Aberrant survival of hippocampal Cajal-Retzius cells leads to memory deficits, gamma rhythmopathies and susceptibility to seizures in adulthood in mice

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Supplementary Tables

Tested parameters		Males		Females	
		Control (N=17)	**Mutant** (N=8)	**Control** (N=9)	**Mutant** (N=9)
Morphology	Fur color	3.8 ± 0.2	4.0 ± 0.0	NA	NA
	Body weight after the test	20.5 ± 0.5	20.2 ± 0.6	18.8 ± 0.8	17.7 ± 0.6
	Body size	149.4 ± 1.4	149.1 ± 1.9	148.8 ± 2.7	148.1 ± 1.6
Physical state	Coat	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Groomed fur	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Marks of biting	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Body	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Whiskers	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Body tone	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Autonomic nervous system	Respiration rate at the beginning of testing	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	Respiration rate during testing	2.0 ± 0.0	1.8 ± 0.3	2.0 ± 0.0	2.0 ± 0.0
	Rectal temperature before testing	36.7 ± 0.2	36.7 ± 0.2	37.3 ± 0.4	37.2 ± 0.3
	Rectal temperature at the end of testing	36.9 ± 0.1	37.0 ± 0.1	37.5 ± 0.1	37.5 ± 0.2
	Salivation	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Pupil size	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.1	1.0 ± 0.1
	Light-Pupil retraction	2.2 ± 0.1	2.5 ± 0.2	2.0± 0.0	2.0± 0.0
Pain sensitivity	Tail pinch	1.8 ± 0.2	1.9 ± 0.3	1.9 ± 0.1	1.7 ± 0.2
	Toe pinch	1.4 ± 0.3	1.5 ± 0.4	1.3 ± 0.2	1.7 ± 0.2
Touch	Pinna touching	2.0 ± 0.0	2.0 ± 0.0	2.1 ± 0.1	2.0 ± 0.0
	Vibrissae touching	2.1 ± 0.1	2.4 ± 0.2	2.4 ± 0.2	2.3 ± 0.2
	Finger approach - Finger slowly approched and held at 1 cm in front of the animal for 5s	2.0 ± 0.0	2.0 ± 0.0	NA	NA
	Head touch - Finger slowly approached until touching the head	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	Touch escape (finger stroke from above)	1.5 ± 0.2	0.9 ± 0.2	1.6 ± 0.3	1.7 ± 0.2
Vision	Touch escape (finger stroke from above)	1.5 ± 0.2	0.9 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Visual placing: hindpaws extension	2.1 ± 0.1	2.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Audition	Auditory response 'clic'	2.9 ± 0.1	3.1 ± 0.1	1.0 ± 0.0	1.0 ± 0.0
	Auditory response 'clap'	2.9 ± 0.1	2.6 ± 0.2	1.0 ± 0.0	1.0 ± 0.0
Motricity Gait	Gait	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Pelvic elevation during forward motion	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	Tail elevation during forward motion	1.5 ± 0.1	1.4 ± 0.2	1.1 ± 0.1	1.2 ± 0.1
Motricity Tonus Strength	Grip strength	67.2 ± 3.9	64.2 ± 3.9	67.3 ± 3.6	75.3 ± 4.7
	Negative geotaxis	4.0 ± 0.0	4.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
	Wire manoeuvre	0.5 ± 0.26	0.9 ± 0.4	0.6 ± 0.24	0.0 ± 0.0
	Hindlimb tone	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0

Motricity Equilibrium	Fall from platform		0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
	Righting reflex: time		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Negative geotaxis		6.5 ± 1.1	4.3 ± 1.1	7.9 ± 2.4	6.5 ± 2.8
	Equilibrium		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Wire maneuver		0.5 ± 0.3	0.9 ± 0.4	NA	NA
SNC tremors	Tremors		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Tremors		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Reflex	Cornea		2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	Hind limb reflex		NA	NA	1.0 ± 0.0	1.0 ± 0.0
	Provoked biting		2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	Hindlimb tone		2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
Reactivity	Pinna touching		2.0 ± 0.0	2.0 ± 0.0	2.1 ± 0.1	2.0 ± 0.0
	Vibrissae touching		2.1 ± 0.1	2.4 ± 0.2	2.4 ± 0.2	2.3 ± 0.2
	Finger approach - Finger slowly approched and held at 1 cm in front of the animal for 5s		2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	Head touch - Finger slowly approached until touching head		2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	Touch escape (finger stroke from above)		1.5 ± 0.2	0.9 ± 0.2	NA	NA
	Touch escape		1.1 ± 0.1	0.9 ± 0.2	1.2 ± 0.1	1.0 ± 0.2
	Resistance to manipulations		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Provoked biting		2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
Activity in home cage	Body position		3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
	Activity		1.9 ± 0.1	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	Awakening		3.8 ± 0.2	4.0 ± 0.0		
	Digging sawdust		0.2 ± 0.1	0.4 ± 0.2	0.4 ± 0.2	0.9 ± 0.2
	Grooming		0.4 ± 0.2	0.1 ± 0.1	0.6 ± 0.2	0.3 ± 0.2
Activity after transfer	Horiz A after transfer		63.3 ± 6.9	63.5 ± 7.0	80.7 ± 6.0	90.0 ± 8.8
	Vertic A after transfer		13.5 ± 1.8	16.3 ± 1.4	18.6 ± 2.4	17.1 ± 1.9
	Horizontal activity	From 0 to 30s	23.3 ± 1.5	25.8 ± 2.6	29.2 ± 2.1	31.6 ± 2.8
		From 30 to 120s	32.3 ± 3.5	40.1 ± 4.0	51.4 ± 4.1	58.4 ± 6.5
	Vertical activity	From 0 to 30s	3.5 ± 0.5	3.3 ± 0.9	4.2 ± 0.9	4.3 ± 0.8
		From 30 to 120s	7.9 ± 0.8	11.5 ± 1.8	14.3 ± 2.0	12.8 ± 1.4
	Palpebral closure		2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	Awakening		4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
Activity after habituation	Palpebral closure		2.0 ± 0.0	2.0 ± 0.0	NA	NA
	Awakening		4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
	Transfer on platform		2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	Descent latency		30.4 ± 0.6	31.0 ± 0.0	31.0 ± 0.0	28.9 ± 2.1
Freezing	Freezing		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Freezing after transfer		NA	NA	0.0 ± 0.0	0.0 ± 0.0
	Negative geotaxis		4.0 ± 0.0	4.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Auditory response 'clic'		2.9 ± 0.1	3.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
	Auditory response 'clap'		2.9 ± 0.1	2.6 ± 0.2	0.1 ± 0.1	0.0 ± 0.0
	Touch escape		1.1 ± 0.1	0.9 ± 0.2	0.2 ± 0.1	0.1 ± 0.1
Grooming	Grooming		0.4 ± 0.2	0.1 ± 0.1	0.6 ± 0.2	0.3 ± 0.2
	Groomings from 0 to 120s		0.5 ± 0.2	0.6 ± 0.2	0.2 ± 0.1	0.8 ± 0.2
Agressivity	Attack to touch		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
	Provoked biting		2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0

Vocalisation	Abnormal vocalisation	NA	NA	0.0 ± 0.0	0.0 ± 0.0
	Vocalisation to tail pinch	NA	NA	0.9 ± 0.1	0.7 ± 0.2
	Vocalisation to toe pinch	NA	NA	0.3 ± 0.2	0.7 ± 0.2
Defecation	Defecation	2.2 ± 0.1	2.0 ± 0.0	1.8 ± 0.1	1.9 ± 0.1
	Defecation	2.0 ± 0.1	2.0 ± 0.3	1.8 ± 0.1	1.8 ± 0.2
	Faeces consistency	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
Urination	Urination	1.0 ± 0.0	1.0 ± 0.0	0.4 ± 0.2	0.6 ± 0.2

Table S1: No changes in pain sensitivity, motricity gait, tonus strength and equilibrium in juvenile *BaxCKO* mutant mice. Primary screening of juvenile (28 to 35 days old) animals, males and females independently tested. The details of the tests and the scores used to evaluate mouse response are in Materials and Methods section. Comparisons between control and mutant animals were performed using the Mann-Whitney U test and no statistically significant differences were observed.

