

<i>S.cerevisiae</i>	1	-----MPEIYGPQPLKPLNTVMRHGFEEQYQSDQLQLQSLAND	37
<i>S.pombe</i>	1	MNDRI SEVSGSSRRARRSVLSYGTTE TGSDRYTENSNIATENGVD TASSMIDGIQSGFPQPRHGFEEYNNAEYINMLEQV	80
human	1	-----MESEMLQSPLLGLGEEDEADLTOWN-----	25
<i>S.cerevisiae</i>	38	FIFYFDDKRHKTNGNPIPEEDKQRDVNRYYPITDWMIMKDRQKTVSAALLCLNLGVDPDPVDMKTHPCARVEAWVDPLN	117
<i>S.pombe</i>	81	FMYMYTDKRHRGVI SKKNAEP-----TETIHDWRMRERLKTVSAALLVCLNIGVDPDPVIKPNPAKYECWIDPFS	151
human	26	LPLAFMKKRHCKEIGES-----KSLAQSWRMKDRMKTVSVALVCLNVGVDPDPDVKTTPCARLECWIDPLS	92
<i>S.cerevisiae</i>	118	FQDSKKAIEQIGKNLQAQYETLSLRTRYKQSLDPCVEDVKRFCNSLRRTSKEDRILFHYNGHGVKPTKSGEIWFVNRGY	197
<i>S.pombe</i>	152	LPASK-ALEAIGKNLQQQYETLSMRTRYRHYLDPAIEEVKKLCIGQRRNAKEERILFHYNGHGVMPPTASGEIWFVNKNY	230
human	93	MGPQK-ALETIGANLQKQYENWQPRARYKQSLDPTVDEVKKLCTSLRRNAKEERVLFHYNGHGVPRPTVNGEVVWFNKNY	171
<i>S.cerevisiae</i>	198	TQYIPVSLYDLQTLWGAPCIFVYDCNSAENILINFQKFVQKRIKDDEEGN-----HDVAAPSPTSAYQDCFQLASCTSD	272
<i>S.pombe</i>	231	TQYIPVSLYDLQSWLGAPCIFVYDCSAAGNIIVNFNRF AEQRDKEALRIAKQNP-----NVLAMPSHTSCIQLAACGPKE	305
human	172	TQYIPLSIYDLQTMGSPSIFVYDCSNAGLIVKSFQKFALQRE-QELEVAAINPNHPLAQMPLPPSMKNCIQLAAC	250
<i>S.cerevisiae</i>	273	LLLMSPELPADLFSCLTTCPIEISIRIFLMQS PLKDSKYKIFFENSTSNQPPGD SKNSFKSKI PNVNI PGMLSDRRTPLG	352
<i>S.pombe</i>	306	TLPMPDLPADLFTSCLTSPIEISVRWYVLQN-----PFPNKLNLNMLLKI PGRLQDRRTPLG	363
human	251	LLPMIPDLPADLFTSCLTTPIKIALRWFCMQKCV-----SLVPGVTLDLIEKI PGRLNDRRTPLG	310
<i>S.cerevisiae</i>	353	ELNWI FTAITDTIAWTS LPRPLFKKLFRRHLMIAALFRNFLLAKRIMPWYNCHPVSDPELPDSITTHPMWKS	432
<i>S.pombe</i>	364	ELNWI FTAITDTIAWNVFPKHLFRRLFRQDLMVAALFRNFLLAERIMLVHSHCPQSSPELPP-THDHPMWN	442
human	311	ELNWI FTAITDTIAWNVLPRLDFQKLFRQDLLVASLFRNFLLAERIMRSYNCTPVSSPRLPP-TYMHAMWQAWD	389
<i>S.cerevisiae</i>	433	LTKIVIDLKN PPATALESQMI LQQQETLQNGGSSKSNAQDTKAGSIQTQSRFAVANLSTMSLVNNPALQSRKSI	512
<i>S.pombe</i>	443	LSQLPMDLDAESKGIAYEYKHS-----	465
human	390	LSQLPTIEEG-----TAFRHSP-----	407
<i>S.cerevisiae</i>	513	QQQLQQQQQQQQFTG FFEQNLTAFELWLKYASNVRHPPQLPIVLQVLLSQVHRI RALVLLSRFLDLGPWAVYLSLSIG	592
