

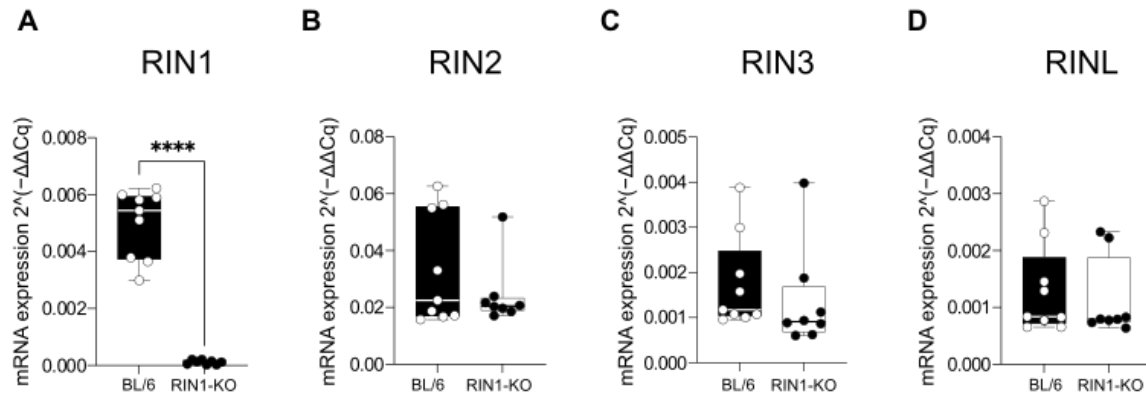


Figure S1. Expression of RIN family members in RIN1-KO mice. (A–D) Box plots show mRNA expression levels of RIN1 (A), RIN2 (B), RIN3 (C) and RINL (D), analysed by RT-qPCR from hippocampi of 4–5-week-old C57BL/6 and RIN1-KO mice. GAPDH expression was used as the reference gene for normalisation. The middle line indicates the median, each symbol represents one animal. BL/6: n = 9 (C57BL/6), RIN1-KO: n = 8. Mann–Whitney test.

