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Supplemental Information

Limiting RyR2 Open Time Prevents Alzheimer's Disease-Related Neuronal Hyperactivity and Memory Loss but Not β-Amyloid Accumulation

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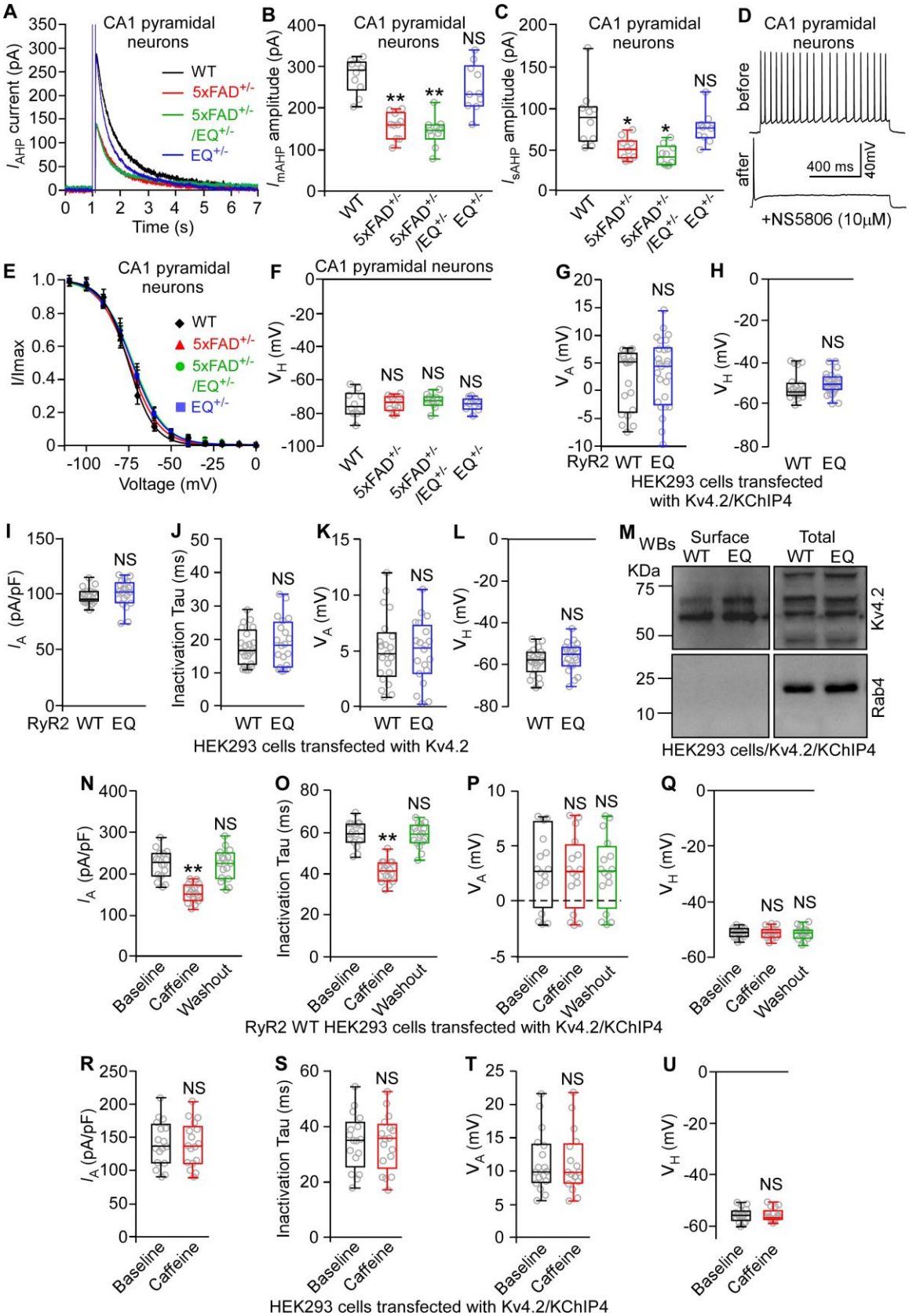
SUPPLEMENTARY FIGURES

