Supplemental Figures

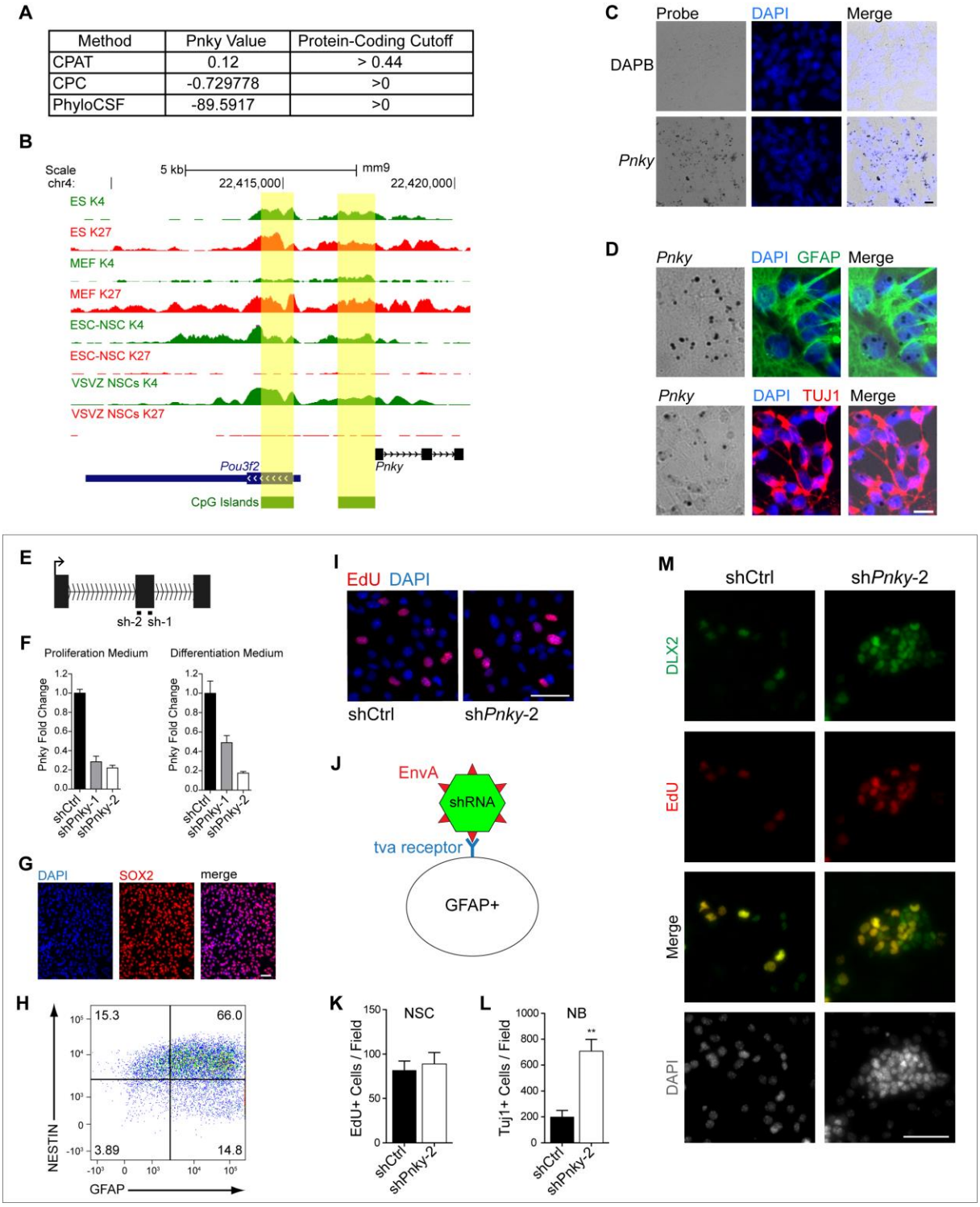

Figure S1: Characterization of the long noncoding RNA *Pnky* and *Pnky* knockdown cultures, related to Figure 1. A) Table of protein-coding scores derived from CPAT, CPC, and

PhyloCSF, and the cut-off for determining that a transcript is likely to encode a protein. B) Genome browser view of the *Pou3f2* and *Pnky* loci with H3K4me3 and H3K27me3 ChIP-seq tracks for embryonic stem cells (ES), mouse embryonic fibroblasts (MEF), ESC-derived neural stem cells (ESC-NSCs), and V-SVZ NSCs. Yellow boxes indicate promoter regions defined by CpG islands. C) *In situ* hybridization using branched DNA probes targeting *Pnky* and negative control bacterial transcript DAPB in V-SVZ-NSC cultures. Nuclei are counter-stained with DAPI. Scale bar = 10 µm. D) *In situ* hybridization using branched DNA probes targeting *Pnky* in V-SVZ-NSC cultures after 4 days of differentiation, immunostained for GFAP (green) or TUJ1 (red). Nuclei are counter-stained with DAPI (blue). Scale bar = 20 µm. E) Schematic of *Pnky* RNA and the regions targeted by sh*Pnky*-1 and sh*Pnky*-2. F) *Pnky* knockdown efficiency in cultures in proliferation medium (left) or differentiation medium (right). Fold change was calculated with the delta-delta Ct method, normalized to shCtrl and *Rplp0* housekeeping gene. Error bars are standard deviation propagated by least-squares method from technical triplicate. G) V-SVZ NSC cultures in proliferation conditions immunostained with SOX2 (red). Nuclei are counter-stained with DAPI (blue). Scale bar = 50 µm. H) FACS plot of GFAP and Nestin expression in GFP+ cells from V-SVZ cultures infected with control lentivirus. I) Pure cultures of V-SVZ-NSCs infected with shCtrl (left) or sh*Pnky*-2 (right) labeled with EdU for 1h and stained for incorporated EdU with Click-iT chemistry. Nuclei are counterstained with DAPI (blue). Scale bar = 50 µm. J) Schematic depicting targeting of shRNA constructs to NSCs with the TVA-EnvA system. K) Quantification of EdU labeling counted from GTVA V-SVZ NSCs infected with control or *Pnky*-knockdown constructs. L) Quantification of TUJ1+ NBs produced from pure cultures of GTVA V-SVZ NSCs after 4d differentiation. M) Pure cultures immunostained with DLX2 (green) and stained for EdU (red) after 3h of labeling in differentiation medium. Nuclei are counterstained with DAPI. Scale bar = 50 µm.

