Table 2 Known and putative autism genes (organized by pathogenesis)

Protein name (function)	Gene symbol/locus	Test availability
Neuronal cell adhesion and/or synapse function		
Neuroligin 3 (synapse formation and function)	*NLGN3X* Xq28	Clinical
Neuroligin 4 (synapse formation and function)	*NLGN4X* Xp22.33	Clinical
Neurexin 1 (transsynaptic binding partner for neuroligins)	*NRXN1* 2p16.3	Research
SH3 and multiple ankyrin repeat domains (organizes post synaptic density and binds neuroligins)	*SHANK3* 22q13	Research
Contactin-associated protein-like 2 (synaptic binding partner for contactin molecules involved in neuronal migration)	*CNTNAP2* 7q36	Research
Contactin 4 and Contactin 3 (neuronally expressed adhesion molecules)	*CNTN4* and *CNTN3* 6p26-p25	Research
Protocadherin 10 (a cadherin-related neuronal receptor: may play a role in the establishment and function of specific cell-cell connections; essential for normal forebrain axon outgrowth)	*PCDH10* 4q28	Research
Neuronal cell adhesion molecule	*NRCAM* 7q31	Research
Neuronal activity regulation		
Methyl CpG-binding protein 1 (CAN methylation-dependent transcriptional repressor)	*MECP2* Xq28	Clinical
Ubiquitin protein ligase E3A	*UBE3A* 15q11-q13	Clinical
Deleted in autism	*DIA1* (c3orf58) 3q	Research
Ataxin 2-binding protein 1	*A2BP1* 16p13	Research
Neurodevelopmental genes		
Engrailed 2 (homeobox gene involved in midbrain and cerebellum development)	*EN2* 7q36	Research
Homeobox A1 (involved in hindbrain development)	*HOXA1* 17p15.3	Clinical
Homeobox B1 (involved in hindbrain development)	*HOXB1* 17q21-q22	Research
Reelin (signaling protein involved in neuron migration)	*RELN* 7q22	Research
WENT2 (signaling proteins involved in embryonic patterning, cell proliferation, and cell determination)	*WNT2* 7q31	Research
FOXP2 (transcription factor involved in embryogenesis and neural functioning)	*FOXP2* 7q31	Research
ARX homeobox gene	*ARX* Xp22.13	Clinical
Patched domain containing 1 gene	*PTCHD1* Xp22.11	Research
Sodium channel		
Sodium channel, voltage-gated, type VII	*SCN7A* 2q	Research
Na+/H+ exchanger isoform 9	*SLC9A9* (*NHE9*) 3q24	Research
Calcium channel		
Calcium channel, voltage-dependent, L type, alpha 1C subunit (Timothy syndrome)	*CACNA1C* 12p13.3	Clinical
Calcium channel, voltage-dependent, alpha 1H subunit	*CACNA1H* 16p13.3	Research
Calcium channel, voltage-dependent, L type, alpha 1F subunit	*CACNA1F* Xp11.23	Clinical
Neurotransmitter genes		
GABA receptor subunits (major inhibitory transmitter receptors in the brain)	*GABRB3, GABRA5, GABRG3* 15q11.2-q12	Research
Serotonin transporter	*SLC6A4* 17q11.1-q12	Clinical
Mitochondrial		
Mitochondrial aspartate/glutamate transporter (mitochondrial function and maintaining ATP levels)	*SLC25A12* 2q24	Research
Other genes		
Oxytocin receptor	*OXTR* 3p26.2	Research
Laminin beta 1	*LAMB1* 7q31.1	Research
RING finger protein 8 (ubiquitin ligase and transcriptional coactivator)	*RNF8* 6p21.3	Research

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Behavioural phenotyping assays for mouse models of autism

Jill L. Silverman, Mu Yang*, Catherine Lord‡ and Jacqueline N. Crawley**

Face validity = strong analogies to the endophenotypes of the human syndrome

Construct validity = the same biological dysfunction that causes the human disease, such as a gene mutation or anatomical abnormality

Predictive validity = analogous response to treatments that prevent or reverse symptoms in the human disease