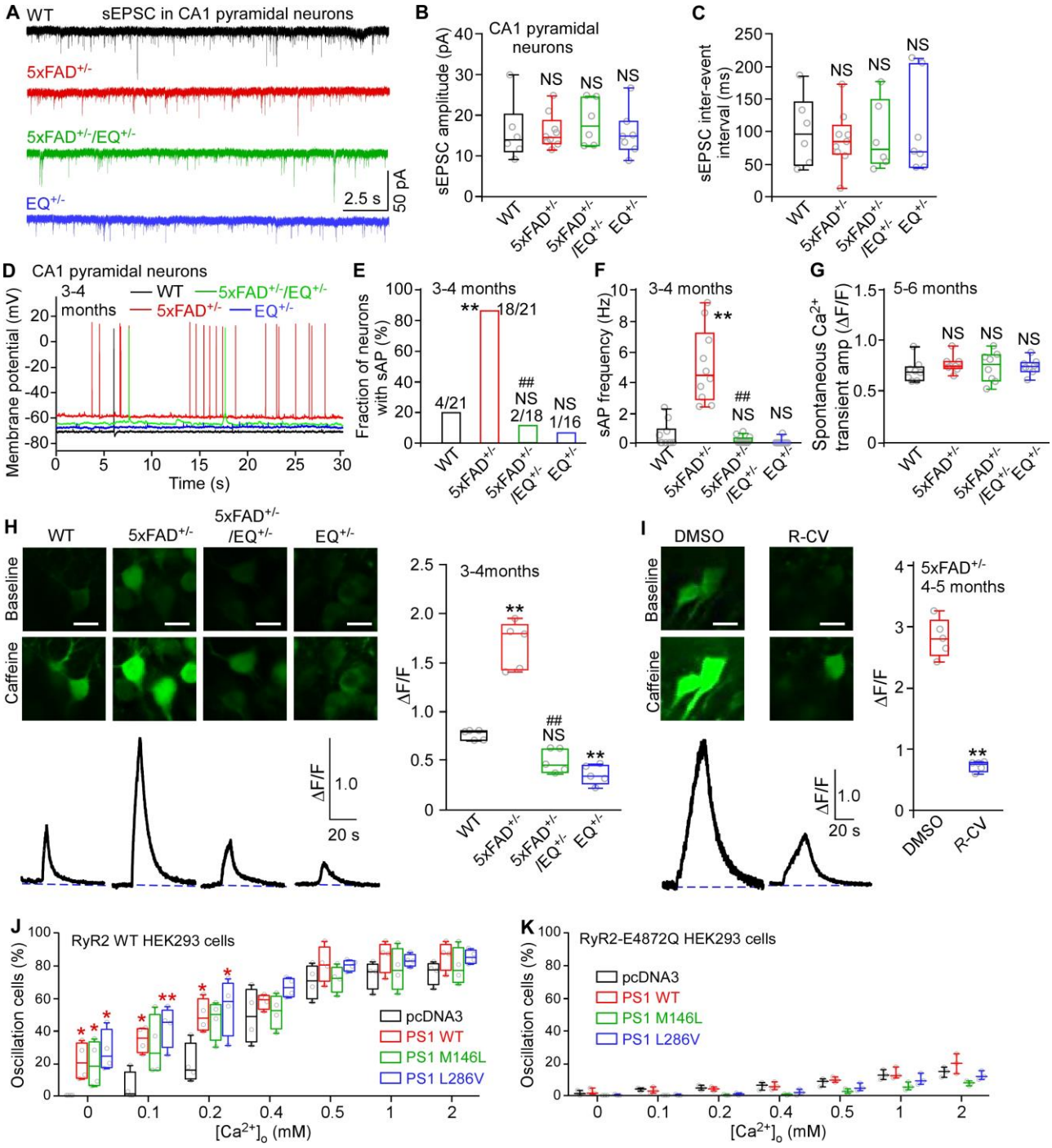
Supplementary Fig. 1



Supplementary Fig. 1. Effects of RyR2-E4872Q$^{+/-}$ on the afterhyperpolarization current (I_{AHP}), A-type K$^+$ current (I_A), and surface expression of Kv4.2, and effect of pharmacological manipulation of RyR2 on I_A. Related to Fig.1.

(**A**) Whole-cell voltage-clamp recording of afterhyperpolarization current (I_{AHP}) from 3-4 months old WT, 5xFAD$^{+/-}$, 5xFAD$^{+/-}$/EQ$^{+/-}$ and EQ$^{+/-}$ CA1 pyramidal neurons. Mean amplitude of I_{mAHP} (**B**) and I_{sAHP} (**C**) recorded from WT (10 mice, 19 neurons), 5xFAD$^{+/-}$ (10 mice, 22 neurons), 5xFAD$^{+/-}$/EQ$^{+/-}$ (10 mice, 22 neurons) and EQ$^{+/-}$ (10 mice, 19 neurons) CA1 neurons. (**D**) Action potential (AP) firing in 3-4 months old 5xFAD$^{+/-}$ mouse CA1 neurons before and after 10 μM NS5806. (**E**) Steady-state inactivation of whole cell A-type K$^+$ currents (I_A) in 3-4 months old WT, 5xFAD$^{+/-}$, 5xFAD$^{+/-}$/EQ$^{+/-}$, and EQ$^{+/-}$ mouse CA1 neurons. (**F**) V_H recorded from WT (10 mice, 30 neurons), 5xFAD$^{+/-}$ (10 mice, 30 neurons), 5xFAD$^{+/-}$/EQ$^{+/-}$ (10 mice, 30 neurons) and EQ$^{+/-}$ (10 mice, 30 neurons) CA1 neurons. Midpoints of I_A voltage dependent activation (V_A) (**G**) and midpoints of I_A voltage dependent inactivation (V_H) (**H**) in Kv4.2/KChIP4 transfected HEK293 cells expressing RyR2 WT (n = 21), RyR2 E4872Q$^{+/-}$ mutation (n = 26). Whole cell I_A (**I**), inactivation time constant Tau (**J**), V_A (**K**) and V_H (**L**) in Kv4.2 (alone) transfected HEK293 cells expressing RyR2 WT (n = 21) or RyR2 E4872Q$^{+/-}$ mutation (n = 20). (**M**) Immunoblotting of whole cell lysate from Kv4.2/KChIP4 transfected HEK293 cells expressing RyR2 WT or RyR2 E4872Q mutation. Whole cell I_A (**N**), inactivation time constant Tau (**O**), V_A (**P**) and V_H (**Q**) in Kv4.2/KCHIP4 transfected HEK293 cells expressing RyR2 WT (n = 16) at baseline, 2 min after application of caffeine (2.5 mM) and 5 min after washout. Whole cell I_A (**R**), inactivation time constant Tau (**S**), V_A (**T**) and V_H (**U**) recorded in Kv4.2/KCHIP4 transfected HEK293 cells (n = 17) at baseline and 2 min after application of caffeine (2.5 mM). Data shown are the median and range (Kruskal-Wallis test with Dunn-Bonferroni post hoc test, Mann-Whitney *U* test, paired t test, Wilcoxon matched-pairs signed rank test, repeated measure ANOVA test with Bonferroni post hoc test and Friedman test with Dunn-Bonferroni post hoc test; *P < 0.05, **P < 0.01 vs WT or baseline, NS, not significant).

