Sensory experience remodels genome architecture in neural circuit to drive motor learning

Tomoko Yamada1,2,6*, Yue Yang1,5,6, Pamela Valnegri1,6, Ivan Juric3, Armen Abnousi4, Kelly H. Markwalder1,4, Arden N. Guthrie1, Abigail Godec1, Anna Oldenborg1, Ming Hu3, Timothy E. Holy3 & Azad Bonni8

Neuronal-activity-dependent transcription couples sensory experience to adaptive responses of the brain including learning and memory. Mechanisms of activity-dependent gene expression including alterations of the epigenome have been characterized1–8. However, the fundamental question of whether sensory experience remodels chromatin architecture in the adult brain in vivo to induce neural code transformations and learning and memory remains to be addressed. Here we use in vivo calcium imaging, optogenetics and pharmacological approaches to show that granule neuron activation in the anterior dorsal cerebellar vermis has a crucial role in a delay tactile startle learning paradigm in mice. Of note, using large-scale transcriptome and chromatin profiling, we show that activation of the motor-learning-linked granule neuron circuit reorganizes neuronal chromatin including through long-distance enhancer–promoter and transcriptionally active compartment interactions to orchestrate distinct granule neuron gene expression modules. Conditional CRISPR knockout of the chromatin architecture regulator cohesin in anterior dorsal cerebellar vermis granule neurons in adult mice disrupts enhancer–promoter interactions, activity-dependent transcription and motor learning. These findings define how sensory experience patterns chromatin architecture and neural circuit coding in the brain to drive motor learning.

We developed a behaviour paradigm that might be represented in the anterior dorsal cerebellar vermis (ADCV). The startle response is an evolutionarily conserved predator-avoiding animal reflex triggered by activation of trigeminal nerve dermatomes9. We induced the startle response by tactile stimulation of the nose of a head-fixed mouse on a treadmill using a motorized animal toy as the stimulus (Fig. 1a). Robust backward mouse locomotion was induced within 20 ms of electrically triggering tactile stimulation of the nose (Fig. 1b), but not of the tail (Extended Data Fig. 1a). When an light-emitting diode (LED) used as the conditioned stimulus (CS) was repeatedly paired with a toy tactile stimulus as the unconditioned stimulus (US), animals learned to move backward in response to the LED cue over several days of training, including in catch trials in response to the CS only (Fig. 1c–e, Extended Data Fig. 1b). These results establish delay tactile startle conditioning as a new motor learning paradigm.

Delivery of the type A γ-aminobutyric acid (GABA A ) receptor agonist muscimol into the ADCV and in particular into the caudal ADCV, but not the contiguous lobule VI, robustly inhibited acquisition of the conditioned startle response in mice, but had little or no effect on the unconditioned response or gait dynamics (Fig. 1f, Extended Data Fig. 1c–g). In an optogenetic approach, silencing of granule neurons10,11 specifically in the ADCV during presentation of the CS inhibited acquisition of the conditioned response in mice (Fig. 1g–i). Little or no change in tactile startle conditioning was observed in distinct types of control mice (Extended Data Fig. 2a).

Direct optogenetic stimulation (optostimulation) of granule neurons12,13 in the ADCV as the CS, together with the tactile stimulus (Fig. 1g, h, Extended Data Fig. 2b), triggered rapid associative motor learning in mice (Fig. 1j). By contrast, optostimulation of granule neurons in lobule IX as the CS, or of distinct types of control mice failed to induce motor learning (Fig. 1j, Extended Data Fig. 2c, d). Together, our data suggest that the ADCV is required and sufficient for associative learning in the delay tactile startle conditioning paradigm. Notably, optostimulation of granule neurons in lobule VI as the CS induced animals to move backward (Extended Data Fig. 2e), suggesting that activation of lobule VI is sufficient, though not essential, to trigger motor learning.

In other experiments, optostimulation of Purkinje cells in the ADCV, but not in lobule IX, as the US led to acquisition of conditioned startle responses (Fig. 1g, h, k). Collectively, our data suggest that cerebellar cortical circuits in the ADCV orchestrate delay tactile startle conditioning.

