nature portfolio

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Last updated by author(s): Jan 26, 2023

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	*Western blot images were acquired using GeneSys v1.8.2.0. Quantitative PCR data was acquired using QuantStudio Real-Time PCR Software v1.3. Live cell imaging of adenovirus infected transgenic A549 cells for FRAP and short-term imaging of biomolecular condensate fusion acquired using Velocity v7 acquisition software. Live imaging for FRAP of transiently expressed 52K-GFP (WT, R/K, P/A) was acquired using MetaMorph v7.7 acquisition software. All other live cell and fixed cell imaging was acquired using ZEN Black v2.3. Images of Coomassie stained SDS-PAGE gels CsCl gradient analyses were captured using the built in camera of a Samsung Galaxy S22 (SM-S901U) running Android v13. Disorder tendency was analyzed using the IUPred2A algorithm (https://iupred2a.elte.hu/). Fold index was obtained using the PLAAC algorithm (http://plaac.wi.mit.edu)6. The fraction of charged residues (FCR), net charge per residue (NCPR), and hydrophobicity were calculated from the Classification of Intrinsically Disordered Ensemble Regions (CIDER) algorithm (http://pappulab.wustl.edu/CIDER/).*
Data analysis	Micrographs and videos were processed and analyzed using FIJI (v1.53f51). Images including micrographs and western blots were assembled in Adobe Illustrator CS6. Fluorescence recovery was analyzed using FIJI (v1.53f51) using the built-in plugins; create spectrum jru v1, combine all trajectories jru v1, normalize trajectories jru v1, batch FRAP fir jru v1, and average trajectories jru v1. Raw data was handled and analyzed using Microsoft Office 2016 Excel. Statistical analysis was performed using GraphPad Prism v9.1.1.

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

All numerical and imaged gel data is available as source data. Unprocessed images will be made available on request.The adenovirus serotype 5 52K protein (aka L1 packaging protein 3) amino acid sequence was obtained from Uniprot (Q6VGV2). The 52K amino acid frequencies were compared to the amino acid frequencies calculated from a curated set of phase separating proteins (PPS proteins) (van Mierlo, G. et al. 2021; DOI: 10.1016/j.celrep.2021.108705). All numerical and imaged gel data is available as source data. Unprocessed images will be made available on request.

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	*No statistical methods were used to predetermine sample size. For image analysis experiments, both full-biological repeats and technical repeats were performed to accommodate for the possibility of experimental variation, and variation within the sample respectively. We have previously shown that this approach is sufficient for reliable phenotype analysis and statistically significant quantification of virus-induced phenotypes (Reyes et al. 2017. DOI: 10.1074/mcp.M117.067116; Price et al. 2022. DOI: 10.1093/nar/gkab896; Price et al. 2022. DOI: 10.1371/journal.ppat.1010797). For analysis of genome accumulation and viral progeny production, 3 repeats were performed. This approach is commonly used in the field, and we have previously shown this approach to be sufficient to determine statistically significant differences in output (Dybas et al. 2021. DOI: 10.1128/mSystems.00468-21; Herrmann et al. 2020. DOI: 10.1038/s41564-020-0750-9).*
Data exclusions	*No data was excluded*
Replication	*In all cases, full biological replicates were performed. For microscopy experiments, full biological replicates each comprising technical replicates were performed to account for the possibility of variability within the sample. Where replicates were pooled, trends were confirmed across all replicates. For image analysis experiments, multiple frames of view were captured for each biological replicate to reduce the impact of variability within the cell monolayers imaged. Protein purification was conducted in small batches, and the phase-separating behavior of the viral 52K protein confirmed to be comparable across batches. All experiments were repeated fully at least once to replicate findings. All attempts at replication were successful.*
Randomization	*All experimental samples (within each full biological repeat) were generated, processed, and analyzed in parallel to control for covariates. For image analysis experiments, random areas of the cell monolayer were selected for image acquisition to acquire technical replicates. Automated selection or sample randomization was not used.*
Blinding	*For quantification of image phenotypes, phenotype categories were determined prior to quantification. Images were blinded and analyzed by an investigator not involved in experiment design or image acquisition. For all other experiments, investigators were not blinded to group allocation during data collection or analysis, as the outputs of these experiments are objective measurements and data collected using predefined protocols.*

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*
Research sample	*State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*
Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were 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. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*

Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were 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 collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
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.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*
Blinding	*Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access & import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*
Disturbance	*Describe any disturbance caused by the study and how it was minimized.*

