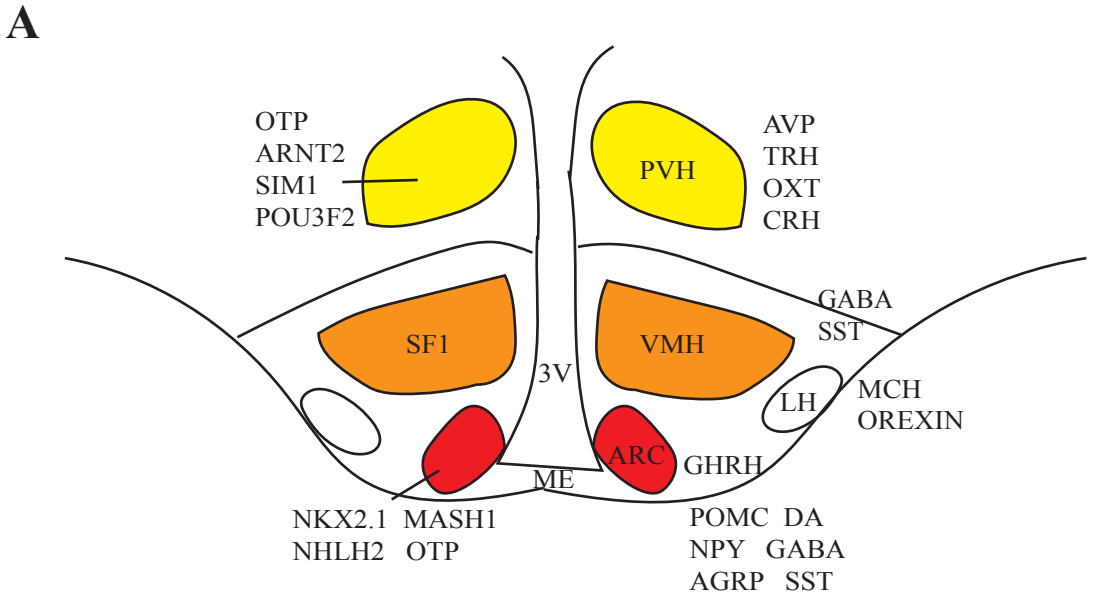
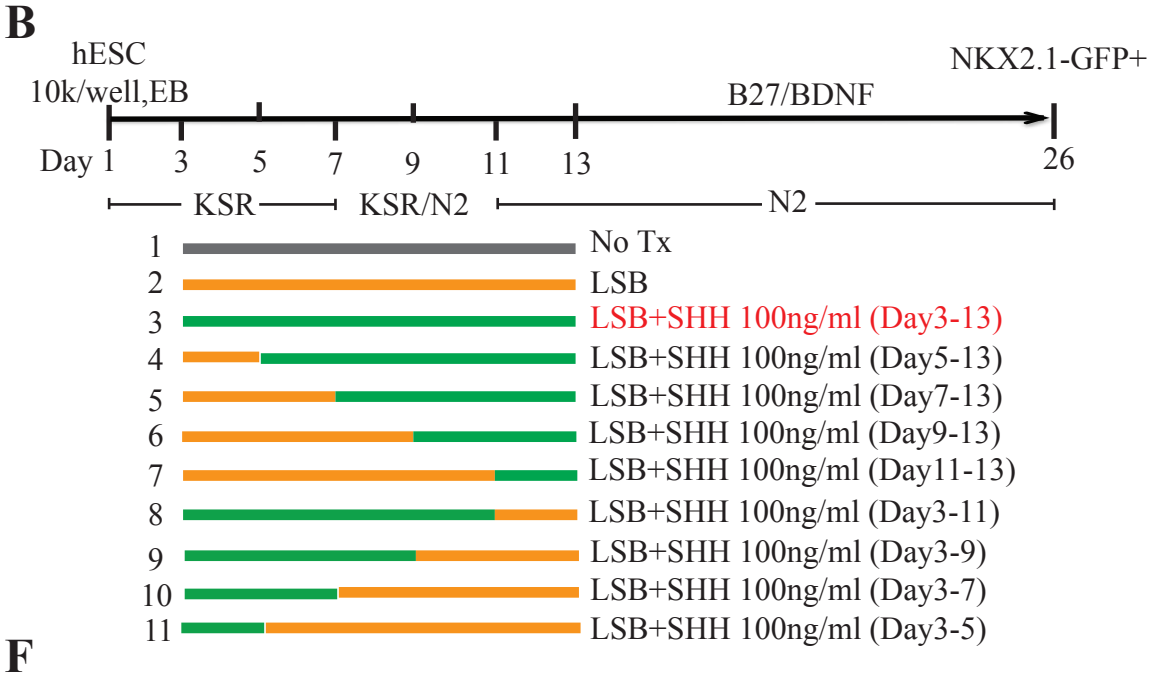
Table S1. qPCR primer list.

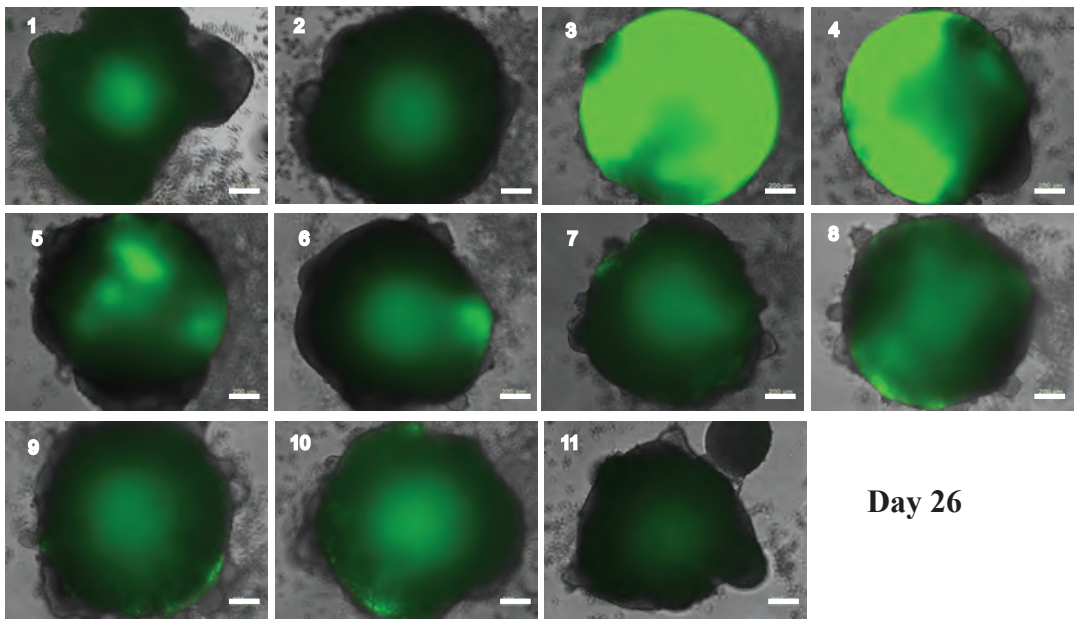
Genes	Forward primer	Reverse primer
NANOG	ACAACTGGCCGAAGAATAGCA	GGTTCCCAGTCGGGTTCAC
PAX6	TCTTTGCTTGGGAAATCCG	CTGCCCGTTCAACATCCTTAG
ASCL1	CAACGCCACTGACAAGAAAG	GGAGCTTCTCGACTTCACCA
NHLH2	GTCCGGACTCAGCATCATTT	ATATTTTCCGGAATCTCCCCT
NKX2.1	AACCAAGCGCATCCAATCTCAAGG	TGTGCCCAGAGTGAAGTTTGGTCT
OTP	GGAACACGTTGGTCGTCTTT	TTCGCCAAGACTCACTACCC
SIM1	CTTTCTGTGTGAAATCCCGAA	CCCACCATGACTGACAACAG
SF1	CCTCGCTATTGTAGATGGGC	GACCTGACTCGTAAACTGCG
NESTIN	GGCGCACCTCAAGATGTCC	CTTGGGGTCCTGAAAGCTG
POMC	GACACTGGCTGCTCTCCAG	AGCAGCCTCCCGAGACA
AGRP	GGATCTGTTGCAGGAGGCTCAG	TGAAGAAGCGGCAGTAGCACGT
NPY	GGTCTTCAAGCCGAGTTCTG	AACCTCATCACCAGGCAGAG
LEPR	ATGTTCCGAACCCCAAGAAT	GGACCACATGTCACTGATGC
SST	CCAGACTCCGTCAGTTTCTGCA	TTCCAGGGCATCATTCTCCGTC
PMCH	ATTGGGGATGAAGAAAACTCAGCT	GACTTGCCAACAAGGTCGGTAG
PCSK1	ACCAGGTGCTGCATATCTCG	CACAATGACTGCACGGAGAC
PCSK2	TTTCGGTCAAATCCTTCCTG	TGCAAAGGCCAAGAGAAGAC
CPE	TAAATTCAGGCTCACCAGGC	CCATCAGCAGGATTTACACG
MC2R	CAGTAGGGGTTACTTGGGCA	CCATCACACTGACCATCCTG
MC3R	GGCTTGATGAAGACCTGCTC	TCAGCCAACACTGCCTAATG
MC4R	CTTATGATGATCCCAACCCG	GTAGCTCCTTGCTTGCATCC
OPRM1	TGGTGGCAGTCTTCATCTTG	GATCATGGCCCTCTACTCCA
OPRD1	GTAGATGTTGGTGGCCGTCT	ATCACCGCGCTCTACTCG
TBP	AACAACAGCCTGCCACCTTA	GCCATAAGGCATCATTGGAC
B2M	TAGCTGTGCTCGGGCTACT	TCTCTGCTGGATGACGCG

