Inhibition of JAK-STAT signaling stimulates adult satellite cell function

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Diminished regenerative capacity of skeletal muscle occurs during adulthood. We identified a reduction in the intrinsic capacity of mouse adult satellite cells to contribute to muscle regeneration and repopulation of the niche. Gene expression analysis identified higher expression of JAK-STAT signaling targets in 18-month-old relative to 3-week-old mice. Knockdown of Jak2 or Stat3 significantly stimulated symmetric satellite stem cell divisions on cultured myofibers. Genetic knockdown of Jak2 or Stat3 expression in prospectively isolated satellite cells markedly enhanced their ability to repopulate the satellite cell niche after transplantation into regenerating tibialis anterior muscle. Pharmacological inhibition of Jak2 and Stat3 activity similarly stimulated symmetric expansion of satellite cells in vitro and their engraftment in vivo. Intramuscular injection of these drugs resulted in a marked enhancement of muscle repair and force generation after cardiotoxic injury. Together these results reveal age-related intrinsic properties that functionally distinguish satellite cells and suggest a promising therapeutic avenue for the treatment of muscle-wasting diseases.

The growth, maintenance and regeneration of skeletal muscle is attributed to the satellite cell, a mitotically quiescent stem cell that resides between the basal lamina and sarcolemma of the muscle fiber1–3. Satellite cells can self-renew, and it is this characteristic that allows adult skeletal muscle to undergo multiple rounds of regeneration without depleting their stem cell pool4–6. However, the functional and structural decline of skeletal muscle in late adulthood is one of the first hallmarks of aging in many organisms7–9. Notably, as an organism ages, a decrease in regenerative capacity is concomitant with a decrease in satellite cell numbers10,11.

The reduced regenerative potential observed in aging skeletal muscle is attributed primarily to changes in the muscle niche. Soluble ligands from the Notch, Wnt and transforming growth factor-β (TGF-β) signaling pathways are deregulated both systemically and within the satellite cell niche12–15. Alterations in the activity of these pathways in combination with fibrosis and an increased immune response result in age-related deficiencies in satellite cell self-renewal and regenerative efficacy16. Notably, a recent publication described intrinsic changes in geriatric satellite cells involving de-repression of p16 (also called Ink4a and Cdkn2a) in mice over 28 months of age17. Other recent studies have implicated the p38-α and p38-β mitogen-activated kinase pathway in the age-related intrinsic changes that occur in aged satellite cells from mice18–20. Moreover, differences in satellite cell number and proliferative capacity have been noted in mouse and rat satellite cells isolated from the skeletal muscle of 3-month-old compared to 7-month-old rodents21,22. Taken together, these findings indicate that age-related changes in satellite cell self-renewal, proliferative and differentiation capacity are likely due to both extrinsic alterations in the microenvironment and intrinsic alterations in cell-autonomous regulatory mechanisms.

To identify age-related intrinsic functional differences in satellite cells, we examined the transcriptional profile and regenerative capacity of satellite cells isolated from mice at different ages. We identified a role for JAK-STAT signaling in mediating this decline by impairing satellite cell function through the stimulation of asymmetric division.

RESULTS

Adult satellite cells exhibit decreased engraftment

To further characterize the intrinsic functional differences in satellite cells as they progress through adulthood, we isolated satellite cells from mice expressing the fluorescent reporter ZsGreen under transcriptional control of Pax7, a canonical transcription factor expressed in satellite cells. We isolated satellite cells from 3-week-old (adolescent), 2- to 4-month-old (young adult) and 18- ± 2-month-old (older adult) Pax7-ZsGreen reporter mice23 by FACS (Supplementary Fig. 1a). We observed that with increasing age of the mice, satellite cells were lower in number, expressed higher levels of ZsGreen/Pax7 and altered their cell surface complement of receptors (Supplementary Fig. 1b–d).

To investigate whether satellite cells intrinsically differ with age in their functional capacity to participate in muscle regeneration, we transplanted 10,000 freshly sorted Pax7-ZsGreen–expressing satellite cells from mice of different ages into regenerating tibialis anterior (TA)
Figure 1 Increasing age negatively affects the engraftment capacity of satellite cells. (a) Experimental schematic outlining the FACS isolation and immediate transplantation into regenerating TA muscle of immunosuppressed mdx mice between the ages of 6 and 8 weeks.

