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Supplemental Information

A Molecular Mechanism for Turning Off IRE1α Signaling during Endoplasmic Reticulum Stress

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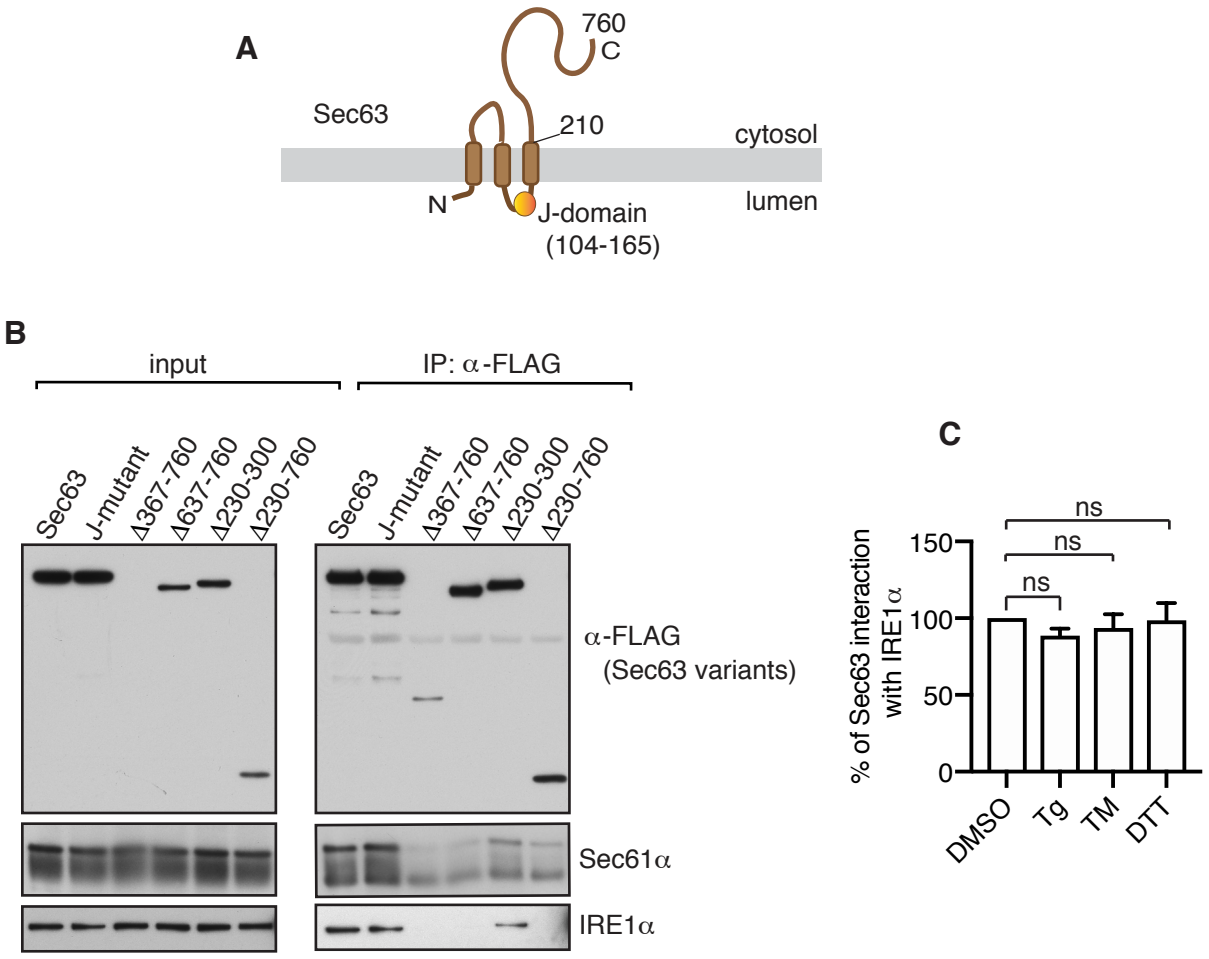

Figure S1, related to Figure 1. IRE1α interacts with Sec63 through the Sec61 translocon
(A) A diagram showing the topology of Sec63.
(B) The cell lysates of the indicated versions of FLAG-tagged Sec63 were immunoprecipitated with anti-FLAG beads and analyzed by immunoblotting for the indicated antigens. The HPD tripeptide in the J-domain was replaced with AAA to create the J-domain mutant of Sec63.
(C) Quantification results of Figure 1E. Error bars represent the standard error of the mean (SEM) from three independent experiments. p-value higher than 0.05 is represented by ns (not significant). The percentage of IRE1α bound to Sec63 in the DMSO treated sample was set as 100%.

