Corresponding author(s): Charles Rice, John Schoggins, Volker Thiel

Last updated by author(s): 6/24/2020

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	flow cytometry:CellCapture v5.0 (Stratedigm)
Data analysis	sequence analysis: Geneious Prime ® 2019.2.3, SeqMan Pro, ApE v2.0.49.10 statistical analyses: GraphPad Prism version 8.3.0 for Windows mRNA-Seq analysis: CLC Genomics Workbench 12.0.3 figures: GraphPad Prism version 8.3.0 for Windows, Adobe Illustrator v22.0.1, and Biorender Premium (2020) flow cytometry: FlowJo v9 (TreeStar), FlowJo v10 (Treestar) immunofluorescence analysis: ImageJ 2.0.0-rc-69/1.52p; Java 1.8.0_172 [64-bit]; Windows 10 10.0

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

The authors declare that the data supporting the findings of this study are available within the article and its Supplementary Information files or are available on request. The RNA-seq data discussed in this publication have been deposited in the Gene Expression Omnibus (GEO) database, https://www.ncbi.nlm.nih.gov/geo (GSE146074). Data used for scRNA-seq analysis of hAEC has been previously reported (Kelly et al. 2020 bioRxiv - full citation in main text). Data used for RNA-seq analysis of LY6E induction in hAEC after viral infection has been previously reported (V'kowski et al. 2020 bioRxiv - full citation in main text). Genbank (https://www.ncbi.nlm.nih.gov/genbank/) accession numbers for viral gene sequences used to generate S protein pseudoparticles: (VSV-G, NC_001560; HCoV-229E S,

X16816; MERS-CoV S, JX869059; SARS-CoV S, AY291315.1; SARS-CoV-2, NCBI Reference Sequence: YP_009724390.1) are available in the section 'VSV pseudoparticles'. The NCBI accession number for generating expression plasmid for C. dromedarius LY6E, XM_031439745.1, is available in Supplementary Information .

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 determine sample size. Sample size was determined to be adequate based on relevant publications (PMID: 19124753, 30190477) experimental consistency and effect, generally 3 independent biological replicates assayed with technical replicates.
Data exclusions	Ly6efl/fl;Vav1-iCre (KO or Ly6e delta HSC) mice were excluded post-mortem if Ly6e gene expression of splenocytes by qPCR equaled that of Ly6efl/fl WT mice. In these mice, Vav1-iCre was silenced which is why we checked both tail genotype and spleen gene expression. In extended data figure 1, one outlier was removed from the CHIKV infection, as the infection control did not work. No infection was observed in the control cells. Since infection was observed in other biological replicates, we assumed a technical error occurred in this replicate and thus removed it.
Replication	Aside from the CHIKV infection, all attempts to reproduce experimental findings were successful. Multiple independent experiments, enumerated in figure legends, were carried out for each assay with similar results.
Randomization	For in vivo experiments, sex and age-matched animals of a given genotype were randomized to experimental groups, regardless of litter. Randomization was otherwise not used as measures were determined to be quantitative and not subjective to bias..
Blinding	Blinding was not performed for group allocation during data collection or analysis outside of liver sample histology as analysis was performed through objective measures that were not subject to bias.

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

-MABI1: I1-Hybridoma ATCC® CRL-2700™
-anti-CD16/CD32 (2.4G2), Tonbo, Cat # 70-0161-U500
-anti-human MERS-CoV spike 1.6c7 (developed and kindly provided by BJ Bosch, no cat # available)
-anti-ACE2 (R&D Systems, AF933)
-anti-LY6E rabbit monoclonal antibody GEN-93-8-1 (Genentech) no catalogue number available
-anti-LY6E mouse monoclonal antibody 4D8.6.7 (Genentech) no catalogue number available
-Peroxidase-AffiniPure Donkey Anti-Rabbit IgG (Jackson Immunoresearch, catalogue number 711-035-152)
-Peroxidase-AffiniPure Donkey Anti-Human IgG (Jackson Immunoresearch, catalogue number 709-035-098)
-Monoclonal Anti-β-Actin-Peroxidase clone AC-15 (Sigma-Aldrich, catalogue number A3854)
-anti-HCoV-229E N protein (Anticuerpo Monoclonal, 1E7, Ingenasa, catalogue number M.30.HCo.I1E7)
-anti-dsRNA, clone J2 (Scicons, catalogue number 10010500)
-donkey anti-mouse AlexaFluor488-conjugated antibody (Jackson Immuno Research, catalogue number 715-545-150)
-anti-HCoV-OC43 N protein (catalogue number MAB9013, Millipore)

