SUPPLEMENTARY INFORMATION

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Fig.S9, related to Fig.5. **1D NMR spectra of purified recombinant cyclophilin B wild type and mutant proteins (K6A/K35A and PentaK-A) indicating that the mutants are folded similarly to wild-type cyclophilin B.**

Supplementary experimental procedures and references

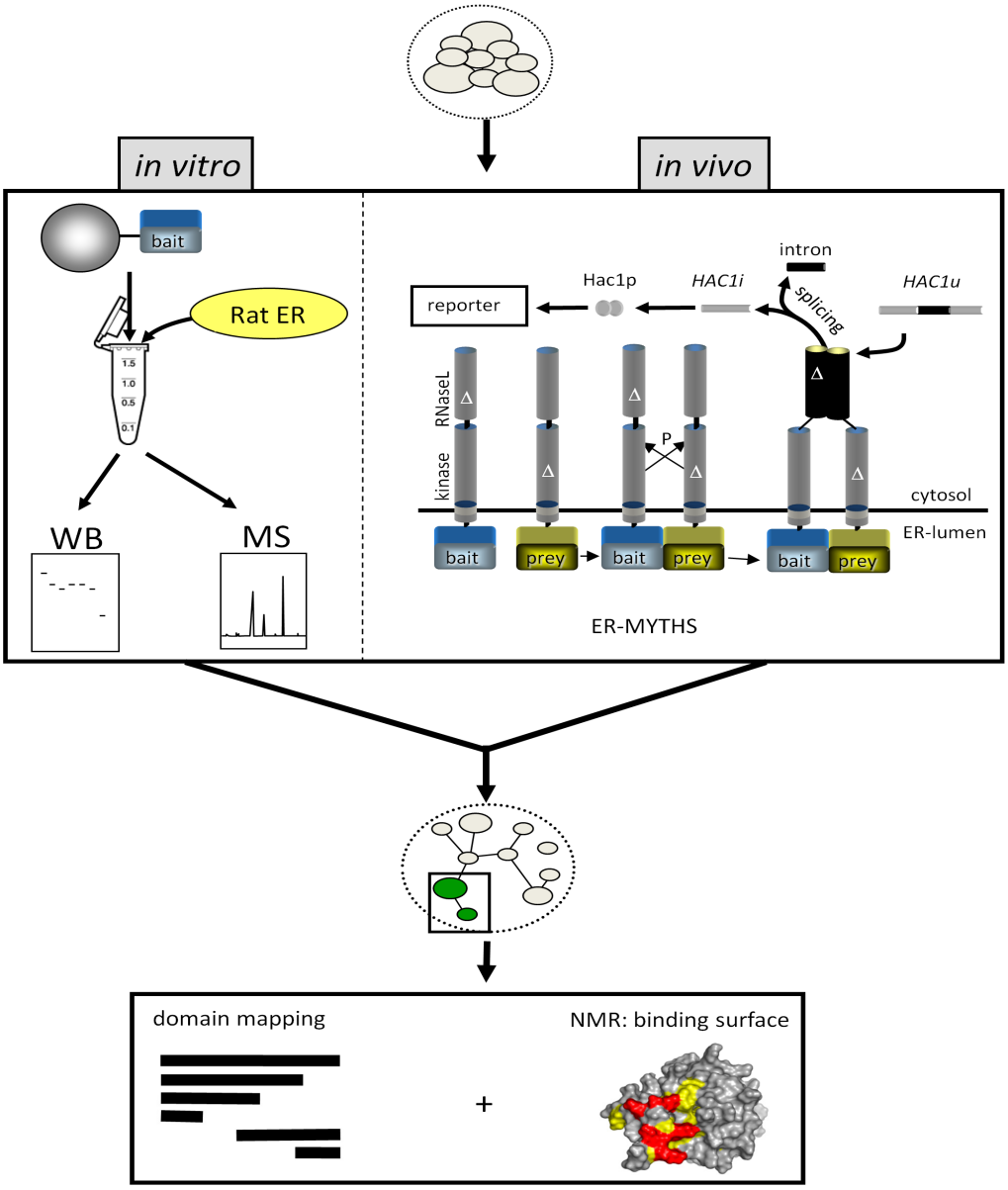


Fig.S1, related to Fig.1. **A combined *in vitro* and *in vivo* approach to dissect the ER multi-chaperone/foldase complex.** Recombinant ER proteins were coupled to a bead matrix and used *in vitro* to purify binding partners from enriched rat ER. Interacting proteins were identified by Western blotting (WB) and mass spectrometry (MS). An ER membrane yeast-two hybrid system (ER-MYTHS) was used to test binary interactions *in vivo* with an *ADE2* growth reporter assay. The results were combined to generate an interaction map showing specific connections between complex members. Molecular details of a specific interaction were determined *in vitro* by domain mapping and NMR.

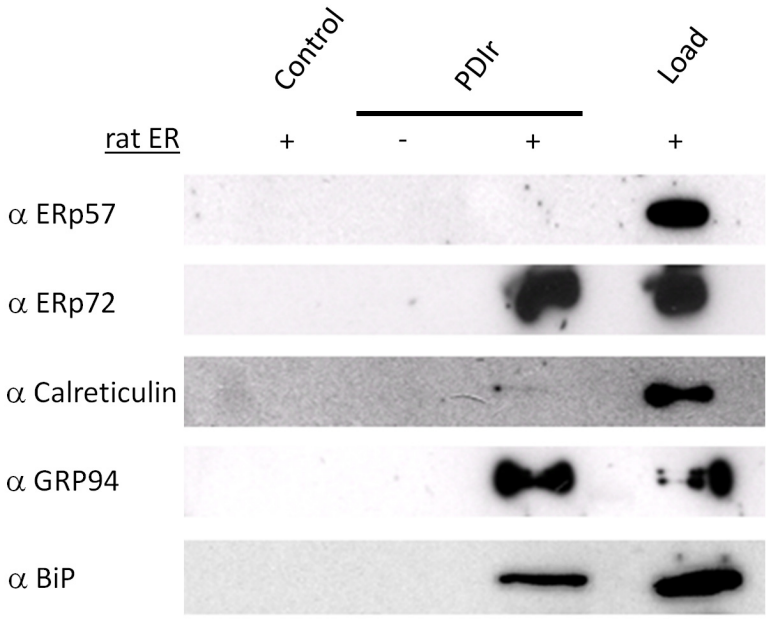


Fig. S2, related to Fig1. **PDIr protein interactions detected by affinity purification.** Western blots identify proteins purified from rat ER by affinity to recombinant PDIr. Load, 20 µg of rat ER; Control, beads blocked with ethanolamine; -, no ER added; +, ER added.

Table SI, related to Fig.1. **Lumenal ER proteins used in ER-MYTHS.**

Protein	*Accession*	*Type*	*localization*
BaP	Q6P6S4	NEF for BiP	ER lumen
BiP	P06761	Chaperone	ER lumen
CANX	P35565	Chaperone	ER membrane
CALR	P27797	Chaperone	ER lumen
CypB	P24368	PPI	ER lumen
ERDJ3	Q6TUG0	Co-Chaperone	ER lumen
ERDJ4	P97554	Co-Chaperone	ER lumen
ERDJ5	Q498R3	Co-Chaperone	ER lumen
ERp19	Q9CQU0	PDI	ER lumen
ERp29	P52555	PDI	ER lumen
ERp46	EDL98253.1	PDI	ER lumen
ERp57	P11598	PDI	ER lumen
ERp72	P38659	PDI	ER lumen
FKBP13	P26885	PPI	ER lumen
FKBP23	Q9Y680	PPI	ER lumen
FKBP60	Q66H94	PPI	ER lumen
FKBP65	Q5U2V1	PPI	ER lumen
GRp170	Q63617	Chaperone	ER lumen
GRp94	Q66HD0	Chaperone	ER lumen
P5	Q15084	PDI	ER lumen
PDI	P07237	PDI	ER lumen
PDIr	Q14554	PDI	ER lumen
Sep15	Q9VVJ7	Redox	ER lumen
TorsinA	O14656	AAA-ATPase	ER lumen
UGGT	Q9JLA3	Glucosyltransferase	ER lumen

