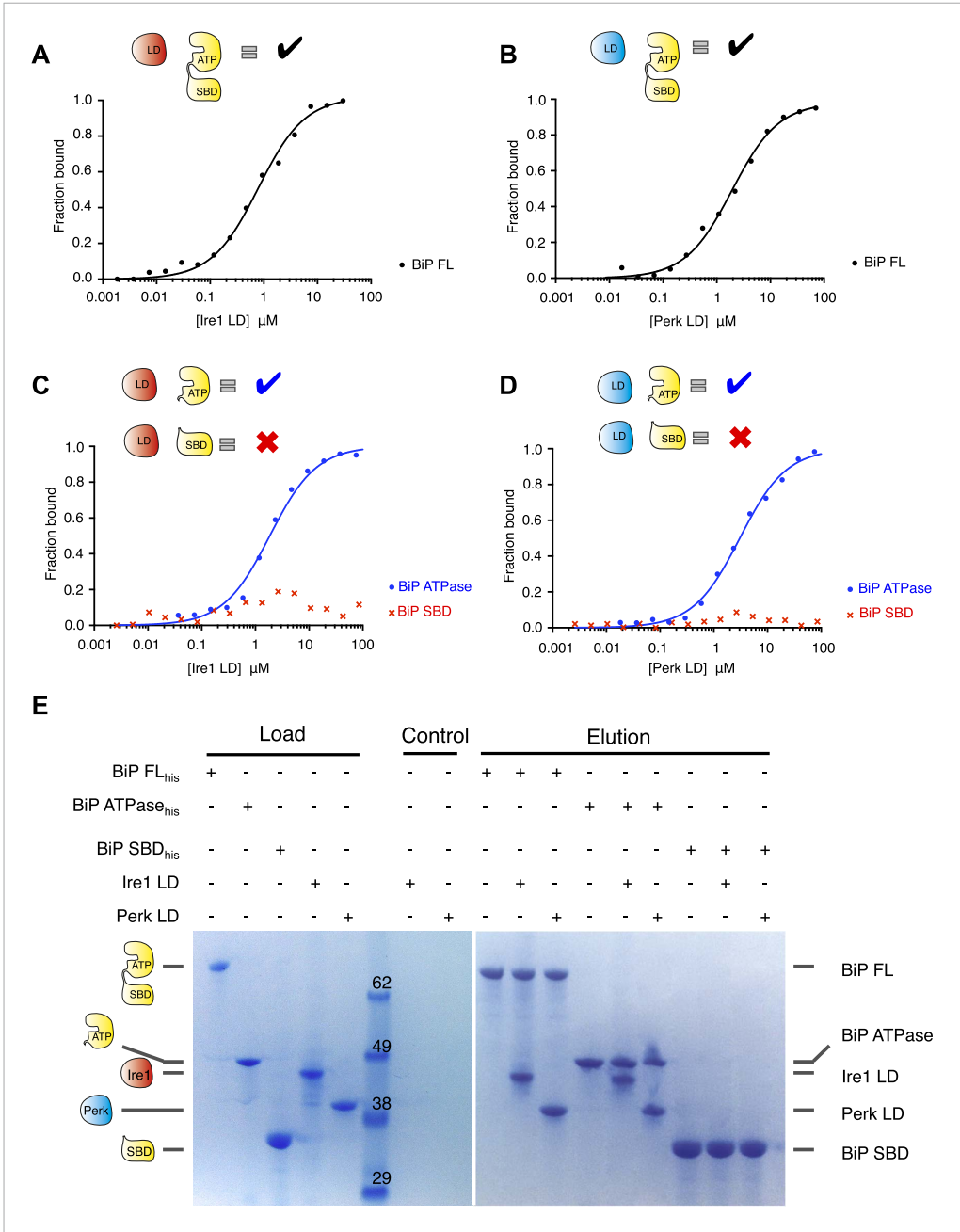


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## Figures and figure supplements

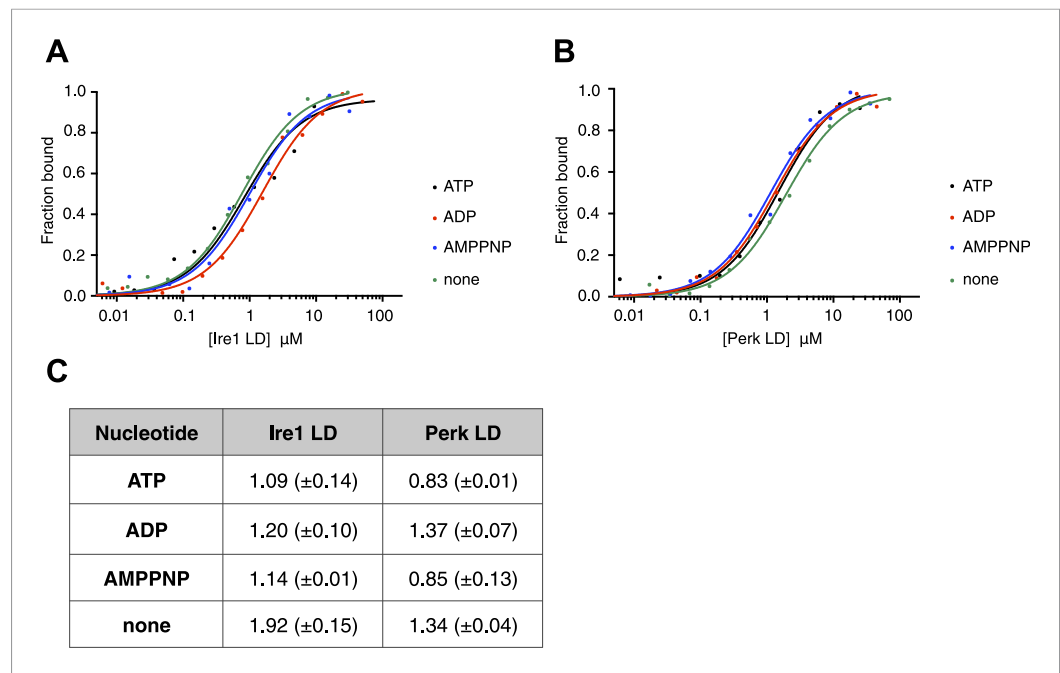
Noncanonical binding of BiP ATPase domain to Ire1 and Perk is dissociated by unfolded protein C<sub>H</sub>1 to initiate ER stress signaling

**Marta Carrara, et al.**



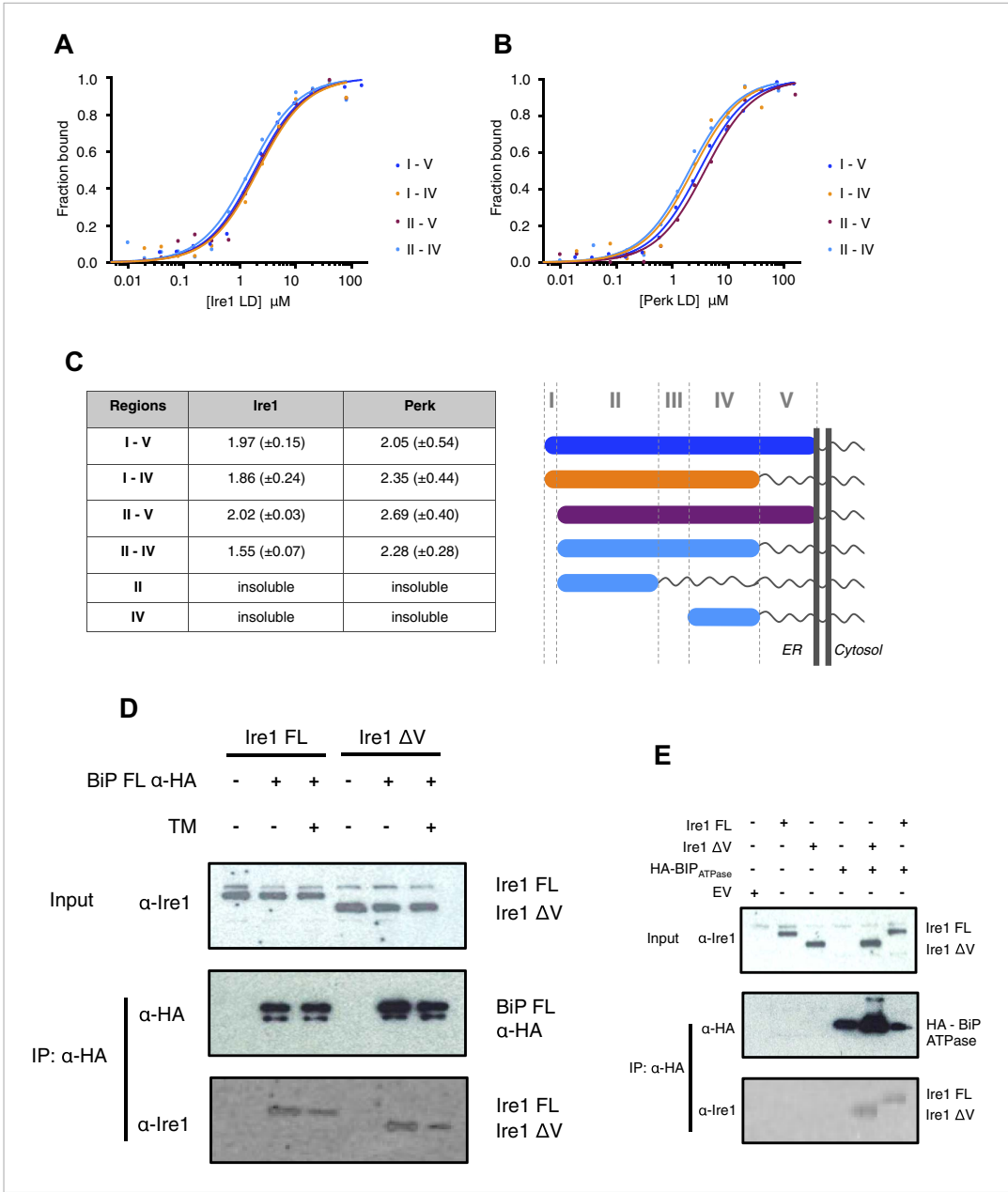
**Figure 1.** Noncanonical binding of BiP ATPase domain to Ire1 and Perk. **(A–B)** Microscale thermophoresis (MST) analysis showing sigmoidal binding curves for interaction between full-length BiP and the complete luminal domains (region I–V) of **(A)** Ire1 luminal domain ( $K_d = 1.33 \mu\text{M}$ ) and **(B)** Perk luminal domain ( $K_d = 1.92 \mu\text{M}$ ). **(C–D)** MST binding curves of interaction between BiP sub-domains (ATPase and substrate binding domain) and **(C)** Ire1 luminal domain (ATPase  $K_d = 1.97 \mu\text{M}$ ; no binding to substrate binding domain) and **(D)** Perk luminal domain (ATPase  $K_d = 2.05 \mu\text{M}$ ; no binding to substrate binding domain). **(E)** Pull down assay showing BiP-luminal domain complexes using His<sub>6</sub>-tagged BiP proteins and luminal domains of Perk and Ire1 visualized by coomassie brilliant blue stained SDS PAGE gel. Ire1 and Perk luminal domains bind to full-length BiP and BiP ATPase domain. No binding to BiP substrate binding domain was observed.

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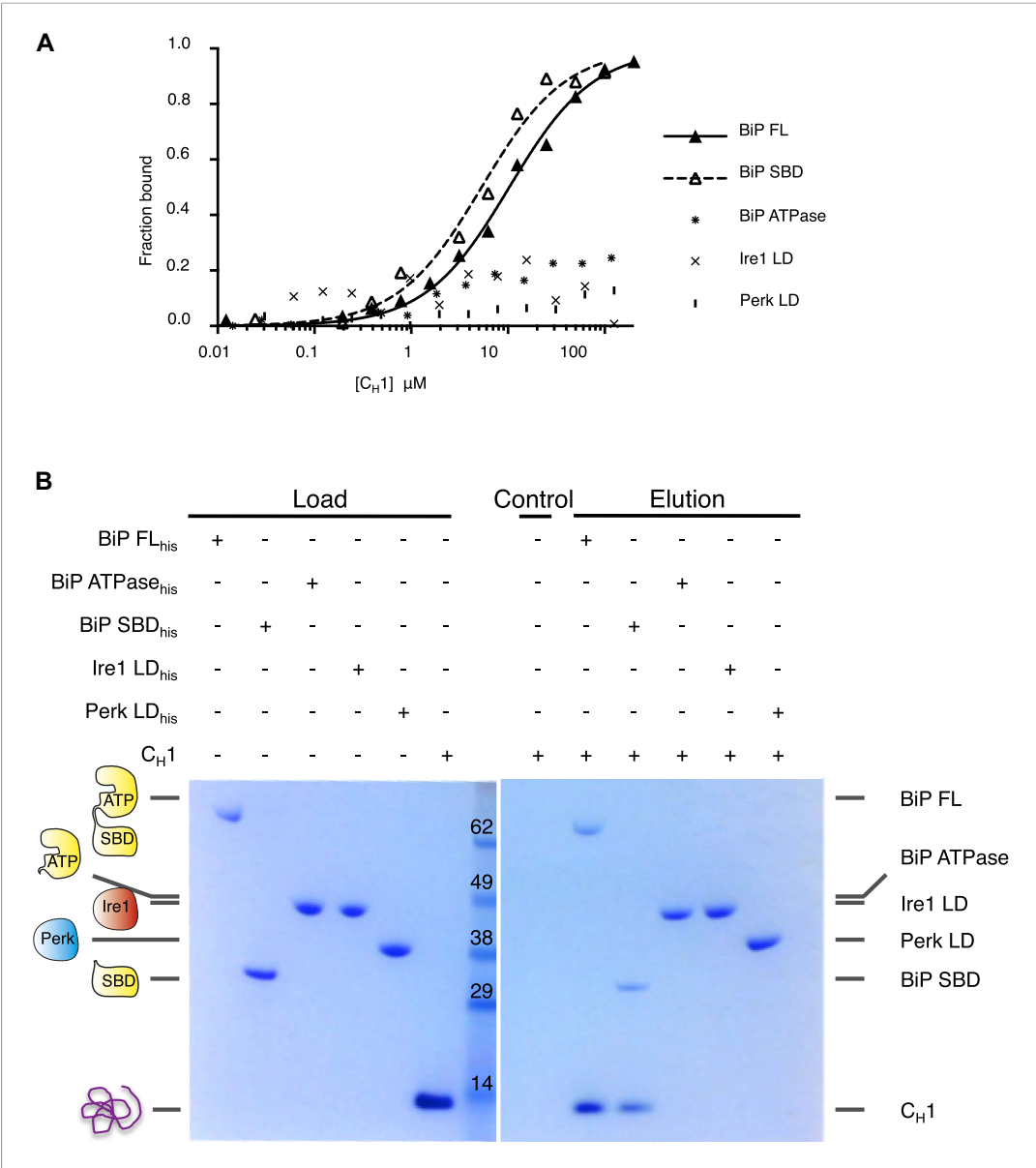
