# Supplemental Materials

***Molecular Biology of the Cell***

Roy et al.

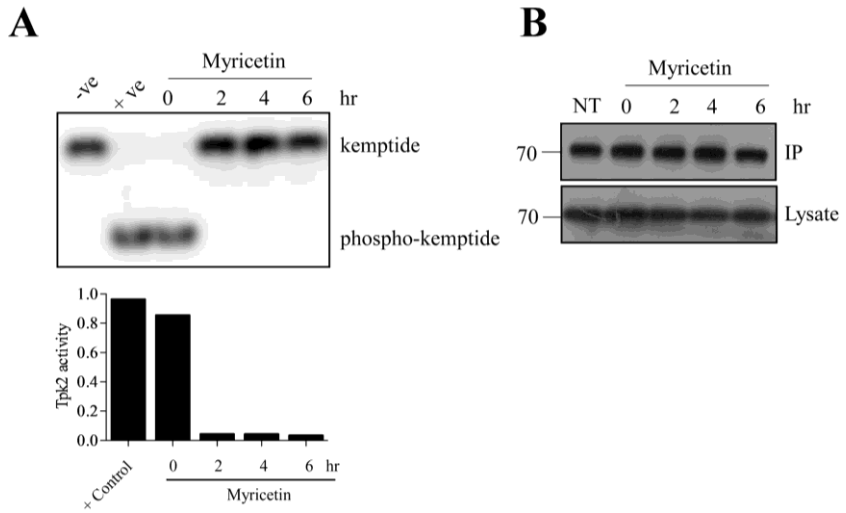


**Figure S1.** Hsp70 impairment inhibits the activity of Tpk2 kinase. (A) Yeast cells transformed with GST-Tpk2 was expressed from GAL promoter in the presence of Hsp70 inhibitor, myricetin (100μM). The cells were harvested at regular time interval (0, 2, 4, 6 hour). The kinase activity of Tpk2 was measured by nonradioactive PKA assay kit (Promega) using kemptide as substrate after isolating GST-Tpk2 kinase by glutathione resin. The phosphorylated and nonphosphorylated kemptide was separated by the agarose gel electrophoresis. The quantification was done according to the manufacturer protocol and plotted as bar diagram. (B) Western Blot of GST-Tpk2 kinase used for the kinase assay in panel (A). The lower panel showed GST-Tpk2 expression in the respective lysate.

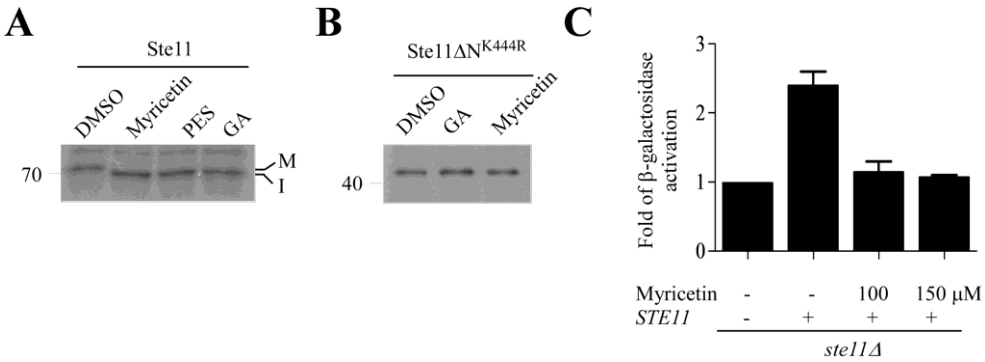


**Figure S2**. Hsp70 facilitates maturation of Ste11 kinase. (A) Pulse labelling of WT Ste11 kinase for 10 min in the presence of DMSO, myricetin (100μM), PES (150μM), and geldanamycin (50μM). Mature (M, phosphorylated) & immature (I, non-phosphorylated) forms are denoted by arrows. (B) Pulse labelling of N-terminal deleted kinase dead mutant of Ste11 kinase, Ste11ΔN$^{K444R}$ in the presence of geldanamycin (50μM) or myricetin (100μM). (C) α-factor induced activation of Ste11 kinase in the presence of myricetin. *ste11Δ* cells were transformed with p316GAL-STE11 and PRE-LacZ (pheromone responsive element fused with β-galactosidase gene). Ste11 kinase was expressed from GAL promoter in the presence of myricetin (100 μM, or 150μM). The cells were treated with DMSO or 5 μM α-factor for two hour and β-galactosidase activity was measured. Fold activation of β-galactosidase activity was plotted. The bar represents the standard error of three independent experiments.

