Inventory of Supplemental Information

Figure S1 linked to Figure 1

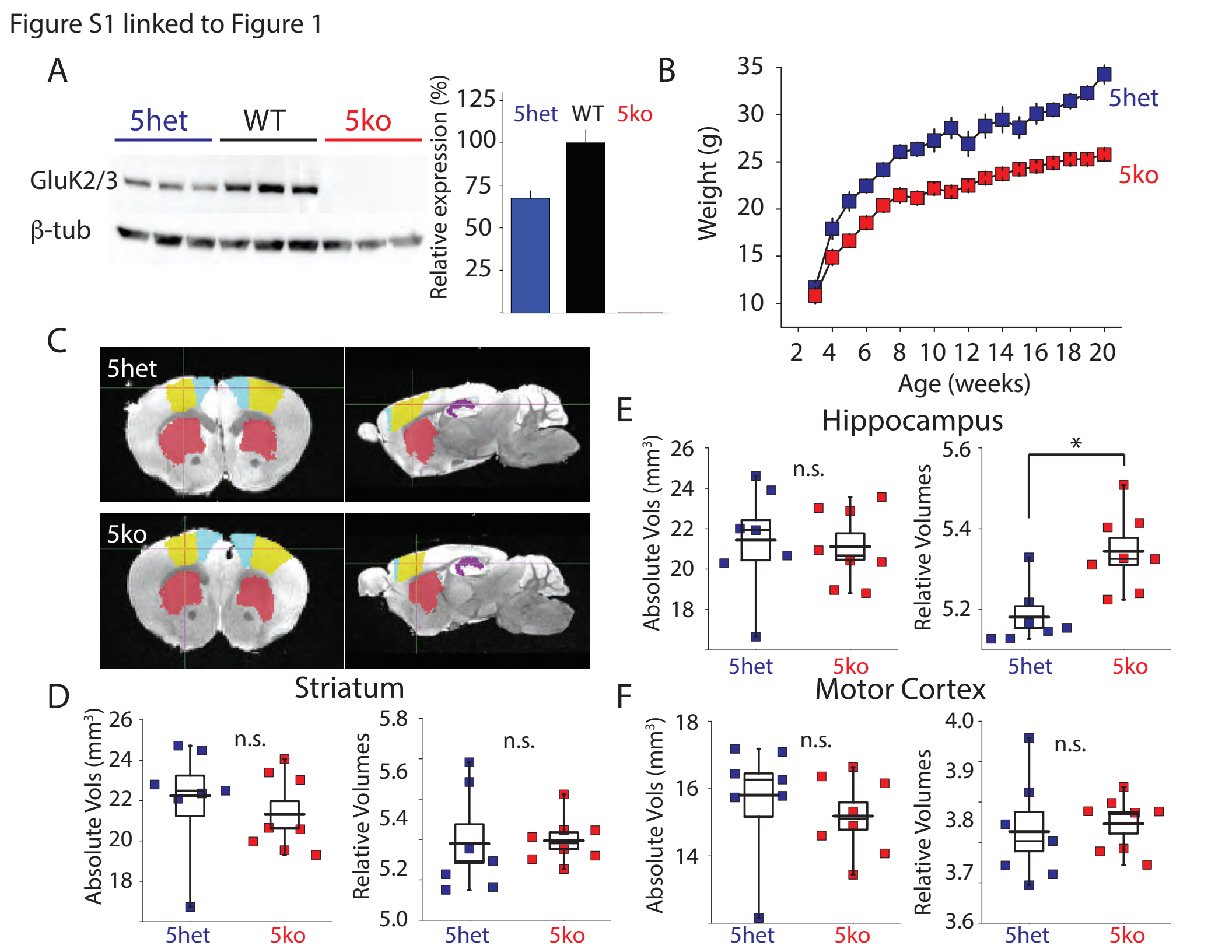
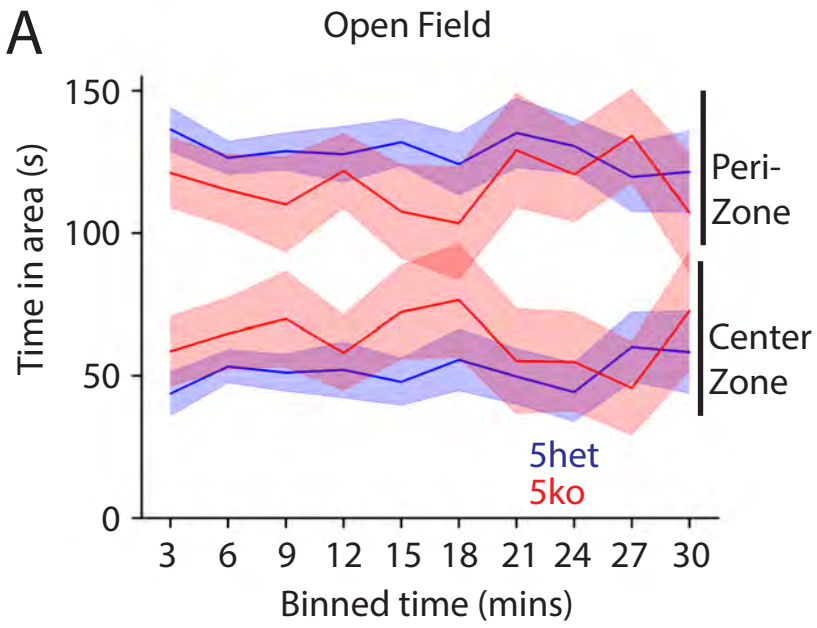
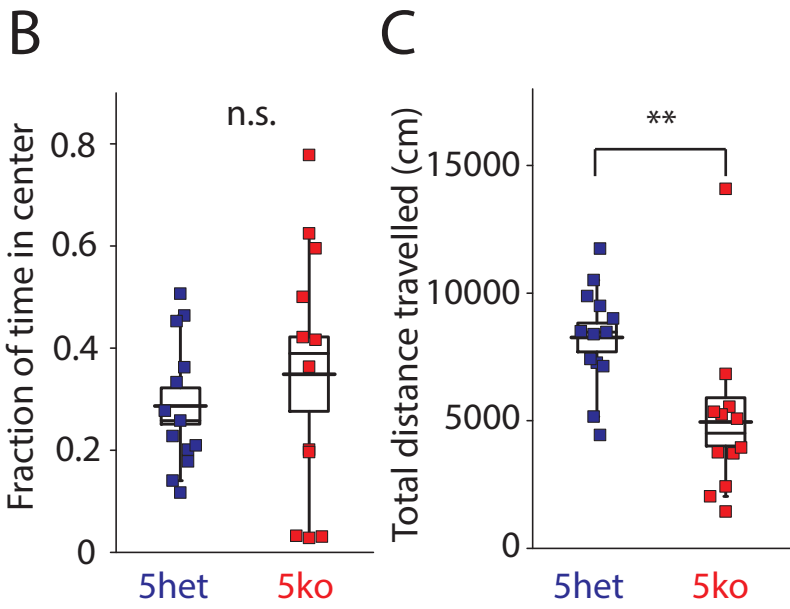


