Expanded View Figures

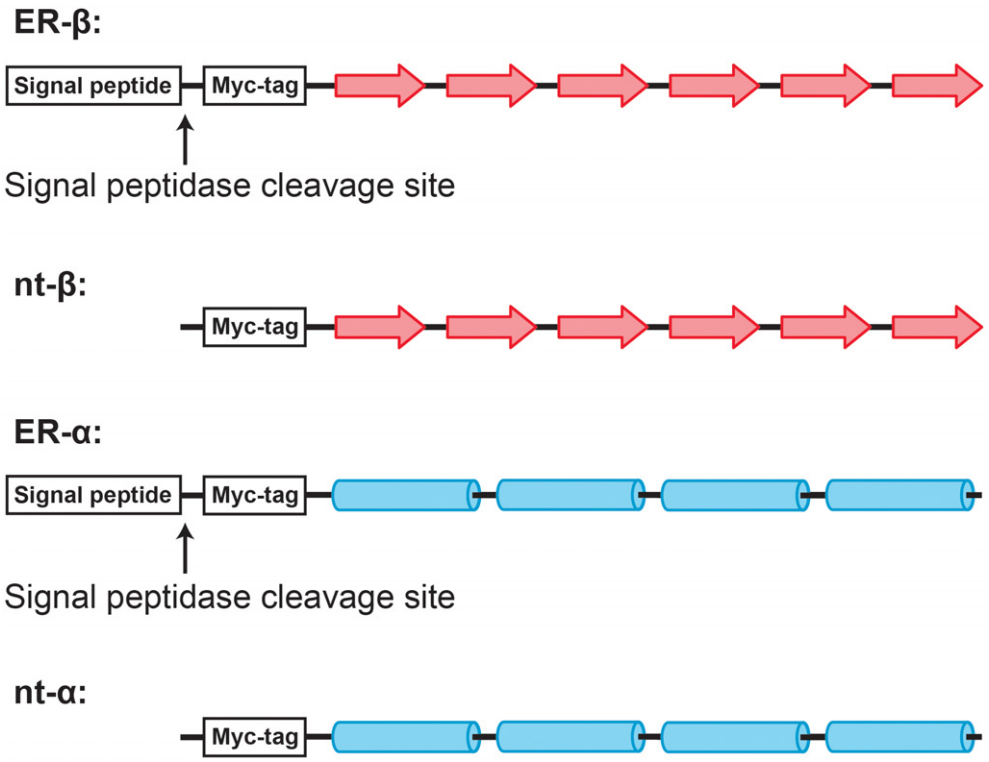

Figure EV1. Design of ER-targeted model proteins.

ER-targeted constructs of β-protein (ER-β) and α-protein (ER-α) were designed by adding an N-terminal signal peptide upstream of the Myc-tag of the non-targeted constructs (nt-β and nt-α, respectively). The model β-proteins were designed to form a β-sheet consisting of six β-strands (indicated by red arrows), and α-proteins were designed to form four-helical bundles (indicated by blue rods). The signal peptidase cleavage sites are indicated by black arrows.