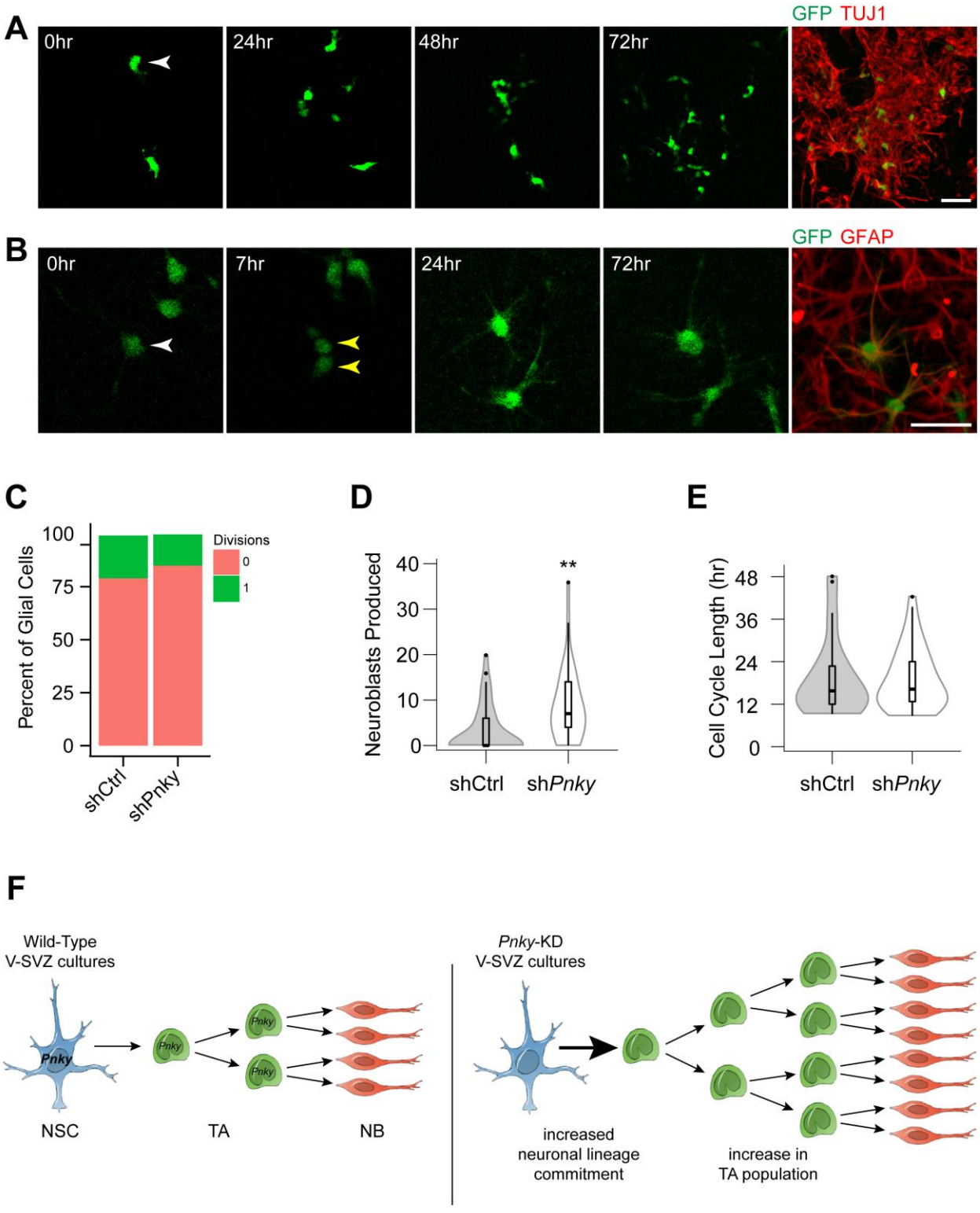

Figure S2: Analysis of time-lapse imaging of V-SVZ cultures, related to Figure 2. A)

Frames from time-lapse movies, demonstrating a neurogenic clone. Immediately after imaging,

plate was fixed and immunostained for GFP (green) and TUJ1 (red). White arrow indicates initial tracked NSC. Scale bar = 50 µm. B) Frames from time-lapse movies, demonstrating a glial clone undergoing one division. White arrow indicates initial tracked NSC and yellow arrows indicate daughter cells resulting from a division. Immediately after imaging, plate was fixed and immunostained for GFP (green) and GFAP (red). Scale bar = 50 µm. C) Bar graph representing the number of glial cell divisions per clone as a proportion of all glial clones. N = 481 shCtrl cells and 272 sh*Pnky* cells. D) Violin plot overlaying box-and-whisker plot representing the total number of progeny produced per initial neurogenic NSC. N = 44 shCtrl and 33 sh*Pnky* progenitors. E) Violin plot overlaying box-and-whisker plot representing the cell cycle length from the first to second divisions of shCtrl and sh*Pnky* cells. N= 27 shCtrl divisions and 45 sh*Pnky* divisions. F) Schematic depicting the role of *Pnky* in postnatal V-SVZ NSCs. Left: Normal lineage progression of neuronal production from V-SVZ NSCs (blue) to DLX2+ transit amplifying cells (TA, green) to TUJ1+ neuroblasts (NB, red). Right: *Pnky*-KD promotes neuronal production. With *Pnky*-KD, a greater proportion of NSCs commit to the neurogenic lineage, and TA cells undergo more cell divisions. **p <0.01, Student's t-test.

