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Sirtuins in Mitochondrial Protein Folding Stress and Neuroinflammation

by

Andrew Widjaja

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Endocrinology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Gian Garriga, Chair
Professor Ellen Robey
Professor Laurent Coscoy

Summer 2022

Abstract

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Systemic aging and neurodegeneration are characterized by the progressive accumulation of aberrant molecules and cellular damage. Tissue homeostasis deteriorates due to stem cell exhaustion, chronic inflammation, and metabolic dysfunction, among other causes. In the following chapters, I present evidence of the beneficial roles that sirtuins (SIRT1-7), NAD^+-dependent deacetylases, play in alleviating these conditions. Further understanding of the molecular mechanisms associated with these conditions would provide potential targets of therapeutic intervention for the alleviation of debilitating diseases, such as hematopoietic stem cell dysfunction and multiple sclerosis.

In Chapter Two, we uncover the importance of the mitochondrial unfolded protein response (UPR^{mt}) in hematopoietic stem cell maintenance. The UPR^{mt} is a cellular protective program that ensures proteostasis in the mitochondria, and it is known to be regulated by SIRT7. We devised three experimental approaches that enabled us to monitor quiescent and proliferating hematopoietic stem cells (HSCs) and provided direct evidence that the UPR^{mt} is activated upon HSC transition from quiescence to proliferation, and more broadly, mitochondrial integrity is actively monitored at the restriction point to ensure metabolic fitness before stem cells are committed to proliferation.

In Chapter Three, I use a mouse model of progressive multiple sclerosis (MS), a chronic autoimmune disease of the central nervous system (CNS), and single-nuclei RNA seq data from MS patients to demonstrate that SIRT2 deficiency may drive an inflammatory phenotype within progressive MS lesions. More importantly, I show that NAD^+ boosting may alleviate MS pathology.

These findings further emphasize the importance of understanding sirtuin biology for the control of homeostasis across multiple systems, such as the hematopoietic and central nervous systems.

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Acknowledgements

A portion of the Introduction was adapted from the journal of Mechanisms of Ageing and Development:
Mu WC, Ohkubo R, Widjaja A, Chen D. The mitochondrial metabolic checkpoint in stem cell aging and rejuvenation. Mech Ageing Dev. 2020;188:111254. doi:10.1016/j.mad.2020.111254

Chapter 2 was adapted from the journal of Aging Cell:
Mohrin M*, Widjaja A*, Liu Y, Luo H, Chen D, 2018. The mitochondrial unfolded protein response is activated upon hematopoietic stem cell exit from quiescence. Aging cell 17, e12756.

Chapter 1. Introduction

1.1 Sirtuins

Sirtuins are NAD^+-dependent class III histone deacetylases (HDACs) that have first been shown to promote health and longevity in yeast (Rogina et al., 2004; Lin et al., 2000; Anderson et al., 2003). In mammals, there are seven sirtuins, SIRT1-7, which localize in different cellular compartments where they combat a wide variety of cellular stressors such as oxidative stress, mitochondrial stress, inflammation, and DNA damage (Bosch-Presegué and Vaquero 2013). SIRT1, SIRT6, and SIRT7 are localized within the nucleus where they influence epigenetics by deacetylating histones (Guarente, 2013). SIRT2 is a cytosolic sirtuin that has been found to modulate cell cycle control and inflammatory responses (Dryden et al., 2003; He et al., 2020). SIRT3-5 act as energy sensors within the mitochondria to regulate metabolic enzymes in order to increase oxidative stress tolerance (Verdin et al., 2010). While the pathways in which sirtuins are involved in vary greatly, the common theme is that sirtuins regulate the aging process. Here, we focus our attention on SIRT7 and SIRT2 and their roles in alleviating mitochondrial stress and neuroinflammation, respectively.

1.2 SIRT7 in Stem Cell Aging and Mitochondrial Maintenance

Stem cell aging contributes to aging-associated tissue degeneration and dysfunction. Recent studies reveal a mitochondrial metabolic checkpoint that regulates stem cell quiescence and maintenance, and dysregulation of the checkpoint leads to functional deterioration of aged stem cells. Here, we present evidence supporting the mitochondrial metabolic checkpoint's role in regulating stem cell aging and demonstrate the feasibility to target this checkpoint to reverse stem cell aging. We discuss the mechanisms by which mitochondrial stress leads to stem cell deterioration. We speculate the therapeutic potential of targeting the mitochondrial metabolic checkpoint for rejuvenating aged stem cells and improving aging tissue functions.

The stem cell theory of aging postulates that aging is the result of the inability of tissue-specific stem cells to repair and maintain tissues. A large body of evidence supports the notion that stem cell functions decline with aging, consistent with the degeneration and dysfunction of aging regenerative tissues (de Haan and Lazare, 2018; Lopez-Otin et al., 2013; Rossi et al., 2008). These observations suggest the importance of stem cells in understanding the biology of aging and in developing effective therapies for treating diseases of aging. Genetic regulators of aging are critical for stem cell maintenance (Chen et al., 2008; Juntilla et al., 2010; Miyamoto et al., 2007; Tothova et al., 2007). Prominently, some genetic regulators of aging become dysregulated during aging and reversion of such dysregulations has been shown to improve the maintenance and function of aged stem cells (Brown et al., 2013; Luo et al., 2017; Mohrin et al., 2015). These findings strongly support the stem cell theory of aging and provide an entry point for understanding the molecular mechanism of stem cell aging and the feasibility of rejuvenating aged stem cells and reversing aging-associated tissue degeneration.

Stem cells in many tissues convert between two metabolically distinctive states: metabolically inactive quiescent state and metabolically active proliferative state. In adult animals, stem cells are mostly quiescent due to a lack of physiological demand to proliferate. Compelling evidence indicates that stem cell quiescence is also a protective mechanism to prevent stem cell death and the depletion of the stem cell pool (Cheung and Rando, 2013; Ren et al., 2017). Quiescent stem cells have fewer mitochondria and primarily rely on glycolysis to

support the energy demand (Ito and Suda, 2014). Upon the transition from quiescence to proliferation, mitochondrial biogenesis occurs and OXPHOS is upregulated to meet the increased energy demand of proliferating cells (Mohrin et al., 2018). This metabolic switch from glycolysis to OXPHOS is also necessary to support stem cell differentiation (Anso et al., 2017; Inoue et al., 2010; Khacho et al., 2016; Tang et al., 2016; Tormos et al., 2011; Vannini et al., 2016; Zhang et al., 2013; Zheng et al., 2016).

Increased mitochondrial biogenesis during the transition from stem cell quiescence to proliferation is inevitably concomitant with increased mitochondrial stress, such as mitochondrial oxidative stress and mitochondrial protein folding stress, which makes the cells prone to death (Mohrin et al., 2018). Thus, mitochondrial health needs to be monitored during the transition from the G0 to G1 phase of the cell cycle, at a cell cycle checkpoint known as the restriction point. Because cells are committed to proliferation once they pass the restriction point, monitoring the mitochondrial health at the restriction point ensures that only cells with healthy mitochondria and metabolic competence can enter the cell cycle.

Recent studies revealed sirtuins, a family of protein deacetylases, as critical regulators of the mitochondrial metabolic checkpoint in stem cells. SIRT3 is a mitochondrial deacetylase that modifies mitochondrial antioxidant SOD2 to reduce oxidative stress (Qiu et al., 2010). It is highly expressed in hematopoietic stem cells (HSCs) and its expression is suppressed in differentiated hematopoietic cells (Brown et al., 2013). SIRT3 deletion results in loss of HSC quiescence and compromised HSC maintenance and function at an old but not young age. SIRT7 represses the expression of mitochondrial ribosomal proteins to regulate the mitochondrial unfolded protein response and to reduce mitochondrial protein folding stress (Mohrin et al., 2015; Shin et al., 2013). SIRT7 deletion leads to loss of HSC quiescence and aspects of HSC aging phenotypes, such as reduced regenerative capacity per cell and myeloid-biased differentiation (Mohrin et al., 2015). Interestingly, the expression of SIRT3 and SIRT7 is reduced in HSCs during aging, and overexpression of SIRT3 or SIRT7 improves the function of aged HSCs (Brown et al., 2013; Chambers et al., 2007; Mohrin et al., 2015). These studies demonstrate that dysregulation of the mitochondrial metabolic checkpoint is an underlying cause of stem cell aging and may be targeted for rejuvenation.

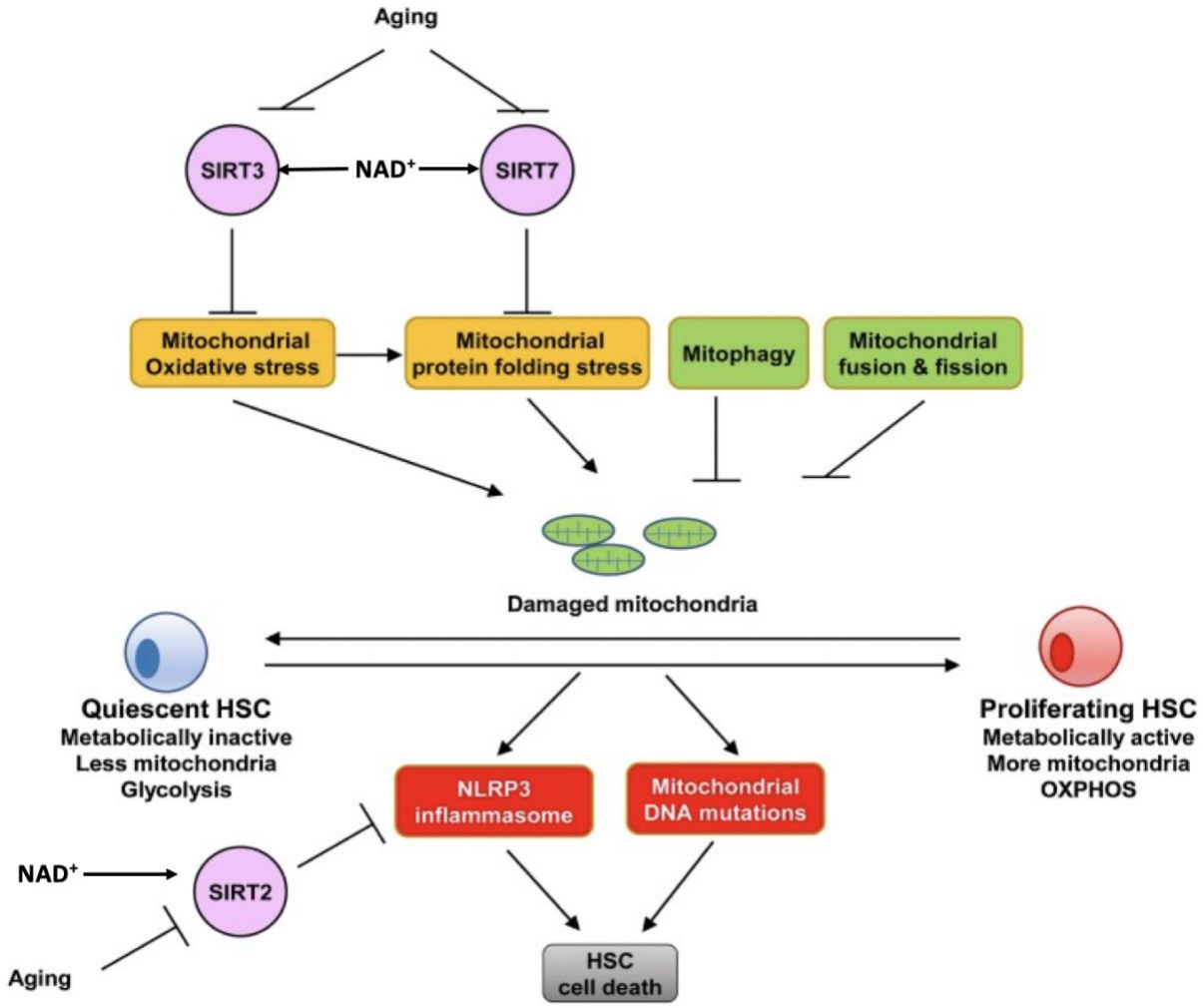

Fig. 1. The mitochondrial metabolic checkpoint regulates stem cell quiescence, maintenance, and aging. Upon stem cell transition from quiescence to proliferation, mitochondrial biogenesis takes place, which is associated with increased mitochondrial oxidative stress and mitochondrial protein folding stress. SIRT3 and SIRT7 govern mitochondrial stress in stem cells. Damaged mitochondria can also be cleared by mitochondrial fusion and fission, and mitophagy. Accumulation of mitochondrial stress results in stem cell death due to DNA mutations and activation of the NLRP3 inflammasome, which is regulated by SIRT2. During aging, the expression of SIRT2, SIRT3, and SIRT7 reduces in stem cells, resulting in the dysregulation of the mitochondrial metabolic checkpoint and loss of stem cell maintenance.

1.3 SIRT2 and Multiple Sclerosis

Multiple sclerosis (MS) is a chronic auto-inflammatory disease of the central nervous system (CNS) affecting motor, cognitive, sensory and bowel functions in patients. About 85% of MS patients experience a relapsing-remitting course of the disease defined by episodes of neurologic disability followed by partial or full recovery, while approximately 15% of patients have a progressive course from onset (Faissner et al., 2019; Reich et al., 2018). There has been much progress in the treatment of relapsing-remitting multiple sclerosis (RRMS); however, these treatments have proven to be insufficient for chronic progressive MS as they provide only partial protection against the neurodegenerative factor of the disease (Fitzner et al., 2010;

Hauser et al., 2020). To date, the anti-CD20 targeted B-cell depleting antibody ocrelizumab is the only approved disease-modifying therapy for the treatment of primary progressive MS (Montalban et al., 2017). Therefore, a better understanding of the progressive form of MS and the discovery of new therapeutic targets are needed.