-goat anti-mouse AlexaFluor488-conjugated antibody (catalogue number A28175, Thermo Fisher Scientific)
-anti-flavivirus group antigen 4G2 (catalogue number MAB10216; RRID: AB_827205, EMD Millipore)
-Goat anti-mouse IgG Cross-adsorbed secondary antibody, AlexaFluor488 (catalogue number A-11001, RRID: AB_2534069, Thermo Fisher Scientific)
-anti-human CD13 Antibody, APC conjugated (clone WM15, BioLegend, catalogue number 301705)
-anti-human DPP4, FITC conjugated (cloneBA5b, BioLegend, catalogue number 302704)
-anti-CD3-PE (145-2C11, Tonbo Biosciences, catalogue number 50-0031)
-anti-CD3-FITC (145-2C11, Tonbo Biosciences, catalogue number 35-0031)
-anti-CD11c-PECy7 (N418, Tonbo Biosciences, catalogue number 60-0114)
-anti-Ly6G-PE (1A8, Tonbo Biosciences, catalogue number 50-1276)
-anti-Ly6G-FITC (1A8, Tonbo Biosciences, catalogue number 35-1276)
-anti-Ly6g-PerCPCy5.5 (1A8, Tonbo Biosciences, catalogue number 65-1276)

Validation

-MABI1: I1-Hybridoma ATCC® CRL-2700™

Lefrancios L, Lyles DS. The interaction of antibody with the major surface glycoprotein of vesicular stomatitis virus. I. Analysis of neutralizing epitopes with monoclonal antibodies. Virology 121: 157-167, 1982. PubMed: 6180550

-anti-ACE2 (R&D Systems, AF933)
Validated by manufacturer by western blot

-anti-LY6E rabbit monoclonal antibody GEN-93-8-1 (Genentech)
Validated by ectopic overexpression and knockout of human LY6E by western blot.

Mar, K. B., Rinkenberger, N. R., Boys, I. N., Eitson, J. L., McDougal, M. B., Richardson, R. B., & Schoggins, J. W. (2018). LY6E mediates an evolutionarily conserved enhancement of virus infection by targeting a late entry step. Nature communications, 9(1), 3603. https://doi.org/10.1038/s41467-018-06000-y

-anti-LY6E mouse monoclonal antibody 4D8.6.7 (Genentech)
Validated in current manuscript by knockout of human LY6E by western blot

-anti-human MERS-CoV spike 1.6c7 (kindly provided by BJ Bosch)
Validated by ability to neutralize MERS-CoV infection and syncytia formation

Widjaja, I., Wang, C., van Haperen, R., Gutiérrez-Álvarez, J., van Dieren, B., Okba, N., Raj, V. S., Li, W., Fernandez-Delgado, R., Grosveld, F., van Kuppeveld, F., Haagmans, B. L., Enjuanes, L., Drabek, D., & Bosch, B. J. (2019). Towards a solution to MERS: protective human monoclonal antibodies targeting different domains and functions of the MERS-coronavirus spike glycoprotein. Emerging microbes & infections, 8(1), 516–530. https://doi.org/10.1080/22221751.2019.1597644

-Monoclonal Anti-β-Actin-Peroxidase clone AC-15 (Sigma-Aldrich, A3854)
Validated by Western blot on manufacturer's website

-Peroxidase-AffiniPure Donkey Anti-Human IgG (Jackson Immunoresearch, catalogue number 709-035-098)
Validated in 4 publications on CiteAb database

