Supplemental Materials

Materials and Methods

Stereology

We used the Cavalieri, the Optical Dissector/Fractionator and Nucleator methods (Stereo-Investigator; MBF Bioscience, Williston, VT) as previously described (Gundersen et al., 1988; West, 1993; Manaye, 2007).

In order to assess volume of the cerebellum, sampling was carried out through the entire cerebellum. Every tenth 40-µm thick sagittal CV stained section was systematically sampled with a random start within the first five sections throughout the entire cerebellum (n=3-4 mice of both sexes/genotype). In order to evaluate number of Purkinje cells (PCs) and their soma size, we collected every twentieth CV stained section from the entire cerebellum. Optical Fractionator with the Disector was placed randomly according to a 100 × 100 µm grid. The counting frame was 30 × 30 µm. An estimation of cell population number was provided using the overall raw counts and mean section thickness. Nucleator was used simultaneously with Optical Fractionator/Disector. We selected a point inside the PC nucleus, the nucleolus, and the Stereo-Investigator system created 6 orthogonal rays. Intersections of the rays with PS soma boundary were marked and soma volume was automatically calculated. On average, 1100 sites were counted per each mouse.

Behavioral tests

Elevated plus maze

Anxiety was evaluated in the elevated plus-maze test. A mouse was placed on the starting platform in the plus maze (San Diego Instruments Inc., San Diego, CA, USA) and behavior was videotaped for 5 min. An experienced observed blind to the group's identity later scored the time spent in the closed and open arms.

Open field test

Locomotor activity was assessed over a 30-min period using activity chambers with infrared beams (San Diego Instruments Inc., San Diego, CA, USA). Horizontal and vertical activity and time spent in the center or along the walls (thigmotaxis) of the chamber were automatically recorded.

Y-Maze test

Spatial working memory was assessed by spontaneous alternation in the y-maze. Mice were placed in the end of one of the three arms and allowed to explore freely for 5 minutes.

Spontaneous alternation was calculated as the number of times the mouse entered three different arms consecutively divided by the total visits.

Spatial recognition memory was assessed in two trials in the y-maze. In the first trial, one of the three arms was blocked and each mouse explored the two open arms for 5 minutes. 30 minutes following the first trial, all three arms were open and mice were allowed to explore for an additional 5 minutes. The amount of time spent exploring the newly unblocked arm during the first two minutes of trial 2 was determined.

Fear Conditioning

Associative memory was assessed using fear conditioning. Mice were placed in a shockbox (Coulbourn Instruments, MA) and baseline freezing behavior was measured for 2 minutes, after which a 20-s white noise tone with a scrambled 2s 0.5 mA footshock coterminating with the tone was delivered. 24 hours following fear conditioning, mice were placed in the shockbox once again, and freezing behavior in response to the context was measured. 48 hours following fear conditioning, mice were placed in a novel environment inside the shockbox, and baseline freezing behavior was measured for 2 minutes. At the beginning of the third minute, a continuous tone was delivered for the remainder of the test. Freezing behavior was recorded automatically using Cleversys Freezescan (Cleversys).

Rotarod

Motor coordination in mice was assessed using the rotarod test (Rotamex 4/8, Columbus Instruments International, Columbus, OH). For training and testing the speed of the rotarod was set to 4 rpm and increased by 0.1 rpm/second. Mice received 5 trials over 3 consecutive days. Mice were allowed to rest for 30 minutes between trials. The data from each group on the last day was averaged and charted.

Supplemental figure legends

SFig.1. Schematic of the Tet-off system

To express mutant DISC1 in the cerebellum, heterozygous Parv2A-tTA2 single transgenic mice (regulatory line) were crossed with homozygous single transgenic TRE-mutant DISC1 mice (responder line). The regulatory line produces tetracycline transactivator (tTA) that binds to the tetracycline regulatory elements (TRE) located upstream of the CMV minimal promoter as a part of the transgene construct of the responder line. Binding of tTA to TRE activates transcription of the transgene (*myc*-tagged mutant DISC1) by the responder line.

SFig.2. **Variable levels of activity of the Parv2A promoter in the cerebellum**

Immunofluorescent co-staining of the brain sections from a control-tdTom mouse with anti-calbindin (green) and anti-mCherry (red; to detect tdTomato expression) antibodies.
Note increased expression of tdTomato in PCs of Lobule II (A) of the anterior cerebellum and decreased expression of tdTomato in PCs of lobule IX (B) of the posterior cerebellum; scale bar – 20 µm.

SFig.3. No genotype-related alterations in sociability

(a) Both control and mutant DISC1 male mice spent significantly more time exploring a novel mouse (grey bar) compared to an inanimate object (black bar); the Y-axis depicts time spent exploring either a live mouse or an object as the percentage of total exploration time; n=12-16 mice per group, * denotes $p<0.05$ vs. object;

(b) Both control and mutant female mice spent significantly more time exploring a novel mouse (grey bar) compared to an inanimate object (black bar); the Y-axis depicts time spent exploring either live mouse or object as the percentage of total exploration time; n=9-13 mice per group, * denotes $p< 0.05$ vs. object.

SFig.4. **No genotype-related alterations in anxiety-like behavior in the elevated plus maze**

We found no significant effects of sex or genotype in % time spent in the open arms of the elevated plus maze; the Y axis shows the time spent in open arms as the percentage of the total time spent in open and closed arms; n=9-16 mice per group.

SFig.5. No genotype-dependent changes in ambulatory activity

We observed no significant effects of genotype or sex on total horizontal activity in open field; the Y axis depicts the number of beams breaks in the activity chambers equipped with infrared beams; the X axis depicts the time intervals (min); n=9-16 mice per group

SFig.6. No genotype-related changes in spatial working memory or spatial recognition

(a) We found not effects of genotype or sex on spontaneous alternation in Y-maze; the Y-axis depicts the number of alternations for each group; alternations were calculated as a ratio of complete triads (non-repetitive visit to all 3 arms of the maze) to total arm visits during 5 minutes.

(b) We detected no genotype or sex effects on time spent in exploring the previously unexplored arm of the Y-maze; mice were initially exposed to 2 arms of the maze with one arm being blocked; 30 minutes later the mice were returned to the maze with all arms accessible for exploration; the Y-axis depicts time spent exploring the previously locked arm as the percentage of time spend exploring all three arms during 2 minutes; n=9-16 mice per group

SFig.7. No genotype-dependent alterations in fear conditioning

(a) We found no effects of genotype or sex on freezing measured immediately after foot shock presentation (training); freezing behavior increases over time for all mice following training to associate a shock with the tone; the Y-axis depicts freezing time as the percentage of total recording time (1 minute); the X-axis depicts one-minute time intervals for sampling of freezing behavior; arrow indicates presentation of shock and tone; n=9-16 mice per group;

(b) We observed no genotype-related difference in context-dependent freezing behavior between control mice and mutant DISC1 mice; the Y-axis depicts freezing time as the percentage of total recording time (5 minutes); n=9-16 mice per group;

(c) We detected a significant effect of sex on cue-dependent freezing behavior, with no differences between mutant DISC1 and control mice; Y-axis depicts freezing time as the percentage of total recording time (5 minutes); n=9-16 mice per group, *** denotes $p<0.001$ vs. cue-dependent freezing in males.

SFig.8. No genotype-dependent changes in motor coordination

All mice spent a similar amount of time on the accelerated rotarod; the Y-axis depicts the latency to fall from the rotating rod; n=9-16 mice per group

SFig.9. No genotype effects on total cerebellar volume or number of PCs

Stereological analyses performed at P21 and P150 did not find any genotype-dependent changes in:

(a) Estimated volume of the cerebellum or

(b) Estimated Purkinje cell population; n=3-4 mice per group

SFig.10. No genotype-related difference in PC dendritic arborization

Representative examples of PCs filled *ex vivo* with Alexa 488 dye in cerebellar slices of control-tdTom (Control) and mutant DISC1-tdTom (Mutant) mice, scale bar - 20 mm; using “Imaris” software-assisted reconstruction of dendritic trees, quantitative analyses found no significant effects of genotype on: (b) area occupied by the dendritic tree; (c) total length of dendritic tree; (d) number of branches; (e) branch length; n=6-10 mice per genotype.

