Normal Morphology of hippocampal synapses in HIP1⁻/⁻ mice. Ultrastructural analysis of wild-type (+/+) and HIP1 (-/-) hippocampal synapses demonstrate that no morphological changes were evident at the pre- (at) and postsynaptic (ds) nerve terminal in mutant synapses. In the presynaptic compartment, the average size of axon terminals was $0.190 \pm 0.018 \ \mu m^2$ in wild-type mice as compared to $0.161 \pm 0.021 \ \mu m^2$ in HIP1⁻/⁻ mice (t-test, $p = 0.09$). The number of synaptic vesicles per $\mu m^2$ was $127.4 \pm 29.2$ in wild-type mice as compared to $135.36 \pm 39.04$ in HIP1⁻/⁻ littermates. Moreover, there was no significant difference in the percentage of docked vesicles ($2.65\% \pm 1.13$ in wild-type mice compared to $3.35 \% \pm 0.39$ in HIP1⁻/⁻ littermates; t-test, $p = 0.37$). In the postsynaptic compartment, the average cross-sectional area and the length of the postsynaptic density (PSD) were $9901.715 \pm 223.13 \ \text{nm}^2$ and $579.17 \pm 24 \ \text{nm}$ in wild-type mice, respectively and $9906.79 \pm 373.05 \ \text{nm}^2$ and $594.78 \pm 20.97 \ \text{nm}$ in HIP1⁻/⁻ littermates, respectively. The scale bar = 250 nm.

Ultrastructural analysis of hippocampal synapses
Mice were deeply anaesthetized with choral hydrate and perfused transcardially with 3% PFA and 0.2% glutaraldehyde in 0.1M phosphate-buffered saline. The brain and spinal cord from wild-type and HIP1⁻/⁻ littermates were dissected and serial sections (50 $\mu m$) through the hippocampus were prepared for electron microscopy analysis. Briefly, sections were osmicated, dehydrated and flat embedded in Epon. Subsequently, thin
sections were cut through a random region of the hippocampus containing CA1 neurons and the adjacent stratum radiatum and analyzed by electron microscopy (Hitachi 7500). Five continuous fields of neuropil, parallel to the CA1 layer of neurons, were photographed, processed in Adobe Photoshop and analyzed on a Macintosh computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). The surface area of the axon terminal, the total number of synaptic vesicles, and the number of docked vesicles at the active zone were determined from a total of 121 wild-type and 163 HIP1⁻/⁻ axon terminals, respectively (n = 2 for each genotype). Moreover, the average cross-sectional area and length of the PSDs were determined from a total of 199 wild-type and 178 HIP1⁻/⁻ PSDs (n = 2 for each genotype). Statistical differences were determined by Student’s t-test.