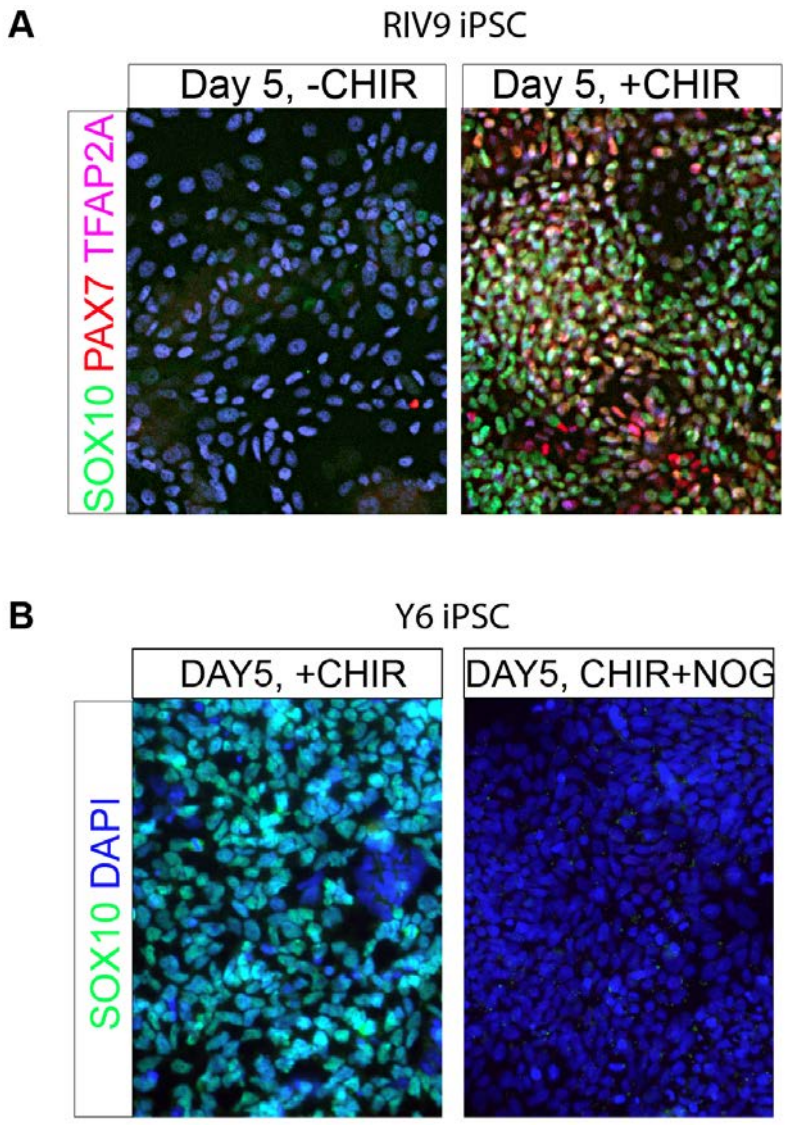

Supplementary figure 1. CHIR 99021 induces neural crest cells in human Y6 iPSCs. SOX10 (green), PAX7 (red) and TFAP2 (violet) triple immunostaining of iPSC RIV9 cultured after dissociation under control (No CHIR) or with CHIR for 5 days. SOX10 staining of day 5 Y6 iPSC cultures treated continuously with 3 μM CHIR and with or without 300 ng/mL NOGGIN from day 0 to day 1.

