Supplementary Information for
Mechanism of Bloom Syndrome complex assembly required for
double Holliday junction dissolution and genome stability.

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Supplementary Materials and Methods

**MultiBAC design, insect cell expression and protein purification**

The BTRR complex and derivatives were cloned into recombinant BACmIDs using the Multibac system (1). Flag-BLM and MBP-BLM were cloned into pFL. BLM-8HIS and TopoIIIα were cloned into pUCDM to create pUCDM-BT. We cloned RMI1 fused with an N-terminal maltose binding protein (MBP) tag and PreScission protease site, and untagged RMI2 into pFL to create pFL-RR. pUCDM-BT and pFL-RR were combined together using Cre recombinase (NEB) to create pFLUCDM-BTRR. These plasmids were transferred by Tn7 mediated transposition into Multibac competent cells. Recombinant BACmids were used to generate baculovirus by standard procedures.

1L High 5 *Trichoplusia ni* cells (1 x 10⁶/ml, Invitrogen) were infected with BTRR (MOI=2.5). After 72 hours, cells were harvested, and resuspended in 3 pellet volumes of hypotonic buffer (20mM Triethanolamine (TEA) pH7.5, 10% glycerol, 75mM NaCl, complete protease inhibitors (Sigma), 1mM EDTA, 1mM DTT), swollen on ice for 20 minutes and lysed with 40 strokes in a dounce homogeniser (pestle A). NaCl levels were increased to 400mM followed by 40 more strokes in dounce using pestle B. Lysates were centrifuged twice at 35,000xg in a T70.1 rotor in a beckman ultracentrifuge, 4°C. Using an AKTA FPLC, supernatant was passed over a 5ml amylose resin column (NEB) followed by extensive washing with buffer A (20mM TEA pH7.5, 10%glycerol, 400mM NaCl). Protein was eluted with 10ml of buffer A + 20mM maltose. Imidazole was added to 10mM and peak fractions were loaded on a 1ml TALON column (Clontech). Bound proteins were washed extensively and then eluted with buffer A + 350mM imidazole. Peak fractions were pooled and dialysed against buffer A for 4 hours. Samples were then either used as is or further purified using 15-35% glycerol gradient centrifugation, 30,000 rpm, 16hr, 4°C, SW30 rotor, in a Beckman ultracentrifuge, or Superdex 200 5/150 GL size exclusion chromatography to remove protein aggregates. Approximate yields of 1-3mg of recombinant BTRR were obtained from 1L of insect cell culture.

SDS-PAGE was performed using the NuPAGE system (Invitrogen) and MOPS buffer. Native PAGE was performed using the Invitrogen Native-Blue system. Gels were stained with colloidal coomassie blue or transferred to immobilon-P (Millipore). The following antibodies were used for Western blotting: goat a-BLM (C19, Santa cruz biotech), rabbit a-TopoIIIα (2), mouse a-RMI1 (B02, Abnova), rabbit a-RMI1 (Proteintech), rabbit anti-Flag (Aviva), mouse anti-Flag (M2, Sigma Aldrich) and mouse a-RMI2 (3).

**Cloning, bacterial expression and purification of isolated BLM N-terminus**

BLM 1-433 and patch mutant were synthesized by Gene Universal (Delaware USA) in the plasmid pMAL-C5x (New England Biolabs). Patch-mutant is encoded be sequence that alters the following underlined residues to alanines: KLIDTIPDDKLLDCGNELLQQRNIRRKLT. The MBP-tagged WT or patch mutant sequence was then subcloned into pFL-BLM to create the MBP-tagged full length versions of these proteins.