(b) The intrinsic contribution of satellite cells to muscle regeneration in 2- to 4-month-old mdx mice (n = 9 for each time point) with respect to increasing age. Images show dystrophin (green), laminin (red) and DAPI (blue) staining in TA muscle from mdx mice 3 weeks after transplantation. Arrowheads designate dystrophin-positive fibers. Data on adolescent mice are shown in Supplementary Figure 2. Scale bars, 100 µm (larger images); 20 µm (inset). (c) Quantification of the percentage of dystrophin-positive fibers after transplantation of age-related satellite cell populations. Values are relative to cell transplants of the percentage of dystrophin-expressing myofibers after transplantation. Arrowheads designate dystrophin-positive fibers. Scale bars, 100 µm (larger images); 20 µm (inset). High-magnification images of Pax7 and ZsGreen along with images from adolescent mice are shown in Supplementary Figure 2. (e) Quantification of the repopulating capacity associated with transplanted satellite cells from the muscle of young adult and older adult mice as evidenced by the number of double-positive Pax7+ZsGreen+ cells in relation to the total number of Pax7+ satellite cells. *P < 0.05, **P < 0.01. The data in c and e are shown as the mean ± s.e.m. All statistical significance was calculated by Student’s t test.

Pathway analysis identifies age-related activation of JAK-STAT signaling

To investigate how age governs the transcriptional profile of satellite cells, we performed a genome-wide expression analysis on freshly sorted satellite cells from mice of different ages (adolescent, n = 3, pooled group of 6; young adult, n = 3, pooled group of 8; and older adult, n = 3, pooled group of 8). Pearson correlation along with principal component analysis conducted between the three satellite cell populations and proliferating myoblasts clustered all satellite cell populations as distinct and markedly different from primary myoblasts (Supplementary Fig. 3a,b).

We next conducted an unbiased DAVID analysis of the satellite cell microarray data across each age group. Gene Ontology (GO) term analysis of genes upregulated (more than twofold) in ZsGreen-expressing cells from adolescent relative to older adult mice identified broad categories of cell cycle regulation, cell division, chromatin organization and striated muscle development (Supplementary Fig. 3c). By contrast, GO term analysis conducted on genes upregulated (more than twofold) in satellite cells from older adult relative to adolescent mice demonstrated enrichment for immune response, vascular development, oxidation reduction and regulation of apoptosis (Supplementary Fig. 3d).

To identify signaling pathways that are differentially activated in adult satellite cells, we conducted gene set enrichment analysis (GSEA) between our data sets of satellite cells from adolescent and older adult mice24. We analyzed robust multiarray average (RMA)-normalized gene sets for satellite cells from adolescent and older adult mice using the Kyoto encyclopedia of genes and genomes (KEGG) suite in GSEA to identify enriched signaling pathways. After GSEA KEGG analysis, we selected the most significantly enriched signaling pathways on the basis of their normalized enrichment score (NESs) (Fig. 2a and Supplementary Fig. 3e). Several of the enriched KEGG signaling pathways in satellite cells from older adult mice have been linked directly to satellite cell function, including Notch signaling25, JAK-STAT signaling26, TGF-β signaling13 and MAPK signaling27.

To further identify signaling pathways involved in satellite cell function that differ with respect to age, we conducted motif analysis in GSEA to identify transcription factor binding sites that are present in the promoters of genes enriched in satellite cells from older adult relative to adolescent mice. Notably, within gene promoters of older adult mice, we found the most significant enrichment (NES > 1.2) for binding sites of STATs, STAT coactivators and activators of JAK-STAT signaling (Supplementary Table 1a).

To confirm enrichment in the expression of genes involved in JAK-STAT signaling with increasing age, we generated global ratio heat maps on the basis of fold changes relative to log 2-transformed RMA
values of adolescent mice from our microarray data sets (Fig. 2b and Supplementary Fig. 4). We validated genes involved in JAK-STAT signaling whose expression increased significantly with respect to age by quantitative PCR (qPCR) analysis on freshly sorted satellite cells from mice of different ages (Fig. 2c). We observed strong upregulation of the JAK-STAT targets Socs3 (9-fold), Bcl2 (5-fold), Bcl6 (5-fold), Pim1 (4-fold) and Myc (3-fold) in satellite cells from older adult relative to young adult or adolescent mice (Fig. 2c). Furthermore, we similarly observed statistically significant higher levels of the JAK-STAT coactivators JunD (30-fold), CebpD (32-fold) and Fos (13-fold), along with the activators of JAK-STAT signaling epidermal growth factor receptor (Egfr) (3-fold), androgen receptor (Ar) (4-fold) and Gp130 (2-fold), in satellite cells from older adult relative to young adult or adolescent mice (Fig. 2c).

