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Reporting Summary

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

Statistics

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

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Vevo 770 (version V3.0.0) acquisition ultrasound software, Nikon Elements BR software (version 4.30.01), Improvision's Volocity software (version 6.3), Quantitative Imaging Corp, QCapture (Version 2.81), BD FACSDiva software (version 6.1.3).
Data analysis	Adobe Photoshop (CS6), ImageJ software (version 1.51C), BD FACSDiva software (version 6.1.3), FlowJo software (version 10), Microsoft Excel (2016), GraphPad Prism (versions 6, 7 and 8), R (version 3.4.4) (Cufflinks (version 2.2.1), samtools (version 0.1.19), MATLAB R2017b (version 9.3.0.713579), Slicer (version 4.8.1).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data availability	
The data generated are available from corresponding authors 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

Life sciences study design

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

Sample size	No statistical methods were used to predetermine sample size. At least 3 samples were used for each group, the minimum to achieve statistical significance, unless otherwise noted.
Data exclusions	No data were excluded from analysis.
Replication	HCMV-infected BLT-L mice assayed for HCMV-specific immune responses represent 10 different human tissue donors with unique HLA types. For the measurement of HCMV-specific IgM and IgG, randomly selected samples were re-run to assess intra-assay variability. All tests showed low inter- and intra-assay variability (<10% CV). Samples assayed by Elispot for IFN-γ were run in triplicate wells (all attempts at replication were successful).
Randomization	No randomization was used to determine how samples/animals were allocated to experimental groups or processed. Groups of mice representing the same human donor were inoculated with the same HCMV strain so that cells from multiple mice could be combined for T cell analyses if necessary.
Blinding	Investigators were not blinded to group allocations or when assessing outcomes. In order assess the HCMV-specific T cell responses elicted by BLT-L mice, information regarding the HLA type of the mouse and infecting strain were needed to ensure that the appropriate reagents (e.g. pentamers, peptides, etc) were utilized in the assays. In addition, in some instances cells were pooled from multiple HLA-matched mice to evaluate HCMV-specific T cell responses (Figure 4D).

Reporting for specific materials, systems and methods

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

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	List of Antibodies: Antibodies were validated by the manufacturer with details in the links provided. Flow Cytometry: • anti-CD3-FITC (clone HIT3a) (3ul/test) BD Biosciences Cat #555339 https://www.biolegend.com/it-it/products/purified-anti-human-cd3-antibody-75 • anti-CD3-AlexaFluor® 700 (clone UCHT1) (3ul/test) BioRad Cat #MCA463A700 https://www.bio-rad-antibodies.com/monoclonal/human-cd3-antibody-ucht1-mca463.html?f=purified • anti-CD3-APC-R700 (clone UCHT1) (3ul/test) BD Biosciences Cat #565119 http://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/apc-r700-mouse-anti-human-cd3-ucht1-also-known-as-ucht-1-ucht-1/p/565119 • anti-CD4-APC-H7 (clone RPA-T4) (3ul/test) BD Biosciences Cat #560158 http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th-1-cells/surface-markers/human/apc-h7-mouse-anti-human-cd4-rpa-t4/p/560158 • anti-CD4-BV™605 (clone RPA-T4) (3ul/test)BD Biosciences Cat #562658 http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th-1-cells/surface-markers/human/bv605-mouse-anti-human-cd4-rpa-t4/p/562658 • anti-CD8-PerCP (clone SK1) (3ul/test) BD Biosciences Cat #347314 http://www.bdbiosciences.com/us/reagents/research/clinical-research---ruo-gmp/single-color-antibodies/percp-mouse-anti-human-cd8-sk1/p/347314 • anti-CD8-APC-Cy7 (clone SK1) (3ul/test) BD Biosciences Cat #557834 http://www.bdbiosciences.com/us/reagents/research/

antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/apc-cy7-mouse-anti-human-cd8-sk1/p/557834

