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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 Authors & Referees 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	Microscopy: IN Cell Analyser 2000 version 5.2-14311 (64-bit) GE Healthcare; RT-qPCR: Biorad CFX Manager V3.1
Data analysis	For the data analyses we used the following tools : ChIP-seq pre-processing : eautils v1.1.2 fastqscreen v0.4.4 bowtie v1.1.1 samtools v1.6 picardtools v1.130 bedtools 2.19.1 spp v1.10.1 macs v2.1.0 idr v1.0 deepTools v2.4.2 ATAC-seq pre-processing : cutadapt v1.8.3 fastqscreen v0.4.4 bowtie v2.2.3 samtools v1.6 picardtools v1.130 bedtools 2.19.1 spp v1.10.1

macs v2.1.0
idr v1.0
deepTools v2.4.2
piq
gem v3.0
hdp

R packages (R v3.4) from CRAN and Bioconductor :
custom R v3.4 scripts
annotate v1.56.2
AnnotationDbi v1.40.0
arcdiagram v0.1.11
bindrcpp v0.2.2
Biobase v2.38.0
BiocGenerics v0.24.0
BiocParallel v1.12.0
BiocStyle v2.6.1
biomaRt v2.34.0
Biostrings v2.46.0
biovizBase v1.26.0
BSgenome v1.46.0
BSgenome.Hsapiens.UCSC.hg19 v1.4.0
ChIPpeakAnno v3.14.0
chromstaR v1.7.2
circlize v0.4.4
clusterProfiler v3.6.0
colorRamps v2.3
ComplexHeatmap v1.18.1
data.table v1.11.4
DBI v1.0.0
DelayedArray v0.4.1
DESeq2 v1.18.1
DiffBind v2.6.6
DOSE v3.4.0
dplyr v0.7.6
dynamicTreeCut v1.63-1
EDASeq v2.14.1
edgeR v3.22.3
EnsDb.Hsapiens.v75 v2.99.0
ensembldb v2.2.2
eulerr v4.1.0
factoextra v1.0.5
FactoMineR v1.41
fastcluster v1.1.25
futile.logger v1.4.3
genefilter v1.60.0
GenomeInfoDb v1.14.0
GenomicAlignments v1.14.2
GenomicFeatures v1.30.3
GenomicRanges v1.30.3
ggdendro v0.1-20
ggplot2 v3.1.0
ggrepel v0.8.0
gplots v3.0.1
graph v1.56.0
gridExtra v2.3
GSEABase v1.40.1
Gviz v1.22.3
heatmaply v0.15.2
hta20transcriptcluster.db v8.7.0
igraph v1.2.2
IRanges v2.12.0
kableExtra v0.9.0
knitr v1.20
lattice v0.20-35
limma v3.36.2
MASS v7.3-51
matrixStats v0.54.0
mgcv v1.8-24
mixOmics v6.3.2
nlme v3.1-137
oligo v1.44.0
oligoClasses v1.40.0
org.Hs.eg.db v3.5.0
oposSOM v2.2.0

pd.hta.2.0 v3.12.2
pheatmap v1.0.10
plotly v4.8.0
plotrix v3.7-4
plyr v1.8.4
preseqR v3.1.2
R.oo v1.22.0
R.utils v2.7.0
randomcoloR v1.1.0
RColorBrewer v1.1-2
ReactomePA v1.22.0
reshape v0.8.7
reshape2 v1.4.3
Rsamtools v1.30.0
RSQLite v2.1.1
rtracklayer v1.38.3
RUVSeq v1.14.0
S4Vectors v0.16.0
ShortRead v1.36.1
SummarizedExperiment v1.8.1
sva v3.26.0
TFBSTools v1.16.0
TxDb.Hsapiens.UCSC.hg19.knownGene v3.2.2
VennDiagram v1.6.20
viridis v0.5.1
viridisLite v0.3.0
WGCNA v1.63
XVector v0.18.0
zoo v1.8-4

