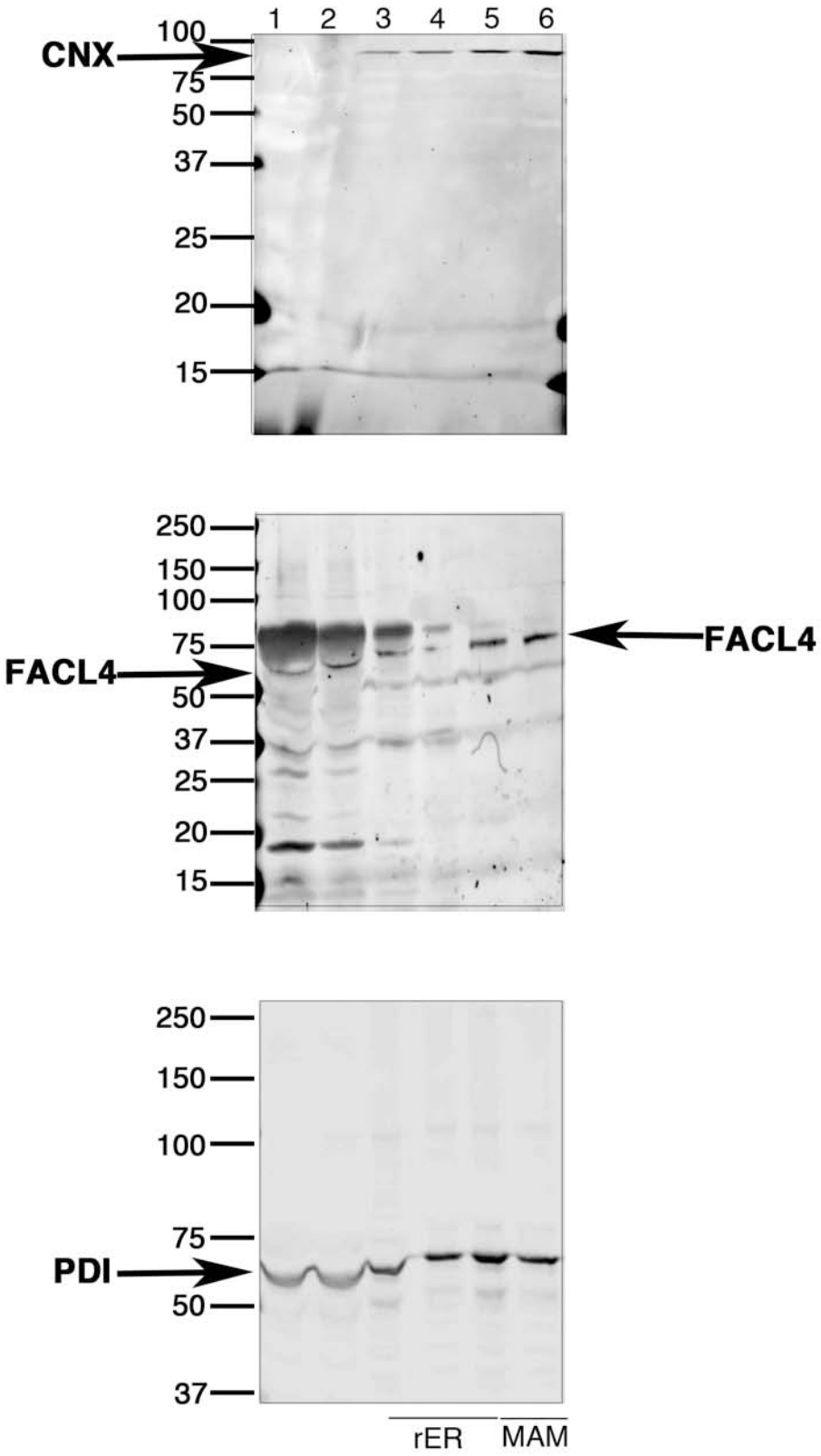
Fig. S1. Full gels for Figure 1B. Representations of the control gels seen in Fig. 1B for calnexin, FACL4, and PDI. Molecular weight markers are shown on the left and arrows indicate the bands of interest.