• anti-CD19-PE-Cy7 (clone SJ25C1) (3ul/test) BD Biosciences Cat #557835 http://www.bdbiosciences.com/us/applications/research/clinical-research/oncology-research/blood-cell-disorders/surface-markers/human/pe-cy7-mouse-anti-human-cd19-sj25c1-also-known-as-sj25-c1/p/557835

• anti-CD25-APC (clone 2A3) (3ul/test) BD Biosciences Cat #340938 http://www.bdbiosciences.com/us/applications/clinical/blood-cell-disorders/asr-reagents/cd25-apc-2a3/p/340938

• anti-CD33-PE (clone P67.6) (3ul/test) BD Biosciences Cat #340679 https://www.bdbiosciences.com/us/applications/research/stem-cell-research/hematopoietic-stem-cell-markers/human/negative-markers/pe-mouse-anti-human-cd33-p676/p/347787

• anti-CD45-APC (clone HI30) (3ul/test) BD Biosciences Cat #555485 http://www.bdbiosciences.com/eu/applications/research/stem-cell-research/cancer-research/human/apc-mouse-anti-human-cd45-hi30/p/555485

• anti-CD45-V500 (clone HI30) (3ul/test) BD Biosciences Cat #560777 http://www.bdbiosciences.com/us/applications/research/stem-cell-research/cancer-research/human/v500-mouse-anti-human-cd45-hi30/p/560777

• anti-CD45RO-FITC (clone UCHL1) (3ul/test) BD Biosciences Cat #555492 http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/regulatory-t-cells/surface-markers/human/fitc-mouse-anti-human-cd45ro-uchl1/p/555492

• anti-CD69-PE (clone FN50) (3ul/test) BD Biosciences Cat #555531 http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/regulatory-t-cells/surface-markers/human/pe-mouse-anti-human-cd69-fn50-also-known-as-fn-50/p/555531

• anti-HLA-DR-PerCP (clone L243) (3ul/test) BD Biosciences Cat #347364 http://www.bdbiosciences.com/us/applications/research/stem-cell-research/mesenchymal-stem-cell-markers-bone-marrow/human/negative-markers/percp-mouse-anti-human-hla-dr-l243/p/347364

• anti-CCR7 (CD197)-PE-Cy7 (clone 3D12) (3ul/test) BD Biosciences Cat #557648 https://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th-2-cells/surface-markers/human/pe-cy7-rat-anti-human-ccr7-cd197/p/557648

• anti-CD38-APC (HB7) (3ul/test) BD Biosciences Cat #340439 http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/regulatory-t-cells/surface-markers/human/apc-mouse-anti-human-cd38-hb7/p/340439

• anti-CD45RA-Pacific Blue (F8-11-13) (3ul/test) BioRad Cat #MCA88PB https://www.bio-rad-antibodies.com/monoclonal/human-cd45ra-antibody-f8-11-13-mca88.html?f=purified

• anti-CD27-PE (M-T271) (3ul/test) BD Biosciences Cat #555441 http://www.bdbiosciences.com/us/applications/research/clinical-research/oncology-research/blood-cell-disorders/surface-markers/human/pe-mouse-anti-human-cd27-m-t271/p/555441

• anti-CD3-BV™421 (UCHT1) (3ul/test) BD Biosciences Cat #562426 http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th-1-cells/surface-markers/human/bv421-mouse-anti-human-cd3-ucht1-also-known-as-ucht-1-ucht-1/p/562426

• anti-CD4-AlexaFluor®488 (clone OKT4) (2uL per test) BioLegend Cat #317420 https://www.citeab.com/antibodies/522291-317420-alexa-fluor-488-anti-human-cd4-okt4-monocl

• anti-CD4-BV™650 (clone OKT4) (1uL per test) BioLegend Cat #317436 https://www.biolegend.com/en-us/products/brilliant-violet-650-anti-human-cd4-antibody-7786

• anti-CD8-BV™510 (clone RPA-T8) (2ul per test) BioLegend Cat #301048 https://www.biolegend.com/en-us/search-results/brilliant-violet-510-anti-human-cd8a-antibody-8000