Microscopy:
IN Cell Investigator 1000 workstation 3.7.2, build 1860

Flow cytometry:
FlowJo V10.2

Other tools :
circos v0.69-3

All the code developped is available in the supplementary material as .html files and hosted on Zenodo (https://zenodo.org, DOI : 10.5281/zenodo.1493872)

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/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

The data and code that support the findings of this study are available from SRA/GEO (Expression data = BioProject PRJNA439263, superserie GSE144397; Sequencing data = BioProject PRJNA439280) and Zenodo (https://zenodo.org, DOI : 10.5281/zenodo.1493872). This archive collapses all the material (including processed data) required to reproduce figures presented 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	The sample size was chosen according to the dynamics of each of the senescence inducer and the quiescence process, which allowed us to capture the different phases of senescence (initiation, establishment and maintenance) for each inducer. Based on extensive literature, OIS of primary fibroblasts in culture occurs rapidly, typically 6-10 days for RAS (Serrano et al., Oncogenic ras Provokes Premature Cell Senescence

Associated with Accumulation of p53 and p16INK4a; Cell, 1997) and 3-5 days for RAF (Zhu et al., Senescence of human fibroblasts induced by oncogenic Raf; Genes and Development 1998 -- Jeanblanc et al; Parallel pathways in RAF-induced senescence and conditions for its reversion; Oncogene 31; 2011). By choosing 6 time points in 2 biologically independent time-series experiments for each OIS inducer, we were able to faithfully capture the general gene expression and chromatin remodeling dynamics. For RS, in contrast, the senescence process occurs with slower kinetics, spanning 3 months for this study, until no cell division occurred as previously described (Shah et al, Lamin B1 depletion in senescent cells triggers large-scale changes in gene expression and the chromatin landscape; Genes and Development 2013., Rai et al, HIRA orchestrates a dynamic chromatin landscape in senescence and is required for suppression of neoplasia; Genes and Development 2014). To capture the entire RS process, we chose 8 time points in 2 biologically independent time-series as described in the main text.

Data exclusions

No data were excluded.

Replication

All performed replications were successful. Similarity between replicates for high-throughput experiments was verified by performing hierarchical clustering and multidimensional scaling approaches such as PCA during the pre-processing. For CRISPRi studies, three independent experiments were performed that were subsequently analyzed by ANOVA and Dunnett's test as indicated in the corresponding figure legends. Flow cytometry experiments (3 independent replicates) are presented as mean +/- standard deviations of less than 20% as shown in Table S10 source data.

Randomization

Sample randomization was not relevant for this study as the main objective was to determine the chromatin-based mechanisms controlling the dynamics of the senescence program as well as to assess its relevance in pertinent disease-models.

Blinding

For high-throughput experiments, generation-acquisition and analysis of experimental data were performed independently by separate individuals (RMZ and PFR). Network analysis was performed independently by JAFR using data generated by RMZ. CRISPRi studies were performed independently by BS and JG. Animal studies were performed independently by DB, MM anad CAS. GSEA studies were performed independently by DB, MM and CAS.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used

Abcam: ab8895 Histone H3K4me1 lot# GR262515-1; ab4729 Histone H3K27ac lot# 150367-1. 5µg antibody per 1-5 million cells for each immunoprecipitation experiment.
Millipore: 07-449 Histone H3K27me3 pAb lot# 2382150; 07-473 Histone H3K4me3 pAb lot# 2384705. 5µg antibody per 1-5 million cells for each immunoprecipitation experiment.
R&D: MAB200 IL1a lot# GX2018071; MAB201 IL1b lot# AWE1118061. 1:100 dilution for microscopy studies.
Santa Cruz: sc-1694 X c-Jun lot# F2916; sc-RELA lot# E0416; sc-604 X Fra 2 lot# G0915. 5µg antibody per 1-5 million cells for each immunoprecipitation experiment.

