Supplemental Material

Control of Muscle Fibro-Adipogenic Progenitors by Myogenic Lineage is Altered in Aging and Duchenne Muscular Dystrophy

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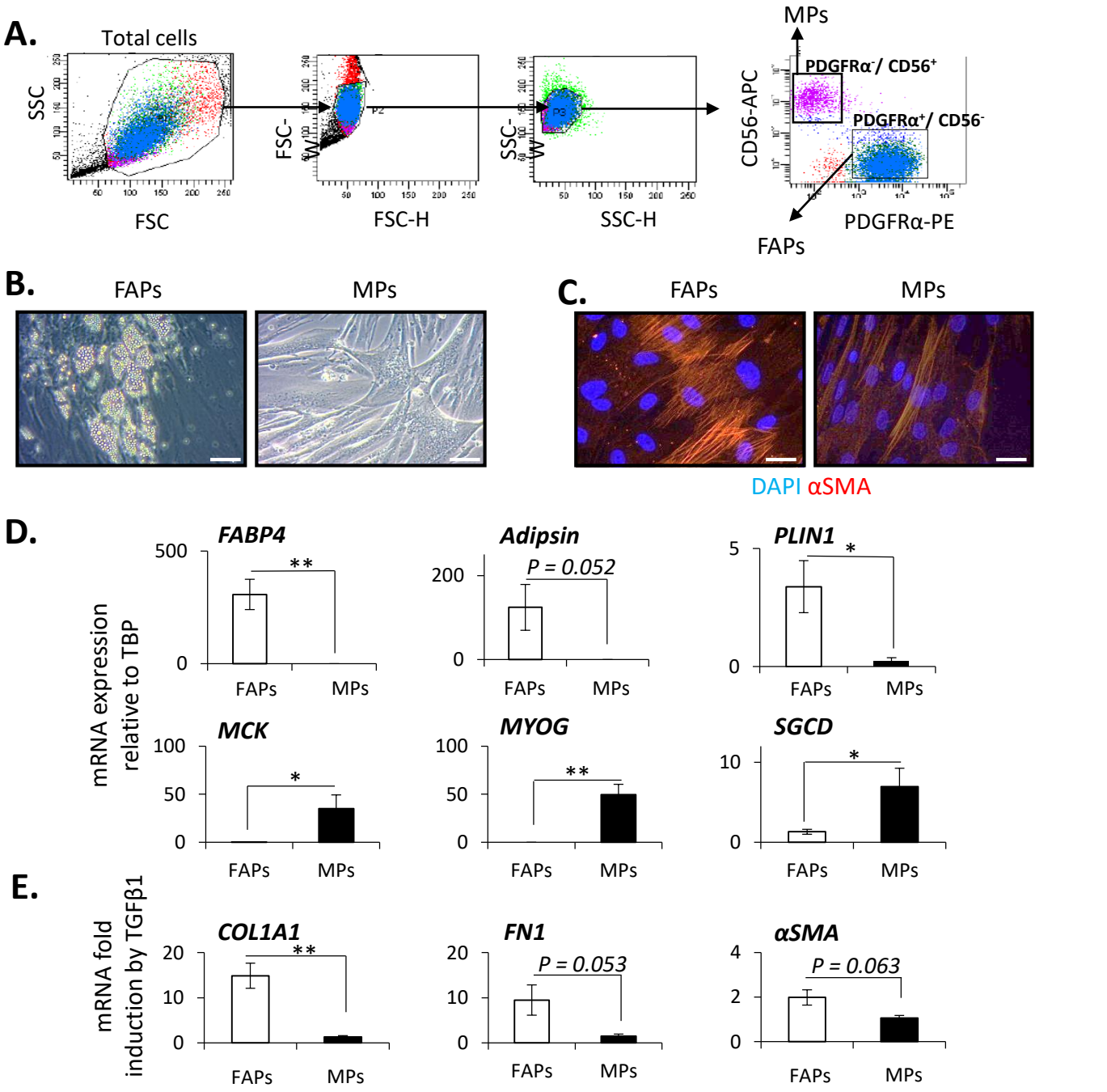
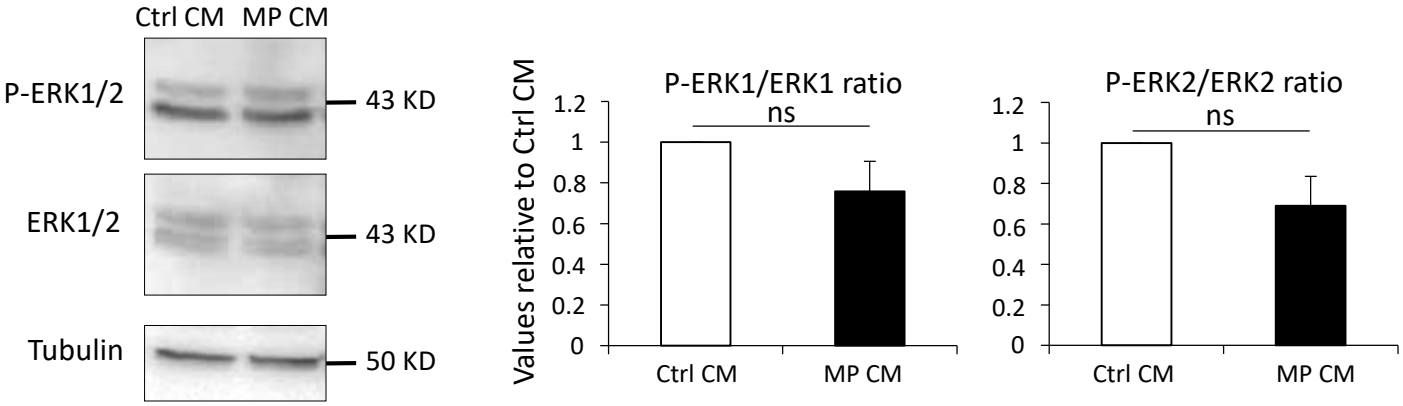
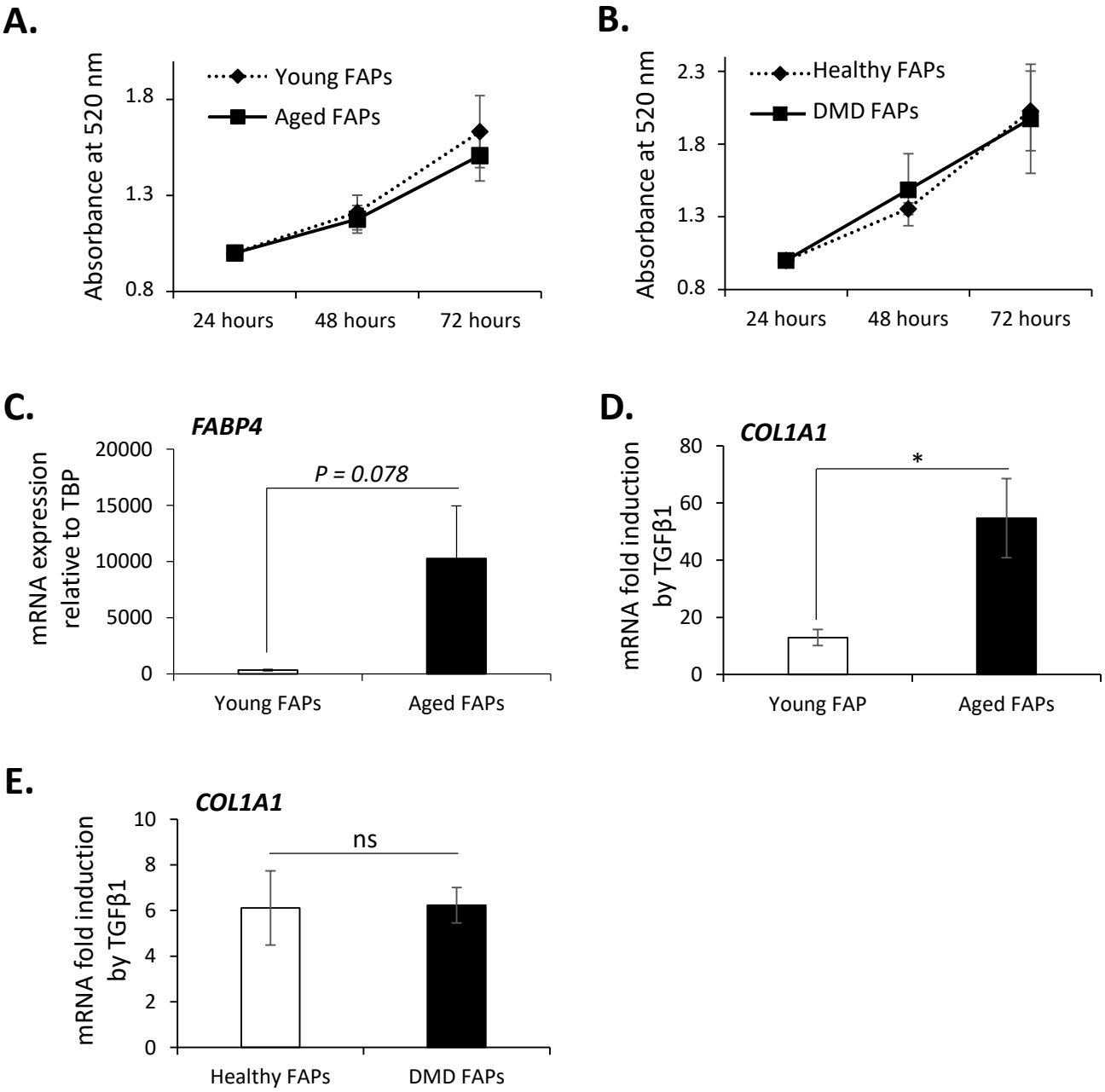


