, N.S.K., Choo, A., Chen, T.S., Salto-Tellez, M., Timmers, L., Lee, C.N., El Oakley, R.M., Pasterkamp, G., de Kleijn, D.P.V., Lim, S.K., 2010. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Res 4, 214–222. https://doi.org/10.1016/j.scr.2009.12.003
65. Lancaster, G.I., Febbraio, M.A., 2014. The immunomodulating role of exercise in metabolic disease. Trends Immunol. 35, 262–269. https://doi.org/10.1016/j.it.2014.02.008
66. Lange, C., Brunswig-Spickenheier, B., Cappallo-Obermann, H., Eggert, K., Gehling, U.M., Rudolph, C., Schlegelberger, B., Cornils, K., Zustin, J., Spiess, A.-N., Zander, A.R., 2011. Radiation Rescue: Mesenchymal Stromal Cells Protect from Lethal Irradiation. PLoS One 6. https://doi.org/10.1371/journal.pone.0014486
67. Laulagnier, K., Motta, C., Hamdi, S., Roy, S., Fauvelle, F., Pageaux, J.-F., Kobayashi, T., Salles, J.-P., Perret, B., Bonnerot, C., Record, M., 2004. Mast cell- and dendritic cell-derived exosomes display a specific lipid composition and an unusual membrane organization. Biochem J 380, 161–171. https://doi.org/10.1042/BJ20031594
68. Lavie, C.J., Church, T.S., Milani, R.V., Earnest, C.P., 2011. Impact of physical activity, cardiorespiratory fitness, and exercise training on markers of inflammation. J Cardiopulm Rehabil Prev 31, 137–145. https://doi.org/10.1097/HCR.0b013e3182122827
69. LaVoy, E.C.P., Fagundes, C.P., Dantzer, R., 2016. Exercise, inflammation, and fatigue in cancer survivors. Exerc Immunol Rev 22, 82–93.
70. Li, C., Lu, L., Zhang, J., Huang, S., Xing, Y., Zhao, M., Zhou, D., Li, D., Meng, A., 2015. Granulocyte colony-stimulating factor exacerbates hematopoietic stem cell injury after irradiation. Cell Biosci 5, 65. https://doi.org/10.1186/s13578-015-0057-3

71. Lorimore, S.A., Rastogi, S., Mukherjee, D., Coates, P.J., Wright, E.G., 2013. The Influence of p53 Functions on Radiation-Induced Inflammatory Bystander-Type Signaling in Murine Bone Marrow. Radiation Research 179, 406–415. https://doi.org/10.1667/RR3158.2
72. Macià i Garau, M., Lucas Calduch, A., López, E.C., 2011. Radiobiology of the acute radiation syndrome. Rep Pract Oncol Radiother 16, 123–130. https://doi.org/10.1016/j.rpor.2011.06.001
73. Marie, P.J., 2008. Transcription factors controlling osteoblastogenesis. Archives of Biochemistry and Biophysics, Highlight Issue: Bone Remodeling: Facts and Perspectives 473, 98–105. https://doi.org/10.1016/j.abb.2008.02.030
74. Martinez-Useros, J., Garcia-Foncillas, J., 2016. Obesity and colorectal cancer: molecular features of adipose tissue. J Transl Med 14. https://doi.org/10.1186/s12967-016-0772-5
75. Mauch, P., Constine, L., Greenberger, J., Knospe, W., Sullivan, J., Liesveld, J.L., Deeg, H.J., 1995. Hematopoietic stem cell compartment: acute and late effects of radiation therapy and chemotherapy. Int. J. Radiat. Oncol. Biol. Phys. 31, 1319–1339. https://doi.org/10.1016/0360-3016(94)00430-S
76. McGlory, C., Devries, M.C., Phillips, S.M., 2017. Skeletal muscle and resistance exercise training; the role of protein synthesis in recovery and remodeling. J. Appl. Physiol. 122, 541–548. https://doi.org/10.1152/japplphysiol.00613.2016
77. Méndez-Ferrer, S., Michurina, T.V., Ferraro, F., Mazloom, A.R., MacArthur, B.D., Lira, S.A., Scadden, D.T., Ma'ayan, A., Enikolopov, G.N., Frenette, P.S., 2010. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 466, 829–834. https://doi.org/10.1038/nature09262
78. Metcalf, D., 2007. Concise review: hematopoietic stem cells and tissue stem cells: current concepts and unanswered questions. Stem Cells 25, 2390–2395. https://doi.org/10.1634/stemcells.2007-0544
79. Morrison, S.J., Spradling, A.C., 2008. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell 132, 598–611. https://doi.org/10.1016/j.cell.2008.01.038
80. Nanji, A.A., Freeman, J.B., 1985. Relationship Between Body Weight and Total Leukocyte Count in Morbid Obesity. Am J Clin Pathol 84, 346–347. https://doi.org/10.1093/ajcp/84.3.346
81. Naveiras, O., Nardi, V., Wenzel, P.L., Hauschka, P.V., Fahey, F., Daley, G.Q., 2009. Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. Nature 460, 259–263. https://doi.org/10.1038/nature08099
82. Ng, J., Shuryak, I., 2014. Minimizing second cancer risk following radiotherapy: current perspectives. Cancer Manag Res 7, 1–11. https://doi.org/10.2147/CMAR.S47220
83. Nielsen, S., Åkerström, T., Rinnov, A., Yfanti, C., Scheele, C., Pedersen, B.K., Laye, M.J., 2014. The miRNA Plasma Signature in Response to Acute Aerobic Exercise and Endurance Training. PLOS ONE 9, e87308. https://doi.org/10.1371/journal.pone.0087308
84. Obesity and overweight [WWW Document], n.d. . World Health Organization. URL http://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed 8.13.18).
85. Ocarino, N.M., Boeloni, J.N., Goes, A.M., Silva, J.F., Marubayashi, U., Serakides, R., 2008. Osteogenic differentiation of mesenchymal stem cells from osteopenic rats subjected to physical activity with and without nitric oxide synthase inhibition. Nitric Oxide 19, 320–325. https://doi.org/10.1016/j.niox.2008.08.004
86. Oliveira, G.P., Porto, W.F., Palu, C.C., Pereira, L.M., Petriz, B., Almeida, J.A., Viana, J., Filho, N.N.A., Franco, O.L., Pereira, R.W., 2018. Effects of Acute Aerobic Exercise on Rats

Serum Extracellular Vesicles Diameter, Concentration and Small RNAs Content. Front Physiol 9. https://doi.org/10.3389/fphys.2018.00532
87. Pagnotti, G.M., Styner, M., 2016. Exercise Regulation of Marrow Adipose Tissue. Front Endocrinol (Lausanne) 7, 94. https://doi.org/10.3389/fendo.2016.00094
88. Park, J., Morley, T.S., Kim, M., Clegg, D.J., Scherer, P.E., 2014. Obesity and cancer--mechanisms underlying tumour progression and recurrence. Nat Rev Endocrinol 10, 455–465. https://doi.org/10.1038/nrendo.2014.94
89. Park, Y.-M., Myers, M., Vieira-Potter, V.J., 2014. Adipose tissue inflammation and metabolic dysfunction: role of exercise. Mo Med 111, 65–72.
90. Pellegrinelli, V., Rouault, C., Rodriguez-Cuenca, S., Albert, V., Edom-Vovard, F., Vidal-Puig, A., Clément, K., Butler-Browne, G.S., Lacasa, D., 2015. Human Adipocytes Induce Inflammation and Atrophy in Muscle Cells During Obesity. Diabetes 64, 3121–3134. https://doi.org/10.2337/db14-0796
91. Raposo, G., Stoorvogel, W., 2013. Extracellular vesicles: Exosomes, microvesicles, and friends. J Cell Biol 200, 373–383. https://doi.org/10.1083/jcb.201211138
92. Ratajczak, J., Miekus, K., Kucia, M., Zhang, J., Reca, R., Dvorak, P., Ratajczak, M.Z., 2006. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. Leukemia 20, 847–856. https://doi.org/10.1038/sj.leu.2404132
93. Rogers, L.Q., Fogleman, A., Trammell, R., Hopkins-Price, P., Vicari, S., Rao, K., Edson, B., Verhulst, S., Courneya, K.S., Hoelzer, K., 2013. Effects of a physical activity behavior change intervention on inflammation and related health outcomes in breast cancer survivors: pilot randomized trial. Integr Cancer Ther 12, 323–335. https://doi.org/10.1177/1534735412449687
94. Salvucci, O., Jiang, K., Gasperini, P., Maric, D., Zhu, J., Sakakibara, S., Espigol-Frigole, G., Wang, S., Tosato, G., 2012. MicroRNA126 contributes to granulocyte colony-stimulating factor-induced hematopoietic progenitor cell mobilization by reducing the expression of vascular cell adhesion molecule 1. Haematologica 97, 818–826. https://doi.org/10.3324/haematol.2011.056945
95. Sano, S., Izumi, Y., Yamaguchi, T., Yamazaki, T., Tanaka, M., Shiota, M., Osada-Oka, M., Nakamura, Y., Wei, M., Wanibuchi, H., Iwao, H., Yoshiyama, M., 2014. Lipid synthesis is promoted by hypoxic adipocyte-derived exosomes in 3T3-L1 cells. Biochem. Biophys. Res. Commun. 445, 327–333. https://doi.org/10.1016/j.bbrc.2014.01.183
96. Schofield, R., 1978. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells 4, 7–25.
97. Schwartz, J., Weiss, S.T., 1991. Host and environmental factors influencing the peripheral blood leukocyte count. Am. J. Epidemiol. 134, 1402–1409.
98. Shao, L., Luo, Y., Zhou, D., 2014. Hematopoietic Stem Cell Injury Induced by Ionizing Radiation. Antioxid Redox Signal 20, 1447–1462. https://doi.org/10.1089/ars.2013.5635
99. Shao, L., Wang, Y., Chang, J., Luo, Y., Meng, A., Zhou, D., 2013. Hematopoietic stem cell senescence and cancer therapy-induced long-term bone marrow injury. Transl Cancer Res 2, 397–411. https://doi.org/10.3978/j.issn.2218-676X.2013.07.03
100. Shen, W., Chen, J., Punyanitya, M., Shapses, S., Heshka, S., Heymsfield, S.B., 2007. MRI-measured bone marrow adipose tissue is inversely related to DXA-measured bone mineral in Caucasian women. Osteoporos Int 18, 641–647. https://doi.org/10.1007/s00198-006-0285-9
101. Shi, X.-F., Wang, H., Kong, F.-X., Xu, Q.-Q., Xiao, F.-J., Yang, Y.-F., Ge, R.-L., Wang, L.-S., 2017. Exosomal miR-486 regulates hypoxia-induced erythroid differentiation of

erythroleukemia cells through targeting Sirt1. Exp. Cell Res. 351, 74–81. https://doi.org/10.1016/j.yexcr.2016.12.023
102. Singh, V.K., Seed, T.M., 2017. A review of radiation countermeasures focusing on injury-specific medicinals and regulatory approval status: part I. Radiation sub-syndromes, animal models and FDA-approved countermeasures. International Journal of Radiation Biology 93, 851–869. https://doi.org/10.1080/09553002.2017.1332438
103. Sol Kim, D., Young Lee, S., Hee Lee, J., Chan Bae, Y., Sup Jung, J., 2015. MicroRNA-103a-3p controls proliferation and osteogenic differentiation of human adipose tissue-derived stromal cells. Exp Mol Med 47, e172. https://doi.org/10.1038/emm.2015.39
104. Styner, M., Pagnotti, G.M., Galior, K., Wu, X., Thompson, W.R., Uzer, G., Sen, B., Xie, Z., Horowitz, M.C., Styner, M.A., Rubin, C., Rubin, J., 2015. Exercise Regulation of Marrow Fat in the Setting of PPARγ Agonist Treatment in Female C57BL/6 Mice. Endocrinology 156, 2753–2761. https://doi.org/10.1210/en.2015-1213
105. Styner, M., Thompson, W.R., Galior, K., Uzer, G., Wu, X., Kadari, S., Case, N., Xie, Z., Sen, B., Romaine, A., Pagnotti, G.M., Rubin, C.T., Styner, M.A., Horowitz, M.C., Rubin, J., 2014. Bone marrow fat accumulation accelerated by high fat diet is suppressed by exercise. Bone 64, 39–46. https://doi.org/10.1016/j.bone.2014.03.044
106. Subra, C., Grand, D., Laulagnier, K., Stella, A., Lambeau, G., Paillasse, M., De Medina, P., Monsarrat, B., Perret, B., Silvente-Poirot, S., Poirot, M., Record, M., 2010. Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins. J. Lipid Res. 51, 2105–2120. https://doi.org/10.1194/jlr.M003657
107. Swift, D.L., Johannsen, N.M., Lavie, C.J., Earnest, C.P., Church, T.S., 2014. The Role of Exercise and Physical Activity in Weight Loss and Maintenance. Prog Cardiovasc Dis 56, 441–447. https://doi.org/10.1016/j.pcad.2013.09.012
108. Szatmári, T., Kis, D., Bogdándi, E.N., Benedek, A., Bright, S., Bowler, D., Persa, E., Kis, E., Balogh, A., Naszályi, L.N., Kadhim, M., Sáfrány, G., Lumniczky, K., 2017. Extracellular Vesicles Mediate Radiation-Induced Systemic Bystander Signals in the Bone Marrow and Spleen. Front Immunol 8, 347. https://doi.org/10.3389/fimmu.2017.00347
109. Taubert, M., Villringer, A., Lehmann, N., 2015. Endurance Exercise as an “Endogenous” Neuro-enhancement Strategy to Facilitate Motor Learning. Front Hum Neurosci 9, 692. https://doi.org/10.3389/fnhum.2015.00692
110. Togliatto, G., Dentelli, P., Gili, M., Gallo, S., Deregibus, C., Biglieri, E., Iavello, A., Santini, E., Rossi, C., Solini, A., Camussi, G., Brizzi, M.F., 2016. Obesity reduces the pro-angiogenic potential of adipose tissue stem cell-derived extracellular vesicles (EVs) by impairing miR-126 content: impact on clinical applications. International Journal of Obesity 40, 102–111. https://doi.org/10.1038/ijo.2015.123
111. Tomasoni, S., Longaretti, L., Rota, C., Morigi, M., Conti, S., Gotti, E., Capelli, C., Introna, M., Remuzzi, G., Benigni, A., 2013. Transfer of growth factor receptor mRNA via exosomes unravels the regenerative effect of mesenchymal stem cells. Stem Cells Dev. 22, 772–780. https://doi.org/10.1089/scd.2012.0266
112. Vainio, H., Kaaks, R., Bianchini, F., 2002. Weight control and physical activity in cancer prevention: international evaluation of the evidence. Eur. J. Cancer Prev. 11 Suppl 2, S94-100.
113. van den Berg, S.M., Seijkens, T.T.P., Kusters, P.J.H., Beckers, L., den Toom, M., Smeets, E., Levels, J., de Winther, M.P.J., Lutgens, E., 2016. Diet-induced obesity in mice diminishes hematopoietic stem and progenitor cells in the bone marrow. FASEB J. 30, 1779–1788. https://doi.org/10.1096/fj.201500175

114. Wang, Y., Schulte, B.A., LaRue, A.C., Ogawa, M., Zhou, D., 2006. Total body irradiation selectively induces murine hematopoietic stem cell senescence. Blood 107, 358–366. https://doi.org/10.1182/blood-2005-04-1418
115. Wardyn, G.G., Rennard, S.I., Brusnahan, S.K., McGuire, T.R., Carlson, M.L., Smith, L.M., McGranaghan, S., Sharp, J.G., 2008. Effects of exercise on hematological parameters, circulating side population cells, and cytokines. Experimental Hematology 36, 216–223. https://doi.org/10.1016/j.exphem.2007.10.003
116. Wen, S., Dooner, M., Cheng, Y., Papa, E., Del Tatto, M., Pereira, M., Deng, Y., Goldberg, L., Aliotta, J., Chatterjee, D., Stewart, C., Carpanetto, A., Collino, F., Bruno, S., Camussi, G., Quesenberry, P., 2016. Mesenchymal stromal cell-derived extracellular vesicles rescue radiation damage to murine marrow hematopoietic cells. Leukemia 30, 2221–2231. https://doi.org/10.1038/leu.2016.107
117. Wilhelm, E.N., Mourot, L., Rakobowchuk, M., 2018. Exercise-Derived Microvesicles: A Review of the Literature. Sports Med 1–15. https://doi.org/10.1007/s40279-018-0943-z
118. Williams, J.P., McBride, W.H., 2011. After the bomb drops: a new look at radiation-induced multiple organ dysfunction syndrome (MODS). Int. J. Radiat. Biol. 87, 851–868. https://doi.org/10.3109/09553002.2011.560996
119. Wren, T.A.L., Chung, S.A., Dorey, F.J., Bluml, S., Adams, G.B., Gilsanz, V., 2011. Bone marrow fat is inversely related to cortical bone in young and old subjects. J. Clin. Endocrinol. Metab. 96, 782–786. https://doi.org/10.1210/jc.2010-1922
120. Wubbolts, R., Leckie, R.S., Veenhuizen, P.T.M., Schwarzmann, G., Möbius, W., Hoernschemeyer, J., Slot, J.-W., Geuze, H.J., Stoorvogel, W., 2003. Proteomic and biochemical analyses of human B cell-derived exosomes. Potential implications for their function and multivesicular body formation. J. Biol. Chem. 278, 10963–10972. https://doi.org/10.1074/jbc.M207550200
121. Xu, J., Lombardi, G., Jiao, W., Banfi, G., 2016. Effects of Exercise on Bone Status in Female Subjects, from Young Girls to Postmenopausal Women: An Overview of Systematic Reviews and Meta-Analyses. Sports Med 46, 1165–1182. https://doi.org/10.1007/s40279-016-0494-0
122. Yáñez-Mó, M., Siljander, P.R.-M., Andreu, Z., Zavec, A.B., Borràs, F.E., Buzas, E.I., Buzas, K., Casal, E., Cappello, F., Carvalho, J., Colás, E., Cordeiro-da Silva, A., Fais, S., Falcon-Perez, J.M., Ghobrial, I.M., Giebel, B., Gimona, M., Graner, M., Gursel, I., Gursel, M., Heegaard, N.H.H., Hendrix, A., Kierulf, P., Kokubun, K., Kosanovic, M., Kralj-Iglic, V., Krämer-Albers, E.-M., Laitinen, S., Lässer, C., Lener, T., Ligeti, E., Linē, A., Lipps, G., Llorente, A., Lötvall, J., Manček-Keber, M., Marcilla, A., Mittelbrunn, M., Nazarenko, I., Nolte-'t Hoen, E.N.M., Nyman, T.A., O'Driscoll, L., Olivan, M., Oliveira, C., Pállinger, É., Del Portillo, H.A., Reventós, J., Rigau, M., Rohde, E., Sammar, M., Sánchez-Madrid, F., Santarém, N., Schallmoser, K., Ostenfeld, M.S., Stoorvogel, W., Stukelj, R., Van der Grein, S.G., Vasconcelos, M.H., Wauben, M.H.M., De Wever, O., 2015. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles 4, 27066.
123. Yeo, R.W., Lai, R.C., Zhang, B., Tan, S.S., Yin, Y., Teh, B.J., Lim, S.K., 2013. Mesenchymal stem cell: an efficient mass producer of exosomes for drug delivery. Adv Drug Deliv Rev 65, 336–341. https://doi.org/10.1016/j.addr.2012.07.001
124. Zaborowski, M.P., Balaj, L., Breakefield, X.O., Lai, C.P., 2015. Extracellular Vesicles: Composition, Biological Relevance, and Methods of Study. Bioscience 65, 783–797. https://doi.org/10.1093/biosci/biv084
125. Zaldivar, F., Wang-Rodriguez, J., Nemet, D., Schwindt, C., Galassetti, P., Mills, P.J., Wilson, L.D., Cooper, D.M., 2006. Constitutive pro- and anti-inflammatory cytokine and growth

factor response to exercise in leukocytes. Journal of Applied Physiology 100, 1124–1133. https://doi.org/10.1152/japplphysiol.00562.2005
126. Zhang, Y., Yu, M., Tian, W., 2016. Physiological and pathological impact of exosomes of adipose tissue. Cell Prolif. 49, 3–13. https://doi.org/10.1111/cpr.12233
127. Zheng, C., Liu, Z., 2015. Vascular function, insulin action, and exercise: an intricate interplay. Trends Endocrinol. Metab. 26, 297–304. https://doi.org/10.1016/j.tem.2015.02.002
128. Zhou, B.O., Yue, R., Murphy, M.M., Peyer, J., Morrison, S.J., 2014. Leptin Receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. Cell Stem Cell 15, 154–168. https://doi.org/10.1016/j.stem.2014.06.008

Chapter 2

Article

The influence of diet-induced obesity and exercise on bone marrow extracellular vesicles in an irradiated mouse model

Matthew Ngu[1], Paul Mensah[1], and Michael De Lisio[1,2]
[1]School of Human Kinetics, [2]Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON.

Running head: Radiation, exercise, and extracellular vesicles
Key words: Hematopoiesis, bone marrow, hematopoietic stem and progenitor cells, exosomes

Corresponding author information:
Dr. Michael De Lisio
University of Ottawa,
Roger Guindon Hall 2058
451 Smyth Road, Ottawa, ON
K1H 8L1
(613) 562-5800 (6987)
Email: mdelisio@uottawa.ca

Abstract

Background:

Radiation therapy is standard care for most patients with cancer; however, the damaging effects of radiation on the hematopoietic system are often dose-limiting. Recent work from our lab, in a preclinical model of radiation damage, indicates that endurance exercise training can enhance hematopoietic recovery, while obesity can impair it. Extracellular vesicles (EVs) have been implicated in regulating hematopoiesis acutely following radiation exposure; however, the long-term, radiation-induced changes to EVs, and the role of exercise and/or obesity at modulating marrow EVs remains unknown. Thus, the purpose of this project was to determine the extent to which obesity and exercise influence the regenerative potential of bone marrow-EVs following radiation.

Method:

Mice were randomly divided into control (n=20; CON) or high fat diet (n=20; HF) groups, then further subdivided into exercise-trained (EX, n=10) or sedentary (SED, n=10). Mice underwent whole-body exposure to a 3 Gy dose of gamma-radiation at age 13 weeks followed by bone marrow collection at 20 weeks of age. EVs were subsequently isolated from the bone marrow by ExoQuick and ultracentrifugation. A non-irradiated, sedentary, control group (n=10) was used to determine the effects of radiation alone. Data was evaluated using repeated-measures three-factor (diet, exercise, time) and two-factor ANOVA. Data was presented as mean ± SEM with $p<0.05$ considered significant.

Results:

Mice on the HF diet had significantly higher body mass and body fat compared to mice on the CON diet ($p<0.05$). Isolated EVs were between 78 and 195 nm and expressed the EV markers Alix, TSG101, and Flotillin by western blot confirming their identity. There was no size differences between groups. The concentration of EVs in irradiated mice was significantly lower compared to EVs from non-irradiated mice ($p<0.01$). Radiation, obesity, exercise, or their combination had no significant effect on HSPC ($Lineage^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}$) content in co-

culture assays. Conversely, EVs from irradiated mice significantly increased the number of CFU-GEMM, CFU-G, and the TOTAL number of colonies compared to EVs from non-irradiated mice ($p<0.01$). However, EVs from the CON+SED, CON+EX, HF+SED, and HF+EX groups did not have a significant effect on colony formation.

Conclusion:

Our findings demonstrate that irradiation can deplete the concentration of bone marrow-EVs and that irradiated bone marrow-EVs can increase the total number of myeloid colonies *in vitro*. These findings suggest that radiation induces myelopoiesis via a mechanism that includes EVs; however, exercise and obesity induce their effects via a different mechanism.