Validation

Antibody validations were performed by antibody suppliers per quality assurance literature provided by each supplier for the applications used in this study (see links below). All antibodies are ChIP-seq certified by the ENCODE project (https://www.encodeproject.org/search/?type=AntibodyLot)
Abcam: 1. ab8895: https://www.abcam.com/histone-h3-mono-methyl-k4-antibody-chip-grade-ab8895.html
2. ab4729: https://www.abcam.com/histone-h3-acetyl-k27-antibody-chip-grade-ab4729.html
Millipore: 1. 07-449: https://www.emdmillipore.com/US/en/product/Anti-trimethyl-Histone-H3-Lys27-Antibody,MM_NF-07-449
2. 07-743: https://www.emdmillipore.com/US/en/product/Anti-trimethyl-Histone-H3-Lys4-Antibody,MM_NF-07-473
R&D: 1. MAB200: https://www.rndsystems.com/products/human-il-1alpha-il-1f1-antibody-4414_mab200
2. MAB201: https://www.rndsystems.com/products/human-il-1beta-il-1f2-antibody-8516_mab201
Santa Cruz: 1. sc-1694X: https://www.scbt.com/p/c-jun-antibody-h-79?requestFrom=search
2. sc-604X: https://www.scbt.com/p/fra-2-antibody-q-20?requestFrom=search
3. sc-372X: https://www.scbt.com/p/nfkappab-p65-antibody-c-20?requestFrom=search

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	primary normal human diploid fibroblasts strain WI38 from ECCAC. GM21 fibroblasts are primary normal human diploid foreskin fibroblasts and were obtained from Coriell Institure. HCT116 was a kind gift provided by A. Relogio. SW480 ACC-313 was obtained from DSMZ. HEK293T cells were from ATCC.
Authentication	ECCAC: Cell count, viability, plating efficiency, Morphology check, Sterility Testing; Mycoplasma Testing by: PCR, DNA Hoechst Stain and culture Isolation. Authentication by: STR Profiling (human cell lines), DNA Barcoding (speciation). ATCC: STR profiling, cell morphology, COI testing. DSMZ: STR profiling. Coriell: STR profiling, cytogenetics.
Mycoplasma contamination	All tested and negative.
Commonly misidentified lines (See ICLAC register)	Not commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Transgenic Eμ-myc mice which spontaneously develop B-cell lymphomas were used for the generation of explated lymphoma cells. Lymphomas with defined genetic defects were generated by intercrossing Eμ-myc transgenic mice to mice carrying loss-of-function alleles at the Suv39h1 locus. 6–8-week-old C57BL/6 ("wild type") female mice were used as recipients for in vivo lymphoma propagation.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal protocols used in this study were approved by the governmental review board (Landesamt Berlin), and conform to the respective regulatory standards.

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

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 *May remain private before publication.*	The data were realeased under SRP136103 on SRA.
Files in database submission	The following files are available : RASOIS_CHIPSEQ_HISTONE_T0_INPUT_REP1.fastq.gz RASOIS_CHIPSEQ_HISTONE_T0_INPUT_REP2.fastq.gz RASOIS_CHIPSEQ_HISTONE_T0_H3K4me1_REP1.fastq.gz RASOIS_CHIPSEQ_HISTONE_T0_H3K4me1_REP2.fastq.gz RASOIS_CHIPSEQ_HISTONE_T0_H3K4me3_REP1.fastq.gz RASOIS_CHIPSEQ_HISTONE_T0_H3K4me3_REP2.fastq.gz RASOIS_CHIPSEQ_HISTONE_T0_H3K27ac_REP1.fastq.gz RASOIS_CHIPSEQ_HISTONE_T0_H3K27ac_REP2.fastq.gz RASOIS_CHIPSEQ_HISTONE_T0_H3K27me3_REP1.fastq.gz RASOIS_CHIPSEQ_HISTONE_T0_H3K27me3_REP2.fastq.gz RASOIS_CHIPSEQ_HISTONE_72H_INPUT_REP1.fastq.gz RASOIS_CHIPSEQ_HISTONE_72H_INPUT_REP2.fastq.gz RASOIS_CHIPSEQ_HISTONE_72H_H3K4me1_REP1.fastq.gz RASOIS_CHIPSEQ_HISTONE_72H_H3K4me1_REP2.fastq.gz RASOIS_CHIPSEQ_HISTONE_72H_H3K4me3_REP1.fastq.gz RASOIS_CHIPSEQ_HISTONE_72H_H3K4me3_REP2.fastq.gz RASOIS_CHIPSEQ_HISTONE_72H_H3K27ac_REP1.fastq.gz RASOIS_CHIPSEQ_HISTONE_72H_H3K27ac_REP2.fastq.gz RASOIS_CHIPSEQ_HISTONE_72H_H3K27me3_REP1.fastq.gz RASOIS_CHIPSEQ_HISTONE_72H_H3K27me3_REP2.fastq.gz RASOIS_CHIPSEQ_HISTONE_144H_INPUT_REP1.fastq.gz RASOIS_CHIPSEQ_HISTONE_144H_INPUT_REP2.fastq.gz

