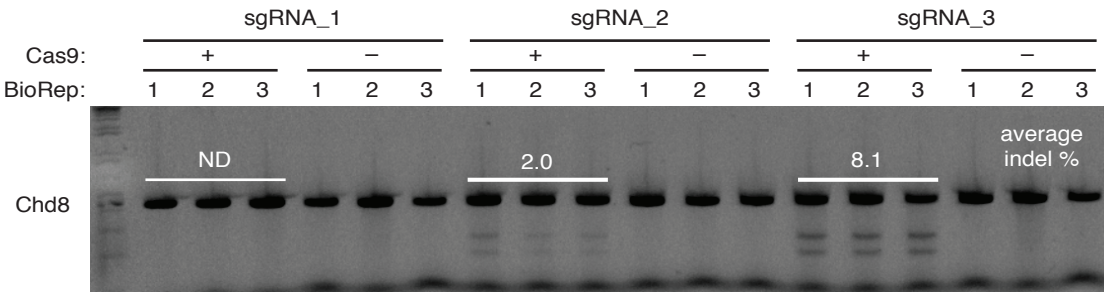

sgRNA_1
sgRNA_2
sgRNA_3
Cas9:
+
–
BioRep:
1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3
Chd8
ND
2.0
8.1
average indel %

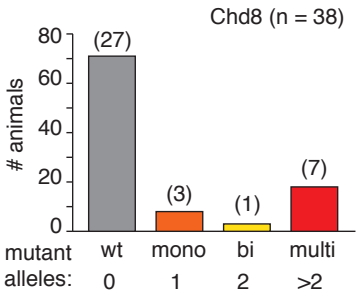

Chd8 (n = 38)
animals
80
60
40
20
0
(27)
(3)
(1)
(7)
mutant alleles:
wt 0
mono 1
bi 2
multi >2

Figure S1. In vitro validation of *Chd8*-targeted sgRNA and quantification of first generation gene edited progeny, Related to Figure 1

(Left) In vitro validation and quantification of *Chd8*-targeted sgRNA efficiency in mouse N2a cells using the SURVEYOR assay for three biological replicates with and without Cas9 co-transfection. (Right) Classification and quantification of mosaic founder genotypes. Mice were classified by having no (WT), one (monoallelic), two (biallelic), or >2 (multiallelic) mutant allele(s). Data are (Left) mean indel percent and (Right) total number of animals.

