LTP recordings in hippocampal slices of reggie-1 k.o. (Flot2-/-) mice.

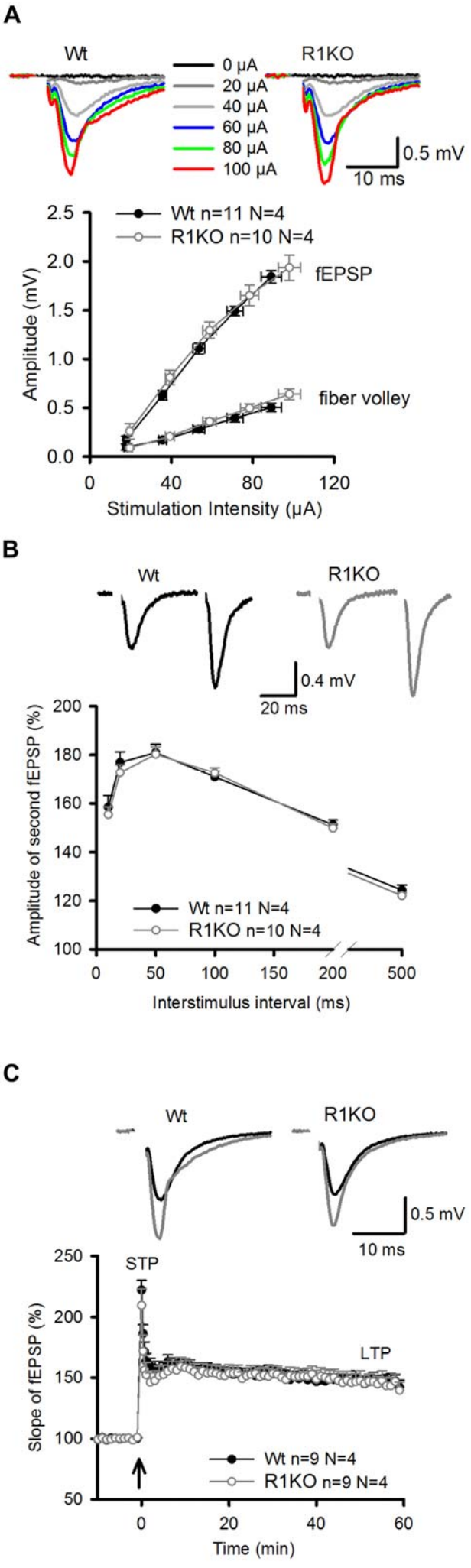
Recordings of LTP in hippocampal slices

Since reggie-1 k.o. (Flot2-/-) neurons, maturated over 10 div in vitro, showed severe defects in PSD-95 trafficking and decreased number of synapses (as determined by co-localisation of FM4-64X, synaptophysin and PSD-95), we expected to detect abnormalities in synaptic transmission and plasticity in the adult reggie-1 k.o. (Flot2-/-) hippocampus.
To analyze if loss of reggie-1 affects the efficacy of synaptic transmission and short- and long-term plasticity in adult stage we recorded field excitatory postsynaptic potentials (fEPSPs) in hippocampal CA1 region of reggie-1 k.o. (Flot2-/-) mice. As a measure of basal synaptic transmission we analysed input output stimulus response curves but found no difference between genotypes (Suppl. Fig. 1 A). No difference between genotypes was detected in the paired-pulse ratio, a measure of presynaptic short-term plasticity, at different interstimulus intervals (Suppl. Fig. 1 B). Short- and long-term plasticity induced by five theta-burst stimulation, was likewise unchanged (Suppl. Fig. 1 C).
Therefore, we suspected that compensatory mechanisms might counteract the genetic loss of reggie-1 in k.o. (Flot2-/-) mice in vivo but not when the neurons of these k.o. mice develop in vitro.

