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Supplementary Online Information for

***Kctd13* deletion reduces synaptic transmission via increased RhoA**

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Supplementary Results

To determine whether alterations in synaptic transmission in *Kctd13* mutants are widespread or limited to hippocampus, we next measured mEPSCs in layer 2/3 pyramidal neurons in somatosensory barrel cortex. mEPSCs in this region also demonstrated decreased frequency (**Extended Data [ED] Fig. 3a-b**) with no change in amplitude (**ED Fig. 3c-d**). These findings suggest that regulation of synaptic function is widespread in *Kctd13* mutants.

While excitatory synaptic transmission is no doubt an important factor in neuropsychiatric disorders, inhibitory synaptic transmission has also been implicated[72,77]. Thus, we next examined miniature inhibitory postsynaptic currents (mIPSCs) in area CA1 of hippocampus. We identified a significant decrease in mIPSC frequency (**ED Fig. 3e-f**) with no change in amplitude (**ED Fig. S3g-h**). Because mIPSCs are typically onto dendritic shafts rather than spines, molecular-scale synaptic alterations are likely to play a role in addition to larger-scale, structural changes. It is also possible that inhibitory synaptic changes represent a homeostatic response to the excitatory synaptic alterations.

ED Figure 4 depicts more extensive body weight examinations. **ED Fig. 4b-c** further depicts these data divided by sex. To examine body weight more systematically, we weighed more than 80 mice of each genotype throughout development and found a very small, yet statistically significant *decrease* in body weight (**ED Fig. 4d**). Thus, *Kctd13* is unlikely to be a major independent driver of *increased* body weight in 16p11.2 deletion.

ED Fig. 4e-g depicts brain weight combined data and also divided by sex. A more thorough examination of brain weight, brain/body weight ratio, and both measures again revealed no change in brain size in *Kctd13* mutants in grouped data or when separated by sex (**ED Fig. 4h-j**).

High resolution, structural MRI of P7 *Kctd13* mutant brains revealed no statistically significant change in the volume of 56 independent brain regions examined (**ED Fig. 5a-b**). 67 mouse brains were provided in total, two images did not register well so they were excluded for this report. After exclusions, group numbers were 21 homozygote *Ktcd13* (♂ n=11, ♀ n=10), 21 Heterozygote (♂ n=11, ♀ n=10), and 23 WT mice. Total brain volume was also unchanged (**ED Fig. 5c**) Additional tests on adult (12-wk-old) mice also revealed no statistically significant change in the volume of 159 different regions (**ED Fig. 5d-e**). The adult MRI cohort consisted of 67 mouse brains with n=23 homozygotes (♂ n=13, ♀ n=10), n=21 heterozygotes (♂ n=11, ♀ n=10), and n=23 WT (♂ n=13, ♀ n=10). Total brain volume in adults was also unchanged (**ED Fig. 5f**). The neuroanatomy was assessed and volume was measured as either absolute volume in mm^3 or relative volume (% total brain volume). This was assessed in all brains and comparisons among *Kctd13* mice and the WT. Multiple comparisons were controlled for by using the False Discovery Rate. We found no differences among the *Ktcd13* (homozygotes or heterozygotes) mice

and the corresponding WT mice in any of our measures in either age group. There was also no difference in total brain volume among the groups (**ED Fig. 5c/f**).

We observed no differences in *Kctd13* mutant cortical layering with additional measures of cortical layer thickness and cell count in P17 mice using the cortical layer selective markers Ctip2 (**ED Fig. 7a-b,i)**, Cux1 (**ED Fig. 7c-d,j)**, Tbr1 (**ED Fig. 7e-f; i)**, Satb2 (**ED Fig. 7g-h,i)**. No differences in cortical layer development of *Kctd13* mutant mice were observed in E15 mice using measures of cortical thickness and cell count with the cortical markers Tbr1 (**ED Fig. 8a-b,g)**, Tbr2 (**ED Fig. 8c-d,h)**, and Tuj1 (**ED Fig. 8e-f,i).**

Measures of anxiety (**ED Fig. 10c-f**), coordination (**ED Fig. 10g**), sensation (**ED Fig. 10h**), olfaction (**ED Fig. 10i**), repetitive grooming (**ED Fig. 10j-l**), social interaction (**ED Fig. 10m-n**), nest building (**ED Fig. 10o-p**), learning/memory (**ED Fig. 10q-x**), and sensorimotor gating (**ED Fig. 10y-z**) were all unchanged in *Kctd13* mutants. It will be of interest to examine whether compound heterozygotes of *Kctd13* and other genes in the 16p11.2 region may lead to synergistic changes in neuronal function, brain development, and behavior.

Supplementary online Table 1. Detailed Statistics

Parameter	Comparison	Results
Fig 1b Western Blot:		
Hippocampus	Genotype	One-way ANOVA. Main effect of Genotype: F(2,11)=201.097, P<0.000001; Post Hoc Test: WT vs HET, P=0.00021, WT vs KO, P=0.00019, HET vs KO, P=0.00017. WT n=6; HET n=4; KO n=4.
Cortex	Genotype	One-way ANOVA. Main effect of Genotype: F(2,13)=45.896, I; WT vs HET, P=0.00079, WT vs KO, P=0.00019, HET vs KO, P=0.00075. WT n=8; HET n=4; KO n=4.
Cerebellum	Genotype	One-way ANOVA. Main effect of Genotype: F(2,11)=291.0828, P <0.000001; Post Hoc Test: WT vs HET, P=0.00017, WT vs KO, P=0.00020, HET vs KO, P=0.00017. WT n=6; HET n=4; KO n=4.
Midbrain	Genotype	One-way ANOVA. Main effect of Genotype: F(2,11)=142.5258, P <0.000001; Post Hoc Test: WT vs HET, P=0.00023, WT vs KO, P=0.00020, HET vs KO, P=0.00017. WT n=6; HET n=4; KO n=4.
All Brain Region	Genotype and Region	RM ANOVA. Main effect of Genotype: $F_{(2,11)}$=251.3974, **P<0.000001**; Main effect of Brain Region: $F_{(3,33)}$=2.8782, ***P=0.05072***; Brain Region * Genotype interaction: $F_{(6,33)}$=0.8286, *p=0.55633*; Post Hoc Test: WT vs HET, ***P=0.00017***, WT vs KO, ***P=0.00019***, HET vs KO**, *P=0.00017***.
Fig 1e I/O Curve:		
I/O Curve	Genotype and Stimulus Intensity	RM ANOVA. Main effect of Genotype: $F_{(2,17)}$=8.999**, P=0.00216**; Main effect of Stimulus Intensity: $F_{(7,119)}$=66.450, ***P*<0.000001**; Stimulus Intensity * Genotype interaction: $F_{(14,119)}$=7.340, ***P*=0.000001**; Post Hoc Test: WT vs HET, ***P=0.00349***, WT vs KO, ***P=0.00417***, HET vs KO, ***P=0.71600***. WT n=19 slices from 6 mice; HET n=14 slices from 7 mice; KO n=20 slices from 6 mice.
Fig 1f mEPSC Amplitude:		
mEPSC Amplitude	Genotype	One-way ANOVA. No differences testing the effect of Genotype: $F_{(2,77)}$=0.142, *P=0.86749*; Post Hoc Test: WT vs HET, *P=0.89538*, WT vs KO, *P=0.87237*, HET vs KO, *P=0.71527*. WT n=26 cells from 9 mice; HET n=24/7; KO n=30/8.
Fig 1g mEPSC Frequency:		
mEPSC Frequency	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,77)}$=7.923, ***P=0.00074***; Post Hoc Test: WT vs HET, ***P=0.000739***, WT vs KO, ***P=0.00445***, HET vs KO, *P=0.35206*. WT n=26 cells from 9 mice; HET n=24/7; KO n=30/8.
Fig 2a Rho GTPase Western Blot:		
RhoA	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,29)}$=5.9422, ***P=0.00687***; Post Hoc Test: WT vs HET, ***P=0.01506***, WT vs KO, ***P=0.04642***, HET vs KO, *P=0.36932*. WT n=14; HET n=11; KO n=7.
Fig 2b Vehicle IO curve with Rhosin:		
Vehicle Treated Slices	Genotype and Stimulus Intensity	RM ANOVA. Main effect of Genotype: $F_{(2,15)}$=4.8173, ***P=0.02422***; Main effect of Stimulus Intensity: $F_{(7,105)}$=86.633, ***P*<0.000001**; Stimulus Intensity * Genotype interaction: $F_{(14,105)}$=3.7822, ***P=0.000001***; Post Hoc Test: WT vs HET, ***P=0.05969***, WT vs KO, ***P=0.02108***, HET vs KO, *P=0.32945*. WT Vehicle n=19 slices from 6 mice; HET Vehicle n=21/6; KO Vehicle n=19/6.
Fig 2c Treatment IO curve with Rhosin:		
Rhosin Treated Slices	Genotype and Stimulus Intensity	RM ANOVA. Main effect of Genotype: $F_{(2,13)}$=3.850 , ***P=0.004908***; Main effect of Stimulus Intensity: $F_{(7,14)}$=97.342, ***P*<0.000001**; Stimulus Intensity * Genotype interaction: $F_{(14,91)}$=3.189, ***P=0.00042***; Post Hoc Test: WT vs HET, ***P=0.03432***, WT vs KO, *P=0.15953*, HET vs KO, *P=0.19837*. WT Rhosin n=18 slices from 5 mice; HET Rhosin n=15/5; KO Rhosin n=20/6.
Fig 2d Vehicle IO curve with C3 Toxin:		
Vehicle Treated Slices	Genotype and Stimulus Intensity	RM ANOVA. Main effect of Genotype: $F_{(1,16)}$=14.8666 , ***P=0.00139***; Main effect of Stimulus Intensity: $F_{(7,112)}$=74.4230, ***P*<0.000001**; Stimulus Intensity * Genotype interaction: $F_{(7,112)}$=9.3004, ***P*<0.000001**; Post Hoc Test: WT vs KO, **P=0.00154**. WT Vehicle n=27 slices from 10 mice; KO Vehicle n=16/8.
Fig 2e Treatment IO curve with C3 Toxin:		
C3 Treated Slices	Genotype and Stimulus Intensity	RM ANOVA. Main effect of Genotype: $F_{(1,15)}$=2.2274 , *P=0.1563*; Main effect of Stimulus Intensity: $F_{(7,105)}$=72.0904, ***P*<0.000001**; Stimulus Intensity * Genotype interaction: $F_{(7,105)}$=1.9819, *P=0.06436*; Post Hoc Test: WT vs KO, *P=0.15646*. WT C3 n=24 slices from 10 mice; KO C3 n=16/8.
Fig 2f Rescue mEPSC with Rhosin Frequency:		
mEPSC Frequency with Rhosin	Genotype and Treatment	Two-way ANOVA. Effect of Genotype: $F_{(1,12)}$=7.927, ***P=0.0156***; Main Effect of Treatment: $F_{(1,12)}$=31.507, ***P=0.0001***; Treatment * Genotype interaction: $F_{(1,12)}$=15.199, ***P=0.0021***; Post Hoc Test: WT vs KO, ***P=0.01503***; Vehicle vs Rhosin; ***P=0.00024***; WT Vehicle vs KO Vehicle, ***P=0.00042***; WT Vehicle vs WT Rhosin, *P=0.31185*; WT Vehicle vs KO Rhosin, *P=0.12838*; KO Rhosin vs WT Rhosin, *P=0.31045*; WT Rhosin vs KO

