File S1

Supporting Materials and Methods

Cell Lysates and Protein Complexes Purification

Ypt1, Ypt31 and Ypt32 proteins expressed in bacteria were purified as described previously (Jones *et al.*, 2000); protein concentration was ~0.5 mg/ml. Yeast cell extracts and purified protein complexes were prepared and analyzed as described previously (Morozova *et al.*, 2006). For purifying TRAPP complexes, cells were grown in SD-Ura medium overnight, and then diluted into SD-Ura-Leu media at OD_{600} of 0.2 to grow overnight. Cells were re-inoculated into 1L medium at OD_{600} of 0.2 to cell OD_{600} of 0.8, and then yeast cells expressing GST-Bet5, GST-Trs85, and GST under the CUP1 promoter were induced with 0.25 mM $CuSO_4$ for 6 h at 26°C. Cell breakage buffers were supplemented with an EDTA-free protease-inhibitors cocktail (Roche Diagnostics). Protein concentrations were determined by a Bio-Rad protein assay (Bio-Rad, Hercules, CA). Ten micrograms of yeast whole cell lysates or 0.2 µg GST-associated complexes were loaded on 10% SDS-polyacrylamide gel electrophoresis (PAGE). Gels were run, and proteins were transferred to polyvinylidene difluoride membranes and subjected to immuno-blot analysis.

GDP Release Assays

GDP release assays were performed as described previously (Morozova *et al.*, 2006). GST-, GST-Bet5-, or GST-Trs85-associated complexes were purified from yeast cell lysates and added as a source of GEF to GDP-release assays with bacterially purified Ypt1, Ypt31 or Ypt32 loaded with ^{3}H-GDP.

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