Figure	Experiment	N	Mean (Ctrl)	Mean (Mut)	SD (Ctrl)	SD (Mut)	p-value	test	Unit of mesurement	Age
1A	Cookie test	11 Ctrl, 11 Mut	232.3 (olfaction) ; 237.0 (motivation)	153.3 ; 157.4	175.5 ; 173.7	110.4 ; 110.0	p=0.2913 for olfaction; p= 0.2657 for motivation	Mann-Whitney	seconds	9 weeks (males)
1A	Cookie test	11 Ctrl, 11 Mut	248.8 (day 1) ; 124.0 (day2)	138.6 ; 67.4	173.9 ; 61.9	102.8 ; 58.6	F(1. 20)=4.466 p=0.0473 for genotype / p=0.027 for Ctrl. p=0.0539 for Mut	RM-two way ANOVA / Wilcoxon matched-pairs signed rank	seconds	9 weeks (males)
1B	Y-maze	14 Ctrl, 14 Mut	30.2 (number of entries) ; 56.51 (alternation)	32.5 ; 56.05	9.5 ; 9.1	11.5 ; 10.9	p=0.6912 for number of entries; p=0.9910 for percentage of alternation	Mann-Whitney	number of entries ; %	9 weeks (males)
1C	Novel object recognition	28 Ctrl, 23 Mut	0.562 (preference) ; 0.124 (discrimination)	0.478 ; -0.0438 ;	0.0493 ; 0.0985	0.0621 ; 0.124	p<0.0001 for preference and p<0.0001 for discrimination index	Mann-Whitney	%, %	Adults (males)
1D	Novel object localization	15 Ctrl, 19 Mut	0.586 (preference); 0.172 (discrimination)	0.474 ; -0.0502	0.0444 ; 0.0887	0.0369 ; 0.0739	p<0.0001 for preference and p<0.0001 for discrimination index	Mann-Whitney	%, %	Adults (males)
1E	Morris Water Maze	15 Ctrl, 19 Mut	20.64	39.28	SE of difference: 7.488		F(1. 31)=6.198 p=0.0184 for Genotype	RM-two way ANOVA	seconds	Adults (males)
1F	Contextual Fear Conditioning	18 Ctrl, 9 Mut	17.5 (training) ; 51.7 (testing)	24.2 ; 55.7	18.1 ; 14.8	11.4 ; 10.2	p=0.0757 for training; p=0.5612 for testing	Mann-Whitney	%	Adults (males)
2A	CRs numbers (DAB staining)	5 Ctrl, 5 Mut	84.34 (HF) ; 58.94 (vDG)	108.06 ; 62.75	6.06 ; 9.5	8.42 ; 11.85	p=0.0018 for HF; p=0.7709 for DG	2-way ANOVA, Sidak's multiple comparison	cell/mm	P24
2B	CRs numbers (DAB staining)	4 Ctrl, 4 Mut	54.32 (HF) ; 38.61 (vDG)	70.85 ; 39.29	2.41 ; 10.73	9.75 ; 8.52	p=0.0348 for HF; p=0.9922 for DG	2-way ANOVA, Sidak's multiple comparison	cell/mm	Adults
2B	CRs numbers (IF P73)	3 Ctrl, 3 Mut	51.34 (HF) ; 37.33 (vDG)	93.25 ; 37.71	7.76 ; 3.52	19.32 ; 12.10	p=0.0009 for HF; p=0.9984 for DG	2-way ANOVA, Sidak's multiple comparison	cell/mm	Adults
2C	TOM-RELN-GABA colocalization	4 Ctrl, 4 Mut	99.15% (TOM+/RELN+/GABA-) ; 0.85% (TOM+/RELN+/GABA+)	99.05% ; 0.95%	0.72 ; 0.72	0.80 ; 0.80	p<0.0001 for controls ; p<0.0001 for mutants	2-way ANOVA, Sidak's multiple comparison	%	Adults
2C	CRs numbers (IF TOM)	4 Ctrl, 4 Mut	58.19 (HF) ; 59.67 (vDG)	82.49 ; 46.09	7.76 ; 3.52	19.32 ; 12.10	p=0.0305 ; p=0.2615	2-way ANOVA, Sidak's multiple comparison	cell/mm	Adults
3C	Power for theta and gamma bands in SLM	17 Ctrl sessions, 16 Mut sessions (from 3 mice/group)	125.69 (theta); 107.66 (gamma)	123.36 (theta); 109.49 (gamma)	2.99 (theta); 2.61 (gamma)	2.64 (theta); 1.41 (gamma)	p=0.0211 (theta. top); p=0.04 (gamma. bottom)	Kruskal-Wallis test	dB	Adults
3D	Power for theta and gamma bands in SP	17 Ctrl sessions, 16 Mut sessions (from 3 mice/group)	116.75 (theta); 99.98 (gamma)	116.37 (theta); 103.05 (gamma)	2.92 (theta); 2.81 (gamma)	2.36 (theta); 1.94 (gamma)	p=0.614 (theta. top); p=0.0055 (gamma. bottom)	Kruskal-Wallis test	dB	Adults
3E	Quantification of CR density along the CA1 HF	13 Ctrl sections, 15 Mut sections (from 3 mice/group)	38.88	52.76	3.95	15.66	p=0.0321	Kruskal-Wallis test	cells/mm	Adults
3F	Correlation between CR number and theta or gamma power	For both theta and gamma: 13 sessions in Ctrl, 15 sessions in Mut (from 3 mice/group). Only sessions from which electrode track was recovered.	Gamma --- SLM: 107.9671; SP: 100.6403 Theta---: SLM: 125.9099 ; SP: 117.2661 CR density---: 38.88	Gamma --- SLM: 109.2713; SP: 102.9070 Theta---: SLM: 123.3406 ; SP: 116.3829 CR density---: 52.76	Gamma --- SLM: 2.3647; SP: 2.5501 Theta---: SLM: 2.9612 ; SP: 2.7687 CR density---: 3.95	Gamma --- SLM: 1.1304; SP: 1.9262 Theta---: SLM: 2.7307 ; SP: 2.4375 CR density---: 15.66	Gamma --- SLM: r=0.17, p=0.381; SP: r=0.39, p=0.04192 Theta--- SLM: r= 0.44. p=0.01933 ; SP: r=0.27. p=0.16933	Pearson correlation	CR density/dB	Adults
