**SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Figure 1 | Old muscle undergoes degeneration following immobilization; negative control for immunofluorescence assays. A**. Immunodetection of embryonic myosin heavy chain (eMyHC, red) and dystrophin (green) in 10-μm old skeletal muscle cryosections. Hoechst labels nuclei (blue). **B.** Negative staining control for immunofluorescence was performed on cryosections with isotype-matched primary antibodies for experimental conditions. Degenerating immobilized muscle is identified by $eMyHC^{+}$ myofiber clusters and "broken" outline of dystrophin immunostaining, which is detected in old, but not in young immobilized tissue; evenly distributed dystrophin pattern signifies good maintenance of young immobilized skeletal muscle. **C.** Additional panels of H&E images, taken from both young old human skeletal muscle sections, are shown. Area of scar tissue formation (quantified in **D**) is compared to the total area of tissue examined (demarcated in the H&E image, shown in **E** and **F**). These results demonstrate that old muscle kept immobile for two weeks had more than 30% scar tissue formation, and that only 2.5% of this degenerated area was repaired with new eMyHC+ myofibers (97.5% remained unrepaired). In contrast, young muscle had less than 2% of scar/degenerating areas (more than 98% of young muscle was maintained). $*P \leq 0.05$, old compared to young (n = 10).

**Supplementary Figure 2 | Immobility-induced muscle atrophy causes an age-specific decline in recovery of muscle size and function. A.** Muscle size (mean myofiber area) pre- and post-2 weeks of lower limb immobilization and 4 weeks of subsequent recovery (retraining) in young and old individuals. Group mean data ± SD. Mean myofiber area was assessed in the quadriceps femoris muscle by muscle biopsy sampling(Aagaard et al, 2001; Suetta et al, 2008). Mechanical muscle

function is depicted in **B-D,** where **B =** concentric contraction strength, **C =** isometric contraction strength and **D =** contractile work. Data represents pre- and post-2 weeks of lower limb immobilization and 4 weeks of subsequent recovery (retraining) in young and old individuals. Maximal concentric, isometric muscle strength and work production were assessed for the quadriceps femoris muscle by use of isokinetic dynamometry (Aagaard et al, 2000; Suetta et al, 2004). In these experiments, not only absolute values, but relative recovery values of muscle strength were improved for young, as compared to old. *, $P \leq 0.05$, 2wk imm. compared to Pre, #, $P \leq 0.05$, 4wk compared to 2 wk and $, $P \leq 0.05$ Young compared to Old.

**Supplementary Figure 3 | Notch activation and Pax upregulation is diminished in regenerating old human skeletal muscle.** Additional panels are provided, which depict cryosections that were analyzed by immunostaining for co-expression of nuclear $Pax7^{+}$/active Notch in resident satellite cells. Pax7 staining is shown in green, active Notch is shown in red. Hoechst labels nuclei (blue).

**Supplementary Figure 4 | Transient treatment with recombinant TGF-β1 diminishes satellite cell capacity to generate de-novo myotubes, in vitro. A.** Activated satellite cells were first cultured for 24hr in the presence of 25ng/ml recombinant TGF-β1, and then subsequently transferred to Differentiation Medium (DMEM + 2% Horse Serum) for 72hr. The formation of de-novo myotubes was analyzed and quantified (**B**), based on the expression of eMyHC (green). Hoechst stains nuclei (blue). Data are means ± s.d., n = 6 for immunodetection of cryosections, n = 6 for Western blotting analysis, n=6 for myogenic culture experiments. *, $P \leq 0.05$ (Young and Old + TGF-β1 compared to Young and Old). **C.**

Myogenic cell populations, derived from isolated satellite cells, were analyzed by immunostaining with desmin (green) and MyoD1 (red) antibodies. Approximately 98% of isolated and cultured cells were satellite cell myogenic progeny.

**Supplementary Figure 5 | Young and old human satellite cells are capable of productive tissue regeneration in young environments, but are similarly inhibited by the aged circulatory milieu.** Isolated satellite cells from young and old human subjects were cultured in the presence of young vs. aged blood sera (isochronic and heterochronic). Cells were cultured for 7 days, followed by BrdU labeling and analysis of myogenic expansion and differentiation (quantified in **A**). **B.** Immunodetection of desmin is shown in red, BrdU shown in green. Hoechst labels nuclei (blue). $n = 6$. *, $P \leq 0.05$ (Y +YS compared to Y + OS, O +YS compared to O +OS). **C.** Young and old myofiber explants were cultured for 7 days in the presence of isochronic sera. Myogenic activation and expansion was analyzed and quantified by immunostaining for Pax7 (green) and desmin (red), depicted in **D**. Hoechst labels nuclei (blue). *, $P \leq 0.05$ (young compared to old). Line represents mean values. $n = 6$. Similar to our published findings in mouse model, the regenerative capacity of young human satellite cells is rapidly attenuated by old human sera, while the regenerative potential of aged human satellite cells is enhanced in the presence of young human sera.