Advancing age is known to significantly influence clinical disease course and pathological processes implicated in progressive MS (Mahad et al., 2015; Minden et al., 2004; Sanai et al., 2016; Scalfari et al., 2011). Diffuse axonal injury and demyelination increase with age and are, thus, more pronounced in patients with progressive disease compared to those with RRMS (Lassmann et al., 2018). Inflammatory demyelination stemming from autoreactive immune cells plays an important role in driving MS pathology and the development of experimental autoimmune encephalomyelitis (EAE), an animal model of MS (Reich et al., 2018; Constantinescu et al., 2011). However, how aging-associated chronic inflammation may exacerbate CNS damage during progressive MS and how it can be alleviated remain important outstanding questions.

Recent investigations have indicated that inflammasomes contribute to the etiology of MS (Govindarajan et al., 2020). The NLR family, caspase activation and recruitment domain (CARD)-containing 3 (NLRP3) sensor has been most closely examined of all inflammasome sensor molecules (Imani et al., 2018; Malhotra et al., 2018; Olcum et al., 2020; Soares et al., 2019). Aberrant activation of the NLRP3 inflammasome has been implicated in the pathogenesis of MS with a particularly heightened activity in progressive MS compared to other forms of the disease (Malhotra et al., 2020; Piancone et al., 2018). Therefore, development of treatments that target inflammasome activation may alleviate chronic inflammatory events and improve functional outcomes of progressive MS patients.

Sirtuin 2 (SIRT2), an NAD^+-dependent deacetylase, is highly expressed in the central nervous system (CNS) and has been reported to regulate a variety of processes including inflammation, metabolism and aging (Fourcade et al., 2018; He et al., 2020; Lee et al., 2019; Luo et al., 2019; Lo Sasso et al., 2014; Misawa et al., 2013). SIRT2 represses NLRP3 inflammasome activation by deacetylating NLRP3 and tubulin, thus preventing inflammasome assembly and transport (He et al., 2020; Misawa et al., 2013). Recent studies have also established a connection between SIRT2 and age-related neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) (Fourcade et al., 2018; Manjula et al., 2020; Yuan et al., 2016). Although SIRT2 has been shown to affect neurological function, oligodendrocyte differentiation and formation of myelin sheath, its focus in the context of MS in particular remains inadequate.

Increasing NAD^+ levels is emerging to be an effective approach to activate sirtuins. CD38, an NAD^+-consuming enzyme, is upregulated as a consequence of aging and neuroinflammation, leading to the decline of NAD^+ and sirtuin activity (Camacho-Pereira et al., 2016; Chini et al., 2020; Guerreiro et al., 2020; Herrmann et al., 2016; Langley et al., 2021; Roboon et al., 2019). 78c is a small molecule CD38 inhibitor and is proving to be a promising NAD^+-boosting therapeutic agent that activates sirtuin signaling-mediated physiological functions (Hogan et al., 2019).

1.4 Benefits of NAD^+-boosting

Tissue regeneration becomes compromised during aging, often originating from mitochondrial dysfunction in adult stem cells; therefore, targeting mitochondrial quality control mechanisms represents a potential treatment strategy for certain age-related diseases. Nicotinamide adenine dinucleotide (NAD^+) is a crucial molecule serving as a regulator of mitochondrial homeostasis, neuroprotection, genomic stability, healthspan and lifespan (Lautrup et al., 2019; Fang et al., 2017; Verdin, 2015; Lou et al., 2019). NAD^+ levels decrease with age and is linked to various age-related pathologies such as skin cancer, cardiovascular disease and neurodegenerative diseases (Johnson et al., 2018; Hershberger et al., 2017; Penberthy et al., 2009). Pharmacological and/or genetic upregulation of NAD^+ levels improves mitochondrial function and alleviates aging deficits in post-mitotic adult tissue, such as the brain (Kerr et al., 2017; Penberthy et al., 2009). While the role of NAD^+ in regulating post-mitotic tissue aging is well established, its role in stem cell aging has only recently gained attention.

Replenishing NAD^+ levels by dietary nicotinamide riboside (NR), a key NAD^+ precursor, increased sirtuin activity, reduced mitochondrial stress within HSCs, and improved the regenerative function of HSCs (Vannini et al., 2019). It significantly expanded the pool of progenitors, without concurrent HSC exhaustion, boosted survival by 80%, and accelerated blood reconstitution after lethal irradiation and HSC transplantation. NR treatment induced the mitochondrial unfolded protein response in aged MuSCs, and mitigated muscle degeneration by decreasing MuSC senescence (Zhang et al., 2016). Treatment with NR was also effective in preventing muscle degeneration in a mouse model of muscular dystrophy. Treatment with NR rejuvenated intestine stem cells (ISCs) from aged mice and reversed an impaired ability to repair gut damage (Igarashi et al., 2019), attenuates NSC senescence and increases the lifespan of aged C57BL/6J mice (Zhang et al., 2016). These findings indicate that the mitochondrial metabolic checkpoint regulation of stem cell aging is a conserved mechanism across tissues and offer a translational path for maintaining tissue homeostasis and treating tissue degenerative diseases.

Chapter 2. The mitochondrial unfolded protein response is activated upon hematopoietic stem cell exit from quiescence

In the previous chapter, I introduced the current knowledge behind sirtuin biology in the context of stem cell aging and neurodegenerative diseases. In this chapter, I will showcase findings that suggest the activation of the mitochondrial unfolded protein response (UPR^{mt}) during hematopoietic stem cell proliferation.

2.1 Abstract

The mitochondrial unfolded protein response (UPR^{mt}), a cellular protective program that ensures proteostasis in the mitochondria, has recently emerged as a regulatory mechanism for adult stem cell maintenance that is conserved in multiple tissues. Despite the emerging genetic evidence implicating the UPR^{mt} in stem cell maintenance, the underlying molecular mechanism is unknown. While it has been speculated that the UPR^{mt} is activated upon stem cell transition from quiescence to proliferation, direct evidence is lacking. In this study, we devised three experimental approaches that enable us to monitor quiescent and proliferating hematopoietic stem cells (HSCs) and provided direct evidence that the UPR^{mt} is activated upon HSC transition from quiescence to proliferation. Our results suggest that mitochondrial integrity is actively monitored at the restriction point to ensure metabolic fitness before stem cells are committed to proliferation.

2.2 Results

Adult stem cells persist throughout the entire lifespan of an organism to repair tissue damage and maintain tissue homeostasis. Among their evolved adaptations are elaborate cellular protective programs that ensure stem cell integrity, tissue homeostasis, and organismal survival (Biteau, Hochmuth & Jasper, 2008; Brown et al., 2013; Ito et al., 2004; Rando, 2006; Renault et al., 2009; Rossi, Jamieson & Weissman, 2008; Rossi et al., 2007; Sahin & Depinho, 2010; Sperka, Wang & Rudolph, 2012; Walter et al., 2015). The mitochondrial unfolded protein response (UPR^{mt}), a cellular protective program that ensures proteostasis in the mitochondria, has recently emerged as a regulatory mechanism for adult stem cell maintenance that is conserved across tissues (Berger et al., 2016; Mohrin et al., 2015; Zhang et al., 2016). This protective program is dysregulated during physiological aging, contributing to the functional deterioration of stem cells, tissue degeneration, and shortened organismal lifespan (Mohrin et al., 2015; Zhang et al., 2016). In addition to the UPR^{mt}, deregulation of compensatory mitochondrial protective programs such as mitophagy and mitochondrial dynamics leads to compromised stem cells, further underscoring the importance of mitochondrial integrity in stem cell maintenance (Ho et al., 2017; Ito et al., 2016; Luchsinger, de Almeida, Corrigan, Mumau & Snoeck, 2016; Vannini et al., 2016).

Despite the emerging genetic evidence implicating the UPR^{mt} in stem cell maintenance, the underlying molecular mechanism is unknown. The UPR^{mt} is a cellular pathway that is activated when cells experience mitochondrial protein folding stress and retrograde signaling from the mitochondria to the nucleus triggers transcriptional activation of nuclear-encoded mitochondrial chaperones and proteases as well as repression of translation to reestablish proteostasis (Haynes, Fiorese & Lin, 2013; Haynes & Ron, 2010; Mohrin et al., 2015; Munch & Harper, 2016; Zhao et al., 2002). Primarily characterized in *C. elegans*, the UPR^{mt} is activated

during a developmental stage when there is a burst of mitochondrial biogenesis (Houtkooper et al., 2013; Lin et al., 2016; Merkwirth et al., 2016; Nargund, Pellegrino, Fiorese, Baker & Haynes, 2012; Pellegrino et al., 2014; Tian et al., 2016). It is therefore speculated that in stem cells, the UPR^{mt} is activated under a physiological condition when mitochondrial biogenesis is induced. Adult stem cells frequently exit the cell cycle and are predominantly found in the quiescent (G0) state, where the number of mitochondria is low and glycolysis is the primary metabolic pathway to support energy production (Folmes, Dzeja, Nelson & Terzic, 2012; Takubo et al., 2013; Warr & Passegue, 2013; Yu et al., 2013). As stem cells transit from quiescence to proliferation, mitochondrial biogenesis is induced to enable metabolic reprogramming from glycolysis to oxidative phosphorylation to meet increasing energy demands. Because a major event during the transition from quiescence to proliferation is mitochondrial biogenesis, this event raises the possibility that the UPR^{mt} is activated during this transition. However, direct evidence is lacking. In this study, we devised three experimental approaches that enable us to monitor quiescent and proliferating stem cells and directly test this hypothesis.

We tested this hypothesis in hematopoietic stem cells (HSCs), immunophenotypically defined as Lin−c-Kit+Sca1+CD150+CD48−. About 90% of HSCs reside in a quiescent state under homeostatic conditions (Pietras, Warr & Passegue, 2011). We isolated HSCs from mouse bone marrow and stimulated them to exit quiescence ex vivo upon culture with cytokines. We first confirmed that HSCs stimulated with cytokines were actively proliferating (Figure 1a) and that mitochondrial mass was increased in HSCs upon proliferation (Figure 1b). Compared to freshly isolated quiescent HSCs, proliferating HSCs stimulated with cytokines exhibited increased RNA levels of mitochondrial chaperones and proteases (Figure 1c). Because mitochondrial biogenesis upon HSC transition from quiescence to proliferation is regulated at the translational level mediated by mTOR (Chen et al., 2008; Gan et al., 2010; Gurumurthy et al., 2010; Morita et al., 2013; Nakada, Saunders & Morrison, 2010), increased expression of mitochondrial chaperones and proteases at the transcriptional level suggests de novo activation of the UPR^{mt}.

We further validated these results by stimulating HSCs to exit quiescence in vivo upon transplantation. Compared to HSCs isolated from untransplanted mice, donor HSCs isolated from transplanted recipient mice 2 weeks post-transplant were actively proliferating (Figure 2a), had increased mitochondrial mass (Figure 2b,c), and increased expression of mitochondrial chaperones and proteases at the transcriptional level (Figure 2d), indicative of activation of the UPR^{mt}.

An alternative approach to model HSC proliferation in vivo is to treat mice with polyinosinic:polycytidylic acid (pIpC), a synthetic double-stranded RNA (dsRNA) mimetic that stimulates the multiple immune signaling pathways that are activated during a viral infection (Walter et al., 2015). Compared to HSCs isolated from untreated mice, HSCs isolated from mice 24 hr after the pIpC treatment showed increased proliferation (Figure 3A) and mitochondrial mass (Figure 3B, C), and induction of the expression of oxidative phosphorylation genes (Figure 3D) and mitochondrial chaperones and proteases (Figure 3E). In contrast, the expression of glycolysis genes (Figure 3D) and ER stress response genes (Figure 3E) was unchanged. These data are consistent with the notion that proliferative HSCs have increased mitochondrial

number, experience a metabolic switch from glycolysis to oxidative phosphorylation, and induce the UPR^{mt} to maintain the mitochondrial homeostasis.

2.3 Discussion

Collectively, these results provide direct evidence that the UPR^{mt} is activated upon HSC transition from quiescence to proliferation (Figures 1 and 2), and more broadly, mitochondrial integrity is actively monitored at the restriction point to ensure metabolic fitness before stem cells are committed to proliferation. Stem cell quiescence is a protective mechanism that prevents cell death and the depletion of the stem cell pool (Cheung & Rando, 2013). Consistent with the activation of the UPR^{mt} at the transition from quiescence to proliferation, dysregulation of the UPR^{mt} results in stem cell death, a reduced stem cell pool, and compromised stem cell self-renewal (Berger et al., 2016; Mohrin et al., 2015; Zhang et al., 2016). Among the induction of the UPR^{mt} is increased expression of SIRT7 (Figure 2i), which alleviates mitochondrial protein folding stress by repressing NRF1 activity and mitochondrial translation, reduces mitochondrial activity and proliferation, and gives cells more time to recover from stress (Mohrin et al., 2015). Failure to do so leads to HSC death. With aging, SIRT7 becomes inactivated, resulting in increased mitochondrial protein folding stress and functional decline (Mohrin et al., 2015). Identifying molecular regulators of the UPR^{mt} in stem cells opens the door for novel therapeutic opportunities for improving stem cell maintenance, enhancing tissue regeneration, and extending lifespan and health span.

