Supplementary Information

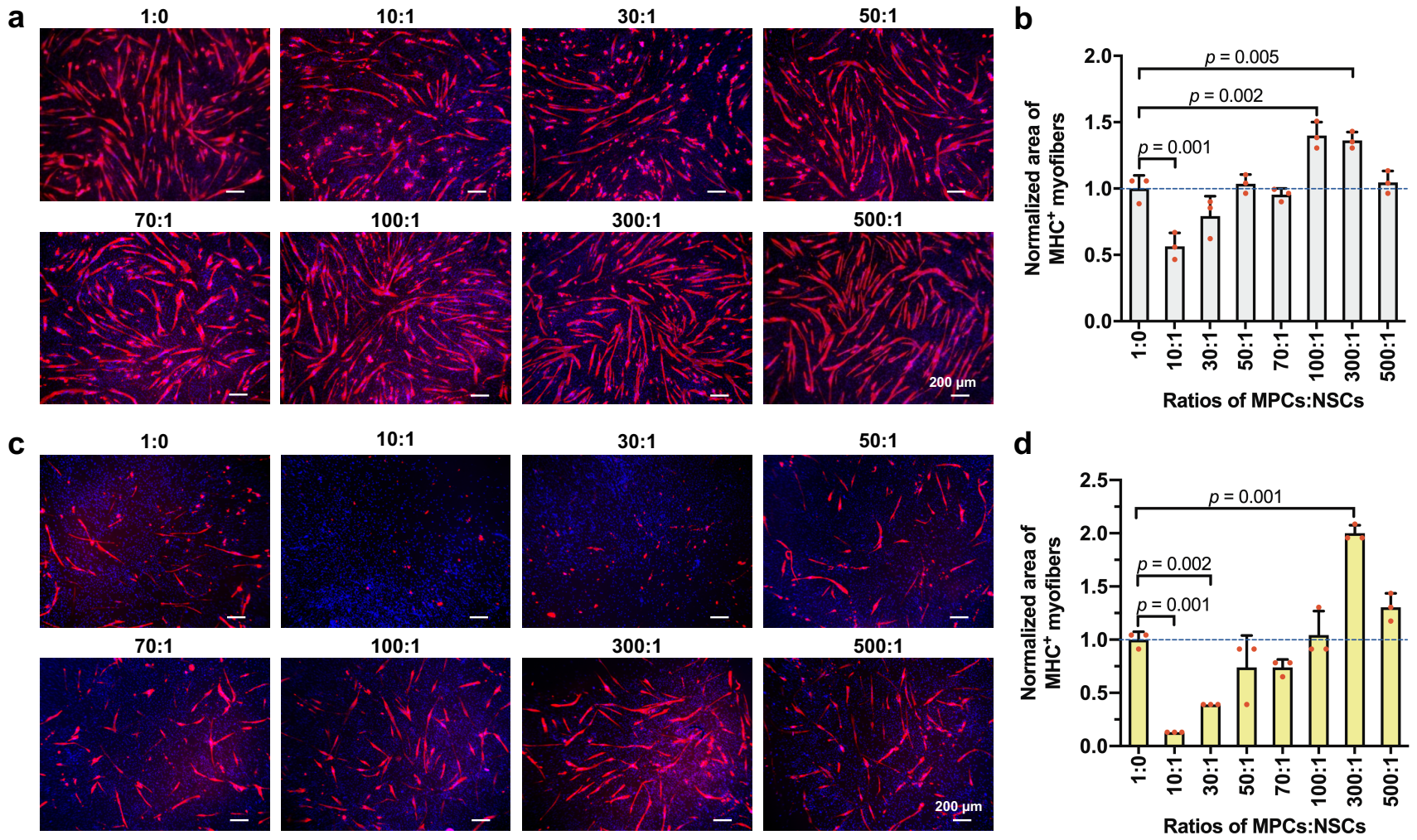
Neural Cell Integration into 3D Bioprinted Skeletal Muscle Constructs Accelerates Restoration of Muscle Function

Kim et al.

Supplementary Table 1. Quantification of human growth factors and cytokines detected in the hMPC- and hNSC-conditioned media.

