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Supplemental Information

Human iPSC-Derived Neural Crest Stem Cells Exhibit Low Immunogenicity

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Supplemental data

MSC differentiation

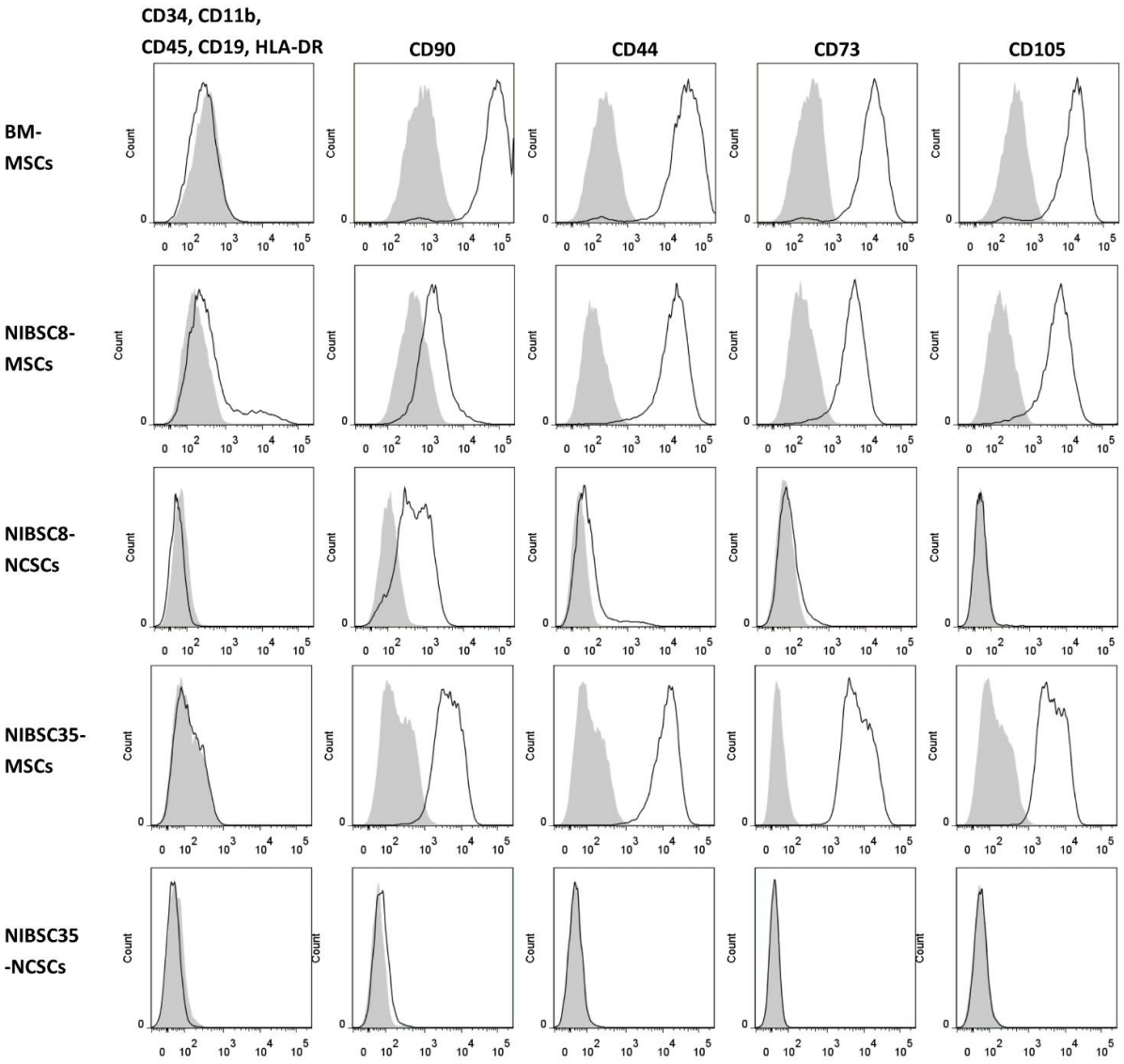


Figure S1. Characterisation of MSCs differentiated from iPSC-NCSCs. Representative histograms of % of cells positive for MSC markers, including CD90, CD44, CD73 and CD105 and MSC negative markers, including CD34, CD11b, CD45, CD19 and HLA-DR (black line vs grey shaded isotype control).

