Wu et al., https://doi.org/10.1083/jcb.201702125

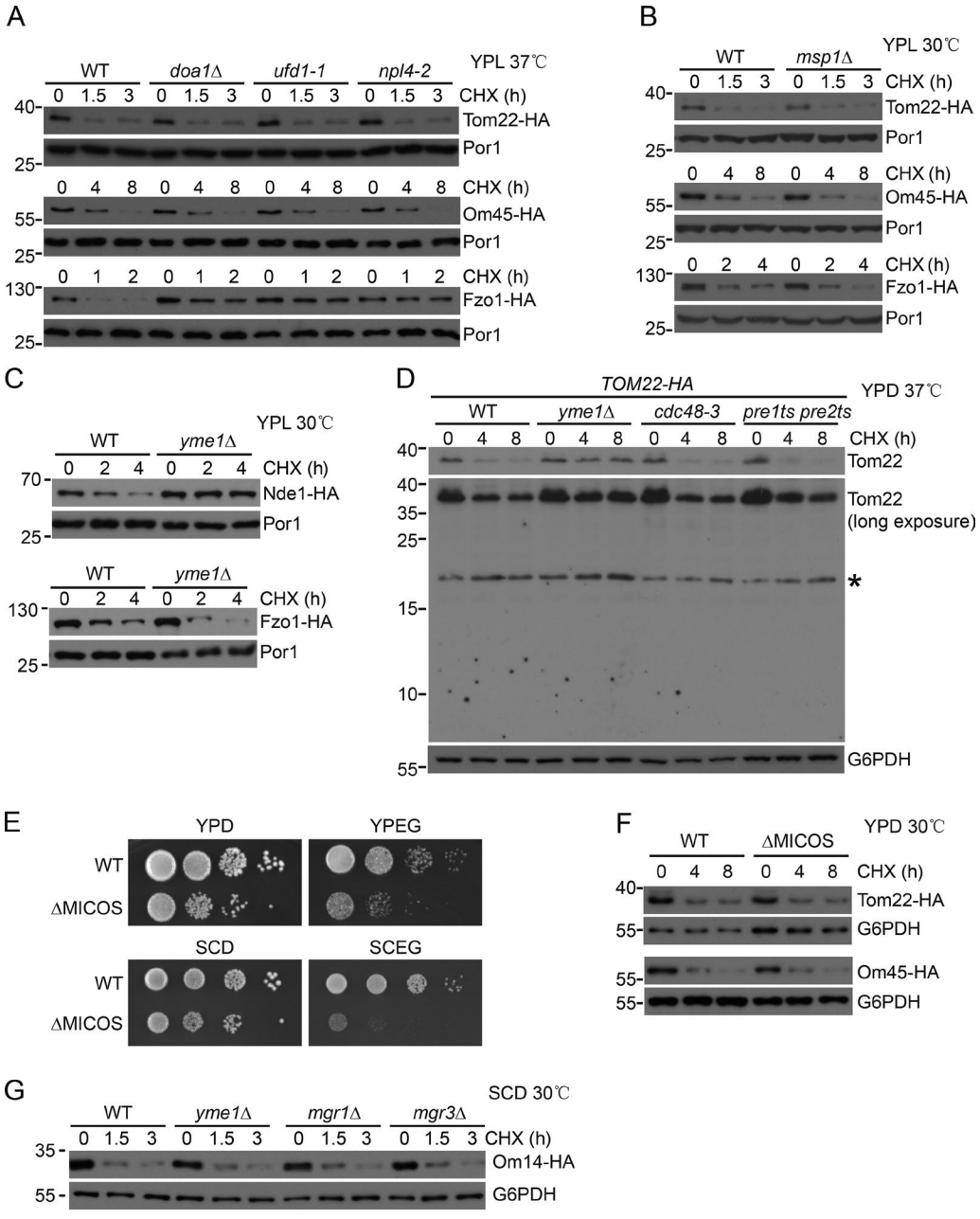

Figure S1. **Degradation of Tom22-HA and Om45-HA was not affected by inhibiting the function of the Doa1-Cdc48$^{Ufd1-Npl4}$ complex or the proteasome, by deletion of *MSP1*, or by deletion of the MICOS complex.** (A) The *TOM22-HA*, *OM45-HA*, and *FZO1-HA* strains in WT, *doa1Δ*, *ufd1-1*, or *npl4-2* background were grown in lactate media (YPL) to log phase at 25°C and then treated with CHX at 37°C (restrictive temperature). *ufd1-1* and *npl4-2* are ts mutations of the Cdc48 adapters Ufd1 and Npl4, respectively. (B) The *TOM22-HA*, *OM45-HA*, and *FZO1-HA* strains in WT or *msp1Δ* backgrounds were grown in lactate media (YPL) to log phase and then treated with CHX and collected at the indicated time points. (C) The *NDE1-HA* and *FZO1-HA* strains in WT or *yme1Δ* backgrounds were treated similarly as in B. (D) The *TOM22-HA* strains in WT, *yme1Δ*, *cdc48-3*, or *pre1ts pre2ts* background were similarly treated as in A. Cell lysates were probed with an antibody recognizing an epitope in the cytoplasmic domain of Tom22. The asterisk indicates nonspecific bands recognized by the antibody. (E) The WT and ΔMICOS (*mic12Δ mic26Δ mic27Δ mic19Δ mic60Δ mic10Δ*) strains were grown in glucose media to log phase and then spotted on glucose (YPD or SCD) or ethanol and glycerol (YPEG or SCEG) plates in a 10-fold serial dilution and then incubated for 2–5 d at 30°C. (F) The *TOM22-H* and *OM45-HA* strains in WT or ΔMICOS background were grown in glucose media (YPD) to log phase and then subject to CHX treatment. (G) The *OM14-HA* strains in WT, *yme1Δ*, *mgr1Δ*, or *mgr3Δ* backgrounds were grown in glucose media (SCD) to log phase and then subject to CHX treatment. Molecular masses are shown in kilodaltons.