Mouse model	Genetic characteristics	Behavioural phenotypes relevant to the symptoms of autism*
Nlgn4	Null mutation in the murine orthologue of the human *NLGN4* gene[43]	• Reduced reciprocal social interactions[43] • Low sociability[43] • Lack of preference for social novelty[43] • Reduced ultrasonic vocalizations[43]
Nlgn3	Homozygous mutation of humanized R451C mutation of the *Nlgn3* gene[44,45]	• No genotype differences in reciprocal social interactions[44,45] • No genotype differences in sociability[44,45] • No genotype differences in preference for social novelty[44] • Reduced ultrasonic vocalizations[44]
	Null mutation in the murine orthologue of the human *NLGN3* gene[41]	• No genotype differences in reciprocal social interactions[41] • Reduced preference for social novelty[41]
Neurexin 1α	Null mutation in the murine *neurexin 1α* generated by deleting the first exon of the gene[46]	• No genotype differences in reciprocal social interactions[46] • No genotype differences in sociability[46] • Impaired nest-building behaviour[46] • Increased repetitive self-grooming[46]
Nlgn1	Null mutation in the murine orthologue of the human *NLGN1* gene[47]	• No genotype differences in reciprocal social interactions[47] • No genotype differences in sociability[47] • No genotype differences in preference for social novelty[47] • Impaired nest-building behaviour[47]
Pten	Conditional null mutation, inactivated in neurons of the cortex and hippocampus, mouse orthologue of the human *PTEN* gene[48]	• Reduced reciprocal social interactions[48] • Low sociability[48] • Impaired nest-building behaviour[48] • Impaired social recognition[48]
	Pten haploinsufficent mutant line in which exon 5, and thus the core catalytic phosphatase domain, is deleted[48]	• Low sociability in females[48]
En2	Null mutation in the murine orthologue of the human *EN2* gene[49,50]	• Reduced reciprocal social interactions[49] • Increased repetitive self-grooming[49] • No genotype differences in sociability, confounded by low activity levels[50]
15q11–13	Duplication in the genomic region on the mouse chromosome 7 homologous to the human genomic region 15q11–13 (REF. 29)	• Low sociability[29] • Ultrasonic vocalizations elevated in pups and reduced in adults[29] • Impaired reversal learning[29]
17p11.2	Duplication in the genomic region of murine chromosome 11 homologous to the human genomic region 17p11.2 (REF. 51)	• Low sociability[51] • No genotype differences in preference for social novelty[51] • Impaired nest-building behaviour[51]
Gabrb3#	Null mutation in the murine orthologue of the human *GABRB3* gene[52]	• Low sociability# (REF. 52) • Lack of preference for social novelty# (REF. 52) • Repetitive stereotyped circling patterns# (REF. 52) • Impaired nest-building behaviour# (REF. 52)
Slc6a4	Null mutation in the murine orthologue of the human serotonin transporter (*SLC6A4*) gene[50]	• Low sociability[50] • Lack of preference for social novelty[50]
	Haploinsufficient mutant line of the human serotonin transporter *SLC6A* gene[48]	• Impaired social recognition[48]
Oxt	Null mutation in the murine *Oxt* gene generated by either a deletion in the first exon[40,53,54] or by deletions in the last two exons[40]	• Impaired social recognition[53] • Reduced pup ultrasonic vocalizations[54] • No genotype differences in sociability[40] • No genotype differences in preference for social novelty[40]
Avpr1b	Null mutation of the murine vasopressin receptor 1b *Avpr1b* gene[55,56]	• Impaired social recognition[55] • Reduced pup ultrasonic vocalizations[56]
Mecp2	Heterozygous mutation in methyl-CpG-binding protein 2 (REFS 39,57,58,59)	• Hindlimb clasping[57,58] • Social avoidance[59] • Impaired social recognition[59] • Reduced social interest in an arena[59]
Fmr1	Null mutant mouse with a targeted mutation in the *Fmr1* gene in three genetic backgrounds: C57BL/6J[38,50,60,61]; hybrid of FVB/NJ x C57BL/6J[62]; and FVB/N-129/OlaHsd[50]	• Increased social approach[60,61] • Reduced reciprocal social interactions[38] • No genotype differences in sociability[62] • No genotype differences in preference for social novelty[62] • Low sociability dependent on genetic background[50] • No genotype differences in preference for social novelty[50]
Tsc	Heterozygous mutation that replaces the second exon in the *Tsc2* gene[63]	• No genotype differences in sociability[63]
	Heterozygous mutation generated by replacing exons 6–8 in the *Tsc1* gene[65]	• Reduced reciprocal social interactions[65] • Impaired nest-building behaviour[65]

