Supporting Information

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SI Methods

Strain Construction. Homologous replacement of DNA was accomplished by lithium acetate transformation of PCR products containing 100 or 500 bp of targeting homology. YES media [5 g/L Difco yeast extract, 250 mg/L of each L-histidine, L-leucine, adenine, uracil, L-lysine, and 3% (wt/vol) glucose] was used in all experiments and FOA media contained 1 g/L 5-fluoroorotic acid.

Yeast Two-Hybrid Analysis. Bait plasmids containing fusion proteins of components of *Schizosaccharomyces pombe* heterochromatin silencing machinery were transformed into EGY48, whereas prey plasmids containing the fusion proteins were transformed into YM3855. See Table S6 for plasmids. Respective bait and prey strains were combined by mating before yeast two-hybrid analysis and β-galactosidase plate and liquid assays were performed in diploid cells.

Protein Cloning and Purification. Full-length Swi6 was cloned into pET30a (Novagen), and Swi6 proteins were purified from *Escherichia coli* Rosetta (DE3) pLysS strains using Cobalt-NTA affinity resin (Clontech) followed by size-exclusion chromatography on a Superdex 200HR 10/300 column (GE Healthcare). Protein was eluted in 25 mM Hepes pH 7.5, 100 mM KCl, and 10% glycerol and stored at −80 °C.

Ers1 fragment 2 was cloned into pMAL (NEB) containing an N-terminal maltose-binding protein (MBP) tag and a C-terminal 6×His-tag. Ers1 fragment 2 proteins were purified from *E. coli* Rosetta (DE3) pLysS strains. Cells were grown to OD_{600} = 0.4 at 37 °C in 2× LB with 50 μg/mL carbenicillin. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a concentration of 0.4 mM to induce protein expression, and cells were incubated overnight at 18 °C. Harvested cells were resuspended in lysis buffer (1× PBS buffer pH 7.5, 300 mM KCl, 10% glycerol, 1 μg/mL pepstatin A, 3 μg/mL leupeptin, 2 μg/mL aprotinin, and 1 mM PMSF). Following sonication, cell debris was removed by centrifugation at 25,000 × *g* for 30 min. Cell lysate supernatants were incubated for 1 h at 4 °C with amylose resin (NEB). Resin was washed with lysis buffer and proteins eluted with lysis buffer containing 40 mM maltose. Proteins were further purified using Cobalt-NTA affinity resin (Clontech). Protein was incubated with resin for 1 h at 4 °C in 20 mM Hepes pH 7.5, 150 mM KCl, 10% glycerol, and 7.5 mM imidazole. Beads were washed with the same buffer and proteins eluted with buffer containing 500 mM imidazole. Purified Ers1 fragment 2 was then dialyzed and stored in 20 mM Hepes pH 7.5, 150 mM KCl, 10% glycerol, and 1 mM DTT.

In Vitro Pull-Down Experiments. To test interactions between recombinant Swi6 and recombinant Ers1 fragment 2, recombinant Swi6 was bound to M2 agarose beads (Sigma) by incubating 3 μg of either 6×His-Swi6-FLAG or 6×His-Swi6(L315D)-FLAG with 25 μL of resin that had been equilibrated with IP buffer (20 mM Hepes-KOH pH 7.5, 200 mM KCl, 2 mM $MgCl_2$, 10% glycerol, 0.1% Tween-20). After 1 h binding at 4 °C, resin was washed three times with IP buffer and then incubated with either 1.2 μg of MBP or 1.2 μg of MBP–Ers1 fragment 2–6×His. After 2 h binding at 4 °C, resin was washed three times with IP buffer. Proteins were eluted by incubating resin with 50 μL of 200 μg/mL 3×-FLAG peptide for 30 min at 4 °C. The samples were boiled at 100 °C for 5 min after the addition of 2× SDS sample buffer and were analyzed by SDS/PAGE and Western using 1:2,000 polyclonal rabbit anti-MBP (Abcam; Ab9084) and 1:1,500 monoclonal rabbit anti-FLAG (Sigma; F7425).

To test interactions between recombinant Swi6 and proteins present in cell lysate, *S. pombe* cultures were grown to OD_{600} = 1.8 at 30 °C, at which point they were washed in lysis buffer (20 mM Hepes-KOH pH 7.5, 200 mM KCl, 10% glycerol, 0.1% Tween-20, 2× EDTA-free complete protease inhibitor; Roche), harvested, then lysed by mechanical disruption using a coffee grinder (3 min) and mortar and pestle (15 min). The frozen powder was resuspended in lysis buffer and cleared by 27,000 × *g* centrifugation for 40 min at 4 °C. Recombinant Swi6 was bound to TALON metal affinity resin (Clontech) by incubating 5 μg 6×His-Swi6-FLAG with 25 μL of resin that had been equilibrated in lysis buffer. After 1 h binding at 4 °C, the TALON resin was washed three times with lysis buffer and then incubated with cleared lysate. After 2 h binding at 4 °C, the TALON resin was washed three times with wash buffer (20 mM Hepes-KOH pH 7.5, 150 mM KCl, 10% glycerol, 0.1% Tween-20, 1× EDTA-free complete, 5 mM imidazole). Proteins were eluted by incubating resin in SDS sample buffer at 100 °C for 5 min and were analyzed by SDS/PAGE and Western blotting using 1:3,000 monoclonal mouse anti-FLAG (Sigma; F3165) and 1:2,500 rabbit polyclonal anti-PSTAIRE (Santa Cruz Biotechnology; SC-53).

Chromatin Immunoprecipitation. ChIP analyses were performed as described (1).

RNA Extraction and RT-qPCR Analyses. This was carried out as described (1), except for the following modifications: 5–10 μg of RNA was used in reverse transcription reactions using 200 units of M-MLV RT (Invitrogen) and strand-specific primers. Following cDNA synthesis at 37 °C for 1 h, the M-MLV RT was inactivated at 80 °C for 10 min and samples were directly analyzed by qPCR. See Table S5 for primers used.

Protein Analysis. Five OD_{A600} of cells grown at 30 °C were harvested and lysed in ice in the presence of 2N NaOH and 7.5% β-mercaptoethanol for 15 min with occasional vortexing. Extracts were treated with 55% TCA for 15 min before maximum speed centrifugation. Pellets were then resuspended in loading buffer (200 mM phosphate buffer, pH 6.8, 8 M urea, 5% SDS, 1 mM EDTA, 100 mM DTT, 0.08% bromophenol blue) and heat denaturated at 70 °C for 15 min. Following centrifugation, soluble fractions were recovered and the concentration was estimated using a Bio-Rad protein assay. Samples were analyzed by standard immunoblotting procedures using 1:3,000 horseradish peroxidase-conjugated antiperoxidase (PAP) (Sigma), 1:3,000 monoclonal anti-FLAG antibody (Sigma), and 1:4,000 polyclonal anti-Swi6 antibody (Abnova).

