

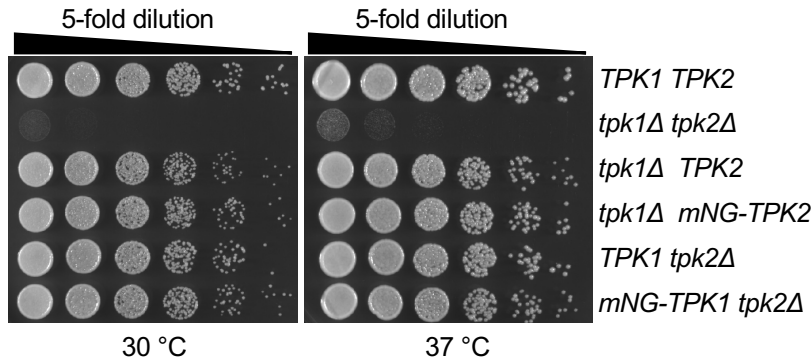
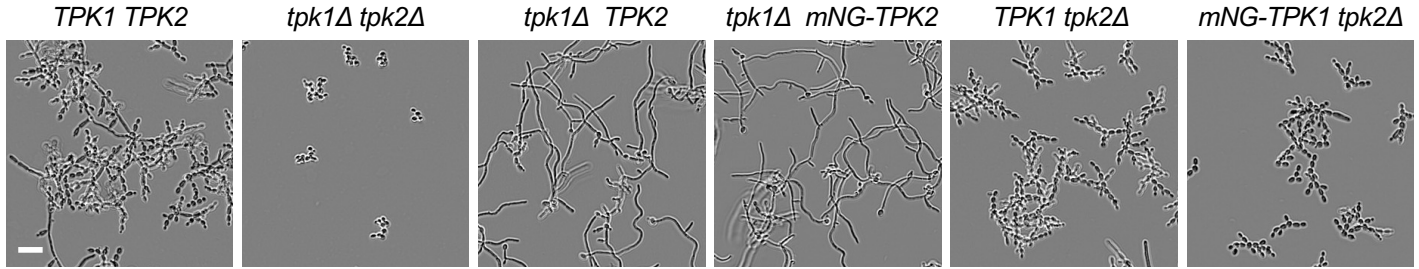
A**B**

Figure S1. Generation of functional mNeonGreen-tagged catalytic subunits of PKA. (A) N-terminal mNeonGreen tagged Tpk1 supports growth comparable to wild-type Tpk1 on solid medium. Cells from overnight cultures were 5-fold serially diluted and spotted on YPD medium. Agar plates were incubated at 30 °C (left) or 37 °C (right) and imaged after 24 hours. **(B)** N-terminal mNeonGreen tagged Tpk2 supports filamentation in liquid medium. Strains were grown under static conditions in the presence of 5 mM *N*-acetylglucosamine at 37 °C for 5 hours prior to imaging. All genotypes are homozygous. Scale bar, 20 μm; mNG, mNeonGreen.

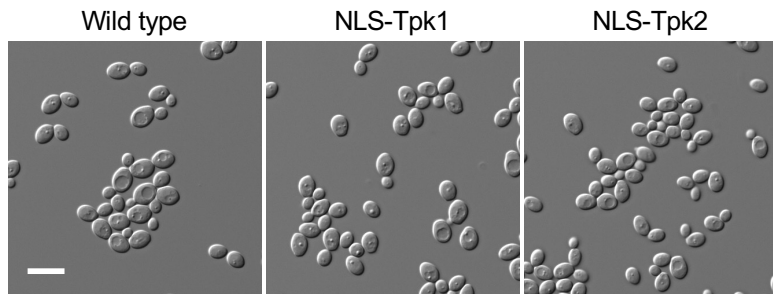


Figure S2. Nuclear deposition of Tpk does not induce filamentation. Strains expressing NLS-Tpk lacking any GFP tag were grown overnight under shaking conditions in YPD medium at 30 °C prior to imaging. Scale bar, 10 μ m.

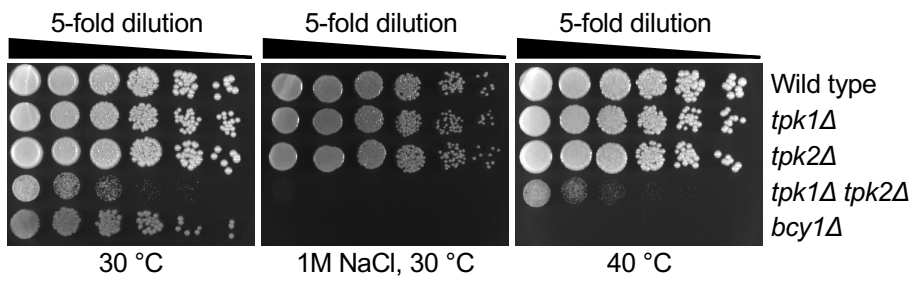


Figure S3. PKA activity is important for stress response. Cells from overnight cultures were 5-fold serially diluted and spotted on YPD medium with or without 1M NaCl. Agar plates were incubated at 30 °C or 40 °C and imaged after 48 hours. This is an independent biological replicate of the experiment shown in Figure 4C.

Table S1: Strains used in this study

Strain Name	Alias	Genotype	Source
CaLC155	SC5314	Clinical isolate	(1)
CaLC239	SN95 Wild type	<i>arg/arg4 his1/his1 URA3/ura3::imm⁴³⁴</i> <i>IRO1/iro1::imm⁴³⁴</i>	(2)
CaLC6118	SN95 <i>CPP-Phluorin</i>	As CaLC239 <i>neut5l::CPP-PHluorin2-SAT1/NEUT5L</i>	This Study
CaLC6489	SN95 <i>bcy1Δ</i>	As CaLC239 <i>bcy1::HygB/bcy1::HygB</i>	This Study
CaLC6699	SN95 <i>tpk2Δ</i>	As CaLC239 <i>tpk1::ARG4/tpk1::ARG4</i>	
CaLC6702	SN95 <i>tpk2Δ</i>	As CaLC239 <i>tpk2::ARG4/tpk2::ARG4</i>	This Study
CaLC6818	SN95 <i>GFP-TPK1</i>	As CaLC239 <i>tpk1::pTPK1-GFP-TPK1-HIS1/tpk1::pTPK1-GFP-TPK1-SAT1</i>	This Study
CaLC6826	SN95 <i>GFP-TPK2</i>	As CaLC239 <i>tpk2::pTPK2-GFP-TPK2-HIS1/tpk2::pTPK2-GFP-TPK2-SAT1</i>	This Study
CaLC6836	SN95 <i>tpk1Δ tpk2Δ</i>	As CaLC239 <i>tpk1::HygB/tpk1::HygB tpk2::HygB/tpk2::HygB</i>	This Study
CaLC6852	SN95 <i>tpk2Δ GFP-TPK1</i>	As CaLC239 <i>tpk1::pTPK1-GFP-TPK1-HIS1/tpk1::pTPK1-GFP-TPK1-SAT1 tpk2::ARG4/tpk2::ARG4</i>	This Study
CaLC6860	SN95 <i>tpk1Δ GFP-TPK2</i>	As CaLC239 <i>tpk2::pTPK2-GFP-TPK2-HIS1/tpk2::pTPK2-GFP-TPK2-SAT1 tpk1::ARG4/tpk1::ARG4</i>	This Study
CaLC6978	SN95 <i>BCY1-GFP</i>	As CaLC239 <i>BCY1-GFP-HIS1/BCY1-GFP-HIS1</i>	This Study
CaLC6985	SN95 <i>GFP-TPK1 VPH1-RFP</i>	As CaLC239 <i>tpk1::pTPK1-GFP-TPK1-HIS1/tpk1::pTPK1-GFP-TPK1-SAT1 VPH1/VPH1-RFP::HygB</i>	This Study
CaLC6987	SN95 <i>GFP-TPK2 VPH1-RFP</i>	As CaLC239 <i>tpk2::pTPK2-GFP-TPK2-HIS1/tpk2::pTPK2-GFP-TPK2-SAT1 VPH1/VPH1-RFP::HygB</i>	This Study
CaLC6993	SN95 <i>BCY1-GFP VPH1-RFP</i>	As CaLC239 <i>BCY1-GFP-HIS1/BCY1-GFP-HIS1 VPH1/VPH1-RFP::HygB</i>	This Study
CaLC7700	SN95 <i>BCY1-GFP VPH1-RFP tpk1Δ</i>	As CaLC239 <i>BCY1-GFP-HIS1/BCY1-GFP-HIS1 VPH1/VPH1-RFP::HygB tpk1::SAT1/tpk1::SAT1</i>	This Study
CaLC7702	SN95 <i>BCY1-GFP VPH1-RFP tpk2Δ</i>	As CaLC239 <i>BCY1-GFP-HIS1/BCY1-GFP-HIS1 VPH1/VPH1-RFP::HygB tpk2::SAT1/tpk2::SAT1</i>	This Study
CaLC7878	SN95 <i>NLS-GFP-TPK1</i>	As CaLC239 <i>tpk1::pTPK1-NLS-GFP-TPK1-HIS1/tpk1::pTPK1-NLS-GFP-TPK1-SAT1</i>	This Study
CaLC7884	SN95 <i>NLS-GFP-TPK2</i>	As CaLC239 <i>tpk2::pTPK2-NLS-GFP-TPK2-HIS1/tpk2::pTPK2-NLS-GFP-TPK2-SAT1</i>	This Study
CaLC8597	SC5314 <i>tpk1Δ</i>	As SC5314 <i>tpk1::SAT1/tpk1::SAT1</i>	This Study
CaLC8599	SC5314 <i>tpk2Δ</i>	As SC5314 <i>tpk2::HygB/tpk2::HygB</i>	This Study
CaLC8601	SC5314 <i>tpk1Δ tpk2Δ</i>	As SC5314 <i>tpk1::SAT1/tpk1::SAT1 tpk2::HygB/tpk2::HygB</i>	This Study