4A	GAD67+ INs (ISH)	3 Ctrl, 4 Mut	229.26 (CA1/CA2) ; 257.69 (CA3) ; 107.99 (DG)	202.63 ; 238.42 ; 95.89	42.54 ; 36.68 ; 7.71	51.58 ; 17.37 ; 28.36	p=0.6947 for CA1/CA2; p=0.8548 for DG; p=0.9578 for CA3	2-way ANOVA, Sidak's multiple comparison	cell/mm2	Adults
4B	GAD67+ INs (ISH)	4 Ctrl, 4 Mut	387.67 for CA1/CA2 ; 378.11 for CA3 ; 222.73 for DG	440.74 ; 378.17 ; 235.76	15.08 ; 22.62 ; 18.86	20.23 ; 27.26 ; 29.14	p=0.0118 for CA1/CA2; p=0.8129 for DG; p>0.9999 for CA3 (p=0.3429 for total)	2-way ANOVA, Sidak's multiple comparison (Mann-Whitney for total)	cell/mm2	P24
4C	SST+ INs (ISH)	3 Ctrl, 3 Mut	65.41 (CA1/CA2) ; 113.57 (CA3) ; 0 (DG)	69.43 ; 114.23 ; 0	7.91 ; 8.12 ; 0	5.78 ; 24.89 ; 0	p=0.9655 for CA1/CA2; p=0.9998 for DG; p>0.9999 for CA3 (p>0.9999 for total)	2-way ANOVA, Sidak's multiple comparison (Mann-Whitney for total)	cell/mm2	P24
4D	NPY+ INs (IF)	4 Ctrl, 4 Mut	29.89 (CA1/CA2) ; 24.15 (CA3) ; 5.8 (DG)	45.1 ; 26.34 ; 10.7	6.65 ; 2.97 ; 2.18	7.13 ; 3.41 ; 3.71	p=0.003 for CA1/CA2; p=0.5889 for DG; p=0.9621 for CA3 (p=0.0017 for total)	2-way ANOVA, Sidak's multiple comparison (Mann-Whitney for total)	cell/mm2	P24
5A	Sholl Analysis (apical dendrites)	8 Ctrl, 7 Mut	4.089	5.184	SE of difference: 0.4524		F(1. 13)= 5.862 p=0.0308 for genotype	RM-two way ANOVA	number of intersections	Adults
5B	Number of spines	6 Ctrl, 6 Mut (apical); 6 Ctrl, 7 Mut (basal)	1.03 (apical) ; 1.33 (basal)	1.59 ; 1.64	0.27 ; 0.237	0.344 ; 0.102	p=0.0260 for apical and 0.0350 for basal	Mann-Whitney	spines/um	Adults
5C	sEPSC	11 Ctrl, 15 Mut	13.1 (amplitude) ; 1.72 (frequency)	13.2 ; 2.04	4.01 ; 0.742	3.12 ; 1.31	p=0.6461 amplitude; p=0.6832 frequency	Mann-Whitney	pA ; Hz	Adults
5D	sIPSC	15 Ctrl, 16 Mut	16.9 (amplitude) ; 4.91 (frequency)	15.2 ; 7.63	4.41 ; 2.95	3.77 ; 3.61	p=0.2995 amplitude; p=0.0405 frequency	Mann-Whitney	pA ; Hz	Adults
6A	Adult seizures	23 Ctrl, 16 Mut	26% (incidence) ; 4.74 (duration) ; 4.35% (mortality) ; 180 (latency)	94% ; 25.1 ; 62.5% ; 24.0	NA ; 10.1 ; 20.9 ; 103	NA ; 29.5 ; 50.0 ; 58.0	p<0.0001 for seizure incidence; p<0.0001 for duration; p=0.0001 for mortality and p<0.0001 for latency	Mann-Whitney	% ; minutes ; % ; minutes	Adults (males)
6B	Duration	23 Ctrl, 16 Mut	4.74	25.1	10.1	29.5	p<0.0001	Mann-Whitney	minutes	Adults (males)
6C	Latency	23 Ctrl, 16 Mut	180 ; 11.4 (responders only)	24 ; 9.64	103 ; 9.36	58 ; 7.38	p<0.0001 ; p=0.8348 (responders only)	Mann-Whitney	minutes	Adults (males)
6D	Juvenile seizures	7 Ctrl, 10 Mut	86% (incidence) ; 13.4 (duration) ; 71.4% (mortality) ; 24.6	100% ; 12.8 ; 70.0 % ; 26.3	0.976 ; 16.0 ; 48.8 ; 42.2	1.87 ; 10.0 ; 48.3 ; 34.3	p=0.4118 for seizure incidence; p=0.7611 for duration; p>0.9999 for mortality and p=0.5503 for latency	Mann-Whitney	% ; minutes ; % ; minutes	Juvenile (males)
7C	type of activity	20 Ctrl slices (4 mice), 20 Mut slices (4 mice)	bursting/seizures: 85%/15% (17/3 out of 20 slices)	bursting/seizures: 45%/55% (9/11 out of 20 slices)	N/A	N/A	p=0.0187	Fisher's exact test	%	Adults
7D	seizure frequency and duration	3 Ctrl slices, 11 Mut slices	0.29 seizures/min (frequency); 3.3.5 s (duration)	0.41 (frequency); 29.1 (duration)	0.08; 5.238	0.24; 20.04	no test because only n=3 for ctrl slices	N/A	seizures/minutes	Adults
7E	seizure propagation	11 Ctrl seizures, 37 Mut seizures	DG ; CA3 ; CA1 ;Sub ; Ent; PRh/Ect: 33. 67. 100. 100. 100. 100 %	DG ; CA3 ; CA1 ; Sub ; Ent; PRh/Ect: 80. 100. 100. 100. 100. 100 %	N/A	N/A	p=0.0011	Chi-square test	seconds	Adults
7F	seizure initiation sites	11 Ctrl seizures, 37 Mut seizures	DG ; CA3 ; CA1 ;Sub ; Ent; PRh/Ect: 0. 27.2. 9.1. 18.2. 9.1. 36.4 %	DG ; CA3 ; CA1 ; Sub ; Ent; PRh/Ect: 24.4. 27..0. 5.4. 18.9. 24.3 %	N/A	N/A	ctrl vs Mut: p=1.000 (cortex vs hippocampal formation); p=0.6065 (Ent vs PRh/Ect); p=0.035 (hippocampal subregions)	Fisher's exact test; Fisher's exact test; Chi-square test	%	Adults

