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Last updated by author(s): 4/30/2024

Reporting Summary

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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 software v8.0.1(LSRII, Fortessa, A3 Lite, and AriaII) for flow cytometry data. Gen5 3.04 for in vitro killing assay data. Living Image 4.4 (PerkinElmer) for bioluminescence flux data. Zen 2.5 (blue edition, Zeiss) software for microscopy data.

Data analysis

FlowJo v10.8.1 for flow cytometry data. Living Image 4.4 (PerkinElmer) for bioluminescence flux data. Statistical analyses were performed using GraphPad Prism (v9.4.0).
For single cell analysis, fastq files were analyzed with Cell Ranger v6.1.2.
No custom code was created for this study.
The code used was deposited to Zenodo (10.5281/zenodo.11672288).

For analysis performed in R, we used the following packages:
#R version 4.3.1 (2023-06-16)
#Platform: aarch64-apple-darwin20 (64-bit)
#Running under: macOS Monterey 12.6.2
#Matrix products: default
#BLAS: /System/Library/Frameworks/Accelerate.framework/Versions/A/Frameworks/vecLib.framework/Versions/A/libBLAS.dylib
#LAPACK: /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRlapack.dylib; LAPACK version 3.11.0
#locale:
[1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
#time zone: America/New_York
#tzcode source: internal

```
#attached base packages:
# [1] stats4    grid      stats     graphics  grDevices utils     datasets  methods   base

#other attached packages:
#  [1] ggnuplot_0.1.0           RColorBrewer_1.1-3       DropletUtils_1.20.0
#[4] SingleCellExperiment_1.24.0 SummarizedExperiment_1.32.0 Biobase_2.62.0
#[7] GenomicRanges_1.54.1       GenomeInfoDb_1.38.5      IRanges_2.36.0
#[10] S4Vectors_0.40.2          BiocGenerics_0.48.1      MatrixGenerics_1.14.0
#[13] matrixStats_1.3.0         glmGamPoi_1.14.0         gridExtra_2.3
#[16] EnhancedVolcano_1.18.0    ggrepel_0.9.5            sctransform_0.4.1
#[19] dplyr_1.1.4               cowplot_1.1.3            fgsea_1.26.0
#[22] patchwork_1.2.0           ggplot2_3.5.1            SeuratObject_5.0.2
#[25] Seurat_4.4.0              rlang_1.1.3              irlba_2.3.5.1
#[28] Matrix_1.6-5

#loaded via a namespace (and not attached):
# [1] RcppAnnoy_0.0.22         splines_4.3.1            later_1.3.2
#[4] bitops_1.0-7             R.oo_1.26.0              tibble_3.2.1
#[7] polyclip_1.10-6          lifecycle_1.0.4          edgeR_3.42.4
#[10] globals_0.16.3          lattice_0.22-6           MASS_7.3-60.0.1
#[13] magrittr_2.0.3          limma_3.56.2             plotly_4.10.4
#[16] remotes_2.5.0           httpuv_1.6.15            spam_2.10-0
#[19] sp_2.1-4                sessioninfo_1.2.2        pkgbuild_1.4.4
#[22] spatstat.sparse_3.0-3   reticulate_1.37.0         pbapply_1.7-2
#[25] abind_1.4-5             pkgload_1.3.4            zlibbioc_1.48.0
#[28] Rtsne_0.17              purrr_1.0.2              R.utils_2.12.3
#[31] RCurl_1.98-1.14         GenomeInfoDbData_1.2.11  listenv_0.9.1
#[34] spatstat.utils_3.0-4.001 goftest_1.2-3           dqrng_0.4.1
#[37] spatstat.random_3.2-3   fitdistrplus_1.1-11      parallelly_1.37.1
#[40] DelayedMatrixStats_1.24.0 leiden_0.4.3.1         codetools_0.2-19
#[43] DelayedArray_0.28.0     scuttle_1.10.3           tidyselect_1.2.1
#[46] farver_2.1.2            spatstat.explore_3.2-7   jsonlite_1.8.8
#[49] ellipsis_0.3.2          progressr_0.14.0         ggridges_0.5.6
#[52] survival_3.5-8          tools_4.3.1              ica_1.0-3
#[55] Rcpp_1.0.12             glue_1.7.0               SparseArray_1.2.3
#[58] usethis_2.2.3           HDF5Array_1.30.0         withr_3.0.0
#[61] fastmap_1.2.0           rhdf5filters_1.14.1      fansi_1.0.6
#[64] digest_0.6.35           R6_2.5.1                 mime_0.12
#[67] colorspace_2.1-0        scattermore_1.2          tensor_1.5
#[70] spatstat.data_3.0-4     R.methodsS3_1.8.2        utf8_1.2.4
#[73] tidyr_1.3.1             generics_0.1.3           data.table_1.15.4
#[76] httr_1.4.7              htmlwidgets_1.6.4        S4Arrays_1.2.0
#[79] uwot_0.2.2              pkgconfig_2.0.3          gtable_0.3.5
#[82] lmtest_0.9-40           XVector_0.42.0           htmltools_0.5.8.1
#[85] profvis_0.3.8           dotCall64_1.1-1          scales_1.3.0
#[88] png_0.1-8               rstudioapi_0.15.0        reshape2_1.4.4
#[91] nlme_3.1-164            cachem_1.1.0             zoo_1.8-12
#[94] rhdf5_2.46.0            stringr_1.5.1            KernSmooth_2.23-22
#[97] parallel_4.3.1          miniUI_0.1.1.1           pillar_1.9.0
#[100] vctrs_0.6.5            RANN_2.6.1               urlchecker_1.0.1
#[103] promises_1.3.0         beachmat_2.18.0          xtable_1.8-4
#[106] cluster_2.1.6          locfit_1.5-9.9           cli_3.6.2
#[109] compiler_4.3.1         crayon_1.5.2             future.apply_1.11.2
#[112] labeling_0.4.3         plyr_1.8.9               fs_1.6.4
#[115] stringi_1.8.4          viridisLite_0.4.2        deldir_2.0-4
#[118] BiocParallel_1.36.0    munsell_0.5.1            lazyeval_0.2.2
#[121] devtools_2.4.5         spatstat.geom_3.2-9      sparseMatrixStats_1.14.0
#[124] future_1.33.2          Rhdf5lib_1.24.0          shiny_1.8.1.1
#[127] ROCR_1.0-11            igraph_2.0.3             memoise_2.0.1
#[130] fastmatch_1.1-4

For code used in python (pyscenic analysis), we used the following packages:
-----
anndata    0.8.0
scanpy     1.7.2
sinfo      0.3.1
-----
MulticoreTSNE      NA
PIL                9.1.0
anndata            0.8.0
anyio              NA
appnope            0.1.3
arrow              1.2.3
attr               21.4.0
babel              2.14.0
backcall           0.2.0
```

```
beta_ufunc          NA
binom_ufunc         NA
bottleneck          1.3.4
brotli              NA
cached_property     1.5.2
certifi             2021.10.08
cffi                1.15.0
charset_normalizer  2.0.12
cloudpickle         2.0.0
colorama            0.4.4
cycler              0.10.0