SFig.11. **No significant effects of genotype on expression of inflammatory markers**

Immunohistochemical staining of the brain sections from control and mutant DISC1 mice detected no genotype-dependent differences in:

(a) Iba1-positive immunoreactivity; control (left panel) and mutant DISC1 (right panel);

(b) GFAP-positive immunoreactivity; control (left panel) and mutant DISC1 (right panel); scale bar – 100 μm

SFig.12. No genotype-dependent changes in the time to peak, decay time and half-width of mEPSCs

Estimation of the miniature excitatory synaptic currents (mEPSCs) from PCs in the presence of 500 nM tetrodotoxin (TTX) didn't find any group differences in the time to peak, half-width or decay time in mutant DISC1-tdTom PCs compared to controls:

(a) averaged values of time to peak,

(b) 90%-10% decay time

(c) half-width, PCs in control-tdTom (Control, n=15 cells, from 8 mice) or mutant DISC1-tdTom (Mutant, n=14 cells from 5 mice)

SFig.13. No significant effects of the genotype on the probability of glutamate release

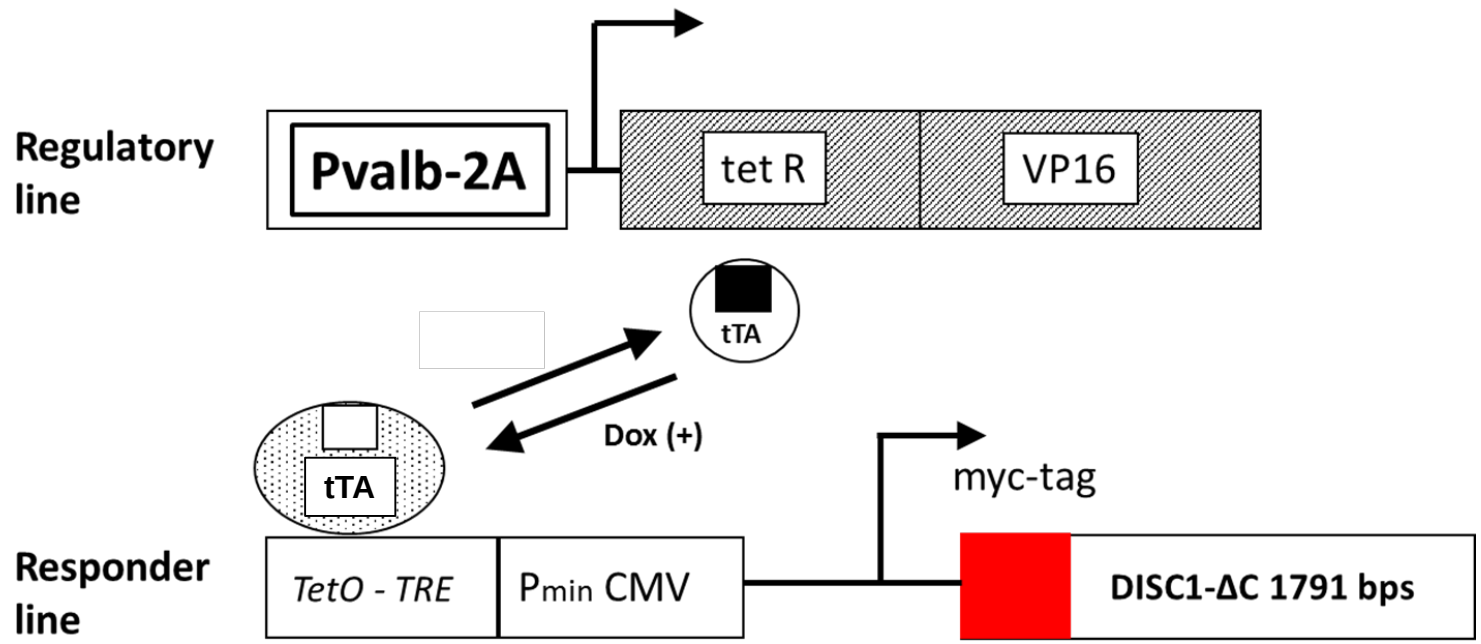
Assessment of the effects of mutant DISC1 on the probability of glutamate release by measuring paired-pulse ratios (PPR) didn't detected differences in the amplitude of evoked EPSCs in PCs of control-tdTom and mutant DISC1-tdTom mice:

(a) Representative traces recorded after parallel fibers stimulation with a paired-pulse (80 ms, left, or 120 ms, right, interval) every 20 s and evoked excitatory postsynaptic currents (EPSCs) were recorded from PCs in control-tdTom (Control, black, n=23 cells from 8 mice) or mutant DISC1-tdTom (Mutant, red, n=16 cells from 4 mice) mice;

(b) Averaged paired-pulse ratios demonstrate no significant changes in mutant DISC1-tdTom after paired-pulse with 80 ms (left) and 120 ms (right) intervals.

Suppl. Table I. Primer sequences used for genotyping.

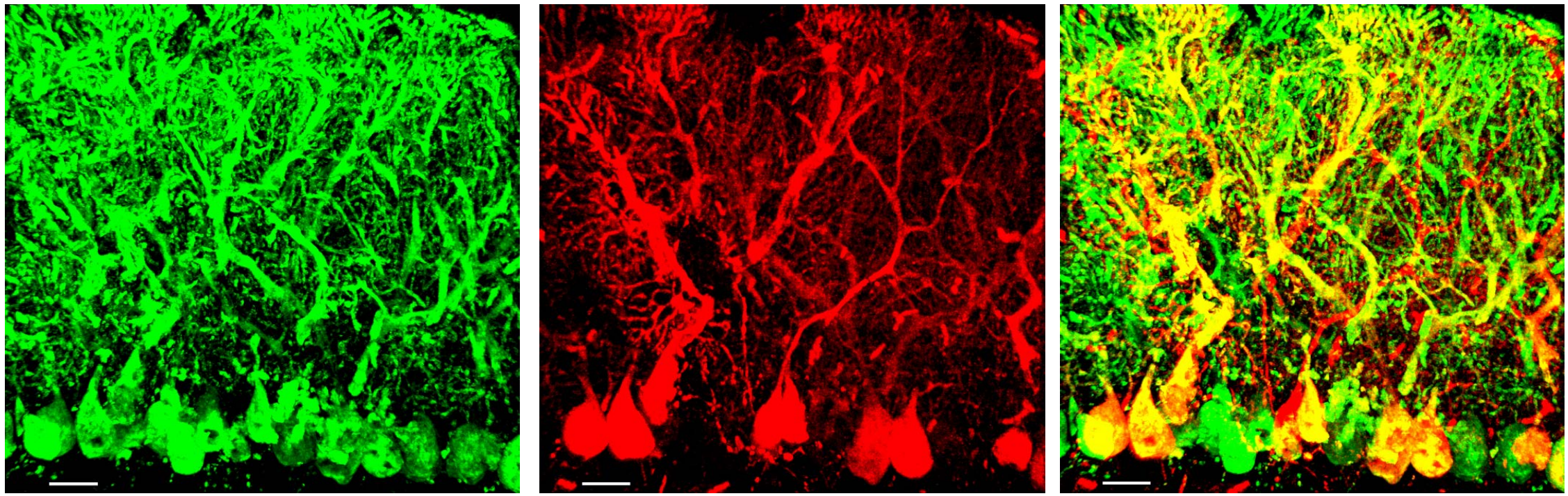
	Gene	Primer name	Primer sequence	Product size
	Mutant DISC1	TRE-CMV-F4	5′-GAC CTC CAT AGA AGA CAC CGG GAC-3′	500bp
		TRE-Hdisc1-R2	5′-TGA GCT GAA TCC CAA AGT GCG CCG-3′	
	internal positive DNA control	Prp-S-98	5’-CCT CTT TGT GAC TAT GTG GAC TGA TGT CGG-3’	750bp
		PrpUT As	5’-GTG GAT ACC CCC TCC CCC AGC CTA GAC C-3’	
	Parv-2AtTA2	tTA2-F	5’-TGG CAA GAC TTT CTG CGG AAC AA-3’	502bp
		tTA2-R	5’-CGT CAG CAG GCA GCA TAT CAA GG-3’	
	internal positive DNA control	GPR141-F	5’-CCT CTT GTG ACC CTA TAC TGG C-3’	628bp
		GPR141-R	5’-CTG GTG GGA TAG TAA GGA GTG G-3’	
	tdTomato	F	5’-TAC GGC ATG GAC GAG CTG TAC AAG TAA-3’	467bp
		R	5’-CAG GCG AGC AGC CAA GGA AA-3’	


Regulatory line
Pvalb-2A
tet R
VP16
tTA
Dox (+)
tTA
myc-tag
Responder line
TetO - TRE
Pmin CMV
DISC1-ΔC 1791 bps

