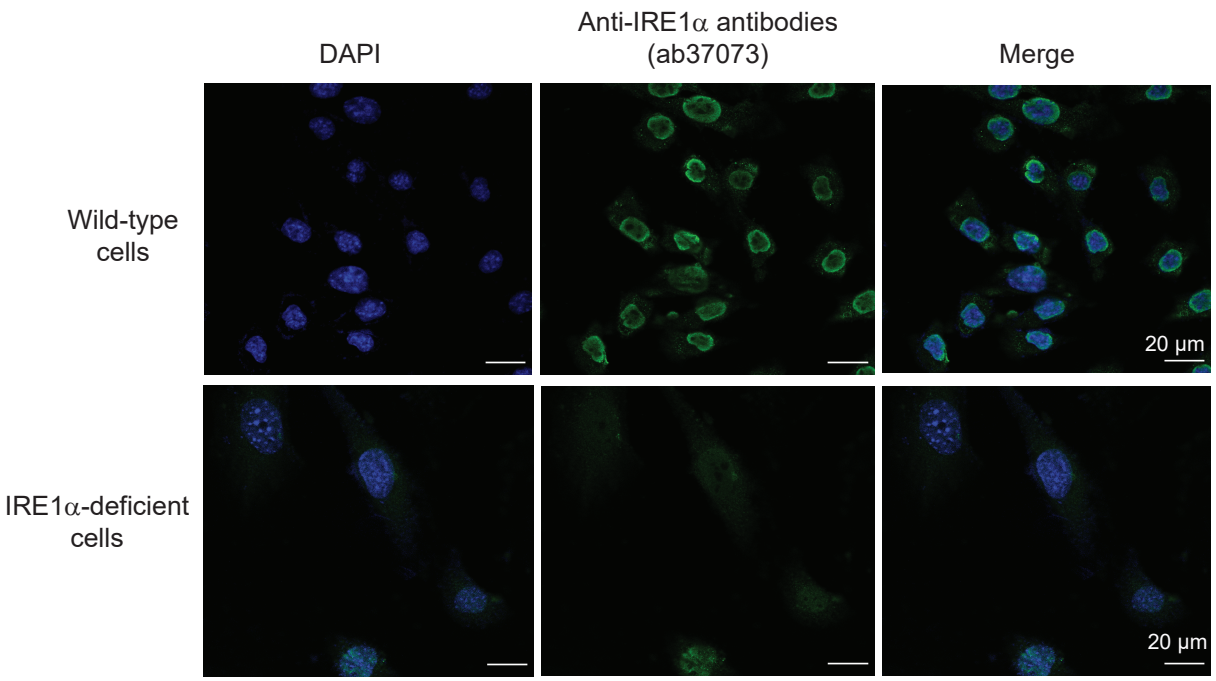
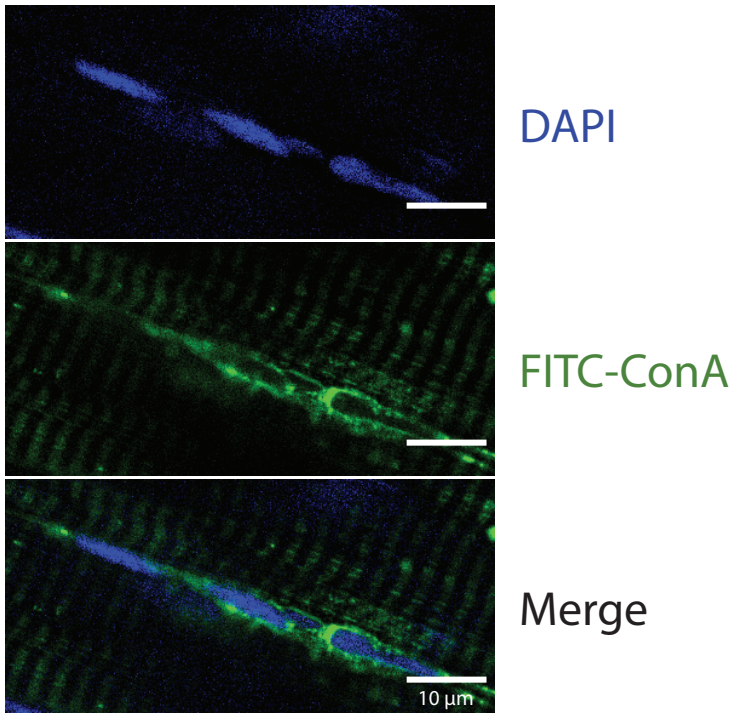
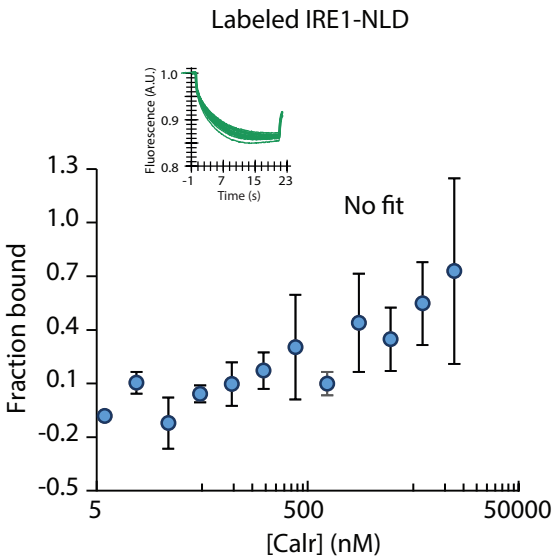


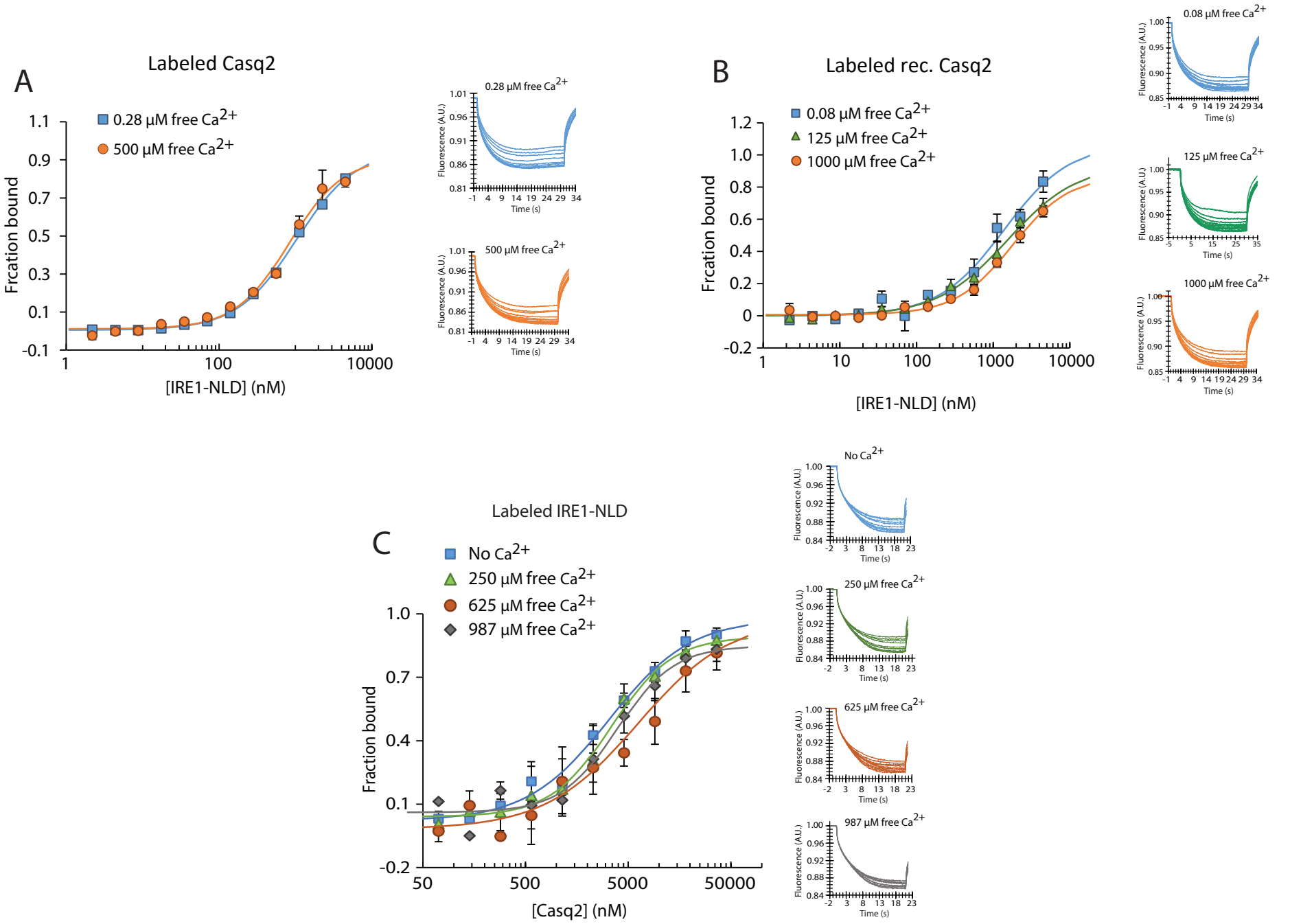
Supplemental Figure S1. Immunostaining of wild-type and IREα-deficient mouse embryonic fibroblasts. Wild-type and *Ern1*$^{-/-}$ mouse embryonic fibroblasts (lack Ire1α) were probed with anti-IRE1α (Abcam, ab37073) antibodies and visualized using a Leica TCS SP5 confocal microscope.



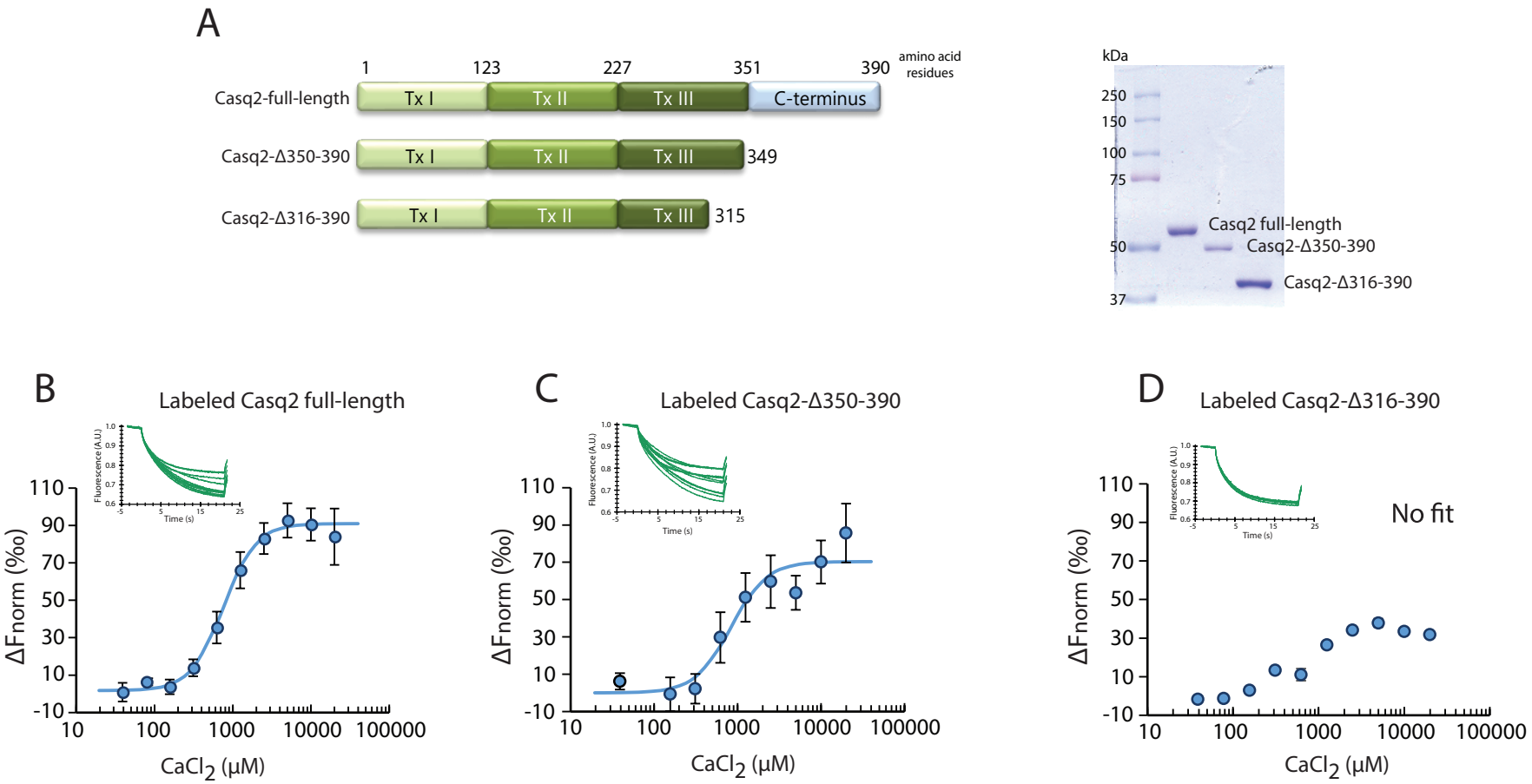
Supplemental Figure S2. Perinuclear ER-like membrane network in skeletal muscle. Longitudinal