

Supplementary Information

Genetic dissection identifies Necdin as a driver gene in a mouse model of paternal 15q duplications

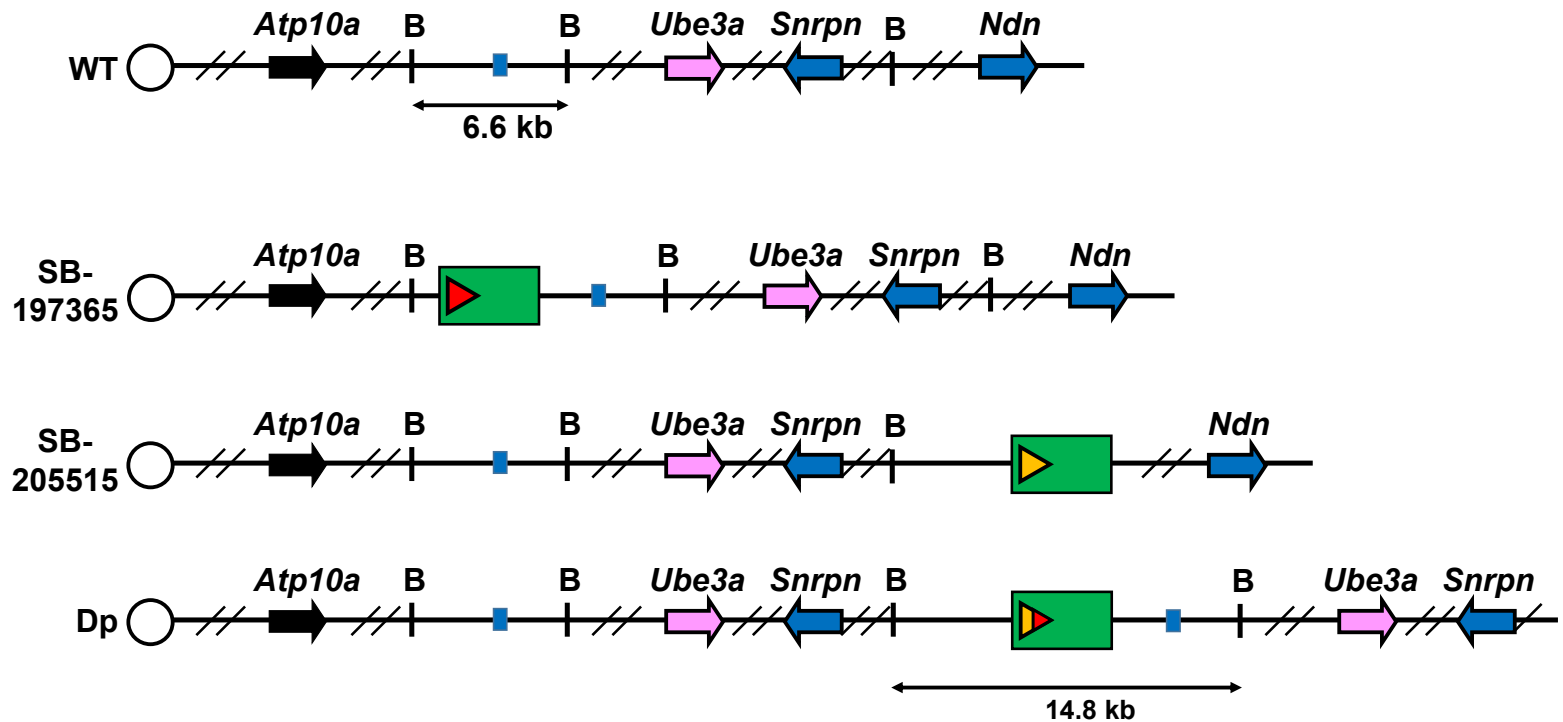
Tamada et al.

