Supplementary Table 1: **Kinase null strains tested for role in Nab2 phosphorylation.**

| Number | Standard Name | Systematic Name | Number | Standard Name | Systematic Name |
|---|---|---|---|---|---|
| 1 | Akl1 | YBR059C | 45 | Pho85 | YPL031C |
| 2 | Ark1 | YNL020C | 46 | Pkh1 | YDR490C |
| 3 | Atg1 | YGL180W | 47 | Pkh2 | YOL100W |
| 4 | Bck1 | YJL095W | 48 | Pkh3 | YDR466W |
| 5 | Bud1 | YGR152C | 49 | Prr1 | YKL116C |
| 6 | Cka2 | YOR061W | 50 | Prr2 | YDL214C |
| 7 | Cmk1 | YFR014C | 51 | Psk1 | YAL017W |
| 8 | Cmk2 | YOL016C | 52 | Psk2 | YOL045W |
| 9 | Dan1 | YJR150C | 53 | Ptk1 | YKL198C |
| 10 | Dbf2 | YGR092W | 54 | Ptk2 | YJR059W |
| 11 | Dbf2 | YGR092W | 55 | Rck1 | YGL158W |
| 12 | Elm1 | YKL048C | 56 | Rck2 | YLR248W |
| 13 | Env7 | YPL236C | 57 | Rim15 | YFL033C |
| 14 | Fmp48 | YGR052W | 58 | Rtk1 | YDL025C |
| 15 | Fpk1 | YNR047W | 59 | Sak1 | YER129W |
| 16 | Fus3 | YBL016W | 60 | Skm1 | YOL113W |
| 17 | Gcn2 | YDR283C | 61 | Slt2 | YHR030C |
| 18 | Gin4 | YDR507C | 62 | Snf1 | YDR477W |
| 19 | Hal5 | YJL165C | 63 | Sps1 | YDR523C |
| 20 | Hog1 | YLR113W | 64 | Ssk2 | YNR031C |
| 21 | Hrk1 | YOR267C | 65 | Ssk22 | YCR073C |
| 22 | Hsl1 | YKL101W | 66 | Ssn3 | YPL042C |
| 23 | Ime2 | YJL106W | 67 | Ste11 | YLR362W |

| | | | | | |
|---|---|---|---|---|---|
| 24 | Ire1 | YHR079C | 68 | Ste20 | YHL007C |
| 25 | Isr1 | YPR106W | 69 | Ste7 | YDL159W |
| 26 | Kcc4 | YCL024W | 70 | Swe1 | YJL187C |
| 27 | Kin1 | YDR122W | 71 | Tda1 | YMR291W |
| 28 | Kin2 | YLR096W | 72 | Tos3 | YGL179C |
| 29 | Kin3 | YAR018C | 73 | Tpk1 | YJL164C |
| 30 | Kin4 | YOR233W | 74 | Tpk2 | YPL203W |
| 31 | Kin82 | YCR091W | 75 | Tpk3 | YKL166C |
| 32 | Kka1 | YJL094C | 76 | Twf1 | YGR080W |
| 33 | Kns1 | YLL019C | 77 | Vhs1 | YDR247W |
| 34 | Ksp1 | YHR082C | 78 | Vps15 | YBR097W |
| 35 | Mck1 | YNL307C | 79 | Yak1 | YJL141C |
| 36 | Mds1 | YMR139W | 80 | Yck2 | YNL154C |
| 37 | Mek1 | YOR351C | 81 | Yck3 | YER123W |
| 38 | Mkk1 | YOR231W | 82 | Ygk3 | YOL128C |
| 39 | Mkk2 | YPL140C | 83 | Ypk1 | YKL126W |
| 40 | Mlp1 | YKL161C | 84 | Ypk2 | YMR104C |
| 41 | Mrk1 | YDL079C | 85 | Uncharacterized ORF | YPL150W |
| 42 | Nnk1 | YKL171W | 86 | Uncharacterized ORF | YBR028C |
| 43 | Npr1 | YNL183C | 87 | Uncharacterized ORF | YNL141C |
| 44 | Pak1 | YIL095W | | | |

