Figure S1. Related to figure 1

A) Coomassie Blue staining of sedimentation standards for the experiment shown in figure 1C. The peak fractions for thyroglobulin (660 kDa), yeast alcohol dehydrogenase (150 kDa), and bovine serum albumin (BSA, 66 kDa), are F26, F12 and F7, respectively. B) Size of 8A-CC inferred by sedimentation. The molecular weights of the standards were plotted as a function of the corresponding peak fractions. Based on these positions, the position of 8A-CC corresponds to approximately 550 kDa. C) Glycerol gradient fractions 4-16 (top-bottom) from the sedimentation analysis of 8A-CC shown in figure 1B, C. Vps8a-FLAG was detected by western blotting with anti-FLAG antibodies. “INPUT” corresponds to 1% of the total eluate. No monomeric Vps8a-FLAG was detected.

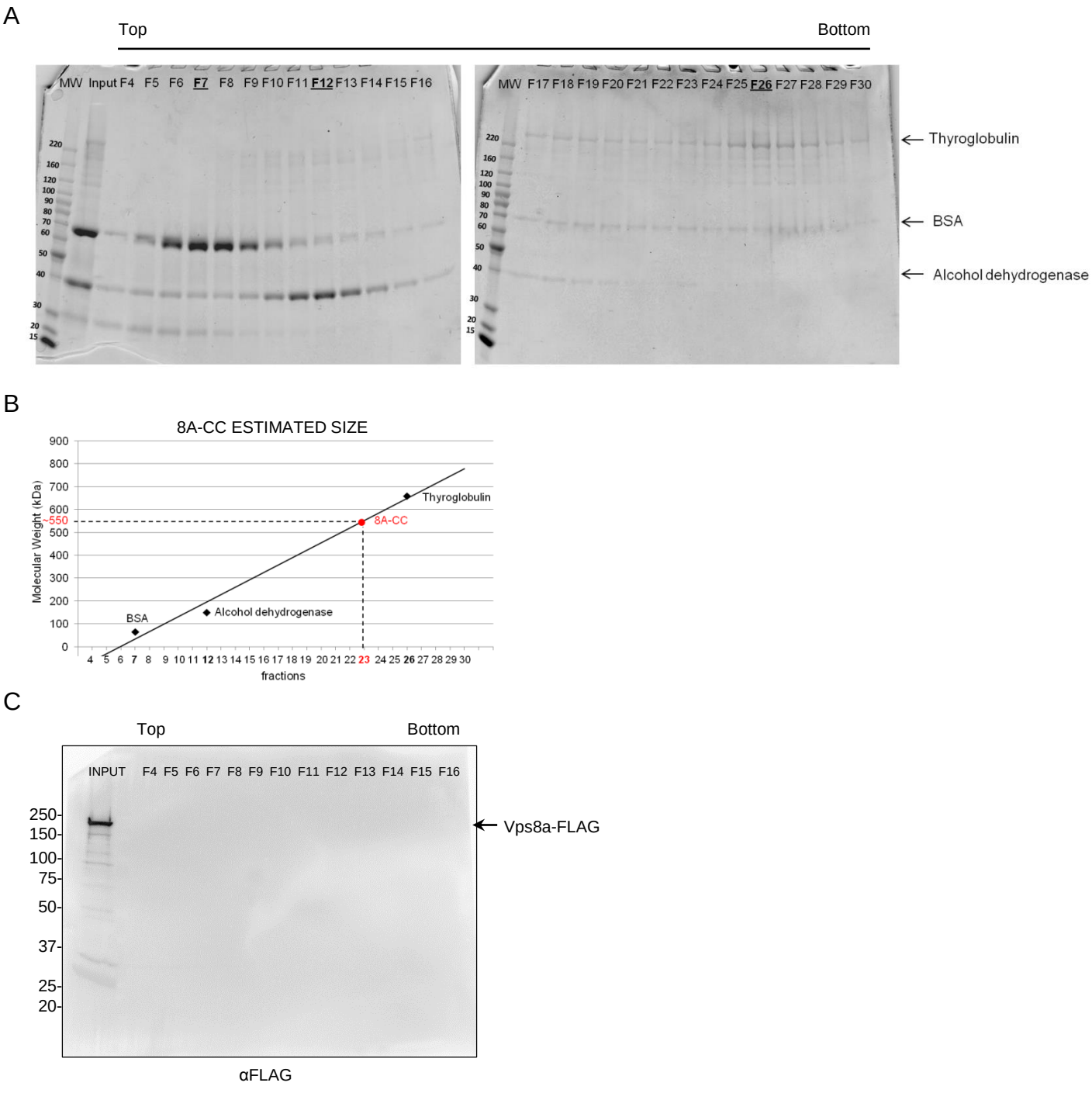


Figure S2. Related to figure 2

A) Immunoprecipitation of Vps8b, Vps8c, Vps8d, Vps8e, Vps8f. Cells were transformed to FLAG-tag the Vps8 paralogs at their endogenous loci. Detergent cell lysates were treated with anti-FLAG beads, and LDS-eluted proteins were analyzed by SDS-PAGE and western blotting with anti-FLAG antibodies. The expected full-length proteins were detected. B) FLAG-tagged Vps8b, Vps8c, Vps8d, Vps8e, Vps8f were immunoisolated from solubilized cryopowders using anti-FLAG beads. Bound proteins were eluted with 3XFLAG peptides, and 25% was loaded on 8% gels for SDS-PAGE followed by silver staining. For each lane, the numbers to the right of the protein bands indicate the Vps8, Vps18, Vps16, Vps11, Vps3, and Vps33 subunits for that complex. All six CORVET subunits were detected for Vps8c, Vps8d, Vps8e, and Vps8f. In the Vps8b-FLAG eluate, the Vps18 subunit (Vps18b) was not clearly detected at the expected size, but may migrate anomalously (note species migrating close to the Vps3 band, unique to this sample). For the Vps8d sample, Vps8d appears to be present in excess of all other subunits. The additional bands common to all samples are non-specific bands captured by the FLAG-beads.