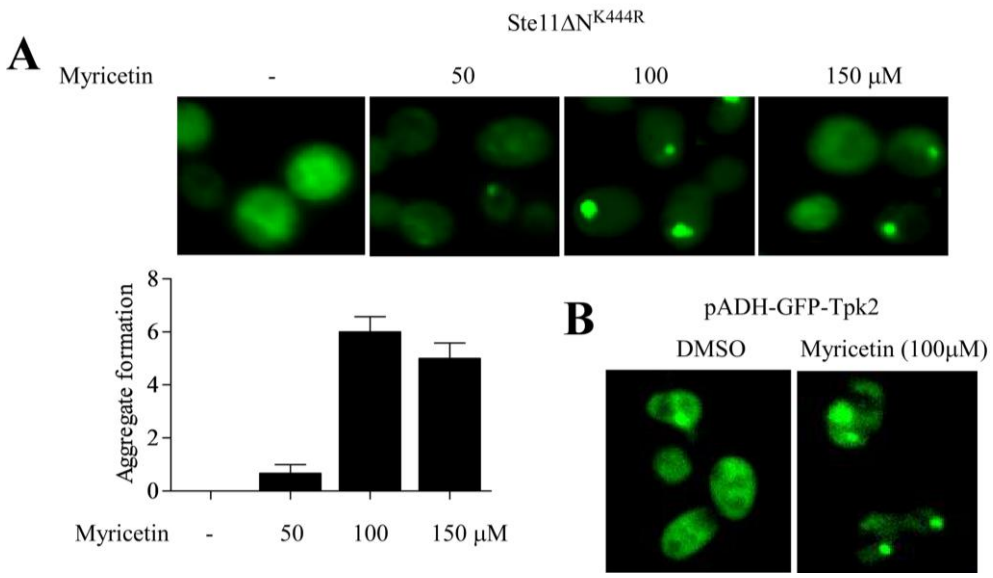


**Figure S3**. Hsp70 inhibition with myricetin generates kinase aggregates in a dose dependent manner. (A) GFP-Ste11ΔN$^{K444R}$ expressing yeast cells were incubated with different concentrations of myricetin (50μM, 100μM, 150μM) for two hour. The kinase aggregates were counted in the microscope using at least three different fields with five cells per field. The propensity of aggregate formation was represented as bar diagram. Bar represents SE, n=3. (B) ADH-GFP-Tpk2 expressing yeast cells were treated with myricetin (100μM) for two hour.

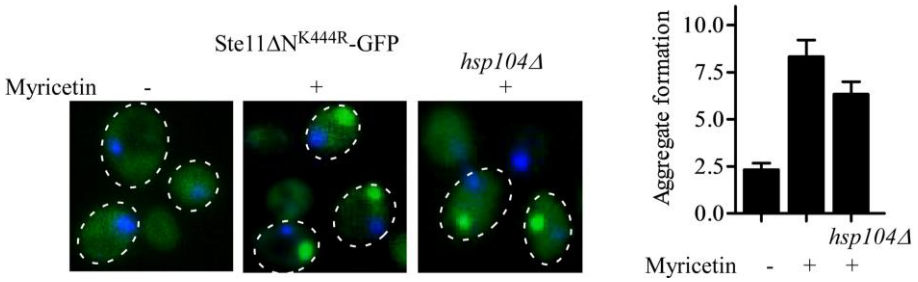


**Figure S4.** IPOD formation does not require Hsp104. GFP-Ste11ΔN$^{K444R}$ kinase was expressed from GAL promoter in WT and *hsp104Δ* cells. The cells were treated with myricetin (100μM) or DMSO for two hour and viewed under fluorescent microscope. The nucleus was stained with Hoechst dye. Kinase inclusions were counted in a minimum of three different fields with an average of five cells per field and plotted as bar diagram. Bar represents standard error of three independent experiments.

