Supplementary Figures

Reduced chromatin accessibility correlates with resistance to Notch activation

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Supplementary figures

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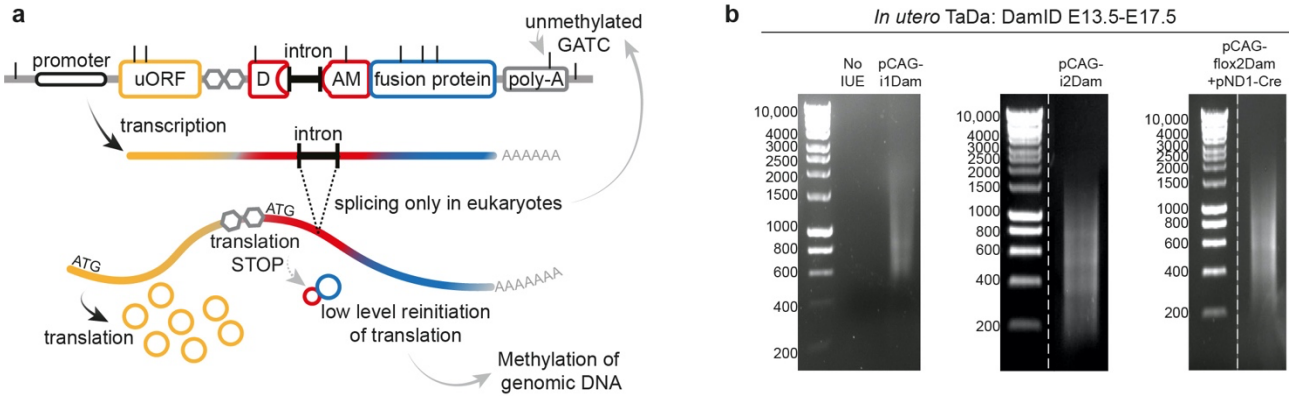
Supplementary Data files

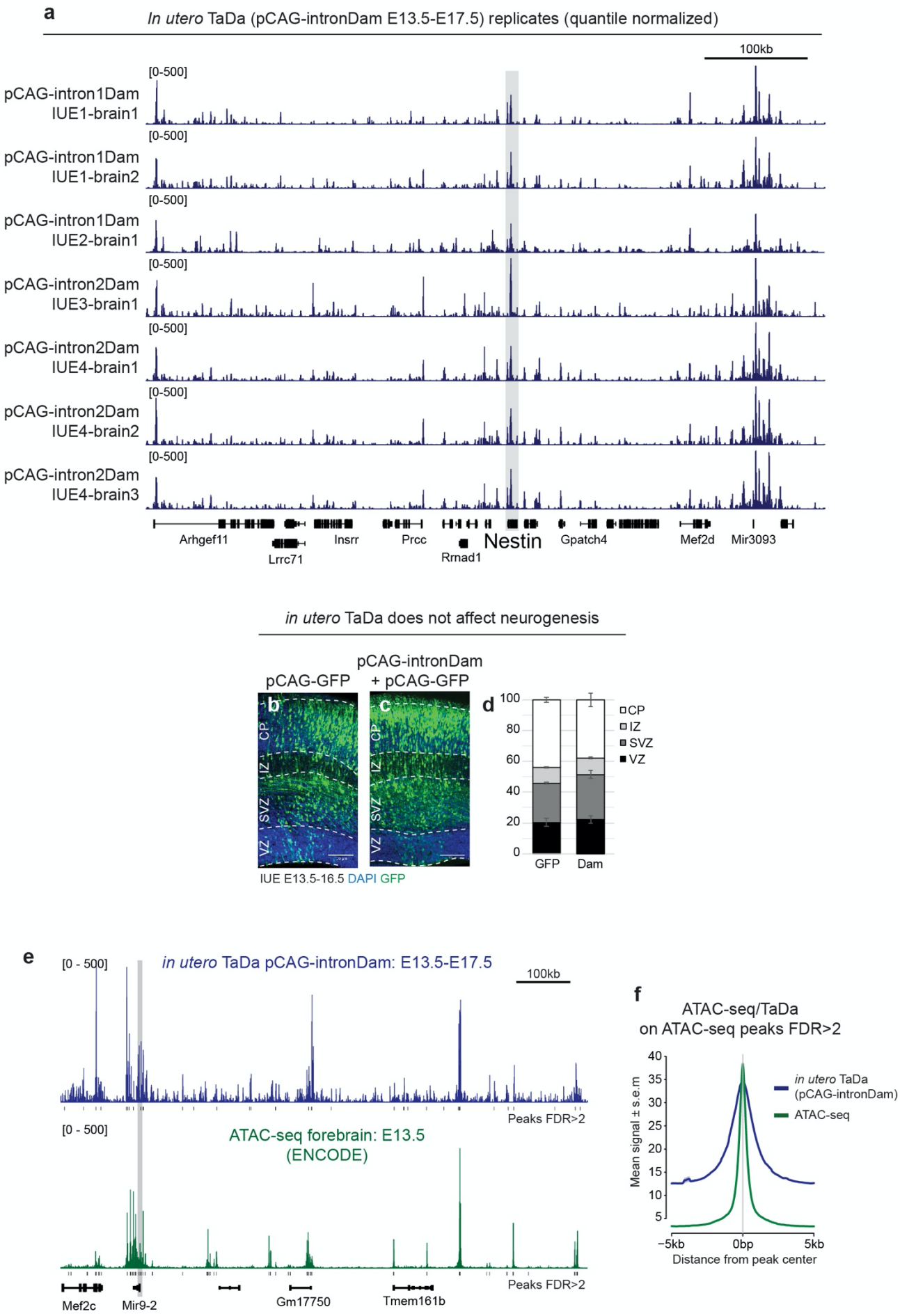
- Supplementary Data 1. Loci bound by NOTCH/RBPJ in RGCs or IPCs and their associated genes. Related to Fig. 2.
- Supplementary Data 2. Overlap of genes bound by RBPJ ChIP-seq, RGC-specific RBPJ TaDa and RNAseq upon NICD overexpression. Related to Supplementary Fig. 5g.
- Supplementary Data 3. Peaks associated with each Notch/RBPJ peak cluster and the associated gene for each peak. Related to Fig. 3.
- Supplementary Data 4. RGC-specific genes determined by bulk RNA-seq that are bound by NOTCH/RBPJ in peak cluster 6. Related to Fig. 4b.

