# Transplantation of Allogeneic PW1$^{pos}$/Pax7$^{neg}$ Interstitial Cells (PICs) Enhance Endogenous Repair of Injured Porcine Skeletal Muscle

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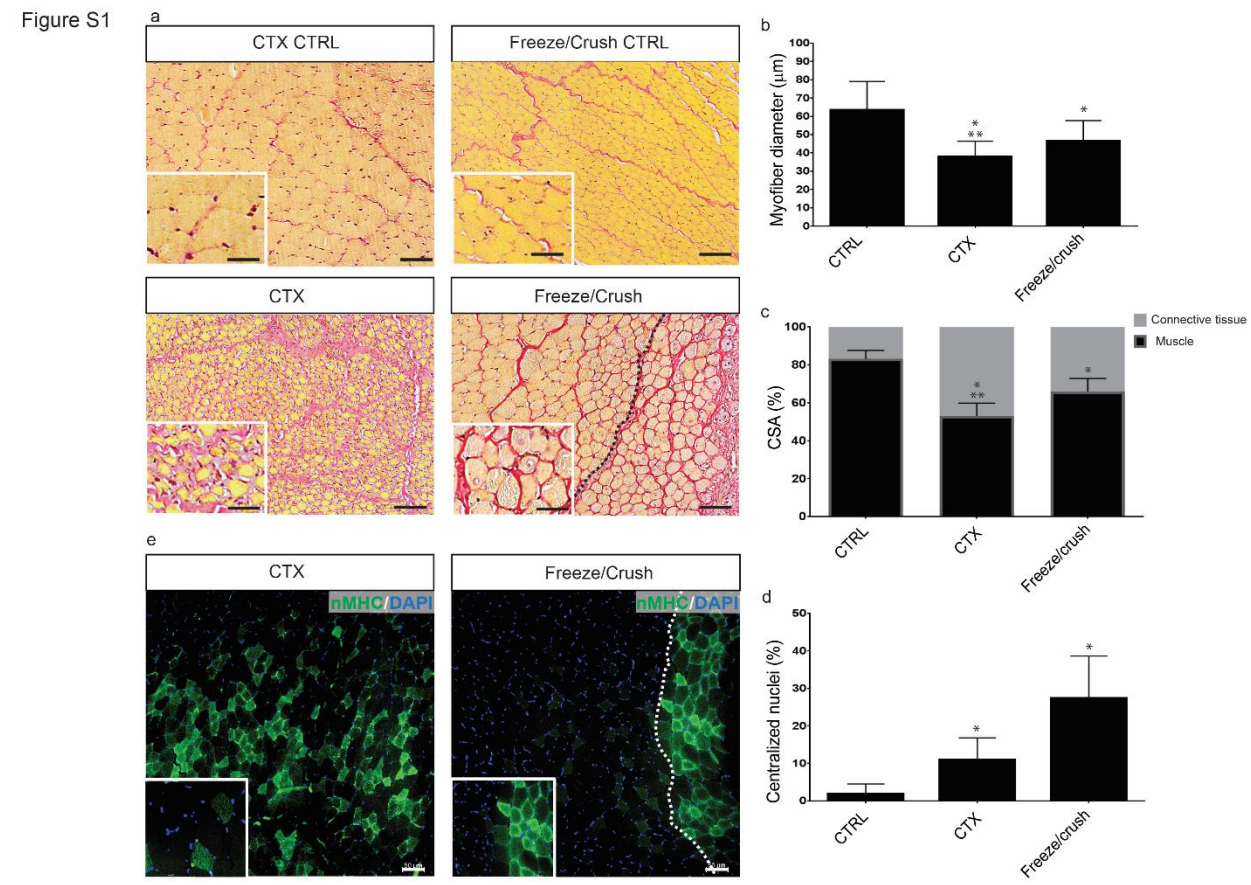


