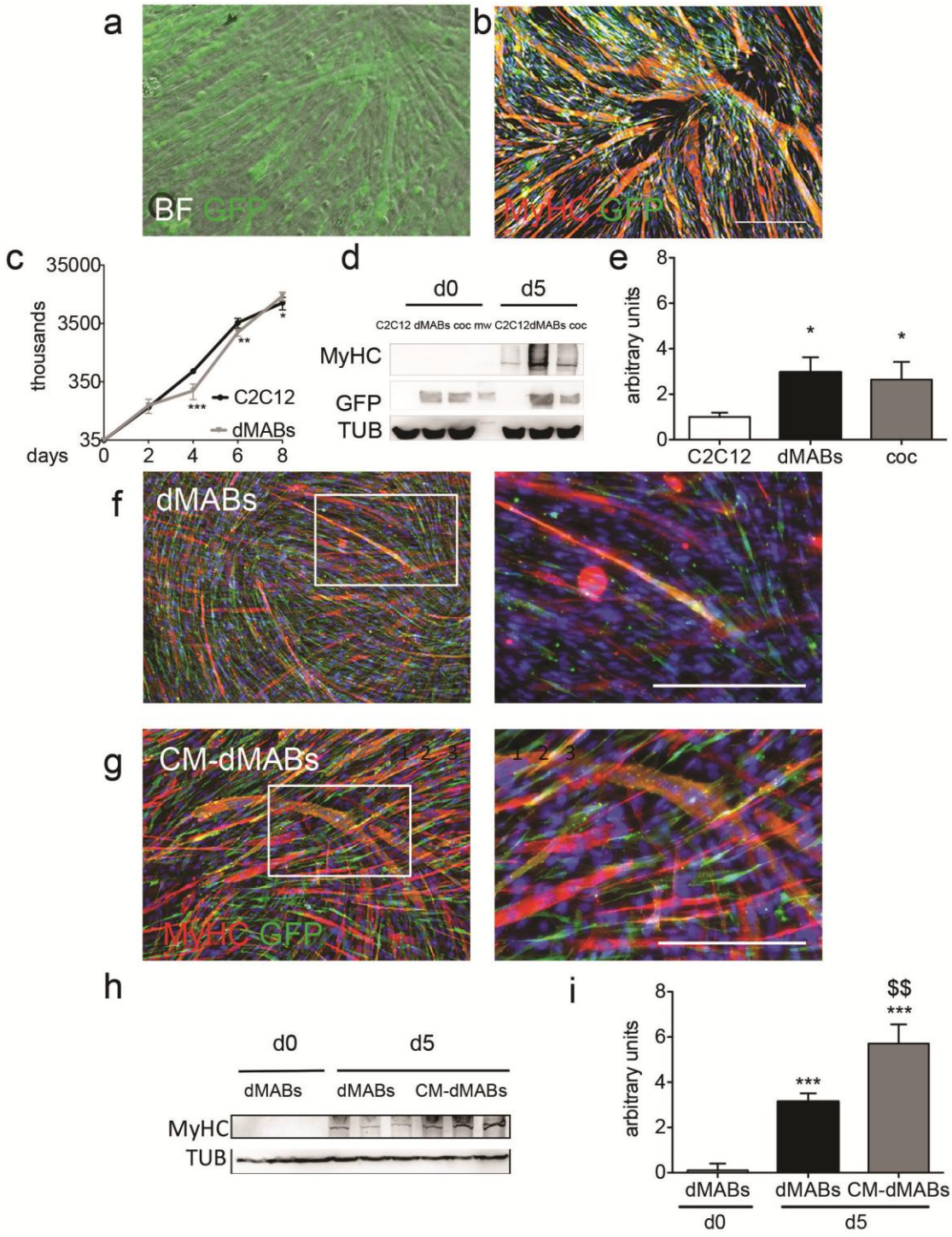
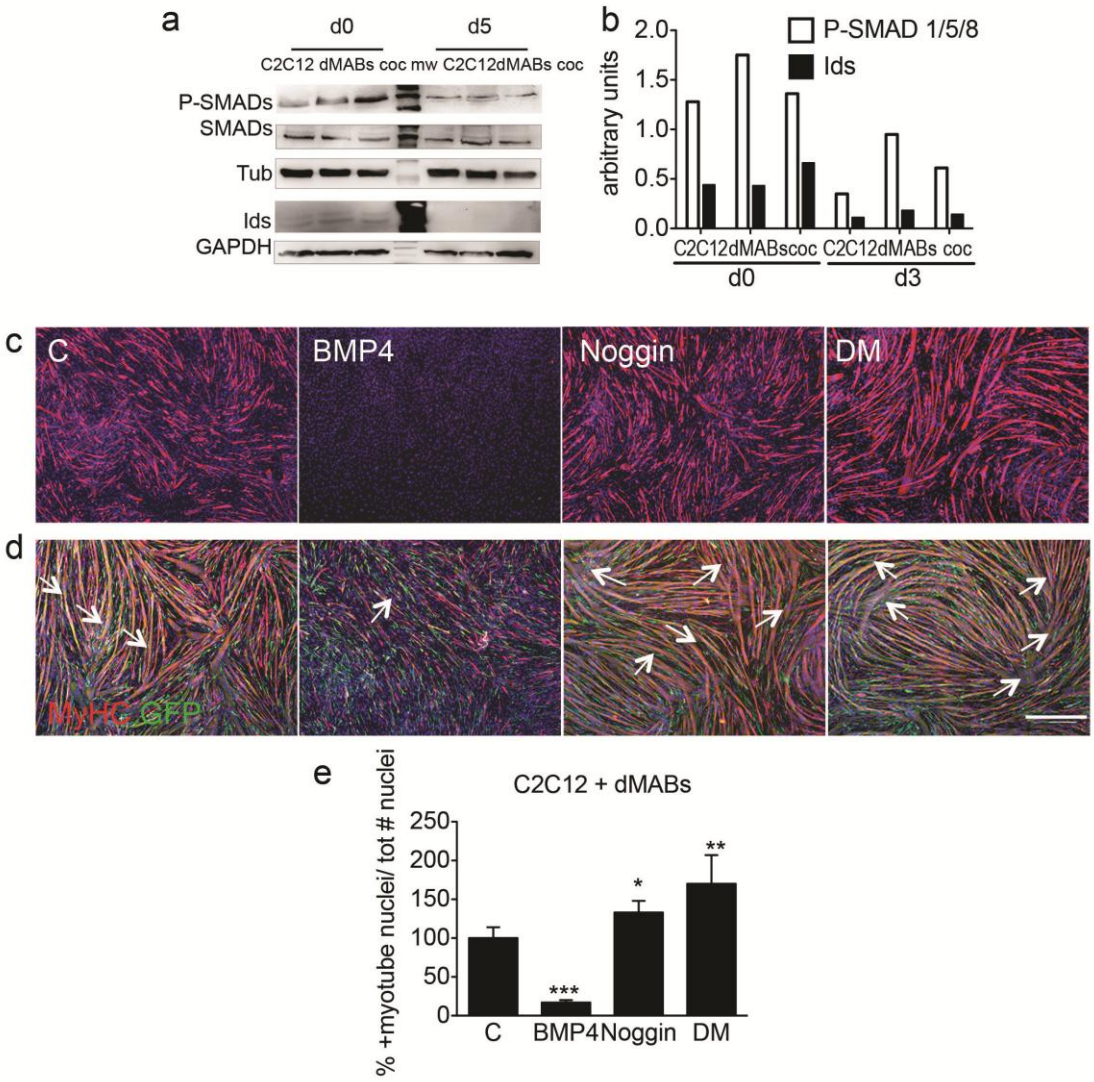
Supplementary Figures



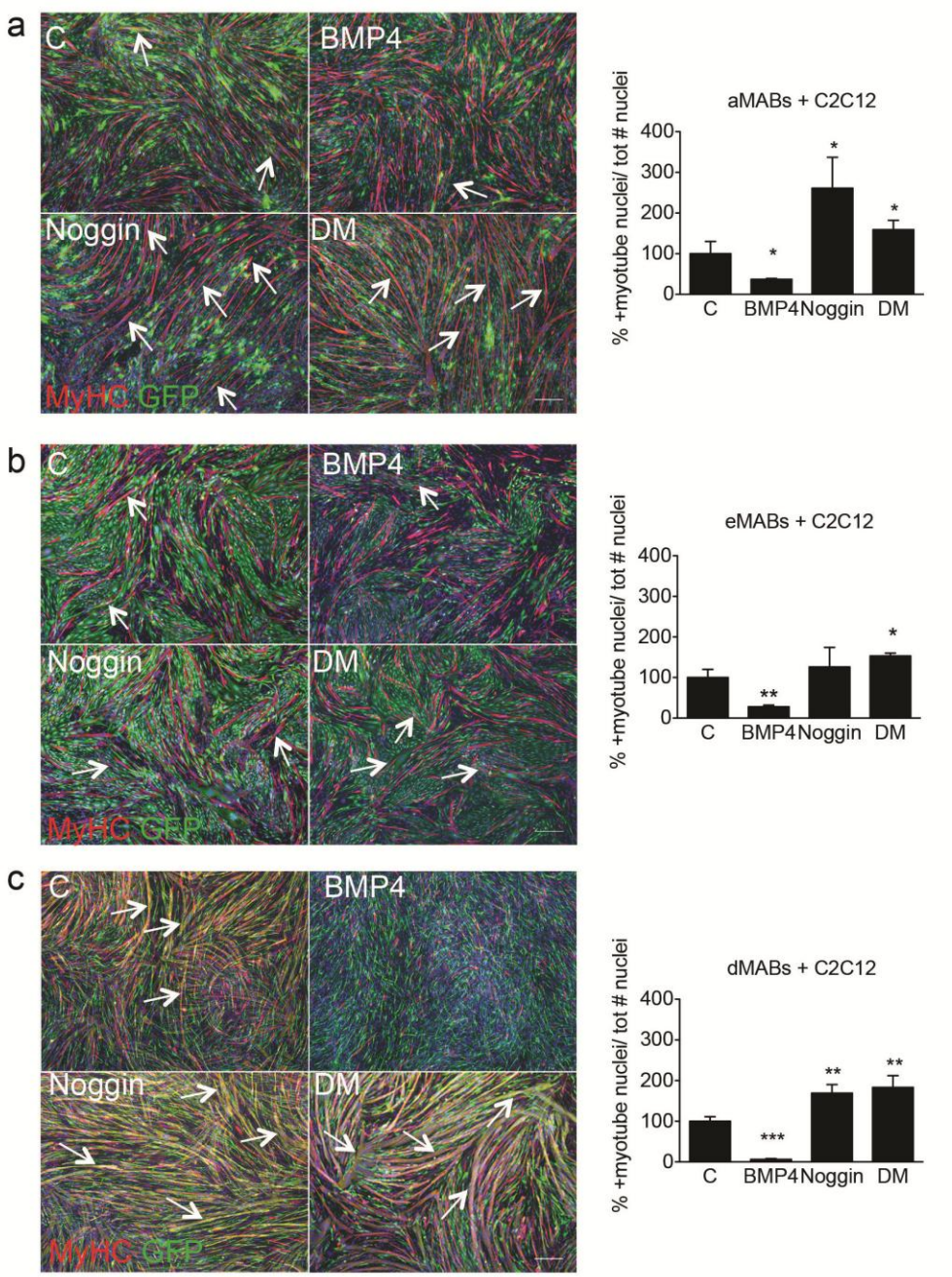
Supplementary Figure S1 Characterization of myogenic potential of dystrophic mesoangioblasts (dMABs). a) Merged images combining phase-contrast to the corresponding GFP signal of GFP^{+} dMABs co-cultured (1:1) with C2C12 in

differentiation medium at day 5 and **b**) IF for MyHC and GFP (respectively in red and green). Nuclei