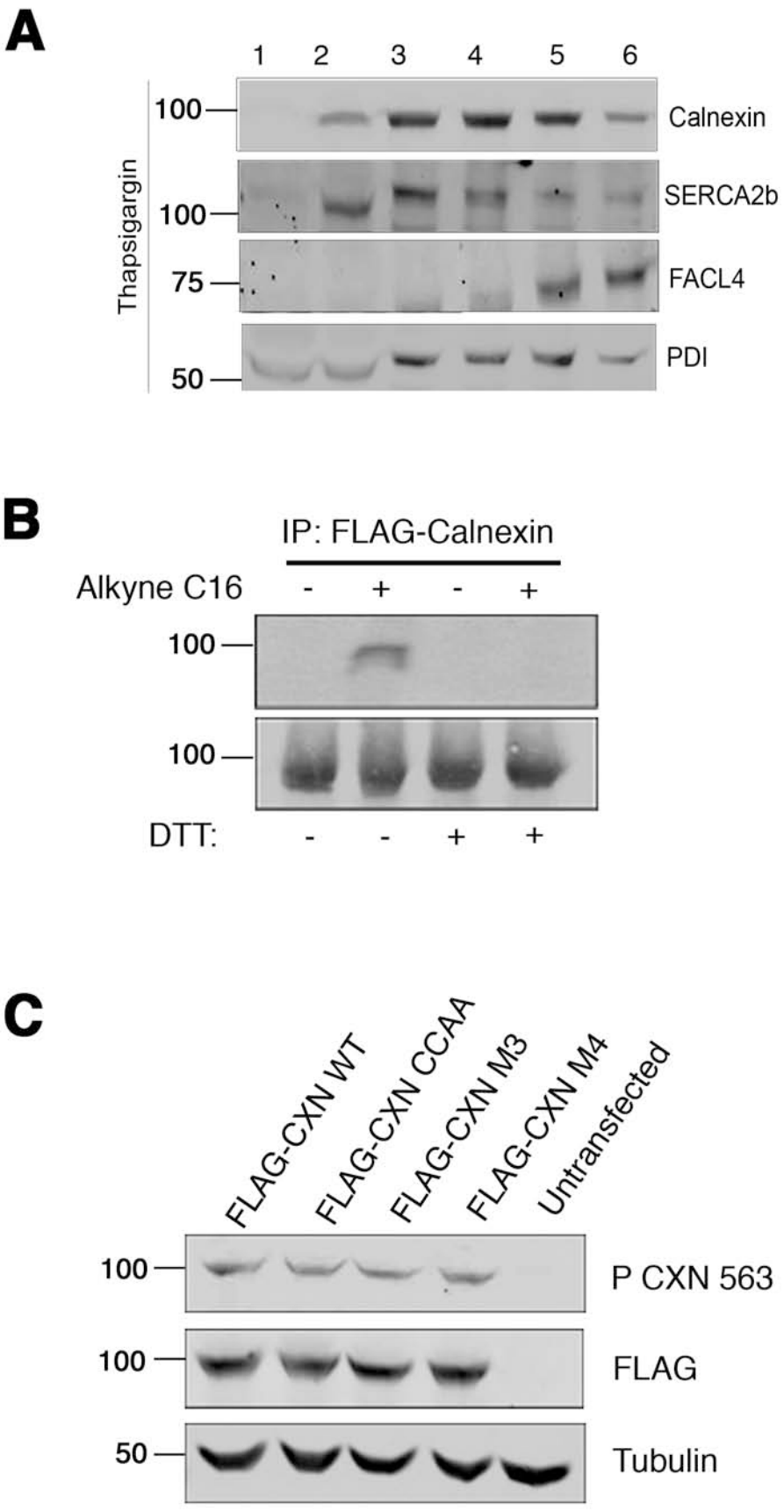
Fig. S2. Additional analysis of calnexin palmitoylation and phosphorylation. A. Optiprep fractionation following thapsigargin treatment. Homogenized HeLa cell lysates were separated via Optiprep following a 4h treatment with 1.5 μM Thapsigargin into 6 fractions. Membrane fractions were analyzed as indicated by SDS-PAGE and Western Blot for PDI (pan-ER), SERCA2b and FACL4 (MAM), as well as calnexin. **B.** Calnexin palmitoylation is reduced during DTT stress. HeLa cells were incubated for 4h with 5 mM DTT and then processed for click chemistry as described (Lynes et al., 2012). **C.** Neither calnexin palmitoylation nor the phosphorylation status of serines 554 and 564 influence serine 583 phosphorylation. Calnexin knockout (ko) MEFs and ko cells transfected with FLAG-tagged wild type CCAA, M3 (S554, 564→A), or M4 (S554, 564→D) calnexin were lysed and processed for Western blot using the phospho-serine 583 antibody.