Peripheral nerve differentiation

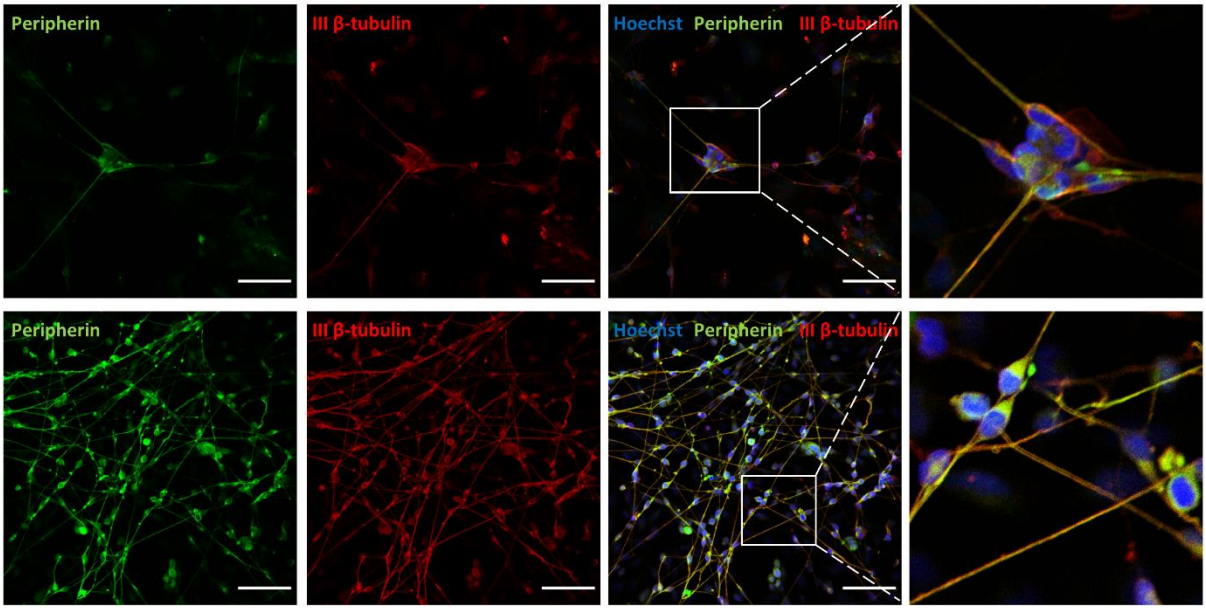


Figure S2. Characterisation of peripheral neurons generated from iPSC-NCSCs. Representative immunofluorescence staining of NIBSC8-NCSC-derived peripheral neurons (top row) and NIBSC35-NCSC-derived peripheral neurons (bottom row) with peripherin (green) and III β-tubulin (red). Hoechst staining was used to label the nuclei of the cells (blue). Scale bar = 100µm.

