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.

- 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
- 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
Diffraction data were collected at Advanced Photon Source (APS), Argonne National Laboratory beamlines, and at National Synchrotron Light Source II (NSLS-II). Diffraction data for MZ1 Fab, and MZ4 Fab were collected at APS 19-ID beamline to a final resolution of 2.05Å, and 2.95Å, respectively, using a Q315r CCD detector. Diffraction data for MZ1-ZIKV E crystals were collected at APS 24-ID-E beamline, and measured using a Dectris Eiger 16M PIXEL detector to a final resolution of 4.2 Å. Diffraction data for MZ4-ZIKV E complex were collected at APS 19-BM beamline, and measured using an ADSC Quantum 210r CCD detector to a final resolution of 4.3 Å. MZ24 Fab diffraction data were collected at beamline NSLS-II AMX 17-ID-1 and measured using a Eiger 9M PIXEL detector to a final resolution of 2.11 Å.

Data analysis
Statistical analyses were performed in Prism (version 8, GraphPad Software), N-Parameter Logistic Regression (nplr) R package version 0.1-7, R (version 3.5.1) and R studio (1.1.442). Data were graphed using Prism software (version 7, GraphPad Software). Flow plots were generated using FlowJo version 9.9.6. Real-time interactions between purified E proteins and antibodies were measured by Biolayer interferometry and analyzed by the FortéBio Data Analysis software 9.0. Structural diffraction data indexing, integration, and scaling were carried out using the HKL2000 suite37. Phenix xtriage (version 1.11.1-2575-0000) was used to analyze all the scaled diffraction data output from HKL2000 (suite 37) and XDS (version Jan 26, 2018 BUILT=20180126), and structure quality was assessed with MolProbity (Phenix suite version 1.11.1-2575-0000). All crystal structures described in this study were solved by molecular replacement using the program Phaser, version 2.139. All structure figures were generated using PyMOL (version 1.3). MZ1-ZIKV E and MZ4-ZIKV E contact residues and previously described flavivirus-neutralizing antibody-E contact residues were identified using PISA (PDBePISA v1.52 [20/10/2014]).

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 associated accession numbers for the coordinates and structure factors reported in this paper are Protein Data Bank ID: 6MTX, 6MTY, 6NIP, 6NIU and 6NIS. The sequences for MZ4, MZ1, MZ2, MZ20, MZ24, MZ54, MZ56 heavy chains and MZ4, MZ1, MZ2, MZ20, MZ24, MZ54, MZ56 light chains have been deposited in GenBank under iD codes MNS23667-MNS23680, respectively. The data that support the findings of this study are available from the corresponding authors upon request. The interim aggregate data of ZPIV trial in Puerto Rico (NCT03008122) are available with permission from Division of Microbiology and Infectious Disease, NIAID, NIH, as this is currently an active, ongoing Phase I study.

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

Monoclonal antibodies were isolated from 1 individual (Participant A), who had the highest ZIKV neutralization titer within the vaccine clinical trial. Upon further analysis, this individual had prior flavivirus exposure. The responses from this individual were compared to the remaining vaccinated individuals within the Phase I trial who were flavivirus naive (N=24), with 5 individuals tested for additional cross-flavivirus neutralization. To determine if these observations were unique to Participant A, the kinetics and magnitude of ZIKV neutralization were compared to flavivirus-experienced individuals (N=34) from Puerto Rico who received the same ZPIV vaccination regimen, 5 of which were further tested for cross-neutralizing response to 7 other flaviviruses (DENV 1-4, WNV, JEV, and YFV). Passive transfer studies of monoclonal antibodies in mice were used to assess protection from in vivo DENV-2 and ZIKV viral replication using two different mouse models. To evaluate the efficacy of the monoclonal antibodies MZ2 and MZ4 against DENV-2 in the Ifnar-/- C57BL/6 weight loss model, three groups of 10 mice each received treatment with 200μg of MZ2, MZ4 or vehicle control prior to virus exposure. In vivo passive protection experiments using MZ4 were performed 3 separate occasions with ZIKV challenge using six to eight-week-old female Balb/c mice (n=5/group/occasion). Mice received 100 μl (200 μg) of a 2 mg ml-1 solution of purified monoclonal antibody prior to ZIKV exposure. To get an idea of the in vivo potency, 30 mice were used in a passive protection experiment with decreasing doses of MZ4 using a 3-fold dilution starting at 100μg (5mg kg-1) to 0.14μg (0.07 mg kg-1).