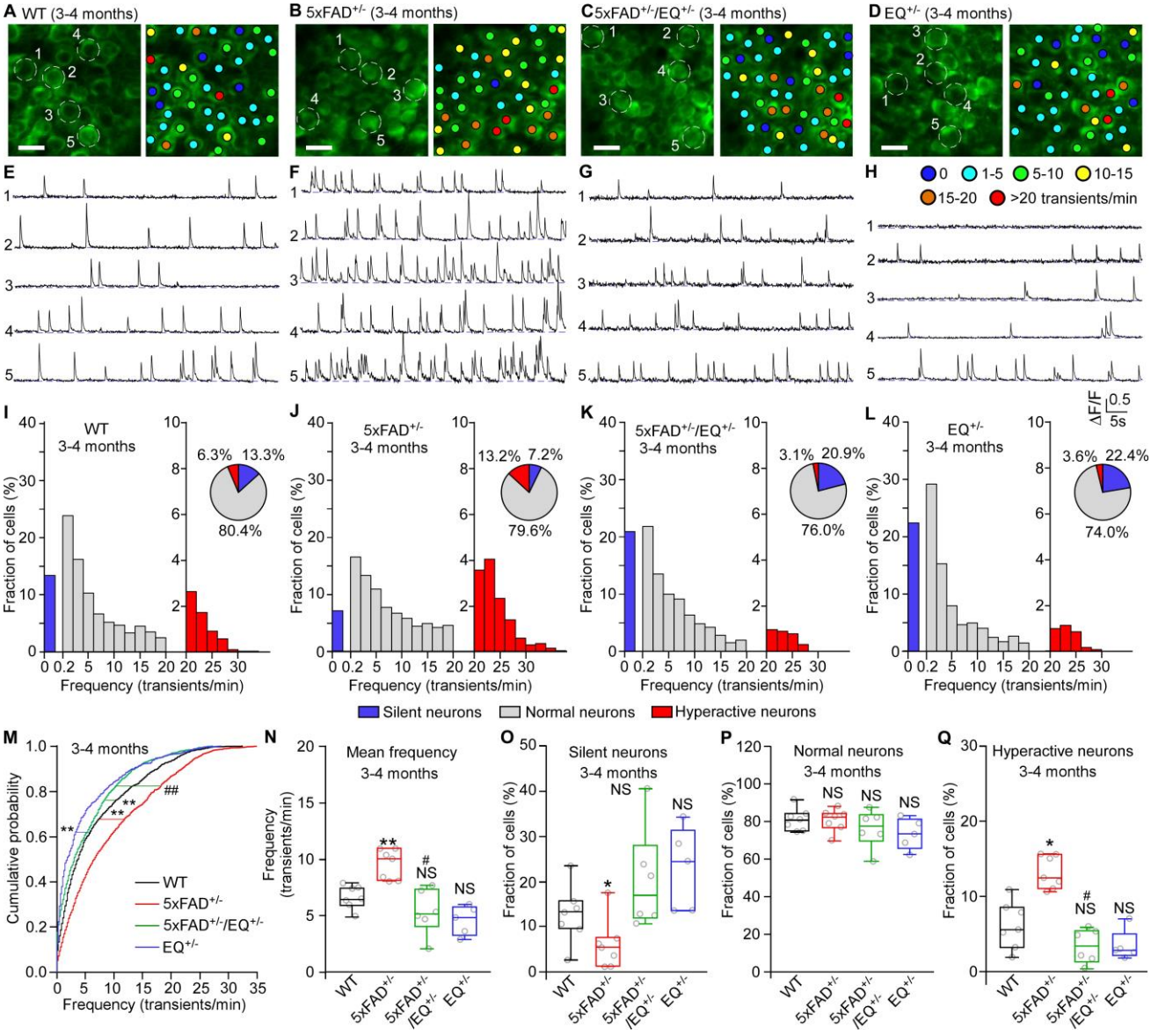
Supplementary Fig. 2



Supplementary Fig. 2. Effects of RyR2-E4872Q$^{+/-}$ on presynaptic activity and spontaneous AP firing and effects of RyR2-E4872Q$^{+/-}$ and *R*-carvedilol on RyR2 function. Related to Fig. 1 and Fig. 2.

(**A**) Whole-cell voltage-clamp recording of spontaneous excitatory post-synaptic currents (sEPSC) in 3-4 months old WT, 5xFAD$^{+/-}$, 5xFAD$^{+/-}$/EQ$^{+/-}$ and EQ$^{+/-}$ mouse CA1 neurons. Amplitude (**B**) and inter-event interval (**C**) of sEPSCs in 3-4 months old WT (6 mice, 11 neurons), 5xFAD$^{+/-}$ (9 mice, 23 neurons), 5xFAD$^{+/-}$/EQ$^{+/-}$ (6 mice, 15 neurons) and EQ$^{+/-}$ (7 mice, 18 neurons) CA1 neurons. (**D**) Spontaneous action potential (sAP) firing in 3-4 months old WT, 5xFAD$^{+/-}$, 5xFAD$^{+/-}$/EQ$^{+/-}$ and EQ$^{+/-}$ mouse CA1 pyramidal neurons. (**E**) Fraction of CA1 pyramidal neurons showing sAP firing. (**F**) sAP firing frequency in WT (6 mice, 21 neurons), 5xFAD$^{+/-}$ (9 mice, 21 neurons), 5xFAD$^{+/-}$/EQ$^{+/-}$ (6 mice, 18 neurons), and EQ$^{+/-}$ (7 mice, 16 neurons) CA1 neurons. (**G**) Amplitude of spontaneous Ca^{2+} transients (*in vivo* Ca^{2+} imaging) in CA1 neurons from 5-6 months old WT (8 mice, 2375 cells), 5xFAD$^{+/-}$ (8 mice, 1708 cells), 5xFAD$^{+/-}$/EQ$^{+/-}$ (8 mice, 2283 cells), and EQ$^{+/-}$ (8 mice, 2392 cells). (**H**) Caffeine-induced Ca^{2+} release in 3-4 months old GCAMP6f-expressing WT (5 mice, 58 neurons), 5xFAD$^{+/-}$ (5 mice, 67 neurons), 5xFAD$^{+/-}$/EQ$^{+/-}$ (5 mice, 68 neurons), and EQ$^{+/-}$ (5 mice, 90 neurons) CA1 neurons, showing GCaMP6f images of CA1 pyramidal neurons of different genotypes before (baseline) and after application of caffeine (40 mM) (top left), fluorescence traces (bottom left), and average data (right). (**I**) Caffeine-induced Ca^{2+} release in 4-5 months old GCAMP6f-expressing 5xFAD$^{+/-}$ mice treated with DMSO (5 mice, 112 neurons) and *R*-CV (5 mice, 113 neurons) for 1 month, showing GCaMP6f images of CA1 pyramidal neurons treated with DMSO or *R*-CV before (baseline) and after application of caffeine (40 mM) (top left), fluorescence traces (bottom left), and average data (right). Fraction of cells displaying spontaneous Ca^{2+} oscillation at different external Ca^{2+} concentrations in HEK293 cells expressing RyR2 WT (**J**) or RyR2-E4872Q mutant (**K**) transfected with control plasmid (pcDNA3), presenilin 1 (PS1)-WT, PS1-M146L or PS1-L286V. Scale bars: 5 μm. Data shown are the median and range (Kruskal-Wallis test with Dunn-Bonferroni post hoc test and Mann-Whitney *U* test; *P < 0.05, **P < 0.01 vs WT or control, $^{\#\#}$P < 0.01, 5xFAD$^{+/-}$/EQ$^{+/-}$ vs 5xFAD$^{+/-}$, NS, not significant).

