

## 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** Data collection for flow cytometry experiments was performed with Becton Dickinson (BD) FACSDiva software (v8.0.1). Data collection from the ViiA 7 real-time PCR system was performed using QuantStudio Real-time PCR software (v1.6.1) and from the StepOne plus real-time PCR system using StepOne software (v2.3).

**Data analysis** R (v4.2.0), Fastqc (v0.11.7), Bowtie (v2.3.4.3), Samtools (v1.9), MarkDuplicates (v2.1.1), deepTools (v3.1.1), MACS2 (v2.1.1), bedGraphToBigWig (v4), ROSE (v1), FeatureCounts (v1.6.3), DESeq2 (v1.26.0), CEMiTool (v1.10.2), clusterProfiler (v3.14.3), STRING (v11.0), immunedeconv (v2.0.0), Seurat (v4.1.0), IGV (v2.5.2), FlowJo (v10.0.5), GraphPad Prism (v8.4.3), inferCNV (v1.10.1) (<https://github.com/broadinstitute/inferCNV>), harmony (v0.1.0), SingleR (v1.6.1), Giotto (V1.0.3), NIS-Elements AR (v5.02.01), and QuPath (v0.3.0). Custom code is available upon reasonable request.

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

RNA-seq and ChIP-seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession number GSE165750. Publicly available RNA-seq data from 498 human neuroblastomas (GSE49711/GSE62564), microarray expression data from 394 neuroblastoma tumors (GSE120572)

and 24 human neuroblastoma cell lines (GSE28019) were accessed through the R2 genomics analysis and visualization platform (<https://hgserver1.amc.nl/cgi-bin/r2/>). Clinical annotations were obtained from GSE49711/GSE62564 regarding MYCN status (MYCN-nonamplified vs. MYCN-amplified, INSS stage [high (stage 4) vs. low (1, 2, 3 and 4s)], risk status (high vs. low) and age (< 18 months vs. ≥ 18 months). Previously published count matrix and metadata of single cell/nuclear RNA-seq data (sc/snRNA-seq) were downloaded from GSE140819, GSM4186961 and GSM4186962. Microarray data of matched neuroblastoma tumors at diagnosis and relapse were obtained from GSE65303. Source data have been provided as Source Data files. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

## 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	No statistical method was used to predetermine sample sizes, but our sample sizes were similar to those reported in previous publications (Debruyne et al., PMID: 31391581, Spranger et al., PMID: 25970248, Burr et al., PMID: 31564637).
Data exclusions	<p>1. Immunotherapy study: Here we provide a brief description of the study design, the data exclusions, and the rationale behind them. Mice bearing subcutaneous NB-9464-H-2Kblo and NB-9464-H-2Kbhi tumors were treated with either (i) anti-PD-1 and anti-CTLA-4, or (ii) isotype-matched control antibodies (n = 15 per group). Tumors from 5 mice per group were harvested on day 36 for FACS analysis and the remaining 10 mice per group were monitored for tumor growth and survival until endpoints were reached. The following numbers of animals from the remaining 10 mice per group were omitted from the final analysis because they a) did not form palpable tumors (n = 1, H-2Kblo anti-PD1 + anti-CTLA4), b) formed palpable tumors but not measurable when alive (tumors were noted to be growing inwards upon euthanasia when endpoints for body condition score and weight loss were attained), (n = 1, H-2Kblo anti-PD1 + anti-CTLA4; n = 1, H-2Kbhi isotype), c) formed tumors that became ulcerated before reaching the volume end point (n = 2, H-2Kblo isotype; n = 1, H-2Kbhi isotype), or d) had tumors that exhibited substantially different growth characteristics compared to the other animals in the cohort (n = 1, H-2Kblo anti-PD1 + anti-CTLA4; n = 2, H-2Kbhi anti-PD1 + anti-CTLA4). Thus, the growth curves and the Kaplan Meier plots presented in Fig. 7f and 7g, and the individual growth curves in Extended Data Fig. 10f represent data from the following numbers of animals: H-2Kblo isotype, n = 8; H-2Kblo anti-PD1 + anti-CTLA4, n = 7; H-2Kbhi isotype, n = 8; and H-2Kbhi anti-PD1 + anti-CTLA4, n = 8. Exclusion criteria were pre-established.</p> <p>2. In the multiplex cytokine assay presented in Fig. 6e, MIP-1b levels were above detection limit in 14/15 samples in this experiment, most likely indicating high background, and hence was excluded from the final heat map.</p>
Replication	To verify the reproducibility of our findings, experiments were performed using at least two biological replicates, unless clearly stated otherwise in the figure legends. All attempts at replication were successful.
Randomization	For the in vivo experiments, mice were randomly allocated to experimental groups. For the remaining studies, randomization for different experimental groups was not relevant as they were performed on uniform biological material, i.e. cell lines procured from commercial sources.
Blinding	The Investigators were not blinded to allocation during experiments and outcome assessment. Blinding was not possible for the in vitro and in vivo studies as these experiments were performed by individual Investigators who were aware of the experimental groups and treatment outcome. Blinding of the Investigator is also not relevant for bioinformatic analyses of large data sets as they are performed using computational algorithms.

