Title: WNT3A promotes myogenesis of human embryonic stem cells and enhances *in vivo* engraftment

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Supplementary Information

Figures S1-S6

Table S1

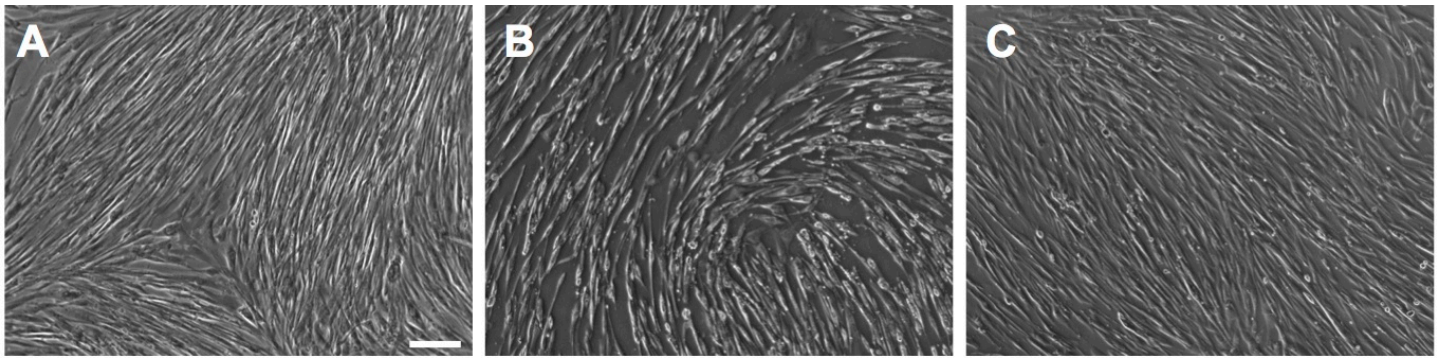

Figure S1. Characterization of cellular morphology of PDGFRA$^+$ cells. Phase contrast images of PDGFRA$^+$ cells undergoing myogenic differentiation in induction medium only **(A)**, L-cell-conditioned induction medium **(B)**, and WNT3A-conditioned induction medium **(C)** for 7 days. Scale bar = 200 μm.

