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Understanding muscle regenerative decline with aging: *new approaches to bring back youthfulness to aged stem cells*

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Abstract

Aging is characterized by the progressive dysfunction of most tissues and organs, which has been linked to the regenerative decline of their resident stem cells over time. Skeletal muscle provides a stark example of this decline. Its stem cells, also called satellite cells, sustain muscle regeneration throughout life, but at advanced age they fail for largely undefined reasons. Here, we discuss current understanding of the molecular processes regulating satellite-cell maintenance throughout life and how age-related failure of these processes contributes to muscle aging. We also highlight the emerging field of rejuvenating biology to restore features of youthfulness in satellite cells, with the ultimate goal of slowing down or reversing the age-related decline in muscle regeneration.

Introduction

Stem cells are rare and specialized cells within a fully differentiated tissue that are essential for host-organ repair and renewal. In an adult organism, stem-cell populations have the unique capacity both to self-renew and to generate progeny that differentiates to replace lost or damaged cells [1, 2]. In most instances, stem cell properties and behavior are determined by the regenerative requirements of the host tissue: high-turnover tissues like the intestine or the hematopoietic system maintain active populations of stem- or progenitor-cell populations, whereas tissues like the skeletal muscle maintain most of their stem cell pool in a quiescent state, activating it only upon injury [3]. The central role played by stem cells in tissue replacement throughout the human lifespan means that their functional decline often results in compromised organ maintenance and regeneration. At the same time, stem-cell based therapies hold immense potential for regenerative medicine to restore or rejuvenate tissues.

Aging is accompanied by a progressive decline in tissue function and increased vulnerability to disease. Current understanding of the aging process centers on the interplay of cell-intrinsic, inter-cellular communication and systemic dysregulations. In this context, the adult stem cell is simultaneously an important integrator of multiple age-related alterations and a key contributor to the progression of age-related tissue dysfunction [4, 5].

Skeletal muscle harbors a population of quiescent stem cells, called satellite cells, that are essential for its extraordinary regenerative capacity. Upon injury, satellite cells exit quiescence and proliferate, giving rise to a population of committed progenitors capable of engaging the myogenic program to generate new myofibers and replace damaged tissue [6, 7]. This remarkable regenerative capacity is greatly affected by the aging process and is associated with an age-related decline in satellite-cell numbers and functionality. Moreover, skeletal-muscle aging is characterized by a loss of mass and decline in muscle strength, a phenotype that broadly defines sarcopenia. Although the involvement of stem cells in the etiology of sarcopenia is debated [8], the maintenance of a healthy population of satellite cells or the exogenous delivery of a rejuvenated progenitor population to the aging muscle has the potential to correct acquired defects that give rise to age-related muscle wasting. Skeletal muscle is thus an attractive model

for the study of stem-cell and tissue aging, as well as a potential future target tissue for stem cell-based rejuvenating interventions.

In this review, we examine and discuss our current understanding of the determinants of satellite-cell aging and their contribution to age-related loss of regenerative capacity in skeletal muscle. We also highlight recent advances in the field pointing to promising rejuvenating interventions that could help restore skeletal-muscle regenerative capacity in the elderly. Finally, we discuss the potential of stem-cell-based interventions as rejuvenating strategies to prevent or delay age-related loss of muscle mass and force.

How muscle stem cells age

Skeletal-muscle aging is accompanied by a considerable reduction in the size of the satellite cell pool. The decline in satellite-cell numbers occurs in the early stages of muscle aging and is likely due to changes affecting the niche and to cell-autonomous alterations that together disturb the proper balance between cell quiescence, proliferation and apoptosis. Increased expression of myofiber-derived FGF2 in old muscles has been shown to bring satellite cells out of quiescence, causing spontaneous mitogenic activity that can contribute to the exhaustion of the satellite-cell pool [9]. Additionally, FGF2 inhibits the expression of sprouty1, an intracellular inhibitor of the ERK/MAPK signaling pathway, and mouse models with satellite-cell-specific deletion of Sprouty1 have defects in the muscle stem-cell pool. Sprouty1-null cycling satellite cells are impaired in their capacity to return to quiescence, succumbing instead to apoptosis [10]. Depletion of resident satellite cells after regenerative events in aged muscles also involves deficiencies in the aged niche microenvironment. A recent study revealed that an age-related decline in Notch activators in the niche provokes satellite cell death by mitotic catastrophe, impairing proliferative expansion of muscle stem cell in aged mice [11]. The idea that stem-cell survival is an important factor in the maintenance of the satellite-cell pool is further supported by the observations that old human satellite cells are susceptible to nuclear apoptosis [12] and that the promotion of anti-apoptotic pathways in aged satellite cells improves muscle regenerative capacity [13].

Although the satellite cell pool diminishes with age, a fraction of the satellite cells survives within the skeletal muscle until very old age. However, even when they are present, these satellite cells have functional defects that undermine their ability to sustain muscle regenerative capacity. The defects in satellite-cell proliferative activity in response to regenerative pressure seen in early stages of the aging process are exacerbated in very old (geriatric) mice. Whereas the early defects are driven by alterations in the environment, at advanced ages quiescent satellite cells are intrinsically changed and become pre-senescent, a state that leads to full senescence in response to regenerative pressure. As a consequence of these defects, old satellite cells show a reduced capacity for activation and expansion after injury, and produce insufficient progeny to sustain muscle regeneration. Moreover, the progeny of muscle stem cells that overcome these limitations have a limited differentiation capacity, with poor myogenic potential and a tendency to commit to alternative lineages. The consequence of these defects in old skeletal muscle is an enhanced fibrotic response to injury. These functional alterations have multiple underlying

molecular causes, including altered signaling cues from the aging environment and intrinsic alterations in genomic integrity and metabolic regulation within the satellite cell (Figure 1, [14-16]).

The Aging Environment

In a young organism, satellite-cell function is tightly regulated by signals originating from the niche and the systemic environment. Alterations to the surrounding milieu have profound effects on satellite cell quiescence, differentiation and self-renewal, and deterioration of the extracellular environment is one of the main factors determining age-related stem-cell dysfunction [17].

The contribution of the aging environment to the decline in satellite-cell function was clearly demonstrated by heterochronic tissue transplant studies, which revealed that the age of the host animal was a key determinant factor of the regenerative success of the transplant [18-20]. Subsequent studies employed heterochronic parabiosis, an experimental model involving the surgically pairing and fusion of the circulatory systems of two mice; this approach demonstrated that the regenerative capacity of the muscle is modulated by exposure to blood from an animal of a different age [21, 22]. A recent study obtained similar results through direct blood exchange between a young mouse and an aged mouse, supporting the established idea that circulatory factors directly affect satellite-cell aging [23].

Since these early discoveries, several signaling molecules, including niche-derived signaling cues and systemic factors, have been identified as mediators of the extrinsic effects of the aging environment on satellite cell function [17]. Changes in the myofiber, the most abundant source of niche signaling, have a significant impact on satellite-cell activity: an age-related increase in TGF β and FGF signaling from the myofiber synergize with a decline in D Δ -driven Notch signaling and a decreased deposition of the ECM protein fibronectin to disrupt satellite-cell activity, contributing to impaired regenerative capacity at old age [9, 24-28]. Changes in TGF β and Notch activity contribute to an imbalance in satellite-cell activation and differentiation cues [11, 24, 25], while increased FGF signaling breaks satellite-cell quiescence leading to stem cell loss [9]. The remaining satellite cells become unresponsive to FGF under regenerative pressure and fail to expand or self-renew [26], a defect that is exacerbated by defective fibronectin deposition in old muscles undergoing regeneration, and the consequent impairment in integrin signaling [27, 28]. Aging also impairs the supportive function of fibro-adipogenic progenitors (FAPs), which fail to induce the matricellular protein WISP1, required for satellite cell expansion and commitment [29].

