Supporting Information

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SI Materials and Methods

Strains, Plasmids, and Growth Conditions. Strains and plasmids used in this work are listed in Tables S1 and S2, respectively. Detailed methods for the construction of the strains and plasmids are described below. The sequences of all primers are shown in Table S3. Transformations and phage transductions were carried out as described (1). *Caulobacter crescentus* strains were grown at 30 °C in peptone yeast extract (PYE). For induction of gene expression in merodiploid strains, exponentially growing cells were treated for 2 h with 0.03% xylose. For BapE overexpression, 0.3% xylose was used to induce expression and 0.02% glucose was used as the uninduced control. To induce DNA damage, we used the DNA crosslinker mitomycin C (MMC) drug at 1 μg/mL unless otherwise noted or UV irradiation (100 J.m^2).

Microscopy and Cell Death Analysis. Images were collected on a Nikon 90i microscope equipped with a Nikon Plan Apo 100×/1.4 phase-contrast objective and a Rolera XR cooled CCD camera and were processed with NIS-Elements software. Samples were placed on agarose-padded slides containing the appropriate medium (PYE). A coverslip was placed on the pad and then sealed with valap (1:1:1 parafin:lanolin:vaseline). DNA was visualized by adding DAPI (live-cell DNA staining) in agarose pads.

Cell death was visualized by fluorescence microscopy using propidium iodide (PI), a membrane-impermeable nucleic acid stain commonly used as a cell death marker. To determine the percentage of dead cells, *Caulobacter* strains were grown in PYE to logarithmic phase. Xylose or MMC was added for 6 h to overnight to induce BapE expression. Samples of cell culture (1 mL) were stained with PI (1 μg/mL) for 1 h at room temperature and imaged by fluorescence microscopy. The number of dead cells was determined by counting the number of red cells (PI-stained cells) and the number of red lysed ghost cells, relative to the total cell population. For this experiment, the total number of bacterial cells used for the quantification of PI staining (Fig. 1*B*) in the indicated strains is the following: ZG488 (n = 375), MT1760 (n = 767), ZG482 (n = 660), ZG66 (n = 541), ZG66 + MMC (n = 160), ZG684 (n = 526), ZG684 +MMC (n = 174), ZG486 (n = 423), and ZG484 (n = 140).

A similar protocol was used to determine the percentage of DIBAC4(3)-stained cells (green). Samples of cell culture (1 mL) were stained with DIBAC4(3) (1 μg/mL), a voltage-sensitive fluorescent dye, for 30 min at room temperature and imaged by fluorescence microscopy. The total number of bacterial cells used for the quantification of DIBAC4(3) staining (Fig. 1*C*) in the indicated strains is the following: ZG488 (n = 532), ZG482 (n = 740), ZG66 (n = 452), ZG66 +MMC (n = 151), ZG684 (n = 426), ZG684 + MMC (n = 285), ZG486 (n = 326), and ZG484 (n = 134).

To classify cells as having high or low levels of GFP-BapE expression, we used fluorescence microscopy and defined cells with bright GFP-BapE foci as "high" and cells with dim diffuse fluorescence as "low."

TUNEL Assay Using FACS Analysis. DNA fragmentation was measured in fixed *Caulobacter* cells using the Deadend fluorometric TUNEL system kit (Promega), which is designed for the specific detection and quantitation of apoptotic cells within a cell population. We measured the fragmented DNA of apoptotic cells by catalytically incorporating fluorescein-12-dUTP at 3′-OH DNA ends using recombinant Terminal Deoxynucleotidyl Transferase (rTdT), which forms a polymeric tail using the principle of the TUNEL (TdT-mediated dUTP nick-end labeling) assay. For our studies, *Caulobacter* strains were grown overnight in PYE. When needed, MMC was added to induce BapE expression. Samples of cell culture (1 mL) were washed twice with 1× PBS before fixation in 1 mL fixation buffer (3% formaldehyde, 150 mM Na_2HPO_4, 30 mM NaH_2PO_4) for 30 min on ice. Cell pellets were washed twice with PBS, resuspended with 70% cold ethanol, stored at −20 °C overnight, washed with equilibration buffer provided with the kit, and resuspended for 1 h at 37 °C in 50 μL staining solution containing equilibration buffer, fluorescein 12-dUTP, and rTdT. Samples were kept in the dark. Reactions were stopped by adding stop buffer (2× SSC) and washed in 1× PBS. The incorporation of fluorescein-12-dUTP–labeled DNA then was quantitated directly by flow cytometry using a LRII flow cytometer (Flow Cytometry Facility, Princeton University). Due to the strong fluorescent green background staining of nonapoptotic cells, which we interpreted as a nonspecific incorporation of fluorescein-12-dUTP, we always performed a control with no rTdT (unstained) in parallel to samples treated with rTdT (stained). All representative data are from two independent experiments.

Growth Curve Experiments. Growth curves were performed in PYE medium. For all experiments, unless mentioned, samples were withdrawn at 60-min intervals over time, and the optical densities of the cultures were recorded at 660 nm.

Mutation Frequency. Mutation frequency was determined as described (2). Wild-type (ZG66) and *bapE-ssrA* (ZG475) strains were grown in PYE until logarithmic phase. A 5-mL batch of culture was exposed to MMC treatment (0.25 μg/mL) for 1 h. Cells were washed to remove MMC before inoculation for overnight incubation. A 200-μL sample of washed treated cells was inoculated in 1 mL of PYE liquid medium and grown overnight to allow mutation fixation. Cultures were plated on PYE medium containing rifampicin (50 μg/mL) to score rifampicin-resistant (Rif^R) mutants and serially diluted onto PYE plates to determine viable cell counts. Mutation frequencies were calculated by dividing the number of Rif^R mutants by the total number of cells.

Real-Time Analysis of Gene Expression. To study the kinetics of the SOS repsonse, cellular mRNA levels of cc1087 (*recA*), cc2590 (*uvrA*), cc3236 (*ruvB*), cc0382, cc1902 (*lexA*), cc0627 (*bapE*), and cc3047 (*rpoD*) genes in ZG66 were determined by quantitative PCR using the primer pairs listed in Table S3. To induce SOS response, MMC drug (1 μg/mL) was applied at an OD_{660} of 0.3, and samples were withdrawn at 15, 30, 45, 60, and 120 min after MMC exposure. Total RNA was extracted using the RNAextraction kit (Qiagen) and DNaseI-treated (DNaseI free; Ambion) for 30 min at 37 °C. cDNA synthesis reactions were performed using the High Capacity cDNA Reverse Trancription (RT) Kit (Applied Biosystems) in a 20-μL final volume, using 150 ng of DNA-free RNA as template. For real-time PCR, 1/40 of RT reaction volume was used in each reaction. Reactions were performed by using the PerfeCTa SYBR Green PCR fast mix (Quanta Bioscience) and run using a 7900HT Fast real-time PCR system machine (Applied Biosystems). Data analysis was performed using the software RQ1 manager (Applied Biosystems).

Viability Assays. Strains ZG66, ZG486, and ZG484 were grown in PYE medium until midlog phase before being treated with MMC (0.5 μg/mL) (ZG66) or induced with xylose (0.03% for ZG486 and 0.3% for ZG484). After 4 h of induction and growth with either MMC or xylose, cells were centrifuged to remove MMC and xylose from the medium and resuspended in fresh PYE

medium containing no MMC and 0.02% glucose for 2 h to stop BapE induction. Control cultures (uninduced) were grown in parallel. Aliquots were removed 2 h after washing, and serial dilutions were plated onto PYE plates to determine viable cell counts (cfu/mL) after 48 h of incubation at 30 °C.