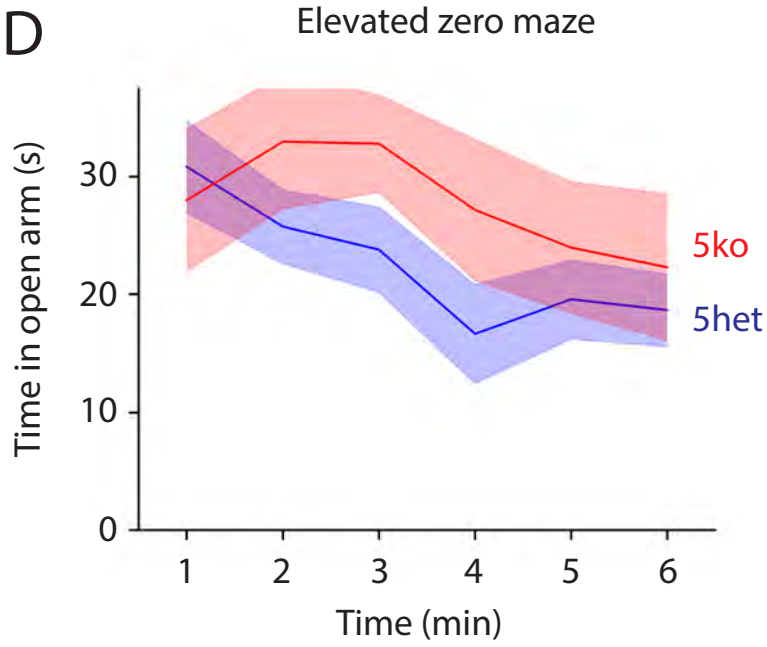
Figure S1 Generation and Basic Characterization of Kainate Receptor 5ko mice

