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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	Flow cytometry data were collected at LSRFortessa X-20 (BD Biosciences). Whole-exome sequencing libraries were constructed using SureSelectXT Mouse All Exon v1 (Agilent Technologies). The libraries were sequenced on HiSeq platform (Illumina) with a 150-bp paired-end read protocol at Macrogen. Bulk RNA-sequencing libraries were prepared for samples with RIN ≥ 7 using NEBNext Ultra II RNA Library Prep Kit for Illumina (New England Biolabs) and NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs) for mouse. The libraries were sequenced on HiSeq platform (Illumina) with a 150-bp paired-end read protocol at Macrogen. Single-cell RNA-sequencing libraries were prepared using the Chromium Next GEM Single Cell 3′ Kit v3.1 (PN-1000268), Chromium Next GEM Chip G Single Cell Kit (PN-1000120), Dual Index Kit TT Set A (PN-1000215), according to manufacturer's instructions (CG000315 Rev E) (10x Genomics). These libraries were sequenced on the NovaSeq 6000 platform (Illumina) with a 150-bp paired-end read protocol at Macrogen.
Data analysis	R version 3.6.1 (https://www.r-project.org) Genomon pipeline version 2.6.2–2.6.3 (https://github.com/Genomon-Project/) CNVkit version 0.9.9 (https://cnvkit.readthedocs.io/en/stable/) edgeR version 3.36.0 (https://bioconductor.org/packages/release/bioc/html/edgeR.html) sva version 3.42.0 (https://bioconductor.org/packages/release/bioc/html/sva.html) GSEA version 4.1.0 (https://www.gsea-msigdb.org/gsea/index.jsp) Cellranger version 5.0.1 (https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger) Seurat version 4.3.0 (https://satijalab.org/seurat) DoubletFinder version 2.0.3 (https://github.com/chris-mcginnis-ucsf/DoubletFinder) ClusterProfiler package version 4.6.2 (https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html)

CellChat version 1.6.1 (http://www.cellchat.org/)
Integrative Genomics Viewer version 2.11.4 (https://software.broadinstitute.org/software/igv/)
FlowJo version 10.7.1 (http://www.flowjo.com/)
SigProfilerMatrixGenerator version 1.1.23 (https://github.com/AlexandrovLab/SigProfilerMatrixGeneratorR)
SigProfilerExtractor version 1.1.0 (https://github.com/AlexandrovLab/SigProfilerExtractor)
CIBERSORTx (https://cibersortx.stanford.edu/)
The original code used for single-cell analyses has been deposited in Github: https://github.com/nccmo/mouse-NK-lymphoma-2024 and in Zenodo: https://doi.org/10.5281/zenodo.13837293.

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

Mouse RNA-seq, WES, and scRNA-seq data have been deposited with links to BioProject accession number PRJDB16976 in the DNA Data Bank of Japan (DDBJ) BioProject database. In humans, WES and RNA-seq data for 49 ENKTCL tumors and 3 normal NK cells from healthy donors were described in our previous study (accession number EGAS00001006906). Publicly available WES and RNA-seq data for 46 ENKTCL tumors and 3 normal tonsils from ENKTCL patients and 1 normal NK cell from healthy donor (accession number SRP057085, SRP049695, and GSE190924) were also obtained from the National Center for Biotechnology Information Sequence Read Archive.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Sex- and gender-based analyses were not performed.
Reporting on race, ethnicity, or other socially relevant groupings	Analyses based on race, ethnicity, or other socially relevant groupings were not performed.
Population characteristics	For WES and RNA-seq analyses, we included data of 49 ENKTCL patients and 3 healthy donors described in our previous study and data of 46 ENKTCL tumors and 3 normal tonsils from ENKTCL patients and 1 normal NK cell from healthy donor obtained from the public databases. For IHC analysis, we included 5 ENKTCL and 1 DLBCL (as a control) patients. Detailed information is available in Supplementary Data 2.
Recruitment	For IHC analysis, ENKTCL and DLBCL (as a control) patients were selectively enrolled and analyzed. For WES and RNA-seq analyses, we included data described in our previous study and data obtained from the public databases.
Ethics oversight	This study was approved by the National Cancer Center Institutional Review Board and the Ethical Committee of Kurume University and performed in accordance with the Declaration of Helsinki. All samples were collected from patients with informed consent without compensation, except for already-collected, anonymized samples whose use was permitted by the institutional review board. We confirmed the acquisition of informed consent in the original publications for the data described in our previous study and obtained from the public databases.

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.

[x] 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	Statistical methods were not used to predetermine sample size. Sample size was determined empirically, at least three independent biological replicates were conducted.
Data exclusions	No data were excluded from analysis.
Replication	All experiments were conducted with at least three biological replicates. All attempts at replication were successful.

Randomization	For in vivo experiments, mice were randomly assigned to each treatment group. Randomization was not relevant to other mouse experiments because samples were analyzed based on genotype. For microscopy experiments, images were taken at random locations on slides. No other experiments were randomized.
Blinding	Investigators were blinded during data collection and analysis wherever possible. For microscopy experiments, samples were blinded before cell counting.

