Figure S1. Loss of satellite number with age. **a**, Representative images of Pax7, laminin and Dapi staining of skeletal muscle cross-sections (scale bar, 50 μm). **b**, Quantification of the number of Pax7+ cells/muscle cross-section averaged from 30 cross sections/mouse (n=5-6 animals/age group). **c**, Quantification of the number of Pax7+ cells/single muscle fibre from adult and aged skeletal muscle. 20-30 single muscle fibres/animal (n=5-6 animals/age group). **d**, FACS profiles for isolation of purified SCs from adult and aged muscle. Cells were positively selected for VCAM-1 (APC) and α7-integrin (Pacific Blue) and negatively selected for CD31, CD45 (both PE conjugated) and propidium iodide (PI). **e**, Representative image of freshly isolated SCs obtained by FACS, plated, immediately fixed and immunostained for Pax7 and Dapi (scale bar 20 μm). **f**, Quantification of the percentage of Pax7+ cells from the selected gate. Note, ~95% of sorted SCs from both adult and aged skeletal muscle are Pax7+ (100% were Myogenin negative). **g**, Relative proportion of SCs sorted from live (PI negative) cells derived from adult and aged muscles. **h**, RT-qPCR analysis for the expression of pax7 in aged relative to adult sorted SCs. Individual experiments were performed in triplicate. Data are from 4-6 adult and aged mice. All data represented as mean ± s.e.m.; *P<0.05 t-test.
Figure S2. Representative images of sorted SCs cultured for 4 days and stained with anti-Pax7, MyoG, MyoD, and activated caspase-3 (aCasp).
Figure S3. Muscle fibre-derived FGF2 increases with age. 

a, Table of expression for 17 different FGF ligands from aged relative to adult isolated single muscle fibers (Fold A/Ad) from duplicate RT-qPCR based array kits (SAbiosciences). N.A. (not attained) designates results whereby Ct values were greater than controls provided by the kit. 

b, Quantification of fgf2 expression in 21 and 24 relative to 5 month of age single muscle fibres. Data are from 3 reactions conducted in triplicate. 

c, Representative images of shows increased fgf2 (brown) in close proximity to Pax7+ (red) SCs in aged muscle. Dapi (blue). And number of fgf2 ‘hotspots’ along the length of single muscle fibres. n>30 fibers/animal, n=3 mice/group. 

d-f, Representative images for FGF2 (red) and laminin (d,f,green) or (e, white) immunostained adult and aged transverse (d, upper panels, f) and longitudinal (d, lower row, e) muscle sections. 

Note the expression of FGF2 along the periphery of aged muscle fibres and the perimysium both of which is not seen after incubation of IgG isotype matched control antibodies used at identical concentrations (d). 

e, Representative longitudinal sections, stained with anti-Pax7, laminin and FGF2. Dapi (blue). Muscle fibre-associated FGF2 (red) in aged muscle fibres identified with anti-laminin (white arrow), SC Pax7+ (green) white box (see Figure 3 for higher mag). 

f, Note the expression of FGF2 primarily in interstitial areas of adult muscle fibres and increased expression in myofibres of aged muscle. 

g, Quantification of FGF2 positive areas in transverse muscle sections, demonstrates increased muscle fibre-associated and decreased interstitial associated FGF2 in aged muscle fibre sections. Note FGF2 protein was not detected on SCs. 

