



Article

Culture Conditions for Human Induced Pluripotent Stem Cell-Derived Schwann Cells: A Two-Centre Study

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Supplementary Material

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Table S1. Primary and secondary antibodies for immunocytochemistry *at MHH*.

Antibodies for immunocytochemistry					
Antibodies	Dilution	Species Raised	Product code	Source	Epitope
GAP43	1:500	Rabbit polyclonal	AB5220	Abcam	Cytoplasm
CDH 19	1:200	Rabbit polyclonal	PA5-101291	Invitrogen	Cytoplasm
SOX10	1:100	Mouse monoclonal	MAB2864	R&D	Nucleus/ Cytoplasm
p75 ^{NTR}	1:200	Mouse monoclonal	AB3125	Abcam	Extracellular
S100B	1:200	Rabbit polyclonal	Z0311	Dako	Cytoplasm
GFAP	1:400	Rabbit polyclonal	G-9269	Sigma	Cytoplasm
GALC	1:500 or 1:200	Rabbit polyclonal	G9152	Sigma	Extracellular

Table S2. Primary and secondary antibodies for immunocytochemistry *at UCL*.

Antibodies for immunocytochemistry – second modified protocol					
Antibodies	Dilution	Species Raised	Product code	Source	Epitope
OCT4	1:200	Goat polyclonal	sc-8629	Santa Cruz Biotechnology	Nucleus
SOX10	1:40	Mouse monoclonal	sc-365692	Santa Cruz Biotechnology	Nucleus/ Cytoplasm
p75 ^{NTR}	1:200	Rabbit monoclonal	8238	Cell Signalling Technology	Extracellular
S100B	1:1	Rabbit polyclonal	GA50461-2	Dako	Cytoplasm
DAPI	1:1000	Fluorescent dye	D9542	Sigma	Nucleus
DyLight 488 Anti-Mouse IgG	1:200	Horse polyclonal	DI-2488	VectorLabs	Secondary
DyLight 549 Anti-Rabbit IgG	1:200	Goat polyclonal	DI-1549	VectorLabs	Secondary
DyLight 594 Anti-Goat IgG	1:200	Horse polyclonal	DI-3094	VectorLabs	Secondary

Table S3. *MHH* primer sequences for RT-qPCR.

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')	Expected amplicon size (bp)
<i>RPLP0</i>	TGGTCTCTTTGACTAATCACC	AGAAGTAAGCCTTTATTCCTT	80
<i>SNAI2</i>	CTGGACACACATACAGTGATTA	CGGTAGTCCACACAGTGAT	121
<i>TFAP2A</i>	GCAGTAGCTGAATTTCTCAAC	CTCTTTGCATATCTGTTTTGTA	96
<i>CDH19</i>	GTAATAGACATCGCTACTGGAA	GATAACTAATGTTCTTCTGGA	15-
<i>NGFR</i>	ACAACCTCATCCCTGTCTAT	AGCTGTTCCACCTCTTGA	87
<i>S100B</i>	GAGACAAGGAAGAGGATGTC	TAAGAAATGGGAAAGCTCAT	150
<i>PMP22</i>	AAATTCTTGCTGGTCTGTG	GTAGGAGTAATCCGAGTTGAG	89
<i>SOX10</i>	GAGAGGGCTCCCCATGTCAGA	GCCCGACTGCAGCTCTGTCTTC	104
<i>c-Jun</i>	ACAATAGGTGCTTATTCTCAA	CTAGGAATTGTCAAAGAGAAGA	94
<i>PLP1</i>	GAGAAAAAGTAAAAGACCGAAG	ACTCTAACAAGCCCATGTC	135
<i>GAP43</i>	CTGAAGAGAACATAGAAGCTGT	AAAGCCATTCTTAGAGTTCA	122

Table S4. *UCL* primer sequences for RT-qPCR.

