SUPPLEMENTAL DATA: 1 TABLE, 4 FIGS, 2 MOVIES

SUPPLEMENTAL TABLE S-I. pMpk1/tMpk1 data for Figure 3

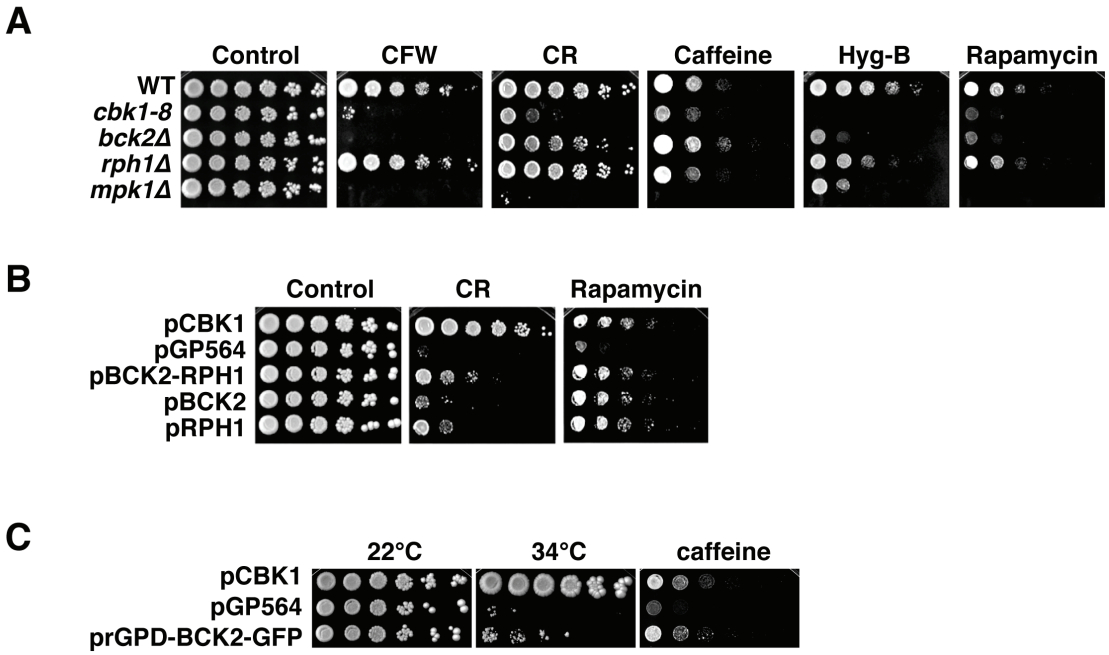
Heat shock (39°C)

Time	WT		*cbk1-8*		*bck2*Δ		*knr4*Δ		*sdp1*Δ		*bck1*Δ
	Ratio	SE	Ratio	SE	Ratio	SE	Ratio	SE	Ratio	SE	Ratio
0	1.4	0.04	2.0	0.55	1.4	0.67	1.2	0.51	1.5	1.0	0.3
15	4.5	0.44	5.0	0.28	4.3	0.17	3.6	0.82	5.4	0.22	0.5
30	4.8	0.7	4.7	1.27	5.0	0.7	3.9	0.35	4.4	0.6	0.4
45	3.4	0.27	3.9	1.55	4.0	0.36	3.3	0.23	3.7	0.66	0.6
60	2.2	0.21	4.5	1.41	3.6	0.9	3.6	0.8	3.6	0.45	0.5
75	2.3	0.26	5.3	0.98	3.8	0.9	4.1	0.3	5.0	0.58	0.5
90	2.5	0.31	7.3	0.63	4.2	0.51	4.7	1.0	5.1	0.93	0.5
105	2.4	0.31	6.5	0.28	3.6	1.0	4.0	1.3	5.2	1.37	0.5
120	2.0	0.14	8.2	0.84	4.5	1.2	5.0	1.65	5.7	1.7	0.53

