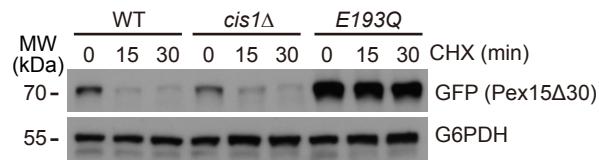


## **Appendix files:**

1. [\*\*Appendix Figure S1.\*\* Cis1 is not required for GFP-Pex15 \$\Delta\$ 30 degradation.](#) (Page 2)
2. [\*\*Appendix Figure S2.\*\* Thermo-stability and sensitivity to limited trypsin digestion of Msp1 and its N-domain mutants.](#) (Page 3)
3. [\*\*Appendix Table S1.\*\* List of non-mitochondrial TA proteins.](#) (Page 4-5)
4. [\*\*Appendix Table S2.\*\* Yeast strains used and generated in this study.](#) (Page 6-14)
5. [\*\*Appendix Table S3.\*\* Plasmids used in this study.](#) (Page 15-17)

## Appendix Figure S1

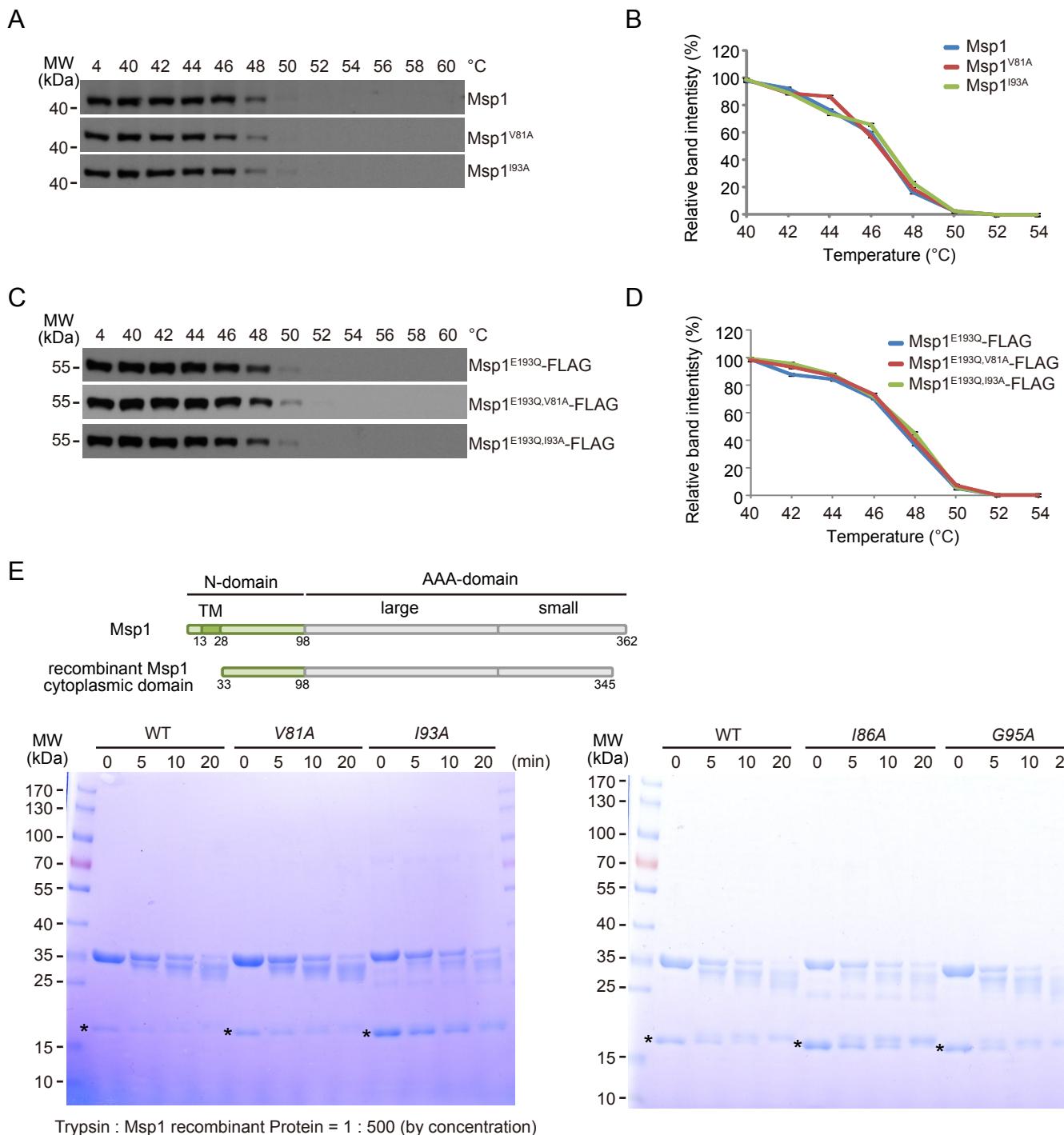


Appendix Figure S1. Cis1 is not required for GFP-Pex15 $\Delta$ 30 degradation.

GFP-Pex15 $\Delta$ 30 was expressed in the indicated strains from a centromeric plasmid under the control of TEF1 promoter.

