Cell Reports, Volume *27*

Supplemental Information

Intrinsic Structural Features of the Human IRE1α Transmembrane Domain Sense Membrane Lipid Saturation

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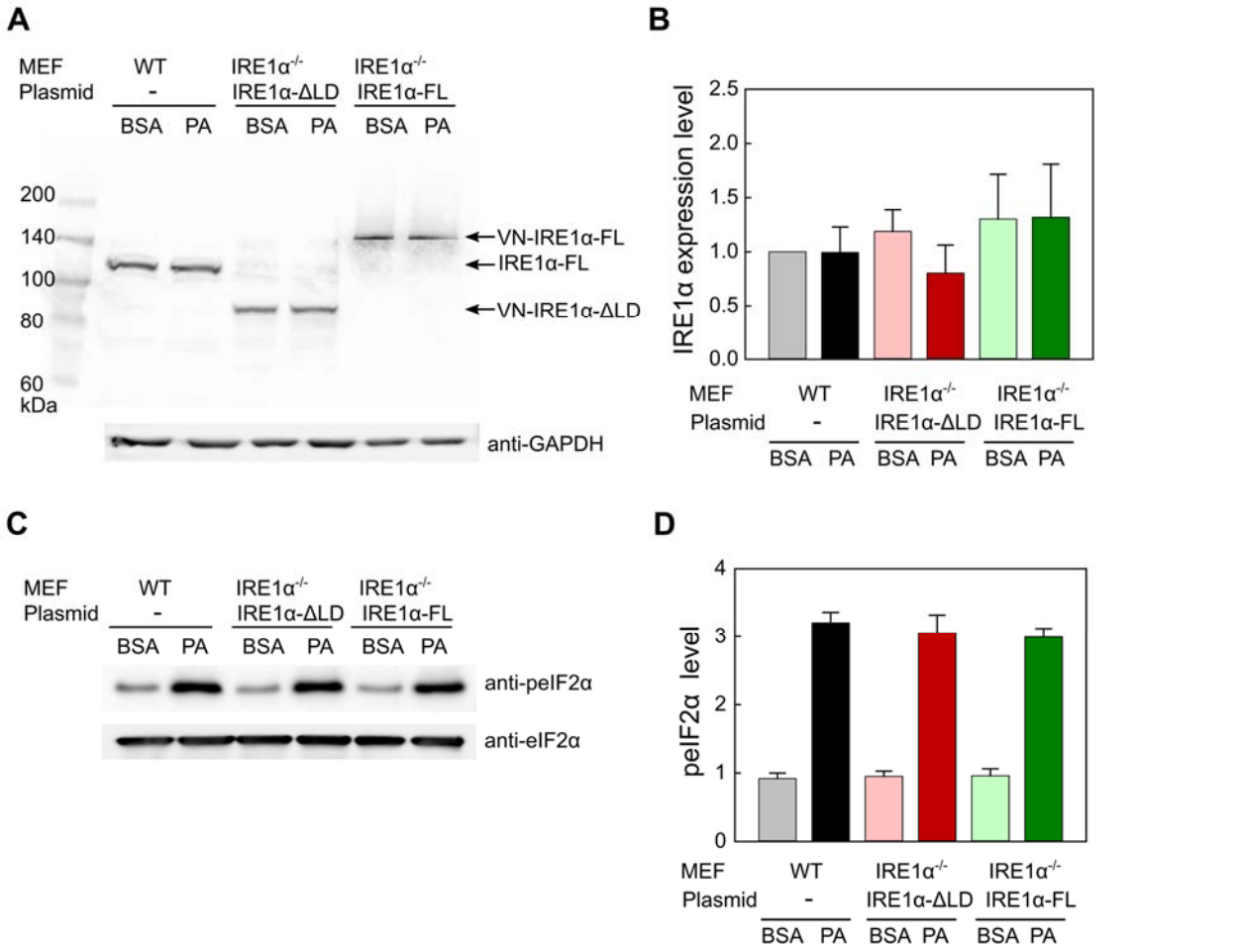

Figure S1. Expression levels of IRE1α-ΔLD and IRE1α-FL proteins in IRE1α$^{-/-}$ MEF cells are comparable to their parental MEF cells. (Related to Figures 2 and 4)

(A) Expression levels of IRE1α-ΔLD and IRE1α-FL proteins in IRE1α$^{-/-}$ MEF. MEF or IRE1α$^{-/-}$ MEF cells were seeded at a density of 3×10^6 cells/mL in each well. IRE1α$^{-/-}$ MEF cells were transfected with VN-IRE1α-ΔLD or VN-IRE1α-FL. After treatment with 2% BSA or 0.4 mM PA for 2 h, cell extracts were collected and subjected to western blot analysis. GAPDH served as a loading control.

