Supplemental Material for Xue et al

List

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Supplemental Methods
Detailed methods for protein purification, analyses of protein-protein and protein-DNA interactions, Mph1 activity assays, 2D gel analyses, DSB repair and crossover assays.

Supplemental Table: Yeast strains and plasmids used in this study

References.
Xue Fig. S1
(GENEDEV/2015/259143)
Xue_Fig. S2
(GENEDEV/2015/259143)

A

|         | YPD                        | MMS 0.001%                  |
|---------|----------------------------|-----------------------------|
| smc6-9  |                            |                             |
| mhf2Δ smc6-9 |                        |                             |
| mhfΔ smc6-9 |                        |                             |

|         | YPD                        | MMS 0.0003%                 |
|---------|----------------------------|-----------------------------|
| smc6-56 |                            |                             |
| mhf1Δ smc6-56 |                       |                             |
| mhf2Δ smc6-56 |                       |                             |
| mhfΔ smc6-56 |                       |                             |

B

|         | YPD                        | MMS 0.03%                  | MMS 0.05%                  |
|---------|----------------------------|-----------------------------|-----------------------------|
| WT      |                            |                             |                             |
| mph1Δ   |                            |                             |                             |
| mhfΔ    |                            |                             |                             |
| mph1Δ mhfΔ |                       |                             |                             |
Xue_Fig. S3
(GENEDEV/2015/259143)

(A) 

scMHF - - - - - + + + + +
Mph1 + + + + + + + + + +
time 0 2 4 6 8 0 2 4 6 8 (min)

(B) 

scMHF - - - - - + + + + +
Mph1 + + + + + + + + + +
time 0 2 4 6 8 0 2 4 6 8 (min)

(C) 

σ Structure (σ)

Holliday Structure (HJ)

linear duplex (2.9 kb)

P2

(D) 

Time (min) 20 0 5 10 15 20 0 5 10 15 20

Mph1 Mph1+scMHF

σ

HJ

P2

% Product

% Product
**A**

ssDNA (90mer) + dsDNA → D-loop

Mph1 (Smc5 or scMHF) → ssDNA (90mer)

**B**

4 min

|        | scMHF | Smc5 | NP | Mph1 |
|--------|-------|------|----|-------|
| 1      | -     | -    | -  | +     |
| 2      | -     | -    | -  | +     |
| 3      | -     | -    | -  | +     |
| 4      | -     | -    | -  | +     |
| 5      | -     | -    | -  | +     |
| 6      | -     | -    | -  | +     |
| 7      | -     | -    | -  | +     |
| 8      | -     | -    | -  | +     |
| 9      | -     | -    | -  | +     |
| 10     | -     | -    | -  | +     |
| 11     | -     | -    | -  | +     |
| 12     | -     | -    | -  | +     |

% D-loop formed

**C**

8 min

|        | scMHF | Smc5 | NP | Mph1 |
|--------|-------|------|----|-------|
| 1      | -     | -    | -  | -     |
| 2      | -     | -    | -  | -     |
| 3      | -     | -    | -  | -     |
| 4      | -     | -    | -  | -     |
| 5      | -     | -    | -  | -     |
| 6      | -     | -    | -  | -     |
| 7      | -     | -    | -  | -     |
| 8      | -     | -    | -  | -     |
| 9      | -     | -    | -  | -     |
| 10     | -     | -    | -  | -     |
| 11     | -     | -    | -  | -     |
| 12     | -     | -    | -  | -     |

% D-loop formed

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**Xue Fig. S4**

(GENEDEV/2015/259143)
Xue_Fig. S5
(GENEDEV/2015/259143)
Xue_Fig. S6
(GENEDEV/2015/259143)
Key residues involved in interaction with FANCM and Mph1

A

hMHF-FANCM

B

scMHF

Xue Fig. S7
(GENEDEV/2015/259143)