cython_runtime      NA
cytoolz             0.11.0
dask                2022.02.0
dateutil            2.8.2
debugpy             1.6.0
decorator           5.1.1
defusedxml          0.7.1
dunamai             1.11.1
entrypoints         0.4
fastjsonschema      NA
fqdn                NA
fsspec              2022.3.0
get_version         3.5.4
h5py                3.6.0
idna                3.3
igraph              0.9.10
importlib_resources NA
ipykernel           6.16.2
ipython_genutils    0.2.0
isoduration         NA
jedi                0.18.1
jinja2              3.1.1
joblib              1.1.0
json5               NA
jsonpointer         2.4
jsonschema          4.17.3
jupyter_server      1.24.0
jupyterlab_server   2.24.0
kiwisolver          1.4.2
legacy_api_wrap     0.0.0
llvmlite            0.38.0
loompy              3.0.7
louvain             0.7.1
markupsafe          2.1.1
matplotlib          3.5.1
matplotlib_inline   NA
mpl_toolkits        NA
natsort             8.1.0
nbformat            5.3.0
nbinom_ufunc        NA
numba               0.55.1
numexpr             2.8.0
numpy               1.21.6
numpy_groupies      0.9.14
packaging           21.3
pandas              1.3.4
parso               0.8.3
pexpect             4.8.0
pickleshare         0.7.5
pkg_resources       NA
prometheus_client   NA
prompt_toolkit      3.0.39
psutil              5.9.0
ptyprocess          0.7.0
pvectorc            NA
pyarrow             0.16.0
pycparser           2.21
pydev_ipython       NA
pydevconsole        NA
pydevd              2.8.0
pydevd_file_utils   NA
pydevd_plugins      NA
pydevd_tracing      NA
pygments            2.11.2
pyparsing           3.0.8
```

```
pyrsistent          NA
pyscenic            0.11.2
pytz                2022.1
requests            2.31.0
rfc3339_validator   0.1.4
rfc3986_validator   0.1.1
scanpy              1.7.2
scipy               1.7.3
send2trash          NA
setuptools          62.1.0
setuptools_scm      NA
sinfo               0.3.1
six                 1.16.0
sklearn             1.0.2
sniffio             1.3.1
socks               1.7.1
sphinxcontrib       NA
storemagic          NA
tables              3.7.0
tblib               1.7.0
terminado           0.13.3
texttable           1.6.4
threadpoolctl       3.1.0
tlz                 0.11.0
toolz               0.11.1
tornado             6.1
traitlets           5.9.0
typing_extensions   NA
unicodedata2        NA
uri_template        NA
urllib3             1.26.9
wcwidth             0.2.5
webcolors           1.13
websocket           1.6.1
yaml                6.0
zipp                NA
zmq                 22.3.0
-----
IPython             7.32.0
jupyter_client      7.2.2
jupyter_core        4.12.0
jupyterlab          3.6.7
notebook            6.4.10
-----
Python 3.7.12 | packaged by conda-forge | (default, Oct 26 2021, 05:59:23) [Clang 11.1.0 ]
Darwin-21.6.0-x86_64-i386-64bit
10 logical CPU cores, i386

For python analysis of RNA velocity, the following packages were used:
-----
anndata     0.8.0
scanpy      1.8.2
sinfo       0.3.1
-----
PIL                     9.0.1
anndata                 0.8.0
appnope                 0.1.2
asttokens               NA
backcall                0.2.0
beta_ufunc              NA
binom_ufunc             NA
cellrank                1.5.1
cffi                    1.15.0
colorama                0.4.4
cycler                  0.10.0
cython_runtime          NA
dateutil                2.8.2
debugpy                 1.5.1
decorator               5.1.1
defusedxml              0.7.1
docrep                  0.3.2
entrypoints             0.4
executing               0.8.3
h5py                    3.6.0
hypergeom_ufunc         NA
igraph                  0.10.0
```

```
ipykernel                6.9.2
ipython_genutils         0.2.0
jedi                    0.18.1
joblib                   1.1.0
kiwisolver               1.4.0
llvmlite                0.38.0
louvain                  0.7.1
matplotlib               3.5.1
matplotlib_inline           NA
mpl_toolkits                NA
natsort                  8.1.0
nbinom_ufunc                NA
numba                   0.55.1
numexpr                  2.8.0
numpy                   1.21.5
packaging                21.3
pandas                   1.4.1
parso                    0.8.3
petsc4py                 3.16.5
pexpect                  4.8.0
pickleshare              0.7.5
pkg_resources               NA
progressbar              4.0.0
prompt_toolkit           3.0.27
psutil                  5.9.0
ptyprocess               0.7.0
pure_eval                0.2.2
pydev_ipython               NA
pydevconsole                NA
pydevd                  2.6.0
pydevd_concurrency_analyser NA
pydevd_file_utils           NA
pydevd_plugins              NA
pydevd_tracing              NA
pygam                    0.8.0
pygments                 2.11.2
pygpcca                  1.0.3
pyparsing                3.0.7
python_utils                NA
pytz                    2022.1
scanpy                   1.8.2
scipy                   1.8.0
scvelo                   0.2.4
seaborn                  0.11.2
setuptools               60.10.0
sinfo                   0.3.1
six                     1.16.0
sklearn                  1.0.2
slepc4py                 3.16.1
sphinxcontrib               NA
stack_data               0.2.0
statsmodels              0.13.2
tables                   3.7.0
texttable                1.6.4
threadpoolctl               3.1.0
tornado                  6.1
tqdm                    4.63.1
traitlets                5.1.1
typing_extensions           NA
wcwidth                  0.2.5
wrapt                   1.14.0
zipp                        NA
zmq                      22.3.0
-----
IPython          8.1.1
jupyter_client   7.1.2
jupyter_core     4.9.2
notebook         6.4.10
-----
Python 3.10.4 | packaged by conda-forge | (main, Mar 24 2022, 17:45:10) [Clang 12.0.1 ]
macOS-12.6.2-x86_64-i386-64bit
10 logical CPU cores, i386
```

