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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.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	No software was used to collect data.
Data analysis	Prism 7 FlowJo v10 Microsoft Excel v14.5.5 GSEA (Java desktop version) IGV v2.3.57 CellProfiler v2.2.0 R v3.2.3 R package ggplot v2.2.1 R package heatmap3 RNASeq: Tophat version 2.0.10 Samtools version 0.1.18 htseq version 0.5.3p9 R package - DESeq2 version 1.10.1 R package - GenomicAlignments version 1.6.3 R package - GOseq v1.22.0

R package - edgeR (v3.16.5)
R package - limma (v3.30.12)

ChIPseq/GROSeq/ATAC-Seq/4C/5C:
R package ChIPQC v1.4.4
bowtie version 0.12.8
Samtools version 0.1.18
Picard version 1.90
MACS2 2.0.10
Homer v4.9.1
ROSE
R version 3.2.3
R package FASTQC v0.9.4
R package - rtracklayer version 1.30.4
R package - Rsamtools version 1.22.0
R package - GenomicRanges version 1.22.4
R package - DESeq2 version 1.10.1
R package - GenomicAlignments version 1.6.3
R package - Soggi version 1.8.0
R package - GenomicInteractions v1.7.1
R package - Gviz v.1.18.2
FASTX- toolkit
Novoalign v3
4CseqPipe software suite version 0.7
my5C software

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 upon request. 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 generated for this study has been deposited at GEO under accession GSE108599.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	No statistical test was used to determine sample size. Three or more biological replicates per group (as indicated in the figure legends) were used to allow statistical inference. In the case of genome-wide sequenced experiments (GRO-seq, ATAC-seq, ChIP-seq) two biological replicates per group were used according to current convention.
Data exclusions	No data were excluded except for standard quality control filtering during sequencing analysis. This includes filtering genes and enhancers with low read counts due to their low statistical power using a DESeq2 independent filtering approach. Standard filtering also involves removing reads aligning to multiple positions and duplicate reads, in order to exclude PCR bias. In the case of the human AML RNA-seq analysis, lowly expressed genes were removed if CPM was smaller than 1 in one of all group samples. In the GRO-seq analysis, analysis of quality of reads indicated that quality of the last 10bp at the 3' end was lower. Hence the last 10bp were excluded from the alignment. Finally, in order to define super-enhancers based on H3K27ac signal, promoter regions were excluded because they also could contain this histone mark and be misinterpreted as enhancers.
Replication	Replication attempts were successful and the results are represented as mean +- SEM as indicated.
Randomization	No randomization was required. Mice used for bone marrow extraction were chosen according to genotype.
Blinding	Investigators were not blinded to group allocation. We considered that blinding was not relevant to this study because we used quantitative assays for all experiments and the computational framework was identical for all samples and replicates.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used

Immunoblot: RAD21 (Abcam ab992) used at 1:1000 dilution, beta-Actin (Santa Cruz sc-69879) used at 1:10000 dilution, c-MYC (Santa Cruz sc-40 9E10) used at 1:1000 dilution. As secondary antibodies we used fluorescent (Alexa Fluor 680) goat anti-rabbit IgG and goat anti-mouse IgG at 1:10000 dilution (Life technologies A21109 and A21057). ChIP: anti-H3 (Abcam ab1791), anti-H3K27ac (Abcam ab4729) and Rad21 (Abcam ab992). For each IP, 1.5 ug of primary antibody was bound to Dynabeads protein G (Invitrogen 10004D) for incubation with sonicated extract. HSPC sorting: Sca-1-BV510 (BD 565507), cKit-PE (eBioscience 12-1171-81), CD16-APC (eBioscience 17-0161-81), CD34-FITC (eBioscience 11-0341-81), SAV-e450 (48-4317-82). HSPC staining: Sca-1-FITC (Biolegend 122505), cKit-Alexa Fluor 700 (eBioscience 56-1172-80), CD11b-APC-Cy7 (BD 557657) and CD16-BV605 (BD 563006). Antibodies for staining and sorting HSPC were used at 1:200. Macrophage staining: CD11b-FITC (BD 01714D) used at 1:100 dilution, F4/80-PE (eBioscience 12-4801-80) used at 1:200 dilution and anti-Mouse Vα 11.1, 11.2 TCR and Vα2 TCR (BD) were used as isotype controls.

