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Supplemental Data

Otud7a Knockout Mice Recapitulate Many Neurological Features of 15q13.3 Microdeletion Syndrome

Jiani Yin, Wu Chen, Eugene S. Chao, Sirena Soriano, Li Wang, Wei Wang, Steven E. Cummock, Huifang Tao, Kaifang Pang, Zhandong Liu, Fred A. Pereira, Rodney C. Samaco, Huda Y. Zoghbi, Mingshan Xue, and Christian P. Schaaf

Material and Methods for Supplementary Figures

Elevated plus maze

Anxiety was assessed, using the elevated plus maze, as described previously [1]. Mice at 10 weeks of age were put at the cross area of the maze in white, facing the open arm. The maze was elevated 50 cm from the floor. Activity data was collected over a 10 min period, using the Fusion software (Omnitech electronics) version 4.75.

Open field activity

Locomotor activity and anxiety level were assessed at 10 weeks of age, using the open field assay, as described previously [1]. Activity in a clear acrylic (40 cm × 40 cm × 30 cm) open field arena was recorded over a 30 min period, using the Fusion software version 3.7.

Light-dark box exploration

Anxiety was also assessed in the light-dark box at 10 weeks of age. The apparatus is composed of two adjoining chambers made of plexiglas: a small enclosed chamber (15 cm × 21 cm × 21 cm) with black walls, and a larger chamber (30 cm × 21 cm × 21 cm) with transparent walls and an open top. The two chambers were connected by a small opening. Mice were placed into the illuminated (750 lux) larger chamber and allowed to explore freely for 10 min. Activity data, including the number and latency of entries, and time spent in each compartment, was collected using the Fusion software version 3.7.

Self-grooming

The self-grooming test was used to evaluate compulsive grooming behaviors, as described previously [1]. Each mouse was placed individually into a standard mouse cage with a thin layer of bedding, habituated for at least 30 min, and was then videotaped for

10 min. Time spent on spontaneous grooming of any part of its face, body, limbs, or tail was quantified and subsequently analyzed.

Holeboard exploration

The holeboard exploration test was used to evaluate repetitive nose-poke behavior. Mice at 10 weeks of age were placed into a clear acrylic (40 cm × 40 cm × 30 cm) chamber with a black 16-hole floorboard. Holeboard exploration data was collected, using the Fusion software version 7.7. The number of total and sequential nose-pokes in a 10 min period was quantified.

Forced swimming test

Depression-related behavior was assessed, using the forced swimming test, as previously described [1]. Mice at 12 weeks of age were placed into a 22 cm diameter circular tank with 17 cm deep water at room temperature for 6 min. Immobility time was defined as the duration in which the percentage of immobility was greater than 88% during any 500 msec period. This was automatically determined using the ANY-Maze Video Tracking System version 4.75 (Stoelting Co., IL).

Three-chamber test

Sociability was assessed at 12 weeks of age, using the three-chamber test, as described previously [1]. After a 10 min habituation period, a sex- and age-matched C57BL/6J mouse was placed under one wire cup, and a lego object of similar size and color was placed under the wire cup in the opposite compartment. Test mice were then allowed to explore freely for another 10 min. Data of time spent in each compartment, and the amount of time spent in close contact with each wire cup in the two phases were determined, using

ANY-Maze version 4.75 and manual scoring. Due to seizure occurrence during the test period, one male null mouse was excluded from data analysis of three-chamber test.

Partition test

Interest in social novelty was assessed using the partition test, as described previously [1]. At 12 weeks of age, each test mouse was housed overnight with an age- and sex-matched C57BL/6J partner mouse in the two separate compartments of a partition cage. The next day, activity at the partition board was measured, first with the familiar overnight partner, followed by an unfamiliar partner, and then back to the original familiar partner, for 5 min each. This was manually scored using a Psion Handheld Computer and Observer XT (Noldus Information Technology, Netherlands).

Conditioned fear

Conditioned fear was used to evaluate learning and memory at 13 weeks of age. On the training day, mice were placed into the isolation cubicles (Coulbourn Instruments, PA) and allowed to explore for 2 min. The conditioned stimulus (CS, a white noise 80 dB sound) was then presented for 30 sec and was followed immediately by a mild foot shock (2 sec, 0.7 mA) that served as the unconditioned stimulus (US). The conditioning pattern (2 min rest followed by 30 sec sound stimulus that was paired with foot shock) was then repeated once. Mice were then returned to their homecages. Timing of CS and US presentations and percentage freezing were monitored and assessed using the freezeframe software (Coulbourn instruments, PA).

Approximately 24h after training, mice were placed back into the original chamber to test for contextual fear conditioning. Freezing was recorded over a 5 min period. One hour later, mice were placed into a new chamber with different contextual cues to test for

auditory cued conditioned fear. White Plexiglas inserts were placed on both the floor and sides of the chamber to alter the shape, texture, and color of the chamber, and vanilla extract was placed in the chamber behind the insert to alter the odor. In addition, light condition in the testing room was changed to dim red light. Mice were brought into the room in transfer cages that contained paper towels instead of bedding, and then placed into the new chamber. Freezing was recorded for 3 min during a "pre-CS" phase, followed by another 3 min while the auditory CS was presented.

Nest building

Nest building was evaluated at 14 weeks of age to test for home cage social behaviors, as described previously [2]. Mice were single-housed after the conditioned fear test and a nestlet (5 cm squares of cotton batting) was placed into the cage around 6:00 PM. The following day between 10-11 AM, nest quality was scored on a 5-point scale by an experimenter who was blind to the genotypes, based on the shape and height of the nests [2].

Novel object recognition

Novel object recognition was used to evaluate the memory of mice at 27 weeks of age. The habituation chamber and test chamber were both transparent plastic chambers (40cm x 24 cm x 20 cm) with no top. The test chamber was surrounded by white boards on three sides (all but the side that faced the observer), with white paper on the bottom. At the side farthest from the observer, three mirrors were placed to assist observation and scoring. Two identical Lego objects for training were placed in the test chamber symmetrically, 5 cm away from the center. Mice were first habituated in the empty chamber for 5 min, and then transferred to the test chamber, in which they were allowed to explore for 5 min. The

test chamber was cleaned with 30% isopropanol between each mouse, to dissipate the odors. Mice were trained for three days. On the fourth day, we tested the mice by replacing one of the objects in the test chamber with a novel Lego object. As a control, on the fifth day, mice were presented the same objects from the training sessions. On all days, the time that mice interacted with each object was manually recorded.