**Supplementary Figure 6 | Treatment of satellite cell cultures with FGF and MEK inhibitor diminishes numbers of satellite cell generated de-novo myotubes, in vitro. A.** Activated satellite cells were first cultured for 24hr in the presence of 10ng/ml recombinant human FGF or 10μM MEKi, and then subsequently transferred to Differentiation Medium (DMEM + 2% Horse Serum) for 72hr. The formation of de-novo myotubes was analyzed and quantified (**B**), based

on the expression of eMyHC (green). Hoechst stains nuclei (blue). Data are means ± s.d., n = 6. *, $P \leq 0.05$ (Young and Old + FGF compared to Young and Old, and Young and Old + MEKi compared to Young and Old).

**Supplementary Figure 7 | Inhibition of Notch by GSI precludes satellite cell regenerative responses, even when MAPK is induced by FGF treatment. A.** Activated satellite cells were cultured for 24hr in the presence of 100nM GSI or GSI + 10ng/ml recombinant human FGF. Myogenic responses were analyzed and quantified (**B**), based on the co-expression of desmin/BrdU Data are means ± s.d., n = 3. *, $P \leq 0.05$ (+ GSI and +GSI/FGF compared to Control alone).

**Supplementary Figure 8 | Ectopic Delta and GSI activate and inhibit Notch (respectively) in human myogenic cells in vitro.** To control for Notch activation and inhibition, freshly-isolated human myogenic cells were cultured in the presence of immobilized Delta ligand (Notch activation + Delta), or gamma secretase inhibitor (Notch activation + GSI). Cell lysates were analyzed by Western blot using an active Notch-specific antibody. Actin immunodetection was used a loading control.

**Supplementary Figure 9 | Simplified experimental scheme of conducted studies.**
A workflow diagram is provided for illustration and explanation of the experimental approaches employed in these studies. Isolation procedures, starting from whole human muscle biopsies, to isolated individual satellite cell populations are described. Assays used at different isolation steps are also indicated, with examples of where they appear in the text and figures.

**Supplementary Table 1 | Myogenic responses from initially-equivalent satellite cells populations of different ages.** An initially-equivalent population of young and old satellite cells (625 cells seeded per well for Yng+Ys, Old+Ys, Old+Os and Ynd+Os), yielded different numbers of myogenic progeny or myoblasts (significantly higher in the young vs. old after 7 days of culture). This age-specific decline is reversed if the aged satellite cells are cultured with young human serum (Old+Ys). Therefore, even with equivalent and clearly myogenic satellite cellular pools, the regenerative outcome of the old cells in an old environment is worse than young cells in a young environment.

