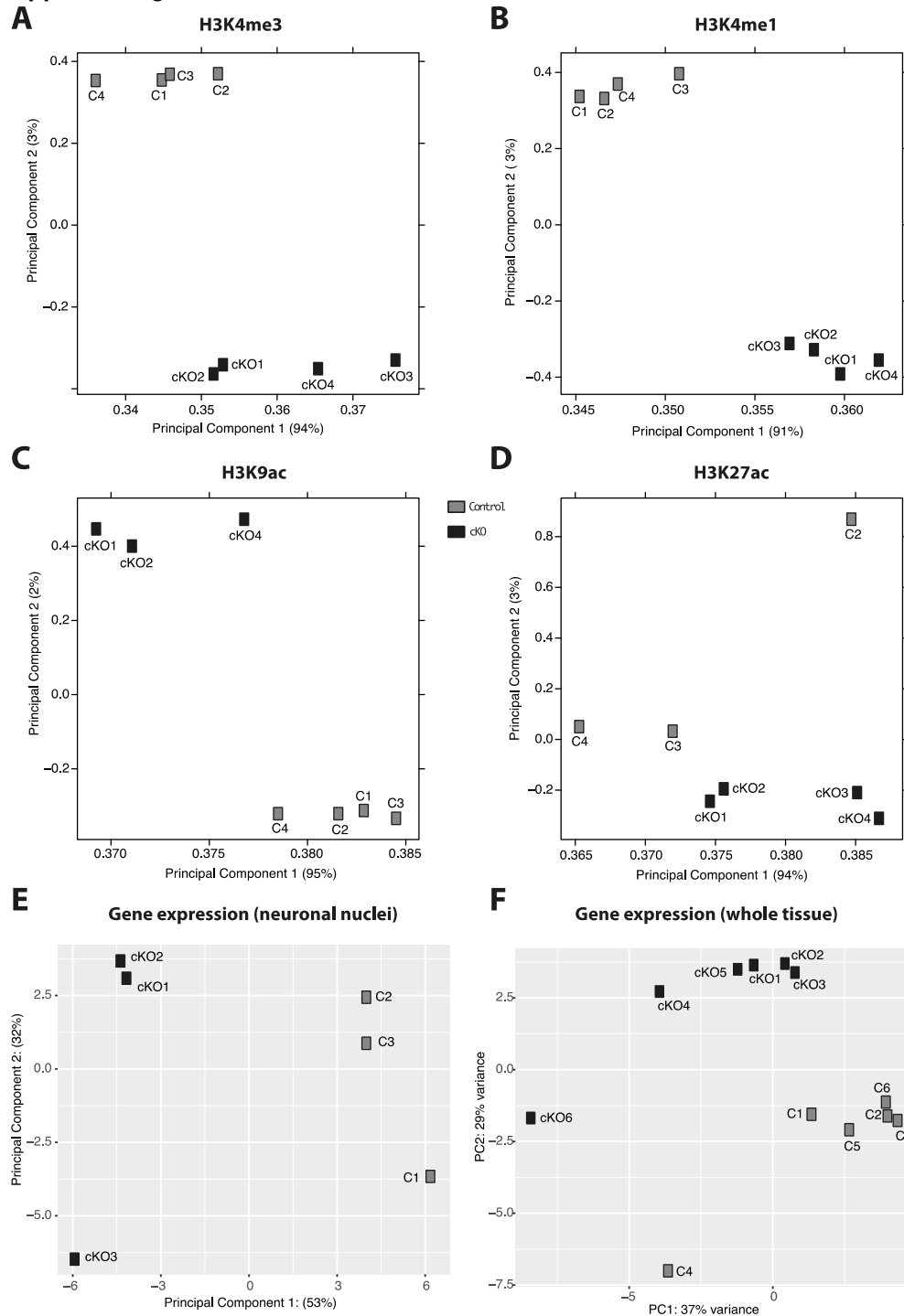


Appendix:
Supplemental Figures 1 – 6

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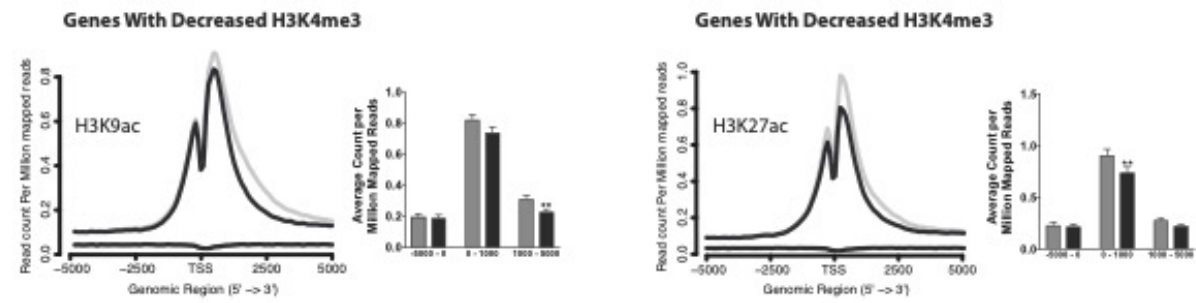
Appendix Fig. S1



