

Supplemental Material

Supplementary Figure legends:

Supplementary Figure I: Photomicrographs of C2C12 cultures before and after differentiation. A) Subconfluent myoblasts in high serum growth media (GM) at day -1. B) Cells after 6 days in low serum differentiation media (DM) showing many multinucleated myotubes.

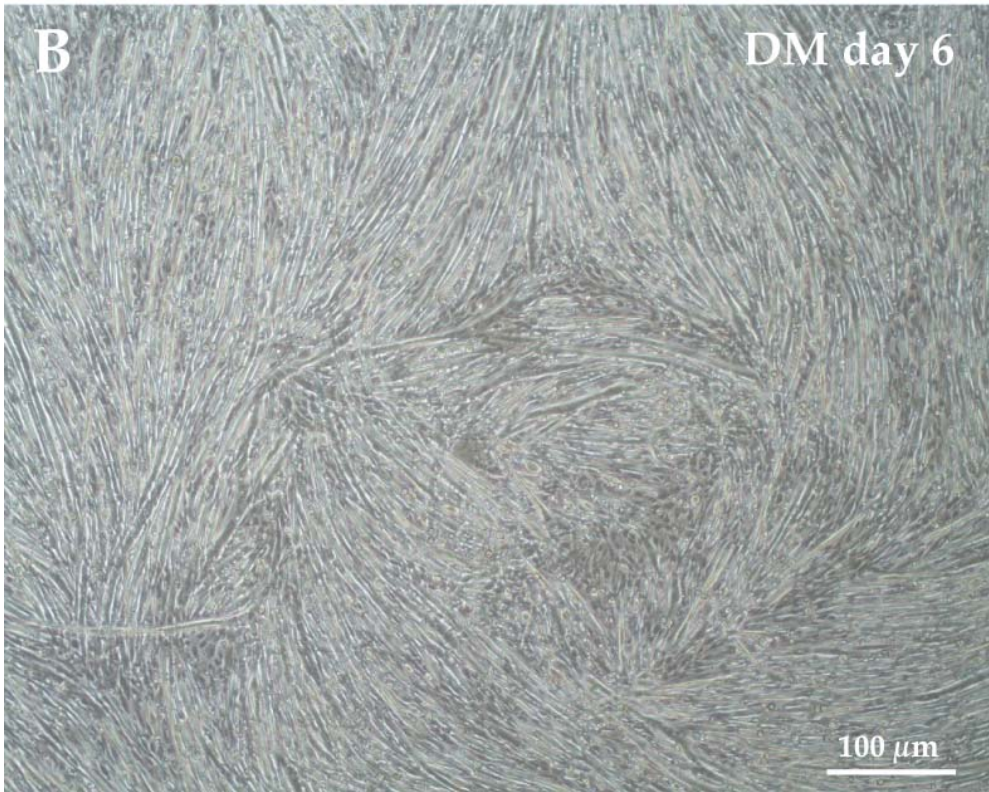
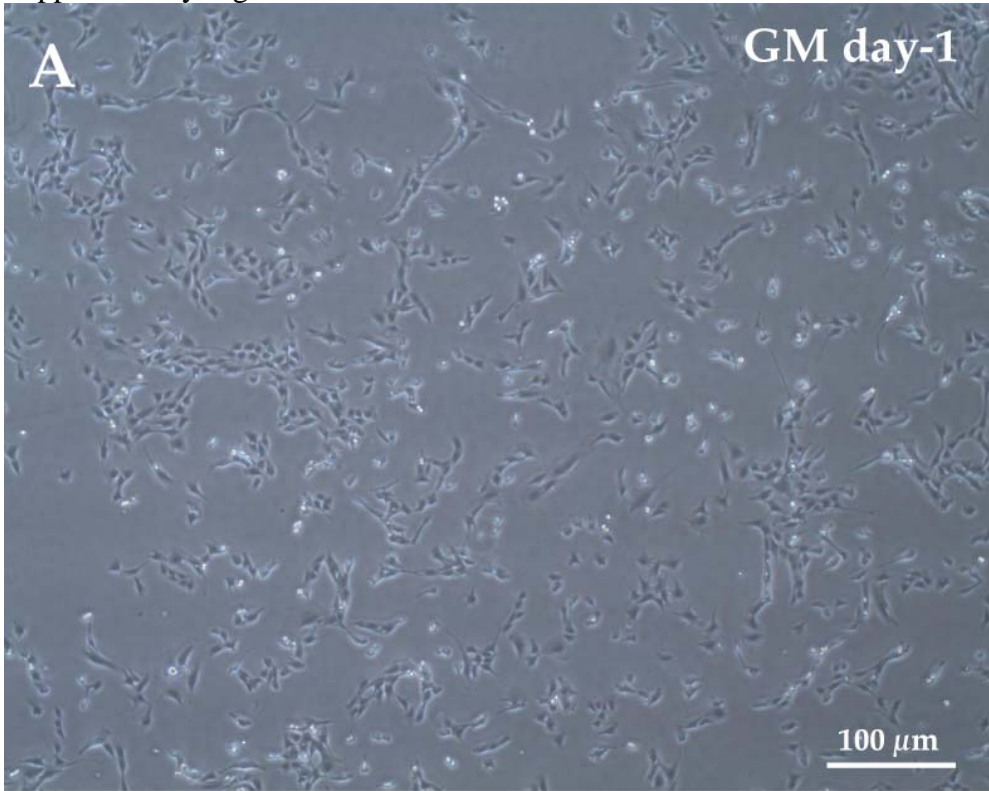
Supplementary Figure II: Western blot to determine the amount of BMP4 secreted by C2C12 cells. Lane 1) Heparin-agarose was used to pull-down BMP4 from C2C12 conditioned differentiation media (CM) after 2 days in culture. Lane 2) Molecular weight markers, the 20 kilodalton (kDa) band is indicated to the left. Lanes 3-7) Known amounts of human recombinant BMP4 (PeproTech) were loaded per lane. The amounts loaded are indicated at the top of each lane.

