Supporting information

Table S1. Primers used in this study.

Primer	Use	Sequence (5' to 3')
JC17	*SAT1* flipper	GGCCCCCCCTCGAGGAAGTT
JC18	*SAT1* flipper	GCTCTAGAACTAGTGGATCT
JC513	5'NCR of *TPK1*	CAGAACATTGTAATTGATGGC
JC514	5'NCR of *TPK1*	AACTTCCTCGAGGGGGGGCCCTTGTTCCCTGTTGTTGTTG
JC515	3'NCR of *TPK1*	AGATCCACTAGTTCTAGAGCTCCATCAACCAACCAACCAA
JC516	3'NCR of *TPK1*	AGGATGTGGATTTATATGGGG
JC517	*TPK1* overlap	TCCGTAGCGTATCGCTAAAT
JC518	*TPK1* overlap	GGTTGATTAAAAAACAAGG
JC521	5'NCR of *TPK2*	AATTGACCGTCCGCACACTA
JC522	5'NCR of *TPK2*	AACTTCCTCGAGGGGGGGCCTGGGCAAAAAAGGGAAGTT
JC523	3'NCR of *TPK2*	AGATCCACTAGTTCTAGAGCCGAAAACGAAACAAAGAGAG
JC524	3'NCR of *TPK2*	AACCATCATCACCACTACCA
JC525	*TPK2* overlap	CAGCACAACGACAAACAGTGA
JC526	*TPK2* overlap	GAGGATAAAGAAGGTCAAAGG
JC545	3'NCR of 2nd *TPK2* allele	AACTTCCTCGAGGGGGGGCCGCTTTTTTAAAAGATTTGGCA
JC546	5'NCR of 2nd *TPK2* allele	AGATCCACTAGTTCTAGAGCAATACTTCCTCGACTTTTAGA
JC555	3'NCR of 2nd *TPK1* allele	AACTTCCTCGAGGGGGGGCCCTCCTTTTTCCATAGATGTCA
JC556	5'NCR of 2nd *TPK1* allele	AGATCCACTAGTTCTAGAGCCAATTTGAAGATTTTTAATCT
JC758	5'NCR of *TPK2*	AAAGGTACCTTAGGCATCACGTGTTTGCT
JC759	5'NCR of *TPK2*	AAAGGGCCCGAAAATGGGCAAAAAAGGGA
JC760	3'NCR of *TPK2*	AAACCGCGGCGAAAACGAAACAAAGAGAGA
JC761	3'NCR of *TPK2*	AAAGAGCTCTCCAGGCACAACTTTCCTTA
JC782	5'NCR of *TPK2*	AAAGGTACCAATTGACCGTCCGCACACTA
JC783	5'NCR of *TPK2*	AAAGGGCCCTGGGCAAAAAAGGGAAGTT
JC784	*TPK2* ORF	AAACCGCGGATGCCAAATCTTTTAAAAAAG
JC785	*TPK2* ORF	AAAGAGCTCCTAAAAGTCGAGGAAGTATTG
JC998	5'NCR and ORF of *TPK2*	AAAGGTACCGTGGTGGTAGTATTAGGCATC
JC999	5'NCR and ORF of *TPK2*	AAAGGGCCCCTAAAAGTCGAGGAAGTATTG
JC1004	3'NCR of *TPK1*	AAACCGCGGTCCATCAACCAACCAACCAA
JC1006	5'NCR and ORF of *TPK1*	AAAGGGCCCTCATCATCGGTAGTAAAA
JC1007	5'NCR and ORF of *TPK1*	AAACTCGAGTTAAAAATCTTCAAATTGATC
JC1023	3'NCR of *TPK1*	AAAGAGCTC TCGTCGACTTTATTATTCTGGTTCA
JC1150	*TPK2*-F for qPCR	AATCCCGTGGCCAAATTTTATG
JC1151	*TPK2*-R for qPCR	TCCATGTGACAGTGCTGACCTC
JC1154	*ALS3*-F for qPCR	TGGCATTTAACGTTGGTGGTTC
JC1155	*ALS3*-R for qPCR	TGCAGCAAAATAGGCTTGGGTA
JC1156	*HWP1*-F for qPCR	AGTGACTGAAGGGCACATTCCA
JC1157	*HWP1*-R for qPCR	TGCAAGACCAACAATAGCAGCA
JC1158	*EFG1*-F for qPCR	AGGAATTGCAAACCCAAGTGCT
JC1159	*EFG1*-R for qPCR	CTTGCTGGTTTGGTTGTCCTTG
JC1160	*NDT80-F* for qPCR	TTGCATCAACAAATGCCACATC
JC1161	*NDT80*-R for qPCR	TCTTGGGTCAAATGAGGGGATT
JC1162	*TEC1-F* for qPCR	CACCTCCTCCAACTCAAGCTCA
JC1163	*TEC1-R* for qPCR	ATTGGTATTCGGAACCGTTGCT
JC1164	*BCR1*-F for qPCR	ACATCCACCACAACAGCCATCT
JC1165	*BCR1*-R for qPCR	TGGTAATGGAGGCAATGGTTTG
JC1193	*ACT1*-F for qPCR	CCAGCCGATTTAGGTTTGGAAG
JC1194	*ACT1*-R for qPCR	CGTTCAGCAATACCTGGGAACA
JC1199	*TPK1*-F for qPCR	GCCAACAGACAACATCAACAGAAT
JC1120	*TPK1*-R for qPCR	TGGACGTGCTATTTTCATTGTCC
JC1203	*RIM101*-F for qPCR	CAACGGTCTTCAAACTGCTGCT
JC1204	*RIM101*-R for qPCR	TCTTCTTCCTCGTCGTCACTGC
JC1205	*BRG1*-F for qPCR	TCCAATCATGCATCCTCAACAA
JC1206	*BRG1*-R for qPCR	CTGGTGTTGGGTTTTGTGGGTA
JC1207	*ROB1*-F for qPCR	ACCTTTGGCTGCACCATTACCT
JC1208	*ROB1*-R for qPCR	TTGCAGCAGTGGTGGTAGTTGA
JC1209	*FLO8*-F for qPCR	CCAATAAACCATCCCCAGCAAT