Introduction

The number of long-term cancer survivors continues to increase; however, long-term, late effects of cancer therapy reduce healthspan and increase the risk of secondary malignancy. Radiation therapy is an effective cancer therapy used on 2/3 of cancer patients (Chen and Kuo, 2017). However, radiation induces acute and persistent damage to hematopoietic stem/progenitor cells (HSPCs), which can result in long-term immunosuppression and the development of radiation-induced hematological cancers (Shao et al., 2014). Confounding the effects of therapy are the increase in the number of cancer survivors who are obese and physically inactive (Courneya et al., 2008). Obesity increases the cycling and myeloid differentiation of HSPCs (Emmons et al., 2017), which increases the risk of hematological malignancies (Lichtman, 2010) and contributes to immunosenescence via premature exhaustion of the HSPC pool (Salvestrini et al., 2019). As such, the increasing number of cancer survivors with obesity creates a critical need to develop a better understanding of how obesity influences the late effects of radiation therapy in the bone marrow, and to develop effective countermeasures.

Recent evidence from our lab suggests that exercise training can mitigate several of the detrimental effects of obesity on HSPCs following radiation exposure (Emmons et al., 2019). These effects were coincident with the preservation of the HSPC niche and increased expression of hematopoietic growth factors in exercise-trained mice (Emmons et al., 2019). However, a complete investigation of exercise-responsive paracrine factors in the bone marrow in irradiated mice has not been undertaken.

EVs, are membrane-bound particles containing proteins, lipids, and miRNA, which are released by cells and are involved in local and distant intercellular communication (Aqmasheh et al., 2017; Butler et al., 2018). EVs have been shown to play a role in controlling hematopoiesis and HSPC maintenance (Chou et al., 2013; Battiwalla and Barrett, 2014; Butler et al., 2018), and *in vivo* and *in vitro r*adiation exposure alters EV cargo (Lehmann et al., 2008; Arscott et al., 2013; Jella et al., 2014). Szatmári (2017) showed that injecting irradiated EVs into non-irradiated mice induced radiation damage that was comparable to direct radiation and

caused a significant reduction in HSPC content (Szatmári et al., 2017). Conversely, Wen and colleagues demonstrated that EVs from non-irradiated mesenchymal stem cells could reverse the damaging effects of radiation on bone marrow stem cells (Wen et al., 2016). Combined, these data suggest that EVs can both transmit and reverse the harmful effects of radiation. Interestingly, in genetic and diet-induced models of obesity, EV content is increased in peripheral blood (Eguchi et al., 2015; Heinrich et al., 2015; Eguchi et al., 2016) similar the effects of acute radiation. Further, EVs derived from obese mice induce increased production of the pro-inflammatory cytokines IL-6 and TNF-α (Deng et al., 2009), while the miRNA profiles of visceral *in vitro* cultured adipose tissue-derived EVs from individuals with obesity contain cargo that is predicted to regulate key inflammatory and fibrotic signalling pathways (Ferrante et al., 2015). These data suggest that obesity may exacerbate the inflammatory effects of radiation exposure, in part via increased concentration and altered cargo of EVs. An acute bout of exercise has been shown to increase EV content in circulation (Frühbeis et al., 2015; Guescini et al., 2015; Bei et al., 2017), and alter EV cargo in a manner that is expected to support erythropoiesis (Aoi et al., 2013). It remains unknown; however, whether exercise-induced EVs directly play a role in hematopoiesis.

Thus, the purpose of this project is to functionally characterize bone marrow-EVs from obese and exercise-trained mice following radiation. By identifying these EVs, we thus can understand, how exercise acts via novel mediators to improve hematopoietic recovery. We hypothesize that bone marrow-EVs from exercise-trained mice will increase HSPC expansion and inhibit obesity-induced myeloid skewing.

Methods

Ethical Approval

Ethical approval was obtained from the University of Ottawa Animal Care Committee.

Mice

A total of 50 male CBA (Jackson Laboratories, Maine, United States) mice were maintained in a 12:12h light-dark schedule with food and water provided *ad libitum* until age 20 weeks.

Diet

At 5 weeks of age, mice were randomly divided into control (n=20; CON) or high-fat diet (n=20; HF) groups. The control diet consisted of 10% kcal fat (D10012M, Research Diets, New Jersey, United States), while the HF diet was composed of 45% kcal from fat (D12451, Research Diets, New Jersey, United States) for 8 weeks to induce obesity as we have previously described (Emmons et al., 2019). Mice were maintained on their respective diets until sacrifice. Body weight and food intake were measured weekly, and fat and lean mass were measured once a month using EchoMRI-900 (EchoMRI LLC, Texas, United States).

Progressive endurance exercise training program

We used a progressive endurance exercise treadmill program that had been previously observed to expand the HSPC pool and decrease MAT (Baker et al., 2011; De Lisio and Parise, 2012; Emmons et al., 2019). At age 9 weeks, mice from CON and HF were sub-divided into exercise (EX, n=10) or sedentary (SED, n=10,) groups. EX mice were exercised 3 days per week for 11 weeks on a motorized treadmill (Exer 3/6 Columbus Instruments, Ohio, United States). Each training session consisted of: (1) A 10-minute warm-up at a speed which began at 8 m/minutes, and increased to 10 m/minutes after 4 weeks, (2) A training period that progressively increased in duration, and speed from 10 m/minutes for 25 minutes to a maximum of 16 m/minutes for 45 minutes , and (3) a 5-minute cool down at a speed of 8 m/min. Exercise sessions occurred at the same time of day, and mice were encouraged to run by stimulation with bristles of a paintbrush as we have found this method to be more effective and humane than electric shock. Non-exercised mice were exposed to treadmill noise, placed in a mock treadmill, and manually manipulated in the same manner/time as exercise-trained mice to control for any stress associated with treadmill exercise. One

CON+SED mouse was removed from the protocol, as it suffered an injury during the 8th week of exercise. Mice continued in their intervention groups after radiation exposure, and until sacrifice.

Total body Irradiation

At 13 weeks of age, all mice underwent whole-body exposure to a 3 Gy dose of gamma-radiation. Radiation was administered as previously described with minor modifications (De Lisio et al., 2013, 2011; De Lisio and Parise, 2012; Emmons et al., 2019). A uniform dose distribution across the radiation field was applied using the X-Rad 320 biological irradiator (Precision X-Ray, Connecticut, United States). Following total body irradiation, mice were provided antibiotic water until sacrifice. A non-irradiated group was also included in our study. This cohort arrived in the facility at age 10 weeks and was maintained on the control diet until sacrifice.

Euthanasia, and bone marrow supernatant collection

At 20 weeks of age, mice were sacrificed by CO_2 asphyxiation, followed by cervical dislocation. Both femurs and tibiae were excised and freed of muscle and fat. Femurs and tibiae were cut at each end, and bones were subsequently flushed with ice-cold phosphate-buffered saline. Flushed marrow was centrifuged at 400g at 4°C for 5 minutes to separate the cellular fraction. The supernatant from the flushed bone samples was collected, snap-frozen, and stored at -80°C.

Isolation of extracellular vesicles

EVs were isolated from the bone marrow supernatant using the ExoQuick-TC® ULTRA for Tissue Culture Media (System Biosciences, California, United States) according to the manufacturer's protocol, with minor modifications. The supernatant was initially centrifuged at 3,000g for 15 minutes at 4°C to remove cellular debris. The supernatant was then added to 200 µL of ExoQuick-TC solution and incubated overnight on ice at 4°C. After incubation, the solution was centrifugation at 3,000g for 10 minutes at 4°C. The EV pellet was then resuspended in 200 µl of Buffer B and stored at -80°C until future application.

Additionally, EVs were isolated from the bone marrow supernatant using differential ultracentrifugation based on the protocol adapted from Lässer (2014). Bone marrow supernatant was thawed and centrifuged at 2,000g for 20 minutes at 4°C. The supernatant was then transferred to a new tube and centrifuged for 40 minutes at 15,000g at 4°C. The supernatant was then spun for 90 minutes at 100,000g at 4°C. The pellet was then resuspended with 100 µL of sterile 1XPBS. The supernatant from the final spin was also saved. Exosome depleted FBS was prepared through ultracentrifugation at 100,000g for 18 hours.

Western blot

EVs were lysed in RIPA lysis buffer containing protease inhibitor (Roche, Basel, Switzerland), and protein concentration was evaluated using the Bradford protein assay kit (Thermo Fisher Scientific, Massachusetts, United States). Equal amounts of protein were loaded, and electrophoresed on a 10% sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel, and transferred to PVDF membranes (Bio-Rad, California, United States). Murine bone marrow cell lysates were used as a control. Primary antibodies against rabbit anti-Alix (Sigma-Aldrich, Missouri, United States), rabbit anti-flotillin-2 (Cell Signaling, Massachusetts, United States), and rabbit anti-Tsg101 (Cell Signaling, Massachusetts, United States) were used at 1:1000 in 5% milk. Membranes were incubated at room temperature for 30 minutes, followed by a 15-minute incubation with mouse anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:1000, Cell Signaling, Massachusetts, United States). Membranes were incubated with the “Pierce™ ECL Western Blotting Substrate” kit (Thermo Fisher Scientific, Massachusetts, United States), and proteins were visualized using the ChemiDoc Imaging System (Bio-Rad, California, United States).

Nanoparticle-tracking analysis

Isolated EVs were applied to the ZetaView (Particle Metrix GmbH, Meerbusch, Germany), which identifies the number and size distribution of vesicles via light-scattering. Isolated EVs from the experimental groups were thawed and diluted in PBS, specifically at

1:250 or 1:500. These dilutions generated concentrations of 10^6–10^9 particle/ml, which is the working range of the instrument. EV mean size and concentration were analysed using the ZetaView software (version 8.02.28, Meerbusch, Germany) using 11 camera positions, a 2-second video length, and a camera frame rate of 30 fps at 21°C.

Hematopoietic Stem/Progenitor Cell Isolation

Bone marrow-HSPC were isolated from 9 weeks old, male, C57BL/6 mice. Mouse euthanasia and bone marrow cell fraction were isolated as described above. Marrow cells were incubated with the following antibodies: anti–Sca-1 (1:200, Biosciences, California, United States), anti–c-kit (1:200 Biosciences, California, United States) and lineage cocktail (1:200) (Ter119, Mac1, Gr1, B220, CD4, and CD8 Biosciences, California, United States). Viability was determined via Zombie Yellow Viability dye (1:200, BioLegend, California, United States). Cell samples were then incubated on ice for one hour and resuspended with Streptavidin-FITC (1:800 Biosciences, California, United States) for one hour on ice. HSPC were sorted using the BeckmanCoulter MoFlo XDP (California, United States) into Iscove's Modified Dulbecco's Medium (IMDM). Compensation and gating strategies were derived from unstained and single stained controls.

Coculture Assay

1500 bone marrow-HSPCs were cultured per well in a 48-well plate for 48 hours at 37°C and 5% CO_2 in StemSpan SFEM Medium (Stemcell Technologies, Vancouver Canada) supplemented with 15% vesicle depleted FBS, 1% penicillin/streptomycin, Ms Recom SCF (Stemcell Technologies, Vancouver Canada), 100 ng/mL, Ms Recom TPO (Stemcell Technologies, Vancouver Canada), 100 ng/mL and Ms Recom Flt3/Flk-2 Ligand (Stemcell Technologies, Vancouver Canada), 100 ng/mL. 100 µL of either the EVs isolated from the ExoQuick kit, ultracentrifugation, the supernatant from ultracentrifugation or non-irradiated EVs were supplemented to the media for the coculture assays. Control cultures were HSPCs cultured without EV supplementation with either 15% non-vesicle depleted FBS supplemented growth media or with 15% vesicle depleted FBS.

Hematopoietic stem/progenitor cell quantification

After a 48 hour coculture, cells were retrieved from the culture plate and were prepared for flow cytometry analysis. Cells were incubated with the following antibodies: anti–Sca-1 (1:200, Biosciences, California, United States), anti–c-kit (1:200 Biosciences, California, United States) and lineage cocktail (1:200) (Ter119, Mac1, Gr1, B220, CD4, and CD8 Biosciences, California, United States). Viability was determined via Zombie Yellow Viability dye (1:200, BioLegend, California, United States). Cell samples were then incubated on ice for one hour and resuspended with Streptavidin-FITC (1:800 Biosciences, California, United States) for one hour on ice. HSPCs were quantified by using the Attune NxT Flow Cytometer (Life Technologies, California, United States). Unstained and fluorescence minus one were used for compensation and gating. Investigators were blinded to groups during quantification.

Colony-forming assay

After the 48 hour coculture with EVs, cells in suspension were retrieved from the culture plate, and cells were counted using the Countess (Thermofisher, Massachusetts, United States. 1000 cells were suspended in MethoCult™ GF M3434 (Stemcell Technologies, Vancouver Canada) medium then plated in duplicate for colony-forming unit assays. After 14 days of culture, colonies were examined and imaged using the EVOS XL Core Configured Microscope (Thermofisher, Massachusetts, United States). Colonies were counted and labelled according to the MethoCult™ GF M3434 manual. Investigators were blinded to groups during quantification.

EV Uptake

EVs were labelled using the PKH67 Green Fluorescent Cell Linker Kit for General Cell Membrane Labeling (Sigma-Aldrich, Missouri, United States) according to the manufacturer's instructions, with minor adjustments. Labelled EVs were combined with sorted HSPCs and uptake was visualized using the Celldiscoverer7 (ZEISS, Oberkochen, Germany) with images taken within the first hour of incubation.

Statistical analysis

Body weight, composition, food intake, and particle size range were analyzed via a repeated-measures three-factor (diet, exercise, time) ANOVA. Comparison of cell quantities in irradiated against non-irradiated EVs was analyzed by an unpaired t-test. All other data were evaluated by two-factor ANOVA and Tukey's post-hoc test using GraphPad Prism 8 (GraphPad Software). Data were presented as mean ± SEM with $p<0.05$ considered significant.

Results

High fat diet-induced changes in body weight and composition and altered food consumption

A schematic of the study is presented in Figure 1a. Body fat percentage was significantly increased in both HF groups compared to both CON groups from age eight to 17 weeks with no effect of exercise ($p<0.05$, Figure 1b). Lean body mass of CON+SED mice at age nine weeks was significantly less than both HF groups, and lean body mass of CON+EX mice at age 13 weeks was significantly less than both HF groups ($p<0.05$, Figure 1c). From age eight weeks old until age 20 weeks, CON+EX and CON+SED mice weighed significantly less compared to mice that were HF+SED or HF+EX, respectively ($p<0.05$, Figure 1d). Food intake and caloric consumption were significantly increased in the HF group compared to CON ($p<0.0001$ diet main effect, Figure 1e, and f).

Characterization of bone marrow extracellular vesicles

Bone marrow-EVs isolated by ultracentrifugation (UC) and ExoQuick (EQ) expressed Alix, Flotillin, and TSG101, which are three markers characteristic of EVs (Figure 2a). There was no significant difference in the mean size of EVs between irradiated and non-irradiated mice **(**Figure 2b). However, the concentration of bone marrow-EVs was significantly lower from irradiated mice (Figure 2c, $P<0.001$). Neither the mean size (Figure 2d) nor quantity (Figure 2e) of EVs was different between diet or exercise conditions. EVs from the bone marrow of irradiated mice under the diet and exercise conditions ranged in size from 78 to 195

nm, which corresponds to the expected sizes for EVs (Figure 2f). We confirmed that bone marrow-EVs could be taken up by HSPCs, which was clearly shown by green dye within HSPCs (Figure 2g).

Irradiated bone marrow-EVs did not negatively impact immature hematopoietic cells or HSPCs *in vitro*

Bone marrow-EVs from irradiated and non-irradiated mice were cocultured with HSPCs for 48 hours, then immature hematopoietic cells (Lin^-) (Figure 3a) and HSPCs (Figure 3b) were quantified by flow cytometry. There was no significant difference in the quantity of Lin^- cells or HSPCs when treated with EVs from irradiated mice compared to non-irradiated EVs.

EVs from obese and or exercise-trained mice do not alter the content of immature hematopoietic cells or HSPCs *in vitro*

Bone marrow-EVs isolated using the ExoQuick-TC kit (EQ), ultracentrifugation (UC) protocol, and supernatant (SU) from the final spin from the ultracentrifugation protocol; from the four experimental groups were cocultured with HSPCs for 48 hours. The number of Lin^- cells (Figure 4a) and HSPCs (Figure 4b) was not affected by bone marrow EQ-EVs from the exercise or diet intervention. The number of Lin^- cells were not significantly affected by U-EVs from the exercise or diet intervention (Figure 4c); however, the number of HSPCs demonstrated a trend for a significance (P=0.064) when cultured with U-EVs from exercise-trained mice with no effect of diet (Figure 4d). Lastly, the number of Lin^- (Figure 4e) and HSPCs (Figure 4f) was not affected by bone marrow SU.

Irradiated bone marrow-EVs promote myeloid lineage colony formation

To determine if irradiated bone marrow-EVs altered HSPC colony formation compared to non-irradiated EVs, bone marrow-EVs from irradiated and non-irradiated mice were cocultured with HSPCs for 48 hours, then cells were subsequently used for colony-forming unit assays. Colony-forming unit-granulocyte, erythrocyte, macrophage, megakaryocyte (CFU-GEMM) colonies were significantly increased when cultured with irradiated EVs

compared to non-irradiated EVs ($P<0.01$; Figure 5a). CFU-granulocyte, macrophage (CFU-GM) were not altered by irradiated EVs (Figure 5b). There was a significant ($P<0.05$) increase in the number of CFU-granulocyte (CFU-G) colonies, with exposure to irradiated bone marrow-EVs (Figure 5c). CFU-macrophage (CFU-M) was also not altered by irradiated EVs (Figure 5d). While the number of burst-forming unit-erythroid (BFU-E) colonies displayed a trend for a significance ($P=0.17$) in the radiated group (Figure 5e). Irradiated EVs also significantly ($P<0.05$) increased the number of CFU-TOTAL (CFU-T) colonies compared to non-irradiated EVs (Figure 5f).

No difference in hematopoietic progenitor colony-forming units by exposure to bone marrow-EVs from obese and/or exercise-trained mice

HSPCs were initially cocultured with EVs from the four experimental groups for 48 hours then used for CFU assays as described above. There was no difference in colony formation between HSPCs co-cultured with bone marrow-EVs derived from the four different groups (Figure 6a-f).

Discussion

Our overall objective was to determine the extent to which long-term changes in hematopoietic regulation of bone marrow-EVs are induced by radiation, and whether obesity- and/or exercise-induced alterations in bone marrow-EVs contribute to alterations in hematopoiesis following radiation. Our results demonstrate that radiation decreased the concentration of bone marrow-EVs, and bone marrow-EVs from irradiated mice increase myeloid lineage colony formation *in vitro*; however, the effects of radiation are not altered by exercise, obesity, or their combination. Together, these data suggest that irradiation increases bone marrow-EVs involved in myelopoiesis, and that mechanisms not related to EVs explain the previously identified differential effects of exercise and obesity on hematopoiesis (Emmons et al., 2019).

Ionizing radiation has been shown to severely damage HSCs, and impair their ability to self-renew, and differentiate (Macià i Garau et al., 2011; Singh and Seed, 2017). Radiation

exposure has also been demonstrated to significantly influence intercellular communication (Jelonek et al., 2016). Irradiated bone marrow-EVs in the context of exercise and diet have yet to be characterized. In the present study, particles were first characterized through the presence of cytosolic proteins: TSG101, Alix and Flotillin; which were all found to be present in ExoQuick and ultracentrifugation isolated EVs. The mean size and range of measured particles fell within the range in which EVs are known to exist (Théry et al., 2018). EVs were also fluorescently labelled and were observed to be taken up by HSPCs. Thus, our results demonstrate the successful isolation of EVs from murine bone marrow through validation by western blot, nanoparticle tracking analysis (NTA), and lipid staining and uptake (Stik et al., 2017; Szatmári et al., 2017; Théry et al., 2018). Interestingly the concentration of irradiated bone marrow-EVs was significantly less compared to non-irradiated bone marrow-EVs. Previous studies have shown that irradiation can increase EV release from cancer and normal cell lines *in vitro* (Lehmann et al., 2008; Arscott et al., 2013; Jella et al., 2014; Al-Mayah et al., 2015). However, these studies measured EV content from culture shortly after or within several days post-radiation. These studies also applied radiation at a higher dosage and EVs were produced from cells *in vitro* and from non-bone marrow cells and tissues. These differences may explain the discrepancies.

Both exercise training and obesity have been shown to confer an effect on hematopoiesis after radiation exposure. Emmons *et al.* used a proteomics approach to investigate the effects of exercise training and obesity on changes to bone marrow cytokines following radiation. Those cytokine data demonstrated that a progressive treadmill exercise program induces a prohematopoietic, anti-inflammatory environment within the bone marrow after radiation exposure, whereas obesity is associated with higher levels of factors that inhibit hematopoiesis (Emmons et al., 2019). Barone and colleagues have demonstrated an increase in EVs in the peripheral blood after a bout of acute exercise (Barone et al., 2016). Similarly, after three weeks of swimming, mice showed increased levels of EVs in the peripheral blood and were more resistant against cardiac ischemia/reperfusion injury (Bei et al., 2017). Exercise is hypothesized to stimulated EV release from platelets, endothelial cells, and

leukocytes and confer benefits to distal organs and resident tissues, including the bone marrow (Bei et al., 2017; Szatmári et al., 2017). However, the effects of exercise and diet after exposure to radiation has not been studied in the context of bone marrow-EVs to date. The effects of radiation on bone marrow-EVs have previously been documented by *in vivo* work by Szatmári and colleagues. Bone marrow-EVs injected into non-irradiated mice significantly depleted the number of HSPCs with no effect seen in either lymphoid or myeloid progenitor populations (Szatmári et al., 2017). To determine if the effects of irradiated EVs are direct on HSPCs or exert their effects indirectly, we used an *in vitro* approach where irradiated EVs were directly applied to isolated HSPCs. Using this reductionist approach, we determined that exposure to irradiated EVs did not alter the number of HSPCs, suggesting that the *in vivo* effects of irradiated EVs may be due to indirect effects on other cells in the HSPC niche.

Wen and colleagues showed that co-culture of irradiated whole bone marrow cells with murine MSC-EVs led to a significant expansion of bone marrow cells and promoted colony formation of irradiated bone marrow cells (Wen et al., 2016). Our results demonstrated that irradiated EVs promote the expansion of CFU-GEMM (common myeloid progenitors), which are primitive blood-forming cells and can differentiate into macrophages, neutrophils, and platelets. The number of CFU-G (granulocyte progenitor) also significantly increased when exposed to irradiated bone marrow-EVs. CFU-G differentiate into neutrophils, and preclinical studies have proposed that neutrophils can have both pro- and anti-tumor effects (Wisdom et al., 2019). Previous *in vivo* work indicated that injecting irradiated EVs into non-irradiated mice did not have an effect on the number of lymphoid, myeloid, megakaryocyte, and erythroid progenitors in the bone marrow (Szatmári et al., 2017). One reason we may have seen myeloid skewing in our study and Szatmári and colleagues did not, could be because of the dose of radiation. Szatmári and colleagues applied 2 Gy of radiation to mice compared to our 3 Gy. Changes to EV activity after radiation exposure could be dose-dependent. Another potential reason Szatmári and colleagues did not see a change, could be because their focus on hematopoietic progenitor cells 24 hours after irradiation. Compared to our work, where we waited 48 hours after co-culture. It is possible that there is a delay in the EVs ability to convey

its effects on marrow cells. Our findings suggest that radiation may be priming bone marrow-EVs in a manner that allows the expansion of these myeloid progenitors. In the disease state, EVs could play a part in maintaining a pathogenic hematopoietic niche. Particularly, by maintaining cells which produce pro-inflammatory cytokines and growth factors, as an environment rich in inflammatory cells and growth factors, certainly potentiates and/or promotes neoplastic risk (Coussens and Werb, 2002).