		Vehicle, ***P*=0.00031**; KO Vehicle vs KO Rhosin, ***P*=0.00025**. WT Vehicle n=12/4; KO Vehicle n=17/4; WT Rhosin n=17/4; KO Rhosin n=18/4.
Fig 3a Embryonic BrdU:		
Embryonic BrdU	Genotype	One-Way ANOVA, Main effect of Genotype: $F_{(2,16)}$=0.152, *P=0.860*; Post Hoc Test : WT vs HET, *P=0.730*, WT vs KO, *P=0.857*, HET vs KO, *P=0.860*. WT n=6; HET n=5; KO n=5.
Fig 3b Embryonic Ki67:		
Embryonic Ki67	Genotype	One-Way ANOVA, Main effect of Genotype: $F_{(2,15)}$=0.284, *P=0.757*; Post Hoc Test : WT vs HET, *P=0.764*, WT vs KO, *P=0.849*, HET vs KO, *P=0.617*. WT n=6; HET n=5; KO n=5.
Fig 3c In Utero Electroporation:		
In Utero Electroporation	Genotype	One-Way ANOVA, Main effect of Genotype: $F_{(2,27)}$=1.6515, *P=0.210542*; Post Hoc Test: WT vs HET, *P=0.724140*, WT vs KO, *P=0.236693*, HET vs KO, *P=0.201759*. WT n=8; HET n=10; KO n=12.
Fig 3d Zebrafish head size:		
Zebrafish Head Size	Genotype	One-Way ANOVA, Main effect of Genotype: $F_{(2,144)}$=2.59, *P=0.07863*; Post Hoc Test: WT vs HET, *P=0.85735*, WT vs KO, ***P*=0.03212**, HET vs KO, *P=0.05271*. WT n=44; HET n=93; KO n=22.
Fig 3e Zebrafish P-Histone H3:		
Zebrafish P-Histone H3	Genotype	One-Way ANOVA, Main effect of Genotype: $F_{(2,66)}$=0.223, *P=0.80046*; Post Hoc Test: WT vs HET, *P=0.76114*, WT vs KO, *P=0.72674*, HET vs KO, *P=0.78943*. WT n=21; HET n=36; KO n=14.
Extended Data Fig 1a PPR:		
Paired Pulse Ratio	Genotype and Inter-Stimulus Interval	RM ANOVA. Main effect of Genotype: $F_{(2,49)}$=0.537, *P*=0.587; Main effect of Inter Stimulus Interval: $F_{(5,245)}$=79.709, ***P*<0.000001**; Inter Stimulus Interval * Genotype interaction: $F_{(10,245)}$=0.820, *p=0.609*.Post Hoc Test for Effect of Inter Stimulus Interval: 30 vs 50, *p=0.98788*; 30 vs 80, ***P*=0.00051**; 30 vs 100, ***P*=0.0002**; 30 vs 200, ***P*=0.00002**; 30 vs 500, ***P*=0.00002**; 50 vs 80**, *P*=0.0063**; 50 vs 100, ***P*<0.000001**; 50 vs 200, ***P*=0.00002**; 50 vs 500**, *P*=0.00002**; 80 vs 100, ***P*=0.00053**; 80 vs 200, ***P*=0.00002**; 80 vs 500, ***P*=0.00001**; 100 vs 200**, *P*=0.00001**; 100 vs 500, ***P*=0.00002**; 200 vs 500, ***P*=0.00028**. WT n=18 slices from 6 mice; HET n=17/7; KO n=17/6.
Extended Data Fig 1b MK801:		
MK-801	Genotype and Bin	RM ANOVA. Main effect of Genotype: $F_{(1,25)}$=0.5900, *P=0.4497;* Post Hoc Test: WT vs KO, *P=0.449894*; Main effect of Bin $F_{(39,975)}$=482.888, ***P*<0.000001***;* Bin* Genotype interaction $F_{(39,975)}$=0.716, *P=0.903825*. WT n=15 cells from 6 mice; KO n=12/6.
Extended Data Fig 1c Total Dendritic Length:		
Total Dendritic Length	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,47)}$=4.9885, ***P*=0.01085**; Post Hoc Test: WT vs HET, *P=0.14351*, WT vs KO, ***P*=0.00463**, HET vs KO, *P=0.06949*. WT n=43/11 neurons/mice, HET n=33/11, KO n=29/6.
Extended Data Fig 1d Sholl Analysis:		
Sholl Analysis		RM ANOVA. Main effect of Genotype: $F_{(2,76)}$=4.003, ***P*=0.02225**; Main effect of Dendritic Branch Point: $F_{(10,760)}$=305.2346, **P<0.00001**; Dendritic Branch Point * Genotype interaction: $F_{(20,760)}$=1.2085, *P=0.2391*; Post Hoc Test: WT vs HET, *P=0.27048*, WT vs KO, ***P*=0.00965**, HET vs KO, *P=0.06014*. WT n=30/11, HET n=31/11, KO n=17/6.
Extended Data Fig 1e Spine Density:		
Total Spine Density	Genotype and Distance From Soma	RM ANOVA. Main effect of Genotype: $F_{(2,70)}$=5.105, ***P*=0.00852**; Main effect of Distance from Soma: $F_{(3,210)}$=19.439, **P<0.0001**; Genotype * Distance from Soma interaction: $F_{(6,210)}$=2.099, *P*=0.054625; Post Hoc Test for all data points: WT vs KO, ***P*=0.03859**; WT vs HET, *P=0.17584*; HET vs KO, *P=0.26083*. WT n=30/11, HET n=31/11, KO n=14/6.
30 µm from Soma	Genotype	One-way Anova: Main effect of Genotype: $F_{(2,70)}$=0.7783, *P=0.46314*; Post Hoc Test for 30 µm: WT vs KO, *P=0.35911*; WT vs HET, *P=0.99786*; HET vs KO, *P=0.62338.*
60 µm from Soma	Genotype	One-way Anova: Main effect of Genotype: $F_{(2,70)}$=1.4759, *P=0.23560*; Post Hoc Test for 60 µm: WT vs KO, *P=0.07158*; WT vs HET, *P=0.55304*; HET vs KO, *P=0.10483*.
90 µm from Soma	Genotype	One-way Anova: Main effect of Genotype: $F_{(2,70)}$=5.145, ***P*=0.00822**; Post Hoc Test for 90 µm: WT vs KO, ***P*=0.00039**; WT vs HET, ***P*=05412**; HET vs KO, ***P*=0.03431**.
120 µm from Soma	Genotype	One-way Anova: Main effect of Genotype: $F_{(2,70)}$=5.8555, ***P*=0.00445**; Post Hoc Test for 120 µm: WT vs KO, ***P*=0.00275**; WT vs HET, ***P*=0.04038**; HET vs KO, *P=0.17640*.
Extended Data Fig 2a Rho GTPase Western Blot		
RhoB	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,29)}$=0.2356, *P=0.7916*. WT n=14; HET n=11; KO n=7.
RhoC	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,20)}$=0.09925, *P=0.9059*. WT n=8; HET n=8; KO n=7.
Rac1	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,21)}$=1.387, *P=0.2718*. WT n=8; HET n=11; KO n=5.
Extended Data Fig 2b Rescue IO curve with Rhosin:		
Rescue with Rhosin	Genotype, Stimulus Intensity, and Treatment	Two-Way RM ANOVA. Main effect of Genotype: $F_{(2,28)}$=1.692, p=0.202; Main Effect of Treatment: $F_{(1,28)}$=6.008, ***P*=0.02074**; Main effect of Stimulus Intensity: $F_{(7,196)}$=186.893,

