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Supplemental Information

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Supplementary Table S1. qPCR Primers

Target Name	Target type	Target size	Target Sequence
SOX10	NC lineage (not neurons)	214 bp	F: TCTGGAGGCTGCTGAACGAA
			R: AAGTGGGCGCTCTTGTAGTG
ASCL1	SAP marker	188 bp	F: TCCCCCAACTACTCCAACGA
			R: GCGATCACCCTGCTTCCAAA
MYCN	SAP marker	123 bp	F: GAGAGGACACCCTGAGCGATTCA
			R: ATGTGGTGACAGCCTTGGTGTTGG
PHOX2B	SAP marker	88 bp	F: ACGCCGCAGTTCCTTACAAA
			R: CTGGTGAAAGTGGTGCGGAT
HAND2	SAP marker	105 bp	F: GGCAGAGATCAAGAAGACCGAC
			R: CGGCCTTTGGTTTTCTTGTCGTT
RET	Neurotrophic factor receptor	200 bp	F: GAGGAGAGACTACTTGGACCTTG
			R: GGGGACAGCGGTGCTAGAAT
α-TH	CA synth. enzyme	196 bp	F: GTGTTCCAGTGCACCCAGTA
			R: ACCAGTACAGCGTGGACAGCTTCT
DβH	CA synth. enzyme	139 bp	F: GCCATCCATTTCCAGCTCCT
			R: TCCAGGCGTCCGCAAAATAG
PNMT	Chromaffin-spec. adrenaline enzyme	200 bp	F: GCCTACCTCCGCAACAACTA
			R: GTCATGGTGATGTCCTCAAAGT
HOXB1	Hindbrain positional	143 bp	F: TGCCCTTCAGAACCTAACACCC
			R: AGCTGCCTTGTGGTGAAGTTGG
HOXB2	Hindbrain positional	75 bp	F: GGCCTCTCCCCTAGCCTACA
			R: GGTGAAAAAATCCAGCTCTTCCT
HOXB3	Hindbrain positional	106 bp	F: CCTTCGTCATGAATGGGATCTG
			R: ATATTCACATCGAGCCCCAGAG
HOXB4	Hindbrain/vagal positional	95 bp	F: GAGCACGGTAAACCCCAATTAC
			R: GAAATTCCTTCTCCAGCTCCAA
OCT4	Pluripotency marker	131 bp	F: AAAGCTCTGCAGAAAGAACTCG
			R: GTCGTTTGGCTGAATACCTTCC
SOX2	Pluripot. & CNP marker	220 bp	F: ATGGACAGTTACGCGCACAT
			R: GCTGCGAGTAGGACATGCTG
GATA3	SAP marker	131 bp	F: TAACATCGACGGTCAAGGCAAC
			R: GTAGGGATCCATGAAGCAGAGG
β2M	Housekeeping	86 bp	F: TGCTGTCTCCATGTTTGATGTATCT
			R: TCTCTGCTCCCCACCTCTAAGT