SFig.1

A

Lobule II



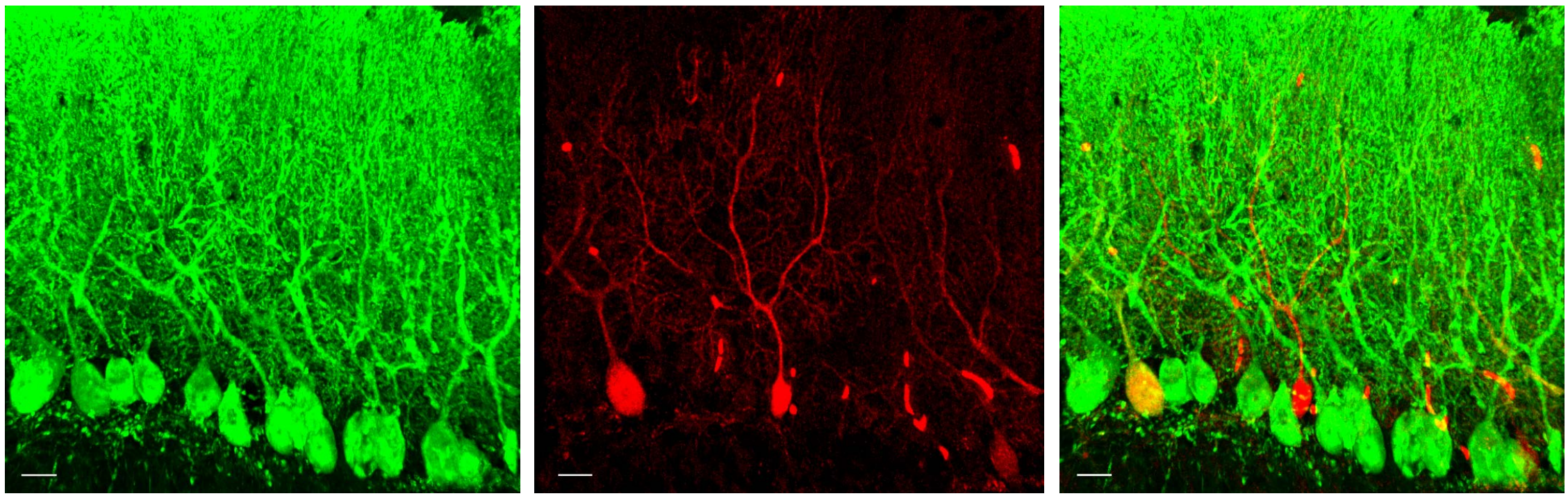
Calbindin

tdTomato

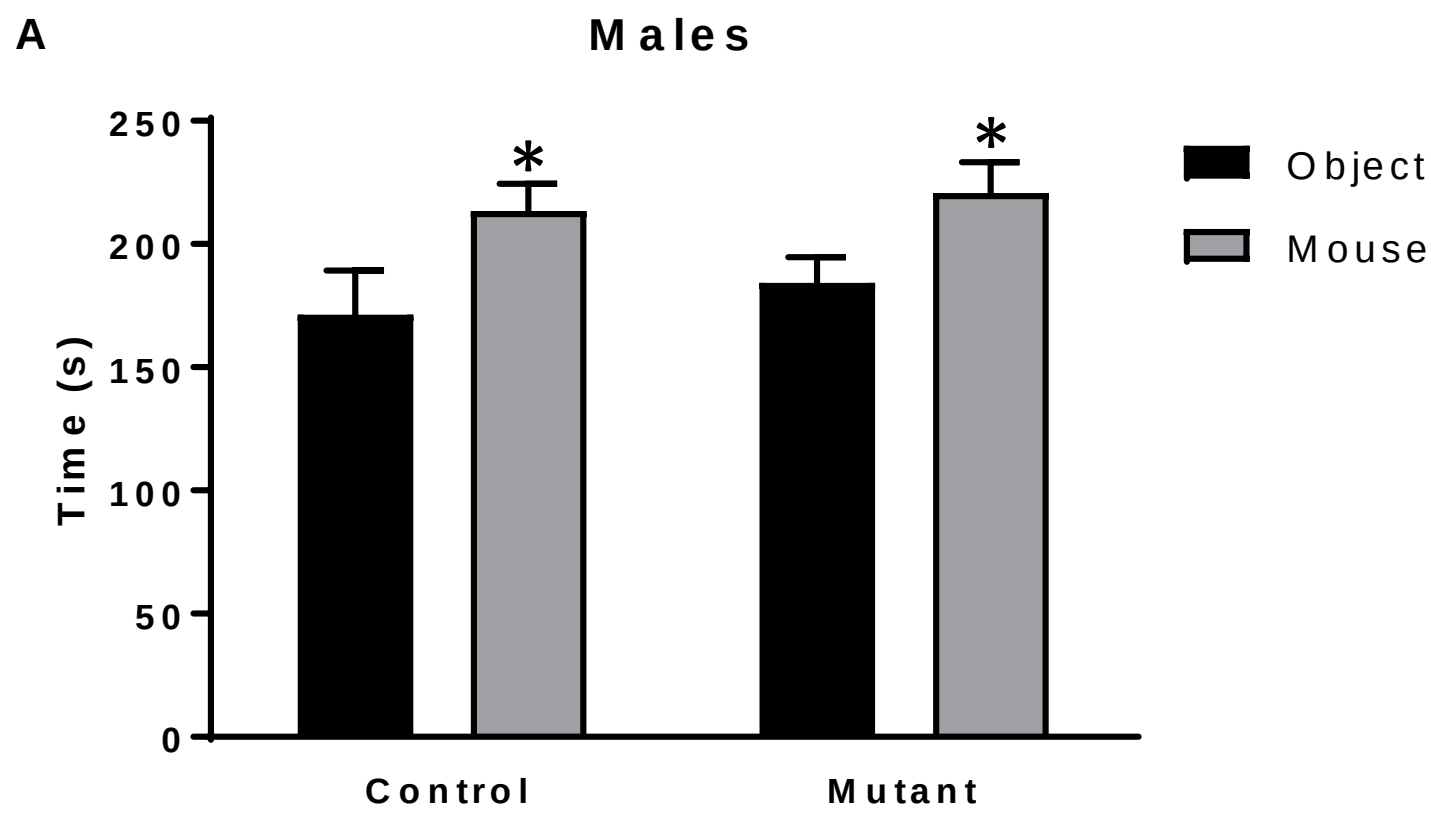
Merged

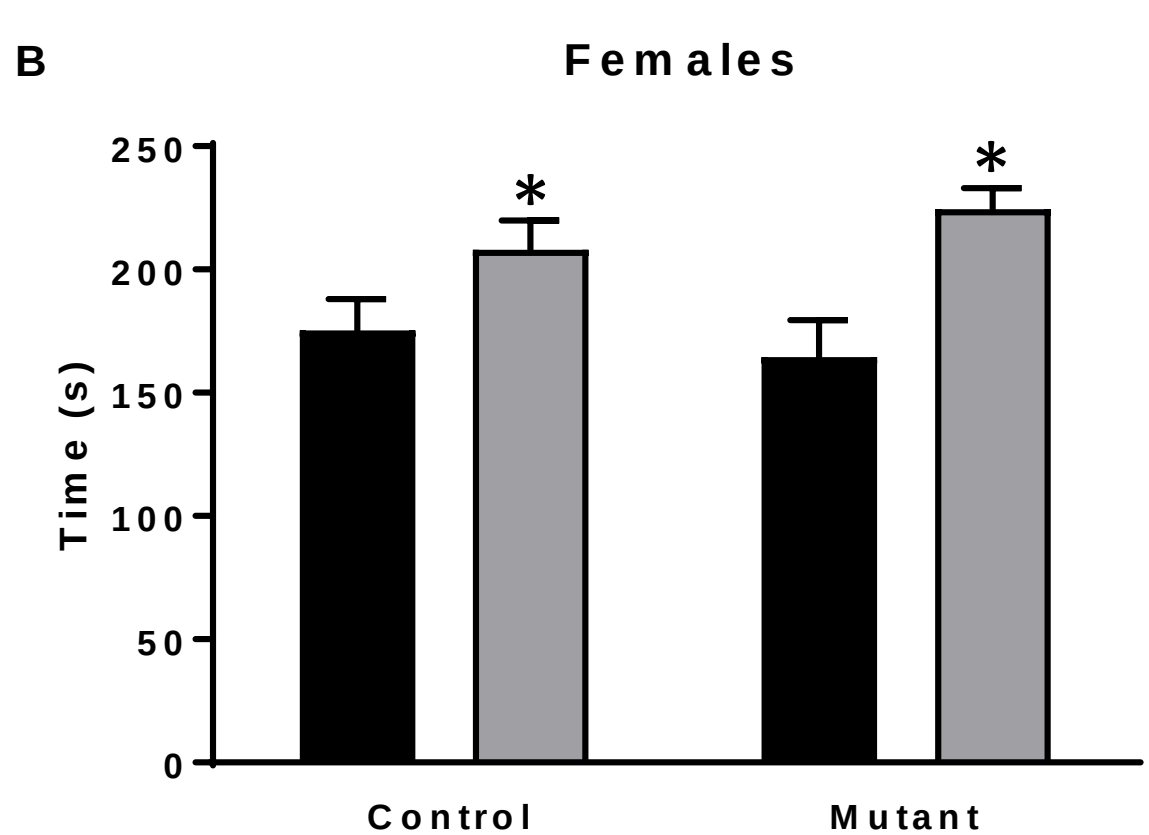
B

Lobule IX

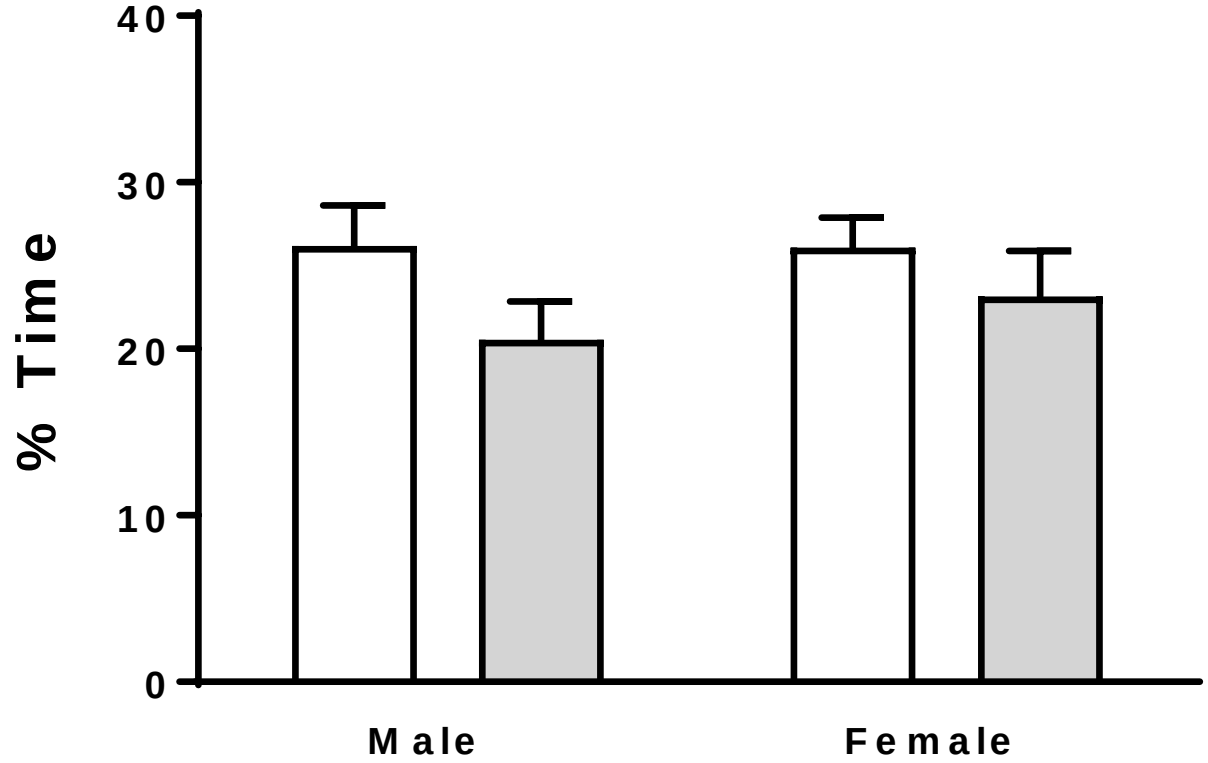


SFig.2

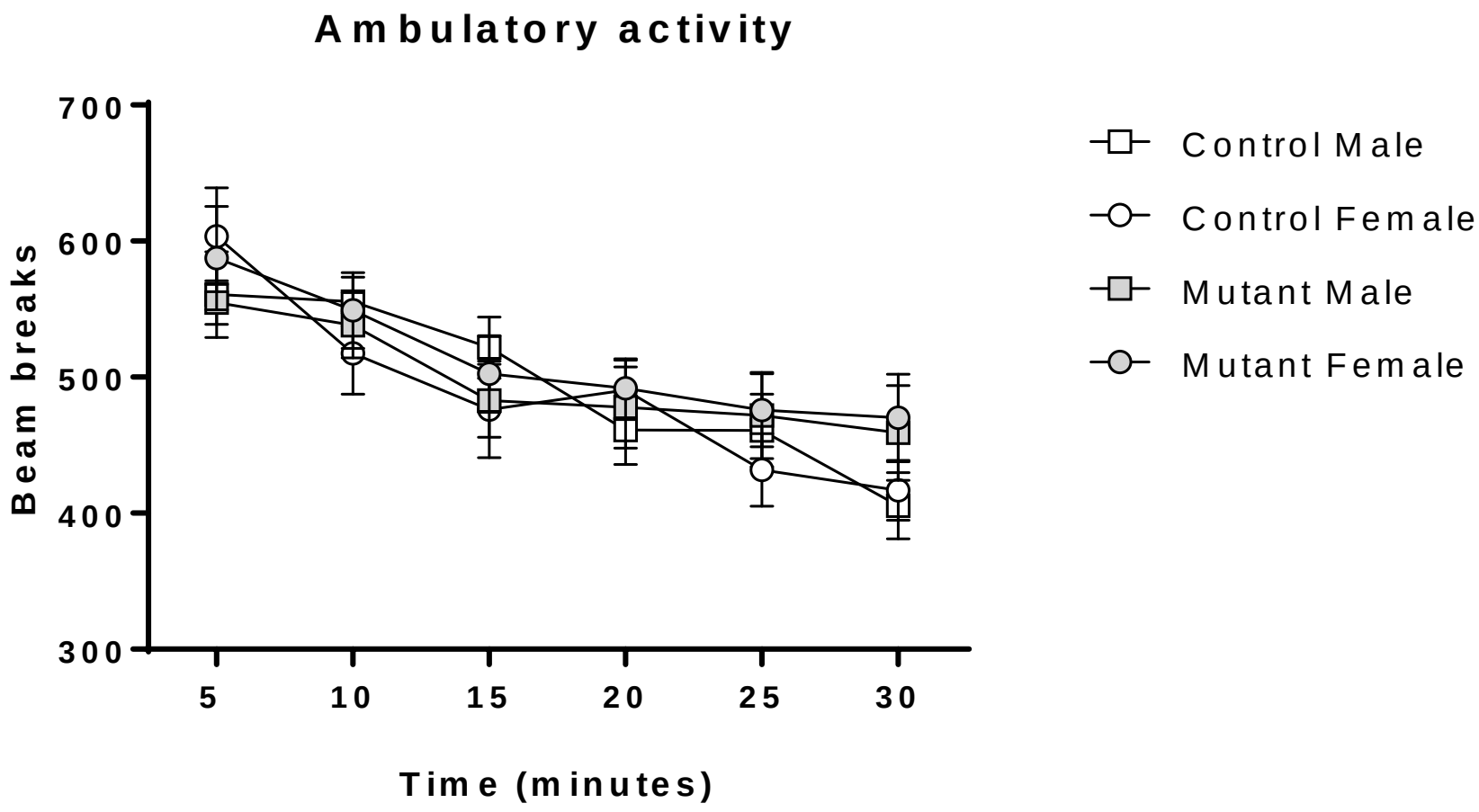