• anti-CD11c-APC (clone S-HCL-3) (2ul per test) BD Biosciences Cat #333144 http://www.bdbiosciences.com/eu/reagents/clinical/reagents/single-antibodies/cd11c-apc-s-hcl-3/p/333144

• anti-CD11c-PE (clone B-LY6) (2ul per test) BD Biosciences Cat #555392 http://www.bdbiosciences.com/eu/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/pe-mouse-anti-human-cd11c-b-ly6/p/555392

• anti-CD14-BV™650 (clone M5E2) (2ul per test) BioLegend Cat #301836 https://www.biolegend.com/en-us/products/brilliant-violet-650-anti-human-cd14-antibody-7819

• anti-CD14-PerCP (clone HCD14) (2ul per test) BioLegend Cat #325632 https://www.biolegend.com/en-us/search-results/percp-anti-human-cd14-antibody-9564

• anti-CD16-PerCP (clone 3G8) (2ul per test) BioLegend Cat #302030 https://www.biolegend.com/en-us/search-results/percp-anti-human-cd16-antibody-4340

• anti-CD19-PerCP (clone HIB19) (2ul per test) BioLegend Cat #302228 https://www.biolegend.com/en-us/products/percp-anti-human-cd19-antibody-4225

• anti-CD56-PerCP (clone HCD56) (2ul per test) BioLegend Cat #318342 https://www.biolegend.com/en-us/search-results/percp-anti-human-cd56-ncam-antibody-8388

• anti-CD80-PE-Cy7 (clone L307.4) (1ul per test) BD Biosciences Cat #561135 https://www.bdbiosciences.com/us/applications/research/b-cell-research/surface-markers/human/pe-cy7-mouse-anti-human-cd80-l3074-also-known-as-l307/p/561135

• anti-CD83-APC (clone HB15E) (3ul per test) BD Biosciences Cat #551073 http://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/apc-mouse-anti-human-cd83-hb15e/p/551073

• anti-CD86-PE-Cy7 (clone 2331 FUN-1) (1ul per test) BD Biosciences Cat #561128 https://www.bdbiosciences.com/us/applications/research/t-cell-immunology/regulatory-t-cells/surface-markers/human/pe-cy7-mouse-anti-human-cd86-2331-fun-1/p/561128

• anti-CD107α-APC (clone H4A3) (1ul per test) BioLegend Cat #328620 https://www.biolegend.com/fr-fr/products/apc-anti-human-cd107a-lamp-1-antibody-5428

• anti-IFN-γ-PE (clone 4S.B3) (3ul per test) BioLegend Cat #502509 https://www.biolegend.com/en-us/products/pe-anti-human-ifn-gamma-antibody-1011

• anti-IFN-γ-FITC (clone 4S.B3) (5ul per test) BioLegend Cat #502505 https://www.biolegend.com/en-us/products/fitc-anti-human-ifn-gamma-antibody-1013

• anti-TNF-α-PE\Dazzle 594 (clone Mab11) (3ul per test) BioLegend Cat #502946 https://www.biolegend.com/en-us/search-results/pe-dazzle-594-anti-human-tnf-alpha-antibody-10268

• anti-HLA-A,B,C-PacificBlue (clone W6/32) (3ul per test) BioLegend Cat #311418 https://www.biolegend.com/fr-ch/products/pacific-blue-anti-human-hla-a-b-c-antibody-3336

• anti-HLA-DR-PE-Cy7 (clone L243) (1ul per test) BioLegend Cat #307616 https://www.biolegend.com/en-us/products/pe-cy7-anti-human-hla-dr-antibody-2862

• Mouse IgG1k Isotype Control-Pacific Blue (MOPC-21) (3 ul per test) BD Biosciences Cat #558120 http://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-

antigens/pacific-blue-mouse-igg1-isotype-control-mopc-21/p/558120

• Mouse IgG1k Isotype Control-PE (clone MOPC-21) (3ul per test) BD Biosciences Cat #559320 http://www.bdbiosciences.com/us/applications/research/intracellular-flow/intracellular-antibodies-and-isotype-controls/anti-human-antibodies/pe-mouse-igg1-isotype-control-mopc-21/p/559320

