

Supplementary Table 1

Figure 1g - BiP ATPase activity with effector proteins (pmol/ μ l/min)

BiP	0.082
BiP+IRE1	0.086
BiP+PERK	0.087
BiP+Sil1	0.107
BiP+Sil1+IRE1	0.080
BiP+Sil1+PERK	0.081
BiP+ERdj3	0.221
BiP+ERdj3+IRE1	0.091
BiP+ERdj3+PERK	0.084
BiP+ERdj3+Sil1	0.386
BiP+ERdj3+Sil1+IRE1	0.081
BiP+ERdj3+Sil1+PERK	0.086

Supplementary Table 2

Figure 2b - BiP ATPase activity with effector proteins and CH1 (pmol/ μ l/min)

BiP	0.082
BiP+CH1	0.126
BiP+Sil1	0.107
BiP+Sil1+ CH1	0.161
BiP+ERdj3	0.221
BiP+ERdj3+CH1	0.358
BiP+ERdj3+Sil1	0.386
BiP+ERdj3+Sil1+CH1	0.506
BiP+ERdj3+Sil1+CH1+ IRE1	0.348

Supplementary Table 3

Figure 2e-h – Binding affinities for BiP and BiP_{V461F} to both folded and misfolded IRE1 LD

	K_d (μ M)
BiP _{WT} + IRE1	0.54 ± 0.06
BiP _{V461F} + IRE1	0.7 ± 0.1
BiP _{WT} + IRE1 _{misfolded}	2.9 ± 0.5
BiP _{V461F} + IRE1 _{misfolded}	no specific binding as data do not fit saturation curve

Supplementary Table 4

Figure 2j - BiP ATPase activity comparing the effects of folded and misfolded IRE1 (pmol/ μ l/min)

BiP	0.082
BiP + IRE1	0.085
BiP + IRE1 _{misfolded}	0.172
BiP + Sil1 + IRE1	0.080
BiP + Sil1 + IRE1 _{misfolded}	0.194
BiP + ERdj3 + IRE1	0.091
BiP + ERdj3 + IRE1 _{misfolded}	0.353
BiP + ERdj3+ Sil1+ IRE1	0.081
BiP + ERdj3 + Sil1+IRE1 _{misfolded}	0.483
BiP+ERdj3+Sil1+IRE1+ IRE1 _{misfolded}	0.336

Supplementary Table 5

Figure 3h-k – Binding affinities for GST tagged BiP_{WT} and BiP_{K294F} to effector proteins

	K _d (μ M)
BiP _{WT} + IRE1	0.66 \pm 0.12
BiP _{K294F} + IRE1	no specific binding - data did not fit saturation curve
BiP _{WT} + PERK	2.6 \pm 0.5
BiP _{K294F} + PERK	no specific binding - data did not fit saturation curve
BiP _{WT} + Sil1	8.4 \pm 1.6
BiP _{K294F} + Sil1	38 \pm 8.0
BiP _{WT} + ERdj3	8.5 \pm 1.0
BiP _{K294F} + ERdj3	17.8 \pm 4.6

Supplementary Table 6

Figure 4 – Binding affinities for GST tagged BiP with effector proteins in the presence of ATP and ADP.

	K_d (μM)
BiP + IRE1 + ATP	1.4 ± 0.17
BiP + IRE1 + ADP	1.5 ± 0.2
BiP + PERK + ATP	1.9 ± 0.3
BiP + PERK + ADP	2.4 ± 0.4
BiP + Sil1 + ATP	1.6 ± 0.3
BiP + Sil1 + ADP	1.8 ± 0.3
BiP + ERdj3 + ATP	4.5 ± 0.9
BiP + ERdj3 + ADP	4.1 ± 0.8

Supplementary Table 7

Figure 5 e-h – IC_{50} and K_i values for both low and high concentration phases in the presence and absence of nucleotides.

	IC_{50} (μM)		K_i (μM)	
	Low phase	High phase	Low phase	High phase
APO	3.67 ± 0.28	121.6 ± 13.5	0.156 ± 0.013	5.2 ± 0.6
ADP	0.064 ± 0.008	102 ± 8.9	0.003 ± 0.0003	4.3 ± 0.4
AMP-PNP	0.57 ± 0.05	14.7 ± 1.0	0.02 ± 0.002	0.6 ± 0.05
ATP	0.034 ± 0.004	4.7 ± 0.15	0.0015 ± 0.0002	0.2 ± 0.01