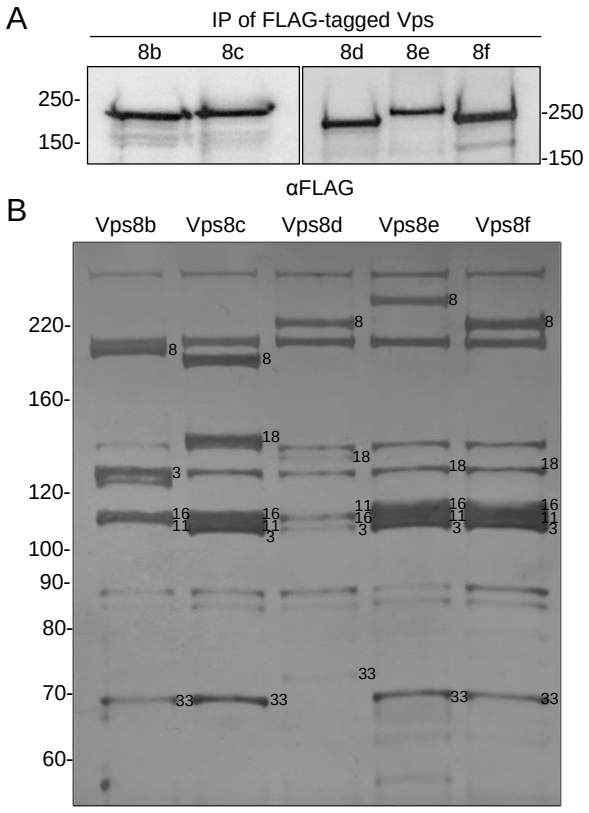
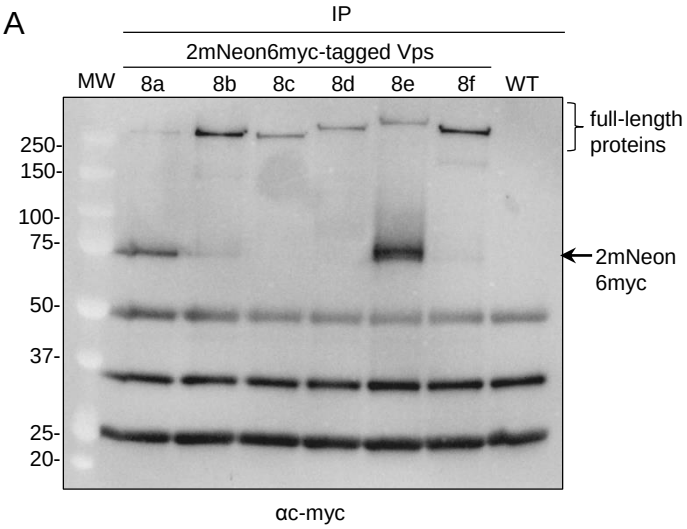


Figure S3. Related to figure 3

A) Immunoprecipitation of mNeon-tagged Vps8 paralogs. Detergent cell lysates were incubated with anti-c-myc beads, and bound proteins eluted with LDS-sample buffer. Samples were analyzed by SDS-PAGE and western blotting with anti-c-myc antibodies. On the right, the black arrow indicates a band whose size corresponds to a cleaved 2mNeon6myc tag, visible in Vps8a and Vps8e samples, while a weaker band is present in Vps8b. The three lower bands, common to all samples, are cross-reactive bands. B) Vps8c puncta are concentrated toward the cell posterior. 30 z-stacks of randomly-chosen cells expressing fluorescently-tagged Vps8c were scored for the absence or presence of concentrated Vps8c signal in the posterior half of the cell. The oral apparatus (OA) was first used to unambiguously define the anterior, and z-projections were then generated to visualize the entire pool of fluorescent puncta within each cell. As shown in the table, ~80% of cells showed distinct posterior accumulation. N/A indicates cells in which the oral apparatus could not be visualized, and such cells were not counted. C) Single frame from a time-lapse movie, with paired differential interference contrast (DIC) image, of a cell endogenously expressing mNeon-tagged Vps8b. Cells were incubated in S-medium for 2h prior to imaging. In favorable focal planes of live cells, Vps8b can be visualized in cytoplasmic puncta, which are more prominent in fixed cells (figure 1B). D) Confocal sections of fixed cells expressing mNeon-tagged Vps8 paralogs, with paired DIC images, in addition to those shown in figure 3B. Arrowheads indicate the Vps8b-labeled phagosomes/phagolysosomes; asterisks indicate localization of Vps8d at contractile vacuoles; white arrows indicate Vps8e vesicles at the cell anterior; black arrows indicate the oral apparatus (OA). Scale bars, 10 μm.