We next characterized how neural circuit activity in the ADCV evolves with learning (Fig. 2a). Granule neurons expressing the calcium indicator GCaMP6f10,11 were robustly activated in the ADCV in response to the CS in mice with motor learning, but not in naive mice (Fig. 2b–f, Extended Data Fig. 3a–c). CS-evoked granule neuron activity in the ADCV in trained mice was observed in the presence or absence of expression of the conditioned response (Fig. 2f, g, Extended Data Fig. 3d). Granule neuron calcium transients were increased in lobule VI with motor learning, but only upon expression of the conditioned response (Fig. 2e, f, Extended Data Fig. 3e).

Purkinje cell dendrites in the ADCV and lobule VI displayed calcium responses to the CS in naive mice, which were significantly enhanced in the ADCV, but not in lobule VI, upon motor learning (Fig. 2h–j, Extended Data Fig. 4). The increase in CS-dependent Purkinje cell calcium transients occurred independently of the conditioned response (Fig. 2i, k). Together, our data suggest that motor learning in the delay tactile startle paradigm triggers transformations in the CS-dependent neural code in granule neuron and Purkinje cell circuits specifically in the ADCV in adult mice.

We next interrogated the molecular mechanisms underlying responses to neural circuit activation in the cerebellum during motor learning. In 52 RNA sequencing (RNA-seq) datasets, comparison of mice subjected to a battery of sensorimotor stimuli versus homecage control mice revealed over 2,000 differentially expressed transcripts (Fig. 3a). A weighted gene co-expression network analysis14 facilitated clustering of the adult cerebellar transcriptome into 26 distinct gene modules, which are represented by distinct colours (Fig. 3b). Upon intersection with cerebellar cell-type-specific expression data15,16, the gene modules were enriched in granule neurons, Bergmann glia, Purkinje cells, molecular layer interneurons or oligodendrocytes (Fig. 3c, Extended Data Fig. 5a).

Sensorimotor stimuli regulated the expression of ten gene modules enriched in granule neurons and Bergmann glia (Fig. 3d, Extended Data Fig. 5b), including genes encoding immediate early transcription

1Department of Neuroscience, Washington University School of Medicine, St. Louis, MO, USA. 2Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan. 3Department of Quantitative Health Sciences, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH, USA. 4MD-PhD Program, Washington University School of Medicine, St. Louis, MO, USA. 5Present address: Department of Neurobiology, Northwestern University, Evanston, IL, USA. 6These authors contributed equally: Tomoko Yamada, Yue Yang, Pamela Valnegri. *e-mail: yamada@md.tsukuba.ac.jp; bonni@wustl.edu

https://doi.org/10.1038/s41586-019-1190-7
Fig. 1 | The ADCV plays a crucial role in delay tactile startle conditioning. 

Factors and cAMP-regulated signalling proteins (light cyan), synaptic proteins (brown) and oxidative phosphorylation proteins (salmon) in granule neurons (Fig. 3e). Silencing of ADCV cortical activity with muscimol reversed the locomotion-induced gene expression changes in the light cyan and midnight blue modules (Extended Data Fig. 5c, d). Importantly, the conditioned stimulus (CS) in the delay tactile startle conditioning task activated the light cyan, midnight blue and salmon modules and repressed the brown gene module (Fig. 3f, Extended Data Fig. 5e–g).

Direct optostimulation of the granule neuron CS pathway in the ADCV rapidly activated the light cyan and midnight blue modules and repressed the brown module (Fig. 3g, h and Extended Data Fig. 6). Optostimulation of granule neurons also modulated expression of enhancer RNA and upstream antisense RNA nearby CS-regulated genes (Fig. 3i, j, Extended Data Fig. 7). In vivo optostimulation of granule neurons in the ADCV followed by chromatin immunoprecipitation with sequencing (ChIP-seq) analyses revealed that H3K27ac enrichment at CS-regulated gene promoters inversely correlated with gene expression upon activation of the CS pathway (Fig. 3i–k, Extended Data Fig. 8). The histone marks H3K4me3 and H3K27me3 exhibited little or no change at CS-regulated gene promoters in response to optostimulation of granule neurons (Fig. 3l, Extended Data Fig. 8c, d). The promoters and enhancers of CS-regulated granule neuron module genes were enriched with distinct activity-dependent transcription factor–binding motifs (Extended Data Fig. 9). Taken together, our results demonstrate that activation of the granule neuron CS pathway triggers distinct epigenetic mechanisms in the ADCV of adult mice in vivo.