Fig. S3. Influence of calnexin presence on mitochondria calcium import, mitochondria membrane potential and ER ability to trigger the unfolded protein response. A. Measurement of cytosolic calcium following thapsigargin-mediated ER calcium release. Calnexin knockout (ko) MEFs and ko cells transfected with FLAG-tagged wild type or CCAA calnexin were loaded with FURA-2. Cells were then treated with 1.5 μM thapsigargin and probe fluorescence was recorded before and after thapsigargin treatment by flow cytometry. The increases of fluorescence for the three conditions were not statistically different from each other. **B**. Plasmid-based measurement of mitochondrial calcium content following thapsigargin. Calnexin wildtype and knockout (ko) MEFs were transfected with a plasmid encoding mitochondria-targeted R-GECO-1. The increase in relative fluorescence units was assayed from three independent experiments following the protocol outlined in Materials and Methods. A representative curve for wild type and knockout cells is shown on the right. **C**. Measurement of mitochondrial membrane potential. Calnexin knockout (ko) MEFs and ko cells transfected with FLAG-tagged wild type or CCAA calnexin were loaded with TMRM and probe fluorescence was recorded by flow cytometry (Statistics: P=0.001 for calnexin wild type, P=0.4135 for calnexin CCAA). **D**. Xbp-1 splicing measured by reverse transcriptase PCR. Calnexin knockout (ko) MEFs and ko cells transfected with FLAG-tagged wild type or CCAA calnexin were treated with 1.5 μM thapsigargin for 16h, followed by analysis of the Xbp-1 mRNA. PCR products were separated on a 7.5% acrylamide gel for Xbp-1 and a 1% acrylamide gel for GAPDH.

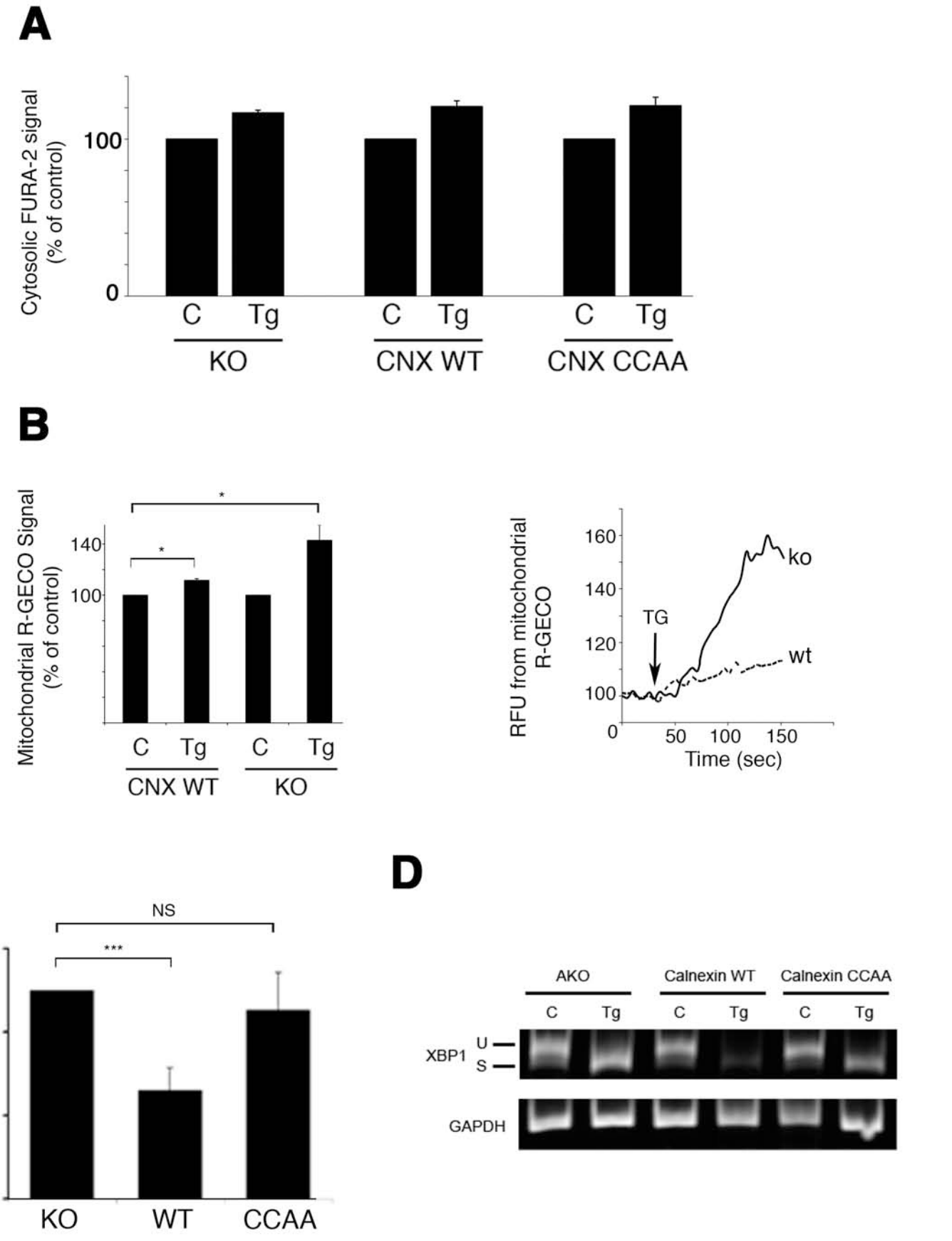
Fig. S4. 2BP promotes the calnexin chaperone activity. A. Calnexin-ERp57 co-immunoprecipitation following 2BP treatment. HeLa cells were treated for 4 h with 100 μM 2BP. DSP-crosslinked lysates (5% inputs) and calnexin immunoprecipitates were analyzed for calnexin and co-immunoprecipitating ERp57. P=0.07. **B.** LDLR surface biotinylation following 2BP treatment. HeLa cells were lysed and processed for Western blot using the LDLR antibody. In parallel, the same set of cells was processed for surface biotinylation and probed for biotinylated surface LDLR.



