

PTEN DELETION IN THE ADULT DENTATE GYRUS INDUCES EPILEPSY

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1 **ABSTRACT**

2 Embryonic and early postnatal promoter-driven deletion of the phosphatase and tensin
3 homolog (PTEN) gene results in neuronal hypertrophy, hyperexcitable circuitry and
4 development of spontaneous seizures in adulthood. We previously documented that
5 focal, vector-mediated PTEN deletion in mature granule cells of adult dentate gyrus
6 triggers dramatic growth of cell bodies, dendrites, and axons, similar to that seen with
7 early postnatal PTEN deletion. Here, we assess the functional consequences of focal,
8 adult PTEN deletion, focusing on its pro-epileptogenic potential. PTEN deletion was
9 accomplished by injecting AAV-Cre either bilaterally or unilaterally into the dentate gyrus
10 of double transgenic PTEN-floxed, ROSA-reporter mice. Hippocampal recording
11 electrodes were implanted for continuous digital EEG with concurrent video recordings in
12 the home cage. Electrographic seizures and epileptiform spikes were assessed manually
13 by two investigators, and correlated with concurrent videos. Spontaneous electrographic
14 and behavioral seizures appeared after focal PTEN deletion in adult dentate granule cells,
15 commencing around 2 months post-AAV-Cre injection. Seizures occurred in the majority
16 of mice with unilateral or bilateral PTEN deletion and led to death in several cases. PTEN-
17 deletion provoked epilepsy was not associated with apparent hippocampal neuron death;
18 supra-granular mossy fiber sprouting was observed in a few mice. In summary, focal,
19 unilateral deletion of PTEN in the adult dentate gyrus suffices to provoke time-dependent
20 emergence of a hyperexcitable circuit generating hippocampus-origin, generalizing
21 spontaneous seizures, providing a novel model for studies of adult-onset epileptogenesis.
22

23 **1. Introduction**

24
25
26 Epilepsy is a complex, multifactorial entity, with both genetic and environmental origins.
27 Among genetic risk factors for epilepsy, dysregulation of the mechanistic target of
28 rapamycin (mTOR) pathway, as takes place in tuberous sclerosis, is well-established.
29 However, the mTOR pathway consists of numerous key enzymes with protean cellular
30 functions. Thus, it is unclear which deficits in the distinct components of the pathway lead
31 to epilepsy. Mutations of the phosphatase and tensin homolog (PTEN) gene, an important
32 upstream negative regulator of the mTOR pathway, are one candidate, motivating studies
33 of consequences of targeted mutations of PTEN in murine models.
34

35 The general strategy for studies of PTEN has been to use promoter-driven Cre expression
36 in PTEN-floxed mice to genetically delete PTEN in particular populations of neurons
37 and/or glia in early development. For example, when deletion is driven by the GFAP
38 promoter, PTEN is deleted in astrocytes and neurons in widespread brain regions during
39 embryonic development. In this situation, there is dramatic brain and neuronal
40 hypertrophy accompanied by progressive development of spontaneous seizures and
41 early mortality (Backman et al., 2001; Fraser et al., 2008; Fraser et al., 2004; Kwon et al.,
42 2003; Kwon et al., 2001). Subsequent studies have assessed the consequences of
43 deleting PTEN in the early postnatal period selectively in newborn granule cells of the
44 dentate gyrus by driving Cre expression either under the Gli1 promoter or via intradentate
45 retroviral injection, for example. These studies also documented dramatic neuronal
46 hypertrophy in the dentate gyrus, including enlarged granule cell bodies, increased

47 dendrite complexity, and aberrant expansion of mossy fiber axon connectivity (Arafa et
48 al., 2019; LaSarge et al., 2015; Pun et al., 2012; Williams et al., 2015). Physiological
49 consequences of these morphological changes have been reported to include the
50 development of spontaneous seizures and the formation of hyperexcitable, pro-epileptic
51 hippocampal circuits (LaSarge et al., 2016; Pun et al., 2012; Santos et al., 2017; Williams
52 et al., 2015).

53

54 In contrast to previous studies of deleting PTEN during development, our lab has focused
55 on consequences of deleting PTEN in fully-mature neurons in adult rodents. Of note, we
56 discovered that both AAV-Cre mediated PTEN deletion in PTEN-floxed adult mice
57 (Gallent & Steward, 2018) and AAV-shRNA mediated PTEN knockdown in adult rats
58 (Steward et al., 2019) results in re-initiation of a growth phenotype in mature cortical
59 neurons involving increases in cell body size and dendritic arborization. More recently,
60 we have expanded our studies to consequences of focal deletion of PTEN in the mature
61 dentate gyrus of adult mice. Injections of AAV-Cre into the dentate gyrus of adult PTEN-
62 floxed mice resulted in focal PTEN deletion, triggering growth of granule cell bodies,
63 elongation of dendrites, robust formation of additional spines (and presumably new
64 synapses) on elongated dendritic segments, and expansion of mossy fiber terminal fields
65 in target areas (Yonan & Steward, 2023). In this collection of studies, casual observations
66 did not reveal spontaneous behavioral seizures out to 6 months following PTEN deletion.
67 However, two mice died during the night after exhibiting no signs of ill health and these
68 sudden deaths might have occurred during a spontaneous seizure. Despite the fact that
69 no seizures were observed in these mice, abnormal physiological activity including
70 electrographic seizures cannot be excluded without continuous recording and monitoring.

71

72 The present study assessed the functional consequences of focal PTEN deletion in the
73 adult dentate gyrus using continuous video-EEG focusing specifically on the development
74 of spontaneous seizures (epileptogenesis). We find that both bilateral and unilateral
75 vector-mediated PTEN deletion in adult PTEN-floxed, ROSA-reporter mice lead to the
76 development of spontaneous seizures beginning around 2 months after AAV-Cre
77 injection. Of note, there were three instances of sudden death during a prolonged seizure
78 (SUDEP). Unlike excitotoxin models of epilepsy, histological assessments revealed no
79 apparent loss of hippocampal neurons in CA3. Thus, focal PTEN deletion provides a
80 novel, toxin/convulsant-free model of adult-onset temporal lobe epilepsy (TLE) in which
81 the pathophysiology is initiated by a localized focus within the hippocampus.

82

83

84 **2. Materials and methods**

85

86 *2.1 Experimental mice*

87

88 Experiments involved two transgenic strains of mice developed in our local breeding
89 colony. The first strain was generated by crossing PTEN-floxed mice carrying lox-p
90 flanked exon 5 of the PTEN gene (RRID: IMSR_JAX:004597) with ROSA26tdTomato
91 (*Rosa^{tdTomato}*) reporter mice having a lox-P flanked STOP cassette in the Rosa locus
92 upstream of a tandem dimer tomato (tdT) fluorescent protein sequence (RRID:

93 IMSR_JAX:007905). This double transgenic strain is designated PTEN^{ff}/Rosa^{tdTomato}. All
94 studies involved mice that were homozygous at the transgenic loci. One other line was
95 created by crossing this strain with Thy1-eYFP mice, originally purchased from the
96 Jackson Labs, to generate mice that were homozygous at the PTEN^{ff}/Rosa^{tdTomato} loci
97 and hemizygous for Thy1-eYFP. For simplicity, these mice will be referred to as PTEN/tdT
98 mice, regardless of their eYFP expression. All the strains used in these studies were
99 generated in our lab, and therefore have different genetic backgrounds than the original
100 mice from Jackson Labs.

101
102

103 *2.2 Surgical procedure, AAV-Cre injections into the dentate gyrus*

104

105 All experimental procedures were approved by the Institutional Animal Care and Use
106 Committee (IACUC) at the University of California, Irvine. Studies involved adult mice (at
107 least 2 months of age at the time of AAV-Cre injection). Briefly, mice were anesthetized
108 with Isoflurane (2-2.5%), placed in a stereotaxic device, and small cranial window was
109 created above the injection site. Using a 10 μ l Hamilton syringe with a pulled glass pipette
110 tip, either a single unilateral or bilateral injection of AAV2-Cre (Vector Bio Labs, 7011) or
111 AAV2-GFP (Vector Bio Labs, 7004) was made at +/-1.3mm lateral and +2.2mm anterior
112 to lambda at a depth of -1.6mm from the cortical surface. Each injection was 0.6 μ l in
113 volume (1x10¹² genome copies (GC)/ml in 1x phosphate-buffered saline (1xPBS, 20mM,
114 pH 7.4) with 5% glycerol) and was performed over 4 minutes. The pipette was left in place
115 for an additional 2 minutes before removal. Following completion of the surgery, mice
116 were allowed to recover for 48 hours in a cage on a 37°C heating pad and then were
117 returned to standard housing conditions. Surgeries were performed in four separate
118 iterations, termed Cohorts, three injected with AAV-Cre and a fourth injected with AAV-
119 GFP (Table 1).

120
121

122 *2.3 Surgical procedure: Bilateral and unilateral intrahippocampal EEG probe* 123 *placement*

124

125 At 4-6 weeks post AAV-Cre injection, mice underwent a second procedure for placement
126 of either bilateral or unilateral intrahippocampal electrodes, as described previously (Chen
127 et al., 2021; Garcia-Curran et al., 2019). Mice were anesthetized with Isoflurane (1.5-
128 2.5%) and placed in a stereotaxic device. A scalp incision was made to expose the skull
129 and the skull was cleaned and dried with a 30% hydrogen peroxide solution. Two burr
130 holes were placed in the skull above the hippocampus in each hemisphere at +/-1.6mm
131 lateral and -1.9 posterior to bregma. Twisted wire electrodes were placed at a depth of -
132 3.0 to -2.2mm below the brain surface (P Technologies, E363/2-2TW/SPC). For mice with
133 a unilateral EEG probe, the electrode was placed into the right hippocampus, contralateral
134 to AAV-Cre injection. Another 2 burr holes were created above on the left side of the
135 cerebellum and right frontal cortex for placement of ground screw electrodes (P
136 technologies, E363/20). Each electrode was then placed into a 6-channel pedestal (P
137 Technologies, MS363). Electrodes, screws, pedestals, and wiring were held in place
138 using a combination of cyanoacrylate and dental cement for creation of a head cap.

139 Following completion of the surgery, mice were allowed to recover in a cage on a 37°C
140 heating pad and then were returned to standard housing conditions until transferred to
141 the continuous recording chambers. Information regarding animal numbers, attrition and
142 exclusions are listed in Table 1.

143
144

145 *2.4 Continuous video EEG recordings*

146

147 At 6-8 weeks post AAV-Cre injection (1-2 weeks following electrode placement), mice
148 were transferred into plexiglass cages and attached to a commutator to allow for free
149 movement during video-EEG recording. Continuous recordings and videos were
150 collected using the Lab Chart EEG analysis software (versions 7 and 8, AD Instruments),
151 as described previously (Chen et al., 2021; Dube et al., 2010). Bilateral recordings were
152 made in mice that received bilateral electrodes. In mice that received unilateral injections
153 and unilateral electrodes, recordings were made on the side contralateral to the injection.
154 Information on recording durations for each cohort of mice is listed in Tables 2-5.

155
156

157 *2.5 EEG analysis and scoring*

158

159 Analyses of EEG recordings were accomplished by investigators blind to animal group.
160 A subset of the EEGs were independently analyzed by two investigators, with excellent
161 concordance. Recordings were scanned for seizures, defined as events lasting more than
162 6 seconds and consisting of EEG polyspikes or sharp-waves (amplitude > 2-fold
163 background) (Chen et al., 2021; Dube et al., 2006; Pitkanen et al., 2002). In addition, the
164 progression of the amplitude and the frequencies of the discharges throughout a given
165 seizure were analyzed, because typical seizures are characterized by increasing
166 amplitude and slowing frequency as the seizure progresses. Finally, seizures required a
167 period of post-ictal depression characterized by a dramatic decrease in EEG amplitude.
168 In mice with bilateral recording electrodes, both ipsilateral and contralateral EEG
169 recordings were scored. In addition to electrographic analyses, videos accompanying
170 seizures were analyzed as described (Dube et al., 2006; Dube et al., 2010). Briefly, we
171 evaluated typical behaviors associated with limbic-onset seizures, including sudden
172 cessation of activity, facial automatisms, head-bobbing, prolonged immobility with staring.
173 These progressed to alternating or bilateral clonus, rearing and falling (Racine). Mice
174 were considered epileptic if they had at least one documented spontaneous seizure as
175 defined above. Data are represented as the percentage of mice within each cohort to
176 develop seizures, cumulative number of seizures for each individual animal over time, the
177 total number of seizures for each mouse, the latency to develop spontaneous seizures,
178 and the average number of seizures per days recorded.

179
180

181 *2.6 Tissue collection and histology*

182

183 At designated endpoints (Tables 2-5), mice were euthanized via intraperitoneal injections
184 of Fatal Plus® (390mg/ml pentobarbital sodium) and were intracardially perfused with 4%

185 paraformaldehyde in phosphate buffer (PFA). Brains were dissected and post-fixed in 4%
186 PFA for 48 hours, cryoprotected in 27% sucrose, and frozen in Tissue-Tek O.C.T.
187 compound. Brains were then cryosectioned at 30 μ m and stored in 1xPBS with 0.1% NaN₃
188 until processed for immunohistochemistry. For mice that were retrieved after being found
189 dead, brains were drop-fixed in 4% PFA and then prepared as above. The area of
190 transduction was visualized by tdTomato expression in PTEN/tdT mice that received
191 AAV-Cre and GFP expression in control mice that received AAV-GFP.

192
193 Sets of sections at 360 μ m intervals were processed for different histological markers. For
194 immunohistochemistry (IHC), sections were washed in tris-buffered saline (1xTBS,
195 100mM Tris, pH 7.4 and 150mM NaCl) then quenched for endogenous peroxidase activity
196 by incubation in 3% H₂O₂ for 15 minutes. Sections were then washed in 1xTBS and
197 blocked in blocking buffer (1xTBS, 0.3% Triton X-100, 5% normal donkey serum (NDS))
198 for 2 hours at room temperature. Sections were then incubated overnight at room
199 temperature in buffer containing primary antibodies for rabbit anti-PTEN (1:250, Cell
200 Signaling Technology 9188, RRID: AB_2253290), rabbit anti-pS6 Ser235/236 (1:250,
201 Cell Signaling Technology 4858, RRID: AB_916156), rabbit anti-Znt3 (1:1500, Millipore
202 ABN994), rabbit anti-cFos (1:1000, Millipore ABE457, RRID: AB_2631318), and mouse
203 anti-GAD67 (1:1000, Millipore MAB5406, RRID: AB_2278725). Sections were then
204 washed in 1xTBS, followed by a 2-hour incubation in buffer containing biotinylated donkey
205 anti-rabbit IgG (1:250, Jackson ImmunoResearch, 711-065-152), then washed again.
206 Visualization was accomplished through incubation in avidin-biotin complex (ABC)
207 reagent (Vectastain Elite kit, catalog #PK-6100; Vector Laboratories) and catalyzed
208 reporter deposition (CARD) amplification with Tyramide-FITC or Tyramide-AMCA.
209 Sections stained for rabbit anti-GFP (1:1500, Novus NB600-308, RRID:10003058) were
210 visualized using Alexa fluor-488 (1:250, Invitrogen A21206). Sections stained for GAD67
211 were visualized by DAB (Vector Laboratories, SK-4100), mounted on slides, dehydrated
212 through graded ethanol, cleared in xylenes, and coverslipped with DPX). All fluorescently
213 labeled sections were then mounted on 0.5% gelatin coated slides and counterstained
214 with Hoechst (1 μ g/mL).