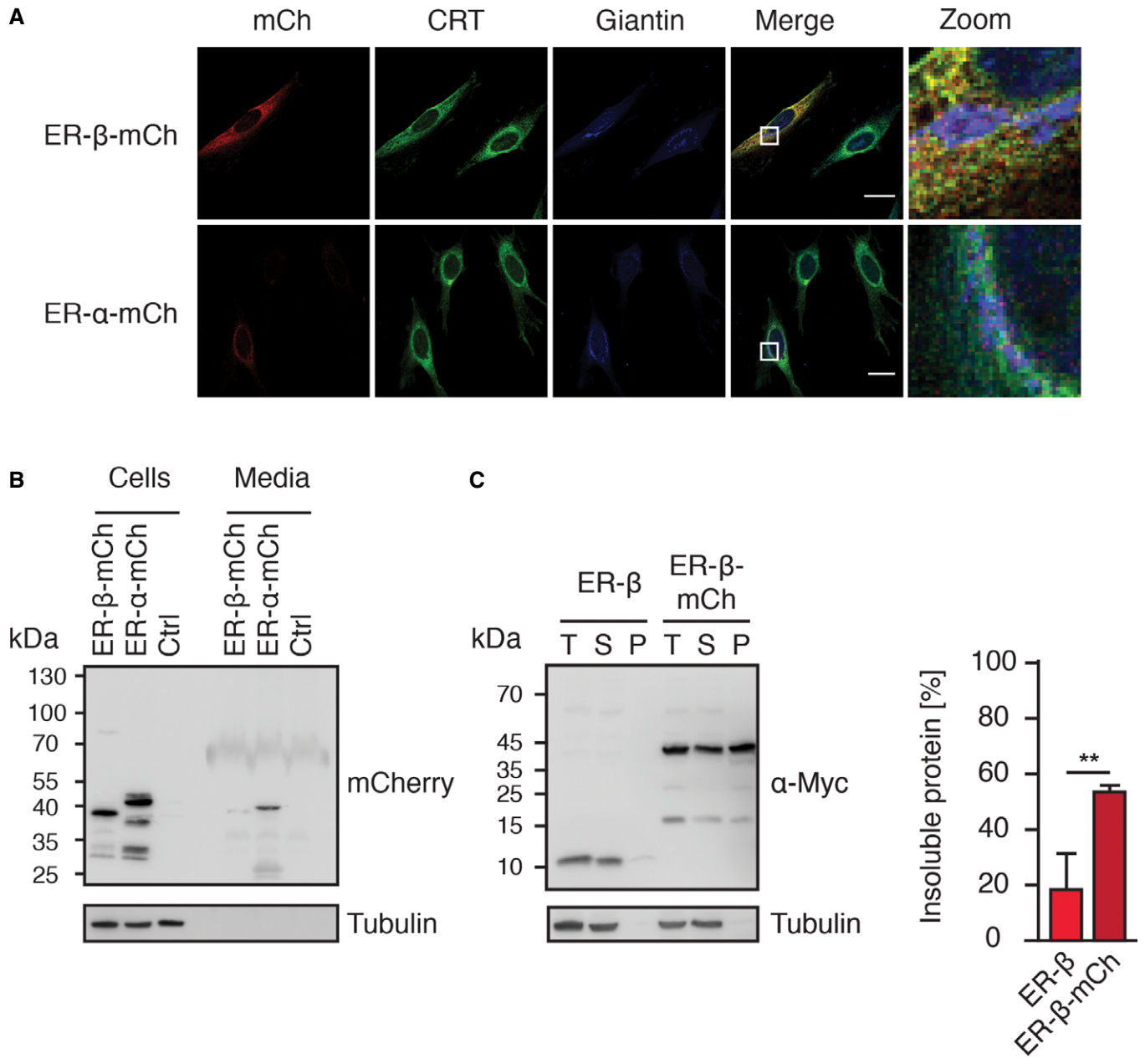

Figure EV2. ER-β-mCh is retained in the ER and is less soluble than ER-β.

A HeLa cells transfected with ER-β-mCh or ER-α-mCh were fixed and stained with anti-calreticulin (green) and anti-giantin (blue) antibodies, followed by fluorescently labeled secondary antibodies and analysis by confocal microscopy. Scale bars represent 30 μm.

B HEK293T cells were transfected with ER-β-mCh or ER-α-mCh. After 48 h, cells and media were collected separately. Proteins from media samples were concentrated by TCA precipitation and equal fractions of total protein from media, and cell samples were analyzed by immunoblotting with anti-mCherry antibody.

C Solubility of ER-β and ER-β-mCh was analyzed 48 h after transfection by fractionation of lysates by centrifugation and immunoblotting with anti-Myc antibody. T, total lysate; S, soluble fraction; P, pellet fraction. Right panel: Protein amounts in the pellet fraction were quantified. Error bars represent SD from three independent experiments. *P*-values are based on Student's *t*-test (unpaired *t*-test). $^{**}P \leq 0.01$.