Supplementary Table 2: ***S. cerevisiae* strains used in this study**

| Strain | Genotype | Reference/Source |
|---|---|---|
| BY4742 | Matα *his3Δ1 leu2Δ0 ura3Δ1 lys2Δ1* | Research Genetics |
| Nab2-TAP | *NAB2-TAP:HIS5* Mata *his3Δ1 leu2Δ0 met15Δ0 ura3Δ1* | (Ghaemmaghami et al., 2003) |
| *bck1Δ* | *BCK1::KAN* Mata *his3Δ1 leu2Δ0 met15Δ0 ura3Δ1* | (Winzeler et al., 1999) |
| *slt2Δ* | *SLT2::KAN* Matα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ1* | (Winzeler et al., 1999) |
| *hog1Δ* | *HOG1::KAN* Mata *his3Δ1 leu2Δ0 met15Δ0 ura3Δ1* | (Winzeler et al., 1999) |
| *ypk1Δ* | *YPK1::KAN* Mata *his3Δ1 leu2Δ0 met15Δ0 ura3Δ1* | (Winzeler et al., 1999) |
| SWY4307 | *NAB2::HIS3* Matα *his3Δ1 leu2Δ0 trp1Δ ura3Δ1* p(AC636) | (Marfatia et al., 2003)<br>(Marfatia et al., 2003) |
| SWY4308 | *NAB2::HIS3* Matα *his3Δ1 leu2Δ0 trp1Δ ura3Δ1* p(SW3579) | This study |
| SWY4309 | *NAB2::HIS3* Matα *his3Δ1 leu2Δ0 trp1Δ ura3Δ1* p(SW3580) | This study |
| *SLT2-TAP* | *SLT2-TAP:HIS5* Mata *his3Δ1 leu2Δ0 met15Δ0 ura3Δ1* | (Ghaemmaghami et al., 2003) |
| *BCK1-TAP* | *BCK1-TAP:HIS5* Mata *his3Δ1 leu2Δ0 met15Δ0 ura3Δ1* | (Ghaemmaghami et al., 2003) |
| *MKK1-TAP* | *MKK1-TAP:HIS5* Mata *his3Δ1 leu2Δ0 met15Δ0 ura3Δ1* | (Ghaemmaghami et al., 2003) |
| *YPK1-TAP* | *YPK1- TAP:HIS5* Mata *his3Δ1 leu2Δ0 met15Δ0 ura3Δ1* | (Ghaemmaghami et al., 2003) |
| *nup42Δ* | *NUP42::KAN* Matα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ1* | (Winzeler et al., 1999) |
| SWY4493 | *NUP42::KAN NAB2::HIS3 Matα his3Δ1 leu2Δ0 trp1Δ ura3Δ1* p(AC636) | This study |
| SWY4494 | *NUP42::KAN NAB2::HIS3 Matα his3Δ1 leu2Δ0 trp1Δ ura3Δ1 p*(SW3579) | This study |
| SWY4495 | *NUP42::KAN NAB2::HIS3 Matα his3Δ1 leu2Δ0 trp1Δ ura3Δ1* p(SW3580) | This study |