Supplementary Information contains

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Supplementary Table 1

Supplementary Figure 1

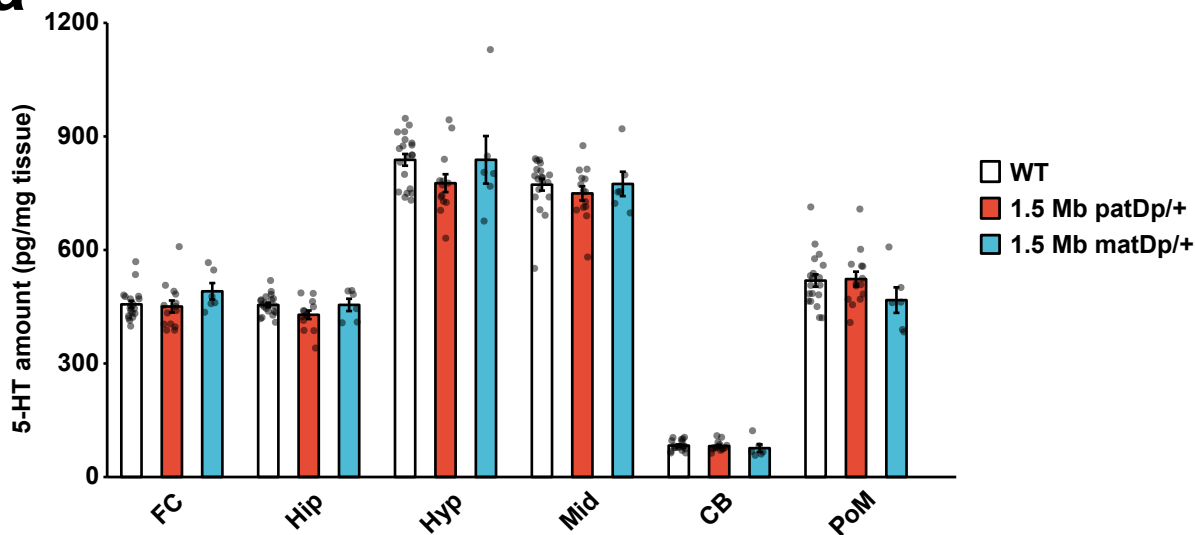


Supplementary Figure 1. Genomic map of 1.5 Mb duplication mice

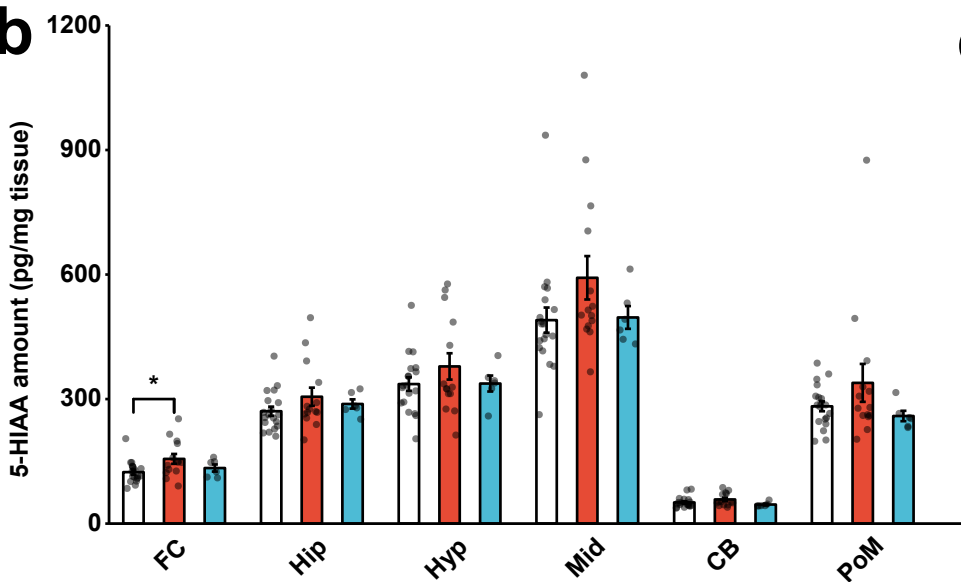
Genomic configuration and restriction map of 1.5 Mb duplication mice in mouse chromosome 7B-C. Mice with a transposon including a loxP sequence insertions between *Atp10a* and *Ube3a* (SB-197365) and between *Snrpn* and *Ndn* (SB-205515) were obtained from the TRACER database⁵⁹. Duplication of the intervening segment (Dp) was obtained by *in vivo* Cre-mediated recombineering *in trans* with an *Hprt-Cre* transgene^{21,22}. Blue box, the probe for Southern blot; B, BsrGI; Green box: transposon; Red and orange triangle; loxP sequence.