GAPDH	Housekeeping	131 bp	F: GTCTCCTCTGACTTCAACAGCG
			R: ACCACCCTGTTGCTGTAGCCAA

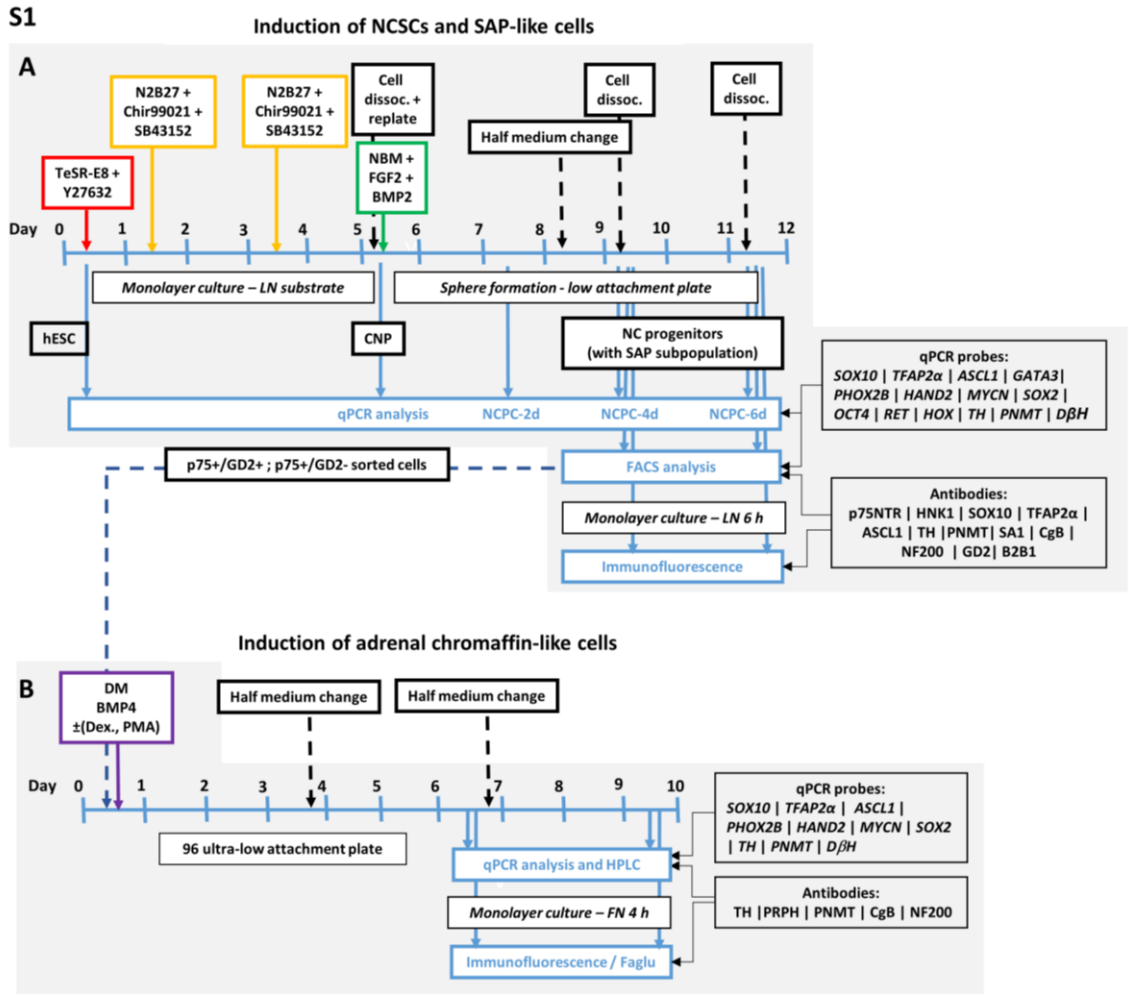
Supplementary Table S2. Primary and secondary antibodies, and probes.