MHF1

α1

α2

α3

α4

HsMHF1 1-MEEBAETEEGRFSYQQRLKAAVHYTVGCLCEEVALDKEMQFSKQTIAAISELTFRQCENFAKLEMFARHAKRTTINTEDVKLLARR
MmMHF1 1-MEEBAETEEGRFSYQQRLKAAVHYTVGCLCEEVALDKEMQFSKQTIAAISELTFRQCENFAKLEMFARHAKRTTINTEDVKLLARR
SpMHF1 1-MEEBAETEEGRFSYQQRLKAAVHYTVGCLCEEVALDKEMQFSKQTIAAISELTFRQCENFAKLEMFARHAKRTTINTEDVKLLARR
ScMHF1 1-MEEBAETEEGRFSYQQRLKAAVHYTVGCLCEEVALDKEMQFSKQTIAAISELTFRQCENFAKLEMFARHAKRTTINTEDVKLLARR

MHF2

α1

α2

α3

α4

HsMHF2 1-MEEBAETEEGRFSYQQRLKAAVHYTVGCLCEEVALDKEMQFSKQTIAAISELTFRQCENFAKLEMFARHAKRTTINTEDVKLLARR
MmMHF2 1-MEEBAETEEGRFSYQQRLKAAVHYTVGCLCEEVALDKEMQFSKQTIAAISELTFRQCENFAKLEMFARHAKRTTINTEDVKLLARR
SpMHF2 1-MEEBAETEEGRFSYQQRLKAAVHYTVGCLCEEVALDKEMQFSKQTIAAISELTFRQCENFAKLEMFARHAKRTTINTEDVKLLARR
ScMHF2 1-MEEBAETEEGRFSYQQRLKAAVHYTVGCLCEEVALDKEMQFSKQTIAAISELTFRQCENFAKLEMFARHAKRTTINTEDVKLLARR

Key residues involved in interaction with FANCM and Mph1
Supplemental Figure legends

**Figure S1. scMHF interacts with Mph1 in vitro.**
(A) scMHF forms a stable complex with Mph1 in vitro. Purified scMHF (lane 1) interacts with Mph1 (lane 2) to form a stable complex (lane 3) that can be purified through size exclusion chromatography. Purified protein species (from size exclusion chromatography) were examined by SDS-PAGE and Coomassie staining.

(B) scMHF does not directly interact with Smc5 in vitro. scMHF was not retained on maltose resin pre-loaded with MBP-Smc5 (lanes 1-3) or MBP (lanes 4-6).

(C) GST-tagged scMHF or GST was incubated with full length Mph1 (lanes 1-6), its helicase domain (residues 1-754; lanes 7-12). The various fractions from the pull-down reactions were examined as in (B). The arrows denote full length Mph1 being pulled down by GST-scMHF (lane 3).

**Figure S2. Removal of scMHF suppresses the MMS sensitivity of smc6-9 and smc6-56, and mhfΔ is epistatic with mph1Δ.**
(A) Experiments were done as Figure 2A-2C.

(B) mhf1Δ mhf2Δ (mhfΔ) exhibits mild sensitivity to MMS and is epistatic with mph1Δ.

**Figure S3. scMHF alone does not influence Mph1 fork regression and branch migration activities in vitro.**
(A) The effect of scMHF on replication fork regression by Mph1 was examined. Mph1 (2 nM) and scMHF (4 nM) were incubated with MRF (15 nM) at 30°C for the indicated times.

(B) The effect of scMHF on DNA branch migration by Mph1 was examined. Mph1 (2 nM) and scMHF (4 nM) were incubated with MHJ (15 nM) at 30°C for the indicated times. The asterisk denotes the 32P label. The results were quantified and plotted. Error bars represent standard deviation from three independent experiments.

(C–D) scMHF does not affect the processing of a sigma structure by Mph1. (C) Schematic of the sigma structure used to assess replication fork regression and HJ branch migration activities. Regression of the fork structure by Mph1 yields a HJ, which can further branch migrate over 2.9 kb to generate a radio-labeled linear product and an unlabeled circular one. The asterisk denotes the 32P label. (D) Mph1 (1 nM) and scMHF (2 nM) were incubated with the sigma structure (0.5 nM) and then analyzed after the indicated incubation times at 30°C. The results were quantified and plotted. Error bars represent standard deviation from three independent experiments.

**Figure S4. scMHF and Smc5 have no effect on Mph1’s D-loop dissociation activity.**
(A) Schematics of Rad51-mediated D-loop formation followed by dissociation by Mph1 were shown.

