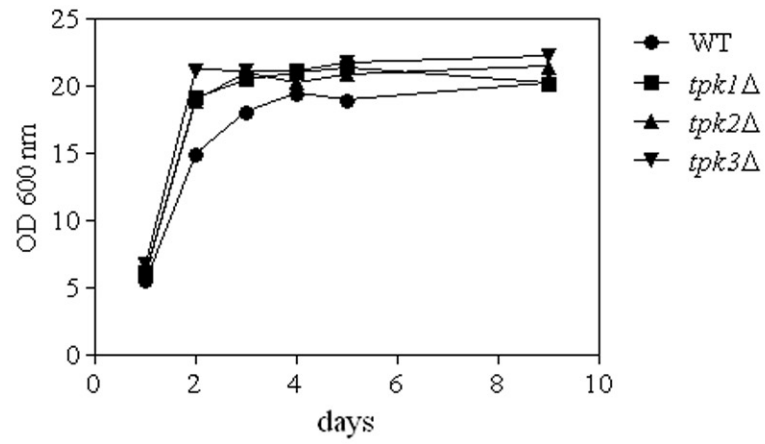
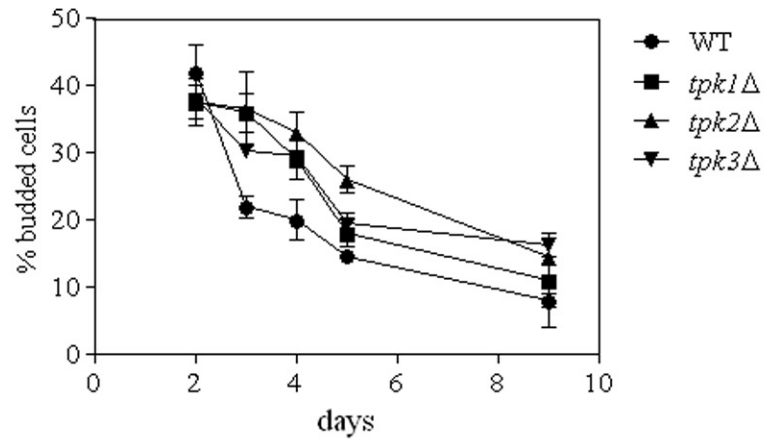
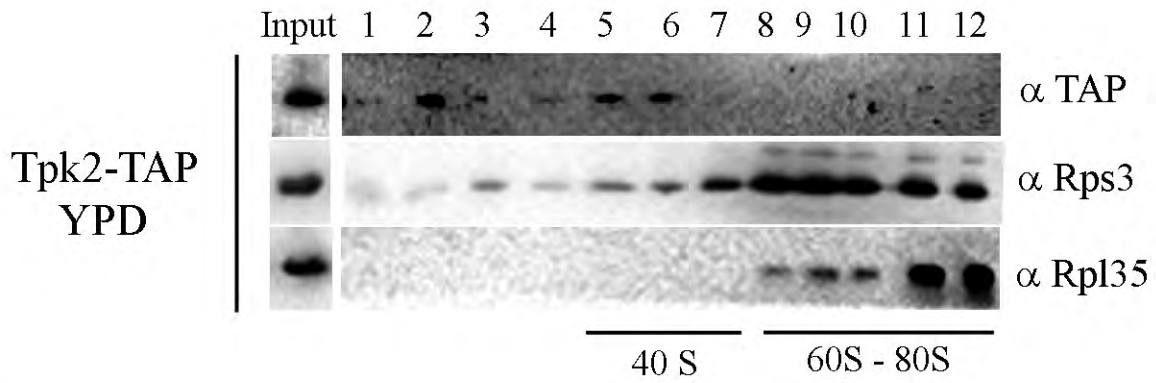
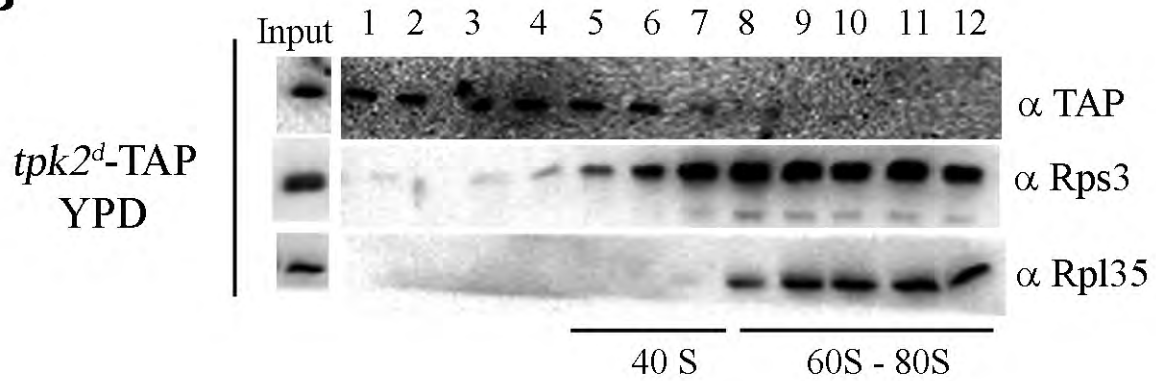