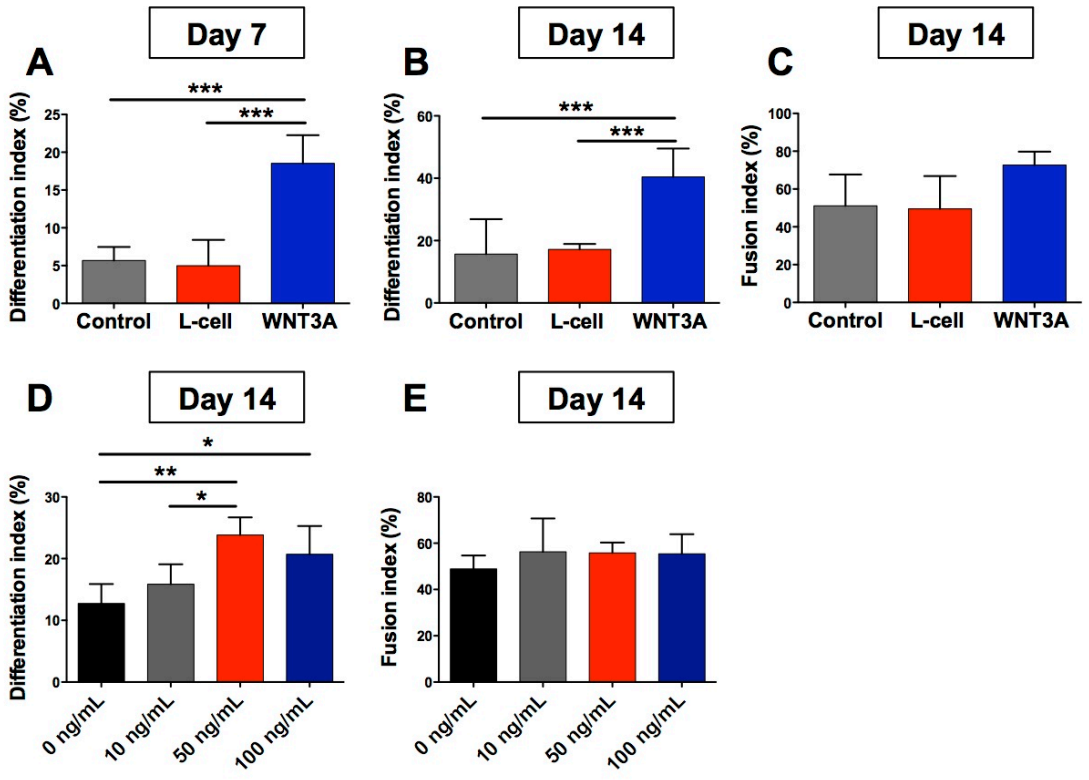

Figure S2. Image analysis demonstrating differentiation and fusion indices of PDGFRA$^+$ cells undergoing myogenic differentiation. Estimated differentiation indices of PDGFRA$^+$ cells undergoing myogenic differentiation in induction medium only (grey), L-cell-conditioned (red), and WNT3A-conditioned (blue) induction media for 7 days **(A)** and 14 days **(B)**. n = 315, 398, and 192 (for day 7) and n = 642, 360, and 923 (for day 14), respectively. **(C)** Estimated fusion indices of differentiated PDGFRA$^+$ cells cultured in induction medium only (grey), L-cell conditioned (red), and WNT3A-conditioned (blue) induction media for 14 days (MF20 positive cells having >3 nuclei). n = 642, 360, and 923, respectively. **(D)** Estimated differentiation indices of PDGFRA$^+$ cells undergoing myogenic differentiation in induction medium only, and medium supplemented with varying amount of recombinant human WNT3A protein for 14 days. n = 1701, 1220, 1069, and 881, respectively. **(E)** Estimated fusion indices of differentiated PDGFRA$^+$ cells cultured in induction medium only, and medium supplemented with varying amount of recombinant human WNT3A protein for 14 days. (MF20 positive cells having >3 nuclei). n = 1701, 1220, 1069, and 881, respectively. *p<0.05, **p<0.01, and ***p<0.001.