Heterochronic parabiosis have identified systemic factors as key regulators of muscle stem-cell function. These include the unconventional Wnt-activating ligand complement component 1q (C1q), the TGF β family member growth differentiation factor 11 (GDF11), and the hormone oxytocin [21, 30-32]. Increased Wnt signaling driven by systemic factors promotes aberrant fibrogenic commitment in aged satellite cells, leading to a fibrotic response upon regenerative pressure [21]. Conversely, oxytocin and GDF11 have been identified as rejuvenating factors, decreased in the circulation of old animals and offer a possible route to improving satellite cell function and muscle regenerative capacity. However, the effects of GDF11 on the muscle and its age-related changes are still debated, with contradictory reports highlighting the need for further investigation [33-37]. Another important circulatory factor affecting satellite-cell function in sarcopenic muscles is the exercise-induced myokine apelin [38]. Apelin levels decrease with age,

and studies in mice show that this has important consequences for healthspan [39]. A recent study revealed an association between apelin signaling and the beneficial effects of exercise, and also identified a positive effect on the regenerative capacity of aged muscle stem cells [38].

Age-related alterations in the immune environment have also emerged in recent years as important contributors to satellite cell impairments in old muscles. Regenerative success depends on a regulated immune response to muscle injury, involving multiple immune cell types and the coordination of pro-inflammatory and anti-inflammatory signaling [40, 41]. Muscle regeneration is critically regulated by macrophages [42, 43] and bone marrow transplant from old donors is sufficient to impair satellite-cell function in young mice, reducing the number of Pax7+ cells and promoting fibrogenic conversion [44]. A potential mediator of these effects is an age-related increase in myeloid-derived TNF α signaling: TNF α -expressing macrophages are present in aged skeletal muscle and old TNF α -null mice show improved satellite-cell activation and myogenic commitment in response to injury [45]. Consistently with these observations, there is evidence that over-activation of the NF κ B pathway (an important TNF α target) in muscle of old mice is sufficient to disrupt satellite-cell function and limit regenerative success [46]. In addition to TNF α -NF κ B signaling, age-related impairments of satellite-cell function are also linked to JAK/STAT signaling. Increased STAT3 activation in old satellite cells compromises symmetric expansion by promoting direct myogenic commitment, and limits regenerative capacity [47]; however, the source of STAT-activating factors in the old muscles is still unknown. The age-related impairment of satellite-cell function is thus likely to be the result of the over-activation of multiple inflammatory pathways acting synergistically. Muscle regenerative capacity is also thought to be limited by age-related impairments to Treg signaling: IL-33-dependent recruitment of Tregs during muscle regeneration is decreased in old mice, due to ineffective production of IL-33 by FAP-like cells, and these defects compromise muscle regenerative capacity [48]. Since one of the functions of Tregs during muscle regeneration is to coordinate the transition between pro-inflammatory and anti-inflammatory macrophage states, this age-related impairment may be an additional way in which chronic activation of inflammatory signaling compromises muscle regeneration in old animals.

Epigenetic Changes and Genomic Stability

Satellite-cell aging is accompanied by genome-wide epigenetic changes that translate abnormal signaling from the aged environment and a life-long accumulation of molecular damage into altered gene expression programs. Comparative epigenomic analysis of young and old satellite cells has revealed complex changes with distinct consequences for quiescent and activated stem cells: while in quiescent satellite cells an age-related increase in repressive chromatin marks can contribute to changes in the expression of genes involved in stem cell self-renewal and lineage commitment [49], in old activated satellite cells permissive chromatin states cause the aberrant induction of developmental pathways that impair stem cell function [50]. Understanding of the impact of these global changes at the level of individual gene expression is still limited; however, epigenetic changes are known to play an important role in the conversion of quiescent satellite cells to a pre-senescent state in geriatric mice [51]. Specific analysis of the p16INK4a locus in satellite cells isolated from geriatric muscles revealed the loss of ubiquitinated H2A, a chromatin repressive mark associated with the polycomb repressor complex 1 (PRC1) [51]. More recently,

the transcriptional repressor Slug, was also found to be down-regulated in aged satellite cells, contributing to the derepression of the p16INK4a gene and the conversion of aged muscle stem cells to a pre-senescent state [52]. Future studies of global chromatin accessibility will likely reveal other changes to specific genomic loci that trigger age-related perturbations in satellite cell function. It should also be remembered that epigenetic alterations can impact the health of neighboring cells in the satellite-cell niche, indirectly contributing to muscle stem cell loss of function. Analysis of histone modifications in whole human skeletal muscle tissues found an age-associated increase in the active enhancer marker H3K27ac. In mouse models, this enhancer activation is associated with the up-regulation of ECM genes during aging, contributing to a decline in myogenic capacity and increased fibrogenic conversion of aged satellite cells [53].

The accumulation of DNA damage is a potential source of genomic instability and an important contributor to global changes in the epigenome with aging [54, 55]. The quiescent satellite cell combines a low risk of replication-induced DNA damage with highly efficient mechanisms of DNA repair [56]. However, satellite cells isolated from aged mice still have an elevated number of foci containing the DNA damage marker γ H2AX [31]. Furthermore, whole-genome sequencing of human satellite cells isolated from individuals of different ages revealed an age-related increase in the somatic mutation burden [57], reinforcing the notion that loss of genomic integrity is an important factor in satellite-cell aging. Knowledge remains limited about how age-related environmental changes converge to drive alterations in the satellite-cell epigenetic landscape. A better understanding of these processes in aged satellite cells could provide important insights into how to expand the muscle stem-cell healthspan.

Autophagic and Metabolic Defects

The life-long accumulation of altered or damaged proteins is an important factor in age-related stem-cell dysfunction, affecting multiple intracellular signaling pathways and ultimately driving genomic instability, senescence and apoptosis [58]. Satellite cells, mostly quiescent throughout life, cannot eliminate toxic organelles and protein aggregates through cell division, and are therefore particularly susceptible to proteostatic stress [59]. As a consequence, mechanisms of intracellular macromolecular clearance, such as autophagy, play a fundamental role in the maintenance of satellite-cell quiescence [58]. Impairment of autophagy causes the accumulation of damaged mitochondria and the generation of high ROS levels, further propagating protein and DNA damage. During aging, a decline in autophagic flux and the consequent increase in ROS levels are directly linked to the epigenetic depression of the p16INK4a locus in old satellite cells and their entry into senescence [60]. The observed decline in defective organelle clearance in aged quiescent satellite cells may be related to an oscillatory rewiring that affects the expression of autophagy genes [61].