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WI38_RS_CHIPSEQ_HISTONE_D11_INPUT_REP1_TRIMMED.fastq
WI38_RS_CHIPSEQ_HISTONE_D11_INPUT_REP2_TRIMMED.fastq
WI38_RS_CHIPSEQ_HISTONE_D42_H3K27ac_REP1_TRIMMED.fastq
WI38_RS_CHIPSEQ_HISTONE_D42_H3K27ac_REP2_TRIMMED.fastq
WI38_RS_CHIPSEQ_HISTONE_D42_H3K4me1_REP1_TRIMMED.fastq
WI38_RS_CHIPSEQ_HISTONE_D42_H3K4me1_REP2_TRIMMED.fastq
WI38_RS_CHIPSEQ_HISTONE_D42_INPUT_REP1_TRIMMED.fastq
WI38_RS_CHIPSEQ_HISTONE_D42_INPUT_REP2_TRIMMED.fastq
WI38_RS_CHIPSEQ_HISTONE_D42_INPUT_REP3_TRIMMED.fastq
WI38_RS_CHIPSEQ_HISTONE_D88_H3K27ac_REP1_TRIMMED.fastq
WI38_RS_CHIPSEQ_HISTONE_D88_H3K27ac_REP2_TRIMMED.fastq
WI38_RS_CHIPSEQ_HISTONE_D88_H3K4me1_REP1_TRIMMED.fastq
WI38_RS_CHIPSEQ_HISTONE_D88_H3K4me1_REP2_TRIMMED.fastq
WI38_RS_CHIPSEQ_HISTONE_D88_INPUT_REP1_TRIMMED.fastq
WI38_RS_CHIPSEQ_HISTONE_D88_INPUT_REP2_TRIMMED.fastq
WI38_RS_CHIPSEQ_HISTONE_D88_INPUT_REP3_TRIMMED.fastq

Genome browser session
(e.g. UCSC)

No longer applicable

Methodology

Replicates

TF ChIP-seq :
For each transcription factor, we generated 4 libraries : 2 corresponding to immuno-precipitated material from MNase-digested chromatin prepared from two different biological replicate, and 2 corresponding matched MNase-digested input libraries.

Histone modification ChIP-seq :
For each time point of the RAS-OIS time-series, we generated 10 libraries: 8 corresponding to immuno-precipitated material from sonicated chromatin using antibodies directed against H3K4me1, H3K4me3, H3K27ac or H3K27me3 each in duplicates, and 2 corresponding input libraries. For each time point of the RAF-OIS and RS time-series, we generated 6 libraries: 4 corresponding to immunoprecipitated material from sonicated chromatin using antibodies against H3K4me1 and H3K27ac, and their corresponting input libraries.

ATAC-seq :
For each time point, we generated 3 libraries, each from different biological replicates.

Sequencing depth

The tables below provides information about layout (paired- or single-end), read length, number of ready-to-analyze reads (after filtering and adapter trimming) and ready-for-peak-calling alignments (de-duplicated, uniquely mapped and not in Encode blacklisted regions) for histone ChIP-seq, TF ChIP-seq and ATAC-seq experiments.