Cells were grown in SCD media to log phase, treated with CHX and collected at the indicated time points.

## Appendix Figure S2



Appendix Figure S2. Thermo-stability and sensitivity to limited trypsin digestion of Msp1 and its N-domain mutants.

(A-D) In (A) and (C), cell extracts were heated at indicated temperatures for 3 minutes and centrifuged to collect supernatants. Equal volume of supernatants was loaded for western blot analysis. In (B) and (D), band intensities were measured by ImageJ and plotted against temperature. Data values represent means  $\pm$  SEM from three independent experiments.

(E) Recombinant proteins of Msp1 cytoplasmic domain and its indicated mutants were digested with 1/500 (by concentration) trypsin at 37 °C for the indicated time. About 15  $\mu$ g recombinant proteins were loaded for SDS-PAGE and Coomassie Blue Staining. Asterisks indicate contaminant proteins.

Table S1 Non-mitochondrial TA proteins

Protein	Localization in WT cells	mitochondrial localization in get3Δmsp1Δ cells	TM gravy	Tail charge	TM segment ( <b>bold</b> ) and C-terminal tail ( <b>red</b> ) sequence
Pex15	peroxisome	Yes	1.7	5.4	<b>VLNKNGLLLTGLLLLCLKKYKSLMAIFKHVPAAFHTVYPQIVGLLKLLASI</b>
Frt1	ER	Yes	2.3	2.9	<b>YFIIDIIAFLLMGGFIVVVKNLLTRFFTR</b>
Sbh1	ER	no	2.8	1.9	<b>VVLFLAVGFIFSVALHVISKVAGKLF</b>
Bos1	ER,Golgi	no	2.8	1.9	<b>LVFWIALILLIIGIYYVLKWLR</b>
Vti1	Golgi-vacuole	no	3.2	1.9	<b>FISYIAHAVLILLILLVLFSKFK</b>
Sso1	plasma membrane	no	3.4	1.9	<b>WLIVFAIHVVVVVVVVPAVKTR</b>
Pgc1	ER	no	1.8	1.1	<b>VHIKLCGWSIAYVIFLFLRTIHFL</b>
Frt2	ER	no	2.6	1.1	<b>IVFLIIIIVICYTFKHLVSHK</b>
Sbh2	ER	no	2.7	1.1	<b>LVVLFLSVGIFSVIALHLLTKFTHII</b>
Sec22	ER	no	2	0.9	<b>ISQYAPIVIVAFFVFLFWWIFLK</b>
Sft1	Golgi	no	2	0.9	<b>SIWRMVGGLALLIFFILYTLFKLF</b>
Prm3	nuclear envelope	no	1.1	0.9	<b>YQGAIFGSFLGAAVTTVLSNLAVKALQN</b>
Kar1	nuclear envelope	no	1.7	0.9	<b>YFLWTICILILLYCNIYVYYRF</b>
YEL073C		no	0.7	0.9	<b>TSGYYAFSTVVVPVLMGNMKVA</b>
YCL007C		no	1.8	0.9	<b>YKLLITLCSSLFVGPLFLKV</b>
Sss1	ER	Yes	1.9	0.9	<b>AVGIGFIAVGHIIGYAIKLIHIPIRYVIV</b>
Ubc6	ER	Yes	2.5	0.9	<b>SMVYIGIAIFLFLVGGLFMK</b>
Vps64	ER	Yes	2.2	0.9	<b>GMLFGVVAISFGLVATAVKQLPQ</b>
Far10	ER and punctate	Yes	2.3	0.9	<b>FTLLTFGTISIGIIAIVFKILSPN</b>
Phm6	vacuole	no	2.8	0.9	<b>LIVVIVLLLYSLTMVGLFYVMTMTKFLF</b>
Scs2	ER	no	2.9	0.9	<b>MGIFILVALLLILVLGWFYR</b>
Snc1	plasma membrane & vesicle	no	3.1	0.9	<b>MCLALVIIILVVIHPVIAVHFSR</b>
Pep12	Golgi-vacuole	no	3.5	0.9	<b>VYLLIVLLVMLLFIFLI</b> <b>MKL</b>
Sso2	plasma membrane	no	3.3	0.9	<b>CLIICFIIFAIIVVVVVVPSV</b> <b>VETRK</b>
Dpm1	ER	no	1.8	0.1	<b>LILFITFWISLFFYVCYQLY</b> <b>HLVF</b>

Table S1 Non-mitochondrial TA proteins (continued)