## REFERENCES

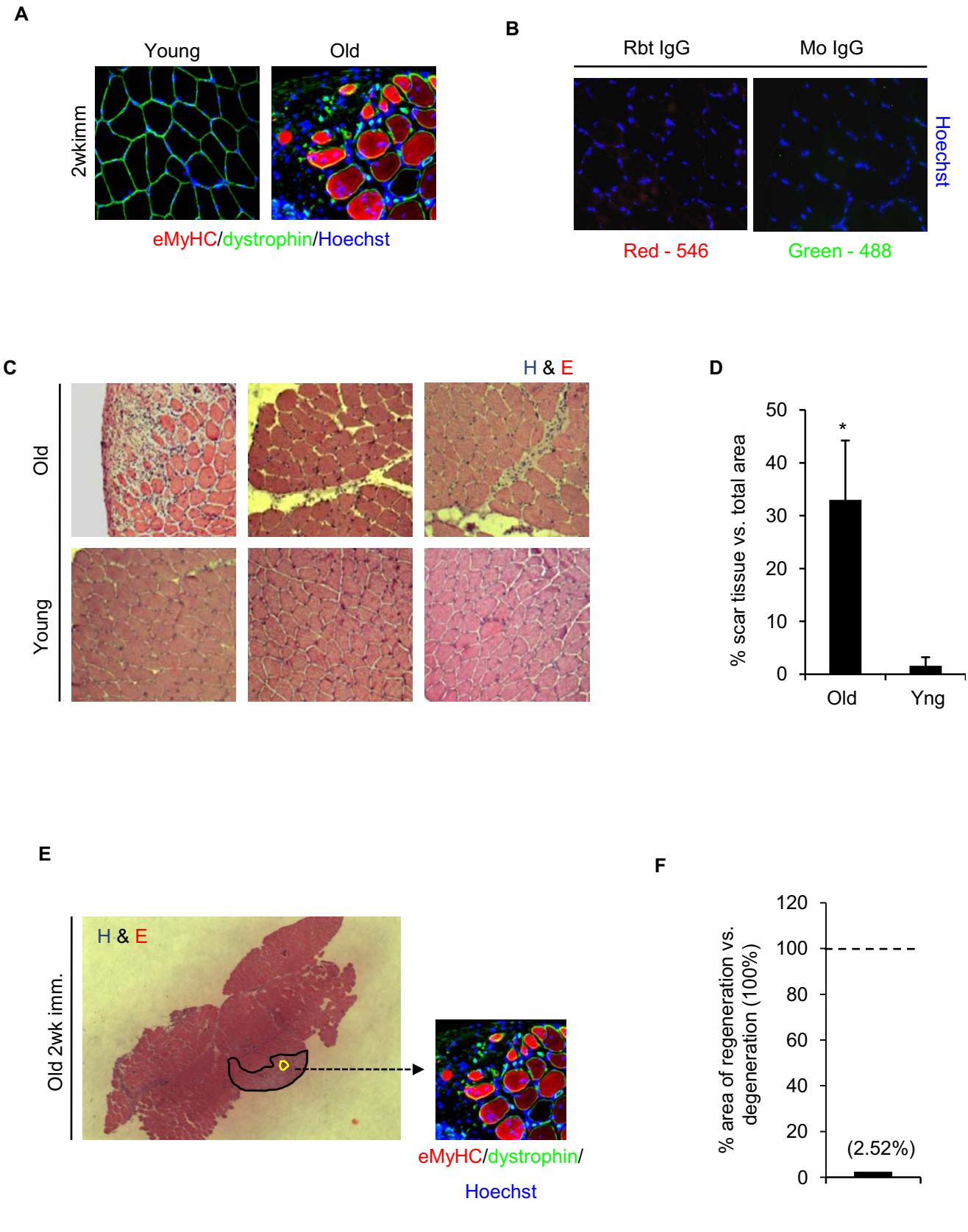
Aagaard P, Andersen JL, Dyhre-Poulsen P, Leffers AM, Wagner A, Magnusson SP, Halkjaer-Kristensen J, Simonsen EB (2001) A mechanism for increased contractile strength of human pennate muscle in response to strength training: changes in muscle architecture. *J Physiol* **534:** 613-623

Aagaard P, Simonsen EB, Andersen JL, Magnusson SP, Halkjaer-Kristensen J, Dyhre-Poulsen P (2000) Neural inhibition during maximal eccentric and concentric quadriceps contraction: effects of resistance training. *J Appl Physiol* **89:** 2249-2257

