Inducible depletion of satellite cells in adult, sedentary mice impairs muscle regenerative capacity without affecting sarcopenia

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A key determinant of geriatric frailty is sarcopenia, the age-associated loss of skeletal muscle mass and strength1,2. Although the etiology of sarcopenia is unknown, the correlation during aging between the loss of activity of satellite cells, which are endogenous muscle stem cells, and impaired muscle regenerative capacity has led to the hypothesis that the loss of satellite cell activity is also a cause of sarcopenia3,4. We tested this hypothesis in male sedentary mice by experimentally depleting satellite cells in young adult animals to a degree sufficient to impair regeneration throughout the rest of their lives. A detailed analysis of multiple muscles harvested at various time points during aging in different cohorts of these mice showed that the muscles were of normal size, despite low regenerative capacity, but did have increased fibrosis. These results suggest that lifelong reduction of satellite cells neither accelerated nor exacerbated sarcopenia and that satellite cells did not contribute to the maintenance of muscle size or fiber type composition during aging, but that their loss may contribute to age-related muscle fibrosis.

Recent estimates indicate that up to one-third of elderly people suffer from frailty, characterized by a common set of symptoms including loss of muscle strength, increased fatigability, modest levels of physical activity and decreased body weight1. The close relationship between loss of muscle strength, increased fatigability, modest levels of physical activity and decreased body weight is a crucial factor in the emergence of geriatric frailty, thus limiting the ability to perform activities of daily living and significantly increasing the risk of falling5,6.

Numerous studies in humans and rodents report a strong correlation between the loss and dysfunction of satellite cells and sarcopenia3,4. Motivated by the idea that the restoration of satellite cell activity will provide a therapeutic basis for treating sarcopenia, a great deal of effort has gone into defining the environmental and cellular changes underlying the loss in satellite cell activity with age7–18. Despite the correlation between declining satellite cell–dependent regenerative capacity and age, no studies to date have tested this relationship directly to determine whether the loss of satellite cell activity causes sarcopenia. We recently developed a genetic mouse model that allows for the specific, inducible depletion of satellite cells in adult skeletal muscle19–21. The Pax7CreER+/;Rosa26DTA/+ (here referred to as Pax7CreER-DTA) strain was generated by crossing Pax7CreER/CreER and Rosa26DTA/DTA strains. Treatment of Pax7CreER-DTA mice with tamoxifen activates Pax7 promoter–driven Cre recombinase only in satellite cells, which activates the gene encoding diphtheria toxin A (DTA) and results in the killing of satellite cells22. We took advantage of this mouse model to directly test the hypothesis that loss of satellite cells, which underlies the well-documented impairment in muscle regenerative capacity21–24, in young adult animals results in muscle wasting with advancing age. If there is a causal relationship between the loss of satellite cell activity and sarcopenia, then we would predict accelerated and exacerbated sarcopenia in muscle with a significantly reduced complement of satellite cells.

