nature portfolio

Corresponding author(s):	Anne Spang
Last updated by author(s):	19.05.2023

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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

~				
5	tat	ŀις	ŤΙ.	\sim

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.
X	A description of all covariates tested
\boxtimes	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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
\boxtimes	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes	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 <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Data analysis

Software microscopy - ZEN 2.6 (Blue edition; widefield microscope); Live Acquisition 2.5 (FEI MORE); FV3000 (FV31S-SW Version 2.5.1) system software (Olympus Fluoview 3000); CellSens (Olympus SpinSR)
Western Blots: captured on a FUSION FX Vilber Lourmat using FusionCapt Advance

Microscopy: Fiji 2.3 and plugins therein; Omero webclient; Deconvolution performed with Huygens pro software 21.10 Statistical analysis data from Western Blots and microscopy - GraphPad Prism 9 and Excel (for MAC 16.72).

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 authors declare that the main data supporting the findings of this study are available within the article and its Supplementary Information files. All other data

supporting the finding University of Basel.	gs of this study	are available from corresponding author upon request. The lipidomics data will be made accessible through open servers of hte			
Human resea	arch part	icipants			
Policy information a	bout <u>studies</u>	involving human research participants and Sex and Gender in Research.			
Reporting on sex a	and gender	Not applicable			
Population charac	cteristics	Not applicable			
Recruitment		Not applicable			
Ethics oversight		Not applicable			
Note that full informat	tion on the app	roval of the study protocol must also be provided in the manuscript.			
Field-spe	cific re	eporting			
Please select the on	e below that	is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.			
X Life sciences	_	Behavioural & social sciences			
or a reterence copy of th	ne document with	all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>			
_ife scien	ices st	udy design			
All studies must disc	close on these	points even when the disclosure is negative.			
Sample size		ras chosen in each case according to experimental design. All cell biology experiments were performed at least in 3 biological repeats. Exact numbers are given in the figure legends.			
Data exclusions	No data was e	xcluded from any analyses reported in this study.			
Replication	At least 3 inde	At least 3 independent experiments were performed per condition. All experiments were shown to be reproducible.			
Randomization	No randomization was used. All the samples were prepared with known composition and contained appropriate controls, which mitigates the influence of co-variates.				
Blinding	Blinding was not used in this study as phenotypes were obvious.				
Behaviou	ıral & s	social sciences study design			
		e points even when the disclosure is negative.			
Study description	iption Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sections quantitative experimental, mixed-methods case study).				
inforn		the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic mation (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For es involving existing datasets, please describe the dataset and source.			
Sampling strategy	Descr	ibe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to			

Data collection

Timing

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.

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.

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. State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no Non-participation participants dropped out/declined participation. If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if Randomization allocation was not random, describe how covariates were controlled.

Ecological, evolutionary & environmental sciences study design

Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, Study description 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.

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. 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

If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, Data exclusions indicating whether exclusion criteria were pre-established.

> 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.

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

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?

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

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.

Ма	terials & experimental systems	Me	ethods
n/a	Involved in the study	n/a	Involved in the study
	X Antibodies	\boxtimes	ChIP-seq
	Eukaryotic cell lines	\boxtimes	Flow cytometry
\boxtimes	Palaeontology and archaeology	\boxtimes	MRI-based neuroimaging
\times	Animals and other organisms		
\boxtimes	Clinical data		
\boxtimes	Dual use research of concern		