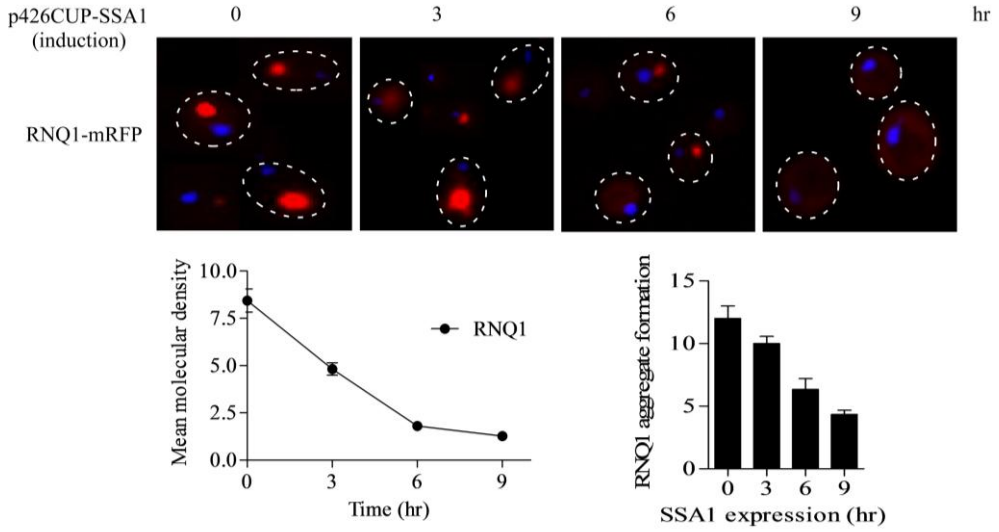


**Figure S5**. Hsp70 overexpression mobilizes RNQ1 inclusions. The RNQ1-mRFP containing yeast cells were transformed with p426CUP-SSA1 plasmid. RNQ1 expression from galactose promoter formed deposits at the peripheral site of yeast cells. SSA1 was expressed from CUP promoter with 300 µM of copper. The fate of the RNQ1 aggregate was observed at regular time interval (0, 3, 6, 9 hour) after expression of SSA1. The aggregates were counted and plotted as bar diagram. The intensity and diameter of the aggregates were measured and plotted as mean molecular density at different time of SSA1 expression.

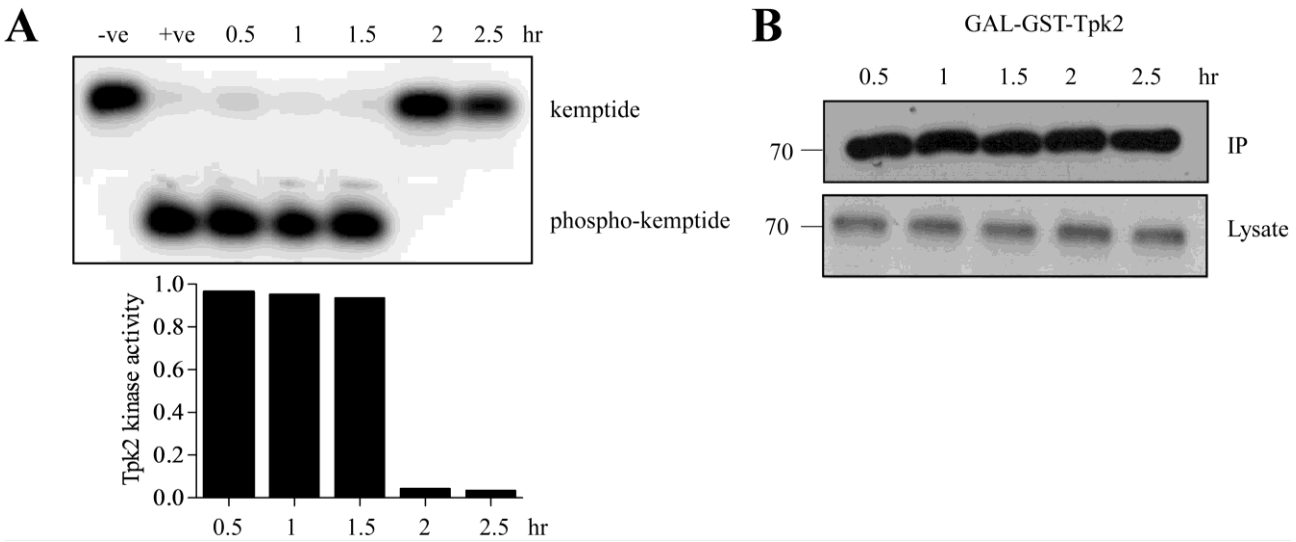


**Figure S6**. Heat stress inactivates Tpk2 kinase activity. (A) GST-Tpk2 expressing yeast cells were incubated at 42 °C for different times. The cells were harvested and Tpk2 kinase activity was measured as described in 'Methods'. The kinase activity was plotted as bar diagram. (B) Western Blot of GST-Tpk2 kinase used for the kinase assay in panel (A). The lower panel showed GST-Tpk2 expression in the lysate.

