Supplementary Methods

***Schizosaccharomyces pombe* strains**

All media and growth conditions unless otherwise stated were as described previously (Moreno et al., 1991). Complete medium (YE), minimal medium (MM) and sporulation-inducing medium (SPA) were used. All strains used are listed in Supplementary Table 1. Deletion (*rec12*$^+$, *eso1*$^+$ and *hos2*$^+$*)* and epitope tagging (*psm3*$^+$ and *eso1*$^+$*)* of endogenous genes were performed by the PCR-based gene-targeting method for *S. pombe* using *kanMX6 (kan*^{r}*), hphMX6 (hyg*^{r}*)* and *natMX6 (nat*^{r}*)* genes as selection markers (Bahler et al., 1998; Sato et al., 2005). The *eso1-H17*, *clr6-1*, *cut9-665*, *cdc25-22*, *rad21-K1, rec8Δ, moa1Δ* and *GFP-3pk-moa1* strains have been described previously (Grewal et al., 1998; Nurse et al., 1976; Samejima and Yanagida, 1994; Tanaka et al., 2000; Watanabe and Nurse, 1999; Yokobayashi and Watanabe, 2005; Yokobayashi et al., 2003). The K->R and K->Q mutations of the *psm3*$^+$ gene were generated by using a PrimeSTAR Mutagenesis Basal Kit (TaKaRa). To generate strains having K->R or K->Q mutations at the chromosomal *psm3*$^+$locus, a *psm3ΔC* (from -750bp to +1000bp) DNA fragment containing mutations was combined with a *nat*^{r} marker, digested by *Sac*I within *psm3ΔC*, and integrated into the endogenous *psm3*$^+$ locus. Correct integration was confirmed by PCR and sequencing. To express the *eso1*$^+$ gene under the *moa1*$^+$ promoter, the ORF of *eso1*$^+$ tagged by the FLAG epitope at the C-terminus was cloned under the *moa1*$^+$ promoter (~750 bp). As a control, an endogenous *eso1*$^+$ promoter was used instead of the *moa1*$^+$ promoter (~1000 bp). The resulting plasmid, carrying a *nat*^{r} marker, was linearized and integrated at the C locus. To express *moa1*$^+$ gene under *spo6*$^+$ promoter (Sakuno et al., 2009), the ORF of *moa1*$^+$ tagged with 3 copies of the Pk epitope at the N-terminus was cloned under the *spo6*$^+$ promoter. The resulting plasmid, carrying a *hyg*^{r} marker, was linearized and integrated at the *lys1*$^+$ locus.

Analysis of Psm3 acetylation

Antibodies raised against the acetylated Psm3 peptides (TIGLK(AcK)DEY) were purified from anti-serum with acetylated-peptide-conjugated CNBr-activated Sepharose and dialysed against PBS. Antibodies were further purified by passing them through non-acetylated-peptide conjugated CNBr-activated beads. To arrest cells at early S phase, cells were cultured in the presence of 12mM Hydroxurea (HU) for 4 hr at 30˚C. To synchronize the cell cycle, temperature-sensitive *cdc25-22* mutant cells were blocked at G2 phase by incubating at 36˚C for 4 hr, and then released at 25˚C. To induce synchronous meiosis, *pat1-114* mutant cells were blocked at G1 phase by culturing in nitrogen-depleted medium for 16 hr at 25˚C and then shifted to 34˚C with adding NH_4Cl (0.25 mg/ml). To monitor cell cycle progression, cell aliquots were fixed and their nuclear number was observed under a microscope. The Flag-tagged Psm3 was immunopresipitated from cell extracts prepared in HB buffer (25mM MOPS (pH7.2), 150mM NaCl, 15mM $MgCl_2$, 15mM EGTA, 60mM ß-glycerophosphate, 0.1mM Na-orthovanadate, 0.1mM NaF, 15mM *p*-nitrophenylphosphate, 1% Triton-X100, 1mM dithiothreitol, 1mM PMSF, 10mM sodium butyrate, complete protease inhibitor

(Roche)) by using anti-Flag M2 monoclonal antibody-conjugated agarose (Sigma) and analyzed by immunoblot probed with anti-Flag M2 (Sigma) and anti-AcPsm3 antibodies.

Sister chromatid cohesion assay

The cells with *cut3*-GFP were incubating at 37˚C for 2 hr, and then the number of cells having two *cut3-GFP* signals in a single nucleus was determined. The temperature-sensitive *cut9-665* mutant cells with *cen2*-GFP were arrested at metaphase by incubating at 36˚C for 4 hr. To visualize tubulin, an mCherry-tagged *atb2*$^{+}$ gene under the *adh15* promoter was integrated at the Z or C locus (Sakuno et al., 2009). The in-focus fluorescent images were obtained with Axio Vision imaging software (Carl Zeiss), and the distance between two *cen2*-GFP signals on the metaphase spindle was measured by Image J software.

Chromatin immunoprecipitation (ChIP) assay

The procedures were carried out essentially as described previously (Yokobayashi et al., 2003). Anti-Rec8, anti-Moa1 and anti-Cnp1 were used for immunoprecipitation. DNA prepared from whole cell extracts or immunoprecipitated fractions was analyzed by quantitative PCR with the ABI PRISM7000 system (Applied Biosystems) using SYBR Premix ExTaq (Perfect Real Time) (Takara). The primers used for PCR were described previously (Ishiguro et al., 2010; Yokobayashi and Watanabe, 2005). We included control IgG immunoprecipitation in each experiment to account for nonspecific binding in the ChIP fractions.