(B–C) scMHF and Smc5 have no effect on Mph1’s D-loop dissociation activity. Assays were performed as described previously (Prakash et al., 2009; Xue et al., 2014). Briefly, D-loops made by Rad51 with a radiolabeled 90-mer ssDNA were incubated with Mph1 (50 nM) (lanes 3, 4); Mph1 (50 nM) with Smc5 (100, 200 nM) (lanes 5, 6); Mph1 (50 nM) with scMHF (tetramer, 50, 100 nM) (lanes 7, 8); Mph1 (50 nM) with scMHF (100 nM) and Smc5 (100, 200 nM) (lanes 9, 10) at 30°C for 4 min (B) or 8 min (C). The results were quantified and plotted. Error bars represent standard deviation from three independent experiments.
Figure S5. scMHF does not bind DNA.
(A) 3D rendering showing that scMHF lacks the positively charged C-terminal tail in hMHF1 and solvent-exposed positively charged residues in both hMHF subunits, features that are germane for DNA engagement by hMHF. PDB 4NDY for hMHF in complex with dsDNA and 3V9R for scMHF were used to generate the vacuum electrostatics (Singh et al., 2010; Tao et al., 2012; Yan et al., 2010). Positively and negatively charged residues are colored blue and red, respectively.

(B–D) scMHF, unlike hMHF, does not bind DNA fork (B), HJ (C), or dsDNA (D). The indicated concentrations of scMHF and hMHF were incubated with the radiolabeled DNA substrates (5 nM each) and analyzed. NP: No protein added.

Figure S6. scMHF does not enhance affinity of Mph1 for DNA.
(A–C) The indicated concentrations of Mph1 and Mph1-scMHF complex were mixed with the radiolabeled DNA substrates (5 nM each) and analyzed. The results were quantified and plotted. Error bars represent standard deviation from at least three independent experiments.

Figure S7. Structural comparison between hMHF and scMHF and sequence alignment of MHF orthologs.
(A) The front view of the structures of hMHF-FANCM based on PDB (4DRB; Tao, et al., 2012) and scMHF (Yang et al., 2012). The tetramer of Mhf1 and Mhf2 are shown in both cases. The two subunits of Mhf1 are green and yellow, while those of Mhf2 are light and dark blue. FANCM is shown as light grey. The human Mhf1 and Mhf2 residues that contribute to FANCM binding are circled. The scMHF residues corresponding to these are also circled.

(B) The sequence alignment among the homologs of Mhf1 and Mhf2 proteins. The alignment was carried out using ClustalW, and illustrated with ESPript. Residues in helix 2 of Mhf1 and helix 3 of Mhf2 that contribute to partner motor protein interactions and selected for mutagenesis are indicated by dots. Conserved regions are boxed. Hs: human, Mm: mouse, Sp: S. pombe, Sc: S. cerevisiae.

Supplemental Methods

Expression and purification of Mph1 and Smc5
Published methods (Prakash et al., 2009; Xue et al., 2014; Zheng et al., 2011) were followed. Briefly, Smc5 and mph1 (754-993) were expressed in E. coli, whereas Hi5 insect cells and recombinant baculoviruses were used for expressing Mph1 and mph1 (1-754). Proteins were purified to near homogeneity by the combination of affinity chromatography, ion exchange, and size exclusion chromatography (Prakash et al., 2009; Xue et al., 2014; Zheng et al., 2011)

