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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

BD FACSDiva (v.8.0.2) was used to collect flow cytometric data. Illumina HiSeq 2500 or HiSeq 4000 Systems were used to collect sequencing data, and BCL files were converted to FASTQ using bcl2fastq2 (v2.17.1.14, v2.18.0, or v2.20.0.422).

Data analysis

FlowJo (v10.5.3) was used for flow cytometric analysis. For bioinformatic analyses, the following software was used:
DESeq2 (v.1.14.1 or v.1.22.2)
Trimmomatic (v.0.36)
Bowtie2 (v.2.2.9 or v.2.3.4)
Salmon (v.0.10.2)
tximport (v.1.8.0 or v.1.10.1)
GenomicAlignments (v1.10.1 or v1.18.1)
MACS2 (v.2.1.1.20160309)
ChipPeakAnno (v.3.8.9)
HOMER (v.4.10.4)
limma (v.3.30.13)
UCSC mm10 Known Gene Annotation Package (v.9)
Irreproducible Discovery Rate (IDR) (v.2.0.2 or v2.0.3)
bedtools2 (v.2.26.0)
bedGraphToBigWig (v.4)
deepTools (v3.2.1)
Gviz (v.1.18.2)
ComplexHeatmap (v.1.18.1 or v.1.99.7)
Cytoscape (v.3.7.1)
R (v.3.3.3 or v.3.5.3)

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

Data generated in this study have been deposited in the Gene Expression Omnibus (GEO Super-Series accession number GSE140044 and and GSE164116).

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	Sample sizes were based on our prior experience in these mouse model systems, which typically require 4-5 mice per experimental group and time point for each treatment condition or genetically altered mouse strain compared with 4-5 control mice. In some cases, fewer control mice were used for experiments that were reproducible across independent experiments. Minimal sample size for human studies was chosen based on previous transcriptome studies on primary human NK cell populations (Schlums et al, Immunity 2015).
Data exclusions	None.
Replication	All experiments for non-high-throughput sequencing projects were performed on at least 3 biological replicates (individual mice). All high-throughput sequencing projects were performed on at least 2 biological replicates (individual mice or human donors).
Randomization	For human studies, experimental groups were not randomized across groups, as control and treatment groups had consisted of the same donors. Age- and sex-matched animals were assigned randomly to control and treatment group, or assigned according to genotype when applicable.
Blinding	For human studies, experiments were not performed in a blinded fashion, as this was not required for the experimental setup. All control and treatment conditions were performed with paired donor samples. For mouse studies, blinding was not performed since mice were grouped by treatment or genotype.

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	The following mouse antibodies were used for flow cytometry: CD3ε (17A2, Tonbo/BioLegend), TCRb (H57-597, Tonbo/BioLegend), CD19 (6D5, BioLegend), F4/80 (BM8, BioLegend), NK1.1 (PK136, BioLegend), Ly49H (3D10, eBioscience/BD Biosciences), CD69 (H1.2F3, BioLegend), pSTAT1 (pSer727, A15158B, BioLegend), pSTAT1 (pTyr701, 4a, BD Biosciences), pSTAT4 (pY693, 38/p-Stat4, BD Biosciences), pSTAT5 (pY694, C71E5, Cell Signaling Technology), and Fixable Viability Dye eFluor™ 506 (eBioscience). The following human antibodies were used for flow cytometry: CD3 (UCHT1, BD Biosciences), CD56 (N901, Beckman Coulter) and CD14 (M5E2, Becton Dickinson), CD57 (HCD57, Biolegend), and NKG2C (134591, R&D). The following mouse antibodies was used for magnetic bead depletion and were purchased from BioXCell: CD3ε (17A2), CD4 (GK1.5), CD8

	(2.43), Ter119 (TER-119), CD19 (1D3), Ly6G (1A8). The following antibodies were used for ChIP-Seq primarily reported in this study: anti-pSTAT5 (#AF2168, R&D Systems, lot# KVE0318071), anti-H3K4me3 (Millipore, 07-473, lot# 3018770).
Validation	Tonbo Biosciences tests all antibodies by flow cytometry. All flow cytometry antibodies from BioLegend, BD Biosciences, Beckman Coulter, Cell Signaling Technology, and R&D have been validated using either human or mouse primary cells or cell lines by flow cytometry. Purified antibodies from BioXCell, R&D, Millipore have been validated by western blot using mouse primary cells or cell lines or human cell lines.