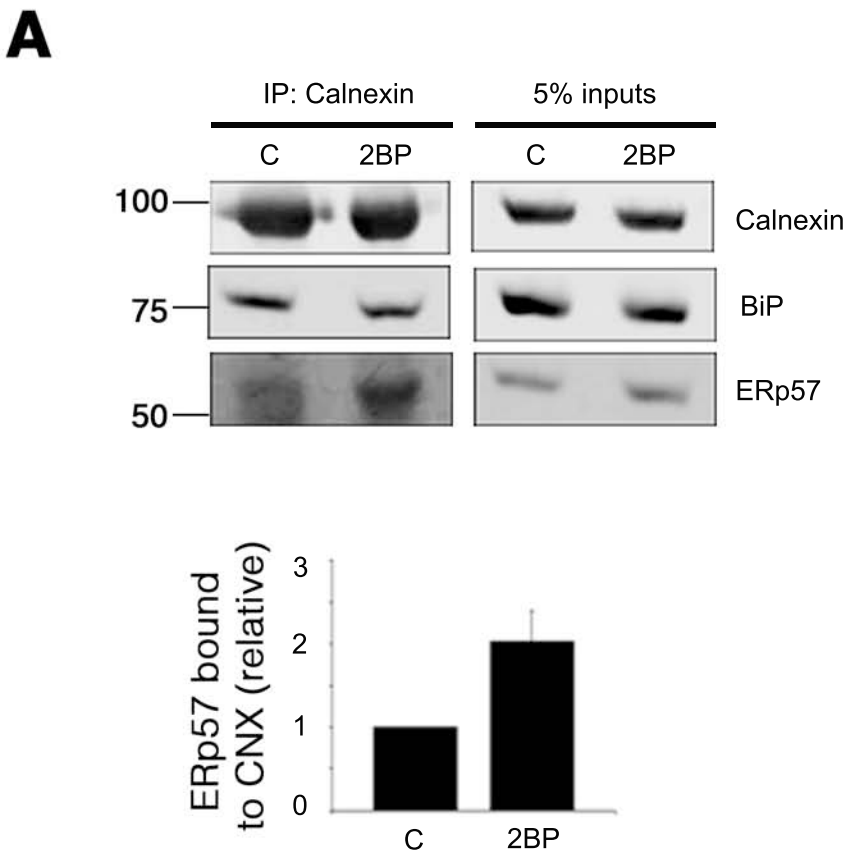
Supplemental Figure 1, Lynes et al.

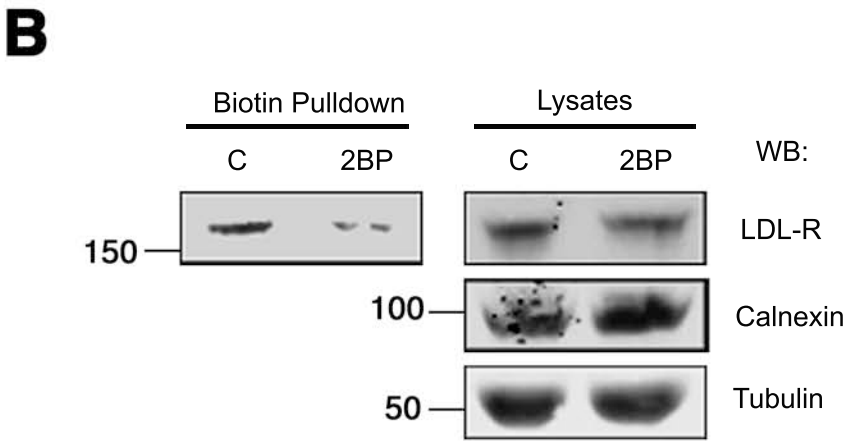


Supplemental Figure 2, Lynes et al.



Supplemental Figure 3, Lynes et al.





Supplemental Figure 4, Lynes et al.