

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	No software was used
Data analysis	Data were analyzed using the following software tools and packages:ATAC-seq: Adapter trimming with cutadapt (v1.18), alignment with Bowtie2 (v2.0.0), duplicate removal with Picard, peak calling with MACS3, annotation with ChIPseeker, and statistical analysis with DESeq2. DNA methylation: Basecalling with Dorado (v0.5.3), alignment and methylation calling with modkit.RNA-seq: Adapter trimming with cutadapt, alignment with STAR (v2.7+), quantification with htseq-count, duplicate removal with Picard, and differential expression analysis with DESeq2.ChIP-seq: Processed with cutadapt, aligned using Bowtie (v1.0.0), peak calling with MACS2 (v2.0.10.20131216).Pathway and motif analyses: GREAT, HOMER, TRRUST, Metascape, GSEA v4.3.2, and BaGFoot (bagfootr) in R.Data visualization and clustering: ggplot2, heatmapper, Metagene2, and IGV for genome browser views.Deconvolution: cellanneal using single-cell data from Ben-Moshe et al.All tools were run with standard or cited parameters unless otherwise stated in the Methods. No custom code was developed or used. Figures were prepared using GraphPad Prism v9.0 and BioRender.

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](#)

SIRT6 TG ATAC-seq GSE294103 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE294103>]
 SIRT6 TG RNA-seq GSE294067 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE294067>]
 SIRT6 TG ChIP-seq GSE294085 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE294085>]
 SIRT6 AAV8 ATAC-seq GSE294084 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE294084>]
 SIRT6 TG Methylome GSE296168 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE296168>]

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

Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

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)

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 size. Sample sizes (typically n = 6–8 animals per group) were selected based on prior experience with similar omics studies to ensure sufficient power for detecting biological differences in chromatin accessibility, methylation, and gene expression. Sample sizes were consistent across experimental groups and are reported in figure legends and Methods.

Data exclusions

No data were excluded from the analyses unless they failed predefined quality control thresholds. For example, ATAC-seq and RNA-seq libraries were excluded if they showed abnormal fragment size distributions or insufficient sequencing depth. These criteria were established prior to analysis and are described in the Methods section.

Replication

All key experiments were independently replicated in multiple biological samples. ATAC-seq, RNA-seq, methylome, and ChIP-seq analyses were performed on multiple animals per group and yielded consistent results across replicates. Western blot validation of histone marks and

RT-qPCR confirmation of expression changes were also repeated successfully. No findings reported in this study failed to replicate.

Randomization

Mice were group-housed in a controlled environment and randomly assigned to experimental groups (WT, SIRT6 TG, or AAV-SIRT6/GFP) using cage-based allocation. All animals were age-matched and housed under identical conditions. Randomization was performed to minimize environmental and handling biases. No additional covariates were controlled beyond age and genotype.

Blinding

Investigators were not blinded to group allocation during sample collection due to the visible phenotypic differences and genotypic knowledge required for proper tissue handling. However, data processing and statistical analysis (e.g., DESeq2, ChIP-seq/ATAC-seq pipelines) were performed using automated, unbiased scripts that do not depend on group identity, reducing the risk of analytical bias.

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 antibodies were used in this study: Anti-H3: Abcam, catalog number #70550 Anti-H4: Abcam, catalog number #10158 Anti-H3K9ac: Abcam, catalog number #32129 Anti-H3K56ac: Cell Signaling Technology, catalog number #4243s Anti-H3K18ac: Abcam, catalog number #1191 Anti-H3K4me3: Cell Signaling Technology, catalog number #9751s Anti-H3K9me3: Abcam, catalog number #176916s Anti-H3K27me3: Cell Signaling Technology, catalog number #c36b11 Anti-H4K16ac: Sigma, catalog number #07-329 Anti-SIRT6: Cell Signaling Technology, catalog number #12486s Anti-GFP: Roche, catalog number #1181460001 HRP-conjugated Anti-Rabbit IgG: Santa Cruz Biotechnology, catalog number #45043 HRP-conjugated Anti-Mouse IgG: Jackson ImmunoResearch, catalog number #115-035-003. H3K27ac Abcam Cat #EP16602. All primary antibodies were diluted 1:1000 in 5% BSA(w/v). Secondary antibodies were diluted 1:15,000 in 5% skim milk (w/v). For ChIP-seq: Anti-H3K9ac – Abcam, catalog number #32129 and Anti-H3K56ac – Epigentek, catalog number #A-4026, H3K27ac Abcam Cat #EP16602. 5 micrograms from each antibody were used.