Data exclusions

No data were excluded from this analysis.

Replication

Binding and neutralization data were calculated from two independent experiments, performed in triplicate. Since there is inherent variability in different neutralization assays, 3 different types of neutralization assays (MN50, PRNT, and FlowNT5) were performed to confirm the observations. Neutralization potencies obtained from each assay correlated for all mAbs tested, and all findings were replicated. The passive protection experiments were performed 3 times with 5 mice each with MZ4 using a dose of 200μg (10 mg kg-1), which yielded 100% protection in all experiments.

Randomization

Flavivirus-naive status at baseline (as determined by neutralization against ZIKV, DENV 1-4, YFV, WNV, and JEV) was a prerequisite for enrolling in the Phase 1 WARAI ZPIV vaccination trial. Prior flavivirus-exposure at baseline was not a prerequisite to enroll in the Puerto Rico ZPIV vaccine clinical trial. Randomization within each trial was not performed. For the in vivo mouse passive protection studies, Ifnar-/- C57BL/6 mice or female Balb/c mice were randomly assigned either treatment or sham groups, respectively, for each study.

Blinding

The Principal Investigators of the Phase I Clinical Trial as well as all other investigators were blinded during each of the trials. Samples from Participant A were chosen for monoclonal antibody isolation based upon high neutralization at peak timepoints and compared to the study participants enrolled in other trials using the same vaccination regimen. Investigators are currently still blinded for the ZPIV Phase I trial in Puerto Rico, study NCT03008122. The subset of 5 individuals for cross-neutralization experiments was randomly selected by a contracted organization who oversees the clinical data collection while keeping the investigators and sponsor blinded. These 5 individuals were randomly selected based upon flavivirus exposure prior to ZPIV vaccination as determined by microneutralization. The Principle Investigators were also blinded to the treatment and sham groups for the in vivo mouse passive protection studies until the studies were complete and results were available.

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

- Antibodies
- Eukaryotic cell lines
- Palaeontology
- Animals and other organisms
- Human research participants
- Clinical data

Methods

- Antibodies
- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

Antibodies

Antibodies not described in this study for the first time and their sources are as follows: Human recombinant EDE1-C8 (Barbas-Spaeth et al., 2016), Human recombinant Z004 (Robbiani et al., 2017), Human recombinant Z3L1 (Wang et al., 2016), Human recombinant VRC01 (Wu et al., 2010), Mouse recombinant 2A10G6 (Deng et al., 2011), Mouse anti-pan-flavivirus E 686-C1 (J.T. Roehrig, CDC, Catalog PUR001, Lot A7031794), Mouse anti-pan-flavivirus E D1-4G2-4-15 (4G2) (Biovest/NCCC, Produced from ATCC Hybridoma D1-4G2-4-15 (ATCC® HB-112™)), Lot G72110, Flow cytometry dilution 1/20, Mouse anti-monkey IgG, HRP conjugated (Southern Biotech, Catalog 4700-05, Lot A3814-M656D, Clone 5B108a, Dilution 1/6000), Mouse anti-human IgG, HRP conjugated (Southern Biotech, Catalog 9040-05, Lot J3314.T085 and L0717-ZC27B, Clone JDC-10, Dilution 1/2800), Goat anti-mouse IgG, HRP conjugated (Southern Biotech, Catalog 1030-05, Lot K5515-5566, Dilution 1/6000), Mouse anti-human CD3, BV510 conjugated (BD Biosciences Cat# 563918, Lot 7037566, Clone SP34-2, Dilution 1/20), Mouse anti-human CD4, BV510 conjugated (BD Biosciences Cat# 562970, Lot 7094727, Clone SK3, Dilution 1/80), Mouse anti-human CD8, BV510 conjugated (BioLegend Cat# 301047, Lot B221676, Clone RPA-T8, Dilution 1/80), Mouse anti-human CD14, BV510 conjugated (BioLegend Cat# 301841, Lot B236875, Clone M5E2, Dilution 1/80), Mouse anti-human CD16, BV510 conjugated (BD Biosciences Cat# 563830, Lot 7103547, Clone 3G8, Dilution 1/160), Mouse anti-human CD56, BV510 conjugated (BD Biosciences Cat# 318339, Lot B205718, Clone HCD56, Dilution 1/40), Mouse anti-human CD19, ECD conjugated (Beckman Coulter Cat# IM2708U, Lot 97, Clone J3-119, Dilution 1/40), Mouse anti-human IgM, PE-Cy5 conjugated (BioLegend Cat# 564230, Lot 7037913, Clone CD19-145, Dilution 1/80), Mouse anti-human IgD, APC-Cy7 conjugated (BioLegend Cat# 348217, Lot B234185, Clone IA6-2, Dilution 1/160), Mouse anti-human IgM, PE-Cy5 conjugated (BD Biosciences Cat# 551079, Lot 7041529, Clone G20-127, Dilution 1/10).