To validate the increase in JAK-STAT expression with age, we quantified the amount of Stat3 phosphorylated on Tyr705 (pStat3) from freshly sorted satellite cells using microcapillary isoelectric focusing. Notably, satellite cell pStat3 protein levels were ~1.6-fold higher in young adult and 2.4-fold higher in older adult mice with respect to the levels in adolescent mice (Fig. 2d and Supplementary Fig. 3f).

**Inhibition of JAK-STAT signaling promotes symmetric expansion**

To investigate the role of JAK-STAT signaling in satellite cell activation and commitment, we cultured isolated single myofibers for 42 or 72 h with siRNAs targeting either Jak2 or Stat3 (ref. 28). Consistent with our FACS analysis, enumeration of the numbers of satellite cells per myofiber revealed that the average satellite cell number per myofiber before *in vitro* culture was lower with age by ~1.6-fold from 2.1 ± 0.39 (mean ± s.e.m.) in young adult to 1.2 ± 0.16 in older adult mice and a further 2.6-fold lower when comparing adolescent (3.2 ± 0.79) to older adult mice (Supplementary Fig. 5a,b).

Satellite stem cells are a subpopulation of satellite cells that are capable of long-term self-renewal and repopulation of the satellite cell niche after transplantation5. Cre-LoxP-mediated lineage tracing using *Mys5-cre* and *R26R-YFP* alleles allows for discrimination between committed satellite myogenic cells that have expressed *Mys5-cre* (YFP+) and the subpopulation (<10%) of satellite stem cells that have never expressed *Mys5-cre* (YFP−). Satellite stem cells can undergo either planar symmetric divisions to give rise to two stem cells, where the orientation of the division is parallel to the basal lamina, or can alternatively undergo an apical–basal asymmetric division to give rise to a stem cell and a committed cell, where the orientation of the division is at a right angle to the basal lamina.

To investigate whether inhibition of JAK-STAT signaling would promote the symmetric expansion of satellite stem cells, we treated single myofibers isolated from young adult mice for 42 h *ex vivo* with siRNA against Stat3 (siStat3) or Jak2 (siJak2) or a scrambled control siRNA and assessed the number of cell doublings and whether or not they occurred...
in a symmetric or asymmetric fashion. Treatment with siStat3 and siJak2 significantly promoted symmetric satellite cell divisions (Fig. 3a) by approximately twofold but had no significant effect on the overall number of dividing satellite cells after 42 h of culture relative to scrambled siRNA–treated controls (Fig. 3b). To investigate whether this effect is maintained in skeletal muscle of older adult mice, we examined the ability of siJak2 and siStat3 to promote planar satellite cell divisions on single myofibers from 18-month-old mice. Myofibers treated with siStat3 displayed an ~2.8-fold higher and siJak2-treated myofibers displayed an ~2.6-fold higher number of planar divisions relative to vehicle-treated controls after 42 h in culture (Supplementary Fig. 5d).

After muscle injury, satellite cells are activated from quiescence, which results in the initiation of expression of the transcription factor MyoD. Expression of MyoD is associated with the acquisition of progenitor status, and subsequent downregulation of Pax7 (Pax7−MyoD+) is followed by differentiation. After 72 h of culture, the average number of Pax7+ cells per myofiber was ~15% higher in adolescent (~8%) and young adult (~76%) mice or after siJak2 treatment in adolescent (~24%) and young adult (~74%) mice compared to scrambled siRNA–treated controls (Fig. 3d).

We also treated single myofibers ex vivo with inhibitors of the JAK-STAT signaling pathway (the Jak2 inhibitor tyrphostin AG 490 (Tyr AG 490) and the Stat3 inhibitor 5,15 diphenylporphrine (5,15 DPP))30,31. Notably, treatment with these inhibitors significantly promoted symmetric satellite cell divisions (Fig. 3e) and had no significant effect on the overall number of dividing satellite cells after 42 h of culture (Fig. 3f).