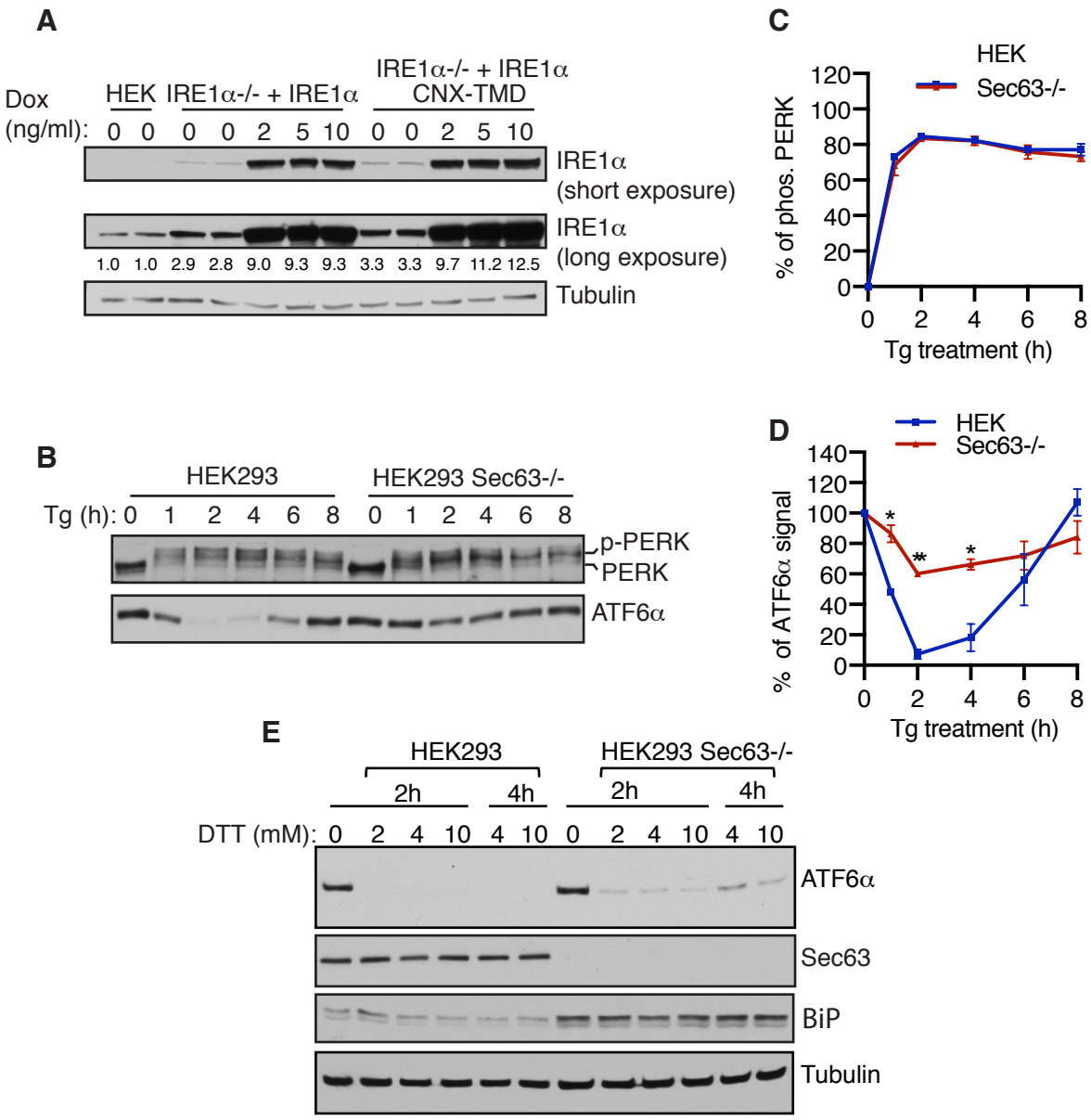

Figure S2, related to Figure 2 and 3. Probing activation and inactivation of PERK and ATF6α in Sec63-/- cells.

(A) The indicated cells were treated without or with the indicated concentration of doxycycline for 24 h. The cells were harvested in 2xSDS sample buffer and analyzed by immunoblotting for the indicated antigens. IRE1 α bands were quantified and values were presented underneath IRE1α immunoblots. IRE1α signals from control HEK293 cells were set as 1.

(B) Wild type HEK293 or Sec63-/- cells were treated with 2.5 μg/ml of Tg for the indicated time points and analyzed by immunoblotting for PERK and ATF6α. The ATF6α bands represent "full length" proteins. The loss of ATF6α signals indicates the cleavage of its N-terminal cytosolic domain during ER stress. Our ATF6α antibodies were not suitable for detecting the cleaved N-terminal cytosolic domain.