215
216

217 *2.7 Extent of transduction of the dentate gyrus*

218

219 To determine the percent area of transduction through the dentate gyrus, sections spaced
220 360 μ m apart were assessed for percentage of the granule cell layer occupied by
221 tdTomato-positive granule cells as in our previous study (Yonan & Steward, 2023). Briefly,
222 images of the dentate gyrus were taken with a 10x objective using an Olympus AX80
223 microscope. Images were imported into NIH ImageJ FIJI, a border was drawn around the
224 Hoechst-positive granule cell layer, the drawn contour was transferred to the tdT-labeled
225 image, and the tdT-positive area within the contour was determined. The percent of the
226 granule cell layer that was tdT-positive throughout the rostro-caudal series of sections
227 was calculated in each mouse. Data are plotted as percent transduction through the
228 length of the dentate gyrus at 360 μ m intervals and percentage transduction of the entire
229 dentate gyrus for each mouse.

230

231 The extent of PTEN deletion within each mouse was qualitatively assessed for
232 transduction of the dentate gyrus and CA1 hippocampal subregion. These include
233 mild/moderate transduction of the CA1 in one or both hippocampi in addition to the
234 dentate gyrus or mistargeting of an injection in which the CA1 was transduced with
235 minimal transduction of the dentate gyrus. Each of these distinctions is noted alongside
236 each mouse in corresponding tables for each Cohort (Tables 2-5). Transduction of the
237 CA1 that was isolated to the needle tract alone was classified as an injection of only the
238 dentate gyrus.

239
240

241 *2.8 Granule cell size measurements*

242

243 Cell body sizes were measured in regions of dense transduction near the injection
244 epicenter. One set of sections for each mouse was stained with Cresyl violet and a
245 separate set of sections was assessed for the region of PTEN deletion through
246 immunohistochemistry and visualization of tdTomato, as described above. A single
247 section at the core of PTEN deletion and transduction was taken for granule cell size
248 measurements. Z-stack images of Cresyl violet stained sections were taken using a 44.4x
249 objective with a 1 μ m step size using the Keyence BZ-X800 microscope and imported into
250 ImageJ FIJI. Sampling was done by measuring 30 cells within the granule cell layer within
251 a 100 x 200 μ m region of interest in the ipsilateral dentate gyrus, and the homologous
252 regions in the contralateral granule cell layer. The mean cross-sectional area for PTEN
253 deleted and PTEN expressing granule cells was determined by first averaging cell sizes
254 from individual mice, then averaging for each hemisphere where n = 6 mice.

255

256 Measurements of the thickness of the molecular layer were taken at the core of
257 transduction using images taken with a 10x objective on an Olympus AX80 microscope
258 as a representation of maximal dendritic length (granule cell dendrites extend to the
259 border of the molecular layer). Measures of molecular layer width from ipsilateral and
260 contralateral sides were then compared.

261

262

263 *2.9 Assessment of mossy fiber projections*

264

265 Sections immunostained for the zinc transporter Znt3 were used to assess alterations in
266 granule cell axonal projections (mossy fibers). Images of both the ipsilateral and
267 contralateral dentate gyrus and CA3 were taken with a 10x objective using an Olympus
268 AX80 microscope and imported into ImageJ FIJI. Measurements were taken of the
269 thickness of the laminae containing mossy fibers as they exited the hilus on both
270 ipsilateral and contralateral sides.

271

272 To detect supra-granular mossy fibers, Znt3 labeled sections at the core of transduction
273 were used. Sections were scored as described by (Hunt et al., 2009): 0=little to no Znt3
274 labeling in granule cell layer; 1=mild Znt3 labeling in granule cell layer; 2=moderate
275 staining in the granule cell layer and punctuate staining in inner molecular layer; 3=dense
276 Znt3 labeling in inner molecular layer.

277 2.10 Statistical methods

278

279 Analyses were conducted without explicit knowledge of experimental group when feasible
280 (in most cases, the area of transduction was obvious due to growth of the dentate gyrus).
281 Graphs were created and statistical analyses were performed using GraphPad Prism
282 Software. One-way analysis of variance (ANOVA) was used to compare seizure
283 outcomes across cohorts. Two-way ANOVA was used for comparison of neuronal
284 morphological measures between contralateral and ipsilateral hemispheres in PTEN/tdT
285 mice. Sidak's multiple comparisons tests were used for comparisons between groups.
286 Relationships between neuronal outcome measures, percent PTEN deletion, or seizure
287 number were assessed by linear regression.

288

289

290 **3. RESULTS**

291

292 3.1 *Effective PTEN deletion in PTEN/tdT mice following unilateral and bilateral AAV-*
293 *Cre injections into the dentate gyrus*

294

295 Injections of AAV-Cre into the dentate gyrus of PTEN/tdT mice at 2 months of age resulted
296 in robust transduction of mature granule cells surrounding the injection site. Figure 1
297 illustrates representative images of mice with bilateral and unilateral AAV-Cre injections
298 collected between 2 and 4 months after injection. Figures 1A and 1B depict the regions
299 of granule cell transduction following bilateral AAV-Cre injection based on tdTomato
300 expression. In both dentate gyri, PTEN immunostaining was absent in the area of tdT
301 expression (Fig. 1C, D). Immunostaining for the phosphorylated form of ribosomal protein
302 S6 (a downstream marker of mTOR activation) revealed robust activation of S6
303 phosphorylation in the regions of PTEN deletion (Fig. 1E, F).

304

305 Figures 1I and 1J depict the regions of transduction following unilateral injection of AAV-
306 Cre into the left dentate gyrus (compare tdT expression in Fig. 1I with the lack of tdT
307 expression in the contralateral, non-injected dentate gyrus in Fig. 1J). PTEN deletion was
308 confirmed by lack of immunostaining in transduced, tdT-positive granule cells (Fig. 1K),
309 but remained intact in granule cells of the contralateral, non-injected dentate gyrus (Fig.
310 1L). Increases in immunostaining for pS6 were evident on the side of PTEN deletion (Fig.
311 1M) in comparison to the contralateral dentate gyrus (Fig. 1N).

312

313

314 3.2 *Spontaneous seizures develop following bilateral PTEN deletion*

315

316 Cohort 1, Bilateral PTEN deletion with bilateral EEG recording: Promoter-driven PTEN
317 deletion in the developing brain results in PTEN deletion throughout the dentate gyri of
318 both hemispheres (Kwon et al., 2001; Matsushita et al., 2016; Pun et al., 2012). To
319 determine if bilateral but focal PTEN deletion in mature granule cells of the adult dentate
320 gyrus results in pro-epileptic alterations to circuit function, recording electrodes were
321 implanted bilaterally into the hippocampi 4-6 weeks after bilateral injections of AAV-Cre.
322 Figure 1G, H illustrate examples of recording electrode tracks.

323 Animal attrition and exclusions: In Cohort 1 (Table 1), one mouse died prior to the EEG
324 electrode placement surgery; tissue for this mouse was unavailable for postmortem
325 analysis because the mouse was found dead by vivarium staff, and the carcass was
326 disposed of. Two of 11 mice (18%) died at 3 and 4 days after electrode probe placement
327 but prior to the initiation of EEG recordings. For 1 mouse, tissue was unavailable for
328 analysis because the mouse was found dead, and the carcass was disposed of. For the
329 remaining mouse, the brain was drop-fixed and was prepared for histology, which
330 revealed appropriate transduction of granule cells in both hemispheres, with no
331 transduction of other hippocampal subregions. The cause of death in these mice is
332 unknown given that death occurred prior to initiation of EEG/video recordings. Another 1
333 of 11 mice (9%) exhibited seizures during the recording period but was excluded following
334 tissue analysis due to a large brain lesion of unknown origin that may have influenced
335 seizure onset and incidence.

336
337 Seizure onset and incidence: Four of five mice in which EEG was recorded (80%)
338 developed spontaneous electrographic and behavioral seizures (Fig. 7A). The first
339 seizures were observed at an average of 90.25 +/- 28.02 days (median = 83 days) post
340 AAV-Cre injection (Fig. 7B). These mice experienced an average of 28.50 +/- 12.61 total
341 seizures (median = 30 seizures) over the recording period (Fig. 2A, 7C, Table 2) with an
342 average of 0.91 +/- 0.098 seizures per day (median = 0.94 seizures per day, Fig. 7D).
343 Mice with bilateral PTEN deletion exhibited seizures that originated in either hippocampus
344 (Fig. 2B, C). The frequency of the seizures did not increase over time (Fig. 2A) and
345 seizures occurred in clusters. This is in contrast to reports with developmental PTEN
346 deletion in which seizures increase in frequency and severity over time (Kwon et al.,
347 2001). In this cohort, one mouse with bilateral PTEN deletion died at 147 days post AAV-
348 Cre injection during a prolonged focal-onset, generalized seizure, after exhibiting a total
349 of 12 seizures during the recording period (Fig. 2A, animal 329-21).

350
351 Interestingly, the majority of mice that were recorded for longer than 2 months after PTEN
352 deletion developed seizures, suggesting an approximately 2-3 month latency period for
353 development of epilepsy. In Cohort 1, two mice were recorded for only two-months and
354 were sacrificed early to verify electrode placement (Table 1). Whether these 2 mice would
355 have developed seizures had they been recorded for longer periods of time is unknown,
356 so these two mice are excluded from the calculation of seizure prevalence.

357
358 Patterns of AAV-Cre transduction in PTEN/tdT mice with bilateral PTEN deletion: There
359 was some variability in transduction efficacy despite the use of consistent stereotaxic
360 coordinates, injection parameters, and vector titers. Table 2 provides detailed
361 descriptions of the pattern of transduction and PTEN deletion in each mouse, specifically
362 the accuracy of transduction of the dentate gyrus and the transduction of other
363 hippocampal subregions. Three mice with bilateral transduction also had mild
364 transduction of the CA1 region in one or both hippocampi, one mouse had bilateral
365 transduction in which targeting in the right hippocampus was predominately in the CA1
366 region, and one mouse with bilateral transduction displayed mild transduction of the left
367 CA1 and targeting in the right hippocampus predominately of the CA1. Of note, this final

368 animal is the one that died during a verified seizure. It remains to be determined how
369 these variations and patterns of PTEN deletion impact circuit and network function.

370

371

372 *3.3 Spontaneous seizures develop following unilateral PTEN deletion*

373

374 Cohort 2: Unilateral PTEN deletion with bilateral EEG recording: Temporal lobe epilepsy
375 in humans often develops from a unilateral seizure focus. To determine if unilateral PTEN
376 deletion is sufficient for seizure development, we carried out a pilot study in which 6 mice
377 received unilateral injections of AAV-Cre into the dentate gyrus followed by placement of
378 bilateral intrahippocampal electrodes. Figures 1O and P illustrate electrode tracks in a
379 mouse with unilateral PTEN deletion.

380

381 Patterns of transduction: In Cohort 2, three of 6 mice (50%) were excluded from analysis
382 due to death (two mice at 1- and 47-days post EEG implantation surgery) or due to
383 incorrect electrode placement (Table 1). Two of three mice included in analysis (Table 3),
384 as well as mice with tissue available postmortem, all displayed appropriate and well
385 targeted transduction of only the dentate gyrus. In these cases, PTEN expression was
386 retained in both the CA1 and CA3. One of three mice included in analysis displayed some
387 transduction of the CA1.

388

389 Seizure onset and frequency: In cohort 2, 2 of 3 mice (67%) developed spontaneous
390 seizures (Fig. 7A) at an average of 67.50 +/- 0.71 days post AAV-Cre injection (median =
391 67.50 days), having an average of 43.00 +/- 11.31 seizures (median = 43 seizures) over
392 the recording period, and an average of 1.60 +/- 1.75 seizures per day (median = 1.60
393 seizures per day) (Fig. 3A, Fig. 7B-D). In mice with unilateral PTEN deletion, seizures
394 consistently originated in the PTEN deleted dentate gyrus and then propagated to the
395 contralateral, PTEN-expressing dentate gyrus (Fig. 3B, C). As seen with bilateral PTEN
396 deletion, seizures were clustered, did not increase in frequency over time, and began
397 more than 2 months post PTEN deletion. In this cohort, animal 305-1 displayed the
398 greatest number of total seizures over time, eventually dying suddenly at 87 days post
399 injection during a prolonged seizure, having experienced a total of 51 seizures during the
400 recording period.

401

402

403 *3.4 Spontaneous seizures develop following unilateral PTEN deletion with contralateral 404 EEG probe placement*

405

406 Cohort 3, unilateral PTEN deletion with unilateral EEG recording: It was noteworthy that
407 several mice in cohorts 1 and 2 died suddenly, and in 2 cases, death occurred during a
408 prolonged seizure. In our previous studies of anatomical consequences of PTEN deletion
409 in the adult dentate gyrus, in which mice survived up to 6 months after PTEN deletion
410 (Yonan & Steward, 2023), behavioral seizures were not observed and there were only
411 two instances of sudden death. Because we had not previously seen high mortality rates
412 in mice with PTEN deletion but no implanted recording electrodes, we wondered whether

413 PTEN deletion and electrode placement within the same regions could synergistically
414 trigger more severe seizures resulting in increased incidence of sudden death.