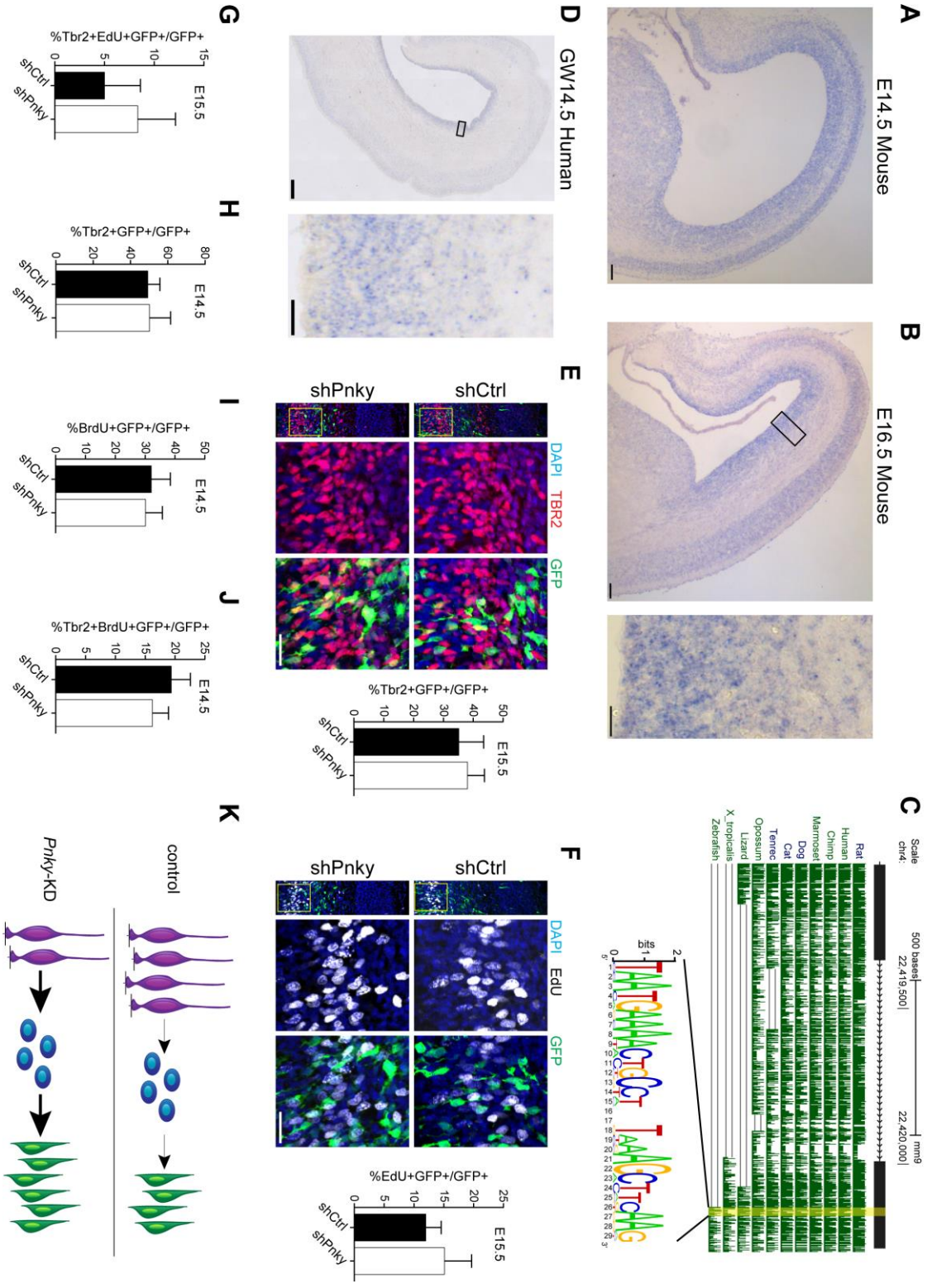

Figure S3: *Pnky* is evolutionarily conserved and expressed in the developing mouse and human cortex, related to Figure 3. A) *In situ* hybridization of *Pnky* in embryonic day 14.5

(E14.5) mouse brain. Scale bar = 100 µm. B) *In situ* hybridization for *Pnky* in E16.5 mouse brain. Scale bar = 100 µm. Black box indicates area expanded at right and in Figure 3A. Scale bar for high magnification image = 25 µm. C) Genome browser view of exons 2 and 3 of mouse *Pnky* and PhastCons scores across indicated vertebrates. The yellow box highlights a sequence conserved to zebrafish. In the sequence logo, a score of 2 bits indicates bases are perfectly conserved across all genomes. D) *In situ* hybridization of *Pnky* in human gestational week 14.5 (GW14.5) brain. Scale bar = 500 µm. Black box indicates area expanded at right and in Figure 3C. Scale bar for high magnification image = 50 µm. E) Left: Cortical sections at E15.5, 2d after electroporation with shCtrl or sh*Pnky*, stained for GFP (Green) and TBR2 (Red), with DAPI nuclear counterstain. Yellow box indicates region expanded in the subsequent panels. Scale bar = 25 µm. Right: quantification of Tbr2+ GFP+ cells as a percentage of total GFP+ cells. F) Left: Cortical sections at E15.5 stained for GFP (green) and EdU (white), with DAPI nuclear counterstain. Yellow box indicates region expanded in the subsequent panels. Scale bar = 25 µm. Right: quantification of EdU+ GFP+ cells as a percentage of total GFP+ cells. G) Quantification of Tbr2+EdU+GFP+ cells as a percentage of total GFP+ cells at E15.5. H) Quantification of Tbr2+GFP+ cells as a percentage of total GFP+ cells at E14.5, 1d after electroporation with shCtrl or sh*Pnky*. I) Quantification of BrdU+GFP+ cells as a percentage of total GFP+ cells at E14.5. J) Quantification of Tbr2+BrdU+GFP+ cells as a percentage of total GFP+ cells at E14.5. K) Schematic depicting the role of *Pnky* in regulating embryonic corticogenesis. Top: Normal lineage progression of neuronal production from radial glial SOX2+ NSCs (purple) to TBR2+ intermediate neurogenic progenitors (blue) to SATB2+ young neurons (green). Bottom: *Pnky*-KD promotes progression through the neurogenic lineage, resulting in a depletion of NSCs and an increase in young neurons. All error bars are standard deviation, n=4 brains of each condition from 3 separate surgeries.

