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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*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	BDFACSDiva software version 8.0.3 and Cytek SpectroFlo sofware version 2.2.0.4 was used for flow-cytometry data collection. Gen5 3.08 software was used for the analysis of in vitro luciferase activity. Living Image version 4.7.3 was used for the analysis of in vivo luciferase activity.
Data analysis	FCS Express 7 Research software version 7.06.0015 was used for flow-cytometry analysis. Incucyte data were analyzed with the Incucyte S3 Basic Analysis software version 2019B Rev3. Microsoft Excel Office365 version 16.57 and GraphPad Prism V9.0.1 were used for data analysis and graphical output. QuantaSoft version v.1.7 was used for ddPCR analysis. R v4.0.2 and Trim Galore! v0.6.0 and Salmon v.0.13.1 were used for the analysis of RNA-sequencing data. Codes can be made available on 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 Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The RNA-sequencing data are available from the Gene Expression Omnibus under the accession number provided in the Data availability statement in this paper. Source data for tumour growth are provided with this paper. The raw and analysed datasets generated during the study are available from the corresponding author on reasonable request.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	This study did not include any human research participants.
Population characteristics	–
Recruitment	–
Ethics oversight	–

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

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	Sample sizes were determined on the basis of similar published studies and of preliminary experiments.
Data exclusions	The BLI signal (total flux) in Fig. 7b, was not computed for three mice on day 28, and for four mice on day 35, as they recorded false positives owing to spillover signal from nearby mice with high tumour burden. The excluded values are indicated in the source data file. No other data were excluded.
Replication	The experimental findings were reproduced in biological and/or technical replicates, as indicated in the figure legends.
Randomization	No randomization was applied. For the in vivo studies, the animals were assigned to the treatment groups to ensure equal tumour burden prior to treatment.
Blinding	For the in vivo experiments, the mice were injected and imaged by an operator who was blinded to the treatment groups. No blinding methods were used for the other experiments.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

Antibody (clone, company, catalogue number, lot number, dilution)
hDLL1 - PE (MHD-314; BioLegend, 346404, B276389, 1:100)
hDLL4 - PE (MHD4-46; BioLegend, 564412, 9288767, 1:100)
hJagged1 - PE (MHJ1-152; BD, 565495, 8270655, 1:100)
hJagged2 - PE (MHJ2-523; BioLegend, 346904, B266746, 1:100)
4-1BBL – PE (5F4, BioLegend, 311504, B261298, 1:100)
SSEA4 – FITC (MC813-70, BD, 560126, 2059243, 1:50)
TRA-1-81 – af647 (TRA-1-81, BD, 560793, 2005011, 1:50)
CD30 – PE (Ber-H8, BD, 550041, 8346901, 1:50)
LNGFR – PE (C401457, BD, 557196, 0342579, 1:100)
CD45 - BV605 (HI30; BioLegend, 304042, B319891, 1:50)
CD3 – BUV737 (UCHT1; BD, 612750, 0213429, 1:50)
TCRab – PE-Cy7 (IP16; Invitrogen, 25-9986-42, 2086337, 1:100)
CD4 – BV785 (SK3; BioLegend, 344642, B342031, 1:200)
CD8a – BUV395 (HIT8a; BD, 740303, 2094040, 1:50)
CD8b – PE (SIDI8BEE; Invitrogen, 12-5273-42, 2252642, 1:50)
CD8ab – APC (2ST8.5H7; BD, 641058, 0220729, 1:50)
CD7 – APC-H7 (M-T701; BD, 564020, 0058101, 1:100)
CD5 – PerCP-Cy5.5 (UCHT2; BioLegend, 300620, B291975, 1:50)
CD56 – BV421 (HCD56; BioLegend, 318328, B310552, 1:50)
CD1a - PE-Cy7 (HI149; BioLegend, 300122, B292845, 1:100)
CD2 – BV711 (RPA-2.10; BD, 740767, 1195655, 1:50)
CD45RA – BV605 (HI100; BioLegend, 304134, B315941, 1:50)
CD45RO – BV421 (UCHL1; BioLegend, 304224, B328292, 1:25)
CD62L – BV711 (DREG-56; BioLegend, 304860, B312134, 1:400)
CCR7 – PE-Cy7 (G043H7; BioLegend, 353226, B322378, 1:25)
CD25 – BB515 (2A3; BD, 564467, 0072491, 1:50)
CD69 – PerCP-Cy5.5 (FN50; BioLegend, 310926, B340058, 1:100)
CD27 – BUV737 (M-T271; BD, 741833, 1021873, 1:25)
CD28 – PE-Cy7 (CD28.2; BioLegend, 302926, B297258, 1:25)
CD16 – BUV737 (3G8; BD, 612786, 0293062, 1:50)
NKG2C – PE (S19005E; BioLegend, 375004, B310323, 1:50)
KIR2D – FITC (NKVFS1; Miltenyi Biotec, 130-098-681, 1321010677, 1:50)
NKp46 – FITC (9E2; BioLegend, 331922, B257991, 1:50)
NKp30 – PerCP-Cy5.5 (P30-15; BioLegend, 325216, B285293, 1:50)
TCRVd2 – PerCP-Cy5.5 (B6; Biolegend, 331424, B304597, 1:50)
CD161 – BV421 (HP-3G10; BioLegend, 339914, B311207, 1:50)
CCR2 - PE (K036C2; BioLegend, 357206, B265080, 1:50)
4-1BB - BV605 (4B4-1; BioLegend, 309822, B305291, 1:100)
biotin-conjugated goat anti-mouse F(ab')2 antibody (polyclonal; Jackson ImmunoResearch, 115-067-003, 153924, 1:100)
streptavidin-PE (BioLegend, 405203, B29004, 1:2500)
streptavidin-APC (BioLegend, 405207, C4317082321203, 1:3000)
anti-pTa (2F1; BD, 552407, 0260427, 1:20)
biotin-labelled anti-mouse IgG1 (RMG1-1; BioLegend, 406604, B270354, 1:100)
Streptavidin-PE (BD, 349023, 0352913, 1:50)
af647-conjugated goat anti-mouse F(ab')2 antibody (polyclonal; Jackson ImmunoResearch, 115-606-072, 147394, 1:100)
CD3z ITAM1 – af488 (EP776(2)Y; Abcam, ab237451, GR3271972-1, 1:50)
CD3z ITAM3 - PE (K25-407.69; BD, 558448,9015752, 1:5)
Annexin-V - APC (Invitrogen, 88-8007-72, 2313139, 1:50)
CD19 – PE-Cy7 (SJ25C1, BioLegend, 363012, B275701, 1:100)
CD107a – BV421 (H4A3, BD, 562623, 1089744, 1:50)
GranzymeB – APC (GB12; Invitrogen, MHGB05, 2291602, 1:100)
IFNg – PE-Cy7 (4S.B3; Invitrogen, 25-7319-82, 2331102, 1:50)
IL-2 – BUV737 (MQ1-17H12; BD, 612836, 1173652, 1:200)
TNFa – PE (Mab11; Invitrogen, 12-7349-82, 2373715, 1:200)
IL-17A – af488 (BL168, BioLegend, 512308, B325567, 1:200)
mCD45 – BV421 (30-F11, BioLegend, 103134, B343558, 1:100)
TIGIT – BV605 (A15153G, BioLegend, 372712, B311443, 1:50)