		P*<*0.0001; Treatment * Genotype interaction: $F_{(2,28)}$=7.145, ***P*=*0.00311***; Stimulus Intensity * Treatment interaction: $F_{(7,196)}$=4.361, ***P*=*0.00016***; Stimulus Intensity * Genotype interaction: $F_{(14,196)}$=1.206, p=0.273; Stimulus Intensity * Treatment * Genotype interaction: $F_{(14,196)}$=5.922, ***P*<*0.0001***; Post Hoc Test: WT vs HET, *P=0.28245*; WT vs KO, *P=0.067946*; HET vs KO, *P=0.29992*; Vehicle vs Rhosin, ***P*=*0.02036***; WT Vehicle vs HET Vehicle, *P=0.13613*; WT Vehicle vs KO Vehicle, *P=0.12365*; HET vehicle vs KO vehicle, *P=0.73059*; WT Rhosin vs HET Rhosin, ***P*=*0.01146***; WT Rhosin vs KO Rhosin, *P=0.26946*; HET Rhosin vs KO Rhosin, *P=0.10303*; WT Vehicle vs WT Rhosin, *P=0.23786*; HET Vehicle vs HET Rhosin**,** ***P*=*0.01005***; KO Vehicle vs KO Rhosin, *P=0.12187*; Het Rhosin vs WT Vehicle, *P=0.16281*; KO Rhosin vs WT Vehicle, *P=0.84659*. WT Vehicle n=19 slices from 6 mice; HET Vehicle n=21/6; KO Vehicle n=19/6; WT Rhosin n=18 slices from 5 mice; HET Rhosin n=15/5; KO Rhosin n=20/6.
Extended Data Fig 2c Rescue with C3 Toxin:		
Rescue with C3	Genotype, Stimulus Intensity, and Treatment	Two-Way RM ANOVA. Main effect of Genotype: $F_{(1,31)}$=13.057, ***P*=*0.00106***; Main Effect of Treatment: $F_{(1,31)}$=1.990, p=0.16829; Main effect of Stimulus Intensity: $F_{(7,217)}$=145.700, ***P*<*0.0001***; Treatment * Genotype interaction: $F_{(1,31)}$=1.6535, *P=0.20801*; Stimulus Intensity * Treatment interaction: $F_{(7,217)}$=2.4435, ***P*=*0.019787***; Stimulus Intensity * Genotype interaction: `$F_{(7,217)}$=8.8268, ***P*<*0.0001***; Stimulus Intensity * Treatment * Genotype interaction: $F_{(7,217)}$=0.7693, *P=0.61368*; Post Hoc Test: WT vs KO, ***P*=*0.000940***; Vehicle vs C3, *P=0.184087*; Treatment * Genotype interaction: WT Vehicle vs KO Vehicle, ***P*=*0.004465***; WT Vehicle vs KO C3, *P=0.129626*; WT Vehicle vs WT C3, *P=0.930337*; WT C3 vs KO Vehicle, ***P*=*0.00659***; WT C3 vs KO C3, *P=0.242180*; KO Vehicle vs KO C3, *P=0.06599*. WT Vehicle n=27 slices from 10 mice; KO Vehicle n=16/8; WT C3 n=24 slices from 10 mice; KO C3 n=16/8.
Extended Data Fig 2d Rescue mEPSC with Rhosin Amplitude:		
mEPSC Amplitude with Rhosin	Genotype and Treatment	Two-way ANOVA. Main Effect of Genotype: $F_{(1,12)}$=0.7754, *P=0.3959*; Main Effect of Treatment: $F_{(1,12)}$=0.0974, *P=0.76035*; Treatment * Genotype interaction: $F_{(1,12)}$=0.1582, *P=0.69779*; Post Hoc Test: WT vs KO, *p=0.39605*; Vehicle vs Rhosin, *P=0.76050*; WT Vehicle vs KO Vehicle, *P=0.80318*; WT Vehicle vs KO Rhosin, *P=0.68448*; WT Vehicle vs WT Rhosin, *P=0.62496*; KO Rhosin vs WT Rhosin, *P=0.73886*; WT Rhosin vs KO Vehicle, *P=0.91547*; KO Vehicle vs KO Rhosin, *P=0.95277*. WT Vehicle n=12 cells from 4 mice; KO Vehicle n=17/4; WT Rhosin n=17/4; KO Rhosin n=18/4.
Extended Data Fig 3a mEPSC Frequency (Layer 2/3 Cortex):		
mEPSC Amplitude (Layer 2/3 Cortex)	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,61)}$=6.0063, ***P*=*0.004158***; Post Hoc Test: WT vs HET, ***P*=*0.003889***, WT vs KO, ***P*=*0.020028***, HET vs KO, *P=0.335538*. (WT n=20 cells from 5 mice; HET n=20/6; KO n=24/5).
Extended Data Fig 3c mEPSC Amplitude (Layer 2/3 Cortex):		
mEPSC Frequency (Layer 2/3 Cortex)	Genotype	One-way ANOVA. No differences testing the effect of Genotype: $F_{(2,61)}$=0.9387, *P=0.396694*; Post Hoc Test: WT vs HET, *P=0.459464*, WT vs KO, *P=0.265203*, HET vs KO, *P=0.942549*. (WT n=20 cells from 5 mice; HET n=20/6; KO n=24/5).
Extended Data Fig 3e mIPSC Frequency (Hippocampus):		
mIPSC Frequency (Hippocampus)	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,13)}$=4.12081, ***P*=*0.041107***; Post Hoc Test: WT vs HET, ***P*=*0.048282***, WT vs KO, ***P*=*0.038481***, HET vs KO, *P=0.726992*. (WT n=20 cells from 5 mice; HET n=20/6; KO n=24/5).
Extended Data Fig 3g mIPSC Amplitude (Hippocampus):		
mIPSC Amplitude (Hippocampus)	Genotype	One-way ANOVA. No differences testing the effect of Genotype: $F_{(2,13)}$=2.2481, *P=0.145039*; Post Hoc Test: WT vs HET, *P=0.826835*, WT vs KO, *P=0.107743,* HET vs KO, *P=0.163902*. (WT n=20 cells from 5 mice; HET n=20/6; KO n=24/5).
Extended Data Fig 4a Body Weight (12 weeks):		
Body Weight	Genotype and Sex	Two-Way ANOVA. Main effect of Genotype: $F_{(2,84)}$=0.942, *P=0.3939*; Main effect of Sex: $F_{(1,84)}$=91.302, **P<0.0001**; Genotype * Sex interaction: $F_{(2,84)}$=1.273, *P=0.28529*; Post Hoc Test Genotype: WT vs HET, *P=0.384541*, WT vs KO, *P=0.486556*, HET vs KO, *P=0.262805*. WT n=23 (males n=11, females n=12); HET n=42 (males n=25, females n=17); KO n=25 (males n=14, females n=11).
Extended Data Fig 4b Female Body Weight (12 weeks):		
Female Body Weight	Genotype	One-Way ANOVA. Main effect of Genotype: $F_{(2,37)}$=0.400, *P=0.672872*; Post Hoc Test Genotype: WT vs HET, *P=0.671702*, WT vs KO, *P=0.591656*, HET vs KO, *P=0.755673*. WT n=12; HET n=17; KO n=11.
Extended Data Fig 4c Male Body Weight (12 weeks):		
Male Body Weight	Genotype	One-Way ANOVA. Main effect of Genotype: $F_{(2,47)}$=1.473, *P=0.239651*; Post Hoc Test Genotype: WT vs HET, *P=0.600948*, WT vs KO, *P=0.192747*, HET vs KO, *P=0.222070*. WT n=11; HET n=25; KO n=14.
Extended Data Fig 4d Body Weight over development:		
Body Weight	Genotype, Sex, and Days	RM ANOVA. Main effect of Genotype: $F_{(2,123)}$=5.075, ***P*=*0.008***; Main effect of Sex: $F_{(1,123)}$=77.120, ***p* <*0.000001***; Main effect of Days: $F_{(18,2214)}$=3066.181, ***P* <*0.000001***; Genotype * Sex interaction: $F_{(2,123)}$=3.219, ***P*=0.043**; Genotype * Sex * Days interaction: $F_{(36,2214)}$=1.566, ***P*=*0.018***; Post Hoc Test (Unequal N HSD): Genotype WT vs HET, ***P*=0.001**, WT vs KO, ***P*=*0.001***, HET vs KO, *P=0.788*; Genotype Males WT vs HET, *P=0.697*, WT vs KO, *P=0.044*, HET vs KO, *P=0.043*; Genotype Females WT vs HET,

