Supplementary Methods

S.1.1. Expression and purification of Mei5

The *S. cerevisiae MEI5* gene was amplified from genomic DNA by PCR, inserted into the bacterial expression plasmid pMAL-c2x (New England Biolabs) and sequenced to ensure no undesired mutations were present. The Mei5 expression plasmid was transformed in *E. coli* BL21 Rosetta(DE3) strain cells, grown at 37°C to an A_{600} of 1.0 prior to the addition of IPTG to 0.4 mM and incubated for 20 h at 16°C. The cells were harvested by centrifugation. All subsequent steps were carried out at 4°C. Cell paste (60 g) was resuspended in Buffer E (50 mM Tris pH 7.5, 1 mM EDTA, 10% sucrose, 0.01% Igepal, 1 mM β-mercaptoethanol, 0.1 mg/mL lysozyme, 1 mM benzamidine, 1 mM PMSF, and protease inhibitors: aprotinin, chymostatin, leupeptin, and pepstatin A at a final concentration of 5 μg/mL) containing 250 mM KCl and subjected to sonication at 3 X 30 second cycles. The extract was clarified by ultracentrifugation (100,000 X g). The lysate was then incubated with 2 mL amylose beads (New England Biolabs) overnight and washed with 20 mL Buffer F (20 mM KH_2PO_4 pH 7.5, 10% glycerol, 0.5 mM EDTA, 0.01% Igepal, and 1 mM β-mercaptoethanol) containing 1 M KCl followed by a wash with 20 mLs of Buffer F containing 300 mM KCl. The protein was eluted with Buffer F containing 300 mM KCl and 10 mM maltose. The eluate was diluted 1:3 with Buffer G (20 mM KH_2PO_4 pH 7.5, 10% glycerol, 0.5 mM EDTA, 0.01% Igepal, and 1 mM dithiothreitol) and loaded onto a 1 mL Source Q column (GE Healthcare). The column was developed with a 30 mL gradient of Buffer G containing 100 – 800 mM KCl and the peak fractions (~350 mM KCl) were pooled, diluted 1:4 with Buffer G and loaded onto a 1 mL Macro Hydroxyapatite column (Bio-Rad). The protein was fractionated with Buffer G containing 100 mM KCl and 0 – 300 mM KH_2PO_4. Peak fractions (~220 mM KH_2PO_4) were pooled, diluted 1:4 with Buffer G and loaded onto a 1 mL Mono Q column (GE Healthcare). The protein was fractionated with a 30

mL gradient of Buffer G containing 100 mM – 500 mM KCl and peak fractions (~320 mM KCl) were pooled and concentrated to 10 mg/mL in a Centricon-30 concentrator (Millipore). Small aliquots of the purified protein were stored at -80°C.

S.1.2. Expression and purification of the MBP-Mei5 N- and MBP-Mei5-C truncation proteins.

The expression plasmid that expressed MBP-Mei5-N truncation protein was constructed with Quickchange site-directed mutagenesis on the MBP-Mei5 expression plasmid using oligonucleotides containing the sequence 5'-CCACTTTAATCAAAATCAATAGAATGGG CTGATATAAAGATTTTCTAGAAAAAGAAATGGAGGC -3' and 5'-GCCTCCATTTCTT TTTCTAGAAAATCTTTATATCAGCCCATTC TATTGATTTTGATTAAAGTGG -3' that introduced a stop codon at residue position 121. The plasmid was sequenced to ensure only the desired mutation was introduced. The expression plasmid that expressed MBP-Mei5-C truncation protein was constructed using the MBP-Mei5 expression plasmid for Quickchange site-directed PCR using oligonucleotides containing the sequence 5'-CCACTTTAATCAAAAT CAATAGAATGGGCGAATTCTATAAAGATTTTCTAGAAAAAGAAATGG-3' and 5'-CC ATTTCTTTTTCTAGAAAATCTTTATAGAATTCGCCCATTCTATTGATTTTGATTAAAG TGG-3' to insert an EcoRI restriction site adjacent to residue 121 while maintaining the reading frame. After purification of the plasmid, restriction digestion with EcoRI removed the 5' half of Mei5. The ligated plasmid was transformed, purified and sequenced to ensure MBP was fused in frame with the C-terminal half of Mei5 (residues 122-222). Both MBP-Mei5-N and MBP-Mei5-C were expressed and purified using the same protocol used for MBP-Mei5.