Caffeine

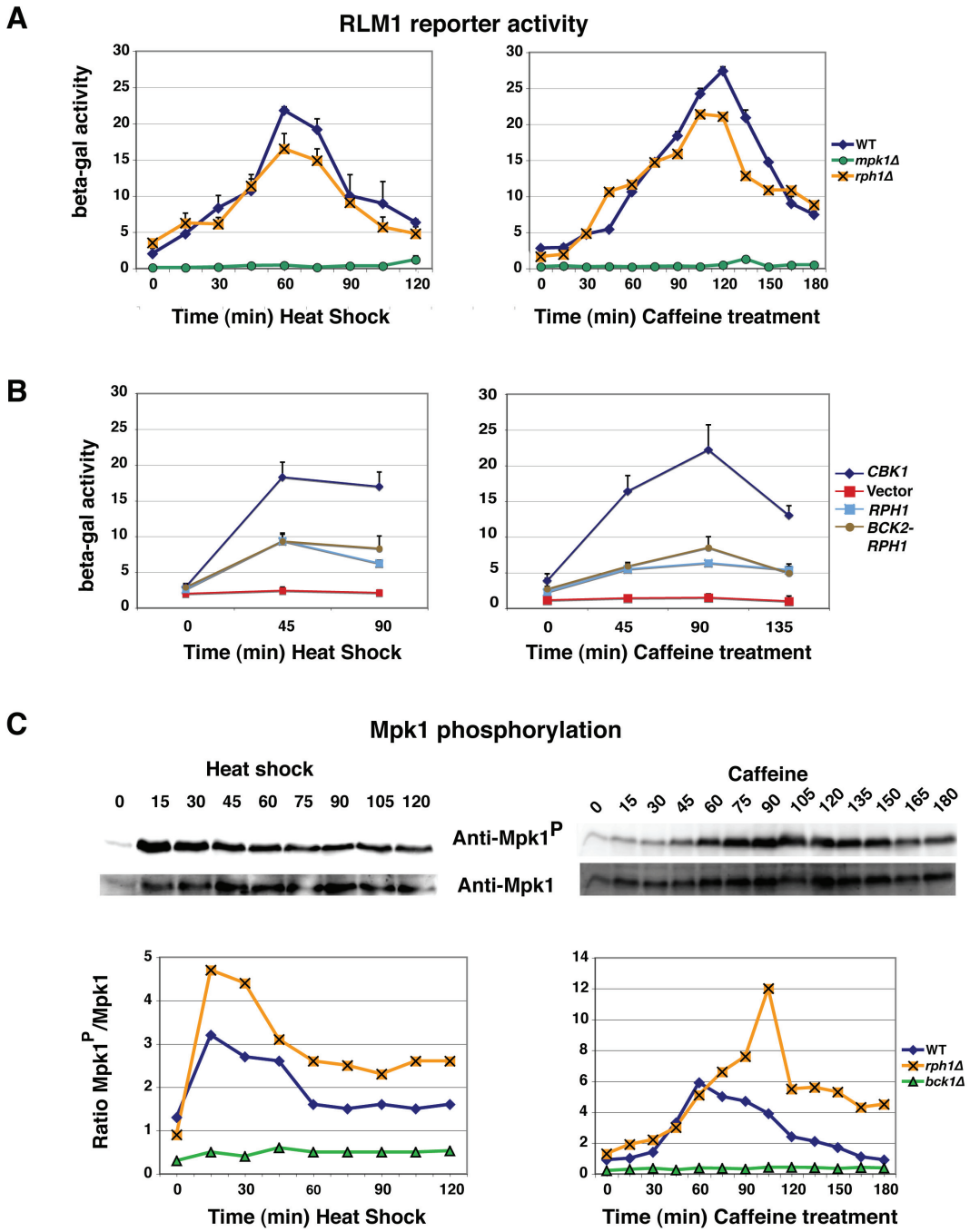
Time	WT		*cbk1-8*		*bck2*Δ		*knr4*Δ		*sdp1*Δ		*bck1*Δ
	Ratio	SE	Ratio	SE	Ratio	SE	Ratio	SE	Ratio	SE	Ratio
0	0.8	0.06	2.4	0.05	1.5	0.55	2.6	2.1	2.5	0.41	0.2
15	1.0	0.02	1.8	0.06	1.8	0.7	3.5	1.8	1.7	0.31	0.3
30	2.0	0.55	3.7	0.25	2.8	1.1	4.6	3	2.4	0.36	0.3
45	3.5	0.17	5.5	0.41	4.6	1.5	6.0	4.7	4.3	0.3	0.2
60	4.5	1.3	9.1	0.4	7.4	0.6	7.0	4.6	3.8	1.0	0.4
75	3.8	1.1	7.7	0.26	9.3	1.7	8.0	4.0	3.6	0.43	0.3
90	3.3	1.3	7.0	0.52	9.8	2.2	9.7	3.2	3.0	0.33	0.3
105	3.0	1.0	6.0	1.0	8.2	2.7	9.2	4.7	3.8	1.0	0.4
120	2.0	0.4	5.4	1.0	6.7	2.3	11.0	3.7	3.0	0.75	0.4
135	1.7	0.43	5.4	0.86	4.8	2.2	12.7	2.4	2.1	0.3	0.4
150	1.4	0.4	6.0	0.09	4.1	2.6	15.7	2.3	1.9	0.45	0.3
165	1.0	0.09	6.6	0.033	3.6	2.8	10.5	1.6	1.4	0.23	0.4
180	0.85	0.06	5.0	0.01	3.4	2.7	7.7	1.3	1.1	0.2	0.4

