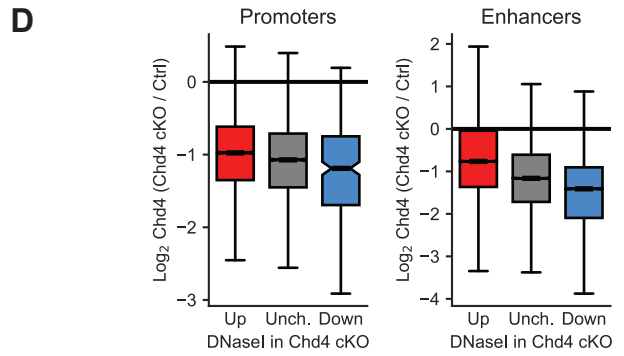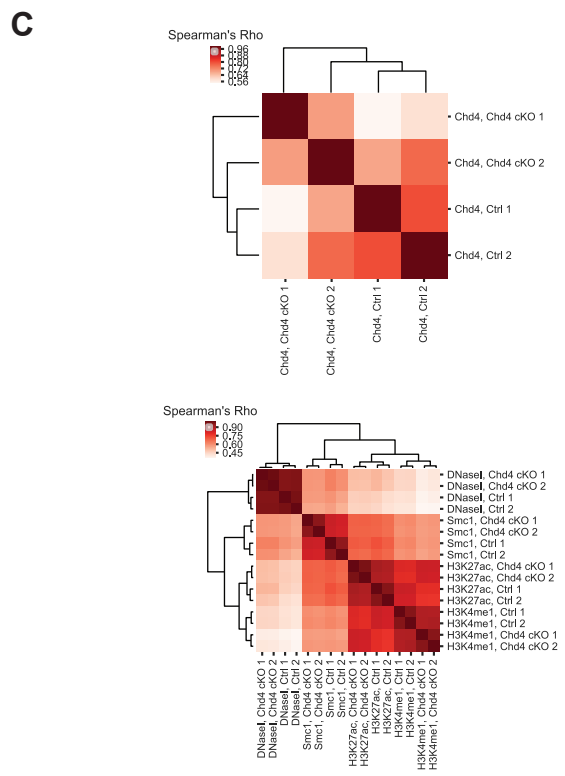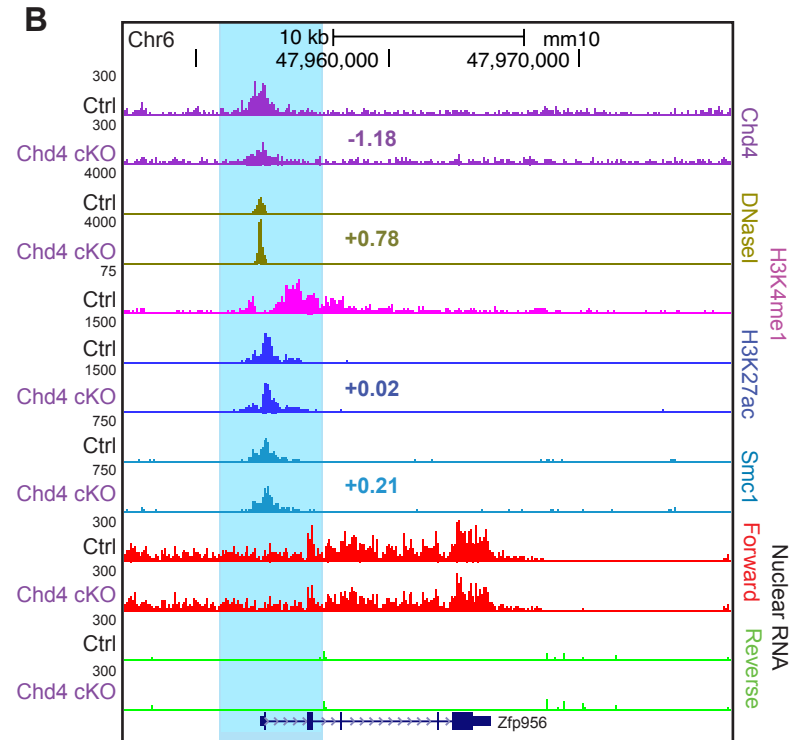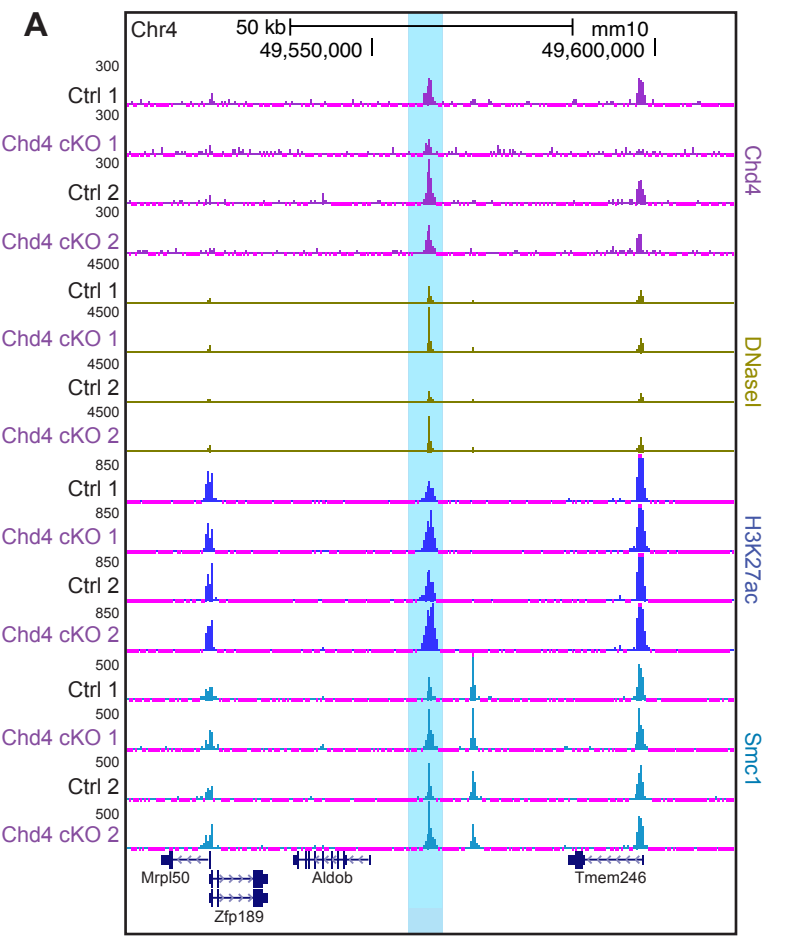Supplementary Information

