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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	No custom codes were used in this study. Commercial softwares used in this study include: Microsoft Excel version 16.26, FACSDiva Software version 6.2, FlowJo v.10.6.1, GraphPad Prism8 version 8.3.0, QuantStudio real time PCR software version 1.1, BioTek Gen5 version 3.03 (used for ELISA plate reading)
Data analysis	GraphPad Prism8 (ver 8.3.0) for Mac OSX was used to perform statistical analysis on the data collected. Detailed data analysis methods are described in the Figure legends and Methods. FlowJo v.10.6.1 was used to analyze flow cytometry data.

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

Data are available upon request. Materials that can be shared will be released via a Material Transfer Agreement.

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 sample size for the study participants used in this study was based on the number of study participants from which the viral sequence was successfully determined. We analyzed samples from all the participants that had tested positive for viral shedding (n=77) by a clinical microbiology lab test (nested-PCR-based) at the time of the clinical challenge studies. From these 77 positive samples, we were able to amplify the region and obtain the selection result from 29 study participants from banked, archived frozen samples (See Figure 1a). All of these study participants and the corresponding analyses are included in the manuscript without any exclusion. 15 mice were used per each group to analyze viral growth kinetics. Of these mice, 5 mice were used each day (Day 2, 4, and 6) for viral titration in the lungs (n=5). Based on the lung viral load data from our previous mouse experiments, a sample size of 4 was expected to have 80% power to detect a 10-fold difference in viral titers between groups at an alpha of 0.05. 6 ferrets were used per each group to analyze viral growth kinetics. Nasal wash samples were collected for viral titration at days 1, 3, 5, and 7 (n=6). Based on the lung viral load data from our previous ferret experiments, a sample size of 6 was expected to have 80% power to detect a 10-fold difference in viral titers between groups at an alpha of 0.05.
Data exclusions	Study participants with no amplifiable amount of RNA were excluded from the analysis. No data were omitted from reporting.
Replication	All in vitro experiments were independently replicated a total of three times (n=3). All data were presented without an exclusion. For in vivo mouse experiments, five animals were used per a group as presented in Figure 2d (n=5). A previous experiment was performed using 3 to 4 mice in a group (n=3 or 4), and the lung viral load was measured using qPCR rather than TCID50 assay. In contrast to the data reported in Figure 2d, the previous experiment failed to detect a statistically significant difference in the viral growth kinetics between the wild-type and mutant viruses. This is potentially due to the insufficient number of animals used (low power) and/or different ways of measuring the viral load (qPCR vs TCID50). The final report of this experiment (Figure 2d) includes 5 animals per group and utilized TCID50, rather than qPCR, according to reviewers' suggestions, and shows a statistically significant difference between groups. For in vivo ferret experiments, six animals were used per group as presented in Figure 2e (n=6). A previous experiment was performed using 3 ferrets in a group (n=3), and the lung viral load was measured using qPCR rather than TCID50 assay. Same as the data reported in Figure 2e, the previous ferret experiment did not show a difference between the growth kinetics of the wild-type and mutant viruses. The final report of this experiment (Figure 2e) includes an increased number of animals for the increased power of the study, and also used TCID50 assay, rather than qPCR, according to a reviewer's suggestion.
Randomization	Study participants were allocated into different groups according to the viral selection results (Fig. 1). Animals were randomized upon arrival and assigned to cages by husbandry staff and the groups were assigned sequentially based on rack numbers.
Blinding	Samples were assigned a numerical code during data collection and unblinded upon the completion of the data collection.

