Supplementary Information

The lncRNA Snhg11 is required for synaptic function, neurogenesis and memory and is downregulated in the dentate gyrus of Down syndrome mouse models

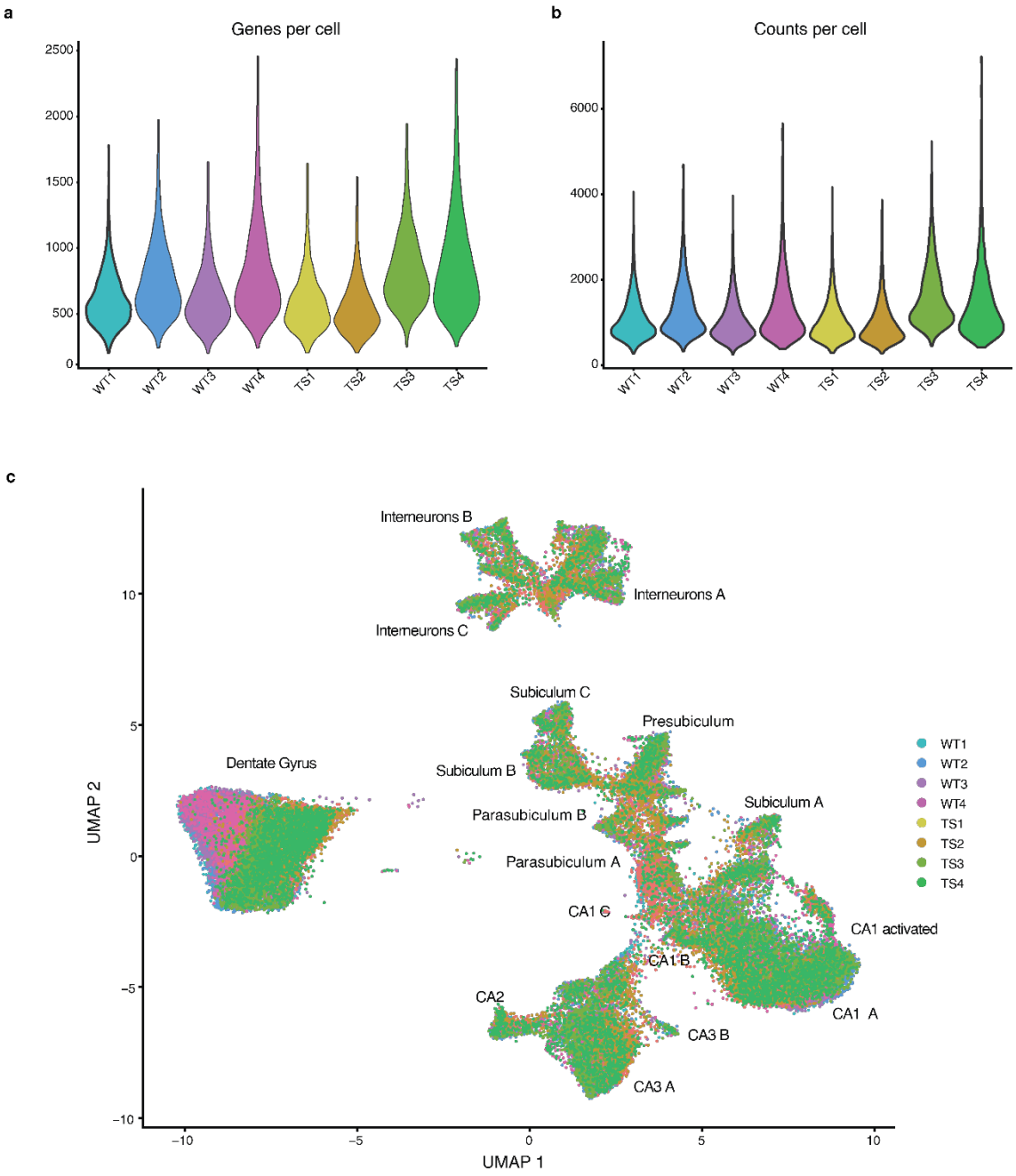
Cesar Sierra[1], Miguel Sabariego[1], Álvaro Fernández-Blanco[1] and Mara Dierssen[1,2,3,*]

[1] Center for Genomic Regulation, The Barcelona Institute for Science and Technology, 08003 Barcelona, Spain;