Animals and other organisms

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

Laboratory animals	The following strains were used, all on the C57BL/6 genetic background: WT CD45.2 (The Jackson Laboratory), WT CD45.1 (B6.SJL; Taconic), Stat1–/–, NKp46-CreERT2 transgenic mice, and Rosa26-lox-STOP-lox-tdTomato.
Wild animals	No wild animals were used in this study.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All mice used in this study were bred and utilized at Memorial Sloan Kettering Cancer Center in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC).

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Peripheral blood was collected from male and female donors between the ages of 25-45.
Recruitment	Samples from healthy donor volunteers were collected at MSKCC. Selection bias may be present, representing only adults ages 25-45.
Ethics oversight	All samples were collected following approval by the MSKCC Institutional Review Board, and donors provided informed written consent.

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.

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE140043

Files in database submission

Paired fastq files, bigWig files (for IP samples only), and BED files of peak ranges are available for the following samples (sample names are preceded by GEO sample ID):

GSM4151996 ChIP_mouse_STAT1KO_UNSTIM_6_H3K4me3_input
GSM4151997 ChIP_mouse_STAT1KO_UNSTIM_6_H3K4me3_IP
GSM4151998 ChIP_mouse_STAT1KO_UNSTIM_9_H3K4me3_input
GSM4151999 ChIP_mouse_STAT1KO_UNSTIM_9_H3K4me3_IP
GSM4152000 ChIP_mouse_STAT1KO_IFNA_6_H3K4me3_input
GSM4152001 ChIP_mouse_STAT1KO_IFNA_6_H3K4me3_IP
GSM4152002 ChIP_mouse_STAT1KO_IFNA_9_H3K4me3_input
GSM4152003 ChIP_mouse_STAT1KO_IFNA_9_H3K4me3_IP
GSM4152004 ChIP_mouse_STAT1KO_IFNA_9a_H3K4me3_input
GSM4152005 ChIP_mouse_STAT1KO_IFNA_9a_H3K4me3_IP
GSM4152006 ChIP_mouse_WT_UNSTIM_1_H3K4me3_input
GSM4152007 ChIP_mouse_WT_UNSTIM_1_H3K4me3_IP
GSM4152008 ChIP_mouse_WT_UNSTIM_4_H3K4me3_input
GSM4152009 ChIP_mouse_WT_UNSTIM_4_H3K4me3_IP
GSM4152010 ChIP_mouse_WT_UNSTIM_3_H3K4me3_input
GSM4152011 ChIP_mouse_WT_UNSTIM_3_H3K4me3_IP
GSM4152012 ChIP_mouse_WT_UNSTIM_6_H3K4me3_input
GSM4152013 ChIP_mouse_WT_UNSTIM_6_H3K4me3_IP
GSM4152014 ChIP_mouse_WT_UNSTIM_7_H3K4me3_input
GSM4152015 ChIP_mouse_WT_UNSTIM_7_H3K4me3_IP
GSM4152016 ChIP_mouse_WT_UNSTIM_8_H3K4me3_input