Emmons *et al.* demonstrated that there were significantly more common lymphoid progenitors (CLP) in exercise-trained mice and there were significantly fewer CLP in obese mice (Emmons et al., 2019). While there was no difference in the number of common myeloid (CMP) progenitors in either diet or exercise condition (Emmons et al., 2019). Our data also demonstrated that neither exercise nor diet had a significant effect on myeloid colony differentiation. This result is in line with what Emmons *et al.* demonstrated with CMP populations from irradiated bone marrow (Emmons et al., 2019)

In conclusion, our results demonstrate that bone marrow-EVs derived from exercise-trained mice do not increase the number of HSPC *in vitro*. Irradiated bone marrow-EVs increase the population of myeloid lineage colonies *in vitro*, which is not reversed by exercised or exacerbated by HF diet. Thus, the effects of irradiated EVs from exercised or HF mice on HSPCs are conferred via alternative mechanisms. Our previous work demonstrated that exercise training partially restores the negative effects of obesity on HSPC and their niche after radiation exposure (Emmons et al., 2019). The present research shows that this effect is not due to EVs, but could be due to changes to cell content, or growth factors or cytokines.

References

Al-Mayah, A., Bright, S., Chapman, K., Irons, S., Luo, P., Carter, D., Goodwin, E., Kadhim, M., 2015. The non-targeted effects of radiation are perpetuated by exosomes. Mutat. Res. 772, 38–45. https://doi.org/10.1016/j.mrfmmm.2014.12.007

Alvarez, M.L., Khosroheidari, M., Kanchi Ravi, R., DiStefano, J.K., 2012. Comparison of protein, microRNA, and mRNA yields using different methods of urinary exosome isolation for the discovery of kidney disease biomarkers. Kidney Int. 82, 1024–1032. https://doi.org/10.1038/ki.2012.256

Aoi, W., Ichikawa, H., Mune, K., Tanimura, Y., Mizushima, K., Naito, Y., Yoshikawa, T., 2013. Muscle-enriched microRNA miR-486 decreases in circulation in response to exercise in young men. Front Physiol 4, 80. https://doi.org/10.3389/fphys.2013.00080

Aqmasheh, S., Shamsasanjan, karim, Akbarzadehlaleh, P., Pashoutan Sarvar, D., Timari, H., 2017. Effects of Mesenchymal Stem Cell Derivatives on Hematopoiesis and Hematopoietic Stem Cells. Adv Pharm Bull 7, 165–177. https://doi.org/10.15171/apb.2017.021

Arscott, W.T., Tandle, A.T., Zhao, S., Shabason, J.E., Gordon, I.K., Schlaff, C.D., Zhang, G., Tofilon, P.J., Camphausen, K.A., 2013. Ionizing radiation and glioblastoma exosomes: implications in tumor biology and cell migration. Transl Oncol 6, 638–648. https://doi.org/10.1593/tlo.13640

Baker, J.M., De Lisio, M., Parise, G., 2011. Endurance exercise training promotes medullary hematopoiesis. FASEB J. 25, 4348–4357. https://doi.org/10.1096/fj.11-189043

Barone, R., Macaluso, F., Sangiorgi, C., Campanella, C., Marino Gammazza, A., Moresi, V., Coletti, D., Conway de Macario, E., Macario, A.J., Cappello, F., Adamo, S., Farina, F., Zummo, G., Di Felice, V., 2016. Skeletal muscle Heat shock protein 60 increases after endurance training and induces peroxisome proliferator-activated receptor gamma coactivator 1 α1 expression. Sci Rep 6. https://doi.org/10.1038/srep19781

Battiwalla, M., Barrett, A.J., 2014. Bone marrow mesenchymal stromal cells to treat complications following allogeneic stem cell transplantation. Tissue Eng Part B Rev 20, 211–217. https://doi.org/10.1089/ten.TEB.2013.0566

Bei, Y., Xu, T., Lv, D., Yu, P., Xu, J., Che, L., Das, A., Tigges, J., Toxavidis, V., Ghiran, I., Shah, R., Li, Y., Zhang, Y., Das, S., Xiao, J., 2017. Exercise-induced circulating extracellular vesicles protect against cardiac ischemia-reperfusion injury. Basic Res Cardiol 112, 38. https://doi.org/10.1007/s00395-017-0628-z

Bryant, A.K., Banegas, M.P., Martinez, M.E., Mell, L.K., Murphy, J.D., 2017. Trends in Radiation Therapy among Cancer Survivors in the United States, 2000–2030. Cancer Epidemiol Biomarkers Prev 26, 963–970. https://doi.org/10.1158/1055-9965.EPI-16-1023

Butler, J.T., Abdelhamed, S., Kurre, P., 2018. Extracellular vesicles in the hematopoietic microenvironment. Haematologica 103, 382–394. https://doi.org/10.3324/haematol.2017.183335

Chen, H.H.W., Kuo, M.T., 2017. Improving radiotherapy in cancer treatment: Promises and challenges. Oncotarget 8, 62742–62758. https://doi.org/10.18632/oncotarget.18409

Chou, S.-H., Lin, S.-Z., Day, C.H., Kuo, W.-W., Shen, C.-Y., Hsieh, D.J.-Y., Lin, J.-Y., Tsai, F.-J., Tsai, C.-H., Huang, C.-Y., 2013. Mesenchymal stem cell insights: prospects in hematological transplantation. Cell Transplant 22, 711–721. https://doi.org/10.3727/096368912X655172

Courneya, K.S., Katzmarzyk, P.T., Bacon, E., 2008. Physical activity and obesity in Canadian cancer survivors: population-based estimates from the 2005 Canadian Community Health Survey. Cancer 112, 2475–2482. https://doi.org/10.1002/cncr.23455

Coussens, L.M., Werb, Z., 2002. Inflammation and cancer. Nature 420, 860–867. https://doi.org/10.1038/nature01322

De Lisio, M., Baker, J.M., Parise, G., 2013. Exercise promotes bone marrow cell survival and recipient reconstitution post-bone marrow transplantation, which is associated with increased survival. Exp. Hematol. 41, 143–154. https://doi.org/10.1016/j.exphem.2012.10.003
De Lisio, M., Parise, G., 2012. Characterization of the effects of exercise training on hematopoietic stem cell quantity and function. J. Appl. Physiol. 113, 1576–1584. https://doi.org/10.1152/japplphysiol.00717.2012
De Lisio, M., Phan, N., Boreham, D.R., Parise, G., 2011. Exercise-induced protection of bone marrow cells following exposure to radiation. Appl Physiol Nutr Metab 36, 80–87. https://doi.org/10.1139/H10-087
Deng, Z., Poliakov, A., Hardy, R.W., Clements, R., Liu, C., Liu, Y., Wang, J., Xiang, X., Zhang, S., Zhuang, X., Shah, S.V., Sun, D., Michalek, S., Grizzle, W.E., Garvey, T., Mobley, J., Zhang, H.-G., 2009. Adipose tissue exosome-like vesicles mediate activation of macrophage-induced insulin resistance. Diabetes 58, 2498–2505. https://doi.org/10.2337/db09-0216
Eguchi, A., Lazic, M., Armando, A.M., Phillips, S.A., Katebian, R., Maraka, S., Quehenberger, O., Sears, D.D., Feldstein, A.E., 2016. Circulating adipocyte-derived extracellular vesicles are novel markers of metabolic stress. J Mol Med 94, 1241–1253. https://doi.org/10.1007/s00109-016-1446-8
Eguchi, A., Mulya, A., Lazic, M., Radhakrishnan, D., Berk, M.P., Povero, D., Gornicka, A., Feldstein, A.E., 2015. Microparticles Release by Adipocytes Act as “Find-Me” Signals to Promote Macrophage Migration. PLOS ONE 10, e0123110. https://doi.org/10.1371/journal.pone.0123110
Elsherbini, A., Bieberich, E., 2018. Ceramide and exosomes: a novel target in cancer biology and therapy. Adv Cancer Res 140, 121–154. https://doi.org/10.1016/bs.acr.2018.05.004
Emmons, R., Ngu, M., Xu, G., Hernández-Saavedra, D., Chen, H., De Lisio, M., 2019. Effects of Obesity and Exercise on Bone Marrow Progenitor Cells following Radiation. Med Sci Sports Exerc. https://doi.org/10.1249/MSS.0000000000001894
Emmons, R., Niemiro, G.M., De Lisio, M., 2017. Hematopoiesis with Obesity and Exercise: Role of the Bone Marrow Niche. Exerc Immunol Rev 23, 82–95.
Emmons, R., Niemiro, G.M., Owolabi, O., De Lisio, M., 2016. Acute exercise mobilizes hematopoietic stem and progenitor cells and alters the mesenchymal stromal cell secretome. J. Appl. Physiol. 120, 624–632. https://doi.org/10.1152/japplphysiol.00925.2015
Ferrante, S.C., Nadler, E.P., Pillai, D.K., Hubal, M.J., Wang, Z., Wang, J.M., Gordish-Dressman, H., Koeck, E., Sevilla, S., Wiles, A.A., Freishtat, R.J., 2015. Adipocyte-derived Exosomal miRNAs: A Novel Mechanism for Obesity-Related Disease. Pediatr Res 77, 447–454. https://doi.org/10.1038/pr.2014.202
Frühbeis, C., Helmig, S., Tug, S., Simon, P., Krämer-Albers, E.-M., 2015. Physical exercise induces rapid release of small extracellular vesicles into the circulation. J Extracell Vesicles 4. https://doi.org/10.3402/jev.v4.28239
Gale, R.P., Armitage, J.O., 2020. Use of molecularly-cloned haematopoietic growth factors in persons exposed to acute high-dose, high-dose rate whole-body ionizing radiations. Blood Rev. 100690. https://doi.org/10.1016/j.blre.2020.100690
Green, D.E., Rubin, C.T., 2014. Consequences of irradiation on bone and marrow phenotypes, and its relation to disruption of hematopoietic precursors. Bone 0, 87–94. https://doi.org/10.1016/j.bone.2014.02.018
Guescini, M., Canonico, B., Lucertini, F., Maggio, S., Annibalini, G., Barbieri, E., Luchetti, F., Papa, S., Stocchi, V., 2015. Muscle Releases Alpha-Sarcoglycan Positive Extracellular Vesicles Carrying miRNAs in the Bloodstream. PLOS ONE 10, e0125094. https://doi.org/10.1371/journal.pone.0125094
Heinrich, L.F., Andersen, D.K., Cleasby, M.E., Lawson, C., 2015. Long-term high fat feeding of rats results in increased numbers of circulating microvesicles with pro-

inflammatory effects on endothelial cells. British Journal of Nutrition 113, 1704–1711. https://doi.org/10.1017/S0007114515001117
Helwa, I., Cai, J., Drewry, M.D., Zimmerman, A., Dinkins, M.B., Khaled, M.L., Seremwe, M., Dismuke, W.M., Bieberich, E., Stamer, W.D., Hamrick, M.W., Liu, Y., 2017. A Comparative Study of Serum Exosome Isolation Using Differential Ultracentrifugation and Three Commercial Reagents. PLOS ONE 12, e0170628. https://doi.org/10.1371/journal.pone.0170628
Hu, M., Lin, W., 2012. Effects of exercise training on red blood cell production: implications for anemia. Acta Haematol. 127, 156–164. https://doi.org/10.1159/000335620
Jella, K.K., Rani, S., O'Driscoll, L., McClean, B., Byrne, H.J., Lyng, F.M., 2014. Exosomes are involved in mediating radiation induced bystander signaling in human keratinocyte cells. Radiat. Res. 181, 138–145. https://doi.org/10.1667/RR13337.1
Jelonek, K., Widlak, P., Pietrowska, M., 2016. The Influence of Ionizing Radiation on Exosome Composition, Secretion and Intercellular Communication. Protein Pept Lett 23, 656–663. https://doi.org/10.2174/0929866523666160427105138
Lehmann, B.D., Paine, M.S., Brooks, A.M., McCubrey, J.A., Renegar, R.H., Wang, R., Terrian, D.M., 2008. SENESCENCE-ASSOCIATED EXOSOME RELEASE FROM HUMAN PROSTATE CANCER CELLS. Cancer Res 68. https://doi.org/10.1158/0008-5472.CAN-07-6538
Lichtman, M.A., 2010. Obesity and the risk for a hematological malignancy: leukemia, lymphoma, or myeloma. Oncologist 15, 1083–1101. https://doi.org/10.1634/theoncologist.2010-0206
Macià i Garau, M., Lucas Calduch, A., López, E.C., 2011. Radiobiology of the acute radiation syndrome. Rep Pract Oncol Radiother 16, 123–130. https://doi.org/10.1016/j.rpor.2011.06.001
Méndez-Ferrer, S., Michurina, T.V., Ferraro, F., Mazloom, A.R., MacArthur, B.D., Lira, S.A., Scadden, D.T., Ma'ayan, A., Enikolopov, G.N., Frenette, P.S., 2010. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 466, 829–834. https://doi.org/10.1038/nature09262
Ng, J., Shuryak, I., 2014. Minimizing second cancer risk following radiotherapy: current perspectives. Cancer Manag Res 7, 1–11. https://doi.org/10.2147/CMAR.S47220
Oblak, A., Jerala, R., 2011. Toll-Like Receptor 4 Activation in Cancer Progression and Therapy [WWW Document]. Clinical and Developmental Immunology. https://doi.org/10.1155/2011/609579
Salvestrini, V., Sell, C., Lorenzini, A., 2019. Obesity May Accelerate the Aging Process. Front Endocrinol (Lausanne) 10. https://doi.org/10.3389/fendo.2019.00266
Shao, L., Luo, Y., Zhou, D., 2014. Hematopoietic Stem Cell Injury Induced by Ionizing Radiation. Antioxid Redox Signal 20, 1447–1462. https://doi.org/10.1089/ars.2013.5635
Shi, X.-F., Wang, H., Kong, F.-X., Xu, Q.-Q., Xiao, F.-J., Yang, Y.-F., Ge, R.-L., Wang, L.-S., 2017. Exosomal miR-486 regulates hypoxia-induced erythroid differentiation of erythroleukemia cells through targeting Sirt1. Exp. Cell Res. 351, 74–81. https://doi.org/10.1016/j.yexcr.2016.12.023
Singh, V.K., Seed, T.M., 2017. A review of radiation countermeasures focusing on injury-specific medicinals and regulatory approval status: part I. Radiation sub-syndromes, animal models and FDA-approved countermeasures. International Journal of Radiation Biology 93, 851–869. https://doi.org/10.1080/09553002.2017.1332438
Stik, G., Crequit, S., Petit, L., Durant, J., Charbord, P., Jaffredo, T., Durand, C., 2017. Extracellular vesicles of stromal origin target and support hematopoietic stem and progenitor cells. J Cell Biol 216, 2217–2230. https://doi.org/10.1083/jcb.201601109
Szatmári, T., Kis, D., Bogdándi, E.N., Benedek, A., Bright, S., Bowler, D., Persa, E., Kis, E., Balogh, A., Naszályi, L.N., Kadhim, M., Sáfrány, G., Lumniczky, K., 2017. Extracellular Vesicles Mediate Radiation-Induced Systemic Bystander Signals in the Bone Marrow and Spleen. Front Immunol 8. https://doi.org/10.3389/fimmu.2017.00347

Tang, Y.-T., Huang, Y.-Y., Zheng, L., Qin, S.-H., Xu, X.-P., An, T.-X., Xu, Y., Wu, Y.-S., Hu, X.-M., Ping, B.-H., Wang, Q., 2017. Comparison of isolation methods of exosomes and exosomal RNA from cell culture medium and serum. Int J Mol Med 40, 834–844. https://doi.org/10.3892/ijmm.2017.3080

Théry, C., Witwer, K.W., Aikawa, E., Alcaraz, M.J., Anderson, J.D., Andriantsitohaina, R., Antoniou, A., Arab, T., Archer, F., Atkin-Smith, G.K., Ayre, D.C., Bach, J.-M., Bachurski, D., Baharvand, H., Balaj, L., Baldacchino, S., Bauer, N.N., Baxter, A.A., Bebawy, M., Beckham, C., Bedina Zavec, A., Benmoussa, A., Berardi, A.C., Bergese, P., Bielska, E., Blenkiron, C., Bobis-Wozowicz, S., Boilard, E., Boireau, W., Bongiovanni, A., Borràs, F.E., Bosch, S., Boulanger, C.M., Breakefield, X., Breglio, A.M., Brennan, M.Á., Brigstock, D.R., Brisson, A., Broekman, M.L., Bromberg, J.F., Bryl-Górecka, P., Buch, S., Buck, A.H., Burger, D., Busatto, S., Buschmann, D., Bussolati, B., Buzás, E.I., Byrd, J.B., Camussi, G., Carter, D.R., Caruso, S., Chamley, L.W., Chang, Y.-T., Chen, C., Chen, S., Cheng, L., Chin, A.R., Clayton, A., Clerici, S.P., Cocks, A., Cocucci, E., Coffey, R.J., Cordeiro-da-Silva, A., Couch, Y., Coumans, F.A., Coyle, B., Crescitelli, R., Criado, M.F., D'Souza-Schorey, C., Das, S., Datta Chaudhuri, A., de Candia, P., De Santana, E.F., De Wever, O., del Portillo, H.A., Demaret, T., Deville, S., Devitt, A., Dhondt, B., Di Vizio, D., Dieterich, L.C., Dolo, V., Dominguez Rubio, A.P., Dominici, M., Dourado, M.R., Driedonks, T.A., Duarte, F.V., Duncan, H.M., Eichenberger, R.M., Ekström, K., EL Andaloussi, S., Elie-Caille, C., Erdbrügger, U., Falcón-Pérez, J.M., Fatima, F., Fish, J.E., Flores-Bellver, M., Försönits, A., Frelet-Barrand, A., Fricke, F., Fuhrmann, G., Gabrielsson, S., Gámez-Valero, A., Gardiner, C., Gärtner, K., Gaudin, R., Gho, Y.S., Giebel, B., Gilbert, C., Gimona, M., Giusti, I., Goberdhan, D.C., Görgens, A., Gorski, S.M., Greening, D.W., Gross, J.C., Gualerzi, A., Gupta, G.N., Gustafson, D., Handberg, A., Haraszti, R.A., Harrison, P., Hegyesi, H., Hendrix, A., Hill, A.F., Hochberg, F.H., Hoffmann, K.F., Holder, B., Holthofer, H., Hosseinkhani, B., Hu, G., Huang, Y., Huber, V., Hunt, S., Ibrahim, A.G.-E., Ikezu, T., Inal, J.M., Isin, M., Ivanova, A., Jackson, H.K., Jacobsen, S., Jay, S.M., Jayachandran, M., Jenster, G., Jiang, L., Johnson, S.M., Jones, J.C., Jong, A., Jovanovic-Talisman, T., Jung, S., Kalluri, R., Kano, S., Kaur, S., Kawamura, Y., Keller, E.T., Khamari, D., Khomyakova, E., Khvorova, A., Kierulf, P., Kim, K.P., Kislinger, T., Klingeborn, M., Klinke, D.J., Kornek, M., Kosanović, M.M., Kovács, Á.F., Krämer-Albers, E.-M., Krasemann, S., Krause, M., Kurochkin, I.V., Kusuma, G.D., Kuypers, S., Laitinen, S., Langevin, S.M., Languino, L.R., Lannigan, J., Lässer, C., Laurent, L.C., Lavieu, G., Lázaro-Ibáñez, E., Le Lay, S., Lee, M.-S., Lee, Y.X.F., Lemos, D.S., Lenassi, M., Leszczynska, A., Li, I.T., Liao, K., Libregts, S.F., Ligeti, E., Lim, R., Lim, S.K., Linē, A., Linnemannstöns, K., Llorente, A., Lombard, C.A., Lorenowicz, M.J., Lörincz, Á.M., Lötvall, J., Lovett, J., Lowry, M.C., Loyer, X., Lu, Q., Lukomska, B., Lunavat, T.R., Maas, S.L., Malhi, H., Marcilla, A., Mariani, J., Mariscal, J., Martens-Uzunova, E.S., Martin-Jaular, L., Martinez, M.C., Martins, V.R., Mathieu, M., Mathivanan, S., Maugeri, M., McGinnis, L.K., McVey, M.J., Meckes, D.G., Meehan, K.L., Mertens, I., Minciacchi, V.R., Möller, A., Møller Jørgensen, M., Morales-Kastresana, A., Morhayim, J., Mullier, F., Muraca, M., Musante, L., Mussack, V., Muth, D.C., Myburgh, K.H., Najrana, T., Nawaz, M., Nazarenko, I., Nejsum, P., Neri, C., Neri, T., Nieuwland, R., Nimrichter, L., Nolan, J.P., Nolte-'t Hoen, E.N., Noren Hooten, N., O'Driscoll, L., O'Grady, T., O'Loghlen, A., Ochiya, T., Olivier, M., Ortiz, A., Ortiz, L.A., Osteikoetxea, X., Østergaard, O., Ostrowski, M., Park, J., Pegtel, D.M., Peinado, H., Perut, F., Pfaffl, M.W., Phinney, D.G., Pieters, B.C., Pink, R.C., Pisetsky, D.S., Pogge von Strandmann, E., Polakovicova, I., Poon, I.K., Powell, B.H., Prada, I., Pulliam, L., Quesenberry, P., Radeghieri, A., Raffai, R.L., Raimondo, S., Rak, J., Ramirez, M.I., Raposo, G., Rayyan, M.S., Regev-Rudzki, N., Ricklefs, F.L., Robbins, P.D., Roberts, D.D., Rodrigues, S.C., Rohde, E., Rome, S., Rouschop, K.M., Rughetti, A., Russell, A.E., Saá, P., Sahoo, S., Salas-Huenuleo, E., Sánchez, C., Saugstad, J.A., Saul, M.J., Schiffelers, R.M., Schneider, R., Schøyen, T.H., Scott, A.,

Shahaj, E., Sharma, S., Shatnyeva, O., Shekari, F., Shelke, G.V., Shetty, A.K., Shiba, K., Siljander, P.R.-M., Silva, A.M., Skowronek, A., Snyder, O.L., Soares, R.P., Sódar, B.W., Soekmadji, C., Sotillo, J., Stahl, P.D., Stoorvogel, W., Stott, S.L., Strasser, E.F., Swift, S., Tahara, H., Tewari, M., Timms, K., Tiwari, S., Tixeira, R., Tkach, M., Toh, W.S., Tomasini, R., Torrecilhas, A.C., Tosar, J.P., Toxavidis, V., Urbanelli, L., Vader, P., van Balkom, B.W., van der Grein, S.G., Van Deun, J., van Herwijnen, M.J., Van Keuren-Jensen, K., van Niel, G., van Royen, M.E., van Wijnen, A.J., Vasconcelos, M.H., Vechetti, I.J., Veit, T.D., Vella, L.J., Velot, É., Verweij, F.J., Vestad, B., Viñas, J.L., Visnovitz, T., Vukman, K.V., Wahlgren, J., Watson, D.C., Wauben, M.H., Weaver, A., Webber, J.P., Weber, V., Wehman, A.M., Weiss, D.J., Welsh, J.A., Wendt, S., Wheelock, A.M., Wiener, Z., Witte, L., Wolfram, J., Xagorari, A., Xander, P., Xu, J., Yan, X., Yáñez-Mó, M., Yin, H., Yuana, Y., Zappulli, V., Zarubova, J., Žėkas, V., Zhang, J., Zhao, Z., Zheng, L., Zheutlin, A.R., Zickler, A.M., Zimmermann, P., Zivkovic, A.M., Zocco, D., Zuba-Surma, E.K., 2018. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 7. https://doi.org/10.1080/20013078.2018.1535750

Wang, Y., Schulte, B.A., LaRue, A.C., Ogawa, M., Zhou, D., 2006. Total body irradiation selectively induces murine hematopoietic stem cell senescence. Blood 107, 358–366. https://doi.org/10.1182/blood-2005-04-1418

Wen, S., Dooner, M., Cheng, Y., Papa, E., Del Tatto, M., Pereira, M., Deng, Y., Goldberg, L., Aliotta, J., Chatterjee, D., Stewart, C., Carpanetto, A., Collino, F., Bruno, S., Camussi, G., Quesenberry, P., 2016. Mesenchymal stromal cell-derived extracellular vesicles rescue radiation damage to murine marrow hematopoietic cells. Leukemia 30, 2221–2231. https://doi.org/10.1038/leu.2016.107

Wisdom, A.J., Hong, C.S., Lin, A.J., Xiang, Y., Cooper, D.E., Zhang, J., Xu, E.S., Kuo, H.-C., Mowery, Y.M., Carpenter, D.J., Kadakia, K.T., Himes, J.E., Luo, L., Ma, Y., Williams, N., Cardona, D.M., Haldar, M., Diao, Y., Markovina, S., Schwarz, J.K., Kirsch, D.G., 2019. Neutrophils promote tumor resistance to radiation therapy. PNAS 116, 18584–18589. https://doi.org/10.1073/pnas.1901562116

Yu, X., Harris, S.L., Levine, A.J., 2006. The regulation of exosome secretion: a novel function of the p53 protein. Cancer Res. 66, 4795–4801. https://doi.org/10.1158/0008-5472.CAN-05-4579

Author Contributions

MN and MD contributed to study design and direction. MN and PM performed experiments. MN and MD contributed to analysis. MN and MD wrote the manuscript. All authors approved final submission.