(B) Quantification of (A). The expression levels of IRE1α-ΔLD and IRE1α-FL proteins were normalized to the BSA-treated endogenous IRE1α sample. Values are reported as mean ± SE (n=3).

(C) The phosphorylation levels of eIF2α. The cell extracts obtained from (A) were used for western blot analysis to detect the total expression levels of eIF2α and phosphorylated eIF2α (peIF2α).

(D) Quantification of (C). The phosphorylated levels of eIF2α were normalized to the total protein levels of eIF2α and then normalized to the BSA-treated endogenous IRE1α sample. Values are mean ± SE (n=2).

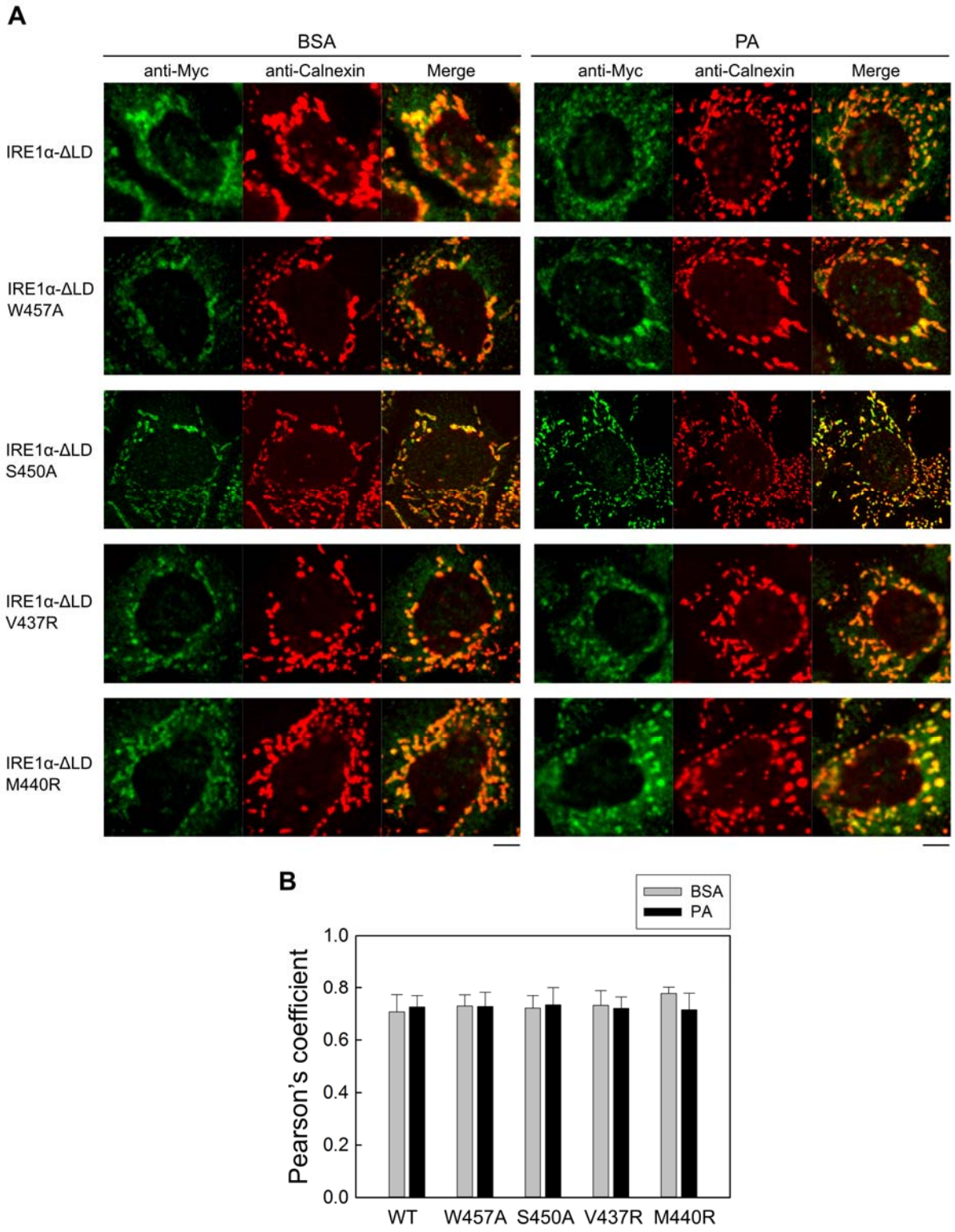

Figure S2. Intracellular localization of IRE1α-ΔLD constructs. (Related to Figures 2 and 3)

