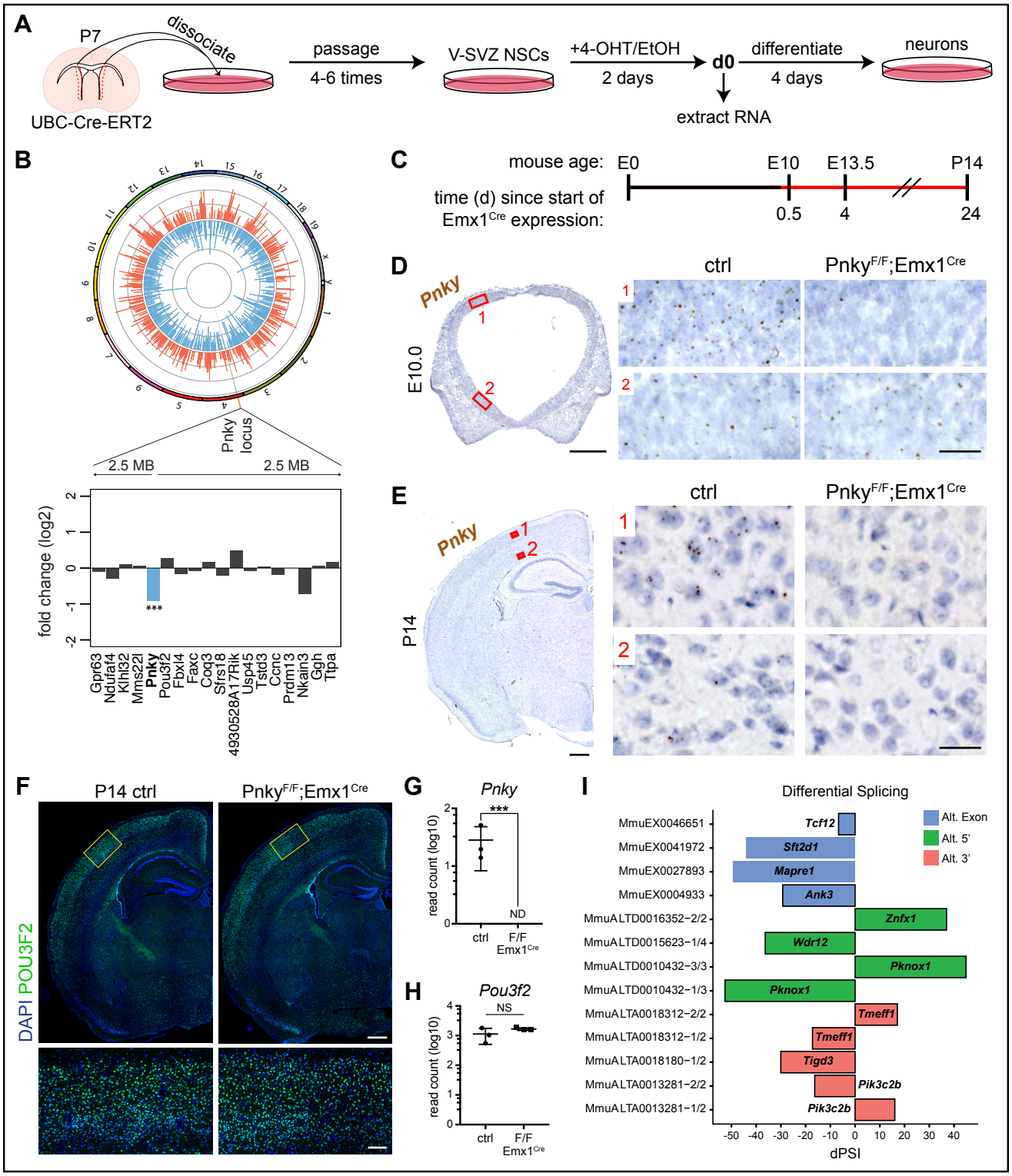

Figure S1: Conditional deletion of *Pnky* in cultured cells and *in vivo*, related to Figure 1.
Quantifications displayed as mean +/- SD. NS = not significant, *** = p < 0.001. **A**, Schematic of V-SVZ cultures. **B**, Circos plot of genomic locations of differentially-expressed genes upon *Pnky*-KD in V-SVZ cultures (Ramos et al., 2015). Expanded region depicts genes within 2.5 MB of *Pnky*. **C**, Schematic of *Emx1*^{Cre} expression during mouse development. **D**, **E**, ISH of *Pnky* (brown puncta) with hematoxylin counterstain (blue). For E10.0 (D), representative coronal section with red boxes to indicate approximate regions of pallium (1) and subpallium (2) enlarged in adjacent panels. Scale bars = 250μm and 25μm (insets). For P14 (E), representative coronal hemisphere with red boxes to indicate approximate regions of upper (1) and deep (2) cortical layers enlarged in adjacent panels. Scale bars = 500μm and 25μm (insets). **F**, IHC of POU3f2 with DAPI nuclear stain (blue). Scale bars = 500μm and 100μm (insets). **G**, **H**, Levels of *Pnky* (G) or *Pou3f2* (H) in E12.5 cortical tissue by RNA-seq. ND = not detected. **I**, Significant differentially-spliced events from biological triplicate samples. Gene names depicted within graph, with specific event names along y-axis. Events with black border = highly supported. Difference in percent spliced in (dPSI) calculated as PSI(Pnky-cKO) – PSI(ctrl).

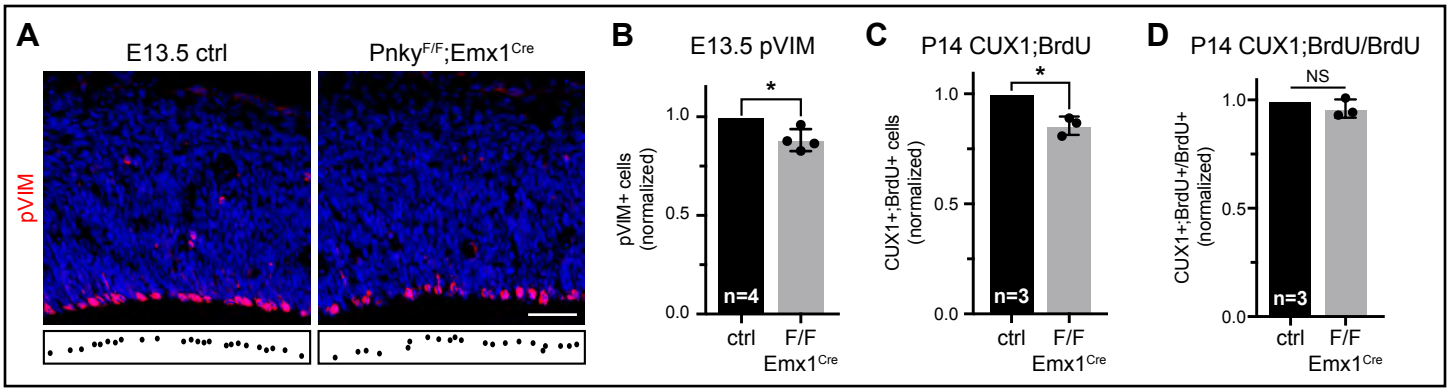

Figure S2: Loss of *Pnky* disrupts cortical development, related to Figure 2.
Quantifications displayed as mean +/- SD from separate biological replicates, normalized to littermate controls. NS = not significant, * = $p < 0.05$, two-tailed ratio paired t test. **A**, pVIM IHC, with DAPI nuclear stain (blue). Locations of pVIM+ cells in VZ depicted below. Scale bar = 50μm. **B**, Quantification of (A). **C**, Quantification of BrdU+;CUX1+ cells in upper layers of P14 cortex. BrdU administered at E15.5. **D**, Quantification of the proportion of BrdU+ cells that also express CUX1 in the P14 cortex. BrdU administered at E15.5.