2.4 Experimental Procedures

Mice

C57BL/6 mice were housed on a 12:12 hr light:dark cycle at 25°C. All animal procedures were in accordance with the animal care committee at the University of California, Berkeley.

Flow cytometry and cell sorting

Bone marrow cells were obtained by crushing the long bones with sterile PBS without calcium and magnesium supplemented with 2% FBS. Lineage staining contained a cocktail of biotinylated anti-mouse antibodies to Mac-1 (CD11b), Gr-1 (Ly-6G/C), Ter119 (Ly-76), CD3, CD4, CD8a (Ly-2), and B220 (CD45R) (BioLegend). For detection or sorting, we used streptavidin conjugated to APC-Cy7, c-Kit-APC, Sca-1-Pacific blue, CD48-FITC, and CD150-PE (BioLegend). For congenic strain discrimination, anti-CD45.1 PerCP and anti-CD45.2 PE-Cy7 antibodies (BioLegend) were used. For assessment of cell cycle analysis, Ki-67 (BioLegend) staining was performed according to the manufacturer's recommendation after cell surface staining. The gates were drawn based on the fluorescence minus one (FMO) control. For mitochondrial mass, bone marrow cells were incubated with 100 nM MitoTracker Green (Invitrogen) for 30 min at 37°C in the dark after cell surface staining. All data were collected on a Fortessa (Becton Dickinson), and data analysis was performed with FlowJo (TreeStar). For cell sorting, lineage depletion or c-kit enrichment was performed according to the manufacturer's instructions (Miltenyi Biotec). Cells were sorted using a Cytopeia INFLUX Sorter (Becton Dickinson). Antibody details are provided in Table S1.

To stimulate HSCs to exit quiescence, freshly isolated HSCs were cultured ex vivo in IMDM (Invitrogen) supplemented with 5% stem cell FBS (Stem Cell Technologies), 1% penicillin/streptomycin, sodium pyruvate, NEAA, l-glutamine (Invitrogen), and cytokines (IL-3 (10 ng/ml), GMCSF (10 ng/ml), SCF (25 ng/ml), IL-11 (25 ng/ml), Flt3L (25 ng/ml), TPO (25 ng/ml) (PeproTech), and EPO (4 U/ml) (R&D)) for 48 hr. Alternatively, to stimulate HSCs to exit quiescence in vivo, 1 × 106 bone marrow cells were transplanted into lethally irradiated recipient mice. Two weeks post-transplantation, donor HSCs were isolated via sorting. To induce in vivo cycling of HSCs, mice were injected intraperitoneally (i.p.) with 5 mg/kg polyinosinic:polycytidylic acid (Sigma) 24 hr prior to analysis.

mRNA analysis

RNA was isolated from cells using TRIzol reagent (Invitrogen). cDNA was generated using qScript™ cDNA SuperMix (Quanta Biosciences). Gene expression was determined by real-time PCR using Eva qPCR SuperMix Kit (BioChain Institute) on an ABI StepOnePlus system. All data were normalized to β-actin expression. PCR primer details are provided in Table S2.

mtDNA/nDNA

The mitochondrial DNA/nuclear DNA (mtDNA/nDNA) ratio was determined by isolating DNA from cells with TRIzol (Invitrogen), as described previously (Lai et al., 2008). The ratio of mtDNA/nDNA was calculated as previously described (Venegas et al., 2011).

Statistical analysis

The number of mice chosen for each experiment is based on the minimum number of mice necessary to have sufficient statistical power and is comparable to published literature for the same assays performed. Mice were randomized to groups, and analysis of mice and tissue samples was performed by investigators blinded to the treatment of the animals. Statistical analysis was performed with Excel (Microsoft). Means between two groups were compared with Student's t test. Error bars represent standard errors. In all corresponding figures, * represents $p < .05$, ** represents $p < .01$, *** represents $p < .001$, and ns represents $p > .05$.

2.5 Figures

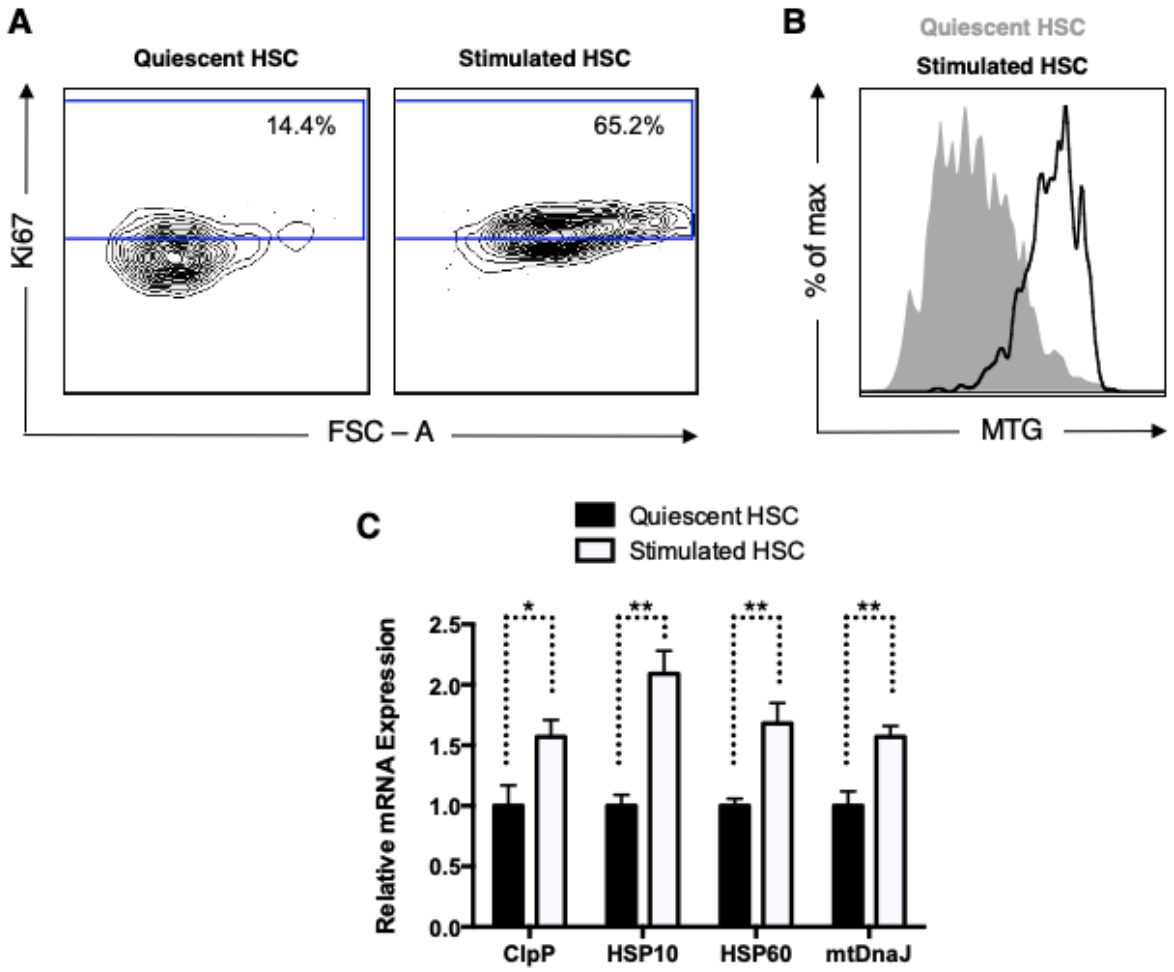

Figure 1. The UPR^{mt} is activated upon HSC transition from quiescence to proliferation ex vivo. **(A)** Cell cycle analysis of HSCs using Ki-67 staining showing increased proliferation in HSCs stimulated by ex vivo culture with cytokines compared to quiescent HSCs freshly isolated from mouse bone marrow. **(B)** MitoTracker Green staining showing increased mitochondrial mass in HSCs stimulated to proliferate via ex vivo culture with cytokines compared to quiescent HSCs freshly isolated from mouse bone marrow. **(C)** qPCR showing increased transcription of mitochondrial chaperones and proteases in HSCs stimulated to proliferate via ex vivo culture with cytokines compared to quiescent HSCs freshly isolated from mouse bone marrow. n = 3

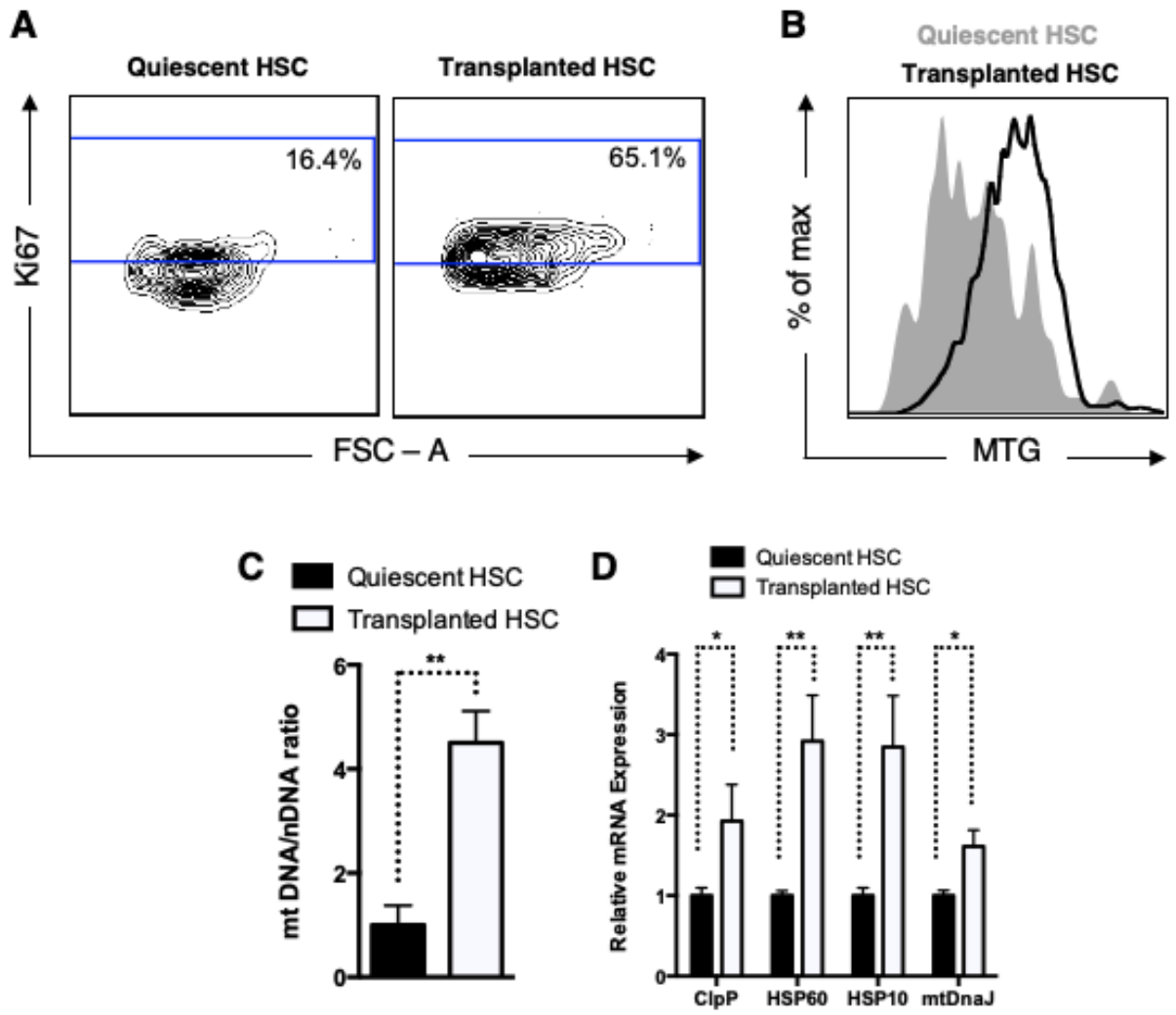

Figure 2. The UPR^{mt} is activated upon HSC transition from quiescence to proliferation in vivo via transplantation. (A) Cell cycle analysis of HSCs using Ki-67 staining showing increased proliferation in HSCs stimulated by in vivo transplantation (2 week post-transplantation) compared to quiescent HSCs freshly isolated from mouse bone marrow. **(B, C)** MitoTracker Green staining **(B)** and quantification of the mitochondrial to nuclear DNA ratio **(C)** showing increased mitochondrial mass in HSCs stimulated to proliferate via in vivo transplantation compared to quiescent HSCs freshly isolated from mouse bone marrow. n = 3. **(D)** qPCR showing increased transcription of mitochondrial chaperones and proteases in HSCs stimulated to proliferate via in vivo transplantation compared to quiescent HSCs freshly isolated from mouse bone marrow. n = 3.

