Supporting Information

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SI Methods.

Protein Expression and Purification. ParC-pET11 and ParE-pET11 were kindly provided by Hiroshi Hiasa, University of Minnesota, Minneapolis (1); *Nde* I and *BamH* I fragments of ParC-pET11 and ParE-pET11 were subcloned into pET-28a vector to provide ParC-pET28a and ParE-pET28a. The constructs MukB-pET28a, MukB-D (566-863)-pET28a, ParC-NTDx (2-482)-pET28b, ParC-NTDb (24-497)-pET28b, and ParC-CTD (497-752)-pET28b were previously described (2, 3). *Nde* I-*EcoR* I fragments of MukB-pET28a and MukB-D-pET28a were subcloned into pET-22b vector to provide MukB-pET22b and MukB-D-pET22b. The coding sequences for MukB (645-804) (4) were amplified by the polymerase chain reaction and subcloned into the vector pET28-LIC (Novagen). MukB-pBB10 (5) and p15sp-B03a (6) were generous gifts from Valentin Rybenkov, University of Oklahoma, Norman. p15sp-B03a is a pACYC184 derived plasmid with the *mukB* gene under the control of its native promoter.

His_6-tagged proteins were expressed in BL21(DE3) cells using the T7 system (7). The cultures were grown in LB broth (700 mL) with kanamycin (10–50 μg/mL) at 37 °C to OD_{600} of 0.4–0.7. Protein overexpression was induced by adding IPTG to a final concentration of 0.5–2 mM. After an additional 3–5 h, cells were harvested by centrifugation and lysed by sonication in lysis buffer (20 mL) (50 mM sodium phosphate, 300 mM NaCl, 10 mM imidazole, 2 mM DTT, pH 8.0). After removing the cell debris by centrifugation, the cell lysates were mixed with Ni-NTA agarose beads (2 mL) (Qiagen) and washed with wash buffer (50 mL) (50 mM sodium phosphate, 300 mM NaCl, 20 mM imidazole, 2 mM DTT, pH 8.0). Bound proteins were then eluted in elution buffer (4–20 mL; 50 mM sodium phosphate, 300 mM NaCl, 250 mM imidazole, 2 mM DTT, pH 8.0). The eluted fractions were dialyzed against Tris buffer (20 mM Tris, 100 mM NaCl, 2 mM DTT, pH 8.0). To produce untagged MukB-D, the His_6 tag was removed by incubation with thrombin (20 U thrombin per mg MukB-D, Amersham Bioscience) at room temperature for approximately 4 h. Then the protein was concentrated by centrifugation in Amicon-15 tubes (Millipore). The concentrated protein was then loaded onto a Superose™12 column coupled to an AKTA™ FPLC system (Amersham Bioscience) running with Tris buffer. The fractions containing the desired protein were pooled and concentrated.

To purify untagged full-length MukB, a ParC-affinity column was used to take advantage of the robust interaction between MukB and ParC. His_6-tagged ParC was first immobilized on Ni-NTA beads by incubating cell lysate of overexpressed ParC (10 mL) with Ni-NTA beads (1.5 mL). The beads were then washed with 25-mL wash buffer three times. Cell lysate (10 mL) containing overexpressed untagged MukB was added to the beads and incubated at 4 °C for 30 min. The beads were washed with wash buffer (25 mL × 3). Part of MukB was eluted off the ParC•Ni-NTA beads with Hi-salt Buffer (10.1 mM sodium phosphate, 1.76 mM potassium phosphate, 437 mM NaCl, 2.68 mM KCl, pH 7.4). The eluted fractions were dialyzed into Tris buffer supplemented with 25% glycerol and concentrated by centrifugation in Amicon-15 and YM-30 microcon tubes (Millipore). The concentrated MukB solution was incubated with fresh Ni-NTA beads again to remove any contaminants.

ParC and ParE were purified as previously described (3). The size and purity of all proteins (>90%) were verified by SDS-PAGE.