The Chromatin Remodeling Enzyme Chd4 Regulates Genome Architecture in the Mouse Brain

Goodman *et al.*
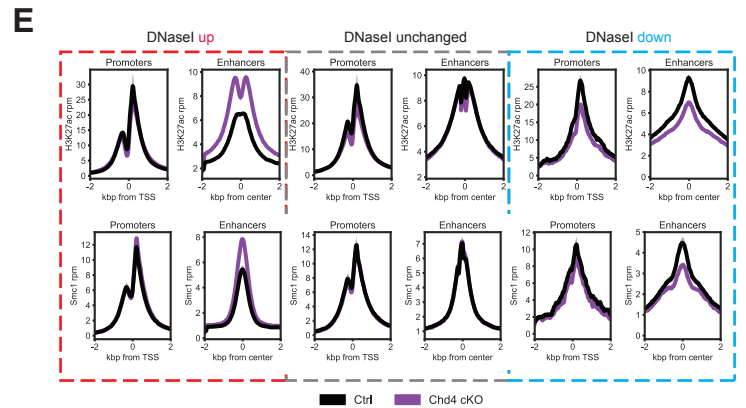
Supp Figure 1

Supplementary Figure 1: Chd4 depletion preferentially modulates enhancer activation and Cohesin binding at enhancers

