Expanded View Figures

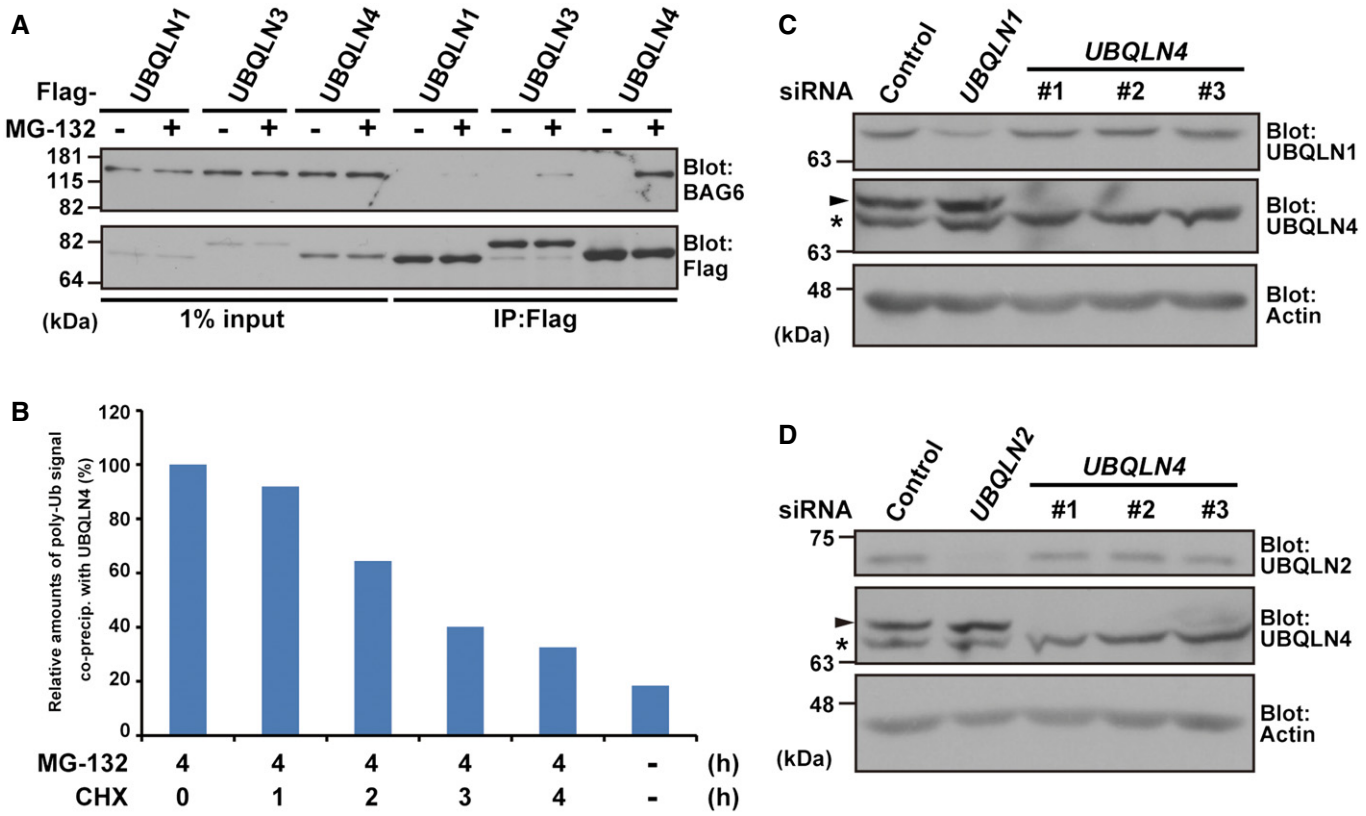

Figure EV1. UBQLN4 possesses a distinct preference over UBQLN1 for endogenous BAG6.

A UBQLN4, but not UBQLN1, interacts with endogenous BAG6 in an MG-132 treatment-dependent manner. Flag-tagged UBQLN1, UBQLN3, and UBQLN4 were expressed in HeLa cells, and the cells were treated with (+) or without (−) 20 μM MG-132 for 4 h. UBQLNs were immunoprecipitated, and the precipitates were blotted with anti-BAG6 and anti-Flag antibodies.

B Polyubiquitinated proteins associated with UBQLN4 are CHX-sensitive. The anti-polyubiquitin signals that co-precipitated with UBQLN4 in Fig 1D were quantified.