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

Sequencing data have been deposited on GEO with accession number GSE268878. The reference genome for single cell RNA sequencing analysis is Human (GRCh38) 2020-A, Human (GRCh38) v5.0.0.

Human research participants

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

Reporting on sex and gender	Healthy human donors of male and female sexes were used in this study. Sex was determined by self reporting information collected by the Penn Human Immunology Core. Study was not designed to detect differences between sexes.
Population characteristics	T cells were isolated from healthy human donors by the Penn Human Immunology Core.
Recruitment	Apheresis product was collected from healthy human donors by the Penn Human Immunology Core. Samples were used without any selection
Ethics oversight	All human studies were in compliance with the Declaration of Helsinki. Apheresis donors were recruited by the Penn Human Immunology Core, which maintains the IRB protocols at the University of Pennsylvania necessary for human cell procurement and distribution of de-identified reagents.

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 not predetermined based on statistical methods, but were chosen based on preliminary data and previously published results. We performed at least three independent experiments for flow cytometry, in vitro cytotoxicity, and real-time metabolic assay experiments; 5-8 NSG mice per treatment group; and 2 independent single-cell profiling experiments. Sample size is stated in each caption.
Data exclusions	Negative OCR values in the real-time metabolic assay was set to zero, as negative values do not represent biological meaning and are appreciated to be technical artifacts when studying 1.5x10^5 T cells per well. Bioluminescence outliers were removed from plotting but included in source data.
Replication	Reported results were replicated across multiple experiments with all replicates generating consistent results. The number of replicates for each experiment are detailed in the figure legends.
Randomization	For all in vivo experiments, treatment groups were randomly selected by the cage number. Randomization was not required for in vitro experiments as each experimental condition was controlled within each T cell donor (e.g. proximal and distal daughter cells from the same donor).
Blinding	In vivo injections were performed in a blinded fashion by a member of the Ellebrecht or Payne laboratories or a staff member of the Human Stem Cell and Xenograft Core of the University of Pennsylvania. For all in vitro experiments, data collection was not blinded, however, data were obtained with objective methodologies (sequencing, flow cytometry). Fully blinded in vitro experiments were not possible due to limited staff availability.