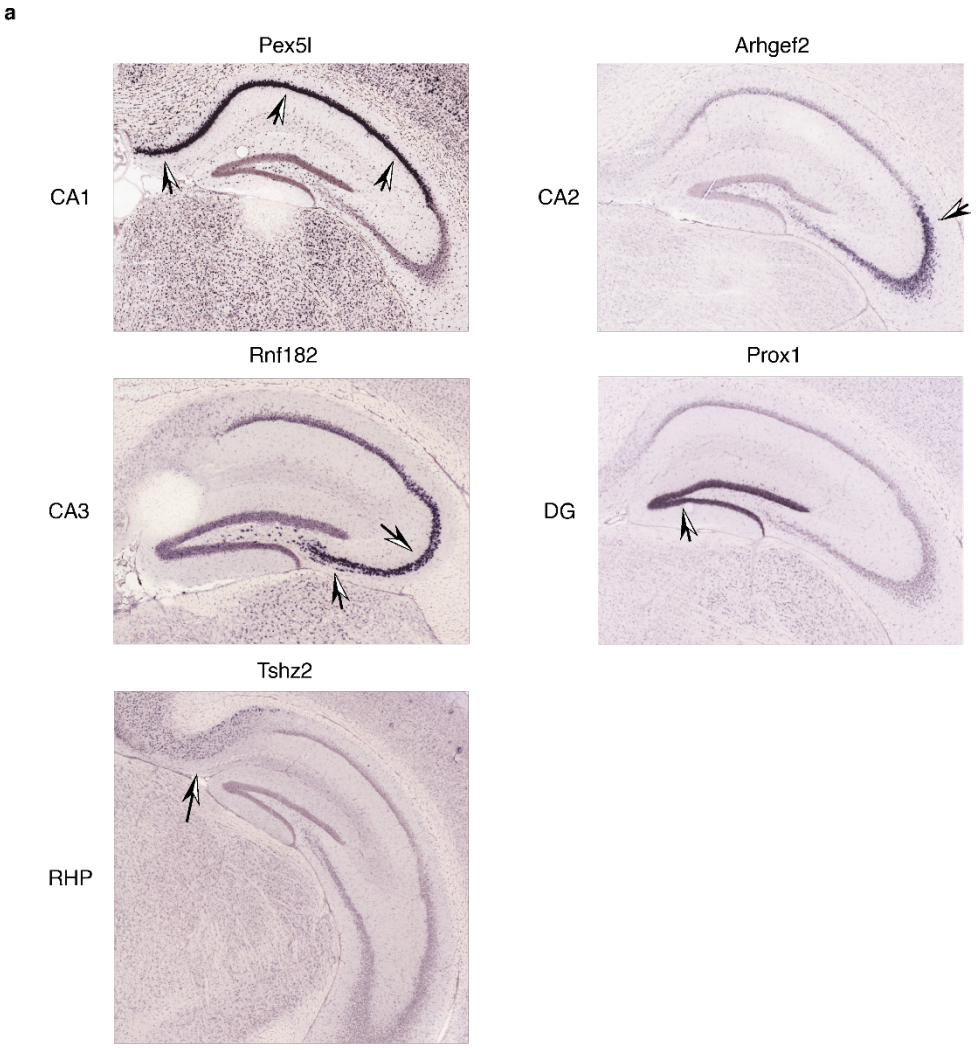
[2] Department of Experimental and Health Sciences, University Pompeu Fabra, 08003 Barcelona, Spain

[3] Biomedical Research Networking Center for Rare Diseases (CIBERER), 08003 Barcelona, Spain