Acknowledgements

The authors gratefully acknowledge the service of Fernando Ortiz the Facility Coordinator of Cell Sorting and Flow Cytometry Core Facility at the Ottawa Hospital. We also thank Dr. Dylan Burger of the Department of Cellular and Molecular Medicine at the University of Ottawa, for providing access to his laboratory to operate the Zetaview Particle Metrix. Lastly, we thankfully acknowledge Dr. Robin Parks of the University of Ottawa and Ottawa Hospital Research Institute and Kathy Poulin for assisting with the western blot protocol.

Funding

American Institute for Cancer Research, and Natural Sciences and Engineering Research Council

Disclosures

The authors have no financial conflicts of interest to disclose.

Figure captions and figures

Figure 1. High-fat diet induces an obesity phenotype which is not reversed by exercise. (a) A schematic of the study timeline. Body fat percentage (b), absolute lean body mass (c) and mouse body weight (d), measured throughout the life of the mice. Mouse food intake measured per gram (e) and calories (f) throughout the life of the mice. The dotted line denotes the age mice were irradiated. Data presented as mean ± SEM. *p < 0.05 significant difference between CON + EX compared with HF + SED and HF + EX. β p< 0.05 significant difference of CON + SED compared with HF + SED and HF + EX. n=9 CON+SED, n=10 CON+EX, n=10 HF+SED, n=10 HF+EX.

Figure 2. Characterization of bone marrow extracellular vesicles. Western blot analysis for Alix, Flotillin, and TSG101 (a) of EVs isolated using the ExoQuick-TC from murine bone marrow. M=Molecular weight marker, CL=Cell lysate, UC=EVs isolated by ultracentrifugation, EQ= EVs isolated by ExoQuick. Using NTA, the mean size of particle was compared between CON and IR measured from each group (b) and the concentration of particles (c). There were significantly fewer particles from IR mice (**P<0.001). Using NTA, the mean size of particle was measured from each group (d) and the concentration of particles (e). Range of particle sizes measured using nanoparticle tracking analysis (f). Uptake of PKH67 stained bone marrow-EVs into HSPCs compared to unstained EVs, imaged by Celldiscoverer 7 (g). Data presented as mean ± SEM. *p <0.05 indicating a main effect of exercise. **p<0.01. n=10 CON, n=9 CON=SED, n=9 CON+SED, n=10 CON+EX, n=10 HF+SED, n=10 HF+EX.

Figure 3. Radiated bone marrow-EVs did not impact hematopoietic stem/progenitor or immature hematopoietic cell content. Bone marrow-EVs from irradiated and non-irradiated mice were cocultured with fluorescence-activated cell sorted (FACS) murine HSPCs for 48 hours. After this 48hour period cells were quantified via flow cytometry. Quantification of viable (a), Lin^- and HSPCs (b). There was no significant change in Lin^- or HSPC populations. Data presented as mean ± SEM. n=10 CON, n=9 IR

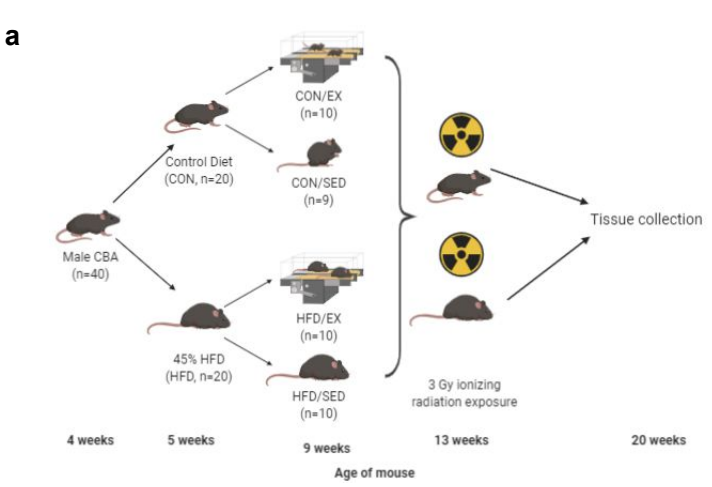
Figure 4. Bone marrow-EVs from exercise-trained or obese mice following radiation do not alter hematopoietic stem/progenitor or immature hematopoietic cell content. Bone

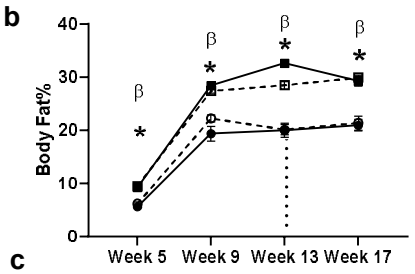
marrow-EVs isolated by ExoQuick or ultracentrifugation and supernatant from ultracentrifugation from the experimental groups were cocultured with FACS murine HSPCs for 48hrs. After these 48 hours, cells were quantified via flow cytometry. Source of EVs retrieved by ExoQuick (EQ), ultracentrifugation (U) and supernatant from ultracentrifugation (SU). Quantification of viable Lin^- (a,c,e) and HSPCs (b,d,f). Lin^- from U cultures showed a trend towards significance at P=0.13 through lifestyle and Lin^- from SU cultures also displayed a trend towards significance above at P=0.15 through diet. Lastly, HSPCs cultured with U approached significance at P=0.064 with this being related to lifestyle. Data presented as mean ± SEM. n=9 CON+SED(EQ), n=10 CON+EX (EQ), n=10 HF+SED (EQ), n=10 HF+EX(EQ), n=4 CON+SED(U,SU), n=4 CON+EX (U,SU), n=4 HF+SED (U,SU), n=4 HF+EX(U,SU).

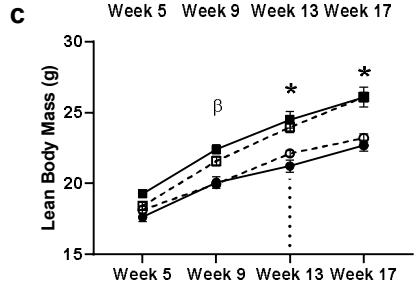
Figure 5. Irradiated bone marrow-EVs promote the expansion of myeloid lineage colonies. HSPCs from ExoQuick isolated bone marrow-EV coculture were plated in methylcellulose at 1,000 cells/ml, and total colony count, and colony type scoring were done after 14 days of culture. Progenitor cell populations were identified and enumerated. CFU-GEMM (a), CFU-GM (b), CFU-G (c), CFU-M (d), BFU-E (e) AND TOTAL (f). Data presented as mean ± SEM. n=5 CON, n=5 IR CFU-GEMM (**P<0.001), CFU-G (*P<0.05) and TOTAL were significantly increased in irradiated groups, while BFU-E displayed a trend towards significance (P=0.17) to increase and CFU-GM and CFUM did not increase.

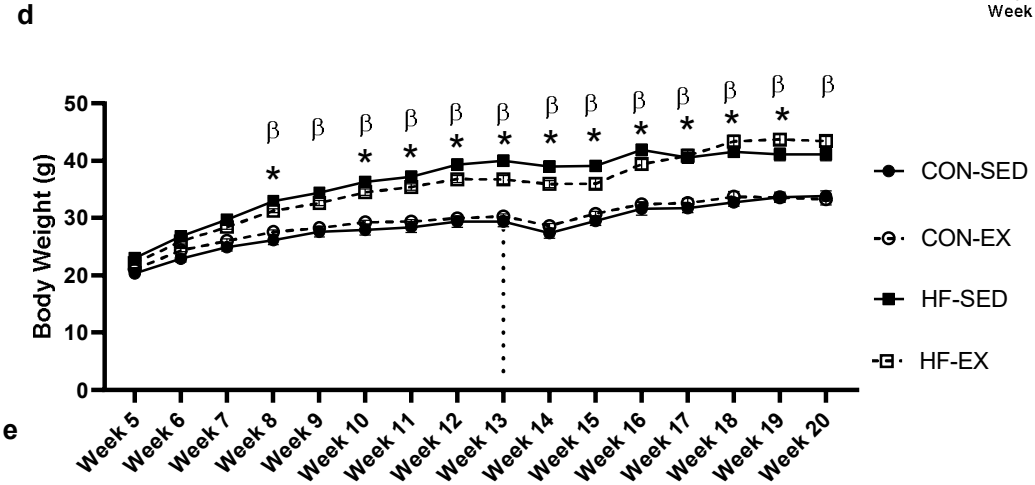
Figure 6. Myeloid progenitor cell populations are not influenced by bone marrow-EVs from exercise or high-fat diet mice. HSPC from ExoQuick isolated BONE MARROW-EV coculture were plated in methylcellulose at 1,000 cells/ml, and total colony count, and colony type scoring were done after 14 days of culture. Progenitor cell populations were identified and enumerated. CFU-granulocyte, erythrocyte, macrophage, megakaryocyte (CFU-GEMM) (a), granulocyte and/or macrophage progenitor cells (CFU-GM) (b), CFU-granulocyte (CFU-G) (c), CFU-macrophage (CFU-M) (d), b-erythroid (BFU-E) (e) and TOTAL (f). Data presented as mean ± SEM. n=5 CON+SED, n=6 CON+EX, n=6 HF+SED, n=6 HF+EX

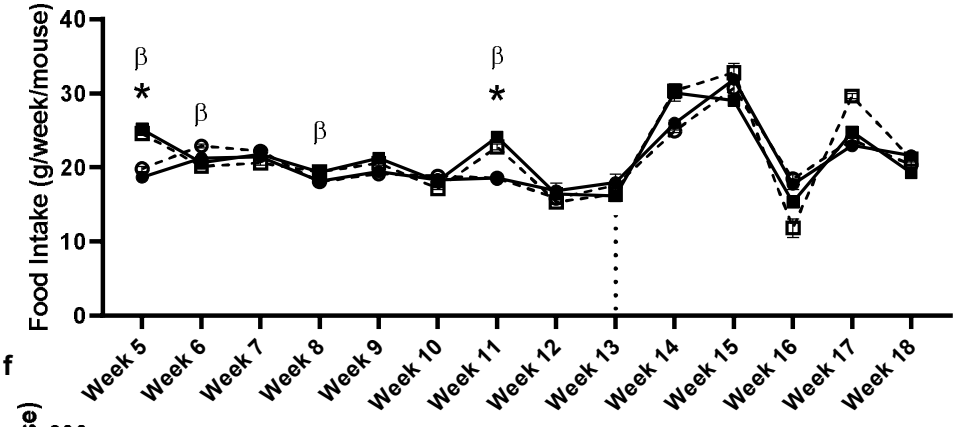
Figure 1


a
CON/EX
(n=10)
Control Diet
(CON, n=20)
CON/SED
(n=9)
Male CBA
(n=40)
HFD/EX
(n=10)
45% HFD
(HFD, n=20)
HFD/SED
(n=10)
Tissue collection
3 Gy ionizing
radiation exposure
4 weeks
5 weeks
9 weeks
13 weeks
20 weeks
Age of mouse


b
Body Fat%
Week 5
Week 9
Week 13
Week 17


c
Lean Body Mass (g)
Week 5
Week 9
Week 13
Week 17


d
Body Weight (g)
CON-SED
CON-EX
HF-SED
HF-EX


e
Food Intake (g/week/mouse)

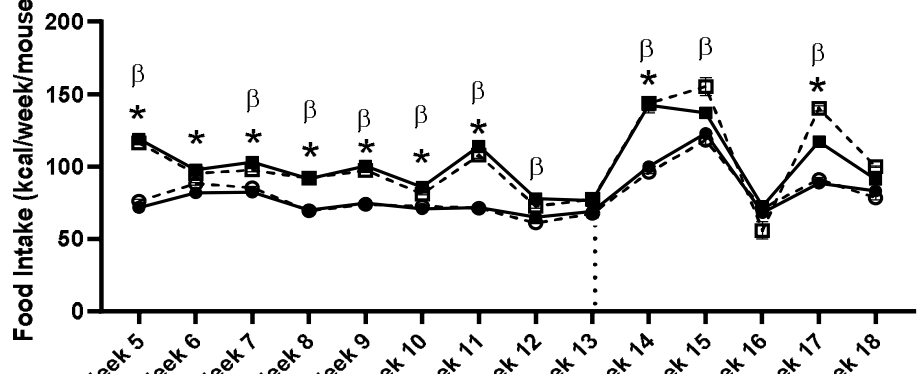

f
Food Intake (kcal/week/mouse)

Figure 2

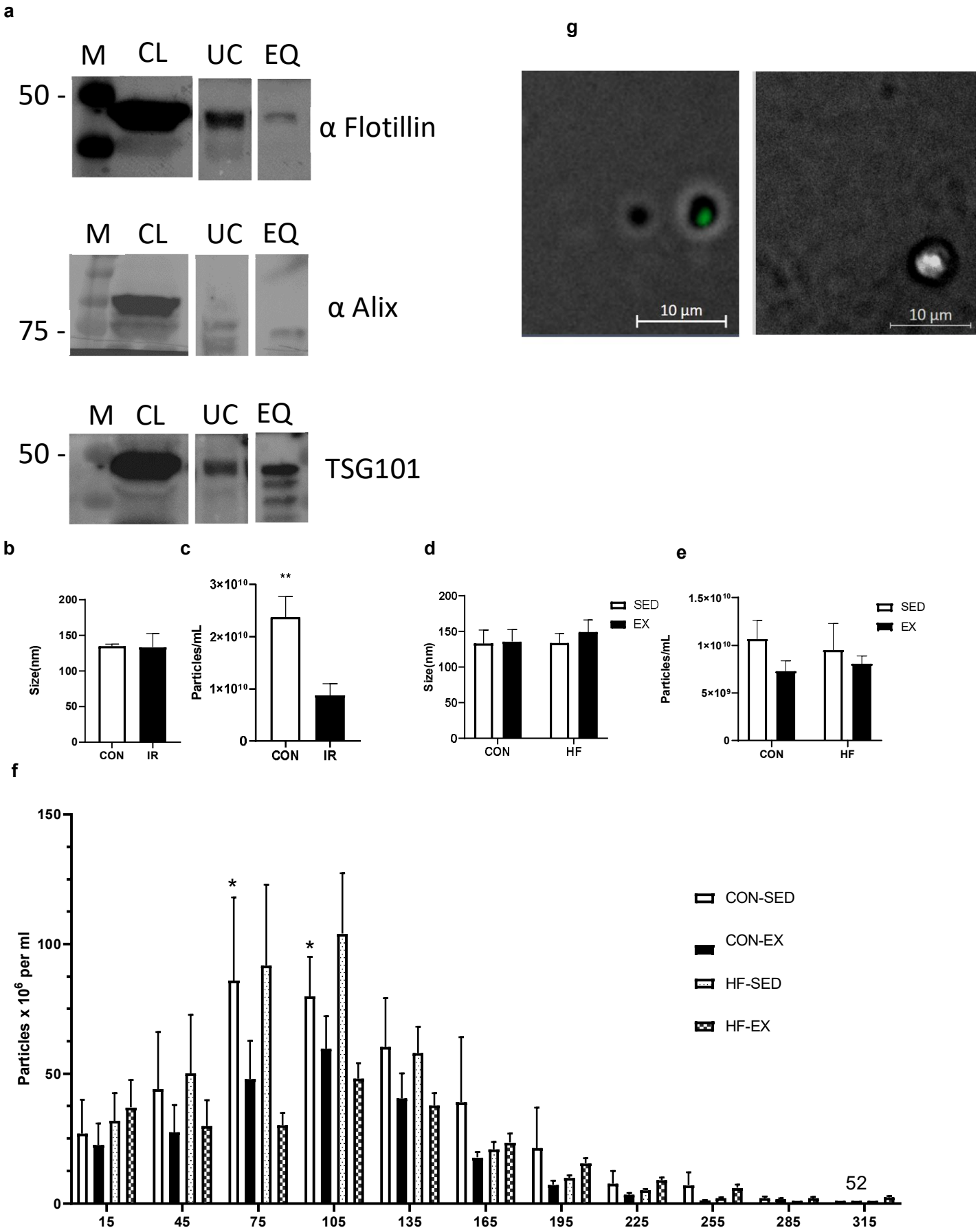
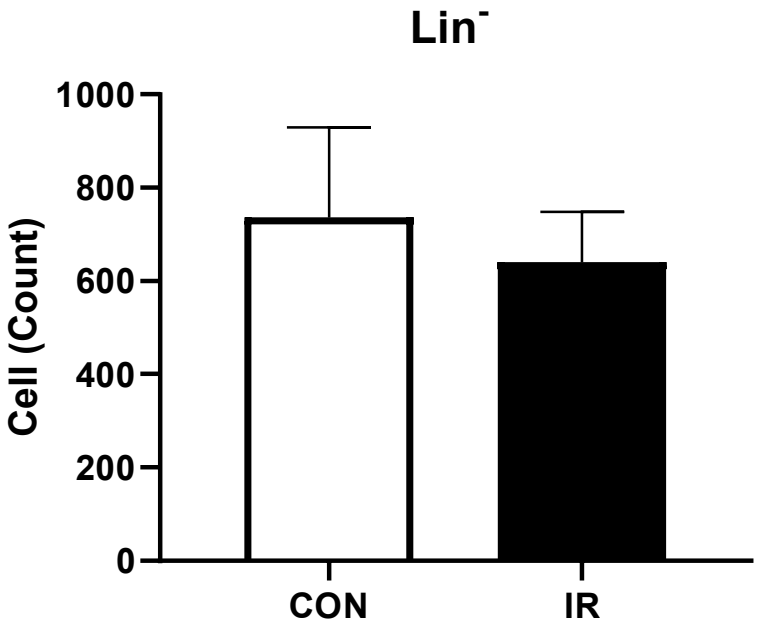

a
M CL UC EQ
50 -
α Flotillin
M CL UC EQ
75 -
α Alix
M CL UC EQ
50 -
TSG101
g
10 µm
10 µm
b
Size(nm)
200
150
100
50
0
CON IR
c
Particles/mL
3×10¹⁰
2×10¹⁰
1×10¹⁰
0
**
CON IR
d
Size(nm)
200
150
100
50
0
SED
EX
CON HF
e
Particles/mL
1.5×10¹⁰
1×10¹⁰
5×10⁹
0
SED
EX
CON HF
f
Particles x 10⁶ per ml
150
100
50
0
*
*
CON-SED
CON-EX
HF-SED
HF-EX
52
15 45 75 105 135 165 195 225 255 285 315
Size (nm)

Figure 3

a


Lin-
1000
800
600
400
200
0
Cell (Count)
CON
IR

b

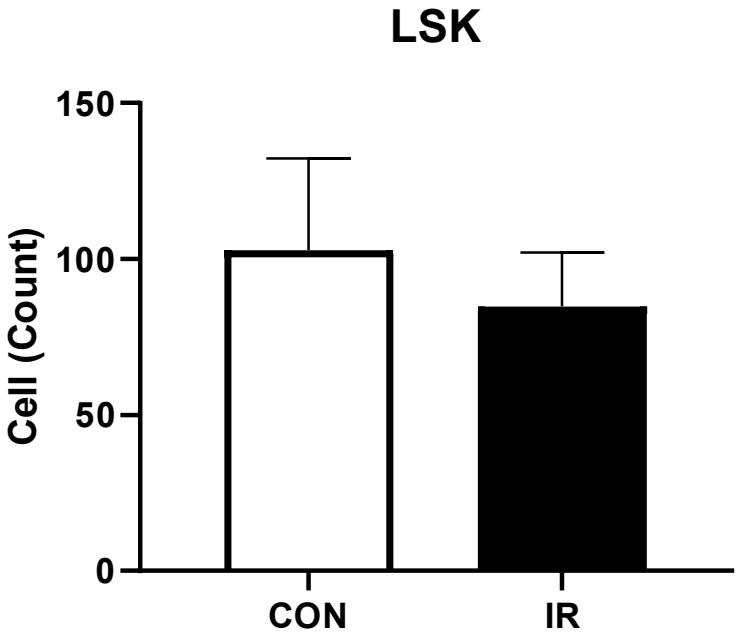

LSK
150
100
50
0
Cell (Count)
CON
IR

Figure 4

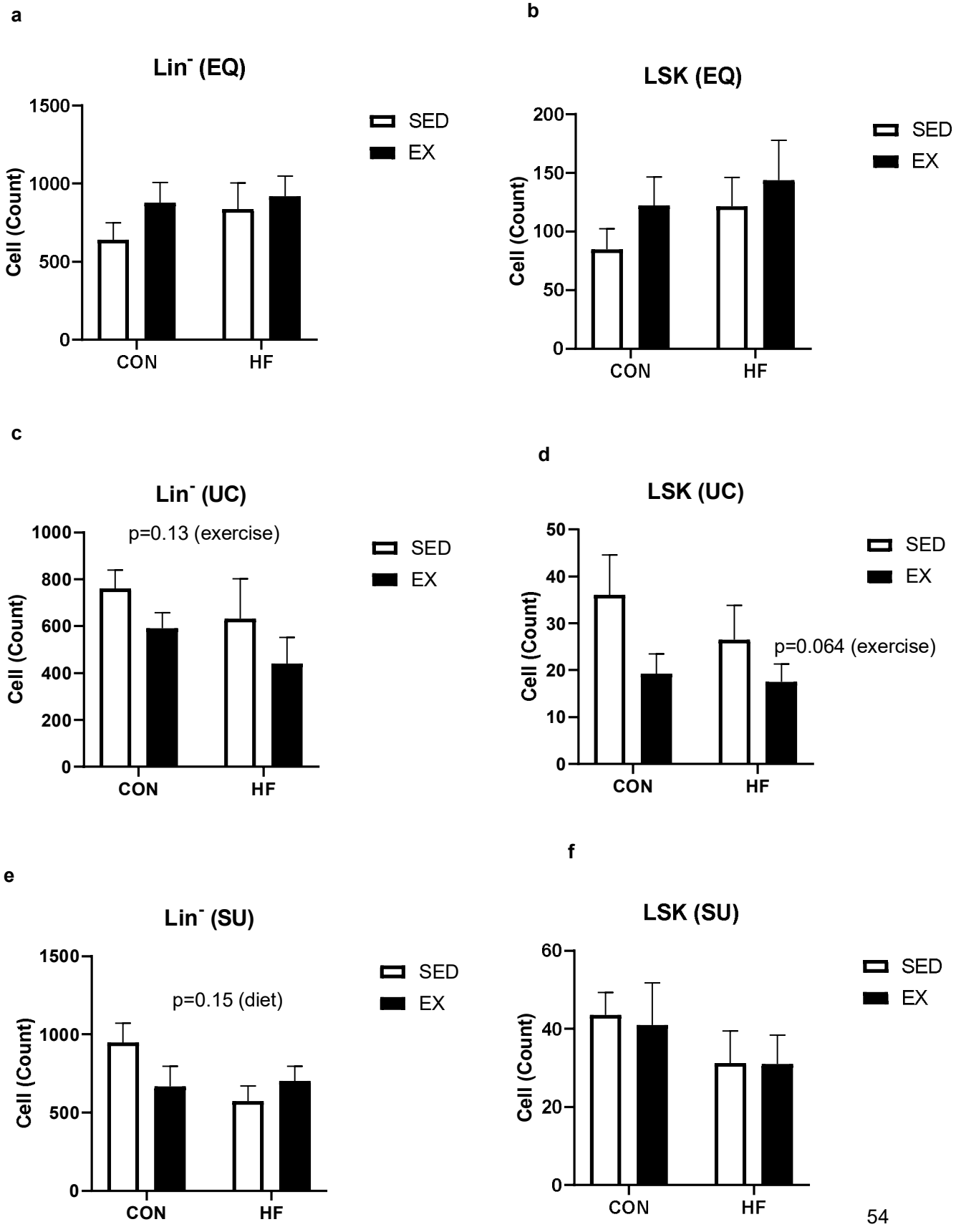

a
Lin- (EQ)
Cell (Count)
1500
1000
500
0
CON
HF
SED
EX
b
LSK (EQ)
Cell (Count)
200
150
100
50
0
CON
HF
SED
EX
c
Lin- (UC)
p=0.13 (exercise)
Cell (Count)
1000
800
600
400
200
0
CON
HF
SED
EX
d
LSK (UC)
Cell (Count)
50
40
30
20
10
0
p=0.064 (exercise)
CON
HF
SED
EX
e
Lin- (SU)
p=0.15 (diet)
Cell (Count)
1500
1000
500
0
CON
HF
SED
EX
f
LSK (SU)
Cell (Count)
60
40
20
0
CON
HF
SED
EX