<i>S.pombe</i>	466	-----FFSEQLTAFEVWL SQGLIRSKPPDQLPLVLQVLLSQVHRLRALILLSKFLDLGVWAVDLALSIG	529
human	408	-----FFAEQLTAFQVWLTMGVENRNPPQLPIVLQVLLSQVHRLRALDLLGRFLDLGPWAVSALS	471
<i>S.cerevisiae</i>	593	IFPYVLKLLQSPAPELKPILVFIWARIMSIDYKNTQSELIKEKGYMYFVTVLVPDWGVNGMSATNGSAMINSGNPL	672
<i>S.pombe</i>	530	IFPYVLKLLQSPAIELKPVLVFIWARILAVDDSS-CQADLLKDNQGYGYFVQILNPNSSIF-----	587
human	472	IFPYVLKLLQSSARELRPLLVFIWAKILAVDSS-CQADLVKDNHGYFLSVLADP-----	525
<i>S.cerevisiae</i>	673	SQNINGPSSRYERQGGNRTSNLGHNNLPFYHSNDTTDEQKAMAVFVLASFVRNFP LGQKNCFSL ELVNKLCFYIDNSEI	752
<i>S.pombe</i>	588	-----PSSNI SEHRAMCAFILSVFCRGFPQQQLACLNPQVLS-HCLSHLNSPD	634
human	526	-----YMPAEHRTMTAFILAVIVNSYHTGQEACLQGNLIA-ICLEQLNDHP	570
<i>S.cerevisiae</i>	753	PLLRLQWCVILLGLLFADNPLNRFVCMNTGAVEILLKSLKDPVPEVRTASIFALKHFI SGFQDAEVI LRLQQEFEEQYQQL	832
<i>S.pombe</i>	635	SLLRQWACLCLISQLWENYSEAKWSGTRDNAHKLAELIVDSVPEVRASVLTAFTTFLG-FPEKTEEV-----	700
human	571	PLLRLQWVAICLGRIQWQNFDSARWCGVRDSAHKELYSLSDPIPEVRCAAVFALGTFVGN	637
<i>S.cerevisiae</i>	833	HSQLQHLQNQSHLQQQSSQQQQHLEQQQMKIEKQIRHCQVMQNQLEVIDLRKLRQIEGNLISILPLINDGSSSLVRKEL	912
<i>S.pombe</i>	701	-----VAVETYIAIAALAALSDASPLVRHEL	726
human	638	-----TTIDHNVAMMLAQLVSDGSPMVRKEL	663
<i>S.cerevisiae</i>	913	VVYFSHIVSRYSNFFIVVVFNDDL EEIKLLEKSDINTRNTSDKYSVSQGSIFYTVMKSLILAEDPFL ENKELSKQVIDY	992
<i>S.pombe</i>	727	VIFLSHFVYVNYKKQLMVVAYESSLADILEKKNHNSISASTIYETVWQAVLVLAADPSIEISLAAEAIN--YVYQ	804
human	664	VVALSHLVVQYESNFCTVALQ----FIEEEKNYALPSPATTEGGSLTPVRDSPCTPRLRSVSSYGNIR--VATAR	737
<i>S.cerevisiae</i>	993	ILLELSAHKELGGPPFAVMEKFLLRSSKAHQTKGFG-----	1028
<i>S.pombe</i>	805	ELRESFLAFLQLPALHKAASLSKDDTNSVTSDPK-----	840
human	738	SLQNL SLTEESGGAVAFSPGNLSTSSASSTLGSPENEELISFETIDKMRRASSYSSLSNLIGVSFNSVYTQIWRVLLH	817
<i>S.cerevisiae</i>	1029	FNSSQVQFVKSSLSRSFSPNERVDNNAFKKEQQQHPKISHPMRTSLAKLFQSLGFSESNSDSTQSSNTSMKSHTSKKGP	1108
<i>S.pombe</i>	841	----PHFPVPSVENKILNRSFSLTRS LKG-----LALSLAGSDRASSELLSLNGENKPAESNL	894
