Supplementary Information

3D culture platform of human iPSCs-derived nociceptors for peripheral nerve modelling and tissue innervation

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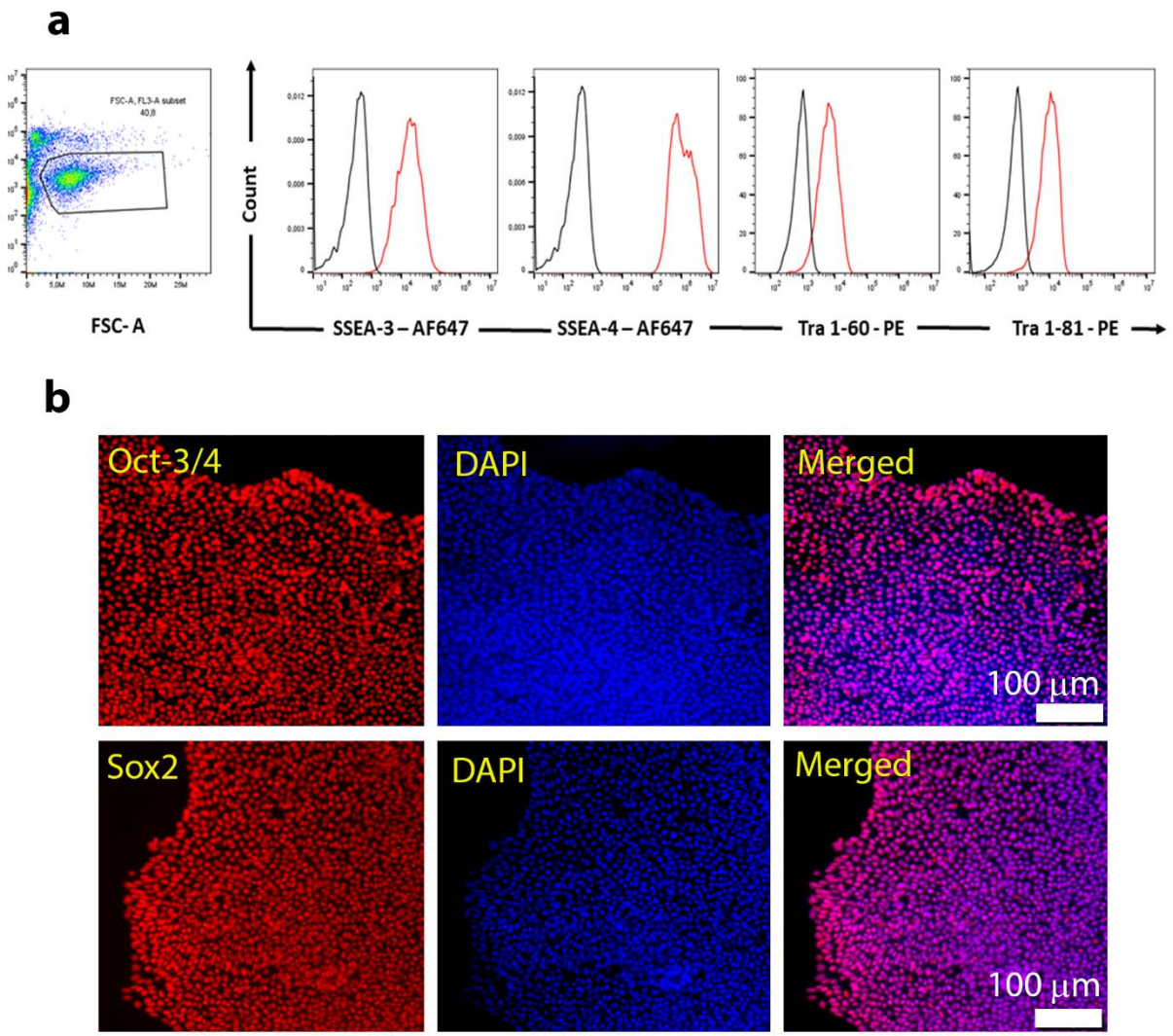