* Correspondence: mara.dierssen@crg.eu; Tel.: +34933160140

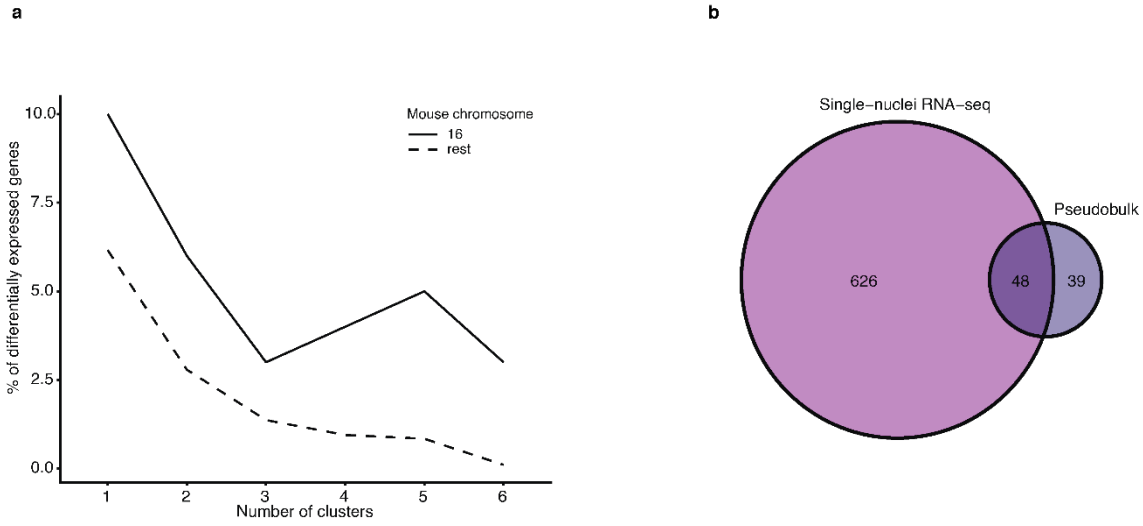


Supplementary Figure 1. 10x library quality control features. a. Violin plot for the number of unique genes detected per cell in each sample. **b.** Violin plot for the number of unique transcripts detected per cell in each sample. **c.** Sample batch effects on cell clusters. Cells are colored by sample (WT1-4, TS1-4) on the UMAP plot. All the clusters are composed of a mixture of cells that originated from each of the eight samples.

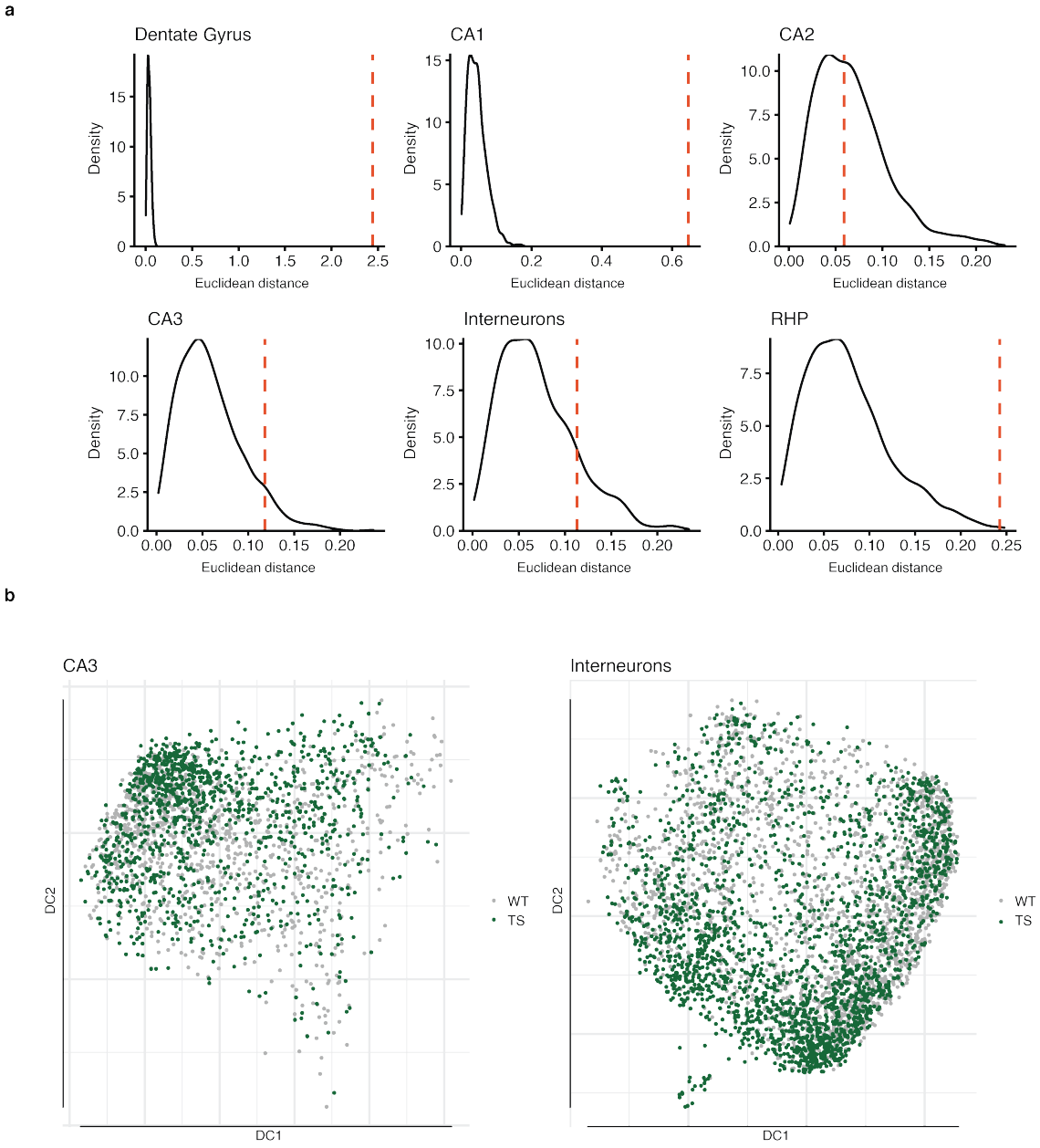


Supplementary Figure 2. Validation of cluster's identity. a. Cross-validation of marker genes for specific hippocampal subpopulations identified in our dataset with the

in situ hybridization images from the Allen Brain Atlas. **b.** Jaccard overlap between cluster markers and enriched genes in each retrohippocampal subregion according to Allen Brain Atlas. p-values are indicated. DG dentate gyrus, RHP retrohippocampal region.



