SUPPLEMENTAL MATERIAL

Endosomal recycling to the surface mediated by Gpa1 and PI3-Kinase is inhibited following glucose starvation

SUPPLEMENTAL FIGURES AND LEGENDS

- Figure S1
  Segmentation and nuclear Mig1 estimates

- Figure S2
  Flow cytometry analysis focussed specifically on transformed cells

- Figure S3
  Localisation of Cos5-GFP

- Figure S4
  Apotome SIM localisation experiments

SUPPLEMENTAL TABLES

- Supplemental Table S1
  Yeast Strains used in this study

- Supplemental Table S2
  Plasmids used in this study

- Supplemental Table S3
  Raw data for bioinformatics included in Figures 2A, 2B, and 6D

SUPPLEMENTAL REFERENCES

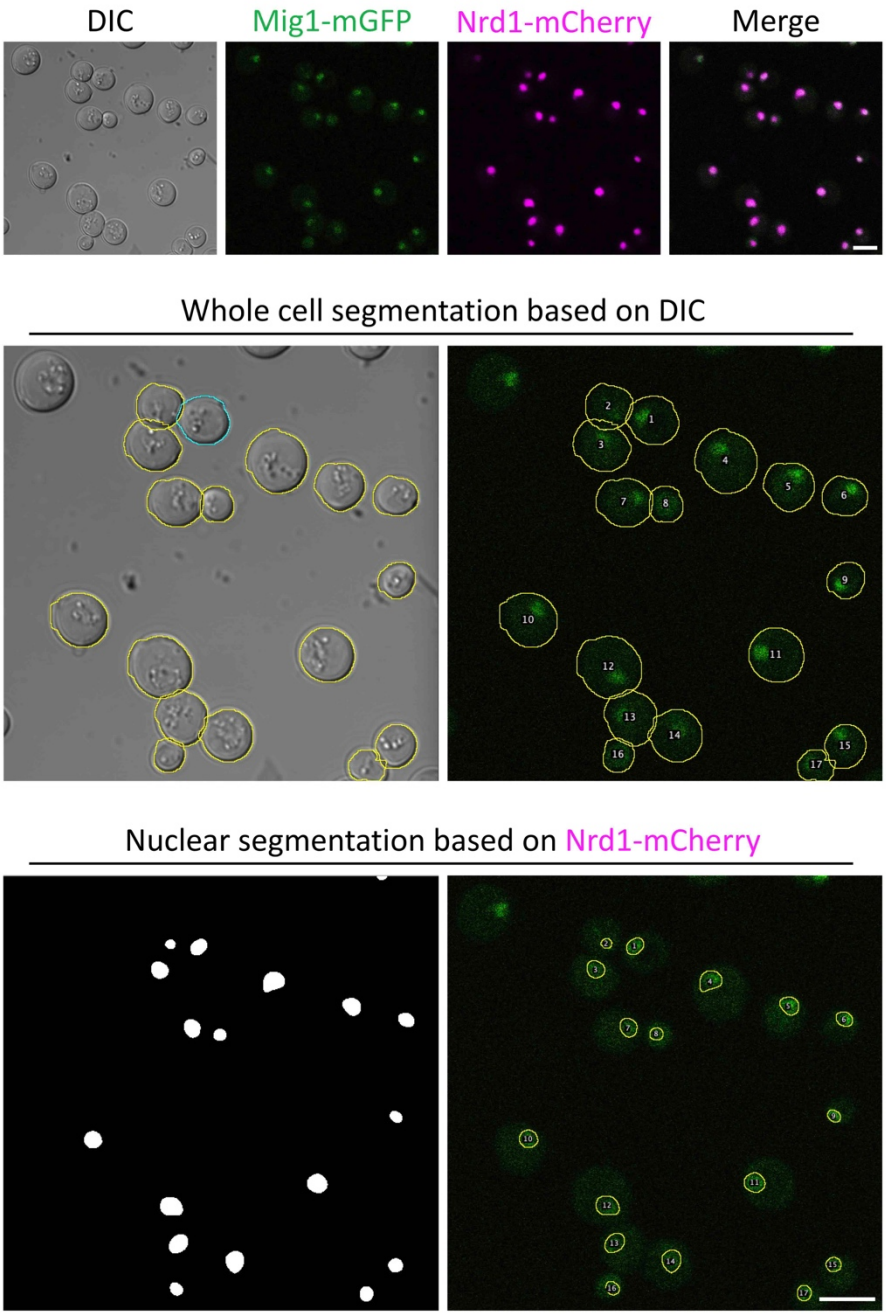

Figure S1: Segmentation and nuclear Mig1 estimates

Wild-type cells stably expressing Mig1-mGFP and Nrd1-mCherry were grown to mid-log phase in SC media containing 2% glucose (upper). Using the Cell Magic Wand Plugin (Fiji) set to roughness = 2.0 whole cells were identified from the DIC image, and these segmenated regions of interest (ROIs) were applied to the green channel image to calculate the overall Mig1-mGFP fluorescence (middle). For nucelar specific localisations, Ostu segemnattion was applied to