Supplementary References

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Supplementary Table 1

Fission yeast strains used in this study

Fig. 1B	PP951	*h^{90} leu1 ade6-M216 psm3-FLAG-kan^{r}*
	PP993	*h^{90} leu1 clr6-1 psm3-FLAG-kan^{r}*
	PJ572	*h^{90} leu1 ade6-M216 hos2Δ::hyg^{r} psm3-FLAG-kan^{r}*
Fig. 1C	PG820	*h^{-} leu1 cdc25-22 cen2^{+}<<lacO-ura4^{+}-kan^{r} his7^{+}<P$_{dis1}$-GFP-lacI-NLS +pREP1*
	PG821	*h^{-} leu1 cdc25-22 cen2^{+}<<lacO-ura4^{+}-kan^{r} his7^{+}<P$_{dis1}$-GFP-lacI-NLS +pREP1-clr6-3pk*
	PG818	*h^{-} leu1 cdc25-22 psm3-FLAG-kan^{r} +pREP1*
	PG819	*h^{-} leu1cdc25-22 psm3-FLAG-kan^{r} +pREP1-clr6-3pk*
Fig. 2A	PH954	*h^{+} pat1-114 psm3-FLAG-kan^{r}*
	PH955	*h^{+} pat1-114 eso1-H17 psm3-FLAG-kan^{r}*
2B	PL485	*h^{+} leu1 rec12Δ::LEU2 nat^{r}-psm3^{+}*
	PL488	*h^{-} leu1 cen2^{+}<<lacO-ura4^{+}-kan^{r} his7^{+}<P$_{dis1}$-GFP-lacI-NLS rec12Δ::LEU2 nat^{r}-psm3^{+}*
	PY343	*h^{-} leu1 rec12Δ::LEU2 rec8::kan^{r}*
	PZ625	*h^{+} leu1 cen2^{+}<<lacO-ura4^{+}-kan^{r} his7^{+}<P$_{dis1}$-GFP-lacI-NLS rec12Δ::LEU2 rec8::kan^{r}*
	PL491	*h^{+} leu1 cen2^{+}<<lacO-ura4^{+}-kan^{r} his7^{+}<P$_{dis1}$-GFP-lacI-NLS rec12Δ::LEU2 eso1-H17 nat^{r}-psm3^{+}*
	PL494	*h^{-} leu1 rec12Δ::LEU2 eso1-H17 nat^{r}-psm3^{+}*
	PJ594	*h^{+} leu1 rec12Δ::LEU2 nat^{r}-psm3(K105RK106R)*
	PJ593	*h^{-} leu1 cen2^{+}<<lacO-ura4^{+}-kan^{r} his7^{+}<P$_{dis1}$-GFP-lacI-NLS rec12Δ::LEU2 nat^{r}-psm3(K105RK106R)*
	PL487	*h^{+} leu1 rec12Δ::LEU2 nat^{r}-psm3(K105QK106Q)*
	PL490	*h^{-} leu1 cen2^{+}<<lacO-ura4^{+}-kan^{r} his7^{+}<P$_{dis1}$-GFP-lacI-NLS rec12Δ::LEU2 nat^{r}-psm3(K105QK106Q)*
2C	PQ607	*h^{+} cnt1^{+}<< kan^{r} -lacO his7^{+}<P$_{dis1}$-GFP-lacI-NLS mei4Δ::ura4-DS/E FY534<<RS FY527<<RS Z::nat^{r}-P$_{adh31}$-tetR-tdTomato dh1L<<tetO-ura4^{+} sad1^{+}-CFP-LEU2 rec8Δ::kan^{r} lys1Δ::P$_{spo5}$-R-T$_{spo5}$-hyg^{r}*
	PS152	*h^{-} leu1 ura4-D18 ade6-M216 mei4Δ::ura4^{+} lys1Δ:: P$_{spo5}$-R-T$_{spo5}$-hyg^{r}*
	PS155	*h^{-} leu1 ura4-D18 ade6-M216 mei4Δ::ura4^{+} rec8Δ::kan^{r} lys1Δ:: P$_{spo5}$-R-T$_{spo5}$-hyg^{r}*
	PG003	*h^{+} leu1 cnt1^{+}<< kan^{r} -lacO his7^{+}<P$_{dis1}$-GFP-lacI-NLS mei4Δ::ura4-DS/E FY534<<RS FY527<<RS Z::nat^{r}-P$_{adh31}$-tetR-tdTomato dh1L<<tetO-ura4^{+} sad1^{+}-CFP-LEU2 eso1-H17 lys1Δ::P$_{spo5}$-R-T$_{spo5}$-hyg^{r}*
	PJ948	*h^{-} ade6-M210 mei4Δ::ura4^{+} eso1-H17 lys1Δ:: P$_{spo5}$-R-T$_{spo5}$-hyg^{r} sad1^{+}-CFP-LEU2*
	PG001	*h^{+} leu1 cnt1^{+}<< kan^{r} -lacO his7^{+}<P$_{dis1}$-GFP-lacI-NLS mei4Δ::ura4-DS/E FY534<<RS FY527<<RS Z::nat^{r}-P$_{adh31}$-tetR-tdTomato dh1L<<tetO-ura4^{+} sad1^{+}-CFP-LEU2 nat^{r}-psm3(K105RK106R) lys1Δ::P$_{spo5}$-R-T$_{spo5}$-hyg^{r}*
	PJ951	*h^{-} leu1 ura4-D18 ade6-M216 mei4Δ::ura4^{+} lys1Δ:: P$_{spo5}$-R-T$_{spo5}$-hyg^{r} sad1^{+}-CFP-LEU2 nat^{r}-psm3(K105RK106R)*
	PG005	*h^{+} leu1 cnt1^{+}<< kan^{r} -lacO his7^{+}<P$_{dis1}$-GFP-lacI-NLS mei4Δ::ura4-DS/E FY534<<RS FY527<<RS Z::nat^{r}-P$_{adh31}$-tetR-tdTomato dh1L<<tetO-ura4^{+} sad1^{+}-CFP-LEU2 nat^{r}-psm3(K105QK106Q)*

