Supplemental information

Methods

small interfering (si) RNA:

Small interfering RNAs (siRNAs) were purchased from Ambion and were stored at -20°C in 5 μM stocks in nuclease free water. Sense oligo sequences were: C8-1: CAGCUCUCCUAAUACGGUUTT, C8-2: CAUUAGGCAAUUAAACGATT, C11-1: GGAUUUAUAAACUACAAGATT, C11-2: GGGUUAACAGCUAUUCAAATT, C12-1: CGGACAAGCUGAACGAACATT, C12-2: GGUUAUCAAGUAUUACCCATT. For transient knockdown, HeLa cells were plated in six-well dishes 24 hours prior to transfection in DMEM+10% FBS with no antibiotics. Cells were approximately 40% confluent at the time of transfection. One hour prior to transfection, DMEM was replaced with Optimem (Invitrogen). Oligonucleotides and Oligofectamine (Invitrogen) were diluted in Optimem and combined as per the manufacturer's directions. Liposomes were added to the wells at a final concentration of 60 nM of each oligonucleotide in a volume of 1 mL per well. Dishes were then incubated at 37°C with 5% CO_2 for 20 hours before supplementation with 1 mL DMEM+10% FBS. At 24 hours post transfection, the medium was replaced with fresh DMEM+10% FBS and dishes were incubated a further 48 hours prior to fixation.

ts045-VSV-G-GFP trafficking assay

siRNA: 24 hours prior to transfection, HeLa cells were plated on glass coverslips in matched 12-well plates in DMEM supplemented with 10% FBS (no antibiotics were

used for the duration of the assay). One hour prior to transfection, DMEM was removed and replaced with 0.5 mL Optimem. Cells were knocked down with 12 pmol siRNA and 2.4 µL Olifofectamine per well. Approximately 20 hours after transfection, 1 mL DMEM+10 % FBS was added to each well. One hour later the medium was exchanged for fresh DMEM+10% FBS and the dishes were returned to the 37°C incubator for 24 hours.

ts045-VSV-G-GFP transfection: VSV-G transfection was performed approximately 48 hours after the start of the knockdown. Prior to transfection, the medium was replaced with 0.5 mL DMEM+10% FBS. Each well of a 12 well dish was transfected with 0.4 µg pEGFP-VSV-G (ts045) (Addgene) and 1 µL Lipofectamine (Invitrogen), each diluted in 250 µL Optimem. This was added to the 0.5 mL DMEM already on the well. Plates were returned to the 37°C incubator for approximately 18 hours.

Temperature shifts: Approximately 60 hours after knockdown and 18 hours after plasmid transfection, dishes were placed in a 39.5°C incubator for 6.5 hours to induce endoplasmic reticulum retention of the ts045-VSV-G-GFP. At this point, control cells were fixed on ice by rinsing with ice-cold phosphate buffered saline (PBS) followed by incubation with ice-cold 4% paraformaldehyde in PBS. The remainder of the cells were shifted to 32°C by replacement of medium with 32°C DMEM+10% FBS containing 10 µg/ml cycloheximide and transferred to a 32°C incubator for 30 minutes. At the end of the 30 minute shift, cells were fixed as described above.

Fluorescence microscopy:

Cells containing ts045-VSV-G-GFP fusions were fixed with 4% paraformaldehyde in PBS as described above, then permeabilized by addition of -80°C 100% methanol and incubation for 20 minutes at -20°C prior to immunofluorescence labelling. All other cells were fixed and permeabilized by incubation for 20 minutes at -20°C after addition of -80°C 4:1 methanol:acetone. In both cases, fixative was removed by serial washes with PBS, then cells were blocked for one hour with 2% FBS, 2% BSA and 0.2% fish skin gelatin in PBS. Primary antibodies were diluted in blocking buffer at the following concentrations: Sec31a 1:250 (BD Diagnostics); ERGIC53 1:125 (Sigma); GM130 1:5000 (ML07) or 1:200 (4A3) (both were kind gifts of Martin Lowe); Mannosidase II 1:500 (kind gift of Kelley Moremen); C12/TTC-15 (Abnova). Secondary antibodies: Alexa488- or Alexa546-labelled anti-mouse or anti-rabbit secondary antibodies (Invitrogen) were diluted 1:250 in blocking buffer containing 4',6-diamidino-2-phenylindole (DAPI) at a concentration of 10 µg/mL. After antibody incubations, slips were washed with three serial dilutions of blocking buffer and a final wash of PBS alone, then mounted on Antifade Gold (Invitrogen). Golgi fragmentation was assessed by epifluorescence microscopy using a Zeiss axioplan microscope fitted with an X-cite series 120Q light source (EXFO Life Sciences) and a Lumenera Infinity 3-1C 1.4 megapixel cooled CCD camera. VSV-G trafficking was monitored on fixed cells by confocal microscopy using a Leica TCS SP2 microscope.

Antibodies:

In addition to the antibodies described above for immunofluorescence, we used rabbit

polyclonal antibodies to His-tagged C2 and –C3 (described previously in Scrivens et al., 2009). For this study, we raised antibodies to a C11 peptide and serum was affinity purified against the antigenic peptide coupled to Sulfolink beads (Thermo Scientific) as per the manufacturer's directions.