415
416 To address this, PTEN/tdT mice (Cohort 3, Tables 1 and 4) received unilateral injections
417 of AAV-Cre into the dentate gyrus followed by unilateral EEG electrode placement into
418 the contralateral dentate gyrus (Fig. 4G, H). As above, AAV-Cre injection resulted in
419 expression of tdTomato in transduced granule cells in the ipsilateral dentate gyrus (Fig.
420 4A vs. 4B), PTEN deletion in transduced cells alone (Fig. 4C vs. 4D), and activation of S6
421 phosphorylation in PTEN deleted granule cells (Fig. 4E vs. 4F). Notably, in Cohort 2,
422 seizures recorded in mice with unilateral AAV-Cre injections always originated in the
423 PTEN deleted dentate gyrus and consistently propagated to the contralateral
424 hippocampus (Fig. 3B, C). Therefore, in this paradigm seizures recorded in the
425 contralateral dentate gyrus are likely propagated from the PTEN-deleted dentate gyrus.

426
427 Seizure onset and incidence: Mice were recorded for 4 months post injection. Seven of
428 seven mice recorded developed spontaneous seizures at varying frequency (Table 4, Fig.
429 5, Fig. 7A). The average time to first seizure in this cohort of mice was 69.29 +/- 5.68 days
430 (median = 71 days) with an average of 28.43 +/- 24.64 total seizures (median = 23
431 seizures) over the recording period, and 1.35 +/- 2.09 seizures per day (median = 0.75
432 seizures per day). One mouse died suddenly at 77 days post AAV-Cre injection during a
433 seizure, having had a total of 12 seizures (Fig. 5A, animal 69-29). These results strongly
434 support the conclusion that unilateral PTEN deletion in the mature dentate gyrus is
435 sufficient to result in the formation of a circuit that eventually leads to spontaneous
436 electrographic and behavioral seizures. While not an entirely isolated circuit, this
437 paradigm mostly eliminates the possibility of a two-hit model of seizure initiation.

438

439

440 *3.5 Unilateral or bilateral AAV-GFP injections do not trigger seizures*

441

442 Recently, a toxic effect of AAV virus injection on dentate gyrus granule cells has been
443 reported (Johnston et al., 2021). Therefore, as a control for the possibility that injections
444 of a control AAV with intrahippocampal EEG probe placement would be sufficient to lead
445 to spontaneous seizures or cell death, PTEN/tdT mice received unilateral or bilateral
446 injections of AAV-GFP into the dentate gyrus followed by continuous video EEG
447 recordings (Table 5). A total of 4 mice were included in this cohort (Cohort 4). Two mice
448 (33%) were excluded following anatomical analysis due to mistargeted viral transduction
449 (Table 1). All survived each surgical procedure, and none of the mice with AAV-GFP
450 injections developed spontaneous seizures (Fig. 6G, H, I).

451

452 Figure 6 illustrates representative images from a mouse with unilateral AAV-GFP
453 injections recorded for 100 days post injection. In Figure 6A, AAV-driven GFP expression
454 is evident throughout the dentate gyrus (green) and is lacking in the contralateral, non-
455 injected dentate gyrus (Fig. 6B). Regardless of injection location, immunostaining for
456 PTEN revealed intact PTEN expression in both hemispheres (Fig. 6C, D) and
457 immunostaining for pS6 is comparable on the two sides of the brain (Fig. 6E, F) indicating
458 no mTOR activation.

459 3.6 Extent of PTEN deletion correlates with seizure outcomes

460

461 Across Cohorts, PTEN deletion resulted in spontaneous seizures in 80, 67, and 100% of
462 mice accessed (Fig. 7A), respectively. Despite our various approaches, there was no
463 significant difference in the average latency to the first spontaneous seizure (Fig. 7B; one-
464 way ANOVA: [F (2,10) = 2.489, p=0.1327]), the average total number of seizures (Fig.
465 7C; one-way ANOVA: [F (2,10) = 0.4214, p=0.6673]), or the average number of seizures
466 per day (Fig. 7D, one-way ANOVA: [F (2,10) = 0.1313, p=0.8785]) across all cohorts. We
467 therefore wondered if the amount of PTEN deletion in an individual animal would influence
468 seizure outcomes. The mice with electrodes positioned contralateral to the PTEN deleted
469 dentate gyrus in Cohort 3 allowed for detailed assessment of area of PTEN deletion
470 without the complication of an implanted recording electrode. The area of transduction in
471 these mice was characterized by a core in which most cells were tdT-positive, with
472 transduction diminishing in the rostral and caudal directions (Fig. 8A). The average
473 percent area transduced throughout the entire length of the dentate gyrus of PTEN/tdT
474 mice was 36.16% +/- 6.79% (Fig. 8B).

475

476 Interestingly, percent transduction was correlated with the total number of seizures in
477 each mouse ($r^2=0.6395$) where total number of seizures experienced over the recording
478 period was greater in mice with larger areas of PTEN deletion within the dentate gyrus
479 (Fig. 8C). Percent transduction was not correlated with the latency to the first seizure
480 ($r^2=0.1728$) or the average number of seizures per day ($r^2=0.06541$) for each mouse (data
481 not shown). In this cohort of mice, 2 of 7 mice had moderate transduction of CA1
482 pyramidal cells (Table 4); seizure number in these mice was within the range of the mice
483 with transduction limited to the dentate gyrus, suggesting that some transduction of the
484 CA1 did not alter seizure development.

485

486

487 3.7 Enlargement of granule cell bodies and processes after PTEN deletion

488

489 As in our other studies, a notable consequence of PTEN deletion was the enlargement of
490 granule cell bodies and processes which was evident at 4 months post AAV-Cre injection.
491 Figure 9A illustrates the enlargement of granule cell somata in the ipsilateral dentate
492 gyrus (left) when compared to granule cells in the contralateral, PTEN-expressing dentate
493 gyrus (right) in Cresyl violet stained sections. Soma cross sectional area was 127.50 +/-
494 15.93 μm^2 for PTEN deleted granule cells vs. 67.96 +/- 14.19 μm^2 for PTEN expressing
495 granule cells (Fig. 9B). This approximately 2-fold increase in granule cell soma size is
496 consistent to what we observed in our previous study (Yonan & Steward, 2023).

497

498 Enlargement of cell body size was accompanied by increases in the width of the molecular
499 layer (Fig. 9D). We have previously confirmed that measurements of molecular layer
500 thickness can serve as an indicator of apical dendrite length, given that dendrites typically
501 extend to the hippocampal fissure (Yonan & Steward, 2023). Molecular layer thickness
502 was 315.90 +/- 44.94 μm in the PTEN deleted dentate gyrus vs. 194.70 +/- 21.02 μm in
503 the contralateral dentate gyrus (Fig. 9E), confirming PTEN-deletion induced expansion of
504 dendritic arbors.

505 To assess whether PTEN deletion led to expansion of mossy fiber projections to CA3,
506 sections at the core of transduction were stained for the zinc vesicular transporter, Znt3.
507 As illustrated in Figure 9G, the collection of mossy fibers as they exit the hilus, which we
508 term the mossy fiber tract (MFT) was notably thicker in the PTEN deleted dentate gyrus
509 compared to the contralateral, PTEN-expressing dentate gyrus (MFT, red lines). Mossy
510 fiber tract thickness was $196.80 \pm 19.30 \mu\text{m}$ in the ipsilateral dentate gyrus vs. $135.0 \pm$
511 $14.80 \mu\text{m}$ in the contralateral dentate gyrus (Fig. 9H). This expansion is again similar to
512 what we observed in our previous study (Yonan & Steward, 2023).

513
514 Two-way ANOVA for all 3 parameters, collectively, revealed an overall significance for
515 ipsilateral vs. contralateral sides [F (1,29) = 96.63, $p < 0.0001$], a significance for
516 measurement location [F (2,29) = 120.6, $p < 0.0001$], and a significant interaction [F (2,29)
517 = 5.816, $p = 0.0075$]. Sidak's multiple comparisons tests were also significant for somata,
518 molecular layer, and mossy fiber tract measurements (see Figure 8 legend for statistics).
519 No correlations were found between somata or process measurements and total seizure
520 number for each animal (Fig. 9C, F, I).

521
522

523 *3.8 Development of supra-granular mossy fibers following PTEN deletion*

524

525 The growth of mossy fiber axons into the inner molecular layer, which indicates the
526 formation of recurrent excitatory connections amongst granule cells, is a hallmark of
527 different models of temporal lobe epilepsy (Althaus & Parent, 2012). We have previously
528 reported the presence of supra-granular mossy fibers in some mice assessed at 4 months
529 post PTEN deletion with greater than 40% transduction of the dentate gyrus (Yonan &
530 Steward, 2023). We were therefore curious if similar ectopic projections would be seen in
531 mice that display spontaneous seizures.

532

533 To quantify this, we used a modified scoring system from (Hunt et al., 2009) where a
534 score of 0 indicates no mossy fibers in the granule cell layer and a score of 3 indicates
535 dense mossy fiber staining in the inner molecular layer. Sections stained for Znt3 at the
536 core of PTEN deletion for each mouse, as well as the contralateral dentate gyrus were
537 assessed according to this metric.

538

539 At 4 months post deletion (Fig. 10A), 3 of 6 mice showed slight Znt3 labeling in the granule
540 cell layer (score of 1), 2 of 6 mice displayed moderate Znt3 labeling in the granule cell
541 layer with mild labeling in the inner molecular layer (score of 2), and 1 of 6 mice showed
542 dense Znt3 labeling in the inner molecular layer (score of 3). Collectively 50% of mice
543 ($n=3$) developed at least moderate Znt3 labeling in the inner molecular layer (score ≥ 2 ,
544 Fig. 10B). All 3 mice had greater than 35% transduction of the dentate gyrus, although
545 two mice with similar percent transductions only showed Znt3 labeling in the granule cell
546 layer that did not extend into the inner molecular layer. Regression analysis of the
547 relationship between percent transduction and mossy fiber score revealed a positive
548 relationship (Fig. 10C, $r^2=0.4563$). Of note, mice with higher mossy fiber scores
549 experienced a greater total number of seizures over the recording period (Fig. 10D,
550 $r^2=0.6244$).

551 3.9 Immunocytochemical evidence for seizures in mice without electrode implantation

552

553 The combined evidence above leaves little doubt that a focal area of PTEN deletion in
554 the dentate gyrus of mature mice leads to the development of spontaneous seizures over
555 time. The only small caveat is the implantation of a recording electrode, which could in
556 theory act synergistically with PTEN deletion to increase excitability. As indirect evidence
557 that seizures do occur following focal PTEN deletion, here we describe incidental findings
558 of increased neuronal activation in a mouse without implanted recording electrodes that
559 was part of an anatomical study in which tissue was collected 4-month post AAV-Cre
560 injection. Of note, we did not monitor or record for behavioral seizures in this mouse.

561

562 We processed sections from this mouse as part of our assessment of s6 phosphorylation
563 as an indicator of mTOR activation (above). In other mice, increases in the
564 phosphorylation of ribosomal protein S6 in our model are confined to the region of PTEN
565 deletion, and do not extend to regions of PTEN expression within the same dentate gyrus,
566 or in the contralateral dentate gyrus (examples in Fig. 1E, F, Fig. 1M, N, Fig. 4E, F). In
567 this mouse, however, there were striking increases in pS6 immunoreactivity in regions
568 beyond the area of PTEN deletion in the ipsilateral dentate gyrus (Fig. 11A; PTEN vs.
569 11C, pS6) including in PTEN expressing granule cells in the contralateral dentate gyrus
570 (Fig. 11B, D). Previous studies have reported prolonged neuronal activation after seizure
571 activity in mouse models of temporal lobe epilepsy (Peng & Houser, 2005), and increased
572 markers of mTOR signaling (Ahmed et al., 2021). Thus, a possible interpretation is that
573 this mouse experienced a seizure in the period prior to tissue collection.

574

575 To further explore this possibility, we stained sections from this mouse for cFOS, which
576 is strongly induced after a seizure; cFOS expression was also dramatically increased in
577 both the ipsilateral (Fig. 11E) and contralateral dentate gyrus (Fig. 11F). In contrast, there
578 are no increases in cFOS expression in dentate granule cells due to PTEN deletion alone
579 (PTEN deleted area outline in Fig. 11H). Moreover, unilateral AAV-Cre injection into Rosa
580 control mice does not trigger any notable increases in cFOS expression within the region
581 of transduction (Fig. 11G). Thus, the dramatic increases in pS6 and cFOS
582 immunoreactivity throughout the hippocampus suggest that this mouse may have
583 experienced a seizure within hours before sacrifice.

584

585

586 3.10 Lack of hippocampal cell death following PTEN deletion

587

588 Epilepsy models that use convulsants to trigger spontaneous seizures following a latent
589 period, like kainic acid and pilocarpine, often result in widespread neuronal death,
590 especially in the CA3 region of the hippocampus (Curia et al., 2008; Drexel et al., 2012).
591 We therefore wondered if seizures induced by adult, vector-mediated PTEN deletion
592 would result in any notable hippocampal cell death. Figure 12 shows sample images from
593 Cresyl violet-stained sections from PTEN/tdT mice injected with AAV-Cre (Fig. 12A) or
594 AAV-GFP (Fig. 12B) near the center of viral transduction. There was no obvious cell loss
595 in any hippocampal region with PTEN deletion (dentate gyrus in Fig. 12f), or in the
596 neighboring CA1 (Fig. 12g) or CA3 (Fig. 12h) compared to the same hippocampal

597 subregions in an AAV-GFP injected control (Fig. 12b-d). Importantly, the PTEN-deleted
598 mouse shown here displayed the most seizures following unilateral PTEN deletion
599 (Cohort 3, animal #68-27, 72 total seizures). There did appear to be fewer large neurons
600 in the hilus in PTEN-deleted mice, which might indicate some loss or shrinkage of mossy
601 cells within the hilus (compare arrowheads in Fig. 12f with Fig. 12b). Loss of mossy cells
602 in the hilus often coincides with the presence of supra-granular mossy fibers and the
603 formation of recurrent excitatory networks, both of which have been found to correlate
604 with seizure frequency and duration (Hester & Danzer, 2013).

605
606

607 *3.11 Decreased immunostaining for GABA within the area of PTEN deletion*

608

609 Interneurons located within the dentate gyrus provide feedforward and feedback inhibition
610 onto granule cells. Loss of inhibition has been implicated in the development of seizures
611 and reductions in the number of interneurons has been reported following developmental
612 PTEN deletion (LaSarge et al., 2021). We therefore wondered if PTEN deletion in mature
613 granule cells would result in decreases in GABA-ergic markers within the dentate gyrus.
614 Immunostaining for glutamic acid decarboxylase, GAD67, a marker of GABA-expressing
615 neurons and synapses, revealed subtle decreases in staining within the area of PTEN
616 deletion in mice exhibiting spontaneous seizures (Fig. 13A) in comparison to the
617 contralateral, PTEN expressing dentate gyrus (Fig. 13B). These decreases in staining
618 could reflect an actual slight decrease in GABAergic innervation or could be due to the
619 fact that immunostaining is diluted as a consequence of the overall enlargement of the
620 dentate gyrus (essentially an increase in the denominator of density/unit area).