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

Unless otherwise specified, antibodies were purchased from BioLegend.
LIPSTIC assay populations were sorted and subsequently phenotyped by staining with CD8-APCH7 (SK1, BD Biosciences, 561423), CD4-BUV805 (SK3, BD Biosciences, 612887), CCR7-APC/Cy7 (G043H7, 353212), CD45RA-BV650 (HI100, 304136), CD45RA-BUV395 (HI100, BD Biosciences, 740298), CD45RO (UCHL1, 304234), CD25-BV711 (M-A251, 356138), CD62L-PE (DREG-56, 304840), and/or CD62L-BV605 (DREG-56, 304834).
For in vivo studies, samples were stained with CD8-APC/Cy5.5 (RFT8, SouthernBiotech 9536-18), CD4-PE/Cy5.5 (RFT4, SouthernBiotech 9522-16), CD3-BV605 (OKT3, 317322), CD19-APC (HIB19, 302212), CD45RA-BUV395 (HI100, 740298), CD45RO-BV785 (UCHL1, 304234), and CD45-PECy7 (QA17A19, 393408).
The following antibodies were used for western blots: rabbit anti-Ikaros (IKZF1) monoclonal antibody (Cell Signaling, 9034S), digital anti-rabbit-HRP (Kindle Biosciences, LLC, R1006), mouse anti-β-actin monoclonal antibody (Cell Signaling, 3700S), and digital anti-mouse-HRP (Kindle Biosciences, LLC, R1005).
The pre-mixed totalseq C human super panel cocktail by Biolegend was used (cat# 900000114, lot# B311489). The dilution and concentration of each antibody in this cocktail is proprietary information owned by Biolegend and not available to us. The following clones are included in the panel (along with their DNA barcode):
The order of the following lines is a follows: id of antibody - Clone name - target specificity - DNA sequence
C0005 2D10 CD80 ACGAATCAATCTGTG
C0006 IT2.2 CD86 GTCTTTGTCAGTGCA
C0007 29E.2A3 CD274B7-H1PD-L1 GTTGTCCGACAATAC
C0008 24F.10C12 CD273B7-DCPD-L2 TCAACGCTTGGCTAG
C0009 2D3 CD275B7-H2ICOSL GTGCATTCAACAGTA
C0020 122 CD270HVEMTR2 TGATAGAAACAGACC
C0021 11C3.1 CD252OX40L TTTAGTGATCCGACT
C0022 5F4 CD137L4-1BBLigand ATTCGCCTTACGCAA
C0023 SKII.4 CD155PVR ATCACATCGTTGCCA
C0024 TX31 CD112Nectin-2 AACCTTCCGTCTAAG
C0026 CC2C6 CD47 GCATTCTGTCACCTA
C0027 113-16 CD70 CGCGAACATAAGAAG
C0028 BY88 CD30 TCAGGGTGTGCTGTA
C0029 BJ40 CD48 CTACGACGTAGAAGA
C0031 5C3 CD40 CTCAGATGGAGTATG
C0032 24-31 CD154 GCTAGATAGATGCAA
C0033 HI186 CD52 CTTTGTACGAGCAAA
C0034 UCHT1 CD3 CTCATTGTAACTCCT
C0046 SK1 CD8 GCGCAACTTGATGAT
C0047 5.1H11 CD56 TCCTTTCCTGATAGG
C0050 HIB19 CD19 CTGGGCAATTACTCG
C0052 P67.6 CD33 TAACTCAGGGCCTAT
C0053 S-HCL-3 CD11c TACGCCTATAACTTG
C0054 581 CD34 GCAGAAATCTCCCTT
C0056 19F2 CD269BCMA CAGATGATCCACCAT
C0058 W6/32 HLA-ABC TATGCGAGGCTTATC
C0061 104D2 CD117c-kit AGACTAATAGCTGAC
C0062 HI10a CD10 CAGCCATTCATTAGG
C0063 HI100 CD45RA TCAATCCTTCCGCTT
C0064 6H6 CD123 CTTCACTCTGTCAGG
C0066 CD7-6B7 CD7 TGGATTCCCGGACTT
C0068 43A3 CD105 ATCGTCGAGAGCTAG
C0069 RCR-401 CD201EPCR GTTTCCTTGACCAAG
C0070 GoH3 mouseCD49f TTCCGAGGATGATCT
C0071 L291H4 CD194CCR4 AGCTTACCTGCACGA
C0072 RPA-T4 CD4 TGTTCCCGCTCAACT
C0073 IM7 CD44 TGGCTTCAGGTCCTA
C0081 M5E2 CD14 TCTCAGACCTCCGTA
C0083 3G8 CD16 AAGTTCACTCTTTGC
C0085 BC96 CD25 TTTGTCCTGTACGCC
C0087 UCHL1 CD45RO CTCCGAATCATGTTG