Protein Purification. Isopropyl β-d-1-thiogalactopyranoside (IPTG) (0.5 mM) was added to a 700-mL LB culture of ZG496 or ZG299 (control experiment) at OD_{600} = 0.5 for 2 h at 37 °C. Cells were harvested, resupended in lysis buffer [50 mM Tris·HCl (pH 8.0), 10 mM imidazole, 300 mM NaCl, 1 mM PMSF, inhibitor protease mixture tablets, 50 U Benzonase], and lysed by using a French Press. The cell lysate was centrifuged at 13,200 × *g* for 30 min at 4 °C. Ni-NTA slurry (Qiagen) was added to the cleared lysate and gently mixed on a rotary shaker for 1 h at 4 °C. The lysate-NiNTA mixture was loaded into a column, and fractions were collected for SDS/PAGE analysis. For washes, imidazole concentration was increased to 20 mM, whereas an imidazole step gradient (100 mM→200 mM→250 mM) was used for elution. Pooled fractions containing BapE-his6 were dialyzed for 36 h against 5% (vol/vol) glycerol, 1 mM $MgCl_2$, 0.5 mM EDTA, 50 mM NaCl, and 10 mM Tris·HCl (pH 8.0) and stored at −80 °C before use. Purity was verified using gel electrophoresis and Coomassie blue staining.

Western Blot Analysis. For BapE protein levels determination, strains ZG474, ZG475, ZG495, ZG685, ZG686, and ZG687 were grown in PYE until midlog phase. When needed, MMC drug (0.2, 0.5, or 1 μg/mL) or 0.3% xylose was added for 8 h. Cultures were spun down, and the pellets were resuspended in lysis buffer [20 mM Tris·HCl (pH 7.5), 150 mM NaCl, 1 mM PMSF, 1 mM EDTA, inhibitor protease mixture tablet, 1% triton] and lysed by using a French Press. Cell lysate was centrifuged at 13,200 × *g* for 30 min at 4 °C. Supernatants were removed, quantitated using a Bradford protein assay, separated by SDS/PAGE (15% gels), and analyzed by Western blot using either polyclonal anti-his antibody (Cell Signaling) or polyclonal anti-Flag antibody (Sigma). For Flag-RecA and BapE-Flag kinetics, strains ZG689 and ZG474 were grown in PYE until midlog phase, and 50-mL samples were taken just before (t = 0) or at different time points (t = 1, 2, 4, and 8 h) after MMC (1 μg/mL) was added. Quantification of BapE protein bands was performed by using Image J software.

In Vitro Nuclease Assays. DNA plasmid pUT18 (3,023 bp; Euromedex) (250 ng) was incubated either alone or in the presence of various concentrations of purified BapE for 10 min at 37 °C. $MgCl_2$ or Proteinase K were added simulteanously in the reaction mix when required. All samples were treated with Proteinase K (2 mg/mL) for 10 min at 37 °C before analysis on a 1% agarose gel. The gel was run at 2 V/cm overnight in 0.5× tris/borate/EDTA (TBE) buffer and stained with ethidium bromide. To test whether BapE nuclease cleaves DNA in a sequence-specific manner, we applied the same protocol to obtain BapE-linearized pUT plasmid. After gel extraction and purification of the BapE-linearized pUT plasmid band, the band was digested for 1 h with EcoRI and analyzed on a 1% agarose gel containing ethidium bromide.

Analysis of DNA Fragmentation by Pulsed-Field Gel Electrophoresis. Pulsed-field gel electrophoresis (PFGE) allows the separation of large DNA molecules. Strains ZG66 and ZG484 were grown in PYE medium supplemented with 0.3% xylose (ZG484) until logarithmic phase (OD_{660} = 0.5). DNA plugs were then prepared from each culture. One volume (50 μL) of either undiluted or 1:1 diluted cell culture was spun down, resuspended in 1 volume of cell suspension buffer [10 mM Tris·HCl (pH 7.5), 20 mM NaCl, 10 mM EDTA], and mixed with 1 volume of 2% low melting point (LMP) agarose. The cell/agarose mixture was kept at 50 °C before being transferred to plug molds and allowed to solidify for 15 min. The solidified agarose plugs were pushed into a 50-mL conical centrifuge tube containing lysozyme solution [10 mM Tris·HCl (pH 7.5), 50 mM NaCl, 0.5% Sarkosyl, 1 mg/mL lysozyme, 1% triton] and incubated for 1 h at 37 °C. The lysozyme solution was removed, and the plugs were rinsed two times with wash buffer [20 mM Tris·HCl (pH 8), 10 mM EDTA], incubated overnight at 50 °C without agitation in Proteinase K reaction buffer [50 mM EDTA (pH 8), 0.5% triton, 1% Sarkosyl, 1 mg/mL Proteinase K], washed four times in wash buffer (containing 1 mM PMSF), and stored in 1× TE buffer (Tris·HCl 10 mM, EDTA 1 mM) at 4 °C. For the electrophoresis set up, a 1% agarose gel was poured, and the plugs were placed into the wells and sealed with 1% LMP agarose. The gel was then placed into a Biorad PFGE Chef Mapper electrophoresis chamber, and ∼2 L of 0.5× TBE was added. The temperature was allowed to equilibrate to 14 °C and, the pump flow was set at 70 (roughly 1 L/min). The run time was 22 h at 6 V/cm with a 50- to 90-s switch time ramp at an angle of 120 °C. The agarose gel was then stained with ethidium bromide and imaged under UV light. Lambda phage concatemers were used as molecular size markers (from the bottom to the top: 48.5, 97, 145.5, 194, 242.5, 291, 339, 388, 436.5, 485, 533, 582, 630, 679, and 727.5 kb).

Details of Strain Constructions. ZG485, ZG486, ZG687, ZG483, ZG484, ZG686, ZG685 were obtained by electoporation of pMR20*Pxyl*, pMR20*Pxyl*-BapE, pMR20*Pxyl*-BapEHis, pJS14*Pxyl*, pJS14*Pxyl*-BapE, pJS14*Pxyl*-BapEHis, and pJS14*Pxyl*-BapEFlag into CB15N ZG66, respectively.

ZG494 was obtained by electroporation of pJS14*Pxyl*-BapE into the localizome CB15N *xyl::popZ-mcherry* strain from the laboratory's localizome strain collection (3).

A ΦCR30 phage lysate prepared from strain MT1758 was used to transduce Δ*sidA* into strain ZG489 to get strain ZG682.

***Replacement of bapE native locus by bapE-flag* (ZG474) *and flag-bapE-ssrA* (ZG475).** ZG474 was obtained by electroporation of the integration vector pNPTS138-*bapE*-Flag into strain CB15N (ZG66). An overnight culture of strain ZG66 *bapE*::pNPTS138-*bapE-flag* was plated on PYE plates containing 3% sucrose. About 15 colonies that had grown on sucrose and were kanamycin (Kan) sensitive were tested by PCR and sequenced to verify the correct integration of *bapE-flag* at the *bapE* locus.The same protocol was used to generate strain ZG475. Integration vector pNPTS138-Flag-BapE-ssrA was electroporated into ZG66 to produce ZG475. It should be noted that cells of strain ZG474 expressing *bapE-flag* as the only copy of *bapE* look wild type under all conditions, indicating that *bapE-flag* is functional.

Replacement of recA native locus by flag-recA* (ZG689) *construct. A similar protocol as described above was used to generate strain ZG689. The integration vector pNPTS138-Flag-RecA was electroporated into ZG66 to produce ZG689. Cells of strain ZG689 expressing Flag-RecA as the only copy of *recA* look wild type under all conditions, indicating that Flag-recA is functional.

***Construction of bapE::gfp-bapE strain* (ZG683).** ZG683 was obtained by electroporation of the integration vector pNPTS138-Gfp-BapE into strain ZG66. Kan-resistant colonies were tested by PCR and sequenced to verify the correct integration of *gfp-bapE* under the endogenous *bapE* promoter.

***Construction of ΔbapE strain* (ZG683).** ZG684 was obtained by electroporation of the integration vector pMCS-2-ΔBapE into strain ZG66. Kan-resistant colonies were tested by PCR and sequenced to verify the correct integration of truncated *bapE* under the endogenous *bapE* promoter.