621
622

623 **4.0 Discussion**

624

625 The goal of this study was to explore whether PTEN deletion and resulting growth of
626 granule cells and alterations in hippocampal circuitry result in the development of
627 spontaneous seizures. The principal findings are: (1) deletion of PTEN in adult dentate
628 gyrus granule cells results in delayed development of recurrent spontaneous seizures
629 (epilepsy) in the majority of mice; seizures were not observed in mice that received AAV-
630 GFP (controls); (2) the latency to the first spontaneous seizure is about two months, (3)
631 spontaneous seizures develop with either a bilateral or unilateral focus of deletion; (4) in
632 contrast to excitotoxin models, epileptogenesis is not associated with obvious neuron loss
633 in CA3. Together these results document a novel, convulsant/toxin-free model of
634 hippocampal/temporal lobe epileptogenesis.

635
636

637 *4.1 Focal, unilateral PTEN deletion is sufficient for the development of spontaneous,* 638 *recurrent hippocampus-origin seizures*

639

640 Continuous video-EEG monitoring with bilateral, intrahippocampal electrodes revealed
641 that either bilateral or unilateral PTEN deletion in the dentate gyrus led to the development
642 of spontaneous electrographic and behavioral seizures in 80% and 67% of mice,

643 respectively. Seizures originated in either hippocampus in mice with bilateral PTEN
644 deletion, but consistently originated in the PTEN deleted dentate gyrus with unilateral
645 PTEN deletion.

646
647 All mice that received unilateral injections of AAV-Cre into the dentate gyrus with
648 recording electrodes positioned in the contralateral hippocampus also developed
649 spontaneous seizures. This largely excludes the possibility that seizure development was
650 due to the combination of PTEN deletion and electrode implantation. Such a
651 combinatorial effect has been suggested with electrode implantation in kainic acid and
652 pilocarpine models of epilepsy (Balzekas et al., 2016; Levesque et al., 2016).

653
654 Importantly, spontaneous seizures developed with as little as 22% transduction of the
655 dentate gyrus on one side, indicating that a relatively small, unilateral focus of PTEN
656 deletion is sufficient for bilateral electrographic and behavioral seizures. This is
657 reminiscent of the situation in human TLE where a unilateral focus can eventually lead to
658 generalized seizures.

659
660 Transduction and PTEN deletion was not restricted to the dentate gyrus in some mice,
661 which could contribute to seizure development. However, 6 mice with well targeted
662 unilateral PTEN deletion developed seizures (1 with bilateral electrodes and 5 with
663 contralateral electrode placement). In addition, seizures were neither more frequent nor
664 more severe in mice with transduction involving the CA1 region in addition to the dentate
665 gyrus.

666
667

668 *4.2 Latent period for seizure development*

669
670 Spontaneous seizures were not observed in mice until about 2 months following PTEN
671 deletion, regardless of pattern of PTEN deletion or electrode placement. This delayed
672 onset of spontaneous seizures, or latent period, suggests progressive network
673 modification over time. Of note, epileptogenesis due to focal PTEN deletion follows a
674 different time course than the commonly used kainic acid and pilocarpine models of TLE,
675 which have relatively short latent periods, ranging from 10-30 and 4-40 days, respectively,
676 before the appearance of spontaneous seizures (see review by (Levesque et al., 2016)).
677 The delayed development of seizures with PTEN deletion is reminiscent of the delayed
678 development of temporal lobe epilepsy (TLE) after some insult or injury involving the
679 hippocampus in humans which can last several years (Buckmaster, 2004).

680
681

682 *4.3 Relationship between seizure development and growth of granule cells*

683
684 PTEN deletion triggers two time-dependent processes; 1) growth of dentate granule cell
685 bodies, elongation of dendrites and new spine formation, and expansion of axonal
686 projections; 2) development of seizures. In our previous study, increases in granule cell
687 body size were statistically significant at 2 months (when seizures appear) but granule
688 cell size continued to increase over time. Dendritic and axonal elongation and alterations

689 in connectivity were not evident until 4 months post-deletion (Yonan & Steward, 2023).
690 Because seizures develop before growth responses are fully developed, seizures may be
691 due to alterations in neuron-intrinsic physiological processes due to PTEN deletion.
692 Alternatively, it is possible that early morphological changes are sufficient to trigger an
693 epileptogenic circuit. Further studies will be required to explore these possibilities.

694

695

696 *4.4 Sudden death during seizures*

697

698 Of the 13 mice that developed seizures, 3 eventually died during a seizure (23%). This is
699 in contrast to reports of high mortality rates that result from increasing seizure severity
700 with PTEN deletion in early development (Kwon et al., 2003; Kwon et al., 2001;
701 Matsushita et al., 2016; Pun et al., 2012; Sunnen et al., 2011). One possible explanation
702 is that seizures in our model are less severe, and thus less likely to lead to respiratory
703 arrest. Other possibilities include some compensatory mechanisms or network properties
704 in mature circuits that attenuate seizure progression.

705

706 Of note, mortality rates in the present study are somewhat higher than in our previous
707 study with the same PTEN deletion model but without electrode implantation (Yonan &
708 Steward, 2023). It is possible that seizures are more severe and/or that mice are more
709 susceptible to respiratory arrest when PTEN deletion and electrode implantation are
710 combined.

711

712

713 *4.5 Supra-granular mossy fibers not required, but may contribute to seizure outcomes*

714

715 Supragranular mossy fibers develop in excitotoxin models of epilepsy and are thought to
716 represent the formation of a recurrent excitatory circuit. Supragranular mossy fibers were
717 noted in studies involving PTEN deletion in early development (Amiri et al., 2012; Kwon
718 et al., 2006; Pun et al., 2012; Sunnen et al., 2011), and were also seen in 40% of mice at
719 4 and 6 months after PTEN deletion in our previous study (Yonan & Steward, 2023). In
720 the present study, all mice with unilateral PTEN deletion with contralateral electrode
721 placement developed seizures and showed some mossy fiber labeling in the granule cell
722 layer, but only 50% of mice had actual supragranular mossy fibers. Thus, spontaneous
723 seizures can occur in the absence of supragranular mossy fibers as reported in studies
724 of developmental PTEN deletion (Pun et al., 2012), but may contribute to seizure
725 progression over time.

726

727

728 *4.6 New model of epileptogenesis and adult-onset epilepsy*

729

730 Various laboratory models have been developed to investigate mechanisms of
731 epileptogenesis, including brain injury, hypoxia and ischemia, kindling, and chemical
732 convulsants [for review see (Buckmaster, 2004)]. Many models including kainic acid and
733 pilocarpine models trigger extensive neuronal death both within and beyond the
734 hippocampus (Curia et al., 2008; Drexel et al., 2012). Whether neuronal death is required

735 for epileptogenesis during development and in adulthood is up for debate (Baram et al.,
 736 2011). For example, febrile status epilepticus (FSE) often results in epilepsy without
 737 widespread cell loss (Dube et al., 2010). Our model of vector-mediated focal PTEN
 738 deletion in the adult dentate gyrus provides another model of epileptogenesis without
 739 apparent neuronal death, which may provide a unique opportunity to define different
 740 mechanisms of adult-onset temporal lobe epilepsy than have not been explored in other
 741 animal models.

742
 743
 744

745 Table 1: Animal numbers, attrition, and exclusions

746

Cohort	Vector	AAV Group	Died post AAV Sx	Died post EEG Sx	Excluded post death/perfusion	Final numbers
1	AAV-Cre	Bilateral, n=11	n=1	n=2	n=1, large brain lesion n=2, ended recording early	n=5
2	AAV-Cre	Unilateral, n=6	n=0	n=2	n=1, incorrect EEG probe	n=3
3	AAV-Cre	Unilateral, n=7	n=0	n=0	n=0	n=7
4	AAV-GFP	Unilateral, n=3 Bilateral, n=3	n=0 n=0	n=0 n=0	n=1, AAV missed n=1, AAV missed	n=4

747
 748
 749

750 Table 2: Cohort 1, Bilateral PTEN-deleted mice with bilateral electrode placement used
 751 for EEG recordings

752

Animal	Strain	AAV-Cre location	EEG location	DPIs recorded	DPI first seizure	Total # seizures	DPI died/sacrificed
307-8	PTEN/tdT	Bilateral ^	Bilateral	43-113	65	42	113
307-9	PTEN/tdT	Bilateral +	Bilateral	43-113	79	27	113
329-20	PTEN/tdT	Bilateral ^	Bilateral	42-187	87	33	190
329-21	PTEN/tdT	Bilateral ^+	Bilateral	42-147	130	12	S.D. 147
329-22	PTEN/tdT	Bilateral ^	Bilateral	42-169	n/a	0	201

753 ^ Bilateral injection with mild/moderate transduction of one/both CA1

754 + Bilateral injection with one injection mistargeted to CA1

755 S.D.= seizure-related death

756
 757
 758

759 Table 3: Cohort 2, Unilateral PTEN-deleted mice with bilateral electrode placement
 760 used for EEG recordings
 761

Animal	Strain	AAV-Cre location	EEG location	DPIs recorded	DPI first seizure	Total # seizures	DPI died/sacrificed
305-1	PTEN/tdT	Unilateral ^	Bilateral	43-87	67	51	S.D. 87
329-18	PTEN/tdT	Unilateral	Bilateral	42-187	68	35	190
329-19	PTEN/tdT	Unilateral	Bilateral	42-169	n/a	0	174

762 ^ Unilateral dentate gyrus injection with mild/moderate transduction of CA1
 763 S.D.= seizure-related death

764
 765

766 Table 4: Cohort 3, Unilateral PTEN-deleted mice with contralateral electrode placement
 767 used for EEG recordings
 768

Animal	Strain	AAV-Cre location	EEG location	DPIs recorded	First Seizure	Total # seizures	DPI died/sacrificed
68-25	PTEN/tdT/Thy1	Unilateral	contralateral	56-127	71	9	127
68-26	PTEN/tdT/Thy1	Unilateral	contralateral	56-127	71	41	127
68-27	PTEN/tdT/Thy1	Unilateral	contralateral	56-127	68	72	127
68-28	PTEN/tdT/Thy1	Unilateral	contralateral	59-113	61	23	113
69-29	PTEN/tdT/Thy1	Unilateral	contralateral	59-77	76	12	S.D. 77
69-30	PTEN/tdT/Thy1	Unilateral ^	contralateral	59-127	75	41	127
69-31	PTEN/tdT/Thy1	Unilateral ^	contralateral	62-127	63	1	127

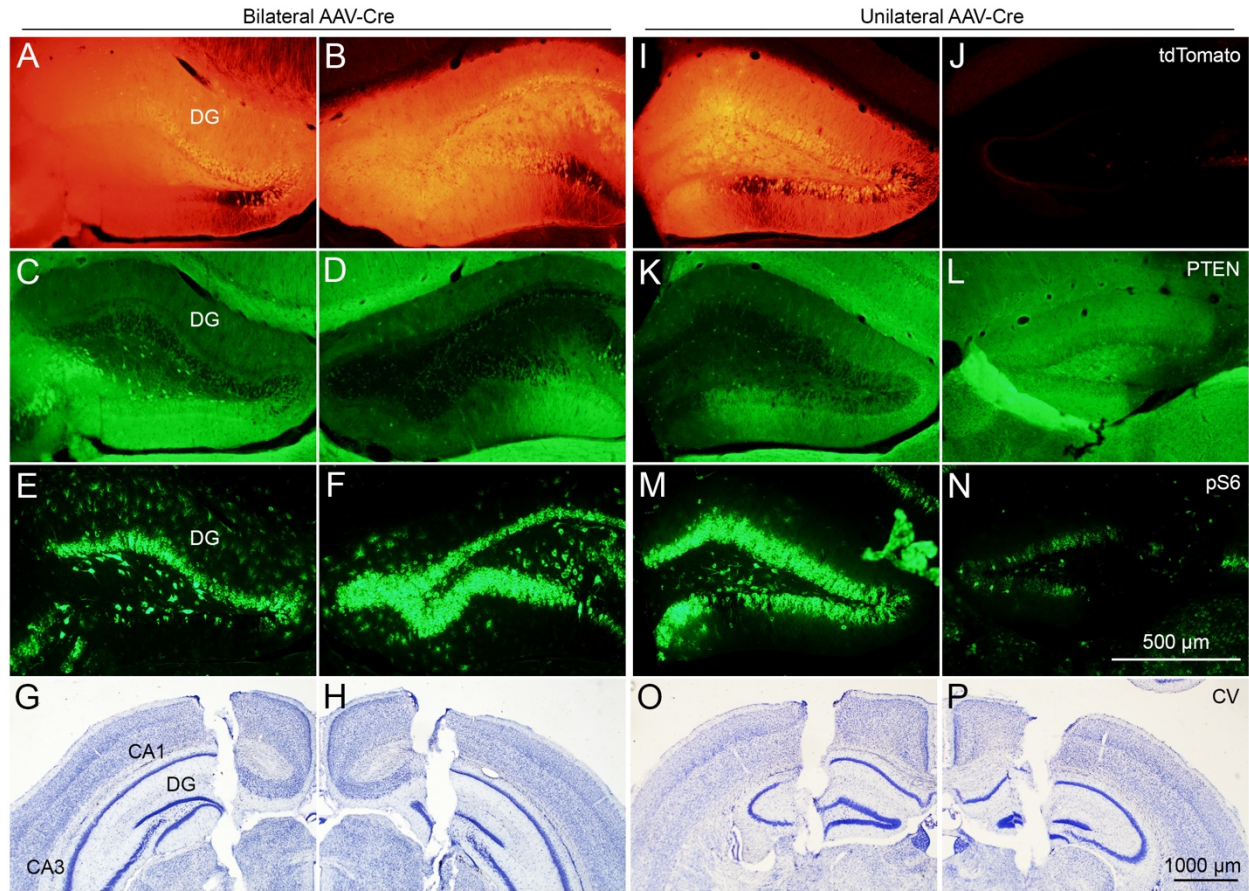
769 ^ Unilateral dentate gyrus injection with mild/moderate transduction of CA1
 770 S.D.= seizure-related death

