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Supplemental information

Silc1 long noncoding RNA is an immediate-early gene promoting efficient memory formation

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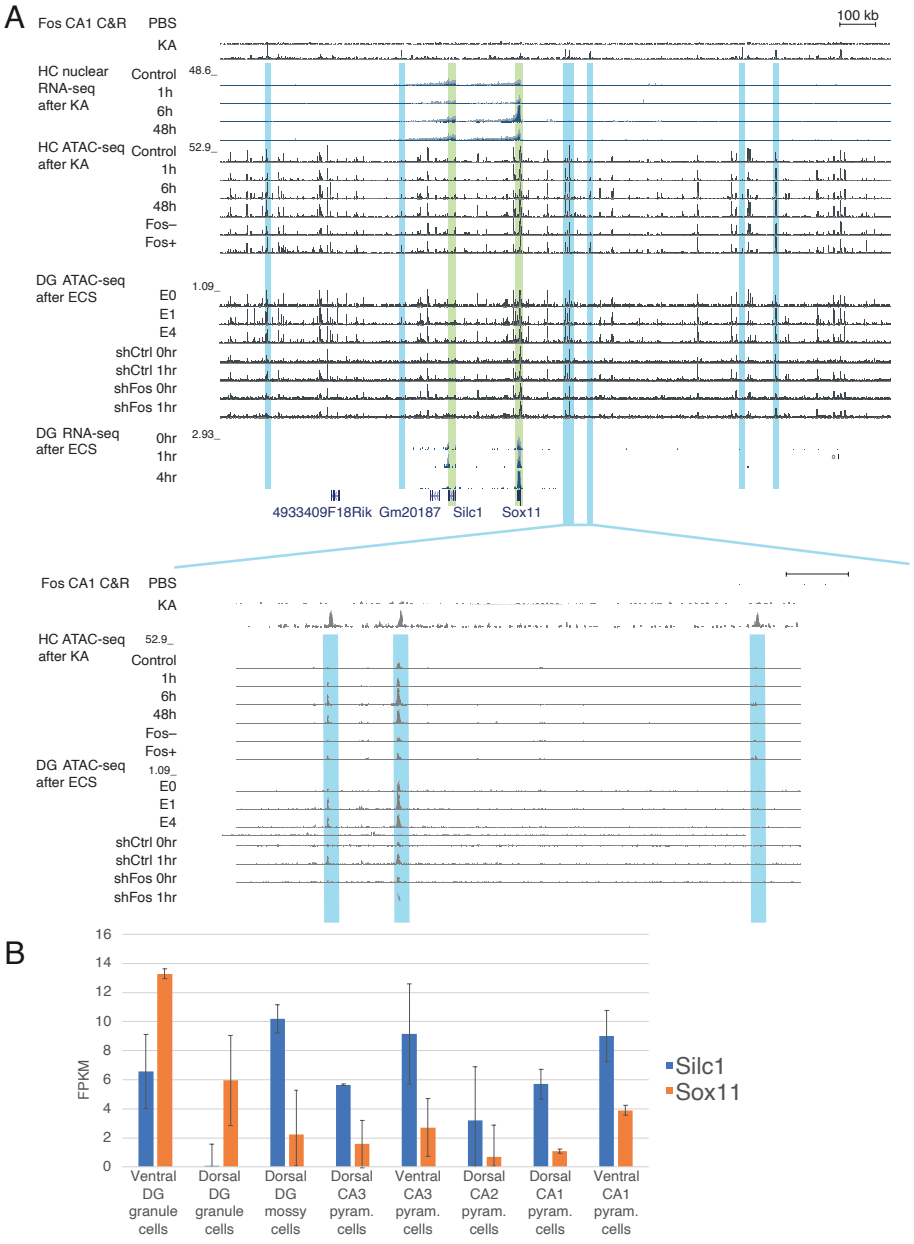

Figure S1. Transcriptomic and epigenomic characterization of the broad Sox11 domain. Related to Figure 1.