		lys1Δ::P_{spo5}-R-T_{spo5}-hyg^r
	PJ950	*h$^-$ leu1 ura4-D18 ade6-M216 mei4Δ::ura4$^+$ lys1Δ:: P_{spo5}-R-T_{spo5}-hyg^r sad1$^+$-CFP-LEU2 nat^r-psm3(K105QK106Q)*
2D	PH980	*h$^+$ leu1 mei4Δ::ura4$^+$ dh1L<<tetO-ura4$^+$ Z::nat^r-P_{adh31}-tetR-tdTomato nat^r-psm3$^+$*
	PH996	*h$^+$ leu1 mei4Δ::ura4$^+$ cut3$^+$<<lacO his7$^+$<Pdis1-GFP-lacI-NLS eso1-H17 nat^r-psm3$^+$*
	PH994	*h$^-$ mei4Δ::ura4$^+$ nat^r-psm3$^+$*
	PH985	*h$^+$ leu1 ade6-M210 mei4Δ::ura4$^+$ dh1L<<tetO-ura4$^+$ Z::nat^r-P_{adh31}-tetR-tdTomato rad21-K1-ura4$^+$ rec8Δ::kan^r*
	PH990	*h$^-$ leu1 ade6-M216 mei4Δ::nat^r mes1-B44 rad21-K1-ura4$^+$ rec8Δ::kan^r*
	PG809	*h$^+$ leu1 ade6-M210 mei4Δ::ura4$^+$ his7$^+$<Pdis1-GFP-lacI-NLS rad21-K1-ura4$^+$ rec8Δ::kan^r*
	PG810	*h$^-$ leu1 ade6-M210 mei4Δ::ura4$^+$ cut3$^+$<<lacO rad21-K1-ura4$^+$ rec8Δ::kan^r*
	PH989	*h$^-$ mei4Δ::ura4$^+$ dh1L<<tetO-ura4$^+$ Z::nat^r-P_{adh31}-tetR-tdTomato eso1-H17*
	PP943	*h$^+$ ade6-M216 mei4Δ::ura4$^+$ eso1-H17*
	PH987	*h$^+$ leu1 mei4Δ::ura4$^+$ cut3$^+$<<lacO his7$^+$<Pdis1-GFP-lacI-NLS eso1-H17*
	PP944	*h$^-$ ade6-M210 mei4Δ::ura4$^+$ eso1-H17*
	PH981	*h$^+$ leu1 mei4Δ::ura4$^+$ dh1L<<tetO-ura4$^+$ Z::nat^r-P_{adh31}-tetR-tdTomato nat^r-psm3(K105RK106R)*
	PH997	*h$^+$ leu1 mei4Δ::ura4$^+$ cut3$^+$<<lacO his7$^+$<Pdis1-GFP-lacI-NLS eso1-H17 nat^r-psm3(K105RK106R)*
	PH970	*h$^-$ leu1 mei4Δ::hyg^r nat^r-psm3(K105RK106R)*
Fig. 3A	PH859	*h$^+$ pat1-114 C::P_{eso1}-eso1-FLAG-T_{eso1}- nat^r*
	PH860	*h$^+$ pat1-114 C::P_{moa1}-eso1-FLAG-T_{spo5}- nat^r*
3B	PJ535	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P_{dis1}-GFP-lacI-NLS rec12Δ::LEU2*
	PY340	*h$^-$ leu1 rec12Δ::LEU2*
	PP871	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P_{dis1}-GFP-lacI-NLS eso1-H17 rec12Δ::LEU2*
	PP874	*h$^-$ leu1 eso1-H17 rec12Δ::LEU2*
	PH861	*h$^-$ leu1 eso1-H17 rec12Δ::LEU2 C::P_{eso1}-eso1-FLAG-T_{eso1}- nat^r*
	PH862	*h$^-$ leu1 eso1-H17 rec12Δ::LEU2 C::P_{moa1}-eso1-FLAG-T_{spo5}- nat^r*
3C	PW632	*h$^+$ pat1-114 3pk-moa1$^+$*
	PJ452	*h$^+$ pat1-114 lys1Δ::P_{spo6}-3pk-moa1-T_{spo5}- hyg^r*
3D	PW670	*h^{90} leu1 GFP-3pk-moa1$^+$*
	PJ449	*h^{90} leu1 ade6-M216 moa1Δ::kan^r lys1Δ::P_{spo6}-GFP-3pk-moa1- T_{spo5}- hyg^r*
3E	PW680	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P_{dis1}-GFP-lacI-NLS rec12Δ::LEU2 moa1Δ::kan^r*
	PX281	*h$^-$ leu1 rec12Δ::LEU2 moa1Δ::kan^r*
	PW662	*h$^-$ leu1 rec12Δ::LEU2 3pk-moa1$^+$*
	PJ446	*h$^-$ leu1 rec12Δ::LEU2 moa1Δ::kan^r lys1Δ::P_{spo6}-3pk-moa1- T_{spo5}-hyg^r*
Fig. 4A	PJ535	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P_{dis1}-GFP-lacI-NLS rec12Δ::LEU2*
	PY340	*h$^-$ leu1 rec12Δ::LEU2*
	PP990	*h$^-$ leu1 rec12Δ::LEU2 clr6-1*
	PP998	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P_{dis1}-GFP-lacI-NLS rec12Δ::LEU2 clr6-1*
	PH871	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P_{dis1}-GFP-lacI-NLS rec12Δ::LEU2 eso1-H17*
	PP874	*h$^-$ leu1 rec12Δ::LEU2 eso1-H17*

