

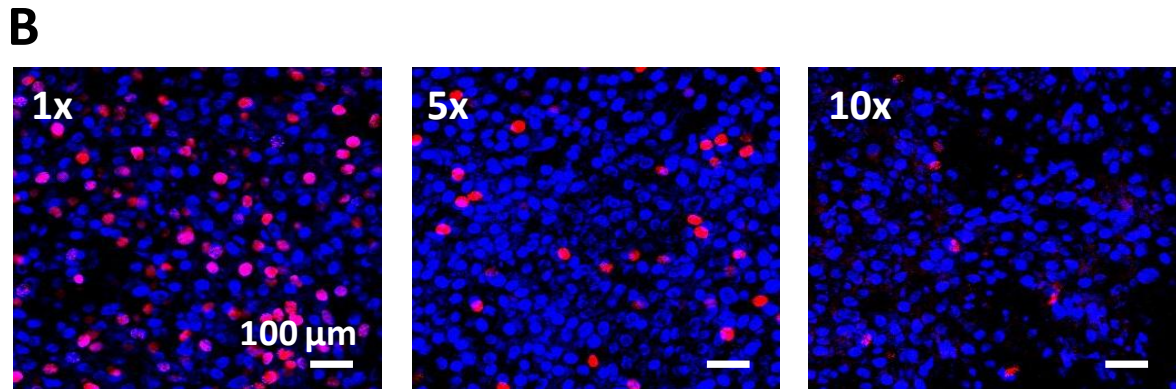
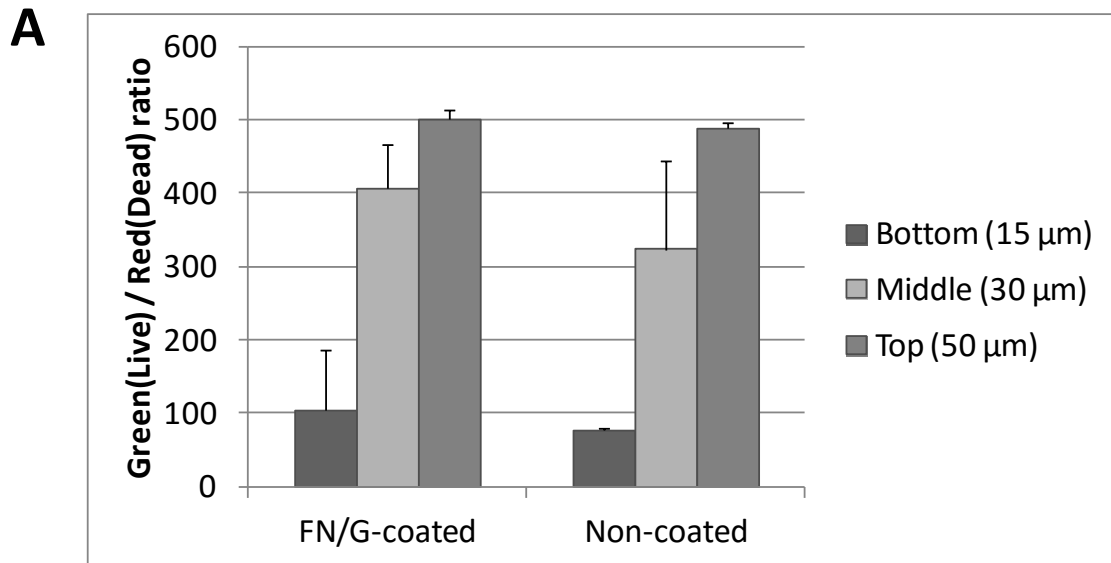
Supporting Information