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')	Expected amplicon size (bp)
<i>RPS18</i>	CAAGAGGGCGGGAGAAC	CGTGGATTCTGCATAATGGTG	66
<i>TBP</i>	ACTTCGTGCCCGAAACG	GTGGTTCGTGGCTCTCTTATC	78
<i>SNAI2</i>	CCAAACTACAGCGAAC	TGAGGATCTCTGGTTG	97
<i>TFAP2A</i>	GAAGCTGTCCACCTAGC	CTTGGCAGGAAATTCGG	61
<i>CDH19</i>	CTTGCTTGGAGCAACAG	ATCTTAGCTGGCCGATG	150
<i>NGFR</i>	GTGAGTGCTGCAAAG	AACGTCACGCTGTC	97
<i>S100B</i>	CTCATCAACAATGAGCTTTC	TCACATTCGCCGTCTC	104
<i>PMP22</i>	GACACGCAACTGATCTC	TGCAGCCATTCGTTTG	91
<i>SOX10</i>	TACACCGACCAGCCATC	GGTCAGAGTAGTCAAACCTGG	109
<i>c-Jun</i>	CCTGATAATCCAGTCC	ATCTGTACAGTTCTTG	82
<i>PLP1</i>	ACCTGCCAGTCTATTG	TGGGAGAACACCATAC	87
<i>GAP43</i>	AGCTCATAAGGCCGCAAC	TCAGCAGCTTGGACATCATC	99
<i>SOX2</i>	GCTCGCAGACCTACATGAAC	ACTTGACCACCGAACCC	102