Auditory Testing

Auditory Brainstem Responses (ABRs) and Distortion Product of the Otoacoustic Emissions (DPOAEs) were measured as previously described [3; 4]. Briefly, 3 month-old mice, 4 WT (♂), 4 HET (♂), 9 KO (♂), 3 WT (♀), 4 HET (♀), and 6 KO (♀), were anesthetized using an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Normal body temperature was maintained throughout the procedure by placing the mice on a heating pad. Pure tone stimuli from 4 kHz to 48 kHz (ABRs) or 8 kHz to 32 kHz (DPOAEs) were generated using Tucker Davis Technologies System 3 digital signal processing hardware and software (Tucker Davis Technologies, Alachua, FL, USA), and the intensity of the tone stimuli was calibrated using a type 4938 1/4″ pressure-field calibration microphone (Brüel and Kjær, Nærum, Denmark). ABR signals were recorded with subcutaneous needle electrodes inserted at the vertex of the scalp, the postauricular region (reference) and the back leg (ground) and the DPOAE distortion-products were captured with a microphone and preamplifier (ER-10B+, Etymotic Research, Inc.). Auditory thresholds were determined by decreasing the sound intensity of each stimulus in 5 dB steps (90 dB to 10 dB for ABRs; 75 dB to 40 dB for DPOAEs) until the lowest sound intensity with reproducible and recognizable ABR waveforms or

2f1-f2 distortion-products was reached. Statistical analysis was performed using Three-way ANOVA with repeated measures.

HeLa cell culture and transfection

HeLa cells were ordered from American Type Culture Collection (ATCC) and cultured in DMEM (Thermo Fisher Scientific, Ca. 11330032) containing 10% FBS and 1% Penicillin/Streptomycin. The cells were tested to be mycoplasma free. Cells were around 90% confluent at the time of transfection. For cells in a 12-well plate, 75 µl Opti-MEM (Invitrogen, Ca. 31985-070) and 3µl Lipofectamine 2000 were mixed and incubated for 5 min. At the same time, 75 µl of Opti-MEM and 400 ng of each plasmid were mixed and incubated for 5 min. Then, the DNA mixture was added to the Lipofectamine mixture and incubated for 20 min, prior to adding them to the cells.

Sholl analysis

Image stacks were imported to the confocal module of Neurolucida 360 (MicroBrightfield, Inc., Williston, VT), and neuronal dendritic trees were traced in interactive mode. Sholl analysis was performed for each traced neuron by automatically calculating the number of dendritic intersections and the dendritic length at 10-µm interval starting from the soma. Soma area was determined by manually defined soma contours and automatic detection of area over a pre-set thickness threshold. Primary neuronal culture and Sholl analysis have been repeated three times. In total, we investigated 27 primary neurons from wild-type and 26 primary neurons from homozygous knockout mice on DIV 14. Data were analyzed blindly to the genotypes. Statistical analysis was performed using Two-way ANOVA with Tukey's multiple comparisons test.

References

1. Yin, J., Wu, C., Yang, H., Xue, M., and Schaaf, Christian P. (2017). Chrna7 deficient mice manifest no consistent neuropsychiatric and behavioral phenotypes. Scientific reports 7.
2. Deacon, R.M.J. (2006). Assessing nest building in mice. Nat Protocols 1, 1117-1119.
3. Xia, A., Song, Y., Wang, R., Gao, S.S., Clifton, W., Raphael, P., Chao, S.-i., Pereira, F.A., Groves, A.K., and Oghalai, J.S. (2013). Prestin regulation and function in residual outer hair cells after noise-induced hearing loss. PloS one 8, e82602.
4. Cai, T., Seymour, M.L., Zhang, H., Pereira, F.A., and Groves, A.K. (2013). Conditional deletion of Atoh1 reveals distinct critical periods for survival and function of hair cells in the organ of Corti. Journal of Neuroscience 33, 10110-10122.