MBP-tagged BLM (residues 1-163 or 1-433) were purified from 1L of *Escherichia coli* strain BL21 (DE3) induced with 0.5mM IPTG for 3 hours at 30°C in Luria broth media. Cells were harvested by centrifugation and lysed by sonication in 20ml of 20mM Tris-HCl pH 8, 150mM NaCl, 10% glycerol, 5mM TCEP, 1mM PMSF and 1mM EDTA. To remove any insoluble materials, the cell lysate was centrifuged at 15,000 g for 30 min and the supernatant fraction was filtered before incubating with Amylose resin (NEB E8021S) for 45 min. The resin was washed with lysis buffer and the bound protein eluted with 20mM Maltose, 20mM TrisHCl pH 8, 150mM NaCl, 10% glycerol, 1mM DTT and loaded onto a 1ml Mono Q column. Bound protein was eluted with a gradient (50-1000mM NaCl). Fractions containing the protein were identified by SDS-polyacrylamide gel electrophoresis, flash frozen and stored at -80°C.
Cloning, bacterial expression and purification of isolated RMI1 N-terminus

Human RMI1 N-terminus (residues 1-213) was cloned in a pET28b His-SUMO plasmid. N-terminally hexa-histidine and SUMO tagged RMI1(1-213) was purified from 5L of Escherichia coli strain BL21 (DE3) induced with 0.3mM IPTG for 5 hours at 25°C in 2xTY media (+50mM kanamycin). Cells were first harvested by centrifugation (4,200 RPM, 4°C, 45 min), then the supernatant was decanted and cells were washed in MilliQ grade water before flash freezing. Cell pellets were thawed and lysed by sonication in lysis buffer (50mM Tris-HCl pH 8, 400mM NaCl, 10% glycerol, 2mM b-mercapto ethanol, 1mM PMSF and 1x Roche Complete Protease inhibitor). To remove any insoluble materials, the cell lysate was centrifuged at 15 000 g for 1hr and the supernatant fraction was filtered before incubating (end-over-end mixing, 2 hours, 4°C) with Ni-NTA resin (Qiagen) pre-equilibrated in lysis buffer followed by washing with 100 column volumes of lysis buffer. Elution was performed in 10 column volumes of elution buffer (25mM Tris-HCl pH=8.0, 400mM NaCl, 2mM b-mercapto ethanol, 400mM imidazole and 1x Roche Complete Protease inhibitor). Cleavage of the N-terminal His-SUMO tag was performed by addition of 70 μl of ULP1 protease (10 mg/ml). To remove imidazole, the sample was loaded onto a 10K MWCO Slide-a-Lyzer dialysis cassette (Thermo Scientific) and dialysed overnight in dialysis buffer (25 mM Tris HCl pH 8.0, 200 mM NaCl, 5% glycerol). The dialysed digested sample was collected and loaded on Ni-NTA resin (Qiagen) pre-equilibrated in dialysis buffer for the removal of the cleaved His-SUMO tag, uncleaved His-SUMO-RMI1(1-213) and the ULP1 protease (which bears an N-terminal His-tag). The untagged or the His-SUMO tagged RMI1(1-213) were collected and concentrated using a Vivaspin 6 column (MWCO 10 kDa) (GE Healthcare). The concentrated sample was purified by size-exclusion chromatography using a Hi-Load Superdex 75 PG 16/600 operating on an ÄKTA Purifier FPLC system (GE Healthcare) and running at 0.35 ml/min in 25 mM Tris HCl pH 8.0, 150 mM NaCl and 1 mM TCEP. Fractions containing the protein were identified by SDS-polyacrylamide gel electrophoresis, flash frozen and stored at -80°C.

Protein analysis:

Size Exclusion Chromatography and Multi Angle Light Scattering (SEC-MALS)

Purified proteins and complexes were separated on an Zenix-C SEC-300 column (Sepax) in (50mM Tris pH 7.5/250mM NaCl/3mM DTT at 0.35 ml/min) attached to a Shimadzu chromatography system. A Dawn Helios II Multi-Angle Light Scattering detector and an Optilab rEX Refractive Index detector recorded the light scattering and refractive index of the samples upon elution from the size exclusion column. The Wyatt software ASTRA was used to analyse the data collected. Standards used for size estimations are the same as in (4).