Coimmunoprecipitation. A total of 200 mL of cells was grown at 30 °C until OD = 1.0–1.5 in all experiments. Cells were harvested by centrifugation, washed with ice-cold water, and resuspended in immunoprecipitation buffer (6 mM Na_2HPO_4, 4 mM NaH_2PO_4, 200 mM $NaC_2H_3O_2$, 5 mM $MgC_2H_3O_2$, 0.25% Nonidet P-40, 2 mM EDTA, 1 mM EGTA, 5% glycerol, 1 mM PMSF, 1.3 mM benzamidine and Roche complete EDTA-free protease inhibitor mixture). The suspension was then slowly dropped into liquid nitrogen to form "pop-corn" and lysis was performed using a ball mill (Retsch; MM400) for 15 min at a 10-Hz frequency. Extracts were then thawed and cleared by centrifugation before precipitation with 25 μL IgG sepharose 6 fast flow beads (Amersham Biosciences) for 2–3 h at 4 °C. For experiments with RNase A (Fermentas) or DNaseI (New England Biolabs), the enzyme was added for 20 min at 25 °C before precipitation in the presence of

5 mM $CaCl_2$ at a concentration of 10 μg/mL extract or 50 unit/mL extract, respectively. Beads were then washed three times in immunoprecipitation buffer, transferred to new tubes, and washed two more times with immunoprecipitation buffer lacking Nonidet P-40 and protease inhibitors. Immunoprecipitated complexes and input extracts were analyzed by standard immunoblotting procedures using 1:3,000 horseradish peroxidase-conjugated antiperoxidase (PAP) (Sigma), 1:3,000 monoclonal anti-myc 9E10 antibody (Covance), 1:3,000 monoclonal anti-FLAG antibody (Sigma), and 1:4,000 polyclonal anti-Swi6 antibody (Abnova).

RNA Immunoprecipitation. A total of 250 mL of cells was grown in YES media at 30 °C until OD = 3–4, with an additional 1% of glucose added at OD of 1.0 to maintain logarithmic growth. Cells were in vivo cross-linked with 0.25% formaldehyde for 15 min at 30 °C and the reaction was quenched by the addition of 0.25 M glycine for 5 min at room temperature. Cells were then harvested by centrifugation, washed two times with ice-cold 1× PBS, and resuspended in RIPA buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 50 mM Tris-HCl pH 8, 2 mM EDTA) supplemented with 1 mM PMSF and 40 unit/mL of murine RNase inhibitor (New England Biolabs). The suspension was then slowly dropped into liquid nitrogen to form pop-corn and lysis was performed using a ball mill (Retsch; MM400) for 15 min at a 15-Hz frequency. Extracts were then thawed and cleared by centrifugation. Precipitation was carried out with 20 μL anti-FLAG M2 agarose resin (Sigma) for 2 h at 4 °C. Beads were subsequently transferred to Eppendorf tubes, rinsed one time with ice-cold RIPA buffer, washed one time with low salt buffer (150 mM NaCl, 10 mM Tris-HCl pH 7.5, 0.5% Triton X-100), two times with high salt buffer (1 M NaCl, 10 mM Tris-HCl pH 7.5, 0.5% Triton X-100), and one more time with low salt buffer for 10 min at room temperature. Then, reversal buffer (10 mM Tris-HCl pH 6.8, 5 mM EDTA, 10 mM DTT, 1% SDS) was added to samples for decross-link at 70 °C for 45 min. Tubes were incubated at 37 °C for 30 min in the presence of 40 μg proteinase K. RNAs were then extracted with acid-equilibrated (pH 4.7) phenol:chloroform and precipitated in 100% ethanol with 30 μg glycogen for 15 min in dry ice. Pellets were washed with 70% ethanol, resuspended in nuclease-free water, and treated with DNaseI (New England Biolabs). RT-qPCR analyses were then performed as described above with strand-specific primers and M-MLV RT (Invitrogen).

1. Rougemaille M, Shankar S, Braun S, Rowley M, Madhani HD (2008) Ers1, a rapidly diverging protein essential for RNA interference-dependent heterochromatic silencing in Schizosaccharomyces pombe. *J Biol Chem* 283:25770–25773.

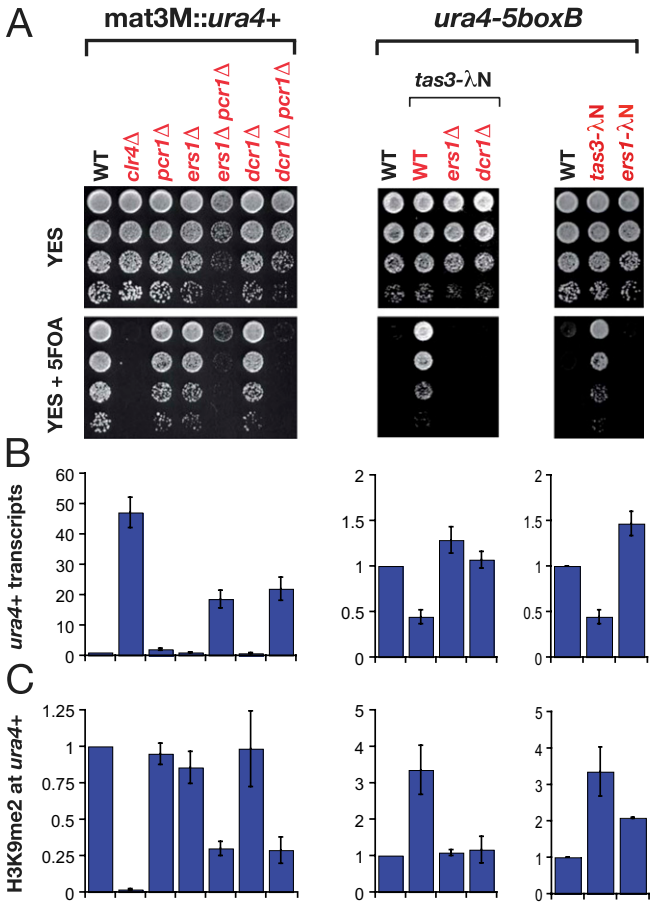

Fig. S1. Ers1 functions in the RNAi-dependent pathway of heterochromatic silencing but does not promote ectopic silencing. (*A*, *Left*) Silencing of a *ura4*$^+$ reporter gene inserted at the mating type locus (mat3M::*ura4*$^+$) is severely compromised in *ers1Δ pcr1Δ* and *dcr1Δ pcr1Δ* cells. (*Center*) Silencing of the endogenous *ura4*$^+$ locus upon Tas3 tethering (*ura4-5boxB tas3-λN*) is completely abolished by the deletion of either *ers1*$^+$ or *dcr1*$^+$. (*Right*) Silencing of the endogeneous *ura4*$^+$ locus is not established upon Ers1 tethering. (*B*) RT-qPCR analysis of *ura4*$^+$ transcript levels normalized to a control transcript *act1*$^+$ and relative to wild type. Error bars represent SD from three experiments. (*C*) ChIP analysis of H3K9me2 levels at the *ura4*$^+$ locus, normalized to *act1*$^+$ and relative to wild type. Error bars represent the deviation from the mean of two experiments.