**Figure 2.** The noncanonical binding of BiP ATPase domain to Ire1 and Perk is independent of nucleotides. **(A–B)** MST analysis showing sigmoidal binding curves for full-length BiP interaction with **(A)** Ire1 luminal domain and **(B)** Perk luminal domain in the presence of 10 mM ATP, ADP, AMPPNP and in the absence of nucleotides. **(C)** List of  $K_d$  values ( $\mu\text{M} \pm \text{SE}$ ) for Ire1 and Perk luminal domain interactions with full-length BiP in the presence of nucleotides for the binding curves represented in **A** and **B**. Binding between luminal domains and BiP was not affected by the presence of the various nucleotides.

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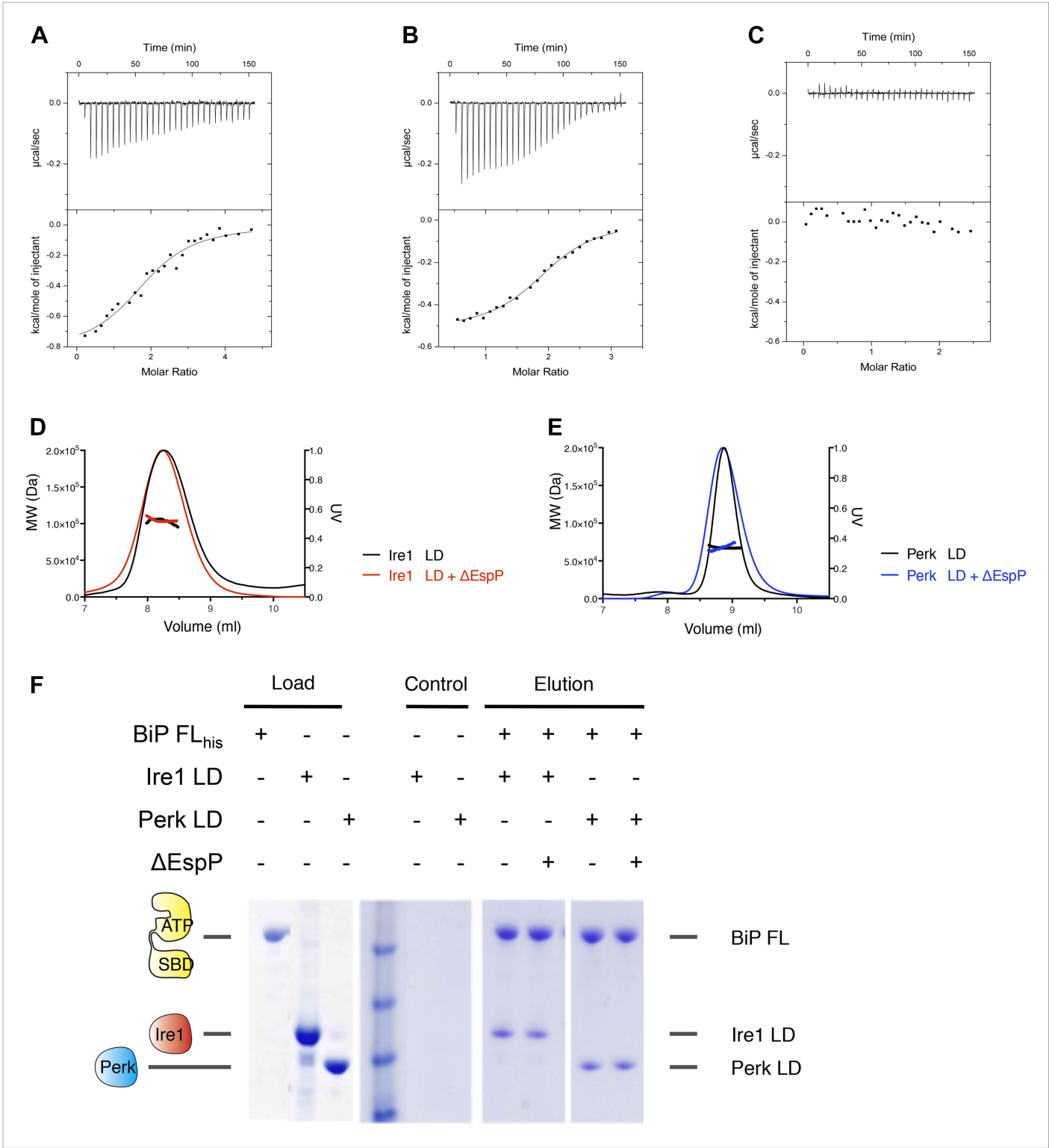


