Figure S1. Spine volume measurements in primary hippocampal neurons that overexpress SHANK2 mutants and wild type

(A) Representative hippocampal neurons transfected with GFP together with either mCherry SHANK2 - wild type (left) or mCherry-SHANK2-R462X mutant (right). Boxed insets show spines in higher magnification. Scale bars, 10µm (main panel), 4µm (insets). (B) SHANK2-WT expression increases spine volume, while this is not the case for the R462X mutant. We determined the relative spine volume of the neurons described in panel (A) (n=3 experiments, 3600 spines from 18 neurons). Values were normalized to the GFP/mCherry - only condition and are shown as mean ± s.d. The P value of the two factorial variance analysis (ANOVA) observed a significant influence after correction for multiple testing (Scheffé) on spine volume for the SHANK2 - WT (P =1.1x 10^{-8}), whereas the R462X mutant behaves like the GFP control. (C) The SHANK2 T1127M missense and the L1008_P1009dup mutant show no significant difference to the GFP control. Values were normalized as indicated in (B) and the pair-wise P values are given (GFP/WT P= 0.0001; GFP/T1127M P= 0.460; GFP/L1008_P1009dup P= 0.79).
Figure S2. Effect of shRNAs directed against rat Shank2 and determination of AMPA and GABA reversal potentials.

(A) HEK 293 cells were co-transfected with rat Shank2, human SHANK2 wild type, rat HA-Shank1 or rat HA-Shank3, together with shRNA#1, #2, co-shRNA or empty vector (#1, binds distal to the PDZ domain; #2 binds proximal and adjacent to the SAM domain). One day after transfection, western blots were performed using anti-Shank2 or anti-HA antibody. shRNA#1 and #2 specifically inhibit rat Shank2 expression but not the expression of human SHANK2 or rat HA-Shank1 or HA-Shank3 used as controls. HA, Hämagglutinin-Tag. (B) Knock-down of Shank2 in primary cortical neurons. Cortical neurons were transfected with Amaxa nucleofection with co-shRNA or shRNA#3. After 6 DIV western blots were performed using anti-Shank2. After normalizing the Shank2 band intensity against the intensity of GAPDH, we observe that shRNA#3 reduces the amount of endogenous Shank2 by half. (C) Determination of AMPA and GABA reversal potentials for CA1 pyramidal cells. Monosynaptic currents were evoked by mono-polar electrical stimulation (0.1 ms pulse) of proximal inputs to identified CA1 pyramidal cells at different membrane potentials. Excitatory (red traces) and inhibitory (black traces) responses were pharmacologically isolated by adding 20 µM bicuculline or 10 µM NBQX to the ASCF, respectively. By plotting the amplitude of the synaptic signals evoked at each membrane potential we interpolated the reversal potentials for AMPA (Eampa = 6.21 mV ± 0.84 mV, n=11) and GABAa (EGABAa = -67 mV ± 3.10 mV, n= 15) responses, as previously described (13). Average values are depicted in the bar plot. Sample traces correspond to averages of 5 responses at each membrane potential. Taking this information into account, we characterized the excitatory and inhibitory drive into the same postsynaptic cells as follows: To isolate AMPAR-mediated spontaneous currents (mEPSCs) neurons were held at EGABAa, and for the isolation of GABAa-R mediated spontaneous currents (mIPSCs), neurons were held at EAMPA.
Figure S3. Quantification of the dendritic complexity of hippocampal neurons overexpressing SHANK2 wildtype and mutants by Scholl analysis.

(A) The total number of intersections is not altered by SHANK2 wild type or mutant overexpression.
(B) No significant difference exists in the distribution of dendritic branches among the different conditions. n=3 experiments, 30 neurons for each condition.
Figure S4. AMPA-GluA2 surface expression is reduced in primary hippocampal neurons overexpressing SHANK2-R462X.

Representative magnifications of dendrites from hippocampal neurons transfected with SHANK2 wild type, R462X mutant or GFP with GluA2 surface staining. SHANK2-R462X GluA2 cluster sizes are reduced compared to the GFP control whereas SHANK2-WT does not significantly increase GluA2 clusters. R462X expression is restricted to the cell soma and primary dendrites. Scale bars: dendrites 2 µm; SHANK2-R462X neuron 10 µm. * P < 0.05; n= 3 experiments, 30 neurons each condition.
SUPPLEMENTARY METHODS

Primer sequence
Cloning of Shank2 (human)
SHANK2_1463 for SalI aatgtcagacagaccattgggagctacgtg
SHANK2_cDNA rev ttccacagcagtttctcag

