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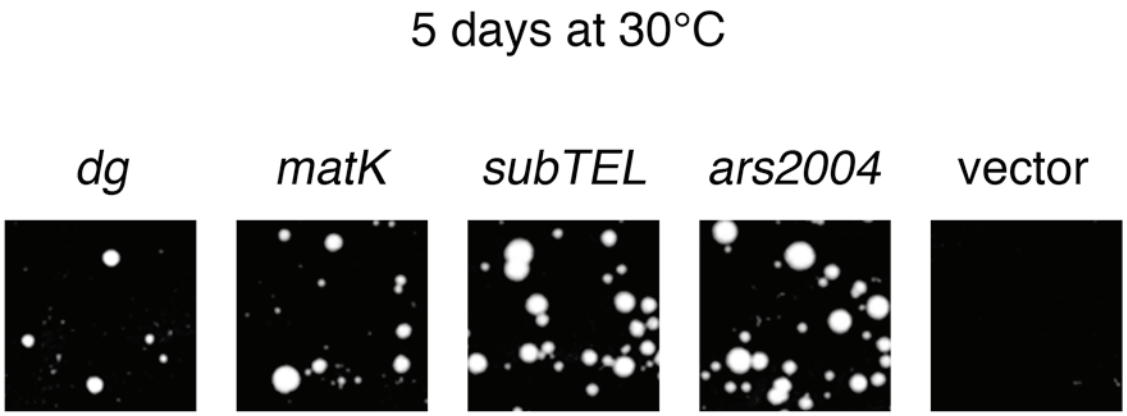

Figure S1 All heterochromatic loci contain autonomously replicating sequence (ARS). To examine the ARS activity of heterochromatic replication origins, fragments corresponding to ars3.0K[1] in the pericentromere (*dg*) and ars2PR[2] in the *mat* locus, which had been previously described, were PCR-cloned into pYC11 carrying *LEU2* gene. For the subtelomeric replication origin, a fragment that contains multiple AT-stretches characteristic in fission yeast replication origins at 21 kb from the right telomere of chromosome 2 was PCR-cloned into pYC11. Each plasmid was introduced into HM123 (h^- *leu1-32*) followed by 5 days incubation at 30°C and pictures were taken. *Ars2004* and vector serve as positive and negative controls, respectively.