CaLC8867	SN95 <i>tpk1Δ</i> <i>pTPK1-GFP-2N1C</i>	As CaLC239 <i>tpk1::pTPK1-GFP-(TPK2N+TPK1C)-HIS1/tpk1::pTPK1-GFP-(TPK2N+TPK1C)-SAT1</i>	This Study
CaLC8869	SN95 <i>tpk1Δ</i> <i>pTPK1-GFP-TPK2</i>	As CaLC239 <i>tpk1::pTPK1-GFP-TPK2-HIS1/tpk1::pTPK1-GFP-TPK2-SAT1</i>	This Study
CaLC8873	SN95 <i>tpk1Δ</i> <i>pTPK1-GFP-1N2C</i>	As CaLC239 <i>tpk1::pTPK1-GFP-(TPK1N+TPK2C)-HIS1/tpk1::pTPK1-GFP-(TPK1N+TPK2C)-SAT1</i>	This Study
CaLC8946	SN95 <i>VPH1-mNG</i>	As CaLC239 <i>VPH1/VPH1-mNG::HygB</i>	This Study
CaLC8948	SN95 <i>VPH1-mNG tpk1Δ</i>	As CaLC239 <i>VPH1/VPH1-mNG::HygB tpk1::ARG4/tpk1::ARG4</i>	This Study
CaLC8950	SN95 <i>VPH1-mNG tpk2Δ</i>	As CaLC239 <i>VPH1/VPH1-mNG::HygB tpk2::ARG4/tpk2::ARG4</i>	This Study
CaLC8966	SN95 <i>tpk1Δ</i> <i>pTPK2-GFP-1N2C</i>	As CaLC239 <i>tpk2::pTPK2-GFP-(TPK1N+TPK2C)-HIS1/tpk2::pTPK2-GFP-(TPK1N+TPK2C)-SAT1 tpk1::HygB/tpk1::HygB</i>	This Study
CaLC8968	SN95 <i>tpk1Δ</i> <i>pTPK2-GFP-TPK1</i>	As CaLC239 <i>tpk2::pTPK2-GFP-TPK1-HIS1/tpk2::pTPK2-GFP-TPK1-SAT1 tpk1::HygB/tpk1::HygB</i>	This Study
CaLC8972	SN95 <i>tpk1Δ</i> <i>pTPK2-GFP-2N1C</i>	As CaLC239 <i>tpk2::pTPK2-GFP-(TPK2N+TPK1C)-HIS1/tpk2::pTPK2-GFP-(TPK2N+TPK1C)-SAT1 tpk1::HygB/tpk1::HygB</i>	This Study
CaLC8995	SN95 <i>tpk1Δ</i> <i>TPK2-GFP</i>	As CaLC239 <i>tpk1::ARG4/tpk1::ARG4 pTPK2-TPK2-GFP-HygB/pTPK2-TPK2-GFP-HygB</i>	This Study
CaLC8997	SN95 <i>tpk2Δ</i> <i>TPK1-GFP</i>	As CaLC239 <i>tpk2::ARG/tpk2::ARG pTPK1-TPK1-GFP-HygB/pTPK1-TPK1-GFP-HygB</i>	This Study
CaLC9013	SN95 <i>mNG-TPK1</i>	As CaLC239 <i>tpk1::pTPK1-mNG-TPK1-HIS1/tpk1::pTPK1-mNG-TPK1-SAT1</i>	This Study
CaLC9015	SN95 <i>mNG-TPK2</i>	As CaLC239 <i>tpk2::pTPK2-mNG-TPK2-HIS1/tpk1::pTPK2-mNG-TPK2-SAT1</i>	This Study
CaLC9049	SN95 <i>pTPK2-GFP-TPK2</i> <i>VPH1-mSC</i>	As CaLC239 <i>tpk2::pTPK2-GFP-TPK2-HIS1/tpk2::pTPK2-GFP-TPK2-HIS1 VPH1-mSC-HygB/VPH1</i>	This Study
CaLC9051	SN95 <i>pTPK2-GFP-TPK2NΔC</i> <i>VPH1-mSC</i>	As CaLC239 <i>tpk2::pTPK2-GFP-TPK2NΔC-HIS1/tpk2::pTPK2-GFP-TPK2NΔC-HIS1 VPH1-mSC-HygB/VPH1</i>	This Study
CaLC9053	SN95 <i>pTPK2-STOP-GFP-TPK2</i> <i>VPH1-mSC</i>	As CaLC239 <i>tpk2::pTPK2-STOP-GFP-TPK2-HIS1/tpk2::pTPK2-STOP-GFP-TPK2-HIS1 VPH1-mSC-HygB/VPH1</i>	This Study
CaLC9055	SN95 <i>pTPK2-STOP-GFP-TPK2NΔC</i> <i>VPH1-mSC</i>	As CaLC239 <i>tpk2::pTPK2-STOP-GFP-TPK2NΔC-HIS1/tpk2::pTPK2-STOP-GFP-TPK2NΔC-HIS1 VPH1-mSC-HygB/VPH1</i>	This Study

CaLC9057	SN95 GFP-2NΔC ^{ΔQ} VPH1-mSC	As CaLC239 <i>tpk2::pTPK2-GFP-TPK2NΔC^{ΔQ}-HIS1/tpk2::pTPK2-GFP-TPK2NΔC^{ΔQ}-HIS1 VPH1/VPH1-mSC::HygB</i>	This Study
CaLC9063	SN95 <i>pTPK2-STOP-GFP-TPK2 VPH1-mSC tpk1Δ</i>	As CaLC239 <i>tpk2::pTPK2-STOP-GFP-TPK2-HIS1/tpk2::pTPK2-STOP-GFP-TPK2-HIS1 VPH1-mSC-HygB/VPH1 tpk1::NAT/tpk1::NAT</i>	This Study
CaLC9103	SN95 CPP-PHluorin <i>vph1Δ</i>	As CaLC239 <i>neut5l::CPP-PHluorin2-SAT1/NEUT5L vph1::HygB/vph1::HygB</i>	This Study
CaLC9154	SN95 CPP-PHluorin <i>tpk1Δ</i>	As CaLC239 <i>neut5l::CPP-PHluorin2-SAT1/NEUT5L tpk1::HygB/tpk1::HygB</i>	This Study
CaLC9156	SN95 CPP-PHluorin <i>tpk2Δ</i>	As CaLC239 <i>neut5l::CPP-PHluorin2-SAT1/NEUT5L tpk2::HygB/tpk2::HygB</i>	This Study
CaLC9158	SN95 CPP-PHluorin <i>tpk1Δ tpk2Δ</i>	As CaLC239 <i>neut5l::CPP-PHluorin2-SAT1/NEUT5L tpk1::HygB/tpk1::HygB tpk2::HygB/tpk2::HygB</i>	This Study
CaLC9458	SN95 <i>mNG-TPK1 NAB2-mSC</i>	As CaLC239 <i>tpk1::pTPK1-mNG-TPK1-HIS1/tpk1::pTPK1-mNG-TPK1-SAT1 NAB2/NAB2-mSc-HygB</i>	This Study
CaLC9460	SN95 <i>mNG-TPK2 NAB2-mSC</i>	As CaLC239 <i>tpk2::pTPK2-mNG-TPK2-HIS1/tpk1::pTPK2-mNG-TPK2-SAT1 NAB2/NAB2-mSc-HygB</i>	This Study
CaLC9478	SN95 <i>VPH1-mNG tpk1Δ tpk2Δ</i>	As CaLC239 <i>VPH1/VPH1-mNG::HygB tpk1::SAT1/tpk1::SAT1 tpk2::SAT1/tpk2::SAT1</i>	This Study
CaLC10330	SN95 <i>mNG-TPK1 PAB1-mSC</i>	As CaLC239 <i>tpk1::mNG-TPK1-HIS1/tpk1::mNG-TPK1-SAT1 PAB1/PAB1-mSC::ARG4</i>	This Study
CaLC10332	SN95 <i>mNG-TPK2 PAB1-mSC</i>	As CaLC239 <i>tpk2::mNG-TPK2-HIS1/tpk1::mNG-TPK2-SAT1 PAB1/PAB1-mSC::ARG4</i>	This Study
CaLC10334	SN95 <i>BCY1-GFP PAB1-mSC</i>	As CaLC239 <i>BCY1-GFP-HIS1/BCY1-GFP-HIS1 PAB1/PAB1-mSC::ARG4</i>	This Study
CaLC10371	SN95 <i>mNG-TPK1 DHH1-mSC</i>	As CaLC239 <i>tpk1::mNG-TPK1-HIS1/tpk1::mNG-TPK1-SAT1 DHH1-mSC::ARG4/DHH1-mSC::HygB</i>	This Study
CaLC10373	SN95 <i>mNG-TPK2 DHH1-mSC</i>	As CaLC239 <i>tpk2::mNG-TPK2-HIS1/tpk1::mNG-TPK2-SAT1 DHH1-mSC::ARG4/DHH1-mSC::HygB</i>	This Study
CaLC10375	SN95 <i>BCY1-GFP DHH1-mSC</i>	As CaLC239 <i>BCY1-GFP-HIS1/BCY1-GFP-HIS1 DHH1-mSC::ARG4/DHH1-mSC::HygB</i>	This Study
CaLC10445	SN95 <i>mNG-TPK1 tpk2Δ</i>	As CaLC239 <i>tpk1::pTPK1-mNG-TPK1-HIS1/tpk1::pTPK1-mNG-TPK1-SAT1 tpk2::HygB/tpk2::HygB</i>	This Study

