

**Extended Data Fig. 3** | **SAFE-based map of the cell and motif enrichment. a**, SAFE-based map of the cell generated from preys with a Pearson correlation score of 0.65 or higher and plotted using Cytoscape with a spring-embedded layout. Each prey is coloured to indicate its primary localization (domain in SAFE terminology) as indicated in the legend. An interactive version of the map can be viewed at humancellmap.org/explore/maps and toggling from NMF to

SAFE on the bottom menu. **b**, Pfam regions or motifs enriched in the indicated SAFE domains. The heat map value represents the log<sub>2</sub>-transformed fold change between the genes localized to the rank and all preys in the dataset. Only compartments or domains with a significant fold change for at least one motif are displayed on the heat map.



Extended Data Fig. 4 | NMF-based correlation map of the cell and motif enrichment. a, NMF-based map of the cell generated from preys with a Pearson correlation score across NMF ranks of 0.9 or higher and plotted using Cytoscape with a spring-embedded layout. Each prey is coloured to indicate its primary localization (rank in NMF terminology) as indicated in the legend. An interactive version of the map can be viewed at humancellmap.org/explore/

maps and toggling from *t*-SNE to correlation on the bottom menu. **b**, Pfam regions or motifs enriched in the indicated NMF ranks. The heat map value represents the log<sub>2</sub>-transformed fold change between the genes localized to the rank and all preys in the dataset. Only compartments/ranks with a significant fold change for at least one motif are displayed on the heat map.



**Extended Data Fig. 5** | **Localization benchmarking and experimental validation. a**, Percentage of genes localized to a previously known compartment for each specificity tier using our NMF and SAFE pipelines, compared with the HPA<sup>1</sup> (www.proteinatlas.org) and the fractionation studies of Christoforou<sup>2</sup> and Itzhak<sup>3</sup>. Specificity tiers were defined by binning GO:CC terms on the basis of their information content (Methods). Tier 1 terms are the most specific, and tier 5 the least specific. **b**, Percentage of preys localized to a previously known compartment relative to the number of baits they were detected with for NMF and SAFE, respectively. **c**, Percentage of preys localized to a previously known compartment relative to the average number of spectral counts they were seen with for NMF and SAFE. Preys were binned by spectral counts. The left tick mark for each data point indicates the lower bound for the bin (inclusive) and the right tick mark the upper bound (exclusive). **d**, Localization prediction validation strategy and examples. Confidence rankings are as defined in Fig. 2d. Representative immunofluorescence images are shown. NMF scores across the defined ranks, categories and compartments are displayed as seen on humancellmap.org with the highest NMF category corresponding to the localization prediction.



Extended Data Fig. 6 | Topology and moonlighting analysis. a-c, Predicted versus annotated proportion of protein exposed to the cytosol or lumen for ER transmembrane proteins. a, Hypothetical examples of proteins with varying proportions of their sequence exposed to the cytosol or lumen. The extent of labelling by cytosolic or lumenal baits should be directly related to proportion of the sequence, and hence lysine residues available for biotinylation, exposed to the respective faces of the membrane that the protein spans. b, All transmembrane domain containing prey proteins localized to the cytosolic face of the ER (NMF compartments 3 and 15) and the lumenal face (NMF compartment 6), were assigned a CLR score on the basis of their NMF profile (313 proteins). The CLR score of a prey is calculated by taking the score in the cytosolic facing compartment/maximum score in that compartment and subtracting the corresponding score in the lumenal compartment. A score closer to 1 would indicate a protein with a signature at the cytosolic face of the ER membrane but little or no signature in the lumen and a score of -1 would indicate the opposite. A similar sequence-based score was calculated as the fraction of the sequence annotated as cytosolic minus the fraction that is lumenal according to UniProt. KTN1 is mis-annotated in UniProt<sup>56</sup> and should have a sequence score of +0.9742. c, Three example of proteins and their topology, CLR and sequence scores. Green examples have predictions matching annotated topology. d, e, Moonlighting and connections between