- Repetitive behaviors
- Excess self grooming
- Novelty preferences
- Executive function/attention
- Memory
- Perseveration/flexibility
- Anxiety/exploration/thigmotaxis

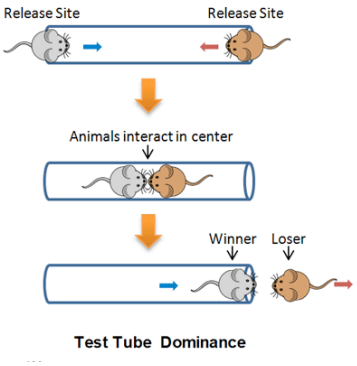
Treatment	Mouse model	Phenotypic improvement
mGluR antagonists, MPEP[88,161,167], fenobam[162]	*Fmr1*$^{-/-}$	• Susceptibility to audiogenic seizures is prevented[161] • Decreased open field hyperactivity[161] • Rescued prepulse inhibition of startle deficit[162] • Rescued abnormal spine morphology[162]
	BTBR	• Reduced repetitive behaviour[88]
mTOR inhibitors, rapamycin[63,77,177,178], RAD001 (REF. 177)	*Pten*	• Prevented and reversed macrocephaly, dendritic and axonal hypertrophy[77] • Improved social interaction time[77] • Increased open field centre time[77] • Reduced duration and frequency of seizures[77]
	Tsc1 null-neuron inactivated in neurons[63,177]	• Improved survival rates[63,177] • Improved neuronal morphology, reduced enlarged neurons and restored myelination[177]
	Tsc1^{GFAP} inactivated in glia[178]	• Improved survival rates and weight gain[178] • Prevented seizures and electroencephalography (EEG) abnormalities[178]
	Tsc2$^{+/-}$ (REF. 63)	• Improved learning and memory on Morris water maze and fear conditioning[63]
Oxytocin[114]	*OXT*$^{-/-}$	• Rescued deficits in social recognition[114]
BDNF[75]	*Fmr1*$^{-/-}$	• Rescued long-term potentiation abnormality[75]
Ampakines, CX546 (REF. 73)	*Mecp2*$^{-/-}$	• Reversed respiratory deficits[73]
mGluR genetic reduction[74]	*Fmr1*$^{-/-}$	• Prevented susceptibility to audiogenic seizures[74] • Rescued abnormal spine morphology[74] • Rescue of exaggerated inhibitory avoidance learning[74]
FMR1 gene replacement[60,61,76]	*Fmr1*$^{-/-}$	• Normalized open field activity[60] • Normalized light–dark anxiety-like behaviour[60] • Rescued abnormal social responses[61] • Rescued increased prepulse inhibition[76]
PAK genetic reduction[92]	*Fmr1*$^{-/-}$	• Normalized open field centre time[92] • Rescued fear-conditioning deficit[92] • Rescued long-term potentiation deficit[92]
MECP2 gene replacement[174,176]	*Mecp2*$^{-/+}$ is an inducible heterozygous transgenic[176]	• Rescued open field deficits[176] • Increased survival and lifespan[174]
	Mecp2/Stop is an *Mecp2* mutant with *Mecp2* conditional activation[174]	• Normalized weights, breathing, gait and activity[174]

Fmr1 KO Mice as a Possible Model of Autistic Features

Maude Bernardet* and Wim E. Crusio

TABLE 1
Phenotypical Checkup of *Fmr1* KO Mice: Behaviors Relevant to Core Symptoms of Autism