Crosslinking-mass spectrometry (XL-MS)

For each crosslinking experiment, ~25–75 µg of BS complex at a concentration of ~0.15–0.4 mg/mL was used. The purified samples were then prepared for XL-MS essentially as described previously (5), using the H12/D12-disuccinimidyl suberate (DSS) crosslinker. For LC-MS/MS, peptides were resuspended in 3% (v/v) acetonitrile, 0.1% (v/v) formic acid and loaded onto a 20 cm x 75 µm inner diameter column packed in-house with 1.9-µm C18AQ particles (Dr Maisch GmbH HPLC) using an Easy nLC-1000 nanoHPLC (Proxeon). Peptides were separated using a linear gradient of 5–30% Buffer B over 120 min at 200 nL/min at 55°C (Buffer A = 0.1% (v/v) formic acid; Buffer B = 80% (v/v) acetonitrile, 0.1% (v/v) formic acid). Mass analyses were performed using a LTQ-Orbitrap Velos Pro mass spectrometer (Thermo Scientific). Following each full-scan MS1 at 30,000 resolution at 200 m/z (300–1700 m/z; 1×10^6 AGC; 250 ms injection time), up to 10 most abundant precursor ions were selected for MS/MS either using CID activation (≥10,000 counts; 2 m/z isolation; 10 ms activation time; activation q = 0.25; NCE = 35; minimum charge state of +3; repeat count 1; dynamic exclusion of 60 s) or HCD activation (100–2000 m/z; 7,500 resolution; 250 ms injection time; NCE = 28; 2.5 m/z isolation window; minimum charge state of +3; repeat count 1; dynamic exclusion of 60 s). Peak lists were generated using the msConvert tool (6) and submitted to the database search program Mascot (Matrix Science). The data was searched with oxidation (M) and carbamidomethyl (C) as variable modifications using a precursor-ion and product-ion mass tolerance of ±15 ppm and ±0.6 Da (±0.02 Da for HCD data), respectively.
The enzyme specificity was trypsin with up to 3 missed cleavages and all taxonomies in the Swiss-Prot database (October 2014; 546,790 entries) were searched. A decoy database of reversed sequences was used to estimate the false discovery rates. To be considered for further analysis, identified peptides had to be top-ranking and statistically significant ($p < 0.05$) according to the Mascot expect metric.

**Analysis of XL-MS data**

Analysis of the XL-MS data was performed with both the xQuest and pLink software (7, 8). In both cases, the protein database used consisted of all the MBP-BS complex components (5 proteins) plus the top 20 contaminants identified (from the Mascot search) in the samples. xQuest search parameters that differ from the default settings: minimum of 4 residues per peptide chain, charge states of +2 to +8 were considered, precursor mass tolerance ±15 ppm, oxidation (M) was set as a variable modification and up to two were allowed, carbamidomethyl (C) was set as a fixed modification, enzyme specificity was trypsin up to two missed cleavages were allowed, search was done in ion-tag mode. pLink search parameters that differ from the default settings: precursor mass tolerance ±15 ppm, product-ion mass tolerance ±20 ppm, variable modifications of oxidation (M) and carbamidomethyl (C), enzyme specificity was trypsin up to two missed cleavages per chain (excluding the site of crosslinking). For the crosslinker settings: crosslinking sites were K and protein N-terminus, isotope shift 12.075 Da, xlink mass-shift 138.068 Da, monolink mass-shift 156.079 Da. For xQuest, only inter-protein and intra-protein crosslinked peptides with the linear discriminant (ld) score of >19 and more than >25, respectively, were considered for further analysis. At these cut-off ld scores, the false discovery rate (FDR), estimated by a target-decoy approach, were determined to be <1% for both the inter-protein and intra-protein crosslinked. For pLINK, the default FDR of 5% was used and only peptides with scores ≤ 1 × 10^{-4} were considered for further analysis. Finally, the spectra of all reported crosslinks were also manually verified. Only crosslinks with at least four fragment ions on both peptide chains addressing the most abundant peaks in the spectrum were retained. This final list of verified crosslinks can be found in **Supplementary Dataset 1**. All raw mass spectrometry data (including spectra) and search results have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (9) with Accession number PXD011078. Crosslinks were mapped onto 2 dimensional plots using xVis (10).