Protein	Localization in WT cells	mitochondrial localization in get3Δmsp1Δ cells	TM gravy	Tail charge	TM segment ( <b>bold</b> ) and C-terminal tail ( <b>red</b> ) sequence
Snc2	plasma membrane & vesicle	no	3.7	0.1	<b>CLFLVVHLLVVHVPIVVHFS</b>
Gos1	Golgi	<b>Yes</b>	<b>2.4</b>	<b>-0.1</b>	<b>AFVLATITTL</b> CILFLFFT <b>W</b>
Sps2	plasma membrane	<b>Yes</b>	<b>1.9</b>	<b>-0.1</b>	<b>IFIDAFKMSVYAVFTVLFSIIF</b>
Ysy6	ER	<b>Yes</b>	<b>2.1</b>	<b>-0.1</b>	<b>WLGILLFLLVGGGVQLQI</b> S <b>YIL</b>
Hlj1	ER	no	3.3	-0.1	<b>MLVLFII</b> FIVLP <b>M</b> I <b>KDYLFS</b>
YDL241w		no	1.3	-0.1	<b>SHSLAKFTI</b> L <b>FMLVLYTIV</b>
YJL119c		no	1.8	-0.1	<b>AITVFFVVFLYINKASVKFFFFFA</b>
Bet1	ER,Golgi	no	1.9	-0.1	<b>GISIKTWL</b> I <b>FFF</b> MVG <b>VGLFFWW</b> WIT
YEL010w		no	1.9	-0.1	<b>LSSFSI</b> LSA <b>VFPDVLF</b> TFLFS
YBR016w	plasma membrane	no	2	-0.1	<b>GCLAACLAALCICCTMDMLF</b>
Nyv1	Golgi-vacuole	no	2.1	-0.1	<b>TLLTFTIILFVSAAFMFY</b> <b>LW</b>
Cyb5	ER	no	2.4	-1.1	<b>GTLVVLAILMLGVAYYLL</b> <b>NE</b>
Use1	ER	no	2.5	-0.1	<b>SYLFYITV</b> FIFM <b>I</b> LG <b>VFTFI</b> III <b>QLFPAL</b>
Sed5	Golgi	no	2.7	-0.1	<b>WLAAKVFFHFVFFVIWVLV</b> N
Tlg1	Golgi	no	3.6	-0.1	<b>CIGLLIVVLIVLLVLAFIA</b>
Syn8	Golgi-vacuole	no	3.6	-0.1	<b>NCVIIHLIVV</b> LLL <b>LLVL</b>
Vam3	vacuole	no	3.6	-0.1	<b>VT</b> LI <b>III</b> VVC <b>MV</b> V <b>LLA</b> V <b>LS</b>
YBL100c	ER	no	1.8	-2.1	<b>AAVRANII</b> C <b>AFFY</b> LC <b>Y</b> <b>CSCDSYES</b>
Sec20	ER	no	1.9	-2.8	<b>LWLLFKFFKG</b> I <b>LVT</b> GL <b>V</b> K <b>SYAGSSSSLQAPS</b> L <b>VLNAPI</b> AT <b>TTTSSATS</b> VEP <b>FASV</b> SAV <b>SSI</b> <b>QRAVDEA</b> V <b>DRIVSHDEL</b>
Ufe1	ER	no	2.1	-1.1	<b>TTYGA</b> I <b>HMGV</b> F <b>ILFL</b> D <b>YVG</b>
Csm4	ER	no	2.5	-1.1	<b>LNSLII</b> V <b>FFISL</b> V <b>FLWV</b> S <b>IEV</b>
Tlg2	Golgi	no	3.2	-8.3	<b>V</b> LLL <b>TLCVIALFFFV</b> ML <b>KPHGGGSGGRNNGSNK</b> YNND <b>DNKTVNN</b> SHDDGSNTHINDEES <b>NLPSIVE</b> VT <b>ESEN</b> DAL <b>DDLL</b>
YML036w		ND	ND	ND	No TM predicted
YDL012c	plasma membrane	ND	ND	ND	No TM predicted

Table S2 Yeast Strains Used and Generated in This Study

Strains generated in the background of BY4741

NO.	Genotype
1	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::kanMX6</i>
2	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::MSP1-FLAG::LEU2</i>
3	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E193Q)-FLAG::LEU2</i>
4	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 cdc48::ADH<sup>P</sup>-FLAG-CDC48::LEU2</i>
5	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 cdc48::ADH<sup>P</sup>-FLAG-cdc48-3 (cdc48-P257L,R387K)::LEU2,kanMX6</i>
6	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
7	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E193Q)::LEU2</i>
8	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1<sup>TM</sup> (ΔI-32)::LEU2</i>
9	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (L122D,L123D)-FLAG::LEU2</i>
10	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (K8A)-FLAG::LEU2</i>
11	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (T9A)-FLAG::LEU2</i>
12	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (I10A)-FLAG::LEU2</i>
13	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (T11A)-FLAG::LEU2</i>
14	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (D12T)-FLAG::LEU2</i>
15	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (L13A,S14A)-FLAG::LEU2</i>
16	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (L16A)-FLAG::LEU2</i>
17	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (V17A,G18A)-FLAG::LEU2</i>
18	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (T19A)-FLAG::LEU2</i>
19	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (I21A,S22A)-FLAG::LEU2</i>
20	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (L23A)-FLAG::LEU2</i>
21	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (Y24A)-FLAG::LEU2</i>
22	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (V27A)-FLAG::LEU2</i>
23	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (R29A,N32A)-FLAG::LEU2</i>
24	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (L304,L31A)-FLAG::LEU2</i>
25	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (V34A,E35A)-FLAG::LEU2</i>
26	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (V67A)-FLAG::LEU2</i>
27	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (L69A)-FLAG::LEU2</i>
28	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (Y72A)-FLAG::LEU2</i>
29	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E73A)-FLAG::LEU2</i>

Table S2 Yeast Strains Used and Generated in This Study (Continued)

