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Corresponding author(s): David J. Lynn

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Reporting Summary

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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	REDcap V14.5.28
Data analysis	No custom code was developed as part of this manuscript. R scripts and processed data for reproducing the analyses can be accessed at https://bitbucket.org/lynnlab/air. Other 3rd party code used: Flow cytometry analysis: FlowJo™ v10.6.1; RNAseq analysis: FastQC v0.11.4; MultiQC v1.8; Trimmomatic version 0.38; HISAT2 v2.1.0; FeatureCounts v.5.0-p2; R v4.2.0; R packages: EdgeR v3.38, svaseq v3.46; fgsea v1.22.0, GSVA v1.44.1; Hmisc v4.7-0. Metagenomic Data Analysis: FastQC v0.11.4; MultiQC v1.8; Cutadapt v1.18; Trimmomatic v0.38; Kraken2 v2.1.1; metaSPAdes v3.15.2; MetaBAT2 v2.15; bowtie2 v2.5.0; GTDB-Tk v2.1.0; PhyloSeq v1.28; DirichletMultinomial v1.38.0; HUMAnN v3.0; SAMtools v1.17 Antimicrobial resistance gene analysis: prodigal v2.6.3; cd-hit v4.8.1. Differential abundance analysis: LinDA as implemented in the R library Microbiome v1.18 Causal mediation analysis: mediation R package v4.5.0. 16S rRNA gene sequence analysis: QIIME2 (release 2023.2); DADA2; ggplot2 v3.3.6 in R version 4.2.0 Mouse data analyses: Prism v10 Confocal: Fiji in ImageJ

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

Data that support the findings of this study have been deposited deposited in the Gene Expression Omnibus (GEO) under accession code "GSE198276" (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE198276) and in the Sequence Read Archive under bioProject accession code PRJNA807448 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA807448). All other data supporting the findings of this study are available within the paper and its supplementary information files or are available via the Lynn Laboratory BitBucket repository (https://bitbucket.org/lynnlab/air). Source data for figures 1-5, extended data figures 2-8 and extended data figure 10 are provided with the paper.

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	Sex was reported by the infant participant's parent/guardian and confirmed via medical records. The expression of the XIST gene in the RNASeq data was used as a way to ensure the correct sex was assigned.
Reporting on race, ethnicity, or other socially relevant groupings	No data on race, ethnicity or other socially relevant groupings is reported.
Population characteristics	Male and female healthy, term, vaginally-born infants were recruited for this study shortly after birth and were followed for the first 15 months of life. Exclusion criteria were <37 weeks' gestation, maternal Body Mass Index (BMI) of >30 (at first trimester antenatal visit), maternal sepsis (defined by laboratory confirmed bacterial infection in blood cultures or CSF) during pregnancy, infant delivered by caesarean section, confirmed sepsis in infant (defined by laboratory confirmed bacterial infection in blood cultures or CSF), a known or suspected disorder of the immune system that would prevent an immune response to the vaccines, such as participant with congenital or acquired immunodeficiency or those receiving systemic immunosuppressive therapy, infant had suspected or confirmed HIV, major congenital abnormality or serious illness, maternal or infant participation in a clinical study that may interfere with participation in this study, anything that would place the individual at increased risk or preclude the individual's full compliance with, or completion of, the study. For immunogenicity analyses, only participants who received at least one vaccine at study visit 1 were included.
Recruitment	Infants were recruited at the Women's and Children's Hospital (WCH) Adelaide, Australia between April 2017 and March 2021 as part of the Antibiotics and Immune Responses (AIR study, a prospective, observational clinical study (ACTRN12617000856314). Potential participants were approached by study research nurses in postnatal wards and invited to participate if the mother and their infant met the study eligibility criteria. Study information had also been previously provided to women delivering at the Women's and Children's Hospital through pamphlets in antenatal care packs. Following written informed consent, stool collection packs were provided to families and medical case notes were reviewed to confirm eligibility criteria and antibiotic exposure status. Ethics approval for the study was obtained from Human Research Ethics Committee of the Women's and Children's Health Network (approval number HREC/17/WCHN/19) and the authors affirm that they have complied with all relevant ethical regulations.
Ethics oversight	Ethics approval for the study was obtained from Human Research Ethics Committee of the Women's and Children's Health Network (approval number HREC/17/WCHN/19) and the authors affirm that they have complied with all relevant ethical regulations.