| | | |
|---|---|---|
| SWY4552 | *NAB2-mCherry:HygB* YRA1-GFP:HIS3 Mata *his3Δ1 leu2Δ0 ura3Δ1* | This study |
| SWY4225 | *NAB2-mCherry:HygB MLP1-GFP:HIS3* Mata *his3Δ1 leu2Δ0 ura3Δ1* | This study |
| SWY4034 | *NAB2-GFP:HIS5 MLP1::KAN* Matα *his3Δ1 leu2Δ0 ura3Δ1* | This study |
| *MEX67-GFP* | *MEX67-GFP:HIS5* Mata *his3Δ1 leu2Δ0 met15Δ0 ura3Δ1* | (Huh et al., 2003) |
| SWY4225 | *NAB2-mCherry:HygB MLP1-GFP:HIS3* Mata *his3Δ1 leu2Δ0 met15Δ0 ura3Δ1* | This study |
| SWY4251 | *NAB2-mCherry:HygB MLP1-GFP:HIS3 SLT2::KAN* Mata *leu2Δ0 ura3Δ1* | This study |
| *mlp1Δ* | *MLP1::KAN* Matα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ1* | (Winzeler et al., 1999) |
| *gfd1Δ* | *GFD1::KAN* Matα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ1* | (Winzeler et al., 1999) |
| pAC1152 | *NAB2::HIS3 p(nab2ΔN LEU2)* Mata *leu2 ura3* | (Marfatia et al., 2003) |

Supplementary Table 3: **Plasmids used in this study**

| Plasmid | Description | Reference/Source |
|---|---|---|
| pAC 636 | *NAB2* CEN *LEU2* AMP | (Green et al., 2002) |
| pSW3579 | *nab2-T178A/S180A* CEN *LEU2* AMP | This study |
| pSW3580 | *nab2-T178E/S180E* CEN *LEU2* AMP | This study |
| Nab2-pGEX2TK | pGEX2TK GST-*NAB2* AMP | (Lee and Aitchison, 1999) |
| pSW3610 | pGEX2TK GST-*nab2 T178A/S180A* AMP | This study |
| pSW3629 | pGEX2TK GST-*nab2 T178E/S180E* AMP | This study |
| pSW3465 | 6x-HIS-*MTR2:MEX67* AMP | This study |
| pSW1319 | GST-Dbp5 AMP | (Alcazar-Roman et al., 2006) |

Supplementary Figure 1: **Poly($A^+$) tail length is unaffected in *slt2*Δ, *nab2-T178A/S180A*, and *nab2-T178E/S180E*.** Total RNA was isolated from wild type, *slt2*Δ, *nab- T178A/S180A*, and *nab2-T178E/S180E* cells grown at 23ºC or after a 1 hr shift to 42ºC. RNA was end-labeled with $^{32}$pCp and T4 RNA ligase, and digested with RNase A and RNase T1 to remove non-poly($A^+$) tracks. Resulting stretches of poly($A^+$) were then resolved by denaturing urea-polyacrylamide gel electrophoresis and visualized by autoradiography. The position of the 70 nucleotide (nt) typical poly($A^+$) tail length is marked on the left.

Supplementary Figure 2: **Differential localization of NPC-associated proteins during heat shock.** Mlp2-GFP is localized in discrete intranuclear foci after shifting to growth at 42°C. Nup49-GFP and Nup60-GFP are localized to the nuclear rim during growth at 25°C and 42°C. Left column shows direct GFP fluorescence, and right column shows DIC.

Supplementary Figure 3: **Formation of intranuclear Nab2-GFP foci is dependent on Mlp1.** Yeast null mutants expressing Nab2-GFP were shifted from growth at 30°C to 42°C for 1 hr. Intranuclear foci formation was evaluated by direct fluorescence microscopy.

Supplementary Figure 4: **Production of heat shock proteins is not affected in *mlp1Δ* cells.** Wild type (lanes 1-2), *nup42Δ* (lane 3-4), *mlp1Δ* (lane 5-6), and *gfd1Δ* (lane 7-8) cells were grown at 23°C, shifted to 42°C for 15 min, and labeled with $^{35}$S methionine for an additional 15 min. Cell lysates were separated by SDS-PAGE and proteins were visualized by autoradiography. Asterisks at the right indicate proteins induced upon heat shock (Hsp104, Hsp82, and Hsp70s respectively.)