**Fig. S1.** ENO2 and translation factor levels in *tpk1*Δ, *tpk2*Δ and *tpk3*Δ versus WT. ENO2-MS2 RT-PCR (A,C) and western blot from protein extracts (B,D) obtained from starved (Exp-Glu) or unstarved (Exp) cells (A,B) and cells grown to stationary phase (SP) and re-fed with YPD for 10 minutes (SP+YPD; C,D). Ponceau staining shows the amount of total proteins loaded in each lane. A representative image of three independent experiments is shown. Arrowheads indicate difference in expression levels pattern between strains.

**A****B**

**Fig. S2.** Growth curve (A) and % of budding cells (B) of *tpk1*Δ, *tpk2*Δ, *tpk3*Δ and WT strain in YPD media.

**A****B**

**Fig. S3.** Kinase activity of Tpk2 is not required for its interaction with 40S subunits. Polysomal profile analysis and immunoblots of 7.5–30% sucrose gradient fractions from *tpk2* $\Delta$  cells expressing Tpk2-TAP or *tpk2<sup>d</sup>*-TAP grown to exponential phase in YPD. The fractions were processed as described in Fig. 3. Input lanes represent 5% of the input fraction.

**Table S1. Strains and plasmids used in this study**

Strain	Genotype	Source
BY4742	<i>MATa his3 leu2 lys2 ura3</i>	<i>Ashe M.</i>
W3031A	<i>MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1</i>	<i>Euroscarf</i>
S288C	<i>MATa his3<math>\square</math>1 leu2<math>\square</math>0 met15<math>\square</math>0 ura3<math>\square</math>0</i>	<i>Open biosystems</i>
<i>tpk2<math>\Delta</math></i>	<i>W3031A TPK2::kanMX4</i>	<i>Euroscarf</i>
<i>tpk3<math>\Delta</math></i>	<i>W3031A TPK3::kanMX4</i>	<i>Euroscarf</i>
<i>TPK1-TAP</i>	<i>S288C TPK1-TAP::HIS3</i>	<i>Open biosystems</i>
<i>TPK2-TAP</i>	<i>S288C TPK2-TAP:: HIS3</i>	<i>Open biosystems</i>
<i>TPK3-TAP</i>	<i>S288C TPK3-TAP:: HIS3</i>	<i>Open biosystems</i>
<i>TPK1-GFP</i>	<i>S288C TPK1-GFP:: HIS3</i>	<i>Invitrogen</i>
<i>TPK2-GFP</i>	<i>S288C TPK2-GFP:: HIS3</i>	<i>Invitrogen</i>
<i>TPK3-GFP</i>	<i>S288C TPK3-GFP:: HIS3</i>	<i>Invitrogen</i>
<i>DCP1-RFP</i>	<i>Mat<math>\square</math> ADE2 his3-11,15 leu 2-3,112 trp 1-1 ura3-1 can1-100 GCD1-P180 DCP1-RFP::NAT</i>	<i>Ashe M.</i>
<i>DCP2-RFP TPK2-GFP</i>	<i>tpk2<math>\Delta</math> + pDCP2-RFP + pTPK2-GFP</i>	<i>This study</i>
