Supporting Methods

Human Pluripotent Stem Cell Culture. hPSCs—H7 and H1 hESCs (WiCell, Madison, WI), WTC and WTB iPSCs (generously donated by Bruce Conklin)—were grown to 70% confluence and passaged using Accutase (Accutase, San Diego, CA) to dissociate to single cells (incubated at 37°C for 5 minutes). Dissociated cells were replated on Matrigel-coated cultureware (hESC-qualified for ESCs and growth factor reduced for iPSCs) at a density of 10,000 cells per cm^2 with 10 μM ROCK inhibitor (Y-27632, Selleckchem, Houston, TX) in mTeSR (StemCell Technologies, Vancouver, Canada).

V2a Interneuron Differentiation. hPSCs were seeded in mTeSR supplemented with 10 μM ROCK inhibitor and dual SMAD inhibitors 0.2 μM LDN193189 and 10 μM SB431542 (StemGent, Cambridge, MA) at 5,000–100,000 cells/cm^2 onto 24-well plates coated with Matrigel. On day 3, medium was changed to mTeSR supplemented with dual SMAD inhibitors only. On day 5, the base medium was switched to neural induction medium (DMEM F:12 (Corning, Corning, NY), N2 supplement (Life Technologies, Carlsbad, CA), L-Glutamine (VWR), 2 μg/ml heparin (Sigma Aldrich, St. Louis, MO), non-essential amino acids (Mediatech INC, Manassas, VA), penicillin-streptomycin (VWR) supplemented with fresh 0.4 μg/ml ascorbic acid (Sigma Aldrich) and 10 ng/ml brain derived neurotrophin factor (BDNF, R&D Systems, Minneapolis, MN)) supplemented with dual SMAD inhibitors and 10 nM–10 μM retinoic acid (Sigma Aldrich). On day 7, dual SMAD inhibition was ceased and 10 nM–10 μM retinoic acid, 10 nM–10 μM pur (EMD Millipore, Darmstast, Germany) and 100 nM–10 μM N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT) were added to the neural induction medium. Medium was changed every 2–3 days throughout the differentiation, with fresh supplements added each time for up to 17 days.

Neuronal Maturation. On day 17 of differentiation, cultures were dissociated with Accutase (45 minutes at 37°C; triturated every 15 minutes) and plated at 100,000 cells/cm^2 on Matrigel-coated µ-slide 8 wells (ibidi, Martinsreid, Germany) or glass coverslips (Warner Instruments, Hamden CT) in neural induction medium containing 100 nM RA, 100 nM pur, and 1 µM DAPT plus 10 µM ROCK inhibitor. Three days later, the medium was switched to neural maturation medium (BrainPhys plus SM1 supplement (Stemcell Technologies (1)) supplemented with 10 ng/ml of BDNF, GDNF, CNTF, and IGF, R&D Systems). Medium was completely changed every 3–4 days for the remainder of the culture duration.

Real Time Quantitative Polymerase Chain Reaction. Samples were lysed and RNA was extracted using the E.Z.N.A. Total RNA Kit (Omega Biotek, Norcross, GA). RNA (500 ng) was reverse-transcribed into cDNA using the iScript cDNA synthesis kit (BioRad, Hercules, CA). RT-qPCR was performed using Fast SYBR Green Master Mix (Life Technologies) and the primers listed in Table S2 were annealed at 61°C on the Step One Plus Real-Time PCR System (Life Technologies). Fold-changes were calculated using the $\Delta\Delta C_t$ method (2). For high-throughput gene expression analysis, Fluidigm was used. Preamplified cDNA samples and primers were mixed with Sso Fast EvaGreen Supermix (BioRad) then loaded onto a 96.96 Dynamic Array integrated fluidic circuit (IFC) and ran on a BioMark HD system.

Flow Cytometry. At day 17 of differentiation, cells were completely dissociated using Accutase and stained with the Transcription Factor Buffer Set, which includes a fixation/permeabilization (FP) and wash/permeabilization (WP) buffer (BD Biosciences, Franklin Lakes, NJ). Dissociated samples were first fixed for 45 minutes at 4°C in the FP buffer followed by a 20 minute block with WP buffer containing 5% normal donkey serum (NDS, Jackson Laboratory, Bay Harbor, ME). Primary antibodies against CHX10 and the proper matching species isotype control were added at the concentration shown in Table S3 into WP

buffer containing 2% NDS and incubated at 4°C for 45 minutes. After two washes with WP buffer, secondary antibodies donkey anti-mouse IgG, Alexa Fluor 488 (Life Technologies), at a dilution of 1:200, were added to WP buffer and incubated at 4°C for 45 minutes. After two washes with WP buffer, samples were passed through a 35-μm filter before assessing with a BD Accuri C6 (BD) cytometer (minimum 20,000 events). Cytometry analysis was performed using FlowJo V10 (Flowjo, Ashland, OR).

***In Vitro* Immunocytochemistry and Imaging.** Samples were fixed using 4% paraformaldehyde (VWR) for 30 minutes and permeabilized using 0.1% Triton-X in PBS for 15 minutes at 4° C before blocking for 1 hour at 4°C with PBS containing 5% NDS. Primary antibodies (Table S3) were diluted in PBS containing 2% NDS and incubated overnight. Samples were washed three times with PBS for 15 minutes at room temperature before incubating with secondary antibodies (Life Technologies) diluted in PBS containing 2% NDS. Hoechst was added to the samples for 10 minutes then washed and imaged using a Zeiss Axio Observer inverted wide-field microscope equipped with an Apotome structured light attachment. An average intensity projection was performed on Z-stack images to create a single two-dimensional image spanning the entire thickness of the observed field. Images were processed using Photoshop.

Single Cell RNAseq. At Day 17 of culture, cells were dissociated with Accutase. Approximately 8,000 cells were prepared for single cell analysis through droplet encapsulation by the Chromium Controller and library preparation with the Chromium Single Cell 3' v1 Library and Gel Bead Kit (10x Genomics, San Francisco, CA). cDNA was sheared using a Covaris S2 sonicator and 12 PCR cycles were run during cDNA amplification. Libraries were sequenced on a NextSeq 500 (Illumina, San Diego, CA). Sequences were demultiplexed and aligned to human reference genome hg19 using the default settings of 10xGenomics *Cellranger* v 1.2. Genes were annotated using Ensembl version 70 (3). After *Cellranger* filtering, >85

million valid reads remained with >70% mapping to the transcriptome. Downstream analysis was performed using *Seurat* (4, 5) and cells not expressing between 500 and 5000 unique genes were removed. A subset of high-variance genes was determined using Seurat's "MeanVarPlot" function (expression cutoff of ≥ 0.25; dispersion cutoff of ≥ 0.50) and used to group cells into clusters (principal components 1–12; cluster resolution parameter = 0.5) (6). The top 20 differentially expressed genes for each cluster were plotted in the heatmap. Raw data is available at SRA under the accession number GSE97564. Gene Ontology analysis was performed on statistically significant differentially expressed genes ($p \leq 0.05$) using PANTHER (7, 8) and GOrilla (9, 10).

Calcium Imaging and Analysis. Cultures were washed with PBS and the medium was replaced with Neurobasal plus Fluo4 AM (10 μM, Life Technologies) for 30 minutes at 37° C. The cultures were then washed with fresh Neurobasal and allowed to recover for an additional 30 minutes at 37°C before recording on a Zeiss Axio Observer. To analyze wide field calcium videos, soma were identified from phase images and selected as regions of interest (ROI) in the corresponding fluorescent green channel. To assess calcium fluctuations, mean fluorescence intensity for each ROI was measured over time at a sampling rate of 2.38 frames per second using MATLAB (MathWorks, Natick, MA) to assess calcium fluctuations within the ROIs. To minimize noise, the median of all the traces was calculated and subtracted from each. A minimum of 20 ROIs were examined for each field of view.

