Supplemental Material

Genetic suppression of precocious transcription termination identifies mutations in essential subunits of the fission yeast cleavage and polyadenylation machinery

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Figures S1 to S9

Table S2

[Table S1 is provided as a separate .xls file]

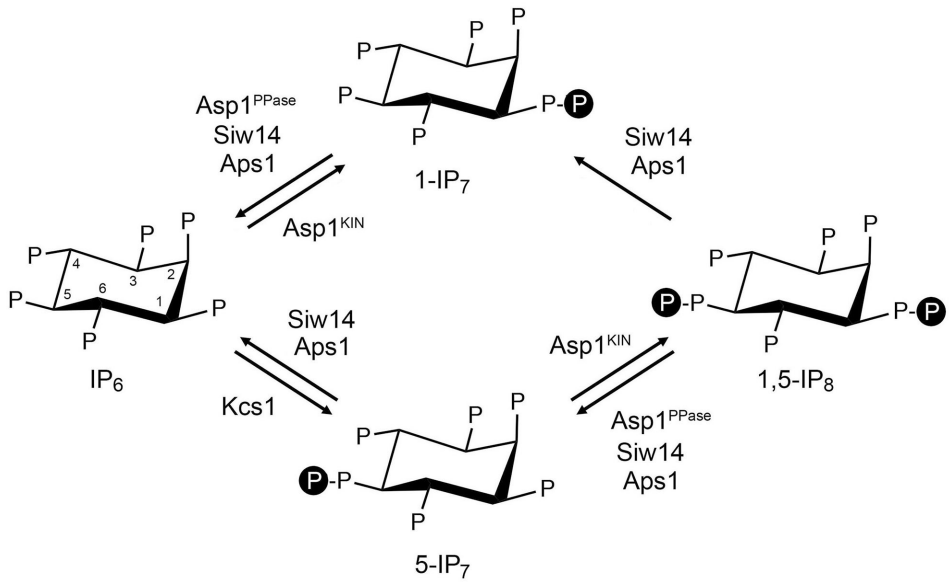

Figure S1. Enzymes of inositol pyrophosphate metabolism in fission yeast.

