

SUPPLEMENTAL METHODS

Constructs for *spslu7+* gene disruptions: The 1139 bp *spslu7+* (SPBC365.05c) ORF was PCR amplified from wild type *S. pombe* (FY528) genomic DNA using gene-specific primers. pBS(KS)-*spslu7+* was created by inserting the BamHI digested PCR product into the BamHI site of pBlueScript(KS) (Stratagene). To create the deletion disruption construct pBS(KS)-*spslu7Δ::KANMX6*, a ~100 bp HincII fragment (with ~35 bp of the sequence from the vector) was deleted from pBS(KS)-*spslu7+* so as to remove the EcoRV restriction site present in the multiple cloning site of pBS(KS). Subsequently a 300 bp *spslu7+* internal EcoRV fragment was replaced by end-filled BamHI/EcoRI restricted ~1.4 Kb *KANMX6* cassette from pFA6A-KANMX6. A ~2.2 Kb *spslu7Δ::KANMX6* BamHI/XhoI fragment from pBS(KS)-*spslu7Δ::KANMX6* was transformed into diploid *S. pombe* cells (FY527 X FY528) in a one-step gene replacement method. Stable G418-resistant *ade+* diploid transformants were isolated and disruption at *spslu7+* locus confirmed by Southern blot analysis.

Constructs for complementation studies: The *spslu7+* ORF + cDNA were excised as BamHI fragment from pBS(KS)-*spslu7+* and ligated to BamHI digested pREP4X to create pREP4X-*spslu7+*. The insert in pDblet-*spslu7+* consisting of *spslu7+* ORF and 1 Kb upstream sequences, was generated by PCR using the primer pair (*spslu7* 5'UTR FP and *spslu7* RP) with wild type FY528 genomic DNA as the template. The PCR fragment was cloned as a blunt fragment into HincII site of the *S. pombe* shuttle vector pDblet.

Constructs for epitope and eGFP-tagging of *spslu7+* and *spslu7* C113A:
pREP41MH-*spslu7+* (Myc-His translation fusion to amino-terminus of SpSlu7) and pREP42

EGFP N-*spslu7*⁺ (EGFP translational fusion to amino-terminus of SpSlu7) was constructed by cloning *spslu7*⁺ ORF cleaved as a BamHI fragment from pBS(KS)-*spslu7*⁺ into BamHI site of pREP41MH-N and pREP42 EGFP-N respectively. While, *spslu7* C113A mutant gene was excised as a BamHI fragment from pBS(KS)-*spslu7mut* C113A plasmid and cloned into BamHI restricted pREP41MH-N or pREP42EGFP-N vectors.

Mini-gene constructs for assessing the dependence of SpSlu7 function on intronic features:

- 1) *rhb1*+I1 wildtype construct: A 147 bp PCR fragment containing the E1-I1-E2 sequence of *rhb1*⁺ gene was amplified with the primer pair, *rhb1*ATG FP and *rhb1* RP using genomic DNA prepared from FY527 wildtype strain, as template. This was then cloned into the EcoRV site of pBS(KS) cloning vector. The resulting clone was named pBS(KS) *rhb1*⁺ E1-I1-E2 from which the *rhb1*⁺ E1-I1-E2 was released as a EcoRI-XhoI fragment and cloned into the yeast shuttle vector pDblet (1) prior to which the pDblet vector was modified to contain the promoter elements in the -587 to -1 bp sequences from the *sptbp1*⁺ locus. The final clone was named pDblet SpPtbp1:*rhb1*⁺ E1-I1-E2 and was used for further analyses.
- 2) *rhb1* I1Δ10 construct: Using the primer pair *rhb1*ATG FP and *rhb1* loopout10E2 RP, a loopout PCR was carried out on FY527 genomic DNA as template. The resulting 130 bp PCR product contained the *rhb1* E1-I1-E2 minigene with a deletion of 10 bp between the BrP to 3'ss. This PCR amplicon was subsequently cloned into pBS(KS) to yield pBS(KS) *rhb1* I1Δ10 and then subcloned into the yeast shuttle vector pDblet SpPtbp1 resulting in the pDblet SpPtbp1:*rhb1* I1Δ10 variant construct. The same enzyme sites used for generation of the wildtype constructs were used for these constructs.