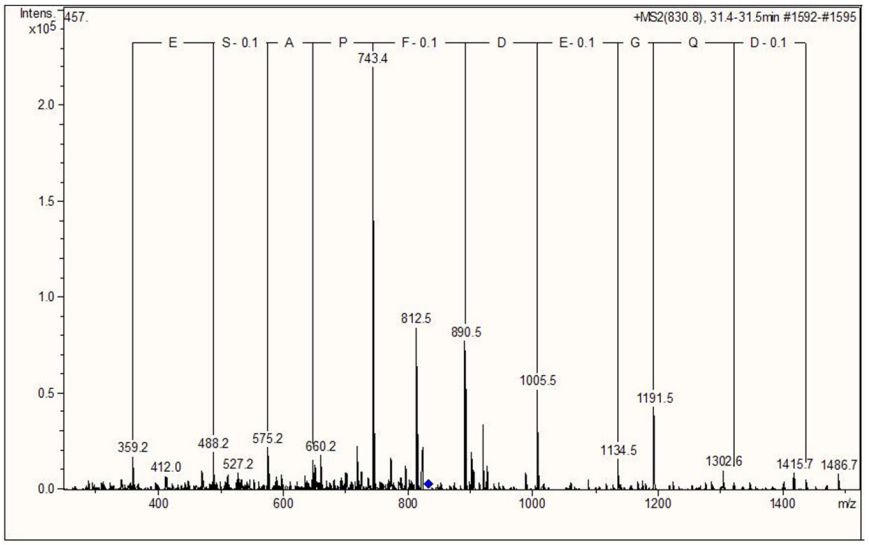
NEF, nucleotide exchange factor. PPI, peptidyl-prolyl cis-trans isomerase. PDI, protein disulfide isomerase. Accession codes are for proteins used in ER-MYTHS experiments. For CANX only the luminal fragment was used. Database for Mascot search was Mammalian.

Table SII, related to Fig.2. **Bait associated proteins identified by mass spectrometry.**

Bait	Protein	Peptides	Number
BiP (P20029)	DnaJc3 (Q9R0T3)	LIGSAEELIR NDNTEAFYK LAEAEDDFKK AEPSVAEYTVR ISILYYQLGDHELSLSEVR FDDGEDPLDAETQQGGGSNPFHR LLAAGQLADALSQFHAAVDGDPDNYIAYYR SNPSENEEKEAQSQLVK	8
	P5 (Q63081)	ILDTGATGR NSYLEVLLK GESPVDYDGGR TGEAIVDAALSALR GSFSEQGINEFLR LAAVDATVNQVLASR NLEPEWAAAATEVK GSTAPVGGGSFPNITPR LYSSSDDVIELTPSNFNR ALDLFSDNAPPPELLEIINEDIAK	10
	GRp170 (Q63617)	TLGGLEMELR FLGDSAAGMAIK EAGTQPQLQIR LPATEKPVLLSK SLAEDFAEQPIK TISSLFGGGTSSDAK LAVMSVDLGSESMK DAVIYPILVEFTR VEFEELCADLFDR DAVITVPAFFNQAER AANSLEAFIFETQDK VAIVKPGVPMEIVLNK LGNTISSLFGGGTSSDAK GQAGPEGVPPAPEEEKK VAIVKPGVPMEIVLNK LYQPEYQEVSTEEQR NINADEAAAMGAVYQAAALSK VLQLINDNTATALSYGVFR	18
	GRp94 (Q66HD0)	LIINSLYK SGYLLPDTK IYFMAGSSR FAFQAEVNR EAESSPFVER SILFVPTSAPR ELISNASDALDK DISTNYYASQK GVVDSDDLPLNVSR EEASDYLELDTIK EEEAIQLDGLNASQIR EEADDEAAVEEEEEEK LISLTDENALAGNEELTVK TETVEEPLEEDETAQEEK FQSSHHSTDITSLDQYVER LTESPCALVASQYGWSGNMER TETVEEPLEEDETAQEEKEEADDEAAVEEEEEEK	17
PDIr (Q14554)	ERp72 (P38659)	DLGLSESGEDVNAAILDESGKK EVSQPDWTPPPEVTLTLTK FDVSGYPTIK FAMEPEEFDSDALR TFDAIVMDPK	5 #
	ERp57 (P11598)	TADGIVSHLK YGVSGYPTLK	2 #
	CALR (P18418)	IKDPDAAKPEDWDER GQTLVVQFTVK	2 #
	P5 (Q63081)	GSTAPVGGGSFPNITPR ALDLFSDNAPPPELLEIINEDIAK HQSLGGQYGVQGFPTIK	3#
	BiP (P06761)	ITPSYVAFTPEGER SQIFSTASDNQPTVTIK SDIDEIVLVGGSTR DNHLLGTFDLTGIPPAPR VTHAVVTVPAYFNDAQR NQLTSNPENTVFDAK TWNDPSVQQDIK TKPYIQVDIGGGQTK	8#
	GRp94 (Q66HD0)	LISLTDENALAGNEELTVK DDEVDVDGTVEEDLGK FAFQAEVNR EEEAIQLDGLNASQIR FQSSHHSTDITSLDQYVER EEASDYLELDTIK DISTNYYASQK	7#
PDI (P07237)	CypB (P24368)	TVDNFVALATGEK DTNGSQFFITTVK	2
ERp29 (P30040)	ERp29 (P52555)	LNMELSEK SLNILTAFR* FDTQYPYGEK GALPLDTVTFYK ILDQGEDFPASELAR* LAENSASSDDLLVAEVGISDYGDK LAENSASSDDLLVAEVGISDYGDKLNMELSEK	7
ERp72 (P13667)	CypB (p24368)	DFMIQGGDFTR TVDNFVALATGEK DTNGSQFFITTVK DKPLKDVIIVDCGK VYFDFQIGDEPVGR	5
	Prdx4 (Q9Z0V5)	GLFIIDDK LVQAFQFTDK QITINDLPVGR	3
FKBP13 (P26885)	ERp57 (P11598)	LNFAVASR QAGPASVPLR YGVSGYPTLK LAPEYEAAATR	4
Cyp B (P23284)	CALR (P18418)	GQTLVVQFTVK AVLGLDLWQVK EQFLDGDAWTNR IDNSQVESGSLEDDWDFLPPK DMHGDSEYNIMFGPDICGPGTK IDNSQVESGSLEDDWDFLPPKK KPEDWDEEMDGEWEPPVIQNPEYK	7
	GRp94 (Q66HD0)	LIINSLYK SGYLLPDTK GLFDEYGSK FAFQAEVNR EAESSPFVER SILFVPTSAPR ELISNASDALDK DISTNYYASQK EFEPLLNWMK GVVDSDDLPLNVSR EEASDYLELDTIK TVWDWELMNDIK DDEVDVDGTVEEDLGK EEEAIQLDGLNASQIR YSQFINFPIYVWSSK DDEVDVDGTVEEDLGKSR LISLTDENALAGNEELTVK LTESPCALVASQYGWSGNMER	18
	BiP (P06761)	ITITNDQNR DAGTIAGLNVMR VEIIANDQGNR FEELNMDLFR NELESYAYSLK ELEEIVQPIISK TWNDPSVQQDIK SDIDEIVLVGGSTR AKFEELNMDLFR TFAPEEISAMVLTK ITPSYVAFTPEGER TKPYIQVDIGGGQTK IINEPTAAAIAYGLDK NQLTSNPENTVFDAK SQIFSTASDNQPTVTIK VTHAVVTVPAYFNDAQR IEWLESHQDADIEDFK IEIESFFEGEDFSETLTR LYGSGGPPPTGEEDTSEKDEL	19
	ERp72 (P38659)	DNDPPIAVAK FDVSGYPTIK TFDAIVMDPK VDATEQTDLAK MDATANDITNDR VEGFPTIYFAPSGDK YGIVDYMVEQSGPPSK DFPEYTFAIADEEDYATEVK QLEPVYTSLGK DLGLSESGEDVNAAILDESGKK DGDDVVILGVFQGVGDPGYLQYQDAANTLR	11
	P5 (Q63081)	GESPVDYDGGR TGEAIVDAALSALR DVVELTDDTFDK LAAVDATVNQVLASR GSTAPVGGGSFPNITPR LYSSSDDVIELTPSNFNR ALDLFSDNAPPPELLEIINEDIAK	7
	CANX (P35565)	APVPTGEVYFADSFDR IADPDAVKPDDWDEDAPSK	2
ERp57 (P30101)	CALR (P18418)	GQTLVVQFTVK AVLGLDLWQVK EQFLDGDAWTNR IDNSQVESGSLEDDWDFLPPK IDNSQVESGSLEDDWDFLPPKK	5