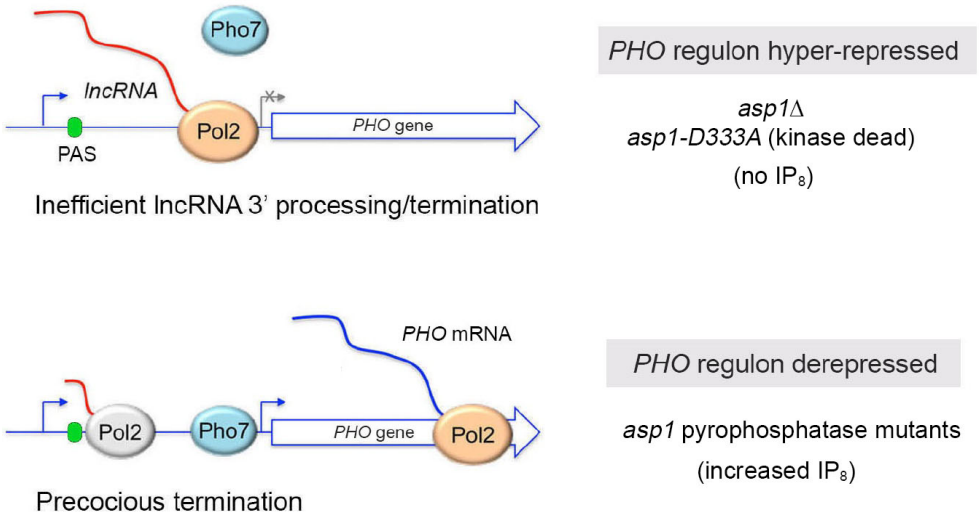

Figure S2. Inositol pyrophosphates modulate lncRNA regulation of fission yeast *PHO* gene expression. The *PHO* regulon genes *pho1*, *pho84*, and *tgp1* are repressed under phosphate-replete conditions via transcriptional interference caused by synthesis of an upstream-flanking long noncoding (lnc) RNA. RNA polymerase II (Pol2) synthesizing the lncRNA traverses the *PHO* gene promoter and interferes with binding of transcription factor Pho7 that activates *PHO* mRNA synthesis. lncRNA interference with *PHO* mRNA synthesis is tunable by genetic maneuvers that affect precocious termination of lncRNA transcription in response to poly(A) signals (PAS) in the 5' region of the lncRNA. Genetically altering IP_8 metabolism affects the *PHO* regulon as shown. (Top panel) The efficiency of lncRNA 3'-processing/termination is diminished in *asp1*Δ and *asp1-D333A* cells that lack IP_8, which results in hyper-repression of *PHO* mRNA synthesis. (Bottom panel) Increased IP_8 levels in *asp1* pyrophosphatase mutants increase precocious lncRNA 3'-processing/termination and thereby derepress *PHO* mRNA synthesis by allowing engagement of Pho7 at the *PHO* mRNA promoters.

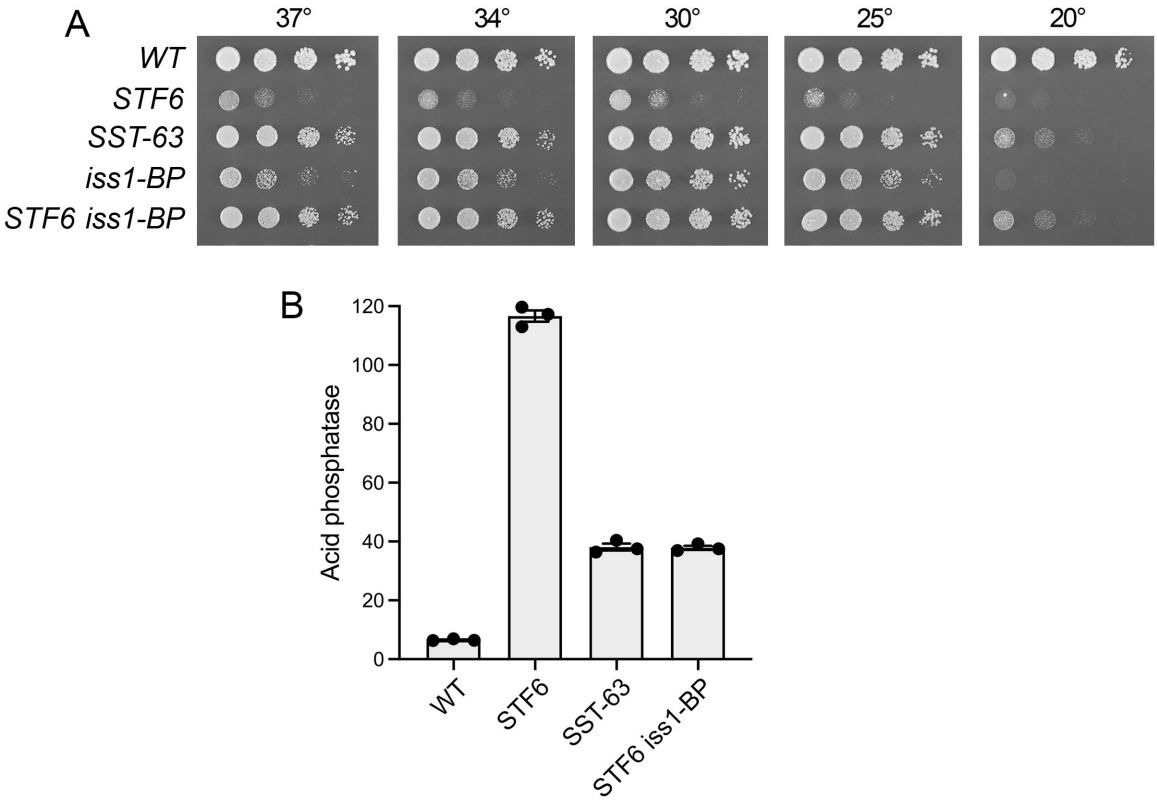

Figure S3. An intron branchpoint mutation in *iss1* suppresses *asp1-STF6*. (A) Serial 5-fold dilutions of strains specified at left were spot tested for growth on YES agar at the indicated temperatures. (B) The indicated strains were assayed for Pho1 acid phosphatase activity. Bar height is the mean of three biological replicates (independent cultures, denoted by dots) ± SEM.