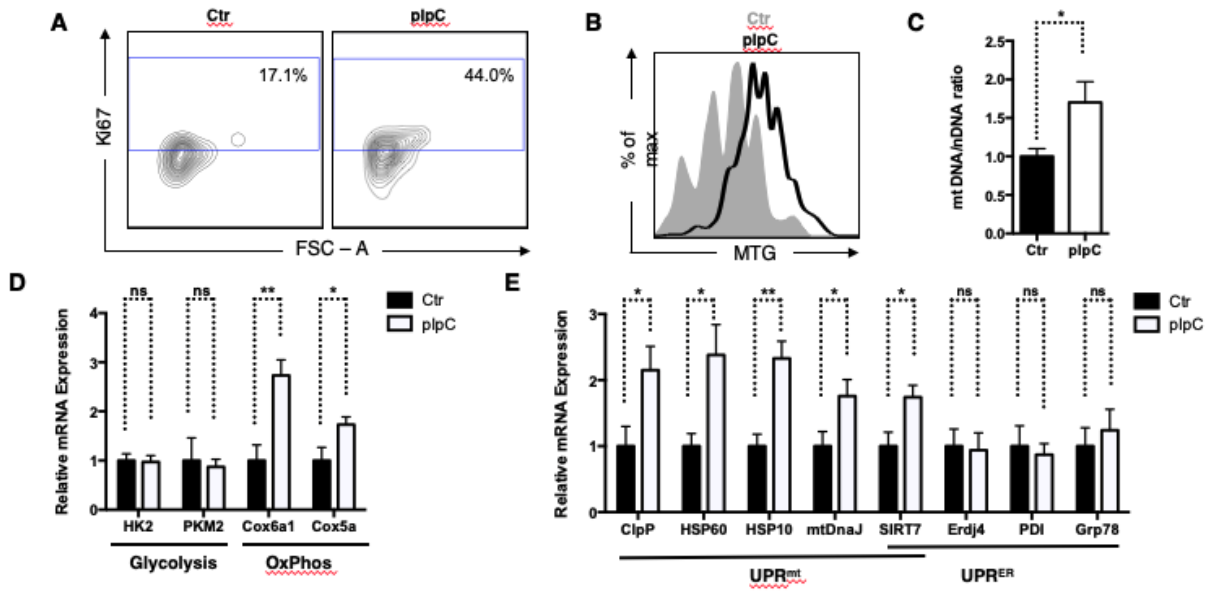

Figure 3. The UPR^{mt} is activated upon HSC transition from quiescence to proliferation in vivo via pIpC injection. (A) Cell cycle analysis of HSCs using Ki-67 staining showing increased proliferation in HSCs stimulated by pIpC treatment (24 hr after treatment) compared to quiescent HSCs isolated from untreated mouse bone marrow. **(B, C)** MitoTracker Green staining **(B)** and quantification of the mitochondrial to nuclear DNA ratio **(C)** showing increased mitochondrial mass in HSCs stimulated to proliferate via pIpC compared to quiescent HSCs isolated from untreated mouse bone marrow. n = 3. **(D, E)** qPCR showing increased transcription of oxidative phosphorylation genes and mitochondrial chaperones and proteases, but not glycolysis genes and ER stress response genes in HSCs stimulated to proliferate via pIpC treatment compared to quiescent HSCs isolated from untreated mouse bone marrow. n = 3

Chapter 3. NAD^+ booster 78c ameliorates multiple sclerosis

In the previous chapter, we provided evidence that the UPR^{mt} is activated when stem cells proliferate as a mechanism of maintaining mitochondrial homeostasis, which is tightly regulated by SIRT7.

Since sirtuins are known to maintain the homeostasis of various other tissues, I next sought to investigate the role of SIRT2 in alleviating neurodegeneration and whether NAD^+-boosting could rescue multiple sclerosis pathology brought on by SIRT2 deficiency.

3.1 Abstract

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS), defined by inflammatory demyelination and neurodegeneration. While the majority of treatments exist for relapsing-remitting multiple sclerosis (RRMS), there is a strong need for the development of therapies for individuals with progressive forms of MS. Although the cause of progressive MS is largely unknown, advancing age significantly influences the clinical disease course and pathological processes. SIRT2, a mammalian sirtuin and protein deacetylase, has been shown to play a large role in regulating inflammatory responses in the central nervous system; therefore, we sought to better understand the importance of SIRT2 in multiple sclerosis disease progression in humans and mice. Analysis of publicly available single-nuclei RNA seq data from white matter tissue of control samples and individuals with progressive MS (Jäkel et al., 2019) indicates that the expression of SIRT2 is decreased in the lesions of MS patients. SIRT2-deficiency increased microglial activation, demyelination, and motor dysfunction in mice induced with experimental autoimmune encephalomyelitis (EAE), a widely used animal model of multiple sclerosis. Treatment with 78c, an NAD^+ booster, attenuated inflammatory demyelination and motor deficits. These results suggest that SIRT2 deficiency may drive an inflammatory phenotype within progressive MS lesions, and that NAD^+ boosting may alleviate MS pathology.

3.2 Results

SIRT2 is downregulated in the lesions of progressive MS patients and EAE mice

To identify the potential genetic factors that drive progressive MS development, we analyzed an snRNA-seq dataset derived from white matter post-mortem tissue from non-neurological control samples and progressive MS patients (Jäkel et al., 2019). Given SIRT2's involvement in regulating neuroinflammatory pathways, we asked whether a change in SIRT2 expression could be associated with MS disease development. Interestingly, among the seven sirtuin family members, the expression of SIRT2 was the most significantly reduced in MS samples than in controls (Figure 1A). Additionally, mice with EAE exhibited lower SIRT2 levels in the subpial region of the cortex (Figure 1B, C), a common area of demyelination in patients with progressive MS (Kutzelnigg et al., 2019). These expression patterns reveal that SIRT2 may particularly have an impact on MS development.

SIRT2 regulates EAE disease progression

To understand the effect of SIRT2 on the progression of MS, we tracked the disease progression of wild-type and Sirt2$^{-/-}$ mice for 17 days following EAE induction. Mice lacking Sirt2 were less resistant to EAE development and started to exhibit signs of disease significantly earlier than wild-type controls (Figure 2A). Concurrently, Sirt2$^{-/-}$ mice began to acquire motor deficits earlier than wild-type mice following EAE induction as demonstrated through a longitudinal rotarod assay (Figure 2B). In the rotarod test, the animal is placed on a rod that rotates at an increasing speed, and the animal must remain upright and not fall off. It is widely used in mouse models of neurodegeneration as it is especially sensitive to motor impairment caused by neurological deficits as opposed to other tests that are more affected by the animal's strength, such as the inverted screen procedure. Overall, these data suggest SIRT2 plays an essential role in maintaining proper motor activity during the development of EAE.

To address how SIRT2 deficiency may exacerbate EAE, we isolated the brains of wild-type and Sirt2$^{-/-}$ mice with or without EAE and stained them for ionized calcium-binding adaptor molecule 1 (Iba1), a microglial and macrophage-specific protein involved in phagocytosis whose expression increases with microglial activation. Although Iba1 levels remained unchanged between young (4-6 months) wild-type and Sirt2$^{-/-}$ control mice, after EAE induction Sirt2$^{-/-}$ mice showed significantly higher microglial activation compared to wild-type mice (Figures 3A and 3B). In addition to microglial activation, a defining characteristic of progressive MS and EAE is diffuse and localized demyelination within cortical grey matter regions (Giralamo et al., 2011). In the subpial zone of the cortex, Sirt2$^{-/-}$ mice had more demyelination than wild-type mice following EAE induction, as indicated by myelin basic protein (MBP) immunostaining (Figures 4A and 4B). These data imply that the worsened EAE disease progression of Sirt2$^{-/-}$ mice may be attributed to increased microglial activation and demyelination.

78c alleviates EAE-induced motor deficit and inflammatory demyelination

To determine whether pharmacologic use of 78c is sufficient to alleviate MS disease symptoms, we administered 78c or a vehicle control intraperitoneally to mice induced with EAE and followed their disease progression daily. 78c treatment significantly attenuated EAE-induced motor deficits as shown with decreased average clinical scoring and improved rotarod performance (Figures 5A, 5B). Treatment with 78c also slightly prevented body weight loss of EAE mice, although it did not reach statistical significance (Figure 5C). After three weeks, cortical tissues were collected and used for subsequent histological staining of myelin and activated microglia. 78c-treated EAE mice showed significantly lower microglial activation compared to vehicle-treated EAE mice (Figures 6A and 6B). Additionally, 78c treatment decreased the severity of EAE-induced demyelination (Figures 7A and 7B). These results provide strong evidence that 78c is effective at rescuing EAE-induced motor deficit by suppressing neuroinflammatory demyelination.

3.3 Discussion

Altogether, our report establishes SIRT2 as a crucial regulator of inflammatory demyelination during EAE, and the decrease of SIRT2 expression in progressive MS lesions suggests its potential involvement in human MS development. In addition to regulating neuroinflammation,

SIRT2 prevents metabolic dysfunction, suppresses tumor growth, and extends lifespan (Gomes et al., 2015; Kim et al., 2011; North et al., 2014); therefore, SIRT2 is increasingly becoming an attractive target for improving healthspan. In addition to SIRT2, SIRT1 also has neuroprotective effects in EAE mice (Shindler et al., 2010; Nimmagadda et al., 2013; McDougald et al., 2018; Khan et al., 2014). Furthermore, genetic variants and the expression of mitochondrial sirtuins, SIRT3-5, have been linked to mitochondrial dysfunction and neurodegeneration in MS patients (Inkster et al., 2013; Rice et al., 2012).

NAD^+ levels have been shown to decrease during neuroinflammation and aging, contributing to functional impairment of sirtuins and further exacerbation of inflammatory conditions (Camacho-Pereira et al., 2016; Guerreiro et al., 2020; Langley et al., 2021). While MS lesions are driven by inflammatory processes, other mechanisms underlying MS progression have been suggested to be affected by NAD^+ levels. For example, NAD^+ supplementation can impact oligodendrocyte lineage cell differentiation and improve the expression of myelin proteins (Jablonksa et al., 2016; Yoon et al., 2016). Additionally, NAD^+ alleviates demyelination by activating autophagy, which in turn regulates the differentiation of pro-inflammatory helper T cell subsets, thereby reducing inflammatory cell infiltration (Wang et al., 2016). Our results support NAD^+ boosting as a potential therapeutic strategy for multiple sclerosis, at least in part through SIRT2, and add to the emerging knowledge around NAD^+ boosting for diseases of aging.

3.4 Experimental Procedures

Mice

SIRT2 knockout mice (C57BL/6) (Bobrowska et al., 2012) were housed on a 12:12 hr light:dark cycle at 25°C and received water and chow ad libitum. All animal procedures were in accordance with the animal care committee at the University of California, Berkeley.

78c drug treatment

78c was administered to C57BL/6 (4-6 months old) mice by intraperitoneal injection (i.p., 10mg/kg/dose) twice daily over a period of 21 days following EAE induction. Control mice received vehicle (5% DMSO, 15% PEG400, 80% of 15% hydroxypropyl-g-cyclodextrin (in citrate buffer pH 6.0)) injections.

EAE induction and clinical evaluation

Mice were housed in standard Makrolon cages in a quiet environment with ad libitum access to water and food. To induce EAE, female mice (4-6 months) were subcutaneously injected at the lower and upper back with a total of 200 ul myelin-oligodendrocyte glycoprotein ($MOG^{35–55}$), a peptide antigen used to immunize the animal against myelin, emulsified in complete Freund's adjuvant (Hooke Laboratories) followed by an intraperitoneal injection of 100 ng of pertussis toxin, which was repeated after 24 hr. Mice were monitored daily for weight and EAE score, using the following scoring scale: 0, no obvious changes; 1, limp tail; 2, limp tail and impaired righting reflex; 3, limp tail and partial paralysis of hind legs; 4, limp tail and complete paralysis of hind legs; and 5, moribund.

Immunohistochemistry

Tissue processing and immunohistochemistry was performed on free-floating sections following standard procedure (Villeda et al., 2011). Briefly, mice were transcardially perfused with 10 ml of PBS with 10 U/ml of heparin and then with 40 ml of PBS with 4% formaldehyde. Brains were extracted and incubated in PBS with 4% formaldehyde at 4°C overnight. Brains were transferred into PBS with 15% sucrose at 4°C for 6 hr and then transferred into PBS with 30% sucrose at 4°C for a day before cutting. Brains were then sectioned coronally at 40 µm with a cryo-microtome (Leica Camera, Inc.) and stored in a cryoprotective medium. Primary antibodies were: rabbit anti-Myelin Basic Protein (MBP) (1:250; Abcam), rabbit anti-Ionized calcium binding adaptor molecule (Iba1) (1:1000; Wako). After overnight incubation, primary antibody staining was revealed using fluorescence conjugated secondary antibodies. Sections were examined at a common area of interest (subpial zone) under a Zeiss LSM710 confocal laser scanning microscope (Carl Zeiss, Inc.), with an average of three sections per animal. Fluorescence intensity and percent area coverage quantification, as well as thresholding of images, was performed in ImageJ. The fluorescence values and percent area coverage were then standardized against the average value of the control group to obtain relative values.

Rotarod assay

Motor coordination was evaluated using a rotarod apparatus (Columbus Instruments). The mice were evaluated on the rotarod where each session consisted of 3 trials with an intertrial interval of 5 mins. The rotation was set at 4 RPM which accelerated over the course of 300 seconds to 40 RPM. The average time (seconds) the subject maintains its balance on the rotarod prior to falling off is recorded as the latency.

snRNA-sequencing analysis

The expression data were downloaded from the gene expression omnibus (GEO) database with accession number GSE118257 (Jäkel et al., 2019). The dataset included 20 samples of isolated single nuclei from white matter post-mortem tissue from 5 non-neurological control samples and post-mortem tissue from 4 progressive Multiple Sclerosis (MS) patients. Cells were quality-filtered, clustered and annotated by the original authors. Differentially expressed gene screening was performed in R using Seurat (v.4.0) and associated packages (Hao et al., 2021) and calculated by Wilcoxon rank-sum test.