Proteins pulled down from rat liver microsomes with respective recombinant protein matrix, and identified by mass spectrometry. PDIr interacting proteins were identified by QTOF. Number = peptides above ID threshold (Mowse score > 45, except #: Spectrum Mill score >10). * = Species specific peptides. Sep15 (Q9VVJ7), ERp19 (O95881), FKBP23 (Q9Y680) did not show binding partners. Database for Spectrum Mill search was Mammalian.

A ILDQGEDFPASELAR



B SLNILTFAR

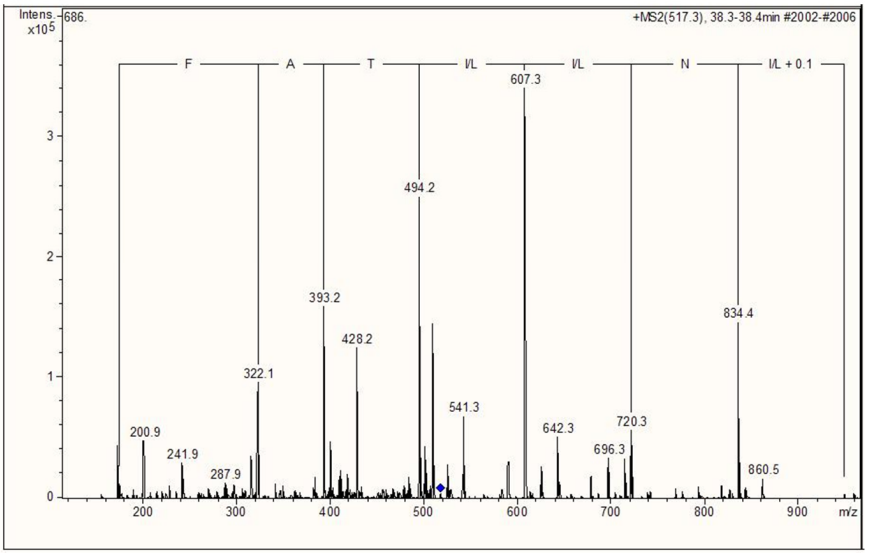
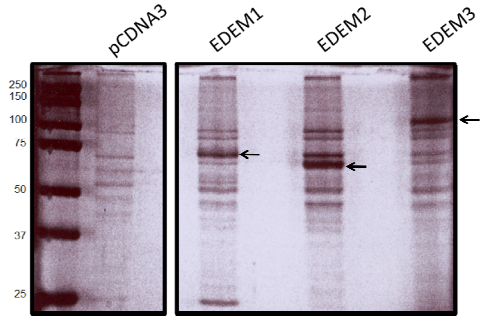


Fig. S3, related to Fig.1. **Rat ERp29-specific peptides identified by MS by affinity purification from rat ER using recombinant human ERp29.** *A* The y-ion series of the peptide ILDQGEDFPASELAR. *B* The y-ion series of the peptide SLNILTFAR. The Mascot scores for the two peptides are 103 and 64, respectively, with expectation values (p-values for random hits) of p = 4.5 e-8 and p = 5.3 e-4. Spectra were generated using Brukers 'Compas Data Analysis' software's amino acid molecular weight ruler on the raw MSMS spectra. The blue diamond indicates the precursor ion.

A



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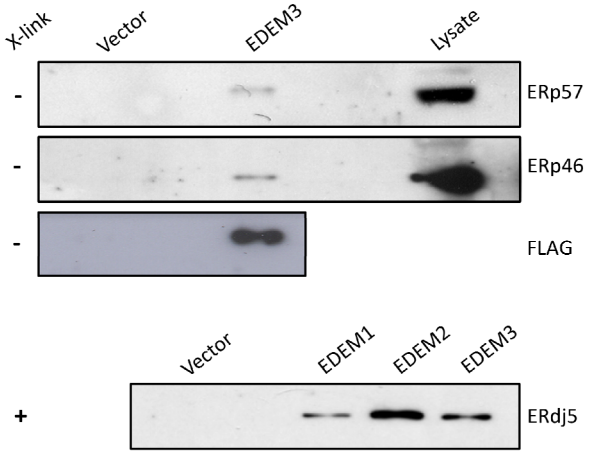

Fig.S4, related to Fig.2. **EDEM purification and co-purified proteins.** *A* SDS-PAGE and Colloidal Coomassie stain of proteins eluted from anti-FLAG affinity purifications of EDEM1, EDEM2, and EDEM3 compared to empty vector control. Arrows indicate bands corresponding to EDEMs. *B* EDEM interactions in the absence and presence of cross-linkers verified by Western blot. Top three panels show affinity purifications in the absence of cross-linkers with FLAG-tagged EDEM3 expressed in HEK293 cells and subsequent blotting of eluates with antibodies against ERp57, ERp46, and FLAG. Bottom panel shows affinity purification of ERdj5 after cross-linking FLAG-tagged EDEM1, 2, and 3.

Table SIII, related to Fig.2. **Proteins associated with epitope-tagged EDEM1, 2, and 3 identified by mass spectrometry.**