were stained in blue with Hoechst. **c**) Growth curves of dMAB and C2C12 cell cultures in the respective growing conditions. **d**) WB for MyHC and GFP normalized for the levels of tubulin (TUB) in C2C12, dMABs and co-cultures (1:1) of dMABs with C2C12 at day 0 and day 5 of differentiation. **e**) Quantification reporting the levels of MyHC normalized for TUB in C2C12, dMABs and co-cultures; n=5, *p<0.05 vs C2C12. Scale bars indicate 500 μm.

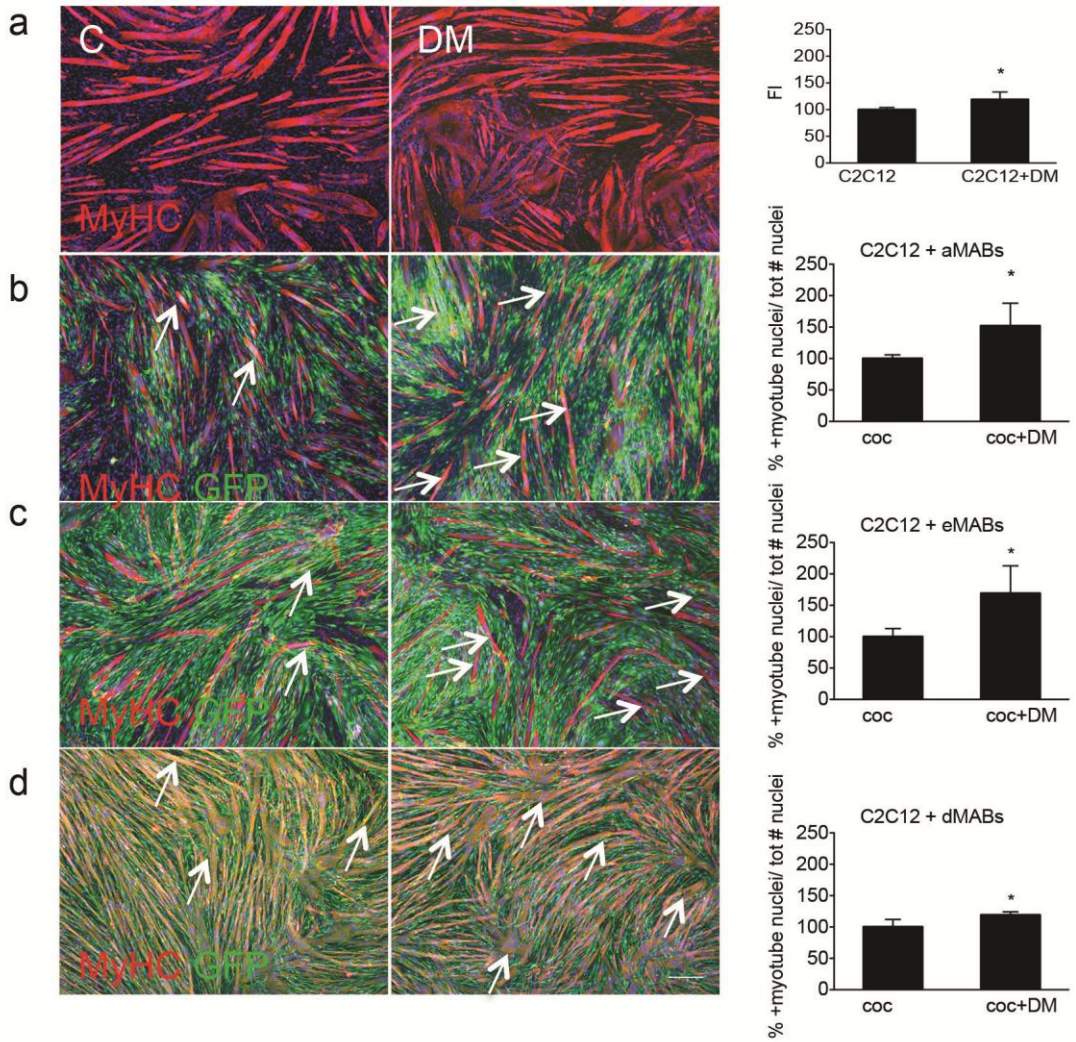
IF for MyHC and GFP (respectively in red and green) in dMABs differentiated by serum starvation (f) and exposed to C2C12 conditioned media (g). Nuclei were stained in blue with Hoechst. h) WB for MyHC normalized for the levels of tubulin (TUB) in C2C12 (1), dMABs (2) and C2C12:dMABs (1:1) co-cultures (3) at day 0 and day 5 of differentiation. **i**) Quantification reporting the levels of MyHC normalized for TUB in C2C12, dMABs and co-cultures; n=5, *p<0.05 vs C2C12.