A
Males
250
200
150
100
50
0
Time (s)
*
*
Object
Mouse
Control
Mutant


B
Females
250
200
150
100
50
0
Time (s)
*
*
Control
Mutant

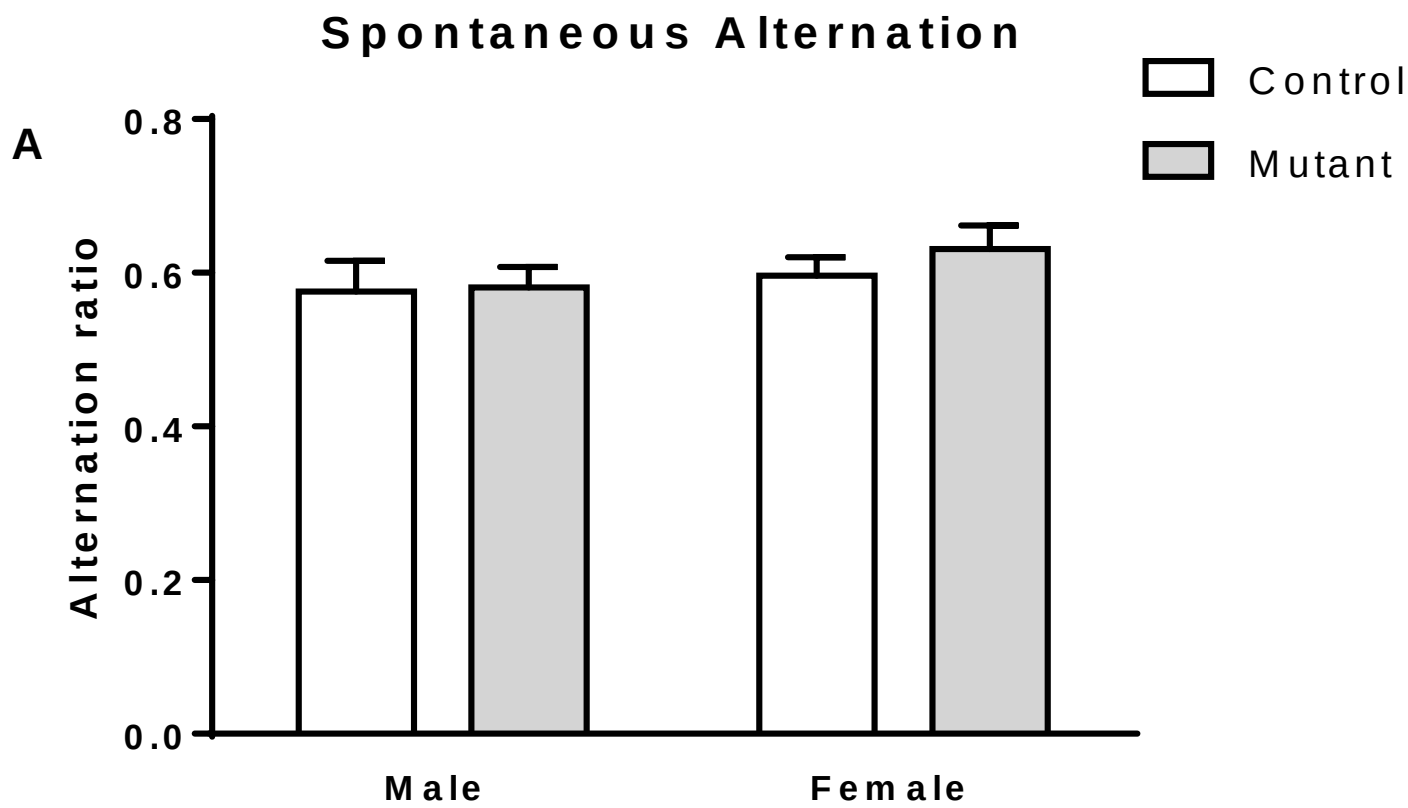
SFig.3

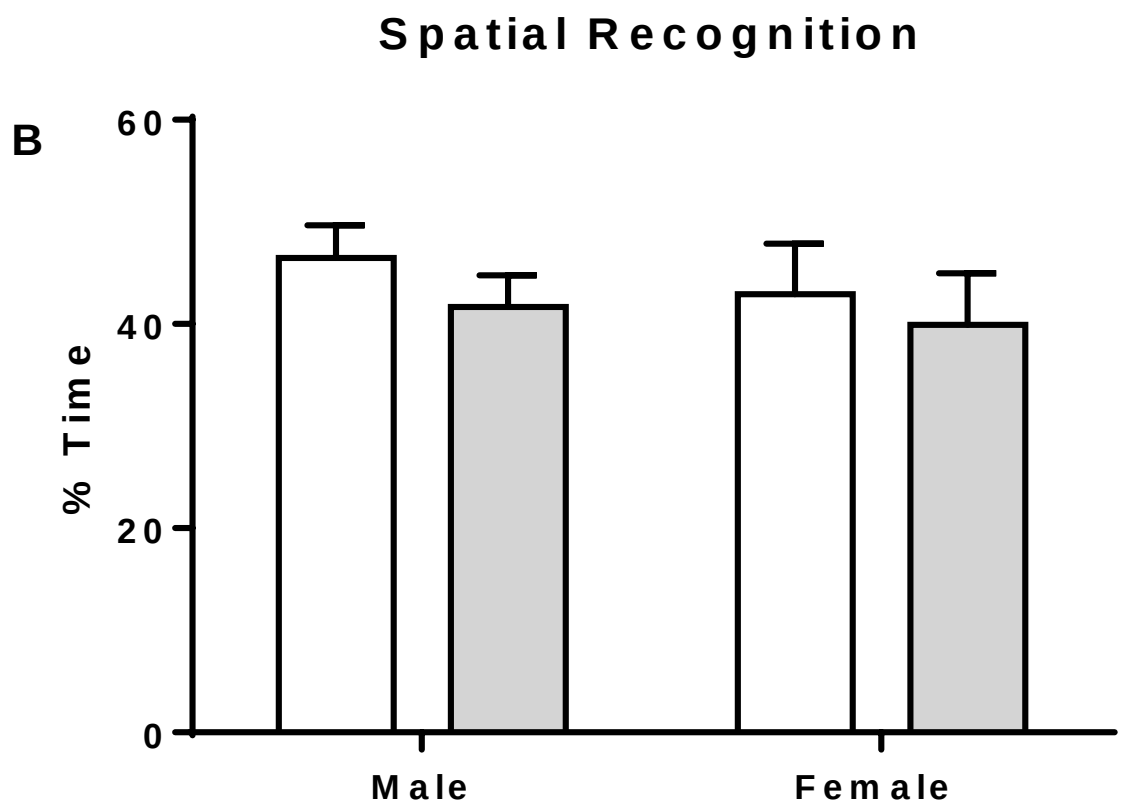


SFig.4


Ambulatory activity
Beam breaks
700
600
500
