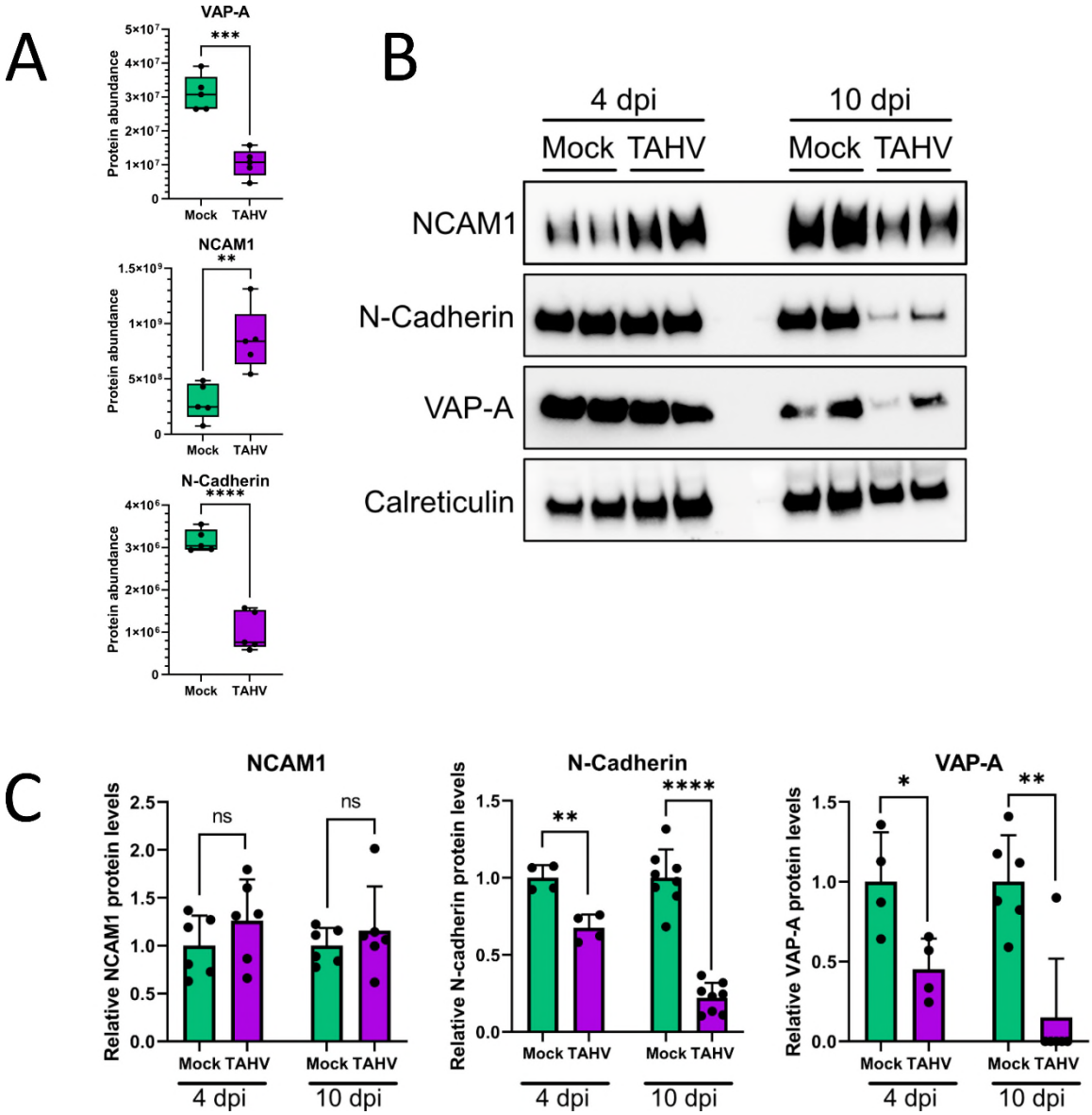
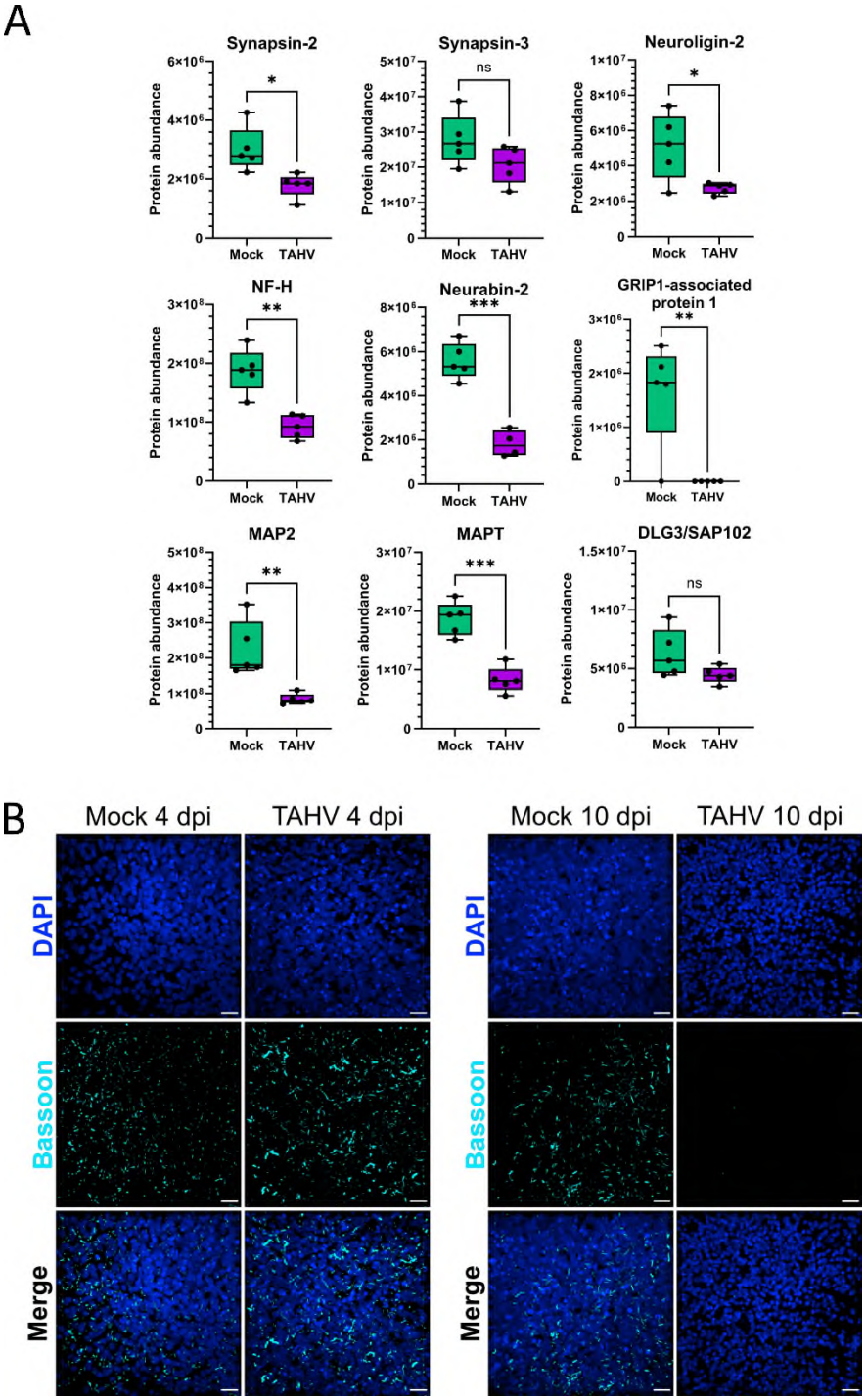
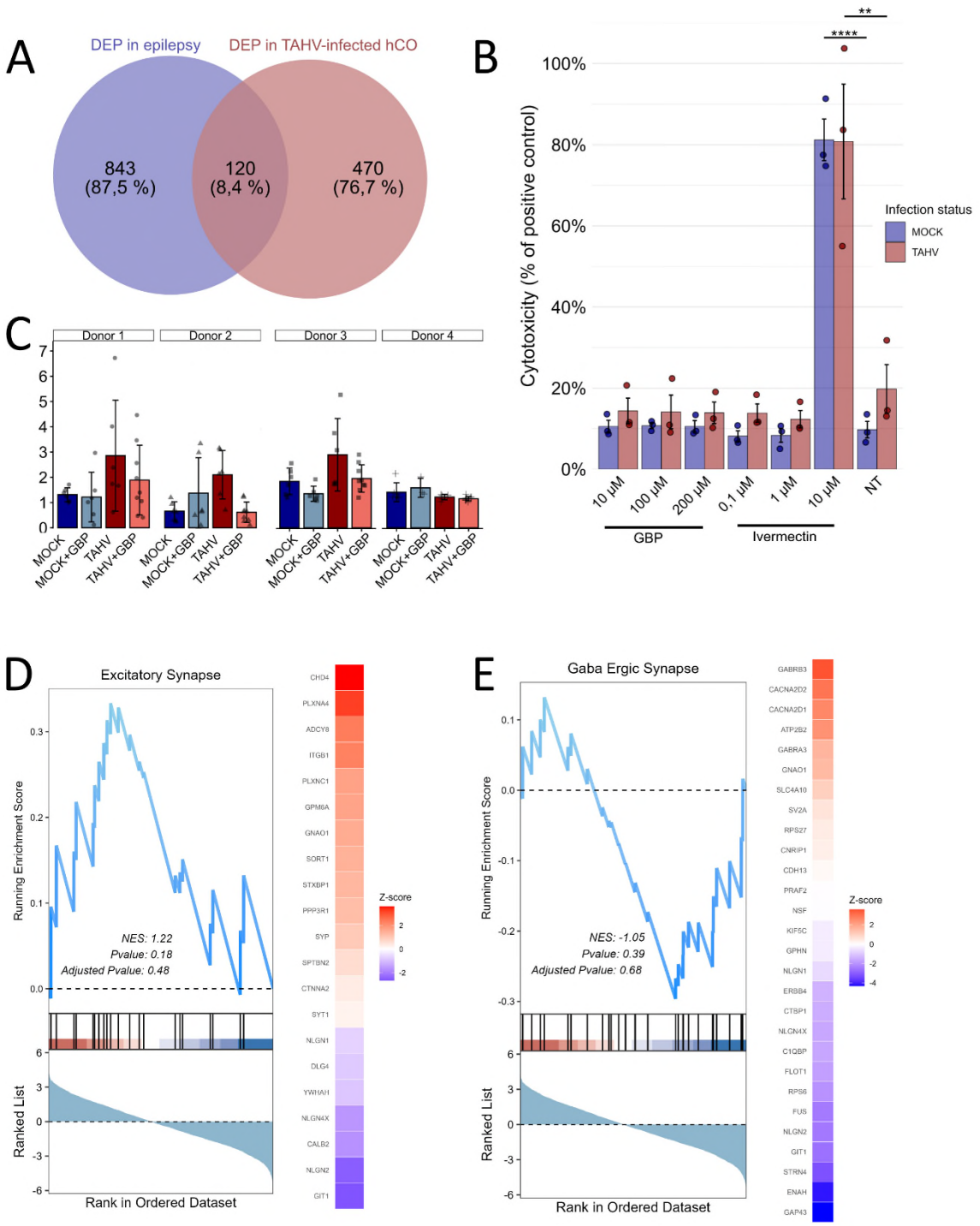
Supplemental Figures and legends



Suppl. Figure S1. Characterization of TAHV-infected hCO. (A) Relative abundance of NCAM1, N-Cadherin, and VAP-A extracted from the mass spectrometry analysis. Unpaired t-test *p* value for NCAM1=0.0052 (**); N-cadherin=<0.0001 (****); VAP-A=0.0001 (***). The differential expression of NCAM1 p values = 0.0037, N-Cadherin p value = 6.99 10^{-5}, VAP-A p value = 0.00018. **(B)** Representative western blot of MOCK and TAHV infected organoids at 4 and 10 dpi. Proteins targeted included NCAM1, N-Cadherin, VAP-A and a loading control, Calreticulin. Each lane represents an individual organoid. **(C)** Western blot protein quantification of NCAM1, N-cadherin and VAP-A normalized to Calreticulin, Calnexin or β-Actin loading controls and normalized to MOCK = 1 at each time point. Data are mean +/- SD from n= 6 biological replicates (6 organoids), except for N-Cadherin and VAP-A conditions at 4 dpi, where 4 organoids were analyzed. Unpaired t-test *p* value for N-Cadherin 4 and 10 dpi = 0.0016 (**) and <0.0001 (****) and VAP-A 4 and 10 dpi = 0.0239 (*) and 0.0013(**), respectively.

