The impact of phosphorylated PTEN at threonine 366 on cortical connectivity and behaviour

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Supplementary Methods

Behavioural experiments

Two cohorts of 12 to 16 week old mice were used in the behavioural experiments (cohort 1: 8 male wt, 5 male *Pten*$^{T366A/T366A}$, 6 female wt, 5 female *Pten*$^{T366A/T366A}$; cohort 2: 5 male wt, 5 male *Pten*$^{T366A/T366A}$, 6 female wt, 5 female *Pten*$^{T366A/T366A}$). Cohort 1 was tested in the Y maze, open field, Barnes maze and conditioned fear tests. Cohort 2 was tested in the Y maze, hanging wire, rotarod, von Frey, Morris water maze, conditioned fear and vibrissae-stimulated reflex tests. All tests were performed during the dark (active) phase of the circadian rhythm with 4 to 7 intervening days. Data from the cohorts were combined in the case of replicated tests.

Y maze test

Spontaneous alternation behaviour, a measure of spatial working memory, exploratory behaviour, and responsiveness to novelty,[1,2] was tested using a Y maze with 34 × 8 × 14 cm arms. Each mouse was tested in a single 5-min trial and spontaneous alternations, sets of three unique arm choices, were recorded. Because mice have the opportunity to perform repeated entries into a single arm, there is a chance performance level of 22% (2/9) for spontaneous alternations.[3,4]

Hanging wire test

The hanging wire test allows for the assessment of grip strength and motor coordination. [5,6] Mice were held so that only their forelimbs contact an elevated metal bar (2 mm diameter, 45 cm long, 37 cm above the floor) held parallel to the table by a large ring stand and let go to hang. Each mouse was given three trials separated by 30s. Each trial was scored as follows and the average for each mouse was calculated: 0 — fell off, 1 — hung onto the wire by two forepaws, 2 — hung onto the wire by two forepaws, but also attempted to climb onto the wire,

3 — hung onto the wire by two forepaws plus one or both hindpaws around the wire, 4 — hung onto the wire by all four paws plus tail wrapped, 5 — escaped (crawled to the ring stand and righted itself or climbed down the stand to the table). Latency to falling off was measured up to a maximum of 30 s.

Rotarod test

Rotarod balancing requires a variety of proprioceptive, vestibular, and fine-tuned motor abilities as well as motor learning capabilities.[7] A Roto-rod Series 8 apparatus (IITC Life Sciences, Woodland Hills, CA) was used which records test results when the animal drops onto the individual sensing platforms below the rotating rod. An accelerating test strategy was used whereby the rod started at 0 rpm and then accelerated to 10 rpm. The mice were tested in two sets of three trials per day for four days, for a total of 24 trials.

Open field test

This test predicts how animals respond when introduced into a brightly illuminated open arena.[8] It is a classic test of "emotionality" used to measure anxiety-like responses of rodents exposed to stressful environmental stimuli (brightly illuminated open spaces) and to capture spontaneous activity measures. The apparatus is a square white plexiglas (50 x 50 cm) open field illuminated to 600 lux in the center. Each animal is placed in the center of the field and several behavioural parameters (distance traveled, velocity, center time, frequency in center) are recorded during a 10-min observation period and analyzed using Noldus Ethovision XT software. Time spent grooming was also assessed.

von Frey test

Mechanical sensitivity was assessed by the application of von Frey filaments of varying forces (0.16, 0.4, 1, 2, 4, 6, 8 g) perpendicularly to the hind paw.[9] If the mouse withdrew its paw, a positive response was recorded. Each filament was tested a total of 10 times.

Vibrissae-stimulated reflex test

This is a test of sensorimotor function. Mice are held by their torso while their vibrissae are brushed along a tabletop. In normal mice, this elicits the placement of a forelimb, ipsilateral to the stimulation side, on the table.[10] Ten trials per side were performed on two days, the number of successful placements for each mouse was tabulated and averaged.

Barnes maze test

This is a spatial memory test [11–13] sensitive to impaired hippocampal function.[14] Mice learn to find an escape chamber (19 x 8 x 7 cm) below 1 of 20 holes (5 cm diameter, 5 cm from perimeter) below an elevated brightly lit and noisy platform (75 cm diameter, elevated 58 cm above floor) using cues placed around the room. Spatial learning and memory were assessed across 4 trials (maximum time was 3 min) and then directly analyzed on the final (fifth) probe test in which the tunnel was removed and the time spent in each quadrant was determined and the percent time spent in the target quadrant (the one originally containing the escape box) was compared with the average percent time in the other three quadrants. This is a direct test of spatial memory as there is no potential for local cues to be used in the mouse's behavioural decision.

