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Supplemental Data

siRNA-Mediated Heterochromatin Establishment Requires HP1 and Is Associated with Antisense Transcription

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Supplemental Experimental Procedures

Yeast strains

All *S. pombe* strains used are listed in Table S1. Deletion and tagging to produce 5FLAG fusion proteins of the endogenous *chp1*$^{+}$ were performed as described previously (Sadaie et al., 2004). For construction of hairpin *ura4h-2 ~ -7* strains, hairpin plasmids linearized by *Bbr*PI digestion were used for transformation with clonNAT (Werner BioAgents) containing medium. To obtain *5-FOA*^{R} background mutants, cells grown in 5-FOA containing medium, were used for transformation.

The *trp1*$^{+}$*::ura4*$^{+}$ allele construction was performed by as described previously (Sadaie et al., 2004). PCR fragments were amplified with primer sets, prITtrp1-1/3, prITtrp1-2/4 and prITtrp1-1/2.

Plasmid construction

Plasmids used in this study are listed in Table S3. For hairpin plasmid construction, *S. pombe adh1*$^{+}$-promoter fragments were amplified by PCR from SPY139 genomic with a primer set prITadh1F/R. And the resultant fragments, which were digested with *Sph*I and *Nde*I, were cloned into *Sph*I-*Nde*I site of pREP1 to obtain pART. To construct hairpin allele *ura4-hX* (X; 2, 3, 4, 5, 6 and 7) hairpin plasmids, pnatMXARTshura4-X, were used. Each *ura4-hX* corresponds to the *ura4*$^{+}$ locus: *h2* is from –130 to 11 (length: 141 bp), *h3* is from 1 to 140 (140 bp), *h4* is from 131 to 410 (280 bp), *h5* is from 401 to 679 (278bp), *h6* is from 674 to 810 (137bp) or *h7* is from 801 to 940 (140bp). The *cox4*-intron fragment and the ura4 fragments were amplified by PCR from SPY139 genomic DNA and pKS-ura4, respectively. Primer pairs to fragments *cox4*-intron, ura4-2, -3, -4, -5, -6 and –7 were prIT0F/R, prIT2F/R, prIT3F/R, prIT4F/R, prIT5F/R, prIT6F/R and prIT7F/R, respectively. *Cox4*-intron and ura4-X fragments were digested with *Bgl*II and *Bam*HI, respectively. The resulting fragments, *Bgl*II-*cox4*-intron-*Bgl*II and *Bam*HI-ura4-X, were ligated by T4-DNA ligase. *Cox4*-ura4-hX fragments were amplified from the ligation mixture by PCR with pimer set, prITXR and prIT0F. Ura4-X fragments were digested with *Xho*I and *Bam*HI, and the resulting fragments were cloned into *Sal*I-*Bam*HI site of pART to construct pARTura4-Xrv. *Cox4*-ura4-X fragments, digested with *Bgl*II and *Sma*I, were cloned into *Bam*HI-*Sma*I site of pARTura4-Xrv to obtain pARTshura4-X. To construct pnatMXARTshura4-X, *Eco*RV-*Xba*I hairpin fragments from pARTshura4-X were cloned into *Eco*RV-*Spe*I site of *natMX*

gene plasmid, pAG25. Each pnatMXARTshura4-X plasmid was used for integration into the *nmt1* terminator region. pREP1-*swi6*$^{+}$ (gift of Marc Bühler) was used for *swi6*$^{+}$-overexpression. pREPNFLAG-dcr1 (Colmenares et al., 2007) was used for *dcr1*$^{+}$-overexpression.

Supplemental References

Colmenares, S.U., Buker, S.M., Bühler, M., Dlakic, M., and Moazed, D. (2007). Coupling of double-stranded RNA synthesis and siRNA generation in fission yeast RNAi. Mol Cell *27*, 449-461.

Sadaie, M., Iida, T., Urano, T., and Nakayama, J. (2004). A chromodomain protein, Chp1, is required for the establishment of heterochromatin in fission yeast. EMBO J *23*, 3825-3835.