- 3) *rhb1* I1+10BrPΔ10 construct: To generate a variant construct, where the *rhb1* intron1 is deleted for 10 bp between the BrP to 3'ss and has these 10 bp inserted just upstream of the BrP, a pair of primers: *rhb1*I1 FP and *rhb1*I1 RP were used which shared an overlap of 10 bp at their respective 5' ends. These 10 bp corresponded to the sequence to be inserted upstream of BrP. The *rhb1*I1 FP was used in combination with *rhb1* loopout10E2 RP (to introduce the 10 bp deletion between BrP and 3'ss) to generate a 64 bp fragment. Additionally, a PCR with the primers *rhb1*ATG FP and *rhb1*I1 RP yielded a 86 bp fragment. These PCR amplicons shared a 10 bp complementary sequence and were then used in combination with the *rhb1*ATG FP and *rhb1* loopout10E2 RP, in overlapping PCR reactions to create a 140 bp long *rhb1* I1+10BrPΔ10 variant fragment. This was then used to generate the pBS(KS) recombinant and then pDblet SpPtbp1: *rhb1* I1+10BrPΔ10 construct following the same steps as described above.
- 4) *nab2*+ E2-I2-E3 wildtype construct: A 257 bp PCR amplicon was generated using the primer pair 7ind2 FP and 7ind2 RP on FY527 genomic DNA. This was then used to generate pDblet SpPtbp1: *nab2*+ E2-I2-E3 construct. The clonings involved were the same as detailed for the *rhb1*+ E1-I1-E2 wildtype construct.
- 5) *nab2* I2+11 construct: To create a variant construct with a 11 bp insertion between the BrP and 3'ss in the *nab2* intron 2, a *nab2* I211loopin RP was designed, which had its 5' end in exon3 and 3' end upstream of BrP and a 11 nt sequence (TTTTTTTTATA from *dim1* intron 1) inserted between the BrP and 3'ss. This primer was used in combination with 7ind2 FP to generate the 122 bp *nab2* I2+11 variant fragment, which was then used to generate pDblet SpPtbp1: *nab2* I2+11 variant construct. The two cloning steps followed were same as detailed before.

6) pDblet SpPtbp1: *bpb-cdc* construct: For this, the primer pair *cdc2_b* F and *cdc2E3* RP were used on the *bpb-cdc* construct (2) as template. The resulting 124 bp PCR product, which had a chimeric intron, with the first 30 nts of *cdc2* intron2 replaced with that of *bpb1* intron1. This amplicon was cloned into pBS(KS) and then pDblet SpPtbp1 to generate pDblet SpPtbp1: *bpb-cdc*, using the same steps as described earlier. All the constructs were sequence verified after cloning into pBS(KS).

Preparation of crude whole cell extracts from *S. pombe* and immunoblot analysis: To assess the expression levels of the wild type and the SpSlu7 C113A mutant protein in *S. pombe*, crude whole cell extracts were prepared from FY527 strains transformed with pREP41MHN-*spslu7*⁺, pREP41MHN-*spslu7*C113A (two independent transformants) and pREP41MHN vector (as a negative control) and *spslu7Δ* strain transformed with pREP41MHN-*spslu7*⁺, grown at 30°C to an O.D. of ~1.0 in absence of thiamine in EMM leu- media. Cells from 10 ml of such a culture was harvested and used for protein extraction (3). The clear crude denatured protein preparation was ~125-150μl of which a 30-40 μl aliquot was resolved on a lane in a 10% SDS-PAGE gel and immunoblotting was performed. The antibodies, monoclonal, anti-His-HRP conjugate (Sigma) was used at 1:3000 dilution. The antibody dilutions and binding was done in 5% non-fat milk prepared in 1X TBS. Incubation was at 25°C for 4 h on a rocking platform. The blots were subsequently developed with SuperSignal West Pico Chemiluminescent Substrate (Pierce).

1. Brun C, Dubey DD, Huberman, JA. 1995. pDblet, a stable autonomously replicating shuttle vector for *Schizosaccharomyces pombe*. *Gene* **164**: 173-177

2. **Sridharan V, Heimiller J, Singh R.** 2011. Genomic mRNA profiling reveals compensatory mechanisms for the requirement of the essential splicing factor U2AF. *Mol Cell Biol* **31**:652-661.
3. **Foiani M, Marini F, Gamba D, Lucchini G, Plevani, P.**1994 The B subunit of the DNA polymerase alpha-primase complex in *Saccharomyces cerevisiae* executes an essential function at the initial stage of DNA replication. *Mol. Cell. Biol.* **14**: 923-933.