(C) Quantification results of PERK phosphorylation in (A). Error bars represent the standard error of the mean (SEM) of three independent experiments. PERK phosphorylation difference in WT cells and Sec63-/- cells was determined by Student's t test. p values were non-significant, as they are higher than 0.05.

(D) Quantification results of ATF6α signal in (A). Error bars represent the standard error of the mean (SEM) of three independent experiments. ATF6α signal difference in WT cells and Sec63-/- cells was determined by Student's t-test. *P<0.05; **P<0.01.

(E) HEK293 or Sec63-/- cells were treated with DTT for the indicated time points with the indicated concentrations. The cells were directly harvested in SDS sample buffer and analyzed by immunoblotting for the indicated antigens.

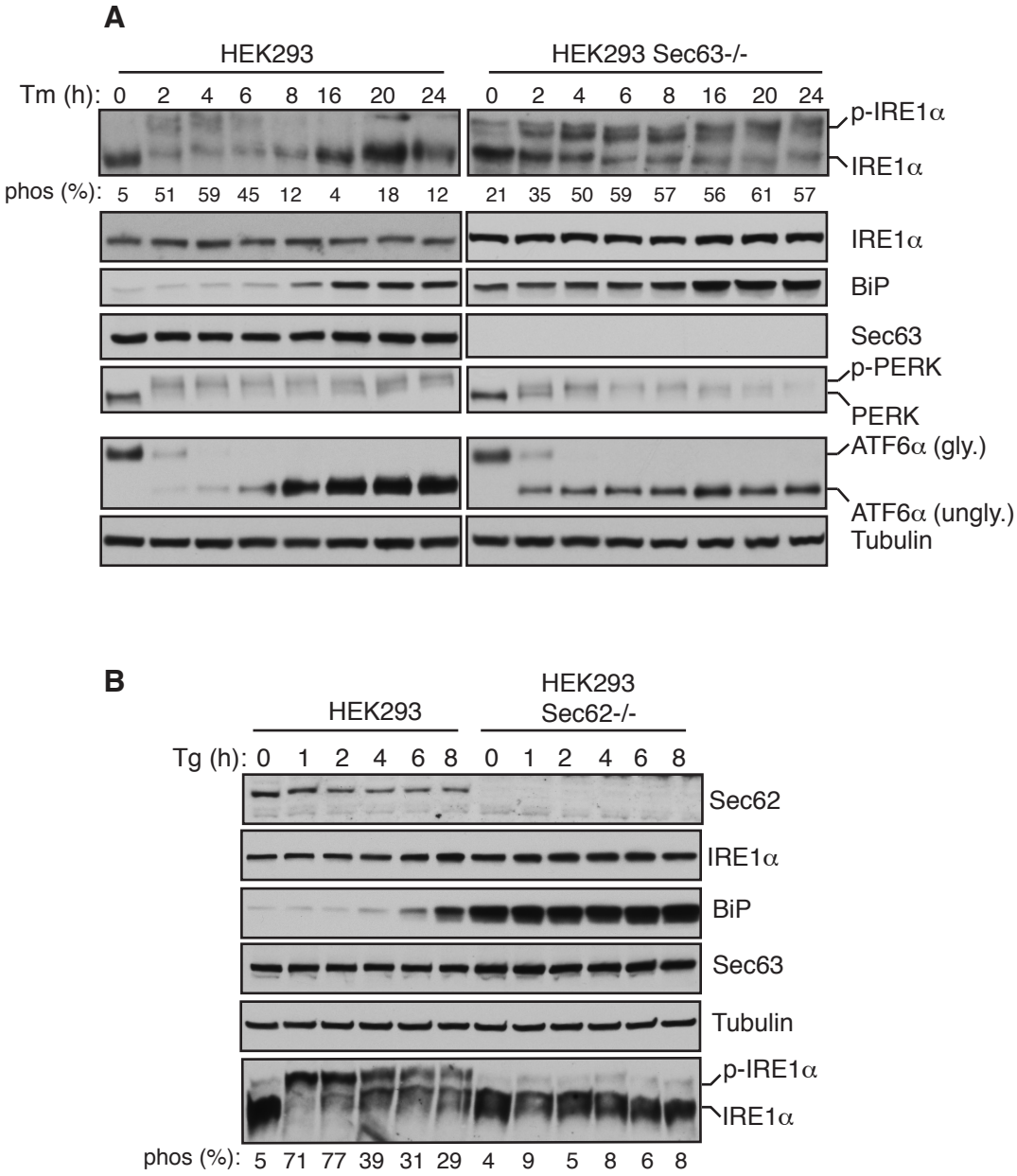

Figure S3, related to Figure 3. IRE1α activation and inactivation in Sec63-/- or Sec62-/- cells.
(A) Wild type HEK293 or Sec63-/- cells were treated with 5 μg/ml of Tm for the indicated time points and analyzed by immunoblotting for the indicated antigens. The percentage of IRE1α phosphorylation is shown underneath phos-tag immunoblots. ATF6 is glycosylated (gly.), but it is unglycosylated (ungly.) during the tunicamycin treatment.
(B) Wild type HEK293 or Sec62-/- cells were treated with 2.5 μg/ml of Tg for the indicated time points and analyzed as in panel A.