Supplemental Figure S1



Supplemental Figure S1. Comparative analysis of *cbk1-8, bck2*Δ*, rph1*Δ and *mpk1*Δ mutant phenotypes. A) 10-fold dilution series of *cbk1-8, bck2*Δ*, rph1*Δ and *mpk1*Δ cells were grown on media containing 100 ug/ml Calcofluor White (CW), 100ug/ml Congo Red (CR), 15mM Caffeine, 50 ug/ml Hygromycin B (Hyg B) 1.2 nM Rapamycin. B) High copy BCK2, RPH1 and BCK2-RPH1 plasmids suppress the Congo Red and Rapamycin sensitivities of *cbk1-8* cells. pCBK1 and empty vector serve as positive and negative controls. C) 10-fold dilution series of *cbk1-8* cells containing a low copy plasmid of GPD-driven Bck2-GFP (pGPD-BCK2-GFP). Moderately overexpressed Bck2 suppresses the temperature sensitivity and caffeine sensitivity of *cbk1-8* cells. The yeast strains and plasmids are listed in Table I and II.

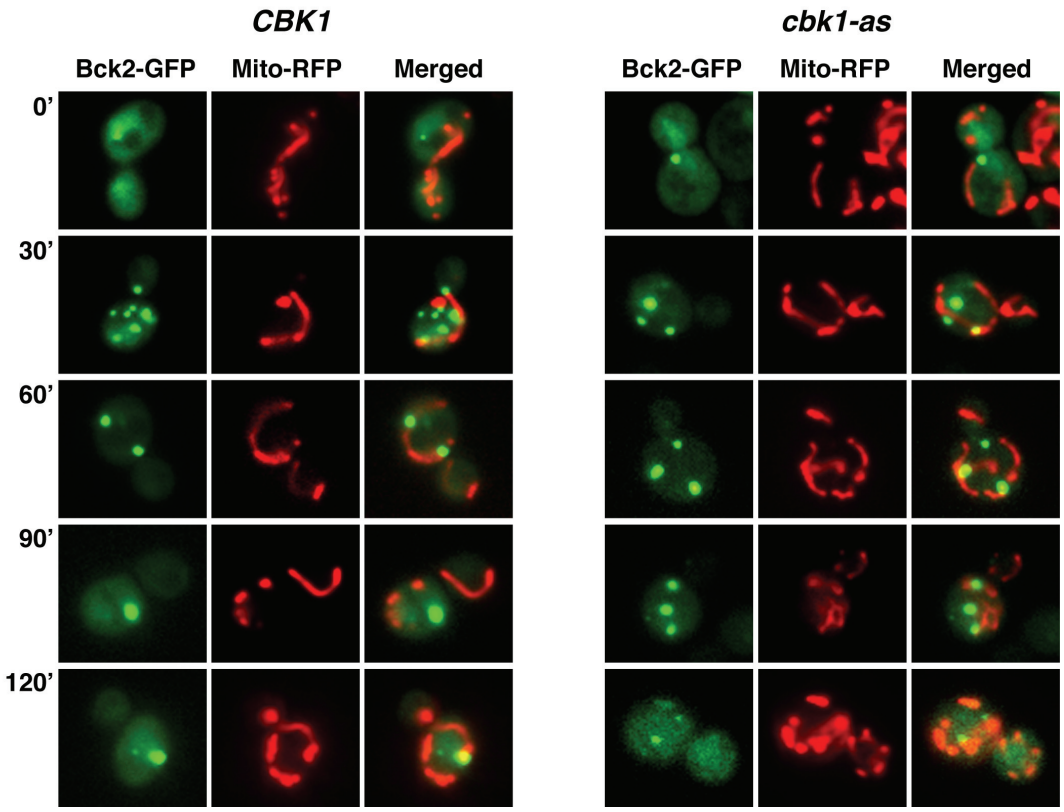
Supplemental Figure S2



Supplemental Figure S2. Role of Rph1 in Rlm1 reporter activation. A) Rlm1 reporter activity from *rph1*Δ cells was quantified and plotted at the designated time points after initiating heat shock (left panel) and caffeine-treatment (right panel), as described

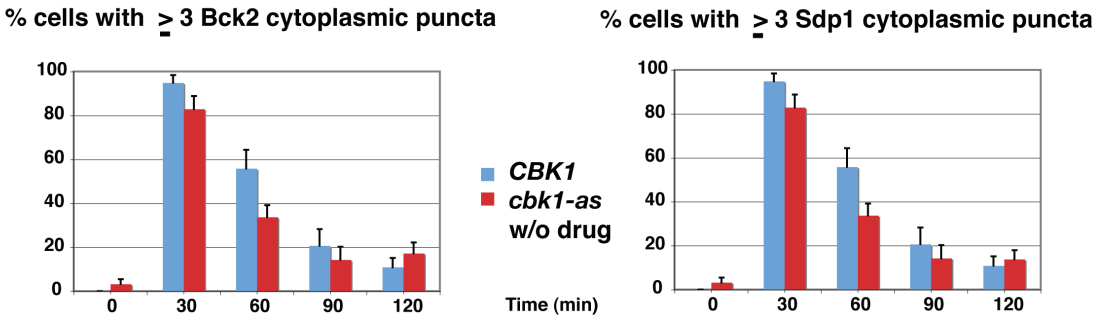
for **Fig. 2**. B) Rph1 overexpression via high copy plasmids partially restores RLM1 reporter activity in heat shocked (left panel) and caffeine-treated (right panel) *cbk1-8* cells. Positive and negative controls (cells containing pCBK1, empty vector) from parallel experiments (from **Fig. 