h, Quantification of FGF2+ cells in isolated skeletal muscle interstitial cells Note a decrease in the expression of FGF2 in aged compared to adult interstitial cells (ICs) (n=1000 cells per condition). Data are from 3 experiments conducted in triplicate. n=5-6 mice/age group. All data represented as mean ± s.e.m.; *P<0.05 t-test.
**Figure S4.** Aged purified muscle fibre (PM) extract contains FGF2 activity. a, Expression of fgf2 in adult and aged fibres obtained to generate PM extract as assessed by RT-qPCR. Note the fold difference in fgf2 expression is similar to that observed for purified single muscle fibres (see Figure 3a and Figure S2a). Data are from 3 reactions conducted in triplicate. b, Quantification of the percentage of muscle fibres that contains FGF2+ regions in aged or adult fibres used to generate PM extract. Data are from 3 separate experiments. c, Quantification of the proportion of cycling sorted SCs cultured in basal media with or without incubation with 100ng/ml FGF2 for 24, 48 and 72 hours. Data are from 2 experiments conducted in triplicate. Data are represented as mean ± s.e.m.; *P<0.05. d, Quantification of quiescent and cycling (Ki67+) aged and adult Pax7+ reserve satellite cells (RSCs) after exposure to aged (Ag) and adult (Ad) interstitial cell protein extract diluted to concentrations found in PM extracts (see panel e). Note no significant difference in the ability of diluted Ad and Ag interstitial protein extract to induce RSCs into cycle. e, Quantification of the number of FGF2+ or FGF2- interstitial cells (ICs) per 1μl of PM extract. Note the extremely low level of ICs in 1μl of PM extract (~5 cells in adult and ~1 cell in aged PM extract). 4μl of extract was the volume added to SCs in vitro to test cell cycle entry. For panels b-e, 300-600 cells counted per condition. f, RSC cell death after exposure to PBS vehicle (V) or niche extracts isolated from Ad and Ag skeletal muscle. g, Quantification of cycling RSCs after exposure to negligible amounts of serum (NS, 3%), 10% horse serum (HS), Ad and Ag serum directly isolated from mice. Note both 10% HS and adult serum can induce RSCs into cycle whereas aged serum is less effective. h, Percentage of cycling aged and adult SCs after exposure to Ad and Ag PM extracts with or without prior treatment with FGF2. n=1000 cells/condition, n=5 animals/group. i, Schematic of the experimental strategy to assess cycling RSCs after exposure to adult and aged PM extract. PM extracts were incubated with RSCs for 24 hours in basal media (3% HS, DMEM). j, Quantification of cycling RSCs SCs after exposure to 40ng/ml FGF2 in basal media k, Quantification of cycling aged and adult RSCs after exposure to Ad and Ag PM extracts with or without prior treatment with FGF2 blocking antibody (FGF2bl) (k) or different dosages of SU5402 (l). m, Quantification of cycling RSCs after adenoviral Cre-mediated deletion of FGFR1 from RSCs (Cre-AV) and upon incubation with vehicle (V) FGF2 (F2) Ag and Ad PM extract. Data in panels e-m, are from a total of 300-600 cells per condition, 4–5 separate experiments. Data represented as mean ± s.e.m.; *P<0.05.
Figure S5. Sprouty1 expression in response to FGF2 and in LRCs. a, Assessment of spry1, 2 and 4 expression in SC progenitors incubated with different dosages of FGF2 in basal media (DMEM 5% HS). Note the specific and dramatic loss of spry1 expression in response to FGF2 supplementation. b, Expression of spry1, spry2, fgfr1, fgfr4 and fgf2 mRNA in aged LRC relative to nonLRC SCs (obtained as described in Figure 1a). For panels a, b, data are from 3 reactions conducted in triplicate. All data are represented as mean ± s.e.m.; *P<0.05 t-test. Ct values for FGF2 from LRCs or nonLRCs were >37 and therefore considered not detectable (ND). c, The Doxycycline (Dox) feeding scheme for H2B-GFP induction in TetO-H2B-GFP; Spry1lacZ+/- mice and chase (20 weeks). d, e, Representative panels for GFP visualization (d) and immunostaining for β gal and Ki67 (e) in LRC and nonLRC SCs. f, Fluorescence intensity of β-gal antibody staining reaction in LRC and nonLRC SCs. Data are significant *P<0.0001 Mann Whitney test n=212 nonLRCs and 168 LRCs, pooled from three 20 month old mice. g, The percentage of Ki67+ LRC and nonLRC SCs. Data represented as mean +/- s.e.m.; *P<0.05.
Figure S6. An FGF2-Spry1 signaling cascade manipulates cell cycle entry of quiescent RSCs. a, Strategy to delete Spry1 in adult SCs and subsequently test SC responsiveness to PM extracts with quantification of cycling (Ki67+) Ctrl or Spry1null SCs cultured for 24 hours, incubated with PBS (V) or 40ng/ml FGF2. b, c, Quantification of cycling Ctrl or Spry1null SCs after 24 hour exposure to adult (Ad) and aged (Ag) PM extracts, with or without prior treatment with FGF2bl (b) or SU5402 (c). d, Strategy to overexpress Spry1 in adult SCs (Spry1OX) and subsequently test SC proliferation (Ki67+) after 24 hour incubation with Adult (Ad) and Aged (Ag) PM extracts. n=300-600 cells/condition performed in triplicate, n=4-6 mice/group (a-d). e, Quantification of cycling RSCs (Pax7+/Ki67+) after adenoviral Cre-mediated deletion of Spry1 from RSCs (Spry1flox) and upon incubation with FGF2. f, Quantification of cycling Spry1flox RSCs after exposure to Ag and Ad PM extract with or without prior incubation with SU5402. g, Quantification of cycling RSCs after adenoviral Cre-mediated over-expression of Spry1 in RSCs (Spry1OX) upon exposure to FGF2. Note the loss of RSC responsiveness to FGF2 upon overexpression of Spry1. DMSO is added to all experiments as a control. h, Quantification of cycling Spry1OX RSCs after exposure to Ag and Ad PM extract. For panels e-h, data are from 3 separate experiments. Total of 300-600 cells counted per condition. Data in panels e-h, represented as mean ± s.e.m.; *P<0.05.
Figure S7. Aging and deletion of Spry1 stimulates expression of the ets factor, erm. a, Schematic of the strategy to specifically delete Spry1 in SCs (Spry1null). b, c, Quantification of spry1 (b) and erm (c) expression in SCs isolated from Ad and Ag control and Spry1null 10 days after Tmx injection. Ct values for spry1 from Spry1 null SCs were >37 and therefore considered not detectable. Data are from 3 reactions conducted in triplicate. All are data represented as mean ± s.e.m.; *P<0.05 t-test.