After 72 h of treatment with inhibitors of JAK-STAT signaling, we observed a statistically significant higher average number of Pax7+ cells per myofiber in young adult mice treated with either 5,15 DPP (40 ± 5 (mean ± s.e.m.) or Tyr AG 490 (31 ± 3) (Fig. 3g and Supplementary Fig. 5c). Inhibition of JAK-STAT signaling had a marginal effect on the numbers of satellite cells per myofiber isolated from adolescent mice. Likewise, we observed a higher average number of Pax7+ cell divisions per myofiber in older adult mice after treatment with 5,15 DPP (27 ± 3) or Tyr AG 490 (29 ± 3) relative to vehicle-treated control mice (15 ± 3) (Fig. 3g and Supplementary Fig. 5c). Isolated myofibers from 18-month-old mice cultured for 42 h displayed a higher average number of planar satellite cell divisions (5,15 DPP, ~2.0-fold increase; Tyr AG 490, ~2.3-fold increase; combination of 5,15 DPP and Tyr AG 490, ~1.7-fold increase) relative to vehicle-treated controls (Supplementary Fig. 5e). After JAK-STAT inhibitor treatment, the percentage of Pax7−MyoD+ cells was decreased in young adult (~30% on average) and, to an even greater
Reduction of Jak2 or Stat3 expression enhances engraftment

We employed FACS to isolate quiescent Pax7-ZsGreen satellite cells from 3-month-old mice, transfected them ex vivo for 3 h on ice with siStat3, siJaks2 or both and then transplanted them into the TA muscles of wild-type (C57BL/6) mice that had been injured with cardiotoxin (CTX) 2 days earlier (Fig. 4a). Twelve days after transplantation, we assessed the engraftment of Pax7 and ZsGreen double-positive (Pax7+/ZsGreen+) cells (Fig. 4b). Knockdown of Stat3 resulted in an ~80% higher degree of engraftment capacity (55 ± 10% (mean ± s.e.m.) with siStat3 treatment compared to ~31 ± 4% with scrambled siRNA control treatment) (Fig. 4c and Supplementary Fig. 6). Likewise, the inhibition of Jak2 (54 ± 7%) or the simultaneous inhibition of both Stat3 and Jak2 (49 ± 7%) resulted in ~80% and ~58% higher engraftment, respectively (Fig. 4d).

To further assess the potential for clinical application of pharmacological inhibition of JAK-STAT signaling, we treated satellite cells from young adult and older adult mice with drug inhibitors to determine their logical inhibition of JAK-STAT signaling, we treated satellite cells from young adult and older adult mice after ex vivo treatment with JAK-STAT inhibitors and transplantation into C57BL/6 mice. After treatment of satellite cells from young adult and older adult mice with JAK-STAT inhibitors, we observed a statistically significant higher percentage of donor (ZsGreen+) satellite cells relative to the number in vehicle-treated control cells (Fig. 4c).

Figure 4 Knockdown of JAK-STAT pathway members ameliorates the engraftment potential of adult satellite cells. (a) Experimental schematic outlining the FACS isolation, treatment ex vivo with scrambled siRNA, siStat3, siJak2 or a combination of siStat3 and siJak2 followed by transplantation into CTX-treated TA muscle of C57BL/6 mice. (b) Representative images (~200 images total per muscle section) from ex vivo siRNA-treated satellite cells. Images show Pax7 (red), ZsGreen (green) and DAPI (blue) staining in TA muscle from C57BL/6 mice 12 d after transplantation. Arrows designate host satellite cells, and the arrowhead designates a donor-derived satellite cell. Scale bar, 50 µm. (c) Quantification of the percentage of ZsGreen+ satellite cells after siRNA knockdown and transplantation. n = 4. **P < 0.01, ***P < 0.001. NS, not significant. Statistical significance was calculated by Student’s t test. The data in c and d are shown as the mean ± s.e.m.

Figure 5 Administration of small-molecule JAK-STAT inhibitors ex vivo before transplantation into young adult mice enhances engraftment. (a) Schematic for ex vivo treatment of freshly sorted satellite cells with JAK-STAT inhibitors before transplantation. (b) Quantification of donor satellite cells from young adult and older adult mice after ex vivo treatment with JAK-STAT inhibitors and transplantation into C57BL/6 mice. (c) Quantification of donor satellite cells from young adult and older adult mice after ex vivo treatment with JAK-STAT inhibitors and transplantation into mdx mice. The data in b and c represent the mean ± s.e.m. n = 3. **P < 0.01, ***P < 0.001. Statistical significance was calculated by Student’s t test.
We performed a serial injury experiment to ensure that the transplanted ZsGreen+ cells were bona fide satellite cells that were capable of self-renewal (Supplementary Fig. 7c). We observed a similar percentage of donor-derived Pax7+ZsGreen+ cells after re-injury, providing conclusive evidence that the transplanted cells were indeed bona fide satellite cells capable of self-renewal (Supplementary Fig. 7d).