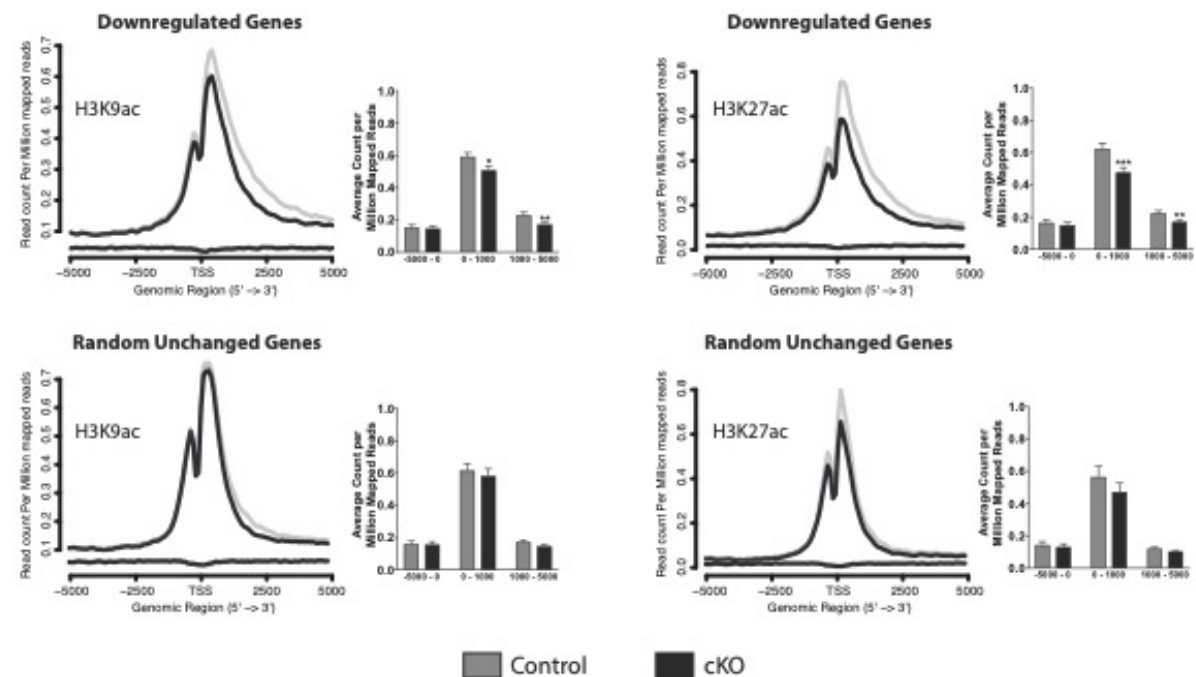
Appendix Fig S1: PCA analysis for the ChIP-seq and RNA-seq datasets generated as part of this study. **A.** Principal component analysis (PCA) plot from H3K4me3 ChIP-Seq experiment. **B.** PCA plot from H3K4me1 ChIP-Seq experiment. **C.** PCA plot from H3K9ac ChIP-Seq experiment. **D.** PCA plot from H3K27ac ChIP-Seq experiment. **E.** PCA plot from RNA-Seq experiment performed from FACS-sorted neuronal (NeuN+) nuclei. **F.** PCA plot from RNA-Seq experiment performed from bulk CA tissue. “n” indicates biological replicates.