Affinity Purification-Mass Spectrometry. His_6-tagged MukB-D was immobilized on Ni-NTA beads and concentrated cell lysate from *mukB*$^-$ strain SH7718 (a gift from Sota Hiraga, Kyoto University, Kyoto, Japan) (8) was loaded on to the resulting column. After SDS-PAGE separation of the eluted proteins, a single band was observed that was not present in the absence of His_6-MukB-D. This band was excised and subjected to mass spectrometry (MS) analysis, and the constituent proteins were identified as ParC and ClpA. The molecular chaperone ClpA was not pursued further, as it binds nonspecifically to hydrophobic regions of proteins (9).

For isolation of complexes containing endogenously expressed MukB, *Escherichia coli* KAT1 (from Sota Hiraga) (10) (*mukB-gfp4*) and wild-type strains were grown in LB culture (4 L; 37 °C) to midlog phase (OD_{600} = ~0.5). The cells were harvested by centrifugation and resuspended in buffer A (10 mL; 10 mM sodium phosphate, 1.76 mM potassium phosphate, 2.7 mM KCl, 137 mM NaCl, 0.2 mg/mL PMSF, 4 μg/mL pepstatin A, 20 μg/mL Dnase I, pH 7.4) supplemented with 1/100 (vol/vol) protease inhibitor cocktail (Sigma). The cells were then lysed by sonication (Microson XL2000, 6 watts, 20 × 10 s) on ice and the resulting lysate was cleared by centrifugation. The lysate was subsequently concentrated twofold by Amicon-15 tube (MWCO 10 KD) and passed through a GD/X glass microfiber filter (1.6 μm; Whatman). The concentrate was then incubated with magnetic beads (8 mg; Dynabeads M-270 Epoxy; Invitrogen) conjugated with a home-raised rabbit anti-GFP antibody as previously described (11). After 1-h incubation (4 °C), the beads were washed with ice-cold buffer B (6 × 1 mL; 10 mM sodium phosphate, 1.76 mM potassium phosphate, 2.7 mM KCl, 137 mM NaCl, 0.1% Tween-20, pH 7.4). The isolated protein complexes were then eluted in ammonium hydroxide solution (3 × 500 μL; 0.5 M NH_4OH, 0.5 mM EDTA) by incubation with shaking for 20 min at RT. The pooled eluates were frozen in liquid nitrogen and dried under reduced pressure. The pellet was subsequently resuspended in lithium dodecyl sulfate (LDS) sample buffer (20 μL; Invitrogen) supplemented with 42 mM DTT and incubated at 70 °C for 10 min. To alkylate reduced cysteines, the resulting samples were incubated with iodoacetamide (4 μL, 1 M; in 200 mM NH_4HCO_3) for 30 min in the dark. The proteins were then separated on a NuPAGE 4–12% Bis-Tris gel (Invitrogen) in 3-(N-morpholino)propanesulfonic acid (MOPS) SDS running buffer (Invitrogen). The resolved proteins were visualized by MS compatible Gelcode Blue stain (Pierce).

Prior to MS analysis, whole gel lanes were sliced into 1-mm pieces on a gel slicer in a ductless fume hood (AC600, Airclean Systems). For each sample, 2–4 gel pieces were pooled together according to the visible protein bands and washed with a destaining solution (2 × 200 μL; 50% acetonitrile, 50 mM NH_4HCO_3). After an additional wash with neat acetonitrile (200 μL), the gel pieces were air-dried in the fume hood. The dried gel pieces were then hydrated with 10-μL sequencing grade trypsin solution (12.5 ng/μL in 25 mM NH_4HCO_3; Promega) and covered with an additional 30 μL of 25 mM NH_4HCO_3 before overnight digestion at 37 °C. The digest was extracted with POROS 20 R2 beads (40 μL; 5 μg/μL in 0.2% TFA, 5% formic acid; Applied Biosystems) and loaded onto a C18 Ziptip (Millipore). TFA (40 μL, 0.1%) was used to wash the gel pieces once. The tip was subsequently washed twice with 0.1% TFA (40 μL). The tryptic peptides were finally eluted with 25% saturated α-Cyano-4-hydroxycinnamic acid (4-HCCA) (3 μL; in 70% acetonitrile, 0.1% TFA) onto a magnetic matrix-assisted laser desorption/ionization (MALDI) target (12).