Supplemental Figure 5: **The nab2-T178E/S180E protein binds Mex67-Mtr2.** 6x-HIS-Mtr2/Mex67 was bound in batch to $Ni^{2+}$ agarose beads at 4°C, and divided equally into nine tubes. Purified recombinant wild type Nab2 (lanes 1-3), nab2-T178A/S180A (lanes 4-6), or nab2-T178E/S180E (lanes 7-9) protein was added to individual tubes in decreasing amounts of 0.5μg, 0.25μg, and 0.1μg. Samples were incubated for 1 hr at 4°C. Beads were washed, and boiled in SDS sample buffer to elute bound proteins. Mex67 was detected by Coomassie stained SDS-PAGE (A), and Nab2 was detected by immunoblotting (B).

**Alcazar-Roman, A. R., Tran, E. J., Guo, S. and Wente, S. R.** (2006). Inositol hexakisphosphate and Gle1 activate the DEAD-box protein Dbp5 for nuclear mRNA export. *Nat. Cell Biol.* **8**, 711-716.

**Ghaemmaghami, S., Huh, W. K., Bower, K., Howson, R. W., Belle, A., Dephoure, N., O'Shea, E. K. and Weissman, J. S.** (2003). Global analysis of protein expression in yeast. *Nature* **425**, 671-672.

**Green, D. M., Marfatia, K. A., Crafton, E. B., Zhang, X., Cheng, X. and Corbett, A. H.** (2002). Nab2p is required for poly(A) RNA export in *Saccharomyces* *cerevisiae* and is regulated by arginine methylation via Hmt1p. *J. Biol. Chem.* **277**, 7752-7760.

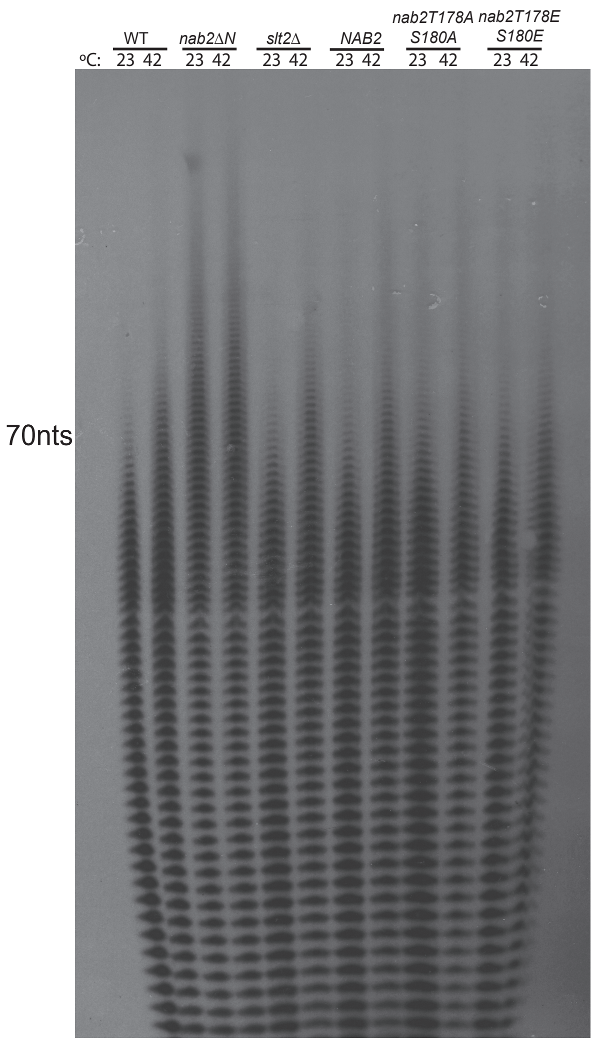
**Huh, W. K., Falvo, J. V., Gerke, L. C., Carroll, A. S., Howson, R. W., Weissman, J. S. and O'Shea, E. K.** (2003). Global analysis of protein localization in budding yeast. *Nature* **425**, 686-691.