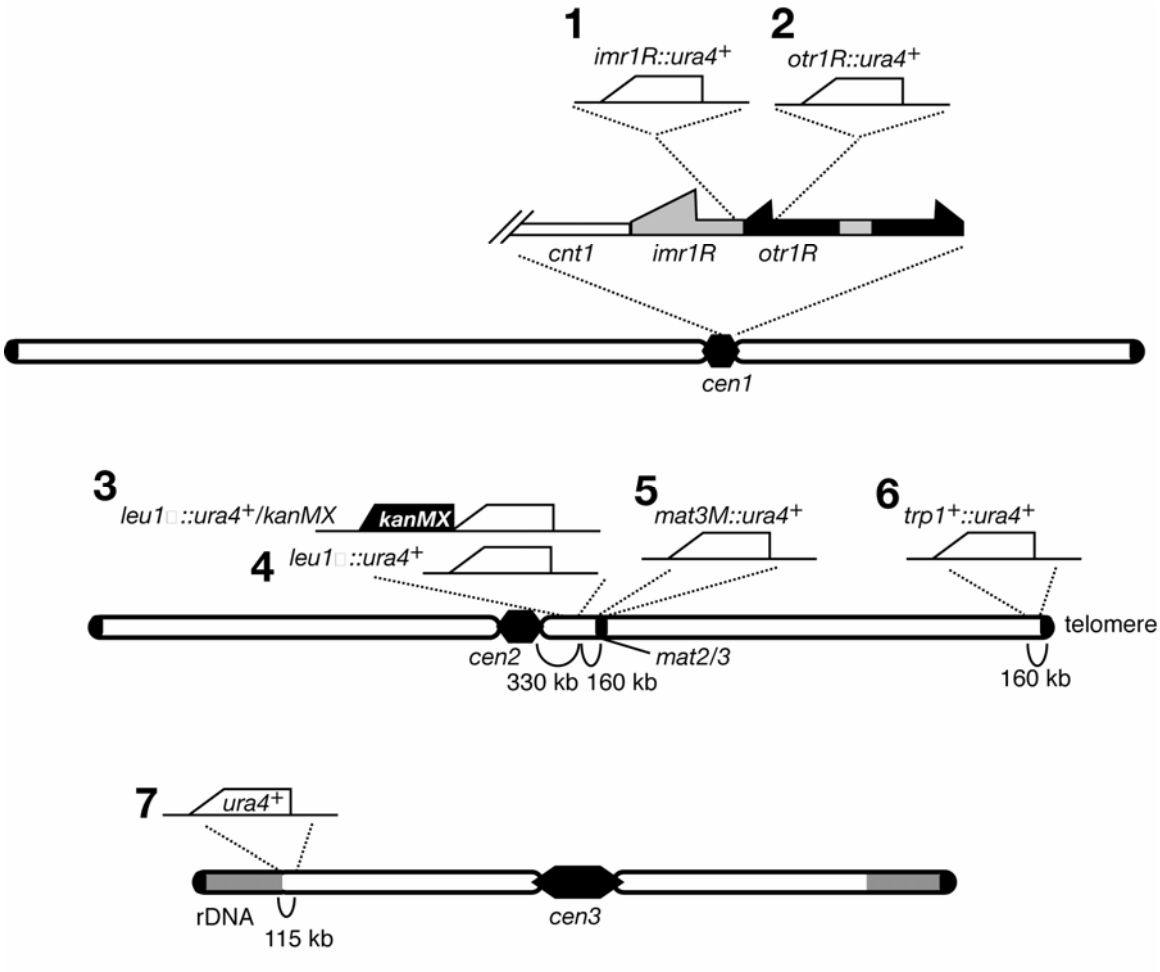

Figure S1. Chromosomal location of hairpin-targeted *ura4*$^+$ genes.
Schematic diagrams of the three chromosomes in *S.pombe*. The location of centromeres (hexagons), centromeric DNA repeats (inset above chromosome I), heterochromatic regions at telomeres and the silent mating type loci (mat2/3), and the 7 *ura4*$^+$ inserts (marked 1 through 7) are indicated. The gray bars on the right and left arms of chromosome III show the locations of rDNA repeat regions. For *ura4*$^+$ and *trp1*$^+$*::ura4*$^+$ loci, distance from a proximal telomere and rDNA are shown, respectively.