S.1.3. Expression and purification of Sae3

A $(HIS)_6$ tag was added to the 3'-end of the *S. cerevisiae SAE3* cDNA by PCR. The PCR product was inserted into the bacterial expression plasmid pET-RSF Duet vector (Novagen) and sequenced to ensure no undesired mutations were present. The pET-RSF-Sae3-$(HIS)_6$ expression vector was then transformed into *E. coli* BL21 Rosetta(DE3) cells, grown at 37°C to an A_{600} of 1.0 prior to the addition of IPTG to 0.4 mM and incubated for 20 h at 16°C. The cells were harvested by centrifugation. All subsequent steps were carried out at 4°C. Cell paste (20 g) was resuspended in 100 mL of Buffer E containing 250 mM KCl and subjected to sonication at 3 X 30 second cycles. The extract was clarified by ultracentrifugation (100,000 x g) and incubated with 2 mL Ni-NTA Sepharose 6 Fast Flow beads (GE Healthcare) and 40 mM imidazole overnight. The matrix was washed with 20 mL Buffer F containing 1 M KCl and 40 mM imidazole followed by a wash with 20 mL of Buffer F containing 300 mM KCl and 40 mM imidazole. The protein was eluted with Buffer F containing 300 mM KCl and 500 mM imidazole. The eluate was diluted 1:3 with Buffer G and loaded onto an 8 mL Macro Hydroxyapatite column. The protein was fractionated with Buffer G containing 0 – 300 mM KH_2PO_4. Peak fractions (~240 mM KH_2PO_4) were pooled, diluted 1:4 with Buffer G and loaded onto a 1 mL Mono Q. The column was developed with a 20 mL gradient of Buffer G containing 100 – 600 mM KCl. Peak fractions (~425 mM KCl) were pooled, diluted 1:4 with Buffer G and loaded onto a 1 mL Mono S (GE Healthcare). The protein was fractionated with a 30 mL gradient of Buffer G containing 100 mM – 1 M KCl and peak fractions (~240 mM KCl) were pooled and concentrated to 15 mg/mL in a Centricon-3 concentrator (Millipore). Small aliquots of the protein were stored at -80°C.

S.1.4. Expression and purification of Mei5-Sae3

The *S. cerevisiae MEI5* gene was amplified from genomic DNA by PCR. The PCR product was inserted into the bacterial expression plasmid pET11c (Novagen) and sequenced to ensure no undesired mutations were present. The Mei5 and Sae3-$(HIS)_6$ expression plasmids were co-transformed into *E. coli* BL21 Rosetta(DE3) cells. The cells were grown at 37°C to an A_{600} of 1.0 followed by the addition of IPTG to 0.4 mM. The cell culture was incubated for 20 h at 16°C followed by centrifugation to harvest the cells. All subsequent steps were carried out at 4°C. Cell paste (60 g) was resuspended in Buffer E containing 250 mM KCl followed by sonication at 3 X 30 second cycles. The extract was clarified by ultracentrifugation (100,000 X *g*) and incubated with 2 mL Ni-NTA Sepharose 6 Fast Flow beads. The matrix was washed with 20 mL Buffer F containing 1 M KCl and 40 mM imidazole followed by a wash with 20 mL of Buffer F containing 300 mM KCl and 40 mM imidazole. The protein was eluted with Buffer F containing 300 mM KCl and 500 mM imidazole and diluted 1:3 with Buffer G to be loaded onto a 1 mL Macro Hydroxyapatite column. The protein was fractionated with Buffer G containing 100 mM KCl and 0 – 300 mM KH_2PO_4. Peak fractions (~150 mM KH_2PO_4) were pooled, diluted 1:3 with Buffer G and loaded onto a 1 mL Mono Q column. The Mono Q was developed with a 20 mL gradient of Buffer G containing 100 – 600 mM KCl and the peak fractions (~160 mM KCl) were pooled, diluted 1:2 with Buffer G and loaded onto a 1 mL Mono S. The protein was fractionated with a 30 mL gradient of Buffer G containing 100 mM – 1 M KCl. Peak fractions (~240 mM KCl) were pooled and concentrated to 10 mg/mL in a Centricon-10 concentrator (Millipore). Small aliquots of the purified protein were stored at -80°C.

S.1.5. Expression and purification of RPA, Rad51 and Rad52

RPA was purified from a bacterial strain that co-expresses the three subunits of this complex as described previously [22] while Rad52 was expressed and purified as described [23].

Rad51 was expressed in BLR(DE3) grown at 37°C to an A_{600} of 1.0 followed by the addition of IPTG to 0.4 mM. The cell culture was incubated for 3 h at 37°C followed by centrifugation to harvest the cells. The purification of Rad51 was performed as previously described [24]. For RPA, Rad52 and Rad51 purifications, the minor modification to use 3 cycles of sonication for 30 seconds was used to lyse the cells rather than a French Press.

