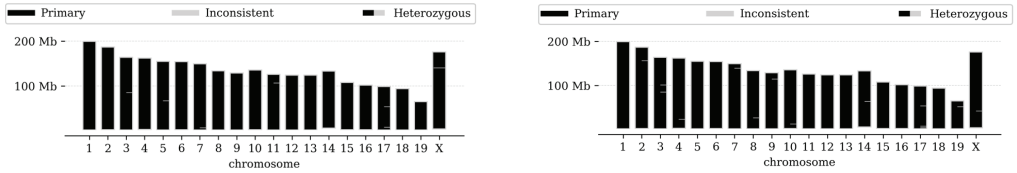
Figure S1

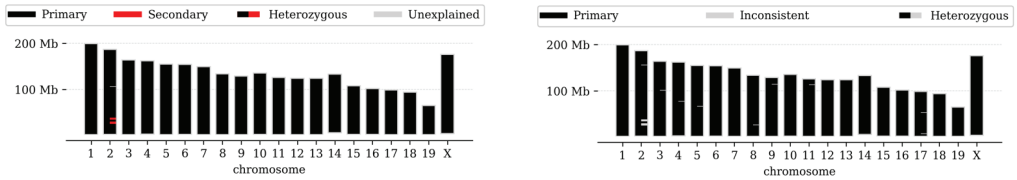
A

control
(behavior/ EEG/ Ca-imaging / cFos/ electrophysiology exp. 2)



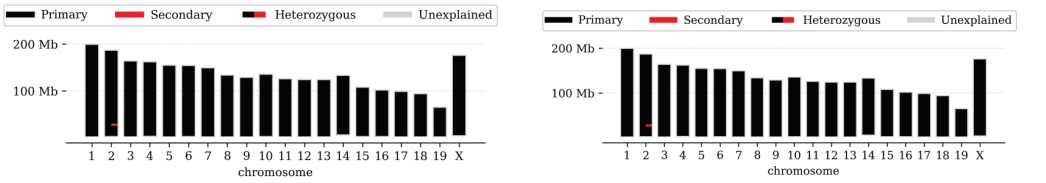
B

Stxbp1 single mutants
(behavior/ EEG/ Ca-imaging / electrophysiology exp. 2)



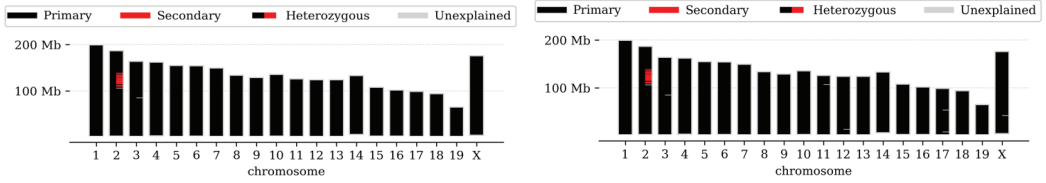
C

Stxbp1 single mutants
(electrophysiology exp. 1 / ICH)



D

Snap25 single mutants
(behavior/ EEG/ Ca-imaging / cFos electrophysiology exp. 2)



E

Snap25 single mutants
(electrophysiology exp. 1 / ICH)