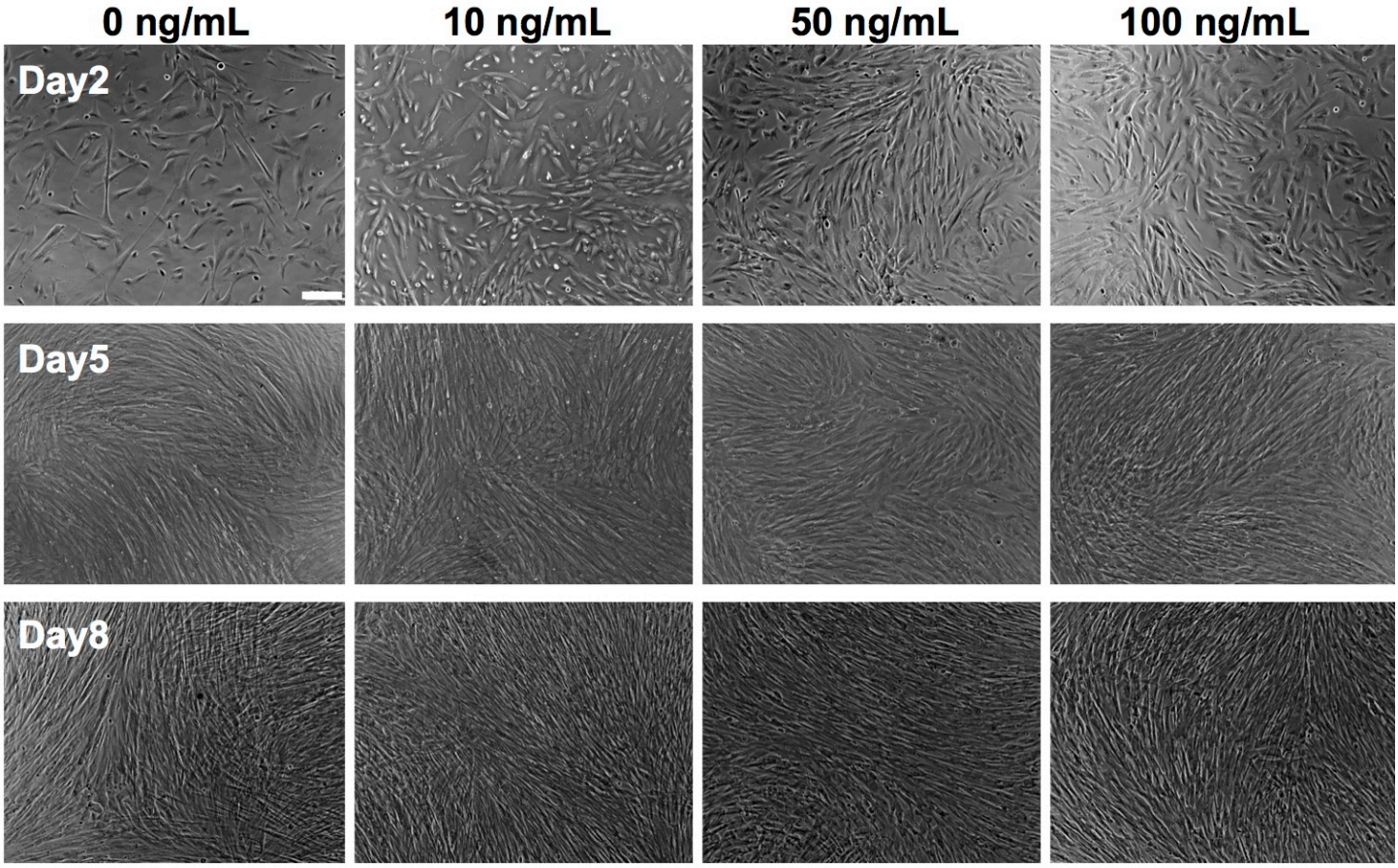

Figure S3. Characterization of cellular morphology of PDGFRA$^+$ cells cultured in the presence of recombinant human WNT3A protein. Phase contrast images of PDGFRA$^+$ cells undergoing myogenic differentiation in induction medium supplemented with 0, 10, 50, and 100 ng/mL of recombinant human WNT3A protein as a function of time. Scale bar = 200 μm.

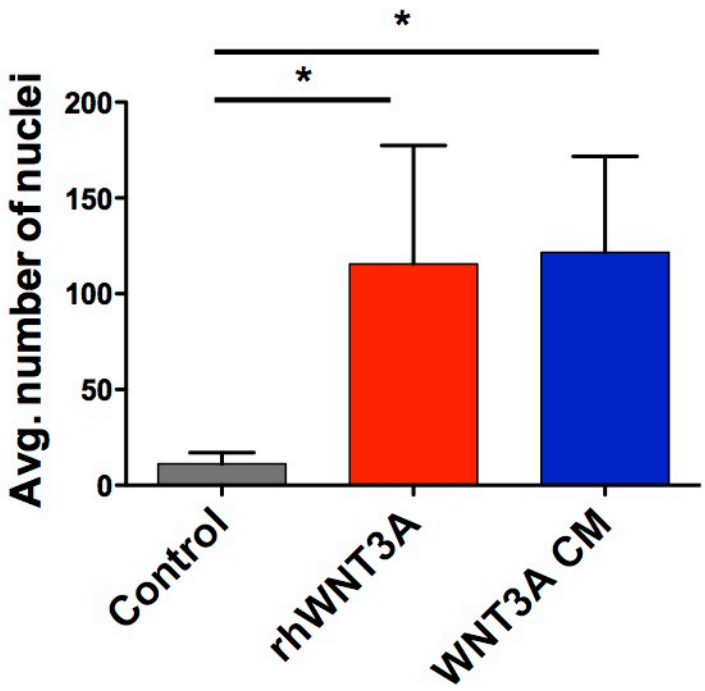

Figure S4. Quantification of engraftment efficiency of PDGFRA^{+} cells. Engraftment efficiency of transplanted cells cultured in induction medium only (grey), induction medium supplemented with 50 ng/mL of recombinant human WNT3A protein (red), and WNT3A-conditioned induction medium (blue) for 14 days *in vitro* prior to the transplantation was represented as the average number of human lamin A/C^{+} cells per each sections.