**Modeling of BLM dimer**

Two molecules of BLM (PDB: 4O3M) were initially docked using Zdock (11) with XL-MS identified crosslinks selected as “contacting residues”. The top 10 predictions were then visually analysed for solutions which fit the crosslink data, and which formed a cluster. The best solution (i.e. highest scoring of the cluster) was re-docked using Rosetta Dock (12) and crosslinks were mapped for visualization to monomer BLM or model dimer BLM using xLink analyzer plugin for UCSF Chimera (13).

**DNA binding and activity assays**

Synthetic oligonucleotide structures were generated as previously described (4). 0.2ng DNA substrates were incubated with increasing amounts of recombinant TopoIIa:RM11:RM2 or BS complex at room temperature for 20 minutes in 1x binding buffer (6mM Tris pH7.5, 75mM NaCl, 12% glycerol, 0.1mM EDTA, 1mM DTT). Reactions were loaded on 5, 6 or 8% acrylamide gels prepared with 0.5xTBE (45 mM Tris-HCl, 45 mM Boric Acid, 1 mM EDTA) and 2.5% glycerol. Samples were run in 0.5xTBE 10V/cm for 90 minutes before being dried between cellophane sheets. Bands were revealed by exposure to film at -80°C.

The ideal double Holliday junction (ideal dHJ) was generated from 4 ssDNA circles as previously described (14). Briefly pDHJS AS+, pDHJS AS-, pDHJS BS+, and pDHJS BS- were transformed into *E.coli strain* BioBlue (Bioline) and phagemids generated by use of M13KO7 helper phage (New England Biolabs). ssDNA was purified using Qiagen Q20 tips and a modified manufacturer recommended protocol for ssDNA purification. Plus and minus strands were annealed and linked together using *Archaeoglobus fulgidus* reverse gyrase purified as described (plasmid for expression kindly provided by Daniela Stock) (15). AN+ ssDNA was linked with BN-, and BN+
ssDNA was linked with AN- to create the first intermediate which were subsequently linearized with Xho1 restriction endonuclease (New England Biolabs). The digested DNAs were then dialyzed into Cre reaction buffer (50 mM Tris, pH 7.5, 33 mM NaCl, 10 mM MgCl2, 1 mM dithiothreitol) and incubated with an empirically determined amount of inhouse purified Cre recombinase at 37 °C for 30 min. The Cre enzyme was then heat-inactivated, and the reactions were cleaned up and concentrated over Qiagen Q20 columns, following manufacturer instructions. These second intermediates (small heterodimers) were then gel-purified from the other Cre reaction products, by a crush, freeze and squeeze method, including centrifugation through Biorad mini-columns. After quantitation, the AN+/BN- and BN+/AN- small heterodimers were annealed and linked with reverse gyrase as described earlier, and then treated with wheatgerm Topoisomerase 1 (Promega) for 10 mins to remove residual supercoiling. This reaction was stopped by addition of EDTA to 12mM, SDS to 0.5% and proteinase K followed by incubation at 37°C for ~30 minutes. The DNA was cleaned up using 1 x phenol/chloroform purification and 1x chloroform purification in a PhaseLock tube (5 Prime) followed by ethanol/NaAc precipitation in the presence of glycogen as a carrier. The integrity of the purified ideal dHJ was confirmed by treatment with restriction endonucleases.

To assay dHJ dissolution, TRR or BS complex or BLM-heterodimer +TRR was incubated with 0.5ng of ideal dHJ in 1x reaction buffer (40mM Tris 7.5, 0.1mM EDTA, 4mM MgCl2, 50mM sodium acetate ±1mM ATP). 16 µl reactions were incubated at 37°C for 30 mins (or indicated times), before addition of 4 µl reaction volume of 5x stop buffer (100mM Tris-HCl (7.5), 100mM MgCl2, 3% SDS, 10mg/ml Proteinase K). Reactions were run for 100 mins at 7V/cm in 1.5% or 1.8% agarose gels prepared in 1x TBE, and post stained with 1x SYBR gold (Invitrogen).

