Supplementary Figure 1

A

NFATc1

NFATc3

NFATc4

RNA expression (Normalized counts)

GM
6h
9h
24h
48h
72h
48h
72h

DM
MT
RC

B

Myogenin

MEF2C

MyHC

STIM1L

Pax7

EGFR

RNA expression (Normalized counts)

GM
6h
9h
15h
24h
48h
72h
48h
72h

DM
MT
RC

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Supplementary Fig. 1. Markers of differentiation during human primary myoblast differentiation. RNA content was analyzed with the nCounter method in different conditions: during proliferation (GM), at the indicated times during differentiation (DM), and in isolated myotubes (MT) and reserve cells (RC) at DM48h and DM72h. A. NFATc1, NFATc3 and NFATc4 expression during human primary myoblast differentiation. Results of the nCounter method (NanoString Technologies): counts were normalized to house-keeping genes; n=4. B. mRNA expressions of four differentiation markers, myogenin, MEF2C, Myosin HC and STIML and two markers of reserve cells, Pax7 and EGFR. Counts are normalized to house keeping genes; n=4. Results are expressed as mean ± SEM. It is worth noting that the clear-cut difference between myotube and reserve cell protein expression suggests a good purity of the myotubes/reserve cells separation, with very little cross-contamination.
Supplementary Figure 2

A

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MyHC

α-Tub

B

EGFP

EGFP-NFATc1

Rescue Experiment

Myotube Fusion

[Graph showing Rescue Experiment results]

[Graph showing Myotube Fusion results]
Supplementary Fig. 2. Expression of EGFP-NFATc1 rescues NFATc1 silencing.

A. Western blot. Myoblasts were transfected with siRNA (control or siNFATc1) and 48 hours later were transfected with plasmids (EGFP or EGFP-NFATc1). Myoblast differentiation was induced 24 hours later for 48h hours. (mean ± standard deviation, n=3).

B. NFATc1 overexpression induced myotube hypertrophy. Myoblasts were transfected with plasmids (EGFP or EGFP-NFATc1) and differentiation was induced 24 hours later as above (mean ± standard deviation, n=3).