Supplementary Figure III: Indirect immunofluorescence staining specificity of the anti-Id3 rabbit monoclonal antibody that was used in this study. A) WT mouse TA muscle section from day 3 post-cardiotoxin injury is shown stained with antibodies against Pax7 (red), Id3 (green), and the nuclear stain DAPI (blue). Arrowheads indicate Pax7⁺ cells strongly stained for Id3. B) Id-mutant mouse (Id3-null) TA muscle section from day 3 post-cardiotoxin injury stained as in panel A showing very little Id3 immunoreactivity. Also note the reduced numbers of Pax7⁺ cells in the Id-mutant compared to the WT tissue.

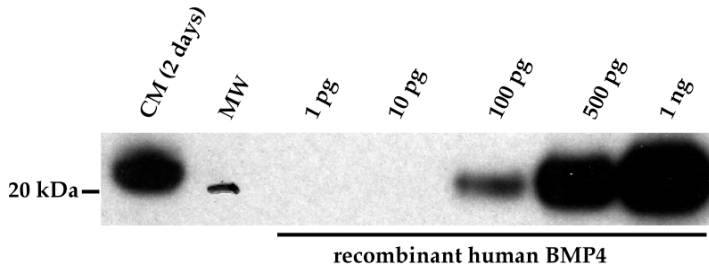
Supplementary Figure IV: Indirect immunofluorescence staining of activated Pax7⁺ satellite cells showing coexpression of phospho-Smad1/5/8 (a,b), Id1 (c,d) or Id3 (e-f) at day 3 post-cardiotoxin injury. White arrows indicate Pax7⁺ cells coexpressing the indicated antigens. The boxes in panels a & b show pSmad1/5/8 staining separated from the green and blue channels. Background staining was increased in order to allow visualization of myofibers in relationship to Pax7⁺ cells.