Details of Plasmid Constructions. ***pNPTS138-BapE-Flag.*** Thirty base pairs upstream of the BapE start codon and 400 bp downstream of the BapE stop codon were PCR-amplified from a genomic DNA preparation of strain CB15N by using overlapping primers (listed in Table S3) that contain the 24-bp sequence coding the Flag-tag

immediately before the BapE stop codon. A mix of these two overlapping PCR fragments (produced by using the combination of the primers CC0627up_short+ CC0627Flag_R and CC0627down_short +CC0627Flag_F) was used as a template for the next PCR using primers CC0627up_short and CC0627 down_short. The generated 800-bp PCR fragment was purified and digested with EcoRI and NheI and ligated into pNPTS138 cut with EcoRI and NheI, creating plasmid pNPTS138-BapE-Flag.

pNPTS138-Flag-BapE-ssrA. Eight hundred base pairs upstream of the BapE start codon and 400 bp downstream of the BapE stop codon were PCR-amplified from a genomic DNA preparation of strain CB15N by using a set of overlapping primers (listed in Table S3): mid-Flag_F and midflag_R, which contain the 24-bp sequence coding the Flag-tag inserted immediately after the BapE start codon, and midssrA_F and midssrA_R, which contain the 42-bp sequence coding the ssrA-tag before the BapE stop codon. First, three overlapping PCR fragments were obtained by using the combination of the primers CC0627up_-800 +midflag_R, midFlag_F + midssrA_R, and midssrA_F + CC0627down_short, respectively. A mix of these three overlapping PCR fragments was then used as a template for another round of PCR, using primers CC0627up_-800 and CC0627down_short. The resulting ~ 1,600-bp PCR fragment was purified and digested with EcoRI and NheI and ligated into pNPTS138 cut with EcoRI and NheI to generate plasmid pNPTS138-Flag-BapE-SsrA.

pMCS-2-ΔBapE. The 3′ end of the *bapE* gene was PCR-amplified from pNPTS-*gfp*-BapE with the SacI-STOP-BapE F and NheI-FLAG-BapEint R primers. The SacI-STOP-BapE F primer truncated the first two codons of BapE and introduced a stop codon before the third codon. The NheI-FLAG-BapEint R primer truncated the last 156 bp of the gene and introduced the FLAG tag and a STOP codon at the end. The amplified 150-bp PCR product was purified, digested with SacI and NheI, and ligated into pMCS-2 (4) to generate pMCS-2-ΔBapE.

pNPTS138-gfp-BapE. *gfp-bapE* was PCR-amplified from the gVGFP-BapE construct, using primer pairs PrBapEGFPstart_F and BapE-end_R (Table S3). An 800-bp region upstream of the BapE start codon and a 400-bp region downstream of the BapE stop codon were PCR-amplified from a genomic DNA preparation of strain CB15N by using the primers CC0627_up−800/PRBapEGFPstartREV and BapEendFOR/CC0627_down+400, respectively (Table S3). A mix of these three overlapping PCR fragments was then used as a template for another round of PCR, using primers CC0627_up−800 and CC0627_down+400. The resulting ~ 2,280-bp PCR fragment was purified and digested with EcoRI and NheI and ligated into pNPTS138 cut with EcoRI and NheI to generate plasmid pNPTS138-GFP-BapE .

pNPTS138-flag-recA. A fragment containing the *flag-recA* fusion was PCR-amplified from a genomic DNA preparation of strain CB15N by using a set of overlapping primers (listed in Table S3): FORRecA_EcoRI (400 bp upstream of *recA* start codon), FORRecAFLAG and REVRecAFLAG (which contain the 24-bp sequence coding the Flag-tag inserted immediately after RecA start codon), and REVRecA_NheI (385 bp downstream of the *recA* start codon). First, two overlapping PCR fragments were obtained by using the combination of the primer pairs FORRecA_EcoRI + REVRecAFLAG and FORRecAFLAG + REVRecA_NheI, respectively. A mix of these two overlapping PCR fragments was then used as a template for another round of PCR, using primers FORRecA_EcoRI and REVRecA_NheI. The resulting ~ 800-bp PCR fragment was purified and digested with EcoRI and NheI and ligated into pNPTS138 cut with EcoRI and NheI to generate plasmid pNPTS138-Flag-RecA.

pENTRY-BapE, pENTRY-BapEHis, pENTRY-BapEFlag. To obtain the entry vector pENTRY-BapE, the BapE ORF (stop codon included) was PCR-amplified from a genomic DNA preparation of strain CB15N by using primers att0627_F and att0627_Rstop (Table S3). The purified PCR product was recombined into the donor vector pDONR223 at its *attP* sites by using bacteria-phage (BP) Clonase II (Invitrogen) as described in the BP reaction protocol provided by Invitrogen. pENTRY-BapEHis and pENTRY-BapEFlag were similarly obtained by using primers att0627_F and att0627HisR and primers att0627_F and att0627FlagR, respectively (Table S3).

pMR20-*Pxyl*-BapE is the construct obtained by an left-right (LR) reaction between the destination vector pMR20-GW (containing the gateway cassette *ccdB*$^+$-*cat*, flanked by *attR* sites) and pENTRY-BapE (containing BapE ORF flanked by *attL* sites) by using LR Clonase (Invitrogen). To verify that the *Caulobacter* strain contained the expression vector with the BapE insert in the Gateway cassette, colonies were patched onto PYE plates containing either Kan and chloramphenicol (Cm) or Kan alone. Strains possessing the expression vector became Cm^S because of replacement of the *cat* gene in the Gateway cassette with the entry vector BapE ORF.

pMR20-*Pxyl*-BapEHis was similarly obtained by an LR reaction between the destination vector pMR20-GW and pENTRY-BapEHis.

pJS14-*Pxyl*-BapE is the construct obtained by LR reaction between the destination vector pJS14-GW (containing the gateway cassette *ccdB*$^+$-*cat*, flanked by *attR* sites) and pENTRY-BapE (containing BapE ORF flanked by *attL* sites) by using LR Clonase (Invitrogen).

pJS14-*Pxyl*-BapEHis and pJS14-*Pxyl*-BapEFlag were similarly obtained by an LR reaction between the destination vector pJS14-GW and pENTRY-BapEHis or pENTRY-BapEFlag, respectively.

pET-BapE-His6. BapE was amplified by PCR from a genomic DNA preparation of CB15N by using primers PET0627-F (which contains an optimized *Escherichia coli* Shine Dlagarno sequence upstream of the BapE start codon) and PET0627-R (which contains the his6-tag before the BapE stop codon) (Table S3). The purified PCR product was digested with EcoRI and XhoI and ligated into the same restriction sites of vector pET28A.

gVGFP-BapE. gVGFP is the destination vector for N-terminal fusion of *gfp* ORF to an ORF of interest under *Pvan* promoter (laboratory collection). gVGFP-BapE was obtained by LR reaction between the destination vector gVGFP (containing the gateway cassette *ccdB*$^+$-*cat*, flanked by *attR* sites under the *Pvan* promoter and in frame with the *gfp* ORF downstream) and pENTRY-BapE (containing the BapE ORF flanked by *attL* sites) by using LR Clonase (Invitrogen). gVGFP-BapE was used as a PCR template to obtain the pNPTS138-GFPBapE construct.

1. Ely B (1991) Genetics of Caulobacter crescentus. *Methods Enzymol* 204:372–384.
2. Galhardo RS, Rocha RP, Marques MV, Menck CF (2005) An SOS-regulated operon involved in damage-inducible mutagenesis in Caulobacter crescentus. *Nucleic Acids Res* 33(8):2603–2614.
3. Werner JN, et al. (2009) Quantitative genome-scale analysis of protein localization in an asymmetric bacterium. *Proc Natl Acad Sci USA* 106(19):7858–7863.
4. Thanbichler M, Iniesta AA, Shapiro L (2007) A comprehensive set of plasmids for vanillate- and xylose-inducible gene expression in Caulobacter crescentus. *Nucleic Acids Res* 35(20):e137.