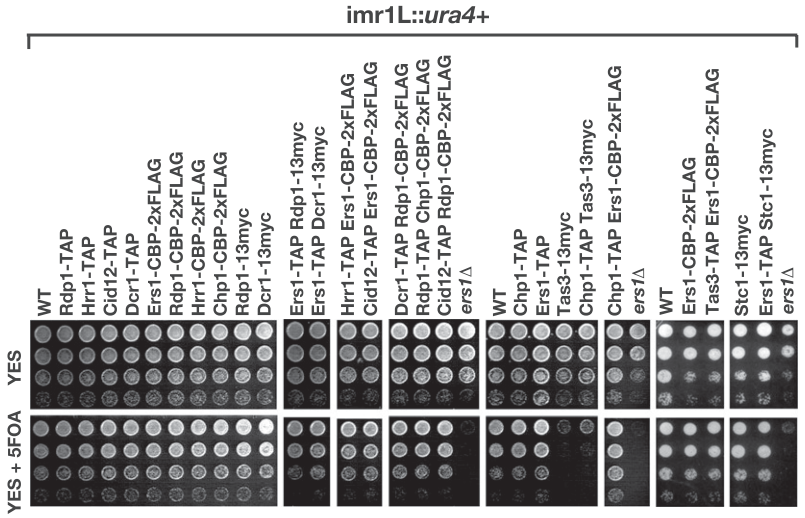

Fig. S2. Tagged strains used for immunoprecipitation experiments. Silencing of a *ura4*$^+$ reporter gene inserted at the centromeric inner repeats (imr1L::*ura4*$^+$) is not affected in most tagged strains, indicating that proteins are functional for centromeric silencing. Note, however, that cells expressing Tas3–13myc are partially defective for silencing. A strain deleted for *ers1*$^+$ is used as a negative control.

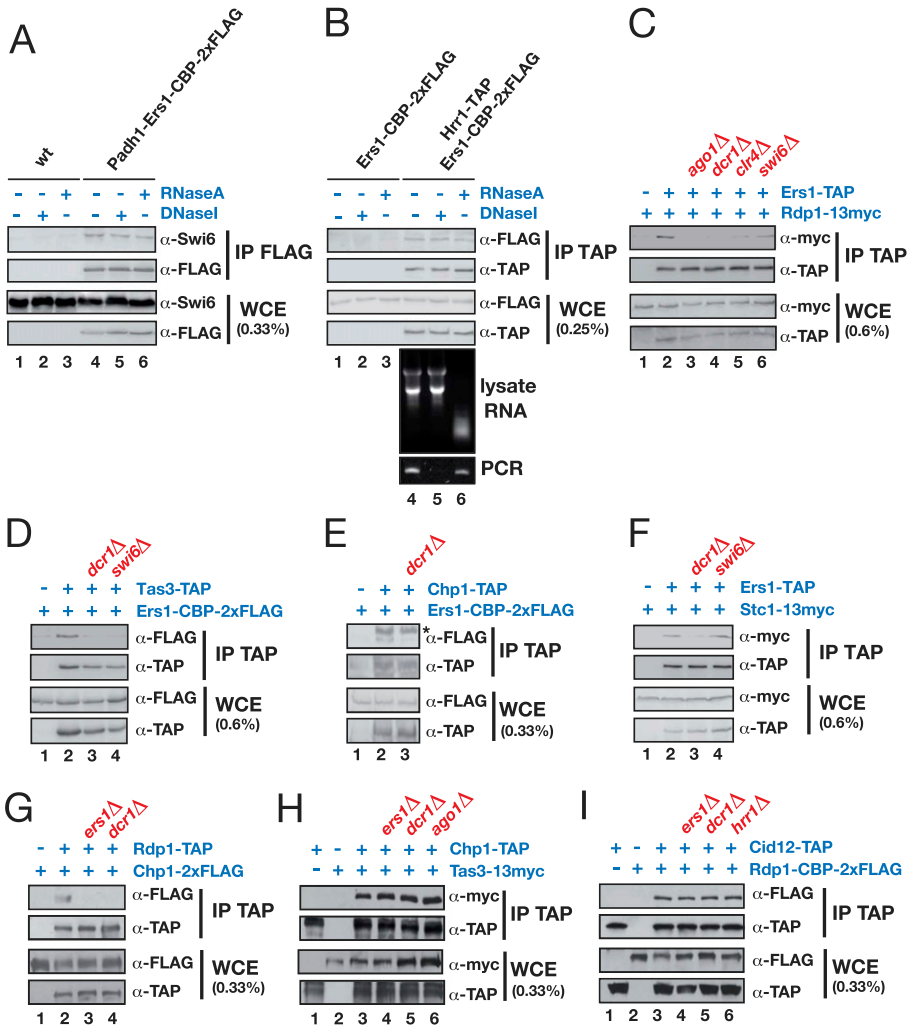

Fig. S3. Ers1 interacts with Swi6 and RNAi factors and is required for the formation of RNAi supercomplexes. Western blots showing that Ers1 associates with Swi6 (*A*) and Hrr1 (*B*) independently of DNA and RNA (compare lanes 4, 5, and 6). Agarose gels indicate the effects of DNaseI and RNaseA treatments of whole-cell extracts (*B*). (*C*, *E*, and *F*) Western blots showing that Ers1 interacts with Rdp1 (*C*), Chp1 (*E*), and Stc1 (*F*) in wild-type and *swi6Δ* cells but not in RNAi mutants (e.g., *ago1Δ* and/or *dcr1Δ*). (*D*) In contrast, Ers1 interacts with Tas3 in a Dcr1- and Swi6-dependent manner. (*E*) Asterisk denotes cross-hybridization with Chp1-TAP. (*G*) Western blot showing that, upon deletion of *ers1*$^+$, Chp1-CBP-2×FLAG does not coprecipitate with Rdp1–TAP. (*H* and *I*) The interactions between Chp1–TAP and Tas3–13myc, as well as Cid12–TAP and Rdp1-CBP-2×FLAG, do not depend on *ers1*$^+$.