Strains generated in the background of BY4741

NO.	Genotype
30	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (I76A)-FLAG::LEU2</i>
31	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (L77A)-FLAG::LEU2</i>
32	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (I80A)-FLAG::LEU2</i>
33	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (V81A)-FLAG::LEU2</i>
34	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (I86A)-FLAG::LEU2</i>
35	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (I88A)-FLAG::LEU2</i>
36	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (F90A)-FLAG::LEU2</i>
37	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (D92A)-FLAG::LEU2</i>
38	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (I93A)-FLAG::LEU2</i>
39	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (G94A)-FLAG::LEU2</i>
40	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (G95A)-FLAG::LEU2</i>
41	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (L96A)-FLAG::LEU2</i>
42	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (D97A)-FLAG::LEU2</i>
43	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E193Q, L122D,L123D)-FLAG::LEU2</i>
44	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E193Q, D12T)-FLAG::LEU2</i>
45	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E193Q, L69A)-FLAG::LEU2</i>
46	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E193Q, Y72A)-FLAG::LEU2</i>
47	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E193Q, E73A)-FLAG::LEU2</i>
48	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E193Q, L77A)-FLAG::LEU2</i>
49	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E193Q, V81A)-FLAG::LEU2</i>
50	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E193Q, I86A)-FLAG::LEU2</i>
51	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E193Q, I93A)-FLAG::LEU2</i>
52	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E193Q, G94A)-FLAG::LEU2</i>
53	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E193Q, G95A)-FLAG::LEU2</i>
54	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E193Q, L96A)-FLAG::LEU2</i>
55	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::MSP1-GFP::LEU2 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
56	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (L122D,L123D)-GFP::LEU2 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
57	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (D12T)-GFP::LEU2 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
58	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (L69A)-GFP::LEU2 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>

Table S2 Yeast Strains Used and Generated in This Study (Continued)

Strains generated in the background of BY4741

NO.	Genotype
59	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (E73A)-GFP::LEU2 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
60	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (V81A)-GFP::LEU2 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
61	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (Y72A)-GFP::LEU2 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
62	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (L77A)-GFP::LEU2 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
63	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (I86A)-GFP::LEU2 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
64	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (I93A)-GFP::LEU2 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
65	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (G94A)-GFP::LEU2 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
66	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (G95A)-GFP::LEU2 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
67	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (L96A)-GFP::LEU2 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
68	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1<sup>TOM70(N)</sup> (TOM70(1-32)-MSP1(33-362))-GFP::LEU2 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
69	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::MSP1-GFP::LEU2</i>
70	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (L122D,L123D)-GFP::LEU2</i>
71	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1<sup>TOM70(N)</sup> (TOM70(1-32)-MSP1(33-362))-GFP::LEU2</i>
72	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::kanMX6</i>
73	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::kanMX6 get3::hphNT1</i>
74	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::MSP1-GFP::LEU2</i>
75	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (L122D,L123D)-GFP::LEU2</i>
76	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1<sup>TOM70(N)</sup> (TOM70(1-32)-MSP1(33-362))-GFP::LEU2</i>
77	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 his3::PEX15<sup>P</sup>-GFP-PEX15::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
78	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 his3::PEX15<sup>P</sup>-GFP-PEX15::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
79	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::SpHIS5 his3::PEX15<sup>P</sup>-GFP-PEX15::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
80	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::SpHIS5 his3::PEX15<sup>P</sup>-GFP-PEX15::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
81	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::MSP1-GFP::LEU2</i>
82	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (L122D,L123D)-GFP::LEU2</i>
83	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (D12T)-GFP::LEU2</i>
84	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (L69A)-GFP::LEU2</i>
85	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (E73A)-GFP::LEU2</i>
86	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (V81A)-GFP::LEU2</i>
87	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (Y72A)-GFP::LEU2</i>

Table S2 Yeast Strains Used and Generated in This Study (Continued)

Strains generated in the background of BY4741

NO.	Genotype
88	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (L77A)-GFP::LEU2</i>
89	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (I86A)-GFP::LEU2</i>
90	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (I93A)-GFP::LEU2</i>
91	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (G94A)-GFP::LEU2</i>
92	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (G95A)-GFP::LEU2</i>
93	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (L96A)-GFP::LEU2</i>
94	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
95	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
96	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::SpHIS5 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
97	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::SpHIS5 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
98	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 his3::UBC6<sup>P</sup>-GFP-UBC6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
99	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 his3::UBC6<sup>P</sup>-GFP-UBC6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
100	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::SpHIS5 his3::UBC6<sup>P</sup>-GFP-UBC6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
101	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::SpHIS5 his3::UBC6<sup>P</sup>-GFP-UBC6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
102	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 his3::VPS64<sup>P</sup>-GFP-VPS64::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
103	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 his3::VPS64<sup>P</sup>-GFP-VPS64::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
104	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::SpHIS5 his3::VPS64<sup>P</sup>-GFP-VPS64::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
105	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::SpHIS5 his3::VPS64<sup>P</sup>-GFP-VPS64::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
106	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 his3::FAR10<sup>P</sup>-GFP-FAR10::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
107	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 his3::FAR10<sup>P</sup>-GFP-FAR10::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
108	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::SpHIS5 his3::FAR10<sup>P</sup>-GFP-FAR10::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
109	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::SpHIS5 his3::FAR10<sup>P</sup>-GFP-FAR10::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
110	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 his3::GOS1<sup>P</sup>-GFP-GOS1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
111	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 his3::GOS1<sup>P</sup>-GFP-GOS1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
112	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::SpHIS5 his3::GOS1<sup>P</sup>-GFP-GOS1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
113	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::SpHIS5 his3::GOS1<sup>P</sup>-GFP-GOS1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
114	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 his3::SPS2<sup>P</sup>-GFP-SPS2::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
115	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 his3::SPS2<sup>P</sup>-GFP-SPS2::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
116	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::SpHIS5 his3::SPS2<sup>P</sup>-GFP-SPS2::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>