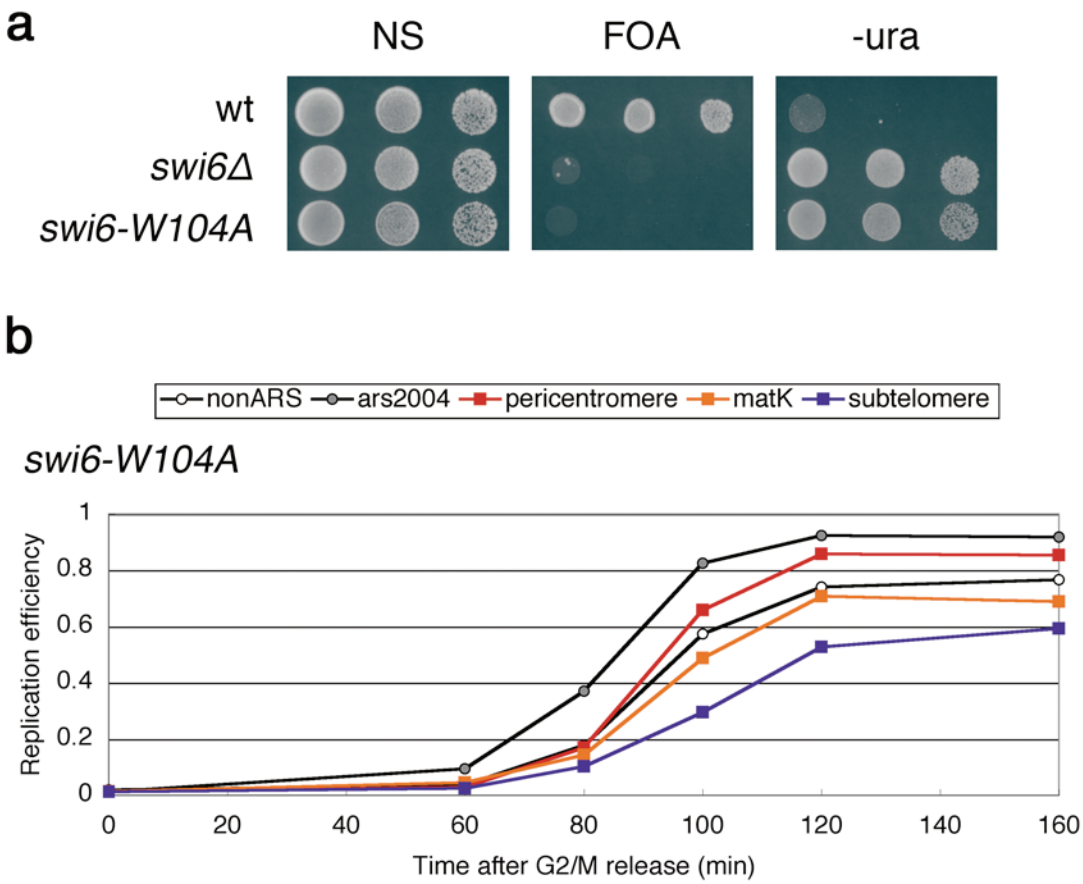

Figure S2 Swi6 promotes early replication at the pericentromere and the *mat* locus in a chromo-domain dependent manner. To express Swi6-W104A mutant protein from the endogenous *swi6*$^{+}$ promoter, the *swi6*$^{+}$ coding sequence with its potential promoter and terminator regions was first cloned into pBluescript (pAL2pBK), and a *hyg*^{r} cassette was introduced into the pAL2pBK plasmid (pAL2pBK-H). The W104A mutation was introduced using site-directed mutagenesis and sequence was confirmed. The resultant plasmid (pAL2W104ApBK-H) was cleaved with *Hpa*I for introduction into downstream of the *swi6* locus in *swi6Δ* cells, and the transformants were isolated using medium containing hygromycin. The expression of Swi6-W104A was confirmed by western blotting with anti-Swi6 antibody (data not shown). (**a**) The point mutation *swi6-W104A* impairs the silencing at the silent *mat* locus. Silencing of a *ura4*$^{+}$ marker inserted at the silent *mat* locus was examined by growth on selective media. Ten-fold-diluted cultures of indicated strains were plated onto nonselective medium (NS), medium containing 5-FOA (FOA) and medium lacking uracil (-ura). (**b**) Chromo-domain of Swi6 is required for early replication at the pericentromere and the *mat* locus. Replication kinetics in *swi6-W104A* cells was analyzed as in Fig. 1**b**.