		P=0.010, WT vs KO, ***P=0.010***, HET vs KO, *P=0.721*. WT n=88 (males n=40, females n=48); HET n=86 (males n=28, females n=58); KO n=82 (males n=36, females n=46).
Extended Data Fig 4e Brain Weight (12 weeks):		
Brain Weight	Genotype and Sex	Two-Way ANOVA. Main effect of Genotype: $F_{(2,84)}$=2.25, *P=0.11201*; Main effect of Sex: $F_{(1,84)}$=0.15, *P*=0.70224; Genotype * Sex interaction: $F_{(2,84)}$=0.03, *P=0.97201*; Post Hoc Test Genotype: WT vs HET, *P=0.16837*, WT vs KO, *P=0.667871*, HET vs KO, *P=0.575430*. WT n=23 (males n=11, females n=12); HET n=42 (males n=25, females n=17); KO n=25 (males n=14, females n=11).
Extended Data Fig 4f Female Brain Weight (12 weeks):		
Female Brain Weight	Genotype	One-Way ANOVA. Main effect of Genotype: $F_{(2,37)}$=1.15, *P=0.328455*; Post Hoc Test Genotype: WT vs HET, *P=0.327035*, WT vs KO, *P=0.411910*, HET vs KO, *P=0.540010*. WT n=12; HET n=17; KO n=11.
Extended Data Fig 4g Male Brain Weight (12 weeks):		
Male Brain Weight	Genotype	One-Way ANOVA. Main effect of Genotype: $F_{(2,47)}$=1.16, *P=0.321719*; Post Hoc Test Genotype: WT vs HET, *P=0.343448*, WT vs KO, *P=0.596384*, HET vs KO, *P=0.384604*. WT n=11; HET n=25; KO n=14.
Extended Data Fig 4h Ratio of Brain/Body Weight (12 weeks):		
Brain Weight/Body Weight Ratio	Genotype and Sex	Two-Way ANOVA. Main effect of Genotype: $F_{(2,84)}$=1.238, *P=0.2952*; Main effect of Sex: $F_{(1,84)}$=186.207, **P<0.0001**; Genotype * Sex interaction: $F_{(1,85)}$=1.234, *P=0.2964*; Post Hoc Test Genotype: WT vs HET, *P=0.7604884*, WT vs KO, *P=0.406251*, HET vs KO, *P=0.491836*. WT n=23 (males n=11, females n=12); HET n=42 (males n=25, females n=17); KO n=25 (males n=14, females n=11.
Extended Data Fig 4i Female Ratio of Brain/Body Weight (12 weeks):		
Female Brain Weight/Body Weight Ratio	Genotype	One-Way ANOVA. Main effect of Genotype: $F_{(2,37)}$=0.001, *P=0.998620*; Post Hoc Test Genotype: WT vs HET, *P=0.967670*, WT vs KO, *P=0.997397*, HET vs KO, *P=0.998987*. WT n=12; HET n=17; KO n=11.
Extended Data Fig 4j Male Ratio of Brain/Body Weight (12 weeks):		
Male Brain Weight/Body Weight Ratio	Genotype	One-Way ANOVA. Main effect of Genotype: $F_{(2,47)}$=2.144, *P=0.128485*; Post Hoc Test Genotype: WT vs HET, *P=0.186192*, WT vs KO, *P=0.070272*, HET vs KO, *P=0.358450*. WT n=11; HET n=25; KO n=14.
Extended Data Fig 5c Total Brain Volume (P7):		
Total Brain Volume	Genotype	One-Way ANOVA, Main effect of Genotype: $F_{(2,63)}$=1.582, *P=0.224991*; Post Hoc Test: WT vs HET, *P=0.301594* , WT vs KO, *P=0.525119*, HET vs KO, *P=0.220494* . [WT n=23 (males n=11, females n=12); HET n=21 (males n=11, females n=10); KO n=21 (males n=11, females n=10)].
Extended Data Fig 5f 12 Week MRI Total Brain Volume		
Total Brain Volume (12 weeks)	Genotype	One-Way ANOVA, Main effect of Genotype: $F_{(2,63)}$=0.78, *P=0.461526*; Post Hoc Test: WT vs HET, *P=0.933318*, WT vs KO, *P=0.513767*, HET vs KO, *P=0.310622*. [WT n=23 (males n=13, females n=10); HET n=21 (males n=11, females n=10); KO n=23 (males n=13, females n=10)].
Extended Data Fig 5b Adult Ki67:		
Ki67	Genotype	One-Way ANOVA, Main effect of Genotype: $F_{(2,17)}$=0.311, *P=0.737*; Post Hoc Test: WT vs HET, *P=0.826*, WT vs KO, *P=0.564*, HET vs KO, *P=0.700*. WT n=9; HET n=11; KO n=7.
Extended Data Fig 6d Adult Doublecortin:		
Doublecortin	Genotype	One-Way ANOVA, Main effect of Genotype: $F_{(2,24)}$=0.025, *P*=0.976; Post Hoc Test WT vs HET, *P=0.864*, WT vs KO, *P=0.987*, HET vs KO, *P=0.980*. WT n=8; HET n=7; KO n=5.
Extended Data Fig 6f Adult BrdU:		
Adult BrdU	Genotype	One-Way ANOVA, Main effect of Genotype: $F_{(2,17)}$=0.8184, *P=0.4578*; Post Hoc Test : WT vs HET, *P=0.516*, WT vs KO, *P=0.899*, HET vs KO, *P=0.336*. WT n=6; HET n=5; KO n=5.
Extended Data Fig 7a P17 CTIP2 Thickness:		
Layer 5	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,22)}$=0.222, *P=0.802586;* Post Hoc Test: WT vs HET, *P=0.724153*, WT vs KO, *P=0.781612*, HET vs KO, *P=0.80008*. WT n=7; HET n=8; KO n=10.
Layer 6	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,22)}$=1.380, *P=0.272499;* Post Hoc Test: WT vs HET, *P=0.818344*, WT vs KO, *P=0.318958*, HET vs KO, *P=0.225015*. WT n=7; HET n=8; KO n=10.
Extended Data Fig 7b P17 CTIP2 Cell count:		
CTIP2 Cell Count	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,22)}$=0.8632, *P=0.435623;* Post Hoc Test: WT vs HET, *P=0.396733*, WT vs KO, *P=0.332829*, HET vs KO, *P=0.741139*. WT n=7; HET n=8; KO n=10.
Extended Data Fig 7c P17 CUX1 Thickness:		