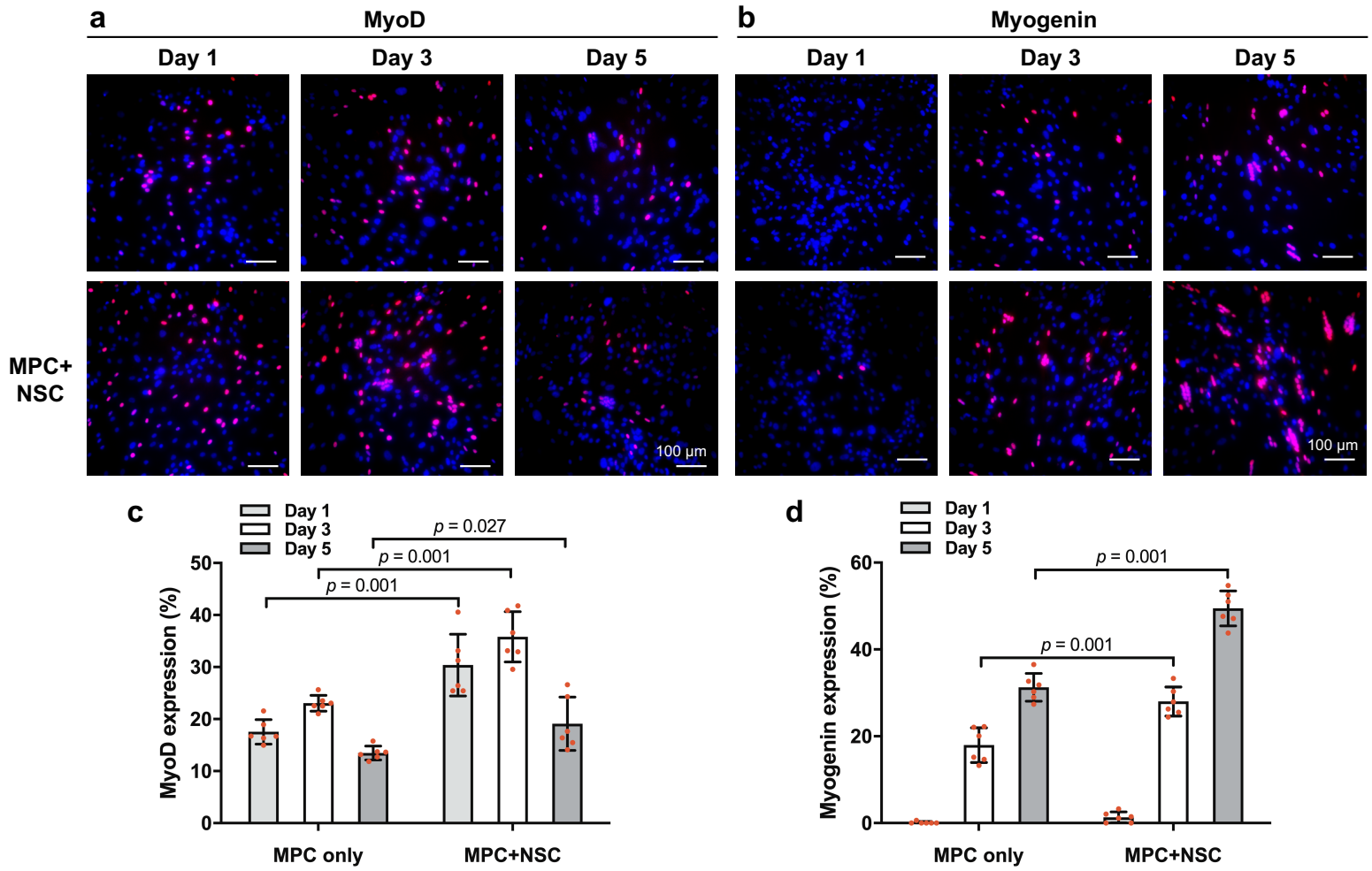
Growth factors/cytokines	MPCs (pg per ml)	NSCs (pg per ml)
BMP-4	0.0*	7.4
EGF	0.0*	6.4
EGF R	250.7	273.0
FGF-2	0.0*	3.4
FGF-4	0.0*	57.3
IGFBP-2	7744.7	25453.9†
Insulin	0.0*	2924.2
OPN	1483.8	3232.5
PDGF-AB	4.9	124.2
PDGF-BB	3.9	70.1
PDGF Rb	81.6	301.7
VCAM-1	829.5	2462.8
VEGF R2	2.9*	11.8

BMP-4: bone morphogenetic protein 4, EGF: epidermal growth factor, EGF R: epidermal growth factor receptor, FGF: fibroblast growth factor, IGFBP-2: insulin-like growth factor-binding protein 2, OPN: osteopontin, PDGF: platelet-derived growth factor, PDGF Rb: platelet-derived growth factor receptor-beta, VCAM-1: vascular cell adhesion protein 1, VEGF R2: vascular endothelial growth factor receptor 2. *Values below the limit of detection, and †values above the highest standards.