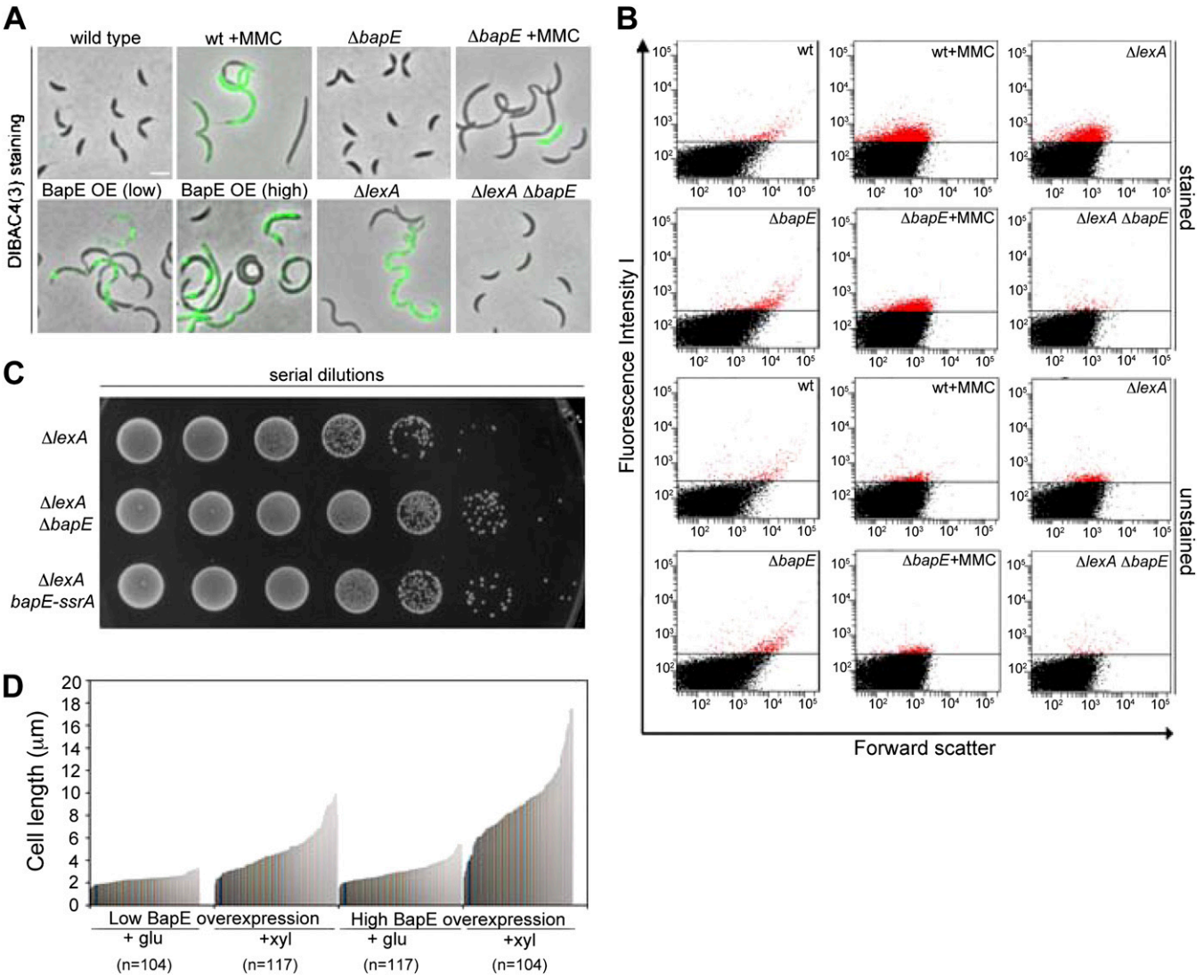

Fig. S1. BapE mediates DNA damage-induced programmed cell death. (*A*) Overlay images of phase contrast and green fluorescence [DIBAC4(3)] of indicated *Caulobacter* cells. (Scale bar, 2 μm.) (*B*) Flow cytometry plots illustrating the detection of DNA strand breaks in apoptotic wild-type and mutant *Caulobacter* cells, grown either with or without MMC, by TUNEL assay. The location of the horizontal threshold line between TUNEL-negative cells (highlighted in black) and TUNEL-positive cells (highlighted in red) was determined on the basis of unstained cells. (*C*) Overnight cultures of strains ZG488 (Δ*lexA*), ZG489 (Δ*lexA bapE-ssrA*), and ZG482 (Δ*lexA* Δ*bapE*) were normalized to the same OD_{660} and serially diluted and spotted onto PYE plates to determine viable cell counts. We obtained the following numbers of cfu/mL: $3.0 \pm 6 \times 10^{+08}$ (Δ*lexA*), $19.0 \pm 10 \times 10^{+08}$ (Δ*lexA* Δ*bapE*), and $12.0 \pm 10^{+08}$ (Δ*lexA* Δ*bapE*). (*D*) Analysis of cell length in cells with high- and low-copy BapE overexpression. Strains ZG687 and ZG686 were grown for 6 h in PYE medium in the presence of xylose to induce BapE expression or glucose to repress BapE expression. Cells were then imaged under the microscope. Length of individual cells was calculated using NIS-Elements software. *n*, number of cells.

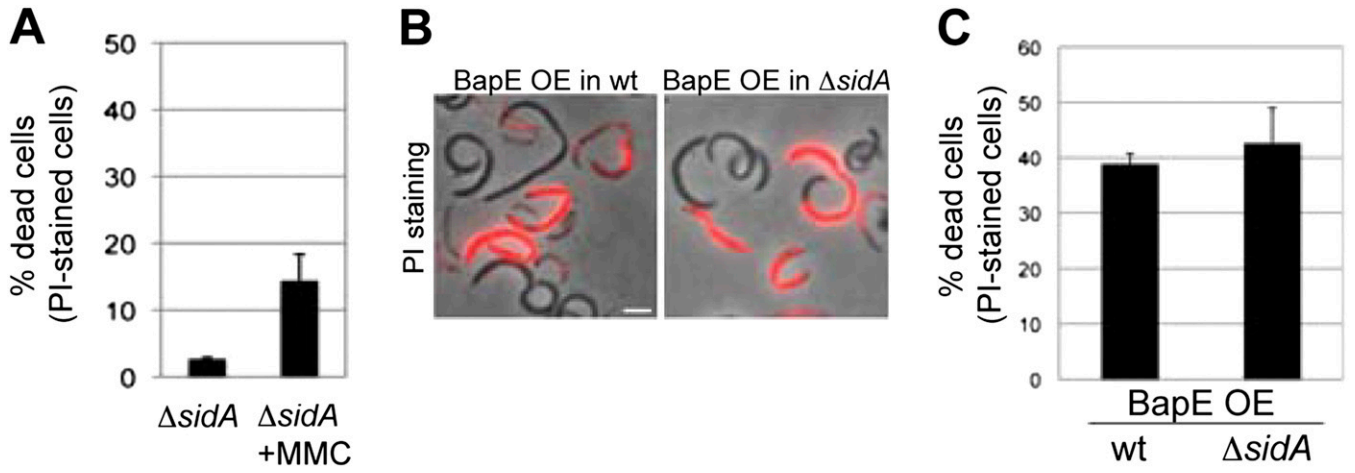

Fig. S2. BapE promotes cell death independently of SidA. (*A*) Quantification of the number of PI-stained cells in strain Δ*sidA* grown without (n = 416) or with MMC (n = 224). Error bars denote the SE. (*B*) Overlay images of phase-contrast images and red fluorescence images (PI) of strains ZG484 (*Left*) and ZG740 (*Right*), taken after xylose induction and PI staining. PI-stained cells indicate dead cells. (Scale bar, 2 μm.) (*C*) Quantification of the number of PI-stained cells in strains ZG484 (n = 339) and ZG740 (n = 340). Error bars denote the SE.