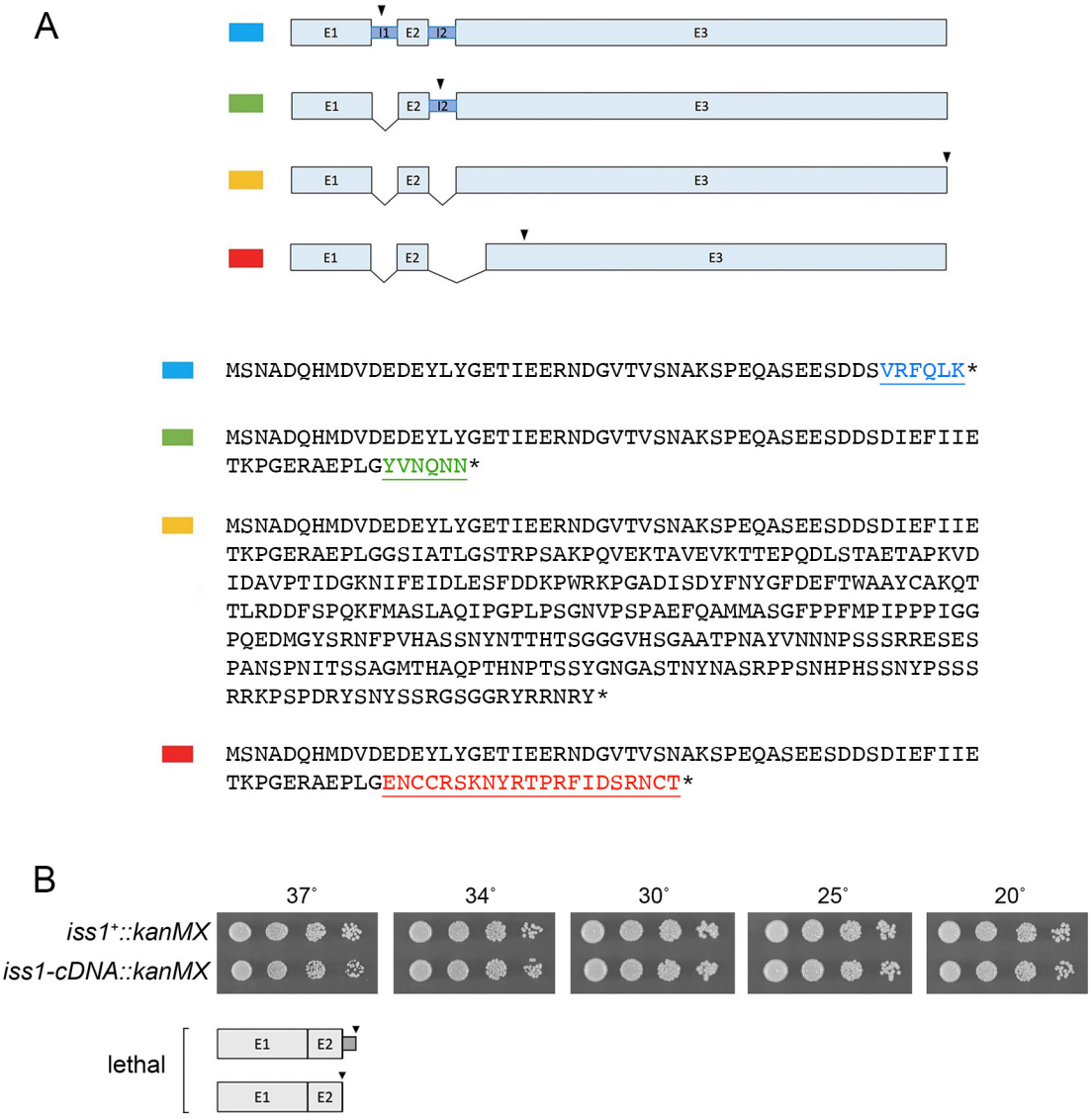

Figure S4. Physical and functional analysis of the four *iss1* transcripts detected in *iss1-BP* cells. (A) The identities of the four *iss1* transcripts present in *iss1-BP* cells, shown here in cartoon form, were established by sequencing the RT-PCR products shown in Fig. 3A. The translation stop codons are denoted by arrowheads. The amino acid sequences of the encoded polypeptides are shown. The underlined C-terminal peptides in colored fonts are non-native appendages resulting from translation of intronic RNA sequences or mis-spliced exon 3 RNA sequence. (B) Diploids in which one chromosomal *iss1*$^{+}$ gene was replaced by 3' *kanMX*-marked alleles comprising: (i) a fully spliced *iss1* cDNA encoding full-length Iss1; (ii) a truncated cDNA encoding the 70-aa translation product denoted in green in panel A; or (iii) a cDNA encoding Iss1-(1-64) were sporulated. Haploid progeny bearing the *iss1-cDNA::kanMX* allele grew normally on YES agar at all temperatures. However, we failed to recover viable kanamycin-resistant haploids expressing the truncated 70-aa or 64-aa Iss1 polypeptides, signifying that these alleles are lethal.

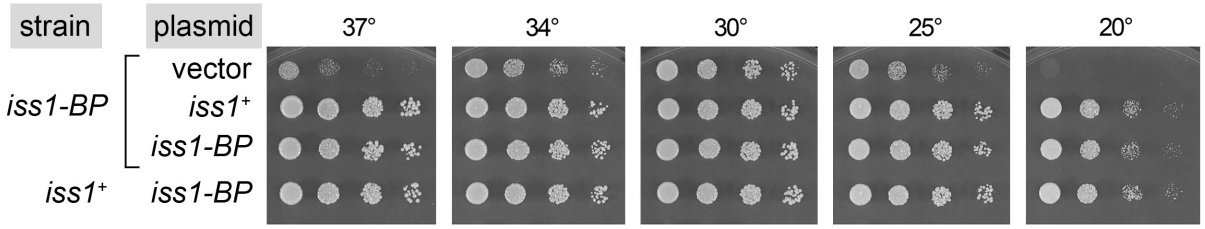