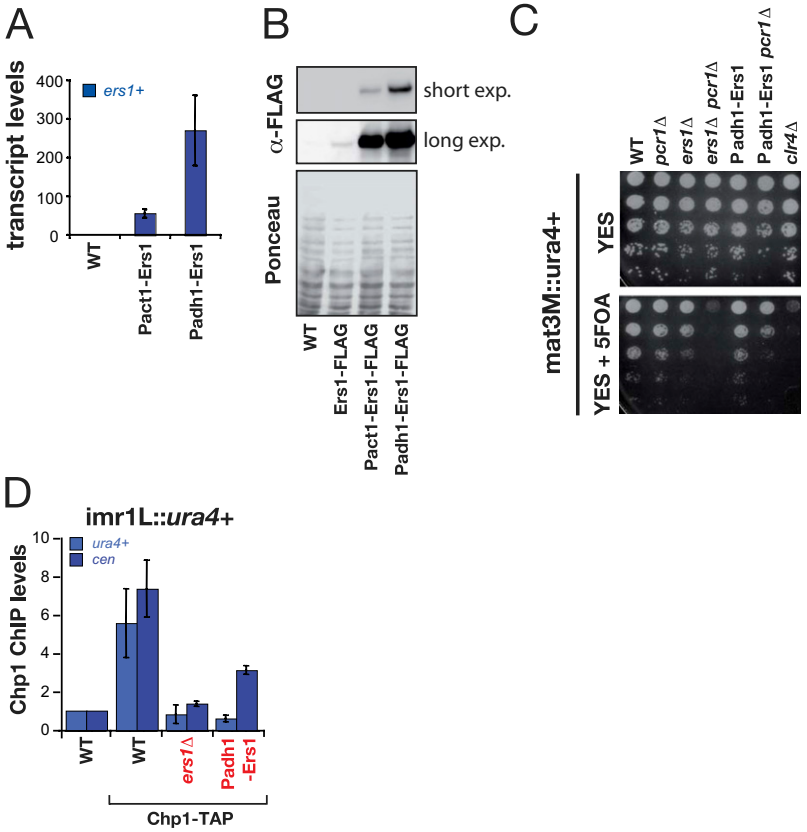

Fig. S4. Overexpression of Ers1 with the *adh1*$^+$ promoter disrupts silencing. (*A*) RT-qPCR analysis of *ers1*$^+$ transcript levels normalized to a control transcript *act1*$^+$ and relative to wild type. Error bars represent SD from three experiments. (*B*) Western blot showing FLAG-tagged Ers1 levels in wild-type cells and upon overexpression with the *act1*$^+$ or *adh1*$^+$ promoters. Shown are short and long exposures as well as a Ponceau staining used as loading control. (*C*) Silencing of a *ura4*$^+$ reporter gene inserted at the mating type locus (mat3M::*ura4*$^+$) is partially defective in cells overexpressing Ers1 and lacking Pcr1. (*D*) ChIP analysis of Chp1–TAP levels at the *ura4*$^+$ and *cen* loci, normalized to *act1*$^+$ and relative to wild type. Error bars represent SD from three immunoprecipitations.

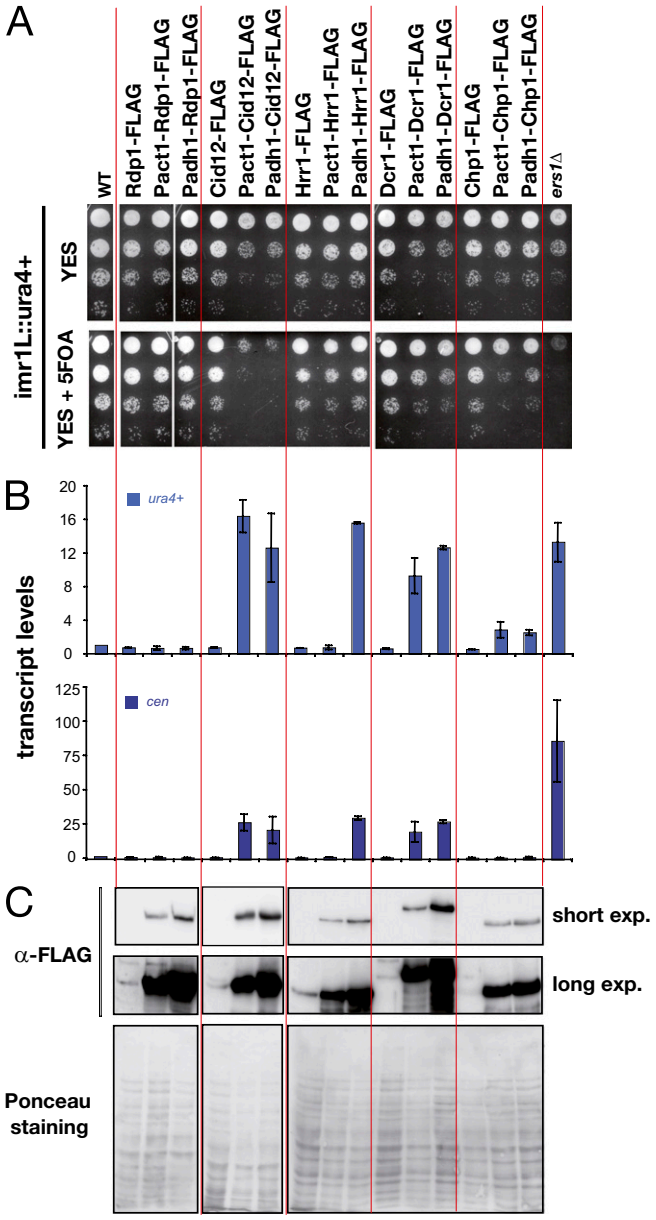

Fig. S5. Effects of overexpressing RNAi factors on pericentromeric silencing. (*A*) Silencing assay of a *ura4*$^+$ reporter gene inserted at the centromeric inner repeats (imr1L::*ura4*$^+$) in cells overexpressing RNAi factors (e.g., Cid12, Rdp1, Hrr1, Dcr1, and Chp1) with the *act1*$^+$ or the *adh1*$^+$ promoter. An *ers1Δ* strain is used as a negative control. (*B*) RT-qPCR analysis of *ura4*$^+$ (*Upper*) and *cen* (*Lower*) transcript levels normalized to a control transcript *act1*$^+$ and relative to wild type. Error bars represent SD from three experiments. (*C*) Western blots showing expression levels of FLAG-tagged RNAi factors in wild-type cells and upon overexpression with the *act1*$^+$ or the *adh1*$^+$ promoter. Shown are short and long exposures as well as a Ponceau staining used as loading control.

