

**Supplementary Table 1 | S. pombe strains****Figure 1C,D**

SK891 *h+* *leu1 mad1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SM001 *h-* *leu1 mad1-RLK/AAA(R580A/L581A/K582A)-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 ST146 *h+* *leu1 mad1-RLK/ALA(R580A/K582A)-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SM090 *h+* *mad1-1-600-GFP<<kanNT3 plo1+-mCherry<<natR nda3-KM311*  
 SM091 *h+* *mad1-1-526-GFP<<kanNT3 plo1+-mCherry<<natR nda3-KM311*

**Figure 1E**

SK842 *h+* *leu1 mad2+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SL905 *h-* *leu1 mad2+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311 mad1-RLK/AAA*  
 SL759 *h-* *leu1 mad3+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SL907 *h-* *leu1 mad3+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311 mad1-RLK/AAA*

**Figure 1F,G**

SK891 *h+* *leu1 mad1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SK893 *h+* *leu1 ade6-M216 mad1Δ::ura4+ plo1+-mCherry<<natR nda3-KM311*  
 SM001 *h-* *leu1 mad1-RLK/AAA-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 ST146 *h+* *leu1 mad1-RLK/ALA-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SM090 *h+* *mad1-1-600-GFP<<kanNT3 plo1+-mCherry<<natR nda3-KM311*  
 SM091 *h+* *mad1-1-526-GFP<<kanNT3 plo1+-mCherry<<natR nda3-KM311*

**Figure 1H**

SK891 *h+* *leu1 mad1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SM001 *h-* *leu1 mad1-RLK/AAA-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 ST146 *h+* *leu1 mad1-RLK/ALA-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SM090 *h+* *mad1-1-600-GFP<<kanNT3 plo1+-mCherry<<natR nda3-KM311*  
 SM091 *h+* *mad1-1-526-GFP<<kanNT3 plo1+-mCherry<<natR nda3-KM311*

**Figure 1J**

SK892 *h+* *leu1 mad1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 ST045 *h-* *bub1-STT/AAA mad1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*

**Figure 1K**

SL901 *h+* *leu1 bub1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SP705 *h-* *leu1 bub1-STT/AAA-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SK892 *h+* *leu1 mad1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 ST045 *h-* *bub1-STT/AAA mad1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*

**Figure 1L**

SL901 *h+* *leu1 bub1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SI437 *h+* *leu1 ade6-M216 (ura4DS/E?) bub1::ura4+ plo1+-GFP<<kanR nda3-KM311*  
 SP705 *h-* *leu1 bub1-STT/AAA-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*

**Figure 2A,B,C**

SK891 *h+* *leu1 mad1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SK893 *h+* *leu1 ade6-M216 mad1Δ::ura4+ plo1+-mCherry<<natR nda3-KM311*  
 SM814 *h+* *ade6-M216 mad1Δ::ura4+ pDUAL-Pnmt81-mis12-mad1+-GFP<<leu1+ plo1+-mCherry<<natR nda3-KM311*  
 SM815 *h+* *ade6-M216 mad1Δ::ura4+ pDUAL-Pnmt81-mis12-mad1-RLK/AAA-GFP<<leu1+ plo1+-mCherry<<natR nda3-KM311*  
 SM827 *h+* *(ade6-M216?) mad1Δ::ura4+ pDUAL-Pnmt81-mad1+-GFP<<leu1+ plo1+-mCherry<<natR nda3-KM311*  
 SM828 *h+* *(ade6-M216?) mad1Δ::ura4+ pDUAL-Pnmt81-mad1-RLK/AAA-GFP<<leu1+ plo1+-mCherry<<natR nda3-KM311*

**Figure 2D,E**

SK891 *h+* *leu1 mad1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SL777 *h+* *leu1 ura4? his2? ade6-M216 plo1+-mCherry<<natR mad1+-GFP<<kanR bub1Δ::ura4+ nda3-KM311*  
 ST045 *h-* *bub1-STT/AAA mad1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SM814 *h+* *ade6-M216 mad1Δ::ura4+ pDUAL-Pnmt81-mis12-mad1+-GFP<<leu1+ plo1+-mCherry<<natR nda3-KM311*  
 ST150 *h+* *ade6-M216 mad1Δ::ura4+ bub1Δ::ura4+ Pnmt81-mis12-mad1-GFP<<leu1+ plo1+-mCherry<<natR nda3-KM311*  
 ST490 *h+* *mad1Δ::ura4+ bub1-STT/AAA Pnmt81-mis12-mad1-GFP<<leu1+ plo1+-mCherry<<natR nda3-KM311*

