Supplemental Supporting Information

Supporting Materials and Methods

Immunofluorescence (IF) was performed using standard procedures (1). Specifically, yeast cells were fixed with 4% (w/v) formaldehyde and were digested with Zymolyase 100T (MP Biomedicals, Solon, OH). After digestion, the cells were placed on polylysine-treated cover slips and were exposed to an anti-hemagglutinin (HA) monoclonal antibody (Covance, Berkeley, CA) for 30 min, followed by incubation for 30 min with an anti-mouse antibody-fluorescein isothiocyanate (FITC) conjugate (Invitrogen, Carlsbad, CA). Cells were visualized using a Nikon Eclipse 80i microscope with a 60X oil immersion objectives. Fluorescent images were acquired using a digital camera and SPOT software (Diagnostics Instruments, Inc., Sterling Heights, MI).

1. Pringle, J. R., Adams, A. E. M., Drubin, D. G. & Haarer, B. K. (1991) *Methods Enzymol* **194,** 565-602.

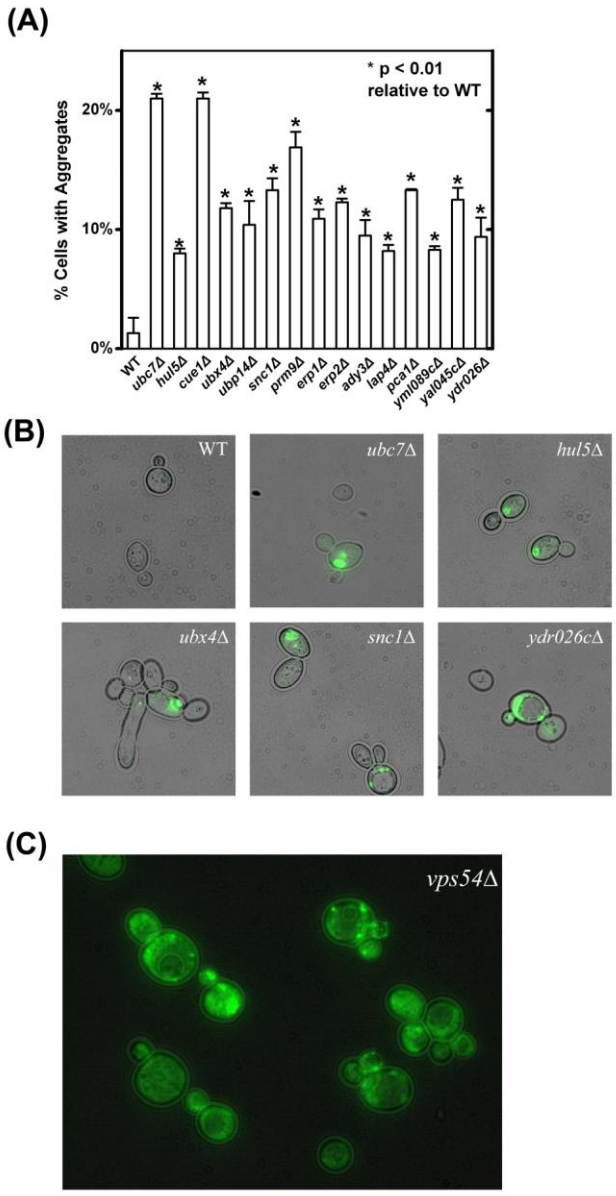

Figure S1. The effect of gene deletions on TPMT*3A-GFP degradation and aggregation in isolated yeast mutants that displayed enhanced fluorescence intensity is shown. (*A*) Increased frequency of TPMT*3A-GFP aggregate formation in *ubc7*Δ, *hul5*Δ, *cue1*Δ, *ubx4*Δ, *ubp14*Δ, *snc1*Δ, *prm9*Δ, *erp1*Δ, *erp2*Δ, *ady3*Δ, *lap4*Δ, *pca1*Δ, *yml089c*Δ, *yal045c*Δ and *ydr026c*Δ strains. A total of 200 cells were counted in each experiment. (*B*) TPMT*3A-GFP was expressed in the parental yeast strain BY4743 (WT) and five gene deletion strains (*ubc7*Δ, *hul5*Δ, *ubx4*Δ, *snc1*Δ and *ydr026c*Δ) that exhibited enhanced TPMT*3A-GFP aggregation on the basis of flow cytometry studies. (*C*) TPMT*3A-GFP formed micro-aggregates in *rpn10*Δ, syn8Δ, *erv46*Δ, *pex22*Δ, *vps54*Δ, *snx4*Δ, *cne1*Δ and *bud14*Δ gene deletion strains. TPMT*3A-GFP aggregates were visualized with epifluorescence microscopy.

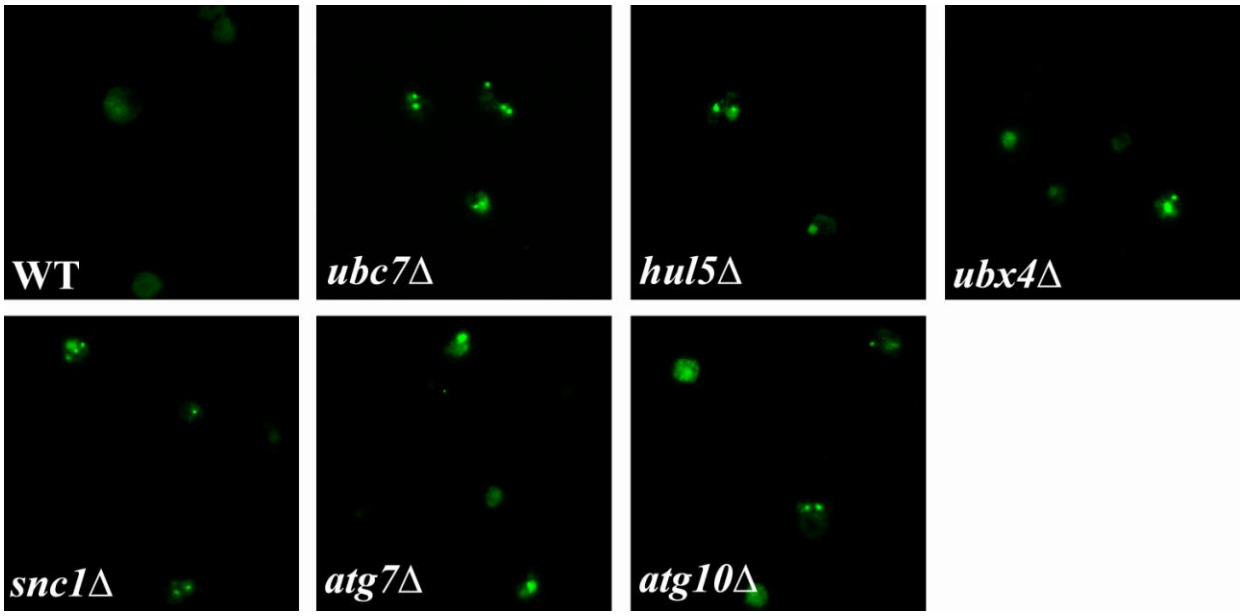

Figure S2. TPMT*3A aggregation in yeast mutants selected for study was not an artifact related to GFP fusion. Immunofluorescence of yeast WT and mutant cells expressing hemagglutinin (HA)-tagged TPMT*3A was performed with anti-HA antibody, followed by an FITC-conjugated anti-mouse secondary antibody. Inclusions were similar in number, size and distributions to those observed with the TPMT*3A-GFP fusion construct.