Validation

All antibodies used in this study are commercially available and widely validated for mouse tissues and for the indicated applications (Western blot or ChIP-seq). Validation was based on manufacturer documentation, prior published studies using the same antibodies, and expected band sizes or signal enrichment. Histone mark antibodies (e.g., H3K9ac, H3K56ac, H3K27ac) showed specific enrichment in ChIP-seq at open chromatin regions as expected, further confirming specificity.

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

Male C57BL/6J OlaHsd mice were used for all experiments. Two main groups were included: Naturally aged mice: Young (5–7 months) and old (18–21 months) wild-type and SIRT6 transgenic (TG) mice maintained on a C57BL/6J OlaHsd background. AAV-injected mice: 5-month-old C57BL/6J OlaHsd male mice purchased from Envigo, Israel were injected via the tail vein with either AAV-SIRT6 or AAV-GFP vectors to induce liver-specific overexpression. All animals were housed under specific pathogen-free (SPF) conditions with controlled temperature and light/dark cycles and had free access to food and water.

Wild animals

This study did not involve wild animals.

Reporting on sex

This study included only male mice across all experimental groups to reduce variability due to sex hormones and to ensure consistency in metabolic and aging-related phenotypes. Sex was explicitly considered in the study design, and all references to "young" and "old" animals refer to male mice. No sex-based analyses were performed, and data were not disaggregated by sex because female subjects were not included in this study. The choice to use only male mice is consistent with previous research in the field aiming to establish foundational mechanisms before expanding to sex comparisons.

Field-collected samples

This study did not involve samples collected from the field.

Ethics oversight

All experiments were conducted in accordance with the Bar-Ilan Institutional Animal Care and Use Committee and approved by the Ministry of Health of Israel.

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, mosaicism, off-target gene editing) were examined.

ChIP-seq

Data deposition

- ☒ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links <i>May remain private before publication.</i>	SIRT6 TG ChIP-seq GSE294085 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE294085]
Files in database submission	The following files are available as part of the GSE294085 submission: Raw sequencing data for all ChIP-seq and input samples are available through the Sequence Read Archive (SRA), linked via BioProject PRJNA1248043. In addition, normalized read counts for ChIP-seq experiments contain peaks data in a merged format for all samples and bigwig for each sample are available.
Genome browser session (e.g. UCSC)	https://genome.ucsc.edu/s/Nagaron/anonymized

Methodology

Replicates	ChIP-seq experiments were performed on liver tissues from young and old WT and SIRT6 TG male mice. Biological replicates were used for each group, with n = 4-6 mice per group for H3K9ac and H3K56ac ChIP-seq and n = 2 for H3K27ac ChIP-seq. Replicates were consistent, and downstream analysis confirmed high reproducibility
Sequencing depth	For both input and IP samples, ChIP-seq libraries were sequenced using Illumina NovaSeq S1, 100 cycles, yielding approximately 31 million reads per sample. The reads were single-end and included 9 bp Unique Molecular Identifiers (UMIs) to improve data resolution.
Antibodies	The antibodies used for ChIP were: Anti-H3K9ac: Abcam, Cat #32129. Anti-H3K56ac: Epigentek, Cat #A-4026. Anti-H3K27ac: Abcam, Cat #EP16602
Peak calling parameters	ChIP-seq reads were aligned uniquely to the mouse genome (mm10) using Bowtie v1.0.0. Peaks were called using MACS2 (v2.0.10.20131216) with default parameters. Input controls were used for peak calling. The top 1,000 variable peaks were used for principal component analysis (PCA). Peaks were annotated and visualized using ChIPseeker and Metagene2.
Data quality	To ensure high data quality, reads shorter than 40 nt were removed after adapter trimming with cutadapt. Duplicate reads were removed using UMI and Picard tools.
Software	Data processing used the following tools: Cutadapt for adapter trimming Bowtie v1.0.0 for alignment MACS2 for peak calling ChIPseeker, Metagene2, and IGV for annotation and visualization R for PCA and statistical analysis