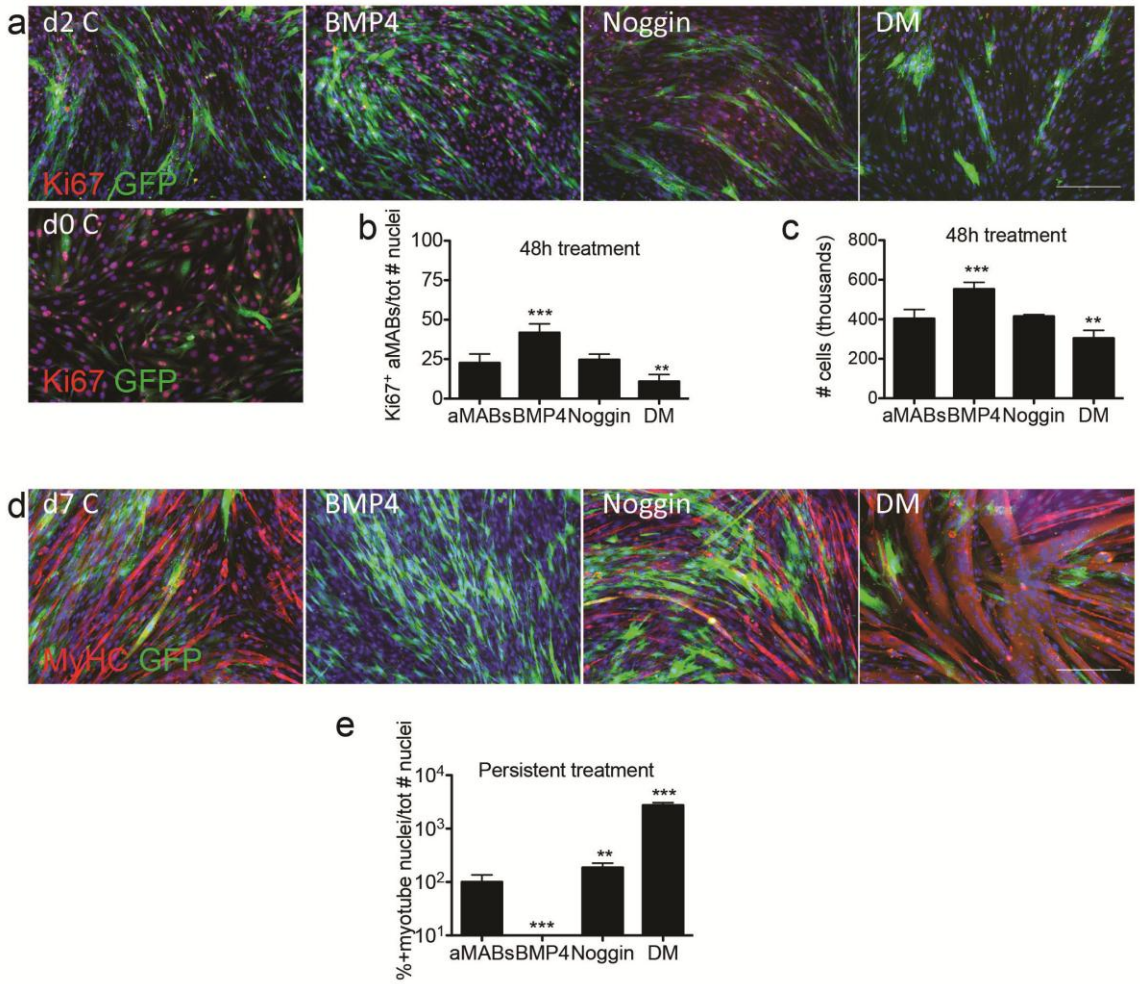
Supplementary Figure S2 Characterization of SMAD and ID proteins during myogenic differentiation of dMABs. WB analysis (**a**) and quantification (**b**) for P-SMAD1/5/8 (indicated as P-SMADs), total SMAD1/5/8 (SMADs), ID proteins normalized for GAPDH in C2C12 (1), dMABs (2) and C2C12:dMABs (1:1) co-cultures (3) at day 0 and day 5 of differentiation. IF analysis for MyHC (red) or GFP (green) of C2C12 (**c**) and GFP^+ dMABs co-cultured with C2C12 cells (**d**) pretreated with 200 ng/ml of BMP4, 100 ng/ml of Noggin or 1 μM of Dorsomorphin (DM) for 48 h and consequently subjected to myogenic differentiation for 5 days. The arrows highlight $GFP^+/MyHC^+$ double-positive myotubes. Nuclei were stained with Hoechst (blue). **e**) Fusion index of chimeric myotubes generated in dMABs co-culture experiments with different stimuli are reported as percentage with respect to control conditions. *p<0.05; **p<0.01; ***p<0.001 vs C. Kruskal Wallis test performed for the analysis scored p=0.0001. Scale bars indicate 500 μm.