	PJ592	*h⁻ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 eso1-H17 clr6-1*
	PH825	*h⁺ leu1 rec12Δ::hyg^r eso1-H17 clr6-1*
	PJ595	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 eso1-H17 nat^r-psm3(K105RK106R)*
	PJ596	*h⁻ leu1 rec12Δ::LEU2 eso1-H17 nat^r-psm3(K105RK106R)*
	PH886	*h⁺ leu1 rec12Δ::hyg^r eso1-H17 clr6-1 nat^r-psm3(K105RK106R)*
	PH889	*h⁻ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 eso1-H17 clr6-1 nat^r-psm3(K105RK106R)*
	PL493	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 eso1-H17 nat^r-psm3(K105QK106Q)*
	PL496	*h⁻ leu1 rec12Δ::LEU2 eso1-H17 nat^r-psm3(K105QK106Q)*
		h⁺ leu1 cnt1⁺<< kan^r -lacO his7⁺<P_dis1-GFP-lacI-NLS mei4Δ::ura4-DS/E FY534<<RS FY527<<RS
4B	PG003	*Z::nat^r-P_adh31-tetR-tdTomato dh1L<<tetO-ura4⁺ sad1⁺-CFP-LEU2 eso1-H17 lys1Δ::P_spo5-R-T_spo5-hyg^r*
	PJ948	*h⁻ ade6-M210 mei4Δ::ura4⁺ eso1-H17 lys1Δ:: P_spo5-R-T_spo5-hyg^r sad1⁺-CFP-LEU2*
		h⁺ cnt1⁺<< kan^r -lacO his7⁺<P_dis1-GFP-lacI-NLS mei4Δ::ura4-DS/E FY534<<RS FY527<<RS
	PG052	*Z::nat^r-P_adh31-tetR-tdTomato dh1L<<tetO-ura4⁺ sad1⁺-CFP-LEU2 eso1-H17 clr6-1 moa1Δ::ura4-D/SE lys1Δ::P_spo5-R-T_spo5-hyg^r*
	PG054	*h⁻ mei4Δ::ura4⁺ eso1-H17 clr6-1 lys1Δ::P_spo5-R-T_spo5-hyg^r Z::P_spo5-R-T_spo5-nat^r (ura4-D18)*
	PG175	*h⁻ ade6-M210 mei4Δ::ura4⁺ eso1-H17 nat^r-psm3(K105QK106Q) lys1Δ::P_spo5-R-T_spo5-hyg^r*
		h⁺ leu1 (ura4-DS/E) cnt1⁺<< kan^r -lacO his7⁺<P_dis1-GFP-lacI-NLS mei4Δ::ura4-DS/E FY534<<RS
	PG178	*FY527<<RS Z::nat^r-P_adh31-tetR-tdTomato dh1L<<tetO-ura4⁺ sad1⁺-CFP-LEU2 eso1-H17 nat^r-psm3(K105QK106Q) lys1Δ::P_spo5-R-T_spo5-hyg^r*
4C	PJ535	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2*
	PY340	*h⁻ leu1 rec12Δ::LEU2*
	PP990	*h⁻ leu1 rec12Δ::LEU2 clr6-1*
	PP998	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 clr6-1*
	PW680	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 moa1Δ::kan^r*
	PX281	*h⁻ leu1 rec12Δ::LEU2 moa1Δ::kan^r*
	PP992	*h⁻ leu1 rec12Δ::LEU2 clr6-1 moa1Δ::kan^r*
	PL401	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 clr6-1 moa1Δ::kan^r*
	PL499	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 moa1Δ::kan^r nat^r-psm3(K105QK106Q)*
	PL502	*h⁻ leu1 rec12Δ::LEU2 moa1Δ::kan^r nat^r-psm3(K105QK106Q)*
		h⁺ leu1 ura4-DS/E cnt1⁺<< kan^r -lacO his7⁺<P_dis1-GFP-lacI-NLS mei4Δ::ura4-DS/E FY534<<RS
4D	PQ638	*FY527<<RS Z::nat^r-P_adh31-tetR-tdTomato dh1L<<tetO-ura4⁺ sad1⁺-CFP-LEU2 moa1Δ::ura4-D/SE lys1Δ::P_spo5-R-T_spo5-hyg^r*
	PS158	*h⁻ ade6-M210 mei4Δ::ura4⁺ moa1Δ::ura4⁺ lys1Δ:: P_spo5-R-T_spo5-hyg^r*
	PG044	*h⁺ leu1 ura4-DS/E cnt1⁺<< kan^r -lacO his7⁺<P_dis1-GFP-lacI-NLS mei4Δ::ura4-DS/E FY534<<RS FY527<<RS Z::nat^r-P_adh31-tetR-tdTomato dh1L<<tetO-ura4⁺ sad1⁺-CFP-LEU2 moa1Δ::ura4-D/SE*

		clr6-1 lys1Δ::P_{spo5}-R-T_{spo5}-hyg^r
	PG047	*h$^-$ leu1 (ura4-D18) mei4Δ::ura4$^+$ moa1Δ::ura4$^+$ clr6-1 lys1Δ:: P_{spo5}-R-T_{spo5}-hyg^r Z::P_{spo5}-R-T_{spo5}-nat^r*
4E	PJ535	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P_{dis1}-GFP-lacI-NLS rec12Δ::LEU2*
	PY340	*h$^-$ leu1 rec12Δ::LEU2*
	PP871	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P_{dis1}-GFP-lacI-NLS rec12Δ::LEU2 eso1-H17*
	PP874	*h$^-$ leu1 rec12Δ::LEU2 eso1-H17*
	PJ593	*h$^-$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P_{dis1}-GFP-lacI-NLS rec12Δ::LEU2 nat^r-psm3(K105RK106R)*
	PJ594	*h$^+$ leu1 rec12Δ::LEU2 nat^r-psm3(K105RK106R)*
	PW680	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P_{dis1}-GFP-lacI-NLS rec12Δ::LEU2 moa1Δ::kan^r*
	PX281	*h$^-$ leu1 rec12Δ::LEU2 moa1Δ::kan^r*
	PP102	*h$^-$ leu1 ade6 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P_{dis1}-GFP-lacI-NLS rec12Δ::LEU2 wpl1Δ::hyg^r*
	PP103	*h$^+$ leu1 rec12Δ::LEU2 wpl1Δ::hyg^r*
	PJ564	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P_{dis1}-GFP-lacI-NLS rec12Δ::LEU2 eso1-H17 wpl1Δ::nat^r*
	PJ565	*h$^-$ leu1 rec12Δ::LEU2 eso1-H17 wpl1Δ::nat^r*
	PL446	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P_{dis1}-GFP-lacI-NLS rec12Δ::LEU2 eso1Δ::ura4$^+$ wpl1Δ::hyg^r*
	PL440	*h$^-$ leu1 rec12Δ::LEU2 eso1Δ::ura4$^+$ wpl1Δ::hyg^r*
	PH892	*h$^-$ leu1 ade6 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P_{dis1}-GFP-lacI-NLS rec12Δ::LEU2 wpl1Δ::hyg^r nat^r-psm3(K105RK106R)*
	PH895	*h$^+$ leu1 rec12Δ::LEU2 wpl1Δ::hyg^r nat^r-psm3(K105RK106R)*
	PP104	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P_{dis1}-GFP-lacI-NLS rec12Δ::LEU2 moa1Δ::kan^r wpl1Δ::hyg^r*
	PP105	*h$^-$ leu1 rec12Δ::LEU2 moa1Δ::kan^r wpl1Δ::hyg^r*
Fig.S1A	PP951	*h^{90} leu1 ade6-M216 psm3-FLAG-kan^r*
	PP960	*h$^-$ leu1 eso1-H17 psm3-FLAG-kan^r*
	PH801	*h^{90} leu1 ade6-M216 nat^r-psm3(K105RK106R)-FLAG-kan^r*
	PH802	*h$^-$ leu1 eso1-H17 nat^r-psm3(K105RK106R)-FLAG-kan^r*
S1B	PJ584	*h$^-$ leu1 nat^r-psm3$^+$*
	PJ577	*h$^-$ leu1 eso1-H17 nat^r-psm3$^+$*
	PJ578	*h$^-$ leu1 eso1-H17 nat^r-psm3(K105Q)*
	PJ579	*h$^-$ leu1 eso1-H17 nat^r-psm3(K106Q)*
	PJ580	*h$^-$ leu1 eso1-H17 nat^r-psm3(K105QK106Q)*
	PJ581	*h$^-$ leu1 eso1-H17 nat^r-psm3(K105R)*
	PJ582	*h$^-$ leu1 eso1-H17 nat^r-psm3(K106R)*
	PJ583	*h$^-$ leu1 eso1-H17 nat^r-psm3(K105RK106R)*
	PL566	*h$^+$ leu1 ura4-D18 eso1Δ::ura4$^+$ nat^r-psm3(K105QK106Q)*
S1C	PJ584	*h$^-$ leu1 nat^r-psm3$^+$*
	PJ587	*h$^-$ leu1 nat^r-psm3(K105QK106Q)*
	PJ590	*h$^-$ leu1 nat^r-psm3(K105RK106R)*