the red Nrd1-mCherry channel to create nuclear ROIs that were then applied to the same Mig1-mGFP fluorescence cannel. Percentage Mig1-mGFP nuclear / total fluorscence was calculated for individual cells across multiple imaging experiemnts (n = 3). The same process was also performed for cells grown in SC media containing raffinose instead of glucose. Scale bar, 5 μM.

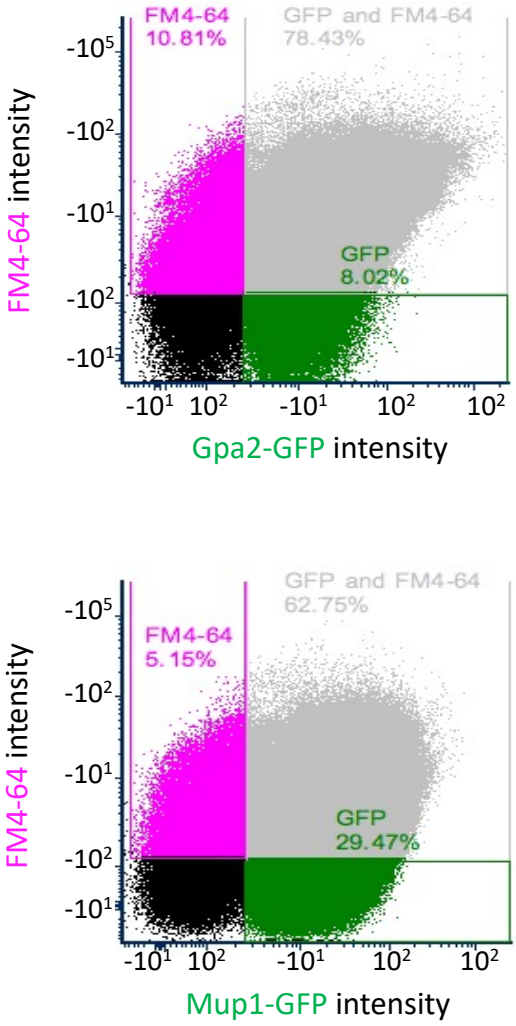

Figure S2: Flow cytometry analysis focussed specifically on transformed cells

Wild-type cells expressing either Gpa2-GFP (lower) or Mup1-GFP (lower) were grown to mid-log phase before preparation for FM4-64 efflux assays (see methods). Briefly, cells were loaded with FM4-64 dye, before excess dye was washed with ice cold media. Flow cytometry measurements of cells upon a return to room temperature media was recorded and gates set to only calculate FM4-64 fluorescence from cells also co-expressing either Gpa2-GFP or Mup1-GFP. A decrease in fluorescence is plotted in Figure 7C, calculated from the mean fluorescence from the first 10 seconds of recording, considered 100%, and then applied to all subsequent measurements over the 10-minute period of continuous flow / measurements.