S1A	Open field	12 Ctrl, 12 Mut	1604	1877	SE of difference: 185.5		F(1. 22)=2.164 p=0.1554 for genotype	RM-two way ANOVA	mm	6 weeks (males)
S1B	Elevated plus maze (EPM)	14 Ctrl, 14 Mut	20.87	15.32	13.74	11.79	p=0.2219	Mann-Whitney	seconds	6 weeks (males)
S1C	3-chambers	12 Ctrl, 12 Mut	140.2 (stranger) ; 91.2 (empty)	155.4 ; 85.8	41.1 ; 38.1	52.1 ; 32.9	p=0.0179 for controls ; p=0.0010 for mutants	RM two-way ANOVA, Sidak's multiple comparison	seconds	8 weeks (males)
S1D	Forced swim test	11 Ctrl, 12 Mut	65.65 (swim) ; 22.09 (climb)	67.73 ; 28.50	16.39 ; 13.55	8.501 ; 21.65	p=0.347 for swim; p=0.5254 for climb	Mann-Whitney	seconds	7 weeks (males)
S1E	Rotarod	12 Ctrl, 12 Mut	528.8	441.3	169.9	161.2	p=0.0848	Mann-Whitney	seconds	8 weeks (males)
S1F	Open field	13 Ctrl, 5 Mut	11.09 (% time in center) ; 23.22 (% distance in center) ; 89.87 (total distance)	8.858 ; 19.79 ; 89.69	6.201 ; 10.88 ; 19.48	3.196 ; 8.416 ; 18.3 1	p=0.7028 for time in center. p=0.7028 for distance in center. p=0.8490 for total distance	Mann-Whitney	% ; %. cm	Adult (males)
S1G	Dark and light	13 Ctrl, 5 Mut	34.3 (% time in light) ; 12.9 (number of entries) ; 34.2 (% distance in light)	25.1 ; 11.2 ; 26.9	16.6 ; 7.34 ; 14.7	16.4 ; 5.67 ; 16.2	p=0.1936 for % time in light; p=0.2274 for number of entries; p=0.1936 % for distance in light	Mann-Whitney	%, number of entries, %	Adult (males)
S2A	Novel object recognition	27 Ctrl, 34 Mut	0.57 (preference) ; 0.141 (discrimination)	0.492 ; -0.0168	0.0372 ; 0.0743	0.0812 ; 0.162	p<0.0001 for preference and p<0.0001 for discrimination index	Mann-Whitney	%, %	Adult (females)
S2B	Novel object localization	22 Ctrl, 23 Mut	0.56 (preference) ; 0.120 (discrimination)	0.472 ; -0.0562	0.0732 ; 0.146	0.0523 ; 0.105	p<0.0001 for preference and p<0.0001 for discrimination index	Mann-Whitney	%, %	Adult (females)
S2C	Morris Water Maze	21 Ctrl, 23 Mut	23.15	46.98	SE of difference: 6.481		F(1. 42)=13.52 p=0.0007 for genotype	RM-two way ANOVA	seconds	Adult (females)
S2D	Contextual Fear Conditioning	12 Ctrl, 18 Mut	11.7 (training) ; 38.4 (testing)	9.19 ; 40.2	13.9 ; 25.4	7.76 ; 23.8	p=0.8265 for training. p=0.2106 for testing	Mann-Whitney	%	Adult (females)
S2E	Open field	7 Ctrl, 13 Mut	12.4 (time in centrer) ; 25.9 (distance in center) ; 113 (total distance)	15.9 ; 28.3 ; 130	12.4 ; 5.19 ; 25.1	15.9 ; 11.0 ; 31.8	p=0.2749 for % time in center; p=0.7573 for % distance in center; p=0.3929 for total distance	Mann-Whitney	% ; %. cm	Adult (females)
S2F	Dark and light	7 Ctrl, 13 Mut	25.3 (number of entries) ; 38.8 (distance in light) ; 32.0 (time in light)	24 ; 42.1 ; 39.2	6.1 ; 7.82 ; 8.37	9.69 ; 15.3 ; 14.7	p=0.3857 for number of entries ; p=0.1207 for distance in light; p=0.1255 time in light	Mann-Whitney	number of entries, %, %	Adult (females)
S3A	CRs numbers (DAB staining)	3 Ctrl, 5 Mut	64.3	109	11.7	26.7	p=0.0357	Mann-Whitney	cell/mm	P24
S3B	WB Reelin	4 Ctrl, 4 Mut	0.133	0.127	0.0508	0.0211	p>0.9999	Mann-Whitney	Total #ELN/α-tubulin	Adult
S4A	SST Adult	4 Ctrl, 4 Mut	42.11 (CA1/CA2) ; 46.01 (CA3) ; 12.58 (DG)	45.67 ; 51.83 ; 18.96	10.33 ; 7.54 ; 4.30	14.06 ; 4.71 ; 3.85	p=0.9113 for CA1/CA2 ; p=0.7086 for CA3 ; p= 0.6471 for DG	2-way ANOVA, Sidak's multiple comparison	cell/mm2	Adult
S4B	NPY adult	5 Ctrl, 5 Mut	67.89 (CA1/CA2) ; 42.31 (CA3) ; 26.99 (DG)	56.18 ; 42.94 ; 20.71	43.76 ; 24.14 ; 13.75	30.51 ; 13.42 ; 12.41	p=0.9527 for CA1/CA2 ; p>0.9999 for CA3 ; p= 0.8519 for DG	2-way ANOVA, Sidak's multiple comparison	cell/mm2	Adult
S5A	Filament lenght	8 Ctrl, 7Mut for apical ; 6 Ctrl, 4 Mut for basal	4210 (apical) ; 3034 (basal)	5194 ; 3308	878 ; 352	764 ; 1269	p=0.0289 for apical ; p=0.9143 for basal	Mann-Whitney	mm	Adult
S5B	Number of branches	6 Ctrl, 4 Mut	55.3 (apical) ; 34.0 (basal)	67.1 ; 37.5	15.9 ; 6.13	15.8 ; 8.58	p=0.244 for apical ; p=0.5333 for basal	Mann-Whitney	number of branches	Adult
S5C	Sholl Analysis (basal dendrites)	5 Ctrl, 4 Mut	9.157	9.893	SE of difference: 1.834		F(1. 7)=0.1609. p=0.7003 for genotype	RM-two way ANOVA	number of intersections	Adult
S5D	mEPSCs	16 Ctrl, 10 Mut	13.3 (amplitude) ; 1.21 (frequency)	11.4 ; 1.47	2.96 ; 0.612	2.32 ; 0.855	p=0.1246 for amplitude ; p=0.5955 for frequency	Mann-Whitney	pA ; Hz	Adult
S5E	Firing frequency	21 Ctrl, 21 Mut	13.23	10.61	SE of difference: 2.614		F(1.000. 20.00)=2.418. p=0.1356 for genotype	RM-two way ANOVA	Hz	Adult
S5F	Resting membrane potential	21 Ctrl, 21 Mut	-59.1	-59.5	6.84	5.34	p=0.9405	Mann-Whitney	mV	Adult
S5G	Action potential threshold	21 Ctrl, 21 Mut	-35.3	-35.8	5.57	3.42	p=0.6585	Mann-Whitney	mV	Adult
S5H	Input resistance	21 Ctrl, 21 Mut	109	91.9	55.9	33.8	p=0.2202	Mann-Whitney	MOhm	Adult
S6A	Adult Seizures	7 Ctrl, 7 Mut	29% (incidence) ; 0.714 (duration) ; 0 % (mortality) ; 90.4 (latency)	86% ; 23.0 ; 42.9 % ; 27.3	0.976 ; 1.25 ; 0.00 ; 50.6	1.35 ; 20.1 ; 53.5 ; 41.6	p=0.0306 for seizure incidence; p=0.0064 for duration; p=0.055 for mortality and p=0.0361 for latency	Mann-Whitney	% ; minutes ; % ; minutes	Adult (females)
S6B	Juvenile Seizures	5 Ctrl, 7 Mut	100% (incidence) ; 18.7 (duration) ; 60.0% (mortality) ; 13.4 (latency)	86% ; 14.8 ; 57.1 % ; 29.8	2.3 ; 13.2 ; 54.8 ; 7.44	2.23 ; 13.6 ; 53.5 ; 40.1	p=0.4241 for seizure incidence; p=0.7866 for duration; p>0.9999 for mortality and p=0.4571 for latency	Mann-Whitney	% ; minutes ; % ; minutes	Juvenile (females)