**Lee, D. C. and Aitchison, J. D.** (1999). Kap104p-mediated nuclear import. Nuclear localization signals in mRNA-binding proteins and the role of Ran and RNA. *J. Biol. Chem.* **274**, 29031-29037.

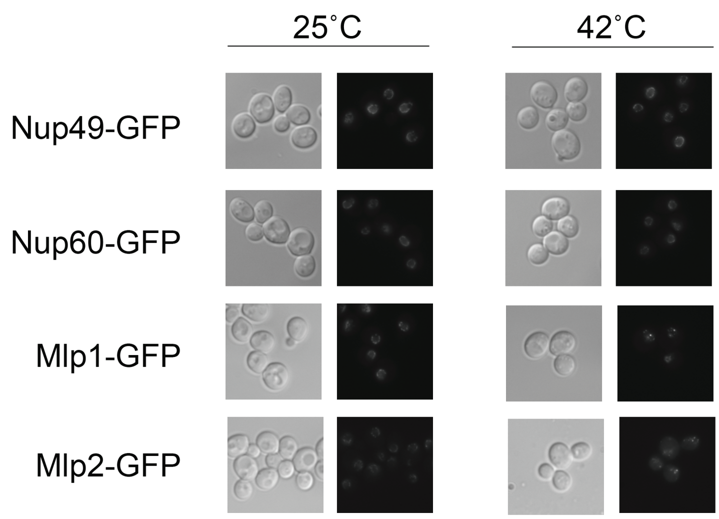
**Marfatia, K. A., Crafton, E. B., Green, D. M. and Corbett, A. H.** (2003). Domain analysis of the *Saccharomyces cerevisiae* heterogeneous nuclear ribonucleoprotein, Nab2p. Dissecting the requirements for Nab2p-facilitated poly(A) RNA export. *J. Biol. Chem.* **278**, 6731-6740.

**Winzeler, E. A., Shoemaker, D. D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J. D., Bussey, H. et al.** (1999). Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* **285**, 901-906.