Figure S1

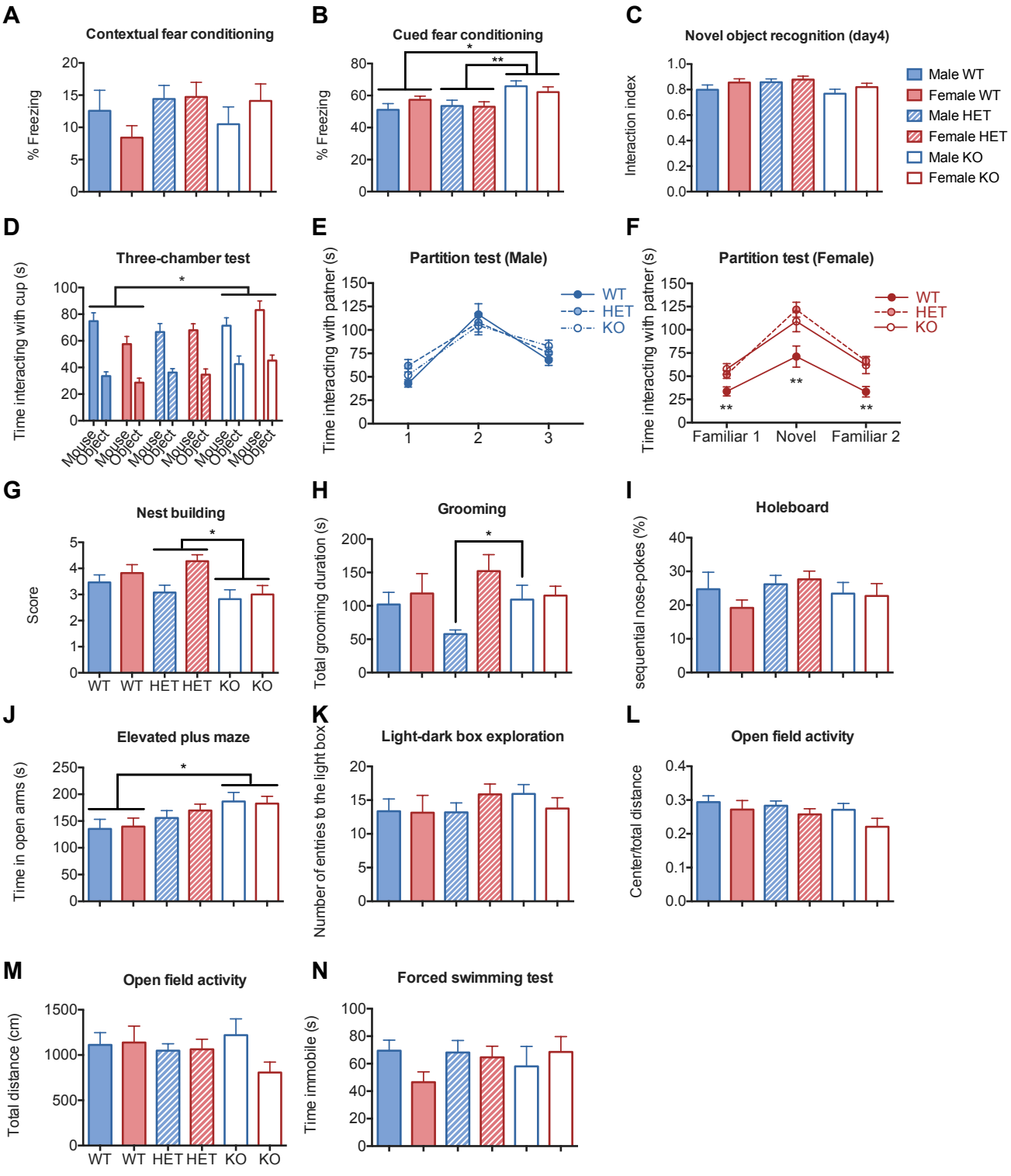
A
Contextual fear conditioning
% Freezing
B
Cued fear conditioning
% Freezing
C
Novel object recognition (day4)
Interaction index
Male WT
Female WT
Male HET
Female HET
Male KO
Female KO
D
Three-chamber test
Time interacting with cup (s)
Mouse
Object
E
Partition test (Male)
Time interacting with patner (s)
WT
HET
KO
F
Partition test (Female)
Time interacting with patner (s)
Familiar 1
Novel
Familiar 2
G
Nest building
Score
WT WT HET HET KO KO
H
Grooming
Total grooming duration (s)
I
Holeboard
sequential nose-pokes (%)
J
Elevated plus maze
Time in open arms (s)
K
Light-dark box exploration
Number of entries to the light box
L
Open field activity
Center/total distance
M
Open field activity
Total distance (cm)
WT WT HET HET KO KO
N
Forced swimming test
Time immobile (s)

Figure S1 Additional behavioral phenotypes of mice deficient in *Otud7a*. Behavioral data from male and female adult mice were shown separately, with males in blue and females in red. **A-B**, Null mice show no impairment in learning and memory in contextual or cued fear conditioning. **C**, Null mice show no significant impairment in novel object recognition. **D**, Null mice do not show reduced sociability in three-chamber test. **E-F**, Neither male or female null mice show reduced social novelty in partition test. **G**, Null mice do not show impairment in nest building compared to wild-type littermates. **H**, Null mice do not show repetitive behavior in self-grooming. **I**, Null mice do not show repetitive behavior in holeboard exploration. **J**, Null mice do not show significant difference in anxiety-like behavior, measured by time in the open arms in elevated plus maze. **K**, Null mice do not show anxiety-like behavior in light-dark box test, measured by number of entries into the light box. **L**, Null mice do not show anxiety-like behavior in open field activity, measured by center/total distance. **M**, Null mice do not show significant difference in open field activity, measured by total distance run. **N**, Null mice do not display depression-like behavior in forced swimming test, measured by percent time immobile. Each point represents the mean ± SEM (*$P < 0.05$, ** $P < 0.01$).