Tandem Affinity Purification:

Tandem affinity purification (TAP)-tagged C2, C2L or C11 were expressed in HEK293T cells. In a typical purification, six 15 cm dishes of cells at 50% confluence were transfected with 20 µg of the expression plasmid using standard Ca_2PO_4 transfection. 18 hours after transfection, cells were washed thoroughly with HBS supplemented with 0.53 mM EDTA, and returned to the incubator in fresh DMEM supplemented with 10% FBS and 1X penicillin/streptomycin. Twenty-four hours after transfection, cells were split two-fold, resulting in twelve 15 cm dishes per purification. Forty-eight hours after transfection, cells were harvested by removing the medium and washing once with 10 mL ice cold PBS and scraping in 500 µl per plate lysis buffer (1 mM EDTA, 50 mM Tris pH 7.2, 150 mM NaCl, 1% Triton X-100, 1X Complete EDTA-free protease inhibitor (Roche), 1 mM NaOV, 2 mM NaF). Lysates were pooled and rocked for 10 minutes at 4 °C. Lysates were then cleared by centrifugation at 16,100 g for 20 minutes and supernatants reserved. TAP-tagged proteins were bound to a 50 µL bed volume of IgG sepharose (GE Healthcare) in batch in 15 mL conical tubes by rocking for three hours at 4°C. Lysates and beads were then transferred to a 10 mL disposable Bio-Rad column for washing. Beads were washed with 3 X 5 mL lysis buffer and 2 X 5 mL Tobacco Etch Virus (TEV) buffer (10 mM Hepes KOH pH 8.0, 150 mM NaCl, 0.1% NP-40, 0.5 mM

EDTA). Beads were then collected from the disposable column by resuspending in TEV buffer and transferring to microfuge tubes, where they were washed twice more in TEV buffer. TEV cleavage was performed by resuspending the IgG beads in 300 uL TEV buffer containing 50 U TEV protease and 1 mM DTT and agitating for 10 hours at 4 °C, followed by 1 hour at 16°C. The supernatant was reserved and the IgG beads were washed three times with 300 µL calmodulin (CAM) binding buffer (10 mM β-mercaptoethanol, 10 mM Hepes-KOH, 150 mM NaCl, 1 mM MgOAC, 1 mM imidazole, 0.1% NP-40, 2 mM $CaCl_2$). The washes and TEV supernatant were pooled, yielding a 1.2 mL sample, cleared by three rounds of centrifugation to remove residual IgG beads, and added to a 35 µL bed volume of CAM beads (New England Biolabs). Proteins were bound by incubation for 3 hours at 4°C, then beads were washed three times with CAM binding buffer and twice with CAM rinsing buffer (10 mM Hepes pH 8.0, 75 mM NaCl, 1 mM MgOAC, 1 mM imidazole, 2 mM $CaCl_2$). Proteins were then eluted in six 35 µL fractions using 50 mM Tris pH 6.8, 25 mM EGTA. Elutions were then subjected to SDS-PAGE followed by silver staining or visualization with Bio-Safe coomassie (Bio-Rad), excision, and mass spectrometry.

Mass Spectrometry.

Proteins associating with TAP-tagged proteins were separated by SDS gel electrophoresis. The gel was subsequently stained with either GelCode Blue Stain (Pierce) or BioSafe Commassie (BioRad) following the manufacturer's instructions. Stained bands were excised from the gel, reduced (10 mM DTT, 10 min) and alkylated (55 mM Iodoacetamide, 30 min) and digested with trypsin (12 ng/µL) overnight. The

resulting peptide digest was treated in one of two ways: (i) peptides were subjected to reverse phase separation, followed by MS-MS on a Bruker HCT Ultra ion trap mass spectrometer. Data files were formatted to mgf files with Bruker Compass Data Analysis software (standard settings) and searched on the Homo sapiens NCBI database (version NCBInr 20090611) using Mascot v. 2.2 (Matrix Sciences). Protein identifications were based on unambiguous peptides with a Mowse score better than 42 (random probability value $p< 0.05$). (ii) peptides were resuspended in 8% acetonitrile, 0.1% formic acid and injected quantitatively for separation on a C18 nanocolumn. Detection and sequencing of the peptides was accomplished by an LTQ ion trap mass spectrometer (Thermo Electron) equipped with an ESI nanosource and operating in positive mode with a voltage of 1.2 kV applied at a liquid junction just upstream of the column. Abundant ions were subjected to pulsed-Q dissociation for ion fragmentation. Peptide sequences thus generated were then used to probe the human genome using SEQUEST from BioWorks 3.3. (Thermo Electron). In some analyses, TAP-C2, -C2L, or C11-associated proteins were excised from a 4% polyacrylamide gel, pH 6.8, as a single band containing all interactors prior to trypsin digestion.