Dendritic cell differentiation

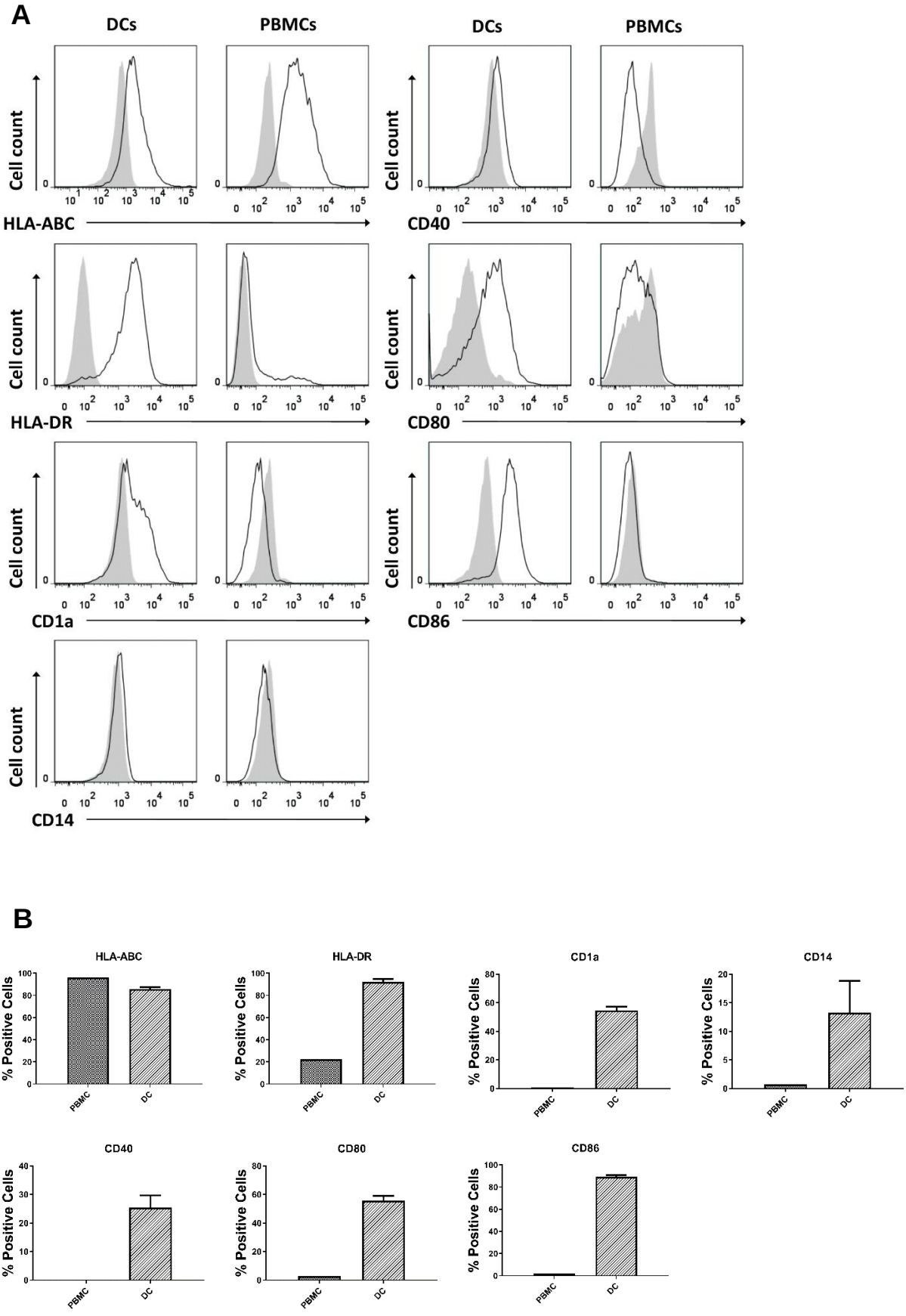