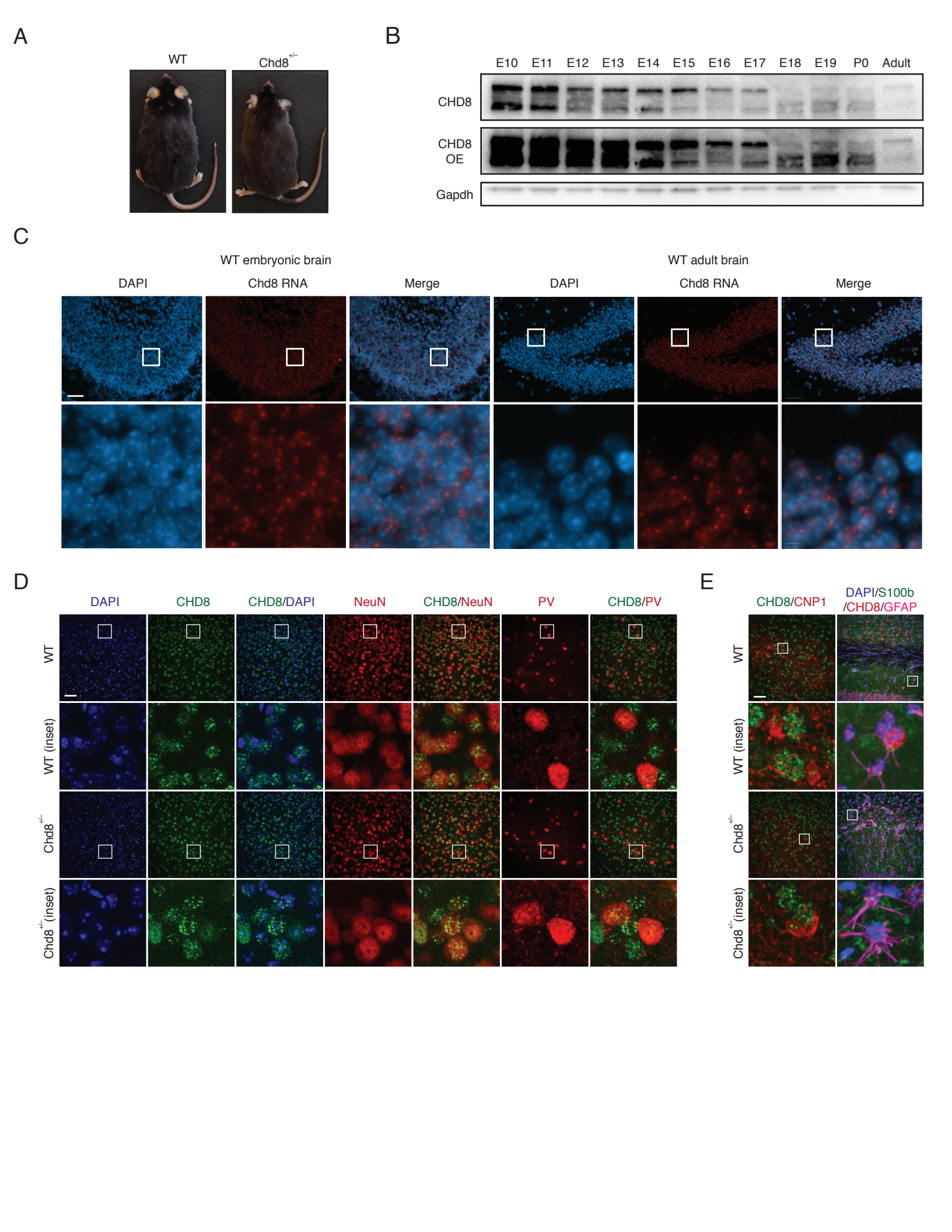

A
WT
Chd8+/–
B
E10 E11 E12 E13 E14 E15 E16 E17 E18 E19 P0 Adult
CHD8
CHD8 OE
Gapdh
C
WT embryonic brain
WT adult brain
DAPI
Chd8 RNA
Merge
DAPI
Chd8 RNA
Merge
D
DAPI
CHD8
CHD8/DAPI
NeuN
CHD8/NeuN
PV
CHD8/PV
WT
WT (inset)
Chd8+/–
Chd8+/– (inset)
E
CHD8/CNP1
DAPI/S100b /CHD8/GFAP
WT
WT (inset)
Chd8+/–
Chd8+/– (inset)

Figure S2. *Chd8*$^{+/-}$ mice are viable and CHD8 is expressed throughout development in most cell types, Related to Figure 1

(A) Representative images of adult male *Chd8*$^{+/-}$ mice and wild-type littermates.

(B) Profiling of CHD8 protein expression throughout development in whole brain samples. Each lane was loaded with 5 μg of total protein with GAPDH as loading control and normalized. An overexposed (OE) image is also shown to better visualize weaker bands.

(C) Representative fluorescence images within the midbrain of wild-type embryonic and adult brain slices probed for *Chd8* mRNA using RNA-FISH. The enclosed region outlines the zoomed region. Scale bar represents 50 μm.

(D-E) Representative immunofluorescence images of somatosensory cortex from male *Chd8*$^{+/-}$ mice and wild-type littermates showing CHD8 is expressed in most major cell types. CHD8 is expressed in mature neurons (NeuN positive), interneurons (parvalbumin (PV) positive), oligodendrocytes (CNP1 positive), and astrocytes (GFAP positive). Scale bar represents 50 μm.