Figure S2

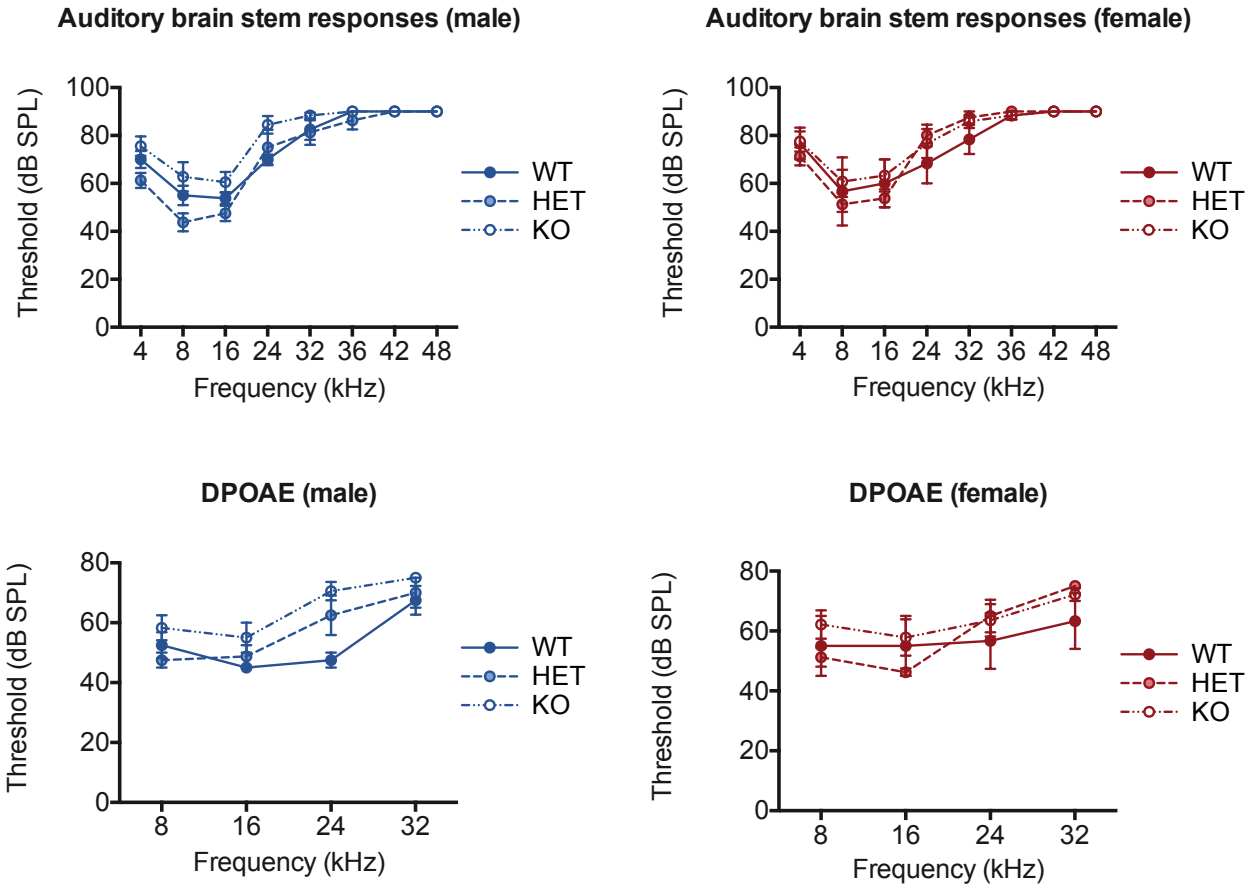
Auditory brain stem responses (male)
Threshold (dB SPL)
100
80
60
40
20
0
4 8 16 24 32 36 42 48
Frequency (kHz)
WT
HET
KO
Auditory brain stem responses (female)
Threshold (dB SPL)
100
80
60
40
20
0
4 8 16 24 32 36 42 48
Frequency (kHz)
WT
HET
KO
DPOAE (male)
Threshold (dB SPL)
80
60
40
20
0
8 16 24 32
Frequency (kHz)
WT
HET
KO
DPOAE (female)
Threshold (dB SPL)
80
60
40
20
0
8 16 24 32
Frequency (kHz)
WT
HET
KO

Figure S2 Hearing ability of *Otud7a* knockout mice is not different from wild-type littermates. Auditory Brainstem Response (ABR) and Distortion Product of Otoacoustic Emissions (DPOAEs) were determined for wild-type and *Otud7a* knockout mice to assess hearing at 3 month-old. Data are presented as mean ± SEM.

Figure S3

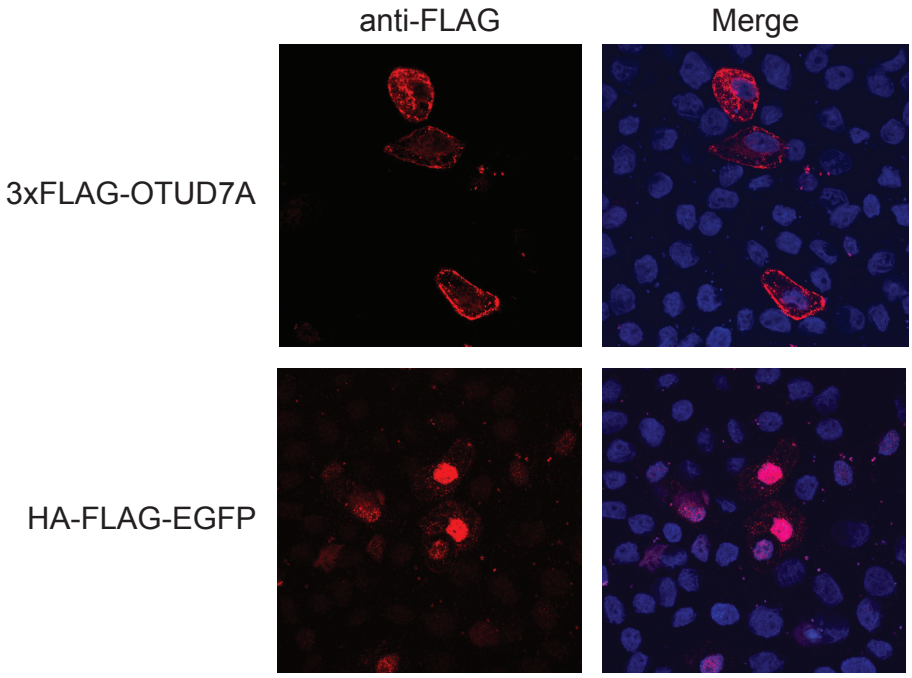

Figure S3 OTUD7A localizes to the membrane compartments in HeLa cells. HeLa cells were ordered from American Type Culture Collection (ATCC) and cultured in DMEM (Thermo Fisher Scientific, Ca. 11330032) containing 10% FBS and 1% Penicillin/Streptomycin. The cells were tested to be mycoplasma free. Cells were around 90% confluent at the time of transfection. For cells in a 12-well plate, 75 µl Opti-MEM (Invitrogen, Ca. 31985-070) and 3µl Lipofectamine 2000 were mixed and incubated for 5 min. At the same time, 75 µl of Opti-MEM and 400 ng of each plasmid were mixed and incubated for 5 min. Then, the DNA mixture was added to the Lipofectamine mixture and incubated for 20 min, prior to adding them to the cells. 3xFLAG-OTUD7A was detected on plasma membrane and other membrane compartments after overexpression in HeLa cells. In the control, HA-FLAG-EGFP localizes to the nucleus and cytoplasm after overexpression in HeLa cells. Over-expressed proteins were stained with FLAG antibody (red), and the nucleus was stained with DAPI (blue).