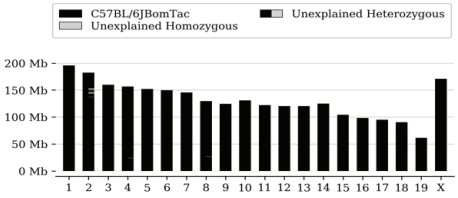


Figure S2

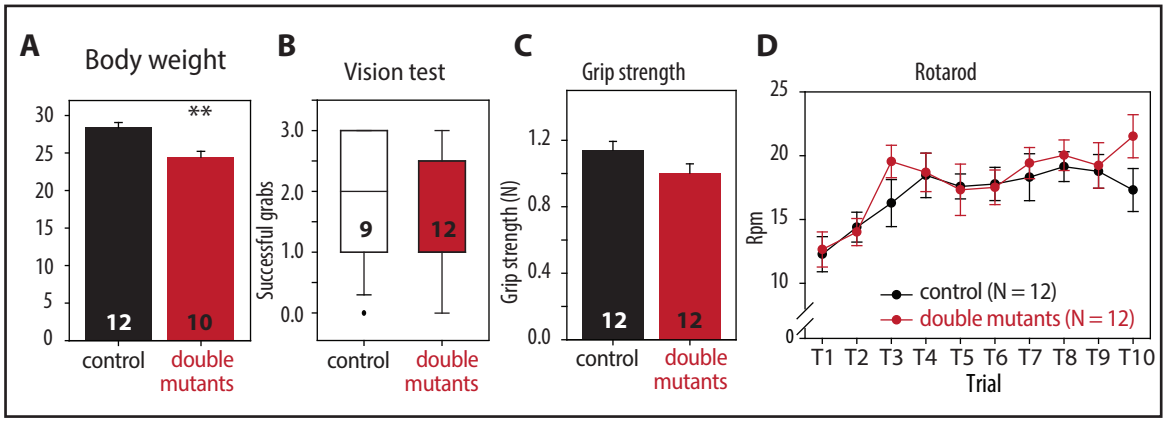

A
Body weight
30
25
20
15
10
5
0
**
12
10
control
double mutants
B
Vision test
Successful grabs
3.0
2.0
1.0
0.0
9
12
control
double mutants
C
Grip strength
Grip strength (N)
1.2
0.8
0.4
0.0
12
12
control
double mutants
D
Rotarod
Rpm
25
20
15
10
0
control (N = 12)
double mutants (N = 12)
T1 T2 T3 T4 T5 T6 T7 T8 T9 T10
Trial