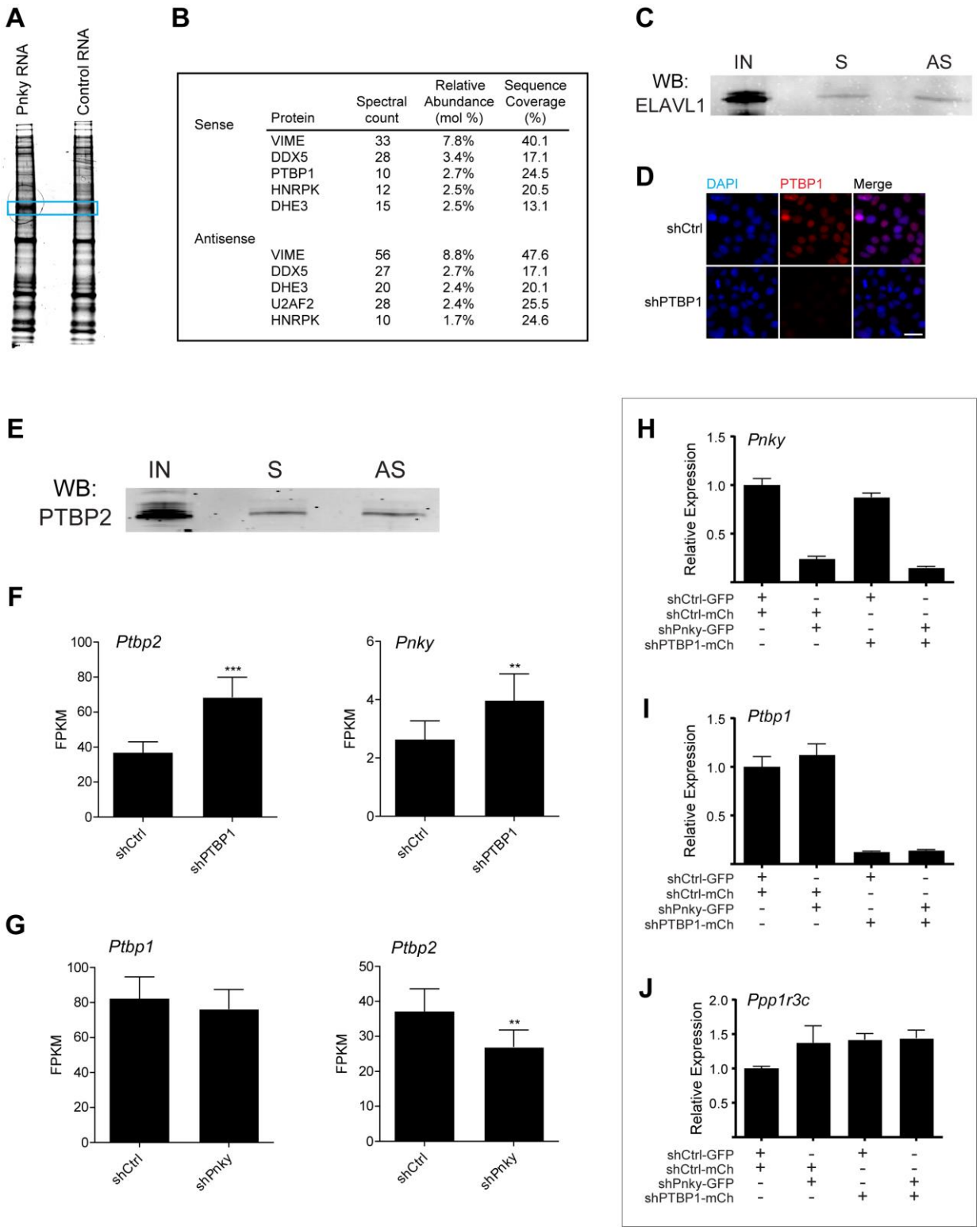

Figure S4: Characterization of the interaction between *Pnky* and PTBP1, related to Figure 4. A) Sypro Ruby-stained SDS-PAGE protein gel of proteins retrieved with biotinylated *Pnky*

RNA or control RNA. Blue box indicates area excised and analyzed by mass spectrometry. B) Top proteins identified by mass spectrometry from excised bands, excluding keratin contaminant peptides. C) Immunoblot for Elavl1 from RNA-pulldown experiment with biotin-labeled sense (S) or control anti-sense (AS) *Pnky* RNA incubated with N2A nuclear extract. D) Immunostaining for PTBP1 (red) in V-SVZ NSC cultures infected with control or shPTBP1. Nuclei are counterstained with DAPI. Scale bar = 25 μm. E) Immunoblot for PTBP2 from RNA-pulldown experiment with biotin-labeled sense (S) or anti-sense (AS) *Pnky* RNA incubated with N2A nuclear extract. F) FPKM values calculated by Cufflinks for *Ptbp2* or *Pnky* upon control or *Ptbp1* knockdown. Error bars represent 95% confidence intervals. **FDR<0.01 ***FDR<0.001. G) FPKM values calculated by Cufflinks for *Ptbp1* or *Ptbp2* upon control or *Pnky* knockdown. Error bars represent 95% confidence intervals. **FDR<0.01. H-J) RT-qPCR detection of expression of indicated gene normalized to GAPDH. Expression in each condition is shown relative to control (shCtrl-GFP, shCtrl-mCherry). Error bars are 95% confidence intervals from 3 separate cultures.

Supplemental Tables

Table S1: Differential Expression of Genes regulated by *Pnky* and PTBP1, related to Figure 4. Genes that are differentially expressed (FDR<.05) in both sh*Pnky* and shPTBP1 cells. Log2 of average fold change over control from two biological replicates is shown.

Table S2: Gene Ontology Terms for *Pnky* and PTBP1 co-regulated genes, related to Figure 4. Gene ontology terms with their DAVID p-value for genes that are differentially expressed upon both *Pnky* and PTBP1 knockdown.

Table S3: Differential Exon Usage for Genes regulated by *Pnky* and PTBP1, related to Figure 4. Differential exon usage upon *Pnky* knockdown or *Ptbp1* knockdown compared to control.

Supplemental Experimental Procedures

ChIP-seq analysis

V-SVZ ChIP-seq library generation and analysis is described in ref. 13 and available in NCBI GEO under accession GSE45282. ESC, ESC-NSC, and MEF ChIP-seq data was originally described in (Mikkelsen et al., 2007), and its analysis described in (Ramos, et al., 2013).