Source Data file

- Source data Fig1j-SupplFig2d.xlsx

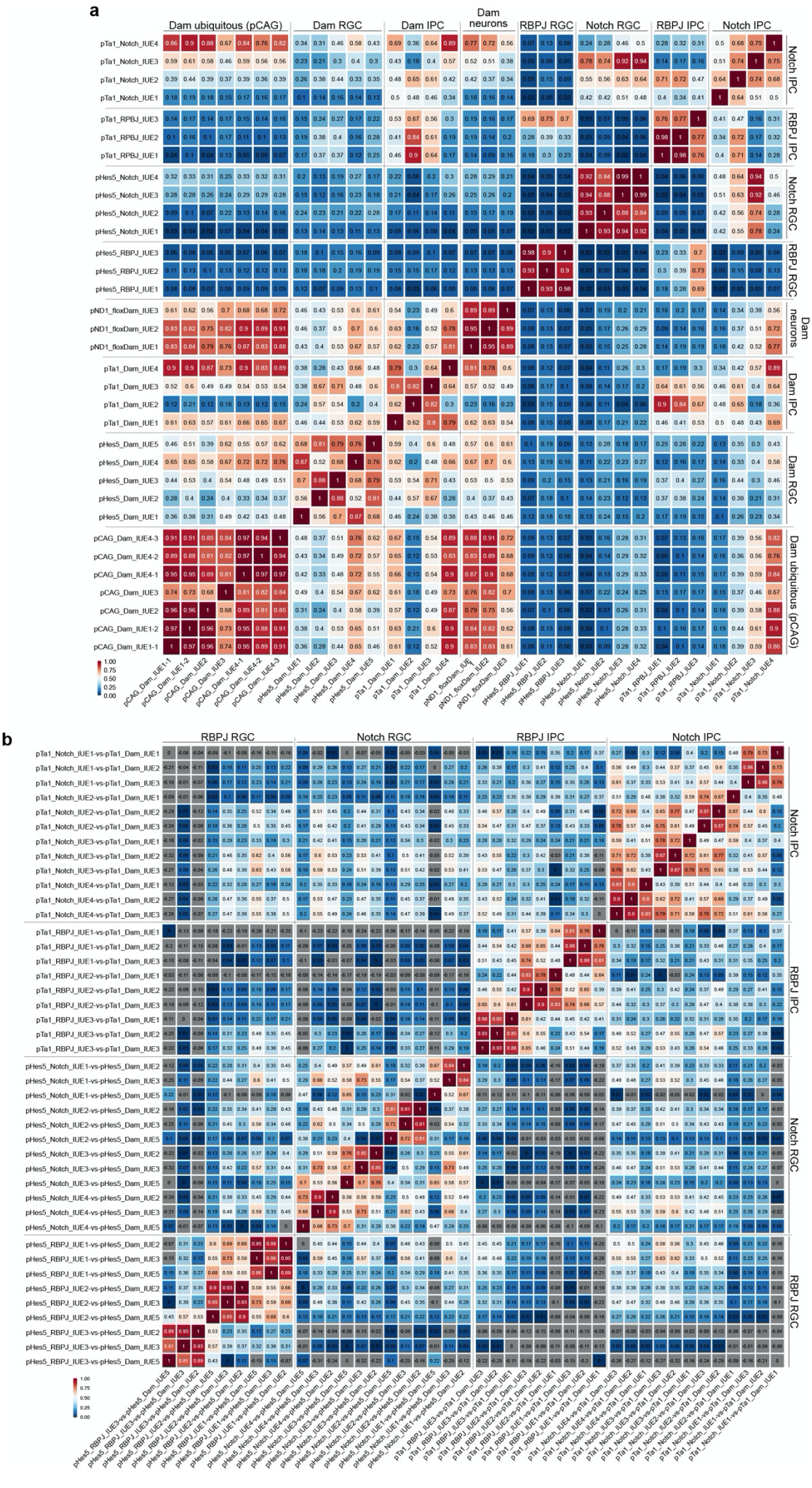


Supplementary Fig. 1. *In vivo* Targeted DamID (TaDa) constructs. a. Schematic of TaDa construct, where an upstream open reading frame (uORF) followed by two stop codons and a frame shift (5'-TAATAAC-3') reduces translation of the downstream open reading frame encoding a Dam fusion protein [13]. Introduction of an intron in the Dam coding sequence prevents expression in bacteria. **b**. TaDa of E17.5 cortex electroporated at E13.5 with the indicated constructs: pCAG-Venus only (lane 1, n=2) or together with pCAG-i1Dam (lane 2, n=3), pCAG-i2Dam (lane 3, n=4) and pCAG-flox2Dam with pNeuroD1-Cre (lane 4, n=3). Ladder is Hyperladder 1 (Bioline).