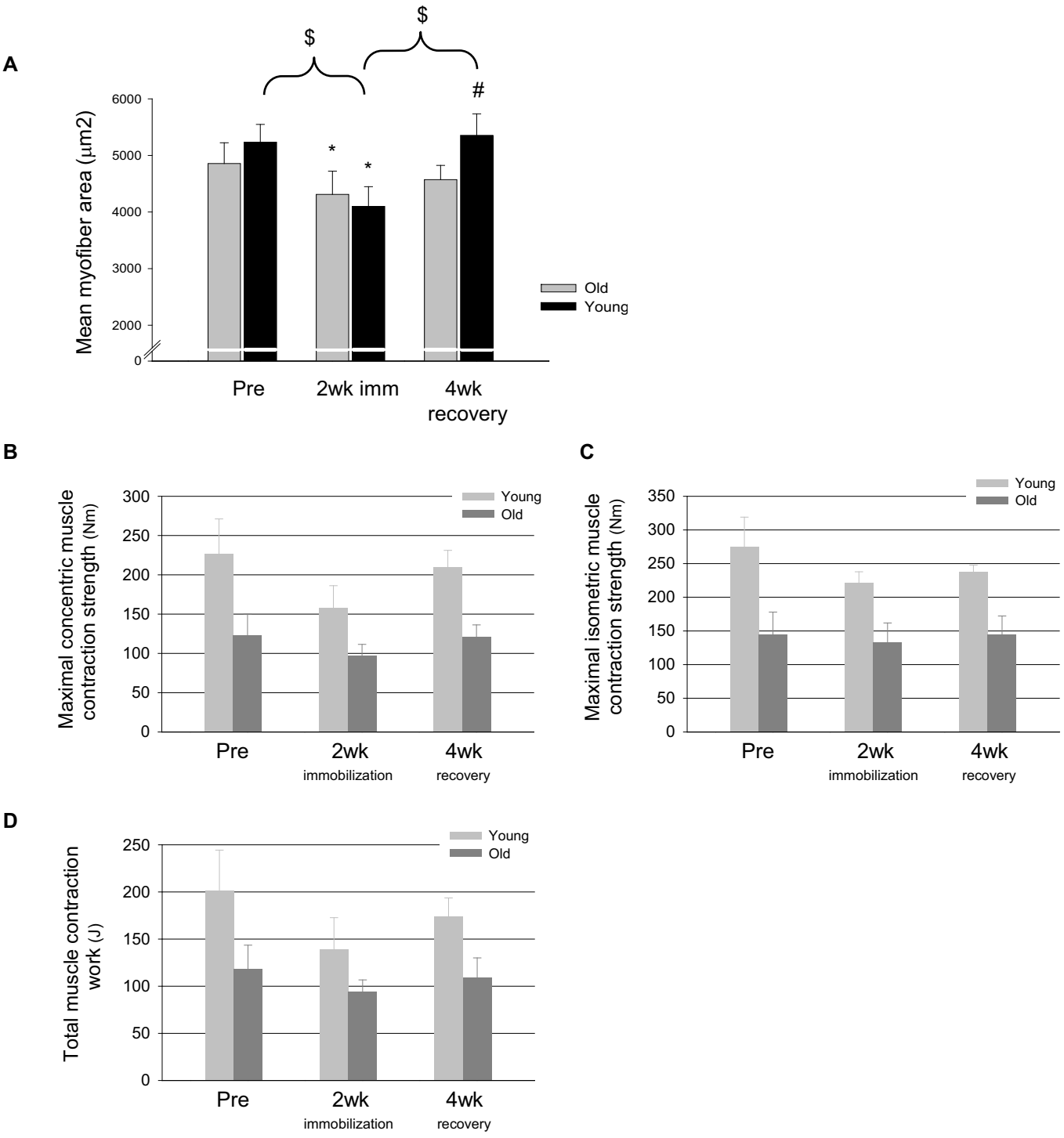
Suetta C, Andersen JL, Dalgas U, Berget J, Koskinen S, Aagaard P, Magnusson SP, Kjaer M (2008) Resistance training induces qualitative changes in muscle morphology, muscle architecture, and muscle function in elderly postoperative patients. *J Appl Physiol* **105:** 180-186

Suetta C, Magnusson SP, Rosted A, Aagaard P, Jakobsen AK, Larsen LH, Duus B, Kjaer M (2004) Resistance training in the early postoperative phase reduces hospitalization and leads to muscle hypertrophy in elderly hip surgery patients--a controlled, randomized study. *J Am Geriatr Soc* **52:** 2016-2022