CaLC10447	SN95 <i>mNG-TPK2 tpk1Δ</i>	As CaLC239 <i>tpk2::pTPK2-mNG-TPK2-HIS1/tpk1::pTPK2-mNG-TPK2-SAT1 tpk1::HygB/tpk1::HygB</i>	This Study
CaLC10572	SN95 <i>NLS-TPK1</i>	As CaLC239 <i>tpk1::pTPK1-NLS-TPK1-HIS1/tpk1::pTPK1-NLS-TPK1-HIS1</i>	This Study
CaLC10575	SN95 <i>NLS-TPK2</i>	As CaLC239 <i>tpk2::pTPK2-NLS-TPK2-HIS1/tpk2::pTPK2-NLS-TPK2-HIS1</i>	This Study

Table S2: Plasmids used in this study

Plasmid ID	Description	Source
pLC963	pV1393-1 (CaCas9/sgRNA entry expression vector, contains NatR gene, targeting <i>NEUT5L</i>)	(3)
pLC1081	CAS9 vector pV1093	(4, 5)
pLC1082	pNAT	(4)
pLC1087	pFA-3HA-SAT1	(6)
pLC1100	pFA-3HA-ARG4	(6)
pLC1181	pDUP3-CPP-PHL2	(7).
pLC1206	pFA-GFP-HIS1	This study
pLC1211	pFA-3HA-HygB	This study
pLC1214	pFA-GFP-HygB	This study
pLC1215	pFA-RFP-HygB	This study
pLC1372	pFA-GFP-TPK1-HIS1-TPK1 3'	This study
pLC1375	pFA-GFP-TPK2-HIS1-TPK2 3'	This study
pLC1402	pFA-GFP-TPK1-SAT1-TPK1 3'	This study
pLC1406	pFA-GFP-TPK2-SAT1-TPK1 3'	This study
pLC1527	pFA-NLS-GFP-TPK1-HIS1	This study
pLC1528	pFA-NLS-GFP-TPK2-HIS1	This study
pLC1529	pFA-NLS-GFP-TPK1-SAT1	This study
pLC1530	pFA-NLS-GFP-TPK2-SAT1	This study
pLC1668	pFA-pTpk1-GFP-2N1C-HIS1	This study
pLC1669	pFA-pTpk1-GFP-2FL-HIS1	This study
pLC1671	pFA-pTpk2-GFP-1N2C-HIS1	This study
pLC1672	pFA-pTpk2-GFP-1FL-HIS1	This study
pLC1673	pFA-pTpk2-GFP-2N-HIS11	This study
pLC1676	pFA-pTpk1-GFP-1N2C-HIS1	This study
pLC1677	pFA-pTpk2-GFP-2N1C-HIS	This study
pLC1682	pFA-pTpk1-GFP-2N1C-SAT1	This study
pLC1683	pFA-pTpk1-GFP-2FL-SAT1	This study
pLC1685	pFA-pTpk1-GFP-1N2C-SAT1	This study
pLC1686	pFA-pTpk2-GFP-1N2C-SAT1	This study
pLC1687	pFA-pTpk2-GFP-1FL-SAT1	This study
pLC1689	pFA-pTpk2-GFP-2N1C-SAT1	This study
pLC1693	pFA-mScarlet-ARG4	This study
pLC1696	pFA-NeonGreen-HygB	This study
pLC1697	pFA-mScarlet-HygB	This study
pLC1699	pFA-mNeonGreen-TPK1-HIS1	This study
pLC1700	pFA-mNeonGreen-TPK2-HIS1	This study
pLC1708	pFA-mNeonGreen-TPK1-SAT1	This study
pLC1709	pFA-mNeonGreen-TPK2-SAT1	This study
pLC1717	pFA-p2-GFP-stop2FL-HIS1	This study
pLC1718	pFA-p2-GFP-stop2N-HIS1	This study
pLC1719	pFA-p2-GFP-Qless2N-HIS1	This study
pLC2041	pFA- NLS-TPK1-HIS1	This study
pLC2042	pFA- NLS-TPK2-HIS1	This study

Table S3: Oligonucleotides used in this study

Oligo ID	Name	Sequence (5'>3')
oLC747	CaTPK2+1132_AB-F	GGACCAGCAGATATCAGAAATC
oLC814	TPK1-984-F	CCTATATTTGTAGTTCTTATCC
oLC1077	CaTPK2-586+Apa1-F	TTGCGGGCCCGCTGAATTTCAAGTTCCATTC
oLC1824	CaTpk1-R	TGCACATCTCAGTCAAATTT
oLC4434	CaPab1+1577-F	AGGTGGATACTATCCAAACC
oLC4435	CaPab1+2688-R	CTCAGATATTATCCTTACTC
oLC5367	CaTPK1AB+102-F	AGGATCACCTACACTAGAAG
oLC5368	CaTPK1AB+309-R	GAACCTGTTCTAATGTTTCG
oLC5370	CaTPK1AB+789-R	TTTGTTGCCACCACTTCAGG
oLC5371	CaTPK2AB+148-F	GTGTCGAGCTCCAACATTAC
oLC5372	CaTPK2AB+278-R	CAGTAGAACGTTTCAGGCAAC
oLC5374	CaTPK2AB+784-R	GGTACCACATAACGTCCATG
oLC5377	CaHygrB+864-F	GCTCAAGGTAGATGTGATGC
oLC5698	CaTPK2-278-AB	CAATACTCGGATTGCAGGTC
oLC5951	HygB+341-R	TTATTGCGTACACGACCTGG
oLC6526	CaDHH1+1224-F	GCTGTTCCAAGACCATTTAG
oLC6527	CaDHH1+2291-R	ACTTTGAGAACACACGTTTC
oLC6916	pLC1869 F (Kpp050)	TTTAAAGTCAATAGGCATTCTCG
oLC6924	CaCas9/for	ATCTCATTAGATTTGGAACCTGTGGGTT
oLC6925	CaCas9/rev	TTCGAGCGTCCCAAACCTTCT
oLC6926	SNR52/F	AAGAAAGAAAGAAAACCAGGAGTGAA
oLC6927	sgRNA/R	ACAAATATTTAAACTCGGGACCTGG
oLC6928	SNR52/N	GCGGCCGCAAGTGATTAGACT
oLC6929	sgRNA/N	GCAGCTCAGTGATTAAGAGTAAAGATGG
oLC6942	LM21	CTAATTAACGTGTGTGTATGGATC
oLC6943	Kpp063	CACAGGATGACGCCTAAC
oLC6970	Kpp065	TGCTCTTGGTGGTACTGC
oLC6971	Kpp068	CATTCTGTTTATTTGAGACTACTCTTG
oLC7318	CaTPK2+209-R	CGAAAAACAAAGCAGACAAGG
oLC7958	HygR check R	CAACAGAAGTAGCAGTCAATTCTGG
oLC7959	HygR check F	GAAGACCATCTACTAGACCAAGAGC
oLC8032	NAT1-3118	CCCAGATGCGAAGTTAAGTGCGCAG
oLC8033	pDUP3-4969	GGATTTAGTTCCATTATGG
oLC8428	SNR52/R_bcy1del	CCAACCAAGGAAGTAGACCCCAAATTAAAAATAGTTTACGC AAGTC
oLC8429	sgRNA/F_bcy1del	GGGTCTACTTCCTTGGTTGGGTTTTAGAGCTAGAAATAGCA AGTTAAA

