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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.

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	Semi-automated EM data collection: SerialEM
Data analysis	All data with the following exceptions were analyzed with GraphPad Prism 7. X-ray crystallography: COOT, PHENIX Cryo-EM: MotionCorr, CTFFIND4, RELION, RELION2, EMAN2 EM image analysis: RELION, EMAN2, FIJI Structure rendering: UCSF Chimera, Blender Flow cytometry: FlowJo 9 Immunogenetics: IMGT V-QUEST Biolayer interferometry: Octet Analysis 9

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

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary information. Coordinates for 441D6 Fab structure and cryo-EM density map have been deposited to the Protein Data Bank (PDB) under PDB ID 5TR8 and EM Data Bank (EMDB) under EMD-7021. HA RBD-np and antibody

sequences are deposited to the GenBank under accession no. MK273069–MK273076 and MK283705–MK283710. Requests for materials should be addressed to M.K. and B.S.G.

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	The number of animals selected for each study was chosen based on our prior experience with similar vaccine regimens. Assuming variance in immune response is proportional to mean for a given group (constant CV of 30%, typical for this type of experiments), a group size of 10 will give 89% power to detect 2-fold differences or 49% power to detect 1.5-fold differences between groups in the magnitude of the immunological parameters based on a two-tailed test of means with alpha set to 0.05 (calculation was performed by 1-way ANOVA pairwise tools at powerandsamplesize.com). A consistent 1.5- to 2-fold difference in immunological parameters is the minimum amount of difference between groups that would be biologically relevant.
Data exclusions	No data has been excluded.
Replication	All analyses for antibody binding, specificity, and virus neutralization assays have been performed at least twice. Many of these these analyses including immunization studies have been repeated three times or more with similar results. All of the data in which we could perform statistical analysis showed that the differences observed were significant and highly consistent across experiments.
Randomization	All animals were age and gender matched and randomly assigned to experimental groups.
Blinding	In vivo challenge studies were done in a blinded manner. All of the serological assays including virus neutralization assays, and structural, biochemical and biophysical characterization of the monoclonal antibodies were not performed in a blinded manner.

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
☐	☒	Animals and other organisms
☐	☒	Human research participants
☐	☒	Clinical data

Methods

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

Antibodies

Antibodies used	All the antibodies used including the ones identified in this study were made recombinantly by cloning antibody heavy and light chains into the mammalian expression vectors. Antibodies were produced in mammalian cells (Expi293 cells) by transient transfection of expression vectors and purified by protein A affinity chromatography. Sequences, specificity and function of the antibodies were verified for each antibody. Antibodies used in flow cytometry were listed below in Flow Cytometry section.
Validation	All the antibodies including the ones identified in this study were tested for their reactivity and specificity by ELISA, BLI or other assays prior to be used in the study.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Expi293 cells (Gibco); Madin-Darby canine kidney (MDCK) cells (ATCC); 293T cells (ATCC); Turkey red blood cells (Lampire biologicals)

Authentication	No authentication was performed.
Mycoplasma contamination	Tested negative.
Commonly misidentified lines (See ICLAC register)	All cells used in the studies were directly purchased from commercial vendors and not extensively passaged. No commonly misidentified cell lines were used in the studies.

Animals and other organisms

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

Laboratory animals	Female 4-6 week old BALB/cAnNTac mice (Jackson Laboratories) were used in the studies in accordance with all federal regulations, NIH guidelines, AAALAC, and IACUC approval.
Wild animals	The studies did not involve wild animals.
Field-collected samples	The studies did not involve samples collected from the field.
Ethics oversight	Institutional Animal Care and Use Committee (IACUC) of the Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health approved all the animal study protocols. The studies were performed in accordance with all federal regulations, NIH guidelines, AAALAC, and IACUC approval.

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	6 years and older, both sexes (VRC 700); ages 18-60 years, both sexes (VRC 310)
Recruitment	All human serum samples for this study were collected with informed consent of volunteers, and approval for this study was obtained under protocol number VRC 700 (Clinicaltrials.gov NCT01262079). All volunteers were at least 6 years of age, healthy, and ranged in age from 7 to 93 years. Human PBMC sample was obtained from VRC 310, a single-site, phase 1, open-label, randomized clinical trial conducted at the National Institutes of Health (NIH) Clinical Center by the NIAID VRC (Clinicaltrial.gov NCT01086657). These studies were approved by the NIAID Intramural Institutional Review Board.
Ethics oversight	Intramural Institutional Review Board (IRB) of the National Institute of Allergy and Infectious Diseases, National Institutes of Health approved all the human studies. U.S. Department of Health and Human Services guidelines for conducting clinical research were followed.

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	VRC 700 (Clinicaltrials.gov NCT01262079); VRC 310 (Clinicaltrial.gov NCT01086657)
Study protocol	VRC 700 (https://clinicaltrials.gov/ct2/show/NCT01262079?term=NCT01262079&rank=1); VRC 310 (https://clinicaltrials.gov/ct2/show/NCT01086657?term=NCT01086657&rank=1)
Data collection	VRC 700 (December 2010–August 2011; Locations, Hope Clinic of the Emory Vaccine Center, Decatur, GA; St. Louis University Doisy Research Center, St. Louis, MO; Cincinnati Children's Hospital Medical Center, Cincinnati, OH; Baylor College of Medicine, Houston, TX); VRC 310 (February 2010–December 2011; Location, National Institutes of Health Clinical Center, Bethesda, MD)
Outcomes	VRC 700 (Primary Outcome Measures, Influenza-specific antibody responses; Secondary Outcome Measures, influenza-specific T-cell responses); VRC 310 (Primary Outcome Measures, adverse events, including clinical, laboratory and local and systemic reactogenicity; Secondary Outcome Measures, immunogenicity as measured by humoral and cellular assays)

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	Mouse peripheral white blood cells were prepared from heparinized whole blood by lysing red blood cells with ACK lysing buffer (Thermo Fisher Scientific). After hemolysis, white blood cells were washed and stained with fluorochrome-conjugated mAbs to mouse CD3-Cy5PE (BD Bioshiences, 553065, clone 145-2C11, 1:300 dilution), CD4-Cy5PE (BD Biosciences, 553654, clone H129.19, 1:300 dilution), CD8-Cy5PE (BD Biosciences, 553034, clone 53-6.7, 1:300 dilution), F4/80-Cy5PE (Biolegend, 1231111, clone BM8, 1:300 dilution), CD19-PE-CF594 (BD Biosciences, 562329, clone 1D3, 1:300 dilution), IgD-BV421 (BD Biosciences, 744291, clone 11-26c.2a, 1:600 dilution). PE- and APC-labeled HA probes were prepared as described previously. AQUA dead cell stain was added for live or dead discrimination (Thermo Fisher Scientific). Samples were analyzed on a LSR II (BD Biosciences) and data analysis was done in FlowJo 9 (TreeStar). To single-cell sort, HA-specific B cells were stained as above and HA+ IgD- B cells were sorted as single cells into 96-well plates using a FACS Aria II (BD Biosciences).
Instrument	BD Biosciences LSR II or FACS Aria II (sorting)
Software	FlowJo (TreeStar)
Cell population abundance	Not applicable.
Gating strategy	Gating strategy was included in the main figure.

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