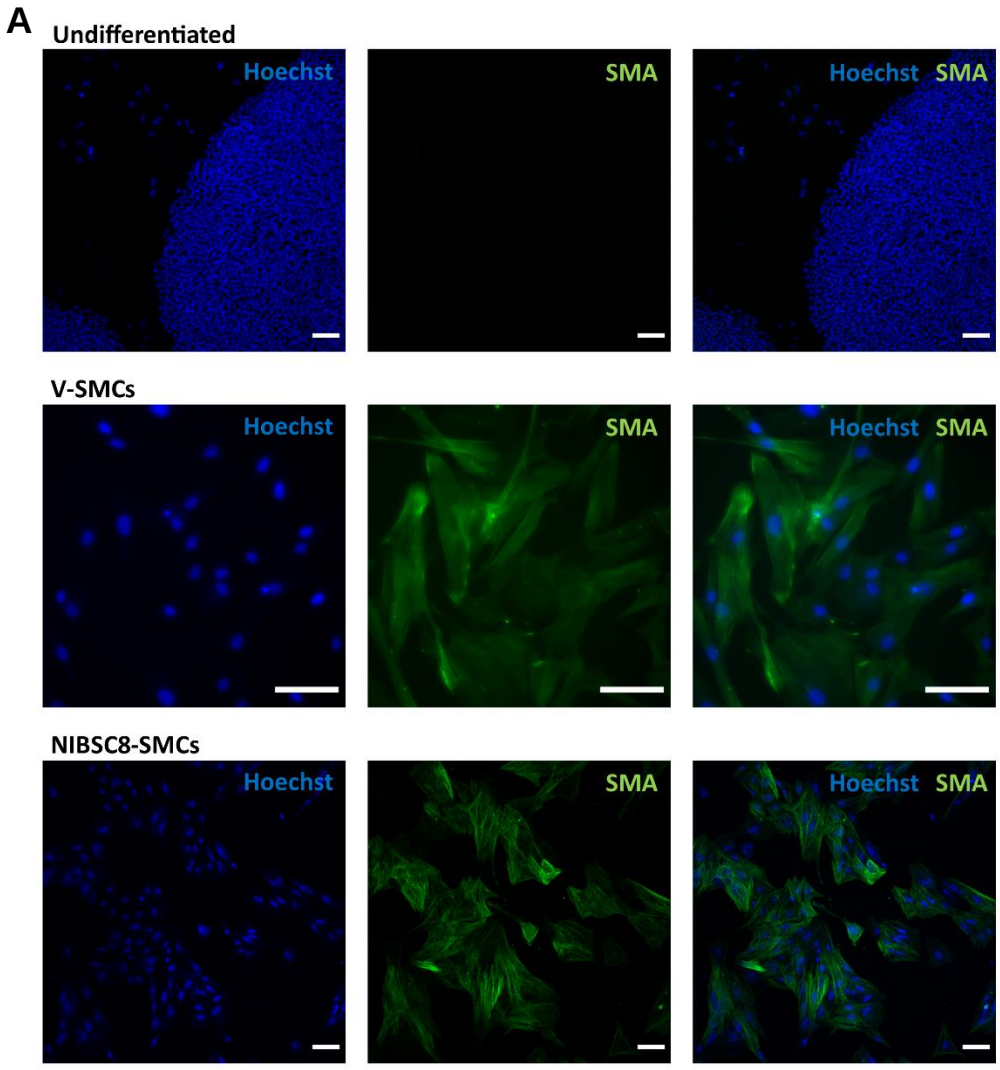