## 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 &amp; experimental systems

## Methods

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input checked="" 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

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

## Antibodies

## Antibodies used

The following commercially-available antibodies were used. Catalog, clone, and lot numbers are provided wherever such information is publicly available. Antibody dilutions are provided in Supplementary Table 7.

From Cell Signaling Technology (Danvers, MA, USA):

1. MYCN (#51705, clone D4B2Y, lot 1)
2. TAP1 (#12341, lot 1)
3. TAP2 (#12259, lot 1)
4. LMP7 (#13635, clone D1K7X, lot 1)
5. NOTCH1 (#3608, clone D1E11, lot 4)
6. cleaved NOTCH1 (#4147, clone D3B8, lot 8)
7. SOX9 (#82630, clone D8G8H, lot 1)
8. AXL (#8661, clone C89E7, lot 4),
9. GAPDH (#2118, clone 14C10, lot 14)
10.  $\beta$ -actin (#3700, clone 8H10D10, lot 16)
11. IRF1 (#8478, clone D5E4, lot 2)
12. VIM (#5741, clone D21H3, lot 1)
13. YAP (#4912, lot 5)
14. Slug (#9585, clone C19G7, lot 6)
15. EZH2 (#5246, clone D2C9, lot 9),
16. SUZ12 (#3737, clone D39F6, lot 6)
17. PRAME (#56426, clone 45084S, lot 1)

From Santa Cruz Biotechnology (Santa Cruz, CA, USA)

1. LMP2 (#271354, clone A-1, multiple lots)
2. PRRX1 (#293386, 1E2, lot A2918)

From Abcam (Cambridge, MA, USA)

1. PHOX2B (#183741, lot GR3227036-3)
2. H3K4me3 (#8580, lot GR3190162-1)
3. H3K27ac (#4729, lot GR3198866-1)
4. PRRX1 antibody (#211292, lot GR3264445-5)

From Millipore (Billerica, MA, USA)

1. H3K27me3 (#07-449, lot 3091919)

From Roche (Basel, Switzerland)

1. Vimentin (#790-2917, clone V9, multiple lots)
2. CD8 (#790-4460, clone SP57, multiple lots)

From Life Technologies Corporation (Eugene, OR, USA)

1. Anti-human Alexa-Fluor 647 (#A21445, lot 2339821)

From R&D Systems (Minneapolis, MA, USA)

1. FN1 (#AF1918, lot UTNO318041)
2. PE-ULBP2 (#FAB1298P, clone 165903, lot LWEO717041)
3. PE-ULBP3 (#FAB1517P, clone 166510, lot ABPX0517121)
4. NKG2D (#MAB139-100, clone 149810, multiple lots)
5. mouse IgG1 isotype control (# MAB002, clone 11711, lot IX2721082)