Appendix Fig. S2

A

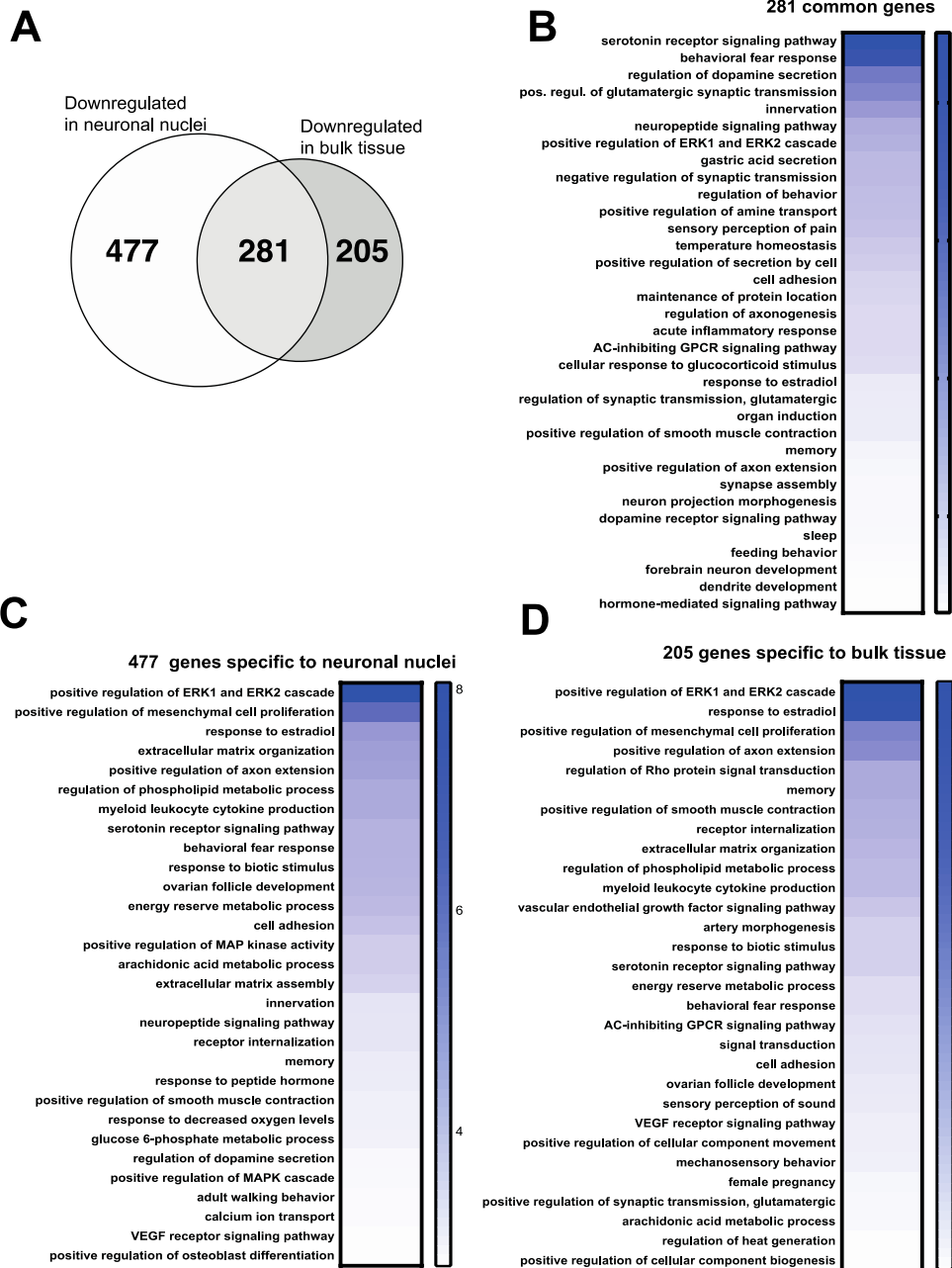


B



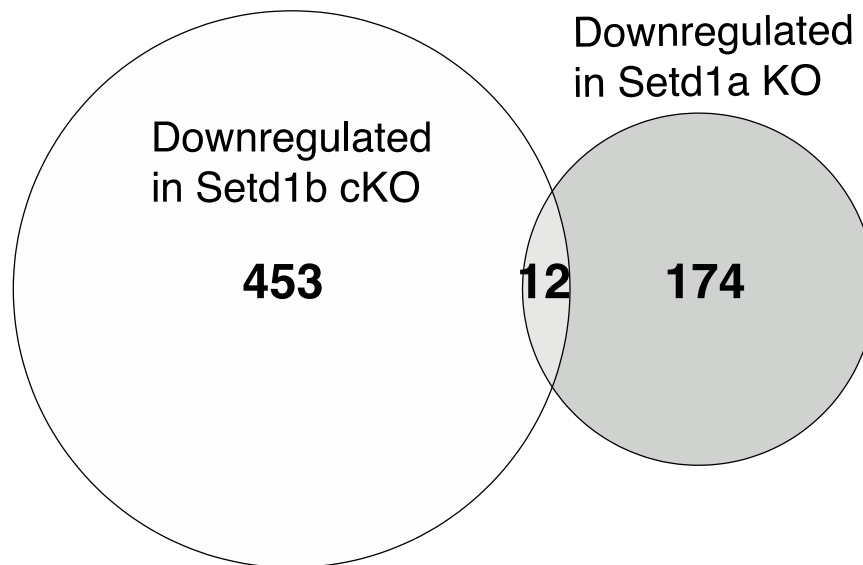
Appendix Fig. S2. Genes downregulated in *Setd1b* cKO mice also exhibit reduced H3K9ac and H3K27ac levels. **A.** Profile plots showing H3K9ac and H3K27ac levels at genes with decreased H3K4me3 in *Setd1b* cKO. **B.** Profile plots showing H3K9ac and H3K27ac levels at downregulated (upper panels) and an equally sized random set of unchanged genes (lower panels) in *Setd1b* cKO. Bar graphs on the right of each profile plot show quantifications of the enrichment of the histone mark at corresponding genomic regions away from TSS (Student t-test: * p -value < 0.05, ** p -value < 0.01, *** p -value < 0.001). Error bars indicate SEM. “n” indicates biological replicates.

Appendix Fig. S3



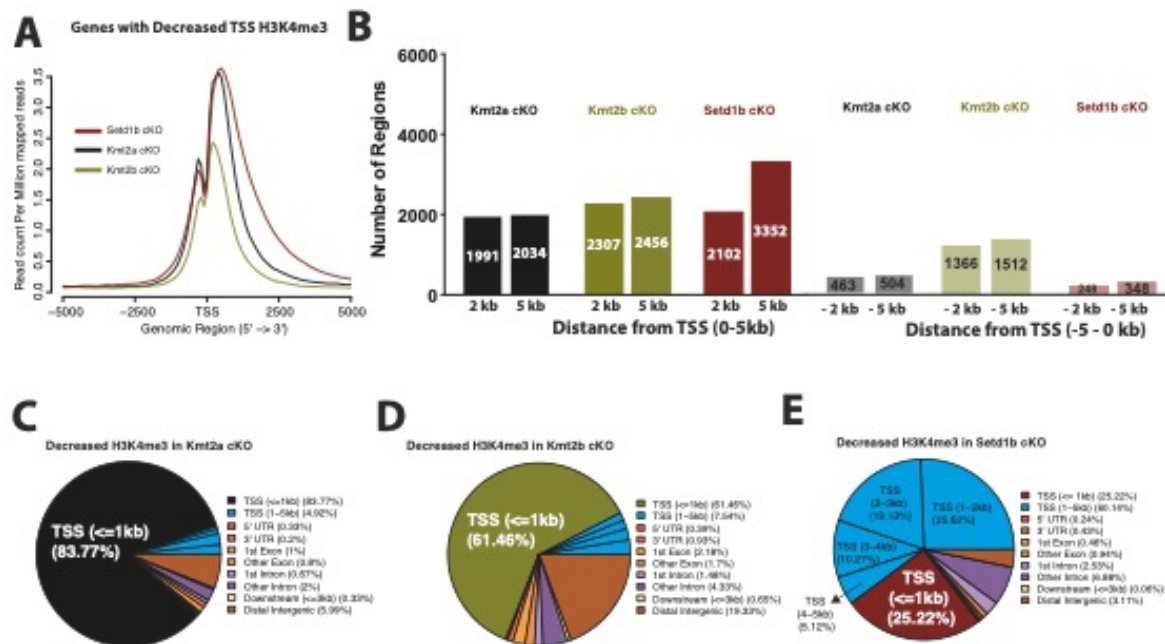
Appendix Fig. S3. Comparison of gene expression changes in *Setd1b* cKO mice detected from cell type specific and bulk tissue RNA-seq. **A.** Venn diagram showing the overlap of genes downregulated in *Setd1b* cKO mice detected via RNA-seq from neuronal nuclei or bulk hippocampal CA tissue. Importantly, the majority of the downregulated genes detected in bulk tissue RNA-seq are also detected in RNA-seq from neuronal nuclei (281 out of 486). Please note that more downregulated genes are detected when neuronal nuclei are analyzed suggesting that some of the differences may be masked by cell type heterogeneity when bulk tissue is analyzed. 205 genes are exclusively detected in the bulk tissue analysis. This might be explained by the fact that especially the cytoplasmatic and synaptic transcriptome is subjected to dynamic changes in post-transcriptional modifications such as RNA-methylation and the regulation by microRNAs, that may even originate from other cells in a paracrine manner [Epple, 2021]. **B-D.** The view that both transcriptomes reflect relevant changes due to the loss of *Setd1b* is supported by GO analysis revealing similar pathways that are linked to neuronal plasticity, and the fact that within the TOP 30, 8 GO-terms are identical, amongst them for example “positive regulation of ERK1 and ERK2 cascade”, “memory” or “positive regulation of axon extension”.

