# nature portfolio

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

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. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted Give <i>P</i> 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 <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 no software was used			
Data analysis GraphPad Prism 9, R-4.5.0			
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 <u>availability of data</u>

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

data are available on request

## 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

Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.

Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).

Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)

Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, 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.

# Field-specific reporting

Please select the one belo	ow that is the best fit for your research	. If you are not sure, read the appropriate sections before making your selection.
<b>x</b> 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 3-6 samples were used for in vitro and in vivo studies. For in vivo studies, a hypothesis-generating sample size was chosen (n=5). This statistical power was sufficient to perform statistical comparison between compared populations.

Data exclusions no data was excluded

Replication replicates were performed (3-6), we confirm that all attempts at replication were successful; Covariates were not used since the study design does not need it

Randomization

none

Blinding

There was no blinding performed since it was not necessary for the study. All results could be interpreted numerically using a software, no bias due to non-blinding could appear.

# 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 & experime	ntal systems Methods
n/a Involved in the study	n/a Involved in the study
<b>▼</b> Antibodies	ChIP-seq
<b>x</b> Eukaryotic cell lines	Flow cytometry
Palaeontology and a	mrchaeology MRI-based neuroimaging
Animals and other o	organisms
Clinical data	
Dual use research o	f.concorn
	Concern
Plants	
Antibodies	
Antibodies used	CDC42 (clone EPR15620, Abcam, UK), pPKCzeta Thr410 (clone S.447.6 Invitrogen, Germany), ZO-1 (clone 1A12, Invitrogen), ICAM-1 (clone R6.5., ThermoFisherScientific, Germany), gm130 (clone D6B1, Cell Signalling, USA), FAK1 (#3285, Cell Signalling, USA), FAK2 (clone YE353, Abcam, UK), FAK2 (clone YE353, Abcam), pFAK2 (Y402) (clone MAB6210, Cell Signaling), tubulin (clone GTU-88, SIGMA), CCR1 (clone MAB145, R&D)
Validation	CDC42 DOI: 10.1073/pnas.2405560121 ab187643, clone EPR15620, 1:100 Abcam, UK
Tandacion	pPKCzeta DOI: 10.1074/jbc.RA119.007660 (Thr410) (PA5-104967, polyclonal, 1:100, Invitrogen, Germany)
	ZO-1 DOI: 10.1007/978-1-0716-3495-0_7 (# 33-9100, clone 1A12, 1:1000, Invitrogen, Germany)
	ICAM-1 DOI: 10.3390/v9040068 (# BMS1011, clone R6.5., 1:1000, ThermoFisherScientific, Germany)
	gm130 DOI: 10.1038/s41467-025-56637-9(#12480, clone D6B1, 1:100, Cell Signalling, USA)
	FAK1 DOI: 10.1038/s41587-024-02143-0 (#3285, polyclonal, 1:1000, Cell Signalling, USA)
	FAK2 DOI: 10.3390/cimb45090446 (ab32571, clone YE353, 1:250, Abcam, UK)
	pFAK2 DOI: 10.1038/s41419-023-05789-x (Y402) (# 592918, clone MAB6210, 1:1000, Cell Signaling)
	gamma tubulin provider states advanced validation, https://www.sigmaaldrich.com/DE/do/product/sigma/t53262crsltid=AfmBOonC

ISPrgBMmKjZlcTlS3LwcysqKzNos4KKSSQqFbNwk-CM5oyb (T6557, clone GTU-88, 1:5000, SIGMA) CCR1 doi: https://doi.org/10.1101/2024.12.05.626954 (#53504, clone MAB145, 1:1000 R&D)

## Eukaryotic cell lines

Policy information about <u>cell lines and Sex and Gender in Research</u>

Cell line source(s)	MUTUI and III BL41 The EBV-negative Burkitt lymphoma cell line BL41 (kind gift from Gilbert Lenoir, IARC, France) and the EBV-positive MUTU cell lines (clones I and III) (kind gift from A. Rickinson, CRUK, Birmingham, UK)
Authentication	cell lines were not authenticated
Mycoplasma contamination	cell lines were tested negative for mycolasma contamination
Commonly misidentified lines (See ICLAC register)	none

## Animals and other research organisms

Policy information about <u>studies involving animals</u>; <u>ARRIVE guidelines</u> recommended for reporting animal research, and <u>Sex and Gender in Research</u>

Laboratory animals	NSG mice (DKFZ in house breeding), 5 animals per group, male sex	
Wild animals	Provide details on animals observed in or captured in the field; report species 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.	
Reporting on sex	findings apply to both sexes	
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	Regierungspräsidium Karlsruhe G-160/22	

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

#### **Plants**

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.

### 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).
- | X | 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	Primary B cells or infected B cells were FACS stained with primary antibodies conjugated with fluorchromes	
Instrument	FACS calibur (Becton, Dickinson and Company)	
Software	FlowJo Software (Becton, Dickinson and Company).	
Cell population abundance	The abundance varied between 1.5% and 80% depending on the antigen to be detected.	
Gating strategy	FSC and SSC gating dead cells were excluded and in case of coculture experiments a second gating was used for cells	
Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.		