A

Control | 12.5µg/ml TBZ

wt

cdc13-117

B

$-$ | p*cdc13*

p*dam1(1-127)*

C

mal3-1

Control | 7µg/ml TBZ

Vector

p*mal3*

p*dam1(1-127)*

D

*mal3-1 Ch16 (ade6-M216)*


Vector | p*mal3* | p*dam1(1-127)*

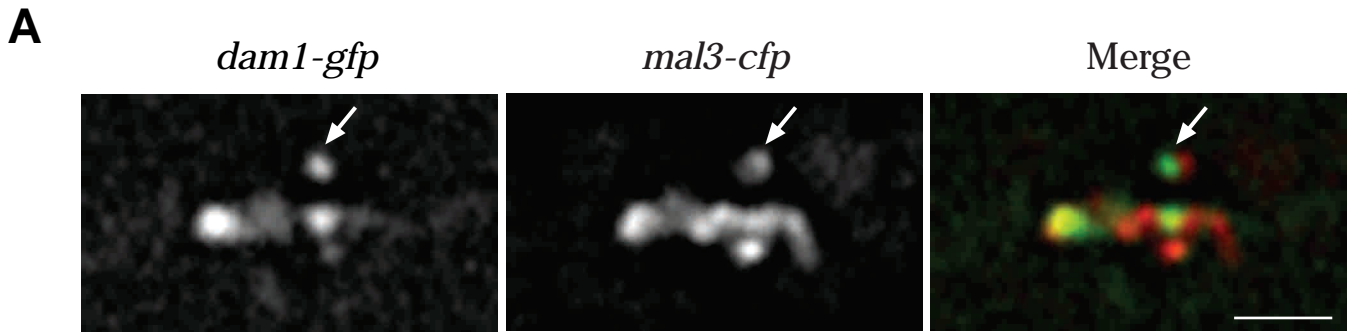Supplementary Figure 1. Sanchez-Perez et al.

Supplementary Figure 1. *dam1(1-127)* suppresses the sensitivity of *cdc13-117* and *mal3-1* cells to thiabendazole.

(A) Wild type or *cdc13-117* cells were plated on rich medium either in the absence or presence of 12.5μg/ml TBZ and incubated for 2 days at 30ºC. (B) *cdc13-117* cells transformed with either empty vector pUR19, pUR19-*dam1*(1-127) or pUR19-*cdc13* were streaked on selective medium either in the absence (control) or presence of 12.5μg/ml TBZ and incubated for 3 days at 30ºC. (C) Serial dilutions of *mal3-1* cells transformed with either empty pUR19 plasmid (Vector), pUR19-*mal3* (p*mal3*), or pUR19-*dam1(1-127)* (p*dam1(1-127)*) were plated either on EMM medium lacking uracil either in the absence (control) or presence of 7μg/ml TBZ. (D) *mal3-1* Ch16 (*ade6-M216*) transformed with the same plasmids as in (A) were plated onto EMM minimal medium lacking uracil and limiting adenine. Colonies were photographed after 3 days at 30ºC and the percentage of chromosome loss per cell division calculated.