Electrophysiological Testing. Neurons (days 27, 41, 63) were recorded in the whole-cell configuration using glass pipette electrodes filled with the internal solution ((mM): 100 K-gluconate, 20 KCl, 10 HEPES, 4 Mg-ATP, 0.3 Na-GTP, 10 Na-phosphocreatine, and 0.2% biocytin; osmolality 300 mOsm), while being perfused with warm artificial cerebrospinal fluid ((mM): 126 NaCl, 26 $NaHCO_3$, 3.0 KCl, 1.25 NaH_2PO_4, 2.0 $CaCl_2$, 2

$MgCl_2$, and 20 dextrose; osmolality 320 mOsm, T = 33ºC; bubbled with 95% O_2 + 5% CO_2 mixture, pH = 7.3–7.35). Resting membrane potential was measured immediately after achieving the whole-cell configuration. Action potentials were elicited by injecting a 1.5s long depolarizing current of 20pA and measuring the voltage response in current clamp (baseline voltage was held between -60 and -70 mV by injecting a small, constant, negative current). Action potentials were analyzed using custom-written software in IgorPro (Wavemetrics, Portland, OR).

Spinal Transplantation. All animal studies were performed in accordance with the Institutional Animal Care and Use Committee at the University of California, San Francisco. Day 17 cultures were dissociated for 45 minutes using Accutase, washed with PBS, resuspended in ice-cold DMEM at ~$5x10^5$ cells/µL, and maintained on ice until transplantation. Female C57Bl/6j SCID mice age 12 weeks were anesthetized using isoflurane and a dorsal laminectomy was performed at T9 to expose the spinal cord. The vertebral column was stabilized, and the dissociated cells were injected at four sites bilaterally in the ventral horns of the spinal cord at the rostral and caudal edges of the laminectomy site (~1.25x10^5 cells/site). The musculature over the exposed spinal cord was sutured closed and the skin was closed with surgical clips. Antibiotics (enrofloxacin) were delivery daily (I.P., 2.5 mg/kg) for 10 days. After 2 weeks, animals were euthanized and transcardially perfused with PBS followed by 4% paraformaldehyde. A 2-cm segment of spinal cord tissue centered over the transplantation site was harvested and post-fixed overnight in 4% paraformaldehyde.

Murine Tissue Processing and Immunofluorescence. Spinal cords were embedded and 20 µm sections were obtained in the sagittal plane. Sections were permeabilized with 0.3% Triton-X in PBS for 15 minutes or ice-cold acetone for 10 minutes. All sections were then blocked with 10% NDS, 5% bovine

serum albumin, and 0.1% Triton-X in PBS. Sections were incubated with primary antibodies found in Table S3. Sections were washed three times and incubated with the appropriate secondary antibodies (1:500) in blocking solution for 1 hour, then mounted with coverslips using Prolong Gold Antifade containing DAPI. Z-stack images (1 μm step size) were acquired using a Zeiss Axio Observer inverted wide-field microscope and an Apotome structured light attachment. For high resolution imaging of synaptic markers, Z-stack imaged were acquired using a Zeiss LSM 880 confocal microscope with Airyscan. Maximum intensity projection was performed on Z-stack images to create a single two-dimensional image spanning the entire thickness of the section.

Image Quantification. Quantification of *in vitro* maturation images was performed by rendering a 3D surface to define the regions positive for NF and β_{III} tubulin using Imaris 8.4 (Bitplane, Belfast, United Kingdom). Data was normalized by observed field area. For CHX10 counts, ImarisColoc was used to identify overlap of $CHX10^+$ cells with $Hoechst^+$ cells. A 3D surface to define the regions of overlap was created to quantify the number of $CHX10^+Hoechst^+$ cells as well as total $Hoechst^+$ cells. Quantification of transplanted V2a interneuron populations was performed by manual counting of 10 evenly spaced sagittal sections spanning the width of the spinal cord in Image J using the Cell Counter plugin.

Statistical Analysis. Statistical analysis was performed using Prism 6 software. The mean and ± standard deviation were calculated for a minimum of three biological replicates for all data unless otherwise noted. Unpaired t-tests were performed when comparing two groups. One-way analysis of variance (ANOVA) followed by appropriate post hoc pairwise comparisons Tukey's tests were used when three or more groups were specified. Specific statistical analysis is mentioned within the corresponding figure legend.

Variances were confirmed to not differ significantly with the Brown-Forsythe test. In all comparisons, significance was defined as $p \leq 0.05$ (denoted as *).