Figure 5

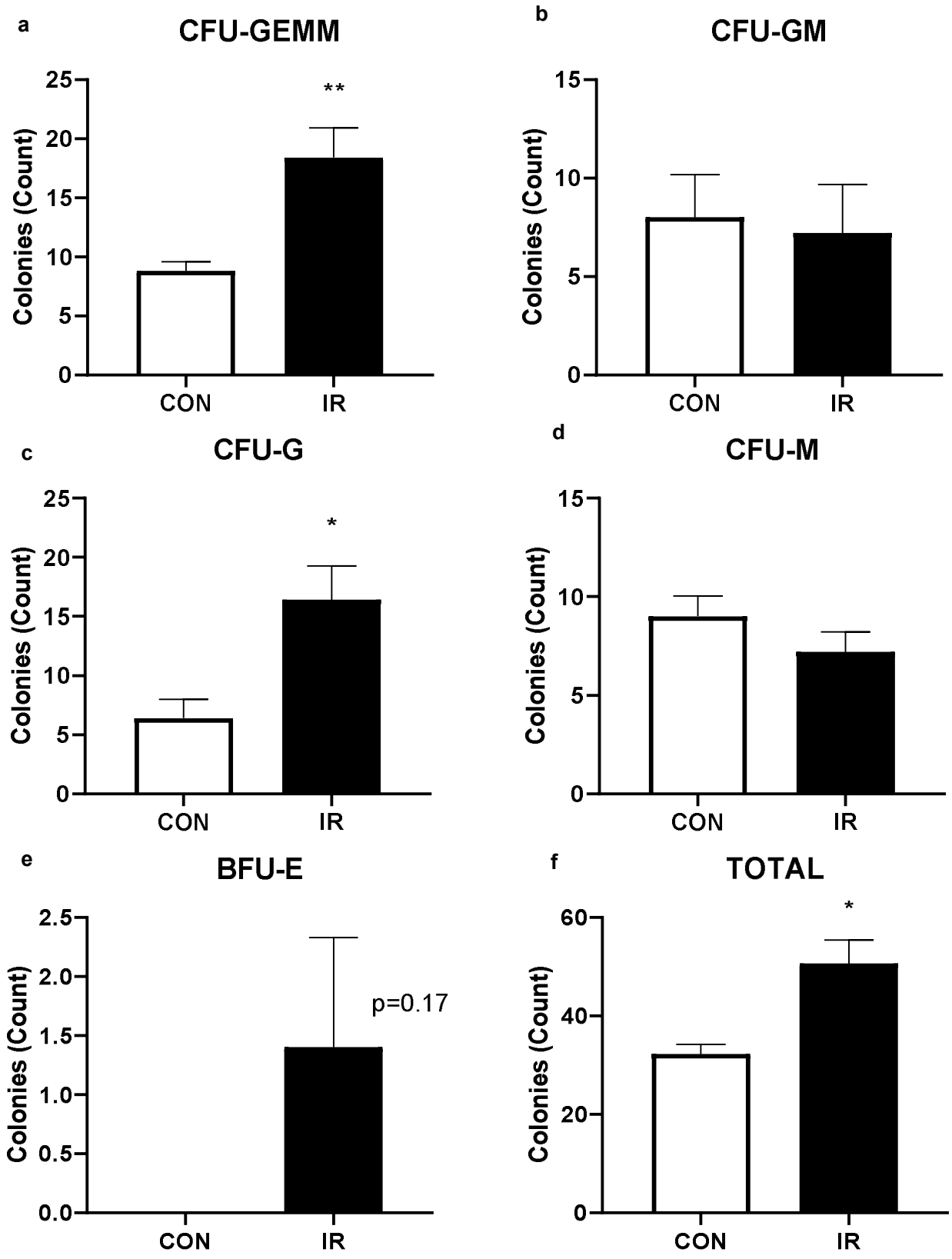
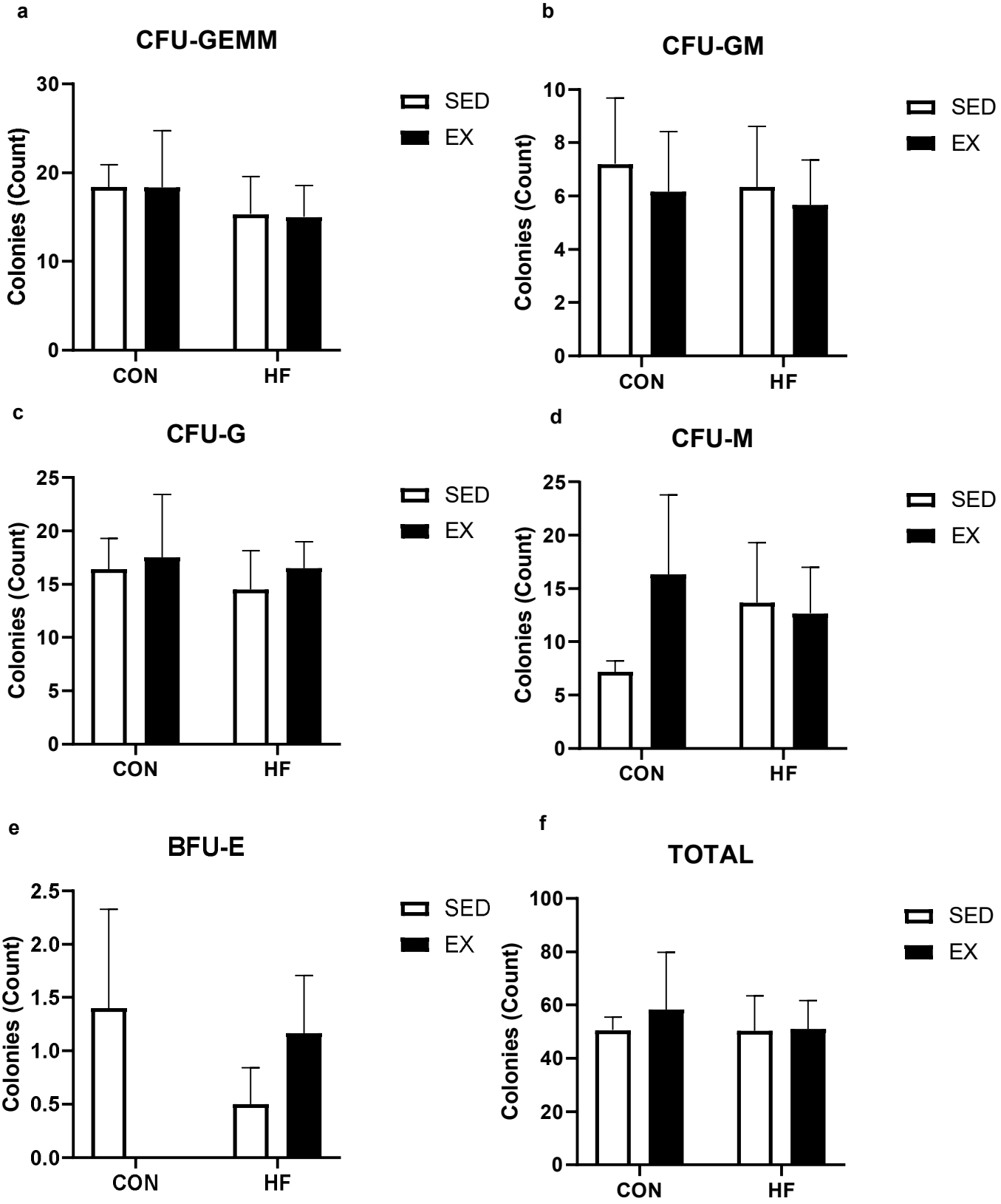

a
CFU-GEMM
Colonies (Count)
25
20
15
10
5
0
**
CON
IR
b
CFU-GM
Colonies (Count)
15
10
5
0
CON
IR
c
CFU-G
Colonies (Count)
25
20
15
10
5
0
*
CON
IR
d
CFU-M
Colonies (Count)
15
10
5
0
CON
IR
e
BFU-E
Colonies (Count)
2.5
2.0
1.5
1.0
0.5
0.0
p=0.17
CON
IR
f
TOTAL
Colonies (Count)
60
40
20
0
*
CON
IR

Figure 6


a
CFU-GEMM
Colonies (Count)
30
20
10
0
CON
HF
SED
EX
b
CFU-GM
Colonies (Count)
10
8
6
4
2
0
CON
HF
SED
EX
c
CFU-G
Colonies (Count)
25
20
15
10
5
0
CON
HF
SED
EX
d
CFU-M
Colonies (Count)
25
20
15
10
5
0
CON
HF
SED
EX
e
BFU-E
Colonies (Count)
2.5
2.0
1.5
1.0
0.5
0.0
CON
HF
SED
EX
f
TOTAL
Colonies (Count)
100
80
60
40
20
0
CON
HF
SED
EX

Chater 3 - Global Discussion

Exposure to radiation at high or low doses can be detrimental to an individual's health. One common source of radiation exposure is through radiation therapy, and it is estimated that 2/3 of cancer treatment plans implement radiation therapy (Chen and Kuo, 2017). With a rise of cancer survivors, the long-term effects of radiation exposure on healthy tissues are becoming a clinical concern (Bryant et al., 2017). In parallel, the number of cancer survivors who are also obese is rising (Courneya et al., 2008). The bone marrow is especially radiosensitive, and obesity is associated with detrimental changes within the marrow. Our lab demonstrated that exercise training can reverse several of the detrimental effects of obesity on bone marrow stem/stromal cell populations following radiation exposure (Emmons et al., 2019). Using a proteomic approach, we demonstrated that exercise training is associated with a prohematopoietic, anti-inflammatory environment within the bone marrow after radiation exposure, whereas obesity is associated with higher levels of factors that inhibit hematopoiesis. One mode of cellular communication that has not been examined in the context of exercise and obesity after radiation exposure is extracellular vesicles (EVs). Thus, the purpose of this research was to functionally characterize bone marrow-EVs from irradiated mice and determine the effects of obese and exercise training on bone marrow-EV function following radiation. Our results demonstrate that radiation decreased the concentration of bone marrow-EVs, and bone marrow-EVs from irradiated mice increase myeloid lineage colony formation *in vitro.* However, the effects of radiation on bone marrow-EVs are not altered by exercise, obesity, or their combination. Together, these data suggest that irradiation alters bone marrow-EVs in a manner that promotes myelopoiesis, and that mechanisms not related to EVs explain the previously identified differential effects of exercise and obesity on hematopoiesis (Emmons et al., 2019).

EVs are nano-sized particles between 30–1000nm that are secreted by various cells and are found abundantly in tissues. Due to their small size EV detection and classification is challenging. The commonly used protocol for isolation of exosomes is ultracentrifugation (UC), although, in recent years, precipitation solutions such as the line of ExoQuick (EQ) kits have

been utilized to precipitate particles in solution. In this research, we isolated EVs using both methods to see if this would affect the quantity, size, and activity of isolated particles. Using the ExoQuick-TC ULTRA kit, we acquired a higher concentration of particles compared to ultracentrifugation ($P< 0.05$) (S4), except for EVs from the HF-SED group. Our results are consistent with those of Tang and colleagues (Tang et al., 2017). Their analysis showed that the concentration of particles obtained using ultracentrifugation was lower than those of the commercial kit (ExoQuick). This lower yield may be caused by vesicle damaged by repeated ultracentrifugation, which causes low particle recovery (Tang et al., 2017). Conversely, isolation by ExoQuick shows higher particle recovery, potentially due to a lack of separation of high-density protein aggregates. The average size of particles was not significantly bigger using the ultracentrifugation method (S5). This outcome is opposite to results seen by Helwa and colleagues, where EVs isolated from ultracentrifugation had a significantly greater diameter than the ExoQuick (Helwa et al., 2017). It has been suggested, that centrifuging at such high speeds can lead to the fusion of particles with contaminants and other proteins, affecting the physical properties of the exosomes. Coculturing with EVs from ExoQuick or ultracentrifugation did not have a significant effect within groups (Figure 4). However, when comparing the number of HSPCs from cocultures with EVs derived from ExoQuick or ultracentrifugation there was a significant reduction ($p<0.05$) except for the CON+SED EVs. This decrease may exist because with ExoQuick isolation there is a possibility that the kit will precipitate non-exosome nanoparticles, such as serum protein aggregates (Alvarez et al., 2012). These aggregates may support HSPCs in culture. Ultracentrifugation can cause a loss of exosomes from the sample, therefore fewer particles are in contact with HSPCs and culture support can be reduced.

Our results demonstrated that at seven weeks post-radiation, mice had significantly depressed bone marrow-EV concentration compared to non-irradiated. Research has shown that irradiation can increase EV release from cancer and normal cell lines *in vitro* (Lehmann et al., 2008; Arscott et al., 2013; Jella et al., 2014; Al-Mayah et al., 2015). However, these studies measured EV content shortly after radiation. These studies also applied radiation at a

higher dosage, EVs were produced from cells *in vitro,* and from non-bone marrow cells and tissues. These differences may explain why the studies showed an increase in EVs after radiation. In the present study, we retrieved bone marrow-EVs seven weeks after radiation. Waiting this length of time may cause changes to the cell's ability to produce, package, and/or release EVs. One proposed cause of the increase of EVs after radiation is the activation of stress-inducible pathways of exosome secretion. Radiation exposure may increase the expression of the Tumor Suppressor-Activated Pathway 6 (TSAP6) protein, which has been implicated in enhancing EV production (Yu et al., 2006). In the context of our study, this pathway may have been down-regulated, have been silenced or associated pathways connected to TSAP6 production may have altered.

Our initial CFU assay, compared irradiated and non-irradiated bone marrow-EVs ability to influence HSPCs function to differentiate down specific myeloid pathways. Our results showed that irradiated EVs protected CFU-GEMM and CFU-G colonies. In a study by Goloviznina and colleagues, they showed that murine HSPCs exposed to MSC-derived EVs *in vitro* favoured myeloid progenitor expansion and skewed hematopoietic repopulation potential (Goloviznina et al., 2016). They demonstrated that EV exposure results in the increased formation of CFU-M (monocyte colony-forming unit) and CFU-G (granulocyte colony-forming unit). This bias towards myeloid expansion was due to EVs binding to toll-like receptor 4 (TLR4) on HSPCs and triggering downstream NF-κB signalling. Activation of this pathway causes the cell to enter the G1 phase of the cell cycle, followed by myeloid expansion. Potentially, in an irradiated mouse, cargo that activates TLR4 ligands is upregulated in bone marrow-EV. One ligand in particular, which activates TLR4 is ceramide (Oblak and Jerala, 2011). Ceramide is one of the lipids critical for exosome formation and is also enriched in these EVs (Elsherbini and Bieberich, 2018).

Our work demonstrated that irradiated bone marrow-EV retrieved *in vivo*, can influence the differentiation of myeloid progenitor cells and that exercise training cannot reduce this effect via EVs. However, we were not able to characterize the cargo of bone marrow-EVs to reveal how they regulate myeloid differentiation. While we previously demonstrated that

exercise training increases cytokines that are associated with a prohematopoietic, anti-inflammatory environment within the bone marrow after radiation exposure (Emmons et al., 2019), the present study suggests that these effects are not induced via EVs but likely through other factors. We also did not include a CON+EX, HF+SED, and HF+EX groups that did not undergo radiation, limiting our ability to characterize how exercise can influence bone marrow-EVs ability to regulate hematopoiesis independent of radiation. The present work used a reductionist approach to examine the direct effects of bone marrow-EVs on HSPCs *in vitro*, which removes the influence of other bone marrow cell populations and factors. Future studies should examine the effect of exercise training on bone marrow-EVs applied *in vivo*, characterization of exercise-trained bone marrow-EV cargo post-irradiation, the inclusion of experimental groups that do not undergo radiation, and examining pathways in EV secretion and composition post-radiation.

Together, our results reveal that neither exercise, obesity, nor their combination alters bone marrow-EVs post-radiation in a manner that regulates HSPC fate directly. Rather the conducive environment for hematopoiesis that exercise provides is likely through other growth factors and cytokines. Lastly, it was demonstrated that irradiated bone marrow-EVs can expand myeloid colonies *in vitro*. This result demonstrates that radiation exposure could promote myeloid skewing through bone marrow-EVs. This skewing may exhaust HSPCs and lead to the development of a pro-inflammatory environment.

References

Al-Mayah, A., Bright, S., Chapman, K., Irons, S., Luo, P., Carter, D., Goodwin, E., Kadhim, M., 2015. The non-targeted effects of radiation are perpetuated by exosomes. Mutat. Res. 772, 38–45. https://doi.org/10.1016/j.mrfmmm.2014.12.007

Alvarez, M.L., Khosroheidari, M., Kanchi Ravi, R., DiStefano, J.K., 2012. Comparison of protein, microRNA, and mRNA yields using different methods of urinary exosome isolation for the discovery of kidney disease biomarkers. Kidney Int. 82, 1024–1032. https://doi.org/10.1038/ki.2012.256

Aoi, W., Ichikawa, H., Mune, K., Tanimura, Y., Mizushima, K., Naito, Y., Yoshikawa, T., 2013. Muscle-enriched microRNA miR-486 decreases in circulation in response to exercise in young men. Front Physiol 4, 80. https://doi.org/10.3389/fphys.2013.00080

Aqmasheh, S., Shamsasanjan, karim, Akbarzadehlaleh, P., Pashoutan Sarvar, D., Timari, H., 2017. Effects of Mesenchymal Stem Cell Derivatives on Hematopoiesis and Hematopoietic Stem Cells. Adv Pharm Bull 7, 165–177. https://doi.org/10.15171/apb.2017.021

Arscott, W.T., Tandle, A.T., Zhao, S., Shabason, J.E., Gordon, I.K., Schlaff, C.D., Zhang, G., Tofilon, P.J., Camphausen, K.A., 2013. Ionizing radiation and glioblastoma exosomes: implications in tumor biology and cell migration. Transl Oncol 6, 638–648. https://doi.org/10.1593/tlo.13640

Baker, J.M., De Lisio, M., Parise, G., 2011. Endurance exercise training promotes medullary hematopoiesis. FASEB J. 25, 4348–4357. https://doi.org/10.1096/fj.11-189043

Barone, R., Macaluso, F., Sangiorgi, C., Campanella, C., Marino Gammazza, A., Moresi, V., Coletti, D., Conway de Macario, E., Macario, A.J., Cappello, F., Adamo, S., Farina, F., Zummo, G., Di Felice, V., 2016. Skeletal muscle Heat shock protein 60 increases after endurance training and induces peroxisome proliferator-activated receptor gamma coactivator 1 α1 expression. Sci Rep 6. https://doi.org/10.1038/srep19781

Battiwalla, M., Barrett, A.J., 2014. Bone marrow mesenchymal stromal cells to treat complications following allogeneic stem cell transplantation. Tissue Eng Part B Rev 20, 211–217. https://doi.org/10.1089/ten.TEB.2013.0566

Bei, Y., Xu, T., Lv, D., Yu, P., Xu, J., Che, L., Das, A., Tigges, J., Toxavidis, V., Ghiran, I., Shah, R., Li, Y., Zhang, Y., Das, S., Xiao, J., 2017. Exercise-induced circulating extracellular vesicles protect against cardiac ischemia-reperfusion injury. Basic Res Cardiol 112, 38. https://doi.org/10.1007/s00395-017-0628-z

Bryant, A.K., Banegas, M.P., Martinez, M.E., Mell, L.K., Murphy, J.D., 2017. Trends in Radiation Therapy among Cancer Survivors in the United States, 2000–2030. Cancer Epidemiol Biomarkers Prev 26, 963–970. https://doi.org/10.1158/1055-9965.EPI-16-1023

Butler, J.T., Abdelhamed, S., Kurre, P., 2018. Extracellular vesicles in the hematopoietic microenvironment. Haematologica 103, 382–394. https://doi.org/10.3324/haematol.2017.183335

Chen, H.H.W., Kuo, M.T., 2017. Improving radiotherapy in cancer treatment: Promises and challenges. Oncotarget 8, 62742–62758. https://doi.org/10.18632/oncotarget.18409

Chou, S.-H., Lin, S.-Z., Day, C.H., Kuo, W.-W., Shen, C.-Y., Hsieh, D.J.-Y., Lin, J.-Y., Tsai, F.-J., Tsai, C.-H., Huang, C.-Y., 2013. Mesenchymal stem cell insights: prospects in hematological transplantation. Cell Transplant 22, 711–721. https://doi.org/10.3727/096368912X655172

Courneya, K.S., Katzmarzyk, P.T., Bacon, E., 2008. Physical activity and obesity in Canadian cancer survivors: population-based estimates from the 2005 Canadian Community Health Survey. Cancer 112, 2475–2482. https://doi.org/10.1002/cncr.23455

Coussens, L.M., Werb, Z., 2002. Inflammation and cancer. Nature 420, 860–867. https://doi.org/10.1038/nature01322