(A) Top: broad ~2Mb region flanking Sox11; Bottom: zoom-in on the indicated region. Shown are (top to bottom): Fos Cut&Run data in the hippocampus (HC) CA1 region [37]; HC nuclear RNA-seq data at the indicated time after KA treatment [37]; ATAC-seq data from the same study; including from sorted Fos-positive and Fos-negative cells; ATAC-seq data from the DG after ECS [27], including a time course and the Fos knockdown experiment; RNA-seq data from the same study. Fos-bound and apparently Fos-regulated regions are shaded. **(B)** Expression of *Sox11* and *Silc1* in data of sorted populations from the HippoSeq dataset (n=3).

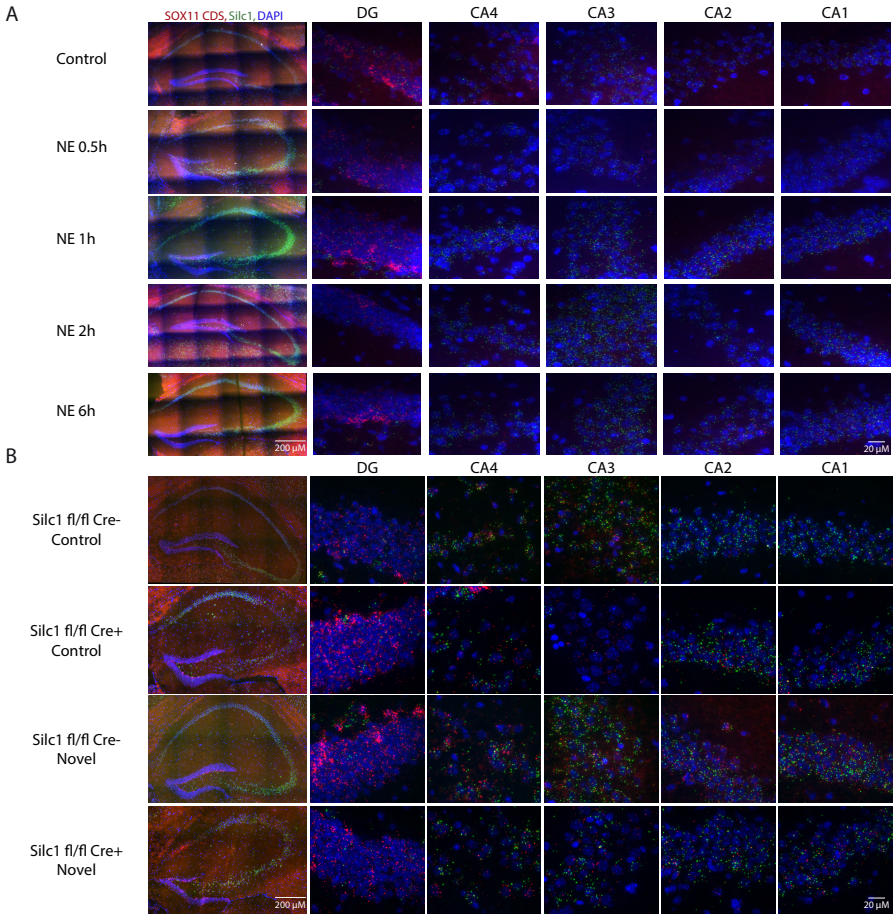

Figure S2. Expression of *Silc1* and *Sox11* at several time points following novel environment (NE) conditions in *Silc1* conditional knockout mice. Related to Figure 2.