C, D Transfection of three independent *UBQLN4* siRNA duplexes (#1, #2, and #3) as in Fig 4A did not affect the expression levels of UBQLN1 (C) or UBQLN2 (D) protein. Arrowheads indicate the UBQLN4 signal, and asterisks indicate a non-specific band. Actin was used as a loading control.

Source data are available online for this figure.

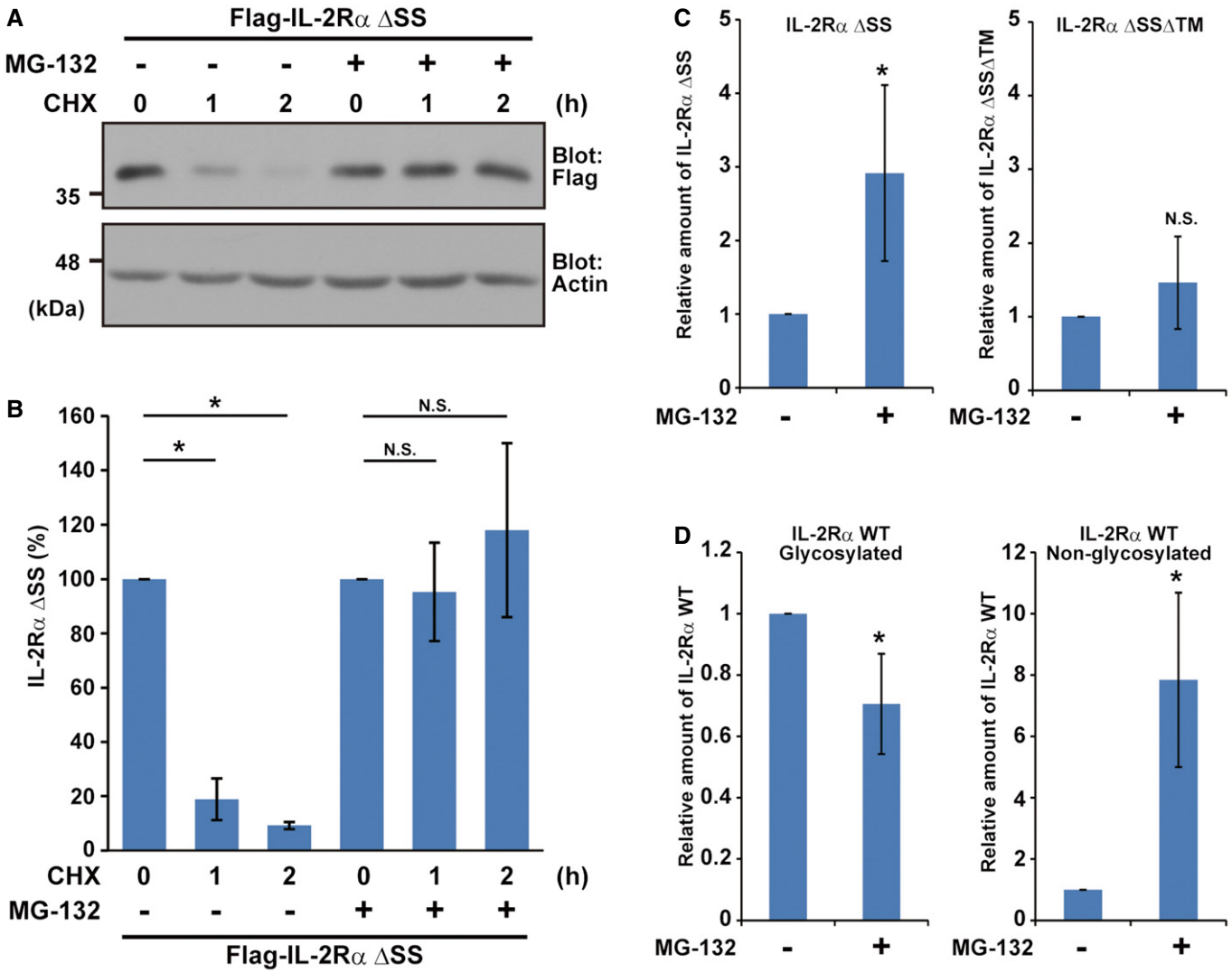

Figure EV2. Defective forms of IL-2Rα protein are stabilized by MG-132.

A, B Mislocalized IL-2Rα protein was stabilized by MG-132. Anti-Flag immunosignals with (+) or without (−) MG-132 were quantified at the indicated time points. The data represent mean ± SD calculated from three independent experiments (n = 3). Statistical analysis by Welch's t-test (*P < 0.01; N.S., non-significant).