From Biolegend (San Diego, CA, USA)

1. PE-HLA (#311406, clone W6/32, lot B265864)
2. PE-MICA/B (#320906, clone 6D4, lot B279674)
3. PE-H-2Kb (#116507, clone AF6-88.5, multiple lots)
4. PE-H-2Kb SIINFEKL (#141603, clone 25-D1.16, lot B254737)
5. PE-mouse IgG2a k isotype control (#400212, clone MOPC-173, lot B298789)
6. FITC-CD8 (#126614, clone YTS156.7.7, multiple lots)
7. PE-CD69 (#104508, clone H1.2F3, multiple lots)
8. PerCP-Cy5.5 CD107a (#328616, clone H4A.3, lot B264869)

9. FITC-CD56 (#362545, clone 5.1H11, multiple lots)
10. AlexaFluor 647-NKp46 (#331910, clone 9E2, B297912)
11. PE-NKG2D (#320805, clone 1D11, multiple lots)
12. BV605-CD45 (#103140, clone 30-F11, multiple lots)
13. BV510-CD8b (#126631, clone YTS156.7.7, multiple lots)
14. PerCP-Cy5.5-CD4 (#100540, clone RM4-5, multiple lots)
15. PE-NK1.1 (#108708, clone PK136, multiple lots)
16. APC-TCRb (# 109211, clone H57-597, multiple lots)
17. Alexa-Fluor 647-HLA (#311416, clone W6/32, multiple lots)
18. APC-HLA-A2 (#343307, clone BB7.2, multiple lots)

From Thermo Fisher Scientific (Waltham, MA, USA)

1. eFluor450-FoxP3 (#48-5773-82, clone FJK-16s, multiple lots)

From Miltenyi Biotec (Bergisch Gladbach, Germany)

1. FITC-TCR Vβ1(#130-120-789, clone REA662, multiple lots)

From Bio X Cell (Lebanon, NH, USA)

1. InVivoMAb anti-PD-1 (BE0273, clone 29 F.1A12, multiple lots)
2. InVivoMAb anti-CTLA-4 (BE0131, clone 9H10, multiple lots)
3. InVivoMAb Rat IgG2a isotype control (BE0089, clone 2A3, multiple lots)
4. InVivoMAb Syrian hamster polyclonal IgG (BE0087, multiple lots)

## Validation

Validation statements for all antibodies listed above can be found through the following links to the manufacturer's website.

From Cell Signaling Technology (Danvers, MA, USA):

1. <https://www.cellsignal.com/products/primary-antibodies/n-myc-d4b2y-rabbit-mab/51705>
2. <https://www.cellsignal.com/products/primary-antibodies/tap1-antibody/12341>
3. <https://www.cellsignal.com/products/primary-antibodies/tap2-antibody/12259>
4. <https://www.cellsignal.com/products/primary-antibodies/psmb8-lmp7-d1k7x-rabbit-mab/13635>
5. <https://www.cellsignal.com/products/primary-antibodies/notch1-d1e11-xp-rabbit-mab/3608>
6. <https://www.cellsignal.com/products/primary-antibodies/cleaved-notch1-val1744-d3b8-rabbit-mab/4147>
7. <https://www.cellsignal.com/products/primary-antibodies/sox9-d8g8h-rabbit-mab/82630>
8. <https://www.cellsignal.com/products/primary-antibodies/axl-c89e7-rabbit-mab/8661>
9. <https://www.cellsignal.com/products/primary-antibodies/gapdh-14c10-rabbit-mab/2118>
10. <https://www.cellsignal.com/products/primary-antibodies/b-actin-8h10d10-mouse-mab/3700>
11. <https://www.cellsignal.com/products/primary-antibodies/irf-1-d5e4-xp-rabbit-mab/8478>
12. <https://www.cellsignal.com/products/primary-antibodies/vimentin-d21h3-xp-rabbit-mab/5741>
13. <https://www.cellsignal.com/products/primary-antibodies/yap-antibody/4912>
14. <https://www.cellsignal.com/products/primary-antibodies/slug-c19g7-rabbit-mab/9585>
15. <https://www.cellsignal.com/products/primary-antibodies/ezh2-d2c9-xp-rabbit-mab/5246>
16. <https://www.cellsignal.com/products/primary-antibodies/suz12-d39f6-xp-rabbit-mab/3737>
17. <https://www.cellsignal.com/products/primary-antibodies/prame-antibody/45084>

