Supplementary figure legends — Aragón *et al.*

Figure S1 | Temporal relation of foci formation and UPR signaling under DTT-induced ER stress. a, Cellular localization of Ire1-mCherry and SpR mRNA decorated with U1A-GFP. **b**, Quantitation of the percentage of Ire1 signal in foci and of co-localization of SpR mRNA in Ire1 foci depected in a histogram (means +/- s.e.m., n = 2-3). Images were taken at indicated times after induction of ER stress with 10 mM DTT.

Figure S2 | Temporal relation of foci formation and UPR signaling under tunicamycin-induced ER stress. a, Cellular localization of Ire1-mCherry and *HAC1* mRNA decorated with U1A-GFP. **b**, Quantitation of the percentage of Ire1 signal in foci and of co-localization of *HAC1* mRNA in Ire1 foci depected in a histogram (means +/- s.e.m., n = 2-5). **c**, Northern blot of *HAC1* mRNA. Splicing percentages (Spl. (%)) are given below the gel. **a**,**c** Samples were taken at indicated times after induction of ER stress with 1 μg/ml tunicamycin.

Figure S3 | Oligomerization of Ire1 is a prerequisite for efficient *HAC1* mRNA recruitment and ER stress signaling. Cellular localization of Sec63-mCherry, Ire1-GFP, and *HAC1* mRNA decorated with U1A-GFP. Imaging was performed in *ire1*Δ yeast treated with 10 mM DTT for 45' and complemented with Ire1 variants expressed from a plasmid either as GFP fusion proteins (left panels) or untagged (right panels). Ire1 variants are wildtype or mutants that are defective in dimerization at interface 1 (if1), 2 (if2) or both (if1/2), as indicated.

Figure S4 | Artificially induced dimerization of Ire1 does not support foci formation. Top, schematic of artificially induced dimerization of Ire1. The AP20187 (Ariad) drug, depicted as black hexagons, facilitates dimerization of the FKBP derived Fv2E modules that replace the ER stress sensing luminal domain. Middle, Viability assay and Northern blot of *ire1*Δ yeast complemented with the Fv2E module containing Ire1 variant in the absence (control) or presence of 1 μM AP20187. Bottom, imaging of Sec63-mCherry and Ire1-Fv2E-GFP before (control) or after a 45' treatment with 1 μM AP20187 either alone or in combination with 10 mM DTT.