Figure S5. Transformation of *iss1-BP* cells with a multicopy plasmid bearing *iss1-BP* under the control of its native promoter rescues the *cs/ts* phenotype. The indicated strains were spot tested for growth on ePMGT-leu agar.

Sample	Total Paired Reads	Mapped Reads
WT (1)	27,673,343	27,232,727 (98%)
WT (2)	28,786,900	28,351,350 (98%)
WT (3)	30,660,670	30,191,101 (98%)
msi2-G252E (1)	27,340,825	26,926,139 (98%)
msi2-G252E (2)	25,955,996	25,373,661 (98%)
msi2-G252E (3)	27,210,772	26,750,298 (98%)
pfs2-E104A (1)	28,386,169	27,927,902 (98%)
pfs2-E104A (2)	21,174,063	20,825,334 (98%)
pfs2-E104A (3)	21,627,266	21,013,771 (97%)
pfs2-V180F (1)	24,537,761	23,538,519 (96%)
pfs2-V180F (2)	32,116,798	31,524,936 (98%)
pfs2-V180F (3)	28,174,588	27,614,810 (98%)
pta1-T524M (1)	30,088,458	29,633,482 (98%)
pta1-T524M (2)	26,253,718	25,876,273 (98%)
pta1-T524M (3)	30,652,726	30,134,629 (98%)
ysh1-L74F (1)	33,096,173	31,546,132 (95%)
ysh1-L74F (2)	31,532,200	29,002,968 (92%)
ysh1-L74F (3)	30,849,501	30,204,941 (98%)
pla1-Y86L (1)	30,632,387	29,138,520 (95%)
pla1-Y86L (2)	26,303,174	24,874,905 (95%)
pla1-Y86L (3)	31,059,216	30,360,281 (98%)
cft1-L249Q (1)	28,746,381	26,180,744 (91%)
cft1-L249Q (2)	24,253,429	23,821,560 (98%)
cft1-L249Q (3)	24,474,070	24,152,994 (99%)