(A) RNAscope FISH assay on hippocampal sections from WT mice. Tissues were hybridized with *Silc1* (green) and Sox11 CDS (red) probes and counterstained with DAPI (blue), and imaged using 20X (Scale bar 200 µm) and 100X oil-immersion objectives (Scale bar 20 µm). Novel environment (NE) performed using the Barnes maze setting and the hippocampus was extracted for coronal sections 0.5, 1, 2 and 6 hr upon stimulus. **(B)** As in A for hippocampus sections from *Silc1*$^{fl/fl}$ mice that were stereotaxic injected in the CA3 region using AAV9 Cre-GFP or AAV9 GFP. Two weeks after injections the mice were exposed to Novel environment (NE) and the hippocampus was extracted for coronal sections after 1 hr of NE.

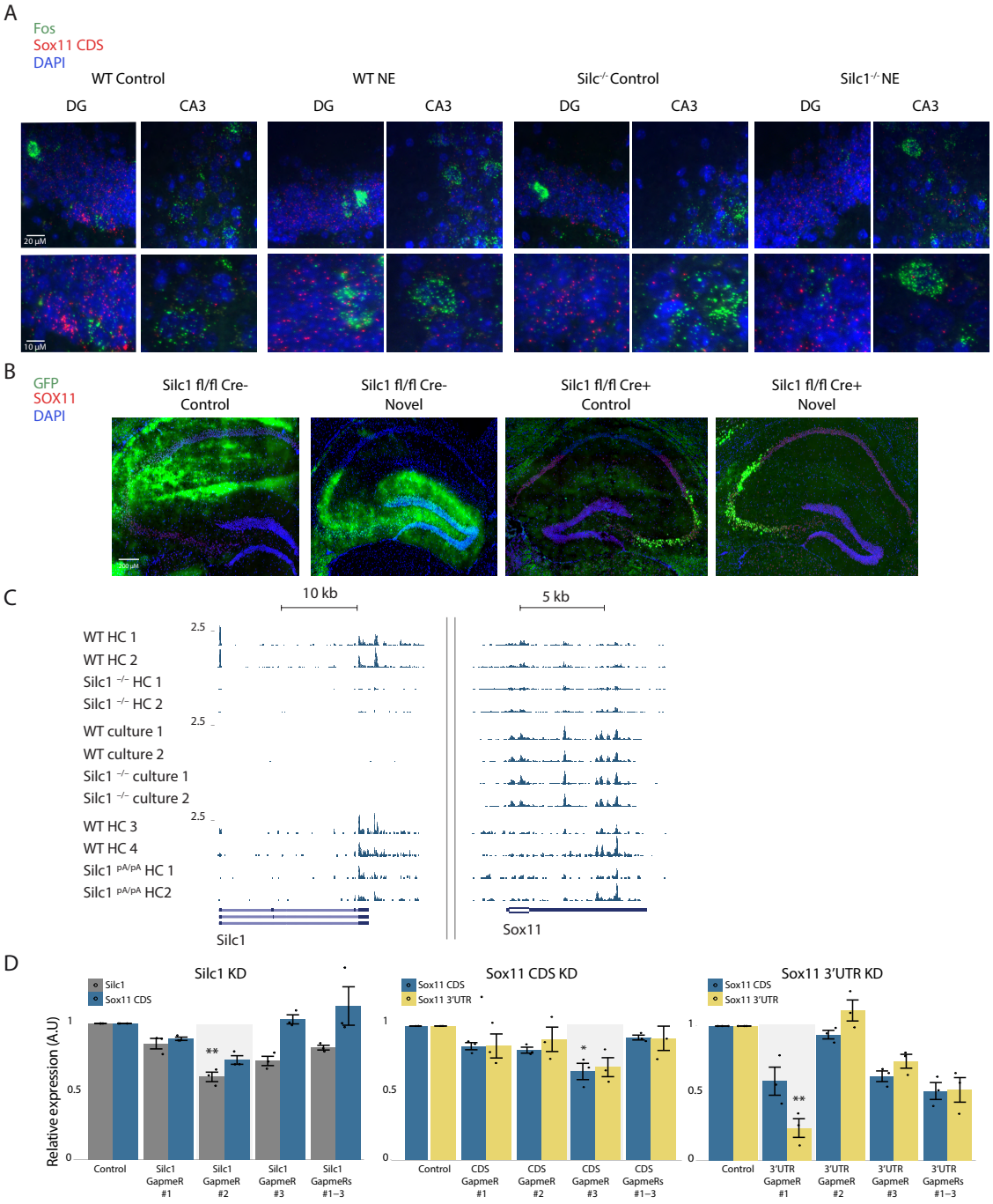

Figure S3. Characterization of gene expression changes in different mouse models. Related to Figure 3.