Figure S3

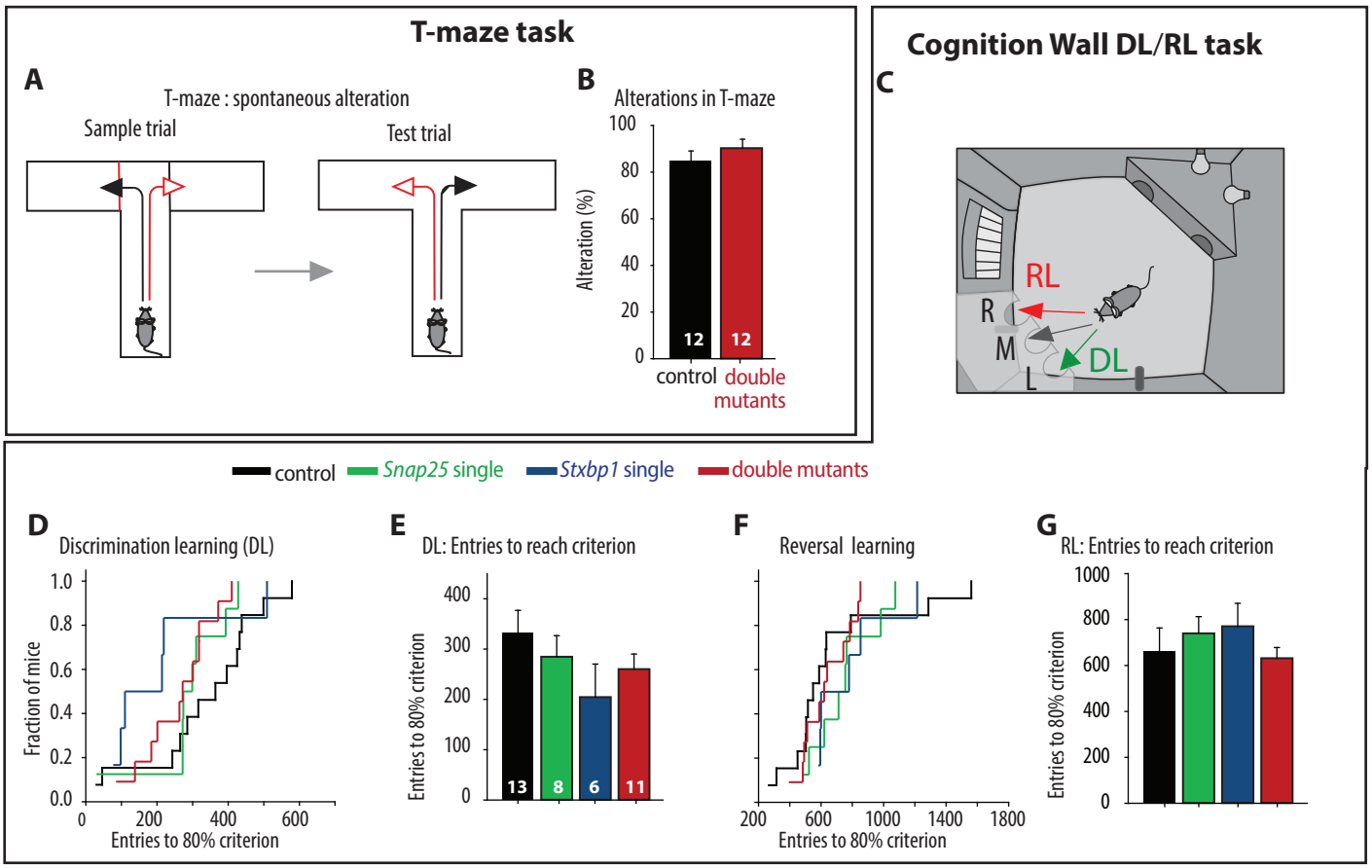

T-maze task
A
T-maze : spontaneous alteration
Sample trial
Test trial
B
Alterations in T-maze
Alteration (%)
100
80
60
40
20
0
12
12
control
double mutants
Cognition Wall DL/RL task
C
RL
R
M
L
DL
control
Snap25 single
Stxbp1 single
double mutants
D
Discrimination learning (DL)
Fraction of mice
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
Entries to 80% criterion
E
DL: Entries to reach criterion
Entries to 80% criterion
400
300
200
100
0
13
8
6
11
F
Reversal learning
200
600
1000
1400
1800
Entries to 80% criterion
G
RL: Entries to reach criterion
Entries to 80% criterion
1000
800
600
400
200
0