ChiP-seq (histone modification)

Treatment Time_point Type Replicate SRA_Accession_Number Layout Length Reads
RAS OIS T0 Input Rep_1 SAMN08743361 Single-end 65 38041601

RAS OIS T0 Input Rep_2 SAMN08743362 Single-end 65 47309670
RAS OIS T0 H3K4Me1 Rep_1 SAMN08743363 Single-end 65 37856793
RAS OIS T0 H3K4Me1 Rep_2 SAMN08743364 Single-end 65 39292372
RAS OIS T0 H3K4Me3 Rep_1 SAMN08743365 Single-end 65 30849188
RAS OIS T0 H3K4Me3 Rep_2 SAMN08743366 Single-end 65 45059087
RAS OIS T0 H3K27Ac Rep_1 SAMN08743367 Single-end 65 43389139
RAS OIS T0 H3K27Ac Rep_2 SAMN08743368 Single-end 65 50108745
RAS OIS T0 H3K27Me3 Rep_1 SAMN08743369 Single-end 65 54520066
RAS OIS T0 H3K27Me3 Rep_2 SAMN08743370 Single-end 65 56634913
RAS OIS 72h Input Rep_1 SAMN08743371 Single-end 65 60506056
RAS OIS 72h Input Rep_2 SAMN08743372 Single-end 65 38735564
RAS OIS 72h H3K4Me1 Rep_1 SAMN08743373 Single-end 65 42497957
RAS OIS 72h H3K4Me1 Rep_2 SAMN08743374 Single-end 65 54511957
RAS OIS 72h H3K4Me3 Rep_1 SAMN08743375 Single-end 65 31470884
RAS OIS 72h H3K4Me3 Rep_2 SAMN08743376 Single-end 65 63652572
RAS OIS 72h H3K27Ac Rep_1 SAMN08743377 Single-end 65 34417706
RAS OIS 72h H3K27Ac Rep_2 SAMN08743378 Single-end 65 41815374
RAS OIS 72h H3K27Me3 Rep_1 SAMN08743379 Single-end 65 60568987
RAS OIS 72h H3K27Me3 Rep_2 SAMN08743380 Single-end 65 45966097
RAS OIS 144h Input Rep_1 SAMN08743381 Single-end 65 30312041
RAS OIS 144h Input Rep_2 SAMN08743382 Single-end 65 37208783
RAS OIS 144h H3K4Me1 Rep_1 SAMN08743383 Single-end 65 28479562
RAS OIS 144h H3K4Me1 Rep_2 SAMN08743384 Single-end 65 40722520
RAS OIS 144h H3K4Me3 Rep_1 SAMN08743385 Single-end 65 34502546
RAS OIS 144h H3K4Me3 Rep_2 SAMN08743386 Single-end 65 33919183
RAS OIS 144h H3K27Ac Rep_1 SAMN08743387 Single-end 65 53361402
RAS OIS 144h H3K27Ac Rep_2 SAMN08743388 Single-end 65 53146990
RAS OIS 144h H3K27Me3 Rep_1 SAMN08743389 Single-end 65 63127594
RAS OIS 144h H3K27Me3 Rep_2 SAMN08743390 Single-end 65 45024907
RAF OIS - Input Rep_1 Single-end 65 59510224
RAF OIS - Input Rep_2 Single-end 65 117108119
RAF OIS T0 H3K4Me1 Rep_1 Single-end 65 69571606
RAF OIS T0 H3K4Me1 Rep_2 Single-end 65 82518823
RAF OIS T0 H3K27Ac Rep_1 Single-end 65 56813680
RAF OIS T0 H3K27Ac Rep_2 Single-end 65 83474318
RAF OIS 48h H3K4Me1 Rep_1 Single-end 65 65728026
RAF OIS 48h H3K4Me1 Rep_2 Single-end 65 57284371
RAF OIS 48h H3K27Ac Rep_1 Single-end 65 66921718
RAF OIS 48h H3K27Ac Rep_2 Single-end 65 62712228
RAF OIS 96h H3K4Me1 Rep_1 Single-end 65 62383811
RAF OIS 96h H3K4Me1 Rep_2 Single-end 65 79081036
RAF OIS 96h H3K27Ac Rep_1 Single-end 65 52809866
RAF OIS 96h H3K27Ac Rep_2 Single-end 65 54812142
RS T0 Input Rep_1 Single-end 65 45048693
RS T0 Input Rep_2 Single-end 65 59034798
RS T0 H3K4Me1 Rep_1 Single-end 65 46682721
RS T0 H3K4Me1 Rep_2 Single-end 65 56317096
RS T0 H3K27Ac Rep_1 Single-end 65 49373674
RS T0 H3K27Ac Rep_2 Single-end 65 77489732
RS 264h Input Rep_1 Single-end 65 56068446
RS 264h Input Rep_2 Single-end 65 65477269
RS 264h H3K4Me1 Rep_1 Single-end 65 46968814
RS 264h H3K4Me1 Rep_2 Single-end 65 60407389
RS 264h H3K27Ac Rep_1 Single-end 65 54612553
RS 264h H3K27Ac Rep_2 Single-end 65 78433916
RS 1008h Input Rep_1 Single-end 65 59626225
RS 1008h Input Rep_2 Single-end 65 74534847
RS 1008h H3K4Me1 Rep_1 Single-end 65 54023390
RS 1008h H3K4Me1 Rep_2 Single-end 65 69148000
RS 1008h H3K27Ac Rep_1 Single-end 65 45453465
RS 1008h H3K27Ac Rep_2 Single-end 65 55334140
RS 2112h Input Rep_1 Single-end 65 62480209
RS 2112h Input Rep_2 Single-end 65 67410952
RS 2112h H3K4Me1 Rep_1 Single-end 65 57473640
RS 2112h H3K4Me1 Rep_2 Single-end 65 56444643
RS 2112h H3K27Ac Rep_1 Single-end 65 58651190
RS 2112h H3K27Ac Rep_2 Single-end 65 46982957