(A) Hep3B cells were transfected with Myc-tagged VN-IRE1α-ΔLD-WT and its corresponding mutants (W457A, S450A, V437R, and M440R) and then treated with 2% BSA or 0.4 mM PA (in 2% BSA) media for 2 h. The cells were fixed and stained with rabbit anti-Myc tag and mouse anti-

calnexin antibodies. Images were taken on an Olympus FV10 confocal microscope using sequential acquisition. Scale bars 7 μm.

(B) Pearson's coefficient of IRE1α-ΔLD variants and calnexin colocalization. Ten cells for each condition were analyzed using Image J software. Data indicate the mean ± SD (n = 10).

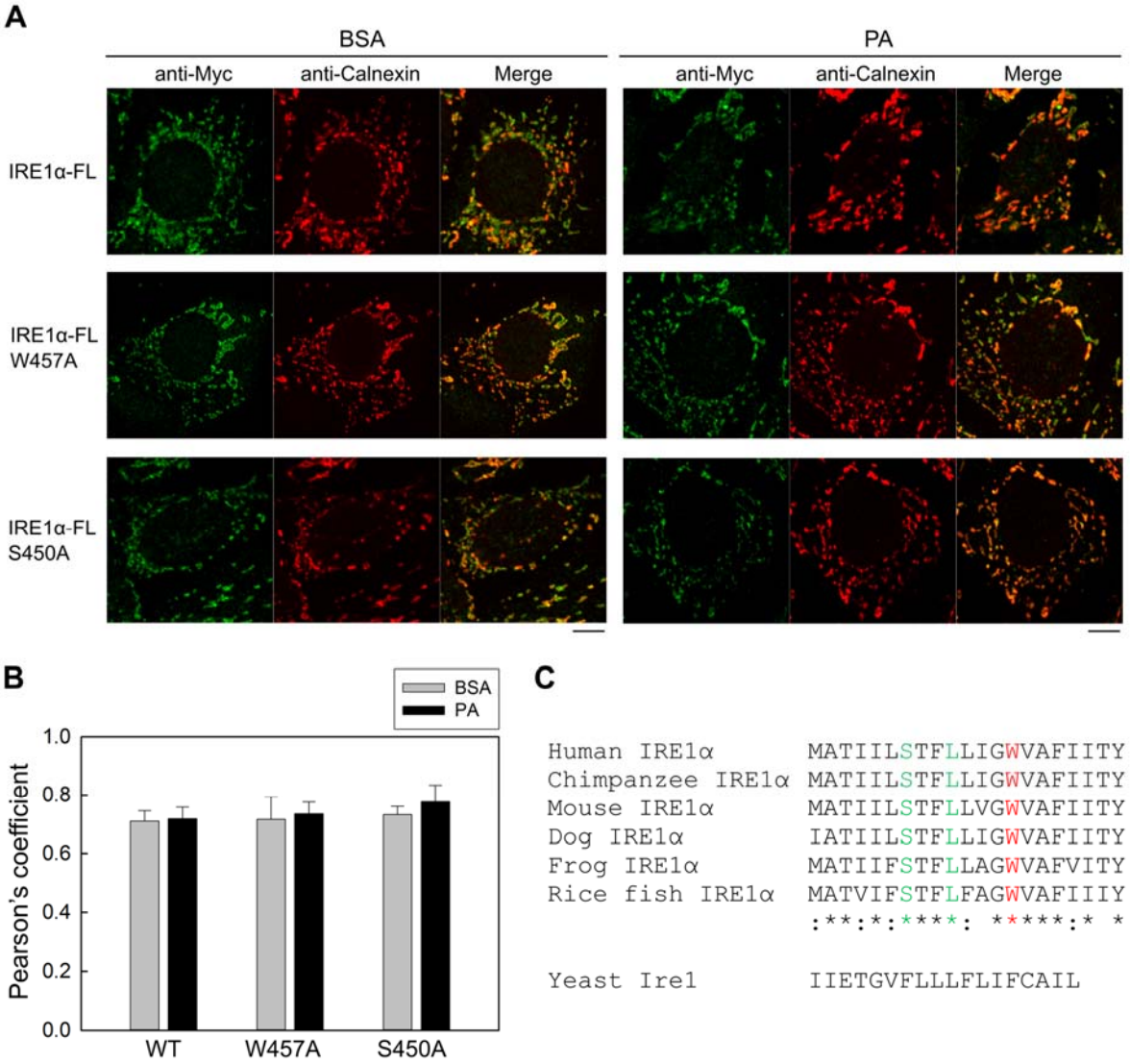

Figure S3. Intracellular localization of IRE1α-FL constructs and TMD sequence comparison of IRE1α. (Related to Figure 4)

(A) Hep3B cells were transfected with Myc-tagged VN-IRE1α-FL-WT and its corresponding mutants (W457A and S450A) and then treated with 2% BSA or 0.4 mM PA for 2 h. The cells were fixed and stained with rabbit anti-Myc tag and mouse anti-calnexin antibodies. Images were collected using an Olympus FV10 confocal microscope (with sequential acquisition). Scale bars 7 μm.

(B) Pearson's coefficient of IRE1α-FL variants and calnexin colocalization. Ten cells for each condition were analyzed using Image J software. Data indicate the mean ± SD (n = 10).

(C) Sequence alignment of IRE1α-TMDs. The TMD sequences were obtained from the Uniprot website (https://www.uniprot.org/). The tryptophan residues are highlighted in red while the serine and leucine residues in the SxxLxxx motif are in green. Note that the TMD of yeast Ire1 is not conserved with IRE1α. The alignment was performed using Cluster Omega.