human	818	LAADPYPEVSDVAMKVLNSIAYKATVNARPQRVLDTSSTLQSA PASPTNKGVIHQAGGSPPASSTSSSLTNDVAKQPV	897
<i>S.cerevisiae</i>	1109	SGLYLLNGNNIYP-----TAETPRFRKHTEPLQLPLNSSF LDYSREYFQEPQMKKQEA	1162
<i>S.pombe</i>	895	NHLTSAKVP GPP-----AFNELEYQSELDMP LTSYLFDWRSKYFTEPQMRPNED	943
human	898	SRDLP SGRPGTTGPAGAQYTPHSHQFPRTRMFDDKGEQ TADDAADDAAGHSFISATVQTGFCDWSARYFAQPVMI	977
<i>S.cerevisiae</i>	1163	DEPGSVEYNARLWRRNRNETIQETQGEKKLSIYGNWSKKLISLNNKSQPKLMKFAQFEDQLITADDRSTITVFDWEK	1242
<i>S.pombe</i>	944	DEPGSICYNQRLWRRNRNEKLIYRTRPLAEYSTNGRWQQLMTFNNTIAPRKL MFHQFEDQLITLGDKDI IQVWDWRNR	1023
human	978	HDLESQIRKEREWRFLRNSRVRRQAQVVIQKGIT-RLDDQIFLNRNPGVPSVVKFHPFTP-CIAVADKDSICFWDWEKGE	1055
<i>S.cerevisiae</i>	1243	TLSKFSNGTPFGTKVTDLKLINEDDSALLLTGSSDGVIKIYRDYQD VDT-FKIVSAWRGLTDMLLT PRSTGLL TEWLQIR	1321
<i>S.pombe</i>	1024	CLNSFKTSASATTNVTDMLLNEDDVALMTGSSDGTIKLYRDYENEK--VELVTSWNLSDLVFGDRNASLLMSWQQNC	1101
human	1056	KLDYFHNGNPRYTRVTAMEYLNQDCCSLLLTATDDGAIRVWKNFADLEKNPEMVTAWQGLSDMLPTTRGAGMVVDWEQET	1135
<i>S.cerevisiae</i>	1322	GSLLTTGDVKVIRVWDAHTETVEVDIPAKTSSLITSLTADQLAGNIFVAGFADGSLRVYDRRLDPRDSMIRRW	1401
<i>S.pombe</i>	1102	GHLLVAGDVVRVIRIWDASKEICYANLPVRSSNSITSLTSDLVGCNIIVAGFSDGVLRVYDKRLPARDSLTDVWKEHS	1178
human	1136	GLLMSSGDVRIVR IWDTDREMKVQDIP TGADSCVTSLSCDSHRS-LIVAGLGDGSI RYDRRMALSECRVMTYREHT--	1211
<i>S.cerevisiae</i>	1402	GVWINNVHLQRGGYREL VSGATNGVVELWDIRSED PVESFVDQNVTSQYGSQQKPTTMTCMQVHEHAPI IATG--TKQIK	1479
<i>S.pombe</i>	1179	-SEIVNVEMQSSGMRELISASSDGEVKLWDIRMNHSLQTFSTDN SG-----LTSLT VHSHAPVYATGSSNQSIK	1246
human	1212	-AWVVKASLQKRPDGHI VSVSVNGDVRIFDPRMPESVNVLIQVKG-----LTALD IHPQADLIACGSVNQFTA	1278
<i>S.cerevisiae</i>	1480	IWTTSGDLLNSFKNSHNNGVTSTLAATGIPKSLSYSSTSDAFLSSMAFHPRMMIAATNSHDSIVNIYKCEDERIDYF--	1557
<i>S.pombe</i>	1247	IWDTLQGNINTFRENPR-----FLNQPKPSSLMCLKFHPPHLLACGDNTDSRVNLYSCTKNEIHTDSP	1310
human	1279	YNSSGELINN I KYDG-----FMGQRVG-AISCLAFHPPHPLAVGSN-DYI SVYSVEKRVR-----	1335
<i>S.cerevisiae</i>	---		
<i>S.pombe</i>	1311	NEF	1313
human	---		

