Supporting Information

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SI Materials and Methods

Cell Culture, Synchronizations, and Treatments. With the exception of Sf9 cells, which were cultured at 27 °C, all cells were cultured at 37 °C and in 5% CO_2 in DMEM (Gibco-BRL) supplemented with 10% FBS (Gibco-BRL), 2 mM L-glutamine (Gibco-BRL), 10,000 U/mL penicillin, and 10 mg/mL streptomycin (Gibco-BRL). Media of U2OS cells expressing GFP-chromatin assembly factor 1 (CAF-1) p150 and the 293Flp-In cell line expressing FLAG-CAF-1 p150 (1) were supplemented with 50 mg/mL G418. HEK-derived 293T Lα +/− cells were cultured as described (2). Sf9 cells were cultured in Grace's insect medium supplemented with 10% FBS (Sigma).

To synchronize cells in G1/S, 2 mM hydroxyurea (HU, Sigma, stock solution in water) was added to the culture media for 16 h. To inhibit methylguanine methyl transferase activity, the cells were pretreated with O^6-benzylguanine for 1 h prior to treatment with 10 μM *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) (Sigma, stock solution in DMSO) for 6 h.

Flow Cytometry. Cells were fixed in ice-cold 70% ethanol before staining with 50 mg/mL propidium iodide (Sigma Aldrich) in PBS containing 0.5 mg/mL RNase A (Amersham). DNA content was analyzed by flow cytometry using CyAn ADP Analyzer and Summit software (Beckman Coulter).

Immunofluorescence. Cells grown on coverslips were fixed in ice-cold 3.7% formaldehyde/PBS for 15 min at 4 °C. Fixation was followed by permeabilization in 0.2% Triton X-100/PBS for 5 min at 4 °C. Blocking was done for 10 min in 3% milk powder in PBS. Overnight incubation with the appropriate primary antibodies diluted in blocking buffer was followed by three washes in blocking solution. Secondary antibodies were used for 1 h. Three washes in PBS were followed by incubation in 0.5 mg/mL DAPI solution (Sigma) and a final rinse in H_2O. Coverslips were mounted onto slides using mounting media (Vector Laboratories).

Slides were observed using a Zeiss LSM710 confocal microscope using sequential scanning mode. Images were processed using ImageJ software.

Cell Extracts. Total cell extracts were prepared by lysing cells in Nonidet P-40 (NP-40) buffer [50 mM Tris·HCl pH 8, 150 mM NaCl, 1% NP-40, 1 mM EDTA, 1 mM PMSF, 1 mM Na_3VO_4, 1 mM NaF, 1× Complete (EDTA free, Roche)] for 1 h on ice followed by centrifugation for 15 min at 14,000 rpm in an Eppendorf centrifuge.

For cross-linking experiments, cells were suspended in 10 mM Pipes pH 8.8, 100 mM NaCl, 300 mM sucrose, 3 mM $MgCl_2$, 1 mM EGTA, 1 mM EDTA, 0.1% Triton X-100 1 mM PMSF, 1 mM Na_3VO_4, 1 mM NaF, 1× Complete for 15 min. One percent formaldehyde/PBS was then added. After 10 min, 100 mM glycine pH 8/PBS was added for 5 min. Cross-linked nuclei were lysed in 50 mM Hepes·KOH pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.1% Triton, 1 mM PMSF, 1 mM Na_3VO_4, 1 mM NaF, 1× Complete and sonicated twice for 8 s at 30% cycle. The lysate was cleared by centrifugation at 10,000 × *g* for 5 min.

Extracts for the supercoiling and mismatch repair assay are described in ref. 3.

Coimmunoprecipiations. Immunoprecipitations were carried out by incubating 1 mg of cell lysate with ProteinG Sepharose 4 Fast Flow (GE healthcare) and the respective antibody in 800 μL NP-40 lysis buffer (50 mM Tris·HCl pH 7.4, 150 mM NaCl, 1% NP-40, 1 mM EDTA, 1 mM PMSF, 1 mM Na_3VO_4, 1 mM NaF, 1× Complete) for 4 h at 4 °C. The immunocomplexes were washed four times with 800 μL of NP-40 lysis buffer, resuspended in 30 μL of 1× SDS loading buffer, and analyzed by Western blotting (WB).