From Santa Cruz Biotechnology (Santa Cruz, CA, USA)

1. <https://www.scbt.com/p/lmp2-antibody-a-1>
2. <https://www.scbt.com/p/prx1-antibody-1e2>

From Abcam (Cambridge, MA, USA)

1. <https://www.abcam.com/phox2b-antibody-epr14423-c-terminal-ab183741.html>
2. <https://www.abcam.com/histone-h3-tri-methyl-k4-antibody-chip-grade-ab8580.html>
3. <https://www.abcam.com/histone-h3-acetyl-k27-antibody-chip-grade-ab4729.html>
4. <https://www.abcam.com/prx-1-antibody-ab211292.html>

From Millipore (Billerica, MA, USA)

1. [https://www.emdmillipore.com/US/en/product/Anti-trimethyl-Histone-H3-Lys27-Antibody,MM\\_NF-07-449](https://www.emdmillipore.com/US/en/product/Anti-trimethyl-Histone-H3-Lys27-Antibody,MM_NF-07-449)

From Roche (Basel, Switzerland)

1. <https://www.citeab.com/antibodies/8955874-790-2917-vimentin-v9-primary-antibody-confirm>
2. <https://www.citeab.com/antibodies/8955616-790-4460-cd8-sp57-rabbit-monoclonal-primary-antibo>

From Life Technologies Corporation (Eugene, OR, USA)

1. <https://www.thermofisher.com/antibody/product/Goat-anti-Human-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21445>

From R&D Systems (Minneapolis, MA, USA)

- [https://www.rndsystems.com/products/human-fibronectin-antibody\\_af1918](https://www.rndsystems.com/products/human-fibronectin-antibody_af1918)
- [https://www.rndsystems.com/products/human-ulbp-2-5-6-pe-conjugated-antibody-165903\\_fab1298p](https://www.rndsystems.com/products/human-ulbp-2-5-6-pe-conjugated-antibody-165903_fab1298p)
- [https://www.rndsystems.com/products/human-ulbp-3-pe-conjugated-antibody-166510\\_fab1517p](https://www.rndsystems.com/products/human-ulbp-3-pe-conjugated-antibody-166510_fab1517p)
- [https://www.rndsystems.com/products/human-nkg2d-cd314-antibody-149810\\_mab139](https://www.rndsystems.com/products/human-nkg2d-cd314-antibody-149810_mab139)
- [https://www.rndsystems.com/products/mouse-igg-1-isotype-control\\_mab002](https://www.rndsystems.com/products/mouse-igg-1-isotype-control_mab002)

From Biolegend (San Diego, CA, USA)