oLC8430	del bcy1 F	TCACTATGTATCTATGTGATTACCTTATCAAATTGGACAATA CGGATTTTTAATTGACCACGATAATTACAAACTTCCCCGTAA CGGTCGACGGATCCCC
oLC8431	del bcy1 R	GGGAAGCTCGTTCATGCTGCCATCTCGTATATACCTGACAA CAAGAAATGTAGTTGAACTTTTGCACCAACCATCCATTATCT CGATGAATTCGAGCTCG
oLC8562	SNR52/R_tpk1Tag	CCCAAACATTGTTGCTAGACCAAATTAATAAGTTTACGC AAGTC
oLC8563	sgRNA/F_tpk1Tag	GTCTAGCAACAATGTTTGGGGTTTTAGAGCTAGAAATAGCA AGTTAAA
oLC8564	tpk1 c tag sense	ATTTGATCGATACCCAGAAGATAAAGATTTGGATTATGGTA TAAGTGGAGTTGAAGACCCATATCGTGATCAATTCCAGGAC TTTGGTCGACGGATCCCC
oLC8565	tpk1 c tag anti	ATTCTCAATGTGGTACTCAGATAAAAACCAATTCTCGCTATT ATGTTTGATTCCAATATTGAACTATTGTGTGTTACACTCCTC GATGAATTCGAGCTCG
oLC8566	oLC8566_tpk1 tag checkF	GTGATATAGAACTCCATATGAACCACC
oLC8567	tpk1 tag checkR	ATGGCAACTTAATAACATCCAATACC
oLC8568	SNR52/R_tpk2Tag	ATACGAGTTTCATCTTTGACCAAATTAATAAGTTTACGCA AGTC
oLC8569	sgRNA/F_tpk2Tag	GTCAAAGATGAAACTCGTATGTTTTAGAGCTAGAAATAGCA AGTTAAA
oLC8570	tpk2 c tag sense	GTCATTGTTTGACCATTATCCAGAAGAACAATTAGACTACG GAAGCCAAGGAGAAGATCCTTATGCTCTGTATTTCTTGAC TTTGGTCGACGGATCCCC
oLC8571	tpk2 c tag anti	TACCGAAAAACAAAGCAGACAAGGAAGAGTCAACATGTTAA GTCATTCGTATAATAACAAAACGAAAATATTAATGTGCATTT CGATGAATTCGAGCTCG
oLC8572	tpk2 tag checkR	CCAGGCGTAAGATATTCTGGGTG
oLC8573	SNR52/R_bcy1Tag	TCTCGTATATACCTGACAACCAAATTAATAAGTTTACGCA AGTC
oLC8574	sgRNA/F_bcy1Tag	GTTGTCAGGTATATACGAGAGTTTTAGAGCTAGAAATAGCA AGTTAAA
oLC8575	bcy1 c tag sense	CGGGTTCCAAAGATTATTGGGTCCTGTTGTGGAGGTATTGA AAGAACAAGACCCTACAAAGAGTCAAGACCCAACCTGCTGG TCATGGTCGACGGATCCCC
oLC8576	bcy1 c tag anti	TAGAAGCCAAGTTTGCAATATATTAGTTACCATGTTTCGCAA AAGGCACGGCCGGATAACTTCAATAATGTTCAACCCATGGT CGATGAATTCGAGCTCG
oLC8577	bcy1 tag check F	GACAAGCTACTGTGGAAGCATTGG
oLC8578	bcy1 tag check R	CTGTGAGAGGGATTCTCGGAATTCTC
oLC8596	tpk1 del sense	CAACCAAAACCAGGATGACATCCATGGAACCAGCAGACAC AAGCATCAGGTCATTAAACGACATCAACTTACAAGAACTTG CCAAGGTCGACGGATCCCC
oLC8597	tpk2 del sense	CTTCACATCACCAAGCTGCATCAACTGAATCAATCCAATTC GGACAGTAATTCTTAAACTCAAACACATCAATGGACAATC ATCGGTCGACGGATCCCC
oLC8604	tpk1 com check anti	ACTCAACTATTGTCTGAATGTGTG
oLC8823	SNR52/R_CaNATMarke r	TCTTACCAGCATCGGATTCCAAATTAATAAGTTTACGC AAGTC
oLC8824	sgRNA/F_CaNATMarke r	GAATCCGATGCTGGTGAAGAGTTTTAGAGCTAGAAATAGCA AGTTAAA

oLC9038	Vph1 C tag F	GGTTGAAAGTATGTCGAAATATTTTGAAGGTGGTGGTTCTG CTTTTGAACCATTTACTTTTAAAGGTTTATTAGACAGTGTTTT AGGTCGACGGATCCCC
oLC9039	Vph1 C tag R	CTTAATTCTTTTTTATAAATTAATTGATGCTTTAATGAATGAA TCTAAACAAGTTAAAACCCCAACCCTATGGTTCTAGGCTC GATGAATTCGAGCTCG
oLC9040	Vph1 C tag check F	CTATGACTATTGGTAATGCATTTGGTCC
oLC9041	Vph1 C tag check R	GAAGAAGGAGTACCTTTAATCTCTTCC
oLC9085	nab2 c tag F	CAGGGATGGTAGTGGCAATAATAACAGTACCTCAAATCGAT CATTTGCTGTTTCTGAAGATCAGATTATGGAACAAGTTGCT CAAGGTCGACGGATCCCC
oLC9086	nab2 c tag R	GGATATACACCTCTTCTACTACTATAACCTCTTCATTCCAGT TATTCAGATTATTTCAATATTGTTCTCTTGTTATTTTATCG ATGAATTCGAGCTCG
oLC9087	nab2 check F	GTGTATGTACCAACATCCTGAAGGAAG
oLC10760	GFP Q F	TGAAGGTGAAGGTGATGCTAC
oLC10761	GFP Q R	CTAAGGTTGGCCATGGAAC
oLC11300	mSc R Asc1	GACTGGCGCGCCCTAGAGCTAATATAATTCATCCATACCAC C
oLC11381	deltpk1 pNAT_F	AGGATGACATCCATGGAACCAGCAGACACAAGCATCAGGT CATTAAACGACATCAACTTACAAGAACTTGCCAATAATGATT TTCCCAAGTCACGACGTT
oLC11382	deltpk1 pNAT_R	TTCTCAATGTGGTACTCAGATAAAAACCAATTCTCGCTATTA TGTTTGATTCCAATATTGAACTATTGTGTGTACACTCCGTG GAATTGTGAGCGGATA
oLC11383	deltpk2 pNAT_F	CAAGCTGCATCAACTGAATCAATCCAATTCGGACAGTAATT CCTTAAACTCAAACACATCAATGGACAATCATCTAATGATTT TTCCCAAGTCACGACGTT
oLC11384	deltpk2 pNAT_R	ACCGAAAAACAAGCAGACAAGGAAGAGTCAACATGTTAA GTCATTCGTATAATAACAAAACGAAAATATTAATGTGCATTG TGGAATTGTGAGCGGATA
oLC11403	del_vph1_F	CTCTCTCTTATCCCTTATCAATACATTTCAAGAAAAAAGGT ATGCCGAGAAAGGAAGAAGCAGTATTCCGTTCCAGCGGATA TGTGGTCGACGGATCCCC
oLC11404	del_vph1_R	CAACCCTATGGTTCTAGGCTTATAAAACACTGTCTAATAAA CCTTTAAAGTAAATGGTTCAAAGCAGAACCACCACCTTC TCGATGAATTCGAGCTCG
oLC11405	SNR52R_delvph1	CCAACCTATACTCGTACCAACAAATTAATAATAGTTTACGCA AGTC
oLC11406	sgRNAF_delvph1	TTGGTACGAGTATAAGTTGGGTTTTAGAGCTAGAAATAGCA AGTTAAA
oLC11407	del vph1 check F	GGTCAGTCGATTGGTTTGCCACAGGGAG
oLC11408	del vph1 check R	CCAAATGCATTACCAATAGTCATAGACC
oLC12593	CaDHH1_NEON F	ATTTGCTGGCTACCCAGGTCAACCTCCACAACCTCCACAA GGTCAACAACAACATGCTCAAGCACAAAATCCTGCTCAACA ATATGGTCGACGGATCCCC
oLC12941	Pab1-mSC F	GTTCAATAACCATTTTGAAGATGCTTTGACTGCTTTTGAAGA GTACAAGAAGTCTGAAGCTGCCGGTAATGCTGAAGAGCAA GCTGGTCGACGGATCCCC
oLC12942	Pab1-mSC R	TAATCTAGATTAATTCTTGGACATCTCCCACTTTCATAAATT CCGTTACAACATCTCCTCTCTGCAATAACAATCAAGTCTTTC GATGAATTCGAGCTCG

oLC12943	Dhh1-mSC R	GCTTTAACTATGGTACAATAAAATACAAGAACAAATGAGG AATTGGCTTTTCAACAACAGCAAAATGGAGGGTGCATAATC TCGATGAATTCGAGCTCG
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Text S1: Supplementary methods including strain construction details and plasmid sequences.

Strain construction:

CaLC6118: To construct the CPP-PHluorin2 strain, the repair template was digested from pLC1181 (pDUP3-CPP-PHL2) by NgoMIV (NEB) and transformed into CaLC239 (7, 8). Transformants were selected by plating onto YPD plates supplemented with 150 µg/mL NAT. Correct integration of the construct at one NEUT5L region by confirmed by PCR using oLC8032 and oLC8033.

CaLC6489: Both alleles of *BCY1* were deleted using a transient CRISPR approach adapted from Min and colleagues (4). The *CaCAS9* cassette was amplified from pLC963 using oLC6924 and oLC6925. The repair cassette was PCR amplified from pLC1211 using oLC8430 and oLC8431. The sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8428 (fragment A), as well as oLC8429 and oLC6927 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929 (5). The repair cassette, sgRNA, and Cas9 DNA were transformed into CaLC239. Transformants were selected by plating onto YPD plates supplemented with 600 µg/mL hygromycin B. Transformants with *BCY1* deletion were distinguishable by their yellow colony color.

