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Supplemental information

A Neuroligin-1 mutation associated with Alzheimer's disease produces memory and age-dependent impairments in hippocampal plasticity

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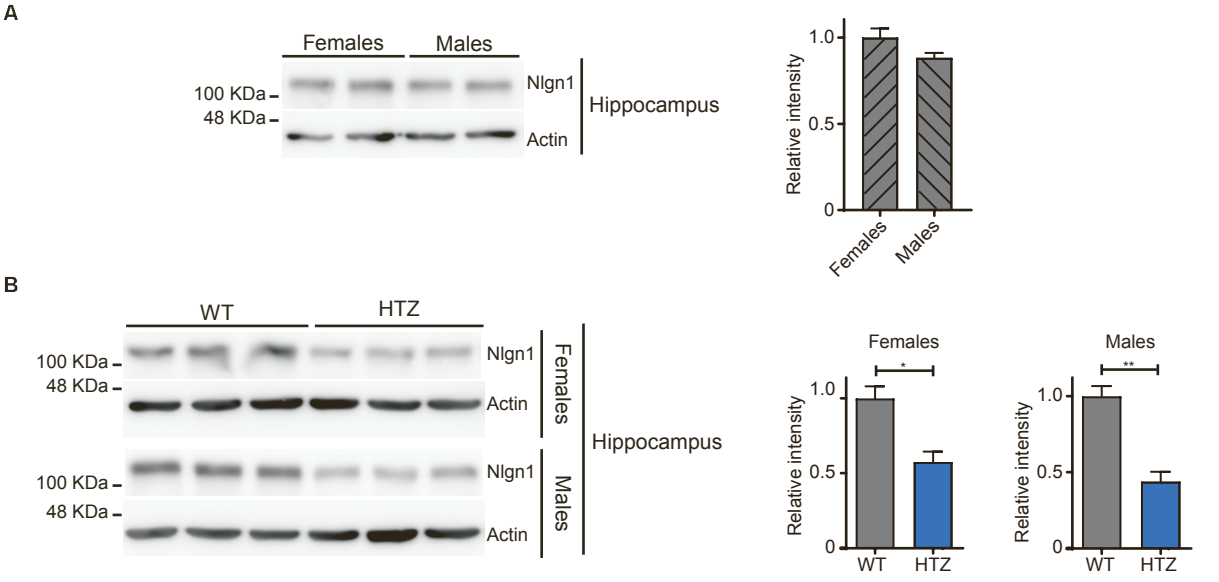
Supplementary Figure S1. Nlgn1 protein levels in female and male Nlgn1 Thr271fs mice, related to Figure 1.

(A) Western-blot experiments showing expression of Nlgn1 in hippocampal lysates from female and male wild-type mice (unpaired t-test: p=0.1202. Normalized intensity to female levels: females 1 ± 0.052; males: 0.882 ± 0.028). (B) Similar decrease in hippocampal Nlgn1 levels in female and male HTZ Nlgn1 Thr271fs mice, compared to wild-type mice (female mice: unpaired t-test: p=0.0156. Normalized intensity to wild-type levels: wild-type 1 ± 0.07; HTZ 0.574 ± 0.06. Male mice: unpaired t-test: p=0.0037. Normalized intensity to wild-type levels: wild-type 1 ± 0.06; HTZ 0.43 ± 0.06). Expression of Nlgn1 and actin were detected with specific antibodies, as indicated. Data are represented as mean +/- SEM.