C0088 EH12.2H7 CD279 ACAGCGCCGTATTTA
C0089 A15153G TIGITVSTM3 TTGCTTACCGCCAGA
C0090 MOPC-21 MouseIgG1kappaisotypeCtrl GCCGGACGACATTAA
C0091 MOPC-173 MouseIgG2akappaisotypeCtrl CTCCTACCTAAACTG
C0092 H1.2F3 MouseIgG2bkappaisotypeCtrl ATATGTATCACGCGA
C0095 RTK4530 RatIgG2bkappaIsotypeCtrl GATTCTTGACGACCT
C0100 2H7 CD20 TTCTGGGTCCCTAGA
C0101 900 CD335NKp46 ACAATTTGAACAGCG
C0102 BM16 CD294CRTH2 TGTTTACGAGAGCCC
C0103 RA3-6B2 CD45R/B220 CCTACACCTCATAAT
C0123 9C4 CD326Ep-CAM TTCCGAGCAAGTATC
C0124 WM59 CD31 ACCTTTATGCCACGG
C0127 NC-08 Podoplanin GGTTACTCGTTGTGT
C0128 16A1 CD140aPDGFRalpha ATGCGCCGAGAATTA
C0129 18A2 CD140bPDGFRbeta CAATGGTTCACTGCC
C0132 AY13 EGFR GCTTAACATTGGCAC
C0134 P1H12 CD146 CCTTGGATAACATCA
C0135 67A4 CD324E-Cadherin ATCCTTCTCCCTTTC
C0136 MHM-88 IgM TAGCGAGCCCGTATA
C0138 UCHT2 CD5 CATTAACGGGATGCC
C0139 B1 TCRdeltagamma CTTCCGATTCATTCA
C0140 G025H7 CD183CXCR3 GCGATGGTAGATTAT
C0141 J418F1 CD195CCR5 CCAAAGTAAGAGCCA
C0142 FUN-2 CD32 GCTTCCGAATTACCG
C0143 G034E3 CD196CCR6 GATCCCTTTGTCACT
C0144 J252D4 CD185CXCR5 AATTCAACCGTCGCC
C0145 Ber-ACT8 CD103IntegrinalphaE GACCTCATTGTGAAT
C0146 FN50 CD69 GTCTCTTGGCTTAAA
C0147 DREG-56 CD62L GTCCCTGCAACTTGA
C0148 G043H7 CD197CCR7 AGTTCAGTCAACCGA
C0149 HP-3G10 CD161 GTACGCAGTCCTTCT
C0151 BNI3 CD152CTLA-4 ATGGTTCACGTAATC
C0152 11C3C65 CD223LAG-3 CATTTGTCTGCCGGT
C0153 SA231A2 KLRG1MAFA CTTATTTCCTGCCCT
C0154 O323 CD27 GCACTCCTGCATGTA
C0155 H4A3 CD107aLAMP-1 CAGCCCACTGCAATA
C0156 DX2 CD95Fas CCAGCTCATTAGAGC
C0158 Ber-ACT35 (ACT35) CD134OX40 AACCCACCGTTGTTA
C0159 L243 HLA-DR AATAGCGAGCAAGTA
C0160 L161 CD1c GAGCTACTTCACTCG
C0161 ICRF44 CD11b GACAAGTGATCTGCA
C0162 10.1 CD64 AAGTATGCCCTACGA
C0163 M80 CD141Thrombomodulin GGATAACCGCGCTTT
C0164 51.1 CD1d TCGAGTCGCTTATCA
C0165 1D11 CD314NKG2D CGTGTTTGTTCCTCA
C0166 6/40c CD66b AGCTGTAAGTTTCGG
C0167 E11 CD35 ACTTCCGTCGATCTT
C0168 QA17A04 CD57Recombinant AACTCCCTATGGAGG
C0169 F38-2E2 CD366Tim-3 TGTCCTACCCAACTT
C0170 MIH26 CD272BTLA GTTATTGGACTAAGG
C0171 C398.4A CD278ICOS CGCGCACCCATTAAA
C0174 TS2/9 CD58LFA-3 GTTCCTATGGACGAC
C0175 NK92.39 CD96TACTILE TGGCCTATAAATGGT
C0176 A1 CD39 TTACCTGGTATCCGT
C0177 NOK-1 CD178Fas-L CCGGTCCTCTGTATT
C0179 K0124E1 CX3CR1 AGTATCGTCTCTGGG
C0180 ML5 CD24 AGATTCCTTCGTGTT
C0181 Bu32 CD21 AACCTAGTAGTTCGG
C0185 TS2/4 CD11a TATATCCTTGTGAGC
C0187 CB3-1 CD79bIgB ATTCTTCAACCGAAG
C0188 ASL-32 CD66ace GGGACAGTTCGTTTC
C0189 C1.7 CD2442B4 TCGCTTGGATGGTAG
C0196 HIR2 CD235ab GCTCCTTTACACGTA
C0205 15-2 CD206MMR TCAGAACGTCTAACT
C0206 7-239 CD169SialoadhesinSiglec-1 TACTCAGCGTGTTTG
C0207 8F9 CD370CLEC9ADNGR1 CTGCATTTCAGTAAG
C0208 S15046E XCR1 AAGACGCATGTCAAC
C0213 MHN1-519 Notch1 AATCTGTAGTGCGTT
C0214 FIB504 integrinbeta7 TCCTTGGATGTACCG
C0215 11C1 CD268BAFF-R CGAAGTCGATCCGTA
C0216 HIP1 CD42b TCCTAGTACCGAAGT
C0217 HA58 CD54 CTGATAGACTTGAGT
C0218 AK4 CD62PP-Selectin CCTTCCGTATCCCTT
C0219 GIR-208 CD119IFNgammaRalphachain TGTGTATTCCCTTGT
C0224 IP26 TCRalpha/beta CGTAACGTAGAGCGA
C0233 MHN3-21 Notch3 CTATTGGACGTATCT