Figure S2

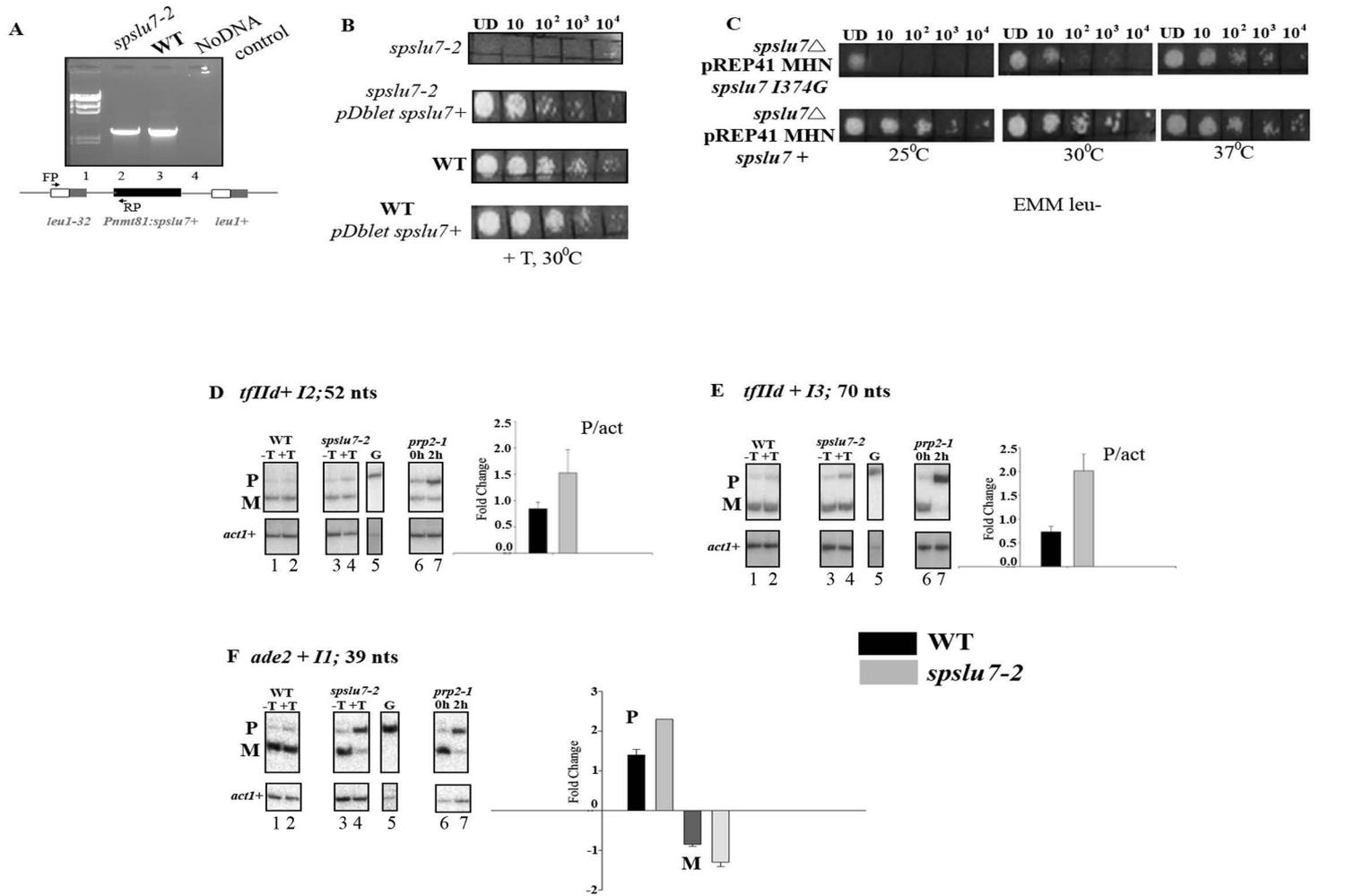


FIG. S2. (A) PCR confirmation of the integration of the *spslu7*⁺ WT (lane 3) and mutant ORF (lane 2) at the *leu1* locus using a forward primer located at the 5'-end of the *leu1*⁺ gene and a reverse primer located at the *Pnmt81* promoter, as shown in the pictorial representation below. DNA marker has been loaded in lane 1 and a PCR with the same primers without the template DNA was run as a control in lane 4. (B) Complementation assay for the rescue of thiamine repression phenotype of the *spslu7-2* mutant. the WT and *spslu7-2* transformed with a plasmid bearing *spslu7*⁺ ORF under its own promoter and growth was checked on thiamine containing EMM leu- medium at 30°C. (C) Growth pattern of *spslu7*Δ cells expressing *nmt41* promoter driven *spslu7*⁺ or *spslu7* I374G from the plasmid pREP41MHN, at 25°C, 30°C and 37°C. Semi-quantitative reverse transcription PCR assays to show splicing status of *tfIID*⁺ I2 (D), I3 (E) and *ade2*⁺ I1 (F). RNA samples are labeled as in Fig. 2. Reverse transcription was done using downstream exon reverse primer followed by limiting cycle PCR in combination with upstream exon forward primer. Pre-mRNA and mRNA levels were calculated by densitometric quantification of the PCR products. The values were normalized to intronless *act1*⁺ levels to obtain the fold change (n=3) of pre-mRNA and message levels in mutant (white bars) compared to wild-type (black bars).