400
300
5
10
15
20
25
30
Time (minutes)
Control Male
Control Female
Mutant Male
Mutant Female

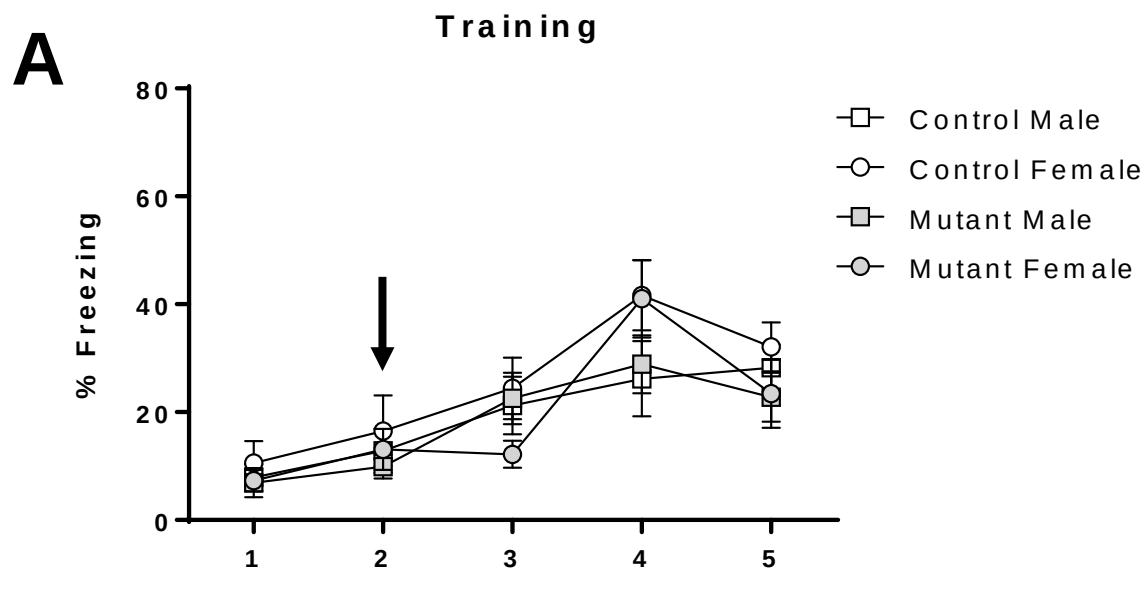
SFig.5

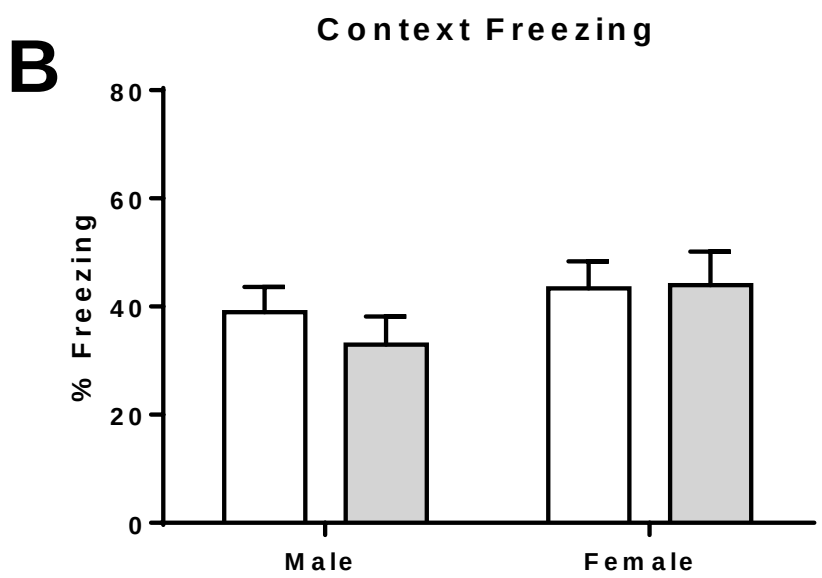

Spontaneous Alternation
A
Alternation ratio
0.8
0.6
0.4
0.2
0.0
Male
Female
Control
Mutant