sections of skeletal muscle were stained with FITC-conjugated Concanavalin A (FITC-ConA) and DAPI.



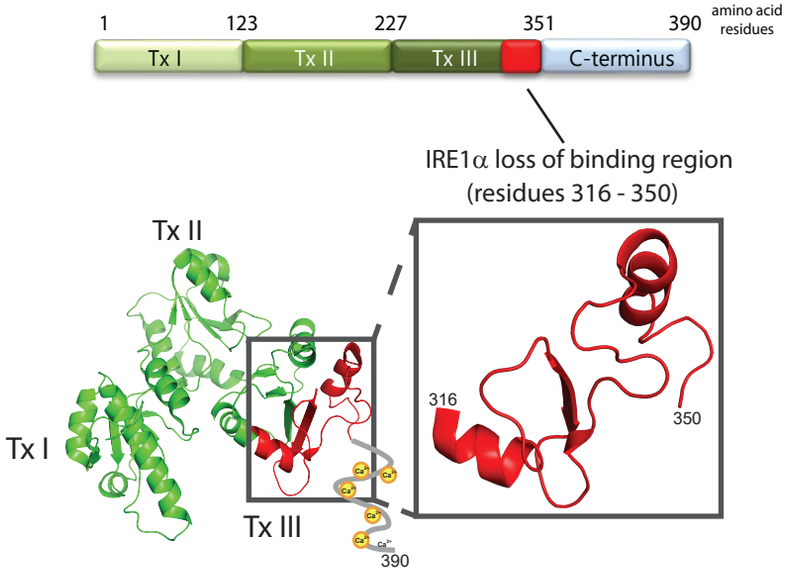
Supplemental Figure S3. Calreticulin does not bind to IRE1 luminal domain. Luminal domain of IRE1 (IRE1-NLD) was covalently labeled with a red fluorescent tag and incubated with increasing amounts of purified calreticulin (Calr) followed by microscale thermophoresis. Normalized microscale thermophoresis time traces are shown above. Each data point is the average of three independent microscale thermophoresis measurements.