GSM4152017 ChIP_mouse_WT_UNSTIM_8_H3K4me3_IP
GSM4152018 ChIP_mouse_WT_UNSTIM_9_H3K4me3_input
GSM4152019 ChIP_mouse_WT_UNSTIM_9_H3K4me3_IP
GSM4152020 ChIP_mouse_WT_UNSTIM_1_STAT5_input
GSM4152021 ChIP_mouse_WT_UNSTIM_1_STAT5_IP
GSM4152022 ChIP_mouse_WT_IFNA_3_H3K4me3_input
GSM4152023 ChIP_mouse_WT_IFNA_3_H3K4me3_IP
GSM4152024 ChIP_mouse_WT_IFNA_6_H3K4me3_input
GSM4152025 ChIP_mouse_WT_IFNA_6_H3K4me3_IP
GSM4152026 ChIP_mouse_WT_IFNA_7_H3K4me3_input
GSM4152027 ChIP_mouse_WT_IFNA_7_H3K4me3_IP
GSM4152028 ChIP_mouse_WT_IFNA_8_H3K4me3_input
GSM4152029 ChIP_mouse_WT_IFNA_8_H3K4me3_IP
GSM4152030 ChIP_mouse_WT_IFNA_9_H3K4me3_input
GSM4152031 ChIP_mouse_WT_IFNA_9_H3K4me3_IP
GSM4152032 ChIP_mouse_WT_IL12IL18_1_H3K4me3_input
GSM4152033 ChIP_mouse_WT_IL12IL18_1_H3K4me3_IP
GSM4152034 ChIP_mouse_WT_IL12IL18_7_H3K4me3_input
GSM4152035 ChIP_mouse_WT_IL12IL18_7_H3K4me3_IP
GSM4152036 ChIP_mouse_WT_IL12IL18_8_H3K4me3_input
GSM4152037 ChIP_mouse_WT_IL12IL18_8_H3K4me3_IP
GSM4152038 ChIP_mouse_WT_IL2IL15_4_H3K4me3_input
GSM4152039 ChIP_mouse_WT_IL2IL15_4_H3K4me3_IP
GSM4152040 ChIP_mouse_WT_IL2IL15_7_H3K4me3_input
GSM4152041 ChIP_mouse_WT_IL2IL15_7_H3K4me3_IP
GSM4152042 ChIP_mouse_WT_IL2IL15_8_H3K4me3_input
GSM4152043 ChIP_mouse_WT_IL2IL15_8_H3K4me3_IP
GSM4152044 ChIP_mouse_WT_IL2IL15_1_STAT5_input
GSM4152045 ChIP_mouse_WT_IL2IL15_1_STAT5_IP
GSM4152046 ChIP_mouse_WT_IL2IL15IL12IL18IFNA_7_H3K4me3_input
GSM4152047 ChIP_mouse_WT_IL2IL15IL12IL18IFNA_7_H3K4me3_IP
GSM4152048 ChIP_mouse_WT_IL2IL15IL12IL18IFNA_8_H3K4me3_input
GSM4152049 ChIP_mouse_WT_IL2IL15IL12IL18IFNA_8_H3K4me3_IP
GSM4152050 ChIP_mouse_WT_MCMVD0_2_H3K4me3_input
GSM4152051 ChIP_mouse_WT_MCMVD0_2_H3K4me3_IP
GSM4152052 ChIP_mouse_WT_MCMVD0_4_H3K4me3_input
GSM4152053 ChIP_mouse_WT_MCMVD0_4_H3K4me3_IP
GSM4152054 ChIP_mouse_WT_MCMVD2_1_H3K4me3_input
GSM4152055 ChIP_mouse_WT_MCMVD2_1_H3K4me3_IP
GSM4152056 ChIP_mouse_WT_MCMVD2_2_H3K4me3_input
GSM4152057 ChIP_mouse_WT_MCMVD2_2_H3K4me3_IP
GSM4152058 ChIP_mouse_WT_MCMVD2_3_H3K4me3_input
GSM4152059 ChIP_mouse_WT_MCMVD2_3_H3K4me3_IP
GSM4152060 ChIP_mouse_WT_MCMVD4_2_H3K4me3_input
GSM4152061 ChIP_mouse_WT_MCMVD4_2_H3K4me3_IP
GSM4152062 ChIP_mouse_WT_MCMVD4_3_H3K4me3_input
GSM4152063 ChIP_mouse_WT_MCMVD4_3_H3K4me3_IP
GSM4152064 ChIP_mouse_WT_MCMVD4_4_H3K4me3_input
GSM4152065 ChIP_mouse_WT_MCMVD4_4_H3K4me3_IP
GSM4152066 ChIP_mouse_WT_MCMVD7_2_H3K4me3_input
GSM4152067 ChIP_mouse_WT_MCMVD7_2_H3K4me3_IP
GSM4152068 ChIP_mouse_WT_MCMVD7_3_H3K4me3_input
GSM4152069 ChIP_mouse_WT_MCMVD7_3_H3K4me3_IP
GSM4152070 ChIP_mouse_WT_MCMVD35_4_H3K4me3_input
GSM4152071 ChIP_mouse_WT_MCMVD35_4_H3K4me3_IP
GSM4152072 ChIP_mouse_WT_MCMVD35_5_H3K4me3_input
GSM4152073 ChIP_mouse_WT_MCMVD35_5_H3K4me3_IP

Genome browser session
(e.g. UCSC)

Custom scripts in R software were used to visualize ChIP-seq signal without the use of a web-based genome browser session.

Methodology

Replicates

For STAT5 ChIP, 4-5 x 10^6 sorted NK (CD3ε- TCRb- CD19- F4/80- NK1.1+) cells from pooled spleens were cultured for 3 hr in RPMI containing 10% fetal bovine serum with 20 ng/mL recombinant mouse IL-2 plus 20 ng/ml recombinant mouse IL-15, or with just media for unstimulated conditions. This replicate was combined with two other replicates previously published (Villarino et al, JEM 2017; GSE100674) with IL-15-stimulated NK cells cultured for 2 hours.