Plasmids:

For those TRAPP subunits cloned from cDNA, the source material was pooled human cDNAs from HEK293T, HeLa and A549 cells. Human C11 /C4orf41 in pENTR201 and human C12/TTC15 in pOTB7 were purchased from OpenBiosystems. pEGFP-VSVG (ts045) was purchased from Addgene. TAP-C2 and –C2L were created by cloning mouse C2 and human C2L, respectively, into the BamHI and EcoRI sites of pcDNA3-

NTAP (kind gift of Anne-Claude Gingras). For TAP-C11 expression, C11 was cloned by LR recombination into pCDNA-TAP-GWY. pCDNA-TAP-GWY consists of the Gateway cloning cassette from pAG424GPD-ccdB-TAP (Alberti et al.Yeast 24(10): 913-919) excised by digestion with XhoI/SpeI, repaired with the Klenow fragment of DNA polymerase, and ligated into the EcoRV site of pcDNA3.1. For HA-C11, C11 was cloned by LR recombination into pcDNA-HA-GWY. pcDNA-HA-GWY consists of the gateway cloning cassette from pAG424GAL-ccdB-HA (Alberti et al.Yeast 24(10): 913-19) cloned into pcDNA3.1 by the same means as pCDNA-TAP-GWY. For V5-C8 or C10 expression, C8 or C10 was cloned by LR cloning into pcDNA3.1/nV5Dest (Invitrogen).

Gateway cloning:

Gene sequences of mammalian TRAPP subunits were amplified with oligonucleotides (see Table 1) flanked by attB sites. For BP recombination reactions, 45 ng of pDONR201, 45 ng of PCR product, 0.5 μl of BP clonase (Invitrogen) and TE buffer were incubated overnight at 25°C in a total volume of 3μl. The entire reaction was transformed into DH5α cells and plated on selective LB agar plates. Clones were checked for the presence of the insert via restriction digest and the insert was verified by DNA sequencing. LR recombination was set up the same as BP recombination except LR recombinase was used.

Cell fractionation following knockdown:

HeLa were plated 24 hours prior to knockdown in 10 cm dish and reached 40% confluence at the time of transfection. One hour before the transfection, the medium

was changed for fresh DMEM+10% FBS. Just prior to transfection, the medium was changed to 7 mL Optimem per dish. 360 pmol of each oligonucleotide (C11-1 or non-specific control) and 72 µL of Oligofectamine were diluted in Optimem and mixed as per the manufacturer's instructions and added to the medium already on the plates. 20 hours after the transfection, 6 mL DMEM+10% FBS was added to each dish. Four hours later, the medium was removed and replaced with fresh DMEM+10% FBS. One day later the cells were trypsinized and split to two 15 cm dishes per knockdown. Cells were harvested 24 hours later (72 hours post transfection) by scraping in a total of 1 mL lysis buffer (150 mM NaCl, 50 mM Tris pH 7.2, 0.5 mM EDTA, 1 mM DTT, 1% Triton X-100, 1 X Complete EDTA-free protease inhibitor (Roche) 1 mM NaOV, 2 mM NaF) for each pair of 15 cm dishes. Lysates were then cleared by centrifugation at 16,100 g at 4°C, and 1 mg of cleared lysate was fractionated in gel filtration buffer (150 mM NaCl, 50 mM Tris pH 7.2, 0.5 mM EDTA, 1 mM DTT) on a Superdex 200 column using an AKTA chromatography system (GE Healthcare).

Co-transfections:

12-18 hours prior to transfection, HEK293T cells were plated at 40% confluency. One or more 15cm dishes were transfected by the calcium phosphate method. In a conical tube, sterile distilled water, 20 µg of each plasmid and 122 µl of 2M $CaCl_2$ were added respectively to make up a volume of 1ml which was then mixed slowly with 1ml of HBS by bubbling. The mixture (2ml in total) was spotted evenly on to a 15 cm dish of HEK293T cells which are left to grow at 37°C in a humidified, 5% CO_2 incubater overnight. 24 hours after transfection, cells were washed with 10ml warm PBS followed

by the addition of 20ml fresh DMEM+10% FBS with 1X penicillin/streptomycin. Transfected cells were harvested 48 hours later on ice by washing with 10ml ice-cold PBS and scraped with 1ml lysis buffer (150mM NaCl, 0.5mM EDTA, 50mM Tris pH 7.2, 1% Triton-X100, 1mM DTT, 1 X Complete EDTA-free protease inhibitor (Roche)). In the case where more than one 15cm dish was transfected, 1ml of lysis buffer was used to harvest all transfected cells. Lysates were homogenized for 1 minute on ice and then centrifuged at maximum speed in a refrigerated microfuge for 10 minutes at 4°C.

Co-immunoprecipitation:

In an eppendorf tube, 500 μg of protein harvested from transfected cells was incubated with 1 μg of antibody rotating overnight at 4°C. Pre-washed agarose beads were resuspended with lysis buffer (150mM NaCl, 0.5mM EDTA, 50mM Tris pH 7.2, 1% Triton-X100, 1mM DTT, 1 X Complete EDTA-free protease inhibitor) to make up a 50% slurry. 10ul bed volume of beads was added to each eppendorf tube. For anti-HA mouse monoclonal and anti-V5 rabbit polyclonal antibody, protein G and protein A agarose beads were used, respectively. After 2 hours of incubation with the beads at 4°C, tubes were centrifuged at 4000 rpm for 2 minutes at 4°C. The supernatant was saved and analyzed by SDS-PAGE to determine the efficiency of the immunoprecipitation. The pellet was washed three times with 1ml of lysis buffer and eventually resuspended with 1x Laemmli sample buffer. Protein samples were boiled for 2 minutes at 95°C, centrifuged at maximum speed for 2 minutes and fractionated by SDS-PAGE.