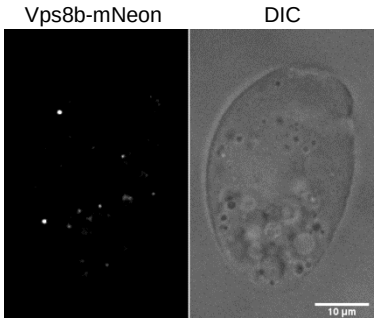
A



B

	N/A (OA not visible)	No posterior pattern	Posterior pattern
n° of cells expressing tagged Vps8c	2	5	23

C



D

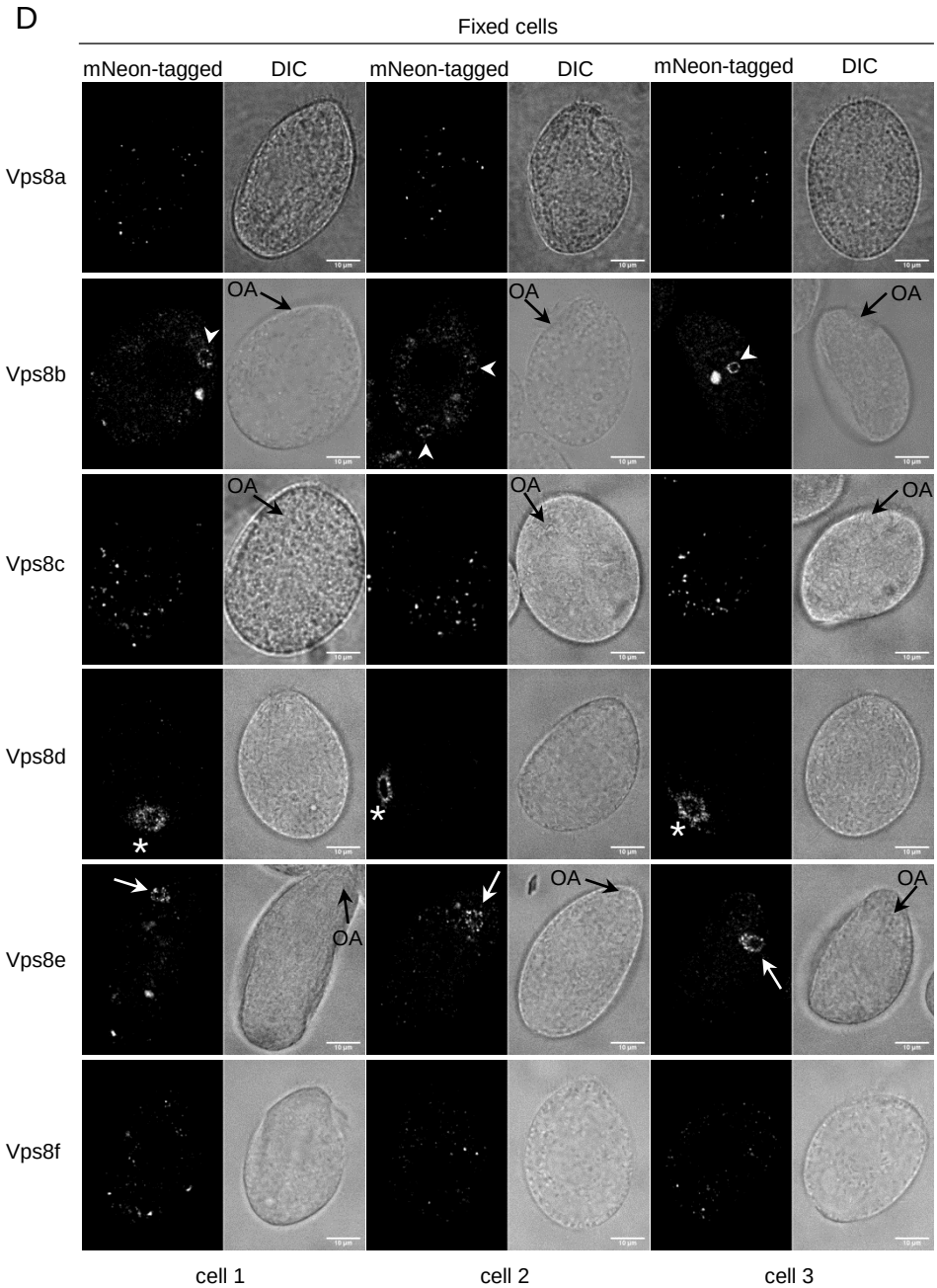
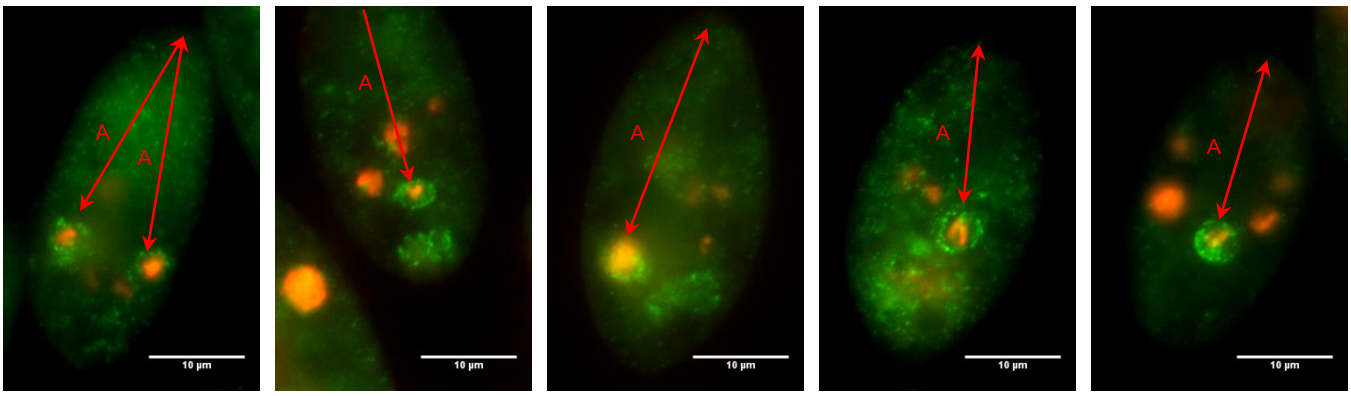