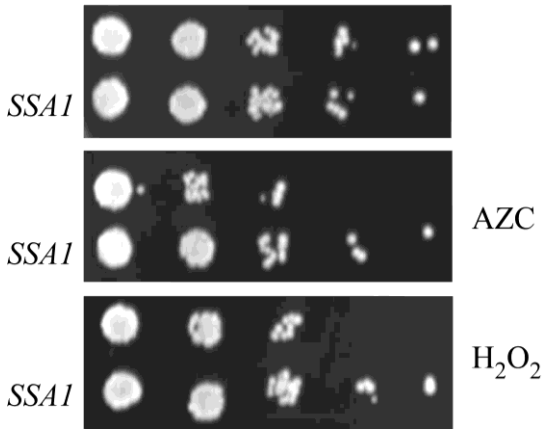


**Figure S7**. Hsp70 rescues cells from proteotoxic stresses. Growth of yeast cells (WT and *SSA1* over-expressed under GPD promoter) in presence of 7 mM azetidine 2-carboxylic acid (AZC) or treated with hydrogen peroxide ($H_2O_2$). 10 fold serially diluted cells were spotted in rich medium (YPD) and incubated at 30°C for two days. For $H_2O_2$ treatment, cells were treated with a low dose of hydrogen peroxide (0.25 mM) for 30 min followed by 5 mM hydrogen peroxide for 45 min. The plates were photographed under "SYNGENE G:BOX" imaging gel dock.

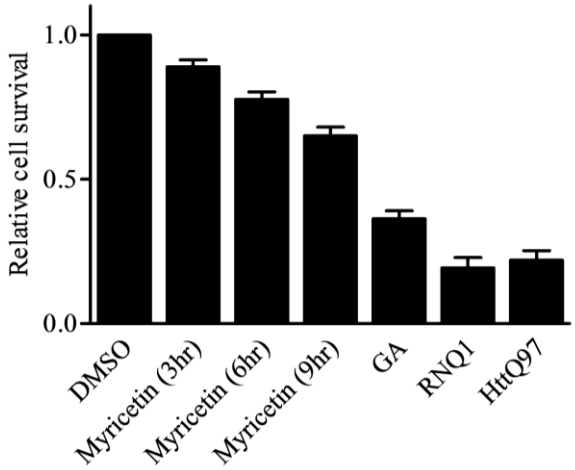


**Figure S8**. Sequestration of misfolded kinases in the IPOD compartment prevents toxicity. Yeast cell viability was measured by MTT assay. Ste11ΔN$^{K444R}$ expressing cells were treated with DMSO, myricetin (100μM) and GA (50μM). Expression of HttQ97 and RNQ1 in yeast cells is more toxic than insoluble kinase inclusions.