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

Antibodies

Antibodies used

The following primary antibodies for viral proteins were used: Adenovirus late protein antibody recognizing Hexon, Penton, Fiber, and protein VI (gift from J. Wilson), species: rabbit, polyclonal, WB 1:10,000. 52K (gift from P. Hearing), species: rabbit, polyclonal, WB 1:10000, IF 1:500. IIIa (gift from P. Hearing), species: rabbit, polyclonal, WB 1:5000, IF 1:500. 100K (gift from P. Hearing), species: rabbit, polyclonal, WB 1:5000. 33K (gift from P. Hearing), species: rabbit, polyclonal, IF 1:500. DBP (gift from A. Levine), species: mouse, clone: B6-8, WB 1:1000, IF 1:400. E1B55K (gift from A. Levine), species: mouse, clone: 58K2AG, WB 1:500, IF 1:250. E4orf6 (gift from D. Ornelles), species: mouse, clone: RSA#3, WB 1:1000, IF 1:250. Protein VII (gift from H. Wodrich), species: rabbit, polyclonal, WB 1:2000, IF 1:500. IVa2 (gift from P. Hearing), species: rabbit, polyclonal, WB 1:2000, IF 1:500. Hexon trimer (Developmental Studies Hybridoma bank, Cat#: TC31-9C12.C9, Lot: N/A), species: mouse, clone: 9C12, IF 1:250. Fiber trimer (Genetex, Cat#: GTX23232, Lot: 821805161), species: mouse, clone: 2A6, IF 1:250. The following primary antibodies for cellular proteins were used: GFP (Abcam, Cat#: ab290, Lot: GR3321614-1), species: rabbit, polyclonal, WB 1:5000. Tubulin (Santa Cruz Biotechnology, Cat#: sc-69969, Lot: D0412), species: mouse, clone: 6A204, WB 1:1000. GAPDH (GeneTex, Cat#: GTX100118, Lot: 43712), species: rabbit, polyclonal, WB 1;5000. Histone H3 (EMD Millipore, Cat#: 06-755, Lot: 24721), species: rabbit, polyclonal, WB 1:5000. Lamin A (Santa-Cruz, Cat#: sc-7293, Lot: L180), species: mouse, clone: 346, IF 1:500. Histone H1 (Abcam, Cat#: ab4269, Lot: GR193594-2), species: mouse, clone AE4, IF 1:500). HSc70 (Abcam, Cat#: ab19136, Lot: GR3238596-11), species: rat, clone 1B5, IF 1:500. FLAG (Sigma Aldrich, Cat#: F3165, Lot: SLCJ3741) species: mouse, clone M2 (see Immunoprecipitation).
For western blotting, Horseradish peroxidase-conjugated (HRP) goat anti-mouse (Jackson Laboratories, Cat#: 115-035-003) or goat anti-rabbit (Jackson Laboratories, Cat#: 111-035-045) secondary antibodies were used at a concentration of 1:5000 and 1:10000 respectively. For IF, the following Alexa Fluor fluorophore-conjugated secondaries were used at a concentration of 1:1000: goat anti-rabbit 488 (Life Technologies, Cat#: A-11008), goat anti-mouse 488 (Life Technologies, Cat#: A-11001), goat anti-mouse 555 (Life Technologies, Cat#: A-21422), goat anti-rat 555 (Life Technologies, Cat#: A-21434) and goat anti-rabbit 647 (Life Technologies, Cat#: A-21245).

