SUPPLEMENTARY INFORMATION

Supplemental Methods:

Whole cell lysate extraction:

H99 and *rpb4*Δ::RPB4myc were grown to mid-log phase in YPD, 30°C, 250 rpm. Cultures were harvested, washed with sterile deionized water, and whole cell lysates were obtained by mechanical disruption. Briefly, pellets were resuspended in 10% volume of lysis buffer (15 mM Hepes pH 7.4, 10 mM KCl, 5 mM MgCl2, 10 µl ml^{-1} HALT protease inhibitor (Thermo Scientific), 1 mM DTT) in microfuge tubes and glass beads were added. Tubes were vortexed for 30 seconds alternating with incubation on ice for a total of 10 minutes. 200 µL ice cold lysis buffer was added and supernatants were transferred to new microfuge tubes. Lysates were cleared by 2 rounds of centrifugation at 14,000 x g, 4°C, 5 minutes.

Western blot Analysis:

50 µg of protein from whole cell lysates (above) were boiled in Laemmli buffer and electrophoresed on 4-12% Tris-glycine polyacrylamide gels. Gels were transferred to nitrocellulose (iBlot, Invitrogen). Membranes were incubated in PBS for 5 minutes followed by incubation with Odyssey Blocking Buffer (LiCOR) for one hour at room temperature or overnight at 4°C. Membranes were probed with mouse anti-myc antibody (1:1000 in 5 ml PBS + 0.1% Tween + 5 ml Odyssey Blocking Buffer) overnight at 4°C. Membranes were washed 4 times, 5 minutes each with shaking in PBS+0.1% Tween. Membranes were probed with IR680-conjugated Donkey anti-mouse antibody (1:10,000 in PBS) for one hour at room temperature. Membranes were washed as above and imaged using the LiCOR Odyssey Imaging system.

CoImmunoprecipitation:

CoImmunoprecipitation was performed using the ProFound c-myc IP/CoIP Application Set (Thermo Scientific) according to manufacturer's protocol with some modifications. Briefly, 600 µg of protein and 10 µl of anti-myc agarose was added to columns and incubated overnight at 4^{o}C with rotation. Flow through was collected. Three washes with PBS with increasing amounts of Tween (0.1%, 0.15%, 0.2%) were collected followed by elution with non-reducing sample buffer provided in the kit. Flow through, washes, and eluates were denatured and electrophoresed on a 4-12% Bis-Tris polyacrylamide gel followed by staining with SimpleBlue (Invitrogen).

Liquid Chromatography Tandem Mass Spectrometry:

LC/MS/MS analyses were performed by the Proteomics core at Fred Hutchinson Cancer Research Center. Gel bands were digested with trypsin, desalted and analyzed with an orbitrap mass spectrometer. The DB search was done using *Cryptococcus neoformans* H99 protein database (Broad Institute of Harvard and MIT (http.www.broadinstitute.org)) after adding common contaminants (i.e. human keratin). The cut-off for peptide ID was error rate ≤ 0.05, and for protein ID, probability score ≥ 0.9.

Supplemental Results:

The Rpb4-myc-tagged strain was created to verify the functional conservation of Rpb4 in *C. neoformans*. To verify that myc-tagged Rpb4 protein was being produced in this strain, we performed a western blot analysis with an anti-myc primary antibody to detect the myc-tag epitope (Fig. S1 A). We observed a band at ~20kDa, the estimated molecular weight of Rpb4,

in the *rpb4*Δ::RPB4myc strain. We also observed a band for GSTmyc in our *E. coli* control lysate, verifying the ability of our antibody to recognize the myc-tag epitope. As expected, we did not observe any bands from our wild type whole cell lysate.

To confirm that Rpb4 in *C. neoformans* is a functional homolog of the *S. cerevisiae* RNA polymerase II subunit Rpb4p, we performed Co-Immunoprecipitations with whole cell lysates from the wild type and the *rpb4*Δ::RPB4myc strains using anti-myc agarose (Fig. S1 B). We observed 3 specific bands in the eluate from the RPB4myc strain that were not present in the eluate from wild type. These bands were excised and analyzed by LC/MS/MS. Our analysis revealed that the two heavier bands corresponded to Rpb1 (CNAG_02166, 196kDa) and Rpb2 (CNAG_01235, 140kDa), the two largest subunits in the RNA polymerase II holoenzyme. These interactions verified the conserved function of Rpb4 in this complex. We also identified Hsp104 (CNAG_07347, 100kDa) as the third band. However, at this point we can not speculate about this association.