Gel filtration:

Approximately 5 mg of protein was fractionated by size exclusion chromatography on a Superdex 200 (10/300 GL) column. Fractions of 0.5 ml were collected in gel filtration buffer (150 mM NaCl, 0.5 mM EDTA, 50 mM Tris pH 7.2, 1mM DTT).

Yeast two hybrid:

The plasmids pGADT7 and pGBKT7 (Clonetech) were modified to be Gateway-compatible by inserting a cassette encompassing *att*P1-*ccd*B-Cam^R-*att*P2 into a repaired SfiI site. Kanamycin resistance was ablated in pGBKT7 and it was further rendered ampicillin resistant by inserting the β-lactamase gene (as a repaired DrdI/AlwNI fragment from pGADT7) into the repaired RsrII/SfoI site. pGADT7 constructs were transformed into AH109 yeast and pGBKT7 constructs were transformed into Y187 yeast. Mating was performed on YPD agar plates overnight at 30°C. Plates were then replicated onto -leucine/-tryptophan to assess mating efficiency. Interactions were scored after replicating to -leucine/-tryptophan/-histidine (TDO) without or with increasing amounts of 3-amino-1,2,4-triazole (3-AT) or to -leucine/-tryptophan/-histidine/-adenine (QDO). Interactions on TDO plates were considered positive if growth was detected at 3-AT levels that could not support growth when a TRAPP-subunit-containing bait or prey plasmid was tested with an empty partner plasmid.

Table 1: List of primers used for PCR

TRAPP subunit	Primers	Sequence	Size (base pair)
C1	forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGACTGTCCACAACCTGTACC	438
	reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAGCCAGCCCGGGCGGAGAAGAAG	
C2	forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTGGGAGCTTCTACTTTG	423
	reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAGCTTAAAAGGTGTTTCTTCC	
C2L	forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCGGTGTGCATCGCGGTG	423
	reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAGCACACCTGTATCATCATCGAC	
C3	forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCGAGGCAGGCGAACCGTG	543
	reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTATTCCTCTCCAGCTGGAAGATTG	
C4	forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCGATTTTTAGTGTGTATG	660
	reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTATGACCCAGGTCCAAAAGTTCC	
C5	forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGAGGCGCGCTTCACGCGC	567
	reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAGCGGCCCTCCAGGGCCCGGTC	
C6a	forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCGGATACTGTGTTGTTTG	522
	reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAGGATTTCGGAATCACCACCTGG	
C6b	forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATGGCGGATGAGGCGTTG	422
	reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTGCAGCTTCTGTATCATCACCTG	
C8	forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGAGAGATCCTAATAATCAAC	4143
	reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTACACATTACTGATGATGATCAG	
C9 N terminal	forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTGCCAGCTGGGGATCAAG	2100
	reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTACTGAACTTCACACACATCTCCTTG	
C9 C terminal	forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATGGATAAGATCCCCCTTCTCTGTG	2931
	reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAGGCCTGCGCCTCCAGGGCACAC	
C10	forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGACGCCTCTGAGGAGCCG	3780
	reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTATGTTACACTGACTTCCAGGACG	
C12	forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGAGGACGCTGGCGGCGGCGAG	2208
	reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAGGCCAGCTTGAGGCACTGTGTG	
C3L	forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTCGCCCTGCACACCG	546
	reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTATTTTTTCCCTCTATATTTTTTCTC	

Supplemental figure legends

Supplemental Figure 1. Conservation of TRAPPC11 and TRAPPC12. BLAST searches were performed with C11 and C12 to identify related proteins which were then used to construct the phylogenetic trees. (A) The phylogenetic tree for C11. Accession numbers for the sequences are: *H. sapiens*, NP_068761.4; *M. musculus*, NP_796214.2; *D. rerio*, NP_955920.2; *D. melanogaster*, NP_728832.2; *C. elegans*, NP_497264.1; *A. thaliana*, NP_201396.4; *O. sativa*, NP_001047920.1; *L. bicolor* , XP_001880173.1; *A. flavus*, XP_002373747.1 (B) The phylogenetic tree for C12. Accession numbers for the sequences are: *H. sapiens*, NP_057114.5; *D. rerio*, NP_001074113.1; *M. musculus*, NP_848926.2; *D. melanogaster*, NP_649255.1; *C. elegans*, NP_508439.2; *A. thaliana*, NP_195692.2; *O. sativa*, NP_001047094.1; *L. bicolor* , XP_001875127.1; *A. flavus*, XP_002374721.1

Supplemental Figure 2. Domain structure of TRAPPC11. The schematic shows the *foie gras* domain (263-522) in yellow and the DUF1683 domain (981-1100) in blue, signatures for C11 and related proteins. Two regions in C11 with limited homology to C10 (I) and yeast Trs130p (II) are also shown. Alignments were performed using BLAST. This extends the region of similarity between C11 and C10, previously reported to start at amino acid 345 in C11 (see Cox *et al.*, 2007), and also demonstrates a statistically significant (E value <0.05) region of homology between C11 and *S. cerevisiae* Trs130p which may be of functional significance. Colouring was performed using Boxshade with black and gray indicating identity and similarity, respectively, in

75% of the sequences. Accession numbers for the sequences are listed in the legend to Supplemental Figure 1.