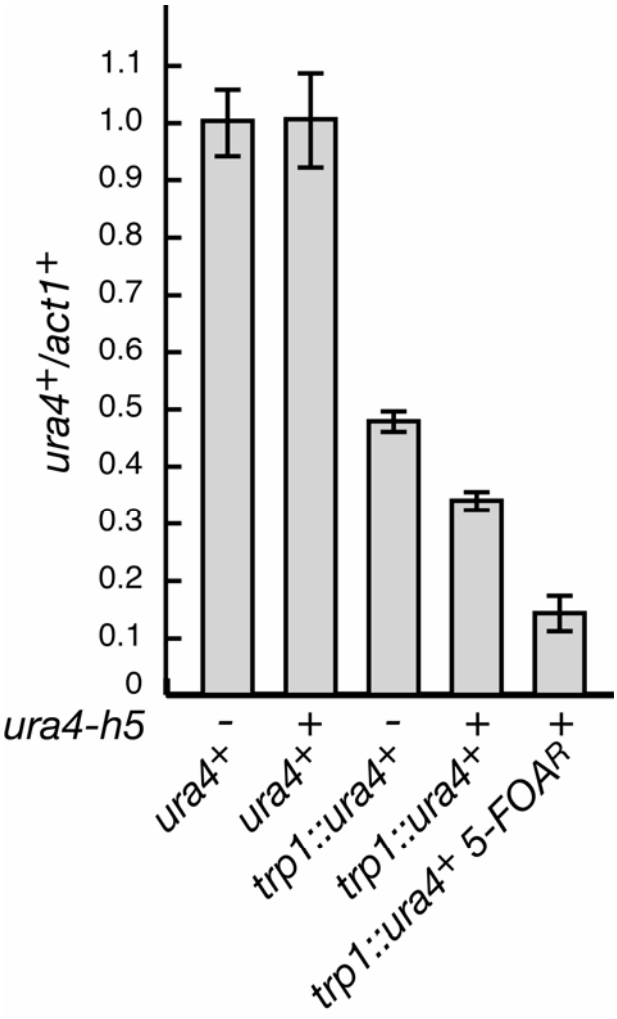

Figure S2. *ura4*$^+$ transcription level in non-silenced clones and silenced clones. The ratio of *ura4*$^+$ versus *act1*$^+$ transcripts (*ura4*$^+$/*act1*$^+$) was determined by quantitative RT-PCR. Total RNA from *ura4*$^+$ and *trp1*$^+$*::ura4*$^+$ cells with or without *ura4h-5* was analyzed. The RNA from silenced clones (*trp1*$^+$*::ura4*$^+$ *ura4h-5 5FOA*^{R})(pREP-Swi6$^+$) was prepared after growth under non-selective conditions (>20 generations). The ratios were normalized to that of the *ura4*$^+$/*act1* ratio in cells without *ura4h-5* hairpin (set to 1.0). Each error bar indicates a standard deviation (n>3).

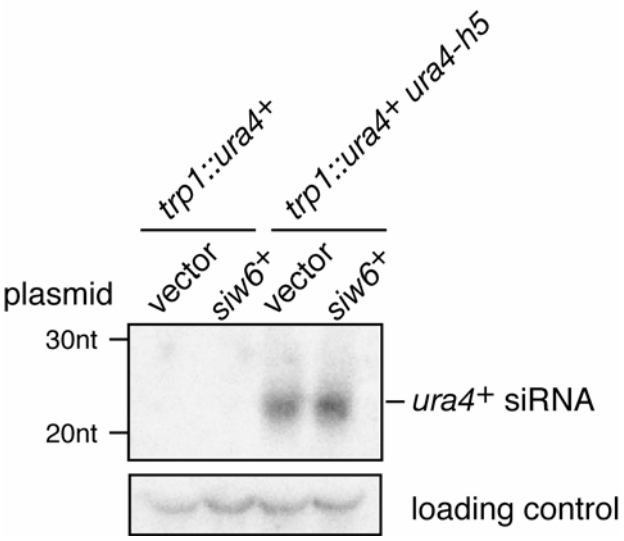

Figure S3. Swi6 overexpression did not affect hairpin siRNA level.
RNA extracted from cells harboring either empty or Swi6-overexpression vector was analyzed using northern blotting as in Figure 1B.