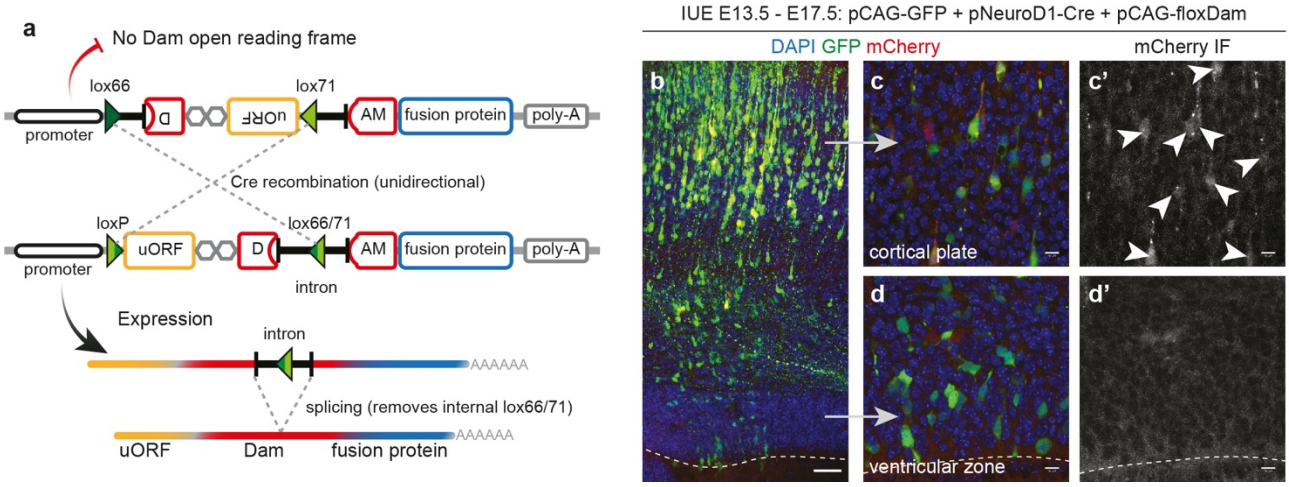


Supplementary Fig. 2. *In utero* Targeted DamID. a. Chromatin accessibility profiles in the genomic region surrounding the *Nestin* locus (grey shading) of individual pCAG-mCherry-i1/2Dam

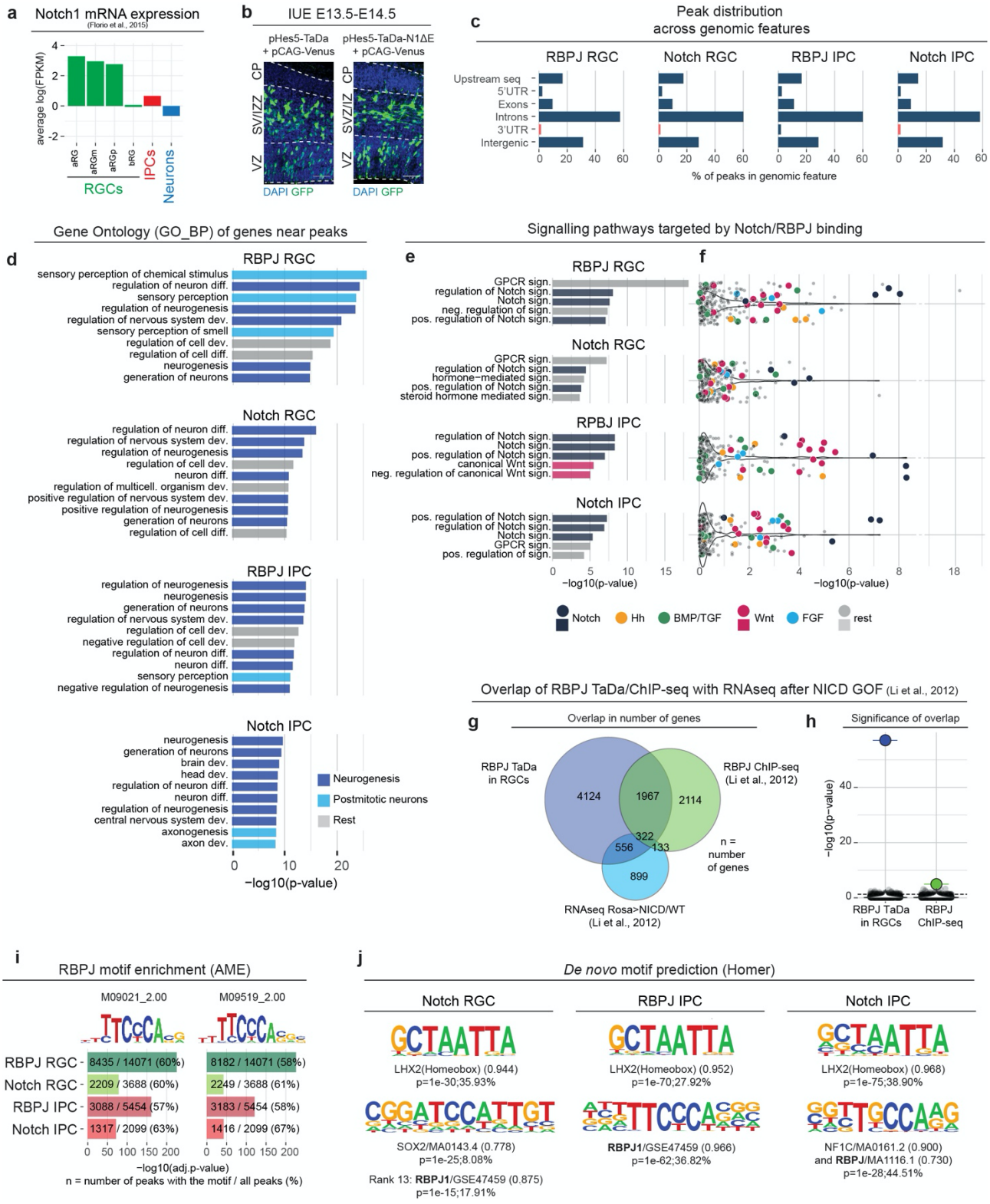
replicates from 7 embryos across 4 litters harvested 96 hours (E17.5) after IUE at E13.5. **b-d**. *In utero* electroporation of pCAG-Venus either alone (**b**) or with pCAG-mCherry-i2Dam (**c**) at E13.5. Coronal sections stained for DAPI (nuclei, blue) and GFP (green) 3 days after IUE (E16.5). Dashed lines mark the VZ, SVZ, IZ and CP. Scale bars are 100μm. Histograms showing the percentage of GFP^+ cells in each indicated region (**d**). Data are presented as mean ± s.e.m. (n = 3 for pCAG-Venus and 2 for pCAG-i2Dam embryos). Source data are provided as a Source Data file. **e,f**. Forebrain ATAC-seq profiles at E13.5 from ENCODE and *in utero* TaDa with pCAG-mCherry-iDam at the *mir9-2* locus (grey shading) (**e**) and mean signal ± s.e.m. (shaded area) plotted over all ATAC-seq peaks at FDR<10^{-2} (**f**).