-donkey anti-mouse AlexaFluor488-conjugated antibody (Jackson Immuno Research, catalogue number 715-545-150)
Validated in 196 publication on CiteAb database

-goat anti-mouse AlexaFluor488-conjugated antibody (catalogue number A28175, Thermo Fisher scientific)
Validated by immunofluorescence by manufacturer and by 6 publications on manufacturer's website

-Goat anti-mouse IgG Cross-adsorbed secondary antibody, AlexaFluor488 (catalogue number A-11001, RRID: AB_2534069, Thermo Fisher Scientific)
Validated by immunflourescence by manufacturer and by 600+ publications on manufacturer's website

-anti-HCoV-229E N protein (Anticuerpo Monoclonal, 1E7, Ingenasa) b
Generation and validation of this antibody by Western blot and ELISA was previously published.

Sastre, P., Dijkman, R., Camuñas, A., Ruiz, T., Jebbink, M. F., van der Hoek, L., Vela, C., & Rueda, P. (2011). Differentiation between human coronaviruses NL63 and 229E using a novel double-antibody sandwich enzyme-linked immunosorbent assay based on specific monoclonal antibodies. Clinical and vaccine immunology : CVI, 18(1), 113–118. https://doi.org/10.1128/CVI.00355-10

-anti-HCoV-OC43 N protein (MAB9013, Millipore)
Validated by manufacturer for immunofluorescence.

-anti-flavivirus group antigen 4G2 (MAB10216; RRID: AB_827205, EMD Millipore)
Validated for immunofluorescence and flow cytometry.

Hanners, N. W., Eitson, J. L., Usui, N., Richardson, R. B., Wexler, E. M., Konopka, G., & Schoggins, J. W. (2016). Western Zika Virus in Human Fetal Neural Progenitors Persists Long Term with Partial Cytopathic and Limited Immunogenic Effects. Cell reports, 15(11), 2315–2322. https://doi.org/10.1016/j.celrep.2016.05.075

-anti-human CD13 Antibody, APC conjugated (clone WM15, BioLegend)
validated by manufacturer on human peripheral blood for flow cytometry

-anti-human DPP4, FITC conjugated (cloneBA5b, BioLegend, catalogue number 302704)
validated by manufacturer on human peripheral blood for flow cytometry

The following antibodies were validated by the manufacturer on mouse splenocytes by flow cytometry:
-anti-CD16/CD32 (2.4G2, Tonbo Biosciences)
-anti-CD3-PE (145-2C11, Tonbo Biosciences)
-anti-CD3-FITC (145-2C11, Tonbo Biosciences)
-anti-CD11c-PECy7 (N418, Tonbo Biosciences)
-anti-Ly6G-PE (1A8, Tonbo Biosciences)
-anti-Ly6G-FITC (1A8, Tonbo Biosciences)
-anti-Ly6g-PerCPCy5.5 (1A8, Tonbo Biosciences)
-anti-CD11b-PECy7 (M1/70, Tonbo Biosciences)
-anti-CD19-PE (6D5, BioLegend)
-anti-CD19-FITC (1D3, Tonbo Biosciences)
-anti-CD19-PECy5 (6D5, BioLegend)
-anti-F4/80-PECy5 (BM8, BioLegend)
-anti-Nkp46-PECy7 (29A1.4, BioLegend)
-anti-CD4-PECy5 (GK1.5, Tonbo Biosciences)
-anti-CD8-PECy7 (53-6.7, Tonbo Biosciences)