Figure S4

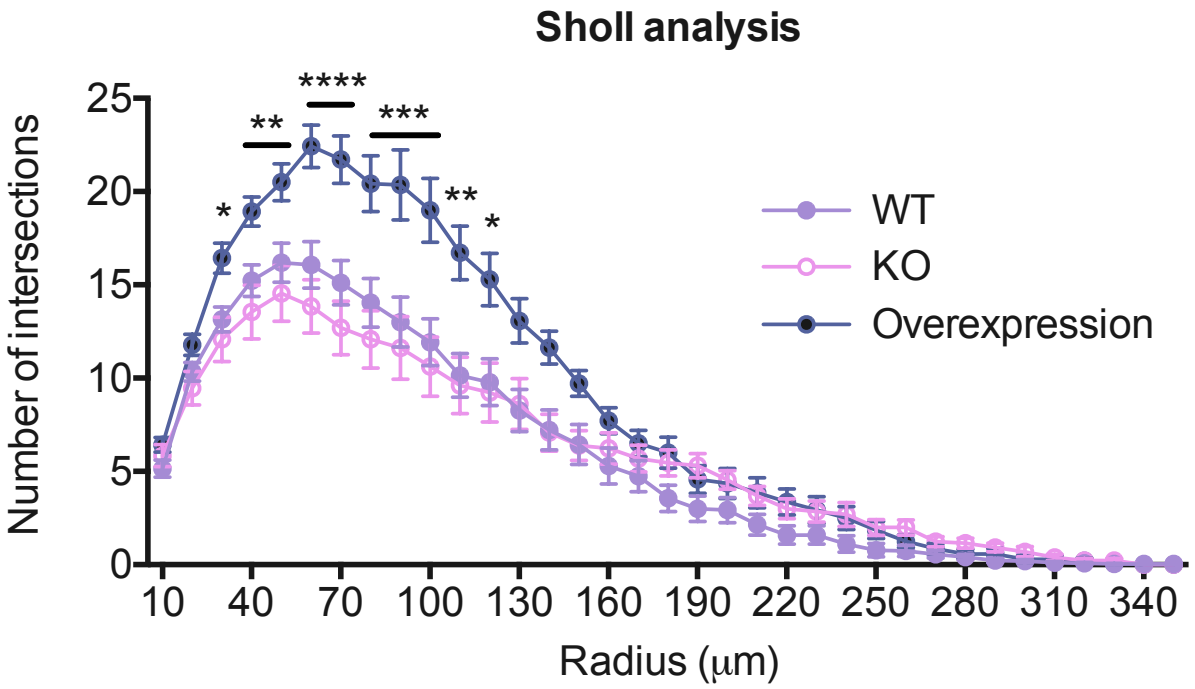
Sholl analysis
Number of intersections
25
20
15
10
5
0
10
40
70
100
130
160
190
220
250
280
310
340
Radius (μm)
WT
KO
Overexpression
*
**
****
***
**
*

Figure S4 Sholl analysis of primary neurons indicates a potential role of Otud7a in dendritic complexity. Image stacks were imported to the confocal module of Neurolucida 360 (MicroBrightfield, Inc., Williston, VT), and neuronal dendritic trees were traced in interactive mode. Sholl analysis was performed for each traced neuron by automatically calculating the number of dendritic intersections and the dendritic length at 10-µm interval starting from the soma. Soma area was determined by manually defined soma contours and automatic detection of area over a pre-set thickness threshold. Primary neuronal culture and Sholl analysis have been repeated three times. In total, we investigated 27 primary neurons from wild-type and 26 primary neurons from homozygous knockout mice on DIV 14. Data were analyzed blindly to the genotypes. Statistical analysis was performed using Two-way ANOVA with Tukey's multiple comparisons test. Primary neurons (n= 26-27) from *Otud7a* null mice showed no significant difference from wild-type, however, overexpression of *Otud7a* (n =18) led to significantly increased dendritic complexity. Data were obtained from 6 *Otud7a* null mice and 3 wild-type littermates. Data are presented as mean ± SEM (*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

Table S1. sgRNA off-target candidates and primers used to amplify the corresponding regions.