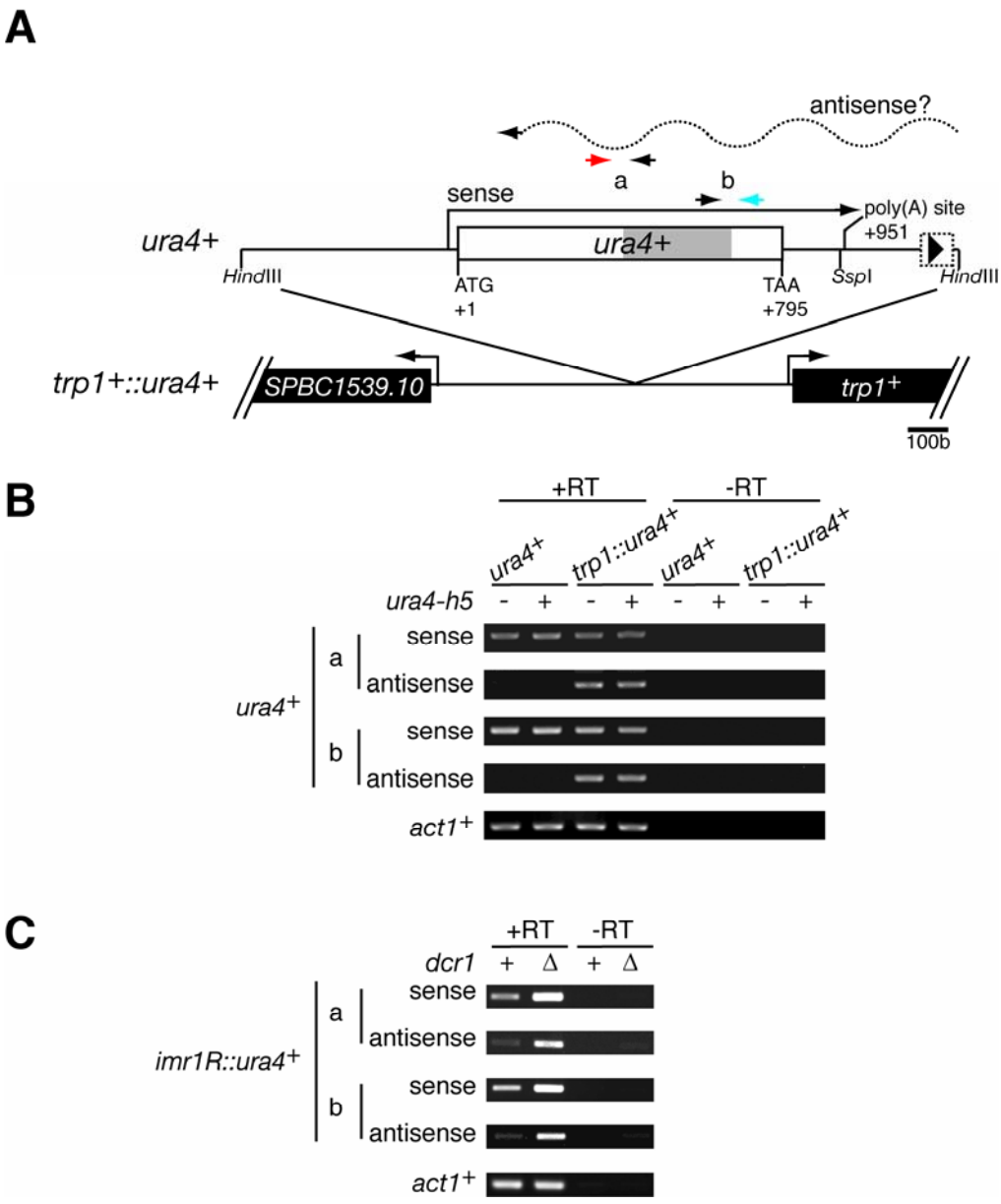

Figure S4. Antisense transcription at *ura4*$^+$ hairpin target loci.
A, Schematic diagram of the *ura4*$^+$ and *trp1*$^+$*::ura4*$^+$ loci. At the *trp1*$^+$*::ura4*$^+$ locus, a 1.8 kb *ura4*$^+$-*Hind* III fragment was inserted into an upstream region of the *trp1*$^+$ gene. Gray box in *ura4*$^+$ coding region shows the *ura4-h5* hairpin target region. Small arrows indicate the location of RT-PCR primer sets (a and b) used in RT-PCR analysis. The red and blue arrows were primers used for cDNA synthesis of anti-sense and sense transcripts, respectively. **B,** Strand-specific RT-PCR detecting an antisense transcript at the *trp1*$^+$*::ura4*$^+$ locus but not the *ura4*$^+$ locus. RT-PCR was performed with equal amount of total RNAs from the indicated strains. As a control, RT-PCR products of *act1*$^+$ in each RNA samples are shown. **C,** Strand-specific RT-PCR at the *imr1R::ura4*$^+$ locus showing the presence of an antisense transcript whose levels are elevated in *dcr1Δ* relative to wild-type cells. RT-PCR was performed with total RNA as in **B**.