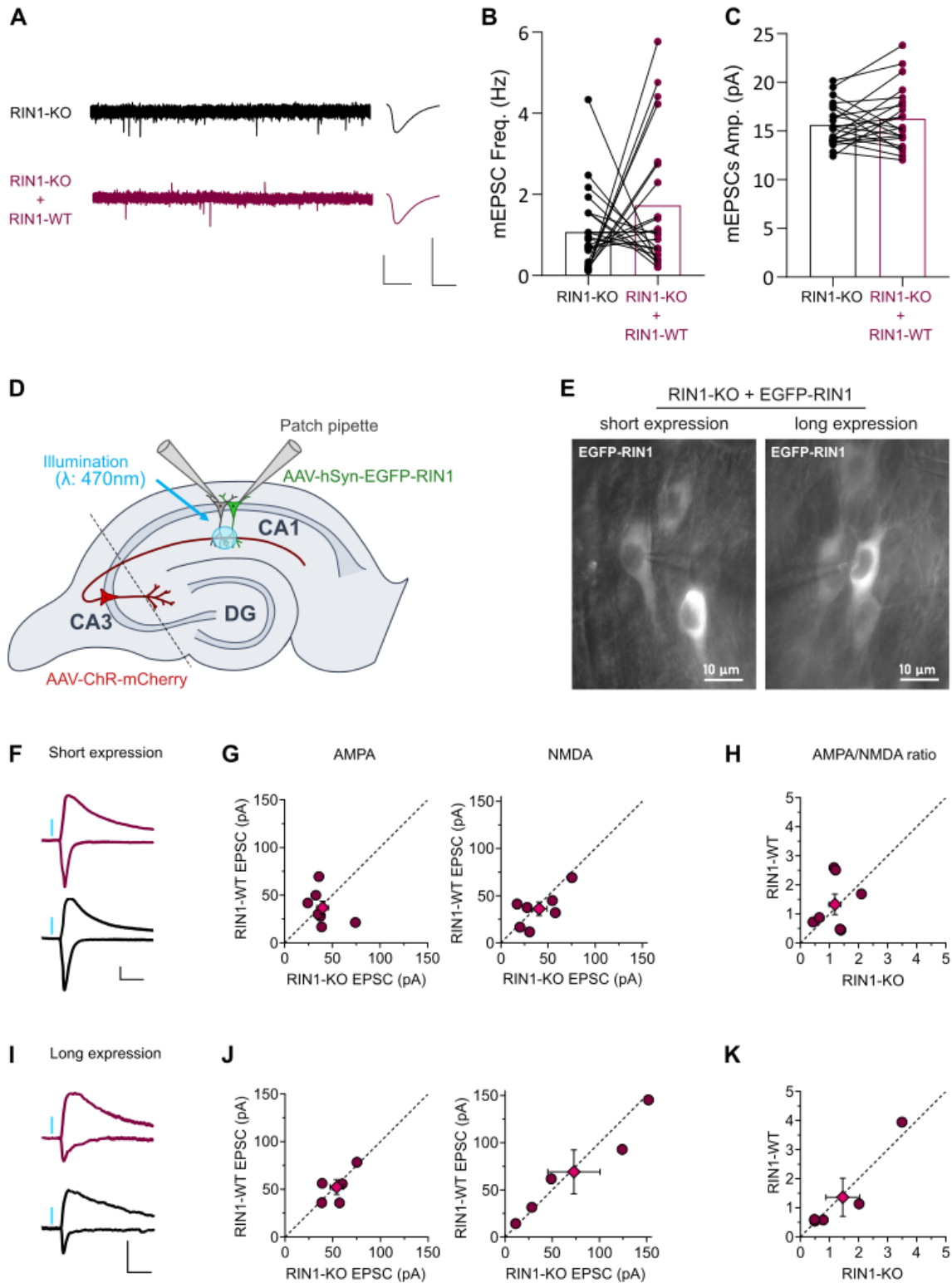


Figure S2. AAV-mediated RIN1 WT expression does not alter basal synaptic transmission or synaptic receptor composition in RIN1-KO CA1 neurons. (A-C) Properties of mEPSCs from CA1 neurons in organotypic hippocampal slices. (A) Recording example (left) and average mEPSC (right) for each genotype. Scale bars: 25 pA, 1 s for full trace, 20 pA and 10 ms for average. (B) mEPSC frequency and (C) amplitude for each neuron pair ($n=5$ animals, $N=22$ pairs). (D) Schematic of the paired whole-cell recording configuration in organotypic hippocampal slice

cultures. AAV-ChR-mCherry was injected into CA3 and EPSCs were evoked by optogenetic stimulation of Schaffer collaterals. Recordings were obtained simultaneously from a RIN1-WT-expressing CA1 neuron and a neighbouring uninfected RIN1-KO neuron. (E) Images of CA1 neurons in slice culture expressing GFP-tagged RIN1-WT after short (5 days) and long (10 days) expression periods. Scale bar: 10 μ m. (F–K) Paired recordings from RIN1-KO CA1 neurons re-expressing RIN1-WT and neighbouring uninfected control cells at DIV12–14, after 5 days (F–H) or 10 days (I–K) of expression. Example traces of EPSCs at -70 and $+40$ mV (F, I); scatter plots of light-evoked AMPA- and NMDA-EPSC amplitudes (G, J) and the corresponding AMPA/NMDA ratios (H, K). Scale bars: 50 pA, 50 ms. Short expression: $n = 4$ animals, $N = 7$ pairs of neurons; long expression $n = 4$ animals, $N = 5$ pairs of neurons. Paired t-test.