Morris water maze test

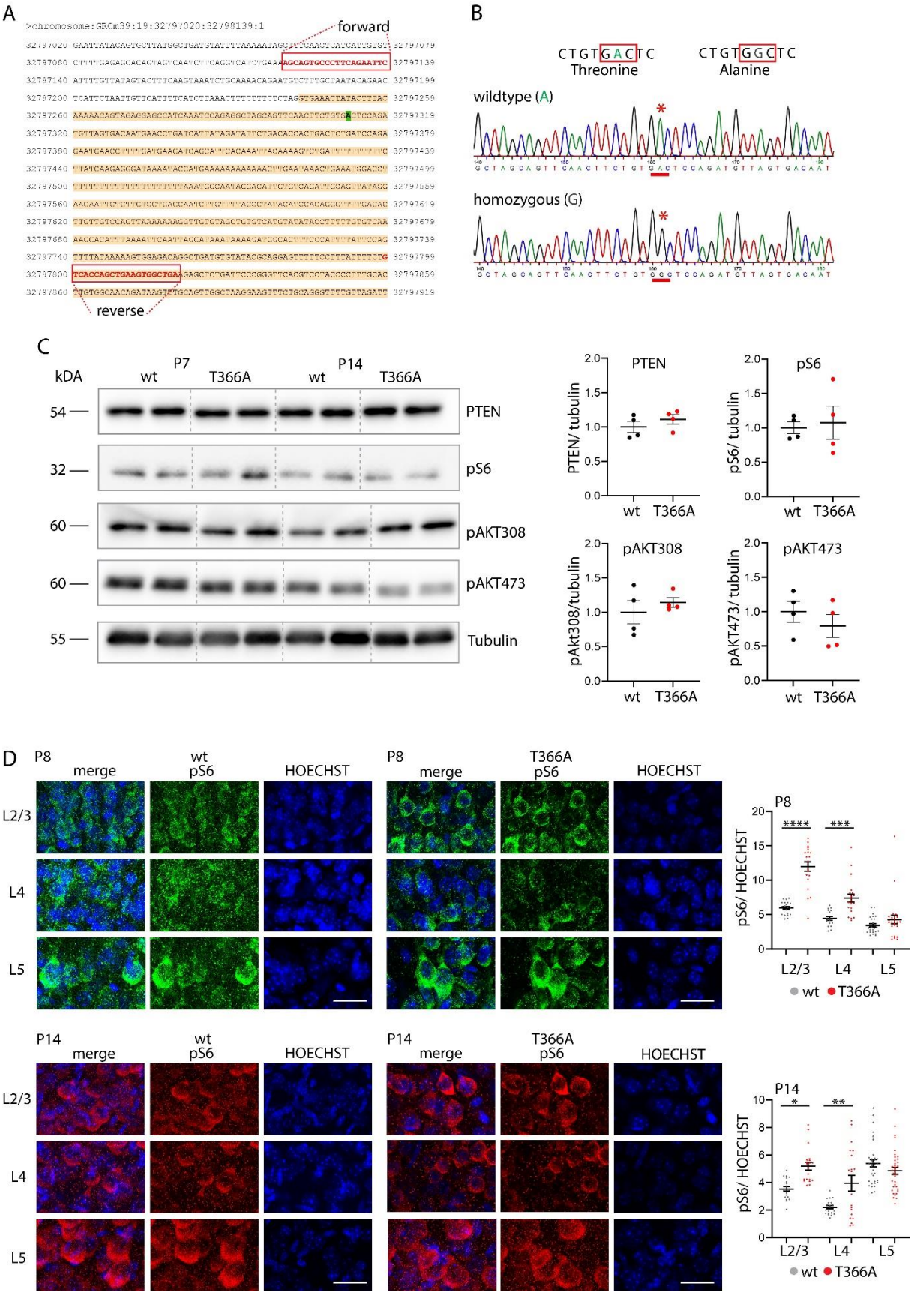
The water maze test is used to assess spatial learning and memory in rodents.[15,16] Mice are placed into a circular tub filled with opaque water and they learn over repeated trials to locate a hidden platform onto which they can sit and escape from the swimming. Each animal underwent two trials per day for four days, with a fixed platform location, and a random start position. After being released into the water, each animal was allowed to swim until the platform is found or 90 s had elapsed, at which point the experimenter gently guided the mouse to the platform. A probe trial was given after the completion of training (day 5), in which the platform was removed from the water maze and the animal was allowed to swim freely for 90s. The amount of time spent in each quadrant, the number of times a mouse entered a quadrant, and the number of times the mouse crossed the platform location (annulus crossings) were recorded using Noldus Ethovision software.