Figure S4. Related to figure 4
Images of cells expressing Vps8b- or Vps8e-mNeon, following ingestion of dsRed-*E. coli*, as in figure 4.

A



B

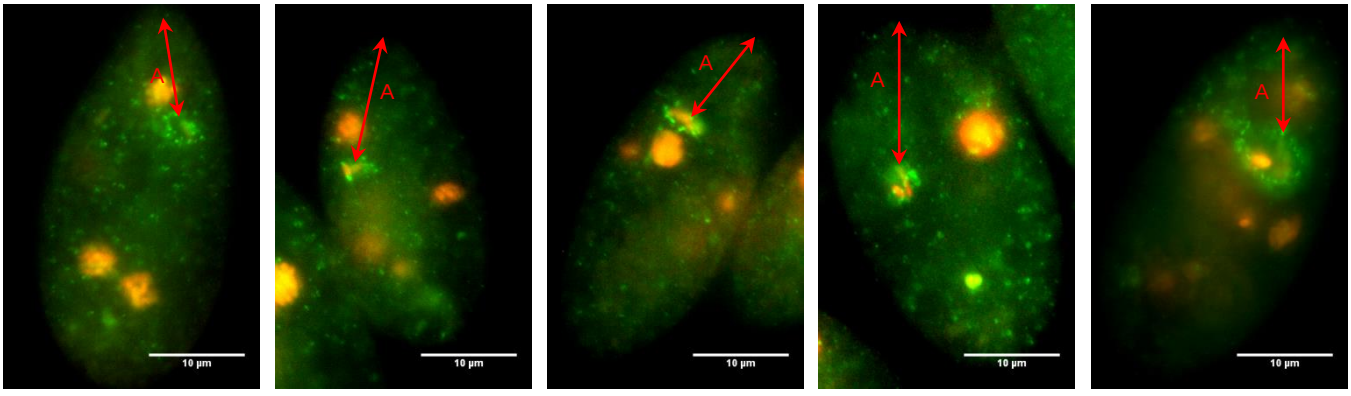


Figure S5. Related to figure 5

A) Confocal section of a fixed cell expressing Vps8e-mCherry, with paired DIC images. The white arrow indicates vesicles at the cell anterior, similar to those observed with the mNeon-tagged version. Scale bar, 10 µm. B) and C) Immunoprecipitation of 3mCherry-tagged Vps8c and Vps8e (B), and 2mCherry-tagged Vps8c (C). Cells were transformed to co-express Vps8a-mNeon together with either Vps8e-3mCherry or Vps8c-3mCherry, or to express Vps8c-2mCherry at the *VPS8A* locus in Vps8c-mNeon-expressing cells. Detergent cell lysates were incubated with anti-HA beads, and bound proteins were eluted with LDS-sample buffer. Samples were subsequently analyzed by SDS-PAGE and western blotting with anti-HA antibodies. In the 3mCherry-Vps8e sample, a band whose size corresponds to 2mCherry2HA is visible (western blot on the left), suggesting that cleavage occurred between the first and second copy of mCherry in the tag. An equivalent band is also visible in the western blot on the right. D) Immunoprecipitation of Vps8a-GFP expressed endogenously in SB281 cells. SB281 has a loss-of-function mutation in the *VPS8A* gene, and expression of the GFP-tagged copy rescues the mutant phenotype(Sparvoli et al., 2018). Detergent cell lysates were treated as in (B), and incubated with anti-GFP beads. Following SDS-PAGE and western blotting with anti-GFP antibodies, only full length protein was detected. E) Maximum intensity projections of confocal z-stacks of fixed cells expressing mNeon-tagged Vps8a (left panel), and GFP-tagged Vps8a (right panel). The DIC images represent confocal cross sections for clarity. The fluorescent puncta for each fusion protein were counted, as summarized in (F). Scale bars, 10 µm. F)Quantification of fluorescent puncta/cell measured in 15 cells/sample, using the Fiji SpotCounter plugin. Error bars represent Standard Deviations. *P*-value (*P*) was determined by two-tailed *t*-test: (n.s.) p=0.8715. Despite the fact that the Vps8a-mNeon fusion undergoes some cleavage, the number of puncta labeled by this protein is not significantly different from uncleaved Vps8a-GFP, suggesting that the distribution of Vps8a-mNeon reflects the full cellular distribution of Vps8a.