Mass spectra were collected on an externally calibrated MALDI orthogonal time of flight mass spectrometer (prOTOF 2000, PerkinElmer Sciex;) (mass range: 800–5000 Da). The data were extracted from the instrument database using the prOTOF extractor (Markus Kalkum). The monoisotopic peaks were then automatically picked using MoverZ (Ronald Beavis) (S/N = 1.5) and manually inspected to generate the mass list. A total of 23 mass lists were obtained, corresponding to the different band positions in the sample lane (MukB-GFP) (Fig. S1). Similarly, 23 mass lists were also generated from the control lane (WT). Each sample mass list, after subtracting the control mass lists of corresponding band positions and the mass values of commonly known contaminants, was uploaded to ProFound (13) for peptide mass fingerprinting [database: NCBI nr 2010/01/04; taxonomy: *Escherichia coli*; mass range: 0–3,000 kDa; pI range: 0–14; digestion: trypsin; max. missed cut: 1; modifications: iodoacetamide (complete) and methionine oxidation (partial); charge state: MH+; monoisotopic mass tolerance: 40 ppm]. Proteins with expectation value ≤0.001 were reported in Table S2. An expectation value of 0.001 corresponds to an approximately 0.1% chance that the match is completely random. For each identified protein, at least two peptides with high MS peak intensity were chosen for tandem mass spectrometry (MS/MS) confirmation on a MALDI linear ion trap mass spectrometer (MALDI LTQ XL, Thermo Scientific) (laser energy: 4.5 μJ; laser shots: 12; μscans: 5; *μ*scans/step: 10; injection waveforms: on; automatic spectrum filter: off; automatic gain control: off; wideband activation: off; isolation width (m/z): 4; normalized collision energy: 35%; activation Q: 0.25; activation time: 250–300 ms). The native data were first converted to DTA format with DTA Creator (Júlio Padovan) and then uploaded to Xproteo (www.Xproteo.com) for protein verification [data type: MS/MS; species: *Escherichia coli* in NCBI nonredundant database (10/16/2006); protein mass: 0–300 KDa; protein PI: 1–14; mixture search: auto; display: top 10 candidates; enzyme: trypsin; max. missed cleavage: 1; mass type: mono; charge state: MH+; precursor tol.: 40 ppm; fragment tol.: 0.5 Da; instrument: MALDI_I_TRAP]. An Xproteo score of 4 corresponds to a true positive rate of 0.99 at false positive rate of 0.05. The higher score means higher true positive and lower false positive rate.

In addition to ParC, EF-Tu was identified in this proteomic analysis (Table S2). Because EF-Tu is the most abundant protein in *E. coli* (14), this finding may be an artifact. However, Graumann and co-workers have recently shown that EF-Tu associates closely with MreB in *Bacillus subtilis*, suggesting that EF-Tu may play a role in establishing the cytoskeleton of prokaryotes (15). We do not detect MreB in these complexes, in spite of the clear demonstration by Madabhushi and Marians that ParC also interacts functionally with MreB (16), nor do we detect ParE.

His_6-Tag Pull-Down Assays. The cell lysate of overexpressed His_6-tagged protein (bait) and untagged protein (prey) were mixed together at roughly equal concentrations and incubated at 4 °C for 60 min. The lysate mixture (600–1,500 μL) was incubated with Ni-NTA agarose beads (100–200 μL) in lysis buffer at 4 °C for 30–60 min on a rotator. Then the supernatant was removed after centrifugation at 13 krpm for 5 s. The beads were washed with lysis buffer (1 mL) at least three times. The proteins bound on the beads were eluted by incubating with elution buffer (100 μL). The sample (10 μL) from the eluted fraction was mixed with an equal amount of loading dye and analyzed by SDS-PAGE. The protein bands were visualized by Coomassie® Brilliant Blue R-250 (BioRad) or Sypro® Ruby protein stain (Invitrogen). Gels were visualized and band intensities quantitated on a Typhoon 9210 imaging system (Amersham Bioscience). For quantitative pull-down experiments, the concentrations of the lysates containing wild-type and mutant MukB-D were adjusted as appropriate to ensure a similar amount of proteins for each experiment. To compare different gels, band intensities were normalized relative to the most concentrated sample containing wild-type MukB. Error bars correspond to the standard deviation of repeated measurements ($n = 3$).