Autophagy is also required for rapid energy mobilization to meet the metabolic demands of satellite-cell activation [62]. The AMPK signaling pathway seems to play an important role in satellite cell fate decisions when autophagy is unable to meet the energy needs of activated cells. Ectopic activation of the AMPK/p27Kip1 pathway enhances autophagy and reduces markers of cell senescence in aged satellite cells, improving their transplantation potential [13]. The close interconnection between proteostatic and metabolic pathways is well illustrated by a recent study

showing that mitochondrial oxidative respiration is fundamental for the functional maintenance of satellite cells. A reduction in the cellular levels of the oxidized form of cellular nicotinamide adenine dinucleotide (NAD⁺) affects the normal mitochondrial protein response (UPR_{mt}), ultimately leading to satellite-cell senescence [63]. Mitochondrial DNA damage can also affect mitochondrial ultrastructure and cell bioenergetics. Levels of α -Klotho, a membrane-bound and circulating hormonal protein, are decreased in satellite cells derived from aged mice, and genetic knockdown of α -Klotho in young muscle stem cells provokes an aged phenotype, consisting of decreased mitochondrial bioenergetics activity, mitochondrial DNA damage, and increased senescence [64]. Replicative senescence of human satellite cells is also associated with the oxidation of glycolytic enzymes, resulting in impaired glucose metabolism and a metabolic shift of energy substrates in senescent muscle stem cells [65].

Interventions for satellite-cell rejuvenation

Considering the combined role of extrinsic and intrinsic determinants on satellite cell aging, interventions that aim to restore the functionality of an aged muscle stem cell are likely to require combinatorial strategies that target age-dependent deregulations in niche signaling and cell-intrinsic alterations simultaneously [66, 67]. The studies described in the previous chapter have contributed promising new approaches to the restoration of regenerative capacity in sarcopenic muscles (Figure 2).

Ex-vivo manipulations of aged satellite cells have proven to be effective strategies to reverse some of the intrinsic alterations limiting their regenerative potential (Figure 2). These manipulations include genetic interventions to silence p16INK4a expression, thereby restoring quiescence and regenerative capacity to the aged satellite cell [51, 52]. Similarly, ex-vivo pharmacological inhibition of p38 MAPK signaling decreases the expression of cell-cycle inhibitors, such as p16INK4a, and restores asymmetric division in satellite cells, contributing to enhanced regenerative potential of aged satellite cells in muscle transplantation experiments [26, 68]. In vivo, local and systemic interventions have also shown promise in reversing age-related satellite cell defects (Figure 2). For example, systemic pharmacological treatments to restore basal autophagy flux preserved quiescence and muscle stem cell regenerative capacity in old muscles [60]. Similarly, systemic delivery of oxytocin restores age-related regenerative capacity in old muscles [32], promoting satellite-cell activation and proliferation, while systemic delivery of WISP1 during a regenerative event improves myogenic commitment and regenerative success [29]. Moreover, systemic delivery of exogenous α -Klotho improves muscle stem cell bioenergetics and improves regenerative capacity in aged animals [64]. Local delivery of fibronectin or β 1-integrin activators also restores satellite cell responsiveness in old mice, enhancing regenerative capacity [27, 28], while intramuscular injection of Wnt inhibitors restore the myogenic potential of old muscle stem cells [21].

Rejuvenating interventions able to target the whole organism have also a positive impact on satellite-cell function during aging. Successful interventions include caloric restriction [69], rapamycin treatment [60], supplementation with the NAD⁺ precursor nicotinamide riboside (NR) [63], senescent cell ablation [70] and in vivo reprogramming [71]. These studies anticipate the

existence of common hallmarks of aging associated with satellite-cell loss of function in old animals, which can be considered common targets for intervention. Consistently, targeting chronic inflammation (a shared feature of several age-related pathologies) through systemic treatment with an inhibitor of NF κ B activation, improves myogenic function in aged satellite cells [46]. The regenerative capacity of old skeletal muscle is also improved by intramuscular or systemic supplementation with IL-33, which reestablishes the recruitment of Tregs into injured muscles [48]. Another related approach is the inhibition of JAK/STAT pathway: isolated satellite cells treated ex-vivo with JAK-STAT inhibitors show improved engraftment in transplantation experiments [47], and intra muscular delivery of STAT inhibitors during regeneration in old mice, also significantly improved muscle regenerative capacity [47]. However, inflammatory pathways are essential regulators of the muscle regenerative response, and pro-inflammatory signaling is essential for the activation phase of satellite-cell function; therefore, caution is advised when using these interventions. For example, inhibition of pro-inflammatory mediators impairs the regenerative response in young animals [72], and IL-6-driven STAT activation may be essential for myogenic commitment during regenerative events [73] and satellite-cell-dependent muscle growth during hypertrophy [74].

Stem-cell-based interventions in muscle aging

The use of stem cells as sources for tissue repair and renewal introduces their potential as rejuvenating interventions [66]. In addition to their primary role as agents of tissue regeneration after injury, satellite cells also contribute to the homeostatic maintenance of muscle fibers [75, 76]. However, debate continues about the contribution of satellite cells to the pathogenesis of sarcopenia. Experiments involving life-long ablation of satellite cells did not produce precocious or exacerbated signs of sarcopenia in sedentary mice [77], leading the authors to conclude that the loss of satellite cells that happens during aging does not contribute the development of sarcopenia. However, in young animals, exercise increases muscle mass through the combined effect on new fiber production in response to exercise-induced fiber damage and the hypertrophy of existing fibers, with both processes depending, at least in part, on satellite-cell function [78, 79]. It is thus possible that the wear and tear associated with daily activity in humans provides a signal for satellite-cell dependent maintenance of muscle mass that was not captured in the ablation study in mice. Moreover, given the functional impairment of satellite cells that persist in the old skeletal muscle, these may be unable to contribute to fiber maintenance in old age. Supporting this view, a recent study showed that preserving functional satellite cells in the old skeletal muscle (through the overexpression of *spry1*) preserves muscle mass and force and attenuates sarcopenia development [80].

Nevertheless, the possible causal relationship of satellite-cell loss-of-function to sarcopenia does not negate the potential of satellite cells as an intervention to restore sarcopenic muscle [81]. The use of healthy muscle stem cells to generate new muscle fibers, and/or to contribute new myonuclei to existing fibers, can be envisioned as a viable strategy to correct the defects that drive age-related muscle wasting, restoring muscle mass and force, and rejuvenating the sarcopenic muscle. In a proof-of-principle study supporting this idea, Hall et al. [82] showed that satellite cells transplanted into injured muscle of young mice (along with the associated muscle

fiber) contribute to an increase in muscle mass and force that persists as the mice age. In this model, the life-long persistence of the enhanced muscle mass results from an increase in myofiber numbers and a progressive myofiber hypertrophy, attributed to myonuclear accretion [82].