S.1.6. DNA substrates

The ϕX 174 (+) viron ssDNA and ϕX 174 RF I dsDNA were purchased from New England Biolabs. The ϕX 174 RF I dsDNA was linearized by digestion with *ApaLI*. All oligonucleotides (Supp. Table 1) were purchased from Integrated DNA Technologies. The oligonucleotides OLH3, OL83 and poly dT of length 10, 20, 40 and 60 nucleotides were 5'-end labeled with [γ-^{32}P]-ATP using T4 polynucleotide kinase (New England Biolabs). The reaction was loaded on a Micro Bio-Spin 30 Column (Bio-Rad) to remove unincorporated [γ-^{32}P] ATP. Oligonucleotide ^{32}P-OLH3 was annealed to OLH3-C to create an 80 bp dsDNA substrate, OLH1 to create a 3' overhang substrate, and OLH5 to create a 40 bp duplex substrate with 40 base 3' ssDNA tails. To anneal the oligonucleotides, equimolar amounts of the indicated oligonucleotides were heated at 95°C for 5 min in Buffer H (10 mM Tris-HCl pH 8.0, 10 mM NaCl, 1 mM EDTA) and slowly cooled to room temperature. The annealed DNA substrates were gel purified on 10% non-denaturing TAE polyacrylamide gels. The substrates were excised from the polyacrylamide gel, electroeluted and filter dialyzed as described [25].

S.1.7. Mei5-Sae3 interacts with Rad51 through Mei5

Rad51 (7 μg) or MBP-Mei5 (7.4 μg) was incubated at 4ºC for 30 min with either Mei5-Sae3 (6.7 μg) or Sae3-$(HIS)_6$ (16 μg) in 30 μL of Buffer C containing 120 mM KCl. The

reactions were combined with Nickel-NTA beads and agitated for 30 min at 4ºC. The supernatant was removed from the beads followed by 3 washes with Buffer C. Equal volumes of SDS loading dye were added to the supernatant and wash fractions while 30 µL was added to the bead fraction. The supernatant, wash and beads were boiled and 8 µL of each was loaded onto a 15% SDS-PAGE gel and subjected to Coomassie Blue staining. As a control, Rad51 or MBP-Mei5 was incubated alone with the Nickel-NTA beads. For some samples, 6 uL of the indicated fraction was loaded onto SDS-PAGE followed by Western analysis.

Supplementary Figure Legend

Supplementary Figure 1. Mei5-Sae3 interacts with Rad51. (A) Sae3-$(HIS)_6$ was mixed with Affi-Gel containing covalently conjugated BSA (panel I lanes 1-3) or Rad51 (panel I lanes 4-6). After a wash, the bound protein was eluted with SDS. The supernatant (S), wash (W), and eluate (E) were separated on a SDS-PAGE gel and stained with Coomassie Blue. (B) MBP-Mei5 or MBP was incubated with Rad51 for 30 min at 4ºC. Amylose beads were added to the reactions for 30 min at 4ºC with agitation to capture protein complexes and analyzed as above. (C) Western analysis using anti-Rad51 antibodies was performed on 6 uL of the indicated fraction from (B) in order to confirm the presence of Rad51.

Supplementary Figure 2. Sequence alignment of Mei5 with murine and *S. pombe* orthologs. Alignment of *S. cerevisiae* Mei5 with murine Sfr1 (A) and *S. pombe* Sfr1 (B). Sequence alignments were performed using ClustalW. Accession numbers for Mei5 and its orthologs are as follows: *S. cerevisiae* Mei5 (AAB68241.1), *S. pombe* Sfr1 (CAB57936.1) and mouse Sfr1 (BAJ25749.1). "*" denotes identical amino acids, ":" denotes conserved amino acids, and "." Denotes semi-conserved amino acids.

Supplementary Figure 3. Sequence alignment of Sae3 with murine and *S. pombe* orthologs. Alignment of *S. cerevisiae* Sae3 with murine Swi5 (A) and *S. pombe* Swi5 (B). Sequence alignments were performed using ClustalW. Accession numbers for Sae3 and its orthologs are as follows: *S. cerevisiae* Sae3 (NP_011947.2), mouse Swi5 (BAJ25750.1) and *S. pombe* Swi5 (CAB52605.1). "*" denotes identical amino acids, ":" denotes conserved amino acids, and "." Denotes semi-conserved amino acids.