Isothermal Titration Calorimetry (ITC). The isothermal titration calorimetry experiments were performed with a VP-ITC microcalorimeter (Microcal) at 20 or 25 °C. The protein samples were dialyzed with degassed PBS buffer (10.1 mM sodium phosphate, 1.76 mM potassium phosphate, 137 mM NaCl, 2.68 mM KCl, pH 7.4) prior to the experiments for at least three times. Full-length ParC [4.4–17.4 μM for MukB-D titrations; 2.3–5.5 μM for MukB (645-804)] was loaded into the calorimetric cell (1.4 mL) and titrated with MukB-D (25.0-176.3 μM) or MukB (645-804) (25.5–49.4 μM) in an injector (300 μL) rotating at 310 rpm. ParC-CTD (10.8–12.1 μM) was titrated with MukB-D (98.4 μM) under the same conditions. MukB-D was chosen as the titrant because it remains soluble at the high concentration necessary for the ITC experiments. The volume of each injection was 5 or 12 μL at 360 or 600 s intervals. Once the heat of addition became constant, subsequent injections were averaged as heats of dilution of the MukB hinge domains and subtracted from the data. The data were then plotted as enthalpy per mole of MukB hinge injected against the molar ratio of MukB hinge to ParC. The parameters of the interaction were obtained by fitting the data using a simple one-site binding model with the Origin™ software provided with the VP-ITC. Because MukB (645-804) was found to be marginally stable at 25 °C, titrations involving this protein were performed at 20 °C.

Topoisomerase Assays. To reconstitute *E. coli* Topo IV in vitro, equal amounts of ParC in stocking buffer A (50 mM Hepes, 400 mM KCl, 1 mM EDTA, 2 mM BME, 20% glycerol, pH 7.5) and ParE in stocking buffer B (50 mM Hepes, 475 mM KCl, 1 mM EDTA, 30% glycerol, pH 7.5) were mixed and placed on ice for 30 min. The reconstituted Topo IV was diluted in dilution buffer (50 mM Hepes, 20 mM KCl, 6 mM $MgCl_2$, 10% glycerol, pH 7.5) to working concentrations. Topo IV (0.5 μL, 15.5 nM or 62 nM) in dilution buffer was mixed with MukB or MukB-D (1 μL, various concentrations) in Tris buffer and incubated on ice once again for 30 min. MukB and MukB-D were added in molar excess to drive complex formation at practically accessible concentrations of topoisomerase. Larger stoichiometric ratios of MukB: ParC were used in the MukB-D experiments to compensate for the lack of a DNA binding domain expected to increase the effective concentrations of the two proteins along the substrate DNA. Reaction buffer [9 μL; 50 mM Tris, 20 mM KCl, 6 mM $MgCl_2$, 1 mM spermidine, 0.1 mg/mL BSA, 1 mM adenosine-5′-triphosphate, 10 mM DTT, 100 ng negatively supercoiled pBR322 or 180 ng *k*DNA (Topogen), pH 7.8] was added and the mixtures were incubated at 30 °C. At the time points indicated, the reaction was quenched by the addition of stopping buffer (2 μL) (10% SDS, 10 mM EDTA) and supplemented with 6 × DNA loading dye (2 μL), then loaded on a 1.2% agarose gel immersed in 0.5 × Tris/Borate/EDTA (TBE) Buffer. The gel was run at about 1 V/cm for 16–18 h and stained with 1 μg/mL ethidium bromide for 30 min. The gel was then scanned on a Typhoon 9210 imaging system. The analysis software ImageQuant (GE Healthcare) was used to quantify the DNA bands. To allow comparison between gels, band intensities are normalized relative to the most concentrated sample (Fig. 3*C*) or longest time point (Fig. 3*D*). Error bars in Fig. 3*D* are the standard deviation of repeated ($n = 3$) experiments. The error in Fig. 3*C* is estimated from the measurements in Fig. 3*D* by linear fitting of error to relative band intensity. The relaxation experiments in Fig. 3 *A* and *B* are representative of trials with a similar but not identical setup. In the control reactions, human Topo II (Topogen) was used in the place of *E. coli* Topo IV; for ease of comparison, a Topo II concentra-

tion (units/mL) at which the Topo II activity in the absence of MukB was similar to that of Topo IV in the same assays was chosen.