Figure S4

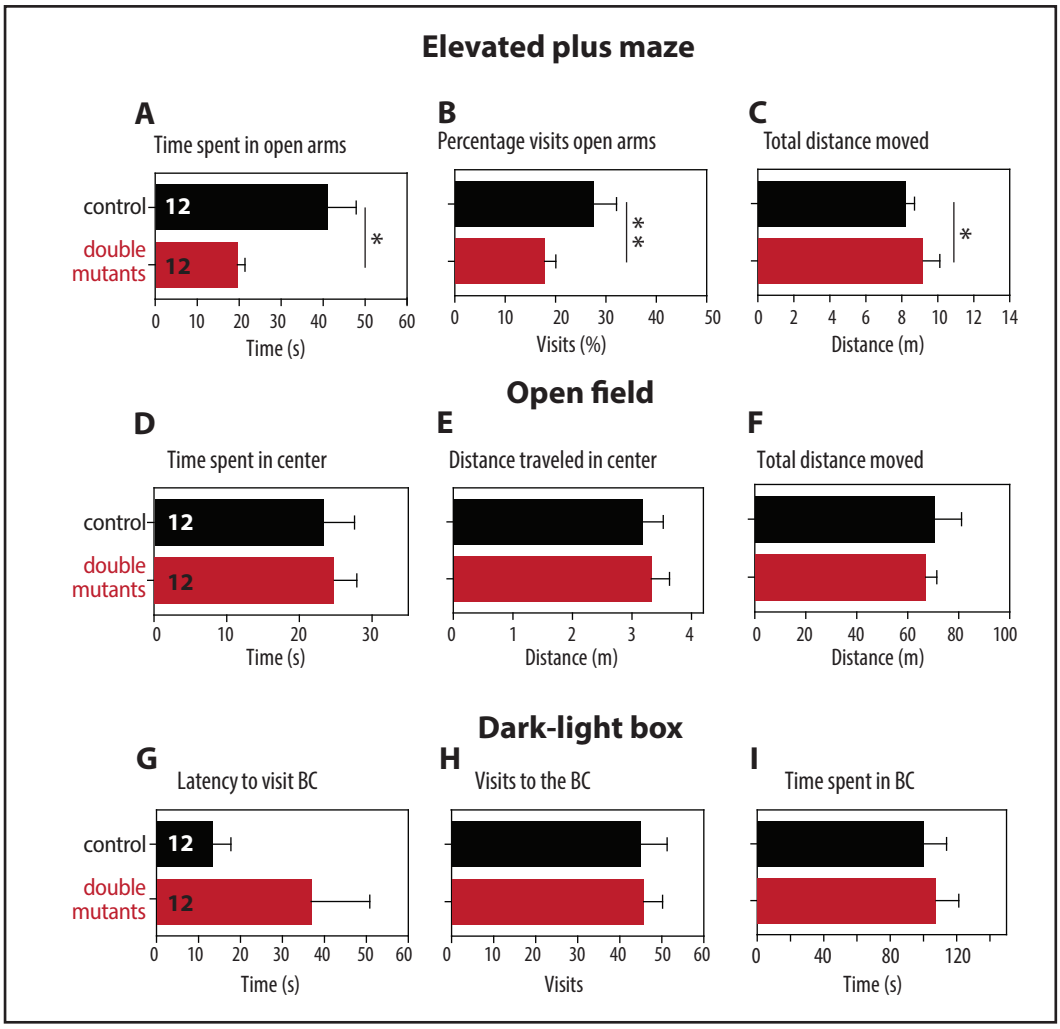

Elevated plus maze
A
Time spent in open arms
control
12
double mutants
12
*
0 10 20 30 40 50 60
Time (s)
B
Percentage visits open arms
**
0 10 20 30 40 50
Visits (%)
C
Total distance moved
*
0 2 4 6 8 10 12 14
Distance (m)
Open field
D
Time spent in center
control
12
double mutants
12
0 10 20 30
Time (s)
E
Distance traveled in center
0 1 2 3 4
Distance (m)
F
Total distance moved
0 20 40 60 80 100
Distance (m)
Dark-light box
G
Latency to visit BC
control
12
double mutants
12
0 10 20 30 40 50 60
Time (s)
H
Visits to the BC
0 10 20 30 40 50 60
Visits
I
Time spent in BC
0 40 80 120
Time (s)

Figure S5

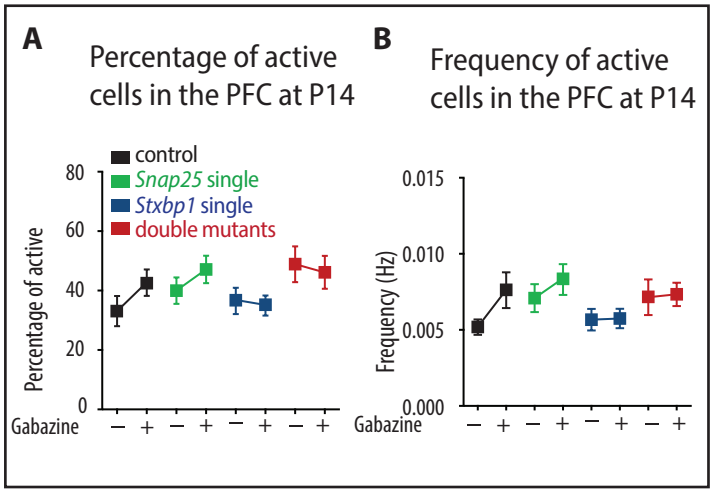

A
Percentage of active cells in the PFC at P14
control
Snap25 single
Stxbp1 single
double mutants
Percentage of active
80
60
40
20
0
Gabazine
– + – + – + – +
B
Frequency of active cells in the PFC at P14
Frequency (Hz)
0.015
0.010
0.005
0.000
Gabazine
– + – + – + – +

