Supplementary Information

Tanc2-mediated mTOR inhibition balances mTORC1/2 signaling in the developing mouse brain and human neurons

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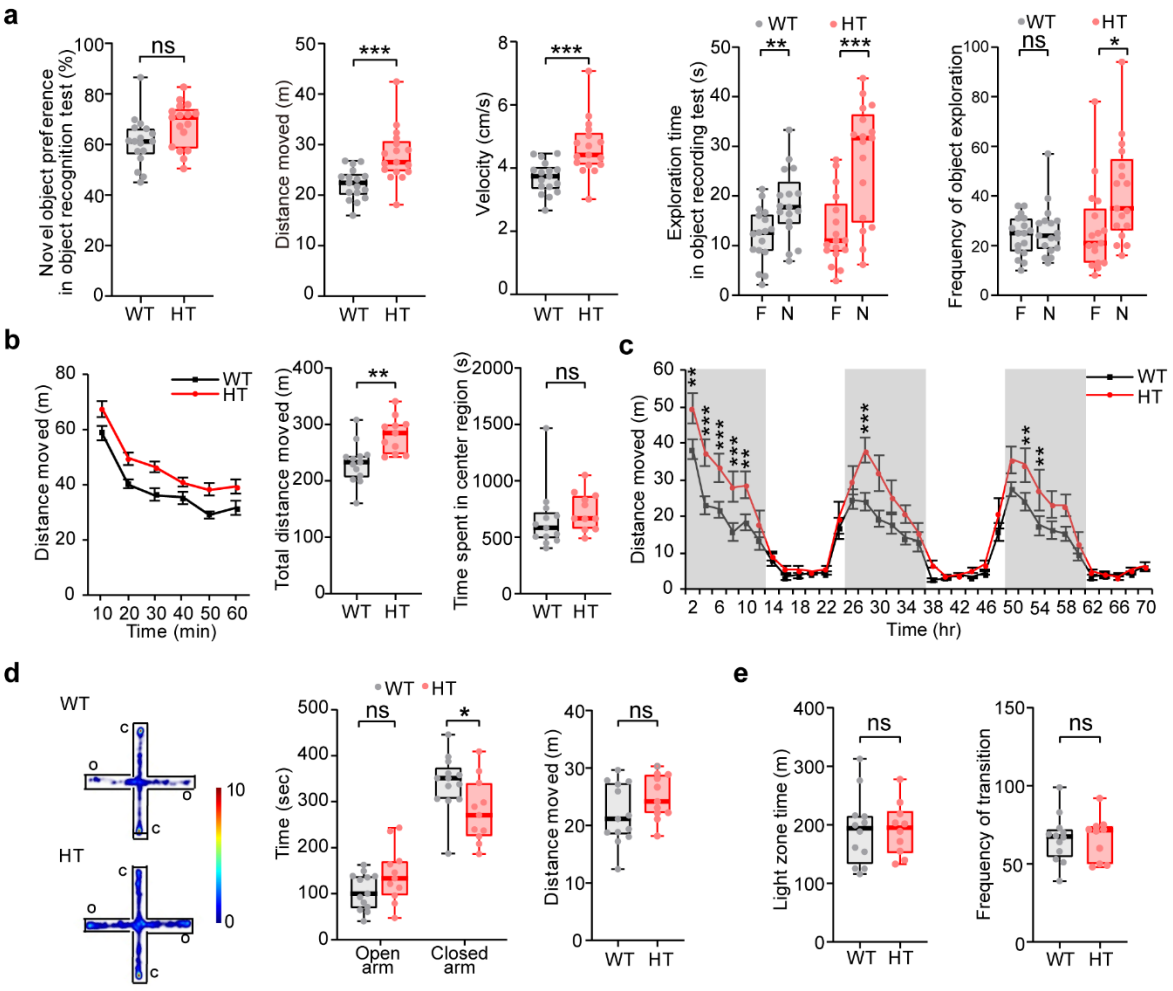
Supplementary figures and legends

Supplementary Fig. 1



Supplementary Fig. 1. Hyperactivity and moderate anxiolytic-like behavior in male *Tanc2*$^{+/-}$ mice.

(a) Normal novel object-recognition memory in *Tanc2*$^{+/-}$ mice (2–5 months; male) in the novel object-recognition test, as shown by novel object preference (% time spent in exploring a novel object relative to the total time spent exploring both familiar and novel objects). F, familiar object; N, new object. Note that these mice also showed hyperactivity in the novel object arena, as supported by the distance moved and movement velocity. The increased frequency of novel object exploration in the mutant mice might be attributable to the increased hyperactivity. Data: minimal, maximal, median, 25%, and 75% values. (n = 17 [WT, HT], *P < 0.05, **P < 0.01, ***P < 0.001, ns, not significant, Student's t-test).

(b) Hyperactivity of *Tanc2*$^{+/-}$ mice (2–5 months; male) in the open-field test, as shown by distance moved over 60 minutes and total distance moved. Note also that the

time spent in the center region of the open field arena was normal in *Tanc2*$^{+/-}$ mice, indicative of normal anxiety-like behavior. Data: mean ± SEM (line graphs), minimal, maximal, median, 25%, and 75% values. (n = 13 [WT], 11 [HT], **P < 0.01, ns, not significant, two-way RM ANOVA and Student's t-test).

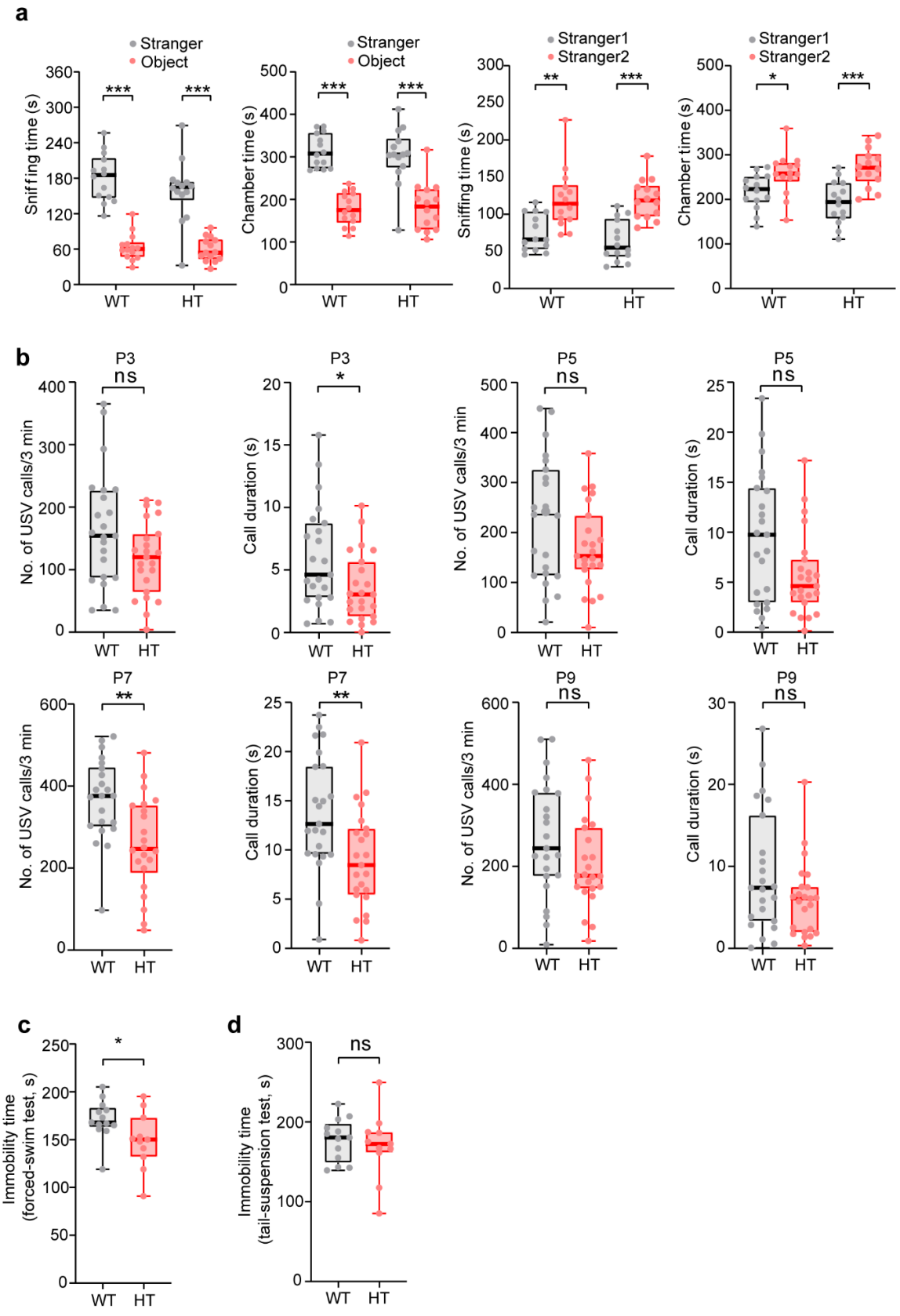
(c) Hyperactivity of *Tanc2*$^{+/-}$ mice (2–5 months; male) in the Laboras test, as shown by distance moved over 72 hours. The shaded areas represent light-off periods. Data: mean ± SEM. (n = 16 [WT, HT], **P < 0.01, ***P < 0.001, two-way RM ANOVA [genotype effect P < 0.05]).

(d) Moderate anxiolytic-like behavior of *Tanc2*$^{+/-}$ mice (2–5 months; male) in the elevated plus-maze test, as shown by time spent in open and closed arms. There was no genotype difference in the total distance moved. Data: minimal, maximal, median, 25%, and 75% values. (n = 13 [WT], 11 [HT], *P < 0.05, ns, not significant, two-way RM ANOVA with Bonferroni test and Student's t-test).

(e) Normal anxiety-like behavior of *Tanc2*$^{+/-}$ mice (2–5 months; male) in the light-dark test, as shown by time spent in the light chamber/zone and frequency of transitions between light and dark chambers. Data: minimal, maximal, median, 25%, and 75% values. (n = 13 [WT], 10 [HT], ns, not significant, Student's t-test).

See Source Data 1 for raw data values and Supplementary Table 1 for statistical details.

Supplementary Fig. 2



Supplementary Fig. 2. Suppressed pup ultrasonic vocalization but normal social interaction, social novelty recognition, and depression-like behavior in male *Tanc2*$^{+/-}$ mice.

(a) Normal social interaction and social-novelty recognition in *Tanc2*$^{+/-}$ mice (2–5 months; male) in the three-chamber social-interaction test, as shown by time spent in the chamber and in sniffing the target (stranger/object). Stranger 1, social stranger mouse; Object, inanimate object; Stranger 2, new social stranger mouse. Data: minimal, maximal, median, 25%, and 75% values. (n = 14 mice [WT, HT], ***P < 0.001, Student's t-test).

(b) Modestly suppressed ultrasonic vocalizations (USVs) in *Tanc2*$^{+/-}$ pups (P3–9; male) separated from their mothers, as shown by the total number of USV calls and the duration of each call. Data: minimal, maximal, median, 25%, and 75% values. n = 23 pups [WT, HT], *P < 0.05, **P < 0.01, ns, not significant, Student's t-test).

(c) Anti-depression-like behavior of *Tanc2*$^{+/-}$ mice (2–5 months; male) in the forced-swim test, as shown by immobility time. Data: minimal, maximal, median, 25%, and 75% values. (n = 13 [WT], 11 [HT], *P < 0.05, Student's t-test).

(d) Normal depression-like behavior of *Tanc2*$^{+/-}$ mice (2–5 months; male) in the tail-suspension test, as shown by immobility time. Data: minimal, maximal, median, 25%, and 75% values. (n = 13 [WT], 11 [HT], ns, not significant, Student's t-test).

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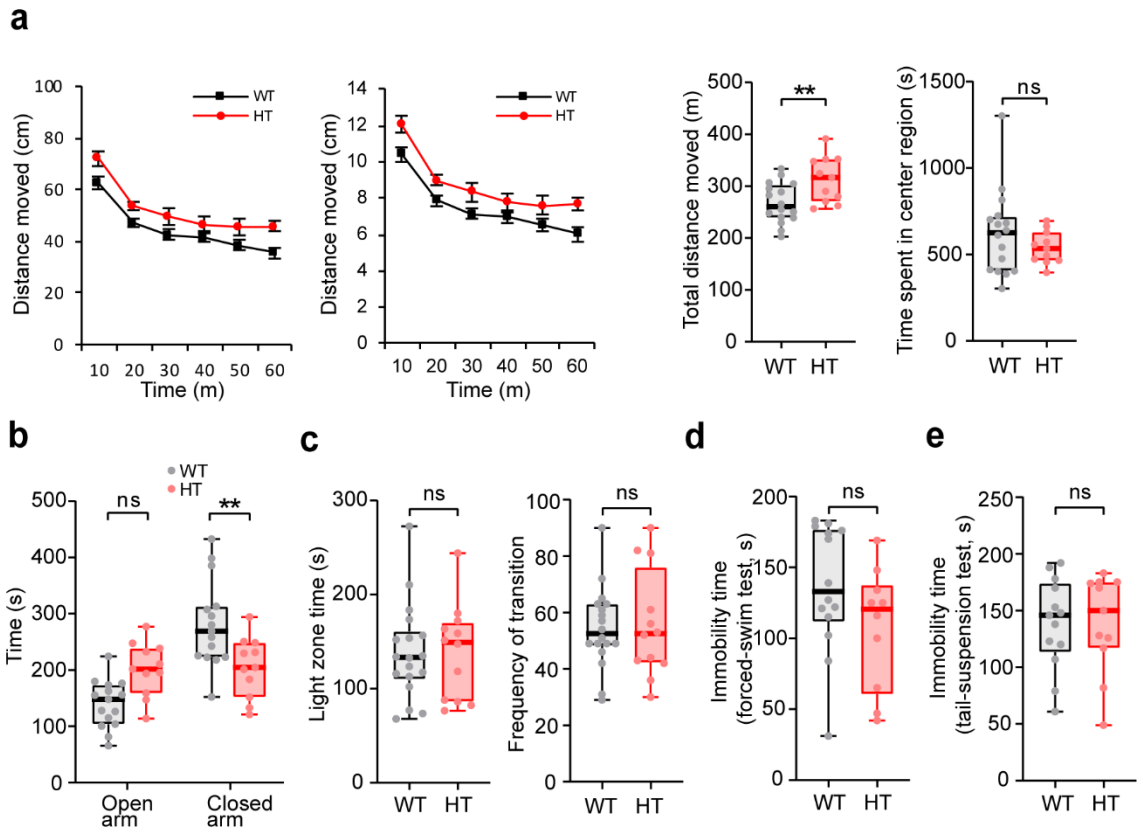
Supplementary Fig. 3



Supplementary Fig. 3. Female *Tanc2*$^{+/-}$ mice display hyperactivity and anxiolytic-like behavior, but normal depression-like behavior.

(a) Hyperactivity of female *Tanc2*$^{+/-}$ mice (2–5 months) in the open-field test, as shown by distance moved over 60 minutes and total distance moved. Note also that the time spent in the center region of the open field arena was normal in female *Tanc2*$^{+/-}$ mice, indicative of normal anxiety-like behavior. Data: mean ± SEM (line gr aphs), minimal, maximal, median, 25%, and 75% values. (n = 16 mice [WT], and 11 mice [HT], **P < 0.01, ns, not significant, two-way RM ANOVA and Student's t-test).