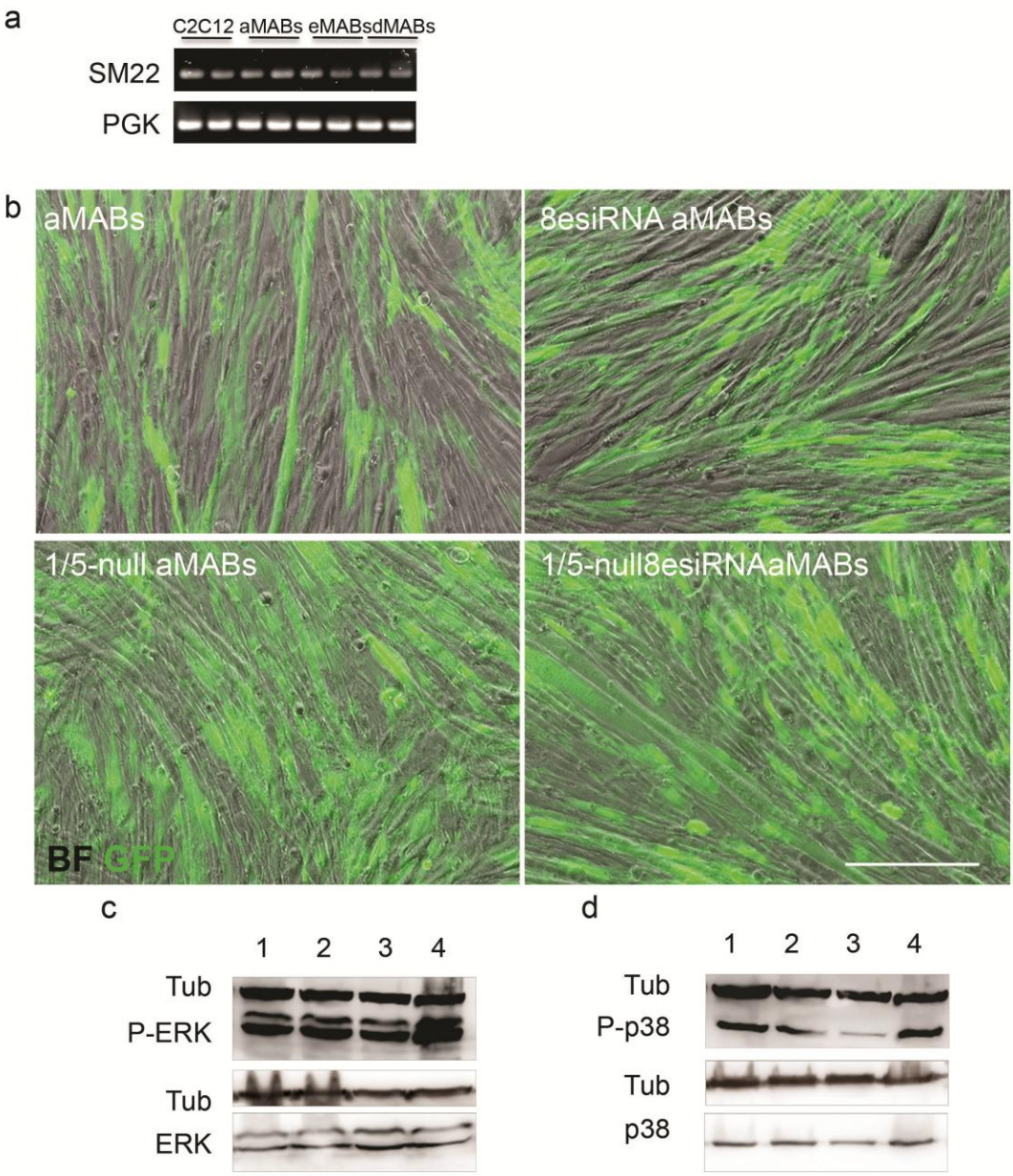
Supplementary Figure S3 MABs pre-conditioning in co-culture experiments. IF analysis for MyHC (red) and GFP (green) in aMABs (**a**) eMABs (**b**) and dMABs (**c**) pre-treated for 48 h with 200 ng/ml of BMP4, 100 ng/ml of Noggin or 1 μM of Dorsomorphin (DM) and consequently induced to differentiate with C2C12 for 5 days by serum starvation. The arrows highlight $GFP^{+}/MyHC^{+}$ double-positive myotubes. Nuclei were stained with Hoechst (blue). On the right panels are reported the fusion index of chimeric myotubes obtained in the different conditions as percentage with respect to control conditions. *p<0.05; **p<0.01; ***p<0.001 vs C. Kruskal Wallis test performed for the quantification scored p=0.0001. Scale bars indicate 500 μm.