Figure S1. Characterization of human iPSCs pluripotency. A) Flow cytometry analysis to the iPSCs surface markers: stage specific embryonic antigen 3 (SSEA-3) and antigen 4 (SSEA-4) (both conjugated with Alexa Fluor 647, AF647) and podocalyxin (Tra 1-60 and Tra 1-81) (both conjugated with phycoerythrin, PE). B) Immunostaining on iPSCs clusters to the pluripotency markers Oct-3/4 (top panel, red) and Sox2 (bottom panel, red), showing the robust expression of these markers on all cells. DAPI is shown in blue and the scale bar is 100 µm.

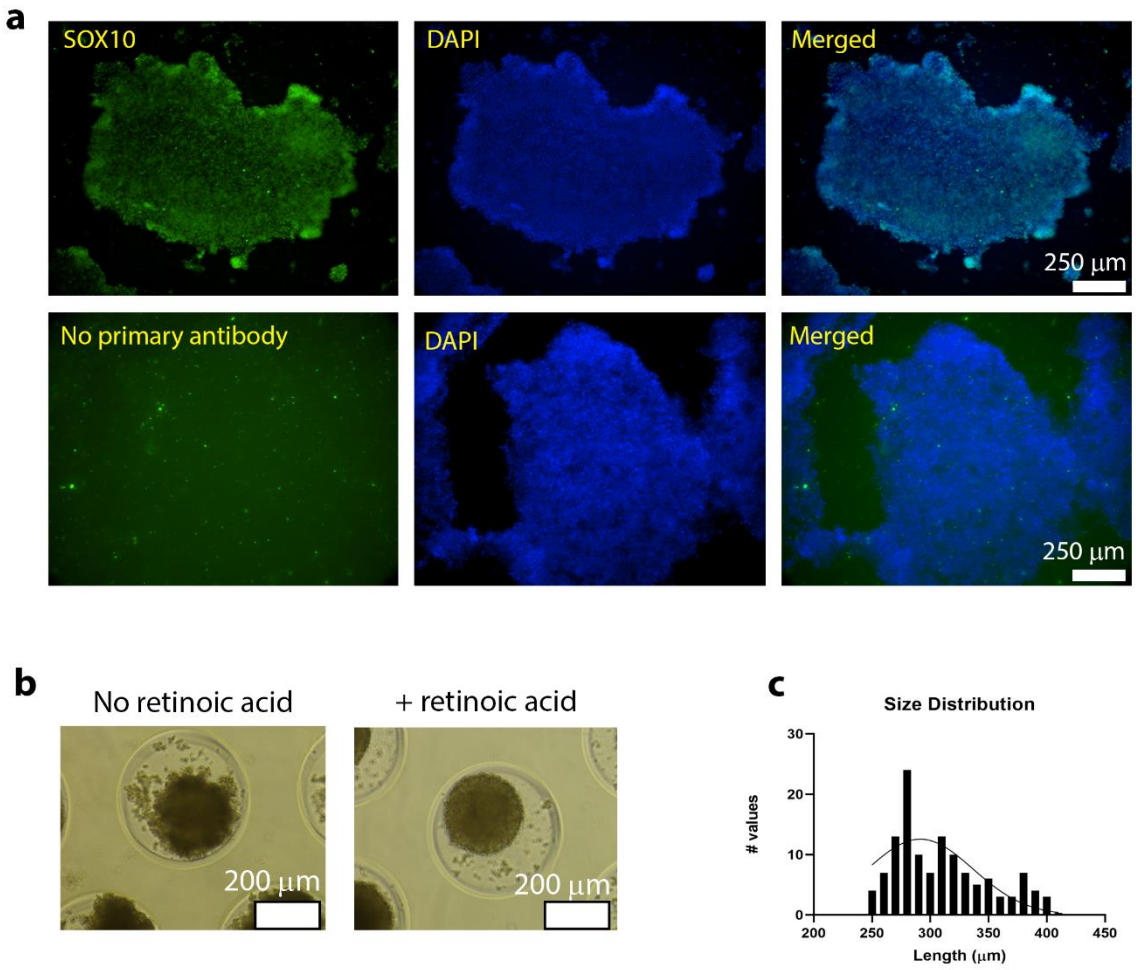

Figure S2. Characterization of neural crest cells obtained from iPSCs neural differentiation. A) SOX10 marker (green, top panel) indicates the acquisition of a neural crest phenotype. On bottom panel, the secondary antibody control (no primary antibody added), indicates the specific binding of the used SOX10 antibody. DAPI is shown in blue and the scale bar is 250 µm. B) Cell clusters at the neural crest stage require retinoic acid for cluster aggregation. The image of the left shows cultures with no retinoic acid added showing their poor integrity. On the right side, cultures with added retinoic acid, evidence good cell aggregation and cluster integrity. The scale bar is 100 µm. C) Size distribution of clusters at the end point of the differentiation process (with retinoic acid).

