Supplementary material

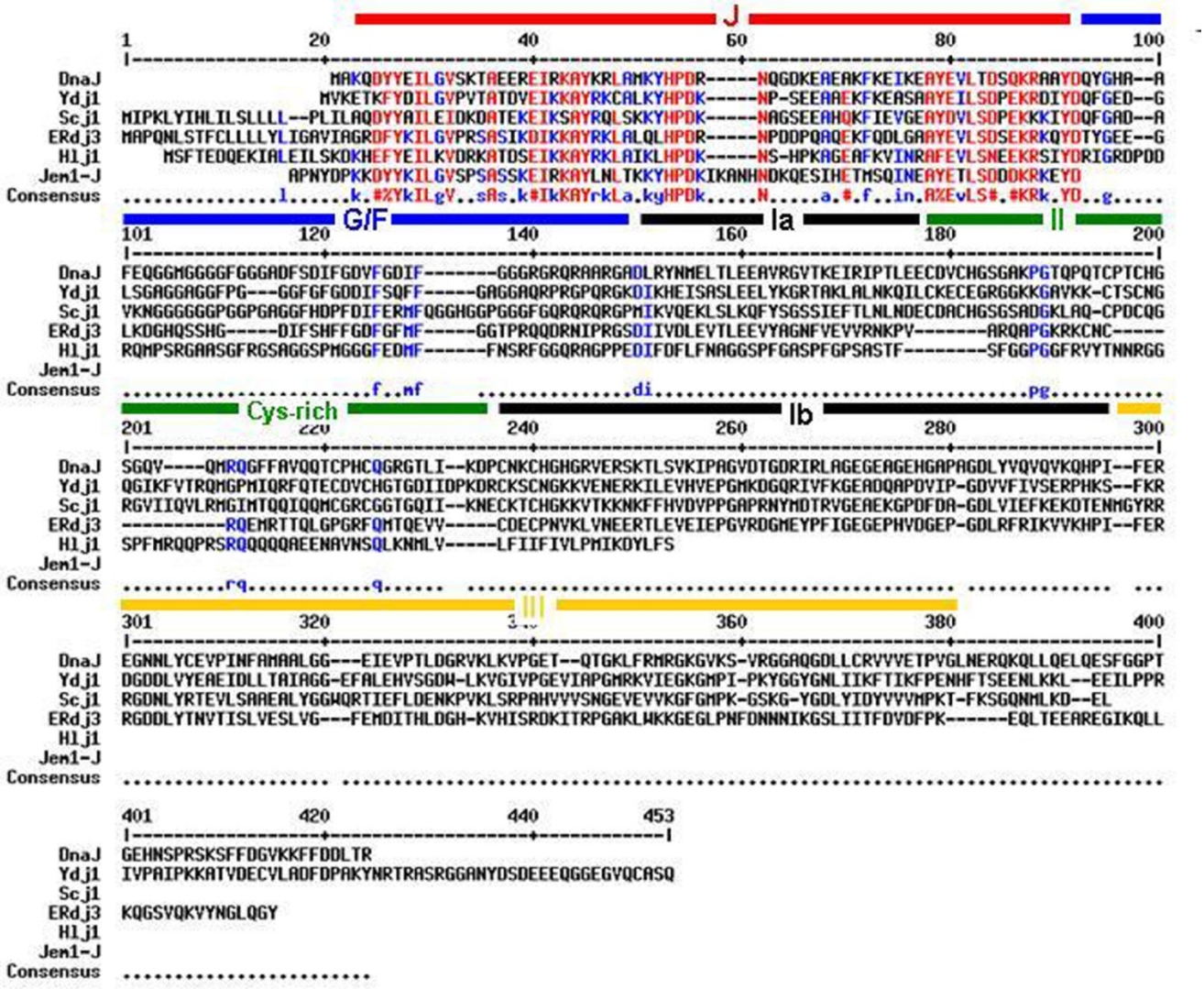

Fig. S1. Sequence comparison of DnaJ, Ydj1, Scj1, ERdj3, Hlj1 and the J domain of Jem1. The protein sequences of DnaJ (AAC73126), Ydj1 (CAA95937), Scj1 (CAA41529), ERdj3 (NP_057390), Hlj1 (NP_013884) and the J domain of Jem1 (amino acids 531-645; NP_012462) were aligned using the software Multalin[1]. The numbers in parantheses indicate NCBI accession numbers. The various domains are represented by: J- J domain; G/F- glycine/ phenylalanine rich domain; Ia and Ib- domain I; II- domain II; III- domain III. Amino acid notations in the row that indicates the consensus sequence include:!- isoleucine or valine; $- leucine or methionine; %- phenylalanine or tyrosine; #- asparagine, aspartate, glutamine or glutamate. Where indicated throughout the main text, % sequence identity was calculated using the program Kalign[2].