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	Human study - We designed and powered the study to describe the impact of antibiotic exposure on the primary outcomes of proportion of infants achieving seroprotective PCV antibody levels at 7 months (following 3 doses of 13vPCV vaccination at 2, 4 and 6 months). Using an assumption that the proportion achieving PCV antibody levels of ≥0.35 ug/ml to common PCV serotypes would be at least 88% in the control

(non-exposed) group, we require 89 individuals per analysis group (exposed vs not exposed) to detect a 20% or greater difference (alpha 0.05) with 90% power. Infants were consented into this study in the first week of life, with the aim of including approximately 200 vaccinated infants (vaccinated cohort) at approximately 6 weeks of age (Study Visit 1). At this visit, we classified participants as either antibiotics exposed, or unexposed. We designed the study to enrol and vaccinate 200 infants with the aim of achieving approximately 100 infants enrolled and vaccinated with no prior antibiotic exposure by 6 weeks of age, and approximately 100 infants enrolled and vaccinated with either direct or maternal intrapartum antibiotic exposure prior to vaccination at 6 weeks of age. This target sample size of 200 allowed for withdrawals and/ or insufficient blood collections for approximately 10%, while ensuring we were adequately powered to detect the difference between exposed and not exposed groups in achieving PCV antibody levels of ≥0.35 ug/ml to common PCV serotypes.

Animal study - Sample size was determined based upon prior experiments evaluating the role of the microbiota in vaccine responses previously undertaken by our team (See Lynn et al. Cell Host & Microbe, 2018, DOI:https://doi.org/10.1016/j.chom.2018.04.009)

Data exclusions

In Figure 4c-d metagenomics data from 13 infants who were withdrawn from the study by study visit 1 were excluded from further analysis. In the analysis shown in Extended Data Fig. 2, only infants with matched samples across 3 timepoints were included in the analysis. No other data that passed QC were excluded from analysis

Replication

The human AIR study was not replicated. Technical replicates were performed on infant samples were sample volume limitations permitted. Replication of data from animal model studies is reported in the figure legends. Replication attempts were successful where animal experiments were performed without issue (e.g. no contamination of germ-free mice). Any experiments where the germ-free status of control mice was breached were excluded and are not included in the manuscript.

Randomization

The clinical study was a prospective, observational study therefore no randomisation was performed. In the animal experiments mice were randomised to treatment groups.

Blinding

In the clinical study, investigators were blinded to antibiotic group exposure allocation during sample collection, processing and analysis. Unblinding occured when all exposure, outcome and covariate data has been entered, second checked for accuracy and the database has been cleaned for spurious or discrepant data (with data queries resolved). Investigators were not blinded to the treatment groups in the animal experiments.

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

Infant flow cytometry analysis: Panel 1 was a pan-leukocyte panel allowing for identification neutrophils, monocytes, dendritic cells, eosinophils, basophils, T cells, B cells, and NK cells. Cells were stained with a cocktail of anti-CD3 FITC (HIT3a, 1:20, BD Biosciences), anti-CD11c PE (B-ly6, 1:20, BD Biosciences), anti-HLA-DR APC-H7 (G46-6, 1:40, BD Biosciences), anti-CD15 V500 (HI98, 1:80, BD Biosciences), anti-CD45 BUV395 (HI30, 1:80, BD Biosciences), anti-CD16 BV421 (3G8, 1:143, BD Biosciences), anti-CD14 AF647 (Mop-9, 1:143, BD Biosciences), anti-CD123 BV711 (9F5, 1:143, BD Biosciences), anti-CD56 PE-Cy7 (5.1H11, 1:40, Biolegend), anti-CD19 BV605 (HIB19, 1:80, Biolegend), anti-CD20 BV786 (2H7, 1:80, Biolegend), anti-FcεRIα PerCP Cy5.5 (AER-37, 1:80, Biolegend), and anti-Siglec8 PE Dazzle (7C9, 1:143, Biolegend). Panel 2 enabled the characterisation of ~20 functional subsets of B and T cells including naïve, central memory and effector memory CD4+ and CD8+ T cells; regulatory T cells; and naïve, memory and plasma B cells and used a cocktail of anti-CD19 PE (HIB19, 1:10, BD Biosciences), anti-CCR7/CD197 AF647 (150503, 1:10, BD Biosciences), anti-CD127 BV421 (A019D5, 1:40, Biolegend), anti-CD38 BV510 (HIT2, 1:40, BD Biosciences), anti-HLA-DR APC H7 (G46-6, 1:40, BD Biosciences), anti-CD3 BUV395 (UCHT1, 1:40, BD Biosciences), anti-CD4 BV605 (RPA-T4, 1:80, BD Biosciences), anti-CD8 Cy7 (RPA-T8, 1:80, BD Biosciences), anti-IgD PerCP Cy5.5 (IA6-2, 1:80, BD Biosciences), anti-CD27 BV711 (L128, 1:143, BD Biosciences) and anti-CD25 PE Dazzle594 (M-A51, 1:80, Biolegend), anti-CD20 BV786 (2H7, 1:80, Biolegend), anti-CD45RA FITC (HI100, 1:143, Biolegend). Panel 3 was designed to assess specific T helper subsets, including Th1, Th2, Th9, Th17, Th22 and NKT cells using anti-CD3 BUV395 (UCHT1, 1:50, BD Biosciences), anti-CD4 BV605 (RPA-T4, 1:100, BD Biosciences), anti-CD8 PE Cy7 (RPA-T8, 1:100, BD Biosciences), anti-CXCR3 BV421 (1C6/CXCR3, 1:100, BD Biosciences), anti-CCR10 APC (1B5, 1:100, BD Biosciences), anti-CCCR6 BV711 (11A9, 1:50, BD Biosciences), anti-CCR4 PECF594 (1G1, 1:50, BD Biosciences), anti-CD45RA FITC (HI100, 1:100, Biolegend) and CD1d-Tet PBS57 PE (1:5,000, NIH)