- <https://www.biolegend.com/en-us/products/pe-anti-human-hla-a-b-c-antibody-1872?GroupID=GROUP28>
- <https://www.biolegend.com/en-us/products/pe-anti-human-mica-micb-antibody-3064?GroupID=GROUP28>
- <https://www.biolegend.com/en-us/products/pe-anti-mouse-h-2kb-antibody-1749?GroupID=GROUP20>
- <https://www.biolegend.com/fr-ch/products/pe-anti-mouse-h-2kb-bound-to-siinfekl-antibody-7247>
- <https://www.biolegend.com/en-us/products/pe-mouse-igg2a-kappa-isotype-ctrl-1401>
- <https://www.biolegend.com/en-us/products/apc-anti-mouse-cd8b-antibody-9055>
- <https://www.biolegend.com/en-us/products/pe-anti-mouse-cd69-antibody-265>
- <https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-human-cd107a-lamp-1-antibody-5365?GroupID=GROUP28>
- <https://www.biolegend.com/en-us/search-results/fitc-anti-human-cd56-ncam-antibody-12074>
- <https://www.biolegend.com/en-us/products/alexa-fluor-647-anti-human-cd335-nkp46-antibody-4578>
- <https://www.biolegend.com/en-us/products/pe-anti-human-cd314-nkg2d-antibody-3016?GroupID=GROUP28>
- <https://www.biolegend.com/en-gb/products/brilliant-violet-605-anti-mouse-cd45-antibody-8721>
- <https://www.biolegend.com/en-us/products/brilliant-violet-510-anti-mouse-cd8b-ly-3-antibody-17356?GroupID=BLG4212>
- <https://www.biolegend.com/fr-ch/search-results/percp-cyanine5-5-anti-mouse-cd4-antibody-4230>
- <https://www.biolegend.com/en-us/products/pe-anti-mouse-nk-1-1-antibody-431?GroupID=GROUP20>
- <https://www.biolegend.com/en-us/products/apc-anti-mouse-tcr-beta-chain-antibody-268>
- <https://www.biolegend.com/en-us/products/alexa-fluor-647-anti-human-hla-a-b-c-antibody-2900>
- <https://www.biolegend.com/de-at/products/apc-anti-human-hla-a2-antibody-8181>

From Thermo Fisher Scientific (Waltham, MA, USA)

- <https://www.thermofisher.com/antibody/product/FOXP3-Antibody-clone-FJK-16s-Monoclonal/48-5773-82>

From Miltenyi Biotec (Bergisch Gladbach, Germany)

- <https://www.miltenyibiotec.com/US-en/products/tcr-vb1-antibody-anti-human-reafinity-rea662.html#gref>

From Bio X Cell (Lebanon, NH, USA)

- <https://bxcell.com/product/invivomab-anti-mouse-cd279-pd-1/>
- <https://bxcell.com/product/m-cd152-m-ctla-4/>
- <https://bxcell.com/product/rat-igg2a-isotype-control/>
- <https://bxcell.com/product/invivomab-polyclonal-syrian-hamster-igg/>

## Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	Human neuroblastoma cell lines (Kelly, NB-L-S, CHP-212, SH-SY5Y, SH-EP, CHLA-20, NB69, SK-N-FI, SK-N-BE2, SMS-KAN, IMR-32) were originally obtained from the Children's Oncology Group biobank. ACN, GI-ME-N, NB-EbC1, SK-N-DZ, NLF were originally obtained from A. Thomas Look and Kimberly Stegmaier at Dana Farber Cancer Institute (DFCI), and murine NB-9464 cells from To-Ha Thai at Beth Israel Deaconess Medical Center, Boston, MA. HEK293T cells were obtained from the American Type Culture Collection (ATCC).
Authentication	All cell lines were authenticated through STR analyses at the DFCI Core facility.
Mycoplasma contamination	All cell lines tested negative for mycoplasma.
Commonly misidentified lines (See <a href="#">ICLAC</a> register)	No 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	6-9-weeks-old female C57BL/6 mice were obtained from Charles River Laboratories. All animals were housed in the AAALAC-accredited animal facility at Dana-Farber Cancer Institute. Dark/light cycle and ambient temperature and humidity were centrally-regulated and animals were closely monitored by resident veterinarians for wellbeing.
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 experiments were performed with approval from the Institutional Animal Care and Use Committee (IACUC) of the Dana-Farber Cancer Institute. Animals were euthanized when tumors reached 2 cm in any dimension based on IACUC criteria for maximum

tumor burden. In none of the experiments was the institutional limit for tumor burden exceeded (<2 cm preceding the day of euthanization).

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

The population characteristics of the seven patients whose tumor samples were analyzed in Figure 2h are detailed in Supplementary Table 5.

### Recruitment

Tumor samples were collected from neuroblastoma patients treated at St Jude Children's Research Hospital.