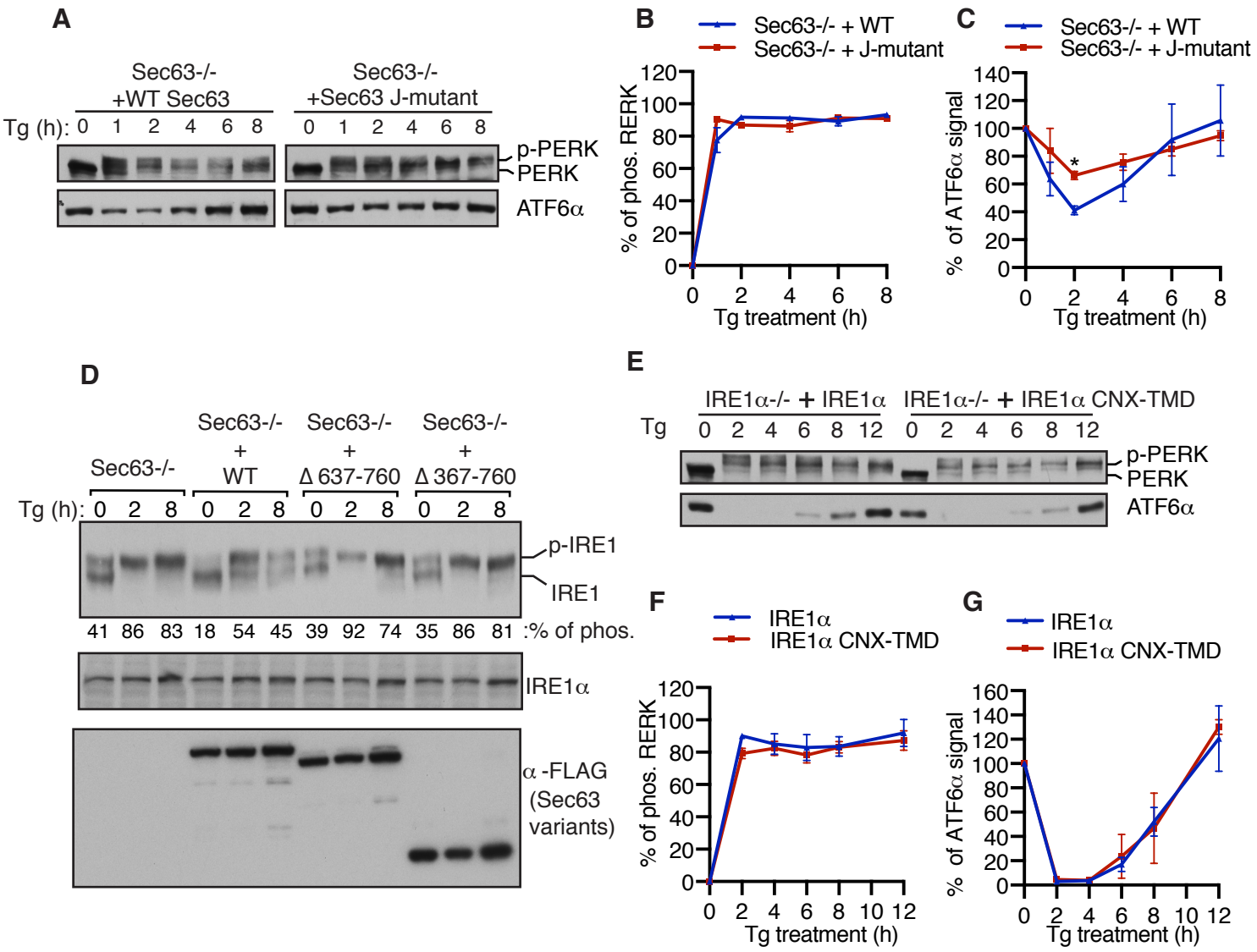

Figure S4, related to Figure 3. Probing activation and inactivation of IRE1α, PERK, and ATF6α in complemented cell lines

(A) Sec63-/- cells complemented with either WT or the J-domain mutant were treated with 2.5 μg/ml of Tg for the indicated time points and analyzed by immunoblotting for PERK and ATF6α.

(B) Quantification results of PERK phosphorylation in (A). Error bars represent the standard error of the mean (SEM) of three independent experiments. PERK phosphorylation difference in WT cells and Sec63-/- cells was determined by Student's *t* test. p values were non-significant, as they are higher than 0.05.