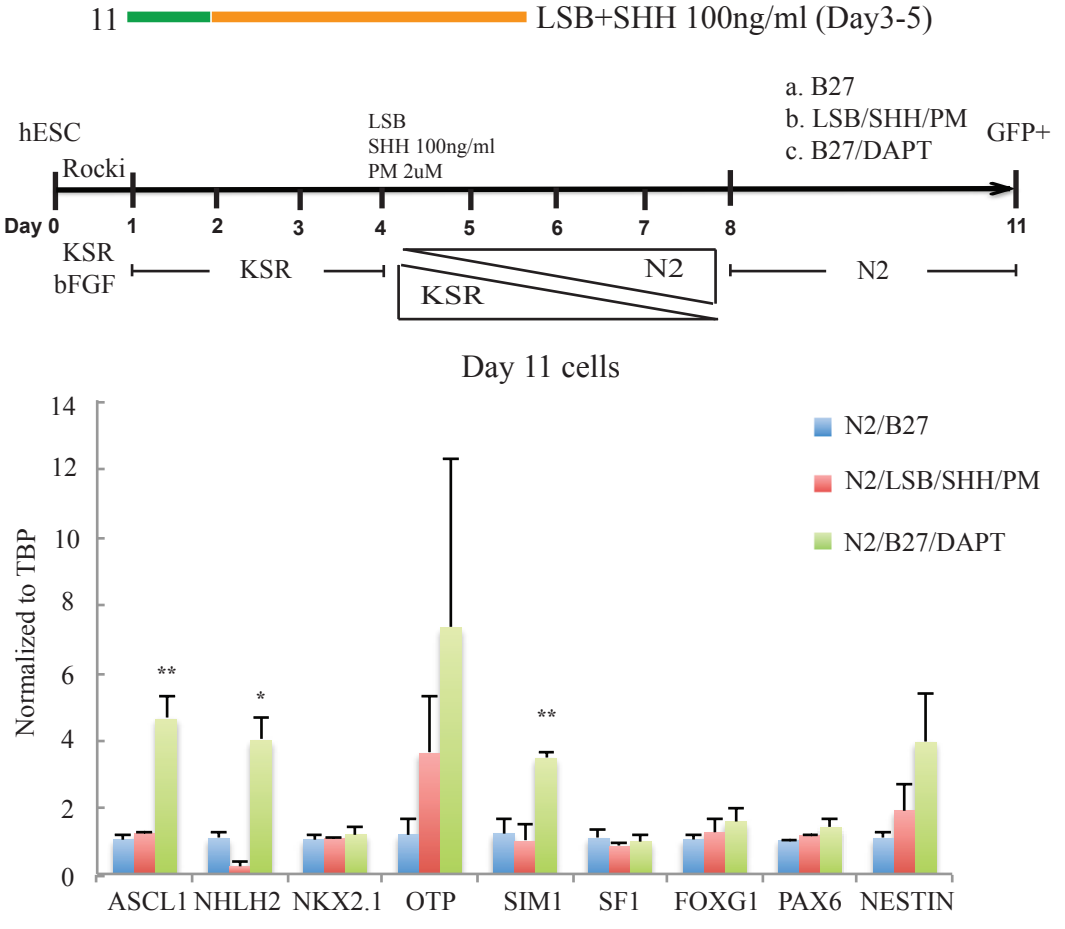
Table S2. List of primary Abs and their concentrations and sources used in this study.

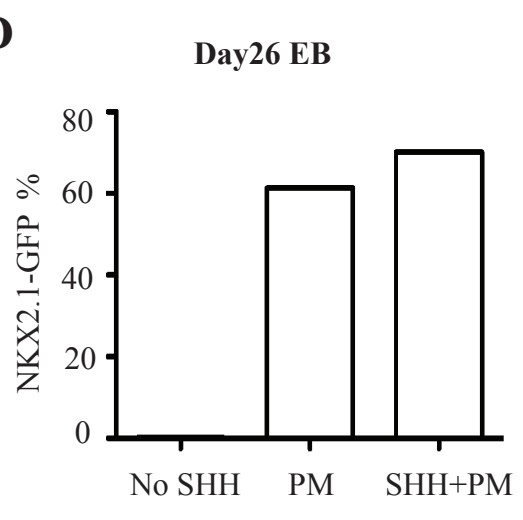
Antibody	Species	Dilution	Source	Catalog No.
αMSH	Sheep	1:1000	Millipore	AB5087
POMC	chicken	1:500	Abcam	ab14064
NPY	Rabbit	1:1500	Novus	npb1-19808
Somatostatin	Rat	1:500	Abcam	ab30788
MCH	Rabbit	1:500	Sigma	M8440
AGRP	Rabbit	1:200	Phoenix Pharmaceuticals	H-003-53
AGRP	Goat	1:500	Neuromics	GT15023
TH	Rabbit	1:500	Abcam	ab76013
GABA	Rabbit	1:500	Sigma	A2052
GAD67	Mouse	1:500	Millipore	MAB5406
Mash1	Rabbit	1:200 WB 1:500	Abcam	ab74065
Pax6	Rabbit	1:100 WB 1:500	Covance	PRB-278P
Nkx2.1(TTF1)	Rabbit	1:200 WB 1:1000	Abcam	ab76013
FoxG1	Rabbit	1:200	Abcam	ab18259
Sox1	Goat	1:500	R&D	AF3369
Nestin	Mouse	1:500	Stemgent	09-0045
β endorphin	Rabbit	1:200	From Sharon Wardlaw	
CPE	Goat	1:300	R&D	AF3587
MAP2	Chicken	1:10,000	Abcam	AB5392
Tra-1-60	Mouse	1:300	Millipore	MAB4360
Tra-1-81	Mouse	1:300	Millipore	MAB4381
Nanog	Goat	1:500	R&D Systems	AF1997
Sox2	Rabbit	1:500	Stemgent	09-0024
Oct4	Rabbit	1:500	Stemgent	09-0023
SSEA4	Mouse	1:300	R&D Systems	MAB1435
p-STAT3	Mouse	1:200 WB 1:1000	Cell signaling	4113
p-STAT3	Rabbit	1:200 WB 1:1000	Cell signaling	9145
p-AKT(T308)	Rabbit	WB 1:1000	Cell signaling	9275
p-AKT(S473)	Rabbit	WB 1:1000	Cell signaling	4060
C-FOS	Rabbit	1:200	Cell signaling	2250
STAT3	Rabbit	WB 1:1000	Cell signaling	12640
AKT	Rabbit	WB 1:1000	Cell signaling	9272
ACTIN	Rabbit	WB 1:1000	Cell signaling	8457