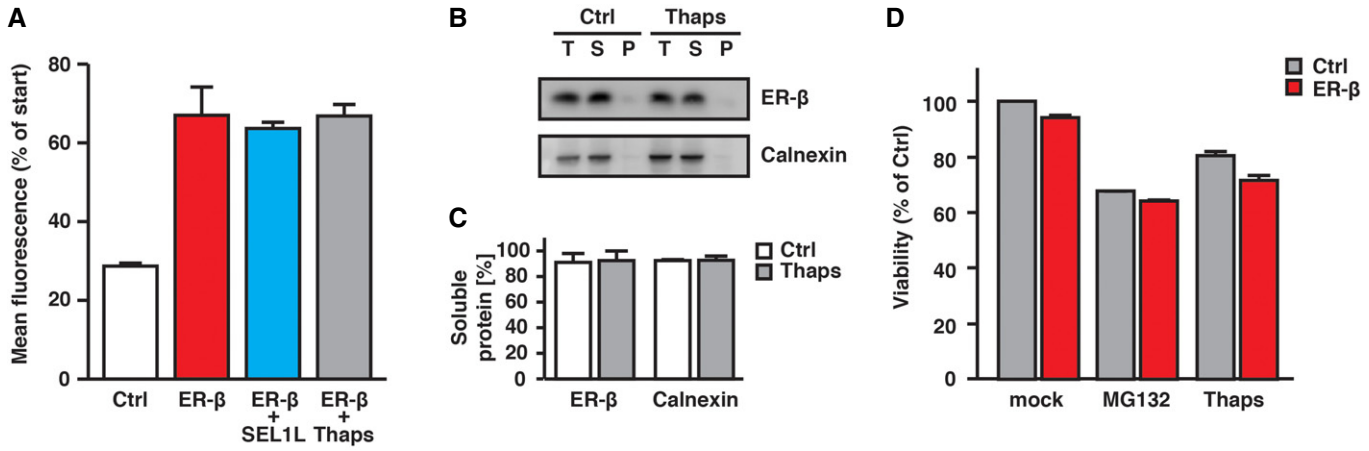

Figure EV3. Thapsigargin does not alter ER-β aggregation, and ER-β expression is not increasing sensitivity toward external stress.

A HEK293T cells were co-transfected with ER-β-mCh or ER-mCh (Ctrl) and either empty pcDNA3.1 or SEL1L in a ratio of 1:1. After 48 h, cells were treated with 1 μM Thapsigargin (Thaps) for 5 h before analysis, where indicated. After recording three images by confocal microscopy, small areas within the ER of cells were repeatedly bleached using the 561 nm laser and images recorded after each bleaching cycle (~22 s). Relative changes in mean fluorescence of total cells after seven bleaching cycles are plotted. Error bars represent SD from three independent experiments. At least five cells per condition per independent repeat were analyzed.

B Solubility of ER-β and endogenous calnexin in HEK293T cells was analyzed 48 h after transfection by fractionation of lysates by centrifugation and immunoblotting with anti-Myc and anti-calnexin antibody. The culture media was replaced 5 h before analysis to DMEM without (Ctrl) or with 1 μM Thapsigargin (Thaps). T, total lysate; S, soluble fraction; P, pellet fraction.

C Quantification of (B). Error bars represent SD from three independent experiments.

D Viability of HEK293T cells expressing ER-β or pcDNA3.1 (Ctrl), as measured by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cells were treated for 16 h with 5 μM MG132 or 1 μM Thapsigargin (Thaps), where indicated. Error bars represent SD from three independent experiments.