We administered vehicle or tamoxifen by intraperitoneal injection to adult (4 months old) male Pax7CreER-DTA mice for 5 consecutive days to effectively deplete satellite cells (Fig. 1) and then allowed the mice to age naturally. Approximately 1 year later, we analyzed a subset of these mice (aged 16–18 months, termed middle aged (MA)) and found that satellite cell numbers did not recover over time. Consistent with previous studies21,23, in muscles that remained substantially depleted (>85% relative to vehicle–treated control mice) of satellite cells, muscle regeneration after injury induced by BaCl2 injection was severely impaired (Fig. 2a). No loss of muscle mass was apparent in hind limb muscle of vehicle- or tamoxifen-treated MA mice except in the soleus muscle (Fig. 2b); however, significant atrophy was apparent in 24-month-old mice (designated as the ‘aged’ group) in both vehicle- and tamoxifen-treated mice (Fig. 2b). Aged vehicle-treated mice had a median lifespan of 23.9 months and a maximum lifespan of 24.6 months; aged tamoxifen-treated mice had a median lifespan of 22.6 months and a maximum lifespan of 24.4 months. Decrements in hind limb muscle mass in the aged mice met criteria for sarcopenia in humans25; that is, appendicular muscle mass was 2 s.d. below that of younger mice (5 months old, 24.4 months, respectively).
Reduction in satellite cell content throughout the lifespan does not appear to affect mean myofiber CSA. (a) Representative plantaris images (from more than 300 total) of Pax7+ cells (satellite cells, white arrowheads) co-stained with DAPI. Scale bars, 100 μm. (b) Quantification of satellite cell counts in hind limb muscles (plantaris, gastrocnemius, TA/EDL and soleus) in vehicle- and tamoxifen-treated Pax7CreER-DTA young and aged mice. Data represent mean ± s.e.m. *P < 0.05, tamoxifen vs. vehicle treatment in mice of the same age, two-factor ANOVA. (c) Mean myofiber CSA of the hind limb muscles. Data represent mean ± s.e.m. N.S., not significant (P = 0.06); †P < 0.05, young vs. aged, independent of treatment, two-factor ANOVA. For young mice, n = 8 mice per treatment group (vehicle and tamoxifen); for aged mice, n = 7 (vehicle) and 8 (tamoxifen) (a–c).

We next determined whether features of sarcopenia were exacerbated in aged mice owing to a lifetime reduction of satellite cells. Analysis of satellite cell abundance in vehicle-treated mice 1 month after injection (young) and 20 months after injection (aged) (Fig. 1a,b) showed a substantial age-associated reduction in satellite cells (Fig. 1b). Tamoxifen administration accelerated this cell loss and resulted in >94% reduction in satellite cells (compared to muscle from vehicle-treated mice) in multiple hind limb muscles 1 month after injection (young), with little recovery in satellite cells per myofiber occurring even after 20 months (aged) (Fig. 1b); on average, satellite cells remained 83% depleted in aged mice (range 64–87%), well below the loss normally associated with aging. Despite the reduction in satellite cell abundance to a degree often associated with severely impaired muscle regeneration, age-associated muscle atrophy, as indicated by changes in both muscle wet weight (Fig. 2b) and mean myofiber cross-sectional area (CSA) (Fig. 1c), occurred to the same extent in all hind limb muscles from both vehicle-treated and satellite cell–depleted mice. Myofiber CSA analyzed by fiber type (Fig. 3) showed age-related atrophy in all muscles except the soleus, which has a higher proportion of slow-twitch fibers (Fig. 3c). In agreement with the well-characterized atrophy of the largest, fast-twitch glycolytic fibers with age26, type 2b fibers showed the greatest degree of atrophy that appeared unaffected by satellite cell abundance (Fig. 3c). Moreover, myofiber size distribution across hind limb muscles showed the characteristic leftward shift owing to overall higher abundance of smaller fibers with age in both treatment groups (Supplementary Fig. 1). Satellite cell depletion also showed no significant effect on fiber