De Lisio, M., Baker, J.M., Parise, G., 2013. Exercise promotes bone marrow cell survival and recipient reconstitution post-bone marrow transplantation, which is associated with

increased survival. Exp. Hematol. 41, 143–154. https://doi.org/10.1016/j.exphem.2012.10.003
De Lisio, M., Parise, G., 2012. Characterization of the effects of exercise training on hematopoietic stem cell quantity and function. J. Appl. Physiol. 113, 1576–1584. https://doi.org/10.1152/japplphysiol.00717.2012
De Lisio, M., Phan, N., Boreham, D.R., Parise, G., 2011. Exercise-induced protection of bone marrow cells following exposure to radiation. Appl Physiol Nutr Metab 36, 80–87. https://doi.org/10.1139/H10-087
Deng, Z., Poliakov, A., Hardy, R.W., Clements, R., Liu, C., Liu, Y., Wang, J., Xiang, X., Zhang, S., Zhuang, X., Shah, S.V., Sun, D., Michalek, S., Grizzle, W.E., Garvey, T., Mobley, J., Zhang, H.-G., 2009. Adipose tissue exosome-like vesicles mediate activation of macrophage-induced insulin resistance. Diabetes 58, 2498–2505. https://doi.org/10.2337/db09-0216
Eguchi, A., Lazic, M., Armando, A.M., Phillips, S.A., Katebian, R., Maraka, S., Quehenberger, O., Sears, D.D., Feldstein, A.E., 2016. Circulating adipocyte-derived extracellular vesicles are novel markers of metabolic stress. J Mol Med 94, 1241–1253. https://doi.org/10.1007/s00109-016-1446-8
Eguchi, A., Mulya, A., Lazic, M., Radhakrishnan, D., Berk, M.P., Povero, D., Gornicka, A., Feldstein, A.E., 2015. Microparticles Release by Adipocytes Act as “Find-Me” Signals to Promote Macrophage Migration. PLOS ONE 10, e0123110. https://doi.org/10.1371/journal.pone.0123110
Elsherbini, A., Bieberich, E., 2018. Ceramide and exosomes: a novel target in cancer biology and therapy. Adv Cancer Res 140, 121–154. https://doi.org/10.1016/bs.acr.2018.05.004
Emmons, R., Ngu, M., Xu, G., Hernández-Saavedra, D., Chen, H., De Lisio, M., 2019. Effects of Obesity and Exercise on Bone Marrow Progenitor Cells following Radiation. Med Sci Sports Exerc. https://doi.org/10.1249/MSS.0000000000001894
Emmons, R., Niemiro, G.M., De Lisio, M., 2017. Hematopoiesis with Obesity and Exercise: Role of the Bone Marrow Niche. Exerc Immunol Rev 23, 82–95.
Emmons, R., Niemiro, G.M., Owolabi, O., De Lisio, M., 2016. Acute exercise mobilizes hematopoietic stem and progenitor cells and alters the mesenchymal stromal cell secretome. J. Appl. Physiol. 120, 624–632. https://doi.org/10.1152/japplphysiol.00925.2015
Ferrante, S.C., Nadler, E.P., Pillai, D.K., Hubal, M.J., Wang, Z., Wang, J.M., Gordish-Dressman, H., Koeck, E., Sevilla, S., Wiles, A.A., Freishtat, R.J., 2015. Adipocyte-derived Exosomal miRNAs: A Novel Mechanism for Obesity-Related Disease. Pediatr Res 77, 447–454. https://doi.org/10.1038/pr.2014.202
Frühbeis, C., Helmig, S., Tug, S., Simon, P., Krämer-Albers, E.-M., 2015. Physical exercise induces rapid release of small extracellular vesicles into the circulation. J Extracell Vesicles 4. https://doi.org/10.3402/jev.v4.28239
Gale, R.P., Armitage, J.O., 2020. Use of molecularly-cloned haematopoietic growth factors in persons exposed to acute high-dose, high-dose rate whole-body ionizing radiations. Blood Rev. 100690. https://doi.org/10.1016/j.blre.2020.100690
Green, D.E., Rubin, C.T., 2014. Consequences of irradiation on bone and marrow phenotypes, and its relation to disruption of hematopoietic precursors. Bone 0, 87–94. https://doi.org/10.1016/j.bone.2014.02.018
Guescini, M., Canonico, B., Lucertini, F., Maggio, S., Annibalini, G., Barbieri, E., Luchetti, F., Papa, S., Stocchi, V., 2015. Muscle Releases Alpha-Sarcoglycan Positive Extracellular Vesicles Carrying miRNAs in the Bloodstream. PLOS ONE 10, e0125094. https://doi.org/10.1371/journal.pone.0125094
Heinrich, L.F., Andersen, D.K., Cleasby, M.E., Lawson, C., 2015. Long-term high fat feeding of rats results in increased numbers of circulating microvesicles with pro-inflammatory effects on endothelial cells. British Journal of Nutrition 113, 1704–1711. https://doi.org/10.1017/S0007114515001117

Helwa, I., Cai, J., Drewry, M.D., Zimmerman, A., Dinkins, M.B., Khaled, M.L., Seremwe, M., Dismuke, W.M., Bieberich, E., Stamer, W.D., Hamrick, M.W., Liu, Y., 2017. A Comparative Study of Serum Exosome Isolation Using Differential Ultracentrifugation and Three Commercial Reagents. PLOS ONE 12, e0170628. https://doi.org/10.1371/journal.pone.0170628

Hu, M., Lin, W., 2012. Effects of exercise training on red blood cell production: implications for anemia. Acta Haematol. 127, 156–164. https://doi.org/10.1159/000335620

Jella, K.K., Rani, S., O'Driscoll, L., McClean, B., Byrne, H.J., Lyng, F.M., 2014. Exosomes are involved in mediating radiation induced bystander signaling in human keratinocyte cells. Radiat. Res. 181, 138–145. https://doi.org/10.1667/RR13337.1

Jelonek, K., Widlak, P., Pietrowska, M., 2016. The Influence of Ionizing Radiation on Exosome Composition, Secretion and Intercellular Communication. Protein Pept Lett 23, 656–663. https://doi.org/10.2174/0929866523666160427105138

Lehmann, B.D., Paine, M.S., Brooks, A.M., McCubrey, J.A., Renegar, R.H., Wang, R., Terrian, D.M., 2008. SENESCENCE-ASSOCIATED EXOSOME RELEASE FROM HUMAN PROSTATE CANCER CELLS. Cancer Res 68. https://doi.org/10.1158/0008-5472.CAN-07-6538

Lichtman, M.A., 2010. Obesity and the risk for a hematological malignancy: leukemia, lymphoma, or myeloma. Oncologist 15, 1083–1101. https://doi.org/10.1634/theoncologist.2010-0206

Macià i Garau, M., Lucas Calduch, A., López, E.C., 2011. Radiobiology of the acute radiation syndrome. Rep Pract Oncol Radiother 16, 123–130. https://doi.org/10.1016/j.rpor.2011.06.001

Méndez-Ferrer, S., Michurina, T.V., Ferraro, F., Mazloom, A.R., MacArthur, B.D., Lira, S.A., Scadden, D.T., Ma'ayan, A., Enikolopov, G.N., Frenette, P.S., 2010. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 466, 829–834. https://doi.org/10.1038/nature09262

Ng, J., Shuryak, I., 2014. Minimizing second cancer risk following radiotherapy: current perspectives. Cancer Manag Res 7, 1–11. https://doi.org/10.2147/CMAR.S47220

Oblak, A., Jerala, R., 2011. Toll-Like Receptor 4 Activation in Cancer Progression and Therapy [WWW Document]. Clinical and Developmental Immunology. https://doi.org/10.1155/2011/609579

Salvestrini, V., Sell, C., Lorenzini, A., 2019. Obesity May Accelerate the Aging Process. Front Endocrinol (Lausanne) 10. https://doi.org/10.3389/fendo.2019.00266

Shao, L., Luo, Y., Zhou, D., 2014. Hematopoietic Stem Cell Injury Induced by Ionizing Radiation. Antioxid Redox Signal 20, 1447–1462. https://doi.org/10.1089/ars.2013.5635

Shi, X.-F., Wang, H., Kong, F.-X., Xu, Q.-Q., Xiao, F.-J., Yang, Y.-F., Ge, R.-L., Wang, L.-S., 2017. Exosomal miR-486 regulates hypoxia-induced erythroid differentiation of erythroleukemia cells through targeting Sirt1. Exp. Cell Res. 351, 74–81. https://doi.org/10.1016/j.yexcr.2016.12.023

Singh, V.K., Seed, T.M., 2017. A review of radiation countermeasures focusing on injury-specific medicinals and regulatory approval status: part I. Radiation sub-syndromes, animal models and FDA-approved countermeasures. International Journal of Radiation Biology 93, 851–869. https://doi.org/10.1080/09553002.2017.1332438

Stik, G., Crequit, S., Petit, L., Durant, J., Charbord, P., Jaffredo, T., Durand, C., 2017. Extracellular vesicles of stromal origin target and support hematopoietic stem and progenitor cells. J Cell Biol 216, 2217–2230. https://doi.org/10.1083/jcb.201601109

Szatmári, T., Kis, D., Bogdándi, E.N., Benedek, A., Bright, S., Bowler, D., Persa, E., Kis, E., Balogh, A., Naszályi, L.N., Kadhim, M., Sáfrány, G., Lumniczky, K., 2017. Extracellular Vesicles Mediate Radiation-Induced Systemic Bystander Signals in the Bone Marrow and Spleen. Front Immunol 8. https://doi.org/10.3389/fimmu.2017.00347

Tang, Y.-T., Huang, Y.-Y., Zheng, L., Qin, S.-H., Xu, X.-P., An, T.-X., Xu, Y., Wu, Y.-S., Hu, X.-M., Ping, B.-H., Wang, Q., 2017. Comparison of isolation methods of exosomes

and exosomal RNA from cell culture medium and serum. Int J Mol Med 40, 834–844. https://doi.org/10.3892/ijmm.2017.3080

Théry, C., Witwer, K.W., Aikawa, E., Alcaraz, M.J., Anderson, J.D., Andriantsitohaina, R., Antoniou, A., Arab, T., Archer, F., Atkin-Smith, G.K., Ayre, D.C., Bach, J.-M., Bachurski, D., Baharvand, H., Balaj, L., Baldacchino, S., Bauer, N.N., Baxter, A.A., Bebawy, M., Beckham, C., Bedina Zavec, A., Benmoussa, A., Berardi, A.C., Bergese, P., Bielska, E., Blenkiron, C., Bobis-Wozowicz, S., Boilard, E., Boireau, W., Bongiovanni, A., Borràs, F.E., Bosch, S., Boulanger, C.M., Breakefield, X., Breglio, A.M., Brennan, M.Á., Brigstock, D.R., Brisson, A., Broekman, M.L., Bromberg, J.F., Bryl-Górecka, P., Buch, S., Buck, A.H., Burger, D., Busatto, S., Buschmann, D., Bussolati, B., Buzás, E.I., Byrd, J.B., Camussi, G., Carter, D.R., Caruso, S., Chamley, L.W., Chang, Y.-T., Chen, C., Chen, S., Cheng, L., Chin, A.R., Clayton, A., Clerici, S.P., Cocks, A., Cocucci, E., Coffey, R.J., Cordeiro-da-Silva, A., Couch, Y., Coumans, F.A., Coyle, B., Crescitelli, R., Criado, M.F., D'Souza-Schorey, C., Das, S., Datta Chaudhuri, A., de Candia, P., De Santana, E.F., De Wever, O., del Portillo, H.A., Demaret, T., Deville, S., Devitt, A., Dhondt, B., Di Vizio, D., Dieterich, L.C., Dolo, V., Dominguez Rubio, A.P., Dominici, M., Dourado, M.R., Driedonks, T.A., Duarte, F.V., Duncan, H.M., Eichenberger, R.M., Ekström, K., EL Andaloussi, S., Elie-Caille, C., Erdbrügger, U., Falcón-Pérez, J.M., Fatima, F., Fish, J.E., Flores-Bellver, M., Försönits, A., Frelet-Barrand, A., Fricke, F., Fuhrmann, G., Gabrielsson, S., Gámez-Valero, A., Gardiner, C., Gärtner, K., Gaudin, R., Gho, Y.S., Giebel, B., Gilbert, C., Gimona, M., Giusti, I., Goberdhan, D.C., Görgens, A., Gorski, S.M., Greening, D.W., Gross, J.C., Gualerzi, A., Gupta, G.N., Gustafson, D., Handberg, A., Haraszti, R.A., Harrison, P., Hegyesi, H., Hendrix, A., Hill, A.F., Hochberg, F.H., Hoffmann, K.F., Holder, B., Holthofer, H., Hosseinkhani, B., Hu, G., Huang, Y., Huber, V., Hunt, S., Ibrahim, A.G.-E., Ikezu, T., Inal, J.M., Isin, M., Ivanova, A., Jackson, H.K., Jacobsen, S., Jay, S.M., Jayachandran, M., Jenster, G., Jiang, L., Johnson, S.M., Jones, J.C., Jong, A., Jovanovic-Talisman, T., Jung, S., Kalluri, R., Kano, S., Kaur, S., Kawamura, Y., Keller, E.T., Khamari, D., Khomyakova, E., Khvorova, A., Kierulf, P., Kim, K.P., Kislinger, T., Klingeborn, M., Klinke, D.J., Kornek, M., Kosanović, M.M., Kovács, Á.F., Krämer-Albers, E.-M., Krasemann, S., Krause, M., Kurochkin, I.V., Kusuma, G.D., Kuypers, S., Laitinen, S., Langevin, S.M., Languino, L.R., Lannigan, J., Lässer, C., Laurent, L.C., Lavieu, G., Lázaro-Ibáñez, E., Le Lay, S., Lee, M.-S., Lee, Y.X.F., Lemos, D.S., Lenassi, M., Leszczynska, A., Li, I.T., Liao, K., Libregts, S.F., Ligeti, E., Lim, R., Lim, S.K., Linē, A., Linnemannstöns, K., Llorente, A., Lombard, C.A., Lorenowicz, M.J., Lörincz, Á.M., Lötvall, J., Lovett, J., Lowry, M.C., Loyer, X., Lu, Q., Lukomska, B., Lunavat, T.R., Maas, S.L., Malhi, H., Marcilla, A., Mariani, J., Mariscal, J., Martens-Uzunova, E.S., Martin-Jaular, L., Martinez, M.C., Martins, V.R., Mathieu, M., Mathivanan, S., Maugeri, M., McGinnis, L.K., McVey, M.J., Meckes, D.G., Meehan, K.L., Mertens, I., Minciacchi, V.R., Möller, A., Møller Jørgensen, M., Morales-Kastresana, A., Morhayim, J., Mullier, F., Muraca, M., Musante, L., Mussack, V., Muth, D.C., Myburgh, K.H., Najrana, T., Nawaz, M., Nazarenko, I., Nejsum, P., Neri, C., Neri, T., Nieuwland, R., Nimrichter, L., Nolan, J.P., Nolte-'t Hoen, E.N., Noren Hooten, N., O'Driscoll, L., O'Grady, T., O'Loghlen, A., Ochiya, T., Olivier, M., Ortiz, A., Ortiz, L.A., Osteikoetxea, X., Østergaard, O., Ostrowski, M., Park, J., Pegtel, D.M., Peinado, H., Perut, F., Pfaffl, M.W., Phinney, D.G., Pieters, B.C., Pink, R.C., Pisetsky, D.S., Pogge von Strandmann, E., Polakovicova, I., Poon, I.K., Powell, B.H., Prada, I., Pulliam, L., Quesenberry, P., Radeghieri, A., Raffai, R.L., Raimondo, S., Rak, J., Ramirez, M.I., Raposo, G., Rayyan, M.S., Regev-Rudzki, N., Ricklefs, F.L., Robbins, P.D., Roberts, D.D., Rodrigues, S.C., Rohde, E., Rome, S., Rouschop, K.M., Rughetti, A., Russell, A.E., Saá, P., Sahoo, S., Salas-Huenuleo, E., Sánchez, C., Saugstad, J.A., Saul, M.J., Schiffelers, R.M., Schneider, R., Schøyen, T.H., Scott, A., Shahaj, E., Sharma, S., Shatnyeva, O., Shekari, F., Shelke, G.V., Shetty, A.K., Shiba, K., Siljander, P.R.-M., Silva, A.M., Skowronek, A., Snyder, O.L., Soares, R.P.,

Sódar, B.W., Soekmadji, C., Sotillo, J., Stahl, P.D., Stoorvogel, W., Stott, S.L., Strasser, E.F., Swift, S., Tahara, H., Tewari, M., Timms, K., Tiwari, S., Tixeira, R., Tkach, M., Toh, W.S., Tomasini, R., Torrecilhas, A.C., Tosar, J.P., Toxavidis, V., Urbanelli, L., Vader, P., van Balkom, B.W., van der Grein, S.G., Van Deun, J., van Herwijnen, M.J., Van Keuren-Jensen, K., van Niel, G., van Royen, M.E., van Wijnen, A.J., Vasconcelos, M.H., Vechetti, I.J., Veit, T.D., Vella, L.J., Velot, É., Verweij, F.J., Vestad, B., Viñas, J.L., Visnovitz, T., Vukman, K.V., Wahlgren, J., Watson, D.C., Wauben, M.H., Weaver, A., Webber, J.P., Weber, V., Wehman, A.M., Weiss, D.J., Welsh, J.A., Wendt, S., Wheelock, A.M., Wiener, Z., Witte, L., Wolfram, J., Xagorari, A., Xander, P., Xu, J., Yan, X., Yáñez-Mó, M., Yin, H., Yuana, Y., Zappulli, V., Zarubova, J., Žėkas, V., Zhang, J., Zhao, Z., Zheng, L., Zheutlin, A.R., Zickler, A.M., Zimmermann, P., Zivkovic, A.M., Zocco, D., Zuba-Surma, E.K., 2018. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 7. https://doi.org/10.1080/20013078.2018.1535750

Wang, Y., Schulte, B.A., LaRue, A.C., Ogawa, M., Zhou, D., 2006. Total body irradiation selectively induces murine hematopoietic stem cell senescence. Blood 107, 358–366. https://doi.org/10.1182/blood-2005-04-1418

Wen, S., Dooner, M., Cheng, Y., Papa, E., Del Tatto, M., Pereira, M., Deng, Y., Goldberg, L., Aliotta, J., Chatterjee, D., Stewart, C., Carpanetto, A., Collino, F., Bruno, S., Camussi, G., Quesenberry, P., 2016. Mesenchymal stromal cell-derived extracellular vesicles rescue radiation damage to murine marrow hematopoietic cells. Leukemia 30, 2221–2231. https://doi.org/10.1038/leu.2016.107

Wisdom, A.J., Hong, C.S., Lin, A.J., Xiang, Y., Cooper, D.E., Zhang, J., Xu, E.S., Kuo, H.-C., Mowery, Y.M., Carpenter, D.J., Kadakia, K.T., Himes, J.E., Luo, L., Ma, Y., Williams, N., Cardona, D.M., Haldar, M., Diao, Y., Markovina, S., Schwarz, J.K., Kirsch, D.G., 2019. Neutrophils promote tumor resistance to radiation therapy. PNAS 116, 18584–18589. https://doi.org/10.1073/pnas.1901562116

Yu, X., Harris, S.L., Levine, A.J., 2006. The regulation of exosome secretion: a novel function of the p53 protein. Cancer Res. 66, 4795–4801. https://doi.org/10.1158/0008-5472.CAN-05-4579

Appendix

Appendix A - Supplementary Figures

S1. Nanoparticle tracking analysis of EVs

Nanoparticle tracking analysis of flushed bone marrow-EVs from CBA mice. Nanoparticle tracking analysis was used to measure the size of the particles, specifically using the ZetaView ParticleMetrix system. This apparatus quantifies vesicles at 11 camera positions. Histogram depicting the size range of the particles (S1a). Representative video of particles within the ZetaView (S1b).

S2. Flow Plot

Representative flow plot for HSPC populations.

S3. Photographs of Mouse Hematopoietic CFUs

The photographs of the classes of mouse hematopoietic progenitor cells detected using MethoCult™ media are shown. Colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte (CFU-GEMM) (a), colony-forming unit-granulocyte, macrophage (CFU-GM) (b), colony-forming unit-granulocyte (CFU-G) (c), colony-forming unit-macrophage (CFU-M) (d) and Burst-forming unit-erythroid (BFU-E) (e).

S4. Comparison of particle concentration between EVs isolated via ExoQuick and Ultracentrifugation

Irradiated EVs isolated via Exoquick (EQ) were compared to EVs isolated by ultracentrifugation (UC) within each group. Comparisons were conducted on particle concentration (particles/mL). The concentration of particles isolated from EQ compared to UC was significantly higher within CON-SED (a), CON-EX (b) and HF-EX (c) groups ($P<0.05$). The concentration was not significantly different within the HF-SED group. Data presented as mean ± SEM. n=9 CON+SED(EQ), n=10 CON+EX (EQ), n=10 HF+SED (EQ), n=10 HF+EX(EQ), n=4 CON+SED(UC), n=4 CON+EX (UC), n=4 HF+SED (UC), n=4 HF+EX(UC). *p <0.05, **p<0.01, ****p<0.0001.

S5. Comparison of mean size between EVs isolated via ExoQuick and Ultracentrifugation

Irradiated EVs isolated via Exoquick (EQ) were compared to EVs isolated by ultracentrifugation (UC) within each group. Comparisons were conducted on particle mean size (nm). The size of particles isolated from EQ compared to UC was not significantly different within any of the groups(a-d). Data presented as mean ± SEM. n=9 CON+SED(EQ), n=10 CON+EX (EQ), n=10 HF+SED (EQ), n=10 HF+EX(EQ), n=4 CON+SED(UC), n=4 CON+EX (UC), n=4 HF+SED (UC), n=4 HF+EX(UC).

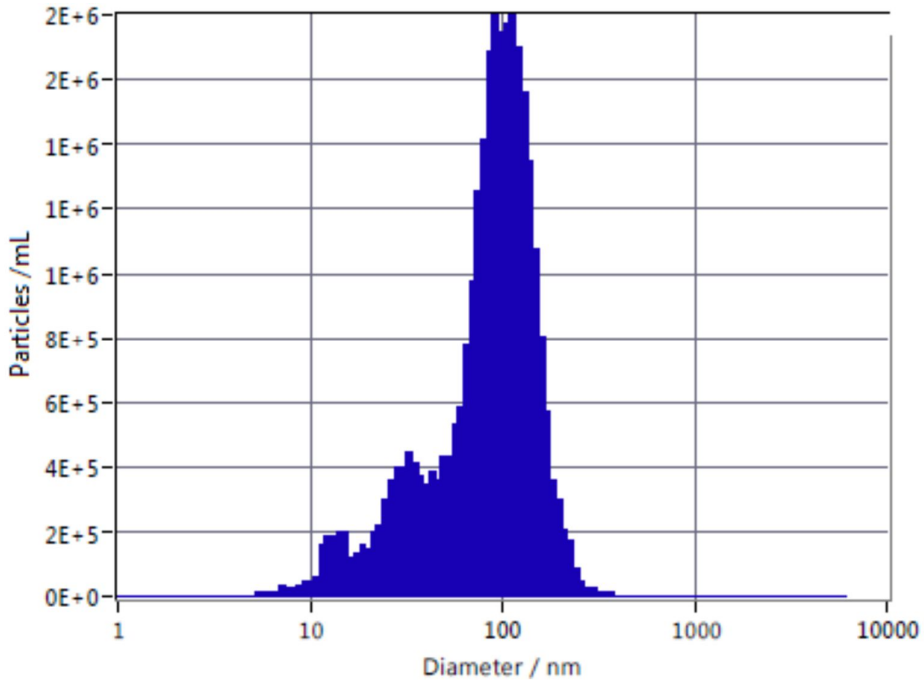
S6. Comparison of HSPC count between EVs isolated via ExoQuick and Ultracentrifugation

Bone marrow-EVs were isolated via Exoquick (EQ) or ultracentrifugation (UC) and cocultured with FACS murine HSPC cells for 48hours. After this 48hour period, cells were quantified via flow cytometry. Comparison of the number of HSPC cells after using EQ or UC EVs. The number of HSPC cells using EQ EVs was significantly higher compared to UC within CON-EX (b), HF-SED (c) and HF-EX (d). Data presented as mean ± SEM. n=9 CON+SED(EQ), n=10 CON+EX (EQ), n=10 HF+SED (EQ), n=10 HF+EX(EQ), n=4 CON+SED(UC), n=4 CON+EX (UC), n=4 HF+SED (UC), n=4 HF+EX(UC). *p <0.05

S7. Irradiation does not promote a sustained modification of body weight nor an increase in body fat. (a) Mouse body weight. Mouse food intake measured per gram (b) and calories (c) throughout the life of the mice. (d) Body fat percentage (e) and absolute lean body mass. Data presented as mean ± SEM. *p < 0.05 significant difference between CON compared IR. **** p< 0.0001 significant difference of CON compared IR. n=9 CON, n=10 IR