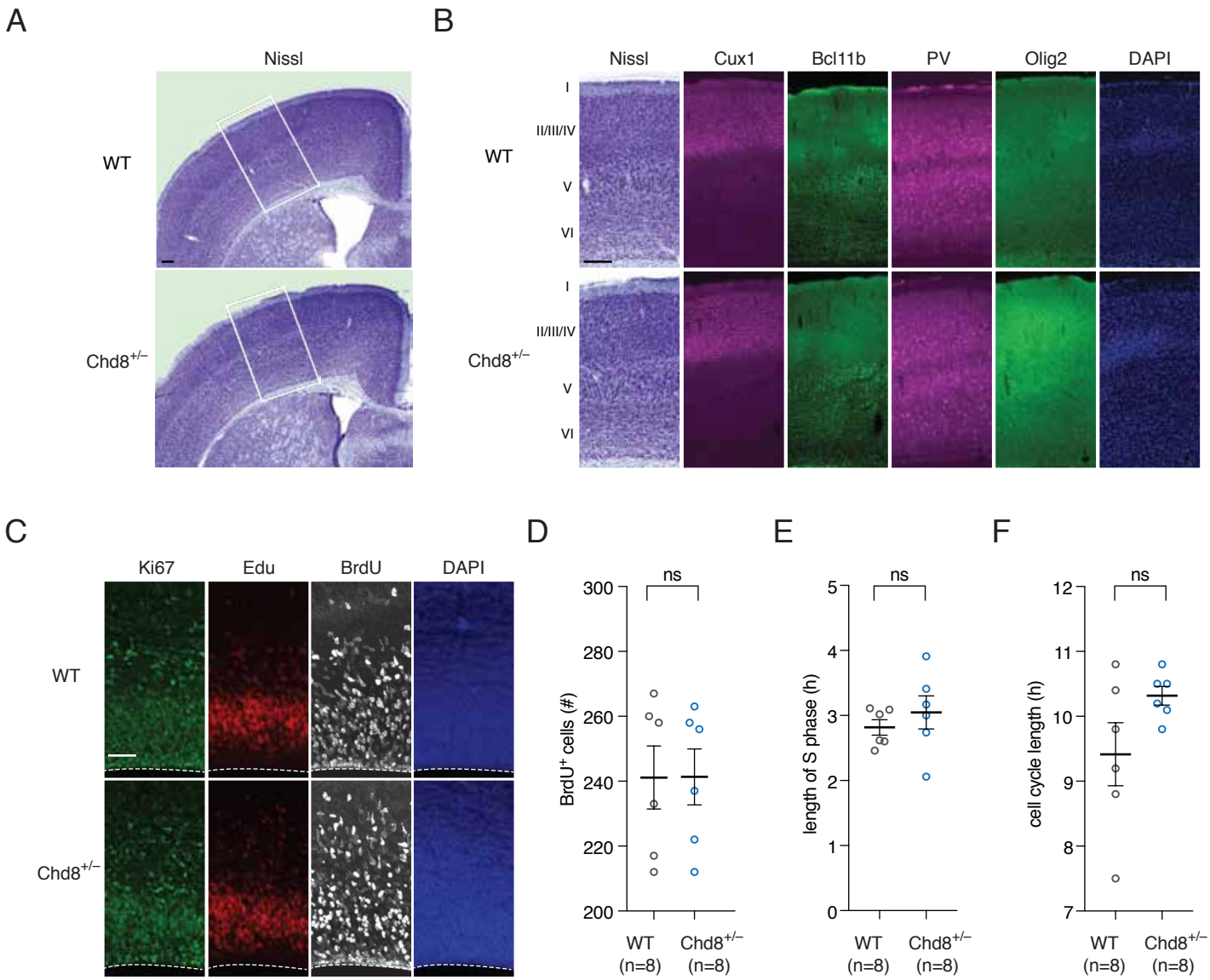

A
Nissl
WT
Chd8+/–
B
Nissl
Cux1
Bcl11b
PV
Olig2
DAPI
WT
Chd8+/–
I
II/III/IV
V
VI
C
Ki67
Edu
BrdU
DAPI
WT
Chd8+/–
D
BrdU+ cells (#)
300
280
260
240
220
200
ns
WT (n=8)
Chd8+/– (n=8)
E
length of S phase (h)
5
4
3
2
1
0
ns
WT (n=8)
Chd8+/– (n=8)
F
cell cycle length (h)
12
11
10
9
8
7
ns
WT (n=8)
Chd8+/– (n=8)

Figure S3. Cortical lamination, major cell types, and mid-stage cortical progenitor number in the somatosensory cortex do not vary between *Chd8*$^{+/-}$ mice and wild-type littermates, Related to Figure 2

(A) Representative Nissl images from (Top) wild-type littermates and (Bottom) *Chd8*$^{+/-}$ mice. The enclosed region highlights the somatosensory cortex and also the region for quantification. Scale bar represents 100 μm.

(B) Representative fluorescence images from the somatosensory cortex of (Top) wild-type littermates and (Bottom) *Chd8*$^{+/-}$ mice. The dotted line highlights the wall of the lateral ventricle. Scale bar represents 100 μm.

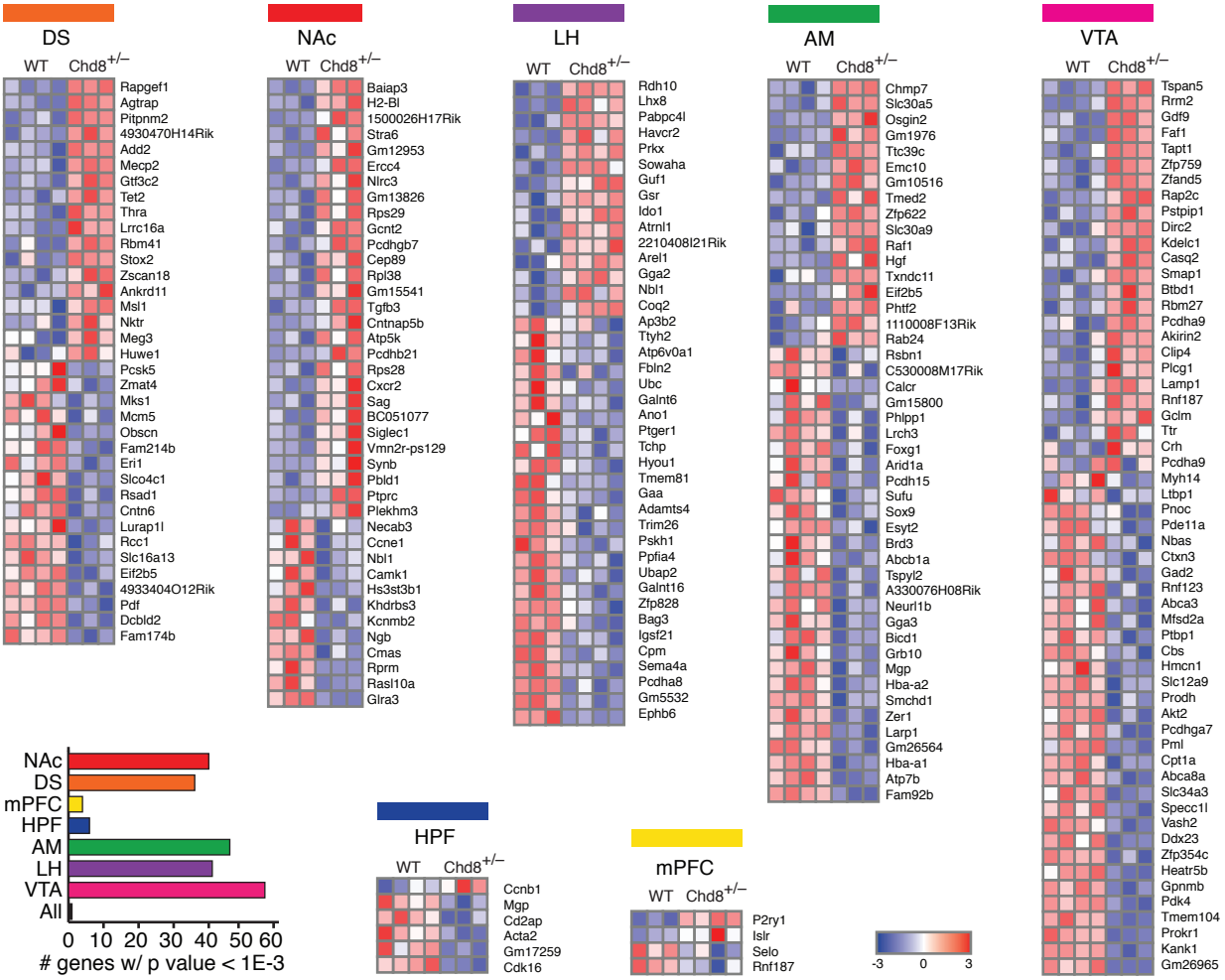
(C) Representative fluorescence images from the somatosensory cortex of (Top) wild-type littermates and (Bottom) *Chd8*$^{+/-}$ mice. Edu and BrdU were injected into timed pregnant dams (E15.5) at 30 and 120 minutes prior to euthanasia, respectively. The dotted line highlights the wall of the lateral ventricle. Scale bar represents 100 μm.

