

## Reporting Summary

Nature Research 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 Research 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 The Illumina short reads were collected by Novogene (Sacramento, CA).

Data analysis Fastp software (0.19.4) tool (<https://github.com/OpenGene/fastp>) to remove the index and barcoded sequences and discard the unpaired reads. Bowtie2 to map all clean short reads to the newest Rattus norvegicus reference genome (Rnor\_6.0). SAMTOOLS to merge and convert alignment files (SAM files). Genome Analysis Toolkit (GATK) to call DNA variants.

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 Research [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 list of figures that have associated raw data
- A description of any restrictions on data availability

Data accession number at NCBI: PRJNA642750 (SRX8786728 and SRX8786729). <https://www.ncbi.nlm.nih.gov/sra/PRJNA642750>.

# 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://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	A total of 128 NIH-HS rats and 48 WI rats were used in the present study. The sample sizes chosen were based on rat colony breeding structure (for the former strain) and resources available in house (for the latter strain). Traditionally, 30 samples per strain are enough to perform such a genetic study.
Data exclusions	Degraded DNA samples were removed from further analysis. To improve data quality, we also removed the DNA variants that had 1) less than 30 total reads for both strains; 2) no genotype clearly scored in one of the strains and 3) mono-polymorphic markers observed in both NIH-HS and WI rats that were different from the reference genome. All DNA variants with 15 reads or more per site were retained for the Y chromosome.
Replication	Two strains of rats were mutually validated to verify the reproducibilities of the experimental findings.
Randomization	NA
Blinding	The strain names were blinded to the service lab for sequencing and to Dr. Weimin Wang for read mapping and variant calling.

## 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		Methods	
n/a	Involved in the study	n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies	<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines	<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology	<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms		
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants		
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data		
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern		

## Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	National Institute of Health heterogeneous stock (NIH-HS rats) and Wistar (WI) rats, half males and half females.
Wild animals	NA
Field-collected samples	NA
Ethics oversight	The use of tissues was approved by the Institutional Animal Care and Use Committee of Washington State University.

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