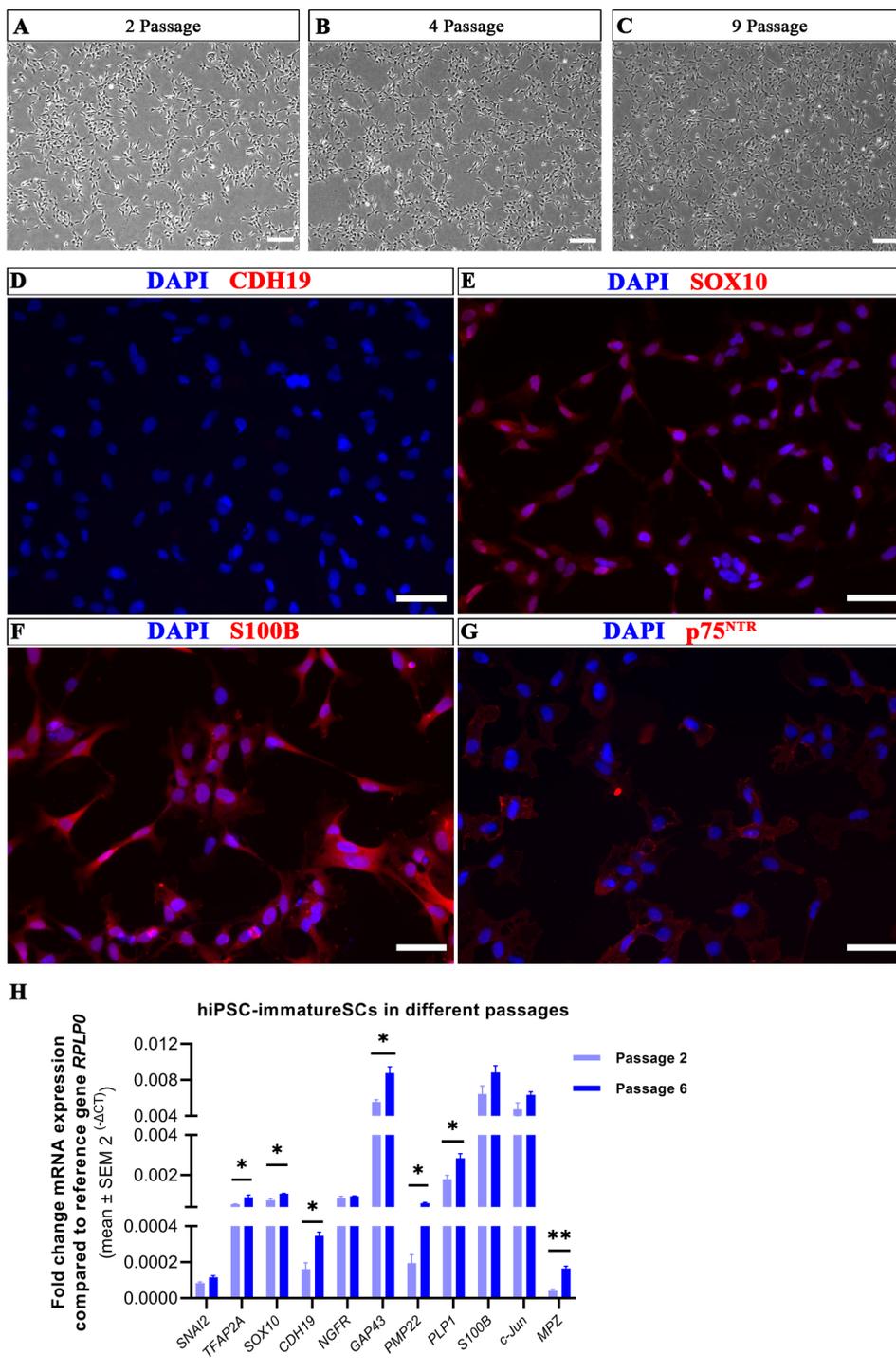


Figure S1. Characterization of MHH hiPSC-immatureSCs morphology, mRNA and protein level when cultured in different passages. (A-C) Representative phase-contrast photomicrographs of cells cultured at passage 2, 4, and 9 with an initial cell density of 2×10^5 . (D-G) Immunocytochemical characterization of hiPSC-immatureSCs from passages 2-9, cells are negative for CDH19 (D) and positive for Schwann cell markers SOX10 (E), S100B (F), and p75^{NTR} (G); cell nuclei were stained with DAPI (blue). (H) Graphical presentation of gene expression analysis of hiPSC-immatureSCs at passage 2 and passage 6 with gene *SNAI2*, *TFAP2A*, *SOX10*, *CDH19*, *NGFR*, *GAP43*, *PMP22*, *PLP1*, *S100B*, *c-Jun*, and *MPZ*. Values were normalized to the reference gene *RPLP0*; Statistical analysis with unpaired t-test, * $P < 0.05$, ** $P < 0.01$, $n = 3$ cell cultures. (A-C) scale bar: 200 μ m. (D-G) Scale bar: 50 μ m.; hiPSC-immatureSCs = human induced Pluripotent Stem Cell derived immature Schwann Cells.