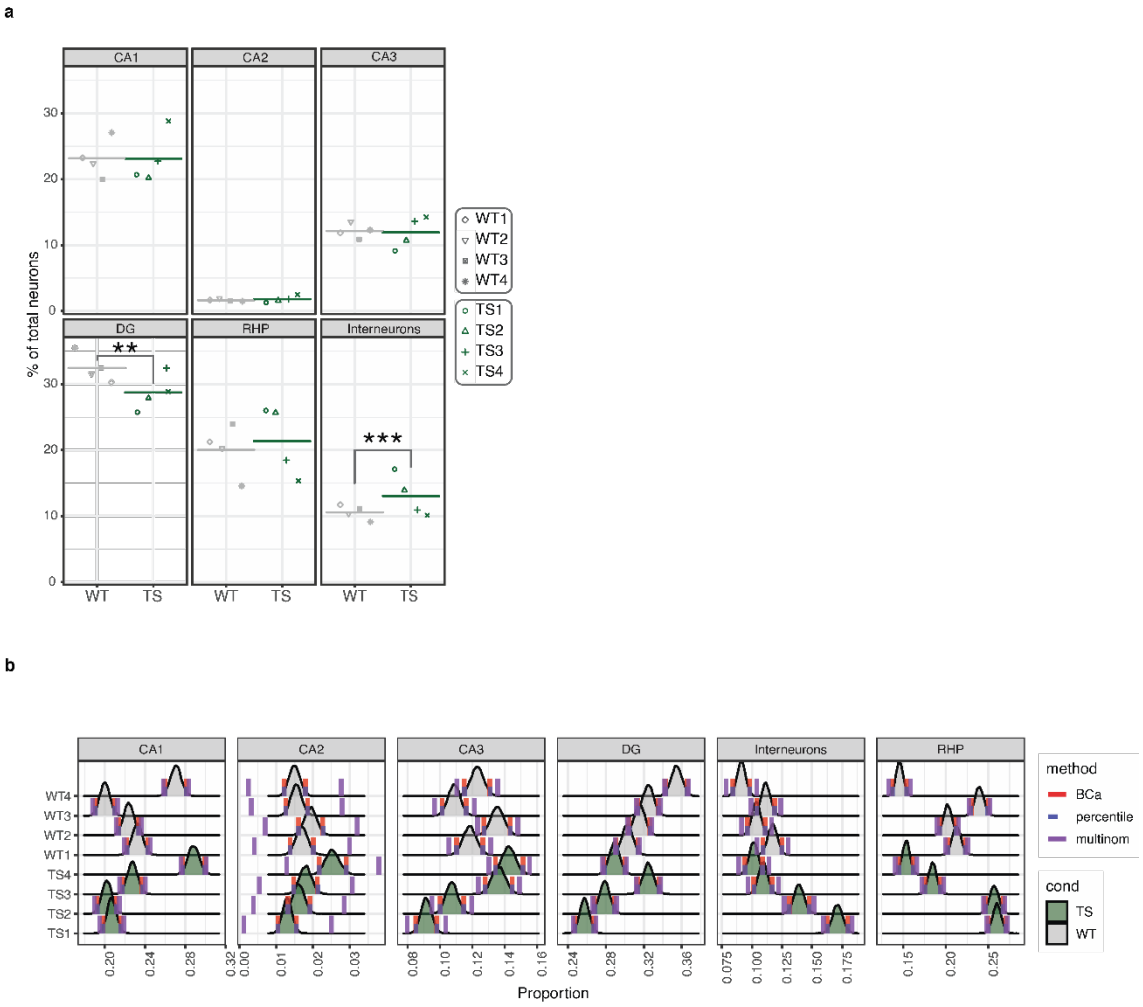
Supplementary Figure 3. a Proportion of chromosome 16 or non-chromosome 16 genes DE in an increasing number of clusters. **b** Venn diagram from DEGs commonly identified in the snRNA-seq and pseudobulk analyses. Most cell-subtype specific DEGs are not captured in pseudobulk analysis



Supplementary Figure 4. Quantification of the Dentate gyrus transcriptomic shift.