sgRNA	off-target candidates	Forward primer	Reverse primer
CTAGCGAGGCGGCATGTAAG	AGAGTGCTTCCTTAGCAAGGCGG	AAGGCAGGAACTCTGCATTG	TCCCGTCTGATCATCCTTTC
	AGACTGCCTACCTAGGGAGGGAG	TGGGTGACTCTTGTCTGTGG	AGTCTGCGGTGAAATGGAAT
	AGAGTGCTTCCCTAGCAAGCCAG	AGTGGGCCTCTTTCTGGAAT	AAGTCATAGCCCAGCAGTGG
	AAAGTGCTTACCCAGCCAGGGAG	GGCAATAGGAACCAGTCCAA	ACAAGCCATTTCTTGGCATC
	AGGGTTCTTCCCTAGAGAGGGAG	GGGATTGATGTTTTGGTGCT	ACACCCAGCTGGAAGCTCTA
	TGGGTCCCTACCTAGCCAGGAAG	TTTCATCATGACCCCTGACA	TTCATTTTGCACAGCTCAGG
	AAGGTCCTTCCTTAGCGAGGCAG	CCAGGTCTTGCACACAGCTA	TGGGGATCAAACTCAAGGTC
	AGGGAGCTTCCCTAGGGAGGAAG	CCCAGTCCTGCTTTAACCAA	ACTTGCCCAAAGTCACCATC
	AGGGTGCCTGACTAGCAAGGCAG	TGACTAGCAAGGCAGCTTGA	ACTCCACACTCTGGCTGAGG
	TGGTTGCTTCCCTAGGGAGGAGG	CATCAGGCAAAACTGACCAA	TAGATGGCCTTGTTGGCTTC
	AGGGTCATTGCCTAGCGAGCTGG	CCTCATTGGCACTTCCTGAT	GTGGGACCTCTTCTGCTCTG
	AAGGTGCTTATCTAGAGAGGCAG	AACCAGGTGTCTGCAAATGA	GCAATGTGGAAGCACCTTTT
	AGGGTGCGTACATAGCGTGGCAG	ATGCTTCTCCAAGGGAGGAT	ATGTGGGCTGCGATCTTATC
	AGGAAGCTTCCCTAGGGAGGGAG	AGTGGGAAACACAGCTCCAG	TCACTTCACCTGACCAGCAC
	ATGGTGATTTCCTAGCTAGGGAG	AAGGGTGGCTTCAGACTTGA	TGACAAGTAGAGGCAGGAGGA
GAAGTGTGTGCGAGGCTCGC	GAAGGGGGAGGGAGGCTCGCTGG	GAGGTGGGATCTTCTGGACA	TGTGGCAGAAGGTGAGTGAG
	GCAGTGCACGCGAGGCTCGCGGG	TGCTAGCGGTTTCCTGACTT	ACAGTGAGGGAGAGCGATGT
	GAGGTGTGTGGGAGGCTGGCTAG	GAGCCACCTTAACCACCGTA	ATTCCACTTTGGTTGCCAAG
	GACGCCTGTGCGGGGCTCGCCGG	GCCATCCTGTCGGTGAAC	ACGCCTCAGTTTTTCCACAC
	GAAGTGTCTGAGAGGCTGGCTGG	ATCTGCCACCCTCACTAAGC	CTGGGCTGACTCAGAAGAGC
	GGAGTGGCTGAGAGGCTCGCTGG	AACCTGTCCCTCATTTGTGC	CACTGCAAACCTGGGAATTT
	TAAGTGTCTGCGATGCTCGCTGG	CAAAACAAGCGTCCTCCATT	AGGAAAAGCTGAGCCAATGA
	GGAATGTGTGCGAGGCTCACAGG	GTGAGGCAGAAGGGTCATGT	AGTGTCTGCATCAGCAGTGG
	GGAGGCTGTGAGAGGCTCGCAAG	CACCAATCCAATTGTGTCGT	GCTTGGTTAGCCTGCTCATT
	GTGGTGTATGCCAGGCTCGCCAG	GGAGGTGACTCCATGGCTAA	TTGCTCACACATTTGCTTCC
	GTGGTGTATGCCAGGCTCGCCAG	GGAGGTGACTCCATGGCTAA	TTGCTCACACATTTGCTTCC
	GTGGTGTATGCCAGGCTCGCCAG	GGAGGTGACTCCATGGCTAA	TTGCTCACACATTTGCTTCC
	GTGGTGTATGCCAGGCTCGCCAG	GGAGGTGACTCCATGGCTAA	TTGCTCACACATTTGCTTCC
	GAAGGGTAGGCGAGGCCCGCTGG	AATGAGCACCGGGAAGTAAA	ACTCGGAGGCTGTGGTTATG
	GAAGTGTCTGTGAGGCGCGCGAG	CCAAGCGTCCTTTACCTCAG	GGCACCTCGAATCTTTGCTA

Table S2. Lipid modification sites predicted in human and mouse OTUD7A and OTUD7B proteins by GPS-lipid software.

ID	Position	Peptide	Type	Score
hOTUD7A	14	PNPTSAE<u>C</u>WAALLHD	S-Palmitoylation	6.60
	137	RSHVASE<u>C</u>NNEQFPL	S-Palmitoylation	2.33
	196	WSTVCTS<u>C</u>KRLLPLA	S-Palmitoylation	1.26
	794	AVGALRP<u>C</u>ATYPQQN	S-Palmitoylation	1.96
hOTUD7B	-	-	-	-
mOTUD7A	14	PNPPSAE<u>C</u>WAALLHD	S-Palmitoylation	7.23
	102	GHKVERP<u>C</u>LQRQDDI	S-Palmitoylation	2.81
	198	WSTVCTS<u>C</u>KRLLPLA	S-Palmitoylation	1.26
	802	TVGALRP<u>C</u>ATYPQQN	S-Palmitoylation	4.43
mOTUD7B	-	-	-	-