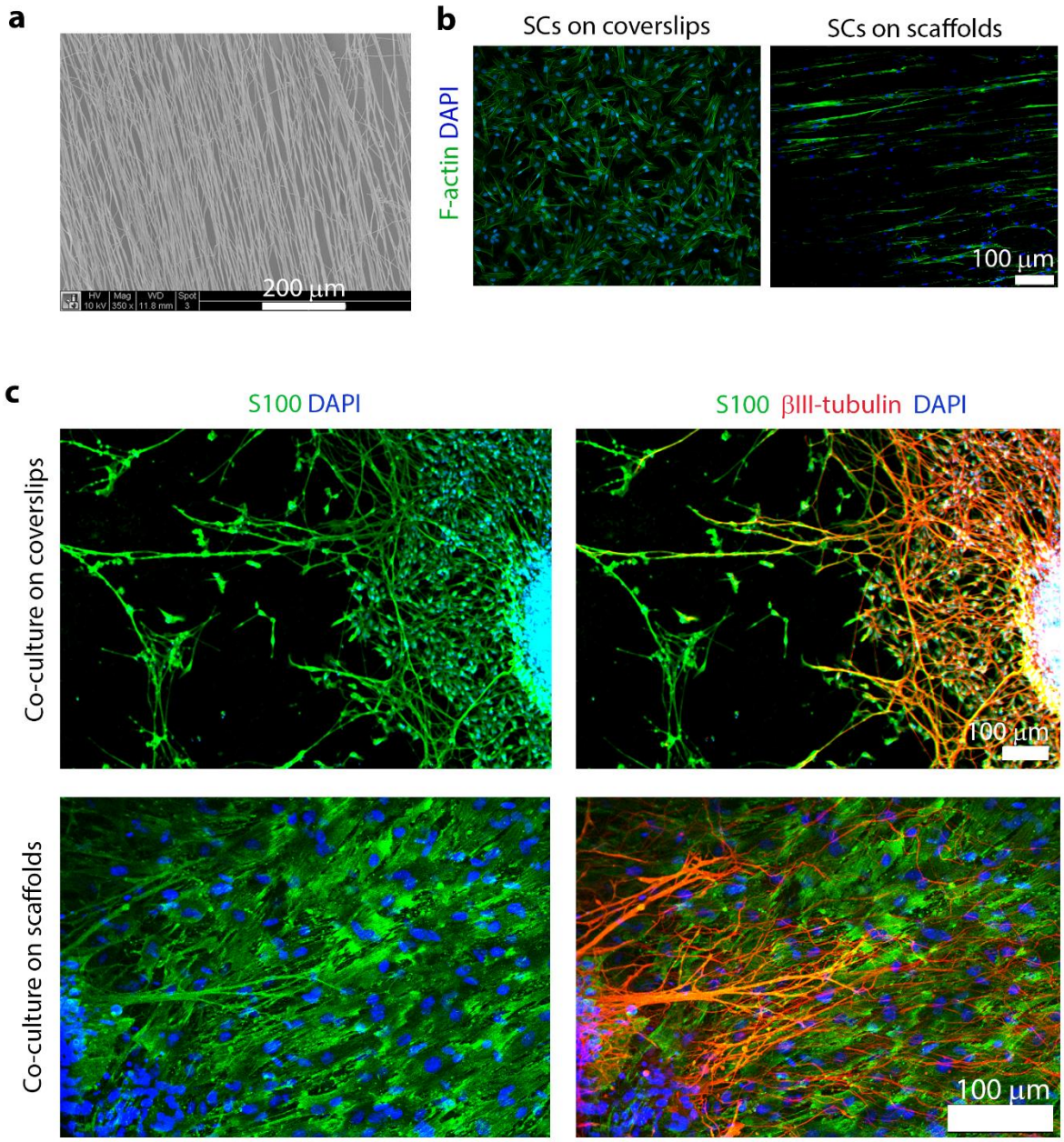

Figure S3. SCs morphology after culture on coverslips or scaffolds. A) SEM micrograph of the scaffold fibers. Scale bar is 200 µm. B) SCs morphology after 7 days of culture on coverslips (left side) and scaffolds (right side). On coverslips, SCs are located randomly and are isotropic, while on scaffolds, SCs are well aligned. F-actin is shown in green and DAPI in blue. Scale bar is 100 µm. C) Co-culture of iPSCs neurospheres with SCs on coverslips (top panel) or scaffolds (bottom panel). SCs (marked by S100 in green) on coverslips cultures tend to group around the neurosphere in large numbers, while few cells remain randomly spread throughout