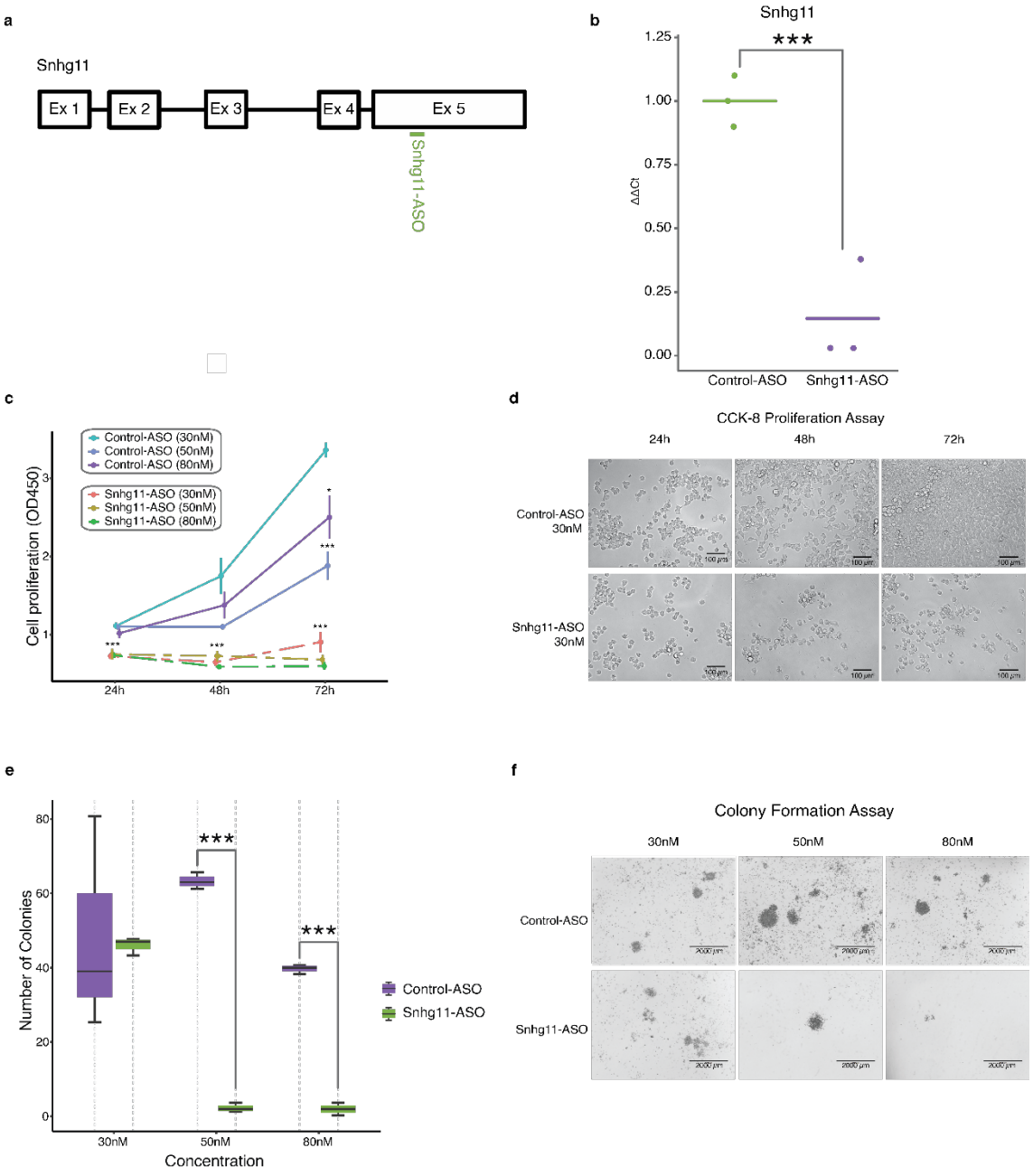
a. Euclidean distance between WT and TS single cells for each cellular subtype. The density plot represents the null distribution of the distances between the single cells

belonging to each cluster and the red line indicates the euclidean distance between the WT and TS centroid of each cluster. Note the different scale in each cellular type **b.** Representation of the two-dimensional embedding of WT (gray) and trisomic (green) cells by Diffusion map from CA3 pyramidal neurons (left) and interneurons (right). RHP retrohippocampal region