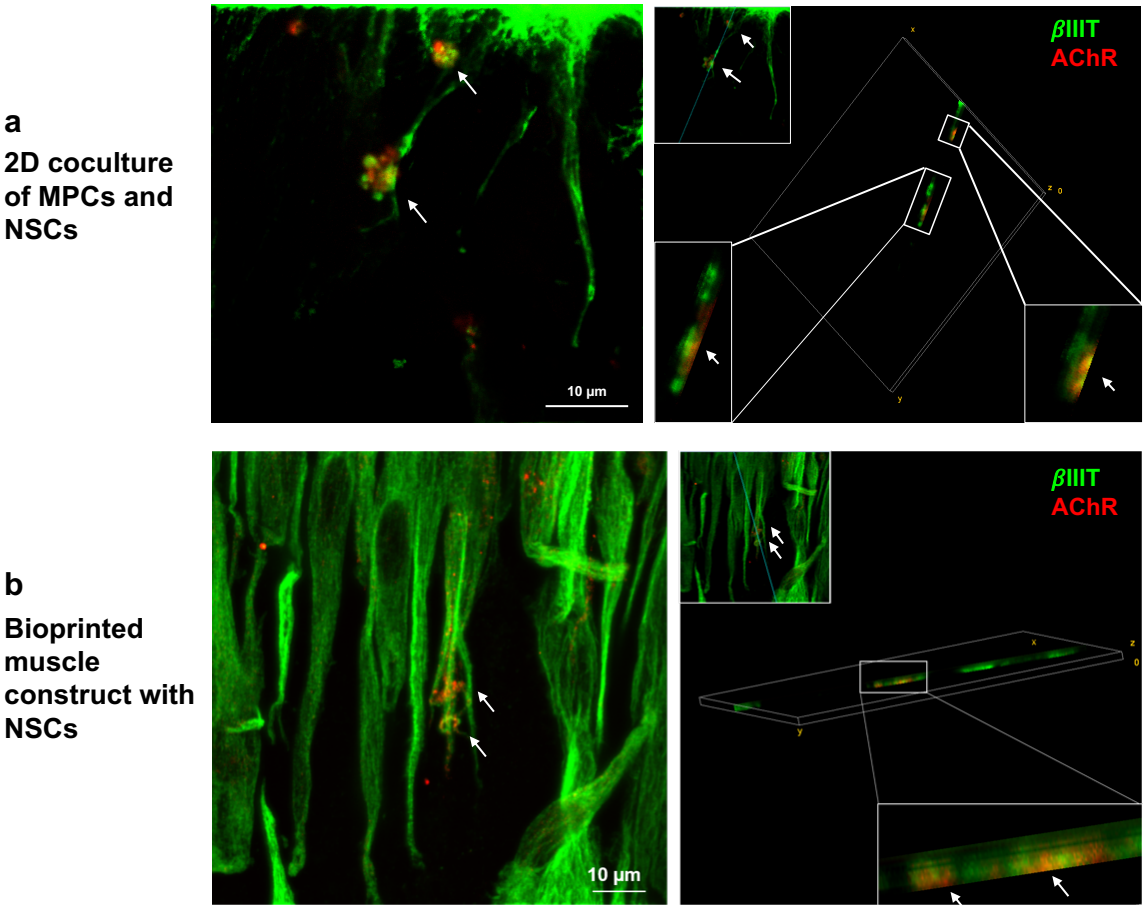


Supplementary Fig. 1. Myotube formation in 2D co-culture of hMPCs and hNSCs. Myotube formation and long-term survival were evaluated in different hMPCs:hNSCs ratios from 1:0 to 500:1 at 5 and 10 days of differentiation. Immunofluorescence for MHC

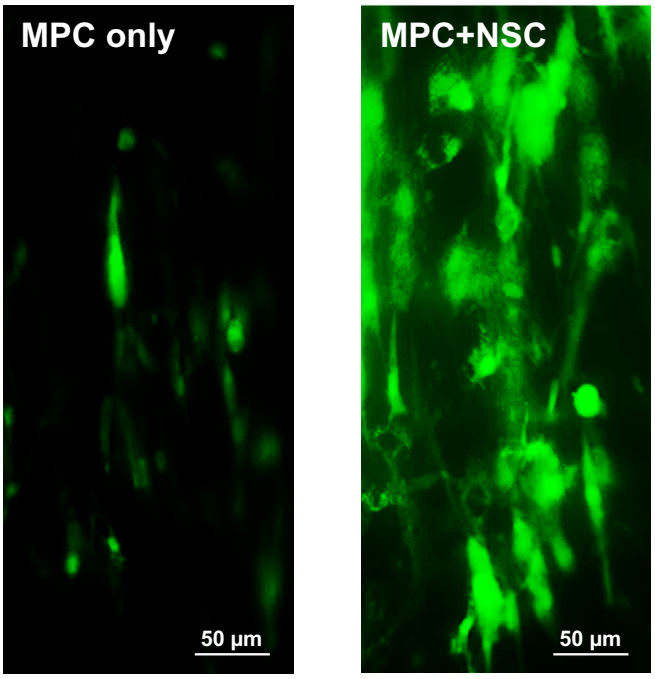
(red)/DAPI (blue) was performed at **a** 5 days and **c** 10 days in culture. The experimental findings were qualitatively reproduced three times. Quantitative data of the area of MHC^+ myofibers (normalized) at **b** 5 days and **d** 10 days in cuture (n = 3 per group). All data are represented as mean ± SD. The p-values by one-way ANOVA followed by Tukey's test are indicated.