	PJ577	*h⁻ leu1 eso1-H17 nat^r-psm3$^+$*
S1D	PH276	*h^{90} leu1 ade6 cut3$^+$<<lacO his7$^+$<Pdis1-GFP-lacI-NLS nat^r-psm3$^+$*
	PH277	*h^{90} leu1 ade6 cut3$^+$<<lacO his7$^+$<Pdis1-GFP-lacI-NLS nat^r-psm3(K105QK106Q)*
	PH278	*h^{90} leu1 ade6 cut3$^+$<<lacO his7$^+$<Pdis1-GFP-lacI-NLS nat^r-psm3(K105RK106R)*
	PH279	*h$^+$ leu1 ade6 cut3$^+$<<lacO his7$^+$<Pdis1-GFP-lacI-NLS eso1-H17*
S1E	PH843	*h$^+$ leu1 cut9-665 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P$_{dis1}$-GFP-lacI-NLS nat^r-psm3$^+$ C::P$_{adh15}$-mCherry-atb2$^+$<< hyg^r*
	PH844	*h$^+$ leu1 cut9-665 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P$_{dis1}$-GFP-lacI-NLS nat^r-psm3(K105QK106Q) C::P$_{adh15}$-mCherry-atb2$^+$<< hyg^r*
	PH845	*h$^+$ leu1 cut9-665 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P$_{dis1}$-GFP-lacI-NLS nat^r-psm3(K105RK106R) C::P$_{adh15}$-mCherry-atb2$^+$<< hyg^r*
	PH226	*h$^?$ leu1 cut9-665 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P$_{dis1}$-GFP-lacI-NLS eso1-H17 Z::P$_{adh15}$-mCherry-atb2$^+$<< nat^r*
S1F	PN43	*h⁻ leu1*
	PP989	*h⁻ leu1 clr6-1*
	PZ612	*h⁻ leu1 eso1-H17*
	PL410	*h⁻ leu1 eso1-H17 clr6-1*
Fig. S2	PJ556	*h⁻ cdc25-22 psm3-FLAG-kan^r*
Fig. S3	PG820	*h⁻ leu1 cdc25-22 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P$_{dis1}$-GFP-lacI-NLS +pREP1*
	PG821	*h⁻ leu1 cdc25-22 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P$_{dis1}$-GFP-lacI-NLS +pREP1-clr6-3pk*
	PG818	*h⁻ leu1 cdc25-22 psm3-FLAG-kan^r +pREP1*
	PG819	*h⁻ leu1cdc25-22 psm3-FLAG-kan^r +pREP1-clr6-3pk*
Fig. S4	PZ621	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P$_{dis1}$-GFP-lacI-NLS*
	PX296	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P$_{dis1}$-GFP-lacI-NLS eso1-H17*
	PH831	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P$_{dis1}$-GFP-lacI-NLS nat^r-psm3$^+$*
	PH832	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P$_{dis1}$-GFP-lacI-NLS nat^r-psm3(K105QK106Q)*
	PH833	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P$_{dis1}$-GFP-lacI-NLS nat^r-psm3(K105RK106R)*
Fig. S5	PH977	*h$^+$/h⁻ leu1/leu1 ade6-M216/ade6-M210 mei4Δ::ura4$^+$/mei4Δ::ura4$^+$ rad21-GFP-kan^r/rad21-GFP-kan^r nat^r-psm3$^+$/nat^r-psm3$^+$*
	PH978	*h$^+$/h⁻ leu1/leu1 ade6-M216/ade6-M210 mei4Δ::ura4$^+$/mei4Δ::ura4$^+$ rad21-GFP-kan^r/rad21-GFP-kan^r nat^r-psm3(K105RK106R)/nat^r-psm3(K105RK106R)*
	PH979	*h$^+$/h⁻ ade6-M216/ade6-M210 mei4Δ::ura4$^+$/mei4Δ::ura4$^+$ rad21-GFP-kan^r/rad21-GFP-kan^r eso1-H17/eso1-H17*
Fig. S6	PL497	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P$_{dis1}$-GFP-lacI-NLS rec12Δ::LEU2 moa1Δ::kan^r nat^r-psm3$^+$*
	PL500	*h⁻ leu1 rec12Δ::LEU2 moa1Δ::kan^r nat^r-psm3$^+$*
	PJ597	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P$_{dis1}$-GFP-lacI-NLS rec12Δ::LEU2 moa1Δ::kan^r nat^r-psm3(K105RK106R)*
	PJ598	*h⁻ leu1 rec12Δ::LEU2 moa1Δ::kan^r nat^r-psm3(K105RK106R)*
	PL499	*h$^+$ leu1 cen2$^+$<<lacO-ura4$^+$-kan^r his7$^+$<P$_{dis1}$-GFP-lacI-NLS rec12Δ::LEU2 moa1Δ::kan^r nat^r-psm3(K105QK106Q)*