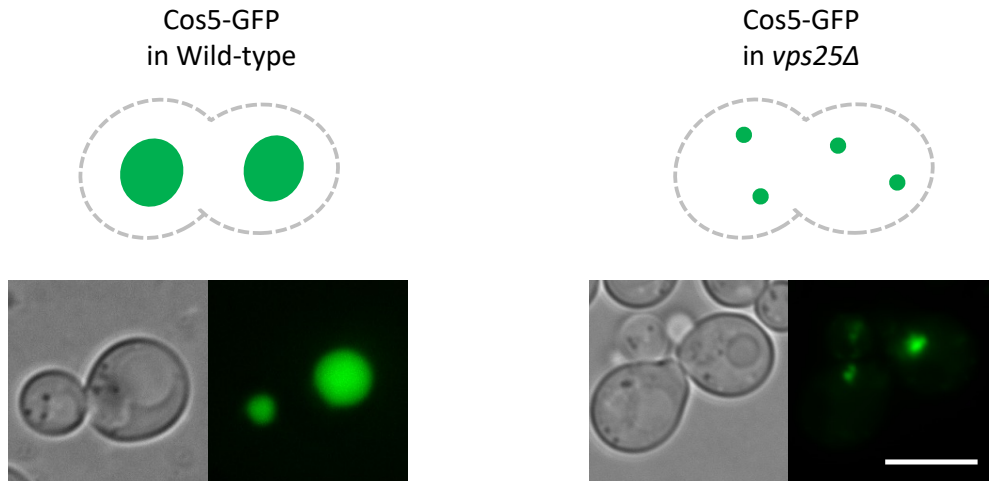

Figure S3: Localisation of Cos5-GFP

Cos5-GFP was expressed from the TDH3 promoter in either wild-type cells (left) or in *vps25*Δ mutants, that are defective in MVB sorting and accumulate cargoes in aberrant endosomes (right). Scale bar, 5 μM.

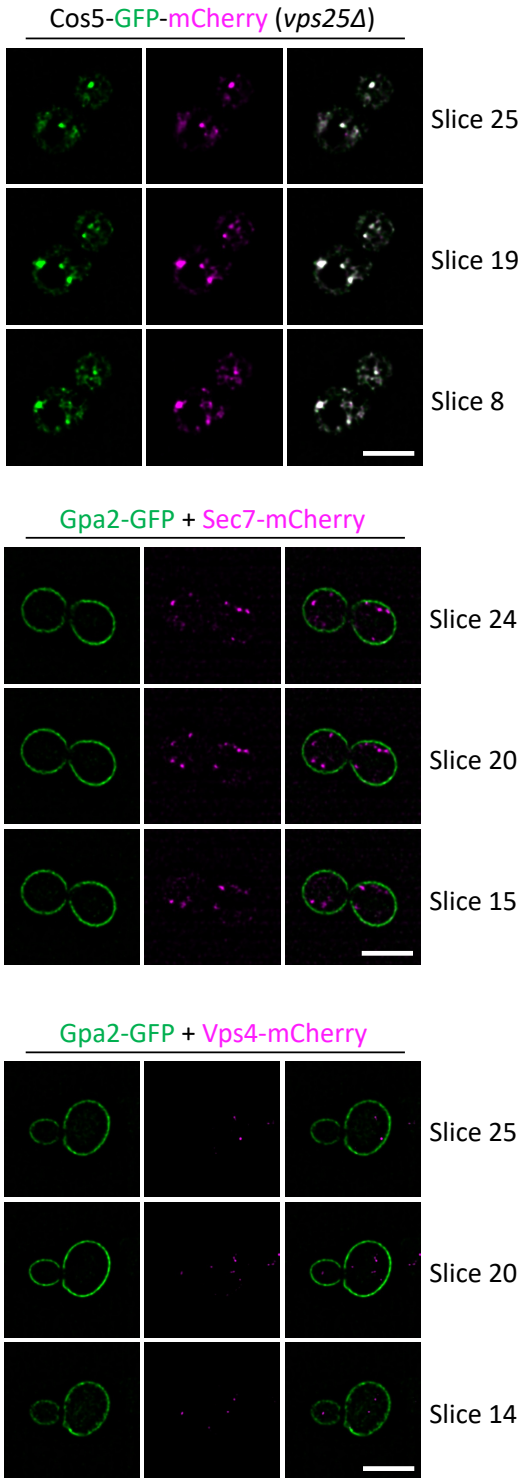

Figure S4: Apotome SIM localisation experiments

4D Apotome SIM was achieved across 42 z-stacks (distance 0.126µm) repeated over 100 time slices, each of 4.3 seconds with no interval period. This approach was used to image: a dual tagged version of Cos5, carrying both GFP and mCherry at the C-terminus was expressed in *vps25Δ* (upper), and wild-type cells co-expressing Gpa2-GFP with either Sec7-mCherry (middle) or Vps4-mCherry (lower). Scale bar, 5 µM.

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