(b) Moderate anxiolytic-like behavior of female *Tanc2*$^{+/-}$ mice (2–5 months) in the elevated plus-maze test, as shown by time spent in open and closed arms. Data: minimal, maximal, median, 25%, and 75% values. (n = 15 mice [WT], and 11 mice [HT], **P < 0.01, ns, not significant, two-way RM ANOVA with Bonferroni test).

(c) Normal anxiety-like behavior of female *Tanc2*$^{+/-}$ mice (2–5 months) in the light-dark test, as shown by time spent in the light chamber and frequency of transitions between light and dark chambers. Data: minimal, maximal, median, 25%, and 75% values. (n = 18 mice [WT], and 12 mice [HT], ns, not significant, Student's t-test).

(d) Normal depression-like behavior of female *Tanc2*$^{+/-}$ mice (2–5 months) in the

forced-swim test, as shown by immobility time. Data: minimal, maximal, median, 25%, and 75% values. (n = 14 mice [WT], and 10 mice [HT], ns, not significant, Student's t-test).

(e) Normal depression-like behavior of *Tanc2*$^{+/-}$ mice (2–5 months; female) in the tail-suspension test, as shown by immobility time. Data: minimal, maximal, median, 25%, and 75% values. (n = 13 mice [WT], and 10 mice [HT], ns, not significant, Student's t-test).

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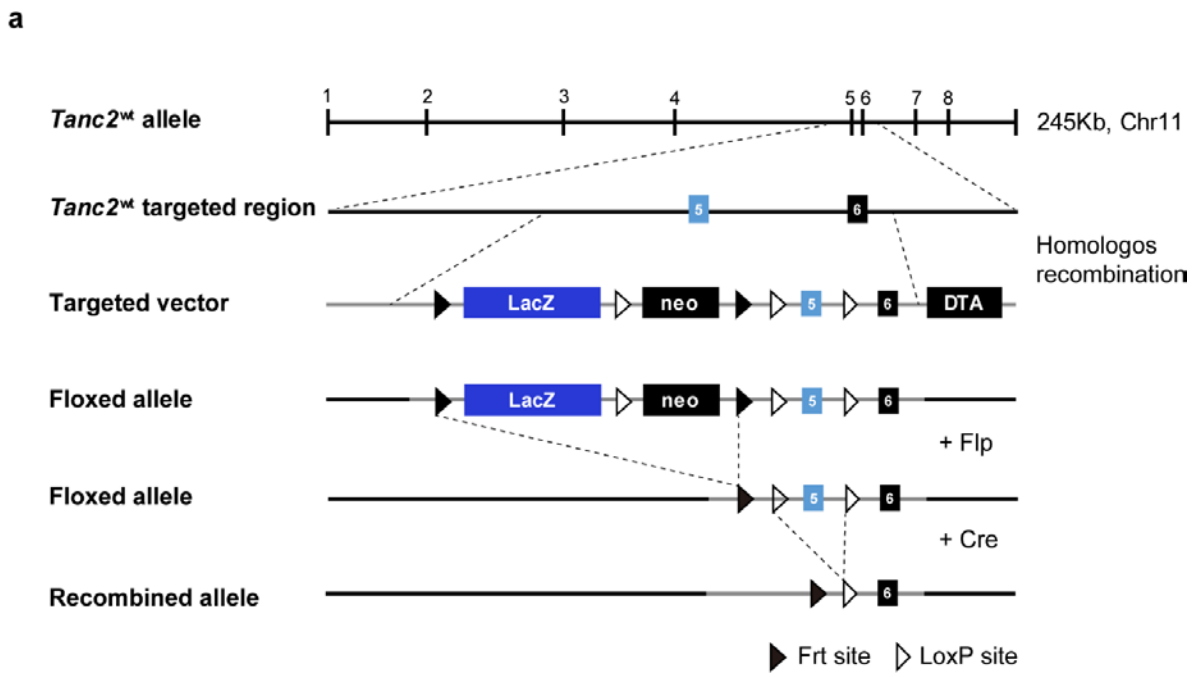
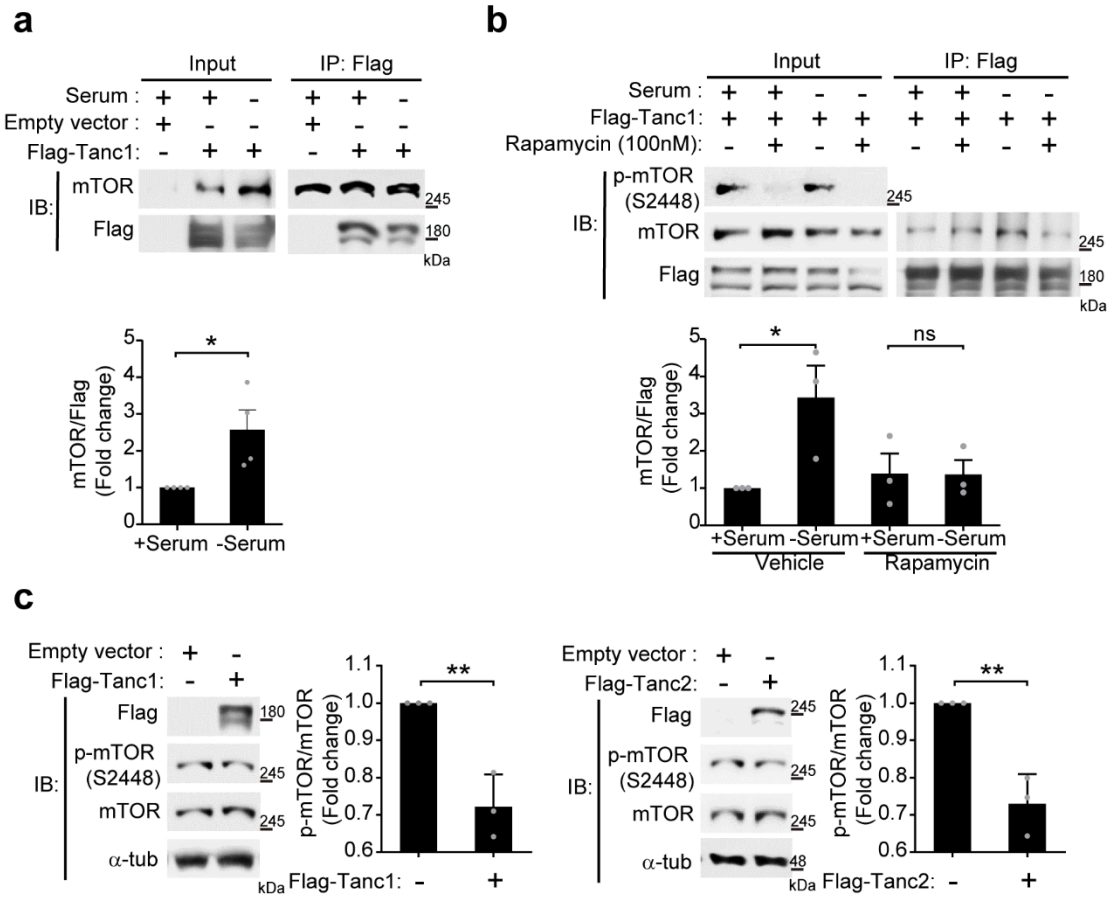
Supplementary Fig. 4



Supplementary Fig. 4. Conditional *Tanc2* KO strategy.

(a) Scheme for the generation of *Tanc2*$^{fl/fl}$ mice carrying floxed exon 5 of the *Tanc2* gene. LacZ, β-galactosidase gene; neo, neomycin resistance cassette; DTA, diphtheria toxin gene; Flp, flippase; Cre, Cre recombinase; Frt site, site for flippase; LoxP site, cleavage site for cre recombinase action.

Supplementary Fig. 5



Supplementary Fig. 5. Tanc1 interacts with mTOR in a serum- and rapamycin-dependent manner and inhibits mTOR activity in heterologous Cells.

(a) Serum starvation (4 hours) promotes the interaction between Tanc1 and mTOR in heterologous cells, as demonstrated by coimmunoprecipitation. HEK293T cells expressing Flag-Tanc1 (human) were incubated with serum (or no serum; control) for 4 hours prior to coimmunoprecipitation and immunoblot experiments. mTOR signals were normalized to Tanc1 signals for quantification. Data: mean ± SD. (n = 4 independent experiments, *P < 0.05, Student's t-test).

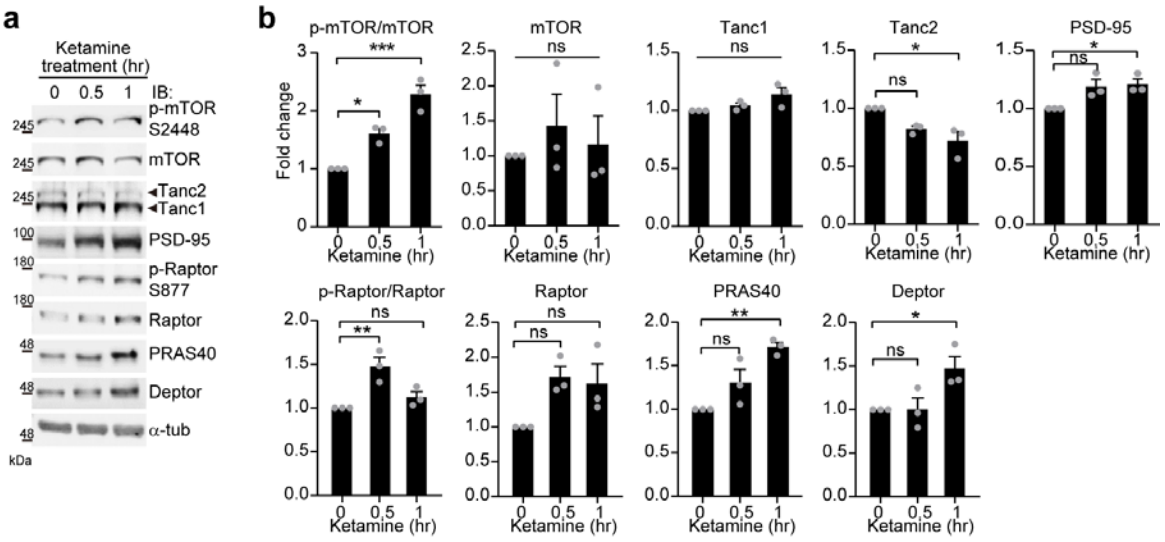
(b) Rapamycin inhibits serum starvation-induced increases in the interaction between Tanc1 and mTOR in heterologous cells. Flag-Tanc1 was expressed in HEK293T cells in the presence of serum. Pretreatment of cells with rapamycin for 2 hours was followed by serum starvation for 4 hours; cell lysates were subjected to coIP and immunoblot experiments. mTOR signals were normalized to Tanc1 signals for quantification. Data: mean ± SD. (n = 3 independent experiments, ***P < 0.05, ns, not significant, Student's t-test).

(c) Overexpression of Tanc1 in HEK293T cells suppresses mTOR activity, as shown

by the ratio of total to phosphorylated (active) mTOR. Tanc2 (human) was also used as a control. Flag-tagged Tanc1/Tanc2 were overexpressed in HEK293T cells, followed by immunoblotting for total and phosphorylated (Ser-2448) mTOR. mTOR signals were normalized to α-tubulin signals. Data: mean ± SD. (n = 3 independent experiments, $^{**}P < 0.01$, Student's t-test).

See Source Data 1 for raw data values and Supplementary Table 1 for statistical details.

Supplementary Fig. 6

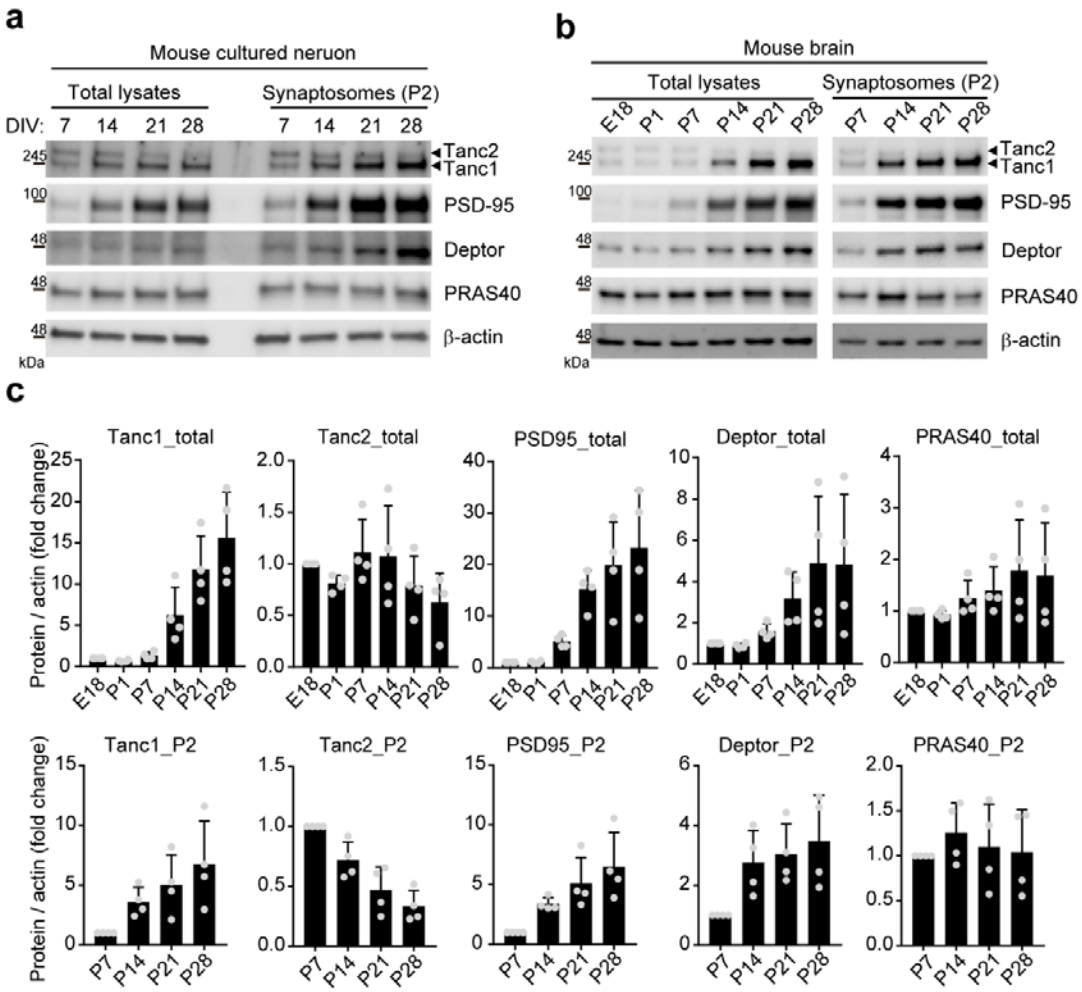


Supplementary Fig. 6. Ketamine induces changes in mTOR activity and synaptic levels of Tanc2-associated and mTORC proteins in the mouse brain.