compartments. d, Primary and secondary localizations of moonlighting preys. Preys with a score of at least 0.15 in each of two non-contiguous NMF compartments were considered to moonlight (a list of non-contiguous compartments is in Supplementary Table 15). The number of preys with a primary localization defined on the vertical axis and a secondary localization defined on the horizontal axis is shown (maximum 18). e, Inter-compartment edges were counted for each NMF rank/compartment. An interaction edge was defined between prey pairs having a correlation score across all NMF compartments of at least 0.9. Edges were then defined as 'intra-compartment' (if the primary localization for the two preys was the same compartment) or 'inter-compartment' (if the primary localization for the two preys was in different compartments) (Supplementary Table 15). Most organelles displayed a much greater proportion of intra-compartment interactions, with the extreme case of the mitochondrial matrix having only 15 inter-compartmental edges out of a total of 37,387 edges. The proportion of inter-compartment edges from the source to each target compartment is shown here. Intercompartmental edges generally conformed with expectations, for example with edges from the chromatin compartment connecting to other nuclear substructures with which they may exchange components. The NMF rank number is shown in brackets next to the source compartment name.



**Extended Data Fig. 7** | **Comparison of prey profiles for LMNA tagged with BiolD, miniTurbo and TurbolD. a**, Spectral counts for significant preys (FDR  $\leq$  0.01) were plotted for LMNA-BiolD versus LMNA-miniTurbo. The average spectral counts value found in controls was subtracted from the

detected spectral counts for each prey and the resulting value plotted. Zero values were set to 0.05 to create values suitable for log-transformation of the axes. **b**, LMNA-BioID versus LMNA-TurboID. **c**, LMNA-miniTurbo vs LMNA-TurboID.



Extended Data Fig. 8 | See next page for caption.

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GFP-CHMP7

C18orf32-GFP

Mitochondria

Mitochondria

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#### $Extended \, Data \, Fig. \, 8 \, | \, Analysis \, of \, mitochondria-ER \, contact \, site$

**candidates. a**, Heat map of genes with a primary localization at the mitochondrial outer membrane and ER membrane/nuclear outer membrane and a secondary localization to the other compartment as computed by NMF. To be included on the heat map, genes required an NMF score of at least 0.15 in the compartments of interest, a score ratio of at least 0.4 between the primary and secondary localization, and a score ratio of at least 2 between the compartments of interest and all other compartments. Bold genes indicate those selected for mitochondrial morphology assays in the following panels. A grey dot on the right side of the plot indicates proteins involved in lipid and cholesterol homeostasis, and a pink dot indicates calcium signalling. **b**, Dot

plot view of BioID data for mito–ER contact site candidates highlighting recovery of mitochondrial fission machinery, mito–ER tethers and outer mitochondrial membrane proteins. Asterisks on the heat map indicate spectral counts for prey genes corresponding to the bait that were ignored by SAINT as peptides from the bait confound accurately evaluating the abundance of itself as an interactor. **c**, Mitochondrial morphology is altered by transient expression of GFP-tagged CHMP7 and C18orf32, as monitored by confocal immunofluorescence microscopy in HeLa cells. Cells were fixed and probed with antibodies directed against GFP and COXIV (Methods). The white box indicates the zoomed area displayed in the rightmost panels. Scale bars, 10 µm.



Extended Data Fig. 9 | Analysis module at humancellmap.org. a, Screenshot of the analysis report for the bait PIK3R1. Red circles indicate the following (1) Baits from the human cellmap are sorted from most similar to least similar as calculated by the Jaccard distance. (2) The ten most similar baits to the query in the humancellmap. (3) The average spectral counts for each prey averaged across all baits in the humancellmap database. (4) Expected localizations of the ten most similar baits. (5) Overlap or similarity metrics between the query bait and the top ten most similar baits in the humancellmap. The distance is the Jaccard distance, with a score of 0 for complete prey overlap and 1 for no overlap. The intersection refers to the number of shared preys, and the union refers to the combined number of preys between the query and the indicated bait. (6) The most specific preys for the query. The specificity score is calculated as the fold enrichment of a previn the query relative to the average across the human cellmap baits used for the comparison. (7) The specificity score calculated against the top ten most similar baits to the query. (8) The specificity score calculated against all baits in the humancellmap. (9) Links to open the heat map or specificity plots at the interactive viewer at ProHits-viz<sup>39</sup>. (10) Links for data downloads. **b**. Specificity plot for RNGTT showing the control-subtracted spectral counts versus the specificity score (calculation of the specificity score is described in the Methods). RNGTT is a nuclear protein involved in mRNA capping previously profiled by BioID7. Humancellmap analysis reported a nuclear localization, with bait-specific interactions including several RNA polymerase II subunits and components of the catalytic