(A) Genome browser snapshot of a region containing the *Aldob* gene locus on chromosome 4 displaying the ChIP-seq profiles of Chd4, H3K4me1, H3K27ac and Smc1 as well as DNaseI-seq from the control and Chd4 cKO cerebellum in biological replicates. Light blue denotes an enhancer upstream of the *Aldob* gene. **(B)** Genome browser snapshot of the promoter of the *Zfp256* gene locus on chromosome 6 displaying the ChIP-seq profile of Chd4, H3K4me1, H3K27ac, and Smc1 as well as DNaseI-seq and nuclear RNA-seq from the control and Chd4 cKO cerebellum. Numbers indicate the Log2 change in signal in the Chd4 cKO cerebellum. **(C)** Clustering of biological replicates from the control and Chd4 cKO cerebellum using Spearman's Rho from Chd4 (top) as well as DNaseI, H3K27ac, H3K4me1, and Smc1 (bottom). **(D)** Change in Chd4 between Chd4 cKO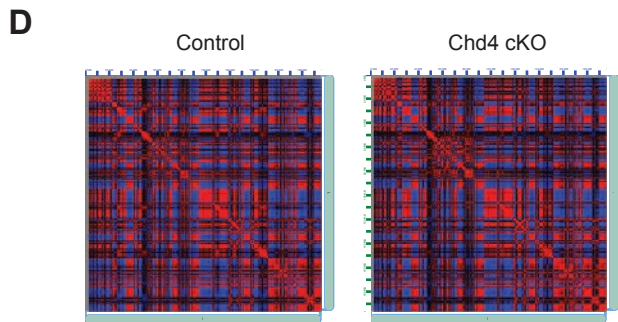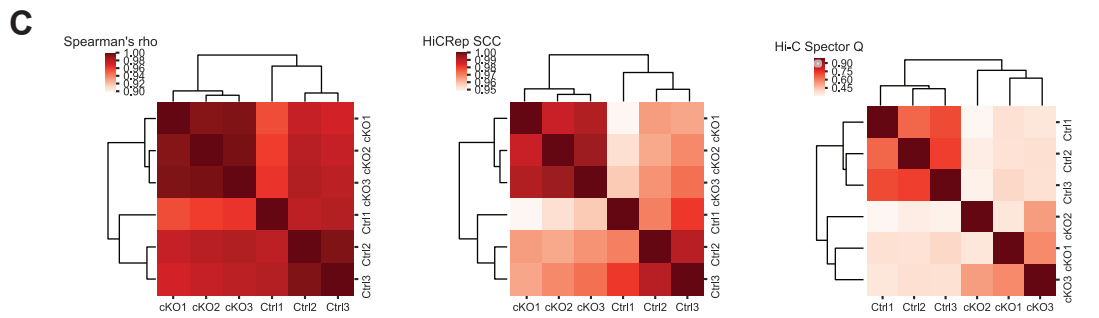 and control cerebellum at promoters and/or enhancers with increased (n=19,389 promoters; n=67,245 enhancers), unchanged (n=15,141 promoters; n=44,661 enhancers), and decreased (n=828 promoters, n=27,750 enhancers) accessibility. **(E)** Aggregate plot of (top) H3K27ac and (bottom) Smc1 read density in the control and Chd4 cKO cerebellum at promoters and enhancers with increased (left), unchanged (middle), and decreased (right) accessibility. P-values for all comparisons in this figure were calculated by the two-sided Kruskal-Wallis H-test for independent samples with Dunn's post hoc T-test and corrected for multiple comparisons by the Bonferroni-Hochberg procedure. ****p<0.001.*

A

chr19 (qA-qD3) 19qA qB qC1 qC3 D2

chr19 (qA-qD3) 19qA qB qC1 qC3 D2

chr19 (qA-qD3)