CaLC6699: Both alleles of *TPK1* were deleted using a transient CRISPR approach adapted from Min and colleagues (4). The *CaCAS9* cassette was amplified from pLC963 using oLC6924 and oLC6925. The repair cassette was PCR amplified from pLC1100 using oLC8596 and oLC8565. The sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8562 (fragment A), as well as oLC8563 and oLC6927 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929 (5). The repair cassette, sgRNA, and Cas9 DNA were transformed into CaLC239. Transformants were selected by plating onto YNB SC plates lacking arginine. Lack of a wild-type allele was verified by PCR using oLC5367 and oLC5370.

CaLC6702: Both alleles of *TPK2* were deleted using a transient CRISPR approach adapted from Min and colleagues (4). The *CaCAS9* cassette was amplified from pLC963 using oLC6924 and oLC6925. The repair cassette was PCR amplified from pLC1100 using oLC8597 and oLC8571. The sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8568 (fragment A), as well as oLC8569 and oLC6927 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929 (5). The repair cassette, sgRNA, and Cas9 DNA were transformed into CaLC239. Transformants were selected by plating onto YNB SC plates lacking arginine. Lack of a wild-type allele was verified by PCR using oLC5371 and oLC5374.

CaLC6818: The *tpk1::pTPK1-GFP-TPK1-HIS1/tpk1::pTPK1-GFP-TPK1-SAT1* strain was constructed in two steps. First, the repair template was digested from pLC1372 using BamHI (NEB) and SacII (NEB) and transformed into CaLC6699 (*tpk1::ARG4/tpk1::ARG4*). Transformants were selected by plating on YNB SC plates lacking histidine. Reintroduction of a *GFP-TPK1* allele downstream of the native *TPK1* promoter was PCR tested using oLC814 and oLC5368. GFP-Tpk1 expression was confirmed by immunoblotting. This strain was then transformed using the repair template digested from pLC1402 using BamHI (NEB) and SacII (NEB). Transformants were selected by plating onto YNB SC plates lacking histidine and supplemented with 150 µg/mL NAT. Presence of two alleles of *GFP-TPK1* was confirmed by arginine auxotrophy and PCR confirmed using oLC814 and oLC6942. Anti-GFP immunoblotting confirmed these strains express more GFP-Tpk1 than the parental strain with one copy of *GFP-TPK1*.

CaLC6826: The *tpk2::pTPK2-GFP-TPK2-HIS1/tpk2::pTPK2-GFP-TPK2-SAT1* strain was constructed in two steps. First, the repair template was digested from pLC1375 using BamHI (NEB) and SacII (NEB) and transformed into CaLC6702 (*tpk2::ARG4/tpk2::ARG4*). Transformants were selected by plating on YNB SC plates lacking histidine. Reintroduction of a *GFP-TPK2* allele downstream of the native *TPK2* promoter was PCR tested using oLC1077 and oLC5372. GFP-Tpk2 expression was confirmed by immunoblotting. This strain was then transformed using the repair template digested from pLC1406 using BamHI (NEB) and SacII (NEB). Transformants were selected by plating onto YNB SC plates lacking histidine and supplemented with 150 µg/mL NAT. Presence of two alleles of *GFP-TPK2* was confirmed by arginine auxotrophy and PCR confirmed using oLC1077 and oLC6942. Anti-GFP immunoblotting confirmed these strains express more GFP-Tpk2 than the parental strain with one copy of *GFP-TPK2*.

CaLC6836: Both alleles of *TPK1* and *TPK2* were deleted using a transient CRISPR approach adapted from Min and colleagues (Min et al. 2016). The CaCAS9 cassette was amplified from pLC963 using oLC6924 and oLC6925. The *tpk1Δ::HYGB* cassette with homology to the *TPK1* locus was PCR amplified from pLC1211 using oLC8596 and oLC8565. The *tpk2Δ::HYGB* cassette with homology to the *TPK2* locus was PCR amplified from pLC1211 using oLC8597 and oLC8571. For *TPK1* the sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8562 (fragment A), as well as oLC6927 and oLC8563 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929. For *TPK2*, the sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8568 (fragment A), as well as oLC6927 and oLC8569 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929. The *tpk1Δ::HYGB* cassette, *tpk2Δ::HYGB* cassette, *TPK1* sgRNA, *TPK2* sgRNA and Cas9 DNA were transformed into CaLC239. Transformants were selected by plating onto YPD plates supplemented with 600 µg/mL hygromycin B. Colonies with homozygous deletion of both genes were identified by slow growth and white color. Lack of a wild-type *TPK1* allele was verified by PCR using oLC5367 and oLC5370. Downstream integration of the cassette was confirmed by PCR using oLC5377 and oLC1824. Lack of a wild-type *TPK2* allele was verified by PCR using oLC5371 and oLC5374. To check for correct integration, the entire *TPK2* locus was amplified using oLC5698 and oLC7318. The wild-type amplicon was 1729bp, whereas the *tpk2Δ::HYGB* amplicon was 2181bp.

CaLC6852: The *tpk1::pTPK1-GFP-TPK1-HIS1/tpk1::pTPK1-GFP-TPK1-SAT1 tpk2::ARG4/tpk2::ARG4* strain was constructed same as CaLC6702, except the *tpk2::ARG4* cassette was transformed into CaLC6818.

CaLC6860: The *tpk2::pTPK2-GFP-TPK2-HIS1/tpk2::pTPK2-GFP-TPK2-SAT1 tpk1::ARG4/tpk1::ARG4* strain was constructed same as CaLC6699 except the *tpk1::ARG4* cassette was transformed into CaLC6826.

CaLC6978: Both alleles of *BCY1* were C-terminally tagged using a transient CRISPR approach adapted from Min and colleagues (4). The CaCAS9 cassette was amplified from pLC963 using oLC6924 and oLC6925. The repair cassette was PCR amplified from pLC1206 using oLC8575 and oLC8576. The sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8573 (fragment A), as well as oLC8574 and oLC6927 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929 (5). The repair cassette, sgRNA, and Cas9 DNA were transformed into CaLC239. Transformants were selected by plating onto YNB SC plates lacking histidine. Lack of the native 3' region of *BCY1* was confirmed by PCR using oLC8577 and oLC8578. Expression of a GFP protein was confirmed by both immunoblot and microscopic analysis.

CaLC6985: To tag *VPH1* C-terminally with RFP, the repair cassette was amplified from pLC1215 using oLC9038 and oLC9039 and transformed into CaLC6818. Transformants were selected by plating on YPD plates supplemented with 600 µg/mL hygromycin B. Correct integration was tested by PCR using oLC9040 and oLC5951.

CaLC6987: This strain was constructed same as CaLC6985 except the repair cassette was transformed into CaLC6826.

CaLC6993: This strain was constructed same as CaLC6985 except the repair cassette was transformed into CaLC6978.

CaLC7700: Both alleles of *TPK1* were deleted using a transient CRISPR approach adapted from Min and colleagues (4). The CaCAS9 cassette was amplified from pLC963 using oLC6924 and oLC6925. The repair cassette was PCR amplified from pLC1087 using oLC8596 and oLC8565. The sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8562 (fragment A), as well as oLC8563 and oLC6927 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929 (5). The repair cassette, sgRNA, and Cas9 DNA were transformed into CaLC6993. Transformants were selected by plating onto YPD plates supplemented with 150 µg/mL NAT. Lack of a wild-type allele was verified by PCR using oLC5367 and oLC5370.

CaLC7702: Both alleles of *TPK2* were deleted using a transient CRISPR approach adapted from Min and colleagues (4). The CaCAS9 cassette was amplified from pLC963 using oLC6924 and oLC6925. The repair cassette was PCR amplified from pLC1087 using oLC8597 and oLC8571. The sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8568 (fragment A), as well as oLC8569 and oLC6927 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929 (5). The repair cassette, sgRNA, and Cas9 DNA were transformed into CaLC6993. Transformants were selected by plating onto YPD plates supplemented with 150 µg/mL NAT. Lack of a wild-type allele was verified by PCR using oLC5371 and oLC5374.

CaLC7878: The *tpk1::pTPK1-NLS-GFP-TPK1-HIS1/tpk1::pTPK1-NLS-GFP-TPK1-SAT1* strain was made in two steps. First, the repair template from pLC1527 was digested using BamHI (NEB) and SacII (NEB) and transformed into CaLC6699. Transformants were selected by plating on YNB SC plates lacking histidine. Reintroduction of one *NLS-GFP-TPK1* allele downstream of the native *TPK1* promoter was PCR tested using oLC814 and oLC5368. This strain was then transformed using the repair template digested from pLC1529 using BamHI (NEB) and SacII (NEB). Transformants were selected by plating onto YPD plates supplemented with 150 µg/mL NAT. Presence of two alleles of *NLS-GFP-TPK1* was confirmed by histidine prototrophy and arginine auxotrophy. This strain showed stronger GFP signal compared to the parental strain with only one *NLS-GFP-TPK1* allele as determined by flow cytometry.