For in vitro H3K4me3 ChIP, 1-2 x 10^5 sorted splenic NK cells (CD3ε- TCRb- CD19- F4/80- NK1.1+) were cultured for 3 hr in RPMI containing 10% fetal bovine serum with either 20 ng/mL recombinant mouse IL-2 plus 20 ng/ml recombinant mouse IL-15 (3 replicates), 20 ng/ml recombinant mouse IL-12 plus 10 ng/ml recombinant mouse IL-18 (4 replicates), 100 IU recombinant mouse IFN-α (5 replicates), a combination of all cytokines (2 replicates), or just media for unstimulated conditions (8 replicates). One replicate pair for WT unstimulated and IL-12/IL-18-stimulated conditions was included from a previously published dataset (Rapp et al, Sci Immunol 2017; GSE106137). ChIP experiments were performed across 8 separate experiments from pooled spleens, each with a corresponding unstimulated pair.

For in vivo H3K4me3 ChIP, splenic Ly49H+ NK cells (CD3ε- TCRb- CD19- F4/80- NK1.1+ Ly49H+ CD69+ (d2 only)) from WT mice infected with MCMV and harvested on days 0 (uninfected; 2 replicates), 2 (3 replicates), 4 (3 replicates), and 7 (2 replicates). For the memory time point, NK cells were harvested at d35 post-infection from NKp46-CreERT2 Rosa26-tdTomato mice that were treated with 4 mg tamoxifen (Sigma) in corn oil by oral gavage one day prior to infection. Memory NK cells were sorted on CD3ε- TCRb- CD19- F4/80- NK1.1+ Ly49H+ tdTomato+ (2 replicates). Each replicate represents one biological replicate harvested from pooled spleens.