Table S1. List of Strains Used in This Study

Strain	Genotype
SPY28	*h⁺ leu1-32 ade6-M216 ura4-D18 imr1*R*(NcoI)::ura4⁺ oriI*
SPY137	*h⁺ leu1-32 ade6-M210 ura4DS/E otr1R(SphI)::ura4⁺*
SPY139	*h⁹⁰ leu1-32 ade6-M216 ura4DS/E mat3M::ura4⁺*
SPY852	*h⁺ leu1-32 ade6-M216 ura4-D18 imr1*R*(NcoI)::ura4⁺ oriI clr4Δ::kanMX*
SPY854	*h⁻ ura4⁺::5BoxB-hphMX tas3⁺::λN-kanMX leu1Δ::ura4-intron-natMX eri1Δ::bleMX*
SPY1491	*h⁻ leu1-32 ade6-M210 ura4⁺*
SPY1641	*h⁺ leu1-32 ade6-M216 ura4-D18 imr1*R*(NcoI)::ura4⁺ oriI ura4h-5/natMX*
SPY1642	*h⁺ leu1-32 ade6-M210 ura4DS/E otr1R(SphI)::ura4⁺ ura4h-5/natMX*
SPY1643	*h⁹⁰ leu1-32 ade6-M216 ura4DS/E mat3M::ura4⁺ ura4h-5/natMX*
SPY1644	*h⁻ leu1-32 ade6-M210 ura4⁺ ura4h-2/natMX*
SPY1645	*h⁻ leu1-32 ade6-M210 ura4⁺ ura4h-3/natMX*
SPY1646	*h⁻ leu1-32 ade6-M210 ura4⁺ ura4h-4/natMX*
SPY1647	*h⁻ leu1-32 ade6-M210 ura4⁺ ura4h-5/natMX*
SPY1648	*h⁻ leu1-32 ade6-M210 ura4⁺ ura4h-6/natMX*
SPY1649	*h⁻ leu1-32 ade6-M210 ura4⁺ ura4h-7/natMX*
SPY1650	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺*
SPY1651	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX*
SPY1652	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX 5FOA^R*
SPY1653	*h⁻ leu1-32 ade6-M210 ura4⁺ chp1::chp1-5FLAGHis10/kanMX*
SPY1654	*h⁻ leu1-32 ade6-M210 ura4⁺ ura4h-5/natMX chp1-5FLAGHis10/kanMX*
SPY1655	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX dcr1Δ::kanMX* (*5FOA^R*)
SPY1656	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX ago1Δ::kanMX* (*5FOA^R*)
SPY1657	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX arb1Δ::kanMX* (*5FOA^R*)
SPY1658	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX arb2Δ::kanMX* (*5FOA^R*)
SPY1659	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX tas3Δ::kanMX* (*5FOA^R*)
SPY1660	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX chp1Δ::kanMX* (*5FOA^R*)
SPY1661	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX rdp1Δ::kanMX* (*5FOA^R*)
SPY1662	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX hrr1Δ::kanMX* (*5FOA^R*)
SPY1663	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX cid12Δ::kanMX* (*5FOA^R*)
SPY1664	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX clr4Δ::kanMX* (*5FOA^R*)
SPY1665	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX rik1Δ::kanMX* (*5FOA^R*)
SPY1666	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX swi6Δ::kanMX* (*5FOA^R*)
SPY1667	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX dcr1Δ::kanMX* (*5FOA^R*)
SPY1668	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5Δ::hphMX* (*5FOA^R*)
SPY1669	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX rdp1-D903A/kanMX* (*5FOA^R*)
SPY1670	*h⁺ leu1-32 ade6-M216 ura4-D18 imr1*R*(NcoI)::ura4⁺ oriI ura4h-5/natMX clr4Δ::kanMX*
SPY1671	*h⁺ leu1-32 ade6-M216 ura4-D18 imr1*R*(NcoI)::ura4⁺ oriI swi6Δ::kanMX*
SPY1672	*h⁺ leu1-32 ade6-M216 ura4-D18 imr1*R*(NcoI)::ura4⁺ oriI ura4h-5/natMX swi6Δ::kanMX*
SPY1673	*h⁻ leu1-32 ade6-M210 ura4DS/E leu1Δ::ura4⁺-intron/kanMX*
SPY1674	*h⁻ leu1-32 ade6-M210 ura4DS/E leu1Δ::ura4⁺-intron/kanMX ura4h-5/natMX*
SPY1675	*h⁻ leu1-32 ade6-M210 ura4⁺ ura4h-4/natMX chp1-5FLAGHis10/kanMX*
SPY1676	*h⁻ leu1-32 ade6-M210 ura4DS/E leu1Δ::ura4⁺-intron*
SPY1677	*h⁻ leu1-32 ade6-M210 ura4DS/E leu1Δ::ura4⁺-intron ura4h-5/natMX*
SPY1678	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX* (*5FOA^R*) [pREP1]
SPY1679	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX* (*5FOA^R*) [pREP1-FLAG-Dcr1]
SPY1680	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX rdp1-D903A/kanMX* (*5FOA^R*) [pREP1]
SPY1681	*h⁻ leu1-32 ade6-M210 ura4DS/E trp1⁺::ura4⁺ ura4h-5/natMX rdp1-D903A/kanMX* (*5FOA^R*) [pREP1-FLAG-Dcr1]