Western Blotting Analysis. Following denaturating SDS-polyacrylamide gel electrophoresis, the proteins were transferred onto Hybond-P PVDF membranes (Amersham). The membranes were blocked with 5% low-fat milk in TBST (0.1% Tween20, 20 mM Tris·HCl pH 7.4, 150 mM NaCl). Incubation with the first antibody was followed by secondary HRP-conjugated IgG. All antibodies were diluted in 5% milk in TBST. The signals were visualized by ECL detection reagents (GE Healthcare).

Far Western Analysis. Protocol described in ref. 4.

Expression and Purification of Recombinant Proteins. ***GST-fragments.*** GST alone (pGEX-2TK), GST-MSH6 fusions (pGEX-2TK plasmids containing different MSH6 fragments) and GST-p150 fusions [pGEX-4T1 plasmids containing different p150 fragments (5)] were expressed in the *Escherichia coli* strain BL21. Cultures were grown at 37 °C with shaking at 250 rpm. Protein expression was induced at OD_{600} 0.6 by the addition of 0.5 mM IPTG at 18 °C for 16–18 h. Bacterial pellets containing the MSH6 fragments were lysed as described in ref. 6, whereas pellets containing the CAF-1 fragments were lysed in 20 mM Tris·HCl pH 8, 150 mM NaCl, 0.1% NP-40, 1 mM EDTA, 1 mM DTT, 0.1 mM PMSF, 10 mg/mL pepstatin and leupeptin followed by sonication for 5 min at 72% (10% cycle) and centrifugation for 5 min at 10,000 × *g*. Supernatants were incubated with Glutathione Sepharose 4 Fast Flow (GE healthcare). The bound GST-fragments were washed with 10 bead volumes of 20 mM Tris·HCl pH 8, 150 mM NaCl, 1 mM EDTA, 10% glycerol, 1 mM DTT, 0.1 mM PMSF, 10 mg/mL pepstatin and leupeptin. Twenty millimolar glutathione was used for elutions. The purified GST-fragments were dialyzed against 25 mM Hepes·KOH pH 7.6, 150 mM NaCl, 0.1 mM EDTA, 10% sucrose, 2 mM 2-mercaptoethanol and 0.1 mM PMSF.

*MutS*α and its variants were generated as described in ref. 7.

CAF-1 trimer and p150 alone. Sf9 cells were coinfected with viruses for his-p150, p60, and p48 or his-p150 alone (8, 9). After 48 h at 27 °C, the cells were harvested, swollen in hypotonic buffer (20 mM Hepes·KOH pH 7.5, 5 mM KCl, 1.5 mM $MgCl_2$, 1 mM DTT, 0.1 mM PMSF, 0.5 mg/mL leupeptin, 1 mg/mL pepstatin) for 20 min followed by Dounce homogenization (B pestle). After centrifugation at 10,000 rpm for 10 min, the pellet was resuspended in buffer A400 (25 mM Tris·HCl pH 7.5, 1 mM EDTA, 10% glycerol, 0.02% NP-40, 400 mM NaCl, 1 mM DTT, 0.1 mM PMSF, 0.5 mg/mL leupeptin, 1 mg/mL pepstatin), incubated for 20 min, and centrifuged again at 10,000 rpm for 20 min. The trimer was purified on a Ni-nitrilotriacetate (Ni-NTA) using 20 mM Tris·HCl pH 8, 150 mM NaCl, 1 mM EDTA, 10% glycerol, 1 mM DTT, 0.1 mM PMSF, 0.5 mg/mL leupeptin, 1 mg/mL pepstatin as buffer and 300 mM imidazole for elution. The purified trimer was dialyzed against buffer A150 (25 mM Tris·HCl pH 7.5, 1 mM EDTA, 10% glycerol, 0.02% NP-40, 150 mM NaCl, 1 mM DTT, 0.1 mM PMSF).