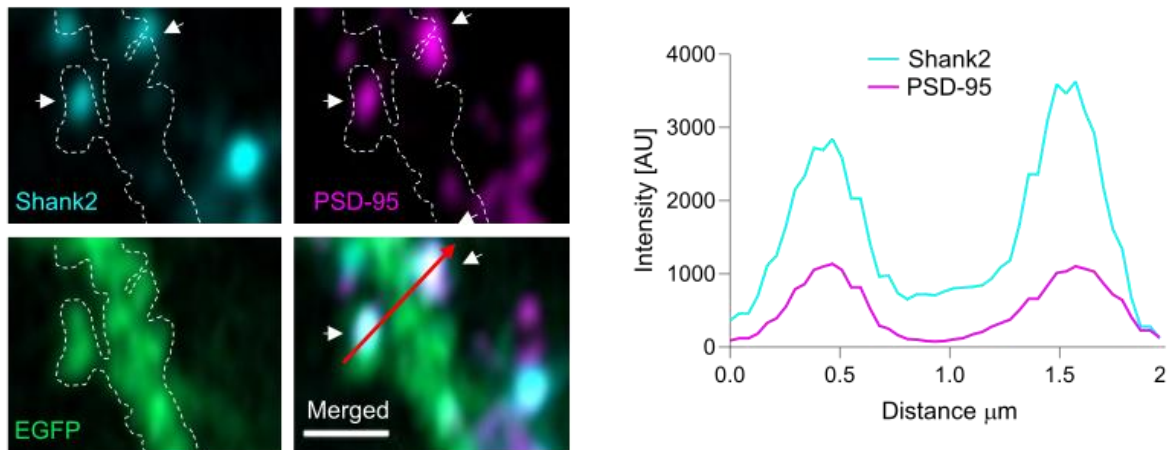
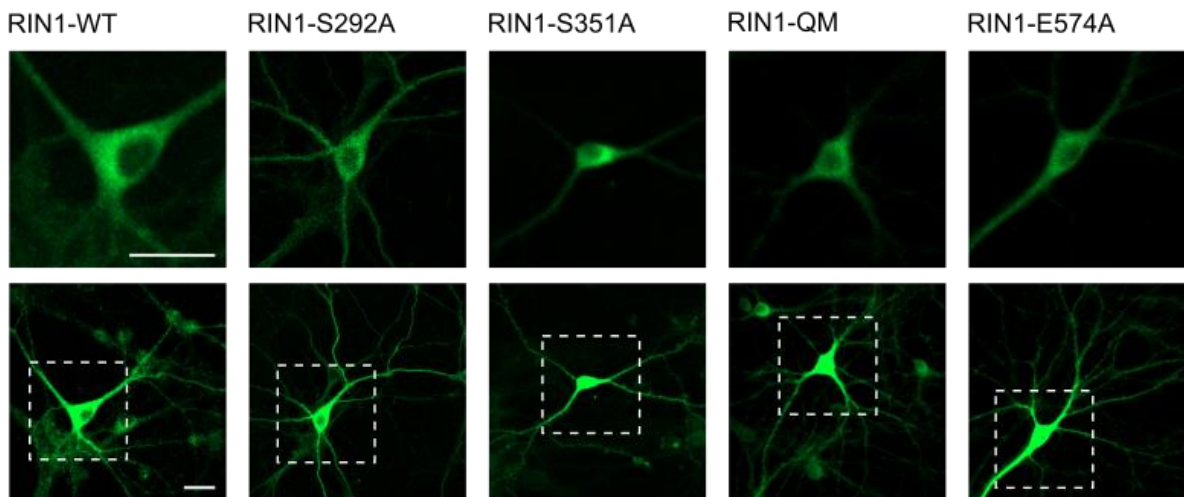
A**B**

Figure S3. RIN1 WT and mutants show cytoplasmic localisation in hippocampal neurons. (A) Representative confocal images of dendritic segments showing EGFP (green), Shank2 (cyan) and PSD-95 (magenta) immunostaining. The GFP signal outlines neuronal morphology. White arrows indicate Shank2 and PSD-95 puncta. The fluorescence intensity profile shows the spatial relationship between Shank2 and PSD-95 signals, along the region marked by the red arrow in the merged image. Scale bar: 1 μm . (B) Representative single Z-section confocal images of soma and dendritic arbour of RIN1-KO hippocampal neurons expressing the indicated EGFP-tagged RIN1 constructs. Z-sections were selected to visualise cytoplasmic distribution and the absence of nuclear signal. Scale bar: 20 μm .