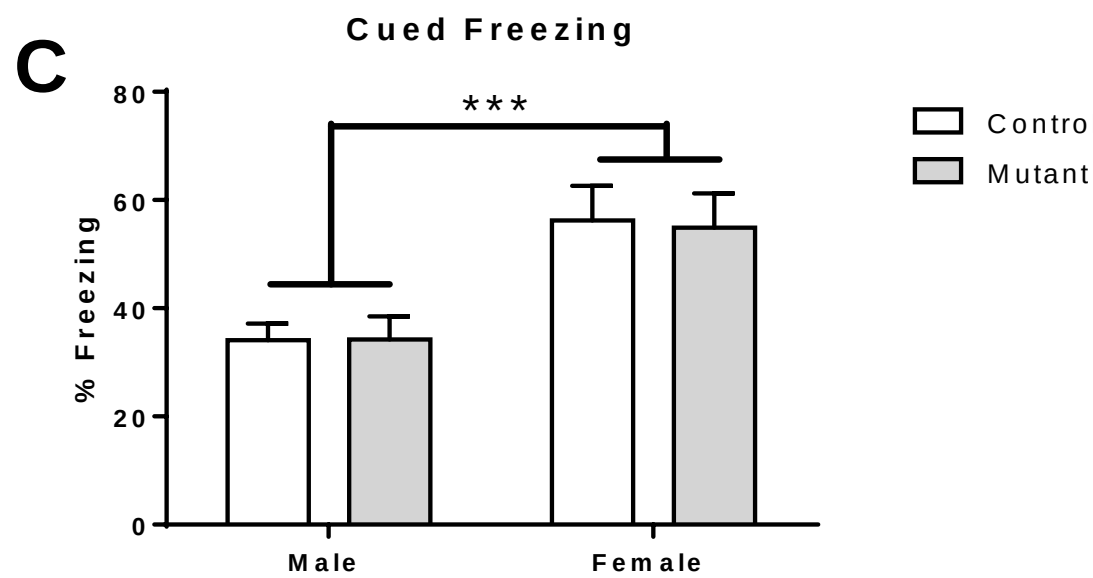

Spatial Recognition
B
% Time
60
40
20
0
Male
Female

SFig.6

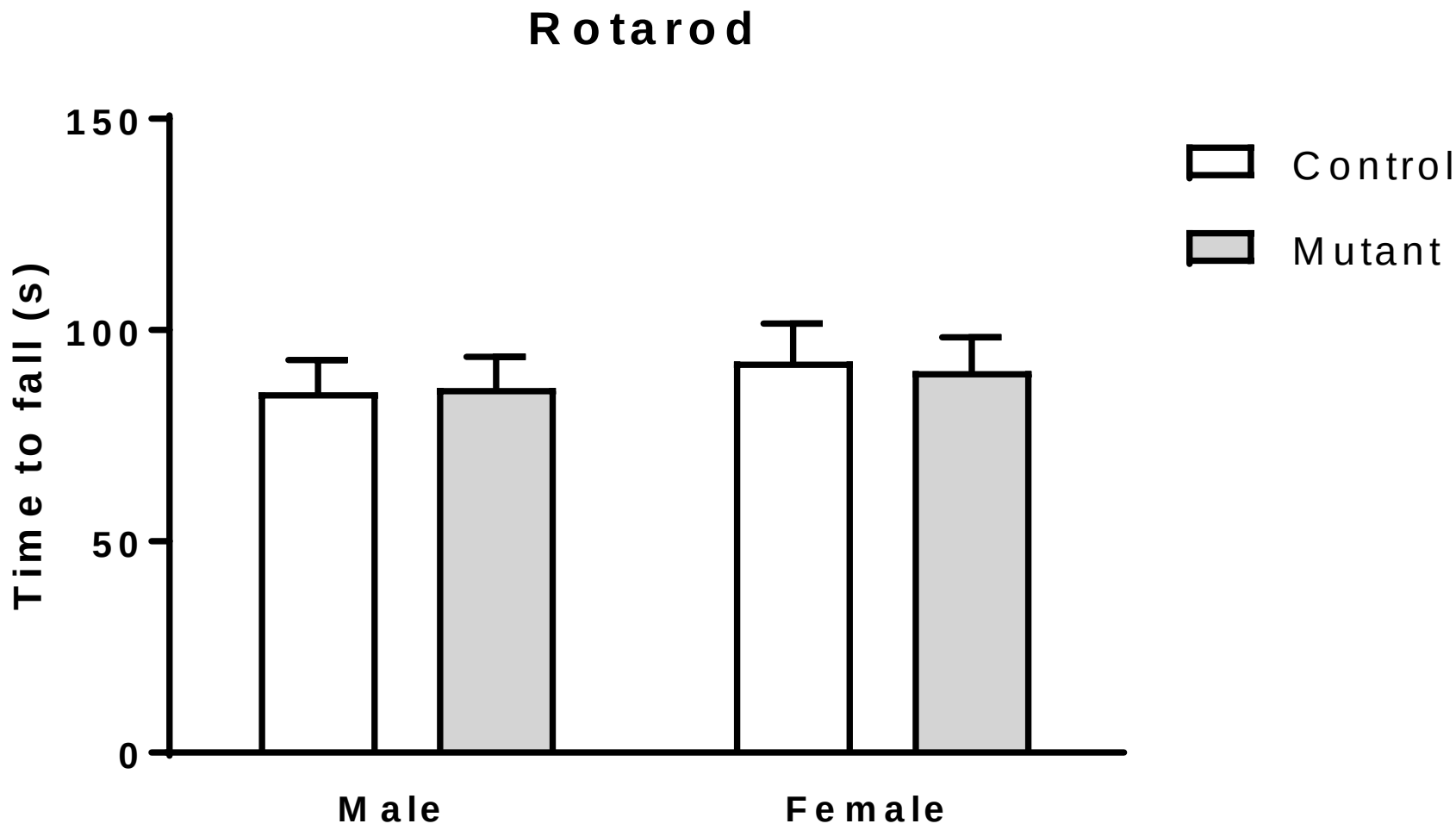
Fear Conditioning





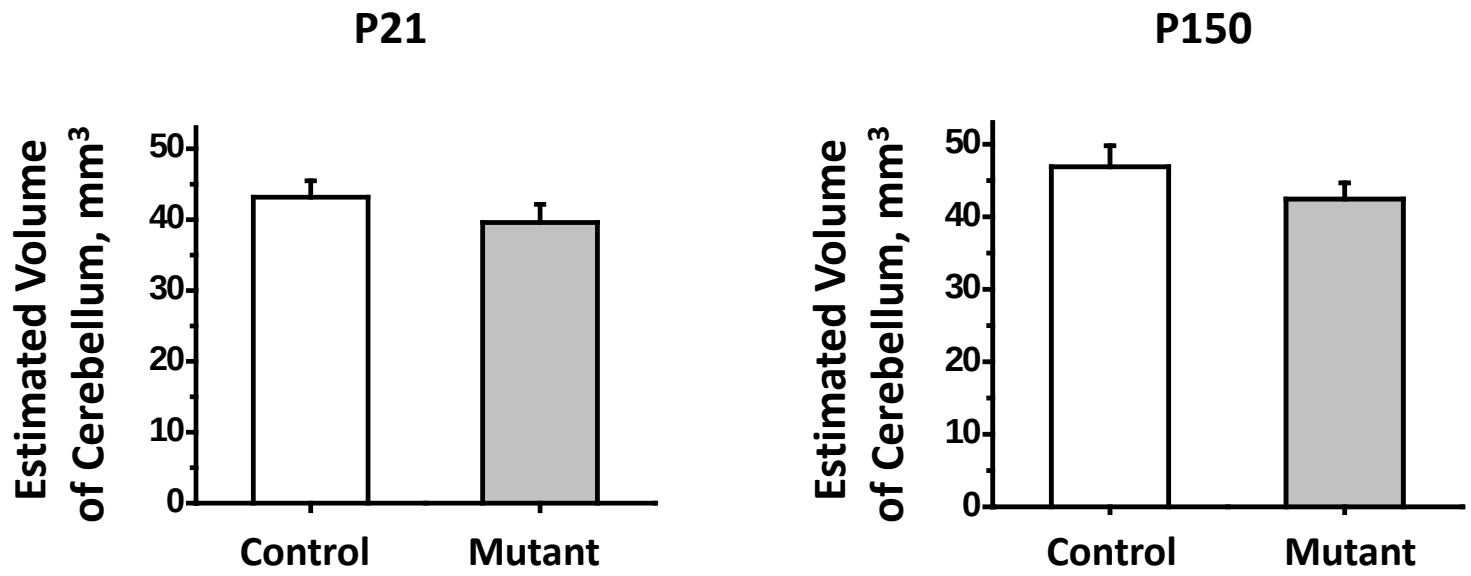


SFig.7

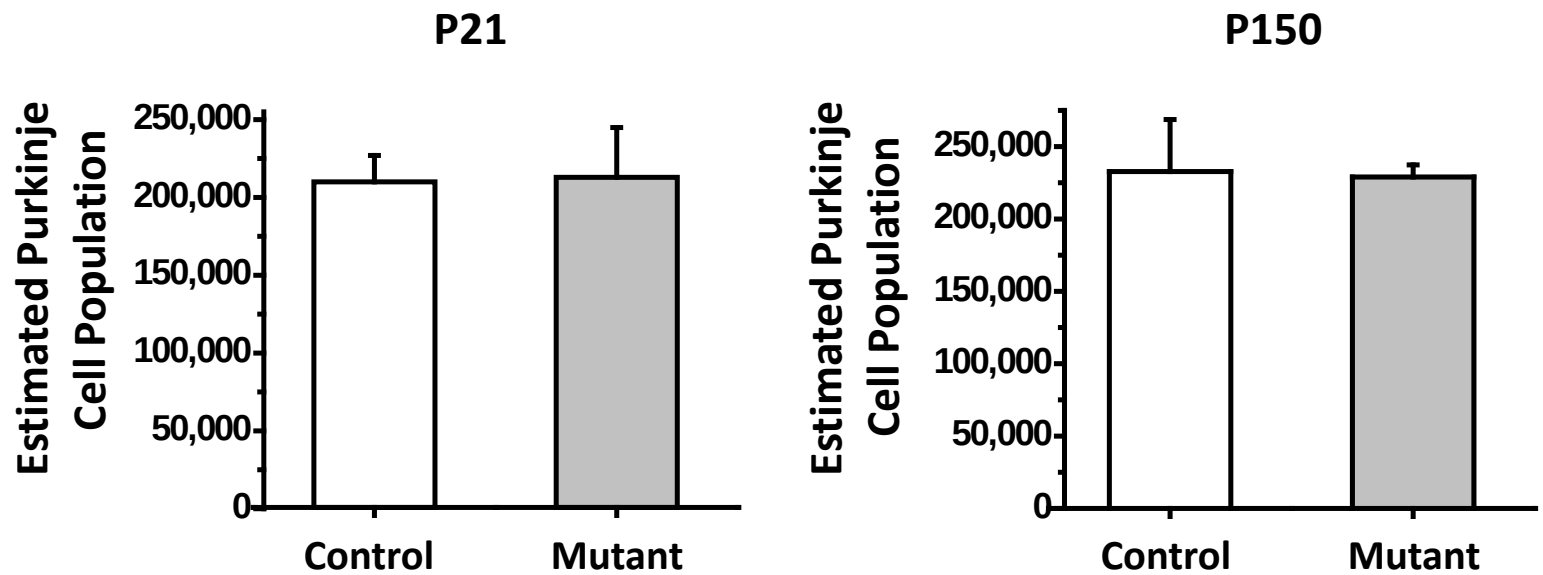

Rotarod
150
100
50
0
Time to fall (s)
Control
Mutant
Male
Female

SFig.8

A

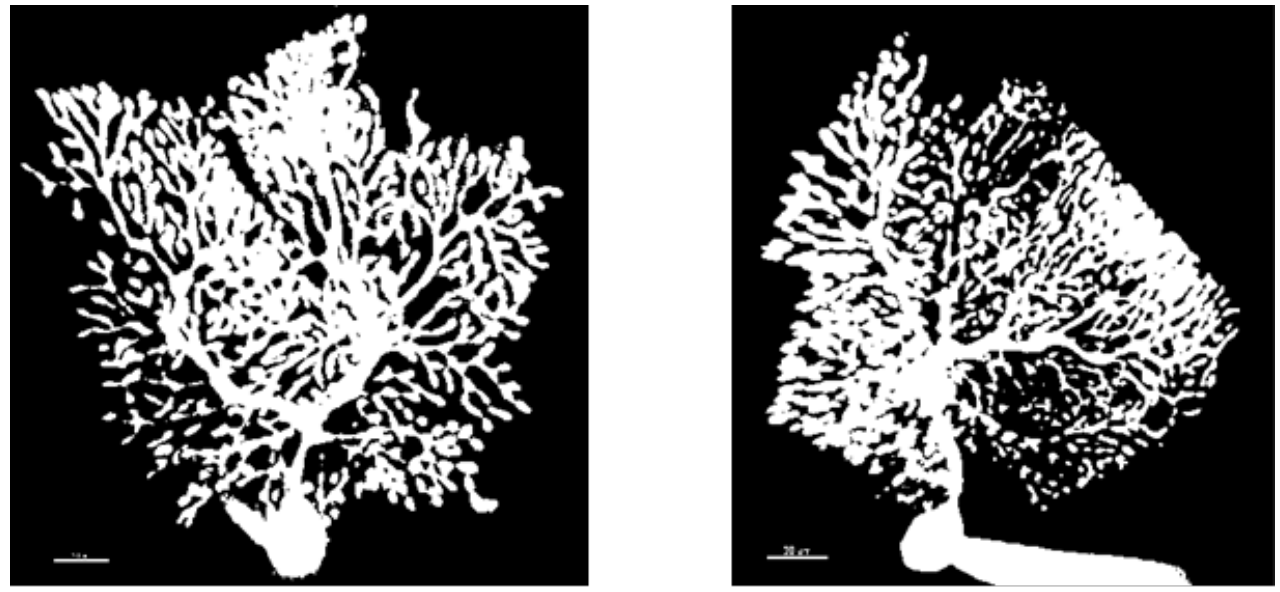

P21
Estimated Volume of Cerebellum, mm3
50
40
30
20
10
0
Control
Mutant
P150
Estimated Volume of Cerebellum, mm3
50
40
30
20
10
0
Control
Mutant

B


P21
Estimated Purkinje Cell Population
250,000
200,000
150,000
100,000
50,000
0
Control
Mutant
P150
Estimated Purkinje Cell Population
250,000
200,000
150,000
100,000
50,000
0
Control
Mutant