Fig. S1. Sequence alignment among Kog1 and the orthologs.
Only Kog1 from *S. cerevisiae* has insertions (Pink); the insertion loops interact with Tor2 in our model structures.

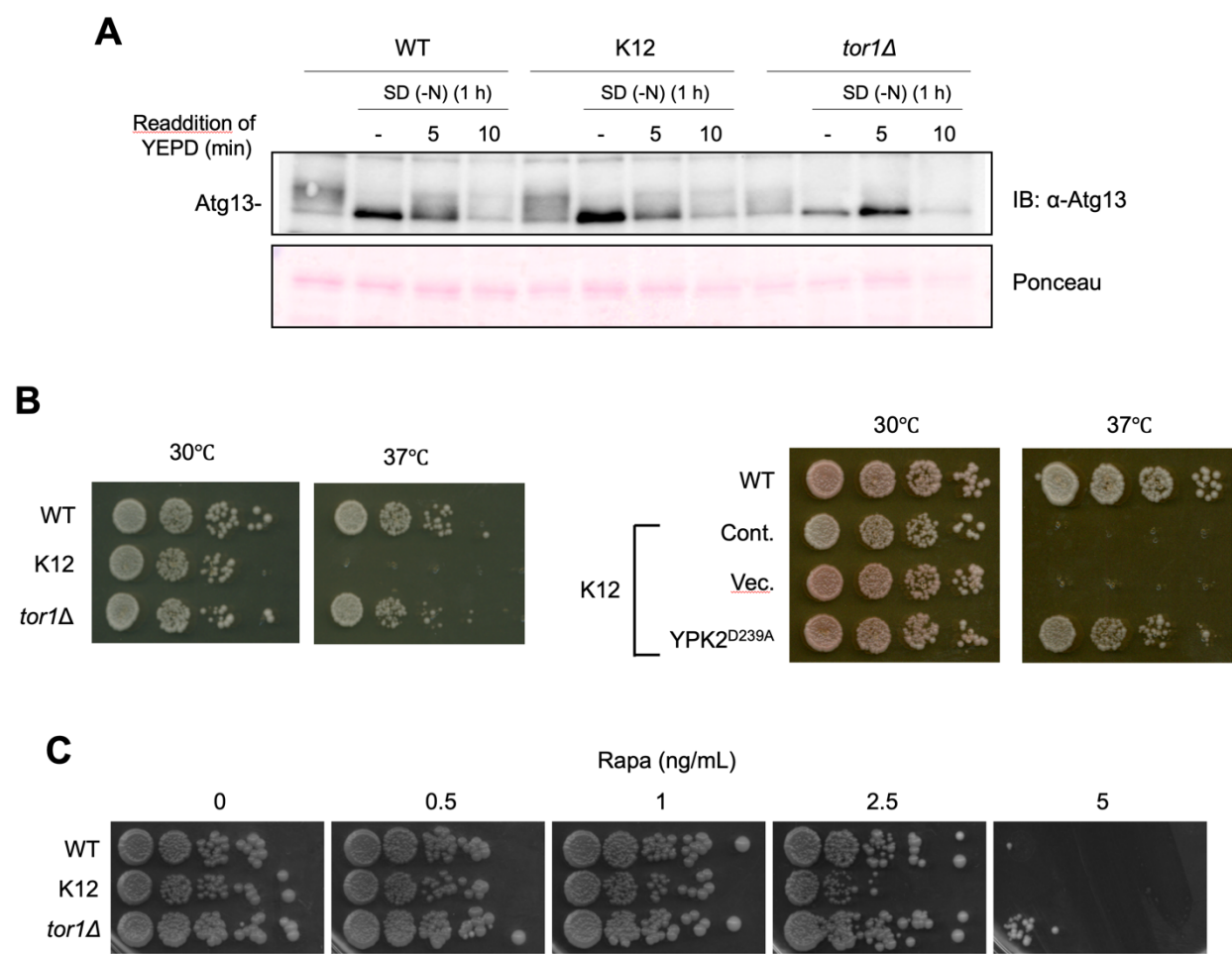


Fig. S2. Additional characteristics of Tor2(K12) mutant.

A. Recovery of Atg13 phosphorylation by re-addition of nutrient after starvation. The wildtype, *tor2*(K12), and *tor1Δ* strains harbouring ATG13 plasmid growing in YEPD at 30 °C were treated by nitrogen starvation (-N) for 1 hour. The phosphorylation states of Atg13 were examined by immunoblotting at 0, 5, and 10 min after re-addition of nutrient. The wildtype and *tor2*(K12) strains were recovered quickly. The recovery of *tor1Δ* strain was slower than others.