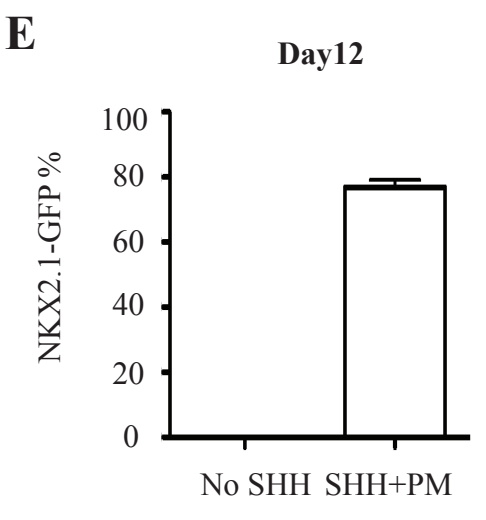


Figure S1. Early activation of SHH signaling and subsequent inhibition of Notch signaling induces the production of hypothalamic NKX2.1 progenitors.
(A) Illution of the adult medial hypothalamus with region-related gene expression of hypothalamic markers based on published data(1, 16-21). ARC: arcuate nucleus, VMN: ventromedial nucleus, PVN: paraventricular nucleus, LH: lateral hypothalamus, ME: median eminence. Markers on the left indicate the transcription factors expressed while markers on the right display the gene expressed in specific hypothalamic nuclei; (B-C) The timing of SHH influences the expression of NKX2.1-GFP in day 26 differntiated EBs. 11 conditions and 9 timings of SHH were indicated in B while the actual expression of NKX2.1-GFP were shown in B for each condit; (D-E) FACS analysis of the percentage of NKX2.1-GFP cells in day 26 EBs treated with no SHH, 2uM Purmorphamine (PM) and SHH 100ng/ml + 2μM PM from days 3 to 13 (D) and day 12 cells. N=1 for each bar; (E) from monolayer feeder-free differentiation with (n=3) or without (n=1) SHH 100ng/ml + 2uM PM (from days 1-8). LSB were used in both cases; (F) qPCR of transcription factor genes in day 11 cells from three conditions (only differ from day 8 to day 11). N=3 for each bar.

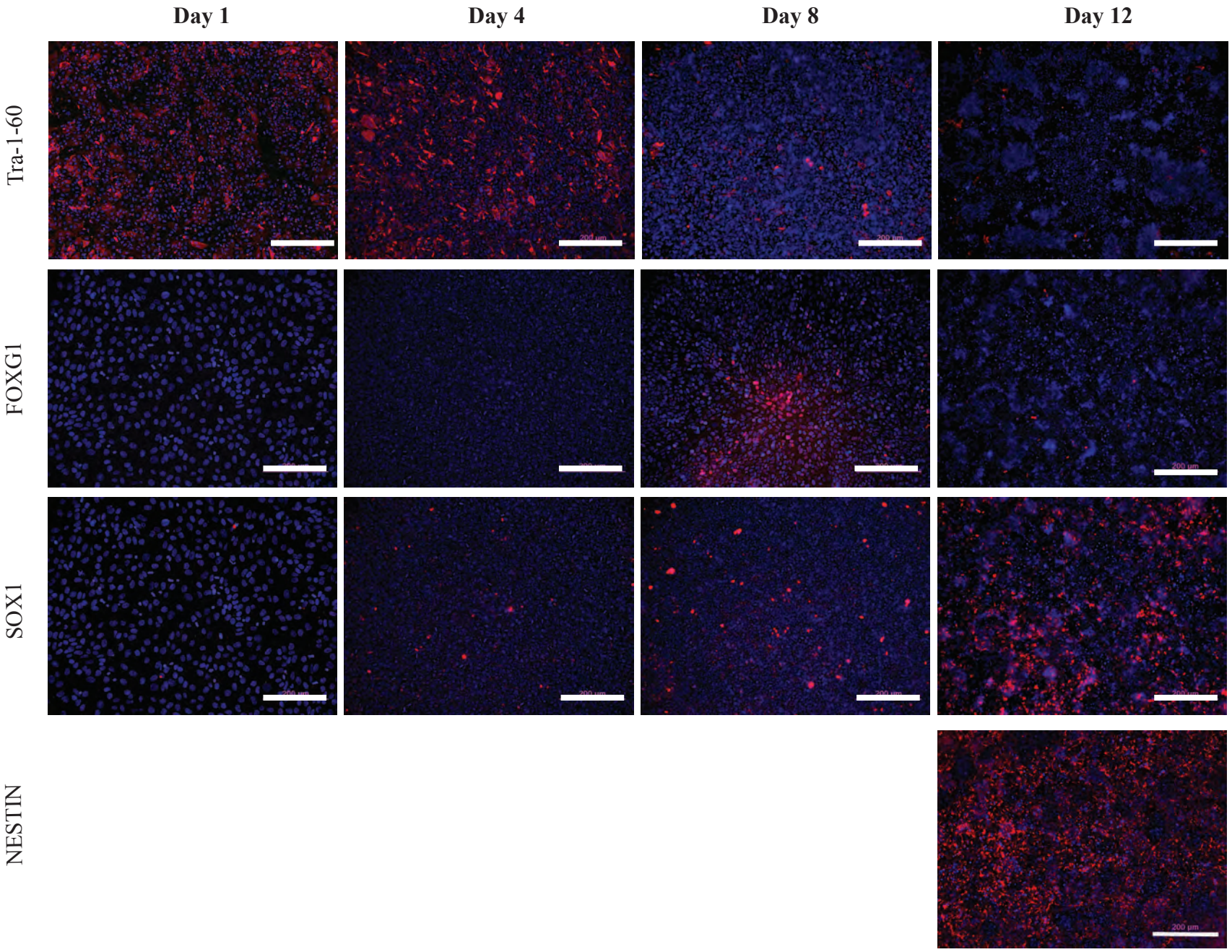


Figure S2. Temporal expression of transcription factors in early hypothalamic neuron induction and patterning.
Immunocytochemical analysis of neuron progenitor markers and stem cell marker in cells of 1, 4, 8 and 12 days of differentiation. Tra-1-60 is a pluripotency marker. FOXG1 is a telencephalon progenitor markers. SOX1 and NESTIN are general markers for neuron progenitors. Scale bar, 200µm.