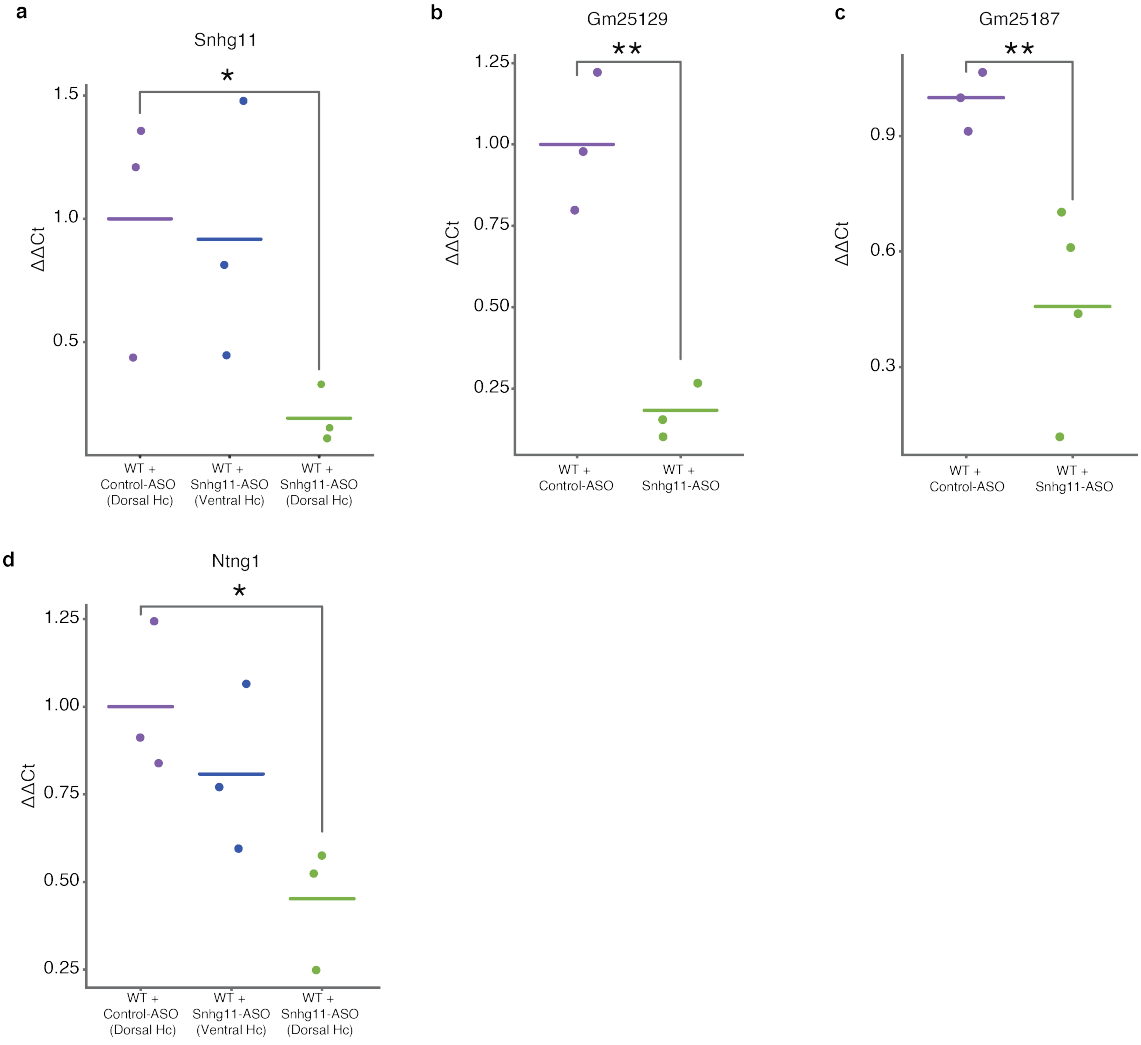
Supplementary Figure 5. Single cell differential composition analysis. a. Dotplot showing the percentage of WT (gray) and TS (green) cells belonging to the main neuronal types. Each symbol represents an individual (TS1-4; WT1-4). ** p < 0.01, *** p < 0.001; Generalized linear model. RHP: retrohippocampal neurons. **b.** Bootstrap

estimated distribution of major hippocampal neuronal types and CA subregion subtypes for each sample. RHP: retrohippocampal neurons. Confidence intervals were estimated with three different methods: multinomial proportions (purple line), bootstrap percentile method (blue line) and bootstrap BCa method (red line).