Primary antibodies	**Target type**	**Host / Isotype**	**Company**	**Dilution**
Anti-Human SOX10	NC lineage (not neurons)	Goat Polyclonal	R&D systems	1:200
Anti-p75NTR (D4B3) XP® Rabbit mAb #8238	NC lineage	Rabbit polyclonal	Cell Signalling	1:1500
Anti-HNK1	NC lineage	Mouse IgM	MCRI	1:200
Anti-AP2α (3B5)	NC lineage	Mouse IgG2b	DSHB	1:200
Anti-GD2 (clone 14.G2a)	SAP marker	Mouse IgG_{2a}	BD Biosciences	0.5 µg / $1x10^6$ cells
Anti-MASH1/Achaete-scute homolog 1 antibody (ab38556)	SAP marker	Rabbit polyclonal	Abcam	1:500
Anti-Peripherin (clone 8G2)	SAP &. neuron marker	Mouse IgG	Millipore	1:500
Anti-SA1	SAP lineage marker	Mouse IgG	DSHB	1:20
Anti-B2B1	Symp. neuron lineage	Mouse IgM	DSHB	1:20
Anti-Ki67 (AFFN-KI67-3E6)	Proliferative marker	Mouse IgG2b	DSHB	1:50
Anti-Neurofilament Heavy antibody (NF421)	Neuron marker	Mouse IgG1	Abcam	1:500
Anti-Neurofilament M (145 kDa) Antibody	“	Rabbit polyclonal	Millipore	1:2000
Anti-TH	Neuron CA synth. enz.	Chicken polyclonal	Abcam	1:1500
Anti-TH	Neuron CA synth. enz.	Rabbit polyclonal	Novus Biologicals	1:1500
Anti-Chromogranin B (ab12242)	Neuroendocrine marker. LDCV	Rabbit polyclonal	Abcam	1:700
Anti-Chromogranin C (ab12241)	Neuroendocrine marker. LDCV	Rabbit polyclonal	Abcam	1:700
Anti-PNMT	Chromaffin-spec. adrenaline enzyme	Rabbit polyclonal	ThermoFisher Sci.	1:700
Anti-Human Nuclear Antigen antibody [235-1] (ab191181)	Human-specific marker	Mouse monoclonal IgG	Abcam	1:200
Anti-Mitochondria antibody [113-1] (ab92824)	Human-specific marker	Mouse monoclonal IgG	Abcam	1:1500
Phospho-Smad1/5 (Ser463/465) (41D10)	BMP signaling	Rabbit monoclonal	Cell Signalling	1:100
Secondary antibodies		**Host / Isotype**	**Company**	**Dilution**
Anti-Goat IgG:Alexa 488		Donkey	ThermoFisher Sci.	1:1000
Anti-Goat IgG:Alexa 594		Donkey	ThermoFisher Sci.	1:1000
Anti-Goat IgG:Alexa 647		Donkey	ThermoFisher Sci.	1:1000
Anti-Sheep IgG:Alexa 594		Donkey	ThermoFisher Sci.	1:1000

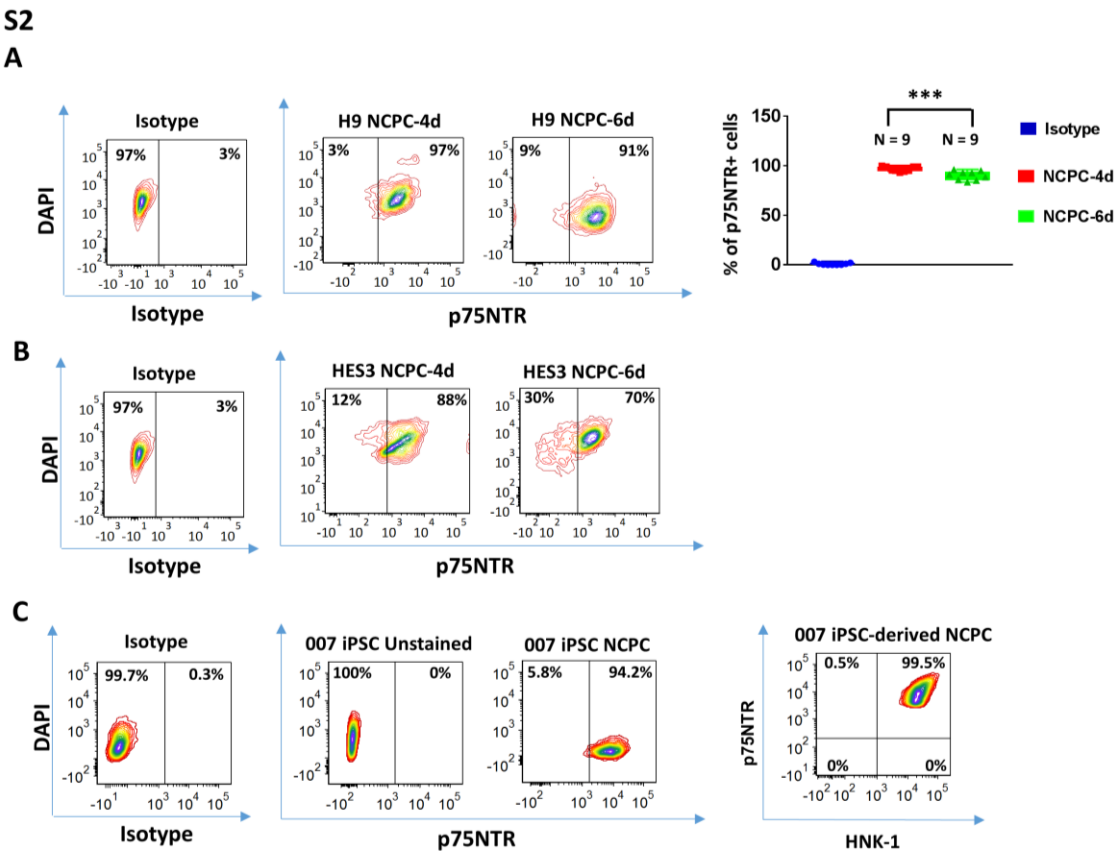
Anti-Rabbit IgG Alexa 488	Donkey	ThermoFisher Sci.	1:1000
Anti-Rabbit IgG Alexa 594	Donkey	ThermoFisher Sci.	1:1000
Anti-Rabbit IgG Alexa 633	Goat	ThermoFisher Sci.	1:1000
Anti-Mouse IgG-specific:Alexa 488	Goat	ThermoFisher Sci.	1:1000
Anti-Mouse IgM- specific:Alexa 488	Goat	ThermoFisher Sci.	1:1000
Anti-Mouse IgG+M Alexa 594	Donkey	Mol. Probes (A21203)	1:1000
Anti-Mouse IgG Alexa 647	Donkey	ThermoFisher Sci.	1:1000
Anti-Chick IgY Alexa 488	Goat	ThermoFisher Sci.	1:1000
Anti-Chick IgY Alexa 568	Goat	ThermoFisher Sci.	1:1000