A
Females
Males
100 KDa
48 KDa
Nlgn1
Actin
Hippocampus
Relative intensity
1.0
0.5
0
Females
Males
B
WT
HTZ
100 KDa
48 KDa
Nlgn1
Actin
Females
Males
Hippocampus
Females
Males
*
**
Relative intensity
1.0
0.5
0
WT
HTZ

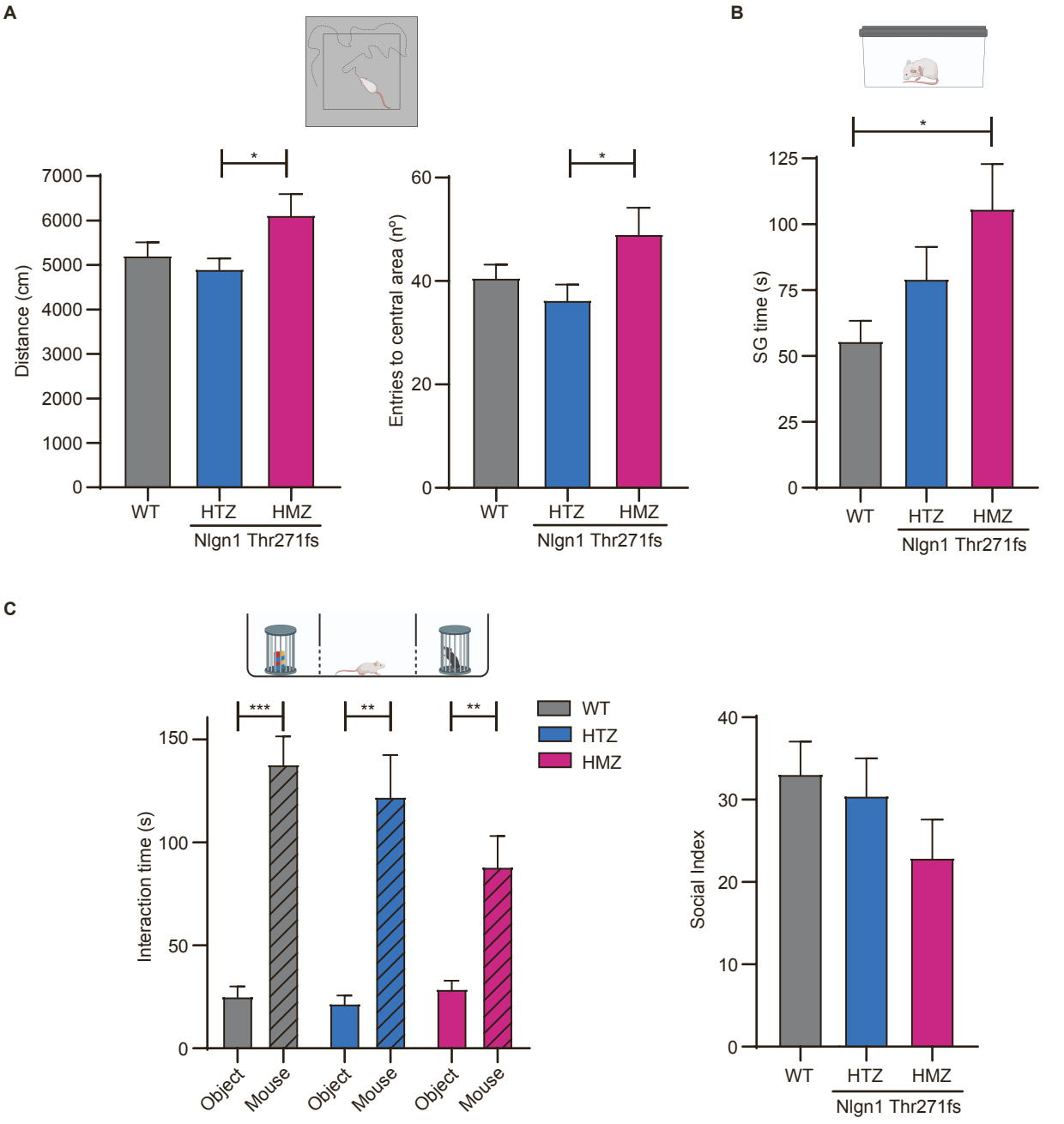
Supplementary Figure S2. Analysis of Nlgn1 Thr271fs mouse in behavioral tests related to neurodevelopmental disorders, related to STAR Methods.