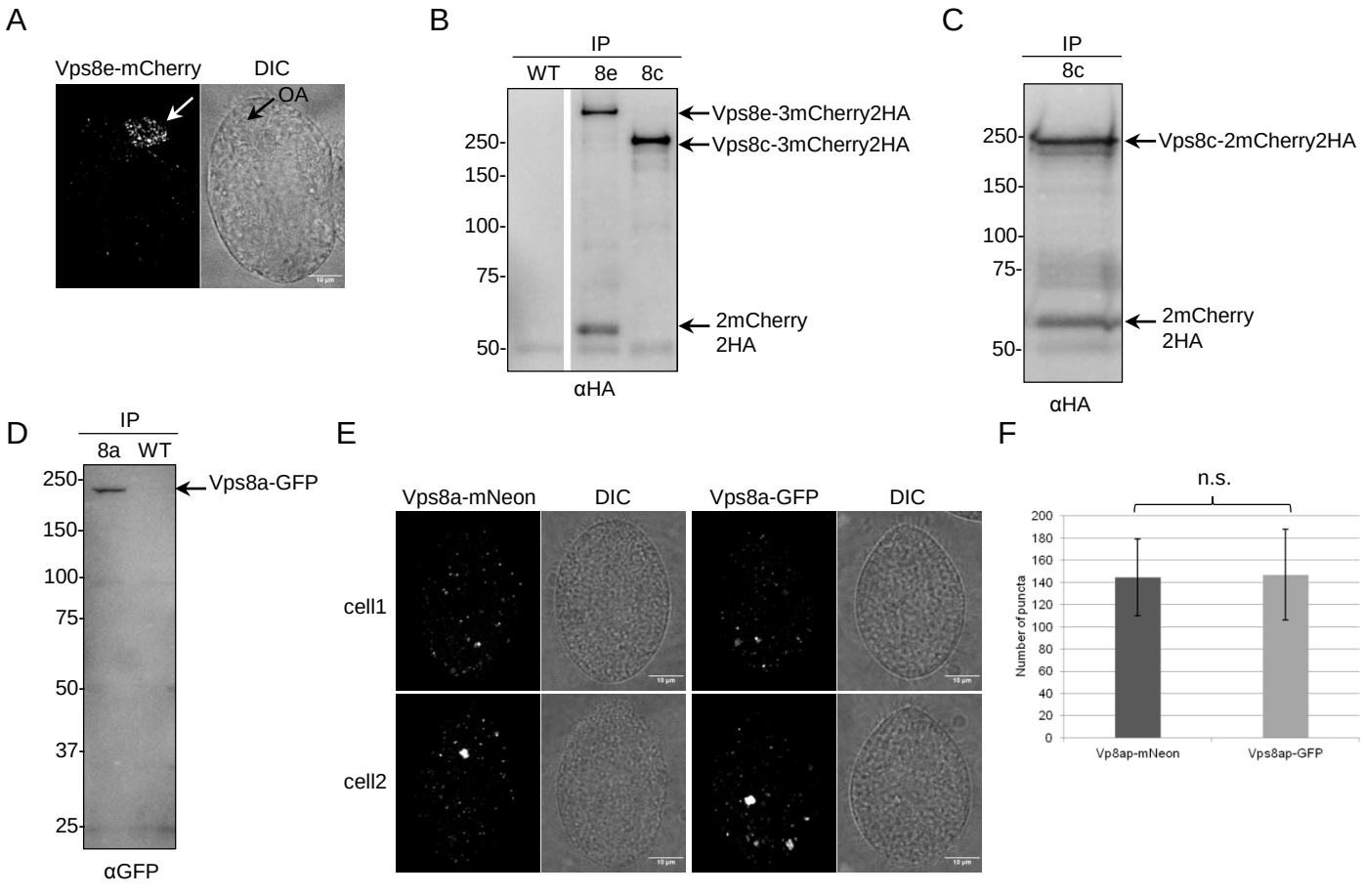
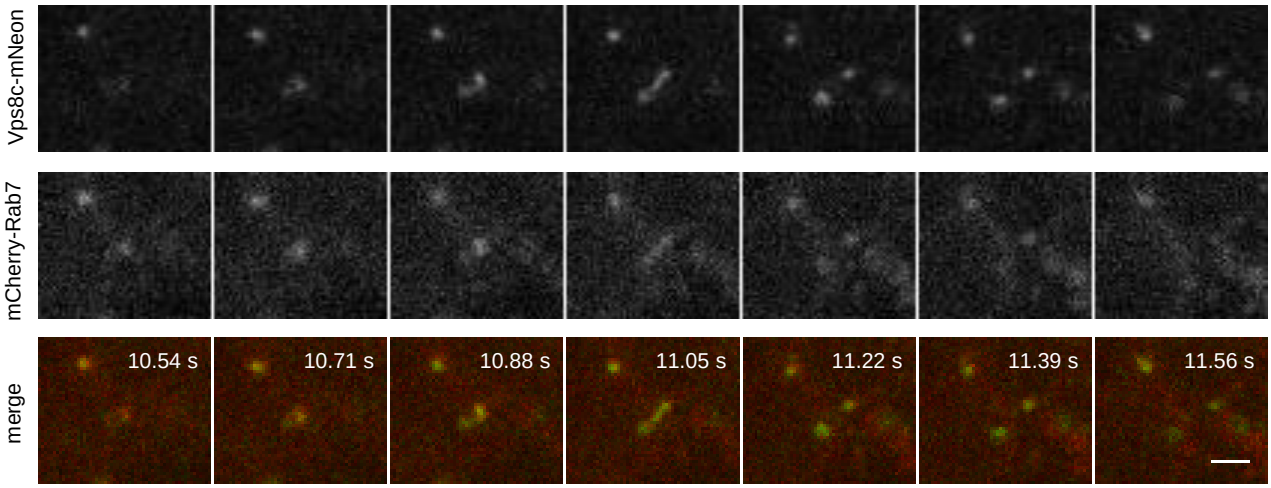