(A) RNAscope Fluorescent *in situ* hybridization (FISH) assay on WT and *Silc1*$^{-/-}$ mice hippocampal sections from HC and NE conditions. Tissues were hybridized with Fos mRNA (green) and Sox11 CDS (red) probes and counterstained with DAPI (blue), and imaged using 100X oil-immersion objectives (Scale bar 20 µm). **(B)** Immunostaining with anti-SOX11 (red) and DAPI (blue) in hippocampi of *Silc1*$^{fl/fl}$ mice that were stereotaxically

injected in the CA3 region using AAV9 Cre-GFP or AAV9 GFP. The GFP signal marks the site of injection. Imaging using 20X objective (Scale bar 200 μm). (**C**) RNA-seq read coverage at the *Silc1* (left) and *Sox11* (right) genomic regions, in the adult hippocampus (HC) or in cultured hippocampal neurons ("culture") from the indicated genetic background. (**D**) Changes in expression of the indicated genes and regions upon the use of the indicated GapmeRs targeting *Silc1* (left) *Sox11* CDS (middle) and *Sox11* 3'UTR (right), as evaluated by qRT-PCR. Levels were normalized to control GapmeR and β-actin for internal control. The GapmeRs selected for further experiments are shaded in gray. 3 biological repeats. Mean ± SEM is shown. P value calculated using unpaired two-sample t-test,* $P < 0.05$, ** $P < 0.005$.