**Figure 2F**

SK891 *h+* *leu1 mad1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SK893 *h+* *leu1 ade6-M216 mad1Δ::ura4+ plo1+-mCherry<<natR nda3-KM311*  
 SM814 *h+* *ade6-M216 mad1Δ::ura4+ pDUAL-Pnmt81-mis12-mad1+-GFP<<leu1+ plo1+-mCherry<<natR nda3-KM311*  
 SM815 *h+* *ade6-M216 mad1Δ::ura4+ pDUAL-Pnmt81-mis12-mad1-RLK/AAA-GFP<<leu1+ plo1+-mCherry<<natR nda3-KM311*  
 SM827 *h+* *(ade6-M216?) mad1Δ::ura4+ pDUAL-Pnmt81-mad1+-GFP<<leu1+ plo1+-mCherry<<natR nda3-KM311*  
 SM828 *h+* *(ade6-M216?) mad1Δ::ura4+ pDUAL-Pnmt81-mad1-RLK/AAA-GFP<<leu1+ plo1+-mCherry<<natR nda3-KM311*  
 ST162 *h-* *mad1+-GFP<<kanR mad2+-mCherry<<natR nda3-KM311*  
 ST167 *h-* *(ura4-D18?) mad2+-mCherry<<natR mad1Δ::ura4+ pDUAL-Pnmt81-mis12-mad1+-GFP<<leu1+ nda3-KM311*  
 ST168 *h-* *(ura4-D18?) mad2+-mCherry<<natR mad1Δ::ura4+ pDUAL-Pnmt81-mis12-mad1-RLK/AAA-GFP<<leu1+ nda3-KM311*  
 ST170 *h-* *(ura4-D18?) mad2+-mCherry<<natR mad1Δ::ura4+ pDUAL-Pnmt81-mad1+-GFP<<leu1+ nda3-KM311*

**Figure 3B,C,F**

SK891 *h+* *leu1 mad1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 ST174 *h-* *leu1 mad1-EDD/QNN(E670Q/D673N/D676N)-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 ST190" *h-* *leu1 mad1-1-661-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*

**Figure 3D,E**

SK891 *h+* *leu1 mad1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SK893 *h+* *leu1 ade6-M216 mad1Δ::ura4+ plo1+-mCherry<<natR nda3-KM311*  
 ST174 *h-* *leu1 mad1-EDD/QNN-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 ST190" *h-* *leu1 mad1-1-661-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*

**Figure 3G**

ST162 *h-* *mad1+-GFP<<kanR mad2+-mCherry<<natR nda3-KM311*  
 ST443 *h+* *leu1 mad1-EDD/QNN-GFP<<kanR mad2+-mCherry<<natR nda3-KM311*  
 ST448 *h-* *leu1 mad1+-GFP<<kanR mad2-R133A-mCherry<<natR nda3-KM311*  
 ST460 *h+* *ade6-M216 leu1 mad1-1-661-GFP<<kanR mad2+-mCherry<<natR nda3-KM311*  
 ST486 *h+* *leu1 mad1-EDD/QNN-GFP<<kanR mad2-R133A-mCherry<<natR nda3-KM311*  
 ST488 *h+* *leu1 mad1-aa1-661-GFP<<kanR mad2-R133A-mCherry<<natR nda3-KM311*

**Figure 3H**

ST167 *h-* *(ura4-D18?) mad2+-mCherry<<natR mad1Δ::ura4+ pDUAL-Pnmt81-mis12-mad1+-GFP<<leu1+ nda3-KM311*  
 ST168 *h-* *(ura4-D18?) mad2+-mCherry<<natR mad1Δ::ura4+ pDUAL-Pnmt81-mis12-mad1-RLK/AAA-GFP<<leu1+ nda3-KM311*  
 ST447 *h+* *ade6-M216 mad1Δ::ura4+ pDUAL-Pnmt81-mis12-mad1+-GFP<<leu1+ mad2-R133A-mCherry<<natR nda3-KM311*

**Figure S1A,C**

SK891 *h+* *leu1 mad1+-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 SM001 *h-* *leu1 mad1-RLK/AAA-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*  
 ST146 *h+* *leu1 mad1-RLK/ALA-GFP<<kanR plo1+-mCherry<<natR nda3-KM311*

SM090 h+ mad1-1-600-GFP<<kanNT3 plo1+mCherry<<natR nda3-KM311  
SM091 h+ mad1-1-526-GFP<<kanNT3 plo1+mCherry<<natR nda3-KM311