Satellite cells and other myogenic progenitor-cell types have been successfully used in young animals to ameliorate the effects of degenerative muscle diseases caused by genetic conditions [Tedesco, 2012]. However, there are no published reports of direct delivery of muscle progenitors into the muscles of old sarcopenic mice. Moreover, while these studies are promising, they have also brought to light several limitations with important implications for the use of cell therapy applied in aged skeletal muscle [66, 81]. Successful engraftment with the current methodology used in preclinical settings to deliver progenitor cells to the muscle requires a concurrent injury, which is not a viable strategy for the treatment of sarcopenic muscles. Moreover, the systemic and local environment of chronic inflammation in old organisms presents a further obstacle to the success of these interventions [66], and would likely worsen survival, engraftment and the myogenic potential of transplanted progenitors. The age-related inflammation and dysregulated immune environment have been consistently demonstrated to have a negative impact on satellite-cell function, limiting their myogenic potential and regenerative capacity, contributing significantly to the development of sarcopenia [44-46]. In a model of Duchene muscular dystrophy (DMD), macrophages have been used to deliver pro-activating signals that enable expansion of myoblasts *in situ* before differentiation is initiated [83]. These experiments, together with the use of pharmacological interventions that promote myoblast migration [84], showed that it is possible to modulate muscle progenitor activity *in situ* to promote muscle repair. Successful cell therapies for sarcopenia will likely require the development of concurrent interventions to provide the pro-repair signals elicited by the injury and to promote myogenesis and the subsequent steps of muscle regeneration that are inhibited by the pro-inflammatory environment.

One possible source of such signals is exercise. In DMD models, the injury signal produced by exercise increases the success of muscle-progenitor engraftment, removing the need for a concurrent injury induced by toxins or chemicals [85]. However, analysis of muscles subjected to overload induced by synergistic ablation suggests that physical exercise is insufficient to induce myofiber hypertrophy in the old skeletal muscle [86], but can improve satellite-cell responses in injury models [87]. Thus, it is likely that in aged animals, the ability of exercise to induce pro-growth and pro-repair signals is not fully functional. Recent work suggests that this is indeed the case, at least for the exercine apelin [38]. The authors showed that young but not aged mice or humans increase apelin levels in response to exercise. Since low apelin levels are associated with sarcopenia and apelin signaling promotes fiber hypertrophy and satellite-cell-mediated muscle repair [38], it is likely that the lack of such signals in aging would blunt the beneficial effects of exercise in promoting myoblast engraftment. It is therefore critical to identify other factors that mediate the beneficial effects of exercise and that could be used as co-adjuvants in skeletal-muscle cell therapy in sarcopenia.

Figure legends:

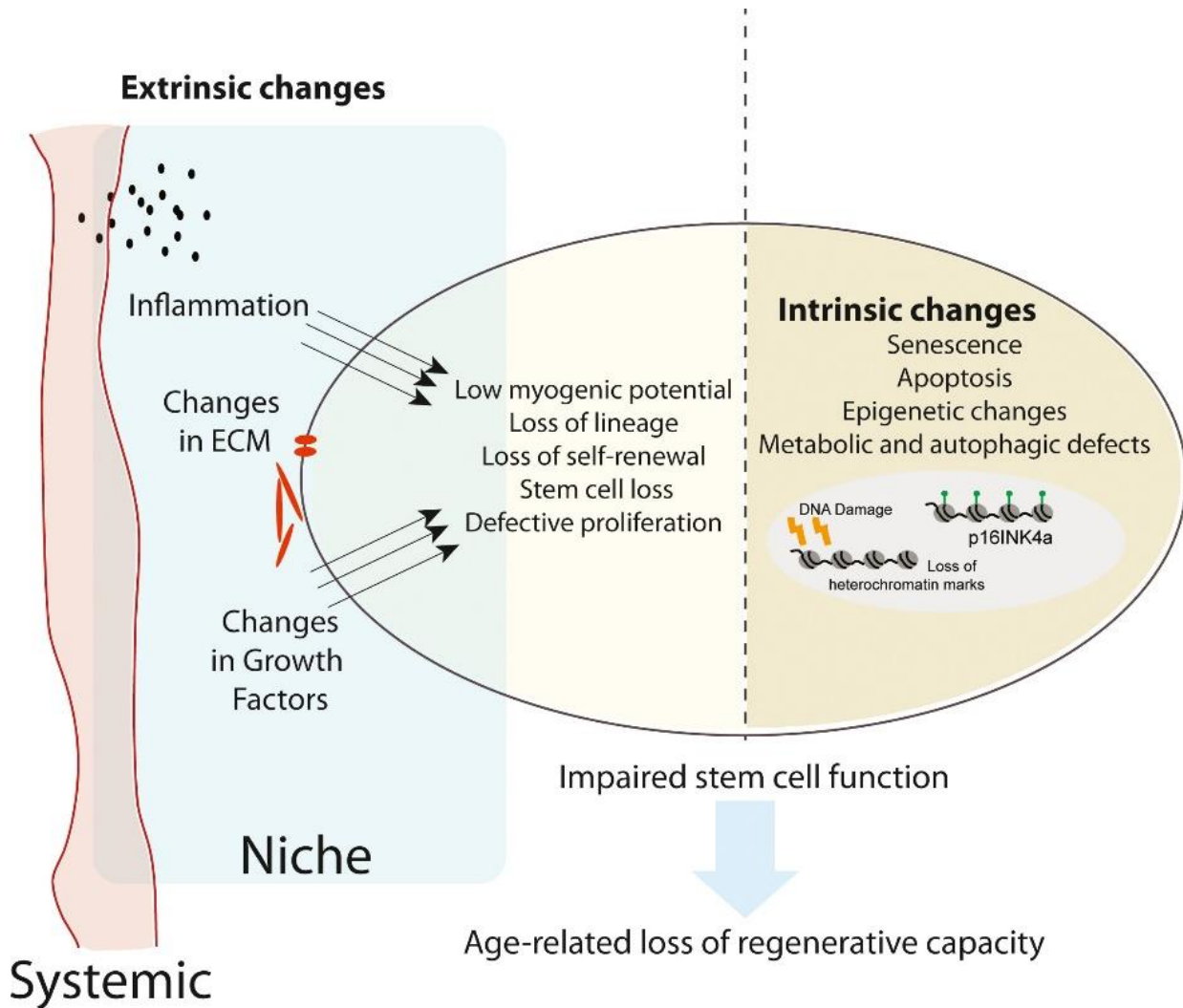


Figure 1: Extrinsic and intrinsic drivers of satellite cell aging.

Changes in niche-derived and systemic signaling molecules, along with intrinsic changes in the satellite cell, contribute to the functional impairments of aged muscle stem cells and the consequent defects in regenerative capacity of the aged skeletal muscle.