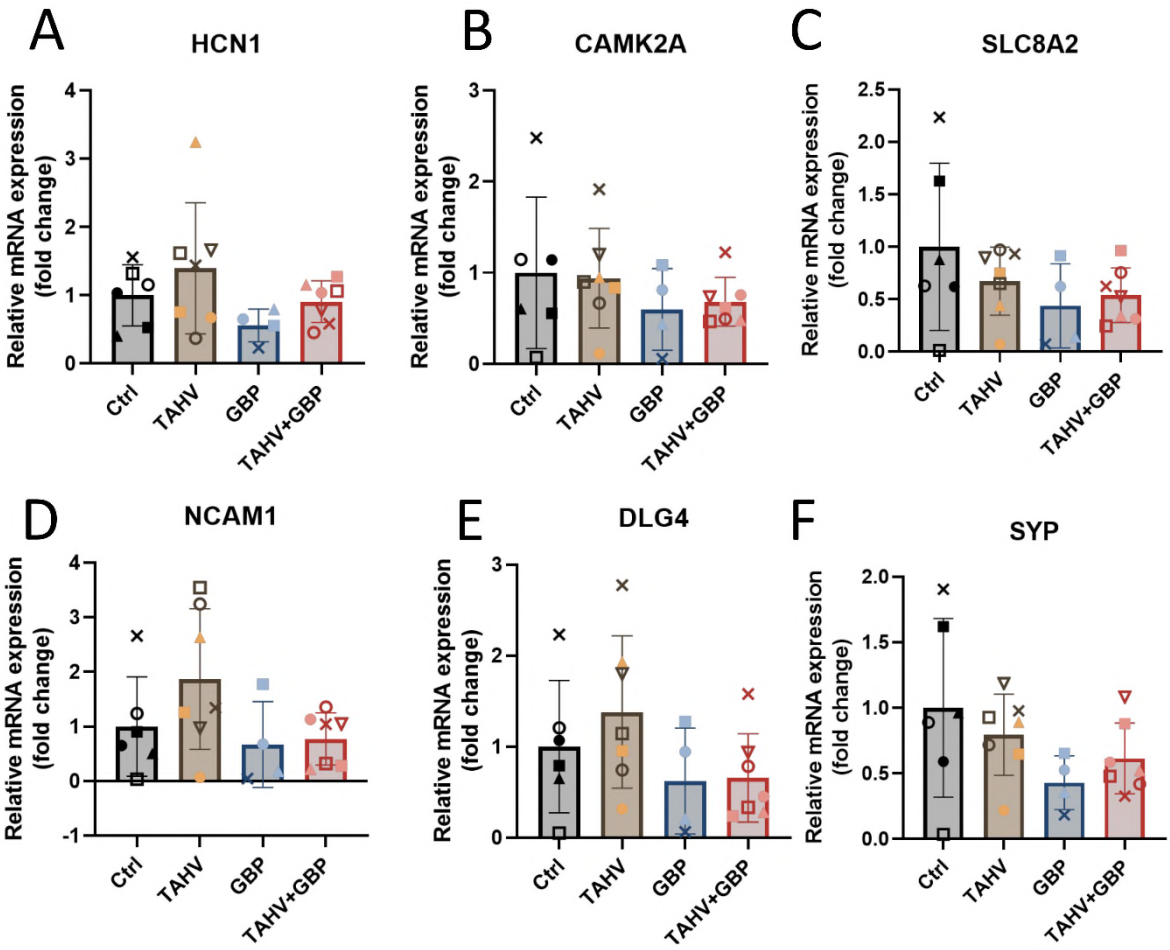


Suppl. Figure S2. Analysis of the expression of proteins associated with neural projections. (A) Expression levels as measured by mass spectrometry for indicated proteins. Unpaired t-test *p* value for Synapsin-2=0.0134 (*); Synapsin-3=0.1104 (ns); Neurabin-2=0.0001 (***); Neuroligin-2=0.0248 (*); Neurofilament heavy chain (NF-H)=0.0011 (**); GRIP1-associated protein 1=0.0051 (**); MAP2=0.0045 (**); Microtubule associated protein Tau (MAPT)=0.0002 (***); DLG3/SAP102=0.0917 (ns). **(B)** Confocal images of organoids infected with 10^5 PFU TAHV or an equal volume of Mock supernatant and stained for a synaptic marker (Bassoon), and nuclei (DAPI). Images were taken at 4 and 10 dpi at 60X magnification. Scale bar: 20 µm.



Suppl. Figure S3. TAHV affects synaptic protein expression and synapse firing. (A) Dysregulated proteomes of TAHV-infected hCO and epilepsy brain tissue overlap. Dysregulated proteins from human hippocampus and cortex brain tissue were obtained and pooled from [58]. **(B)** LUHMES-derived neurons infected with TAHV (MOI 1) were either non-treated (TAHV), treated with 10, 100 or 200 µM GBP or with 0.1, 1 or 10µM or ivermectin for 1 day. Cytotoxicity was assessed by LDH assay 24 hpi. The data correspond to the mean +/- SD from three independent experiments. Cytotoxicity under MOCK or TAHV infection after gabapentin or ivermectin treatment (0.1–10 µM). Statistical analysis was performed separately for each infection condition using one-way ANOVA with Dunnett's post hoc test versus NT. Mean ± SEM. **: $p < 0.01$; ****: $p<0.0001$ versus NT. n=3 biological replicates (3