Layer 2/3	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,22)}$=0.171, *P=0.844013;* Post Hoc Test: WT vs HET, *P=0.907442*, WT vs KO, *P=0.680241*, HET vs KO, *P=0.854843*. WT n=7; HET n=9; KO n=9.
Layer 4	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,22)}$=1.367, *P=0.275636;* Post Hoc Test: WT vs HET, *P=0.232292*, WT vs KO, *P=0.383204*, HET vs KO, *P=0.433842*. WT n=7; HET n=9; KO n=9.
Extended Data Fig 7d P17 CUX1 Cell count:		
Cux1 Cell Count	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,22)}$=2.2387, *P=0.130327 ;* Post Hoc Test: WT vs HET, *P=0.115922*, WT vs KO, *P=0.115267*, HET vs KO, *P=0.660193*. WT n=7; HET n=9; KO n=9.
Extended Data Fig 7e P17 TBR1 Thickness:		
Layer 2/3	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,22)}$=1.571, *P=0.230184;* Post Hoc Test: WT vs HET, *P=0.206753*, WT vs KO, *P=0.637221*, HET vs KO, *P=0.213870*. WT n=7; HET n=8; KO n=10.
Layer 5	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,22)}$=1.092, *P=0.352944;* Post Hoc Test: WT vs HET, *P=0.285996*, WT vs KO, *P=0.784512*, HET vs KO, *P=0.373000*. WT n=7; HET n=8; KO n=10.
Layer 6	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,22)}$=0.495, *P=0.616206;* Post Hoc Test: WT vs HET, *P=0.564280*, WT vs KO, *P=0.527680*, HET vs KO, *P=0.699315*. WT n=7; HET n=8; KO n=10.
Extended Data Fig 7f P17 TBR1 Cell count:		
TBR1 Cell Count	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,22)}$=1.4330, *P=0.260013;* Post Hoc Test: WT vs HET, *P=0.203185*, WT vs KO, *P=0.352657*, HET vs KO, *P=0.421523*. WT n=7; HET n=8; KO n=10.
Extended Data Fig 7g P17 SATB2 Cell count:		
SATB2 Cell Count	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,21)}$=1.7862, *P=0.192138 ;* Post Hoc Test: WT vs HET, *P=0.172252*, WT vs KO, *P=0.582604* , HET vs KO, *P=0.203926*. WT n=7 ; HET n=8; KO n=9.
Extended Data Fig 7h P17 Cortical thickness:		
Cortical Thickness	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,21)}$=1.338, *P=0.283955;* Post Hoc Test: WT vs HET, *P=0.530166*, WT vs KO, *P=0.363614* , HET vs KO, *P=0.281402*. WT n=7 ; HET n=8; KO n=9.
Extended Data Fig 8a E15 TBR1 Thickness:		
TBR1 Thickness	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,15)}$=0.1145, *P=0.892568 ;* Post Hoc Test: WT vs HET, *P=0.841831*, WT vs KO, *P=0.773501* , HET vs KO, *P=0.874162*. WT n=7; HET n=6 ; KO n=5.
Extended Data Fig 8b E15 TBR1 Cell count:		
TBR1 Cell Count	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,15)}$=1.292, *P=0.303687;* Post Hoc Test: WT vs HET, *P=0.804485*, WT vs KO, *P=0.208347* , HET vs KO, *P=0.289661*. WT n=7; HET n=6; KO n=5.
Extended Data Fig 8c E15 TBR2 Thickness:		
TBR2 Thickness	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,11)}$=0.6467, *P=0.542568;* Post Hoc Test: WT vs HET, *P=0.482814*, WT vs KO, *P=0.681760*, HET vs KO, *P=0.506413*. WT n=6; HET n=5; KO n=3.
Extended Data Fig 8d E15 TBR2 Cell count:		
TBR2 Cell Count	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,11)}$=1.148, *P=0.352568;* Post Hoc Test: WT vs HET, *P=0.624609*, WT vs KO, *P=0.290677*, HET vs KO, *P=0.280855*. WT n=6; HET n=5; KO n=3.
Extended Data Fig 8e E15 TUJ1 Thickness:		
Tuj1 Thickness	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,15)}$=0.8977, *P=0.428316;* Post Hoc Test: WT vs HET, *P=0.392853*, WT vs KO, *P=0.583002*, HET vs KO, *P=0.444947*. WT n=7; HET n=5; KO n=6.
Extended Data Fig 8f E15 TUJ1 Intensity:		
Tuj1 Intensity	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,15)}$=2.827, *P=0.090807;* Post Hoc Test: WT vs HET, *P=0.075355*, WT vs KO, *P=0.253716*, HET vs KO, *P=0.252449*. WT n=7; HET n=5; KO n=6.
Extended Data Fig 9c Zebrafish RhoA Western Blot:		
Total RhoA Levels	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,33)}$=3.5887, *P=0.038885;* Post Hoc Test: WT vs HET, *P=0.641048*, WT vs KO, ***P=0.043282***, HET vs KO, ***P=0.048624***. WT n=12; HET n=12; KO n=12.
Extended Data Fig 9d RhoA Developmental Profile:		
Total RhoA Levels at E15	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,32)}$=0.9163, *P=0.410223*; Post Hoc Test: WT vs HET, *P=0.413038*, WT vs KO, *P=0.661119*, HET vs KO, *P=0.420873*. WT n=11; HET n=16; KO n=8.
Total RhoA Levels at P7	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,27)}$=0.48339, *P=0.621925*; Post Hoc Test: WT vs HET, *P=0.900836*, WT vs KO, *P=0.396664*, HET vs KO, *P=0.590961*. WT n=10; HET n=14; KO n=6.

Total RhoA Levels at P18	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,29)}$=4.2365, ***P=0.024313;*** Post Hoc Test: WT vs HET, ***P=0.030324***, WT vs KO, ***P=0.032890***, HET vs KO, *P=0.706593*. WT n=11; HET n=10; KO n=11.
Total RhoA Levels at 4-6 weeks	Genotype	One-way ANOVA. Main effect of Genotype: $F_{(2,29)}$=5.9422, ***P=0.00687***; Post Hoc Test: WT vs HET, ***P=0.01506***, WT vs KO, ***P=0.04642***, HET vs KO, *P=0.36932*. WT n=14; HET n=11; KO n=7.