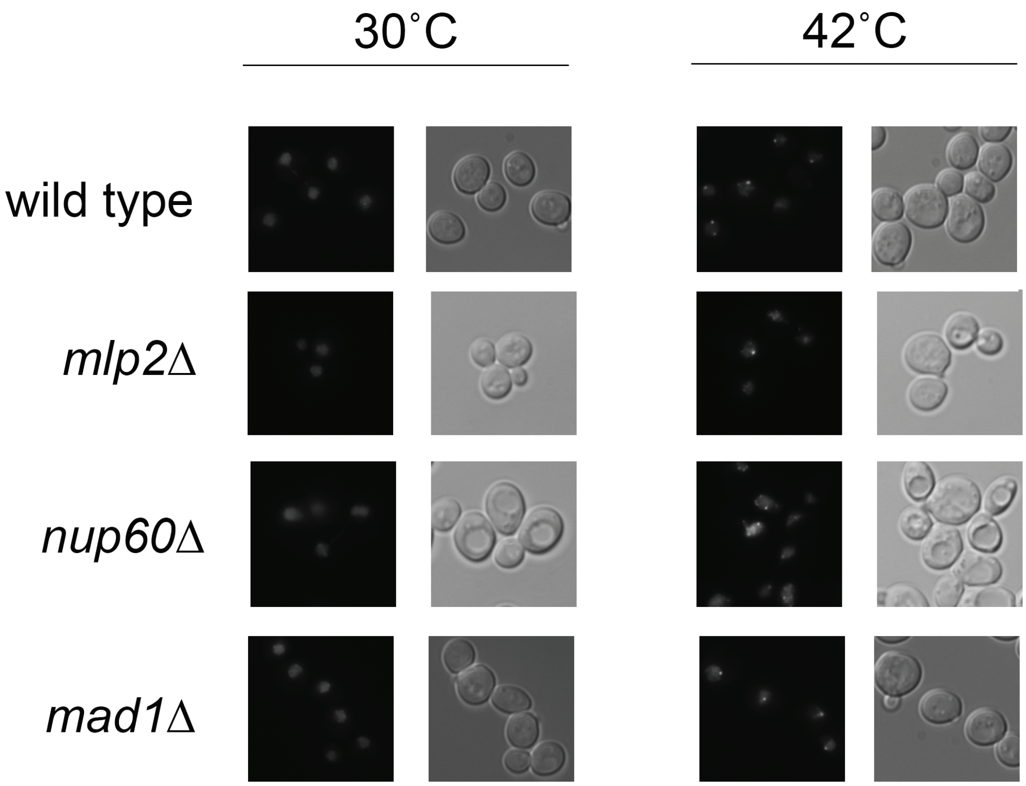
Supplemental Figure 1



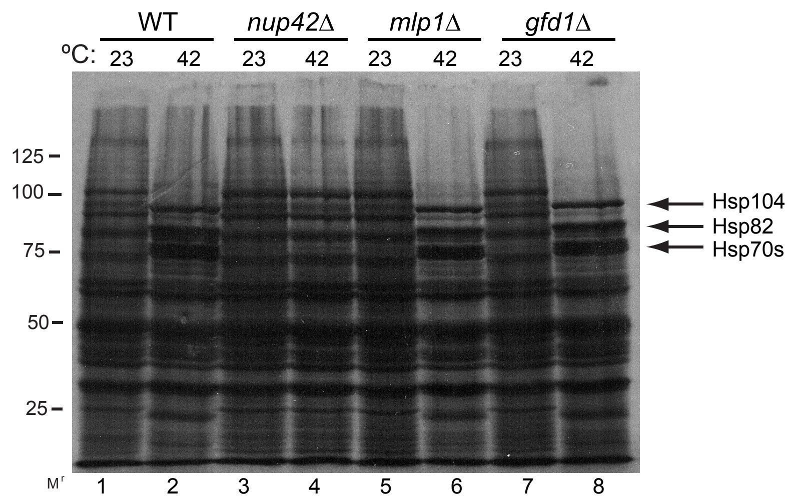
Supplemental Figure 2



Supplemental Figure 3

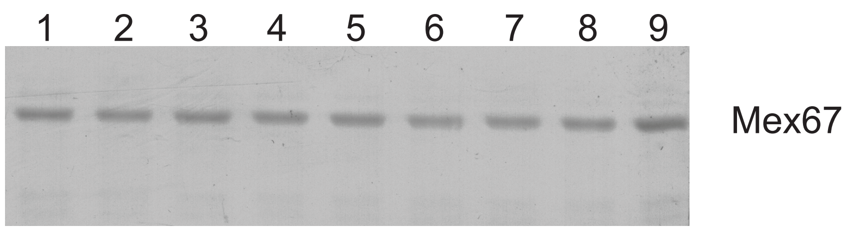


# Supplemental Figure 4



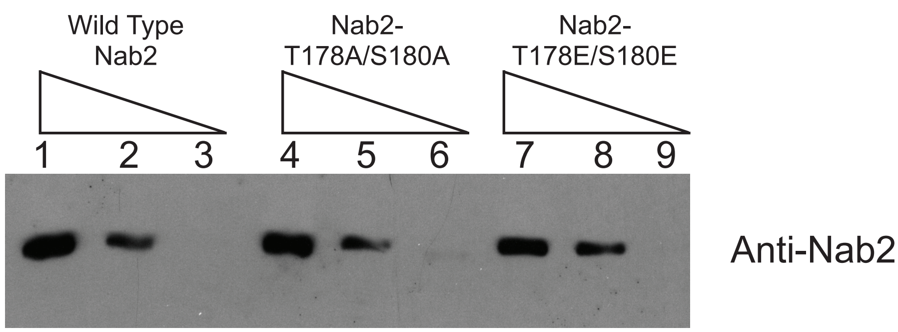
Supplemental Figure 5

A




Coomassie Stain

B




Western Blot