Methods

Mouse husbandry. All experiments were conducted on 5-7 week old male and female mice from the C57Bl/6 background. *Pten*$^{loxP/loxP}$ mice[1] and *Rictor*$^{loxP/loxP}$ mice[2] were previously described. *Rptor*$^{loxP/loxP}$ mice were purchased from the Jackson Laboratory (Stock# 013188). Double floxed mice were generated by crossing *Pten*$^{loxP/loxP}$ with either *Rptor*$^{loxP/loxP}$ or *Rictor*$^{loxP/loxp}$. To generate forebrain specific knockout mice (fb-KO), *Pten*$^{loxP/loxP}$ mice, *Pten-Rptor* $^{loxP/loxP}$ mice or *Pten-Rictor* $^{loxP/loxP}$ were crossed with mice expressing *Cre* recombinase under the control of the α subunit of the calcium/calmodulin-dependent protein kinase II (*Camk2α*) promoter to then produce *Pten* fb-KO, *Pten-Rptor* fb-DKO and *Pten-Rictor* fb-DKO mice, respectively. Mice were weaned at the third postnatal week and genotyped by Transetyx using real-time PCR. Mice were kept on a 12h/12h light/dark cycle (lights on at 7:00 am) and had access to food and water *ad libitum.* Animal care and experimental procedures were approved by the institutional animal care and use committee (IACUC) at Baylor College of Medicine, according to NIH Guidelines.

Electroencephalographic (EEG) Recordings. EEG recordings were performed as previously described[3]. Briefly, mice were anesthetized 0.02ml/g with Avertin (1.25% tribromoethanol/amyl alcohol solution, i.p. injection). Teflon-coated silver wire electrodes were implanted bilaterally into the subdural space over frontal and parietal cortices. After a 72 hr post-surgical recovery period, freely moving mice were recorded for 24 hr per day. Digitized videoEEG data were obtained

daily for 2 weeks from mice at 5-7 weeks of age. EEG data (interictal spikes and seizure frequency) were interpreted by analysts with expertise in epilepsy who were blinded to genotype. Recordings were visually inspected in their entirety. Electrographic seizure activity was verified by inspection of the concurrent video with documentation of motor seizure episodic behavior consisting of arrest, clonic limb movements, tonic posture, and tonic elevation of the tail (Straub tail).

Electrophysiology. The experimenter was blind to the genotype. Briefly, animals were decapitated after being anaesthetized with isoflurane (Henry Schein Animal Health, Dublin, OH, USA). The brain was rapidly removed from the skull and fixed on the vibroslicer stage (VT 1000S, Leica Microsystems, Buffalo Grove, IL) with cyanoacrylate glue. Acute 300 µm-thick slices were cut in the horizontal plane in ice cold cutting-solution containing 87 mM NaCl, 25 mM $NaHCO_3$, 25 mM glucose, 75 mM sucrose, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 0.5 mM $CaCl_2$ and 7 mM $MgCl_2$ (equilibrated with 95% O_2–5% CO_2 gas mixture, pH 7.3-7.5). Slices were incubated for 20 min at 32°C and then stored at room temperature in a holding bath containing oxygenated standard ACSF (125 mM NaCl, 25 mM $NaHCO_3$, 25 mM glucose, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, and 1 mM $MgCl_2$, equilibrated with 95% O_2-5% CO_2) for at least 20 min, before being transferred to a recording chamber mounted on the stage of an upright microscope (Examiner D1, Carl Zeiss, Oberkochen, Germany). The slices were perfused with oxygenated ACSF (2 ml/min) and maintained at 32°C with a Peltier feedback device (TC-324B, Warner Instruments). Spontaneous excitatory

post synaptic currents (sEPSCs) were recorded in the presence of the $GABA_A$ receptor antagonist Picrotoxin (100 μM; Sigma-Aldrich, USA). Whole-cell recordings were performed using conventional patch-clamp techniques and CA1 neurons were visually identified by infrared differential interference contrast video microscopy on the stage of the upright microscope.