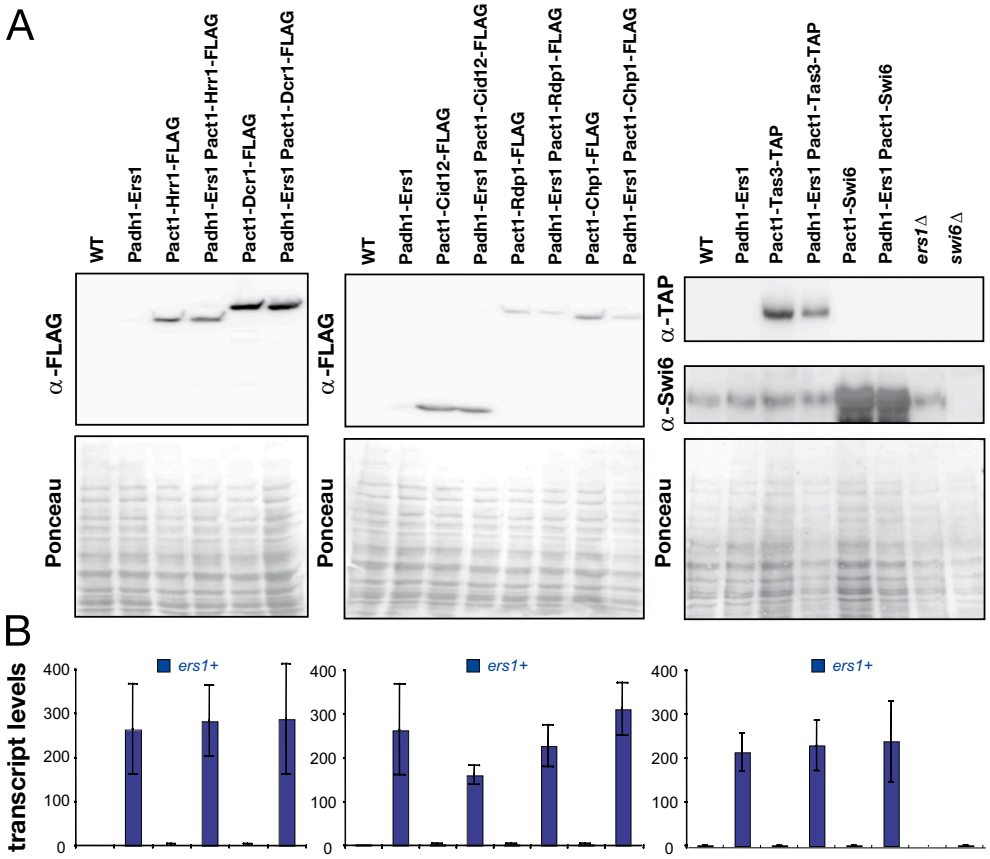

Fig. S6. Levels of RNAi factors and Swi6 upon overexpression. (*A*) Western blots showing expression levels of FLAG or TAP-tagged RNAi factors and Swi6 overexpressed with the *act1*$^+$ promoter. Shown is a short exposure and a Ponceau staining used as loading control. (*B*) RT-qPCR analysis of *ers1*$^+$ transcript levels normalized to a control transcript *act1*$^+$ and relative to wild type. Error bars represent SD from three experiments.

Table S1. Yeast two-hybrid fold over empty prey values for Fig. 1*A*

	Baits								
Preys	*empty*	*ago1*	*chp1*	*tas3*	*cid12*	*hrr1*	*rdp1*	*dcr1*	*ers1*
empty	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
ago1	0.9	1.1	1.2	1.3	1.4	1.3	1.1	1.4	1.4
chp1	0.6	1.2	0.4	0.6	0.4	0.3	0.7	1.2	0.3
tas3	0.8	0.2	**46.8**	0.9	**6.3**	1.2	**49.1**	0.1	0.5
cid12	0.1	1.4	0.2	0.5	0.6	0.1	**2.8**	0.8	0.1
hrr1	1.0	0.7	0.5	0.5	**4.3**	1.3	**17.3**	1.0	1.6
rdp1	1.5	1.0	0.9	0.8	0.4	1.0	0.5	1.1	1.1
dcr1	0.4	0.8	0.3	0.8	0.1	0.8	0.3	0.3	1.3
ers1	0.7	1.2	0.7	0.6	0.8	0.6	1.3	0.6	1.4

The fold β-galactosidase activity over the β-galactosidase activity of the empty prey was calculated for each bait–prey set. Values in bold are interactions meeting the following threshold: twofold enrichment in β-galactosidase activity over the empty prey.

Table S2. Yeast two-hybrid fold over empty prey values for Fig. 1*B* with Ers1 fragments as bait

	Prey																					
Baits	*empty*		*clr4*		*raf1*		*raf2*		*rik1*		*swi6*		*ago1*		*chp1*		*tas3*		*cid12*		*hrr1*		*rdp1*		*dcr1*	
Empty	1.0		0.5	(0.4)	0.6	(0.1)	0.6	(0.8)	0.2	(0.0)	0.0	(0.3)	0.7	(0.4)	0.6	(0.1)	0.8	(0.4)	1.0	(0.3)	1.1	(0.6)	0.7	(0.2)	0.3	(0.1)
Fragment 1	1.0		0.9	(0.0)	0.9	(0.0)	1.4	(0.8)	0.1	(0.3)	**3.4**	**(1.5)**	1.0	(0.1)	1.2	(0.2)	1.8	(0.3)	1.9	(0.3)	2.0	(0.0)	1.4	(0.5)	0.6	(0.1)
Fragment 2	1.0		0.6	(0.1)	0.7	(0.3)	−0.1	(0.3)	−1.1	(1.8)	**7.4**	**(0.3)**	**2.2**	**(0.6)**	1.4	(0.5)	1.4	(0.1)	1.5	(0.3)	2.0	(1.1)	1.3	(0.1)	0.8	(0.0)
Fragment 3	1.0		1.2	(0.2)	1.5	(0.4)	1.3	(0.6)	1.4	(0.8)	1.7	(1.0)	1.5	(0.4)	1.3	(0.0)	1.0	(0.3)	1.3	(0.1)	1.5	(0.2)	1.5	(0.1)	1.4	(0.8)
Fragment 4	1.0		0.9	(0.0)	1.5	(0.6)	0.4	(0.1)	−0.8	(1.6)	0.8	(0.0)	1.5	(0.7)	**2.3**	**(0.9)**	**2.3**	**(0.5)**	**3.0**	**(0.2)**	**3.2**	**(0.9)**	3.1	(0.3)	0.9	(0.0)
Fragment 5	1.0		0.9	(0.2)	1.3	(0.1)	0.7	(0.2)	0.8	(1.8)	1.2	(0.6)	1.4	(0.4)	1.9	(0.6)	1.8	(0.0)	**3.4**	**(1.0)**	**3.8**	**(0.1)**	2.6	(0.0)	1.5	(0.5)
Fragment 6	1.0		0.4	(0.2)	1.0	(0.3)	0.3	(0.0)	0.9	(1.2)	0.2	(0.7)	**2.2**	**(0.3)**	1.7	(0.4)	2.0	(0.6)	**3.6**	**(1.4)**	**3.7**	**(0.9)**	2.8	(1.0)	2.3	(0.0)
Fragment 7	1.0		1.1	(0.0)	1.0	(0.5)	0.6	(0.5)	1.4	(1.3)	1.0	(0.3)	**2.1**	**(0.6)**	1.9	(0.6)	**2.1**	**(0.4)**	**2.4**	**(0.3)**	**2.6**	**(0.8)**	2.4	(0.3)	1.7	(0.6)
Fragment 8	1.0		0.8	(0.2)	1.1	(0.2)	0.7	(0.1)	1.0	(0.1)	0.9	(0.1)	1.1	(0.4)	0.6	(0.0)	0.9	(0.2)	0.9	(0.3)	1.1	(0.5)	1.0	(0.3)	0.8	(0.2)

The fold β-galactosidase activity over the β-galactosidase activity of the empty prey was calculated from two independent experiments. The mean of the two experiments for that bait–prey set is displayed above. The value in the parentheses is the deviation from the mean for the two experiments. Values in bold are interactions meet the following threshold: twofold enrichment in β-galactosidase activity over the empty prey in both experiments.