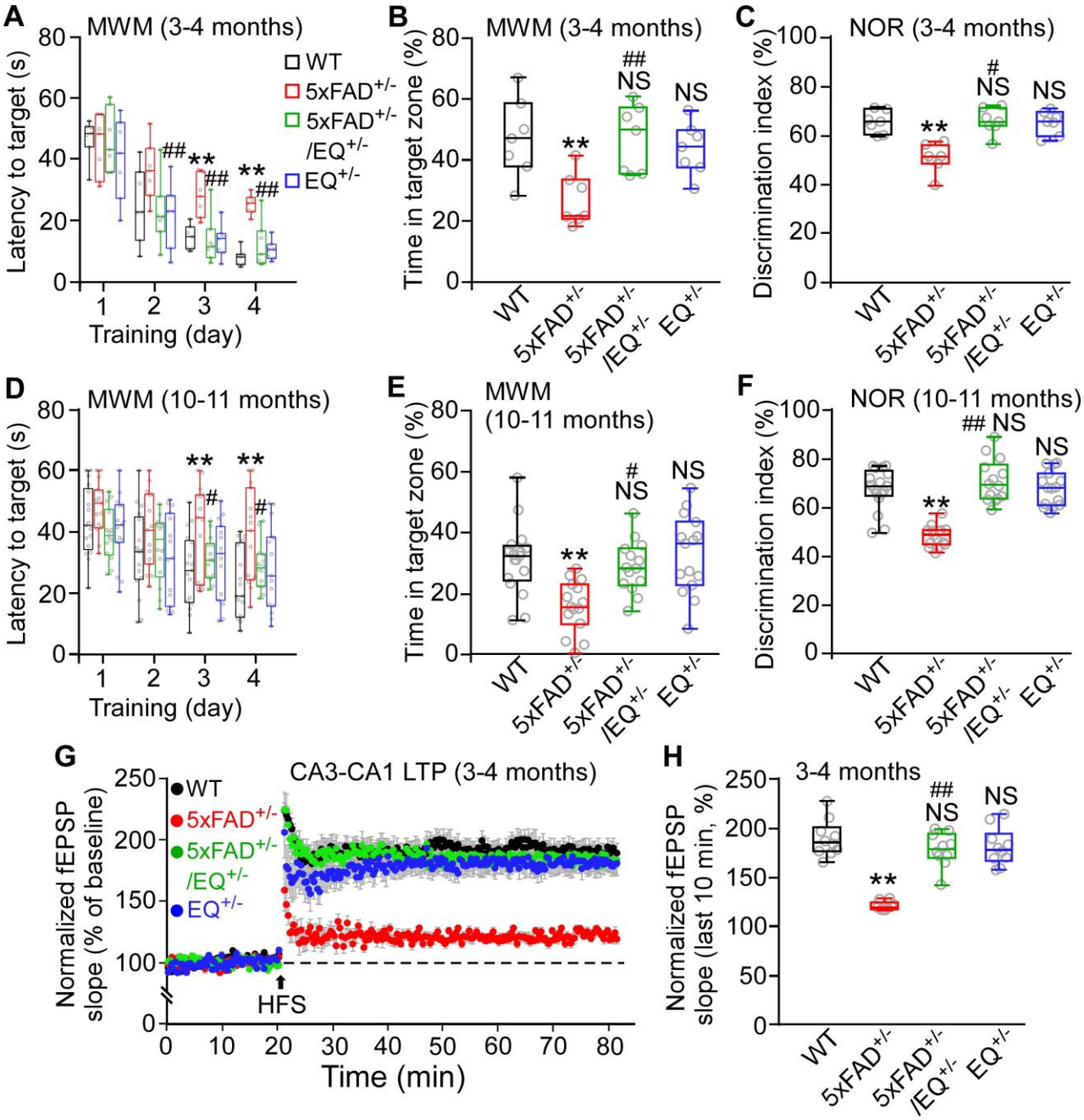
Supplementary Fig. 3



Supplementary Fig. 3. The RyR2 E4872Q$^{+/-}$ mutation prevents neuronal hyperactivity of 5xFAD$^{+/-}$ hippocampal CA1 neurons *in vivo*. Related to Fig.2.

Two-photon *in vivo* Ca^{2+} images of hippocampal CA1 region of 3-4 months old WT (**A**), 5xFAD$^{+/-}$ (**B**), 5xFAD$^{+/-}$/EQ$^{+/-}$ (**C**) and EQ$^{+/-}$ (**D**) mice. The colored dots indicate the number of Ca^{2+} transients per minute. (**E**-**H**) Ca^{2+} traces of the five neurons circled in **A**-**D,** respectively. Histograms showing the frequency distribution of Ca^{2+} transients in WT (**I**, 7 mice, 1756 cells), 5xFAD$^{+/-}$ (**J**, 7 mice, 1466 cells), 5xFAD$^{+/-}$/EQ$^{+/-}$ (**K**, 6 mice, 1089 cells) and EQ$^{+/-}$ (**L**, 5 mice, 715 cells). Pie charts show the relative proportions of silent, normal, and hyperactive neurons. (**M**) Cumulative probability functions showing frequency distributions of spontaneous Ca^{2+} transients in CA1 region of WT (black), 5xFAD$^{+/-}$ (red), 5xFAD$^{+/-}$/EQ$^{+/-}$ (green) and EQ$^{+/-}$ (blue) mice (Kruskal-Wallis test with Dunn's multiple comparisons test; **P < 0.0001 vs WT. $^{\#\#}$P < 0.0001 5xFAD$^{+/-}$/EQ$^{+/-}$ vs 5xFAD$^{+/-}$, NS, not significant). (**N**) Mean Ca^{2+} transient frequency in WT, 5xFAD$^{+/-}$, 5xFAD$^{+/-}$/EQ$^{+/-}$ and EQ$^{+/-}$ mice CA1 region as defined previously (Busche, 2018; Busche et al., 2012; Busche et al., 2008). Percentage of silent (**O**), normal (**P**) and hyperactive (**Q**) cells in WT, 5xFAD$^{+/-}$, 5xFAD$^{+/-}$/EQ$^{+/-}$ and EQ$^{+/-}$ mice CA1 region. Note that analyses of frequency distributions (**I** to **M**) were performed using cells pooled from all animals, while analyses of mean frequency and fraction of silent, normal, and hyperactive cells (**N** to **Q**) were based on data from individual animals. Scale bars: 10 μm. Data shown are the median and range (Kruskal-Wallis test with Dunn-Bonferroni post hoc test; *P < 0.05, **P < 0.01 vs WT, $^{\#}$P < 0.05 5xFAD$^{+/-}$/EQ$^{+/-}$ vs 5xFAD$^{+/-}$, NS, not significant).