Supplementary Figure 2

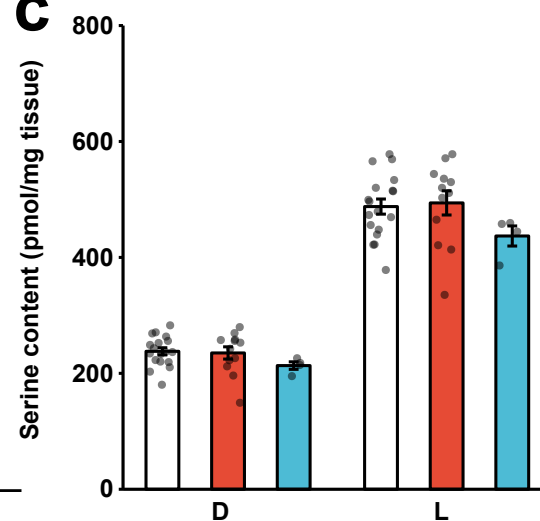
a



b



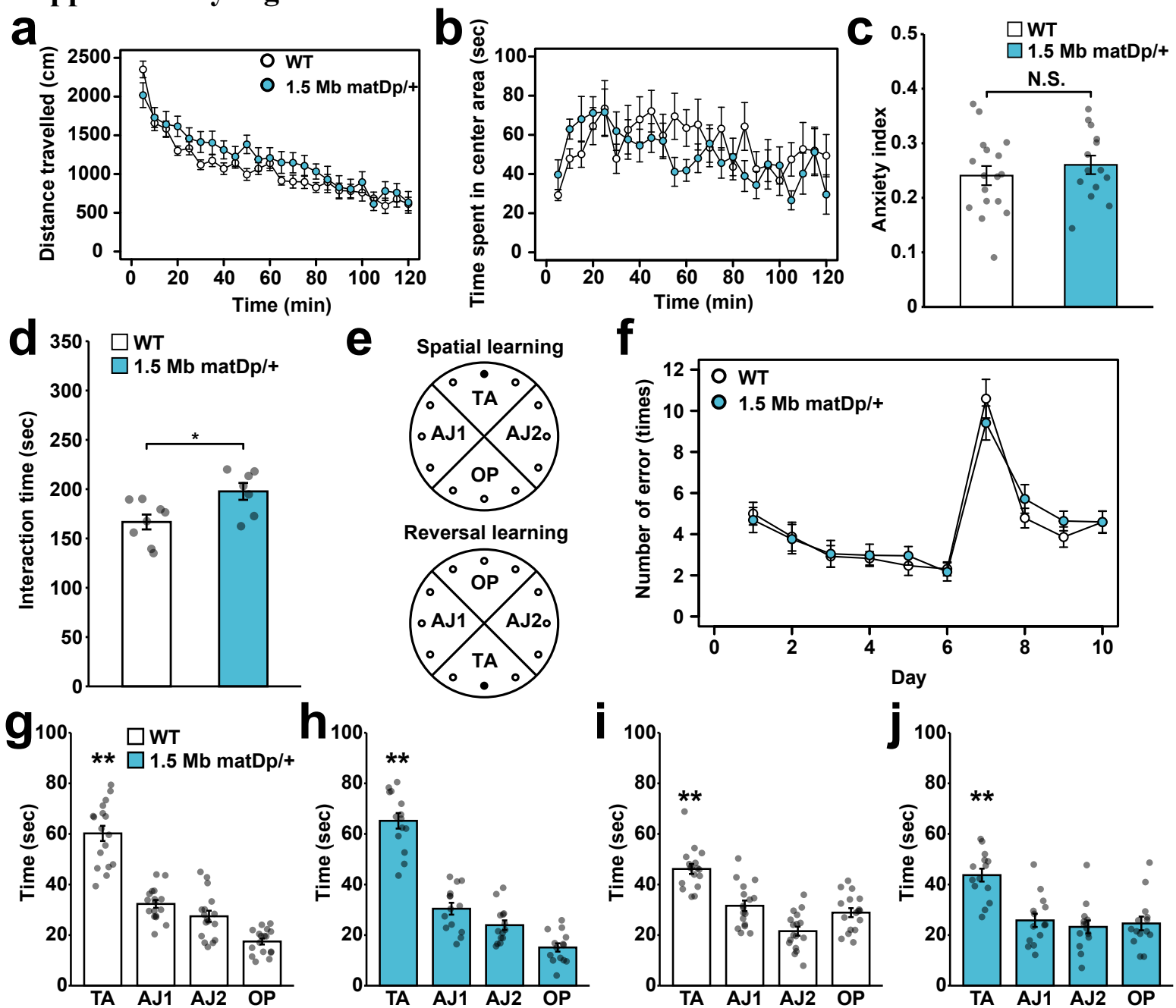
c



Supplementary Figure 2. Duplication of 1.5 Mb does not affect serotonin and serine amount in the brain

(a-b) Quantification of serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in 6 brain regions using HPLC. FC: frontal cortex, Hip: hippocampus, Hyp: hypothalamus, Mid: midbrain, CB: cerebellum, PoM: pons and medulla. N = 19 (WT; 5-HIAA; all brain regions, 5-HT; FC, Mid, CB, PoM), 20 (WT; 5-HT; Hip, Hyp), 13 (*1.5 Mb patDp/+*; 5-HT; Hip, Hyp), 14 (*1.5 Mb patDp/+*; 5-HIAA; all brain regions, 5-HT; FC, Mid, CB, PoM), 6 (*1.5 Mb matDp/+*) biologically independent mice. 5-HIAA : WT vs. *1.5 Mb patDp/+* : $p = 0.028$. * $p < 0.05$ (Tukey-Kramer test). **(c)** Quantification of D- and L- serine in frontal cortices. N=18, 12, 4 biologically independent mice for WT, *1.5 Mb patDp/+* and *matDp/+*, respectively. Data are represented as mean $\pm$ s.e.m.

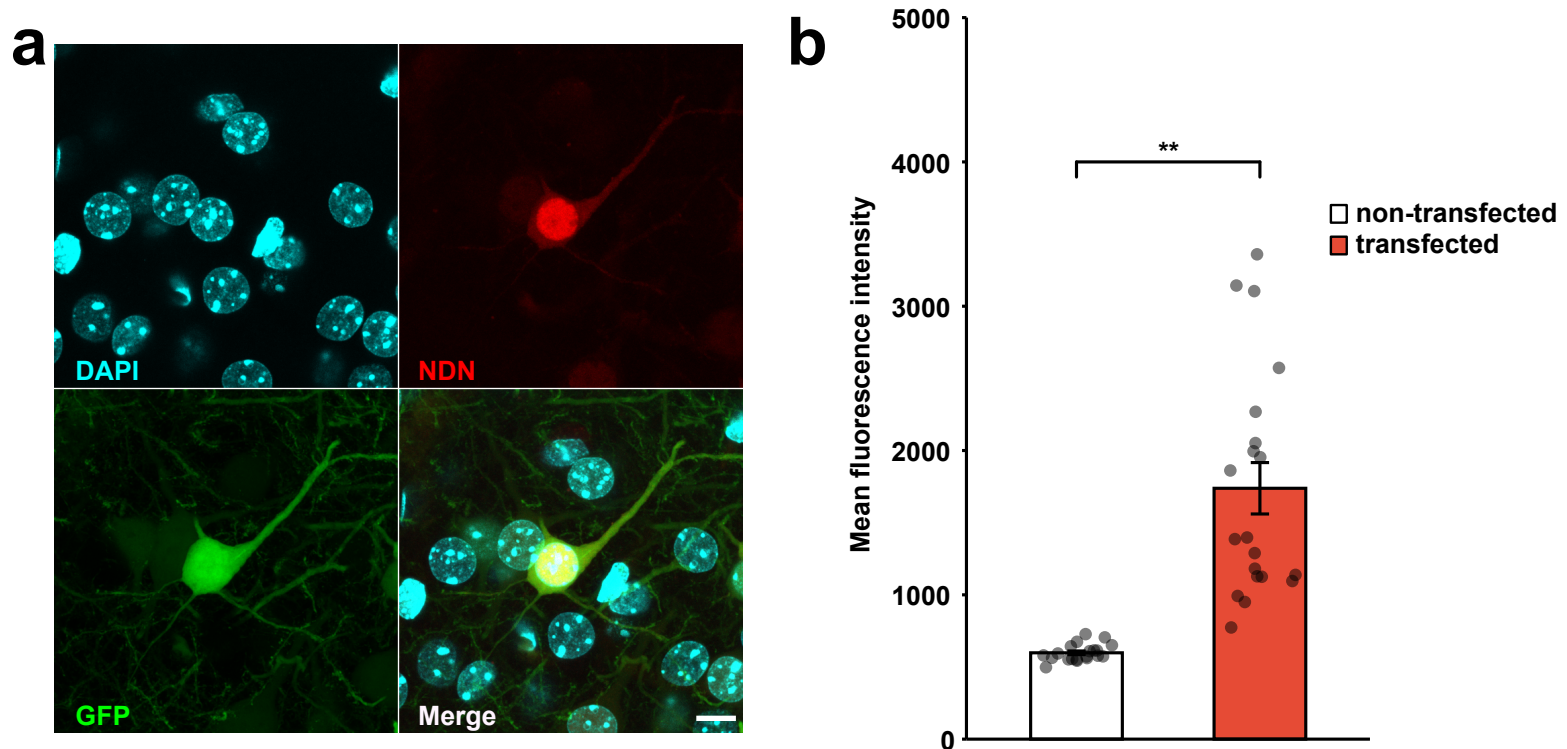
Supplementary Figure 3



Supplementary Figure 3. Maternal duplication of 1.5 Mb does not induce ASD-like behaviors

