nature portfolio

Corresponding author(s):	Kay Hofmann
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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>.

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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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
×	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>
x	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
x	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
x	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 statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

AMC assays: Magellan 7.1 SP1

Data analysis

Diffraction data processing: XDS 20210205

Phasing, model building and refinement: PHENIX 1.20_4478 , PHASER 2.8.3 $\,$

CCP4 8.0m (contains Refmac 5.8.0267 and AceDRG 222)

Model building: Coot 0.9.8.3

Visualization by PyMol 2.5.0 and ChimeraX 1.3

SAD Analysis: SHELX C/D/E 2013/2 and HKL2MAP 0.4e-beta

MAFFT: v7.487 HHSEARCH: 1.5.1

Clans: No versioning, obtained from http://ftp.tuebingen.mpg.de/pub/protevo/CLANS/ in Jan/2021

pftools: v2.3 with pfsearch v3.

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 <u>data availability statement</u>. 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 X-ray structures of DrT1-VTD1 and Wc-VTD in its apo and ubiquitin-bound form have been deposited at the PDB database under the accession numbers 8ADD, 8ADC and 8ADB, respectively. The X-ray structures of M48 and AvrPphB are publicly available at the PDB database under the accession numbers 2J7Q and 1UKF, respectively. The data underlying the findings of this study are available in this article and its Supplementary Information or are available from the corresponding author upon reasonable request

Human research participants

Reporting on sex and gender	n/a
Population characteristics	n/a
Recruitment	n/a

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

n/a

Policy information about studies involving human research participants and Sex and Gender in Research.

Field-specific reporting

Ethics oversight

Please select the one belo	w 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	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	replicates (numbers are given in the text, typically N=3), but the replicates were only used for noise reduction of the time curves showing AMC-release.
Data exclusions	No data was excluded from the analysis.
Replication	All experiments documented by gel- and blot-images were performed at least twice (on different days, true biological replicates) with ident

results. All numerical measurements were also performed multiple times with biological replicates (typically N=3). The exact replicate numbers are given in the text. All replication attempts were successful.

Randomization Since no inter-group comparisons and statistical tests have been performed, randomization was not relevant in this study

Blinding Since no inter-group comparisons and statistical tests have been performed, blinding was not relevant in this study

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 Methods n/a Involved in the study x Antibodies x ChIP-seq x Flow cytometry x Animals and other organisms x Clinical data

Dual use research of concern