• Mouse IgG1k Isotype Control-APC (clone MOPC-21) (3ul per test) BD Biosciences Cat #555751 http://www.bdbiosciences.com/eu/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/apc-mouse-igg1-isotype-control-mopc-21/p/555751

• Mouse IgG2ak Isotype Control-FITC (clone G155-178) (3ul per test) BD Biosciences Cat #555573 http://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/fitc-mouse-igg2a-isotype-control-g155-178/p/555573

• Mouse IgG2a Isotype Control-PerCP (clone X39) (3 ul per test) BD Biosciences Cat #349054 https://www.bdbiosciences.com/eu/reagents/research/clinical-research---ruo-gmp/single-color-antibodies/percp-mouse-iggsub2asub-kappa-isotype-control-x39/p/349054

• Rat IgG2ak Isotype Control-PE-Cy7 (clone R35-95) (3ul per test) BD Biosciences Cat #557855 http://www.bdbiosciences.com/us/applications/research/intracellular-flow/intracellular-antibodies-and-isotype-controls/anti-human-antibodies/pe-cy7-rat-igg2a-isotype-control-r35-95/p/557855

Immunohistochemistry (IHC)/Immunofluorescence (IF)

• anti-CC10-unconjugated (clone E-11) (IF, 1:500 dilution) Santa Cruz Biotech Cat #SC-365992 https://www.scbt.com/scbt/product/cc10-antibody-e-11

• anti-CD3-unconjugated (clone SP7) (IHC, 1:150 dilution) ThermoFisher Cat #MA1-90582 https://www.thermofisher.com/antibody/product/CD3e-Antibody-clone-SP7-Monoclonal/MA1-90582

• anti-CD4-unconjugated (clone SP35) (IHC, 1:100 dilution) Genway Biotech Cat #GWB-B38C3A (product discontinued). Positive control-human tonsil tissue.

• anti-CD8-unconjugated (clone SP16) (IHC, 1:50 dilution) Biocare Medical Cat #CRM 311 C https://biocare.net/wp-content/uploads/PDF%20Data%20Sheets/311.pdf

• anti-CD11c-unconjugated (clone 5D11) (IHC, 1:100 dilution) Leica Biosystems Cat #CD11C-563-L-CE https://www.leicabiosystems.com/ihc-ish-fish/immunohistochemistry-ihc-antibodies-novocastra-reagents/primary-antibodies/products/cd11c/

• anti-CD20-unconjugated (clone L26) (IHC, 1:100 dilution) Biocare Medical Cat #CM004C https://biocare.net/product/cd20-antibody/

• anti-CD34-unconjugated (clone QBEnd10) (IHC, 1:200 dilution; IF, 1:100 dilution) Agilient Cat #M716501-2 https://www.agilent.com/store/en_US/Prod-M716501-2/M716501-2

• anti-mCD34-unconjugated (clone MEC 14.7) (IHC, 1:200 dilution) Novus Biologicals Cat #NB600-1071 https://www.novusbio.com/products/cd34-antibody-mec-147_nb600-1071

• anti-CD45-unconjugated (clones 2B11 and PD7/26) (IHC, 1:350 dilution) Agilent Cat #M070101-2 https://www.agilent.com/store/en_US/Prod-M070101-2/M070101-2

• anti-mCD45-unconjugated (clone 30-F11) (IHC, 1:200 dilution) BD Biosciences Cat #550539 http://www.bdbiosciences.com/eu/applications/research/stem-cell-research/cancer-research/human/purified-rat-anti-mouse-cd45-30-f11/p/550539

• anti-CD56-unconjugated (clone 123C3) (IHC, 1:50 dilution) Agilent Cat #M730429-2 https://www.agilent.com/store/en_US/Prod-M730429-2/M730429-2

