nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Bulk RNA sequencing data were generated using standard Illumina sequencing platforms and manufacturer-provided software. Protein binding microarray (PBM) experiments and qPCR data were acquired using instrument-specific proprietary software. No custom software or code was used for data collection. RNA seq data: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi PBM data:https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE298334
Data analysis	The code produced to analyze the data is available on GitHub: https://github.com/Istevant/Enh13_mutations.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data is available in the manuscript or the supplementary materials. RNA-seq data and PBM data have been deposited in the Gene Expression Omnibus under accession numbers GSE291809 (Review access token: gluxiykcvhgvnub) and GSE298334 (Review access token: stkvqkmenrudrif). After publication, the material will be made availible with no access token

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	This research did not involve human participants.
Reporting on race, ethnicity, or other socially relevant groupings	This research did not involve human participants.
Population characteristics	This research did not involve human participants.
Recruitment	This research did not involve human participants.
Ethics oversight	This research did not involve human participants.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	No formal statistical method was used to predetermine sample sizes. Instead, sample sizes were selected based on common practice for comparable assays and on ensuring reproducibility across independent experiments. All key experiments were performed with a minimum of three independent biological replicates. When increased variability was observed between replicates, we performed additional independent repeats to confirm results. Technical replicates are indicated in the Methods/figure legends. Exact n values and what n represents are provided in each figure legend.
Data exclusions	No data were excluded from the analyses. qPCR data was analyzed for outliers and outliers were removed only if recommended by the Graphed Prism software.
Replication	All experiments were independently repeated with similar results. At least ten different animals of each genotype from all mouse strains were analysed. Immunostaining experiments were performed on multiple biological replicates. RNA sequencing, and qPCR analyses were performed using independent biological samples.
Randomization	Randomization was not applicable to this study, as experimental groups were defined by genotype or developmental stage.
Blinding	Blinding was not performed, as groups were defined by genotype or experimentally induced conditions that were apparent during analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

The following primary antibodies were used in this study: Goat α SOX9 (R&D systems, AF3075, lot:WL0523091), Rabbit α FOXL2 (Abcam, ab246511, lot:1067642-41), Mouse α DDX4 (Abcam, ab27591, lot:1067533-24), Rat α GCNA1 (TRA98) (Abcam,ab82527, lot:1098726-9), Rat αNr5a1 Ad4(BP/SF-1) (Cosmo-Bio, KAL-KO610, lot: 1B1F10)

Validation

All primary antibodies used in this study have been validated by the manufacturers or in previous studies for use in mouse tissues and for immunofluorescence applications. Specificity was further supported by reproducible staining patterns consistent with known expression domains and by extensive prior use in the field.
SOX9- https://www.rndsystems.com/products/human-sox9-antibody_af3075
FOXL2-https://www.abcam.com/en-us/products/primary-antibodies/foxl2-antibody-epr23523-68-ab246511
TRA98-https://www.abcam.com/en-us/products/primary-antibodies/gcna1-antibody-tra98-ab82527
DDX4-https://www.abcam.com/en-us/products/primary-antibodies/ddx4-mvh-antibody-mabcam27591-ab27591
SF1- https://search.cosmobio.co.jp/cosmo_search_p/search_gate2/docs/KAL_/KO610.20111014.pdf

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK293T and COS7 cells used in this study. Cell lines were purchased from ATCC.
HEK: CRL-3216 (Human)
COS7: CRL-1651 (Cercopithecus aethiops)

Authentication

None of the cell lines were authenticated in our lab.

Mycoplasma contamination

Cells lines were not tested for mycroplasma contamination.

Commonly misidentified lines
(See ICLAC register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

All mice were maintained under specific pathogen–free (SPF) conditions in the Bar-Ilan University animal facility. Animals were housed at a temperature of 20–24°C with a relative humidity of 35–65%, under a 12-hour light/12-hour dark cycleThis study involved testing on mus musculus. All mice strains were maintained on a C57BL/6J genetic background. We analyzed these mice at E11.5, E12.5, E13.5, E15.5, P1 and 6 weeks.

Wild animals

This study did not involve wild animals.

Reporting on sex

This study investigated sex determination in mice. Genetic sex was determined by PCR genotype for embryonic and adult mice as described in the methods section of the paper. Additionally, gonadal sex phenotype was reported at length for both XY and XX mice of each genotype.

Field-collected samples

This study did not involve samples collected from the field

Ethics oversight

All animals were maintained with appropriate husbandry according to Bar Ilan University ethics protocols 11-02-2020, 1-11-2020, and 2306-117-11.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*