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		# Supplementary Videos:	0

Reporting Checklist for Nature Neuroscience

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. For more information, please read Reporting Life Sciences Research.

Please note that in the event of publication, it is mandatory that authors include all relevant methodological and statistical information in the manuscript.

Statistics reporting, by figure

- Please specify the following information for each panel reporting quantitative data, and where each item is reported (section, e.g. Results, & paragraph number).
- Each figure legend should ideally contain an exact sample size (n) for each experimental group/condition, where n is an exact number and not a range, a clear definition of how n is defined (for example x cells from x slices from x animals from x litters, collected over x days), a description of the statistical test used, the results of the tests, any descriptive statistics and clearly defined error bars if applicable.
- For any experiments using custom statistics, please indicate the test used and stats obtained for each experiment.
- Each figure legend should include a statement of how many times the experiment shown was replicated in the lab; the details of sample collection should be sufficiently clear so that the replicability of the experiment is obvious to the reader.
- For experiments reported in the text but not in the figures, please use the paragraph number instead of the figure number.

Note: Mean and standard deviation are not appropriate on small samples, and plotting independent data points is usually more informative. When technical replicates are reported, error and significance measures reflect the experimental variability and not the variability of the biological process; it is misleading not to state this clearly.

		TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
	FIGURE NUMBER	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #
example	1a	one-way ANOVA	Fig. legend	9, 9, 10, 15	mice from at least 3 litters/group	Methods para 8	error bars are mean +/- SEM	Fig. legend	p = 0.044	Fig. legend	F(3, 36) = 2.97	Fig. legend
example	results, para 6	unpaired t-test	Results para 6	15	slices from 10 mice	Results para 6	error bars are mean +/- SEM	Results para 6	p = 0.0006	Results para 6	t(28) = 2.808	Results para 6

		TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
	FIGURE NUMBER	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #
+ -	1C	DESeq exact test on negative binomial distributions	Methods, under "DNase-seq alignments, peak calling, and differential signal tests"	3	cerebellae from 4-6 mice	Methods, under "DNase-seq library generation"	Mean per DHS site per stage	Supp. Table 2	FDR < .05 threshold	Fig. 1 legend, Supp. Table 2 has p-values per site		Supp. Table 2
+ -	S2C, E,F	two-sided Mann-Whitney	Methods, under "Cell type enriched gene expression comparisons"	3 for DNase-seq, 2 for ChIP-seq	cerebellae from 4-6 mice	Methods, under "DNase-seq library generation" and "Chromatin immunoprecipitation"	Median and distribution	Fig. S2C,E, F	p = 4.1e-11, p = 85e-4, p = 1.8e-115, p = 1.1e-126, p = 5.4e-57, p = 1.2e-51	FIg. S2C,E,F	W = 1292109, W = 2515024, W = 9795393, W = 1673682, W = 1509642, W = 3005509	Not included
+ -	S3D	DESeq exact test on negative binomial distributions	Methods, under "DNase-seq alignments, peak calling, and differential signal tests"	3	independent CGN cultures	Methods, under "DNase-seq library generation"	Mean per DHS site per timepoint	Supp. Table 3	FDR < .05 threshold	Fig. S3 legend, Supp. Table 3 has p-values per site		Supp. Table 3
+ -	S5B	Cuffdiff delta method on negative binomial distributions	Methods, under "RNA-seq alignments and differential expression tests"	3	cerebellae of at least 2 mice	Methods, under "RNA isolation and sequencing"	Mean FPKM estimates per gene per stage	Supp. Table 4	FDR < .05 threshold	Fig. S5 legend, Supp. Table 4 has p-values per gene		Supp. Table 4