(D) Quantification of BrdU+ cells in E15.5 *Chd8*$^{+/-}$ mice compared to wild-type littermate embryos 120 minutes after injection of BrdU into pregnant dams [WT (n = 6 sections from 3 embryos) 240 ± 10 BrdU^{+} cells SEM; *Chd8*$^{+/-}$ (n = 6 sections from 3 embryos) 241 ± 9 BrdU^{+} cells SEM, two-tailed t-test p-value = 0.990].

(E) Quantification of S-phase cell cycle length in E15.5 *Chd8*$^{+/-}$ mice compared to wild-type littermate cortical progenitors [WT (n = 6 sections from 3 embryos) 9.4 ± 0.5 hours SEM; *Chd8*$^{+/-}$ (n = 6 sections from 3 embryos) 10.3 ± 0.1 hours SEM, two-tailed t-test p-value = 0.107].

(F) Quantification of total cell cycle length in E15.5 *Chd8*$^{+/-}$ mice compared to wild-type littermate cortical progenitors [WT (n = 6 sections from 3 embryos) 2.8 ± 0.1 hours SEM; *Chd8*$^{+/-}$ (n = 6 sections from 3 embryos) 3.0 ± 0.3 hours SEM, two-tailed t-test p-value = 0.430].

A


DS
WT Chd8+/–
Rapgef1
Agtrap
Pitpnm2
4930470H14Rik
Add2
Mecp2
Gtf3c2
Tet2
Thra
Lrrc16a
Rbm41
Stox2
Zscan18
Ankrd11
Msl1
Nktr
Meg3
Huwe1
Pcsk5
Zmat4
Mks1
Mcm5
Obscn
Fam214b
Eri1
Slco4c1
Rsad1
Cntn6
Lurap1l
Rcc1
Slc16a13
Eif2b5
4933404O12Rik
Pdf
Dcbld2
Fam174b
NAc
WT Chd8+/–
Baiap3
H2-Bl
1500026H17Rik
Stra6
Gm12953
Ercc4
Nlrc3
Gm13826
Rps29
Gcnt2
Pcdhgb7
Cep89
Rpl38
Gm15541
Tgfb3
Cntnap5b
Atp5k
Pcdhb21
Rps28
Cxcr2
Sag
BC051077
Siglec1
Vmn2r-ps129
Synb
Pbld1
Ptprc
Plekhm3
Necab3
Ccne1
Nbl1
Camk1
Hs3st3b1
Khdrbs3
Kcnmb2
Ngb
Cmas
Rprm
Rasl10a
Glra3
LH
WT Chd8+/–
Rdh10
Lhx8
Pabpc4l
Havcr2
Prkx
Sowaha
Guf1
Gsr
Ido1
Atrnl1
2210408I21Rik
Arel1
Gga2
Nbl1
Coq2
Ap3b2
Ttyh2
Atp6v0a1
Fbln2
Ubc
Galnt6
Ano1
Ptger1
Tchp
Hyou1
Tmem81
Gaa
Adamts4
Trim26
Pskh1
Ppfia4
Ubap2
Galnt16
Zfp828
Bag3
Igsf21
Cpm
Sema4a
Pcdha8
Gm5532
Ephb6
AM
WT Chd8+/–
Chmp7
Slc30a5
Osgin2
Gm1976
Ttc39c
Emc10
Gm10516
Tmed2
Zfp622
Slc30a9
Raf1
Hgf
Txndc11
Eif2b5
Phtf2
1110008F13Rik
Rab24
Rsbn1
C530008M17Rik
Calcr
Gm15800
Phlpp1
Lrch3
Foxg1
Arid1a
Pcdh15
Sufu
Sox9
Esyt2
Brd3
Abcb1a
Tspyl2
A330076H08Rik
Neurl1b
Gga3
Bicd1
Grb10
Mgp
Hba-a2
Smchd1
Zer1
Larp1
Gm26564
Hba-a1
Atp7b
Fam92b
VTA
WT Chd8+/–
Tspan5
Rrm2
Gdf9
Faf1
Tapt1
Zfp759
Zfand5
Rap2c
Pstpip1
Dirc2
Kdelc1
Casq2
Smap1
Btbd1
Rbm27
Pcdha9
Akirin2
Clip4
Plcg1
Lamp1
Rnf187
Gclm
Ttr
Crh
Pcdha9
Myh14
Ltbp1
Pnoc
Pde11a
Nbas
Ctxn3
Gad2
Rnf123
Abca3
Mfsd2a
Ptbp1
Cbs
Hmcn1
Slc12a9
Prodh
Akt2
Pcdhga7
Pml
Cpt1a
Abca8a
Slc34a3
Specc1l
Vash2
Ddx23
Zfp354c
Heatr5b
Gpnmb
Pdk4
Tmem104
Prokr1
Kank1
Gm26965
NAc
DS
mPFC
HPF
AM
LH
VTA
All
0 10 20 30 40 50 60
genes w/ p value < 1E-3
HPF
WT Chd8+/–
Ccnb1
Mgp
Cd2ap
Acta2
Gm17259
Cdk16
mPFC
WT Chd8+/–
P2ry1
Islr
Selo
Rnf187
-3 0 3