**Injection of JAK-STAT inhibitors enhances repair**

Our experiments raise the possibility that pharmacological inhibition of JAK-STAT signaling in vivo may be a potential therapeutic approach to stimulate muscle regeneration. Therefore, we injured the TA muscles of young adult and older adult C57BL/6 mice with CTX to initiate regeneration, which we followed with direct intramuscular (i.m.) injection of JAK-STAT pathway inhibitors (Fig. 6a). We observed higher minimal fiber Feret (a measure of myofiber diameter) in the skeletal muscle of both young adult and older adult mice after i.m. injection of JAK-STAT inhibitors (Fig. 6b and Supplementary Fig. 8a). We also observed a lower number of developmental myosin heavy chain (devMyHC+) fibers in the muscle of young adult and older adult mice treated with JAK-STAT signaling inhibitors (Supplementary Fig. 8b). This lower number of devMyHC+ myofibers was more pronounced in the muscle of older adult mice, where the proportion of devMyHC+ myofibers was 83% ± 10% (mean ± s.e.m.) with vehicle treatment, 48% ± 7.3% with 5,15 DPP, 40% ± 12% with Tyr AG 490 and 30% ± 8.2% with both 5,15 DPP and Tyr AG 490 (Supplementary Fig. 8b).

Furthermore, we examined macrophage infiltration (CD11b), connective tissue formation ( Sirius Red) and general muscle architecture (H&E stain). Injection of JAK-STAT inhibitors resulted in a lower degree of macrophage infiltration (Supplementary Fig. 9a) and Sirius Red staining and better muscle architecture as evidenced by H&E staining relative to vehicle-treated controls (Supplementary Fig. 9b,c). We also found a ~50% and ~100% higher total number of satellite cells in young adult mice and older adult mice, respectively (Fig. 6c), as well as a higher number of satellite cells that did not express MyoD (Pax7+MyoD−) relative to vehicle-treated controls (Supplementary Fig. 8d). Furthermore, we observed a higher proportion of satellite cells that lacked MyoD expression (Pax7−MyoD−) after treatment with JAK-STAT inhibitors relative to vehicle-treated controls (Supplementary Fig. 8d).

To address whether JAK-STAT inhibition functionally improves the performance of damaged skeletal muscle, we injured the extensor digitorum longus (EDL) muscle from young adult mice with CTX, which we followed with i.m. injection of JAK-STAT inhibitors 3 d after injury (Fig. 6d). Treatment with JAK-STAT inhibitors resulted in significantly higher peak force observed over time relative to vehicle-treated controls (Fig. 6e). Under control conditions, the maximal force of regenerating EDL muscle measured was 11.3 ± 0.8
DISCUSSION
Changes in the microenvironment and other extrinsic factors are thought to be the major cause of the progressive decline in skeletal muscle regenerative capacity throughout adulthood. However, derepression of p16 (Ink4a) has been shown to intrinsically shift satellite cells into deep quiescence in mice over 28 months of age. In addition, overactivation of the p38-α and p38-β mitogen-activated kinase pathway also contributes to diminished function in satellite cells from aged (24 month) mice. Therefore, satellite cells undergo profound cell-autonomous alterations, potentially as a result of changes to their microenvironment, that result in altered functional capacity, even after transplantation into a young environment. Our results suggest that intrinsic changes in satellite cell functional capacity begin at a much younger age.

Our data further demonstrate that one distinguishing feature of satellite cells as they age is the concerted activation of the JAK-STAT pathway. Known activators of JAK-STAT signaling include leukemia inhibitory factor (Lif), basic fibroblast growth factor (bFGf, also called Fgf2), interleukin-6 (IL-6), platelet-derived growth factor (PDGf), EGF and androgen[33,34]. The JAK-STAT signaling pathway is involved in skeletal muscle differentiation, whereby Stat3 activation mediated by extracellular interleukins (IL-6 and Lif) affects MyoD and promotes myoblast differentiation[35]. Furthermore, previous reports have demonstrated activation of the JAK-STAT signaling pathway in satellite cells of skeletal muscle from aged humans[36]. JAK-STAT signaling pathway members include suppressor of cytokine signaling 3 (Socs3), c-Myc, Fos, JunB Cebp- and Bcl6, all of which are upregulated in satellite cells from young adult and older adult mice compared to satellite cells isolated from adolescent mice. Interestingly, further analysis of recent transcriptional data comparing satellite cells from young adult mice to those of geriatric mice also showed a significant increase in the expression of JAK-STAT signaling.