Appendix Fig. S4

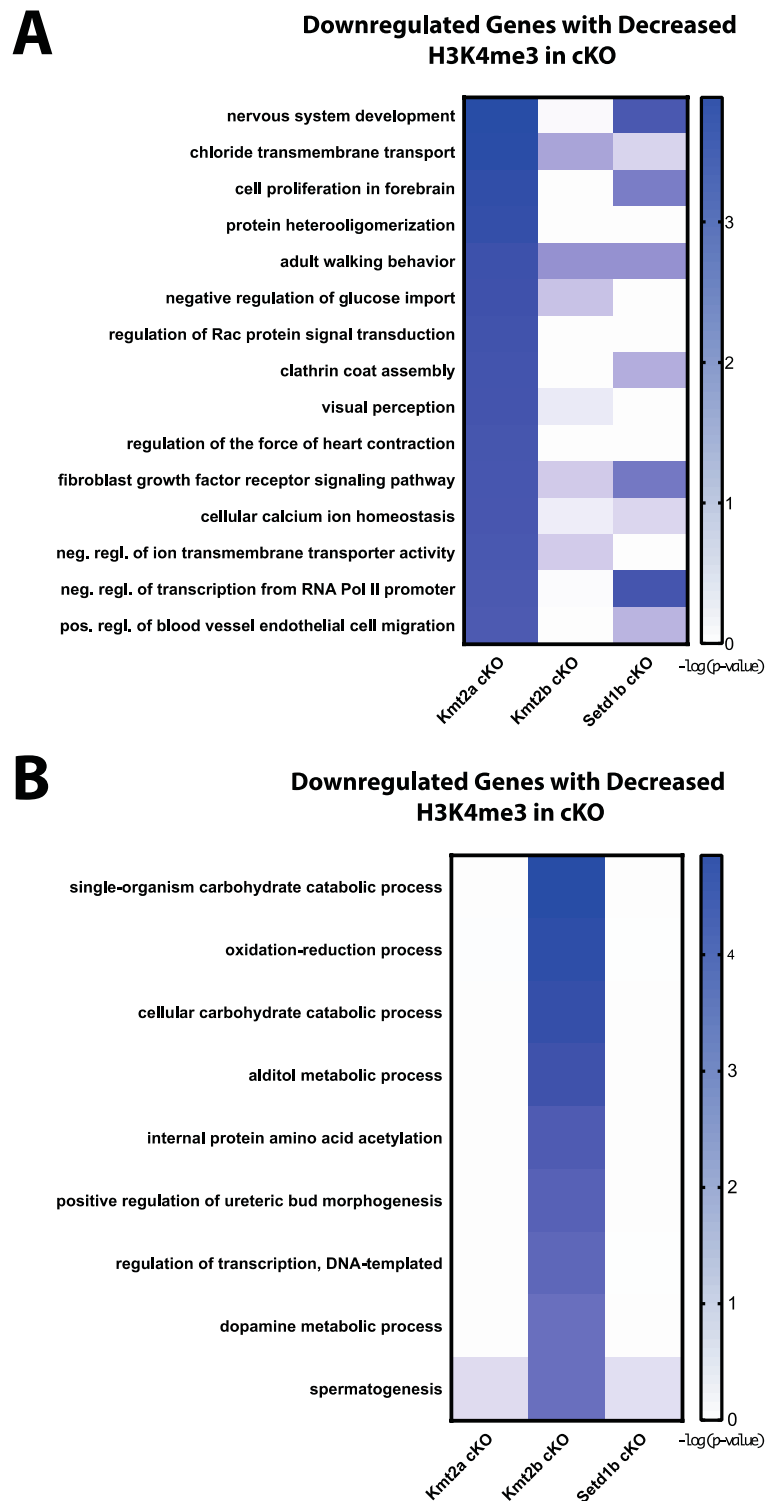


Appendix Fig. S4: Comparison of the genes downregulated in *Setd1a* heterozygous mutant and *Setd1b* cKO mice. Venn diagram showing genes downregulated in *Setd1a* heterozygous mutant vs *Setd1b* cKO mice. Please note that genes affected in the different mutant mice are very different. The data from *Setd1a* heterozygous mutant mice stems from a recent publication by Mukai et al., 2019 (PMID:31606247). Care has to be taken since in that study cortical tissue from heterozygous mice constitutively lacking *Setd1a* was analyzed, while our data stems from the hippocampus of conditional knockout mice.

Appendix Fig. S5.