Control

Chd4 cKO

0 — 61.43Mb

61.43Mb

B

C

Spearman's rho

HiCRep SCC

Hi-C Spector Q

D

Control   Chd4 cKO

E

Chr4

102.90   103.43Mb

102.90   103.43Mb

Chr4

Control   Chd4 cKO

Contacts
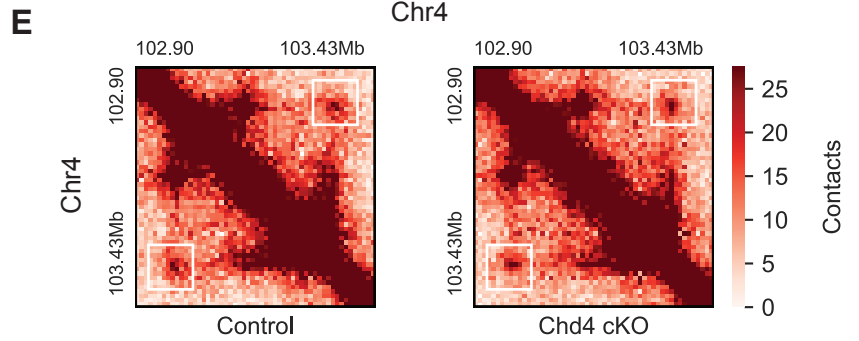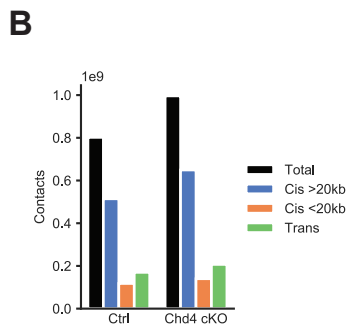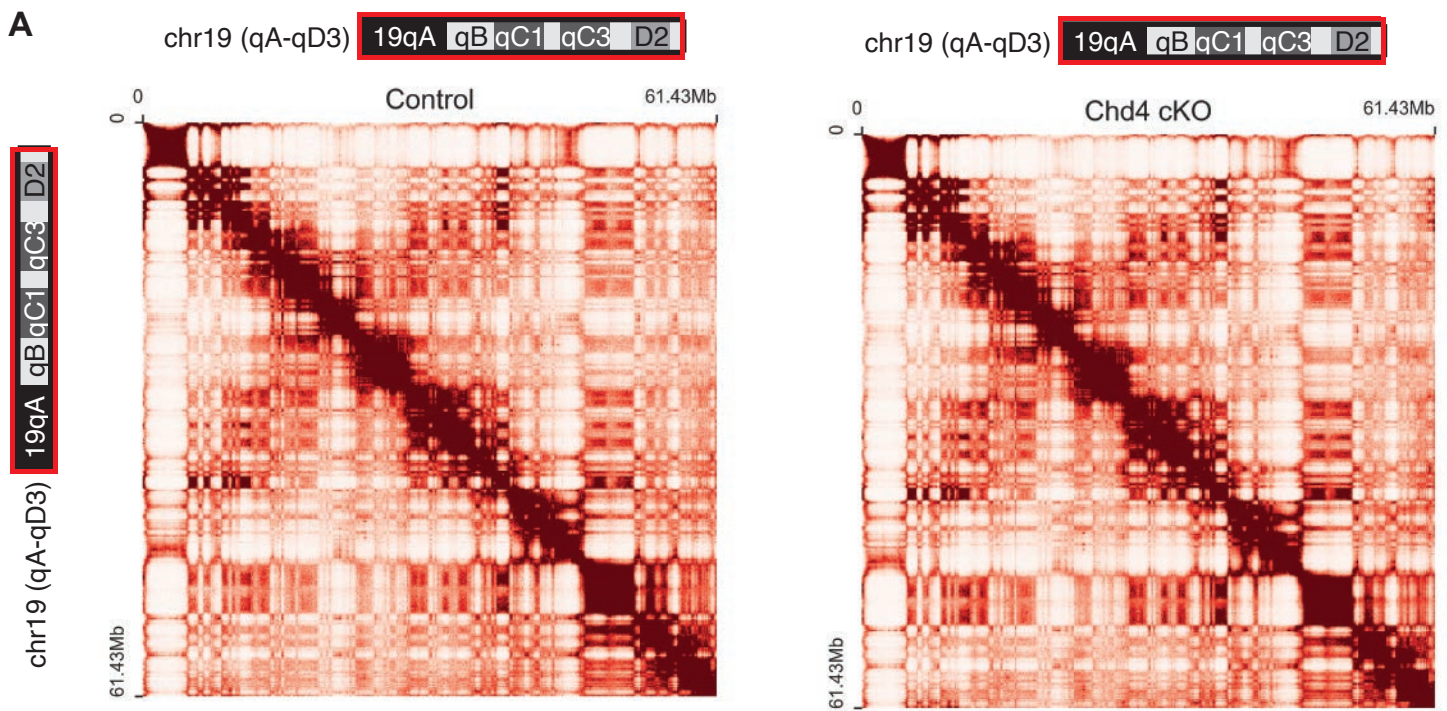
Supp Figure 2

Supplementary Figure 2: Hi-C in the cerebellum of control and Chd4 cKO mice reveals principles of genomic architecture

(A) Hi-C contact matrices of chromosome 19 in the control and Chd4 cKO cerebellum. **(B)** Distribution of pooled Hi-C contacts in the control (ctrl) and Chd4 cKO cerebellum. **(C)** Clustering of Hi-C contacts in 1Mb bins by Spearman's Rho (left), HiCRep (middle), and Hi-C Spector (right) of the three biological replicates from the control and Chd4 cKO cerebellum. **(D)** Juicebox browser snapshot of the Pearson's correlation matrix of chromosome 1. **(E)** Hi-C contact matrices of a region on chromosome 4 depicted small-scale features such as contact domains and genomic loops (highlighted).