To determine whether the three-dimensional organization of chromatin architecture might be regulated in a dynamic manner in the brain, we employed in situ chromosome conformation capture with high-throughput sequencing (Hi-C) and proximity ligation-assissted ChIP-seq (PLAC-seq), the latter to enrich for promoter-centric interactions at H3K4me3-marked promoters (Fig. 4a). Model-based analysis of long-range chromatin interactions from PLAC-seq (hereafter, MAPS) identified 22,430 long-distance promoter-centric interactions in optostimulated and control mice, enriched for regulatory regions of the genome marked by H3K27ac or CTCF occupancy (Extended Data Fig. 10a). At the locus of the activity-dependent gene Nr1a1, promoter interactions with enhancers within 50 kilobases (kb) upstream from the transcription start site (TSS) and in particular with the 30-kb-distal enhancer increased upon granule neuron activation (Fig. 4b). In genome-wide analyses, enhancer–promoter interactions at the CS-induced light cyan and midnight blue module genes were increased and in particular with distal enhancers, whereas enhancer–promoter interactions were reduced in the CS-repressed brown gene module in the ADCV upon optostimulation (Fig. 4c, d). Enhancer–promoter interactions correlated with the levels of H3K27ac at gene promoters, but not with the levels of H3K27ac at proximal versus distal
enhancers, upon optostimulation of ADCV granule neurons in adult mice (Extended Data Fig. 10b–f).

Consistently with PLAC-seq analyses, DNA fluorescence in situ hybridization (FISH) analyses using probes targeting the \(\text{Nr}_{4a}3\) gene and its distal enhancer located 500 kb upstream revealed that the distance between the \(\text{Nr}_{4a}3\) enhancer and the \(\text{Nr}_{4a}3\) gene was reduced upon optostimulation (Fig. 4e, f, Extended Data Fig. 10g, h). Together, these data suggest that activation of the granule neuron CS pathway strengthens long-distance enhancer–promoter interactions and tightly co-regulates enhancer–promoter interactions and gene transcription in vivo.

Genomic loci with increased H3K27ac levels in the ADCV upon optostimulation had enhanced association with transcriptionally active A compartments (Fig. 4g–i, Extended Data Fig. 10i). However, genes with decreased H3K27ac levels had reduced association with active A compartments (Fig. 4i, Extended Data Fig. 10i). Interaction frequencies of genomic loci across chromosomes was also augmented with increased H3K27ac in optostimulated ADCV granule neurons (Fig. 4j, Extended Data Fig. 10j). These results suggest that activated genes might be recruited to active transcriptional compartments to achieve efficient gene expression upon activation of the CS pathway in vivo (Fig. 4k).

We next characterized mechanisms underlying activity-dependent chromatin architecture remodelling in vivo and its role in motor learning. The protein complex cohesin is required in loop formation (Fig. 5a), but its role in the brain has remained largely unexplored. The occupancy of the core cohesin subunit Rad21 at enhancers and promoters correlated with changes in H3K27 acetylation enrichment in the ADCV upon optostimulation (Fig. 5b–d). We employed a conditional CRISPR approach to induce knockout of Rad21 in ADCV granule neurons in adult mice (Fig. 5e–g, Extended Data Fig. 10k), bypassing developmental effects of cohesin inactivation (21). Of note, conditional
CRISPR Rad21 knockout significantly reduced enhancer–promoter interactions in the ADCV of mice undergoing delay tactile startle learning (Fig. 5h, i). Conditional CRISPR Rad21 knockout attenuated induction of the midnight blue gene module in the ADCV upon delay tactile startle conditioning (Fig. 5j, Extended Data Fig. 10f). Accordingly, conditional CRISPR Rad21 knockout in ADCV granule neurons significantly impaired acquisition of the conditioned startle response in mice (Fig. 5k). Collectively, these results suggest that cohesin-dependent reorganization of chromatin architecture and activation of transcription has a critical role in associative motor learning in mice.