Figure S3

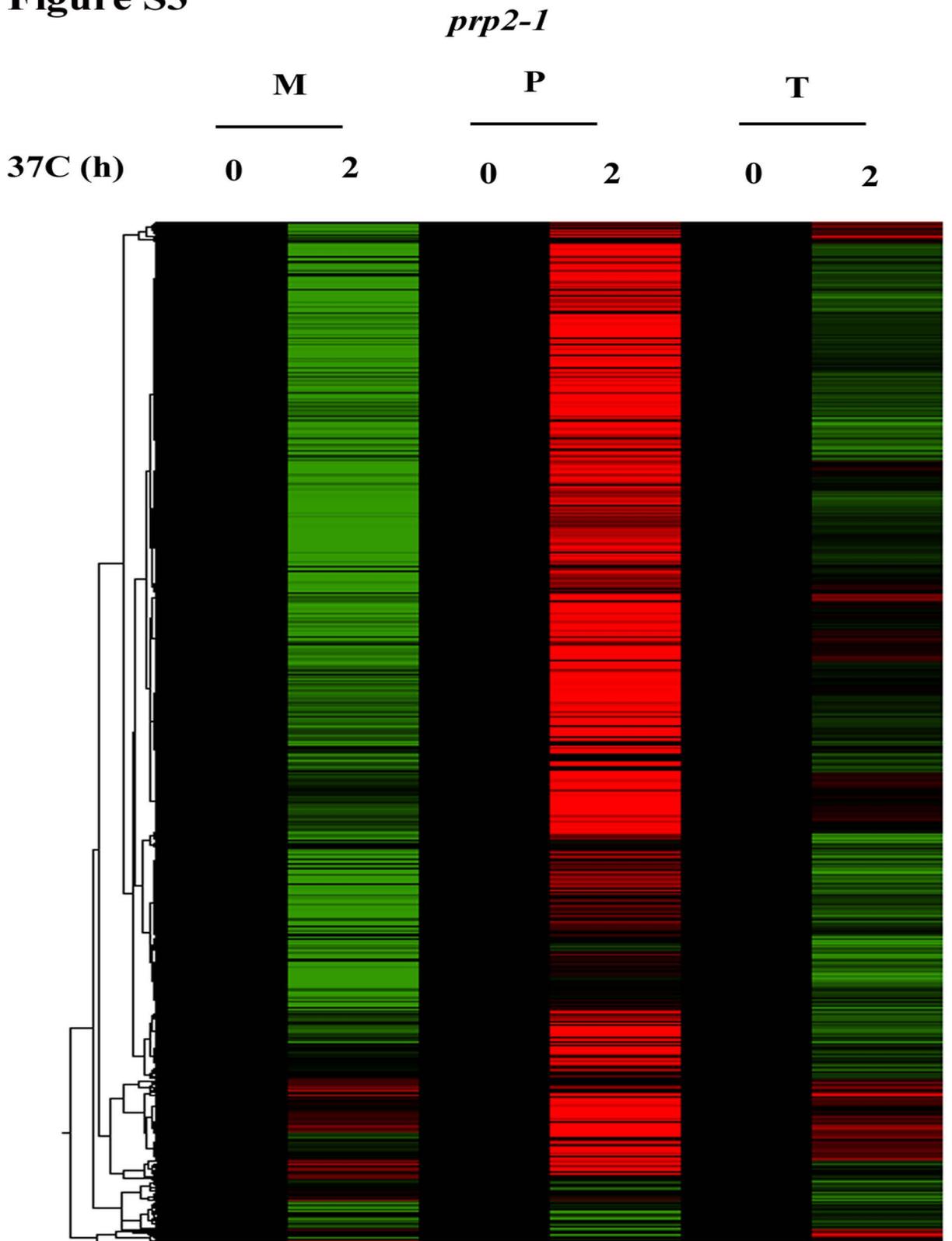


FIG. S3. Heatmap representing the splicing status of pombe introns in *prp2-1* mutant grown at non-permissive temperature for 2 h. Each horizontal row depicts the splicing status of an individual pombe intron.