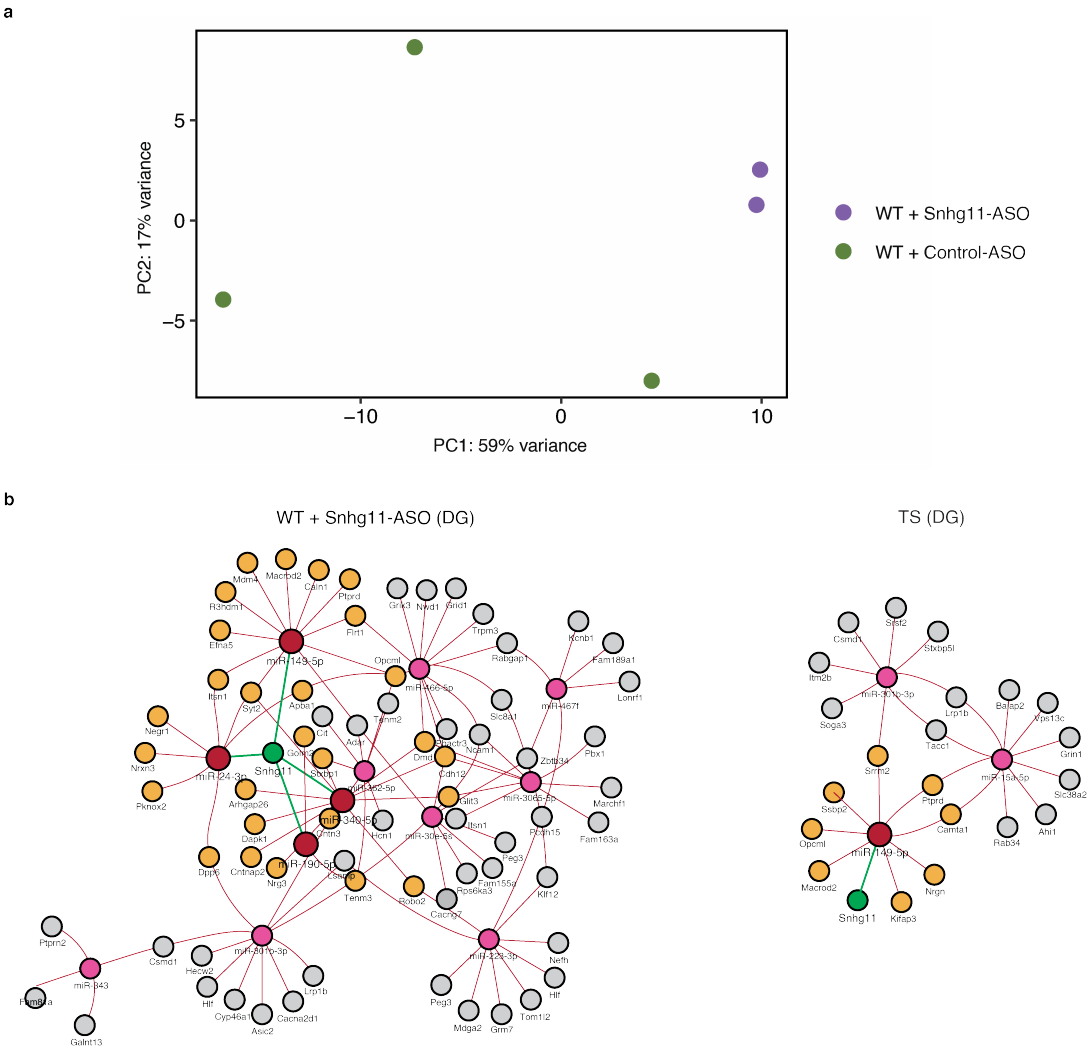


Supplementary Figure 6. In vitro validation of ASO-mediated Snhg11 knockdown. **a.** Exonic target of the designed ASO. **b.** RT-qPCR analysis of Snhg11 expression in N2a cells 72 hours after transfection. *** p<0.001; Student t-test. **c.** CCK8 quantification of cell proliferation for 72 hours after transfection of Control-ASO or Snhg11-ASO. * p< 0.05, *** p<0.001; TukeyHSD test **d.** Representative images of the proliferative state of cells transfected either with 30nM control-ASO or with 30nM Snhg11-ASO after 24, 48 and 72 hours. **e.** Colony count of transfected cells in the Colony Formation Assay. On

the boxplots the horizontal line indicates the median, the box indicates the first to third quartile of expression and whiskers indicate 1.5 × the interquartile range. *** p<0.001; Two factor ANOVA followed by TukeyHSD. **f.** Representative images of the N2a colonies 21 days after being transfected with increasing concentrations (30nM, 50nM and 80nM) of Control-ASO or Snhg11-ASO.