Sample pairs	Pearson Coefficient
WT (1) vs (2)	0.987
WT (1) vs (3)	0.987
WT (2) vs (3)	0.987
msi2-G252E (1) vs (2)	0.988
msi2-G252E (1) vs (3)	0.988
msi2-G252E (2) vs (3)	0.988
pfs2-E104A (1) vs (2)	0.988
pfs2-E104A (1) vs (3)	0.987
pfs2-E104A (2) vs (3)	0.986
pfs2-V180F (1) vs (2)	0.985
pfs2-V180F (1) vs (3)	0.986
pfs2-V180F (2) vs (3)	0.989
pta1-T524M (1) vs (2)	0.988
pta1-T524M (1) vs (3)	0.989
pta1-T524M (2) vs (3)	0.988
ysh1-L74F (1) vs (2)	0.988
ysh1-L74F (1) vs (3)	0.989
ysh1-L74F (2) vs (3)	0.988
pla1-Y86L (1) vs (2)	0.988
pla1-Y86L (1) vs (3)	0.988
pla1-Y86L (2) vs (3)	0.987
cft1-L249Q (1) vs (2)	0.985
cft1-L249Q (1) vs (3)	0.983
cft1-L249Q (2) vs (3)	0.986

Figure S6. RNA-seq read counts for triplicate biological replicates (left panel) and data reproducibility between biological replicates (right panel).

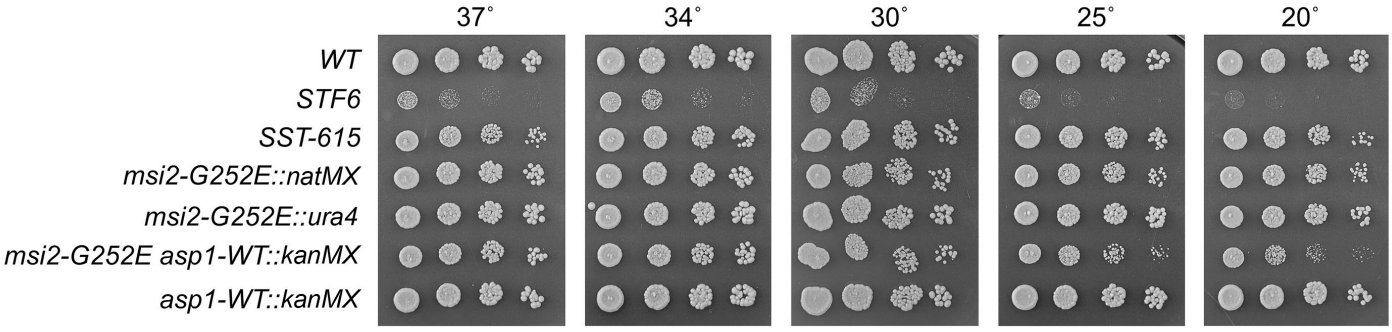

Figure S7. Growth of marked *msi2-G252E* strains. Serial 5-fold dilutions of strains specified at left were spot tested for growth on YES agar at the indicated temperatures.