### Ethics oversight

All human tumor specimens were obtained under an IRB-approved protocol of St Jude Children's Research Hospital and informed consent was obtained from all subjects. Participants were not compensated for enrollment in the study.

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=GSE165750>

### Files in database submission

ChIPseq\_Original\_Submission  
Raw files:  
5Y-par-H3K27me3-1.fastq.gz  
5Y-par-H3K27me3-2.fastq.gz  
5Y-par-input-K27me3.fastq.gz  
5Y-LDK-res-H3K27me3-1.fastq.gz  
5Y-LDK-res-H3K27me3-2.fastq.gz  
5Y-LDK-res-input-K27me3.fastq.gz  
SHEP-H3K27me3-1.fastq.gz  
SHEP-H3K27me3-2.fastq.gz  
SHEP-input-K27me3.fastq.gz  
5Y-par-H3K4me3-1.fastq.gz  
5Y-par-H3K4me3-2.fastq.gz  
5Y-par-input-K4me3.fastq.gz  
5Y-LDK-res-H3K4me3-1.fastq.gz  
5Y-LDK-res-H3K4me3-2.fastq.gz  
5Y-LDK-res-input-K4me3.fastq.gz  
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SHEP-H3K4me3-2.fastq.gz  
SHEP-input-K4me3.fastq.gz  
5Y-par-H3K27ac-1.fastq.gz  
5Y-par-H3K27ac-2.fastq.gz  
5Y-par-input-K27ac.fastq.gz  
5Y-LDK-res-H3K27ac-1.fastq.gz  
5Y-LDK-res-H3K27ac-2.fastq.gz  
5Y-LDK-res-input-K27ac.fastq.gz  
SHEP-H3K27ac-1.fastq.gz  
SHEP-H3K27ac-2.fastq.gz  
SHEP-input-K27ac.fastq.gz  
Processed files:  
norm\_5Y-par-H3K27ac\_merged.noNeg.sorted.bw  
norm\_5Y-LDK-res-H3K27ac\_merged.noNeg.sorted.bw  
norm\_SHEP-H3K27ac\_merged.noNeg.sorted.bw  
norm\_5Y-par-H3K27me3\_merged.noNeg.sorted.bw  
norm\_5Y-LDK-res-H3K27me3\_merged.noNeg.sorted.bw  
norm\_SHEP-H3K27me3\_merged.noNeg.sorted.bw  
norm\_5Y-par-H3K4me3\_merged.noNeg.sorted.bw  
norm\_5Y-LDK-res-H3K4me3\_merged.noNeg.sorted.bw  
norm\_SHEP-H3K4me3\_merged.noNeg.sorted.bw  
5Y-par\_H3K27ac\_vs\_5Y-par\_input\_peaks.narrowPeak  
5Y-LDK-res\_H3K27ac\_vs\_5Y-LDK-res\_input\_peaks.narrowPeak  
SHEP\_H3K27ac\_vs\_SHEP\_input\_peaks.narrowPeak  
5Y-par\_H3K4me3\_vs\_5Y-par\_input\_peaks.narrowPeak  
5Y-LDK-res\_H3K4me3\_vs\_5Y-LDK-res\_input\_peaks.narrowPeak  
SHEP\_H3K4me3\_vs\_SHEP\_input\_peaks.narrowPeak

5Y-par\_H3K27me3\_vs\_5Y-par\_input\_peaks.broadPeak  
 5Y-LDK-res\_H3K27me3\_vs\_5Y-LDK-res\_input\_peaks.broadPeak  
 SHEP\_H3K27me3\_vs\_SHEP\_input\_peaks.broadPeak  
 RNA-seq\_original\_submission  
 Raw files:  
 Kelly\_1.fastq.gz  
 SY5Y-Res\_1.fastq.gz  
 CHP212\_1.fastq.gz  
 NBLS\_1.fastq.gz  
 SHEP\_1.fastq.gz  
 SY5Y-par\_1.fastq.gz  
 CHP212\_2.fastq.gz  
 NBLS\_2.fastq.gz  
 SHEP\_2.fastq.gz.sam  
 SY5Y-par\_2.fastq.gz  
 Kelly\_2.fastq.gz  
 SY5Y-Res\_2.fastq.gz  
 processed file:  
 Normalized\_cell\_lines\_with\_each\_rep\_TPM.txt

Genome browser session  
 (e.g. [UCSC](#))

No longer applicable.