Figure S4

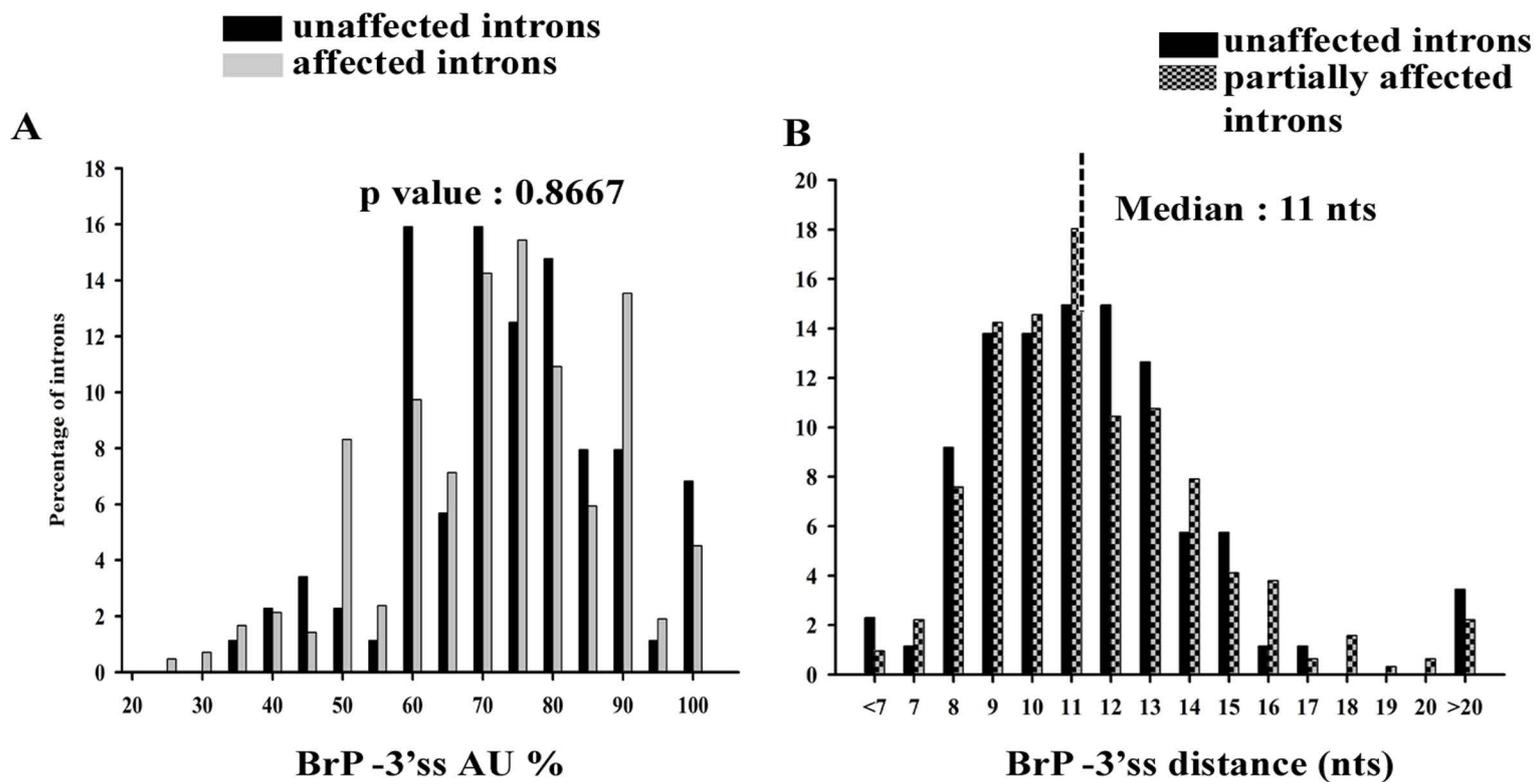
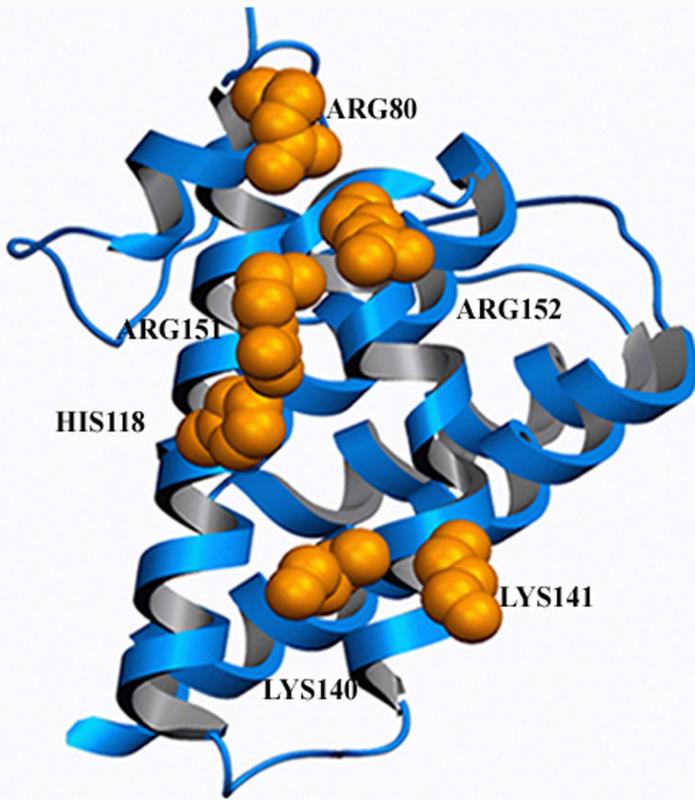
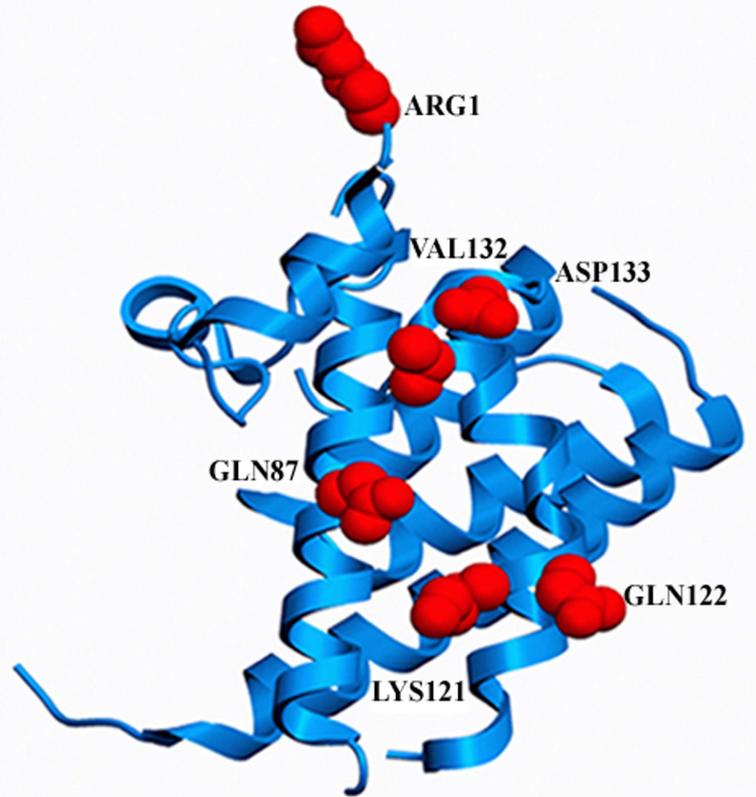