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 772
 773

774 Table 5: Cohort 4, Control mice used for EEG recordings
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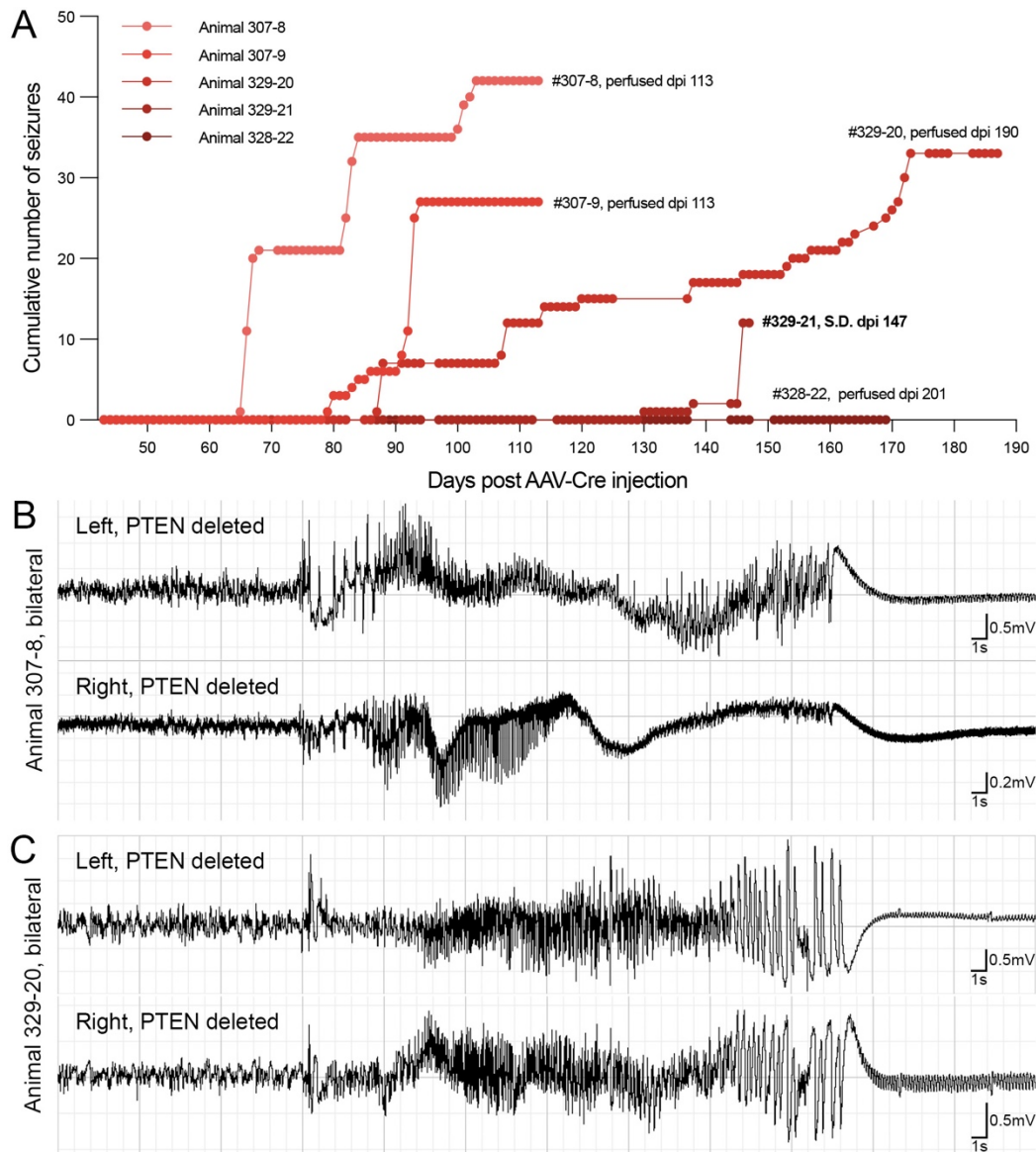
Animal	Strain	AAV-GFP location	EEG location	DPIs recorded	First seizure	Total # seizures	DPI sacrificed
322-13	PTEN/tdT	Unilateral ^	Bilateral	44-100	n/a	0	100
323-16	PTEN/tdT	Unilateral ^	Bilateral	44-100	n/a	0	100
322-14	PTEN/tdT	Bilateral ^+	Bilateral	44-100	n/a	0	100
323-17	PTEN/tdT	Bilateral ^	Bilateral	44-100	n/a	0	100

776 ^ Injection with mild/moderate transduction of one/both CA1
 777 + Injection with one injection mistargeted to CA1



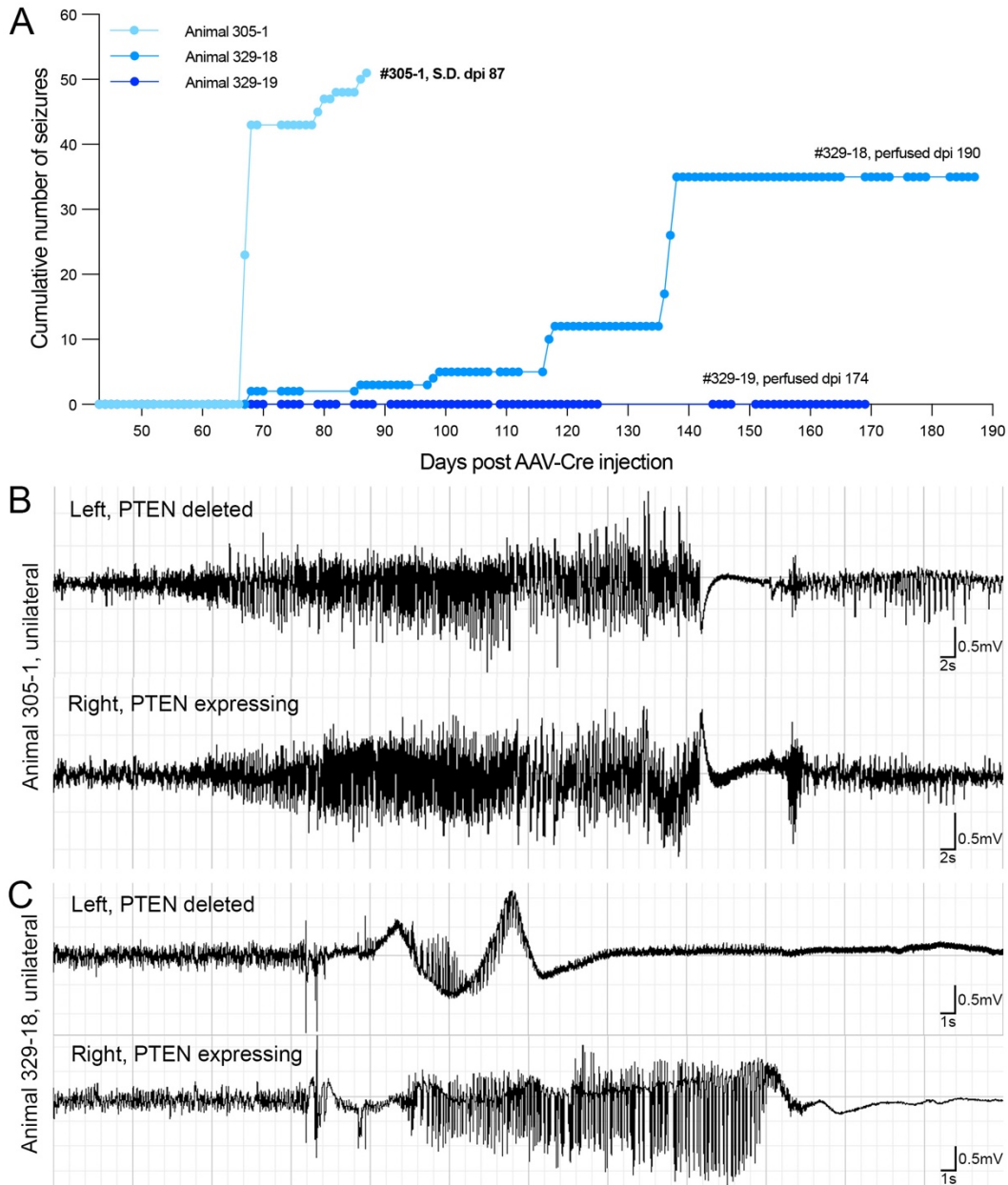
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780 **Figure 1. Effective PTEN deletion and mTOR activation in PTEN/tdT mice following**
781 **bilateral and unilateral AAV-Cre injections into the dentate gyrus.** A-B) tdTomato
782 expression in transduced dentate granule cells following bilateral AAV-Cre injection in a
783 PTEN/tdT mouse. C-D) Immunostaining for PTEN reveals deletion in tdT positive granule
784 cells. E-F) Immunostaining for phospho-S6 indicates activation of mTOR in PTEN deleted
785 granule cells. G-H) Cresyl violet stained sections reveal bilateral electrode placement into
786 the hippocampus. I-J) tdT expression in the transduced dentate gyrus (left) and lack of
787 tdT expression in the non-injected dentate gyrus (right) of a PTEN/tdT mouse following
788 unilateral AAV-Cre injection. K-L) PTEN deletion in the ipsilateral dentate gyrus. Note, the
789 preservation of PTEN expression in the contralateral dentate gyrus. M-N) pS6
790 immunoreactivity in the PTEN deleted and PTEN expressing dentate gyrus of the same
791 mouse. O-P) Location of bilateral, intrahippocampal electrodes in the same mouse.



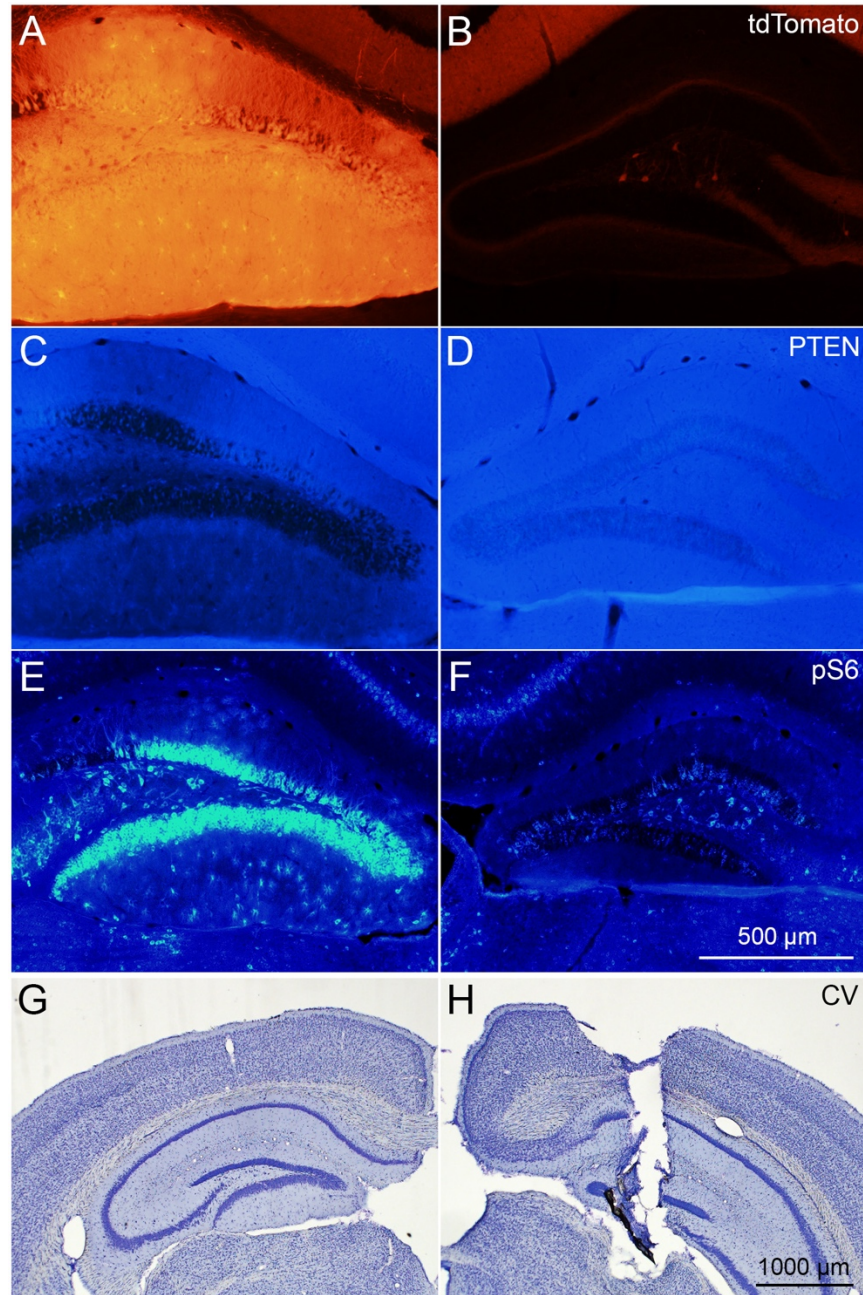
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Figure 2. EEG recordings in PTEN/tdT mice (Cohort 1) following bilateral AAV-Cre injections into the dentate gyrus. A) Cumulative number of seizures for each mouse over time following bilateral PTEN deletion. B-C) Representative EEG recordings from two mice with bilateral PTEN deletion in the dentate gyrus. Note the typical progression of seizure activity in both hippocampi followed by a period of post ictal depression.



