Supplemental Materials

Molecular Biology of the Cell

Ushioda et al.

Supplemental Information

Supplemental Figure Titles and Legends

Figure S1. Expression levels of ERdj5 and QQQ (A-C) Western blot analyses were performed 24 h after transfection of HEK293T cells with indicated construct.(A) After the transfection with ERdj5/WT, Western blot were performed with endogenous ERdj5 and Actin antibody. (B) QQQ and ERdj5/WT or ERdj5 mutants were detected by A1AT, FLAG and Actin antibody after the transfection. (C) After the transfection with indicated amount of A1AT cDNA, QQQ expression levels were anlyzed by A1AT antibody. Transfected HEK293T cells were incubated with [^{35}S]methionine-cysteine in the presence of Cst.

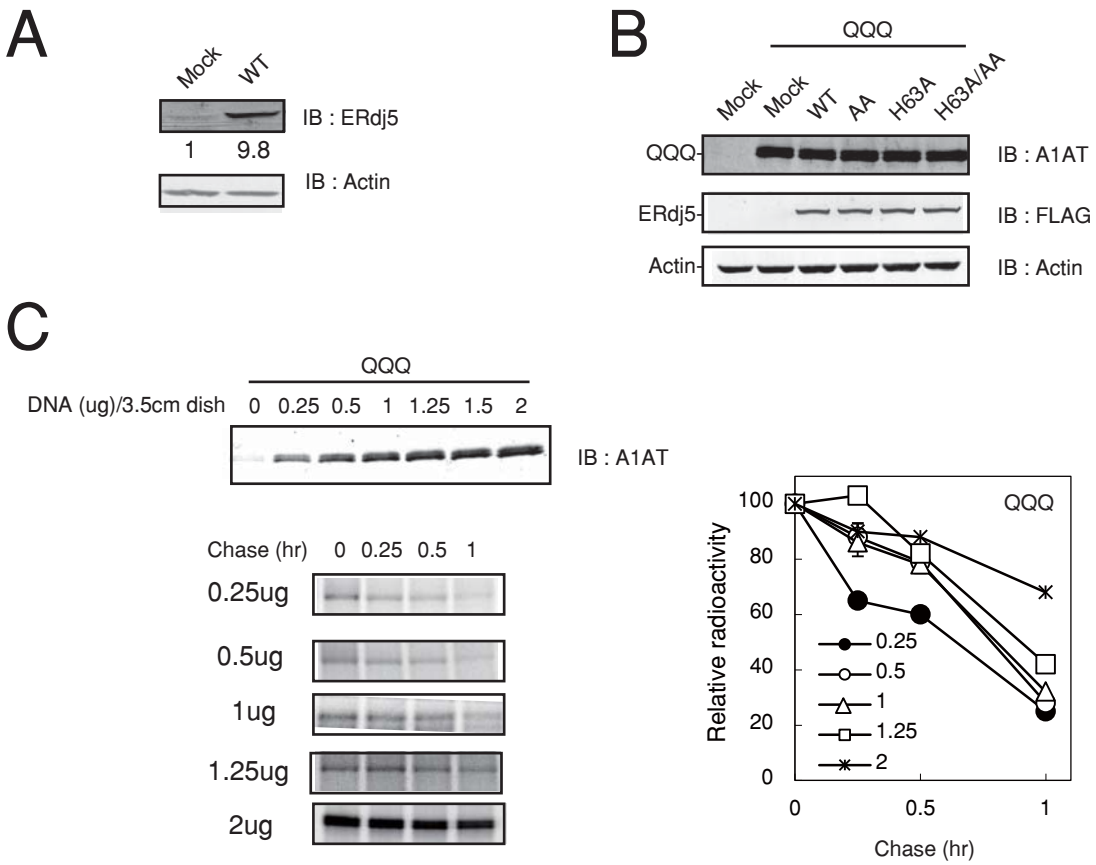
Figure S2. ERdj5-mediated promotion of Tyr/T373K ERAD (A) Pulse-chase analysis of HEK293T cells overexpressing Tyr/T373K and WT-, H63A-, or ΔTrx4-ERdj5-FLAG. Cells were pulse-chased for the indicated times 24 h after transfection. (B) Pulse-chase analysis of the effect of Cst on ERdj5-mediated promotion of Tyr/T373K ERAD. Twenty-four hours after transfection, HEK293T cells were pretreated with 1 mg/ml Cst for 1 h and then pulse-chased with [^{35}S]methionine-cysteine in the presence of Cst. Data represent the mean ± SD of n = 3 independent experiments.

Figure S3. ERdj5/mC4 accelerates ERAD of NHK similarly to ERdj5/WT Pulse-chase analysis of HEK293T cells overexpressing NHK and WT or the mC4 mutant of ERdj5, in which the CXXC motif of the Trx4 domain was mutated to AXXA. Cells were pulse-chased for the indicated times 24 h after transfection. Data represent the mean ± SD of n = 3 independent experiments.