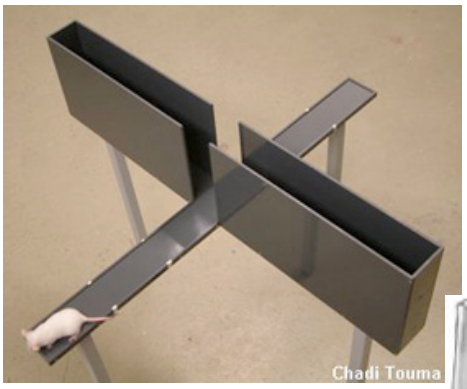
Test	Background	Result	Ref.
Inappropriate social interactions			
Mirrored chamber test	B6	KO < WT for % time in the mirrored chamber	[94]
Tube test of social dominance	B6	KO < WT vs. unfamiliar WT the first time KO = WT vs. unfamiliar WT the third day KO = WT vs. familiar WT	[94]
Social interaction test	B6	KO vs. WT: Active social behavior: KO > WT Passive social behavior: KO < WT KO vs. KO, WT vs. WT: Sniffing and receptive behavior: KO > WT KO vs. C3H, WT vs. C3H: KO < WT	[94] [95]
Crawley test	B6	KO = WT	[94]
Influence of cage familiarity on response to unfamiliar social partners	B6	In an unfamiliar cage: KO = WT; in a familiar cage: KO < WT during the first 5 min, KO > WT after 20 min	[94]
Perseverance			
Water maze reversal learning:			
Hidden-platform condition	B6	KO = WT	[97,98]
	B6	Escape latencies: KO > WT	[82,89,96]
	B6	Path length: KO > WT	[96]
	B6	Number of trials: KO > WT	[98]
	B6	Rate of learning: KO = WT, KO>WT	 [96] [89]
Visible-platform condition	B6	Escape latencies: KO > WT KO = WT	 [96] [82]
E-shaped water maze reversal learning	B6	KO = WT	[89]
Plus-shaped water maze reversal learning	B6	Escape latencies: KO = WT, but rate of learning: KO < WT	[98]



Test Tube Dominance

TABLE 2
Phenotypical Checkup of *Fmr1* KO Mice: Behaviors Relevant to Variable Symptoms of Autism

Test	Background	Result	Ref.
Anxiety			
Elevated plus maze	FVB	KO = WT	[100]
	B6	KO = WT	[91,107]
	FVBxB6	KO = WT	[107]
	FVBxB6	KO less anxious than WT	[83]
Thigmotaxis in open-field	B6	KO < WT	[94,101]
	FVBxB6	KO < WT	[83]
Boli in open-field	B6	KO < WT	[94]
Light-dark exploration	B6	Transitions between compartments: KO > WT Time spent in both compartments: KO = WT	[82,101]
Corticosterone response to acute stress	B6	Males: Sham and 15 min: KO = WT 0 min: KO < WT 60 min: KO > WT Females: Sham, 0 and 60 min: KO = WT 15 min: KO < WT	[104]
	B6	Males: No stress, 30 min stress: KO = WT 2 h stress: KO > WT	[103]
Conditioned emotional response	B6	KO = WT	[98]
Learning and memory			
Cross-shaped water maze	FVB	Correct trials: KO < WT	[102]
	B6	Escape latencies: KO = WT	[98]
		Correct trials: KO < WT	[98]
		KO = WT	[102]
Changing position of platform in water maze	B6	KO = WT	[97,98]
E-shaped water maze	B6	KO = WT	[89]
Morris water maze training: Hidden-platform condition	B6	Escape latencies: KO = WT	[96,97,101]
		KO > WT	[89]
		KO > WT the first four trials	[82]
	FVBxB6	Escape latencies: KO > WT	[83]
	B6	Rate of learning: KO = WT	[82,89,102]
	FVB	Rate of learning: KO < WT	[102]
Visible-platform condition	B6	Escape latencies: KO = WT	[82,96]
Radial maze	B6	Working memory: KO = WT	[91]
	FVBxB6	Working memory: KO < WT the first 6 days; reference memory: KO < WT; strong choice design: KO = WT	[83]
Barnes maze	FVBxB6	KO = WT; during probe test: KO < WT	[83]
Fear conditioning: context and conditioned cue	FVB	KO = WT	[102]
	B6	KO = WT	[98,101,102]
	B6	KO < WT	[97]
Trace fear conditioning	B6	KO < WT	[100]
Conditioned eyelid blink reflex	B6	KO < WT	[109]