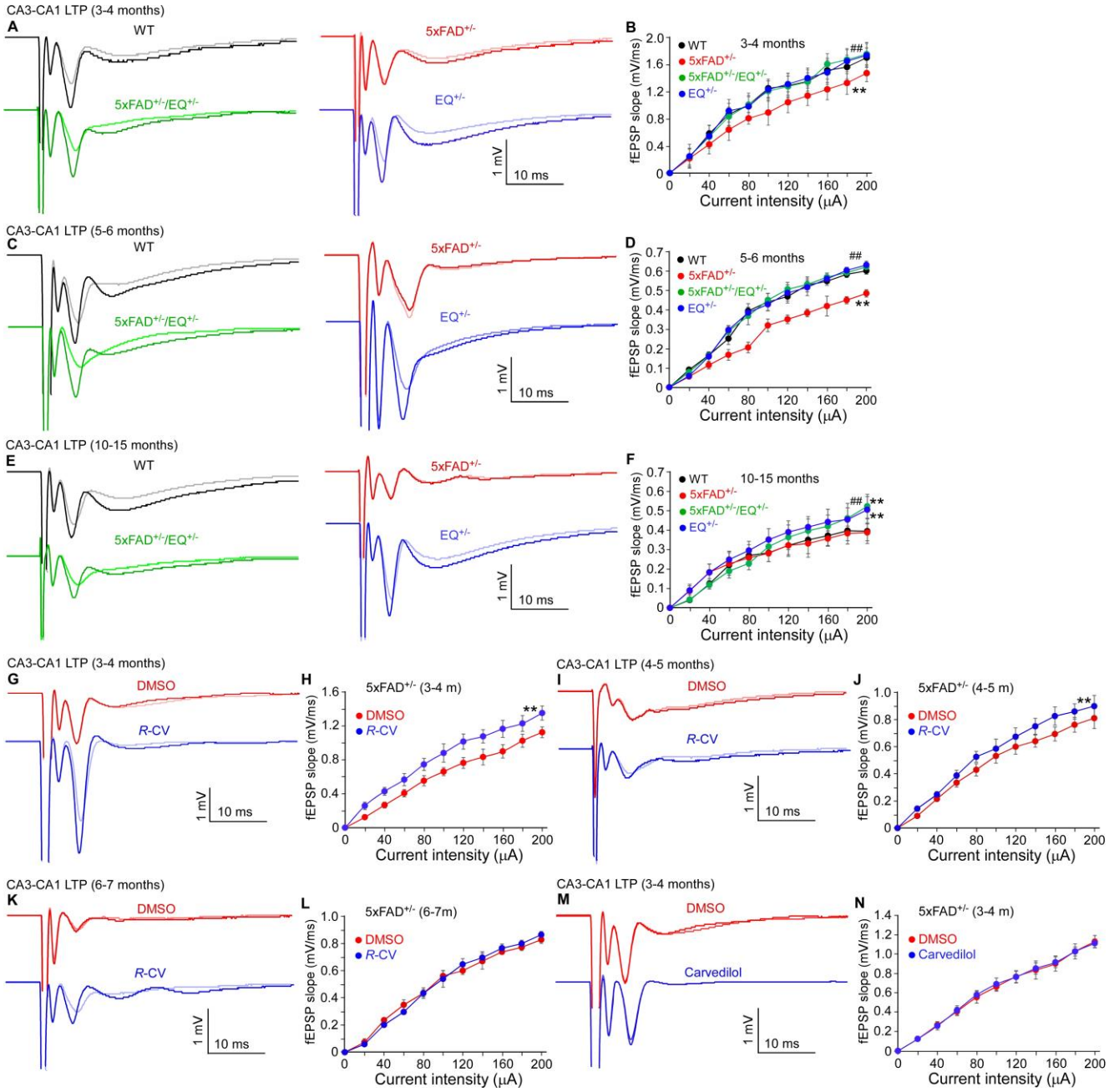
Supplementary Fig. 4



Supplementary Fig. 4. The E4872Q$^{+/-}$ mutation prevents memory loss and LTP impairment in both young and aged 5xFAD$^{+/-}$ mice. Related to Fig. 3.

(**A**) The latency to reach the target platform of 3-4 months old WT (n = 7), 5xFAD$^{+/-}$ (n = 7), 5xFAD$^{+/-}$/EQ$^{+/-}$ (n = 7) and EQ$^{+/-}$ (n = 7) mice in the Morris Water Maze (MWM) test. (**B**) The time spent in the target quadrant. (**C**) The percentage of time spent on the novel object during the Novel Object Recognition (NOR) test in 3-4 months old WT (n = 7), 5xFAD$^{+/-}$ (n = 7), 5xFAD$^{+/-}$/EQ$^{+/-}$ (n = 7) and EQ$^{+/-}$ (n = 7) mice. (**D**) The latency to reach the target platform of 10-11 months old WT (n = 17), 5xFAD$^{+/-}$ (n = 15), 5xFAD$^{+/-}$/EQ$^{+/-}$ (n = 14) and EQ$^{+/-}$ (n = 15) mice in the Morris Water Maze (MWM) test. (**E**) The time spent in the target quadrant. (**F**) The percentage of time spent on the novel object during the Novel Object Recognition (NOR) test in 10-11 months old WT (n = 17), 5xFAD$^{+/-}$ (n = 15), 5xFAD$^{+/-}$/EQ$^{+/-}$ (n = 14) and EQ$^{+/-}$ (n = 15) mice. (**G**) Effect of 100 Hz high frequency stimulation (HFS) on mean CA3-CA1 fEPSP slope in hippocampal slices from 3-4 months old WT (10 mice, 20 slices), 5xFAD$^{+/-}$ (10 mice, 20 slices), 5xFAD$^{+/-}$/EQ$^{+/-}$ (10 mice, 20 slices) and EQ$^{+/-}$ (10 mice, 20 slices). Data were normalized to the mean fEPSP slope in slices from 20 min baseline recording. (**H**) The averaged normalized fEPSP slope recorded between 50-60 min after HFS. Data shown are the median and range (Kruskal-Wallis test with Dunn-Bonferroni post hoc test; **P < 0.01 vs WT, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01 5xFAD$^{+/-}$/EQ$^{+/-}$ vs 5xFAD$^{+/-}$, NS, not significant).