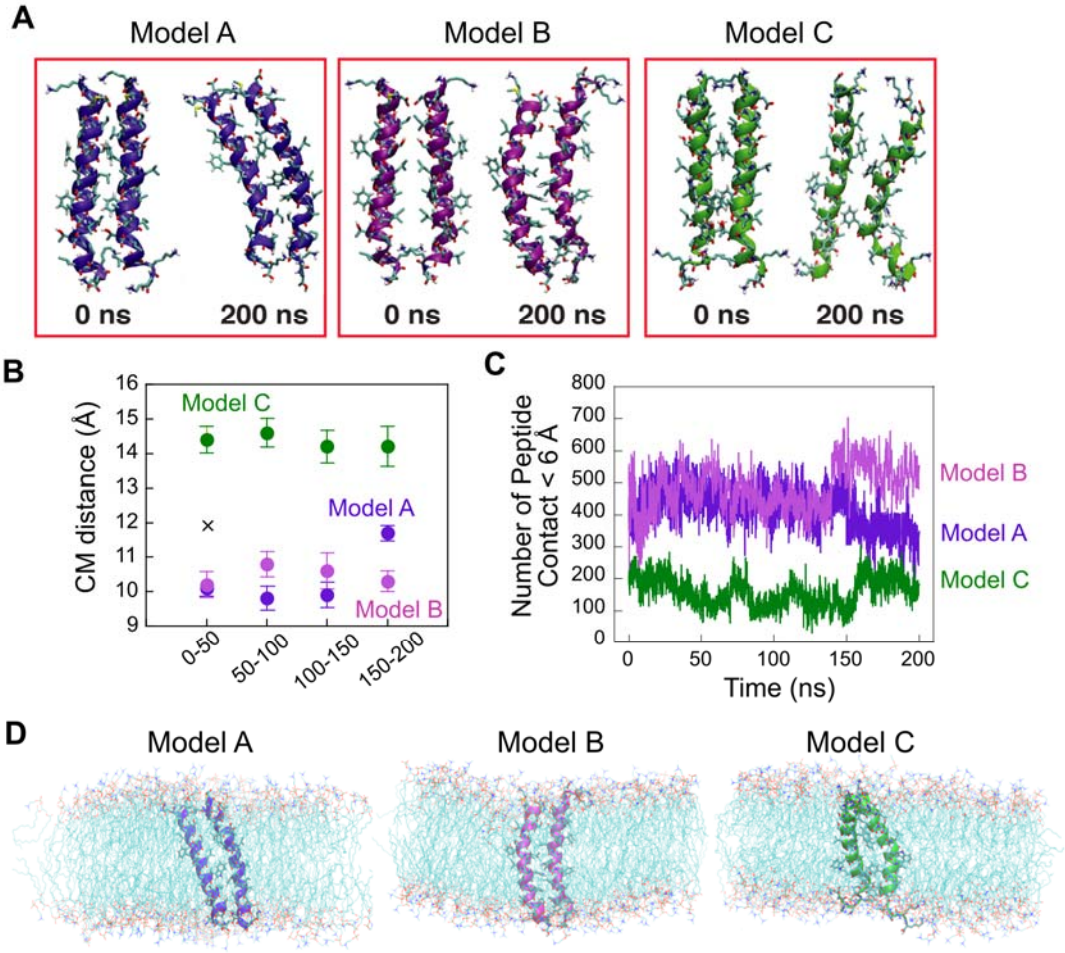

Figure S4. MD simulations of the IRE1α-TMD peptides in models A, B, and C. (Related to Figure 5)

(A) Initial (0 ns) and final (200 ns) conformations of models A, B, and C. The peptide backbone is shown as a cartoon. The lipids and water are not shown for clarity.

(B) Average distance between the center of mass (CM) of two TMD peptides in models A (purple), B (magenta), and C (green). The initial distance between the two peptides in the three models is indicated by an "X". The average distance was calculated by dividing the simulation into 4 blocks of 50 ns time steps. Values are mean ± SD, with n = 2500.

(C) Time evolution of the number of atomic contacts between the two TMD peptides in models A, B, and C. The number of contacts corresponds to the number of non-hydrogen atoms from the two TM peptides that come within 6 Å of each other.