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
☒	☐	Plants

Methods

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

Antibodies

Antibodies used

For Flow Cytometry, the following anti-mouse fluorochrome-conjugated antibodies were used.
Alexa Fluor 488 anti-mouse/human CD11b (Flow cytometry) BioLegend #101217, M1/70
Alexa Fluor 647 anti-mouse/human CD45R/B220 (Flow cytometry) BioLegend #103226, RA3-6B2
Alexa Fluor 647 anti-mouse CD117 (c-Kit) (Flow cytometry) BioLegend #105818, 2B8
Alexa Fluor 647 anti-mouse/human Ki67 (Flow cytometry) BioLegend #151206 11F6
APC anti-mouse CD122 (IL-2Rβ) (Flow cytometry) BioLegend #123214, TM-β1
APC anti-mouse SiglecH (Flow cytometry) BioLegend #129612, 551
Biotin anti-mouse TER-119/Erythroid Cells Antibody (Flow cytometry) BioLegend #116204, TER-119
Biotin anti-mouse CD3ε Antibody (Flow cytometry) BioLegend #100304, 145-2C11
Biotin anti-mouse CD19 Antibody (Flow cytometry) BioLegend #115504, 6D5
Biotin anti-mouse Ly-6G/Ly-6C (Gr-1) (Flow cytometry) BioLegend #108404, RB6-8C5
Brilliant Violet 421 anti-mouse/human CD11b (Flow cytometry) BioLegend #101251, M1/70
Brilliant Violet 421 anti-mouse Ly-6G/Ly-6C (Gr-1) (Flow cytometry) BioLegend #108434, RB6-8C5
Brilliant Violet 421 anti-mouse CD3ε (Flow cytometry) BioLegend #100341, 145-2C11
Brilliant Violet 421 anti-mouse CD11c (Flow cytometry) BioLegend #117343, N418
Brilliant Violet 421 anti-mouse CD226 (DNAM-1) (Flow cytometry) BioLegend #133615, TX42.1
Brilliant Violet 421 anti-mouse KLRG1 (MAFA) (Flow cytometry) BioLegend #138414, 2F1/KLRG1
Brilliant Violet 605 anti-mouse CD4 (Flow cytometry) BioLegend #100451, GK1.5
Brilliant Violet 605 Streptavidin (Flow cytometry) BioLegend #405229
Brilliant Violet 785 anti-mouse CD19 (Flow cytometry) BioLegend #115543, 6D5
Brilliant Violet 785 anti-mouse TER-119/Erythroid Cells (Flow cytometry) BioLegend #116245, TER-119
Brilliant Violet 785 anti-mouse CD69 (Flow cytometry) BioLegend #104543, H1.2F3
Brilliant Violet 785 anti-mouse CD62L (Flow cytometry) BioLegend #104440, MEL-14
Brilliant Violet 785 anti-mouse NK1.1 (Flow cytometry) BioLegend #108749, PK136
BV786 anti-mouse CD43 (Flow cytometry) BD Bioscience #740857, S7
FITC anti-mouse CD122 (IL-2Rβ) (Flow cytometry) BioLegend #123208, TM-B1
PE anti-mouse CD45.2 (Flow cytometry) BioLegend #109808, 104
PE anti-mouse CD186 (CXCR6) (Flow cytometry) BioLegend #151104, SA051D1
PE anti-mouse CD146 (Flow cytometry) BioLegend #134704, ME-9F1
PE anti-mouse I-A/I-E (Flow cytometry) BioLegend #107608, M5/114.15.2
PE anti-mouse IFN-γ (Flow cytometry) BioLegend #505808, XMG1.2
PE anti-mouse Rat IgG1,k (Flow cytometry) BioLegend #400408, RTK2071
PE anti-mouse Eomes (Flow cytometry) BioLegend #157706, W17001A
PE anti-mouse Rat IgG2b,λ (Flow cytometry) BioLegend #403804, G013B8
PE anti-mouse/human T-bet (Flow cytometry) BioLegend #644810, 4B10
PE anti-mouse mouse IgG1,k (Flow cytometry) BioLegend #400114, MOPC-21
PE anti-mouse Ly6C (Flow cytometry) BioLegend #128008, HK1.4
PE/Cyanine7 anti-mouse CD8a (Flow cytometry) BioLegend #100722, 53-6.7
PE/Cyanine7 anti-mouse/human KLRG1 (MAFA) (Flow cytometry) BioLegend #138416, 2F1/KLRG1

PE/Cyanine7 anti-mouse CD49a (flow cytometry) BioLegend #142608, HMα1
PerCP/Cyanine5.5 anti-mouse NK-1.1 (Flow cytometry) BioLegend #108728, PK136
PerCP/Cyanine5.5 anti-mouse Ly-6G/Ly-6C (Gr-1) (Flow cytometry) BioLegend #108428, RB6-8C5
PerCP/Cyanine5.5 anti-mouse CD183 (CXCR3) (Flow cytometry) BioLegend #126514, CXCR3-173