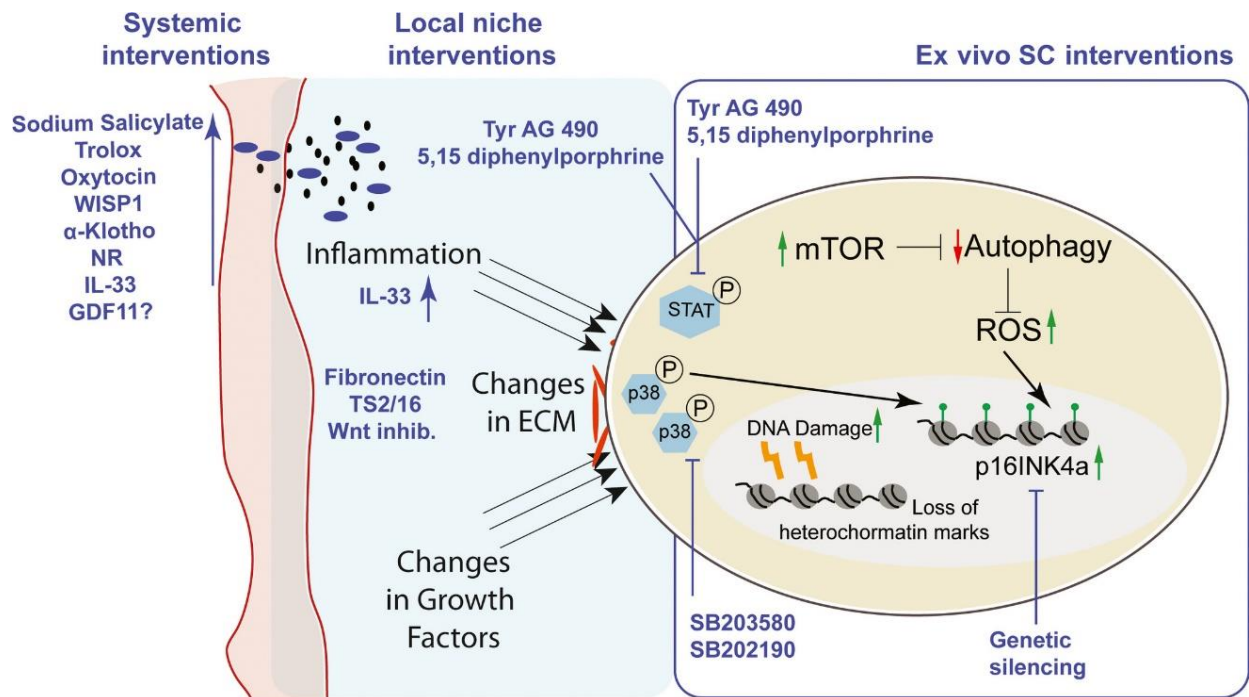


Figure 2: Interventions for satellite cell rejuvenation.

Rejuvenation of the regenerative capacity of aged skeletal muscle can be achieved through multiple interventions, including ex-vivo rejuvenation of the satellite cell pool prior to transplantation, intra muscular delivery of agents that regulate niche signaling to improve satellite cell function, and systemic treatments that target satellite cell and niche specific age-related alterations.

References

1. Fuchs, E. & Chen, T. (2013) A matter of life and death: self-renewal in stem cells, *EMBO Rep.* **14**, 39-48.
2. Weissman, I. L. (2000) Stem cells: Units of development, units of regeneration, and units in evolution, *Cell.* **100**, 157-168.
3. Sanchez Alvarado, A. & Yamanaka, S. (2014) Rethinking differentiation: stem cells, regeneration, and plasticity, *Cell.* **157**, 110-9.
4. Lopez-Otin, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. (2013) The hallmarks of aging, *Cell.* **153**, 1194-217.
5. Sharpless, N. E. & DePinho, R. A. (2007) How stem cells age and why this makes us grow old, *Nature reviews Molecular cell biology.* **8**, 703-13.
6. Relaix, F. & Zammit, P. S. (2012) Satellite cells are essential for skeletal muscle regeneration: the cell on the edge returns centre stage, *Development.* **139**, 2845-2856.
7. Yin, H., Price, F. & Rudnicki, M. A. (2013) Satellite cells and the muscle stem cell niche, *Physiological reviews.* **93**, 23-67.

8. Snijders, T. & Parise, G. (2017) Role of muscle stem cells in sarcopenia, *Curr Opin Clin Nutr.* **20**, 186-190.
9. Chakkalakal, J. V., Jones, K. M., Basson, M. A. & Brack, A. S. (2012) The aged niche disrupts muscle stem cell quiescence, *Nature.* **490**, 355-60.
10. Shea, K. L., Xiang, W., LaPorta, V. S., Licht, J. D., Keller, C., Basson, M. A. & Brack, A. S. (2010) Sprouty1 regulates reversible quiescence of a self-renewing adult muscle stem cell pool during regeneration, *Cell Stem Cell.* **6**, 117-29.
11. Liu, L., Charville, G. W., Cheung, T. H., Yoo, B., Santos, P. J., Schroeder, M. & Rando, T. A. (2018) Impaired Notch Signaling Leads to a Decrease in p53 Activity and Mitotic Catastrophe in Aged Muscle Stem Cells, *Cell Stem Cell.* **23**, 544-556 e4.
12. Fulle, S., Sancilio, S., Mancinelli, R., Gatta, V. & Di Pietro, R. (2013) Dual role of the caspase enzymes in satellite cells from aged and young subjects, *Cell Death Dis.* **4**, e955.
13. White, J. P., Billin, A. N., Campbell, M. E., Russell, A. J., Huffman, K. M. & Kraus, W. E. (2018) The AMPK/p27(Kip1) Axis Regulates Autophagy/Apoptosis Decisions in Aged Skeletal Muscle Stem Cells, *Stem Cell Reports.* **11**, 425-439.
14. Sousa-Victor, P. & Munoz-Canoves, P. (2016) Regenerative decline of stem cells in sarcopenia, *Mol Aspects Med.* **50**, 109-117.
15. Sousa-Victor, P., Garcia-Prat, L. & Munoz-Canoves, P. (2018) New mechanisms driving muscle stem cell regenerative decline with aging, *Int J Dev Biol.* **62**, 583-590.
16. Brack, A. S. & Munoz-Canoves, P. (2016) The ins and outs of muscle stem cell aging, *Skeletal muscle.* **6**.
17. Sousa-Victor, P., Garcia-Prat, L., Serrano, A. L., Perdiguero, E. & Munoz-Canoves, P. (2015) Muscle stem cell aging: regulation and rejuvenation, *Trends Endocrinol Metab.* **26**, 287-96.
18. Carlson, B. M. & Faulkner, J. A. (1989) Muscle transplantation between young and old rats: age of host determines recovery, *The American journal of physiology.* **256**, C1262-6.
19. Gutmann, E. & Carlson, B. M. (1976) Regeneration and transplantation of muscles in old rats and between young and old rats, *Life sciences.* **18**, 109-14.
20. Roberts, P., McGeachie, J. K. & Grounds, M. D. (1997) The host environment determines strain-specific differences in the timing of skeletal muscle regeneration: cross-transplantation studies between SJL/J and BALB/c mice, *Journal of anatomy.* **191 (Pt 4)**, 585-94.
21. Brack, A. S., Conboy, M. J., Roy, S., Lee, M., Kuo, C. J., Keller, C. & Rando, T. A. (2007) Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis, *Science.* **317**, 807-10.
22. Conboy, I. M., Conboy, M. J., Wagers, A. J., Girma, E. R., Weissman, I. L. & Rando, T. A. (2005) Rejuvenation of aged progenitor cells by exposure to a young systemic environment, *Nature.* **433**, 760-4.
23. Rebo, J., Mehdipour, M., Gathwala, R., Causey, K., Liu, Y., Conboy, M. J. & Conboy, I. M. (2016) A single heterochronic blood exchange reveals rapid inhibition of multiple tissues by old blood, *Nat Commun.* **7**, 13363.
24. Carlson, M. E., Hsu, M. & Conboy, I. M. (2008) Imbalance between pSmad3 and Notch induces CDK inhibitors in old muscle stem cells, *Nature.* **454**, 528-32.