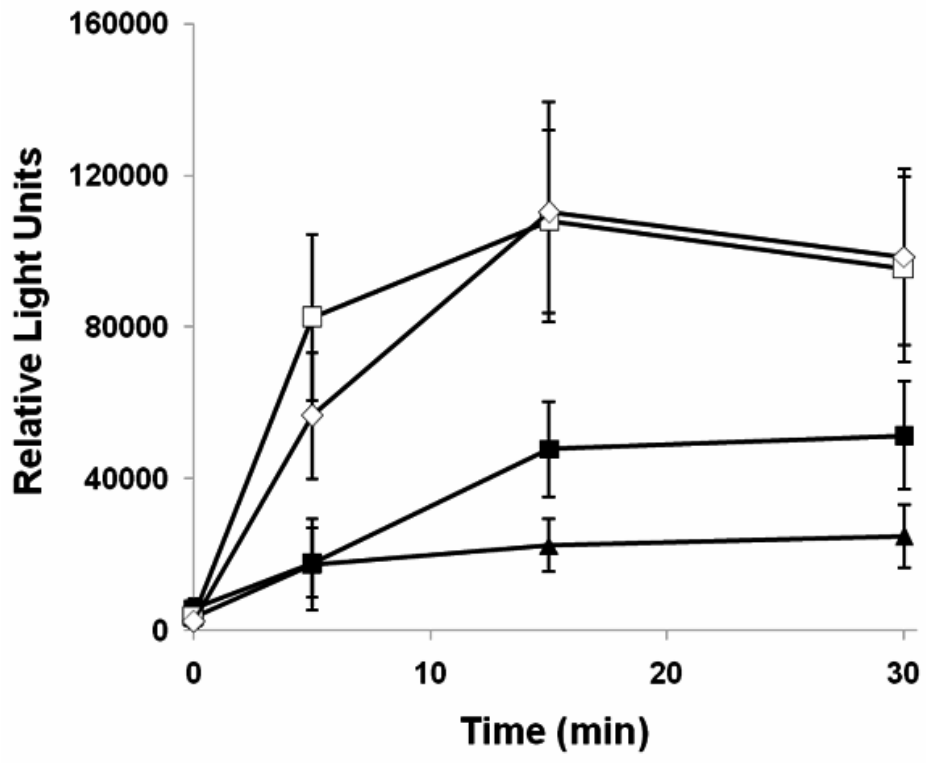

Fig. S2: Ydj1 enhances the ability of BiP to refold denatured substrates. The refolding of chemically-denatured firefly luciferase was monitored at 30ºC in reactions containing 1 mM ATP in the absence (▲) or presence of 2 μM BiP either without (■) or with 2 μM Ydj1 (□) or 2 μM ERdj3 (◇). Data represent the means of a minimum of three independent experiments ± standard errors.

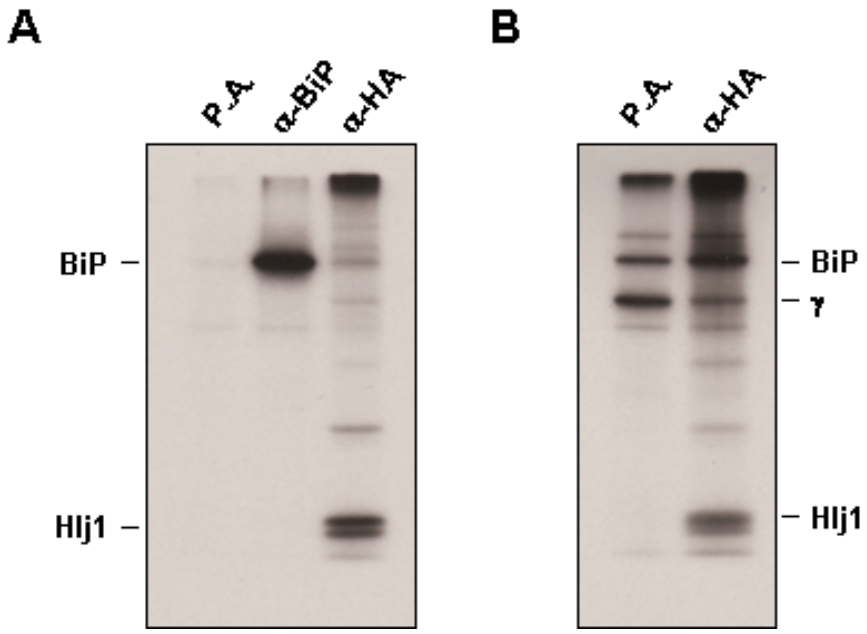

Fig. S3: Hlj1 interacts poorly with BiP and γHC in the mammalian ER. (A) COS cells were co-transfected with cDNAs encoding BiP and HA-tagged Hlj1. Metabolically labeled, DSP cross-linked cell lysates were immunoprecipitated with anti-HA or anti-BiP antibodies, or Protein A Sepharose alone. Isolated proteins were separated by denaturing gel electrophoresis. **(B)** COS cells were co-transfected with cDNAs encoding γHC and HA-tagged Hlj1. Cell lysates were immunoprecipitated with Protein A Sepharose beads which directly bind to the γHC, or with anti-HA antibodies. Samples were analyzed as described in (A).

