nature portfolio

Corresponding author(s): Hans Langedijk

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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 *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	SEC-MALS data were collected using Chromeleon 7.2.8.0 DSF data was collected with QuantStudio Real-Time PCR system software
Data analysis	ELISA and VNA (PRNT) titers were calculated with in-house custom made R scripts (R version 3.4.3) based on Gen5™ Data Analysis Software Graph Pad Prism version 10 SEC-MALS data were analyzed using Chromeleon 7.2.8.0 and Astra 8.0.0.19 (Wyatt) Statistical analyses for preclinical experiments were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA) BLI data was analyzed using FortéBio Data Analysis 12.0 software (FortéBio) C3 map was processed using the DeepEMhancer tool within Cosmic2 Gateway (v2.2.0) Motion correction, CTF-estimation, blob particle picking, and particle extraction was performed in cryoSPARC Live v3.2.0 Cryo-EM structure was build and checked using ChimeraX (v1.6.1) and MolProbity (v4.5), and refined iteratively using Phenix (v1.20), Coot (v1.2), and ISOLDE (v1.4).

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 coordinates and EM maps generated in this study have been deposited in the Protein Data Bank and the Electron Microscopy Data Bank under accession codes: PDB 9B2X, EMD-44117. All other data are available in the source data file published with this manuscript.

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	Sera from a human clinical trial were used. The study design of this Phase 2b Study to Assess the Efficacy, Immunogenicity and Safety of an Ad26.RSV.preF-based Regimen in the Prevention of RT PCR-confirmed RSV-mediated Lower Respiratory Tract Disease in Adults Aged 65 Years and Older (NCT03982199) will be published elsewhere and referred to in the text.
Reporting on race, ethnicity, or other socially relevant groupings	Sera from a human clinical trial were used. The study design of this Phase 2b Study to Assess the Efficacy, Immunogenicity and Safety of an Ad26.RSV.preF-based Regimen in the Prevention of RT PCR-confirmed RSV-mediated Lower Respiratory Tract Disease in Adults Aged 65 Years and Older (NCT03982199) will be published elsewhere and referred to in the text.
Population characteristics	Sera from a human clinical trial were used. The study design of this Phase 2b Study to Assess the Efficacy, Immunogenicity and Safety of an Ad26.RSV.preF-based Regimen in the Prevention of RT PCR-confirmed RSV-mediated Lower Respiratory Tract Disease in Adults Aged 65 Years and Older (NCT03982199) will be published elsewhere and referred to in the text.
Recruitment	Sera from a human clinical trial were used. The study design of this Phase 2b Study to Assess the Efficacy, Immunogenicity and Safety of an Ad26.RSV.preF-based Regimen in the Prevention of RT PCR-confirmed RSV-mediated Lower Respiratory Tract Disease in Adults Aged 65 Years and Older (NCT03982199) will be published elsewhere and referred to in the text.
Ethics oversight	Sera from a human clinical trial were used. The study design of this Phase 2b Study to Assess the Efficacy, Immunogenicity and Safety of an Ad26.RSV.preF-based Regimen in the Prevention of RT PCR-confirmed RSV-mediated Lower Respiratory Tract Disease in Adults Aged 65 Years and Older (NCT03982199) will be published elsewhere and referred to in the text.

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	Sample size of the different animal studies described in the manuscript are determined based on power calculations. Historical data for the investigated or similar vaccines were used to guide statistical powering. Sample sizes were calculated using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA)
Data exclusions	All data is included and shown
Replication	Binding and neutralization antibody titers were always measured as technical duplicates. Screen for RSV preF-stabilizing substitutions and related experiments were performed in biological triplicate. Three cryo-EM datasets were collected. All attempts at reproduction were successful.
Randomization	Mice were randomly assigned to groups. For all other experiments samples or wells, were allocated randomly.
Blinding	Investigators were not blinded during ELISA and VNA data collection, but serum samples were randomly mixed onto the measurement plates for ELISA measurements to minimizing group specific biases in data collection. Investigators were blinded during cryo-EM analysis; only 1 protein design was tested so no group blinding was performed. Investigators were not blinded during Octet, DSF, SEC and FACS experiments, since there was no preference towards use of any of the stabilizing substitutions or vaccine candidates and hence there was no scope for bias in unblinded testing.

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

CR9501 5 µg/mL (produced in house)
RSV90 5 µg/mL (produced in house)
RSD5-GL 5 µg/mL (produced in house)
ADI-15644 5 µg/mL (produced in house)
Goat anti-mouse IgG-HRP (Biorad Cat172-1011) 1:2000 or 1:40000
Mab8262 clone 133-1H (Merck) 1:2000
Goat anti-human IgG HRP Jackson ImmunoResearch (109-035-098) 1:2000 or 1:3750
Anti-human CW800 (Rockland; 609-145-002) 1:10.000

Validation

All antibodies have been published and validated in the respective publications.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Human embryonic kidney cell line expi293F (Thermo Fisher; A14527)
A549 cells were received from ATCC (CCl-185), a working cell bank was prepared directly.
VERO cells were received from the WHO (WHO10-87; 880101), a working cell bank was prepared directly.

Authentication

Authentication was performed by supplier

Mycoplasma contamination

All cell lines were tested as being mycoplasma negative by supplier.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study

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

Mouse studies: BALB/c mice, female, 6- to 8-weeks-old at the start of the study
Four cynomolgus macaques (2M/2F, approximately 7 years of age at study start)

Wild animals

The study did not involve wild animals.

Reporting on sex

Mouse studies were only performed in female animals. For a careful comparison of induced immune responses between (combinations of) different vaccine candidates, as was the main objective of studies described in the current manuscript, it is important to keep the groups of experimental animals as homogeneous as possible. Therefore, experimental groups (in rodent experiments) were mostly comprised of inbred animals, from the same strain and age, as well as from the same sex. By reducing the variation, group sizes can be smaller, requiring fewer animals to address a specific research question. In the human population, more heterogenous immune responses can be expected. In addition to sex differences, many other factors (eg, genetic background, pre-exposure history, underlying health conditions, and age) contribute to this heterogeneity. Therefore, in later stages of vaccine development, human testing in a heterogenous population comprised of both sexes is part of the vaccine development program. The study in Cynomolgus macaques was performed in two males and two female animals.

Field-collected samples

Study did not involve samples collected from the field.

Ethics oversight	The clinical study was approved by the ethics committee or institutional review board at each participating center and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All participants provided written informed consent that allows use of the samples in this study. An independent data and safety monitoring committee monitored safety outcomes throughout the trials. The African green monkey study was conducted at the Wake Forest School of Medicine test facility and approved by the IACUC of Wake Forest University (WFU). The cynomolgus macaque study was performed by Alpha Genesis and approved by the IACUC of Alpha Genesis. Mouse studies were conducted at Janssen Vaccines and Prevention B.V. according to the Dutch Animal Experimentation Act and the Guidelines on the Protection of Animals for scientific purposes by the Council of the European Committee after approval by the Centrale Commissie Dierproeven and the Dier Experimenten Commissie of Janssen Vaccines and Prevention B.V. Project license: AVD213002020-10024. Approval codes: RSV23MM01-10024, RSV23MM09-10024, and RSV23MM12-10024

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	n.a.
Study protocol	n.a.
Data collection	n.a.
Outcomes	n.a.

Plants

Seed stocks	n.a.
Novel plant genotypes	n.a.
Authentication	n.a.