(A) Open field. Total distance and entries to the center area are shown (one-way ANOVA with Tukey's post-hoc analysis. $F_{(2.56)}$=3.398, p=0.040, wild-type vs HTZ p=0.799, wild-type vs HMZ p=0.1771, HTZ vs HMZ p=0.035. Distance (cm): wild-type 5206 ± 303.5; HTZ 4902 ± 244.6; HMZ 6116 ± 476.7). Entries to the central area (one-way ANOVA with Tukey's post-hoc analysis. $F_{(2.56)}$=3.078, p=0.053, wild-type vs HTZ p=0.685, wild-type vs HMZ p=0.279, HTZ vs HMZ p=0.043. Entries: wild-type 40.56 ± 2.64; HTZ 36.26 ± 3.07; HMZ 49.00 ± 5.16). Wild-type, n=18; HTZ, n=23; HMZ, n=18.

(B) Increased self-grooming time (s) in HMZ Nlgn1 Thr271fs mice (one-way ANOVA with Tukey's post-hoc analysis. $F_{(2.56)}$=3.041, p=0.0558, wild-type vs HTZ p=0.417, wild-type vs HMZ p=0.044, HTZ vs HMZ p=0.320. Wild-type 55.53 ± 7.81; HTZ 79.19 ± 12.26; HMZ 105.80 ± 17.13). Wild-type, n=16; HTZ, n=26; HMZ, n=17.

(C) Social interaction in the three-chamber test. Graphs show time interacting with the mouse or the object (left) and social index (right). Differences in interaction time with new and familiar object within each genotype (wild-type, mouse vs object paired t-test p=0.0001; HTZ, mouse vs object paired t-test p=0.0019; HMZ, mouse vs object paired t-test p=0.008.Time (s) interacting with the object: Wild-type 25.04 ± 5.02; HTZ 21.66 ± 4.02; HMZ 28.80 ± 4.12; time (s) interacting with the mouse: Wild-type 138.0 ± 13.50; HTZ 122.0 ± 20.44; HMZ 88.11 ± 15.04). Social index (one-way ANOVA analysis. $F_{(2.26)}$=1.26, p=0.299. Wild-type 33.08 ± 3.98; HTZ 30.44 ± 4.58; HMZ 22.92 ± 4.67). Wild-

type, n=10; HTZ, n=11; HMZ, n=8. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Data are represented as mean +/- SEM.


A
Distance (cm)
7000
6000
5000
4000
3000
2000
1000
0
*
WT
HTZ
HMZ
Nlgn1 Thr271fs
Entries to central area (nº)
60
40
20
0
*
WT
HTZ
HMZ
Nlgn1 Thr271fs
B
SG time (s)
125
100
75
50
25
0
*
WT
HTZ
HMZ
Nlgn1 Thr271fs
C
Interaction time (s)
150
100
50
0
***
**
**
WT
HTZ
HMZ
Object
Mouse
Object
Mouse
Object
Mouse
Social Index
40
30
20
10
0
WT
HTZ
HMZ
Nlgn1 Thr271fs

Supplementary Figure S3. Permanence of specific memory deficits in HTZ Nlgn1 Thr271fs mice at an old age, related to Figures 3 and 4.

(A) Fear conditioning in old-mice. Freezing time (s) during conditioning (left) (two-way repeated measures ANOVA with Sidak's post-hoc analysis on genotype. Interaction x genotype $F_{(2.88)}$=0.120, p= 0.886; genotype $F_{(1.44)}$=0.241 p=0.625). Response during context presentation (middle) (unpaired t-test p=0.531. Wild-type, 106.3 ± 20.41; HTZ, 91.90 ± 12.30). Response during tone presentation (right) (unpaired t-test p=0.539. Wild-type, 32.29 ± 4.77; HTZ, 28.40 ± 4.13). Wild-type, n=21; HTZ, n=27. Age of mice, 15.28 ± 0.19 months.