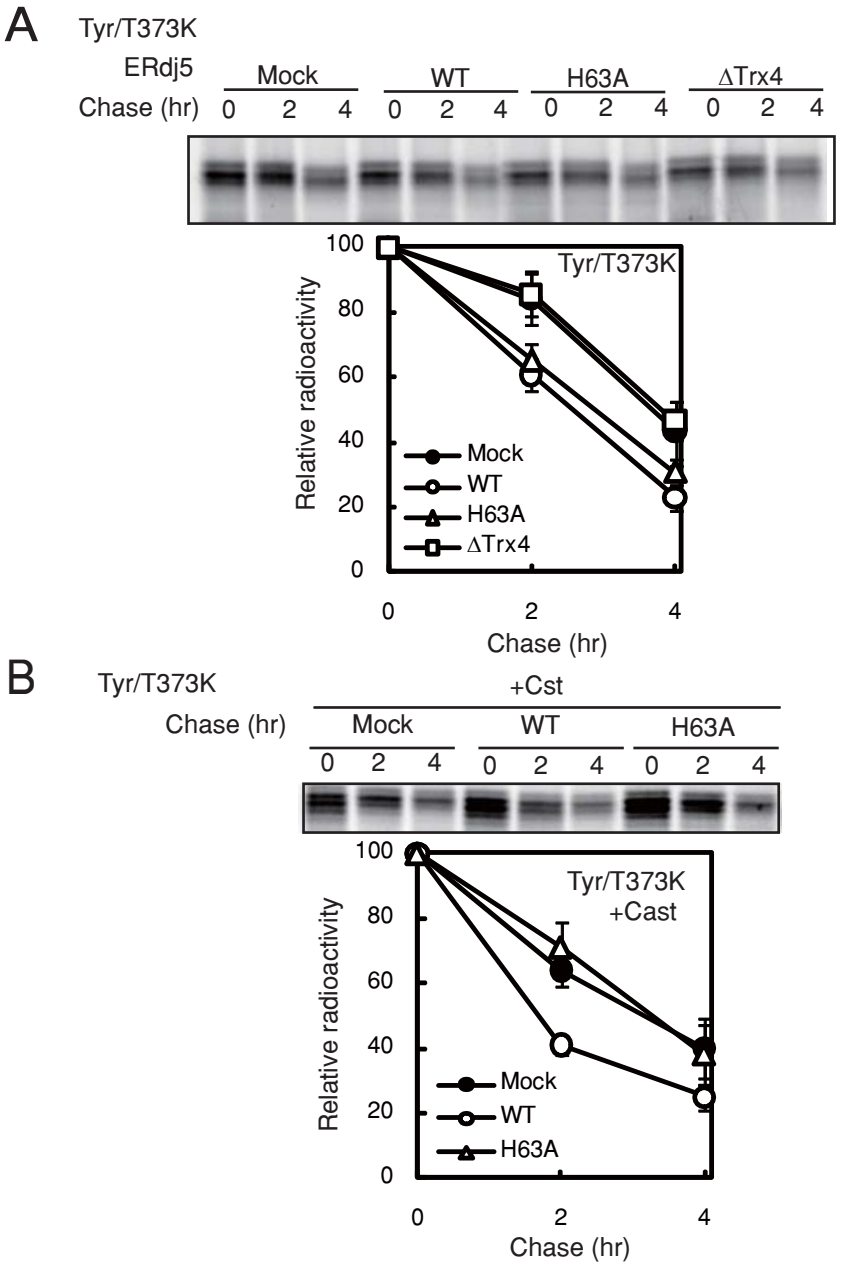
Figure S4. The dominant negative effect of the ERdj5/AA mutant on ERAD of the QQQ mutant is suppressed by knockdown or mutation (H63A) of endogenous ERdj5 (A) Western blot analyses of endogenous ERdj5 72 h after transfection of HEK293T cells with non-specific (NS) or ERdj5-specific siRNA. (B) Western blot analysis of exogenous mouse ERdj5/WT and ERdj5/AA in HEK293T cells transfected with ERdj5-specific siRNA. (C) Pulse-chase analysis of siRNA-transfected HEK293T cells overexpressing the QQQ mutant and exogenous mouse ERdj5/WT or ERdj5/AA. Pulse chasing for the indicated times was performed 24 h after transfection. (D) Pulse-chase analysis of HEK293T cells overexpressing the QQQ mutant and exogenous mouse ERdj5/WT, ERdj5/AA, or ERdj5/H63A/AA. Pulse chasing for the indicated times was performed 24 h after transfection. (C, D) Data represent the mean ± SD of n = 3 independent experiments.