C0236 RTK2071 RatIgG1kappaisotypeCtrl ATCAGATGCCCTCAT
C0238 RTK2758 RatIgG2akappaIsotypeCtrl AAGTCAGGTTCGTTT
C0241 HTK888 ArmenianHamsterIgGIsotypeCtrl CCTGTCATTAAGACT
C0245 STA CD106 TCACAGTTCCTTGGA
C0246 TU27 CD122IL-2Rbeta TCATTTCCTCCGATT
C0247 1A1 CD267TACI AGTGATGGAGCGAAC
C0248 HAE-1f CD62E CTCCCTGTGGCTTAA
C0352 AER-37 (CRA-1) FcepsilonRIalpha CTCGTTTCCGTATCG
C0353 HIP8 CD41 ACGTTGTGGCCTTGT
C0355 4B4-1 CD1374-1BB CAGTAAGTTCGGGAC
C0356 MIH24 CD254TRANCERANKL TCCGTGTTAGTTTGT
C0358 GHI/61 CD163 GCTTCTCCTTCCTTA
C0359 HB15e CD83 CCACTCATTTCCGGT
C0360 108-17 CD357GITR ACCTTTCGACACTCG
C0363 G077F6 CD124IL-4Ralpha CCGTCCTGATAGATG
C0364 WM15 CD13 TTTCAACGCCCTTTC
C0366 12G5 CD184CXCR4 TCAGGTCCTTTCAAC
C0367 TS1/8 CD2 TACGATTTGTCAGGG
C0368 11A8 CD226DNAM-1 TCTCAGTGTTTGTGG
C0369 TS2/16 CD29 GTATTCCCTCAGTCA
C0370 201A CD303BDCA2 GAGATGTCCGAATTT
C0371 P1E6-C5 CD49b GCTTTCTTCAGTATG
C0373 5A6 CD81TAPA-1 GTATCCTTCCTTGGC
C0374 MEM-108 CD98 GCACCAACAGCCATT
C0375 M1310G05 IgGFc CTGGAGCGATTAGAA
C0384 IA6-2 IgD CAGTCTCCGTAGAGT
C0385 TS1/18 CD18 TATTGGGACACTTCT
C0386 CD28.2 CD28 TGAGAACGACCCTAA
C0387 1D3 TSLPRTSLP-R CAGTCCTCTCTGTCA
C0389 HIT2 CD38 TGTACCCGCTTGTGA
C0390 A019D5 CD127IL-7R GTGTGTTGTCCTATG
C0391 HI30 CD45 TGCAATTACCCGGAT
C0393 S-HCL-1 CD22 GGGTTGTTGTCTTTG
C0394 CY1G4 CD71 CCGTGTTCCTCATTA
C0395 MIH43 B7-H4 TGTATGTCTGCCTTG
C0396 BA5b CD26 GGTGGCTAGATAATG
C0399 7C9C20 CD204 TAGCGAGCCAGATGT
C0400 BV9 CD144VE-Cadherin TCCACTCATTCTGTA
C0402 HI149 CD1a GATCGTGTTGTGTTA
C0406 12C2 CD304Neuropilin-1 GGACTAAGTTTCGTT
C0407 5-271 CD36 TTCTTTGCCTTGCCA
C0420 HP-MA4 CD158KIR2DL1S1S3S5 TATCAACCAACGCTT
C0433 8C11 CD325N-Cadherin CCTTCCCTTTCCTCT
C0437 4C7 aCD207 CGATTTGTATTCCCT
C0575 TS2/7 CD49a ACTGATGGACTCAGA
C0576 9F10 CD49d CCATTCAACTTCCGG
C0577 AD2 CD73 CAGTTCCTCAGTTCG
C0581 3C10 TCRValpha72 TACGAGCAGTATTCA
C0582 B6 TCRVdelta2 TCAGTCAGATGGTAT
C0583 B3 TCRVgamma9 AAGTGATGGTATCTG
C0584 6B11 TCRValpha24 AACTTCTGTGGTAGC
C0590 NKTA255 CD305LAIR1 ATTTCCATTCCCTGT
C0591 15C4 LOX-1 ACCCTTTACCGAATA
C0592 DX27 CD158bKIR2DL2L3NKAT2 GACCCGTAGTTTGAT
C0597 9E9A8 CD209DC-SIGN TCACTGGACACTTAA
C0599 DX9 CD158e1KIR3DL1NKB1 GGACGCTTTCCTTGA
C0600 UP-R1 CD158fKIR2DL5 AAAGTGATGCCACTG
C0801 P30-15 CD337NKp30 AAAGTCACTCTGCCG
C0802 P44-8 CD336NKp44 GGGCAATTAGCGAGT
C0828 413D12 CD307dFcRL4 CGATTTGATCTGCCT
C0829 509f6 CD307eFcRL5 TCACGCAGTCCTCAA
C0830 162.1 CD319CRACC AGTATGCCATGTCTT
C0845 3B2/TA8 CD99 ACCCGTCCCTAAGAA
C0853 50C1 CLEC12A CATTAGAGTCTGCCA
C0863 1D6 CD257BAFFBLYS CAGAGCACCCATTAA
C0864 NT-7 CD352NTB-A AGTTTCCACTCAGGC
C0867 DX22 CD94 CTTTCCGGTCCTACA
C0870 A12 (7D4) CD150SLAM GTCATTGTATGTCTG
C0894 MHK-49 Iglightchainkappa AGCTCAGCCAGTATG
C0895 M3/38 Mac-2 GATGCAATTAGCCGG
C0896 GHI/75 CD85jILT2 CCTTGTGAGGCTATG
C0897 EBVCS-5 CD23 TCTGTATAACCGTCT
C0898 MHL-38 Iglightchainlambda CAGCCAGTAAGTCAC
C0899 BB7.2 HLAA2 GAACATTTCCGACAA
C0901 7B11 GARPLRRC32 AGGTATGGTAGAGTA
C0902 6-434 CD328Siglec-7 CTTAGCATTTCACTG