(a-c) Open field test in 1.5 Mb matDp/+ mice. Mean values in 5-min segments are plotted including (a) locomotor activity and (b) time spent in the center area. (c) Anxiety index is calculated as distance in the center area divided by total distance during the first 30 min. N=17 (WT) and 14 (*1.5 Mb matDp/+*) biologically independent mice. (d) Reciprocal social interaction test. The interaction time for each pair of mice is shown. N=8 (WT), 7 (*1.5 Mb matDp/+*) pairs. WT vs. *1.5 Mb matDp/+* : $p = 0.018$. ** $p < 0.05$ (t-test). (e-j) Spatial and reversal learning memory was evaluated by the Barnes maze test. (e) A schematic view of 2 learning paradigms. TA: target quadrant, AJ1/2: adjacent quadrant and OP: opposite quadrant. A black filled circle indicates the correct hole with an escapable box under the hole. (f) The number of errors until reaching the correct target is shown. Spatial and reversal learning was conducted on days 1 to 6 and 7 to 10, respectively. (g, h) Results of the probe test on day 6 (g: WT, h: *1.5 Mb matDp/+*). WT : TA vs. AJ1/AJ2/OP : $p < 0.0001$, *1.5 Mb matDp/+* : TA vs. AJ1/AJ2/OP: $p < 0.0001$. ** $p < 0.01$ (Dunnett's test). (i, j) Results of the reversal probe test on day 10. N=17 (WT) and 14 (*1.5 Mb matDp/+*) biologically independent mice. WT : TA vs. AJ1/AJ2/OP : $p < 0.0001$, *1.5 Mb matDp/+* : TA vs. AJ1/AJ2/OP: $p < 0.0001$. ** $p < 0.01$ (Dunnett's test). Data are represented as mean $\pm$ s.e.m. N.S.: not statistically significant.

Supplementary Figure 4

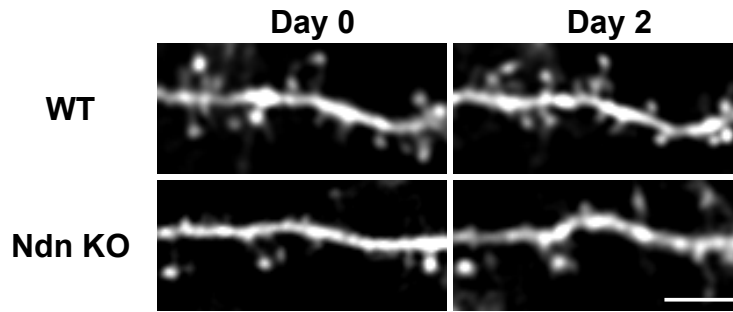


Supplementary Figure 4. Quantification of the expression level of *Ndn* in transfected neurons by *in utero* electroporation

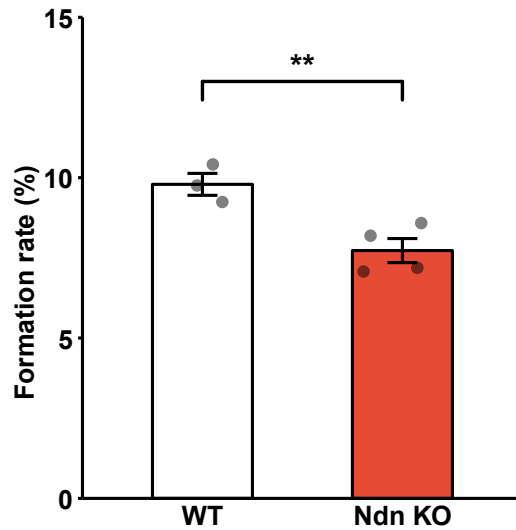
(a) Representative images of *Ndn* transfected cortical neurons. *Ndn* expressing plasmid and GFP plasmid were transfected to cortical neurons by *in utero* electroporation on E15.5. Immunohistochemistry by using NDN antibody was performed on postnatal day 3 weeks old. An arrow and arrowhead indicate transfected and non-transfected neurons, respectively. **(b)** Mean fluorescence intensity per pixel of NDN signal in each nucleus was measured in non-transfected and transfected neurons (N= 20 neurons from 2 biologically independent mice for each group). Non-transfected vs. transfected: $p < 0.0001$ ** $p < 0.01$ (t-test). Data are represented as mean $\pm$ s.e.m. Scale bar in **a**, 10 μ m.