CaLC7884: The *tpk2::pTPK1-NLS-GFP-TPK1-HIS1/tpk2::pTPK1-NLS-GFP-TPK1-SAT1* strain was made in two steps. First, the repair template from pLC1528 was digested using BamHI (NEB) and SacII (NEB) and transformed into CaLC6702. Transformants were selected by plating on YNB SC plates lacking histidine. Reintroduction of one *NLS-GFP-TPK2* allele downstream of the native *TPK2* promoter was PCR tested using oLC1077 and oLC5372. This strain was then transformed using the repair template digested from pLC1530 using BamHI (NEB) and SacII (NEB). Transformants were selected by plating onto YPD plates supplemented with 150 µg/mL NAT. Presence of two alleles of *NLS-GFP-TPK1* was confirmed by histidine prototrophy and arginine auxotrophy. This strain showed stronger GFP signal compared to the parental strain with only one *NLS-GFP-TPK1* allele as determined by flow cytometry.

CaLC8597: This strain was constructed same as CaLC7700 except the repair cassette was transformed into CaLC155.

CaLC8599: Both alleles *TPK2* were deleted using a transient CRISPR approach adapted from Min and colleagues (Min et al. 2016). The CaCAS9 cassette was amplified from pLC963 using oLC6924 and oLC6925. The *tpk2Δ::HYGB* cassette with homology to the *TPK2* locus was PCR amplified from pLC1211 using oLC8597 and oLC8571. For *TPK2*, the sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8568 (fragment A), as well as oLC6927 and oLC8569 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929. The repair cassette, sgRNA and Cas9 DNA were transformed into CaLC155. Transformants were selected by plating onto YPD plates supplemented with 600 µg/mL hygromycin B. Lack of a wild-type *TPK2* allele was verified by PCR using oLC5371 and oLC5374.

CaLC8601: Both alleles of *TPK1* and *TPK2* were deleted using a transient CRISPR approach adapted from Min and colleagues (Min et al. 2016). The CaCAS9 cassette was amplified from pLC963 using oLC6924 and oLC6925. The *tpk1Δ::SAT1* cassette with homology to the *TPK1* locus was PCR amplified from pLC1087 using oLC8596 and oLC8565. The *tpk2Δ::HYGB* cassette with homology to the *TPK2* locus was PCR amplified from pLC1211 using oLC8597 and oLC8571. For *TPK1* the sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8562 (fragment A), as well as oLC6927 and oLC8563 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929. For *TPK2*, the sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8568 (fragment A), as well as oLC6927 and oLC8569 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929. The *tpk1Δ::SAT1* cassette, *tpk2Δ::HYGB* cassette, *TPK1* sgRNA, *TPK2* sgRNA and Cas9 DNA were transformed into CaLC239. Transformants were selected by plating onto YPD plates supplemented with 150 µg/mL NAT and 600 µg/mL hygromycin B. Colonies with homozygous deletion of both genes were identified by slow growth and white color. Lack of a wild-type *TPK1* allele was verified by PCR using oLC5367 and oLC5370. Lack of a wild-type *TPK2* allele was verified by PCR using oLC5371 and oLC5374.

CaLC8867: The *tpk1::pTPK1-GFP-(TPK2N+TPK1C)-HIS1/tpk1::pTPK1-GFP-(TPK2N+TPK1C)-SAT1* strain was constructed in two steps. First, the repair template from pLC1668 was digested using BamHI (NEB) and SacII (NEB) and transformed into CaLC6699. Transformants were selected by plating on YNB SC plates lacking histidine. Reintroduction of one *GFP-(TPK2N+TPK1C)* hybrid allele downstream of the native *TPK1* promoter was PCR tested using oLC814 and oLC10761. This strain was then transformed using the repair template digested from pLC1682 using BamHI (NEB) and SacII (NEB). Transformants were selected by plating onto YNB SC plates lacking histidine and supplemented with 150 µg/mL NAT. Presence of two alleles of *GFP-(TPK2N+TPK1C)* was confirmed by arginine auxotrophy and PCR confirmed using oLC6943 and oLC8604. Anti-GFP immunoblotting and fluorescence microscopy confirmed that this strain expresses more GFP compared to the parental strain with only one *GFP-(TPK2N+TPK1C)* allele.

CaLC8869: The *tpk1::pTPK1-GFP-TPK2-HIS1/tpk1::pTPK1-GFP-TPK2-SAT1* strain was constructed in two steps. First, the repair template from pLC1669 was digested using BamHI (NEB) and SacII (NEB) and transformed into CaLC6699. Transformants were selected by plating on YNB SC plates lacking histidine. Reintroduction of one *GFP-TPK2* allele downstream of the native *TPK1* promoter was PCR tested using oLC814 and oLC10761. This strain was then transformed using the repair template digested from pLC1683 using BamHI (NEB) and SacII (NEB). Transformants were selected by plating onto YNB SC plates lacking histidine and supplemented with 150 µg/mL NAT. Presence of two alleles of *GFP-TPK2* was confirmed by arginine auxotrophy and PCR confirmed using oLC6943 and oLC8604. Anti-GFP immunoblotting and fluorescence microscopy confirmed that this strain expresses more GFP compared to the parental strain with only one *GFP-TPK2* allele.

CaLC8873: The *tpk1::pTPK1-GFP-(TPK1N+TPK2C)-HIS1/tpk1::pTPK1-GFP-(TPK1N+TPK2C)-SAT1* strain was constructed in two steps. First, the repair template from pLC1676 was digested using BamHI (NEB) and SacII (NEB) and transformed into CaLC6699. Transformants were selected by plating on YNB SC plates lacking histidine. Reintroduction of one *GFP-(TPK1N+TPK2C)* hybrid allele downstream of the native *TPK1* promoter was PCR tested using oLC814 and oLC10761. This strain was then transformed using the repair template digested from pLC1685 using BamHI (NEB) and SacII (NEB). Transformants were selected by plating onto YNB SC plates lacking histidine and supplemented with 150 µg/mL NAT. Presence of two alleles of *GFP-(TPK1N+TPK2C)* was confirmed by arginine auxotrophy and PCR confirmed using oLC6943 and oLC8604. Anti-GFP immunoblotting and fluorescence microscopy confirmed that this strain expresses more GFP compared to the parental strain with only one *GFP-(TPK1N+TPK2C)* allele.

CaLC8946: To tag *VPH1* C-terminally with mNeonGreen, the repair cassette was amplified from pLC1696 using oLC9038 and oLC9039 and transformed into CaLC239. Transformants were selected by plating on YPD plates supplemented with 600 µg/mL hygromycin B. Upstream integration of the cassette was confirmed by PCR using oLC9040 and oLC7958 and downstream integration was confirmed using oLC7959 and oLC9041.

CaLC8948: This strain was constructed same as CaLC8946 except the repair cassette was transformed into CaLC6699.

CaLC8950: This strain was constructed same as CaLC8946 except the repair cassette was transformed into CaLC6702.

CaLC8966: The *tpk2::pTPK2-GFP-(TPK1N+TPK2C)-HIS1/tpk2::pTPK2-GFP-(TPK1N+TPK2C)-SAT1 tpk1::HygB/tpk1::HygB* strain was constructed in three steps. First, the repair template from pLC1671 was digested using BamHI (NEB) and SacII (NEB) and transformed into CaLC6702. Transformants were selected by plating on YNB SC plates lacking histidine. Reintroduction of one *GFP-(TPK1N+TPK2C)* hybrid allele downstream of the native *TPK2* promoter was PCR tested using oLC10760 and oLC10761 for presence of GFP coding sequence and 3' integration was PCR confirmed using oLC6916 and oLC8572. This strain was then transformed using the repair template digested from pLC1686 using BamHI (NEB) and SacII (NEB). Transformants were selected by plating onto YNB SC plates lacking histidine and supplemented with 150 µg/mL NAT. Presence of two alleles of *GFP-(TPK1N+TPK2C)* was confirmed by arginine auxotrophy and PCR confirmed using oLC6943 and oLC8572 for 3' integration. Anti-GFP immunoblotting and fluorescence microscopy confirmed that this strain expresses more GFP compared to the parental strain with only one *GFP-(TPK1N+TPK2C)* allele.

Next, both alleles of *TPK1* were deleted using a transient CRISPR approach adapted from Min and colleagues (Min et al. 2016). The CaCAS9 cassette was amplified from pLC963 using oLC6924 and oLC6925. The repair cassette was PCR amplified from pLC1211 using oLC8596 and oLC8565. The sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8562 (fragment A), as well as oLC6927 and oLC8563 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929. The repair cassette, sgRNA, and Cas9 DNA were transformed into CaLC239. Transformants were selected by plating onto YPD plates supplemented with 600 µg/mL hygromycin B. Lack of a wild-type *TPK1* allele was verified by PCR using oLC5367 and oLC5370.

CaLC8968: The *tpk2::pTPK2-GFP-TPK1-HIS1/tpk2::pTPK2-GFP-TPK1-SAT1 tpk1::HygB/tpk1::HygB* strain was constructed in three steps. First, the repair template from pLC1672 was digested using BamHI (NEB) and SacII (NEB) and transformed into CaLC6702. Transformants were selected by plating on YNB SC plates lacking histidine. Reintroduction of one *GFP-TPK1* allele downstream of the native *TPK2* promoter was PCR tested using oLC10760 and oLC10761 for presence of GFP coding sequence and 3' integration was PCR confirmed using oLC6916 and oLC8572. This strain was then transformed using the repair template digested from pLC1687 using BamHI (NEB) and SacII (NEB). Transformants were selected by plating onto YNB SC plates lacking histidine and supplemented with 150 µg/mL NAT. Presence of two alleles of *GFP-TPK1* was confirmed by arginine auxotrophy and PCR confirmed using oLC6943 and oLC8572 for 3' integration. Anti-GFP immunoblotting and fluorescence microscopy confirmed that this strain expresses more GFP compared to the parental strain with only one *GFP-TPK1* allele. Both alleles of *TPK1* were subsequently deleted as described for CaLC8966.