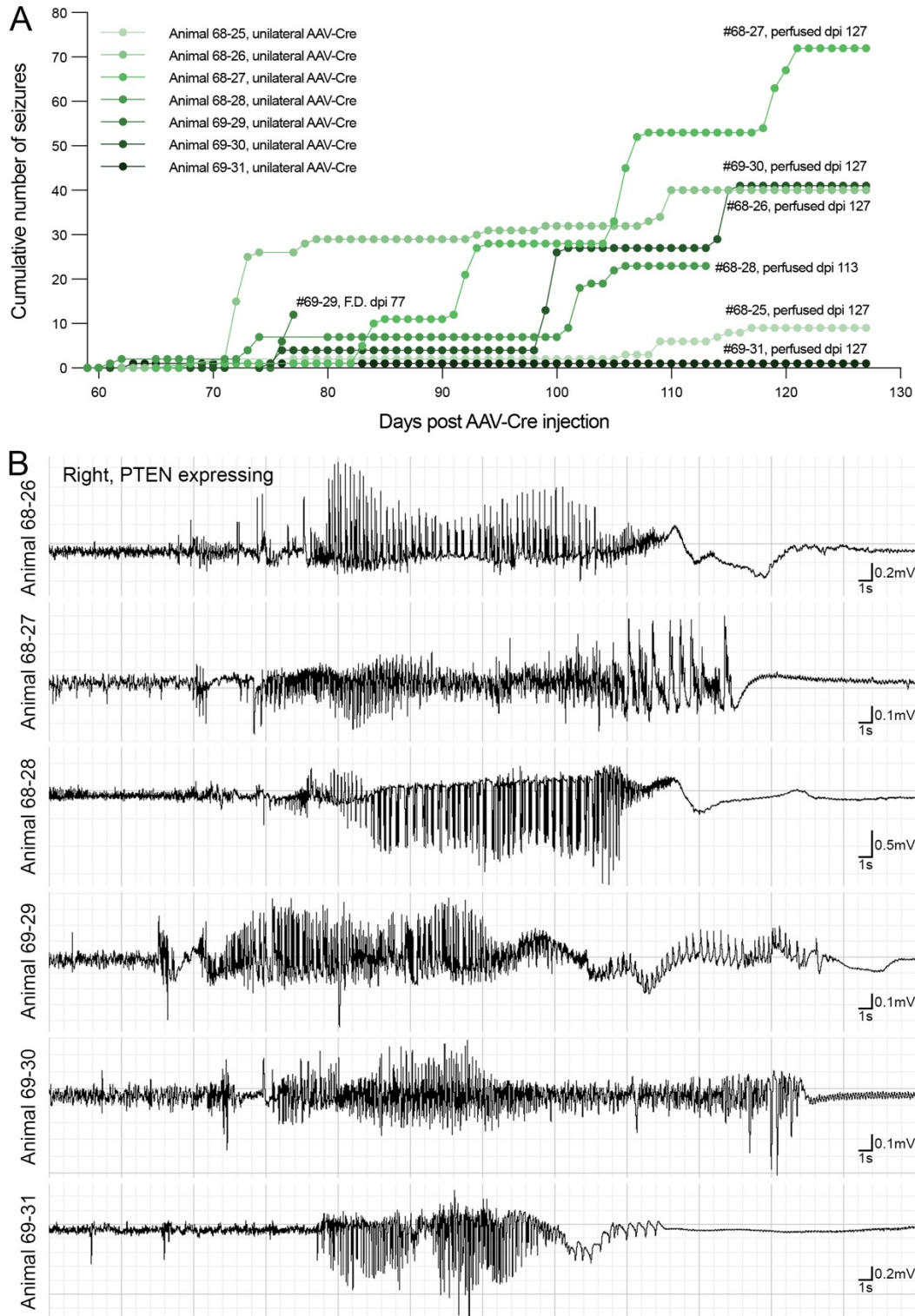
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Figure 3. EEG recordings in PTEN/tdT mice (Cohort 2) following unilateral AAV-Cre injections into the dentate gyrus. A) Cumulative number of seizures for each mouse over time with unilateral PTEN deletion. B-C) Representative EEG recordings from two mice with unilateral PTEN deletion in the dentate gyrus. Note, seizure activity is initiated in the PTEN deleted hippocampus then propagates to the contralateral hippocampus.

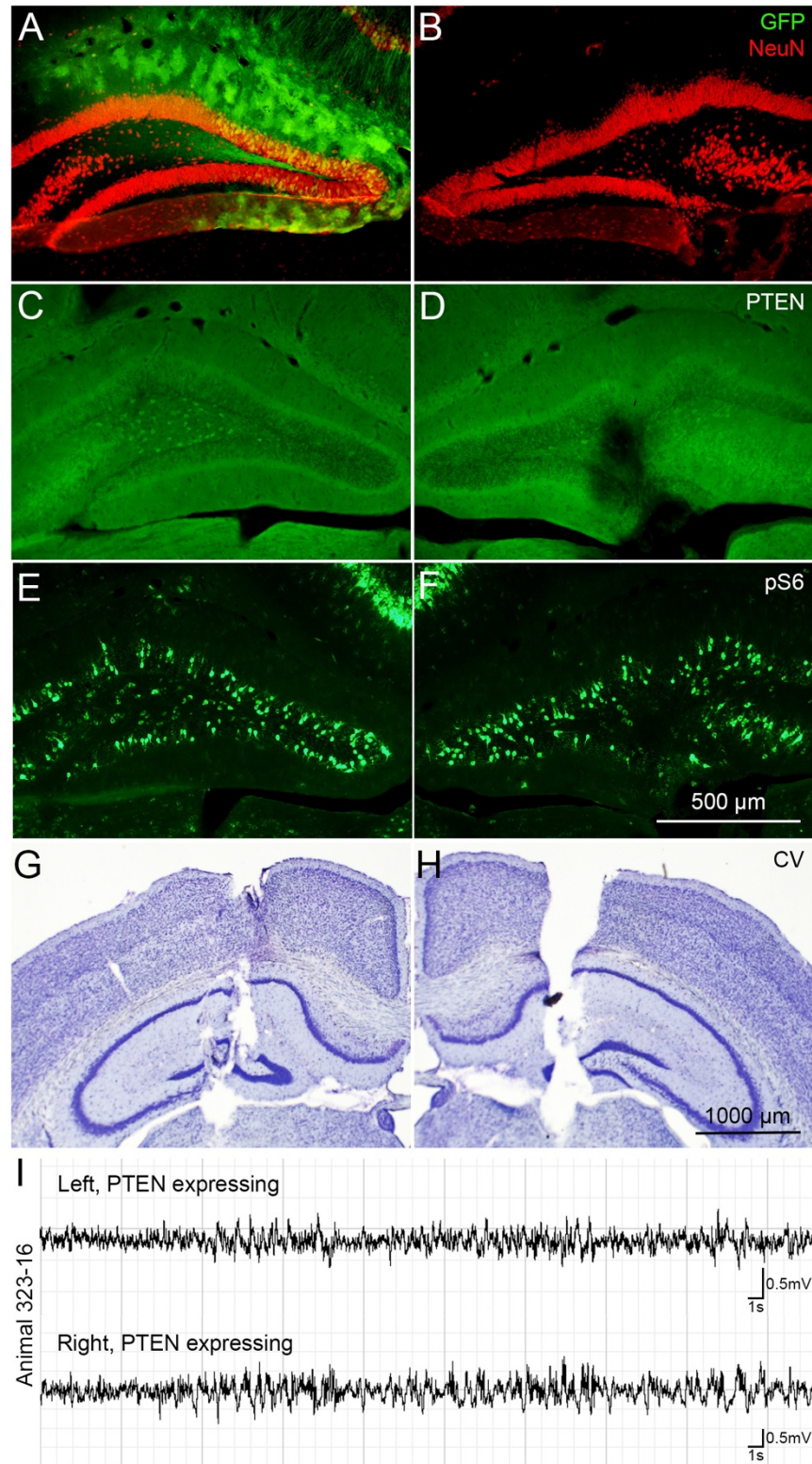


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810 **Figure 4. Unilateral PTEN deletion in the dentate gyrus of a PTEN/tdT mouse**
811 **(Cohort 3) with EEG placement in the contralateral dentate gyrus.** A) tdTomato
812 expression in transduced granule cells of the ipsilateral dentate gyrus following unilateral
813 AAV-Cre injection into a PTEN/tdT mouse. B) Lack of tdT expression in non-transduced
814 cells in the contralateral dentate gyrus. C) PTEN deletion in tdT positive granule cells. D)
815 Preservation of PTEN expression in the contralateral dentate gyrus. E) Increased
816 phosphorylation of ribosomal protein S6 in PTEN deleted granule cells. F) pS6
817 immunoreactivity in the contralateral dentate gyrus. G) Cresyl violet stained section at the
818 core of transduction and PTEN deletion. H) Cresyl violet stained section showing
819 unilateral electrode placement into the contralateral hippocampus.



820
821 **Figure 5. EEG recordings in PTEN/tdT mice (Cohort 3) following unilateral AAV-Cre**
822 **injections into the dentate gyrus and placement of EEG electrode into contralateral**
823 **hippocampus.** A) Cumulative number of seizures recorded in the contralateral, PTEN-
824 expressing dentate gyrus for each mouse over time. B) Representative EEG recordings
825 from all mice with unilateral PTEN deletion in the dentate gyrus.

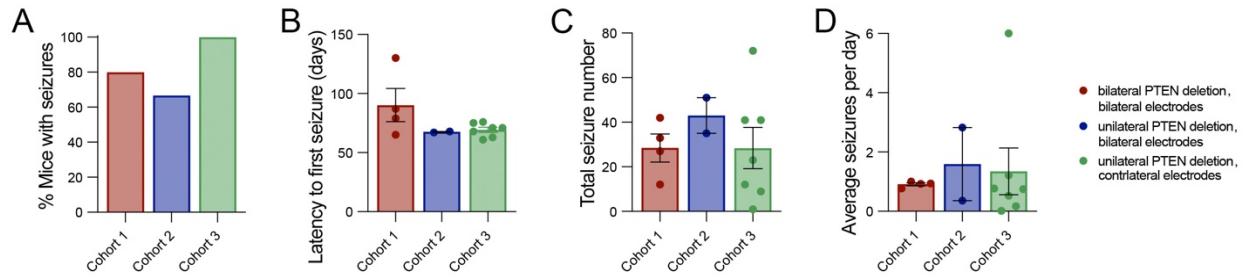


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828 **Figure 6. EEG recordings following AAV-GFP injection into the dentate gyrus of**
829 **PTEN/tdT mice (Cohort 4).** A) GFP expression in the ipsilateral (left) dentate gyrus of a
830 PTEN/tdT mouse following unilateral AAV-GFP injection. B) Lack of GFP expression in

831 the contralateral dentate gyrus. C-D) Preservation of PTEN expression in both
 832 hippocampi following unilateral AAV-GFP injection. E-F) pS6 immunoreactivity in the
 833 same control mouse. G-H) Cresyl violet stained sections show placement of bilateral
 834 electrodes in a control mouse. I) Representative EEG recordings from a mouse with AAV-
 835 GFP injection.

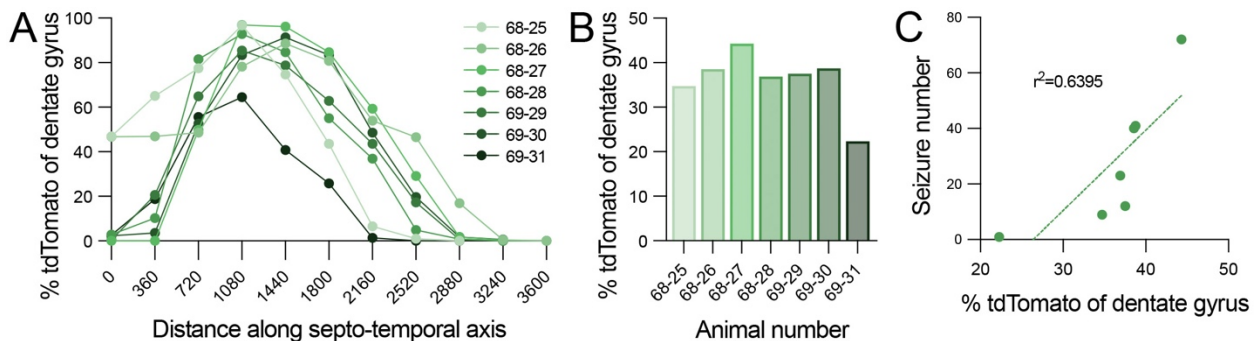
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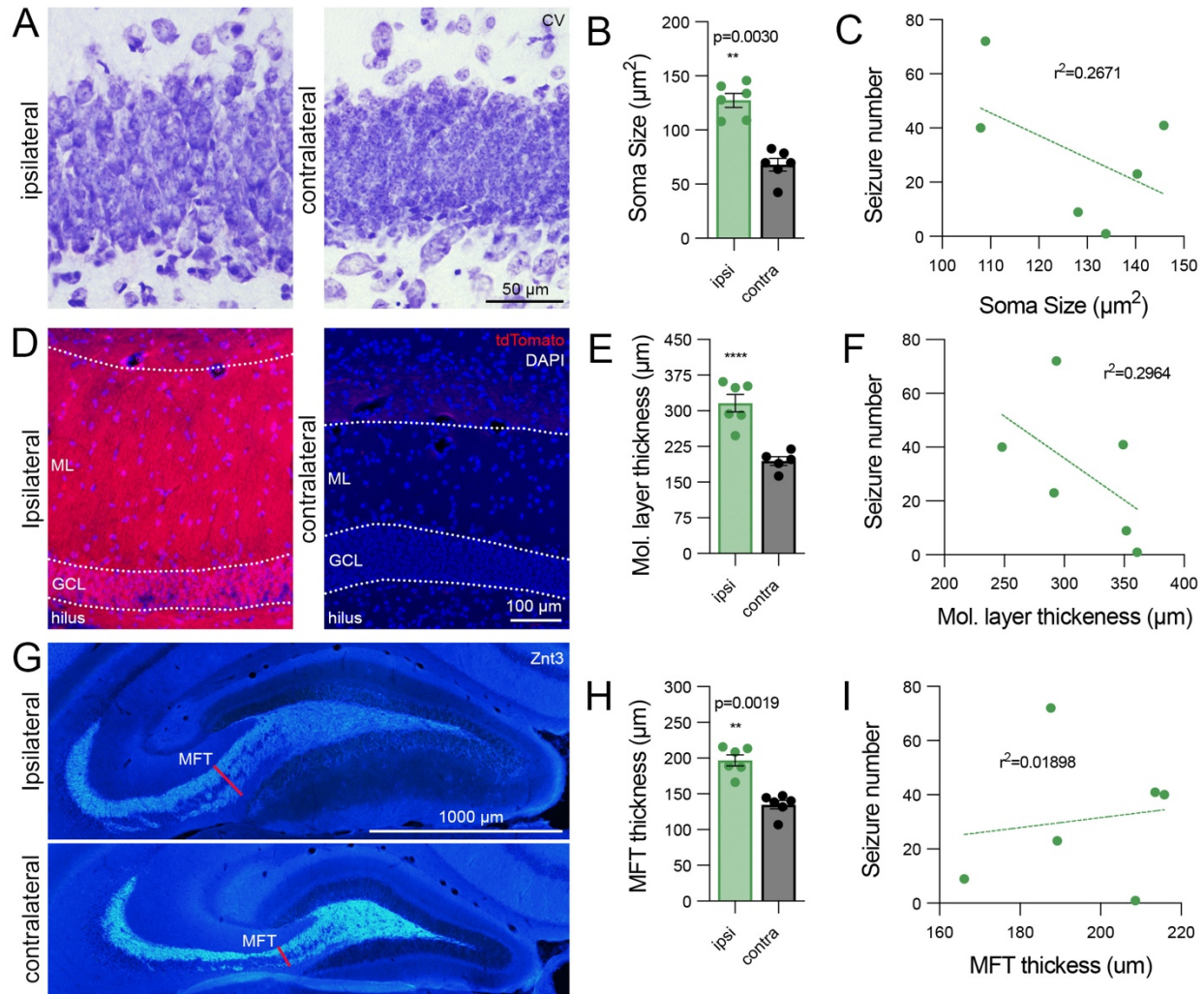
841 **Figure 7. Seizure incidence across cohorts following PTEN deletion.** A) Percent of
 842 mice within each cohort that displayed spontaneous seizures over the recording period.
 843 Mice with bilateral PTEN deletion and bilateral electrodes are shown in red (Cohort 1),
 844 mice with unilateral PTEN deletion and bilateral electrodes shown in blue (Cohort 2),
 845 and mice with unilateral PTEN deletion and contralateral electrode placement shown in
 846 green (Cohort 3). B) Latency to the first spontaneous seizure for each mouse within
 847 each cohort. C) Total seizure number across cohorts. D) Average number of seizures
 848 per day following PTEN deletion.