(a and b) Ketamine induces mTOR activation in the mouse brain (P13–14; 10 mg/kg; i.p.), as shown by the time-dependent increases in the phosphorylation of mTOR and the mTORC1 protein Raptor in crude synaptosomes. Note that synaptic levels of other mTORC1/2 proteins (PRAS40 and Deptor) and PSD-95 also increased, whereas those of Tanc2 (but not Tanc1) were modestly decreased. Data: mean ± S D. (n = 3 independent experiments, *P < 0.05, **P < 0.01, ***P < 0.001, ns, not significant, one-way ANOVA with Bonferroni test).

See Source Data 1 for raw data values and Supplementary Table 1 for statistical details.

Supplementary Fig. 7



Supplementary Fig. 7. Temporal changes in total levels and synaptic enrichment of Tanc2, Deptor, and Tanc1 in cultured mouse hippocampal neurons and the mouse brain.

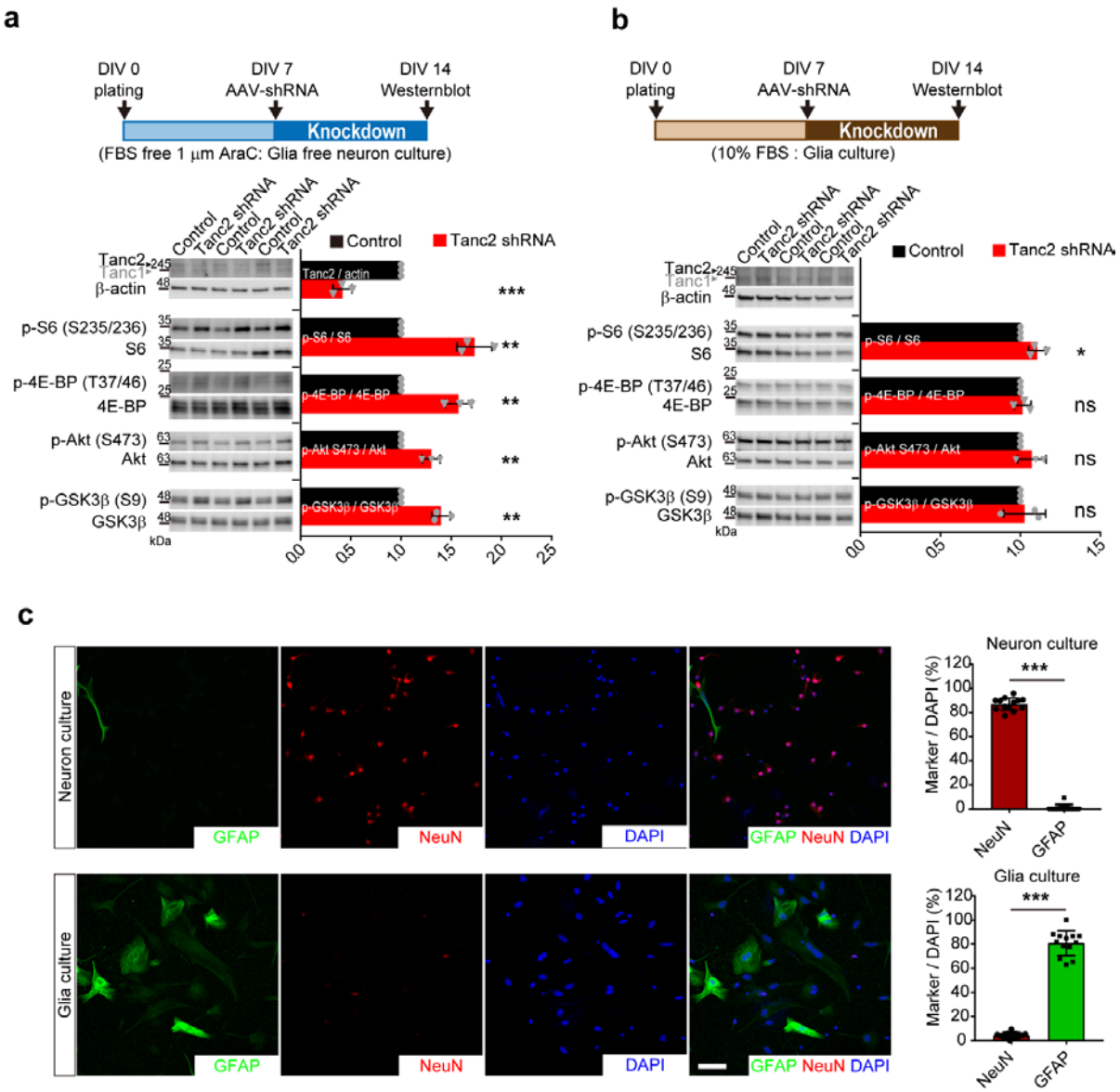
(a) Western blot analyses of whole lysates from cultured mouse hippocampal neurons show Tanc2 protein levels that are largely stable across developmental stages (DIV/days in vitro 7–28) and stronger relative to Tanc1 at early stages, while Deptor and Tanc1 protein levels gradually increase. Western blot analyses of crude synaptosomes from cultured mouse hippocampal neurons show largely unchanged synaptic levels of Tanc2 proteins across the developmental stages, which contrasts with the increasing synaptic levels of Deptor and Tanc1 proteins. Total and synaptic levels of PRAS40 (a component of mTORC1) were largely unchanged across developmental stages. PSD-95 was used as a positive control for stage-dependent increases in total expression and synaptic enrichment, and β-actin was used as a

loading control.

(b and c) Western blot analyses of whole lysates from the mouse brain (in vivo results) show Tanc2 protein levels that are largely stable across developmental stages (E/embryonic day 18–P/postnatal day 28) and higher relative to Tanc1 at early stages, while Deptor and Tanc1 protein levels gradually increase. Western blot analyses of crude synaptosomes from the mouse brain show decreasing synaptic levels of Tanc2 proteins across the developmental stages, which contrasts with the increasing synaptic levels of Deptor and Tanc1 proteins. Total and synaptic levels of PRAS40 were largely unchanged across developmental stages. PSD-95 was used as a positive control for stage-dependent increases in total expression and synaptic enrichment, and β-actin was used as a loading control. Data: mean ± SD. (n = 4 independent experiments).

See Source Data 1 for raw data values and Supplementary Table 1 for statistical details.

Supplementary Fig. 8



Supplementary Fig. 8. Acute Tanc2 knockdown in cultured mouse neurons, but not glial cells, leads to mTORC1/2 hyperactivity.

(a) Acute knockdown of Tanc2 in cultured mouse neurons by infection with AAV-shRNA (DIV7–14) leads to increased phosphorylation levels of S6 (S235/236), 4E-BP (T37/46), Akt (S473), and GSK3β (S9), indicative of mTORC1 and mTORC2 hyperactivity. Data: mean ± SD. (n = 3 independent experiments, **P < 0.01, ***P < 0.001, Student's t-test).

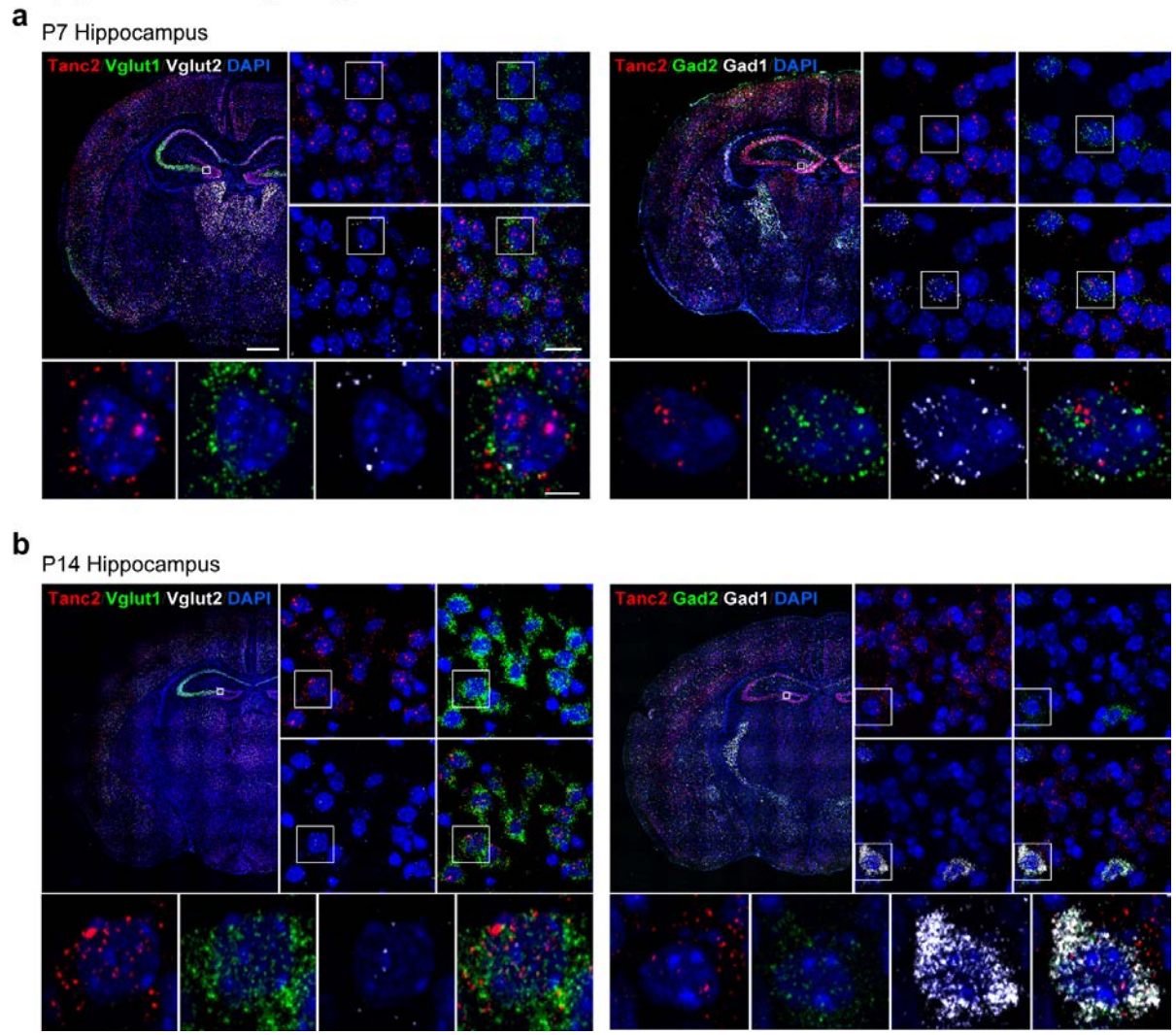
(b) Acute knockdown of Tanc2 in cultured mouse glial cells by infection with AAV-shRNA (DIV7–14) does not affect the phosphorylation levels of S6 (S235/236), 4E-

BP (T37/46), Akt (S473) or GSK3β (S9), except for a small increase in p-S6, suggesting that Tanc2 mainly suppresses mTORC1/2 signaling in neurons rather than glial cells. Note that Tanc2 expression is much weaker relative to Tanc1. Data: mean ± SD. (n = 3 independent experiments, *P < 0.05, ns, not significant, Student's t-test).

(c) Control co-immunofluorescence staining showing that cultured neurons are positive for NeuN (neuronal marker) but not for GFAP (astrocytic marker) and that cultured glia are positive for GFAP but not for NeuN. DAPI was used for nuclear staining. Scale bar, 50 µm. Data: mean ± SEM. (n = 13 images for neuron and glial cultures, ***P < 0.001, Student's t-test).

See Source Data 1 for raw data values and Supplementary Table 1 for statistical details.

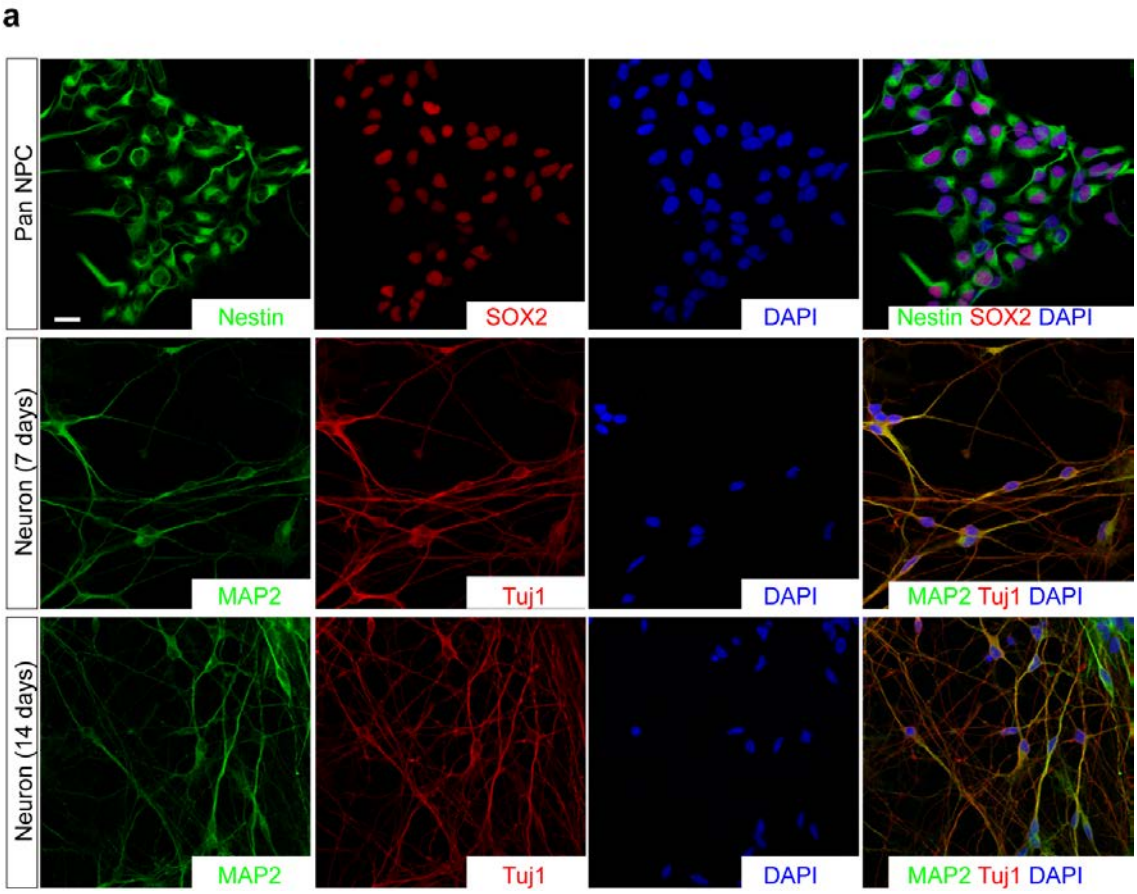
Supplementary Fig. 9



Supplementary Fig. 9. Tanc2 mRNA expression in both glutamatergic and GABAergic neurons in the mouse brain.

(a and b) Tanc2 expression is observed in both Vglut1/2-positive glutamatergic neurons and Gad1/2-positive GABAergic neurons in the cortical and hippocampal regions of the mouse brain at P7 and P14 (hippocampal examples are shown), as revealed by fluorescent in situ hybridization (FISH). Images in the bottom lines of each figure panel indicate enlarged single cells to clearly show the co-expression of Tanc2 with the indicated markers in the same cell. Scale bars, 500 μm (left), 20 μm (right), and 5 μm (bottom). Three independent experiments yielded similar results.

Supplementary Fig. 10



Supplementary Fig. 10. Characterization of human NPCs and neurons by immunofluorescence staining.