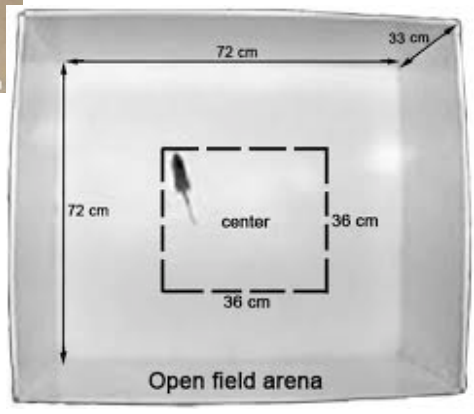


TABLE 2 (continued)

Test	Background	Result	Ref.
Learning and memory (continued)			
Passive avoidance (latency to enter dark compartment)	B6	KO = WT	[82]
	FVB	KO = WT	[108]
Lever press escape/avoidance task	B6	KO < WT	[113]
Instrumental conditioning	B6	Conditioning learning : KO = WT	[73]
		Devaluation of reward and omission of lever press : KO > WT	
Olfactory learning and memory tasks	FVBxB6	KO = WT	[83]
Novel object task	FVBxB6	KO = WT	[83]
	FVB	KO < WT	[114]
Motor abilities			
Rotarod motor coordination and balance	B6	KO = WT	[101]
Aggression			
Neutral cage aggression test	B6	KO = WT	[91]
Hyperactivity			
Open field activity	B6	KO > WT	[91,94,101]
	B6	KO = WT	
	FVBxB6	KO = WT	[107]
	FVB	KO = WT	[107]
	FVB	KO = WT before 18 min	[100]
		KO > WT after 18 min	[108]
Activity cage	FVB	KO > WT	[114]
Motor activity test	B6	KO > WT	[82]
Idiosyncratic responses to sensory stimuli			
Auditory startle response	B6	KO = WT, but increased response with Fmr1gene containing YAC	[101]
	B6	KO > WT at 70 and 80 dB; KO < WT at 120 dB	[107]
	B6	KO < WT at higher intensities, interaction between genotype and intensity	[73]
	FVB	KO < WT	[110]
	FVB	KO = WT under 110 dB; KO< WT from 110 dB and above	[108]
	FVBxB6	KO > WT at 80 dB; KO < WT at 100, 110, and 120 dB	[83]
	FVBxB6	KO = WT	[83]
Prepulse inhibition	B6	KO > WT	[73]
	B6	KO > WT at 67 dB (2 dB above background noise)	[107]
	FVB	KO > WT	[110]
Audiogenic seizures (AS)	FVB	KO after long loud sound and after age 10 weeks	[110]
		KO >> WT (143 ± 5 days)	[115]
		KO >> WT (45 days and under)	[108]
	B6 and FVBxB6	KO display AS, WT do not (21 days)	[83]
	FVB	KO >> WT (30 days)	[83]
Hot plate and tail-flick test	FVB	KO = WT	[100]

Shank mutant mice as an animal model of autism

Juyoun Yoo, Joseph Bakes, Clarrisa Bradley, Graham L. Collingridge and Bong-Kiun Kaang

The Shank family of scaffolding proteins (also known as ProSAP, cortBP, SSTRIP, Synamon and Spank) consists of three major isoforms—Shank1, Shank2 and Shank3—all of which are present in the brain, though with very different patterns of expression. Shank1 is expressed throughout most of the brain, except the striatum, being particularly highly expressed in the cortex and the hippocampus. Shank2 and 3 are also present in the cortex and hippocampus. Shank2 is almost absent in the thalamus and striatum, while Shank3 seems to be dominantly expressed in those regions. In the cerebellum, Shank2 is restricted to Purkinje cells, while Shank3 is restricted to granule cells [48].