Murine flow cytometry analysis:
GC/Tfh assessment: CD3 (142-C11, 1:300, BD Biosciences), CD4 (RM4, 1:300, BD Biosciences), CD8 (53-6.7, 1:600, BD Biosciences), CD19 (1D3, 1:300, BD Biosciences), CD38 (90, 1:600, Biolegend), CD44 (IM7, 1:600, BD Biosciences), CD62L (MEL-14, 1:600, BD

Biosciences), CD95 (J02, 1:300, BD Biosciences), CD138 (281-2, 1:600, BD Biosciences), B220 (RA3-6B2, 1:600, BD Biosciences), CXCR5 (L138D7, 1:200, Biolegend), GL7 (GL7, 1:600, BD Biosciences), IgD (11-26c.2a, 1:300, Biolegend), IgM (R6-60.2, 1:300, BD Biosciences), PD-1 (RMP1-30, 1:300, BD Biosciences), streptavidin-PECF549 (1:400, Biolegend).

CD86, CD80 and MCHII activation: CD19 (ID3, 1:200, BD Biosciences), CD3 (145-2C11, 1:150. BD Biosciences), CD8 (53-6.7, 1:200, BD Biosciences), NK1.1 (PK136, 1:200, BD Biosciences), Ly6G (1A8, 1:600, BD Biosciences), B220 (RA3-6B2, 1:3000, BD Biosciences), CD11b (M1/70, 1:500, BD Biosciences), CD11c (HL3, 1:400, BD Biosciences), MHCII (M5/114.15.2, 1:150, Miltenyi), CD86 (PO3.3, 1:150, Miltenyi), CD80 (16-10A1, 1:150, Tonbo Biosciences, San Diego, USA).

Immunofluorescence staining: CD16/32 (1:100, BD Biosciences), CD21/35 clone 7E9 (1:200, Biolegend), GL7 (1:100, Biolegend) CD3 clone 17A2 (1:200, Biolegend), IgD clone 11-26c.2a (1:200, Biolegend).

Validation

No specific validation experiments were conducted in house. All antibodies were commercially sourced and validated by the companies. A certificate of analysis and technical data sheet is available for each antibody utilised from the suppliers website.

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	C57BL/6J SPF and GF mice. Young mice (aged ~3 weeks), dams (aged 8-16 weeks) and pups from birth to 21 days were used as described in the manuscript.
Wild animals	This study did not involve wild animals.
Reporting on sex	Equal numbers of each sex were incorporated into the experimental design for this study.
Field-collected samples	This study did not involve field collected samples.
Ethics oversight	SAHMRI Animal Ethics Committee.