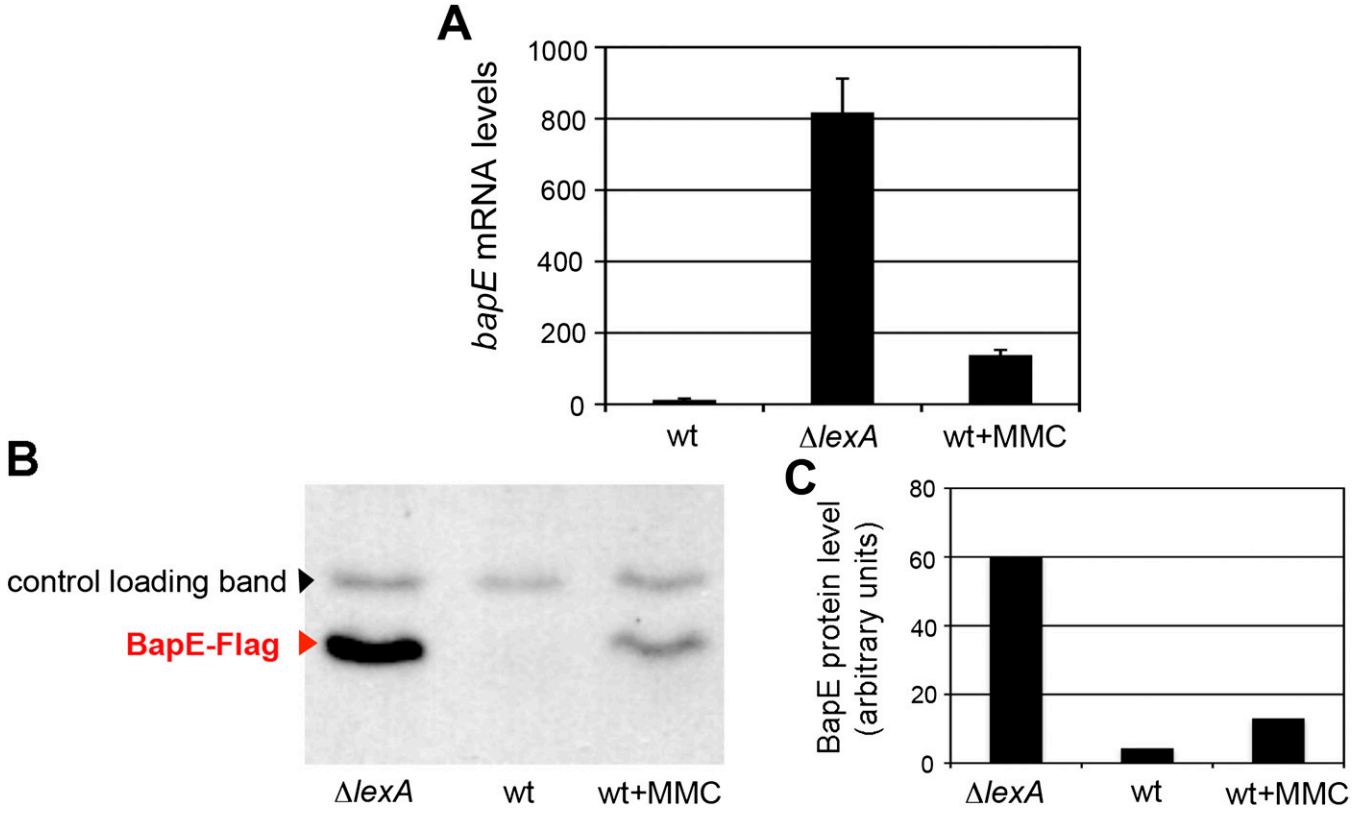

Fig. S3. BapE mRNA and protein levels increase upon DNA damage. (*A*) Cellular mRNA levels of *bapE* (relative to *rpoD*, taken as an endogenous reference) were measured from midlog-phase cultures of wild-type (ZG66) and Δ*lexA* (ZG488) cells by quantitative PCR using the primer pairs listed in Table S3. MMC (1 μg/mL) was added for 30 min. Error bars denote the SE. (*B*) Western blot analysis performed on cell lysates of strain ZG474 (*bapE::bapE-flag*) grown with or without MMC (1 μg/mL, added for 4 h) and strain ZG495 (Δ*lexA bapE::bapE-flag*) with an anti-Flag antibody. The red arrowhead shows the BapE-Flag band (13 kDa); the black arrowhead shows a loading control band. (*C*) Quantification of BapE protein level given in arbitrary units. BapE band intensity was normalized to the loading control band in strains described in *B* using Image J software.