RNA-seq analysis

Published RNA-seq datasets were downloaded from GSE29184 and GSE36026, and abundance estimation was performed using Cufflinks v2.1.1. Experiments were done in biological duplicates. RNA was extracted using TRIzol and loaded onto RNeasy columns (QIAGEN) for on-column DNase treatment. Strand-specific, poly(A) selected cDNA libraries were generated using TruSeq Stranded mRNA kit (illumina) according to the manufacturer's protocol. Library validation and normalization were performed using RT-PCR and Quant-iT PicoGreen (Invitrogen). Cluster generation and high-throughput sequencing were performed on a HiSeq 2500 (Illumina), using the paired-end 100 bp protocol. Reads were aligned to the mouse genome mm9, using Tophat v2.0.10 with the following arguments: -p 6 --library-type fr-firststrand. Differential expression was assessed using Cuffdiff v2.1.1 with the following arguments: -b genome.fa -u --library-type fr-firststrand. A transcriptome index that includes all UCSC genes and the *Pnky* sequence was used for Tophat and Cufflinks. Alternative splicing was analyzed using DEXSeq v1.8.0, using a FDR threshold of 0.01.

V-SVZ NSC cultures and differentiation assay

The brain of postnatal day 5-7 (P5-7) mice was removed from the skull and placed in ice-cold L15 media and a 0.5 mm thick coronal slab was obtained. V-SVZ was dissected and

dissociated with 0.25% trypsin with occasionally agitation for 20 min at 37°C, and resuspended in N5 medium (DMEM/F12 with Glutamax, 5% Fetal Bovine Serum, N2 supplement, 35 µg/mL bovine pituitary extract, 20 ng/mL EGF, 20 ng/mL FGF, antibiotic/antimycotic). For most experiments, C57/B6 mice were used. For GTVA experiments, the GFAP-TVA mouse (Holland and Varmus, 1998) was used.

Cells were split 1:2 to passage 5 or 6 before switching to differentiation medium (DMEM/F12 with Glutamax, 2% FBS, N2 supplement, 35 µg/mL bovine pituitary extract, antibiotic/antimycotic). For analysis of cell fate, cells were fixed with 4% PFA and stained with anti-TUJ1 (Covance, 1:1000), anti-GFAP (Dako, 1:500), or anti-DLX2 (1:500). For EdU incorporation assays, 10µM EdU was added to culture medium for 1 hour (Figure 1H, S1K) or 3 hours (Figure 1K) before fixing.

Nuclear fractionation

~10 million V-SVZ NSCs were resuspended in 2 mL PBS, 2 mL nuclear isolation buffer (1.28 M sucrose, 40 mM Tris, pH 7.5, 20 mM $MgCl_2$, 4% Triton X-100), 6 mL of water, and incubated on ice for 20 mins with frequent mixing. Nuclei were then pelleted at 2500xg for 15 mins, resuspended in lysis buffer (150 mM KCl, 25 mM Tris pH 7.5, 5 mM EDTA, 0.5% Igepal, 0.5 mM DTT) and incubated on ice for 30 mins. For whole cell lysate, cell pellet was resuspended in lysis buffer and incubated on ice for 30 mins. RNA was extracted using Trizol LS according to manufacturer's instructions. 1 µg of RNA was used for first-strand synthesis with the Transcriptor First Strand cDNA Synthesis kit (Roche) using oligo-dT primers. cDNA was used for qPCR with Sybr Green master mix (Roche) and analyzed by a Light Cycler 480 (Roche).

Branched DNA *in situ* hybridization

Branched DNA *in situ* was performed on adult tissue according to manufacturer's instructions using the RNAScope 2.0 high definition BROWN kit (ACD). For V-SVZ cultures, cells were grown on laminin and poly-d-lysine coated Labtek 8-well chamber slides and fixed with 4% PFA. *In situ* was performed on cells according to manufacturer's instructions using the RNAScope 2.0 high definition BROWN kit with the following modification: Pretreat solution 2 was added to cells and they were steam treated for 10 mins. No protease was used.

FACS-purified V-SVZ lineage and microarray

Purification of the V-SVZ lineage and subsequent custom lncRNA microarray hybridization has been described in (Ramos et al., 2013) and (Pastrana et al., 2009). Raw data is available at NCBI GEO under accession GSE45282. Processed data is available at http://neurosurgery.ucsf.edu/danlimlab/lncRNA/

Knockdown lentiviral constructs

All shRNA sequences were designed using the Dharmacon siDESIGN tool. shRNA oligos were ordered from ELIM Biosystems, annealed, and ligated into the PSICO-R vector (Ventura et al., 2004), which carries a GFP or mCherry marker. shRNA targeting luciferase was used as a control. To establish purified infected cultures, GFP+ cells or GFP+, mCherry+ double positive cells were sorted using a FACS Aria II.

Time-lapse imaging

Cultures were established by trypsinization and subsequent mixing of infected GFP+ cultures (shCtrl or sh*Pnky*) with wildtype, uninfected cultures at a ratio of 1:200 to give ~15 GFP+ cells/high powered field and cultured in proliferation media for 8 hours to allow cells to adhere to the plate. Cultures were switched to differentiation medium and imaged on a Leica

SP5 inverted confocal microscope fitted with a Life Imaging Services microscope temperature control system. Cultures were maintained at 37°C and 5% CO_2, 21% O_2 and 8 optical sections were taken every 15 minutes for 3 days. Optical sections were summed and movies assembled with ImageJ. Cell fate was determined by morphology and representative fields were confirmed by fixing and staining for TUJ1 and GFAP. Cell cycle length was quantified as the amount of time between the first division of an NSC and the next division of each daughter that divided.

Human Fetal Tissue

Fetal cortical tissue was collected from elective pregnancy termination specimens at the San Francisco General Hospital, usually within 2h of the procedure. Research protocols were approved by the Committee on Human Research (institutional review board) at the University of California, San Francisco.

***In situ* hybridization**

In situs were performed as described (Wallace and Raff, 1999). Briefly, DIG-labeled RNA probes were synthesized using the T7 high yield RNA synthesis kit (NEB) and a Digoxigenin RNA labeling mix (Roche), and probes were hybridized overnight at 65 degrees. After washing, anti-digoxigenin Fab fragment (Roche) was added and incubated overnight at 4°C. Slides were washed and color reaction was carried out in 10% polyvinyl alcohol with NBT/BCIP (Roche) for 24-48 hours.