## Methodology

Replicates

For each mark assessed, two biological replicates were performed. All ChIP-seq data are derived from the analysis of both replicates.

Sequencing depth

ChIP-seq libraries were generated using the NEBNext Ultra II DNA Library Prep kit (E7645), following the manufacturer's instructions. Starting DNA material ranged from 18 to 25ng, and PCR amplification (9 to 10 cycles) was performed using NEBNext Multiplex Oligos for Illumina (E7335 and E7500) with distinct indices to allow for multiplexing of up to 10 samples to be run on the Illumina NextSeq 500 for 75 bases in single-read mode.

Sample ID\_total reads\_uniquely mapped reads  
 5Y-par-H3K27me3-1.fastq.gz 67528019 46381712  
 5Y-par-H3K27me3-2.fastq.gz 53085299 36606967  
 5Y-par-input-K27me3.fastq.gz 68202469 47821300  
 5Y-LDK-res-H3K27me3-1.fastq.gz 54746852 37291562  
 5Y-LDK-res-H3K27me3-2.fastq.gz 51941998 35602319  
 5Y-LDK-res-input-K27me3.fastq.gz 72090938 49295499  
 SHEP-H3K27me3-1.fastq.gz 52496710 36951720  
 SHEP-H3K27me3-2.fastq.gz 43805468 30892765  
 SHEP-input-K27me3.fastq.gz 64431513 45408008  
 5Y-par-H3K4me3-1.fastq.gz 44376992 35799322  
 5Y-par-H3K4me3-2.fastq.gz 46113286 36971093  
 5Y-par-input-K4me3.fastq.gz 55869118 38738391  
 5Y-LDK-res-H3K4me3-1.fastq.gz 50501595 39709290  
 5Y-LDK-res-H3K4me3-2.fastq.gz 47937690 38318691  
 5Y-LDK-res-input-K4me3.fastq.gz 53489838 36167507  
 SHEP-H3K4me3-1.fastq.gz 52932407 42033497  
 SHEP-H3K4me3-2.fastq.gz 45911633 36615039  
 SHEP-input-K4me3.fastq.gz 58647552 40930970  
 5Y-par-H3K27ac-1.fastq.gz 49068554 39557949  
 5Y-par-H3K27ac-2.fastq.gz 44700213 36123449  
 5Y-par-input-K27ac.fastq.gz 78478490 56040153  
 5Y-LDK-res-H3K27ac-1.fastq.gz 30025527 24294665  
 5Y-LDK-res-H3K27ac-2.fastq.gz 40028948 32389142  
 5Y-LDK-res-input-K27ac.fastq.gz 49730292 34748522  
 SHEP-H3K27ac-1.fastq.gz 40382350 32542078  
 SHEP-H3K27ac-2.fastq.gz 38664129 31240395  
 SHEP-input-K27ac.fastq.gz 53891531 3852630

Antibodies

The following antibodies were used: H3K4me3 (Abcam #8580, lot GR3190162-1); H3K27ac (Abcam #4729, lot GR3198866-1); H3K27me3 (Millipore #07-449, lot 3091919)

Peak calling parameters

Samples were aligned to the human genome (build hg19, GRCh37.75) with Bowtie2 (v3.4.3) with default parameters. Next, non-duplicate reads that mapped to the reference chromosomes were retained using Samtools (v1.9) and MarkDuplicates (v2.1.1) from Picard tools. Peaks were identified with MACS2 (2.1.1) for narrow peaks with the parameters "--q 0.01--call-summits" and for broad peaks with the parameters "--broad-cutoff 0.01".