	LAG3 – PE-Cy7 (11C3C65, BioLegend, 369310, B306813, 1:100) PD1 – BV711 (EH12.2H7, BioLegend, 329928, B303708, 1:100)
Validation	All antibodies are validated for use in flow cytometry. Data are available on the manufacturer's website. All used antibodies are commercially available. All antibodies were titrated.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	OP9-mDLL1 were obtained from Dr Riviere, 3T3-CD19 were previously generated in the Sadelain Lab, TiPS cell lines were generated in the Sadelain Lab, OP9 were purchased from ATCC, K562-mbIL21-41BBL were generated at Fate Therapeutics Inc., and H1 were obtained from the Studer Lab.
Authentication	Certificates of authentication were provided with the cell lines from ATCC. No other authentication was performed. Morphology and properties pertinent to the experiments, such as antigen or Notch-ligand expression, were confirmed routinely by flow cytometry.
Mycoplasma contamination	The cell lines were tested for mycoplasma every 2 months. TiPS lines were tested for mycoplasma prior to each differentiation.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Male 6–8 week-old NOD/SCID/IL-2Ry-null (NSG) mice were obtained from the Jackson Laboratory, with average weight of 25–28 grams (data provided by the Jackson Laboratory).
Wild animals	The study did not involve wild animals.
Reporting on sex	Sex was not considered in the study design. The findings apply to both sexes.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal experiments were conducted in accordance with protocols approved by the MSKCC Institutional Animal Care and Use Committee (IACUC) and following National Institutes of Health (NIH) guidelines for animal welfare.

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

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	Cells during in vitro differentiation were harvested from cell culture vessels, washed and stained in facs buffer and analysed fresh. Cells retrieved from bone marrow, spleen and blood were washed and stained in PBS with 2mM EDTA, and analysed fresh. For intracellular cytokine detection cells were fixated and stained as per instructions of BD cytofix/perm.
Instrument	Fortessa X-20 (BD) or 5-laser Aurora (Cytek BioSciences).
Software	FACSDiva software (BD) and SpectroFlo software (Cytek Biosciences).
Cell population abundance	The purity of the sorted samples was confirmed by flow cytometry.

Gating strategy

The lymphocyte gate was drawn based on forward and side scatter; doublets were excluded and dead cells were excluded with a viability stain. Where indicated, cells were gated on CD45+ and CD7+ expression. Unstained and stained PBMC and TiPS samples were used for positive/negative gating.

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