Table S2: Summary of experimental data, parameters, sex and statistical analysis.

Aberrant survival of hippocampal Cajal-Retzius cells leads to memory deficits, gamma rhythmopathies and susceptibility to seizures in adulthood in mice

Martina Riva[1,2], Stéphanie Moriceau[3], Annunziato Morabito[4#], Elena Dossi[5#], Candela Sanchez-Bellot[6#], Patrick Azzam[1,2#], Andrea Navas-Olive[6], Beatriz Gal[6,7], Francesco Dori[1,2], Elena Cid[6], Fanny Ledonne[2], Sabrina David[8], Fabrice Trovero[8], Magali Bartolomucci[9], Eva Coppola[2], Nelson Rebola[4], Antoine Depaulis[9], Nathalie Rouach[5], Liset Menendez de la Prida[6], Franck Oury[3] and Alessandra Pierani [1,2,10*]

Supplementary Figures

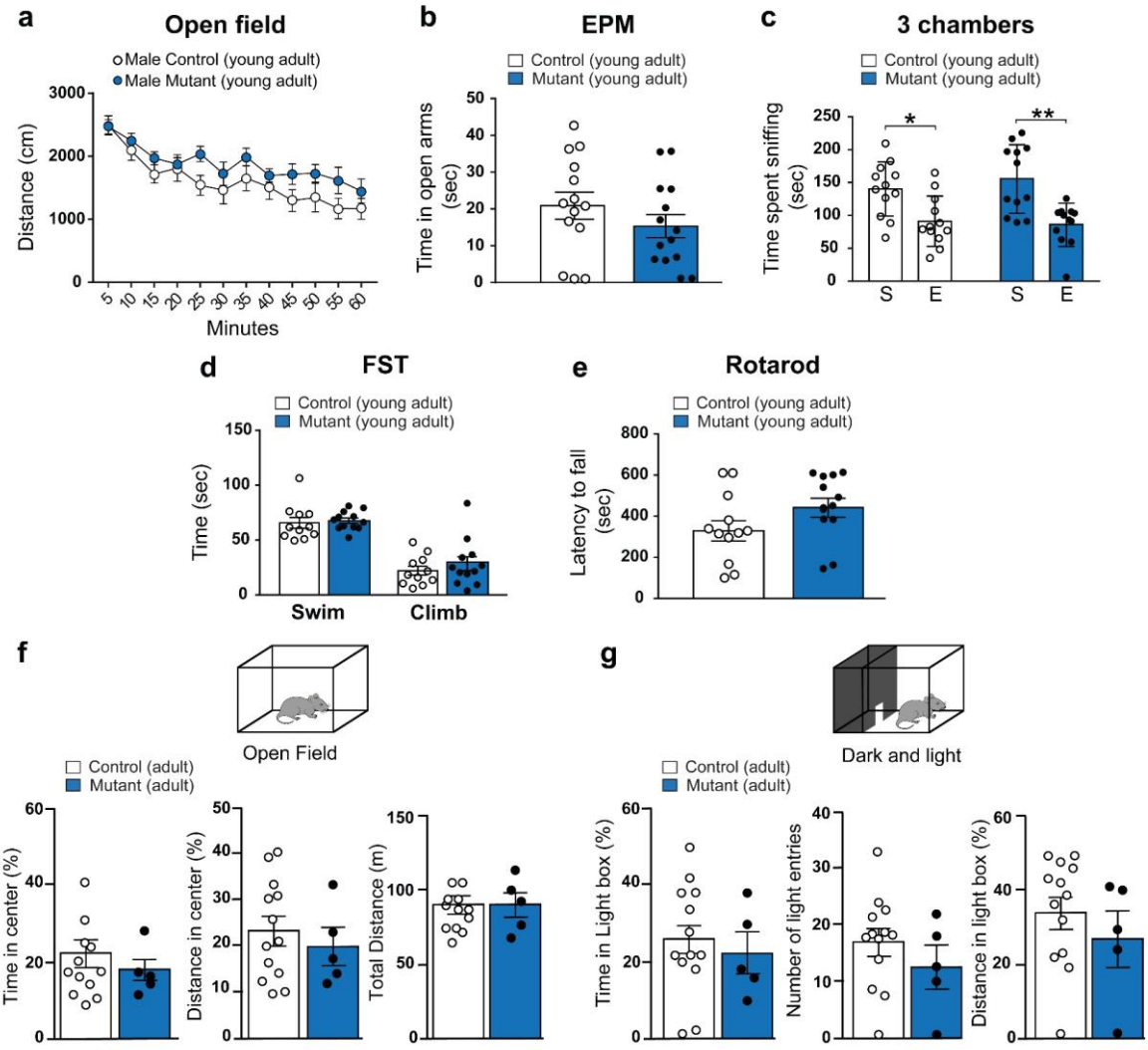

a
Open field
Male Control (young adult)
Male Mutant (young adult)
Distance (cm)
Minutes
b
EPM
Control (young adult)
Mutant (young adult)
Time in open arms (sec)
c
3 chambers
Control (young adult)
Mutant (young adult)
Time spent sniffing (sec)
S
E
d
FST
Control (young adult)
Mutant (young adult)
Time (sec)
Swim
Climb
e
Rotarod
Control (young adult)
Mutant (young adult)
Latency to fall (sec)
f
Open Field
Control (adult)
Mutant (adult)
Time in center (%)
Distance in center (%)
Total Distance (m)
g
Dark and light
Control (adult)
Mutant (adult)
Time in Light box (%)
Number of light entries
Distance in light box (%)

Figure S1

Fig. S1: *BaxCKO* mutant mice show normal locomotion, anxiety and sociability. a Open field test (OF) performed on young adults (6 weeks-old) male animals showing no differences in traveled distance between controls (n=12) and mutant (n=12) animals ($F(1, 22) = 2.164$; $p=0.1554$ RM Two-way ANOVA). **b** Elevated plus maze (EPM) performed on young adults (6 weeks-old) male animals showing no differences in time spent in the open arms (OA) between controls (n=14) and mutant (n=14) animals ($p=0.2219$ Mann-Whitney test). **c** 3-chamber sociability test performed on young adults (8 weeks-old) male animals. Both controls (n=12) and mutant (n=12) spent significantly more time sniffing the cage with a stranger (S) then the empty cage (E) ($p=0.0179$ for controls and $p=0.0010$ for mutants, RM Two-way ANOVA, post hoc Sidak's multiple comparison test). **d** Forced swim test (FST) performed on young adults (7 weeks-old) male animals showing no differences in the time spent swimming or climbing between controls (n=12) and mutant (n=12) animals ($p=0.347$ for swim, $p=0.5254$ for climb, Mann-Whitney test). **e** Rotarod test performed on young adults (8 weeks-old) male animals indicating no differences in latency to fall between controls (n=12) and mutant (n=12) animals ($p=0.0848$ Mann-Whitney test). **f** OF test performed in adult male (n=5 mutants and n=13 controls, Mann-Whitney test) animals. % of the time spent in the center versus the periphery ($p=0.7028$), % of the distance traveled in the center versus the periphery ($p=0.7028$) and the Total distance (m) ($p=0.8490$) were measured. No differences were detected between controls and mutants. **g** Light/Dark transition Test (L/DT) performed in adult males (n=5 mutants and n=13 controls, Mann-Whitney test). % time spent in the light compartment ($p=0.1936$), the number of entries in the light compartment ($p=0.2274$) and % of the distance traveled ($p=0.1936$) were measured. No differences were detected between controls and mutants. Asterisks indicate statistical significance (**, $p<0.01$). All data are presented as Mean ± SEM.