C0908 H131 TCRVbeta131 TTATGGACGTATGGT
C0912 CG4 GPR56 GCCTAGTTTCCGTTT
C0918 3D12 HLA-E GAGTCGAGAAATCAT
C0920 ASL-24 CD82 TCCCACTTCCGCTTT
C0944 BB27 CD101BB27 CTACTTCCCTGTCAA
C0985 17A12 CD360IL-21R GAGGATGATGCCATG
C1046 S5/1 CD88C5aR GCCGCATGAGAAACA
C1051 mAb 84 Podocalyxin GAGCCGGTATAATGC
C1052 KF29 CD224 CTGATGAGATGTCAG
C1056 T5-39 CD258LIGHT ACTTCCCTGTAGAAA
C1057 JD3 DR3TRAMP GAGTTCCCTCAGTTC
C0956 APC-Streptavidin APC-Streptavidin GGTAACTCTGGTAGC

Validation

Unless specified otherwise, antibodies were purchased from Biolegend.
CD8-APCH7 (SK1, BD Biosciences, 561423):https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-h7-mouse-anti-human-cd8.561423
CD4-BUV805 (SK3, BD Biosciences, 612887):https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv805-mouse-anti-human-cd4.612887
CD45RA-BUV395 (HI100, BD Biosciences, 740298): https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv395-mouse-anti-human-cd45ra.740298
CD45RO (UCHL1, 304234): https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-human-cd45ro-antibody-7973
CD25-BV711 (M-A251, 356138): https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-human-cd25-antibody-13762
CD62L-PE (DREG-56, 304840): https://www.biolegend.com/en-us/products/pe-anti-human-cd62l-antibody-653?GroupID=BLG10034
CD8-APC/Cy5.5 (RFT8, SouthernBiotech 9536-18): https://www.southernbiotech.com/mouse-anti-human-cd8-apc-cy5-5-rft8-9536-18
CD4-PE/Cy5.5 (RFT4, SouthernBiotech 9522-16): https://www.southernbiotech.com/mouse-anti-human-cd4-pe-cy5-5-rft4-9522-16
CD3-BV605 (OKT3, 317322): https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-human-cd3-antibody-7666
CD19-APC (HIB19, 302212): https://www.biolegend.com/en-us/products/apc-anti-human-cd19-antibody-715
CD45RA-BUV395 (HI100, BD Biosciences, 740298): https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv395-mouse-anti-human-cd45ra.740298
CD45RO-BV785 (UCHL1, 304234): https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-human-cd45ro-antibody-7973?GroupID=GROUP658
CD45-PECy7 (QA17A19, 393408): https://www.biolegend.com/en-us/products/pe-cyanine7-anti-human-cd45-recombinant-antibody-16253
TCR alpha/beta-BV421 (IP26, 306722): https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-human-tcr-alpha-beta-antibody-8526

All antibodies were listed by the respective vendor as validated for flow cytometry.