(a) Morphological characterization of pan-NPCs by immunofluorescence staining for Nestin and SOX2 (NPC markers) and pan-neurons at DIV7 and DIV14 by staining for MAP2 and Tuj1 (neuronal markers). Scale bar, 20 μm. Three independent experiments yielded similar results.

Supplementary table 1. Statictical analyis

Figure		Assay Performed	Comparison (unless stated otherwise, WT vs HT)	Sex	Age	n	Parameter (Unit)	Variable		Descriptive statistics (Average ± SE or SD)	Statistical test and significance			
											Statistical test	Significance		
1	a	Morris water maze		Male	2-5 months	WT = 20 HT = 20	Latency to escape (s)	Training	Day 1	WT = 52.20 ± 1.92 HT = 54.78 ± 2.44	Two-way ANOVA repeated measures Bonferroni's multiple comparison test	p = 0.9999, ns	Interaction: F(4, 76) = 2.39, p = 0.0581 Tanc2: F(1, 19) = 11.37, p = 0.0032 Time: F(4, 76) = 45.54, p < 0.0001	
									Day 2	WT = 38.55 ± 3.08 HT = 49.10 ± 2.28		p = 0.0180, *		
									Day 3	WT = 31.90 ± 3.80 HT = 46.33 ± 2.88		p = 0.0005, ***		
									Day 4	WT = 21.7 ± 3.01 HT = 38.21 ± 3.96		p = 0.0001, ***		
									Day 5	WT = 20.95 ± 2.58 HT = 29.56 ± 3.40		p = 0.0821, ns		
							Time spent in quadrant (s)	Probe test	Target	WT = 30.62 ± 2.42 HT = 19.86 ± 2.21	Two-way ANOVA repeated measures Bonferroni's multiple comparison test	p = 0.0001, ***	Interaction: F(3, 152) = 8.959, p < 0.0001 Tanc2: F(1, 152) = 2.607, p = 0.0986 Quadrant: F(3, 152) = 29.7, p < 0.0001	
									A Left	WT = 11.10 ± 1.30 HT = 13.13 ± 1.77		p = 0.9999, ns		
									A Right	WT = 10.54 ± 1.26 HT = 17.60 ± 1.97		p = 0.0278, *		
									Opposite	WT = 7.95 ± 1.38 HT = 9.86 ± 1.93		p = 0.9999, ns		
							Number of crossing (count)			WT = 3.90 ± 0.47 HT = 2.35 ± 0.50	Mann-Whitney U	p = 0.0128, *	U = 110	Two-tailed
							Swimming speed (cm/s)			WT = 14.29 ± 0.72 HT = 12.27 ± 0.89	Student's t-test	p = 0.0896, ns	t(38) = 1.745	Two-tailed
	b	Electrophysiology		Male	7-8 weeks	WT = 12 slices/ 5 mice HT = 10 slices/ 5 mice	Hippocampus, HFS, 5 min , LTP (%)			WT = 152.3 ± 4.96 HT = 132.1 ± 5.02	Student's t-test	p = 0.0100, *	t(20) = 2.8440	Two-tailed
	c	Electrophysiology		Male	7-8 weeks	WT = 10 slices/ 3 mice HT = 10 slices/ 3 mice	Input-output ratio		0.5	WT = 0.1508 ± 0.0151 HT = 0.1264 ± 0.0270	Two-way ANOVA repeated measures Bonferroni's multiple comparison test	p > 0.9999, ns	Interaction: F(10, 180) = 0.07672, p > 0.9999 Tanc2: F(1, 18) = 0.06271, p = 0.8051 Fiber Volley: F(10, 180) = 269.9, p < 0.0001	
									0.75	WT = 0.2782 ± 0.0145 HT = 0.2636 ± 0.0204		p > 0.9999, ns		
									1	WT = 0.3647 ± 0.0122 HT = 0.3648 ± 0.0143		p > 0.9999, ns		
									1.25	WT = 0.4580 ± 0.0113 HT = 0.4524 ± 0.0122		p > 0.9999, ns		
									1.5	WT = 0.5269 ± 0.0130 HT = 0.5303 ± 0.0151		p > 0.9999, ns		
									1.75	WT = 0.5905 ± 0.0195 HT = 0.5912 ± 0.0176		p > 0.9999, ns		
									2	WT = 0.6551 ± 0.0191 HT = 0.6542 ± 0.0226		p > 0.9999, ns		
									2.25	WT = 0.7085 ± 0.0242 HT = 0.7045 ± 0.0286		p > 0.9999, ns		
									2.5	WT = 0.7745 ± 0.0233 HT = 0.7660 ± 0.0332		p > 0.9999, ns		
									2.75	WT = 0.8250 ± 0.0284 HT = 0.8160 ± 0.0395		p > 0.9999, ns		
									3	WT = 0.8761 ± 0.0333 HT = 0.8695 ± 0.0411		p > 0.9999, ns		
	d					WT = 10 slices/ 3 mice HT = 10 slices/ 3 mice	Hippocampus, PPR	Inter-pulse interval (ms)	25 msec	WT = 167.8 ± 3.77 HT = 159.8 ± 6.43	Two-way ANOVA repeated measures Bonferroni's multiple comparison test	p > 0.9999, ns	Interaction: F(5, 90) = 1.408, p = 0.2291 Tanc2: F(1, 18) = 0.01536, p = 0.9027 Time: F(5, 90) = 164.7, p < 0.0001	
									50 msec	WT = 173.2 ± 2.23 HT = 174.8 ± 3.93		p > 0.9999, ns		
									100 msec	WT = 141.3 ± 4.43 HT = 136.9 ± 2.46		p > 0.9999, ns		
									200 msec	WT = 110.7 ± 1.57 HT = 109.7 ± 2.41		p > 0.9999, ns		
									300 msec	WT = 110.7 ± 1.57 HT = 109.7 ± 2.41		p > 0.9999, ns		
	e	Electrophysiology		Male	4-5 weeks	WT = 12 slices/ 6 mice HT = 12 slices/ 5 mice	Hippocampus, HFS, 5 min , LTP (%)			WT = 142.8 ± 4.52 HT = 132.4 ± 3.21	Student's t-test	p = 0.1041, ns	t(22) = 1.6950	Two-tailed
	f	Electrophysiology		Male	3-4 weeks	WT = 10 slices/ 4 mice HT = 13 slices/ 5 mice	Input-output ratio		0.5	WT = 0.0620 ± 0.0062 HT = 0.0746 ± 0.0105	Two-way ANOVA repeated measures Bonferroni's multiple comparison test	p > 0.9999, ns	Interaction: F(6, 126) = 2.029, p = 0.0665 Tanc2: F(1, 21) = 1.551, p = 0.2267 Fiber Volley: F(6, 126) = 157, p < 0.0001	
									1	WT = 0.2561 ± 0.0695 HT = 0.2006 ± 0.0494		p > 0.9999, ns		
									1.5	WT = 0.4417 ± 0.0802 HT = 0.3295 ± 0.0654		p > 0.9999, ns		
									2	WT = 0.5944 ± 0.0933 HT = 0.4473 ± 0.0693		p > 0.9999, ns		
									3	WT = 0.8305 ± 0.107 HT = 0.6588 ± 0.0829		p > 0.9999, ns		
									4	WT = 1.0239 ± 0.1167 HT = 0.8215 ± 0.0871		p = 0.6209, ns		
									5	WT = 1.2235 ± 0.1273 HT = 0.9914 ± 0.0960		p = 0.3592, ns		
	g					WT = 11 slices/ 4 mice HT = 13 slices/ 5 mice	Hippocampus, PPR	Inter-pulse interval (ms)	20 msec	WT = 167.8 ± 3.77 HT = 159.8 ± 6.43	Two-way ANOVA repeated measures Bonferroni's multiple comparison test	p = 0.6200, ns	Interaction: F(4, 88) = 1.388, p = 0.2447 Tanc2: F(1, 22) = 0.1803, p = 0.6752 Time: F(4, 88) = 149.3, p < 0.0001	
									50 msec	WT = 173.2 ± 2.23 HT = 174.8 ± 3.93		p > 0.9999, ns		
									100 msec	WT = 155.2 ± 2.08 HT = 159.4 ± 3.42		p > 0.9999, ns		
									200 msec	WT = 141.3 ± 4.43 HT = 136.9 ± 2.46		p > 0.9999, ns		
									500 msec	WT = 110.7 ± 1.57 HT = 109.7 ± 2.41		p > 0.9999, ns		
	h	Electrophysiology		Male	2-3 weeks	WT = 8 slices/ 6 mice HT = 9 slices/ 5 mice	Hippocampus, 5 Hz, 5 min NMDAR LTD (%)			WT = 152.3 ± 4.96 HT = 132.1 ± 5.02	Student's t-test	p = 0.0134, *	t(15) = 2.8040	Two-tailed
	i	Electrophysiology			3-4 weeks	WT = 9 slices/ 5 mice HT = 7 slices/ 4 mice	Hippocampus, DHPG (50 μM), 5 min mGluR LTD (%)			WT = 67.6 ± 3.15 HT = 70.4 ± 4.11	Student's t-test	p = 0.6005, ns	t(14) = 0.5358	Two-tailed
	a	Western blotting		Male	P14	WT = 8 HT = 8	Protein levels normalized to α-tubulin (Fold change)	Whole brain	TANC2	WT = 1.19 ± 0.12 (SD) HT = 0.80 ± 0.11 (SD)	Mann-Whitney U	p = 0.0281, *	U = 11	Two-tailed
						WT = 6 HT = 6			PSD-95	WT = 1.00 ± 0.19 (SD) HT = 1.02 ± 0.19 (SD)	Student's t-test	p = 0.8455, ns	t(10) = 0.2	Two-tailed
						WT = 8 HT = 8			mTOR	WT = 1.00 ± 0.12 (SD) HT = 1.27 ± 0.16 (SD)	Mann-Whitney U	p = 0.1304, ns	U = 17	Two-tailed
									p-mTOR	WT = 0.99 ± 0.13 (SD) HT = 5.19 ± 1.34 (SD)	Student's t-test	p = 0.0076, **	t(14) = 3.1130	Two-tailed
						WT = 4 HT = 4			Akt	WT = 1.00 ± 0.05 (SD) HT = 0.91 ± 0.02 (SD)	Student's t-test	p = 0.1814, ns	t(6) = 1.5120	Two-tailed
									p-Akt	WT = 1.00 ± 0.07 (SD) HT = 2.81 ± 0.54 (SD)	Student's t-test	p = 0.0166, *	t(6) = 3.2890	Two-tailed
									GSK3β	WT = 1.00 ± 0.09 (SD) HT = 1.06 ± 0.04 (SD)	Student's t-test	p = 0.5677, ns	t(6) = 0.6044	Two-tailed
									p-GSK3β	WT = 1.00 ± 0.14 (SD) HT = 1.80 ± 0.16 (SD)	Student's t-test	p = 0.0109, *	t(6) = 0.3636	Two-tailed
						WT = 6 HT = 6			PKCa	WT = 1.00 ± 0.2 (SD) HT = 1.06 ± 0.04 (SD)	Student's t-test	p = 0.8332, ns	t(10) = 0.2162	Two-tailed
									p-PKCa	WT = 1.00 ± 0.14 (SD) HT = 1.80 ± 0.16 (SD)	Student's t-test	p = 0.1291, ns	t(10) = 1.654	Two-tailed
						WT = 4 HT = 4			S6	WT = 0.99 ± 0.03 (SD) HT = 1.08 ± 0.05 (SD)	Student's t-test	p = 0.2486, ns	t(6) = 1.2780	Two-tailed
									p-S6	WT = 1.00 ± 0.15 (SD) HT = 1.10 ± 0.17 (SD)	Mann-Whitney U	p = 0.6857, ns	U = 6	Two-tailed
									4EBP	WT = 1.00 ± 0.15 (SD) HT = 1.46 ± 0.05 (SD)	Student's t-test	p = 0.0340, *	t(6) = 2.7340	Two-tailed
									p-4EBP	WT = 1.00 ± 0.15 (SD) HT = 1.77 ± 0.06 (SD)	Mann-Whitney U	p = 0.0286, *	U = 0	Two-tailed
	b	Western blotting		Male	P14	WT = 4 HT = 4	Protein levels normalized to α-tubulin (Fold change)	Whole brain	ERK1/2	WT = 1.00 ± 0.14 (SD) HT = 0.93 ± 0.14 (SD)	Student's t-test	p = 0.7544, ns	t(6) = 0.3276	Two-tailed
									p-ERK1/2	WT = 0.99 ± 0.10 (SD) HT = 1.26 ± 0.21 (SD)	Mann-Whitney U	p = 0.3429, ns	U = 4	Two-tailed
									p38	WT = 1.06 ± 0.12 (SD) HT = 1.10 ± 0.04 (SD)	Student's t-test	p = 0.7909, ns	t(6) = 0.2772	Two-tailed
									p-p38	WT = 1.00 ± 0.05 (SD) HT = 0.93 ± 0.15 (SD)	Student's t-test	p = 0.7084, ns	t(6) = 0.3923	Two-tailed
									TSC1	WT = 1.00 ± 0.02 (SD) HT = 0.99 ± 0.12 (SD)	Student's t-test	p = 0.9413, ns	t(6) = 0.0767	Two-tailed
									TSC2	WT = 1.00 ± 0.07 (SD) HT = 0.76 ± 0.05 (SD)	Student's t-test	p = 0.0286, *	t(6) = 2.8640	Two-tailed
									PTEN	WT = 1.00 ± 0.13 (SD) HT = 1.43 ± 0.20 (SD)	Student's t-test	p = 0.1276, ns	t(6) = 1.7670	Two-tailed
									p-PTEN	WT = 1.00 ± 0.15 (SD) HT = 1.10 ± 0.16 (SD)	Student's t-test	p = 0.6444, ns	t(6) = 0.4858	Two-tailed
									PI3K	WT = 1.00 ± 0.07 (SD) HT = 1.04 ± 0.05 (SD)	Student's t-test	p = 0.6791, ns	t(6) = 0.4346	Two-tailed
									p-PI3K	WT = 1.31 ± 0.41 (SD) HT = 1.17 ± 0.26 (SD)	Student's t-test	p = 0.9286, ns	t(6) = 0.0933	Two-tailed
	c	Western blotting		Male	P28	WT = 4 HT = 4	Protein levels normalized to β-actin (Fold change)	Whole brain	TANC2	WT = 1.00 ± 0 (SD) HT = 0.48 ± 0.13 (SD)	Student's t-test	p = 0.0003, ***	t(6) = 7.351	Two-tailed
									PSD-95	WT = 1.19 ± 0.12 (SD) HT = 0.80 ± 0.11 (SD)		p = 0.0281, *	U = 11	Two-tailed
									mTOR	WT = 1.00 ± 0 (SD) HT = 1.09 ± 0.24 (SD)		p = 0.4522, ns	t(6) = 0.8038	Two-tailed
									p-mTOR	WT = 1.00 ± 0 (SD) HT = 1.08 ± 0.20 (SD)		p = 0.4681, ns	t(6) = 0.7743	Two-tailed
									Akt	WT = 1.00 ± 0 (SD) HT = 0.92 ± 0.17 (SD)		p = 0.4369, ns	t(6) = 0.8328	Two-tailed
									p-Akt	WT = 1.00 ± 0 (SD) HT = 1.01 ± 0.19 (SD)		p = 0.8585, ns	t(6) = 0.186	Two-tailed
									GSK3β	WT = 1.00 ± 0 (SD) HT = 1.01 ± 0.35 (SD)		p = 0.9271, ns	t(6) = 0.0953	Two-tailed
									p-GSK3β	WT = 1.00 ± 0 (SD) HT = 1.11 ± 0.46 (SD)		p = 0.6413, ns	t(6) = 0.4904	Two-tailed
									PKCa	WT = 1.00 ± 0.09 (SD) HT = 1.02 ± 0.19 (SD)		p = 0.5677, ns	t(6) = 0.6044	Two-tailed