ChiP-seq (TFs)

Time_point Type Replicate Layout Length Reads Alignments
T0 Input Rep_1 Single-end 65 42840252 35011622
T0 Input Rep_2 Single-end 65 34500046 28307013
T0 FOSL2 Rep_1 Single-end 65 55715710 31545379
T0 FOSL2 Rep_2 Single-end 65 35034473 20466185

144h Input Rep_1 Single-end 65 75634598 59880455
144h Input Rep_2 Single-end 65 59306037 47325250
144h RELA Rep_1 Single-end 65 37551904 24333615
144h RELA Rep_2 Single-end 65 44850364 33750411
144h JUN Rep_1 Single-end 65 40945212 26473673
144h JUN Rep_2 Single-end 65 32274528 19167796

ATAC-seq
Treatment Time_point Replicate Layout Length Reads Alignments
RAS OIS T0 Rep_1 Paired-end 2x101 74534822 39804932
RAS OIS T0 Rep_2 Paired-end 2x101 46122085 17544844
RAS OIS T0 Rep_3 Paired-end 2x101 38712979 12711927
RAS OIS 24h Rep_1 Paired-end 2x101 63306191 29332882
RAS OIS 24h Rep_2 Paired-end 2x101 43307168 25032681
RAS OIS 24h Rep_3 Paired-end 2x101 35181249 16625638
RAS OIS 48h Rep_1 Paired-end 2x101 60920142 30598003
RAS OIS 48h Rep_2 Paired-end 2x101 43819122 23073114
RAS OIS 48h Rep_3 Paired-end 2x101 45120149 17365235
RAS OIS 72h Rep_1 Paired-end 2x101 55433753 30551768
RAS OIS 72h Rep_2 Paired-end 2x101 47996427 17792443
RAS OIS 72h Rep_3 Paired-end 2x101 30462130 13121630
RAS OIS 96h Rep_1 Paired-end 2x101 52450780 32405688
RAS OIS 96h Rep_2 Paired-end 2x101 39834082 16115231
RAS OIS 96h Rep_3 Paired-end 2x101 30172425 17857274
RAS OIS 144h Rep_1 Paired-end 2x101 61205776 23149209
RAS OIS 144h Rep_2 Paired-end 2x101 57408561 19270130
RAS OIS 144h Rep_3 Paired-end 2x101 39939712 19270130
RAF OIS T0 Rep_1 Paired-end 2x101 26297748 14551180
RAF OIS T0 Rep_2 Paired-end 2x101 26817643 14287781
RAF OIS 12h Rep_1 Paired-end 2x101 29267962 10408678
RAF OIS 12h Rep_2 Paired-end 2x101 31414016 10645220
RAF OIS 24h Rep_1 Paired-end 2x101 35312617 8045152
RAF OIS 24h Rep_2 Paired-end 2x101 35235884 14121641
RAF OIS 48h Rep_1 Paired-end 2x101 28574637 15278426
RAF OIS 48h Rep_2 Paired-end 2x101 47686021 20395405
RAF OIS 72h Rep_1 Paired-end 2x101 39422022 19988101
RAF OIS 72h Rep_2 Paired-end 2x101 36094240 20978858
RAF OIS 96h Rep_1 Paired-end 2x101 48186884 18071059