Figure S1

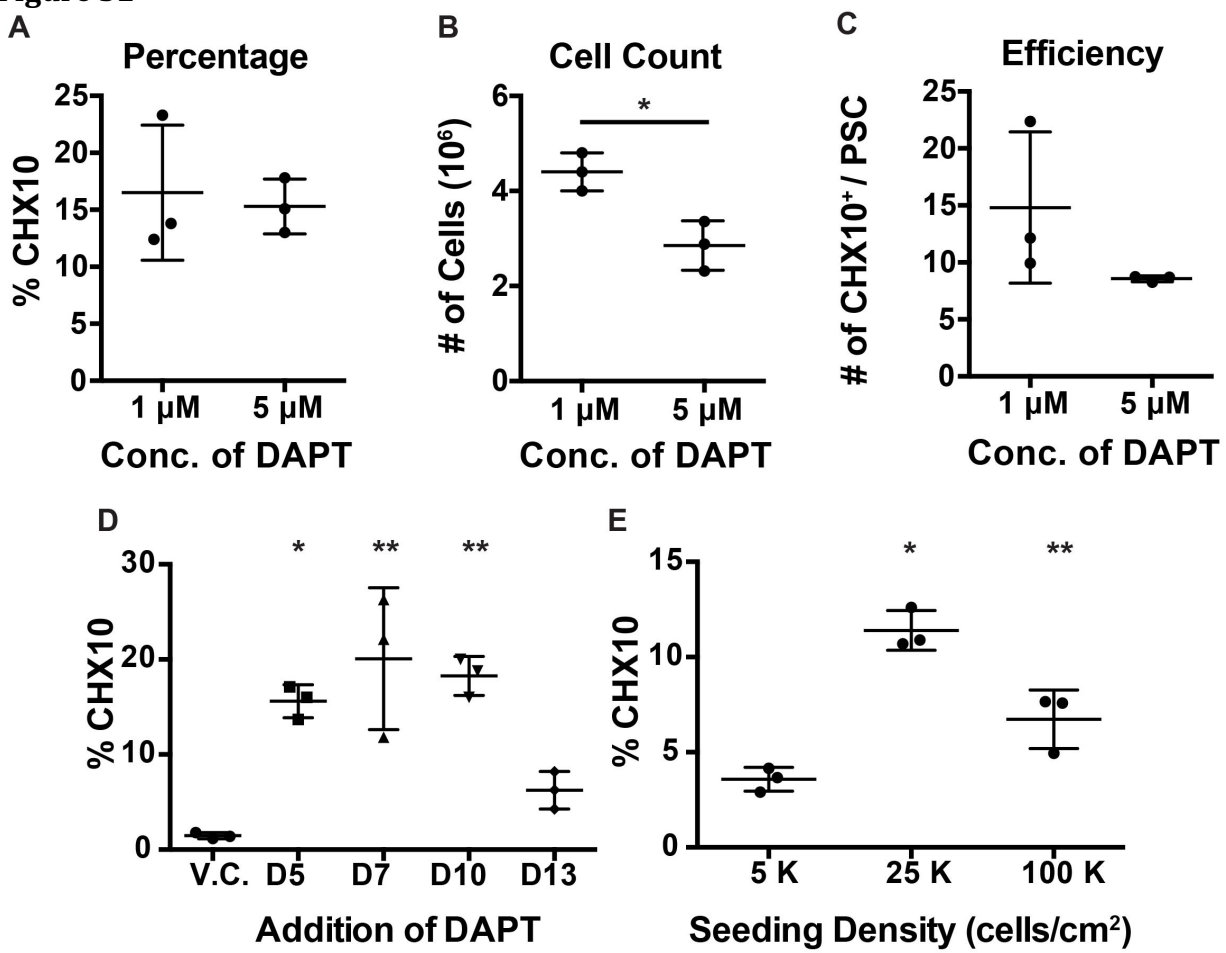

Fig. S1: DAPT concentration affects V2a interneuron yield. *(A)* Flow cytometry analysis of CHX10 expression at Day 17 with 1 μM and 5 μM of DAPT. *(B)* Total number of cells per 24-well culture at day 17 of V2a interneuron differentiation with 1 μM and 5 μM DAPT (using 100 nM RA and 100 nM pur) is significantly different ($p<0.05$, unpaired t-test). *(C)* Total number of $CHX10^+$ cells at day 17 per input pluripotent stem cell. *(D)* Flow cytometry analysis of CHX10 at day 17 when DAPT was added on day 5, day 7, day 10, day 13, or vehicle control (V.C., DMSO). CHX10 expression adding DAPT on day 5 was significantly different (* = $p<0.05$, one-way ANVOA and Tukey post hoc comparison) than V.C. CHX10 expression adding DAPT on day 7 and day 10 was significantly different (** = $p<0.05$, one-way ANVOA and Tukey post hoc comparison) than V.C. and day 13. *(E)* Flow cytometry analysis of CHX10 at day 17 using three different initial seeding densities. CHX10 expression at a seeding density of 25k was significantly different (* = $p<0.05$, one-way ANVOA and Tukey post hoc comparison) compared to 5k and 100k. CHX10 expression at a seeding density of 100k was significantly different (** = $p<0.05$, one-way ANVOA and Tukey post hoc comparison) compared to 5k. Data represented as mean ± standard deviation.

Figure S2

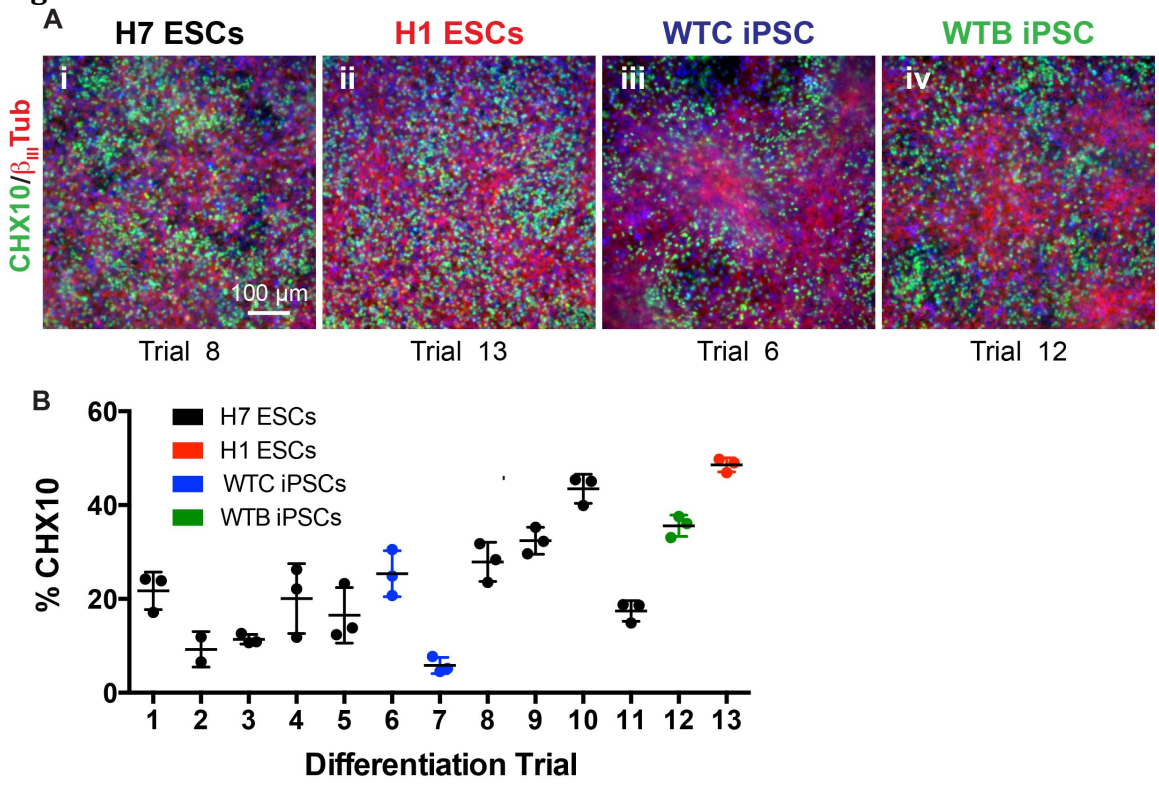

Fig S2: V2a interneuron differentiation is effective in multiple PCS lines. *($A_{i\text{-}iv}$)* Immunostaining for CHX10 (green), β_{III} tubulin (red), and labeled nuclei (blue) of hPSCs differentiated using the V2a interneuron protocol. Scale bar is 100µm. *(B)* Percentage of $CHX10^{+}$ cells from multiple independent V2a interneuron differentiations in various PSC lines. Data represented as mean ± standard deviation.

Figure S3

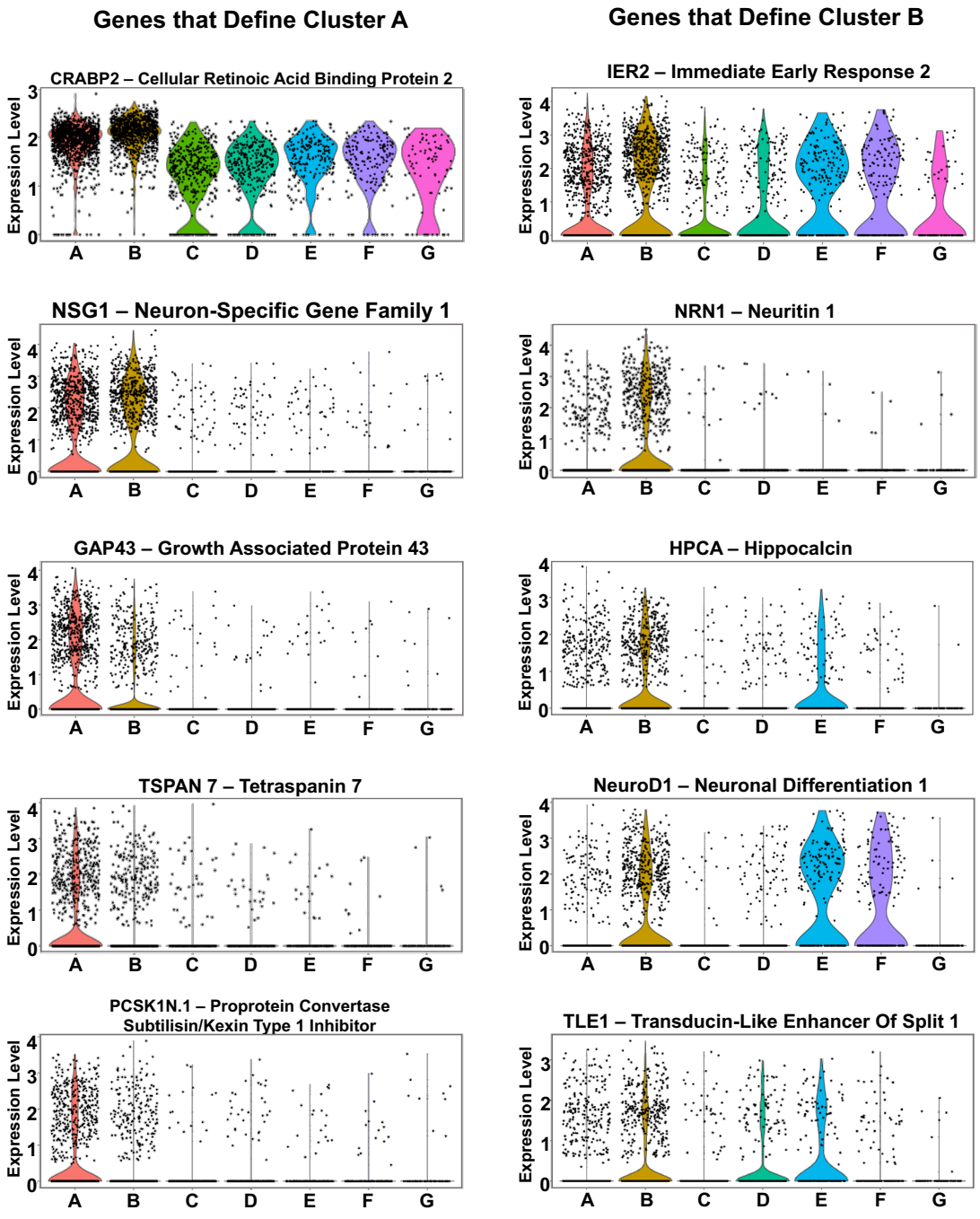

Fig. S3: Select violin plots of genes that identify cluster A and cluster B. Representative genes for cluster A and B chosen from the top 50 differentially expressed genes from the A:B pair-wise comparison.