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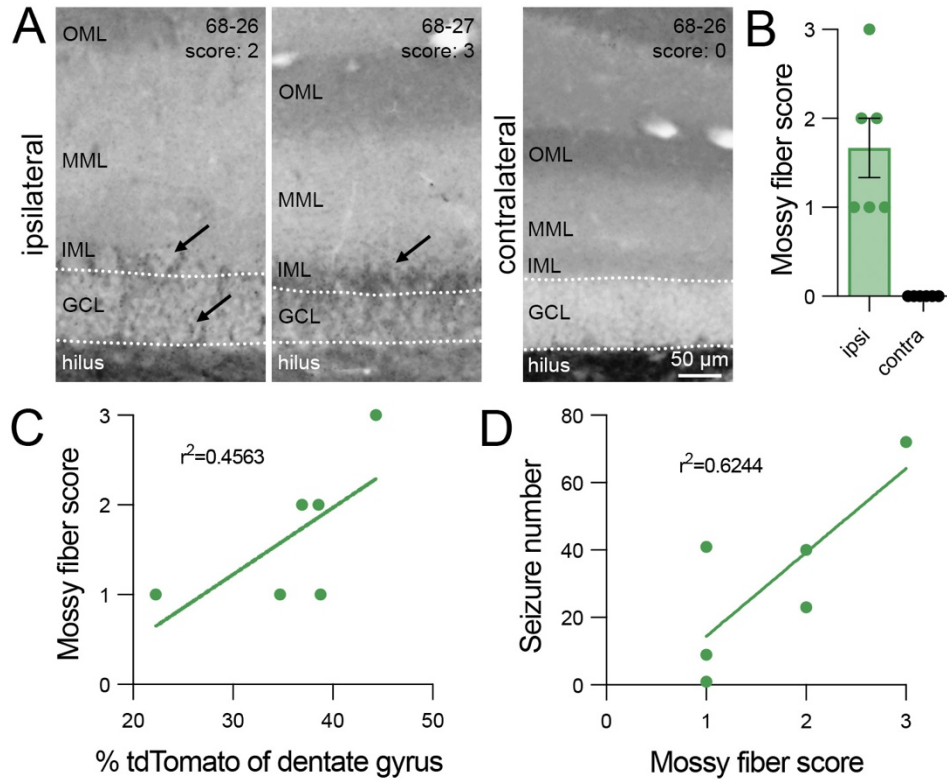


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854 **Figure 8. Relationship between PTEN deletion of the dentate gyrus and seizure**
 855 **number.** A) Percent transduction of granule cells based on tdTomato expression by AAV-
 856 Cre over the septo-temporal axis of the ipsilateral dentate gyrus for each mouse in Cohort
 857 3. B) Percent transduction of the entire ipsilateral dentate gyrus of the same mice. C)
 858 Relationship between percent transduction of the dentate gyrus for each mouse and
 859 cumulative number of seizures reported over the recording period, $r^2 = 0.6395$.

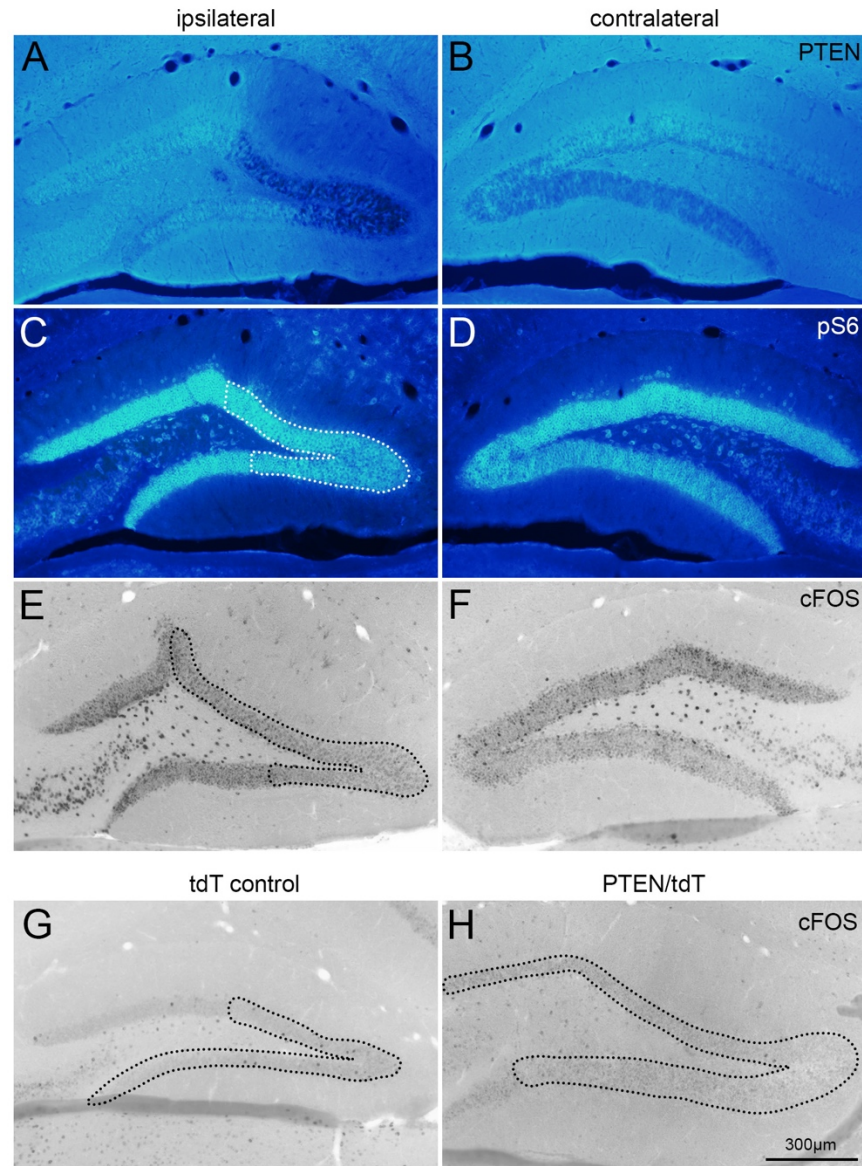


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 861 **Figure 9. PTEN deletion triggers growth of granule cell bodies and processes.** A)
 862 Representative Cresyl violet stained sections from the ipsilateral (left) and contralateral
 863 (right) dentate gyrus showing enlarged granule cell bodies. B) Average cell body size from
 864 the ipsilateral and contralateral dentate gyrus. Note each dot represents the average of
 865 30 granule cells for an individual mouse. Sidak's multiple comparisons for ipsilateral vs.
 866 contralateral sides in PTEN/tdT mice: $p=0.0030$. C) Relationship between soma size and
 867 seizure number for each animal, $r^2= 0.2671$. D) Images of the molecular layer from the
 868 ipsilateral and contralateral dentate gyrus from the same mouse showing enlargement of
 869 the molecular layer, suggestive of increased apical dendrite length. E) Molecular layer
 870 thickness measured at the core of transduction for each mouse and the corresponding
 871 contralateral molecular layer. Sidak's multiple comparisons for ipsilateral vs. contralateral
 872 sides in PTEN/tdT mice: $p<0.0001$. F) Relationship between molecular layer thickness
 873 and seizure number, $r^2= 0.2964$. G) Znt3 labeling in the ipsilateral and contralateral
 874 dentate gyrus reveals enlargement of mossy fiber tract projections to the CA3. H)
 875 Measurements of mossy fiber tract thickness as it exits the hilus for each mouse (red lines
 876 labeled MFT in G). Sidak's multiple comparisons for ipsilateral vs. contralateral sides in
 877 PTEN/tdT mice: $p=0.0019$. I) Relationship between mossy fiber tract thickness and
 878 seizure number, $r^2= 0.01898$.



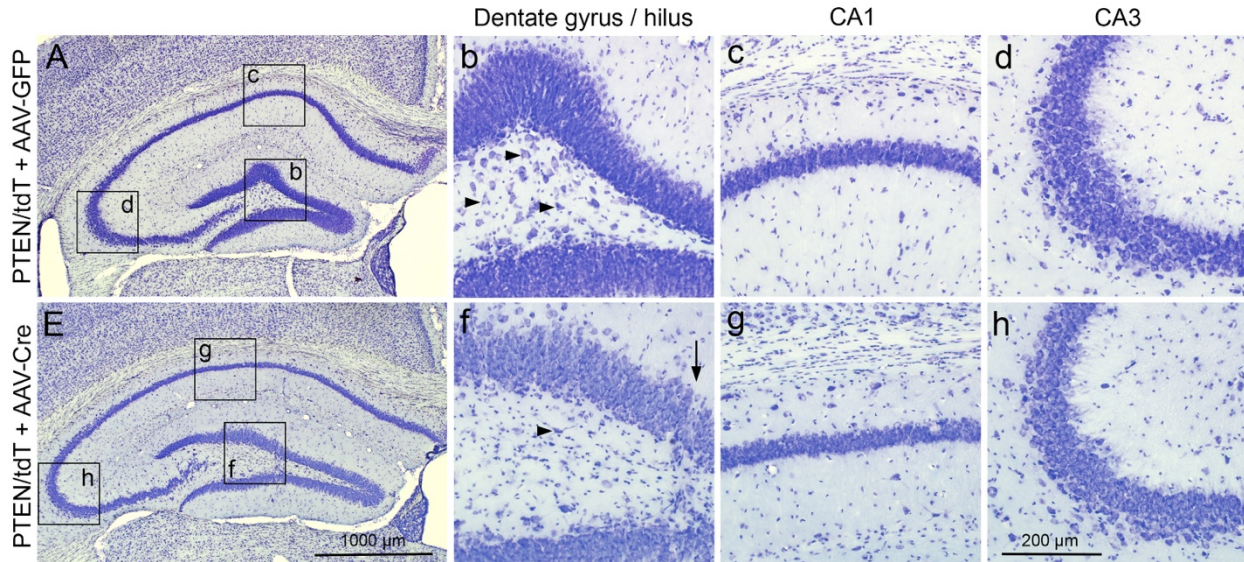
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881 **Figure 10. Presence of supragranular mossy fibers following PTEN deletion**
882 **correlates with seizure number for each animal.** A) Znt3 labeling in the granule cell
883 layer and inner molecular layer reveals presence of supragranular mossy fibers following
884 PTEN deletion (arrows) that are not present in the contralateral dentate gyrus. B) Mossy
885 fiber score for the transduced and contralateral dentate gyrus of each mouse shows
886 variability in the presence of supra-granular mossy fibers. Note representative scores are
887 depicted in the top right corner of panel A. C) Relationship between percent transduction
888 (PTEN deletion) and mossy fiber score, $r^2= 0.4563$. D) Relationship between mossy fiber
889 score and total seizure number, $r^2= 0.6244$. Scoring scale: 0 - little to no Znt3 labeling in
890 GCL, 1 - mild Znt3 labeling in GCL, 2 - moderate Znt3 labeling in GCL and mild labeling
891 in IML, 3 - dense Znt3 labeling in IML.



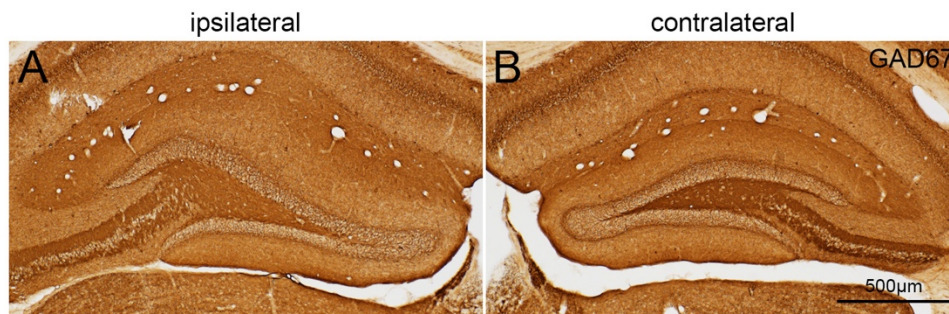
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894 **Figure 11. cFOS expression in the dentate gyrus of PTEN/tdT and control mice at 4**
895 **months following unilateral AAV-Cre injection.** A) Effective PTEN deletion in the
896 ipsilateral dentate gyrus of a PTEN/tdT mouse at 4 months after AAV-Cre injection. B)
897 PTEN expression is maintained in the contralateral dentate gyrus. C) Increased
898 phosphorylation of ribosomal protein S6 in the area of PTEN deletion (outlined in white)
899 and in PTEN expression granule cells of the ipsilateral dentate gyrus. D) Increased
900 phospho S6 in the contralateral dentate gyrus of the same mouse. E) Ipsilateral dentate
901 gyrus of the same PTEN/tdT mouse showing increased cFos expression within and
902 beyond the regions of PTEN deletion. F) Increased cFos expression in the contralateral
903 dentate gyrus. G) Injections of AAV-Cre in a tdT control mouse does not result in
904 increased cFOS expression (area of transduction outlined in black). H) PTEN deletion
905 alone does not trigger increased cFOS expression (area of transduction and PTEN
906 deletion outlined in black).
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Figure 12. Lack of neuronal death in major hippocampal subregions following PTEN deletion. A) PTEN/tdT mouse injected with AAV-GFP into the dentate gyrus shows presence of neuronal cell bodies in the dentate gyrus and hilus (b), the CA1 (c), and the CA3 (d). B) Lack of neuronal cell death in the PTEN-deleted dentate gyrus (f), CA1 (g), and CA3 (h) following AAV-Cre injection in a PTEN/tdT mouse. Note fewer large neurons in the hilus in f (arrowheads vs. multiple arrowheads in b). Injection tract is observable in the PTEN deleted dentate gyrus in panel f.