Aged skeletal myofibers secrete heightened levels of bFGF that impede quiescence and ultimately affect the functional capacity of resident satellite cells[37]. It is also plausible that extrinsic activators such as bFGF, interleukins, PDGF or EGF present in adult skeletal muscle may also act on satellite cells to alter their intrinsic functional capacity through activation of JAK-STAT signaling. Chronic inflammation is a common characteristic of diseased (dystrophic) and/or aged skeletal muscle. Increased infiltration of inflammatory cells in combination with circulating proinflammatory cytokines (interleukins or tumor necrosis factor-α (TNF-α)) have a detrimental effect on tissue regenerative potential[38]. The JAK-STAT signaling pathway has a critical role in the transduction of extracellular signals from cytokines and growth factors that are involved in proliferation, migration, apoptosis, survival and oncogenesis[39]. Therefore, another possible cause of increasing JAK-STAT signaling in adulthood is increasing levels of inflammatory cells in aging muscle tissue.

Our data suggest that activation of JAK-STAT signaling in satellite cells profoundly inhibits their capacity to undergo symmetric stem cell expansion. Previous studies from our lab have indicated that regulation of the level of symmetric stem cell division by Wnt7a-Fzd7 signaling is a central mechanism regulating satellite cell homeostasis and that enhancing symmetric stem cell division markedly augments muscle repair[40-42]. The Par polarity complex, consisting of Par3, Par6 and aPKC, has a central role in integrating Wnt and Notch signaling into the regulation of asymmetric stem cell divisions[43]. Interestingly, MAPK and p38 kinases are activated in satellite cells in a Par complex-dependent manner[12,18,19,27]. Furthermore, the Par complex is implicated in the activation of aPKC and Stat3 in breast cancer stem cells[44]. These findings suggest that Jak2-Stat3 signaling has a role in the function of the Par polarity complex.

Our experiments provide strong support for the hypothesis that with increasing age, activation of JAK-STAT signaling in satellite cells substantially contributes to myogenic commitment and results in their regenerative deficiency. Our data support recent reports indicating that aged satellite cells exhibit enhanced myogenic commitment[18,19,37]. We found that treatment with small-molecule inhibitors of JAK-STAT signaling resulted in increased numbers of satellite cells, enhanced muscle repair and enhanced functional performance. Although i.m. injection of JAK-STAT inhibitors may also act on other cells in the tissue milieu, our experiments nevertheless indicate that the enhancement of muscle repair can be stimulated using small drugs that have been identified on the basis of their ability to modulate the function of satellite cells.

Therefore, we conclude that aberrant JAK-STAT signaling is an important contributor to satellite cell dysfunction in adulthood. Together these results reveal intrinsic differences that distinguish young and adult satellite cells and suggest a promising therapeutic approach for the stimulation of muscle regeneration.

METHODS
Methods and any associated references are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
F.D.P. designed and carried out experiments, analyzed results and wrote the manuscript. J.v.M. designed and conducted experiments and analyzed results. C.F.B., N.A.D., H.Y. and N.C.C. conducted experiments and interpreted data. D.H.W. conducted experiments. J.F. provided expertise in physiological analysis. M.A.R. designed and carried out experiments, analyzed results and wrote the manuscript and provided financial support.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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ONLINE METHODS

Animal experiments. For this study, we used the following mouse lines bred in our animal facility: Pax7-ZsGreen mice, mdx mice (C57BL/10ScSn-Dmdmdx/J), Jackson Laboratories, Bar Harbor, Maine, #001801) and SV129 mice (Charles River Laboratories, St. Constant, QC). All mice were housed under standard conditions and allowed access to food and water ad libitum. All experiments were performed in accordance with University of Ottawa guidelines for animal handling and animal care determined by the University of Ottawa Animal Care Committee.