Complementation Assay. The mutations were systematically introduced into the MukB coding sequence of the low-copy number plasmid p15sp-B03a (Table S1). After DNA sequencing verification (17), the plasmid was transformed into the *mukB*$^{-}$ strain SH7718 (from Sota Hiraga) with electroporation according to the manual (BioRad). The transformants were grown on a LB agar plate with chloramphenicol at RT for 2–3 days. Single colonies were then streaked onto two fresh plates and incubated at the permissive (22 °C) or restrictive (37 °C) temperature to assay their ability to complement the growth deficiency of SH7718 at restricted temperature.

Velocity Sedimentation Assay. Apparent molecular weights were determined by velocity sedimentation on a Beckman Optima™ XL-I analytical ultracentrifuge at 20 °C. The MukB-D (2.5 μM) and MukB-D-D692A (5 μM) were dialyzed in PBS. Data were obtained at 230 nm with an An-60Ti rotor (Beckman) at 50,000 rpm. Calculations were performed on the UltraScan LIMS cluster at the Bioinformatics Core Facility at the University of Texas Health Science Center at San Antonio and the Lonestar cluster at the Texas Advanced Computing Center supported by NSF Teragrid Grant #MCB070038 (to Borries Demeler). The solvent density and the partial specific volume of the proteins were calculated with UltraScan II.

Gel-Mobility Assay. Full-length MukB (19.4 μL, 1.9 μM) or MukB-D692A (18 μL, 2.05 μM) was mixed with pUC19 (50 ng) and incubated at 4 °C (10–15 min) in the reaction buffer (20 mM Hepes, 137 mM NaCl, 1 mM DTT, 2 mM EDTA, 5% glycerol, pH 7.4). The samples were supplemented with 6 × DNA loading dye (4 μL), then loaded on a 1.5% agarose gel immersed in 1 × TBE buffer. The gel was run for 1 hr at 90 V and stained with ethidium bromide for 30 min.

Circular Dichroism (CD) Spectroscopy. CD spectra were obtained on a Jasco J-715 spectropolarimeter. The protein solutions were dialyzed against PBS buffer with 1 mM TCEP. Concentrations were then determined using the method of Edelhoch (18). Wavelength dependence of $[\Theta]$ was monitored in 1-nm increments with a 4-s response time at 4 °C. Thermal stability was assessed by monitoring the temperature dependence of $[\Theta]_{222}$ from 10–70 °C (2 °C increments, 90-s equilibration time). Helical content was calculated by the method of Chen et al. (19). The T_m of the thermal unfolding transition is estimated from the maximum of the first derivative curve.

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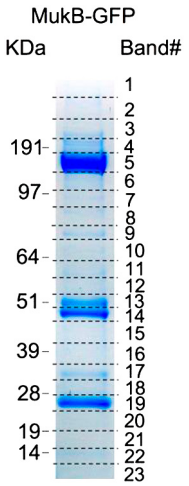


Fig. S1. MukB associated proteins were identified by mass spectrometry. Gel slices from different band positions were digested with trypsin before MS analysis. Detailed information for proteins with a low expectation value (≤0.001) is shown in Table S2.

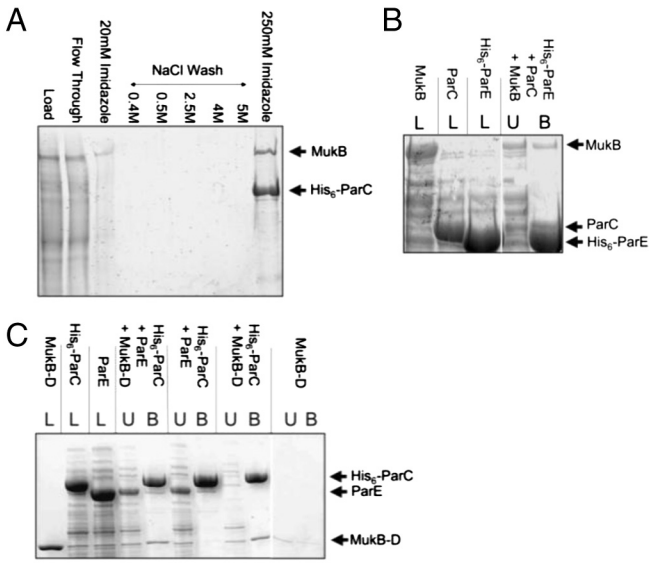
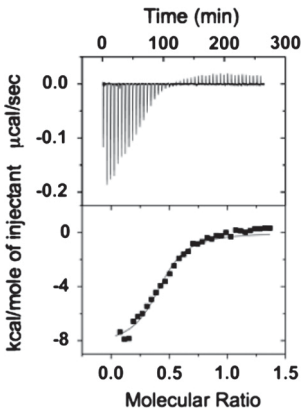