Figure 1 Reduction in satellite cell content throughout the lifespan does not appear to affect mean myofiber CSA. (a) Representative plantaris images (from more than 300 total) of Pax7+ cells (satellite cells, white arrowheads) co-stained with DAPI. Scale bars, 100 μm. (b) Quantification of satellite cell counts in hind limb muscles (plantaris, gastrocnemius, TA/EDL and soleus) in vehicle- and tamoxifen-treated Pax7CreER-DTA young and aged mice. Data represent mean ± s.e.m. *P < 0.05, tamoxifen vs. vehicle treatment in mice of the same age, two-factor ANOVA. (c) Mean myofiber CSA of the hind limb muscles. Data represent mean ± s.e.m. N.S., not significant (P = 0.06); †P < 0.05, young vs. aged, independent of treatment, two-factor ANOVA. For young mice, n = 8 mice per treatment group (vehicle and tamoxifen); for aged mice, n = 7 (vehicle) and 8 (tamoxifen) (a–c).
Age-associated fiber type–specific atrophy appears unaffected by reduction in satellite cell content. Fiber-type specific CSA of plantaris, gastrocnemius (gastroc), tibialis anterior and EDL (TA/EDL) and soleus muscles of vehicle- and tamoxifen-treated Pax7<sup>CreERT2</sup>DTA young, MA and aged mice. (a, b) Representative images from more than 450 plantaris (a) and 450 soleus (b) images showing myosin heavy chain types 1 (pink), 2a (green), 2b (orange) and 2x (unstained) fibers and dystrophin (white) ringing each fiber. Scale bars, 100 µm. (c) Quantification of mean CSA by fiber type in the hind limb muscles. Mean CSA is provided for fiber types that comprise ≥2% of total fibers in a given muscle (see Supplementary Fig. 1b for fiber type frequencies). Data represent mean ± s.e.m. *P < 0.05, aged vs. young mice; †P < 0.05, aged vs. MA mice; ‡P < 0.05, MA vs. young mice; independent of treatment, two-factor ANOVA. n = 8 (young, vehicle); 8 (young, tamoxifen); 4 (MA, vehicle); 5 (MA, tamoxifen); 7 (aged, vehicle) or 8 (aged, tamoxifen) mice.

Figure 3 Age-associated fiber type–specific atrophy appears unaffected by reduction in satellite cell content. Fiber-type specific CSA of plantaris, gastrocnemius (gastroc), tibialis anterior and EDL (TA/EDL) and soleus muscles of vehicle- and tamoxifen-treated Pax7<sup>CreERT2</sup>DTA young, MA and aged mice. (a, b) Representative images from more than 450 plantaris (a) and 450 soleus (b) images showing myosin heavy chain types 1 (pink), 2a (green), 2b (orange) and 2x (unstained) fibers and dystrophin (white) ringing each fiber. Scale bars, 100 µm. (c) Quantification of mean CSA by fiber type in the hind limb muscles. Mean CSA is provided for fiber types that comprise ≥2% of total fibers in a given muscle (see Supplementary Fig. 1b for fiber type frequencies). Data represent mean ± s.e.m. *P < 0.05, aged vs. young mice; †P < 0.05, aged vs. MA mice; ‡P < 0.05, MA vs. young mice; independent of treatment, two-factor ANOVA. n = 8 (young, vehicle); 8 (young, tamoxifen); 4 (MA, vehicle); 5 (MA, tamoxifen); 7 (aged, vehicle) or 8 (aged, tamoxifen) mice.

Type distribution (Supplementary Fig. 2): at 24 months, only the plantaris and soleus muscles showed an age-dependent shift in the relative frequency of different fast-twitch (type 2) fibers that was independent of satellite cell abundance.

In addition to atrophy of individual myofibers, a decline in the number of myofibers and a reduction in single fiber–specific force generation (force per unit area) are associated with sarcopenia. We measured these characteristics in the plantaris muscle, as it showed little recovery of satellite cells with age and was therefore most severely depleted (Fig. 1b). We did not observe a loss of myofibers at 24 months in either vehicle- or tamoxifen-treated mice (Fig. 4a). Analyses of isolated myofibers (Fig. 4b) showed that the number of myonuclei per 100 µm of myofiber length was not changed with age (Fig. 4c). We performed myonuclear counts on single, fixed myofibers of intermediate size (1,000–2,500 µm<sup>2</sup>), which make up greater than 80% of all myofibers, and our results were consistent with a recent study reporting no myonuclear loss with age in fibers in this size range<sup>27</sup>. Moreover, myonuclear number appeared to be unaffected by depletion of satellite cells (Fig. 4c), suggesting that the maintenance of myonuclei is not linked to satellite cell abundance. This conclusion was further supported by analysis of BrdU-labeled myonuclei (Fig. 4d). We provided mice with BrdU via drinking water for 2 weeks to identify nuclei that had undergone DNA replication, then killed the mice and quantified fusion of labeled nuclei into myofibers. Although myonuclear addition was very infrequent during the 2-week labeling (approximately 0.1% of myofibers from vehicle-treated young and aged mice contained a labeled myonucleus), no labeled myonuclei were detected in plantaris muscle after tamoxifen treatment (Fig. 4d).