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	ACTRN12617000856314.
Study protocol	This study was an observational study and not a randomised controlled trial, as such the study protocol was not published in advance.
Data collection	Infants were recruited at the Women's and Children's Hospital (WCH) Adelaide, Australia between April 2017 and March 2021.
Outcomes	The primary objective of the AIR study was to determine whether antibody responses to the PCV13 vaccine at 7 months of age are different between antibiotic exposed and unexposed groups. Specifically comparing the following: 1) Percentage of participants achieving a seroprotective antibody response (greater than or equal to 0.35 micrograms/ml) against pneumococcal polysaccharide serotypes in the PCV13 vaccine at approximately 7 months of age. 2) Geometric mean concentrations in micrograms/ml of anti-PPS IgG as measured by serum assay at approximately 7 months of age. As secondary outcomes, we also compared PCV13 vaccine responses at 15 months of age, as well as antibody responses to the Infanrix Hexa® 6-in-1 vaccine (6-in-1, DTPa-HepB-IPV-Hib), and the Rotarix® oral rotavirus vaccine (ORV) in antibody exposed and unexposed infants. Multivariable linear regression adjusting for baseline pre-vaccination titres and sex was used to test for an association between antibiotic exposure and IgG GMCs for each of the vaccine antigens assessed. Multivariable logistic regression was used to test for an association between antibiotic exposure and the proportion of infants achieving a seroprotective response. The seroprotective threshold for each vaccine antigen is listed in Table S1. Statistical significance was defined was $P < 0.05$.

Plants

Seed stocks: N/A

Novel plant genotypes: N/A

Authentication: N/A

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 human flow cytometry analysis, 450 µl of infant blood was transferred from a BD Vacutainer K3 EDTA tube into a sterile 10 mL tube within 3 hours of collection and red blood cells were lysed with addition of 4.5 mL of 1X BD Pharm Lyse for 15 min at room temperature. Cells were then pelleted by centrifugation at 200 x g for 5 min, supernatant aspirated and cell pellet resuspended in 10 mL flow cytometry buffer and centrifuged again. Cells were then resuspended in 150 µl flow cytometry buffer and divided equally into 3 tubes for staining. Fc receptors were first blocked Human TruStain FcX (Biolegend, San Diego, USA) in Brilliant Stain Buffer (BD) for 10 min on ice. Three panels were designed for same day flow cytometry analysis (see Supplementary Table 3 for complete list of cell subsets identified on each panel). After each antibody cocktail was added, samples were incubated in the dark on ice for 30 min before being washed and resuspended in 300 µl of flow cytometry buffer. Panel 1 was added to a BD Trucount FACS tube (BD), while panels 2 and 3 were added to round bottomed 5 mL FACS tubes alongside 10 µl liquid counting beads (BD). Counts were generated as previously described 49. Dead cells were stained by addition of DNA binding dye DAPI prior to running on a flow cytometer and were excluded from analysis.

Mouse flow cytometry analysis:
Single cell suspensions of murine spleen or lymph nodes were prepared by mechanical dissociation and filtration through a 70 µm nylon filter (Merck Millipore, Burlington, USA). Cells were stained with Fixable Viability Stain 780 (BD Biosciences) and murine FC blocking antibody Clone 2.4G2 (1:100, BD Biosciences) in PBS for 15 minutes. Cells were washed before staining with fluorochrome-conjugated antibodies in FACS buffer (PBS, 0.1% BSA, 2 mM EDTA) for staining with antibody cocktails from 1 of 2 panels (detailed in methods section). Surface antibody cocktails were added directly after staining for 20 minutes at 4C. Data were acquired on BD FACS Symphony and analyzed using FlowJo v10.

Instrument: FACSymphony™ (BD) flow cytometer

Software: FlowJo™ v10.6.1

Cell population abundance: BD Trucount FACS tubes were used to enumerate cells.

Gating strategy

Gating strategy for the human pan-leukocyte panel. Beads from each Trucount tube were recorded from FSCxSSC and then PEhi. For all analyses, cells were gated by singlet discrimination, followed by live, CD45+ and leukocytes. Leukocytes were split into SSC high and low populations. Eosinophils and neutrophils were gated on SSC low and high populations, respectively. T cells were gated on SSC low CD3hi and CD3- cells were further defined as CD19+CD20+ B cells. The CD19- population was gated on to define CD16-CD56+ NK cells. CD56- cells were further gated on CD16 and CD14 expression to identify monocyte populations. Remaining CD14-CD16- cells were further gated on HLA-DR and CD123 expression to identify CD123+HLA-DR- Basophils. HLA-DR+ cells were gated on CD11c and CD123 in order to identify various DC populations. CD3+ T cells were gated further on their CD56 expression to identify CD56+CD3+ NKT-like cells.