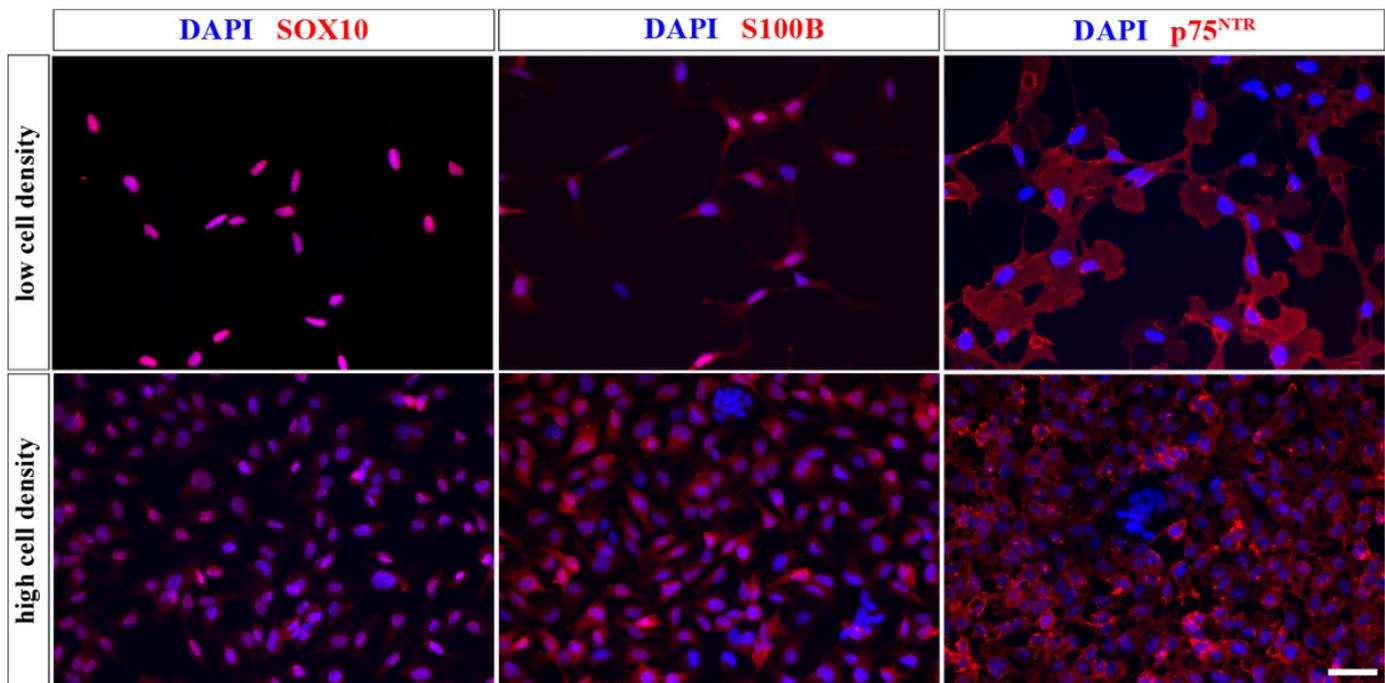


Figure S2. Morphology changes of hiPSC-immatureSCs cultured with different cell densities. Low cell density stands for a seeding density of 2.5×10^4 cells / 24-well, and high cell density stands for a seeding density of 5×10^4 cells / 24-well. The cells in both cultures were immunopositive for SOX10, S100B, and p75^{NTR}. Cell nuclei were stained with DAPI (Blue). Scale bar: 50 μ m. hiPSC-immatureSCs = human induced Pluripotent Stem Cell derived immature Schwann Cells.

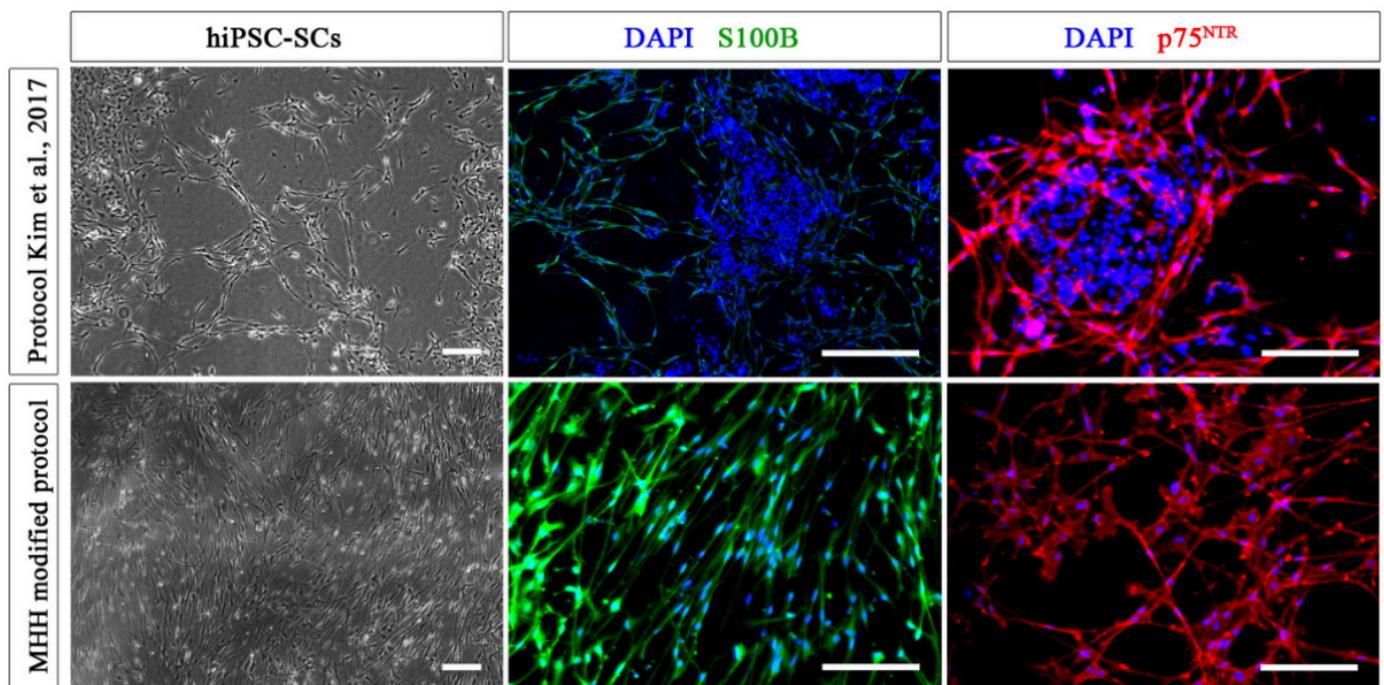


Figure S3. Microscopic comparison of hiPSC-SCs derived from the original and the MHH modified protocol. Phase-contrast photomicrographs represent hiPSC-SCs differentiated by using the unmodified protocol according to [25] and the MHH modified

protocol. Immunocytochemical staining was performed to detect Schwann cell markers S100B and p75^{NTR}. Cell nuclei were stained with DAPI (Blue). Scale bar: 200 μm . hiPSC-SCs = human induced Pluripotent Stem Cell derived Schwann Cells.