FIG. S4. (A) AU% (X-axis) between the BrP to 3'ss excluding the 3'ss in unaffected (black bar) and affected (grey bar). P-value determined through unpaired Student's t-test is indicated. (B) The intron distribution (Y axis) for the various BrP -3' ss distances for unaffected (black) and partially affected (checkered) introns and the median of this distance in both intron classes is shown.



ScPrp18 Δ 79 structure



SpPrp18 modeled structure

FIG. S5. Comparison of the modeled SpPrp18 structure with the crystal structure of ScPrp18. The 6 amino acid residues that occupy positions equivalent to the charged amino acids of ScPrp18 are indicated in the modeled SpPrp18 structure.

spslu7-2 X prp1-4 : three point cross

spslu7::KanMX6 (A) *leu1::Pnmt81:spslu7I374G* (B) *prp1+* (C)
spslu7+ (a) *leu1-32* (b) *prp1-4* (c)

Predicted outcome :

Parental ditype (PD) : ABC (2) abc (2)

TI (tetratype I) : ABC, Abc (non-viable), aBC, abc

TII (tetratype II) : ABC, **ABc(double mutant)**, abC, abc

TIII (tetratype III) : ABC, AbC (non-viable), aBc, abc

Non-parental ditype I (NPD I) : Abc (2) (non-viable) aBC(2)

Non-parental ditype II (NPD II) : ABc (2) (double mutant) abC(2)

Non-parental ditype III (NPD III) : AbC (2) (non-viable) aBc(2)

WT X prp1-4 : two point cross

leu1::Pnmt81:spslu7+ (A) *prp1+* (B)
leu1-32 (a) *prp1-4* (b)

Predicted outcome :

Parental ditype (PD) : AB, AB, ab, ab

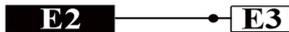
Tetratype : Ab, aB, AB, ab

Non-parental ditype (NPD) : Ab, Ab, aB, aB

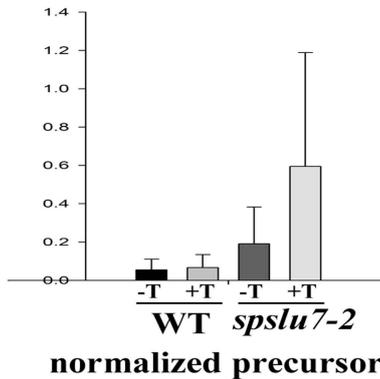
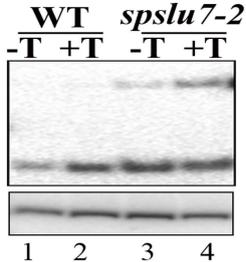
FIG. S6. Summary of the expected spore patterns upon dissecting *spslu7-2 X prp1-4* (top panel) and *WT X prp1-4* (bottom panel).

Figure S7

A *cdc2+* E2-I2-E3
wildtype



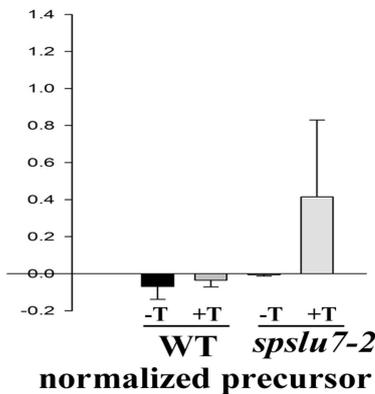
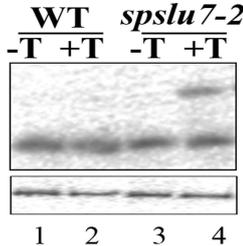
72 nts, BrP-3'ss: 12 nts
AU% (5'ss to Brp): 69%