**Figure 3.** Core interaction between BiP ATPase and luminal domains occurs via region II-IV of luminal domains. **(A–B)** MST binding curves of interaction between BiP ATPase and different length constructs of **(A)** Ire1 luminal domain and **(B)** Perk luminal domain. **(C)** List of  $K_d$  values ( $\mu\text{M} \pm \text{SE}$ ) for BiP ATPase interaction with the various Ire1 and Perk luminal domain constructs (based on regions I–V) for binding curves represented in **A** and **B**. The luminal domain region II–IV, is solely responsible for binding to BiP proteins and regions I and V are dispensable in this interaction. **(D)** Co-immunoprecipitation experiment in which HEK293T cells were co transfected with either Ire1 mutant lacking region V (Ire1ΔV; Δ390–430) or full-length Ire1, along with HA-tagged BiP, in the absence or presence of ER stress (TM = 5  $\mu\text{M}$ ; 4 hr tunicamycin). Immunoprecipitating with HA peptide and then immunoblotting with Ire1 specific antibody reveals an interaction between BiP and both full-length and mutant Ire1 that is missing region V (Ire1 ΔV), which is reduced after ER stress. This interaction in cells reinforces the in vitro data. **(E)** Co-immunoprecipitation experiment similar to **(D)**, but cells were co transfected with HA-tagged BiP ATPase and were not subjected to ER stress with tunicamycin.