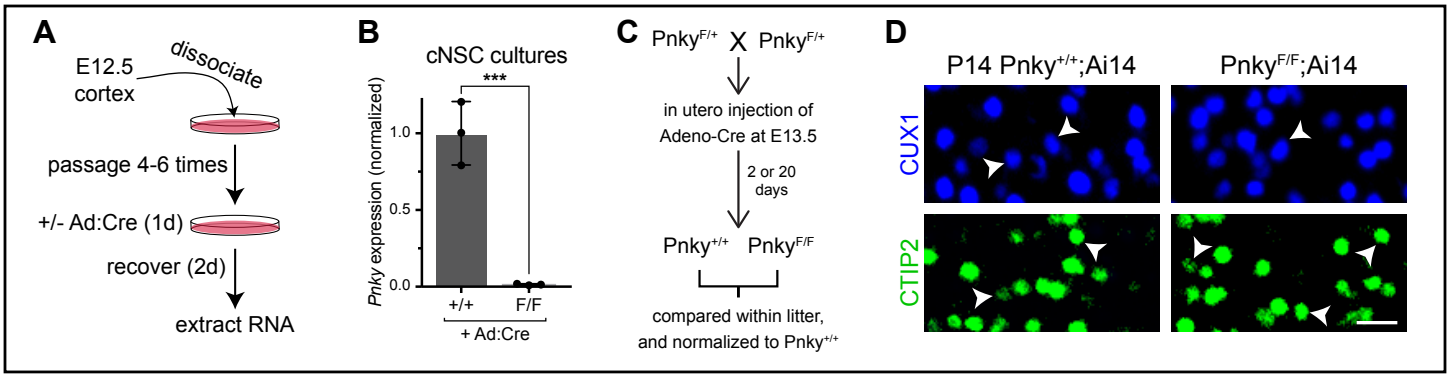

Figure S3: *Pnky* deletion from a small cohort of cells in the developing cortex, related to Figure 3.
A, Schematic of cNSC culture derivation to test Ad:Cre-mediated deletion of *Pnky*. **B**, *Pnky* expression in Ad:Cre-treated cNSC cultures, quantified by qRT-PCR. Mean +/- SD from separate biological replicates, normalized to paired uninfected controls and to average $Pnky^{+/+}$ +Ad:Cre value. *** = $p < 0.001$, two-tailed ratio paired t test. **C**, Schematic of genetic crosses used to produce littermates for *in utero* experiments. **D**, CTIP2 and CUX1 IHC at P14, following *in utero* injection of Ad:Cre at E13.5. Arrowheads = tdTomato+ cells, as shown in (Fig. 3F). Scale bar = 20μm.

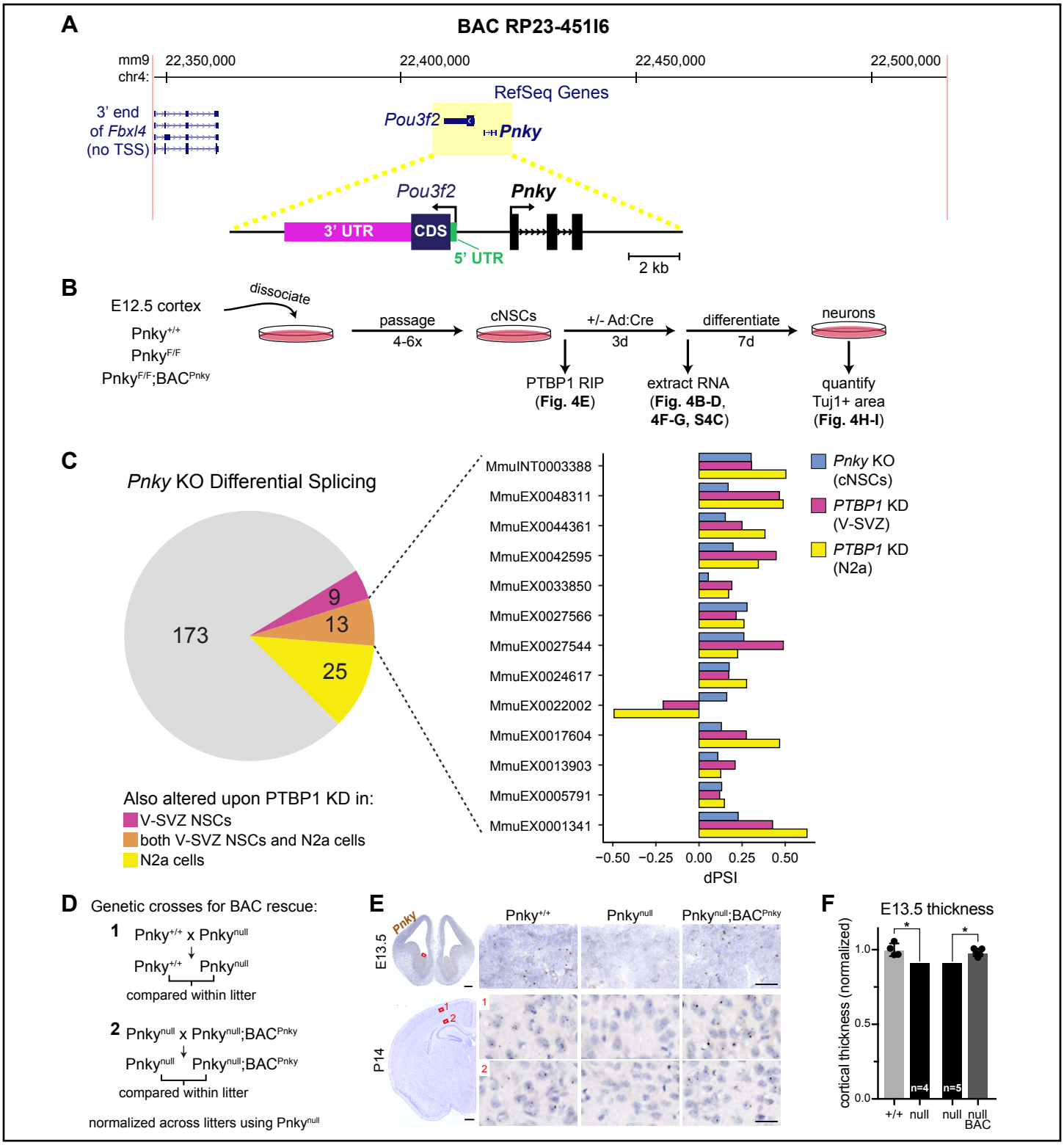

Figure S4: Loss of *Pnky* is rescued by transgenic expression from a BAC, related to Figure 4.
Quantifications displayed as mean +/- SD. * = p < 0.05, ** = p < 0.01, two-tailed ratio paired t test. **A**, Diagram of the BAC used to generate BAC^{Pnky} transgenic mouse line. Coding sequence (CDS) of *Pou3f2* was removed, leaving untranslated regions (UTRs) intact. **B**, Schematic depicting cNSC culture experiments in the figures indicated. **C**, Overlap of differentially-spliced events upon *Pnky*-KO or PTBP1-KD (Raj et al., 2014; Ramos et al., 2015). Difference in percent spliced in (dPSI) = PSI(experimental) – PSI(ctrl). **D**, Diagram of genetic crosses for BAC rescue experiments. **E**, ISH of *Pnky* (brown puncta) in E13.5 and P14 sections, with hematoxylin counterstain (blue). For E13.5, representative coronal section (also in Fig. 4J) with red box to indicate approximate regions of subpallium enlarged in adjacent panels. Scale bars = 250μm and 25μm (insets). For P14, representative coronal hemisphere with red boxes to indicate the approximate regions of upper (1) and deep (2) cortical layers enlarged in adjacent panels. Scale bars = 500μm and 25μm (insets). **F**, Quantification of cortical thickness. Littermate biological replicates, normalized as indicated in (D).