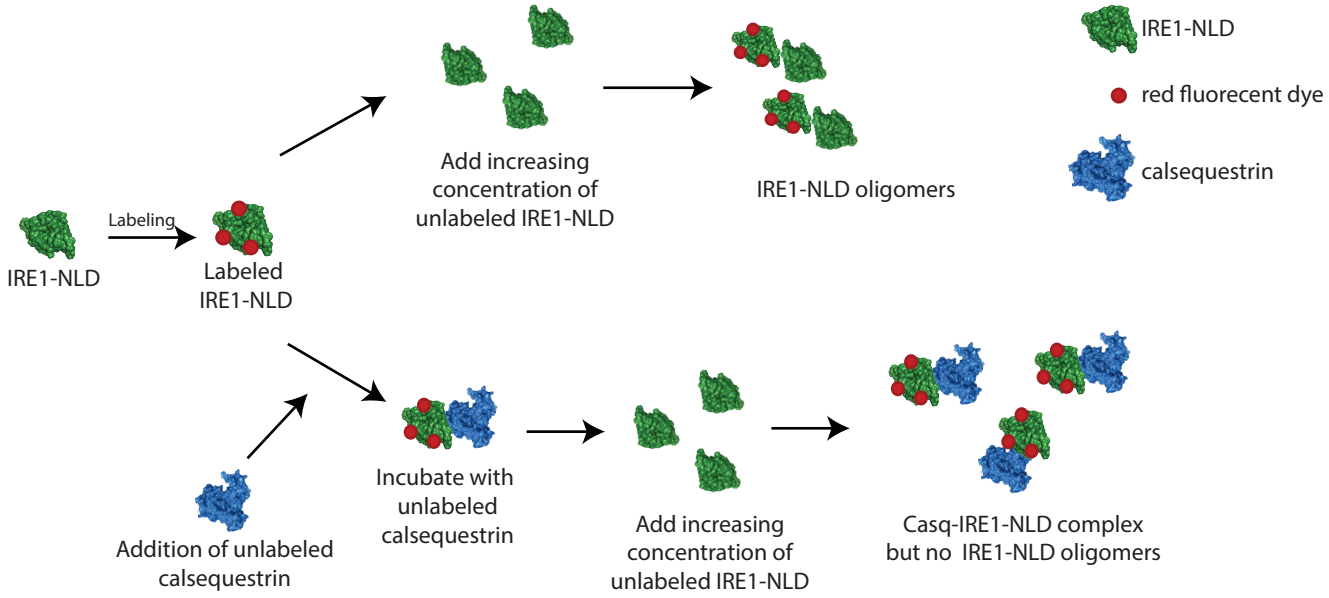
Supplemental Figure S4. Calsequestrin-IRE1-NLD interaction in the presence of Ca^{2+}. A. Cardiac calsequestrin (Casq2) protein was covalently labeled with a red fluorescent tag and incubated in the presence of different Ca^{2+} concentrations as indicated in the Figure followed by microscale thermophoresis analysis. **B.** Labeled recombinant cardiac calsequestrin (rec. Casq2) protein was incubated with increasing Ca^{2+} concentrations as indicated in the Figure followed by microscale thermophoresis.
C. Fluorescent labeled N-terminus luminal domain of IRE1α (IRE1-NLD) protein was incubated with native cardiac Casq2 and increasing Ca^{2+} concentrations as indicated in the Figure followed by microscale thermophoresis. Normalized time traces are shown in the graph. Normalized microscale thermophoresis time traces are shown to the right of the graphs. In the Figure, each data point is the average of three independent microscale thermophoresis measurements.



Supplemental Figure S5. Ca^{2+} binding to cardiac calsequestrin. A. Schematic representation of truncated calsequestrin protein used for label free microscale thermophoresis analysis shown in *B, C* and *D*. The C-terminus truncations of cardiac calsequestrin with deleted residues 350 to 390 (*Casq2 Δ350-390*) or residues 316 to 390 (*Casq2 Δ316-390*). *Right panel:* Coomassie blue stained SDS-PAGE of purified full-length and truncated recombinant cardiac calsequestrin used for microscale thermophoresis analysis. **B, C, D.** Recombinant cardiac calsequestrin (**B**), residues 350 to 390 truncated calsequestrin (*Casq2 Δ350-390*) (**C**) or residues 316 to 390 truncated protein (*Casq2 Δ316-390*) (**D**) were incubated with increasing concentration of Ca^{2+}. Each data point is the average of three to six independent microscale thermophoresis measurements.



Supplemental Figure S6. A model of the third thioredoxin-like domain in cardiac calsequestrin binding to the ER luminal domain of IRE1α binding. Schematic representation of the cardiac calsequestrin with red labeled 316-350 region of the Δ316-390 protein. Tx, thioredoxin domains. PDB ID: 2VAF.



Supplemental Figure S7. Schematic representation of the IRE1α dimerization assay.