Validation

Adenovirus late protein antibody (gift from J. Wilson): Recognizes Hexon (band of approx. 110 kDa), Penton (band of approx. 65 kDa), Fiber (band of approx. 61 kDa), and protein VI (band of approx. 25 kDa) in adenovirus infected whole cell lysates but not uninfected lysates (Kozarsky et al. 1996. DOI: 10.1038/ng0596-54; Herrmann et al. 2020. DOI: 10.1038/s41564-020-0750-9).
52K (gift from P. Hearing): Recognizes 52K (band of approx. 52kDa) on western blots of adenovirus infected whole cell lysates, but not uninfected whole cell lysates (Ostapchuk et al. 2005. DOI: 10.1128/JVI.79.5.2831-2838.2005).
IIIa (gift from P. Hearing): Recognizes IIIa (band of approx. 65 kDa) on western blots of adenovirus infected whole cell lysates or adenovirus particles, but not uninfected whole cell lysates (Ma & Hearing. 2011. DOI: 10.1128/JVI.00467-11).
100K (gift from P. Hearing): Recognizes 100K (band of approx. 100 kDa) on western blots of adenovirus infected whole cell lysates, but not uninfected whole cell lysates. Also recognizes 100K in whole cell lysates from uninfected cells that ectopically express 100K (Yan et al. 2016. DOI: 10.1038/srep22464).
33K (gift from P. Hearing): Recognizes 33K (band of approx. 33 kDa) on western blots of adenovirus infected whole cell lysates and nuclear extracts, but not uninfected whole cell lysates and nuclear extracts, or whole cell lysates and nuclear extracts from cells infected with a Δ33K adenovirus (Wu et al. 2013. DOI: 10.1128/JVI.00652-13).
DBP (gift from A. Levine): Recognizes DBP (band of approx. 72 kDa) on western blots of adenovirus infected whole cell lysates, but not uninfected whole cell lysates. Immunofluorescence staining shows localization to viral replication compartments in H5ts107-infected cells grown at the permissive temperature of 32°C but not in H5ts107-infected cells grown at the non-permissive temperature of 39.5° C (Reich et al. 1983. DOI: 10.1016/0042-6822(83)90274-x).
E1B-55K (gift from A. Levine): Recognizes E1B-55K (band of approx. 55 kDa) on western blots of adenovirus infected whole cell lysates, but not uninfected whole cell lysates. Immunofluorescence staining shows localization to the nucleus in adenovirus infected cells but not uninfected cells (Sarnow et al. 1982. DOI: 10.1016/0042-6822(82)90054-x).
E4orf6 (gift from D. Ornelles): Recognizes E1B-55K (band of approx. 17 kDa) on western blots of adenovirus infected whole cell lysates, but not uninfected whole cell lysates (Marton et al. 1990. DOI: 10.1128/JVI.64.5.2345-2359.1990). This antibody was also shown to recognize a fusion protein of E4orf6 and DBP generated during infection (Price et al. 2022. DOI: 10.1371/journal.ppat.1010797).
Protein VII (gift from H. Wodrich): Recognizes pre-protein VII and protein-VII (band of approx. 22 kDa and 19 kDa respectively) on western blots of adenovirus infected whole cell lysates but not uninfected lysates (Johnson et al. 2004. DOI: 10.1128/JVI.78.12.6459-6468.2004). Immunofluorescence staining of adenovirus infected cells recognizes incoming viral genomes (Komatsu et al. 2015. DOI: 10.1371/journal.pone.0137102).
IVa2 (gift from P. Hearing): Recognizes IVa2 (band of approx. 50 kDa) on western blots of adenovirus infected whole cell lysates, but not uninfected whole cell lysates (Ostapchuk et al. 2005. DOI: 10.1128/JVI.79.5.2831-2838.2005).
Hexon trimer Developmental Studies Hybridoma bank, Cat#: TC31-9C12.C9): Validated previously by (Varghese et al. 2004. DOI: 10.1128/JVI.78.22.12320-12332.2004). Cannot detect heat-denatured hexon on western blots. TC31-9C12.C9 immunoprecipitated the complete set of proteins normally associated with intact Ad5 particles; in contrast, TC31-27F11.C2 (a mAb isolated from the same fusion that generated TC31-9C12.C9) pulls down only free hexon protein. While TC31-9C12.C9 neutralizes Ad5, TC31-27F11.C2 doesn't. For a list of citations and additional information see supplier's website.
Fiber trimer (Genetex, Cat#: GTX23232): Validated by the supplier. Recognizes only trimers (high molecular weight bands of 180 -