A
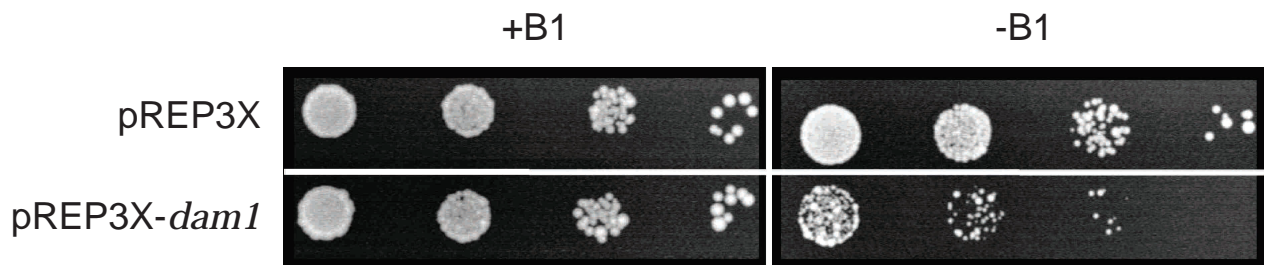
B
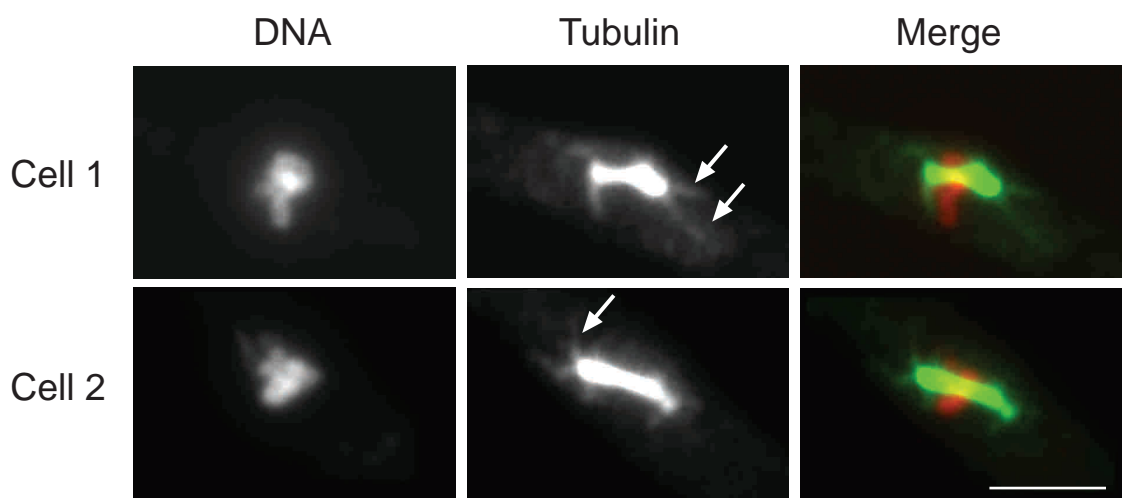
Supplementary Figure 2. Overexpression of *dam1* arrests cells in mitosis.

(A) Full length *dam1* was expressed from the wild type regulatable *nmt1* promoter. Ten fold serial dilutions (10^4 - 10^1) of wild type cells transformed with either empty vector (pREP1) or pREP1-*dam1* were plated on minimal medium lacking leucine either in the presence (+B1) or absence (-B1) of thiamine for 5 days at 30ºC. (B) Photomicrographs of two wild type cells overexpressing *dam1*. Fixed cells were stained with DAPI (DNA) and the anti-tubulin antibody TAT1 (tubulin). Scale bar = 2μm.