**Figure S1B**

SL894 h+ leu1 ade6-M210 ark1+-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SL891 h+ leu1 ark1+-GFP<<kanR plo1+mCherry<<natR nda3-KM311 mad1-RLK/AAA  
SL900 h- leu1 bub1+-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SL898 h- leu1 bub1+-GFP<<kanR plo1+mCherry<<natR nda3-KM311 mad1-RLK/AAA  
SM092 h- leu1 ade6-M216 bub3+-S(GGGGS)3-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SL358 h- leu1 bub3+-S(GGGGS)3-GFP<<kanR plo1+mCherry<<natR nda3-KM311 mad1-RLK/AAA

**Figure S1D**

SK842 h+ leu1 mad2+-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SL905 h- leu1 mad1Δ::mad1-RLK/AAA mad2+-GFP<<kanR plo1+mCherry<<natR nda3-KM311

**Figure S1F**

SP784 h+ leu1 rpl42::cyhR(sP56Q) bub1Δ::rpl42+hphNT1  
PX938 h- leu1 bub1+-GFP<<kanR  
SP297 h- leu1 bub1-STT/AAA-GFP<<kanR

**Figure S1G**

SK442 h+ leu1 bub1+-GFP<<kanR plo1+mCherry<<natR  
SP705 h- leu1 bub1-STT/AAA-GFP<<kanR plo1+mCherry<<natR nda3-KM311

**Figure S1H**

SK892 h+ leu1 mad1+-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST045 h- bub1-STT/AAA mad1+-GFP<<kanR plo1+mCherry<<natR nda3-KM311

**Figure S1I**

SK713 h- leu1 plo1+mCherry<<natR mad2+-GFP<<kanR nda3-KM311  
ST053 h+ leu1 bub1-STT/AAA mad2+-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SL759 h- leu1 mad3+-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST051 h+ leu1 bub1-STT/AAA mad3+-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SM092 h- leu1 ade6-M216 bub3+-S(GGGGS)3-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST050 h- leu1 bub1-STT/AAA bub3+-S(GGGGS)3-GFP<<kanR plo1+mCherry<<natR nda3-KM311

**Figure S2B**

SK891 h+ leu1 mad1+-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SK893 h+ leu1 ade6-M216 mad1Δ::ura4+ plo1+mCherry<<natR nda3-KM311  
ST141 h+ leu1 ade6-M216 GCN4(250-277)-mad1-306-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST441 h+ leu1 GCN4(250-277)-mad1-458-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST408 h- leu1 GCN4(250-277)-mad1-564-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST417 h+ leu1 ade6-M216 GCN4(250-277)-mad1-585-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311

**Figure S2C,D**

SK891 h+ leu1 mad1+-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST141 h+ leu1 ade6-M216 GCN4(250-277)-mad1-306-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST442 h+ leu1 GCN4(250-277)-mad1-458-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST408 h- leu1 GCN4(250-277)-mad1-564-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST417 h+ leu1 ade6-M216 GCN4(250-277)-mad1-585-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311

**Figure S2E**

ST162 h- mad1+-GFP<<kanR mad2+mCherry<<natR nda3-KM311  
ST748 h- leu1 (ade6-M216?) GCN4(250-277)-mad1-306-676-GFP<<kanR mad2+mCherry<<natR nda3-KM311  
ST750 h+ leu1 (ade6-M216?) GCN4(250-277)-mad1-458-676-GFP<<kanR mad2+mCherry<<natR nda3-KM311  
ST752 h- leu1 (ade6-M216?) GCN4(250-277)-mad1-564-676-GFP<<kanR mad2+mCherry<<natR nda3-KM311  
ST754 h+ leu1 (ade6-M216?) GCN4(250-277)-mad1-585-676-GFP<<kanR mad2+mCherry<<natR nda3-KM311

**Figure S2F**

SK891 h+ leu1 mad1+-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SK893 h+ leu1 ade6-M216 mad1Δ::ura4+ plo1+mCherry<<natR nda3-KM311  
ST141 h+ leu1 ade6-M216 GCN4(250-277)-mad1-306-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST142 h+ leu1 ade6-M216 GCN4(250-277)-mad1-306-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST441 h+ leu1 GCN4(250-277)-mad1-458-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST442 h+ leu1 GCN4(250-277)-mad1-458-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST408 h- leu1 GCN4(250-277)-mad1-564-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST409 h- leu1 ade6-M216 GCN4(250-277)-mad1-564-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST417 h+ leu1 ade6-M216 GCN4(250-277)-mad1-585-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST419 h- leu1 ade6-M216 GCN4(250-277)-mad1-585-676-GFP<<kanR plo1+mCherry<<natR nda3-KM311