Table S2 Yeast Strains Used and Generated in This Study (Continued)

Strains generated in the background of BY4741

NO.	Genotype
117	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::SpHIS5 his3::SPS2<sup>P</sup>-GFP-SPS2::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
118	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
119	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
120	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::SpHIS5 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
121	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::SpHIS5 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
122	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 his3::SSS1<sup>P</sup>-GFP-SSS1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
123	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 his3::SSS1<sup>P</sup>-GFP-SSS1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
124	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::SpHIS5 his3::SSS1<sup>P</sup>-GFP-SSS1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
125	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::SpHIS5 his3::SSS1<sup>P</sup>-GFP-SSS1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
126	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::MSP1-FLAG::LEU2 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
127	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (E193Q)-FLAG::LEU2 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
128	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (D12T)-FLAG::LEU2 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
129	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (L69A)-FLAG::LEU2 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
130	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (Y72A)-FLAG::LEU2 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
131	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (E73A)-FLAG::LEU2 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
132	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (L77A)-FLAG::LEU2 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
133	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (V81A)-FLAG::LEU2 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
134	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (I86A)-FLAG::LEU2 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
135	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (I93A)-FLAG::LEU2 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
136	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (G94A)-FLAG::LEU2 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
137	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (G95A)-FLAG::LEU2 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
138	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (L96A)-FLAG::LEU2 his3::FRT1<sup>P</sup>-GFP-FRT1::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
139	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::MSP1-FLAG::LEU2 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
140	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (E193Q)-FLAG::LEU2 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
141	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (D12T)-FLAG::LEU2 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
142	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (L69A)-FLAG::LEU2 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
143	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (Y72A)-FLAG::LEU2 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
144	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (E73A)-FLAG::LEU2 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
145	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (L77A)-FLAG::LEU2 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>

Table S2 Yeast Strains Used and Generated in This Study (Continued)

Strains generated in the background of BY4741

NO.	Genotype
146	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (V81A)-FLAG::LEU2 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
147	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (I86A)-FLAG::LEU2 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
148	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (I93A)-FLAG::LEU2 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
149	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (G94A)-FLAG::LEU2 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
150	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (G95A)-FLAG::LEU2 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
151	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::msp1 (L96A)-FLAG::LEU2 his3::YSY6<sup>P</sup>-GFP-YSY6::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
152	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 his3::PEX15<sup>P</sup>-GFP-PEX15::kanMX4 pex3::PEX3-mRFP::hphNT1</i>
153	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 his3::PEX15<sup>P</sup>-GFP-PEX15::kanMX4 pex3::PEX3-mRFP::hphNT1 get3::KLURA3</i>
154	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 his3::PEX15<sup>P</sup>-GFP-PEX15::kanMX4 pex3::PEX3-mRFP::hphNT1 msp1::SpHIS5</i>
155	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 his3::PEX15<sup>P</sup>-GFP-PEX15::kanMX4 pex3::PEX3-mRFP::hphNT1 get3::KLURA3 msp1::SpHIS5</i>
156	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 get3::hphNT1 msp1::SpHIS5 his3::FRT1<sup>P</sup>-GFP-frt1 (K593N, R598Q)::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>
157	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (D12A)-FLAG::LEU2</i>
158	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (D12E)-FLAG::LEU2</i>
159	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::msp1 (D12K)-FLAG::LEU2</i>
160	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 cim3::cim3-1 (G551A)::kanMX6</i>
161	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 cim3::cim3-1 (G551A)::kanMX6 msp1::hphNT1</i>
162	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 cim3::cim3-1(G551A)::kanMX6 msp1::MSPI-FLAG::LEU2</i>
163	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 cim3::cim3-1(G551A)::kanMX6 msp1::msp1 (E193Q)-FLAG::LEU2</i>
164	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 pex19::KLURA3 ho::TEF1P-MTS-TagBFP (mtBFP)::natNT2</i>
165	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 pex19::KLURA3 msp1::kanMX6 ho::TEF1P-MTS-TagBFP (mtBFP)::natNT2</i>
166	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 pex19::KLURA3 get3::kanMX6 ho::TEF1P-MTS-TagBFP (mtBFP)::natNT2</i>
167	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 msp1::kanMX6 get3::hphNT1 pex19::KLURA3 ho::TEF1P-MTS-TagBFP (mtBFP)::natNT2</i>
168	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 pex19::KLURA3 msp1::MSPI-FLAG::LEU2 ho::TEF1P-MTS-TagBFP (mtBFP)::natNT2</i>
169	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 pex19::KLURA3 msp1::msp1 (E193Q)-FLAG::LEU2 ho::TEF1P-MTS-TagBFP (mtBFP)::natNT2</i>
170	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 pex19::KLURA3 msp1::msp1 (D12T)-FLAG::LEU2 ho::TEF1P-MTS-TagBFP (mtBFP)::natNT2</i>
171	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 pex19::KLURA3 msp1::msp1 (Y72A)-FLAG::LEU2 ho::TEF1P-MTS-TagBFP (mtBFP)::natNT2</i>
172	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 pex19::KLURA3 msp1::msp1 (V81A)-FLAG::LEU2 ho::TEF1P-MTS-TagBFP (mtBFP)::natNT2</i>
173	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 pex19::KLURA3 msp1::msp1 (I86A)-FLAG::LEU2 ho::TEF1P-MTS-TagBFP (mtBFP)::natNT2</i>
174	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 pex19::KLURA3 msp1::msp1 (I93A)-FLAG::LEU2 ho::TEF1P-MTS-TagBFP (mtBFP)::natNT2</i>