CaLC8972: The *tpk2::pTPK2-GFP-(TPK2N+TPK1C)-HIS1/tpk2::pTPK2-GFP-(TPK2N+TPK1C)-SAT1 tpk1::HygB/tpk1::HygB* strain was constructed in three steps. First, the repair template from pLC1677 was digested using BamHI (NEB) and SacII (NEB) and transformed into CaLC6702. Transformants were selected by plating on YNB SC plates lacking histidine. Reintroduction of one *GFP-(TPK2N+TPK1C)* hybrid allele downstream of the native *TPK2* promoter was PCR tested using oLC10760 and oLC10761 for presence of the GFP coding sequence and 3' integration was PCR confirmed using oLC6916 and oLC8572. This strain was then transformed using the repair template digested from pLC1689 using BamHI (NEB) and SacII (NEB). Transformants were selected by plating onto YNB SC plates lacking histidine and supplemented with 150 µg/mL NAT. Presence of two alleles of *GFP-(TPK2N+TPK1C)* was confirmed by arginine auxotrophy and PCR confirmed using oLC6943 and oLC8572 for 3' integration. Anti-GFP immunoblotting and fluorescence microscopy confirmed that this strain expresses more GFP compared to the parental strain with only one *GFP-(TPK2N+TPK1C)* allele. Both alleles of *TPK1* were subsequently deleted as described for CaLC8966.

CaLC8995: Both alleles of *TPK2* were C-terminally tagged using a transient CRISPR approach adapted from Min and colleagues (4). The CaCAS9 cassette was amplified from pLC963 using oLC6924 and oLC6925. The repair cassette was PCR amplified from pLC1214 using oLC8570 and oLC8571. The sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8568 (fragment A), as well as oLC8569 and oLC6927 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929 (5). The repair cassette, sgRNA, and Cas9 DNA were transformed into CaLC6699. Transformants were selected by plating onto YPD plates supplemented with 600 µg/mL hygromycin B. Lack of the native 3' region of *TPK2* was confirmed by PCR using oLC747 and oLC8572.

CaLC8997: Both alleles of *TPK1* were C-terminally tagged using a transient CRISPR approach adapted from Min and colleagues (4). The CaCAS9 cassette was amplified from pLC963 using oLC6924 and oLC6925. The repair cassette was PCR amplified from pLC1214 using oLC8564 and oLC8565. The sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8562 (fragment A), as well as oLC8563 and oLC6927 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929 (5). The repair cassette, sgRNA, and Cas9 DNA were transformed into CaLC6702. Transformants were selected by plating onto YPD plates supplemented with

600 µg/mL hygromycin B. Lack of the native 3' region of *TPK1* was confirmed by PCR using oLC8566 and oLC8567.

CaLC9013: The *tpk1::pTPK1-mNG-TPK1-HIS1/tpk1::pTPK1-mNG-TPK1-SAT1* strain was constructed in two steps. First, the repair template was digested from pLC1699 using BamHI (NEB) and SacII (NEB) and transformed into CaLC6699. Transformants were selected by plating on YNB SC plates lacking histidine. Reintroduction of a *mNG-TPK1* allele downstream of the native *TPK1* promoter was PCR tested using oLC6916 and oLC8604 for 3' integration. The presence of *TPK1* orf was confirmed by PCR using oLC5367 and oLC5370. This strain was then transformed using the repair template digested from pLC1708 using BamHI (NEB) and SacII (NEB). Transformants were selected by plating onto YNB SC plates lacking histidine and supplemented with 150 µg/mL NAT. Presence of two alleles of *mNG-TPK1* was confirmed by arginine auxotrophy and PCR confirmed using oLC6943 and oLC8604 for 3' integration. Upstream integration was confirmed using oLC8566 and oLC6942.

CaLC9015: The *tpk2::pTPK2-mNG-TPK2-HIS1/tpk2::pTPK2-mNG-TPK2-SAT1* strain was constructed in two steps. First, the repair template was digested from pLC1700 using BamHI (NEB) and SacII (NEB) and transformed into CaLC6702. Transformants were selected by plating on YNB SC plates lacking histidine. Reintroduction of a *mNG-TPK2* allele downstream of the native *TPK2* promoter was PCR tested using oLC6916 and oLC8572 for 3' integration. The presence of *TPK2* orf was confirmed by PCR using oLC5371 and oLC5374. This strain was then transformed using the repair template digested from pLC1709 using BamHI (NEB) and SacII (NEB). Transformants were selected by plating onto YNB SC plates lacking histidine and supplemented with 150 µg/mL NAT. Presence of two alleles of *mNG-TPK2* was confirmed by arginine auxotrophy and PCR confirmed using oLC6943 and oLC8572 for 3' integration. Upstream integration was confirmed using oLC747 and oLC6942.

CaLC9049: The *tpk2::pTPK2-GFP-TPK2-HIS1/tpk2::pTPK2-GFP-TPK2-HIS1 VPH1-mSC-HygB/VPH1* strain was constructed in three steps. First, to tag *VPH1* C-terminally with mScarlet, the targeting cassette was amplified from pLC1696 using oLC9038 and oLC9039 and transformed into CaLC239. Transformants were selected by plating on YPD plates supplemented with 600 µg/mL hygromycin B. Upstream integration of the cassette was confirmed by PCR using oLC9040 and oLC7958 and downstream integration was confirmed using oLC7959 and oLC9041.

Next, both alleles of *TPK2* were deleted using a transient CRISPR approach adapted from Min and colleagues (4). The CaCAS9 cassette was amplified from pLC963 using oLC6924 and oLC6925. The repair cassette was PCR amplified from pLC1082 using oLC11383 and oLC11384. The sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8568 (fragment A), as well as oLC8569 and oLC6927 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929 (5). The repair cassette, sgRNA, and Cas9 DNA were transformed into the strain above. Transformants were selected by plating onto YPD plates supplemented with 150 µg/mL NAT. Lack of a wild-type allele was verified by PCR using oLC5371 and oLC5374.

Finally, the two copies of *GFP-TPK2* allele was introduced using a transient CRISPR approach adapted from Min and colleagues (4). The CaCAS9 cassette was amplified from pLC963 using oLC6924 and oLC6925. The repair template was digested from pLC1375 using BamHI (NEB) and SacII (NEB). The sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8823 (fragment A), as well as oLC8824 and oLC6927 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929 (5). Transformants were selected by plating onto YNB SC plates lacking histidine. Presence of two alleles of *GFP-TPK2* was confirmed by NAT sensitivity. Expression of the GFP-TPK2 fusion protein was confirmed by anti-GFP immunoblotting.

CaLC9051: This strain was constructed same as CaLC9049 except in the final step the repair cassette containing the N-terminus of TPK2 (*GFP-TPK2NΔC*) was digested from pLC1673 using BamHI (NEB) and SacII (NEB) for transformation.

CaLC9053: This strain was constructed same as CaLC9049 except in the final step the repair cassette containing an extra translation stop codon just upstream of the start codon (*STOP-GFP-TPK2*) was digested from pLC1717 using BamHI (NEB) and SacII (NEB) for transformation.

CaLC9055: This strain was constructed same as CaLC9049 except in the final step the repair cassette containing the N-terminus of TPK2 with an extra translation stop codon just upstream of the start codon (*STOP-GFP-TPK2NΔC*) was digested from pLC1718 using BamHI (NEB) and SacII (NEB) for transformation.

CaLC9057: This strain was constructed same as CaLC9049 except in the final step the repair cassette containing the N-terminus of *TPK2* lacking its N-terminal poly glutamine PolyQ tract (*GFP-TPK2NΔC^{ΔQ}*) was digested from pLC1719 using BamHI (NEB) and SacII (NEB) for transformation.

CaLC9063: Both alleles of *TPK1* were deleted using a transient CRISPR approach adapted from Min and colleagues (4). The *CaCAS9* cassette was amplified from pLC963 using oLC6924 and oLC6925. The repair cassette was PCR amplified from pLC1082 using oLC11381 and oLC11382. The sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8562 (fragment A), as well as oLC8563 and oLC6927 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929 (5). The repair cassette, sgRNA, and Cas9 DNA were transformed into CaLC9049. Transformants were selected by plating onto YPD plates supplemented with 150 μg/mL NAT. Lack of a wild-type allele was verified by PCR using oLC5367 and oLC5370.

CaLC9103: Both alleles of *VPH1* were deleted using a transient CRISPR approach adapted from Min and colleagues (4). The *CaCAS9* cassette was amplified from pLC963 using oLC6924 and oLC6925. The repair cassette was PCR amplified from pLC1211 using oLC11403 and oLC11404. The sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC11405 (fragment A), as well as oLC11406 and oLC6927 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929 (5). The repair cassette, sgRNA, and Cas9 DNA were transformed into CaLC6118. Transformants were selected by plating onto YPD plates supplemented with 600 μg/mL hygromycin B. Lack of a wild-type allele was verified by PCR using oLC11407 and oLC11408.