Supplementary Figure 5

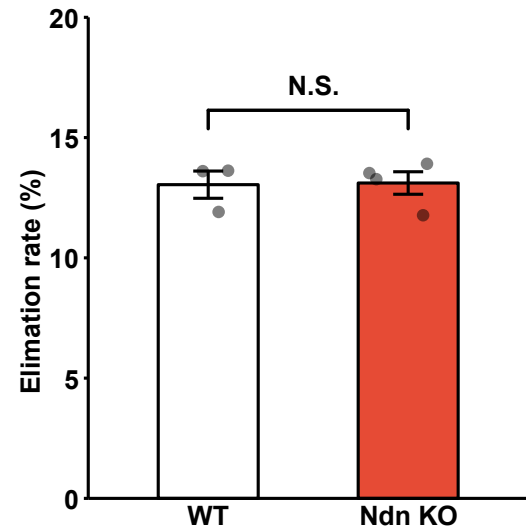
a



b



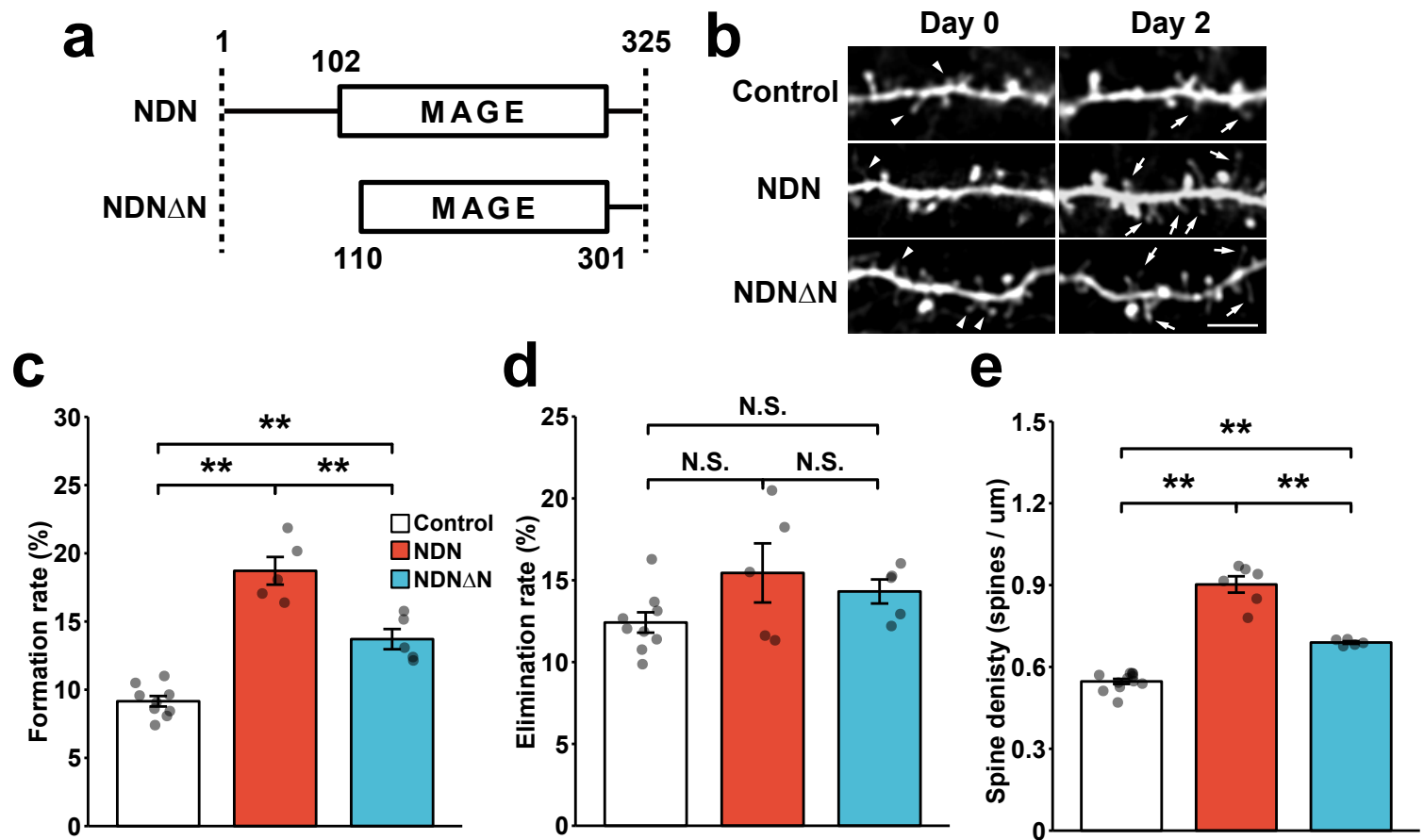
c



Supplementary Figure 5. *Ndn* KO mice exhibit decreased spine formation rate

(a) Representative images of dendrites in WT and *Ndn* KO mice. (b) Spine formation rate and (c) elimination rate calculated from WT and *Ndn* KO mice, visualized by *in utero* electroporation of EGFP plasmid. Arrows and arrowheads indicate newly formed and eliminated spines, respectively. The scale bar is 5 μ m. N = 3 (WT) and 4 (*Ndn* KO) biologically independent mice. Formation rate : WT vs. *Ndn* KO : $p = 0.0099$, Elimination rate : $p = 0.929$. ** $p < 0.01$ (t-test). Data are represented as mean $\pm$ s.e.m. N.S.: not statistically significant.