B. Cell growth at high temperature (37°C). Left: Cell growth of the wildtype, *tor2*(K12) and *tor1Δ* strains at 37°C. *tor2*(K12) strain is sensitive at higher temperature (37 °C). The figure of 30 °C data is the same as that of control data in Fig. 5A, because these experiments were conducted at the same time with a shared control. Right: Cell growth of *tor2*(K12) strain with YPK2 mutant D239A). YPK2^{D239A} mutant has been reported to constantly activate TORC2 signal pathway(Kamada et al., 2005). By the mutation of YPK2, temperature sensitivity of *tor2*(K12) strain was suppressed. This result indicates the temperature sensitivity is caused by decreased TORC2 activity of *tor2*(K12) strain.

C. Cell growth under different dilution series of rapamycin (Rapa). Cell growth of the wildtype, *tor2*(K12) and *tor1Δ* strains were observed under 0, 0.5, 1, 2.5, and 5 g/mL rapamycin. *tor2*(K12) strain is more sensitive to rapamycin than *tor1Δ* strain.

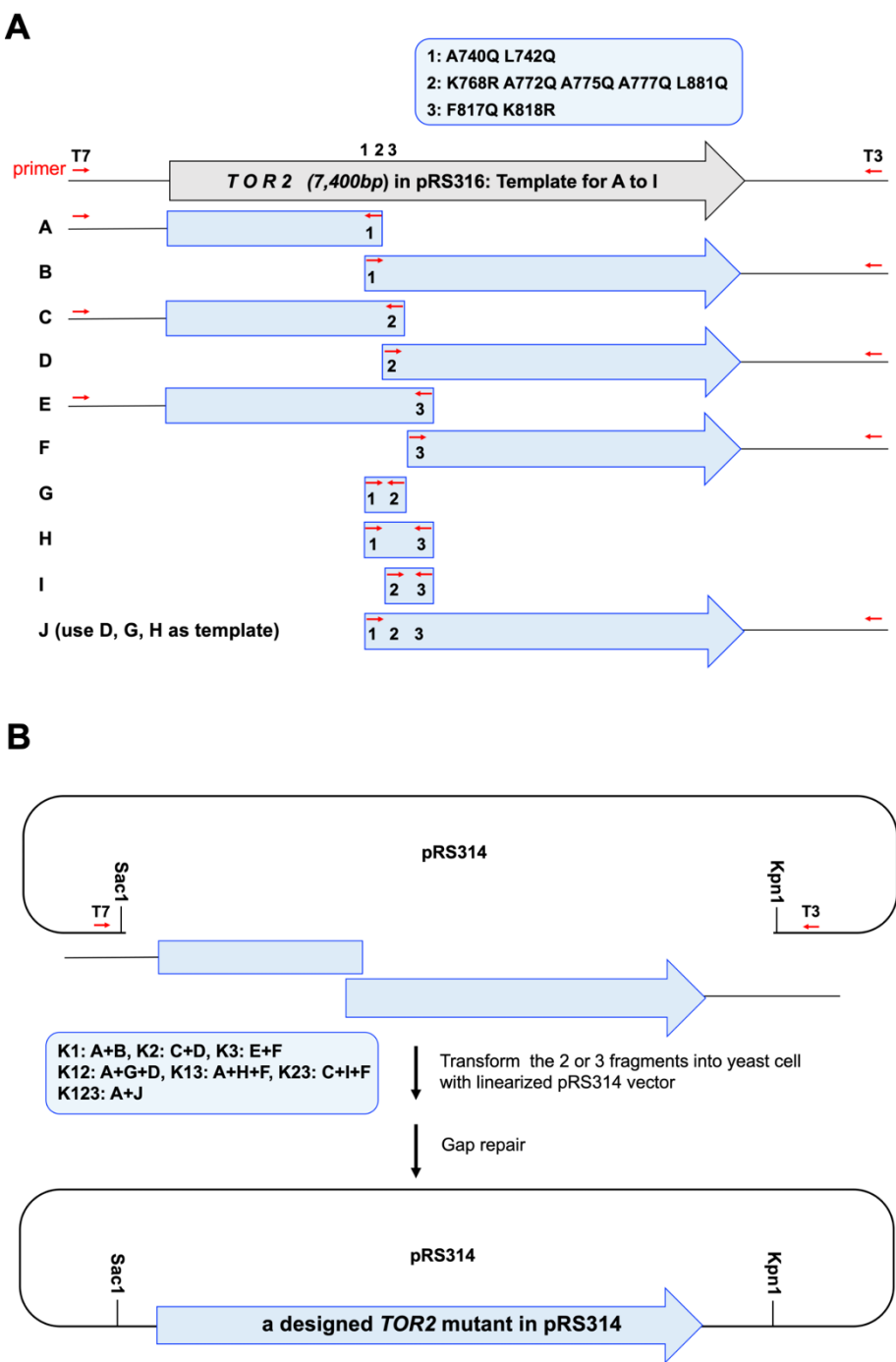


Fig. S3. Protocol to construct Tor2 mutant plasmids.
A. gene fragments of designed Tor2 mutants were amplified by PCR using DNA primers.
B. the gene fragments were mixed with linearized pRS314 vector and transformed into yeast cells. The plasmids of designed Tor2 were extracted from the transformed yeast cells.

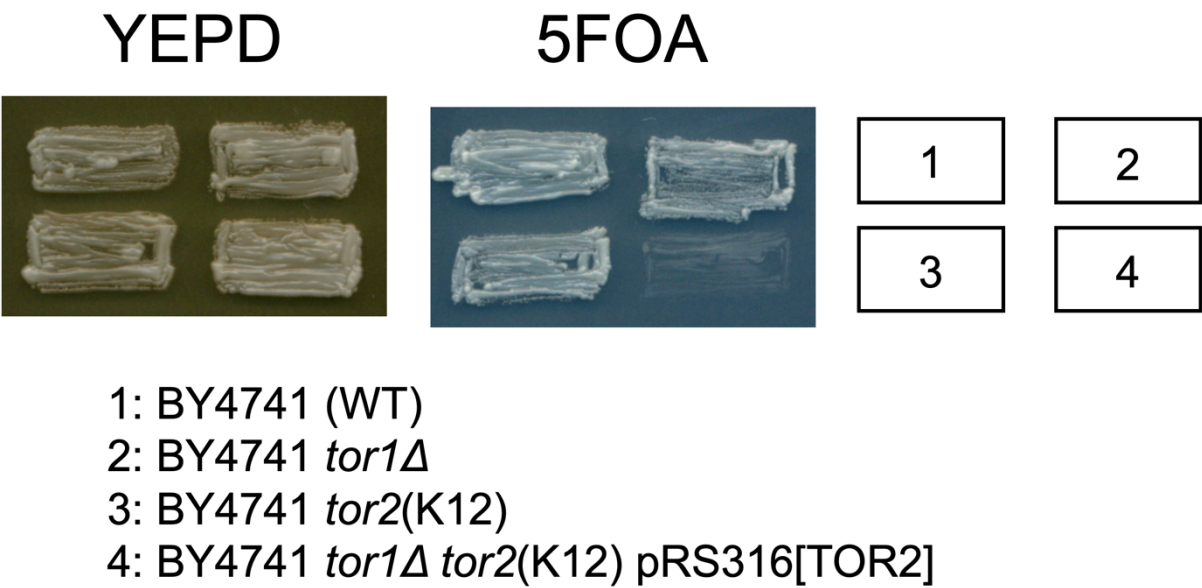


Fig. S4. Cell-based assay of *tor2*(K12) strain derived from BY4741.
Cell-based activity assay indicated *tor2*(K12) strain derived from the BY4741 also have no TORC1 activity and keep TORC2 activity, as is the case with that from the W303 (Fig. 3C).

Table S1. Yeast strains used in this study.