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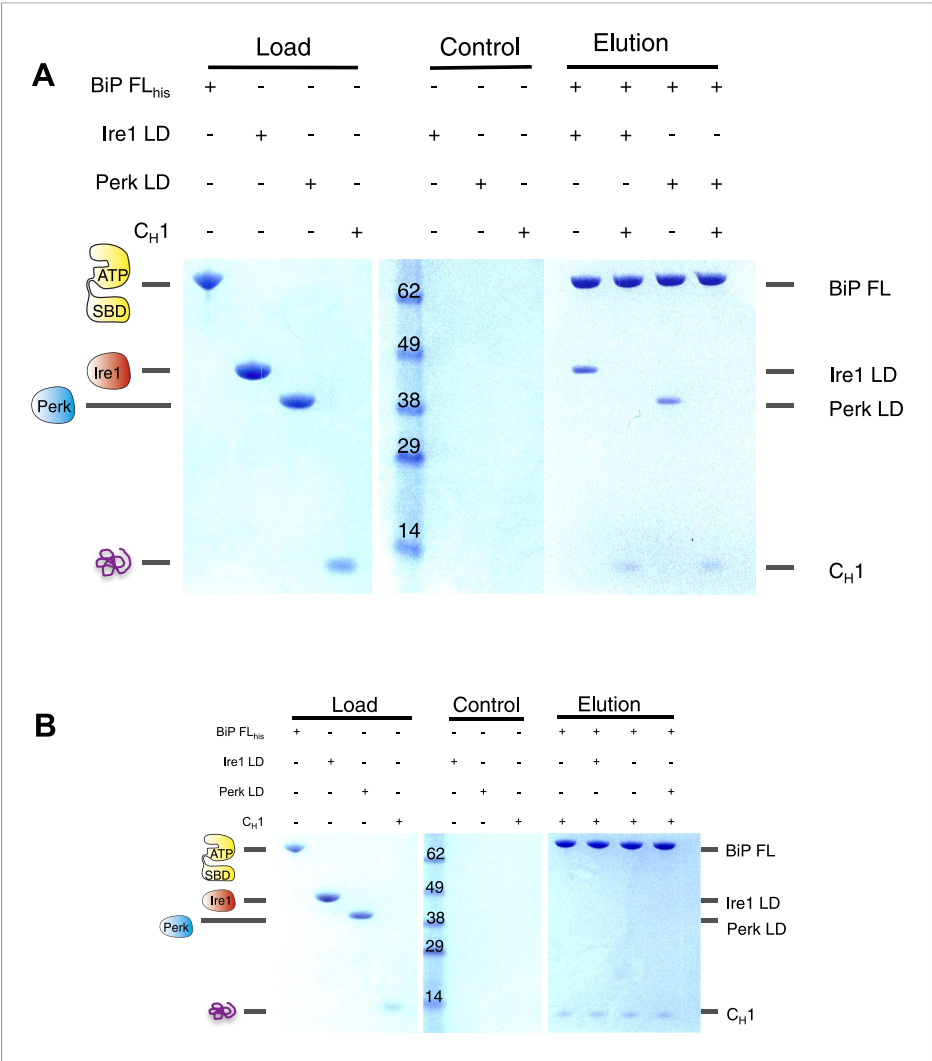
DOI: 10.7554/eLife.03522.007



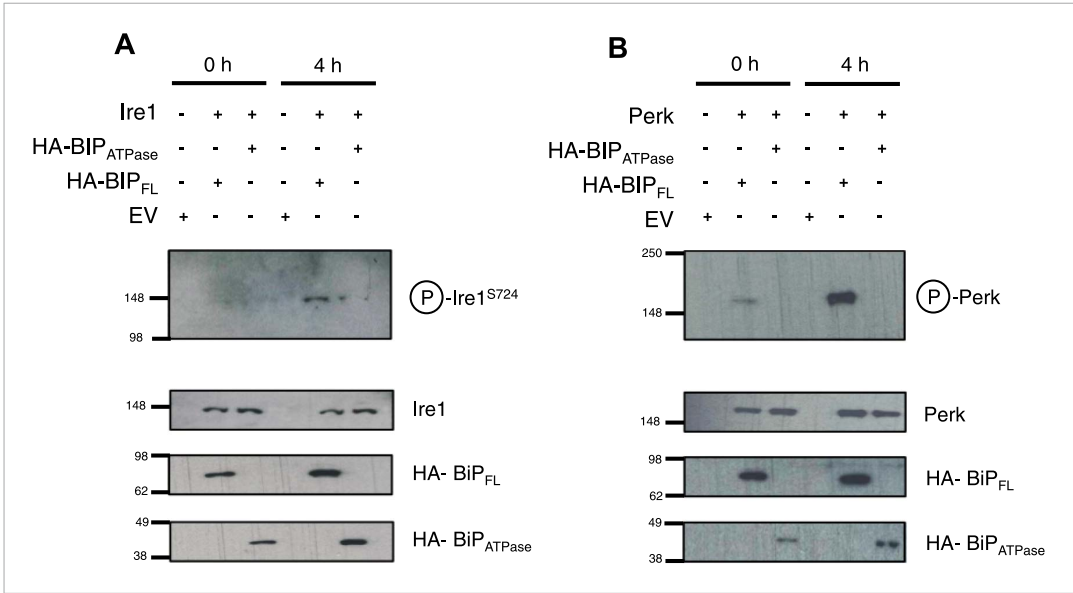
**Figure 4—figure supplement 1.** Assessing the role of unfolded protein peptide mimic (ΔEspP) in UPR stress sensing. (A–C) ITC binding curves for ΔEspP titration into (A) Ire1 luminal domain ( $K_d = 6.4 \mu\text{M}$ ;  $N = 1.97$ ), (B) Perk luminal domain ( $K_d = 9.3 \mu\text{M}$ ;  $N = 1.98$ ), (C) full-length BiP (no binding). (D) SEC MALS analyses showing Ire1 luminal domain is dimeric in solution ( $\text{MW} = 104.5 \text{ kDa}$ ) and the addition of ΔEspP has no effect on Ire1 luminal domain oligomeric state ( $\text{MW} = 103.2 \text{ kDa}$ ). (E) SEC MALS analyses showing Perk luminal domain is dimeric in solution ( $\text{MW} = 67.8 \text{ kDa}$ ) and the addition of ΔEspP has no effect on Perk luminal domain oligomeric state ( $\text{MW} = 67.6 \text{ kDa}$ ). (F) Pull down assay to test the effect of ΔEspP on His<sub>6</sub>-tagged full-length BiP-luminal

Figure 4—figure supplement 1. continued on next page