Bait	Protein	Peptides	Number
EDEM1 (Q925U4)	EDEM1 (Q925U4)	YSGAFPPQLR DSTVQVFEATIR VLGSLLSAHR LLGDSTFEWVAR VINSSSNCNRVPDER RYSLPLK	6
	GRp94 (P14625)	DDEVDVDGTVEEDLGK FAFQAEVNR ELISNASDALDK SILFVPTSAPR GVVDSDDLPLNVSR SGYLLPDTK FQSSHHPTDITSLDQYVER	7
	ERp72 (P13667)	IDATSASVLASR FDVSGYPTIK FDVSGYPTLK	3
	CANX (P27824)	APVPTGEVYFADSFDR IPDPEAVKPDDWDEDAPAK	2
	ERp57 (P30101)	LAPEYEAAATR YGVSGYPTLK IFRDGEEAGAYDGPR TADGIVSHLK LNFAVASR VVVAENFDEIVNNENK LSKDPNIVIAK EATNPPVIQEEKPK	8
	PDI (P07237)	VDATEESDLAQQYGVR YKPESEELTAER	2
	P5 (Q15084)	TGEAIVDAALSALR NLEPEWAAAASEVK LAAVDATVNQVLASR GSFSEQGINEFLR GSTAPVGGGAFPTIVER	5
	CALR (P27797)	EQFLDG DGWTSR IDNSQVESGSLEDDWDFLPPKK DPDASKPEDWDER	4
EDEM2 (Q8BJT9)	EDEM2 (Q8BJT9)	VECGFATIK ATGDPTLLELGR VVGGLLSAHLLSK MESFFLAETVK ISKVECGFATIK TPLLSCPSQPFTSK SDIGLVGNHIDVLTGK AGVEVEAGWPCSGPLLR SGPWEPQSGPATLSSPANQPR EGYPLRPELIESAMYLYR KAGVEVEAGWPCSGPLLR	11
	GRp94 (P14625)	DDEVDVDGTVEEDLGK FAFQAEVNR LIINSLYK ELISNASDALDK SILFVPTSAPR GVVDSDDLPLNVSR FQSSHHPTDITSLDQYVER SGYLLPDTK LSLNIDPDAK	9
	ERp72 (P38659)	IDATSASVLASR RFDVSGYPTIK FDVSGYPTLK FHHTFSTEIAK	4
	CANX (P355565)	APVPTGEVYFADSFDR TPELNLDQFHDK IPDPEAVKPDDWDEDAPAK KIPNPDFFEDLEPFR IVDDWANDGWGLK	5
	ERp57 (P11598)	LAPEYEAAATR YGVSGYPTLKIFR RDGEEAGAYDGPR TADGIVSHLK FLQDYFDGNLK SEPIPESNDGPVK EATNPPVIQEEKPK	7
	P5 (Q63081)	LYSSSDDVIELTPSNFNR TGEAIVDAALSALR NLEPEWAAAASEVK NLEPEWAAAASEVKEQTK IFQKGESPVDYDGGR GESPVDYDGGR NSYLEVLLK GSFSEQGINEFLR GSTAPVGGGAFPTIVER	9
	CALR (Q96L12)	EQFLDGDGWTSR IDNSQVESGSLEDDWDFLPPK IDNSQVESGSLEDDWDFLPPKK IKDPDASKPEDWDER	4
	ERp46 (Q8NBS9)	VDCTAHSDVCSAQGVR EYVESQLQR	2
EDEM3 (Q2HXL6)	EDEM3 (Q2HXL6)	MGVSLIHLK ALDFLWEK QVEVLLSDK EGSIILDAIR VQLVQHAIQAASSIDAEDGLR ATGDPYYLEVGK AVQIISHPFFGR FTGATIFEEYAR AYVLLGDDSFLER TLIENLNK VVLTAGPAQFGLDLSK VRGQEPSRGDVDDALGK DVNLDNDVVVSVFETNIR DSGVGAGIDSYYEYLLK KDSGVGAGIDSYYEYLLK KALDFLWEK GQEPSRGDVDDALGK	17
	GRp94 (P14625)	FAFQAEVNR ELISNASDALDK SILFVPTSAPR GVVDSDDLPLNVSR	4
	ERp72 (P38659)	IDATSASVLASR RFDVSGYPTIK VDATAETDLAK VDATAETDLAKR FDVSGYPTLK	5
	ERp57 (P30101)	RLAPEYEAAATR YGVSGYPTLK TADGIVSHLK LNFAVASR FLQDYFDGNLK SEPIPESNDGPVK LSKDPNIVIAK EATNPPVIQEEKPK	8
	P5 (Q15084)	TGEAIVDAALSALR LAAVDATVNQVLASR IFQKGESPVDYDGGR KGESPVDYDGGR GSTAPVGGGAFPTIVER	5
	ERp46 (Q8NBS9)	VDCTAHSDVCSAQGVR LFKPGQEAVK VDCTQHYELCSGNQVR EYVESQLQR TLAPTWEELSK FVLSQAKDEL	6

Number = peptides above ID threshold (Mowse score > 45, except #: Spectrum Mill score >10).

Table SIV, related to Fig.2. **Interactions detected by ER-MAP.**

Detection Method	Bait	Prey	MS	WB	ER-MYTHS	PMID
AP	BiP	DnaJC3	+	nab	na	17567950
	BiP	GRp170	+	+	-	16962589
	BiP	GRp94	+	+	-	
	BiP	P5	+	nab	-	19887585
	CypB	BiP	+	+	ne	18946027
	CypB	CALR	+	+	ne	20801878
	CypB	CANX	+	nab	ne	20801878
	CypB	ERp72	+	+	ne	
	CypB	GRp94	+	+	ne	18946027
	CypB	P5	+	nab	ne	
	ERp57	CALR	+	+	-	10436013
	ERp72	CypB	+	+	ne	
	ERp72	Prdx4	+	nab	na	
	FKBP13	ERp57	+	nd	-	
	PDI	CypB	+	-	ne	12204109
	PDIr	BiP	+	+	ne	
	PDIr	CALR	+	-	ne	12584332
	PDIr	ERp57	+	-	ne	
	PDIr	ERp72	+	+	ne	
	PDIr	GRp94	+	+	ne	
	PDIr	P5	+	nab	ne	
FLAG-IP	EDEM1	CALR	+	nd	nd	
	EDEM1	CANX	+	nd	nd	12610305
	EDEM1	ERdj5	-	+	nd	18653895
	EDEM1	ERp57	+	nd	nd	
	EDEM1	ERp72	+	nd	nd	
	EDEM1	GRp94	+	nd	nd	
	EDEM1	P5	+	nd	nd	
	EDEM1	PDI	+	nd	nd	
	EDEM2	CALR	+	nd	nd	
	EDEM2	CANX	+	nd	nd	
	EDEM2	ERdj5	-	+	nd	
	EDEM2	ERp46	+	nd	nd	
	EDEM2	ERp57	+	nd	nd	
	EDEM2	ERp72	+	nd	nd	
	EDEM2	GRp94	+	nd	nd	
	EDEM2	P5	+	nd	nd	
	EDEM3	ERp46	+	+	nd	
	EDEM3	ERp57	+	+	nd	
	EDEM3	ERp72	+	nd	nd	
	EDEM3	GRp94	+	nd	nd	
	EDEM3	P5	+	nd	nd	

Y2H	BiP	BiP	-	na	+	9756927
	BiP	BaP	-	nab	+	12356756
	BiP	ERdj3	-	nab	+	18923428
	BiP	ERdj5	-	nab	+	18653895
	BiP	SEP15	-	nab	+	
	ERdj3	ERdj3	ne	na	+	19090675
	ERdj3	ERdj4	ne	na	+	
	ERdj3	ERdj5	ne	na	+	
	ERdj3	FKBP60	ne	na	+	
	ERdj3	GRp94	ne	na	+	
	ERdj3	P5	ne	na	+	
	ERdj3	torsinA	ne	na	+	
	ERdj3	UGGT	ne	na	+	
	ERdj4	CANX	ns	na	+	
	ERdj4	ERdj5	ns	na	+	
	ERdj4	FKBP60	ns	na	+	
	ERdj4	GRp94	ns	na	+	
	ERdj4	P5	ns	na	+	
	ERdj5	ERdj5	ns	na	+	
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	ERdj5	P5	ns	na	+	
	ERp19	BiP	-	-	+	
	ERp19	FKBP65	-	nab	+	
	ERp29	BiP	-	-	+	15865205
	ERp29	FKBP23	-	-	+	
	ERp57	CANX	-	nab	+	14988724
	ERp72	ERp72	-	-	+	
	FKBP23	BiP	-	-	+	14960307
	FKBP23	FKBP23	-	-	+	
	GRp94	torsinA	ne	na	+	
	SEP15	UGGT	-	-	+	16129668
AP and Y2H	ERp29	ERp29	+	+	+	17267685
	SEP15	ERp72	-	+	+	
Literature only	EDEM3	PRDX4	-	nab	nd	19887585
	ERp29	CANX	-	nd	-	15865205
	ERp29	ERp72	-	-	-	15865205
	ERp29	GRp94	-	-	-	15865205
	ERp46	PRDX4	-	nab	na	19887585
	PDI	PDI	-	-	ne	15169950

na = not applicable; nd = not done; ne = not expressed; ns = not soluble; nab = no suitable antibody. AP = recombinant affinity purification, Y2H = ER-Membrane Yeast 2-Hybrid System (ER-MYTHS). Literature only = interactions reported in the literature, but missed using ER-MAP. PMID = Pubmed IDs, MS (affinity purification results by mass spectrometry), WB (affinity purification results by western blotting), ER-MYTHS (interactions detected by Y2H).