200kDa) on western blots of Ad2 and Ad5 fiber proteins that were not boiled during processing. Positive immunofluorescence with Ad5 fiber mutant H5ts142 infected cells at 32ºC but not at 39.5°C. For additional information see supplier's website.
GFP (Abcam, Cat#: ab290): Validated by both the supplier and independent research groups. Recognizes GFP on western blots of whole cell lysates from cells expressing GFP. Recognized GFP expressed in cells when used for immunofluorescence staining. For a list of citations and additional information see supplier's website.
Tubulin (Santa Cruz Biotechnology, Cat#: sc-69969): Validated by both supplier and independent research groups. Recognizes tubulin (band of approx. 55 kDa) on western blots of A-431 and MCF7 whole cell lysates. Immunofluorescence staining of methanol-fixed HeLa cells showing cytoskeletal localization. For a list of citations and additional information see supplier's website.
GAPDH (GeneTex, Cat#: GTX100118): Validated by both supplier and independent research groups. Recognizes GAPDH (band of approx. 37 kDa) on western blots of various whole cell lysates. Immunofluorescence staining of paraformaldehyde-fixed HeLa cells shows localization to the endoplasmic reticulum, cytoplasm, and nucleus. For a list of citations and additional information see supplier's website.
Histone H3 (EMD Millipore, Cat#: 06-755): Validated by both supplier and independent research groups. Recognizes Histone H3 (band of approx. 17 kDa) on western blots of various whole cell lysates. Immunofluorescence staining of HeLa cells shows localization to the nucleus. For a list of citations and additional information see supplier's website.
Lamin A (Santa-Cruz, Cat#: sc-7293): Validated by both supplier and independent research groups. Recognizes Lamin A/C (band of approx. 65 kDa) on western blots of 87 MG, PC-3, C2C12, CCD-1064Sk, and FHs 173We whole cell lysates. Immunofluorescence staining of methanol-fixed HS68 cells shows localization to the nuclear envelope. For a list of citations and additional information see supplier's website.
Histone H1 (Abcam, Cat#: ab4269): Validated by both supplier and independent research groups. Recognizes Histone H1 isoforms (bands of approx. 27-32 kDa) on western blots of various whole cell lysates or recombinant Histone H1 protein. Immunofluorescence staining of methanol-fixed HeLa cells shows localization to the nuclear envelope.
HSc70 (Abcam, Cat#: ab19136): Validated by both supplier and independent research groups. Recognizes Hsc70 (band of approx. 72 kDa) on western blots of various whole cell lysates or recombinant Hsc70 protein. Immunofluorescence staining of paraformaldehyde-fixed, heat-shocked HeLa cells shows localization to the nucleus and nucleoli. For a list of citations and additional information see supplier's website.
In addition to the validation mentioned above, all antibodies raised against viral proteins were validated for the application used in this study by comparing infected and uninfected samples (See main article Figures 1-4 and Extended Data Figures). Antibodies raised against adenovirus late proteins (recognizing Hexon, Penton, Fiber, and VI) IIIa, 52K, and VII were also validated by recognition of packaged and/or incomplete particles (see Extended Data Figure 8a). The antibody raised against 52K was further validated in this study by recognition of 52K (band of approx. 52kDa) on western blots of adenovirus infected whole cell lysates, but not uninfected or pm8001 (Δ52K) adenovirus infected whole cell lysates (see figure 2j).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	All cell lines were obtained from the American Type Culture Collection (ATCC). Primary like human bronchial epithelial cells HBEC3-KT (Cat#: ATCC CRL-4051), 293 (Cat#: ATCC CRL-1573), 293T (Cat#: ATCC CRL-3216), A549 cells (Cat#: ATCC CCL-185).
Authentication	Cell lines were authenticated by the American Type Culture Collection (ATCC). This authentication includes assessment of cell morphology, karyotyping, and short tandem repeat profiling. For more information visit https://www.atcc.org.
Mycoplasma contamination	All cell lines tested negative for mycoplasma using the LookOut Mycoplasma PCR Detection Kit (Sigma-Aldrich). Testing was carried out on a routine basis twice a year.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used as part of this study.

Palaeontology and Archaeology

Specimen provenance	Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.
Specimen deposition	Indicate where the specimens have been deposited to permit free access by other researchers.
Dating methods	If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

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

Animals and other organisms

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

Laboratory animals	For laboratory animals, report species, strain, sex and age OR state that the study did not involve laboratory animals.

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	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

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	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☒	☐	Public health
☒	☐	National security
☒	☐	Crops and/or livestock
☒	☐	Ecosystems
☒	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☒	☐	Demonstrate how to render a vaccine ineffective
☒	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐	Increase transmissibility of a pathogen
☒	☐	Alter the host range of a pathogen
☒	☐	Enable evasion of diagnostic/detection modalities
☒	☐	Enable the weaponization of a biological agent or toxin
☒	☐	Any other potentially harmful combination of experiments and agents

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

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	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	*Identify the instrument used for data collection, specifying make and model number.*

Software	*Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*
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	*Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*

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

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s)	*Specify: functional, structural, diffusion, perfusion.*
Field strength	*Specify in Tesla*
Sequence & imaging parameters	*Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
Area of acquisition	*State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software	*Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*
Normalization	*If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*
Normalization template	*Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*
Noise and artifact removal	*Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*
Volume censoring	*Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

Statistical modeling & inference

Model type and settings	*Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*
Effect(s) tested	*Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)	*Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*
Correction	*Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.