

**Fig. S1. Images of wild-type and *ssp2A* cells stained with calcofluor after nitrogen starvation.** Nitrogen starvation accelerates entry into mitosis and cell cycle arrest in G1 phase with a small cell size. Wild-type cells arrested with a small cell size, whereas *ssp2A* cells showed a clear delay in mitotic entry and cells were much longer during the first 4 hours of nitrogen starvation. Scale bar: 10  $\mu$ m.

**Fig. S2. Rst2 subcellular localization.** Rst2-GFP was observed by fluorescence microscopy in wild-type and *ssp2A* cells during exponential growth and 4 hours after nitrogen starvation. Rst2-GFP localized to the nucleus in both strains in the presence or in the absence of nitrogen. Nuclei were stained with Hoechst. Scale bar: 10  $\mu$ m.

**Table S1.** Genes not properly induced after nitrogen starvation in *ssp2* mutant cells.

**Table S2.** Genes not properly repressed after nitrogen starvation in *ssp2* mutant cells.

**Table S3.** Fission yeast strains.

Fig S1

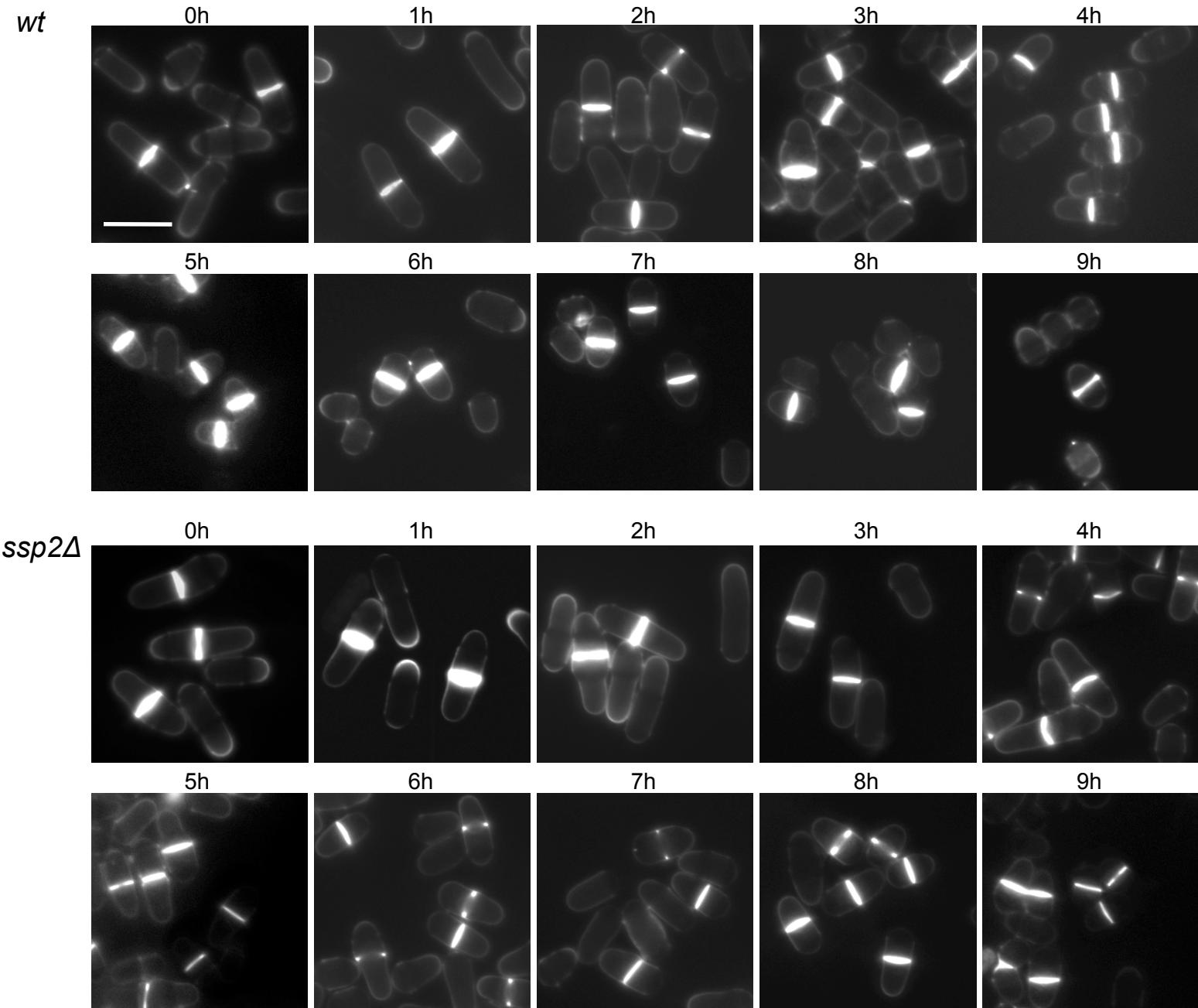
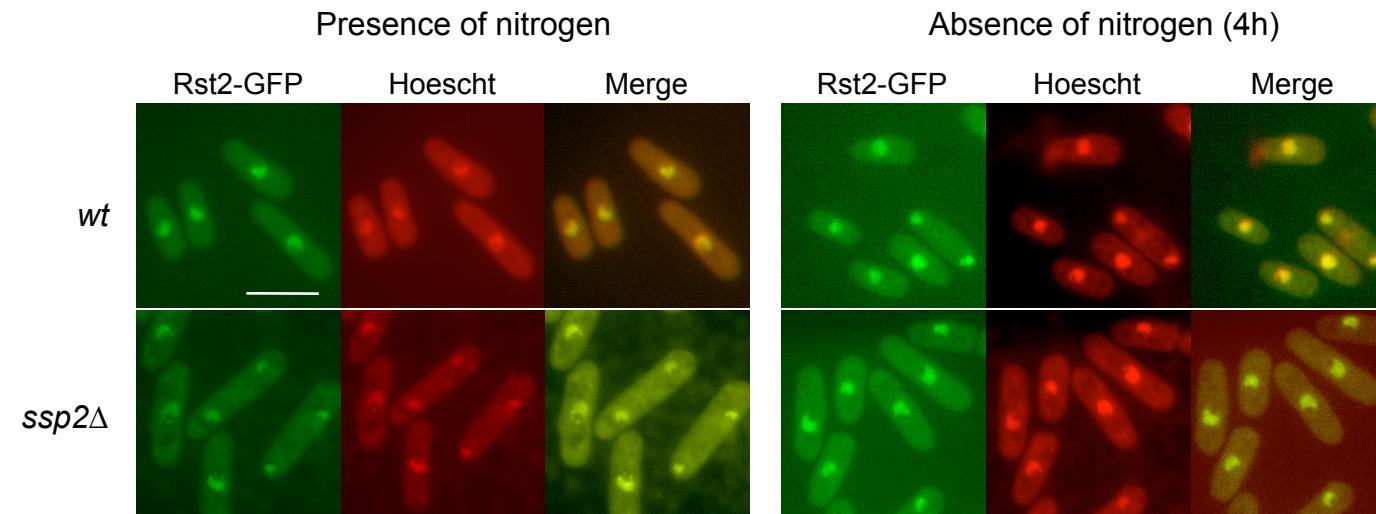