B

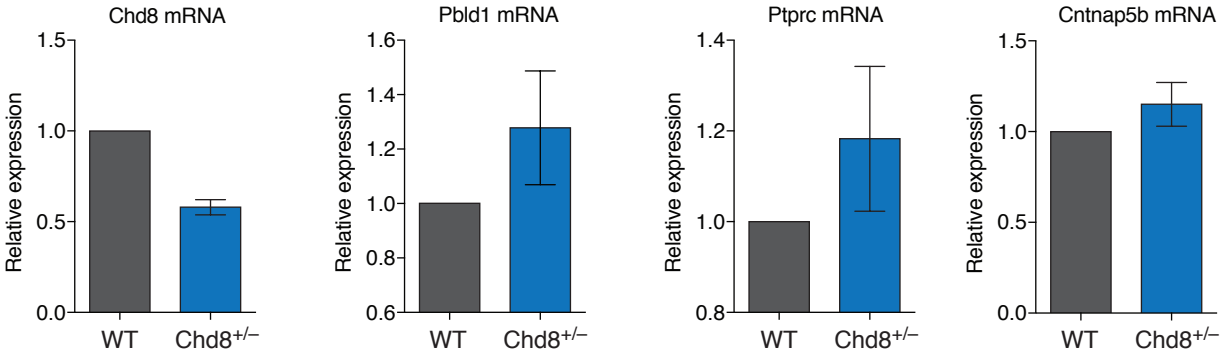

Chd8 mRNA
Relative expression
1.5
1.0
0.5
0.0
WT
Chd8+/–
Pbld1 mRNA
Relative expression
1.6
1.4
1.2
1.0
0.8
0.6
WT
Chd8+/–
Ptprc mRNA
Relative expression
1.4
1.2
1.0
0.8
WT
Chd8+/–
Cntnap5b mRNA
Relative expression
1.5
1.0
0.5
0.0
WT
Chd8+/–

Figure S4. RNA sequencing analysis on microdissected tissue from *Chd8*$^{+/-}$ mice, Related to Figure 3

(A) Heatmap visualizations and quantification of differentially expressed genes with nominal p-values < 1.00E-3. Genes were hierarchically clustered using average linkage of Pearson's correlation statistic.

(B) RT-qPCR validation of *Chd8* mRNA reduction as well as select differentially expressed genes identified in RNA sequencing libraries. Samples were independent from those used for RNA sequencing. Data represents the mean ± standard deviation of mRNA fold change in *Chd8*$^{+/-}$ mice compared to wild-type littermates [*Chd8* (n=4), *Pbld1* (n=5), and *Ptprc* (n=4), *Cntnap5b* (n=4)] relative to *Actb*.