Validation

All antibodies are commercially available and have been tested in mouse for the experiments they were used.

Animals and other organisms

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

Laboratory animals

Specific pathogen free male and female laboratory-bred mice of the indicated genotype on a mixed B6/129 background were used at 6-10 weeks of age.

Wild animals

No wild animals involved.

Field-collected samples

The study did not involve collecting samples from the field.

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.

GSE108599
reviewers token to access the data: spszqeogzlqnhqn
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE108599

Files in database submission

GSM2905558 WT_unstimulated_Rad21_ChIP_rep1
GSM2905559 WT_LPS_1h_Rad21_ChIP_rep1
GSM2905560 WT_LPS_6h_Rad21_ChIP_rep1
GSM2905561 WT_unstimulated_Rad21_ChIP_rep2
GSM2905562 WT_LPS_1h_Rad21_ChIP_rep2
GSM2905563 WT_LPS_6h_Rad21_ChIP_rep2
GSM2905564 WT_unstimulated_Rad21_input_rep1
GSM2905565 WT_LPS_1h_Rad21_input_rep1
GSM2905566 WT_LPS_6h_Rad21_input_rep1
GSM2905567 WT_unstimulated_Rad21_input_rep2
GSM2905568 WT_LPS_1h_Rad21_input_rep2
GSM2905569 WT_LPS_6h_Rad21_input_rep2
GSM2913383 Rad21-/-_unstimulated_H3_ChIP_rep1
GSM2913384 Rad21-/-_LPS_1h_H3_ChIP_rep1

GSM2913385 Rad21-/-_LPS_6h_H3_ChIP_rep1
GSM2913386 WT_unstimulated_H3_ChIP_rep1
GSM2913387 WT_LPS_1h_H3_ChIP_rep1
GSM2913388 WT_LPS_6h_H3_ChIP_rep1
GSM2913389 Rad21-/-_unstimulated_H3_ChIP_rep2
GSM2913390 Rad21-/-_LPS_1h_H3_ChIP_rep2
GSM2913391 Rad21-/-_LPS_6h_H3_ChIP_rep2
GSM2913392 WT_unstimulated_H3_ChIP_rep2
GSM2913393 WT_LPS_1h_H3_ChIP_rep2
GSM2913394 WT_LPS_6h_H3_ChIP_rep2
GSM2913395 Rad21-/-_unstimulated_H3_K27Ac_input_rep1
GSM2913396 Rad21-/-_LPS_1h_H3_K27Ac_input_rep1
GSM2913397 Rad21-/-_LPS_6h_H3_K27Ac_input_rep1
GSM2913398 WT_unstimulated_H3_K27Ac_input_rep1
GSM2913399 WT_LPS_1h_H3_K27Ac_input_rep1
GSM2913400 WT_LPS_6h_H3_K27Ac_input_rep1
GSM2913401 Rad21-/-_unstimulated_H3_K27Ac_input_rep2
GSM2913402 Rad21-/-_LPS_1h_H3_K27Ac_input_rep2
GSM2913403 Rad21-/-_LPS_6h_H3_K27Ac_input_rep2
GSM2913404 WT_unstimulated_H3_K27Ac_input_rep2
GSM2913405 WT_LPS_1h_H3_K27Ac_input_rep2
GSM2913406 WT_LPS_6h_H3_K27Ac_input_rep2
GSM2913407 Rad21-/-_unstimulated_H3K27Ac_ChIP_rep1
GSM2913408 Rad21-/-_LPS_1h_H3K27Ac_ChIP_rep1
GSM2913409 Rad21-/-_LPS_6h_H3K27Ac_ChIP_rep1
GSM2913410 WT_unstimulated_H3K27Ac_ChIP_rep1
GSM2913411 WT_LPS_1h_H3K27Ac_ChIP_rep1
GSM2913412 WT_LPS_6h_H3K27Ac_ChIP_rep1
GSM2913413 Rad21-/-_unstimulated_H3K27Ac_ChIP_rep2
GSM2913414 Rad21-/-_LPS_1h_H3K27Ac_ChIP_rep2
GSM2913415 Rad21-/-_LPS_6h_H3K27Ac_ChIP_rep2
GSM2913416 WT_unstimulated_H3K27Ac_ChIP_rep2
GSM2913417 WT_LPS_1h_H3K27Ac_ChIP_rep2
GSM2913418 WT_LPS_6h_H3K27Ac_ChIP_rep2