(**A**) Representative Western blot of striatal synaptosome samples from 5het, WT and 5ko mice. Signal for GluK2/3 is completely eliminated in 5ko samples. Right panel shows quantification from multiple experiments showing no signal in 5ko samples and a reduction in expression in 5het compared to WT. (**B**) Growth curves for male mice. Tracking two cohorts of male mice over five months we found that the 5ko mice had consistently lower body weights into adulthood compared to the 5het mice, although no elevated mortality was observed during the perinatal period (**C**) Representative MRI image scans of brains from 5het (top) and 5ko (bottom). Regions of interest (striatum, motor cortex and hippocampus) are colored with masks (**D**) Analysis of absolute volumes and relative volumes of striatum, hippocampus (**E**), and motor cortex (**F**).
Analysis of several regions including the striatum (ventral and dorsal regions), hippocampus and motor cortex (primary and secondary) demonstrated that the absolute volumes did not show any significant difference between strains. However, we did find that the relative volume of the hippocampus was significantly increased in proportion to total brain volume in 5ko mice.

Figure S2 linked to Figure 1







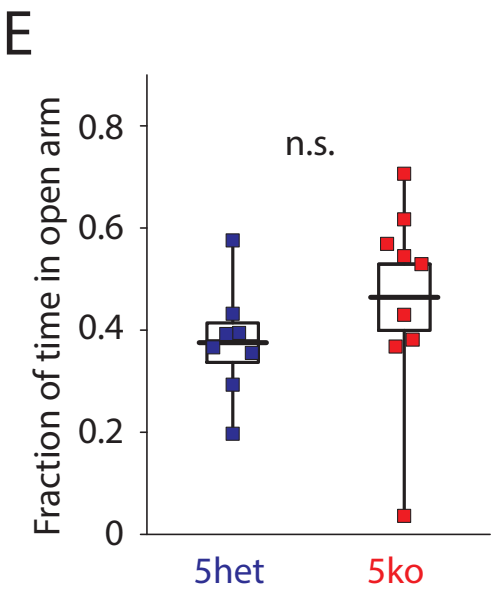


Figure S2 5ko mice do not demonstrate elevated anxiety

(**A**) Timecourse analysis of time spent in each zone of the open field (center or periphery) for 5het and 5ko mice (**B**) Analysis of fraction of total time spent in center zone (**C**) Analysis of total distance travelled during the test. 5ko mice had significantly decreased travel distances (**D**) Timecourse of elevated zero maze test analyzing time in the open arm (**E**) Fraction of time spent in the open arm.