Figure S3. Dendritic cell generation from monocytes. (A) Representative histograms of % of cells positive for DC markers, including HLA-ABC, HLA-DR, CD1a, CD14, CD40, CD80 and CD86. Percentages are of total PBMCs and monocyte-derived DCs (black line vs grey shaded isotype control). (B) Graphs of % cells positive for DC markers in PBMCs and DCs. Error bars represent ±SEM (n = 3 technical replicates for DCs and n=1 technical replicate for PBMCs).

Smooth muscle cell differentiation



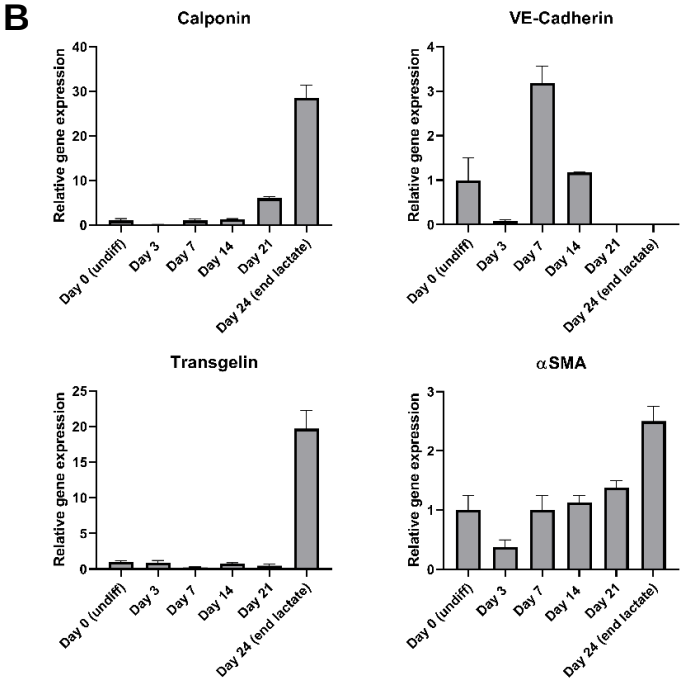


Figure S4. Characterisation of smooth muscle cells (SMCs) generated from iPSCs. (A) Representative immunofluorescence staining with smooth muscle actin (SMA, green) and Hoechst staining (blue) for undifferentiated iPSCs (NIBSC8), vessel-derived SMCs (v-SMCs) and NIBSC8-SMCs. Scale bar = 100µm. (B) cDNA was prepared from iPSCs at day 0 (undiff) and iPSC-SMCs at day 4 day 3, day 7, day 14, day 21 and day 24 (end of lactate=end of enrichment phase) of SMC differentiation and qPCR was performed for key SMC markers, including α*SMA*, *CALPONIN, TRANSGELIN* and for the endothelial marker *VE-CADHERIN.* Error bars represent ±SEM (n = 2 biological replicates). Results were normalised to *GAPDH* expressed as fold change gene expression relative to the undifferentiated control.