B *bpb1+* E1-I1-E2
wildtype



46 nts, BrP-3'ss: 9 nts
AU% (5'ss to Brp): 78%



C *bpb-cdc*



48 nts, BrP-3'ss: 12 nts
AU% (5'ss to Brp): 74.2%

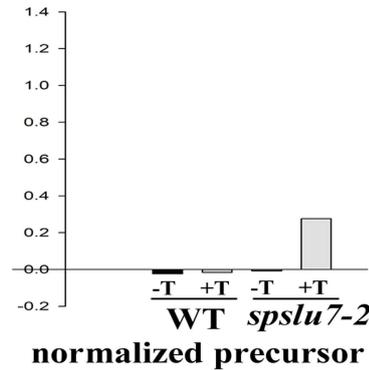
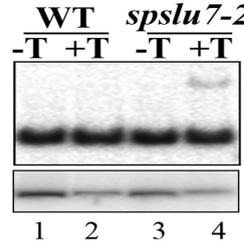


FIG. S7. Representative semi-quantitative RT-PCR assays to assess splicing status of endogenous *cdc2+* I2 (A), *bpb1+* I1 (B) and the *bpb-cdc* chimeric construct (C). Total RNA from WT and mutant cells grown in absence (-T, lanes 1 and 3 respectively) or presence (+T, lanes 2 and 4 respectively) of thiamine for 28 h were used. cDNAs primed with a 3' exon reverse primer (for endogenous transcripts) or mini-transcript specific reverse primer (for chimeric construct) was used with a forward primer in the 5' exon for limiting PCR cycles. Change in normalized pre-mRNA levels for each sample (n=3 for *cdc2+* I2 and *bpb1+* I1) has been plotted as bar graphs. Increased pre-mRNA accumulation in *spslu7-2* -T and +T samples for *cdc2+* I2 indicates higher dependency for this intron on SpSlu7 when compared to *bpb1+* I1. This increased dependency is reduced to levels of *bpb1+* I1 in the *bpb-cdc* chimeric construct.

Table S1

Gene/ intron	Size (nts)	5'ss sequence	3'ss sequence	BrP to 3'ss distance	Py(n) tract
<i>tfIIId+ I1</i>	255	GTAAGT	TAG	17	multiple Py(4) upstream of BrP
<i>tfIIId+ I2</i>	52	GTACGT	TAG	n.a	single Py(4) near 5'ss
<i>tfIIId+ I3</i>	70	GTAAGT	TAG	14	single Py(6) downstream BrP
<i>ade2+ I1</i>	39	GTAAGA	CAG	n.a	none
<i>ade2+ I2</i>	383	GTATGT	TAG	13	multiple Py(4-8) upstream of BrP Py(4) and Py(5) downstream BrP
<i>naa10+ I1</i>	86	GTAAGT	TAG	10	single Py(4) upstream of BrP
<i>naa10+ I2</i>	315	GTAAGT	CAG	16	multiple Py(4-8) upstream of BrP
<i>naa10+ I3</i>	47	GTATGT	TAG	11	single Py(5) upstream of BrP single Py(4) downstream BrP
<i>phospholipase I3</i>	55	GTAAGT	TAG	11	single Py(6) downstream BrP
<i>phospholipase I4</i>	115	GTATGA	TAG	17	multiple Py(4) upstream of BrP single Py(4) downstream BrP
<i>rad24+</i>	407	GTATGT	CAG	9	multiple Py(4-7) upstream of BrP
<i>SPAC19B12+ I3</i>	43	GTATGA	TAG	n.a	single Py(4) near 3'ss
<i>dim1+ I2</i>	81	GTACGC	TAG	19	single Py(9) downstream BrP
<i>rhb1+ I1</i>	59	GTAAGC	TAG	17	single Py(8) downstream BrP
<i>naa25+ I4</i>	40	GTAAGT	TAG	11	single Py(11) upstream of BrP
<i>nab2+I2</i>	46	GTAAGT	CAG	9	none
<i>SPAC 13G7.11+ I2</i>	101	GTACGT	AAG	11	Py(4) and Py(7) upstream of BrP
<i>cdc2+I2</i>	72	GTAAGT	AAG	12	single Py(5) upstream of BrP single Py(6) downstream BrP
<i>bpb1+I1</i>	46	GTAAGT	TAG	9	Two Py(4) upstream of BrP single Py(5) downstream BrP

Supplementary Table 2: Oligonucleotides used for the study.