Figure S6

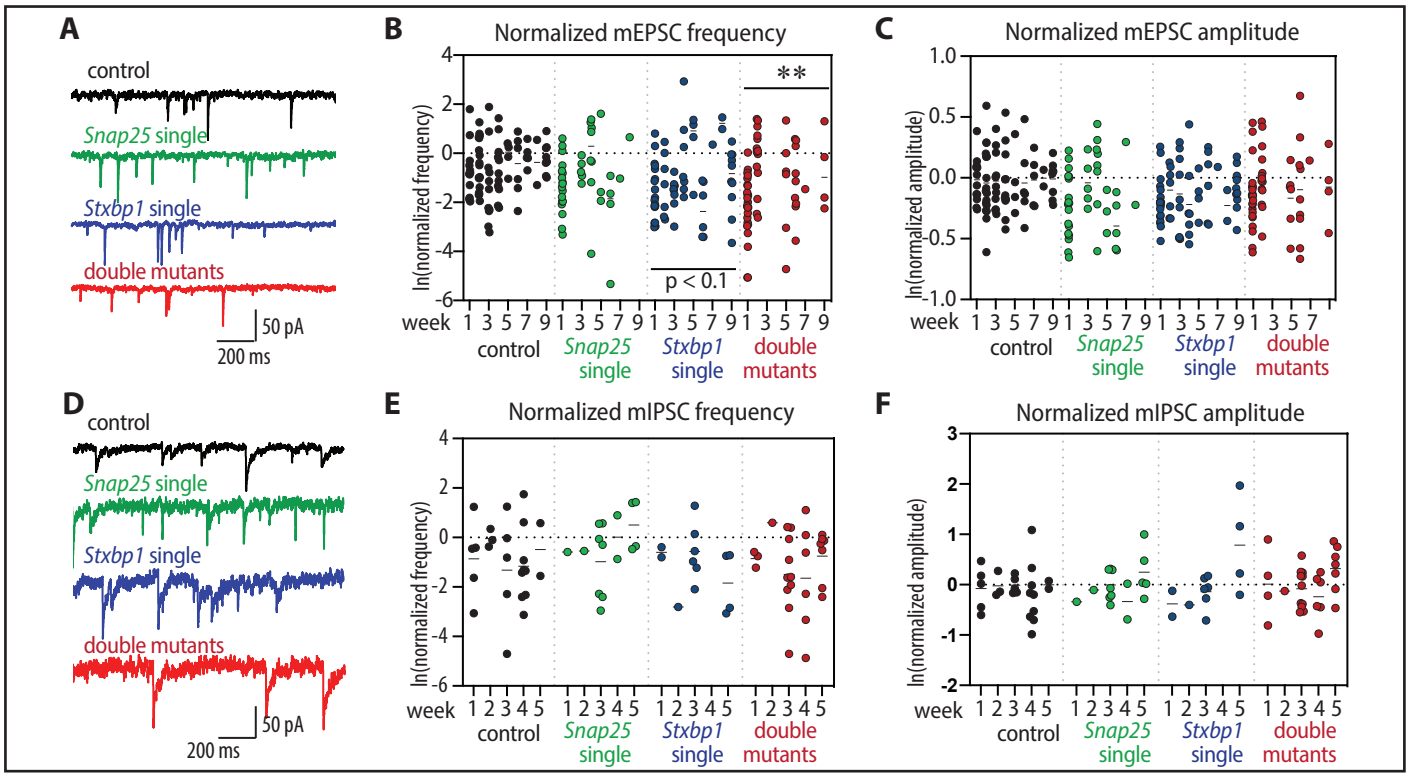