2								p-PKCa		WT = 1.00 ± 0.16 (SD) HT = 1.14 ± 0.14 (SD)		p = 0.0109, *	t(6) = 0.3.636	Two-tailed
								S6		WT = 1.00 ± 0 (SD) HT = 1.04 ± 0.12 (SD)		p = 0.4753, ns	t(6) = 0.7614	Two-tailed
								p-S6		WT = 1.00 ± 0 (SD) HT = 0.92 ± 0.15 (SD)		p = 0.3993, ns	t(6) = 0.907	Two-tailed
								4EBP		WT = 1.00 ± 0 (SD) HT = 0.92 ± 0.19 (SD)		p = 0.4761, ns	t(6) = 0.7599	Two-tailed
								p-4EBP		WT = 1.00 ± 0 (SD) HT = 0.97 ± 0.26 (SD)		p = 0.8519, ns	t(6) = 0.195	Two-tailed
	d	Western blotting		Male	P52	WT = 6 HT = 6	Protein levels normalized to β-actin (Fold change)	TANC2	Whole brain	WT = 1.00 ± 0 (SD) HT = 0.42 ± 0.08 (SD)	Student's t-test	p < 0.0001, ***	t(10) = 16.87	Two-tailed
								PSD-95		WT = 1.00 ± 0 (SD) HT = 0.97 ± 0.21 (SD)		p = 0.8255, ns	t(10) = 0.2264	Two-tailed
								mTOR		WT = 1.00 ± 0 (SD) HT = 1.04 ± 0.21 (SD)		p = 0.6558, ns	t(10) = 0.4594	Two-tailed
								p-mTOR		WT = 1.00 ± 0 (SD) HT = 0.92 ± 0.17 (SD)		p = 0.3062, ns	t(10) = 1.078	Two-tailed
								Akt		WT = 1.00 ± 0 (SD) HT = 0.13 ± 0.27 (SD)		p = 0.26, ns	t(10) = 1.194	Two-tailed
								p-Akt		WT = 1.00 ± 0 (SD) HT = 0.83 ± 0.38 (SD)		p = 0.3275, ns	t(10) = 1.029	Two-tailed
								GSK3β		WT = 1.00 ± 0 (SD) HT = 0.98 ± 0.11 (SD)		p = 0.7703, ns	t(10) = 0.3001	Two-tailed
								p-GSK3β		WT = 1.00 ± 0 (SD) HT = 0.97 ± 0.05 (SD)		p = 0.2735, ns	t(10) = 1.159	Two-tailed
								PKCa		WT = 1.00 ± 0.09 (SD) HT = 0.95 ± 0.15 (SD)		p = 0.5353, ns	t(10) = 0.6421	Two-tailed
								p-PKCa		WT = 1.00 ± 0.14 (SD) HT = 0.92 ± 0.13 (SD)		p = 0.2121, ns	t(10) = 1.333	Two-tailed
								S6		WT = 1.00 ± 0 (SD) HT = 0.95 ± 0.15 (SD)		p = 0.4457, ns	t(10) = 0.7939	Two-tailed
								p-S6		WT = 1.00 ± 0 (SD) HT = 0.85 ± 0.32 (SD)		p = 0.3054, ns	t(10) = 1.08	Two-tailed
								4EBP		WT = 1.00 ± 0 (SD) HT = 0.93 ± 0.19 (SD)		p = 0.4366, ns	t(10) = 0.8104	Two-tailed
								p-4EBP		WT = 1.00 ± 0 (SD) HT = 0.86 ± 0.07 (SD)		p = 0.0017, **	t(10) = 4.256	Two-tailed
	f	Western blotting	GFP vs GFP-cre	male	P14	WT = 6 HT = 6	Protein levels normalized to β-actin (Fold change)	TANC2		GFP = 1.00 ± 0 (SD) GFP-Cre = 0.55 ± 0.08 (SD)	Student's t-test	p = 0.0006, ***	t(10) = 4.972	Two-tailed
								mTOR		GFP = 1.00 ± 0 (SD) GFP-Cre = 1.06 ± 0.06 (SD)		p = 0.3207, ns	t(10) = 1.045	Two-tailed
								p-mTOR		GFP = 1.00 ± 0 (SD) GFP-Cre = 1.53 ± 0.20 (SD)		p = 0.0242, *	t(10) = 2.652	Two-tailed
								Akt		GFP = 1.00 ± 0 (SD) GFP-Cre = 0.96 ± 0.07 (SD)		p = 0.6930, ns	t(10) = 0.4064	Two-tailed
								p-Akt		GFP = 1.00 ± 0 (SD) GFP-Cre = 1.58 ± 0.12 (SD)		p = 0.0007, ***	t(10) = 4.837	Two-tailed
								GSK3β		GFP = 1.00 ± 0 (SD) GFP-Cre = 1.01 ± 0.04 (SD)		p = 0.8213, ns	t(10) = 0.2319	Two-tailed
								p-GSK3β		GFP = 1.00 ± 0 (SD) GFP-Cre = 1.67 ± 0.14 (SD)		p = 0.0008, ***	t(10) = 4.725	Two-tailed
								S6		GFP = 1.00 ± 0 (SD) GFP-Cre = 0.78 ± 0.07 (SD)		p = 0.0215, *	t(10) = 2.72	Two-tailed
								p-S6		GFP = 1.00 ± 0 (SD) GFP-Cre = 1.68 ± 0.28 (SD)		p = 0.0364, *	t(10) = 2.414	Two-tailed
								4EBP		GFP = 1.00 ± 0 (SD) GFP-Cre = 1.40 ± 0.14 (SD)		p = 0.0191, *	t(10) = 2.791	Two-tailed
								p-4EBP		GFP = 1.00 ± 0 (SD) GFP-Cre = 2.21 ± 0.40 (SD)		p = 0.0133, *	t(10) = 3.004	Two-tailed
	h	Western blotting	GFP vs GFP-cre	male	P28	WT = 6 HT = 6	Protein levels normalized to β-actin (Fold change)	TANC2		GFP = 1.00 ± 0 (SD) GFP-Cre = 0.48 ± 0.05 (SD)	Student's t-test	p = 0.0006, ***	t(10) = 23.95	Two-tailed
								mTOR		GFP = 1.00 ± 0 (SD) GFP-Cre = 1.1 ± 0.23 (SD)		p = 0.2796, ns	t(10) = 1.143	Two-tailed
								p-mTOR		GFP = 1.00 ± 0 (SD) GFP-Cre = 1.06 ± 0.17 (SD)		p = 0.3743, ns	t(10) = 0.93	Two-tailed
								Akt		GFP = 1.00 ± 0 (SD) GFP-Cre = 0.99 ± 0.13 (SD)		p = 0.9727, ns	t(10) = 0.0351	Two-tailed
								p-Akt		GFP = 1.00 ± 0 (SD) GFP-Cre = 1.13 ± 0.32 (SD)		p = 0.3343, ns	t(10) = 1.014	Two-tailed
								GSK3β		GFP = 1.00 ± 0 (SD) GFP-Cre = 1.04 ± 0.15 (SD)		p = 0.548, ns	t(10) = 0.6218	Two-tailed
								p-GSK3β		GFP = 1.00 ± 0 (SD) GFP-Cre = 0.97 ± 0.34 (SD)		p = 0.8376, ns	t(10) = 0.2104	Two-tailed
								S6		GFP = 1.00 ± 0 (SD) GFP-Cre = 1.29 ± 0.13 (SD)		p = 0.0003, ***	t(10) = 5.379	Two-tailed
								p-S6		GFP = 1.00 ± 0 (SD) GFP-Cre = 1.54 ± 0.78 (SD)		p = 0.1198, ns	t(10) = 1.701	Two-tailed
								4EBP		GFP = 1.00 ± 0 (SD) GFP-Cre = 0.69 ± 0.12 (SD)		p = 0.0001, ***	t(10) = 5.947	Two-tailed
								p-4EBP		GFP = 1.00 ± 0 (SD) GFP-Cre = 0.64 ± 0.13 (SD)		p < 0/0001, ***	t(10) = 6.432	Two-tailed
3	b	Electorphysiology (Local field current clamp)			7-8 weeks	WT vehicle = 10 slices/ 5 mice WT rapamycin = 14 slices/ 6 mice HT vehicle = 12 slices/ 5 mice HT rapamycin = 15 slices/ 7 mice	Hippocampus, 100 Hz, 5min HFS LTP (%)			WT vehicle = 158 ± 6.28 WT rapamycin = 159 ± 5.79 HT vehicle = 130 ± 3.33 HT rapamycin = 151 ± 3.76	Two-way ANOVA Bonferroni's multiple comparison test	WT vehicle vs HT vehicle, p = 0.0025; WT vehicle vs WT rapamycin, ns; HT vehicle vs HT rapamycin, p = 0.0171; WT vehicle vs HT rapamycin, ns	Interaction F(1, 47) = 4.242, p = 0.0450 F(1, 47) = 13.49, p = 0.0006 Treatment F(1, 47) = 5.028, p = 0.0297	Tanc2
	c	Morris water maze	WT vehicle vs HT vehicle vs WT rapamycin vs HT rapamycin [5 mg/kg]	male	2-4 months	WT vehicle = 16 mice WT rapamycin = 15 mice HT vehicle = 11 mice HT rapamycin = 15 mice	Latency to escape (s)	Training	Day 1	WT vehicle = 51.56 ± 2.27 WT rapamycin = 50.73 ± 2.13 HT vehicle = 54.18 ± 2.28 HT rapamycin = 52.16 ± 2.36	Two-way ANOVA repeated measures Bonferroni's multiple comparison test	WT vehicle vs HT vehicle, ns; WT vehicle vs WT rapamycin, ns; HT vehicle vs HT rapamycin, ns; WT vehicle vs HT rapamycin, ns	Interaction F(12, 212) = 1.895, p = 0.0260 Treatment F(3, 53) = 3.274, p < 0.0281 Time F(4, 212) = 50.47, p <0.0001	
									Day 2	WT vehicle = 35.27 ± 2.95 WT rapamycin = 38.67 ± 14.34 HT vehicle = 40.55 ± 5.43 HT rapamycin = 44.64 ± 3.08		WT vehicle vs HT vehicle, ns; WT vehicle vs WT rapamycin, ns; HT vehicle vs HT rapamycin, ns; WT vehicle vs HT rapamycin, ns		
									Day 3	WT vehicle = 28.27 ± 3.86 WT rapamycin = 31.64 ± 4.31 HT vehicle = 49.15 ± 2.73 HT rapamycin = 31.49 ± 2.91		WT vehicle vs HT vehicle, p = 0.0005; WT vehicle vs WT rapamycin, ns; HT vehicle vs HT rapamycin, p = 0.0030; WT vehicle vs HT rapamycin, ns		
									Day 4	WT vehicle = 24.98 ± 3.66 WT rapamycin = 28.16 ± 3.19 HT vehicle = 33.10 ± 4.45 HT rapamycin = 27.31 ± 3.93		WT vehicle vs HT vehicle, ns; WT vehicle vs WT rapamycin, ns; HT vehicle vs HT rapamycin, ns; WT vehicle vs HT rapamycin, ns		
									Day 5	WT vehicle = 22.96 ± 3.41 WT rapamycin = 23.07 ± 2.01 HT vehicle = 38.7 ± 4.23 HT rapamycin = 23.38 ± 2.93		WT vehicle vs HT vehicle, p = 0.0148; WT vehicle vs WT rapamycin, ns; HT vehicle vs HT rapamycin, p = 0.0116; WT vehicle vs HT rapamycin, ns		
							Number of crossing (count)	Probe test	Day 5	WT vehicle = 3.13 ± 0.47 WT rapamycin = 2.20 ± 0.40 HT vehicle = 1.10 ± 0.41 HT rapamycin = 2.73 ± 0.36	One-way ANOVA Bonferroni's multiple comparison test	WT vehicle vs HT vehicle, p = 0.0269; WT vehicle vs WT rapamycin, ns; HT vehicle vs HT rapamycin, ns; WT vehicle vs HT rapamycin, ns	Interaction F(3, 53) = 3.210, p = 0.0302	
							Swimming speed (cm/s)			WT vehicle = 18.91 ± 0.72 WT rapamycin = 19.76 ± 1.11 HT vehicle = 16.32 ± 0.70 HT rapamycin = 18.30 ± 0.69	One-way ANOVA Bonferroni's multiple comparison test	WT vehicle vs HT vehicle, ns; WT vehicle vs WT rapamycin, ns; HT vehicle vs HT rapamycin, ns; WT vehicle vs HT rapamycin, ns	Interaction F(3, 53) = 2.604, p = 0.0614	
	d	Open field test	WT vehicle vs HT vehicle vs WT rapamycin vs HT rapamycin [5 mg/kg]	Male	7-8 weeks	WT vehicle = 15 mice WT rapamycin = 16 mice HT vehicle = 12 mice HT rapamycin = 14 mice	Total distance moved (m)		60 minutes	WT vehicle = 159.65 ± 4.41 WT rapamycin = 140.70 ± 4.86 HT vehicle = 184.37 ± 7.56 HT rapamycin = 149.08 ± 6.19	Two-way ANOVA Bonferroni's multiple comparison test	WT vehicle vs HT vehicle, p = 0.0243; WT vehicle vs WT rapamycin, ns; HT vehicle vs HT rapamycin, p = 0.0006; WT rapamycin vs HT rapamycin, ns; WT vehicle vs HT rapamycin, ns	Interaction F(1, 53) = 2.082, p = 0.1549 F(1, 53) = 8.553, p = 0.0051 Treatment F(1, 53) = 22.97, p < 0.0001	Tanc2
	e	EPM				WT vehicle = 16 mice WT rapamycin = 19 mice HT vehicle = 12 mice HT rapamycin = 15 mice	Time spent in arm (s)	Open arm		WT vehicle = 108.08 ± 6.93 WT rapamycin = 109.38 ± 6.99 HT vehicle = 141.35 ± 11.65 HT rapamycin = 106.86 ± 11.71	One-way ANOVA Bonferroni's multiple comparison test	WT vehicle vs HT vehicle, ns; WT vehicle vs WT rapamycin, ns; WT rapamycin vs HT vehicle, ns; HT vehicle vs HT rapamycin, ns; WT rapamycin vs HT rapamycin, ns; WT vehicle vs HT rapamycin, ns	Interaction F(3, 58) = 2.107, p = 0.1092	
								Closed arm		WT vehicle = 355.08 ± 9.76 WT rapamycin = 367.09 ± 10.01 HT vehicle = 308.74 ± 12.5 HT rapamycin = 365.45 ± 18.76		WT vehicle vs HT vehicle, ns; WT vehicle vs WT rapamycin, ns; WT rapamycin vs HT vehicle, p = 0.0339; HT vehicle vs HT rapamycin, ns; WT rapamycin vs HT rapamycin, ns; WT vehicle vs HT rapamycin, ns	Interaction F(3, 58) = 3.259, p = 0.0278	
								Closed arm		WT vehicle = 355.08 ± 9.76 WT rapamycin = 367.09 ± 10.01 HT vehicle = 308.74 ± 12.5 HT rapamycin = 365.45 ± 18.76	One-way ANOVA Tukey's multiple comparison test	WT vehicle vs HT vehicle, ns; WT vehicle vs WT rapamycin, ns; WT rapamycin vs HT vehicle, p = 0.0339; HT vehicle vs HT rapamycin, p = 0483; WT rapamycin vs HT rapamycin, ns; WT vehicle vs HT rapamycin, ns	Interaction F(3, 58) = 3.259, p = 0.0278	
5	a	Western blotting				n=3	p-mTOR/mTOR levels in HEK293 cells normalized to untreated p-mTOR/mTOR levels	p-mTOR/mTOR	Untreat	1.01 ± 0.07 (SD)	One-way ANOVA Bonferroni's multiple comparison test		F(7, 16) = 22.88, p < 0.0001	
									Flag-Empty	0.85 ± 0.09 (SD)		p = 0.7274, ns		
									Flag-hTanc2a	0.53 ± 0.05 (SD)		p = 0.0005, ***		
									Flag-hTanc2b	0.74 ± 0.06 (SD)		p = 0.0624, ns		
									Flag-hTanc2c	0.94 ± 0.04 (SD)		p > 0.9999, ns		
									Rapamycin 1 nM	0.71 ± 0.08 (SD)		p = 0.0342, *		
									Rapamycin 10 nM	0.31 ± 0.03 (SD)		p < 0.0001, ***		
									Rapamycin 100 nM	0.14 ± 0.01 (SD)		p < 0.0001, ***		
							p-S6/S6 levels in HEK293 cells normalized to untreated p-S6/S6 levels	p-S6/S6	Untreat	0.96 ± 0.02 (SD)			F(7, 16) = 27.35, p < 0.0001	
									Flag-Empty	1.20 ± 0.12 (SD)		p = 0.6487, ns		
									Flag-hTanc2a	0.41 ± 0.09 (SD)		p = 0.0054, **		
									Flag-hTanc2b	0.94 ± 0.18 (SD)		p > 0.9999, ns		
									Flag-hTanc2c	0.89 ± 0.11 (SD)		p > 0.9999, ns		
									Rapamycin 1 nM	0.08 ± 0.03 (SD)		p < 0.0001, ***		
									Rapamycin 10 nM	0.01 ± 0.004 (SD)		p < 0.0001, ***		
									Rapamycin 100 nM	0.003 ± 0.003 (SD)		p < 0.0001, ***		
	b	In vitro kinase assay (Western blotting)				n=3	Protein levels normalized to mTORC only (Fold change)		mTORC	1.00 ± 0 (SD)	One-way ANOVA Bonferroni's multiple comparison test	mTORC S6K ATP vs mTORC S6K ATP Tanc2, p > 0.9999, ns	F(4, 10) = 1.352, p = 0.3172	
									mTORC ATP	0.99 ± 0.16 (SD)				
									mTORC S6K	0.76 ± 0.12 (SD)				
									mTORC S6K ATP	1.89 ± 0.74 (SD)				
									mTORC S6K ATP Tanc2	1.03 ± 0.31 (SD)				
						n=3	Protein levels normalized to mTORC, S6K, and ATP (Fold change)		mTORC S6K	0.01 ± 0.004 (SD)	One-way ANOVA Bonferroni's multiple comparison test	mTORC S6K vs mTORC S6K ATP, p=0.0001; mTORC S6K ATP vs mTORC S6K ATP Tanc2, p = 0.0473, *	F(2, 6) = 51.47, p = 0.0002	
									mTORC S6K ATP	1.00 ± 0.00 (SD)				
									mTORC S6K ATP Tanc2	0.66 ± 0.21 (SD)				
	c	In vitro kinase assay				n=3	Protein levels normalized to mTORC only (Fold change)		mTORC	1.00 ± 0 (SD)	One-way ANOVA Bonferroni's multiple comparison test	mTORC Akt ATP vs mTORC Akt ATP Tanc2, p > 0.9999, ns	F(4, 10) = 0.6426, p = 0.6444	
									mTORC ATP	1.21 ± 0.38 (SD)				
									mTORC Akt	1.19 ± 0.19 (SD)				
									mTORC Akt ATP	1.27 ± 0.47 (SD)				