Supplemental figure 1. Sorting of FAPs and MPs by flow cytometry and characterization of their differentiation potentials. (A) Adherent cells prepared from human skeletal muscle samples were amplified and submitted to flow cytometry sorting. FAPs are positive for PDGFRα and negative for CD56, while MPs are positive for CD56 and negative for PDGFRα. **(B)** FAPs and MPs were cultured in differentiation medium for 10 days. FAP-derived adipocytes with lipid droplets (scale bar: 10 µm) and MP-derived multinucleated myotubes (scale bar: 20 µm) were visualized with phase-contrast microscopy. **(C)** FAPs and MPs were cultured in differentiation medium supplemented with 5 ng/ml TGFβ1 for 5 days, fixed, immuno-labeled with anti-αSMA antibody and stained with DAPI (scale bar: 10 µm). **(D)** FAPs and MPs were cultured in differentiation medium for 10 days, and mRNA expressions of the adipogenic markers *FABP4*, *Adipsin* and *PLIN1* and the myogenic markers *MCK, MYOG* and *SGCD* were measured by quantitative Q-PCR (n= 5 healthy and young donors). **(E)** FAPs and MPs were cultured in differentiation medium supplemented with 5 ng/ml TGFβ1 for 5 days, and mRNA expressions of the fibrogenic markers *COL1A1*, *FN1* and *ACTA2* were measured by quantitative Q-PCR (n=3 donors). Fold inductions by TGFβ-1 are presented. ** P < 0.01; * P < 0.05; *p* values close to significance are indicated.