**Fig. S1.** ***Comparison of two porcine models of skeletal muscle injury.*** (**a**) HVG staining of CTRL, CTX and freeze/crush-injured paraffin-embedded muscle, showing connective tissue (red) and skeletal muscle (yellow), scale bar 100µm. Inset shows 40x magnification of centralized nuclei, scale bar 20µm. Dotted line demarcates the restricted injury area observed in freeze/crush-injured muscle only. (**b**) Myofiber diameter per 100 myofibers in CTRL, CTX and freeze/crush-injured muscle, n=2 animals per group. Data are mean ± SD, **P*<0.0001 vs. CTRL, ** *P*<0.0001 vs. freeze/crush-injured muscle. (**c**) Quantification of the ratio of skeletal muscle to connective tissue CSA of CTRL, CTX and freeze/crush-injured muscle, determined from five fields of view per muscle, n=2 animals per group. Data are mean ± SD, **P*<0.0001 vs. CTRL, ** *P*=0.018 vs. freeze/crush-injured muscle. (**d**) Number of centralized nuclei in CTRL, CTX and freeze/crush-injured muscle determined from five fields of view per muscle, n=2 animals per group. Data are mean ± SD, **P*<0.0001 vs. CTRL (**e**) Immunohistochemical staining indicates a diffuse pattern of nMHC expression in CTX-injured muscle compared to restricted expression in freeze/crush-injured muscle outlined by the white dotted line, scale bar 50µm.