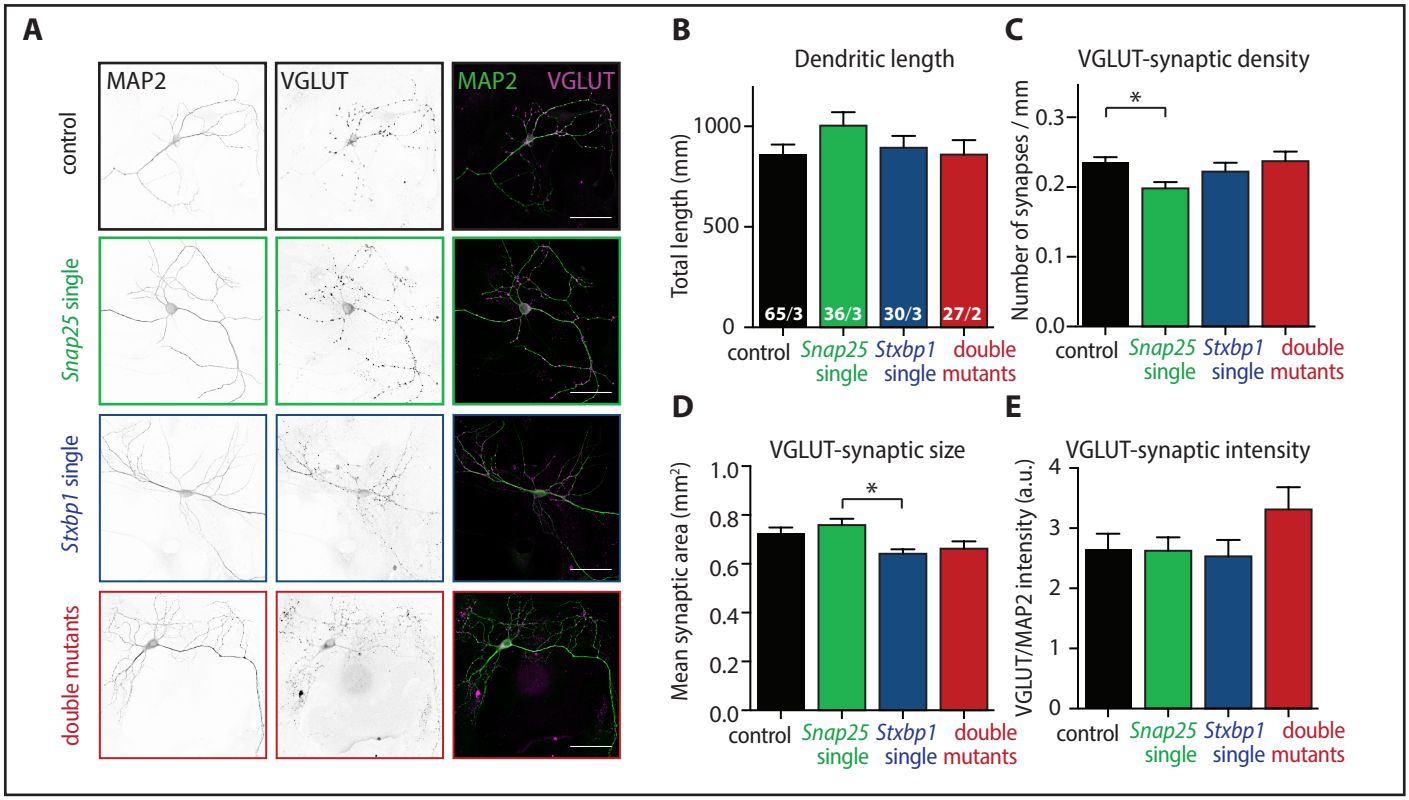
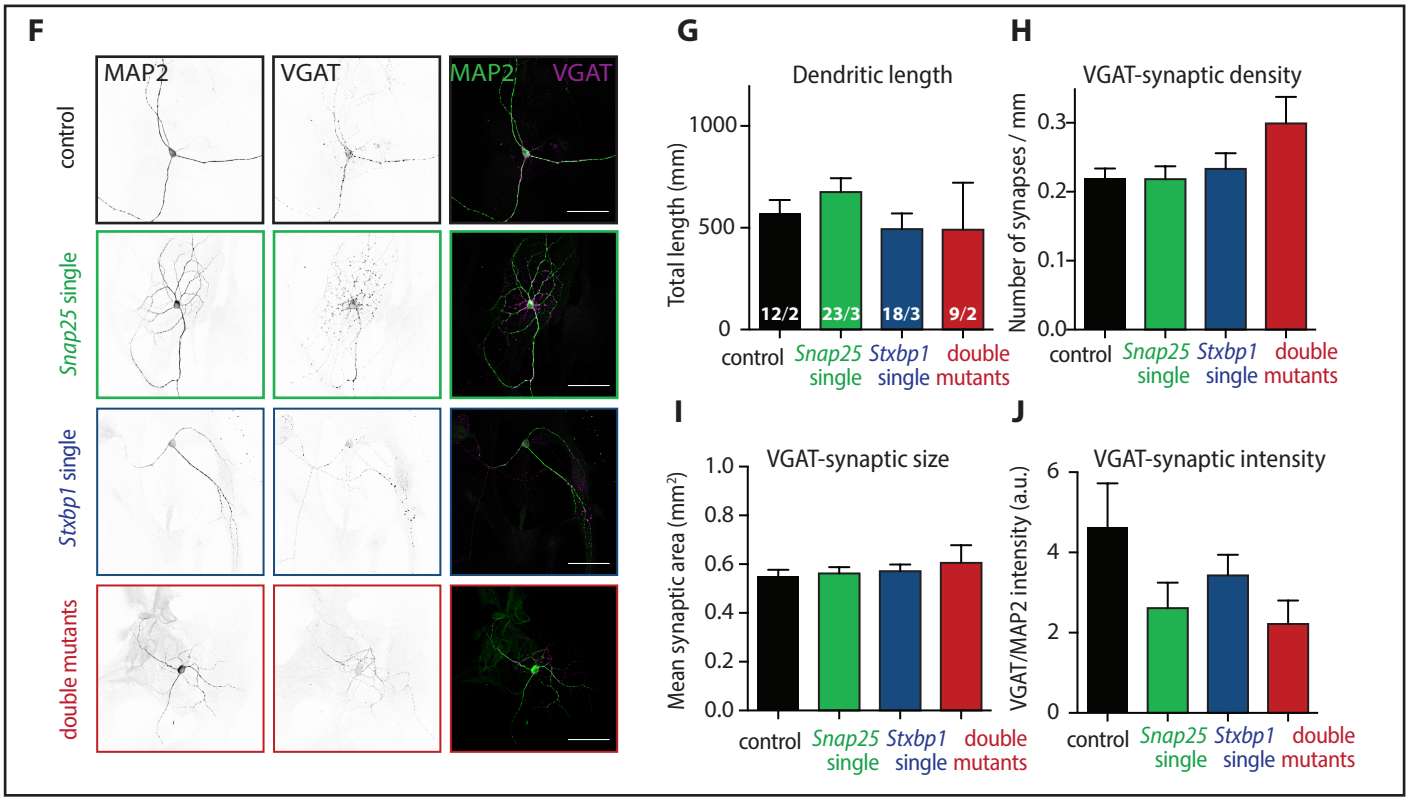