Method	Interactions	Confirmed by literature
AP-total	22	11
AP-MS	21	11
AP-WB	12	6
FLAG-total	21	2
FLAG-MS	19	1
FLAG-WB	4	1
Y2H-total	33	10
Total interactions	75	22
Literature coverage : 79%		

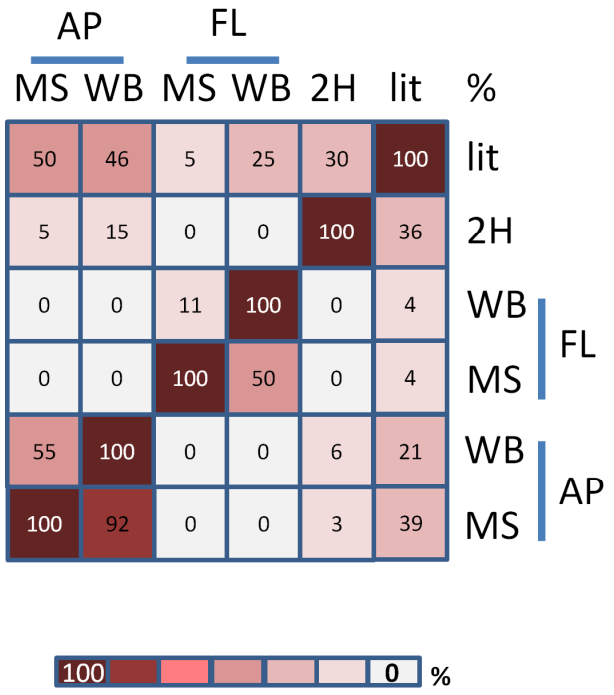


Fig. S5, related to Fig.2. **Method performance in ER-MAP.** Upper panel: Interactions picked up by the individual detection methods in ER-MAP, related to the interaction coverage by the literature. Lower panel: Cross-comparison of individual ER-MAP detection methods in relation to method overlap and literature evidence, expressed in percentage (%). The degree of overlap is visually interpreted in shades of red (declining intensities represent a lower overlap, dark red 100%, white 0%). AP (bacterial affinity purification); FL (FLAG-tag affinity purification): MS (mass spectrometry); WB (western blotting); 2H (ER membrane yeast two-hybrid system); lit (interactions reported in the literature).

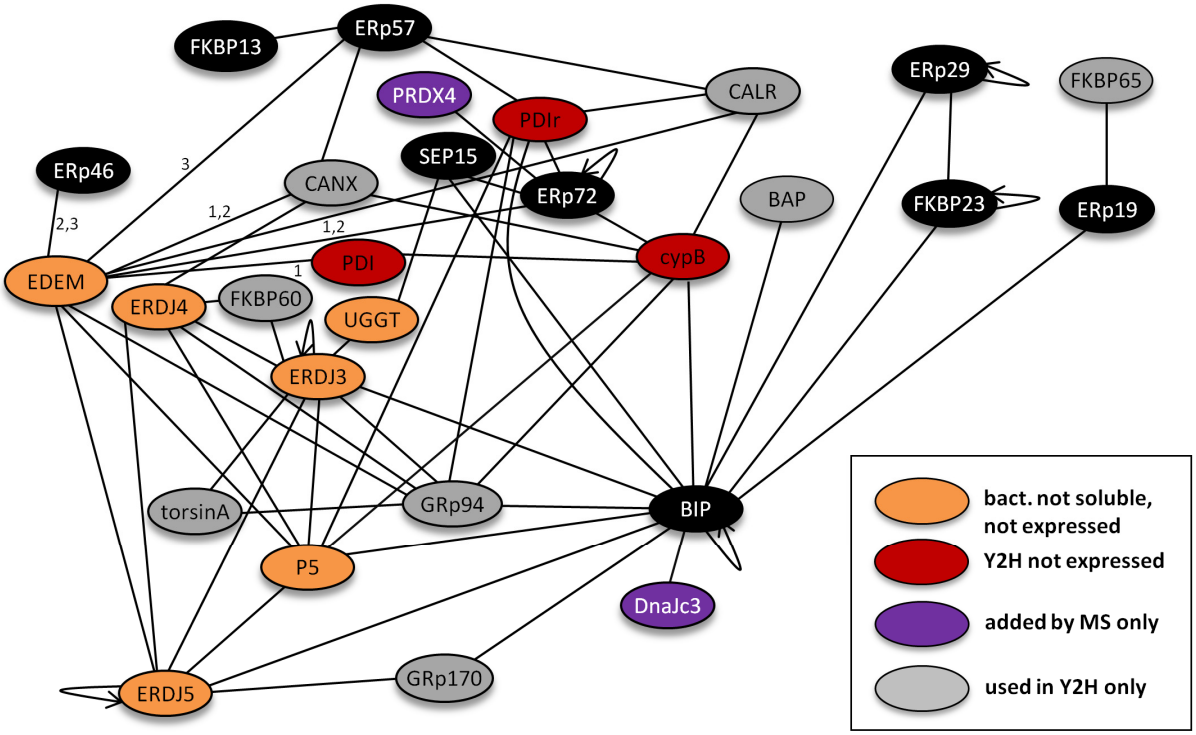


Fig. S6*A*, related to Fig.2. **Suitability of proteins for different detection methods used in ER-MAP.** Combined map illustrating proteins not suitable for Y2H or AP. ER protein nodes with color indicate restricted use. Protein fusion not expressed in yeast two-hybrid system (RED), bacterial fusion proteins for affinity purification not expressed or insoluble (ORANGE), proteins subsequently added to the network by MS detection that were therefore not used in Y2H or AP (PURPLE), proteins used in Y2H only (GREY). Black nodes indicate proteins used in both Y2H and AP.