In this study, we define how sensory experience transduced through a granule neuron pathway triggers dynamic remodelling of chromatin architecture and neural circuit activity in the ADCV of the adult mouse brain to orchestrate motor learning. Our study provides novel insights into the chromatin mechanisms engaged by sensory experience that induce neural code transformations in mice. Furthermore, the finding
Fig. 4 | Activation of granule neuron CS pathway promotes enhancer–promoter interactions and compartmentalization in vivo. a, Schematic of PLAC-seq (left). A MAPS-normalized contact map at the Pax6 gene locus in the ADCV (right). b, Promoter-centric interactions at the Nrk1 locus at 10 kb resolution upon optostimulation of ADCV granule neurons. Classes indicate viewpoint. c, d, CS-regulated promoter interactions with enhancers (*P = 0.014, **P = 0.0035, ***P = 0.0003, two-sided Wilcoxon signed rank test, n = 77, 39, 174 enhancer–promoters for light cyan, midnight blue, brown) or activated promoter interactions with distal or proximal enhancers (d, ***P = 0.00057, two-sided Wilcoxon signed rank test, n = 22, 38, multi-enhancer–promoters for proximal, distal) upon optostimulation of granule neurons. e, f, Promoter-centric interactions at the Nrk4a3 locus and DNA FISH probes recognizing the distal Nrk4a3 enhancers and Nrk4a3 gene together with the DNA dye Hoechst (e, f, left, n = 169 nuclei). Distance between the Nrk4a3 enhancers and gene upon optostimulation of granule neurons (Figs. 4f, h). g, h, Genome organization of A/B compartments in chromosome 1 using the Pearson correlation matrix or first eigenvector (λ) of Hi-C contacts. h, Change in compartment strength (λ) and H3K27ac levels along chromosome 1 upon optostimulation of granule neurons. i, Compartment strength at genomic loci with changes in H3K27ac levels upon optostimulation of granule neurons (***P = 0.0018, 0.0023 for up (≥0.585), down (< −0.585), one-way ANOVA with Bonferroni post hoc test). j, Inter-chromosomal normalized interaction frequency between genomic loci with changes in H3K27ac levels (**P = 0.0056, one-way ANOVA with Bonferroni post hoc test). k, A model of activity-dependent regulation of chromatin architecture at activated (blue) or repressed (green) genomic loci. a, b, e, One-sided P value from zero-truncated Poisson distribution with Benjamin–Hochberg post hoc test. a–f, n = 2 biological replicates. c, d, f, Box plots show median, quartiles (box) and range (whiskers). g–j, n = 3 biological replicates. i, j, Data show mean ± s.e.m.

Fig. 5 | The core cohesin subunit Rad21 is required for activity-dependent transcription and motor learning in mice. a, Cohesin is a chromatin architectural protein that regulates enhancer–promoter interactions. b, UCSC genome browser tracks at the Fox or Kcnkl1 locus. c, d, Profiles of Rad21 density at promoters (left), enhancers (middle) or flanking CTCF-bound insulators (right) of genes with increased H3K27ac (c) or reduced H3K27ac (d) upon optostimulation of granule neurons. e, f, Schematic of AAV delivery approach to knock out Rad21 in ADCV granule neurons; e, mCherry labelled cerebellum from adult mice expressing Cas9 in granule neurons (n = 25 mice). Scale bar, 200 μm. g, Conditional CRISPR knockout of Rad21 (Rad21-cKO) in ADCV granule neurons in adult mice significantly downregulated Rad21 mRNA levels in the ADCV, normalized to levels in lobule IX (**P = 4.7 × 10⁻⁶, two-tailed t-test, n = 4, 6 mice for control, Rad21-cKO). h, i, Aggregate peak analysis identified enhancer–promoter interactions identified using MAPS analyses of PLAC-seq data. Hi-C interactions are normalized to the mean interactions in the lower-left (LL) corner. Heat maps of the 200 kb surrounding region (h) and bar plots of the peak 10 kb bin normalized to the lower-left bins (i) upon conditional CRISPR knockout of Rad21 in ADCV granule neurons (n = 2 biological replicates). j, The ADCV of conditional CRISPR Rad21-knockout or control mice undergoing delay tactile startle conditioning or the home cage control condition was dissected and subjected to RNA-seq analyses (Mann–Whitney–Wilcoxon test, n = 2, 2, 5 mice for home-control, home-Rad21-cKO, US + CS control, US + CS Rad21-cKO). k, Performance of conditional CRISPR Rad21 knockout or control animals in delay tactile startle conditioning (**P = 0.025, 0.040 for days 3, 4, two-way repeated measures ANOVA with Sidak’s post hoc test, n = 10, 11 mice for control, Rad21-cKO). In all panels, data show mean and shading or error bars denote s.e.m.
of activity-dependent chromatin reorganization specifically in granule
neurons highlights the key role of granule neurons in learning and
memory.

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https://doi.org/10.1038/s41586-019-1190-7.

Received: 1 May 2018; Accepted: 4 April 2019;
Published online 8 May 2019.