subunit of the PP4 phosphatase, as previously reported<sup>57,58</sup>. **c**, Exploratory analysis of FAM171A1 reveals links to the cytoskeleton. FAM171A1 was predicted by our NMF and SAFE analyses to localize to the cell junction and plasma membrane. Consistent with this prediction, its BioID profile when screened as a bait was most similar to junctional and plasma membrane baits, whereas baitspecific preys included several cytoskeletal proteins, in line with a previous study<sup>59</sup> that reported a reduction of actin stress fibres after knockdown of FAM171A1. d, Specificity plot of MTFR2 showing the high specificity of proteins involved in mitochondrial dynamics. MTFR2 was associated with the mitochondrial outer membrane and peroxisome as a prey protein, with a weak signature at the mitochondrial inner membrane or mitochondrial intermembrane space. When profiled as a bait, the analysis module reports that it is most similar to peroxisomal baits, followed by mitochondrial outer and inner membrane baits, supporting its predicted localization. Interactions with MTFR1, SLC25A46 and VPS13D were found to be highly specific to MTFR2, consistent with the mitochondrial fragmentation previously observed after overexpression of GFP-MTFR2<sup>60</sup>. e, BRD3 relocalization after JQ1 treatment. BirA-tagged BRD3 was treated with vehicle or IO1 for 24 h (data from ref.<sup>25</sup>) and analysed using the analysis module at humancellmap.org. The Jaccard indices (1-Jaccard distance) for the top 20 most similar baits were used to create networks in Cytoscape<sup>41</sup> using an edge-weighted spring-embedded layout. Humancellmap baits are coloured on the basis of their expected localization to chromatin or the nucleolus.



Extended Data Fig. 10 | BirA\*-Flag and GFP-BirA\*-Flag control stable cell line, and LMNA-BirA\*-Flag and AIFM1-BirA\*-Flag bait stable cell line immunofluorescence. Cell lines were probed by confocal immunofluorescence microscopy in HEK293 Flp-In T-REx stable cells to assay for localization of the fusion construct and general biotinylation. Cells were fixed and then probed with an antibody to the Flag epitope and streptavidin for biotinylated proteins (Methods). The green channel represents nuclear or mitochondrial staining, the red channel denotes Flag and the blue channel represents streptavidin (biotinylated proteins). Scale bars, 10 µm.

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Last updated by author(s): 20/4/2021

# **Reporting Summary**

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#### **Statistics**

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	firmed
	$\square$	The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement
	$\square$	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.
$\boxtimes$		A description of all covariates tested
	$\square$	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
$\boxtimes$		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
 The Orbitrap Elite Hybrid Ion Trap-Orbitrap mass spectrometer was operated with Xcalibur 2.0 software.

 Data analysis
 Samples analyzed on the Orbitrap Elite Hybrid Ion Trap-Orbitrap mass spectrometer were converted to mzML using ProteoWizard (3.0.4468) and analyzed using the iProphet pipeline implemented within ProHits (4.0). Spectra were analyzed separately using Mascot (2.3.02; Matrix Science) and Comet (2012.01 rev.3). The resulting Comet and Mascot search results were individually processed using the Trans-Proteomic Pipeline (TPP; Linux version, v0.0 Development trunk rev 0, Build 201303061711). SAINTexpress analysis was performed using version exp3.6.1. Cytoscape version 3.6.1 was used for network creation. R was version 3.3.3, and the CBA R package was version 0.2-18. The Matlab (version 9.4) implementation of SAFE (version 1.5) was used. Python 3.9.2 and the scikit-learn package 0.18.1 were used for NMF analysis. t-SNE was performed using the Matlab script (versionless) available at http://lvdmaaten.github.io/tsne. Custom code used for the analysis of SAINTexpress results is available on GitHub at github.com/knightjdr/cellmap-scripts.

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. 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

Datasets consisting of raw files and associated peak lists and results files have been deposited in ProteomeXchange through partner MassIVE (http:// proteomics.ucsd.edu/ProteoSAFe/datasets.jsp) as complete submissions. Additional files include the sample description, the peptide/protein evidence and the complete SAINTexpress output for each dataset, as well as a "README" file that describes the dataset composition and the experimental procedures associated with each submission. The different datasets generated here were submitted as independent entries.