A
control
Snap25 single
Stxbp1 single
double mutants
50 pA
200 ms
B
Normalized mEPSC frequency
ln(normalized frequency)
**
p < 0.1
week
control
Snap25 single
Stxbp1 single
double mutants
C
Normalized mEPSC amplitude
ln(normalized amplitude)
D
control
Snap25 single
Stxbp1 single
double mutants
50 pA
200 ms
E
Normalized mIPSC frequency
ln(normalized frequency)
F
Normalized mIPSC amplitude
ln(normalized amplitude)

Figure S7


A
MAP2
VGLUT
MAP2 VGLUT
control
Snap25 single
Stxbp1 single
double mutants
B
Dendritic length
Total length (mm)
1000
500
0
65/3
36/3
30/3
27/2
control
Snap25 single
Stxbp1 single
double mutants
C
VGLUT-synaptic density
Number of synapses / mm
0.3
0.2
0.1
0.0
*
D
VGLUT-synaptic size
Mean synaptic area (mm²)
1.0
0.8
0.6
0.4
0.2
0.0
*
E
VGLUT-synaptic intensity
VGLUT/MAP2 intensity (a.u.)
4
3
2
1
0


F
MAP2
VGAT
MAP2 VGAT
control
Snap25 single
Stxbp1 single
double mutants
G
Dendritic length
Total length (mm)
1000
500
0
12/2
23/3
18/3
9/2
control
Snap25 single
Stxbp1 single
double mutants
H
VGAT-synaptic density
Number of synapses / mm
0.3
0.2
0.1
0.0
I
VGAT-synaptic size
Mean synaptic area (mm²)
1.0
0.8
0.6
0.4
0.2
0.0
J
VGAT-synaptic intensity
VGAT/MAP2 intensity (a.u.)
6
4
2
0

SUPPLEMENTARY FIGURE LEGENDS:

Supplementary Figure 1 MiniMUGA genetic background analysis. (A) Control (*Stxbp1*$^{+/+}$*Snap25*$^{+/+}$) samples from laboratory 1 were of excellent quality and analysis revealed the presence of 96.4% C57BL/6J substrain diagnostic SNP probes as a primary background. **(B)** *Stxbp1* single samples from laboratory 1 were inbred (96.5% consistency with C57BL/6J diagnostic markers) with the presence of multiple 129-strains markers in one sample and unexplained secondary background in the second sample. **(C)** *Stxbp1* single samples from laboratory 2 contained multiple C57BL6 sub-strains background markers, with dominant C57BL/6JBomTac sub-strain and several clusters with 129-strains markers. **(D)** Two *Snap25* single samples from laboratory 1 were C57BL/6J inbred with the presence of multiple 129-strains markers. (E) *Snap25* single samples from laboratory 1 was C57BL/6JBomTac inbred with the presence of unexplained secondary background.

