

Reporting Summary

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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 |
|-------------------------------------|--|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's d , Pearson's r), 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 Cryo-EM data collection used the following software: RELION-3.1, MotionCor2, CTFIND-4.1, cryoSPARC 3.2, and crYOLO. Mass spectrometry data were collected using a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with a nanospray ion source. Phosphorimaging of radioactive samples was acquired on Typhoon FLA7000 (GE Healthcare). Flow cytometry data were collected using a Beckton Dickinson LSRII or LSRFortessa.

Data analysis Cryo-EM data analysis and structural modeling used the following software: RELION-3.1, COOT (release 0.9.4.1), PHENIX (release 1.19.2-4158), ChimeraX (version 1.3), and CoLabFold implementation of AlphaFold2 (AlphaFold2_advanced.ipynb notebook). Mass spectrometry raw files were processed using Proteome Discoverer (version 2.1, Thermo Scientific). MS/MS spectra were searched against mammals, UniProt Fasta database using the Sequest search engine within Proteome Discoverer (version 2.1, Thermo Scientific). Scaffold 4 Version 4.8.9 (www.proteomesoftware.com) was used to analyze mass spectrometry data. FlowJo (version 10.8.0) was used to analyze flow cytometry data.

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](#)

Data generated in this study are available in the main article, supplementary materials, or in public repositories: EMD-25994 and EMD-26133 of the EMDB (www.ebi.ac.uk/emdb); 7TM3 and 7TUT of the PDB (www.rcsb.org). Source data for all gels can be found in Supplementary Fig. 1. The gating strategy for flow cytometry experiments is shown in Supplementary Fig. 2. Source data for Fig. 4a is provided in Supplementary Table 1. Source data for Extended Data Fig. 7f is provided in Supplementary Table 4. In addition, we made use of a previously published structural model (accession 6T59 of the PDB: <https://www.rcsb.org/structure/6t59>) and the UniProt Fasta databases (<https://www.uniprot.org/proteomes>) for *Canis lupus familiaris* (downloaded on 30/03/2021) and *Oryctolagus cuniculus* (downloaded on 09/02/2021).

Field-specific reporting

Please select the one below that is 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/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	No sample size calculations were performed. Biochemical experiments and flow cytometry experiments were repeated on independent days to verify reproducibility. Flow cytometry measurements included a minimum of 20,000 live cells expressing the reporter. In cases where no difference was observed (less than 10% difference) or where the magnitude of the effect was large (greater than 200%) and the experiment was internally controlled, a single repeat on a separate day was sufficient for verifying reproducibility. When the effect was more modest, at least two repeats were performed to verify that the same result was obtained in each case. Statistical analysis was performed in these cases to assess whether comparisons were different from each other or not. Cryo-EM structures were each determined from a single sample. The mass spectrometry experiment was performed once, with the key hits or non-hits validated by immunoblotting of independently prepared samples to verify reproducibility of the result.
Data exclusions	No data were excluded from the analysis.
Replication	Reproducibility and reliability of the findings has been ensured in several ways. In most cases, biochemical experiments in vitro and functional assays in cells were performed on separate and fully independent occasions and verified to give the same result as the example shown in the figure. This applies to each of the following main figure panels, with the number of repeats in parentheses: 1b (3), 1c (2), 2c (3), 2d (2), 3b (2), 3d (3), 4b (2), 4d (2), 4e (2), 4f (2). The same applies to the following Extended Data Fig. panels: 1d (2), 1e (2), 5b (2), 7b (2), 7c (2), 7d (2), 7f (3), 7g (2), 7h (2), 9b (2), 9e (3), 9g (2), 9h (2), 9i (2). Statistical tests (two-tailed Student's t-test) was performed on the results of Fig. 3d and Extended Data Fig. 7f to verify the significance of the observed difference. Cryo-EM structures were each determined from a single sample. The mass spectrometry experiment (Fig. 4a) was performed once, with the key hits or non-hits validated by immunoblotting of multiple independent samples (e.g., Fig. 4b, Extended Data Fig. 7a and others). The following Extended Data Fig. panels were performed once as displayed, but with individual key segments of the experiment reproduced as parts of other experiments: 1a, 1b, 1c, 1f, 5c, 5d, 5e, 5f, 7a, 7e, 9c, 9d. For example, the crosslinking in Extended Data Fig. 1e was reproduced as part of an earlier experiment titrating crosslinker concentration, another earlier experiment varying crosslinking time, and an experiment testing the importance of substrate length (which is shown in Extended Data Fig. 1a using radioactive detection rather than immunoblotting). In this way, each of these results can be considered to have been reproduced at least once, even if the displayed experiment was not formally repeated. No attempts at replication failed.
Randomization	Samples were not randomized for the functional assays because there is nothing to randomize. The different conditions being compared within any given experiment derive from a single common stock of reagent or a single culture of cells.
Blinding	Blinding is not technically or practically feasible for either the functional assays or for structure determination.