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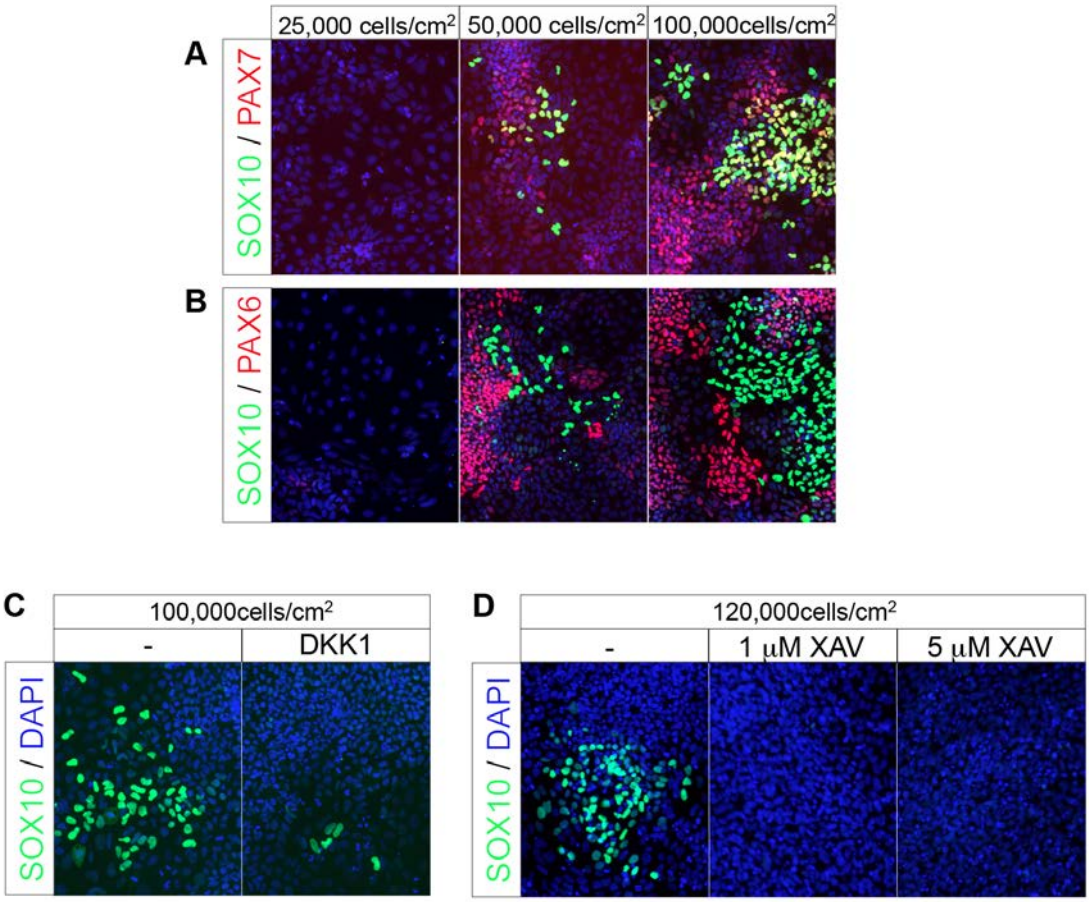

Supplementary figure 2. Spontaneous induction of SOX10$^+$, PAX7$^+$ and PAX6$^+$ cells in high-density cultures. A, B. Dissociated hESCs were plated at the indicated densities and cultured for 5 days in the base medium for NC induction without the administration of CHIR. SOX10/PAX7 (A) and SOX10/PAX6 (B) double labeling were performed. C. Day 5 cultures at the indicated initial seeding density were treated with or without 100 ng/mL DKK1 for 5 days and were stained with SOX10 antibody. D. Differentiating hESCs cultured in base differentiation medium without CHIR were treated with or without XAV 939 (XAV) at the indicated concentrations. Day 5 cultures were collected for SOX10 immunofluorescence.