Figure S2

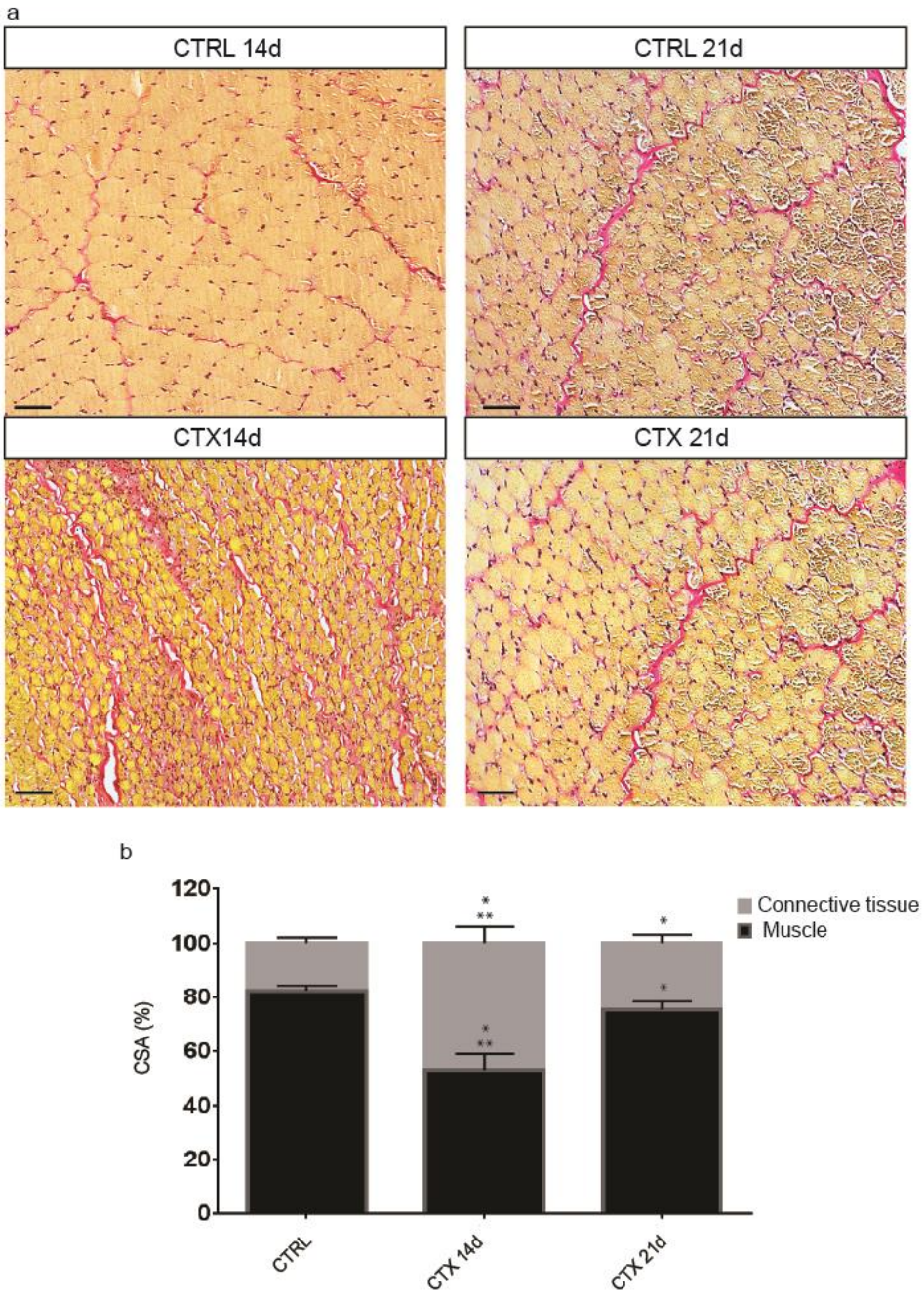


**Fig. S2.** ***CTX-injured muscle undergoes significant regeneration 21 days post-injury.*** (**a**) Representative image of HVG stained CTX-injured paraffin-embedded muscle 21 days post-injury compared to 14 days post-CTX injury, scale bar 100µm. (**b**) Quantification of the ratio of skeletal muscle to connective tissue CSA in CTRL, CTX: 14 days and CTX: 21 days post-injury determined from five fields of view per muscle, n=2 animals per group. Data are mean ± SD, **P*<0.01 vs. CTRL, ***P*<0.0001 vs. CTX: 21 days post-injury, n=2.