Appendix Fig. S5. A. Profile plot representing, in wild type mice, the average H3K4me3 distribution around TSS of all genes that display decreased H3K4me3 in *Setd1b* cKO, *Kmt2a* cKO or *Kmt2b* cKO neurons. **B.** Bar plots representing the number of genomic regions in distance to the TSS that show significantly decreased H3K4me3 in *Kmt2a* cKO, *Kmt2b* cKO and *Setd1b* cKO neurons binned into 0-2 kb and 0-5 kb upstream or downstream of the TSS. Note that more regions are detected in *Setd1b* cKO neurons when the region more distant to the TSS (0-5kb) is analyzed. **C-E.** Pie charts depicting the genomic distribution of regions with decreased H3K4me3 in *Kmt2a* cKO (C), *Kmt2b* cKO (D) and *Setd1b* cKO (E) neurons.



Appendix Fig. S6: Functional categories affected in *Kmt2a* and *Kmt2b* cKO mice. A. Heat map showing functional GO category analysis for genes downregulated in *Kmt2a* cKO mice. Enrichment of the same categories is also shown for *Kmt2b* and *Setd1b* cKO mice. **B.** Heat map showing functional GO category analysis for genes downregulated in *Kmt2b* cKO mice. Enrichment of the same categories is also shown for *Kmt2a* and *Setd1b* cKO mice. Please note that the GO categories affected in *Kmt2a* or *Kmt2b* cKO mice differ substantially from those affected in *Setd1b* cKO mice. All analyses are based on comparable RNA-seq data generated from bulk hippocampal CA region.

Expanded View Figures

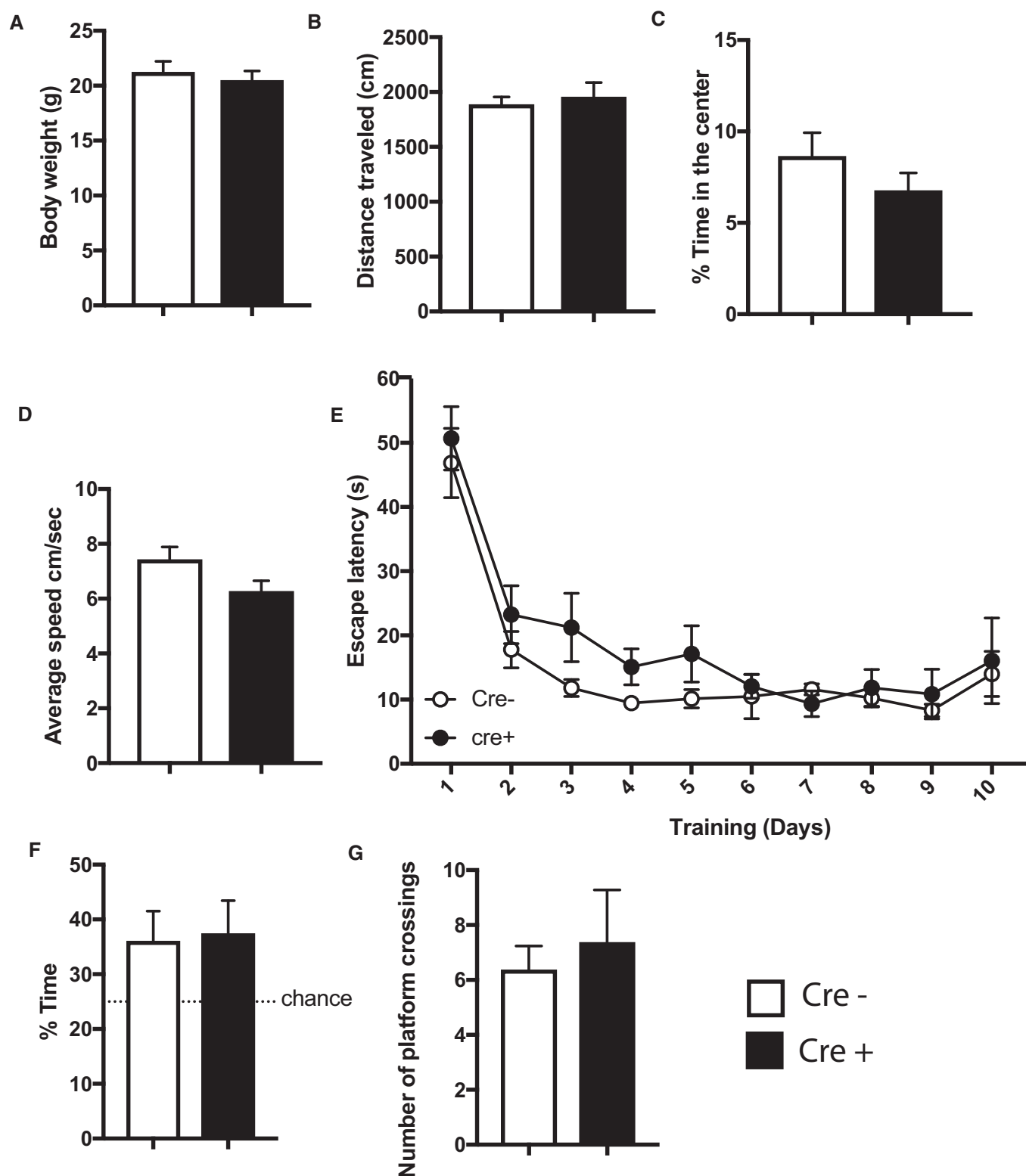
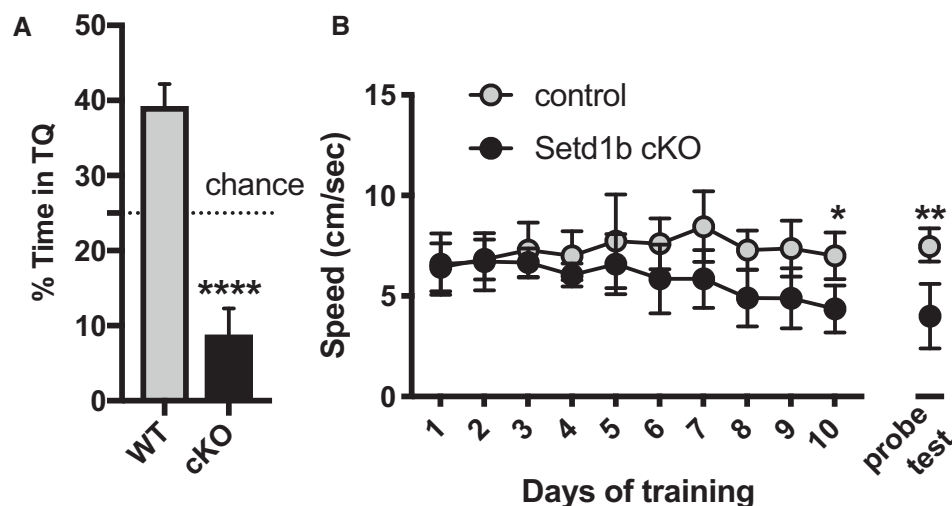


