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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☒	☐	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	semi-automated software EM-TOOLS (TVIPS)
Data analysis	MotionCor2, Gctf v1.06, Relion 2.1-beta-1, Gautomatch v0.56, PSIPRED v3.3, SWISS-MODEL, CCP4 v7.0, Phenix 1.13 (including MolProbity) and Phenix 1.15 (used for calculating the B factors), Chimera 1.12, ChimeraX v0.7, Coot 0.8.8, MacPyMOL 1.3.

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The three reported cryo-EM density maps have been deposited in the EM Data Bank under the accession codes EMD-4753, EMD-4751 and EMD-4752. The corresponding atomic models have been deposited in the Protein Data Bank under the accession codes 6R87, 6R84 and 6R86.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-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 applied to pre-determine sample sizes. Single particle analyses averaged 31,832, 52,740 and 115,812 particles for the reported three reconstructions and all reached overall high resolution (3.4 - 3.6 Å).
Data exclusions	No biochemical data were excluded from the analysis.
Replication	Critical biochemical experiments were successfully and reliably reproduced. Cryo-EM sample preparation and image data collection of Vms1 Q295L ΔVIM and Vms1 ΔVIM pullouts reported in the paper were performed once (n = 1). More than two grids were used for each data collection. Moreover, preparation of Vms1 Q295L and Vms1 ΔVIM pullouts with or without fixing reagent had similar results. The quality check of the purified sample (Extended Data Figure 1a) was performed once (n = 1) serving as a confirmation before the cryo-EM analysis. In addition, the absence of Cdc48 in Vms1 ΔVIM was reported previously (Izawa et al., 2017). In vitro release assays in Figure 2c-d were biologically repeated twice (n = 2); release assays in Figure 3f and Extended Data Figure 3l were both performed once (n = 1) as the results were similar in the different strains and each of them covered two combinations of protein concentrations. In vivo assays in Figure 2f-g, Figure 3g-i, Extended Data Figure 2g-j and Extended Data Figure 3h, 3m-n were all biologically repeated twice (n = 2). The growth analysis of vms1Δ arb1::degron strain (Extended Data Figure 3k, left) was biologically repeated twice (n = 2), and the Arb1 protein level (Extended Data Figure 3k, right) was checked once (n = 1). Representative data from one experiment are shown in the paper.
Randomization	No randomization was required for the reported experiments as no allocation into experimental groups were needed. For single particle analyses, particles were divided into two random seeds for reconstruction in order to calculate the resoluion via fourier shell correlation. Each final maps are joined reconstructions.
Blinding	Blinding to group allocation was not required for the reported experiments as no allocation into experimental groups were needed.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Mouse monoclonal anti-Myc antibodies
Source:
1) Izawa et al. 2017 (home made)
2) Santa Cruz Biochemistry (Cat.# sc-40; Lot# K2707)

Mouse monoclonal anti-HA antibody
Source: Santa Cruz Biochemistry (Cat.# sc-7392; Lot# 10616)

Rabbit polyclonal anti-HA antibody
Source: Santa Cruz Biochemistry (Cat.# -805; Lot# A0807)

Mouse monoclonal anti-GFP antibody
Source: Roche (Cat.# 11814460001; Lot# 36405300)

Mouse monoclonal anti-FLAG M2-Peroxidase (HRP) antibody
Source: Sigma-Aldrich (Cat.# A8592)

Rabbit polyclonal anti-Rip1 antibodies
Source:
1) Izawa et al. 2017 (home made)
2) Sawamura et al. 2014 DOI:10.1016/j.bbrc.2013.12.084 (a gift from Masatoshi Esaki)

Mouse monoclonal anti-Rpl3 antibody
Source: Developmental Studies Hybridoma Bank

Validation

All the employed commercial antibodies were verified by their manufacturers, including the specie specificity, noisy signaling, and applications. The data of validations are available on the manufacturers' website with the indicated Cat #. Home-made antibodies were validated and used in the indicated published papers.