Supporting material

"mTOR Inhibition Suppresses Established Epilepsy in a Mouse Model of Cortical Dysplasia" (Nguyen *et al*., 2014)

Supplemental methods

Video-electroencephalography (EEG)

Electrode implantation: Mice were anesthetized with a ketamine/xylazine/acepromazine mixture (obtained from Baylor College of Medicine Center for Comparative Medicine) and positioned in a stereotaxic frame. Four stainless steel electrodes (Plastics One, Roanoke, VA, USA) were placed bilaterally over the cortex; one in each of the left and right anterior cortex (2.0 mm posterior and 2.2 mm lateral to bregma) and one in each of the left and right posterior cortex (5.0 mm posterior and 2.2 mm lateral to bregma). A reference electrode was placed anterior to bregma and a ground electrode was placed in the cervical paraspinous area. Mice were allowed to recover for at least four days before video-EEG recording.

Video-EEG analysis: All recordings were reviewed for epileptiform activity including interictal spikes [fast (<200 msec) single events of high amplitude (2X baseline)], repetitive spike and polyspike activity [trains of spikes, polyspikes, or spike-and-slow wave discharges lasting <10 sec], and seizures [repetitive spike or spike-and-slow wave activity lasting ≥10 sec]. Because subclinical polyspike and seizure activity was nearly continuous in older NS-*Pten* KO mice, we were unable to count the frequency of individual events. Therefore, we quantified the percent time animals spent in epileptiform activity. To quantify time spent in epileptiform activity, we selected a representative 30-min sample (Fig. 1) or 10-sec epochs every 15 min over 3 hours (Fig. 4) of EEG recording after a 1-hour acclimation period and counted the amount of

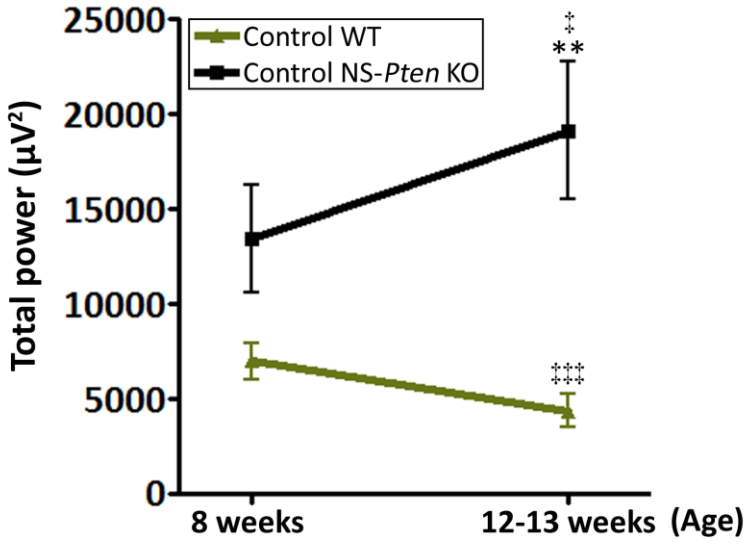
time (in sec) spent in epileptiform activity. Data were reported percentages of total observation time. Video-EEG recordings were also reviewed for motor seizures [electrographic seizures accompanied by motor changes involving myoclonic jerks, tonic-clonic activity, wild running, and loss of postural control (rearing and falling)], and the number of events per recording session per mouse was reported as events/hour. EEG signals were digitized at a sampling rate of 250 Hz and filtered between 0.5 and 70 Hz for automated spike detection and total power analysis using LabChart 7 software (AD Instruments, Colorado Springs, CO, USA). For automated analysis, spikes were defined as waveforms of negative polarity ≥500 μV and within 60 msec in width from the middle of the peak. The number of spikes in a 90-min epoch of recording after a 30-min acclimation was counted and reported as spikes/sec. Total power in the frequency range 0.5–50 Hz was analyzed using Fast Fourier Transformation in ten 10-sec samples randomly chosen from baseline EEG activity and averaged. EEG analyses were conducted by an investigator blinded to genotype and treatment. Multiple investigators (LHN, ALB, ARG, CNS, AEA) independently reviewed the EEG recordings.

Antibodies

The primary antibodies used for western blotting were as follows: p-S6 (S240/244) (1:1000), p-AKT (S473) (1:200), GFAP (1:1000, Cell Signaling, Danvers, MA, USA), IBA1 (1:1000, Wako, Cambridge, MA, USA), p-S6 (S240) (1:100, Dako, Carpinteria, CA, USA), and NeuN (1:1000, Millipore, Billerica, MA, USA). The primary antibodies used for immunohistochemistry were as follows: p-S6 (S240/244) (1:1000), p-AKT (S473) (1:200), GFAP (1:1000, Cell Signaling, Danvers, MA, USA), IBA1 (1:1000, Wako, Cambridge, MA,

USA), p-S6 (S240) (1:100, Dako, Carpinteria, CA, USA), and NeuN (1:1000, Millipore, Billerica, MA, USA).

Figure S1: Changes in total EEG power with age in NS-*Pten* KO and WT mice



Total EEG power in NS-*Pten* KO and WT mice was quantified using Fast Fourier Transformation analysis. No differences between NS-*Pten* KO and WT total power were observed at postnatal week 8. By postnatal weeks 12-13, there was a significant decrease in WT total power and increase in NS-*Pten* KO total power. n=5-6 mice per group; **$p<0.01$ (compared to age-matched WT), ‡ $p<0.05$, ‡‡‡ $p<0.001$ (compared to postnatal weeks 8) by two-way repeated measured ANOVA with Bonferroni post-hoc test, error bars = ±SEM.