the coverslip. On the scaffold cultures, SCs are tightly packed and remain well aligned over the whole scaffold. In both culture systems, axons (marked by βIII-tubulin in red) tend to follow and intimately associated with SCs. In coverslips, neurite projection is short and radial, while in scaffolds the neurites are long and aligned with the SCs direction. In both panels, DAPI is shown in blue and the scale bar is 100 µm.

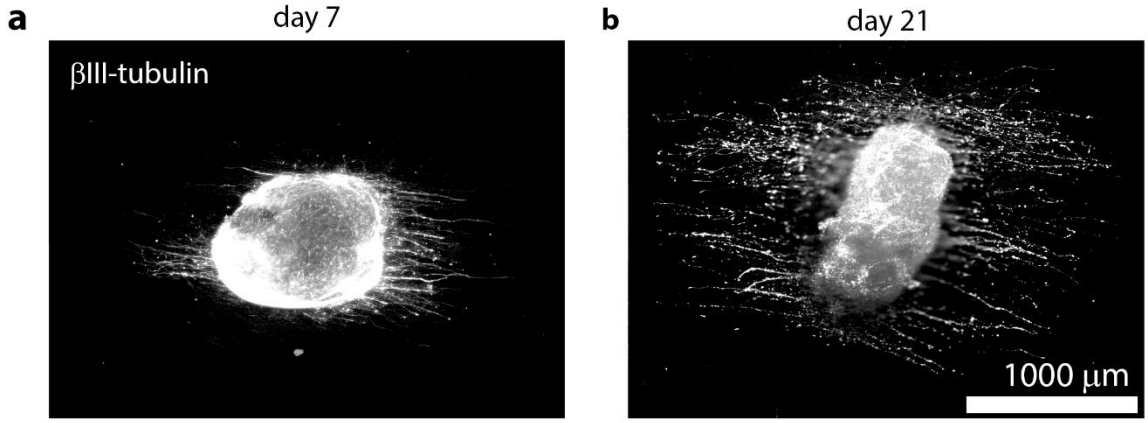

Figure S4. iPSCs neurosphere morphology on scaffolds without coating or seeded SCs. A) iPSCs neurosphere after 7 days of culture and B) 21 days of culture. βIII-tubulin is shown in white and the scale bar is 1000 µm.