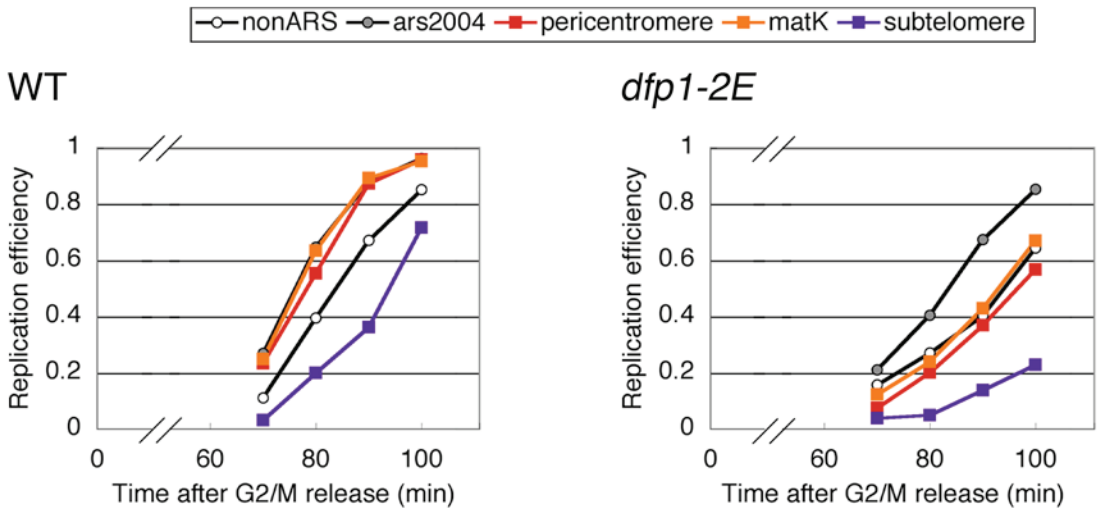

Figure S3 Replication delays at the pericentromere and the *mat* locus in *dfp1-2E* cells. The endogenous *dfp1*$^{+}$ gene was replaced with *dfp1-2E* and replication kinetics of indicated loci was examined as described in Fig. 1**b**.

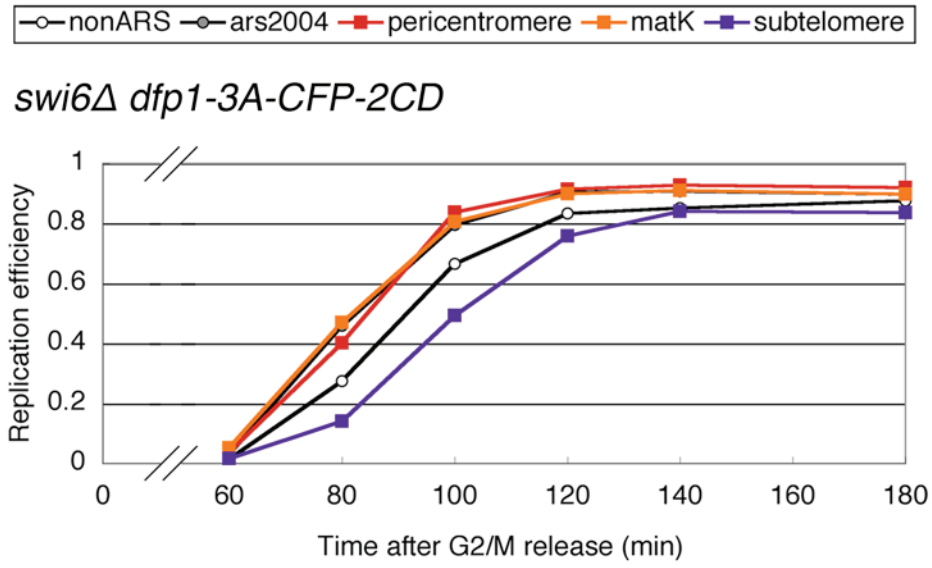

Figure S4 Tethering of Dfp1-3A restores early replication at the pericentromere and the *mat* locus in *swi6Δ* cells. Dfp1-3A was fused with CFP and two tandem copies of chromo-domain (CD) of Swi6 and expressed from the native *dfp1*$^{+}$ promoter in *swi6Δ* cells. Replication kinetics of indicated loci were analyzed as described in Fig. 1**b**.