FRET-based DNA unwinding assays were performed as previously described (16).

**Peptide arrays**

Peptide arrays were made from 20mer peptides derived from the N-terminal half of BLM (residues 1-655). Starting from the N terminus, each 20mer peptide in the array was advanced from the previous one by 1 residue in the C-terminal direction. Peptides were synthesized on cellulose membranes using an Intavis Multipep Peptide Synthesizer (Intavis Bioanalytical Instruments AG, Cologne Germany) at the Francis Crick Institute Peptide Chemistry Service. The peptides were synthesized using 9 fluorenylmethyloxycarbonyl for temporary α-amino group protection. Protecting groups used are Pbf (2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl) for arginine, OtBu (t-butyl ester) for glutamic acid and aspartic acid, Trt (trt) for asparagine, glutamine, histidine, and cysteine tBu (t-Butyl) for serine, threonine and tyrosine and Boc (t-butoxycarbonyl) for lysine and tryptophan. Each amino acid was coupled by activating its carboxylic acid group with diisopropylcarbodiimide (DIC) in the presence of hydroxybenzotriazole (HOBT). Individual aliquots of amino acids were spotted on to a cellulose membrane which has been derivatised to have 8 to 10 ethylene glycol spacers between the cellulose and an amino group. Synthesis was accomplished by cycles of coupling of amino acids, washing then removal of the temporary α-amino protecting group by piperidine followed by more washing. Once the required number of cycles of coupling and deprotection and washing had been completed, the membranes were treated with a solution of 20 ml containing 95% trifluoroacetic acid, 3% trisopropylsilane and 2% water for four hours. Following this treatment membranes were washed 4 times with dichloromethane, 4 times with ethanol, and twice with water to remove side chain protecting groups and TFA salts and once again with ethanol for easier drying. Membranes were allowed to dry then stored at -20 deg C before use. The membranes were activated in 50% methanol before blocking in 4% milk powder in 100mM NaCl, 20mM Tris HCl pH7.5, 0.1% Tween 20 (TBS-T). The BLM membranes were then incubated with His-SUMO-tagged RMI1 (residues 1-213) in 1% milk in TBS-T overnight, and bound protein was detected by incubation with anti-RMI1 polyclonal antibody (Novus NBP158133) and chemiluminescence. Subsequently, the membrane was imaged under UV transillumination to reveal the exact location of each peptide.

**Fluorescence polarization experiments**

Fluorescence anisotropy experiments were performed using a PHERAstar FS plate reader (BMG Labtech) equipped with a fluorescence polarization optic module with an excitation of 485 nm and
emission of 520 nM. Measurements of fluorescence anisotropy were acquired at 25 °C using 384-wells, low flange, NBS plates (Corning). Experiments were performed as follows, 60μl of purified His<sub>6</sub>-SUMO-RM1 (residues 1-213) at starting concentration of 200μM in Buffer FP (25mM TRIS HCl pH = 8.0, 250mM NaCl, 1mM DTT), were serially diluted (1:2 dilutions in Buffer FP) and immediately added to 10μl of labeled BLM<sub>27-46</sub> peptide (fluorescein iso-thiocyanate (FITC)-aminohexanoic acid (eahx) spacer -LSKPKFSG<sub>46</sub>KKKTSSDDN-CONH<sub>2</sub> or FITC-eahx-LSKPKFSG<sub>GGG</sub>KKKTSSDDN-CONH<sub>2</sub>) at starting concentration of 200nM in buffer FP. Reaction mix was incubated for 15min prior to FP measurements.