Figure S1

a



b

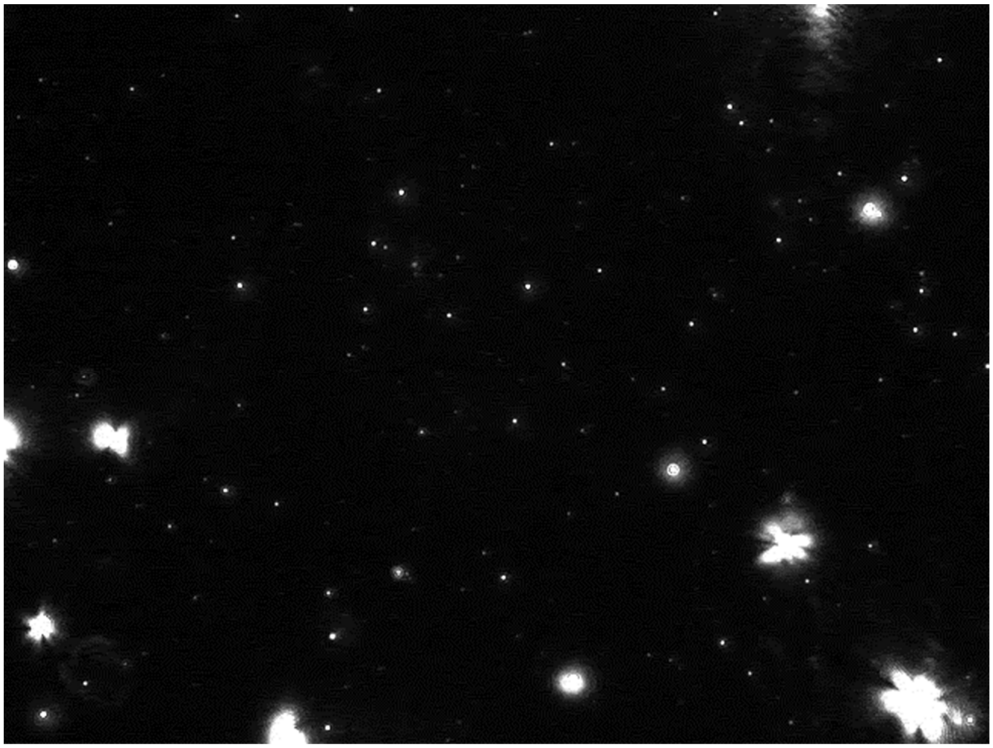


Figure S2

a

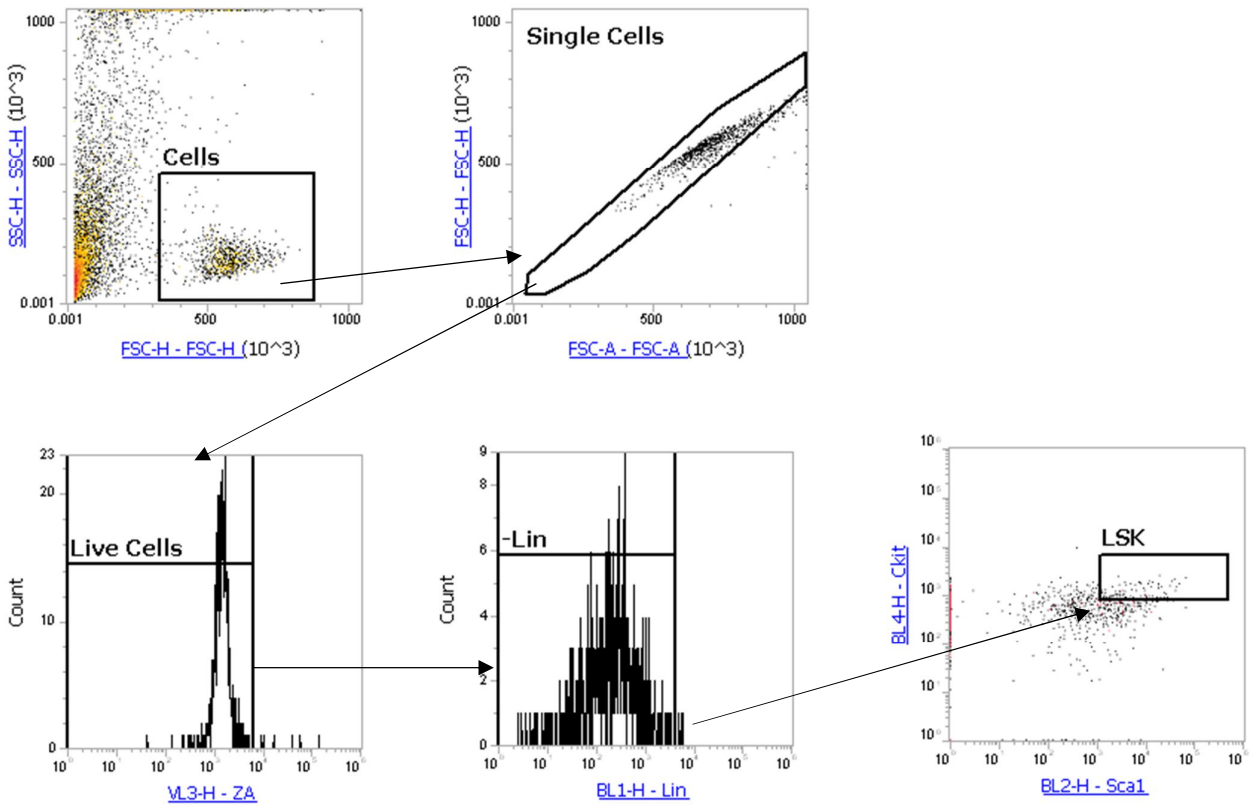

Cells
SSC-H - SSC-H (10^3)
FSC-H - FSC-H (10^3)
Single Cells
FSC-H - FSC-H (10^3)
FSC-A - FSC-A (10^3)
Live Cells
Count
VL3-H - ZA
-Lin
Count
BL1-H - Lin
LSK
BL4-H - CKit
BL2-H - Sca1

Figure S3

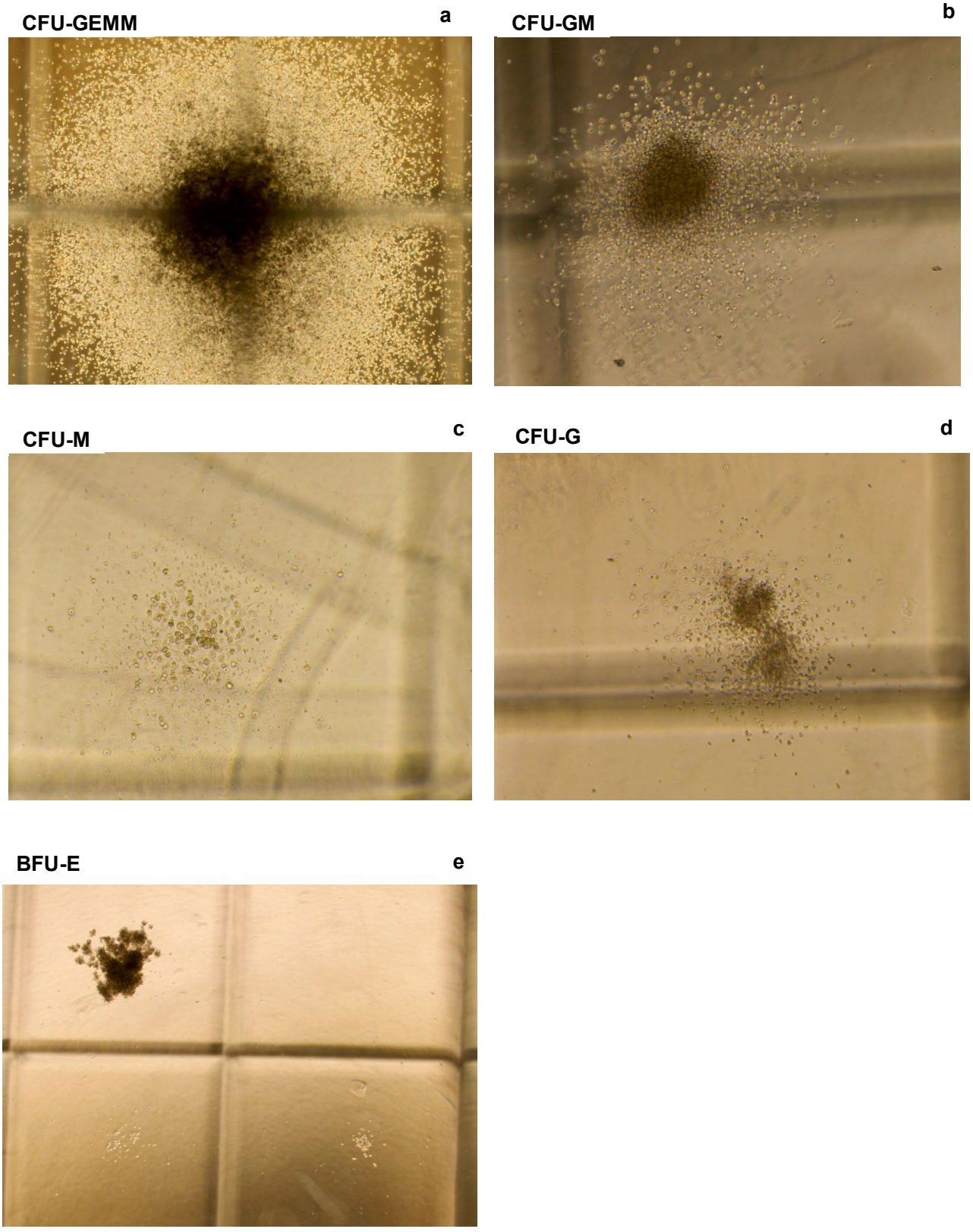


Figure S4

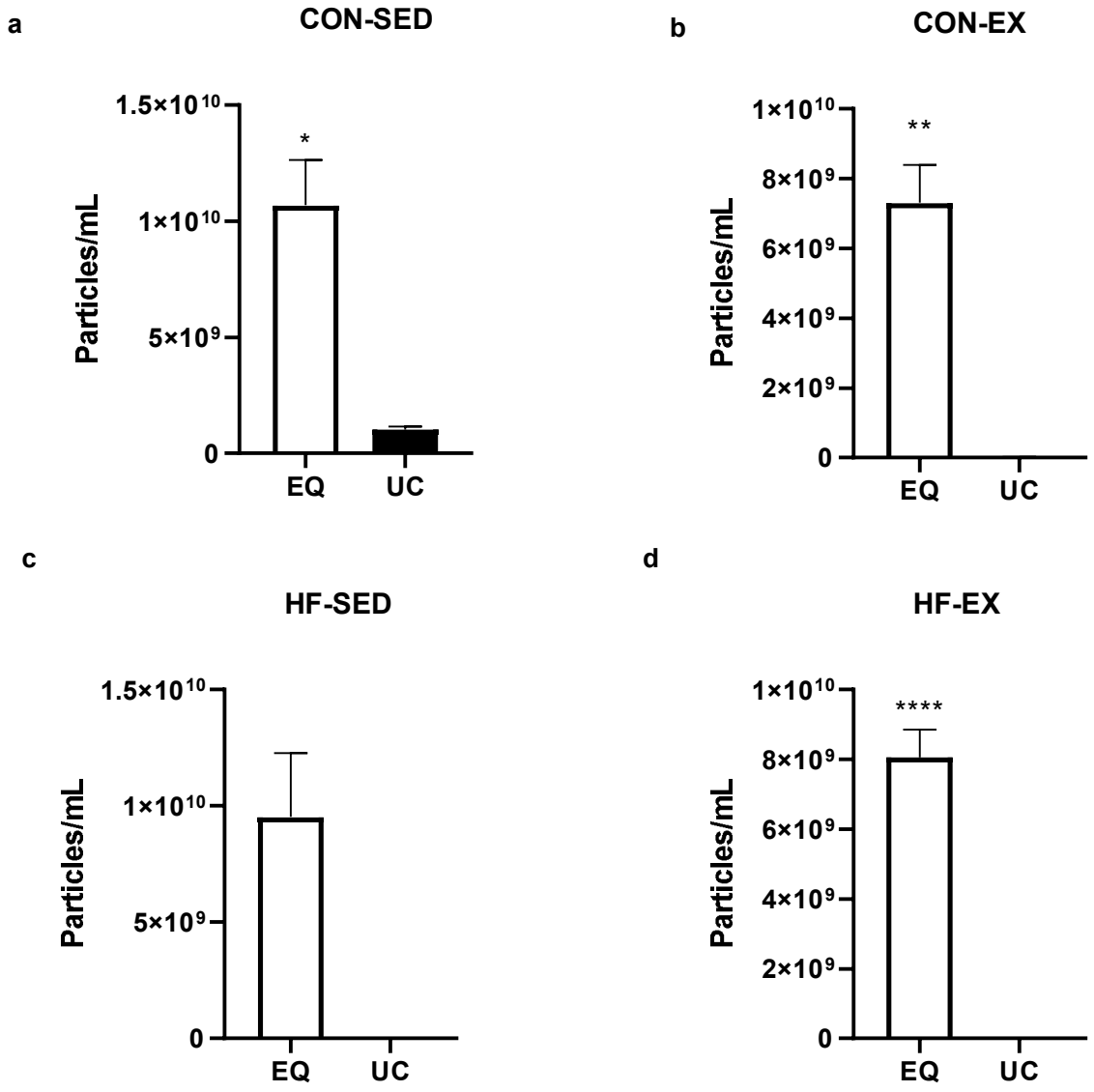

a
CON-SED
Particles/mL
1.5×10^10
1×10^10
5×10^9
0
*
EQ
UC
b
CON-EX
Particles/mL
1×10^10
8×10^9
6×10^9
4×10^9
2×10^9
0
**
EQ
UC
c
HF-SED
Particles/mL
1.5×10^10
1×10^10
5×10^9
0
EQ
UC
d
HF-EX
Particles/mL
1×10^10
8×10^9
6×10^9
4×10^9
2×10^9
0
****
EQ
UC

Figure S5

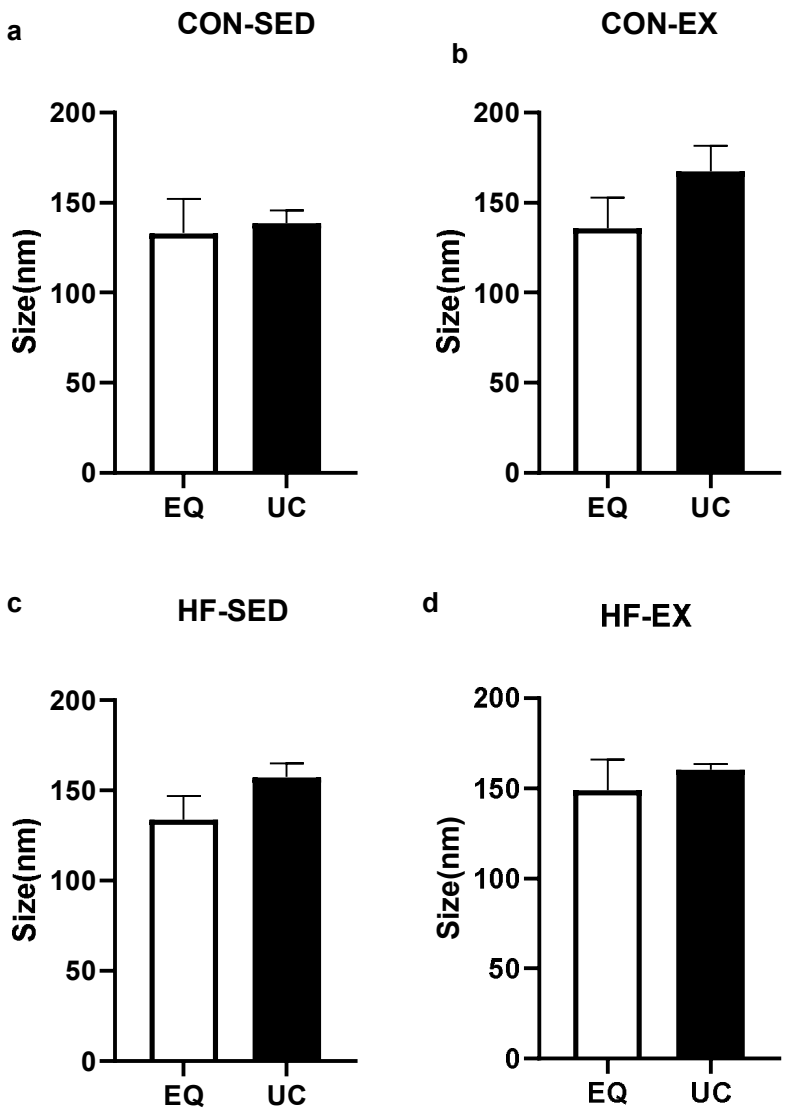


Figure S6

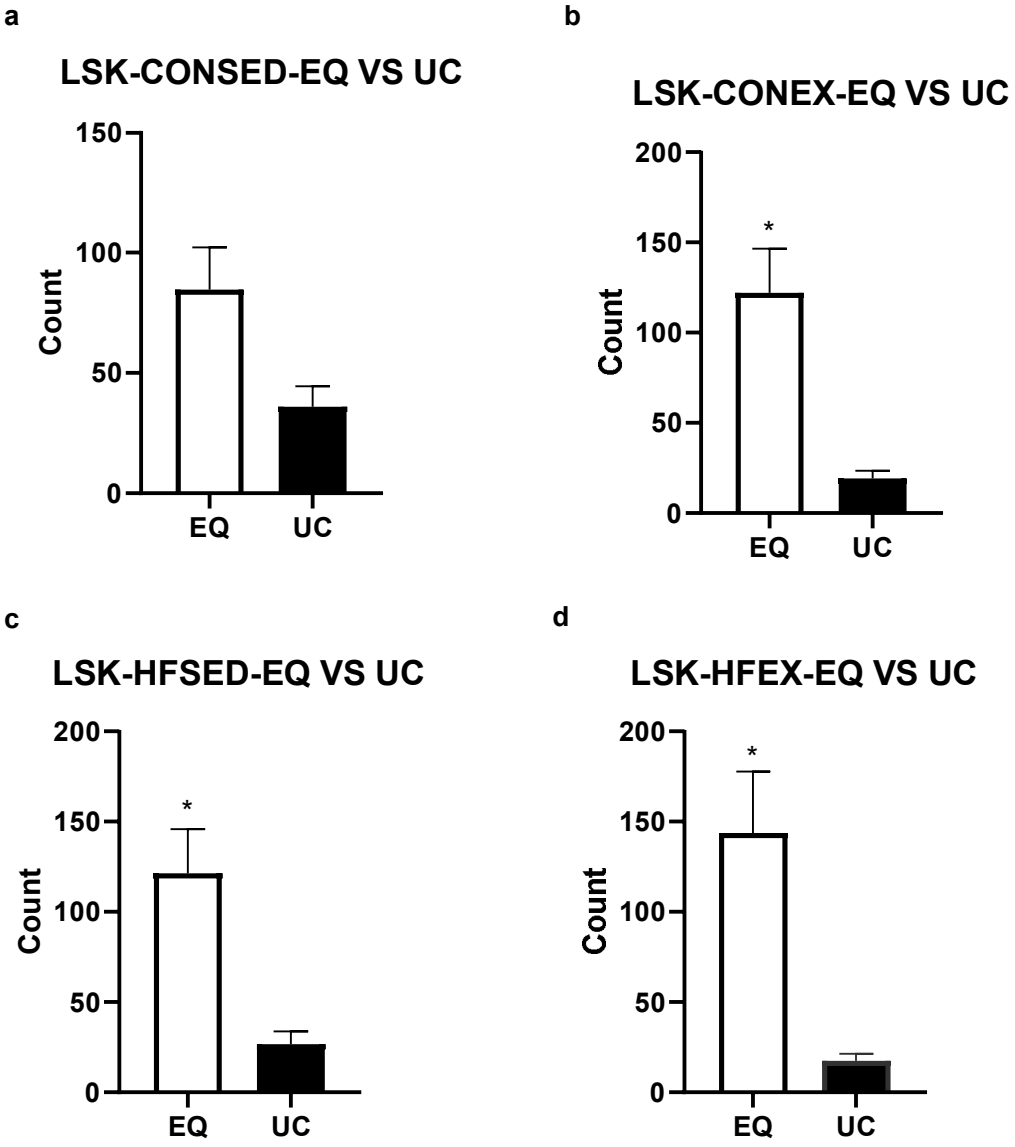
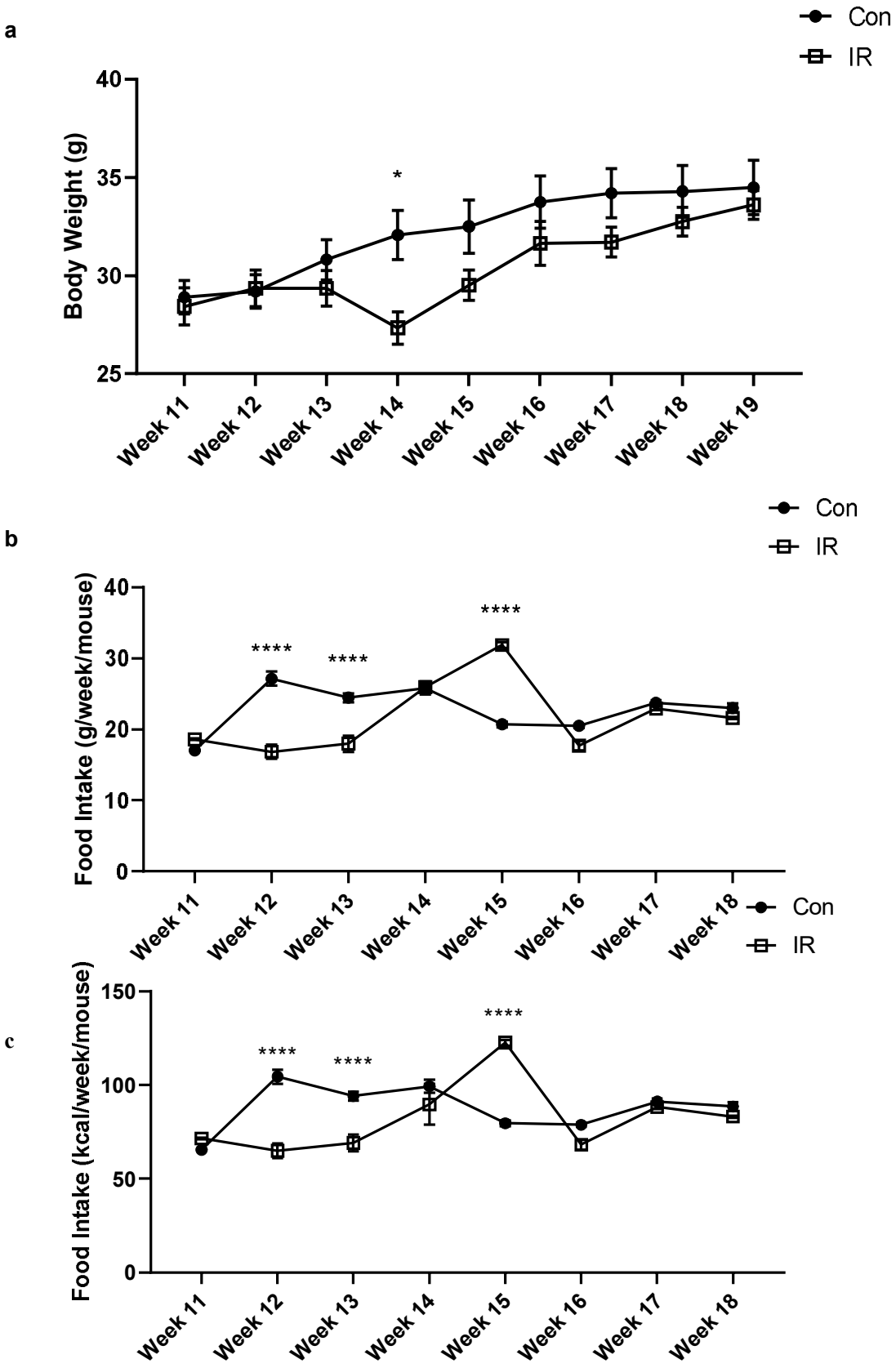
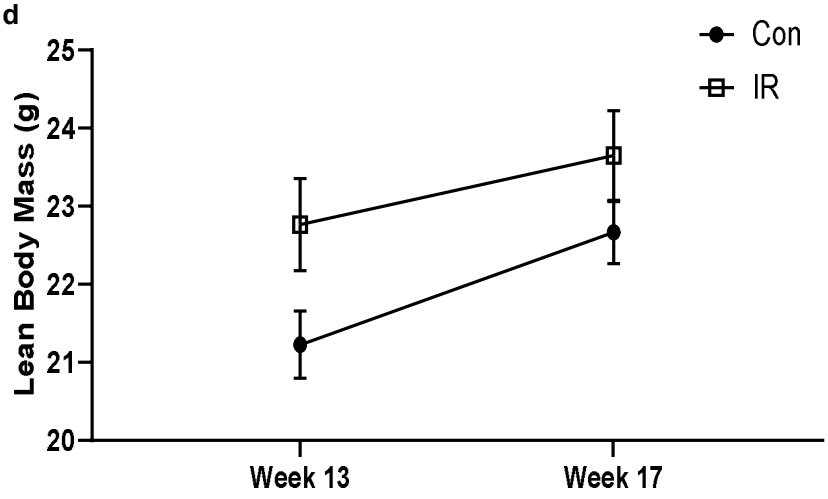

a
LSK-CONSED-EQ VS UC
Count
150
100
50
0
EQ
UC
b
LSK-CONEX-EQ VS UC
Count
200
150
100
50
0
*
EQ
UC
c
LSK-HFSED-EQ VS UC
Count
200
150
100
50
0
*
EQ
UC
d
LSK-HFEX-EQ VS UC
Count
200
150
100
50
0
*
EQ
UC