Statistical Analysis

The number of mice chosen for each experiment is based on the minimum number of mice necessary to have sufficient statistical power and is comparable to published literature for the same assays performed. Mice were randomized to groups and analysis of mice and tissue samples were performed by investigators blinded to the treatment of the animals. Statistical analysis was performed with Excel (Microsoft). Means between two groups were compared with Student's t-test. Error Bars represent standard errors. In all corresponding figures, * represents $p<0.05$. ** represents $p<0.01$. *** represents $p<0.001$. ns represents $p>0.05$. Wilcoxon rank-sum test for single-nuclei RNA sequencing analysis was performed using the Seurat R package (v.4.0).

3.5 Figures

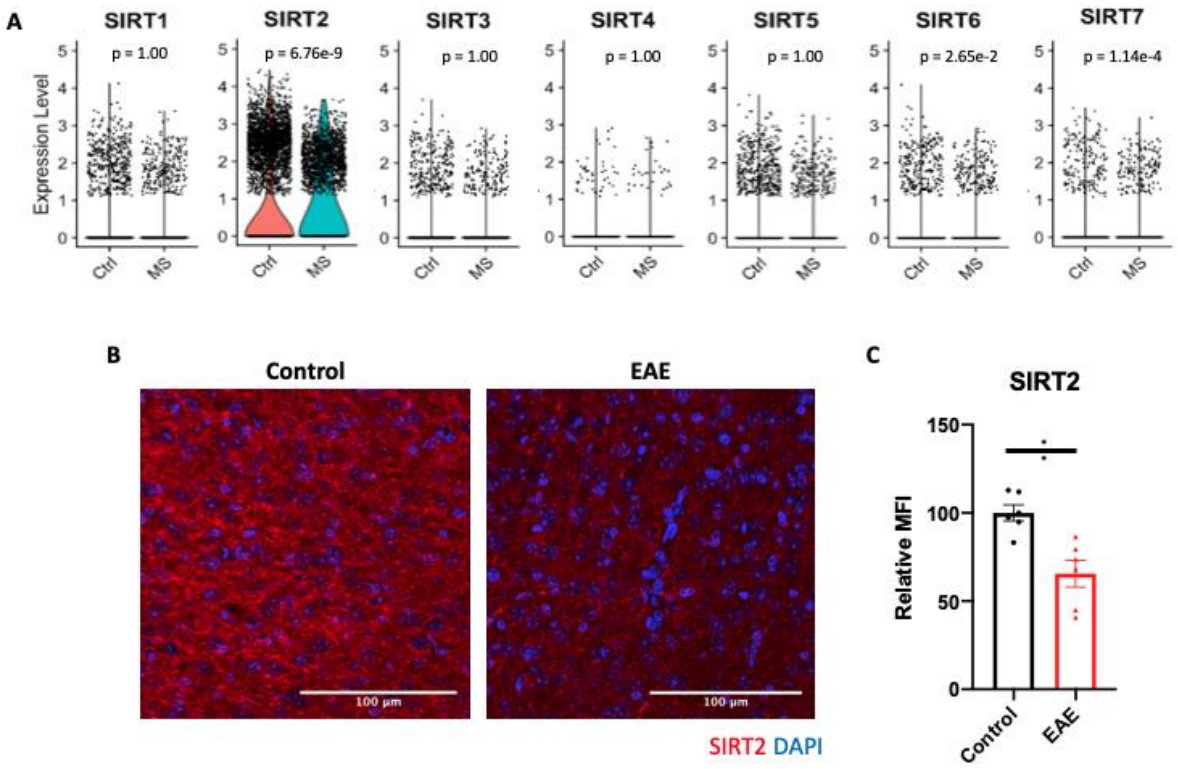

Figure 1. SIRT2 is downregulated in the lesions of progressive MS patients and EAE mice
(A) Violin plot comparing normalized expression values of seven sirtuins (SIRT1-7) between control (ctrl) and MS samples in all recovered cells in the snRNA-seq dataset. Each dot represents the gene expression levels in one cell. Wilcoxon rank-sum test.
(B and C) Representative images **(B)** and quantification **(C)** of SIRT2 immunostaining in the subpial zone (n = 6 mice per group).

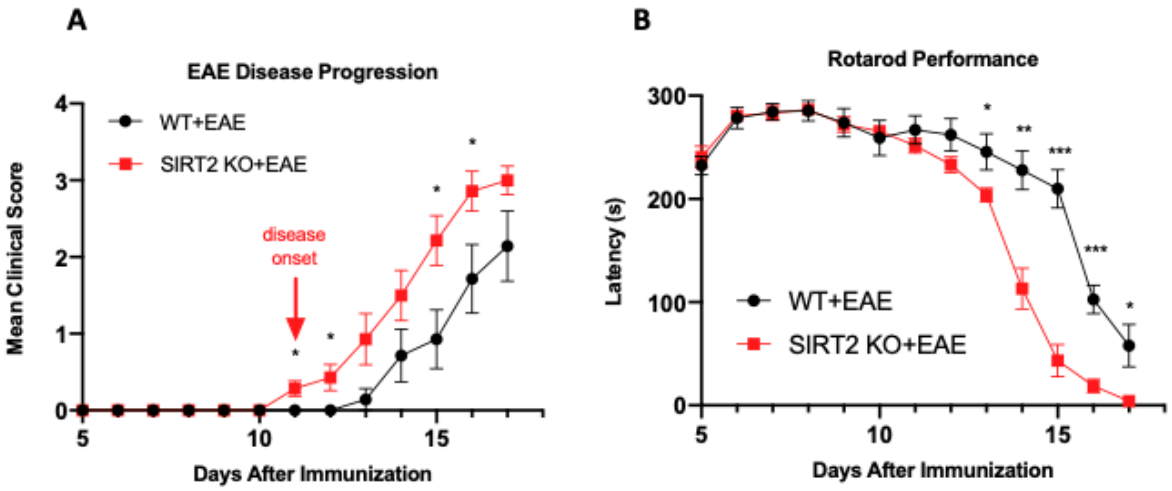

Figure 2. SIRT2 regulates EAE disease progression
(A) Average clinical score per day of EAE-induced wild-type and SIRT2 KO mice. Disease scores are presented as mean ± SEM for each group (n = 7 per group).
(B) Rotarod performance tests were performed daily following EAE induction in wild-type and SIRT2KO mice (n = 7 per group).
Error bars represent SE. Student's t test. *: p<0.05. **: p<0.01. ***: p<0.001.

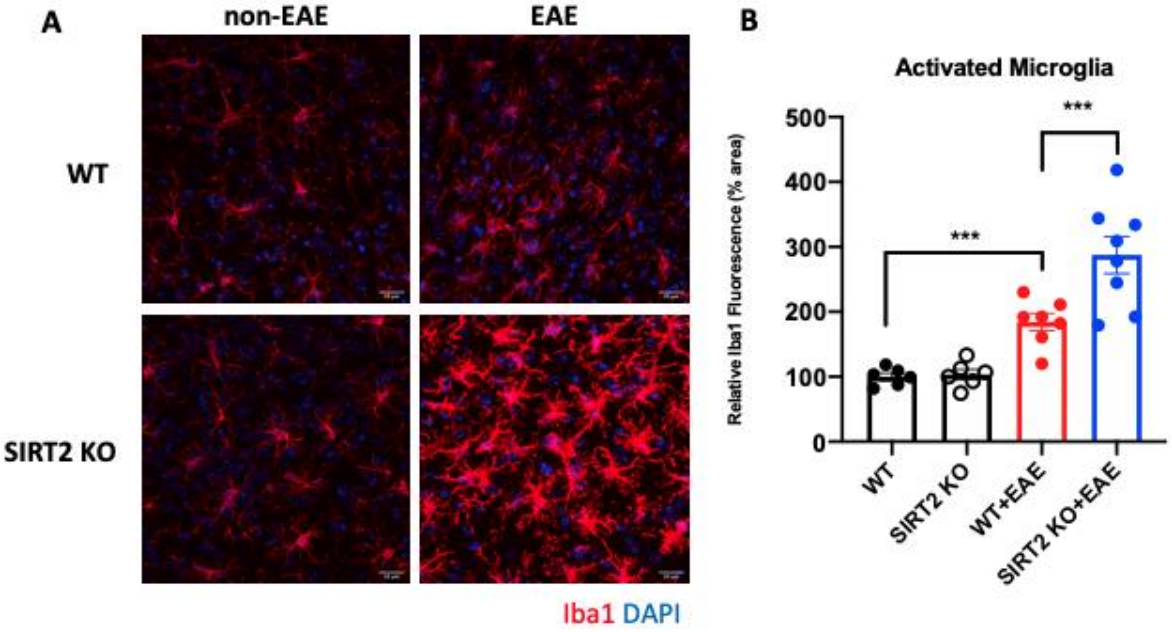

Figure 3. SIRT2-deficiency increases EAE-induced microglial activation
(A and B) Representative images **(A)** and quantification **(B)** of Iba1 immunostaining in the subpial zone of EAE-induced wild-type and SIRT2 KO mice (n = 6-8 mice per group). Error bars represent SE. Student's t test. *: p<0.05. **: p<0.01. ***: p<0.001.

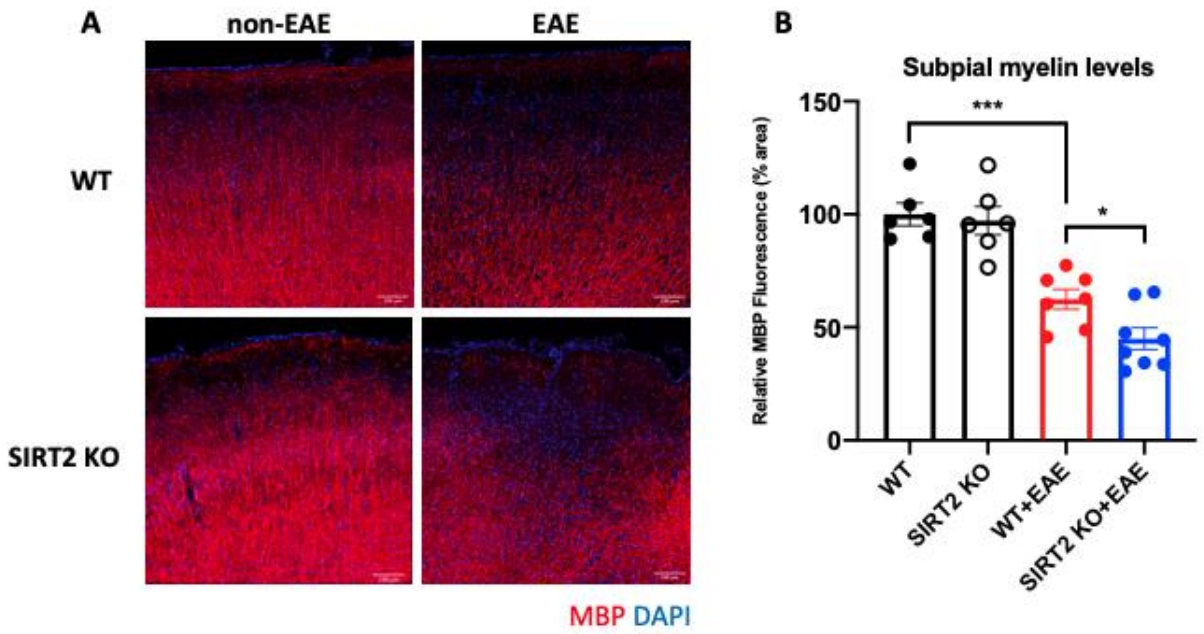

Figure 4. SIRT2-deficiency increases EAE-induced demyelination
(A and B) Representative images **(A)** and quantification **(B)** of MBP immunostaining in the subpial zone of EAE-induced wild-type and SIRT2 KO mice (n = 6-8 mice per group). Error bars represent SE. Student's t test. *: $p<0.05$. **: $p<0.01$. ***: $p<0.001$.