JC1210	*FLO8*-R for qPCR	TGCCAGCACTTGTAGGTTGTGA
JC1211	*GAL4*-F for qPCR	AAACTTGCATCCTGCGAGAGTG
JC1212	*GAL4*-R for qPCR	AGGGGTTTGATTTGGATTGACG

Sequences complementary to the *SAT1* flipper are underlined.

Table S2. Plasmids used in this study

Plasmid	Relevant insert	Enzymes used	Reference
pSFS2A			[1]
pYSJ6	3' NCR of 1st *TPK2* allele	SacII, SacI	pSFS2A
pYSJ10	5' NCR of 1st *TPK2* allele	KpnI, ApaI	pYSJ6
pYEN2	5' NCR of 1st *TPK2* allele and ORF	KpnI, ApaI	pYSJ6
pYSJ25	3' NCR of 2nd *TPK2* allele	SacII, SacI	pSFS2A
pYSJ29	5' NCR of 2nd *TPK2* allele	KpnI, HindIII	pYSJ25
pYEN37	3' NCR of *TPK1* allele	SacII, SacI	pSFS2A
pYSJ122	5' NCR and ORF of *TPK1* allele	ApaI, XhoI	pYEN37

Reference

1. Reuß O, Vik A, Kolter R, Morschhäuser J. The *SAT1* flipper, an optimized tool for gene disruption in *Candida albicans*. Gene 2004; 341:119-27.