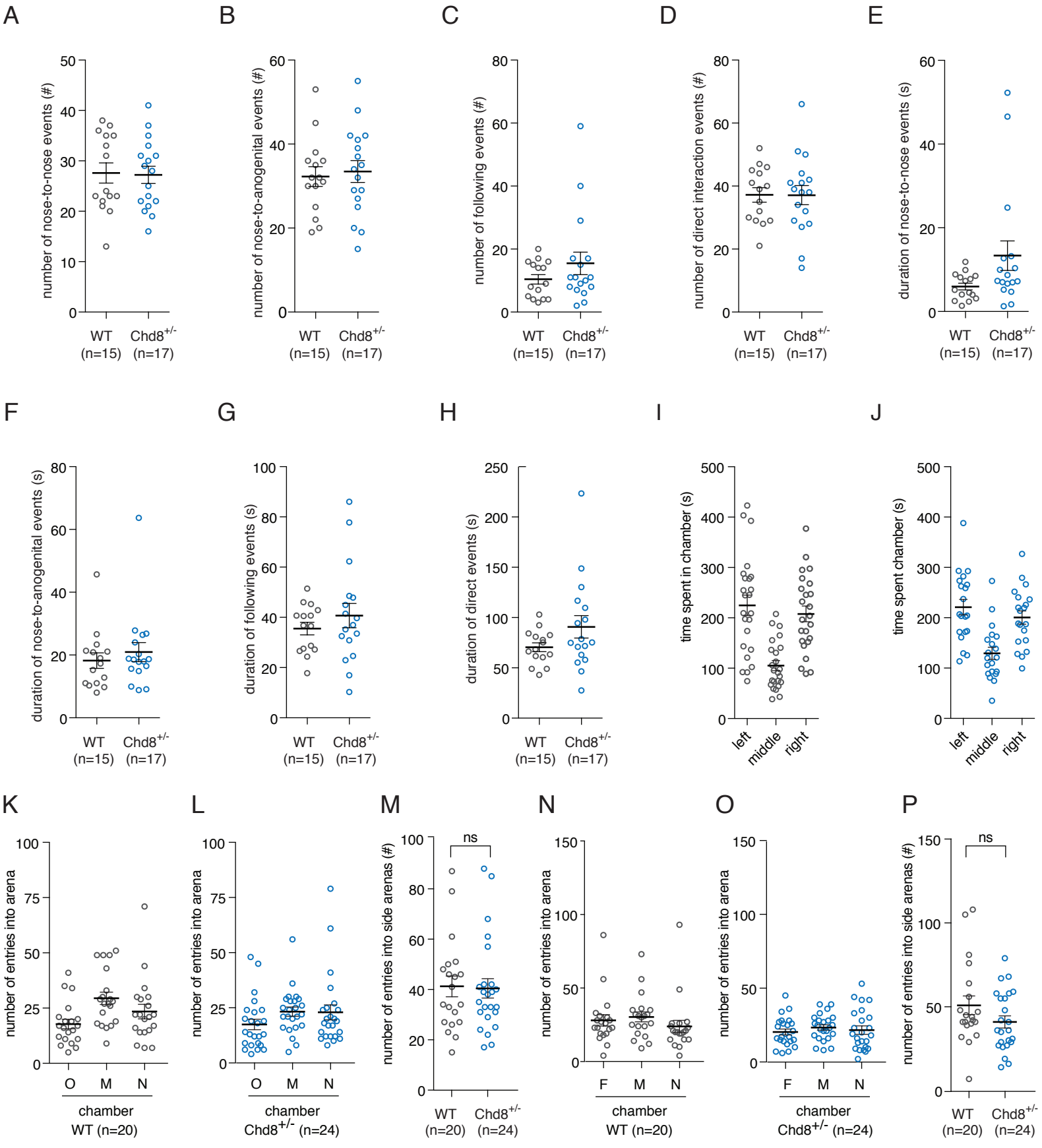

A
number of nose-to-nose events (#)
WT (n=15)
Chd8+/- (n=17)
B
number of nose-to-anogenital events (#)
C
number of following events (#)
D
number of direct interaction events (#)
E
duration of nose-to-nose events (s)
F
duration of nose-to-anogenital events (s)
G
duration of following events (s)
H
duration of direct events (s)
I
time spent in chamber (s)
left
middle
right
J
time spent chamber (s)
K
number of entries into arena
O
M
N
chamber
WT (n=20)
L
Chd8+/- (n=24)
M
number of entries into side arenas (#)
ns
N
F
O
P

Figure S5. Behavioral characterization of *Chd8*$^{+/-}$ mice using juvenile social play and the three-chambered social approach test, Related to Figure 5

(A-D) By categorizing the reciprocal play behavior we found no difference in the total number of interactive events for each category between genotypes: (A) nose-to-nose events [WT (n = 15) 28 ± 2 events SEM; *Chd8*$^{+/-}$ (n = 17) 27 ± 2 events SEM, one-way ANOVA with Bonferroni post hoc test p-value > 0.05], (B) nose-to-anogenital sniffing events [WT (n = 15) 32 ± 2 events SEM; *Chd8*$^{+/-}$ (n = 17) 33 ± 3 events SEM, one-way ANOVA with Bonferroni post hoc test p-value > 0.05], (C) following behavior events [WT (n = 15) 10 ± 2 events SEM; *Chd8*$^{+/-}$ (n = 17) 15 ± 4 events SEM, one-way ANOVA with Bonferroni post hoc test p-value > 0.05], (D) direct interaction events [WT (n = 15) 37 ± 2 events SEM; *Chd8*$^{+/-}$ (n = 17) 37 ± 3 events SEM, one-way ANOVA with Bonferroni post hoc test p-value > 0.05].

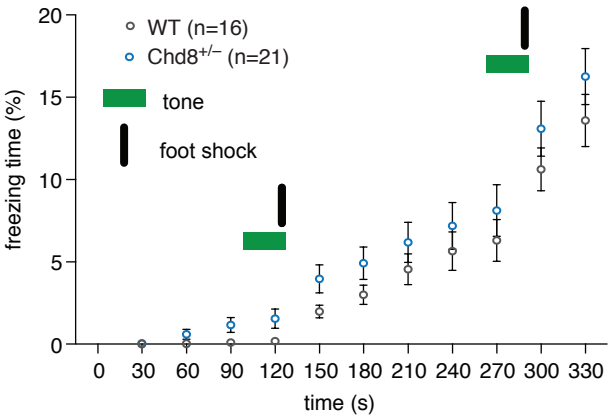
(E-H) By categorizing the reciprocal play behavior we found no difference in the total duration of interaction for each category between genotypes: (E) nose-to-nose duration [WT (n = 15) 10.8 ± 0.9 s SEM; *Chd8*$^{+/-}$ (n = 17) 16 ± 2 s SEM, one-way ANOVA with Bonferroni post hoc test p-value > 0.05], (F) nose-to-anogenital sniffing duration [WT (n = 15) 18 ± 2 s SEM; *Chd8*$^{+/-}$ (n = 17) 21 ± 3 s SEM, one-way ANOVA with Bonferroni post hoc test p-value > 0.05], (G) following behavior duration [WT (n = 15) 6.0 ± 0.8 s SEM; *Chd8*$^{+/-}$ (n = 17) 13 ± 4 s SEM, one-way ANOVA with Bonferroni post hoc test p-value > 0.05], (H) direct interaction duration [WT (n = 15) 36 ± 2 s SEM; *Chd8*$^{+/-}$ (n = 17) 41 ± 5 s SEM, one-way ANOVA with Bonferroni post hoc test p-value > 0.05].

(I-J) In the habituation phase of the three-chambered assay (I) wild-type mice and (J) *Chd8*$^{+/-}$ littermates exhibited no preference for a specific chamber.

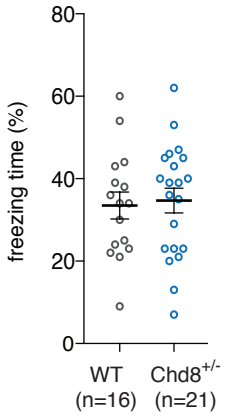
(K-M) In the sociability phase of the three-chambered assay (K) wild-type mice and (L) *Chd8*$^{+/-}$ littermates exhibited no difference in (M) locomotor activity [WT (n = 20) 41 ± 4 entries SEM; *Chd8*$^{+/-}$ (n = 24) 40 ± 4 entries SEM, two-tailed t-test p-value = 0.889].

(N-P) In the sociability phase of the three-chambered assay (N) wild-type mice and (O) *Chd8*$^{+/-}$ littermates exhibited no difference in (P) locomotor activity [WT (n = 20) 52 ± 6 entries SEM; *Chd8*$^{+/-}$ (n = 24) 42 ± 4 s SEM, two-tailed t-test p-value = 0.131].