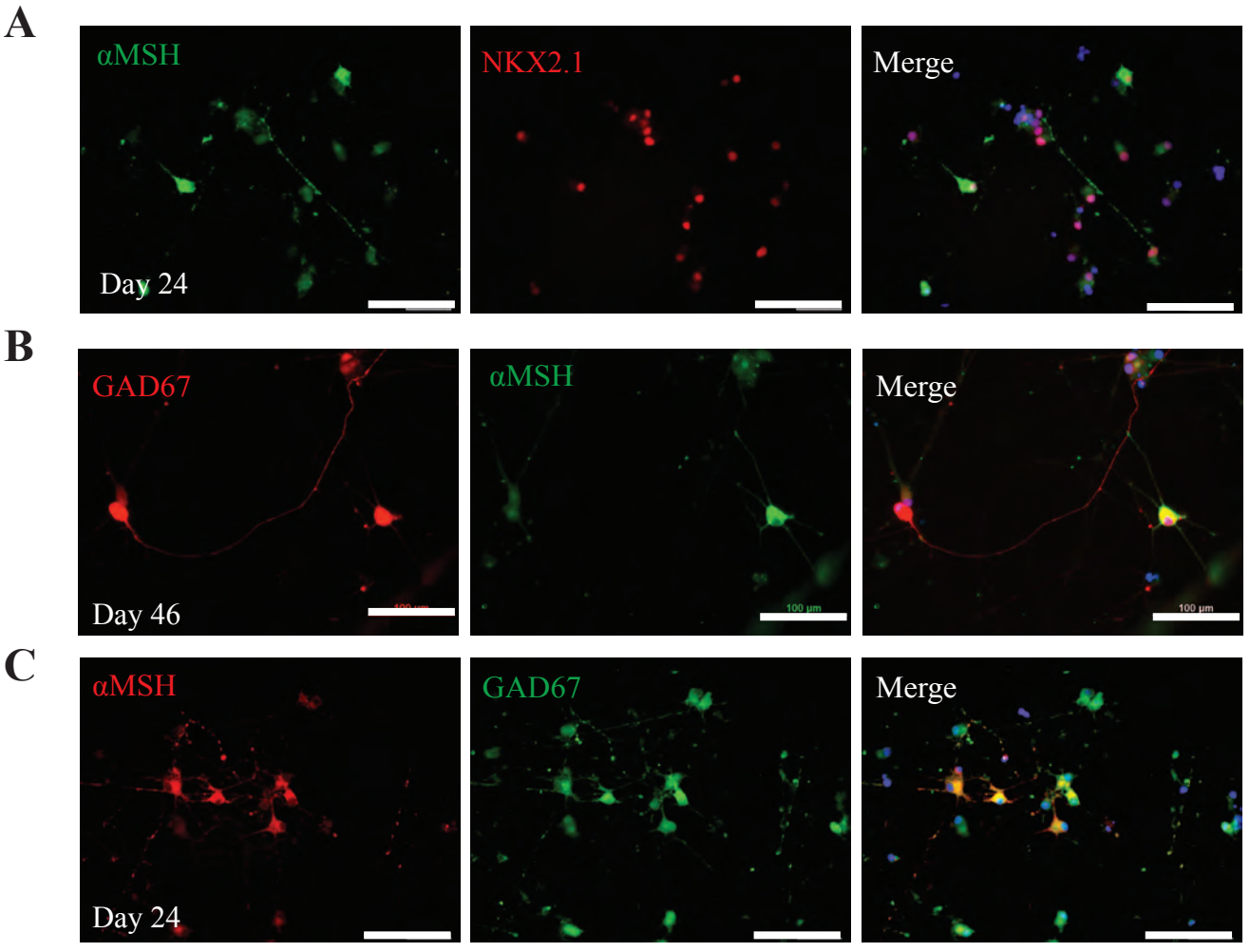


Figure S3. hESC-derived neurons express hypothalamic neuron markers.
(A) Immunocytochemical analysis of day 24 differentiated neurons with alphaMSH and NKX2.1; (B) Immunostaining of day 46 neurons with αMSH and GABA; (C) Immunocytochemical analysis of day 24 neurons with alphaMSH and GAD67. Scale bar,100μm.

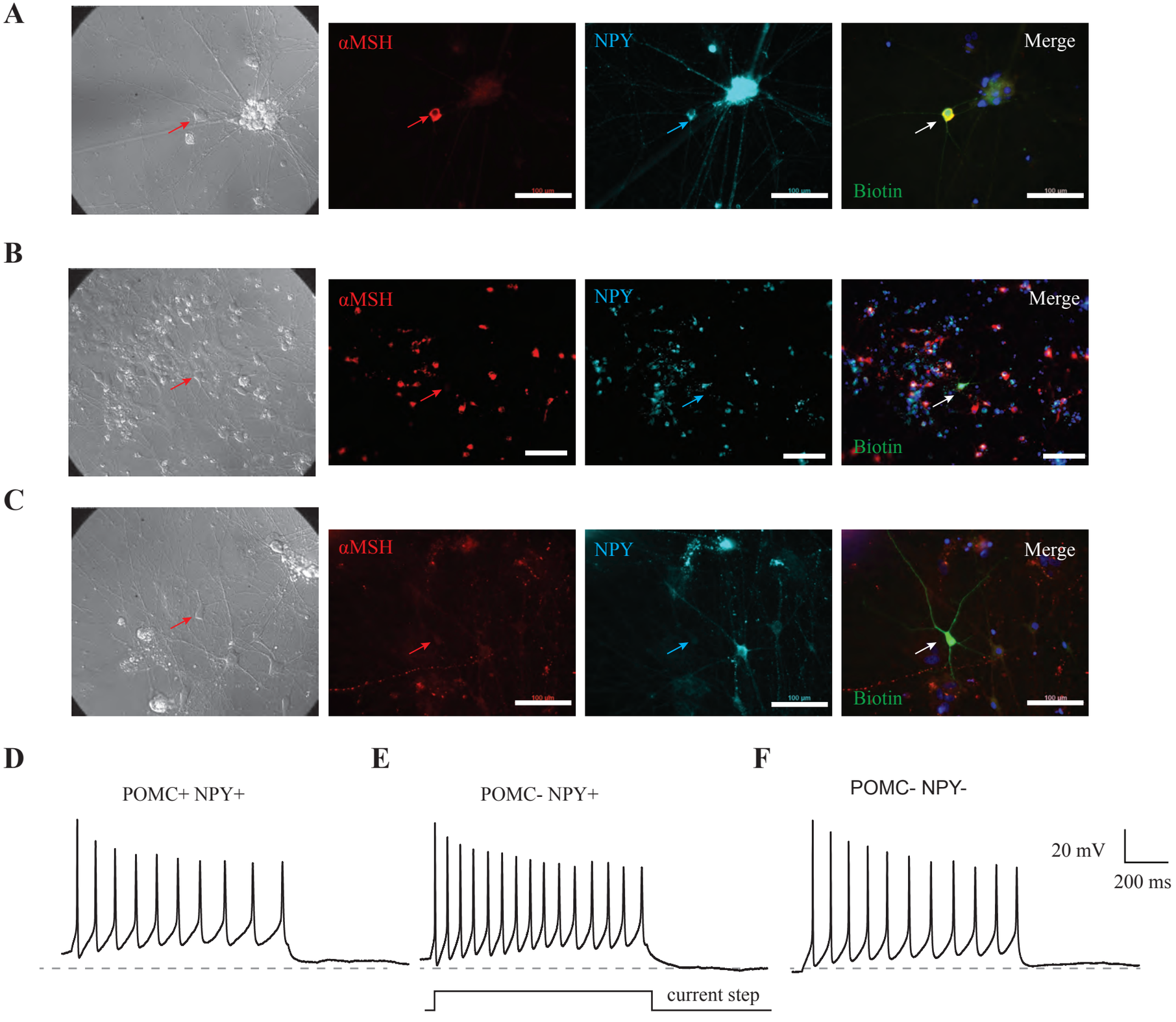
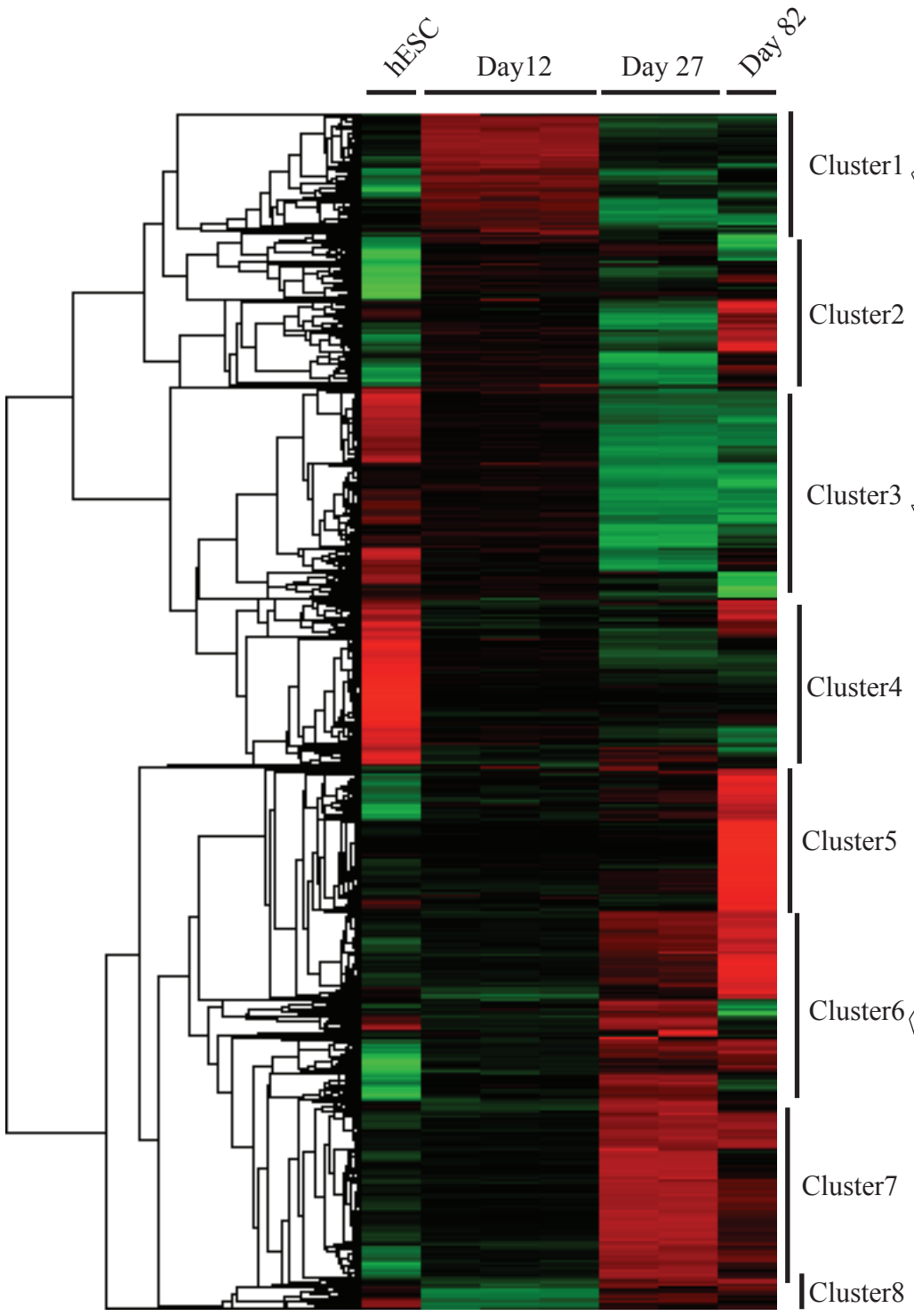