+ -	S6A	Cuffdiff delta method on negative binomial distributions	Methods, under "RNA-seq alignments and differential expression tests"	3	independent CGN cultures	Fig. S6 legend, Methods	Mean FPKM estimates per gene per timepoint	Supp. Table 4	FDR < .05 threshold	Fig. S6 legend, Supp. Table 4 has p-values per gene		Supp. Table 4
+ -	2A	two-sided Mann-Whitney	Fig. legend and Methods	3	cerebellae of 4-6 mice for DNase-seq ; cerebellae of at least 2 mice for RNA-seq	Methods		Full distribution in Fig.	p = 5e-31 ; p = 2.7e-34	Fig. 2A	W = 2785888 ; W = 10503097	Not included
+ -	2B	two-sided Mann-Whitney	Fig. legend and Methods	3	independent CGN cultures	Methods		Full distribution in Fig.	p = 1e-29 ; p = 1.4e-12	Fig. 2B	W = 849325 ; W = 11824268	Not included
+ -	2G	unpaired t-test	Fig. legend	3	transfections per timepoint	Fig. legend, Methods under "Luciferase reporter assays"	error bars are mean +/- SEM	Fig. legend	p = .0012 for Cbln3 at +3DIV vs. +6DIV ; p = 6e-6 for Cbln3 vs. pGL3	Fig. legend	df = 4 t = 8.30, 32.3	Fig. Legend
+ -	3C	unpaired t-test	Fig. legend	5 for gRNAs with CAS9-VP64, 8 for site #1 controls, 10 for site #2 controls	independent CGN infections per virus (from at least 2 independent CGN cultures)	Methods, under "Cas9-based RNA-guided gene activation", Fig. Legend	error bars are mean +/- SEM	Fig. legend	p = .0028 for site 1, p = .00022 for site 2	Fig. legend	df =11, df = 13 t = 5.5 for site 1, t =12.3 for site 2	Fig. Legend
+ -	4A	two-sided Mann-Whitney	Fig. legend and Methods	3	cerebellae of 4-6 mice for DNase-seq ; cerebellae of at least 2 mice for RNA-seq	Methods		Full distribution in Fig.	p = .03 ; NS	Fig. 4A	W = 7942012 ; W = 200725	Not included
+ -	4B	two-sided Mann-Whitney	Fig. legend and Methods	3	independent CGN cultures	Methods		Full distribution in Fig.	p = .003 ; NS	Fig. 4B	W = 2598576	Not included
+ -	6B	DESeq exact test on negative binomial distributions	Methods, under "H3K27ac and Zic ChIP-seq analysis"	2	cerebellae of 3 P7 mice or 2 P60 mice per ChIP	Methods, under "Chromatin Immunoprecipitation"	Mean ChIP signal per peak per condition	Supp. Table 10	FDR < .05 threshold	Fig. legend, p-values per peak in Supp. Table 10		Supp. Table 10
+ -	7C	unpaired t-test	Fig. legend	3	independent CGN cultures transfected per construct per timepoint	Methods, under "Luciferase reporter assays"	error bars are mean +/- SEM	Fig. legend	p = .0026 ; p = .0042	Fig. legend	t = 6.7 , 5.8 df = 4	Fig. Legend

+ -	8A	two-sided Mann-Whitney	Fig. legend	2 for ChIP-seq, 3 for RNA-seq	cerebellae of 3 P7 mice or 2 P60 mice per ChIP ; cerebellae of at least 2 mice for RNA-seq	Methods		Full distribution in Fig.	p = 2.27e-15; p = 6.75e-10	in Fig. 8A	W = 50271364 ; W = 26209340	Not included
+ -	8C,D	linear regression	Fig. legend	2 (Zic KD and control) or 3 (normal CGN diff)	independent CGN cultures	Methods, under "RNA isolation and sequencing"	Pearson's r included	All data points in Fig.	p = 4.622e-11; p < 2.2e-16	removed to highlight correlation only	F = 57.95, 80 df; F = 102.2, 141 df	Not included

Representative figures

1. Are any representative images shown (including Western blots and immunohistochemistry/staining) in the paper?

   If so, what figure(s)?

Immunostaining in Fig. 1A
Immunostaining in Fig. S2A
Immunostaining in Fig. S3A
Western blot in Fig. S9B
All genome browser images except Fig. 1B

2. For each representative image, is there a clear statement of how many times this experiment was successfully repeated and a discussion of any limitations in repeatability?

   If so, where is this reported (section, paragraph #)?

Biological replicate numbers are listed in figure legends and in Results for each set of sequencing experiments. Representative browser images were used for clarity; in each case we are highlighting regions initially called significant by statistical tests that included all available replicates.

The immunostaining images and western blot serve as our representation of previously published data (listed in references) to verify we are observing the same results and therefore are not reproduced in this manuscript.

Statistics and general methods

1. Is there a justification of the sample size?

   If so, how was it justified?

   Where (section, paragraph #)?

   Even if no sample size calculation was performed, authors should report why the sample size is adequate to measure their effect size.

Sample size was adequate to measure effect size based on statistical tests selected ahead of each experiment and previous experience with high-throughput sequencing data replication. Power calculations were not performed ahead of time.
This is noted in Methods under "statistical analysis".

2. Are statistical tests justified as appropriate for every figure?

   Where (section, paragraph #)?

t-tests used for bar plots are justified by normal distributions; DESeq, Cuffdiff, and Mann-Whitney tests justified in Methods section under "DNase-seq alignments, peak calling, and differential signal tests", "RNA-seq alignments and differential expression tests", and "Relationship between differential DHS and gene expression changes", respectively.

   a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?