Supplementary Fig. 5

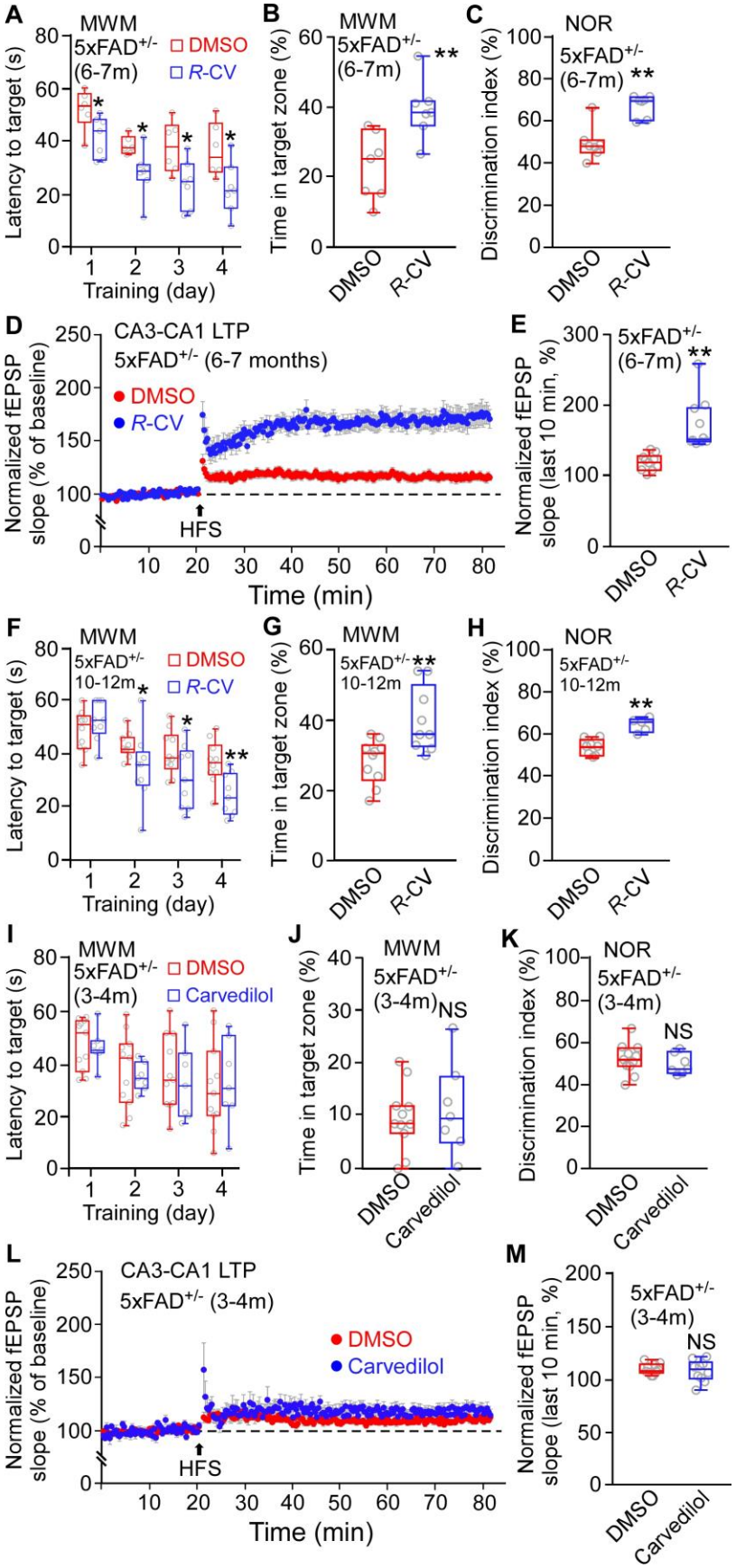


Supplementary Fig. 5. Effect of the RyR2 E4872Q$^{+/-}$ mutation, carvedilol, and *R*-carvedilol on the input-output relationship in the mouse hippocampal CA3-CA1 pathway. Related to Fig. 3 and Fig. 5.