25. Conboy, I. M., Conboy, M. J., Smythe, G. M. & Rando, T. A. (2003) Notch-mediated restoration of regenerative potential to aged muscle, *Science*. **302**, 1575-7.
26. Bernet, J. D., Doles, J. D., Hall, J. K., Kelly Tanaka, K., Carter, T. A. & Olwin, B. B. (2014) p38 MAPK signaling underlies a cell-autonomous loss of stem cell self-renewal in skeletal muscle of aged mice, *Nature medicine*. **20**, 265-71.
27. Lukjanenko, L., Jung, M. J., Hegde, N., Perruisseau-Carrier, C., Migliavacca, E., Rozo, M., Karaz, S., Jacot, G., Schmidt, M., Li, L., Metairon, S., Raymond, F., Lee, U., Sizzano, F., Wilson, D. H., Dumont, N. A., Palini, A., Fassler, R., Steiner, P., Descombes, P., Rudnicki, M. A., Fan, C. M., von Maltzahn, J., Feige, J. N. & Bentzinger, C. F. (2016) Loss of fibronectin from the aged stem cell niche affects the regenerative capacity of skeletal muscle in mice, *Nature medicine*. **22**, 897-905.
28. Rozo, M., Li, L. & Fan, C. M. (2016) Targeting beta1-integrin signaling enhances regeneration in aged and dystrophic muscle in mice, *Nature medicine*. **22**, 889-96.
29. Lukjanenko, L., Karaz, S., Stuelsatz, P., Gurriaran-Rodriguez, U., Michaud, J., Dammone, G., Sizzano, F., Mashinchian, O., Ancel, S., Migliavacca, E., Liot, S., Jacot, G., Metairon, S., Raymond, F., Descombes, P., Palini, A., Chazaud, B., Rudnicki, M. A., Bentzinger, C. F. & Feige, J. N. (2019) Aging Disrupts Muscle Stem Cell Function by Impairing Matricellular WISP1 Secretion from Fibro-Adipogenic Progenitors, *Cell Stem Cell*. **24**, 433-446 e7.
30. Naito, A. T., Sumida, T., Nomura, S., Liu, M. L., Higo, T., Nakagawa, A., Okada, K., Sakai, T., Hashimoto, A., Hara, Y., Shimizu, I., Zhu, W., Toko, H., Katada, A., Akazawa, H., Oka, T., Lee, J. K., Minamino, T., Nagai, T., Walsh, K., Kikuchi, A., Matsumoto, M., Botto, M., Shiojima, I. & Komuro, I. (2012) Complement C1q activates canonical Wnt signaling and promotes aging-related phenotypes, *Cell*. **149**, 1298-313.
31. Sinha, M., Jang, Y. C., Oh, J., Khong, D., Wu, E. Y., Manohar, R., Miller, C., Regalado, S. G., Loffredo, F. S., Pancoast, J. R., Hirshman, M. F., Lebowitz, J., Shadrach, J. L., Cerletti, M., Kim, M. J., Serwold, T., Goodyear, L. J., Rosner, B., Lee, R. T. & Wagers, A. J. (2014) Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle, *Science*. **344**, 649-52.
32. Elabd, C., Cousin, W., Upadhyayula, P., Chen, R. Y., Chooljian, M. S., Li, J., Kung, S., Jiang, K. P. & Conboy, I. M. (2014) Oxytocin is an age-specific circulating hormone that is necessary for muscle maintenance and regeneration, *Nat Commun*. **5**, 4082.
33. Egerman, M. A., Cadena, S. M., Gilbert, J. A., Meyer, A., Nelson, H. N., Swalley, S. E., Mallozzi, C., Jacobi, C., Jennings, L. L., Clay, I., Laurent, G., Ma, S., Brachat, S., Lach-Trifillieff, E., Shavlakadze, T., Trendelenburg, A. U., Brack, A. S. & Glass, D. J. (2015) GDF11 Increases with Age and Inhibits Skeletal Muscle Regeneration, *Cell metabolism*. **22**, 164-74.
34. Hinken, A. C., Powers, J. M., Luo, G., Holt, J. A., Billin, A. N. & Russell, A. J. (2016) Lack of evidence for GDF11 as a rejuvenator of aged skeletal muscle satellite cells, *Aging cell*. **15**, 582-4.
35. Schafer, M. J., Atkinson, E. J., Vanderboom, P. M., Kotajarvi, B., White, T. A., Moore, M. M., Bruce, C. J., Greason, K. L., Suri, R. M., Khosla, S., Miller, J. D., Bergen, H. R., 3rd & LeBrasseur, N. K. (2016) Quantification of GDF11 and Myostatin in Human Aging and Cardiovascular Disease, *Cell metabolism*. **23**, 1207-15.
36. Poggioli, T., Vujic, A., Yang, P., Macias-Trevino, C., Uygur, A., Loffredo, F. S., Pancoast, J. R., Cho, M., Goldstein, J., Tandias, R. M., Gonzalez, E., Walker, R. G., Thompson, T. B., Wagers, A. J., Fong, Y. W. & Lee, R. T. (2016) Circulating Growth Differentiation Factor 11/8 Levels Decline With Age, *Circulation research*. **118**, 29-37.