Figure 4—figure supplement 1. Continued  
domain complexes. ΔEspP has no visible effect upon BiP-luminal domain complexes.  
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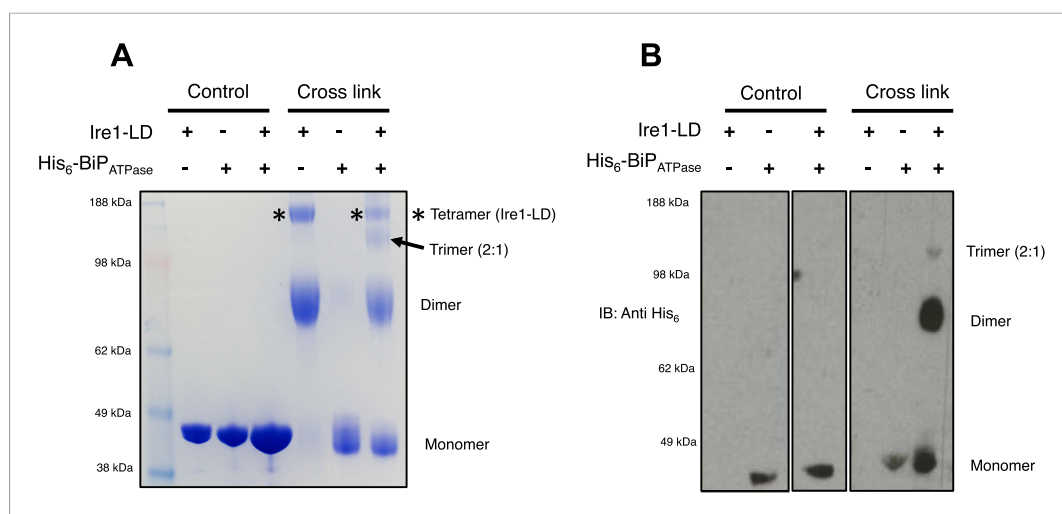
**Figure 5.** The unfolded protein C<sub>H</sub>1 dissociates the noncanonical interaction between BiP ATPase domain and the luminal domain of Ire1 or Perk. **(A)** Pull down assay assessing the effects upon addition of unfolded protein C<sub>H</sub>1 to His<sub>6</sub>-tagged full-length BiP-luminal domain complexes. C<sub>H</sub>1 disrupts BiP-luminal domain interaction and causes the complexes to dissociate. **(B)** When His<sub>6</sub>-tagged full-length BiP is initially incubated with C<sub>H</sub>1 and then subsequently Ire1 and Perk luminal domains are added, we see no binding between luminal domains and BiP indicating that luminal domains and C<sub>H</sub>1 binding to BiP are mutually exclusive.  