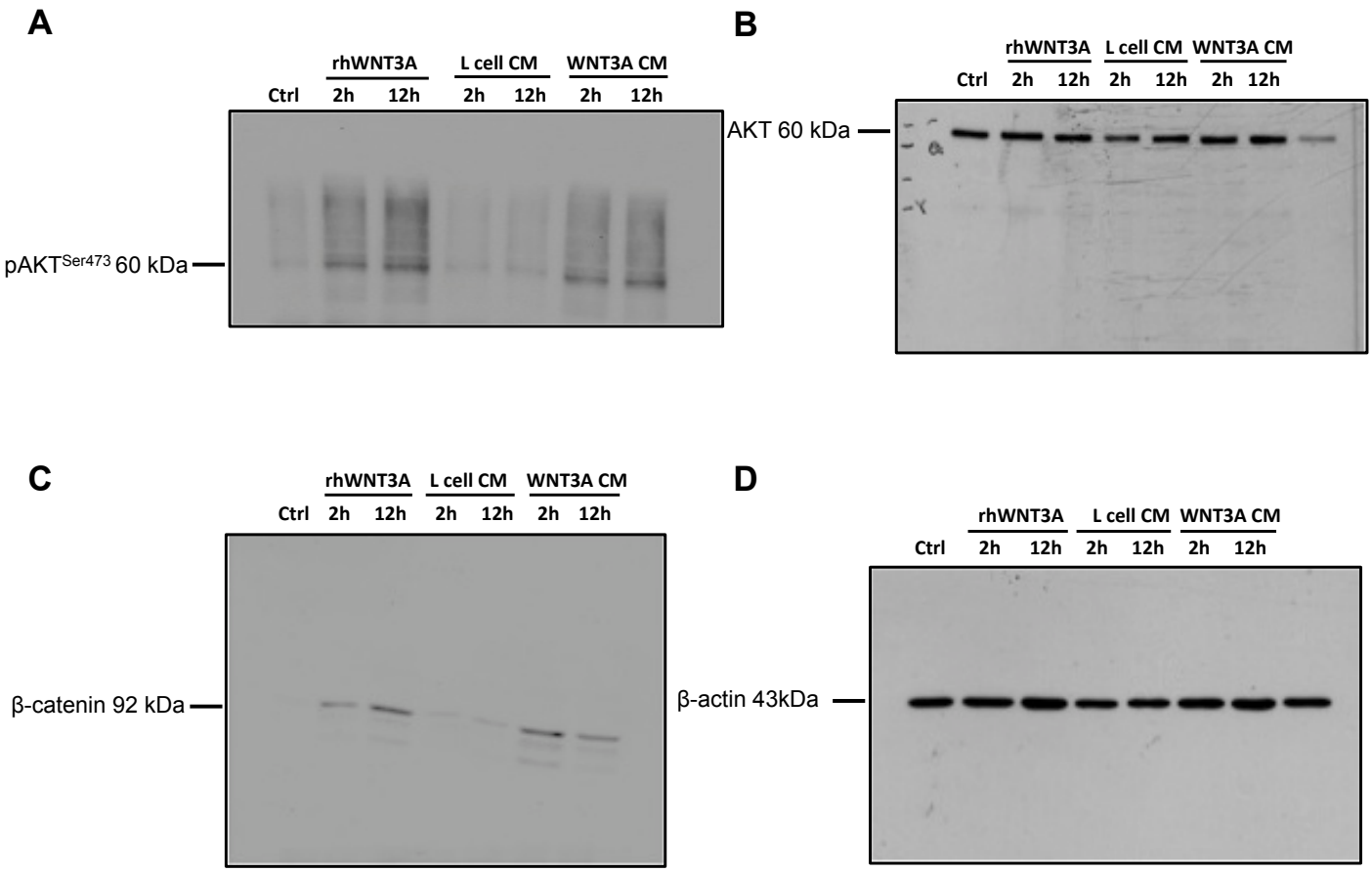

Figure S5. Full-length Western blot images for Figure 4B and 5D. (A) PhosphoAKT^{Ser473} of WNT3A-conditioned induction medium (WNT3A CM) treated (Fig. 4B) and recombinant human WNT3A (rhWNT3A) protein-treated (Fig. 5D) cells. (B) Total AKT of WNT3A CM-treated (Fig. 4B) and rhWNT3A-treated (Fig. 5D) cells. (C) Active β-catenin of WNT3A CM-treated (Fig. 4B) and rhWNT3A protein-treated (Fig. 5D) cells. (D) β-actin of WNT3A CM-treated (Fig. 4B) and rhWNT3A protein-treated (Fig. 5D) cells. Ctrl: Control for rhWNT3A. L cell CM: Control for WNT3A CM.

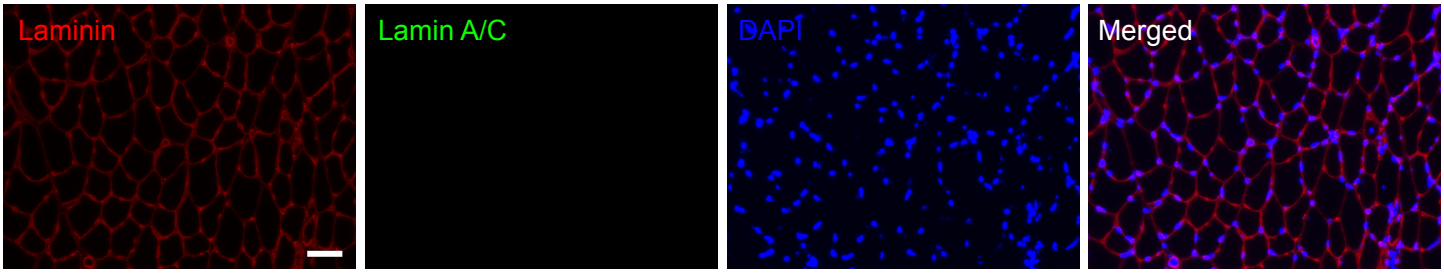

Figure S6. Immunofluorescence staining for human-specific lamin A/C in negative control. TA muscle section from NOD/SCID mice injected with the same volume of PBS devoid of any cells was stained with lamin A/C to determine its specificity. Scale bar = 20 μm.

Gene	Primer Sequence (5' to 3')