Table S: Plasmids used in this study

| Plasmid | Description | Reference |
|---|---|---|
| pSte11ΔN$^{K444R}$ | Ste11ΔN$^{K444R}$ expression plasmid | (Flom *et al.*, 2008) |
| pRS423-myc-Ub | Myc epitope tagged UB14 with CUP1 promoter | (Nakatsukasa *et al.*, 2008) |
| p416GPD | Ura3-based expression plasmid, GPD promoter | (Mumberg *et al.*, 1995) |
| p413GPD | His 3-based expression plasmid, GPD promoter | (Mumberg *et al.*, 1995) |
| p416 TEF | Ura3- based expression plasmid,TEF promoter | (Mumberg *et al.*, 1995) |
| pXP116 | ADH-GFP1 in YCplac33 | (Pan and Heitman, 2002) |
| pXP287 | pGAL1-GST-TPK2$^{K99R}$ in YEplac195 | (Pan and Heitman, 2002) |
| pRS316 GAL1-GFP-Ste11ΔN$^{K444R}$ | GFP tagged expression plasmid,GAL promoter | (Theodoraki *et al.*, 2012) |
| pCUP426 SSA1 | Ura3-based expression plasmid,CUP promoter | (McClellan and Brodsky, 2000) |
| p416GPD SSA1 | Ura3-based expression plasmid, GPD promoter (HindIII+Xho1) | This study |
| p416TEF SSA1 | Ura3-based expression plasmid.TEF promoter (HindIII+Xho1) | This study |
| ADH-TPK2-GFP | GFP-Tpk2,ADH promoter in YCplac33 ( Xba1+BamH1) | This study |
| p416GPD-Ste11 | Ste11 expression plasmid (BamH1+Xho1) | This study |
| pRS315 GAL1-GFP-Ste11ΔN$^{K444R}$ | GFP tagged expression plasmid,GAL promoter | This study |
| pGAL1-GST-TPK2 | GST tagged Tpk2 in YEplac195 ,GAL promoter | This study |
| pGAL-GST-TPK2$^{S80A,T224A}$ | Autophosphorylation defective Tpk2 mutant,YEplac195,GAL promoter | This study |
| p316GAL-Ste11 | His-tag Ste11 expression plasmid, GAL promoter, (BamH1+Not1) | This study |
| GAL1-RFP-Ste11ΔN$^{K444R}$ | RFP-tagged expression plasmid, GAL promoter | This study |
| pRS416Gal-Q97-GFP | GFP-tagged expression plasmid, Gal promoter | (Kaganovich *et al.*, 2008) |
| pESC Leu CHFP-VHL | CHFP-tagged expression plasmid, Gal promoter | (Kaganovich *et al.*, 2008) |
| pESC Leu CHFP-Ubc9$^{ts}$ | CHFP tagged expression plasmid,Gal promoter | (Kaganovich *et al.*, 2008) |
| pRS316Gal-RFP | Ura3 based expression plasmid, RFP tagged (BamH1+Spe1) | This study |
| pRS316Gal-RFP-Hsp42 | RFP-tagged expression plasmid,Gal promoter (Spe1+Not1) | This study |
| pRS316Gal-RFP-Hsp104 | RFP-tagged expression plasmid,Gal promoter (Spe1+Not1) | This study |
| pRS315 GAL1-RFP-Ste11ΔN$^{K444R}$ | RFP-tagged expression plasmid,Gal promoter (Spe1+Not1) | This study |

## Supplementary Methods

### Pheromone assay

Yeast cells were co-transformed with p316GAL-Ste11 and PRE-LacZ plasmid. PRE-LacZ contains LacZ gene under the control of pheromone response element (PRE). Cells were grown in synthetic drop out media containing glucose and then transferred into 2% raffinose. Ste11 kinase was induced with 2% galactose at $OD_{600}$ of 0.3 in the presence of Hsp70 inhibitor, myricetin (100 µM and 150 µM respectively). After 4 hr of induction, cells were treated with α-factor (5 µM) for 2 hr. The cells were then harvested and washed with water. The cell pellets were resuspended in extract buffer (HEPES-KOH, pH7.4, 150 mM NaCl, 1 mM EDTA, 10% glycerol, 5mM DTT, 1 mM PMSF, 1X protease inhibitor cocktail) and lysed in bead beater at 4°C. The supernatants were collected by centrifugation at 13,500 rpm for 15 minutes at 4°C and the protein was estimated by Bradford method. β-galactosidase assay was performed using equal amount of protein. Total reaction volume was made upto 100 µl with Z buffer (60 mM $Na_2HPO_4$, 40mM $NaH_2PO_4$, 10mM KCl, 1mM $MgSO_4$, 50 mM β-ME, pH7.0). The reactions were initiated by the addition of 25 µl of 0.1% MUG (4-methyl umbelliferyle β-D-galactopyronoside) and incubated for 15 minutes at room temperature. The reactions were stopped by the addition of 30 µl of 1(M) $Na_2CO_3$. The β-galactosidase activity was measured by using excitation at 360 nm and emission at 460 nm in multimode multiplate reader

(Thermo Scientific). Fold induction of β-galactosidase activity was evaluated by normalizing absorbance at 460 nm with total protein content measured by Bradford method.

## MTT assay

The cell viability was estimated according to the method of Hodgson *et al.* (Hodgson *et al.*, 1994). An inoculum from an overnight culture was incubated at 30°C with constant shaking until late exponential phase was reached. Aliquots (1.0 ml) containing approximately 1 x $10^8$ cells/ml were pipetted into microcentrifuge tubes. The cells (500µl) were mixed with 50µl of 5 mg/ml MTT (Sigma) and incubated at 30°C for 2 hour. To each tube was added 500µ1 of propan-2-ol containing 0.04 M HC1. The mixture was vigorously vortexed to remove MTT-Formazan from the cells and then centrifuged at 11,600×g for 2 min. The absorbance of the supernatants was measured at 570 nm against cell-free controls treated in an identical manner.

## Supplemental References


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