	PL502	*h⁻ leu1 rec12Δ::LEU2 moaΔ1::kan^r nat^r-psm3(K105QK106Q)*
	PH806	*h⁻ leu1 rec12Δ::LEU2 clr6-1 moa1Δ::kan^r nat^r-psm3⁺*
	PH812	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 clr6-1 moa1Δ::kan^r nat^r-psm3⁺*
	PH808	*h⁻ leu1 rec12Δ::LEU2 clr6-1 moa1Δ::kan^r nat^r-psm3(K105RK106R)*
	PH814	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 clr6-1 moa1Δ::kan^r nat^r-psm3(K105RK106R)*
	PH807	*h⁻ leu1 rec12Δ::LEU2 clr6-1 moa1Δ::kan^r nat^r-psm3(K105QK106Q)*
	PH813	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 clr6-1 moa1Δ::kan^r nat^r-psm3(K105QK106Q)*
	PL425	*h⁻ leu1 rec12Δ::LEU2 clr6-1 moa1Δ::kan^r rec8Δ::nat^r*
	PL426	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 clr6-1 moa1Δ::kan^r rec8Δ::nat^r*
Fig. S7	PL487	*h⁺ leu1 rec12Δ::LEU2 nat^r-psm3(K105QK106Q)*
	PL490	*h⁻ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 nat^r-psm3(K105QK106Q)*
	PL568	*h⁻ leu1 rec12Δ::LEU2 eso1Δ::ura4⁺ nat^r-psm3(K105QK106Q)*
	PL576	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 eso1Δ::ura4⁺ nat^r-psm3(K105QK106Q)*
Fig. S8	PL499	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 moa1Δ::kan^r nat^r-psm3(K105QK106Q)*
	PL502	*h⁻ leu1 rec12Δ::LEU2 moaΔ1::kan^r nat^r-psm3(K105QK106Q)*
	PH807	*h⁻ leu1 rec12Δ::LEU2 clr6-1 moa1Δ::kan^r nat^r-psm3(K105QK106Q)*
	PH813	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 clr6-1 moa1Δ::kan^r nat^r-psm3(K105QK106Q)*
	PL573	*h⁻ leu1 rec12Δ::LEU2 moaΔ1::kan^r eso1Δ::ura4 nat^r-psm3(K105QK106Q)*
	PL579	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 moa1Δ::kan^r eso1Δ::ura4 nat^r-psm3(K105QK106Q)*
	PH865	*h⁻ leu1 rec12Δ::LEU2 clr6-1 moa1Δ::kan^r eso1Δ::hyg^r nat^r-psm3(K105QK106Q)*
	PH866	*h⁺ leu1 cen2⁺<<lacO-ura4⁺-kan^r his7⁺<P_dis1-GFP-lacI-NLS rec12Δ::LEU2 clr6-1 moa1Δ::kan^r eso1Δ::hyg^r nat^r-psm3(K105QK106Q)*

Supplemental Figure S1.

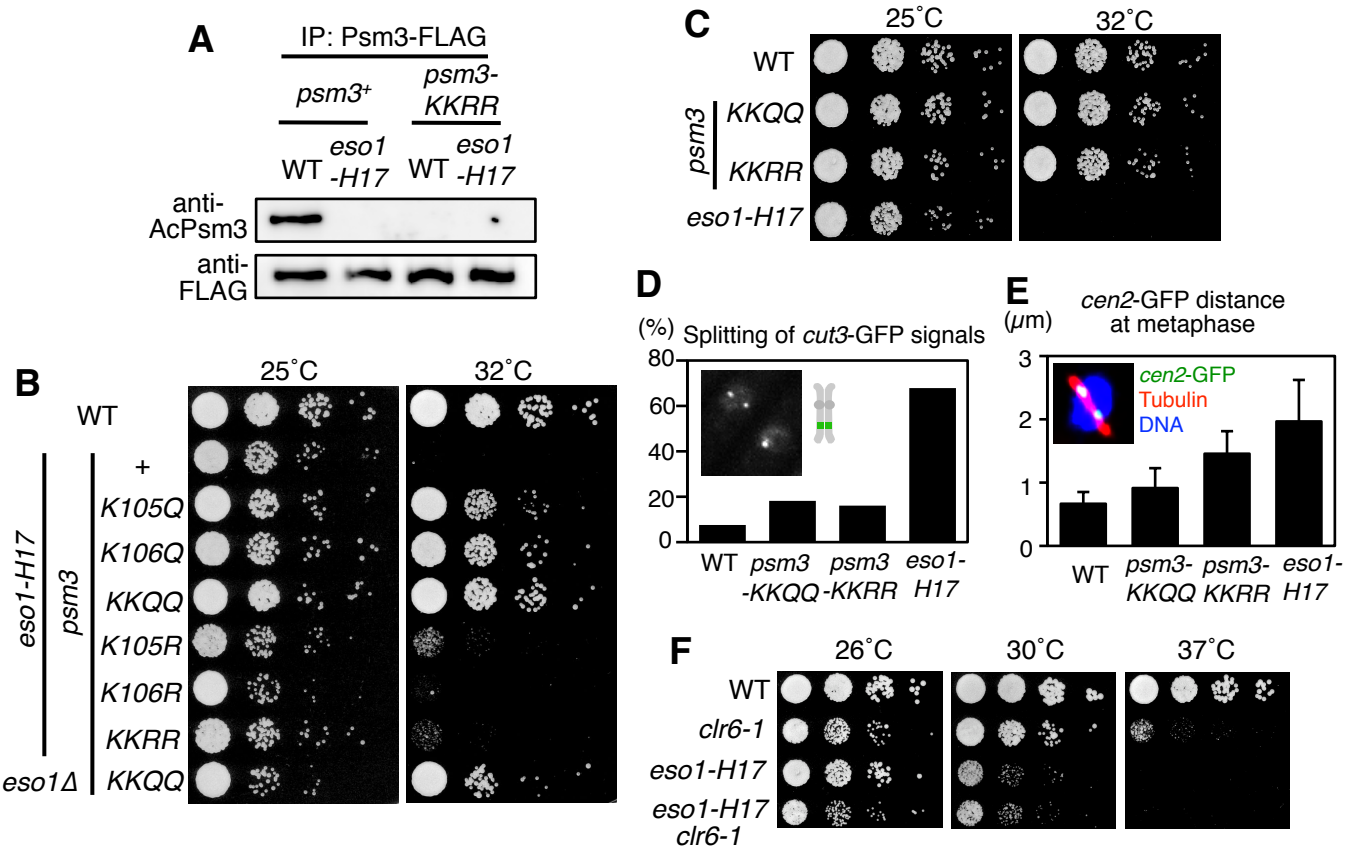

Figure S1. Psm3-K105/K106 is the acetylation target of Eso1 in mitotic cells

(A)The indicated cells were cultured at 26˚C. Immunopurified Psm3-FLAG proteins from the cell extracts were analyzed by immunoblot using anti-AcPsm3 and anti-FLAG antibodies.

(B)Serial dilutions of the indicated cells were spotted onto yeast extract (YE) plates and incubated at 25˚C and 32˚C.

(C)Serial dilutions of the indicated cells were spotted onto yeast extract (YE) plates and incubated at 25˚C and 32˚C.