Fig S2



**Table S1.** Genes not properly induced after nitrogen starvation in *ssp2* mutant cells.

Genes	GO Annotation (Biological Process described in GeneDB)
<b>Metabolism</b>	
<i>mae2</i>	Malate metabolic process
<i>SPBC19C7.04c</i>	Biological process
<i>SPBC1E8.05</i>	Biological process
<i>SPCC1322.10</i>	Biological process
<i>SPCC306.11</i>	Biological process
<i>SPBPB7E8.01</i>	Biological process
<i>adg2</i>	Biological process
<i>adg1</i>	Biological process
<i>pcr2</i>	Ubiquinol-cytochrome-c reductase complex core
<i>mug120</i>	Proteolysis
<i>SPAC869.04</i>	Nitrogen compound metabolic process
<i>pex7</i>	Protein targeting to peroxisome
<i>SPCC736.13</i>	Catalytic activity
<i>atg22</i>	Autophagy
<i>SPCC1322.04</i>	Protein amino acid glycosylation
<i>cyc1</i>	Organelle ATP synthesis coupled electron transport
<i>SPCC584.01c</i>	Sulfate assimilation
<i>ssa2</i>	ATP catabolic process
<b>Transporters</b>	
<i>SPBC23G7.13c</i>	Urea transporter
<i>SPAC869.03c</i>	Urea transporter
<i>SPAC3H1.06c</i>	Membrane transporter (predicted)
<i>mac1</i>	Membrane anchored protein
<i>SPBC1683.01</i>	Inorganic phosphate transporter
<i>SPBC3H7.02</i>	Sulfate transporter
<i>ght5</i>	Hexose transporter
<i>ght8</i>	Hexose transporter
<i>ght1</i>	Hexose transporter
<i>SPCC794.04c</i>	Amino acid transporter
<i>SPCC285.05</i>	Purine nucleoside transporter
<b>Others</b>	
<i>ssn6</i>	Transcriptional corepressor
<i>SPAC1705.03c</i>	Intracellular signal transduction
<i>scw1</i>	RNA-binding protein
<b>Conjugation and cell cycle</b>	
<i>ste11</i>	Conjugation with cellular fusion
<i>ste9</i>	Regulation of conjugation with cellular fusion
<i>rgs1</i>	Regulation of pheromone-dependent signal transduction
<i>ste7</i>	Conjugation with cellular fusion
<i>pat1</i>	Negative regulation of conjugation with cellular fusion
<i>mei2</i>	Positive regulation of meiosis
<i>rad25</i>	Regulation of mitotic cell cycle
<i>scr1</i>	Regulation of transcription

<i>mfm1</i>	Conjugation with cellular fusion
<i>mfm2</i>	Conjugation with cellular fusion
<i>mfm3</i>	Conjugation with cellular fusion
<i>mam1</i>	Conjugation with cellular fusion
<i>mam3</i>	Agglutination involved in conjugation with cellular fusion
<i>matmc_1</i>	Conjugation with cellular fusion
<i>matmc_2</i>	Conjugation with cellular fusion
<i>slp1</i>	Activation of anaphase-promoting complex activity
<i>oca2</i>	Regulation of cell cycle
<i>swi2</i>	Mating type switching

#### *Cell wall or spore biogenesis*

<i>pho1</i>	Fungal-type cell wall
<i>SPCC757.12</i>	Fungal-type cell wall biogenesis
<i>pxl1</i>	Negative regulation of Rho GTPase activity,contractile ring contraction involved in cytokinesis
<i>par2</i>	Regulation of septation initiation signaling
<i>mpg1</i>	Cell size control checkpoint,fungal-type cell wall biogenesis
<i>exg1</i>	Cell wall organization and biogenesis
<i>SPAC11E3.13c</i>	Cell wall organization and biogenesis
<i>SPAC19B12.02c</i>	Cell wall organization and biogenesis
<i>SPAPB1E7.04c</i>	Ascospore wall chitin catabolic process,cell separation during cytokinesis
<i>psy1</i>	Ascospore formation
<i>cfh4</i>	fungal-type cell wall polysaccharide biosynthetic process

#### *Cytokinesis and cell separation*

<i>ace2</i>	Cytokinetic cell separation
<i>adg3</i>	Cytokinetic cell separation
<i>eng1</i>	Cytokinetic cell separation
<i>rgf3</i>	Primary cell septum disassembly
<i>mid2</i>	Cytokinetic cell separation
<i>SPBC27.04</i>	Barrier septum formation involved in cell cycle cytokinesis
<i>agn1</i>	Cell septum edging catabolic process
<i>sep1</i>	Barrier septum formation