Validation

All antibodies from commercial sources undergo validation using flow cytometry, western blot, chromatin immunoprecipitation, immunofluorescence, immunohistochemistry, and/or biofunctional assays to ensure specificity and to provide clarity for research uses. Specifically, mouse anti-human CD3, BV510 conjugated (Clone SP34-2), mouse anti-human CD16, BV510 conjugated (Clone 3G8), mouse anti-human IgG, BV785 conjugated (Clone G18-145), mouse anti-human IgM, PE-Cy5 conjugated (Clone G20-127) and mouse anti-human IgD, APC-Cy7 conjugated (Clone IA6-2) were thoroughly validated by Biolegend. Each antibody was tested using the following criteria: 1. Staining of 1-3 target cell types with either single- or multi-color analysis detailed in the QC specification (including positive and negative controls). The tested cells can be primary cells and/or transfected cell models with relevant controls using multiple immunoassays to ensure biological accuracy. BD also performs multiplexing with additional antibodies to interrogate antibody staining in multiple cell populations. Mouse anti-human CD8, BV510 conjugated (Clone RPA-T8), mouse anti-human CD14, BV510 conjugated (Clone M5E2), mouse anti-human CD56, BV510 conjugated (Clone SK3) underwent stringent testing and validation by BD Biosciences to assure that it generates a high-quality conjugate with consistent performance and specific binding activity. The validation process included testing on a combination of primary cells, cell lines and/or transfected cell models with relevant controls using multiple immunoassays to ensure biological accuracy. BD also performs multiplexing with additional antibodies to interrogate antibody staining in multiple cell populations. Mouse anti-human CD8, BV510 conjugated (Clone RPA-T8), mouse anti-human CD14, BV510 conjugated (Clone M5E2), mouse anti-human CD56, BV510 conjugated (Clone SK3) and mouse anti-human IgD, APC-Cy7 conjugated (Clone IA6-2) were thoroughly validated by Biolegend. Each antibody was tested using the following criteria: 1. Staining of 1-3 target cell types with either single- or multi-color analysis detailed in the QC specification (including positive and negative controls). The tested cells can be primary cells and/or cell lines known to be positive or negative for the target antigen; 2. Each batch product is validated by QC testing with a series of dilutions to make sure the product is working within expected antibody titer range; 3. Each batch is compared to an internally established "gold standard" to maintain batch-to-batch consistency; 4. When applicable, our products are side-by-side tested with our competitors’ products to make sure that BioLegend’s products exceed or are at least the same quality; 5. For most tandem dye-conjugated products, color compensation is examined in order to verify tandem integrity.