(D) Average conformations of the peptides in the POPC lipid bilayer in models A, B, and C calculated over the last 50 ns of the simulation. The water molecules are not shown for clarity. The peptide backbone is shown as a cartoon and the aliphatic chains of the lipids are colored in cyan, the oxygen in red, the nitrogen in blue, and the phosphorus in brown.

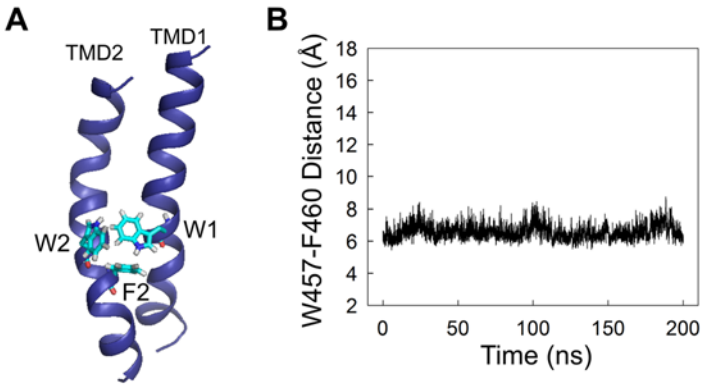

Figure S5. MD simulation analysis of model A. (Related to Figure 5)

(A) Aromatic interaction between Trp457 and Phe460 in the simulation of model A. A representative structure of the T-shaped stacking interaction formed by Trp457 in TMD1 (W1) and Phe460 in TMD2 (F2) is shown. Note that Trp457 in TMD2 (W2) is in-close proximity with the other Trp457 in TMD1 (W1). The red, blue, white, and cyan colors represent oxygen, nitrogen, hydrogen, and carbon atoms, respectively.

(B) Distance between the CM of the indole ring of Trp457 and the benzene ring of Phe460.