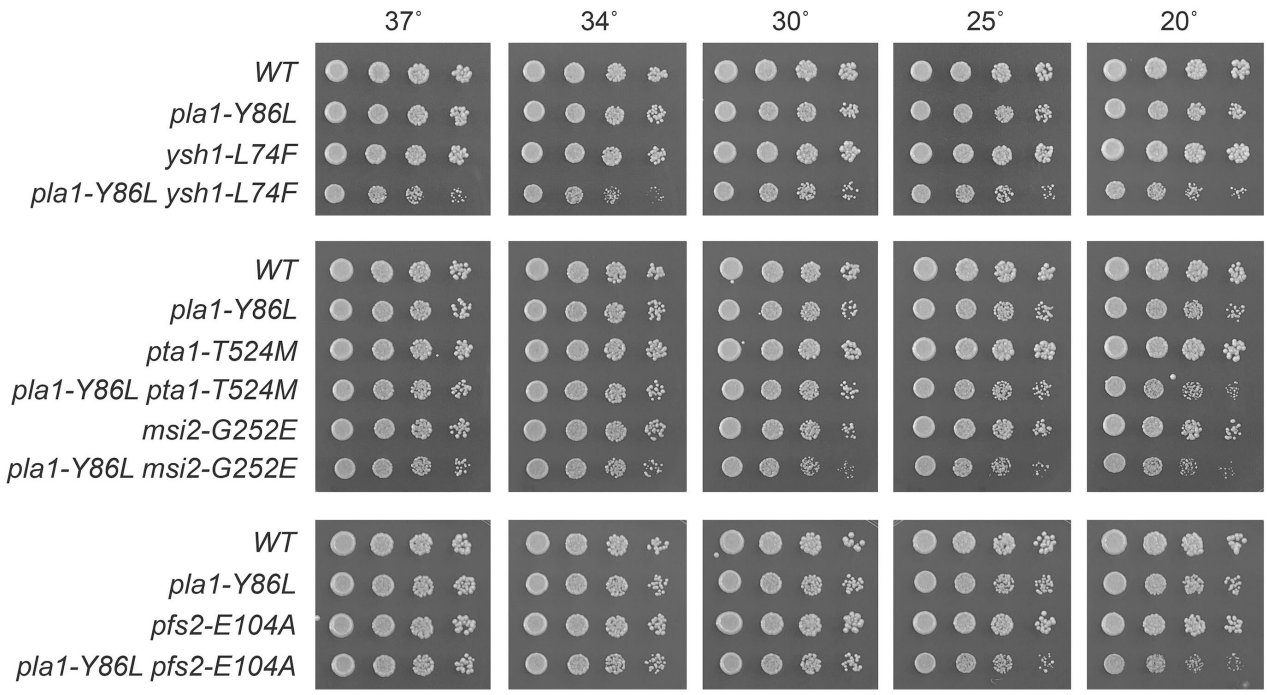

Figure S8. Effect of combining *pla1-Y86L* with *ysh1-L74F*, *pta1-T524M*, *msi2-G252E*, and *pfs2-E104A*. Serial 5-fold dilutions of strains specified at left were spot tested for growth on YES agar at the indicated temperatures.