(*5FOA^R*): derivative from *5FOA^R* epi-allele strain.

Table S2. List of Oligonucleotides Used in This Study

Name		Sequence
prlTadh1F	5'-	CGAATGGCATGCCGATATCCAACTAAGAAAATGGC
prlTadh1R	5'-	CAAGACATATGATTCTCTTGCTTAAAGAAAAGCGAAG
prlT0F	5'-	GCTCTAGAGAAGATCTCCAAAACGGTAAGTCCTTTGAAAG
prlT0R	5'-	GACTAGTGAAGATCTCTTTACAACTAAAAGAATGTTAGTG
prlT2F	5'-	GACTAGTCCGGGATCCTAGCATCCATAACTTTGCTTTTAAAC
prlT2R	5'-	GCCCCCGGGGGAGCTCGAGATTGATTTTACCATCCCAGTT
prlT3F	5'-	GACTAGTCCGGGATCCATGGATGCTAGAGTATTTCAAAGC
prlT3R	5'-	GCCCCCGGGGGAGCTCGAGATTTCGGATTTCTTCGTCAAATC
prlT4F	5'-	GACTAGTCCGGGATCCAATCCGAAATCTTAGAATTGGTAG
prlT4R	5'-	GCCCCCGGGGGAGCTCGAGTCTTTGAGGCCTTGTATAATAC
prlT5F	5'-	GACTAGTCCGGGATCCCGCGCTACCGCAGTTTACAATC
prlT5R	5'-	GCCCCCGGGGGAGCTCGAGCCTCAAAGAAGTTGGTTTAC
prlT6F	5'-	GACTAGTCCGGGATCCGTAGCGATATCATCATTGTTGGTC
prlT6R	5'-	GCCCCCGGGGGAGCTCGAGTACATTAGTCTTTTTTTAATG
prlT7F	5'-	GACTAGTCCGGGATCCTTTAGTCGCTACATAAAATTTTAC
prlT7R	5'-	GCCCCCGGGGGAGCTCGAGACTAATGTAAAATTTTTTTGG
prlTtrp1-1	5'-	GCCATCTTATCTATTTAGAG
prlTtrp1-2	5'-	CATTTTCAGCTTGTTATAATTG
prlTtrp1-3	5'-	TCCTGTGTGAAATTGTTATCCGCTATAATAAAGTTGTAAACCAAATGAC
prlTtrp1-4	5'-	GTCGTGACTGGGAAAACCCTGGCGATTAACAGTTTTAAATGAACCGAC
prlT49	5'-	TTCGACAACAGGATTACGACC
prlT57	5'-	TGATTTTACCATCCCAGTTTAACTATGCTTCGTCGGCATC
prlT58	5'-	GCACATGTCGTGTTTTCTTACCGTATTGTCCTACCAAGAA
prlT59	5'-	CTTTTTTGCTTGGATCGAAATTAAAGGTTTAAAAGCAAAG
prlT60	5'-	AGTATTTCAAAGCTATTCAGCTAGAGCTGAGGGGATGAAA
prlT61	5'-	AAATCCCATTGCCAAGGAATTGTTGGCTTTGATGGAAGAA
prlT62	5'-	AAGCAAAGCAACTTGTCAGTCGCGGTCGATTTGACGAAGA
prlT63	5'-	CTTAGAATTGGTAGATAAAATTGGACCCTATGTCTGTGTT
prlT64	5'-	TGTTATCAAGACACATATTGACGTTGTCGAGGATTTCGAC
prlT65	5'-	GACCAGGATATGGTAGAAAAACTGGTGGCCTTAGGTAAA
prlT66	5'-	AAAAGCATCGTTTTCTTATCTTTGAGGATCGCAAATTCGC
prlT67	5'-	CGCAGACATTGGAAATACCGTCAAGCTACAATATGCATCT