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	L929 cells (Source : 85011425 (Sigma, ECACC, 2017); authenticated 04/2019 by Thiel lab) 17Cl-1 cells (gift from Stanley Sawicki; not available commercially, authenticated 04/2019 by Thiel lab) Huh7 cells (gift from Volker Lohman, University of Heidelberg; not available commercially, authentication was done in Heidelberg) VeroB4 and VeroE6 (obtained from Marcel Müller, Charité, Berlin; authentication done in Berlin) Huh7.5 cells (Originate from C. Rice lab, authenticated by Schoggins lab in 2018) A549 cells (ATCC cat CCL-185, authenticated by Schoggins lab in 2018) BHK-21 (DSMZ collection # ACC61) BHK-G43 cells were generated as described before (PMID: 15831958) and kindly provided by Gert Zimmer. Cells originated from a commercial source, DSMZ collection # ACC61. STAT1-/- fibroblasts (gift from J.-L. Casanova, authenticated by Schoggins lab in 2018) 293LTV were obtained from Cell Biolabs, Inc (cat#LTV-100) HCT-8 (ATCC cat CCL-244)
Authentication	Thiel lab cell lines: Profiling of cell line was done using highly-polymorphic short tandem repeat loci (STRs). Fragment analysis was done on an ABI3730xl (Life Technologies) and the resulting data were analyzed with GeneMarker software (Softgenetics). Schoggins lab cell lines: Common human cells lines were authenticated with STR analysis using the ATCC Cell Line Authentication service. The identity of Huh7 cells has been verified by a Mulitiplex human cell line authentication test via SNP profiling (Multiplexion, Immenstaad, Germany). VeroB4 and VeroE6 were authenticated by CytB sequencing using published species-specific primers (primers from: Irwin DM, Kocher TD, Wilson AC (1991) Evolution of the cytochrome b gene of mammals. J Mol Evol 32: 128–144.) BHK-21 and BHK-G43 were obtained from commercial sources and thus not secondarily authenticated.
Mycoplasma contamination	Thiel lab: all cell lines in our laboratory (including L929 cells, 17Cl-1 cells, Huh7 cells, VeroB4 and VeroE6, A549 cells, STAT1-/- fibroblasts, BHK-21 and BHK-G43) are routinely screened for mycoplasma contamination and were tested negative. Schoggins lab: All cell lines are routinely tested for mycoplasma using a PCR based assay and were tested negative (Vendor GeM Mycoplasma Detection Kit from Sigma Cat.#MP0025-1KT).
Commonly misidentified lines (See ICLAC register)	none

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Ly6etm1a ES cells were obtained from the EUCOMM consortium59consortium59 and microinjected into C57BL/6J blastocysts by the UTSW Transgenic Technology Center. Chimeric mice with germline transmission were bred to obtain Ly6etm1a/+ offspring. Ly6etm1a/+ mice were crossed with FLPe-expressing mice (B6N.129S4-Gt(ROSA)26Sortm1(FLP1)Dyn/J, #16226, Jackson

	Laboratories) to obtain Ly6efl/+ offspring, which were bred to homozygosity. Conditional Ly6efl/fl mice were bred to Vav1-iCre transgenic mice (B6.Cg-Commd10Tg(Vav1-icre)A2Kio/J, #008610, Jackson Laboratories) to obtain Ly6eΔHSC/+ (Ly6efl/+; Vav1-iCre) offspring. Ly6eΔHSC/+ mice were bred to obtain Ly6eΔHSC (Ly6efl/fl; Vav1-iCre) offspring, which harbor a deletion of exon 3 and 4 in hematopoietic stem cells (HSC). Experimental animals were obtained by crossing Ly6eΔHSC and Ly6efl/fl mice. Littermates were used for the majority of experiments when possible; otherwise mice of similar age (within 2 weeks) were used. Sample size was chosen based on published studies and no randomization or blinding was performed aside from scoring of liver histology. Genotype was confirmed by PCR of genomic DNA in-house or outsourced to Transnetyx. Ablation of Ly6e was confirmed by qPCR in immune cells from spleen, liver, or bone marrow. Animal studies were carried out in specific-pathogen-free barrier facilities managed and maintained by the UTSW Animal Resource Center. Ifnar-/- mice on a C57BL/6J background were a kind gift from N. Yan. . Facilities are maintained at an acceptable range of 68-79°F at a humidity of 30-70% on a 12 hour dark/12 hour light cycle.
Wild animals	Study did not involve wild animals
Field-collected samples	study did not involve samples collected from the field
Ethics oversight	ll procedures used in this study complied with federal and institutional guidelines enforced by the UTSW Institutional Animal Care and Use Committee (IACUC) and were granted institutional approval after veterinary and committee review.