pET30a-hp150. The his-tagged CAF1-p150 (10) was expressed in the *E. coli* strain Rosetta 2 (DE3, Novagen). Cultures were grown

at 37 °C with shaking at 250 rpm. Protein expression was induced at OD_{600} 0.6 by addition of 0.4 mM IPTG at 30 °C for 3 h. The bacterial pellet was lysed in 20 mM Tris·HCl pH 8, 150 mM NaCl, 0.05% NP-40, 0.2 mM PMSF, 1 mg/mL pepstatin and leupeptin and lysosyme. Sonication for 3 min at 10% was followed by centrifugation for 10 min at 10,000 rpm. The supernatant was stored at −80 °C. The lysate was centrifuged for 30 min at 14,000 rpm and incubated with Ni-NTA beads. Bound CAF1-p150 was washed with 10 beads volumes of 50 mM Tris·HCl pH 8, 250 mM NaCl, 0.05% NP-40, 0.2 mM PMSF, 1 mg/mL pepstatin and leupeptin, 20 mM imidazole; and 250 mM imidazole was used for elution. Purified his-tagged CAF1-p150 was dialyzed against 20 mM Hepes·KOH pH 7.8, 5 mM potassium actetate, 0.5 mM $MgCl_2$, 50 mM NaCl, 0.1 mM PMSF.

Dephosphorylations. Proteins were treated with λ-protein phosphatase (λ-PPase) for 30 min at 30 °C using 1× λ-PPase reaction buffer and 2 mM $MnCl_2$ provided by the supplier (New England Biolabs). To inhibit the λ-phosphatase activity, 5 mM p-nitrophenyl phosphate and 0.5 mM sodium orthovanadate were added.

Statistical Analyses. Quantification of supercoiling and DNA mismatch repair efficiencies was carried out using ImageQuant TL software. Supercoiling represents the number of all topoisomers (excluding the open/nicked forms Ir/II) relative to the total amount of DNA based on the ethidium bromide (EtBr)-stained gels. Error bars represent standard deviation of three independent experiments.

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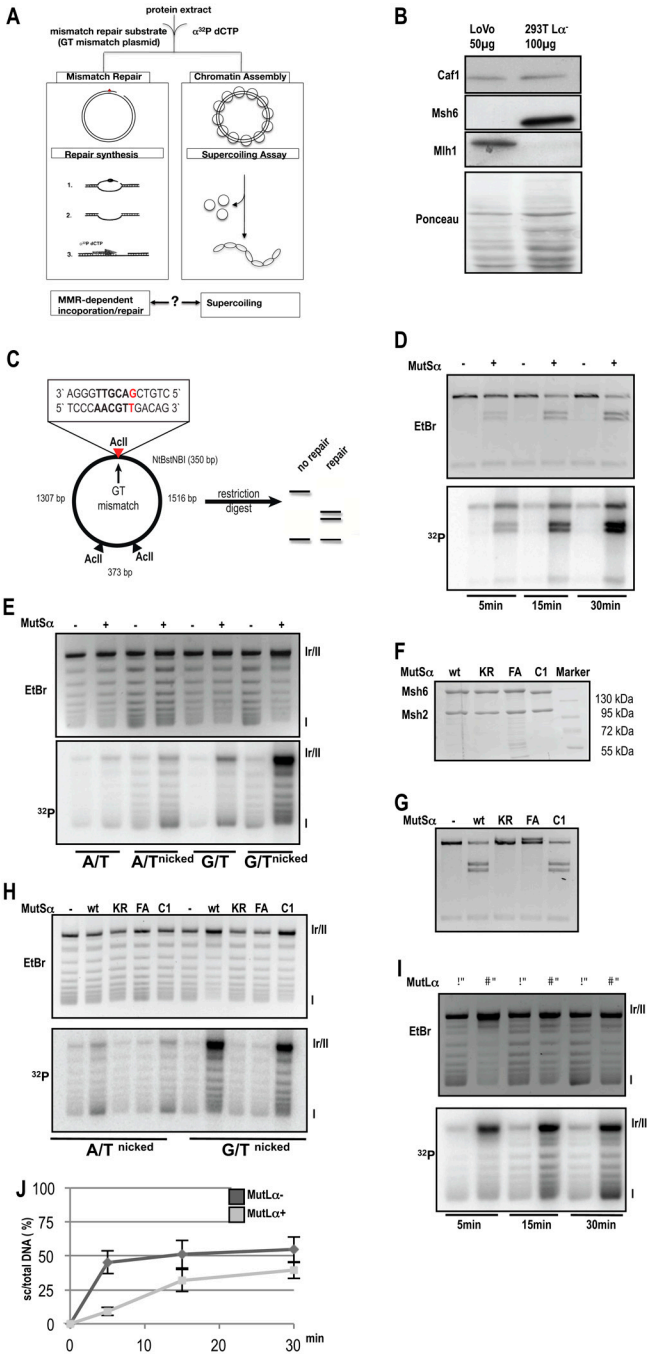