Supplemental Figure 3. Conservation and domain structure of TRAPPC12/TTC-15. A multiple sequence alignment was performed using human, bovine, *Danio* and *Xenopus* TTC-15. Conserved residues are highlighted in black and residues common to at least 2 sequences are highlighted in gray. The four tetratricopeptide (TPR) repeats are boxed in four different colours. It should be noted that high conservation is seen not only in the TPR region but in the region immediately amino-terminal to the TPR domain. Homology is reduced even further upstream and more ancient species such as *Arabidopsis*, *Anopholes* and *Drosophila* have a truncated TTC-15 missing this region, suggesting it has evolved a function in higher eukaryotes. Colouring was performed using Boxshade with black and gray indicating identity and similarity, respectively, in 50% of the sequences. Accession numbers for the sequences are listed in the legend to Supplemental Figure 1 with the following additions: *X. tropicalis*, NP_001006869.1; *B. taurus*, NP_001095760.1.

Supplemental Figure 4. TRAPPC2L and TRAPPC10 co-fractionate in two high molecular weight peaks in C10-transfected cells. HEK293T cells were transfected with V5-C10 and fractionated by size exclusion chromatography. Fractions were probed with anti-C2L and -V5 antibodies. The ability of excess C10 to draw the low molecular weight pool of C2L into a higher molecular weight fraction is consistent with their strong interaction by yeast two hybrid and supports the notion that C2L mediates interactions

with C10 and perhaps other high molecular weight TRAPP proteins. A similar effect on C2 was not seen upon C10 overexpression.

Supplemental Figure 5. Knockdown of TRAPPC11. HeLa cells were transfected with HA-C11 and then treated with a non-specific siRNA (NS) or two different siRNAs against C11 (C11-1, C11-2). To estimate the level of knockdown, increasing amounts of lysate from the non-specific siRNA sample were fractionated (μg of protein loaded are shown above each lane). The samples were analyzed by western blotting using anti-HA IgG. Both siRNAs against C11 reduce the levels of HA-C11 by ~90%. The coomassie-stained gel is shown as a loading control.

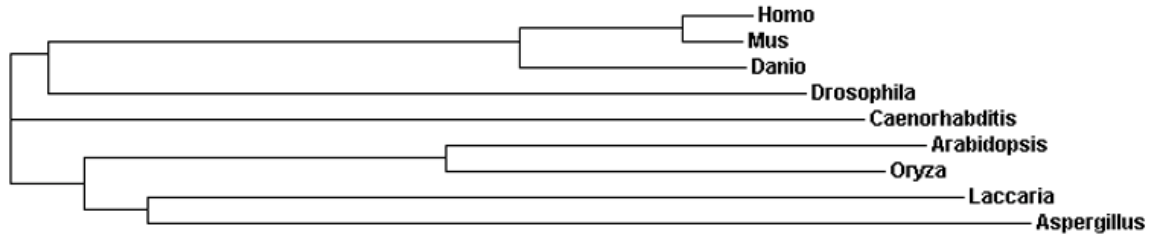
Supplemental Figure 6. A view of the cells from Figure 4B in the main text showing the regions used for the higher magnification. The bars represent 10 μm.

Supplemental Figure 7. A view of the cells from Figure 5 in the main text showing the regions used for the higher magnification. The bars outside of the boxed region represent 10 μm.

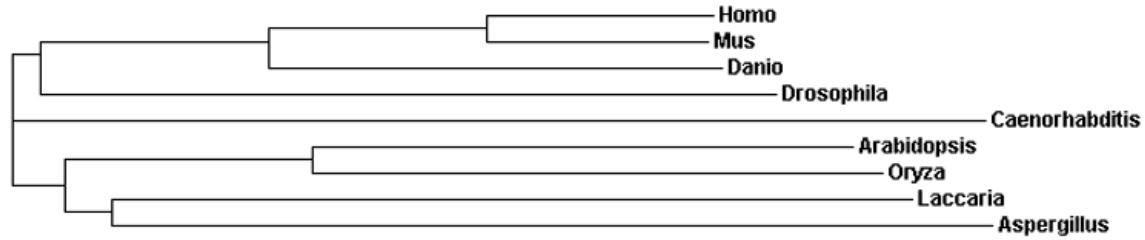
Supplemental Figure 8. TRAPPC3L is a novel mammalian TRAPP protein. (**A**) A BLAST search using human C3 revealed a related sequence called TRAPPC3L (accession number NP_001132916). The human C3 and C3L proteins were aligned and coloured by conserved residues (black) or similar residues (gray). (**B**) Lysates from cells expressing FLAG-C3L (panels b-e) or FLAG-C3L and V5-C10 (panel c) were

immunoprecipitated with antibody recognizing C2 (panel b), V5 (panel c), C11 (panel d) or C12 (panel e). A control without antibody is shown in panel a. The immunoprecipitates were then probed for the presence of C3L using anti-FLAG antibody. The asterisk in panel e indicates the FLAG-C3L band while the dark band above it is the IgG light chain from the mouse serum used in the immunoprecipitation.