Finally, myofiber-specific force was reduced 31% with age (aged versus young) (Fig. 4e), comparable to the force decrement reported in myofibers isolated from adult humans aged 65–85 years using a similar permeabilized fiber preparation<sup>28</sup>; however, the decrease in specific force did not seem to be affected by satellite cell abundance. We also functionally tested single myofibers from the extensor digitorum longus (EDL) muscle from tamoxifen- or vehicle-treated aged mice (Supplementary Fig. 3a–c). As with the plantaris muscle, no differences in specific or absolute force or single-fiber CSA were apparent as a result of satellite cell depletion in the EDL. Further, overall loss of function with age, as measured by grip strength, was not altered by satellite cell depletion (Supplementary Fig. 3d). The loss of 45–59% raw grip strength in the aged mice (compared to the young group) did not differ with treatment and is consistent with loss reported in humans with age<sup>29,30</sup>.

Although age-related myofiber atrophy and weakness were not altered by lifelong satellite cell reduction, we observed a change in the muscle fiber environment. Extracellular matrix (ECM) surrounding myofibers, quantified by Sirius Red staining of collagens, was higher with age and reduced satellite cell content in the plantaris muscle (Fig. 4f–h). We also assessed ECM accumulation via wheat germ agglutinin (WGA) staining of glycosaminoglycans, which showed that this component of the ECM was also more abundant with age and satellite cell depletion (Supplementary Fig. 4a–c). WGA staining of other hind limb muscles showed excess accumulation of ECM with age that was further exacerbated by a reduction in satellite cell content specifically in the plantaris and tibialis anterior–EDL complex (TA/EDL) muscles (Supplementary Fig. 4c). These observations support our recent findings that satellite cells regulate the myofiber environment by signaling to fibroblasts<sup>19</sup>. Although our previous work illustrated an additive effect of satellite cell depletion and functional overload on ECM deposition in young adult mice<sup>19</sup>, the present study shows that long-term depletion of satellite cells in...
sedentary mice contributes to dysregulation of the ECM in old age. Moreover, the present work extends previous findings to suggest that satellite cells limit fibrosis preferentially in fast muscles.

In summary, although the understanding of satellite cell function in muscle regeneration continues to be refined, our results suggest that the loss of satellite cell–dependent regenerative capacity neither accelerates nor exacerbates sarcopenia. Moreover, our findings have broader implications for the study of tissue homeostasis by showing that skeletal muscle, much like the pancreas, kidney and liver, apparently employs cellular mechanisms that do not necessarily require stem cell participation for tissue maintenance under nonstress conditions. However, the loss of satellite cells may adversely affect overall muscle quality, potentially contributing to the increase in fibrosis observed in aged skeletal muscle. One limitation of the current findings stems from the sedentary nature of the mice. The satellite cell requirement for muscle maintenance in more physically active mice remains to be determined; however, the sedentary nature of the mice in the current study is reflective of low physical activity levels of older adults in the United States.

The findings of the current study have clinical importance, as they draw a clear distinction between therapeutic strategies involving replenishment of muscle satellite cells that may effectively treat degenerative myopathies, such as dystrophies and cachexia, and those that treat sarcopenia. Although degenerative conditions are likely to benefit from a satellite cell–based therapy, our results support a recent review’s conclusion that treatment for sarcopenia should focus on the myofiber and motor neuron.

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


ONLINE METHODS

General experimental approaches. All experimental procedures were performed in a blinded fashion for treatment, but not for age. No data points, samples or mice were excluded from the study. Animals were randomized as stated below for vehicle or tamoxifen treatment.