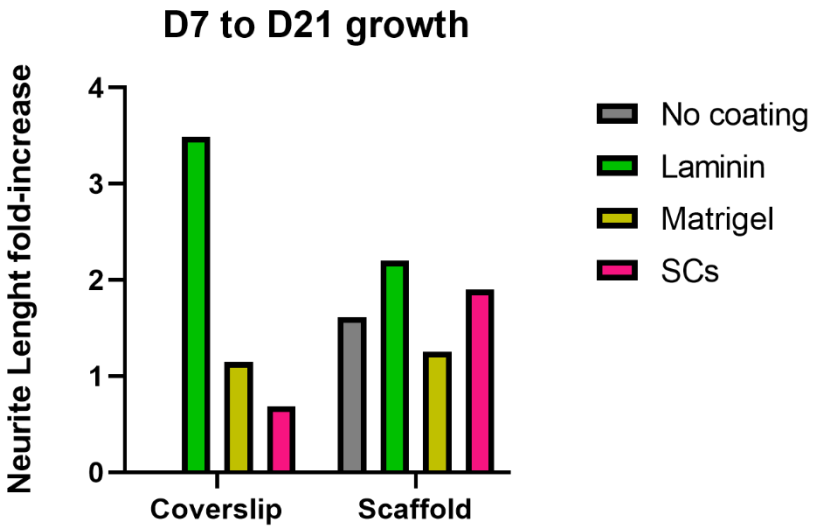

Figure S5. Neurosphere neurite growth increment from day 7 to day 21 in coverslips and scaffolds. The bars represent the ratio between the mean length at day 21 and the mean length at day 7. As visible, the laminin condition was the one that promoted the largest growth increment between these time points.