General statements from Biolegend: “The specificity and sensitivity of each antibody is thoroughly validated in the New Product Development stage. This is done by staining multiple target cells with either single- or multi-color analysis or by other testing approaches. The QC specifications and testing SOPs and gold standard for each product are then developed”. In general, each product is tested using the following criteria: 1. Staining of 1-3 target cell types with either single- or multi-color analysis detailed in the QC specification (including positive and negative controls). The tested cells can be primary cells and/or cell lines known to be positive or negative for the target antigen; 2. Each batch product is validated by QC testing with a series of dilutions to make sure the product is working within expected antibody titer range. 3. Each batch is compared to an internally established “gold standard” to maintain batch-to-batch consistency.

Mouse anti-human IgG2, (Biovest/NCCC, Produced from ATCC Hybridoma D1-4G2-4-15 (ATCC® HB-112™) has been described for reactivity and specificity in Henchal et al., 1982. (Henchal, E.A., Gentry, M.K., McCown, J.M. & Brandt, W.E. Dengue virus-specific and Flavivirus group determinants identified with monoclonal antibodies by indirect immunofluorescence. Am. J. Trop. Med. Hyg. 31, 830-836 (1982)).
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) | D1-4G2-4-15 mouse hybridoma (ATCC #HB-112), C6/36 (ATCC #CRL-1660), Vero (ATCC #CCL-81), Expi293F (ThermoFisher Scientific #A14527), DS-2 (ThermoFisher Scientific #R69007), U937-DC-SIGN (ATCC #CRL-3253) and K562 (ATCC #CCL-243) cell lines were utilized in this study.

Authentication | All cell lines were authenticated using short-tandem repeat analysis.

Mycoplasma contamination | Cell lines were not recently tested for mycoplasma contamination.

Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used in this study.

Animals and other organisms

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

Laboratory animals | Six to eight week old female Balb/c mice and Ifnar-/- knock-out C57BL/6 female mice were used in this study.

Wild animals | This study did not involve wild animals.

Field-collected samples | This study did not involve samples collected from the field.

Ethics oversight | This study was approved by the Institutional Animal Care and Use Committees (IACUCs) at both the Beth Israel Deacon Medical Center and the U.S. Army Medical Research Institute of Infectious Diseases. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics | These Phase I ZIKV vaccine clinical trials were described previously in Modjarrad, K., et al. "Preliminary aggregate safety and immunogenicity results from three trials of a purified inactivated Zika virus vaccine candidate: phase 1, randomised, double-blind, placebo-controlled clinical trials", The Lancet 391, 563-571 (2018). Men and women, ages 18 to 49 years, were recruited to participate these vaccination trials. Individuals within the BIDMC and WRAIR studies, NCT02937233, NCT02963909, respectively, were enrolled if they had no history of flavivirus infection or vaccination. In addition, individuals within the WRAIR study had to demonstrate a lack of serologic flavivirus neutralization. Individuals within the Puerto Rican trial were not screened for flavivirus history. In addition, individuals with serologic evidence of human immunodeficiency virus, hepatitis B or C infection were excluded from study participation, along with pregnant or breast-feeding women.

Recruitment | Men and women, ages 18 to 49 years, were recruited to participant these 3 ZPIV vaccination trials. Individuals within the BIDMC and WRAIR studies, NCT02937233, NCT02963909, respectively, were enrolled if they had no history of flavivirus infection or vaccination. In addition, individuals within the WRAIR study had to demonstrate a lack of serologic flavivirus neutralization. Individuals within the Puerto Rican trial were not screened for prior flavivirus infection or vaccination history. For further details see Modjarrad, K., et al. "Preliminary aggregate safety and immunogenicity results from three trials of a purified inactivated Zika virus vaccine candidate: phase 1, randomised, double-blind, placebo-controlled clinical trials", The Lancet 391, 563-571 (2018).

Ethics oversight | These studies were approved by the Beth Israel Deaconess Medical Center (BIDMC), Walter Reed Army Institute of Research (WRAIR), St. Louis University (STL), and Quorum Central (now Advarra Central) Institutional Review Boards and written informed consent was obtained from all participants.

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 | NCT 02937233, NCT02963909, and NCT03008122

Study protocol | The full trial protocol is published at Modjarrad, K., et al. "Preliminary aggregate safety and immunogenicity results from three trials of a purified inactivated Zika virus vaccine candidate: phase 1, randomised, double-blind, placebo-controlled clinical trials", The Lancet 391, 563-571 (2018).