Mouse model. The Pax7CreER strain was generated by placing an zros-CreERTM-FRT-Neo-FRT cassette into the ClaI site of the Pax7 gene 3′-UTR following the stop codon in exon 9 (ref. 40). Mice are on a mixed C57BL/6-129 background strain. Mice were housed in a temperature- and humidity-controlled room and maintained on a 14:10 h light/dark cycle with food and water ad libitum. Mice were not housed in a barrier or pathogen-free facility and were not screened for tumors. Adult (4 months of age) male Pax7CreER-DTA mice were distributed randomly into groups and administered by intraperitoneal (IP) injection vehicle (15% ethanol in sunflower seed oil) or tamoxifen (2.0 mg d−1) for 5 consecutive days, 2 h before lights out. After 1 month (5 months old, young), 12 months (16–18 months old, middle aged (MA)) or 20 months (24 months old, aged), vehicle- and tamoxifen-treated mice were killed (n = 4–8 mice per group). A subset of mice was provided with BrdU at a concentration of 0.8 µg ml−1 in their drinking water for 2 weeks before sacrifice. All animal procedures were conducted in accordance with institutional guidelines approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

BaCl2-induced muscle injury. MA mice were anesthetized with isoflurane and the tibialis anterior (TA) injected with either 50 µl of 1.2% BaCl2 solution in PBS or sterile PBS. After 7 d, TA muscles were collected and processed for histchemistry.

Histochemistry and immunohistochemistry. Muscles were mounted at resting length, covered in OCT compound (Tissue Tek, Sakura, Torrance, CA) and frozen in liquid nitrogen-cooled isopentane and stored at −80 °C until cryosectioning (7 µm). For Pax7 (satellite cells) immunohistochemistry, muscle sections were fixed in 4% PFA and subject to epitope retrieval using sodium citrate (10 mM, pH 6.5) at 90 °C for 20 min. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in PBS followed by an additional blocking step with Mouse-on-Mouse Blocking Reagent (Vector Laboratories, Burlingame, CA). Incubation with anti-Pax7 antibody (1:100, Developmental Studies Hybridoma Bank, Iowa City, IA) was followed by incubation with the biotin-conjugated secondary antibody and streptavidin-HRP included within the Tyramide Signal Amplification kit (#T20935, Invitrogen, Carlsbad, CA). Sections were co-stained with DAPI (10 nM, Invitrogen) and mounted within the Tyramide Signal Amplification kit (#T20935, Invitrogen, Carlsbad, CA). Incubation with anti-Pax7 antibody (1:100, Developmental Studies Hybridoma Bank, Iowa City, IA) was followed by incubation with the biotin-conjugated secondary antibody and streptavidin-HRP included within the Tyramide Signal Amplification kit (#T20935, Invitrogen, Carlsbad, CA). Sections were co-stained with DAPI (10 nM, Invitrogen) and mounted within the biotin-conjugated secondary antibody and streptavidin-HRP included within the Tyramide Signal Amplification kit (#T20935, Invitrogen, Carlsbad, CA). Sections were co-stained with DAPI (10 nM, Invitrogen) and mounted with Vectashield fluorescent mounting medium. For fiber typing, unfixed sections were incubated in antibodies to myosin heavy chain (MyHC) types I, IIA and IIB, respectively. Developmental Studies Hybridoma Bank) in addition to dystrophin (1:50, VPD505, Vector), MyHC type 2x expression was inferred from unstained fibers. Fluorescence-conjugated secondary antibodies to various mouse immunoglobulin subtypes (Gt anti-Ms IgG2b AF647, 1:250, #A21242, Gt anti-Ms IgG1 AF488 1:500, #A21211, Gt anti-Ms IgM AF555, 1:250, #A21426; Invitrogen) were applied to the MyHC expression and dystrophin. Sections were post-fixed in 4% PFA before mounting. For extracellular matrix accumulation, muscle sections were fixed in 4% PFA and then incubated with Texas Red conjugated wheat germ agglutinin (WGA, W21405, Invitrogen). Basic muscle morphology and regeneration were assessed with hematoxylin and eosin staining, and collagen content was assessed with Sirius Red staining according to standard protocols. For BrdU detection, unfixed slides were incubated in an antibody to dystrophin followed by Gt anti-Ms secondary antibody conjugated to Texas Red (#610-109-121, Rockland Immunocinmicals Inc., Gilbertsville, PA). Sections were then fixed in absolute methanol, treated with 2 N HCl to denature DNA and neutralized with 0.1 M borate buffer (BORAX, pH 8.5). BrdU antibody (Ms anti-BrdU Roche, #11170376001; 1:100) incubation was followed by biotin-conjugated Gt anti-Ms secondary antibody (Biotin Gt Anti-Ms IgG Jackson ImmunoResearch, #115-065-205; 1:1000) and streptavidin-FITC (SA-5001, Vector; 1:100). Sections were post-fixed in PFA and co-stained with DAPI.

