Table S1. Ion Intensities of Selected[1] Phosphorylated Peptides Detected by Mass Spectrometry, Related to **Figure 1**

Residue	Peptide[2]	Hrr25 alone		Coexpression		
		Phosphorylated Intensity[3]	Unmodified Intensity	Phosphorylated Intensity	Unmodified Intensity	Change in sensitivity[4]
Hrr25 S17	K.IGS#GSFGDIYHGTNLISGEEVAIK.L	3.57E+05	1.53E+07	3.34E+04	2.90E+07	0.049
Hrr25 S19	K.IGSGS#FGDIYHGTNLISGEEVAIK.L	2.47E+06	1.53E+07	1.68E+05	2.90E+07	0.036
Hrr25 S325	K.GDLNANSNAASASNS#TDNK.S	5.20E+04	2.86E+06	8.23E+05	4.34E+07	1.04
Hrr25 S330	K.GDLNANSNAASASNSTDNKS#ETFNK.I	3.97E+06	6.80E+06	7.75E+06	1.65E+07	0.804
Hrr25 S377	K.QQTILNNNAASS#LPEELLNALDK.G	5.38E+06	7.15E+06	3.37E+05	7.94E+06	0.056
Mam1 S214	K.ENIEDLTIEIDS#IIETNHQK.K	-	-	1.83E+06	1.41E+06	-
	R.KENIEDLTIEIDS#IIETNHQK.K	-	-	6.70E+05	6.55E+05	-
	K.RKENIEDLTIEIDS#IIETNHQK.K	-	-	8.44E+05	2.34E+05	-
	K.RKENIEDLTIEIDS#IIETNHQKK.R	-	-	1.91E+05 [5]	3.28E+04	-

[1]Peptides chosen were those in which both the phosphorylated and unmodified peptide were detected in both Hrr25 and coexpressed samples.

[2]Periods (.) indicate trypsin cleavage sites. Pound symbols (#) indicate phosphorylated residue.

[3]Peak intensities listed are those of the (+2) ion unless otherwise noted.

[4]Change in sensitivity = $(\text{phosphorylated}_{coex}/\text{unmodified}_{coex})/(\text{phosphorylated}_{Hrr25}/\text{unmodified}_{Hrr25})$. A number below 1 indicates that detected phosphorylation at this site was reduced when Hrr25 was coexpressed with Mam1.

[5](+3) ion.

Table S2. Molecular Mass Determinations from Sedimentation Equilibrium Centrifugation/ Multi-Angle Light Scattering, Related to **Figure 1**

Protein/Complex	Method[1]	Observed MW (kDa)	Calculated MW (kDa)[2]	Oligomeric state	Reference
Csm1 full-length	AUC	43.2 +/- 0.9	43.5	2 Csm1	(Corbett et al., 2010)
$Csm1^{69-190}$	AUC	29.1 +/- 2.3	28.2	2 Csm1	(Corbett et al., 2010)
Csm1:Lrs4 full-length	AUC	172.3 +/- 4.7	165.6	4 Csm1:2 Lrs4	(Corbett et al., 2010)
$Csm1:Lrs4^{1-102}$	AUC	108.5 +/- 3.0	111.0	4 Csm1:2 Lrs4	(Corbett et al., 2010)
$Csm1:Mam1^{221-290}$	AUC	50.3 +/- 2.7	51.5	2 Csm1:1 Mam1	this work
$Csm1^{69-181}/Mam1^{221-290}$	AUC	32.8 +/- 1.6	33.9	2 Csm1:1 Mam1	this work
$Hrr25^{1-394}$ K38R	SEC-MALS	43.6 +/- 1.5	45.8	1 Hrr25	this work
$Hrr25^{1-394}$ K38R:$Mam1^{87-191}$	AUC	57.2 +/- 2.8	58.6	1 Hrr25:1 Mam1	this work
$Hrr25^{1-494}$ K38R:$Mam1^{87-191}$	SEC-MALS	69.9 +/- 1.2	70.1	1 Hrr25:1 Mam1	this work
His-$Hrr25^{1-394}$ K38R: $Mam1^{87-302}$:$Csm1:Lrs4^{1-102}$	AUC	258 +/- 37 [3]	258.5	2 Hrr25:2 Mam1:4 Csm1:2 Lrs4	this work

[1] The molecular mass was measured either by equilibrium analytical ultracentrifugation (AUC) or by size exclusion chromatography/multi-angle light scattering (SEC-MALS).

[2] 'Calculated MW' is the expected molecular mass of a complex with the stoichiometry listed in 'Oligomeric State'.

[3] Data showed evidence of aggregation or self-association (non-linearity of r^2 *vs.* $\log(A_{280})$ plot, especially at low speed), so curves were truncated to minimize the impact of aggregated species on the molecular weight estimate.

Table S3. Data Collection and Refinement Statistics, Related to **Figure 2**

Data collection	**Csm1:Mam1$^{221\text{-}290}$ Native**	**Csm1:Mam1$^{221\text{-}290}$ F243M S-anomalous**
Resolution (Å)	50 – 3.05	50 – 4.4
Wavelength (Å)	0.9795	1.771
Space Group	$P3_121$	$P3_121$
Unit Cell Dimensions (a, b, c) Å	101.85, 101.85, 225.08	101.89, 101.89, 224.72
Unit Cell Angles (α,β,γ) °	90, 90, 120	90, 90, 120
I/σ (last shell)	5.5 (1.4)	3.5 (1.3)
[1]R_{sym} (last shell)	0.091 (0.721)	0.216 (1.196)
[2]R_{meas} (last shell)	0.125 (0.991)	0.263 (1.448)
Completeness (last shell) %	98.6 (99.7)	99.6 (100.0)
Number of reflections	60821	50297
unique	26073	9017
Multiplicity (last shell)	2.3 (2.4)	5.6 (5.9)
Refinement		
Resolution (Å)	50 – 3.05	
No. of reflections	26017	
working	24705	
free	1312	
[3]R_{work} (last shell) (%)	21.32 (36.77)	
[3]R_{free} (last shell) (%)	26.02 (39.13)	
Structure/Stereochemistry		
No. of atoms	2829	
r.m.s.d. bond lengths (Å)	0.010	
r.m.s.d. bond angles (°)	1.394	
Ramachandran favored/allowed (%)	98.2%/100.0%	
[4]MolProbity score/percentile	2.77/87th	
[5]Protein Data Bank ID	4EMC	

[1]$R_{sym} = \sum\sum_j |I_j - \langle I \rangle| / \sum I_j$, where I_j is the intensity measurement for reflection j and $\langle I \rangle$ is the mean intensity for multiply recorded reflections.

[2]$R_{meas} = \sum_h [\sqrt{(n/(n\text{-}1))} \sum_j [I_{hj} - \langle I_h \rangle] / \sum_{hj} \langle I_h \rangle$

where I_{hj} is a single intensity measurement for reflection h, $\langle I_h \rangle$ is the average intensity measurement for multiply recorded reflections, and n is the number of observations of reflection h.

[3]$R_{work,\ free} = \sum||F_{obs}| - |F_{calc}||/|F_{obs}|$, where the working and free R-factors are calculated using the working and free reflection sets, respectively.

[4]Calculated by the MolProbity structure validation server (http://molprobity.biochem.duke.edu).

[5]Coordinates and structure factors deposited at the Protein Data Bank (http://www.pdb.org).