A

dam1-gfp   mal3-cfp   Merge

B

wt   Δmal3

dam1-gfp

C

wt   Δdam1

mal3-cfp

D
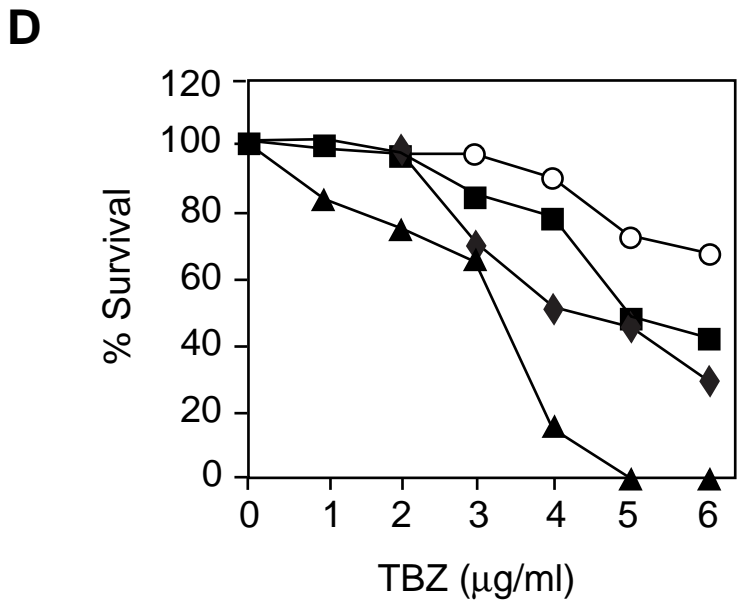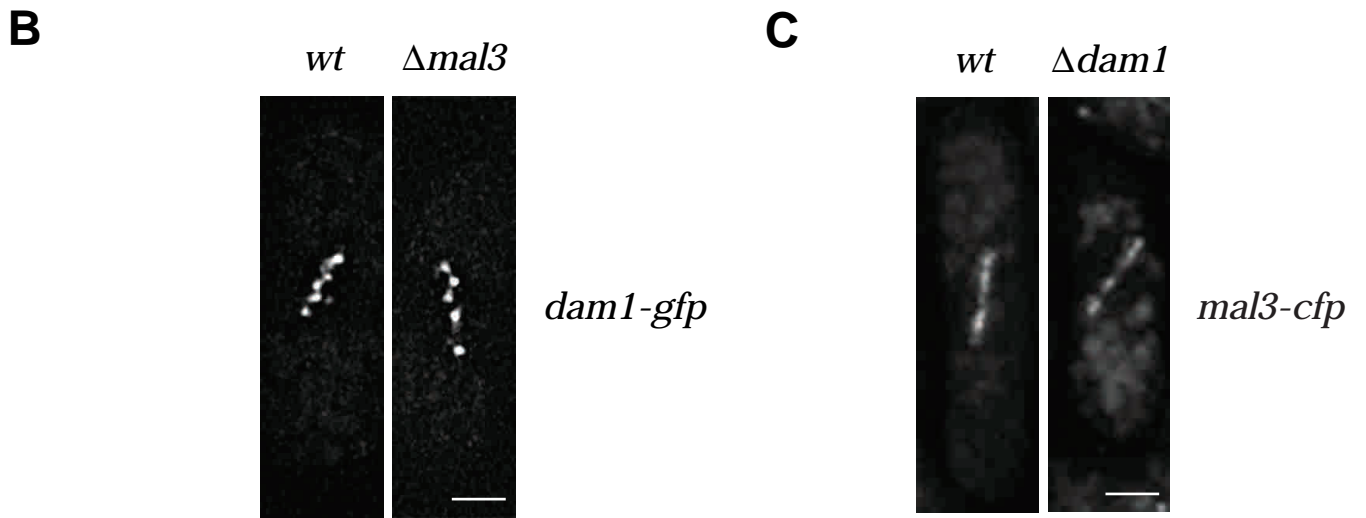
Supplementary Figure 3. Sanchez-Perez et al.

Supplementary Figure 3. Dam1 and Mal3 act independently in mitosis.