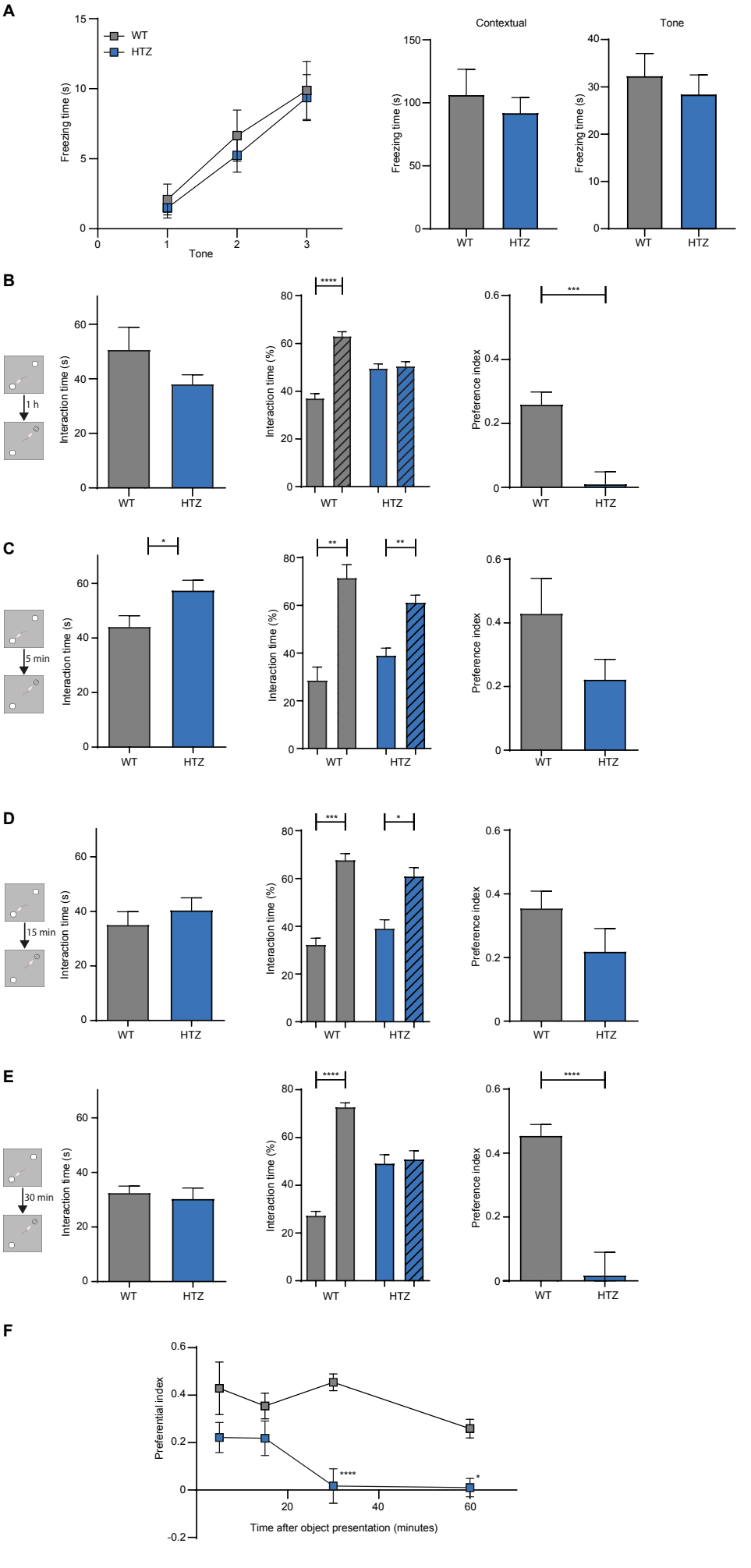
(B) Recognition memory in old-mice. Interaction time (s) during exploration (left) (unpaired t-test p=0.1429. Wild-type, 50.61 ± 8.31; HTZ, 38.06 ± 3.398). Interaction time (%) with objects one hour after exploration (center). Differences in interaction time with new and familiar object within each genotype (wild-type, familiar vs novel object paired t-test p<0.0001; HTZ, familiar vs novel object paired t-test p=0.8155. Wild-type, familiar 37.03 ± 1.96, novel 62.97 ± 1.96; HTZ, familiar 49.54 ± 1.92, novel 50.46 ± 1.92. Corresponding time in s: Wild-type, familiar 23.62 ± 3.42, novel 37.69 ± 4.14; HTZ, familiar 22.72 ± 1.81, novel 24.61 ± 3.31). Preference index (right) (unpaired t-test p=0.0002. Wild-type, 0.25 ± 0.03; HTZ, 0.01 ± 0.03). Wild-type, n=12; HTZ, n=14. Age of mice, 15.15 ± 0.25 months.