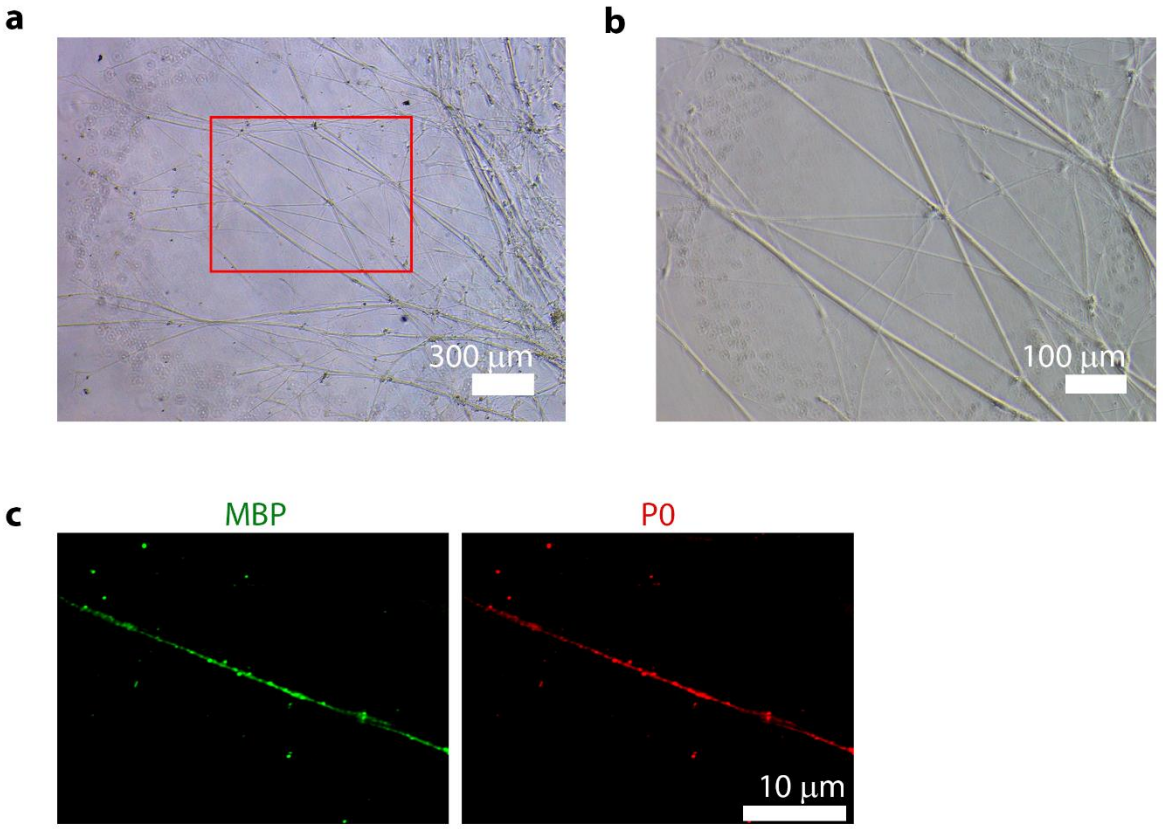

Figure S6. Myelin morphology and characterization on 21 day iPSCs/SCs cultures. A) Brightfield image of iPSCs/SCs co-culture on coverslips, depicting the existence of several myelin segments. Scale bar is 300 µm. B) Detailed view of the area highlighted by the red box in A). Scale bar is 100 µm. Myelin segments, which appear thick, straight and gleamy, can be easily distinguished from unmyelinated axons that look thin, irregular and less reflecting. C) High magnification view of a myelin segment, immunostained for MBP (green) and protein P0 (red).

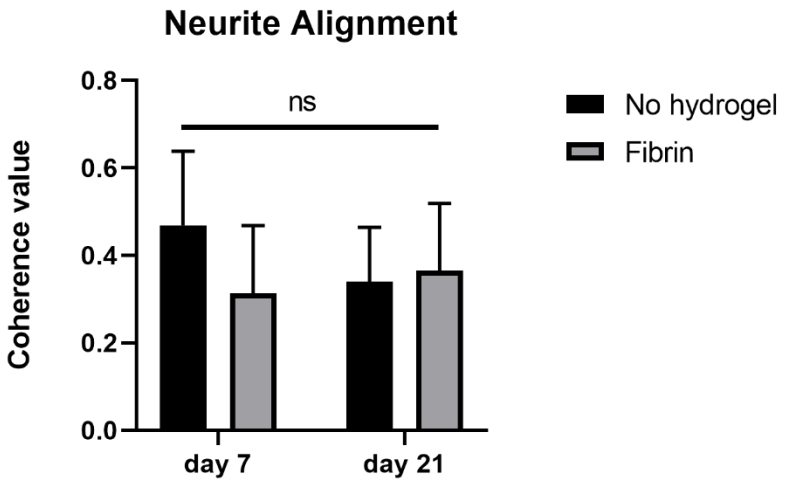

Figure S7. Neurite alignment measurement on neurosphere/SCs scaffold co-cultures with (black bars) and without (gray bars) fibrin embedding over 7 (left side) and 21 days (right side). The values represent the coherence of identical ROIs containing the neurites, where 0 is full isotropy and 1 is full anisotropy. The bar graphs are shown as mean ± SD. We performed this experiment once, and took over 5 images per sample (n = 4). Statistics were performed with two-way ANOVA followed by a Tukey's HSD post-hoc test, where ns is $p > 0.05$.