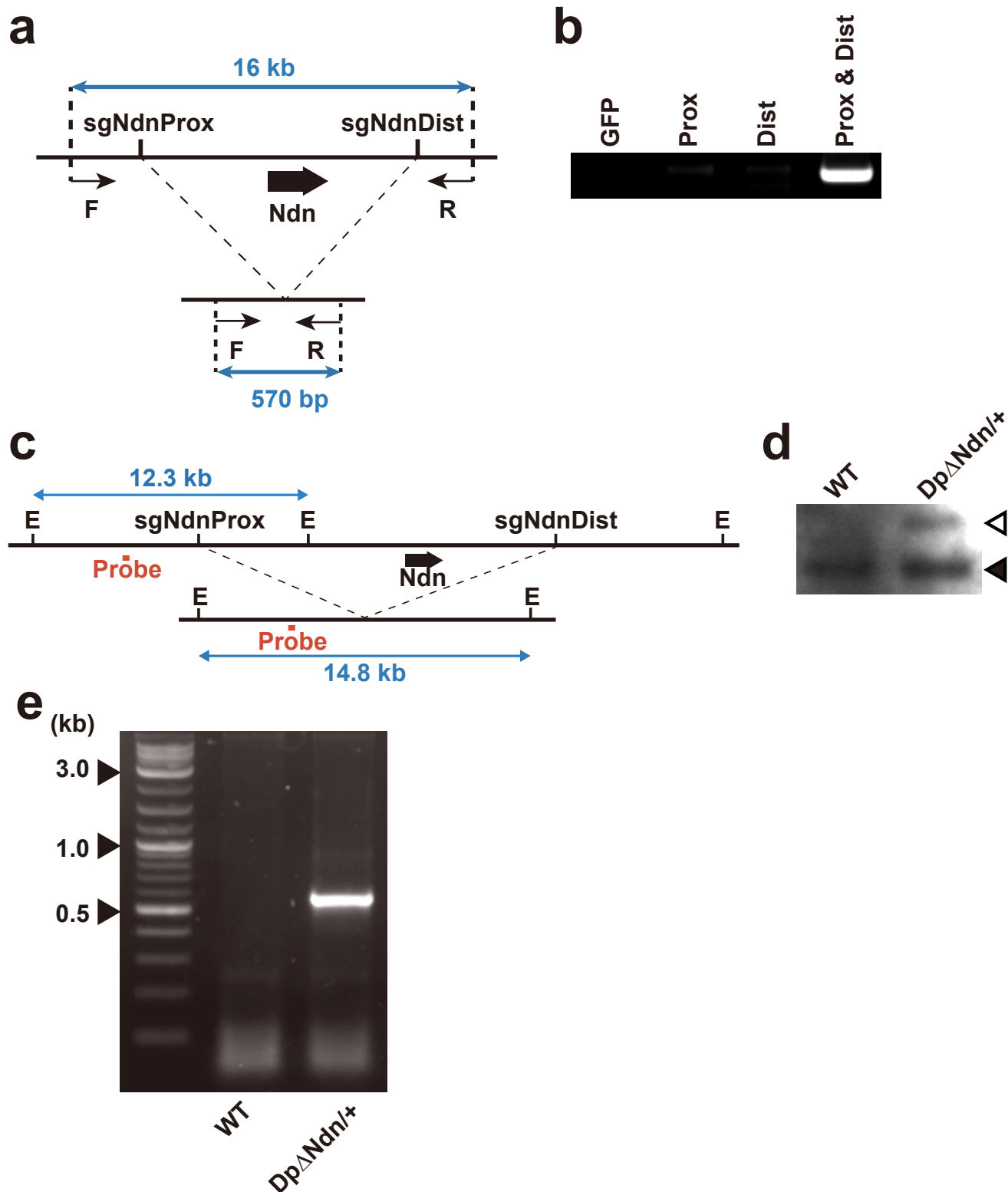
Supplementary Figure 6



Supplementary Figure 6. N-terminal of NDN partially contributes to dendritic spine formation

(a) A schematic protein structure of intact NDN (325 amino acids) and NDN Δ N (110-325). This NDN Δ N is reported to lack binding affinities and changes transcriptional activities. MAGE domain was identified using the database of protein domains, PROSITE (<http://prosite.expasy.org/>). **(b)** Representative images of dendritic spines electroporated with each construct of control, NDN, and NDN Δ N. Arrows and arrowheads indicate newly formed and eliminated spines within 2 days, respectively. **(c)** Spine formation rate, **(d)** elimination rate, and **(e)** density with overexpression of each plasmid are shown. Data of control and NDN are the same as in Figure 3c-e. Formation rate : Control vs. NDN : $p < 0.0001$, Control vs. NDN Δ N : $p = 0.0003$, NDN vs. NDN Δ N : $p = 0.0005$, Elimination rate : Control vs. NDN : $p = 0.115$, Control vs. NDN Δ N : $p = 0.398$, NDN vs. NDN Δ N : $p = 0.765$. Density: Control vs. NDN : $p < 0.0001$, Control vs. NDN Δ N : $p < 0.0001$, NDN vs. NDN Δ N : $p < 0.0001$. Arrows and arrowheads indicate newly formed and eliminated spines, respectively. A scale bar indicates 5 μ m. (c-e), $N = 4$ (control) and 5 (NDN and NDN Δ N) biologically independent mice. ** $p < 0.01$ (Tukey-Kramer test). Data are represented as mean $\pm$ s.e.m. N.S.: not statistically significant.

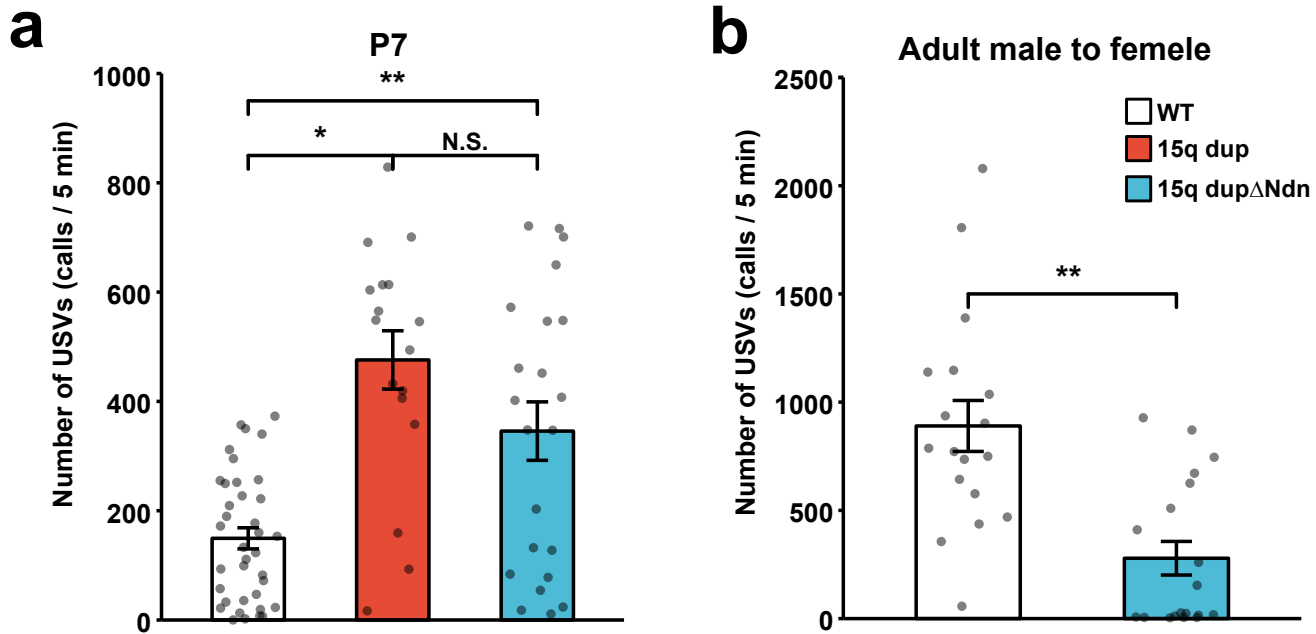
Supplementary Figure 7



Supplementary Figure 7. Validation of guide RNA for *15q dupΔNdn* and evaluation of genomic deletion