Figure EV1.

Figure EV1. Behavioral analysis of mice expressing CamKII-driven Cre recombinase.

- A Transgenic mice expressing Cre under control of the CamKII promoter were subjected to behavior testing ($n = 8$, Cre+) comparing them with wild-type mice from the same breeding colony that did not express Cre ($n = 8$; Cre-). No difference was observed in body weight.
- B-C The distance traveled in the open field test (B) and the time spent in the center of the arena (C) were similar amongst groups.
- D No difference in the swimming speed was observed among groups when subjected to the water maze test.
- E Escape latency during water maze training was similar in Cre- and Cre+ mice.
- F-G During the probe test performed after 10 training days, Cre- and Cre+ mice showed similar performance when time spent in the target quadrant (F) and the number of platform crossings (G) were analyzed.

Data information: Bar graphs indicate mean, Error bars indicate $\pm$ SEM. "n" indicates biological replicates.

**Figure EV2. Analysis of memory recall cannot be interpreted in *Setd1b* cKO mice.**

- A, B (A) Twenty-four hours after the completion of the 10 day-training procedure in the water maze paradigm (See Fig 1G) mice were subjected to a probe test. The performance in the probe test, as measured by the time spent in the target quadrant (TQ), is impaired in *Setd1b* cKO mice (Control: $n = 15$, cKO: $n = 15$, **** $P < 0.0001$; Student's t -test). These results have to be interpreted with care, however, since faulty interpretation about memory recall is possible in this experiment since the *Setd1b* cKO mice not even being able to form the memory during the training (See Fig 1G and H). It is moreover important to note that *Setd1b* cKO mice spent even less time in TQ than would be predicted by chance alone. To address the issue we analyzed the swim speed during the entire experiment (B) and noticed that the swim speed was similar in control and *Setd1b* cKO mice across the first 5 days of training. There was however a trend for reduced swim speed in *Setd1b* cKO mice from training day 6 to 10. Although we did not detect significant differences in the swim speed when we analyzed the average values across all training days, a more detailed analysis revealed that swim speed was significantly impaired in *Setd1b* cKO mice at training day 10 (* $P = 0.016$; Student's t -test) and also during the probe test (** $P = 0.001$; Student's t -test). These data may help to explain why the *Setd1b* cKO mice appear to avoid the target quadrant during the one trial 60 s probe test session. The training session consists of a sequence of 4×60 s sessions and mice are always placed into the pool from 4 different locations. During the probe test, that consists of a trial of one 60-s session, animals are always placed in the quadrant opposite to the target quadrant, that is, the quadrant that is the farthest away from the target quadrant. While the reason for the reduced swim speed after 9 days of training is unclear, at present these data do not affect the conclusion that *Setd1b* cKO mice exhibit impaired learning ability.

Data information: Bar graphs/dots indicate mean, Error bars indicate $\pm$ SEM. "n" indicates biological replicates.