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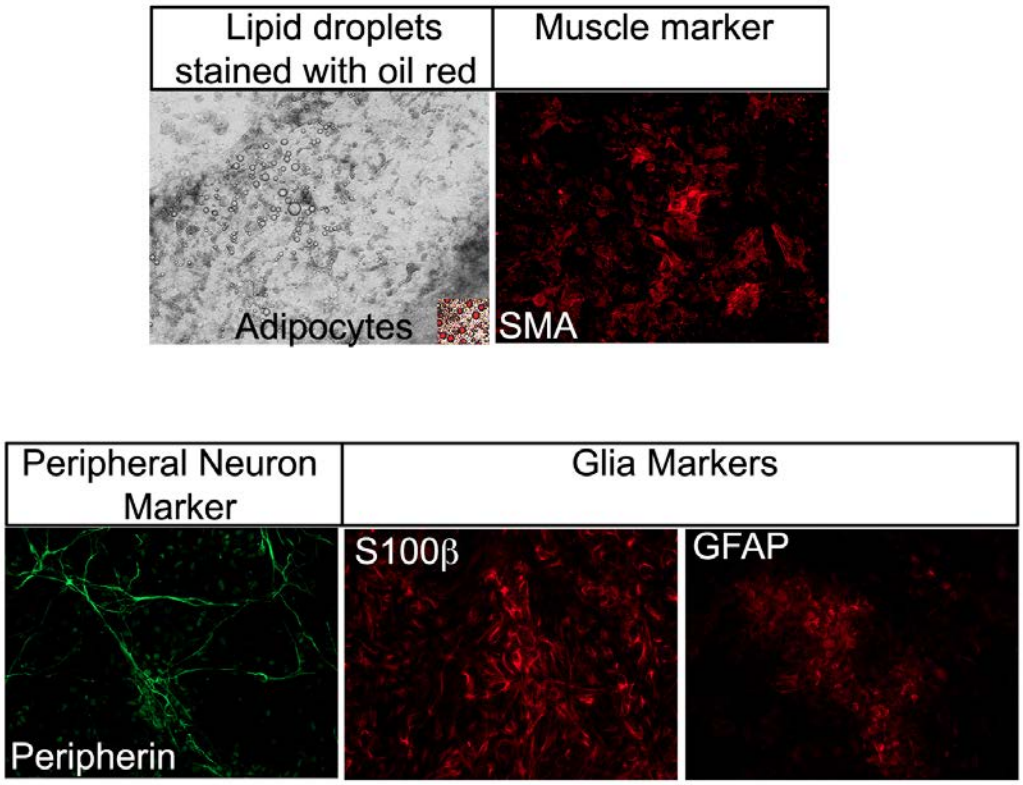

Supplementary figure 3. Terminal neural crest derivatives derived from WNT3a induced neural crest cells. Dissociated hESCs were induced for 5 days in Wnt3a (10 ng/ml) prior to being assayed for their ability to produce neural crest derivatives. Assays were specific to the derivatives of interest.

