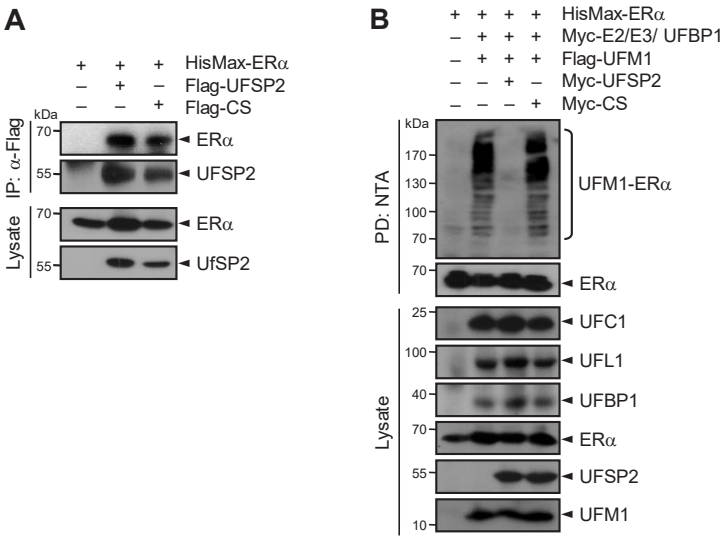
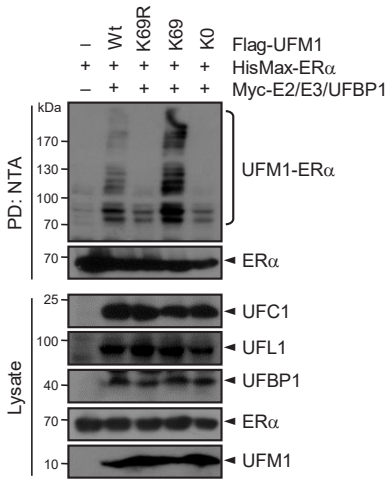
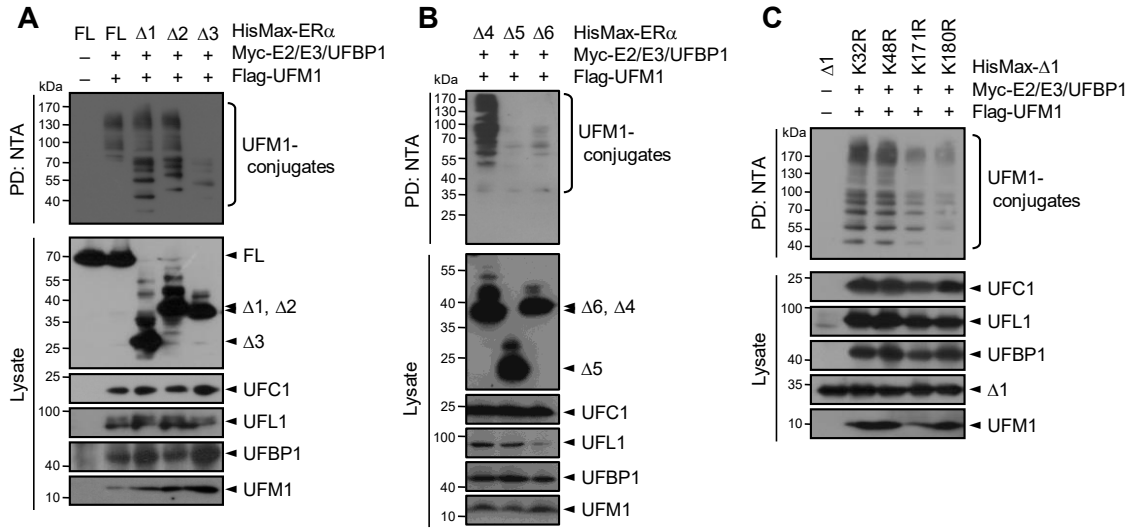


Supplementary Fig. S1. Reversal of ERα ufmylation by UFSP2. (A) HisMax-ERα was expressed in HEK293T cells with Flag-UFSP2 or Flag-CS. Cell lysates were subjected to immunoprecipitation with anti-Flag antibody followed by immunoblot with anti-Flag and anti-Xpress antibodies. CS denotes a catalytically inactive form of UFSP2. (B) Experiments were performed as in Fig. 1D, but in the presence of Myc-UFSP2 or Myc-CS. Cell lysates were subjected to pull-down with NTA resins, followed by immunoblot with anti-Flag and anti-Xpress antibodies.



Supplementary Fig. S2. Formation of poly-UFM1 chains on ERα via K69-linked isopeptide bonds. HisMax-ERα, Myc-UFC1 (E2), Myc-UFL1 (E3), and Myc-UFBP1 were expressed in HEK293T cells with Flag-tagged UFM1 (Wt) or its K69 variants (see text). Cell lysates are subjected to pull-down with NTA resins followed by immunoblot with anti-Flag and anti-Xpress antibodies.



Supplementary Fig. S3. Identification of ufmylation sites in ERα fragments. (A) HisMax-tagged ERα (FL) and its fragments, Δ1-Δ3, were expressed with UFM1-conjugating system in HEK293T cells. Cell lysates were subjected to pull-down with NTA resins followed by immunoblot with anti-Flag and anti-Xpress antibodies. (B) Experiments were carried out as above, but by expressing ERα fragments, Δ4-Δ6. (C) HisMax-tagged fragment Δ1 and its variants carrying K32R, K48R, K171R, and K180R mutations were expressed with UFM1-conjugating system in HEK293T cells. Pull-down analysis was then performed using anti-Flag antibody.