Figure S1

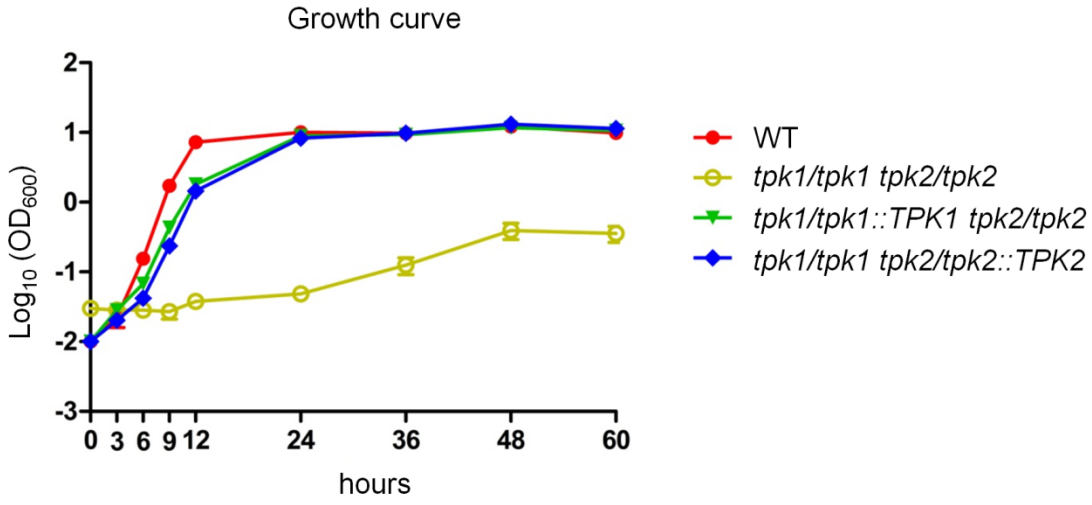

Figure S1. Reintroducing *TPK1* or *TPK2* gene complements the growth defects of the *tpk1/tpk1 tpk2/tpk2* mutant.

Growth curves of the indicated *C. tropicalis* strains. Cells were grown overnight in YPD at 30°C, washed twice with dH_2O, diluted to 0.01 OD_{600}, (except for the *tpk1/tpk1 tpk2/tpk2* mutant (YEN1), which was diluted to 0.03 OD_{600} with fresh YPD medium), and incubated at 30°C for 60 h with shaking at 200 rpm. The OD_{600} of strains was measured via microplate spectrophotometer at the indicated time points. The experiments were performed in triplicate and the values represent the mean ± standard error of the mean (SEM).