Fig. S1. (*A*) To study the influence of mismatch repair (MMR) on chromatin assembly, an in vitro system was sought that allows the study of MMR and chromatin assembly in a single assay. In the scheme shown in this figure, MMR efficiency was assessed by a restriction enzyme-based assay and the incorporation of a radiolabeled nucleotide, whereas the supercoiling assay, which gave information about chromatin assembly, was followed by agarose gel electrophoresis and autoradiography. (*B*) Western blotting of the extracts used in this study. LoVo extracts lack MutSα, whereas 293T-Lα^{-} extracts lack MutLα. The amounts were adjusted to contain comparable amounts of CAF-1. (*C*) Schematic representation of the G/T substrate. The three *Acl*I restriction sites and the restriction pattern before and after repair are indicated. The *Acl*I restriction site indicated by an arrow contains the G/T mismatch, which renders the site refractory to cleavage. Repair of the G/T mismatch to A/T restores the bona fide *Acl*I site. (*D*) Kinetics of G/T to A/T repair of a G/T^{nicked} substrate incubated with LoVo extracts supplemented (+) or not (–) with purified recombinant MutSα. The EtBr-stained agarose gel (*Upper*) and the corresponding autoradiogram (^{32}P, *Lower*) after digest with *Acl*I are shown. (*E*) Representative example of supercoiling assay of A/T, A/T^{nicked}, G/T, or G/T^{nicked} substrates after incubation with LoVo extracts supplemented (+) or not (–) with purified recombinant MutSα. The EtBr-stained agarose gel (*Upper*) and the corresponding autoradiogram (^{32}P, *Lower*) are shown. (*F*) Coomassie blue-stained SDS-PAGE showing the relative amounts of the MutSα wild type or its variants KR, FA, and C1 used to complement the LoVo extracts. (*G*) G/T to A/T repair of a G/T^{nicked} substrate incubated with LoVo extracts supplemented (+) or not (–) with purified recombinant MutSα, either wild type, or its KR, FA, or C1 variants. The figure shows an EtBr-stained agarose gel after digest with *Acl*I. Quantitation shows repaired DNA relative to the total DNA as a percentage, where A/T and A/T^{nicked} were set to 100%. Three independent experiments were used to evaluate the standard deviation. (*H*) Representative supercoiling assay of A/T^{nicked} and G/T^{nicked} substrates after incubation with LoVo extracts supplemented with purified recombinant MutSα, either wild type or its variants. EtBr-stained DNA gel (*Upper*) and its autoradiogram (*Lower*) are shown. (*I*) Kinetics of supercoiling of the G/T^{nicked} substrate upon incubation with MMR-deficient extracts of 293T Lα^{-} cells, supplemented (+) not (–) with purified recombinant MutLα. A representative EtBr-stained DNA gel (*Upper*) and its autoradiogram (*Lower*) are shown. (*J*) Quantitation of the experiment described in *I* above. The data were collected from three independent experiments. Error bars represent standard deviation from the mean.