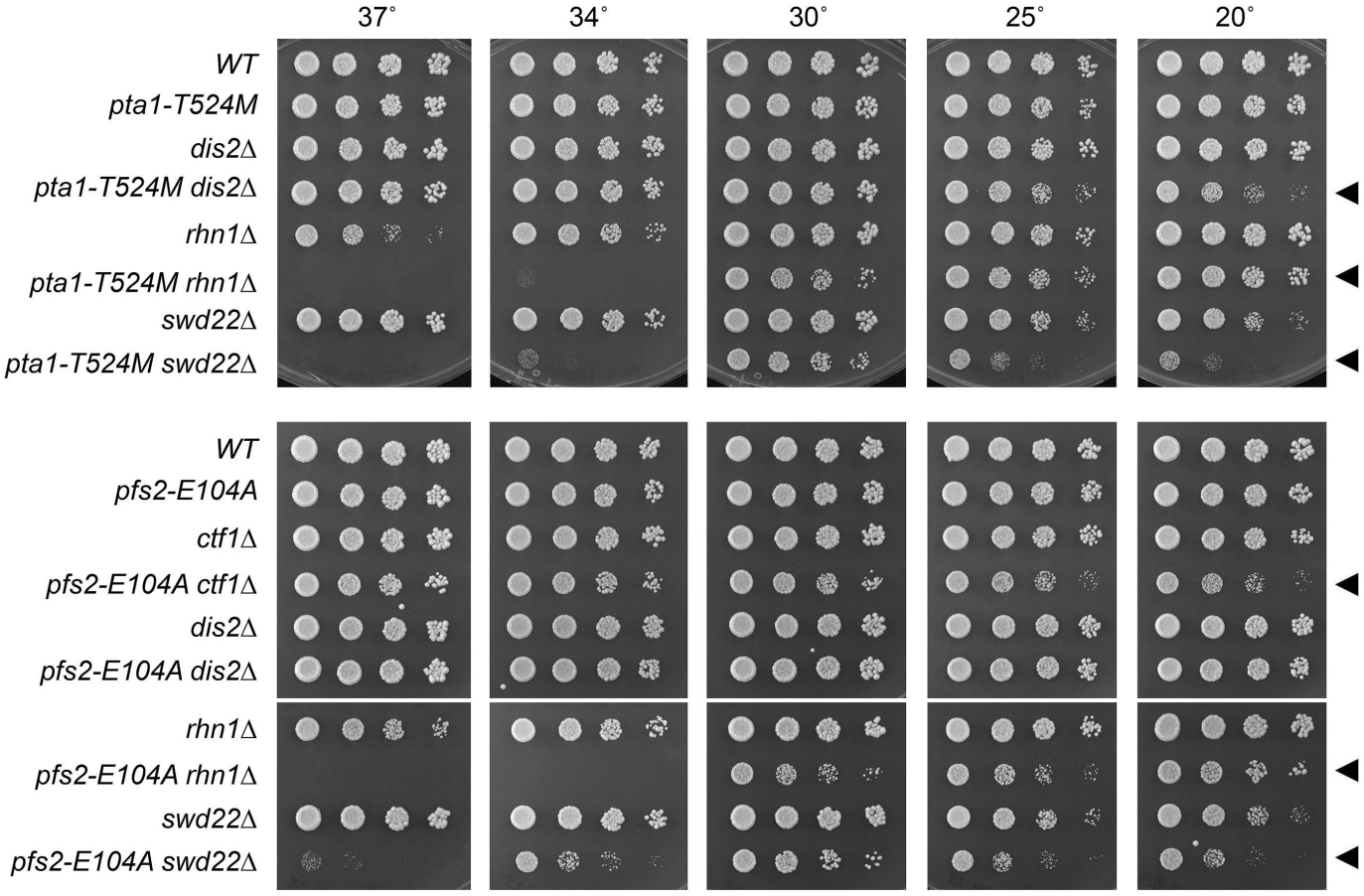

Figure S9. Genetic interactions of *pta1-T524M* and *pfs2-E104A*. Serial 5-fold dilutions of strains specified at left were spot tested for growth on YES agar at the indicated temperatures.

Table S2. List of *S. pombe* strains used in this study.

Strain	Genotype	Source
AGP263	*h- pla1-Y86L::kanMX*	PMC10578478
AGP264	*h+ pla1-Y86L::kanMX*	PMC10578478
AS1985	*h- ppn1Δ:: natMX*	PMC6233144
AS1980	*h- swd22Δ:: hygMX*	PMC6233144
AS2020	*h+ dis2Δ::ura4+*	PMC6233144
AS2218	*h+ dis2Δ::natMX*	PMC6233144
AS1823	*h- ctf1Δ::ura4+*	PMC6233144
AGP169	*h- ctf1Δ::kanMX*	PMC6233144
AS2307	*h+ ssu72-C13S::hygMX*	PMC4574753
AGP120	*h- rhn1Δ::natMX*	PMC6233144
AGP170	*h- rhn1Δ::kanMX*	PMC6233144
AGP139	*h+ rpb1-T4A$_{29}$::natMX*	PMC6233144
AS1887	*h- asp1Δ::kanMX*	PMC6895273
AS2183	*h- asp1Δ::natMX*	PMC6895273
BS848	*h+ STF6::hygMX [asp1-W386*]*	PMC10865970
BS851	*h+ SST-63 asp1-STF6::hygMX iss1-BP gls2-N263T*	This study
BS858	*h+ asp1-STF9::hygMX [asp1-W493*]*	PMC10865970
BS860	*h+ asp1-STF9::hygMX cft1-L249Q*	This study
BS1025	*h+ iss1-BP*	This study
BS1246	*h- iss1^{+}::kanMX*	This study
BS1247	*h+ iss1-cDNA::kanMX*	This study
BS1335	*h- asp1-STF6::hygMX iss1-BP*	This study
BS1363	*h- msi2-G252E::natMX*	This study
BS1468	*h- pfs2-E104A::ura4MX*	This study
BS1467	*h- pfs2-E104A::natMX*	This study
BS1472	*h- pta1-T524M::ura4MX*	This study
BS1471	*h- pta1-T524M::natMX*	This study
BS1570	*h^{0} ppn1Δ::natMX ysh1-L74F::ura4MX*	This study
BS1571	*h^{0} ssu72-C13S ysh1-L74F::ura4MX*	This study
BS1478	*h+ ysh1-L74F::ura4MX*	This study
ASY-255	*h^{0} pla1-Y86L::kanMX ppn1Δ::natMX*	This study
ASY-256	*h+ pla1-Y86L::kanMX swd22Δ::hygMX*	This study
ASY-258	*h- pla1-Y86L::kanMX dis2Δ::natMX*	This study
ASY-263	*h^{0} pla1-Y86L::kanMX ctf1Δ::ura4+*	This study
ASY-264	*h^{0} pla1-Y86L::kanMX ssu72-C13S::hygMX*	This study
ASY-265	*h+ pla1-Y86L::kanMX rhn1Δ::natMX*	This study
ASY-267	*h- pla1-Y86L::kanMX rpb1-T4A$_{29}$::natMX*	This study
ASY-288	*h- pla1-Y86L::kanMX msi2-G252E::natMX*	This study
ASY-289	*h+ pla1-Y86L::kanMX pfs2-E104A::ura4MX*	This study
ASY-290	*h+ pla1-Y86L::kanMX pta1-T524M::ura4MX*	This study
ASY-296	*h^{0} pla1-Y86L::kanMX ysh1-L74F::ura4MX*	This study
IAP-157	*h^{0} rpb1-T4A$_{29}$::natMX pfs2-E104A::ura4MX*	This study
IAP-158	*h^{0} ssu72-C13S::hygMX pta1-T524M::ura4MX*	This study