Figure S3 linked to Figure 4

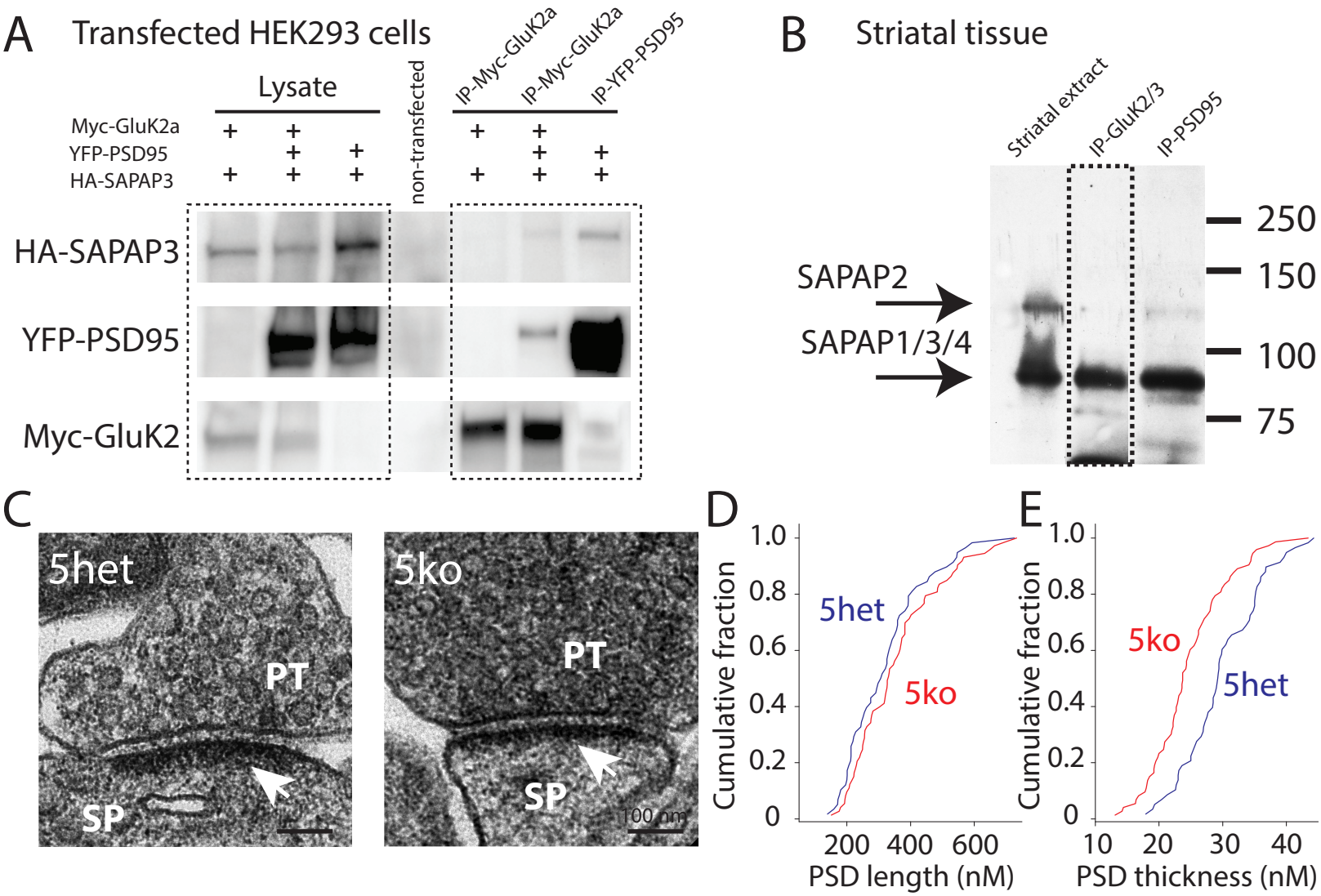


Figure S3 Kainate receptors are in synaptic complexes with the OCD-associated protein Sapap and PSD thickness is reduced in 5ko mice

(**A**) Representative co-immunoprecipitation from cells transfected with myc-GluK2a, YFP-PSD95 & HA-Sapap3. Co-IP of HA-Sapap3 by myc-GluK2a is only observed when PSD95 is co-transfected. Lysis buffer contains 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton and 1 mM EDTA. After cell lysis, protein concentrations were measured using a BCA assay. 600 µg of protein samples were used for the co-IP experiment. In the western blot, each lysate lane was loaded with 20 µg of protein. For the IP panel, approximately half of the co-IP-ed samples were loaded. (**B**) Representative co-immunoprecipitation of endogenous Sapap with GluK2 and PSD95 from striatal homogenates demonstrating that these excitatory synaptic proteins exist in the same complex in vivo. (**C**) Representative electron micrographs of striatal synapses in 5het and 5ko mice. (**D & E**) Analysis of PSD length and thickness demonstrated significant thinning of the PSD in 5ko mice.