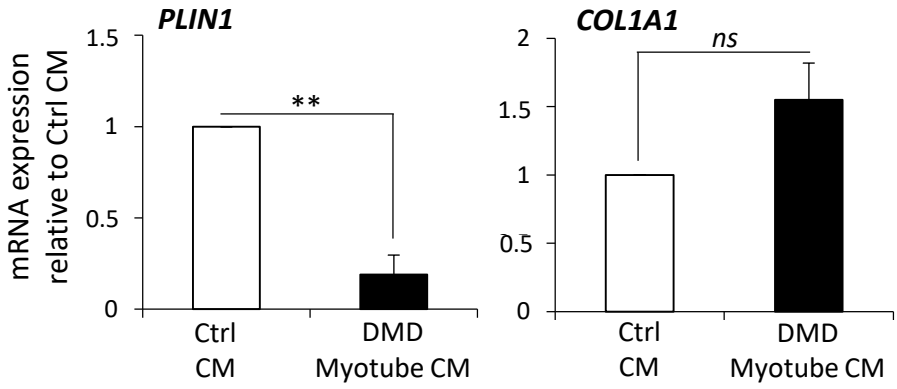


Supplemental figure 2. The stimulation of FAP proliferation by MPs is non-dependent of ERK1/2 pathway. FAPs proliferated with control conditioned medium (Ctrl CM) or MP conditioned medium (MP CM). 15 minutes after addition of the conditioned medium, total proteins were extracted. Expression level of phosphorylated ERK1/2, total ERK1/2 and tubulin were assessed by western-blot (left panel). Immunoblot signals were quantified and normalized with total ERK1/2 signals (right panels) (n=3 donors). ns= non-significant

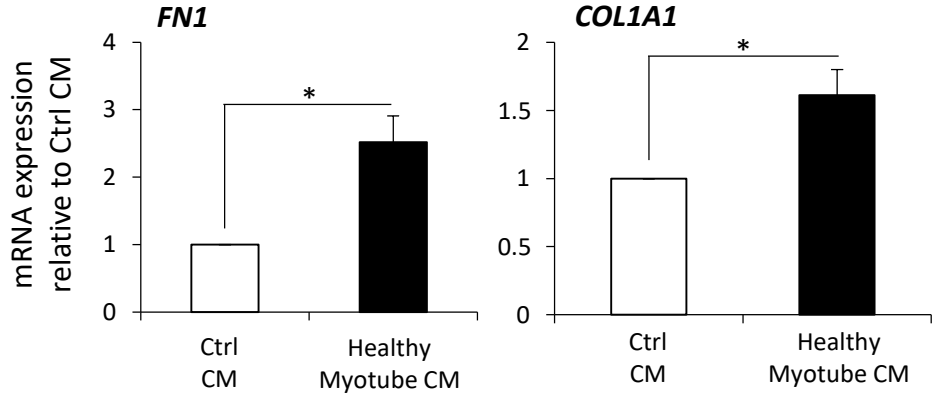


Supplemental figure 3. Proliferation rate and differentiation potentials of DMD and aged FAPs. Proliferation of young and aged FAPs **(A)**, or healthy and DMD FAPs **(B)** was measured by MTT assays 24 hours, 48 hours and 72 hours after plating (n=4 donors per group). **(C)** Young and aged FAPs were differentiated for 10 days in differentiation medium. Expression of *FABP4* was measured by quantitative Q-PCR (n=4 donors per group). Young and aged FAPs **(D)**, or healthy and DMD FAPs **(E)** were cultured in differentiation medium supplemented with 5 ng/ml TGFβ1 for 5 days. Expression of *COL1A1* was measured by quantitative Q-PCR. Fold inductions by TGFβ-1 are presented (n=4 donors per group). * $P < 0.05$; ns= non-significant; *p* values close to significance are indicated.

A.



B.



Supplemental figure 4. Regulation of DMD FAP differentiation by DMD or Healthy myotube conditioned-medium. DMD FAPs were cultured in the presence of control conditioned medium (Ctrl CM) or conditioned medium from DMD myotubes (DMD Myotube CM) **(A)** or from Healthy myotubes (Healthy Myotube CM) **(B)** for 10 days in differentiation medium. Expression of *COL1A1, FN1* and *PLIN1* was measured by quantitative Q-PCR (n=3 healthy donors and n=3 DMD donors). * $P < 0.05$; ** $P < 0.01$; ns= non-significant.