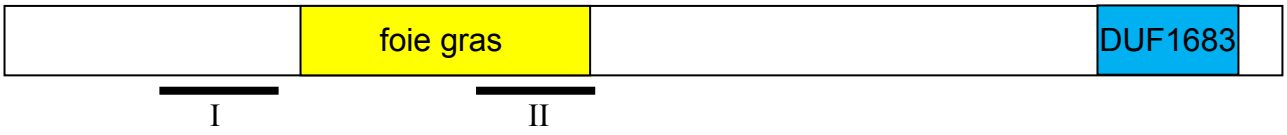
A



B



Supplemental Figure 1



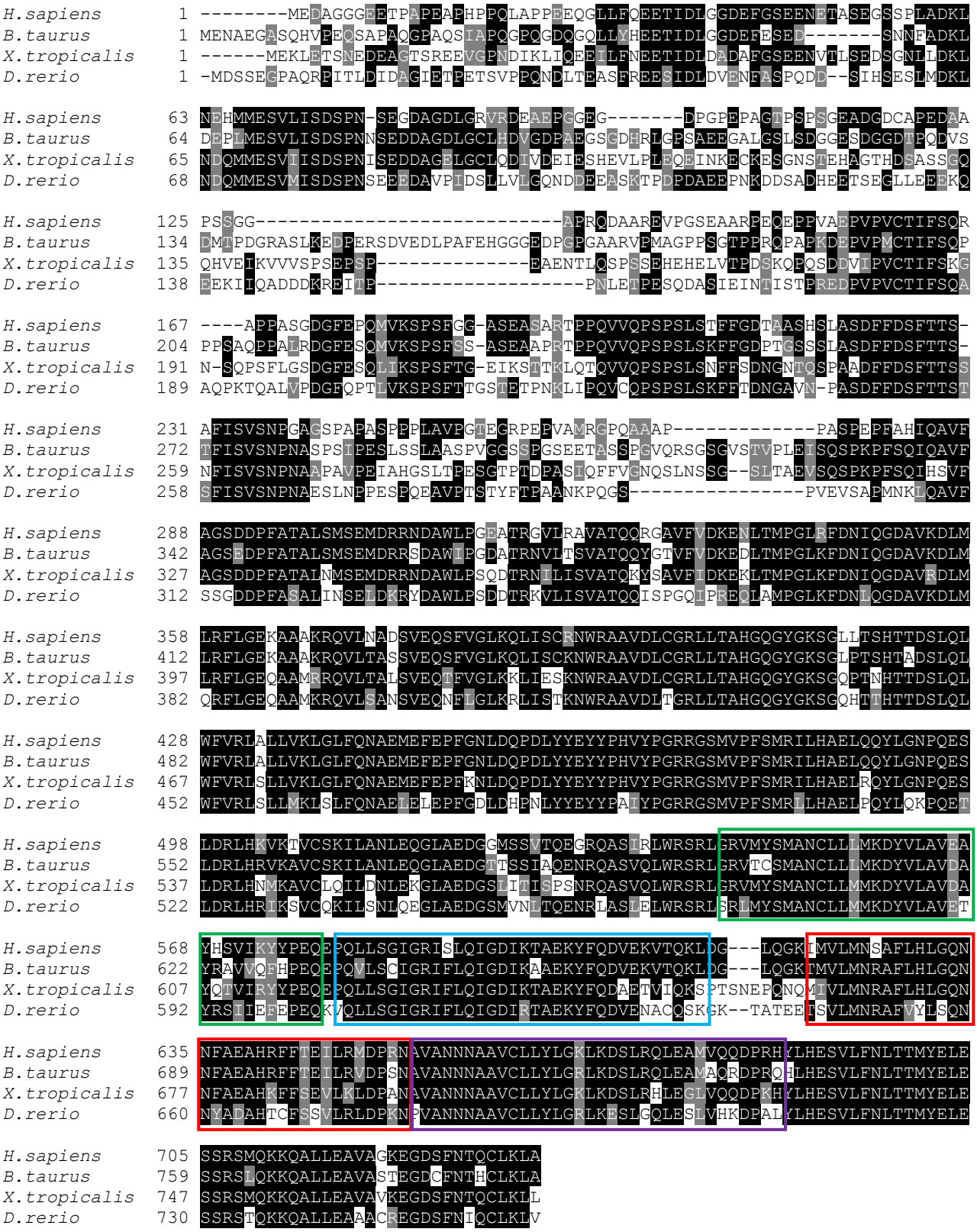
I

```
C11   136 AVVLIQKKTPLPPGEDVIASERAA------ALCNACELSGKSLFVLPHTDHLVGYII 186
C10   724 AHVLRCSHVTLEPGANQITFRTQAKEPGTYTLRQLCASVGSVWFVLPHIYPIVQYDV 780

C11   195 HAQTYYYTEIRRVKSHKEFLNKTTHQLLFVRHQFKIAFESELKQDTQNALKNY 247
C10   463 HATIEMYTSIGRIRSAKFVGKDLAEFYMRKKAPQKAEIY------LQGALKNY 509
```