Figure S8. Long term stimulation of FGF-signaling impacts satellite cell fate. a, b, The percentage of MyoG+ (a) and aCasp+ (b) cells from aged Ctrl and Spry1null SCs after 6 weeks of Tmx administration and cultured for 4 days. n=300 cells/ condition performed in triplicate, n=5 mice/group.
Figure S9. Short-term stimulation of FGF-signaling accelerates aged skeletal muscle regeneration. a, Schematic of the strategy to specifically delete Spry1 in SCs (Spry1null) followed by injury with 1.2% BaCl. b, Quantification of fibre size (μm^2) 10 days after injury in adult and aged Ctrl (− CreER) and 10 day deleted Spry1null skeletal muscle. Data are from 4–6 mice per group. All data represented as mean ± s.e.m.; *P<0.005.
**Figure S10.** Long term inhibition of FGF-signaling impacts skeletal muscle regenerative outcome. **a,** Representative H&E images of 30 day regenerated skeletal muscles from adult and aged mice injected (IP) with beads containing vehicle (DMSO) or SU5402 six weeks prior to muscle injury with 1.2% BaCl2. **b,** Quantification of fibre size after 30 days regeneration (30d) relative to contralateral uninjured control (0d) in adult (b) and aged (c) mice injected with DMSO or SU5402 beads prior to injury. **d,** Quantification of Pax7+ SC number at 0d and 30d after injury in adult (d) and aged (e) regenerated muscles from mice injected with DMSO or SU5402 beads prior to injury. Data are from 4-6 mice/condition/age. All data are represented as mean ± s.e.m.; *P<0.05.

**Figure S11.** Model of FGF2/FGFR1/Spry1 mediated loss of SC quiescence during aging. In adult muscle, SCs are retained in a quiescent state. The niche, the differentiated myofiber, (depicted by pink box) is non-stimulatory and SCs express growth factor inhibitors, such as Spry1. During aging, FGF2 increases (depicted in bold) in the muscle stem cell niche, this causes SCs to break quiescence and proliferate, leading to the eventual a loss of self-renewal function in favour of apoptosis or differentiation and eventual diminution of the SC pool. Aged SCs that retain stem cell function undergo fewer divisions (i.e. LRCs) throughout adult life and express higher levels of FGF inhibitor, Spry1.