Figure S4. Neurons made from hypothalamic NKX2.1+ cells fire action potentials.
Representative current clamp recordings and post hoc identification of POMC+ or NPY+ neurons (days 24 to 33). After recording, cultures were fixed and stained for αMSH, NPY and recorded cells (biotin+) cells identified with streptavidin-alexa-488.(A) POMC+NPY+; (B) POMC-NPY+; (C) POMC-NPY- neurons. Left column of images: DIC image (40x) of live cells prior to recording. Arrows indicate the recorded neurons.Scale bar, 100μm; (D-F) Action potential firing in the neurons identified in (A–C). Membrane potential traces in response to a 1s current step, as shown under trace (E), were recorded. The amplitude of the current step was dependent on the individual cell. (D) POMC+NPY+ (neuron in A), (E) POMC- NPY+ (neuron in B),(F) POMC-NPY- (neuron in C). Dashed lined = -60 mV. Total number of identified cells recorded: POMC+NPY+ N= 3, POMC-NPY+ N= 2, POMC-NPY- N= 4.

A



GOTERM_BP_FAT

Term	Count	%	P-Value
GO:0045449~regulation of transcription	325	20.61	2.14E-15
GO:0035295~tube development	52	3.30	1.60E-11
GO:0045941~positive regulation of transcription	95	6.02	6.68E-11
GO:0010628~positive regulation of gene expression	97	6.15	6.74E-11
GO:006350~ transcription	256	16.23	1.16E-10
GO:0045165~cell fate commitment	34	2.16	3.32E-08
GO:0060284~regulation of cell development	41	2.60	3.85E-07
GO:0045449~regulation of nervous system development	38	2.41	1.43E-06

GOTERM_BP_FAT

Term	Count	%	P-Value
GO:0006412~translation	147	6.02	7.77E-42
GO:0007049~cell cycle	249	10.20	1.42E-40
GO:0022402~cell cycle process	197	8.07	1.57E-37
GO:000278~mitotic cell cycle	150	6.14	5.01E-37
GO:0022403~cell cycle phase	157	6.43	1.11E-34
GO:0007067~mitosis	98	4.01	4.53E-28
GO:0051301~cell division	113	4.63	1.84E-25
GO:006260~DNA replication	83	3.40	3.54E-23

PATHWAY ANALYSIS

Category	Term	Count	%	P-Value
KEGG_PATHWAY	hsa04722: Neurotrophin signaling pathway	26	1.085	5.79E-04
KEGG_PATHWAY	hsa04912: GnRH signaling pathway	19	0.793	0.009
PANTHER_PATHWAY	P04379: Beta3 adrenergic receptor	10	0.418	0.003
PANTHER_PATHWAY	P04391: Oxytocin receptor mediated	15	0.626	0.006
PANTHER_PATHWAY	P04374:5HT2 type receptor mediated	15	0.668	0.009
PANTHER_PATHWAY	P05915:Opioid proenkephalin pathway	10	0.418	0.010
PANTHER_PATHWAY	P05917: Opioid proopiomelanocortin	10	0.418	0.013
PANTHER_PATHWAY	P04385:Histamine H1 receptor mediated	11	0.459	0.019
PANTHER_PATHWAY	P04378: Beta2 adrenergic receptor	11	0.459	0.030
PANTHER_PATHWAY	P04386: Histamine H2 receptor mediated	8	0.334	0.030
PANTHER_PATHWAY	P00015: Circadian clock system	6	0.251	0.030
PANTHER_PATHWAY	P05916: Opioid prodynorphin pathway	9	0.376	0.031