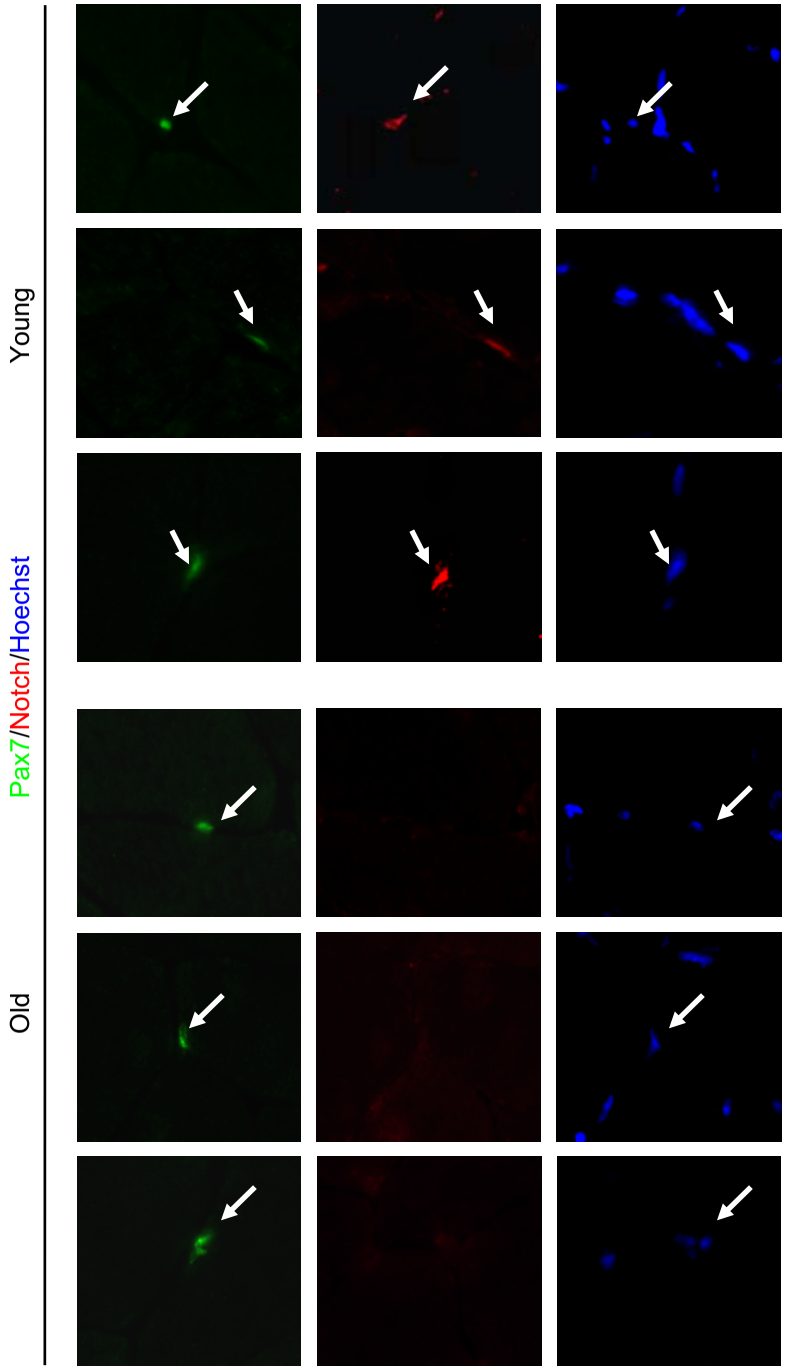
Supplementary Figure 1



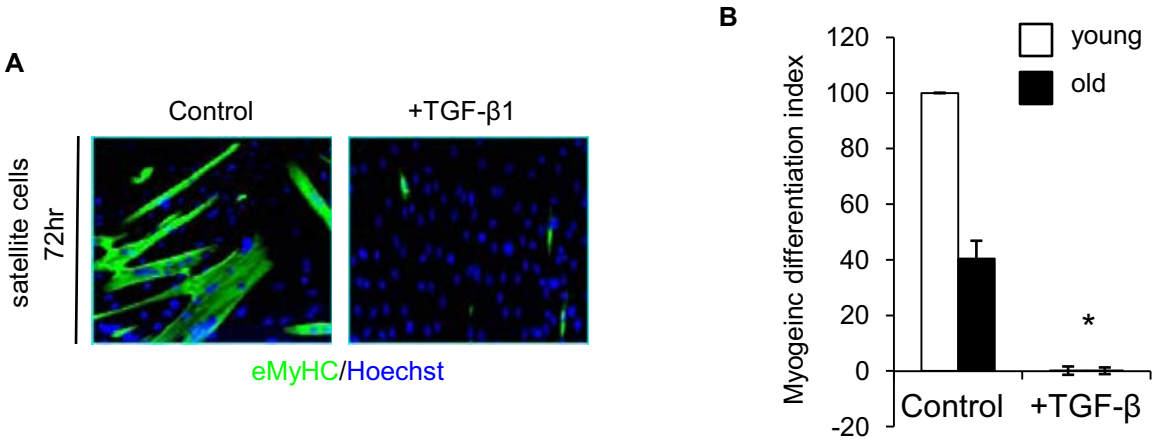
Supplementary Figure 2

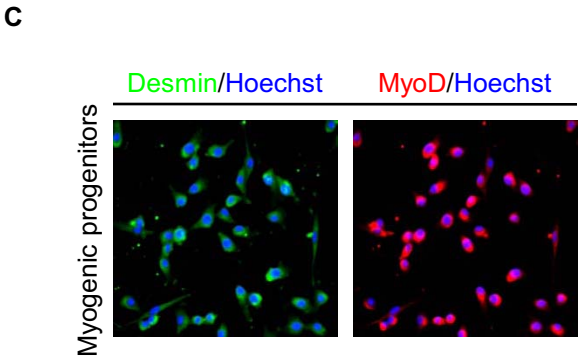


Supplementary Figure 3

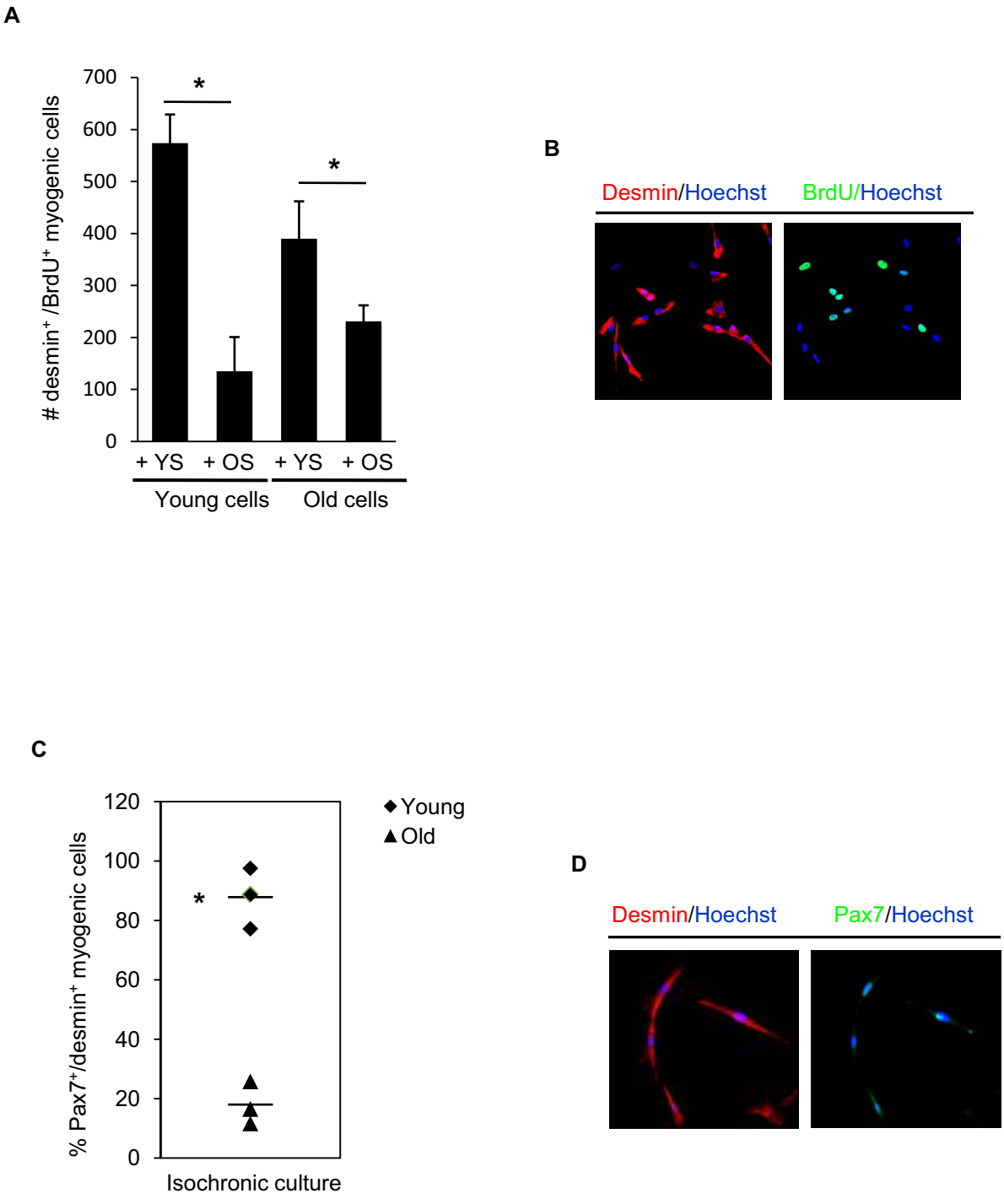