We utilized the *rpb4*Δ::RPB4myc strain to determine the localization of Rpb4 during optimal conditions and following a shift to host-temperature. We observed that Rpb4myc shifted from the nucleus during optimal conditions, to the cytoplasm after the temperature shift. We speculated that this cytoplasmic accumulation was related to the role in enhanced degradation. However, it is possible that the myc-tag interfered with the function of Rpb4. To confirm that the myc-tag did not alter the function of Rpb4 in degradation, we analyzed the stability of *RPL2* in the *rpb4*Δ::RPB4myc strain following a shift to 37^{o}C (Fig. S1 C). The half-life of *RPL2* in the *rpb4*Δ::RPB4myc strain was consistent with our results obtained with the wild type (<20 minutes), whereas the half-life of *RPL2* in the parental *rpb4*Δ strain was stabilized. These results verified that the RPB4myc construct restored Rpb4 function to the *rpb4*Δ strain.

We determined that signaling through Pkh202 triggered enhanced degradation of RP transcripts immediately following a shift to host-temperature. We were interested in identifying downstream targets of Pkh2 that may also affect this response. It was previously shown that Pkh1-dependent activation of Pkc1 influences P-body formation and mRNA degradation during nutrient stress in *S. cerevisiae* (Luo et al., 2011). To begin investigating the downstream components of the signaling cascade that mediates stress-responsive changes in mRNA degradation, we measured RP transcript decay in the *bck*1Δ mutant, which is null for the MAPKKK in the PKC-MPK1 signaling pathway. RP transcript decay in the *bck1*Δ mutant was identical to wild type (Fig. S2), suggesting that Pkh2-02 influences host-temperature induced RP transcript degradation independent of the PKC-dependent MAP kinase cascade.

Supplementary Figure Legends

Figure S1. Complementation by Rpb4-myc. A. Western blot analysis verifying the protein production of myc-tagged Rpb4. GSTmyc is a positive control from *E. coli* extract containing c-Myc-tagged GST B. Eluted fractions from coimmunoprecipitation reactions with anti-c-myc agarose. Arrows point to bands that were present in the *rpb4*Δ::RPB4myc eluate and absent in the wild type. Rpb1 and Rpb2 are subunits of RNA polymerase II; Hsp104 is a heat shock protein. C. Stability of *RPL2* following a shift to host temperature. Stability of *RPL2* in the *rpb4*Δ::RPB4myc strain is comparable to the wild type indicating that Rpb4myc rescues the defect in destabilization in the *rpb4*Δ mutant strain.

Figure S2. Signaling through Pkh2-02, independent of the PKC-dependent MAP Kinase Cascade, mediates destabilization of RP transcripts during host-temperature adaptation. Wild type and *bck1*Δ cultures were transcriptionally arrested and simultaneously shifted to 37^{o}C

for one hour. Stability of *RPL2* was analyzed by northern blot. Expression of all transcripts was normalized to ribosomal RNA and plotted on log2 graphs. All data presented is representative of 3 biological replicates.