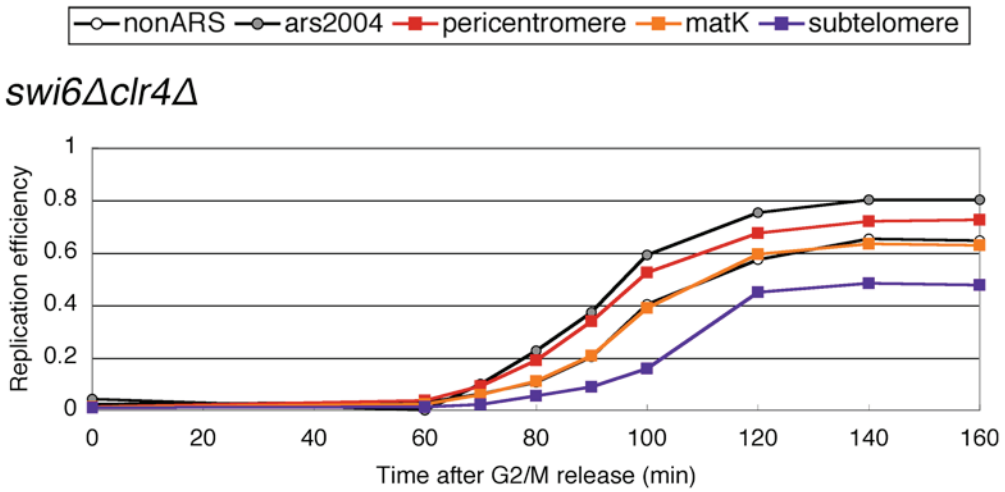

Figure S5 Deletion of the *clr4*$^{+}$ in *swi6Δ* background restores early replication at the pericentromere but not at the *mat* locus. Replication kinetics of indicated loci in *swi6Δclr4Δ* double mutant cells were analyzed as described in Fig. 1**b.**