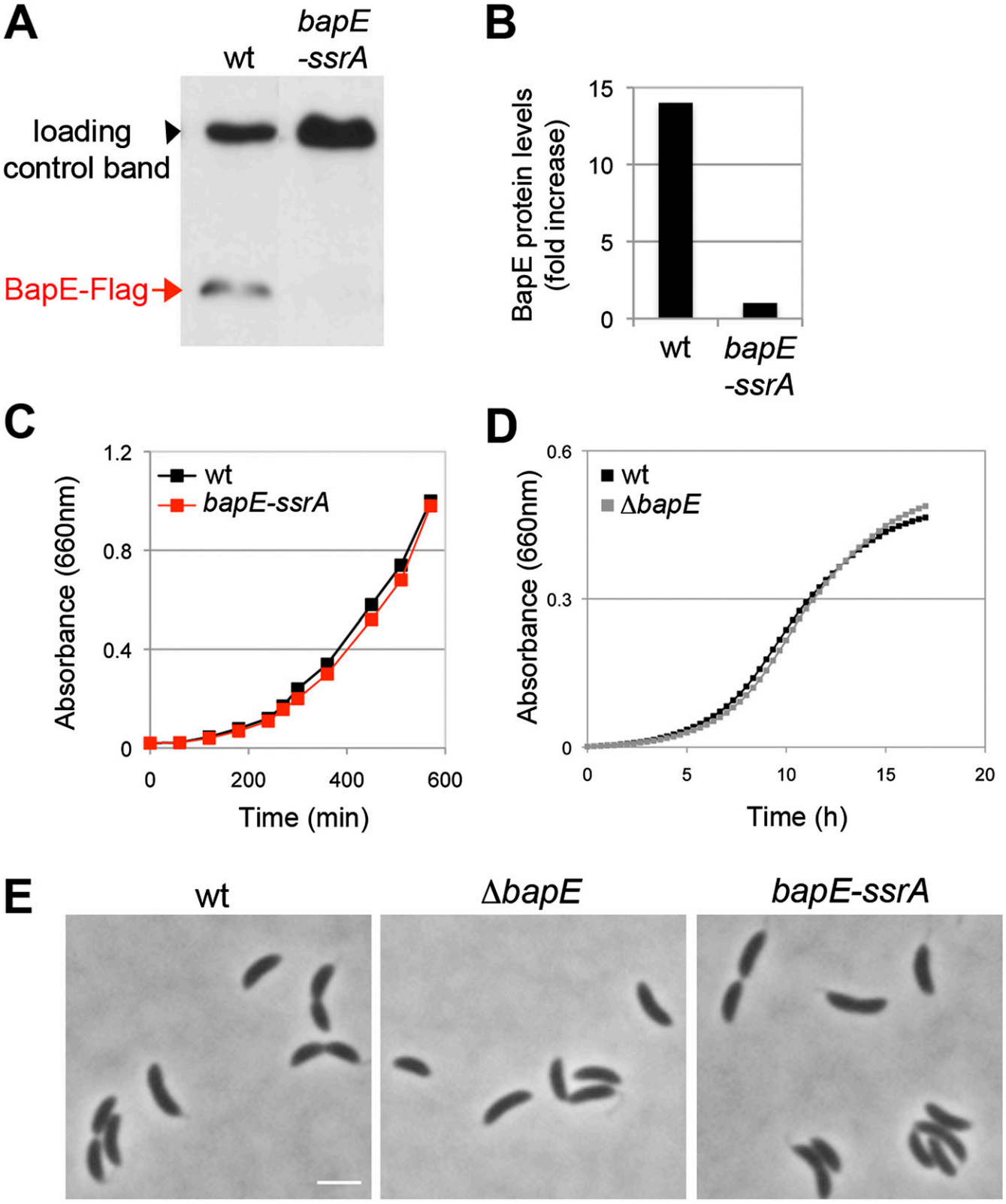

Fig. S4. BapE is not essential under normal growth conditions. (*A*) The *bapE-ssrA* fusion protein is unstable. Western blot analysis of Flag-tagged BapE protein in strains ZG474 (*bapE-flag*) and ZG475 (*flag*-bapE-*ssrA*), using an anti-Flag antibody. The addition of the SsrA-tag at the BapE C terminus reduces BapE protein abundance. The red arrow shows BapE-Flag band (~13 kDa); the black arrowhead shows a loading control band. (*B*) Quantification of BapE protein levels (fold increase) in strains described in *A* was performed using Image J software. (*C*) Growth curves of wild type (ZG66) (black squares) and *bapE*::*flag-bapE-ssrA* (ZG475) (red squares). Samples were withdrawn at 30- and 60-min intervals for absorbance measurements at 660 nm. (*D*) Growth curves of wild type (ZG66) (black squares) and Δ*bapE* (ZG684) (gray squares). Samples were withdrawn at 20-min intervals for absorbance measurements at 660 nm using an automated microplate reader. (*E*) Microscopy phase images of ZG66, ZG475, and ZG684 strains grown under normal growth conditions. (Scale bar, 2 μm.)

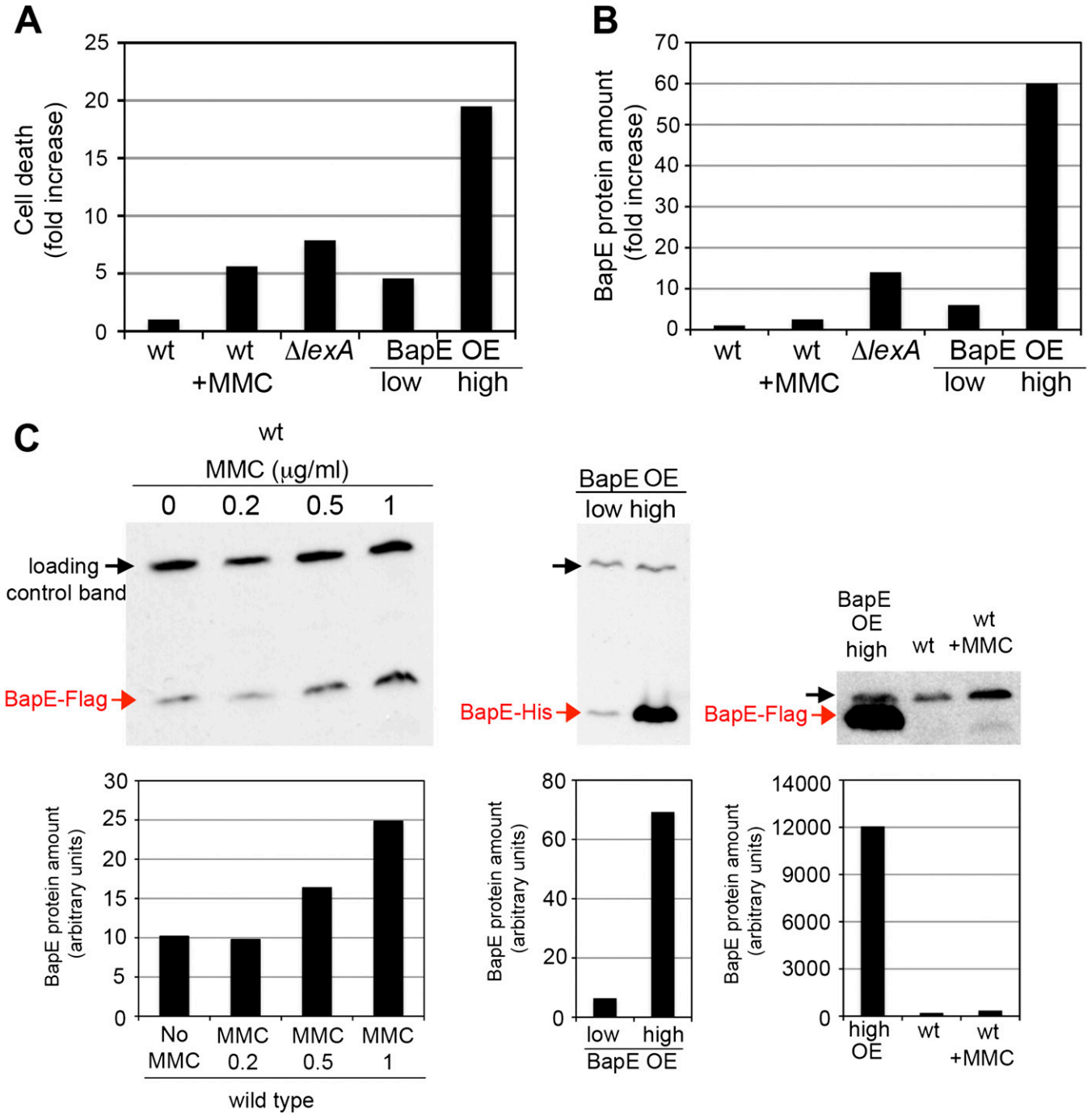

Fig. S5. BapE protein expression levels correlate with the levels of cell death induced. (*A*) Fold increase in the number of dead cells (PI-stained cells) in wild-type (treated either with or without MMC), Δ*lexA*, low-BapE overexpression, and high-BapE overexpression strains. The values were obtained on the basis of the quantification results shown in Fig. 1*B* and represent the ratio of the number of dead cells (%) in the indicated strains to the number of dead cells (%) in the wild-type strain (with no MMC treatment) taken as a reference. The fold increase in cell death is then plotted for each strain on the *y* axis in Fig. 3*A* as a function of BapE protein levels (see *B* and *C*). (*B*) Fold increase in BapE protein amount in wild-type (ZG66) (treated either with or without 1 μg/mL MMC), Δ*lexA* (ZG488), low-BapE overexpression (ZG687), and high-BapE overexpression (ZG686) strains. The values were obtained by quantifying the intensity of the BapE band on the Western blot performed in the indicated strains (Fig. S5*C*) and by comparing BapE band intensity in the indicated strains to BapE band intensity in the wild-type strain (without MMC treatement) taken as a reference. The fold increase in BapE protein level is then plotted for each strain on the *x* axis in Fig. 3*A*. (*C*) Examination of BapE protein levels by Western blotting in different strain backgrounds. (*Left*) ZG474 *(bapE::bapE-flag)* treated with increasing concentrations MMC (0, 0.2, 0.5, and 1 μg/mL). (*Center*) ZG687 (low-BapE overexpression, P*xyl-bapEhis*) and ZG686 (high-BapE overexpression, P*xyl-bapEhis*). (*Right*) ZG685 (high-BapE overexpression, P*xyl-bapEflag*) and ZG474 (*bapE::bapE-flag)* (either without or with 1 μg/mL MMC). Western blots were performed by using either an anti-Flag or an anti-His antibody. Red arrows show the BapE-Flag and BapE-His bands (~13 kDa); black arrows indicate a loading control band. Quantification of BapE protein levels using Image J software is shown below each blot. In all blots, BapE band intensity values (given in arbitrary units) are normalized to the loading control band.