Probes	**Target type**	Product size	**Company**	**Accession Number**
HOXA2	Hindbrain positional	107	ThermoFisher Sci.	Hs00534579_m1
HOXA5	Vagal positional	127	ThermoFisher Sci.	Hs00430330_m1
HOXA7	Trunk positional	131	ThermoFisher Sci.	Hs00600844_m1
HOXA10	Trunk positional	52	ThermoFisher Sci.	Hs00172012_m1
HOXB7	Trunk positional	66	ThermoFisher Sci.	Hs04187556_m1
TFAP2α	NC lineage	73	ThermoFisher Sci.	Hs01029413_m1
β2M	Housekeeper	64	ThermoFisher Sci.	Hs00187842_m1

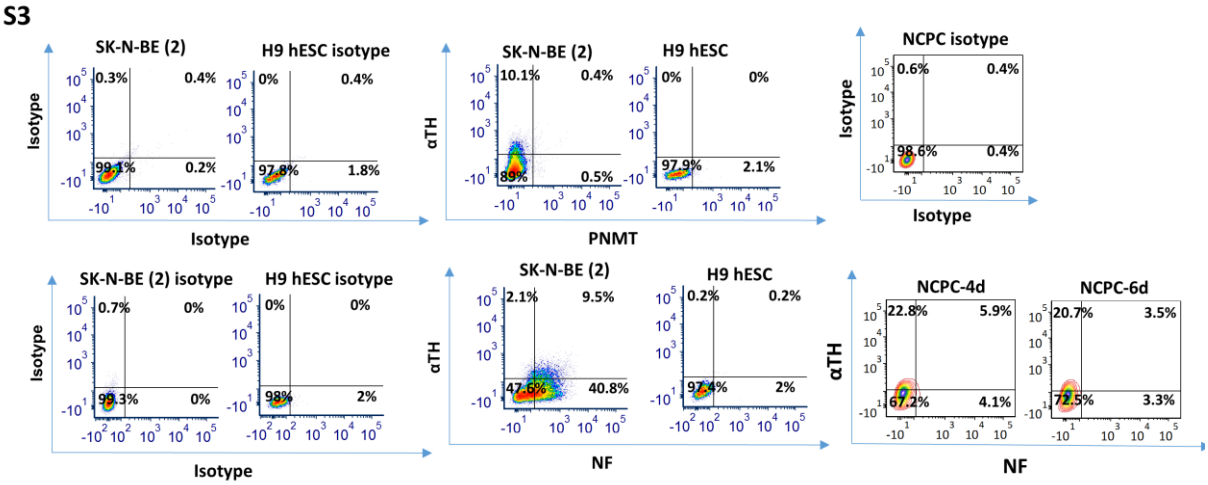
Supplementary Figures



Supplementary Figure S1. (A & B) Schematic illustration of the differentiation protocol of human pluripotent cells to NCPC/SAP-like cells and further to chromaffin-like cells. Detailed extension of Figure 1A, B. **Related to time points in Figure 1**.

