

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	BORIS software, v.7.10.5
Data analysis	Code for Figure 5 has been deposited in GitHub here: https://github.com/adidust4/Offspring-genetic-diversity-Chd8-haploinsufficiency All other data was analyzed in SPSS v29.0.2.0.

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](#)

The data that support the findings of this study are openly available³⁹ in Figshare at DOI 10.6084/m9.figshare.31395966.

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 N/A

Reporting on race, ethnicity, or other socially relevant groupings N/A

Population characteristics N/A

Recruitment N/A

Ethics oversight N/A

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](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Behavioural & social sciences study design

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

Study description	Controlled experimental quantitative observational study in genetically modified mice assessing behavioral phenotypes and litter composition across strains
Research sample	The research sample includes Chd8 heterozygous B6 dams and their genetically diverse offspring. The offspring consist of B6-B6 and 15 B6-Collaborative Cross WT and Chd8 heterozygous males and females that were observed during the preweaning period from postnatal day 1-21 with behaviors reported. Adult behaviors of the offspring were previously reported in Tabbaa, Knoll, and Levitt (2023) and the effect sizes are further analyzed here in relation to developmental measures reported for the first time here.
Sampling strategy	For cohort's one and two, litter observations consisted of recording twelve snapshots of behavior conducted at 5-minute intervals over a one-hour period during the preweaning period. Tally marks corresponding to observed behaviors were recorded during each spot checked. For Cohort 3, the duration of litter behaviors and the frequency of nest exits were recorded for two offspring strain groups that were also observed in Cohort 2 (B6-B6 and B6-CC12). During postnatal day (P) 4, P5, P11, P12, P18, and P19, observations for each cage took place over one hour and involved a researcher live scoring maternal behaviors for three 5-minute observation periods spaced fifteen minutes apart. The number of litters per strain included in Cohort's 1 and 2 were based off the number of litters needed to generate n=8 mice per group of strain, Chd8 genotype and sex at weaning for adult behavioral testing reported in Tabbaa, Knoll, and Levitt (2023). The number of litters generated in Cohort 3 was based off the variability in measured behaviors from Cohort's 1 and 2.
Data collection	For Cohort's 1 and 2, pen and paper was used to record tally marks totaling spot checks for each behavior and litter across each postnatal day. Tally marks were summed for each postnatal day and used for data analysis for Cohorts 1 and 2. The average percent frequency of each behavior for each litter was then calculated across each time bin and analyzed for differences across offspring strain groups. For Cohort 3, the duration of litter behaviors and the frequency of nest exits were scored live during postnatal days 4, 5, 11, 12, 18, and 19 for three 5-minute observation periods spaced fifteen minutes apart using BORIS software. During all recording sessions for all cohorts, the researcher was blind to the strain of the offspring. For all cohorts, the litter size, sex ratio, and percentage of Chd8 heterozygous mice were recorded at weaning.
Timing	Data for cohort's 1 and 2 were collected from November 2019-March 2020. Data for cohort 3 was collected from August-October 2023.
Data exclusions	Analyses that tested group differences in behaviors across P1-21 included average behavioral frequencies for litters that had at least 2 postnatal day observations during each postnatal week (PNW) to meet enough litters for statistical analyses. Most litters had at least 3 observation days per PNW (see Supplementary Data 1). Analyses that tested group differences in behaviors across PNWs included average frequencies of dam behaviors per litter for litters that had at least 3 postnatal day (PND) observations per PNW. The total number of observations, as well as the number of litters, included in the analyses of specific postnatal periods are listed in Supplementary Figure 1A and Supplementary Data 1. For pup developmental milestone analyses, not all litters were observed at the end of PNW3 and thus only litters that displayed each behavior were included in analyses for age differences across strains. The percentage of litters excluded in the developmental milestone analysis is listed in Supplementary Figure 4. Extreme outliers, defined in SPSS as values more than 3 times the first to third interquartile range, were removed if only one outlier was identified per strain

group and a maximum of one outlier was removed per group.

Non-participation

Litters that died prior to weaning dropped out of the study.

Randomization

Litter size and the ratio of males and Chd8 heterozygous mice per litter as well as dam parity were covaried in statistical analyses to control for these variables across strain groups.

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 |

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

B6-Chd8^{+/−} females mated with males from 15 Collaborative Cross (CC) strains and B6 produced genetically diverse (i.e., B6-CC) and identical (i.e., B6-B6) F1 litters comprised of WT and Chd8^{+/−} offspring. C57BL/6J (B6) mice that were heterozygous for Chd8 (Chd8^{+/−}) were originally received from Dr. Feng Zhang (MIT). This B6-Chd8 mouse line was generated through Cas9-mediated germline editing followed by germline transmission and inheritance. Chd8^{+/−} mice in this study are descendants from one founder with germline transmission of a loss-of-function Chd8 allele containing a 7-nucleotide deletion in exon 1, resulting in a 50% reduction in CHD8 protein expression at embryonic day 18 compared to WT littermates³². B6-Chd8^{+/−} dams were bred with B6 males and males from 15 Collaborative Cross (CC) strains, all obtained from The Jackson Laboratory (Bar Harbor, ME), at 6–8 weeks of age and allowed to habituate to the colony for at least one week before breeding. Sires were from strains that included CC001/Unc (Stock No. 21238), CC002/Unc (Stock No. 21236), CC007/Unc (Stock No. 29625), CC010/GeniUnc (Stock No. 21889), CC012/GeniUnc (Stock No. 28409), CC013/GeniUnc (Stock No. 21892), CC016/GeniUnc (Stock No. 24684), CC022/GeniUnc (Stock No. 25424), CC024/GeniUnc (Stock No. 21891), CC025/GeniUnc (Stock No. 18857), CC028/GeniUnc (Stock No. 25126), CC032/GeniUnc (Stock No. 20946), CC044/Unc (Stock No. 26426), CC057/Unc (Stock No. 24683), CC075/Unc (Stock No. 27293), and the reference strain C57BL/6J (Stock No. 000664). For cohort's 1 and 2, litters were observed from postnatal day 1–21. For cohort 3, litters were observed on postnatal days 4, 5, 11, 12, 18, and 19.

Wild animals

N/A

Reporting on sex

Sex information is reported including in all data files. Pre-weaning pup measures from litter observations consisted of litters containing males and females, of which the ratio of males and females for each litter is reported. Postweaning traits are analyzed separately for males and females and reported.

Field-collected samples

N/A

Ethics oversight

All experimental procedures were approved by the University of Southern California and Children's Hospital Los Angeles Institutional Animal Care and Use Committees under protocols #11844-CR011 and #430, respectively.

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

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A