Genome browser session
(e.g. UCSC)

Not available.

Methodology

Replicates

Two biological replicates were performed per experiment.

Sequencing depth

ChIP-Seq libraries were sequenced along with input libraries as single end 50bp reads. Reads were aligned to mouse genome mm9 using Bowtie version 0.12.8 with default parameters. Reads aligning to multiple positions in the genome were discarded from the analysis.

Sample Total Reads Aligned Reads Aligned Reads% Duplicates Duplicates%
KO0 H3 Rep1 22609822 15305559 67.69429233 1071078 6.997967209
KO0 H3 Rep2 27119309 18032998 66.49504971 1148925 6.371236774
KO0 Input Rep1 33288347 23591678 70.87068036 1285842 5.450405011
KO0 Input Rep2 31358682 22124333 70.55249643 1385029 6.260206805
KO0 K27 Rep1 25278150 21875600 86.53956085 2317774 10.59524767
KO0 K27 Rep2 27781144 23593503 84.92631909 2787525 11.81479918
KO1 H3 Rep1 25387465 16905434 66.58968905 1258427 7.443920103
KO1 H3 Rep2 40703436 27296325 67.06147609 2138808 7.83551632
KO1 Input Rep1 29805767 21093075 70.76843552 1113557 5.279253973
KO1 Input Rep2 37127228 26389556 71.07871344 1985574 7.524090212
KO1 K27 Rep1 29849706 25585312 85.71378224 2767563 10.81699922
KO1 K27 Rep2 28677463 24273019 84.64144475 3002956 12.37158015
KO6 H3 Rep1 31154307 21193449 68.02734851 1417149 6.686731357
KO6 H3 Rep2 21022077 13748800 65.4017203 808317 5.879182183
KO6 Input Rep1 22672509 16028248 70.69463728 808907 5.046758698
KO6 Input Rep2 34350009 24004428 69.88186815 1513077 6.303324537
KO6 K27 Rep1 27106962 23415327 86.38122929 2600942 11.1078611
KO6 K27 Rep2 21982406 18628349 84.74208419 1918015 10.29621573
WT0 H3 Rep1 28664543 19819582 69.14319897 1441557 7.273397592
WT0 H3 Rep2 29712633 20463358 68.87090081 1429656 6.986419335
WT0 Input Rep1 24275431 17342299 71.43971615 865354 4.989845925
WT0 Input Rep2 39174296 28740889 73.36670198 1745459 6.073086327
WT0 K27 Rep1 21350236 18711541 87.64090945 2041258 10.90908547
WT0 K27 Rep2 30454900 26496451 87.00225908 3333386 12.58049993
WT1 H3 Rep1 31286852 21357477 68.2634258 1566038 7.332504677
WT1 H3 Rep2 45213347 30459749 67.36893201 2797620 9.184645612
WT1 Input Rep1 41142016 30028759 72.98805921 1730887 5.764097677
WT1 Input Rep2 22939630 16515463 71.99533297 906780 5.490490942