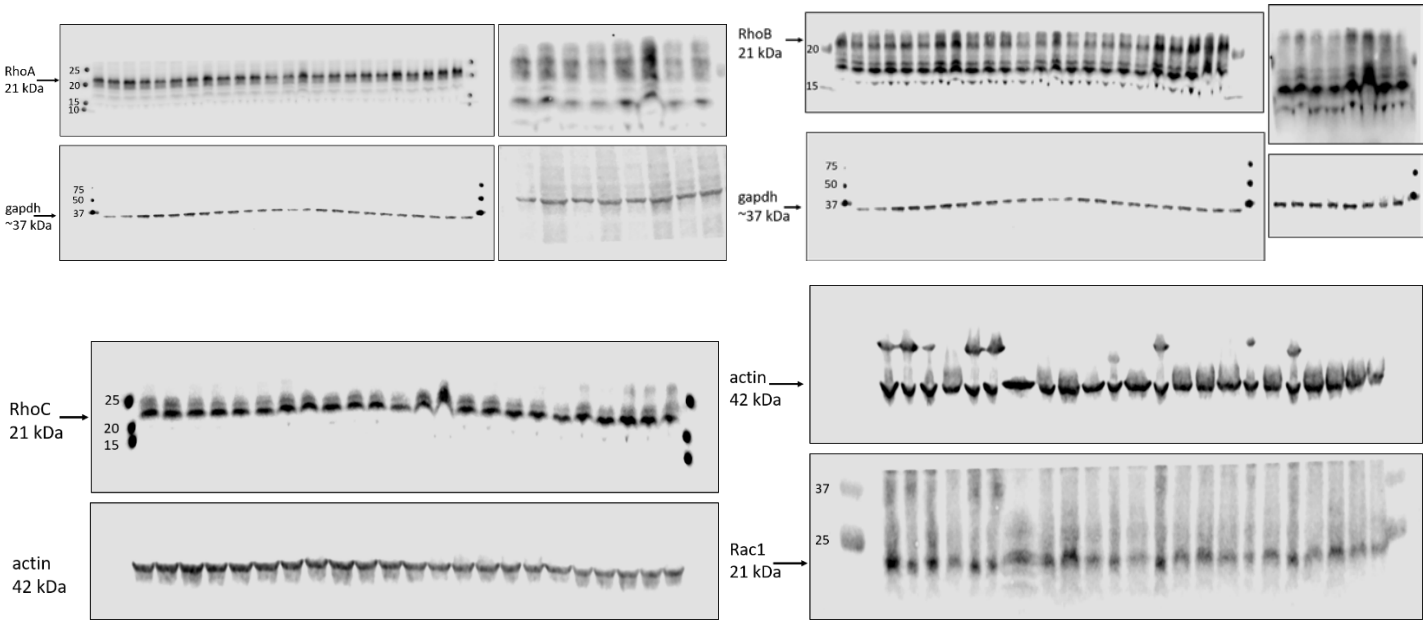
Parameter	Comparison	Results
Extended Data Fig 10a-b Locomotor habituation		
Average beam breaks	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,61)}$=1.062, *P*=0.307; **main effect of genotype: $F_{(2,61)}$=4.795; *P*=0.012**, sex X genotype interaction: $F_{(2,63)}$=1.506, *P*=0.230. **Post-hoc: Newman-Keuls test: WT vs HET *P*=0.023, WT vs KO *P*=0.018, HET vs KO *P*=0.631.**
Total beam breaks	Sex, genotype and bin	3 way ANOVA; main effect of sex: $F_{(1,61)}$=1.062, *P*=0.307; **main effect of genotype: $F_{(2,61)}$=4.795, *P*=0.012**; **main effect of bin: $F_{(23,1403)}$=111.244, *P*=0.0001**; sex X genotype interaction: $F_{(2,61)}$=1.506, *P*=0.230; sex X bin interaction: $F_{(23,1403)}$=1.284, *P*=0.166; genotype x bin interaction: $F_{(46,1403)}$=0.982, *P*=0.509; sex X genotype X bin interaction: $F_{(46,1403)}$=1.313, *P*=0.080. **Post-hoc: Newman-Keuls test: WT vs HET *P*=0.023, WT vs KO *P*=0.018, HET vs KO *P*=0.631.**
Extended Data Fig 10c-d Elevated plus maze		
Distance travelled	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,62)}$=0.543, *P*=0.584; main effect of genotype: $F_{(2,62)}$=2.480, *P*=0.120; sex X genotype interaction: $F_{(2,62)}$=0.648, *P*=0.508
Time in open arms/time in both arms	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,62)}$=1.195, *P*=0.340; main effect of genotype: $F_{(2,62)}$=0.473, *P*=0.494; sex X genotype interaction: $F_{(2,62)}$=1.509, *P*=0.229
Entries to open arms/entries to both arms	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,62)}$=0.506, *P*=0.605; main effect of genotype: $F_{(2,62)}$=0.004, *P*=0.953; sex X genotype interaction: $F_{(2,62)}$=2.762, *P*=0.071
Extended Data Fig 10e Light/dark		
Total Activity	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,61)}$=1.770, *P*=0.188; main effect of genotype: $F_{(2,62)}$=0.933, *P*=0.399; sex X genotype interaction: $F_{(2,61)}$=1.206, *P*=0.304
Latency to enter dark side	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,61)}$=3.262, *P*=0.076; main effect of genotype: $F_{(2,62)}$=0.169, *P*=0.846; sex X genotype interaction: $F_{(2,61)}$=0.016, *P*=0.984
Time in light side	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,61)}$=2.449, *P*=0.122; main effect of genotype: $F_{(2,62)}$=2.221, *P*=0.117; sex X genotype interaction: $F_{(2,61)}$=2.791, *P*=0.069
Time in dark side	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,61)}$=2.464, *P*=0.122; main effect of genotype: $F_{(2,61)}$=2.213, *P*=0.118; sex X genotype interaction: $F_{(2,61)}$=2.780, *P*=0.070
Extended Data Fig 10f Open field		
Distance travelled	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,61)}$=2.266, *P*=0.137; main effect of genotype: $F_{(2,62)}$=2.514, *P*=0.089; sex X genotype interaction: $F_{(2,61)}$=0.707, *P*=0.497
Time in periphery	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,61)}$=0.009, *P*=0.925; main effect of genotype: $F_{(2,62)}$=0.122, *P*=0.886; sex X genotype interaction: $F_{(2,61)}$=0.889, *P*=0.416
Time in center	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,61)}$=0.269, *P*=0.606; main effect of genotype: $F_{(2,62)}$=2.253, *P*=0.114; sex X genotype interaction: $F_{(2,61)}$=0.252, *P*=0.778
Time center/time in periphery	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,61)}$=0.001, *P*=0.972; main effect of genotype: $F_{(2,62)}$=1.349, *P*=0.267; sex X genotype interaction: $F_{(2,61)}$=0.520, *P*=0.597
Extended Data Fig 10g Rotarod		
Latency to fall	Sex, genotype and trial	3-way rmANOVA; **main effect of sex: $F_{(1,61)}$=22.607, *P*=0.0001**; main effect of genotype: $F_{(2,61)}$=1.496, *P*=0.232; **main effect of trial: $F_{(7,427)}$=29.585, *P*=0.0001**; sex X genotype interaction: $F_{(2,61)}$=0.107, *P*=0.899; sex X trial interaction: $F_{(7,427)}$=1.853, *P*=0.076; genotype x trial interaction: $F_{(14,427)}$=1.017, *P*=0.435; sex X genotype X trial interaction: $F_{(14,427)}$=1.046, *P*=0.406
Extended Data Fig 10h Hot plate		
Latency to lick the paw	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,61)}$=0.632, *P*=0.430; main effect of genotype: $F_{(1,62)}$=0.713, *P*=0.494; sex X genotype interaction: $F_{(2,61)}$=0.565, *P*=0.572
Extended Data Fig 10i Olfactory food finding		
Time to find food	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,63)}$=2.180, *P*=0.145; main effect of genotype: $F_{(2,63)}$=0.552, *P*=0.579; sex X genotype interaction: $F_{(2,63)}$=1.080, *P*=0.346
Extended Data Fig 10j-k Grooming		
Time	Sex and genotype	2 way ANOVA; **main effect of sex: $F_{(1,61)}$=4.600, *P*=0.036**; main effect of genotype: $F_{(2,62)}$=1.700, *P*=0.191; sex X genotype interaction: $F_{(2,61)}$=0.291, *P*=0.749
Bouts	Sex and genotype	2 way ANOVA; **main effect of sex: $F_{(1,61)}$=5.160, *P*=0.027**; main effect of genotype: $F_{(2,62)}$=0.659, *P*=0.521; sex X genotype interaction: $F_{(2,62)}$=0.791, *P*=0.458
Extended Data Fig 10l Marble burying		
Marbles buried	Sex and genotype	2 way ANOVA; main effect of sex: F(1,61)=2.019, *P*=0.157; main effect of genotype: F(1,62)=2.335, *P*=0.105; sex X genotype interaction: F(2,61)=0.005, *P*=0.996
Extended Data Fig 10m Three-chamber sociability test - *Social preference*		