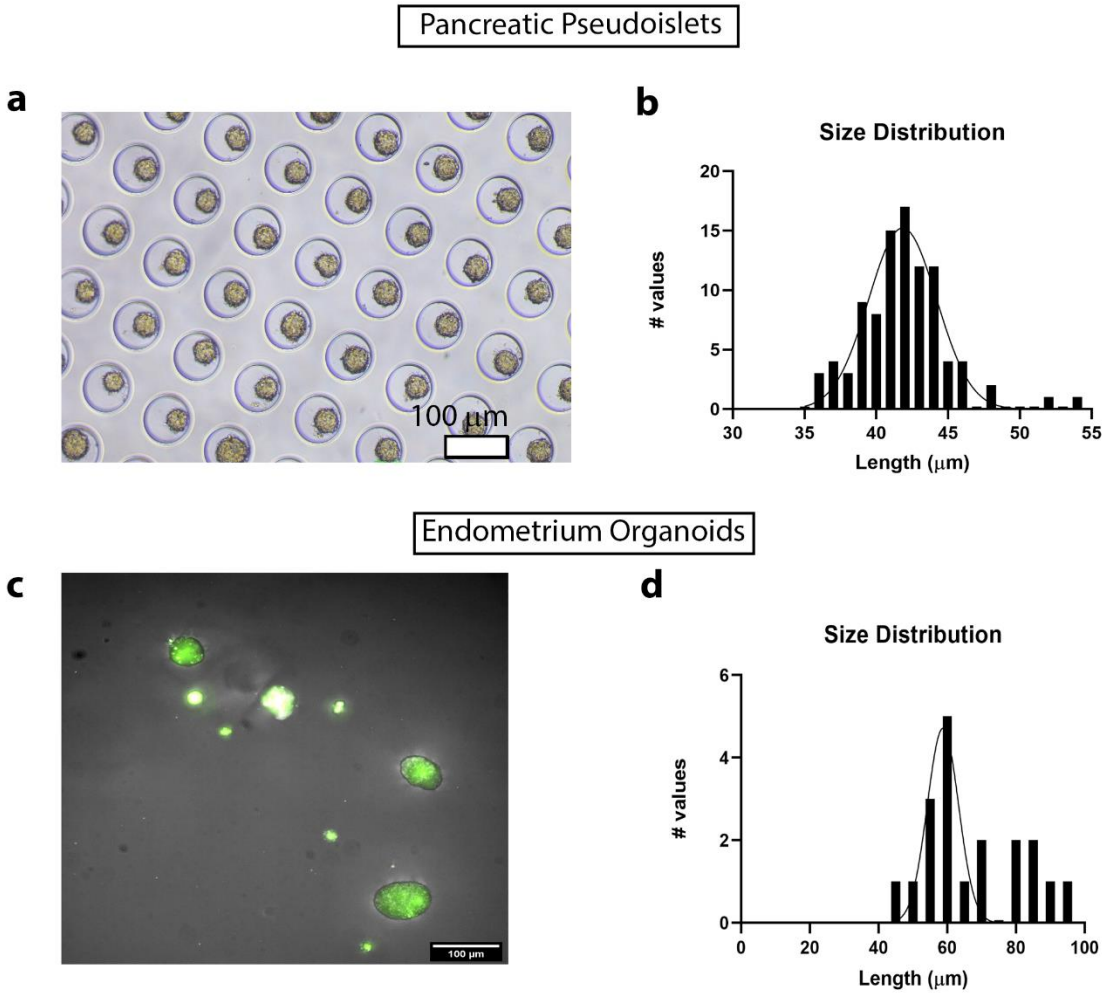

Figure S8. Generation and characterization of tissue spheroids for innervation within the peripheral nerve platform. A) Pancreatic pseudoislets made from alpha TC and INS1E cells and B) correspondent size distribution. C) Endometrium organoids formed from GFP^+ Ishikawa cells within matrigel domes and D) respective size distribution. All scale bar are 100 µm.