For single-cell RNA-sequencing, the following anti-mouse fluorochrome-conjugated antibodies and hashtags were used.
TruStain FcX PLUS anti-mouse CD16/32 BioLegend #156604, S17011E
TotalSeq-B0090 Mouse IgG1, κ isotype control BioLegend #400185, MOPC-21
TotalSeq-B0301 anti-mouse Hashtag 1 BioLegend #155831, M1/42; 30-F11
TotalSeq-B0302 anti-mouse Hashtag 2 BioLegend #155833, M1/42; 30-F11

For immunohistochemical analysis, the following antibodies were used.
Anti-human KLRG1 R&D Systems #MAB70293, 2388C
Anti-human CD49a Cell Signaling Technology #15574, E9K2J
Anti-human CXCR3 BD Bioscience #557183, 1C6
Anti-human CXCR6 Abcam #ab273116, EPR24686-113
Anti-human CD11c Abcam #ab52632, EP1347Y

For in vivo treatment experiments, anti-mouse/human KLRG-1 antibody (BioXcell BE0201), polyclonal Syrian hamster IgG control (BioXcell BE0087), anti-mouse CXCL16 antibody (R&D Systems MAB503), and Rat IgG2A isotype control (R&D systems MAB006) were used.

Validation

All antibodies used in this paper are commercially available.
BioLegend: https://www.biolegend.com/ja-jp/quality/quality-control
BioXcell: https://bioxcell.com/
R&D systems: https://www.rndsystems.com/
Cell Signaling Technology: https://www.cellsignal.com/
Abcam: https://www.abcam.co.jp/

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

B6.129P2-Trp53tm1Brn/J (Trp53flox/flox, catalog number: 008462) and B6.Cg-Ncr1tm1.1(icre)Viv/Or (Ncr1-iCre, catalog number: EM05625) were purchased from The Jackson Laboratory and The Institut national de la santé et de la recherche médicale, respectively. LMP1stop conditional knock-in mice were kindly provided from Dr. Yasui. C57BL/6-SR-PSOX (Cxcl16 knockout) mice (nbio 196) at 8 weeks old were obtained from the Laboratory Animal Resource Bank of the National Institute of Biomedical Innovation, Health and Nutrition. Female C57BL/6 mice (8 weeks old) were obtained from Charles River Laboratories Japan. C57BL/6-SR-PSOX mice were used at 8 weeks old for transplantation experiments, while C57BL/6 mice were used at 8 and 60 weeks old. For Trp53flox/flox, Trp53-/-, LMP1+, Trp53-/-LMP1+ mice, we used naturally bred cohorts for long-term observation. Additionally, 8-week-old mice from these strains were used for pre-tumor analyses.
All mice were maintained under specific pathogen-free conditions and maintained on a 12-h light/dark cycle at room temperature (22 ± 0.5 °C) with constant humidity (55 ± 10%). Mice were monitored daily and euthanized when they appeared moribund or lost > 25% of their body weight.

Wild animals

This study did not involve wild animals.

Reporting on sex

Male and female mice were sacrificed for lymphoma model experiments. Drug experiments were only done in female mice.

Field-collected samples

This study did not involve samples collected from the field.

Ethics oversight

All mouse experiments were approved by the Animals Committee for Animal Experimentation of the National Cancer Center (approval ID: T17-081) and met the Guidelines for Proper Conduct of Animal Experiments (the Science Council of Japan).

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

Flow Cytometry

Plots

Confirm that:

- [x] The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- [x] 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).
- [x] All plots are contour plots with outliers or pseudocolor plots.
- [x] A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	SP and BM cells from the femur and tibia were filtered through a 70 µm mesh filter to obtain single cell suspensions. The samples were then resuspended in PBS containing 3% fetal bovine serum (FBS; Biosera). The suspension was layered over Histopaque (Sigma-Aldrich) and centrifuged at 400 × g for 20 min. The interphase was subsequently washed with 3% FBS-containing PBS. SG and other site tumor samples were processed using Tumor Dissociation Kit (Miltenyi Biotec) and the GentleMACS dissociator (Miltenyi Biotec), following the manufacturer's instructions. After centrifugation at 300 × g for 5 min at 4°C, the samples were resuspended in red cell lysis buffer (156 mM NH4Cl, 20 mM NaHCO3, and 0.1 mM EDTA [Nacalai Tesque]) and incubated for 5 min at room temperature. The samples were then centrifuged again, with the supernatant being discarded. PB was treated with 700 µL of red cell lysis buffer for 5 min at room temperature. After centrifugation at 300 × g for 5 min at room temperature, the supernatant was removed. Lysis and washing were repeated twice.
Instrument	For analysis, LSRFortessa X-20 was used. For sorting, FACSAria II or III Cell Sorter was used.
Software	FCS files were analyzed using FlowJo version 10.7.1.
Cell population abundance	Cell subset frequencies were calculated from event counts for each gate.
Gating strategy	For all flow cytometry experiments, gates were drawn based on unstained, single-strained, and fluorescence-minus-one (FMO) controls. The gating strategy is demonstrated in Supplementary Fig. 9.

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