(**A**) fEPSP traces recorded from 3-4 months old WT, 5xFAD$^{+/-}$, 5xFAD$^{+/-}$/EQ$^{+/-}$ and EQ$^{+/-}$ mouse hippocampal slices before (lighter color) and after (darker color) LTP induction. (**B**) Input-output relationships between fEPSP slope and stimulus intensity measured from CA3-CA1 pathway in 3-4 months old WT (10 mice, 20 slices), 5xFAD$^{+/-}$ (10 mice, 20 slices), 5xFAD$^{+/-}$/EQ$^{+/-}$ (10 mice, 20 slices), and EQ$^{+/-}$ (10 mice, 20 slices) mouse hippocampal slices. (**C**) fEPSP traces recorded from 5-6 months old WT, 5xFAD$^{+/-}$, 5xFAD$^{+/-}$/EQ$^{+/-}$ and EQ$^{+/-}$ mouse hippocampal slices before (lighter color) and after (darker color) LTP induction. (**D**) Input-output relationships between fEPSP slope and stimulus intensity measured from CA3-CA1 pathway in 5-6 months old WT (10 mice, 20 slices), 5xFAD$^{+/-}$ (10 mice, 20 slices), 5xFAD$^{+/-}$/EQ$^{+/-}$ (10 mice, 20 slices), and EQ$^{+/-}$ (10 mice, 20 slices) mouse hippocampal slices. (**E**) fEPSP traces recorded from 10-15 months old WT, 5xFAD$^{+/-}$, 5xFAD$^{+/-}$/EQ$^{+/-}$, and EQ$^{+/-}$ mouse hippocampal slices before (lighter color) and after (darker color) LTP induction. (**F**) Input-output relationships between fEPSP slope and stimulus intensity measured from CA3-CA1 pathway in 10-15 months old WT (10 mice, 20 slices), 5xFAD$^{+/-}$ (10 mice, 20 slices), 5xFAD$^{+/-}$/EQ$^{+/-}$ (10 mice, 20 slices), and EQ$^{+/-}$ (10 mice, 20 slices) mouse hippocampal slices. Data shown are the mean ± SD. (Two-way ANOVA with Bonferroni post hoc test; **P < 0.01 compared to WT, $^{\#\#}$P < 0.01 5xFAD$^{+/-}$/EQ$^{+/-}$ vs 5xFAD$^{+/-}$). (**G**) fEPSP traces recorded from hippocampal slices of 3-4 months old 5xFAD$^{+/-}$ mice treated with DMSO or *R*-carvedilol (*R*-CV) before (lighter color) and after (darker color) LTP induction. (**H**) Input-output relationships between fEPSP slope and stimulus intensity measured from hippocampal slices of 3-4 months old 5xFAD$^{+/-}$ mice treated with DMSO (10 mice, 20 slices) or *R*-CV (10 mice, 20 slices). (**I**) fEPSP traces recorded from hippocampal slices of 4-5 months old 5xFAD$^{+/-}$ mice treated with DMSO or *R*-CV before (lighter color) and after (darker color) LTP induction. (**J**) Input-output relationships between fEPSP slope and stimulus intensity measured from hippocampal slices of 4-5 months old 5xFAD$^{+/-}$ mice treated with DMSO (10 mice, 20 slices) or *R*-CV (10 mice, 20 slices). (**K**) fEPSP traces recorded from hippocampal slices of 6-7 months old 5xFAD$^{+/-}$ mice treated with DMSO or *R*-CV before (lighter color) and after (darker color) LTP induction. (**L**) Input-output relationships between fEPSP slope and stimulus intensity measured from hippocampal slices of 6-7 months old 5xFAD$^{+/-}$ mice treated with DMSO (10 mice, 20 slices) or *R*-CV (10 mice, 20 slices). (**M**) fEPSP traces recorded from hippocampal slices of 3-4 months old 5xFAD$^{+/-}$ mice treated with DMSO or carvedilol before (lighter color) and after (darker color) LTP

induction. (**N**) Input-output relationships between fEPSP slope and stimulus intensity measured from hippocampal slices of 3-4 months old 5xFAD$^{+/-}$ mice treated with DMSO (10 mice, 20 slices) or carvedilol (10 mice, 20 slices). Data shown are the mean ± SD (Two-way ANOVA with Bonferroni post hoc test; **P < 0.01 compared to the DMSO group).

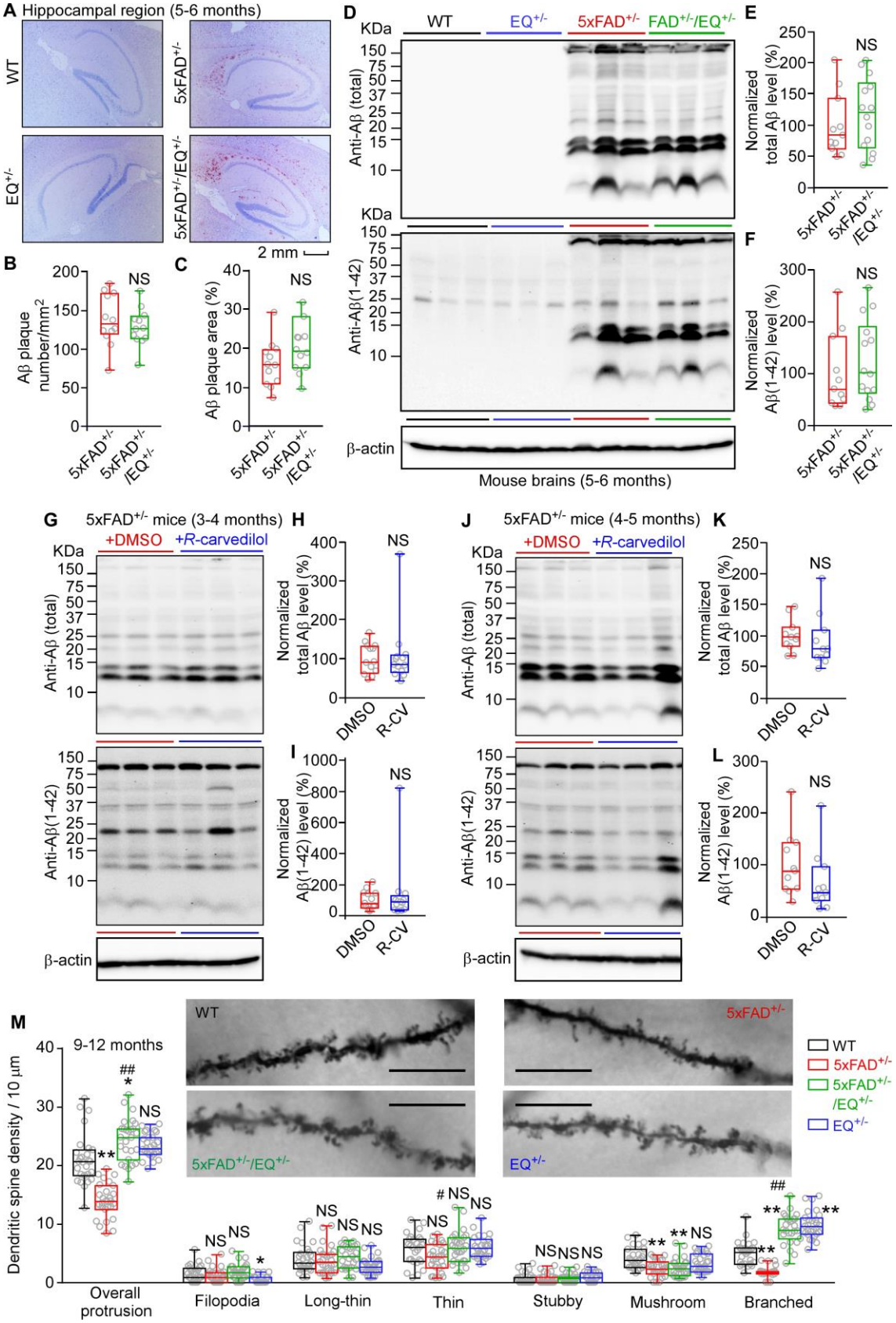
Supplementary Fig. 6



Supplementary Fig. 6. *R*-carvedilol but not carvedilol racemic mixture rescues memory loss and LTP deficit in 5xFAD$^{+/-}$ mice. Related to Fig. 5.