Sequencing depth

All reads were paired-end with lengths of 50 bp. After trimming and filtering for low-quality reads, these were the read numbers for each sample (first number shows total number of filtered read pairs, second number shows number of concordantly aligned read pairs):
ChIP_mouse_STAT1KO_UNSTIM_6_H3K4me3_input: 45233347, 44067276
ChIP_mouse_STAT1KO_UNSTIM_6_H3K4me3_IP: 43872620, 41850740
ChIP_mouse_STAT1KO_UNSTIM_9_H3K4me3_input: 49111878, 43660926
ChIP_mouse_STAT1KO_UNSTIM_9_H3K4me3_IP: 54373346, 41790007
ChIP_mouse_STAT1KO_IFNA_6_H3K4me3_input: 49758206, 48159891
ChIP_mouse_STAT1KO_IFNA_6_H3K4me3_IP: 44031334, 41844718
ChIP_mouse_STAT1KO_IFNA_9_H3K4me3_input: 65642306, 56847076
ChIP_mouse_STAT1KO_IFNA_9_H3K4me3_IP: 64749912, 47633226
ChIP_mouse_STAT1KO_IFNA_9a_H3K4me3_input: 50900496, 47446035
ChIP_mouse_STAT1KO_IFNA_9a_H3K4me3_IP: 53158385, 45992963
ChIP_mouse_WT_UNSTIM_1_H3K4me3_input: 57615188, 55947441
ChIP_mouse_WT_UNSTIM_1_H3K4me3_IP: 42883798, 40933952
ChIP_mouse_WT_UNSTIM_4_H3K4me3_input: 45583080, 41869736
ChIP_mouse_WT_UNSTIM_4_H3K4me3_IP: 41604442, 38129650
ChIP_mouse_WT_UNSTIM_3_H3K4me3_input: 74110467, 71739155
ChIP_mouse_WT_UNSTIM_3_H3K4me3_IP: 86149162, 83256048
ChIP_mouse_WT_UNSTIM_6_H3K4me3_input: 54791551, 53463964
ChIP_mouse_WT_UNSTIM_6_H3K4me3_IP: 44792436, 42995551
ChIP_mouse_WT_UNSTIM_7_H3K4me3_input: 44883699, 43068178
ChIP_mouse_WT_UNSTIM_7_H3K4me3_IP: 49142753, 45857688
ChIP_mouse_WT_UNSTIM_8_H3K4me3_input: 50846078, 47581680
ChIP_mouse_WT_UNSTIM_8_H3K4me3_IP: 51479728, 48782474
ChIP_mouse_WT_UNSTIM_9_H3K4me3_input: 27867793, 24913047
ChIP_mouse_WT_UNSTIM_9_H3K4me3_IP: 54002732, 42638999
ChIP_mouse_WT_UNSTIM_1_STAT5_input: 36890417, 31797818
ChIP_mouse_WT_UNSTIM_1_STAT5_IP: 37699714, 18471901
ChIP_mouse_WT_IFNA_3_H3K4me3_input: 67258735, 64901834
ChIP_mouse_WT_IFNA_3_H3K4me3_IP: 85469212, 82485029
ChIP_mouse_WT_IFNA_6_H3K4me3_input: 44357730, 43072762
ChIP_mouse_WT_IFNA_6_H3K4me3_IP: 45604260, 43689740
ChIP_mouse_WT_IFNA_7_H3K4me3_input: 43510699, 42531132
ChIP_mouse_WT_IFNA_7_H3K4me3_IP: 48001647, 45145287
ChIP_mouse_WT_IFNA_8_H3K4me3_input: 57849731, 53199933
ChIP_mouse_WT_IFNA_8_H3K4me3_IP: 54378361, 49453599
ChIP_mouse_WT_IFNA_9_H3K4me3_input: 48123748, 43354052
ChIP_mouse_WT_IFNA_9_H3K4me3_IP: 48188861, 38205143
ChIP_mouse_WT_IL12IL18_1_H3K4me3_input: 55532890, 52759788
ChIP_mouse_WT_IL12IL18_1_H3K4me3_IP: 43963022, 41461815
ChIP_mouse_WT_IL12IL18_7_H3K4me3_input: 49799893, 48805234
ChIP_mouse_WT_IL12IL18_7_H3K4me3_IP: 41769130, 40394337
ChIP_mouse_WT_IL12IL18_8_H3K4me3_input: 55881436, 51915786
ChIP_mouse_WT_IL12IL18_8_H3K4me3_IP: 54222541, 49911335
ChIP_mouse_WT_IL2IL15_4_H3K4me3_input: 55367152, 50654240
ChIP_mouse_WT_IL2IL15_4_H3K4me3_IP: 41865602, 37990709
ChIP_mouse_WT_IL2IL15_7_H3K4me3_input: 49695467, 48679096
ChIP_mouse_WT_IL2IL15_7_H3K4me3_IP: 39226168, 37795774
ChIP_mouse_WT_IL2IL15_8_H3K4me3_input: 52849758, 49378664
ChIP_mouse_WT_IL2IL15_8_H3K4me3_IP: 47348120, 43789735
ChIP_mouse_WT_IL2IL15_1_STAT5_input: 38969495, 34972889
ChIP_mouse_WT_IL2IL15_1_STAT5_IP: 22758219, 13259916
ChIP_mouse_WT_IL2IL15IL12IL18IFNA_7_H3K4me3_input: 38399557, 34686817
ChIP_mouse_WT_IL2IL15IL12IL18IFNA_7_H3K4me3_IP: 43023275, 36101979
ChIP_mouse_WT_IL2IL15IL12IL18IFNA_8_H3K4me3_input: 46443381, 43150522
ChIP_mouse_WT_IL2IL15IL12IL18IFNA_8_H3K4me3_IP: 58738987, 54513173
ChIP_mouse_WT_MCMVD0_2_H3K4me3_input: 50871694, 49721550
ChIP_mouse_WT_MCMVD0_2_H3K4me3_IP: 53783326, 51666720
ChIP_mouse_WT_MCMVD0_4_H3K4me3_input: 39751142, 38230388
ChIP_mouse_WT_MCMVD0_4_H3K4me3_IP: 42776020, 26641240
ChIP_mouse_WT_MCMVD2_1_H3K4me3_input: 32836191, 24097087
ChIP_mouse_WT_MCMVD2_1_H3K4me3_IP: 47474143, 44040562
ChIP_mouse_WT_MCMVD2_2_H3K4me3_input: 39301295, 38440770
ChIP_mouse_WT_MCMVD2_2_H3K4me3_IP: 44985113, 42176774
ChIP_mouse_WT_MCMVD2_3_H3K4me3_input: 44029048, 41542165

