Supplemental Materials

Molecular Biology of the Cell

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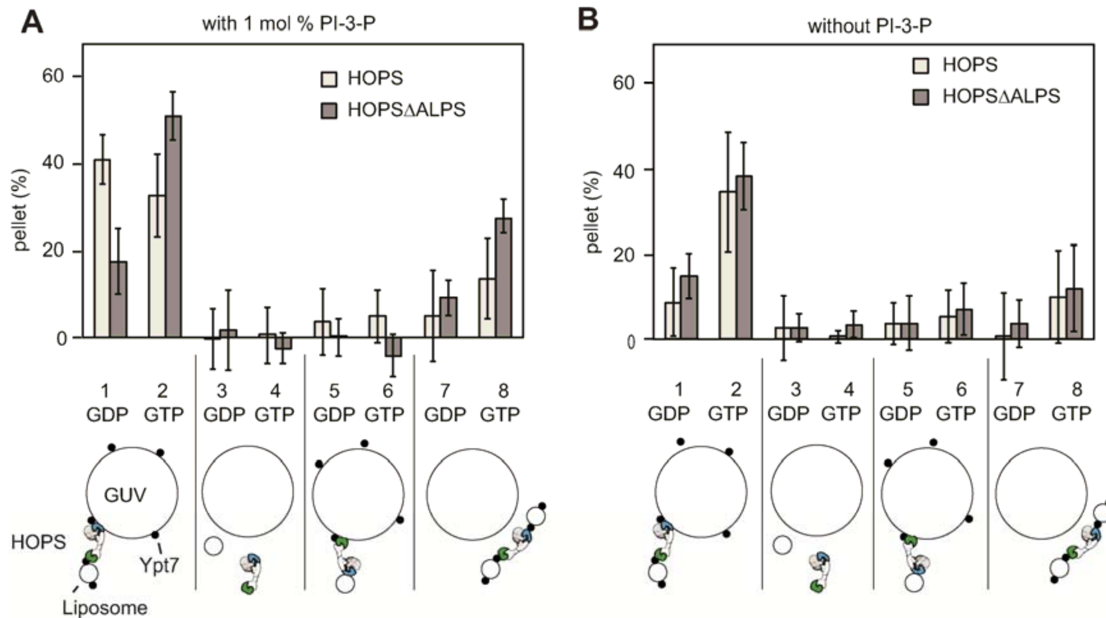

Figure 2 - Figure supplement 1

Analysis of small and large liposomes in HOPS-mediated tethering.

(A, B) HOPS-dependent tethering needs Ypt7 on both membranes. Assays were performed with small (SUV, liposomes) and giant unilamellar vesicles (GUV) as indicated in the cartoons underneath the charts. When liposomes or GUVs carried DOGS-NTA, Ypt7 (indicated as a black dot) could bind and recruit HOPS as illustrated. HOPS may also bind just to liposomes to tether them independently of bound Ypt7. The following concentrations were used: 0.25 mM GUVs, 0.125 mM liposomes and 250 nM wild-type HOPS (light gray bars) or HOPSΔALPS (dark gray bars). In lanes 1 and 2, GUVs and liposomes carried Ypt7-GDP or GTP, whereas in columns 5-8 either GUVs or liposomes had Ypt7

on their surface. After incubation of GUVs and liposomes with HOPS and Ypt7 and brief centrifugation (3 min, 1000 *g*) the remaining fluorescence in the supernatant was measured. To determine the apparent clustering of liposomes with GUVs, the signal for liposome-liposome tethering was subtracted from the GUV-liposome tethering signal. In part **(B)**, PI-3-P was not included, when GUVs and liposomes were made. Error bars are representative for n=3 experiments.

Figure legends (Source data)

Figures 1C-F; Figures 2B-E; Figures 5D,E:

Tethering was measured at different times by pelleting liposomes as described. The signal in the supernatant was measured in a fluorimeter and set relative to the total fluorescence signal of the reaction, which was determined in parallel. Values of the signal in the supernatant of each reaction were added up and the mean was calculated, resulting one measurement for one tethering event (left part of source file). The total measurements equal n=3. Based on the signal in the supernatant, the amount of pelleted liposomes was determined including the standard deviation (right part of Figure).

Figure 2 – supplement 1 A,B

After incubation of GUVs and liposomes with HOPS and Ypt7 and brief centrifugation (3 min, 1000 *g*) the remaining fluorescence in the supernatant was measured. GUV+ indicates that GUVs carried Ypt7 due to the presence of DOGS-NTA, Lip+ indicates that liposomes carried Ypt7. The wild-type HOPS and HOPSΔALPS are shown next to each other with their three measurements, out of which the standard deviation was calculated. To determine the apparent clustering of liposomes with GUVs, the signal for liposome-liposome tethering was subtracted from the GUV-liposome tethering signal, which is shown on the right side of the figure. In part (E), PI-3-P was not included, when GUVs and liposomes were made (in the source data labeled as without PI-3-P). Error bars are representative for n=3 experiments.

Figures 2E

OD_{400} values of three independent *vps11-1* vacuole fusion reactions of HOPS wild-type and the corresponding mutants were determined after the reaction. The data of three replicates is shown.