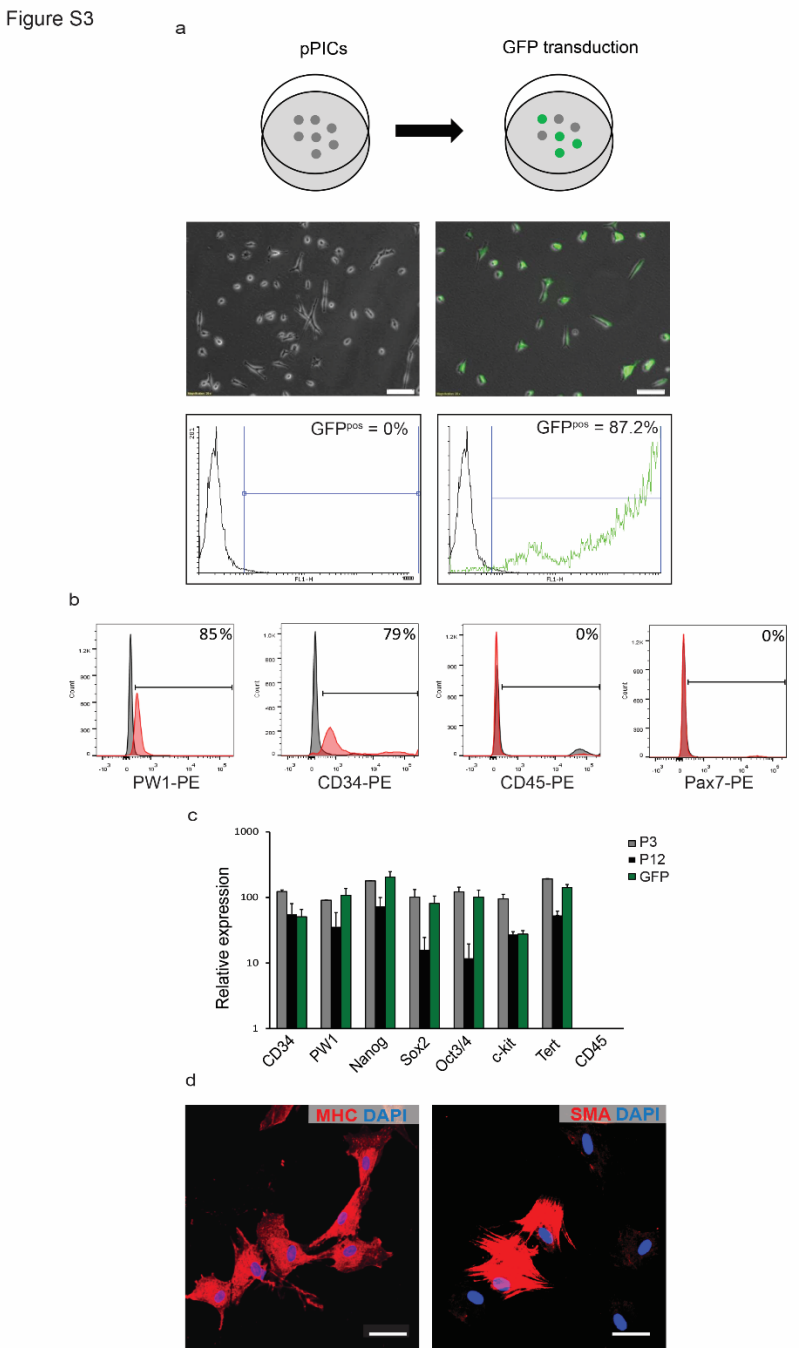


**Fig. S3.** ***GFP$^{pos}$ pPICs retain their phenotype and myogenic differentiation potential.*** (**a**) Porcine PICs were transduced with a GFP lentiviral construct at an efficiency of 87% as determined by flow cytometric analysis. (**b**) Flow cytometry confirmed GFP$^{pos}$ pPICs expressed PW1 and CD34 while being negative for CD45 and Pax7. (**c**) qRT-PCR profile of GFP$^{pos}$ pPICs compared to unlabelled pPICs confirms maintenance of pPIC phenotype. (**d**) Porcine PICs were subjected to differentiation media and display bi-potent muscle differentiation potential as demonstrated by expression of MHC and SMA (both red). DAPI stain nuclei in blue. Scale bar 50μm.