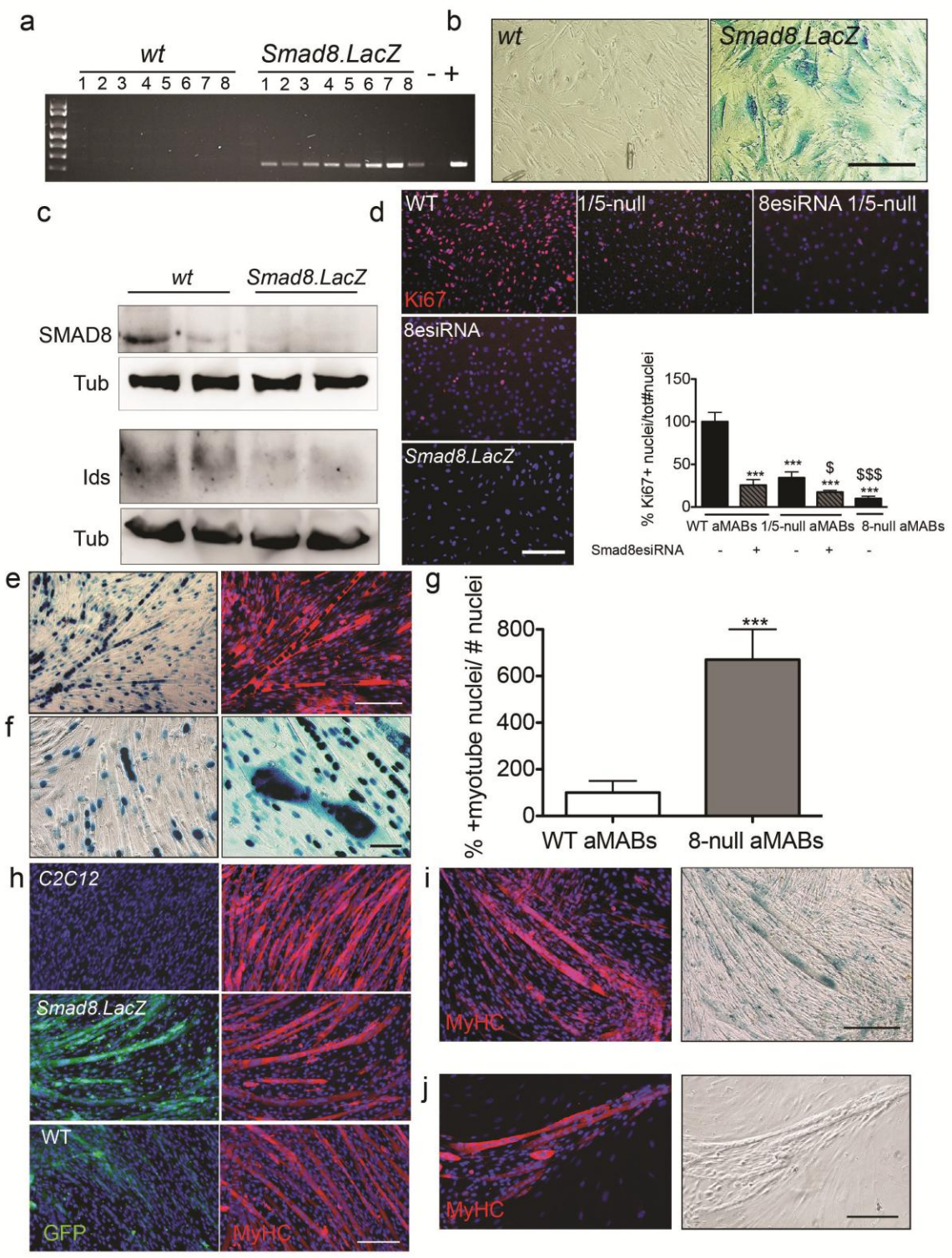
Supplementary Figure S4 Effect of treatment with DM on MABs in differentiation medium. IF analysis at day 5 for MyHC (red) and GFP (green) in C2C12 (**a**) and aMABs (**b**), eMABs (**c**) and dMABs (**d**) cocultured with C2C12 cells and treated for 48 h with 1 μM Dorsomorphin (DM) during differentiation conditions. Nuclei were stained with Hoechst (blue). In the right panels the fusion index of chimeric myotubes obtained in the different conditions is indicated as percentage with respect to control conditions. *$p<0.05$ vs untreated controls. Scale bars indicate 500 μm.



Supplementary Figure S5 Effect of transient and permanent treatments of SMAD activator and inhibitors in aMABs. **a**) IF analysis for Ki67 (red) and GFP (green) of GFP^+ aMABs in co-cultures with C2C12 cells treated for 48 h with 200 ng/ml of BMP4, 100 ng/ml of Noggin or 1 μM of Dorsomorphin (DM) respectively, and cultivated in proliferation conditions for 48 hours; staining at day 0 is shown as control. Ki67 positive cells (**b**) and cell numbers (**c**) are also reported. **d)** IF analysis for MyHC (red) and GFP (green) of GFP^+ aMABs in co-cultures with C2C12 after a permanent treatment with the same molecules in (**a**) and subjected to myogenic differentiation for 5 days. **e**) Fusion index of chimeric myotubes generated in (**d**) are reported as percentage with respect to control conditions. **p<0.01; ***p<0.001 vs controls. Kruskal Wallis test performed for both the analyses scored p=0.0001. Scale bars indicate 500 μm.