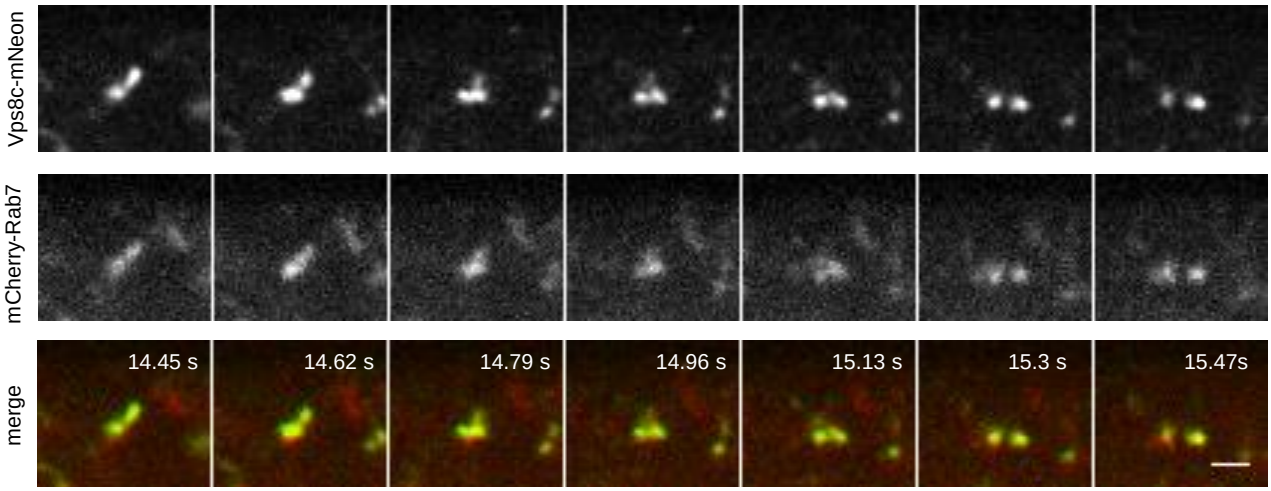
Figure S6. Related to figure 7

A) and B) Live images of selected areas of a cell co-expressing Vps8c-mNeon (top) and mCherry-Rab7 (middle)(bottom=merge). The two panels show events of Rab7/Vps8c-tubule outgrowth and apparent fission, occurring at different time points in different regions of the same cell. The sequential images for each fluorescent channel were simultaneously acquired at 0.17 sec/frame interval. The time intervals corresponding to the appearance and subsequent split of the tubulovesicular structures in the video, are indicated in white in each merged frame (see the selected frames in the full Movie 7). Scale bar, 2 μm. C) Rab4b shares the transcriptional profile of mucocyst-associated genes. The expression profile of *RAB4B* (black line) is similar to that of mucocyst-related genes, here illustrated by *VPS8A*, *SOR4*, *STX7L1*, and *APM3*. Transcription profiles were downloaded from http://tfgd.ihb.ac.cn, based on sampling from growing cultures (low Ll, medium Lm, and high Lh culture density), starvation over 24 hr (S0–S24), and time points during conjugation (C0–C18). For clarity in plotting, each trace was normalized to that gene's maximum expression level.