IAP-159	*h^0 rpb1-T4A$_{29}$::natMX pta1-T524M::ura4MX*	This study
IAP-160	*h^0 rpb1-T4A$_{29}$::natMX ysh1-L74F::ura4MX*	This study
JTB1015	*h+ msi2-G252E::natMX pfs2-E104A::ura4MX*	This study
JTB1016	*h- msi2-G252E::natMX pta1-T524M::ura4MX*	This study
JTB1037	*h+ ysh1-L74F::ura4MX pfs2-E104A::natMX*	This study
JTB1039	*h^0 ysh1-L74F::ura4MX msi2-G252E::natMX*	This study
JTB1041	*h- ysh1-L74F::ura4MX pta1-T524M::natMX*	This study
JTB1043	*h- pfs2-E104A::natMX pta1-T524M::ura4MX*	This study
ASY-308	*h+ asp1Δ::kanMX pfs2-E104A::ura4MX*	This study
ASY-309	*h- asp1Δ::kanMX ysh1-L74F::ura4MX*	This study
ASY-310	*h^0 asp1Δ::kanMX pta1-T524M::natMX*	This study
ASY-313	*h^0 asp1Δ::natMX pla1-Y86L::kanMX*	This study
ASY-316	*h- msi2-G252E::natMX rhn1Δ::kanMX*	This study
ASY-317	*h+ msi2-G252E::natMX dis2Δ::ura4+*	This study
ASY-318	*h- pfs2-E104A::natMX dis2Δ::ura4+*	This study
ASY-319	*h- pfs2-E104A::natMX ctf1Δ::kanMX*	This study
ASY-320	*h- pfs2-E104A::natMX rhn1Δ::kanMX*	This study
ASY-323	*h^0 pfs2-E104A::natMX swd22Δ::hygMX*	This study
ASY-324	*h- pta1-T524M::natMX rhn1Δ::kanMX*	This study
ASY-325	*h^0 pta1-T524M::natMX swd22Δ::hygMX*	This study
ASY-326	*h+ pta1-T524M::natMX dis2Δ::ura4+*	This study
ASY-332	*h+ ysh1-L74F::ura4MX dis2Δ::natMX*	This study
ASY-333	*h^0 ysh1-L74F::ura4MX ctf1Δ::kanMX*	This study
ASY-334	*h^0 ysh1-L74F::ura4MX rhn1Δ::kanMX*	This study
ASY-335	*h^0 ysh1-L74F::ura4MX swd22Δ::hygMX*	This study

The strains are *leu1-32*, *ura4-D18*, *his3-D1*, and either *ade6-m216 or ade6-m210*. Mating types were determined by mixing cells with each of the parental *h+* and *h-* strains on malt agar and observing tetrads after 24-48 h of incubation. *h^0* means that no tetrads or zygotes were detected after 48 h.