Antibody validation information provided by Biolegend:
Antibody validation is a critical step in the journey towards obtaining consistent reproducibility in science. To ensure they are both specific and sensitive, we validate our antibodies through a variety of methods including:
Testing on multiple cell and tissue types with a variety of known expression levels.
Validation in multiple applications as a cross-check for specificity and to provide additional clarity for researchers.
Comparison to existing antibody clones.
Using cell treatments to modulate target expression, such as phosphatase treatment to ensure phospho-antibody specificity.

Antibody validation information provided by BD Biosciences:
Antibody specificity
BD Biosciences identifies key targets of interest in scientific research and develops its own specific antibodies or collaborates with top research scientists around the world to license their antibodies. We then transform these antibodies into flow cytometry reagents by conjugating them to a broad portfolio of high-performing dyes, including our vastly popular portfolio of BD Horizon Brilliant™ Dyes. A world-class team of research scientists helps ensure that these reagents work reliably and consistently for flow cytometry applications.
The specificity is confirmed using multiple methodologies that may include a combination of flow cytometry, immunofluorescence, immunohistochemistry or western blot to test staining on a combination of primary cells, cell lines or transfectant models.
All flow cytometry reagents are titrated on the relevant positive or negative cells. To save time and cell samples for researchers, test size reagents are bottled at an optimal concentration with the best signal-to-noise ratio on relevant models during the product development. To ensure consistent performance from lot-to-lot, each reagent is bottled to match the previous lot MFI.

Antibody validation information provided by Southern Biotech:
The success of any immunoassay hinges on the quality of the antibody reagents that are used. At SouthernBiotech, we produce all of our antibodies in-house and validate them with techniques including:
ELISA
FLISA
Flow cytometry
Western blot
Immunohistochemistry
Immunocytochemistry
Immunoprecipitation
By maintaining complete control over our antibody production workflow, we provide competitive pricing and next-day delivery, backed by fast and comprehensive technical support, giving you total confidence in your experimental results.

Validation information provided by Cell Signaling:

IKZF1 antibody: Ikaros (clone: D10E5) rabbit monoclonal antibody cat#9034: https://www.cellsignal.com/products/primary-antibodies/ikaros-d10e5-rabbit-mab/9034
beta-Actin β-Actin (8H10D10) mouse monoclonal antibody cat#3700: https://www.cellsignal.com/products/primary-antibodies/b-actin-8h10d10-mouse-mab/3700

Kindle Biosciences:
Digital anti mouse HRP (cat#: R1005): https://kindlebio.com/products/29-digital-anti-mouse-hrp.html

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Lenti-X 293T cells were purchased from Takara (Cat#632180). Nalm6 cells were provided by Dr. Michael Milone, originally obtained from DSMZ. K562 cells were obtained from ATCC (CCL-243).
Authentication	Nalm-6 cells were periodically authenticated by flow cytometry after staining with anti-CD19 antibody. Lenti-X 293T cell were not further authenticated since they were directly obtained from the vendor. K562 cells expressing CD64 were authenticated by staining for antibodies bound to CD64.
Mycoplasma contamination	All cells were periodically tested for mycoplasma contamination. None of the cell lines were contaminated.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study

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	NSG mice (male, 6-8 weeks) were provided by SCXC core at University of Pennsylvania. Mice were housed in a barrier facility with a 12 hour light-dark cycle at a temperature of 20-23 degrees Celsius with a humidity ranging from 30-70%.
Wild animals	No wild animals were used in this study
Reporting on sex	Only male NSG mice were used in this study.
Field-collected samples	No field-collected samples were used in this study
Ethics oversight	All studies involving animals were performed under a protocol approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

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	Sample preparation is described in the Methods.
Instrument	BD LSR II, Fortessa, or A3 Lite
Software	FlowJo (Tree Star, V10.8.1), BD FACSDiva software v8.0.1
Cell population abundance	Port-sort purity was performed by flow cytometry for one experiment and determined to be >85%.
Gating strategy	Lymphocytes and singlets were gated as shown in Ext. Data. Fig 2f for all T cell experiments. For LIPSTIC assay experiments, LIPSTIC positivity was determined as greater than the signal of CAR T cells incubated without target. Cell division was

determined by CTV dilution, with zero division signal determined by CTV-stained resting CAR T cells incubated without target.

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