		(Western blotting)						mTORC Akt ATP Tanc2	1.39 ± 0.26 (SD)						
						n=3	Protein levels normalized to mTORC, S6K, and ATP (Fold change)		mTORC Akt	0.41 ± 0.05 (SD)	One-way ANOVA Bonferroni's multiple comparison test	mTORC Akt vs mTORC Akt ATP, p<0.0001; mTORC Akt ATP vs mTORC Akt ATP Tanc2, p = 0.0006, ***	F(2, 6) = 204.6, p < 0.0001		
									mTORC Akt ATP	1.00 ± 0.00 (SD)					
									mTORC Akt ATP Tanc2	0.76 ± 0.02 (SD)					
6	a	Confocal microscopy				n=24 cells	Pearson's correlation coefficient between CFP-Tanc2 and YFP-mTOR		+serum	0.27 ± 0.02	One-way ANOVA Bonferroni's multiple comparison test	+serum vs -serum 4 hr, p < 0.0001;+serum vs +serum 4hr, p < 0.0001; +serum vs +serum 24hr, p > 0.9999; -serum 4 hr vs +serum 4 hr, p = 0.8761; -serum 4 hr vs +serum 24 hr, p < 0.0001; +serum 4hr vs +serum 24hr, p < 0.0001	F(3, 92) = 60.12, p < 0.0001		
									-serum 4 hr	0.66 ± 0.02					
									+serum 4 hr	0.61 ± 0.02					
									+serum 24 hr	0.32 ± 0.02					
	b	Confocal microscopy				n=24 cells	Pearson's correlation coefficient between CFP-Tanc2 and YFP-mTOR	Vehicle	+serum	0.23 ± 0.02	Student's t-test	p < 0.0001, ***	t(46) = 11.17	Two-tailed	
									-serum 4 hr	0.65 ± 0.02					
								Rapamycin	+serum	0.29 ± 0.02	Student's t-test	p = 0.5988, ns	t(46) = 0.5299	Two-tailed	
									-serum 4 hr	0.31 ± 0.02					
	c	Coimmunoprecipitation (Western blotting)				n=4	mTOR/Tanc2 ratio in HEK293T cells (Fold change)		Serum +	1.00 ± 0.00 (SD)	Student's t-test	p = 0.0158, *	t(6) = 3.329	Two-tailed	
									Serum -	2.15 ± 0.34 (SD)					
	d	Coimmunoprecipitation (Western blotting)				n=3	mTOR/Tanc2 ratio in HEK293T cells (Fold change)	Vehicle	+serum	1.00 ± 0.00 (SD)	Student's t-test	p < 0.0001, ***	t(4) = 29.66	Two-tailed	
									-serum 4 hr	2.18 ± 0.04 (SD)					
								Rapamycin	+serum	1.35 ± 0.20 (SD)	Student's t-test	p = 0.5988, ns	t(4) = 29.66	Two-tailed	
									-serum 4 hr	1.46 ± 0.24 (SD)					
	e	Coimmunoprecipitation using P2 fraction (Western blotting)			P13-14	n=4	Ratio (Fold change)	mTOR/Tanc	0 hr	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test	p = 0.0241, * p = 0.011, *	F(2, 6) = 7.748, p = 0.011		
									0.5 hr	0.73 ± 0.06 (SD)					
									1 hr	0.69 ± 0.08 (SD)					
								PSD-95/Tanc	0 hr	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test	p > 0.9999, ns p = 0.3317, ns	F(2, 6) = 1.319, p = 0.3144		
									0.5 hr	1.03 ± 0.15 (SD)					
									1 hr	1.20 ± 0.07 (SD)					
						n=5		Tanc/mTOR	0 hr	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test	p = 0.1345, ns p = 0.0140, *	F(2, 12) = 5.367, p = 0.0216		
									0.5 hr	0.79 ± 0.25 (SD)					
									1 hr	0.67 ± 0.10 (SD)					
								PSD-95/mTOR	0 hr	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test	p > 0.9999, ns p = 0.3092, ns	F(2, 12) = 0.9637, p = 0.4091		
									0.5 hr	1.04 ± 0.06 (SD)					
									1 hr	0.12 ± 0.09 (SD)					
7	b	Western blotting			DIV 14	n=4	Ratio (Fold change)	Tanc2 / actin	Control	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test	p = 0.0002, *** p > 0.9999, ns p < 0.0001, *** p > 0.9999, ns	F(4, 15) = 25.89, p < 0.0001		
									Tanc2 shRNA	0.31 ± 0.13 (SD)					
									Deptor shRNA	1.12 ± 0.17 (SD)					
									Tanc2 & Deptor shRNA	0.23 ± 0.16 (SD)					
									Tanc1 shRNA	1.03 ± 0.25 (SD)					
								Deptor / actin	Control	1.00 ± 0.00 (SD)		p > 0.9999, ns p = 0.0033, ** p = 0.0012, ** p = 0.3681, ns	F(4, 15) = 16.16, p < 0.0001		
									Tanc2 shRNA	0.98 ± 0.17 (SD)					
									Deptor shRNA	0.41 ± 0.08 (SD)					
									Tanc2 & Deptor shRNA	0.33 ± 0.12 (SD)					
									Tanc1 shRNA	1.25 ± 0.38 (SD)					
								Tanc1 / actin	Control	1.00 ± 0.00 (SD)		p > 0.9999, ns p > 0.9999, ns p = 0.14, ns p < 0.0001, ***	F(4, 15) = 20.45, p < 0.0001		
									Tanc2 shRNA	1.10 ± 0.08 (SD)					
									Deptor shRNA	1.10 ± 0.17 (SD)					
									Tanc2 & Deptor shRNA	1.22 ± 0.22 (SD)					
									Tanc1 shRNA	0.43 ± 0.07 (SD)					
								p-S6 / S6	Control	1.00 ± 0.00 (SD)		p = 0.0091, ** p = 0.8660, ns p = 0.0096, ** p = 0.0426, *	F(4, 15) = 6,478 p = 0.0031		
									Tanc2 shRNA	2.29 ± 0.57 (SD)					
									Deptor shRNA	1.31 ± 0.33 (SD)					
									Tanc2 & Deptor shRNA	2.28 ± 0.63 (SD)					
									Tanc1 shRNA	1.95 ± 0.47 (SD)					
								p-4E-BP / 4E-BP	Control	1.00 ± 0.00 (SD)		p = 0.0056, ** p > 0.9999, ns p = 0.0398, * p = 0.8059, ns	F(4, 15) = 5.664, p = 0.0055		
									Tanc2 shRNA	2.39 ± 0.72 (SD)					
									Deptor shRNA	1.11 ± 0.20 (SD)					
									Tanc2 & Deptor shRNA	2.05 ± 0.61 (SD)					
									Tanc1 shRNA	1.47 ± 0.29 (SD)					
								p-mTOR / mTOR	Control	1.00 ± 0.00 (SD)		p = 0.4619, ns p > 0.9999, ns p = 0.0228, * p = 0.9187, ns	F(4, 15) = 3.121, p = 0.0469		
									Tanc2 shRNA	1.25 ± 0.20 (SD)					
									Deptor shRNA	1.07 ± 0.24 (SD)					
									Tanc2 & Deptor shRNA	1.49 ± 0.13 (SD)					
									Tanc1 shRNA	0.19 ± 0.34 (SD)					
								p-Akt (T308) / Akt	Control	1.00 ± 0.00 (SD)		p > 0.9999, ns p = 0.8083, ns p > 0.9999, ns p > 0.9999, ns	F(4, 15) = 1.976, p = 0.1500		
									Tanc2 shRNA	0.93 ± 0.14 (SD)					
									Deptor shRNA	1.23 ± 0.17 (SD)					
									Tanc2 & Deptor shRNA	1.16 ± 0.20 (SD)					
									Tanc1 shRNA	0.80 ± 0.46 (SD)					
								p-Akt (S473) / Akt	Control	1.00 ± 0.00 (SD)		p = 0.0192, * p > 0.9999, ns p = 0.0018, ** p > 0.9999, ns	F(4, 15) = 8.58, p = 0.0008		
									Tanc2 shRNA	1.68 ± 0.28 (SD)					
									Deptor shRNA	1.17 ± 0.15 (SD)					
									Tanc2 & Deptor shRNA	1.92 ± 0.51 (SD)					
									Tanc1 shRNA	0.97 ± 0.24 (SD)					
								pGSK3β / GSK3β	Control	1.00 ± 0.00 (SD)		p = 0.0002, *** p > 0.9999, ns p = 0.0034, ** p > 0.9999, ns	F(4, 15) = 14.53, p < 0.0001		
									Tanc2 shRNA	1.55 ± 0.19 (SD)					
									Deptor shRNA	0.93 ± 0.08 (SD)					
									Tanc2 & Deptor shRNA	1.41 ± 0.12 (SD)					
									Tanc1 shRNA	1.11 ± 0.19 (SD)					
	d	Western blotting			DIV 28	n=4	Ratio (Fold change)	Tanc2 / actin	Control	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test	p < 0.0001, *** p = 0.8578, ns p < 0.0001, *** p > 0.9999, ns	F(4, 15) = 34.27, p < 0.0001		
									Tanc2 shRNA	0.37 ± 0.15 (SD)					
									Deptor shRNA	0.89 ± 0.02 (SD)					
									Tanc2 & Deptor shRNA	0.32 ± 0.15 (SD)					
									Tanc1 shRNA	1.00 ± 0.14 (SD)					
								Deptor / actin	Control	1.00 ± 0.00 (SD)		p > 0.9999, ns p = 0.004, ** p = 0.0306, * p = 0.0199, *	F(4, 15) = 16.94, p < 0.0001		
									Tanc2 shRNA	0.99 ± 0.05 (SD)					
									Deptor shRNA	0.50 ± 0.05 (SD)					
									Tanc2 & Deptor shRNA	0.62 ± 0.16 (SD)					
									Tanc1 shRNA	1.40 ± 0.34 (SD)					
								Tanc1 / actin	Control	1.00 ± 0.00 (SD)		p = 0.6206, ns p > 0.9999, ns p = 0.0562, ns p < 0.0001, ***	F(4, 15) = 25.82, p < 0.0001		
									Tanc2 shRNA	0.88 ± 0.15 (SD)					
									Deptor shRNA	0.94 ± 0.12 (SD)					
									Tanc2 & Deptor shRNA	0.78 ± 0.08 (SD)					
									Tanc1 shRNA	0.31 ± 0.10 (SD)					
								p-S6 / S6	Control	1.00 ± 0.00 (SD)		p > 0.9999, ns p = 0.007, ** p = 0.0196, * p < 0.0001, ***	F(4, 15) = 19.21 p < 0.0001		
									Tanc2 shRNA	1.19 ± 0.13 (SD)					
									Deptor shRNA	1.85 ± 0.35 (SD)					
									Tanc2 & Deptor shRNA	1.74 ± 0.21 (SD)					
									Tanc1 shRNA	2.78 ± 0.55 (SD)					
								p-4E-BP / 4E-BP	Control	1.00 ± 0.00 (SD)		p = 0.1126, ns p = 0.0073, ** p = 0.024, * p = 0.0257, *	F(4, 15) = 4.407, p = 0.0149		
									Tanc2 shRNA	1.42 ± 0.30 (SD)					
									Deptor shRNA	1.65 ± 0.15 (SD)					
									Tanc2 & Deptor shRNA	1.55 ± 0.10 (SD)					
									Tanc1 shRNA	1.54 ± 0.41 (SD)					
								p-mTOR / mTOR	Control	1.00 ± 0.00 (SD)		p = 0.1394, ns p = 0.0362, * p = 0.0148, * p = 0.0164, *	F(4, 15) = 4.077, p = 0.0197		
									Tanc2 shRNA	0.82 ± 0.07 (SD)					
									Deptor shRNA	0.76 ± 0.18 (SD)					
									Tanc2 & Deptor shRNA	0.73 ± 0.06 (SD)					
									Tanc1 shRNA	0.73 ± 0.12 (SD)					
								p-Akt (T308) / Akt	Control	1.00 ± 0.00 (SD)		p > 0.9999, ns p = 0.0898, ns p = 0.0627, ns p > 0.9999, ns	F(4, 15) = 4.659, p = 0.0121		
									Tanc2 shRNA	1.02 ± 0.16 (SD)					
									Deptor shRNA	1.28 ± 0.15 (SD)					
									Tanc2 & Deptor shRNA	1.29 ± 0.22 (SD)					
									Tanc1 shRNA	0.94 ± 0.14 (SD)					
								p-Akt (S473) / Akt	Control	1.00 ± 0.00 (SD)		p > 0.9999, ns p < 0.0001, *** p < 0.0001, *** p = 0.0052, **	F(4, 15) = 39.84, p < 0.0001		
									Tanc2 shRNA	1.06 ± 0.11 (SD)					
									Deptor shRNA	1.53 ± 0.03 (SD)					
									Tanc2 & Deptor shRNA	1.73 ± 0.04 (SD)					
									Tanc1 shRNA	1.27 ± 0.17 (SD)					
								pGSK3β / GSK3β	Control	1.00 ± 0.00 (SD)		p = 0.1376, ns p = 0.0036, ** p = 0.002, ** p < 0.0001, ***	F(4, 15) = 15.56, p < 0.0001		
									Tanc2 shRNA	1.19 ± 0.01 (SD)					
									Deptor shRNA	1.35 ± 0.13 (SD)					
									Tanc2 & Deptor shRNA	1.37 ± 0.15 (SD)					
									Tanc1 shRNA	1.64 ± 0.17 (SD)					
8	c	Western blotting			Day 14	n=4	Ratio (Fold change)	Tanc2 / actin	Control	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test	p < 0.0001, *** p < 0.0001, ***	F(2, 9) = 87.04, p < 0.0001		
									Tanc2 shRNA1	0.27 ± 0.05 (SD)					
									Tanc2 shRNA2	0.27 ± 0.14 (SD)					
								p-S6 / S6	Control	1.00 ± 0.00 (SD)		p = 0.0584, ns p = 0.0243, *	F(2, 9) = 5.59 p = 0.0263		
									Tanc2 shRNA1	1.51 ± 0.17 (SD)					
									Tanc2 shRNA2	1.62 ± 0.45 (SD)					
								p-4E-BP / 4E-BP	Control	1.00 ± 0.00 (SD)		p = 0.0288, * p = 0.0124, *	F(2, 9) = 7.34, p = 0.0129		
									Tanc2 shRNA1	1.65 ± 0.40 (SD)					
									Tanc2 shRNA2	1.77 ± 0.34 (SD)					
								p-mTOR / mTOR	Control	1.00 ± 0.00 (SD)		p = 0.3150, ns p = 0.104, ns	F(2, 9) = 2.62, p = 0.1266		
									Tanc2 shRNA1	1.47 ± 0.39 (SD)					
									Tanc2 shRNA2	1.68 ± 0.64 (SD)					
								p-Akt (S473) / Akt	Control	1.00 ± 0.00 (SD)		p = 0.2209, ns p = 0.0321, *	F(2, 9) = 11.41, p = 0.0034		
									Tanc2 shRNA1	0.77 ± 0.29 (SD)					
									Tanc2 shRNA2	1.37 ± 0.09 (SD)					
								pGSK3β / GSK3β	Control	1.00 ± 0.00 (SD)		p = 0.03, * p = 0.0343, *	F(2, 9) = 5.83, p = 0.0237		
									Tanc2 shRNA1	1.97 ± 0.64 (SD)					
									Tanc2 shRNA2	1.94 ± 0.46 (SD)					
	a	Novel object test		Male	2-3 months	WT = 17 HT = 17		Preference index (%)		WT = 61.12 ± 2.23	Student's t-test	p = 0.0529, ns	t(32) = 2.0100	Two-tailed	
										HT = 67.60 ± 2.07					
								Distance moved (cm)		WT = 2221.67 ± 69.83	Mann-Whitney U	p = 0.0005, ***	U = 47	Two-tailed	
										HT = 2789.45 ± 122.54					
								Speed (cm/s)		WT = 3.70 ± 0.11	Mann-Whitney U	p = 0.0006, ***	U = 48	Two-tailed	
										HT = 4.64 ± 0.20					
								Exploration time (s)	Familiar object vs novel object	WT	Student's t-test	p = 0.0073, **	t(32) = 2.867	Two-tailed	
										F = 12.11 ± 1.26					
										N = 18.31 ± 1.60					
										HT	Student's t-test	p = 0.0002, ***	t(32) = 4.258	Two-tailed	
										F = 13.08 ± 1.66					
										N = 27.26 ± 0.15					
								Frequency of object exploration (count)	Familiar object vs novel object	WT	Mann-Whitney U	p = 0.9120, ns	U = 141	Two-tailed	
										F = 24.17 ± 1.83					
										N = 26.00 ± 2.44					
										HT	Mann-Whitney U	p = 0.0114, *	U = 72	Two-tailed	
										F = 26.35 ± 4.00					
										N = 41.47 ± 4.64					
									10 minutes	WT = 58.95 ± 2.63		p =0.0436, *			
										HT = 67.53 ± 2.95					
									20 minutes	WT = 40.13 ± 1.61		p = 0.0212, *			
										HT = 49.48 ± 2.15					