RAF OIS 96h Rep_2 Paired-end 2x101 26334924 14285924
RS T0 Rep_1 Paired-end 2x101 91177629 51493139
RS T0 Rep_2 Paired-end 2x101 72154211 39009043
RS 144h Rep_1 Paired-end 2x101 71995259 37128433
RS 144h Rep_2 Paired-end 2x101 55514354 30809657
RS 264h Rep_1 Paired-end 2x101 66136812 24124425
RS 264h Rep_2 Paired-end 2x101 42269183 19146787
RS 432h Rep_1 Paired-end 2x101 78244574 43206109
RS 432h Rep_2 Paired-end 2x101 51179557 26928449
RS 624h Rep_1 Paired-end 2x101 72994913 39904342
RS 624h Rep_2 Paired-end 2x101 71198400 37646575
RS 792h Rep_1 Paired-end 2x101 89290229 47648487
RS 792h Rep_2 Paired-end 2x101 65137444 33909314
RS 1008h Rep_1 Paired-end 2x101 50860918 26647540
RS 1008h Rep_2 Paired-end 2x101 88035448 44748971
RS 1368h Rep_1 Paired-end 2x101 78526407 29045817
RS 1368h Rep_2 Paired-end 2x101 70776924 30060238
RS 2112h Rep_1 Paired-end 2x101 54652255 27609161
RS 2112h Rep_2 Paired-end 2x101 67278185 26232430
ESCAPE_ATAC_pBABE_D8_REP1_R1_TRIMMED_2.fastq 116586303* 45511000* *Total reads, addition of R1 and R2
ESCAPE_ATAC_pBABE_D8_REP1_R2_TRIMMED_2.fastq
ESCAPE_ATAC_pBABE_D8_REP3_R1_TRIMMED_2.fastq 85493815* 24126700*
ESCAPE_ATAC_pBABE_D8_REP3_R2_TRIMMED_2.fastq
ESCAPE_ATAC_RAS_D8_REP1_R1_TRIMMED_2.fastq 44243235* 18454900*
ESCAPE_ATAC_RAS_D8_REP1_R2_TRIMMED_2.fastq
ESCAPE_ATAC_RAS_D8_REP3_R1_TRIMMED_2.fastq 83418999* 27419700*
ESCAPE_ATAC_RAS_D8_REP3_R2_TRIMMED_2.fastq
ESCAPE_ATAC_RAS_D14_REP1_R1_TRIMMED_2.fastq 53178385* 22577345*
ESCAPE_ATAC_RAS_D14_REP1_R2_TRIMMED_2.fastq
ESCAPE_ATAC_RAS_D14_REP3_R1_TRIMMED_2.fastq 197047170* 79025100*
ESCAPE_ATAC_RAS_D14_REP3_R2_TRIMMED_2.fastq

Antibodies

Abcam: ab8895 Histone H3K4me1 lot# GR262515-1; ab4729 Histone H3K27ac lot#150367-1
Millipore: 07-449 Histone H3K27me3 pAb lot # 2382150; 07-473 Histone H3K4me3 pAb lot#2384705
Santa Cruz: sc-1694 X c-Jun lot# F2916; sc-RELA lot# E0416; sc-604 X Fra 2 lot# G0915

Peak calling parameters

Below, we give the command lines and parameters used to process ChIP-seq data. Note : the IDR pipeline (generation of

Peak calling parameters

self-pseudo-replicates, pooled pseudo-replicates) is not described here.