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

1. Goat anti-human IgG (HRP-conjugated) (polyclonal; Abcam; catalog no. Ab 205630)
2. Goat anti-mouse IgG (HRP-conjugated) (polyclonal; ThermoFisher; catalog no. A28177)
3. Goat anti-rabbit IgG (HRP-conjugated) (polyclonal; ThermoFisher; catalog no. A16110)
4. CR6261 (clone number CR6261; provided by Janssen Pharmaceutical Companies)
5. CR9114 (clone number CR9114; Creative Biolabs; catalog no. PABX-119)
6. FI6V3 (clone number FI6V3; Creative Biolabs; catalog no. PABL-214)
7. 70-1F02 (clone number 70-1F02; provided by Dr. Rafi Ahmed, Emory Vaccine Center)
8. C179 (clone number C179; Takara Bio; catalog no. M145)
9. CT149 (clone number CT149; Creative Biolabs; catalog no. PABL-213)
10. EM-4C04 (clone number EM-4C04; provided by Dr. Rafi Ahmed, Emory Vaccine Center)
11. 2-12C (clone number 2-12C; provided by Dr. Alain Townsend, University of Oxford, United Kingdom)
12. Anti-influenza nucleoprotein (NP) antibody (clone number A1; MilliporeSigma; catalog no. MAB8257)

Validation

1. Goat anti-human IgG (HRP-conjugated) (Abcam; catalog no. Ab 205630) - Cross-adsorbed to Human IgM and IgA, to minimize non-specific signal
2. Goat anti-mouse IgG (HRP-conjugated) (ThermoFisher; catalog no. A28177) - minimal cross-reactivity with rabbit, rat, human, bovine, guinea pig and donkey IgG
3. Goat anti-rabbit IgG (HRP-conjugated) (ThermoFisher; catalog no. A16110) - cross- adsorbed against bovine, goat, human,
4. CR6261: Throsby, M., et al. (https://www.ncbi.nlm.nih.gov/pubmed/19079604),
   Ekiert, D.C., et al. (https://www.ncbi.nlm.nih.gov/pubmed/19251591)
5. CR9114: Dreyfus, C., et al. (https://www.ncbi.nlm.nih.gov/pubmed/22878502)
6. FI6V3: Corti, D., et al. (https://www.ncbi.nlm.nih.gov/pubmed/21798894)
7. 70-1F02: Wrammert, J., et al. (https://www.ncbi.nlm.nih.gov/pubmed/21220454)
8. C179: Okuno, Y., et al. (https://www.ncbi.nlm.nih.gov/pubmed/7682624)
9. CT149: Wu, Y., et al. (https://www.ncbi.nlm.nih.gov/pubmed/26196962)
10. EM-4C04: Wrammert, J., et al. (https://www.ncbi.nlm.nih.gov/pubmed/21220454)
11. 2-12C: Huang, K.Y., et al. (https://www.ncbi.nlm.nih.gov/pubmed/26011643)
12. Anti-influenza nucleoprotein (NP) antibody (https://www.who.int/influenza/gisrs_laboratory/manual_diagnosis_surveillance_influenza/en/)

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	MDCK, Vero, and A549 cells are purchased from ATCC. Sf9 insect cells are purchased from Thermo Fisher Scientific.
Authentication	not authenticated
Mycoplasma contamination	not tested for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	not commonly misidentified lines

Animals and other organisms

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

Laboratory animals	Mouse - BALB/cJ (7-8-week-old female, The Jackson Laboratory, Stock no. 000651) Ferret (6-7-month-old female, TRIPLE F FARMS)
Wild animals	not used
Field-collected samples	not used
Ethics oversight	All animal experiments were conducted under protocols approved by the Animal Care and Use Committee (ACUC) at the National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Health (NIH).