Mutagenesis
SHANK2-Mut-R462X_F gtttctgggcatccct
tgaggtacgatg cg
SHANK2-Mut-R462X_R cgcatcgtacctc aagggatgcccagaaac
SHANK2-Mut-L1008_P1009dup_F gcggtgattttgccatt gc
SHANK2-Mut-L1008_P1009dup_R gaggagggatgcggaatggc
SHANK2-Mut-T1127M_F ccaccacctcgagacacagactatetc
SHANK2-Mut-T1127M_R gagatagtgttgtgctcaggtgttg

Cloning of Shank2 (Rat)
SHANK2-Rat-BamHI TAGGAT CCGGTGATTTATTGGCTGTGATGATG
SHANK2-Rat-NotI TGCGGCCGCTTATCTGTCCAGCAGCTGTTTC

shRNAs
Shank2-si#1-fw1 gatcccc GGATAAACCGGAAGAGATTtcaagaga
Shank2-si#1-fw2 tatctctc ttccggtttatcctttttggaaa
Shank2-si#1-bw1 agcttttcca aaaaGGATAAACCGGAAGAGATTtctcttgaa
Shank2-si#1-bw2 tatctctcgggtttatccggg
Shank2-si#2-fw1 gatcccc GGAATTGAGCAAAGAGATTttcaagaga
Shank2-si#2-fw2 aatctctc ttggcattcattcctttttggaaa
Shank2-si#2-bw1 agcttttcca aaaaGGAATTGAGCAAAGAGATTtctcttgaa
Shank2-si#2-bw2 aatctctcgggtttatccggg

Mouse behavior

Open field and novel object recognition. In order to measure overall motor activity, mice were tested in an open
field. All the mice were run in the same wooden arena measuring 50 x 50 x 50 (h) cm. Mice were placed in a corner
of an open field and allowed to run around freely for a duration of 10 minutes while their motor activity was
monitored using a video camera placed 2 m above the center of the arena. After 10 minutes, a novel object was placed
in the center of the open field for another 5 minutes. The time which the mice spent in the center was monitored
during the first 10 and the last 5 minutes.

Light-dark box. The light-dark-box consists of an open white compartment 30 x 20 x 20 cm with an additional
illumination, joined by a 3 x 3 cm opening to a dark box (painted black with a lid) 15 x 20 x 20 cm. The animals are
placed in the middle of the light side facing away from the opening. The time spent on the dark side (all 4 feet) is
measured for five minutes test duration.

Puzzle box. This test is slightly modified from 16. The puzzle box consists two divided compartments (a brightly-lit
start zone and a smaller covered goal zone) within a Plexiglas white box. Animals were introduced into the start zone
and trained to move into the goal zone through a narrow underpass (about 4 cm wide) located under the barrier.
Animals underwent a total of 11 trials over 4 consecutive days, with three trials per day on the first three days, and
two trials on the last day. On day 1 (habitation), during trial 1, the underpass was un-blocked and the barrier had an
open door over the location of the underpass. On trial 2 and trial 3, the barrier had no doorway and the animals had to
enter the goal zone via the small under pass. On day 2 (burrowing puzzle), Trial 4 was identical to trial 2 and trial 3. On trial 5 and trial 6, however, the underpass was filled with sawdust and the animals had to dig through the sawdust. On day 3 (plug puzzle), the animals had to repeat trial 6 first as trial 7. And on trial 8 and trial 9, the animals were presented with the underpass blocked by a cardboard plug that mice had to pull with teeth and paws to enter the goal zone. Trial 10 on day 4 is again to repeat trial 9. At the end of the test, on trial 11, the task is to repeat trial 1 on the first day.

Amaxa nucleofection

Cortical neurons from rat E18 were transfected directly after preparation at DIV0 according to the manual of the Amaxa Rat Neuron Nucleofector Kit. 30 µg of control-shRNA or shRNA#3 were used for transfection of 2 x 10^6 neurons that were then plated in a 6 cm dish.

Western blot

Proteins were separated by SDS-PAGE (6%, 8%) and blotted onto a poly-vinylidene difluoride (PVDF, Immobilon-FL, Millipore) membrane. Non-specific bindings were blocked with the Odyssey Blocking Buffer (diluted 1:2 with TBS, Licor). The mCherry-SHANK2, the rat Shank2, the HA-Shank1 and Shank3 constructs were transfected into HEK293 cells and analyzed by western blotting using a mouse anti-SHANK2 (1:2000; NeuroMab, Antibodies Inc.) or anti-HA antibody (Sigma, monoclonal HA-7) and secondary donkey anti-mouse IRDye800CW antibody (1:15000, Licor). Signals were detected with the Odyssey infrared imager (Licor).