ChIP_mouse_WT_MCMVD2_3_H3K4me3_IP: 46714944, 42595387
ChIP_mouse_WT_MCMVD4_2_H3K4me3_input: 34346823, 32949940
ChIP_mouse_WT_MCMVD4_2_H3K4me3_IP: 41593003, 38776115
ChIP_mouse_WT_MCMVD4_3_H3K4me3_input: 41768025, 39665828
ChIP_mouse_WT_MCMVD4_3_H3K4me3_IP: 50964850, 47121017
ChIP_mouse_WT_MCMVD4_4_H3K4me3_input: 39344629, 37504279
ChIP_mouse_WT_MCMVD4_4_H3K4me3_IP: 55372425, 51822028
ChIP_mouse_WT_MCMVD7_2_H3K4me3_input: 58133706, 56972123
ChIP_mouse_WT_MCMVD7_2_H3K4me3_IP: 45169138, 42838230
ChIP_mouse_WT_MCMVD7_3_H3K4me3_input: 41086766, 39490099
ChIP_mouse_WT_MCMVD7_3_H3K4me3_IP: 44758162, 42604343
ChIP_mouse_WT_MCMVD35_4_H3K4me3_input: 32672839, 31748162
ChIP_mouse_WT_MCMVD35_4_H3K4me3_IP: 39974301, 38640211
ChIP_mouse_WT_MCMVD35_5_H3K4me3_input: 40112280, 35606163
ChIP_mouse_WT_MCMVD35_5_H3K4me3_IP: 35740009, 33040844

Antibodies

The following antibodies were used for ChIP-Seq primarily reported in this study: anti-pSTAT5 (#AF2168, R&D Systems, lot# KVE0318071), anti-H3K4me3 (Millipore, 07-473, lot# 3018770).

Peak calling parameters

Reads were trimmed for adaptors and removal of low quality reads using Trimmomatic (v.0.36). Trimmed reads were mapped to the Mus musculus genome (mm10 assembly) using Bowtie2 (v2.2.9 or v2.3.4). For STAT5 ChIP, peak calling was performed on each IP-input paired sample using MACS2 (v2.1.1.20160309), using arguments "-f BAM -p 0.05 -m 2 50", for single-end peak calling. For H3K4me3 ChIP, peak calling was performed on each IP-input paired sample with MACS2, with arguments "–BAMPE –q 0.05".

Data quality

For STAT5 ChIP, Irreproducible discovery rate (IDR) calculations were performed on all pairs of replicates using an oracle peak list called from merged replicates. A total of 1,201 peaks passed an IDR threshold of 0.05.

For H3K4me3 ChIP, peak that exhibited a q-value score higher than the 25th percentile were retained. Samples that yielded less than 6000 of these filtered peaks were not considered.

Software

DESeq2 (v.1.14.1 or v.1.22.2)
Trimmomatic (v.0.36)
Bowtie2 (v.2.2.9 or v.2.3.4)
GenomicAlignments (v1.10.1 or v1.18.1)
MACS2 (v.2.1.1.20160309)
ChipPeakAnno (v.3.8.9)
UCSC mm10 Known Gene Annotation Package (v.9)
Irreproducible Discovery Rate (IDR) (v.2.0.2 or v2.0.3)
bedtools2 (v.2.26.0)
bedGraphToBigWig (v.4)
deepTools (v3.2.1)
Gviz (v.1.18.2)
ComplexHeatmap (v.1.18.1 or v.1.99.7)
Cytoscape (v.3.7.1)
R (v.3.3.3 or v.3.5.3)

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

Spleens were dissociated using glass slides and filtered through a 100 μm strainer. Red blood cells in spleen were lysed using ACK lysis buffer. NK cells were enriched before cell sorting by incubation of whole splenocytes with the following antibodies: CD3ε (17A2), CD4 (GK1.5), CD8 (2.43), Ter119 (TER-119), CD19 (1D3), Ly6G (1A8) (BioXCell). This was followed by magnetic depletion using goat anti-rat beads (Qiagen). For human samples, PBMC were isolated by Ficoll gradient purification before sorting.

Instrument

BD LSR II was used for data collection, and BD Aria II was used for cell sorting.

Software

BD FACSDiva (v.8.0.2) was used for data collection, and FlowJo (v10.5.3) for data analysis.

Cell population abundance

Cell populations were sorted to >95% purity post sort, as determined by flow cytometry.

Gating strategy

Depleted splenocytes were sorted for NK cells, gated as CD3ε- TCRb- CD19- F4/80- NK1.1+ among the live gate (using Live/Dead stain). For in vivo H3K4me3 ChIP, depleted splenocytes were sorted for Ly49H+ NK cells, gated as CD3ε- TCRb- CD19- F4/80- NK1.1+ Ly49H+ CD69+ (d2 only) and TdTom+ (d35 only). Human NK cells were sorted from PBMC, gated as DAPI- CD14- CD3- CD56+.

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