**Cell-lines**

All cells were grown in humidified incubators supplied with 5% CO<sub>2</sub> and maintained at 37 °C, with regular testing to verify mycoplasma-free status using a LookOut Mycoplasma PCR Detection Kit (Sigma-Aldrich). 293FT cells were obtained from Thermo Fisher Scientific and cultured in DMEM supplemented with 10% foetal calf serum (FCS; Thermo Fisher Scientific), 2 mM glutamine (Lonza), 1% MEM non-essential amino acids (Thermo Fisher Scientific) and 500 µg/ml Geneticin (G418; Thermo Fisher Scientific). RPE-1 cells (CRL-4000) were purchased from the ATCC, and cultured in Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific) supplemented with 10% FCS and 100 u/ml penicillin/100 mg/ml streptomycin (Lonza). RPE-1 BLM<sup>+</sup> cells expressing GFP or GFP-BLM were generated as described previously (17). Briefly, RPE-1 cells were incubated in lentiviral media for 48 hr, before selection in fresh medium containing 500 µg/ml G418 for up to 7 days. GFP-positive cells were sorted by FACS to generate stable lines for further study, followed by verification by western blotting using the following antibodies at the indicated dilutions: BLM (A300-110A, Bethyl Laboratories, 1/2000), GFP (11814460001, Roche, 1/5000), RMI1 (NB100-1720, Novus Biologicals, 1/1000), RPA2 (ab10359, Abcam, 1/10,000) and TOP3A (14525-1-1AP, Proteintech, 1/1000). Sister chromatid exchange assays were performed and scored as previously described (17). Images were acquired using a ZEISS Axio Observer Z1 widefield microscope fitted with an Axiocam MRm camera and a Plan-Apochromat 100x/1.4 oil objective, running on ZEN Pro 2012 software.

**MUS81-GEN1 knockdown experiments and measurement of segmented chromosomes**

siRNAs were transfected using Lipofectamine RNAiMAX (Thermo Fisher Scientific) according to the manufacturer's instructions. The following siRNAs were used: siCtrl (targeting firefly luciferase, with sequence 5'-CGUACCGGAUACUUCGA-3'), and siPOOLs targeting MUS81 and GEN1 from siTOOLs Biotech. Knockdown was determined at 48 hr post transfection by Western blotting using anti-MUS81 (sc-53382, Santa Cruz Biotechnology, 1/200) or anti-GEN1 (18); 1/500). Clonogenic survival was determined by growing cells for up to 14 days followed by staining with Coomassie Brilliant Blue R 250 as described previously (Shorocks et al., Nat. Commun. 2021). Images of segmented chromosomes were acquired using a ZEISS Axio Observer Z1 widefield microscope fitted with an Axiocam MRm camera and a Plan-Apochromat 100x/1.4 oil objective, running on ZEN Pro 2012 software. Segmented chromosomes were scored as per (19).

**Co-immunoprecipitation experiments**

Flag-tagged Topoisomerase III<sub>α</sub> or RMI1 and RMI1-fragments A-E are cloned into pDEST-FRT/3xFlag by Gateway cloning from pDONR221-entry vectors (described in Deans and West, 2009(20)). Topo III<sub>α</sub> fragments A-G were generated by inverse PCR and relegation in pDONR221-Topo III<sub>α</sub>. Flag III<sub>α</sub> fragments Z1-Z4 were cloned by regular PCR into pDONR221 and then transferred to pDEST-FRT3xFlag by Gateway cloning (Invitrogen). 1 μg of each plasmid (or appropriate controls) was transfected using Fugene HD at a 2.5:1 ratio into 2x10<sup>6</sup> cells HEK293 cells plated in 10cm dishes. Two days later, cells were lysed for 30 min at 4°C in buffer K100 (50 mM KPO4 [pH 7.5], 100 mM NaCl, 10% glycerol, 1 mM DTT, 1 mM MgCl<sub>2</sub>, 0.1% Triton X-100, 5 mM NaF, complete protease inhibitor) containing 50 U/ml benzonase (Promega). The NaCl concentration was increased to 250 mM, and the lysates were cleared at 16,000 × g for 30 min. The supernatant was taken and used for immunoprecipitation with 25 μl of Flag M2 agarose (Sigma). After 3 hr mixing, beads were washed four times with K250 and once with 50 mM NH4(CO<sub>3</sub>)<sub>2</sub> and were eluted with 500 mM NH4OH (pH 11.0), 0.5 mM EDTA. Samples were dried in...
a speedvac and resuspended in 1 x LDS loading buffer (Invitrogen). Western blotting was performed using the following primary antibodies: from HRP-conjugated α-FLAG-M2 from Sigma, α-BLM (C-18) from Santa Cruz, α-RMI2 (Xu et al., 2008), α-RMI1 (14630-1-AP) and α- Topo IIIα (14525-1-AP) from Proteintech. HRP-coupled α-goat, or protein A were used as secondary detection reagents.