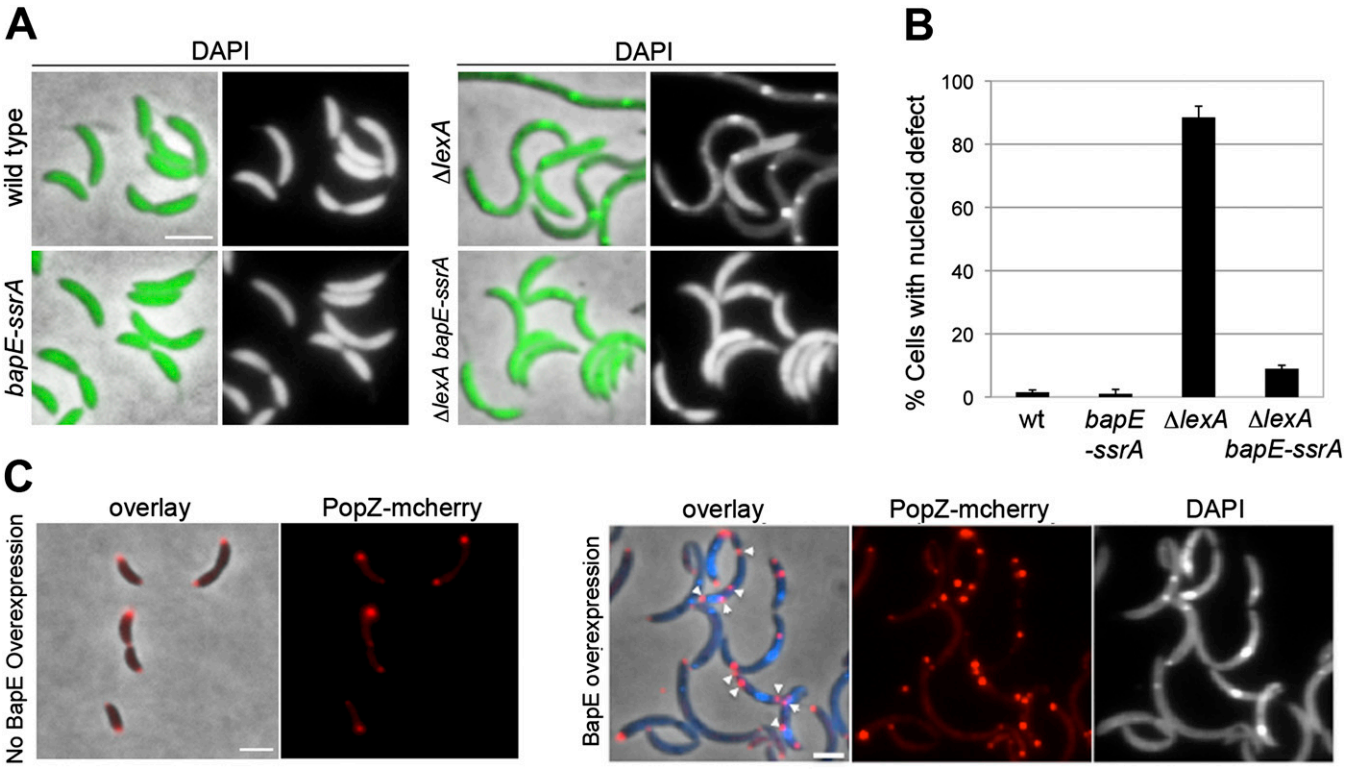

Fig. S6. BapE perturbs chromosome organization. (*A*) Strains ZG66 (wild type, *Upper Left*), ZG475 (*bapE-ssrA*, *Lower Left*), ZG488 (Δ*lexA*, *Upper Right*) and ZG489 (Δ*lexA bapE-ssrA*, *Lower Right*) were stained with DAPI to image chromosome morphology. Overlayed phase/fluorescence (*Left*) and fluorescence images (*Right*) are shown for each strain. (*B*) Quantification of defects in chromosome organization from the experiment in *A*. (*C*) Localization of PopZ-mCherry in BapE nonoverexpressing cells (*Left*) and in BapE-overexpressing cells (*Right*). (Scale bar is 2 μm.) Strain ZG494 was grown in PYE medium containing xylose to induce both PopZ-mCherry and BapE expression. PopZ-mCherry localization and nucleoid morphology were examined by fluorescence microscopy on an agarose pad containing DAPI. PopZ-mCherry forms multiple ectopic foci in DNA-free regions in 11 ± 2% of BapE-overexpressing cells. Arrowheads denote the presence of PopZ-mCherry ectopic foci. (Scale bar, 2 μm.)

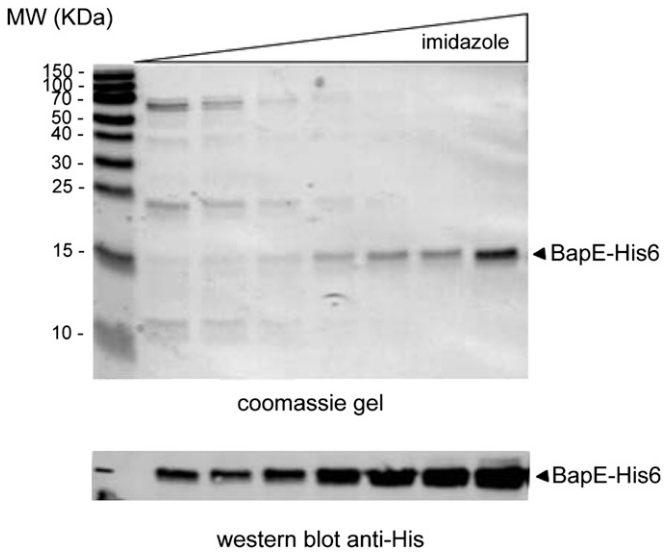

Fig. S7. Purification of BapE-His6 protein. Analysis of eluate fractions containing BapE-His_6. (*Upper*) Coomassie blue-stained gel of eluate fractions obtained after applying a gradient of imidazole: 100 mM (lanes 1–3), 200 mM (lanes 4–6), and 250 mM (lanes 7–8). (*Lower*) Western blot analysis of eluate fractions described above using an anti-His antibody. Arrowheads indicate BapE-His_6 protein (13 kDa).

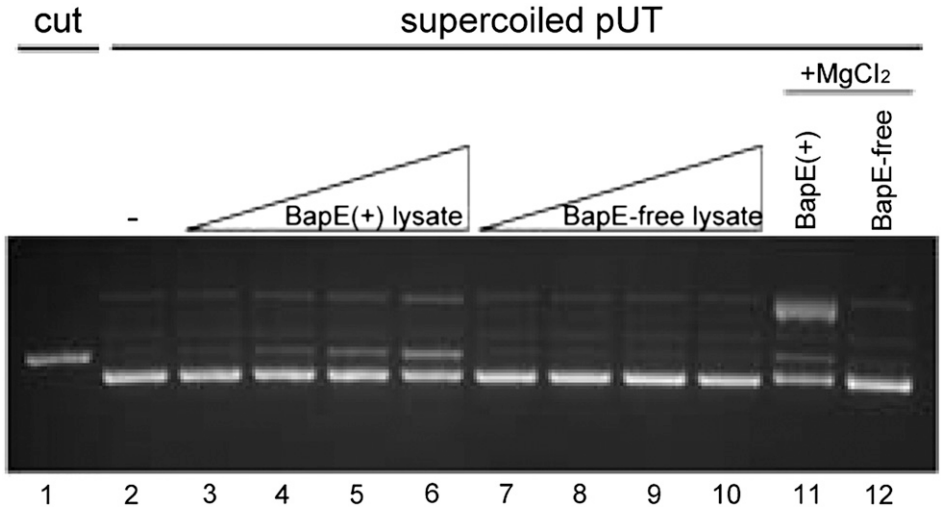

Fig. S8. BapE-free lysate exhibits no DNA endonuclease activity. Supercoiled plasmid DNA (pUT) was incubated alone (lane 2), with increasing concentrations of a dialyzed eluate fraction containing BapE-His_6 protein (from ZG496 strain culture) (lanes 3–6 and 11), with increasing concentrations of a dialyzed eluate fraction of BapE-free lysate (from ZG299 strain culture) (lanes 7–10 and 12), and with $MgCl_2$ (2 mM) (lanes 11–12). EcoRI-cut pUT plasmid is shown as a control of linear state (lane 1).