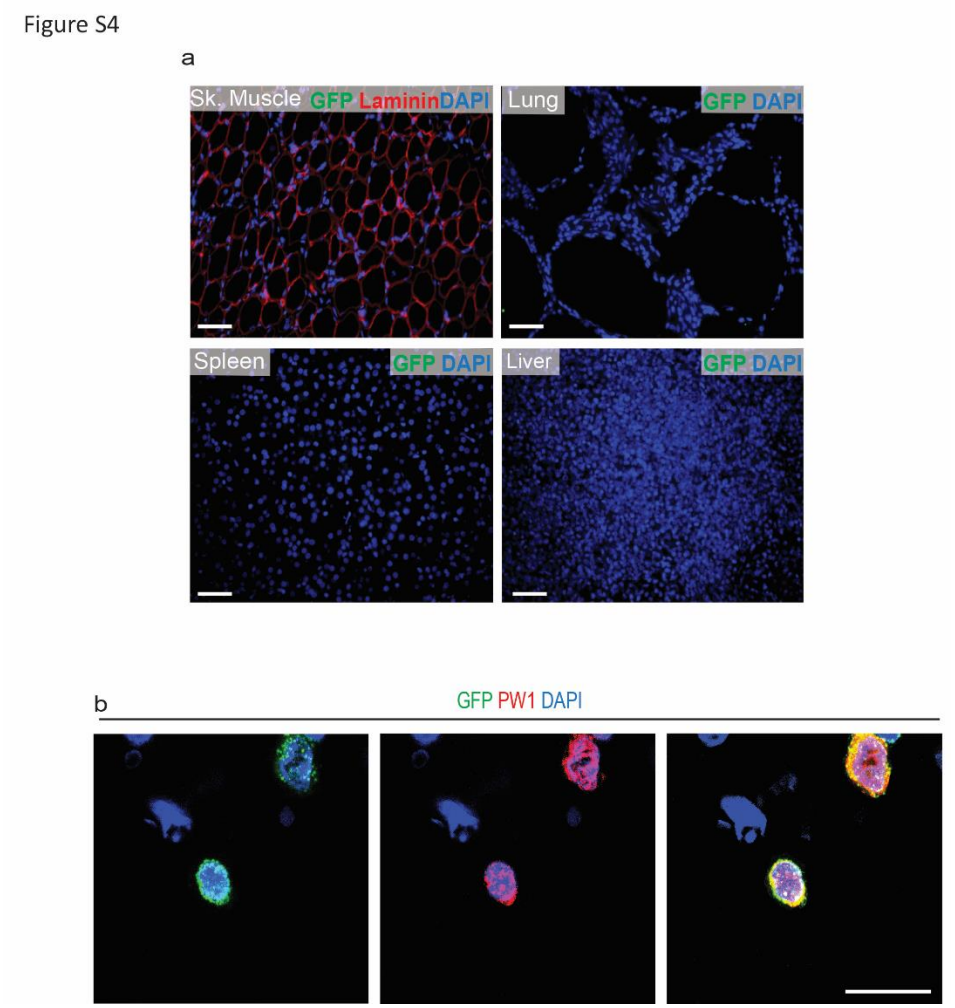


**Fig. S4.** ***Evaluation of GFP$^{pos}$ pPIC distribution in host tissues***. (**a**) No donor GFP$^{pos}$ PICs were identified in PBS-CTRL skeletal muscle, or the lung, spleen or liver examined from animals transplanted with allogeneic GFP$^{pos}$ pPICs 14 days post-transplantation, scale bar 50µm. (**b**) Co-expression of GFP and PW1 confirmed that the interstitial nuclei were donor GFP$^{pos}$ pPICs, scale bar 10µm. DAPI stain nuclei in blue.