For mouse *Pnky,* the full-length transcript was cloned using SMARTer RACE cDNA amplification kit with RNA isolated from V-SVZ NSC cultures and cloned into the PGEM-Teasy vector (Promega). For human *Pnky*, a fragment corresponding to bp 367-1592 was cloned and used as an *in situ* probe.

***In utero* electroporation**

In utero electroporation was performed on E13.5 embryos from timed-pregnant wildtype Swiss-Webster mice (Simonsen labs) as described (Saito, 2006). Constructs used were PsicoR-shLuciferase (shCtrl), and PsicoR-sh*Pnky*-2 (sh*Pnky*). Embryos were harvested 24 or 48 hours later. One hour before harvesting, mice were given a single injection of either BrdU or EdU. Brains were fixed in 4% PFA, washed in PBS, and then equilibrated in 20% sucrose before embedding in 1:1 OCT:20% sucrose and sectioning on a Cryostat.

1-3 non-adjacent coronal sections per brain were imaged for quantification. Optical sections through the dorso-lateral telencephalon containing GFP+ electroporated cells were acquired at constant separation on a Leica SP5 Upright Confocal microscope. Three to four optical sections through the center were summed using ImageJ. Four animals from three separate surgeries were quantified for each experiment. For quanification of GFP+ cells in each zone, sections were costained with Nestin and Dcx. VZ was defined as NESTIN+DCX-, and CP was NESTIN-DCX+.

Antibodies

Goat anti-SOX2 (Santa Cruz Biotechnology), chicken anti-NESTIN (Aves), mouse anti-NESTIN (Millipore), rabbit anti-GFAP (DakoCytomation), chicken anti-GFP (Abcam), goat anti-GFP (Abcam), mouse anti-TUJ1 (Covance), mouse anti-PTBP1 (Invitrogen), guinea pig anti-DCX (Millipore), rabbit anti-SATB2 (Abcam), rabbit anti-TBR2 (Abcam), chicken anti-TBR2 (Millipore), rat anti-BrdU (Abcam). Guinea pig anti-DLX2 is described in (Kuwajima et al., 2006). For FACS-analysis of infected V-SVZ cultures, directly conjugated 555-Nestin and 647-GFAP (BD Pharmigen) were used. For Western Blot analysis, PTBP2 antibody is described in (Polydorides et al., 2000). Anti-Elavl1 (HuR) antibody was mouse anti-HuR (Santa Cruz 3A2).

RNA Immunoprecipitation Assay (RIP)

RIP assay was performed as described in (Rinn et al., 2007). ~10^7 V-SVZ cells or N2A cells were trypsinized and resuspended in 2 ml PBS, 6 ml water, 2 ml nuclear isolation buffer (1.28 M sucrose, 40 mM Tris pH 7.5, 20 mM MgCl2, 4% Triton X-100) and incubated on ice for 20 mins. Nuclei were then pelleted at 2500 x g for 20 mins at 4°C. Nuclear pellets were resuspended in 1 mL RIP buffer (150 mM KCl, 25 mM Tris pH 7.4, 5 mM EDTA, 5 mM MgCl2, 0.5% NP-40 0.5 mM DTT, PI tablet (Roche), and 100 u/ml RNAse OUT (Invitrogen)), and sheared with 20 strokes in a dounce homogenizer. Nuclear debris was pelleted by centrifugation at max speed for 10 mins at 4°C. Nuclear lysate was split into two aliquots and 8 ug antibodies were added. Antibodies used were anti-PTBP1 (Invitrogen) and anti-FLAG (Sigma). IP was performed rotating at 4°C overnight. The next day, 50 µL pre-washed Protein G Dynabeads were added and incubated for an additional 2 hours. Beads were washed on magnetic rack 3x with RIP buffer, and resuspended in Trizol after final wash. Trizol extraction was carried out according to manufacturers' instructions, cDNA was made using the Transcriptor First Strand cDNA synthesis kit (Roche) with both oligo-dT and random hexamer primers. Transcripts were detected with qPCR as described above.

RNA-pulldown

Biotinylated RNA pulldown was performed as described in (Hacisuleyman et al., 2014). To generate biotinylated RNA, *Pnky* sense and antisense were cloned into the PGEM-Teasy vector (Promega), which contains a T7 promoter. Biotinylated RNA was synthesized using the T7 High Yield Synthesis Kit (NEB) and biotinylated UTP (Roche). RNA probes were purified using the RNeasy mini kit (Qiagen). Immediately before use, 30 pmol RNA was resuspended in RNA structure buffer (10 mM HEPES pH 7, 10 mM MgCl2), heated to 65°C, then slow-cooled to 4°C in a thermocycler. RNA was run on a 1% agarose gel to verify integrity and correct size.

~10^7 V-SVZ-NSCs or N2A cells were pelleted and nuclei obtained as described for RIP protocol. To generate lysate, nuclear pellet was resuspended in RIP buffer with 1% NP-40 and

rotated for 30 mins at 4°C, and then debris was cleared by centrifugation at max speed for 30 mins. For preclear, 40 µL/pulldown MyOne T1 beads (Invitrogen) were washed/prepared according to manufacturer's instructions and added to the lysate. Mixture was rotated at 4°C for 1 hour, beads were removed with magnetic rack and discarded. Precleared lysate was diluted 1:2 in RIP buffer without NP-40 (Final concentration of NP-40 = 0.5%), and probes and yeast tRNA (final concentration = 0.1 µg/µL) (Invitrogen) were added. Binding reaction was carried out overnight at 4°C. The next day, 40 µL pre-washed MyOne T1 beads were added for an additional 1 hour. Tubes were added to the magnetic rack and washed 3 x 10 mins at 4°C on the rack with wash buffer (RIP buffer with 1% NP-40 and 300 mM KCl). After final wash, 1X NuPage running buffer was added and beads were boiled for 10 mins to elute protein.