B

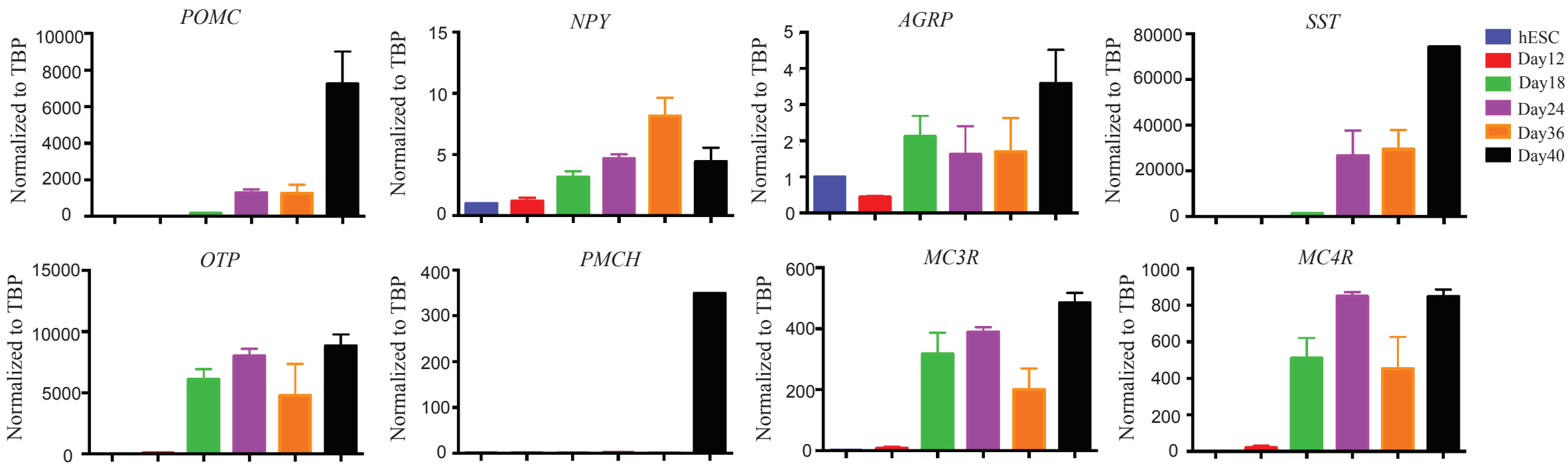


Figure S5. RNA sequencing of differentiated neurons reveals hypothalamic gene signatures.
(A) Heatmap from hierarchical clustering analysis of RNA sequencing results from undifferentiated hESC; day12 neuron progenitors; days 27 and 82 of neuronal differentiation. Gene Ontology and pathway analysis were performed for genes in eight major clusters defined by the gene expression patterns according to all samples. Data are shown for Gene Ontology results for clusters 1 (highly expressed in day 12 progenitor cells) and 3 (highly expressed in hESCs), and pathway analysis for the top hits in cluster 6 (highly expressed in differentiated neurons); (B) Temporal expression of hypothalamic transcripts during Nkx2.1 GFP/W-hESC neuronal differentiation. qPCR analysis of RNA samples from hESC, and cells at 12, 18, 24, 36 and 40 days of neuronal differentiation. N=3 for each bar.