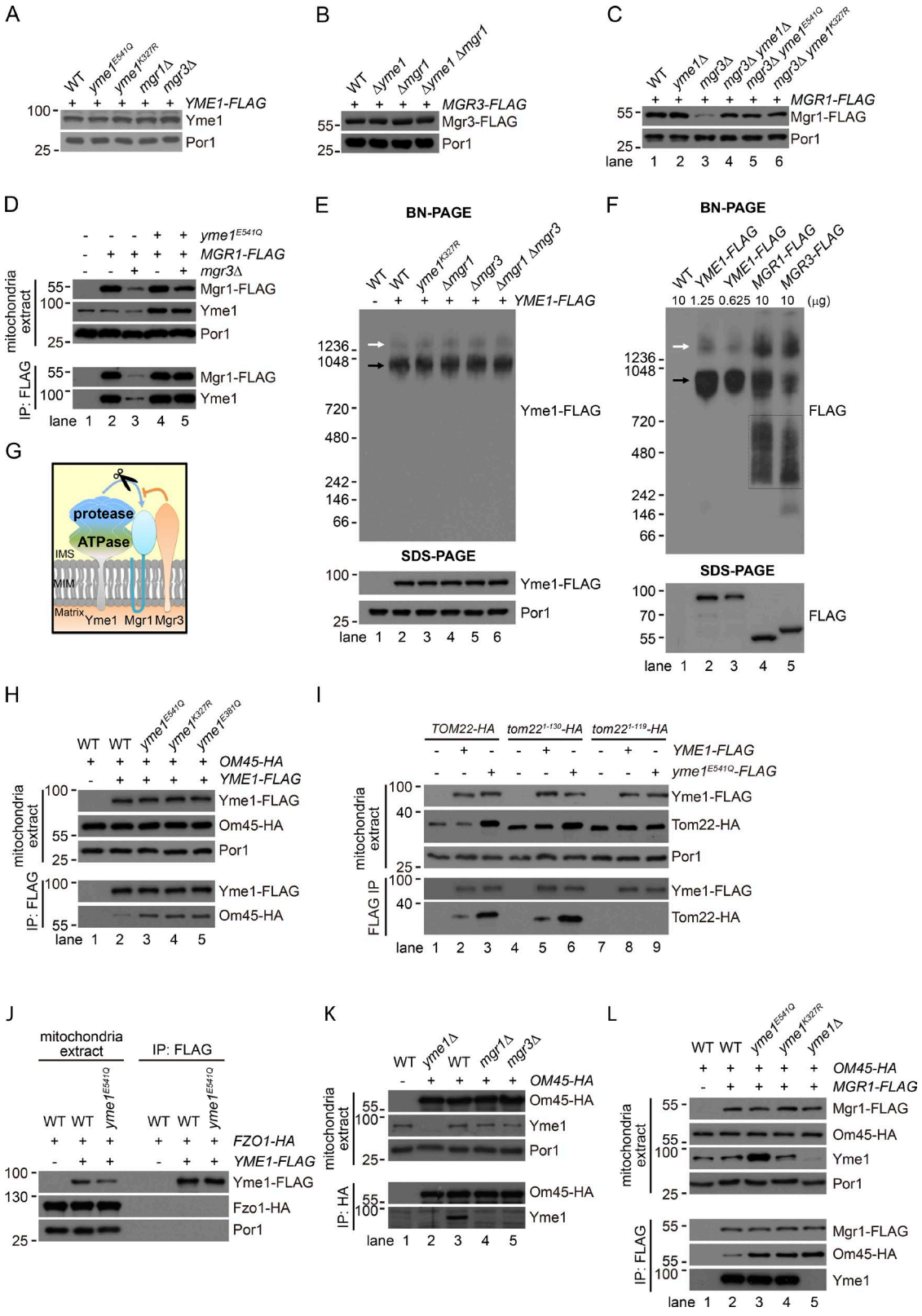

Figure S2. **Characterization of the Yme1$^{-Mgr1-Mgr3}$ complex and IP analysis of substrate interaction with the Yme1 complex.** (A–C) Whole-cell lysates from the indicated WT and mutant strains were analyzed by SDS-PAGE. (D) Digitonin-solubilized mitochondrial extracts from the indicated WT and mutant strains were subject to anti-FLAG IP and analyzed by SDS-PAGE. (E and F) Digitonin-solubilized mitochondrial extracts from the indicated WT and mutant strains were analyzed by BN-PAGE and SDS-PAGE. The major and minor Yme1-containing complexes are pointed by black and white arrows, respectively. The loading amounts of mitochondrial lysates in F were adjusted as indicated. (G) Working model of the Yme1$^{-Mgr1-Mgr3}$ complex. (H–L) Digitonin-solubilized mitochondrial extracts from the indicated WT and mutant strains were subject to anti-FLAG or anti-HA IP and analyzed by SDS-PAGE. In H, I, and L, 5 µl out of 100 µl immunoprecipitates were loaded for the anti-HA blots. In J and K, 20 µl out of 100 µl immunoprecipitates were loaded for the anti-HA, anti-Por1 (J), and anti-Yme1 (K) blots.