Image quantification. Images were captured at 10x and 20x with an upright microscope (AxioImager M1; Zeiss, Götttingen, Germany). Fiber type-specific CSA was quantified using a newly developed, automated image segmentation algorithm41,42 that identifies fiber types by MyHC isoform expression combined with fiber boundary detection using dystrophin immunohistochemistry. All other images were quantified with Zeiss AxioVision rel. software (v4.8). Satellite cell abundance was assessed using Pax7 staining and only those cells that were Pax7+ and DAPI+ were counted. Fibers were classified as BrdU+ with a BrdU+ and DAPI+ nucleus within the dystrophin border. WGA and Sirius Red staining were quantified using threshold intensity programs within the imaging software. Investigators were blinded to treatment (vehicle or tamoxifen) but not age during image quantification and analysis.

Single myofiber contractility. Measurement of permeabilized muscle fiber contractility was performed as previously described43. Muscles were removed from the animal and immediately placed in ice-cold relaxing solution (pCa −9.0); composed of (mM): HEPES, 90; Mg (total), 10.3; Mg2+, 1.0; EGTA, 50; ATP, 8.0; CrP, 10.0; NaN3, 1.0; Na (total), 36; K (total), 125; pH 7.1). Bundles of fibers were dissected from whole muscles, placed in skinnng solution (mM): potassium propionate, 125; imidazole, 20; EGTA, 5; MgCl2, 2; ATP, 2; pH 7.0. The storage solution was composed of (mM): potassium propionate, 125; imidazole, 20; EGTA, 5; MgCl2, 2; ATP, 2; gycercol, 50% (v/v); pH 7.0) for 30 min and then in storage solution for 16 h at 4 °C and assayed immediately or stored at −80 °C until use. Individual myofibers (n = 8 myofibers per mouse; 3–8 mice per group) were pulled from bundles in relaxing solution and secured at one end to a force transducer (Aurora Scientific, Model 403, Ontario, Canada) and at the other end to a servomotor (Aurora Scientific). The length of the whole fiber was adjusted to obtain a sarcomere length of 2.5 µm using laser diffraction techniques. The average fiber CSA was calculated assuming an elliptical cross-section, with diameters obtained at five different positions along the fiber from high-magnification images of the top and the side views. Maximum isometric force (F0) of the fiber was elicited by immersing the fiber in a high-concentration Ca2+ solution (pCa −4.5) containing: (mM): HEPES, 90; Mg (total), 8.12; Mg2+, 1.0; EGTA, 50; Ca2+ (total), 50; ATP, 8.0; CrP, 10.0; NaN3, 1.0; Na (total), 36; K (total), 125; pH 7.1). Specific force (sf0) was calculated by dividing F0 by CSA.

Myonuclear number. Plantaris muscles were fixed in situ at resting length in 4% PFA for 48 h. Single myofibers were isolated by 40% NaOH digestion as previously described42. Single myofibers were stained with DAPI and nuclei from 15–25 myofibers per animal (n = 4–8 mice per group) within a given segment were counted by z-stack analysis using the AxioImager M1 microscope. AxioVision software was used to measure myonuclear number per myofiber segment.