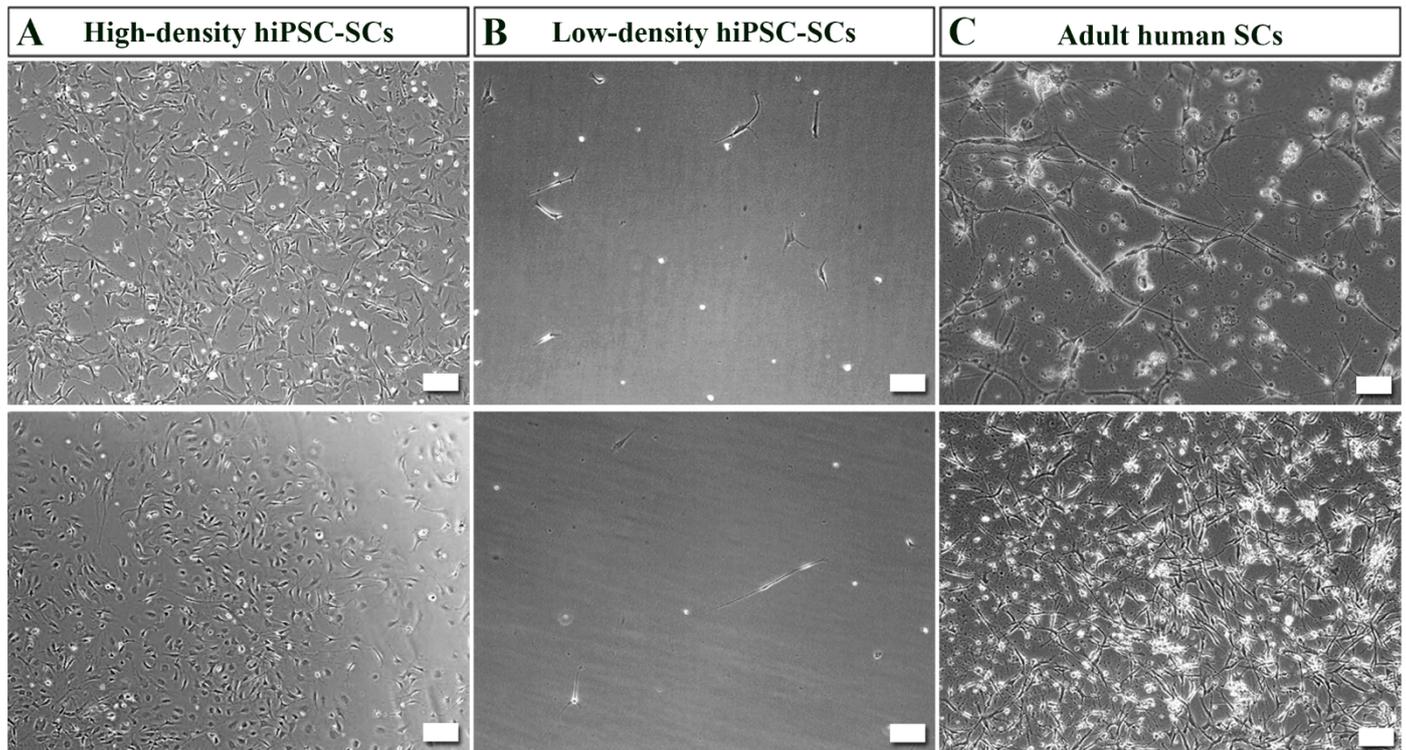


Figure S4. Phase contrast micrographs showing morphological change between high-density hiPSC-SCs (A), low-density hiPSC-SCs (B) and adult human Schwann cells cultured at UCL (C). Low cell density stands for a seeding density of 1763 cells / cm^2 , and high cell density stands for a seeding density of 14101 cells / cm^2 . Adult human Schwann cells in (C) were seeded in a density of 3.7×10^4 cells / cm^2 . Upper and lower panels show examples from two different differentiation procedures for the differentiated Schwann cells while the adult human Schwann cells images are from two separate flasks from one cell line. Low density plating produces cells more closely resembling the primary Schwann cell culture, being longer and more bipolar in shape. Scale bar = 100 μm . hiPSC-SCs were differentiated from hiPSC-SCPs for 7 days in SCDM. hiPSC-SCPs = human induced Pluripotent Stem Cell derived Schwann Cell Precursor cells; hiPSC-SCs = human induced Pluripotent Stem Cell derived Schwann Cells.