Expression and purification of (His)_6-tagged scMHF and mutant
The budding yeast MHF1 and MHF2 genes were introduced into the pETDuet vector with a (His)_6 tag sequence at the N-terminus of Mhf2. E. coli BL21:DE3 Rosetta cells harboring pETDuet-Mhf1/(His)_6-Mhf2 were grown at 37°C to OD600 = 0.8, and protein expression was induced by the addition of 0.2 mM IPTG and incubation at 16°C for 16 h. Cells were harvested by centrifugation and all the subsequent steps were carried out at 0 to 4°C. For lysate preparation, a cell pellet (10 g, from 2 L of culture) was suspended in 50 ml K buffer (20 mM
Expression and purification of GST- and (His)$_6$ double-tagged scMHF
The *MHF1* and *MHF2* genes were introduced into the pGex-BICIS vector to construct pGex-BICIS-GST-Mhf2/(His)$_6$-Mhf1. Cell growth, protein induction, and cell lysate preparation were as above. The clarified lysate was incubated with 2 ml glutathione Sepharose 4 Fast Flow resin (GE Healthcare) for 1 h. The glutathione resin was washed with 3 x 30 ml K buffer containing 150 mM KCl, 5 mM ATP, and 10 mM MgCl$_2$ and 20 ml of K buffer containing 150 mM KCl. The bound GST-Mhf2/(His)$_6$-Mhf1 was eluted in K buffer containing 150 mM KCl and 20 mM reduced glutathione. The eluate was mixed gently with 2 ml of Nickel-NTA resin (Qiagen) for 2 h. The resin was washed sequentially with 3 x 30 ml K buffer containing 300 mM KCl, 3 x 30 ml K buffer containing 150 mM KCl, and 20 ml K buffer containing 150 mM KCl and 200 mM imidazole. Bound scMHF was eluted with 10 ml K buffer containing 150 mM KCl, 3 x 30 ml K buffer containing 150 mM KCl, and 20 ml K buffer containing 150 mM KCl and 20 mM imidazole. Bound scMHF was eluted with 10 ml K buffer containing 150 mM KCl and 200 mM imidazole, concentrated to 0.5 ml (Amicon 10K concentrator, Millipore), and fractionated in a Superdex 200 gel filtration column (24 ml) in K buffer containing 150 mM KCl. Fractions containing highly purified, doubly-tagged scMHF (~5 mg protein) were pooled, concentrated to 5 mg/ml, and stored in small aliquots at -80°C.

Expression and purification of untagged scMHF
Untagged *MHF1* and *MHF2* genes were introduced into the pRSFDuet vector. Cell growth, protein induction, and cell lysate extract preparation were performed as above. The clarified lysate was fractionated in a 5 ml Q Sepharose column with a 100 ml gradient of 150-600 mM KCl in K buffer. Fractions containing the scMHF peak were pooled (~300 mM KCl) and applied onto a 1 ml column of hydroxyapatite (BioRad), which was developed with a 30 ml gradient of 0-400 mM KH$_2$PO$_4$ in K buffer. The scMHF peak fractions were pooled (~130 mM KH$_2$PO$_4$), diluted with an equal volume of K buffer, and then fractionated in a 1 ml DEAE (Pharmacia Biotech) column with a 30 ml gradient of 150-600 mM KCl in K buffer. The scMHF peak fractions were pooled (~240 mM KCl), diluted with an equal volume of K buffer, and further fractionated in a 1 ml Mono Q column with a 30 ml gradient of 150-600 mM KCl in K buffer. The scMHF peak fractions were pooled (~300 mM KCl), concentrated to 0.5 ml, and fractionated in a Superdex 200 gel filtration column (24 ml) in K buffer containing 150 mM KCl. Fractions containing highly purified scMHF (1 mg protein) were pooled, concentrated to 2 mg/ml, and stored in small aliquots at -80°C.

Affinity pull-down assay

KH$_2$PO$_4$, pH 7.4, 10% glycerol, 0.5 mM EDTA, 0.01% Igepal, 1 mM DTT) with 150 mM KCl and 5 µg/ml each of aprotinin, chymostatin, leupeptin and pepstatin, and then subject to sonication (three 1 min pulses). The crude cell lysate was clarified by ultracentrifugation (100,000Xg for 90 min) and then mixed gently with 2 ml of Nickel-NTA resin (Qiagen) for 2 h. The resin was washed sequentially with 3 x 30 ml K buffer containing 300 mM KCl, 3 x 30 ml K buffer containing 150 mM KCl, and 20 ml K buffer containing 150 mM KCl and 20 mM imidazole. The tagged scMHF was eluted with 10 ml K buffer containing 150 mM KCl and 200 mM imidazole and concentrated to 0.5 ml (Amicon 10K concentrator, Millipore) and fractionated in a Superdex 200 gel filtration column (24 ml) in K buffer containing 150 mM KCl. Fractions containing highly purified scMHF (10 mg protein) were pooled, concentrated to 8 mg/ml, and stored in small aliquots at -80°C. The scMHF mutant (mhf1-R36A/L44A/S54A, mhf2-E70A/L75E) was purified using the same procedure with a similar yield.
The reactions consisted of 5 µg of tagged Smc5, scMHF (wild-type or mutant), Mph1 and test proteins in 30 µl K buffer (20 mM KH₂PO₄, pH 7.4, 10% glycerol, 0.5 mM EDTA, 0.01% Igepal, 1 mM DTT) and 150 mM KCl and were incubated at 4°C for 30 min. The reaction mixtures were incubated with 10 µl of Nickel-NTA agarose (for (His)₅-tagged proteins), glutathione Sepharose (for GST-tagged proteins), amylose agarose (for MBP-tagged proteins; NEB), or anti-FLAG agarose (for FLAG-tagged proteins; Sigma) for 30 min at 4°C. After washing with the same buffer, bound proteins were eluted with 20 µl of 2% SDS. Ten percent of the supernatant (S) and elution (E) fractions, and 2% of the wash (W) fraction were analyzed by 4-20% gradient SDS-PAGE, followed by staining with Coomassie Blue.