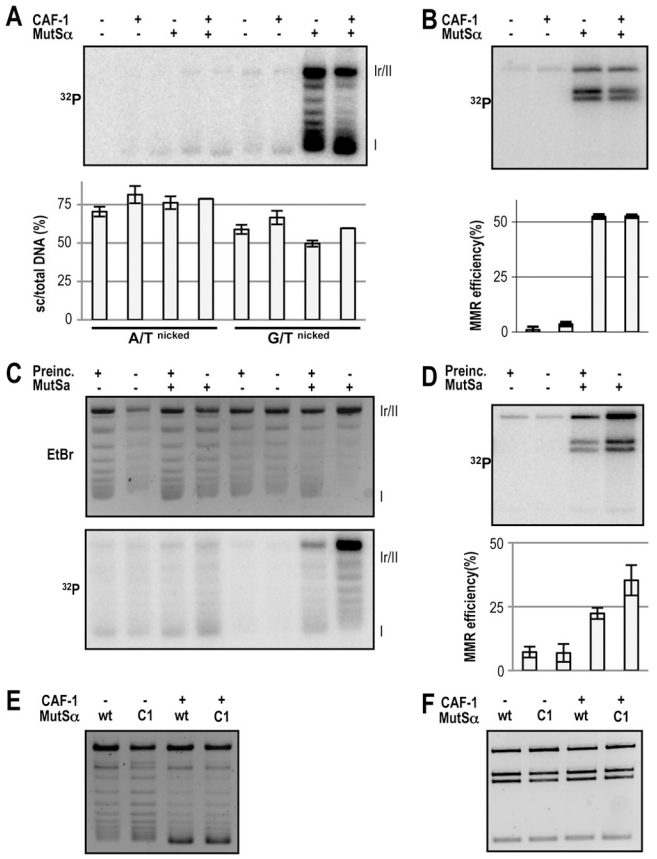

Fig. S2. (*A*) Representative supercoiling assay of a G/T^{nicked} substrate upon incubation with LoVo extracts supplemented (+) or not (–) with recombinant MutSα and/or CAF-1 as indicated. The autoradiogram (*Upper*) of Fig. 2*A* is shown. Quantitation addresses the relative percentage of all topoisomers versus total DNA. Data from three independent experiments are shown. The error bars represent the standard deviation from the mean (*Lower*). (*B*) G/T to A/T repair of a G/T^{nicked} substrate incubated with LoVo extracts supplemented (+) or not (–) with purified recombinant MutSα and/or CAF-1. The figure shows an autoradiogram of an agarose gel. (*Lower*) Quantitation of the above experiment. The data are from three independent experiments. Error bars represent standard deviation from the mean. (*C*) Representative supercoiling assay of a G/T^{nicked} substrate upon incubation with LoVo extracts supplemented (+) or not (–) with recombinant MutSα. Where indicated (+), the substrate was preincubated with the extract for 10 min prior to the addition of MutSα. EtBr-stained DNA gel (*Upper*) and its autoradiogram (*Lower*) are shown. (*D*, *Upper*) G/T to A/T repair of a G/T^{nicked} substrate incubated with LoVo extracts supplemented (+) or not (–) with purified recombinant MutSα. Where indicated (+), the substrate was preincubated with the extract for 10 min prior to the addition of MutSα. The figure shows an autoradiogram of an agarose gel. (*Lower*) Quantitation of the above experiment. The data are from three independent experiments. Error bars represent standard deviation from the mean. (*E*) Representative supercoiling assay of a G/T^{nicked} substrate upon incubation with LoVo extracts supplemented with recombinant MutSα wild type or C1 mutant and/or CAF-1 as indicated. The figure shows an EtBr-stained agarose gel. (*F*) G/T to A/T repair of a G/T^{nicked} substrate incubated with recombing MutSα wild type or C1 mutant and/or CAF-1 as indicated. The figure shows an EtBr-stained agarose gel.