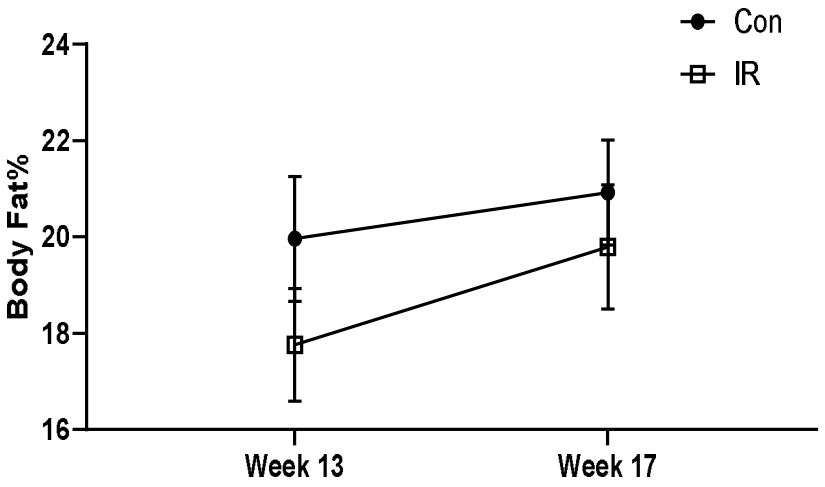
Figure S7


a
Con
IR
Body Weight (g)
40
35
30
25
*
Week 11
Week 12
Week 13
Week 14
Week 15
Week 16
Week 17
Week 18
Week 19
b
Con
IR
Food Intake (g/week/mouse)
40
30
20
10
0
****
****
****
Week 11
Week 12
Week 13
Week 14
Week 15
Week 16
Week 17
Week 18
Con
IR
c
Food Intake (kcal/week/mouse)
150
100
50
0
****
****
****
Week 11
Week 12
Week 13
Week 14
Week 15
Week 16
Week 17
Week 18

d


Con
IR
Lean Body Mass (g)
25
24
23
22
21
20
Week 13
Week 17

e


Con
IR
Body Fat%
24
22
20
18
16
Week 13
Week 17

Appendix B – Diet Composition

Table 1. Formulation of the D10012M diet

Class description	Ingredients	Grams	Kcal
Protein	Casein, Lactic, 30 Mesh	140.00 g	560
Protein	L-Cystine	1.80 g	7.2
Carbohydrate	Corn Starch	495.69 g	1983
Carbohydrate	Maltodextrin 10	125.00 g	500
Carbohydrate	Sucrose, Fine Granulated	100.00 g	400
Fibre	Solka Floc, FCC200	50.00 g	0
Fat	Soybean Oil, USP	40.00 g	360
Mineral	S10022M	35.00 g	0
Vitamin	V10037	10.00 g	40
Vitamin	Choline Bitartrate	2.50 g	0
Anti-oxidant	tert-Butylhydroquinone	0.008 g	0
Total		1000	3850

Table 2. Caloric information of the D10012M diet

Class description	gm%	kcal%
Protein:	14.2	14.7
Fat:	4	9.4
Carbohydrate:	73.1	75.9
Total		100
Kcal/gm	3.85	

Table 3. Formulation of the D12451 diet

Class description	Ingredients	Grams	Kcal
Protein	Casein, Lactic, 30 Mesh	200.00 g	800
Protein	L-Cystine	3.00 g	12
Carbohydrate	Sucrose, Fine Granulated	172.80 g	691
Carbohydrate	Maltodextrin 10	100.00 g	400
Carbohydrate	Corn Starch	72.80 g	291
Fiber	Solka Floc, FCC200	50.00 g	0
Fat	Lard	177.50 g	1598
Fat	Soybean Oil, USP	25.00 g	225
Mineral	S10026B	10.00 g	0
Mineral	DiCalcium Phosphate	13	0
Mineral	Calcium Carbonate	5.5	0
Mineral	Potassium Citrate, 1 H2O	16.5	0
Vitamin	Choline Bitartrate	2.00 g	0
Vitamin	V10001C	10.00 g	40
Dye	Dye, Red FD&C #40, Alum. Lake 35-42%	0.05 g	
Total:		858.15 g	4057

Table 4. Caloric information of the D12451 diet

Class description	gm%	kcal%
Protein:	24	20
Fat:	24	45
Carbohydrate:	41	35
Total		100
Kcal/gm	4.73	

Appendix C – Protocols used in Thesis

Acquisition of bone marrow supernatant for EV isolation

1. Sacrifice mouse by using CO2 and dislocation. Note time of death
2. Spray or dunk mouse with 70% ethanol
3. Dissect mouse and remove bottom half of fur and skin
4. Obtain Femurs and Tibias
5. Remove all non-adherent tissues and store in ice cold PBS, 4mLs in 5mL tube. (Entire length incl. head of femur) from both sides. Remove any attached muscle (via gentle Kim-wipe rubbing). Place bones into 2mL of PBS solution in a 65 mm dish and place on ice
6. Flush bone marrow with 500uL 1xPBS buffer (into a 1.5mL centrifuge tube) until bone is white (no red inside bone) - ~2-4 flushes total
7. Spin at 400g at 4°C for 5mins
8. Save supernatant in a new 1.5mL centrifuge tube and flash freeze using liquid nitrogen
9. Store in -80°C freezer

Isolation of extracellular vesicles by ExoQuick-TC

1. With flushed bone marrow supernatant, centrifuge at 3,000gfor 15 minutes at 4°C
2. Transfer the supernatant to a new tube
3. Add the appropriate volume of ExoQuick-TC to the supernatant
   a. For every 500µL of supernatant add 100µL ExoQuick-TC
4. Mix well by inverting or flicking the tube
5. Incubate on ice for at least 12hrs or overnight at 4°C. The tubes do not need to be rotated during the incubation period.
6. Centrifuge the ExoQuick-TC/biofluid mixture at 3,000gfor 10 minutes at 4°C
7. EVs may appear as a beige or white pellet at the bottom of the tube
8. Carefully aspirate off the supernatant. Spin down any residual ExoQuick solution and remove all traces of fluid by aspiration
9. Do not to disturb the precipitated EVs in the pellet
10. Resuspend the pellet in 200 µl of Buffer B

Isolation of extracellular vesicles by Ultracentrifugation

1. Supernatant from flushed bone marrow is centrifuged at 2,000gfor 20 min at 4°C
2. After 2,000g spin, supernatant was then transferred to new tube, and centrifuged 40 min at 15,000gat 4°C
3. After 15,000g spin, supernatant transferred to a new tube and spun for 90 min at 100,000gat 4°C
4. Pellets was resuspended with 100 µL of sterile 1XPBS
5. Supernatant from final spin was saved

EV Depletion from FBS by Ultracentrifugation

1. Warm up ultracentrifugation machine for 1 hour before using
2. Fill designated ultracentrifuge tube with 3mL of FBS
    - Ensure proper balance in rotor
    - Typically fill 6 tubes, to acquire 18mL at end
3. Set machine for 100,000g for 18 hours at 4°C
4. After an hour has passed, ensure centrifuge is still spinning
    - If not warmed up, machine will stop within an hour
    - Must restart if this occurs
5. Next day, retrieve supernatant
6. Do not disturb the pellet
    - Pellet has appearance of brown jelly like material
7. Filter collected supernatant with a 0.2µm filter
8. FBS can be stored for 1 week at 4°C

EV Protein Analysis

Extraction of Protein from EVs

1. Dilute concentrated Biorad reagent 1:5 in PBS bring to room temperature in the dark
2. Prepare standards and blank
    a. Serial dilution of 2µg/µL BSA stock in PBS
    b. Concentrations: 0, 0.125, 0.25, 0.5, 1, 2
3. Apply equal volume of RIPA buffer supplemented with protease inhibitor
    a. If sample is 100uL add 100uL of RIPA
4. Pipette 5uL of each sample or standard into a 96 well plate
5. Pipette 250µL of 1:5 Biorad reagent into wells
    a. Pop bubbles
6. Place plate in plate reader
7. Open Gen5 software and select “BRAD” program

Western Blot

Day 1:

1. Retrieve samples from -800C; thaw on ice
2. Aliquot sample into pcr tubes
3. Add 7.5uL of loading dye
4. Get ladder from freezer and thaw
5. Load sample into wells
6. Set frames and cassette with short plate facing inwards; remove comb
7. Transfer assemblies to take with red facing the red (+) electrode
8. Fill assembly with 1x Running Buffer; pipette in and out of wells to clean; check for leaks
9. Load samples; standard; and ladder
    a. 10uL ladder

b. see WB excel document for samples and standard volumes to load

10. Fill tank with remaining 1x RB to 4 gels line
11. Set voltage to 90V for 30min @ room temp then set for 120V for 2hours
    a. Turn on; look for bubbles
12. Cut membrane (PVDF) the size of filter paper; activate with methanol for 5mins on shaker- wash approximately 10 times with ddH2O (or until no bubbles appear on the methanol)

13. Make water table; prep gel blot assembly- wet each layer with 1x TB
    a. Black cassette face; sponge; filter paper; gel; membrane; filter paper; sponge; clear cassette face
14. Once step 9 is complete- remove glass plates; carefully remove short glass; remove stacking gel from top; cut the corner with the ladder *keep track of gels
15. Place the gel on the filter paper; then membrane directly on top; use roller to remove air bubbles once you've added filter paper and sponge
16. Black side of cassette to black (-) electrode of tank
    move to cold room
17. Fill tank with 1x TB to the blotting line
18. Run at 120V for 60 min at 40C
19. Disassemble gel; remove membrane; cut the membrane in the same corner as the gel
20. Rinse membrane with dH2O
21. Place in Ponceau buffer for 5 minutes
22. Visualize membrane
23. Rinse Ponceau off with water (3minuts x 4)
24. Block with 5% milk for 60min room temperature on shaker
25. 10Ab: follow manufacturers antibody directions and dilutions depending on the specific sample/proteins – in block; incubate overnight in cold room (40C) on shaker

a. Flotillin 1:1000 in %5 BSA in TBST
b. Alix 1:1000 in %5 BSA in TBST

Day 2:

1. Remove 10Ab
2. Wash 5x3min in 1x TBST . Place on shaker each time
3. 20 HRP conjugated Ab was diluted at 1:10000 in 5% milk. Membrane was incubated for 1hourat room temp
4. Wash 5x3min in 1x TBST
5. ECL detection: use equal amounts of both solutions in kit – 5 mins
6. Remove from ECL and blot on kim wipe – place in plastic sleeve
7. Image on BioRad

a. Colorimetric – auto
b. Chemiluminescence – auto

Removal of Primary:

1. Wash Flottilin membrane with TBST 3x3mimutes
2. The add 7mL of stripping buffer. Place on shaker for 20minutes at room temperature
3. Wash membrane with TBST 3x3mimutes
4. Apply 20 HRP conjugated Ab was diluted at 1:10000 in 5% milk. To ensure no residual primary
5. Wash 5x3min in 1x TBST
6. ECL detection: use equal amounts of both solutions in kit – 5 mins
7. Remove from ECL and blot on kim wipe – place in plastic sleeve
8. Image on BioRad
    1. Colorimetric – auto
    2. Chemiluminescence – auto
9. After imaging block with 5% milk for 60min room temperature on shaker
10. 10Ab: follow manufacturers antibody directions and dilutions depending on the specific sample/proteins – in block; incubate overnight in cold room (40C) on shaker

1. TSG101 1:1000 in %5 BSA in TBST

Day 3:

1. Remove 10Ab
2. Wash 5x3min in 1x TBST . Place on shaker each time
3. 20 HRP conjugated Ab was diluted at 1:10000 in 5% milk. Membrane was incubated for 1hourat room temp
4. Wash 5x3min in 1x TBST
5. ECL detection: use equal amounts of both solutions in kit – 5 mins
6. Remove from ECL and blot on kim wipe – place in plastic sleeve
7. Image on BioRad
   a. Colorimetric – auto
   b. Chemiluminescence – auto

Preparation of EV samples for Nanoparticle-tracking analysis

1. Turn on Zetaview machine first. Allow to warm up for 5 minutes
2. Open Zetaview software
3. Press cancel on first pop up
4. Wash system using ddH2O
   a. Use 10mL syringe to push water through
   b. Aim for less than 10 particles
5. Click cell quality check
6. Focus machine: by injecting bead sample 1mL
   a. Inject 700µL
7. Set exposure value. Samples from animal is 80-92
8. Clear system with ddH2O
9. Inject 700µL of sample
   a. Depending on sample, ensure to dilute no less than 1:100
   b. Remember to incorporate this value when calculating final concentration
10. Analyze sample
11. Select file location to be saved
12. Select the default SOP
13. Frame rate “15” for measuring microvesicle and “30” for exosome
14. Ensure the exposure value is correct
15. Then click analyse
16. Wash machine with ddH2O between every sample

Hematopoietic Stem Progenitor Cell Isolation

Mouse Dissection to obtain Bone Marrow Cells

1. Sacrifice mouse by using CO2 and dislocation. Note time of death
2. Spray or dunk mouse with 70% ethanol
3. Dissect mouse and remove bottom half of fur and skin
4. Obtain Femurs and Tibias
5. Remove all non-adherent tissues and store in ice cold PBS, 4mLs in 5mL tube. (Entire length incl. head of femur) from both sides. Remove any attached muscle (via gentle Kim-wipe rubbing). Place bones into 2mL of PBS solution in a 65 mm dish and place on ice
6. Flush bone marrow with 500uL 5%FBS buffer until bone is white (no red inside bone) - ~2-4 flushes total

Preparation of cells for FACS or Flow Analysis (LSK Stain)

1. Aliquot samples into respective tubes (Table 1)
2. Spin down sample – 400g x 4°C x 5mins
3. Remove supernatant. Re-suspend cell in appropriate cocktail (see Table 1)
4. Incubate on ice for 1hourin dark
5. Quench with 1mL of PBS
6. Spin at 400g x 4°C x 5mins
7. Remove supernatant. Re-suspend in 200uL of diluted streptavidin-FITC
8. Incubate on ice for 1hourin dark
9. Quench with 1mL of PBS
10. Spin at 400g x 4°C x 5mins
11. Remove supernatant. Re-suspend cells in 2mL 5%FBS
12. Filter cells through a 50um filter into a 5ml round-bottom polypropylene tube (Falcon 352063)
13. Place samples on ice and cover samples to keep in dark
14. Bring samples to FACS or Flow machine to be sorted

Bring extra 5%FBS to dilute sample if sample is found to be to concentrated

Sample tubes to be prepared

Sample Name	Dilution (In 5% FBS)
Unstained	N/A
Lineage – Single (1uL of each AB)	1:200
Sca 1 – Single	1:200
Ckit – Single	1:200
Streptavidin-FITC	1:800
Live/Dead (100uL per sample)	1:300
Full Stain	1:200 (per ab)

Coculture Assay

Culture Media

StemSpan SFEM Medium (STEMCELL Technologies)
15% vesicle depleted FBS
1% penicillin/streptomycin
Ms Recom SCF 100 ng/mL
Ms Recom TPO 100 ng/mL
Ms Recom Flt3/Flk-2 Ligand 100 ng/mL

LSK Cell Culture

1. Sort HSPC cells into tubes filled with 3mL of culture media + 1% Pen/Strep
2. Plate 1500 HSPC cells into 48 well plate
3. Add 100µL EVs to each well
4. Top up well to 1mL with culture media
5. Culture for 48hours at 37°C 5% CO2

Cell Harvest

1. After 48hour culture, retrieve suspended cells from wells, and aliquot in respective 1 5mL tube

2. Rinse each well with 1mL of StemSpan SFEM Medium
3. Centrifuge cells at 400g for 5-10 min at 4°C
4. Prepare cells according to “Preparation of cells for FACS (LSK Stain)”

Colony-forming assay

1. After 48hour culture, retrieve suspended cells from wells, and aliquot in respective 15mL tube
2. Rinse each well with 1mL of StemSpan SFEM Medium
3. Centrifuge cells at 400g for 5-10 min at 4°C
4. Count cells using CellCountess
    - 10uL of Cell + 10uL tryphan blue. Mix
    - Dispence 10uL into the CellCountess slide
    - Let slide settle for 30 seconds
    - Insert into countess
    - (Dilute cell sample if concentrated)
5. Keeps cells on ice
6. Thaw the required number of pre-aliquoted tubes of complete MethoCult™ medium at room temperature (15 - 25°C) or overnight at 2 - 8°C
7. Prepare culture dishes by placing 2 x 35 mm culture dishes with lids inside a 100 mm Petri dish with a lid. Add a third 35 mm culture dish without a lid as a water dish. This set of dishes is sufficient for one duplicate assay
8. Dilute the cells with IMDM + 2% FBS (or IMDM supplemented with 0.1% BSA for serum-free conditions) to 10X the final concentration(s) required for plating. We will be plating 1000 cells

Example: To achieve a final plating concentration of 1 x 103 cells per dish, prepare a cell suspension of 1 x 104cells per mL. When it is difficult to anticipate the correct plating cell concentration, use 2 or more cell concentrations that differ by 2- to 3-fold. Example: 2 x 103 cells per dish and 1 x 103 cells per dish.

9. For a duplicate assay, ADD 0.3 mL of diluted cells to a pre-aliquoted 3 mL MethoCult™ tube. For a triplicate assay, add 0.4 mL of diluted cells to a 4 mL MethoCult™ tube.This 1:10 (v/v) ratio of cells:medium gives the correct viscosity to ensure optimal CFU growth and morphology.
10. Vortex the tube vigorously to mix the contents thoroughly
11. Let the tube stand for at least 5 minutes to allow the bubbles to rise to the top
12. To dispense the MethoCult™ mixture containing cells into culture dishes, attach a sterile 16 gauge Blunt-End Needle to a sterile 3 mL luer lock syringe
    Note: For each tube plated, use a new sterile disposable 3 mL syringe fitted with a new 16 gauge
    Blunt-End Needle to prevent contamination between samples
    Note: Do not use pipettes to dispense methylcellulose as the volume dispensed will not be accurate. Syringes and large bore blunt-end needles should be used for accurate dispensing of viscous methylcellulose medium and to prevent needle-stick injuries

13. To expel the air from the syringe, place the needle below the surface of the solution and draw up approximately 1 mL. Gently depress the plunger and expel medium completely. Repeat until no air space is visible

14. Draw up the MethoCult™ mixture containing cells into the syringe and dispense a volume of 1.1 mL into each 35 mm dish as follows:
While holding the syringe containing the MethoCult™ and cells in one hand, remove the lid of a 35 mm dish with the opposite hand. Position the syringe over the center of the dish without touching the syringe to the dish. Dispense 1.1 mL and replace the lid

Note: Do not expel the medium to the "0" mark on the syringe when dispensing. For example, measure from 1.5 mL to 0.4 mL rather than 1.1 mL to 0 mL.

15. Distribute the medium evenly across the surface of each 35 mm dish by gently tilting and rotating the dish to allow the medium to attach to the wall of the dish on all sides
Note: If any medium contacts the lid of the 35 mm dish while distributing the medium across the surface of the dish, replace the lid to minimize the risk of contamination

16. Place the culture dishes into the outer dish (e.g. 100 mm Petri dish, 245 mm square dish or other cultureware of an appropriate size with a loose-fitting lid). Add approximately 3 mL of sterile water to the uncovered 35 mm dish(es).
Note: Using a 100 mm Petri dish with lid (or other cultureware with a loose-fitting lid) and water dish(es) helps maintain humidity and minimize contamination during culture and handling
17. Incubate at 37°C, in 5% CO2 with ≥ 95% humidity. Incubation times are dependent on the MethoCult™ medium formulation. MethoCult™ GF M3434 7-10 Days for Note: Proper culture conditions are critical for optimal CFU growth. Use of water-jacketed incubators with water pan in chamber and routine monitoring of temperature and CO2 levels is recommended. A suitable additive (i.e. copper sulfate crystals) can be added to the water pan to inhibit microbial growth.
18. If cultures cannot be counted after the recommended incubation time, refill water dishes, if required, and transfer cultures to an incubator maintained at 33°C in 5% CO2 with ≥ 95% humidity. Colony growth will be slowed; count as soon as possible, preferably within 1 week
Note: Most CFUs will have reached a maximal size (cells per colony) by the recommended incubation time. The lower incubation temperature will not completely inhibit proliferation or prevent cell death but will assist in maintaining colony morphology.

EV Uptake

Labelling EVs with PKH67

1. Obtain isolated extracellular vesicles, obtained from specific isolation technique (ExoQuick or Ultracentrifugation)
2. 100uL of Diluent C is added to the extracellular vesicles
3. In a separate microcentrifuge tube, 1µl of PKH67 dye is added to 100uL of Diluent C
4. Add extracellular vesicles + Diluent C solution to the Diluent C + PKH67 solution
5. Mix thoroughly with gentle pipetting (up and down)
6. Incubate at room temperature for 4mins. No more than 5 minutes
7. Add 100uL of 10% exosome-depleted fetal bovine serum in DMEM OR IMDM to bind the excess dye
8. Conduct ultracentrifugation for at 1hour@ 100,000g@ 4°C
9. Remove supernatant. Resuspend with 1mL of 1XPBS and spin at 1hour@ 100,000g@ 4°C

10. Remove supernatant. Resuspend with 1mL of 1XPBS and spin at 1hour@ 100,000g@ 4°C
11. Resuspend in 100µL of PBS
12. Stained extracellular vesicles can now be used for co-culture
    Avoid long-term storage of stained extracellular vesicles
    Store at -20°C or 2-8°C on ice, no longer than 24hrs
    Co-Culture ofHSPCwith Labelled EVs

1. LSK cell which had been stained as previously mentioned, were FACS sorted
2. Subsequently, after sorting,1500 HSPC cells were plated in a 48-well plate supplemented with StemSpan SFEM Medium (STEMCELL Technologies) supplemented with 15% vesicle depleted FBS, 1% penicillin/streptomycin, Ms Recom SCF, 100 ng/mL, Ms Recom TPO, 100 ng/mL and Ms Recom Flt3/Flk-2 Ligand, 100 ng/mL.
3. The cells were placed in the incubator for two hours, at 37°C and 5% CO2
4. 100µL of PKH67 stained EVs were combined with HSPC cells after two hours
5. Uptake was visualized using the Celldiscoverer 7 and images were taken within the first hour