nature portfolio

Corresponding author(s):	Jai S. Rudra, Janice J. Endsley
Last updated by author(s):	January 22, 2022

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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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St	at	151	ICS

For a	all statistical ar	alyses, 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 stateme	ent on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	The statis Only comm	tical test(s) used AND whether they are one- or two-sided non tests should be described solely by name; describe more complex techniques in the Methods section.
\boxtimes	A descript	zion of all covariates tested
	A descript	cion of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	A full desc	cription of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) ition (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	For null h	ypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>es as exact values whenever suitable.</i>
\boxtimes	For Bayes	ian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes	For hierar	chical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\boxtimes	Estimates	of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated
'		Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.
Sof	ftware an	d code
Polic	cy information	about <u>availability of computer code</u>
Da	ita collection	BD FACSDIVA v9
Da	ita analysis	FlowJo v10, BD FACSDiva v9, ReactomePA package79 (version 1.34.0) in R Studio v4.0.5, GraphPad Prism version 9
		g 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 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 <u>availability of data</u>

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

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

The transcriptomic data discussed in this publication are accessible through Zenodo at https://doi.org/10.5281/zenodo.588839682.

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Please select the or	ne 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 t	he document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf					
Life scier	ices study design					
All studies must dis	close on these points even when the disclosure is negative.					
Sample size	Experiments included 4-10 animals per treatment, based on power calculation guided by previous/preliminary results					
Data exclusions	No experimental data was excluded. Missing data points did occur as a result of technical issues (e.g. flow cytometry probe clog) that resulted in sample loss					
Replication	Animal experiments to assess Trm were conducted three times using two different peptide synthesis batches with similar results					
Randomization	Animals were from a single cohort purchased from a commercial vendor and randomly assigned to treatment					
Blinding	Investigators were not blinded during collection or analysis due to the nature of the treatments including requirement for knowledge of priming dose to ensure proper boost.					

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	Methods			
n/a Involved in the study	n/a Involved in the study			
Antibodies	ChIP-seq			
Eukaryotic cell lines	Flow cytometry			
Palaeontology and archaeology	MRI-based neuroimaging			
Animals and other organisms	•			
Human research participants				
Clinical data				
Dual use research of concern				
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Antibodies

Antibodies used

BV711 anti-CD45 (BD Biosciences, 563709)

Fixable Viability Dye eFluor 506 (eBioscience, 65-0866-14)

CD3 (BUV395, BD Biosciences, 563565)

CD4 (BUV496, BD Biosciences, 612952)

CCR7 (PE-Dazzle 594, Biolegend, 120122)

CD44 (PE-Vio770, Miltenyi Biotec, 130-102-377)

CD62L (Brilliant Violet 786, BD Biosciences, 564109)

CD69 (APC, Biolegend, 104514)

CD49a (VioBright FITC, Miltenyi Biotec, 130-107-592)

CD103 (Brilliant Violet 605, BD Biosciences, 748257)

CD127 (Brilliant Violet 650, BioLegend, 135043)

CXCR3 (PerCP-Cy5.5, BioLegend, 126514)

CXCR6 (PE, BioLegend, 151104)

IFN-γ (Brilliant Violet 605, Biolegend, 505840)

TNF- α (APC, Biolgened, 506108))

IL-2 (Brilliant Violet 711, Biolegend, 503837)

IL-17A (PE, Biolegend, 506904)

MHC-II Ag85B tetramer (I-A(b) FQDAYNAAGGHNAVF), NIH Tetramer Core Facility

Validation

All antibodies were purchased directly from reputable vendors (listed above) and came with quality certificates of validation. Additional validation was performed using ultracomp beads to assess quality and performance

Animals and other organisms

Policy informat	ion about studies	s involving animals	; ARRIVE gui	iidelines recomm	nended for re	porting animal r	research
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Laboratory animals Female C57BL/6 mice supplied by Jackson Labs, 6-8 weeks old

Wild animals study did not involve wild animals

Field-collected samples | study did not utilize field collected samples

Ethics oversight University of Texas Medical Branch 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 Lung and spleen cells isolated through disruption including use of DNASE and collagenase in processing (full details are included in manuscript methods).

Instrument Becton Dickinson LSR II, Fortessa

Software BD FACSDiva and FlowJo v10 software

Cell population abundance Sorting experiments were not performed. For analytical flow cytometry, all events in isolated tissue were collected which

averaged 10 million in lung and 2 million in spleen which is more enriched in lymphocytes versus lung.

Gating strategy

Gating was performed by selection of FSC/SSC characteristics of live leukocytes, selection of singlets, selection of viable cells excluding a viability dye, followed by additional downstream gating to select CD3+CD4+ populations. Analysis of activation, memory, or tissue resident phenotype was subsequently determined from viable CD3+CD4+ singlets.

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