Patch pipettes were pulled from borosilicate glass capillaries (World Precision Instruments, Inc., Sarasota, FL) and filled with a solution containing (in mM) 110 K-gluconate, 10 KCl, 10 HEPES, 10 Na_2-phosphocreatine, 2 Mg_3-ATP, and 0.2 Na_3-GTP; pH was adjusted to 7.2 and osmolarity to 290 mOsm using a vapor pressure osmometer (Vapro*5600,* ELITechGroup Wescor, South Logan, Utah, USA). When filled with the internal solution, patch pipettes had a resistance of 3–5 MΩ before seal formation. Spontaneous inhibitory post synaptic currents (sIPSCs) were recorded in the presence of 2 mM kynurenic acid (Sigma-Aldrich, USA). In this case the internal solution composition was identical, except that potassium gluconate was replaced by KCl. Recordings were performed with a Multiclamp 700B (Molecular Devices), low-pass filtered at 2 Hz and sampled at 20 kHz with a Digidata 1440A interface, and analyzed off-line with pClamp10 software (Molecular Devices, San Jose, CA). CA1 pyramidal cells were clamped at -70 mV and spontaneous currents were recorded with a background activity protocol. The stability of whole-cell recordings can be influenced by changes in series resistance (R_s). To ensure that R_s remained stable during recordings, passive electrode-cell parameters were monitored throughout the experiments by analyzing passive current relaxations induced by 10 mV hyperpolarizing steps

from a holding potential of −70 mV applied before and after spontaneous currents recording. Variation in R_s >20% led to the rejection of the experimental data. Spontaneous currents were digitally filtered at 1.5 kHz and analyzed off-line. Automated sEPSC and sIPSCs analysis was performed automatically with Clampfit software. A further visual inspection of detected signals allowed us to reject noise artifacts. A 5 min period was used to evaluate sEPSCs and sIPSCs frequency and mean sEPSC and sIPSC amplitude. Intrinsic excitability of CA1 pyramidal neurons was recorded in current-clamp configuration. Current steps were injected (500 ms) and the average action potentials (APs) number over the entire current pulse was used to construct input-output curve.

Behavior Tests. Male and female 5-6 week-old mice were used for behavior experiments. Similar numbers of male and female mice for each genotype were included. No differences were found between males and females for the tested behaviors (data not shown). To control for odor cues, apparatuses were thoroughly cleaned with ethanol, dried, and ventilated between testing of individual mice. Behavior experiments used littermates as controls and were carried out and analyzed with the experimenter blinded to genotype.

Three-Chamber Sociability and Social Novelty. Tests were performed as previously described[4,5]. Sex and age matched control stranger mice were habituated to a cylindrical wire cup for 30 min once a day for 2 days prior to testing. On the testing day, the experimental mice were allowed to freely explore a 60 × 40 × 23 cm Plexiglas arena divided into three equally sized,

interconnected chambers (left, neutral, right) for 10 min (habituation). Immediately after habituation, a stranger mouse was placed in the wire cup and introduced to the test mouse. Another empty cup was placed at the opposite end of the chamber and the test mouse was allowed to interact either with the empty wire cup or stranger mouse for 10 min (sociability). Time spent interacting (sniffing, crawling upon) with either the empty cup or the stranger mouse contained in the other cup as well as time spent in each chamber was recorded using the AnyMaze software, by independent observers. Empty cup placement in the left or right chamber during the sociability period was counterbalanced between trials. Finally, for the social novelty session, a second stranger mouse was introduced into the previously empty wire cup while the first mouse remained in the chamber as the familiar mouse for this session. Time spent in each chamber as well as time spent interacting with either mouse was recorded by independent observers using the automated AnyMaze software.

Reciprocal Social Interaction. The procedure was adapted from a previously described protocol[4]. Experimental mice were paired with age and sex matched control stranger mice. Prior to the test, all mice were isolated in home cages for 1 hour. An experimental mouse and a paired stranger mouse were placed in a 25 × 25 × 25 cm Plexiglas arena with a single layer of corncob bedding together for 10 min. Social interaction behavior including close following, touching, nose-to-nose sniffing, nose-to-anus sniffing, and/or crawling over/under each other were scored by the investigator and calculated as total time spent in contact.

T-maze alternation task. The spontaneous alternation task was conducted as previously described[6]. The apparatus was a black wooden T-maze with 25 cm high walls and each arm was 30 cm long and 9 cm wide. A removable central partition was used during the sample phase but not the test phase of each trial. Guillotine doors were positioned at the entrance to each goal arm. At the beginning of the sample phase, both doors were raised, and the mouse was placed at the end of the start arm facing away from the goal arms. Each mouse was allowed to make a free choice between the two goal arms; after its tail had cleared the door of the chosen arm, the door was closed, and the mouse was allowed to explore the arm for 30 sec. The mouse was then returned to the end of the start arm, with the central partition removed and both guillotine doors raised, signaling the beginning of the test phase. Again, the mouse was allowed to make a free choice between the two goal arms. This sequence (trial) was repeated 20 times. Trials that were not completed within 120s were terminated and disregarded during analysis. Correct alteration between trails was expressed as % alteration = (number of correct alteration)/20 x 100%.

