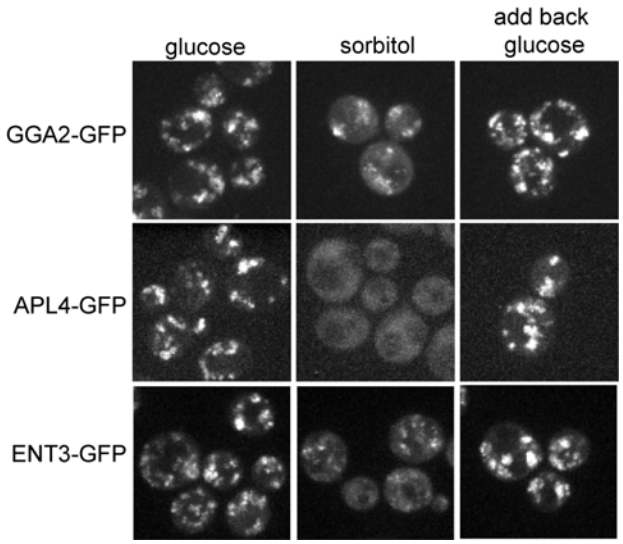
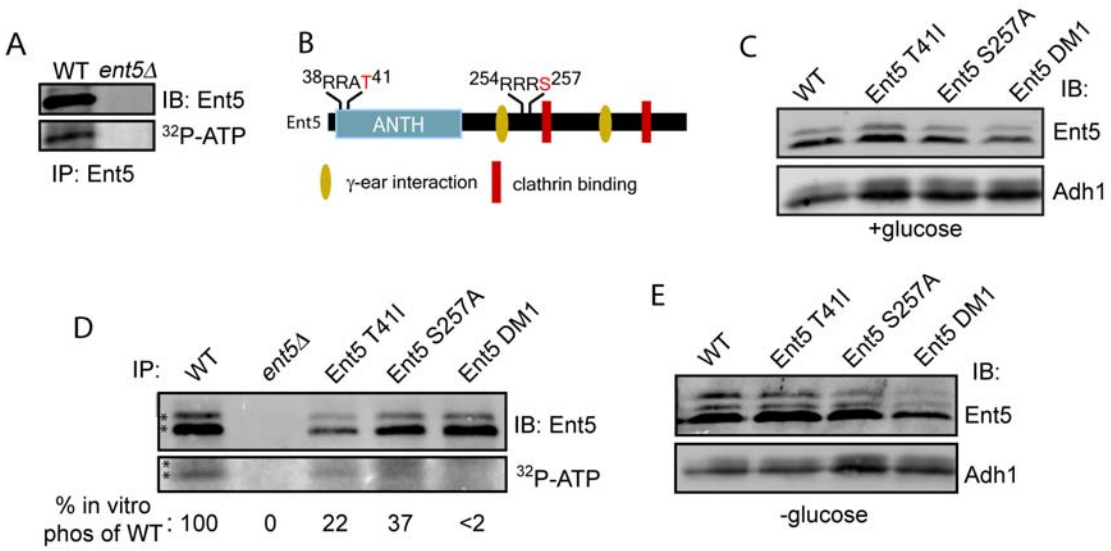
Supplemental Figure 1



Supplemental Figure 1. Glucose regulates localization of all adaptors. Fluorescence microscopy of adaptors in cells expressing Gga2-GFP (DLY4), the AP-1 subunit Apl4-GFP (DLY36) or Ent3-GFP (DLY6). Cells were grown in the presence of glucose for 2 hours then transferred to water with 2% glucose (Left) or into 2% sorbitol (Center) or first washed into 2% sorbitol for 15 minutes and then into 2% glucose (Right).

Supplemental Figure 2



Supplemental Figure 2. Ent5 is a substrate for PKA. (A) Ent5 was immunoprecipitated from wt or *ent5Δ* cells (GPY2731) and incubated with bovine PKA and ^{32}P-ATP. The samples were then subject to SDS-PAGE, dried, and exposed to a phosphorscreen (bottom panel). Alternatively, immunoprecipitated samples were probed with Ent5 antibody (top panel). (B) Ent5 contains two consensus PKA phosphorylation (RRxS/T) sites. The putative phosphorylated residue is indicated in red. (C) Expression of Ent5 consensus site mutants. Immunoblot of Cell lysates of wt, *ENT5 S257A* (DLY20), *ENT5 T41I* (DLY19), or double mutant *ENT5 DM1* (DLY21) in the presence of glucose. (D) Ent5 can be phosphorylated on both consensus sites. *In vitro* phosphorylation of Ent5 mutants. Cells expressing *ENT5 T41I*, *ENT5 S257A*, or double mutant *ENT5 DM1* were immunoprecipitated and assayed as in (A). (E) Neither PKA consensus sites contribute significantly to Ent5 hyperphosphorylation in the absence of glucose. Immunoblot of Cell lysates from glucose-starved cells expressing Ent5 PKA mutants *ENT5 S257A*, *ENT5 T41I*, or double mutant *ENT5 DM1*.