5xFAD$^{+/-}$ mice were treated with DMSO or *R*-carvedilol (*R*-CV, 3.2 mg/kg/day) for 1 month, starting at 5-6 months or 9-11 months of age after the onset of neuronal hyperactivity and memory loss. (**A**) The latency to reach the target platform of 6-7 months old 5xFAD$^{+/-}$ mice treated with DMSO (n=6) or *R*-CV (n=7) in MWM test. (**B**) The time spent in the target quadrant. (**C**) The percentage of time spent on the novel object during the NOR test in 6-7 months old 5xFAD$^{+/-}$ mice treated with DMSO (n=6) or *R*-CV (n=7). (**D**) Effect of 100 Hz high frequency stimulation (HFS) on mean CA3-CA1 fEPSP slope in brain slices from 6-7 months old 5xFAD$^{+/-}$ mice treated with DMSO (10 mice, 20 slices) or *R*-CV (10 mice, 20 slices). (**E**) The averaged normalized fEPSP slop recorded between 50-60 min after HFS from 6-7 months old 5xFAD$^{+/-}$ mice treated with DMSO or *R*-CV. (**F**) The latency to reach the target platform of 10-12 months old 5xFAD$^{+/-}$ mice treated with DMSO (n=13) or *R*-CV (n=12) in MWM test. (**G**) The time spent in the target quadrant. (**H**) The percentage of time spent on the novel object during the NOR test in 10-12 months old 5xFAD$^{+/-}$ mice treated with DMSO (n=8) or *R*-CV (n=5). 5xFAD$^{+/-}$ mice were treated with DMSO or carvedilol (3.2 mg/kg/day) for 1 month, starting at 2-3 months of age before the onset of neuronal hyperactivity and memory loss. (**I**) The latency to reach the target platform of 3-4 months old 5xFAD$^{+/-}$ mice treated with DMSO (n=11) or carvedilol (n=7) in MWM test. (**J**) The time spent in the target quadrant. (**K**) The percentage of time spent on the novel object during the NOR test in 3-4 months old 5xFAD$^{+/-}$ mice treated with DMSO (n=11) or carvedilol (n=7). (**L**) Effect of 100 Hz high frequency stimulation (HFS) on mean CA3-CA1 fEPSP slope in brain slices from 3-4 months old 5xFAD$^{+/-}$ mice treated with DMSO (10 mice, 20 slices) or carvedilol (10 mice, 20 slices). (**M**) The averaged normalized fEPSP slop recorded between 50-60 min after HFS from 3-4 months old 5xFAD$^{+/-}$ mice treated with DMSO or carvedilol. Data shown are the median and range (Mann-Whitney *U* test; *P < 0.05, **P < 0.01 vs DMSO group, NS, not significant).

Supplementary Fig. 7



Supplementary Fig. 7. Effect of the E4872Q$^{+/-}$ mutation and *R*-carvedilol on Aβ-accumulation and effect of E4872Q$^{+/-}$ on CA1 pyramidal neuron apical dendritic spine density and morphology. Related to Fig. 6 and Fig. 7.

(**A**) Aβ deposition in the hippocampus in 5-6 months old WT, EQ$^{+/-}$, 5xFAD$^{+/-}$ and 5xFAD$^{+/-}$/EQ$^{+/-}$ mouse brains. (**B**) Averaged Aβ plaque number per mm^2 in 5-6 months old 5xFAD$^{+/-}$ (12 mice, 36 slices) and 5xFAD$^{+/-}$/EQ$^{+/-}$ (12 mice, 36 slices) mouse hippocampal region. (**C**) Percentage of hippocampal area showing positive Aβ staining. (**D**) Immunoblotting analysis of brain tissue homogenates from 5-6 months old WT, EQ$^{+/-}$, 5xFAD$^{+/-}$, and 5xFAD$^{+/-}$/EQ$^{+/-}$ mice. (**E**) Normalized total Aβ levels and (**F**) Normalized Aβ (1-42) levels in WT (n=9), EQ$^{+/-}$ (n=12), 5xFAD$^{+/-}$ (n=11), and 5xFAD$^{+/-}$/EQ$^{+/-}$ (n=14) brains. (**G-L**) 5xFAD$^{+/-}$ mice were treated with DMSO or *R*-carvedilol (*R*-CV) (3.2 mg/kg/day) for 1 month, starting at 2-3 months or 3-4 months of age before or after the onset of neuronal hyperactivity and memory loss. (**G**) Immunoblotting analysis of brain tissue homogenates from 3-4 months old 5xFAD$^{+/-}$ mice treated with DMSO or *R*-CV. (**H**) Normalized total Aβ levels and (**I**) Normalized Aβ (1-42) levels in 5xFAD$^{+/-}$ mice treated with DMSO (n=13) or *R*-CV(n=13) at the age of 3-4 months old. (**J**) Immunoblotting using brain tissue homogenates from 4-5 months old 5xFAD$^{+/-}$ mice treated with DMSO or *R*-CV. (**K**) Normalized total Aβ levels and (**L**) Normalized Aβ (1-42) levels in 5xFAD$^{+/-}$ mice treated with DMSO (n=11) or *R*-CV (n=11) at the age of 4-5 months old. Data shown are the median and range (Mann-Whitney *U* test; NS, not significant). (**M**) Apical dendritic spine density in 9-12 months old WT (3 mice, 27 dendrites), 5xFAD$^{+/-}$ (3 mice, 27 dendrites), 5xFAD$^{+/-}$/EQ$^{+/-}$ (3 mice, 27 dendrites), and EQ$^{+/-}$ (3 mice, 27 dendrites) CA1 neurons. Upper panel: Golgi staining images, scale bars:10 μm. Lower panel: densities of overall protrusions and different types of dendritic spines. Data shown are the median and range (Kruskal-Wallis test with Dunn-Bonferroni post hoc test; *P < 0.05, **P < 0.01 vs WT, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01 5xFAD$^{+/-}$/EQ$^{+/-}$ vs 5xFAD$^{+/-}$, NS, not significant).