Mass spectrometry

Selected SDS PAGE-separated bands were excised and in-gel digested with trypsin according to the established protocols (Jim nez et al., 2001). LC MS analyses of tryptic peptides utilized LTQ Orbitrap Velos mass spectrometer (Thermo Scientific, San Jose, CA) equipped with a NanoLC Ultra System (Eksigent, Dublin, CA), as described (Roan et al., 2014). MS/MS data were interrogated with Mascot 2.2.04 search engine (Matrix Science) following their conversion to the .mgf format with an aid of Mascot Deamon (Matrix Science): Rodentia taxonomy of UniProt database (release 2014_01, 26206 entries) was searched. Dynamic modifications included sulfoxide oxidatation at Met, deamidation at Asn or Gln, and Gln to pyro-Glu conversion at N-terminus. Carbamidomethyl at Cys was included as a static modification. One missed tryptic cleavage was allowed. Precursor and fragment ion mass tolerances were set to 5 ppm and 0.2 Da, respectively. A target-decoy strategy with a 0.045 target false discovery (FDR) rate was used for protein identification (Elias and Gygi, 2007). Relative abundances of proteins in the sense and antisense samples were estimated on the basis of two independent approaches: spectral counting (Liu et al., 2004; Lundgren et al., 2010) and the

exponentially modified protein abundance indices (emPAI) . Spectral counting utilized peptides identified at or above identity. Protein molar content (mol %) was calculated as described by (Ishihama et al., 2005) using emPAI values generated by the Mascot algorithm: only peptides identified at or above homology threshold are included. (http://www.matrixscience.com/help/quant_empai_help.html).

RT-qPCR Analysis:

Sequences for some primers were found with PrimerBank (Wang et al., 2012). For averaging qPCR results over biological replicates in double KD experiments, the method suggested in (Willems et al., 2008) was used.

***Pnky* sequences:**

>Human *PINKY*

TAAGCAGTGGTATCAACGCAGAGTACATGGGGAGGCGCCAGGGGCCCGGTTGGCGCGAA
CGCCGGGTTCCGAGCACCCTGGGCTTCCTTGTCTGCCTCCCAGCGCGGCACCTCTTCGG
GGCTCCCGAAACCTGAGCTCTCGCTGGTTTTAGGTCCAGACGGGGGCCTCTCCACCGGTT
CCTCCCCCGCCCCGGGCTCTGGGGCCCATTCTTTGGGCTGACCCTGTCAGGGCAGAGTC
CGCGCGTCTGCCTGCCATTCTCCGCCCGCATAAAAGCACGTTGAAGGTGTCTCGGGCAGA
CACCTCCAGGTTTTGAATCAGTTTATTCCCTTTCACTGTTCAAAGCAGCTGTTCAAATACAC
AGGCTGCTTACGTTGACGTGGAGAGGATTTCAAACAACGCTAAAATGCTTTGAACTGACAA
GGTGTCTTGATATCTCCCTCACTCCATCCAGCACAGCTCCTCGAGATCACTCGCTAGGACA
ATGGCTGAGCAGGCGATTCGTGCGGGCCTCGCCACCTCGGGGCGCGGACTGCGGGGTG
TCCTAAGCCCCTTCCGCAAGGACAGGATGGAGGCACCTGTAAGGAGATGCTGGCGCCAC
CCCAGCTTCTCCCAGGTCCGGAGGAACCTCTACTCAGTCAATACCCTGAGCTGGACTTGT
CTGAAGAAACGGAGCCGACTCCCTCTTGCCGGGGTGCGCTGAGTGGAGGGGAAACATCC
TCGAATAACAGAACTACACCAAAAAGACACCCATGTTATCTCTCACACTTTCACACTCCTCG

AGATAGTGAGCCGGACCTGGGTCTTAGTAGCACCCAGTACCTTGACACAAACCTCCCAAAT
TTCCACCTGAGTAACAGTTATGGGGTCAGTCCATGCACTGTAACTTGAACTCTAATTTATTA
ACTATTTCATCTAGTAAACACACTCACACCATATATAAAATAGCATTTATTTATTTCTATATAC
CAGGAGTTGGCAGAAAACCCACCGTGACCACTCCCATACATTGAGCTGGAGGCACACAAT
TACTAAAACAGAGGTGAGATGGTATTCATTTGATCTTAATTTTTTCTTATTTATGTAGTCCCA
GGATAATAGAAATCAGGAAACAAAAGAAAACAAAGAATTTTCTGAGGAGATGGCCATTGGG
GGAGTGGAGGTAGCAGCTGGTTTAAACCTAAGTAAAACTAGAAAAAGAAACTGCTGTTTCC
TTTTTCTTATATCCACCTTAGAGGATCATGTTTGAACGTCCCTACTCCTCCTCCTCTTTTTAA
AAAGCCTTGTCTCAGTCATTCATTCCTGTGCTTCCTGCTCTTCTGCTAGACCCCAGCAGCT
GTTTGATTTGGTGAGGCCCCCCTCCAACCTCTGAGTGGAACTTCTTTTCTAAGGGCCTGCA
GAATGTCAAAACTGAGGCTCTGGCTTCGGAGCTAGAGCTTTGAACAGCCAATCCACACAAA
AAGGCAGCTGGCTGCTTTAATGAAAACTGCTATAAAGCTTCAAGAACTTTAGCCTTGGGGG
ATGCATTTATAAGGAACATGGAAAATGCATTTCCAAGTTGCTGGTTCTTGGGAGAGGCATA
ATAAACATTTACC

>Mouse *Pinky*

GGGAGAAGCAACTTCCTCTGGTCTTCTGGAGGTGTAACCTACGTGCCCAGTAGGATATACT
GCCGGGTTGTGAAATGTCCACGCCTCTCCCCAACTGTCTTCCTTCCCAGCCCTCCGGGCT
TGGCTTTCTTGCTTCCCCAGGAGTTCAAATCTCCAACCTGCGGAAGAATTCAGCTGCTTGA
AAGGACTTAAGGCAGTGTGCGGAGGACATCTCCTTTCTCCGCCAGTAAAGAGAGCTGTTC
AAAGACCGAGGCTGCTTACGATGACGTGGAGAGGATTTCAAACAACCTTAACAGGCTTTGA
ACTGACAAGAAGCGGTGATATCTGCCTCACTCTGGCACAGCTCCTCCAGTGCACTTGCTAG
GACAATGGCTGAGAAAGCACTTGGTGCTGGCCTcTCTGCCGGGGGCGAGGACTGCCCAAA
CCCCAGAGACCCTAAGGCAGGAGTTGCTGCACTaCAATGGAGAGGTCTTGTcTTAGGCTGC
ACCTCAGGTTCTcTCAGATcTTCTGGAAGGGCGTTATTCAGCGGTACTGTGGTTGCGCTGT
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AGTcTACAGAATGTCAAAACTGAGGCTCCGACCTCAGAGCTACAGCTTTAAGGACCACTCC