a
Dam ubiquitous (pCAG)
Dam RGC
Dam IPC
Dam neurons
RBPJ RGC
Notch RGC
RBPJ IPC
Notch IPC
Dam ubiquitous (pCAG)
Dam IPC
Dam RGC
Dam neurons
RBPJ RGC
RBPJ IPC
Notch IPC
Notch RGC
b
RBPJ RGC
Notch RGC
RBPJ IPC
Notch IPC
Notch IPC
RBPJ IPC
Notch RGC
RBPJ RGC

Supplementary Fig. 3. **Genome-wide Pearson correlation matrices**. Pearson correlation coefficients of linear correlations between all replicates across the four experimental conditions (see Fig. 2a) and the Dam-only conditions (see Fig. 5a) based on 500bp bins across the genome (**a**); and between the normalised datasets (see Fig. 2a) based on the peak coordinates identified across the four experimental conditions (**b**).

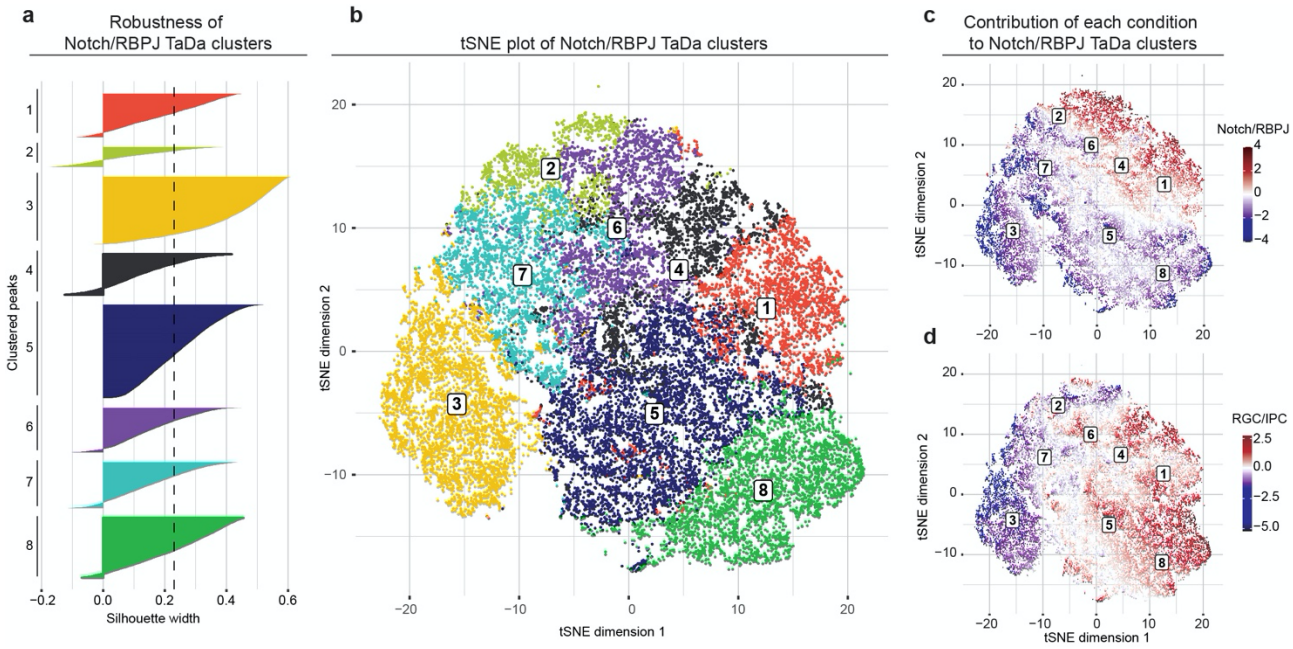


Supplementary Fig. 4. Cre-inducible construct (floxDam). **a**. Unidirectional Cre-induced recombination reconstitutes the Dam coding sequence and the internal Lox66/71-site is removed from the transcript by splicing. **b-d**. *In utero* electroporation of pNeuroD1-Cre with pCAG-floxDam and pCAG-Venus at E13.5; coronal sections at E17.5 stained for DAPI (nuclei, blue), GFP (green) and mCherry (uORF, red). Dashed lines mark the apical side of the VZ. Arrowheads indicate mCherry-expressing cells. Scale bars are 50µm (**b**) and 10µm (**c,d**).