Figure S5

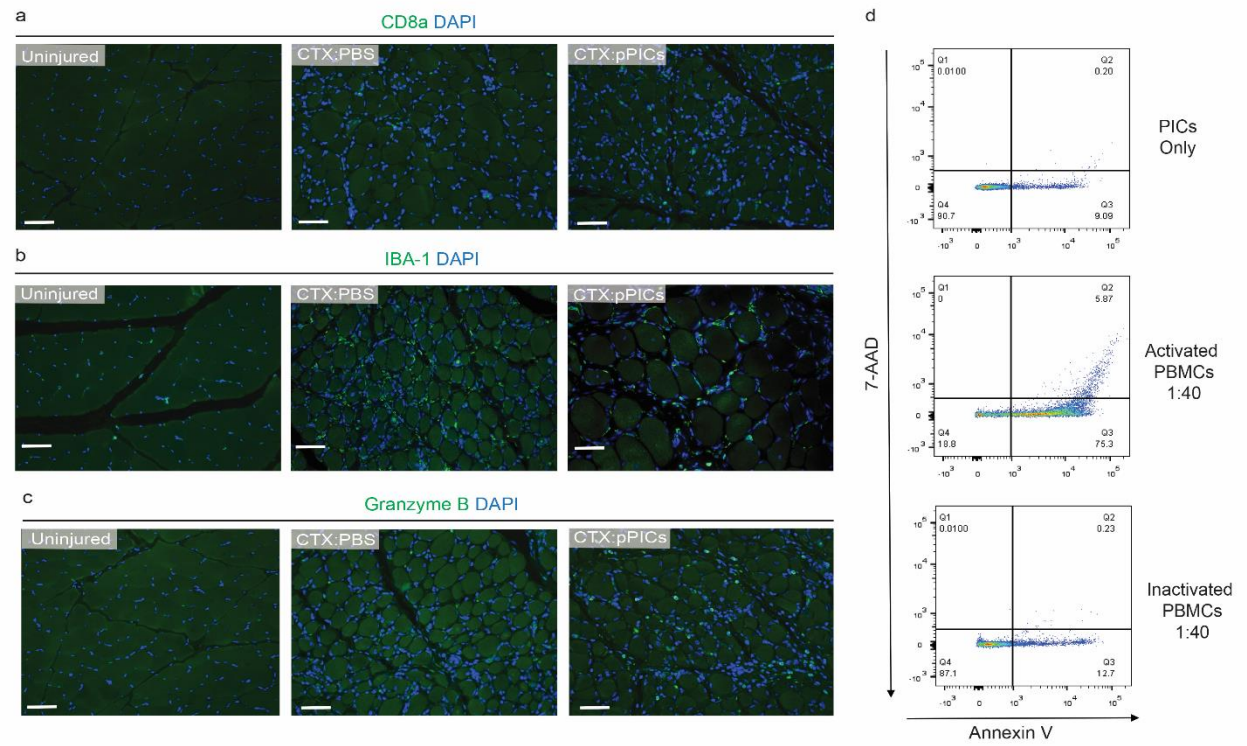


**Fig. S5.** ***Allogeneic pPICs are cleared by immune cells*** (**a**) Representative micrographs of skeletal muscle sections stained for (**a**) the cytotoxic T-lymphocyte marker, CD8a, (**b**) the macrophage marker, IBA-1, (**c**) the NK cell and lymphocyte marker, Granzyme B at 14 days post-injury, scale bar 50µm. (**d**) Representative Annexin V/7-AAD flow cytometry dot plots of pPICs only, co-culture of pPICs with activated porcine PBMCs, and co-culture of pPICs with inactivated porcine PBMCs. Apoptosis was measured by 7-AAD and Annexin V.