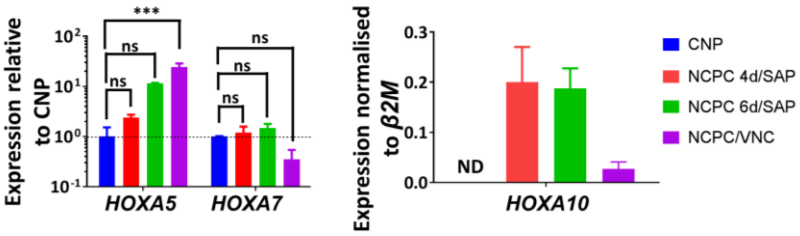


Supplementary Figure S2. **Induced human pluripotent cells express NC markers reliably and similarly in several cell lines. Related to Figure 1B. (**A) Proportion of p75NTR+ cells was very reproducible (9 separate NCPC-4d and -6d derivations of H9 cells). FACS analysis of (B) HES3 hESCs at NCPC-4d and -6d and (C) NCPC-6d only of 007 iPSC showing NCPC marker, p75NTR, similar to H9-derived cells. The p75NTR+ 007 cell population was used to gate for HNK-1 expression. Representative plots: NCPC-4d; N = 10 independent experiments and NCPC-6d; N = 10 independent experiments for HES3 hESC and NCPC-6d; N=4 independent experiments for 007 iPSC. Error bars represent mean ± SEM. ns- not significant, *P > 0.05, **P > 0.01, ***P > 0.001, ****P > 0.0001.



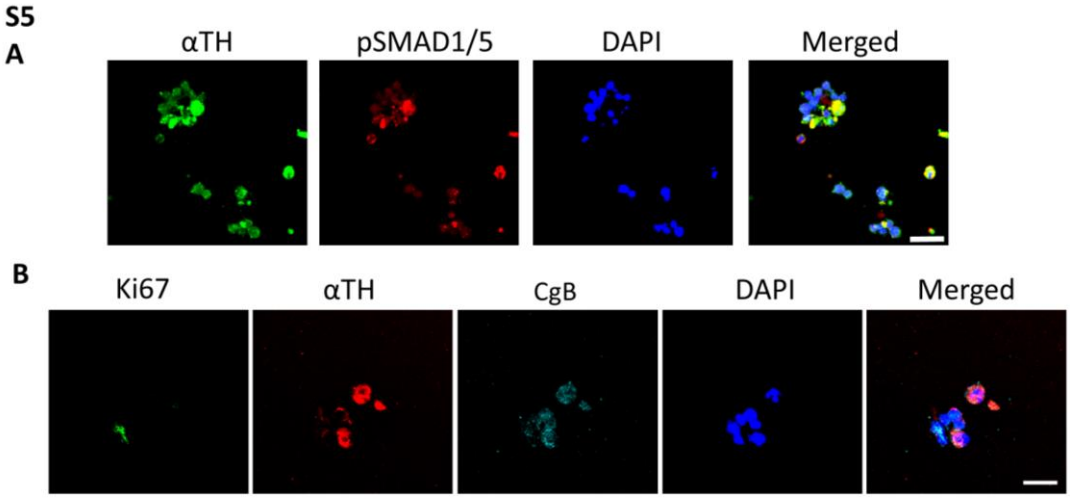
Supplementary Figure S3. Neuroblastoma line SK-N-BE (2), negative control cells (H9 hESC) and NCPC-4d and 6d FACS analysed for αTH, NF-200 kDa and PNMT. Related to Figure 2 and 4. A representative FACS analysis of SK-N-BE (2) showing the expression of αTH and NF-200 kDa with no expression of PNMT. H9 hESC showing no expression of αTH, NF-200 kDa and PNMT. NCPC-4d and 6d showing an expression for αTH and NF-200kDa. DAPI was used to gate for cells. N = 3 independent experiments for both cell types.