Table S2 Yeast Strains Used and Generated in This Study (Continued)

Strains generated in the background of BY4741

NO.	Genotype
175	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 pex19::KIURA3 get3::hphNT1 msp1::MSP1-FLAG::LEU2 ho::TEF1P-MTS-TagBFP (mtBFP)::natNT2</i>
176	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 pex19::KIURA3 get3::hphNT1 msp1::msp1 (E193Q)-FLAG::LEU2 ho::TEF1P-MTS-TagBFP (mtBFP)::natNT2</i>
177	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 pex19::KIURA3 get3::hphNT1 msp1::msp1 (D12T)-FLAG::LEU2 ho::TEF1P-MTS-TagBFP (mtBFP)::natNT2</i>
178	<i>MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 pex19::KIURA3 get3::hphNT1 msp1::msp1 (V81A)-FLAG::LEU2 ho::TEF1P-MTS-TagBFP (mtBFP)::natNT2</i>

Table S2 Yeast Strains Used and Generated in This Study (Continued)

Strains generated in other strain backgrounds

NO.	Genotype	Strain Background
1	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 msp1::MSPI-FLAG::LEU2</i>	W303-1A
2	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 msp1::msp1 (E193Q)-FLAG::LEU2</i>	W303-1A
3	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (90TAG)-FLAG::kanMX4</i>	W303-1A
4	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (90TAG)-GFP::kanMX4 ho::TEF1<sup>P</sup>-MTS-TagBFP (mtBFP)::natNT2</i>	W303-1A
5	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 msp1::kanMX4</i>	W303-1A
6	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 90TAG)-FLAG::kanMX4</i>	W303-1A
7	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 166TAG)-FLAG::kanMX4</i>	W303-1A
8	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 167TAG)-FLAG::kanMX4</i>	W303-1A
9	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 168TAG)-FLAG::kanMX4</i>	W303-1A
10	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 169TAG)-FLAG::kanMX4</i>	W303-1A
11	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 199TAG)-FLAG::kanMX4</i>	W303-1A
12	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 201TAG)-FLAG::kanMX4</i>	W303-1A
13	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 203TAG)-FLAG::kanMX4</i>	W303-1A
14	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 205TAG)-FLAG::kanMX4</i>	W303-1A
15	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 207TAG)-FLAG::kanMX4</i>	W303-1A
16	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 209TAG)-FLAG::kanMX4</i>	W303-1A
17	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 241TAG)-FLAG::kanMX4</i>	W303-1A
18	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 243TAG)-FLAG::kanMX4</i>	W303-1A
19	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 2TAG)-FLAG::kanMX4</i>	W303-1A
20	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 3TAG)-FLAG::kanMX4</i>	W303-1A
21	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 5TAG)-FLAG::kanMX4</i>	W303-1A
22	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 6TAG)-FLAG::kanMX4</i>	W303-1A
23	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 7TAG)-FLAG::kanMX4</i>	W303-1A
24	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 8TAG)-FLAG::kanMX4</i>	W303-1A
25	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 9TAG)-FLAG::kanMX4</i>	W303-1A
26	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 10TAG)-FLAG::kanMX4</i>	W303-1A
27	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 11TAG)-FLAG::kanMX4</i>	W303-1A
28	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 13TAG)-FLAG::kanMX4</i>	W303-1A

Table S2 Yeast Strains Used and Generated in This Study (Continued)