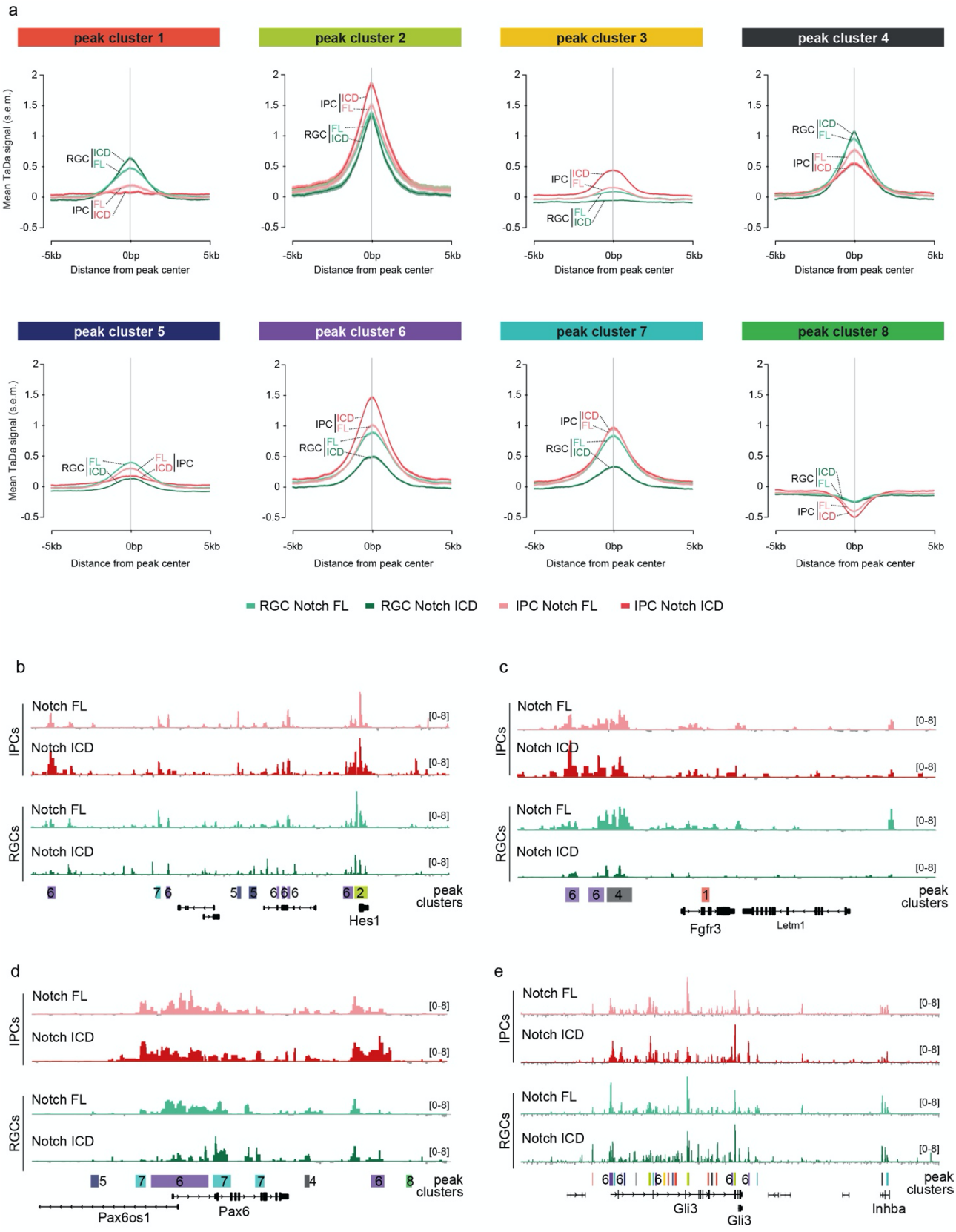


Supplementary Fig. 5. Cell-type specific *in utero* TaDa of Notch and RBPJ. **a.** Notch1 mRNA expression (average log(FPKM) values) in RGCs, IPCs and neurons from bulk RNA-seq [34]. **b**. IUE at E13.5 of pCAG-Venus with Dam or N1ΔE-Dam under control of pHes5, stained at E14.5 for DAPI (nuclei, blue) and GFP (Venus, green). Dashed lines mark the VZ, SVZ/IZ and CP. Scale bars are 50µm. **c**. Relative peak distribution in relation to genomic features. **d-f**. Gene ontology analysis of genes associated with peaks, showing all terms from GO_BP (Biological Processes) (**d**), or terms containing "signalling" (**e,f**). Specific categories or signalling pathways are highlighted in the indicated colours. P-values derive from the enrichment model as implemented in the broadenrich command of the chipenrich package. **g,h**. Overlap of genes differentially expressed in the cortex upon NICD overexpression (RNA-seq [37], light blue) and bound by RBPJ (*in utero* TaDa

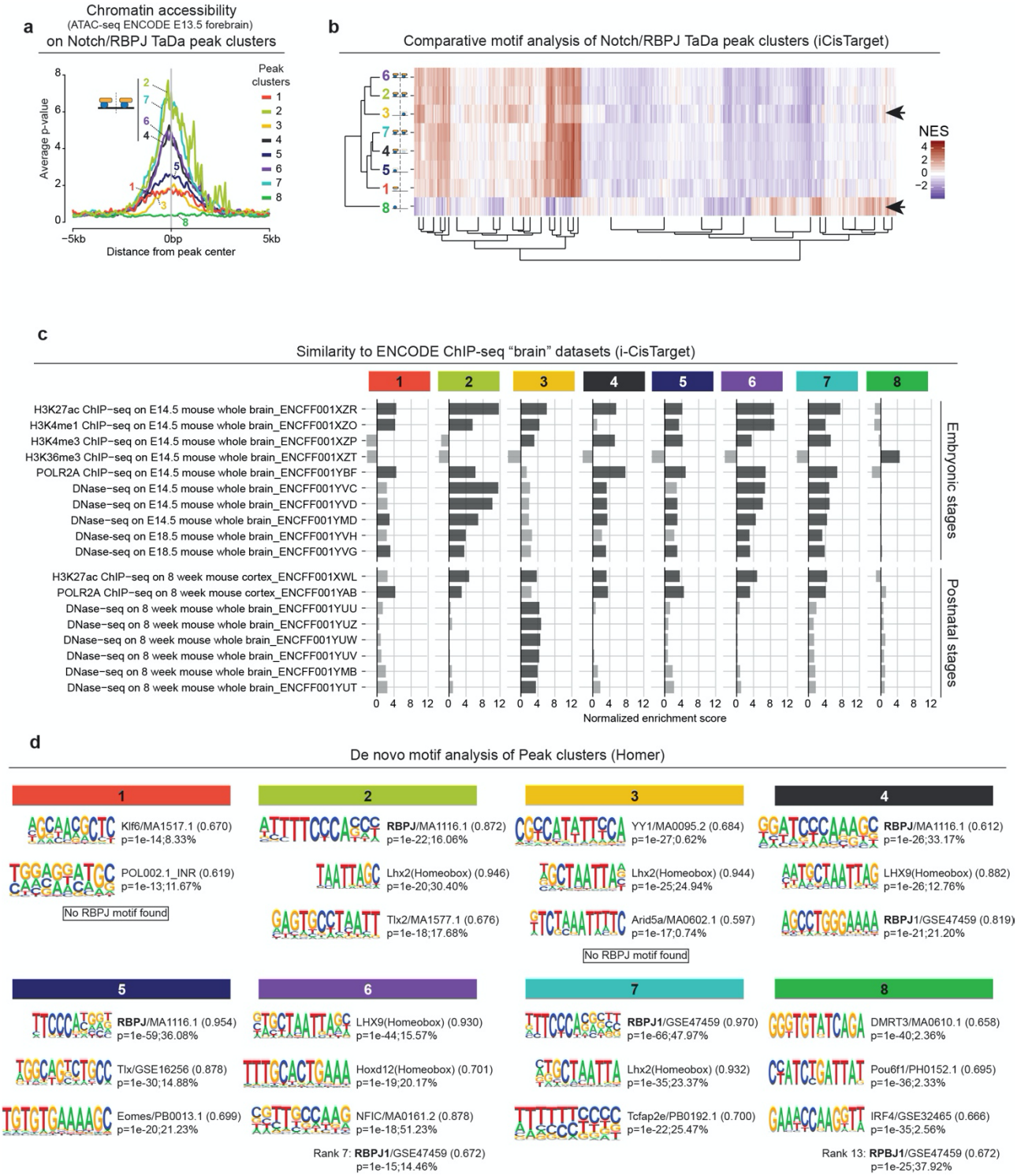
in RGCs, green, or RBPJ ChIP-seq [37], dark blue (**g**). Significance values of the observed overlaps compared to expected overlap for these gene numbers and sizes; p-values calculated via hypergeometric test (**h**). **i**. RBPJ motif enrichment; p-values calculated via Fisher's exact test implemented in AME of the MEME suite. **j.** *De novo* motif prediction; p-values calculated via Homer based on a binomial distribution.