Supplementary Figure V: Reduced numbers of Pax7⁺ cells are also seen in ischemic TA muscles of Id-mutants compared to WT mice from another injury model created by femoral artery excision (FAE). A) TA muscle from WT littermate mice was harvest at day 5 post-FAE surgery and stained for Pax7 (red) and mounted with DAPI. B) Id-mutant TA muscle at day 5 post-FAE was also stained for Pax7 and mounted with DAPI. Note that there are fewer Pax7⁺ cells in the Id-mutant tissue compared to WT tissue similar to what we observed after the cardiotoxin injury (Figure 5).

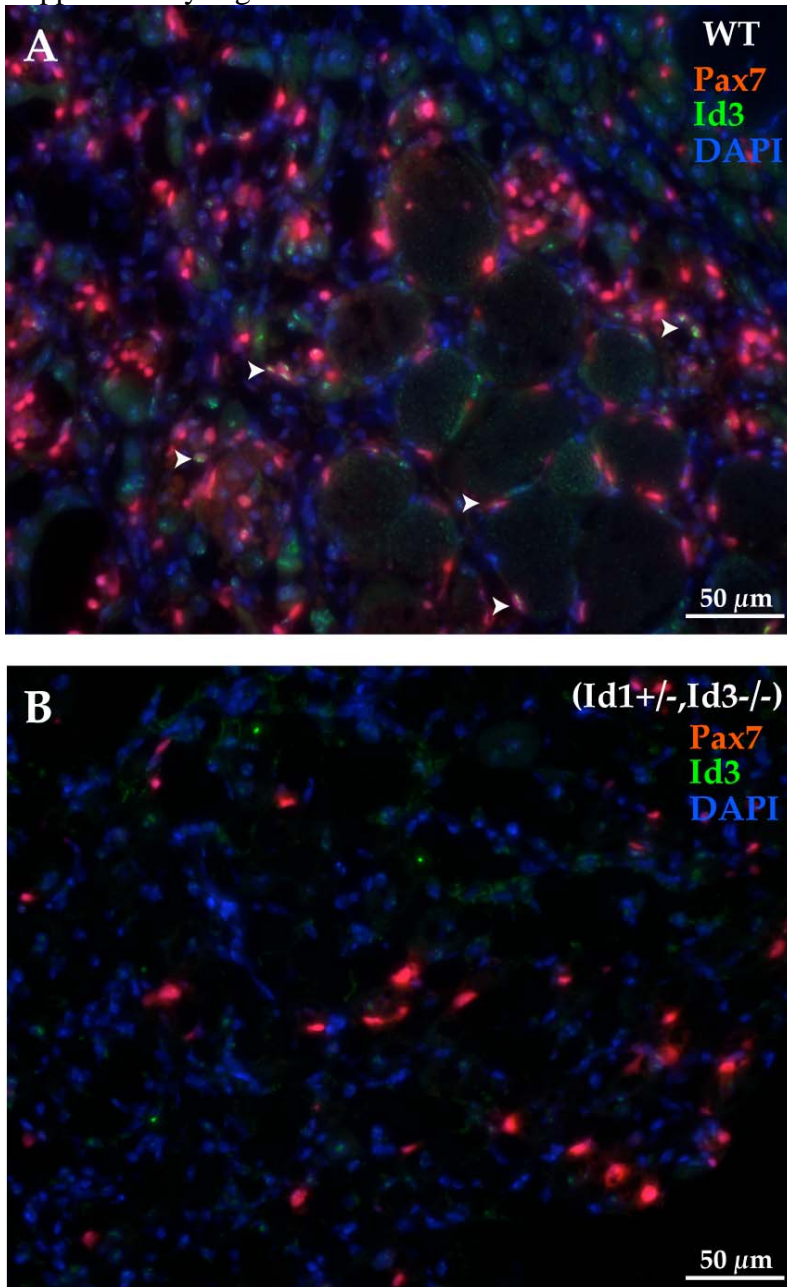
Supplementary Figure I:



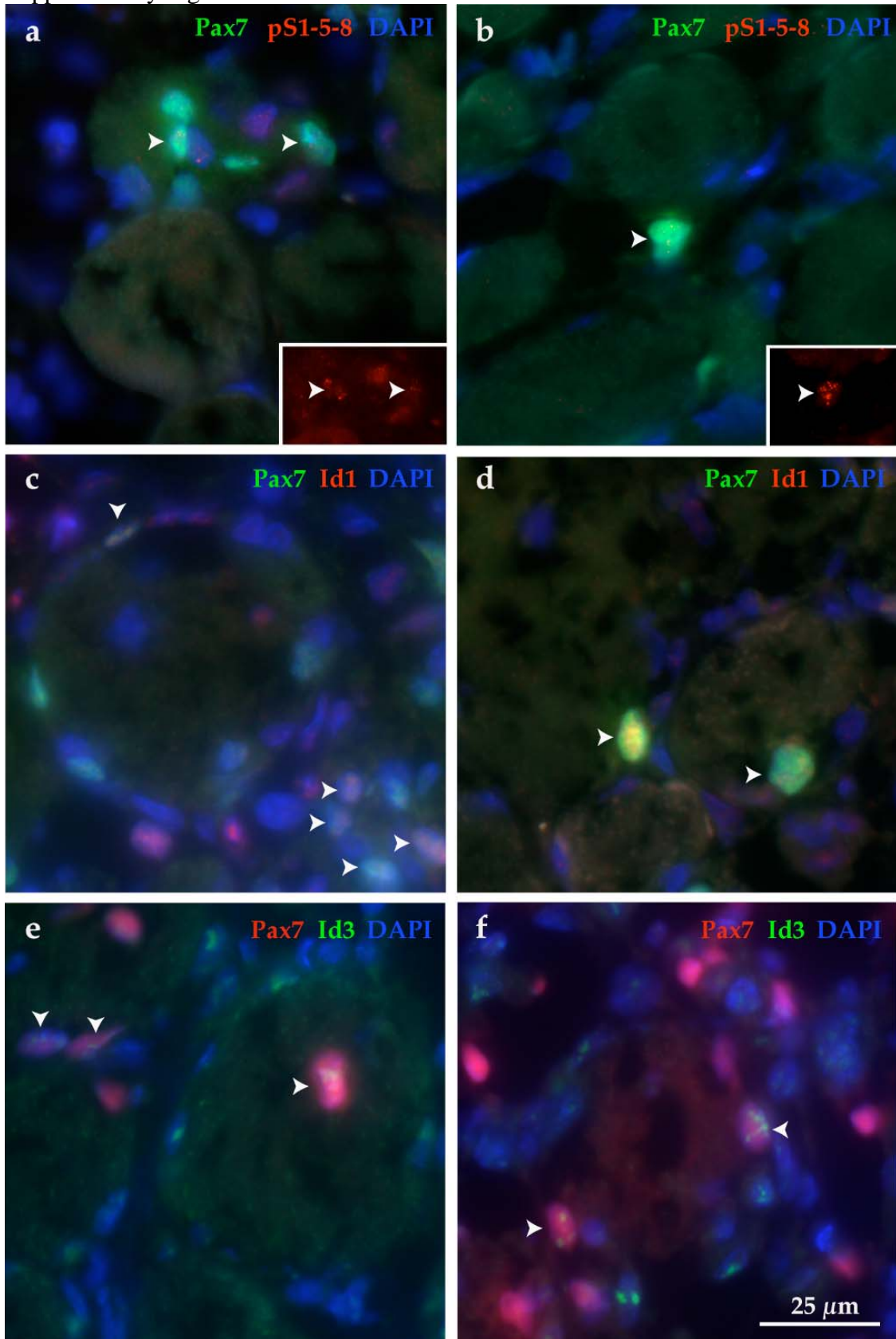
Supplementary Figure II:



Supplementary Figure III:



Supplementary Figure IV:



Supplementary Figure V:

