

Supplementary Materials for

H3 acetylation selectively promotes basal progenitor proliferation and neocortex expansion

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Figs. S1 to S10

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Other Supplementary Material for this manuscript includes the following:

Tables S1 to S9

Fig. S1

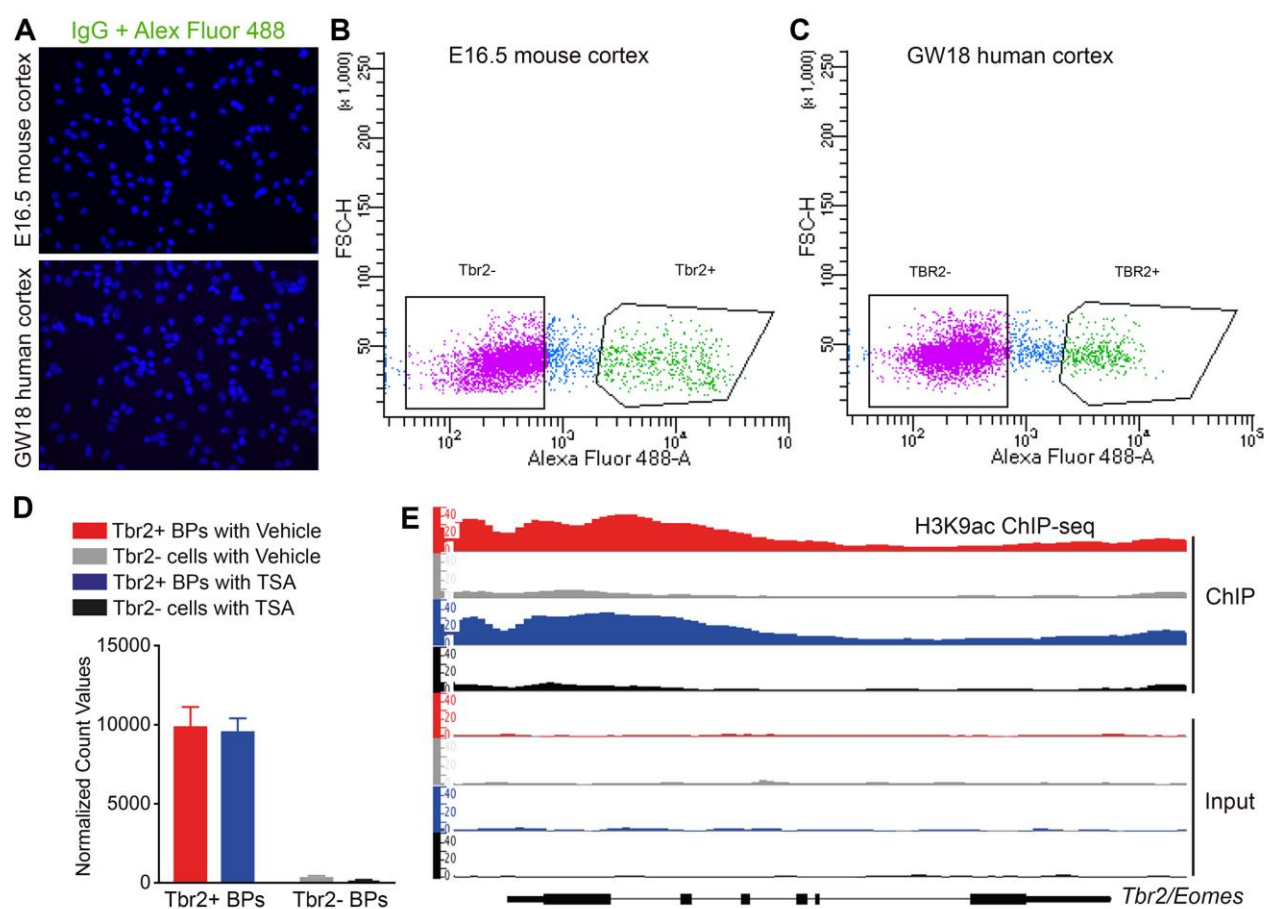


Fig. S1. Purification and characterization of Tbr2+ BPs.

(A) Images showed stained samples as isotype control (see also Fig. 1B/C). (B, C) Representative plot showing sorting gates for Tbr2+ cells and Tbr2- cells from mouse cortex at E16.5 (B) and human cortex at GW18 (C). (D, E) Purity of the sorted Tbr2+ BPs and Tbr2- cells was further proven by RNA-Seq (D) and H3K9ac ChIP-Seq (E). Tbr2+ BPs had much higher levels of Tbr2 RNA expression (D) and much higher levels of H3K9ac at Tbr2 gene locus (E) compared to Tbr2- cells.

Fig. S2

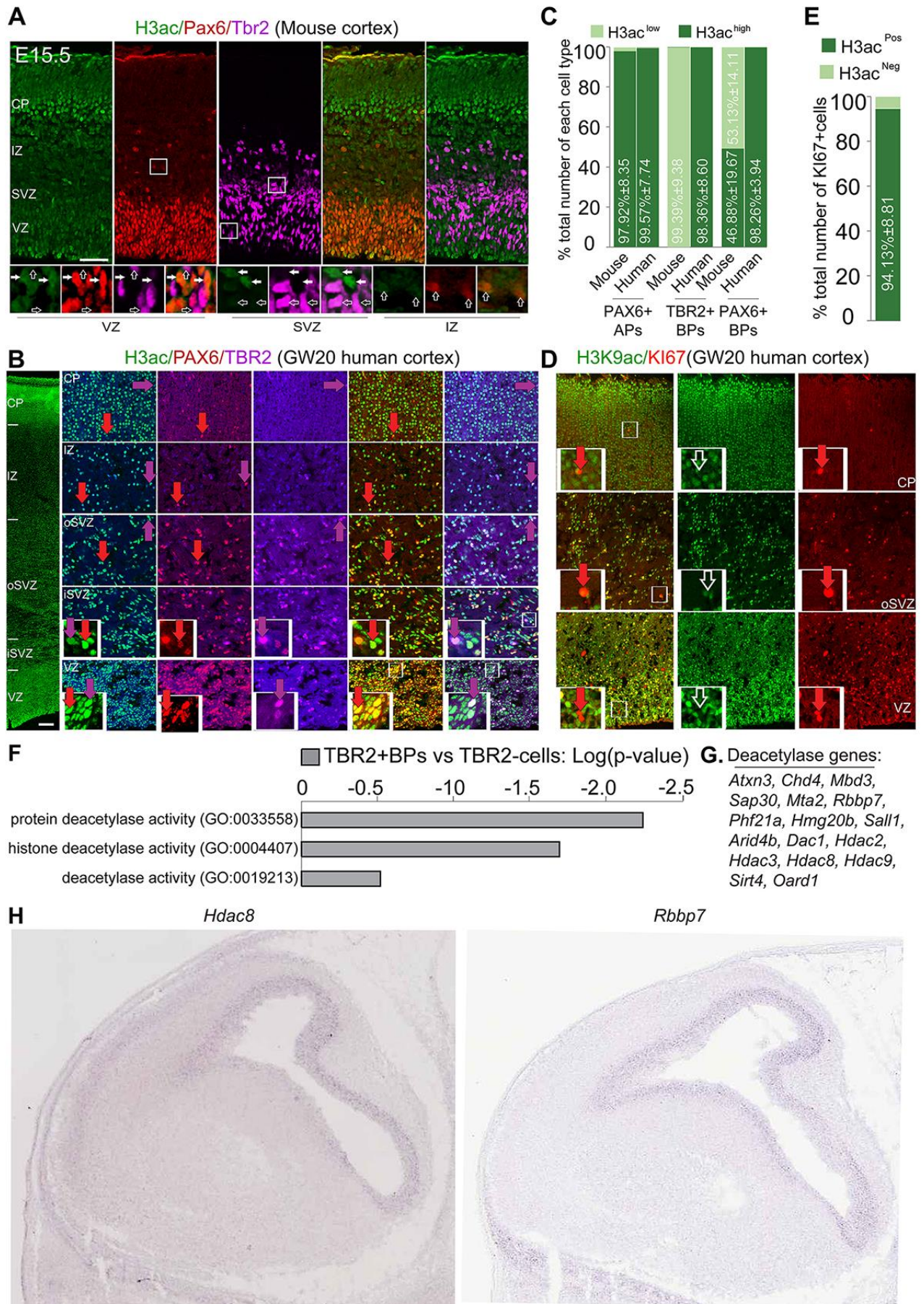


Fig. S2. Histone H3 is acetylated differently in basal progenitors in murine and human developing cortex.

(A) Images of triple IHC with cortical section from mouse embryo at E15.5 stained with antibodies against: H3ac, Pax6, and Tbr2. The lower panels showing selected frames at VZ and IZ at higher magnification reveal Pax6^{high+}, Tbr2^{low+} APs are H3ac^{high+} cells (filled arrows), whereas many Pax6^{high+} BPs in IZ and all Tbr2^{high+} BPs are H3ac^{low+} cells (empty arrows). (B) Images showing triple immunolabeling with antibodies against H3ac, Pax6 and Tbr2 and human fetal cortex at GW20 demonstrate that both the Pax6⁺ APs and Pax6⁺ BPs (arrows in red) and the Tbr2⁺ BPs (arrows in magenta) are highly immunoreactive with H3ac. (C) Statistical analyses of IHC assay (shown in A and B) comparing the level of H3ac (H3ac^{high} and H3ac^{low}) in progenitor subtypes in developing mouse and human cortex confirm the high level of H3ac mark in both APs and BPs in humans. (D, E) IHC (D) and statistical analyses (E) of GW20 human cortical sections with H3K9ac and Ki67 antibodies revealed that not all H3K9ac⁺ cells co-express Ki67. (F) Graphical representation of Gene ontology analysis with terms related to protein deacetylases. (G) List of the genes identified in mouse BPs that functionally fall under protein/histone deacetylation processes. (H) Micrographs showing *in situ* hybridization obtained from GenePaint database, and indicating examples of the identified protein/histone deacetylation genes with distinctive expression in the developing mouse cortical subventricular zone. Abbreviations: BPs, Basal progenitors; vRGs, ventricular radial glial progenitors; IPs, intermediate progenitors; oRGs, outer sub-ventricular radial glial progenitors; VZ, ventricular zone; SVZ, subventricular zone; iSVZ, inner subventricular zone; oSVZ, outer subventricular zone; IZ, intermediate zone; CP, cortical plate. Scale bars = 50 μ m.

Fig. S3

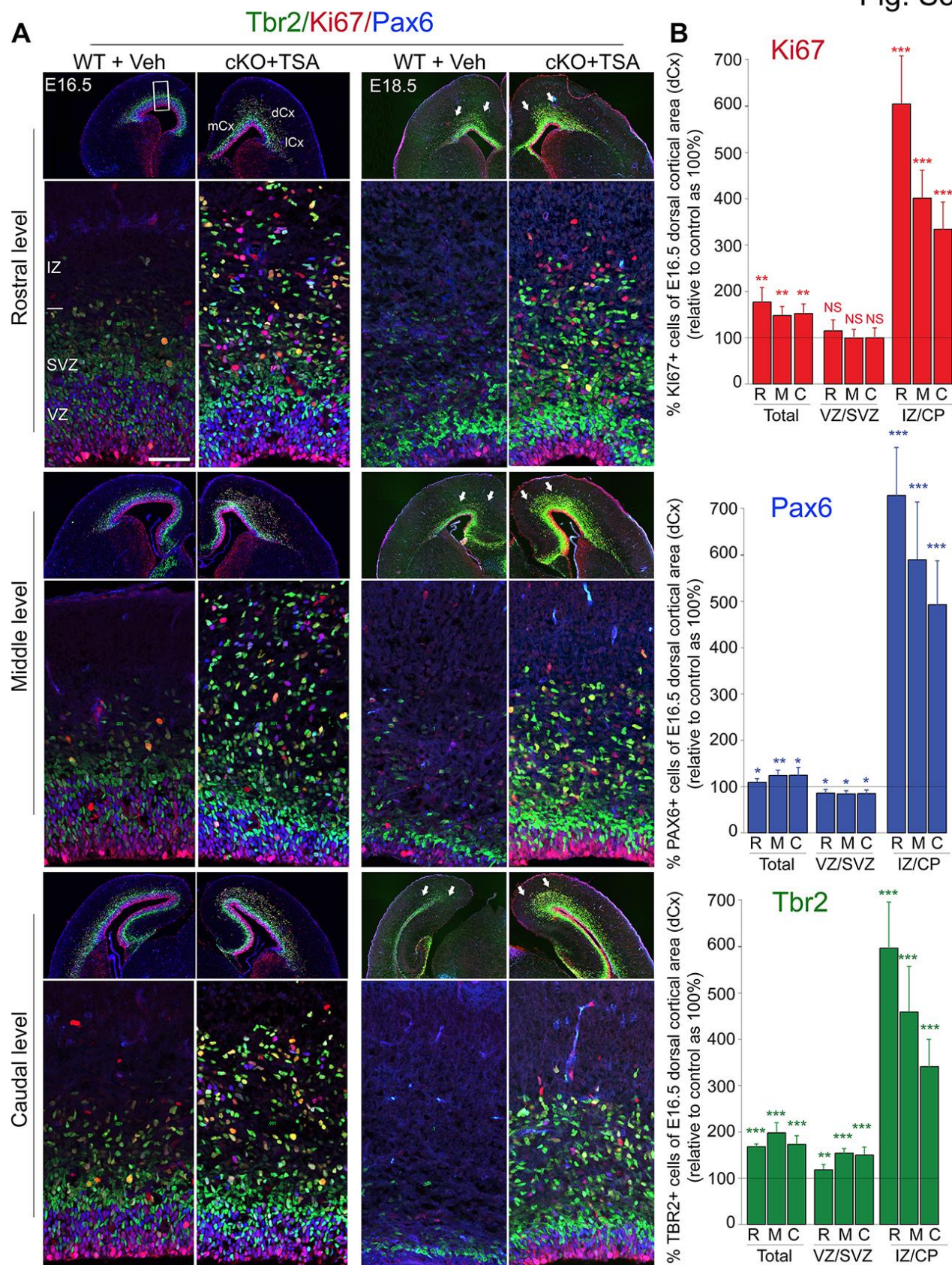


Fig. S3. Area-restricted increases of Tbr2+, Pax6+, Ki67+ BPs in HDACi-treated cortex.

(A) Merged confocal micrographs of fluorescent immunostaining for Pax6 (APs and BPs), Tbr2 (BPs), and Ki67 (actively-proliferating progenitors in cell cycle) with rostral, middle and caudal levels from unfolded areas of Veh-treated WT and TSA-treated Baf155cKO cortex at stages E16.5 and E18.5. In each level, *upper rows* present coronal mouse brain sections while *lower rows* present confocal images of an area in dorsal pallium. The arrows point the border between dCx/mCx, which corresponds to the selected medNcx region at E18.5 in the previous study (Vaid et al., 2018), and contains a high proportion of Tbr2+, Pax6+, Ki67+ BPs. (B) Statistical analyses of immunostainings shown in A for dorsal cortex (dCx) at E16.5. Values are presented as mean $\pm$ SEM (*P < 0.05, **P < 0.01, ***P < 0.005). Abbreviations: BPs, Basal progenitors; vRGs, ventricular radial glial progenitors; IPs, intermediate progenitors; VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate. Scale bars = 50 μ m.

Fig. S4

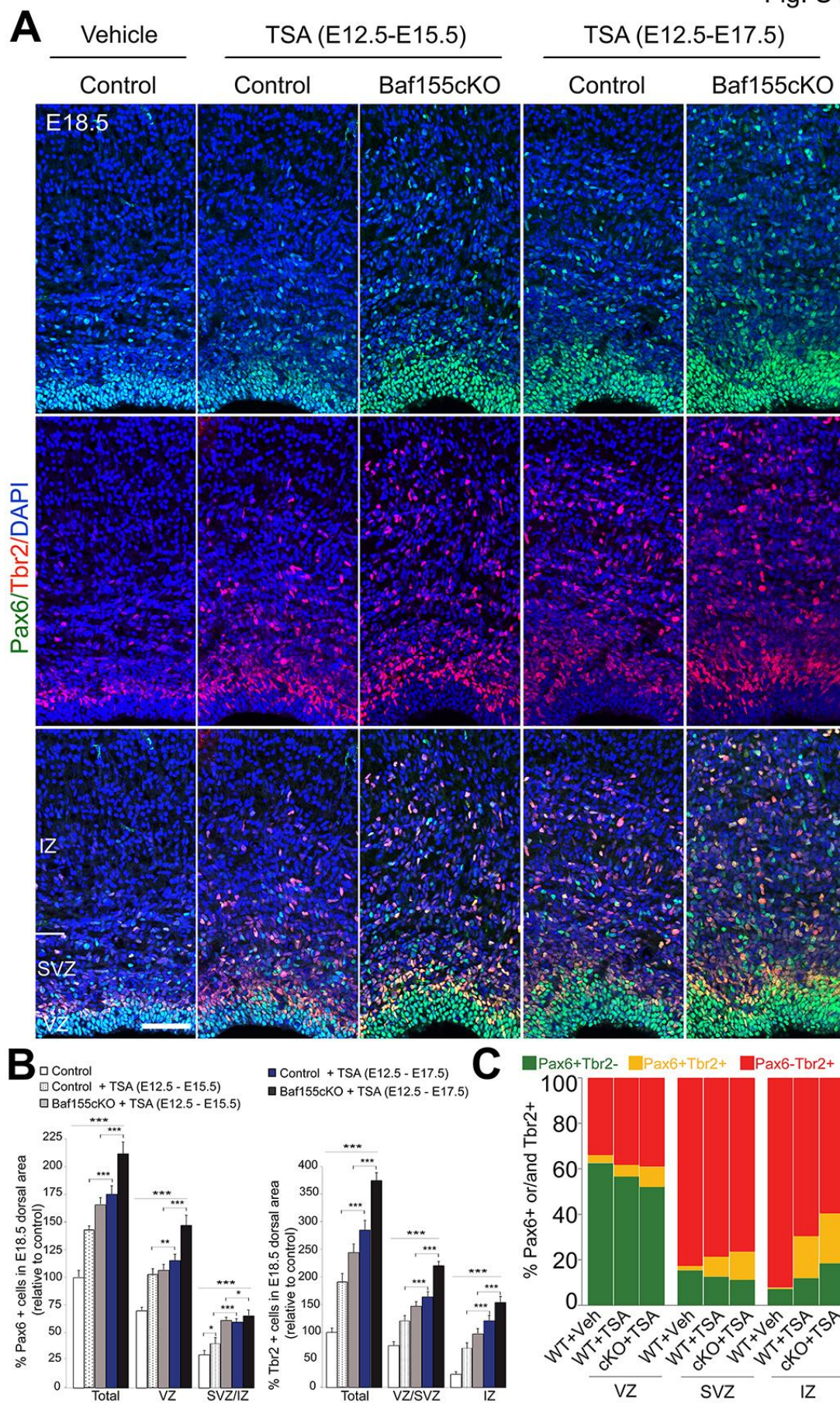


Fig. S4. HDAC inhibition increase the genesis of BPs in dose-dependent fashion.

(A) Immunofluorescence micrographs showing the quantity and distribution of Pax6 and Tbr2 at E18.5 in control and Baf155cKO cortices, with or without two regimens of HDACi treatment. (B) Statistical analysis for dorsal cortical area revealed that more Pax6⁺ BPs and Tbr2⁺ BPs were found in E18.5 cortex treated with TSA for E13.5-E17.5 than those in those injected with TSA for E13.5-E15.5. Notably, the TSA treatment also led to increased number of Pax6⁺vRGs at E18.5. (C) Proportions of cortical progenitors expressing Pax6 and/or Tbr2 in VZ, SVZ, and IZ from dorsal area of WT+ Veh, WT+ TSA and Baf155cKO+ TSA cortex at E16.5. Values are presented as mean $\pm$ SEM (*P < 0.05, **P < 0.01, ***P < 0.005). Abbreviations: BPs, Basal progenitors; vRGs, ventricular radial glial progenitors; IPs, intermediate progenitors; VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate. Scale bars = 50 μ m.

Fig. S5. Increased level of H3ac promotes genesis and proliferation of BPs in developing mouse cortex.

(A, B) Increasing H3 acetylation by other HDAC inhibitors such as VPA (A) and SAHA (B) also increased the number of Tbr2⁺, Pax6⁺ BPs in both WT and Baf155cKO developing cortex. Values are presented as mean $\pm$ SEM (*P < 0.05, **P < 0.01, ***P < 0.005). Abbreviations: BPs, Basal progenitors; vRGs, ventricular radial glial progenitors; IPs, intermediate progenitors; VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate. Scale bars = 50 μ m.

Fig. S6

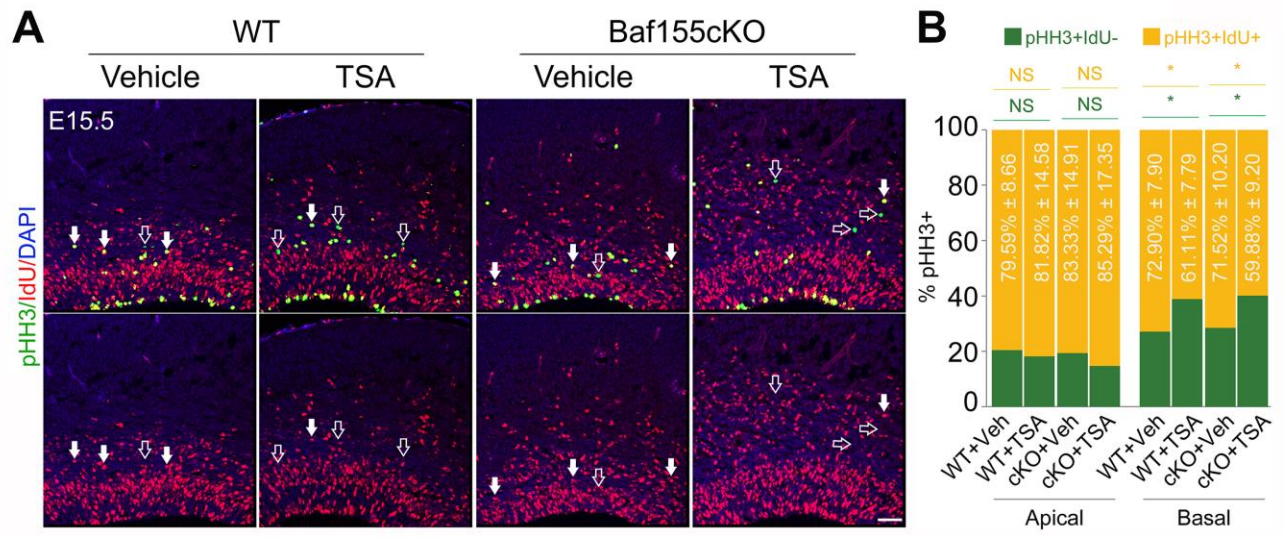


Fig. S6. H3 acetylation specifically promotes proliferation of basal progenitors

(A, B) 4 hours-IdU pulse-labeling is to trace the progression of cortical progenitors within the S-M phases. (A) Double IHC analysis with antibodies against IdU and pHH3 (markers for cells in the late G2-M phases) to label APs (apical surface- located IdU+/pHH3+) and BPs (basally located IdU+/pHH3+, filled arrows), which already passed through S phase and entered into late G-M phases. (B) Proportion of BPs, which entered the late G2-M phases in TSA-treated cortices is smaller compared with that in Veh-treated cortices. Values are presented as mean $\pm$ SEM (*P <0.05, **P <0.01, ***P <0.005). Abbreviations: VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate. Scale bars = 50 μ m.

Fig. S7

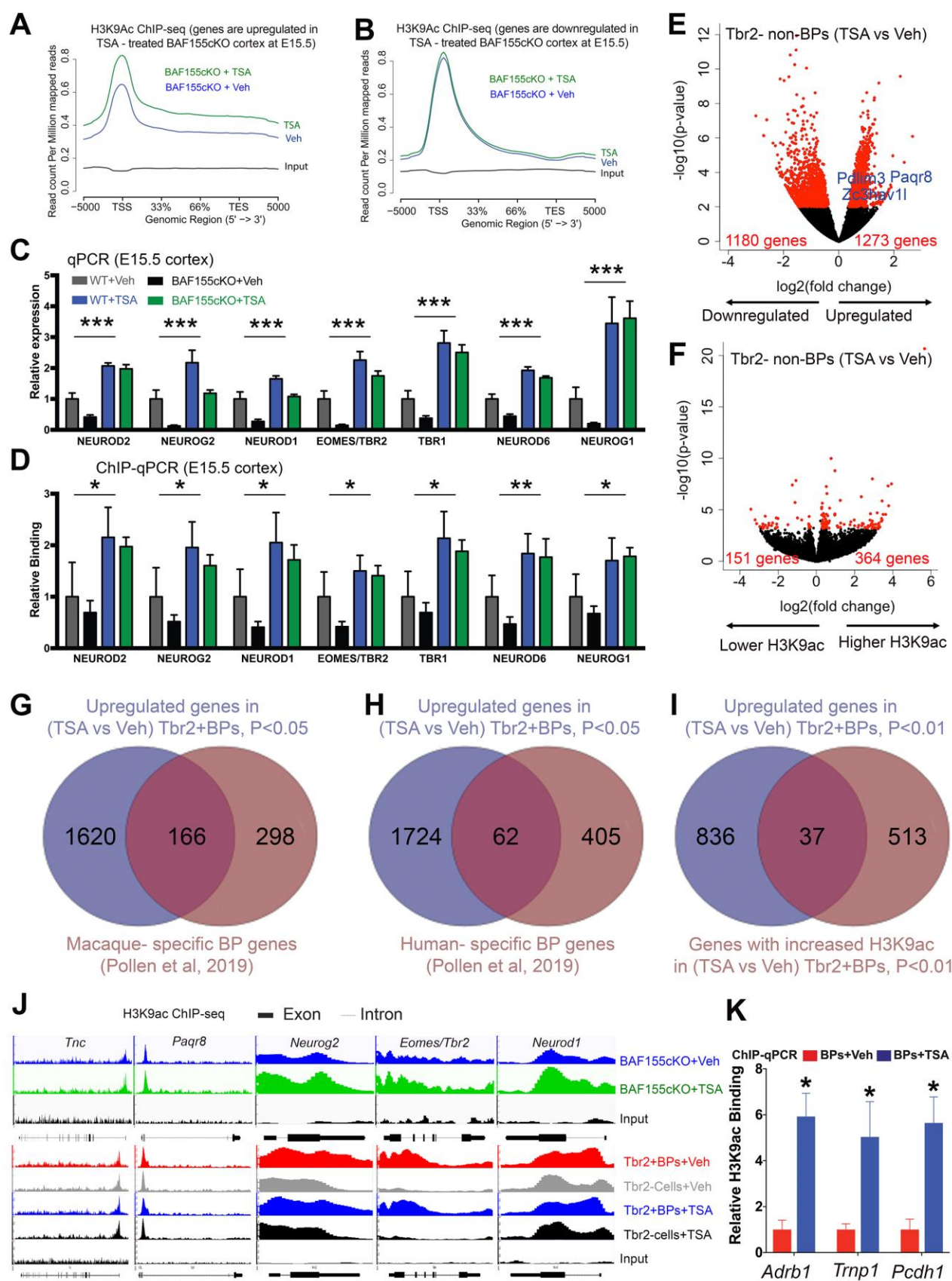


Fig. S7. HDAC inhibition causes H3K9ac-linked upregulation of gene expression in Baf155cKO developing cortex, Tbr2+ BPs and Tbr2- cells.

(A, B) H3K9ac is increased at loci of upregulated genes (A), but not at those of downregulated genes (B) in TSA treated Baf155cKO cortex. (C, D) Graphs of qPCR to examine gene expression (C) and ChIP-qPCR to quantify H3K9ac level (D). TSA treatment leads to increase in H3K9ac levels at promoters of neurogenesis-related genes (D) and their expression (C) both in WT and in Baf155cKO. ChIP-qPCR and qPCR results for a selection of genes are shown (WT_Veh: n = 3, WT_TSA: n = 4, Baf155 cKO_Veh: n = 4, Baf155 cKO_TSA: n = 4; general TSA effect: mixed linear model p -value < 0.001; general effect of Baf155 knockdown: Two-way ANOVA WT_Veh vs Baf155 cKO_Veh p < 0.01). (E, F) Volcano plots showing statistically significant changes (Paired Student's t -test < 0.01, FC > 1.2) visualized by our RNA-Seq (E) and H3K9ac ChIP-Seq (F) analyses of Tbr2- cells in TSA vs. Vehicle experiments (see also Fig. 5C/D for Tbr2+ BPs). (G, H) Overlap between the upregulated genes in TSA-treated Tbr2+ BP genes and BP/IP genes, which were recently identified specifically for macaque (G), and human (H) (Pollen et al., 2019). Notably, the TSA treatment provoked expression of a large set of macaque BP genes and in less extend, human BP genes in Tbr2+ BPs in the developing mouse cortex. (I) Overlap between up-regulated genes in RNA-seq and genes with increased H3K9ac in ChIP-seq studies of Tbr2+ BPs upon the TSA treatment. (J) Genome browser views of the distribution of H3K9ac along representative BP-enriched genes in Baf155cKO cortex, Tbr2+ BPs, Tbr2- cells, which were treated with either TSA and or vehicle as control. It should be noted that the bulk level of H3K9ac at their loci was unaltered in Tbr2+ BPs and Tbr2- cells in response to TSA treatment. In Baf155cKO cortex, the increased level of H3K9ac at these loci is due to expanded pool of BPs upon the treatment of TSA. (K) The increased level of H3K9ac at gene loci of *Adrb1*, *Trnp1*, *Pcdh1* in ChIP-seq upon TSA treatment were validated in ChIP-qPCR.

Fig. S8

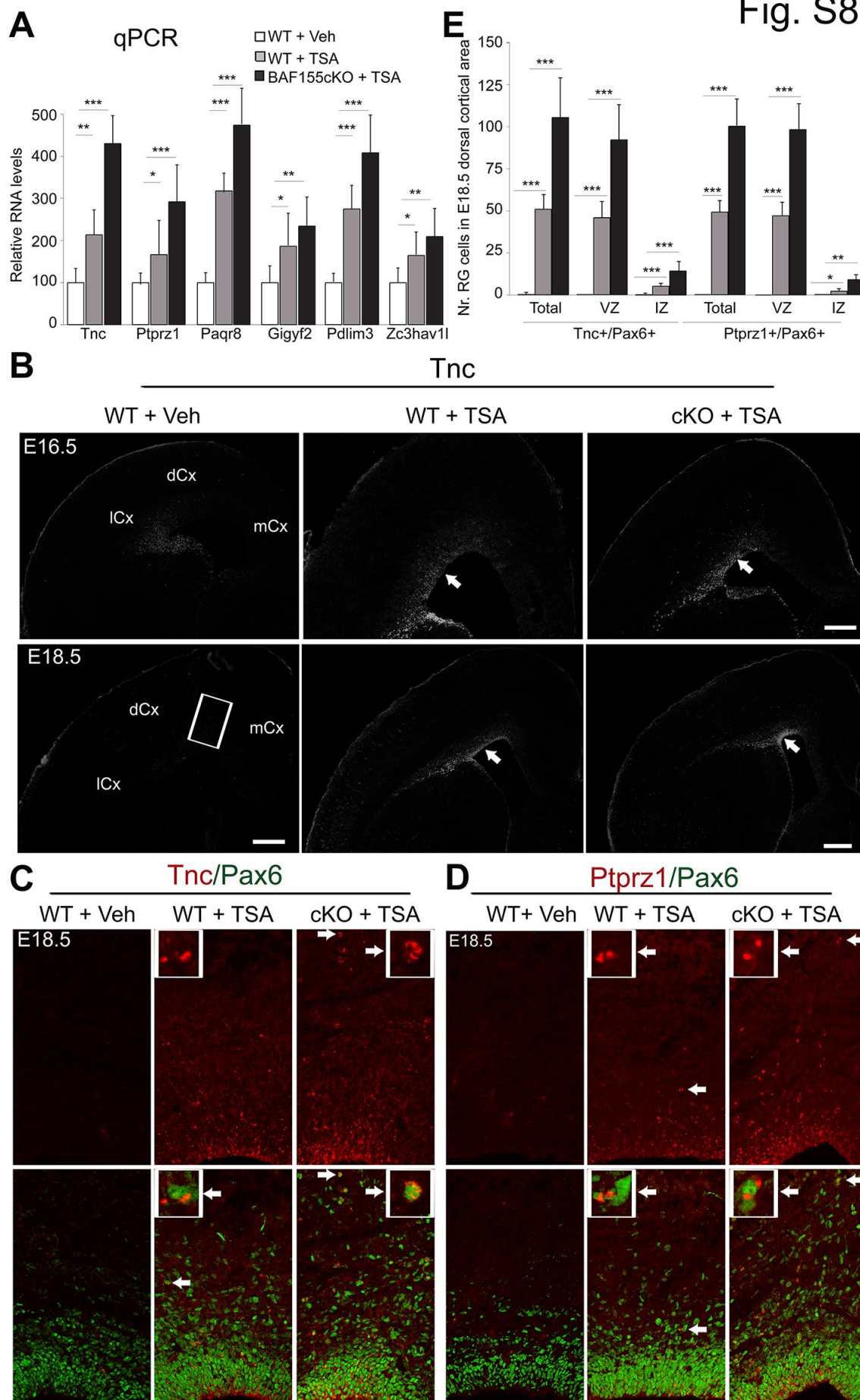


Fig. S8. HDAC inhibition causes upregulation of bRG-enriched genes in developing mouse cortex.

(A) qPCR analysis was used to confirm the upregulated expression of human-enriched bRG markers in TSA-treated cortex as compared to vehicle-treated control. (B-E) Among bRG-enriched genes, expression of *Tnc* and *Ptprz1* have been well characterized in the developing cortex of both human and mouse (Pollen et al., 2015). *Tnc* and *Ptprz1* antibodies labeled subsets of APs and BPs/bRGs in the entire human cortex, whereas in mouse their expression was detected only in a sub-population of Pax6⁺ APs in the lateral cortex (ICx, B) (Pollen et al., 2015). The increased expression of *Tnc* (B/C) and *Ptprz1* (D) in TSA-treated WT and Baf155cKO cortex at E16.5 and E18.5 was revealed by immunolabeling (B-D) and quantification (E). Notably, the expression of these human bRG markers *Ptprz1* and *Tnc* was found in Pax6⁺ APs in VZ of dorsal cortex (dCX, white filled arrows in B) and Pax6⁺ bRGs (white filled arrows in C, D) in TSA-treated cortex. Uppers images show the expression in arrow-pointed cells in lower pictures at higher magnification. Values are presented as mean $\pm$ SEM (*P <0.05, **P <0.01, ***P <0.005). Abbreviations: l/d/mCx, lateral/dorsal/medial cortex. Scale bars = 100 μ m.

Fig. S9

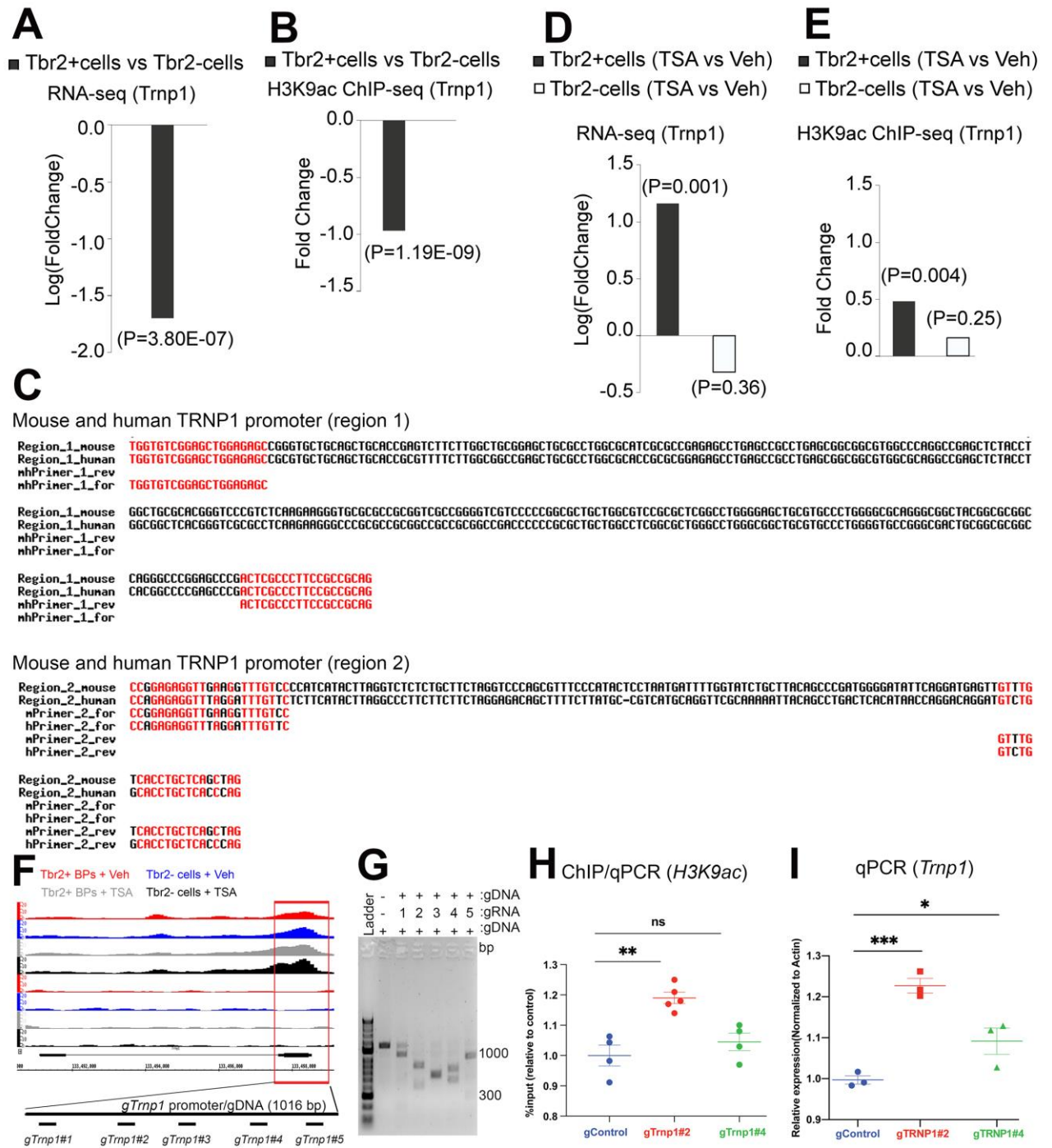


Fig. S9. In vitro testing of Trnp1 gRNAs.

(A/B) Tbr2⁻ cells have higher basal levels of H3K9ac at *Trnp1* promoter (A) and higher basal expression (B) of this gene compared to Tbr2⁺ BPs. (C) DNA sequence alignment for two ortholog regions of mouse (m) and human (h) *Trnp1* promoter and primers, which were used in CHIP/qPCR experiment (see also Fig. 6C). Note that mhPrimer_1 set was used to amplify the region 1 of both mouse and human *Trnp1* promoter. The mPrimer_2 and hPrimer_2 sets, which have similar sequence, were used to amplify the region 2. (D/E) TSA treatment significantly increases H3K9ac levels at *Trnp1* promoter (D) and upregulates its expression (E) specifically in Tbr2⁺ BPs but not in Tbr2⁻ cells. (F/G) Analysis of the Cas9 cutting efficiency guided by various gRNAs targeting the *Trnp1* promoter. (F) Depiction of different gRNAs targeting various regions in *Trnp1* promoter that were used for testing. (G) Agarose gel showing the cutting efficiency of each tested gRNA-Cas9 complex on a 1016-bp-long PCR product of *Trnp1* promoter region. (H/I) ChIP/qPCR (H) and qPCR (I) analyses indicate that transfection of Neuro2A cells with gTrnp1#2 and #4 increases H3K9ac level at *Trnp1* promoter (H) and upregulates its expression (I). Values are presented as means $\pm$ SEMs (*P <0.05, **P <0.01, ***P <0.005).

Fig. S10

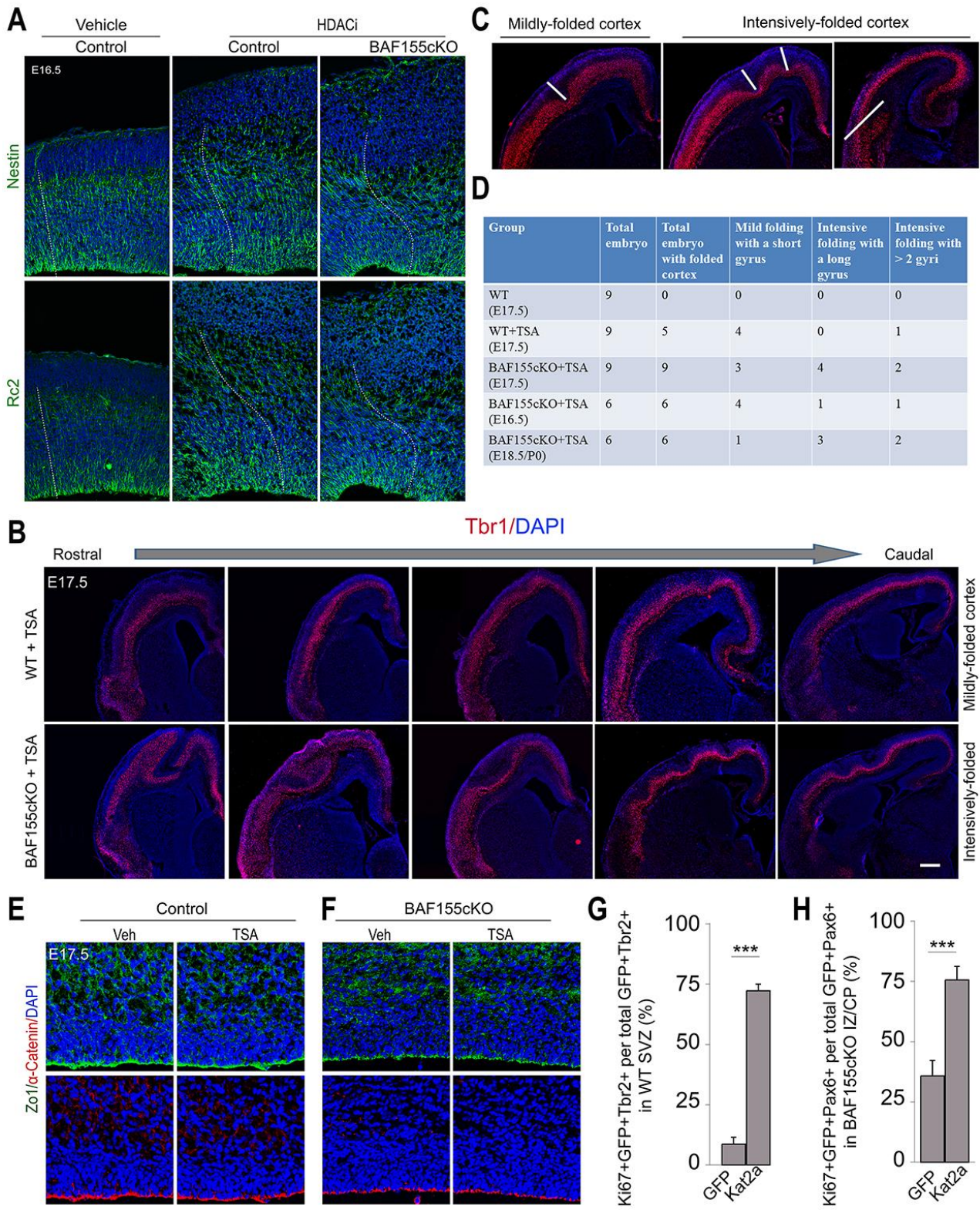


Fig. S10. Augmented level of H3K9ac by HDAC inhibition and overexpression of Kat2a increase basal progenitor proliferation and can induce cortical folding. (A) Staining of RG fibers (Nestin and Rc2) in Veh-treated control, TSA-treated-control and TSA-treated Baf155cKO cortex revealed an increase and divergence of radial fibers at basal sides upon TSA treatment (radial processes indicated by white-dashed lines on confocal images). (B) Cortical tissue was processed for Tbr1 immunostaining. Examples with different forms of cortical folding were shown from rostral cortex to caudal cortex at E17.5. The mildly-folded and intensively-folded cortices were usually found in TSA - treated WT embryos and TSA - treated Baf155cKO embryos, respectively. (C) The folding in length was measured from the pial surface to the end of the innervated point outlined by white lines. Cortical folding phenotypes were scored as a mild folding being shorter than 200 μ m in length (leftmost image in B) and intensive folding with a gyrus deeper than 200 μ m from the surface or with more than two gyri (right images). (D) Overview of cortical phenotypes observed after TSA or Veh-treatment. In total, nine Veh-treated control, nine TSA-treated control and nine TSA-treated *Baf155cKO* brains at E17.5, six brains at E16.5 and six brains at E18.5/P0 of TSA-treated Baf155cKO mutants were used to examine folding phenotypes. Six coronal sections per brain covering the rostral, middle-level and caudal cortex were processed for Tbr1 IHC. Cortex was scored as “mild folding” or “intensive folding”, if at least two sections met the above criteria. (E/F) Staining of Zo1 and α -Catenin in treated control (E), and Baf155cKO cortex (F) revealed an intact VZ basement membrane upon TSA treatment. (G-H) E13.5 cortices of WT or Baf155cKO embryos were electroporated with eGFP or Kat2a-ires-eGFP, followed by quantification of Tbr2+/Ki67+ proliferating BPs/IPs (G) and Pax6+/Ki67+ proliferating BPs/bRGs (H) at E15.5. Statistical analyses revealed that the overexpression of Kat2a led to increase the proliferation of BPs. Scale bars = 100 μ m.

- **Table S1:** Differential gene expression between TSA- and vehicle-treated Baf155cKO embryonic cortex (as a Supplemental Spreadsheet).
- **Table S2:** Differential binding of H3K9ac at promoters between TSA- and vehicle-treated Baf155cKO embryonic cortex (as a Supplemental Spreadsheet).
- **Table S3:** Differential gene expression between Tbr2+ BPs in TSA- and vehicle-treated embryonic cortex (as a Supplemental Spreadsheet).
- **Table S4:** Differential binding of H3K9ac at promoters between Tbr2+ BPs in TSA- and vehicle-treated embryonic cortex (as a Supplemental Spreadsheet).
- **Table S5:** Differential gene expression between Tbr2- cells in TSA- and vehicle-treated embryonic cortex (as a Supplemental Spreadsheet).
- **Table S6:** Differential binding of H3K9ac at promoters between Tbr2- cells in TSA- and vehicle-treated embryonic cortex (as a Supplemental Spreadsheet).
- **Table S7:** Comparisons of the upregulated genes in TSA-treated Tbr2+ BP genes and BP/IP genes, which were recently identified specifically for macaque and human (Pollen et al., 2019) (as a Supplemental Spreadsheet).
- **Table S8:** List of primers for qPCR and ChIP-qPCR (as a Supplemental Spreadsheet).
- **Table S9:** statistical analyses (as a Supplemental Spreadsheet).