(C-E) Recognition memory at short-time intervals in old mice. (C) Recognition memory at 5 minutes. Interaction time (s) during exploration (left) Unpaired t-test p=0.0248. Wild-type, 44.08 ± 4.11; HTZ, 57.45 ± 3.73). Interaction time (%) with objects (center). Differences in interaction time with new and familiar object within each genotype (wild-type, familiar vs novel object paired t-test p=0.0032;

HTZ, familiar vs novel object paired t-test p=0.0045 Wild-type, familiar 28.57 ± 5.56, novel 71.43 ± 5.56; HTZ, familiar 38.91 ± 3.18, novel 61.09 ± 3.18. Corresponding time in s: Wild-type, familiar 11.46 ± 2.80, novel 25.21 ± 2.88; HTZ, familiar 15.24 ± 2.20, novel 21.87 ± 1.49). Preference index (right) (unpaired t-test p=0.1077. Wild-type, 0.42 ± 0.11; HTZ, 0.22 ± 0.06). Wild-type, n=11; HTZ, n=13. Age of mice, 17.58 ± 0.30 months. (D) Recognition memory at 15 minutes. Interaction time (s) during exploration (left) (unpaired t-test p=0.4327. Wild-type, 35.04 ± 4.88; HTZ, 40.41 ± 4.56). Interaction time (%) with objects (center). Differences in interaction time with new and familiar object within each genotype (wild-type, familiar vs novel object paired t-test p=0.0001; HTZ, familiar vs novel object paired t-test p=0.0119. Wild-type, familiar 32.28 ± 2.69, novel 67.72 ± 2.69; HTZ, familiar 39.07 ± 3.63, novel 60.93 ± 3.63. Corresponding time in s: Wild-type, familiar 9.11 ± 1.27, novel 19.20 ± 2.12; HTZ, familiar 11.84 ± 1.81, novel 17.33 ± 1.38). Preference index (right) (unpaired t-test p=0.645. Wild-type, 0.35 ± 0.05; HTZ, 0.21 ± 0.07). Wild-type, n=10; HTZ, n=12. Age of mice, 17.88 ± 0.28 months. (E) Recognition memory at 30 minutes. Interaction time (s) during exploration (left) (unpaired t-test p=0.4327). Wild-type, 32.49 ± 2.49; HTZ, 30.34 ± 3.97. Interaction time (%) with objects (center). Differences in interaction time with new and familiar object within each genotype (wild-type, familiar vs novel object paired t-test p<0.0001; HTZ, familiar vs novel object paired t-test p=0.818. Wild-type, familiar 27.28 ± 1.76, novel 72.72 ± 1.76; HTZ, familiar 49.14 ± 3.62, novel 50.86 ± 3.62. Corresponding time in s: Wild-type, familiar 9.23 ± 1.37, novel 23.83 ± 2.42; HTZ, familiar 16.42 ± 2.13, novel 16.79 ± 1.77). Preference index (right)

(unpaired t-test p<0.0001. Wild-type, 0.45 ± 0.03; HTZ, 0.01 ± 0.07). Wild-type, n=11; HTZ, n=10. Age of mice, 18.45 ± 0.29 months.