A


WT (n=16)
Chd8+/– (n=21)
tone
foot shock
freezing time (%)
time (s)
0
5
10
15
20
0 30 60 90 120 150 180 210 240 270 300 330

B


freezing time (%)
0
20
40
60
80
WT (n=16)
Chd8+/- (n=21)

C

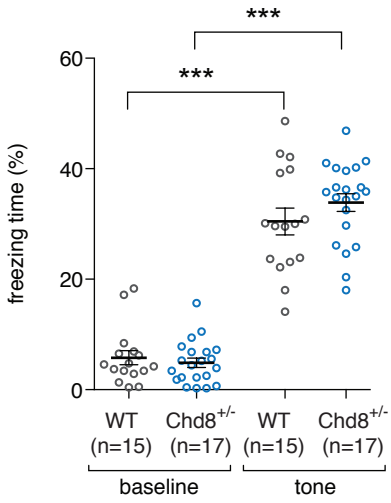

***
***
freezing time (%)
0
20
40
60
WT (n=15)
Chd8+/- (n=17)
WT (n=15)
Chd8+/- (n=17)
baseline
tone

Figure S6. *Chd8*$^{+/-}$ mice display normal behavior during contextual and tone based fear conditioning, Related to Figure 6

(A) In the training phase of contextual fear learning *Chd8*$^{+/-}$ mice exhibited a similar percentage of freezing behavior compared to wild-type littermates [repeated-measures two-way ANOVA with Bonferroni post hoc test, adjusted p-value > 0.05 for each time point].

(B) During the contextual fear conditioning we observed no difference in freezing time between *Chd8*$^{+/-}$ mice and wild-type littermates [WT (n = 16) 34 ± 3 % SEM; *Chd8*$^{+/-}$ (n = 21) 35 ± 3 % SEM, two-tailed t-test p-value = 0.788].

(C) During the tone fear conditioning we observed no difference in freezing time between *Chd8*$^{+/-}$ mice and wild-type littermates [WT (n = 16) 31 ± 2 % SEM; *Chd8*$^{+/-}$ (n = 21) 34 ± 2 % SEM, two-tailed t-test p-value = 0.231]. We did observe a significant difference between freezing time between the baseline and tone conditions for both genotypes [WT (n=16) baseline 6 ± 1 % SEM; tone 30 ± 2 % SEM, two-tailed t-test p-value < 0.001; *Chd8*$^{+/-}$ (n=21) baseline 4.9 ± 0.9 % SEM; tone 34 ± 2 % SEM, two-tailed t-test p-value < 0.001].

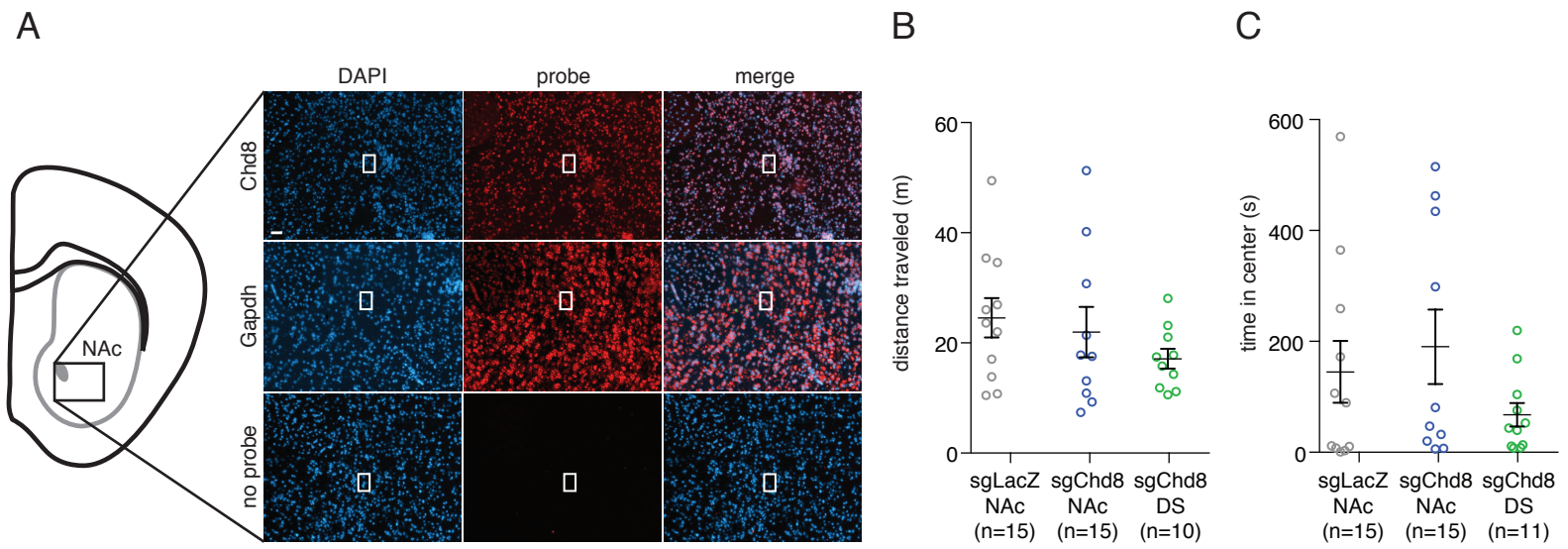

A
DAPI
probe
merge
Chd8
Gapdh
no probe
NAc
B
distance traveled (m)
60
40
20
0
sgLacZ NAc (n=15)
sgChd8 NAc (n=15)
sgChd8 DS (n=10)
C
time in center (s)
600
400
200
0
sgLacZ NAc (n=15)
sgChd8 NAc (n=15)
sgChd8 DS (n=11)

Figure S7. Perturbation of *Chd8* in adult Cas9 knockin mice, Related to Figure 7

(A)(Left) Schematic of a coronal brain section highlighting the NAc and DS. (Right) Representative fluorescence images within the NAc of wild-type adult brain slices probed for *Chd8* or *Gapdh* mRNA using RNA-FISH. The dotted circle represents the anterior commissure and the enclosed region outlines the zoomed region. Scale bar represents 50 μm.