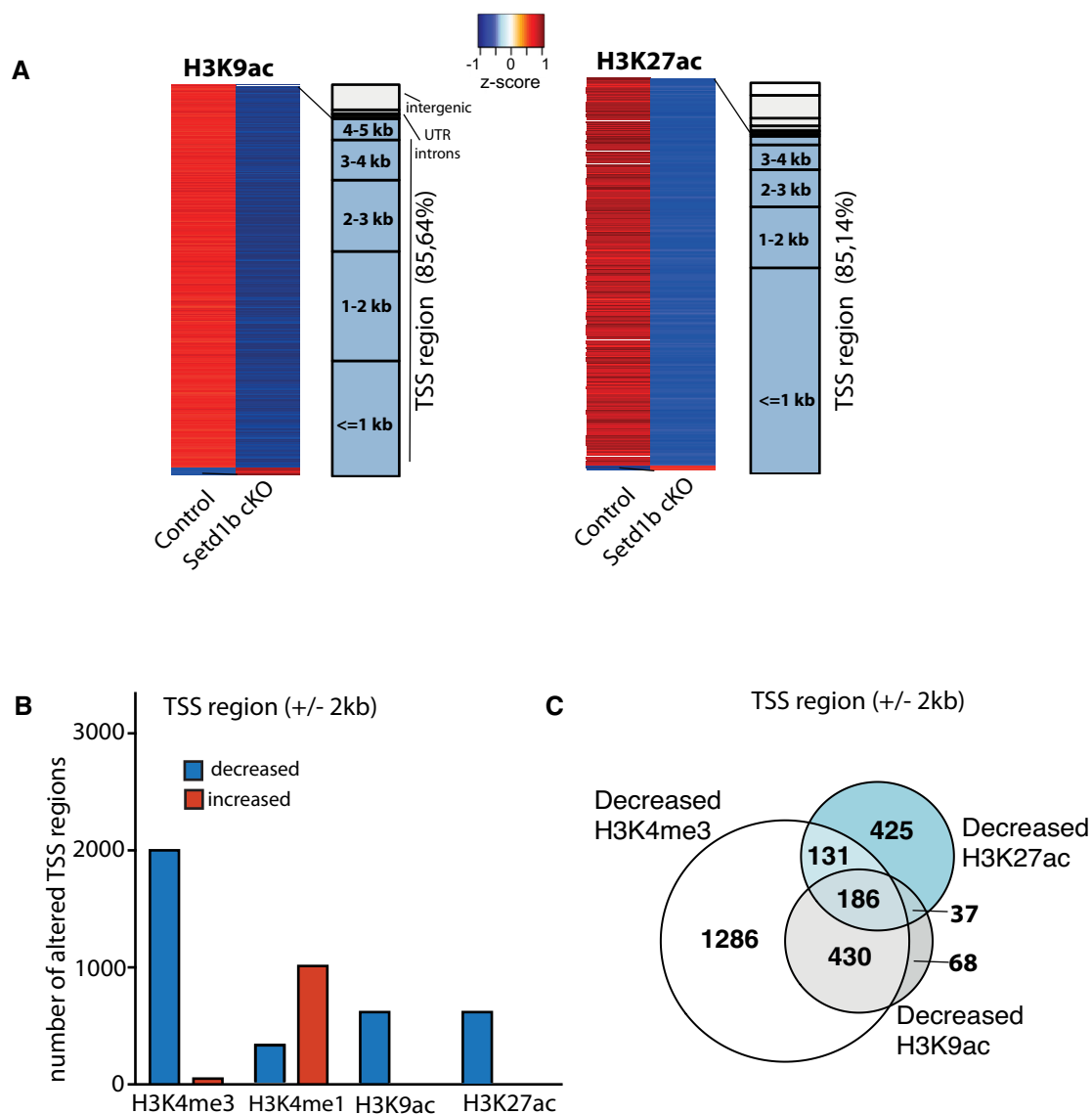


Figure EV3. Decreased H3K9ac and H3K27ac in *Setd1b* cKO mice.

- A** Left panel: Heat map showing genomic regions with differentially bound H3K9ac sites in the close vicinity of TSS (± 2 kb) in neuronal nuclei from *Setd1b* cKO mice and the overall genomic locations of altered H3K9ac levels (Control: $n = 4$; *Setd1b* cKO: $n = 3$). Right panel shows the same analysis for H3K27ac (Control: $n = 3$; *Setd1b* cKO: $n = 4$).
- B** Bar chart showing the number of genes with significantly decreased and increased H3K9ac and H3K27ac marks at the TSS region (± 2 kb). Data for H3K4me3 and H3K4me1 are shown for comparison. As expected, the most affected histone mark is H3K4me3.
- C** Venn diagram showing that most of the sites exhibiting decreased H3K9ac at the TSS also exhibit reduced H3K4me3, while this is not the case for H3K27ac. TSS, transcription start site.

Figure EV4. Sorting neuronal and non-neuronal nuclei for RNA-Seq analysis.

Nuclei from the hippocampal CA region were subjected to FANS as depicted in Fig 2A.

- A Representative images showing nuclei that were sorted using the neuronal marker NeuN. Note that no NeuN positive nuclei are detected in the NeuN (–) fraction confirming the purity of the approach. Scale bar: 50 μ m.
- B Gating strategy for NeuN (+) and NeuN (–) nuclei sorting.
- C RNA-sequencing ($n = 2$ /group) was performed from NeuN (+) and NeuN (–) nuclei and a differential expression analysis was performed. Heat map shows 836 genes specifically enriched in NeuN (+) nuclei when compared to NeuN (–) nuclei. The criteria to select those genes were: adjusted P -value < 0.01, base mean > 150, fold change > 5.
- D GO-term analysis showing that the top 10 enriched biological processes and molecular functions for the 836 genes enriched in NeuN (+) nuclei all represent specific neuronal processes.
- E Normalized expression values obtained from the RNA-seq experiment showing the expression of selected genes known to be enriched in neurons.
- F Normalized expression values of genes that are known to be enriched in non-neuronal cells including glia cells. Error bars indicate SEM.

Data information: Bar graphs indicate mean, Error bars indicate $\pm$ SEM. “ n ” indicates biological replicates.