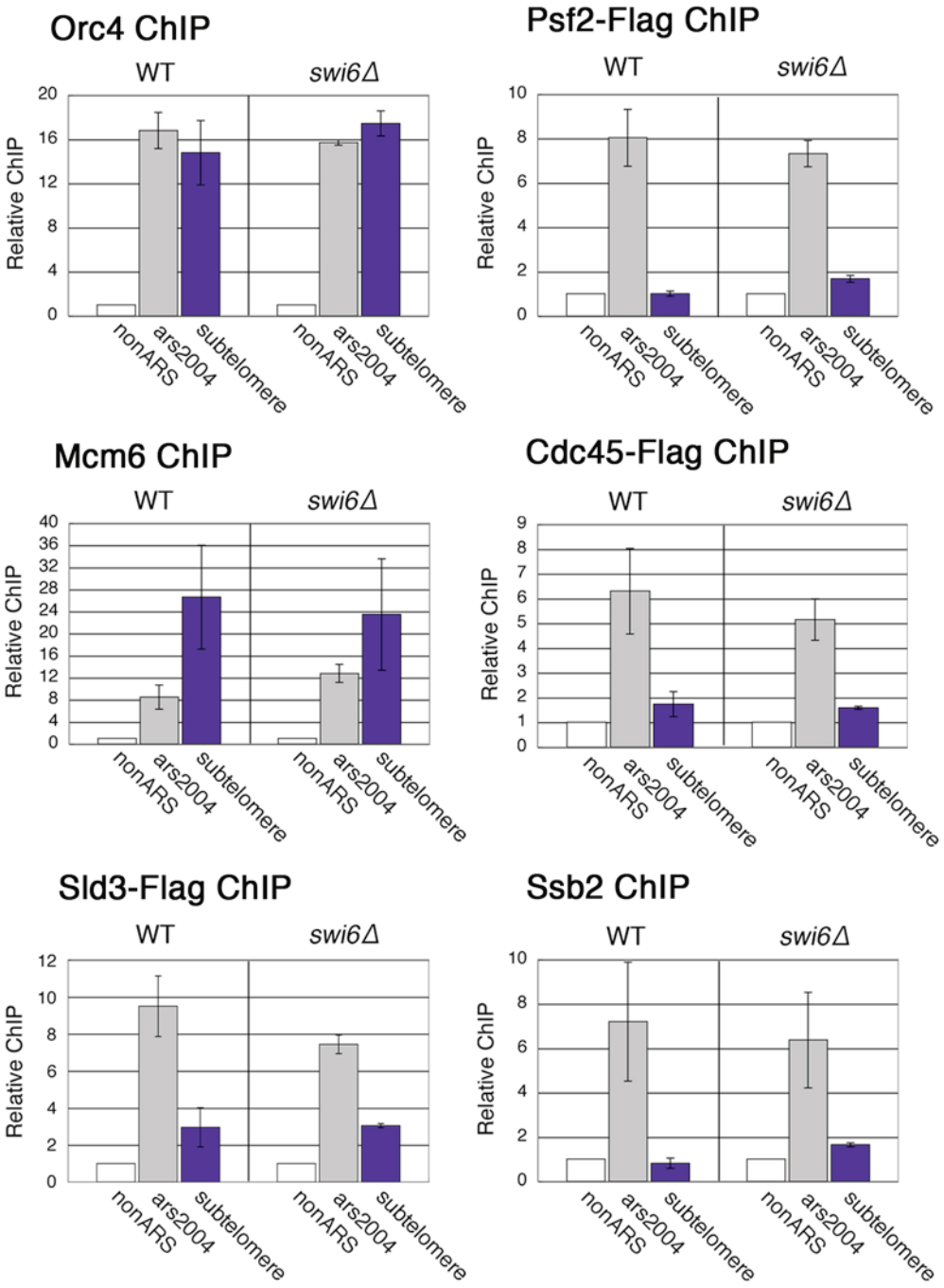

Figure S6 Initiation of replication at the subtelomeric ARS is inhibited after pre-RC formation in both wild type and *swi6Δ* cells. ChIP samples in Fig. 2 **b**-**g** were examined by quantitative real-time PCR using the subtelomeric primers shown in Fig. 1**a**. Error bars represent standard deviations (n=3).

Supplementary References

1. Smith, J. G. et al. Replication of centromere II of *Schizosaccharomyces pombe*. *Mol. Cell Biol.* **15**, 5165-5172 (1995).
2. Olsson, T., Ekwall, K. & Ruusala, T. The silent P mating type locus in fission yeast contains two autonomously replicating sequences. *Nucleic Acids Res.* **21**, 855-861 (1993).