Fig. S2. The robust interaction between MukB and ParC does not interfere with the ParC-ParE interaction. (*A*) Stringent wash of MukB•ParC complex with high concentrations of salt (NaCl, 0.4–5 M). (*B*) His_6-ParE with untagged ParC and MukB. (*C*) His_6-ParC with untagged MukB-D and ParE. The lane labels for parts *B* and *C* are as follows: L: lysate; B: bound to the Ni-NTA resin; U: unbound.



Proteins	K_d (μM)	ΔH (kcal/mol)	N (stoichiometry)
MukB-D ParC	0.4 ± 0.2	-7.9 ± 4.3	0.6 ± 0.3
MukB-D ParC-CTD	0.5 ± 0.1	-5.1± 1.2	1.0 ± 0.1
MukB (645-804) ParC	0.5 ± 0.3	-7.2 ± 6.9	0.5 ± 0.2

Fig. S3. (*Top*) Representative isothermal titration calorimetry measurement of the binding affinity between full-length ParC and MukB-D protein (PBS buffer; 25 °C). (*Bottom*) Table showing composite binding data for MukB-ParC interactions. Parameters were obtained by fitting the ITC data to a one-site binding model; error shown is the standard deviation of repeated measurements ($n = 3$). Because MukB (645-804) was found to be marginally stable at 25 °C, the titrations involving this protein were performed at 20 °C.

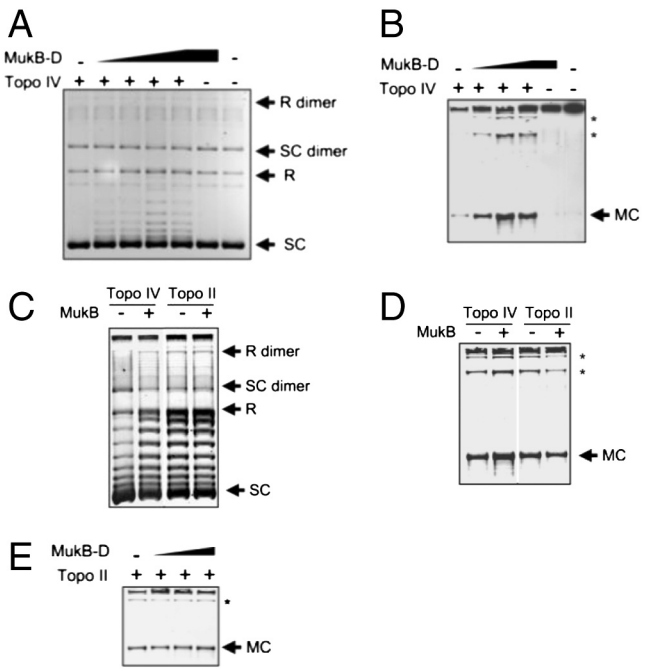


Fig. S4. MukB-D alone is sufficient to stimulate Topo IV activity. (*A*) Topo IV (3.0 nM) is incubated with pBR322 (100 ng) at 30 °C, along with MukB-D at various concentrations (lanes 1–5: 0, 48, 95, 190, 380 nM, respectively). The position of negatively supercoiled plasmids is indicated by SC, and the position of completely relaxed plasmids is indicated by R. Between them are incompletely relaxed topoisomers. (*B*) Topo IV (0.74 nM) is incubated with *k*DNA (180 ng) at 30 °C, along with MukB-D at various concentrations (lanes 1–4: 0, 48, 95, 190 nM, respectively). Larger stoichiometric ratios of MukB-D: ParC were used in this experiment to compensate for the lack of a DNA binding domain expected to increase the effective concentrations of the two proteins along the substrate DNA molecule. The position of decatenated mini circles is indicated by MC. The asterisks (*) indicate incompletely decatenated products. In addition, at high concentrations of MukB-D, decatenation appears to be inhibited, suggesting that large excesses of MukB-D may lead to a less active complex. (*C*) Topo IV (3.0 nM) or *Homo sapiens* Topo II (0.29 U/μL) was incubated with pBR322 (100 ng) in the absence or presence of MukB (39 nM) at 30 °C. (*D*) Topo IV (0.74 nM) or Topo II (0.071 U/μL) was incubated with *k*DNA (180 ng) in the absence or presence of MukB (39 nM) at 30 °C. (*E*) Topo II (0.095 U/μL) is incubated with *k*DNA (180 ng) at 30 °C, along with MukB-D at various concentrations (lanes 1–4: 0, 48, 95, 190 nM, respectively).