Conditioned fear test

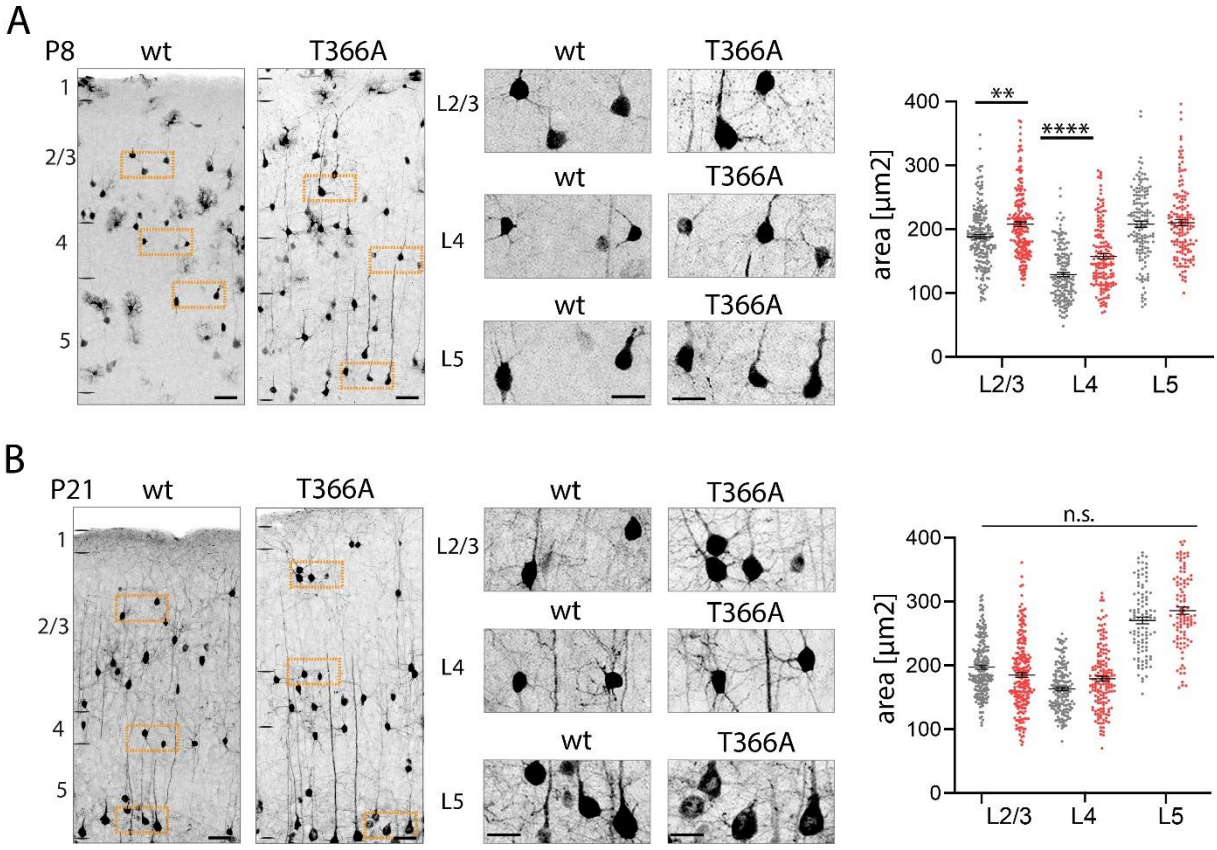
In this procedure, mice learn to associate a novel environment (context) and a previously neutral stimulus (conditioned stimulus, a tone) with an aversive foot shock stimulus.[17] It allows the assessment of both hippocampus-dependent and amygdala-dependent learning processes in the same mouse. [18,19] Conditioned animals, when exposed to the conditioned stimuli, tend to refrain from all but respiratory movements by freezing. Freezing responses can be triggered by exposure to either the context in which the shock was received (context test) or the conditioned stimulus (CS+ test). Briefly, mice were habituated to the system (Freeze Monitor, Med Associates, VT) to measure baseline freezing behaviour on day 1 (5 min trial) and then on day

2 were conditioned with two 0.6 mA foot shocks given in the final 2 s of cue exposure (30s, 3000Hz, 80dB sound + white light) in a 6 min trial. On day 3, contextual conditioning (as determined by freezing behaviour) was measured in a 5 min trial in the chamber where the mice were trained (context test). The following day, the mice were tested for cued conditioning (CS+ test). The mice were placed in a novel context for 3 min, after which they were exposed to the conditioned stimuli (light + tone) for 3 min. For this test, the chamber was disguised with new walls (white opaque plastic creating a circular compartment in contrast to a clear plastic square compartment) and a new floor (white opaque plastic in contrast to metal grid). Freezing behaviour (i.e., the absence of all voluntary movements except breathing) was measured in all sessions by real-time digital video recordings calibrated to distinguish between subtle movements, such as whisker twitches, tail flicks, and freezing behaviour. Freezing behaviour is indicative of the formation of an association between the particular stimulus (either the environment or the tone) and the shock; i.e. that learning has occurred.