Supplementary Figure 4

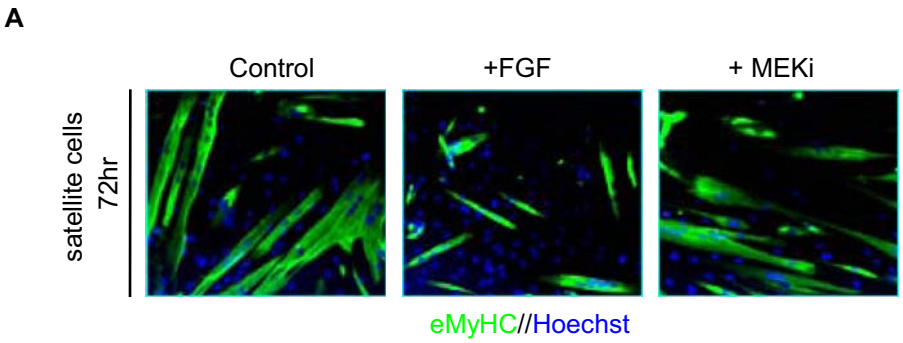


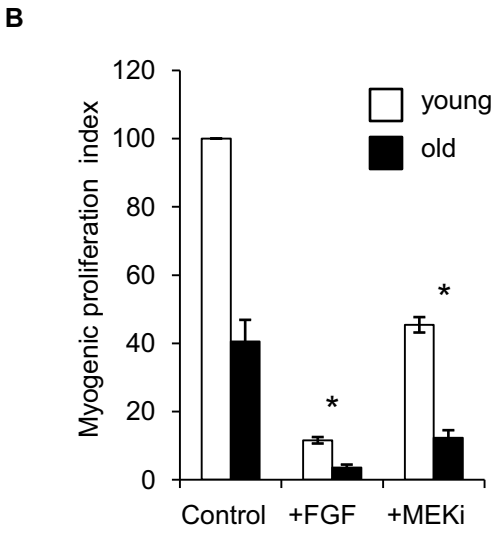



Supplementary Figure 5

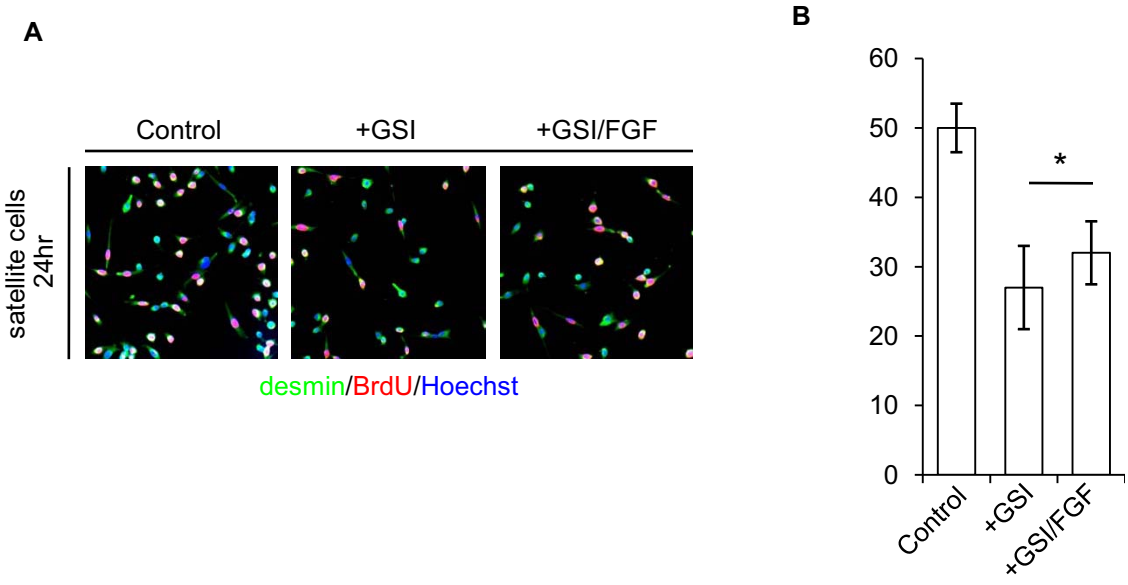


Supplementary Figure 6