Immunohistochemistry and FACS isolation. Immunohistochemistry was performed as described previously4,45 using the following antibodies: Pax7 (undiluted), MyHC (MF20, undiluted) and dystrophin (7A10, undiluted) from BioScience; CXC4R1 (BD Bioscience, 1 µg/million cells); M-cadherin (Millipore, 1 µg/million cells); laminin (Sigma, 1:1,000); ZsGreen (Clontech, 1:200); devMyHC (Leica, 1:50); and MyoD (C-20 Santa Cruz, 1:200), along with the appropriate secondary antibodies (Molecular Probes, BD Bioscience, 1:1,000). Skeletal muscles from a mixture of male and female heterozygous Pax7-ZsGreen mice were isolated at the following defined stages: 3 weeks (adolescent, hind limb), 2–4 months (young adult, hind limb) and 18 ± 2 months (older adult, hind limb). Muscles were aseptically dissected, minced and digested in a dispase and collagenase solution (1 mg/ml collagenase and 4 mg/ml Dispase II, Roche) for 25 min at 37°C. The digested muscle slurry was triturated briefly after 15 min of incubation and returned to the incubator. The slurry was subsequently diluted with PBS containing 10% FBS and 2 mM EDTA (FACS sorting medium), filtered through 70-µm Netwell cell strainers (Costar) and pelleted at 1,700 rpm for 5 min (Thermo IEC Centra CL2). Cell pellets were gently resuspended in FACS buffer and incubated and agitated with primary antibodies and, when appropriate, secondary antibodies for 90 min on ice. Cells were subsequently washed in FACS buffer, pelleted and filtered through 30-µm filters (Miltenyi Biotech) before FACS. Cells were separated on a MoFlo cytometer (DakoCytomation) equipped with three lasers. Sorting gates were strictly defined on the basis of the age of the sample or, in the case of a negative control for Pax7-ZsGreen mice, wild-type SV129 mice. We routinely stained FACS-sorted cells in these experiments, and ~99% of the cells expressed Pax7.

NanoPro immunoassay. Male and female ZsGreen-expressing satellite cells were isolated by FACS from adolescent, young adult and older adult mice and pelleted by centrifugation at 8,000 r.p.m. for 5 min at 4°C. Cell lysis was performed with bicine and CHAPS lysis buffer (20 mM Bicine, pH 7.5, and 0.6% CHAPS) supplemented with 1× DMSO and Aquous Inhibitor Mixes (ProteinSimple). Cells were lysed for 30 min on ice, and lysates were cleared by centrifugation at 14,800 r.p.m. for 15 min at 4°C. Analysis of satellite cell lysates by capillary isoelectric focusing using the NanoPro 1000 was performed according to the manufacturer’s protocol. Immunoprophing was performed using pStat3 Tyr705 (Cell Signaling, 9145, 1:50) and Hsp70 (Abcam, ab5439, 1:50) antibodies. Chemiluminescent signals were analyzed and quantified with Compass software (ProteinSimple).

Myofiber culture, satellite cell transplantation and muscle injury. Myofiber culture and transplantation of freshly sorted satellite cells was performed as described previously28,42. Enumeration of satellite cells was conducted on at least 15 fibers per replicate at 72 h. These experiments were conducted in at least biological triplicate from each condition at each age time point using male mice (for example, over 45 myofibers in total per condition). Furthermore, at 0 h, a mixture of male and female heterozygous Pax7-ZsGreen mice were isolated by FACS-sorting at the following defined stages: 3 weeks (adolescent, hind limb), 2–4 months (young adult, hind limb) and 18 ± 2 months (older adult, hind limb). Muscles were aseptically dissected, minced and digested in a dispase and collagenase solution (1 mg/ml collagenase and 4 mg/ml Dispase II, Roche) for 25 min at 37°C. The digested muscle slurry was triturated briefly after 15 min of incubation and returned to the incubator. The slurry was subsequently diluted with PBS containing 10% FBS and 2 mM EDTA (FACS sorting medium), filtered through 70-µm Netwell cell strainers (Costar) and pelleted at 1,700 rpm for 5 min (Thermo IEC Centra CL2). Cell pellets were gently resuspended in FACS buffer and incubated and agitated with primary antibodies and, when appropriate, secondary antibodies for 90 min on ice. Cells were subsequently washed in FACS buffer, pelleted and filtered through 30-µm filters (Miltenyi Biotech) before FACS. Cells were separated on a MoFlo cytometer (DakoCytomation) equipped with three lasers. Sorting gates were strictly defined on the basis of the age of the sample or, in the case of a negative control for Pax7-ZsGreen mice, wild-type SV129 mice. We routinely stained FACS-sorted cells in these experiments, and ~99% of the cells expressed Pax7.