Data collection | Participants for the BIDMC and WRAIR trials were enrolled between Nov. 2016 and Jan. 2017. Participants were assessed on days 1, 4, 8, 15, 29, 32, 36, 43, and 157 following ZPIV vaccination for ZIKV neutralization. Data were collected and masked to study clinicians. Interim results were published in Modjarrad, K., et al. "Preliminary aggregate safety and immunogenicity results from..."
Outcomes

The primary outcomes for each trial was designed to evaluate safety, reactogenicity, and immunogenicity of ZPIV through the primary timepoint of day 57. Participants were assessed on days 1, 4, 8, 15, 29, 32, 36, 43, and 157 57 following ZPIV vaccination for ZIKV neutralization. Interim results were published in Modjarrad, K., et al. "Preliminary aggregate safety and immunogenicity results from three trials of a purified inactivated Zika virus vaccine candidate: phase 1, randomised, double-blind, placebo-controlled clinical trials", The Lancet 391, 563-571 (2018). Overall the vaccine caused only mild or moderate reactogenicity, and by day 57 92% of vaccine recipients seroconverted. Microneutralization geometric mean titers peaked at day 43 and exceeded protective thresholds observed in prior animal models. For the Puerto Rican trial, group assignment still remains masked to study clinicians as this study has not yet reached completion. Study clinical staff have remained blinded to individual-level results from the interim analysis.

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

Cryopreserved PBMCs were thawed in warm medium containing benzonase, then washed with phosphate-buffered saline (PBS) and stained for viability using Invitrogen Aqua Live/Dead stain. Cells were incubated at 4°C for 30 minutes with a cocktail of antibodies including: CD3 BV510 (BD Biosciences), CD4 BV510 (BD Biosciences), CD8 BV510 (BioLegend), CD14 BV510 (BioLegend), CD16 BV510 (BD Biosciences) and CD56 BV510 (BioLegend) as dump channel markers, and CD19 ECD (Beckman Coulter), IgG BV785 (BioLegend), IgD APC-Cy7 (BioLegend) and IgM PE-Cy5 (BD Biosciences). ZIKV E and DENV-2 E were tetramerized and conjugated to BV421 (BioLegend) and BV650 (BioLegend), respectively. To obtain monoclonal antibodies that target quaternary epitopes, primary staining also included live whole ZIKV virions (Paraiba_01) followed by secondary staining using 4G2 (Biovest) conjugated to APC (Thermofisher).

Instrument

FACSAria (Becton Dickinson)

Software

FlowJo, version 9.9.6

Cell population abundance

Less than 5% of the total B cell population was specific for either ZIKV or DENV-2. This information was not used within the analysis, as all whole ZIKV virions, ZIKV E and DENV-2 E triple positive B cells were sorted into lysis buffer, sequenced, cloned and characterized.

Gating strategy

CD19+/IgG+/IgD-/IgM- B cells reactive to ZIKV-E, DENV-E, or whole ZIKV virions were sorted directly into lysis buffer at one cell per well into PCR plates using a FACSAria (Becton Dickinson) and stored at −80°C until subsequent reverse transcription. We focused on sequencing and cloning B cell receptors (BCRs) from DENV-2 E positive B cells with and without cross-reactivity with ZIKV E or whole ZIKV virions. The same sorting strategy was performed from PBMCs obtained from Participant A at week 0, prior to ZPIV vaccination, to determine if MZ4-family members were present prior to vaccination, and at week 2, 2 weeks following first ZPIV vaccination, where high neutralization titers against ZIKV and the 4 DENV serotypes were observed. Sorting at week 8 was performed on a different day compared to PBMCs from Participant A at week 0 and week 2, and the ZIKV-naïve donor. Increased frequencies of antigen-specific and cross-reactive B cells were detected against all antigens (whole ZIKV virions, ZIKV E, and DENV-2 E) between week 0 and week 2 (Extended Data Fig. 1b).

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