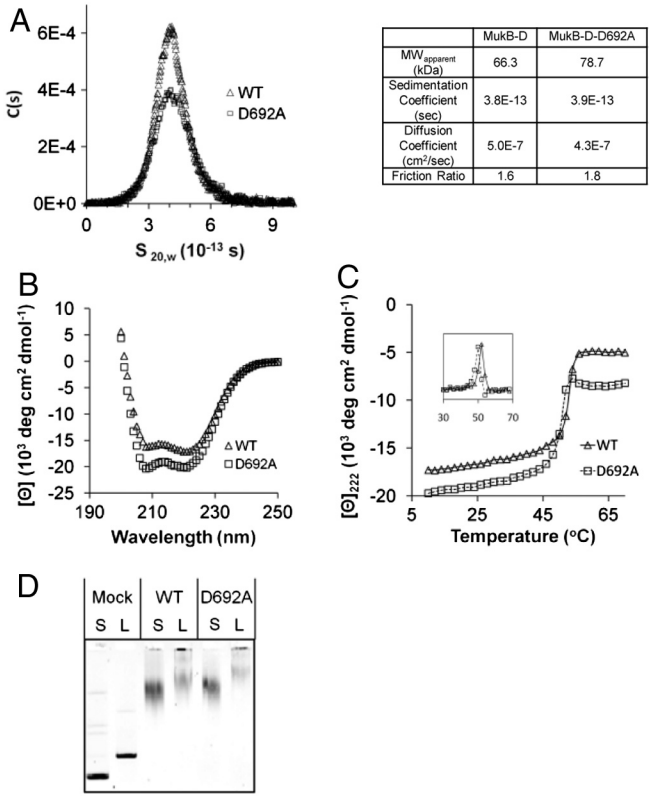


Fig. S5. Biophysical characterization of MukB proteins and their mutants. (*A*) Velocity sedimentation of MukB-D (2.5 μM) and MukB-D-D692A (5 μM). C(s): continuous distributions; $S_{20,w}$: sedimentation coefficient. (*B*) CD spectra of MukB-D (triangles, 13 μM) and MukB-D-D692A (squares, 10 μM) at 4 °C in PBS buffer. (*C*) Temperature dependence of the CD signal of the same proteins at 222 nm, MukB-D (6 μM) and MukB-D-D692A (10 μM). (*D*) In vitro DNA binding of MukB proteins. The proteins were incubated with different forms of pUC19 (S: supercoiled; L: linearized). WT: full-length MukB; D692A: MukB-D692A.