<i>DCP2-RFP TPK3-GFP</i>	<i>tpk3<math>\Delta</math> + pDCP2-RFP + Ptpk3-GFP</i>	<i>This study</i>
<i>DCP2-RFP tpk2<sup>d</sup>-GFP</i>	<i>tpk2<math>\Delta</math> + pDCP2-RFP + ptpk2<sup>d</sup>-GFP</i>	<i>This study</i>
<i>DCP2-RFP tpk3<sup>d</sup>-GFP</i>	<i>tpk3<math>\Delta</math> + pDCP2-RFP + Ptpk3-GFP + ptpk3<sup>d</sup>-GFP</i>	<i>This study</i>
<i>eIF4E-RFP</i>	<i>Mata ADE2 his3-11,15 leu 2-3,112 trp 1-1 ura3-1 can1-100 GCD1-S180 CDC33-RFP::NAT</i>	<i>Ashe M.</i>
<i>DCP2-CFP</i>	<i>Mat<math>\square</math> ADE2 his3-11,15 leu 2-3,112 trp 1-1 ura3-1 can1-100 GCD1-P180 DCP2-CFP::TRP</i>	<i>Ashe M.</i>
<i>DCP2-CFP eIF4E-RFP</i>	<i>MAT<math>\square</math> ADE2 his3-11,15 leu2-3 112 trp1-1 ura3-1 can1-100 GCD1-S180 DCP2-CFP-TRP CDC33-RFP::NAT</i>	<i>This study (1)</i>
<i>DCP2-CFP eIF4E-RFP TPK1-GFP</i>	<i>MAT<math>\square</math> ADE2 his3-11,15 leu2-3 112 trp1-1 ura3-1 can1-100 GCD1-S180 DCP2-CFP-TRP CDC33-RFP::NAT TPK1-GFP::HIS</i>	<i>This study (2)</i>
<i>DCP2-CFP eIF4E-RFP TPK2-GFP</i>	<i>MAT<math>\square</math> ADE2 his3-11,15 leu2-3 112 trp1-1 ura3-1 can1-100 GCD1-S180 DCP2-CFP-TRP CDC33-RFP::NAT TPK2-GFP::HIS</i>	<i>This study (2)</i>
<i>DCP2-CFP eIF4E-RFP TPK3-GFP</i>	<i>MAT<math>\square</math> ADE2 his3-11,15 leu2-3 112 trp1-1 ura3-1 can1-100 GCD1-S180 DCP2-CFP-TRP CDC33-RFP::NAT TPK3 GFP::HIS</i>	<i>This study (2)</i>
<i>DCP2-CFP eIF4E-RFP tpk1<math>\Delta</math></i>	<i>MAT<math>\square</math> ADE2 his3-11,15 leu2-3 112 trp1-1 ura3-1 can1-100 GCD1-S180 DCP2-CFP-TRP CDC33-RFP::NAT tpk1::URA3</i>	<i>This study (4)</i>
<i>DCP2-CFP eIF4E-RFP tpk2<math>\Delta</math></i>	<i>MAT<math>\square</math> ADE2 his3-11,15 leu2-3 112 trp1-1 ura3-1 can1-100 GCD1-S180 DCP2-CFP-TRP CDC33-RFP::NAT tpk2::URA3</i>	<i>This study (4)</i>
<i>DCP2-CFP eIF4E-RFP tpk3<math>\Delta</math></i>	<i>MAT<math>\square</math> ADE2 his3-11,15 leu23 112 trp1-1 ura3-1 can1-100 GCD1-S180 DCP2-CFP-Trp CDC33-RFP::NAT tpk3::URA3</i>	<i>This study (4)</i>
<i>DCP2-YFP eIF4E-CFP ENO2-MS2</i>	<i>MATa ADE2 his3-11,15 leu2-3 112 ura3-1 can1-100 GCD1-P180 DCP2-YFP-KanMX CDC33-CFP::TRP ENO2-MS2 + pCP-mCh-MS2</i>	<i>Ashe M. (3)</i>