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

n/a	Included in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

n/a	Included in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used

A table of all antibodies used in this study, their catalog numbers, and their specific conditions for use, are provided in Supplementary Table 3.

Validation

Each antibody was validated for specificity against the human antigen by the manufacturer or in earlier published work as follows:

Anti-CCDC47 - validated by manufacturer (Bethyl) and in ref. 10 against human protein by both IP and IB.
 Anti-Asterix - validated by manufacturer (Invitrogen) and ref. 10 against human protein by IP and IB.
 Anti-RPL8 - validated by manufacturer (Abcam) against human protein by both IP and IB.
 Anti-Sec61alpha - Validated in ref. 55 for IB and in ref. 10 for IP. Antigen is 100% conserved in human and canine proteins.
 Anti-Sec61beta - Validated in ref. 55 for IB and in ref. 10 for IP. Antigen is 100% conserved in human and canine proteins.
 Anti-FLAG epitope tag - Validated by manufacturer (Sigma) for IB.
 Anti-TMEM147 - Validated for IB by manufacturer (Invitrogen) against mouse and in ref. 11 against human protein.
 Anti-NOMO1 - Validated for IB by manufacturer (Invitrogen) and in ref. 11 against human protein.
 Anti-Nicalin - Validated for IP and IB by manufacturer (Bethyl) and in ref. 11 against human protein.
 Anti-TMCO1 - Validated for IB by manufacturer (Invitrogen) and in ref. 11 against human protein.
 Anti-OPT1 - Validated for IB against human protein by manufacturer (Invitrogen).
 Anti-EMC2 - Validated for IB against human protein by manufacturer (Proteintech) and in Ref. 17.
 Anti-Strep tag - Validated for IB and IP by manufacturer (Abcam).
 Anti-Sec61gamma - Validated for IB against human protein by manufacturer (Proteintech).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HEK293 FRT/TO TRex cells were from Invitrogen. HEK293-derived Asterix and CCDC47 knockout cell lines were from Chitwood & Hegde, Nature, 564:630-34 (reference 10 in the paper) The HEK293-derived TMCO1 knockout cell line was from McGilvray et al., Elife, 9:e56889 (reference 11 in the paper) Fluorescent reporter cell lines were derived from parental HEK293-FRT/TO TRex cells and are from references 10, 14, and 17 in the paper (Chitwood & Hegde, Nature, 564:630-34; Chitwood et al., Cell, 175:1507-1519; Guna et al., Science, 359:470-473).

Authentication

Cell lines were not authenticated beyond ensuring the presence of known antibiotic resistance markers within their genomes (by growth in the relevant antibiotics) and by their unique FRT site downstream of a doxycycline-inducible promoter as determined by the ability to integrate fluorescent reporters at this site.

Mycoplasma contamination

Cell lines were negative for mycoplasma. They are tested monthly.

Commonly misidentified lines (See ICLAC register)

None used.

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

Samples consisted of HEK293-derived cell lines that stably expressed a fluorescent protein reporter or were transiently transfected with the desired reporter. Where indicated in the Methods, they were first treated with siRNAs or drugs. The

	cells were collected in ice-cold PBS, washed and resuspended in PBS supplemented with 2% FCS and 1 µg/ml DAPI (Thermo Fisher Scientific). Cells were passed through 70-µm filter immediately prior to analysis using Beckton Dickinson LSRII or LSRFortessa instrument. A total of at least 20,000 fluorescent and live (negative for DAPI stain) cells were collected.
Instrument	Beckton Dickinson LSRII or LSRFortessa.
Software	FlowJo (version 10.8.0).
Cell population abundance	A total of at least 20,000 live cells (negative for DAPI stain) that also were positive for the fluorescent protein reporter (either GFP or RFP) were analyzed.
Gating strategy	Gating was used only to exclude dead cells (as judged by staining by DAPI) and cells that did not express the reporter (as judged by lack of fluorescence of a translation reporter, either GFP or RFP). Further gating was not used, and the raw scatter plots of the entire cell population after the above exclusions is shown in Extended Data Fig. 9g for several representative experiments.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.