Retinoic acid maintains human skeletal muscle progenitor cells in an immature state

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As myoblasts can be partially transdifferentiated into osteoblasts and adipocytes when cultured in the appropriate medium [1,2], we assessed the effect of RA on these two differentiation programs. Confluent myoblasts were exposed to adipogenic or osteogenic stimuli in the presence or absence of 10^{-7} M RA for three days. Adipocyte and osteoblast differentiation were then assessed by visual analysis (lipid vesicle accumulation and alkaline phosphatase staining, respectively) (Fig. s2a) and RT-qPCR quantification of the expression of *ALPL* and osterix (osteoblast lineage markers) (Fig. s2b) and *CEBP* alpha and *AP2* (adipocyte markers) (Fig. s2c). Both osteoblast and adipocyte transdifferentiation were markedly reduced in myoblasts incubated with RA compared to controls (DMSO alone) (Fig. s2). These data suggest that RA also inhibits myoblast transdifferentiation into adipocytes and osteoblasts.

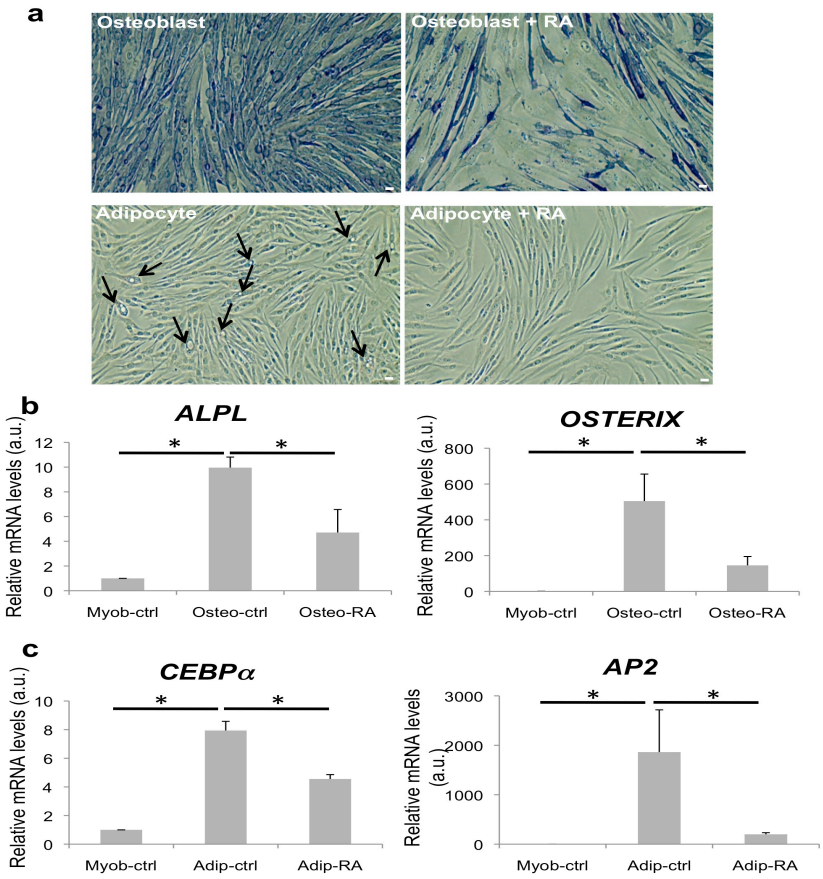

Figure s2: **Retinoic acid inhibits myoblast transdifferentiation into adipocytes and osteoblasts.**

Human myoblasts treated or not with 10^{-7}M RA were transdifferentiated into osteoblasts or adipocytes, using the appropriate culture media for three days (100 ng/ml BMP2 for osteoblast differentiation or Preadipocyte Differentiation Medium/Adipocyte Nutrition Medium (PromoCell) for adipocyte differentiation). (A) Upper panels: osteoblasts were identified by alkaline phosphatase expression (in blue). Lower panels: black arrows show lipid vesicles of adipocytes (Oil Red O staining). (B) (C) RT-qPCR assessment of the expression of (B) osteoblast (*ALPL* and osterix) and (C) adipocyte (*CEBPa* and *AP2*) markers in myoblasts grown in proliferating medium, osteoblast medium or in adipocyte medium without RA (Myob-ctrl; Osteo-ctrl; Adip-ctrl) and with RA (Myob-RA; Osteo-RA; Adip-RA). Data are shown as fold induction relative to control myoblasts cultured in growth medium (Myob-ctrl) without RA (vehicle; 0.1% DMSO), which was set at 1. $P \leq 0.05$ (*). Scale bars: 10μM.

1. Agley CC, Rowlerson AM, Velloso CP, Lazarus NR, Harridge SD (2013) Human skeletal muscle fibroblasts, but not myogenic cells, readily undergo adipogenic differentiation. J Cell Sci 126:5610-5625

2. Asakura A, Komaki M, Rudnicki M (2001) Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. Differentiation 68:245-253