PAX3	F-TAC AGG TCT GGT TTA GCA AC
	R-GATCTGACACAGCTTGTGGA
PAX7	F-ACC CCT GCC TAA CCA CAT C
	R-GCG GCA AAG AAT CTT GGA GAC
MYF5	F-TTC TCC CCA TCC CTC TCG CT
	R-AGC CTG GTT GAC CTT CTT CAG
MYOD	F-AGC ACT ACA GCG GCG ACT C
	R-TAG TAG GCG CCT TCG TAG CA
DESMIN	F-GAA GCT GCT GGA GGG AGA G
	R-ATG GAC CTC AGA ACC CCT TT
MYOG	F-CAG CTC CCT CAA CCA GGA G
	R-GCT GTG AGA GCT GCA TTC G
MYH1	F-TCT TGG ACA TTG CTG GCT TT
	R-TCC ACT CAA TGC CTT CCT TC
CD34	F-TTT GCT TGC TGA GTT TGC TG
	R-ATT TGA AAA TGT TCC CTG GGT
FLK1	F-CCT GTA TGG AGG AGG AGG AA
	R-CGG CTC TTT CGC TTA CTG TT
CD56	F-TGG CTG GGA ACA ATA TCC AC
	R-AGC CAG CAG ATT ACA ATG C
WNT3A	F-CTG CCA GGA GTG TAT TCG CAT C
	R-GAG AGC CTC CCC GTC CAC AG
CCND1	F-GCC GTC CAT GCG GAA GAT
	R-CCT CCT CCT CGC ACT TCT GT
AXIN2	F-GCG ATC CTG TTA ATC CTT ATC AC
	R-AAT TCC ATC TAC ACT GCT GTC
18s	F-CCC TGT AAT TGG AAT GAG TCC ACT T
	R-ACG CTA TTG GAG CTG GAA TTA C

Table S1. List of primers used for quantitative PCR.