(A) Image of a *dam1-gfp mal3-cfp* cell in mitosis. Arrow shows that Mal3 and Dam1 frequntly co-localise in mitosis. Scale bar = 1μm (B) Mitotic *dam1-gfp* or *Δmal3 dam1-gfp* cells were visualised by fluorescence microscopy. Scale bar = 1 μm. (C) Mitotic *mal3-cfp* or *Δdam1 mal3-cfp* cells were visualised by fluorescence microscopy. Scale bar = 1μm. (D) 500 cells from log phase cultures of wild type (open circles), *Δdam1* (closed squares), *Δmal3* (Closed diamonds) or *Δdam1 Δmal3* (closed triangles) cells were plated onto fresh medium containing various concentrations of TBZ in triplicate. Cell viability was expressed as the percentage of colonies formed relative to the control plate.
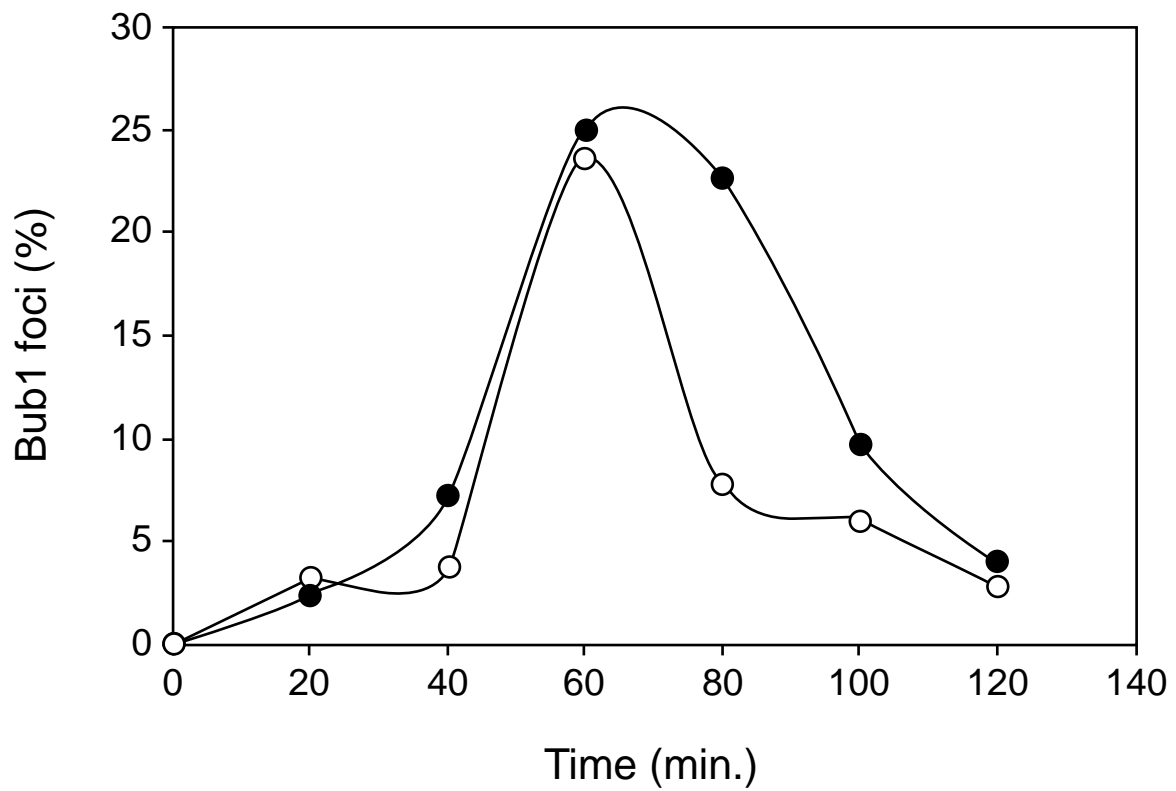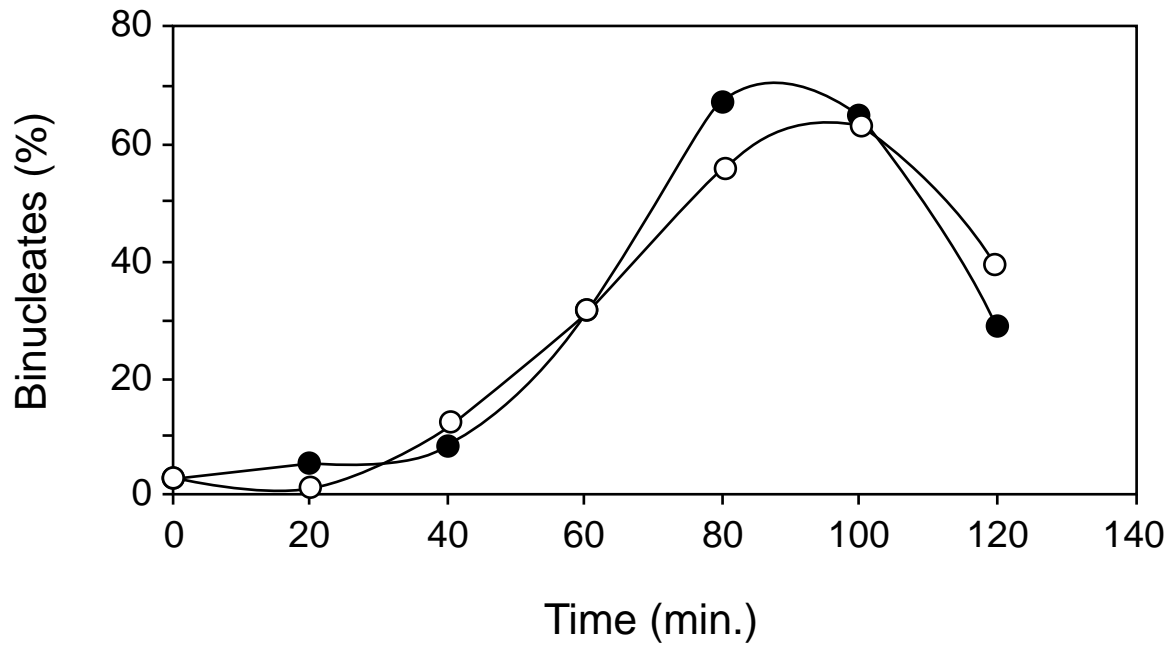
Supplementary Figure 4. Sanchez-Perez et al.