**Table S2.** Genes not properly repressed after nitrogen starvation in *ssp2* mutant cells.

<b>Conjugation, cell cycle and response to stress</b>	
<i>sro1</i>	Cellular response to stress
<i>rds1</i>	Response to stress
<i>SPBC577.10</i>	20S proteasome component beta 7, regulation of cell cycle
<i>fkh1</i>	Conjugation with cellular fusion
<i>cda1</i>	Ascospore wall assembly
<i>ubc4</i>	Ubiquitin conjugating enzyme Ubc4, cyclin catabolic process
<i>isp5</i>	Conjugation with cellular fusion
<i>mug168</i>	Meiosis
<i>SPAC3C7.02c</i>	Cellular response to heat
<i>SPAC11D3.01c</i>	Cellular response to stress
<i>SPAC4G8.01c</i>	Cellular response to stress
<i>SPAC23H3.15c</i>	Cellular response to oxidative stress
<i>gpd1</i>	Cellular response to osmotic stress
<i>SPBC660.06</i>	Cellular response to stress
<i>vip1</i>	Cellular response to stress
<i>yak3</i>	Cellular response to stress
<i>zym1</i>	Cellular response to stress
<i>SPAC9E9.09c</i>	Cellular response to stress
<i>plr1</i>	Cellular response to stress
<i>SPBC1683.06c</i>	Cellular response to stress
<i>fnx1</i>	Cellular response to stress
<b>Transporters and permeases</b>	
<i>fur4</i>	Cation transport
<i>ctr5</i>	Copper ion transport
<i>SPAC23D3.12</i>	Phosphate transmembrane transport
<i>mfs1</i>	Transmembrane transport
<i>SPBC1683.12</i>	Nicotinamide mononucleotide transmembrane transport
<i>SPBC1683.12</i>	Nicotinamide mononucleotide transmembrane transport
<i>ctr4</i>	Plasma membrane copper ion transport
<i>SPCC74.04</i>	Amino acid transmembrane transport
<i>SPBC13A2.04c</i>	Dipeptide transmembrane transport
<b>Histones</b>	
<i>hta2</i>	DNA repair
<i>hhf1</i>	Chromatin assembly or disassembly
<i>hht1</i>	Chromatin assembly or disassembly
<i>hhf2</i>	Nucleosome assembly
<i>hht2</i>	Nucleosome assembly
<i>hht3</i>	Nucleosome assembly
<i>hhf3</i>	Nucleosome assembly
<i>obr1</i>	Chromatin silencing at silent mating-type cassette
<b>Metabolism</b>	
<i>SPAC1002.12c</i>	Glutamate catabolic process
<i>urg2</i>	Nucleoside metabolic process
<i>urg1</i>	Riboflavin biosynthetic process
<i>hpt1</i>	Nucleoside metabolic process
<i>SPAPB1A11.03</i>	Cellular response to oxidative stress
<i>SPAC3G9.11c</i>	Cellular amino acid catabolic process

<i>aru1</i>	Urea cycle
<i>SPAC1F7.09c</i>	Allantoin catabolic process
<i>snz1</i>	Pyridoxine biosynthesis protein
<i>SPAC1039.02</i>	Nucleotide catabolic process
<i>uga1</i>	Glutamate metabolic process
<i>SPAC922.07c</i>	Cellular aldehyde metabolic process
<i>SPAC869.06c</i>	Biological process
<i>SPAC869.01</i>	Biological process
<i>eno102</i>	Glycolysis
<i>mal1</i>	Maltose catabolic process
<i>SPCC61.06</i>	L-methionine biosynthetic process from L-homoserine via cystathione
<i>SPBC12C2.07c</i>	Spermidine biosynthetic process
<i>ura4</i>	pyrimidine nucleotide biosynthetic process
<i>thi2</i>	thiamine biosynthetic process
<b>Translation initiation and elongation factors</b>	
<i>tif1</i>	Translational initiation
<i>tif512</i>	Translational frameshifting
<i>efla-a</i>	Translational elongation
<i>efla-b</i>	Translational elongation
<i>efla-c</i>	Translational elongation
<b>Others</b>	
<i>btf3</i>	Cotranslational protein targeting to membrane
<i>aes1</i>	Enhancer of RNA-mediated gene silencing
<i>srp7</i>	SRP-dependent cotranslational protein targeting to membrane
<i>mmf1</i>	Mitochondrial genome maintenance
<i>SPAC922.04</i>	Biological process
<b>Ribosomal proteins and rRNA</b>	
<i>ubi1</i>	Ribosome biogenesis
<i>rps1102</i>	40S ribosomal protein S11
<i>rps602</i>	40S ribosomal protein S6
<i>rps1401</i>	40S ribosomal protein S14
<i>rps2401</i>	40S ribosomal protein S24
<i>rps7</i>	40S ribosomal protein S7 (predicted)
<i>SPAC13G6.07c</i>	40S ribosomal protein S6
<i>rps102</i>	40S ribosomal protein S3a (predicted)
<i>rps2201</i>	40S ribosomal protein S15a
<i>rps23</i>	40S ribosomal protein S23
<i>rps1101</i>	40S ribosomal protein S11
<i>rps002</i>	40S ribosomal protein S0B"
<i>rps801</i>	40S ribosomal protein S8
<i>rps1701</i>	40S ribosomal protein S17
<i>rps1002</i>	40S ribosomal protein S10
<i>rps1601</i>	40S ribosomal protein S16
<i>rps902</i>	40S ribosomal protein S9
<i>rps401</i>	40S ribosomal protein S4
<i>rps1901</i>	40S ribosomal protein S19
<i>rps3</i>	40S ribosomal protein S3
<i>rps1501</i>	40S ribosomal protein S15
<i>rps1702</i>	40S ribosomal protein S17
<i>rps2</i>	40S ribosomal protein S2