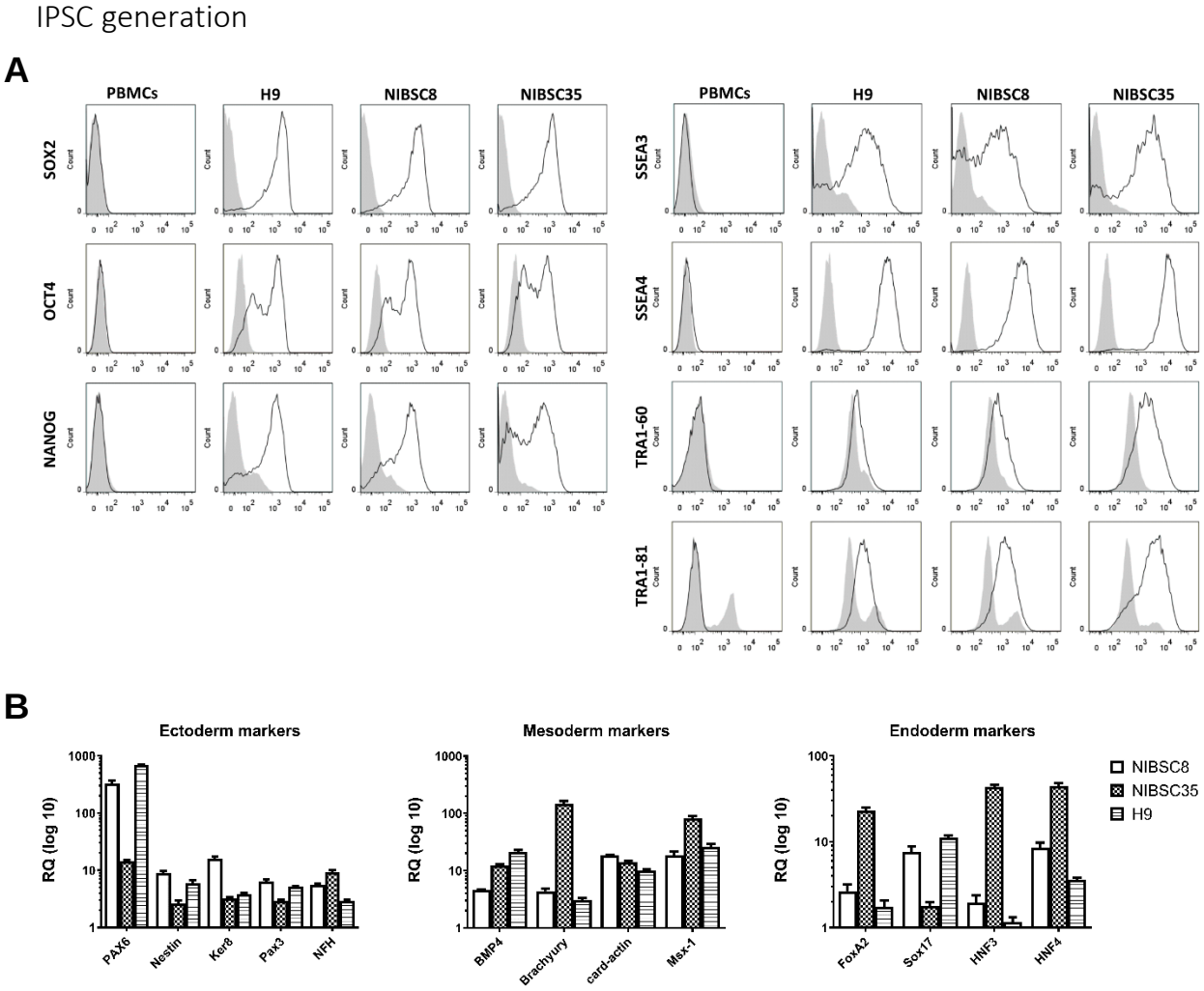


Figure S5. NIBSC8 and NIBSC35 iPSC characterisation. (A) Representative histograms of % of cells positive for pluripotency markers, including SOX2, OCT4, NANOG, SSEA3, SSEA4, TRA1-60 and TRA1-81. Percentages are of total PBMCs, H9 ES cells, NIBSC8 iPSCs and NIBSC35 iPSCs (black line vs grey shaded isotype control). (B) qPCR data from EBs, subject to 14 days of spontaneous differentiation, for expression of germ layer specific genes. The fold change in expression relative to the pluripotent control is shown (relative quantification, RQ). Error bars represent ±SEM (n = 3 biological replicates). Results were normalised to *GAPDH* expressed as fold change gene expression relative to the undifferentiated control.

Supplemental tables

Name	HLA-A	HLA-B	HLA- DRB1
IPSC lines:			
NIBSC8	*03 *23	*15 *44	*07 *13
NIBSC35	*02 *24	*08	*04 *15
PBMCs:			
Donor i	*26	*38 *35	*04 *04
Donor ii	*32 *29	*44 *27	*01 *08

Table S1: HLA types of stimulator and responder cell lines. The number after the asterisk represents an HLA allele (a blank number means that the line is homozygous for that allele). Antigen mismatch for NIBSC8 vs donor i is 6/6, mismatch for NIBSC8 vs donor ii is 5/6, mismatch for NIBSC35 vs donor i is 5/6 and mismatch for NIBSC35 vs donor ii is 6/6.