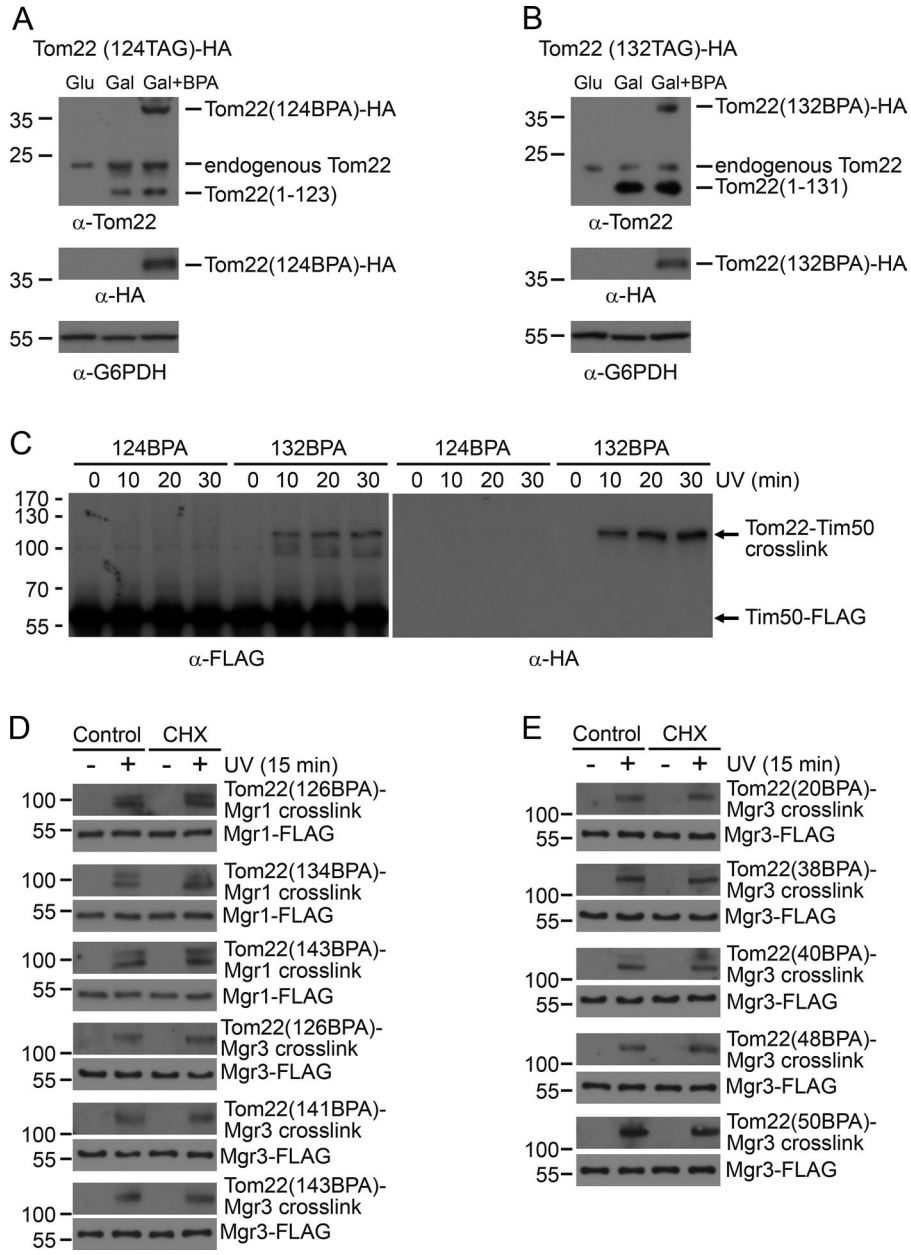

Figure S3. **Validation of photo cross-linking between Tom22 and Tim50 and photo cross-linking of Tom22 (BPA)-HA with Mgr1 and Mgr3 in the presence or absence of CHX.** (A and B) WT strains were transformed with two plasmids to express Tom22-HA with an amber (TAG) codon at site 124 or 132 under the control of the repressible GAL1 promoter. Cells were grown in repressive glucose media (Glu) to log phase and then switched to inducing galactose media in the absence or presence of BPA (Gal or Gal + BPA) for 16–18 h. Whole-cell extracts were analyzed by SDS-PAGE using anti-Tom22, anti-HA, and anti-G6PDH antibodies. Endogenous Tom22, truncated forms of Tom22 (Tom22 [1–123] or Tom22 [1–131]), and full-length Tom22-HA with BPA incorporated at sites specified by the amber codon are indicated. (C) The *TIM50-FLAG* strains expressing Tom22-HA with BPA incorporated at sites 124 or 132 were irradiated with UV for the indicated time periods. Whole-cell extracts were prepared and subject to anti-FLAG IP as described in the Site-specific in vivo photo cross-linking and IP section of Materials and methods. About 5 µl out of 100 µl immunoprecipitates were loaded for the anti-HA blots. Note that BPA at site 132 but not at site 124 of Tom22 cross-linked to Tim50. (D and E) The *MGR1-FLAG yme1*^{E541Q} or *MGR3-FLAG yme1*^{E541Q} strains expressing Tom22-HA with BPA incorporated at the indicated sites were untreated or pretreated with CHX for 5 min and then irradiated with UV for 15 min in the same media. Cells were analyzed as in Fig. 4 (B and C). Molecular masses are shown in kilodaltons.

Table S1 is a separate Excel document listing strains and primers used in this study.