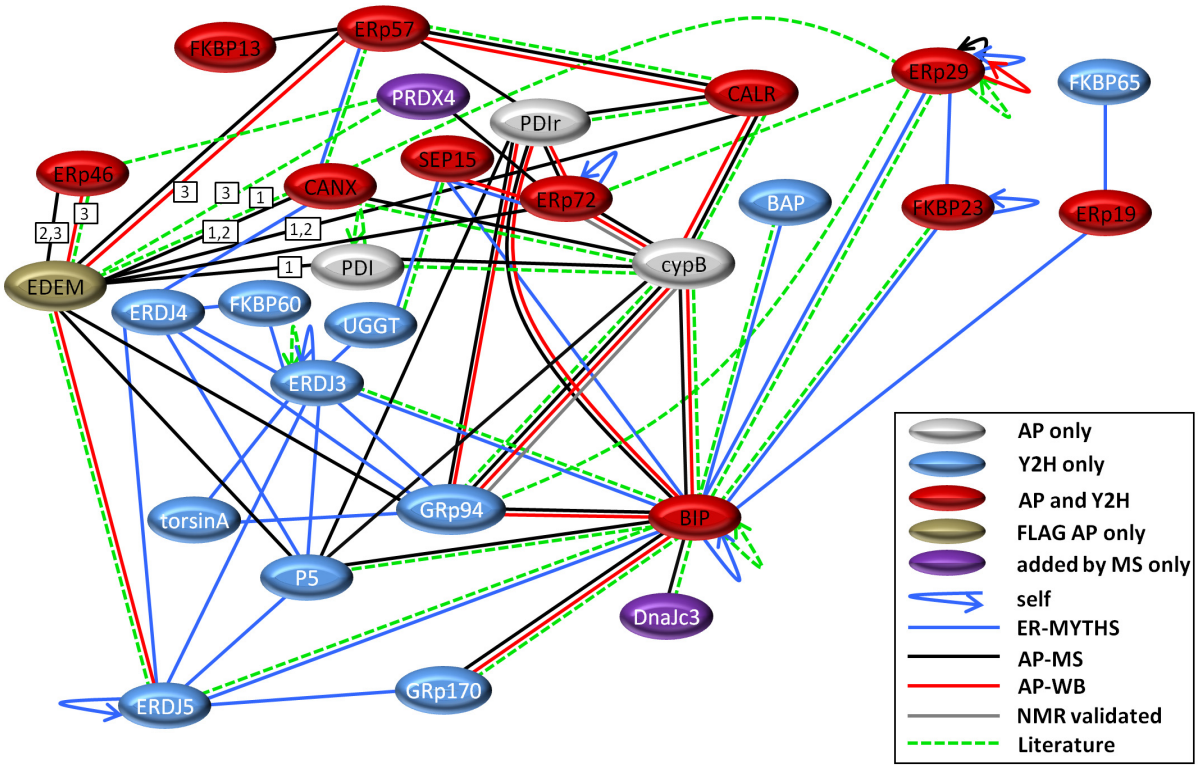
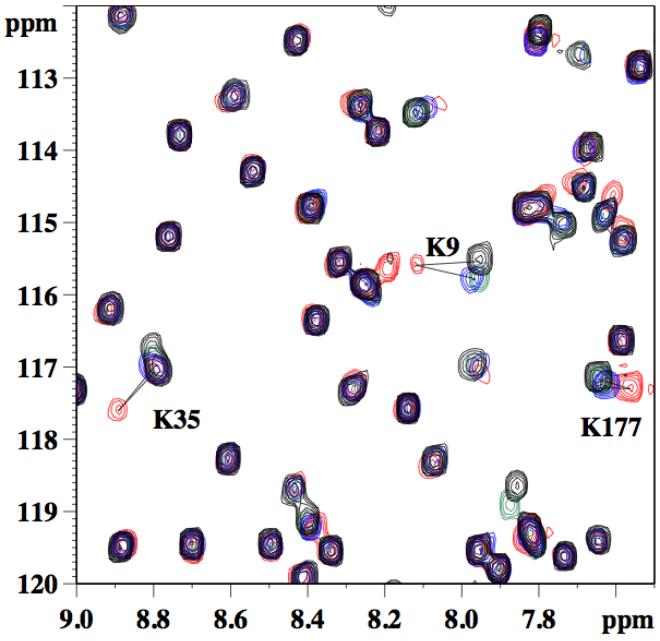


Fig. S6*B*, related to Fig.2. **Suitability of proteins for different detection methods used in ER-MAP.** Protein distribution between methods in ER-MAP. Proteins used in affinity purification only (GREY), Y2H only (BLUE), both AP and Y2H (RED), FLAG epitope affinity purification only (OLIVE), and proteins subsequently added to the network by MS detection that were therefore not used in Y2H or AP (PURPLE). Edge color represents interactions detected by ER-MYTHS (BLUE), AP-MS (affinity purification results by mass spectrometry) (BLACK), AP-WB (affinity purification results by western blotting) (RED), interactions validated by NMR experiments (GREY), and interactions already validated in the literature (GREEN).

A



B

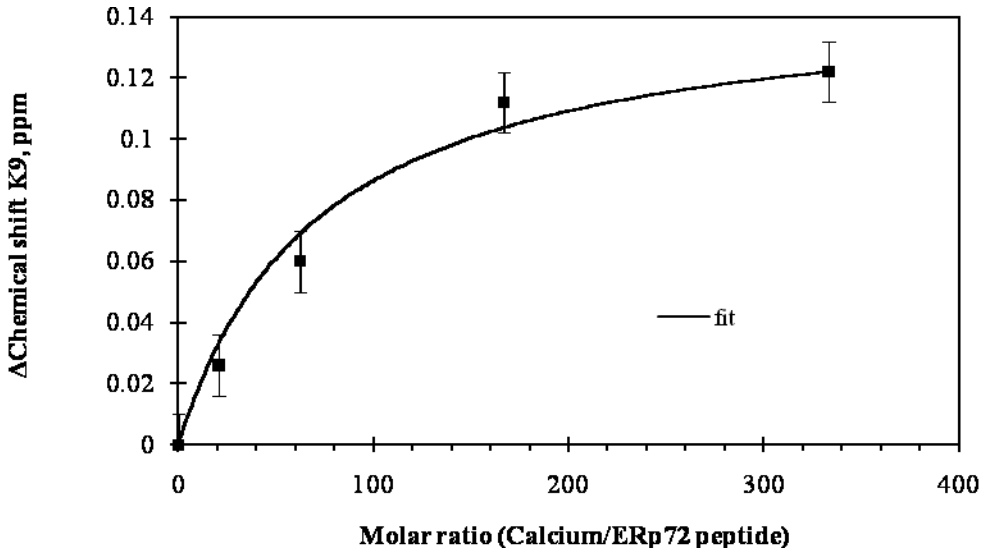


Fig.S7, related to Fig.3. **Calcium titration against the 36 amino acid N-terminal peptide of ERp72 bound to ^{15}N labeled cyclophilin B.** *A* ^{1}H-^{15}N heteronuclear single quantum correlation (HSQC) spectrum for 0.25 mM cyclophilin B (Black), 2:1 cyclophilin B: ERp72 peptide (Red), 2:1 cyclophilin B: ERp72 peptide in the presence of 30 mM $CaCl_2$ (Blue), pure cyclophilin B in the presence of 30 mM $CaCl_2$ (Green). *B* Calcium induced release of ERp72 peptide from cyclophilin B determined by chemical shift change of K-9 for calcium concentrations of 2.5, 7.5, 20 and 40 mM.