Figure S4

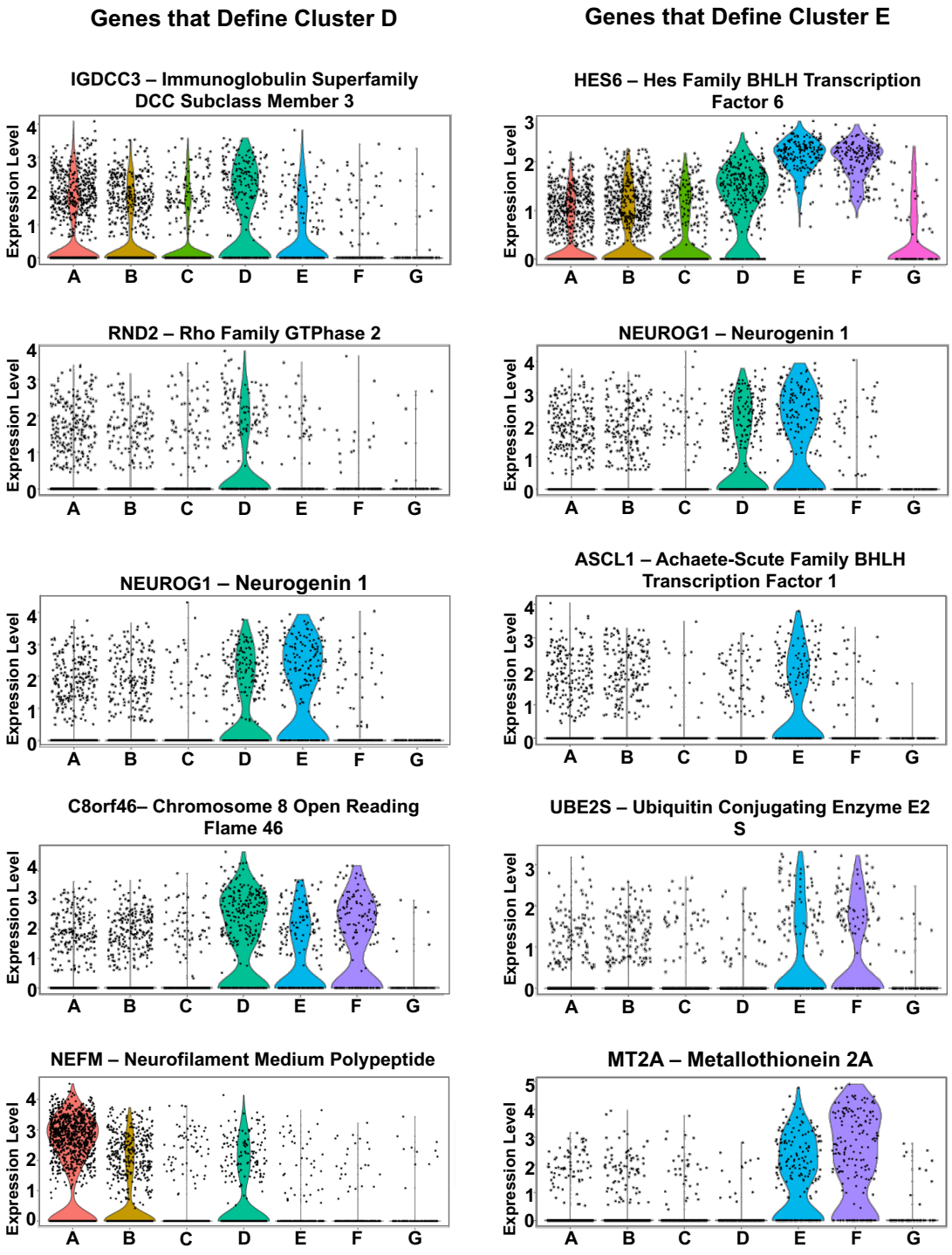

Fig. S4: Select violin plots of genes that identify cluster D and cluster E. Representative genes for cluster D and E chosen from the top 50 differentially expressed genes from the D:B and E:B pair-wise comparison, respectively.

Figure S5

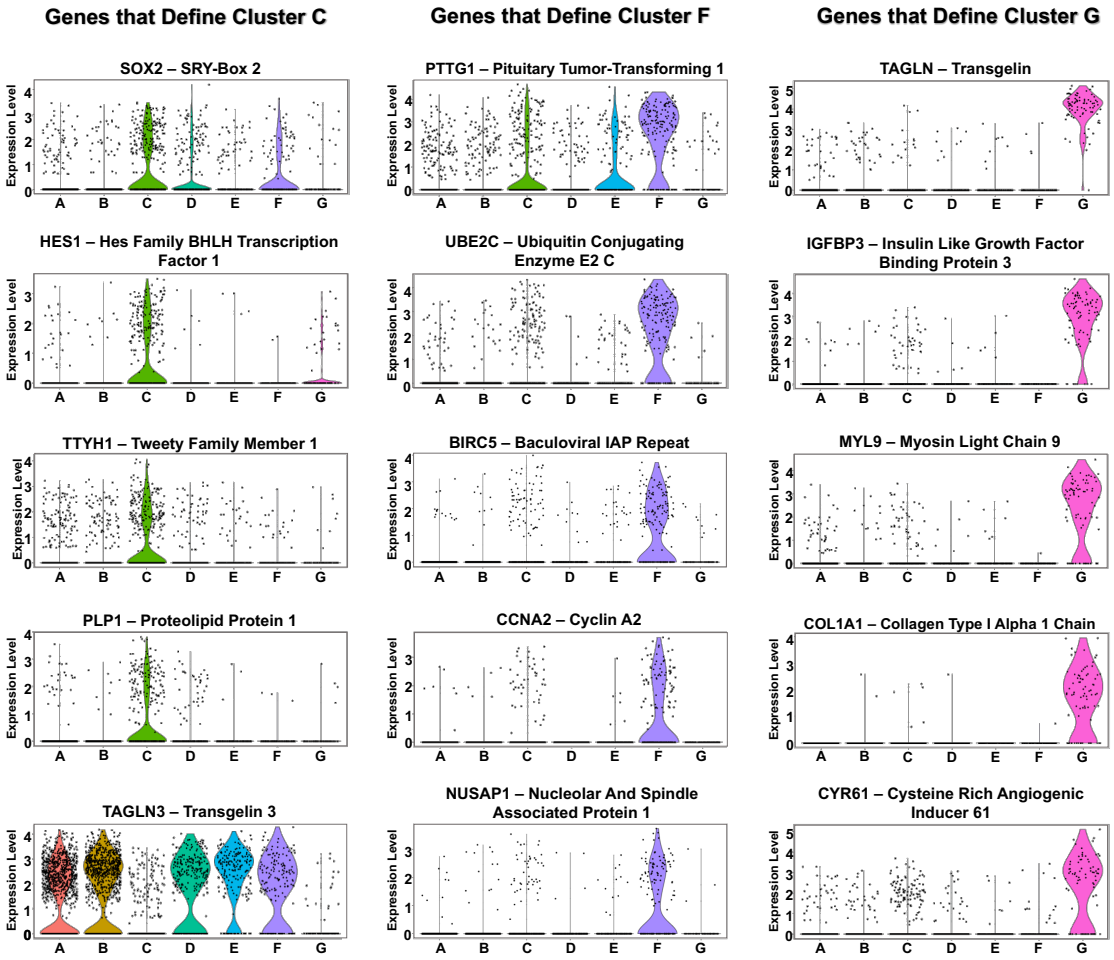

Fig. S5: Select violin plots of genes that identify cluster C, cluster F and, cluster G. Representative genes for cluster C, F, and G chosen from the top 50 differentially expressed genes from the C:B, F:B, and G:B pair-wise comparison, respectively.