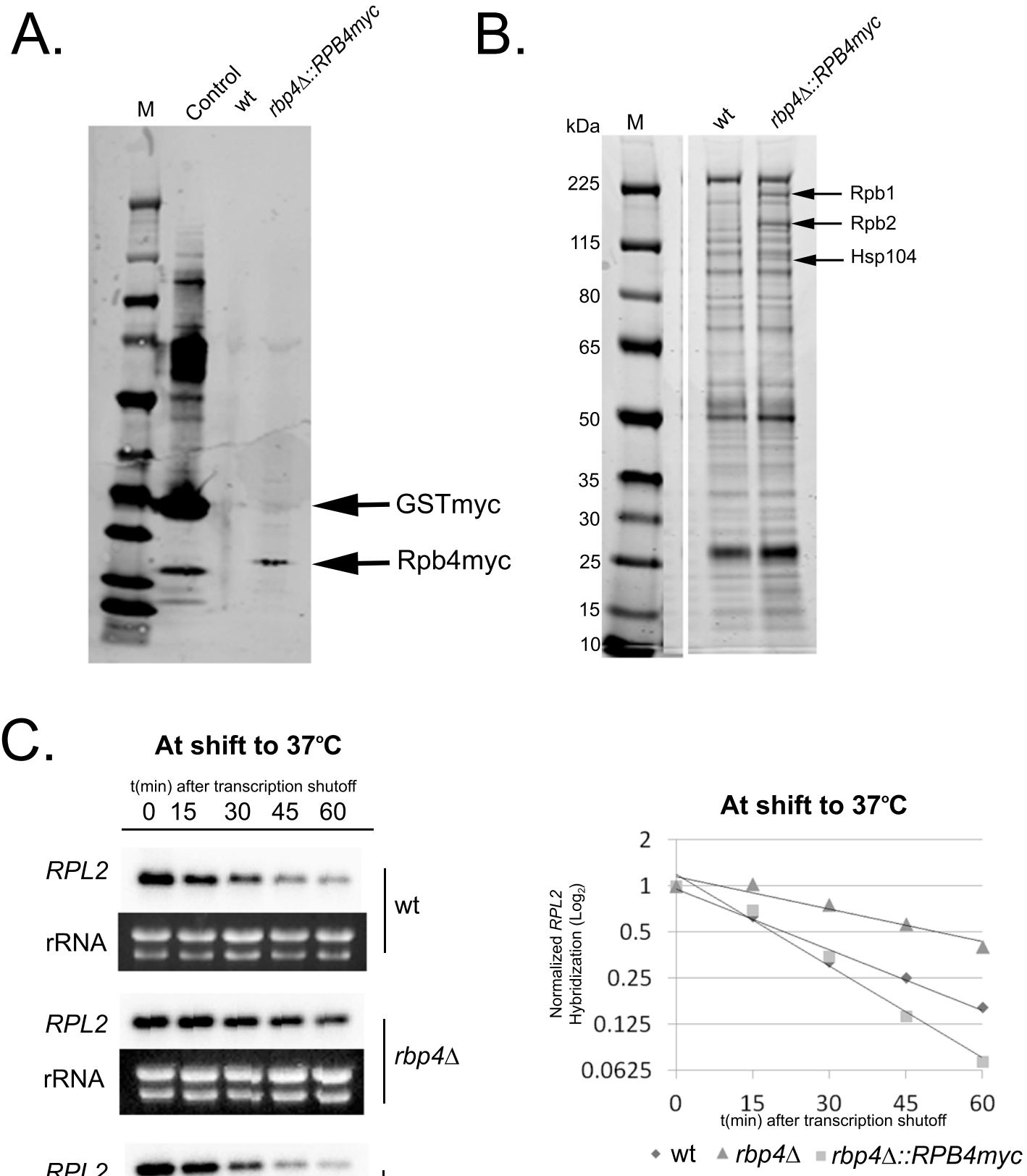
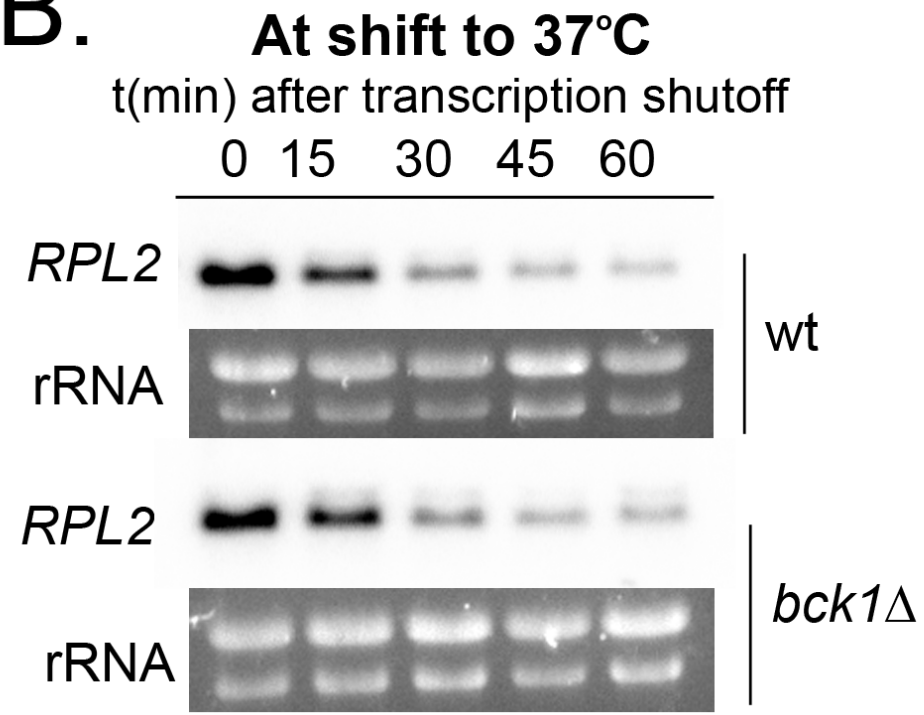

A.
M
Control
wt
rbp4Δ::RPB4myc
GSTmyc
Rpb4myc
B.
kDa
M
wt
rbp4Δ::RPB4myc
225
115
80
65
50
35
30
25
15
10
Rpb1
Rpb2
Hsp104
C.
At shift to 37°C
t(min) after transcription shutoff
0 15 30 45 60
RPL2
rRNA
wt
rbp4Δ
rbp4Δ::RPB4myc
At shift to 37°C
Normalized RPL2 Hybridization (Log2)
2
1
0.5
0.25
0.125
0.0625
0
15
30
45
60
t(min) after transcription shutoff
wt
rbp4Δ
rbp4Δ::RPB4myc

Figure S1

B.