Supplementary Figure 4. Bub1 associates to kinetochores for longer in the absence of Dam1. Log phase *bub1-gfp cdc11-cfp* and *Δdam1 bub1-gfp cdc11-cfp* cells were synchronised in G2 and released into rich medium. The percentage of binucleate cells (top panel) or cells with non-SPB associated Bub1 dots (bottom panel) was measured at each time point (n=300).

Supplementary Table I. *S.pombe* **orthologues of the** *S.cerevisiae* **DASH complex**

S.c. protein	*S.p.* gene / chromosomal locus	BLAST Score	Proposed *S.p.* name	Protein Length (a.a.)
S.c. Dam1	SPAC589.08c	0.0008	S.p. Dam1	155
S.c. Duo1	SPBC32F12.08c	0.89*	S.p. Duo1	172
S.c. Ask1	SPBC27.02c	2.4 x e-09	S.p. Ask1	307
S.c. Spc34	SPAC8C9.17c	0.0073	S.p. Spc34	164
S.c. Spc19	SPCC1223	0.0098	S.p. Spc19	**
S.c. Dad1	SPAC16A10.05c	0.00049	S.p. Dad1	85
S.c. Dad2(Hsk1)	*hos2*	5.4 x e-05	S.p. Dad2	94
S.c. Dad3	SPAC14C4.16	2.3 x e-06	S.p. Dad3	86
S.c. Dad4(Hsk2)	SPBC3B9	0.00039	S.p. Dad4	**
S.c. Hsk3	*hos3*	0.012	S.p. Hsk3	133

* *S.p.*Duo1 shows limited sequence homology to *S.c.*Duo1 but is required for binding of *S.p.*Dam1 to kinetochores (see text)

** *S.p.*Spc19 and *S.p.*Dad4 have not been annotated as ORFs in the *S.pombe* GeneDB database.

Supplementary Table II.   Strains used in this study

Strain no.	Genotype	Reference
PR109	*h-*	our stocks
JM2767	*h- cdc13-117*	our stocks
UFYS0203	*h- mal3-1 ura4-D6 Ch16[ade6-M216]*	our stocks
JM2881	*h- dam1::kanR*	this study
JM2952	*h- dam1::hygR*	this study
JM2949	*h- duo1::hygR*	this study
JM3016	*h- spc34::kanR*	this study
JM2948	*h- ask1::hygR*	this study
JM3074	*h- dad1::hygR*	this study
JY741DH2	*h- hos2::ura4 ade6-M216*	H. Aiba
JY741DH3	*h- hos3::ura4 ade6-M216*	H. Aiba
JM2959	*h+ dam1-GFP:kanR*	this study
KC63	*h- dad1-GFP:kanR his2-245*	this study
KC56	*h- dad1-GFP:kanR nuf2-cfp:kanR*	this study
JM3146	*h- dad1-GFP:kanR ndc80-cfp:kanR*	this study
JM2940	*h- dam1-GFP:kanR ndc80-CFP:kanR*	this study
JM3075	*h- dam1-GFP:kanR duo1::hygR cdc11-CFP:kanR*	this study
JM3077	*h- dam1-GFP:kanR ask1::hygR cdc11-CFP:kanR*	this study
JM3049	*h+ dam1-GFP:kanR spc34::hygR cdc11-CFP:kanR*	this study
JM3079	*h- dam1-GFP:kanR dad1::hygR cdc11-CFP:kanR*	this study
JM2939	*h+ dam1::kanR ade6-M210 [Ch16 (ade6-M216 bub1::ura4)]*	this study
JM2886	*h? dam1::kanR lys1:lacO his7:GFP-NLS-lacI*	this study
JM2590	*h+ cdc13-GFP(LEU2):cdc13-117*	M. Yanagida
JM3029	*h- cdc13-GFP(LEU2):cdc13-117 dam1::hygR*	this study
JM2763	*h- ndc80-GFP:kanR cdc11-CFP:kanR*	our stocks
JM2888	*h? dam1::hygR ndc80-GFP:kanR cdc11-CFP:kanR*	this study
AE304	*h+ mad2-GFP(LEU2) ura4+*	T. Matsumoto
MA071	*h- mad2-GFP:kanR his3-237*	our stocks
JM2967	*h- dam1::hygR mad2-GFP(LEU2)*	this study
MA239	*h+ bub1-GFP:kanR*	our stocks
JM3082	*h- dam1::hygR bub1-GFP:kanR*	this study
AE148	*h- mad2::ura4*	T. Matsumoto