(C) Quantification results of ATF6α signal in (A). Error bars represent the standard error of the mean (SEM) of three independent experiments. ATF6α cleavage difference in WT cells and Sec63-/- cells was determined by Student's *t* test. *P<0.05.

(D) Sec63-/- cells transiently expressing either WT or Sec63 mutants were treated with 2.5 μg/ml of Tg for the indicated time points and analyzed by immunoblotting. The percentage of IRE1α phosphorylation is shown underneath phos-tag immunoblots

(E) IRE1α -/- cells complemented with either WT or IRE1α CNX-TMD were treated with 2.5 μg/ml of Tg for the indicated time points and analyzed by immunoblotting for PERK and ATF6α.

(F) PERK phosphorylation in (E) was quantified as in (B), but error bars represent the standard error of the mean (SEM) of two independent experiments.

(G) ATF6α signal in (E) was quantified as in (B), but error bars represent the standard error of the mean (SEM) of two independent experiments.

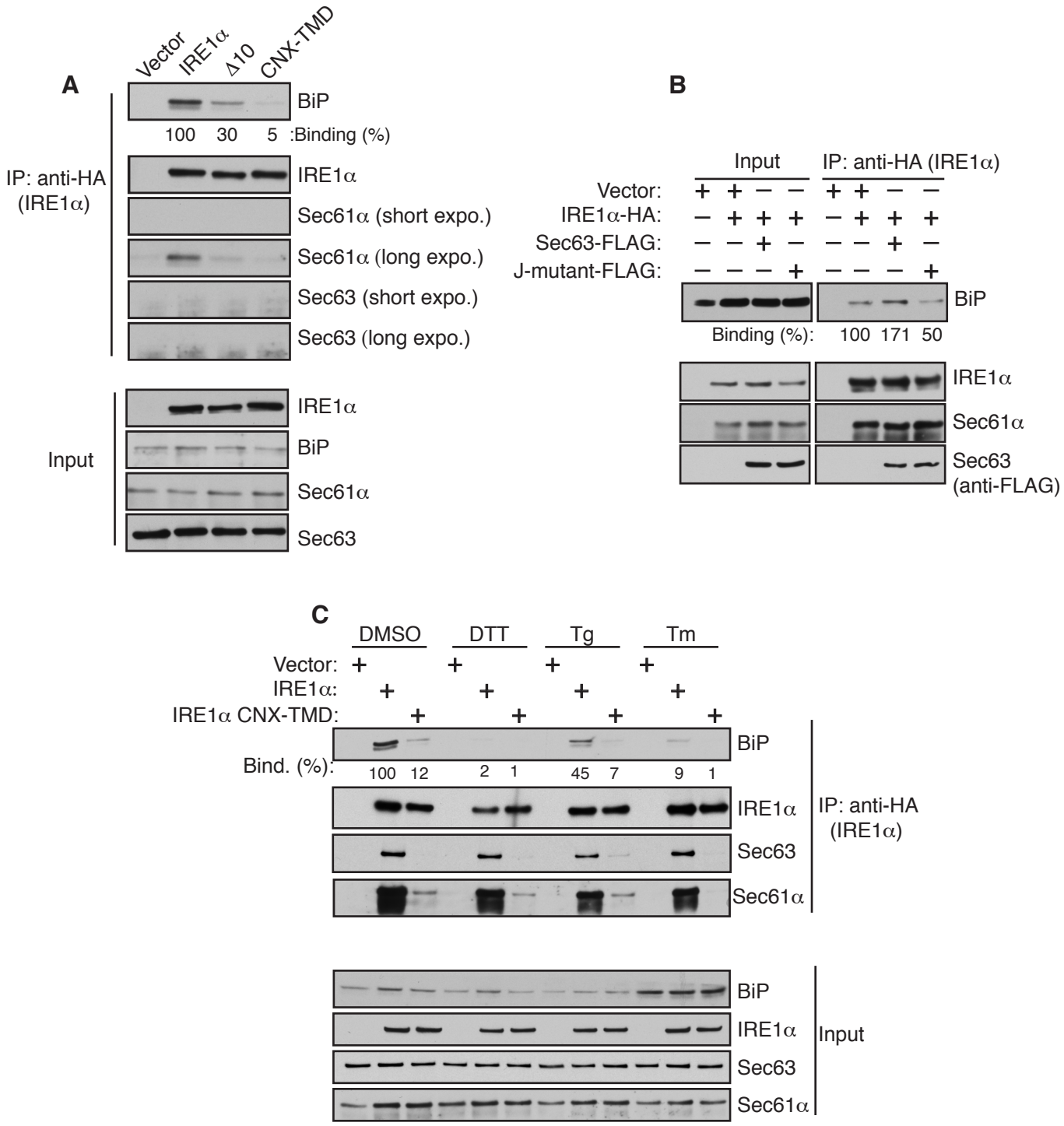

Figure S5, related to Figure 4. Sec61/Sec63-mediated BiP binding to IRE1α in cells.
(A) HEK293 Sec63-/- cells complemented with wild type Sec63 were transiently transfected with either IRE1 α-HA or its variants. The cell lysates were prepared using the buffer containing NP40/deoxycholate followed by immunoprecipitation with anti-HA magnetic beads and analyzed by immunoblotting for the indicated antigens. The amount of BiP binding to wild type IRE1α was taken as 100%.