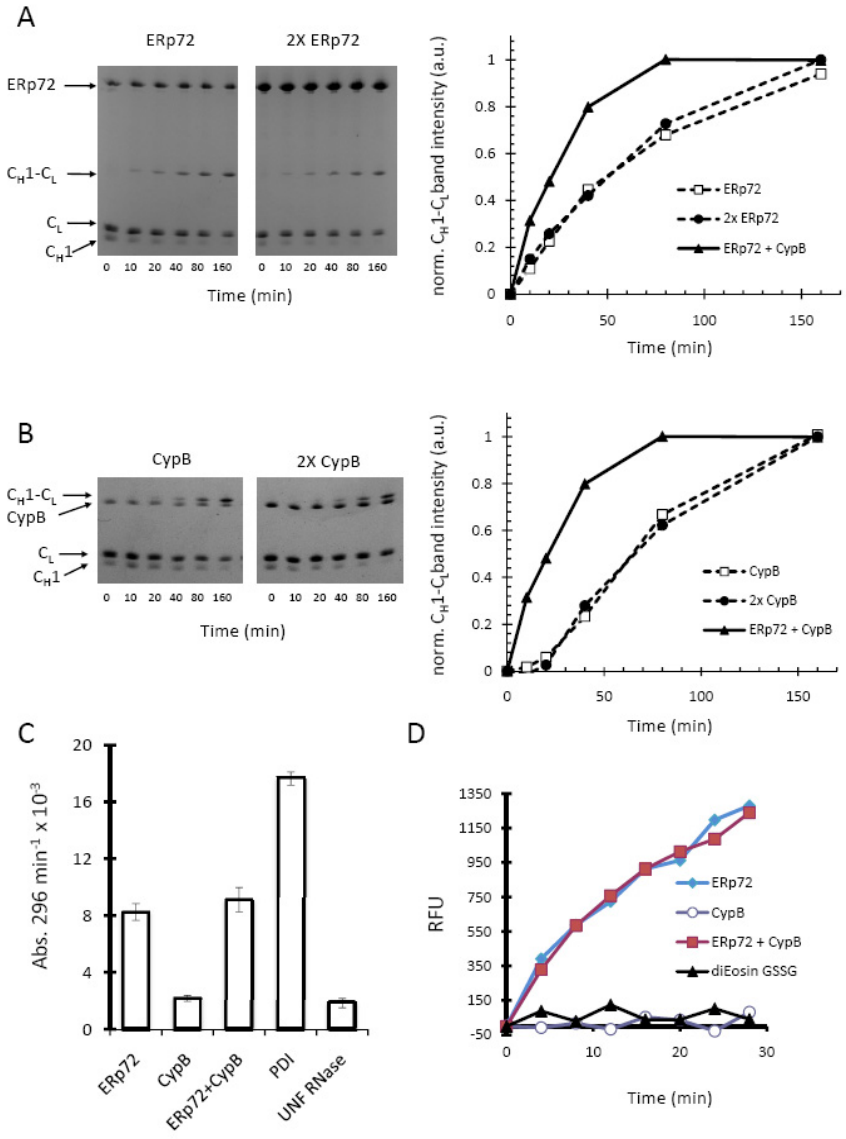


Fig.S8, related to Fig.4. **Doubling the concentration of ERp72** *A* **or cyclophilin B** *B* **does not enhance the rate of C_H1-C_L heterodimer assembly.** Assembly was monitored as described in experimental procedures with 5 μM and 10 μM of each protein. Quantified results are plotted on the right panels compared to ERp72/cyclophilin B combined (5 μM of each). *C* The activity of ERp72 to refold RNaseA *in vitro* is not inhibited by cyclophilin B. Refolding of unfolded and reduced RNaseA was monitored continuously by hydrolysis of 2'3' cCMP as previously described (Lyles and Gilbert, 1991), see methods. *D* The reductase activity of ERp72 towards the small molecule fluorescent substrate Di-Eosin-GSSG (1) is identical in the presence (red) or absence (blue) of cyclophilin B.

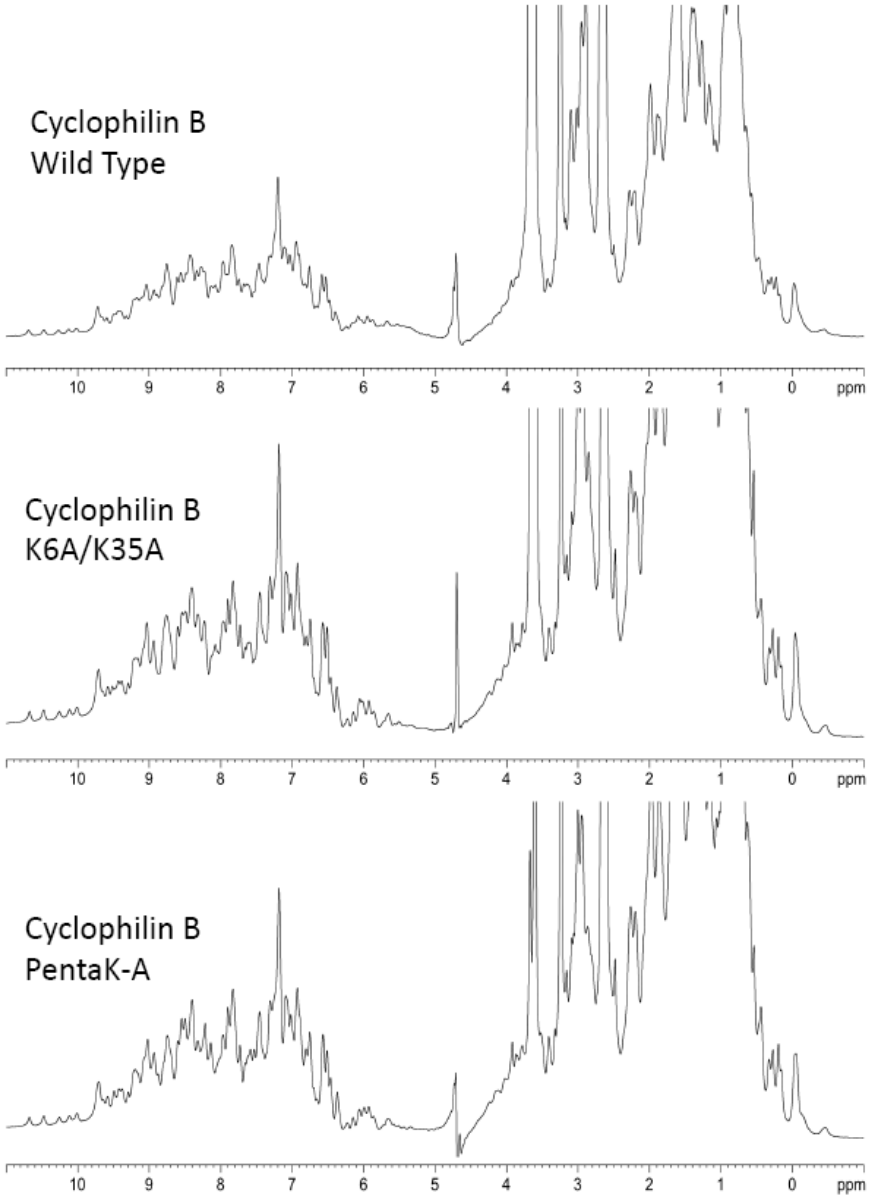


Fig.S9, related to Fig.5. **NMR spectra of purified recombinant cyclophilin B wild type and mutant proteins.** K6A/ K35A cyclophilin B, and PentaK-A (K4A/ K5A/ K6A/ K9A/ K35A) cyclophilin B spectra are essentially identical to each other and to wild-type cyclophilin B, illustrating that the proteins are similarly folded.

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Plasmid constructions -- To prepare clones for bacterial expression, cDNAs were cloned into pGEX-6P-1 (GE Healthcare) using *BamHI* and *NotI* restriction sites, as follows: rat ERp72 (E25-T639), human cyclophilin B (D33-E216), human FKBP23 (Q24-L222), human PDI (D18-L508), human ERp57 (A24-L505), human ERp29 (L33-L261), and human FKBP13 (A25-L142). Human ERp19 (S24-L172) and PDIr (A24-L519) were cloned into pGEX-6P-1 using *BglII* and *NotI* sites. Plasmid pUJ4 encoding mouse BiP with a C-terminal 6HIS tag was a generous gift of Johannes Buchner. Mouse EDEM1, 2, and 3 were cloned into pCDNA3 (Invitrogen) as *EcoRV–NotI* (EDEM1), *BglII–NotI* (EDEM2) or *BamHI–NotI* (EDEM3) fragments. EDEM1 and 2 coding sequences were amplified by PCR with the addition of a C-terminal double flag tag (DYKDHDGDYKDHD) followed by a stop codon, whereas for EDEM3 the double flag tag was added before 'KDEL' at the C-terminus. Cyclophilin B mutants were prepared by Quickchange Lightning site-directed mutagenesis (Stratagene) with mismatched primers and verified by DNA sequencing. For the two-hybrid study, genes were cloned without STOP codon in pDONR221 using Gateway cloning technology (InVitrogen). Genes were then introduced into the two-hybrid destination vectors pMYTHS3 (Ire1 K702R), and pMYTHS4 (Ire1 D995A) using Gateway cloning technology.

Antibodies used in this study -- α-ERp72 and α-GRp170 from Michael Green (St. Louis University, St. Louis, MO, USA); α-ERp57 and α-calreticulin from Daniel Tessier (BRI/NRC, Montreal, Canada); α-PDI from Stressgen, (Ann Arbor, Michigan, USA); α-

GRp94 from Christopher Nicchita (Duke University, Durham, North Carolina, USA); α-cyclophilin B, α-ERp19 and α-ERp29 from Abcam (Cambridge, MA, USA); α-BiP from Linda Hendershot (St. Jude's Children's Research Hospital, Memphis, TN, USA). Other antibodies used for Western blots of EDEM eluates were mouse monoclonal M2 anti-FLAG (Sigma), rabbit polyclonal anti-ERp46 (Abcam), rabbit polyclonal anti-ERp57 (Thermo Scientific), rabbit polyclonal anti-ERdj5 (Abnova).