(F) Preference index at different delays (mixed-effects model with Sidak's post-hoc analysis. $F_{(1, 85)}$=31.98, p<0.0001. P-values< 0.05: 30 minutes: WT vs HTZ p<0.0001; HTZ vs HMZ p=0.035. 60 minutes: HTZ vs HMZ p=0.0186). * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. Data are represented as mean +/- SEM.


A
WT
HTZ
Freezing time (s)
Tone
Contextual
Tone
B
1 h
Interaction time (s)
Interaction time (%)
Preference index
****
***
C
5 min
*
**
**
D
15 min
***
*
E
30 min
****
****
F
Preferential index
Time after object presentation (minutes)
****
*

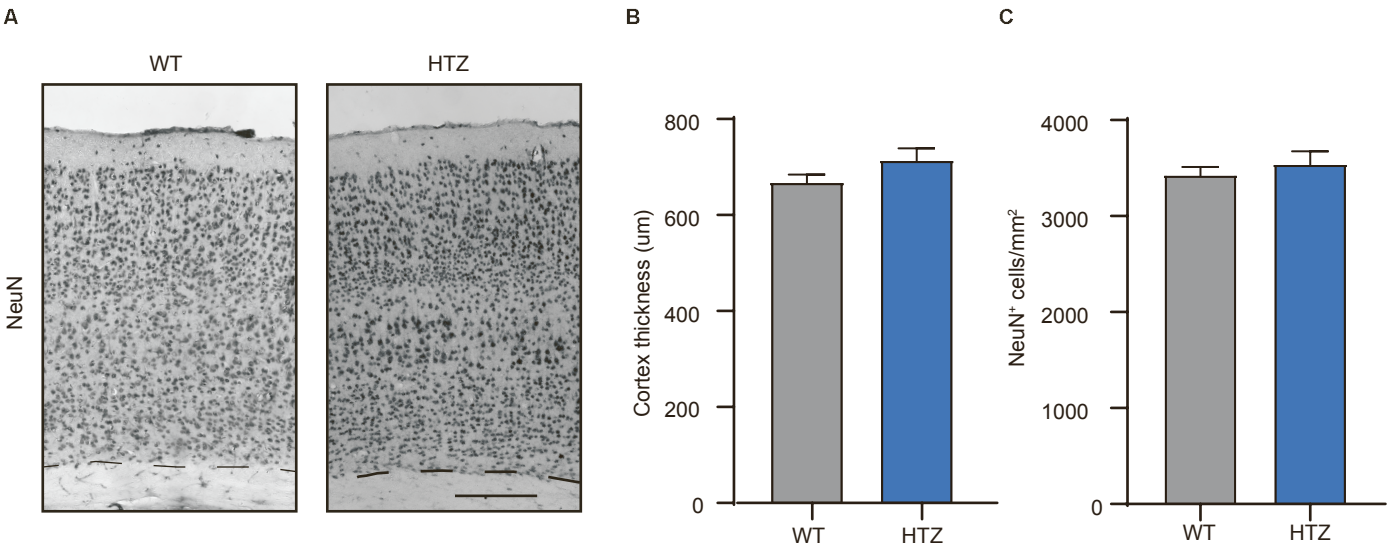
Supplementary Figure S4. Neuronal density in HTZ Nlgn1 Thr271fs mice, related to Figure 7.

(A) NeuN staining in the cortex of wild-type and HTZ Nlgn1 Thr271fs mice. Scale bar, 200 μm.

(B) Cortical thickness (unpaired t-test p=0.1925. Wild-type, 668.01 ± 16.41; HTZ, 714.34 ± 24.62, thickness in μm).

(C) Neuronal density (unpaired t-test p=0.5123. Wild-type, 3429.46 ± 82.71; HTZ, 3541.74 ± 132.64, cells/mm^2).

(B-C) Three independent animals per condition, 5 brain slices per animal. Age of mice, 12.34 ± 0.23 months. Data are represented as mean +/- SEM.


A
WT
HTZ
NeuN
B
Cortex thickness (um)
800
600
400
200
0
WT
HTZ
C
NeuN+ cells/mm2
4000
3000
2000
1000
0
WT
HTZ