Table S3. Statistic analysis of behaviors in *Otud7a* mutant mice

Behavioral assay	Measurement	Statistical test	Comparison	Statistics	Defrees of freedom	*P* value	Post hoc		Adjusted *P* value	Interpretation	Fig.
Developmental delay	The first day of negative geotaxis	Kruskal-Wallis Test	Factor: Genotype	14.560	2	<0.001	Dunn's multiple comparison test	WT vs. HET	>0.999	KO has significant developmental delay in negative geotaxis.	Fig. 2b
								WT vs. KO	<0.001		
								HET vs. KO	0.003		
	The first day of cliff aversion	Kruskal-Wallis Test	Factor: Genotype	14.760	2	<0.001	Dunn's multiple comparison test	WT vs. HET	>0.999	KO has significant developmental delay in cliff aversion.	
								WT vs. KO	<0.001		
								HET vs. KO	0.005		
	The first day of incisor eruption	Kruskal-Wallis Test	Factor: Genotype	7.794	2	0.020	Dunn's multiple comparison test	WT vs. HET	>0.999	KO has significant developmental delay in incisor eruption.	
								WT vs. KO	0.029		
								HET vs. KO	0.046		
	The first day of incisor growth	Kruskal-Wallis Test	Factor: Genotype	7.041	2	0.030	Dunn's multiple comparison test	WT vs. HET	0.205	KO has significant developmental delay in incisor growth.	
								WT vs. KO	0.030		
								HET vs. KO	0.913		
	The first day of eye lid opening	Kruskal-Wallis Test	Factor: Genotype	5.188	2	0.075				KO is not significantly delayed in eye lid opening.	
	The first day of ear opening	Kruskal-Wallis Test	Factor: Genotype	3.399	2	0.183				KO is not significantly delayed in ear opening.	
Ultrasonic vocalization	Number of vocalizations	Two-Way ANOVA with repeated measures	Factor1: Genotype	8.946	2, 61	<0.001	Tukey's multiple comparison test	PND2 WT vs. HET	0.954		Fig. 2c
								PND2 WT vs. KO	0.357	KO is not significantly different in vocalizaiton on postnatal day 2.	
								PND2 HET vs. KO	0.414		
								PND4 WT vs. HET	0.843		
								PND4 WT vs. KO	0.345	KO is not significantly different in vocalizaiton on postnatal day 4.	
								PND4 HET vs. KO	0.568		
								PND6 WT vs. HET	0.198		
								PND6 WT vs. KO	0.018	KO has significantly reduced vocalizations on postnatal day 6.	
								PND6 HET vs. KO	0.381		
								PND8 WT vs. HET	0.463		
								PND8 WT vs. KO	0.030	KO has significantly reduced vocalizations on postnatal day 8.	
								PND8 HET vs. KO	0.214		
								PND10 WT vs. HET	0.011	HET has significantly reduced vocalizations on postnatal day 10.	
								PND10 WT vs. KO	0.012	KO has significantly reduced vocalizations on postnatal day 10.	
								PND10 HET vs. KO	0.972		
			Factor2 (repeated): Day	3.647	4, 244	0.007					
			Interaction (F1 x F2)	0.702	8, 244	0.690					
Conditioned fear	Freezing in contextual fear conditioning (%)	Two-Way ANOVA	Factor1: Genotype	1.403	2, 93	0.251				KO is not significantly different in contextual fear conditioning.	Sup Fig. 1a
			Factor2: Sex	0.001	1, 93	0.971					
			Interaction (F1 x F2)	1.110	2, 93	0.334					
	Freezing in cued fear conditioning (%)	Two-Way ANOVA	Factor1: Genotype	6.160	2, 93	0.003	Tukey's multiple comparison test	WT vs. HET	0.953		Sup Fig. 1b
								WT vs. KO	0.022	KO has significantly increased cued fear conditioning.	
								HET vs. KO	0.005		
			Factor2: Sex	0.068	1, 93	0.795					
			Interaction (F1 x F2)	1.025	2, 93	0.363					
Novel object recognition	Interaction index (Ratio of time interacting with novel/familiar object)	Two-Way ANOVA	Factor1: Genotype	3.050	2, 95	0.052					Sup Fig. 1c
			Factor2: Sex	2.948	1, 95	0.089					
			Interaction (F1 x F2)	0.207	2, 95	0.814					
Rotarod	Latency to fall (s)	Three-Way ANOVA with repeated measures	Factor1: Genotype	20.857	2, 80	<0.001	Tukey's multiple comparison test	WT vs. HET	>0.999		Fig. 2e-f
								WT vs. KO	<0.001	KO has significantly reduced motor coordination.	
								HET vs. KO	<0.001		
			Factor2: Sex	0.664	1, 80	0.418					
			Factor3 (repeated): Trial	19.649	7, 560	<0.001					
			Interaction (F1 x F2)	2.315	2, 80	0.105					
			Interaction (F1 x F3)	2.068	14, 560	0.012	Tukey's multiple comparison test	WT vs. HET	0.980		

								WT vs. KO	<0.001	KO has significantly reduced motor learning	
								HET vs. KO	<0.001		
			Interaction (F2 x F3)	0.755	7, 560	0.626					
			Interaction (F1x F2 x F3)	2.417	14, 560	0.003					
Acoustic startle	Response amplitude	Two-Way ANOVA	Factor1: Genotype	35.970	2, 92	<0.001	Tukey's multiple comparison test	WT vs. HET	0.002		Fig. 2g
								WT vs. KO	<0.001	KO has significant acoustic startle deficit.	
								HET vs. KO	<0.001		
			Factor2: Sex	4.910	1, 92	0.029					
			Interaction (F1 x F2)	1.678	2, 92	0.192					
Prepulse inhibition	Prepulse inhibition at 74 dB prepulse intensity (%)	Two-Way ANOVA	Factor1: Genotype	2.071	2, 98	0.132				KO is not significantly different in prepulse inhibition at 74 db prepulse intensity.	Fig. 2h-i
			Factor2: Sex	2.125	1, 98	0.148					
			Interaction (F1 x F2)	0.887	2, 98	0.415					
	Prepulse inhibition at 78 dB prepulse intensity (%)	Two-Way ANOVA	Factor1: Genotype	1.397	2, 98	0.252				KO is not significantly different in prepulse inhibition at 78 db prepulse intensity.	
			Factor2: Sex	1.863	1, 98	0.176					
			Interaction (F1 x F2)	2.354	2, 98	0.101					
	Prepulse inhibition at 82 dB prepulse intensity (%)	Two-Way ANOVA	Factor1: Genotype	7.916	2, 98	0.001					
			Factor2: Sex	8.362	1, 98	0.005					
			Interaction (F1 x F2)	4.060	2, 98	0.020	Split data by sex				
		One-Way ANOVA on males	Factor: Genotype	1.719	2, 47	0.191					
		One-Way ANOVA on females	Factor: Genotype	10.240	2, 50	<0.001	Tukey's multiple comparison test	WT vs. HET	0.860		
								WT vs. KO	0.001	Female KO has significant prepulse inhibition deficit at 82 db prepulse intensity.	
								HET vs. KO	0.001		
Three-chamber test	Time interacting with cups (s)	Three-Way ANOVA with repeated measures	Factor1: Genotype	4.236	2, 91	0.017	Tukey's multiple comparison test	WT vs. HET	>0.999		Sup Fig. 1d
								WT vs. KO	0.022	KO has significantly increased interaction with cups on both sides.	
								HET vs. KO	0.072		
			Factor2: Sex	0.158	1, 91	0.692					
			Factor3 (repeated): Side	169.108	1, 91	<0.001					
			Interaction (F1 x F2)	2.227	2, 91	0.114					
			Interaction (F1 x F3)	0.135	2, 91	0.874					
			Interaction (F2 x F3)	0.001	1, 91	0.978					
			Interaction (F1x F2 x F3)	1.396	2, 91	0.253					
Partition test	Time interacting with partition board (s)	Three-Way ANOVA with repeated measures	Factor1: Genotype	8.244	2, 89	0.001					Sup Fig. 1e-f
			Factor2: Sex	7.438	1, 89	0.008					
			Factor3 (repeated): Partner mouse	101.076	2, 178	<0.001					
			Interaction (F1 x F2)	4.270	2, 89	0.017	Split data by sex				
			Interaction (F1 x F3)	0.294	4, 178	0.881					
			Interaction (F2 x F3)	2.446	2, 178	0.090					
			Interaction (F1x F2 x F3)	2.725	4, 178	0.031	Split data by sex				
		Two-Way ANOVA with repeated measures on males	Factor1: Genotype	0.427	2, 43	0.655					
			Factor2 (repeated): Partner mouse	41.423	2, 86	<0.001					
			Interaction (F1 x F2)	1.267	4, 86	0.290				Male KO is not significantly different in interest in social novelty.	
		Two-Way ANOVA with repeated measures on females	Factor1: Genotype	10.322	2, 46	<0.001	Tukey's multiple comparison test	WT vs. HET	<0.001		
								WT vs. KO	0.002	Female KO has significantly increased interaction with partners in general.	
								HET vs. KO	0.876	Female HET has significantly increased interaction with partners in general.	
			Factor2 (repeated): Partner mouse	66.470	2, 92	<0.001					
			Interaction (F1 x F2)	1.806	4, 92	0.134				Female KO is not significantly different in interest in social novelty.	
Nest building	Scores of nest	Two-Way ANOVA	Factor1: Genotype	3.927	2, 92	0.023	Tukey's multiple comparison test	WT vs. HET	0.986		Sup Fig. 1g
								WT vs. KO	0.068		

