Poster: H3K27m3 mediates repression of network excitability genes post *status epilepticus*

Panel 1: H3K27m3 is enriched over silenced developmental genes in the naïve hippocampus, consistent with its canonical function

(A) Consensus peaks were generated from replicates of naïve hippocampus for H3K27m3 (n=8 replicates), EZH2 (n=4 replicates), and SUZ12 (n=4 replicate). Peaks were clustered based on their co-localization.

(B) Normalized heatmaps showing average H3K27m3, EZH2, and SUZ12 signal (RPKM) across all clusters of PRC2 and H3K27m3 co-localization as identified in (A). Heatmaps are centered at the peak summit and extend ± 1kb. Regions a and b are expanded for ease of viewing.

(C) Barplot depicting the fraction of genes annotated to co-localization regions identified in (A) that were detected by RNA sequencing relative to the total number of genes annotated to that respective region in the naïve hippocampus. Genes were defined as detected if they showed a count of at least 5 transcripts per million (TPM) in at least 1 sample.

(D) Violin plot showing mean expression of genes (TPM) annotated to co-localization regions identified in (A) in naïve hippocampus. Y-axis is plotted as $\log_2$(mean RNA expression). Median and quartiles are depicted in overlayed boxplots. Outliers are plotted as black dots.

(E) KEGG terms identified by ontology analysis of genes annotated to each co-localization regions identified in (A) in naïve hippocampus. Dot color is scaled to -$\log_{10}$(p adj.) and size is scaled to the Odds Ratio.

Panel 2: H3K27m3 is deposited at pre-marked and novel loci

(A) Volcano plot showing differential enrichment analysis of H3K27m3 peaks between naïve (n=8) and 4d. post-SE (n=9) hippocampi. We identified n=3187 differentially enriched or depleted peaks (FDR < 0.05). Blue dots represent peaks enriched 4d. post-SE (n = 2487). Red dots represent peaks depleted 4d. post-SE (n = 700).

(B) Normalized heatmaps and metaplots (RPKM) of average H3K27m3 signal in naïve (n=8) and 4d. post-SE hippocampi (n=9) across differentially enriched and depleted peaks. Plots are centered on the peak summit and extend ± 1kb. Metaplots represent the average signal across all peaks in either differentially depleted (light blue) or enriched (dark blue) peaks per condition.

(C) Euler plot showing the co-localization of H3K27m3 peaks enriched 4d. post-SE with H3K27m3 peaks in the naïve hippocampus. We identified n=1353 enriched peaks that co-localized with H3K27m3 peaks in the naïve hippocampus corresponding to H3K27m3 enrichment at pre-marked H3K27m3 loci. Conversely, we found n=1134

enriched peaks that did not co-localize with H3K27m3 peaks in the naïve hippocampus, corresponding to de novo H3K27m3 deposition which we refer to as novel loci.

(D) Normalized heatmaps and metaplots (RPKM) of average H3K27m3 signal in naïve and 4d. post-SE hippocampi across H3K27m3 enriched peaks at pre-marked and novel loci. Plots are centered on the peak summit and extend ± 1kb. Metaplots represent the average signal across all peaks in either *de novo* (light blue) or established (dark blue) loci per condition.

(F) Violin plots comparing average H3K27m3 signal between naïve (light blue) and 4d. post-SE (dark blue) at pre-marked and novel loci. H3K27m3 signal is plotted as $\log_2$(mean counts). Median and quartiles are depicted in overlayed boxplots. Outliers are plotted as black dots.

(G) Violin plots comparing $\log_2$(fold-change) of H3K27m3 between pre-marked and novel loci. Novel loci have higher FC compared to pre-marked loci (Wilcoxon rank sum test with continuity correction, p-value = 1.38×10^{-218}). Median and quartiles are depicted in overlayed boxplots. Outliers are plotted as black dots.

Panel 3: Integration of CUT&RUN and RNA-seq reveals complex associations of H3K27m3 and expression

(A) Genomic distribution of H3K27m3 peaks enriched 4d. post-SE. Promoters were defined as within 3kb upstream to 200bp downstream of the gene transcription start site (TSS).

(B) Volcano plot showing differential gene expression between naïve (n=3) and 4d. post-SE (n=3) hippocampi. We identified n=6225 differentially expressed genes (p adj. < 0.05, fold-change (FC) threshold = 1.2). Blue dots genes induced 4d. post-SE (n = 3482). Red dots represent genes repressed 4d. post-SE (n = 2743).

(C) Violin plots comparing RNA expression of genes annotated to H3K27m3 peaks enriched post-SE, in naïve (light blue) and 4d. post-SE (dark blue), separated by pattern of RNA expression post-SE (induced, no change, repressed). RNA expression is plotted as $\log_2$(mean RNA expression). Median and quartiles are depicted in overlayed boxplots. Outliers are plotted as black dots.

(D) Normalized heatmaps and metaplots (RPKM) of average H3K27m3 signal in naïve and 4d. post-SE hippocampi across H3K27m3 enriched genes post-SE, clustered by RNA expression pattern. Plots are centered on the transcription start site (TSS) and extend ± 3kb. Metaplots represent the average signal across all plotted regions for each RNA expression cluster.

(E-H) Average H3K27m3 CUT&RUN signal tracks in naïve and 4d. post-SE hippocampi for representative genes in each RNA expression cluster (F: induced, G: no change,

H: repressed, I: undetected). Gene diagrams in black are in the positive (+) strand while gene diagrams in gray are in the negative (-) strand.

(I) Distribution of RNA expression patterns of genes annotated to pre-marked and novel H3K27m3 enriched peaks 4d. post-SE. Distributions between pre-marked and novel enriched peaks are significantly different (p-value = 1.04×10^{-43}).

Panel 4: H3K27m3 marked and repressed genes are associated with network excitability

(A-B) Top ontological terms identified by ontology analysis of H3K27m3 marked repressed genes against (A) KEGG database, (B) Gene Ontology (GO): Biological Function. Genelists are separated into pre-marked and novel loci. Dot color is scaled to $-\log_{10}$(p adj.) and size is scaled to the Odds Ratio.

Panel 5: Neuronal signaling pathways repressed by H3K27m3 are expressed in distinct cell populations

(A,B) snRNA-sequencing in the pilocarpine model of epilepsy (2) shows that repressed pathways related to network excitability are expressed in distinct populations in the hippocampus. Repressed Ca2+ channel genes are expressed broadly in CA1 and DG. Interestingly, the most highly expressed potassium channel (KCNC2) is expressed almost exclusively in inhibitory interneurons and is repressed post-SE.