prlT68	5'-	TCTGGTGTGTACAAAATTGCTTCTTGGGCTCATATCACAA
prlT69	5'-	CAAATTGCCATACAGTGCCAGGCGAGGGTATTATACAAGG
prlT70	5'-	AGTTGGTTTACCTTTGGGACGTGGTCTCTTGCTTTTGGCT
prlT71	5'-	CTGAAATGTCTTCCAAAGGCTCTTTGGCTACTGGTTCCTA
prlT72	5'-	TACACAGAGAAAACCTTAGAATGGTTTGAGAAGCATACCG
prlT73	5'-	CCGATTTTTGCTTTGGCTTTATAGCTGGTCGTCGATTTC
prlT74	5'-	TTCCTAACCTTCAAAGCGACTACATAACTATGTCCCCTGG
prlT75	5'-	TGGTATCGGCTTGGATGTTAAAGGAGACGGGCTGGGACAG
prlT76	5'-	CAGCAATATCGTACTCCTGAAGAAGTGATTGTAAACTGCG
prlT77	5'-	CATCATTGTTGGTCGTGGAGTCTATGGAGCTGGTCGTAAT
prlT78	5'-	TGTTGTCGAAGCCAAGAGATATAGAGAAGCTGGTTGGAAG
prlT79	5'-	TATCAGCAAAGACTTTCTCAGCATTAAAAAAAGACTAATG
prlT80	5'-	TTTTTTGGTTGGTTATTGAAAAAGTCGATGCCTTGTTTGC
prlT81	5'-	GTTTGTTTTCCTAGGCGTTTTATGTCAGAAGGCATTTAGA
prlT82	5'-	AGTATACAAGTACTCTTTGGTAAAATTTTATGTAGCGACT
prlT102	5'-	GAAGTACCCCATTGAGCACGG
prlT103	5'-	CAATTTCACGTTCGGCGGTAG
prlT105	5'-	CTGCCGATACATTTTAGGGCT
prlT106	5'-	CAGTACTGGCAAGGGAGACA
prlT107	5'-	TGGTAATACGTACTAGCTCTCG
prlT108	5'-	AACTAATTCATGGTGATTGATG
prlT181	5'-	GAGTCATCTTCTCACGGTTGG

prIT182	5'-	TCCTACGTTGGTGATGAAGC
prIT205	5'-	AATACCGTCAAGCTACAATATGCATCTGGTG
prIT206	5'-	GGTTTTCTCTGTGTAGGAACCAGTAGCC
prIT207	5'-	AACCCTCAGCTTTGGGTCTT
prIT208	5'-	TTTGCATACGATCGGCAATA

Table S3. List of Plasmids Used in this Study

Plasmid	Original name	Purpose
pDM914	pREPNFLAG-dcr1	*dcr1*$^{+}$ overexpression
pDM1153	pnatMXARTshura4-2	Hairpin expression (*ura4-h2*)
pDM1154	pnatMXARTshura4-3	Hairpin expression (*ura4-h3*)
pDM1155	pnatMXARTshura4-4	Hairpin expression (*ura4-h4*)
pDM1156	pnatMXARTshura4-5	Hairpin expression (*ura4-h5*)
pDM1157	pnatMXARTshura4-6	Hairpin expression (*ura4-h6*)
pDM1158	pnatMXARTshura4-7	Hairpin expression (*ura4-h7*)
pDM1159	pREP1-siw6^{+}	*swi6*$^{+}$ overexpression
pDM1160	pREP1	Control vector