C, D Anti-Flag immunosignals of IL-2Rα ΔSS and IL-2Rα ΔSSΔTM (C) as well as those of low-mobility (indicated as glycosylated) and high-mobility (indicated as non-glycosylated) signals of IL-2Rα WT (D) (shown in Fig 3G as a representative example) were quantified. The data represent mean ± SD calculated from four independent experiments (n = 4). Statistical analysis by Welch's t-test (*P < 0.05; N.S., non-significant).

Source data are available online for this figure.

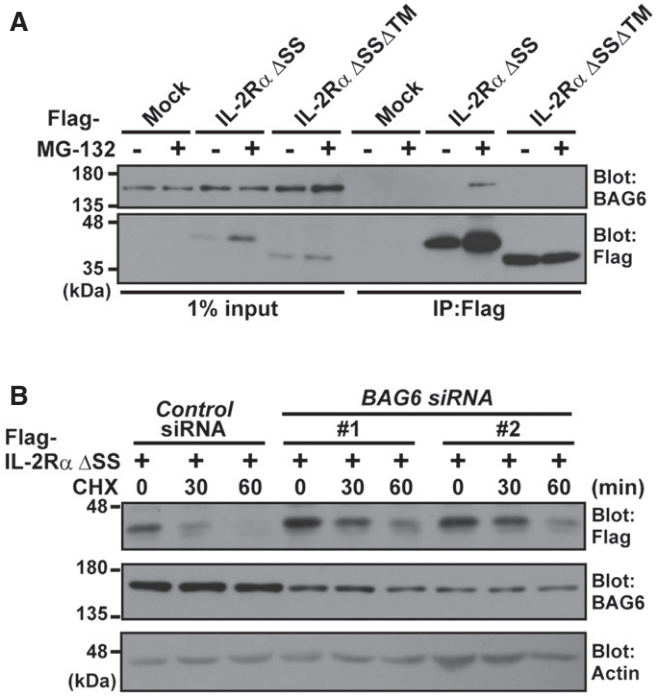

Figure EV3. BAG6 is involved in the degradation of mislocalized IL-2Rα ΔSS.

A BAG6 interacts with misassembled IL-2Rα through its TMD. HeLa cells expressing Flag-tagged IL-2Rα ΔSS or IL-2Rα ΔSSΔTM were treated with (+) or without (−) 10 μM MG-132 for 4 h, and then, Flag-IL-2Rα substrates were immunoprecipitated and their co-precipitation with endogenous BAG6 was analyzed with an anti-BAG6 antibody.

B BAG6 associates with the mislocalized IL-2Rα ΔSS degradation pathway. HeLa cells were transfected with two distinct siRNA duplexes for BAG6 (*BAG6* siRNA#1 and #2) or control siRNA. At 48 h after siRNA transfection, Flag-tagged IL-2Rα ΔSS was detected as in Fig 4A. Anti-Flag immunosignals in control or *BAG6* siRNA-treated cells were detected at the indicated time points. Actin was used as a loading control.

Source data are available online for this figure.