Supplementary Table 1 *S. pombe* strains used in this study

Strain	Genotype		Figures and Supplementary figures
HM123	h^-	*leu1-32*	S1
HM664	h^-	*ura4-D18::ura4$^+$nmt1-TK$^+$*	used for transformation
HM683	h^+	*ura4-D18::ura4$^+$nmt1-TK$^+$*	used for transformation
HM1182	h^{90}	*cdc25-22 ura4-D18:: ura4$^+$nmt1-TK$^+$ his2 Kint2::ura4$^+$*	1**b**, **c**, **d**, 3**d**, S3
HM1183	h^{90}	*cdc25-22 ura4-D18:: ura4$^+$nmt1-TK$^+$ his2 Kint2::ura4$^+$ swi6Δ::kanMX6*	1**b**, **c**, **d**, 3**d**, 4**c**
HM1418	h^-	*ura4-D18::ura4$^+$nmt1-TK$^+$ lys1Δ::(dfp1$^+$-CFP-2CD hphMX6)*	transformant
HM1420	h^{90}	*cdc25-22 ura4-D18:: ura4$^+$nmt1-TK$^+$ his2 Kint2::ura4$^+$ swi6Δ::kanMX6 lys1Δ::(dfp1$^+$-CFP-2CD hphMX6)*	4**a**, **b**, **c**
HM1423	h^{90}	*cdc25-22 ura4-D18:: ura4$^+$nmt1-TK$^+$ his2 Kint2::ura4$^+$ lys1Δ::(dfp1$^+$-CFP-2CD hphMX6)*	4**d**
HM1460	h^-	*ura4-D18::ura4$^+$nmt1-TK$^+$ lys1Δ::(CFP-2CD hphMX6)*	transformant
HM1467	h^{90}	*cdc25-22 ura4-D18:: ura4$^+$nmt1-TK$^+$ his2 Kint2::ura4$^+$ swi6Δ::kanMX6 lys1Δ::(CFP-2CD hphMX6)*	4**a**, **b**, **c**
HM1471	h^{90}	*cdc25-22 ura4-D18:: ura4$^+$nmt1-TK$^+$ his2 Kint2::ura4$^+$ lys1Δ::(CFP-2CD hphMX6)*	4**d**
HM1482	h^{90}	*cdc25-22 ura4-D18:: ura4$^+$nmt1-TK$^+$ his2 Kint2::ura4$^+$ swi6Δ::kanMX6 clr4Δ::kanMX6*	S5
HM1588	h^{90}	*cdc25-22 ura4-D18:: ura4$^+$nmt1-TK$^+$ Flag-cdc45::kanMX6 his2 Kint2::ura4$^+$*	2**c**, **f**, **g**, S6
HM1589	h^{90}	*cdc25-22 ura4-D18:: ura4$^+$nmt1-TK$^+$ Flag-cdc45::kanMX6 his2 Kint2::ura4$^+$ swi6Δ::kanMX6*	2**c**, **f**, **g**, S6
HM1590	h^{90}	*cdc25-22 ura4-D18:: ura4$^+$nmt1-TK$^+$ psf2-5Flag::kanMX6 his2 Kint2::ura4$^+$*	2**b**, **e**, S6
HM1591	h^{90}	*cdc25-22 ura4-D18:: ura4$^+$nmt1-TK$^+$ psf2-5Flag::kanMX6 his2 Kint2::ura4$^+$ swi6Δ::kanMX6*	2**b**, **e**, S6
HM1826	h^+	*ura4-D18::ura4$^+$nmt1-TK$^+$ dfp1-3A::kanMX6*	transformant
HM1828	h^+	*ura4-D18::ura4$^+$nmt1-TK$^+$ dfp1-2E::kanMX6*	transformant
HM1841	h^{90}	*cdc25-22 nda4-108 ura4-D18::ura4$^+$nmt1-TK$^+$ sld3-5Flag::kanMX6 his2 Kint2::ura4$^+$*	2**d**, S6
HM1843	h^{90}	*cdc25-22 nda4-108 ura4-D18::ura4$^+$nmt1-TK$^+$ sld3-5Flag::kanMX6 his2 Kint2::ura4$^+$ swi6Δ::kanMX6*	2**d**, S6
HM1853	h^{90}	*cdc25-22 ura4-D18::ura4$^+$nmt1-TK$^+$ dfp1-3A::kanMX6 his2 Kint2::ura4$^+$*	3**c**, **d**
HM1857	h^{90}	*cdc25-22 ura4-D18::ura4$^+$nmt1-TK$^+$ dfp1-2E::kanMX6 his2 Kint2::ura4$^+$*	S3
HM1899	h^+	*ura4-D18::ura4$^+$nmt1-TK$^+$ lys1Δ::(dfp1-3A-CFP-2CD hphMX6)*	transformant
HM1934	h^{90}	*cdc25-22 ura4-D18::ura4+nmt1-TK$^+$ his2 Kint2::ura4$^+$ swi6Δ::kanMX6 lys1Δ::(dfp1-3A-CFP-2CD hphMX6)*	S4
HM1994	h^{90}	*ura4-DS/E Kint2::ura4$^+$ swi6Δ::kanMX6*	S2**a**
HM2608	h^{90}	*cdc25-22 ura4-D18::ura4+nmt1-TK$^+$ his2 Kint2::ura4$^+$ swi6Δ::kanMX6::(swi6-W104A hphMX6)*	S2**b**
HM2613	h^{90}	*ura4-DS/E Kint2::ura4$^+$ swi6Δ::kanMX6::(swi6-W104A hphMX6)*	S2**a**
TNF2518	h^{90}	*ura4-DS/E Kint2::ura4$^+$*	S2**a**

Supplementary Table 2 Primers used in this study

Locus	Name	Sequence	Source
ars2004	ars2004-66-F	5'-CGGATCCGTAATCCCAACAA-3'	Hayashi *et al*., 2007
	ars2004-66-R	5'-TTTGCTTACATTTTCGGGAACTTA-3'	
nonARS	nonARS-70-F	5'-TACGCGACGAACCTTGCATAT-3'	Hayashi *et al*., 2007
	nonARS-70-R	5'-TTATCAGACCATGGAGCCCATT-3'	
dg (pericentromere)	dg-108-F	5'-TCCAAATGTCGCATGAACACTC-3'	Hayashi *et al*., 2007
	dg-108-R	5'-CTTTTTTGGGAATACATTGGGTTT-3'	
mat K locus	matK-108-F	5'-TCTTCCCTGCGTTGGACTTC-3'	This study
	matK-108-R	5'-CACCCTACCATCCGTGTTACCT-3'	
subtelomere	TEL-59-F	5'-CAGAAGAGACTACAGAGGCGGTTT-3'	This study
	TEL-59-R	5'-GGATGCCTTATCTGCGACCA-3'	