

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 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ | 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | 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

Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.

Data analysis

Yeast display titration curve fitting were done by the standard non-cooperative Hill equation, fitted by nonlinear least-squares regression with two additional parameters describing the curve by using Python 3.7 similarly common methods eg doi.: 10.1016/j.cell.2020.08.012; 10.1038/nprot.2006.94;

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 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 RBD-62 Cryo-EM data are available at EMD-12187 and PDB 7BH9.

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](https://www.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	Describe how sample size was determined, detailing any statistical methods used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.
Data exclusions	Describe any data exclusions. If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Replication	The number of replicates is clearly identified when relevant. There was a high level of reproducibility among yeast display expression levels and binding affinities.
Randomization	Describe how samples/organisms/participants were allocated into experimental groups. If allocation was not random, describe how covariates were controlled OR if this is not relevant to your study, explain why.
Blinding	Describe whether the investigators were blinded to group allocation during data collection and/or analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	HEK293T cells (Weizmann Institute of Science cell line repository), HEK-293T-ACE2 (GenScript M00770); VeroE6 (C1008, Institute Pasteur, France), Expi293F cells (ThermoFisher), S.cerevisiae EBY100 (Invitrogen)
Authentication	Provided by the supplier.
Mycoplasma contamination	All used cell lines were negatively tested for mycoplasma contamination by Hy-mycoplasma detection PCR kit (HyLabs, Ky 50341)
Commonly misidentified lines (See ICLAC register)	Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Golden Syrian hamsters (Mesocricetus auratus) were housed at BIOQUAL, Inc. Hamsters were 6- 8 weeks old on day of infection and were randomized into groups of 3 female and 3 male hamsters each per group.
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Wild animals

Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.

Field-collected samples

For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

Ethics oversight

Animal studies were reviewed and approved by the Animal Care and Use Committee of the Vaccine Research Center, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda USA

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

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

Sample preparation is described in detail in the Method section. *S.cerevisiae* EBY100 cells expressing the RBD variant/s were washed by an ice-cold PBSB buffer (PBS with 1 g/L BSA) and incubated with various concentrations of CF-640 labeled ACE2 protein. The sample volume was adjusted (1 – 100 ml) to avoid the ligand depletion as well as an equilibrium incubation time (1 – 12 h). The cells were subsequently washed (PBSF), passed through the cell strainer, and analyzed by cytometry. The HEK293T cells were washed twice with PBS (Biological Industries, REF 02-023-1A) and directly analyzed on BD Accuri™ C6 Plus Flow Cytometer, BD Biosciences, USA. The gating strategy is shown in Figure S2.

Instrument

The sorting experiments were performed on S3e Cell Sorter (BioRad, USA, serial no 777BR2079). The binding data were acquired on S3e Cell Sorter (BioRad, USA) and BD Accuri™ C6 Flow Cytometer (BD Biosciences, USA).

Software

Analyses were done by ProSort™ Software (v. 1.6) or by CFlow Plus Software (v. 1.0.227.4)

Cell population abundance

Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.

Gating strategy

Gating strategies are described in detail in the Supplementary material Figure S2.

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