A Contact domains

1553 3951 2292

Ctrl   Chd4 cKO

B Loops

2985 5057 3483

Ctrl   Chd4 cKO

C Contact domains

Count

Loop   Comp.

D Loop domains

387 1866 499

Ctrl   Chd4 cKO

E Comp. domains

1166 2085 1793

Ctrl   Chd4 cKO

F

Rep 1

28.26   Chr13   29.08Mb

Chr13

Control

28.26   Chr13   29.08Mb

Chr13

Chd4 cKO

log2 o/e contacts

Rep 2

28.26   Chr13   29.08Mb

Chr13

Control

28.26   Chr13   29.08Mb

Chr13

Chd4 cKO

log2 o/e contacts

Rep 3

28.26   Chr13   29.08Mb

Chr13

Control

28.26   Chr13   29.08Mb

Chr13

Chd4 cKO

log2 o/e contacts

G

Loop domains   r=0.64
Log_2 Intra-domain Contacts (Chd4 cKO/Ctrl)
Log_2 DNaseI (Chd4 cKO/Ctrl)
Density

Comp. domains   r=0.61
Log_2 Intra-domain Contacts (Chd4 cKO/Ctrl)
Log_2 DNaseI (Chd4 cKO/Ctrl)
Density

H

Loop domains   r=0.58
Log_2 Intra-domain Contacts (Chd4 cKO/Ctrl)
Log_2 H3K27ac (Chd4 cKO/Ctrl)
Density

Comp. domains   r=0.55
Log_2 Intra-domain Contacts (Chd4 cKO/Ctrl)
Log_2 H3K27ac (Chd4 cKO/Ctrl)
Density

I

Loop domains   r=0.65
Log_2 Intra-domain Contacts (Chd4 cKO/Ctrl)
Log_2 Smc1 (Chd4 cKO/Ctrl)
Density

Comp. domains   r=0.58
Log_2 Intra-domain Contacts (Chd4 cKO/Ctrl)
Log_2 Smc1 (Chd4 cKO/Ctrl)
Density

J

TADtree TADs

Intra-domain Contacts   0.49   0.46   0.52

DNaseI   H3K27ac   Smc1

Spearman's rho

K

Contact domains   r=0.11
Log_2 Intra-domain Contacts (Chd4 cKO/Ctrl)
Log_2 H2A.Z (Chd4 cKO/Ctrl)
Density

Loop domains   r=0.07
Log_2 Intra-domain Contacts (Chd4 cKO/Ctrl)
Log_2 H2A.Z (Chd4 cKO/Ctrl)
Density

Comp. domains   r=0.13
Log_2 Intra-domain Contacts (Chd4 cKO/Ctrl)
Log_2 H2A.Z (Chd4 cKO/Ctrl)
Density

L Contact domains

Log2 Insulation (Chd4 cKO/Ctrl)

Up   Unch.   Down

DNaseI in Chd4 cKO

M

Eigenvalue Correlation

Ctrl   0.8   0.79   0.12   -0.17
Chd4 cKO   0.79   0.78   0.13   -0.18

DNaseI   H3K27ac   H3K27me3   H3

Spearman's rho

N

r=0.98

Eigenvalue (Chd4 cKO)