SFig.9

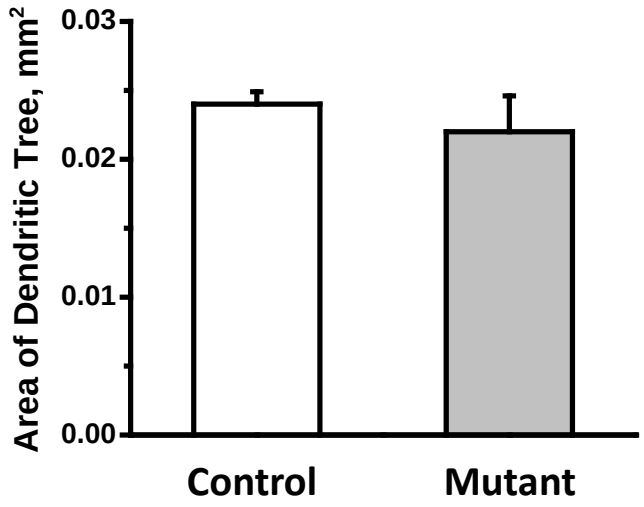
A



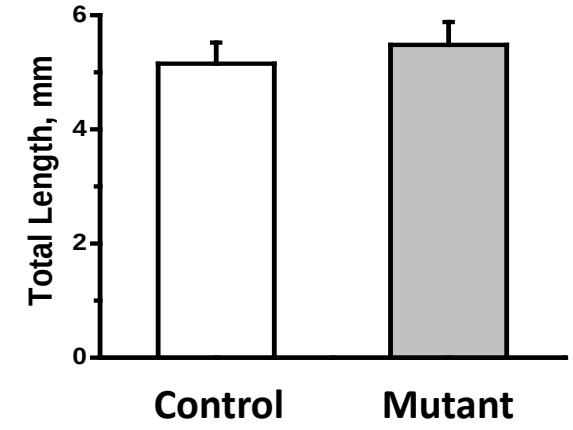
Control

Mutant

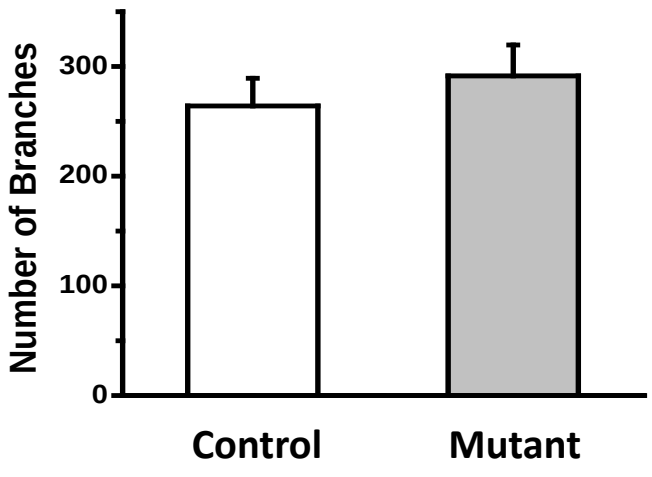
B



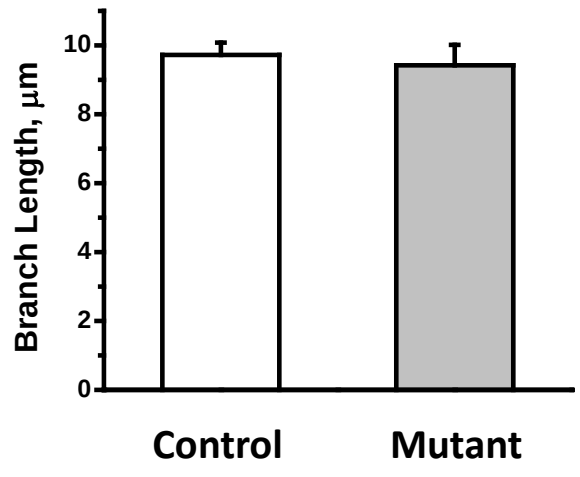
C



D

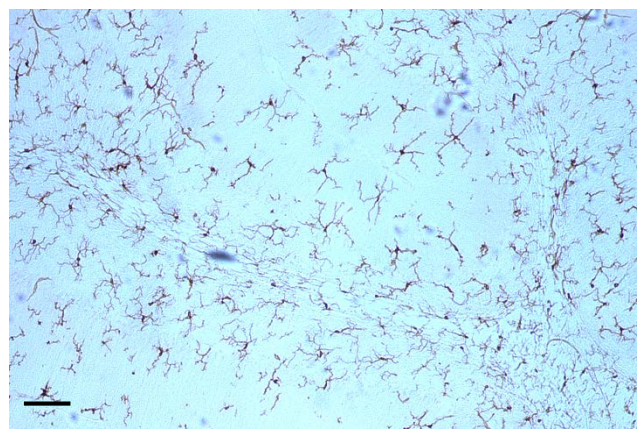


E

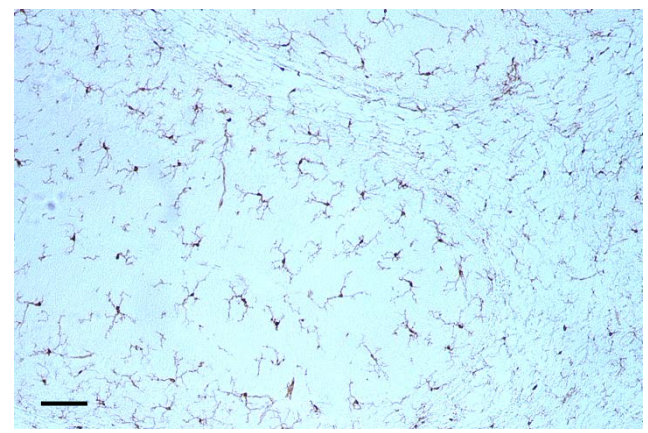


SFig.10

A



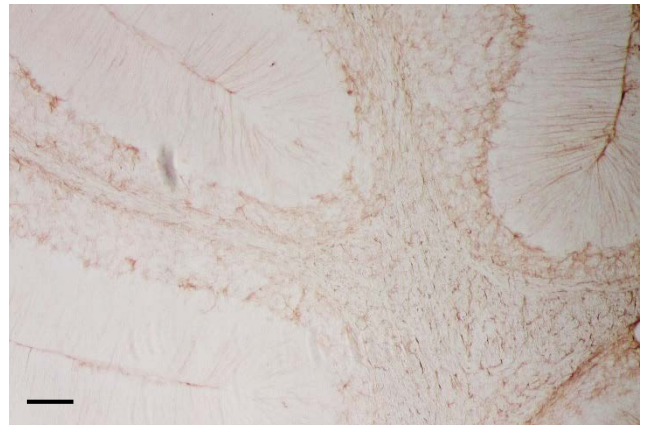
Control



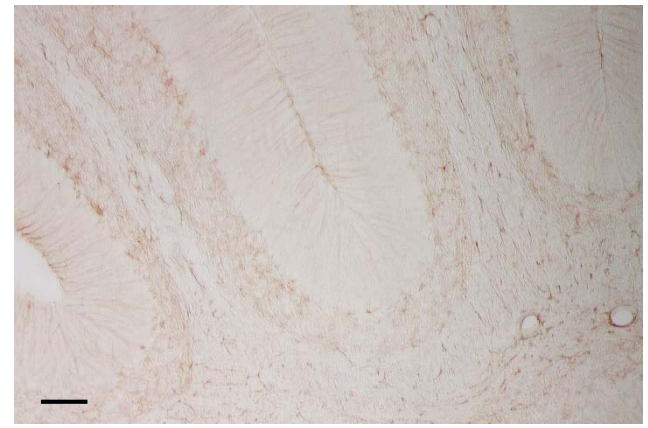
Mutant

Iba1

B



Control