**Competition pull-down assay**

0.7 µM Smc5 was incubated with 1.5 µM MBP-mph1 (754-993) or MBP in the absence of scMHF, or in the presence of 1.5 µM or 7.5 µM scMHF tetramer in K buffer (20 mM KH₂PO₄, pH 7.4, 10% glycerol, 0.5 mM EDTA, 0.01% Igepal, 1 mM DTT) containing 150 mM KCl at 4°C for 30 min. The reaction mixtures were incubated with 10 µl of amylose agarose for 30 min at 4°C. After washing with the same buffer, bound proteins were eluted with 20 µl of 2% SDS. Each fraction was analyzed by 4-20% gradient SDS-PAGE, followed by staining with Coomassie Blue.

**In vitro assays for Mph1 activities in replication fork regression and DNA branch migration**

These assays were performed with ³²P-labeled DNA substrates as described (Prakash et al., 2009; Xue et al., 2014; Zheng et al., 2011). In Figure S3A and S3B, Mph1 (2 nM) and scMHF (4 nM) were incubated on ice for 10 min in 10 µl buffer A (25 mM Tris-HCl, pH 7.5, 1 mM DTT, 100 µg/ml BSA, 45 mM KCl, 1 mM MgCl₂, 2 mM ATP, 15 mM phosphocreatine and 30 units/ml of creatine phosphokinase), followed by the incorporation of the MRF (Fig. S3A, 15 nM, (Xue et al., 2014)) or MHJ (Fig. S3B, 15 nM, (Xue et al., 2014)) substrate, and incubated at 30°C. In Figure 4 and Figure 5, the indicated concentrations of Mph1, scMHF (or mutant) and Smc5 were incubated with 5 nM of MRF (Figs. 4A and 5A) or MHJ (Figs. 4B and 5B) at 30°C for 4 min. The reaction mixtures were deproteinized by treatment with SDS (0.5% final) and proteinase K (0.5 mg/ml) for 5 min at 37°C before being resolved in an 8% polyacrylamide gel in TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0). In Figure S3D, Mph1 (1 nM) was incubated with scMHF (2 nM) on ice for 10 min in 10 µl buffer A, followed by the incorporation of the sigma structure substrate (0.5 nM) and incubation at 30°C. The reaction mixtures were deproteinized with SDS and proteinase K as above before being resolved in a 1% agarose gel in TBE buffer. Gels were dried and analyzed in the PhosphorImager (Bio-Rad).

**D-loop dissociation assay**

The D-loop reaction was conducted as described previously (Prakash et al., 2009; Xue et al., 2014; Zheng et al., 2011). Briefly, ³²P-labeled 90-mer ssDNA (2.4 µM nucleotides) was incubated with Rad51 (0.8 µM) to assemble the Rad51-ssDNA nucleoprotein filament. Following the incorporation of Rad54 (150 nM), RPA (200 nM), and pBluescript replicative form I dsDNA (36 µM base pairs), the reaction was incubated at 30°C for 4 min before the incorporation of Mph1 (50 nM), or Mph1 (50 nM) with Smc5 (100-200 nM), or Mph1 (50 nM) with scMHF (tetramer, 50-100 nM), or Mph1 (50 nM) with scMHF (100 nM) and Smc5 (100-200 nM). The reaction mixtures were incubated at 30°C for 4 or 8 min, deproteinized as above,
and resolved in 0.9% agarose gels in TBE buffer. Gels were dried and analyzed in the Phosphorimager.