Microarray and bioinformatic analyses. FACS-purified ZsGreen-expressing cells representing one biological replicate were obtained from pooled populations of male and female muscles from adolescent (n = 6), young adult (n = 8) and older adult (n = 8) mice. Experiments were conducted in biological triplicate. Total RNA was extracted from each replicate using the Picopure RNA isolation kit (Applied Biosystems) and concentrated using the RNA Clean and Concentrator-5 kit (Zymo Research) according to the manufacturer’s instructions. qPCR analysis was conducted as described previously4 with the following primer sequences, 5′ to 3′: JunD exon 1 (forward) (A) ATCTTGGGCTGTCAACTCT, JunD ex 1 reverse (B) AACTGCTCAGGTTGGCGTAG, Bcl2 ex 1 (F) GGAGCTCAGGA GGAGATGT, Bcl2 ex 2 (R) TTCCAATGTCCTCCTCTGCT, For ex 1 (A) ATCTGGTGGTTCGTCAGGC, For ex 2 (R) CCAGAGACACCATCTCAAG, Myc ex 2 (F) TCCTGTACCT CTCGCCATT, Myc ex 3 (R) GTGTGGTCCTCCTCCACAG, Egf ex 1 (F) AACAATGATTTCTGTGCAG, Egf ex 2 (R) GTGCTCGTTGTTGTCAG, Gsea1 ex 3 (F) CCAACAGACCCATCTTCAG, Gsea1 ex 4 (R) CCAAAGATCGACAACCAAC, Ar ex 3 (R) ATCTGGTGGTTCGTCAGGC, Gsea1 ex 3 (F) CCAACAGACCCATCTTCAG, Gsea1 ex 4 (R) CCAAAGATCGACAACCAAC, Ar ex 3 (R) ATCTGGTGGTTCGTCAGGC, Gsea1 ex 3 (F) CCAACAGACCCATCTTCAG, Gsea1 ex 4 (R) CCAAAGATCGACAACCAAC, Ar ex 3 (R) ATCTGGTGGTTCGTCAGGC, Gsea1 ex 3 (F) CCAACAGACCCATCTTCAG, Gsea1 ex 4 (R) CCAAAGATCGACAACCAAC, Ar ex 3 (R) ATCTGGTGGTTCGTCAGGC, Gsea1 ex 3 (F) CCAACAGACCCATCTTCAG, Gsea1 ex 4 (R) CCAAAGATCGACAACCAAC, Ar ex 3 (R) ATCTGGTGGTTCGTCAGGC, Gsea1 ex 3 (F) CCAACAGACCCATCTTCAG, Gsea1 ex 4 (R) CCAAAGATCGACAACCAAC. Sample RNA quality was assessed on the Agilent Bioanalyzer 2100 using the RNA 6000 nanochip (Agilent Technologies, Santa Clara). Microarray target preparation was achieved using the WT Expression kit (Ambion) and the Genechip terminal labeling kit (Affymetrix). The microarray data were collected using Genechip mouse gene 1.0 ST arrays (Affymetrix). R 2.10.0 with the XPS library was used to import CEL files, RMA normalize and perform DBAG (detection background) calls. Anti-genomic background and the metacore exon level definition were used. The RMA expression values are log2 transformed. Probe annotation was extracted from Ensembl v62 using the biomart R package and used to map transcript cluster ID’s to genes. The five most Gogene v1.0ST microarray data sets are referred to as adolescent, young adult and older adult for subsequent methods of analysis. Genes that met the criteria of a log-fold change of greater than one (that is, twofold cutoff) were used for further analysis by DAVID (http://david.abcc.ncifcrf.gov/home.jsp)47,48 or GSEA49. The NGS score corresponds to a weighted Kolmogorov-Smirnov statistic and reflects the degree to which a signaling pathway is overrepresented at the extremities of the ranked list24. All microarray data have been deposited in GEO and are available in the series GSE47401.

Statistical analyses. A minimum of three replicates were analyzed for each experiment presented. Data are shown as the mean ± s.e.m. (Microsoft Excel). Statistical analyses were conducted using the Student’s t test method of determining inference based on small samples. *P < 0.05, **P < 0.01, ***P < 0.001.

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Corrigendum: Inhibition of JAK-STAT signaling stimulates adult satellite cell function

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In the version of this article initially published online, the third sentence of the Abstract read “Gene expression analysis identified higher expression of JAK-STAT signaling targets in 3-week-old relative to 18-month-old mice,” when it should have read “Gene expression analysis identified higher expression of JAK-STAT signaling targets in 18-month-old relative to 3-week-old mice.” The error has been corrected for the print, PDF and HTML versions of this article.