Yes, under Methods there is a "statistical analysis" section and each experiment has its specific statistical test listed in the corresponding figure legend.

b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)?

Where is this described (section, paragraph #)?

t-tests were used for comparison of normal distributions (most bar plots with variance reflecting experimental variability), Mann-Whitney test used for non-normal distributions (fold-change gene expression distributions and chromatin-based signals in some cases were found to be non-normal), and exact tests used for non-normally distributed sequencing data (negative binomial distributions used). This is described in Methods, under each specific technique description.

c. Is there any estimate of variance within each group of data?

Is the variance similar between groups that are being statistically compared?

Where is this described (section, paragraph #)?

Variance estimates are quantified in the DESeq and Cuffdiff statistical packages across sequencing depths for identifying differential DNase, RNA, and ChIP signals. This is mentioned in Methods under "Bioinformatic analyses".

d. Are tests specified as one- or two-sided?

All tests presented are two-sided and this is noted in Methods.

e. Are there adjustments for multiple comparisons?

Sequencing experiment analyses were corrected to False Discovery Rates (FDR) by the Benjamini-Hochberg method.

3. Are criteria for excluding data points reported?

Was this criterion established prior to data collection?

Where is this described (section, paragraph #)?

The only criteria used to exclude data points was technical failure. For example, if our internal control gene Gapdh Ct value was outside predefined normal range for qPCR measures of cDNA, all related samples were excluded and the experiment was repeated.

4. Define the method of randomization used to assign subjects (or samples) to the experimental groups and to collect and process data.

If no randomization was used, state so.

Where does this appear (section, paragraph #)?

No particular randomization techniques used. Sequencing samples were collected in random order. This is mentioned under "statistical analysis".

5. Is a statement of the extent to which investigator knew the group allocation during the experiment and in assessing outcome included?

If no blinding was done, state so.

Where (section, paragraph #)?

No blinding was performed.

6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?

Where (section, paragraph #)?

Yes, this is included in Methods under "Cerebellar immunostaining".

7. Is the species of the animals used reported?

Where (section, paragraph #)?

Yes, this is included in Methods under "Cerebellar immunostaining". (mice)

8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported?

Where (section, paragraph #)?

Yes, this is included in Methods under "Cerebellar immunostaining". (C57BL/6Ncrl mice).

9. Is the sex of the animals/subjects used reported?

Where (section, paragraph #)?

Yes, this is included in Methods under "Cerebellar immunostaining" and "Isolation of cerebellar nuclei". We used litters of both male and female mice.

10. Is the age of the animals/subjects reported?

    Where (section, paragraph #)?

Yes, this is reported throughout the manuscript and in Methods as the ages define our postnatal developmental stages.

11. For animals housed in a vivarium, is the light/dark cycle reported?

    Where (section, paragraph #)?

Yes, reported in Methods under "Cerebellar immunostaining". (14/10h light/dark).

12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?

    Where (section, paragraph #)?

Yes, reported in Methods under "Cerebellar immunostaining". (4-5 animals per cage).

13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?

    Where (section, paragraph #)?

No behavioral experiments were performed.

14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?

    Where (section, paragraph #)?

No previous history to report.

    a. If multiple behavioral tests were conducted in the same group of animals, is this reported?

       Where (section, paragraph #)?

No behavioral experiments were performed.

15. If any animals/subjects were excluded from analysis, is this reported?

    Where (section, paragraph #)?

No animals were excluded from analysis. Samples were pooled from multiple animals as reported in Methods.

    a. How were the criteria for exclusion defined?

       Where is this described (section, paragraph #)?

N/A

    b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.

       Where is this described (section, paragraph #)?

N/A

Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?

Yes, we used commercial antibodies with the exception of the rabbit anti-Zic1/2 antibody, which we extensively validated by a) Western blot before and after knockdown of Zic1 or Zic2 and b) Western blot and ChIP-seq with the antibody in cortex, which does not express Zic1/2 (Fig. S6).

    a. Is antibody catalog number given?

       Where does this appear (section, paragraph #)?

Yes, catalog numbers are included in Methods under "Cerebellar immunostaining" , "CGN immunostaining", and "Chromatin immunoprecipitation" sections.

b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?

Where does this appear (section, paragraph #)?

Zic1/2 antibody validation data is presented in Supp. Fig. 6 and referenced in Results, under "Zic transcription factors bind developmentally regulated DHS sites and promote neuronal maturation". Commercial antibodies externally validated.

2. If cell lines were used to reflect the properties of a particular tissue or disease state, is their source identified?

Where (section, paragraph #)?

No cell lines were used, just primary CGN cultures.

a. Were they recently authenticated?