	5Het n = 18	5ko n = 12
Stride time	381 ± 8.50 ms	404 ± 9.28 ms
Stride length	5.34 ± 0.120 cm	5.66 ± 0.130 cm
Swing duration	135 ± 4.38 ms	141 ± 5.74 ms
Stance length	5.34 ± 0.120 cm	5.66 ± 0.129 cm

Legend for Table 1: Gait analysis of 5het and 5ko mice

A detailed kinematic gait analysis was performed to quantify parameters of the phase of stride. Mice were placed on a treadmill, and parameters of their stride were extracted by measuring the digitized fore and hind paw patterns as the mouse moved at a fixed walking velocity. We initially found that 5ko mice could not remain on the treadmill at a velocity of 17 cm s^{-1} or higher (0 out of 6 mice), unlike 5het mice (6 out of 6). However, 5ko mice could remain on the treadmill at a speed of 14 cm s^{-1} (6 out of 6). For that reason, a fixed treadmill speed 14 cm s^{-1} was used for all subsequent tests. At this speed parameters of stride duration were compared between 5het and 5ko mice. Gait analysis was performed using a DigiGait Imaging system (Mouse Specifics, Quincy, MA, USA).

Experimental Procedures for Supplemental Data:

MRI and analysis of brain volumes

To prepare samples for MRI scanning mice (4 month old males) were deeply anesthetized and perfused intracardially with 4% paraformaldehyde (PFA). After perfusion, brains were carefully removed from the skull, post-fixed in 4% PFA for 3 hrs before being transferred to PBS for 3 days. Brains were then soaked in 1/100 Magnevist (containing 5mM Gadolinium MR contrast agent) in PBS for 7 days before they were extracted from the PBS + Gadolinium 5mM solution and were placed in a 15 cc plastic tube full of perfluoropolyether (Fomblin®, Solvay Solexis, Inc, Thorofare, NJ). This perfluorinated oil has a multiple functions: 1) it preserves the sample from air; 2) it reduces air to tissue susceptibility artifacts and 3) it allows acquisition of images with no "background" signal (the oil is made out of fluorine and is "invisible" when imaging with MRI at proton frequencies).

After placing the tube with the sample in the scanner (7 Tesla Bruker ClinScan MRI) a 4 channel phased array coil was used for acquisition of the images. First, three localization slices (axial, sagittal coronal) were obtained. Higher order field map shimming was run to improve magnetic field homogeneity across the whole brain and finally using a three dimensional (3D) gradient echo sequence (GRE) with TR/TE of 60 msec/2.6 sec, flip angle of 15 the final data set was acquired. The 3D-GRE scan was run exploiting a GRAPPA parallel acquisition scheme that reduced the overall acquisition time by a factor of 2 (total time for each brain ~ 4 hours). The geometrical parameters were adjusted to obtain an isotropic spatial resolution of ~80 μm.

Kainic Acid Injection

To confirm the functional ablation of kainate receptors in these mice, we examined susceptibility to kainic acid-induced seizures. Mice were injected intra-peritoneal (IP) with various doses of kainic acid and seizure severity was determined on a modified Racine scale by observation. 20 mg/kg kainic acid induced behavioral seizures (scores of 4 or above) in five out of eight WT animals, whereas all three 5ko mice injected were unaffected and therefore scored zero at this dose. 30 mg/kg kainic acid induced maximum seizure severity in all WT mice and in five of six 5het mice, whereas 5ko mice again failed to display any behavioral seizures at this dose. At 40mg/kg, a dose that resulted in seizure and rapid death in all WT (n=2) and 5het mice (n=3) tested, 5ko mice did not display any behavioral seizures; increasing the dose to 50mg/kg caused behavioral seizures in 3 out of 6 of the 5ko mice (5het and WT mice were not tested this dose). Therefore, the 5ko mice have a relatively large shift in reduced seizure susceptibility compared to the modest change in the 5het mice or the previously studied single GluK2 ko mice (Mulle et al., 1998). Together these results demonstrate that the loss of all kainate receptors has a significant effect on the threshold for chemically induced seizures, and that the kainic acid model of seizures is primarily induced by activation of central kainate receptors, rather than other glutamate receptors, at doses that cause maximal seizure activity in WT mice.