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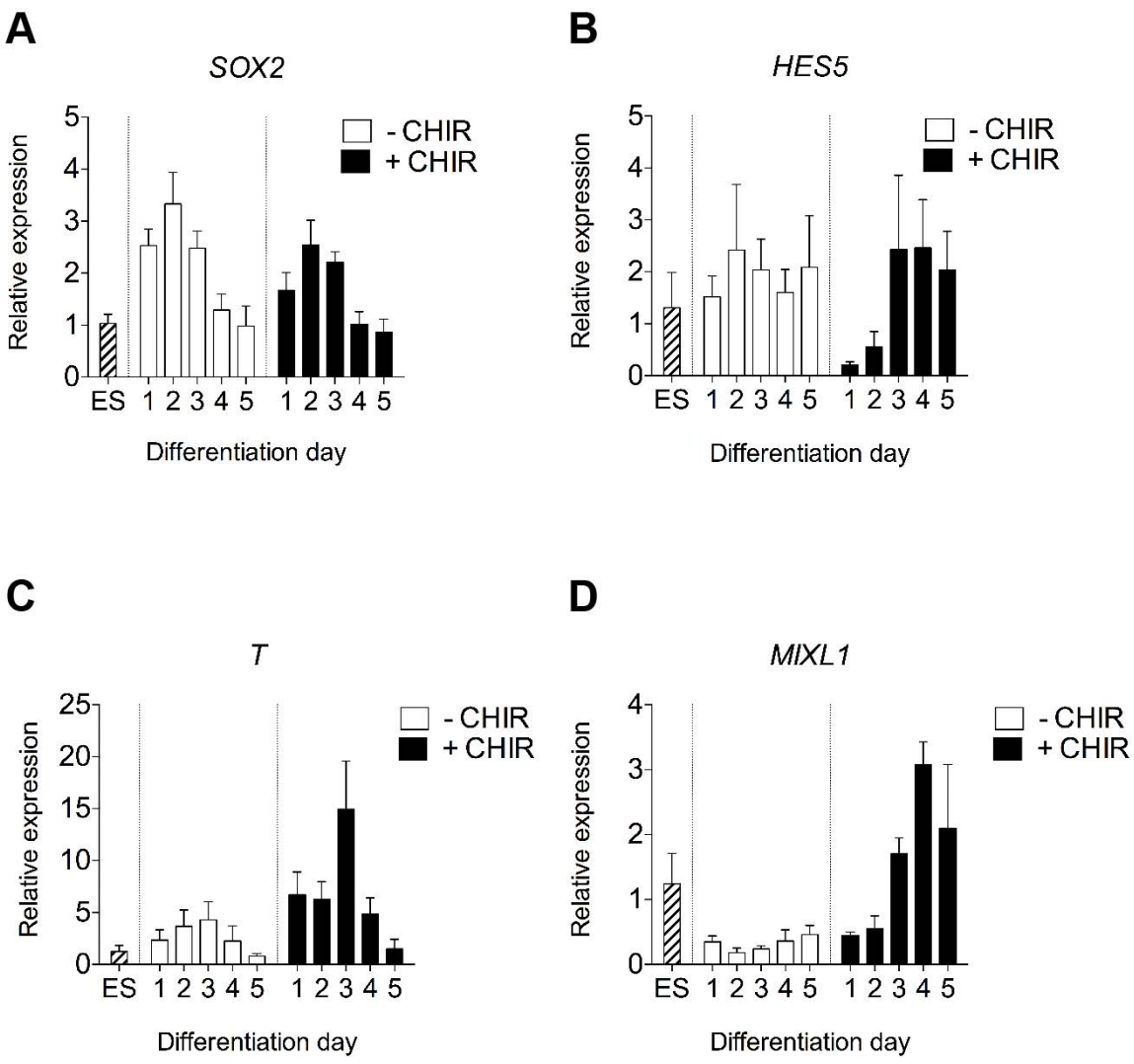

Supplementary figure 4. Expression of neural and mesoderm associated transcripts during human NC induction. A-D. Time course real time PCR assays of *SOX2*, *HES5*, *T* and *MIXL1* during human NC induction.

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Supplementary table 1. Human primer sequences for real-time PCR of gene markers during ESC differentiation.