37. Harper, S. C., Brack, A., MacDonnell, S., Franti, M., Olwin, B. B., Bailey, B. A., Rudnicki, M. A. & Houser, S. R. (2016) Is Growth Differentiation Factor 11 a Realistic Therapeutic for Aging-Dependent Muscle Defects?, *Circulation research*. **118**, 1143-50; discussion 1150.
38. Vinel, C., Lukjanenko, L., Batut, A., Deleruyelle, S., Pradere, J. P., Le Gonidec, S., Dortignac, A., Geoffre, N., Pereira, O., Karaz, S., Lee, U., Camus, M., Chaoui, K., Mouisel, E., Bigot, A., Mouly, V., Vigneau, M., Pagano, A. F., Chopard, A., Pillard, F., Guyonnet, S., Cesari, M., Burlet-Schiltz, O., Pahor, M., Feige, J. N., Vellas, B., Valet, P. & Dray, C. (2018) The exerkinic apelin reverses age-associated sarcopenia, *Nature medicine*. **24**, 1360-1371.
39. Rai, R., Ghosh, A. K., Eren, M., Mackie, A. R., Levine, D. C., Kim, S. Y., Cedernaes, J., Ramirez, V., Procissi, D., Smith, L. H., Woodruff, T. K., Bass, J. & Vaughan, D. E. (2017) Downregulation of the Apelinergic Axis Accelerates Aging, whereas Its Systemic Restoration Improves the Mammalian Healthspan, *Cell reports*. **21**, 1471-1480.
40. Perdiguero, E., Sousa-Victor, P., Ruiz-Bonilla, V., Jardi, M., Caelles, C., Serrano, A. L. & Munoz-Canoves, P. (2011) p38/MKP-1-regulated AKT coordinates macrophage transitions and resolution of inflammation during tissue repair, *J Cell Biol*. **195**, 307-22.
41. Tidball, J. G. (2017) Regulation of muscle growth and regeneration by the immune system, *Nature reviews Immunology*. **17**, 165-178.
42. Arnold, L., Henry, A., Poron, F., Baba-Amer, Y., van Rooijen, N., Plonquet, A., Gherardi, R. K. & Chazaud, B. (2007) Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis, *J Exp Med*. **204**, 1057-69.
43. Juhas, M., Abutaleb, N., Wang, J. T., Ye, J., Shaikh, Z., Sriworarat, C., Qian, Y. & Bursac, N. (2018) Incorporation of macrophages into engineered skeletal muscle enables enhanced muscle regeneration, *Nature biomedical engineering*. **2**, 942-954.
44. Wang, Y., Wehling-Henricks, M., Welc, S. S., Fisher, A. L., Zuo, Q. & Tidball, J. G. (2019) Aging of the immune system causes reductions in muscle stem cell populations, promotes their shift to a fibrogenic phenotype, and modulates sarcopenia, *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. **33**, 1415-1427.
45. Wang, Y., Welc, S. S., Wehling-Henricks, M. & Tidball, J. G. (2018) Myeloid cell-derived tumor necrosis factor- α promotes sarcopenia and regulates muscle cell fusion with aging muscle fibers, *Aging cell*. **17**, e12828.
46. Oh, J., Sinha, I., Tan, K. Y., Rosner, B., Dreyfuss, J. M., Gjata, O., Tran, P., Shoelson, S. E. & Wagers, A. J. (2016) Age-associated NF- κ B signaling in myofibers alters the satellite cell niche and re-strains muscle stem cell function, *Aging*. **8**, 2871-2896.
47. Price, F. D., von Maltzahn, J., Bentzinger, C. F., Dumont, N. A., Yin, H., Chang, N. C., Wilson, D. H., Frenette, J. & Rudnicki, M. A. (2014) Inhibition of JAK-STAT signaling stimulates adult satellite cell function, *Nature medicine*.
48. Kuswanto, W., Burzyn, D., Panduro, M., Wang, K. K., Jang, Y. C., Wagers, A. J., Benoist, C. & Mathis, D. (2016) Poor Repair of Skeletal Muscle in Aging Mice Reflects a Defect in Local, Interleukin-33-Dependent Accumulation of Regulatory T Cells, *Immunity*. **44**, 355-67.
49. Liu, L., Cheung, T. H., Charville, G. W., Hurgo, B. M., Leavitt, T., Shih, J., Brunet, A. & Rando, T. A. (2013) Chromatin modifications as determinants of muscle stem cell quiescence and chronological aging, *Cell reports*. **4**, 189-204.
50. Schworer, S., Becker, F., Feller, C., Baig, A. H., Kober, U., Henze, H., Kraus, J. M., Xin, B., Lechel, A., Lipka, D. B., Varghese, C. S., Schmidt, M., Rohs, R., Aebersold, R., Medina, K. L., Kestler, H. A., Neri, F., von Maltzahn, J., Tumpel, S. & Rudolph, K. L. (2016) Epigenetic stress

responses induce muscle stem-cell ageing by Hoxa9 developmental signals, *Nature*. **540**, 428-432.

51. Sousa-Victor, P., Gutarra, S., Garcia-Prat, L., Rodriguez-Ubreva, J., Ortet, L., Ruiz-Bonilla, V., Jordi, M., Ballestar, E., Gonzalez, S., Serrano, A. L., Perdiguero, E. & Munoz-Canoves, P. (2014) Geriatric muscle stem cells switch reversible quiescence into senescence, *Nature*.

52. Zhu, P., Zhang, C., Gao, Y., Wu, F., Zhou, Y. & Wu, W. S. (2019) The transcription factor Slug represses p16(Ink4a) and regulates murine muscle stem cell aging, *Nat Commun*. **10**, 2568.

53. Zhou, J., So, K. K., Li, Y., Li, Y., Yuan, J., Ding, Y., Chen, F., Huang, Y., Liu, J., Lee, W., Li, G., Ju, Z., Sun, H. & Wang, H. (2019) Elevated H3K27ac in aged skeletal muscle leads to increase in extracellular matrix and fibrogenic conversion of muscle satellite cells, *Aging cell*. **18**, e12996.

54. Behrens, A., van Deursen, J. M., Rudolph, K. L. & Schumacher, B. (2014) Impact of genomic damage and ageing on stem cell function, *Nature Cell Biology*. **16**, 201-207.

55. Ermolaeva, M., Neri, F., Ori, A. & Rudolph, K. L. (2018) Cellular and epigenetic drivers of stem cell ageing, *Nat Rev Mol Cell Bio*. **19**, 594-610.

56. Vahidi Ferdousi, L., Rocheteau, P., Chayot, R., Montagne, B., Chaker, Z., Flamant, P., Tajbakhsh, S. & Ricchetti, M. (2014) More efficient repair of DNA double-strand breaks in skeletal muscle stem cells compared to their committed progeny, *Stem cell research*. **13**, 492-507.

57. Franco, I., Johansson, A., Olsson, K., Vrtacnik, P., Lundin, P., Helgadottir, H. T., Larsson, M., Revechon, G., Bosia, C., Pagnani, A., Provero, P., Gustafsson, T., Fischer, H. & Eriksson, M. (2018) Somatic mutagenesis in satellite cells associates with human skeletal muscle aging, *Nat Commun*. **9**, 800.

58. Garcia-Prat, L., Sousa-Victor, P. & Munoz-Canoves, P. (2017) Proteostatic and Metabolic Control of Stemness, *Cell Stem Cell*. **20**, 593-608.

59. Garcia-Prat, L., Munoz-Canoves, P. & Martinez-Vicente, M. (2016) Dysfunctional autophagy is a driver of muscle stem cell functional decline with aging, *Autophagy*. **12**, 612-3.

60. Garcia-Prat, L., Martinez-Vicente, M., Perdiguero, E., Ortet, L., Rodriguez-Ubreva, J., Rebollo, E., Ruiz-Bonilla, V., Gutarra, S., Ballestar, E., Serrano, A. L., Sandri, M. & Munoz-Canoves, P. (2016) Autophagy maintains stemness by preventing senescence, *Nature*. **529**, 37-42.

61. Solanas, G., Peixoto, F. O., Perdiguero, E., Jordi, M., Ruiz-Bonilla, V., Datta, D., Symeonidi, A., Castellanos, A., Welz, P. S., Caballero, J. M., Sassone-Corsi, P., Munoz-Canoves, P. & Benitah, S. A. (2017) Aged Stem Cells Reprogram Their Daily Rhythmic Functions to Adapt to Stress, *Cell*. **170**, 678-692 e20.

62. Tang, A. H. & Rando, T. A. (2014) Induction of autophagy supports the bioenergetic demands of quiescent muscle stem cell activation, *EMBO J*. **33**, 2782-97.

63. Zhang, H., Ryu, D., Wu, Y., Gariani, K., Wang, X., Luan, P., D'Amico, D., Ropelle, E. R., Lutolf, M. P., Aebbersold, R., Schoonjans, K., Menzies, K. J. & Auwerx, J. (2016) NAD(+) repletion improves mitochondrial and stem cell function and enhances life span in mice, *Science*. **352**, 1436-43.

64. Sahu, A., Mamiya, H., Shinde, S. N., Cheikhi, A., Winter, L. L., Vo, N. V., Stolz, D., Roginskaya, V., Tang, W. Y., St Croix, C., Sanders, L. H., Franti, M., Van Houten, B., Rando, T.