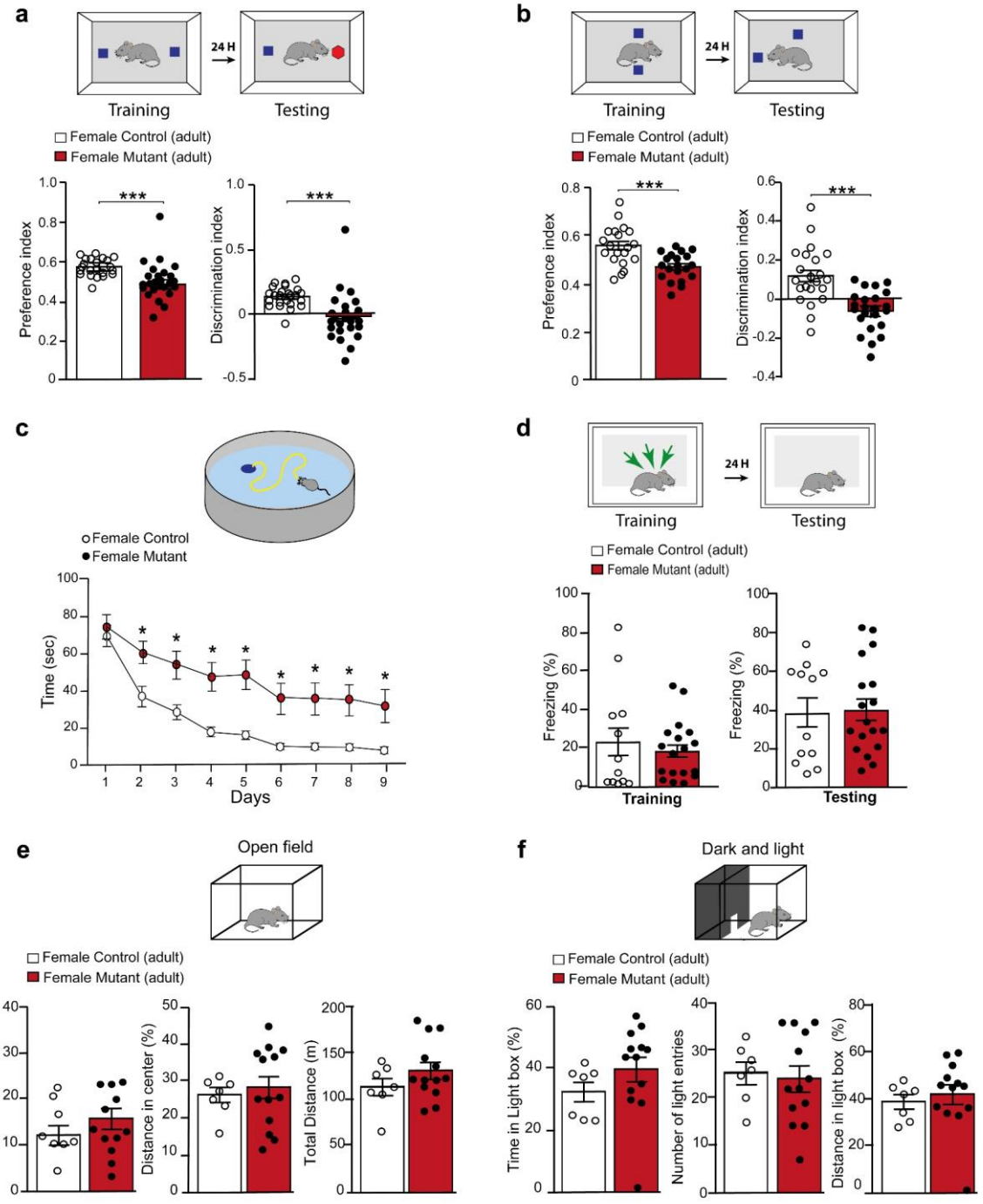

a
Training
24 H
Testing
Female Control (adult)
Female Mutant (adult)
Preference index
Discrimination index
***
b
Training
24 H
Testing
Female Control (adult)
Female Mutant (adult)
Preference index
Discrimination index
***
c
Female Control
Female Mutant
Time (sec)
Days
d
Training
24 H
Testing
Female Control (adult)
Female Mutant (adult)
Freezing (%)
Training
Testing
e
Open field
Female Control (adult)
Female Mutant (adult)
Time in center (%)
Distance in center (%)
Total Distance (m)
f
Dark and light
Female Control (adult)
Female Mutant (adult)
Time in Light box (%)
Number of light entries
Distance in light box (%)

Figure S2

Fig. S2: Adult *BaxCKO* mutant female mice exhibit deficits in hippocampal associated memory. **a** NOR test performed on three independent groups of adult female mutant animals and their control littermates (n=34) and controls (n=27). Preference and Discrimination index were measured 24 h after the training phase to assess memory performance. Mutant animals showed impairments in discriminating the new object ($p<0.0001$ and $p<0.0001$ for preference and discrimination index respectively, Mann-Whitney test). **b** NOL test performed on two independent groups of adult female mutant animals (n=22) and their controls littermates (n=23). Preference and Discrimination index were measured 24 h after the training phase. Mutant animals showed impairments in discriminating the new located object ($p<0.0001$ and $p<0.0001$ for preference and discrimination index respectively, Mann-Whitney test). **c** MWM test performed in adult female (n=23 mutants and n=21 controls) mice for 9 days. Mutant animals required more time to find the hidden platform ($F(1, 42) = 13.52$, $p=0.0007$ for Genotype, RM Two-way ANOVA). **d** Contextual Fear Conditioning (CFC) performed on females (mutant, n=18; control, n=12) from two independent groups. Percent freezing time was recorded during the training (as control for basal level, $p=0.8265$, Mann-Whitney test) and testing phases (to assess memory performance, $p=0.2106$, Mann-Whitney test). No differences were detected between control and mutant animals. **e** OF test performed in adult female mutant (n=13) and control (n=7) animals (Mann-Whitney test). % of the time spent in the center versus the periphery ($p=0.2749$), % of the distance traveled in the center versus the periphery ($p=0.7573$) and the Total distance (m) ($p=0.3929$) were measured. No differences were detected between controls and mutants. **f** Light/Dark transition Test (L/DT) performed in adult females (n=13 mutants and n=7 controls, Mann-Whitney test). % time spent in the light compartment ($p=0.1255$), number of entries in the light compartment ($p=0.3857$) and % of the distance traveled ($p=0.1207$) were measured. No differences were detected between controls and mutants. All tests were performed on 3 months-old female mice. Asterisks indicate statistical significance (*, $p<0.05$; ***, $p<0.001$). All data are presented as Mean ± SEM.