Figure S6

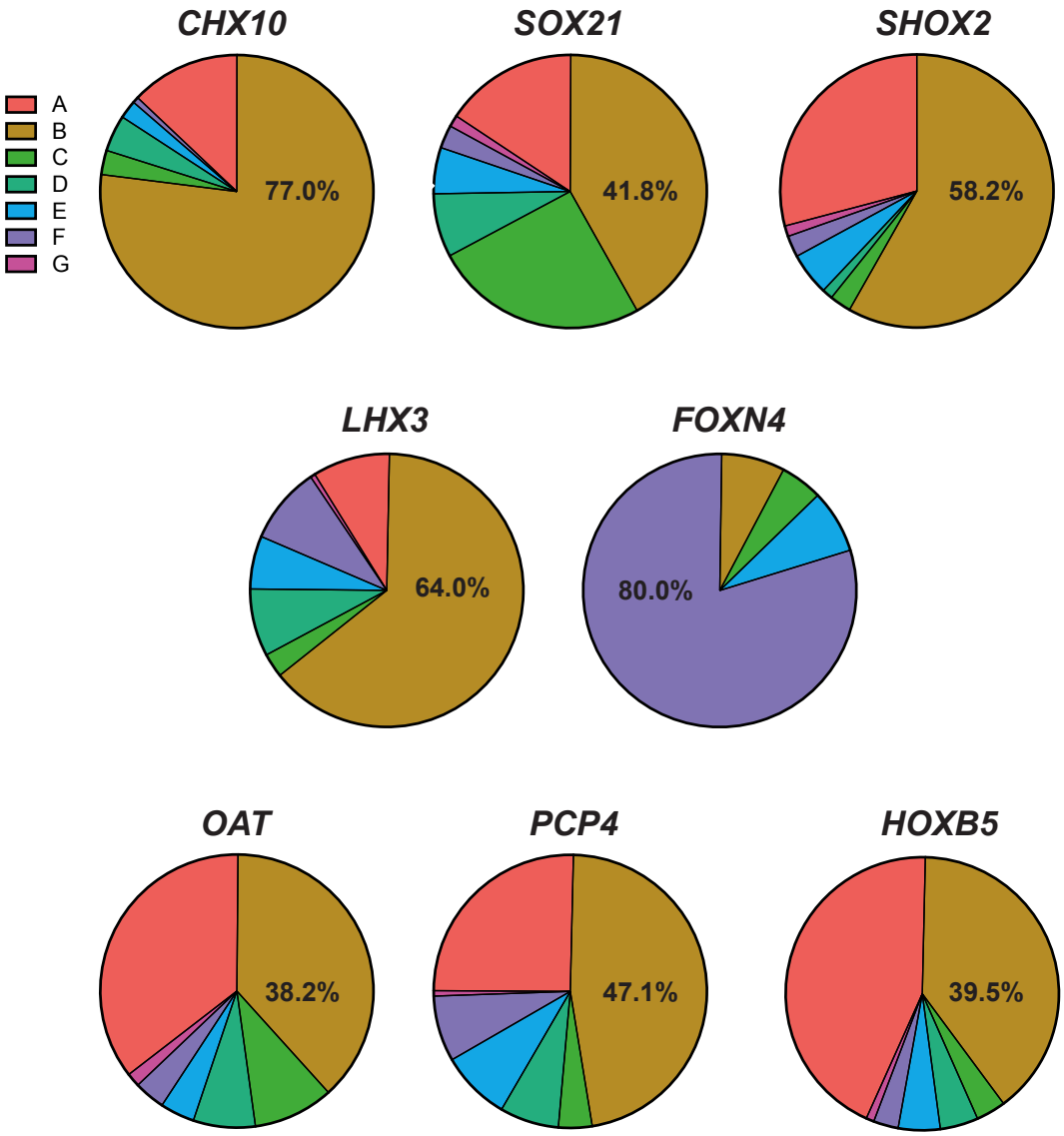

Fig. S6: Additional V2a interneuron genes and their cluster identity. Each pie chart reports the number of cells with at least one read for the gene and its cluster identity. The percentage of cells found within cluster B is labeled on the chart for all genes except FOXN4, which labels the percentage of cells found in cluster F.

Figure S7

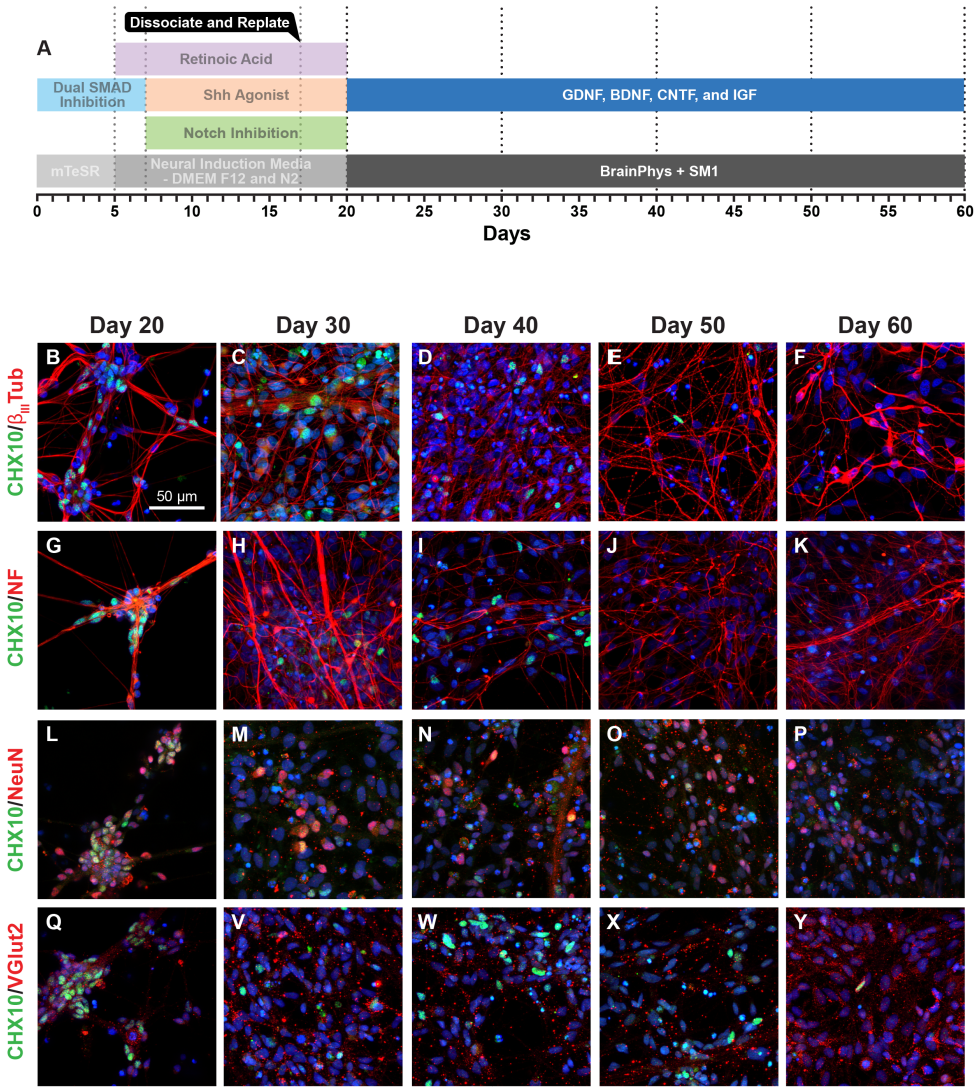

Fig. S7: V2a interneuron maturation *in vitro*. *(A)* Timeline of V2a interneuron maturation cultures. *(B-Y)* Immunostaining for CHX10 (green) and nuclei labeling (blue) of V2a interneurons on day 20, 30, 40, 50 and 60 of culture. *(B-F)* Immunostaining for β_{III} tubulin (red). *(G-K)* Immunostaining for neurofilament (NF, red). *(L-P)* Immunostaining for NeuN (red). *(Q-Y)* Immunostaining of vesicular glutamate transporter 2 (VGLUT2, red). Scale bar = 50µm.