(B) HEK293 cells were co-transfected with IRE1α-HA and empty vector or Sec63-FLAG or J-domain mutant of Sec63-FLAG. The cell lysates were immunoprecipitated with an anti-HA antibody and analyzed by immuno-blotting for the indicated antigens. The amount of BiP binding to IRE1α that was co-transfected with empty vector was taken as 100%.
(C) HEK293 IRE1α-/- cells stably expressing IRE1α-HA or IRE1α-CNX-TMD-HA were treated with DMSO for 2 h, 4 mM DTT for 2 h, 5 μg/ml Tg for 2 h, or 10 μg/ml Tm for 4 h. The treated cells were harvested and analyzed as in A. The amount of BiP binding to wild type IRE1α in unstressed cells was taken as 100%.

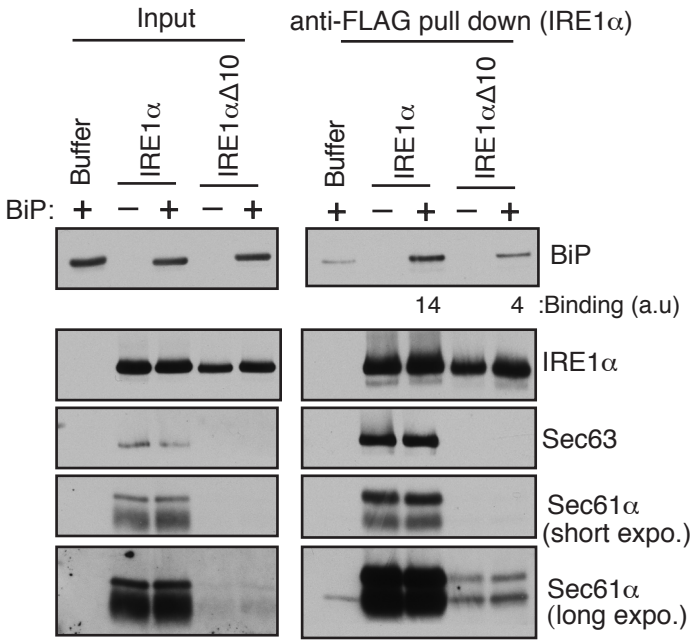

Figure S6, related to Figure 5. Sec61/Sec63-mediated BiP binding to IRE1α *in vitro*.
The purified IRE1α/Sec61/Sec63 complex or IRE1αΔ10 was incubated with or without BiP in the presence of ATP. After incubation, IRE1α was immunoprecipitated using anti-FLAG beads. A negative control reaction was performed by mixing the buffer, BiP, and ATP, followed by immunoprecipitation with anti-FLAG beads. The samples were analyzed by immunoblotting for the indicated antigens. Note that IRE1α Δ10 contains a residual amount of the Sec61 translocon (long exposure blot), suggesting that weak binding between BiP and IRE1α Δ10 may be due to the presence of a small amount of Sec61/Sec63 in the sample. BiP bands were quantified and presented as arbitrary units (a.u) after subtracting the buffer background.

Figure 1B (similar experiment)

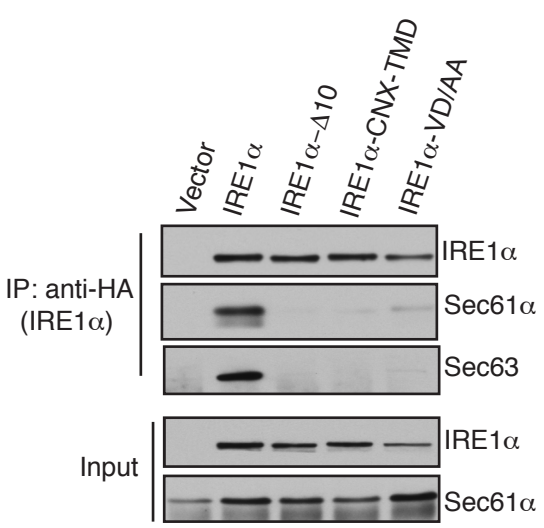

Figure 1C (similar experiment)