Figure S6

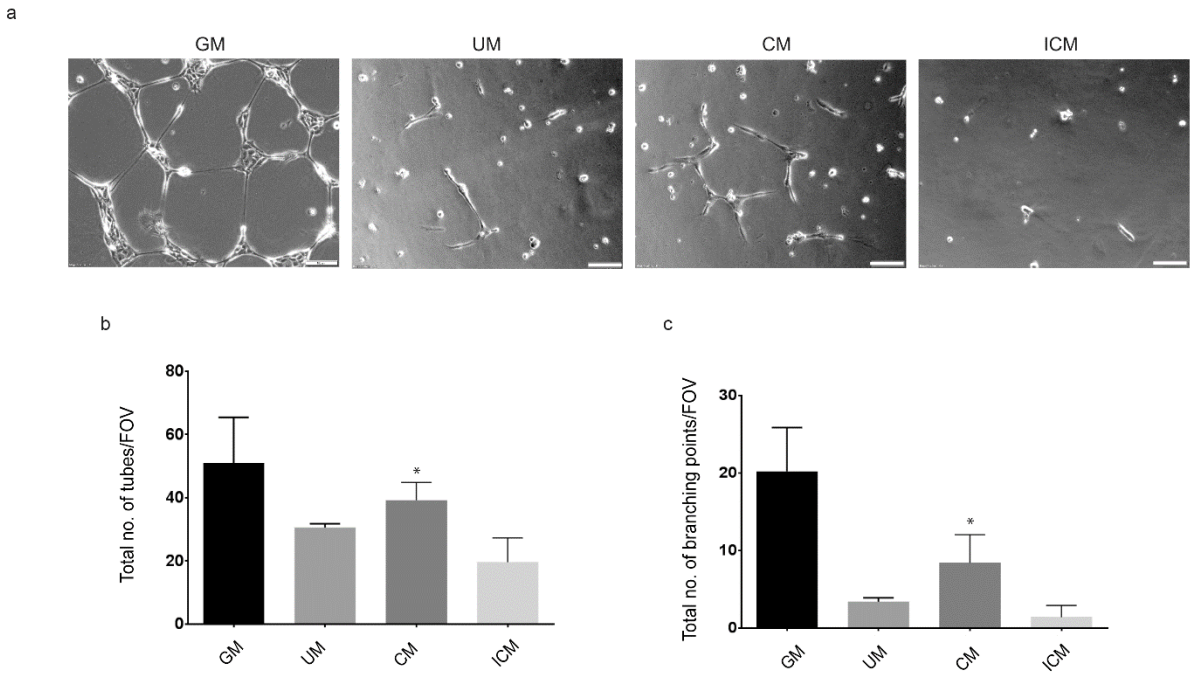


**Fig. S6.** ***pPIC conditioned media promotes in vitro angiogenesis.*** (**a**) Representative micrographs of HUVECs subjected to a 24h angiogenesis assay supplemented with endothelial growth media (GM), unconditioned media (UM), 24h pPIC conditioned media (CM) or heat inactivated conditioned media (ICM), scale bar 100µm. Endothelial network formation was quantified based on parameters including number of capillaries per field of view (**b**), and total number of branching points per field of view (**c**), which were found to be significantly increased ($p<0.05$) where HUVECs were exposed to 24h pPIC conditioned media compared to heat-inactivated conditioned media.