Table S3. Yeast two-hybrid fold over empty prey values for Fig. 1*B* with Ers1 fragments as prey

	Baits																					
Preys	*empty*		*clr4*		*raf1*		*raf2*		*rik1*		*swi6*		*ago1*		*chp1*		*tas3*		*cid12*		*hrr1*		*rdp1*		*dcr1*	
Fragment 1	0.6	(0.1)	0.9	(0.1)	0.8	(0.4)	0.5	(0.2)	1.2	(0.2)	1.1	(0.4)	0.4	(0.2)	1.4	(0.5)	1.0	(0.2)	1.3	(0.5)	0.6	(0.0)	1.2	(0.1)	0.7	(0.5)
Fragment 2	0.4	(0.0)	2.4	(1.8)	0.7	(0.4)	4.4	(4.2)	1.2	(0.1)	0.9	(0.2)	0.6	(0.1)	1.0	(0.1)	1.1	(0.6)	0.8	(0.2)	0.7	(0.0)	0.5	(0.0)	0.6	(0.3)
Fragment 3	0.5	(0.1)	0.5	(0.1)	1.4	(0.6)	1.2	(0.3)	0.9	(0.7)	0.6	(0.0)	2.2	(1.7)	0.8	(0.1)	**6.7**	**(0.9)**	1.3	(0.7)	0.6	(0.1)	0.8	(0.1)	−0.3	(0.6)
Fragment 4	0.5	(0.1)	0.6	(0.2)	1.1	(0.3)	0.9	(0.5)	0.9	(0.1)	0.5	(0.2)	0.4	(0.1)	0.5	(0.1)	0.5	(0.1)	0.5	(0.5)	0.5	(0.1)	0.6	(0.1)	0.4	(0.2)
Fragment 5	0.8	(0.0)	0.8	(0.0)	1.2	(0.6)	1.2	(1.1)	0.8	(0.1)	0.7	(0.1)	0.7	(0.3)	0.8	(0.3)	0.7	(0.0)	0.6	(0.2)	0.3	(0.0)	0.8	(0.0)	0.8	(0.6)
Fragment 6	0.5	(0.0)	0.7	(0.1)	0.5	(0.1)	0.8	(1.2)	0.7	(0.3)	0.9	(0.3)	0.7	(0.2)	0.9	(0.2)	0.8	(0.2)	0.6	(0.0)	0.3	(0.0)	0.6	(0.0)	0.5	(0.3)
Fragment 7	0.5	(0.1)	0.4	(0.2)	0.4	(0.2)	0.1	(0.9)	0.5	(0.2)	0.5	(0.1)	0.4	(0.0)	0.5	(0.1)	0.6	(0.3)	0.8	(0.1)	0.3	(0.1)	0.7	(0.2)	0.5	(0.3)
Fragment 8	0.6	(0.3)	0.6	(0.4)	0.3	(0.2)	0.8	(0.9)	0.5	(0.1)	0.5	(0.4)	0.6	(0.5)	1.3	(1.0)	0.5	(0.2)	0.4	(0.2)	0.3	(0.2)	0.5	(0.2)	0.6	(0.3)
Empty	1.0		1.0		1.0		1.0		1.0		1.0		1.0		1.0		1.0		1.0		1.0		1.0		1.0	

See Table S2.

Table S4. *Schizosaccharomyces pombe* strains used in this study

Strain	Genotype	Source
PM021	P(h$^+$), ura4-DS/E, ade6-210, leu1-32, imr1L(NcoI)::ura4$^+$	D. Moazed*
PM102	h90, *ura4-DS/E, ade6-M210, leu1-32, mat3M::ura4*$^+$	D. Moazed
PM104	M(h$^-$), *ura4*$^+$*::5BoxB-hphR, tas3*$^+$*::λN-kanR*	D. Moazed
PM251	P(h$^+$), *ura4-DS/E, ade6-210, leu1-32, imr1L(NcoI)::ura4*$^+$*, otr1R(SphI)::ade6*$^+$	K. Ekwall†
PM333	PM102, *clr4::kanMX*	(1)
PM369	PM251, Ers1-TAP<*kanMX*>	This study
PM408	PM102, *ers1::natMX*	This study
PM410	PM102, *dcr1::natMX*	This study
PM412	PM104, *ers1::natMX*	This study
PM414	PM104, *dcr1::natMX*	This study
PM416	PM251, *ers1::natMX*	This study
PM418	PM251, *dcr1::natMX*	This study
PM526	PM102, *pcr1::kanMX*	This study
PM552	PM251, Chp1-TAP<*kanMX*>	This study
PM554	PM251, *ers1::natMX* Chp1-TAP<*kanMX*>	This study
PM568	PM251, Ers1-CBP-2×FLAG<*kanMX*>	This study
PM592	PM102, *ers1::natMX pcr1::kanMX*	This study
PM594	PM102, *dcr1::natMX pcr1::kanMX*	This study
PM613	M(h$^-$), *ura4*$^+$*::5BoxB-hphR*	D. Moazed
PM614	M(h$^-$), *ura4*$^+$*::5BoxB-hphR, ers1*$^+$*::λN-kanR*	This study
PM644	PM251, Rdp1-13myc<*hygMX*>	This study
PM646	PM251, Ers1-TAP<*kanMX*> Rdp1-13myc<*hygMX*>	This study
PM648	PM251, *dcr1::natMX* Ers1-TAP<*kanMX*> Rdp1-13myc<*hygMX*>	This study
PM658	PM251, Sap61-CBP-2×FLAG<*kanMX*>	This study
PM663	PM251, Cid12-TAP<*hygMX*>	This study
PM677	PM251, Hrr1-CBP-2×FLAG<*kanMX*>	This study
PM679	PM251, Chp1-CBP-2×FLAG<*kanMX*>	This study
PM684	PM251, *dcr1::natMX* Ers1-CBP-2×FLAG<*kanMX*>	This study
PM689	PM251, Tas3-13myc<*hygMX*>	This study
PM691	PM251, Dcr1-13myc<*hygMX*>	This study
PM696	PM251, Ers1-TAP<*kanMX*> Dcr1-13myc<*hygMX*>	This study
PM702	PM251, *ers1::natMX* Hrr1-CBP-2×FLAG<*kanMX*>	This study
PM712	PM251, Hrr1-TAP<*hygMX*> Ers1-CBP-2×FLAG<*kanMX*>	This study
PM716	PM251, Rdp1-CBP-2×FLAG<*kanMX*>	This study
PM718	PM251, *ers1::natMX* Rdp1-CBP-2×FLAG<*kanMX*>	This study
PM760	PM251, *dcr1::natMX* Hrr1-TAP<*hygMX*> Ers1-CBP-2×FLAG<*kanMX*>	This study
PM775	PM251, *ago1::natMX* Ers1-TAP<*kanMX*> Rdp1-13myc<*hygMX*>	This study
PM777	PM251, *clr4::natMX* Ers1-TAP<*kanMX*> Rdp1-13myc<*hygMX*>	This study
PM842	PM251, *clr4::natMX*	This study
PM843	PM251, *clr4::natMX* Ers1-CBP-2×FLAG<*kanMX*>	This study
PM845	PM251, *ago1::natMX*	This study
PM847	PM251, *ago1::natMX* Ers1-CBP-2×FLAG<*kanMX*>	This study
PM849	PM251, *ers1::natMX* Chp1-CBP-2×FLAG<*kanMX*>	This study
PM853	PM251, Cid12-TAP<*hygMX*> Rdp1-CBP-2×FLAG<*kanMX*>	This study
PM862	PM251, Dcr1-TAP<*hygMX*> Rdp1-CBP-2×FLAG<*kanMX*>	This study
PM864	PM251, Chp1-TAP<*hygMX*> Ers1-CBP-2×FLAG<*kanMX*>	This study
PM868	PM251, Rdp1-TAP<*hygMX*> Chp1-CBP-2×FLAG<*kanMX*>	This study
PM872	PM251, Chp1-TAP<*kanMX*> Tas3-13myc<*hygMX*>	This study
PM886	PM251, *dcr1::natMX* Cid12-TAP<*hygMX*> Rdp1-CBP-2×FLAG<*kanMX*>	This study
PM892	PM251, <*natMX*>-Pact1-Ers1	This study
PM894	PM251, <*natMX*>-Pact1-Ers1-CBP-2×FLAG<*kanMX*>	This study
PM896	PM251, <*natMX*>-Padh1-Ers1	This study
PM898	PM251, <*natMX*>-Padh1-Ers1-CBP-2×FLAG<*kanMX*>	This study
PM900	PM251, *dcr1::natMX* Chp1-TAP<*hygMX*> Ers1-CBP-2×FLAG<*kanMX*>	This study
PM902	PM251, *ers1::natMX* Rdp1-TAP<*hygMX*> Chp1-CBP-2×FLAG<*kanMX*>	This study
PM904	PM251, *dcr1::natMX* Rdp1-TAP<*hygMX*> Chp1-CBP-2×FLAG<*kanMX*>	This study
PM906	PM251, *ers1::natMX* Chp1-TAP<*kanMX*> Tas3-13myc<*hygMX*>	This study
PM908	PM251, *dcr1::natMX* Chp1-TAP<*kanMX*> Tas3-13myc<*hygMX*>	This study
PM910	PM251, *ers1::natMX* Dcr1-TAP<*hygMX*> Rdp1-CBP-2×FLAG<*kanMX*>	This study
PM912	PM251, *hrr1::natMX* Dcr1-TAP<*hygMX*> Rdp1-CBP-2×FLAG<*kanMX*>	This study
PM914	PM251, *ers1::natMX* Cid12-TAP<*hygMX*> Rdp1-CBP-2×FLAG<*kanMX*>	This study
PM916	PM251, *hrr1::natMX* Cid12-TAP<*hygMX*> Rdp1-CBP-2×FLAG<*kanMX*>	This study
PM917	PM251, ago1*::natMX* Chp1-TAP<*kanMX*> Tas3-13myc<*hygMX*>	This study