Figure S8

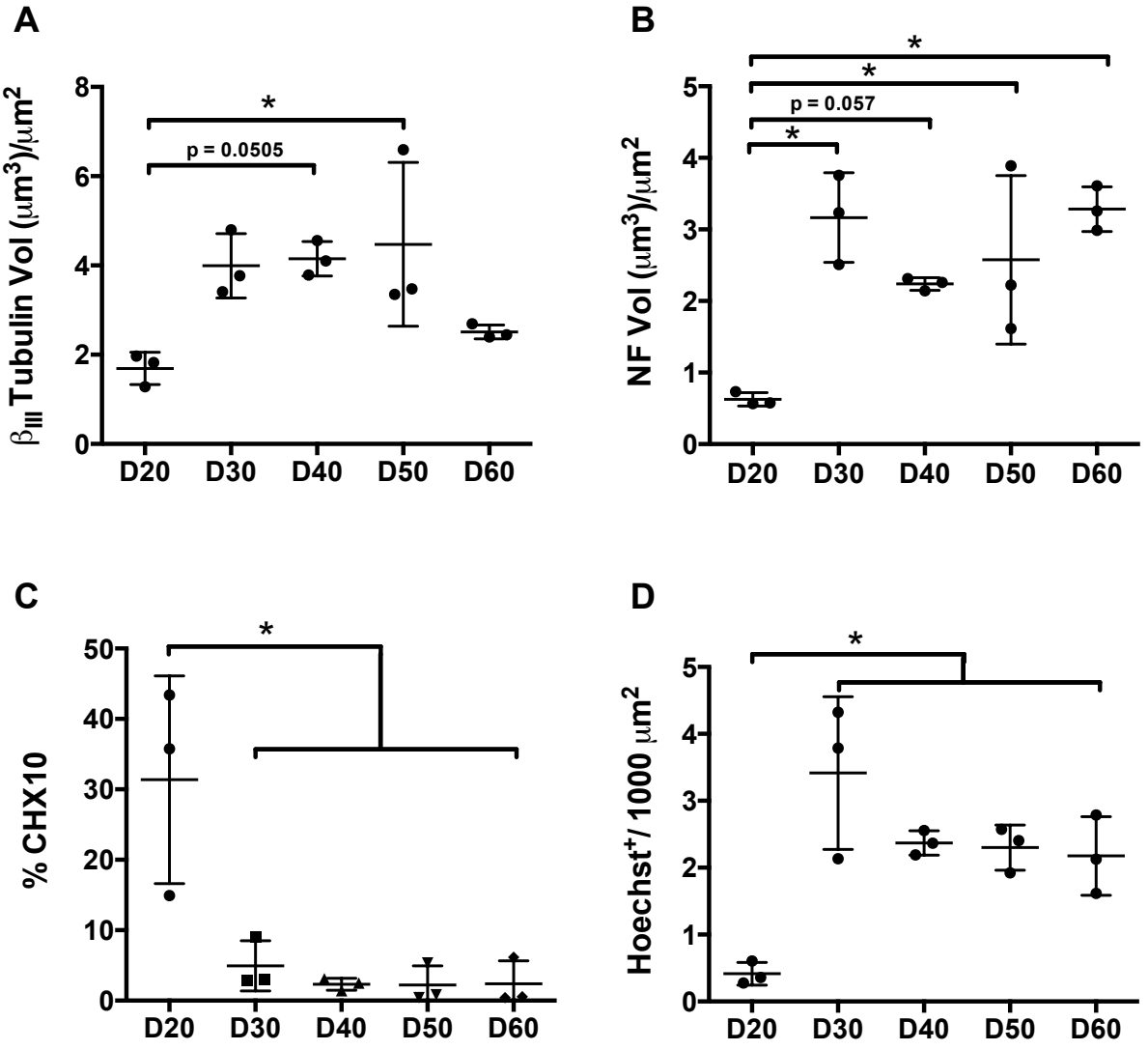

Fig S8: Quantification of *in vitro* maturation cultures. *(A)* Volume of β_{III} tubulin throughout culture duration normalized by observation view area. Day 50 volume was greater than day 20 volume ($p<0.05$, one-way ANOVA and Tukey post hoc comparison) *(B)* Volume of neurofilament throughout culture duration normalized by observation view area. Day 60, day 50, and day 30 volume was greater than day 20 volume ($p<0.05$, one-way ANOVA and Tukey post hoc comparison) *(C)* Percent CHX10$^+$ cells throughout culture duration. Day 20 percentage was greater than all other time points Day 50 volume was greater than day 20 volume ($p<0.05$, one-way ANOVA and Tukey post hoc comparison). *(D)* Number of Hoechst$^+$ cells throughout culture duration normalized by observation view area. Day 30 through day 60 were greater than day 20 Day 50 volume was greater than day 20 volume ($p<0.05$, one-way ANOVA and Tukey post hoc comparison).

Figure S9

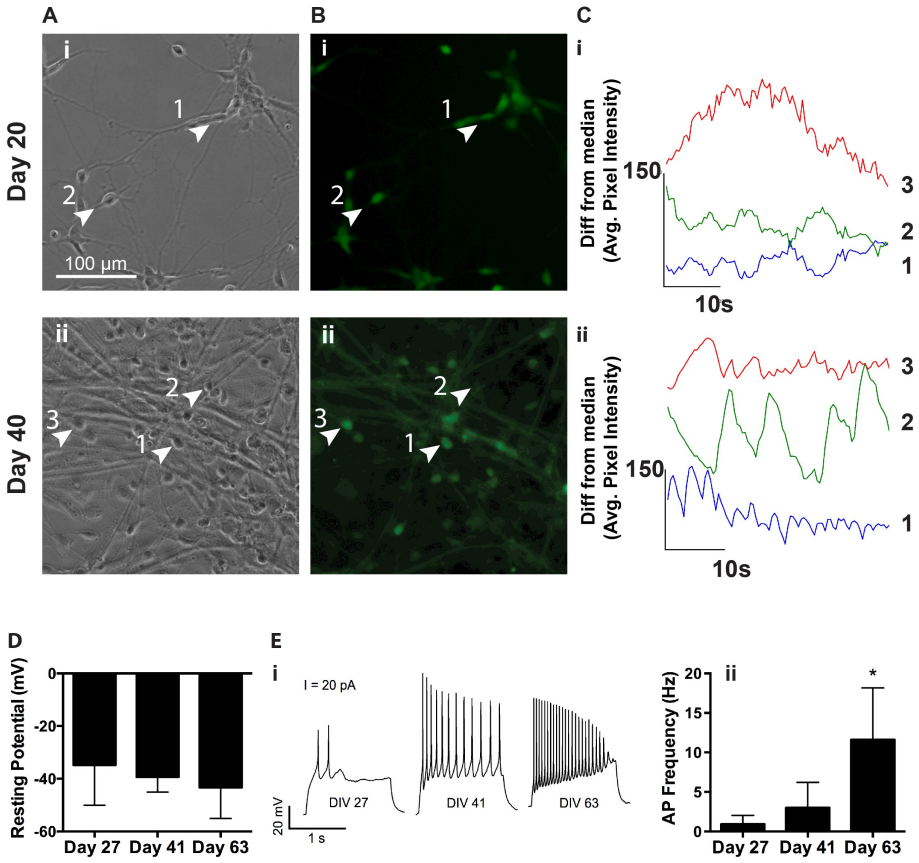

Fig. S9: Electrophysiological properties show increased maturity with culture duration. *(A_{i}-$_{ii}$)* Representative phase images of cultures on days 20 and 40. *(B_{i}-$_{ii}$)* Representative fluorescent images of the calcium indicator Fluo4-loaded cells. White arrowheads indicate somas where the regions of interest were selected for calcium imaging. *(C_{i-ii})* Representative traces of fluorescence intensity over time. *(D)* Resting membrane potential of current-clamp patched neurons at Day 27, 41, and 63. *(E_{i-ii})* Representative action potential firing in response to 20 pA current stimulation at Day 27, 41, and 63 (i). Average action potential frequency at Day 27, 41, and 63. Action potential frequency on Day 63 is significantly different ($p<0.05$, one-way ANOVA and Tukey post hoc comparison) than Day 27 and 41 (ii). n = 5, 6, and 9 for Day 27, 41, and 63, respectively. Error bars indicate standard error of the mean.