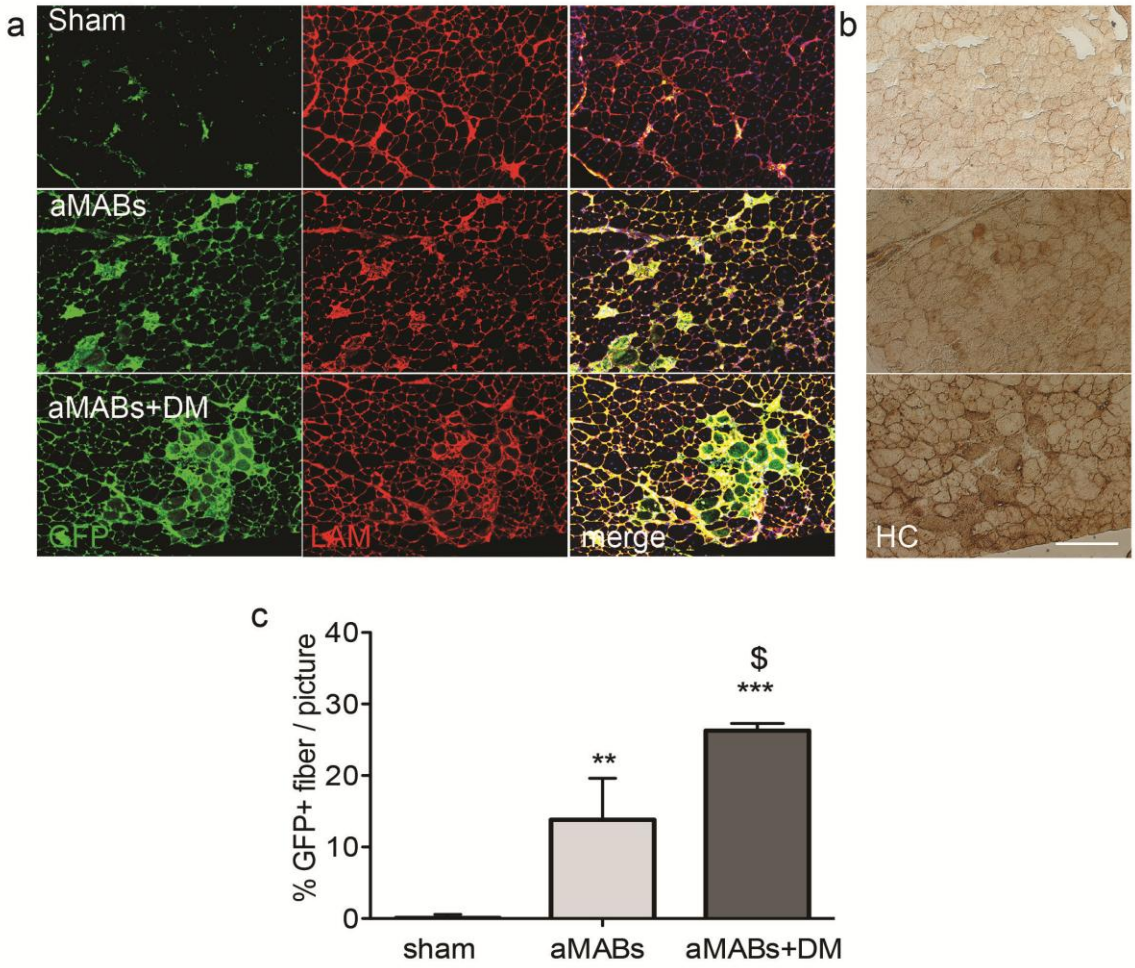


Supplementary Figure S6 Characterization of *Smad1*/5-*null* aMABs. (a) RT-PCR analysis of SM22 expression in C2C12 cells, aMABs, eMABs and MABs. Phosphoglycerate kinase 1 (PGK) was used as loading control. The GFP^+ signal (**b**) is revealed in live imaging of aMABs (upper left panel)**,** aMABs silenced for *Smad8* (upper right panel), *Smad1*/5-*null* aMABs (lower left panel) and *Smad1*/5-*null* aMABs silenced for *Smad8* (lower right panel), co-cultured (1:1) with C2C12 cells at day 5 from serum starvation. Scale bars indicate 500 μm. WB analysis of P-ERK (**c**) and and P-p38 (**d**) in aMABs (1), *Smad1/5-null* aMABs (2), and 1/5-*null* 8esiRNA aMABs (3) 8esiRNA aMABs (4). Total level of ERK and p38 as well as tubulin (TUB) are reported.