S4

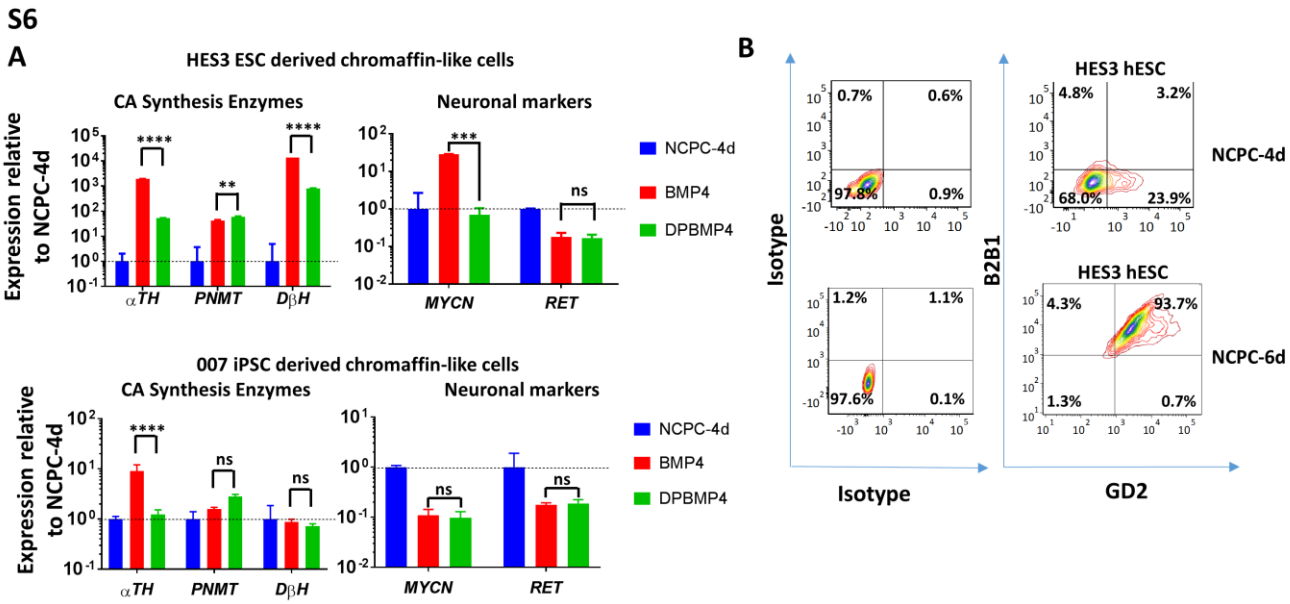


Supplementary Figure S4. NCPC/SAP express higher number (trunk) *HOX* genes compared to NCPC/vagal NC. Related to Figure 2. QPCR analysis of NCPC/SAP showing the increased expression of *HOXA7* and *HOXA10* trunk positional markers compared to NCPC/vagal NC with elevated *HOXA5* vagal positional marker. N = 3 independent experiments. Error bars represent mean ± SEM. ns- not significant, *$P > 0.05$, **$P > 0.01$, ***$P > 0.001$, ****$P > 0.0001$.