Transcript	Forward	Reverse
ACTB	TGAACCCCAAGGCCAACCGC	GACCCCGTCACCGGAGTCCA
AXIN2	AGTCAGCAGAGGGACAGGAA	AGCTCTGAGCCTTCAGCATC
CHD1/E-CADHERIN	GGATGTGCTGGATGTGAATG	CTCAAAATCCTCCCTGTCCA
CTNNB1	TCCCACTGGCCTCTGATAAA	AACTAGTCGTGGAATGGCAC
ΔNp63	GGAAAACAATGCCCAGACTC	GTGGAATACGTCCAGGTGGC
DLX5	ACGCTAGCTCCTACCACCAG	TTTGCCATTCACCATTCTCA
FOXD3	GCATCTGCGAGTTCATCAGC	CGTTGAGTGAGAGGTTGTGG
GATA3	GAGGAGGTGGATGTGCTTTT	CAGGGTAGGGATCCATGAAG
HES5	CTCAGCCCCAAAGAGAAAAA	TAGTCCTGGTGCAGGCTCTT
MIXL1	AGCTGCTGGAGCTCGTCTTC	TGGAAGGATTTCCCACTCTG
MSX1	CTGCACCCTCCGCAAACACA	AGGCTGAGCGAGCTGGAGAA
MSX2	CGGTCAAGTCGGAAAATTCA	GAGGAGCTGGGATGTGGTAA
NANOG	CAAAGGCAAACAACCCACTT	TCTGCTGGAGGCTGAGGTAT
NR2F1	CGAGTACAGCTGCCTCAAAG	TACTGGCTCCTCACGTACTC
OCT4	GAAGGATGTGGTCCGAGTGT	GTGAAGTGAGGGCTCCCATA
PAX3	AATTACTCAAGGACGCGGTC	TTCTTCTCGCTTTCCTCTGC
PAX6	CACCTACAGCGCTCTGCCGC	CCCGAGGTGCCCATTGGCTG
PAX7	TGACAGCTTCATGAATCCGG	GATGGAGAAGTCAGCCTGTG
SIX1	TCAGCTCCAAGACTCTCTGC	ACAAGCTGCAAAAATGTTCC
SNAI2	CAGACCCTGGTTGCTTCAAG	GAGCCCTCAGATTTGACCTG
SOX2	TCAAGCGGCCCATGAATGCC	AGCCGCTTAGCCTCGTCGAT
SOX10	CTCTGGAGGCTGCTGAA	TGGGCTGGTACTTGTAGTC
T	AGGCTCCCGTCTCCTTCAGCAA	TGGCTGGTGATCATGCGCTGT
TFAP2A	GATCCTCGCAGGGACTACAG	TACCCGGGTCTTCTACATGC
ZIC1	GTCCTACACGCATCCCAGTT	GCGATAAGGAGCTTGTGGTC
ZIC3	AAGTCTTTCAAGGCGAAGTA	GTCACAGCCTTCAAATTCAC

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Supplementary table 2. Short-hairpin RNA sequences and information for knockdown experiments in hESCs.

Transcripts	ID	RNA consortium Clone ID	Target sequence	Match region	Knockdown efficiency*
CTNNB1	shRNA1	TRCN0000003844	CGCATGGAAGAAATAGTTGAA	CDS	62%
CTNNB1	**shRNA2**	**TRCN0000314921**	**TCTAACCTCACTTGCAATAAT**	**CDS**	**82%**
CTNNB1	shRNA3	TRCN0000010824	CCATTGTTTGTGCAGCTGCTT	CDS	46%
PAX6	shRNA1	TRCN0000016123	GCAAGAATACAGGTATGGTTT	CDS	74%
PAX6	shRNA2	TRCN0000016125	CGTCCATCTTTGCTTGGGAAA	CDS	68%
PAX6	**shRNA3**	**TRCN0000246162**	**ATACGCACTGTTGGTACAATT**	**3UTR**	**97%**

*Knockdown efficiency was measured by real-time PCR in undifferentiated hESCs and differentiated ESC cultures at day 5 for *CTNNB1* and *PAX6* respectively. The species with the highest knockdown were bolded and selected for functional analyses.

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Supplementary table 3. Antibodies used for immunofluorescence.

Protein/Gene	Source	Catalog no.	Antibody type	Dilution
PAX7	Developmental hybridoma bank	Pax7	Mouse IgG1	1:50
PAX6	Developmental hybridoma bank	Pax6	Mouse IgG1	1:100
TFAP2A	Developmental hybridoma bank	3B5	Mouse IgG2b	1:50
ISL1	Developmental hybridoma bank	Isl1/2	Mouse IgG2b	1:100
SOX10	Vivian Lee	-	Rabbit	1:3000
POU5F1 (OCT4)	Santa Cruz Biotechnology	SC-5279	Mouse IgG2b	1:250
T (BRACHYURY)	R&D Systems	AF2085	Goat	1:200
HuC/D	Molecular probe	A-21271	Mouse IgG2b	1:300
S100β	Thermo-Scientific	MA1-2505	Mouse IgG1	1:100
MITF	R&D Systems	AF5769	Goat	1:50
PRPH	Millipore	AB1530	Rabbit	1:200
TUJ1	Covance	MMS-435P-250	Mouse IgG2a	1:2000
GFAP	Covance	SMI-22R	mouse IgG2b	1:500

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