Contextual Fear Conditioning. Experiments were performed as previously described[2,3,7,8]. Briefly, mice were habituated to the conditioning chamber for 20 min daily for two days. On the training day, mice were place in the chamber for 2 min (Naïve) and then received two foot shocks (0.7 mA, 2 sec, 90 sec apart). The mice were then left in the chamber for another 1 min (post-shock) before being

returned to their home cages. Twenty-four hours later, mice were put back into the chamber for 5 min. Real-time video was recorded and analyzed using FreezeView as we previously described[2,3,7,8].

Novel Object Recognition. Novel object recognition was performed as we previously described[9] with small modifications. Briefly, mice were habituated to a black Plexiglas rectangular chamber (31 x 24 cm, height 27 cm) for 20 min under dim ambient light for 3 days before testing. One the first day, test mice were allowed to explore two identical objects in the testing chamber for 10 min and then returned to the home cage. On the second day (24 hours), test mice were again placed in the testing chamber and presented with one object used on the previous day (familiar object) and a novel object for 10 min. The novel object has the same height and volume, but different shape and appearance. Exploration of the objects was defined as sniffing of the objects (with nose contact or head directed to the object) within at 2 cm radius of the objects. Discrimination Index (DI) was computed as (Novel Object Exploration Time – Familiar Object Exploration Time/Total Exploration Time) X 100.

Western Blotting. Western blotting was performed as we previously described[2,7,9,10]. The hippocampus and cortex from control and knockout mice were isolated from 5-7 week-old mice. Tissue was homogenized in cold lysis buffer [200 mM HEPES, 50 mM NaCl, 10% Glycerol, 1% Triton X-100, 1 mM EDTA, 50 mM NaF, 2 mM Na_3VO_4, 25 mM β-glycerophosphate, and EDTA-free

complete ULTRA tablets (Roche, Indianapolis, IN)], and centrifuged at 13,000 × *g* for 10 min. The supernatants were collected and resolved on SDS–PAGE (6-10%) and transferred onto nitrocellulose membranes (Pall, Port Washington, NY).

Antibodies. Antibodies against pten (1:2000 #9188), raptor (1:1000 #2280), Rictor (1:1000 #2114), p-S6 (1:2000, Ser240/244 #5364), p-Akt (Ser473, 1:1000, #9271), total S6 (1:1000 #2217), total Akt (1:1000 #9272), p-PKC (Ser657, 1:2000, #9371) total PKCα (1:1000 #2056), p-NDRG1 (1:1000, Thr346, #3217), total NDRG1 (1:1000 #9408) were purchased from Cell Signaling and Technology Laboratories (Danvers, MA) and β-actin (1:5000 #1501) from Millipore (Temecula, CA).

Non-targeted metabolomics analysis. Cortex samples from 5-7 weeks old mice were freshly dissected and snap frozen at -80^{o}C until processed. Sample preparation and analysis were done by Metabolon, Inc. (Durham, North Carolina, USA). Briefly, samples were prepared using the automated MicroLab STAR® system from Hamilton Company. Samples were placed briefly on a TurboVap® (Zymark) to remove the organic solvent. The sample were stored overnight under nitrogen before preparation for analysis. The resulting fractions were analyzed by two separate reverse phase (RP)/UPLC-MS/MS methods with positive ion mode electrospray ionization (ESI), RP/UPLC-MS/MS with negative ion mode ESI, and HILIC/UPLC-MS/MS with negative ion mode

ESI. All methods utilized a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. Raw data was extracted, peak-identified and QC processed using Metabolon's hardware and software. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. The raw data counts were used for analysis. Data points were normalized to the control group and expressed as fold changes and the statistics were based on normalized values.

Electron transport chain (ETC) analysis. ETC enzyme activity was determined as previously described[11,12]. After sonication, protein concentration was determined by Bradford assay and ETC complexes and citrate synthase (CS) activity was measured using a Tecan Infinite M200 microplate plate reader. The enzyme activity data were normalized to the protein concentration.