S5

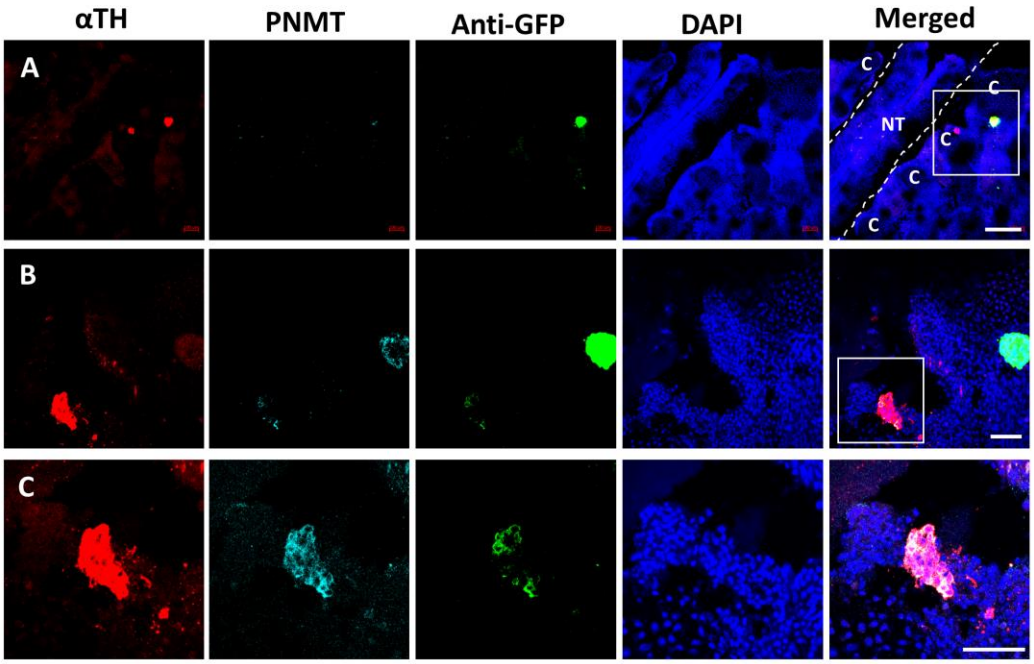


Supplementary Figure S5. The downstream signaling effector of BMP4, SMAD1/5, is activated using 50 pg/mL of BMP4 in NCPC/SAP differentiation to chromaffin-like cells and these cells have limited proliferative capacity. Related to Figure 3. (A) Immunofluorescence of chromaffin-like cells in vitro showing the expression of pSMAD1/5, and αTH. Scale bar 20 µm. (B) Immunofluorescence of nuclear Ki67 in αTH+/CgB+ chromaffin-like in a cell aggregate after 9 days chromaffin differentiation. Note that Ki67 signal here is in cells with low αTH and CgB. Scale bar 20 µm.



Supplementary Figure S6. HES3 hESC- and 007 iPSC-derived in chromaffin induction conditions increase expression of mRNA of CA synthesizing enzymes and decrease expression of *RET* and HES3 hESC-derived NCPC cells express markers of SAP and pro-neuronal differentiation. Related to Figure 3 and 5. (A) CA enzyme mRNAs (especially with BMP4 alone) were upregulated similarly in HES3 and 007s (and H9s) with the exception of *DβH* in 007s. Neuronal differentiation marker *MYCN* was reduced in 007s paralleling that in H9s in contrast to upregulation with BMP4 in HES3 cells. The upregulation of *RET* mRNA seen in H9 cells was not shown in these cell lines. N = 3 independent experiments. Error bars represent mean ± SEM. ns- not significant, *$P > 0.05$, **$P > 0.01$, ***$P > 0.001$, ****$P > 0.0001$. (B) FACS analysis of HES3 NCPC-4d and -6d cells (initially gated by p75NTR expression). GD2 (SA lineage marker) appears in fewer NCPC-4d cells compared to H9 cells at the same stage, but later (NCPC-6d) many more HES3 GD2+ cells co-express the pro-neuronal marker B2B1, compared to H9-derived cells. Representative FACS plots: NCPC-4d, N = 10 and NCPC-6d, N = 10.

S7



Supplementary Figure S7. Transplanted NCPC/SAP cells *in vivo* integrate, migrate and differentiate into cells expressing chromaffin markers, αTH and PNMT. Related to Figure 7. (row A) Human GFP+ NCPC/SAP-like cells grafted into 2 day quail embryo trunk displace latero-ventrally and show SAP and chromaffin markers. Frontal-oblique section of ventral neural tube (NT) and somitic vertebral cartilage (C) in 4-day transplant. Scale bar 50 µm. (row B) Boxed area in A is enlarged (next section ventral to A) to show human cells stained for αTH and PNMT and identified with anti-GFP. Scale bar 250 µm. (row C) Enlargement of area boxed in B showing co-expression of SAP and chromaffin markers. Scale bar 250 µm.