• anti-CD68-unconjugated (clone KP1) (IHC, 1:200 dilution) Agilent Cat #M081401-2 https://www.agilent.com/store/en_US/Prod-M081401-2/M081401-2

• anti-Cytokeratin 19-unconjugated (clone EPR1579Y) (IHC, 1:100 dilution) Abcam Cat #ab76539 https://www.abcam.com/cytokeratin-19-antibody-epr1579y-ab76539.html

• anti-Cytokeratin 19-unconjugated (clone A53-B/A2) (IF, 1:100 dilution) Abcam Cat #ab7754 https://www.abcam.com/cytokeratin-19-antibody-a53-ba2-ab7754.html

• anti-Cytokeratin 19-unconjugated (clone KRT19) (IF, 1:500 dilution) Novus Biologicals Cat #NB100-687 https://www.novusbio.com/products/cytokeratin-19-antibody_nb100-687

• anti-mCytokeratin 19-unconjugated (clone EPNCIR127B) (IHC, 1:200 dilution) Abcam Cat #ab133496 https://www.abcam.com/cytokeratin-19-antibody-epncir127b-ab133496.html

• anti-Vimentin-unconjugated (clone V9) (IHC, 1:1,000 dilution, IF, 1:250 dilution) Abcam Cat #ab8069 https://www.abcam.com/vimentin-antibody-v9-cytoskeleton-marker-ab8069.html

• anti-HCMV Glycoprotein B-unconjugated (rabbit polyclonal) (IF, 1:500 dilution) Sino Biological Cat #10202-RP01 https://www.sinobiological.com/CMV-gB-Antibody-g-2881.html

• anti-HCMV IE-unconjugated (clone 6F8.2) (IHC, 1:2,000 dilution) Millipore Cat #MAB8131 https://www.emdmillipore.com/US/en/product/Anti-Cytomegalovirus-Antibody-immediate-early-clone-6F8.2,MM_NF-MAB8131

• anti-HCMV IE/E-unconjugated (clones DDG9 and CCH2) (IHC, 1:200 dilution) Cell Marque Cat #213M-15-ASR http://www.cellmarque.com/cmsial-ifu/ASR21321030.pdf

• anti-HCMV Late-unconjugated (clone QB1/06) (IHC, 1:50 dilution) Abcam Cat #ab49211 (product discontinued). Positive control-CMV infected tissue.

• Anti-Zika NS2B-unconjugated (rabbit polyclonal) (IF, 1:50 dilution) GeneTex Cat #GTX133308 https://www.genetex.com/Product/Detail/Zika-virus-NS2B-protein-antibody/GTX133308

• anti-MERS-CoV nucleocapsid-unconjugated (rabbit polyclonal) (IF: 1:200 dilution) LS Bio Cat #LS-C483529 https://www.lsbio.com/antibodies/mers-coronavirus-nucleocapsid-antibody-elisa-flow-icc-if-immunofluorescence-ihc-ip-wb-western-ls-c483529/496447

• anti-RSV-unconjugated (goat polyclonal) (IF, 1:500 dilution) Meridian Life Science Cat #B65860G https://meridianlifescience.com/bioSpecs/B65860G.pdf

• anti-RSV-unconjugated (goat polyclonal) (IF, 1:500 dilution) Abcam Cat #ab20745 https://www.abcam.com/respiratory-syncytial-virus-antibody-ab20745.html

• anti-β-Tubulin IV-unconjugated (clone ONS.1A6) (IF, 1:500 dilution) Sigma-Aldrich Cat #T7941 https://www.sigmaaldrich.com/catalog/product/sigma/t7941?lang=en&region=US

• Anti-Goat IgG heavy and light chains-AlexaFluor®594 (donkey polyclonal) (1:500 dilution) Invitrogen Cat #A-11058 https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11058