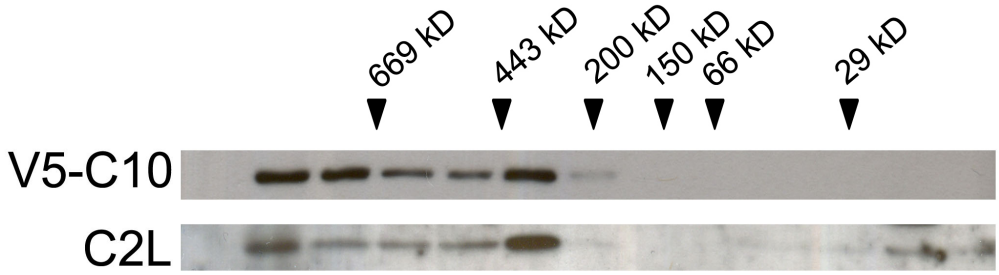
II

```
A.thaliana             441 --AKRVQDSLQIIAWLKRSYESFTNLKAQ------RMAALCAFEVAREYFDLADPNNAKF
O.sativa               427 --AERFQDSYEIIALFRKAYESFRSLGAT------RMASACSGGMAIEYYAAGDFSNAKQ
H.sapiens              421 LKERNVVHSEIIITLLSNAVAQFKKYKCP------RMKSHLMVQMGEEYYYAKDYTKALK
M.musculus             421 LKERSVVHSEIIIALLSNAVAQFKKYKCP------RMKSHLMVQMGEEYYYAKDYTKALK
D.rerio                421 VKERDVLHSELIIALLSNAVAQFKKYKCP------RMKSHLMVQMGEEYYHAKDYTKALK
D.melanogaster         435 DQERSYNHSAAIIALLSQAMAQFKIYKCL------RFRKKLAIDMAEEYLKSGDHAKALT
L.bicolor              492 ANEKKVEHLAIILELYTKAYELFKKYTPASSQGQGRLTLWIAYRIAQTYHQSGKFDMAVR
S.cerevisiae Trs130p   401 ------VFQENFLTLTKEILSLFNKCEGK----RQRIVDILSIEIGLLYYQGKKYEEAVS

A.thaliana             494 FFDIAANLYRQEGWVTLLWEVLGYLRE---------------------------------
O.sativa               479 LFDGVAGLYRQEGWATLLWENLGYLRE---------------------------------
H.sapiens              475 LLDYVMCDYRSEGWWTLLTSVLTTALK---------------------------------
M.musculus             475 LLDYVMCDYRSEAWWTLLTSILTTALK---------------------------------
D.rerio                475 LLDYVMCDYRTERWWSLLTSIVCTALK---------------------------------
D.melanogaster         488 LYSLMLPDYRQEKWTTIFTDVLLKTLR---------------------------------
L.bicolor              552 FFERIARTYRREKWYTMLRPLLSTWYT---------------------------------
S.cerevisiae Trs130p   451 LFLSCYEYYTQTNWNSIGLKILQVFIDSLSHCPKLDVLQIDGESVSASAVLTNAFLNILK

A.thaliana             521 -CSRNLD-----------ALKDFVEFSLEMVALPVTSYENS-----------------
O.sativa               506 -CARKLK-----------SLKDFISYSLEMAALPLFSGSGQ-----------------
H.sapiens              502 -CSYLMA-----------QLKDYITYSLELLGRASTLKD--------------------
M.musculus             502 -CSYLMA-----------QLKDYITYSLELLGRASTLKD--------------------
D.rerio                502 -CSYLMG-----------QVKDYITYSMELVGRASILSE--------------------
D.melanogaster         515 -CALLSG-----------SVADYIACSVEALSLRHQSDQSERILI-------------
L.bicolor              579 -CAQQLG-----------DVELSICLLIEMLGHDSVESEDP-----------------
S.cerevisiae Trs130p   511 LCKDNDSKEIWWKKFMDLQMKNNIHLMYPLDGLFEVTLNSKVHLARANVSAIEVNLKS
```