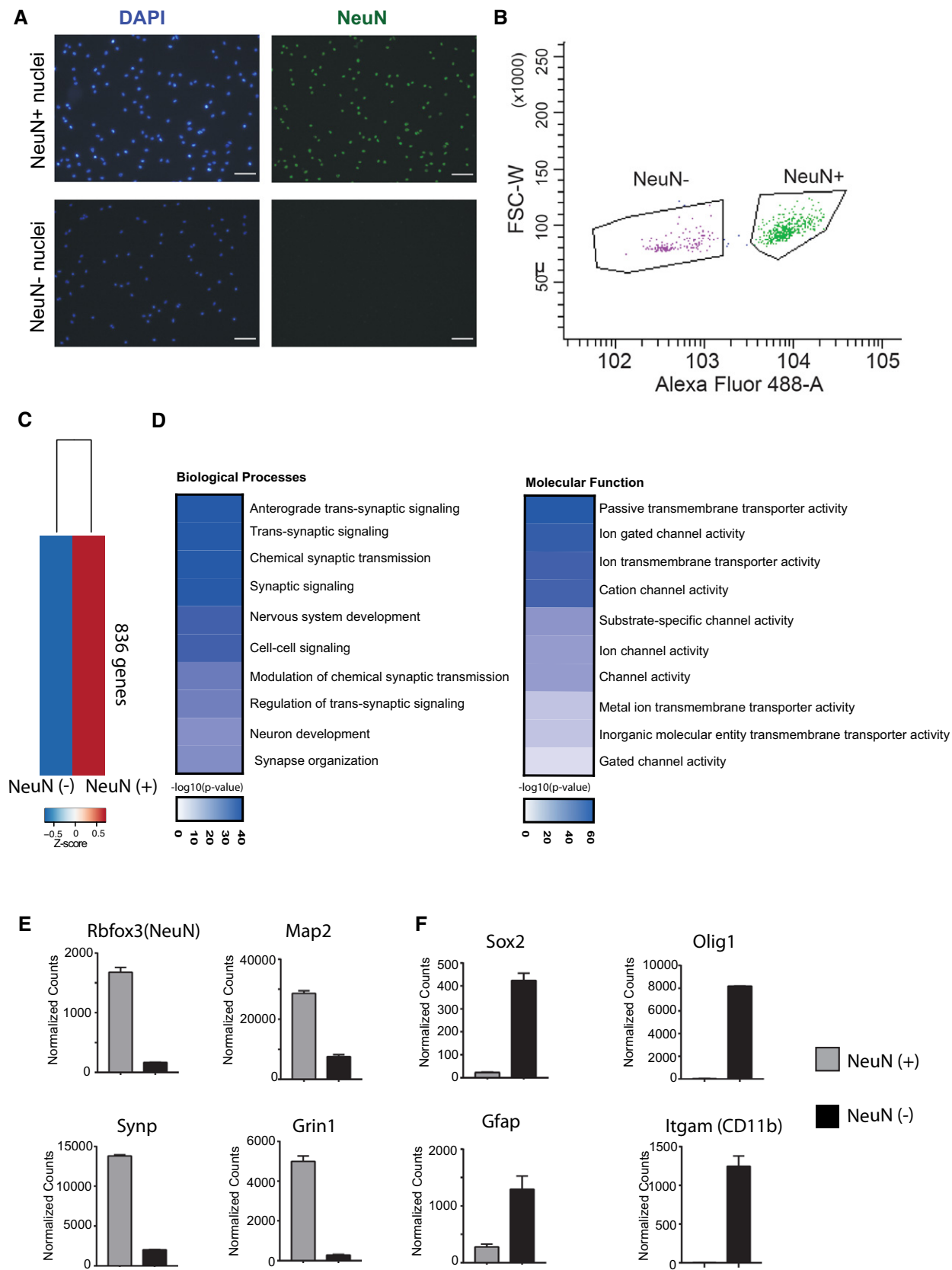


Figure EV4.

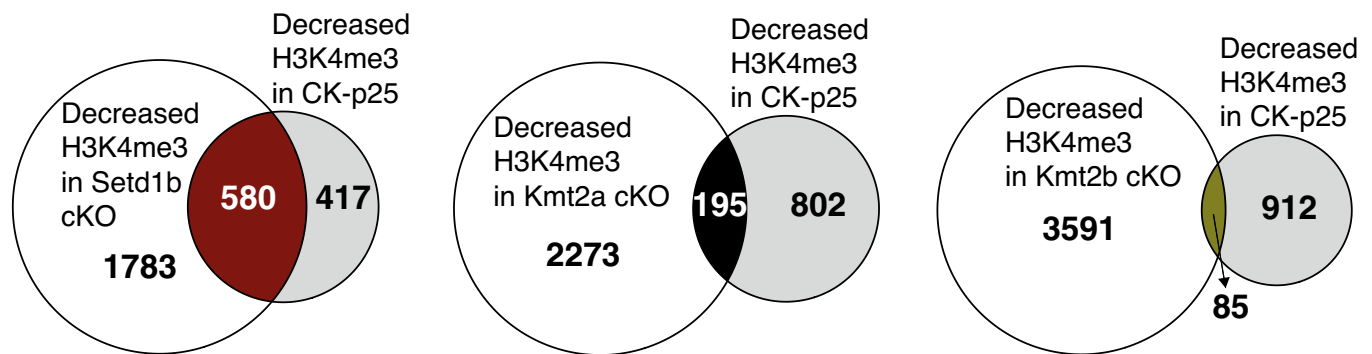


Figure EV5. Comparison of H3K4me3 changes in *Setd1b* cKO, *Kmt2a* cKO, *Kmt2b* cKO, and CK-p25 mice.

Overlap of TSS regions (± 2 kb) with decreased H3K4me3 in each of the three KMT cKO mice with those decreased in mouse model for Alzheimer's disease (CK-p25 mice) (Gjoneska et al, 2015). The pattern of overlapping regions (*Setd1b* > *Kmt2a* > *Kmt2b*) is in agreement with our suggested role of the 3 KMTs in the regulation of neuronal genes important for memory function. Left panel: A highly significant overlap was only seen in case of *Setd1b* cKO mice (580 out of 997 regions; hypergeometric test: P -value = 0). Middle panel: The overlap between *Kmt2a* cKO and CK-p25 is much smaller (195 TSS regions) but still remains significant according to the hypergeometric test (P -value = 0.00000292). Right panel: The overlap between *Kmt2b* cKO and CK-p25 is in turn negligible (85 TSS regions) and is not significant (hypergeometric test: P -value = 1). It is important to note that we re-analyzed our ChIP-seq data from *Kmt2a*, *Kmt2b*, and *Setd1b* cKO mice together with the data from the CK-p25 mice and selected only genomic regions which showed H3K4me3 signal in all datasets to allow for a reliable comparison. Thus, the total numbers of H3K4me3 regions that differ between conditions are slightly different to our results reported, for example, in Fig 4A. This is also due to the fact that Gjoneska et al. analyzed bulk hippocampal tissue, whereas our experiments are based on sorted hippocampal neurons from the CA region. Nevertheless, the fact that the overlap is still substantial with *Setd1b* cKO and lower or negligible in the other two KMT cKOs supports the view that *Setd1b* is particularly important for regulating genes important for synaptic plasticity and memory function.