supernatants of LUHMES cells from different experiments) per condition, and n=2 technical replicates per biological replicate. Mock: Ivermectin 10µM vs NT pval = 3.87e-07 ; TAHV: Ivermectin 10µM vs NT pval = 0.00154. **(C)** Barplot representing the neuronal firing rate of OPABs during TAHV infection and GBP treatment, separated by donors. Each point corresponds to the firing rate of a 1 min recording from one OPAB. For each donor, n=2 biological replicates (OPABs) (Mock, TAHV and Mock+GBP) or n=3 (TAHV+GBP). For each biological replicate, n=3 technical replicates (different recordings). **(D)** Enrichment plot of "excitatory synapse" term. **(E)** Enrichment plot of "Gabaergic synapse" term. NES, p-value, and FDR q-value were determined using the GSEA R function.



Suppl. Figure S4. Expression of mRNAs in the mouse brain. (A-F) RT-qPCR of neuronal mRNAs from mouse brains. Each data point represents an individual mouse. Relative gene expression was normalized to a designated control mouse and to the reference genes *Gapdh* and *Hmbs*. No statistical significance was observed. Biological replicates (homogenized mice brain): control (n=6), TAHV (n=7), GBP (n=4), TAHV+GBP (n=7). For each biological replicate, n=2 technical replicates (2 RT-qPCR reactions).