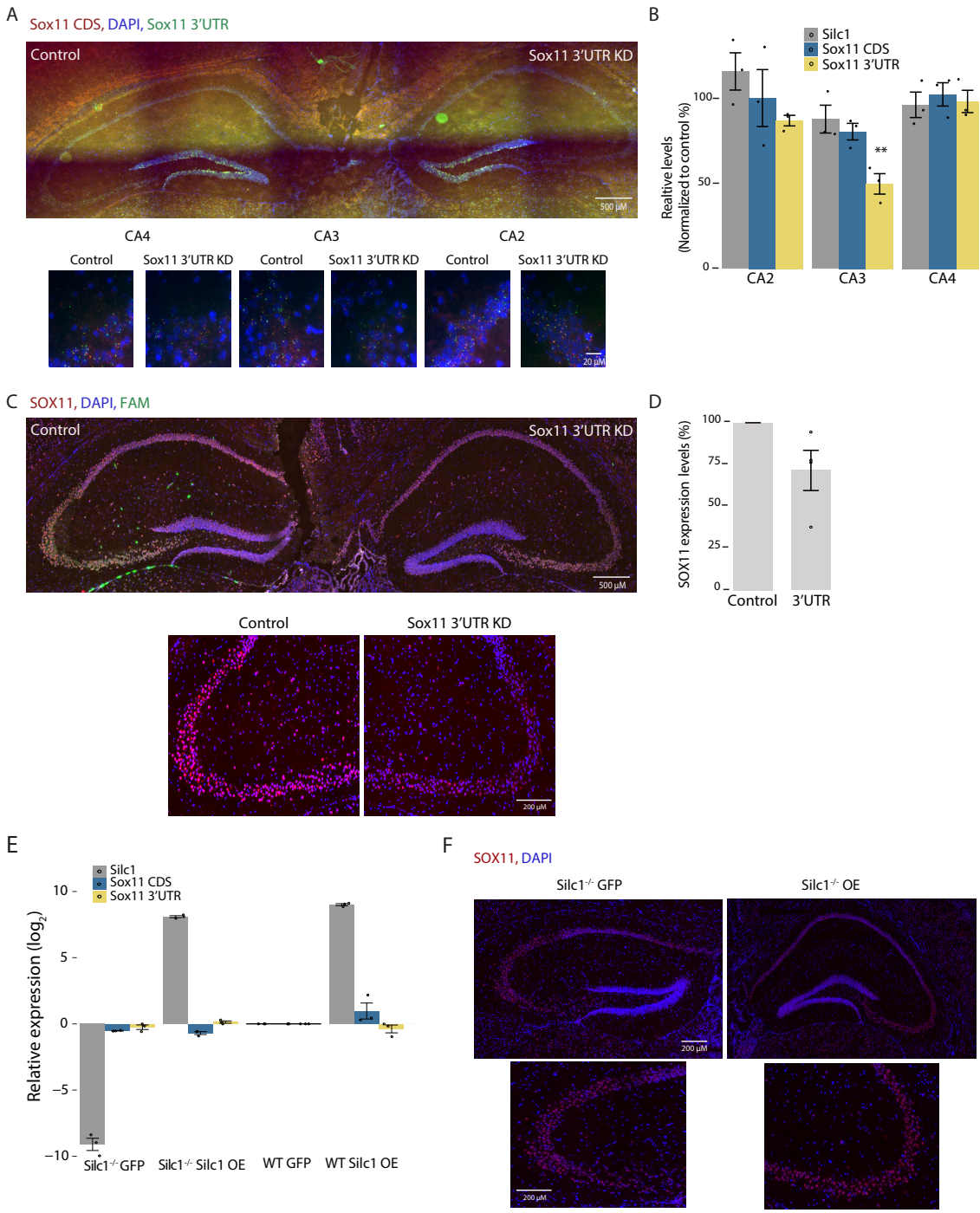

Figure S4. Expression of *Sox11* after *Sox11* 3' UTR KD by injection of GapmeRs into the CA3 region and *Silc1* over-expression in *Silc1*$^{-/-}$ mice. Related to Figure 4.

(A) Control-FAM (left) or Sox11 3' UTR (right) GapmeRs were injected into the CA3 region. 5 days later, the hippocampus was extracted for coronal sections. RNAscope analysis of *Sox11* expression using Sox11 3' UTR(green), Sox11 CDS (red) probes, and DAPI. Imaging was done using 20X (Scale bar 200 µm) and 100X oil-immersion objectives (Scale bar 20 µm). **(B)** RNAscope quantification of the number of green and

red dots, normalized to control GapmeR, performed using IMARIS software. 12 images of non-overlapping fields per biological repeat were quantified; 3 biological repeats. Mean ± SEM is shown. P value calculated using an unpaired two-sample t-test, * P < 0.05. **(C)** Immunostaining with anti-SOX11 (red) and DAPI (blue) in hippocampi of *Sox11* 3' UTR KD mice. FAM signal marks the injection site of the control GapmeR. Imaging using 20X objective (Scale bar 200 μm). **(D)** Quantification of 3 biological repeats of hippocampal staining, normalized to the injection of control GapmeR. Mean ± SEM. **(E)** qRT-PCR quantifications of *Silc1* and *Sox11* after injection of AAV9-Silc1 or AAV9-GFP into the CA3 region of WT and *Silc1*$^{-/-}$ mice. Levels were normalized to WT mice injected with AAV9-GFP, and β actin was used as an internal control. **(F)** Immunostaining with anti-SOX11 (red) and DAPI (blue) in hippocampi of *Silc1*$^{-/-}$ mice injected with Silc1 OE AAV. Imaging using 20X objective (Scale bar 200 μm).