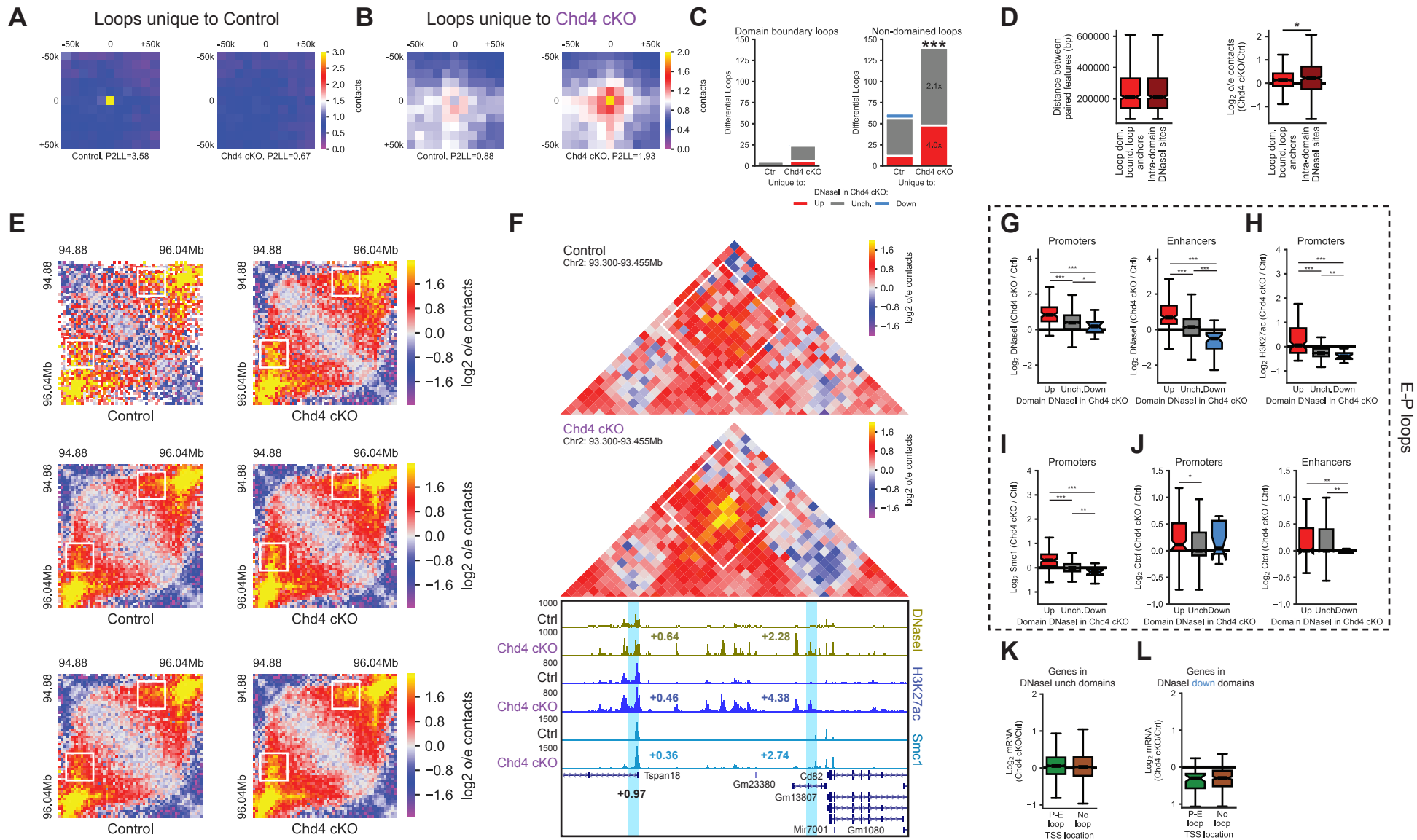Eigenvalue (Ctrl)

Density

Supp Figure 3

Supplementary Figure 3: Chd4 loss alters interactions within contact domains

(A-B) Overlap of contact domains and loops identified in the control and Chd4 cKO cerebellum. **(C)** Distribution of loop and compartmental domains identified among all contact domains. Chi-squared test. ****p<0.001* **(D-E)** Overlap of loop (D) and compartmental (E) domains identified in the control and Chd4 cKO cerebellum. **(F)** Hi-C contact matrix of a contact domain on chromosome 13 and the flanking region as in Figure 2A from individual biological replicates of the control and Chd4 cKO cerebellum. **(G-I)** Density plots comparing the change in (G) DNaseI, (H) H3K27ac, and (I) Smc1 within a loop (left) or compartmental (right) domain and the change in interactions in that domain between the Chd4 cKO and control cerebellum. Pearson's r, *p<0.001*. **(J)** Correlation of the change in intra-domain contacts (Chd4 cKO/Ctrl) with the change in epigenomic marks (Chd4 cKO/Ctrl) among TADs identified by TADtree. **(K)** Density plots comparing the change in H2A.Z within all (left), loop (middle) or compartmental (right) domain and the change in interactions in that domain between the Chd4 cKO and control cerebellum. Pearson's r, *p<0.001*. **(L)** Change in insulation strength of domain boundaries among domains with increased ($Log_2FC>0.585$), unchanged ($-0.585<Log_2FC<0.585$), and decreased ($-0.585<Log_2FC$) accessibility in the Chd4 cKO cerebellum. Points represent average of domains in individual biological replicates (n=3). **(M)** Correlation of eigenvalue with epigenomic marks in 150kb bins. **(N)** Density plot comparing the eigenvalue of a bin in the control cerebellum to its eigenvalue in the Chd4 cKO cerebellum. Pearson's r, *p<0.001*.