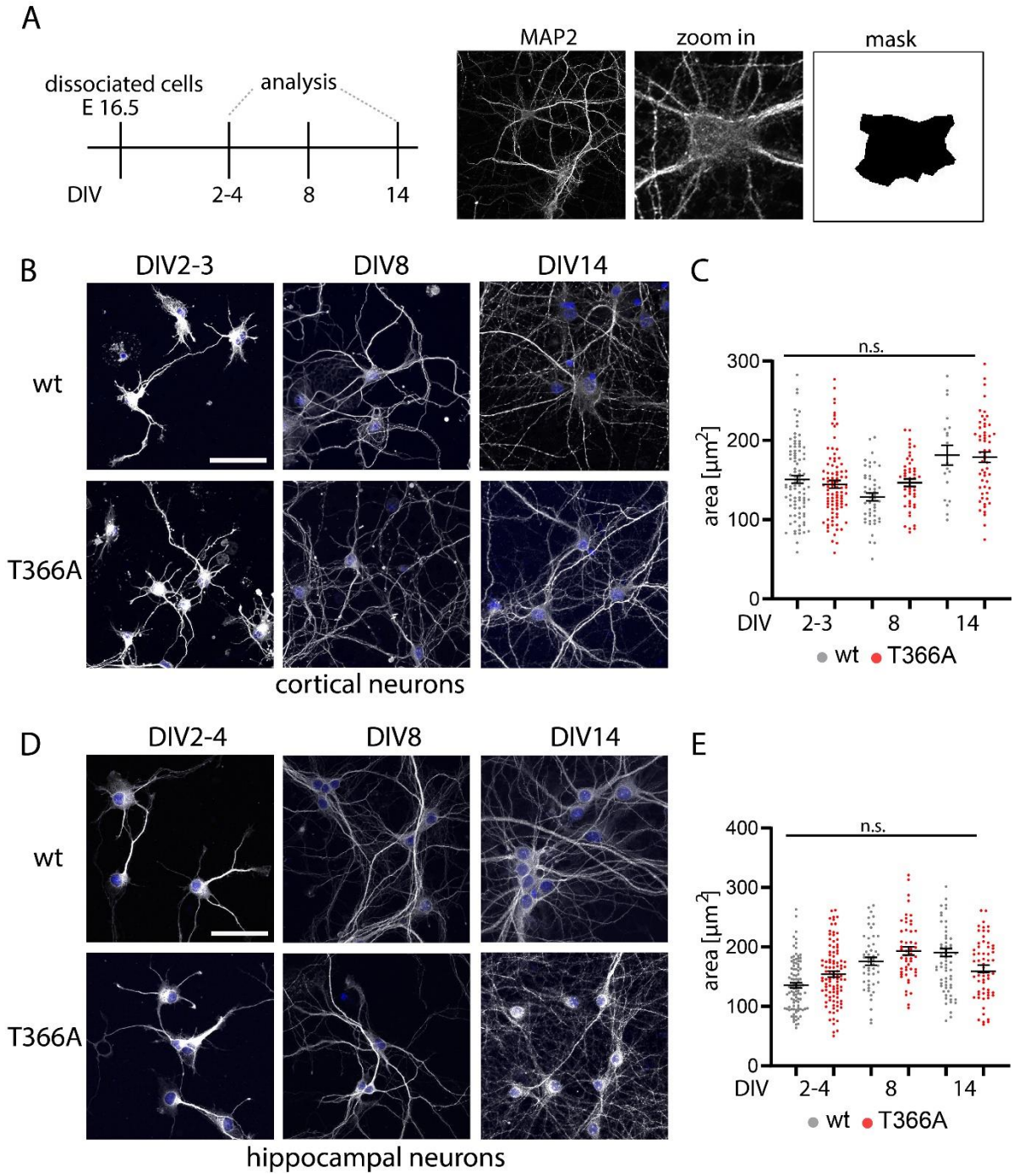
Supplementary Figures



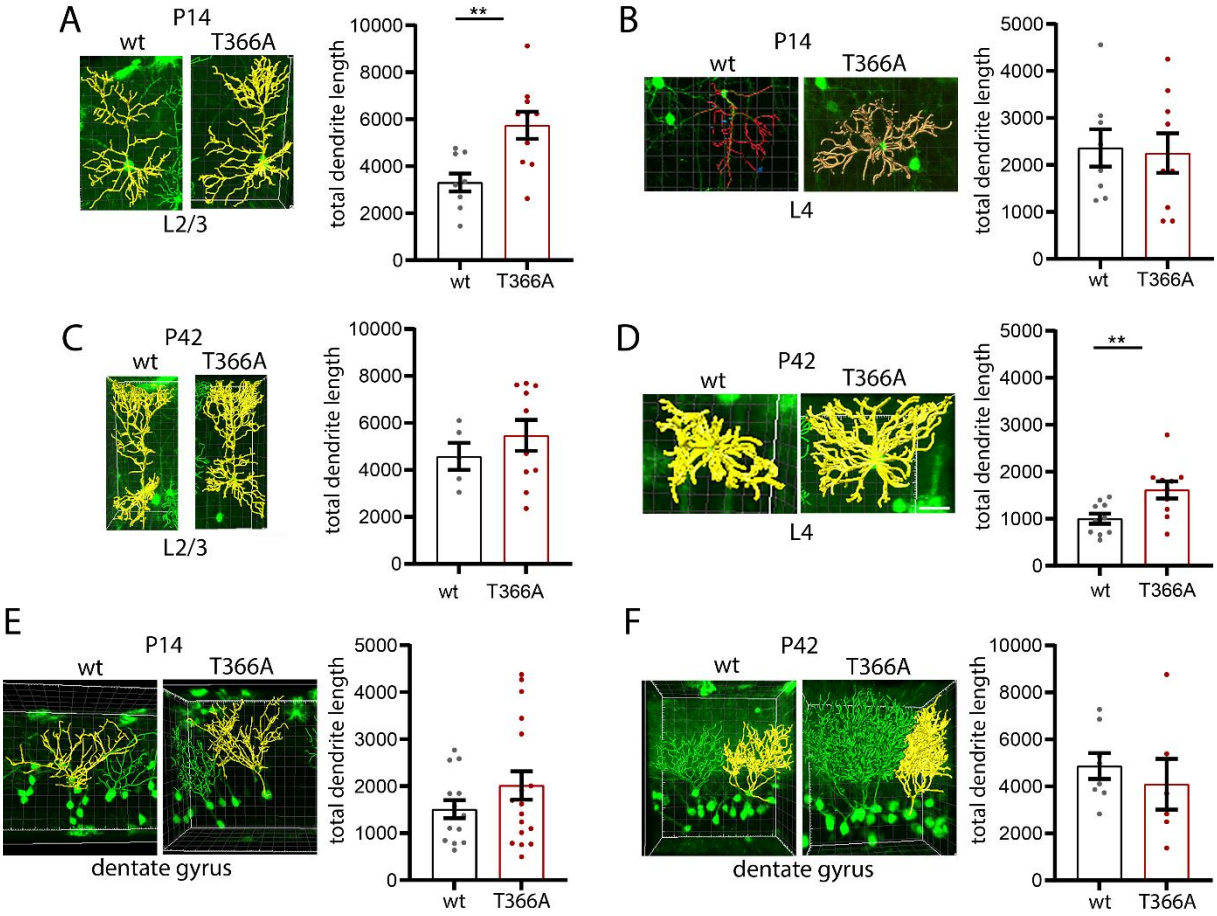
Supplementary Figure 1 Sequence and protein levels of *Pten*$^{T366A/T366A}$. (A) Sequence of T366A mutation depicting location of forward and reverse primer and the substitution (A) from threonine (GAC) to alanine (GGC) highlighted in color at position 32797312 on chromosome 19. **(B)** Example sequences from wt and *Pten*$^{T366A/T366A}$ brains showing the location of the mutation at bp 160 when sequenced with the forward primer. **(C)** Protein levels of Pten, pS6, pAkt374 and pAkt308 in forebrain lysates from P7 and P14 brains of wt and *Pten*$^{T366A/T366A}$ mice. Samples from two brains each stage and genotype. Molecular weight protein ladder is in kilodaltons. Quantification from P7 to P14 is shown in graphs. **(D)** pS6 fluorescence levels of single cortical neurons from layers 2/3, 4 and 5 from wt and *Pten*$^{T366A/T366A}$ mice at P8 and P14. Quantification in graphs. Data from two mice each genotype and stage. Data shown as average +/- S.E.M. Statistical analysis with Student's t-test and one-way ANOVA, ****$p<0.0001$. Analyses details in **Supplementary Tables 1 and 2.** Scale bars 40 µm.