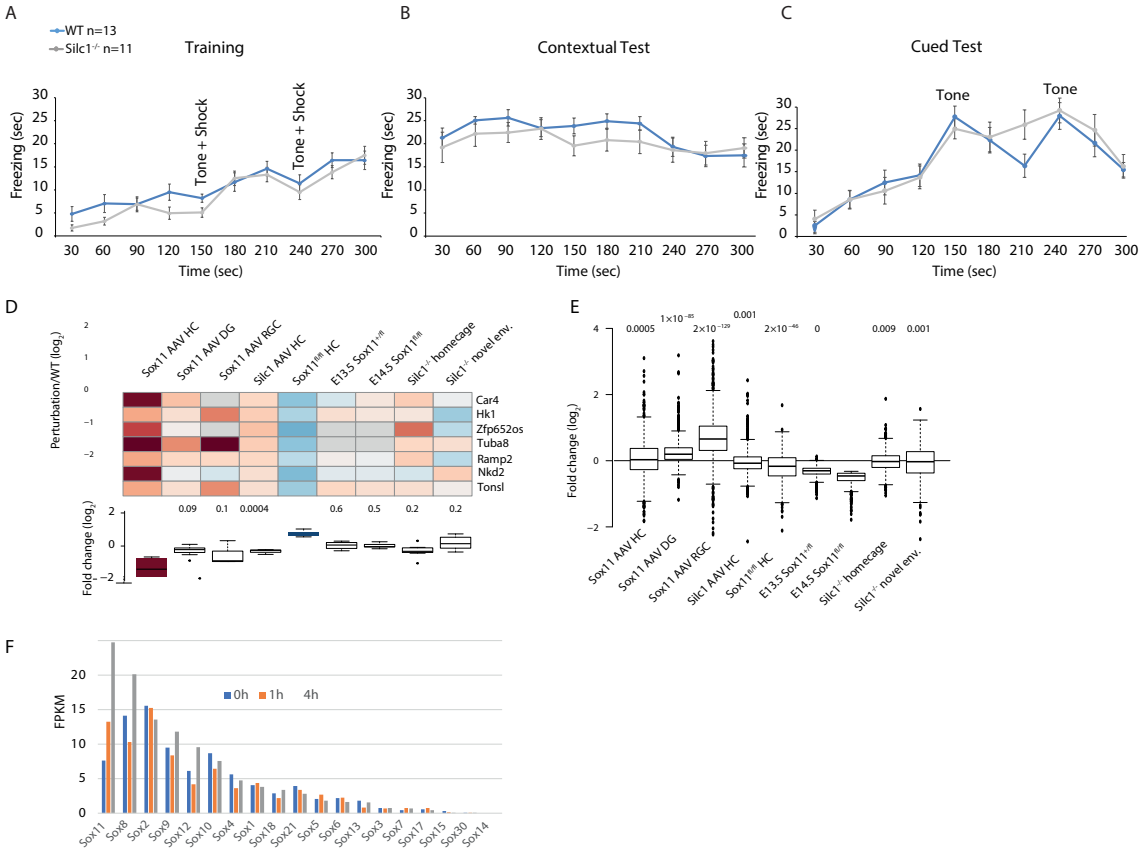

Figure S5. *Silc1*$^{-/-}$ mice showed no alteration in freezing responses during fear conditioning and subsequent recall tests and characterization of genes regulated by *Sox11* and *Silc1* by RNA-seq. Related to Figures 5 and 6.

(A-C) Freezing behavior of *Silc1*$^{-/-}$ (13) mice compared to WT (13) littermates over Fear Conditioning **(A)**, Context Test **(B)** and Cue Test **(C)**. Data represent mean +/- SEM (error bars). Two-way ANOVA, for Gene (Between-Subjects), Time (30 sec intervals; Within-Subjects with repeated measures), and their interaction (Gene × Time) indicated no difference between the genotypes in this type of learning and its recall [Gene (main effect): Conditioning- $F_{(1,24)}$=2.294; p=0.143. Context- $F_{(1,22)}$=0.839; p=0.370. Cue- $F_{(1,22)}$=0.210; p=0.839. **(D)** Genes negatively regulated by Sox11 in the hippocampus**.** As in **Fig. 6**, for the seven genes negatively regulated by Sox11. **(E)** Distribution of the changes in gene expression for the comparisons shown in Fig. 7 for the genes that are significantly reduced in the E13.5 *Sox11*$^{fl/fl}$ Cre+ dentate neuroepithelium. P-values for each group obtained using Wilcoxon rank-sum test are shown above each boxplot. **(F)** As in Fig. 1C, for the different genes in the Sox family of TFs. The RNA-seq data are from the mouse dentate gyrus and the indicated time after ECS.