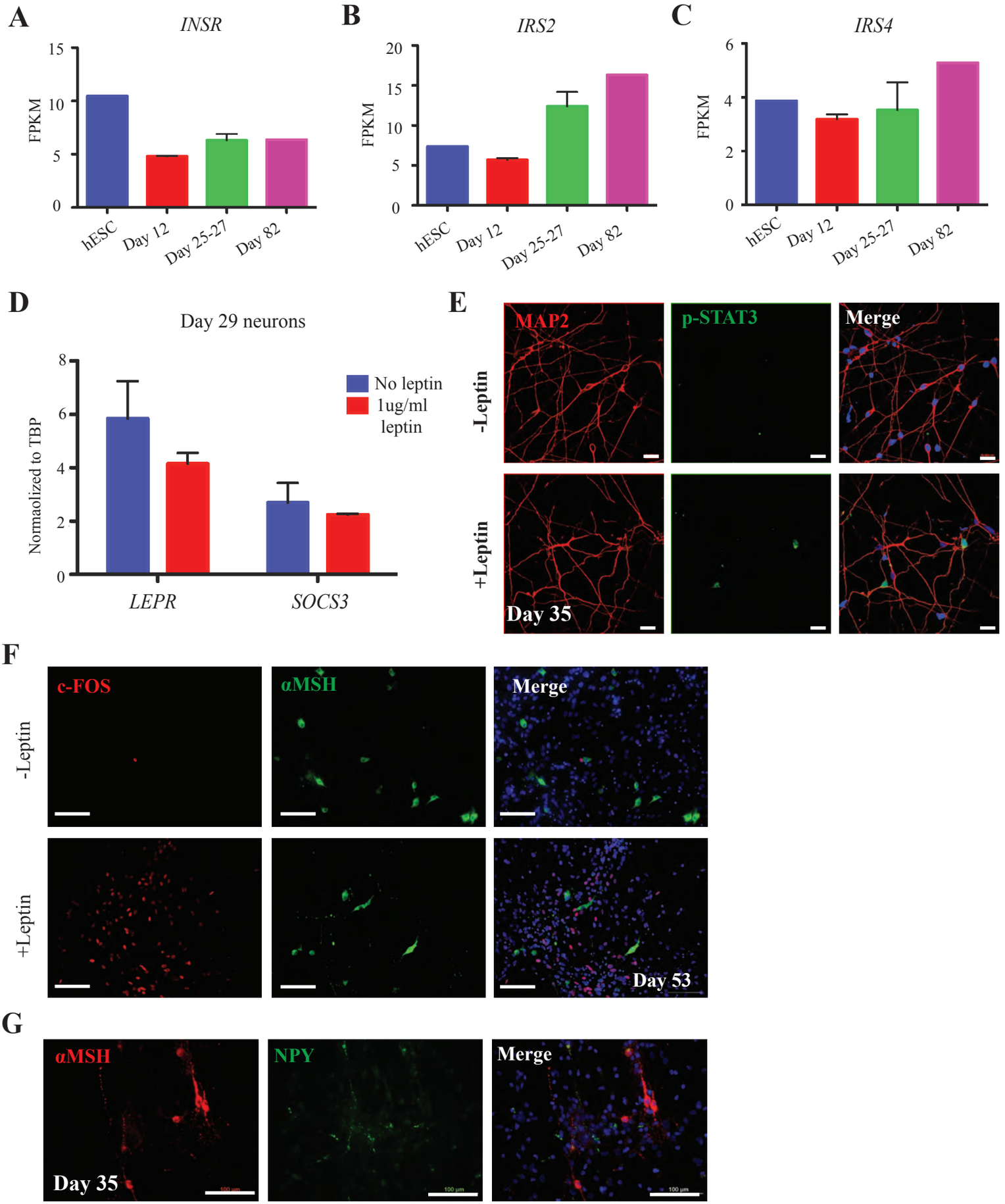
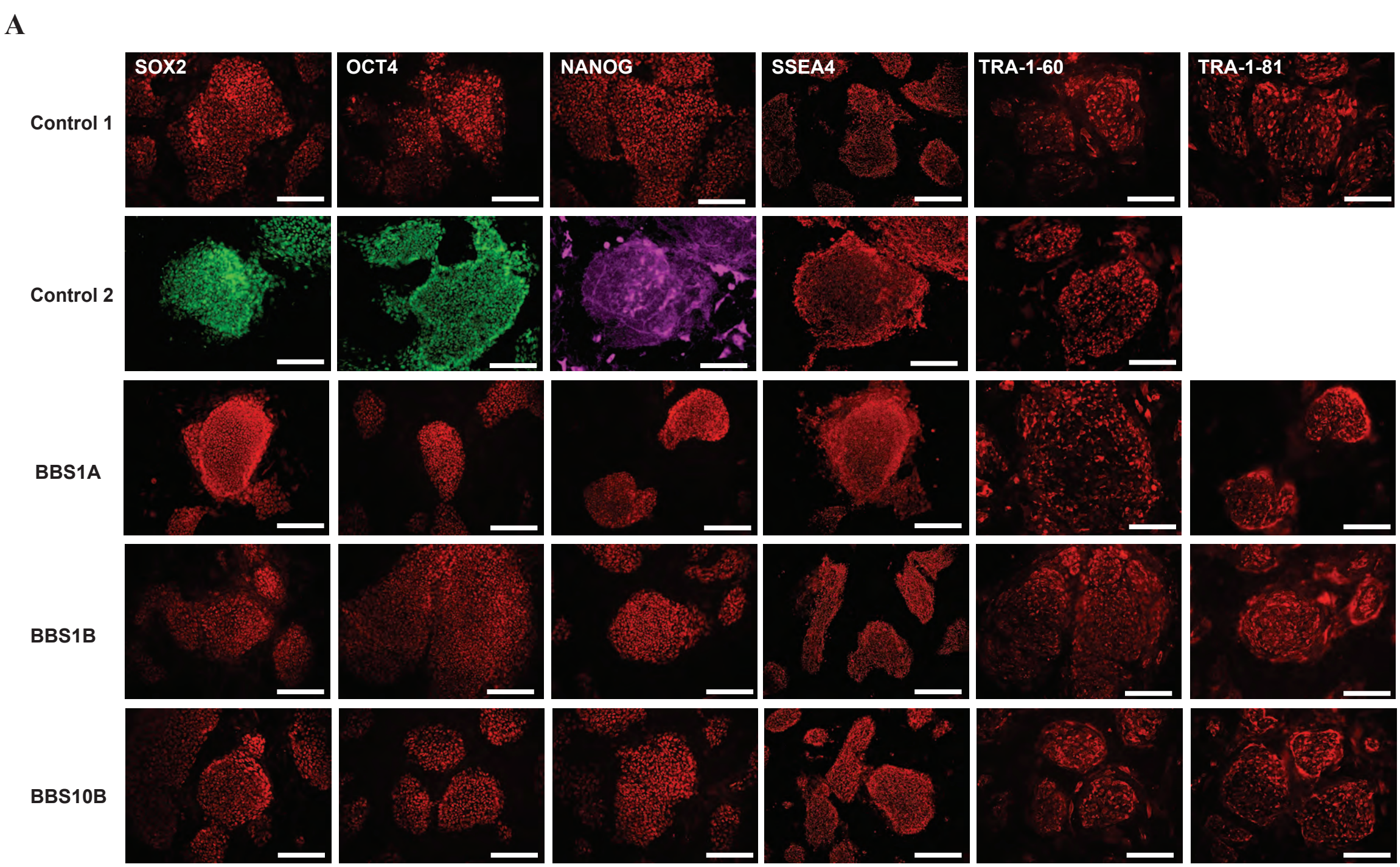
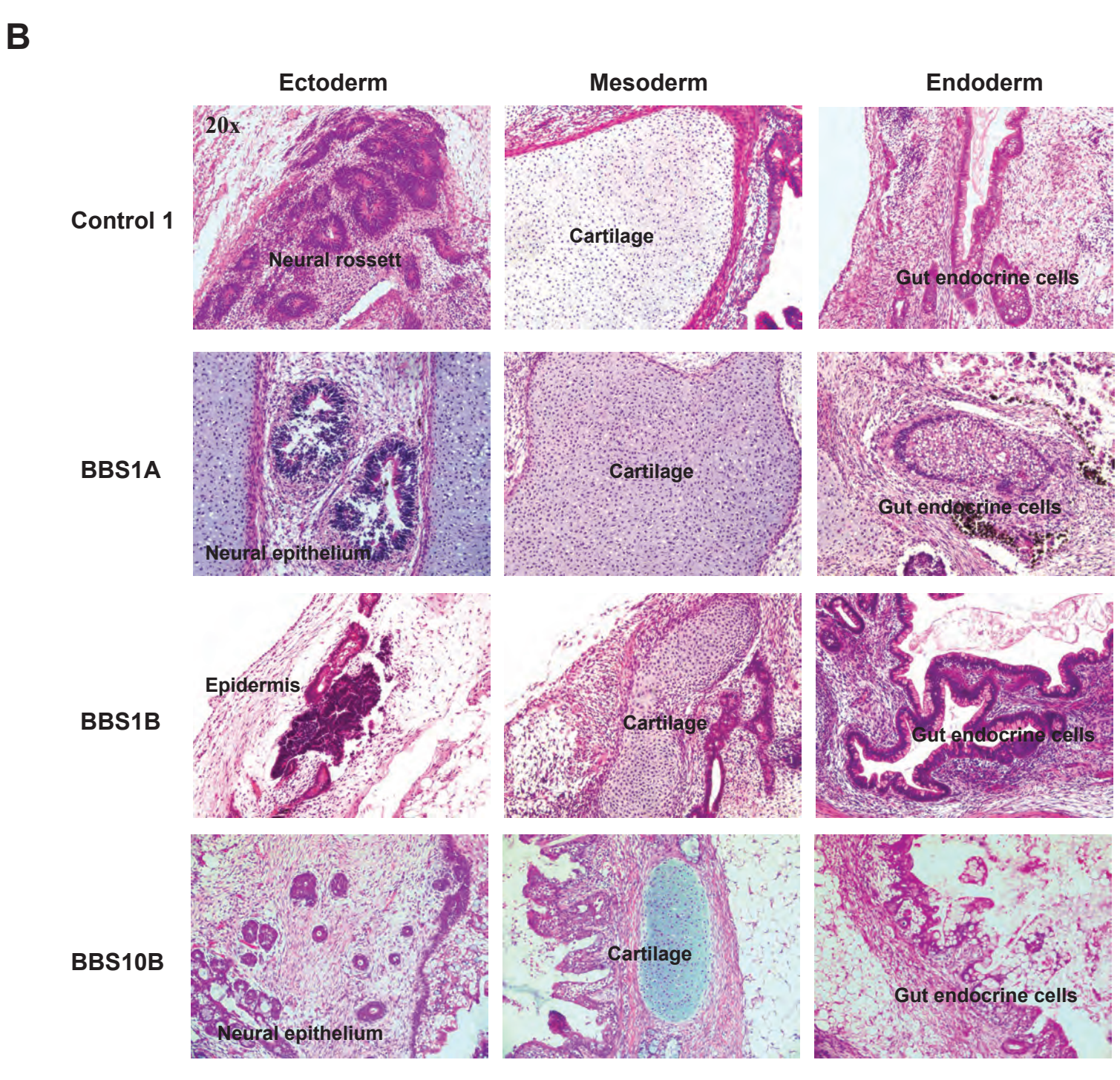


Figure S6. Mouse cortical astrocyte co-culture induces leptin responsiveness of differentiated hypothalamic neurons. (A-C) RNA-seq data for insulin signaling molecules INSR, IRS2 and IRS4 expression in hESC, 12 (n=3), 25-27 (n=3) and 82 days of differentiated cells; (D) qPCR analysis of LEPR and SOCS3 in leptin-treated day 29 differentiated neurons (n=3); (E) Immunostaining for p-STAT3 and MAP2 in leptin treated day 35 neurons. Scale bar, 20μm; (F) Immunocytochemical analysis for c-FOS and αMSH in day 53 hESC-derived hypothalamic neurons co-cultured with mouse astrocytes. Scale bar, 100μm; (G) Immunostaining of αMSH and NPY in day 35 mouse astrocyte co-cultured neurons. Scale bar, 100μm.