In conclusion, the genetics of ASD have led to a focus on the glutamatergic synapse. Based on available evidence there does not appear to be a single causal deficit but rather various alterations in pre- and postsynaptic function, including changes in synaptic plasticity. Perhaps the most exciting results to emerge are the findings that, in mouse models, it is possible to reverse behavioural and physiological deficits with pharmacological treatments, indicating that the underlying cause may not necessarily be an irreversible developmental abnormality but rather an ongoing synaptopathy. Thus, a greater understanding of the glutamatergic synapse in rodent models of autism should aid in the development of effective therapies for ASD.

Induced chromosome deletions cause hypersociability and other features of Williams–Beuren syndrome in mice

Hong Hua Li[1], Madhuri Roy[2†], Unsal Kuscuoglu[1†], Corinne M. Spencer[3†], Birgit Halm[1], Katharine C. Harrison[1], Joseph H. Bayle[4], Alessandra Splendore[1], Feng Ding[1], Leslie A. Meltzer[2], Elena Wright[1], Richard Paylor[3], Karl Deisseroth[2,5], Uta Francke[1,6]*

The neurodevelopmental disorder Williams–Beuren syndrome is caused by spontaneous ~1.5 Mb deletions comprising 25 genes on human chromosome 7q11.23. To functionally dissect the deletion and identify dosage-sensitive genes, we created two half-deletions of the conserved syntenic region on mouse chromosome 5G2. Proximal deletion (PD) mice lack *Gtf2i* to *Limk1*, distal deletion (DD) mice lack *Limk1* to *Fkbp6*, and the double heterozygotes (D/P) model the complete human deletion. Gene transcript

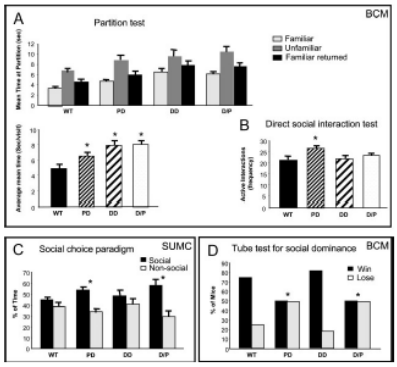


Figure 5. Deletion mice have abnormal social behaviour.

A. Partition test measures the time a test mouse spends at the partition that separates it from a partner mouse during three sessions: with a familiar partner, an unfamiliar partner and finally the familiar partner returned. The mean time at the partition per approach to the partition is shown for each test (top panel). The mean time per approach averaged across all three tests (bottom panel) shows that PD, DD and D/P mice exhibited greater social interest at the partition than WT. $N = 12–17$ per genotype, both sexes.

B. In a direct social interaction test, only PD mice showed an increased frequency of interactions during the 10 min test period. $N = 12–17$ per genotype.

C. In the social choice test, mice are placed in a three-chambered apparatus with a stimulus mouse in one chamber, and the percentage of time spent in the 'social' versus the 'non-social' chambers is recorded. Male PD and D/P demonstrated increased sociability. $N = 8–10$ per genotype.

D. Abnormal social dominance behaviour of PD and D/P in a tube test. Tested mice are released into a tube against a control mouse. The one who backs out of the tube first is considered the loser. $N = 12–17$ per genotype.