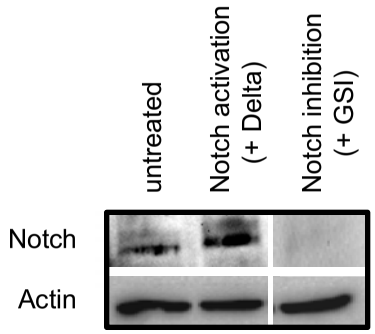


Supplementary Figure 7

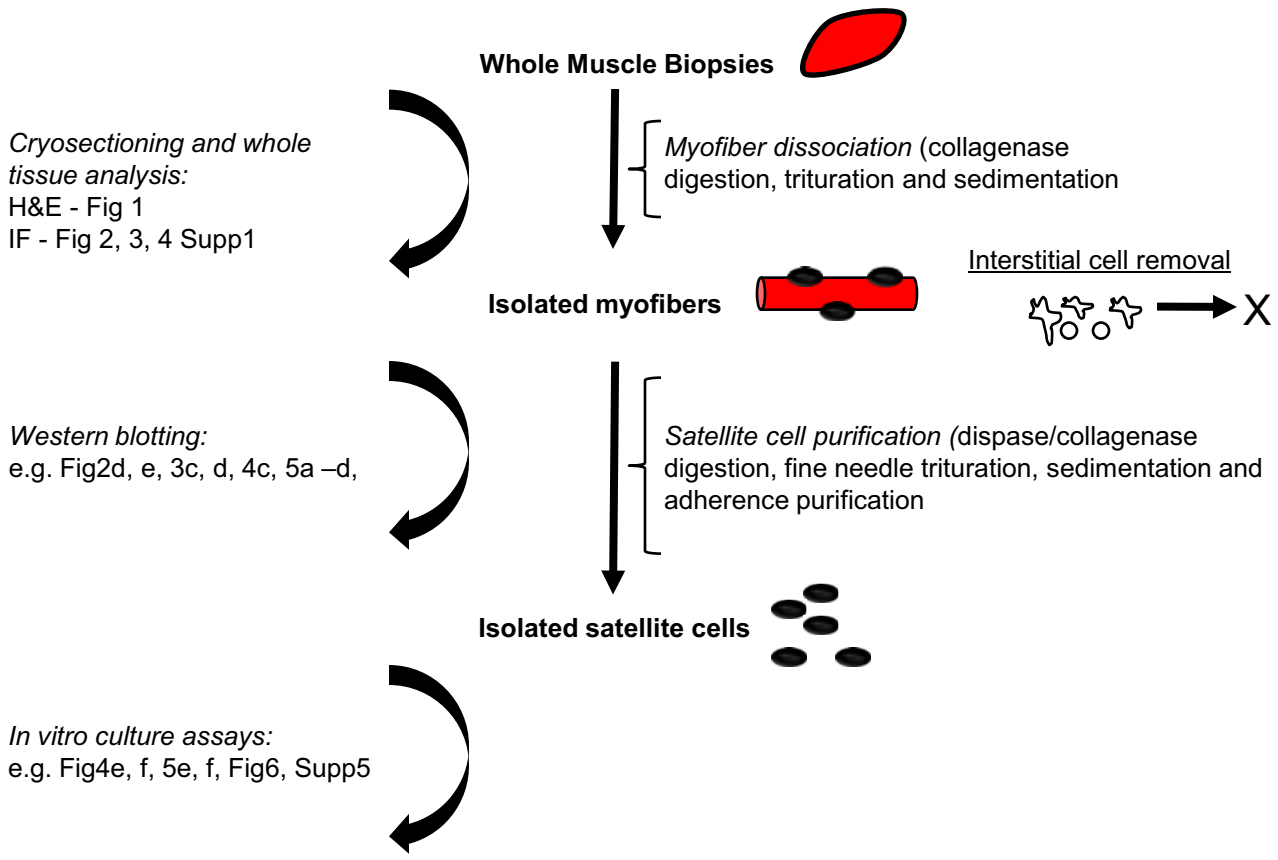


Supplementary Figure 8



Supplementary Figure 9

Simplified experimental scheme:



Supplementary Table 1

Myogenic responses from initially equivalent populations of different ages (625 satellite cells/well)

| *In vitro* condition | % desmin$^+$/BrdU$^+$ |
|---|---|
| Yng+Ys | 72.4 |
| Old+Ys | 67.9 |
| Old+Os | 35.0 |
| Yng+Os | 23.5 |