(D)The number of cells with two *cut3*-GFP dots in an interphase nucleus was counted in the indicated strains (n > 100). Representative picture showing the *cut3*-GFP signals is shown.

(E)The distance between *cen2*-GFP marked at the centromere was measured in the indicated cells arrested at metaphase (by *cut9-665*). Representative picture showing the *cen2*-GFP signals on the metaphase spindle (mCherry-tubulin) is shown. Error bars represent SD (n > 20). Note that centromere cohesion is mildly impaired in the metaphase-arrested *psm3-KKRR* cells.

(F)Serial dilutions of the indicated cells were spotted onto yeast extract (YE) plates and incubated at the indicated temperatures.

Supplemental Figure S2.

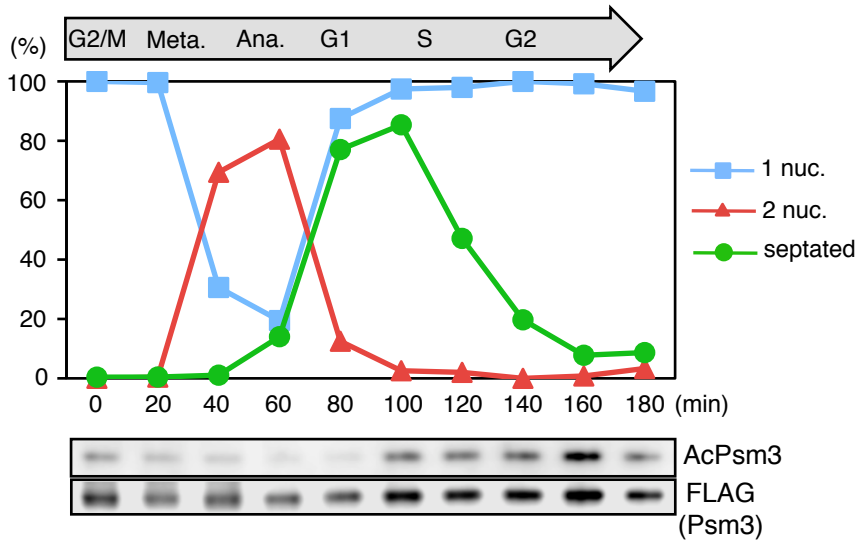

Figure S2. Acetylation of Psm3-K106 increases during S phase and declines during anaphase toward G1 phase

Immunopurified Psm3-FLAG proteins from synchronously cultured cell extracts were analyzed by immunoblot using anti-AcPsm3 and anti-FLAG antibodies.

Note that, although the Psm3 protein level does not fluctuate during the cell cycle, acetylation increases during S phase and declines during anaphase toward G1 phase.

Supplemental Figure S3.

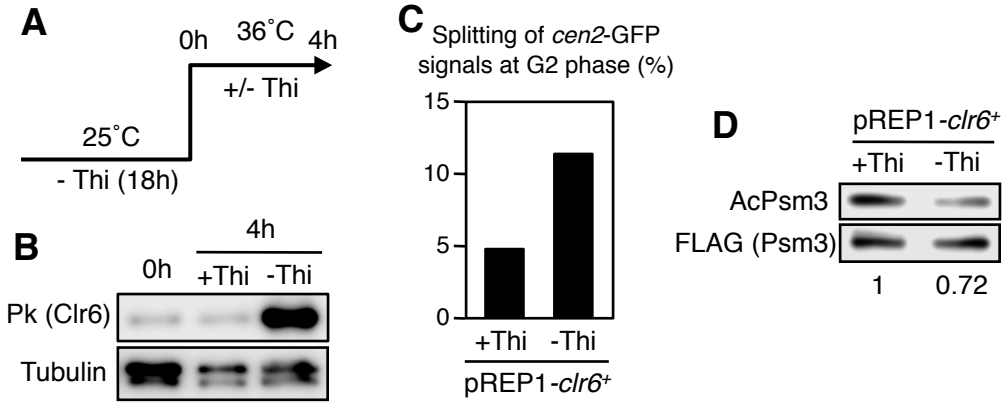

Figure S3. Transient expression of Clr6 during G2 phase decreases Psm3 acetylation and sister chromatid cohesion

(A) *cdc25-22 cen2*-GFP cells carrying pREP1-*clr6*$^{+}$ were cultured at 25˚C in the absence of thiamine for 18 hr and shifted to 36˚C for 4 hr with or without adding thiamine to arrest at G2 phase. (B) Immunoblots of Pk-tagged Clr6 indicate that Clr6 is overexpressed only after shift to 36˚C in this condition. (C) The number of cells with two *cen2*-GFP dots was counted (n > 190). (D) Acetylation status of Psm3 was analyzed by immunoblot as in Figure 1B. The ratios of signal intensities of bands representing AcPsm3 and FLAG (Psm3) were used to calculate the relative acetylation values shown.

Supplemental Figure S4.

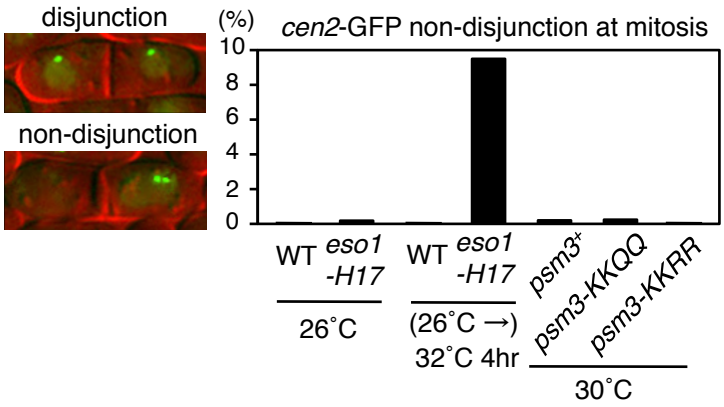

Figure S4. Acetylation at Psm3-K105/K106 is dispensable for equational division at mitosis, although centromeric cohesion at metaphase is partly impaired
Segregation of *cen2*-GFP at/after mitotic anaphase was examined in the indicated cells under the indicated conditions (n > 300).

Supplemental Figure S5.