Data quality

- 1) Peaks overlapping regions with known artefact regions (<http://mitra.stanford.edu/kundaje/akundaje/release/blacklists/>) were blacklisted out.
- 2) Antibody enrichment was assessed using the plotFingerprint command from deepTools (v3.0.2).
- 3) Correlation of replicates was assessed with the deepTools command "multiBigwigSummary BED-file" using all bigwigs and identified peaks as input.
- 4) broad peaks with 4-fold enrichment, 5% FDR after blacklisting



5Y-par\_H3K27ac\_vs\_5Y-par\_input\_peaks.narrowPeak 71923  
 5Y-LDK-res\_H3K27ac\_vs\_5Y-LDK-res\_input\_peaks.narrowPeak 92252  
 SHEP\_H3K27ac\_vs\_SHEP\_input\_peaks.narrowPeak 80287  
 5Y-par\_H3K4me3\_vs\_5Y-par\_input\_peaks.narrowPeak 30217  
 5Y-LDK-res\_H3K4me3\_vs\_5Y-LDK-res\_input\_peaks.narrowPeak 38231  
 SHEP\_H3K4me3\_vs\_SHEP\_input\_peaks.narrowPeak 57822  
 5Y-par\_H3K27me3\_vs\_5Y-par\_input\_peaks.broadPeak 5729  
 5Y-LDK-res\_H3K27me3\_vs\_5Y-LDK-res\_input\_peaks.broadPeak 6757  
 SHEP\_H3K27me3\_vs\_SHEP\_input\_peaks.broadPeak 3495

## Software

R (v4.2.0), Fastqc (v0.11.7), Bowtie2 (v2.3.4.3), Samtools (v1.9), MarkDuplicates (v2.1.1), deepTools (v3.1.1), MACS2 (v2.1.1), bedGraphToBigWig (v4), Rstudio (v1.1.456), ROSE (v1), IGV (v2.5.2). Custom code is available upon reasonable request.

## 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

Adherent cells were disassociated using Accutase (Invitrogen). For each staining reaction 1 million live cells were placed in a 12 x 75 mm polystyrene round bottom tube (Falcon), resuspended in 100  $\mu$ l 1x PBS and stained with the Zombie near-infrared (Zombie NIR) viability dye (BioLegend) at a 1:1,000 dilution for 15 minutes at RT. Cells were then washed once in FACS buffer (0.5% BSA in 1x PBS), resuspended in 100  $\mu$ l of FACS buffer and incubated in 5  $\mu$ l of Human TruStain FcX™ (Fc receptor blocking solution, BioLegend) for 10 minutes at RT. Next, appropriate volumes of conjugated fluorescent primary antibodies at predetermined optimum concentrations were added and incubated on ice for 20 minutes in the dark. Cells were then washed once in 2 ml of FACS buffer by centrifugation at 1500 rpm for 5 minutes. Finally, cell pellets were resuspended in 400  $\mu$ l of FACS buffer for analysis.

#### Instrument

Cells were analyzed using FACSCanto II [Becton Dickinson(BD)] and LSRFortessa (BD) flow cytometers.

#### Software

Data collection was performed using FACSDiva software (BD). Data was analyzed using FlowJo software (BD).

#### Cell population abundance

Murine NB-9464 cells were sorted based on H-2Kb cell surface expression using fluorescence-activated cell sorting (FACS) analysis. Purity of post-sort populations was determined by FACS for H-2Kb.

#### Gating strategy

Murine NB-9464 cells were sorted based on H-2Kb cell surface expression using fluorescence-activated cell sorting (FACS) analysis. A logscale expression value of 10e3 was used as a threshold to gate H-2Kblo (<10e3) and H-2Kbhi ( $\geq$ 10e3) cell populations. H-2Kblo and H-2Kbhi populations were expanded to NB9464-H-2Kblo and NB9464-H-2Kbhi cell lines.

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