• Anti-Rabbit IgG heavy and light chains-AlexaFluor®488 (goat polyclonal) (1:500 dilution) Invitrogen Cat #A-11008 https://

www.thermofisher.com/antibody/product/A-11008.html?CID=AFLAG-A-11008
• Anti-Rabbit IgG heavy and light chains-AlexaFlour®568 (goat polyclonal) (1:500 dilution) Invitrogen Cat #A-11036 https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11036
• Anti-Mouse IgG heavy and light chains-AlexaFlour®488 (goat polyclonal) (1:500 dilution) Invitrogen Cat #A-11001 https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11001
• Anti-Mouse IgG heavy and light chains-AlexaFlour®568 (goat polyclonal) (1:500 dilution) Invitrogen Cat #A-11004 https://www.thermofisher.com/order/genome-database/details/antibody/A-11004.html?CID=AFLBC-A-11004
• Anti-Mouse IgG heavy and light chains-AlexaFlour®488 (donkey polyclonal) (1:500 dilution) Invitrogen Cat #A-21202 https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21202
• Rat IgG2ak Isotype Control-unconjugated (clone KLH/G2a-1-1) (dilution dependent on primary antibody concentration) Novus Biologicals Cat #NBP1-51104 https://www.novusbio.com/products/igg2a-isotype-control-klh-g2a-1-1_nbp1-51104
• Negative control mouse IgG1k-unconjugated (clone DAK-GO1) (dilution dependent on primary antibody concentration) Agilent Cat #X093101-2 https://www.agilent.com/store/en_US/Prod-X093101-2/X093101-2
• Negative control mouse IgG2ak-unconjugated (clone DAK-GO5) (dilution dependent on primary antibody concentration) Agilent Cat #X094301-2 https://www.agilent.com/store/en_US/Prod-X094301-2/X094301-2
• Negative control rabbit Ig fraction-unconjugated (rabbit polyclonal Ig) (dilution dependent on primary antibody concentration) Agilent Cat #X090302-8 https://www.agilent.com/store/en_US/Prod-X090302-8/X090302-8

| Validation | The specificity of the antibodies purchased from commercial sources (BD Biosciences, Biolegend, Biorad, Novus Biological, Santa Cruz Biotech, ThermoFisher, Genway Biotech, Biocare Medical, Leica Biosystems, Abcam, Sino Biological, Millipore, Cell Marque, Genetex, LS Bio, Meridian Life Sciences Invitrogen, Agilent, Sigma-Aldrich) were validated by the manufacturer as noted on their website (links provided above for each antibody). |

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Vero CCL-81, MRC-5, and ARPE-19 cells were purchased from ATCC
Authentication	Cell lines were authenticated by morphological identification and virus susceptibility profiles.
Mycoplasma contamination	Cell lines were tested negative for mycoplasma by the supplier.
Commonly misidentified lines (See ICLAC 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	Lung-only mice (LoM) were constructed using 13-18 week old male and female NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ mice (NSG; The Jackson Laboratory, Bar Harbor, ME) mice. BLT-Lung (BLT-L) mice were constructed using 10-15 week old male and female NSG mice.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve field-collected samples.
Ethics oversight	Animal studies were carried out according to protocols approved by the Institutional Use and Care Committee at UNC-Chapel Hill and in adherence to the NIH Guide for the Care and Use of Laboratory Animals.

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 for the flow cytometric analysis of peripheral blood and tissues from humanized mice is detailed in the |

Sample preparation	Materials and Methods.
Instrument	Flow cytometry data was collected on BD LSRFortessa or BD FACSCanto instruments using BD FACSDiva software (version 6.1.3)
Software	Flow cytometry data was analyzed with BDFACSDiva (version 6.1.3) or FlowJo software (version 10)
Cell population abundance	No cell populations were sorted by flow cytometry.
Gating strategy	For ICS and pentamer staining analyses, we first gated on singlets based on side scatter (SSC) area and height. Cell populations were gated based on their forward scatter (FSC) and SSC profiles. Gates to define positive and negative populations were defined by isotype and fluorescent minus one (FMO) controls when appropriate.

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