Specificity	Fluorochromes	Isotype	Clone	Staining dilution	Manufacturer, catalogue number
Surface markers:					
CD271/p75	PE	mouse IgG1	C40-1457	1:20	BD, 557196
HNK-1	APC	mouse IgG2a	NK-1	1:10	BD, 560845
CD3	Pacific Blue™	mouse IgG1	UCHT1	1:50	BD, 558117
CD4	APC-Cy™7	mouse IgG1	RPA-T4	1:50	BD, 557871
CD8	PerCP-Cy™5.5	mouse IgG1	RPA-T8	1:50	BD, 560662
HLA-ABC	PE	mouse IgG1	G46-2.6	1:25	BD, 560964
HLA-DR	APC	mouse IgG2a	L243	1:50	BioLegend, 307610
HLA-DR, DP, DQ	Alexa Fluor® 647	mouse IgG2a	Tu39	1:50	BD, 563591
CD40	V450	mouse IgG1	5C3	1:50	BD, 561219
CD80	PE-Cy™7	mouse IgG1	L307.4	1:50	BD, 561135
CD86	PerCP-Cy™5.5	mouse IgG1	2331	1:50	BD, 561129
CD1a	PE	mouse IgG1	HI149	1:10	BD, 555807
CD14	FITC	mouse IgG2a	M5E2	1:10	BD, 555397
SSEA3	Alexa Fluor® 647	rat IgM	MC-631	1:33	BD, 561145
SSEA4	PerCP-Cy™5.5	mouse IgG3	MC813-70	1:20	BD, 561565
TRA1-60	PE	mouse IgM	TRA-1-60	1:12.5	BD, 560884
TRA1-81	FITC	mouse IgM	TRA-1-81	1:5	BD, 560194
Human MSC Analysis Kit:					BD, 562245
CD90	FITC	mouse IgG1	5E10	1:20	n/a
CD105	PerCP-Cy™5.5	mouse IgG1	266	1:20	n/a
CD73	APC	mouse IgG1	AD2	1:20	n/a
CD44	PE	mouse IgG2b	G44-26	1:20	n/a
CD34	PE	mouse IgG1	581	1:20	n/a
CD11b	PE	mouse IgG1	ICRF44	1:20	n/a

CD19	PE	mouse IgG1	HIB19	1:20	n/a
CD45	PE	mouse IgG1	HI30	1:20	n/a
HLA-DR	PE	mouse IgG2a	G46-6	1:20	n/a
Intracellular markers:					
Ki-67	PE-Cy™7	mouse IgG1	B56	1:20	BD, 561283
SOX2	V450	mouse IgG1	O30-678	1:20	BD, 561610
OCT4	PerCP-Cy™5.5	mouse IgG1	40/Oct-3	1:25	BD, 560794
NANOG	Alexa Fluor® 647	mouse IgG1	N31-355	1:20	BD, 561300

Table S2: Antibodies used for flow cytometry. Summary of all antibodies used throughout this study for flow cytometry analysis.

No.	Primer	Primer Sequence	Annealing Temperature	PCR Product size
Pluripotency markers:				
3.	Oct4-F	GAC AGG GGG AGG GGA GGA GCT AGG	57 (°C)	143 bp
4.	Oct4-R	CTT CCC TCC AAC CAG TTG CCC CAA AC		
5.	Sox2-F	GGG AAA TGG GAG GGG TGC AAA AGA GG	57 (°C)	151 bp
6.	Sox2-R	TTG CGT GAG TGT GGA TGG GAT TGG TG		
7.	Nanog-F	TGC CTC ACA CGG AGA CTG TC	58 (°C)	65 bp
8.	Nanog-R	AGG GCT GTC CTG AAT AAG CA		
Housekeeping gene:				
13.	GAPDH-F	ACG AAT TTG GCT ACA GCA ACA GGG	56 (°C)	188 bp
14.	GAPDH-R	TCT ACA TGG CAA CTG TGA GGA GG		
Germ layer-specific markers:				
13.	PAX3-F	CGT CTC CAA GAT CCT GTG C	56 (°C)	91 bp
14.	PAX3-R	AGG CGT TGT CAC CTG CTT		
15.	PAX6-F	CCA GAA AGG ATG CCT CAT AAA GG	58 (°C)	50 bp
16.	PAX6-R	TCT GCG CGC CCC TAG TTA		
17.	KER8-F	TGA GGT CAA GGC ACA GTA CG	60 (°C)	161 bp
18.	KER8-R	TGA TGT TCC GGT TCA TCT CA		
19.	NFH-F	TGA ACA CAG ACG CTA TGC GCT CAG	58 (°C)	400 bp
20.	NFH-R	CAC CTT TAT GTG AGT GGA CAC AGA G		
21.	NESTIN-F	TGC GGG CTA CTG AAA AGT TC	58 (°C)	63 bp
22.	NESTIN-R	TGT AGG CCC TGT TTC TCC TG		
23.	BRACHYURY-F	TGC TTC CCT GAG ACC CAG TT	58 (°C)	121 bp
24.	BRACHYURY-R	GAT CAC TTC TTT CCT TTG CAT C		
25.	BMP4-F	CTG CAA CCG TTC AGA GGT C	58 (°C)	91 bp
26.	BMP4-R	TGC TCG GGA TGG CAC TAC		
27	Card-actin F	TCT ATG AGG GCT ACG CTT TG	50 (°C)	630 bp
28.	Card-actin R	CCT GAC TGG AAG GTA GAT GG		
29.	MSX-1-F	CCT TCC CTT TAA CCC TCA CAC	62 (°C)	287 bp
30.	MSX-1-R	CCG ATT TCT CTG CGC TTT TC		