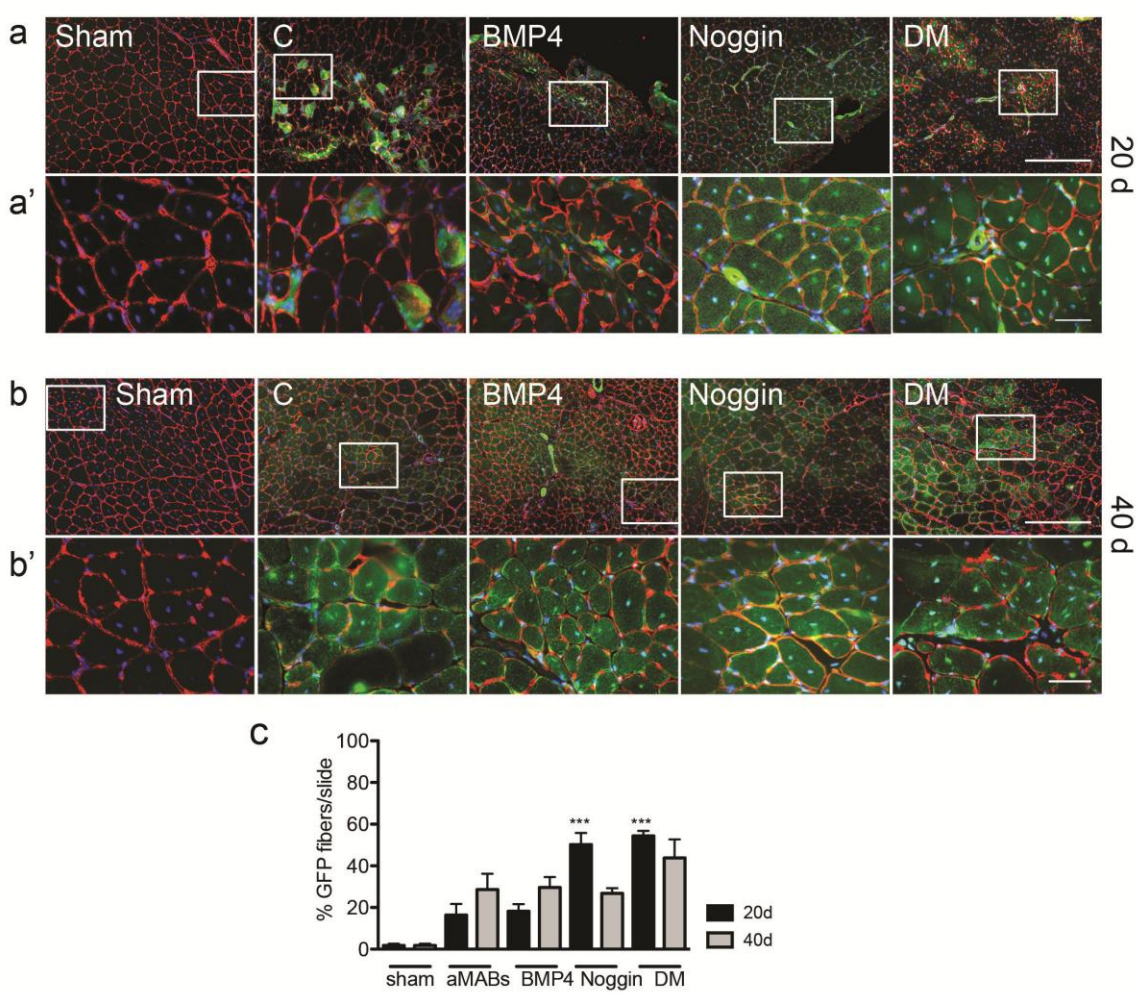


Supplementary Figure S7 Homozygous *Smad8.LacZ* aMABs characterization. PCR analysis (**a**) and X-gal staining (**b**) confirmed the presence of the active beta

galactosidase (beta-gal) gene in homozygous *Smad8.LacZ* aMABs. Homozygous *Smad8.LacZ* aMABs lack the expression of *Smad8* as shown by WB analysis (**c**). This affects the levels of Id proteins, resulting in reduced proliferation ability as shown by the diminished number of Ki67 positive cells compared to wt aMABs (**d**). *Smad8.LacZ* aMABs and wt aMABs cocultures with *nLacZ* C2C12 formed myotubes expressing MyHC under myogenic induction (**e**). However, *Smad8.LacZ* aMABs chimeric myotubes elicited a 6-fold increase in the number of nuclei as compared with controls (**f** and **g**). *Smad8.LacZ* aMABs transfected with Sleeping Beauty transposon carrying GFP showed higher number of GFP positive myoutubes compared to wt aMABs in co-culture experiments (**h**). *Smad8.LacZ* aMABs fused with C2C12 cells and chimeric myotubes expressed beta-gal and MyHC (**i**). In addition, *Smad8.LacZ* aMABs exposed to C2C12-conditioned medium were able to generate myotubes (j). Data are representative of 3 independent experiments and values are expressed as mean ± sd; ***$p<0.001$ vs controls (C); \$$p<0.05$; \$\$\$$p<0.001$ vs *Smad1/5-null* aMABs. Scale bars indicate 50 μm.



Supplementary Figure S8 SMAD-perturbed aMAB engraftment in a chronic model of muscle degeneration/regeration. (a) IF analysis for GFP (green) and laminin (LAM, in red) of *Sgca-null* muscles injected with GFP^+ aMABs (indicated as aMABs, middle panels) or GFP^+ aMABs pretreated with Dorsomorphin (indicated as aMABs+DM, lower panels) for 48 h. Upper panels showed sham treated muscles. Nuclei were stained with Hoechst. **b**) Histochemical analysis of GFP (brown) expression in injected muscles. **c**) Quantification for the GFP^+ fibers in the untreated aMAB or DM-treated aMAB injected muscles compared to sham. **$p<0.01$ vs aMABs injected mice. Scale bars indicate 500 μm.



Supplementary Figure S9 SMAD-perturbed aMAB engraftment in acute model of muscle regeneration. Examples of IF analysis (a, a', b and b') for GFP (green) and laminin (LAM, in red) of CTX-*injured* TA muscles. Mice were injected only once after the first CTX injury with GFP^+ aMABs (C) or GFP^+ aMABs transiently pre-treated for 48 h with BMP4 (BMP4), Noggin (Noggin) and Dorsomorphin (DM) or sham-operated (Sham). Magnification of areas indicated in **a** and **b** are reported in **a'** and **b'** respectively. The staining was performed after 20 days from a first CTX injury (**a**, **a'**) and 20 days after a second CTX treatment (40 days from the first CTX damage). Nuclei were stained with Hoechst. **c**) Quantification for the GFP^+ fibers in the untreated aMAB or treated aMAB injected muscles. **p<0.01 vs aMABs injected mice. Scale bars indicate 500 μm.