	b	Open field test		Male	7-8 weeks	WT = 13 HT = 11	Distance moved (m)	Light off (0 lux)	30 minutes	WT = 36.29 ± 2.22 HT = 46.33 ± 1.58	Two-way ANOVA repeated measures Bonferroni's multiple comparison test	p = 0.0107, *	Interaction: F(5, 110) = 0.4458, p = 0.8155 Tanc2: F(1, 22) = 12.78, p = 0.0017 Time: F(5, 110) = 88.29, p < 0.0001		
									40 minutes	WT = 35.25 ± 2.10 HT = 40.99 ± 1.79		p = 0.4236, ns			
									50 minutes	WT = 29.28 ± 1.37 HT = 38.34 ± 2.24		p = 0.0280, *			
									60 minutes	WT = 34.54 ± 2.69 HT = 39.16 ± 2.71		p = 0.1020, ns			
							Total distance moved (m)		60 minutes	WT = 231.47 ± 10.06 HT = 281.86 ± 96.59	Student's t-test	p = 0.0017, **	t(22)= 3.575	Two-tailed	
							Time spent in center region (s)		60 minutes	WT = 651.6 ± 76.00 HT = 733.7 ± 53.17	Mann-Whitney U	p = 0.1339, ns	U = 45	Two-tailed	
Supplementary Fig. 1	c	Laboras		Male	2-3 months	WT = 16 HT = 16	Distance moved (m)	Time (hr)	2	WT = 38.54 ± 2.75 HT = 49.688 ± 4.14	Two-way ANOVA repeated measures Bonferroni's multiple comparison test	p > 0.0010, **	Interaction: F(34, 510) = 3.07, p < 0.0001 Tanc2: F(1, 15) = 16.05, p = 0.0011 Time: F(34, 510) = 34.39, p < 0.0001		
									4	WT = 23.04 ± 2.53 HT = 37.49 ± 3.59		p > 0.0001, ***			
									6	WT = 21.79 ± 2.01 HT = 33.29 ± 3.86		p = 0.0005, ***			
									8	WT = 15.69 ± 2.47 HT = 28.11 ± 4.00		p = 0.0001, ***			
									10	WT = 18.37 ± 2.34 HT = 28.46 ± 3.75		p = 0.0051, **			
									12	WT = 13.34 ± 2.41 HT = 17.88 ± 3.58		p > 0.9999, ns			
									14	WT = 7.98 ± 1.58 HT = 9.06 ± 1.41		p > 0.9999, ns			
									16	WT = 3.33 ± 0.67 HT = 5.53 ± 1.01		p > 0.9999, ns			
									18	WT = 3.87 ± 0.69 HT = 5.39 ± 0.85		p > 0.9999, ns			
									20	WT = 4.36 ± 0.60 HT = 4.69 ± 0.42		p > 0.9999, ns			
									22	WT = 4.25 ± 0.52 HT = 5.62 ± 0.91		p > 0.9999, ns			
									24	WT = 16.65 ± 3.15 HT = 19.47 ± 0.41		p > 0.9999, ns			
									26	WT = 24.66 ± 2.65 HT = 29.82 ± 3.88		p > 0.9999, ns			
									28	WT = 23.99 ± 2.40 HT = 38.10 ± 3.32		p < 0.0001, ***			
									30	WT = 19.25 ± 2.36 HT = 31.87 ± 4.94		p < 0.0001, ***			
									32	WT = 17.84 ± 2.64 HT = 25.29 ± 4.34		p = 0.1710, ns			
									34	WT = 14.18 ± 1.69 HT = 20.56 ± 2.28		p = 0.5561, ns			
									36	WT = 13.18 ± 2.70 HT = 15.56 ± 2.39		p > 0.9999, ns			
									38	WT = 2.51 ± 0.48 HT = 6.70 ± 0.92		p > 0.9999, ns			
									40	WT = 3.19 ± 0.71 HT = 3.46 ± 0.50		p > 0.9999, ns			
									42	WT = 4.01 ± 0.50 HT = 3.86 ± 0.57		p > 0.9999, ns			
									44	WT = 3.26 ± 0.44 HT = 4.94 ± 0.74		p > 0.9999, ns			
									46	WT = 4.30 ± 0.46 HT = 6.65 ± 1.26		p > 0.9999, ns			
									48	WT = 15.71 ± 2.49 HT = 20.73 ± 4.00		p > 0.9999, ns			
									50	WT = 27.52 ± 1.92 HT = 35.50 ± 3.51		p = 0.0901, ns			
									52	WT = 24.14 ± 2.48 HT = 34.14 ± 4.60		p = 0.0058, **			
									54	WT = 17.46 ± 2.58 HT = 27.12 ± 5.41		p = 0.0094, **			
									56	1WT = 6.38 ± 2.46 HT = 23.13 ± 3.65		p = 0.3753, ns			
									58	WT = 15.36 ± 1.97 HT = 22.96 ± 2.98		p = 0.1438, ns			
									60	WT = 9.58 ± 1.62 HT = 12.71 ± 2.77		p > 0.9999, ns			
									62	WT = 3.27 ± 0.69 HT = 4.85 ± 0.74		p > 0.9999, ns			
									64	WT = 3.74 ± 0.70 HT = 4.13 ± 0.75		p > 0.9999, ns			
									66	WT = 3.82 ± 0.58 HT = 3.25 ± 0.57		p > 0.9999, ns			
									68	WT = 4.41 ± 0.47 HT = 5.16 ± 0.70		p > 0.9999, ns			
									70	WT = 6.37 ± 0.78 HT = 6.25 ± 1.36		p > 0.9999, ns			
	d	Elevated pluse maze		Male	2-3 months		Time spent in arm (s)		Open arm	WT = 104.98 ± 10.97 HT = 140.98 ± 18.30	Student's t-test	p = 0.0946, ns	t(22) = 1.747	Two-tailed	
									Closed arm	WT = 340.70 ± 16.97 HT = 279.32 ± 21.05		p = 0.0316, *	t(22) = 2.296	Two-tailed	
							Distance moved (m)			WT = 22.00 ± 1.44 HT = 24.91 ± 1.18	Student's t-test	p = 0.1417, ns	t(22) = 1.524	Two-tailed	
	e	Light-dark box test		Male	2-3 months	WT = 13 HT = 10	Light zone (s)			WT = 191.19 ± 17.38 HT = 192.98 ± 14.30	Student's t-test	p = 0.9836, ns	t(20) = 0.0208	Two-tailed	
							Frequency of tranfition (count)			WT = 66.16 ± 4.47 HT = 66.30 ± 4.49	Student's t-test	p = 0.9391, ns	t(20) = 0.0773	Two-tailed	
Supplementary Fig. 2	a	3-chamber test		Male	2-3 months	WT = 14 HT = 14	Sniffing time (s)	Stranger vs Object	WT	O = 63.60 ± 6.12 S = 181.26 ± 10.84	Student's t-test	p < 0.0001, ***	t(26) = 9.45	Two-tailed	
									HT	O = 59.46 ± 5.40 S = 158.85 ± 14.25	Student's t-test	p < 0.0001, ***	t(26) = 6.539	Two-tailed	
							Chamber time (s)		WT	O = 178.33 ± 10.44 S = 314.38 ± 10.55	Student's t-test	p < 0.0001, ***	t(26) = 9.161	Two-tailed	
									HT	O = 182.72 ± 15.16 S = 302.61 ± 17.93	Student's t-test	p < 0.0001, ***	t(26) = 5.104	Two-tailed	
							Sniffing time (s)	Stranger 1 vs Stranger 2	WT	S1 = 75.50 ± 6.60 S2 = 120.31 ± 10.90	Student's t-test	p = 0.0016, **	t(26) = 3.515	Two-tailed	
									HT	S1 = 64.15 ± 7.39 S2 = 120.65 ± 7.09	Student's t-test	p < 0.0001, ***	t(26) = 5.512	Two-tailed	
							Chamber time (s)		WT	S1 = 217.72 ± 10.62 S2 = 257.03 ± 12.87	Student's t-test	p = 0.0263, *	t(26) = 2.355	Two-tailed	
									HT	S1 = 193.32 ± 12.91 S2 = 271.35 ± 11.56	Student's t-test	p < 0.0001, ***	t(26) = 4.501	Two-tailed	
	b	Pup ultrasonic vocalization		Pup	P3	WT = 23 HT = 23	Call duration (s)			WT = 5.93 ± 0.87 HT = 3.58 ± 0.58	Student's t-test	p = 0.0274, *	t(44) = 2.281	Two-tailed	
							Call number (count)			WT = 163.39 ± 19.67 HT = 117.34 ± 12.5	Student's t-test	p = 0.0501, ns	t(44) = 2.051	Two-tailed	
					P5	WT = 23 HT = 23	Call duration (s)			WT = 9.55 ± 1.38 HT = 5.70 ± 0.89	Mann-Whitney U	p = 0.0554, ns	U = 177	Two-tailed	
							Call number (count)			WT = 225.11 ± 26.59 HT = 170.04 ± 18.19	Student's t-test	p = 0.0873, ns	t(44) = 1.749	Two-tailed	
					P7	WT = 23 HT = 23	Call duration (s)			WT = 13.83 ± 1.25 HT = 9.02 ± 1.05	Student's t-test	p = 0.0047, **	t(44) = 2.982	Two-tailed	
							Call number (count)			WT = 396.52 ± 20.88 HT = 261.65 ± 24.31	Student's t-test	p = 0.0017, **	t(42) = 3.358	Two-tailed	
					P9	WT = 23 HT = 23	Call duration (s)			WT = 9.32 ± 1.59 HT = 6.03 ± 0.97	Mann-Whitney U	p = 0.1628, ns	U = 200.5	Two-tailed	
							Call number (count)			WT = 266.26 ± 30.23 HT = 207.21 ± 23.65	Student's t-test	p = 0.1229, ns	t(44) = 1.573	Two-tailed	
	c	Forced swimming test		Male	2-3 months	WT = 13 HT = 11	Immobility time (s)			WT = 171.07 ± 5.75 HT = 149.00 ± 8.89	Student's t-test	p = 0.0431, *	t(22) = 2.147	Two-tailed	
	d	Tail suspension test		Male	2-3 months		Immobility time (s)			WT = 158.38 ± 6.15 HT = 150.27 ± 12.79	Student's t-test	p = 0.4543, ns	t(22) = 0.6005	Two-tailed	
Supplementary Fig. 3	a	Open field test		Female	7-8 weeks	WT = 16 HT = 11	Distance moved (m)	Light off (0 lux)	10 minutes	WT = 62.34 ± 2.48 HT = 72.11 ± 2.79	Two-way ANOVA repeated measures Bonferroni's multiple comparison test	p =0.0274, *	Interaction: F(5, 125) = 0.8727, p = 0.5020 Tanc2: F(1, 25) = 7.9840, p = 0.0091 Time: F(5, 125) = 75.43, p < 0.0001		
									20 minutes	WT = 47.07 ± 1.55 HT = 53.60 ± 1.81		p = 0.3375, ns			
									30 minutes	WT = 42.46 ± 1.91 HT = 49.77 ± 3.11		p = 0.1976, ns			
									40 minutes	WT = 41.61 ± 1.96 HT = 46.34 ± 0.36		p = 0.9921, ns			
									50 minutes	WT = 38.50 ± 2.19 HT = 45.35 ± 3.08		p = 0.2701, ns			
									60 minutes	WT = 35.45 ± 2.33 HT = 45.82 ± 2.23		p = 0.0158, *			
							Speed (cm/s)	Light off (0 lux)	10 minutes	WT = 62.34 ± 2.48 HT = 72.11 ± 2.79	Two-way ANOVA repeated measures Bonferroni's multiple comparison test	p =0.0240, *	Interaction: F(5, 125) = 0.8693, p = 0.5039 Tanc2: F(1, 25) = 7.6160, p = 0.0107 Time: F(5, 125) = 76.91, p < 0.0001		
									20 minutes	WT = 47.07 ± 1.55 HT = 53.60 ± 1.81		p = 0.3084, ns			
									30 minutes	WT = 42.46 ± 1.91 HT = 49.77 ± 3.11		p = 0.2184, ns			
									40 minutes	WT = 41.61 ± 1.96 HT = 46.34 ± 0.36		p > 0.9999, ns			
									50 minutes	WT = 38.50 ± 2.19 HT = 45.35 ± 3.08		p = 0.3171, ns			
									60 minutes	WT = 35.45 ± 2.33 HT = 45.82 ± 2.23		p = 0.0247, *			
							Total distance moved (m)		60 minutes	WT = 267.46 ± 9.70 HT = 313.03 ± 1.34	Student's t-test	p = 0.0091, **	t(25)= 2.8260	Two-tailed	
							Time spent in center region (s)		60 minutes	WT = 622.2 ± 61.7 HT = 539.4 ± 23.81	Mann-Whitney U	p = 0.4221, ns	U = 71	Two-tailed	
	b	Elevated pluse maze				WT = 15 HT = 11	Time spent in arm (s)		Open arm	WT = 139.83 ± 10.90 HT = 201.73 ± 14.41	Two-way ANOVA Bonferroni's multiple comparison test	p = 0.0555, ns	Interaction: F(1, 48) = 18.56, p < 0.0001 Tanc2: F(1, 48) = 0.2230, p = 0.6389 Arm: F(1, 48) = 20.12, p < 0.0001		
									Closed arm	WT = 281.76 ± 19.77		p = 0.0082, **			