Gating strategy for the human B and T cell panel. For all analyses, cells were gated by singlet discrimination, live and for leukocytes using SSCxFSC. CD3+ T cells were gated on CD4+ and CD8+ expression. CD4+ T cells were then defined as CD45RA-HLA-DR+ CD4+ T cells, CCR7+CD45RA+ Naïve CD4+ T cells, CCR7+CD45RA- CD4+ central memory T cells, CCR7-CD45RA- CD4 effector memory T cells. CD4+ T cells were also gated on CD127 and CD25 expression to identify CD127-CD25+ Tregs, which were further gated for CD45RA and HLA-DR expression to identify CD45RA-HLADR+ Tregs. CD8+ T cells were then defined as CD45RA-HLADR+ CD8+ T cells, CCR7+CD45RA+ naïve CD8+ T cells (naïve T cells), CCR7+CD45RA- CD8+ central memory T cells

(Tcm), and CCR7-CD45RA- CD8 effector memory T cells (Tem). The CD3- population from b was gated further on CD20 and CD19 so that CD20-CD19- cells could be gated out and CD19+ B cells could then be gated for. These were further divided into a CD38 and CD20 gate to identify CD38+CD20- plasma cells and an IgD and CD27 gate to identify IgD-CD27+ Memory B cells and IgD+ naïve B cells.

Gating strategy for the extended human B and T cell panel. Cells were gated by singlet discrimination, live cells and for lymphocytes using SSCxFSC. NKT cells were identified using a tetramer specific for CD1d. CD3+ T cells were gated on CD4+ and CD8+ expression. CD4+ T cells were then defined as CCR4+ CCR3+ Th1 cells and CCR4+ CD4 T cells. This CCR4+ gate was used to define CCR4+CCR6- Th2 cells and CCR4+CCR6+ cells. The CCR4+CCR6+ cells were further gated to identify CCR10 +CCR6+ Th22 cells and CCR10-CCR6+ Th17 cells. Th17 cells could be further distinguished by identifying the CXCR3- population of Th17 cells. CD45RA- T cells were identified using the CD4+ T cell gate. This CD45RA- T cell gate was used to identify CCR4-CXCR3+ Th1 memory cells (mTh1) and a CCR4+ memory population that was further gated to identify CCR4 +CCR6- Th2 memory cells (mTh2). The CD8+ T cell gate was further gated on the CD45RA- population to identify CD8+ memory T cells (mCD8). This population was further gated to identify CCR10+CCR4- CD8+ mTc1 cells. Finally, the mCD8 population was also gated to identify a CCR6+CCR10- population.

Gating strategy for the mouse GC B cells panel. For all analyses, lymphocytes were gated by singlet discrimination, followed by live discrimination. Live cells were gated on B220 and CD19 to identify CD19+ B220+ B cells. These cells were gated on IgD and IgM to identify IgD- B cells. IgD- B cells were gated on GL7 and Fas to identify GL7+Fas+ germinal centre B cells. GC B cells were gated on CRM where biotinylated CRM, (Fina Biosolutions, Rockville, USA) was detected using streptavidin-PECF549 antibody to identify CRM+GC B cells.

Gating strategy for the mouse T follicular helper (Tfh) panel. For all analyses, lymphocytes were gated by singlet discrimination, followed by live discrimination. Live cells were gated on CD3 and CD19 to identify CD3+ cells. CD3+ cells were gated on CD4 and CD8 to identify CD4+ T cells. CD4+ T cells were gated on CD44 and CD62 to identify CD44hiCD62L T cells. CD44hiCD62L T cells were gated on CXCR5 and PD1 to identify CXCR5+PD-1+ Tfh cells.

Gating strategy for the mouse cell activation panel. For all analyses, lymphocytes were gated by singlet discrimination, followed by live discrimination. Live cells were gated on CD19 and CD3 and B220 to gate out B cells. Cells were gated on CD8 to gate out CD8+ T cells. Remaining cells were gated on CD3 to gate out CD4+ T cells. Non- B and T cells were gated on Ly6G to gate out neutrophils and NK1.1 to gate out NK cells. Remaining cells were gated on CD11b and CD11c to identify CD11b +CD11c- Monocytes and CD11c+ DCs. DCs were gated on B220 and MHCII to identify MHCIIloB220+ pDCs and MHCII+B220- cDCs. cDCs were gated on CD8 and CD11b to identify cDC1 (MHCII+B220-CD11b-CD8a+) and cDC2 (MHCII+B220-CD11b +CD8a-) cDC populations. DCs were also gated on CD11c and MHCII to identify (CD11chiMHCIIlo) rDCs and (CD11chiMHCIIhi mDCs).

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