Growth Defects and Impaired Cognitive–Behavioral Abilities in Mice with Knockout for *Eif4h*, a Gene Located in the Mouse Homolog of the Williams-Beuren Syndrome Critical Region

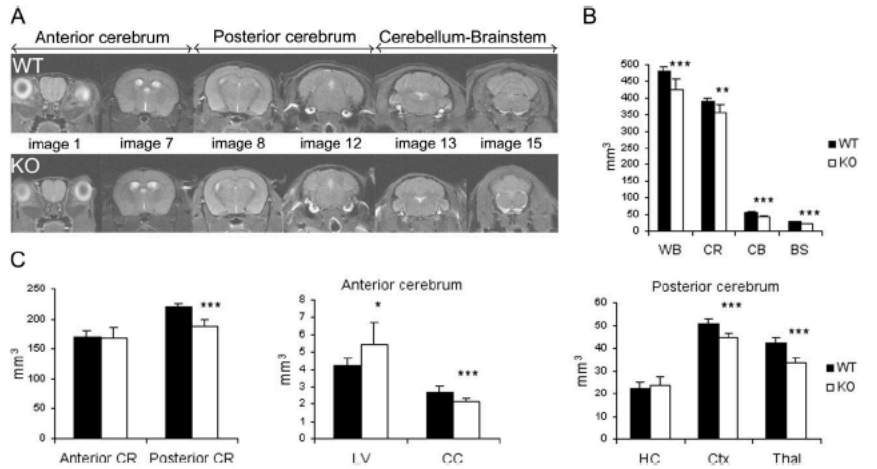


Figure 5. Brain MRI analysis. **A:** Typical T2-weighted images from wild-type (WT; **upper panel**) and *Etl4b* knockout mice (KO; **lower panel**). Fifteen contiguous coronal sections were acquired with a 0.85-mm thickness. Volume of anterior cerebrum was calculated from the first to the seventh image, volume of posterior cerebrum from image 8 to 12 and volume of cerebellum and brainstem from image 13 to 15. **B:** Volume measurement of whole brain and selected regions: WB, whole brain; CR, cerebrum; CB, cerebellum; BS, brain stem. $^{**}P < 0.01$; $^{***}P < 0.001$. **C:** Volume measurements of the anterior and posterior cerebrum and respective selected structure: LV, lateral ventricles; CC, corpus callosum; HC, hippocampus; Ctx, cortex; Thal, thalamic-hypothalamic nuclei. $^{*}P < 0.05$; $^{***}P < 0.001$. ($n = 7$ WT, 9 KO). Error bars represent SD. The statistical analysis was performed using the Student's *t*-test. Values of $P < 0.05$ were considered significant.