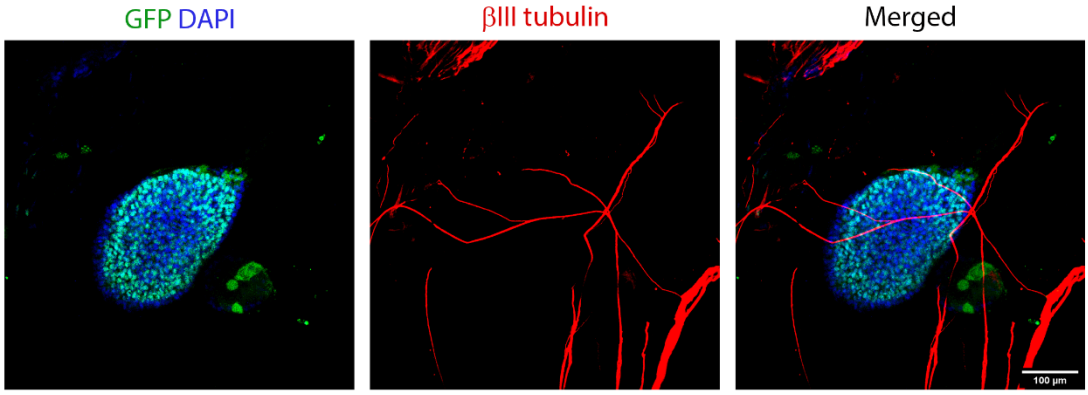

Figure S9. Endometrium organoid innervation in a 10-day co-culture platform. The organoids retain their shape as seen in the image, showing spatial correlation of GFP (in green) and DAPI (in blue). Nociceptor neurites are seen in the image on the middle by βIII-tubulin immunostaining (in red), and as demonstrated in the merged image (right hand side) these neurites interact and surround the endometrium spheroid, to establish innervation. Scale bar is 100 µm.

Human	PHOX2B	Forward	TACGCCGCAGTTCCTTACAA
Human	PHOX2B	Reverse	GAAGACCCTTTCCAGCTCTTT
Human	ETS1	Forward	GCAGAATGAGCTACTTTGTGGA
Human	ETS1	Reverse	TTGCTAGGTCCTTGCCTCA

Table S1. Used primers for qPCR of PHOX2B and ETS1 genes.

Cell #	Cm (pF)	RMP (mV)	Overshoot (mV)	AP amplitude (mV)	I step
#1	24,0	-44,2	55,4	99,6	200 pA
#2	11,9	-40,6	67,2	107,8	200 pA
#3	14,0	-60,3	-9,1	51,2	200 pA
#4	13,0	-38,5	24,1	62,6	200 pA
#5	20,0	-44,7	-8,7	36,0	200 pA
#6	16,0	-37,5	13,1	50,6	200 pA
#7	13,0	-42,8	6,1	48,9	200 pA
#8	18,0	-44,1	38,6	82,7	200 pA

Table S2. Patch clamp results from 40 DIV dissociated iPSCs-derived nociceptors.

Target protein	Source	Used dilution
βIII tubulin	Sigma-Aldrich, T8578	1:500
S100	Sigma-Aldrich, S2644	1:100
Myelin Basic Protein (MBP)	Thermo Fisher Scientific, PA1-46447	1:50
Phox2B	Santa Cruz Biotechnology, sc-376997	1:50
ETS1	Santa Cruz Biotechnology, sc-55581	1:50
Substance P	Abcam, ab14184	1:100
CGRP	Abcam, ab22560	1:500
TRPV-1	Alomone Labs, ACC-030	1:100
BRN3A	Merck Millipore, MAB1585	1:100
Myelin Protein Zero (P0)	Novus Biologicals, NB100-1607	1:50
Insulin	Abcam, ab7842	1:50
Glucagon	Bio-Techne, NBP1-67575	1:750

Table S3. List of used primary antibodies.

Host	Target	Conjugated fluorophore	Source	Dilution
Goat	Mouse IgG	488	Thermo Fischer Scientific	1:500
Goat	Mouse IgG	568	Thermo Fischer Scientific	1:500
Goat	Mouse IgG	647	Thermo Fischer Scientific	1:500
Goat	Rabbit IgG	488	Thermo Fischer Scientific	1:500
Goat	Rabbit IgG	568	Thermo Fischer Scientific	1:500
Goat	Guinea Pig IgG	647	Thermo Fischer Scientific	1:500
Donkey	Sheep IgG	488	Thermo Fischer Scientific	1:500

Table S4. List of used secondary antibodies.