Supplementary Information

Strains and plasmids. The strains were used in this study are listed in Supplementary Table I. All strains are *leu1-32* and *ura4-D18*. Most deletion and tagged strains were constructed using the one-step replacement method. For complementation assays, localization studies and MudPIT, the open reading frames of *sws1*$^+$, *rlp1*$^+$ and *rdl1*$^+$ were cloned between the *Nde*I and *BamH*I restriction sites of appropriate pREP1, pREP42X or pREP41X vectors. 2xmyc- and GFP- containing pREP41x vectors have been described elsewhere (Craven et al., 1998). The pREP42X vector used for co-immunoprecipitations and mass-spectrometry contains two protein A copies and one myc epitope fused to the N-terminal region of the corresponding proteins. All plasmid-derived fusion proteins were N-terminal tagged. Site-directed mutagenesis were performed with the Stratagene QuikChange kit.

Media and growth conditions. Growth media, general biochemical and genetic methods for fission yeast and staining with DAPI have been described elsewhere. Cells were grown at 32ºC in YES medium (0.5% yeast extract, 3% glucose, supplements) unless indicated otherwise. For HU, CPT and MMS treatment, cells were plated onto YES plates containing the indicated concentrations of drugs. For gamma irradiation, cells were plated and plates were irradiated with a 3.0-Gys/min ^{137}Cs source at room temperature and then placed at 32ºC. For UV light treatment, cells were plated and irradiated at 250 nm with a Stratalinker (Stratagene). For HU arrest and release experiments, exponentially growing cells were incubated in YES in the presence of 12mM HU for 5 hours. Cells were then washed and stained with DAPI at the indicated time points to detect the presence of “cut” cells.

Recombination assays. Quantification of mitotic recombination within the *ade6*$^{-}$ heteroalleles was done as described (Osman et al., 1996). Briefly, 10^5 cells were plated on YEA supplemented with guanine (which inhibits the growth of auxotrophs requiring adenine) to select for *ade*$^{+}$ recombinants. The cell titer was determined by appropriate dilution and plating onto YEA. The *ade*$^{+}$ recombinants were plated onto media lacking uracil to determine the proportion of deletion (*ade*$^{+}$ *ura*$^{-}$) and conversion (*ade*$^{+}$ *ura*$^{+}$) types. Recombination frequencies are mean values from three independent assays, and in each assay four independent colonies were tested. The statistical significance of differences in recombination frequencies was determined with two-sample *t*-test.

SWS1 RNA interference (RNAi). 3HASWS1 was cloned into pCDNA3 (Invitrogen). Two SWS1 19-nucleotide-long regions (gcagttgagtgacatatta for SWS1RNAi3, and cttggaagttccagtaaaa for SWS1RNAi4) were selected and cloned into pSuper. HeLa cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% calf serum and 100 mg/ml penicillin and streptomycin. $0.5x10^6$ cells were cultured overnight before co-transfected with 3HASWS1 and pSuper or 3HASWS1 and pSuper SWS1RNAi. Effectene (QIAGEN) was used for all transfections. Parallel transfection with pCMU-β-galactosidase was used to determine that more than 90% of cells got transfected. Cells were harvested at 24, 48 and 72 h post-transfection for Western analysis.

Western analysis of *S. pombe*. Whole-cell extracts were prepared from exponentially growing cells and processed for Western blot analysis as described elsewhere (Noguchi et al., 2003). EDTA was excluded from the buffers. For TAP tag protein precipitation, protein extracts were mixed with immunoglobulin G-Sepharose beads and incubated for 2 h at 4ºC. Sepharose beads were collected and washed three times in lysis buffer. Sws1

and Rdl1 tagged with a tandem affinity purification tag were detected with peroxidase-antiperoxidase reagent (PAP at 1:1,000 dilution; Sigma). 2myc-tagged Sws1 and Rlp1 were incubated with anti-myc antibody (9E10 at 1:1,000 dilution; Santa Cruz Biotechnology) and then detected with horseradish peroxidase (HRP)-conjugated anti-mouse (1:5,000 dilution; Promega). Enhanced chemiluminescence detection (PIERCE) was used to visualize proteins.

Western analysis of HeLa cells. 3HASWS1, FLAGRAD51D and mycXRCC2 were cloned into pCDNA3 (Invitrogen). HeLa cells were lysed in 20 mM HEPES, pH 7.4, 150 mM NaCl, 5% glycerol, 0.1% NP-40, 0.1% b-mercaptoethanol, 0.5 mM phenylmethylsulphonyl fluoride, and 5 μg/ml leupeptin, pepstatin, and aprotinin. Lysates were cleared by centrifugation at 10000x g for 10 minutes. Protein concentration of the supernatants was determined using Bradford reagent (Bio-Rad, Hercules, CA). Cell lysate, 100 μg, was resolved on 12% acrylamide-SDS gels. Proteins were co-precipitated using Sigma anti-FLAG-M2 affinity gel for 2 hours at 4ºC. Beads were collected and washed three times with lysis buffer. Immunoblots were incubated in anti-HA (1:5000 dilution; Novus Biologicals), anti-myc (1:1000 dilution; Covance), anti-FLAG (1:1000 diltuion; Sigma anti-FLAG-M2) or anti-tubulin (1:1000 dilution; Santa Cruz) followed by horseradish peroxidase-conjugated anti-rabbit or anti-mouse (Promega, Madison WI). Chemoluminescence (Pierce, Rockford, IL) was used to detect the respective proteins.

Immunofluorescence. HeLa cells were grown on cover slides overnight before being transfected with pSuper or pSuper SWS1RNAi. Cells were cultured for additional 65 h, then either not treated or subject to 10Gy γ-irradiation and cultured for additional 5 hours. Then, *in situ* fractionation was performed prior to fixation. Cells were fixed in 4%

formaldehyde, permeabilized in 0.5% Triton X-100, blocked with 10% calf serum, and then incubated with Rad51 antibody (Abcam 13E4, 1:150) overnight in 4ºC followed by Alexa-fluor 488-conjugated anti-mouse immunoglobulin G (Molecular Probes, 1:1000) for 1 h at room temperature. Cells were counterstained with 4', 6'-diamidino-2-phenylindole (DAPI) and mounted in 70% glycerol. Gray-scale images were processed using Adobe Photoshop 7.0. Three hundred and fifty cells were counted for each sample. Cells containing two or more distinct foci were scored as positive.

Sequence accession numbers. *S. pombe* Sws1 (CAC37510), *C. albicans* Sws1 (XP_712296), *A. thaliana* Sws1 (NP_567942), *M. musculus* SWS1 (CAI35328), *H. sapiens* SWS1 (XP_058899), *S. pombe* Rdl1 (CAB11212), *A. thaliana* RAD51D (Q9LQQ2).

Supplementary references

Craven, R.A., Griffiths, D.J., Sheldrick, K.S., Randall, R.E., Hagan, I.M. and Carr, A.M. (1998) Vectors for the expression of tagged proteins in Schizosaccharomyces pombe. *Gene*, **221**, 59-68.

Noguchi, E., Noguchi, C., Du, L.L. and Russell, P. (2003) Swi1 prevents replication fork collapse and controls checkpoint kinase Cds1. *Mol Cell Biol*, **23**, 7861-7874.