Primer Name	Sequence
spact1 FP	5' GCTGCTCAATCTTCCTCCCTTG 3'
spact1 RP	5' GGTCCGCTCTCATCATACTCTT 3'
ade2 E1 FP	5' ATGGCTTCAGTGCGAGAAAC 3'
ade2 E2 RP	5' GGATATCAACAAGTTTACCCT 3'
ade2 FP	5' GGGTAAACTTGTTGATATCCTTTG 3'
ade2 RP-1	5' GACCAGCATTGTTACCA 3'
bpb1 F Nde	5'GCGCATATGGCTGAATTCAAGGTCAGTAGGG 3'
bpb1 F Nde	5'GCAGGATCCCTCATTCCATTTGCATCAGG 3'
cdc2.2 F	5'GCAGGATCCCTCATTCCATTTGCATCAGG 3'
cdc2 E3 RP	5' GATACAACCTTTGATTCAGC 3'
dim1 FP	5' GCACGTTGATCAAGCAATT 3'
dim1 RP	5' GATCTCTACCAAATCGAATG 3'
7ind2 FP (nab2 I2)	5' GTATGGTCCTGAGCAGTGAAAT 3'
7ind2 RP (nab2 I2)	5' GGCCGCCATGTCATATTTCTGCTTT 3'
7ind3 FP (SPAC13G7.11 I2)	5' CTTCAATTCTGGTGTCAACA 3'
7ind3 RP (SPAC13G7.11 I2)	5' GGGCACGACTCAAATTAAG 3'
leu1 FP	5' GCGCAATTTCAACAATTCCTATG 3'
naa10 E1 FP	5' CGGCCAGCTCGTATAAGTG 3'
naa10 E2RP	5' GCATAGGCCAAGAAATAGCATG 3'
naa10 E2FP	5' CT AAACGTCTCATGGTTCAAAG 3'
naa10 E3 RP	5' GCAAAGTATCACGATAAAGATGA 3'
naa10 E3 FP	5' AAGAGCCATGGTTCGAAGTTTAT 3'
naa10 E4 RP	5' GCATAAGCATCTTCACCATC 3'
naa10 lariat FP	5' CCTTGATATTTTCGGTCCATTCC 3'
naa10 lariat RP	5' GGATATATCCGTAATCTACTTCGCTC 3'
naa25 FP	5' CGTTTTCTCTTTTCCAAACTGGG 3'
naa25 RP	5' GCTGGTTGACAACGCTTTC 3'
nab2 I2 loopin 20 RP	5' CTTGACACACTGTATTATAAAAAAAAAACGTTAGTAATAGTT 3'
phos E3 FP	5' ATCTATC CCTGTTACCGTCAA 3'
phos E4 RP	5' GTGAAAATCCGCCAATTAATAT 3'
phos E4 FP	5' GGATTCCTAGTGATCGCATATTA 3'
phos E5 RP	5' GGGTATCTCTTTAGCAACTCG 3'
phos lariat FP	5' GTGAAATGGTTTTCACTTTTC 3'
phos lariat RP	5' GCAATGACTAGCGAGTTAAC 3'
Pnmt RP	5' CGATAATTTTCTGATCCTCTAGA 3'
pREP81 FP	5' GTTTTCTAGAATTATTGTTGTCTCTTTATGGTTGG 3'
pREP81 RP	5' CAGCGTATGATTGCTTTTAAATATTTAATTTTCATC 3'
rad24 FP	5' ATGTCTACTACTTCTCGTGAA 3'
rad24 RP-1	5' CAGTGGAGGCAACGCTTTTCATG 3'
rfp FP	5' ATTCGCTTCTGGCTATGT 3'
rfp RP	5' AATAACGACAGCAGTAAGGT 3'
rhb1 FP	5' GCTGTTCTTGGCAGTCGTTTC 3'
rhb1 RP	5' GGTTTTCAACATATTGCACCG 3'

rhb1ATG E1FP	5' ATGGCTCCTATTAATCTCGTAG 3'
rhb1 I1 FP	5' TTTTTTTAAAGAAGTTTATGCGCAAATAG 3'
rhb1 I1 FP	5' TTAAAAAAAAA TCAAACGCTTACCT 3'
rhb1 loopout E2RP	5' ACATATTGCACCGTTAAACTAATGGTTAGTA 3'
snu1	5' GCTGCAGAACTCATGCCAGGTAAGT 3'
snu2	5' GAACAGATACTACACTTGATC 3'
snu4	5' GTTGGAGCGGTCAGGGTAATAG 3'
snu5	5' GATTACAAAACTATACAGTCAAATTAGCAC 3'
snu6	5' CTCTGTATCGTTTCAATTTGACC 3'
spslu7 FP	5' CCGCTCGAGGCATAGATGGGTGCAAC 3'
spslu7 5'UTR FP	5' CCGCTCGAGGCATAGATGGGTGCAAC 3'
spslu7 RP	5' CGGGATCCATCATAAGTCCCCTTCAAC 3'
spslu7 C113A FP	5' TATCGAAAGGGTGCTGCTGAGAATTGTGGAGCC 3'
spslu7 C113A RP	5' GGCTCCACAATTCTCAGCAGCACCCCTTTCGATA 3'
spslu7 I374X FP	5' CCGAAAACCTTAGTTNKGTTGAAGGGG 3'
spslu7 I374X RP	5' CCCCTTCAACMNNACTAAGTTTTTCGG 3'
tfIIId FP-1	5' GTGTATCTGGCATTGTTCCAACCCTTC 3'
tfIIId+ RP	5' GGGTTGTATTCTGCATTACG 3'
tfIIId E2FP	5' CGTAATGCAGAATACAACCC 3'
tfIIId E3RP	5' AAAGTACCGTGGGAGTAAGC 3'
tfIIId E3FP	5' GGCTTACTCCCACGGTACTTT 3'
tfIIId E4RP	5' GACAATACTGGATAAATGGCTTC 3'