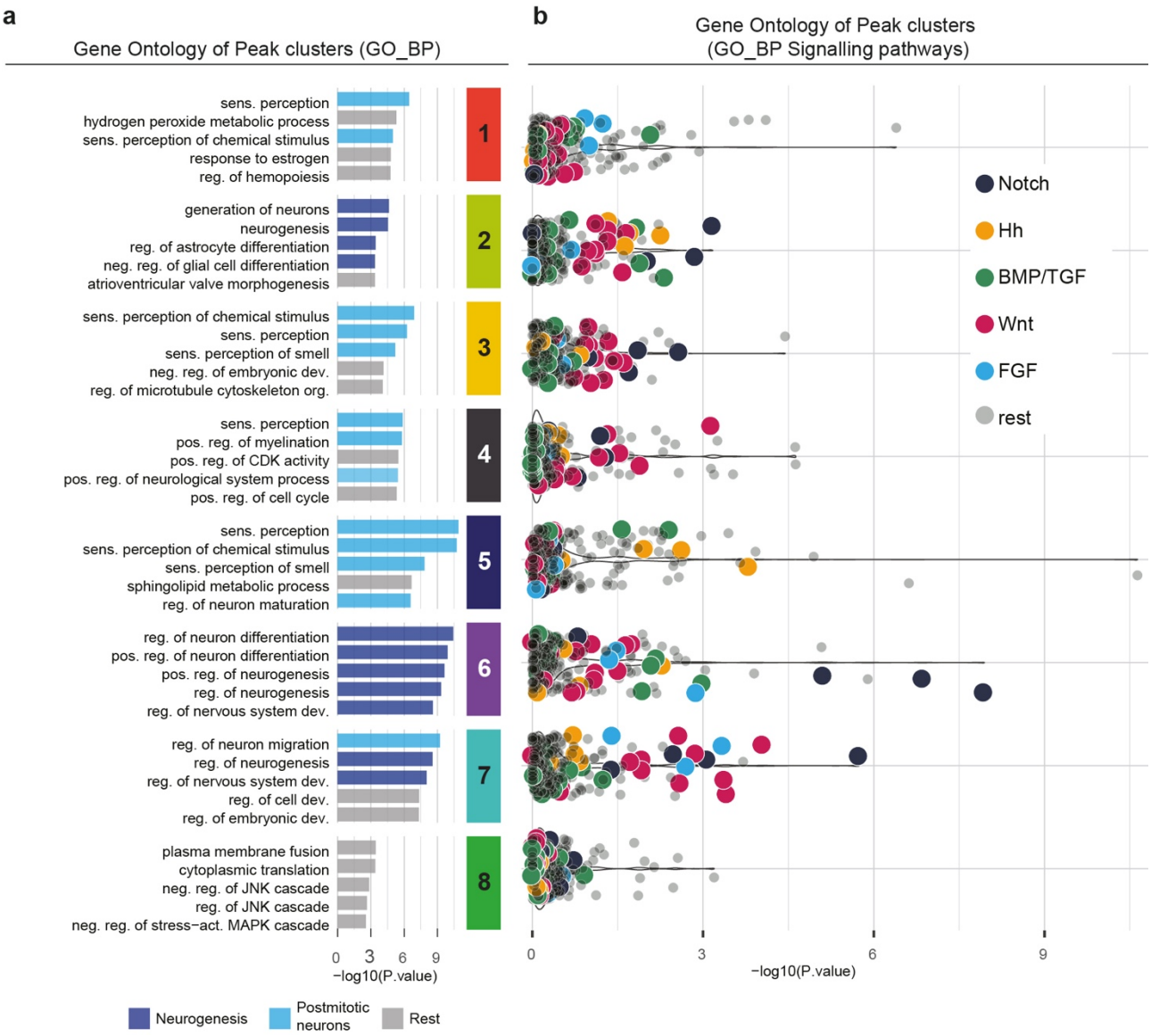
Supplementary Fig. 6. k-means clustering for Notch/RBPJ peak regions. a. Silhouette plot for the clustering. Silhouettes are sorted according to distance of a peak to the centre of the neighbouring cluster which determines order in Fig. 3a. **b**. tSNE plot representing distribution of peaks by dimensionality reduction of aggregated binding intensities of peaks. Colours indicate cluster affiliation. **c,d**. Ratios of summed binding intensities for assayed proteins (Notch/RBPJ) (**c**) and cell types (RGC/IPC) (**d**) overlaid on tSNE evaluates dimension reduction according to these two factors.