Strains generated in other strain backgrounds

NO.	Genotype	Strain Background
29	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 15TAG)-FLAG::kanMX4</i>	W303-1A
30	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 17TAG)-FLAG::kanMX4</i>	W303-1A
31	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 20TAG)-FLAG::kanMX4</i>	W303-1A
32	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 21TAG)-FLAG::kanMX4</i>	W303-1A
33	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 22TAG)-FLAG::kanMX4</i>	W303-1A
34	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 23TAG)-FLAG::kanMX4</i>	W303-1A
35	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 25TAG)-FLAG::kanMX4</i>	W303-1A
36	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 26TAG)-FLAG::kanMX4</i>	W303-1A
37	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 29TAG)-FLAG::kanMX4</i>	W303-1A
38	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 32TAG)-FLAG::kanMX4</i>	W303-1A
39	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 38TAG)-FLAG::kanMX4</i>	W303-1A
40	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 41TAG)-FLAG::kanMX4</i>	W303-1A
41	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 47TAG)-FLAG::kanMX4</i>	W303-1A
42	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 50TAG)-FLAG::kanMX4</i>	W303-1A
43	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 53TAG)-FLAG::kanMX4</i>	W303-1A
44	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 56TAG)-FLAG::kanMX4</i>	W303-1A
45	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 59TAG)-FLAG::kanMX4</i>	W303-1A
46	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 62TAG)-FLAG::kanMX4</i>	W303-1A
47	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 65TAG)-FLAG::kanMX4</i>	W303-1A
48	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 68TAG)-FLAG::kanMX4</i>	W303-1A
49	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 71TAG)-FLAG::kanMX4</i>	W303-1A
50	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 77TAG)-FLAG::kanMX4</i>	W303-1A
51	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 80TAG)-FLAG::kanMX4</i>	W303-1A
52	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 83TAG)-FLAG::kanMX4</i>	W303-1A
53	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 86TAG)-FLAG::kanMX4</i>	W303-1A
54	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 89TAG)-FLAG::kanMX4</i>	W303-1A
55	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 92TAG)-FLAG::kanMX4</i>	W303-1A
56	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 95TAG)-FLAG::kanMX4</i>	W303-1A
57	<i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3::GAL1<sup>P</sup>-msp1 (E193Q, 98TAG)-FLAG::kanMX4</i>	W303-1A

Table S3 Plasmids used in this study

No	plasmid name	parent vector	insert composition		
			promoter	terminator	ORF
1	pRS426-TEF1p-HA-UB	pRS426	TEF1p	CYCt	HA-UBIQUITIN
2	pRS416-MSP1p-MSP1-GFP	pRS416	MSP1p	ADH1t	MSP1-GFP
3	NAT-TEF1p-GFP-PEX15Δ30	p417-CYC1	TEF1p	CYCt	GFP-PEX15 (1-353aa)
4	NAT-TEF1p-GFP-PEX15(C)-TOM5 (OTS)	p417-CYC1	TEF1p	CYCt	GFP-PEX15 (1-324aa)-TOM5 (23-50aa)
5	NAT-TEF1p-GFP-PEX15(C)-GEM1 (OTS)	p417-CYC1	TEF1p	CYCt	GFP-PEX15 (1-324aa)-GEM1 (629-662aa)
6	NAT-TEF1p-GFP-PEX15(C)-FIS1 (OTS)	p417-CYC1	TEF1p	CYCt	GFP-PEX15 (1-324aa)-FIS1 (126-155aa)
7	NAT-TEF1p-GFP-PEX15(C)-TOM6 (OTS)	p417-CYC1	TEF1p	CYCt	GFP-PEX15 (1-324aa)-TOM6 (27-61aa)
8	NAT-TEF1p-GFP-PEX15(C)-TOM22 (OTS)	p417-CYC1	TEF1p	CYCt	GFP-PEX15 (1-324aa)-TOM22 (73-119aa)
9	NAT-TEF1p-GFP-GEM1	p417-CYC1	TEF1p	CYCt	GFP-GEM1
10	NAT-TEF1p-GFP-GEM1-Ins	p417-CYC1	TEF1p	CYCt	GFP-GEM1 (1-628aa)-PEX15 (312-324aa)-GEM1 (629-662aa)
11	NAT-TEF1p-GFP-FIS1	p417-CYC1	TEF1p	CYCt	GFP-FIS1
12	NAT-TEF1p-GFP-FIS1-Ins	p417-CYC1	TEF1p	CYCt	GFP-FIS1 (1-125aa)-PEX15 (312-324aa)-FIS1 (126-155aa)
13	pRS426-GAL1p-GFP-3xHA-PEX15Δ30	pRS426	GAL1p	CYCt	GFP-3xHA-PEX15 (1-353aa)
14	pRS416-GAL1p-GFP-3xHA-PEX15Δ30	pRS416	GAL1p	CYCt	GFP-3xHA-PEX15 (1-353aa)
15	NAT-TEF1p-GFP-PEX15	p417-CYC1	TEF1p	CYCt	GFP-PEX15
16	NAT-TEF1p-GFP-FRT1	p417-CYC1	TEF1p	CYCt	GFP-FRT1
17	NAT-TEF1p-GFP-SBH1	p417-CYC1	TEF1p	CYCt	GFP-SBH1
18	NAT-TEF1p-GFP-BOS1	p417-CYC1	TEF1p	CYCt	GFP-BOS1
19	NAT-TEF1p-GFP-VTI1	p417-CYC1	TEF1p	CYCt	GFP-VTI1
20	NAT-TEF1p-GFP-SSO1	p417-CYC1	TEF1p	CYCt	GFP-SSO1
21	NAT-TEF1p-GFP-PGC1	p417-CYC1	TEF1p	CYCt	GFP-PGC1
22	NAT-TEF1p-GFP-FRT2	p417-CYC1	TEF1p	CYCt	GFP-FRT2
23	NAT-TEF1p-GFP-SBH2	p417-CYC1	TEF1p	CYCt	GFP-SBH2
24	NAT-TEF1p-GFP-SEC22	p417-CYC1	TEF1p	CYCt	GFP-SEC22
25	NAT-TEF1p-GFP-SFT1	p417-CYC1	TEF1p	CYCt	GFP-SFT1
26	NAT-TEF1p-GFP-PRM3	p417-CYC1	TEF1p	CYCt	GFP-PRM3
27	NAT-TEF1p-GFP-KAR1	p417-CYC1	TEF1p	CYCt	GFP-KAR1
28	NAT-TEF1p-GFP-YEL073C	p417-CYC1	TEF1p	CYCt	GFP-YEL073C
29	NAT-TEF1p-GFP-YCL007C	p417-CYC1	TEF1p	CYCt	GFP-YCL007C
30	NAT-TEF1p-GFP-SSS1	p417-CYC1	TEF1p	CYCt	GFP-SSS1