2**) are included for reference. C) Rph1 is not essential for Mpk1 Thr^{190}/Tyr^{192} phosphorylation. Mpk1 Thr^{190}/Tyr^{192} phosphorylation levels in *rph1*Δ cells (FLY3278) were monitored by quantitative immunoblots at various times after heat shock initiation and caffeine treatment. The ratios of phospho-Mpk1 to total Mpk1 protein were plotted. Data from wild type and *bck1*Δ cells (from **Fig. 3**) were plotted as positive and negative controls.

Supplemental Figure S3



Supplemental Figure S3. Colocalization of Bck2 and mitochondria during heat shock. Parallel experiment to **Fig. 9A** showing the timing of Bck2 colocalization with mitochondria upon heat shock. Cells expressing Bck2-GFP and Mito-RFP (pHCRED) were treated and monitored as described for **Fig. 9A**. Heat shock and Cbk1 inhibition (1NA-PP1 addition) were done simultaneously in *cbk1-as* cells. All images represent single optical sections.

Supplemental Figure S4



Supplemental Figure S4. **Additional controls for Fig. 9.** The percentage of Bck2 and Sdp1 puncta were quantified in heat shocked *cbk1-as* cells in the absence of 1NA-PP1. In the absence of 1NA-PP1, the phenotypes of *cbk1-as* cells are similar to that of wild type cells. Data from wild type cells (from **Fig. 9**) are included as a reference.

Supplemental Movie 1. The images from the experiment in **Fig. 5B** are presented as a movie (3 frames/second).

Supplemental Movie 2. Rotating images of the 3D model in **Fig. 7B**.