**Figure S3A**

SM822 h+ plo1+mCherry<<natR mad1+-GFP<<kanR cdc25-22  
SK671 h- lys1 hph<<ark1-as3 (L166A, S229A) plo1+mCherry<<natR mad1+-GFP<<kanR nda3-KM311  
SK676 h- lys1 hph<<ark1-as3 (L166A, S229A) plo1+mCherry<<natR bub1+-GFP<<kanR nda3-KM311

**Figure S3B**

SL355 h+ leu1 (ade6-M216?) nda3-KM311 bub1+-HA<<hph bub3+S(GGGGS)3-GFP<<kanR plo1+mCherry<<natR

**Figure S3C**

ST162 h- mad2+mCherry<<natR mad1+-GFP<<kanR nda3-KM311  
ST167 h- (ura4-D18?) mad2+mCherry<<natR mad1Δ::ura4+ pDUAL-Pnmt81-mis12-mad1+-GFP<<leu1+ nda3-KM311  
ST168 h- (ura4-D18?) mad2+mCherry<<natR mad1Δ::ura4+ pDUAL-Pnmt81-mis12-mad1-RLK/AAA-GFP<<leu1+ nda3-KM311  
ST170 h- (ura4-D18?) mad2+mCherry<<natR mad1Δ::ura4+ pDUAL-Pnmt81-mad1+-GFP<<leu1+ nda3-KM311  
ST171 h- (ura4-D18?) mad2+mCherry<<natR mad1Δ::ura4+ pDUAL-Pnmt81-mad1-RLK/AAA-GFP<<leu1+ nda3-KM311

**Figure S4A**

SP903 h- leu1 mad1-L533A-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SP965 h? leu1 ade6-M216 mad1-F592A-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SP986 h+ leu1 mad1-R593A-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SP983 h+ leu1 mad1-V596A-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SP967 h+ leu1 mad1-G601A-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST115 h+ leu1 ade6-M216 mad1-R613A-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SP476 h+ leu1 ade6-M216 mad1-S616A-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SP511 h+ leu1 mad1-P661A-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SP513 h+ leu1 mad1-P661A/S633G-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
SP597 h- leu1 mad1-ELK/AAA(E670A/L672A/K674A)-GFP<<kanR plo1+mCherry<<natR nda3-KM311  
ST148 h- leu1 mad1-ELK/AAA plo1+mCherry<<natR nda3-KM311

**Figure S4B,D**

SK891	h+	<i>leu1 mad1+-GFP&lt;&lt;kanR plo1+-mCherry&lt;&lt;natR nda3-KM311</i>
ST174	h-	<i>leu1 mad1-EDD/QNN-GFP&lt;&lt;kanR plo1+-mCherry&lt;&lt;natR nda3-KM311</i>
ST190"	h-	<i>leu1 mad1-1-661-GFP&lt;&lt;kanR plo1+-mCherry&lt;&lt;natR nda3-KM311</i>

**Figure S4C**

ST162	h-	<i>mad1-GFP&lt;&lt;kanR mad2+-mCherry&lt;&lt;natR nda3-KM311</i>
ST443	h+	<i>leu1 mad1-EDD/QNN-GFP&lt;&lt;kanR mad2+-mCherry&lt;&lt;natR nda3-KM311</i>
ST460	h+	<i>ade6-M216 leu1 mad1-1-661-GFP&lt;&lt;kanR mad2+-mCherry&lt;&lt;natR nda3-KM311</i>

**Figure S4G**

SK443	h-	<i>leu1 plo1+-mCherry&lt;&lt;natR bub1+-GFP&lt;&lt;kanR</i>
ST764'	h-	<i>(leu1?) mad1-aa1-661 bub1+-GFP&lt;&lt;kanR plo1+-mCherry&lt;&lt;natR nda3-KM311</i>
ST766	h-	<i>leu1 (ura4-D18?) mad1-EDD/QNN bub1+-GFP&lt;&lt;kanR plo1+-mCherry&lt;&lt;natR nda3-KM311</i>

**Figure S4H**

JY265	h-	<i>leu1</i>
SP010	h-	<i>leu1 mad1Δ::ura4+</i>
ST812	h+	<i>leu1 rpl42::cyhR(sP56Q) mad1-aa1-661</i>
ST812'	h+	<i>leu1 rpl42::cyhR(sP56Q) mad1-aa1-661</i>
ST481	h+	<i>leu1 mad1-EDD/QNN</i>