								HET vs. KO	0.029	KO has significantly reduced nest building ability (compared to HET).	
			Factor2: Sex	5.256	1, 92	0.024					
			Interaction (F1 x F2)	1.714	2, 92	0.186					
Self-grooming	Total grooming duration (s)	Two-Way ANOVA	Factor1: Genotype	0.088	2, 90	0.916					Sup Fig. 1h
			Factor2: Sex	5.775	1, 90	0.018					
			Interaction (F1 x F2)	3.428	2, 90	0.037	Split data by sex				
		One-Way ANOVA on males	Factor: Genotype	3.850	2, 45	0.029	Tukey's multiple comparison test	WT vs. HET	0.093		
								WT vs. KO	0.943		
								HET vs. KO	0.043	Not sure	
		One-Way ANOVA on females	Factor: Genotype	0.874	2, 45	0.424				Female KO is not significantly different in grooming duration.	
Holeboard exploration	Sequential nose-pokes (%)	Two-Way ANOVA	Factor1: Genotype	1.393	2, 81	0.254				KO is not significantly different in holeboard exploration.	Sup Fig. 1i
			Factor2: Sex	0.344	1, 81	0.559					
			Interaction (F1 x F2)	0.588	2, 81	0.558					
Grip strength	Grip strength (g)	Two-Way ANOVA	Factor1: Genotype	3.596	2, 95	0.031	Tukey's multiple comparison test	WT vs. HET	0.322		Fig. 2d
								WT vs. KO	0.026	KO has significantly reduced grip strength.	
								HET vs. KO	0.404		
			Factor2: Sex	4.668	1, 95	0.033					
			Interaction (F1 x F2)	0.185	2, 95	0.831					
Elevated plus maze	Time in open arms (s)	Two-Way ANOVA	Factor1: Genotype	4.477	2, 92	0.014	Tukey's multiple comparison test	WT vs. HET	0.207		Sup Fig. 1j
								WT vs. KO	0.010	KO has significantly increased axiety level in elevated plus maze.	
								HET vs. KO	0.294		
			Factor2: Sex	0.157	1, 92	0.693					
			Interaction (F1 x F2)	0.197	2, 92	0.821					
Light-dark box exploration	Number of entries to the light box	Two-Way ANOVA	Factor1: Genotype	0.437	2, 93	0.647				KO is not significantly different in axiety level.	Sup Fig. 1k
			Factor2: Sex	0.004	1, 93	0.949					
			Interaction (F1 x F2)	1.080	2, 93	0.344					
Open field activity	Center/total distance ratio	Two-Way ANOVA	Factor1: Genotype	1.603	2, 93	0.207				KO is not significantly different in axiety level.	Sup Fig. 1l
			Factor2: Sex	3.965	1, 93	0.049					
			Interaction (F1 x F2)	0.282	2, 93	0.755					
	Total distance traveled (cm)	Two-Way ANOVA	Factor1: Genotype	0.333	2, 93	0.717				KO is not significantly different in total activity.	Sup Fig. 1m
			Factor2: Sex	1.338	1, 93	0.250					
			Interaction (F1 x F2)	1.785	2, 93	0.174					
Forced swimming test	Time immobile (%)	Two-Way ANOVA	Factor1: Genotype	0.369	2, 93	0.693				KO is not significantly different in depression-like behaviors.	Sup Fig. 1n
			Factor2: Sex	0.435	1, 93	0.511					
			Interaction (F1 x F2)	1.310	2, 93	0.275					
Hearing ABR test	Threshold (dB SPL)	Three-Way ANOVA with repeated measures	Factor1: Genotype	3.072	2, 25	0.064				KO is not significantly different in auditory brain stem responses.	Sup Fig. 2
			Factor2: Sex	0.338	1, 25	0.566					
			Factor3 (repeated): Frequency	11.817	5, 75	0.038					
			Interaction (F1 x F2)	0.236	2, 25	0.791					
			Interaction (F1 x F3)	2.554	2, 25	0.098					
			Interaction (F2 x F3)	0.677	1, 25	0.418					
			Interaction (F1x F2 x F3)	0.515	2, 25	0.604					
Hearing DPOAE test	Threshold (dB SPL)	Three-Way ANOVA with repeated measures	Factor1: Genotype	2.460	2, 24	0.107				KO is not significantly different in DPOAE.	Sup Fig. 2
			Factor2: Sex	0.446	1, 24	0.511					
			Factor3 (repeated): Frequency	99.622	7, 168	0.000					
			Interaction (F1 x F2)	0.666	2, 24	0.523					
			Interaction (F1 x F3)	2.231	14, 168	0.064					
			Interaction (F2 x F3)	0.922	7, 168	0.491					
			Interaction (F1x F2 x F3)	0.311	14, 168	0.992					