- A., Barchowsky, A. & Ambrosio, F. (2018) Age-related declines in alpha-Klotho drive progenitor cell mitochondrial dysfunction and impaired muscle regeneration, *Nat Commun.* **9**, 4859.
65. Baraibar, M. A., Hyzewicz, J., Rogowska-Wrzesinska, A., Bulteau, A. L., Prip-Buus, C., Butler-Browne, G. & Friguet, B. (2016) Impaired energy metabolism of senescent muscle satellite cells is associated with oxidative modifications of glycolytic enzymes, *Aging.* **8**, 3375-3389.
66. Neves, J., Sousa-Victor, P. & Jasper, H. (2017) Rejuvenating Strategies for Stem Cell-Based Therapies in Aging, *Cell Stem Cell.* **20**, 161-175.
67. Bengal, E., Perdiguero, E., Serrano, A. L. & Munoz-Canoves, P. (2017) Rejuvenating stem cells to restore muscle regeneration in aging, *F1000Research.* **6**, 76.
68. Cosgrove, B. D., Gilbert, P. M., Porpiglia, E., Mourkioti, F., Lee, S. P., Corbel, S. Y., Llewellyn, M. E., Delp, S. L. & Blau, H. M. (2014) Rejuvenation of the muscle stem cell population restores strength to injured aged muscles, *Nature medicine.* **20**, 255-64.
69. Cerletti, M., Jang, Y. C., Finley, L. W., Haigis, M. C. & Wagers, A. J. (2012) Short-term calorie restriction enhances skeletal muscle stem cell function, *Cell Stem Cell.* **10**, 515-9.
70. Chang, J., Wang, Y., Shao, L., Laberge, R. M., Demaria, M., Campisi, J., Janakiraman, K., Sharpless, N. E., Ding, S., Feng, W., Luo, Y., Wang, X., Aykin-Burns, N., Krager, K., Ponnappan, U., Hauer-Jensen, M., Meng, A. & Zhou, D. (2016) Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice, *Nature medicine.* **22**, 78-83.
71. Ocampo, A., Reddy, P., Martinez-Redondo, P., Platero-Luengo, A., Hatanaka, F., Hishida, T., Li, M., Lam, D., Kurita, M., Beyret, E., Araoka, T., Vazquez-Ferrer, E., Donoso, D., Roman, J. L., Xu, J., Rodriguez Esteban, C., Nunez, G., Nunez Delicado, E., Campistol, J. M., Guillen, I., Guillen, P. & Izpisua Belmonte, J. C. (2016) In Vivo Amelioration of Age-Associated Hallmarks by Partial Reprogramming, *Cell.* **167**, 1719-1733 e12.
72. Ho, A. T. V., Palla, A. R., Blake, M. R., Yucel, N. D., Wang, Y. X., Magnusson, K. E. G., Holbrook, C. A., Kraft, P. E., Delp, S. L. & Blau, H. M. (2017) Prostaglandin E2 is essential for efficacious skeletal muscle stem-cell function, augmenting regeneration and strength, *Proceedings of the National Academy of Sciences of the United States of America.* **114**, 6675-6684.
73. Tierney, M. T., Aydogdu, T., Sala, D., Malecova, B., Gatto, S., Puri, P. L., Latella, L. & Sacco, A. (2014) STAT3 signaling controls satellite cell expansion and skeletal muscle repair, *Nature medicine.*
74. Serrano, A. L., Baeza-Raja, B., Perdiguero, E., Jordi, M. & Munoz-Canoves, P. (2008) Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy, *Cell metabolism.* **7**, 33-44.
75. Keefe, A. C., Lawson, J. A., Flygare, S. D., Fox, Z. D., Colasanto, M. P., Mathew, S. J., Yandell, M. & Kardon, G. (2015) Muscle stem cells contribute to myofibres in sedentary adult mice, *Nat Commun.* **6**, 7087.
76. Pawlikowski, B., Pulliam, C., Betta, N. D., Kardon, G. & Olwin, B. B. (2015) Pervasive satellite cell contribution to uninjured adult muscle fibers, *Skeletal muscle.* **5**, 42.
77. Fry, C. S., Lee, J. D., Mula, J., Kirby, T. J., Jackson, J. R., Liu, F., Yang, L., Mendias, C. L., Dupont-Versteegden, E. E., McCarthy, J. J. & Peterson, C. A. (2015) Inducible depletion of satellite cells in adult, sedentary mice impairs muscle regenerative capacity without affecting sarcopenia, *Nature medicine.* **21**, 76-80.

78. Fry, C. S., Lee, J. D., Jackson, J. R., Kirby, T. J., Stasko, S. A., Liu, H., Dupont-Versteegden, E. E., McCarthy, J. J. & Peterson, C. A. (2014) Regulation of the muscle fiber microenvironment by activated satellite cells during hypertrophy, *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. **28**, 1654-65.
79. Egner, I. M., Bruusgaard, J. C. & Gundersen, K. (2016) Satellite cell depletion prevents fiber hypertrophy in skeletal muscle, *Development*. **143**, 2898-906.
80. Liu, W., Klose, A., Forman, S., Paris, N. D., Wei-LaPierre, L., Cortes-Lopez, M., Tan, A., Flaherty, M., Miura, P., Dirksen, R. T. & Chakkalakal, J. V. (2017) Loss of adult skeletal muscle stem cells drives age-related neuromuscular junction degeneration, *Elife*. **6**.
81. Naranjo, J. D., Dziki, J. L. & Badylak, S. F. (2017) Regenerative Medicine Approaches for Age-Related Muscle Loss and Sarcopenia: A Mini-Review, *Gerontology*. **63**, 580-589.
82. Hall, J. K., Banks, G. B., Chamberlain, J. S. & Olwin, B. B. (2010) Prevention of muscle aging by myofiber-associated satellite cell transplantation, *Science translational medicine*. **2**, 57ra83.
83. Bencze, M., Negroni, E., Vallese, D., Yacoub-Youssef, H., Chaouch, S., Wolff, A., Aamiri, A., Di Santo, J. P., Chazaud, B., Butler-Browne, G., Savino, W., Mouly, V. & Riederer, I. (2012) Proinflammatory macrophages enhance the regenerative capacity of human myoblasts by modifying their kinetics of proliferation and differentiation, *Molecular therapy : the journal of the American Society of Gene Therapy*. **20**, 2168-79.
84. Lafreniere, J. F., Mills, P., Tremblay, J. P. & El Fahime, E. (2004) Growth factors improve the in vivo migration of human skeletal myoblasts by modulating their endogenous proteolytic activity, *Transplantation*. **77**, 1741-7.
85. Bouchentouf, M., Benabdallah, B. F., Mills, P. & Tremblay, J. P. (2006) Exercise improves the success of myoblast transplantation in mdx mice, *Neuromuscular disorders : NMD*. **16**, 518-29.
86. Ballak, S. B., Jaspers, R. T., Deldicque, L., Chalil, S., Peters, E. L., de Haan, A. & Degens, H. (2015) Blunted hypertrophic response in old mouse muscle is associated with a lower satellite cell density and is not alleviated by resveratrol, *Exp Gerontol*. **62**, 23-31.
87. Joannis, S., Nederveen, J. P., Baker, J. M., Snijders, T., Iacono, C. & Parise, G. (2016) Exercise conditioning in old mice improves skeletal muscle regeneration, *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. **30**, 3256-68.

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