JM2883	*h- mad2::ura4 dam1::kanR*	this study
JM2323	*h- bub1::ura4*	K. Hardwick
JM2884	*h- bub1::ura4 dam1::kanR*	this study
JM2565	*h- lys1:nmt1-gfp-atb2*	our stocks
JM2568	*h- lys1:nmt1-gfp-atb2 mad2::ura4*	our stocks
JM2887	*h- lys1:nmt1-gfp-atb2 dam1::kanR*	this study
JM3043	*h+ lys1:nmt1-gfp-atb2 mad2::ura4 dam1::kanR*	this study
JM2962	*h- mal3::ura4*	our stocks
JM2965	*h- mal3::ura4 dam1-GFP:kanR*	this study
JM2885	*h+ mal3::ura4 dam1::kanR*	this study
DM443	*h+ pkl1::ura4 ade6-M216 his3-D1*	R. McIntosh
DM227	*h- klp2::ura4 ade6-M210 his3-D1*	R. McIntosh
ZC263	*h- klp3::ura4 ade6- his3-237*	Z. Cande
DM474	*h- tea2::his3 ade6- his3-D1*	R. McIntosh
MA060	*h+ klp5::ura4 ade6-M216*	our stocks
MA101	*h- klp6::ura4 his7-366*	our stocks
JM3190	*h+ klp5::ura4 lys1:nmt1-gfp-atb2*	this study
JM3191	*h- dam1::LEU2 lys1:nmt1-gfp-atb2*	this study
JM3155	*h+ klp5::ura4 mad2-GFP:kanR*	this study
JM3167	*h- dam1::LEU2 mad2-GFP:kanR*	this study
JM3014	*h- klp5::ura4 lys1:lacO his7:GFP-NLS-lacI cdc11-CFP:kanR*	this study
JM3060	*h+ dam1::LEU2 lys1:lacO his7:GFP-NLS-lacI cdc11-CFP:kanR*	this study
SP540	*h- mis6-302 ura4+ ade6-M216*	M. Yanagida
SP920	*h- mis12-537*	M. Yanagida
SP525	*h- cnp1::ura4 lys1:cnp1-1*	M. Yanagida
ANF254-3A	*h+ nuf2-4 leu1+ ura4+*	Y. Hiraoka
VFY852	*h- mal2-1 ade6-M210*	U. Fleig
KC181	*h- mis6-302 dad1-gfp:kanR*	this study
KC179	*h- mis12-537 dad1-gfp:kanR*	this study
KC200	*h- cnp1::ura4 lys1:cnp1-1 dad1-gfp:kanR*	this study
KC199	*h+ nuf2-4 dad1-gfp:kanR*	this study
KC201	*h+ mal2-1 dad1-gfp:kanR*	this study
KC186	*h- mis12-537 dam1::kanR*	this study
KC188	*h+ cnp1::ura4 lys1:cnp1-1 dam1::hygR*	this study
KC158	*h+ mis12-537 ask1::kanR his2-245*	this study
KC190	*h+ cnp1::ura4 lys1:cnp1-1 ask1::hygR his2-245*	this study

JM3145	*h+ dad1-GFP ndc80-CFP*	this study
MA147	*h- mal3-CFP:kanR*	our stocks
JM3152	*h- dam1::hygR mal3-CFP:kanR*	this study
JM3143	*h- dam1-GFP:kanR mal3-CFP:kanR*	this study
JM3185	*h- dam1::hygR dad1-GFP*	this study
JM3186	*h- dam1::hygR dad1-GFP ndc80-CFP*	this study
JM3174	*h- nmt1-GFP-dam1:kanR*	this study

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All strains are *leu1-32 ura4-D18* unless otherwise stated. *ade6- is either ade6-M210 or ade6-M216.*