Cell proliferation

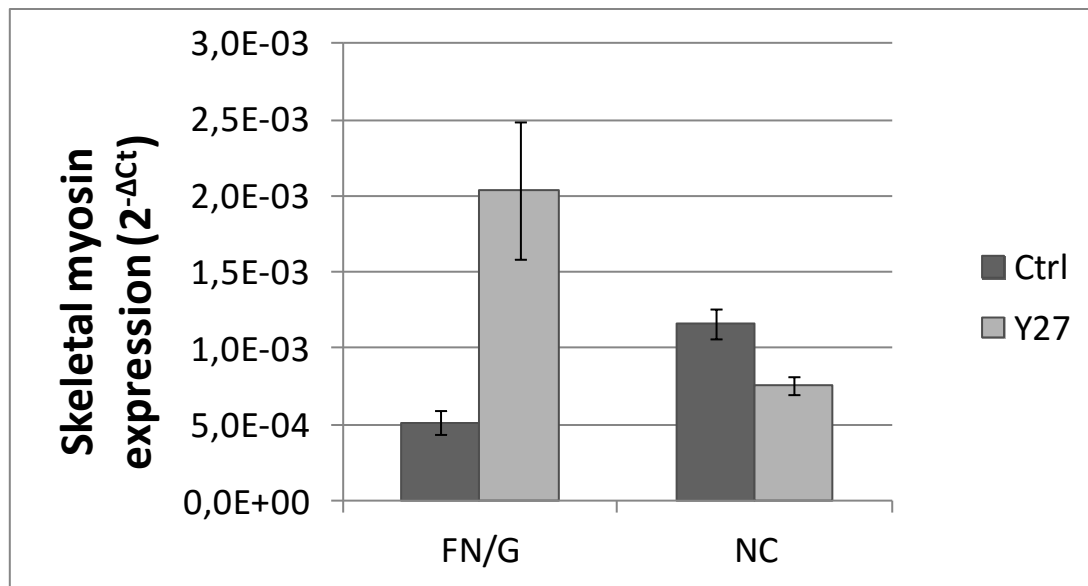
Cell proliferation was assessed by the 5-ethynyl-2'-deoxyuridine (EdU) assay (Click-iT EdU Imaging Kit, Invitrogen). FN/G-coated constructs were built and cultured for 1 day in GM, then incubated for 1 h at 37°C with EdU diluted at 1/1000 in cell culture medium. The detection was carried out following the manufacturer instructions. At the end, nuclei were counter-stained with Hoechst 33432 (Invitrogen). The images of EdU and Hoechst-labeled nuclei were taken using a confocal microscope, approximately in the middle of each construct.

Quantitative RT-PCR

FN/G-coated and non-coated constructs at Day 6 of differentiation were lysed directly in the inserts. Total RNA was isolated using PureLink RNA Micro Kit (Invitrogen) and cDNA was synthesized from 2 µg of total RNA using the High-Capacity RNA-to-cDNA Kit (Applied Biosystems) following the manufacturer's instructions. The expression level of skeletal myosin (heavy polypeptide 2) mRNA was quantified on an ABI Prism 7500 (Applied Biosystems) using TaqMan Gene Expression Master and Mix TaqMan Gene Expression Assay (Assay ID: Mm01332564_m1, Applied Biosystems) following the manufacturer's instructions. Each sample was amplified in duplicate at 50°C for 2 minutes and 95°C for 10 minutes followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. Data were collected with Step One V.2.1 software and analyzed using the threshold cycle (Ct) relative quantification method. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene was used for data normalization. Values are given as $2^{-\Delta Ct} = 2^{-[Ct(X) - Ct(GAPDH)]}$ where Ct(X) is the value for gene X and Ct(*GAPDH*) that of reference gene.



S1 Fig. Cell viability and proliferation. (A) Live (green)/Dead (red) fluorescence ratio in 10x FN/G-coated and non-coated constructs after 24h of culture in GM (n=2). (B) Cell proliferation in FN/G-coated constructs after 24h of culture in GM. Scale bar: 100 μm .



S2 Fig. Effect of Y27 on skeletal myosin expression. Skeletal myosin gene expression has been quantified after 6 days of differentiation (n=2).