Where is this information reported (section, paragraph #)?

N/A

Data deposition

Data deposition in a public repository is mandatory for:

a. Protein, DNA and RNA sequences
b. Macromolecular structures
c. Crystallographic data for small molecules
d. Microarray data

Deposition is strongly recommended for many other datasets for which structured public repositories exist; more details on our data policy are available here. We encourage the provision of other source data in supplementary information or in unstructured repositories such as Figshare and Dryad.

1. Are accession codes for deposit dates provided?

Where (section, paragraph #)?

All sequencing data deposited to GEO under accession # GSE60731

GEO information added after Methods section.

Computer code/software

Any custom algorithm/software that is central to the methods must be supplied by the authors in a usable and readable form for readers at the time of publication. However, referees may ask for this information at any time during the review process.

1. Identify all custom software or scripts that were required to conduct the study and where in the procedures each was used.

Small, mostly interactive scripts were used for sequencing data analysis, including annotating each DHS site with it's nearest reference gene, differential testing, and computing and plotting gene expression changes linked to DNase/ChIP-seq sites.

2. Is computer source code/software provided with the paper or deposited in a public repository? Indicate in what form this is provided or how it can be obtained.

The short scripts are mostly non-functional without our local directory structure and files (i.e., they are far from stand-alone software). However, upon request we can provide supplementary files that contains various scripts used in the manuscript.

Human subjects

1. Which IRB approved the protocol?

Where is this stated (section, paragraph #)?

N/A, no human subjects used.

2. Is demographic information on all subjects provided?

   Where (section, paragraph #)?

   N/A

3. Is the number of human subjects, their age and sex clearly defined?

   Where (section, paragraph #)?

   N/A

4. Are the inclusion and exclusion criteria (if any) clearly specified?

   Where (section, paragraph #)?

   N/A

5. How well were the groups matched?

   Where is this information described (section, paragraph #)?

   N/A

6. Is a statement included confirming that informed consent was obtained from all subjects?

   Where (section, paragraph #)?

   N/A

7. For publication of patient photos, is a statement included confirming that consent to publish was obtained?

   Where (section, paragraph #)?

   N/A

fMRI studies

For papers reporting functional imaging (fMRI) results please ensure that these minimal reporting guidelines are met and that all this information is clearly provided in the methods:

1. Were any subjects scanned but then rejected for the analysis after the data was collected?

   N/A, no fMRI studies.

   a. If yes, is the number rejected and reasons for rejection described?

      Where (section, paragraph #)?

      N/A

2. Is the number of blocks, trials or experimental units per session and/or subjects specified?

   Where (section, paragraph #)?

   N/A

3. Is the length of each trial and interval between trials specified?

   N/A

4. Is a blocked, event-related, or mixed design being used? If applicable, please specify the block length or how the event-related or mixed design was optimized.

   N/A

5. Is the task design clearly described?

   Where (section, paragraph #)?

   N/A

6. How was behavioral performance measured?	N/A
7. Is an ANOVA or factorial design being used?	N/A
8. For data acquisition, is a whole brain scan used? If not, state area of acquisition.	N/A
a. How was this region determined?	N/A
9. Is the field strength (in Tesla) of the MRI system stated?	N/A
a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?	N/A
b. Are the field-of-view, matrix size, slice thickness, and TE/TR/ flip angle clearly stated?	N/A
10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?	N/A
11. Is the coordinate space for the anatomical/functional imaging data clearly defined as subject/native space or standardized stereotaxic space, e.g., original Talairach, MNI305, ICBM152, etc? Where (section, paragraph #)?	N/A
12. If there was data normalization/standardization to a specific space template, are the type of transformation (linear vs. nonlinear) used and image types being transformed clearly described? Where (section, paragraph #)?	N/A
13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?	N/A
14. Were any additional regressors (behavioral covariates, motion etc) used?	N/A
15. Is the contrast construction clearly defined?	N/A
16. Is a mixed/random effects or fixed inference used?	N/A
a. If fixed effects inference used, is this justified?	N/A
17. Were repeated measures used (multiple measurements per subject)?	N/A
a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?	N/A

18. If the threshold used for inference and visualization in figures varies, is this clearly stated?	N/A
19. Are statistical inferences corrected for multiple comparisons?	N/A
a. If not, is this labeled as uncorrected?	N/A
20. Are the results based on an ROI (region of interest) analysis?	N/A
a. If so, is the rationale clearly described?	N/A
b. How were the ROI's defined (functional vs anatomical localization)?	N/A
21. Is there correction for multiple comparisons within each voxel?	N/A
22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?	N/A

Additional comments

Additional Comments	