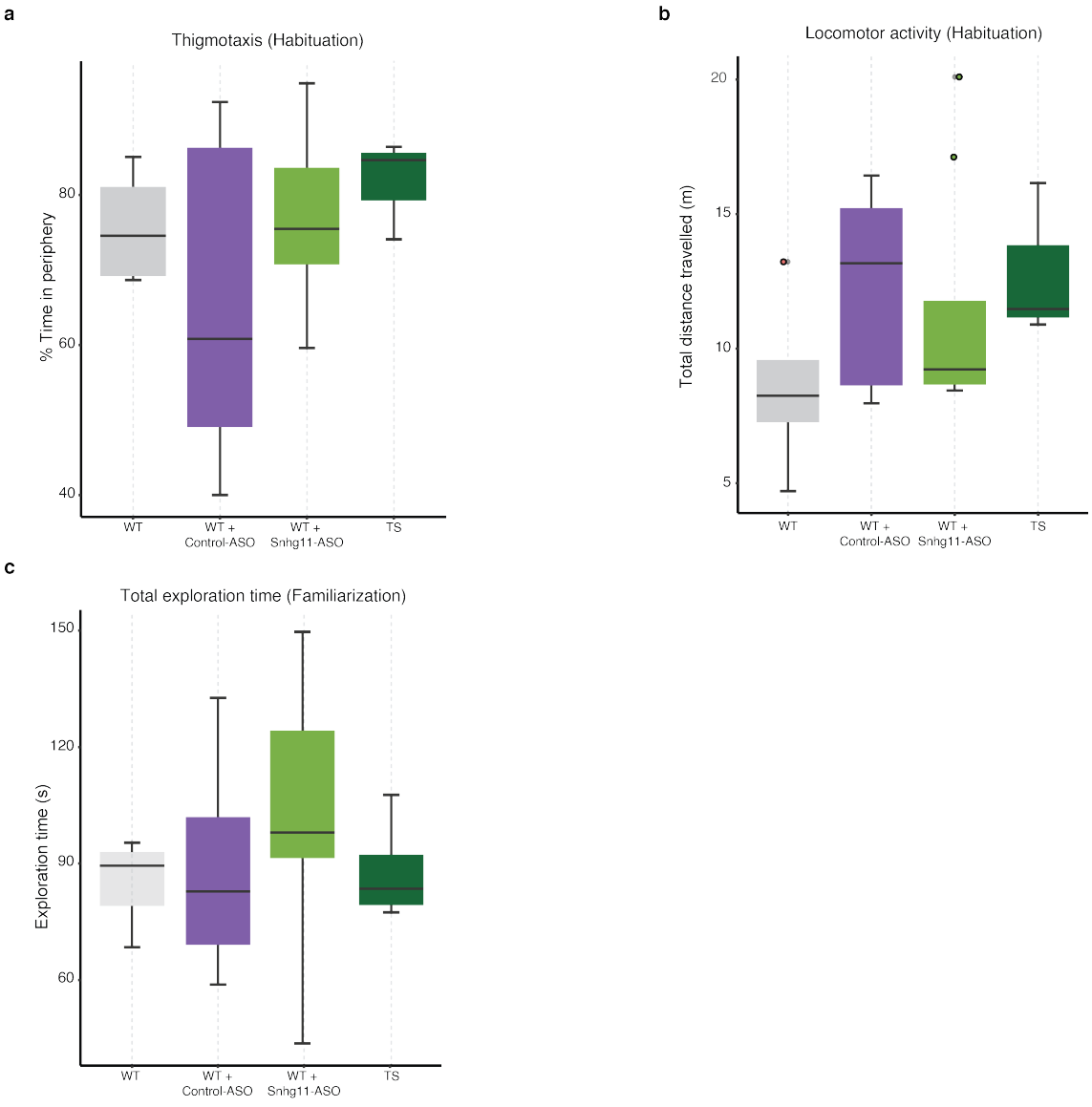


Supplementary figure 7. Gene expression analyses upon Snhg11 knockdown. RT-qPCR analysis of **a.** Snhg11 expression in the dorsal and ventral hippocampus of injected animals, **b.** Gm25129 snoRNA expression in the dorsal hippocampus of injected animals, **b.** Gm25187 snoRNA expression in the dorsal hippocampus of injected animals and **d.** RT-qPCR analysis of Ntng1 expression in the dorsal and ventral hippocampus of injected animals.



Supplementary Figure 8. RNA-seq upon Snhg11 knockdown. a. PCA plot of dorsal hippocampi gene expression data obtained via RNA-seq from three WT animals injected with Control-ASO and two WT animals injected with Snhg11-ASO. **b.** Network analysis

of miRNAs with enriched targets among downregulated genes in the dorsal hippocampus of WT animals injected with Snhg11-ASO (left) and in the trisomic DG (right). Snhg11 is indicated in green and its direct miRNA interactors are colored in red. Coregulated target genes are colored in yellow.



Supplementary Figure 9. Novel object recognition performance in the habituation and familiarization sessions in WT, WT injected either with Control-ASO or Snhg11-ASO and TS mice. Boxplots depicting the experimental variables: **a**. Percentage of time in periphery (Thigmotaxis) during habituation, **b**. Total distance traveled in the habituation session and **c**. Total exploration time recorded in the familiarization session.