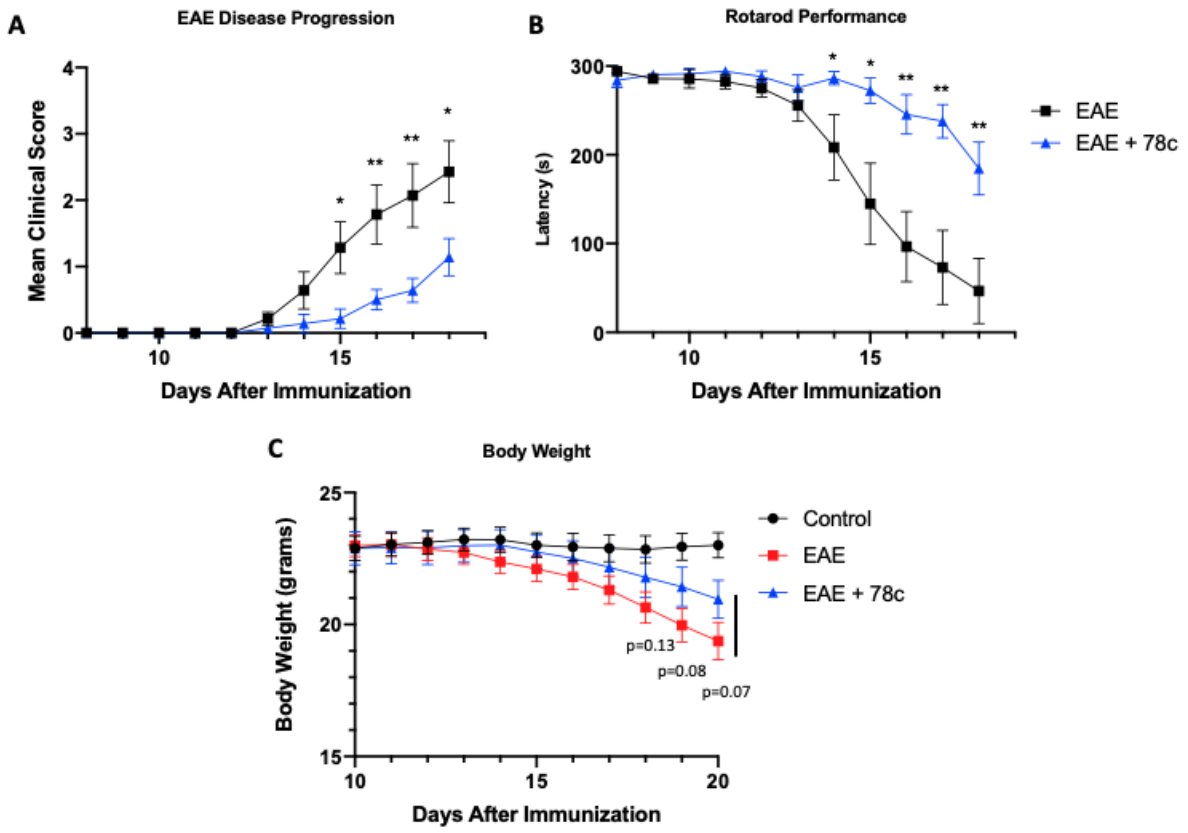

Figure 5. 78c alleviates EAE-induced motor deficit and disease progression
(A) Average clinical score per day of EAE-induced mice treated with either a vehicle control or 78c. Disease scores are presented as mean ± SEM for each group (*n* = 7 per group).
(B) Rotarod performance tests were performed daily following EAE induction in mice treated with a vehicle control or 78c (*n* = 7 per group).
(C) Body weight of control mice and EAE mice treated with a vehicle control or 78c (n = 6-7 per group).
Error bars represent SE. Student's t test. *: p<0.05. **: p<0.01. ***: p<0.001.

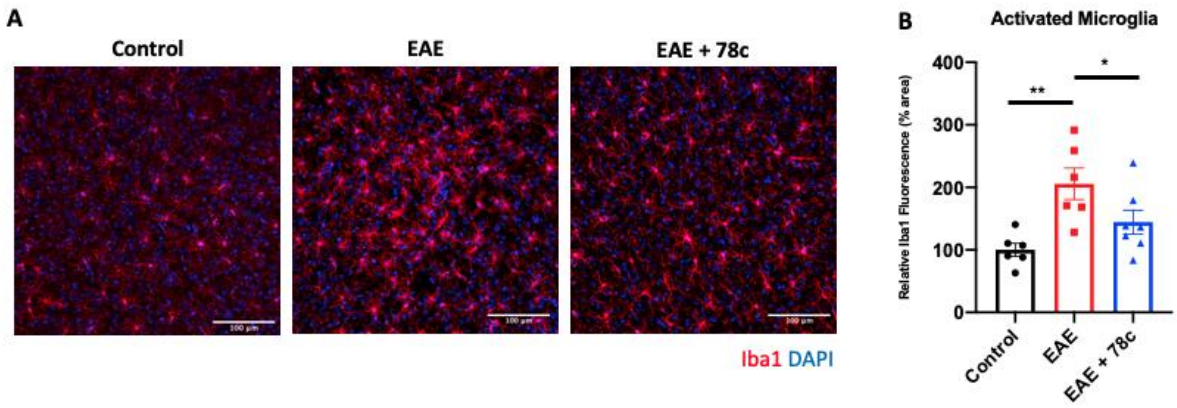

Figure 6. 78c alleviates EAE-induced microglial activation
(A and B) Representative images **(A)** and quantification **(B)** of Iba1 immunostaining in the subpial zone of EAE-induced mice treated with either a vehicle control or 78c. (*n* = 6-7 per group).
Error bars represent SE. Student's t test. *: p<0.05. **: p<0.01. ***: p<0.001.

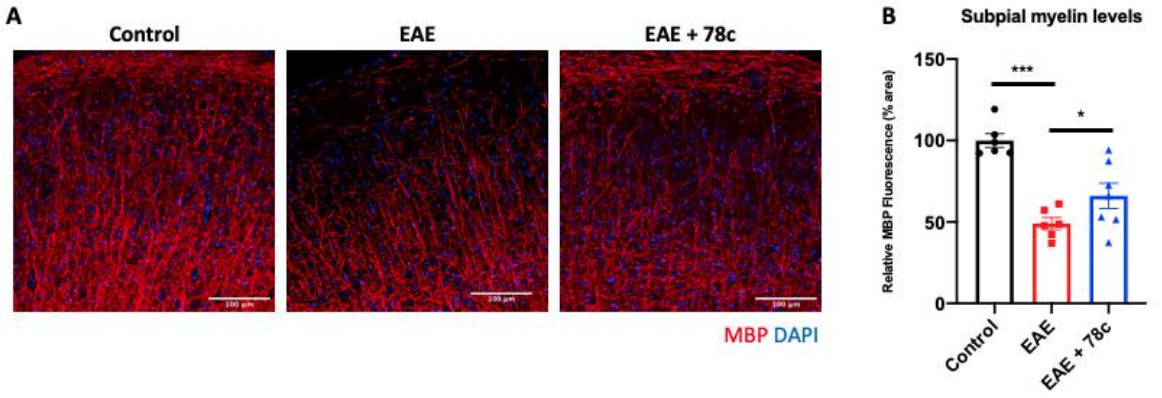

Figure 7. 78c alleviates EAE-induced demyelination
(A and B) Representative images **(A)** and quantification **(B)** of MBP immunostaining in the subpial zone (n = 6-7 per group).
Error bars represent SE. Student's t test. *: p<0.05. **: p<0.01. ***: p<0.001.

Chapter 4. Conclusion

4.1 Concluding remarks

In Chapter 2, we delve into the mechanisms underlying stem cell maintenance, specifically the mitochondrial unfolded protein response (UPR^{mt}). We found that when hematopoietic stem cells (HSCs) exit quiescence in response to stressors, the UPR^{mt} is activated in order to maintain mitochondrial homeostasis. Previous studies have linked sirtuins, such as SIRT7, to the regulation of this critical process and provided evidence that a lack of SIRT7 drives HSC aging. Altogether, this study highlights the importance of sirtuins in maintaining proper stem cell function in response to different stressors and aging.

In Chapter 3, we focused on another sirtuin, SIRT2, and its role in regulating neuroinflammation in the context of progressive multiple sclerosis (MS). Consistent with other reports of SIRT2's ability to suppress inflammation, mice lacking SIRT2 were more susceptible to disease progression and paralysis induced by EAE, a model for MS. More importantly, treatment with 78c, a NAD^+ booster, abrogated the neuroinflammatory phenotypes present in EAE mice.

Studies in both chapters emphasize the importance of sirtuins in maintaining tissue homeostasis in the contexts of aging and disease. While many studies have suggested increasing sirtuin activity via NAD^+-boosting may improve disease outcomes, it's effects in human aging-associated diseases are still not fully understood.

4.2 Future Implications for NAD^+ modulation

NAD^+ for Human Healthspan

The most common pharmacological method of NAD^+ upregulation is through the supplementation of the NAD^+ precursor, nicotinamide riboside (NR). NR supplementation in rodents ameliorates neuronal and metabolic dysfunction, but human data are sparse. Clinical trials have already begun for the use of NR supplementation and data shows that the treatment is well-tolerated by healthy individuals while increasing their NAD^+ levels (Martens et al., 2018; Elhassan et al., 2019). Current trials are testing the efficacy of NR supplementation in patients with Alzheimer's Disease (AD). In a pilot study with low sample sizes, researchers could not find a significant difference between treated and non-treated patients when testing their cognitive function. Although their results were inconclusive, studies with larger sample sizes will be performed before conclusions are made about the efficacy of NR supplementation on aging-related disorders, such as AD. Moving forward, perhaps 78c can be another promising drug to upregulate NAD^+ levels in humans.

Stem cell-aging disorders

Many diseases arise, in part, as a result of stem cell aging; therefore, there may be potential benefits of NR supplementation in slowing or preventing the development of such aging-related pathologies. For example, aged HSCs have an increased propensity of establishing a distinct subpopulation of blood cells containing somatic mutations which confer a competitive advantage upon transplantation (Jaiswal et al., 2019). This aging-related phenomenon, termed clonal hematopoiesis, is linked to an increased risk of developing hematologic malignancies and

cardiovascular disease (Genovese et al., 2014; Kusne et al., 2022). Although it is currently unknown how HSCs first acquire these mutations, studies suggest that mitochondrial dysfunction leads to genomic instability (Fakouri et al., 2018). It would be interesting to assess whether NR supplementation could decrease the clonality of aged HSCs by maintaining proper mitochondrial homeostasis, and thus lower the risk of DNA damage-induced malignant transformation.

4.3 References

Anderson RM, Latorre-Esteves M, Neves AR, et al. Yeast life-span extension by calorie restriction is independent of NAD fluctuation. Science. 2003;302(5653):2124-2126. doi:10.1126/science.1088697

Anso E, Weinberg SE, Diebold LP, Thompson BJ, Malinge S, Schumacker PT, Liu X, Zhang Y, Shao Z, Steadman M, Marsh KM, Xu J, Crispino JD, Chandel NS, 2017. The mitochondrial respiratory chain is essential for haematopoietic stem cell function. *Nature cell biology* 19, 614–625.

Berger, E., Rath, E., Yuan, D., Waldschmitt, N., Khaloian, S., Allgauer, M., Staszewski, O., Lobner, E.M., Schottl, T., Giesbertz, P., et al. (2016). Mitochondrial function controls intestinal epithelial stemness and proliferation. Nature communications 7, 13171.

Biteau, B., Hochmuth, C.E., and Jasper, H. (2008). JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging Drosophila gut. Cell stem cell 3, 442-455.

Bobrowska A, Donmez G, Weiss A, Guarente L, Bates G. SIRT2 ablation has no effect on tubulin acetylation in brain, cholesterol biosynthesis or the progression of Huntington's disease phenotypes in vivo. PLoS One. 2012;7(4):e34805.

Bosch-Presegué L, Vaquero A. Sirtuins in stress response: guardians of the genome. Oncogene. 2014. 33, 3764–3775.

Brown K, Xie S, Qiu X, Mohrin M, Shin J, Liu Y, Zhang D, Scadden DT, Chen D, 2013. SIRT3 reverses aging-associated degeneration. *Cell Rep* 3, 319–327.

Camacho-Pereira J, Tarragó MG, Chini CCS, Nin V, Escande C, Warner GM, et al. CD38 Dictates Age-Related NAD Decline and Mitochondrial Dysfunction through an SIRT3-Dependent Mechanism. Cell Metab. 2016;23(6):1127-39.

Chambers SM, Shaw CA, Gatza C, Fisk CJ, Donehower LA, Goodell MA, 2007. Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. *PLoS biology* 5, e201.

Chen, C., Liu, Y., Liu, R., Ikenoue, T., Guan, K.L., Liu, Y., and Zheng, P. (2008). TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. J Exp Med 205, 2397-2408.

Cheung, T.H., and Rando, T.A. (2013). Molecular regulation of stem cell quiescence. Nature reviews Molecular cell biology 14, 329-340.

Chini CCS, Peclat TR, Warner GM, Kashyap S, Espindola-Netto JM, de Oliveira GC, et al. CD38 ecto-enzyme in immune cells is induced during aging and regulates NAD. Nat Metab. 2020;2(11):1284-304.