Dataset 1 (see Supplementary Table 2): Go\_BioID\_humancellmap\_HEK293\_lowSDS\_core\_dataset\_2019 MassIVE ID MSV000084359 and PXD015530

Dataset 2 (see Supplementary Table 2): Go\_BioID\_humancellmap\_HEK293\_ highSDS\_core\_dataset \_2019 MassIVE ID MSV000084360 and PXD015531

Dataset 3 (see Supplementary Table 18): Go\_BioID\_humancellmap\_HEK293\_prediction\_2019 MassIVE ID MSV000084369 and PXD015554

Dataset 4 (see Supplementary Table 17): Go\_BioID\_humancellmap\_HEK293\_ER-mito\_candidates\_2019 MassIVE ID MSV000084357 and PXD015528

Negative control samples were deposited in the Contaminant Repository for Affinity Purification44 (CRAPome.org) and assigned samples numbers CC1100 to CC1185 (see Supplementary Table 2); this will be part of the next release of the database.

The BioGRID human database v3.5.169 was downloaded on 13/2/2019 (https://downloads.thebiogrid.org/BioGRID/Release-Archive/BIOGRID-3.5.169/). Human gene annotations were downloaded from Gene Ontology (GO) on 15/2/2019 (GO version date 1/2/2019, http://release.geneontology.org/2019-02-01/annotations/ goa\_human.gaf.gz). The GO hierarchy (release date 1/2/2019) was downloaded from GO on 15/2/2019 (http://release.geneontology.org/2019-02-01/ontology/gobasic.obo). The UniProt database release 2019\_2 was downloaded on 21/2/2019 (ftp.uniprot.org/pub/databases/uniprot/previous\_releases/release-2019\_02/ knowledgebase/uniprot\_sprot-only2019\_02.tar.gz). The IntAct human database release 2018\_11\_30 was downloaded on 13/2/2019 (ftp.ebi.ac.uk/pub/databases/ intact/2018-11-30/psimitab/intact.txt). Human protein domain annotations and motifs were retrieved from Pfam (version 32) on 21/2/2019 (ftp.ebi.ac.uk/pub/ databases/Pfam/releases/Pfam32.0/proteomes/9606.tsv.gz). ProteomicsDB was queried for protein expression information on 1/14/2020 using their API. Text mining data was downloaded from the Compartments database on 1/21/20 (https://compartments.jensenlab.org/Downloads).

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## Life sciences study design

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

Sample size	The decision of performing biological duplicates for the BioID data comes from cost-benefit analysis based on our scoring approach. SAINTexpress treats each biological replicate separately, and the values are averaged to a final SAINT score, which is then used for FDR calculation. This strategy ensures that the proximal interactions we are reporting have been detected confidently across both replicates (Choi H, et. al. Nat Methods, PMID: 21131968, Teo G, et. al. J Proteomics. PMID: 24513533). When we devised the SAINT scoring approach, we evaluated different experimental designs and combinations of scores. The method selected here (using biological duplicates and reporting of only those interactions detected with confidence across both experiments) is more stringent than the other strategies explored (such as selecting the best 2 of 3 replicates or jointly analyzing all replicates), but as a drawback is prone to false negatives. This aspect is mitigated here by the fact that our BioID experiments resulted in a high reproducibility across the biological replicates (average R^2 of 0.95 across the entire dataset), further bolstering the selection of 2 biological replicates for this dataset. For microscopy assessments, a sample size of three was chosen to ensure sufficient statistical power (0.8) at a p-value of 0.05 for the genes under testing. Statistics, including power, are in the source data for Figure 3.
Data exclusions	Baits selected as compartment markers were excluded from analysis if they did not pass quality control. Quality control consisted of ensuring they localized to the correct compartment by immunofluorescence of the tagged protein and GO enrichment analysis of identified interactors following mass spectrometry. 42 of the 234 baits were excluded using these criteria.
Replication	Mass spectrometry sample reproducibility was assessed by R^2 of the spectral counts for identified interactors. These results are in Supplementary Tabe 2, Sheet C. All replication attempts were successful and the overall R^2 was 0.95. Each experiment for mitochondrial morphology was performed in n = 3 biological independent experiments and all replication attempt were successful.
Randomization	The order of MS sample acquisition was randomized between replicates to obviate effects from sample carryover. Randomization of microscopy acquisition was not intentionally randomized as this is not effected by non-randomization effects.
Blinding	The analysis of MS data was not blinded. Initially QC analysis required manual assessment and knowledge of each bait gene and its results, making blinding infeasible. Subsequent analysis of protein localization was prey-centric, making the bait samples they were identified with, and blinding of those samples, irrelevant. Quantification of mitochondrial morphology defects when transiently transfecting constructs in HeLa cells were not blinded. However, the follow-up quantification of defects in primary fibroblasts were blinded. The absence of blinding in the initially transient experiments was an oversight, but as the effect sizes were large and the follow-up blinded validation in primary fibroblasts supported the results in transient transfections, we feel the shortcomings of not-blinding were mitigated.