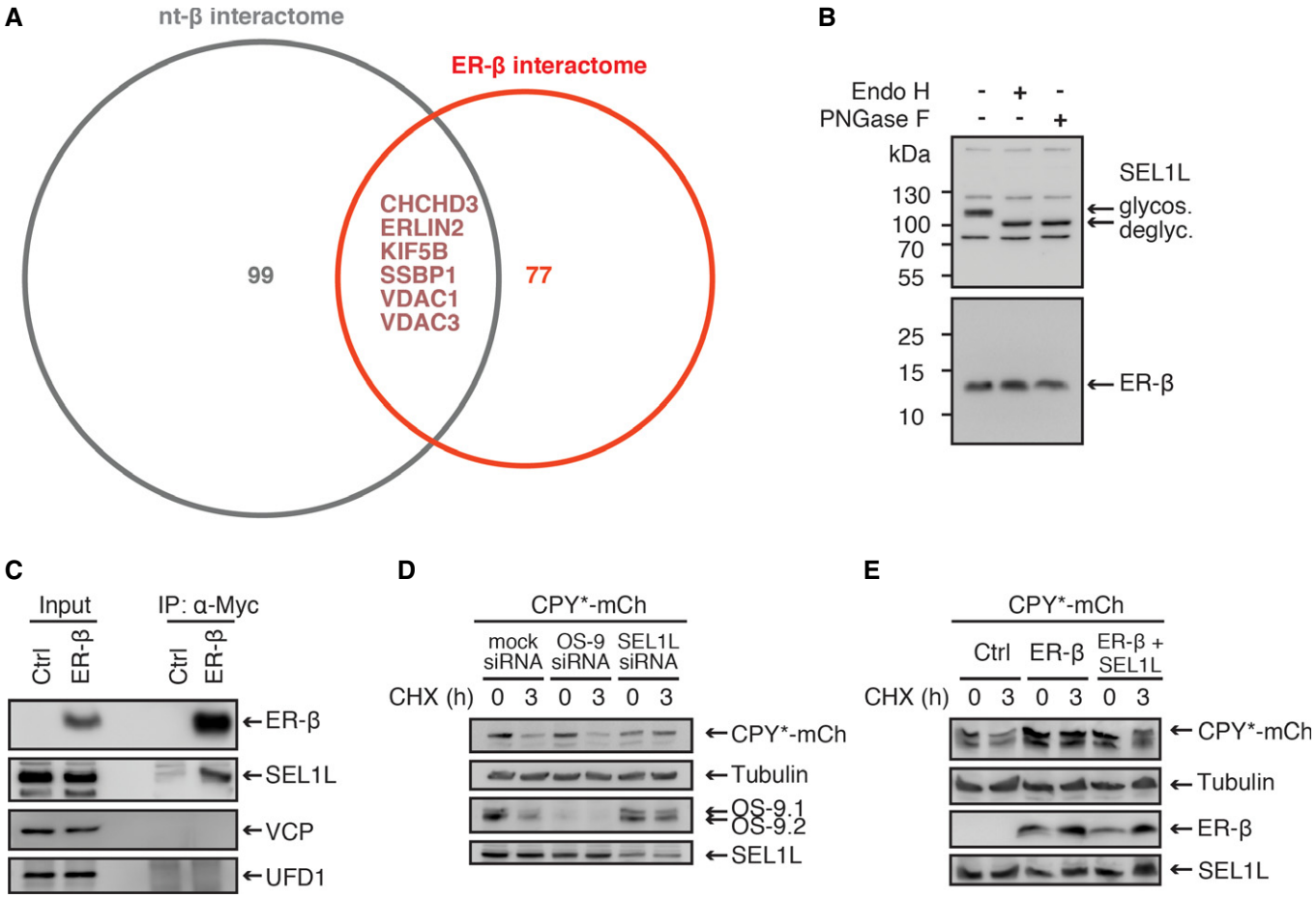

Figure EV4. ER-β interacts with a distinct set of cellular proteins and is not glycosylated.

A Comparison of the interactors of nt-β (Olzscha *et al*, 2011; 105 proteins, represented by gray circle) and the identified interactors of ER-β (83 proteins, represented by red circle) revealed six common interactors. The gene names of the common interactors are indicated.

B Lysates from HEK293T cells transfected with ER-β were treated with Endo H or PNGase F. Samples were then analyzed by immunoblotting with anti-SEL1L and anti-Myc antibodies, using 8% and 12% Bis–Tris gels for SEL1L and ER-β, respectively.

C Immunoblotting against SEL1L, VCP, and UFD1 after immunoprecipitation of ER-β as in Fig 5B.

D, E Immunoblots corresponding to Fig 5E and F.