31.	HNF3-F	GAC AAG TGA GAG AGC AAG TG	56 (°C)	237 bp
32.	HNF3-R	ACA GTA GTG GAA ACC GGA G		
33.	HNF4-F	TCT CAT GTT GAA GCC ACT GC	51 (°C)	501 bp
34.	HNF4-R	GGT TTG TTT CTC GGG TTG A		
35.	SOX17-F	ACG CCG AGT TGA GCA AGA	58 (°C)	82 bp
36.	SOX17-R	TCT GCC TCC TCC ACG AAG		
37.	FOXA2-F	GGG AGC GGT GAA GAT GGA	58 (°C)	92 bp
38.	FOXA2-R	TCA TGT TGC TCA CGG AGG AGT		
NCSC markers:				
39.	AP2-F	AAC ATG CTC CTG GCT ACA AAA	56 (°C)	71 bp
40.	AP2-R	AGG GGA GAT CGG TCC TGA		
41.	SOX9-F	GTA CCC GCA CTT GCA CAA C	56 (°C)	74 bp
42.	SOX9-R	TCT CGC TCT CGT TCA GAA GTC		
43.	p75-F	TCA TCC CTG TCT ATT GCT CCA	56 (°C)	99 bp
44.	p75-R	TGT TCT GCT TGC AGC TGT TC		
SMC markers:				
45.	VE-CADHERIN-F	GAA ACA GAG CCC AGG TCA TTA	59 (°C)	773 bp
46.	VE-CADHERIN-R	GAT GGT GAG GAT GCA GAG TAA G		
47.	αSMA-F	GAT CTG GCA CCA CTC TTT CTA C	59 (°C)	486 bp
48.	αSMA-R	CAG GCA ACT CGT AAC TCT TCT C		
49.	CALPONIN-F	ATG TCC TCT GCT CAC TTC AAC	59 (°C)	420 bp
50.	CALPONIN-R	CAC GTT CAC CTT GTT TCC TTT C		
51.	TRANSGELIN-F	GAA GAA AGC CCA GGA GCA TAA	59 (°C)	410 bp
52.	TRANSGELIN-R	CCA GGA TGA GAG GAA CAG TAG A		

Table S3: Primers for qPCR analysis. Summary of all qPCR primers used throughout this study. All primers were purchased from IDT®, Integrated DNA Technologies.

Specificity	Immunised animal	Clone	Staining dilution	Manufacturer, catalogue number
Primary antibody:				
Anti-α smooth muscle Actin (Alexa Fluor® 488)	Mouse	1A4	1:100	Abcam, ab184675
Anti-Peripherin	Rabbit	(Polyclonal)	1:50	MilliporeSigma, AB1530
Anti-β-Tubulin Isotype III	Mouse	SDL.3D10	1:50	MilliporeSigma, T5076
Secondary antibody:				
FITC Anti-rabbit	Goat	IgG	1:200	Abcam, ab97050
Alexa Fluor 647 Anti-mouse	Goat	IgG	1:200	Abcam, ab150115

Table S4: Antibodies used for ICC. Summary of all antibodies used throughout this study for ICC analysis.