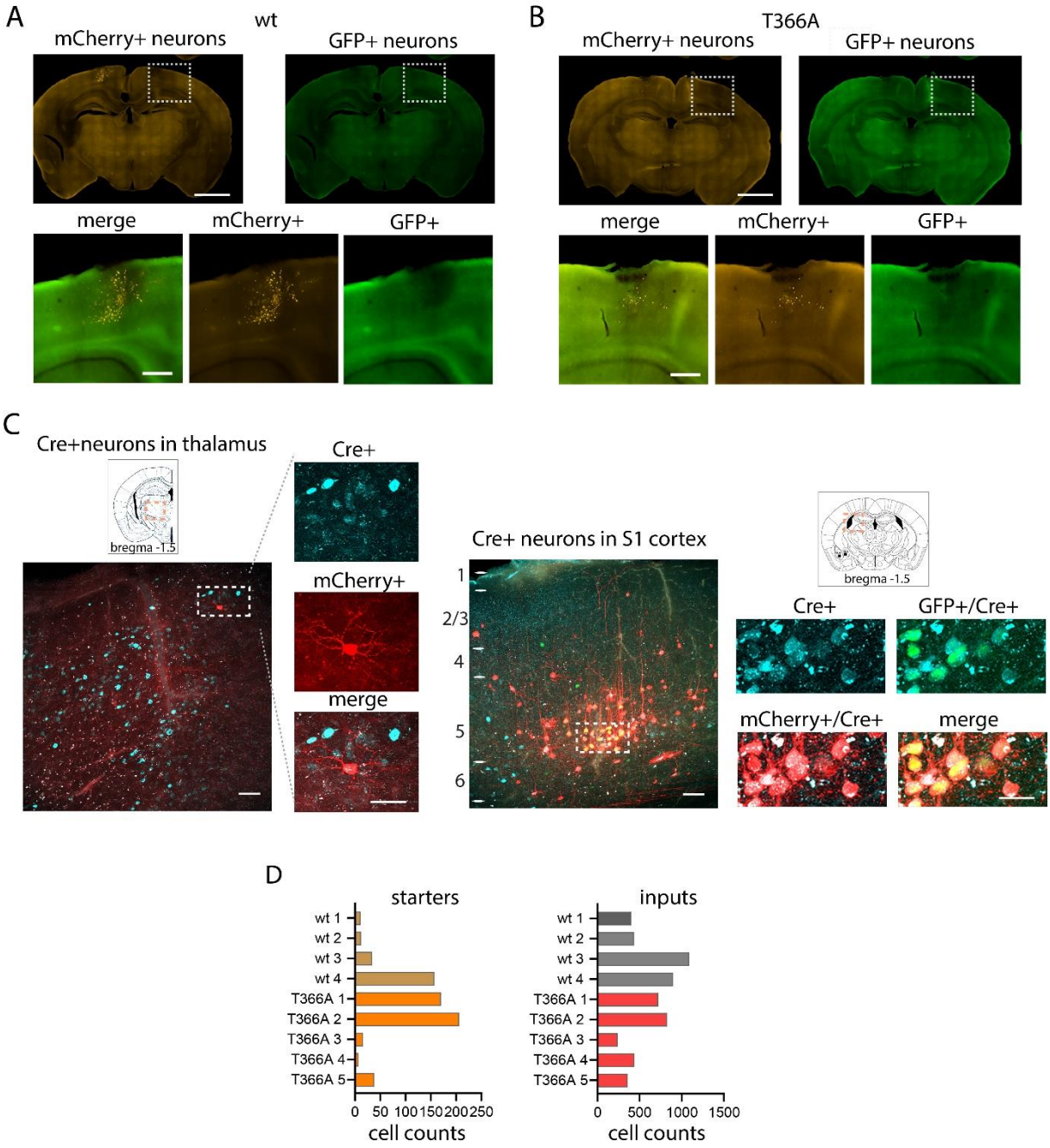
Supplementary Figure 2 Soma size in *Pten*$^{T366A/T366A}$ cortical neurons at P8 and P21. (A, B) Images showing GFP expression in S1 cortex at P8 **(A)** and P21 **(B)**. Zoom-ins showing somata from pyramidal neurons in L2/3 and L5, and neurons in L4. Graphs showing quantitative analysis for soma size in L2/3 to L5 neurons. Each dot accounts for one cell. Data shown as average +/- S.E.M. Statistical analysis with one-way ANOVA, ****$p<0.0001$. For analysis details see **Supplementary Table 4.** Scale bars 100 µm, 50 µm in zoom-ins.



