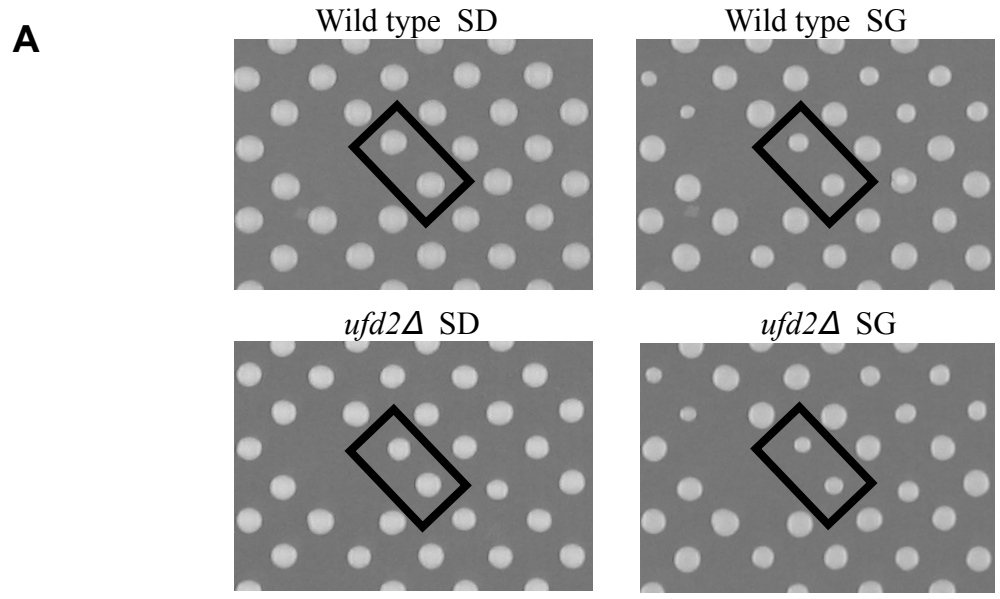
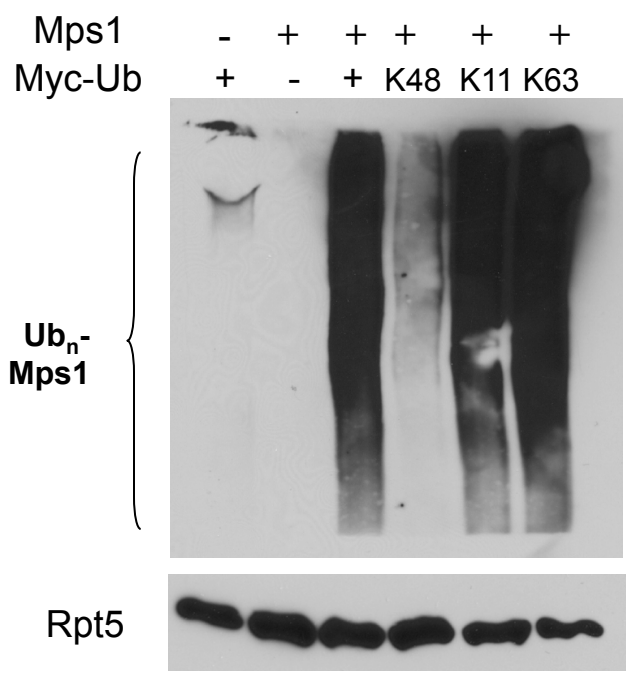
Supplementary Information

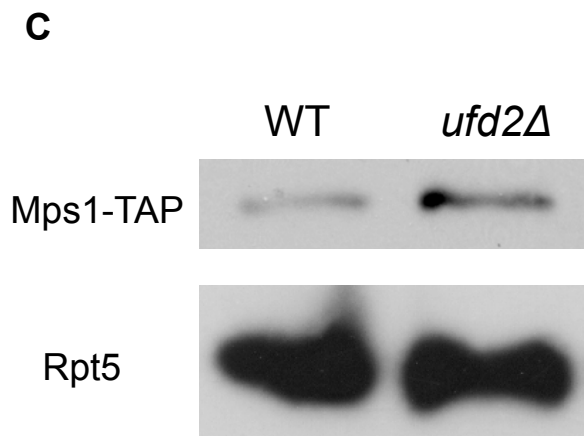
Figure S1. **Mps1 is tied to the Ufd2 pathway.** *A,* Representative images from the plasmid overexpression screen. Each of ~5280 yeast ORFs regulated by the *GAL1* promoter was transformed separately into wild-type Y8835 and *ufd2Δ* cells. Transformants were grown on media containing either glucose (SD, expression off) or galactose (SG, expression on). Each ORF is represented by two spots on the plate to reduce false positives. After 2 days for SD plates and 3 days for SG plates, colony sizes were scored for possible hits. Boxed spots are *MPS1* on each plate. Strains and growth conditions are labeled at the top of each image. *B,* Mps1 ubiquitylation requires Lys-48 for Ub chain synthesis. GST-His6-tagged Mps1 was co-transformed with the plasmid expressing Myc-tagged Ub alleles into wild-type cells. Mps1 ubiquitylation pattern was detected as described in Figure 2C. Loading was ascertained by anti-Rpt5 blot. *C,* endogenous Mps1 levels in unsynchronized wild-type and *ufd2Δ* cells. Unsynchronized yeast cells were grown to similar densities and harvested for protein extraction. Immunoblot analysis of endogenously expressed Mps1-TAP was performed as described in Figures 1 and 3. *D,* Mps1 overexpression promotes chromosome maintenance. Moderate Mps1 expression was achieved with a yeast 2 micron-containing high copy plasmid pE512 that bears Mps1 under the regulation of its own endogenous promoter. The plasmid bearing Mps1 and the vector plasmid were transformed to wild-type cells. The sectoring assay was done as described in Figure 4C.

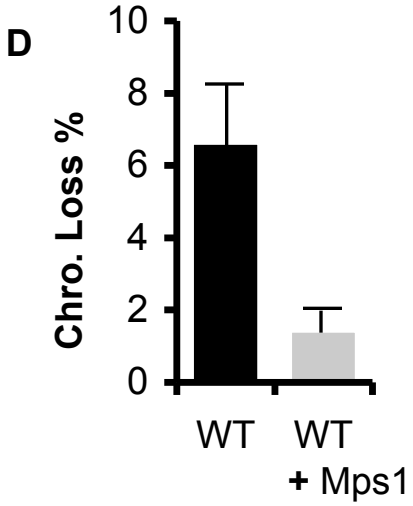
Figure S2. **UFD2a-deficiency causes mitotic delay in NB-1 cells.** SH-SY5Y (SH), NB-1 (NB) cells were treated with monastrol, and after 0, 15, 30, 45, or 60 min post release from monatsrol mitotic cells were characterized as having monopolar, metaphase, or anaphase spindles, or as intermediates in the recovery from monastrol. The bar graph represents the percentage of each phenotype at 0, 15, 30, 45, or 60 min post release from monastrol, error bars representing standard deviation of triplicate samples where at least 100 mitotic cells were scored per replicate. Images above the bar graph show examples of each phenotype; blue, DNA (Hoechst); green, microtubules (α-Tubulin); red, centrosomes (γ-Tubulin); bar = 5 μm.

Supplementary Fig. S1









Supplementary Fig. S2