Read filtering and trimming :
fastq-mcf -C 500000 -q 30 -l 25 -S --qual-mean 30 -max-ns 1 -o out.fastq ILLUMINA_PRIMER_INDEX_N.fasta in.fastq

Alignments with best-match parameters :
bowtie -v 2 -m 1 --best --strata --S mm10.fa in.fastq | samtools view -Sb - > out.bam

Sort aligments :
samtools sort in.bam -o out.bam -T in.tmp

Remove PCR / optical duplicates :
java -jar picard.jar MarkDuplicates REMOVE_DUPLICATES=FALSE METRICS_FILE=out.txt INPUT=in.bam OUTPUT=out.bam
samtools view -h -F 0x400 in.bam | samtools view -hSb - -o out.bam

Remove alignments in Encode blacklisted regions :
intersectBed -v -abam in.bam -b encode_blacklist_mm10.bed > out.bam

Compute the average fragments length :
Rscript run_spp_nodups.R -rf -c=in.bam -odir=./ -savp=out.pdf -out=out.txt -s=-100:5:1500 -x=-500:85

Peak calling (NOTE : the IDR pipeline is not detailed here) :
macs2 callpeak --tempdir ./ -t in_chip.bam -c in_control.bam --name out --nomodel --extsize 100 --gsize mm -p 1e-1

Data quality

To ensure data quality we used the following approaches :
- Follow the percentage of discarded reads at each step of the pipeline (% of adapter contamination, % of low quality reads, % of ambiguously mapped reads, % of optical / PCR duplicates, % of alignments inside Encode blacklisted regions, potential cross-contamination) using FastQC and FastqScreen.
- Compare the in silico estimated fragment length distribution profiles obtained with spp with the electropherogram obtained with the Tapestation.
- Evaluate the closeness of peaks set obtained for biological replicated with the IDR pipeline.
- Evaluate the consistency of biological replicate using unsupervised / multivariate analytical strategies (principal component analysis, hierarchical bi-clustering, genome-wide pair-wise correlation computation).
- Evaluate the library saturation after peak calling.

Software

To process / analyze our ChIP-seq we used the following tools :
fastq-mcf v1.04.803, bowtie v1.1.1, samtools v1.6, picardtools v1.130, bedtools 2.19.1, spp v1.10.1, macs v2.1.0, idr v1.0, custom R scripts and Bioconductor packages (Gviz, ComplexHeatmap, ChIPpeakAnno, preseqR).

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Senescence was assessed two days after drug removal by SABG activity and standard cell cycle analysis using 5-bromo-2'-deoxyuridine (FITC Mouse Anti-BrdU antibody, Clone B44, BD Biosciences)/propidium iodide (BrdU/PI)-based flow cytometric measurement (FACS Calibur™, BD Biosciences) as described previously (refs 40, 42).

Instrument

The data were acquired using FACS Calibur™ (BD Biosciences).

Software

Flow cytometry data was analysed using FlowJo™ V10.2 (Becton, Dickinson and Company).

Cell population abundance

Lymphoma cells coexpressing cJun4A and GFP or expressing GFP only were sorted for GFP+ signal using a BioRad S3e cell sorter. The sorting method reaches above 95% abundance of the sorted cell population in the post-sort fraction.
The purity (% GFP+) of the samples was determined before the senescence-induction experiments by flow cytometry using the Guava easycyte 12HT system. Dead cells were excluded by propidium iodide staining. The % GFP+ cells among PI- cells were as follows: Lymphoma #1 empty vector 98.9%, lymphoma #1 cJun4A 82.6%; lymphoma #2 empty vector 99.8%, lymphoma #2 cJuna4A 95.2%; lymphoma #3 empty vector 99.9%, lymphoma #3 cJun4A 97.3%

Gating strategy	Single 2n-4n nuclei populations were gated via an FL2-Area vs. FL2-Width plot (FL2 meausring Propidium Iodide fluorescence). Cell cycle phase fractions were clearly separable.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.