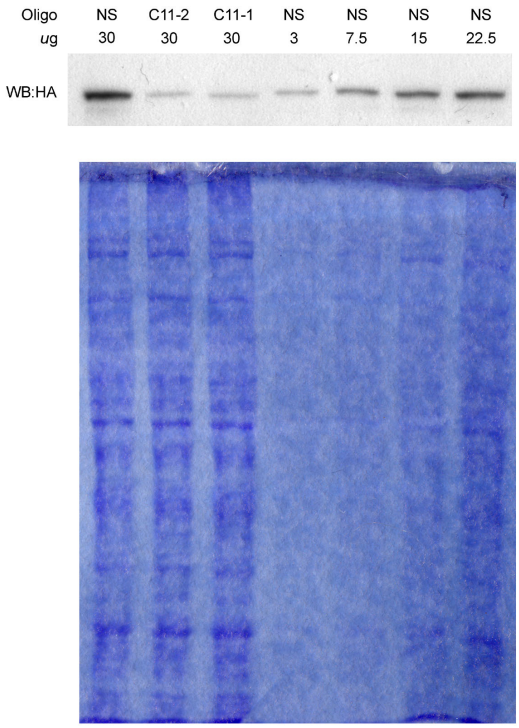
Supplemental Figure 2



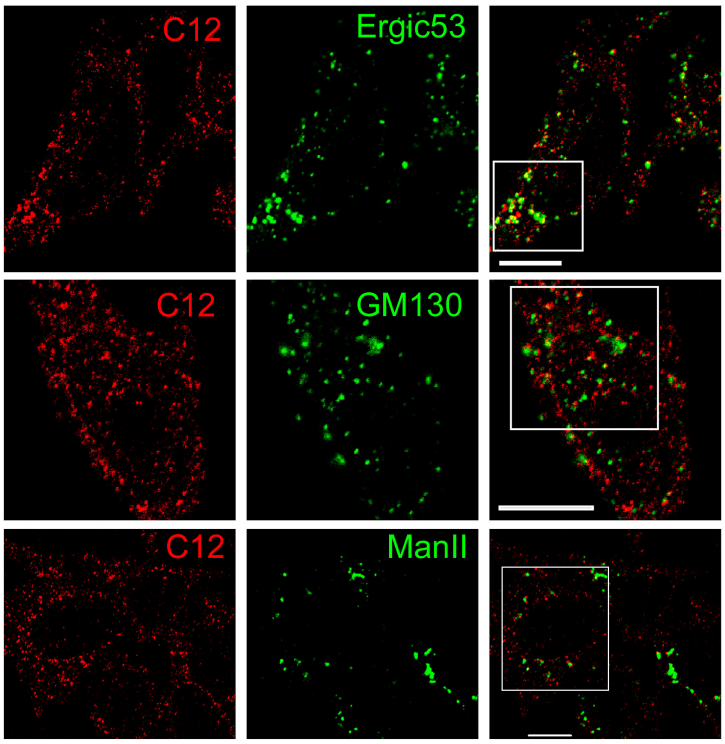
Supplemental Figure 3



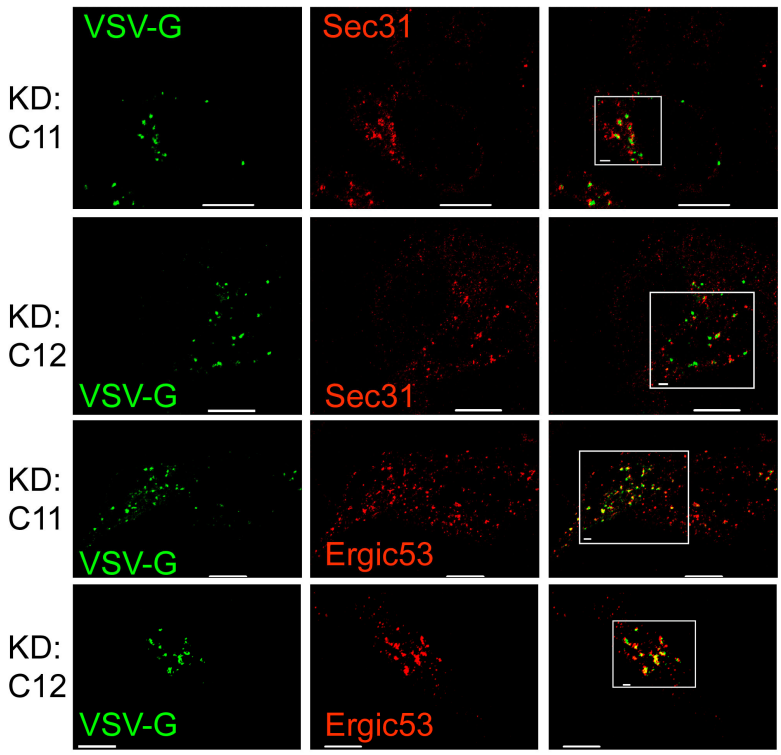
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6

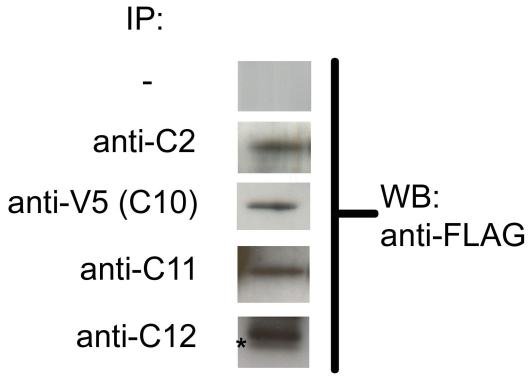


Supplemental Figure 7

A

```
C3      1 MSRQANRGTESKKMSSELFTLTYGALVTQLCKDYENDEDVNKQLDKMGFNIGVRLIEDFL
C3L     1 MSRPAHRRPEYHKINKDLFVLTYGALVAQLCKDYEKDEDVNQYLDKMGYGIGTRLVEDFL

C3     61 ARSNVGRCHDFRETADVIAKVAFKMYLGITPSITNWSPAGDEFSLILENNPLVDFVELPD
C3L    61 ARSCVGRCHSYSEIIDIIAQVAFKMYLGITPSVTCNNSSKNEFSLILEKNPLVEFVEELP

C3    121 N-HSSLIYSNLLCGVLRGALEMVQMAVEAKFVQDTLKGDGVTEIRMRFIRRIEDNLPAGE
C3L   121 AGRSSLCYCNLLCGIIRGALEMVHLAADVTFLQDRLKGDSVTEIGITFLKKRDEKKYRGK

C3    180 E
C3L   181 K
```

B



Supplemental Figure 8