**DNA mobility shift assay**

These assays were performed with $^{32}$P-labeled DNA substrates as described (Prakash et al., 2009; Xue et al., 2014; Zheng et al., 2011). In Figure S5B, scMHF (20, 40, 80, 120, 160 nM) was incubated with 5 nM MRF substrate at 30°C for 10 min in 10 µl of buffer D (30 mM Tris-HCl, pH 7.5, 1 mM DTT, 100 µg/ml BSA, and 50 mM KCl). In Figure S5C, hMHF or scMHF (25, 50, 75, and 100 nM) was incubated with a mixture of MRF, MHJ, and dsDNA substrates (30 nM each) at 30°C for 10 min in 10 µl of buffer D. In Figure S5D, hMHF or scMHF (30, 60 and 90 nM) was incubated with dsDNA substrate (30 nM) at 30°C for 10 min in 10 µl of buffer D. In Figure S6, Mph1 or Mph1-scMHF complex (10, 20, 40, and 80 nM) was incubated with MRF and dsDNA (5 nM each in Fig. S6A), MHJ and dsDNA (5 nM each in Fig. S6B) or D-loop structure (5 nM in Fig. S6C) at 30°C for 10 min in 10 µl of buffer D. The reaction mixtures were resolved in 6.5% polyacrylamide gels in TBE buffer. Gels were dried and analyzed in the PhosphorImager.

**Co-immunoprecipitation in yeast**

Yeast strains containing chromosomally tagged proteins were grown to mid-log phase and harvested. Yeast cells were lysed in TBT buffer (20 mM HEPES, pH 7.4, 110 mM potassium acetate, 2 mM MgCl$_2$, 0.1% Tween-20) supplemented with 100-300 mM NaCl, 0.5% Tween-20, 1 mM dithiothreitol (DTT), protease inhibitors (pepstatin and phenyl-methylsulfonyl fluoride or PMSF) and DNase I with glass beads by vortexing (MP Biomedicals). Lysates were incubated with either agarose (Calbiochem) or sepharose (Pierce) beads conjugated with Protein A or G and indicated antibodies on a rotating wheel for 2-4 hours at 4°C to immunoprecipitate tagged proteins. After stringent washing with lysis buffer (4-7 times), the bound proteins were eluted in the SDS-PAGE loading dye and analyzed by Western Blotting using indicated antibodies.

**2D gel analysis**

Experiments were performed as described previously (Choi et al., 2010). In brief, cells were synchronized in G2 by adding nocodazole to the final concentration of 10 mg/ml together with DMSO at 1% for 2.5 h. Cells were then released from nocodazole arrest into YPD medium containing MMS at a final concentration of 0.033% at 30°C. At the indicated times after release, cells were collected for fluorescence-activated cell sorting (FACS) analysis and purification of DNA intermediates. The DNA samples were digested with HindIII and EcoRV and separated by 2D gel electrophoresis followed by Southern blotting with a probe against ARS305.

**Measurements of crossover frequency and DSB repair assays**

This method is described in (Prakash et al., 2009). In brief, CO frequency was determined as a ratio of the intensity of the band corresponding to gene conversion with crossover to the intensity of bands corresponding to gene conversion both with and without crossover. We measured the frequency of crossovers among the recombination products 8 h after HO induction. DNA isolated from cells was digested with EcoRI enzyme and separated on 0.8% agarose gel. DNA was transferred to Nylon+ membrane and hybridized with a MATa probe corresponding to 200 bp on each side of HO cut site. To calculate kinetics of repair we normalized the DNA amount using DNA probe specific for APA1 gene.
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### Supplemental Table: Yeast strains and plasmids used in this study