<i>rpl15</i>	60S ribosomal protein L15
<i>rpl3702</i>	60S ribosomal protein L37
<i>rpl2702</i>	60S ribosomal protein L27
<i>rpl39</i>	60S ribosomal protein L39
<i>rpl6</i>	60S ribosomal protein L6
<i>rpl1702</i>	60S ribosomal protein L17
<i>rpl1201</i>	60S ribosomal protein L12.1/L12A
<i>rpl1202</i>	60S ribosomal protein L12.1/L12A
<i>rpl2802</i>	60S ribosomal protein L27a.2/L28A
<i>rpl124</i>	60S ribosomal protein L24
<i>rpl2002</i>	60S ribosomal protein L20
<i>rpl1001</i>	60S ribosomal protein L11
<i>rpl2102</i>	60S ribosomal protein L21
<i>rpl501</i>	60S ribosomal protein L5
<i>rpl1502</i>	60S ribosomal protein L15b
<i>rpl1901</i>	60S ribosomal protein L9
<i>rpl2001</i>	60S ribosomal protein L20a
<i>rpl301</i>	60S ribosomal protein L3
<i>rpl114</i>	60S ribosomal protein L14 (predicted)
<i>rpl1002</i>	60S ribosomal protein L10
<i>rpl22</i>	60S ribosomal protein L22
<i>rpl4101</i>	60S ribosomal protein L41
<i>rpl4301</i>	60S ribosomal protein L37a
<i>rpl2501</i>	60S ribosomal protein L25
<i>rpl1803</i>	60S ribosomal protein L8
<i>rpl1601</i>	60S ribosomal protein L13/L16
<i>rpl26</i>	60S ribosomal protein L26
<i>rpl802</i>	60S ribosomal protein L8
<i>rpl1701</i>	60S ribosomal protein L17
<i>rpl8</i>	60S ribosomal protein L7a (L8)
<i>rpl401</i>	60S ribosomal protein L2
<i>rpl1102</i>	60S ribosomal protein L11
<i>rpl21</i>	60S ribosomal protein L21
<i>rpl3602</i>	60S ribosomal protein L36
<i>rpl2801</i>	60S ribosomal protein L128B 27a
<i>rpl402</i>	60S ribosomal protein L2
<i>rpp201</i>	60S acidic ribosomal protein P2A subunit
<i>rpl1901</i>	60S ribosomal protein L19
<i>rpl35</i>	60S ribosomal protein L35
<i>rpl902</i>	60S ribosomal protein L9
<i>rpl2402</i>	60S ribosomal protein L24
<i>SPAC11D3.01c</i>	5s rRNA
<i>5SrRNA</i>	5SrRNA
<i>SPRRNA.12</i>	5S rRNA
<i>SPRRNA.13</i>	5S rRNA
<i>SPRRNA.16</i>	5S rRNA
<i>SPRRNA.17</i>	5S rRNA
<i>SPRRNA.18</i>	5S rRNA
<i>SPRRNA.19</i>	5S rRNA
<i>SPRRNA.28</i>	5S rRNA
<i>SPRRNA.29</i>	5S rRNA
<i>SPRRNA.30</i>	5S rRNA
<i>SPRRNA.38</i>	5S rRNA