Table S4. Cont.

Strain	Genotype	Source
PM972	PM251, *ago1::natMX* Ers1-TAP<*kanMX*> Dcr1-13myc<*hygMX*>	This study
PM974	PM251, *rdp1::natMX* Ers1-TAP<*kanMX*> Dcr1-13myc<*hygMX*>	This study
PM976	PM251, *clr4::natMX* Ers1-TAP<*kanMX*> Dcr1-13myc<*hygMX*>	This study
PM986	PM251, <*natMX*>-Padh1-Ers1 Chp1-TAP<*kanMX*>	This study
PM989	PM251, <*natMX*>-Padh1-Ers1 Chp1-CBP-2×FLAG<*kanMX*>	This study
PM991	PM251, <*natMX*>-Padh1-Ers1 Rdp1-CBP-2×FLAG<*kanMX*>	This study
PM993	PM251, <*natMX*>-Padh1-Rdp1-CBP-2×FLAG<*kanMX*>	This study
PM996	PM251, <*natMX*>-Padh1-Ers1 Rdp1-TAP<*hygMX*> Chp1-CBP-2×FLAG<*kanMX*>	This study
PR001	PM251, <*natMX*>-Padh1-Ers1 Dcr1-TAP<*hygMX*> Rdp1-CBP-2×FLAG<*kanMX*>	This study
PR003	PM251, <*natMX*>-Padh1-Ers1-TAP < kan*MX*> Rdp1-13myc<*hygMX*>	This study
PR004	PM251, <*natMX*>-Padh1-Ers1-TAP < kan*MX*> Dcr1-13myc<*hygMX*>	This study
PR005	PM251, <*hygMX*>-Padh1-Ers1	This study
PR007	PM102, <*hygMX*>-Padh1-Ers1	This study
PR008	PM102, <*hygMX*>-Padh1-Ers1 pcr1::kan*MX*	This study
PR009	PM251, <*hygMX*>-Pact1-Rdp1-CBP-2×FLAG<*kanMX*>	This study
PR010	PM251, <*natMX*>-Pact1-Cid12-CBP-2×FLAG<*kanMX*>	This study
PR011	PM251, <*natMX*>-Padh1-Cid12-CBP-2×FLAG<*kanMX*>	This study
PR012	PM251, <*natMX*>- Pact1-Hrr1-CBP-2×FLAG<*kanMX*>	This study
PR013	PM251, <*natMX*>- Padh1-Hrr1-CBP-2×FLAG<*kanMX*>	This study
PR014	PM251, <*hygMX*>- Pact1-Dcr1-CBP-2×FLAG<*kanMX*>	This study
PR015	PM251, <nat*MX*>- Pact1-Dcr1-CBP-2×FLAG<*kanMX*>	This study
PR016	PM251, <nat*MX*>- Padh1-Dcr1-CBP-2×FLAG<*kanMX*>	This study
PR017	PM251, <nat*MX*>- Pact1-Chp1-CBP-2×FLAG<*kanMX*>	This study
PR018	PM251, <hyg*MX*>- Padh1-Chp1-CBP-2×FLAG<*kanMX*>	This study
PR023	PM251, <nat*MX*>-Padh1-Ers1 <*hygMX*>-Pact1-Rdp1-CBP-2×FLAG<*kanMX*>	This study
PR024	PM251, <hyg*MX*>-Padh1-Ers1 <*natMX*>-Pact1-Cid12-CBP-2×FLAG<*kanMX*>	This study
PR025	PM251, <nat*MX*>-Padh1-Ers1 <*hygMX*>-Pact1-Hrr1-CBP-2×FLAG<*kanMX*>	This study
PR026	PM251, <nat*MX*>-Padh1-Ers1 <*hygMX*>-Pact1-Dcr1-CBP-2×FLAG<*kanMX*>	This study
PR027	PM251, <hyg*MX*>-Padh1-Ers1 <*natMX*>-Pact1-Dcr1-CBP-2×FLAG<*kanMX*>	This study
PR028	PM251, <nat*MX*>-Padh1-Ers1 <*hygMX*>-Pact1-Chp1-CBP-2×FLAG<*kanMX*>	This study
PR063	PM251, *swi6*::hyg*MX*	This study
PR069	PM251, <kan*MX*>-Pact1-Swi6	This study
PR071	PM251, Tas3-TAP<*kanMX*> Ers1-CBP-2×FLAG<*kanMX*>	This study
PR075	PM251, <*hygMX*>-Pact1-Tas3-TAP<*kanMX*>	This study
PR076	PM251, <nat*MX*>-Padh1-Ers1 <kan*MX*>-Pact1-Swi6	This study
PR077	PM251, *swi6*::hyg*MX* Chp1-CBP-2×FLAG<*kanMX*>	This study
PR078	PM251, *swi6*::hyg*MX* Hrr1-CBP-2×FLAG<*kanMX*>	This study
PR079	PM251, *swi6*::hyg*MX* Rdp1-CBP-2×FLAG<*kanMX*>	This study
PR080	PM251, *swi6*::nat*MX* Tas3-TAP<*kanMX*> Ers1-CBP-2×FLAG<*kanMX*>	This study
PR081	PM251, *swi6*::nat*MX* Hrr1-TAP<*hygMX*> Ers1-CBP-2×FLAG<*kanMX*>	This study
PR082	PM251, *swi6*::nat*MX* Ers1-TAP < kan*MX*> Rdp1-13myc<*hygMX*>	This study
PR084	PM251, *swi6*::nat*MX* Dcr1-TAP<*hygMX*> Rdp1-CBP-2×FLAG<*kanMX*>	This study
PR085	PM251, <nat*MX*>-Padh1-Ers1 <*hygMX*>-Pact1-Tas3-TAP<*kanMX*>	This study
PR088	PM251, Stc1-13myc<*hygMX*>	This study
PR089	PM251, *dcr1*::nat*MX* Tas3-TAP<*kanMX*> Ers1-CBP-2×FLAG<*kanMX*>	This study
PR090	PM251, *swi6*::nat*MX* Ers1-TAP < kan*MX*> Dcr1-13myc<*hygMX*>	This study
PR097	PM251, *ago1*::nat*MX* Hrr1-TAP<*hygMX*> Ers1-CBP-2×FLAG<*kanMX*>	This study
PR098	PM251, *clr4*::nat*MX* Hrr1-TAP<*hygMX*> Ers1-CBP-2×FLAG<*kanMX*>	This study
PR099	PM251, Ers1-TAP < kan*MX*> Stc1-13myc<*hygMX*>	This study
PR100	PM251, *swi6*::nat*MX* Ers1-TAP < kan*MX*> Stc1-13myc<*hygMX*>	This study
PR101	PM251, *dcr1*::nat*MX* Ers1-TAP < kan*MX*> Stc1-13myc<*hygMX*>	This study
PR102	PM251, <nat*MX*>-Ers1*fragment2Δ*-CBP-2×FLAG<*kanMX*>	This study
PR104	PM251, <nat*MX*>-Padh1-Ers1*fragment2Δ*-CBP-2×FLAG<*kanMX*>	This study