Constantinescu CS, Farooqi N, O'Brien K, Gran B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). Br J Pharmacol. 2011;164(4):1079-106.

de Haan G, Lazare SS, 2018. Aging of hematopoietic stem cells. *Blood* 131, 479–487.
Dryden SC, Nahhas FA, Nowak JE, Goustin AS, Tainsky MA. Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle. Mol Cell Biol. 2003;23(9):3173-3185. doi:10.1128/MCB.23.9.3173-3185.2003
Elhassan YS, Kluckova K, Fletcher RS, Schmidt MS, Garten A, Doig CL, Cartwright DM, Oakey L, Burley CV, Jenkinson N, Wilson M, Lucas SJE, Akerman I, Seabright A, Lai YC, Tennant DA, Nightingale P, Wallis GA, Manolopoulos KN, Brenner C, Philp A, Lavery GG. Nicotinamide Riboside Augments the Aged Human Skeletal Muscle NAD+ Metabolome and Induces Transcriptomic and Anti-inflammatory Signatures. Cell Rep. 2019 Aug 13;28(7):1717-1728.e6. doi: 10.1016/j.celrep.2019.07.043. PMID: 31412242; PMCID: PMC6702140.
Faissner S, Gold R. Progressive multiple sclerosis: latest therapeutic developments and future directions. Ther Adv Neurol Disord. 2019;12:1756286419878323.
Fakouri N.B., Hou Y., Demarest T.G., Christiansen L.S., Okur M.N., Mohanty J.G., Croteau D.L., Bohr V.A. Towards Understanding Genomic Instability, Mitochondrial Dysfunction and Aging. FEBS J. 2018 doi: 10.1111/febs.14663.
Fang, E. F. et al. NAD(+) in aging: molecular mechanisms and translational implications. *Trends Mol. Med.* **23**, 899–916 (2017).
Fitzner D, Simons M. Chronic progressive multiple sclerosis - pathogenesis of neurodegeneration and therapeutic strategies. Curr Neuropharmacol. 2010;8(3):305-15.
Folmes, C.D., Dzeja, P.P., Nelson, T.J., and Terzic, A. (2012). Metabolic plasticity in stem cell homeostasis and differentiation. Cell stem cell 11, 596-606.
Fourcade S, Outeiro TF, Pujol A. SIRT2 in age-related neurodegenerative disorders. Aging (Albany NY). 2018;10(3):295-6.
Gan, B., Hu, J., Jiang, S., Liu, Y., Sahin, E., Zhuang, L., Fletcher-Sananikone, E., Colla, S., Wang, Y.A., Chin, L., et al. (2010). Lkb1 regulates quiescence and metabolic homeostasis of haematopoietic stem cells. Nature 468, 701-704.
Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, Chambert K, Mick E, Neale BM, Fromer M, Purcell SM, Svantesson O, Landén M, Höglund M, Lehmann S, Gabriel SB, Moran JL, Lander ES, Sullivan PF, Sklar P, Grönberg H, Hultman CM, McCarroll SA. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014 Dec 25;371(26):2477-87. doi: 10.1056/NEJMoa1409405. Epub 2014 Nov 26. PMID: 25426838; PMCID: PMC4290021.
Girolamo F, Errede M, Longo G, Annese T, Alias C, Ferrara G, et al. Defining the role of NG2-expressing cells in experimental models of multiple sclerosis. A biofunctional analysis of the neurovascular unit in wild type and NG2 null mice. PLoS One. 2019;14(3):e0213508.
Girolamo F, Ferrara G, Strippoli M, Rizzi M, Errede M, Trojano M, et al. Cerebral cortex demyelination and oligodendrocyte precursor response to experimental autoimmune encephalomyelitis. Neurobiol Dis. 2011;43(3):678-89.
Gomes P, Fleming Outeiro T, Cavadas C. Emerging Role of Sirtuin 2 in the Regulation of Mammalian Metabolism. Trends Pharmacol Sci. 2015;36(11):756-68.
Govindarajan V, de Rivero Vaccari JP, Keane RW. Role of inflammasomes in multiple sclerosis and their potential as therapeutic targets. J Neuroinflammation. 2020;17(1):260.
Guarente L. Calorie restriction and sirtuins revisited. Genes Dev. 2013;27(19):2072-2085. doi:10.1101/gad.227439.113

Guerreiro S, Privat AL, Bressac L, Toulorge D. CD38 in Neurodegeneration and Neuroinflammation. Cells. 2020;9(2).

Gurumurthy, S., Xie, S.Z., Alagesan, B., Kim, J., Yusuf, R.Z., Saez, B., Tzatsos, A., Ozsolak, F., Milos, P., Ferrari, F., et al. (2010). The Lkb1 metabolic sensor maintains haematopoietic stem cell survival. Nature 468, 659-663.

Hao Y, Hao S, Andersen-Nissen E, Mauck WM, Zheng S, Butler A, et al. Integrated analysis of multimodal single-cell data. Cell. 2021;184(13):3573-87.e29

Hauser SL, Cree BAC. Treatment of Multiple Sclerosis: A Review. Am J Med. 2020;133(12):1380-90.e2.

Haynes, C.M., and Ron, D. (2010). The mitochondrial UPR - protecting organelle protein homeostasis. Journal of cell science 123, 3849-3855.

Haynes, C.M., Fiorese, C.J., and Lin, Y.F. (2013). Evaluating and responding to mitochondrial dysfunction: the mitochondrial unfolded-protein response and beyond. Trends in cell biology 23, 311-318.

He M, Chiang HH, Luo H, Zheng Z, Qiao Q, Wang L, et al. An Acetylation Switch of the NLRP3 Inflammasome Regulates Aging-Associated Chronic Inflammation and Insulin Resistance. Cell Metab. 2020;31(3):580-91.e5.

Herrmann MM, Barth S, Greve B, Schumann KM, Bartels A, Weissert R. Identification of gene expression patterns crucially involved in experimental autoimmune encephalomyelitis and multiple sclerosis. Dis Model Mech. 2016;9(10):1211-20.

Hershberger, K., Martin, A. & Hirschey, M. Role of NAD^+ and mitochondrial sirtuins in cardiac and renal diseases. *Nat Rev Nephrol* **13,** 213–225 (2017) doi:10.1038/nrneph.2017.5

Ho, T.T., Warr, M.R., Adelman, E.R., Lansinger, O.M., Flach, J., Verovskaya, E.V., Figueroa, M.E., and Passegue, E. (2017). Autophagy maintains the metabolism and function of young and old stem cells. Nature 543, 205-210.

Hogan KA, Chini CCS, Chini EN. The Multi-faceted Ecto-enzyme CD38: Roles in Immunomodulation, Cancer, Aging, and Metabolic Diseases. Front Immunol. 2019;10:1187.

Houtkooper, R.H., Mouchiroud, L., Ryu, D., Moullan, N., Katsyuba, E., Knott, G., Williams, R.W., and Auwerx, J. (2013). Mitonuclear protein imbalance as a conserved longevity mechanism. Nature 497, 451-457.

Igarashi M, Miura M, Williams E, Jaksch F, Kadowaki T, Yamauchi T, Guarente L, 2019. NAD(+) supplementation rejuvenates aged gut adult stem cells. Aging Cell 18, e12935.

Imani D, Azimi A, Salehi Z, Rezaei N, Emamnejad R, Sadr M, et al. Association of nod-like receptor protein-3 single nucleotide gene polymorphisms and expression with the susceptibility to relapsing-remitting multiple sclerosis. Int J Immunogenet. 2018;45(6):329-36.

Inkster B, Strijbis EM, Vounou M, Kappos L, Radue EW, Matthews PM, et al. Histone deacetylase gene variants predict brain volume changes in multiple sclerosis. Neurobiol Aging. 2013;34(1):238-47.

Inoue S, Noda S, Kashima K, Nakada K, Hayashi J, Miyoshi H, 2010. Mitochondrial respiration defects modulate differentiation but not proliferation of hematopoietic stem and progenitor cells. *FEBS letters* 584, 3402–3409.

Ito K, Suda T, 2014. Metabolic requirements for the maintenance of self-renewing stem cells. *Nat Rev Mol Cell Biol* 15, 243–256.

Ito, K., Hirao, A., Arai, F., Matsuoka, S., Takubo, K., Hamaguchi, I., Nomiyama, K., Hosokawa, K., Sakurada, K., Nakagata, N., et al. (2004). Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. Nature 431, 997-1002.

Ito, K., Turcotte, R., Cui, J., Zimmerman, S.E., Pinho, S., Mizoguchi, T., Arai, F., Runnels, J.M., Alt, C., Teruya-Feldstein, J., et al. (2016). Self-renewal of a purified Tie2+ hematopoietic stem cell population relies on mitochondrial clearance. Science 354, 1156-1160.

Jablonska B, Gierdalski M, Chew LJ, Hawley T, Catron M, Lichauco A, et al. Sirt1 regulates glial progenitor proliferation and regeneration in white matter after neonatal brain injury. Nat Commun. 2016;7:13866.

Jaiswal S, Ebert BL. Clonal hematopoiesis in human aging and disease. Science. 2019 Nov 1;366(6465):eaan4673. doi: 10.1126/science.aan4673. PMID: 31672865; PMCID: PMC8050831.

Jäkel S, Agirre E, Mendanha Falcão A, van Bruggen D, Lee KW, Knuesel I, et al. Altered human oligodendrocyte heterogeneity in multiple sclerosis. Nature. 2019;566(7745):543-7.

Johnson S, Imai SI. NAD $^{+}$ biosynthesis, aging, and disease. *F1000Res*. 2018;7:132. Published 2018 Feb 1. doi:10.12688/f1000research.12120.1

Juntilla MM, Patil VD, Calamito M, Joshi RP, Birnbaum MJ, Koretzky GA, 2010. AKT1 and AKT2 maintain hematopoietic stem cell function by regulating reactive oxygen species. *Blood* 115, 4030–4038.

Kerr, J. S. et al. Mitophagy and Alzheimer's disease: cellular and molecular mechanisms. *Trends Neurosci.* 40, 151–166 (2017).

Khacho M, Clark A, Svoboda DS, Azzi J, MacLaurin JG, Meghaizel C, Sesaki H, Lagace DC, Germain M, Harper ME, Park DS, Slack RS, 2016. Mitochondrial Dynamics Impacts Stem Cell Identity and Fate Decisions by Regulating a Nuclear Transcriptional Program. *Cell stem cell* 19, 232–247.

Khan RS, Dine K, Das Sarma J, Shindler KS. SIRT1 activating compounds reduce oxidative stress mediated neuronal loss in viral induced CNS demyelinating disease. Acta Neuropathol Commun. 2014;2:3.

Kim HS, Vassilopoulos A, Wang RH, Lahusen T, Xiao Z, Xu X, et al. SIRT2 maintains genome integrity and suppresses tumorigenesis through regulating APC/C activity. Cancer Cell. 2011;20(4):487-99.

Kusne Y, Xie Z, Patnaik MM. Clonal hematopoiesis: Molecular and clinical implications. Leuk Res. 2022 Feb;113:106787. doi: 10.1016/j.leukres.2022.106787. Epub 2022 Jan 10. PMID: 35091334.

Kutzelnigg A, Lucchinetti CF, Stadelmann C, Brück W, Rauschka H, Bergmann M, et al. Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain. 2005;128(Pt 11):2705-12.

Langley MR, Choi CI, Peclat TR, Guo Y, Simon WL, Yoon H, et al. Critical Role of Astrocyte NAD. J Neurosci. 2021;41(41):8644-67.

Lassmann H. Pathogenic Mechanisms Associated With Different Clinical Courses of Multiple Sclerosis. Front Immunol. 2018;9:3116.

Lautrup, S., Sinclair, D. A., Mattson, M. P. & Fang, E. F. NAD^+ in brain aging and neurodegenerative disorders. *Cell Metab*. 30, 630–655 (2019).
Lee IH. Mechanisms and disease implications of sirtuin-mediated autophagic regulation. Exp Mol Med. 2019;51(9):1-11.
Lin SJ, Defossez PA, Guarente L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. Science. 2000;289(5487):2126-2128. doi:10.1126/science.289.5487.2126
Lin, Y.F., Schulz, A.M., Pellegrino, M.W., Lu, Y., Shaham, S., and Haynes, C.M. (2016). Maintenance and propagation of a deleterious mitochondrial genome by the mitochondrial unfolded protein response. Nature 533, 416-419.
Lo Sasso G, Menzies KJ, Mottis A, Piersigilli A, Perino A, Yamamoto H, et al. SIRT2 deficiency modulates macrophage polarization and susceptibility to experimental colitis. PLoS One. 2014;9(7):e103573.
Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G, 2013. The hallmarks of aging. *Cell* 153, 1194–1217.
Lou, G. et al. Mitophagy and neuroprotection. *Trends Mol. Med.* (2019).
Luchsinger, L.L., de Almeida, M.J., Corrigan, D.J., Mumau, M., and Snoeck, H.W. (2016). Mitofusin 2 maintains haematopoietic stem cells with extensive lymphoid potential. Nature 529, 528-531.
Luo H, Chiang HH, Louw M, Susanto A, Chen D, 2017. Nutrient Sensing and the Oxidative Stress Response. *Trends Endocrinol Metab* 28, 449–460.
Luo H, Mu WC, Karki R, Chiang HH, Mohrin M, Shin JJ, et al. Mitochondrial Stress-Initiated Aberrant Activation of the NLRP3 Inflammasome Regulates the Functional Deterioration of Hematopoietic Stem Cell Aging. Cell Rep. 2019;26(4):945-54.e4.
Mahad DH, Trapp BD, Lassmann H. Pathological mechanisms in progressive multiple sclerosis. Lancet Neurol. 2015;14(2):183-93.
Malhotra S, Costa C, Eixarch H, Keller CW, Amman L, Martínez-Banaclocha H, et al. NLRP3 inflammasome as prognostic factor and therapeutic target in primary progressive multiple sclerosis patients. Brain. 2020;143(5):1414-30.
Malhotra S, Sorosina M, Río J, Peroni S, Midaglia L, Villar LM, et al. NLRP3 polymorphisms and response to interferon-beta in multiple sclerosis patients. Mult Scler. 2018;24(11):1507-10.
Manjula R, Anuja K, Alcain FJ. SIRT1 and SIRT2 Activity Control in Neurodegenerative Diseases. Front Pharmacol. 2020;11:585821.
Martens, C.R., Denman, B.A., Mazzo, M.R. et al. Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD+ in healthy middle-aged and older adults. Nat Commun 9, 1286 (2018) doi:10.1038/s41467-018-03421-7
McDougald DS, Dine KE, Zezulin AU, Bennett J, Shindler KS. SIRT1 and NRF2 Gene Transfer Mediate Distinct Neuroprotective Effects Upon Retinal Ganglion Cell Survival and Function in Experimental Optic Neuritis. Invest Ophthalmol Vis Sci. 2018;59(3):1212-20.
Merkwirth, C., Jovaisaite, V., Durieux, J., Matilainen, O., Jordan, S.D., Quiros, P.M., Steffen, K.K., Williams, E.G., Mouchiroud, L., Tronnes, S.U., et al. (2016). Two Conserved Histone Demethylases Regulate Mitochondrial Stress-Induced Longevity. Cell 165, 1209-1223.