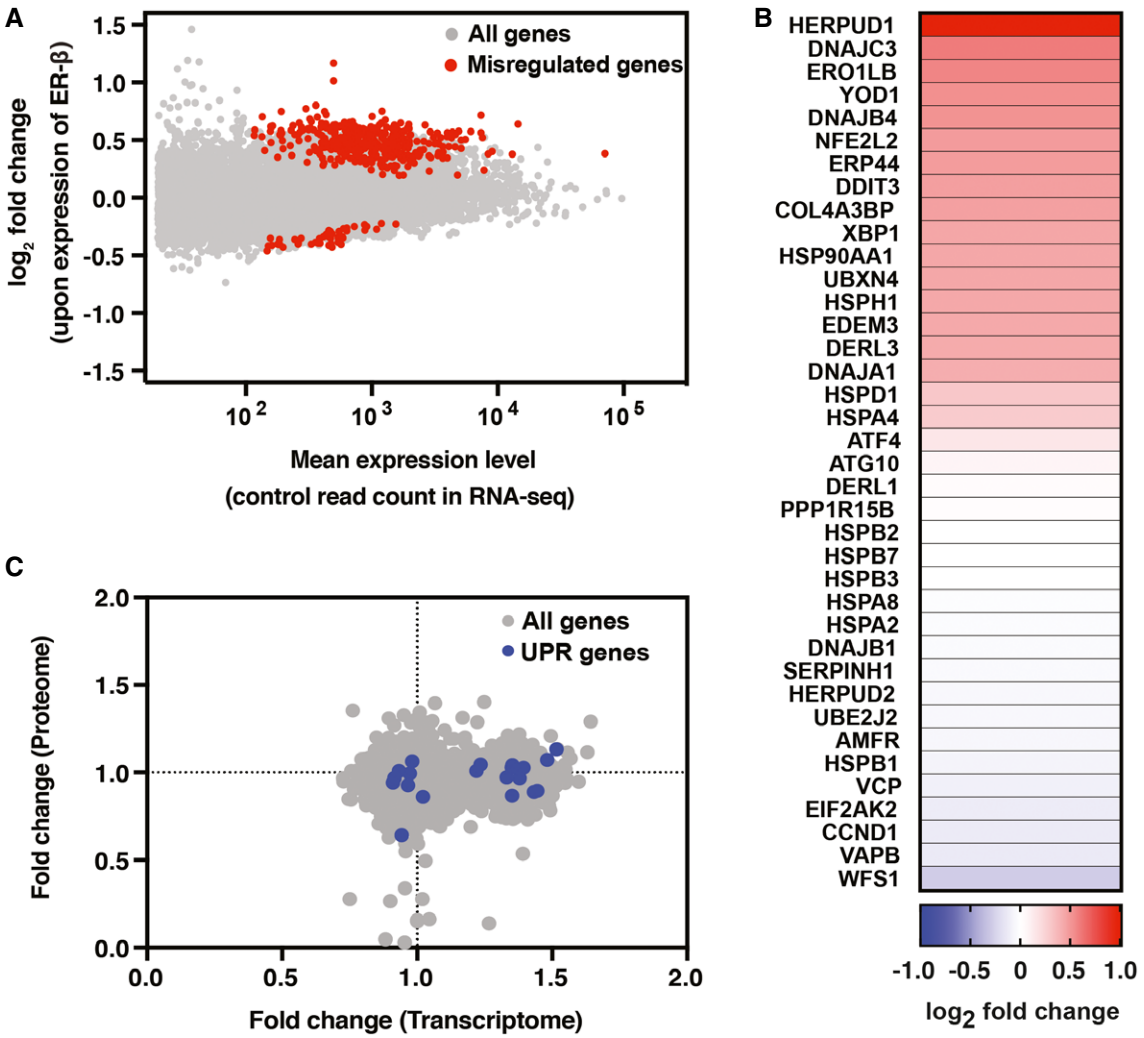

Figure EV5. ER-β expression leads to UPR transcriptional response.

A Changes in gene expression of all annotated genes after ER-β expression in HEK293T cells are plotted against mean expression levels under empty vector control conditions. Data obtained by RNA-seq analysis are shown as means from three independent cell cultures. Genes with significant misregulation are shown in red (adjusted *P*-values < 0.05, on the basis of DESeq2 analysis, as outlined in Materials and Methods).

B A heat map of UPR target genes upon ER-β expression as identified by RNA-seq analysis. The UPR target genes were defined in an earlier study (Raina *et al*, 2014).

C Scatter plot of the protein- and transcript-level changes upon ER-β expression. Misregulated UPR target genes are highlighted in blue.