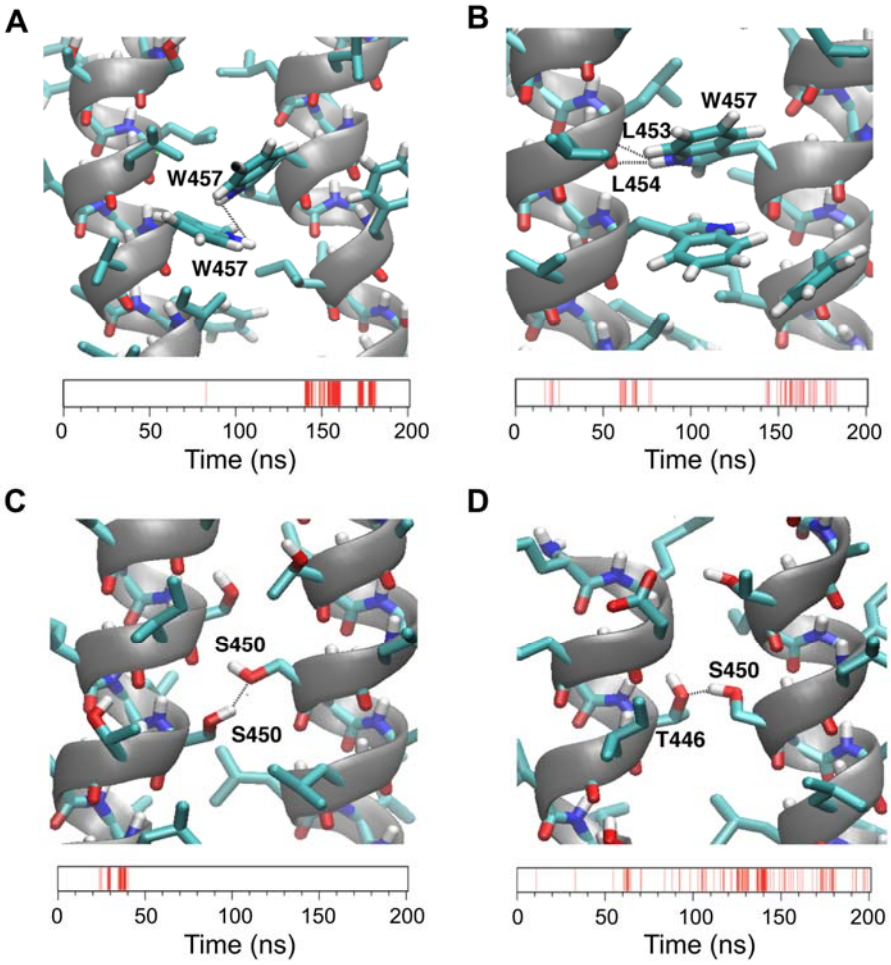

Figure S6. H-bonds detected during the simulation of model B and persistence map of the H-bonds. (Related to Figure 5)

(A) A representative example of H-bond formed by the NH indole group of Trp457 that emerged from the simulation.

(B) A representative example of H-bonds between NH of the indole group of the Trp457 residue and the carbonyl groups of the Leu453 and Leu454 residues.

(C) A representative example of the H-bond between the OH groups on the side chains of the Ser450 residues.

(D) A representative example of the H-bond between the OH groups on the side chains of the Ser450 and Thr446 residues. The red, blue, white, and cyan colors represent oxygen, nitrogen, hydrogen, and carbon atoms, respectively. The presence of H-bond is represented by the red sticks in the persistence map (lower panel).

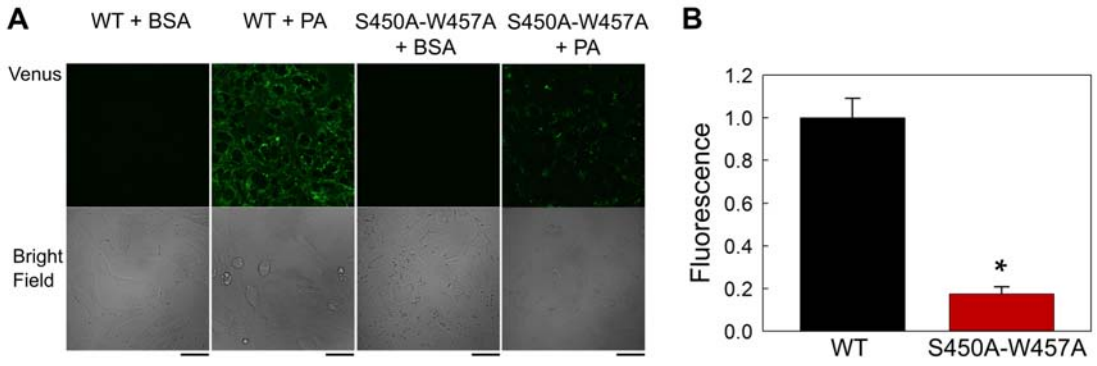

Figure S7. BiFC analysis of IRE1α-WT-FL and its double mutant W457A-S450A. (Related to Figure 6)

(A) IRE1α$^{-/-}$ MEF cells expressing IRE1α-WT-FL or its double mutant W457A-S450A were treated with 2% BSA or 0.4 mM PA in 2% BSA media for 2 h. Venus fluorescence was detected with confocal microscopy. Bars 42μm

(B) Quantification of Venus fluorescence in (A). The CTCF values were calculated and the fluorescence fold-change upon PA treatment was normalized to WT. Values are reported as mean ± SD (n = 3), *p < 0.001 (Student's t-test).