Supp Figure 4

Supplementary Figure 4: Chd4 regulates loop domain boundary loop strength

(A) Hi-C contact matrix of a loop domain on chromosome 10 and the flanking region as in Figure 3A, with a loop domain boundary loop highlighted by a white box, from individual biological replicates of the control and Chd4 cKO cerebellum. **(B-D)** Change in DNaseI (B), Smc1 (C), and Ctcf (D) at promoters and/or non-promoters underlying loop domain boundary loops at domains with increased (n=229 promoters, n=1,343 non-promoters), unchanged (n=711 promoters, n=5,336 non-promoters), or decreased (n=26 promoters, n=241 non-promoters) accessibility in the Chd4 cKO. P-values for all comparisons in this figure were calculated by the two-sided Kruskal-Wallis H-test for independent samples with Dunn's post hoc T-test and corrected for multiple comparisons by the Bonferroni-Hochberg procedure. *$p<0.01$, ***$p<0.0001$.*

A Loops unique to Control

Control, P2LL=3.58

Chd4 cKO, P2LL=0.67

B Loops unique to Chd4 cKO

Control, P2LL=0.88

Chd4 cKO, P2LL=1.93

C

Domain boundary loops

Non-domained loops

DNaseI in Chd4 cKO:
Up Unch. Down

D

Distance between paired features (bp)

Log₂ o/e contacts (Chd4 cKO/Ctrl)

Loop dom. bound. loop anchors
Intra-domain DNaseI sites

E

94.88 96.04Mb

Control

Chd4 cKO

log2 o/e contacts

F

Control
Chr2: 93.300-93.455Mb

Chd4 cKO
Chr2: 93.300-93.455Mb

log2 o/e contacts

DNaseI
Ctrl 1000
Chd4 cKO 1000   +0.64   +2.28
H3K27ac
Ctrl 800
Chd4 cKO 800   +0.46   +4.38
Smc1
Ctrl 1500
Chd4 cKO 1500   +0.36   +2.74

Tspan18   Cd82
Gm23380
Gm13807
+0.97
Mir7001   Gm1080

G

Promoters

Enhancers

Log₂ DNaseI (Chd4 cKO / Ctrl)

Domain DNaseI in Chd4 cKO

H

Promoters

Log₂ H3K27ac (Chd4 cKO / Ctrl)

Domain DNaseI in Chd4 cKO

I

Promoters

Log₂ Smc1 (Chd4 cKO / Ctrl)

Domain DNaseI in Chd4 cKO

J

Promoters

Enhancers

Log₂ Ctcf (Chd4 cKO / Ctrl)

Domain DNaseI in Chd4 cKO

E-P loops

K

Genes in DNaseI unch domains

Log₂ mRNA (Chd4 cKO/Ctrl)

P-E loop   No loop
TSS location

L

Genes in DNaseI down domains

Log₂ mRNA (Chd4 cKO/Ctrl)

P-E loop   No loop
TSS location

Supp Figure 5

Supplementary Figure 5: Chd4 coordinates intra-domain loop strength and gene expression