ER-MYTHS Screen -- We have developed ER-MYTHS to be able to detect interactions between ER proteins based on the use of the type I transmembrane kinase Ire1p. Upon interaction of N-terminal generated Ire1p-fusions, the Ire1p kinase domains oligomerize, transphosphorylate and activate their C-terminal RNAseL domains, which specifically splice the mRNA of the transcriptional activator *HAC1*. The expression of the Hac1p splice variant leads to the interaction-specific expression of a reporter. The ER-MYTHS plasmids contain DNA fragments for the *IRE1* promoter and *IRE1* transmembrane/cytosolic domain in the pGREGs vector backbone (2). To be able to assay homo-oligomerizing proteins in ER-MYTHS, a trans-activating system was created by introducing inactivating Ire1p mutations in either the kinase (K702R) or RNase (D995A) domains. These mutations disable the auto-activation that would otherwise occur if Ire1p were fused to homotypic proteins. When fused to interacting proteins the Ire1p mutants are able to phosphorylate and activate each other in *trans*. Gateway (Invitrogen) cloning cassettes were introduced into the mutant vectors between the promoter and cytosolic fragment of *IRE1*, creating pMYTHS3 (K702R) and pMYTHS4 (D995A). Appropriate cDNAs in pMYTHS3 and pMYTHS4 were transformed into

YGJ574 (*MAT**a** ade2 trp1-901 leu2-3,112 Δire1::natR CYC1(2xupre)-LacZ::kanMX*, *CYC1(2xupre)-ADE2*) and YGJ575 (*MAT ade2 trp1-901 leu2-3,112 Δire1::natR CYC1(2xupre)-LacZ*, *CYC1(2xupre)-ADE2)*. Transformants were selected on SD-leu for pMYTHS3 plasmids and SD-trp for pMYTHS4. A mating-based two-hybrid interaction grid was setup to test all possible binary interactions for proteins as listed in Supplementary Table SIII. *MAT***a** and *MAT*α cultures were arrayed in 384 well plates in such a way that each *MAT***a** culture was mated with each individual *MAT*α culture in YPD medium overnight at 30°C. The mating matrix was then replicated in quadruplicate onto SD agar -leu-trp to select for diploid yeast containing both bait and prey. Diploids were screened for interactions by plating on SD-leu-trp-ade-inositol and incubated for 2 days at 30°C before replicating onto SD-leu-trp-ade-inositol for a second time. Interactions are indicated by the activation of the upre-coupled *ADE2* reporter which allows growth on media lacking adenine. Positive interactions were determined by growth compared to controls.

Protein Production -- 1L cultures of *E.coli* BL21-CodonPlus-RIL (Stratagene) were grown in LB supplemented with 100 μg/ml of ampicillin to an OD_{600} of 0.4-0.6. Recombinant protein expression was induced by addition of 1mM IPTG, followed by growth for another 4 hours at 30°C in a shaking incubator before harvesting. Cells were broken in PBS (pH 7.4) and GST-fusion proteins were purified by affinity chromatography on glutathione-Sepharose 4B resin (Amersham Biosciences). The GST tag was removed by cleavage with Prescission Protease (GE), leaving the N-terminal extension Gly-Pro-Leu-Gly-Ser. BiP with C-terminal 6HIS tag was purified by affinity

chromatography with Ni-NTA resin (Qiagen) as previously described (Knarr et al, 1999), and subsequently by size exclusion chromatography in 40 mM HEPES, pH 7.0, 400 mM NaCl, 5mM $CaCl_2$. Purified drosophila Sep15 was a generous gift from Sean Taylor.

Covalent Coupling of Proteins to Bead Matrix -- 0.5-2 mg of purified recombinant protein was coupled to 100 µl of ice cold 1 mM HCl-washed NHS-activated Sepharose 4B for 2 hours at 4°C in a total volume of 500 µl of HA buffer (115 mM potassium acetate, 20 mM HEPES pH 7.0, 0.75 mM $CaCl_2$, 0.1% TritonX-100) with rotation. After coupling, beads were blocked with 500 µl of 0.5 M NaCl, 0.5 M ethanolamine, pH 8.3 for 30 min with rocking at room temperature. Beads were washed 4X with 500 µl of HA buffer before short-term storage at 4°C.

Crosslinking and FLAG Coimmunoprecipitation -- Four 10cm-dishes per construct were seeded with HEK293 cells on day one, transfected on day 2 and then harvested for immunoprecipitation 40h after transfection. The lipophilic cross-linking agent DSP (dithiobis[succinimidylpropionate]) from Thermo Scientific was used to stabilize transient interactions. Cells were harvested by trypsin treatment, washed three times with 25mM HEPES-KOH pH 8.3 and 125mM KCl, then resuspended in the same solution at 10^6 cells per mL. They were incubated in a final concentration of 150µg/mL of DSP for 1h on ice. Cross-linking was quenched by addition of a final concentration of 100mM glycine and 40mM N-ethylmaleimide during incubation on ice for 15 minutes. Cells were then washed with TBS prior to lysis and immunoprecipitation. Cross-linking was reversed by reducing samples by boiling at 100^{0}C for 5mins in the presence of

Laemmeli buffer (final concentration of β-mercaptoethanol was of 2%). 1mL of the HEPES-acetate buffer (25mM HEPES pH 7.0, 115 mM potassium acetate) with 1% Triton and one protease inhibitor cocktail tablet (Roche Complete EDTA-) per 50 mL was used to lyse the cells. The membrane fraction was then removed by a 30min centrifugation at 14000rpm at 4°C. The soluble fraction was precleared for 1h by incubation with protein G Sepharose beads (40 μL 50% slurry washed in lysis buffer) (GE Healthcare) with head-over-tail rotation at 4°C. Samples were centrifuged at 15000rpm for 15mins at 4°C and the soluble supernatant was incubated for 3h with anti-FLAG beads (40μL of 50% slurry washed in lysis buffer) (Sigma EZview Red ANTI-FLAG M2 Affinity Gel) with rotation at 4°C. Supernatant was removed and kept at -20°C. Beads were then washed three times with 1mL of 0.5% Triton HEPES-acetate with 1 protease inhibitor cocktail per 50mL, the supernatant was rapidly removed each time. Beads were then eluted with 40 μL of 3X FLAG peptide (Sigma) used at 150ng/μl.

RNaseA Refolding Assay -- 3 mg of RNaseA from bovine pancreas (Sigma) was reduced and unfolded in a volume of 500 μl of 0.1 M Tris-HCl (pH 8.0) containing 6M guanidine HCl and 140 mM DTT for 20 minutes at room temperature. The unfolded enzyme was desalted with a NAP-5 column into 0.1% formic acid. Concentration of RNaseA was determined spectrophotometrically using a molar absorbance at 278 nm of 9800 $cm^{-1}M^{-1}$. RNase refolding was monitored by hydrolysis of 2'3' cCMP essentially as previously described (3). Briefly, 11.4 μM RNAseA was refolded using 6.4 μM of ERp72, cyclophilin B, PDI, or a combination of ERp72 and cyclophilin B in 200 μl of 0.1 M Tris-Acetate, pH 8.0 containing 0.5 mM oxidized glutathione and 2 mM reduced

glutathione. Folding was initiated by the addition of reduced RNaseA to each folding mixture, and Abs. 296 was monitored in a 96 well plastic UV compatible plate (SARSTEDT, Numbrecht, Germany) using a Molecular Devices Spectramax M5 plate reader at 25°C. Folding rate was determined by the slope of the absorbance curve between 30-600 seconds from initiation of the refolding reaction. Results shown are the mean slope for 3 separate experiments, and error bars reflect the standard deviation from the mean.

Di-Eosin-GSSG Reductase Assay -- Reductase activity of ERp72 in the absence and presence of cyclophilin B was assayed with the self-quenching fluorescent substrate Di-Eosin-GSSG. Identical reductase activity of ERp72 was observed in the presence or absence of cyclophilin B. Assays were performed as previously published for PDI (1).

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