Minden SL, Frankel D, Hadden LS, Srinath KP, Perloff JN. Disability in elderly people with multiple sclerosis: An analysis of baseline data from the Sonya Slifka Longitudinal Multiple Sclerosis Study. NeuroRehabilitation. 2004;19(1):55-67.

Misawa T, Takahama M, Kozaki T, Lee H, Zou J, Saitoh T, et al. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. Nat Immunol. 2013;14(5):454-60.

Miyamoto K, Araki KY, Naka K, Arai F, Takubo K, Yamazaki S, Matsuoka S, Miyamoto T, Ito K, Ohmura M, Chen C, Hosokawa K, Nakauchi H, Nakayama K, Nakayama KI, Harada M, Motoyama N, Suda T, Hirao A, 2007. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. *Cell stem cell* 1, 101–112.

Mohrin M, Shin J, Liu Y, Brown K, Luo H, Xi Y, Haynes CM, Chen D, 2015. Stem cell aging. A mitochondrial UPR-mediated metabolic checkpoint regulates hematopoietic stem cell aging. *Science* 347, 1374–1377.

Mohrin M, Widjaja A, Liu Y, Luo H, Chen D, 2018. The mitochondrial unfolded protein response is activated upon hematopoietic stem cell exit from quiescence. *Aging cell* 17, e12756.

Montalban X, Hauser SL, Kappos L, Arnold DL, Bar-Or A, Comi G, et al. Ocrelizumab versus Placebo in Primary Progressive Multiple Sclerosis. N Engl J Med. 2017;376(3):209-20.

Morita, M., Gravel, S.P., Chenard, V., Sikstrom, K., Zheng, L., Alain, T., Gandin, V., Avizonis, D., Arguello, M., Zakaria, C., et al. (2013). mTORC1 controls mitochondrial activity and biogenesis through 4E-BP-dependent translational regulation. Cell metabolism 18, 698-711.

Munch, C., and Harper, J.W. (2016). Mitochondrial unfolded protein response controls matrix pre-RNA processing and translation. Nature 534, 710-713.

Nakada, D., Saunders, T.L., and Morrison, S.J. (2010). Lkb1 regulates cell cycle and energy metabolism in haematopoietic stem cells. Nature 468, 653-658.

Nargund, A.M., Pellegrino, M.W., Fiorese, C.J., Baker, B.M., and Haynes, C.M. (2012). Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. Science 337, 587-590.

Nimmagadda VK, Bever CT, Vattikunta NR, Talat S, Ahmad V, Nagalla NK, et al. Overexpression of SIRT1 protein in neurons protects against experimental autoimmune encephalomyelitis through activation of multiple SIRT1 targets. J Immunol. 2013;190(9):4595-607.

North BJ, Rosenberg MA, Jeganathan KB, Hafner AV, Michan S, Dai J, et al. SIRT2 induces the checkpoint kinase BubR1 to increase lifespan. EMBO J. 2014;33(13):1438-53.

Olcum M, Tastan B, Kiser C, Genc S, Genc K. Microglial NLRP3 inflammasome activation in multiple sclerosis. Adv Protein Chem Struct Biol. 2020;119:247-308.

Pellegrino, M.W., Nargund, A.M., Kirienko, N.V., Gillis, R., Fiorese, C.J., and Haynes, C.M. (2014). Mitochondrial UPR-regulated innate immunity provides resistance to pathogen infection. Nature 516, 414-417.

Penberthy WT, Tsunoda I. The importance of NAD in multiple sclerosis. *Curr Pharm Des*. 2009;15(1):64–99. doi:10.2174/138161209787185751

Piancone F, Saresella M, Marventano I, La Rosa F, Santangelo MA, Caputo D, et al. Monosodium Urate Crystals Activate the Inflammasome in Primary Progressive Multiple Sclerosis. Front Immunol. 2018;9:983.

Pietras, E.M., Warr, M.R., and Passegue, E. (2011). Cell cycle regulation in hematopoietic stem cells. The Journal of cell biology 195, 709-720.

Qiu X, Brown K, Hirschey MD, Verdin E, Chen D, 2010. Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. *Cell Metab* 12, 662–667.

Rando, T.A. (2006). Stem cells, ageing and the quest for immortality. Nature 441, 1080-1086.

Reich DS, Lucchinetti CF, Calabresi PA. Multiple Sclerosis. N Engl J Med. 2018;378(2):169-80.

Ren R, Ocampo A, Liu GH, Izpisua Belmonte JC, 2017. Regulation of Stem Cell Aging by Metabolism and Epigenetics. *Cell Metab* 26, 460–474.

Renault, V.M., Rafalski, V.A., Morgan, A.A., Salih, D.A., Brett, J.O., Webb, A.E., Villeda, S.A., Thekkat, P.U., Guillerey, C., Denko, N.C., et al. (2009). FoxO3 regulates neural stem cell homeostasis. Cell stem cell 5, 527-539.

Rice CM, Sun M, Kemp K, Gray E, Wilkins A, Scolding NJ. Mitochondrial sirtuins--a new therapeutic target for repair and protection in multiple sclerosis. Eur J Neurosci. 2012;35(12):1887-93.

Roboon J, Hattori T, Ishii H, Takarada-Iemata M, Le TM, Shiraishi Y, et al. Deletion of CD38 Suppresses Glial Activation and Neuroinflammation in a Mouse Model of Demyelination. Front Cell Neurosci. 2019;13:258.

Rogina B, Helfand SL. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. Proc Natl Acad Sci U S A. 2004;101(45):15998-16003. doi:10.1073/pnas.0404184101

Rossi, D.J., Bryder, D., Seita, J., Nussenzweig, A., Hoeijmakers, J., and Weissman, I.L. (2007). Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature 447, 725-729.

Rossi, D.J., Jamieson, C.H., and Weissman, I.L. (2008). Stems cells and the pathways to aging and cancer. Cell 132, 681-696.

Sahin, E., and Depinho, R.A. (2010). Linking functional decline of telomeres, mitochondria and stem cells during ageing. Nature 464, 520-528.

Sanai SA, Saini V, Benedict RH, Zivadinov R, Teter BE, Ramanathan M, et al. Aging and multiple sclerosis. Mult Scler. 2016;22(6):717-25.

Scalfari A, Neuhaus A, Daumer M, Ebers GC, Muraro PA. Age and disability accumulation in multiple sclerosis. Neurology. 2011;77(13):1246-52.

Shin J, He M, Liu Y, Paredes S, Villanova L, Brown K, Qiu X, Nabavi N, Mohrin M, Wojnoonski K, Li P, Cheng HL, Murphy AJ, Valenzuela DM, Luo H, Kapahi P, Krauss R, Mostoslavsky R, Yancopoulos GD, Alt FW, Chua KF, Chen D, 2013. SIRT7 represses Myc activity to suppress ER stress and prevent fatty liver disease. *Cell reports* 5, 654–665.

Shindler KS, Ventura E, Dutt M, Elliott P, Fitzgerald DC, Rostami A. Oral resveratrol reduces neuronal damage in a model of multiple sclerosis. J Neuroophthalmol. 2010;30(4):328-39.

Soares JL, Oliveira EM, Pontillo A. Variants in NLRP3 and NLRC4 inflammasome associate with susceptibility and severity of multiple sclerosis. Mult Scler Relat Disord. 2019;29:26-34.

Sperka, T., Wang, J., and Rudolph, K.L. (2012). DNA damage checkpoints in stem cells, ageing and cancer. Nature reviews Molecular cell biology 13, 579-590.

Takubo, K., Nagamatsu, G., Kobayashi, C.I., Nakamura-Ishizu, A., Kobayashi, H., Ikeda, E., Goda, N., Rahimi, Y., Johnson, R.S., Soga, T., et al. (2013). Regulation of glycolysis by Pdk

functions as a metabolic checkpoint for cell cycle quiescence in hematopoietic stem cells. Cell stem cell 12, 49-61.

Tang Y, Luo B, Deng Z, Wang B, Liu F, Li J, Shi W, Xie H, Hu X, Li J, 2016. Mitochondrial aerobic respiration is activated during hair follicle stem cell differentiation, and its dysfunction retards hair regeneration. *PeerJ* 4, e1821.

Tian, Y., Garcia, G., Bian, Q., Steffen, K.K., Joe, L., Wolff, S., Meyer, B.J., and Dillin, A. (2016). Mitochondrial Stress Induces Chromatin Reorganization to Promote Longevity and UPR(mt). Cell 165, 1197-1208.

Tormos KV, Anso E, Hamanaka RB, Eisenbart J, Joseph J, Kalyanaraman B, Chandel NS, 2011. Mitochondrial complex III ROS regulate adipocyte differentiation. *Cell metabolism* 14, 537–544.

Tothova Z, Kollipara R, Huntly BJ, Lee BH, Castrillon DH, Cullen DE, McDowell EP, Lazo-Kallanian S, Williams IR, Sears C, Armstrong SA, Passegue E, DePinho RA, Gilliland DG, 2007. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell* 128, 325–339.

Tripathi RB, Rivers LE, Young KM, Jamen F, Richardson WD. NG2 glia generate new oligodendrocytes but few astrocytes in a murine experimental autoimmune encephalomyelitis model of demyelinating disease. J Neurosci. 2010;30(48):16383-90.

Vannini N, Campos V, Girotra M, Trachsel V, Rojas-Sutterlin S, Tratwal J, Ragusa S, Stefanidis E, Ryu D, Rainer PY, Nikitin G, Giger S, Li TY, Semilietof A, Oggier A, Yersin Y, Tauzin L, Pirinen E, Cheng WC, Ratajczak J, Canto C, Ehrbar M, Sizzano F, Petrova TV, Vanhecke D, Zhang L, Romero P, Nahimana A, Cherix S, Duchosal MA, Ho PC, Deplancke B, Coukos G, Auwerx J, Lutolf MP, Naveiras O, 2019. The NAD-Booster Nicotinamide Riboside Potently Stimulates Hematopoiesis through Increased Mitochondrial Clearance. Cell stem cell 24, 405–418.e407.

Vannini, N., Girotra, M., Naveiras, O., Nikitin, G., Campos, V., Giger, S., Roch, A., Auwerx, J., and Lutolf, M.P. (2016). Specification of haematopoietic stem cell fate via modulation of mitochondrial activity. Nature communications 7, 13125.

Verdin, E. NAD(+) in aging, metabolism, and neurodegeneration. *Science* **350**, 1208–1213 (2015).

Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, et al. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature. 2011;477(7362):90-4.

Walter, D., Lier, A., Geiselhart, A., Thalheimer, F.B., Huntscha, S., Sobotta, M.C., Moehrle, B., Brocks, D., Bayindir, I., Kaschutnig, P., et al. (2015). Exit from dormancy provokes DNA-damage-induced attrition in haematopoietic stem cells. Nature 520, 549-552.

Wang X, Li B, Liu L, Zhang L, Ma T, Guo L. Nicotinamide adenine dinucleotide treatment alleviates the symptoms of experimental autoimmune encephalomyelitis by activating autophagy and inhibiting the NLRP3 inflammasome. Int Immunopharmacol. 2021;90:107092.

Warr, M.R., and Passegue, E. (2013). Metabolic makeover for HSCs. Cell stem cell 12, 1-3.

Yoon H, Kleven A, Paulsen A, Kleppe L, Wu J, Ying Z, et al. Interplay between exercise and dietary fat modulates myelinogenesis in the central nervous system. Biochim Biophys Acta. 2016;1862(4):545-55.

Yu, W.M., Liu, X., Shen, J., Jovanovic, O., Pohl, E.E., Gerson, S.L., Finkel, T., Broxmeyer, H.E., and Qu, C.K. (2013). Metabolic regulation by the mitochondrial phosphatase PTPMT1 is required for hematopoietic stem cell differentiation. Cell stem cell 12, 62-74.

Yuan F, Xu ZM, Lu LY, Nie H, Ding J, Ying WH, et al. SIRT2 inhibition exacerbates neuroinflammation and blood-brain barrier disruption in experimental traumatic brain injury by enhancing NF-κB p65 acetylation and activation. J Neurochem. 2016;136(3):581-93.

Zhang H, Ryu D, Wu Y, Gariani K, Wang X, Luan P, D'Amico D, Ropelle ER, Lutolf MP, Aebersold R, Schoonjans K, Menzies KJ, Auwerx J, 2016. NAD(+) repletion improves mitochondrial and stem cell function and enhances life span in mice. Science 352, 1436–1443.

Zhang Y, Marsboom G, Toth PT, Rehman J, 2013. Mitochondrial respiration regulates adipogenic differentiation of human mesenchymal stem cells. *PloS one* 8, e77077.

Zhao, Q., Wang, J., Levichkin, I.V., Stasinopoulos, S., Ryan, M.T., and Hoogenraad, N.J. (2002). A mitochondrial specific stress response in mammalian cells. The EMBO journal 21, 4411-4419.

Zheng X, Boyer L, Jin M, Mertens J, Kim Y, Ma L, Ma L, Hamm M, Gage FH, Hunter T, 2016. Metabolic reprogramming during neuronal differentiation from aerobic glycolysis to neuronal oxidative phosphorylation. *eLife* 5.