Figure S10

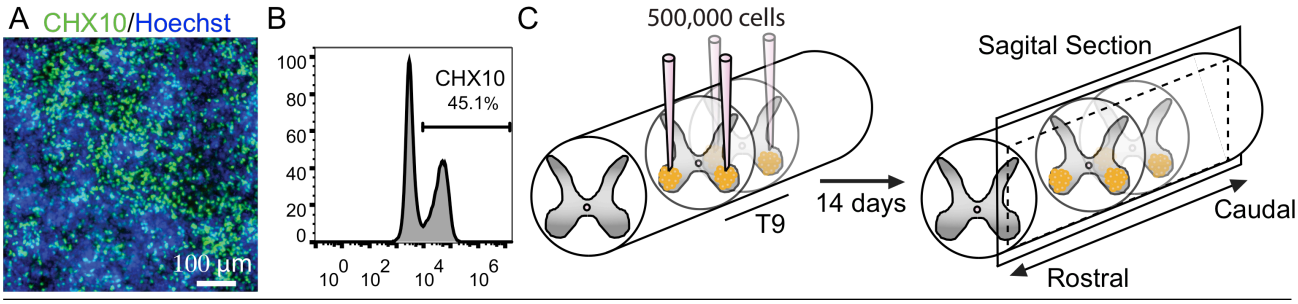

Fig. S10: Schematic of *in vivo* cell transplantation. *(A)* Immunostaining of CHX10 (green) and Hoeschst labeling of nuclei (blue) in V2a interneuron cultures on day 17. *(B)* Flow cytometry analysis of CHX10 in V2a interneuron cultures used for transplantation. *(C)* Schematic of cell transplantation into the adult murine spinal cord and sectioning of harvested spinal cord tissue at 2 weeks post-transplantation.

Figure S11

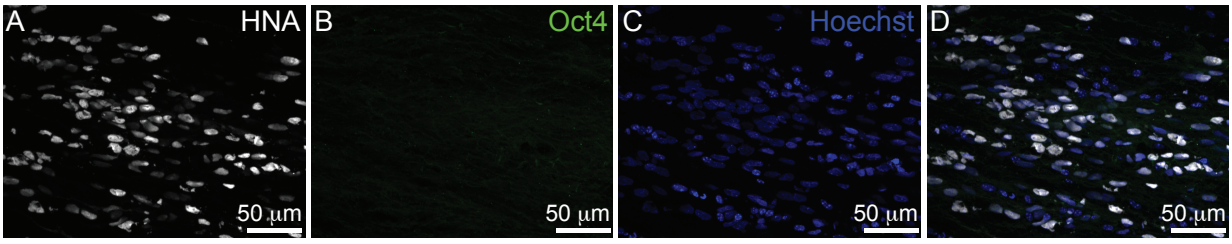

Fig. S11: Transplanted cells do not express Oct4. *(A-D)* Stem121 (white), Oct4 (green), and Hoechst labeled nuclei (blue) immunostaining of transplanted V2a interneurons.

Figure S12

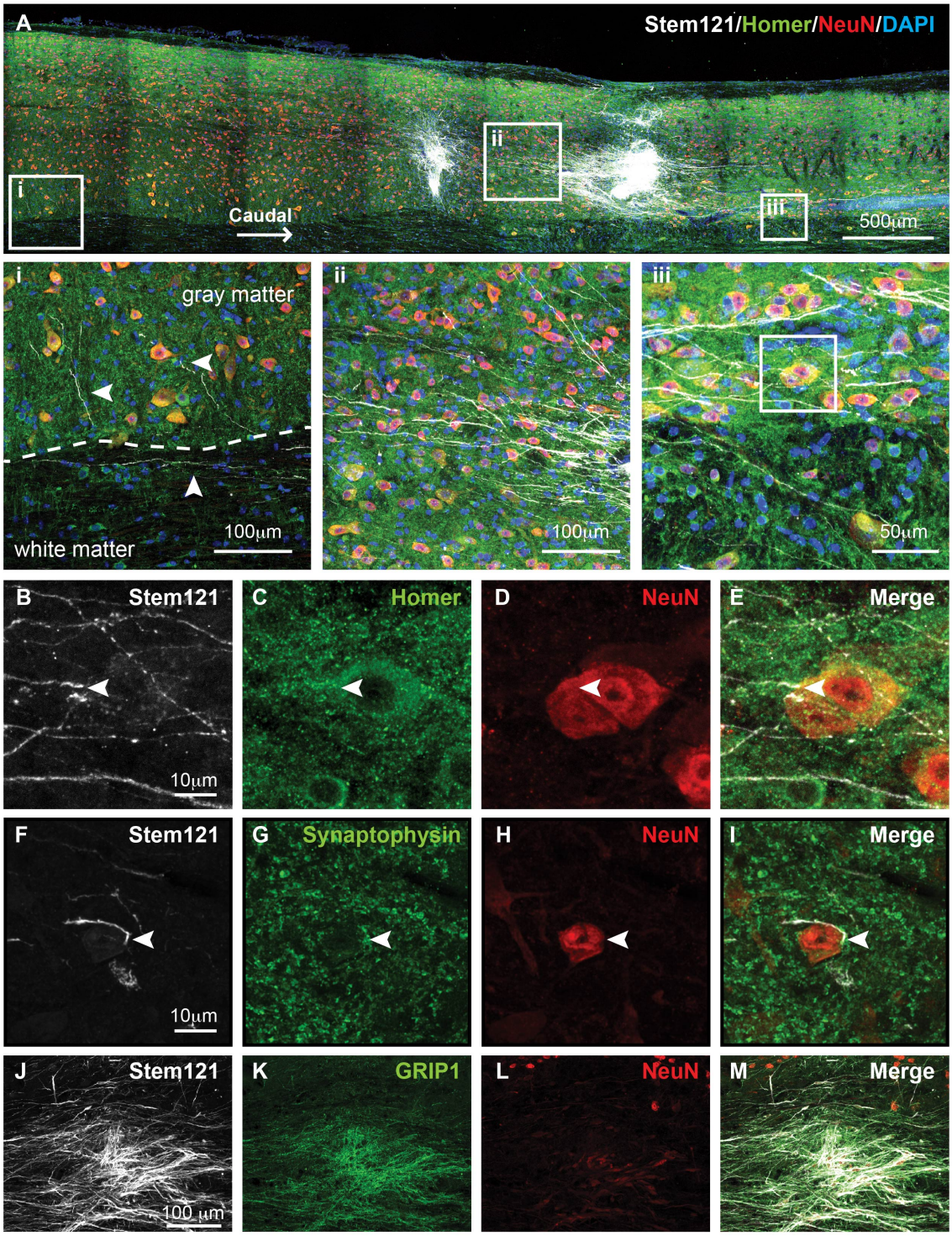

Fig. S12: Transplanted cells extend projections and form synapses with host neurons

(A_{i-iii}) Stem121 (white), Homer (green) and NeuN (red) immunostaining of transplanted V2a interneurons. (i) Inset of tissue rostral to the transplantation site. Horizontal arrows point to neurites that have extended into the gray matter. Vertical arrows point to neurites that have further extended into the white matter. (ii) Inset of tissue between the two transplantation sites. (iii) Inset of tissue caudal to

the transplantation site. Box highlights the area shown in B-E. *(B-E)* Stem121 (white), Homer (green) and NeuN (red) immunostaining of transplanted V2a interneurons. Arrows point to colocalization of Stem121, Homer, and NeuN. *(F-I)* Stem121 (white), synaptophysin (green) and NeuN (red) immunostaining of transplanted V2a interneurons. Arrowheads point to colocalization of Stem121, synaptophysin, and NeuN. *(J-M)* Stem121 (white), NeuN (red), and GRIP1 (green) immunostaining of transplanted V2a interneurons.