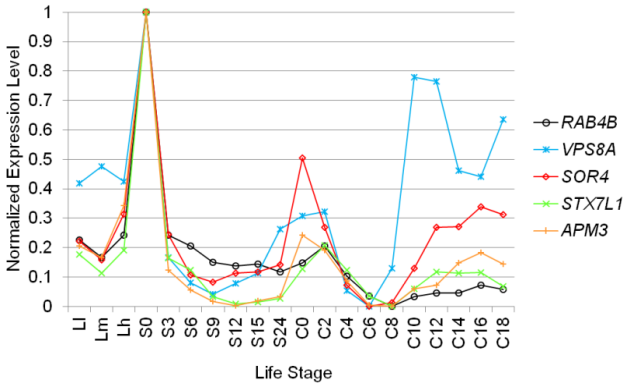
A



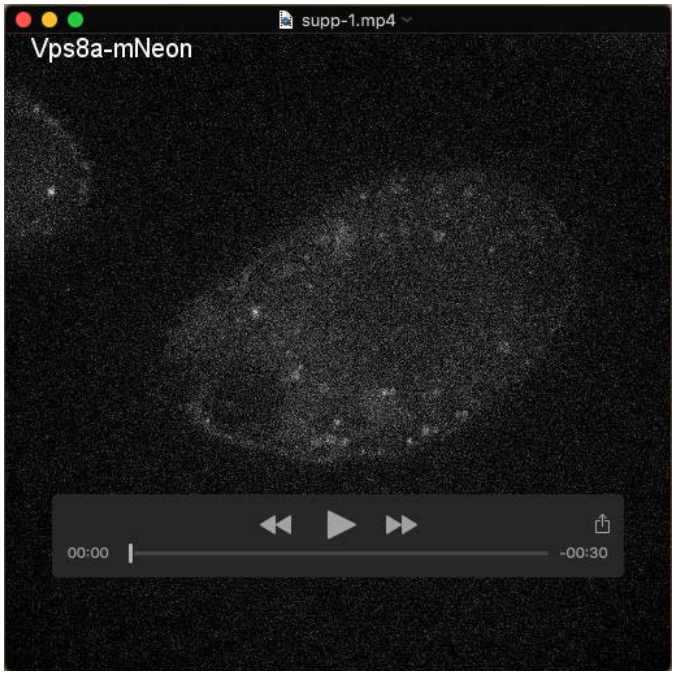
B



C

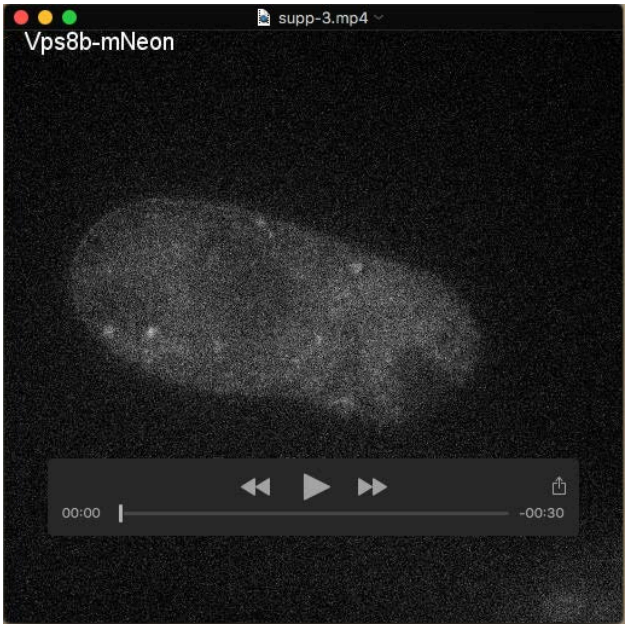


Supplemental movies



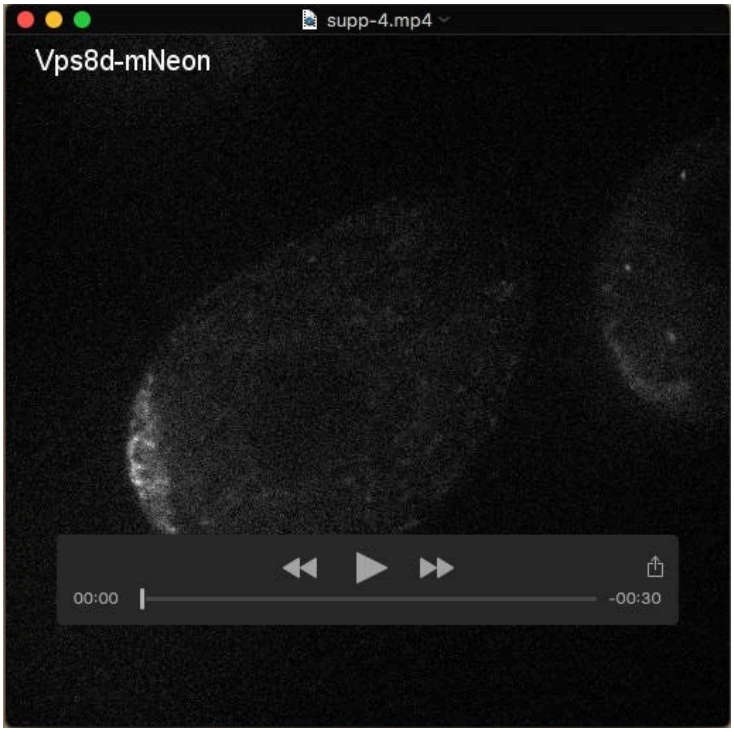
Movie 1: Vps8a-mNeon expressing cell
300 frames were collected at 0.17 sec intervals
For this movie and those below, live *Tetrahymena* cells expressing individual mNeon-tagged Vps8 paralogs at their endogenous loci, or co-expressing Vps8c-mNeon and mCherry-Rab7, were transferred to S medium for 2 h prior to imaging. For cells expressing Rab7, expression was induced by including 1 µg/ml $CdCl_2$ in the S medium. Cells were immobilized using a low melting agarose pad, and imaged with a Marianas Yokogawa confocal microscope as described in Materials and Methods. 300 and 200 frames were collected at 0.17 sec/interval for movies 1-6 and for movie 7, respectively. Movies were speeded up at 10 frames/sec. A single cell is shown in each movie. *Tetrahymena* cells are highly mobile, and difficult to fully immobilize. In movies 3 and 5, the cells can be seen spinning on their long axis within the agarose. In movies 1 and 7, the cell anterior is on the left; in movies 3, 4, 6 the anterior is on the right; in movies 2 and 5 the anterior is pointing down. Mobile vesicles are visible in the cells expressing tagged Vps8a, Vps8c and Vps8e, and less evident in cells expressing Vps8b. Cells expressing Vps8c show labeled tubulovesicular structures, visible in movies 2 and 7. In the latter, circles and squares highlight the outgrowth and fission events for which still images are shown in figure S6A, B. Localization of Vps8d to the water-pumping contractile vacuole, present near the cell posterior, can be seen in movie 3. Localization of Vps8b to large structures resembling phagosomes/food vacuoles can be seen in movie 4. Vps8f could not be clearly detected in living cells as shown in movie 6, instead, autofluorescence within food vacuoles was visible. The movies were minimally manipulated, to adjust brightness and contrast as needed to reveal the localization of the tagged proteins, whose endogenous expression levels are uniformly low