Supplementary Figure 2: Body weight and general behavior in double mutants. (**A)** Body weight was measured in 8 weeks old animals. (**B)** Vision test scored number of successful grabs to reach for the platform. (**C)** Grip strength test scored the amount of force that mice applied grasping a pull bar. (**D)** Rotarod test measured the maximum rpm reached per trial.

Supplementary Figure 3: Assessment of different aspects of cognition. (A) Representation of T-maze spontaneous alteration protocol consists of two trials: sample and test trial during which animal's working memory was assessed. (**B)** Percentage of alteration in the T maze for control and double mutants. (**C)** Representation of CognitionWall task protocol for assessment of discrimination (DL) and reversal (RL) learning. (**D)** Kaplan-Meier survival curves shows the fraction of control (black), *Snap25* single (green), *Stxbp1* single (blue) and double mutants

(red) that reached the 80% criterion as a function of hole entries during the DL phase. (**E)** Average number of entries made to reach the 80% criterion during DL phase. **(F)** Kaplan-Meier survival curves shows the fraction of control (black), *Snap25* single (green), *Stxbp1* single (blue) and double mutants (red) that reached the 80% criterion as a function of hole entries during the RL phase. (**G)** Average number of entries made to reach the 80% criterion during RL phase.

Supplementary Figure 4: Assessment of anxiety-related behavior in double mutants. (A) Time spent in open arms of elevated plus maze (EPM). (**B)** Percentage of visits to the open arms of EPM. (**C)** Total distance moved in the EPM. (**D)** Time spent in the central part of the OF. (**E)** Distance traveled in center of the OF. (**F)** Total distance moved. (**G)** Latency to visit bright compartment (BC) of the dark-light box. (**H)** Number of visits to the BC. (**I)** Time spent in BC.

Supplementary Figure 5: Calcium imaging of PFC- brain slice. (A-B) Percentage of active cells (A) and frequency of their activity (B) at baseline and after application of gabazine in PFC-brain slices from control, *Snap25* single-, *Stxbp1* single-, and double mutants.

Supplementary Figure 6: Assessment of spontaneous release in excitatory and inhibitory hippocampal autaptic neurons. (A) Typical spontaneous release traces in control, *Snap25* single-, *Stxbp1* single- and double- mutants glutamatergic neurons (**B)** Normalized frequency of spontaneous release (mEPSC) of glutamatergic neurons per week. mEPSC frequency was significantly lower in double mutants glutamatergic neurons compared to control group. (**C)** Normalized amplitude of mEPSC per week. **D)** Typical spontaneous release traces in control-, *Stxbp1* single-, *Snap25* single- and double mutants GABA-ergic neurons (**E)** Normalized

frequency of spontaneous release (mIPSC) of GABA-ergic neurons per week. (**F)** Normalized amplitude mIPSC per week. ** p< 0.01.

Supplementary Figure 7: Morphological analysis of ICH-stained dissociated hippocampal neurons. A) Dissociated hippocampal neurons were stained for morphological marker (MAP2) and glutamatergic marker (VGLUT). Examples represent control, *Snap25* single-, *Stxbp1* single- and double- mutants neurons. **B-E)** Quantification of several morphological parameters in glutamatergic neurons: B) Total dendritic length. C) Synaptic density. D) Synaptic size and E) Synaptic intensity. **F)** Dissociated hippocampal neurons were stained for morphological marker (MAP2) and GABAergic marker (VGAT). Examples represent control, *Snap25* single-, *Stxbp1* single- and double- mutants neurons. **B-E)** Quantification of several morphological parameters in GABAergic neurons: B) Total dendritic length. C) Synaptic density. D) Synaptic size and E) Synaptic intensity.