Table S1: Top differentially expressed genes for each cluster from heatmap

	Cluster A	Cluster B	Cluster C	Cluster D	Cluster E	Cluster F	Cluster G
1	NEFL	CRABP2	VIM	C8orf46	HES6	UBE2C	TAGLN
2	NEFM	NRN1	FGFBP3	RGS16	MT1X	HMGB2	IGFBP3
3	STMN4	SNCG	ZFP36L1	GADD45G	CKB	MT2A	ACTA2
4	TUBB2B	STMN2	HES1	ARL4D	VSX1	HES6	ANXA1
5	PMEL	GNAS	MGST1	NEUROG1	MT2A	PTTG1	CTGF
6	GAP43	HOXB5	PLP1	HES6	GLRX	MT1X	NPPB
7	RTN1	PRR24	DLK1	TFDP2	NEUROG1	BIRC5	MYL9
8	MLLT11	CRABP1	TTYH1	GADD45A	CDH13	CCNA2	S100A11
9	TCEAL7	NEUROD1	SOX2	DLL3	PPP1R17	CCNB2	TPM1
10	GNG3	NSG1	ID3	PSTPIP1	ASCL1	NEK2	IGFBP5
11	RP11-834C11.4	PCP4	GPC3	MFNG	NEUROD1	CDC20	CALD1
12	AP1S2	HOTAIRM1	GSN	PHLDA1	DOK5	NUSAP1	CYR61
13	CLDN5	ISG15	GNG5	PRDX1	RASD1	TUBB4B	HSPB1
14	TSPAN7	COTL1	ARL4A	BTG1	FAM162A	PBK	SPARC
15	UCHL1	GLRX	NPC2	ELAVL4	B2M	CDKN3	ANXA2
16	CPE	DUSP1	MAD2L1	VIM	STC1	CCNB1	TNFRSF12A
17	SCG5	GNG3	HMGB2	RASD1	DLL3	CDK1	MYL12A
18	TM2D3	DANCR	SMS	ARL4A	PPP1R14C	CCNA1	LGALS1
19	HERPUD1	FOS	MEST	SH3BGRL3	GADD45G	VSX1	B2M
20	KLHL35		PTTG1	SOX2	BID	GPC3	C8orf4

Table S2: Primers used for quantitative polymerase chain reaction

Gene Symbol	Gene Description	Lineage	Alias	Forward Primer Sequence	Reverse Primer Sequence
RPS18	Ribosomal Protein S18	Ribosomal protein	*18S*	CTTCCACAGGAGGCCTACAC	CTTCGGCCCACACCCTTAAT
CHX10	Ceh-10 Homeodomain-Containing Homolog	V2a interneurons	*VSX2*	CGGCGACACAGGACAATCTT	CCTGTATCCTGTCTTCCGGC
FOXN4	Forkhead Box N4	p2 domain		CGTACAGCTGTCTGATCGCC	GGAGCCGCTCATCTTGTTCT
GATA3	GATA Binding Protein 3	V2b interneurons		TTGTGCTCGGAGGGTTTCTT	CAGCACAGGCTGCAGGAATA
OLIG2	Oligodendrocyte Lineage Transcription Factor 2	Progenitor motor neurons		CGCATCCAGATTTTCGGGTC	AAAAGGTCATCGGGCTCTGG
HB9	Homeobox Protein HB9	Committed motor neurons	*MNX1*	TCTCTTAACGGGAAGGGGCA	CTAATTCAGGGCGCTCTCGG
PAX6	Paired Box 6	Early neural		GAGCGAGCGGTGCATTTG	TCAGATTCCTATGCTGATTGGTGAT
TUBB3	Beta 3 Class III Tubulin	Immature neurons		GAACCCCAGGCAGCTAGAC	ACTGATGACTTCCCAGAACTGT
POU5F1	POU Class 5 Homeobox 1	Pluipotency	*OCT4*	ATGCATTCAAACTGAGGTGCCT	AACTTCACCTTCCCTCCAACCA
NES	Nestin	Early neural		CCACCCTGCAAAGGGAATCT	GGTGAGCTTGGGCACAAAAG
SOX14	SRY (Sex Determining Region Y)-Box 14	V2a interneurons		GAACCCTTGCACTCCCTACC	TCGATGTATGGCCGCTTCTC
SIM1	Single-Minded Family BHLH Transcription Factor 1	V3 interneurons		GGCTCTCACCGGCAGTATTT	TGAGCCATTACAGCCCAAGG
RBFOX3	Hexaribonucleotide Binding Protein 3 Neuronal Nuclei (NeuN)	Neuronal Nuclei		ACGATCGTAGAGGGACGGAA	AATTCAGGCCCGTAGACTGC
NEFL	Neurofilament (NF) Light Chain	Neurons		CATCAGCGCTATGCAGGACA	GTCTCCTCGCCTTCCAAGAG
PDGFRA	Platelet-derived growth factor receptor, alpha peptide	Progenitor oligodendrocyte		CTGGACACTGGGAGATTCGG	CACGGCCTCCAATGATCTCT
CSPG4	Chondroitin Sulfate Proteoglycan 4	Progenitor oligodendrocyte		CACTCAGGACGAAGGAACCC	GGAGCAATACGGTACCCTGG
SOX10	SRY (Sex Determining Region Y)-Box 10	oligodendrocyte precursor		CACAAGAAAGACCACCCGGA	AAGTGGGCGCTCTTGTAGTG
GFAP	Glial Fibrillary Acidic Protein	Astrocytes		CAGTTATCAGGAGGCGCTGG	TTTGCCCCCTCGAATCTGC
THY1	Thy-1 Cell Surface Antigen	Retina		TGGATTAAGGATGAGGCCCG	TGGGGAGGTGCAGTCTGTAT
IRBP	Interphotoreceptor Retinoid-Binding Protein	Retina and photo receptor cells	*RBP3*	TATCTACAACCGCCCCTCCA	CTGGTGAGGACCACCACATC
CRX	Cone-Rod Homeobox	Retina		CCTTCTGACAGCTCGGTGTT	TGGTGTACTTCAGCGGTCAC

Table S3: Antibodies used for flow cytometry and immunostaining

Antibody Target	Species	Vendor	Cat. Number	Dilution
CHX10	Mouse	Santa Cruz Biotechnology	sc-374151	1:1000
CHX10	Sheep	Abcam	ab16141	1:500
β_{III} Tubulin	Rabbit	BioLegend	MRB-435P	1:1000
Vesicular Glutamate Transporter 2 (VGlut 2)	Rabbit	Synaptic Systems	135 403	1:500
Neurofilament (NF)	Mouse	Developmental hybridoma studies bank	2H3	1:20
Neurofilament 200	Rabbit	Sigma-Aldrich	N4142	1:200
LHX3 (LIM3)	Mouse	Developmental hybridoma studies bank	67.4E12	1:500
OLIG2	Rabbit	Millipore (EMD)	AB9610	1:500
HB9	Mouse	Developmental hybridoma studies bank	81.5C10	1:20
Stem 121	Mouse	Clontech	Y40410	1:500
Human Nuclear Antigen	Mouse	Millipore (EMD)	MAB1281	1:200
GRP1	Rabbit	Abcam	ab25963	1:200
GABA	Rabbit	Sigma-Aldrich	A2052	1:2000
NeuN	Chicken	Millipore (EMD)	abN91	1:500
Oct-3/4	Goat	Santa Cruz Biotechnology	sc-8629	1:100
Ms isotype	Mouse	R&D Systems	MAB002	
NeuN	Rabbit	Millipore (EMD)	ABN91	1:1000
Synaptophyin	Rabbit	Synaptic Systems	101 002	1:200
HOMER	Rabbit	GeneTex	GTX103278	1:100

Supporting References

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