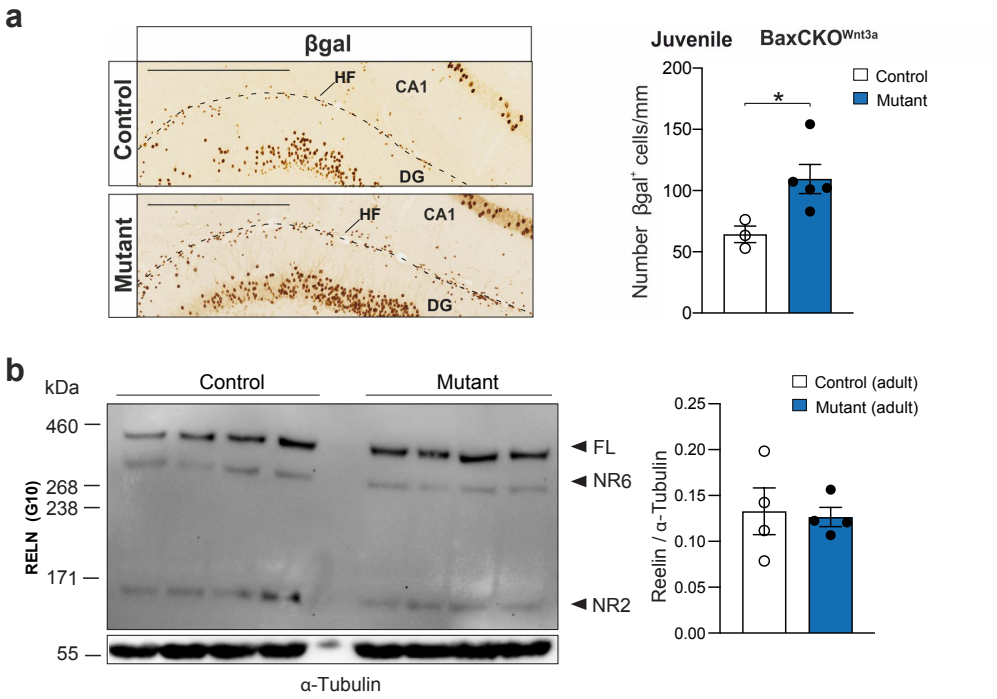


Figure S3

Fig. S3: Enhanced survival of CRs in the hippocampus of juvenile *BaxCKO*^{Wnt3aCre} mutants. a Coronal sections of control and *BaxCKO*^{Wnt3acre} (all the samples are carrying the *Tau*^{nlsLacZ} reporter) hippocampal region at P24 immunostained for βgal **(**left panels). Quantification of βgal^{+} cells in HF of control and mutant animals (right panels) showing increased number of CRs in mutants (controls: 64.25 ± 6.76 cells/mm; mutants: 109.5 ± 11.92 cells/mm, p=0.0357; Mann-Whitney test, n=3 controls and n=5 mutants). **b** Western blotting using an anti-Reln (G10) and anti-αtubulin (as internal control) showing no differences in the total amount of RELN protein in the hippocampus of adult controls and *BaxCKO* mice (p>0.9999; Mann-Whitney test, n=4 controls and n=4 mutants). All data are presented as Mean ± SEM.

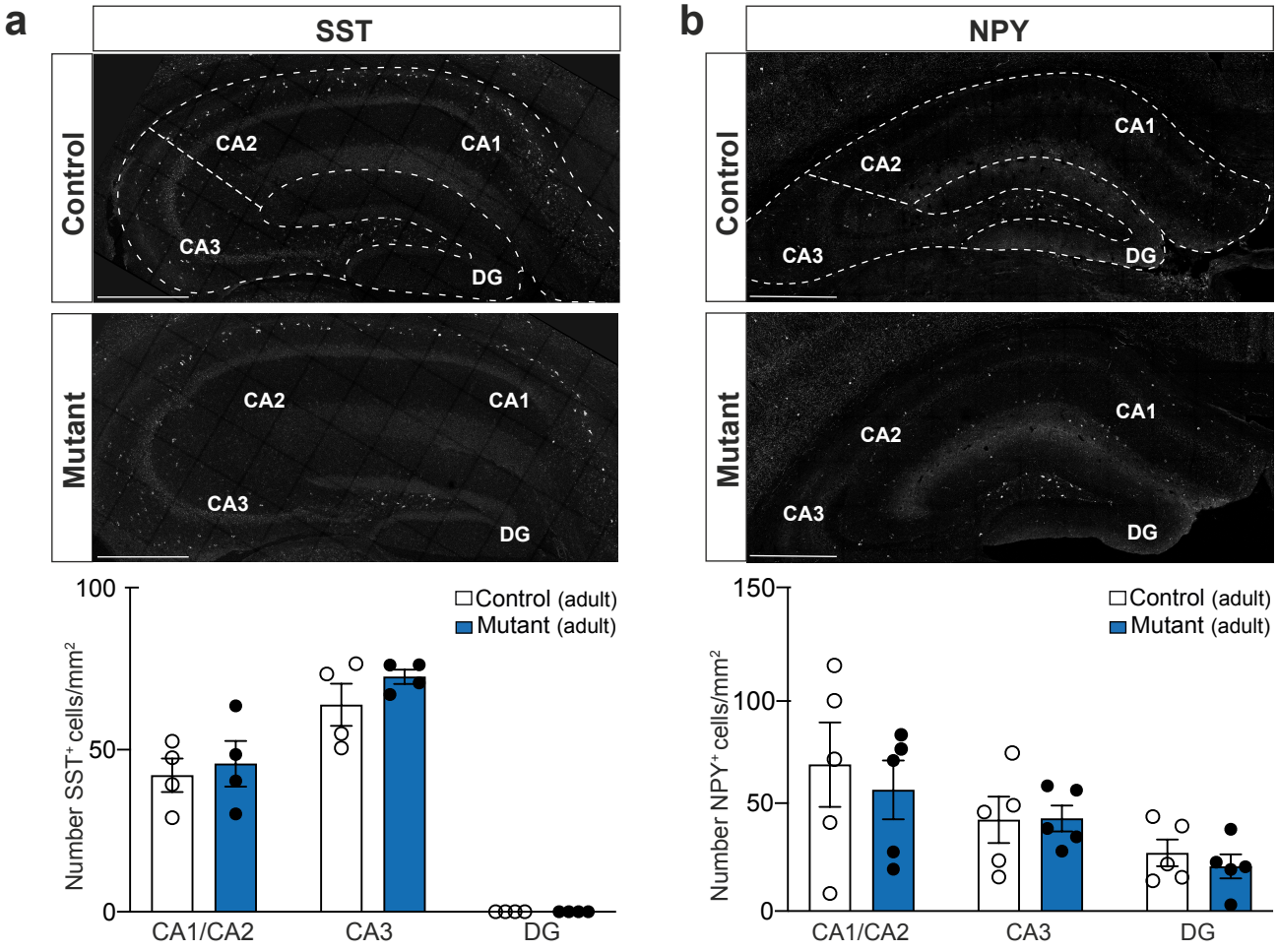

a
SST
Control
CA2
CA1
CA3
DG
Mutant
CA2
CA1
CA3
DG
100
50
0
Number SST+ cells/mm2
Control (adult)
Mutant (adult)
CA1/CA2
CA3
DG
b
NPY
Control
CA1
CA2
CA3
DG
Mutant
CA1
CA2
CA3
DG
150
100
50
0
Number NPY+ cells/mm2
Control (adult)
Mutant (adult)
CA1/CA2
CA3
DG

Figure S4

Fig. S4: Unaffected number of interneurons in the adult hippocampus. a Immunofluorescence of coronal sections from adult control and *BaxCKO* brains stained for SST (top panels). Quantification of SST^+ cells in the different hippocampal regions of control and mutant animals showing no differences ($p=0.9291$ for CA1/CA2; $p=0.4788$ for CA3; $p>0.9999$ for DG, two-way ANOVA, Sidak's multiple comparison test, n=4 controls and n=4 mutants). **b** Immunofluorescence of coronal sections from adult control and *BaxCKO* brains stained for NPY (top panels). Quantification of NPY^+ cells in the different hippocampal regions of control and mutant animals showing no differences ($p=0.9527$ for CA1/CA2; $p>0.9999$ for CA3; $p=0.8519$ for DG, two-way ANOVA, Sidak's multiple comparison test, n=5 controls and n=5 mutants). Dotted lines represent the subdivisions in different regions: CA1/CA2, CA3 and DG. Scale bar: 500 µm. All data are presented as Mean ± SEM.