(B-C) In the open field test, sgChd8-NAc (n = 15), sgChd8-DS (n = 11), and sgLacZ-NAc (n = 15) AAV injected mice (B) traveled the same distance and (C) spent the same amount of time in the center. Data are means ± SEM; one-way ANOVA with Bonferroni post hoc test p-value > 0.05.

Table S1. Oligos used in this study

Oligo	Sequence (5'-3')		Usage
sgChd8-1	AGCTGTTTTACTGGTCGGCT	sgRNA	In vitro validation
sgChd8-2	AATGGATACACCTGGTCGAA	sgRNA	In vitro validation
sgChd8-3	CAATGGATACACCTGGTCGA	sgRNA	In vitro validation, mouse generation, and viral injection
sgScn2a-1	ACAGCTTCCGCTTCTTTACC	sgRNA	In vitro validation and mouse generation
sgScn2a-2	CAATCAGTGCTGGTACCGCC	sgRNA	In vitro validation
sgScn2a-3	AAGGACGAAGACGACGAAAA	sgRNA	In vitro validation
sgKctd13-1	GCCGGCTGCGGCCGAATGCT	sgRNA	In vitro validation and mouse generation
sgKctd13-2	AGGGGCTTCAGACTGTACGA	sgRNA	In vitro validation
sgKctd13-3	CACCACGCTGCGCACCCTCA	sgRNA	In vitro validation
sgKatnal2-1	AATAATTTACCGTCACGAAG	sgRNA	In vitro validation
sgKatnal2-2	ATTTACCGTCACGAAGTGGA	sgRNA	In vitro validation and mouse generation
sgKatnal2-3	TTCTTCCCTCCACTTCGTGA	sgRNA	In vitro validation
sgGit1-1	GGCCACTTTGCACTTGCGCA	sgRNA	In vitro validation and mouse generation
sgGit1-2	CGGCCACTTTGCACTTGCGC	sgRNA	In vitro validation
sgGit1-3	GCACACGCTTGCCAGCAACG	sgRNA	In vitro validation
U6-F	GAGGGCCTATTTCCCATGATTC	Primer	In vitro validation
U6-sgChd8-R	AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAA CGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACT CGACCAGGTGTATCCATTGCGGTGTTTCGTCCTTTCCACAAG	Primer	In vitro validation
U6-sgScn2a-R	AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAA CGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACG GTAAAGAAGCGGAAGCTGTCGGTGTTTCGTCCTTTCCACAA G	Primer	In vitro validation
U6-sgKctd13-R	AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAA CGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACA GCATTCGGCCGCAGCCGGCGGTGTTTCGTCCTTTCCACAAG	Primer	In vitro validation
U6-sgKatnal2-R	AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAA CGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACT CCACTTCGTGACGGTAAATCGGTGTTTCGTCCTTTCCACAAG	Primer	In vitro validation
U6-sgGit1-R	AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAA CGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACT GCGCAAGTGCAAAGTGGCCGGTGTTTCGTCCTTTCCACAAG	Primer	In vitro validation
Chd8-F	GACAACCATCTCAGTCAGCTCCTA	Primer	In vitro validation (SURVEYOR assay)
Chd8-R	TCCTCTATGAAAGCACAGTTCACC	Primer	In vitro validation (SURVEYOR assay)
Scn2a-F	TGTAGCACTTTCTTATGCGAGGAG	Primer	In vitro validation (SURVEYOR assay)
Scn2a-R	AATAATGGCTCCATTCCCTTATCA	Primer	In vitro validation (SURVEYOR assay)
Kctd13-F	CGGAGTAGCTGTGGAGAGTGG	Primer	In vitro validation (SURVEYOR assay)
Kctd13-R	AAGGATTGGGGAAAGAGAGAGATT	Primer	In vitro validation (SURVEYOR assay)
Katnal2-F	CTGGGTGTCTTTCTCTGTGTGACT	Primer	In vitro validation (SURVEYOR assay)
Katnal2-R	TACATGGACAAGAAAGGCAGGATA	Primer	In vitro validation (SURVEYOR assay)
Git1-F	TGAAGTGCTAAACAGGACACATGA	Primer	In vitro validation (SURVEYOR assay)
Git1-R	TACTTTGCCCTGATGAACTCTGAC	Primer	In vitro validation (SURVEYOR assay)
Chd8-F	GAGGAATCCGACAACCATCTCAGTCAGCTCCTA	Primer	Mouse genotyping (EcoRI/HindIII restriction sites)
Chd8-R	GAGAAGCTTTCCTCTATGAAAGCACAGTTCACC	Primer	Mouse genotyping (EcoRI/HindIII restriction sites)
Chd8-F	ACAGCCAACGGTGGAAAAGT	Primer	Single nuclei analysis (Illumina sequencing)
Chd8-R	CTGGACAATGCGCTGAACA	Primer	Single nuclei analysis (Illumina sequencing)
T7	TAATACGACTCACTATAGGG	Oligo	T7 promoter for in vitro transcription of sgRNA
T7-sgChd8-3-R	AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAAC GGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACTC GACCAGGTGTATCCATTGCCCTATAGTGAGTCGTATTA	Oligo	sgChd8-3 for in vitro transcription of sgRNA