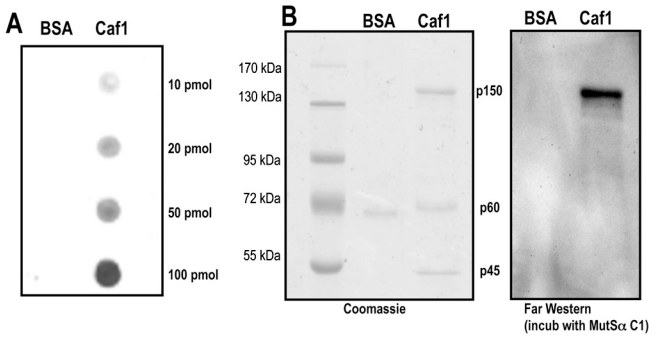

Fig. S3. (*A*) Far Western analysis 1. The indicated amounts of the recombinant purified CAF-1 heterotrimer were spotted onto a membrane. The membrane was blocked and subsequently incubated with purified recombinant MutSα. Hybridization with an anti-MSH6 antibody showed that CAF-1 interacts with MutSα directly. BSA was used as the negative control. (*B*) Far Western analysis 2. The three subunits of the recombinant purified CAF-1 heterotrimer were separated on SDS-PAGE and electrotransferred onto a membrane. The membrane was blocked and subsequently incubated with purified recombinant variant C1 of MutSα. Hybridization with an anti-MSH6 antibody showed that MutSα lacking the N-terminal 80 amino acids of MSH6 containing the PIP motif still interacts directly with the 150 kDa subunit of CAF-1. BSA was used as the negative control.

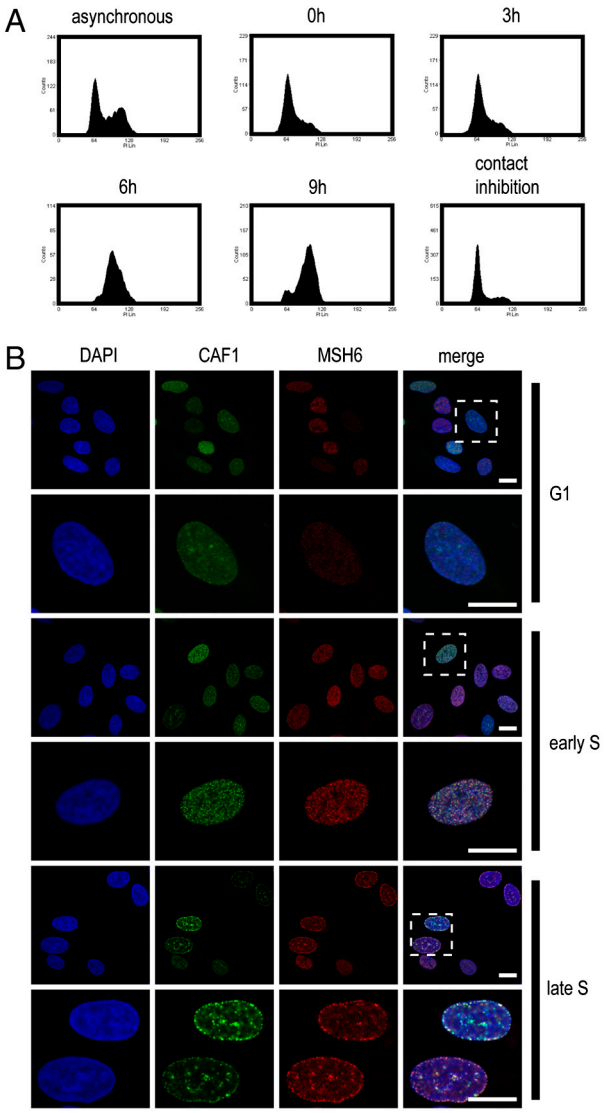

Fig. S4. (*A*) U2OS cells were synchronized with 2 mM hydroxyurea (HU). FACS analyses were carried out at the indicated time points after release. Asynchronous- and contact-inhibited cells were used as controls. (*B*) HU synchronized U2OS cells stably expressing GFP-p150 were stained 6–9 h post release with an anti-MSH6 (red) antibody. In the merged pictures, colocalization appears yellow (relates to Fig. 4).