Grip strength. Mice were held by the base of the tail and were allowed to grab with forelimb paws the horizontal bar of the grip-strength apparatus (Grip Strength Meter, #1027SM, Columbus Instruments, Columbus, OH) in a pronated manner while being held horizontally. The mice were then pulled away gently until the grip was released and the maximal force achieved by the animal was recorded (in N); hind limbs were kept free from the apparatus, and the average of three trials was reported. Data are reported as mean grip strength/body weight. All mice were tested by the same person.

Statistical analysis. All data were analyzed with SigmaPlot v12.0 software (Systat Software, San Jose, CA) via a two-factor ANOVA (factors: treatment (vehicle or tamoxifen) × age (young, MA or aged)) or simple two-tailed t-tests for each dependent variable under consideration. Assumptions for statistical analyses were met (i.e., normal distribution, equal variance). If a significant interaction was detected, an appropriate post hoc analysis was employed to determine the source of the significance. Sample size was determined by a power analysis with an expected s.d. of 0.001, power of 0.8 and alpha of 0.05. Statistical significance was accepted at P < 0.05. Data are reported as mean ± s.e.m.; brackets in figures are used to help visualize significant main effects.

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Supplemental Figure 1: Muscle fiber size is reduced with age. Binned distribution of fiber cross-sectional area (CSA) of plantaris, gastrocnemius, TA/EDL and soleus muscles of vehicle- and tamoxifen-treated Pax7\textsuperscript{CreER}-DTA young (5 month) and aged (24 month) mice shown in Fig. 3.
Supplemental Figure 2: Muscle fiber type appears unaffected by reduced satellite cell content. Relative frequency of different fiber types in the plantaris, gastrocnemius, TA/EDL and soleus muscles of young (5 month) and aged (24 month) mice. Data are presented as mean relative fiber type frequency ± SEM. † Significant difference between Young and Aged, independent of treatment ($P < 0.05$) as measured by a two-factor ANOVA (factors: Age (young/aged) and Treatment (vehicle/tamoxifen)).
Supplemental Figure 3. Specific force, absolute force and cross-sectional area (CSA) of permeabilized fibers from the EDL appear unaffected by reduced satellite cell content in aged mice; grip strength of the fore limb is reduced in aged mice but unaffected by reduced satellite cell content. (a) Specific force (KN · m⁻²), (b) absolute maximum isometric force (mN) or (c) fiber cross-sectional area (µm²) of permeabilized single fibers from the EDL of vehicle (V)- and tamoxifen (T)-treated Pax7CreER-DTA aged (24 months) mice. Data are presented as mean specific force ± SEM and measured by a student’s t-test (vehicle/tamoxifen). N = 3 vehicle, 5 tamoxifen, 8 fibers/mouse, 24–40 fibers/group. (d) Fore limb grip strength (N) in young and aged mice. Data are presented as mean grip strength per body weight ± SEM. N = 3–5 mice/group. † Significant difference between Young and Aged in the same treatment group (P < 0.05) as measured by a two-factor ANOVA (factors: Age (young/aged) and Treatment (vehicle/tamoxifen)).
Supplemental Figure 4. Increased extracellular matrix (ECM) accumulation with age in the soleus and gastrocnemius muscles and increased ECM accumulation with age and reduced satellite cell content in plantaris and TA/EDL muscles. Representative images of vehicle- (a) and tamoxifen-treated (b) plantaris muscle ECM assessed by wheat germ agglutinin (WGA) staining that binds glycosaminoglycans. Scale bar = 100µm. (c) Quantification of WGA staining in vehicle- and tamoxifen-treated Pax7\textsuperscript{CreER}_{DTA} young (5 month) and aged (24 month) mice presented as a percentage of the total cross-sectional area. Data are presented as mean % area ± SEM. † Significant difference between Young and Aged, independent of treatment (p < 0.05); * Significant difference between vehicle and tamoxifen (P < 0.05) as measured by a two-factor ANOVA (factors: Age (young/aged) and Treatment (vehicle/tamoxifen)).