Material and Methods
Electrophysiology

3-4 months old wt and reggie-1 k.o. (Flot2-/-) mice were used for acute hippocampal field electrophysiology. Mouse was decapitated quickly after cervical dislocation and the brain extracted into ice cold dissection artificial cerebrospinal fluid (ACSF) containing (in mM): 2.5 KCl, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 1.5 $MgSO_4$, 2 $CaCl_2$, 25 glucose, 250 sucrose. The brain was dissected into equal hemispheres glued on object mounting platform and sectioned into 350 µm thick sagittal slices using vibroslicer (Leica, VT 1200S). The slices were collected in a resting chamber containing ACSF in which 250 mM sucrose was replaced with 120 mM NaCl (pH = 7.35-7.4) and incubated for at least 1.5 h to recover before recordings. Recordings performed in submerged chamber (Warner instruments RC-27L), supplied with continuously bubbled ACSF with a solution exchange of 3-5 ml per min at room temperature (22-24°C). An upright microscope (Olympus, BX61WI) was used for the positioning of slices to have access to the CA1 region of the hippocampus for recordings and electrode placement. Electrodes were made from glass capillaries (Hilgenberg) using micropipette puller Sutter P-1000 (Sutter Instruments). Stimulating (1-1.5 MΩ) and recording (1.5-2.5 MΩ) electrodes filled with ACSF were placed in stratum radiatum of the CA1 region and field excitatory postsynaptic potentials (fEPSPs) were recorded. Basal stimulation of 0.2 ms pulses were delivered at 0.05 Hz at the stimulation intensity which induced approximately 30-50% of the maximal responses. After 10 minutes of stabile baseline recordings stimulus response curves were made as a measure of basal excitatory synaptic transmission. Stimulation intensity was increased by 20 µA steps until the maximal fEPSP was obtained. The Amplitudes of fEPSPs and presynaptic volleys were plotted vs increasing stimulation intensity as a measure of basal synaptic function. Paired pulse facilitation (PPF) protocol was used to test short-term plasticity and facilitation was calculated as a ratio of the amplitude of the second response compared to the first. Two pulses at time intervals 10, 20, 50, 100, 200 and 500 ms were delivered at a stimulation intensity which induced one third of the maximal responses. For short intervals (10 and 20 ms), the first fEPSPs were digitally subtracted before measurements of the second fEPSPs. Each value measured is an average of five consecutive stimulations repeated every 20 sec for stimulus responses and every 30 sec for PPF measurements. To test synaptic plasticity we induced long-term potentiation (LTP) using theta burst stimulation (TBS) protocol. Five theta burst stimuli were delivered every 20 sec, each stimulus containing 8 bursts at 5 Hz, each burst consisting of 4 pulses at 100 Hz. For LTP experiments the stimulation intensity was selected to elicit 50% of its maximum amplitude, and basal stimulation was monitored at 0.05 Hz for 10 min before and 1 h after LTP induction. LTP was evaluated 1 h after induction and calculated as % increase of fEPSP slope 50-60 min after TBS as compared to the initial -10-0 min of baseline. The data were recorded at a sampling rate of 10 kHz, low-pass filtered at 3 kHz and analyzed using PatchMaster software and EPC9 amplifier (Heka Elektronics). SigmaPlot (Systat), software was used for data analyses and presentation. Data were statistically evaluated using two-way ANOVA with repeated measures. For comparisons of two groups, statistical significance was tested by two-tailed unpaired Student's t-test. Values are depicted as mean ± SEM. n and N indicate the number of tested slices and mice, respectively.

Supplemental figure: LTP in hippocampal slices



Adult reggie-1 k.o. (Flot2-/-) mice display normal levels of basal synaptic transmission, short- and long-term synaptic plasticity.

(A) Basal synaptic transmission is not altered in adult reggie-1 k.o. (Flot2-/-) mice. Representative examples of fEPSPs recorded with increasing stimulation intensities from 0 to 100 µA for each genotype. The relationship between the stimulus intensity and amplitude of fEPSPs (two-way RM ANOVA, P = 0.66) and between stimulus intensity and amplitude of presynaptic volleys (two-way RM ANOVA, P = 0.29) reveal normal levels of basal synaptic transmission in reggie-1 k.o. (Flot2-/-).

(B) Normal levels of paired-pulse facilitation in reggie-1 k.o. (Flot2-/-) mice. Representative traces of paired-pulse facilitation at 50 ms interstimulus interval for each genotype. The relationship between the interstimulus interval and paired-pulse facilitation, given as ratio of the second to the first response show no changes in facilitation of the second response in reggie-1 k.o. (Flot2-/-) mice (two-way RM ANOVA, P = 0.65).

(C) Reggie-1 k.o. (Flot2-/-) mice shows normal levels of short and long-term potentiation. Representative fEPSPs responses (each average of 30) recorded 0-10 min before (black) and 50-60 min after (gray) LTP induction for each genotype. The graph shows the induction of normal STP (t-test P = 0.48) and LTP (t-test P = 0.83) by delivering five TBS stimulation (indicated by arrow) in reggie-1k.o. (Flot2-/-) mice. Each value is an average of 3 consecutive time points recorded every 20 s. The mean slope of the fEPSP recorded 0-10 min before TBS application is taken as 100%.