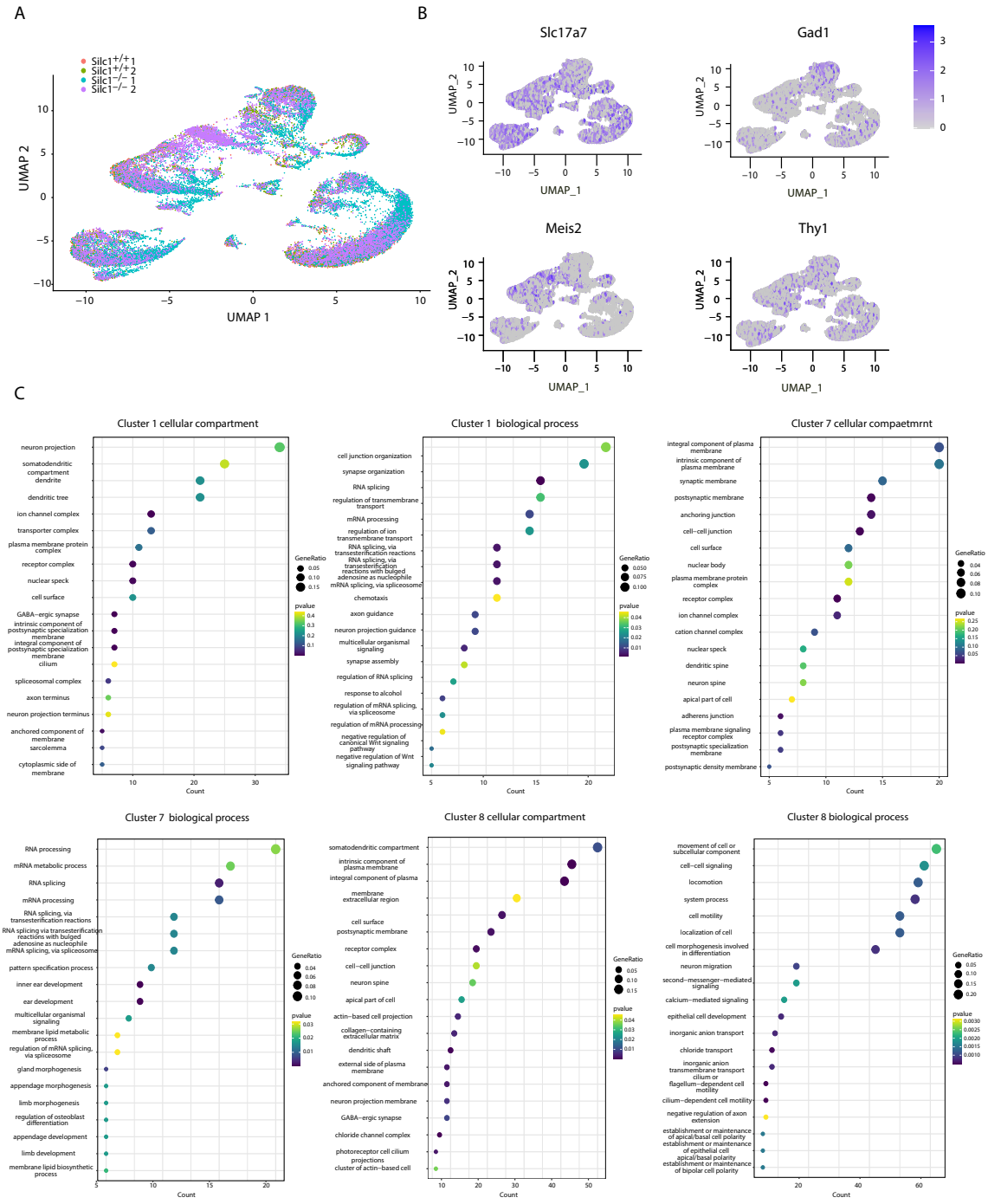

Figure S6. Characterization of genes regulated by *Sox11* and *Silc1* by RNA-seq. Related to Figure 6.

(A) UMAP visualization of the single-nucleus RNA-seq expression data, color-coding the sample from which each cell originated. **(B)** Expression levels of the indicated genes projected onto the UMAP visualization. **(C)** GO cellular compartment and biological process terms enriched in the genes significantly down-regulated in Silc1$^{-/-}$ cells compared to WT cells in the indicated clusters.