At shift to 37°C
t(min) after transcription shutoff
0 15 30 45 60
RPL2
rRNA
wt
RPL2
rRNA
bck1Δ

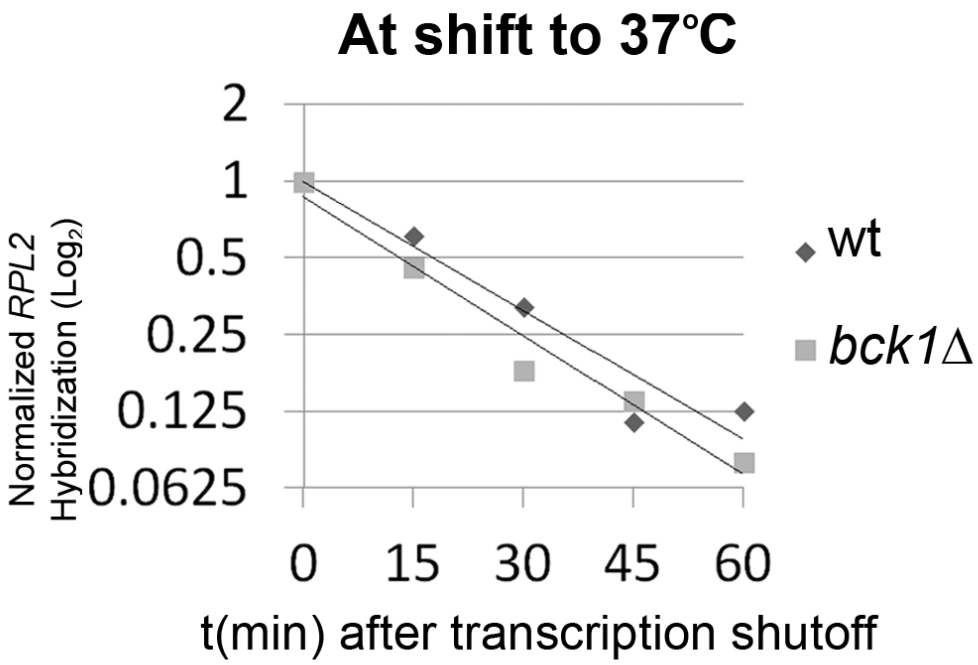

At shift to 37°C
Normalized RPL2 Hybridization (Log2)
2
1
0.5
0.25
0.125
0.0625
0 15 30 45 60
t(min) after transcription shutoff
wt
bck1Δ

Figure S2

Supplementary Table 1. Primers used in this study.

Primer name	Sequence
5'RPB4up-XbaI	TAATAATCTAGAGGCTTGTACTCTGGCTCCTGC
3' RPB4up-BglII	TAATAAAGATCTTTGTAATATGCGGATGGATGTCG
5'RPB4down-MunI	TAATAACAATTGAGAGGAGGGACTTTCCTGG
3'RPB4down-XhoI	TAATAACTCGAGGGAGAATCTGCTAGAAGTTGG
5'NAT-BglII	TAATAAAGATCTGCTGCGAGGATGTGAGCTGG
3'NAT-MunI	TAATAACAATTGAAGCTTATAGAAGAGATGTAGAAACTAGC
5'RPB4-KO-PCR	ACGCGATGTTGACGGTCC
3'RPB4-KO-PCR	GAACGATTTGCCTTCTCTGG
5'RPB4-KO-screen	CGACTGTGAAGCCAACGC
3'RPB4-KO-screen	TTGAGATTCAAAACTTATGAAGG
5'RPB4complement-MunI	TAATAACAATTGCATACACCTAGACAAGCAGGG
3'RPB4complement-MunI	TAATAACAATTGAGACTGTAGATGTGCCGATCG
Rpb4-myc	CGGTGTTCAGCCCGAGCAGAAGCTTATCTCTGAGGAGGACCTTTAGATTCTAGTCAAAGG
Rpb4-myc-RC	CCTTTGACTAGAATCTAAAGGTCCTCCTCAGAGATAAGCTTCTGCTCGGGCTGAACACCG
RPL8 RNaseH cleavage	GATGAGACCGGCTTTTTGACC