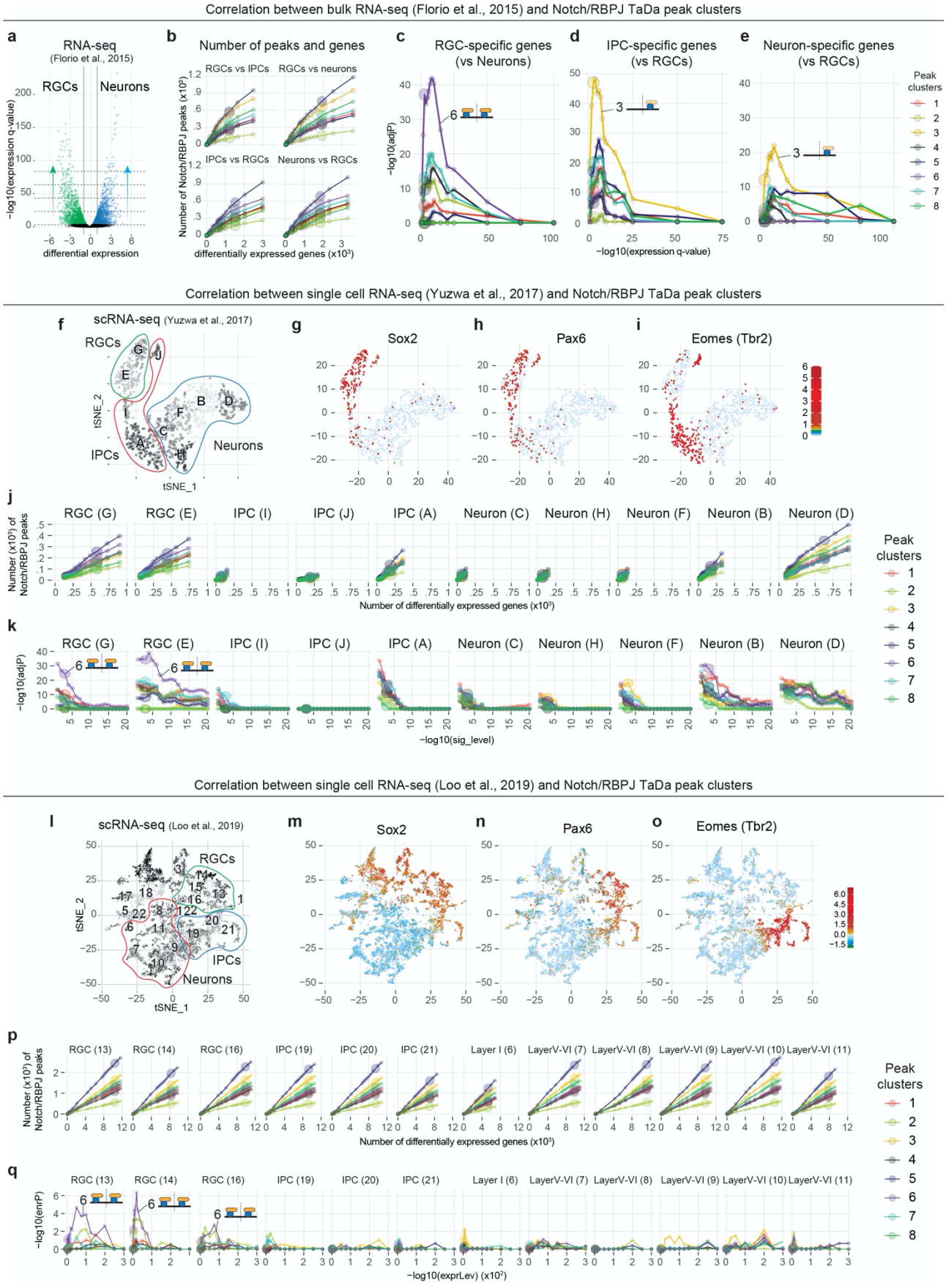
Supplementary Fig. 7. **TaDa in RGCs and IPCs with full-length Notch**. **a**. Average signal intensity (±s.e.m, shaded area) in RGCs (green) and IPCs (red) on peaks from the indicated Notch/RBPJ peak clusters for full-length Notch TaDa (Notch FL) or NICD TaDa (Notch ICD). **b-e**. Binding profiles for TaDa in RGCs or IPCs with full-length (FL) Notch and Notch ICD near RGC-specific Notch target genes *Hes1* (**b**), *Fgfr3* (**c**), *Pax6* (**d**) and *Gli3* (**e**) associated with cluster 6 peaks.



Supplementary Fig. 8. Properties of Notch/RBPJ peak clusters. **a**. Average p-value from E13.5 ATAC-seq (values from ENCODE [31]) plotted on NOTCH/RBPJ peak clusters. **b**. Normalized enrichment scores (NES) and hierarchical clustering of motifs detected by i-CisTarget [47] under peaks of each NOTCH/RBPJ peak cluster. **c**. Similarity in genome-wide distribution between peaks of each cluster and the indicated datasets from ENCODE [31], as determined by i-CisTarget [47]. Dark grey indicates significant similarity. **d**. *De novo* motif analysis of sequences under the peaks of each cluster. P-values calculated via Homer based on a binomial distribution. Top 3 position weight matrices are shown, with the name of the best matching motif and its similarity score, p-value of the motif and percentage of peaks where the motif is detected.