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	All primary cells were maintained in 10% FBS/10 mM HEPES/1 mM sodium pyruvate/2 mM L-glutamine/1x p-s/RPMI (cRPMI2) unless otherwise indicated. Primary bone marrow derived macrophages (BMDM) were prepared by isolating marrow from femur/tibia, lysing red blood cells, and culturing hematopoietic stem cells in M-CSF for 5 days. Viable cells were quantified using trypan blue exclusion. BMDM were plated at 2.5 x 104 cells per well in non-treated 24-well tissue culture plates. Cells were infected the next day with 0.1 MOI MHV-A59-GFP and isolated 6 hours post-infection for analysis by flow cytometry. Splenocytes were prepared by mashing whole spleens against a 70 µM cell strainer. Red blood cells were removed by using RBC lysis buffer (Tonbo Biosciences). Viable cells were quantified using trypan blue exclusion. For in vitro infection, splenocytes were plated at 1x106 cells per well in non-treated 6-well tissue culture plates. Cells were infected the next day with 1 MOI MHV-A59-GFP and isolated 6 hours post-infection. Infected cells were stained with a fluorescent viability dye (Ghostdye Violet 450, Tonbo Biosciences) and fixed in 1% PFA/PBS. The next day, fixed cells were treated with anti-CD16/CD32 (Tonbo Biosciences) and then stained with antibodies that recognize surface lineage markers and analyzed by flow cytometry. Livers were perfused by PBS injection into the inferior vena cava and out of the portal vein. Blanched livers were minced with sterile scissors and mashed against a 70 µM cell strainer. Hepatocytes and large debris were pelleted at 60 x g for 1 minute, 22° C, with no brake. Intrahepatic immune cell (IHIC) and residual hepatocyte containing supernatants were pelleted at 480 x g for 8 minutes, 22°C, with max brake and resuspended in 37.5% Percoll (Sigma-Aldrich)/cRPMI2. Remaining hepatocytes were separated from IHIC by centrifugation at 850 x g for 30 minutes, 22°C, with no brake. Red blood cells were removed from the resulting pellet with RBC lysis buffer (Tonbo Biosciences). Viable cells were quantified using trypan blue exclusion. IHIC and splenocytes (4 x 105) from infected or mock-infected mice were stained with a fluorescent viability dye (Ghostdye Violet 450, Tonbo Biosciences), treated with anti-CD16/CD32 (Tonbo Biosciences), stained with antibodies that recognize surface lineage markers, and then fixed in 1% PFA/PBS. Fixed volumes of cell suspensions were analyzed by flow cytometry the next day. Absolute cell counts were determined by multiplying final number of lineage marker-positive cells by dilution factor used to normalize cell counts for antibody staining. The flow gating strategy for liver and spleen immune cells is included in Extended Data Figure 9.
Instrument	1000 Flow Cytometer with a A600 96-well plate high throughput extension
Software	CellCapture software (Stratedigm)
Cell population abundance	MHV infection affected cell abundance. Range of cell numbers recovered includes PBS and MHV-injected mice. From liver: CD4+ T cells - 150-1200 CD8+ T cells - 55-2000 macrophages - 39-879 dendritic cells - 50-1977 neutrophils - 0-190

B cells - 29-465
NK cells - 42-1291

in spleen:
CD4+ T cells - 273-1350
CD8+ T cells -190-1119
macrophages - 87-533
dendritic cells - 87-774
neutrophils - 58-846
B cells- 1413-5300
NK cells - 65-400

Gating strategy

Antibody-stained suspensions of isolated intrahepatic immune cells or splenic immune cells were first analyzed by forward scatter/side scatter to remove debris events, then gated for exclusion of a viability dye, and finally for selection of single cell events. A dump-gate strategy was used to identify immune cell populations as indicated in the diagram. Boundaries of gates were determined by use of single color control samples.

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