<i>DCP2-YFP eIF4E-CFP ENO2-MS2 Tpk1Δ</i>	<i>MATa ADE2 his3-11,15 leu2-3 112 ura3-1 can1-100 GCD1-P180 DCP2-YFP-KanMX CDC33-CFP::TRP ENO2-MS2 tpk1:URA3 + pCP-mCh-MS2</i>	<i>This study (4)</i>
<i>DCP2-YFP eIF4E-CFP ENO2-MS2 Tpk2Δ</i>	<i>MATa ADE2 his3-11,15 leu2-3 112 ura3-1 can1-100 GCD1-P180 DCP2-YFP-KanMX CDC33-CFP::TRP ENO2-MS2 tpk2:URA3 + pCP-mCh-MS2</i>	<i>This study (4)</i>
<i>DCP2-YFP eIF4E-CFP ENO2-MS2 Tpk1Δ</i>	<i>MATa ADE2 his3-11,15 leu2-3 112 ura3-1 can1-100 GCD1-P180 DCP2-YFP-KanMX CDC33-CFP::TRP ENO2-MS2 tpk3:URA3 + pCP-mCh-MS2</i>	<i>This study (4)</i>

(1). *CDC33-RFP DCP2-CFP* (*CDC33* encodes eIF4E) was constructed by crossing *CDC33-RFP* strain with *DCP2-RFP* strain (Oshima and Takano, 1980).

(2). *CDC33-RFP DCP2-CFP TPK1-GFP*, *CDC33-RFP DCP2-CFP TPK2-GFP* and *CDC33-RFP DCP2-CFP TPK3-GFP* were obtained by transformation of *CDC33-RFP DCP2-CFP* by epitope tagging (Huh et al., 2003).

(3). MS2 sequences were inserted into the 3'UTR of the *ENO2* gene (Haim et al., 2007).

(4). Deletions in each *tpkΔ* were constructed by one-step disruption technique (Rothstein, 1991).

**Table S2. Plasmids used in this study**

<b>Plasmids</b>	<b>Description</b>	<b>Source</b>
<i>pTPK2-GFP</i>	<i>pTD46, CEN, LEU2, TPK2 promoter, TPK2-GFP</i>	<i>This study</i>
<i>pTPK3-GFP</i>	<i>pTD49, CEN LEU2, TPK3 promoter, TPK3-GFP.</i>	<i>This study</i>
<i>ptpk2<sup>d</sup>-GFP</i>	<i>pTD55, CEN, LEU2, TPK2 promoter, TPK2<sup>K99M</sup>-GFP.</i>	<i>This study</i>
<i>ptpk3<sup>d</sup>-GFP</i>	<i>pTD49, CEN, LEU2, TPK3 promoter, TPK3<sup>K117M</sup>-GFP.</i>	<i>This study</i>
<i>ptpk2<sup>d</sup>-TAP</i>	<i>pTD55, CEN, LEU2, TPK2 promoter, TPK2<sup>K99M</sup>-TAP.</i>	<i>This study</i>
<i>pTPK2-TAP</i>	<i>pTD46, CEN, LEU2, TPK2 promoter, TPK2-TAP</i>	<i>This study</i>
<i>pCP-mCh-MS2</i>	<i>CEN, HIS3, mCh-MS2</i>	<i>Ashe M.</i>
<i>pDCP2-RFP</i>	<i>pRP1156, CEN, TRP, DCP2 promoter, DCP2-GFP</i>	<i>Parker R.</i>