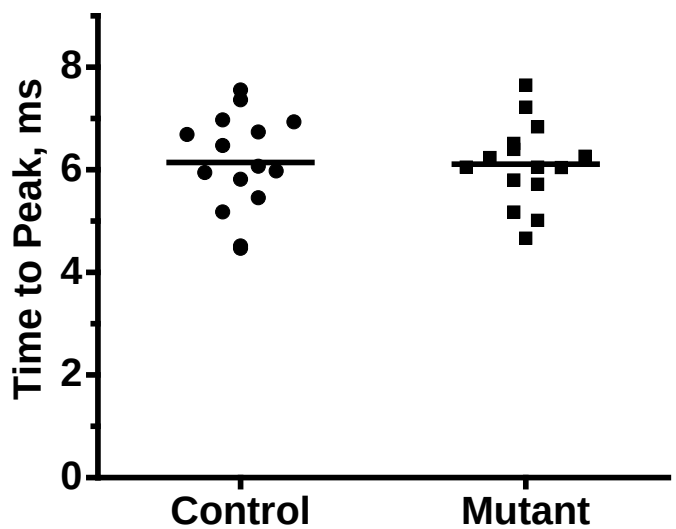
Mutant

GFAP

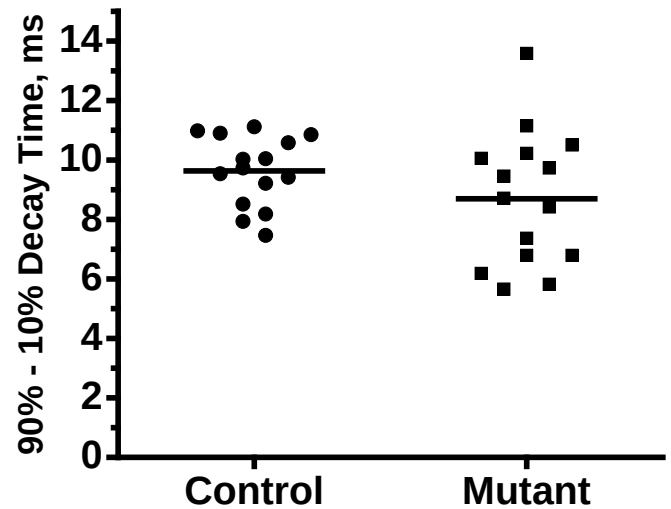
SFig.11

mEPSC KINETICS

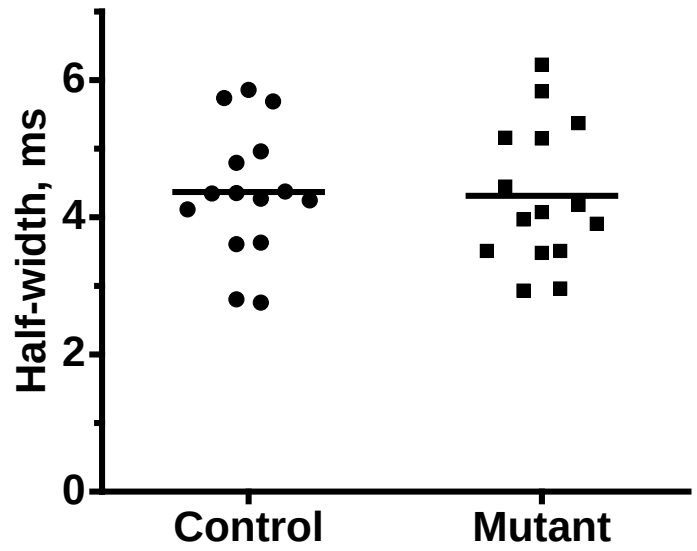
A



B



C

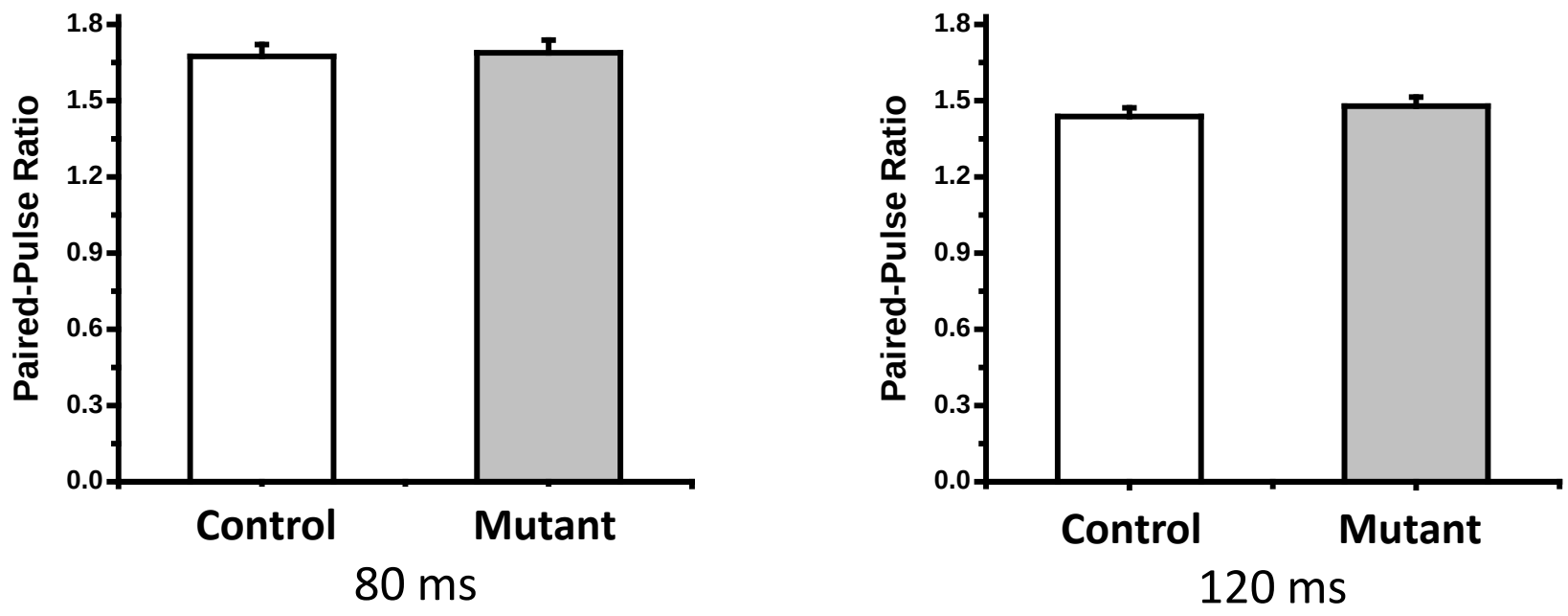


SFig.12

A


Control-tdTom
Mutant DISC1-tdTom
100 pA
100 ms
100 pA
100 ms
80 ms
120 ms

B


Paired-Pulse Ratio
1.8
1.5
1.2
0.9
0.6
0.3
0.0
Control
Mutant
80 ms
Paired-Pulse Ratio
1.8
1.5
1.2
0.9
0.6
0.3
0.0
Control
Mutant
120 ms

SFig.13