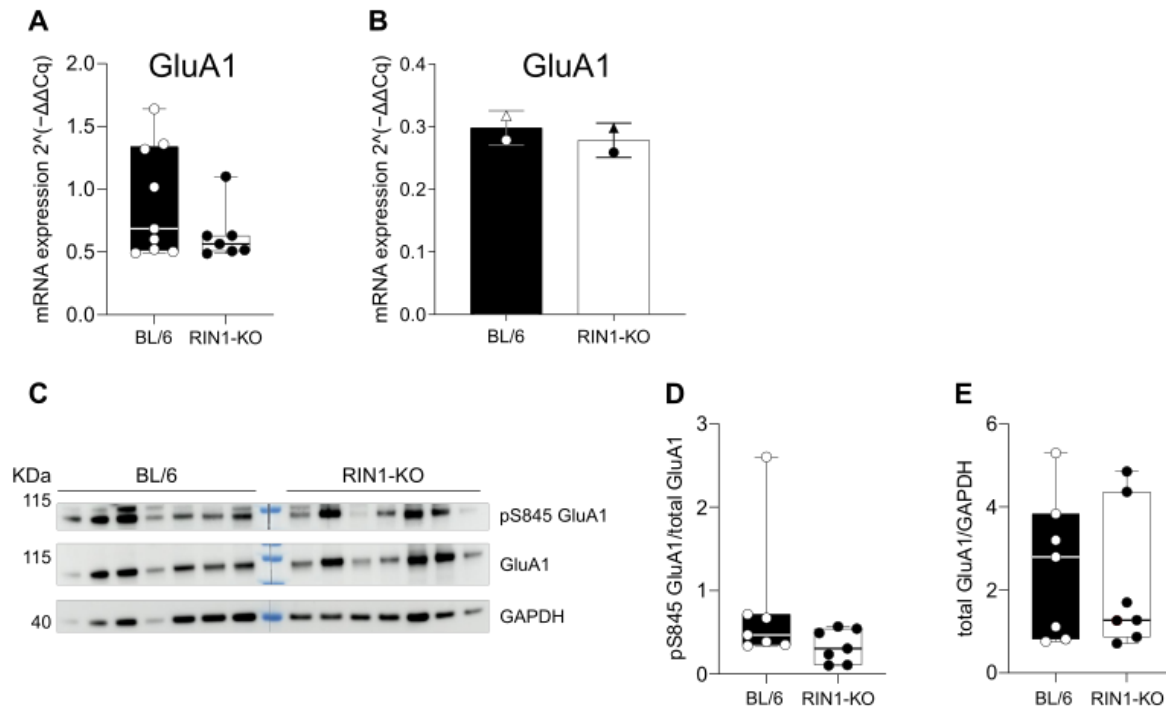


Figure S4. GluA1 expression and phosphorylation at S845A are unaffected in RIN1-KO mice.

(A-B) mRNA expression levels analysed by RT-qPCR for GluA1 in (A) from isolated hippocampi of 4–5-week-old C57BL/6 (n=9 animals) and RIN1-KO mice (n=8 animals) and (B) from dissociated hippocampal cultures of C57BL/6 and RIN1-KO mice at DIV15. n=2 independent cultures per genotype. GAPDH expression was used as the reference gene for normalization. Each symbol represents an independent animal or culture. (A) Paired t test, (B) Mann-Whitney test. (C) Western blot analysis probed with GluA1- and pS845-GluA1-specific antibodies. GAPDH was used as a loading control. Densitometric quantification of the total GluA1/GAPDH ratio (D) and pS845-GluA1/total GluA1 ratio (E). Each symbol represents one animal. n=7 animals per genotype. (C, D) Mann-Whitney test.

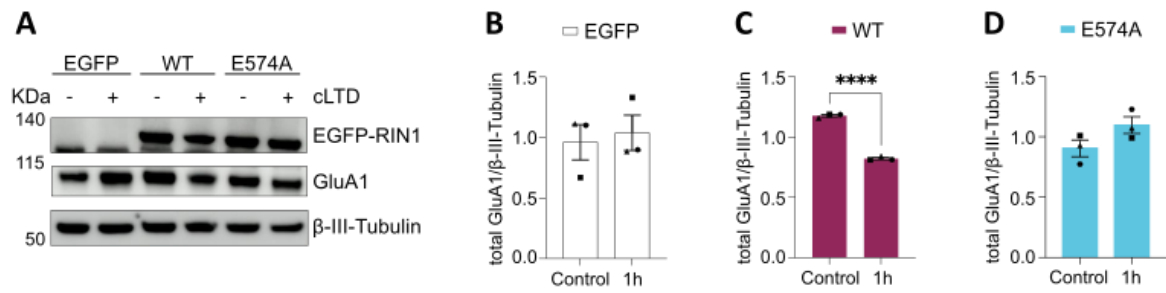


Figure S5. RIN1 GEF activity is required for GluA1 degradation during cLTD. (A) RIN1-KO dissociated hippocampal neurons transduced with EGFP, RIN1-WT, or RIN1-E574A were subjected to cLTD and harvested 1 h after induction. Lysates were probed for Western blot analysis with GFP-, and GluA1-specific antibodies. β III-tubulin was used as a loading control. (B-D) Bar graph shows densitometric analysis of the total GluA1/ β III-tubulin ratio of RIN1-KO neurons transduced with EGFP, RIN1-WT and RIN1-E574A. Welch's test, $n = 3$.