(a) A schema of deletion detection PCR. Two guide RNAs (sgNdnProx, sgNdnDist) were designed adjacent to the *Ndn* gene. F and R indicate PCR primers used for evaluating genomic deletion. **(b)** Genomic PCR of Neuro2a transfected with guide RNA expressing vectors. **(c)** Design of Southern blot probe. E: EcoRI site. Upper: WT and lower deleted alleles. **(d)** Southern blot using mouse tail genome. White triangle: deletion specific band (14.8 kb) and black triangle: WT band (12.3 kb). Similar results were obtained in 3 biologically independent mice (Source Data). **(e)** Genomic PCR using the tail genome of WT and *15q dupΔNdn* mice. Similar results were obtained in more than 3 biologically independent mice. The primer pair shown in **(a)** was used for PCR.

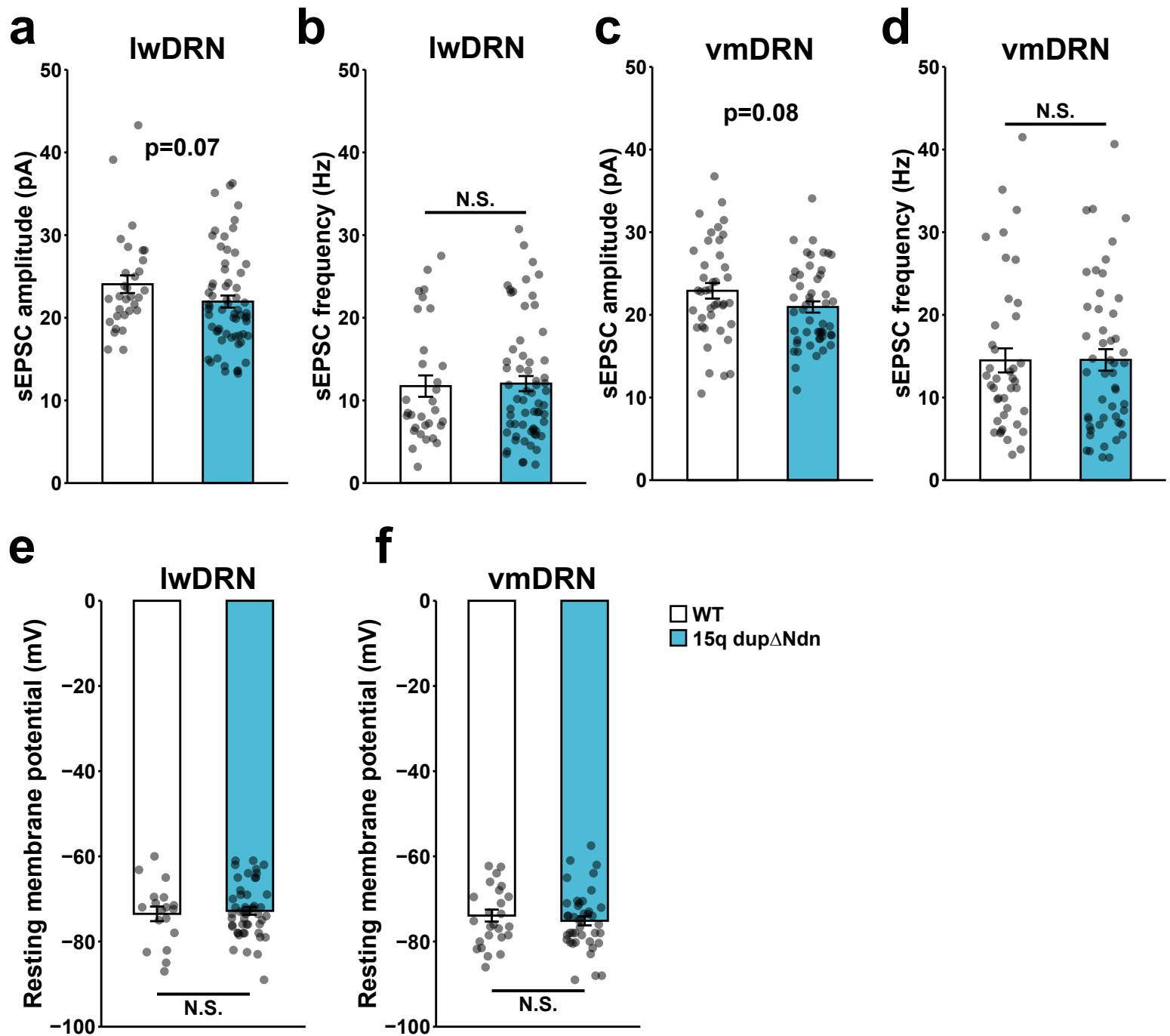
Supplementary Figure 8



Supplementary Figure 8. Altered social communications in paternal *15q dup* mice were retained in paternal *15q dupΔNdn* mice

(a) Maternal isolation-induced pup USV (P7). N = 37, 17, 22 for WT, *15q dup*, *15q dupΔNdn*, respectively. Both male and female mice were used for this analysis. P7 USV: WT vs. *15q dup* : $p = 0.0115$, WT vs. *15q dupΔNdn* : $p < 0.0001$, *15q dup* vs. *15q dupΔNdn* : $p = 0.2578$. * $p < 0.05$, ** $p < 0.01$ (Steel-Dwass test). **(b)** Female-induced male USV in adult mice. N = 18 and 19 for WT and *15q dupΔNdn* male mice, respectively. Adult USV: WT vs. *15q dupΔNdn* : $p = 0.0002$. ** $p < 0.01$ (Mann-Whitney U test). Data are represented as mean $\pm$ s.e.m. N.S.: not statistically significant.