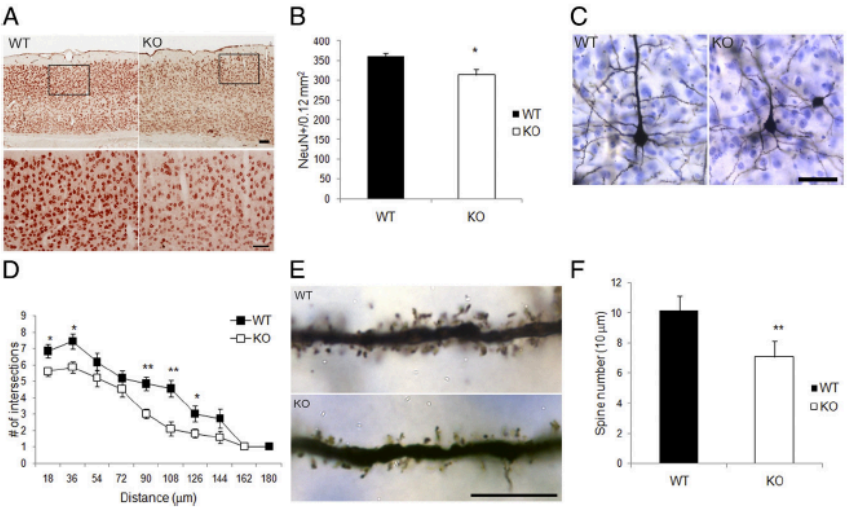


Figure 6. Immunohistological and morphological characterization of *Etl4b* knockout brains. **A:** NeuN immunostaining on representative sections of the posterior cortex of knockout (KO) and wild-type (WT) brains. Original magnification ×4, **upper** part; ×20, of the **lower** part (from boxed area above). **B:** NeuN^{+} cells were counted on four images of 0.12 mm^2 ($n = 3$ WT, 3 KO). $^{*}P < 0.05$. **C:** Example of Golgi-stained cortical neuron (original magnification ×20). **D:** Sholl analysis illustrating differences in dendritic complexity. The values are the average of 12 neurons for each genotype ($n = 3$ WT, 3 KO). $^{*}P < 0.05$, $^{**}P < 0.01$. **E:** Golgi preparation showing dendrites from WT and KO (original magnification ×100). **F:** Spine density >10 μm length. The values are the average of 15 dendrites for each genotype ($n = 3$ WT, 3 KO). $^{**}P < 0.01$. Error bars represent SEM. The statistical analysis was performed using the Student's *t*-test. Values of $P < 0.05$ were considered significant. Scale bars = 50 μm (**A** and **C**) and 10 μm (**E**).

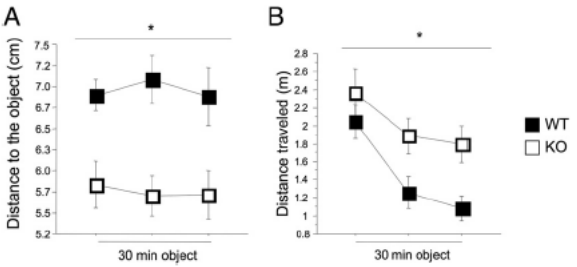


Figure 8. Novelty test. **A:** Distance to the object of wild-type (WT) and knockout (KO) mice ($n = 20$ WT, 20 KO). *$P < 0.05$. **B:** Locomotor activity: distance traveled when the novel object was introduced in the center of the arena. *$P < 0.05$. Statistical computations were done using Statview 5.0. Values of $P < 0.05$ were considered significant.

Novelty Induced Hyper-Activity

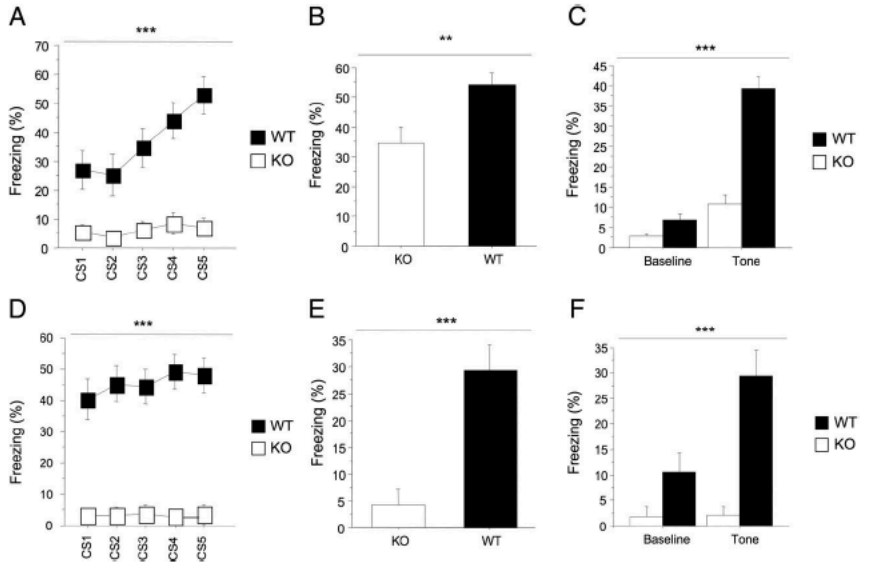


Figure 10. Fear conditioning tasks. Wild-type (WT) and knockout mice (KO) ($n = 20$ WT, 20 KO) were subjected to two different protocols: delay (**A–C**) and trace (**D–F**) fear conditioning. **A** and **D:** Percentage of freezing during the training sessions. ***$P < 0.001$. **B** and **E:** Percentage of freezing during the context test, 24 hours later. **$P < 0.01$; ***$P < 0.001$. **C** and **F:** Percentage of freezing during the tone test, 24 hours later. Baseline indicated the period without tone. ***$P < 0.001$. Statistical computations were done using Statview 5.0. Values of $P < 0.05$ were considered significant.