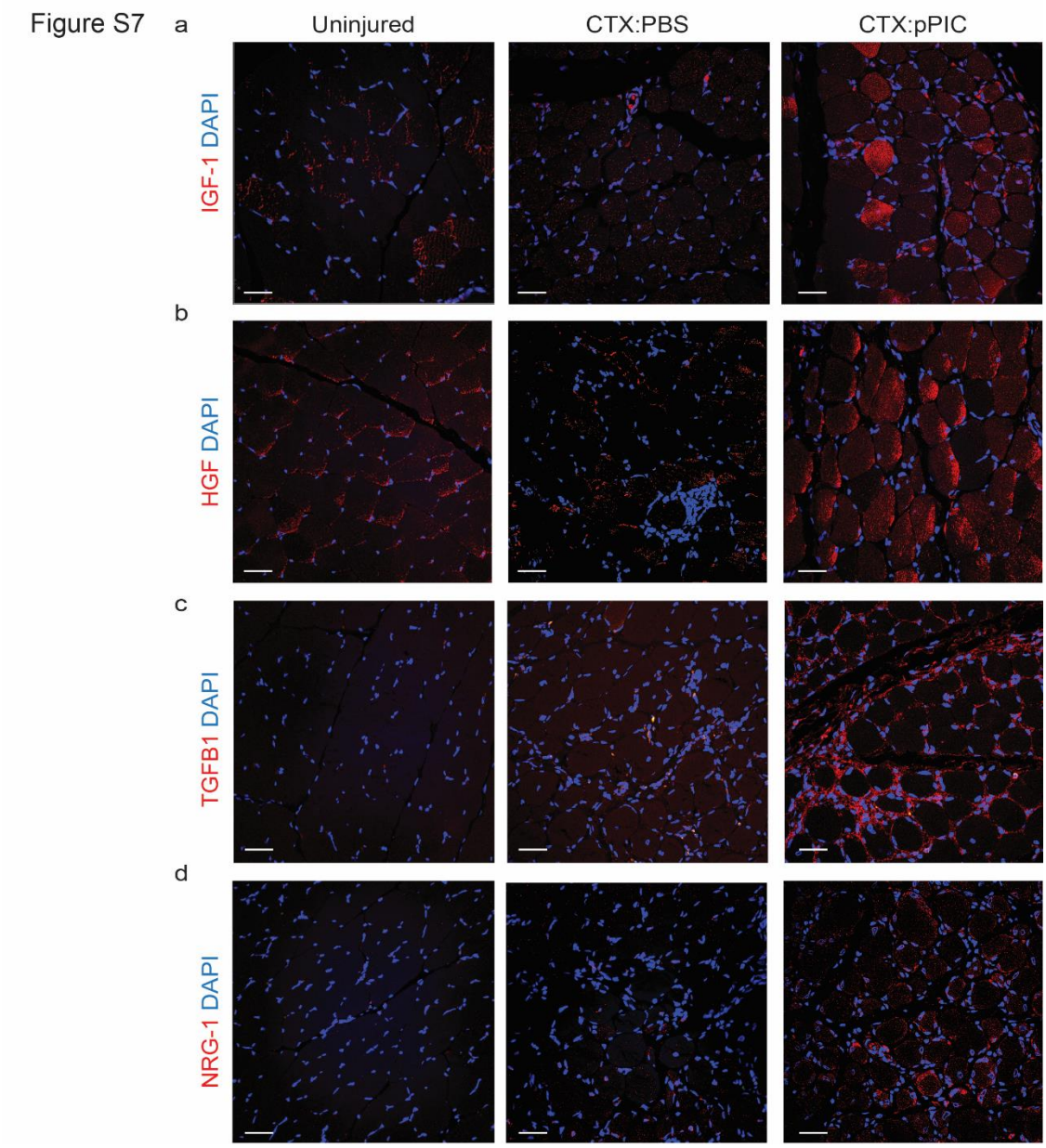


**Fig. S7.** ***Evaluation of growth factor expression in pPIC-treated muscle***. The growth factors IGF-1, HGF, TGFβ1, NRG-1, were screened in uninjured, CTX:PBS and CTX:pPICs-treated muscle at 14 days post-injury, n=5 animals per group. Scale bar 50μm.

**Table S1.** Volcano plot dataset listing all genes that were significantly upregulated or downregulated by pPICs, which had undergone 24h of myogenic differentiation compared to undifferentiated pPICs.

| Genes Upregulated in Differentiated vs. Undifferentiated pPICs | | |
|---|---|---|
| **Gene Symbol** | **Fold Regulation** | **p-value** |
| INHBA | 22.284 | 0.000488 |
| SPP1 | 8.8333 | 0.0123 |
| IL8 | 5.3279 | 0.001386 |
| BMP4 | 5.2978 | 0.02984 |
| IGF1 | 4.9029 | 0.120844 |
| POSTN | 3.8978 | 0.001753 |
| TGFB2 | 3.7774 | 0.007102 |
| BDNF | 3.3156 | 0.007734 |
| CCL2 | 3.0488 | 0.081075 |
| FGF9 | 2.7595 | 0.092373 |
| VEGFd | 2.7526 | 0.010973 |
| GDF15 | 2.7265 | 0.036191 |
| AFGF | 2.6136 | 0.11545 |
| NTF3 | 2.3613 | 0.155942 |
| IGF2 | 2.3254 | 0.141795 |
| IL1A | 2.3105 | 0.138098 |
| PDGFRc | 2.134 | 0.020948 |
| INHBB | 2.0393 | 0.155071 |
| Neuregulin 1 | 2.0185 | 0.045671 |
| **Genes Downregulated in Differentiated vs. Undifferentiated pPICs** | | |
| **Gene Symbol** | **Fold Regulation** | **p-value** |
| IL6 | -10.5331 | 0.013034 |
| PGF | -3.9006 | 0.067643 |
| GPI | -3.45 | 0.026743 |
| HGF | -3.1783 | 0.077588 |
| NOGGIN | -3.0586 | 0.140538 |
| BMP1 | -2.7257 | 0.35925 |
| LIF | -2.2785 | 0.277737 |
| SCF | -2.0049 | 0.067705 |