Mitochondrial DNA copy (mtDNA) number. mtDNA copy number was quantified as previously published described[13]. Briefly, a SYBR Green-based qPCR assay with primers specific to tRNA region of mtDNA and β2-microglobulin region of nuclear DNA was used for the quantification of mtDNA.

Antisense Oligonucleotide (ASO) Synthesis. ASOs consist of 20 chemically modified nucleotides, five 2' -O-methoxyethyl-modified nucleotides at each end separated by ten DNA nucleotides in the center. The backbone of the ASOs consists of a mixture of modifications from 5- to 3- are: 1-PS (Phosphorothioate), 4-PO (phosphodiester), 10-PS, 2-PO and 2-PS. Rictor-ASO (GTTCACCCTATACATTACCA) targeting mouse Rictor mRNA and a Control-ASO (CCTATAGGACTATCCAGGAA) were developed and synthesized by Ionis Pharmaceutical as previously described[14]. The algorithm Bowtie[15] was used to determine potential off-targets for the Rictor-ASO and confirmed that Rictor-ASO binds with 100% complementarity (zero mismatch) to the mouse *Rictor* transcript and does not bind to any other mRNA with full complementary in the mouse transcriptome.

Intracerebroventricular (ICV) ASO Injection. Surgical site was sterilized with betadine and 70% alcohol. Buprenorphine 1mg/kg was administered subcutaneously 1 hour before surgery for pain control. Mice were anaesthetized with 3% isoflurane for 10 min before placing on a computer-guided stereotaxic instrument (Kopf instruments) fully integrated with the Franklin and Paxinos35 mouse brain atlas through a control panel. Anesthesia was continuously delivered (2% isoflurane) throughout the surgery. A midline incision was cut on the skull skin and a small hole was drilled through the skull above the right lateral ventricle. *Rictor* ASO (500 μg) was diluted in PBS and then ICV-delivered (10 μl) using a Hamilton syringe and glass needles. Control mice received either control-

ASO (500 μg) or the same volume (10 μl) of PBS. The coordinates used for ICV injection were: AP= - 0.2 mm, ML = 1 mm, DV = - 3 mm. The needle was left for 2 min on the site of injection. The incision was manually closed with sutures. Mice were maintained on a 39 °C isothermal pad while anaesthetized and during recovery.

qRT-PCR. Total RNA was extracted from freshly dissected tissue using RNeasy Plus Universal Mini Kit (Qiagen). 2μg total RNA was used for reverse transcription (Superscript VILO cDNA synthesis kit, Invitrogen). qRT-PCR was performed using PowerUp SYBR Green Master Mix (Thermo Fisher Scientific) according to the manufacturer's protocol. mRNA levels of target genes were normalized to GAPDH RNA and data are presented as fold-change relative to control levels. Primer set sequences are listed in **Extended Data Table 1.**

Statistical analyses. No statistical methods were used to pre-determine sample sizes, but our sample sizes are selected based on previous studies published in the field (see Life Science Reporting Summary for references). Animals in the same litter were randomly assigned to different treatment groups in various experiments. No animals or data points were excluded from the analysis. Normality testing and F-tests of homogeneity of variances were performed before choosing statistical tests. Statistics were based on the two-sided Student's *t*-test or Mann-Whitney Rank Sum test for two-group comparisons (for data sets that were not normally distributed). One-way ANOVA followed uncorrected Fisher's

LSD method for pairwise comparisons analysis was performed for multiple comparisons, unless otherwise indicated. $P < 0.05$ was considered significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Extended Data References

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Supplementary Table 1. Sequence of primers used in qRT-PCR experiments

Primer name	Primer Sequence 5' –> 3'
Rictor F	TCGATCTGACCGAGAACCTT
Rictor R	GTTATTCAGATGGCCCAG
Nisch F	CTGACCCCGCCCCCT
Nisch R	ACCTGGATGACATAAACCGTGT
Pus7l F	GGAAGCGAGGCACCGTGA
Pus7l R	GGTGAGGTTTTTATGCTGCCC
GM6943 F	AGTGCAGGCCATCTACAAGA
GM6943 R	TTGTCGGTTCGATTCTCGGG
Gm9265F	GCTCGAGCAGAAGGTCAAGT
Gm9265 R	AGGGACATTTTGCCTGCAC
Gm9295 F	TGTTTGACACCAACAGTGTCAGC
Gm9295 R	CTTGTAGATGGCCTGCACTATCTC
GM5954 F	TAGTGCAGGCCATCTACAAGA
GM5954 R	TTGTCTGTTCGCTTCTCGG