CaLC9154: Both alleles of *TPK1* were deleted as CaLC8966 except the repair cassette was transformed into CaLC6118.

CaLC9156: This strain was constructed same as CaLC8599 except the repair cassette was transformed into CaLC6118.

CaLC9158: This strain was constructed same as CaLC6836 except the repair cassettes was transformed into CaLC6118.

CaLC9458: To tag *NAB2* C-terminally with mScarlet, the targeting cassette was amplified from pLC1697 using oLC9085 and oLC9086 and transformed into CaLC9013. Transformants were selected by plating on YPD plates supplemented with 600 μg/mL hygromycin B. Upstream integration of the cassette was confirmed by PCR using oLC9087 and oLC11300.

CaLC9460: This strain was constructed same as CaLC9048 except the repair cassette was transformed into CaLC9015.

CaLC9478: Both alleles of *TPK1* and *TPK2* were deleted using a transient CRISPR approach adapted from Min and colleagues (Min et al. 2016). The *CaCAS9* cassette was amplified from pLC963 using oLC6924 and oLC6925. The *tpk1Δ::SAT1* cassette with homology to the *TPK1* locus was PCR amplified from

pLC1087 using oLC8596 and oLC8565. The *tpk2Δ::SAT1* cassette with homology to the *TPK2* locus was PCR amplified from pLC1087 using oLC8597 and oLC8571. For *TPK1* the sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8562 (fragment A), as well as oLC6927 and oLC8563 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929. For *TPK2*, the sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8568 (fragment A), as well as oLC6927 and oLC8569 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929. The *tpk1Δ::SAT1* cassette, *tpk2Δ::SAT1* cassette, *TPK1* sgRNA, *TPK2* sgRNA and Cas9 DNA were transformed into CaLC6118. Transformants were selected by plating onto YPD plates supplemented with 150 µg/mL NAT. Colonies with homozygous deletion of both genes were identified by slow growth and white color. Lack of a wild-type *TPK1* allele was verified by PCR using oLC5367 and oLC5370. Lack of a wild-type *TPK2* allele was verified by PCR using oLC5371 and oLC5374.

CaLC10330: To tag *PAB1* C-terminally with mScarlet, the targeting cassette was amplified from pLC1693 using oLC12941 and oLC12942 and transformed into CaLC9013. Transformants were selected by plating on YNB SC plates lacking arginine. Upstream integration of the cassette was confirmed by PCR using oLC4434 and oLC6971 and downstream integration was confirmed using oLC6970 and oLC4435.

CaLC10332: This strain was constructed same as CaLC10330 except the repair cassette was transformed into CaLC9015.

CaLC10334: This strain was constructed same as CaLC10330 except the repair cassette was transformed into CaLC6978.

CaLC10371: Both alleles of *DHH1* was C-terminally with mScarlet in two steps. First, the targeting cassette was amplified from pLC1693 using oLC12943 and oLC12593 and transformed into CaLC9013. Transformants were selected by plating on YNB SC plates lacking arginine. Upstream integration of the cassette was confirmed by PCR using oLC6526 and oLC6971 and downstream integration was confirmed using oLC6970 and oLC6527. This strain was subsequently transformed using the repair template amplified from pLC1697 using oLC12943 and oLC12593. Transformants were selected by plating on YNB SC plates lacking arginine and supplemented with 600 µg/mL hygromycin B. Upstream integration of the cassette was confirmed by PCR using oLC6526 and oLC7958 and downstream integration was confirmed using oLC7959 and oLC6527. Lack of the native 3' region of *DHH1* was confirmed by PCR using oLC6526 and oLC6527.

CaLC10373: This strain was constructed same as CaLC10371 except the repair cassette was transformed into CaLC9015.

CaLC10375: This strain was constructed same as CaLC10371 except the repair cassette was transformed into CaLC6978.

CaLC10445: Both alleles of *TPK2* were deleted as CaLC8599 except the repair cassette was transformed into CaLC9013.

CaLC10447: Both alleles of *TPK1* were deleted as CaLC8966 except the repair cassette was transformed into CaLC9015.

CaLC10572: The *tpk1::pTPK1-NLS-TPK1-HIS1/tpk1::pTPK1-NLS-TPK1-HIS1* strain was made using two steps. First, both alleles of *TPK1* were deleted as CaLC9063 except the repair cassette was transformed into CaLC239. Next, the two copies of *NLS-TPK1* allele was introduced using a transient a CRISPR approach adapted from Min and colleagues (4). The *CaCAS9* cassette was amplified from pLC963 using

oLC6924 and oLC6925. The repair template was digested from pLC2041 using BamHI (NEB) and SacII (NEB). The sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8823 (fragment A), as well as oLC8824 and oLC6927 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929 (5). The repair cassette, sgRNA, and Cas9 DNA were transformed into the *TPK1* deletion strain. Transformants were selected by plating onto YNB SC plates lacking histidine. Presence of two alleles of *NLS-TPK1* was confirmed by NAT sensitivity.

CaLC10575: The *tpk2::pTPK2-NLS-TPK2-HIS1/tpk2::pTPK2-NLS-TPK2-HIS1* strain was made using two steps. First, both alleles of *TPK2* were deleted as described for CaLC9049 except the repair cassette was transformed into CaLC239. Next, the two copies of *NLS-TPK2* allele was introduced using a transient a CRISPR approach adapted from Min and colleagues (4). The *CaCAS9* cassette was amplified from pLC963 using oLC6924 and oLC6925. The repair template was digested from pLC2042 using BamHI (NEB) and SacII (NEB). The sgRNA fusion cassette was made by PCR amplifying from pLC1081 with oLC6926 and oLC8823 (fragment A), as well as oLC8824 and oLC6927 (fragment B), at which point fusion PCR was performed on the fragments using the nested primers oLC6928 and oLC6929 (5). The repair cassette, sgRNA, and Cas9 DNA were transformed into the *TPK2* deletion strain. Transformants were selected by plating onto YNB SC plates lacking histidine. Presence of two alleles of *NLS-TPK2* was confirmed by NAT sensitivity.

Plasmid sequences:

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TPK1 promoter; GFP+GGGlinker; TPK1 ORF; HIS1 marker (replaced with a SAT1 marker in pLC1402); TPK1 3' HR

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TPK2 promoter; GFP+GGGlinker; TPK2 ORF; HIS1 marker (replaced with a SAT1 marker in pLC1406); TPK2 3' HR

>pLC1527_pFA-NLS-GFP-TPK1-HIS

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TPK1 promoter; SV40 NLS; GFP+GGGGlinker; *TPK1* ORF; *HIS1* marker (replaced with a *SAT1* marker in pLC1529); *TPK1* 3' HR

> pLC1528_pFA-NLS-GFP-TPK2-HIS

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TPK2 promoter; SV40 NLS; GFP+GGGGlinker; *TPK2* ORF; *HIS1* marker (replaced with a *SAT1* marker in pLC1530); *TPK2* 3' HR

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TPK1 promoter; GFP+GGGlinker; TPK2N+TPK1C coding sequence; HIS1 marker (replaced with a SAT1 marker in pLC1682); TPK1 3' HR

[illegible]

>pLC1676_pFA-pTpk1-GFP-1N2C-HIS

[illegible]

TPK1 promoter; GFP+GGGglinker; TPK1N+TPK2C coding sequence; HIS1 marker (replaced with a SAT1 marker in pLC1685); TPK1 3' HR

>pLC1673_pFA-pTpk2-GFP-2N-HIS

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TPK2 promoter: GFP+GGGLinker: TPK2N coding sequence: HIS1 marker: TPK2 3' HR

>pLC1671_pFA-pTpK2-GFP-1N2C-HIS

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TPK2 promoter; GFP+GGGlinker; TPK1N+TPK2C coding sequence; HIS1 marker (replaced with a SAT1 marker in pLC1686);
TPK2 3' HR

>pLC1672_pFA-pTpk2-GFP-1FL-HIS

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TPK2 promoter; GFP+GGGGlinker; TPK1 coding sequence; HIS1 marker (replaced with a SAT1 marker in pLC1687); TPK2 3' HR

>pLC1677_pFA-pTpK2-GFP-2N1C-HIS

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TPK2 promoter; GFP+GGGlinker; TPK2N+TPK1C coding sequence; HIS1 marker (replaced with a SAT1 marker in pLC1689);
TPK2 3' HR

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TPK2 promoter; Stop codon; GFP+GGGlinker; TPK2 ORF; HIS1 marker; TPK2 3' HR

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TPK2 promoter; Stop codon; GFP+GGGlinker; TPK2N coding sequence; HIS1 marker; TPK2 3' HR

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TPK2 promoter; GFP+GGGlinker; TPK2N^{4Q} coding sequence; HIS1 marker; TPK2 3' HR

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Linker+mNeonGreen; *HygB^R* marker

[illegible]

[illegible]

TPK1 promoter; SV40 NLS+linker; TPK1 ORF; HIS1 marker; TPK1 3' HR

>pLC2042_pFA-NLS-TPK2-HIS

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TPK2 promoter; SV40 NLS+linker; TPK2 ORF; HIS1 marker; TPK2 3' HR

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