Supplementary Figure 3 Soma size in *Pten*$^{T366A/T366A}$ primary cortical and hippocampal neurons. (A) Soma size was analyzed in primary cell culture from E16.5 hippocampal and cortical neurons with MAP2 immunostaining. (**B-D**) Example images from cortical neurons (**B**) and hippocampal neurons (**D)** at DIV2-3, DIV8 and DIV14. Graphs showing analysis for cortical (**C**) and hippocampal (**E**) neurons. Each dot in graphs accounts for one cell. Data shown as average +/- S.E.M. Two pregnant mice each genotype and cell type, cortices/ hippocampi of 5-8 embryos were pooled. Statistical analysis with one-way ANOVA, details in **Supplementary Table 6**. Scale bars 50 µm.



Supplementary Figure 4 Dendritic lengths of cortical and dentate gyrus neurons in *Pten*$^{T366A/T366A}$ and wt mice. (A-D) Images showing reconstruction of dendritic length of L2/3 pyramidal neurons (**A, C**), and of L4 neurons (**B, D**) in *Pten*$^{T366A/T366A}$ and wt mice at P14 and at P42. (**E, F**) Images of reconstruction of dentate gyrus at P14 (**E**) and P42 (**F**) in wt and *Pten*$^{T366A/T366A}$ mice. Data from three brains each genotype. Statistical analysis was performed with unpaired t-test, $**p<0.01$. Analysis details in **Supplementary Table 7**.



Supplementary Figure 5 Injection sites and transsynaptic Cre expressing neurons in thalamus and cortex. (A, B) Images showing injection sites in S1 cortex in wt and *Pten*$^{T366A/T366A}$ mice. (**C**) Example images showing Cre expressing neurons in thalamus and in S1 cortex. Cre expressing neurons are double labeled with mCherry in thalamus and with GFP and mCherry in S1 cortex. **(D)** Percentages of starter and input neurons in brains analysed. Scale bars in A, B, 500 µm, in zoom-ins 200 µm; in C, 100 µm, 50 µm in zoom-in.

Supplementary Tables

Supplementary Table 1 Protein expression in *Pten*$^{T366A/T366A}$ and wt mice

Genotype	PTEN, P7-P14	pS6, P7-P14	pAKT308, P7-P14	pAKT473, P7-P14
wt	0.3±0.05	0.6±0.05	0.72±0.1	1.0±0.2
***Pten*$^{T366A/T366A}$**	0.5±0.06	0.6±0.1	0.8±0.05	0.8±0.2
P-value	0.1071 (n.s.)	0.7769 (n.s.)	0.4682 (n.s.)	0.3921 (n.s.)

Values are percentage mean ± standard error, data from two mice from each genotype and age. Statistical analysis unpaired t-test. P-value indicates significance level for comparison between wt and *Pten*$^{T366A/T366A}$ whole brain lysates.

Supplementary Table 2 pS6 levels in *Pten*$^{T366A/T366A}$ and wt mice

Genotype	L2/3 - P8	L4 - P8	L5 - P8	L2/3 - P14	L4 - P14	L5 - P14
Wt (number neurons)	6.0±0.2 (20)	4.4±0.3 (17)	3.4±0.3 (24)	3.5±0.2 (20)	2.2±0.1 (19)	5.4±0.3 (35)
***Pten*$^{T366A/T366A}$ (number neurons)**	12.0±0.7 (20)	7.4±0.6 (20)	34.3±0.6 (25)	5.2±0.3 (20)	3.9±0.6 (20)	4.9±0.3 (36)
P-value	>0.0001 ****	0.0042 **	<0.9999 (n.s.)	0.0460 *	0.0284 *	<0.9999 (n.s.)

Values are percentage mean ± standard error, data from two mice each genotype. Statistical analysis one-way ANOVA, *$p<0.05$. P-values indicate significance level for comparison between wt and *Pten*$^{T366A/T366A}$ neurons.