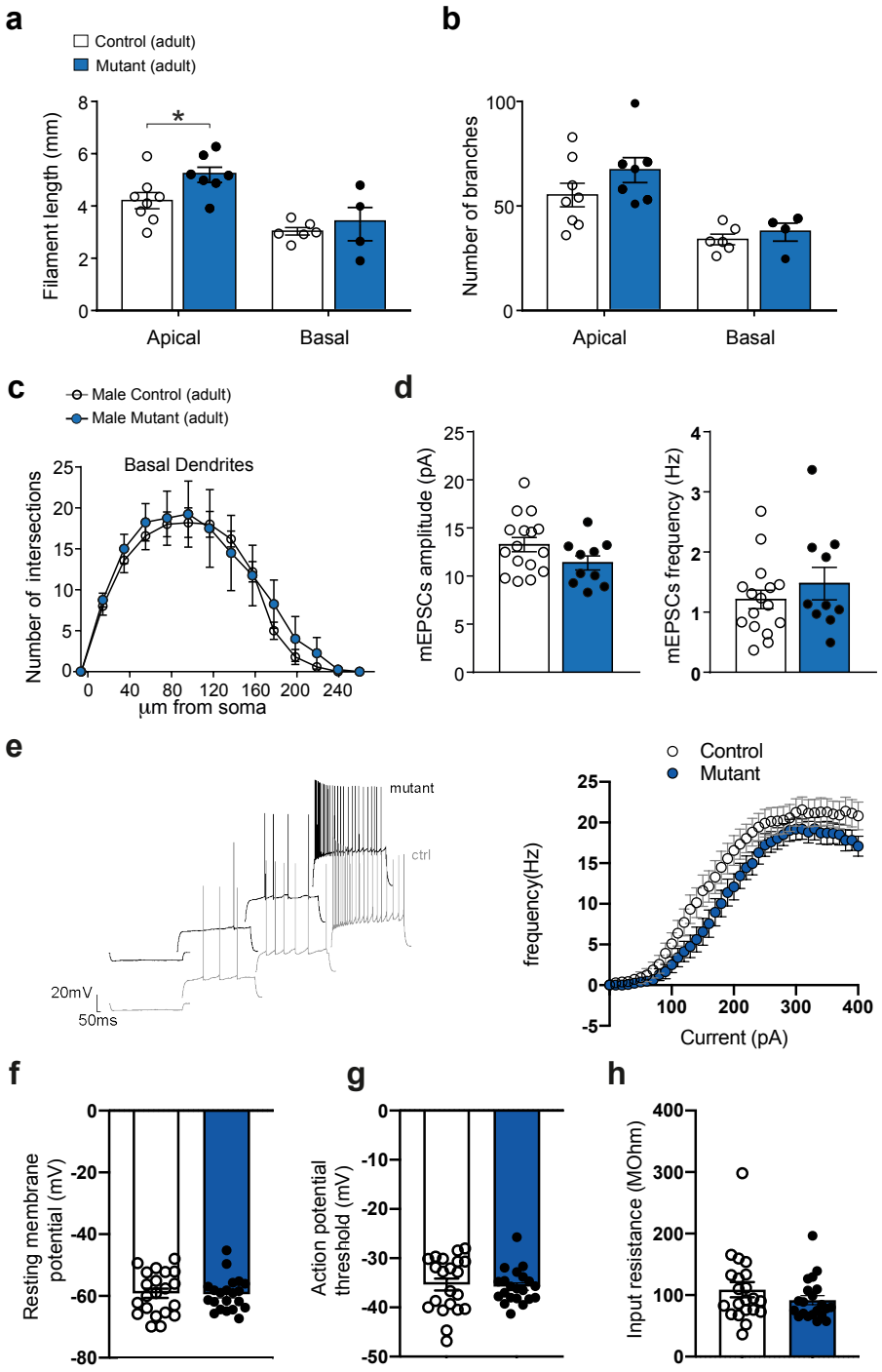
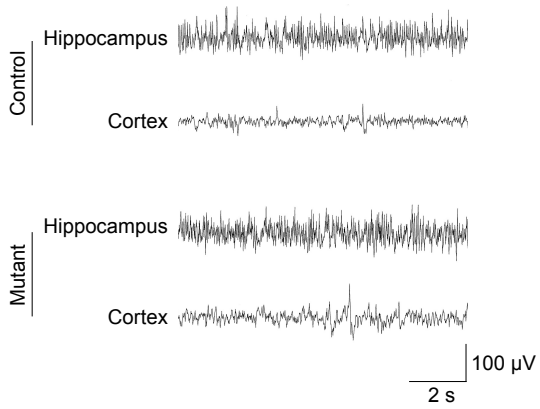

a
Control (adult)
Mutant (adult)
Filament length (mm)
Apical
Basal
b
Number of branches
Apical
Basal
c
Male Control (adult)
Male Mutant (adult)
Basal Dendrites
Number of intersections
µm from soma
d
mEPSCs amplitude (pA)
mEPSCs frequency (Hz)
e
mutant
ctrl
20mV
50ms
Control
Mutant
frequency(Hz)
Current (pA)
f
Resting membrane potential (mV)
g
Action potential threshold (mV)
h
Input resistance (MOhm)

Figure S5

Fig. S5: Analysis of CA1 pyramidal neurons morphology. a Increased filament length of apical ($p=0.0289$), but not basal ($p=0.9143$) dendrites in adult mutant animals (Mann-Whitney test, n=8 controls and 7 mutants for apical, n=6 controls and 4 mutants for basal dendrites). **b** No changes detected in the total number of branches ($p=0.2440$ for apical and 0.5333 for basal dendrites, Mann-Whitney test). **c** No changes detected in complexity (number of intersections) of basal dendrites (n=6 controls and 4 mutants, $F(1, 7)=0.1609$; $p=0.7003$ for genotype, RM Two-way ANOVA). **d** Summary plot of mEPSC amplitude and frequency estimated from individual CA1 pyramidal neurons from control and mutant mice. No statistically significant difference was observed between the two groups ($p=0.1246$ and 0.5955, respectively; n = 16 and 10 in controls and mutants, Mann-Whitey test). **e** *Left*: Representative current-clamp traces showing changes in the membrane potential of CA1 pyramidal neurons in response to somatic current injection steps of increasing amplitude (-90 pA,160 pA,170 pA and 290 pA) in both control and mutant mice. *Right*: Firing frequency as function of injected current showing no differences between control and mutant mice ($F(1, 20)=2.418$; $p=0.1356$ for genotype, RM two-way ANOVA). **f** Summary plot of resting membrane potential showing no differences between control and mutant mice ($p=0.9405$, Mann-Whitney test). **g** Same as F but for action potential threshold. Values were not statistically different between the two groups ($p=0.6585$, Mann-Whitney test). **h** Same as F but for input resistance ($p=0.2202$, Mann-Whitney test). Asterisks indicate statistical significance (*, $p<0.05$). All data are presented as Mean ± SEM.

a



b **Adult females**

Mouse Strain	Seizure incidence	Duration (min)	Mortality	Latency (min)
Control	2/7	0.71±0.47	0/7 0%	90.43±19.13
Mutant	6/7*	23.00±7.60 *	3/7 42.86%	27.29±15.74*

c **Juvenile females**

Mouse Strain	Seizure incidence	Duration (min)	Mortality	Latency (min)
Control	5/5	18.65±5.92	3/5 60%	13.40±3.32
Mutant	6/7	14.79±5.13	4/7 57.14%	29.79±15.14

Figure S6

Fig. S6: Survival of CRs increases susceptibility to develop tonic-clonic seizures upon kainate injection in adult female mice. a Example of electroencephalographic recording in hippocampus and parietal cortex (Cortex) in control and mutant juvenile mice**. b** Results of kainate injection in adult female mice. Seizure incidence, duration, latency but not mortality, were increased in mutant females compared to littermate controls (p=0.0306, 0.0064, 0.0361 and 0.055 respectively; Mann Whitney test). **c** No change in any analyzed parameter was detected for juvenile females (p=0.4241 for seizure incidence; p=0.7866 for duration; p>0.9999 for mortality and p=0.4571 for latency, Mann Whitney test). Asterisks indicate statistical significance (*, $p<0.05$). Values are expressed as Mean ± SEM.