											HT = 204.60 ± 16.17				
	c	Light-dark box test			3-4 months	WT = 18 HT = 12	Light zone (s)			WT = 138.59 ± 11.30 HT = 139.09 ± 14.56	Student's t-test	p = 0.9790, ns	t(28) = 0.0266	Two-tailed
							Frequency of tranfition (count)			WT = 54.38 ± 3.35 HT = 55.83 ± 5.56	Student's t-test	p = 0.8151, ns	t(28) = 0.2361	Two-tailed
	d	Forced swimming test				WT = 14 HT = 10	Immobility time (s)			WT = 136.00 ± 11.97 HT = 107.1 ± 13.5	Student's t-test	p = 0.1276, ns	t(22) = 1.5840	Two-tailed
	e	Tail suspension test				WT = 13 HT = 10	Immobility time (s)			WT = 138.69 ± 11.13 HT = 139.00 ± 13.12	Student's t-test	p = 0.9858, ns	t(22) = 0.0180	Two-tailed
Supplementary Fig. 5	a	Coimmunoprecipitation (Western blotting)				n = 4	mTOR/Tanc1 ratio in HEK293T cells (Fold change)		Serum +	1.00 ± 0.00 (SD)	Student's t-test	p = 0.0258, *	t(6) = 2.945	Two-tailed
									-serum 4 hr	2.57 ± 0.53 (SD)				
	b					n = 3	mTOR/Tanc1 ratio in HEK293T cells (Fold change)	Vehicle	+serum	1.00 ± 0.00 (SD)	Student's t-test	p = 0.0467, *	t(4) = 2.843	Two-tailed
									-serum 4 hr	3.42 ± 0.85 (SD)				
								Rapamycin	+serum	1.38 ± 0.53 (SD)	Student's t-test	p = 0.9735, ns	t(4) = 0.0353	Two-tailed
									-serum 4 hr	1.36 ± 0.38 (SD)				
	c	Western blotting	Tanc1 overexpression (Empty vs Tanc1)					p-mTOR/mTOR		Empty = 1.00 ± 0.00 (SD) Tanc1 = 0.72 ± 0.05 (SD)	Student's t-test	p = 0.0051, **	t(4) = 5.562	Two-tailed
			Tanc2 overexpression (Empty vs Tanc2)					p-mTOR/mTOR		Empty = 1.00 ± 0.00 (SD) Tanc2 = 0.72 ± 0.04 (SD)	Student's t-test	p = 0.0041, **	t(4) = 5.907	Two-tailed
Supplementary Fig. 6	b	Western blotting (P2 fraction)				n=3	Protein levels normalized to α-tubulin (Fold change)	p-mTOR/mTOR	0 hr	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test		F(2, 6) = 36.97, p = 0.0004	
									0.5 hr	1.68 ± 0.25 (SD)		p = 0.0130, *		
									1 hr	2.02 ± 0.32 (SD)		p = 0.0003, ***		
								mTOR	0 hr	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test		F(2, 6) = 0.3685, p = 0.7064	
									0.5 hr	1.42 ± 0.45 (SD)		p = 0.8546, ns		
									1 hr	1.16 ± 0.40 (SD)		p > 0.9999, ns		
								Tanc1	0 hr	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test		F(2, 6) = 3.735, p = 0.0884	
									0.5 hr	1.04 ± 0.02 (SD)		p = 0.6933, ns		
									1 hr	1.02 ± 0.5 (SD)		p > 0.0820, ns		
								Tanc2	0 hr	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test		F(2, 6) = 9.172, p = 0.0150	
									0.5 hr	0.82 ± 0.02 (SD)		p = 0.0824, ns		
									1 hr	0.71 ± 0.07 (SD)		p = 0.0120, *		
								PSD-95	0 hr	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test		F(2, 6) = 7.057, p = 0.0265	
									0.5 hr	1.19 ± 0.05 (SD)		p = 0.0649, ns		
									1 hr	1.21 ± 0.04 (SD)		p = 0.0434, *		
								p-Raptor/Raptor	0 hr	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test		F(2, 6) = 12.54, p = 0.0072	
									0.5 hr	1.47 ± 0.10 (SD)		p = 0.0087, **		
									1 hr	1.12 ± 0.06 (SD)		p = 0.5771, ns		
								Raptor	0 hr	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test		F(2, 6) = 0.9666, p = 0.4326	
									0.5 hr	1.17 ± 0.11 (SD)		p > 0.9999, ns		
									1 hr	1.42 ± 0.17 (SD)		p = 0.6469, ns		
								PRAS40	0 hr	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test		F(2, 6) = 14.92, p = 0.0047	
									0.5 hr	1.30 ± 0.15 (SD)		p = 0.1272, ns		
									1 hr	1.71 ± 0.05 (SD)		p = 0.0038, **		
								Deptor	0 hr	1.00 ± 0.00 (SD)	One-way ANOVA Bonferroni's multiple comparison test		F(2, 6) = 5.93, p = 0.0379	
									0.5 hr	1.00 ± 0.13 (SD)		p > 0.9999, ns		
									1 hr	1.47 ± 0.14 (SD)		p = 0.0488, *		
Supplementary Fig. 7	c	Western blotting				n=3	Protein levels normalized to β-actin (Fold change)	Tanc1_total	E18	1.00 ± 0.00 (SD)				
									P1	0.68 ± 0.09 (SD)				
									P7	1.33 ± 0.43 (SD)				
									P14	6.22 ± 3.34 (SD)				
									P21	11.8 ± 4.05 (SD)				
									P28	15.62 ± 5.54 (SD)				
								Tanc2_total	E18	1.00 ± 0.00 (SD)				
									P1	0.81 ± 0.07 (SD)				
									P7	1.11 ± 0.32 (SD)				
									P14	1.07 ± 0.49 (SD)				
									P21	0.78 ± 0.28 (SD)				
									P28	0.62 ± 0.28 (SD)				
								PSD-95_total	E18	1.00 ± 0.00 (SD)				
									P1	1.1 ± 0.22 (SD)				
									P7	5.10 ± 1.16 (SD)				
									P14	15.2 ± 3.72 (SD)				
									P21	19.83 ± 8.45 (SD)				
									P28	23.25 ± 11.21 (SD)				
								Deptor_total	E18	1.00 ± 0.00 (SD)				
									P1	0.88 ± 0.11 (SD)				
									P7	1.59 ± 0.36 (SD)				
									P14	3.18 ± 1.28 (SD)				
									P21	4.90 ± 3.23 (SD)				
									P28	4.82 ± 3.39 (SD)				
								PRAS40_total	E18	1.00 ± 0.00 (SD)				
									P1	0.92 ± 0.08 (SD)				
									P7	1.25 ± 0.33 (SD)				
									P14	1.39 ± 0.45 (SD)				
									P21	1.78 ± 0.98 (SD)				
									P28	1.68 ± 1.01 (SD)				
								Tanc1_P2	P7	1.00 ± 0.00 (SD)				
									P14	3.60 ± 1.22 (SD)				
									P21	5.01 ± 2.50 (SD)				
									P28	6.75 ± 3.59 (SD)				
								Tanc2_P2	P7	1.00 ± 0.00 (SD)				
									P14	0.71 ± 0.15 (SD)				
									P21	0.46 ± 0.19 (SD)				
									P28	0.33 ± 0.13 (SD)				
								PSD-95_P2	P7	1.00 ± 0.00 (SD)				
									P14	3.38 ± 0.53 (SD)				
									P21	5.08 ± 2.17 (SD)				
									P28	6.51 ± 2.84 (SD)				
								Deptor_P2	P7	1.00 ± 0.00 (SD)				
									P14	2.75 ± 1.07 (SD)				
									P21	3.03 ± 1.00 (SD)				
									P28	3.48 ± 1.52 (SD)				
								PRAS40_P2	P7	1.00 ± 0.00 (SD)				
									P14	1.25 ± 0.33 (SD)				
									P21	1.09 ± 0.47 (SD)				
									P28	1.04 ± 0.47 (SD)				
Supplementary Fig. 8	a	Western blotting	Control vs Tanc2 shRNA		DIV14	n=3	Protein levels normalized to b-actin (Fold change)	Tanc2/actin	Control	1.00 ± 0.00 (SD)	Student's t-test	p = 0.0004, ***	t(4) = 10.66	
									Tanc2 shRNA	0.41 ± 0.09 (SD)				
								p-S6/S6	Control	1.00 ± 0.00 (SD)	Student's t-test	p = 0.0019, **	t(4) = 7.27	
									Tanc2 shRNA	1.73 ± 0.17 (SD)				
								p-4E-BP/4E-BP	Control	1.00 ± 0.00 (SD)	Student's t-test	p = 0.0018, **	t(4) = 7.4	
									Tanc2 shRNA	1.57 ± 0.13 (SD)				
								p-Akt/Akt	Control	1.00 ± 0.00 (SD)	Student's t-test	p = 0.0045, **	t(4) = 5.761	
									Tanc2 shRNA	1.30 ± 0.09 (SD)				
								p-GSK3β/GSK3β	Control	1.00 ± 0.00 (SD)	Student's t-test	p = 0.0017, **	t(4) = 7.456	
									Tanc2 shRNA	1.39 ± 0.09 (SD)				
	b							p-S6/S6	Control	1.00 ± 0.00 (SD)	Student's t-test	p = 0.0273, *	t(4) = 3.397	
									Tanc2 shRNA	1.10 ± 0.05 (SD)				
								p-4E-BP/4E-BP	Control	1.00 ± 0.00 (SD)	Student's t-test	p = 0.6689, ns	t(4) = 0.4607	
									Tanc2 shRNA	1.01 ± 0.05 (SD)				
								p-Akt/Akt	Control	1.00 ± 0.00 (SD)	Student's t-test	p = 0.2364, ns	t(4) = 1.392	
									Tanc2 shRNA	1.07 ± 0.08 (SD)				
								p-GSK3β/GSK3β	Control	1.00 ± 0.00 (SD)	Student's t-test	p = 0.7421, ns	t(4) = 0.3528	
									Tanc2 shRNA	1.02 ± 0.13 (SD)				
	c	Immunocytochemistry	NeuN vs GFAP			n=13	Marker / DAPI (%)	Neuron culture	NeuN	86.98 ± 5.09 (SD)	Student's t-test	p < 0.0001, ***	t(24) = 54.13	
									GFAP	1.03 ± 2.6 (SD)				
								Glia culture	NeuN	4.01 ± 2.4 (SD)	Student's t-test	p < 0.0001, ***	t(24) = 26.06	
									GFAP	80.77 ± 10.34 (SD)				

Supplementary table 2. Primers used in this study

Mouse line	Primer name	Sequence (5'-)
Tanc2 $^{+/-}$	Tanc2$^{+/-}$-F	GGTTAGATAGGAAATAACCATCA
	WT-B	ATGTGGCATATAAAGTTATACCTC
	Tanc2$^{+/-}$-B	TCCCCACAACGGGTTCTTCTG
Tanc2 $^{fl/fl}$	Tanc2-fl-F	CTGTGCATGAAAGCTTCTGG
	Tanc2-fl-B	TCTCCACCTCCCTGGTACTG