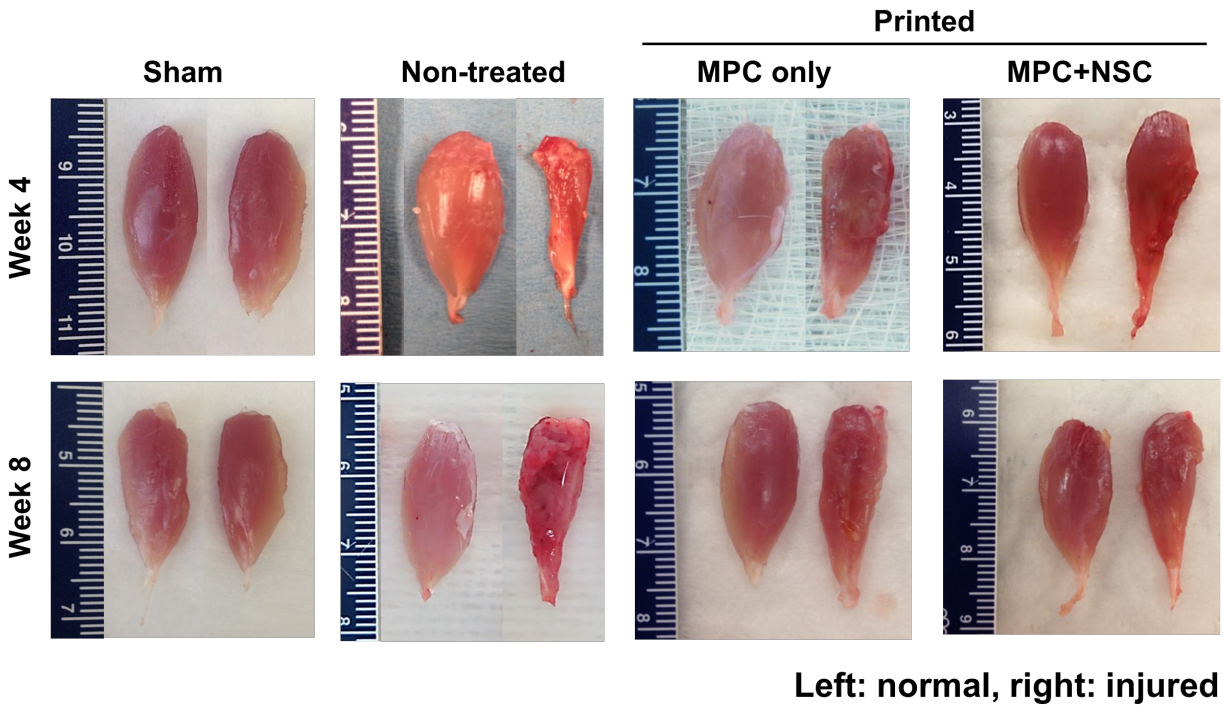
Supplementary Fig. 2. Immunofluorescence for **a** myoD and **b** myogenin at 1, 3, and 5 days in culture. The experimental findings were qualitatively reproduced three times. Quantification of **c** myoD and **d** myogenin expression in MPC and MPC+NSC (300:1) (n = 6 per group and time point). All data are represented as mean ± SD. The p-values by two-way ANOVA followed by Tukey's test are indicated.



Supplementary Fig. 3. Neuromuscular junction formation. Immunofluorescence for βIIIT (green)/AChR (red) on (A) 2D co-culture and (B) bioprinted skeletal muscle construct (MPC+NSC). *Z*-stack confocal microscopy image (arrows, $\beta IIIT^{+}$ $AChR^{+}$ neuromuscular junctions). The experimental findings were qualitatively reproduced three times.



Supplementary Fig. 4. Calcium uptake images of the bioprinted skeletal muscle constructs (MPC only vs. MPC + NSC). Green fluorescence indicates intracellular calcium ions. The experimental findings were qualitatively reproduced three times.



Supplementary Fig. 5. Gross appearance of TA muscles in the rat model of TA defect injury. The muscle defect was created by excising 40% of the TA muscle of the left leg; bioprinted skeletal muscle constructs were implanted into the defect sites. Harvested TA muscles of left legs (defective TA, right) and right legs (contralateral normal TA, left) at 4 and 8 weeks after implantation. The experimental findings were qualitatively reproduced three times.