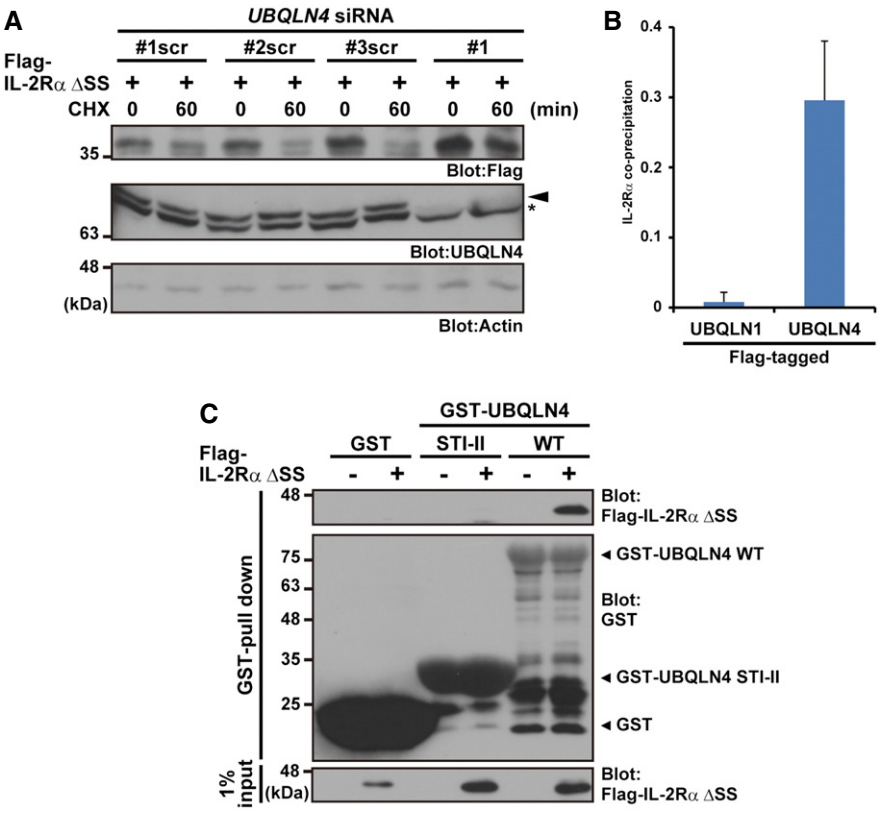

Figure EV4. UBQLN4-mediated metabolism for mislocalized proteins.

A HeLa cells were transfected with three independent scrambled siRNA duplexes (*UBQLN4* siRNA#1scr, #2scr, and #3scr) as negative controls for the *UBQLN4* siRNA#1-#3 experiments shown in Fig 4A. The arrowhead indicates the UBQLN4 signal, and the asterisk indicates a non-specific band. Actin was used as a loading control.

B T7-tagged IL-2Rα ΔSS signals (shown in Fig 6B as a representative example) were quantified. The data represent mean ± SD calculated from three independent experiments (n = 3).

C Purified UBQLN4 WT protein co-precipitated with Flag-IL-2Rα ΔSS, while the STI-II fragment of UBQLN4 did not. Bacterially expressed GST-UBQLN4 WT and GST-STI-II were incubated with extracts of HeLa cells expressing Flag-tagged IL-2Rα ΔSS and treated with MG-132 for 4 h. After *in vitro* GST pull-down, the precipitates were probed with anti-Flag and anti-GST antibodies.

Source data are available online for this figure.

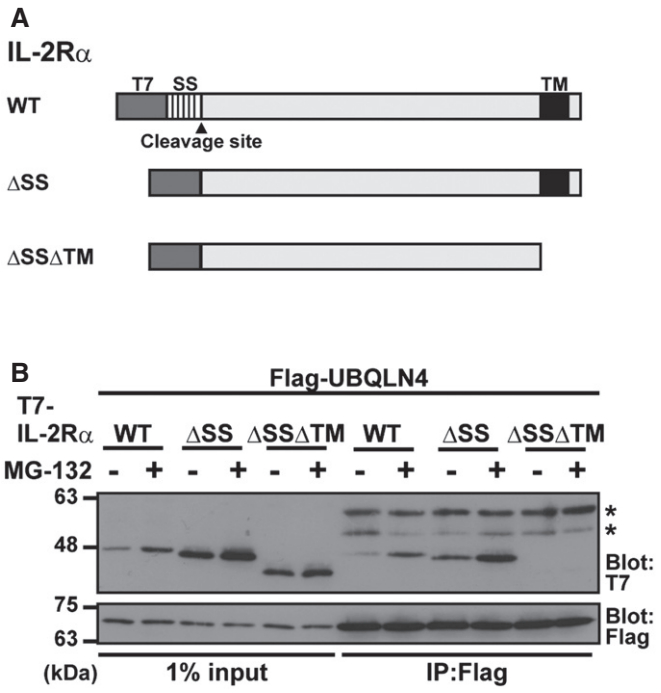

Figure EV5. UBQLN4 recognizes ER assembly defective forms of IL-2Rα.

A Schematic representation of the IL-2Rα proteins used in this experiment. WT: wild type, ΔSS: SS-deleted mutant, and ΔSSΔTM: SS- and TM-deleted mutant. Note that the position of the T7-tag in WT IL-2Rα is upstream (N-terminal) of the SS: thus, SS cleavage by signal peptidase that occurs immediately after ER luminal incorporation would result in the loss of the T7 signal.

B A series of T7-tagged IL-2Rα substrates were expressed in HeLa cells with Flag-tagged UBQLN4 and treated with (+) or without (−) 10 μM MG-132 for 4 h. Flag-UBQLN4 was immunoprecipitated, and its co-precipitation with IL-2Rα was analyzed. In contrast to the case in Fig 5, glycosylated bands of WT IL-2Rα were never detected, since T7-tagged SS for ER targeting is cleaved off immediately after its successful co-translational insertion into the lumen of the ER. Thus, only cytoplasmic defective forms of IL-2Rα can be detected as T7-positive polypeptides in this experiment. Asterisks indicate non-specific signals.

Source data are available online for this figure.