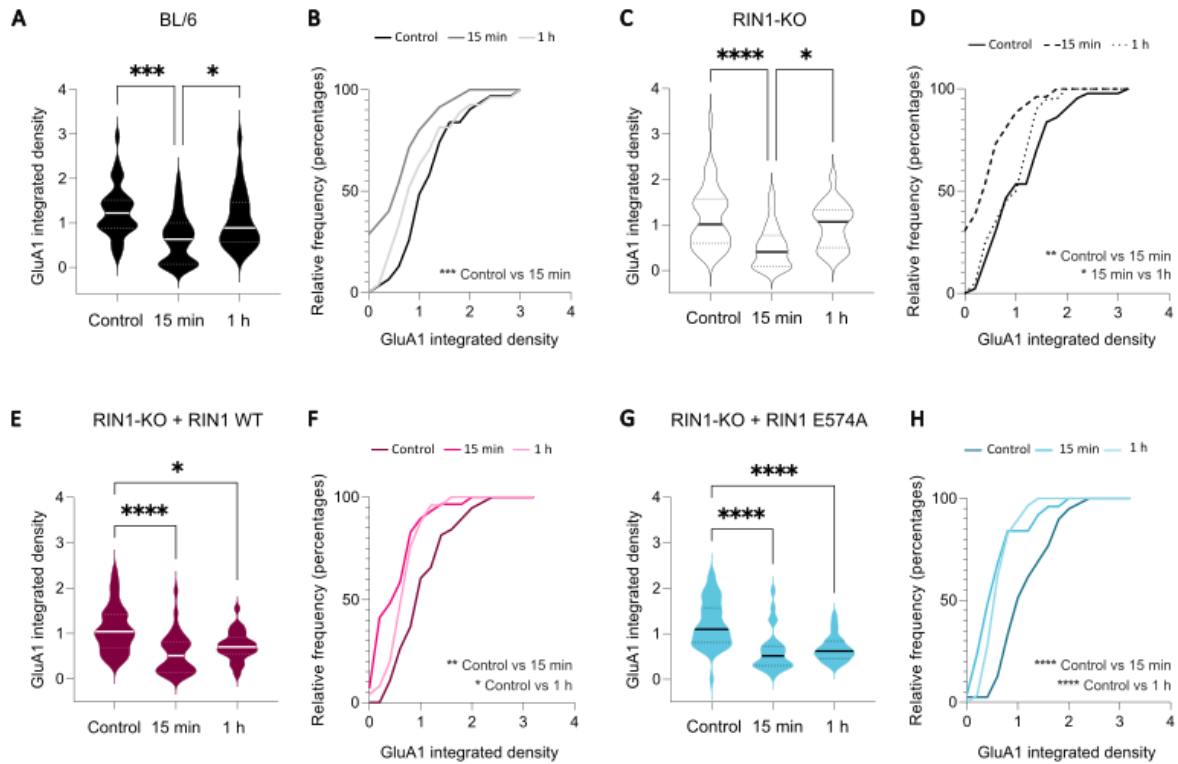


Figure S6. cLTD-induced extrasynaptic GluA1 recovery is impaired by RIN1-E574A but partially preserved by RIN1-WT re-expression. Dissociated hippocampal neurons from BL/6 (A, B), RIN1-KO (C, D), RIN1-KO expressing RIN1-WT (E, F), or RIN1-KO expressing RIN1-E574A (G, H) were subjected to cLTD induction, and surface GluA1 levels were assessed by an antibody specific for the N-terminal domain of GluA1. (A, C, E, G) Violin plots show changes in extrasynaptic GluA1 mean integrated density at baseline, 15 min, and 1 h after cLTD induction. $n = 3$. (A) $N = 31$ (Control), 35 (15 min) dendrites, 27 (1h); (C) $N = 43$ (Control), 26 (15 min), 20 (1h) dendrites; (E) $N = 38$ (Control), 29 (15 min), 25 (1h) dendrites; (G) $N = 39$ (Control), 25 (15 min), 29 (1h) dendrites. (A, C, E, G) Kruskal–Wallis test followed by Dunn’s post hoc test. (B, D, F, H) Cumulative frequency distributions of extrasynaptic GluA1 integrated density at baseline, 15 min, and 1 h after cLTD induction. $n = 3$, N as in the violin plots. Kolmogorov–Smirnov test. Asterisks over violin plots indicate per-observation comparisons while asterisks on cumulative distributions indicate comparisons between timepoints.