For GFP-trap experiments (Figure 7), plasmids were transfected into 293FT cells using Lipofectamine 2000 (Thermo Fisher Scientific) according to the manufacturer’s instructions. For preparation of lysates for immunoprecipitations (IPs), cells were washed in phosphate-buffered saline (PBS), and lysed in IP buffer (150 mM NaCl, 100 mM KCl, 1 mM EDTA, 0.2% Igepal CA-630, 10% glycerol, 5 mM NaF, 20 mM HEPES-KOH, pH 7.5), supplemented with cOmplete EDTA-free protease inhibitor cocktail. Lysates were sheared by 5 strokes of a 19G needle, cleared by centrifugation, and were then incubated with 15 µl of GFP-Trap magnetic agarose beads (ChromoTek) for 2 hr with end-to-end mixing at 4 °C. Immunoglobulin-antigen complexes were washed five times with IP buffer before elution in 2X SDS sample buffer for SDS-PAGE.
Supplemental Figure S1: Location of monolinks (red lines) and “within peptide” crosslinks (orange lines) are evenly distributed across BS complex components. Color coding of domains as per Figure 1.
Supplemental Figure S2: a) Cartoon of BLM -dimer (brown) overlayed on RECQ1 dimer (2WWY, cyan). b-c) alternative cartoon overlayed with surface views of RECQ1 and BLM dimers showing RecA1 (royal blue), RecA2 (red) of first monomer, and RecA1’ (dim blue) and RecA2’ (orange-red) domains of second monomer. RQC and HRDC domains have been dimmed to white. DNA is shown from crystal structures in black. d) EM images of BLM “hexamer” from Karow et al, 1999 can be reinterpreted as dimers, with similarity to BLM model (dotted outline, right) shown directly above in c.
Supplemental Figure S3: control experiments that confirm dimerization or protein:protein interactions are not mediated by the SUMO or MBP tags attached to RMI1 or BLM fragments used in Figures4f or 5e of the main text.

a) SEC comparing MBP-BLM (dark blue) and MBP-PP-BLM untreated (light blue) or treated (pink) with Prescission protease to cleave between the tag and the BLM fragment. A small amount of monomer and multimer are revealed post cleavage (fractions 4 and 1/2 respectively) but the predominant peak remains a dimer form (fraction 3).

b) Nickel binding and elution of HIS-RMI(1-213) shows that it binds to BLM N-terminus after cleavage of MBP tag. MBP tag does not co-elute, and SUMO tag on RMI1 used in main Figure 5e is not necessary for in the interaction.
Supplemental Figure S4: Coimmunoprecipitation of full length, or fragments of a) RMI1 or b) Topollα with other components of the BS complex. Left is the schematic (to scale) of each gene showing domain structure as indicated, and the corresponding fragments used in immunoprecipitation experiments. Blue and orange lines represents 20 amino acid moving average of BLOSUM64 score from alignment of 96 RMI1 or 87 Topollα homologs. Right panels are the Western blots of Flag-immunoprecipitates. A dash indicates “no transfection” control lane. Data shown in panel (a) is derived from the same experimental data conducted in Deans & West, 2009 Molecular Cell.
Supplemental Figure S5: Western blots indicating level of expression and knockdown for experiments shown in Figure 7 of the main text.
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