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Table S5. Primers used in this study

Primers	Sequence	Experiments
P581: *ura4*$^+$ fwd	5′-CAGCAATATCGTACTCCTGAA-3′	qPCR
P582: *ura4*$^+$ rev	5′-ATGCTGAGAAAGTCTTTGCTG-3′	qPCR
P638: *act1*$^+$ fwd	5′-AACCCTCAGCTTTGGGTCTT-3′	qPCR
P639: *act1*$^+$ rev	5′-TTTGCATACGATCGGCAATA-3′	qPCR
P972: *U2* fwd	5′-GGCTTAGATCAAGTGTAGTATCTGT-3′	qPCR
P978: *U2* rev	5′-CAACTCAAACCAAAAACAAG-3′	qPCR
P1044: *cen* fwd	5′-CCATCACCACTTTCATCTCC-3′	qPCR
P1045: *cen* rev	5′-CAGGATACCTAGACGCACAA-3′	qPCR
PR37: *ers1*$^+$ fwd	5′-CAGCAATTCAAAGTCATAGTG-3′	Diagnostic PCR
PR47: *ers1*$^+$ rev	5′-TGTCCTGATCGTTATTGGCT-3′	qPCR
PR49: *ers1*$^+$ fwd	5′-CTACTGTTTACAACGGGAGTTTC-3′	qPCR
PR188: *ers1*$^+$ rev	5′-CAAGCTCTAAACATTCTACACT-3′	Diagnostic PCR

Table S6. Yeast two-hybrid plasmids used in this study

Vector	Insert	Strain	Source
pEG202	*Ers1 fragment 1*	BHM1809	This study
pEG202	*Ers1 fragment 2*	BHM1810	This study
pEG202	*Ers1 fragment 3*	BHM1811	This study
pEG202	*Ers1 fragment 4*	BHM1812	This study
pEG202	*Ers1 fragment 5*	BHM1813	This study
pEG202	*Ers1 fragment 6*	BHM1814	This study
pEG202	*Ers1 fragment 7*	BHM1815	This study
pEG202	*Ers1 fragment 8*	BHM1816	This study
pEG202	*clr4*	BHM1744	This study
pEG202	*raf1*	BHM1719	This study
pEG202	*raf2*	BHM1745	This study
pEG202	*rik1*	BHM1759	This study
pEG202	*swi6*	BHM1717	This study
pEG202	*ago1*	BHM1689	This study
pEG202	*chp1*	BHM1671	This study
pEG202	*tas3*	BHM1678	This study
pEG202	*cid12*	BHM1672	This study
pEG202	*hrr1*	BHM1675	This study
pEG202	*rdp1*	BHM1676	This study
pEG202	*dcr1*	BHM1673	This study
pEG202	*ers1*	BHM1674	This study
pEG202	*No insert*	BHM1757	(1)
pJSC401	*Ers1 fragment 1*	BHM1817	This study
pJSC401	*Ers1 fragment 2*	BHM1818	This study
pJSC401	*Ers1 fragment 3*	BHM1819	This study
pJSC401	*Ers1 fragment 4*	BHM1820	This study
pJSC401	*Ers1 fragment 5*	BHM1821	This study
pJSC401	*Ers1 fragment 6*	BHM1822	This study
pJSC401	*Ers1 fragment 7*	BHM1823	This study
pJSC401	*Ers1 fragment 8*	BHM1824	This study
pJSC401	*clr4*	BHM1670	This study
pJSC401	*raf1*	BHM1751	This study
pJSC401	*raf2*	BHM1712	This study
pJSC401	*rik1*	BHM1752	This study
pJSC401	*swi6*	BHM1760	This study
pJSC401	*ago1*	BHM1710	This study
pJSC401	*chp1*	BHM1687	This study
pJSC401	*tas3*	BHM1679	This study
pJSC401	*cid12*	BHM1686	This study
pJSC401	*hrr1*	BHM1680	This study
pJSC401	*rdp1*	BHM1683	This study
pJSC401	*dcr1*	BHM1684	This study
pJSC401	*ers1*	BHM1681	This study
pJSC401	*No insert*	BHM1682	(1)

1. Golemis, et al. (2009) Interaction trap/two-hybrid system to identify interacting proteins. *Curr Protoc Protein Sci*, Chapter 19: Unit 19.2.