| Strain/Plasmid | Genotype | Sources |
|---------------|----------|---------|
| T538-2        | MATa MPH1-3FLAG::HIS3 | (Chen et al., 2009) |
| T294-11B      | MATa SMC5-13MYC::HIS3 | lab collection |
| X3921-9C      | MPH1-3FLAG::HIS3 MHF1-TAP::TRP | this study |
| X5606-3D      | SMC5-13MYC::HIS3 MHF1-TAP::TRP | this study |
| T783          | MATa mhf1Δ::KAN | this study |
| X4253-5C      | mhf1Δ::KAN smc6-P4-13MYC::HIS3 | this study |
| X4253-20A     | mhf1Δ::URA3 mhf1Δ::KAN smc6-P4-13MYC::HIS3 | this study |
| X4179-14D     | MATa mhf2Δ::KAN smc6-P4-13MYC::HIS3 | this study |
| T382-P4       | MATa smc6-P4-13MYC::HIS3 | (Chen et al., 2009) |
| 1483-2Ca      | MATa mhf1Δ::KAN | (Chen et al., 2009) |
| X1787-4A      | MATa mhf1Δ::KAN smc6-P4-13MYC::HIS3 | (Chen et al., 2009) |
| T773          | MATalpha mhf2Δ::KAN | this study |
| X3095-17C     | mhf1Δ::URA3 mhf2Δ::KAN smc6-P4-13MYC::HIS3 | this study |
| X3089-10A     | mhf1Δ::URA3 mhf1Δ::KAN | this study |
| X3087-2A      | mhf1Δ::URA3 mhf2Δ::KAN | this study |
| X3092-4A      | mhf1Δ::URA3 rad51Δ::LEU2 | this study |
| W3111-1C      | MATa rad51Δ::LEU2 | R. Rothstein |
| X5804-9B      | MPH1-FLAG::HIS3 | this study |
| X3999-6D      | mhf1Δ::URA3 mhf2Δ::KAN | lab collection |
| X4091-2D      | mhf1Δ::URA3 mhf1Δ::URA3 mhf2Δ::KAN | this study |
| pXZ216        | pOAD-MPH1 | (Chen et al., 2009) |
| pXZ477        | pOAD-mph1-S1-S3 (754-993 a.a.) | this study |
| pXZ194        | pOAD-MPH1 | (Duan et al., 2009) |
| X6550-11C     | MHH1-Strep::KAN MPH1-FLAG::HIS3 | this study |
| X6551-1C      | mhf1-RLS-Strep::KAN mhf2-EL MPH1-FLAG::HIS3 | this study |
| X6553-4C      | mhf1-RLS-Strep::KAN mhf2-EL smc6-P4::HIS3 | this study |
| X6552-8B      | MHH1-Strep::KAN smc6-P4::HIS3 | this study |
| X6553-2C      | mhf1-RLS-Strep::KAN mhf2-EL | this study |
| X6759-4A      | smc6-9::NAT | this study |
| X6759-8B      | mhf2Δ::KAN smc6-9::NAT | this study |
| X6759-8A      | mhf1Δ::URA3 mhf2Δ::KAN smc6-9::NAT | this study |
| X6758-2-13B   | smc6-56-13myc::HIS3 | this study |
| X6758-2-15C   | mhf1Δ::URA3 smc6-56-13myc::HIS3 | this study |
| X6758-2-15A   | mhf2Δ::KAN smc6-56-13myc::HIS3 | this study |
| X6758-2-12A   | mhf1Δ::URA3 mhf2Δ::KAN smc6-56-13myc::HIS3 | this study |
| pXZ503        | pOBD-MHF1 | this study |
| pXZ504        | pOBD-MHF2 | this study |
| pETDuet-Mhf1/His6-Mhf2 | this study |
| pRSFDuet-Mhf1/Mhf2 | this study |
| pETDuet-mhf1-RLS/His6-mhf2-EL | this study |
| pFastBac HTb-Mph1 | this study |
| pFastBac HTb-Mph1(1-754aa) | this study |
| pMAL-TEV-Mph1(754-993aa) | this study |
| pMAL-TEV-Smc5 | this study |
| pFastBac Dual-HA-Mhf1/Myc-Mhf2 | this study |
| pGex-BICIS-GST-Mhf2/His6-Mhf1 | this study |
Strains are derivatives of W1588-4C, a $RAD5$ variant of W303 ($MATa\ ade2-1\ can1-100\ ura3-1\ his3-11,15\ leu2-3,112\ trp1-1\ rad5-535$ (Zhao and Blobel, 2005)). Only one strain for each genotype is listed, but at least two independent spore clones of each genotype were used in all the experiments.