## 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		Me	Methods	
n/a	Involved in the study	n/a	Involved in the study	
	Antibodies	$\boxtimes$	ChIP-seq	
	Eukaryotic cell lines	$\boxtimes$	Flow cytometry	
$\boxtimes$	Palaeontology	$\boxtimes$	MRI-based neuroimaging	
$\boxtimes$	Animals and other organisms			
$\boxtimes$	Human research participants			
$\boxtimes$	Clinical data			

#### Antibodies

Antibodies used	Antibody information is provided in Supplementary Table 21.
Validation	Validation statements provided by the manufacturers are listed below.
	For primary antibodies:
	Mouse anti-FLAG (Sigma, F3165) (https://api.sigmaaldrich.com/deepweb/assets/sigmaaldrich/quality/spec/120/274/F3165- BULK.pdf)
	Rabbit anti-FLAG (Sigma, F7425) (https://www.sigmaaldrich.com/catalog/product/sigma/F7425?lang=en&region=US)
	Mouse anti-g-tubulin (Sigma, T6557) (https://www.sigmaaldrich.com/catalog/product/sigma/t6557?lang=en&region=US) Mouse anti-a-tubulin (Sigma,T9026) (https://www.sigmaaldrich.com/catalog/product/sigma/t9026?lang=en&region=US) Mouse anti-TOMM20 (Millipore Sigma, MABT166) (https://www.emdmillipore.com/CA/en/product/Anti-Tom20-Tomm20- Antibodycolone-2E8 1 MM_NE-MABT166)
	Mouse anti-GFP (Roche, CAT# 11814460001) (https://www.sigmaaldrich.com/catalog/product/roche/11814460001?
	lang=en&region=CA)
	Rabbit anti-GFP (abcam, ab183734) (https://www.abcam.com/gfp-antibody-epr14104-ab183734.html) Rabbit anti-Giantin (abcam, ab24586) (https://www.abcam.com/giantin-antibody-9b6-golgi-marker-ab37266.html)
	Rabbit anti-GRP/8 BiP (abcam, ab21685) (https://www.abcam.com/grp/8-bip-antibody-ab21685.html)
	Mouse anti-Cytochrome C (BD Biosciences, 556432) (https://www.bdbiosciences.com/eu/applications/research/apoptosis/ purified-antibodies/ourified-mouse-anti-cytochrome-c-6h2b4/o/556432)
	Rabbit anti-NPM1 (One World Lab, AP2834a) (https://www.abcepta.com/products/AP2834a-NPM1-Antibody-N-term)
	Mouse anti-b-tubulin (DSHB at the University of Iowa, E7) (https://dshb.biology.uiowa.edu/E7_2)
	Mouse anti-CD63 (EXBIO, 11-343) (https://www.exbio.cz/research-product/antibodies/cd-and-related-antigens/anti-hu-cd63-
	purified) Mouse anti-CD107a (CVDIO_10_C71) (https://www.aukia.az/racearch.product/antihadias/ad.and.related.aptigane/anti-hu
	Mouse anti-CD10/a (EXBIO, 10-6/1) (https://www.exbio.cz/research-product/antibodies/cd-and-related-antigens/anti-nu-
	Mouse anti-UBF (Santa Cruz Biotechnology, sc-13125) (https://www.scbt.com/p/ubf-antibody-f-9)
	Rabit anti-ERGIC53 (Santa Cruz Biotechnology, sc-66880) (https://www.scbt.com/p/ergic-53-antibody-h-245) Rabbit anti-COXIV (Cell Signaling Technology, 4850) (https://www.cellsignal.com/products/primary-antibodies/cox-iv-3e11-
	rabbit-mab/4850) Rabbit anti-CAT (Cell Signaling Technology, 12980) (https://www.cellsignal.com/products/primary-antibodies/catalase-d4p7b-xp-
	Rabbit anti-NEFL (Cell Signaling Technology, 2837) (https://www.cellsignal.com/products/primary-antibodies/neurofilament-l- c28e10-rabbit-mab/2837)
	Rabbit anti-CANX (Cell Signaling Technology, 2679) (https://www.cellsignal.com/products/primary-antibodies/calnexin-c5c9-rabbit-mab/2679)
	Rabbit anti-RAB11 (Cell Signaling Technology, 5589) (https://www.cellsignal.com/products/primary-antibodies/rab11-d4f5-xp-rabbit-mab/5589)
	Rabbit anti-FBL (Cell Signaling Technology, 2639) (https://www.cellsignal.com/products/primary-antibodies/fibrillarin-c13c3-rabbit-mab/2639)
	Rabbit anti-EEA1 (Cell Signaling Technology, 3288) (https://www.cellsignal.com/products/primary-antibodies/eea1-c45b10- rabbit-mab/3288)
	Rabbit anti-KRT5 (Cell Signaling Technology, 25807) (https://www.cellsignal.com/products/primary-antibodies/keratin-5-d4u8q-
	Rabbit anti-KRT17 (Cell Signaling Technology, 4543) (https://www.cellsignal.com/products/primary-antibodies/keratin-17-d73c7-
	rabbit-mab/4543)
	Rabbit anti-LAMTOR1/C11orf59 (Cell Signaling Technology, 8975) (https://www.cellsignal.com/products/primary-antibodies/ lamtor1-c11orf59-d11h6-xp-rabbit-mab/8975)
	Rabbit anti-Rab5 (Cell Signaling Technology, 3547) (https://www.cellsignal.com/products/primary-antibodies/rab5-c8b1-rabbit-mab/3547)
	Rabbit anti-LAMP1 (Cell Signaling Technology, 9091) (https://www.cellsignal.com/products/primary-antibodies/lamp1-d2d11-xp-rabbit-mab/9091)
	For secondary antibodies:

Goat anti-Rabbit coupled to Alexa Fluor 488 (Invitrogen, A-11034) (https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11034)

Goat anti-Mouse coupled to Alexa Fluor 555 (Invitrogen, A-21422) (https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21422)

Goat anti-Rabbit coupled to Alexa Fluor 555 (Invitrogen, A-21428) (https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21428)

Goat anti-Mouse coupled to Alexa Fluor 488 (Invitrogen, A-11029) (https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11029)

Goat anti-Rat coupled to Alexa Fluor 488 (Invitrogen, A-11006) (https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11006)

Goat anti-Rabbit coupled to Alexa Fluor 488 (Invitrogen, A-11008) (https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11008)

Goat anti-Mouse coupled to Alexa Fluor 594 (Invitrogen, A-11032) https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11032)

Goat anti-Rabbit coupled to Alexa Fluor 594 (Invitrogen, A-11037) (https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11037)

Goat anti-Rat coupled to Alexa Fluor 647 (Invitrogen, A-21247) (https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21247)

Streptavidin coupled to Alexa Fluor 647 (Invitrogen, S32357) (https://www.thermofisher.com/order/catalog/product/S32357#/ S32357)

Phalloidin coupled to Alexa Fluor 647 (Invitrogen, A22287) (https://www.thermofisher.com/order/catalog/product/A22287#/ A22287)

Phalloidin coupled to Alexa Fluor 488 (Invitrogen, A12379) (https://www.thermofisher.com/order/catalog/product/A12379#/ A12379)

Concanavalin A coupled to AlexFluor 647 (Invitrogen, C21421) (https://www.thermofisher.com/order/catalog/product/C21421#/ C21421)

#### Eukaryotic cell lines

Policy information about <u>cell lines</u>	
Cell line source(s)	HEK293 Flp-In T-REx cells were obtained from Invitrogen. HeLa cells were from ATCC. Primary fibroblasts were from the cell bank at Montreal Children's hospital.
Authentication	HEK293 Flp-In T-Rex were authenticated by STR analysis with The Center for Applied Genomics Genetic Analysis Facility (Sick Kids Hospital, Toronto). HeLa cells and primary fibroblasts were not independently authenticated
Mycoplasma contamination	Cell lines were routinely monitored for mycoplasma contamination as assessed by a commercial kit (MycoAlert, Lonza).
Commonly misidentified lines (See <u>ICLAC</u> register)	No commonly misidentified cell lines were used in this study.