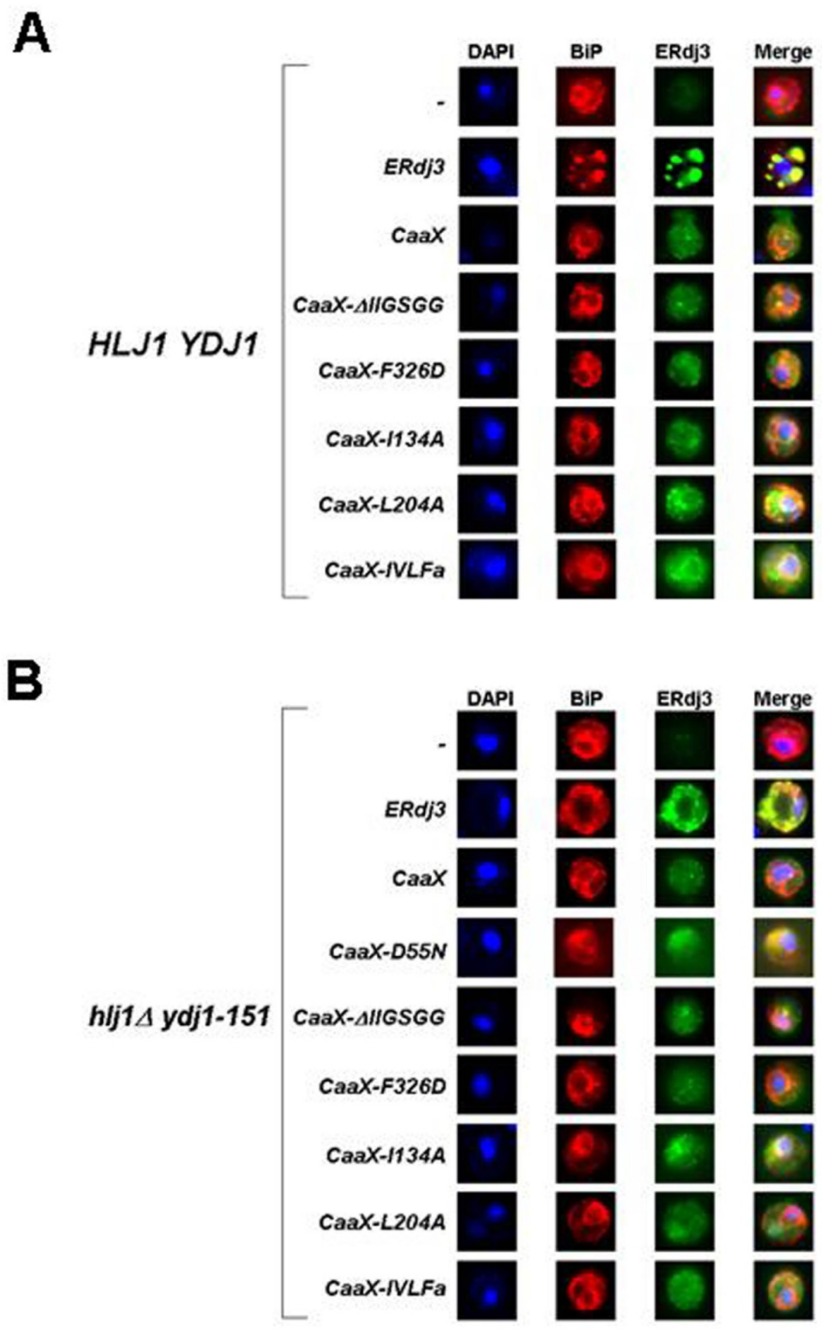

Fig. S4. Expression of the various wild-type and mutant ERdj3 proteins in yeast. Wild-type *HLJ1YDJ1* and mutant *hlj1Δydj1-151* yeast strains containing an empty vector (-), or expression vectors for an ER-targeted form of ERdj3 (*ERdj3*), an ER-tethered cytosolically localized form of full-length ERdj3 (CaaX) or CaaX mutants (*CaaX-D55N, CaaX-ΔII-GSGG, CaaX-F326D, CaaX-L204A* and *CaaX-IVLFa*) were stained with antibodies against ER-lumenal Kar2p (TRITC-labeled secondary antibody) and ERdj3 (FITC-labeled secondary antibody). DAPI was used to visualize the nuclear DNA. Indirect immunofluorescence microscopy was performed as previously described[3].

Supplementary References

1. Corpet, F. (1988) *Nucleic Acids Res* **16**, 10881-10890.
2. Labarga, A., Valentin, F., Andersson, M. and Lopez, R. (2007) *Nucleic Acids Research Web Services Issue 2007*.
3. Coughlan, C.M., Walker, J.L., Cochran, J.C., Wittrup, K.D., and Brodsky, J.L. (2004) *J Biol Chem* **279**, 15289-15297.