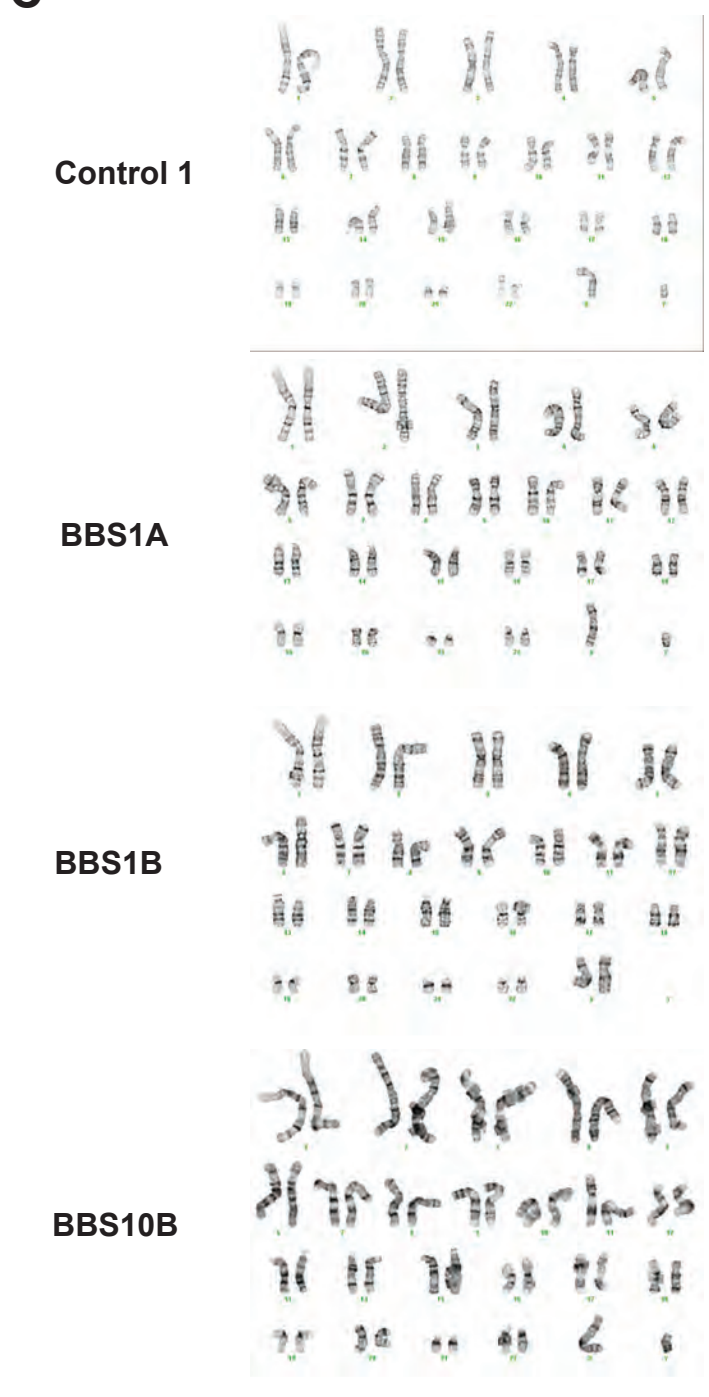


Figure S7. All control and BBS iPSCs are pluripotent and have normal karyotypes.
(A) Immunocytochemistry analysis of two control (Control 1, Control 2) and three BBS (BBS1A, BBS1B, BBS10B) iPSCs with pluripotency markers as indicated. Scale bar, 200μm; (B) H&E staining of control and BBS iPSC-derived teratoma sections. We identified cell types representing all three germ layers in all iPSC-derived terotomas; Images were taken with 20x objective lens; (C) Karyotyping of control and BBS iPSC lines. All of these lines had normal karyotypes.

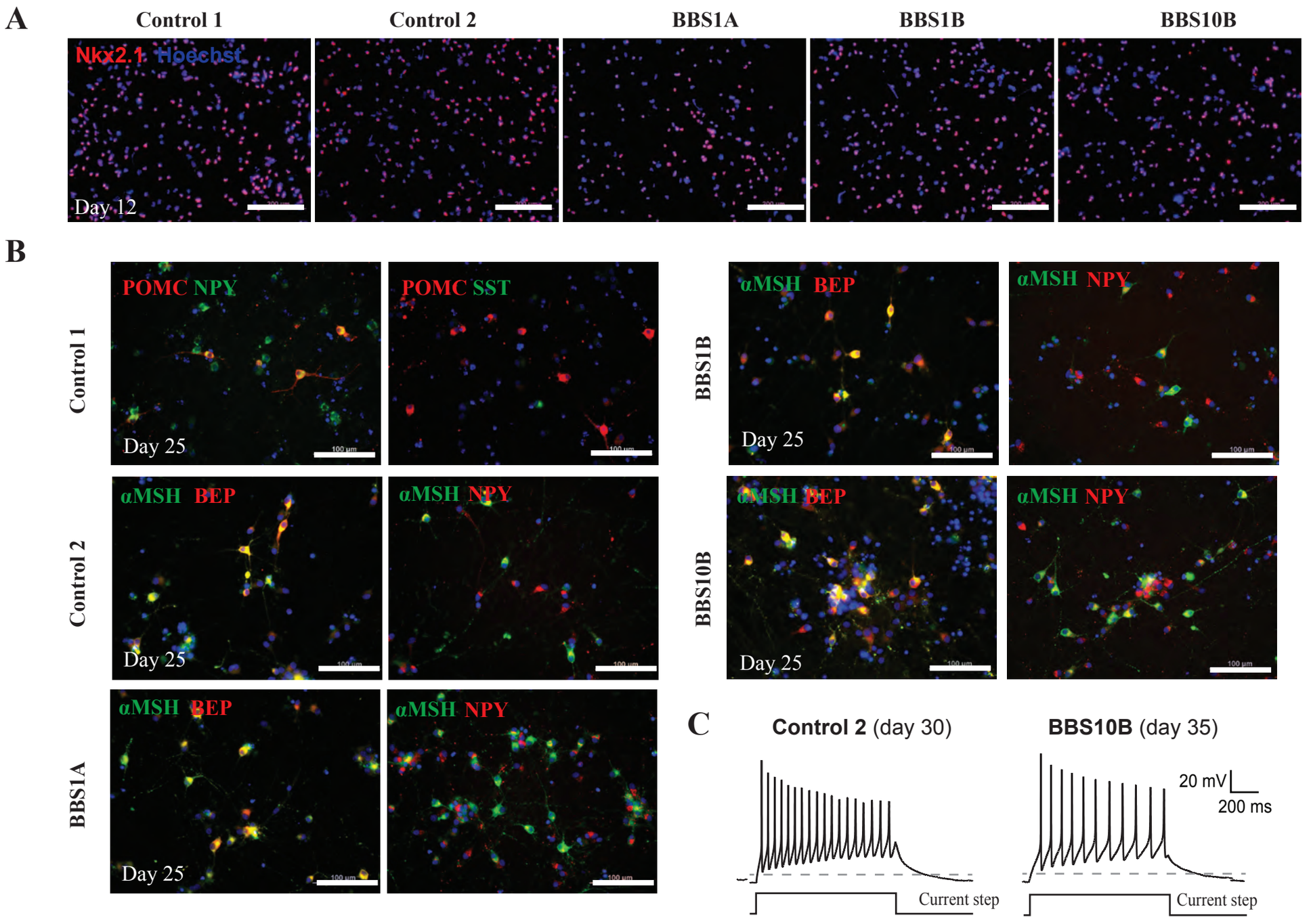


Figure S8. Efficient generation of hypothalamic neurons from human iPSCs.
(A) Immunostaining of NKX2.1 in Day 12 progenitors derived from five independent iPSC lines: Control1, Control 2, BBS1A, BBS1B and BBS10B; Scale bar, 200μm; (B) Immunostaining of day 25 neurons with hypothalamic neuron markers. Scale bar, 100μm; (C) Action potential firing in Control 2 (day 30) and BBS10B (day 35) iPSC-derived neurons. Membrane potential traces in response to a 1s current step, as shown under trace, were recorded. Dashed lined = -60 mV.