Supplementary Table 3 Behaviour in *Pten*$^{T366A/T366A}$ and wt mice

Genotype	**Open field distance travelled (cm)**	**Open field center time (s)**	**Hanging wire**	**Vibrissae stimulation**	**grooming**	**rotarod**	
Wt	48.4±196.2	49.9±5.8	4.7±0.1	5.2±0.8	16.7 (mean)	8.3±0.3 (day 1)	8.9±0.6 (day 4)
***Pten*$^{T366A/T366A}$**	48.2±207,1	52.0±6.4	4.7±0.1	1.5±0.5	12.3 (mean)	14.0±0.9 (day 1)	14.3±0.6 (day 4)
P-value[a]	0.9442 (n.s.)	0.7342 (n.s.)	0.9590 (n.s.)	0.0002 ***	[b] >0.05 *	>0.9999 (n.s.)	>0.9999 (n.s.)
	Y maze females			**Conditioned freezing females**			
	Successful alternations	*Arm entries*	*Spontaneous alterations*	*Habituation*	*Context*	*Pre-cues*	*cues*
Wt	18.0±2.1	30.6±3.2	59.7±3.5	0.3±0.1	16.8±5.4	4.1±1.7	32.8±7.0
***Pten*$^{T366A/T366A}$**	18.8±1.8	30.4±3.0	62.3±2.7	0.3±0.1	20.3±7.0	9.7±4.4	39.6±9.9
P-value[a]	>0.9999 (n.s.)	>0.9999 (n.s.)	>0.9999 (n.s.)	>0.9999 (n.s.)	>0.9999 (n.s.)	>0.9999 (n.s.)	>0.9999 (n.s.)
	Y maze male			**Conditioned freezing males**			
	Successful alternations	*Arm entries*	*Spontaneous alterations*	*Habituation*	*Context*	*Pre-cues*	*cues*
Wt	18.2±1.7	28.3±2.8	65.5±2.5	0.5±0.1	23.6±14.4	6.7±1.9	40.0±4.9
***Pten*$^{T366A/T366A}$**	13.0±1.5	25.1±2.8	52.4±1.7	0.7±0.1	16.8±4.0	8.8±2.4	41.2±5.7
P-value[a]	>0.9999 (n.s.)	>0.9999 (n.s.)	0.0030 **	>0.9999 (n.s.)	0.0145 *	>0.9999 (n.s.)	>0.9999 (n.s.)
	Barnes maze		**Water maze**				
	Target quadrant	*Other quadrants*	*Target quadrant*	*Other quadrants*			
Wt	**40.2±25.4**	**19.9±1.8**	**44.4±4.7**	**18.5±1.6**			
***Pten*$^{T366A/T366A}$**	27.3±3.7	24.2±1.3	38.0±6.9	20.7±2.3			
P-value	0.114 (n.s.), [c] 0.008 ***	>0.9999 (n.s.), [c] 0.0265 *	>0.9999 (n.s.), [c] 0.0006 ***	>0.9999 (n.s.), [d] 0.0024 **			
	Van Frey						
	0.16	0.4	1	2	4	6	8
Wt	1.3±0.5	2.6±0.4	3.0±0.8	4.1±0.8	5.4±0.8	6.4±0.7	5.6±0.9
***Pten*$^{T366A/T366A}$**	1.4±0.5	3.4±0.8	4.2±0.8	5.1±0.7	7.3±0.5	7.4±0.7	7.2±0.6
P-value[a]	>0.9999 (n.s.)	>0.9999 (n.s.)	>0.9999 (n.s.)	>0.9999 (n.s.)	>0.9999 (n.s.)	>0.9999 (n.s.)	>0.9999 (n.s.)

Values are percentage mean ± standard error, data from three mice each genotype. Statistical analysis one-way ANOVA and unpaired t-test, *$p<0.05$. P-value indicates significance level for comparison between, [a] wt and *Pten*$^{T366A/T366A}$ groups, [b] Analysis with Wilcoxon test, [c] between wt target – wt other quadrants, [d] between wt target *Pten*$^{T366A/T366}$ other quadrants.

Supplementary Table 4 Cortical layers and proliferation of cortical progenitor neurons in *Pten*$^{T366A/T366A}$ and wt mice

Genotype	L1 (%)	L2/3 (%)	L4 (%)	L5 (%)	L6 (%)	Neuron count
Wt, NeuN	2.5±0.6	28.4±2.3	13.0±0.6	23.3±2.4	30.7±1.5	6293 NeuN
***Pten*$^{T366A/T366A}$, NeuN**	2.5±0.5	32.7±1.5	14.5±2.3	21.9±1.3	28.2±2.4	8014 NeuN
P-value	>0.9999 (n.s.)	0.4328 (n.s.)	>0.9999 (n.s.)	>0.9999 (n.s.)	>0.9999 (n.s.)	
Wt, Cux1	-	86.6±2.4	-	-	-	5282 Hoechst, 4486 Cux1
***Pten*$^{T366A/T366A}$, Cux1**	-	90.5±1.1	-	-	-	3517 Hoechst, 3172 Cux1
P-value		0.2107 (n.s)				
Wt, FoxP2	-	-	-	-	83.3±2.3	2324 Hoechst, 1928 FoxP2
***Pten*$^{T366A/T366A}$, FoxP2**	-	-	-	-	79.3±1.5	2769 Hoechst, 2196 FoxP2
P-value					0.1945 (n.s.)	
Wt, Ctip2	-	-	-	58.3±2.8	-	1822 Hoechst, 1070 CTIP2
***Pten*$^{T366A/T366A}$, Ctip2**	-	-	-	56.0±3.4	-	1345 Hoechst, 765 CTIP2
P-value				0.6056 (n.s.)		
E11/13-P1: wt	9.9±1.9	39.1±3.8	31.8±3.7	13.5±2.8	6.8±2.1	6837 BrdU
E11/13-P1: *Pten*$^{T366A/T366A}$	5.8±0.7	35.0±2.8	37.7±2.1	15.8±1.7	5.5±2.0	8864 BrdU
P-value	>0.9999 (n.s.)	>0.9999 (n.s.)	0.3008 (n.s.)	>0.9999 (n.s.)	>0.9999 (n.s.)	
E13/15-P8: wt	2.1±0.5	64.8±4.1	20.4±3.6	9.2±1.3	3.3±0.9	7852 BrdU
E13/15-P8: *Pten*$^{T366A/T366A}$	1.5±0.7	58.1±4.2	19.4±3.8	7.2±1.0	9.4±3.3	5376 BrdU
P-value	>0.9999 (n.s.)	0.4874 (n.s.)	>0.9999 (n.s.)	>0.9999 (n.s.)	0.6381 (n.s.)	

Values are percentage mean ± standard error, data from two mice (layers), three mice (proliferation) each genotype. Statistical analysis two-way ANOVA for NeuN and proliferation, t-test for layer comparison. P-values indicate significance level for comparison between wt and *Pten*$^{T366A/T366A}$ groups.

