# 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>.

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For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	nfirmed
	$\boxtimes$	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	$\boxtimes$	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	$\boxtimes$	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.
$\boxtimes$		A description of all covariates tested
$\boxtimes$		A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
$\boxtimes$		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)
$\boxtimes$		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
$\times$		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\boxtimes$		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

-SlideBook v.6.0 (3i-Intelligent Imaging) was used to acquire confocal fluorescent images of Legionella-infected Hek293T cells. -Image Studio v.5.2 (LI-COR Biosciences) was used to acquire western blots.

-Mass spec data were acquired using Orbitrap Fusion Lumos Tribrid Mass Spectrometer (catalog no. IQLAAEGAAPFADBMBHQ; Thermo Scientific).

Data analysis

-Confocal fluorescent images were contrasted, overlaid and analyzed using ImageJ v.1.53 (NIH).

-Western blots were contrasted using contrasted using Image Studio v.5.2 (LI-COR Biosciences).

-Mass specs spectra were recorded using Thermo Xcalibur Software v.4.4 (catalog no. OPTON-30965; Thermo Scientific) and Tune application v.3.0 (Thermo Scientific). Raw files were searched using Proteome Discoverer Software 2.3. Spectra were searched with the SEQUEST HT or Comet (v.2019.01.1) search engine. Figures in the text were prepared in Adobe Illustrator.

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.

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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

Data is available from the corresponding author at the reader's request.

### Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race, ethnicity and racism</u>.

Reporting on sex and gender	No applicable.
Reporting on race, ethnicity, or other socially relevant groupings	No applicable.
Population characteristics	No applicable.
Recruitment	No applicable.
Ethics oversight	No applicable.

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. I	you are not sure, read the appropriate sections before making your selection.
X Life sciences	Behavioural & social sciences	Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <a href="mailto:nature.com/documents/nr-reporting-summary-flat.pdf">nature.com/documents/nr-reporting-summary-flat.pdf</a>

### Life sciences study design

Blinding

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

Blinding was not relevant to this study.

Sample size

No sample size calculations were preformed prior to the beginning of experiments. The sample size for all experiments was carried out based on consistency and to demonstrate differences between samples. To estimate significance of data being reported, at least 60 LCVs were selected for each condition.

Data exclusions

No data was excluded.

All the experiments were repeated several times on different timing using independent biological samples.

For all the fluorescent images, fields were selected randomly.

# 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.

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

#### **Antibodies**

#### Antibodies used

- 1. Rabbit polyclonal anti-Legionell, home made; dilution 1:500 for immunofluorescence.
- 2. mouse monoclonal anti-HA, Sigma-Aldrich, Cat# H9658; dilution 1:1000 for immunofluorescence; dilution 1:10000 for Western blot.
- 3. Mouse monoclonal anti-ubiquitin, clone P4D1, BioLegend, Cat# 646302; dilution 1:2000 for Western blot.
- 4. Rabbit polyclonal anti-Flag, Proteintech, Cat# 20543-1-AP; dilution 1:2000 for Western blot.
- 5. Alexa Fluor 488-conjugated Donkey anti-Rabbit IgG, Invitrogen, Cat# A-21206; dilution 1:1000 for immunofluorescence.
- 6. Alexa Fluor 488-conjugated Donkey anti-mouse IgG, Invitrogen, Cat# A-21202; dilution 1:1000 for immunofluorescence.
- 7. Alexa Fluor 568-conjugated Donkey anti-Rabbit IgG, Invitrogen, Cat# A-10042; dilution 1:1000 for immunofluorescence. 8. Alexa Fluor 647-conjugated Donkey anti-Rabbit IgG, Invitrogen, Cat# A-31573; dilution 1:1000 for immunofluorescence.
- 9. Alexa Fluor 680-conjugated Donkey anti-Mabbit 1gG, Invitrogen, Cat# A-31373, dilution 1:1000 for Immunority and 9. Alexa Fluor 680-conjugated Donkey anti-mouse 1gG, Invitrogen, Cat# A-10038; dilution 1:10000 for Western blot.
- 10. Donkey anti-rabbit RDye 800CW igG, LI-COR, Cat# 926-32213; dilution 1:10000 for Western blot.

#### Validation

All antibodies were validated by Western blot for specificity and validation reports of the commercially available antibodies are provided on the manufacturer's website:

- 1. PMID: 31682223; PMID: 31690664.
- 2. https://www.sigmaaldrich.com/US/en/product/sigma/h9658
- 3. https://www.biolegend.com/en-us/products/purified-anti-ubiquitin-antibody-6021
- 4. https://www.ptglab.com/products/Flag-Tag-Antibody-20543-1-AP.htm
- 5. https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21206
- 6. https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A21202
- 7. https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A10042
- $8. \ https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31573$
- $9. \ https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A10038$
- 10. https://www.licor.com/bio/reagents/irdye-800cw-donkey-anti-rabbit-igg-secondary-antibody

### Eukaryotic cell lines

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

Cell line source(s)	Hek293T cells
Authentication	Cell lines used were not authenticated by researchers in this study.
Mycoplasma contamination	Cell lines were routinely tested for mycoplasma contamination by mycoplasma detection kit and confirmed that they were negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.