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Acknowledgements We thank members of the Bonni laboratory for helpful
discussions and critical reading of the manuscript, Y. Tanabe for genotyping,
and M. Muratani and Tsukuba i-Laboratory for sequencing. Supported by NIH
grants NS041021 (A.B.) and U54DK107977 (M.H.), the Mathers Foundation
(A.B.), Program to Disseminate Tenure Tracking System by MEXT (T.Y.) and
JSPS KAKENHI Grant-in-Aid for Young Scientists 17H04981 (T.Y.).

Reviewer information Nature thanks Timothy Ebner and the other anonymous
reviewer(s) for their contribution to the peer review of this work.

Author contributions T.Y., YY, PV, and A.B. designed the study and wrote the
manuscript. T.Y., YY, JJ, AA, and M.H. performed RNA-seq, ChIP-seq, Hi-C,
PLAC-seq and bioinformatics analyses. YY, PV, KH, ANG, AG, AO, and
TEH performed mouse behavior, optogenetics, CRISPR genetics and in vivo
imaging analyses.

Competing interests The authors declare no competing interests.

Additional information Extended data is available for this paper at https://doi.org/10.1038/s41586-019-1190-7.

Supplementary information is available for this paper at https://doi.org/
10.1038/s41586-019-1190-7.

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Software and code

Policy information about availability of computer code

Data collection

Behavior, electrophysiology, or calcium imaging data was acquired using Clampex (10.7, Molecular Devices), High-speed Data Acquisition (Di-2108, Dataq Instruments), or Prairie View Imaging software (Bruker).

Data analysis

Behavior, electrophysiology, or calcium imaging data was analyzed using Matlab (R2015a, MathWorks). RNA-Seq, ChiP-Seq, DHS, HiC, and PLAC-Seq data was analyzed using HISAT2/Bowtie2 on the public server at usegalaxy.org or bwa mem for genome alignment, EdgeR on the public server at bioinf-galaxian.erasmusmc.nl/galaxy for differential gene expression and ChiP-Seq signals, WGCNA using the R package, DAVID Bioinformatics Resources for gene ontology, MACS2 for peak calling, Homer for transcription factor motif analyses, UCSC genome browser for visualizing ChiP-Seq and RNA-Seq datasets, MAPS for PLAC-Seq analyses, Juicebox for visualizing HiC and PLAC-Seq datasets, and Juicer (HiCCUPS or Eigenvector) or FIRE for HiC analyses.

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ChiP-Seq, RNA-seq, DHS, HiC, and PLAC-Seq data are available in the Gene Expression Omnibus (GEO) database under the reference number GSE127995.
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**

Sample sizes were determined by the standards of the field. All statistical tests were made between groups with similar sample sizes. For animal experiments, 3 to 20 mice per group were used. For ChIP-Seq, RNA-Seq, DHS, HiC, and PLAC-Seq experiments, 2-4 biological replicates per group were used.

**Data exclusions**

In calcium imaging experiments testing how neural coding transforms following successful learning, two mice that failed to acquire conditioned responses after 8 days of delay tactile startle conditioning were excluded. In HiC analyses, promoter-enhancer interactions greater than 5kb were considered due to the resolution limits of chromatin conformation approaches. In MAPS analyses of PLAC-seq data, intra-chromosomal bins between 20kb and 1Mb were used to identify long-range chromatin interactions, since the majority of raw count frequency beyond 1Mb was extremely sparse. No other data was excluded.

**Replication**

Biological replicates were performed for all experiments and only reproducible results are reported.

**Randomization**

All mice were allocated into sex-matched, littermate-matched experimental groups.

**Blinding**

Investigators were not blinded to allocation during experiments and outcome assessment. Animal behavior experiments were automated and did not require blinding.

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**Materials & experimental systems**

| n/a | Involved in the study |
|-----|-----------------------|
| ☐   | Antibodies            |
| ☑   | Eukaryotic cell lines |
| ☑   | Palaeontology         |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |
| □   | Clinical data         |

**Methods**

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq              |
| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging |

**Antibodies**

Antibodies used: histone H3K4me3 (Abcam ab8580), histone H3K27ac (Abcam ab4729), histone H2A.Z (Abcam ab4174), histone H3K27me3 (Abcam ab6002), CTCF (Millipore 07-729), Rad21 (Abcam ab992), Calbindin (Millipore AB1778), GFP (Abcam ab13970), and DsRed (Clontech 632496)

**Validation**

All antibodies are commercially available and have been tested in mice.