Movie 2: Vps8c-mNeon expressing cell
300 frames were collected at 0.17 sec intervals



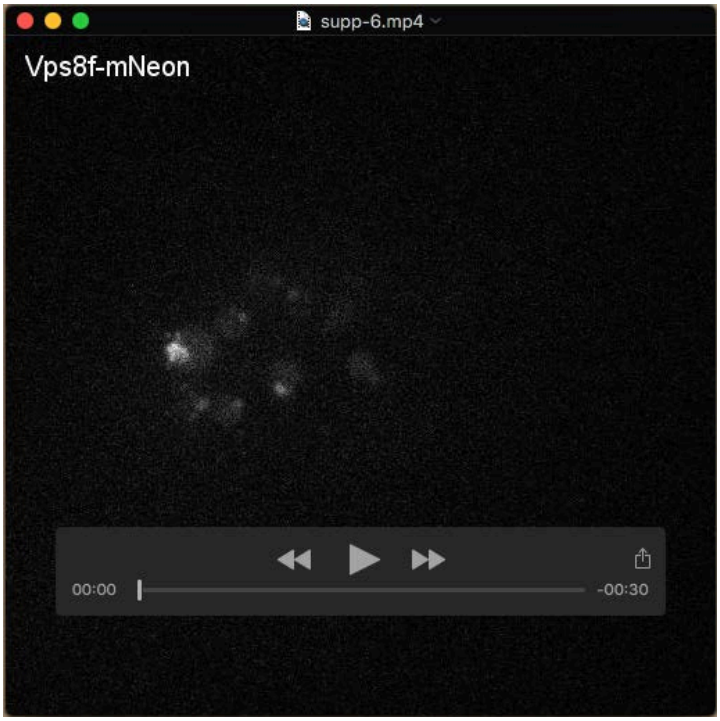
Movie 3: Vps8b-mNeon expressing cell
300 frames were collected at 0.17 sec intervals

Journal of Cell Science • Supplementary information

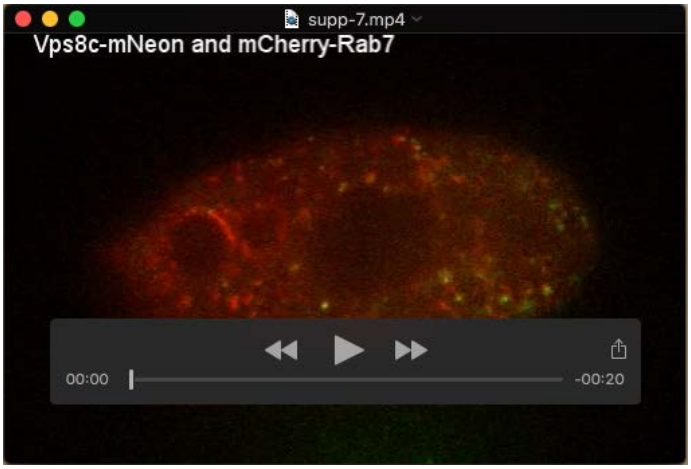


Movie 4: Vps8d-mNeon expressing cell
300 frames were collected at 0.17 sec intervals

Movie 5: Vps8e-mNeon expressing cell
300 frames were collected at 0.17 sec intervals



Movie 6: Vps8f-mNeon expressing cell
300 frames were collected at 0.17 sec intervals



Movie 7: Cell co-expressing Vps8c-mNeon and mCherry-Rab7
200 frames were collected at 0.17 sec intervals

Journal of Cell Science • Supplementary information

Table S1. Gene IDs of CORVET subunits

TTHERM_ID	Gene Name
00290710	*VPS8A*
00393150	*VPS8B*
00716100	*VPS8C*
00532700	*VPS8D*
00691590	*VPS8E*
00384890	*VPS8F*
01141590	*VPS3A*
000777289	*VPS3B*
01205260	*VPS11*
00370870	*VPS16A*
00294740	*VPS16B*
00242090	*VPS18A*
00463740	*VPS18B*
00292270	*VPS18C*
00410250	*VPS18D*
00355020	*VPS33A*
000077749	*VPS33B*

Table S2. Description of *Tetrahymena* strains used in this study

Click here to Download Table S2

Table S3. Primers used in this study

Click here to Download Table S3