<i>SPRRNA.34</i>	5S rRNA
<i>SPRRNA.36</i>	5S rRNA
<i>SPRRNA.31</i>	5S rRNA
<i>SPRRNA.32</i>	5S rRNA
<i>SPRRNA.33</i>	5S rRNA
<i>SPRRNA.39</i>	5S rRNA
<i>5S rRNA</i>	5S rRNA
<i>SPRRNA.26</i>	5S rRNA
<i>SPRRNA.04</i>	5S rRNA
<i>SPRRNA.06</i>	5S rRNA
<i>rRNA</i>	rRNA
<i>U3snRNA</i>	small nuclear RNA U3

**Table S3.** Fission yeast strains

Strain	Genotype	Source
<i>wt</i>	<i>h-</i> 972	P. Nurse
<i>ssp2-HA</i>	<i>h- ssp2-HA:KanMX6</i>	This study
<i>amk2-TAP</i>	<i>h- amk2-TAP:KanMX6</i>	This study
<i>cbs2-TAP</i>	<i>h- cbs2-TAP:KanMX6</i>	This study
<i>ssp2-HA amk2-TAP</i>	<i>h- ssp2-HA:KanMX6 amk2-TAP:KanMX6</i>	This study
<i>ssp2-HA cbs2-TAP</i>	<i>h- ssp2-HA:KanMX6 cbs2-TAP:KanMX6</i>	This study
<i>ssp1Δ</i>	<i>h- ssp1::ura4+ ura4-d18</i>	This study
<i>ssp2Δ</i>	<i>h+ ssp2::ura4+ ura4-d18</i>	This study
<i>ssp2Δ pREP3X-ste11+</i>	<i>h- ssp2::ura4+ ura4-d18 leu1-32 pREP3x-ste11+</i>	This study
<i>amk2Δ</i>	<i>h+ amk2::KanMX4</i>	YGR/C/NBRP*
<i>cbs2Δ</i>	<i>h+ cbs2::KanMX4</i>	YGR/C/NBRP*
<i>rst2-gfp</i>	<i>h- rst2-gfp:KanMX6</i>	Lab stock
<i>rst2-gfp ssp2Δ</i>	<i>h+ ssp2::ura4+ rst2-gfp:KanMX6 ura4-d18</i>	This study
<i>rst2-HA</i>	<i>h- rst2-HA:KanMX6</i>	Lab stock
<i>rst2-HA ssp2Δ</i>	<i>h+ rst2-HA:KanMX6 ssp2::ura4+ ura4-d18</i>	This study
<i>ste11-gfp</i>	<i>h- ste11-gfp:KanMX6</i>	Lab stock
<i>ste11-gfp ssp2Δ</i>	<i>h+ ste11-gfp:KanMX6 ssp2::ura4+ ura4-d18</i>	This study
<i>ssp2-gfp</i>	<i>h- ssp2-gfp:KanMX6</i>	This study
<i>ssp2-gfp ssp1Δ</i>	<i>h- ssp2-gfp:KanMX6 ssp1::ura4+ ura4-d18</i>	This study
<i>amk2-gfp</i>	<i>h- amk2-gfp:KanMX6</i>	This study
<i>cbs2-gfp</i>	<i>h- cbs2-gfp:KanMX6</i>	This study
<i>ssp2T189A</i>	<i>h- ssp2::ura4+ ura4-d18 leu1-32::pJK148-</i>	This study

	<i>ssp2T189A</i>	
<i>ssp2T189A-gfp</i>	<i>h- ssp2::ura4+ ura4-d18 leu1-32::pJK148-ssp2T189A-gfp:KanMX6</i>	This study
<i>ssp2T189E</i>	<i>h- ssp2::ura4+ ura4-d18 leu1-32::pJK148-ssp2T189E</i>	This study
<i>ssp2T189E ssp1Δ</i>	<i>h- ssp1::ura4+ ura4-d18 leu1-32::pJK148-ssp2T189E</i>	This study
<i>ssp2+</i>	<i>h- ssp2::ura4+ ura4-d18 leu1-32::pJK148-ssp2+</i>	This study

\* Yeast Genetic Resource Center of Japan supported by the National BioResource Project (YGRG/NBRP; <http://yeast.lab.nig.ac.jp/nig/>)