Figure S5. BiP co-immunoprecipitates with NHK or the QQQ mutant in HEK293T cells Twenty-four hours after transfection of HEK293T cells with BiP and NHK or the QQQ mutant, supernatants of cell lysates prepared in a 1% NP40 lysis buffer were subjected to immunoprecipitation (IP) with an anti-BiP antibody. Immunoprecipitants were immunoblotted (IB) with anti-BiP or anti-A1AT antibodies. The graph shows the relative degree of binding of BiP to NHK or the QQQ mutant. Data were normalized to the result for the QQQ mutant and represent the mean ± SD of n = 3 independent experiments.

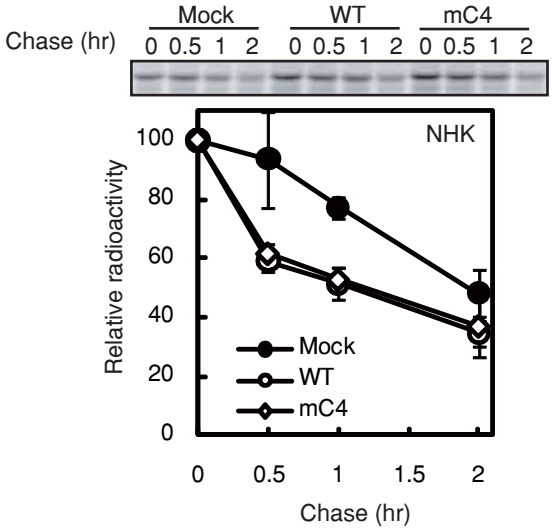
Supplementary Figure 1



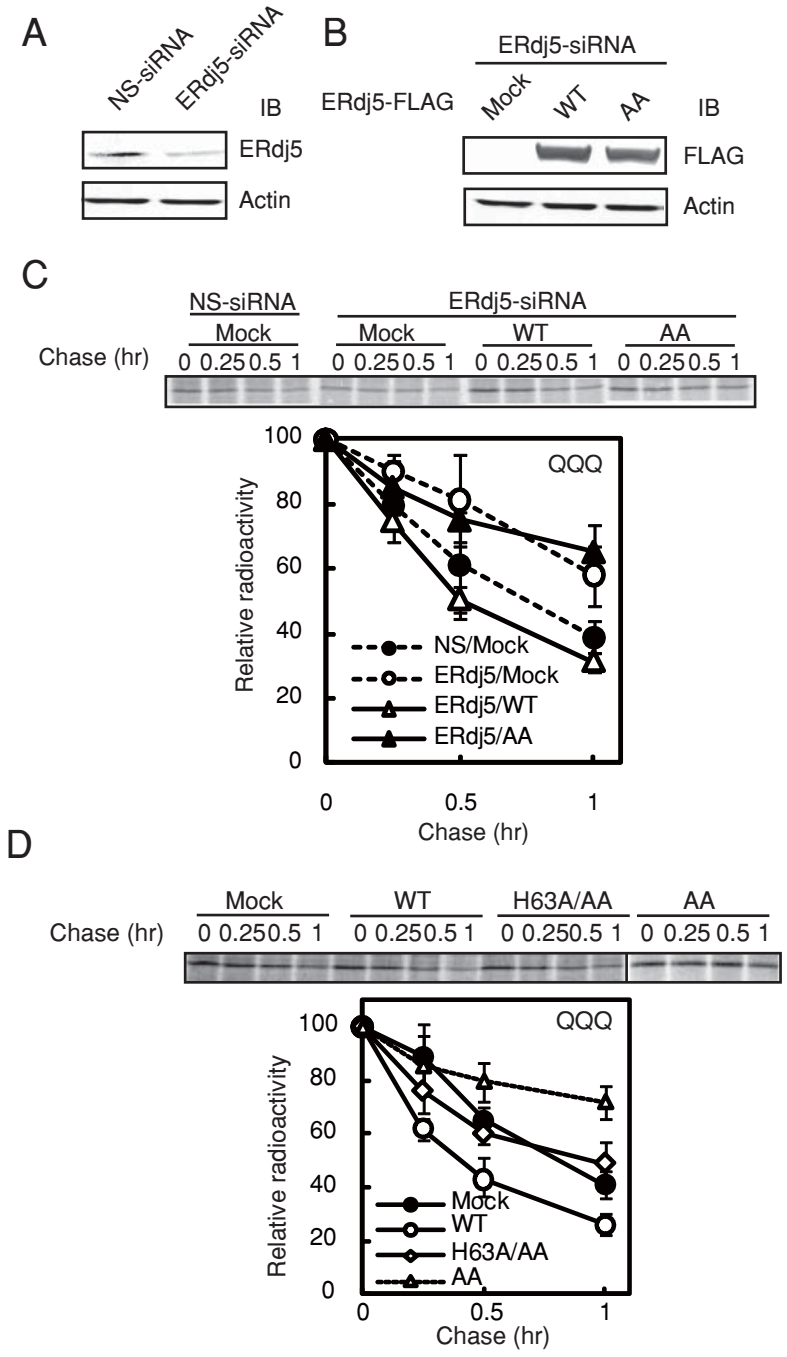
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5