**Animals and other organisms**

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**Laboratory animals**

6-12 weeks old mice (mus musculus) of both sexes on a mixed background were used.

**Wild animals**

not applicable

**Field-collected samples**

not applicable

**Ethics oversight**

All animal experiments were done according to protocols approved by the Animal Studies Committee of Washington University.
Ethics oversight

School of Medicine in accordance with the National Institutes of Health guidelines.

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ChIP-seq

Data deposition

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PN6 H3K4me3 home1, PN6 H3K4me3 home2, PN6 H3K4me3 opto1, PN6 H3K4me3 opto2, PN6 H3K27ac home1, PN6 H3K27ac home2, PN6 H3K27ac opto1, PN6 H3K27ac opto2, PN6 H3K27me3 home1, PN6 H3K27me3 home2, PN6 H3K27me3 opto1, PN6 H3K27me3 opto2, PN6 H2A.z home1, PN6 H2A.z home2, PN6 H2A.z opto1, PN6 H2A.z opto2, PN6 H2A.z opto3, PN6 CTCF home1, PN6 CTCF home2, PN6 CTCF home3, PN6 CTCF home4, PN6 CTCF opto1, PN6 CTCF opto2, PN6 CTCF opto3, PN6 CTCF opto4, PN6 Rad21 home1, PN6 Rad21 opto1

Genome browser session

(e.g. UCSC)

N/A

Methodology

Replicates

2-4 biological replicates were performed for all ChIP-Seq experiments. All replication attempts were successful

Sequencing depth

36bp paired-end reads were generated for all ChIP-Seq experiments.

Stats: Condition; total # of paired-end fragments; uniquely mapped paired-end fragments

PN6 H3K4me3 home1 26609170 22276973
PN6 H3K4me3 home2 29270907 23363574
PN6 H3K4me3 opto1 27595064 22663638
PN6 H3K4me3 opto2 28612043 21707440
PN6 H3K27ac home1 27285187 23967809
PN6 H3K27ac home2 24853568 21679983
PN6 H3K27ac opto1 28243874 25004217
PN6 H3K27ac opto2 25362900 21603725
PN6 H3K27me3 home1 22578964 17303533
PN6 H3K27me3 home2 26523437 21035529
PN6 H3K27me3 home3 24609405 19019379
PN6 H3K27me3 opto1 25587942 20063674
PN6 H3K27me3 opto2 30983079 24391815
PN6 H3K27me3 opto3 25021827 18799939
PN6 H2A.z home1 29914410 24115624
PN6 H2A.z home2 35044605 28767504
PN6 H2A.z home3 29257380 24434444
PN6 H2A.z opto1 29964135 25088776
PN6 H2A.z opto2 31705781 26188674
PN6 H2A.z opto3 31705781 26188674
PN6 CTCF home1 26656517 21563083
PN6 CTCF home2 26854777 21394138
PN6 CTCF home3 26545783 19548334
PN6 CTCF home4 24151141 18092971
PN6 CTCF opto1 30161606 23876679
PN6 CTCF opto2 23803862 18551819
PN6 CTCF opto3 23415452 17347855
PN6 CTCF opto4 23357342 16615928
PN6 Rad21 home1 35840764 26020692
PN6 Rad21 opto1 3857840 26600264
PN6 input home 20382945 17265984
PN6 input opto 15681266 13265137

Antibodies

histone H3K4me3 (Abcam ab8580), histone H3K27ac (Abcam ab4729), histone H2A.z (Abcam ab4174), histone H3K27me3 (Abcam ab6002), CTCF (Millipore 07-729), Rad21 (Abcam ab992)

Peak calling parameters

Reads were aligned to the mm10 reference genome using Bowtie2 and peaks were called using MACS2 on the public server at usegalaxy.org using default parameters.

Data quality

Using a FDR<0.05, score>=100: 39,624 peaks for H3K27ac; 23,459 peaks for CTCF; 58,043 peaks for H2A.z, and 21,689 peaks for H3K4me3 were identified

Software

Reads were aligned to the mm10 reference genome using Bowtie2 and peaks were called using MACS2 on the public server
Software at usegalaxy.org. Differential binding with optogenetic stimulation was analyzed using EdgeR on the public server at bioinf-galaxian.erasmusmc.nl/galaxy.