Figure S2

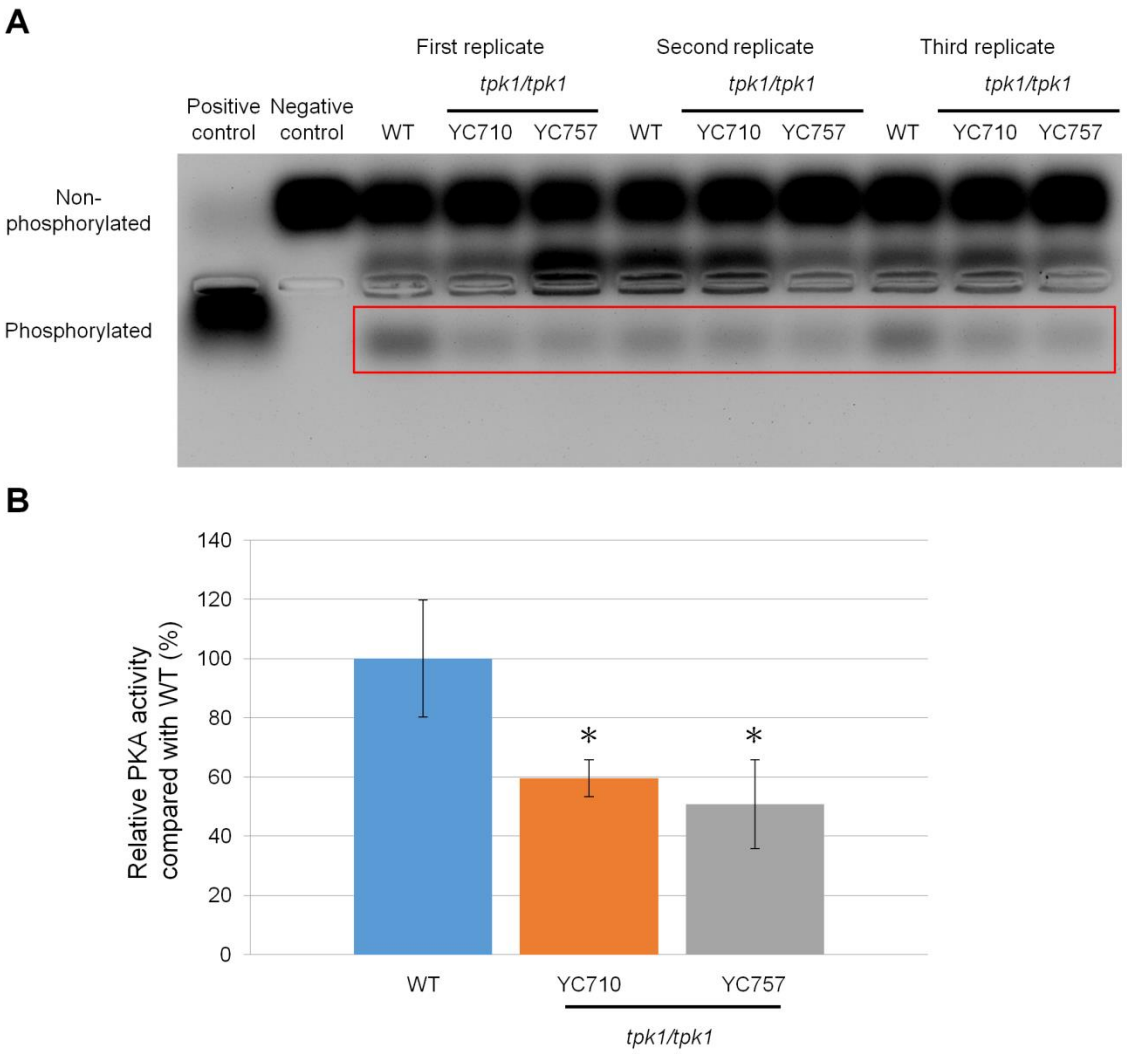

Figure S2. Loss of *TPK1* results in lower PKA activity.

(A) Protein kinase A activity of the indicated strains (red rectangles). Cells were grown overnight in YPD at 30°C and washed twice with dH_2O. Crude protein extracts of the samples were isolated. PKA activity assays were carried out using the PepTag assay for non-radioactive detection of cAMP-dependent protein kinase kit. **(B)** Quantification of PKA activity in panel A (red rectangles) using Image J software. The experiments were performed in triplicate and the values indicate the mean ± standard deviation (SD). Asterisks indicate statistically significant differences compared with wild type using the T test ($P < 0.05$).

Figure S3

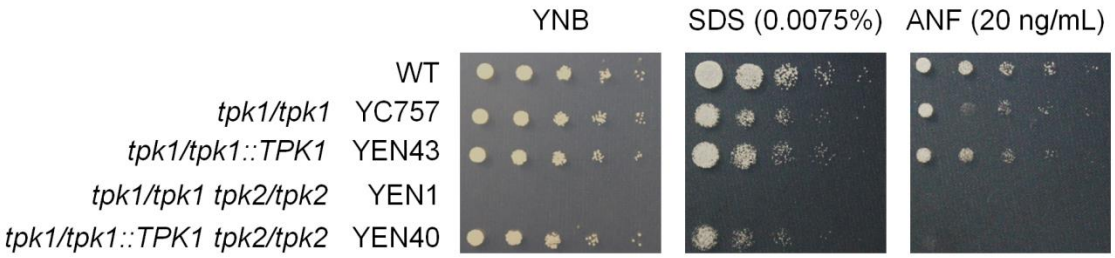

Figure S3. Introducing *TPK1* in the *tpk1/tpk1* or *tpk1/tpk1 tpk2/tpk2* mutant restores stress responses.

Growth of the indicated strains exposed to various stresses. Cells were grown overnight in YPD at 30°C (except the *tpk1/tpk1 tpk2/tpk2* mutant, which was grown for two days), washed twice with dH_2O, and diluted to 0.2 OD_{600}. Samples were five-fold serially diluted, spotted onto YNB medium containing the indicated chemicals, and incubated at 30°C for 24 h. ANF, anidulafungin; SDS, sodium dodecyl sulfate.