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	All the study participants were healthy and young (18-43 years old, Average age 29.4) with no health issues. While these recruitment criteria were imperative considering the nature of the intervention (influenza virus challenge) and to minimize the risk of adverse events (AEs), it is possible that the clinical observations and analyses of the current report may not be fully representative of the larger population as a whole. Detailed population characteristics for the primary clinical trials can be found from ClinicalTrials.gov and our previous

	publications. - ClinicalTrials.gov Identifiers NCT01646138 and NCT01971255 - Memoli, M.J. et al. Clin Infect Dis 60, 693-702 (2015) - Memoli, M.J. et al. MBio 7, e00417-00416 (2016)
Recruitment	Detailed recruitment strategy for the primary clinical trials can be found from ClinicalTrials.gov and our previous publications. - ClinicalTrials.gov Identifiers NCT01646138 and NCT01971255 - Memoli, M.J. et al. Clin Infect Dis 60, 693-702 (2015) - Memoli, M.J. et al. MBio 7, e00417-00416 (2016)
Ethics oversight	Primary clinical trials were approved by the National Institute of Allergy and Infectious Diseases Institutional Review Board and conducted in accordance with the provisions of the Declaration of Helsinki and good clinical practice guidelines.

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	ClinicalTrials.gov Identifiers for the primary clinical trials from which samples analyzed in this study were derived: NCT01646138, NCT01971255
Study protocol	Detailed study protocols for the primary clinical trials can be found from ClinicalTrials.gov and our previous publications. - ClinicalTrials.gov Identifiers NCT01646138 and NCT01971255 - Memoli, M.J. et al. Clin Infect Dis 60, 693-702 (2015) - Memoli, M.J. et al. MBio 7, e00417-00416 (2016)
Data collection	The primary clinical trials (ClinicalTrials.gov Identifiers NCT01646138 and NCT01971255) were conducted using an inpatient setting at the National Institutes of Health Clinical Center (Bethesda, MD, USA). These studies were conducted from 2012 to 2015.
Outcomes	Primary outcome measure: - Number (or percentage) of patients with Mild to Moderate Influenza Disease (MMID) Secondary outcome measures: - Clinical disease severity score - Duration of shedding (Days) - Duration of symptoms (Days) - Number of symptoms - Number of participants with Influenza Symptoms Participants were evaluated daily for the symptoms by study physicians. A validated participant-directed questionnaire, called FLUPRO, was also used to measure the clinical disease severity score. Participants had 4 follow-up visits over 8 weeks. The viral shedding was determined from the nasal wash samples collected daily using BioFire FilmArray Respiratory Panel. Detailed outcomes for the primary clinical trials can be found from ClinicalTrials.gov and our previous publications. - ClinicalTrials.gov Identifiers NCT01646138 and NCT01971255 - Memoli, M.J. et al. Clin Infect Dis 60, 693-702 (2015) - Memoli, M.J. et al. MBio 7, e00417-00416 (2016)

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	MDCK cells were infected at 1 multiplicity of infection (MOI) of wild-type (A388) or mutant (V388) H1N1pdm viruses generated by reverse genetics. 24 hours after infection, cell culture supernatant was discarded, cells were washed twice with PBS and treated with Trypsin-EDTA (catalog no. 25200056; ThermoFisher) for 10min at 37°C. The trypsin-treated cells were harvested, and the trypsin was neutralized by adding an equal volume of PBS supplemented with 5% FBS. After centrifugation (300 x g, 5min), the supernatant was discarded, and cells were fixed by resuspending in Fixative Solution (catalog no. R37814; ThermoFisher) for 15min at room temperature. Fixed cells were filtered using 40µm cell strainer (catalog no. 352340; Corning

	Life Sciences, USA) to remove clumped cells. Filtered cells were spun (300 x g, 5min), the supernatant was removed, and the cells were washed twice with flow cytometry buffer (1% BSA, 0.1% sodium azide in PBS) using centrifugation (300 x g, 5min), and resuspended in the flow cytometry buffer. Detailed method for the staining is described in the Methods.
Instrument	Becton Dickinson LSR II
Software	FACSDiva Software (version 6.2), FlowJo v.10.6.1,
Cell population abundance	At least 1.5x10E4 events were acquired for each sample.
Gating strategy	Small debris and dead cells that have low SSC/FSC values were excluded from analysis. Anti-influenza nucleoprotein (NP) antibodies conjugated with APC were used to stain influenza-infected cells. Cells with positive for the APC signal were gated and used for analysis.

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