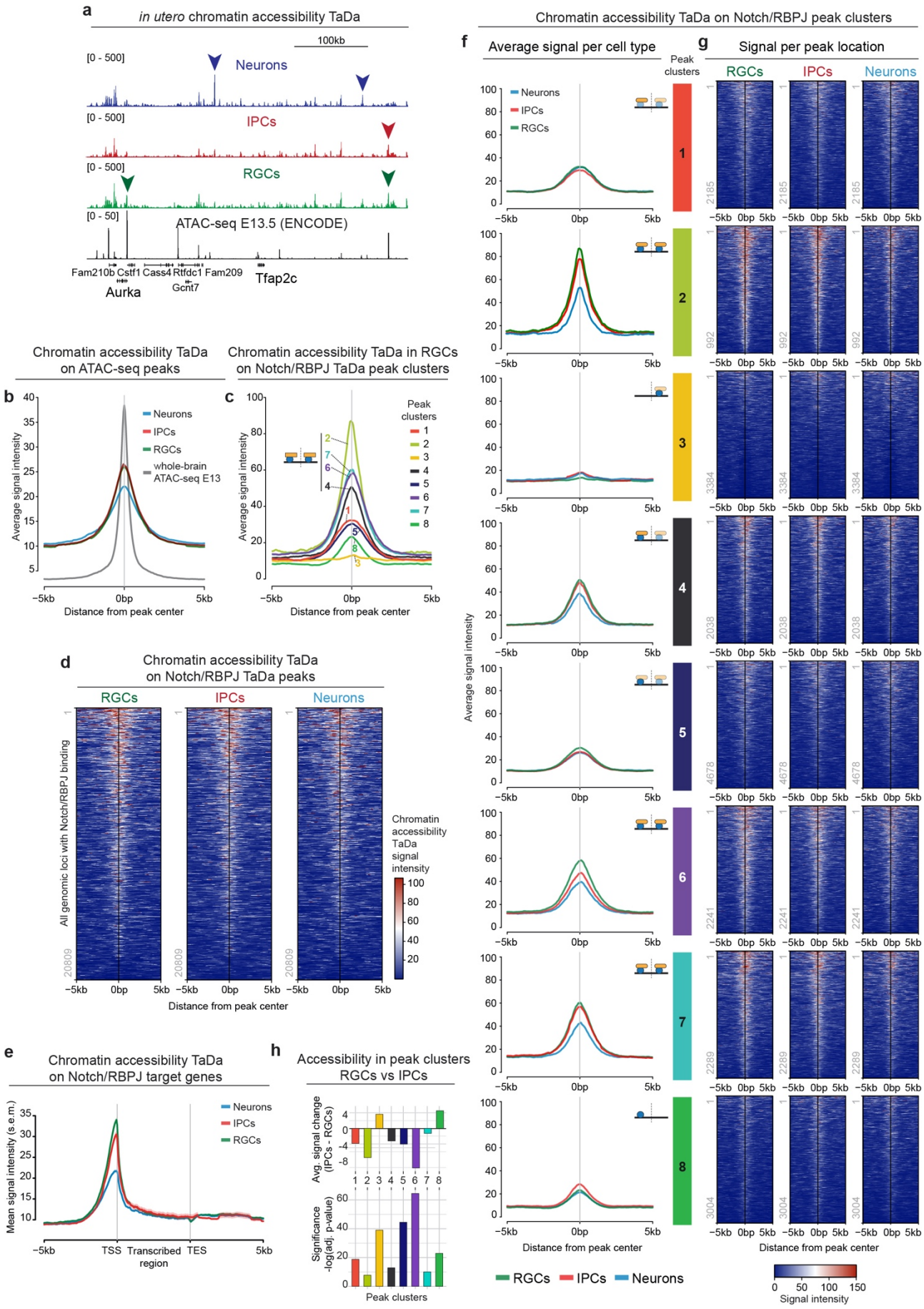


Supplementary Fig. 9. Gene ontology analysis of NOTCH/RBPJ-bound clusters. a,b. Gene ontology analysis of genes associated with peaks in each Notch/RBPJ peak cluster, showing all terms from GO_BP (Biological Processes) (**a**), or terms containing "signalling" (**b**). Specific categories or signalling pathways are highlighted in the indicated colours. P-values derive from the enrichment model as implemented in the broadenrich command of the chipenrich package.



Supplementary Fig. 10. **Genome-wide comparison of cell-type specific expression data and Notch/RBPJ binding patterns**. **a**. Genes differentially expressed in RGCs compared to neurons from bulk RNA-seq. **b**. Number of genes differentially expressed in RGCs, IPCs or neurons (x-axis) at decreasing q-values (datapoints; q=0.05 is enlarged), and numbers of peaks from each peak cluster (y-axis) associated with the differentially expressed genes. **c-e**. Enrichment of Notch/RBPJ

peak clusters near genes differentially expressed at different q-values in RGCs compared to neurons (**c**), IPCs compared to RGCs (**d**) and neurons compared to RGCs (**e**). **f-q**. tSNE-plot of single-cell RNA-seq from Yuzwa et al. [35] (**f-i**) or Loo et al. [50] (**l-o**) indicating the different scSeq clusters and the cell-type annotations (**f,l**), or the expression level of the indicated marker genes (**g-i,m-o**). **j,p**. Number of genes differentially expressed in the cells from the indicated scSeq cluster compared to all other cells (x-axis) at decreasing q-values (datapoints; q=0.05 is enlarged) and the number of peaks from each peak cluster (y-axis) associated with the differentially expressed genes. Note that some scSeq clusters from Yuzwa et al (**j**) (I,J,C,H,F) have very few genes specifically expressed in their cells. **k,q**. Enrichment of Notch/RBPJ peak clusters near genes differentially expressed in the indicated scSeq cluster at different q-values.



Supplementary Fig. 11. Cell-type specific *in utero* chromatin accessibility TaDa. a. I*n utero* chromatin accessibility profiles in RGCs, IPCs and post-mitotic neurons and of E13.5 forebrain ATAC-seq (ENCODE) at the *Tfap2c* locus. Arrowheads indicate cell-type specific differences in

signal intensity. **b-g**. Average signal intensity (**b,c,e,f**) or signal intensity (**d,g**) in RGCs (**c**) or in RGCs, IPCs and neurons (**b,d-g**) on ATAC-seq peaks (FDR<10^{-2}) (**b**), on peaks from the indicated Notch/RBPJ peak clusters (**c,f,g**), on all genomic loci with NOTCH or RBPJ binding (**d**), or across all Notch/RBPJ target genes and the 5kb upstream and downstream genomic region (**e**). **h**. Change in average signal (bottom) and its associated adjusted p-value (top) of chromatin accessibility TaDa from RGCs to IPCs (compare to signal in (**f**)) on the indicated peak clusters. P-values calculated via Wilcoxon rank sum test with Bonferroni correction for multiple testing.