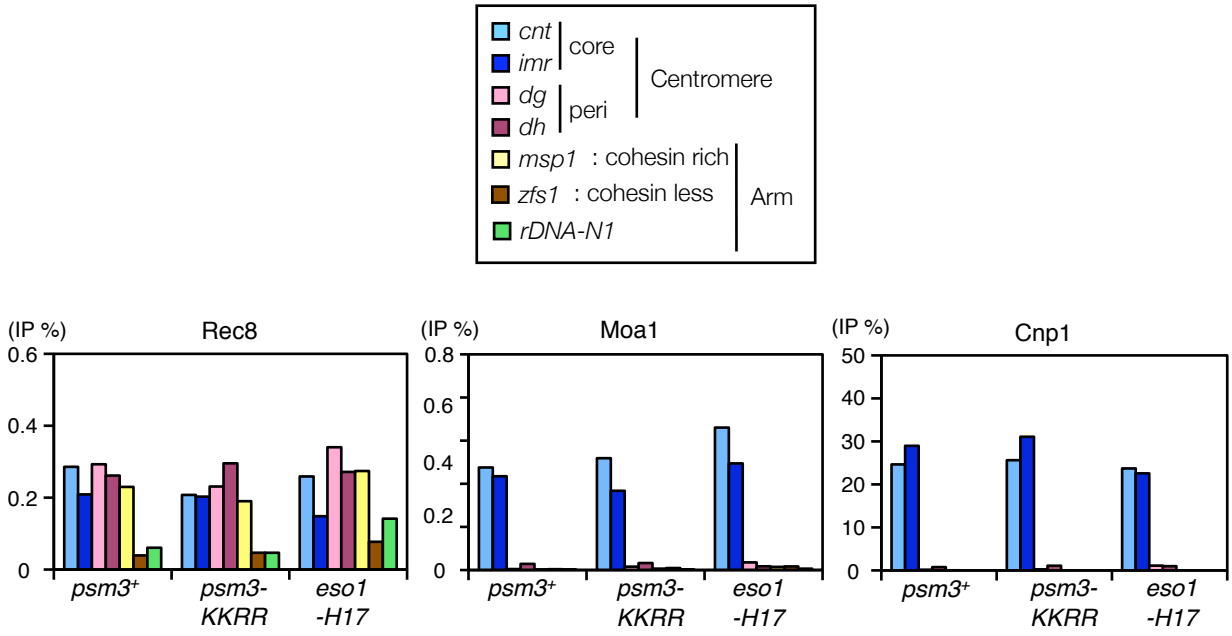

Figure S5. Localization of Rec8 and Moa1 is intact in *psm3-KKRR* and *eso1-H17* cells in meiosis I

ChIP assay was used to measure Rec8, Moa1 and Cnp1 throughout core centromere (*cnt* and *imr)*, pericentric (*dg* and *dh*), arm (*mps1*and *zfs1*) and rDNA (*rDNA-N1*) regions in the indicated strains arrested at prometaphase I by *mei4Δ* mutation. Average of two polymerase chain reaction (PCR) amplifications.

Supplemental Figure S6.

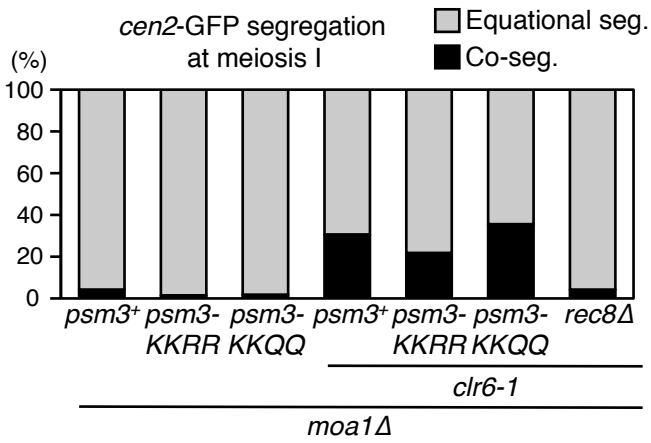

Figure S6. The mutation of *clr6-1* can suppress *moa1Δ* even in the background of Psm3-acetylation mutations

Segregation of heterozygous *cen2*-GFP at meiosis I was examined in the indicated *rec12Δ* zygotes at 30˚C (n > 150).

Supplemental Figure S7.

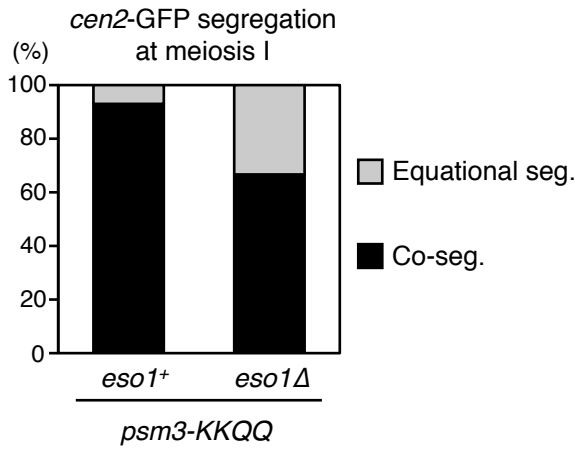

Figure S7. *eso1Δ* is not completely suppressed by *psm3-KKQQ* in meiosis
Segregation of heterozygous *cen2*-GFP at meiosis I was examined in the indicated *rec12Δ* zygotes at 26˚C (n > 160).

Supplemental Figure S8.

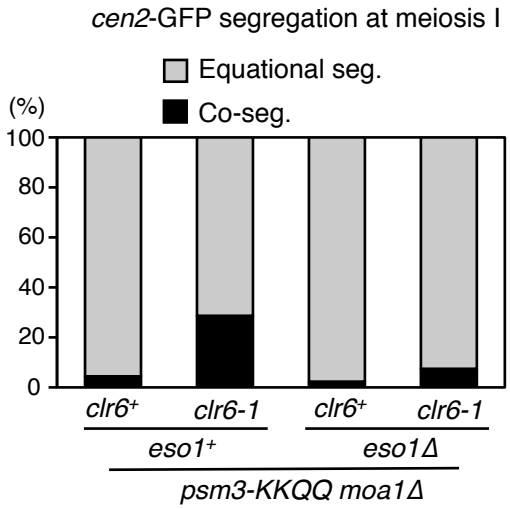

Figure S8. Acetylation of the non-Psm3-K105/K106 substrate counteracted by *clr6-1* in *moa1Δ* background is largely dependent on Eso1
Segregation of heterozygous *cen2*-GFP at meiosis I was examined in the indicated *rec12Δ* zygotes at 30˚C (n > 120).