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Figure 13. GAD67 immunoreactivity following vector-mediated PTEN deletion. A) Decreased GAD67 immunoreactivity in the granule cell layer, molecular layer, and hilus in the area of PTEN deletion. B) Control GAD67 labeling in the contralateral dentate gyrus.

933 **REFERENCES**

934

935 Ahmed, M. M., Carrel, A. J., Cruz Del Angel, Y., Carlsen, J., Thomas, A. X., Gonzalez, M. I.,
936 Gardiner, K. J., & Brooks-Kayal, A. (2021). Altered Protein Profiles During Epileptogenesis
937 in the Pilocarpine Mouse Model of Temporal Lobe Epilepsy. *Front Neurol*, *12*, 654606.
938 <https://doi.org/10.3389/fneur.2021.654606>

939

940 Althaus, A. L., & Parent, J. M. (2012, Sep 20). Pten-less dentate granule cells make fits. *Neuron*,
941 *75*(6), 938-940. <https://doi.org/10.1016/j.neuron.2012.09.008>

942

943 Amiri, A., Cho, W., Zhou, J., Birnbaum, S. G., Sinton, C. M., McKay, R. M., & Parada, L. F. (2012,
944 Apr 25). Pten deletion in adult hippocampal neural stem/progenitor cells causes cellular
945 abnormalities and alters neurogenesis. *J Neurosci*, *32*(17), 5880-5890.
946 <https://doi.org/10.1523/JNEUROSCI.5462-11.2012>

947

948 Arafa, S. R., LaSarge, C. L., Pun, R. Y. K., Khademi, S., & Danzer, S. C. (2019, Jan). Self-reinforcing
949 effects of mTOR hyperactive neurons on dendritic growth. *Exp Neurol*, *311*, 125-134.
950 <https://doi.org/10.1016/j.expneurol.2018.09.019>

951

952 Backman, S. A., Stambolic, V., Suzuki, A., Haight, J., Elia, A., Pretorius, J., Tsao, M. S., Shannon,
953 P., Bolon, B., Ivy, G. O., & Mak, T. W. (2001, Dec). Deletion of Pten in mouse brain causes
954 seizures, ataxia and defects in soma size resembling Lhermitte-Duclos disease. *Nat*
955 *Genet*, *29*(4), 396-403. <https://doi.org/10.1038/ng782>

956

957 Balzekas, I., Hernandez, J., White, J., & Koh, S. (2016, May 27). Confounding effect of EEG
958 implantation surgery: Inadequacy of surgical control in a two hit model of temporal lobe
959 epilepsy. *Neurosci Lett*, *622*, 30-36. <https://doi.org/10.1016/j.neulet.2016.04.033>

960

961 Baram, T. Z., Jensen, F. E., & Brooks-Kayal, A. (2011, Jan). Does acquired epileptogenesis in the
962 immature brain require neuronal death. *Epilepsy Curr*, *11*(1), 21-26.
963 <https://doi.org/10.5698/1535-7511-11.1.21>

964

965 Buckmaster, P. S. (2004, Oct). Laboratory animal models of temporal lobe epilepsy. *Comp Med*,
966 *54*(5), 473-485. <https://www.ncbi.nlm.nih.gov/pubmed/15575361>

967

968 Chen, K. D., Hall, A. M., Garcia-Curran, M. M., Sanchez, G. A., Daglian, J., Luo, R., & Baram, T. Z.
969 (2021, Mar). Augmented seizure susceptibility and hippocampal epileptogenesis in a
970 translational mouse model of febrile status epilepticus. *Epilepsia*, *62*(3), 647-658.
971 <https://doi.org/10.1111/epi.16814>

972

973 Curia, G., Longo, D., Biagini, G., Jones, R. S., & Avoli, M. (2008, Jul 30). The pilocarpine model of
974 temporal lobe epilepsy. *J Neurosci Methods*, *172*(2), 143-157.
975 <https://doi.org/10.1016/j.jneumeth.2008.04.019>

976

- 977 Drexel, M., Preidt, A. P., & Sperk, G. (2012, Oct). Sequel of spontaneous seizures after kainic
978 acid-induced status epilepticus and associated neuropathological changes in the
979 subiculum and entorhinal cortex. *Neuropharmacology*, 63(5), 806-817.
980 <https://doi.org/10.1016/j.neuropharm.2012.06.009>
981
- 982 Dube, C., Richichi, C., Bender, R. A., Chung, G., Litt, B., & Baram, T. Z. (2006, Apr). Temporal lobe
983 epilepsy after experimental prolonged febrile seizures: prospective analysis. *Brain*,
984 129(Pt 4), 911-922. <https://doi.org/10.1093/brain/awl018>
985
- 986 Dube, C. M., Ravizza, T., Hamamura, M., Zha, Q., Keebaugh, A., Fok, K., Andres, A. L., Nalcioglu,
987 O., Obenaus, A., Vezzani, A., & Baram, T. Z. (2010, Jun 2). Epileptogenesis provoked by
988 prolonged experimental febrile seizures: mechanisms and biomarkers. *J Neurosci*,
989 30(22), 7484-7494. <https://doi.org/10.1523/JNEUROSCI.0551-10.2010>
990
- 991 Fraser, M. M., Bayazitov, I. T., Zakharenko, S. S., & Baker, S. J. (2008, Jan 24). Phosphatase and
992 tensin homolog, deleted on chromosome 10 deficiency in brain causes defects in
993 synaptic structure, transmission and plasticity, and myelination abnormalities.
994 *Neuroscience*, 151(2), 476-488. <https://doi.org/10.1016/j.neuroscience.2007.10.048>
995
- 996 Fraser, M. M., Zhu, X., Kwon, C. H., Uhlmann, E. J., Gutmann, D. H., & Baker, S. J. (2004, Nov 1).
997 Pten loss causes hypertrophy and increased proliferation of astrocytes in vivo. *Cancer*
998 *Res*, 64(21), 7773-7779. <https://doi.org/10.1158/0008-5472.CAN-04-2487>
999
- 1000 Gallent, E. A., & Steward, O. (2018, May). Neuronal PTEN deletion in adult cortical neurons
1001 triggers progressive growth of cell bodies, dendrites, and axons. *Exp Neurol*, 303, 12-28.
1002 <https://doi.org/10.1016/j.expneurol.2018.01.005>
1003
- 1004 Garcia-Curran, M. M., Hall, A. M., Patterson, K. P., Shao, M., Eltom, N., Chen, K., Dube, C. M., &
1005 Baram, T. Z. (2019, Nov/Dec). Dexamethasone Attenuates Hyperexcitability Provoked by
1006 Experimental Febrile Status Epilepticus. *eNeuro*, 6(6).
1007 <https://doi.org/10.1523/ENEURO.0430-19.2019>
1008
- 1009 Hester, M. S., & Danzer, S. C. (2013, May 22). Accumulation of abnormal adult-generated
1010 hippocampal granule cells predicts seizure frequency and severity. *J Neurosci*, 33(21),
1011 8926-8936. <https://doi.org/10.1523/JNEUROSCI.5161-12.2013>
1012
- 1013 Hunt, R. F., Scheff, S. W., & Smith, B. N. (2009, Feb). Posttraumatic epilepsy after controlled
1014 cortical impact injury in mice. *Exp Neurol*, 215(2), 243-252.
1015 <https://doi.org/10.1016/j.expneurol.2008.10.005>
1016
- 1017 Johnston, S., Parylak, S. L., Kim, S., Mac, N., Lim, C., Gallina, I., Bloyd, C., Newberry, A., Saavedra,
1018 C. D., Novak, O., Goncalves, J. T., Gage, F. H., & Shtrahman, M. (2021, Jul 14). AAV
1019 ablates neurogenesis in the adult murine hippocampus. *Elife*, 10.
1020 <https://doi.org/10.7554/eLife.59291>

- 1021
- 1022 Kwon, C. H., Luikart, B. W., Powell, C. M., Zhou, J., Matheny, S. A., Zhang, W., Li, Y., Baker, S. J.,
1023 & Parada, L. F. (2006, May 4). Pten regulates neuronal arborization and social
1024 interaction in mice. *Neuron*, 50(3), 377-388.
1025 <https://doi.org/10.1016/j.neuron.2006.03.023>
1026
- 1027 Kwon, C. H., Zhu, X., Zhang, J., & Baker, S. J. (2003, Oct 28). mTor is required for hypertrophy of
1028 Pten-deficient neuronal soma in vivo. *Proc Natl Acad Sci U S A*, 100(22), 12923-12928.
1029 <https://doi.org/10.1073/pnas.2132711100>
1030
- 1031 Kwon, C. H., Zhu, X., Zhang, J., Knoop, L. L., Tharp, R., Smeyne, R. J., Eberhart, C. G., Burger, P.
1032 C., & Baker, S. J. (2001, Dec). Pten regulates neuronal soma size: a mouse model of
1033 Lhermitte-Duclos disease. *Nat Genet*, 29(4), 404-411. <https://doi.org/10.1038/ng781>
1034
- 1035 LaSarge, C. L., Pun, R. Y., Muntifering, M. B., & Danzer, S. C. (2016, Dec). Disrupted hippocampal
1036 network physiology following PTEN deletion from newborn dentate granule cells.
1037 *Neurobiol Dis*, 96, 105-114. <https://doi.org/10.1016/j.nbd.2016.09.004>
1038
- 1039 LaSarge, C. L., Pun, R. Y. K., Gu, Z., Riccetti, M. R., Namboodiri, D. V., Tiwari, D., Gross, C., &
1040 Danzer, S. C. (2021, May). mTOR-driven neural circuit changes initiate an epileptogenic
1041 cascade. *Prog Neurobiol*, 200, 101974.
1042 <https://doi.org/10.1016/j.pneurobio.2020.101974>
1043
- 1044 LaSarge, C. L., Santos, V. R., & Danzer, S. C. (2015, Mar). PTEN deletion from adult-generated
1045 dentate granule cells disrupts granule cell mossy fiber axon structure. *Neurobiol Dis*, 75,
1046 142-150. <https://doi.org/10.1016/j.nbd.2014.12.029>
1047
- 1048 Levesque, M., Avoli, M., & Bernard, C. (2016, Feb 15). Animal models of temporal lobe epilepsy
1049 following systemic chemoconvulsant administration. *J Neurosci Methods*, 260, 45-52.
1050 <https://doi.org/10.1016/j.jneumeth.2015.03.009>
1051
- 1052 Matsushita, Y., Sakai, Y., Shimmura, M., Shigeto, H., Nishio, M., Akamine, S., Sanefuji, M.,
1053 Ishizaki, Y., Torisu, H., Nakabeppu, Y., Suzuki, A., Takada, H., & Hara, T. (2016, Mar 10).
1054 Hyperactive mTOR signals in the proopiomelanocortin-expressing hippocampal neurons
1055 cause age-dependent epilepsy and premature death in mice. *Sci Rep*, 6, 22991.
1056 <https://doi.org/10.1038/srep22991>
1057
- 1058 Peng, Z., & Houser, C. R. (2005, Aug 3). Temporal patterns of fos expression in the dentate gyrus
1059 after spontaneous seizures in a mouse model of temporal lobe epilepsy. *J Neurosci*,
1060 25(31), 7210-7220. <https://doi.org/10.1523/JNEUROSCI.0838-05.2005>
1061
- 1062 Pitkanen, A., Nissinen, J., Nairismagi, J., Lukasiuk, K., Grohn, O. H., Miettinen, R., & Kauppinen,
1063 R. (2002). Progression of neuronal damage after status epilepticus and during

1064 spontaneous seizures in a rat model of temporal lobe epilepsy. *Prog Brain Res*, 135, 67-
1065 83. [https://doi.org/10.1016/S0079-6123\(02\)35008-8](https://doi.org/10.1016/S0079-6123(02)35008-8)
1066
1067 Pun, R. Y., Rolle, I. J., Lasarge, C. L., Hosford, B. E., Rosen, J. M., Uhl, J. D., Schmeltzer, S. N.,
1068 Faulkner, C., Bronson, S. L., Murphy, B. L., Richards, D. A., Holland, K. D., & Danzer, S. C.
1069 (2012, Sep 20). Excessive activation of mTOR in postnatally generated granule cells is
1070 sufficient to cause epilepsy. *Neuron*, 75(6), 1022-1034.
1071 <https://doi.org/10.1016/j.neuron.2012.08.002>
1072
1073 Santos, V. R., Pun, R. Y. K., Arafa, S. R., LaSarge, C. L., Rowley, S., Khademi, S., Bouley, T.,
1074 Holland, K. D., Garcia-Cairasco, N., & Danzer, S. C. (2017, Dec). PTEN deletion increases
1075 hippocampal granule cell excitability in male and female mice. *Neurobiol Dis*, 108, 339-
1076 351. <https://doi.org/10.1016/j.nbd.2017.08.014>
1077
1078 Steward, O., Coulibay, A., Metcalfe, M., Yonan, J. M., & Yee, K. M. (2019). AAVshRNA-mediated
1079 PTEN knockdown in adult neurons attenuates activity-dependent immediate early gene
1080 induction. *Exp Neurol*. <https://doi.org/https://doi.org/10.1016/j.expneurol.2019.113098>
1081
1082
1083 Sunnen, C. N., Brewster, A. L., Lugo, J. N., Vanegas, F., Turcios, E., Mukhi, S., Parghi, D.,
1084 D'Arcangelo, G., & Anderson, A. E. (2011, Nov). Inhibition of the mammalian target of
1085 rapamycin blocks epilepsy progression in NS-Pten conditional knockout mice. *Epilepsia*,
1086 52(11), 2065-2075. <https://doi.org/10.1111/j.1528-1167.2011.03280.x>
1087
1088 Williams, M. R., DeSpenza, T., Jr., Li, M., Gullledge, A. T., & Luikart, B. W. (2015, Jan 21).
1089 Hyperactivity of newborn Pten knock-out neurons results from increased excitatory
1090 synaptic drive. *J Neurosci*, 35(3), 943-959. [https://doi.org/10.1523/JNEUROSCI.3144-](https://doi.org/10.1523/JNEUROSCI.3144-14.2015)
1091 [14.2015](https://doi.org/10.1523/JNEUROSCI.3144-14.2015)
1092
1093 Yonan, J. M., & Steward, O. (2023, Aug). Vector-mediated PTEN deletion in the adult dentate
1094 gyrus initiates new growth of granule cell bodies and dendrites and expansion of mossy
1095 fiber terminal fields that continues for months. *Neurobiol Dis*, 184, 106190.
1096 <https://doi.org/10.1016/j.nbd.2023.106190>
1097
1098
1099
1100