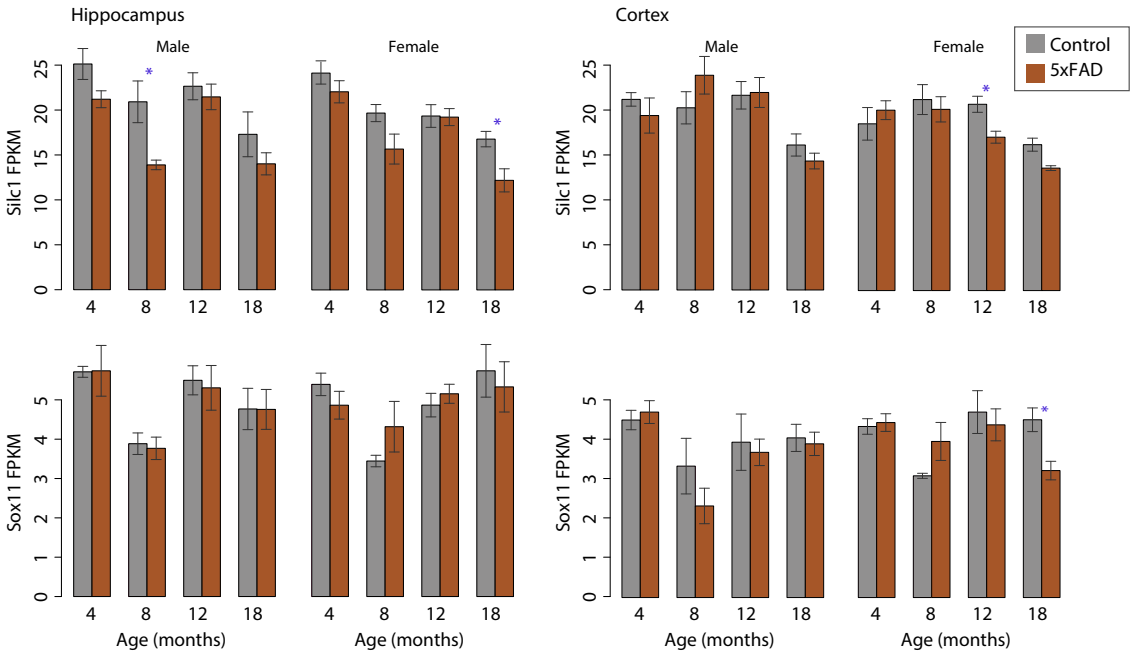

Figure S7. Changes in expression *Silc1* and *Sox11* during aging and in a model of Alzheimer's disease. Related to Discussion.

Expression of *Silc1* (top) and *Sox11* (bottom) in the indicated brain region extracted from control WT mice and from 5xFAD mice at the indicated age, data from[66]. Blue asterisks denote P<0.05 for the comparison between Control and 5xFAD mice, two-sided Wilcoxon rank-sum test.