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Reporting Summary

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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	All MS data was acquired on a Thermo Fisher Scientific Q-Exactive Plus mass spectrometer using the Thermo software Xcalibur (4.2.47) and Tune (2.11 QF1 Build 3006).
Data analysis	Raw mass spectrometry data were searched using MaxQuant (version 1.6.11.0) and scored using MIST (available at https://github.com/kroganlab/mist) and SAINT (version 3.6.3). Custom scripts were designed to map interacting proteins to drugs and compounds (avaialble at https://github.com/momeara/BioChemPantry/tree/master/vignette/COVID19). Data on virus assays were analyzed using GraphPad Prism version 7.00 for Mac (GraphPad Software, La Jolla California USA, www.graphpad.com). High-confidence protein-protein interactions were visualized using Cytoscape version 3.7.1. The over-representation analysis (ORA) was performed using the enricher function of clusterProfiler package in R with default parameters. Protein E sequences were aligned using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/).

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 availability: The mass spectrometry raw data and search results files generated during the current study are available in the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD018117 (https://www.ebi.ac.uk/pride/archive/projects/PXD018117) and PPI networks have also been uploaded to NDEx (https://public.ndexbio.org/#/network/43803262-6d69-11ea-bfdc-0ac135e8bacf). All data generated or analyzed during this study are included

in this published article (and its supplementary information files).

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	It is an accepted practice in the field of large-scale interactomics (via AP-MS), that biological triplicate measurements are sufficient for measuring high confidence interactions using the methods and software performed in this study. At least three biological replicates were independently prepared for affinity purification. All antiviral experiments at Mount Sinai were performed in triplicate. For experiments performed at the Institut Pasteur: A sample size of n=3 was chosen for each treatment assessing the effect of drug treatment on viral RNA and infectious units following infection in pre-treated cells. In control-treated cells, n=6. This sample size was chosen as it is sufficient in medium-throughput screening in order to identify differences in viral replication vitro following drug treatment compared to control conditions. A sample size of n=6 was chosen for assessing the effect of drug treatment on cell viability (alamar blue). This was because this larger sample size enabled us to identify any outliers.
Data exclusions	On cell viability assays performed at the Institut Pasteur, replicates were excluded if they were considered outliers (if cell viability was significantly different compared to other replicates). No other data were excluded from the study.
Replication	Reproducibility between bioreplicates can be measured by the degree of variance explained by matching LC-MS feature identifications (peptide and charge) between replicates. We used standard artMS procedures. First, LC-MS features were identified and quantified by MaxQuant in each LC-MS run. Next, the strength of effect was measured as a correlation coefficient (Pearson's r) between each pair of LC-MS runs, pairing individual feature intensities between runs by their peptide and charge identifications. Correlation patterns between LC-MS runs from biological replicates are clustered here along the x and y axes, showing both high correlation coefficients (near 1.0) as well as a trend for most same-bait replicates to cluster by similarity with each other, indicating consistent and bait-specific results. For virus assays, all finding were replicated a minimum of 2 times at Mount Sinai. At the Institut Pasteur, drug screening of positive hits were confirmed by replication of the experiment at least once.
Randomization	Sample randomization is not relevant to our study because experimental groups do not exist. Moreover, AP-MS samples were processed and collected on the same instruments in a short time frame (roughly 3 weeks time). Therefore instrument performance did not have time to drift. QCloud was used to control instrument longitudinal performance during the project.
Blinding	Blinding is not relevant to the AP-MS data because our data are acquired and processed systematically with established scoring algorithms, excluding human bias. For viral assays at Institut Pasteur, Investigators were blinded to group allocation by defining each drug with a number. The name of each drug (numbered 1 to 66) were not revealed to investigators during the screening. Additionally, different investigators were involved at different stages of the process (pre-treatment, infection, data collection, analysis).

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	Anti-strep antibody, Qiagen # 34850 (1:2,500). Anti-mouse-HRP conjugate, BioRad # 1706516 (1:20,000)

	Poly-clonal anti-SARS-CoV-NP antisera produced in a single rabbit, Garcia-Sastre lab at Mount Sinai (1:10,000). Due to the current shelter-in-place order we were unable to identify the lot numbers of commercially available antibodies.
Validation	Use of the anti-strep antibody and anti-mouse HRP conjugate by western blot only detects signal in cell lysate from cells expressing Strep-tagged fusion proteins.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK-293T/17 cells were procured from the UCSF Cell Culture Facility, now available through UCSF's Cell and Genome Engineering Core (https://cgec.ucsf.edu/cell-culture-and-banking-services); cell line collection listed here: https://ucsf.app.box.com/s/6xkydeqhr8a2xes0mbo2333i3k1lndqv (CCLZR076) . Vero E6 cells used at Mount Sinai and Institut Pasteur were purchased from ATCC (VERO C1008 [Vero 76, clone E6, Vero E6] (ATCC® CRL-1586™))
Authentication	STR analysis by the Berkeley Cell Culture Facility on August 8, 2017 authenticates our HEK-293T/17 cells with 94% probability. The African green monkey kidney epithelial Vero E6 (ATCC CRL-1586) is derived from ATCC, and thus is already authenticated.
Mycoplasma contamination	Cells were tested on July 3, 2019 using the MycoAlertTM Mycoplasma Detection Kit (Lonza LT07-318) and were negative: B/A ratio < 1 (no detected mycoplasma). Vero E6 cells: The cell line was tested for mycoplasma contamination prior to commencement of experiments and was negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.