Antibodies

Sampling strategy

Data collection

Reproducibility

Randomization

Blinding

Antibodies used

HRP-conjugated anti-mouse, 1:10,000; Invitrogen 31430, anti-HA primary antibody (1:5,000, Eurogentec 16B12), or anti-Pgk1 primary antibody (1:5,000, Invitrogen clone 22C5D8), beta-COP (1:500; CM1; hybridoma supernatant; gift from Dr. Felix Wieland, Heidelberg University, Heidelberg, Germany), monoclonal mouse anti-CLIMP63 (1:1,000, G1/296 gift from the Hauri lab), GM130 antibody (1:1,000, Cell Signalling 12480S), TOM20 antibody (1:200, Santa Cruz sc-17764), anti-Arf1 (1:2,500, Abnova MAB10011), anti-actin (1:100,000, Sigma-Aldrich MAB1501), HRP-conjugated secondary antibody (1:10,000; anti-rabbit, Sigma-Aldrich A0545 or anti-mouse, Sigma-Aldrich A0168), anti-GFP antibody (1:100, 6556 Abcam), goat anti-rabbit secondary antibody coupled with 10 nm gold (1:100; BBI solutions EM.GAR10/2); Secondary Alexa-Fluor 568 (1:500; anti-mouse A10037 and anti-rabbit A10042, Invitrogen)

Validation

All primary antibodies, except anti-B-COP and anti-CLIMP63, are commercially available and validated by the manufacturer. In addition, most of the antibodies have been used by other research groups previously as referenced in the associated manuscript. Anti-B-COP and anti-CLIMP63 were validated by the Wieland and Hauri labs, respectively, and have been widely used by the scientific community.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HeLa alpha cell lines obtai

HeLa alpha cell lines obtained from ATCC and HeLa ARF1 KO cells were kind gift from Prof. Martin Spiess and are further referenced here: Pennauer M, Buczak K, Prescianotto-Baschong C, Spiess M. Shared and specific functions of Arfs 1-5 at the Golgi revealed by systematic knockouts. J Cell Biol. 2022 Jan 3;221(1):e202106100. doi: 10.1083/jcb.202106100. Epub 2021

Nov 8. PMID: 34749397; PMCID: PMC8579194.

Authentication Standard cell lines, authenticated by ATCC. Recently (2021), the cell lines' identities were authenticated by STR analysis by

Microsynth AG (Balgach Switzerland).

Mycoplasma contamination We routinely test our cell lines for mycoplasma contamination. All cell lines were negative.

Commonly misidentified lines (See ICLAC register)

No commonly misidentified cell lines were used

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,

port.

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

rovided.

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.

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.

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 livest	ock				
Ecosystems					
Any other significar	nt area				
Experiments of concer	n				
Does the work involve an	of these experiments of concern:				
No Yes					
Demonstrate how	o render a vaccine ineffective				
Confer resistance t	o therapeutically useful antibiotics or antiviral agents				
Enhance the virule	nce of a pathogen or render a nonpathogen virulent				
Increase transmissi	bility of a pathogen				
Alter the host rang	e of a pathogen				
Enable evasion of o	iagnostic/detection modalities				
Enable the weapon	ization of a biological agent or toxin				
Any other potentia	ly harmful combination of experiments and agents				
ChIP-seq					
D 1 1 111					
Data deposition					
Confirm that both raw	and final processed data have been deposited in a public database such as <u>GEO</u> .				
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 public	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 submissi	on Provide a list of all files available in the database submission.				
Genome browser session (e.g. <u>UCSC</u>)	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 as 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 la number.				
Peak calling parameters	S 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.				

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.

Software

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

ARF1 KO HeLa alpha cells transfected with either ARF1-EGFP, ARF1-11-EGFP or empty EGFP vector were seeded in 12-well plates at a density of 5500 cells/well. Every 24h for 6 consecutive days, cells from one well for each cell line were trypsinized

and resuspended in PBS complemented with 2% FCS.

Instrument BD LSR Fortessa Analyzer

Software FlowJo Version 10.9.0

Cell population abundance GFP intensity of 100.000 cells were measured per sample (single cells were 97-98% of the initial cell population).

Gating strategy

To discard the cell debri from the initial cell population FSC/SSC gating was applied. Single cells were determined by SSC-A/SSC-W and by FSC-A/FSC-W gating. From the single cell population GFP+ cells were gated by GFP-A/SSC-A and the GFP+ cells

were counted in each sample.

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 & infer	rence	
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 <u>Eklund et al. 2016</u>)	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		

Functional and/or effective connectivity		
Graph analysis		
Multivariate modeling or predictive analysis		
Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).		
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.