Strain	Genotype	Source
W303a	<i>Mataade2 his3 leu2 trp1 ura3 can1</i>	Laboratory stock
W303α	<i>Mataαade2 his3 leu2 trp1 ura3 can1</i>	Laboratory stock
BY4741	<i>Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ1</i>	Laboratory stock
YYK332	BY4741 <i>tor1Δ::KanMX</i>	Laboratory stock
YYK799	W303α <i>tor1Δ::KanMX</i> pRS313 ^[HA-TOR1] pRS316 ^[Flag-KOG1]	This study
YYK1411	W303α <i>tor2Δ::HIS3</i> pRS316[TOR2]	This study
YYK1412	W303a <i>tor1Δ::KanMX tor2Δ::HIS3</i> pRS316[TOR2]	This study
YYK1414	W303α <i>tor2Δ::HIS3</i> pRS314[TOR2 ^{WT}]	This study
YYK1418	W303a <i>tor1Δ::KanMX tor2Δ::HIS3</i> pRS314[TOR2 ^{WT}]	This study
YYK1448	W303α <i>tor2Δ::HIS3</i> AVO3 ^{Flag::HphMX} pRS316[TOR2]	This study
YYK1449	W303a <i>tor1Δ::KanMX tor2Δ::HIS3</i> AVO3 ^{Flag::HphMX} pRS316[TOR2]	This study
YYK1464	W303α <i>tor2Δ::HIS3</i> AVO3 ^{Flag::HphMX} pRS314 ^[HA-TOR2^{WT}]	This study
YYK1465	W303α <i>tor2Δ::HIS3</i> AVO3 ^{Flag::HphMX} pRS314 ^[HA-TOR2 K123]	This study
YYK1467	W303α <i>tor2Δ::HIS3</i> pRS314 ^[HA-TOR2^{WT}] pRS316 ^[Flag-KOG1]	This study
YYK1468	W303α <i>tor2Δ::HIS3</i> pRS314 ^[HA-TOR2 K123] pRS316 ^[Flag-KOG1]	This study
YYK1470	W303a <i>tor1Δ::KanMX tor2Δ::HIS3</i> pRS314 ^[HA-TOR2^{WT}] pRS316 ^[Flag-KOG1]	This study
YYK1499	W303α <i>tor2Δ::HIS3</i> pRS314[TOR2 K12]	This study
YYK1500	W303α <i>tor2Δ::HIS3</i> pRS314[TOR2 K13]	This study
YYK1501	W303α <i>tor2Δ::HIS3</i> pRS314[TOR2 K23]	This study
YYK1502	W303a <i>tor1Δ::KanMX tor2Δ::HIS3</i> pRS314[TOR2 K13]	This study
YYK1503	W303a <i>tor1Δ::KanMX tor2Δ::HIS3</i> pRS314[TOR2 K23]	This study
YYK1528	W303α <i>tor2Δ::HIS3</i> AVO3 ^{Flag::HphMX} pRS314 ^[HA-TOR2 K12]	This study
YYK1530	W303α <i>tor2Δ::HIS3</i> pRS314 ^[HA-TOR2 K12] pRS316 ^[Flag-KOG1]	This study
YYK1551	BY4741 <i>tor2::BleMX::TOR2 K12</i>	This study
YYK1553	BY4741 <i>tor1Δ::NatMX tor2::BleMX::TOR2 K12</i>	This study
YYK1580	W303a <i>tor1Δ::KanMX tor2Δ::HIS3</i> pRS315 ^[HA-TOR1] pRS314[TOR2 K12] pRS316 ^[Flag-KOG1]	This study

Table S2. Plasmids used in this study.

<i>Name</i>	<i>Source</i>
pRS316[TOR2]	Laboratory stock
pRS314[TOR2]	Laboratory stock
pRS314[TOR2 K1]	This study
pRS314[TOR2 K2]	This study
pRS314[TOR2 K3]	This study
pRS314[TOR2 K12]	This study
pRS314[TOR2 K13]	This study
pRS314[TOR2 K23]	This study
pRS314[TOR2 K123]	This study
pRS314 ^[HA] TOR2]	Laboratory stock
pRS314 ^[HA] TOR2 K12]	This study
pRS314 ^[HA] TOR2 K123]	This study
pRS313 ^[HA] TOR1]	Laboratory stock
pRS315 ^[HA] TOR1]	Laboratory stock
pRS316 ^[Flag] KOG1]	Laboratory stock
YE _p 352[<i>ATG13</i>]	Laboratory stock
YC _{plac33} ^[HA] <i>SCH9</i>]	Laboratory stock
YE _p 352[MPK1 ^[HA]]	Laboratory stock
YE _p 352[YPK2 ^{D239A}]	Kamada et al. 2005
BYP9689	NBRP Yeast
BYP9689[TOR2(K12) H3Δ]	This study

Table S3. DNA primers used in this study.