Supplementary Figure 9



Supplementary Figure 9. Electrophysiological analyses of paternal *15q dup Δ Ndn* mice in the dorsal raphe nucleus

(a-d) Comparisons between WT and paternal *15q dup Δ Ndn* mice for sEPSC amplitude and frequency in 5-HT neurons recorded in the vmDRN and lwDRN. N = 41 (WT; vmDRN), 50 (*15q dup Δ Ndn*; vmDRN), 31 (WT; lwDRN), 63 (*15q dup Δ Ndn*; lwDRN) neurons from biologically independent 3-5 mice. **(e-f)** The resting membrane potentials of 5-HT neurons in the vmDRN and lwDRN. N = 26 (WT; vmDRN), 41 (*15q dup Δ Ndn*; vmDRN), 18 (WT; lwDRN), 48 (*15q dup Δ Ndn*; vmDRN) neurons from biologically independent 2-4 mice. Statistical testing was conducted using the Mann-Whitney U test and all parameters between genotypes did not reach the statistically significant level ($p > 0.05$). Data are represented as mean $\pm$ s.e.m. N.S.: not statistically significant.

Supplementary Table 1 : Primer sequences

Primer name	Related figure	Sequence (5' to 3')	Used for
1.5 Mb Dp_Fw	Fig1a	AAGTAGATGTCCTAACTGACTTGC	Genotyping
1.5 Mb Dp_Rv	Fig1a	AGCTTTGAATACCATAACCAACAAA	Genotyping
Gapdh_Fw	Fig1d, 5c	ACGGGAAGCTCACTGGCATGGCCTT	qPCR
Gapdh_Rv	Fig1d, 5c	CATGAGGTCCACCACCCTGTTGCTG	qPCR
Herc2_Fw	Fig1d, 5c	TGGCCCTTTAAGCCCCAAT	qPCR
Herc2_Rv	Fig1d, 5c	GCCTATTAACCCCCAACCTATG	qPCR
Gabra5_Fw	Fig1d	TCCAAACATCCCCAAAGAGC	qPCR
Gabra5_Rv	Fig1d	GAGAGGTGGCCCCCTTTTATC	qPCR
Gabrb3_Fw	Fig1d, 5c	GAATGTTGTCTTCGCCACAGGT	qPCR
Gabrb3_Rv	Fig1d, 5c	ACCCACGAGAGGATTGTGATCA	qPCR
Atp10a_Fw	Fig1d, 5c	CGCACTCACCATCTGCAATACA	qPCR
Atp10a_Rv	Fig1d, 5c	CGTGAACCTCCGAAGGAAATCT	qPCR
Ube3a_Fw	Fig1d, 5c	TCTGCTGCTGCTATGGAAGA	qPCR
Ube3a_Rv	Fig1d, 5c	CACATTCCACGTTAGGTGACA	qPCR
Ube3a_ATS_Fw	Fig1d, 5c	GGCACCCCTTGTTTGAAACTT	qPCR
Ube3a_ATS_Rv	Fig1d, 5c	GCTCATGACCCTGTCCTTTC	qPCR
Snord116_Fw	Fig1d, 5c	TGGATCTATGATGATTCCCAG	qPCR
Snord116_Rv	Fig1d, 5c	TGGACCTCAGTTCCGATGAG	qPCR
Snord115_Fw	Fig1d, 5c	GGGTCAATGATGACAACCCAATG	qPCR
Snord115_Rv	Fig1d, 5c	GGGCCTCAGCGTAATCCTATTG	qPCR
lpw_Fw	Fig1d, 5c	TCACCACAACACTGGACAAAA	qPCR
lpw_Rv	Fig1d, 5c	TGCTGCTACACAGGAAAGAGG	qPCR
Snrpn_Fw	Fig1d, 5c	GCAAAACAGCCAGAACGTGAA	qPCR
Snrpn_Rv	Fig1d, 5c	GCACACGAGCAATGCCAGTAT	qPCR
Ndn_Fw	Fig1d, 5b, 5c	GTATCCCAAATCCACAGTGC	qPCR, ddPCR
Ndn_Rv	Fig1d, 5b, 5c	TAACTCTCCAGGGCCTTCTT	qPCR, ddPCR
Magel2_Fw	Fig1d, 5b, 5c	TTGGTGCCACTTTCTGTGCTC	qPCR, ddPCR
Magel2_Rv	Fig1d, 5b, 5c	GGCAGGAAAGGTCTCTGATGTG	qPCR, ddPCR
Mktn3_Fw	Fig1d, 5c	AGAGCATTCTGTGCTTCGCCT	qPCR
Mktn3_Rv	Fig1d, 5c	TACTGAAGCAAGAGCCAACGGT	qPCR
Ndn_deletion_Fw	Fig5A, FigS7a, b, e	TAGTCCCAGGTTACCAAGGC	Genotyping, deletion detection PCR
Ndn_deletion_Rv	Fig5A, FigS7a, b, e	CCTCCACATGAGTCTGCTG	Genotyping, deletion detection PCR
Htr1a_Fw	Fig5b	CAACTATCTCATCGGCTCCT	ddPCR
Htr1a_Rv	Fig5b	GTCCTCTTGTTACGTCATGTC	ddPCR
Herc2_ddPCR_Fw	Fig5b	ATAGACACAGATGACTATGCTC	ddPCR
Herc2_ddPCR_Rv	Fig5b	GTTTCCATCTTAGTGTCTGTC	ddPCR
Peg12_Fw	Fig5b, 5c	TTTTCCCCCTTGTTTCTGCTG	qPCR, ddPCR
Peg12_Rv	Fig5b, 5c	TGTCCCTGGAGTGCCATTAT	qPCR, ddPCR
Cyfp1_Fw	Fig5c	CTTGACACCCAGCGAAAAAC	qPCR
Cyfp1_Rv	Fig5c	TACTCACACTCCCATCCATCAG	qPCR
Chrna7_Fw	Fig5c	CTTGATAGCACAGTACTTCG	qPCR
Chrna7_Rv	Fig5c	CCATGCACACCAATTTCAG	qPCR