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**Figure 6.** BiP deletion mutants, lacking the substrate binding domain, attenuate UPR signaling. **(A)** Ire1 was co expressed with either full-length BiP (HA-BiP<sub>FL</sub>) or BiP ATPase domain, lacking the substrate binding domain (HA-BiP<sub>ATPase</sub>), and challenged with tunicamycin (5  $\mu$ M) over 0 hr and 4 hr time points in Ire1<sup>-/-</sup> cells. S724 phosphorylated Ire1 was measured as an indicator of UPR signaling (Ali et al., 2011) using pIre1<sup>S724</sup> antibody (ICR). Cells expressing full-length BiP and Ire1 were fully able to respond to induced ER stress, whilst expression with BiP ATPase domain attenuated UPR signaling. EV = empty vector. **(B)** Similar to **(A)**, but expressing Perk full-length in Perk<sup>-/-</sup> cells and using pPerk antibody (Santa Cruz).

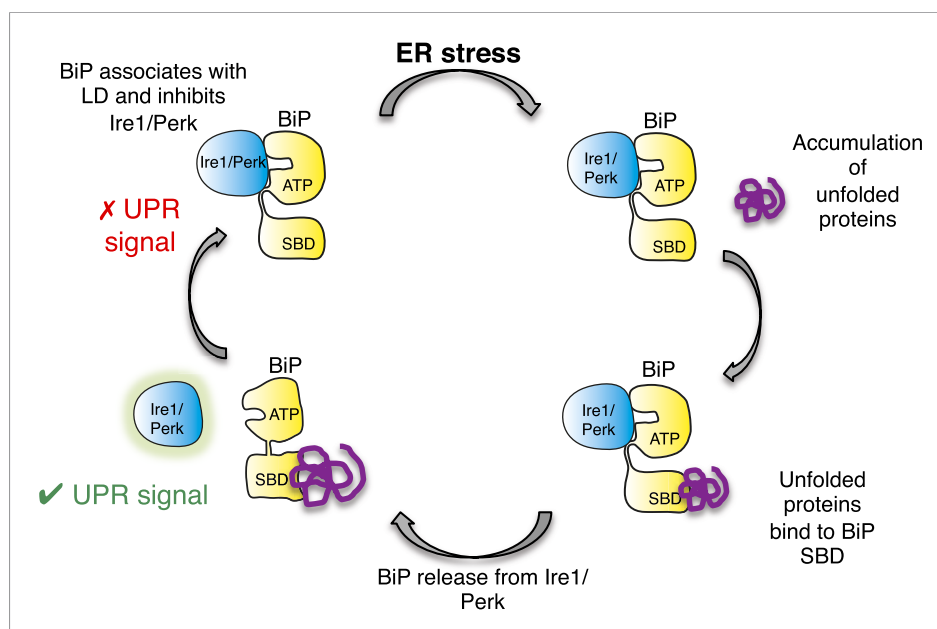
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**Figure 7.** BiP impedes Ire1 LD tetramer formation. **(A)** Ire1 luminal domain (LD; regions II–IV) and His<sub>6</sub>-tagged BiP ATPase domain proteins, both individually and in 1:1 molar ratio mixture, were visualized as control lanes on a 4–12% Bis-Tris SDS-PAGE gel. The same proteins were then subjected to EGS cross linker for 1 hr, after which the reaction was quenched and samples were visualized along side control lanes. In the presence of cross linker, Ire1 LD forms dimer and tetramer species; when in a mixture with BiP ATPase, there is a reduction in the corresponding tetramer band (\*). Also, a band appears that is consistent in size with a trimer species. **(B)** Samples from **(A)** were immunoblotted using anti-His<sub>6</sub> antibody, which detects His<sub>6</sub>-tagged BiP ATPase domain protein. Since BiP ATPase protein is monomeric in the absence or presence of cross linker, BiP ATPase forms hetero dimer and hetero trimer with Ire1 LD. The binding of BiP to Ire1 reduces the size of the tetramer (\*) that is exclusively formed by Ire1 LD, leading to the conclusion that BiP inhibits Ire1 LD tetramer formation.

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**Figure 8.** Allosteric model of UPR induction. In the absence of misfolded protein, BiP interacts with UPR luminal domains, which acts to repress the UPR signal. Upon ER stress, unfolded protein binds to the canonical BiP substrate binding domain, which in turn causes the noncanonical BiP-luminal domain interaction to dissociate, ultimately leading to UPR signal activation/propagation.

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