Expanded View Figures

Figure EV1. CRISPR/Cas9 deletion of Trim28 in NPCs results in upregulation of ERVs and nearby genes.

A Differential expression analysis of individual TEs in mouse NPCs upon CRISPR/Cas9-mediated disruption of Trim28 was performed with DESeq2 (described in Materials and Methods).

B The upregulated transcription of MMERVK10C elements was validated by qRT–PCR. Columns show an average of the individual data points.

C Genes located in the close vicinity of upregulated MMERVK10C elements were significantly upregulated. X-axis indicate the window-of-inclusion for genes located close to an MMERVK10C. Boxplot hinges represent first and third quartile, and the median is indicated by the central band. Whiskers extend to 1.5 times the interquartile range.

D RNA-seq differential expression analysis of protein coding genes performed with DESeq2 (described in Materials and Methods) in mouse NPCs upon CRISPR/Cas9-mediated disruption of Trim28.

E, F PCA analysis of gene expression was unable to distinguish the Trim28-KO cells from Ctrl, while PCA analysis of TE expression was. The spread of the groups is visualized with opaque ellipses.

Source data are available online for this figure.
Figure EV2. Deletion of Trim28 in adult neurons in vivo does not result in an upregulation of ERVs.

A A schematic of the workflow targeting Trim28 in the forebrain of Stop-Cas9-GFP knock-in mice using AAV vectors expressing the gRNA and a nuclear RFP reporter as well as an AAV vector expressing Cre, specifically in neurons by the Synapsin promoter. 8 weeks after injection, the animals were analyzed either by IHC or by DNA/RNA-sequencing following nuclei isolation by FACS.

B Gene editing efficiency was evaluated by amplicon sequencing of the respective targeted sequences. DNA was isolated from 50,000 RFP⁺ nuclei per animal, one animal per group was analyzed. Black bars indicate % of frameshift mutations.

C, D Neuron-specific editing of the Trim28-loci resulted in a robust loss of the Trim28 protein in neurons, as evaluated by IHC where the expression of Trim28 in RFP⁺ cells was quantified and is displayed as mean ± SEM. Approximately 550 RFP⁺/GFP⁺ cells per animal and group was evaluated. Scale bar 30 µm.

E Differential expression analysis of TEs in adult neurons from the Stop-Cas9-GFP knock-in mice upon Trim28-KO was done by using TEtranscripts and DESeq2 (described in Materials and Methods).

F A schematic of the workflow targeting Trim28 in adult neurons in the forebrain of Trim28-flox mice (+/- and +/+) by co-injecting AAV vectors expressing Cre or nuclear GFP under the control of the Synapsin promoter. 8 weeks after injection, the animals were analyzed either by IHC or by RNA-sequencing following nuclei isolation by FACS.

G RNA-seq analysis of the isolated GFP⁺ nuclei revealed a loss of the Trim28 transcript. Boxplot hinges represent first and third quartile, and the median is indicated by the central band. Whiskers extend to 1.5 times the interquartile range.

H TE differential expression analysis on the adult neurons in the Trim28-flox animals was done using TEtranscripts and DESeq2 (described in Materials and Methods).

I IHC for Trim28, GFP, and the neuronal marker NeuN revealed a complete neuronal loss of the protein Trim28 in the Trim28-KO animals (Trim28-flox mice (+/-)). Scale bar 75 µm.
Figure EV2.
Figure EV3. Differential gene expression upon loss of Trim28.

A RNA-seq analysis of Trim28 expression in the adult cortex of Emx1-Cre/Trim28-KO animals (Emx1-Cre(+/−), Trim28-flox(+/−)). Boxplot hinges represent first and third quartile, and the median is indicated by the central band. Whiskers extend to 1.5 times the interquartile range.

B Differential TE expression analysis for individual TEs in the adult cortex of Emx1-Cre/Trim28-KO animals compared to their Cre-negative control litter mates was performed with DESeq2 (described in Materials and Methods).

C Expression of genes nearby upregulated full length MMERVK1OCs were not upregulated in the Emx1-Cre/Trim28-KO animals. X-axis indicate the window-of-inclusion for genes located close to an MMERVK1OC (test performed with DESeq2, Log2FC > 0). Boxplot hinges represent first and third quartile, and the median is indicated by the central band. Whiskers extend to 1.5 times the interquartile range.

D No transcriptional readthrough into nearby genes from upregulated MMERVK1OCs were observed, exemplified with an UCSC screenshot of the same location in the genome as shown in Fig 1G.

E Differential gene expression performed with DESeq2 [see Materials and Methods] in the adult cortex of Emx1-Cre/Trim28-KO animals compared to their control litter mates.

F Differential gene expression performed with DESeq2 [see Materials and Methods] in the adult forebrain upon AAV.Cre/Trim28-KO compared to controls.

G Venn diagrams showing significantly up- and downregulated genes in the Emx1-Cre/Trim28-KO and the AAV.Cre/Trim28-KO animals, as well as the genes overlapping in between them.

H GO term analysis of the overlapping upregulated genes between Emx1-Cre/Trim28-KO and the AAV.Cre/Trim28-KO animals.
Figure EV3.
Figure EV4. Cell type marker expression projected onto the UMAP plot of Emx1-Cre/Trim28 cell clusters.

A An overview of the defined cell clusters from single cell RNA-seq of Emx1-Cre/Trim28-flox animals in an UMAP plot.
B–H The projected expression of two cell markers per cluster on the UMAP.

Source data are available online for this figure.
Figure EV5. Iba1+ densities and inflammatory/viral defense genes upon Trim28-KO.

A The number of Iba1+ cells did not differ in the cortex of Emx1-Cre/Trim28-KO animals and controls, here shown as mean ± SEM, unpaired t-test. Iba1+ cells were counted in 20 photographs (20x objective) per animal, control n = 3 and KO n = 2.

B Immunohistochemical analysis for the microglia marker upon Trim28-KO in mature neurons in vivo (AAV.Syn-Cre/Trim28-KO animals) revealed no difference in Iba1 morphology between Trim28-KO animals and controls. Scale bar 75 μm.

C Inflammatory genes were chosen from 216 genes annotated in the immune response GO term (GO:0006954) if none of the confidence intervals overlapped zero. Three genes were significantly downregulated upon the Emx1-Cre/Trim28-KO and are labeled in red: Bmp6, Cd163, and Ptgdr (P adj < 0.05). n = 3 per condition. Error lines showing 95% confidence intervals. The list of viral defense genes was retrieved from (Liu et al, 2018). None of these were significantly different upon the Emx1-Cre/Trim28-KO.

Source data are available online for this figure.
Expression of inflammatory genes in Emx1-Cre/Trim28-flox animals

Expression of viral defence genes in Emx1-Cre/Trim28-KO

Figure EVS.