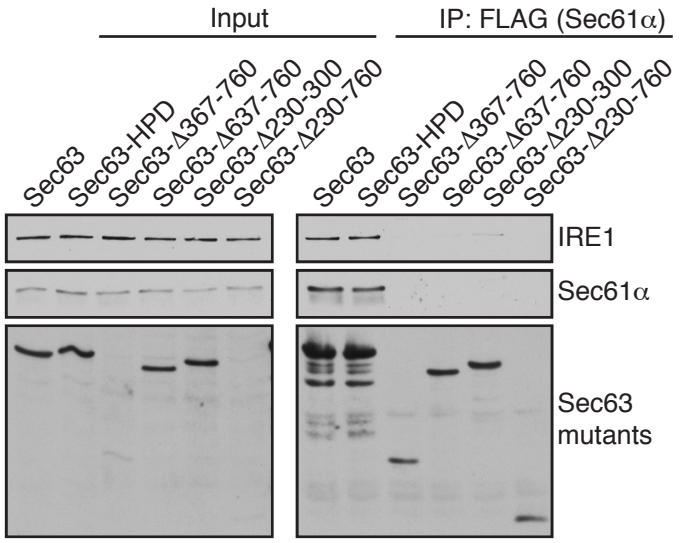

Figure 1D (similar experiment)

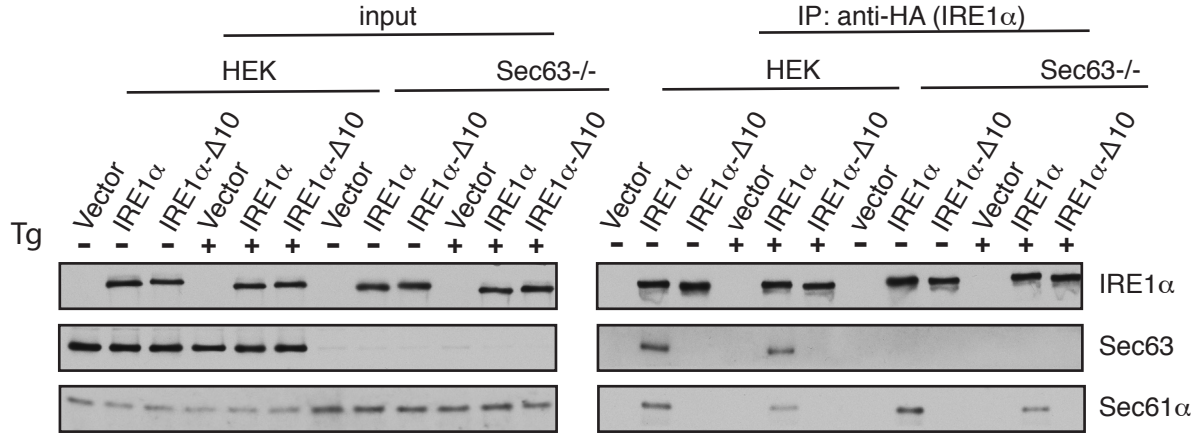

Figure 4A (replicate experiment)

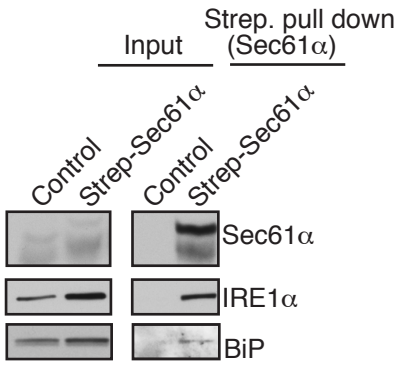

Figure 4B (replicate experiment)

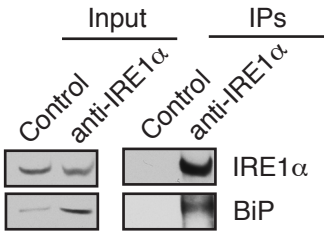

Figure S7: Experiments related to Figure 1 and Figure 4. The experiments were carried out as described in the legend of either Figure 1 or Figure 4.

Figure 3A (replicate experiments)