Name	Sequence
AVO3-F2	GGGACAAAAGGCCGGCTAATTTTACACGTCGGATCCCCGGGTTAATTAA
AVO3-R1rc	CTATATACATTTATACATGCGGCCCTTTTTTGCTGAATTCGAGCTCG
AVO3-4991	CAATACCGCAATGACGATGACTCCATCG
AVO3-5935rc	GTAAGTCACGTGAAATATTTCCCTGTGGC
TOR2seq-3104	GGCAGATTGAGTTCTGTCAACCCCG
TOR2-961	GGCAGATTGAGTTCTGTCAACCCCG
TOR2-3578rc	CAAACGAACAGTTCCACGCCTGATATGAGG
T3-PCR	CCAAGCGCGCAATTAACCCTCACTAAAGGG
T7-PCR	TGAGCGCGCGTAATACGACTCACTATAGGG
TOR2seq701	GGGCGATGTCAGAATATTTACCCGG
TOR2seq1200	GTCATATTGGTAAAATATCCTTTGT
TOR2seq1701	CTAAGTTGGAATACAGGAGACATGC
TOR2seq2203	GAAGAACATCGATATGAATGCTGCA
TOR2seq2706	CAGATGCCCAAATTTTGATTCAAGTG
TOR2seq3208	GGAGGAAAGTGCAACTCTATTATGT
TOR2seq3703	CGACATCGCATTGCTAATGCAAGGG
TOR2seq4206	CTTTGGTTACTTTTGGGCCGAATCT
TOR2seq4702	GAAGACCAAAGAAGATTGGCAAGAA
TOR2seq5203	GGAAACTTGGTATGAAAACTTCAA
TOR2seq5702	GATAAACGTCTAACTATGAGAGAAA
TOR2seq6201	GATTGAGCAATCCAGATTTCGATCCT
TOR2seq6705	CTGATCTAGGTAAGGCTCATCCGCA

<i>Name</i>	<i>Sequence</i>
TOR2seq7201	GCCAAAAC TACTATCTGCGCATGAT
TOR2seq7704	CGGAGGGACAAGATCTTTATAAGGT
TOR2seq8205	CCGAAGAAGAAGTTCAAAGGGTGGA
TOR2L527Q-1	TTACTGTCGATATCCCAATCTGGTGAAAAA
TOR2L527Q-1rc	TTTTTCACCAGATTGGGATATCGACAGTAA
TOR2I568Q-2	GAATCTAATGACGATCAAACAGATGCCCAA
TOR2I568Q-2rc	TTGGGCATCTGTTTGATCGTCATTAGATTC
TOR2D606EK611R-3	ACATTGAGCATGAGGAGTCGTCTGTCAGACGTCTGGCAGCATTAAACG
TOR2D606EK611R-3rc	CGTTAATGCTGCCAGACGTCTGACAGACGACTCCTCATGCTCAATGT
TOR2A740QL742Q-4	CAAAAAAAAAAGGAGGAAAGTCAAAC TCAGCTGTGTACGCTGATAAATTC
TOR2A740QL742Q-4rc	GAATTTATCAGCGTACACAGCTGAGTTTGACTTTCCTCCTTTTTTTTTTG
TOR2K768RtoL781Q-5	GACGTCATTCTTCCGCGGTGCCAGGATCAATCATCTCAAGTACAATCCACCGCTCAA AAGGTTTTGGGTGAAC
TOR2K768RtoL781Q-5rc	GTTCACCCCAAAACCTTTTGAGCGGTGGATTGTACTTGAGATGATTGATCCTGGCACCGCGGAAGAATGACGTC
TOR2F817QK818R-6	GACCAATCAAAC TCTCAAAGAAGAGATGCCGCCTTAAC
TOR2F817QK818R-6rc	GTTAAGGCGGCATCTCTTCTTTGAGAGTTTGATTGGTC
TOR2 922	CATAAAGAGCATAGICATTAAGATCAAATAGTTATC
TOR2 8529rc	GTTAGTAACGTCACGCTCGGAACTAAACATTAAIG