Table S3 Plasmids used in this study (Continued)

No	plasmid name	parent vector	insert composition			ORF
			promoter	terminator		
31	NAT-TEF1p-GFP-UBC6	p417-CYC1	TEF1p	CYCt		GFP-UBC6
32	NAT-TEF1p-GFP-VPS64	p417-CYC1	TEF1p	CYCt		GFP-VPS64
33	NAT-TEF1p-GFP-FAR10	p417-CYC1	TEF1p	CYCt		GFP-FAR10
34	NAT-TEF1p-GFP-PHM6	p417-CYC1	TEF1p	CYCt		GFP-PHM6
35	NAT-TEF1p-GFP-SCS2	p417-CYC1	TEF1p	CYCt		GFP-SCS2
36	NAT-TEF1p-GFP-SNC1	p417-CYC1	TEF1p	CYCt		GFP-SNC1
37	NAT-TEF1p-GFP-PEP12	p417-CYC1	TEF1p	CYCt		GFP-PEP12
38	NAT-TEF1p-GFP-SSO2	p417-CYC1	TEF1p	CYCt		GFP-SSO2
39	NAT-TEF1p-GFP-DPM1	p417-CYC1	TEF1p	CYCt		GFP-DPM1
40	NAT-TEF1p-GFP-SNC2	p417-CYC1	TEF1p	CYCt		GFP-SNC2
41	NAT-TEF1p-GFP-GOS1	p417-CYC1	TEF1p	CYCt		GFP-GOS1
42	NAT-TEF1p-GFP-SPS2	p417-CYC1	TEF1p	CYCt		GFP-SPS2
43	NAT-TEF1p-GFP-YSY6	p417-CYC1	TEF1p	CYCt		GFP-YSY6
44	NAT-TEF1p-GFP-HLJ1	p417-CYC1	TEF1p	CYCt		GFP-HLJ1
45	NAT-TEF1p-GFP-YDL241W	p417-CYC1	TEF1p	CYCt		GFP-YDL241W
46	NAT-TEF1p-GFP-YJL119C	p417-CYC1	TEF1p	CYCt		GFP-YJL119C
47	NAT-TEF1p-GFP-BET1	p417-CYC1	TEF1p	CYCt		GFP-BET1
48	NAT-TEF1p-GFP-YEL010W	p417-CYC1	TEF1p	CYCt		GFP-YEL010W
49	NAT-TEF1p-GFP-YBR016W	p417-CYC1	TEF1p	CYCt		GFP-YBR016W
50	NAT-TEF1p-GFP-NYV1	p417-CYC1	TEF1p	CYCt		GFP-NYV1
51	NAT-TEF1p-GFP-CYB5	p417-CYC1	TEF1p	CYCt		GFP-CYB5
52	NAT-TEF1p-GFP-USE1	p417-CYC1	TEF1p	CYCt		GFP-USE1
53	NAT-TEF1p-GFP-SED5	p417-CYC1	TEF1p	CYCt		GFP-SED5
54	NAT-TEF1p-GFP-TLG1	p417-CYC1	TEF1p	CYCt		GFP-TLG
55	NAT-TEF1p-GFP-SYN8	p417-CYC1	TEF1p	CYCt		GFP-SYN8
56	NAT-TEF1p-GFP-VAM3	p417-CYC1	TEF1p	CYCt		GFP-VAM3
57	NAT-TEF1p-GFP-YBL100C	p417-CYC1	TEF1p	CYCt		GFP-YBL100C
58	NAT-TEF1p-GFP-SEC20	p417-CYC1	TEF1p	CYCt		GFP-SEC20
59	NAT-TEF1p-GFP-UFE1	p417-CYC1	TEF1p	CYCt		GFP-UFE1
60	NAT-TEF1p-GFP-CSM4	p417-CYC1	TEF1p	CYCt		GFP-CSM4

Table S3 Plasmids used in this study (Continued)

No	plasmid name	parent vector	insert composition			ORF
			promoter	terminator		
61	NAT-TEF1p-GFP-TLG2	p417-CYC1	TEF1p	CYCt		GFP-TLG2
62	NAT-TEF1p-GFP-YML036W	p417-CYC1	TEF1p	CYCt		GFP-YML036W
63	NAT-TEF1p-GFP-YDL012C	p417-CYC1	TEF1p	CYCt		GFP-YDL012C
64	NAT-TEF1p-mRFP-6xHA-PEX15 $\Delta$ 30	p417-CYC1	TEF1p	CYCt		mRFP-6xHA-PEX15 (1-353aa)