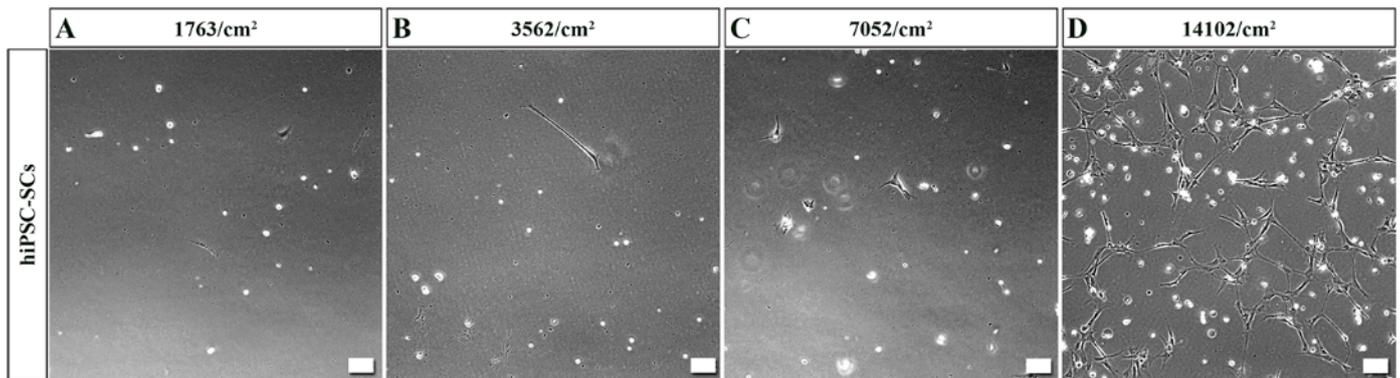


Figure S5. Phase contrast micrographs showing UCL hiPSC-SCPs plating density prior to hiPSC-SC differentiation and hiPSC-SC survival after 7 days. (A) 1763/cm²; (B) 3526/cm²; (C) 7052/cm²; (D): 14102/cm²; Scale bar = 100µm. Survival of hiPSC-SCs may be increased by increasing plating density of hiPSC-SCPs 8-fold. hiPSC-SCPs = human induced Pluripotent Stem Cell derived Schwann Cell Precursor cells.

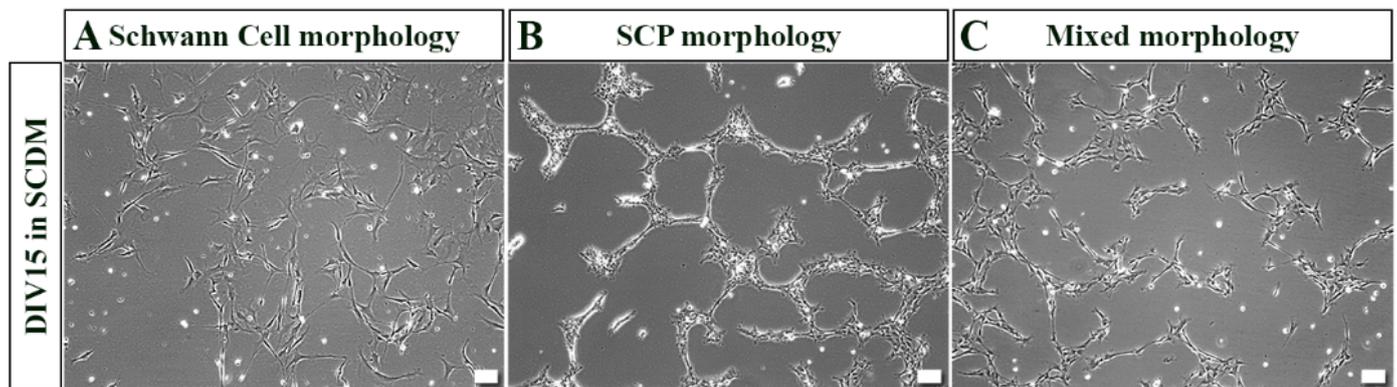


Figure S6. Morphology of UCL hiPSC-SCs at DIV 15 in UCL-SCDM. Differing morphologies can be seen with the long, bipolar morphology seen in Schwann cells evident in (A). (B) shows cells closer in morphology to the hiPSC-SCPs (dense, small and closely associated with one another) while (C) shows a mixture of these cell types. The changes in gene expression seen between DIV 7 and DIV 15 in figure 9 (RT-qPCR results) could be due to mixed populations of cell types appearing by DIV 15. UCL-SCDM = Schwann cell differentiation medium as used by the UCL team. Scale bar = 100µm.