(A-B) Aggregate peak analysis of loops unique to the control (A) or Chd4 cKO (B) cerebellum. P2LL, Peak-to-lower-left. **(C)** Location of loops unique to the control or Chd4 cKO cerebellum among domain boundary loops (left) or those not overlapping domain boundaries (right) among domains with increased, unchanged, or decreased accessibility in the Chd4 cKO cerebellum. Inset values indicate the linear fold-change of non-domained loops in domains with increased or unchanged accessibility present in the Chd4 cKO cerebellum compared to the control cerebellum. Chi-square test. ***p<0.001* **(D)** (Left) Distance between loop domain boundary loop anchors (n=613) and a distance-controlled random sample of intra-domain DNaseI peak pairs (n=613) and (right) the change in Hi-C contacts at loop domain boundary loops among domains with increased accessibility (n=613) and the random sampling of intra-domain DNaseI peak pairs among domains with increased accessibility upon conditional Chd4 knockout (n=613). *p = 0.01* (see methods) **(E)** Hi-C contact matrix of a loop domain on chromosome 4 and the flanking region as in Figure 4A, with an intra-domain enhancer-promoter (E-P) loop highlighted by a white box, from individual biological replicates of the control and Chd4 cKO cerebellum. **(F)** (Top) Hi-C contact matrix of an E-P loop on chromosome 2 and the flanking region, with the loop highlighted by a white box. (Bottom) Genome-browser snapshot of the region corresponding to the Hi-C contact matrix displaying the ChIP-seq profiles of H3K27ac, Smc1, and Ctcf as well as DNaseI-seq from the control and Chd4 cKO cerebellum. Blue denotes the the loop anchors and regions of the insets. Numbers indicate the Log2 change in signal in the Chd4 cKO cerebellum, including that of mRNA for *Tspan18*. **(G-J)** Change in DNaseI (G), H3K27ac (H), Smc1 (I), and Ctcf (J) at promoters and/or enhancers underlying intra-domain E-P loops in domains with increased (n=107 promoters, n=183 enhancers), unchanged (n=795 promoters, n=1,682 enhancers), or decreased (n=19 promoters, n=45 enhancers) accessibility in the Chd4 cKO cerebellum. Two-sided Kruskal-Wallis H-test for independent samples with Dunn's post hoc T-test and corrected for multiple comparisons by the Bonferroni-Hochberg procedure. *p<0.01, **p<0.001, ***p<0.0001.* **(K-L)** Change in mRNA of genes at intra-domain E-P loops (green) or underlying no detectable loop (brown) in domains with unchanged (K, n=1,342 at loops, n=11,307 not at loop) or decreased (L, n=50 at loops, n=261 not at loop) accessibility in the Chd4 cKO.

A

Loop domains — Log₂ H3K27ac (P60/P7) vs Log₂ Intra-domain Contacts (Chd4 cKO/Ctrl), r=-0.38

Compartmental domains — Log₂ H3K27ac (P60/P7) vs Log₂ Intra-domain Contacts (Chd4 cKO/Ctrl), r=-0.34

B

Loop domains — Log₂ P60 H3K27ac (Chd4 cKO/Ctrl) vs Log₂ Intra-domain Contacts (Chd4 cKO/Ctrl), r=0.53

Compartmental domains — Log₂ P60 H3K27ac (Chd4 cKO/Ctrl) vs Log₂ Intra-domain Contacts (Chd4 cKO/Ctrl), r=0.52

C

Spearman's Rho heatmap:
H3K27ac P60, Chd4 cKO 1
H3K27ac P60, Chd4 cKO 2
H3K27ac P60, Ctrl 1
H3K27ac P60, Ctrl 2

D

Contact domains — Log₂ P60 H3K27ac (Chd4 cKO/Ctrl) vs Log₂ P22 H3K27ac (Chd4 cKO/Ctrl), r=0.71

Loop domains — Log₂ P60 H3K27ac (Chd4 cKO/Ctrl) vs Log₂ P22 H3K27ac (Chd4 cKO/Ctrl), r=0.73

Compartmental domains — Log₂ P60 H3K27ac (Chd4 cKO/Ctrl) vs Log₂ P22 H3K27ac (Chd4 cKO/Ctrl), r=0.69

E

Contact domains — Log₂ H3K27ac (P60/P7) vs Eigenvalue (Chd4 cKO-Ctrl), r=-0.43

Loop domains — Log₂ H3K27ac (P60/P7) vs Eigenvalue (Chd4 cKO-Ctrl), r=-0.43

Compartmental domains — Log₂ H3K27ac (P60/P7) vs Eigenvalue (Chd4 cKO-Ctrl), r=-0.42

Supp Figure 6

Supplementary Figure 6: Dysregulated contact domains exhibit developmental regulation

(A-B) Density plots comparing the change in loop (A) or compartmental (B) domain contacts between the Chd4 cKO and control cerebellum and the change in H3K27ac in that domain in the cerebellum between P7 to P60. Pearson's r, *p<0.001*. **(C)** Clustering of biological replicates of H3K27ac from the control and Chd4 cKO cerebellum at P60 using Spearman's Rho. **(D)** Density plots comparing the change in H3K27ac at P60 between the control and Chd4 cKO cerebellum to the change in H3K27ac at P22 between the control and Chd4 cKO cerebellum among all (left), loop (middle), or compartmental (right) domains. Pearson's r, *p<0.001*. **(E)** Density plots comparing the change in eigenvalue of all (left), loop (middle) or compartmental (right) domains between the Chd4 cKO and control cerebellum and the change in H3K27ac in that domain in the cerebellum between P7 to P60. Pearson's r, *p<0.001*.