Table S1. Primers used for alanine scan of MukB

Primer	Sequence
A686–689 (+)	GCA GCC GCT GCG TAT GAC GAC GTT AGC CTG GAA GAT GCG C
A694–697 (+)	GCC GCA GCT GCG GCG CCG TAC TTC TCA GCG CTG TAT G
A721–724 (+)	GCA GCT GCG GCC GAA GGC TTG ACC GAT TGC CCG GAA G
A742–745 (+)	GCA GCC GCT GCG GAC AGC GTG TTC AGC GTT GAT GAG CTG G
A749–752 (+)	GCC GCA GCT GCG GAG CTG GAA AAA GCG GTA GTG GTG AAA ATC G
A788–791 (+)	GCC GCT GCA GCG GCC GAG CGT GAA GTG CTT TCC GAA C
A686–689 (–)	CGC AGC GGC TGC CAG CAC ACC ACC AAA ACG CTC CGC C
A694–697 (–)	CGC AGC TGC GGC AAC GTC GTC ATA AAT TTC TGA CAG CAG CAC ACC
A721–724 (–)	GGC CGC AGC TGC TAC CTG TGA CAG ATC TGG CAC CAC GAT G
A742–745 (–)	CGC AGC GGC TGC CGG ATC TCC TTC GAT CAG ATA GAG ATC TTC C
A749–752 (–)	CGC AGC TGC GGC CAC GCT GTC ATC GAA TGA CTG CGG ATC
A788–791 (–)	CGC TGC AGC GGC AAT ACG GCT TTC ACG CGC AGC ACG ACC
D692A	G CTG TCA GAA ATT TAT GAC GCC GTT AGC CTG GAA GAT GC
E725A	CT GAA CAC CTG GCA GGC TTG ACC GAT TGC C
E738A	G GAA GAT CTC TAT CTG ATC GCA GGA GAT CCG CAG TC
D746A	CCG CAG TCA TTC GAT GCC AGC GTG TTC AGC G
E753A	GC GTG TTC AGC GTT GAT GCG CTG GAA AAA GCG G
D764A	GTG GTG AAA ATC GCC GCT CGT CAG TGG CG
MukB9 Ant	CCAGTTCGACGCGACGGC

Table S2. Proteomic analysis of the protein complex containing MukB

Band#	Protein identified	GI#	#matched peptides from MS	Sequence coverage, %	Expectation value*, ≤0.001	Peptides confirmed by MS/MS	Xproteo score†, MS/MS
1	MukB	2851431	23	22	8E-10	NA	NA
2	MukB	2851431	43	38	9E-16	NA	NA
3	MukB	2851431	56	43	5E-16	NA	NA
4	MukB	2851431	70	50	1E-22	NA	NA
5	MukB	2851431	57	46	5E-13	YEADLDELQIR; SLTLINWNGFFAR STTMAAFVTALIPDLTLLHFR FGGVLLSEIYDDVSLEDAPYFSALYGPSR	25
6	MukB	2851431	61	49	5E-23	NA	NA
7	MukB	2851431	35	34	7E-13	NA	NA
8	MukB	2851431	26	23	4E-9	NA	NA
9	ParC	147106	25	41	5E-10	SYAIDPITLPSAR; ALPFIGDGLKPVQR YPLVDGQGNWGAPDDPK TTLDQLLDIVQGPDYPTEAEIITSR	17
10	MukB	2851431	14	14	3E-5	NA	NA
11	None	NA	NA	NA	NA	NA	NA
12	None	NA	NA	NA	NA	NA	NA
13	MukF	450465	22	66	5E-10	NA	NA
14	MukF	450465	26	64	4E-11	YSVAEIFDSIDLTQR; IISWGQQSIDLWIGYDR	9
15	EF-Tu	49258331	8	34	4E-4	AGENVGVLLR; AFDQIDNAPEEK QVGVPYIIVFLNK; GITINTSHVEYDTPTR ILELAGFLDSYIPEPER; AIDKPFLLPIEDVFSISGR TKPHVNVGTIGHVDHGKTTLTAAITTVLAK	16
16	None	NA	NA	NA	NA	NA	NA
17	GFPuv4‡	7415879	14	60	1E-5	FEGDTLVNR; AEVKFEGDTLVNR FSVSGEGEGDATYGK; GIDFKEDGNILGHK LEYNYNSHNVYITADK GEELFTGVVPILVELDGDVNGHK	41
18	None	NA	NA	NA	NA	NA	NA
19	MukE	26107350	14	60	1E-3	APEGFFYLRPR; LAQALANPLFPALDSALR LANEGIFTQQELYDELLTLADEAK	28
20	None	NA	NA	NA	NA	NA	NA
21	None	NA	NA	NA	NA	NA	NA
22	ACP§	349815	3	33	NA	IIGEQLGVK; KIIGEQLGVK ITTVQAAIDYINGHQA	19
23	None	NA	NA	NA	NA	NA	NA

*An expectation value of 0.001 approximately means 0.1% chance that the match is completely random.
†Xproteo score of 4 means a true positive rate of 0.99 at false positive rate of 0.05. The higher score means higher true positive and lower false positive rate.
‡Data were searched against all taxa.
§Acyl carrier protein (ACP) was identified directly by tandem mass spectrometry.