

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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ | 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | 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

Data analysis

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](#)

The authors declare that the findings of this study are available within the main and supplemental figures and materials provided with the manuscript. For any further information, please email the corresponding author Dr. David Dowling at david.dowling@childrens.harvard.edu.

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	No sex-based analyses were performed.
Reporting on race, ethnicity, or other socially relevant groupings	Socially relevant categorization was not performed.
Population characteristics	Peripheral blood was collected from healthy adult volunteers (n = ~15), and represent the general population.
Recruitment	Participants were voluntarily recruited via public dispersal of recruitment flyers around the Longwood Medical Area.
Ethics oversight	Blood from healthy, adult volunteers was collected after written informed consent with approval from the Ethics Committee of Boston Children's Hospital, Boston, MA (protocol number X07-05-0223). All ethical regulations relevant to human research participants were followed.

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](https://www.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 size for in vitro stimulations and in vivo studies were chosen empirically based on the results of previous studies.
Data exclusions	A single mouse splenocyte replicate used in splenocyte restimulation/spectral flow cytometry and ELISpot was excluded due to a technical error during splenocyte processing. This is noted in the Data Deposition.
Replication	Murine studies were not replicated, but benchmarking positive and negative controls were used for each experiment. In vitro sample replicates (i.e. multiple donors for primary cell stimulations, or experimental replicates for secondary cell culture and stimulations) were acquired in batches across multiple experiment days.
Randomization	For splenocyte restimulation and ELISpot experiments, and the experiments shown in Figures 6 & 9, and Supplementary Figure 7, 8, 9, & 10D-E, vaccine formulations administered to the mice were randomized within and across mouse cages. ELISpot wells were evaluated randomized. Randomization was not performed on the remaining assays. However, all experiments were carried out in an unbiased manner to prevent potential biases in the experimental groups. Statistics were not calculated until each experiment was complete and all data had been verified to be accurate.
Blinding	For splenocyte restimulation and ELISpot experiments, and the experiments shown in Figures 6 & 9, and Supplementary Figure 7, 8, 9, & 10D-E, the investigator performing immunizations was blinded to the vaccine formulations. ELISpot wells were evaluated blinded to mouse immunization status. Blinding was not performed on the remaining assays. However, all experiments were carried out in an unbiased manner to prevent potential biases in the experimental groups. Statistics were not calculated until each experiment was complete and all data had been verified to be accurate.

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	We list the specific information of the fluorochrome-tagged antibodies for flow cytometry run on splenocytes in "Supplementary Table 2". For flow cytometry run on MoDCs, all fluorochromes are listed in the Materials and Methods section.
Validation	Commercial antibodies were either titrated in-house or used according to the manufacturer's information, and this is noted in the manuscript where applicable.

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	Female, 6-8 week old C57BL/6 (#000664), BALB/c (#000651), and TLR3-/- (#005217) mice were purchased from The Jackson Laboratory.
Wild animals	Not applicable.
Reporting on sex	Only mice of the female sex were used in experiments.
Field-collected samples	Not applicable.
Ethics oversight	Mice were housed under specific pathogen-free conditions at Boston Children's Hospital, in individually ventilated cages with standard corncob bedding, ad libitum access to autoclaved water and irradiated chow, and environmental enrichment. The animal facility maintained a 12-hour light/12-hour dark cycle, and ambient temperature of ~22°C, and relative humidity of ~50 ± 10 %. All the procedures were approved under the Institutional Animal Care and Use Committee (IACUC) and operated under the supervision of the Department of Animal Resources at Children's Hospital (protocol no. 00002499).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	Not applicable.
Novel plant genotypes	Not applicable.
Authentication	Not applicable.

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 splenocyte preparation, harvested spleens were mechanically dissociated and filtered through a 70µm cell strainer. After centrifugation, cells underwent treatment with 1 mL of ammonium-chloride-potassium lysis buffer for 2 minutes at room temperature. Subsequently, splenocytes were washed and plated in a 96-well U-bottom plate at a density of 2e+6 cells/well, and rested overnight at 37°C in T Cell Media (TCM) comprising RPMI 1640 supplemented with 10% heat-inactivated FBS, penicillin (100 U/mL), streptomycin (100 mg/mL), 2-mercaptoethanol (55 mM), nonessential amino acids (60 mM), HEPES (11 mM), and L-glutamine (800 mM) (all Gibco).

For MoDC preparation, MoDCs were differentiated from PBMCs as described in the Methods. Differentiated MoDCs were harvested and plated in a 96-well U-bottom plate at a density of 2e+5 cells/well, or a 48-well plate at a density of 1e+6 cells/well, in RPMI 1640 supplemented with IL-4 (500 IU/mL), GM-CSF (1000 IU/mL; both Miltenyi), and autologous platelet-poor plasma (PPP) or FBS (Cytiva Hyclone).

Instrument

For flow cytometry performed on splenocytes, Sony ID7000 spectral cell analyzer (Sony Biotechnology Inc.) was used. For flow cytometry performed on MoDCs, LSRFortessa flow cytometer (BD Biosciences) was used.

Software

FlowJo v10.10 (FlowJo LLC) was used for analyses.

Cell population abundance

No sorting was performed.

Gating strategy

Gating strategy is supplied in Supplementary Figures.

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