Figure S4

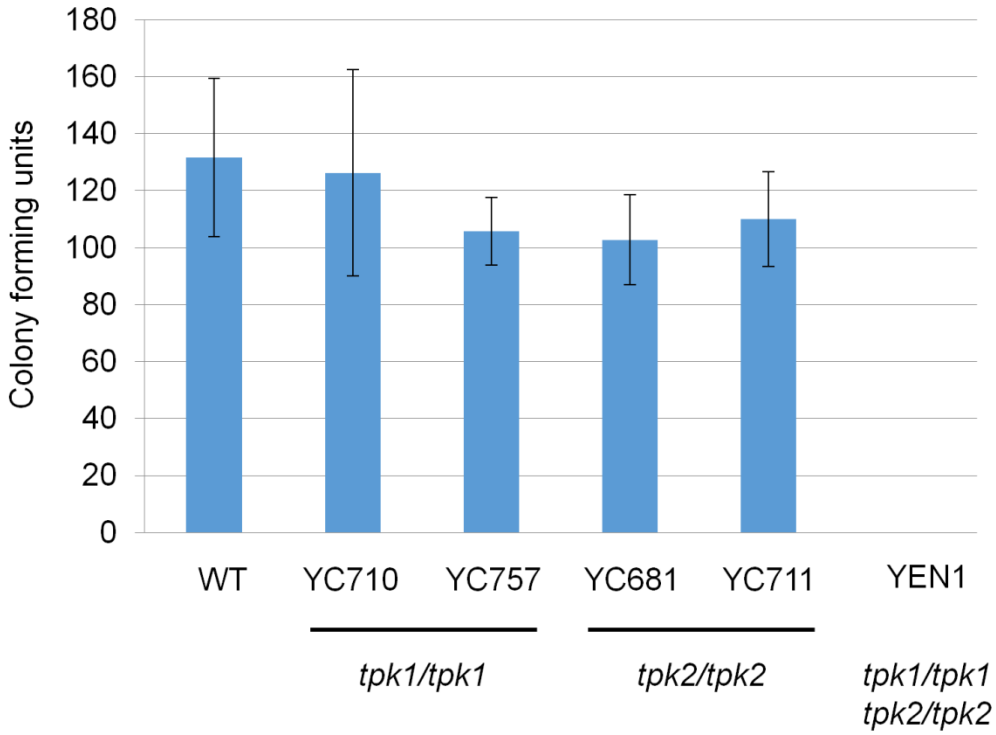

Figure S4. Neither *TPK1* nor *TPK2* has effects on adherence to polystyrene.

Adherence of the indicated strains on a 96-well plate. Cells were grown overnight in YPD at 30°C (except the *tpk1/tpk1 tpk2/tpk2* mutant, which was grown for two days), washed twice with PBS, and adjusted to 0.5 OD_{600} in SC medium. Then, 200 µL of each strain was inoculated into 96-well polystyrene plates and incubated for 90 min at 37°C and 200 rpm. The wells were then washed twice with PBS to remove non-adhered cells. The adhered cells were vigorously resuspended in water and plated on YPD medium to determine the colony-forming units.