Time spent in quadrants	Sex, genotype and interaction target (inanimate vs social)	3-way ANOVA; main effect of sex: $F_{(1,122)}$=1.282, *P*=0.260; main effect of genotype: $F_{(2,122)}$=0.159, *P*=0.853; **main effect of target: $F_{(1,122)}$=84.610, *P*=0.0001**; sex X genotype interaction: $F_{(2,122)}$=0.592, *P*=0.555; sex X target interaction: $F_{(1,122)}$=6.547, *P*=0.012; genotype x target interaction: $F_{(2,122)}$=0.094, *P*=0.910; sex X genotype X target interaction: $F_{(2,122)}$=0.882, *P*=0.416
Extended Data Fig 10n Caged-conspecific social interaction		
Time spent sniffing	Sex, genotype and trial (inanimate vs social)	3-way rmANOVA; **main effect of sex: $F_{(1,61)}$=8.446, *P*=0.005**; main effect of genotype: $F_{(2,61)}$=0.714, *P*=0.494; **main effect of trial: $F_{(1,61)}$=50.016, *P*=0.0001**; sex X genotype interaction: $F_{(2,61)}$=1.762, *P*=0.180; sex X trial interaction: $F_{(1,61)}$=10.148, *P*=0.002; genotype x trial interaction: $F_{(2,61)}$=1.007, *P*=0.371; sex X genotype X trial interaction: $F_{(2,61)}$=0.780, *P*=0.463
Extended Data Fig 10o Nest building		
Increase in nest height	Sex, genotype and time	3-way rmANOVA; main effect of sex: $F_{(1,63)}$=0.132, *P*=0.718; main effect of genotype: $F_{(2,63)}$=1.645, *P*=0.207; **main effect of time: $F_{(2,126)}$=21.707, *P*=0.0001**; sex X genotype interaction: $F_{(2,63)}$=2.500, *P*=0.0905; sex X time interaction: $F_{(2,126)}$=0.205, *P*=0.815; genotype x time interaction: $F_{(4,126)}$=0.170, *P*=0.954; sex X genotype X time interaction: $F_{(4,126)}$=1.823, *P*=0.129
Increase in nest width	Sex, genotype and time	3-way rmANOVA; main effect of sex: $F_{(1,63)}$=0.140, *P*=0.709; main effect of genotype: $F_{(2,63)}$=0.9025, *P*=0.411; **main effect of time: $F_{(2,126)}$=11.637, *P*=0.0001**; sex X genotype interaction: $F_{(2,63)}$=1.923, *P*=0.155; sex X time interaction: $F_{(2,126)}$=0.441, *P*=0.644; genotype x time interaction: $F_{(4,126)}$=0.493, *P*=0.741; sex X genotype X time interaction: $F_{(4,126)}$=1.292, *P*=0.277
Extended Data Fig 10q-r Novel object recognition		
Sniffing an object in a new location	Sex, genotype and object	3-way ANOVA; **main effect of sex: $F_{(1,174)}$=5.121, *P*=0.025**; main effect of genotype: $F_{(2,174)}$=1.435, *P*=0.241; **main effect of object: $F_{(2,174)}$=15.121, *P*=0.0001**; sex X genotype interaction: $F_{(2,174)}$=0.218, *P*=0.804; sex X object interaction: $F_{(2,174)}$=20.861, *P*=0.060; genotype x object interaction: $F_{(2,174)}$=0.730, *P*=0.573; sex X genotype X object interaction: $F_{(4,174)}$=0.687, *P*=0.602
Sniffing novel object	Sex, genotype and object	3-way ANOVA; main effect of sex: $F_{(1,174)}$=3.685, *P*=0.057; main effect of genotype: $F_{(2,174)}$=885, *P*=0.414; **main effect of object: $F_{(2,174)}$=34.313, *P*=0.0001**; sex X genotype interaction: $F_{(2,174)}$=0.485, *P*=0.617; **sex X object interaction: $F_{(2,174)}$=3.105, *P*=0.018**; genotype x object interaction: $F_{(2,174)}$=1.081, *P*=0.367; sex X genotype X object interaction: $F_{(4,174)}$=0.709, *P*=0.587
Extended Data Fig 10s Fear conditioning – *Context*		
% Freezing	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,63)}$=1.800, *P*=0.184; main effect of genotype: $F_{(2,63)}$=1.516, *P*=0.227; sex X genotype interaction: $F_{(2,63)}$=1.917, *P*=0.156
Extended Data Fig 10t Fear conditioning - *Cued*		
% Freezing	Sex, genotype and tone	3-way ANOVA; main effect of sex: $F_{(1,63)}$=0.103, *P*=0.749; main effect of genotype: $F_{(2,63)}$=0.721, *P*=0.490; **main effect of tone: $F_{(1,63)}$=910.619, *P*=0.0001**; sex X genotype interaction: $F_{(2,63)}$=1.060, *P*=0.353; **sex X tone interaction: $F_{(1,63)}$=0.0001, *P*=0.993**; genotype x tone interaction: $F_{(2,63)}$=0.176, *P*=0.839; sex X genotype X tone interaction: $F_{(2,63)}$=0.934, *P*=0.398
Extended Data Fig 10u-v Morris water maze - *Training*		
Latency	Sex, genotype and day	3-way rmANOVA; main effect of sex: $F_{(1,63)}$=0.297, *P*=0.588; main effect of genotype: $F_{(2,63)}$=0.715, *P*=0.493; **main effect of day: $F_{(9,567)}$=36.141, *P*=0.0001**; sex X genotype interaction: $F_{(2,63)}$=0.597, *P*=0.553; sex X day interaction: $F_{(9,567)}$=0.792, *P*=0.624; genotype x day interaction: $F_{(18,567)}$=1.388, *P*=0.131; sex X genotype X day interaction: $F_{(18,567)}$=0.866, *P*=0.620
Speed	Sex, genotype and day	3-way rmANOVA; main effect of sex: $F_{(1,63)}$=0.209, *P*=0.649; main effect of genotype: $F_{(2,63)}$=0.278, *P*=0.758; **main effect of day: $F_{(9,567)}$=8.291, *P*=0.0001**; sex X genotype interaction: $F_{(2,63)}$=0.121, *P*=0.886; sex X day interaction: $F_{(9,567)}$=3.778, *P*=0.0001; genotype x day interaction: $F_{(18,567)}$=1.836, *P*=0.0001; sex X genotype X day interaction: $F_{(18,567)}$=0.443, *P*=0.978
Extended Data Fig 10w Morris water maze - *Probe trial*		
Time thigmotaxis	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,63)}$=0.127, *P*=0.723; main effect of genotype: $F_{(2,63)}$=0.617, *P*=0.543; sex X genotype interaction: $F_{(2,63)}$=2.527, *P*=0.088
Time spent in quadrants	Sex, genotype and quadrant	3-way ANOVA; main effect of sex: $F_{(1,252)}$=0.0001, *P*=1.000; main effect of genotype: $F_{(2,252)}$=0.0001, *P*=1.000; **main effect of quadrant: $F_{(3,252)}$=44.679, *p*=0.0001**; sex X genotype interaction: $F_{(2,252)}$=0.0001, *P*=1.000; sex X quadrant interaction: $F_{(3,252)}$=0.987, *P*=0.399; genotype x quadrant interaction: $F_{(6,252)}$=0.606, *P*=0.723; **sex X genotype X quadrant interaction: $F_{(6,252)}$=2.281, *P*=0.037**
Extended Data Fig 10x Morris water maze - *Visible platform*		
Latency	Sex, genotype and trial	3-way rmANOVA; main effect of sex: $F_{(1,63)}$=2.618, *P*=0.111; main effect of genotype: $F_{(2,63)}$=0.953, *P*=0.391; **main effect of trial: $F_{(2,126)}$=6.104, *P*=0.003**; sex X genotype interaction: $F_{(2,63)}$=1.572, *P*=0.216; sex X trial interaction: $F_{(4,126)}$=0.471, *P*=0.626; genotype x trial interaction: $F_{(4,126)}$=0.536, *P*=0.710; sex X genotype X trial interaction: $F_{(4,126)}$=0.353, *P*=0.841
Extended Data Fig 10y-z Pre-pulse inhibition		
% inhibition	Sex, genotype and decibel	3-way rmANOVA; main effect of sex: $F_{(1,62)}$=1.678, *P*=0.200; main effect of genotype: $F_{(2,62)}$=0.806, *P*=0.451; **main effect of trial: $F_{(3,186)}$=98.497, *P*=0.0001**; sex X genotype interaction: $F_{(2,62)}$=0.318, *P*=0.729; sex X trial interaction: $F_{(3,186)}$=0.327, *P*=0.806;