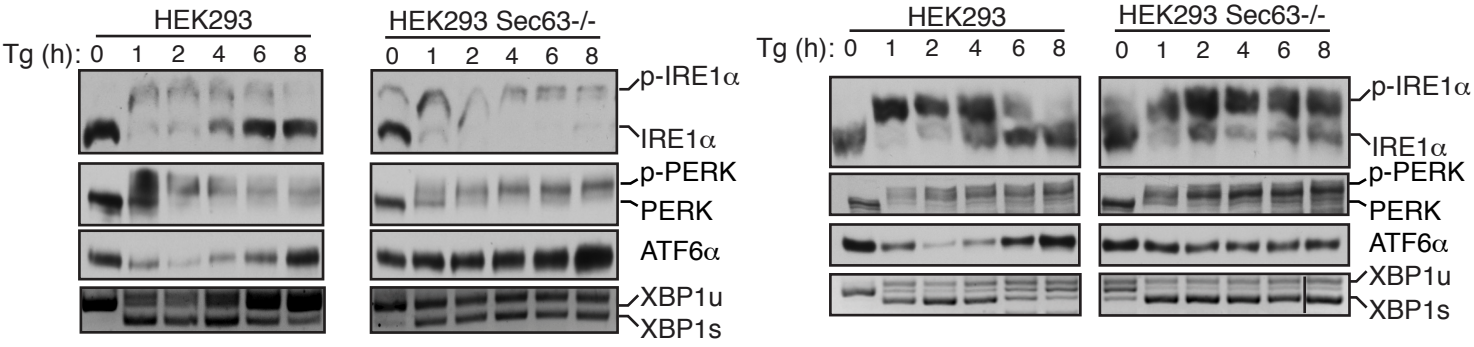

Figure 3B (replicate experiments)

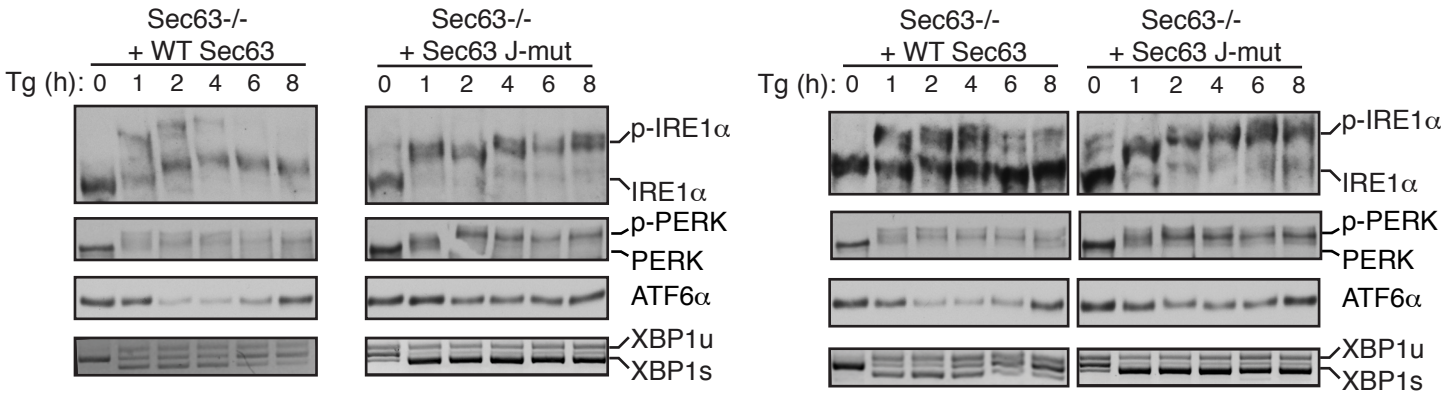

Figure 3C (replicate experiments)

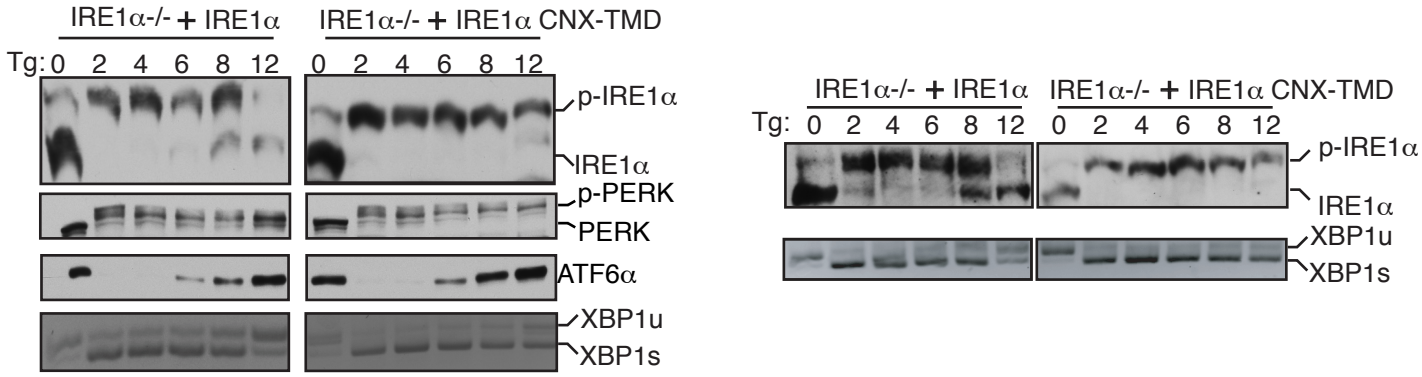

Figure S8: Replicate experiments related to Figure 3. The experiments were carried out as described in the legend of Figure 3.