Figure S5

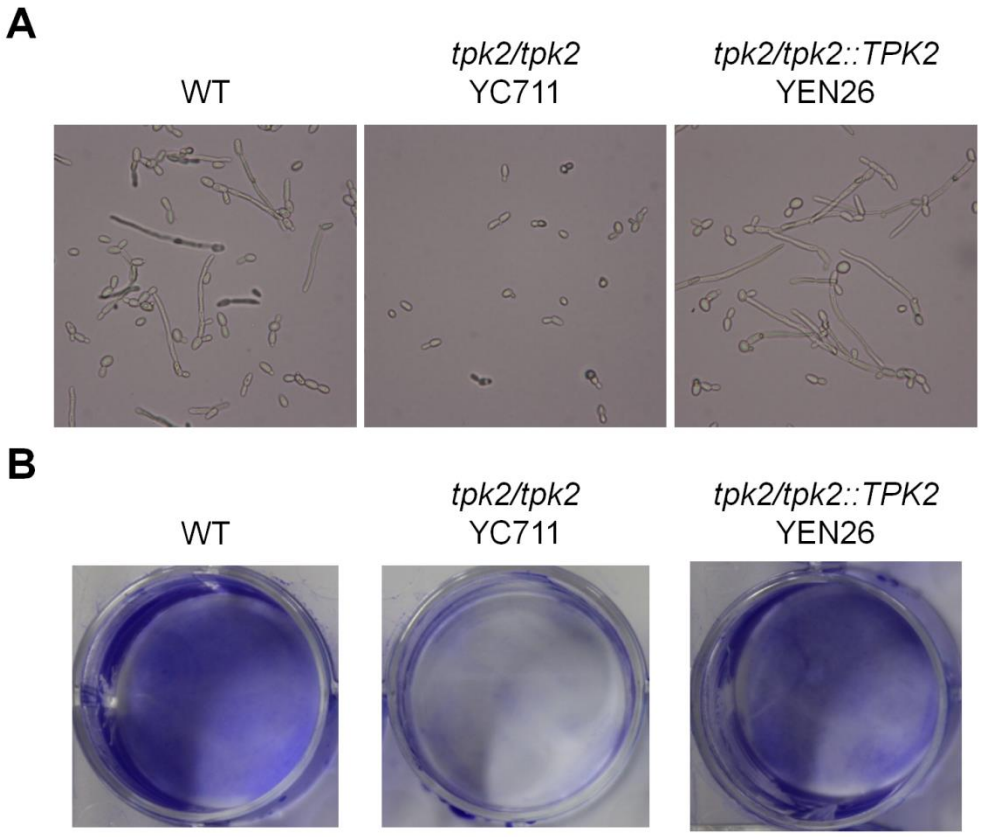

Figure S5. ***TPK2*** **complemented strain rescues hyphal growl and biofilm formation.** **(A)** Hyphal growth of the indicated strains in SC medium with 10 mM GlcNAc. Cells were grown overnight in YPD at 30°C, washed twice with dH_2O, diluted to 0.2 OD_{600} with fresh SC medium with 10 mM of GlcNAC, and incubated at 37°C and 200 rpm for 4 h. **(B)** Cells were grown in YPD medium overnight at 30°C, washed twice with dH_2O, and diluted to 0.5 OD_{600} in SC medium. Afterwards, 2 mL of each sample were inoculated into a 12-well polystyrene plate for 90 min at 37°C and 200 rpm. The wells were then washed twice with PBS buffer, inoculated with 2 mL of fresh SC medium, and incubated for 24 h at 37°C and 200 rpm. The wells were washed twice with PBS, stained with 0.05% crystal violet, and photographed.

Figure S6

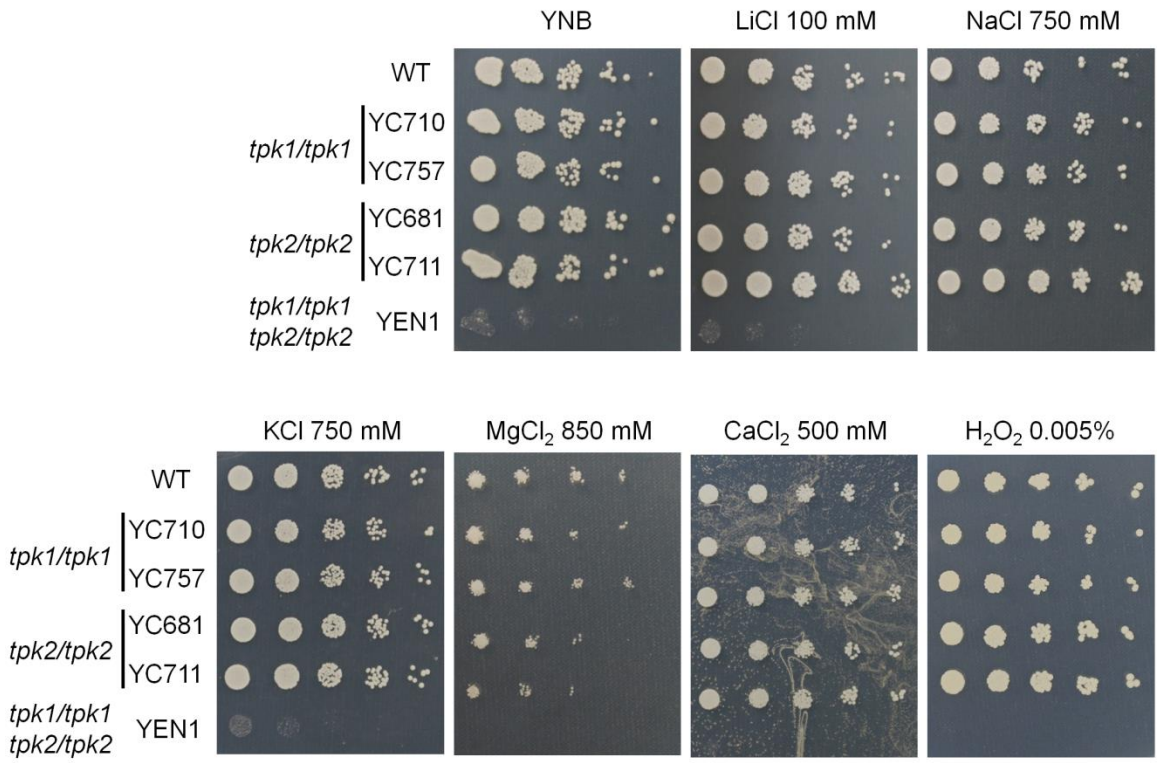

Figure S6. *C. tropicalis* Tpk2 has a specific role in magnesium stress response.

Growth of the indicated *C. tropicalis* strains exposed to various stresses (LiCl, NaCl, KCl, $MgCl_2$, $CaCl_2$, or H_2O_2). Cells were grown overnight in YPD at 30°C (except the *tpk1/tpk1 tpk2/tpk2* mutant, which was grown for two days) and washed twice with dH_2O. Samples were five-fold serially diluted using 0.2 OD_{600} as the initial concentration, spotted onto YNB medium containing the indicated chemicals, and incubated at 30°C for 48 h.