Supplementary Table III.   Oligonucleotides used in this study

dam1:: kanR and dam1:hygR

F:TGTTAAAAATTGTATATAAATCGTAAAATTGAATGATCATTATGATTCCGTT
GTTTCATTATCCAACCTGATATGCTAGGCGGATCCCCGGGTTAATTAA
R:AACGTCGGAAGAAAAATGTTCCTGCTGCTGCTGCCGAAAGCGCTGTAGAAC
ATCTACATCTCACCCAAAGTCGCGAAAACGAATTCGAGCTCGTTTAAAC

dam1::LEU2

F:TGTTAAAAATTGTATATAAATCGTAAAATTGAATGATCATTATGATTCCGTT
GTTTCATTATCCAACCTGATATGCTAGGCGAGGAGAACTTTCTAGTATAT
R:AACGTCGGAAGAAAAATGTTCCTGCTGCTGCTGCCGAAAGCGCTGTAGAAC
ATCTACATCTCACCCAAAGTCGCGAAAACCGACTACGTCGTTAAGGCC

dam1 (1-153):kanR

W:GGATGATAATGTGAGTGAATT
X:GGGGATCCGTCGACCTGCAGCGTACGACTAATCGTGAGAAATAACACCAC
Y:GTTTAAACGAGCTCGAATTCATCGATTAAGCAAGGGAGACTGGTT
Z:TGAAGATTGGAGAAGCTATTA

dam1 (1-127):kanR

W:GGATGATAATGTGAGTGAATT
X:GGGGATCCGTCGACCTGCAGCGTACGAATCGTGAGAAATAACACCAC
Y:GTTTAAACGAGCTCGAATTCATCGATTAAGCAAGGGAGACTGGTT
Z:TGAAGATTGGAGAAGCTATTA

dam1-GFP:kanR

F:ATTTTGCAACTGCAGACGAAACATTTGCTACAAATGACACTTCTTTCAGAAC
GGCCAGAAACCTATTCCGCTTCCAGACGGATCCCCGGGTTAATTAA
R:GATTGGAGAAGCTATTACATATGAGTTTACTGAACGCTTTCTAGAACTTTTA
AAGCAATTGTCTACAGTTCACTTCGCTGGAATTCGAGCTCGTTTAAAC

nmt1-GFP-dam1:kanR

F:GCAGAAAAAGTCGCATAGTTTCCATTACCCACACGGTCATCTCACGAAGAG
AATTCTGAGTCAATTTTGAGGGTTGATGGAATTCGAGCTCGTTTAAAC
R:GGGATTGCATTCTCACTTTCTATTTTCACATTATCAACATTTTCTAAAGGATT
TTGATAGCTTTCTGGTATTTCTCCATTTTGTATAGTTCATCCATGC

dad1::hygR

F:CTTGATTGTAACCTTATGATAAACTTTGGCTCATGCTTGACTTTGACACTAGC
CATTAACATACCATGTCCTCAGAAAATCGGATCCCCGGGTTAATTAA
R:TGTAATATGTAAGTTAATTTAAAAGAATCCTGACCTTTGACCAATTTTTGAG
GACGCTATTAAATTTATAGATTCATAAAGAATTCGAGCTCGTTTAAAC

dad1-gfp:kanR

W: CTATGCTCGTACGGAAGC
X: GGGGATCCGTCGACCTGCAGCGTACGAAGGTGCATCTAGCATTTCA
Y:GTTTAAACGAGCTCGAATTCATCGATCTCCTGACCAGCAGTATCC
Z:AGGTGAATGACAGCTGGTC

spc34:: kanR and *spc34::hygR*

W:GTATTGGGAGTAAGCCGATC
X:GGGGATCCGTCGACCTGCAGCGTACGAGGAAGGCTATTTGCGTTGG
Y:GTTTAAACGAGCTCGAATTCATCGATCTGATGTAGCAGCAATAGAC
Z:GCAAGGAATGAACGCAATGC

duo1:: kanR and *duo1:: hygR*

W:GGTTGAGTGGTTTCACGAGTG
X:GGGGATCCGTCGACCTGCAGCGTACGAGACTTCTAACCCAAACTCAC
Y:GTTTAAACGAGCTCGAATTCATCGATGTGTGCAATTTAAGCCTAAC
Z:GTAATGCACATAACTAACTCTTT

ask1:: kanR **and** ***ask1:: hygR***

W:GTTATTTGCTTTCTTAGTTT

X:GGGGATCCGTCGACCTGCAGCGTACGAGCACTGCTGAAGTTATCACC

Y:GTTTAAACGAGCTCGAATTCATCGATGGTGAAATTTATGGCGAACTGC

Z:TTATCAGGGTTCGTCAACTCC