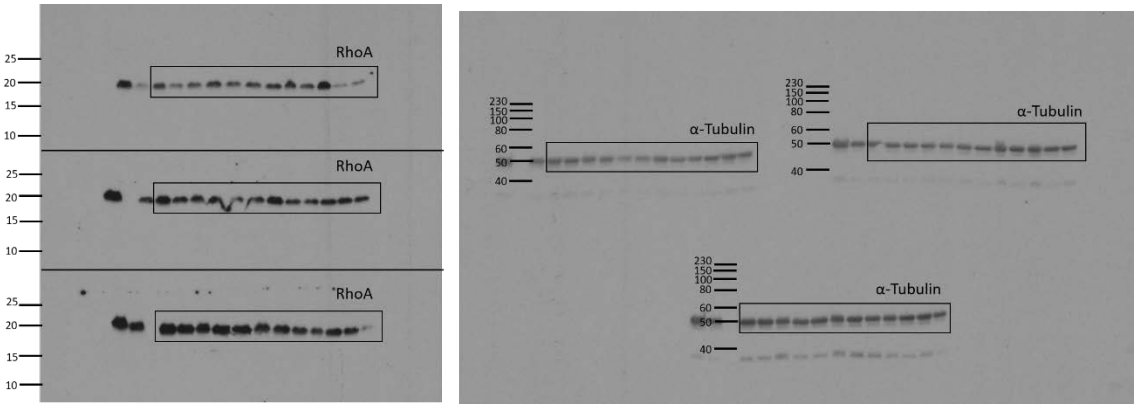
		genotype x trial interaction: $F_{(6,186)}$=0.661, *P*=0.681; sex X genotype X trial interaction: $F_{(6,186)}$=0.117, *P*=0.994
Startle response	Sex and genotype	2 way ANOVA; main effect of sex: $F_{(1,62)}$=1.180, *P*=0.282; main effect of genotype: $F_{(2,62)}$=0.487, *P*=0.617; sex X genotype interaction: $F_{(2,62)}$=0.067, *P*=0.936

Supplementary Online Figure S1. Blot Source Images

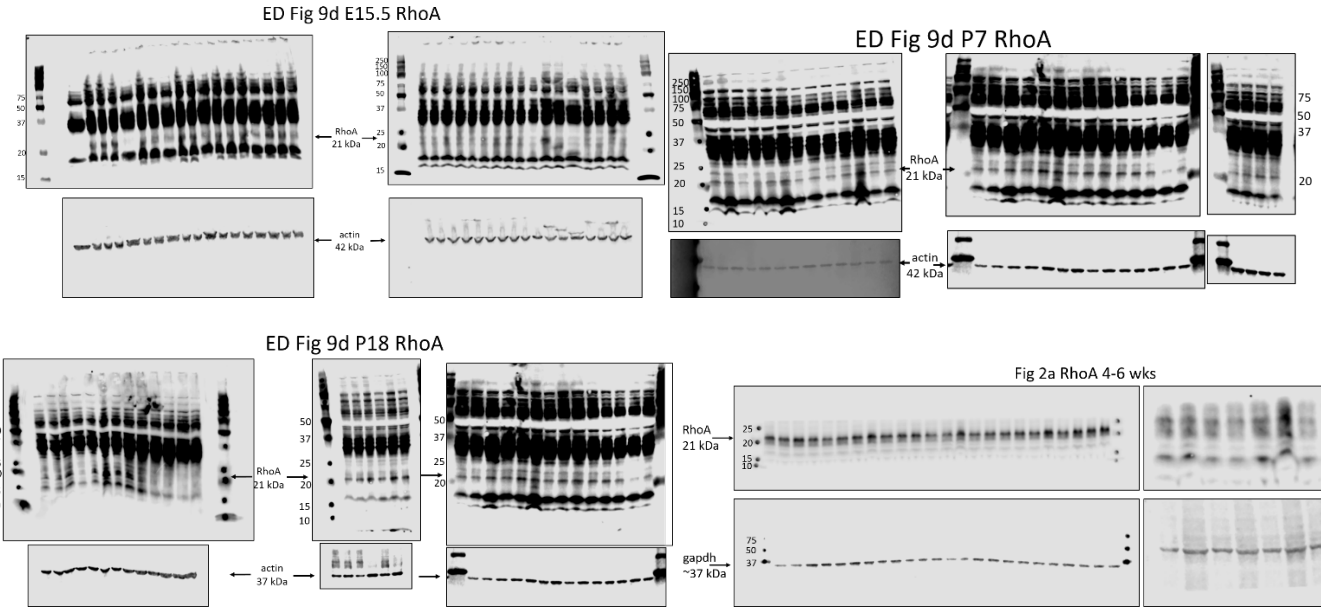
Fig 2a & ED Fig 2a



ED Fig 9a



ED Fig 9d



Main text paragraphs with extra references

When *Kctd13* was selectively and globally reduced or deleted in mice, we found no change in multiple measures of brain size, embryonic cell proliferation, neurogenesis, or cortical layering/migration. Given that previous studies of cultured cells, of brain slice culture, and of IUE overexpression of WT or active RhoA suggest effects on cell proliferation and migration[64–67], we determined the developmental time course of increased RhoA expression in *Kctd13* mutant brains. *Kctd13* deletion does not lead to elevated RhoA until after P7 (Extended Data Fig. 9d). Thus, compensation for loss of *Kctd13* or other mechanisms maintaining RhoA levels may be present during early brain development. Because increased RhoA in *Kctd13* mutants occurs later in development, our data do not challenge the role that RhoA may play in early brain development including neurogenesis or cortical layering/migration.

We propose several potential explanations for why our data differ from a previous study of *kctd13* in zebrafish and of *Kctd13* in mice[8]. First, the previous study used shRNA and morpholinos, approaches that may have off-target effects[68–72] not controlled for in previous experiments[8]. Second, previous IUE work in mice knocked down levels of *Kctd13* in only a subset of neural progenitors; this may have resulted in 'competition' with nearby WT neural progenitors for resources modulating embryonic neurogenesis. Third, both shRNA IUE in mice and morpholino approaches in zebrafish may not result in the same compensation as in genetic knockout[73]. Alternatively, other genes in the 16p11.2 region may act alone, in combination, or in concert with *Kctd13* to alter brain size.

None of the 29 genes in the 16p11.2 region has yet been individually implicated in human autism spectrum disorders[74], causing geneticists and others to suggest that this recurrent deletion syndrome is polygenic in nature rather than the result of deletion of a single gene within the region. Thus, we hypothesized that *Kctd13* deletion alone would not be sufficient to induce multiple behavioural abnormalities with face validity to autism or associated neuropsychiatric disorders such as attention-deficit/hyperactivity disorder. Indeed, extensive behavioural analysis of *Kctd13* mutants largely supported this hypothesis. We did identify increased locomotor activity as the primary altered behavioural phenotype in *Kctd13* mutants (Extended Data Fig. 10a, b). This phenotype may have some face validity for the approximately 20% incidence of attention-deficit/hyperactivity disorder in patients with 16p11.2 (ref. 75). Additional behavioural results are in Extended Data Fig. 10.

Extra references

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