nature portfolio

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Reporting Summary

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

Statistics

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

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

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

Software and code

Policy information about availability of computer code

Data collection	BD FACSDiva LSR II, Perkin Elmer Wizard2 Automatic Gamma Counter, Bio Rad ChemiDoc XRS+ and Image Lab, Molecubes Beta-cube scanner, SpectraMax 190 Microplate reader, ImmunoSpot S6 Universal M2
Data analysis	GraphPad Prism, Microsoft Excel, FlowJo, Bio Rad Image Lab, MIM.

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

Source data are provided with this paper. The data generated in this study are provided in the Supplementary Information/Source Data file.

Research involving human participants, their data, or biological material

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

Reporting on sex and gender	This study did not use human research participants.
Reporting on race, ethnicity, or other socially relevant groupings	This study did not use human research participants.
Population characteristics	This study did not use human research participants.
Recruitment	This study did not use human research participants.
Ethics oversight	This study did not use human research participants.

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 determined on case by case basis relative to the experiment being conducted. In general, a powered number was used to yield statistically significant results.
Data exclusions	3 data points were excluded from the biodistribution experiment shown in Supplementary Figure 3 using the ROUT method with Q=0.1. The data is shown with and without exclusions and the full data set is provided in the Source Data File.
Replication	Replicates were determined on case by case basis relative to the experiment being conducted. All flow cytometry and Western blot assays were repeated at least 3 independent times and yielded reproducible results. in vivo cytotoxicity was repeated twice for optimization and yielded reproducible results. Variations of mouse immunogenicity experiments including ELISA and T cell polyfunctionality were repeated twice with different control groups and biological replicates yielding reproducible results. Mouse imaging was repeated with both biological and technical replicates and various controls with reproducible results. NHP experiments were conducted with smaller group sizes and less replicates given the nature of the studies but data was reproducible across n = 2 NHPs. NHP ELISA and ELISPOT experiments were repeated at least twice with reproducible results.
Randomization	For all mouse experiments, mice were randomly assigned to a cage upon receipt and remained in those groups for experimentation.
Blinding	For imaging studies, blinding to groups was not performed during data collection. Blinding was performed during image analysis in which ROIs were drawn on the CT image to prevent subjective interpretation. In other experiments such as flow cytometry, western blot, ELISA, and ELISPOT, results were obtained through automated and quantitative analysis, minimizing subjective interpretation.

Reporting for specific materials, systems and methods

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

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	COX IV Mouse mAb (Cell Signaling Technology, 11967s), SARS-CoV-2 Spike S2 Mouse mAB (Sino Biological, Cat# 40590-MM05, Clone 05), FITC Rat Anti-Mouse CD107a (BD, Cat# 553793, Clone 1D4B), PerCP-Cy5.5 Anti-Mouse CD4 (Biolegend, Cat# 100434, Clone GK1.5), Pacific Blue Anti-Mouse CD8a (Biolegend, Cat# 100725, Clone 53-6.7), Aquablue LIVE/DEAD (ThermoFisher, Cat# L34966), Brilliant Violet 605 Anti-Mouse CD3e (Biolegend, Cat# 100351, Clone 145-2C11), BV786 Rat Anti-Mouse IL4 (BD, Cat# 564006, Clone 11B11), APC Rat Anti-Mouse IL-2 (BD, Cat# 554429, Clone JES6-5H4), Alexa Fluor 700 Rat Anti-Mouse IFN-γ (BD, Cat# 557998, Clone XMG1.2), APC/Cy7 Anti-Mouse IL-17A (Biolegend, Cat# 506940, Clone TC11-18H10.1), PE Anti-Mouse/Human IL5 (Biolegend, Cat#504304, Clone TRFK5), PE-Cy7 Rat Anti-Mouse TNF (BD, Cat# 557644, Clone MP6-XT22). Additional antibodies used in supplementary experiments are provided in tables in the Supplementary Information.
Validation	Antibody validation conducted by source company. We did not do any additional antibody validation outside of confirming the appropriate molecular weight of the expected products detected by Western Blot and having appropriate experimental controls.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293T is a cell line from embryo kidney tissue. DC2.4 cells are immortalized murine dendritic cells isolated from C57BL/6 mice.
Authentication	HEK293T cells were purchased from ATCC and validated by ATCC. DC2.4 cells were purchased from Sigma Aldrich and validated by Sigma-Aldrich
Mycoplasma contamination	Immortalized cells used in experiments from early passage. No mycoplasma testing was performed.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cells 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	Balb/c mice, female, adult (8-12 weeks) Rhesus macaque, all male, 9 years (S2P-eDHFR NHP 1), 17 years (S2P-eDHFR NHP 2) and 20 years (C. diff)
Wild animals	This study did not involve wild animals.
Reporting on sex	Results are not correlated to sex.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	The University of Pennsylvania-University Laboratory Animal Resources (ULAR) and IACUC organizations oversaw the use of vertebrate animals for this study. We followed the ULAR/IACUC approved mouse protocol (IACUC 805477, 803941) and Nonhuman primate protocol (IACUC 805073) as well as employed best laboratory practices to ensure the safety of the animals and researchers involved in this study. These protocols align with the ethics standards set forth by the Animal Welfare Act (AWA) and the "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.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

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	For HEK293T and DC2.4: Cells were lifted using versene (EDTA), centrifuged, and the soluble phase was removed by vacuum. The remaining cell pellet was resuspended in cold FACS buffer (5% FBS in PBS) and incubated with an AF647 labeled antibody for 1hour at 4 degrees Celsius. The cells were then centrifuged again and the FACS buffer was removed by vacuum. The cells were resuspended in fresh FACS buffer and kept on ice until analysis. For mouse splenocytes: spleens were collected, processed as single cells, filtered, centrifuged, and red blood cells were lysed in ACK lysis buffer. Cells were then fixed in 1% PFA (in FACS buffer) and kept on ice until analysis.
Instrument	BD LSR II
Software	BD FlowJo
Cell population abundance	For HEK293T and DC2.4: 10,000 events were recorded For T cell polyfunctionality: 450,000 events were recorded For in vivo cytotox: 25,000 events were recorded For mouse LN: at least 1,000,000 events were recorded
Gating strategy	For fluorescence: cells were gated into "Live" and "Single Cell" populations, which is standard practice. We used positive and negative fluorescent controls to validate fluorescent signal detection. For T cell reactivity: splenocytes were first gated into "Live" and "Single Cell" populations. Next cells were gated by Live/Dead staining. Cells were then gated on CD3+ to select for T cells. T cells were then gated for CD4 and CD8 to isolate cytotoxic and helper T cells for analysis. For mouse LN flow: splenocytes were gated into single cell populations. T cells, B cells, and NK cells were dumped using antibodies against various markers and a single fluorophore. Live cells were isolated using a L/D dye. Macrophages and DC cell populations were analyzed using markers against F4/80 and CD11c, respectively. Macrophages and DC cells were probed for TMP-JF646 and S2-488 positivity.

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