ACACAAAGAGGCAGCTGGTTGCTTTAATGAAAACTGCCTTTAAGCTTCAAGAACTGAGGCC

TTGGGGAATCCATTTATAAGGAGCCTAGAAAATGCATTTCCAAGTTGTATGTTcTTAGGAGA

TACAAAATAAAATTTACCTGAAAAAATTAATTGT

shRNA sequences:

Luciferase_F:

T**GAGCTGTTTCTGAGGAGCC**TTCAAGAGA**GGCTCCTCAGAAACAGCTC**TTTTTTC

Luciferase_R:

TCGAGAAAAAA**GAGCTGTTTCTGAGGAGCC**TCTCTTGAA**GGCTCCTCAGAAACAGCTC**

Pnky_sh1_F:

T**GGACAATGGCTGAGAAAGC**TTCAAGAGA**GCTTTCTCAGCCATTGTCC**TTTTTTC

Pnky_sh1_R:

TCGAGAAAAAA**GGACAATGGCTGAGAAAGC**TCTCTTGAA**GCTTTCTCAGCCATTGTCC**

Pnky_sh2_F:

T**GATGACGTGGAGAGGATTT**TTCAAGAGA**AAATCCTCTCCACGTCATC**TTTTTTC

Pnky_sh2_R:

TCGAGAAAAAA**GATGACGTGGAGAGGATTT**TCTCTTGAA**AAATCCTCTCCACGTCATC**

shPTBP1_F:

T**GGGTGAAGATCCTGTTCAA**TTCAAGAGATTGAA**CAGGATCTTCACCCTTTTTTC**

shPTBP1_R:

TCGAGAAAAAA**GGGTGAAGATCCTGTTCAA**TCTCTTGAA**TTGAACAGGATCTTCACCC**

qPCR Primer Sequences:

Pnky_F1: TCTCCTTTCTCCGCCAGTAA

Pnky_R1: CACCGCTTCTTGTCAGTTCA

Pnky_F2: GCAGGAGTTGCTGCACTACA

Pnky_R2: GTACCGCTGAATAACGCCCT

GAPDH_F: GGGAAATTCAACGGCACAGT

GAPDH_R: AGATGGTGATGGGCTTCCC

U1_F: ACGAAGGTGGTTTTCCCAG

U1_R: GTCCCCCACTACCACAAA

B-Act_F: CTAAGGCCAACCGTGAAAAG

B-Act_R: ACCAGAGGCATACAGGGACA

PTBP1_F: AGTGCGCATTACACTGTCCA

PTBP1_R: CTTGAGGTCGTCCTCTGACA

Igfbp5_F: CCCTGCGACGAGAAAGCTC

Igfbp5_R: GCTCTTTTCGTTGAGGCAAACC

Ppp1r3c_F: TGATCCATGTGCTAGATCCACG

Ppp1r3c_R: ACTCTGCGATTTGGCTTCCTG

Ntsr2_F: TTCACCGCGCTCTATTCGC

Ntsr2_R: AGGGGTAGTGGGACCACAC

Scrg1_F: CCTTGGGCTAACTTTGCTGTT

Scrg1_R: TGGACATTTGCATCTATCAGCTT

Supplemental References

Elias, J.E., and Gygi, S.P. (2007). Target-decoy search strategy for increased confidence in large-scale protein identifications by mass spectrometry. Nat Methods *4*, 207–214.

Holland, E.C., and Varmus, H.E. (1998). Basic fibroblast growth factor induces cell migration and proliferation after glia-specific gene transfer in mice. Proc. Natl. Acad. Sci. U.S.a. *95*, 1218–1223.

Ishihama, Y., Oda, Y., Tabata, T., Sato, T., Nagasu, T., Rappsilber, J., and Mann, M. (2005). Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. Mol. Cell Proteomics *4*, 1265–1272.

Kuwajima, T., Nishimura, I., and Yoshikawa, K. (2006). Necdin promotes GABAergic neuron differentiation in cooperation with Dlx homeodomain proteins. Journal of Neuroscience *26*, 5383–5392.

Liu, H., Sadygov, R.G., and Yates, J.R. (2004). A model for random sampling and estimation of relative protein abundance in shotgun proteomics. Anal. Chem. *76*, 4193–4201.

Lundgren, D.H., Hwang, S.-I., Wu, L., and Han, D.K. (2010). Role of spectral counting in quantitative proteomics. Expert Rev Proteomics *7*, 39–53.

Mikkelsen, T.S., Ku, M., Jaffe, D.B., Issac, B., Lieberman, E., Giannoukos, G., Alvarez, P., Brockman, W., Kim, T.-K., Koche, R.P., et al. (2007). Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature *448*, 553–560.

Rinn, J.L., Kertesz, M., Wang, J.K., Squazzo, S.L., Xu, X., Brugmann, S.A., Goodnough, L.H., Helms, J.A., Farnham, P.J., Segal, E., et al. (2007). Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell *129*, 1311–1323.

Ventura, A., Meissner, A., Dillon, C.P., McManus, M., Sharp, P.A., Van Parijs, L., Jaenisch, R., and Jacks, T. (2004). Cre-lox-regulated conditional RNA interference from transgenes. Proc. Natl. Acad. Sci. U.S.a. *101*, 10380–10385.

Wang, X., Spandidos, A., Wang, H., and Seed, B. (2012). PrimerBank: a PCR primer database for quantitative gene expression analysis, 2012 update. Nucleic Acids Res *40*, D1144–D1149.

Willems, E., Leyns, L., and Vandesompele, J. (2008). Standardization of real-time PCR gene expression data from independent biological replicates. Anal. Biochem. *379*, 127–129.