WT1 K27 Rep1 27223264 23657327 86.90114088 2515333 10.6323635
WT1 K27 Rep2 36933781 31620977 85.61532598 3841506 12.14859996
WT6 H3 Rep1 25821798 17739240 68.69870177 1239530 6.987503411
WT6 H3 Rep2 32259224 21773607 67.49575563 1475326 6.775753783
WT6 Input Rep1 28525927 20282270 71.10117754 1141329 5.627225158
WT6 Input Rep2 29495520 21047517 71.35835205 1404818 6.674507021
WT6 K27 Rep1 34666003 29973863 86.46472165 3722803 12.42016419
WT6 K27 Rep2 32982649 28158509 85.37370361 3513445 12.47738295
Sample Name Total Reads Aligned Reads Aligned Reads % Duplicates Reads Duplicate %
WT0 Rad21 Rep1 48273783 35238650 72.99749017 4012543 11.3868
WT1 Rad21 Rep1 56406004 39204817 69.50468783 4217228 10.7569
WT6 Rad21 Rep1 109991223 76631453 69.67051635 6167779 8.0486
WT0 Input Rep1 59364171 41486953 69.88550889 2405021 5.7971
WT1 Input Rep1 80533810 55761614 69.24000491 3658310 6.5606
WT6 Input Rep1 67528746 45837468 67.87845283 3005451 6.5568
WT0 Rad21 Rep2 80939929 57562029 71.11697491 21695972 37.6915
WT1 Rad21 Rep2 33049137 25007481 75.66757643 1918994 7.6737
WT6 Rad21 Rep2 86963760 60705101 69.80505558 19573540 32.2436
WT0 Input Rep2 38490800 27276533 70.86507165 897809 3.2915
WT1 Input Rep2 18655009 13465319 72.18071565 373078 2.7707
WT6 Input Rep2 37297789 26057953 69.86460511 835909 3.2079

Antibodies

H3: ab1791 Abcam
H3K27ac: ab4729 Abcam
Rad21: ab992 Abcam

Peak calling parameters

ChIP-Seq Peaks were identified by MACS2 using input libraries using default parameters. RAD21 consensus peaks were derived by taking the intersection of RAD21 peaks identified in each biological replicate. The 3 time points contain 53762, 43710, 37037 peaks respectively (5%FDR).

Data quality

Reads were aligned to the mouse genome mm9 using Bowtie version 0.12.8 with default parameters. Reads aligning to multiple positions in the genome were discarded from the analysis. Quality of the ChIP-Seq libraries were assessed using ChIPQC . Duplicate reads were identified using Picard MarkDuplicates and excluded from the downstream analysis.

Software

R package ChIPQC v1.4.4
bowtie version 0.12.8
Samtools version 0.1.18
Picard version 1.90
MACS2 2.0.10
R version 3.2.3
R package - rtracklayer version 1.30.4
R package - Rsamtools version 1.22.0
R package - GenomicRanges version 1.22.4
R package - DESeq2 version 1.10.1

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

Bone marrow was extracted from femurs and tibiae using 25G needles and complete DMEM media. Total bone marrow cells, bone marrow derived macrophages and sorted HSPCs were stained with the corresponding antibodies diluted in PBS-2%FCS for 20 minutes in the dark at 4 degrees.

Instrument

FACSAria Fusion

Software

BD FACSDiva 8.0
FlowJo v10
Prism 7

Cell population abundance

HSPC populations were sorted from Lineage negative bone marrow cells. LSK population was usually 1-3% of the lineage

Cell population abundance	negative population. cKit+ Sca1- were ~30% of the lineage negative, and within this population CMP and GMP were usually 20-30% each.
Gating strategy	Cells were first gated for live cells (FSC/SSC) and gated to exclude doublets. To exclude lineage-positive cells, total bone marrow cells were first stained with biotin-lineage antibodies (CD4, CD8, B220, CD19, NK1.1, CD11b, Ter119, GR-1) and subsequently depleted with streptavidin beads (Miltenyi 130-048-102), further confirmed by streptavidin-negative gating (SAV-e450). LSK were Sca-1 and cKit positive. The Kit-positive Sca-1 negative population was gated, and from this gate CD16-intermediate CD34-positive were sorted as CMP, and CD16-high CD34-positive were sorted as GMP.

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