Supplementary Table 5 Soma size of cortical and dentate gyrus neurons in brain sections of *Pten*$^{T366A/T366A}$ and wt mice

Genotype	L2/3 (µm²)	Neuron count	L4 (µm²)	Neuron count	L5 (µm²)	Neuron count	Dentate gyrus (µm²)	Neuron count
P8, wt	188.2±3.3	203	128.8±3.4	155	207.9±4.7	146	-	-
P8, *Pten*$^{T366A/T366A}$	207.8±3.7	203	157.5±4.1	155	210.1±4.7	146	-	-
P-value	0.0020 **		<0.001 ****		>0.9999 (n.s.)		-	-
P14, wt	163.5±1.9	411	131.9±2.3	201	240.0±4.7	179	109.8±29.11	89
P14, *Pten*$^{T366A/T366A}$	195.4±2.1	411	173.9±2.9	201	147.7±4.7	181	143.5±24.9	141
P-value	>0.0001 ****		>0.0001 ****		>0.9999 (n.s.)		<0.001 ****	
P21, wt	197.2±2.9	218	163.1±2.8	159	270.6±5.3	101	-	-
P21, *Pten*$^{T366A/T366A}$	184.7±5.4	218	179.2±3.9	159	285.9±5.7	101	-	-
P-value	0.2102 (n.s.)		0.0517 (n.s.)		0.4076 (n.s.)		-	-
P42 wt	131.1±1.7	319	91.4±2.2	146	253.4±4.7	197	106.2±29.4	253
P42, *Pten*$^{T366A/T366A}$	130.0±1.9	319	95.6±2.2	146	241.7±4.3	225	96.8±26.2	366
P-value	>0.9999 (n.s.)		>0.9999 (n.s.)		>0.1071 (n.s.)		0.0986 (n.s.)	

Values are percentage mean ± standard error, data from three mice each genotype. Statistical analysis with one-way ANOVA, *$p<0.05$, unpaired t-test for data dentate gyrus, *$p<0.05$. P-values indicate significance level for comparison between wt and *Pten*$^{T366A/T366A}$ groups.

Supplementary Table 6 Soma size of cortical and hippocampal neurons in primary cell culture of *Pten*$^{T366A/T366A}$ and wt mice

Genotype (neuron count)	2-4 DIV (µm²)	Neuron count	8 DIV (µm²)	Neuron count	14 DIV (µm²)	Neuron count
Wt, cortical neurons	150.7±4.9	92	128.6±4.9	48	181.4±12.5	19
***Pten*$^{T366A/T366A}$, cortical neurons**	144.6±4.6	92	146.6±4.7	48	178.69±6.5	56
P-value	>0.9999 (n.s.)		0.6966 (n.s.)		>0.9999 (n.s.)	
Wt, hippocampal neurons	135.6±4.1	99	175.9±6.6	50	190.5±6.5	60
***Pten*$^{T366A/T366A}$, hippocampal neurons**	154.2±4.9	100	193.3±7.0	51	214.6±6.4	60
P-value	0.0923 (n.s.)		>0.9999 (n.s.)		0.0904 (n.s.)	

Values are percentage mean ± standard error, data from two pregnant females each genotype, 5-8 embryos pooled. Statistical analysis with one-way ANOVA. P-values indicate significance level for comparison between wt and *Pten*$^{T366A/T366A}$ groups.

Supplementary Table 7 Dendrite lengths and dendritic arborisation of cortical and hippocampal neurons in *Pten*$^{T366A/T366A}$ and wt mice

Genotype (neuron count)	L2/3 (μm²)	P-value	L4 (μm²)	P-value	Dentate gyrus (μm²)	P-value
P14, wt (9 cortex, 10 DG)	3310±380.2	0.0032 **	2364±397.9	0.8523 (n.s.)	1509±191.9	0.1947 (n.s.)
P14, *Pten*$^{T366A/T366A}$ (10 cortex, 18 DG)	5742±578.2	-	2253±421.0	-	2018±302.6	-
Sholl analysis		<0.0001****		<0.0001****		<0.0001****
P42 wt (8 cortex, 8 DG)	4582±573.0	0.4024 (n.s.)	1002±106.3	0.0097 **	4870±550.7	0.5031 (n.s.)
P42, *Pten*$^{T366A/T366A}$ (10 cortex, 6 DG)	5471±659.0	-	1614±182.8		4092±108.3	-
Sholl analysis		<0.0001****		<0.0001****		<0.0001****, interaction >0.9999 (n.s.)

Values are percentage mean ± standard error, data from three mice each genotype. Statistical analysis for dendritic length with unpaired t-test, *$p<0.05$, two-way Anova for Sholl analysis, ****$p<0.0001$. P-values indicate significance level for comparison between wt and *Pten*$^{T366A/T366A}$ groups.

Supplementary Table 8 Presynaptic input to S1 cortex in *Pten*$^{T366A/T366A}$ and wt mice

Genotype	Local S1	Long-range	Visual cortices	S2 cortex	Motor cortices	thalamus	contralateral S1
Wt %	71.5±3.2	38.1±3.2	22.4±5.9	5.0±1.7	32.3±7.3	26.4±5.1	13.8±7.6
Wt, neuron count	2811	751	175	31	267	193	85
***Pten*$^{T366A/T366A}$ %**	76.4±6.2	23.3±6.3	11.0±3.9	2.6±1.6	14.2±3.1	50.3±7.5	19.2±5.2
***Pten*$^{T366A/T366A}$, neuron count**	2580	536	51	15	76	235	128
P-value	>0.9999 (n.s.)	0.4553 (n.s.)	0.1350 (n.s.)	0.330 (n.s.)	0.0440 *	0.0437 *	0.5613 (n.s.)

Values are percentage mean ± standard error, data from four mice for wt, five mice for *Pten*$^{T366A/T366A}$. Statistical analysis with unpaired t-test, *$p<0.05$. P-values indicate significance level for comparison between wt and *Pten*$^{T366A/T366A}$ brain areas.

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