Table S1. Strains used in this study

Strains	Relevant genotype or description	Source or reference
Caulobacter strains		
ZG66	CB15N (NA1000) wild type	Laboratory collection
ZG684	CB15N Δ*bapE*	This work
ZG474	CB15N *bapE::bapE-flag*	This work
ZG475	CB15N *bapE::flag-bapE-ssrA*	This work
ZG488	CB15N Δ*lexA*	This work
ZG495	CB15N Δ*lexA bapE::bapE-flag*	This work
ZG489	CB15N Δ*lexA bapE::flag-bapE-ssrA*	This work
MT1758	CB15N Δ*sidA*	(1)
MT1760	CB15N Δ*lexA* Δ*sidA*	(1)
ZG682	CB15N Δ*lexA* Δ*sidA bapE::flag-bapE-ssrA*	This work
ZG482	CB15N Δ*lexA* Δ*bapE*	This work
ZG481	CB15N Δ*lexA* Δ*sidA* Δ*bapE*	This work
ZG683	CB15N *bapE::gfp-bapE*	This work
ZG483	CB15N pJS14-*Pxyl*	This work
ZG484	CB15N pJS14-*Pxyl- bapE*	This work
ZG740	CB15N Δ*sidA* pJS14-*Pxyl*-BapE	This work
ZG685	CB15N pJS14-*Pxyl*-BapEFlag	This work
ZG686	CB15N pJS14-*Pxyl*-BapEHis	This work
ZG485	CB15N pMR20-*Pxyl*	This work
ZG486	CB15N pMR20-*Pxyl*-BapE	This work
ZG687	CB15N pMR20-*Pxyl*-BapEHis	This work
ZG494	CB15N *xylX::popZ-mCherry* pJS14-*Pxyl*-BapE	This work
ZG689	CB15N *recA::flag-recA*	This work
E. coli strains		
DH5α	*F^- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17(r_K^- m_K^+), λ–*	Laboratory collection
BL21(DE3)	*F^- ompT gal dcm lon hsdS_B(r_B^- m_B^-) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])*	Laboratory collection
ZG496	BL21 pET28-BapEHis6	This work
ZG299	BL21 pET28-His6	Laboratory collection

1. Modell JW, Hopkins AC, Laub MT (2011) A DNA damage checkpoint in Caulobacter crescentus inhibits cell division through a direct interaction with FtsW. *Genes Dev* 25(12):1328–1343.

Table S2. Plasmids used in this study

Plasmids	Relevant genotype or description	Source or reference
pMR20-*Pxyl*	RK2-based Tet^R broad range host vector, contains xylose inducible promoter	(1)
pJS14-*Pxyl*	pBBR1-based Tet^R broad range host vector, contains xylose inducible promoter	Lab collection
pMR20-*Pxyl*-GW	pMR20 containing gateway cassette (*att-ccdB-Cm*)	Lab collection
pMR20-*Pxyl*-BapE	pMR20 carrying BapE under control of *Pxyl*	This work
pMR20-*Pxyl*-BapEHis	pMR20 carrying BapE-*his6* under control of *Pxyl*	This work
pJS14-*Pxyl*-GW	pJS14 containing gateway cassette (*att-ccdB-Cm*)	Lab collection
pJS14-*Pxyl*-BapE	pJS14 carrying BapE under control of *Pxyl*	This work
pJS14-*Pxyl*-BapEHis	pJS14 carrying BapE-*his6* under control of *Pxyl*	This work
pJS14-*Pxyl*-BapEFlag	pJS14 carrying BapE-*flag* under control of *Pxyl*	This work
pNPTS138	Kan^R integration vector, derivative of pLITMUS28 with *sacB*$^+$ and oriT	Lab collection
pNPTS138-BapE-Flag	EcoRI-NheI fragment containing BapE-*flag and flanking regions* in pNPTS138	This work
pNPTS138-Flag-BapE-ssrA	EcoRI-NheI fragment containing flag-BapE-*ssrA and flanking regions* in pNPTS138	This work
pNPTS138-GFPBapE	EcoRI-NheI fragment containing *gfp*-BapE *and flanking regions* in pNPTS138	This work
pNPTS138-Flag-RecA	EcoRI-NheI fragment containing flag-*recA and flanking regions* in pNPTS138	This work
pMCS-2	Integrating vector	(2)
pMCS-2-ΔBapE	SacI-NheI fragment containing truncated BapE in pMCS-2	This work
pET28A+	for IPTG-inducible protein expression in *E. coli*	Novagen
pET28-BapEHis6	EcoRI-XhoI fragment containing BapE*his6* in pET28A+	This work
pENTRY-BapE	Contains *att*-BapE-*att*	This work
pENTRY-BapEHis	Contains *att*-BapE-*his-att*	This work
pENTRY-BapEFlag	Contains *att*-BapE-*flag-att*	This work

1. Roberts RC, Mohr CD, Shapiro L (1996) Developmental programs in bacteria. *Curr Top Dev Biol* 34:207–257.
2. Thanbichler M, Iniesta AA, Shapiro L (2007) A comprehensive set of plasmids for vanillate- and xylose-inducible gene expression in Caulobacter crescentus. *Nucleic Acids Res* 35(20): e137.

Table S3. Primers used in this study

Primer	Sequence
CC0627_upshort	CTGGAATTCGTGCGCCAGAAGAGAACA
CC0627_up-800	GGGAATTCCGCCTTCACGCCCACCCGGC
CC0627_down+400	CTAGGCTAGCACCCTCCTCTAAACGGAACC
CC0627-flag_F	GTCGCCTGCTGGTCGCGAGACTACAAGGACGACGACGACAAGTGAAGGGGCTTGGCACCC
CC0627-flag_R	GGGTGCCTAAGCCCCTTCACTTGTCGTCGTCGTCCTTGTAGTCTCGCGACCAGCGGCGAC
5′ MIDFLAG	CTTTTGGACTACAAGGACGACGACGACAAGGGGCTTGGTATGTCCGC
3′MIDFLAG	CTTGTCGTCGTCGTCCTTGTAGTCCAAAAGTTCGCGTTATGTTCTCT
5′ MIDSSRA	GCAGCAAACGACAACTTCGCAGAGGAGGACGCAGTTGCTGCATGAAGGGGC
3′MIDSSRA	CATGCAGCAACTGCGTCCTCCTCTGCGAAGTTGTCGTTTGCTGCTCGCGACCAGAGGCG
FOR RecA_EcorI	GGAATTCGCCAGCGCAGCCTCGCTC
FOR RecAFLAG	GATCAACAATGGACTACAAGGACGACGACGACAAGACAAGTCAGGCGGCTTTG
REV RecAFLAG	CAAAGCCGCCTGACTTGTCTTGTCGTCGTCGTCCTTGTAGTCCATTGTTGATC
REV_RecA_NheI	CTAGCGCTAGCGAGAGACCAGGAGATTATCG
SacI-STOP-BapE_F	CATGAGCTCTAACTTGGTATGTCCGCCAACGC
NheIFLAGBapEint_R	CATGCTAGCTTACTTGTCATCGTCGTCCTTGTAGTCTCGAGCTATCCCGCAATGCG
PRBapEGFPstart_F	CCAGAAGAGAACATAACGCGAACTTATGGTGAGCAAGGGCGAGGAGCTG
PRBapEGFPstart_R	CAGCTCCTCGCCCTTGCTCACCATAAGTTCGCGTTATGTTCTCTTCTGG
BapEend_F	TGGTCGCGATGAAGGGGC
BapEend_R	GCCCCTTCATCGCGACCA
Pxyl	GGTCAGACAACCTACTTGCCG
qCC0627_F	GGTATCCGACAAGCAAGTGG
qCC0627_R	AGCTTGTAGAGCGGCACATC
qlexA_F	GTCTTGGAAGTCCAGGGTGA
qlexA_R	CCAAAGATCCGGGTTTCATA
qrpoD_F	CTCTATGCGATCAACAAGCG
qrpoD_R	ATAGGCCTTGAGGAACTCGC
qcc0382_F	ACCCGTTCTACGAGGCCTAT
qcc0382_R	GACCTTCTCGGGATCGAAAT
qcc3236_F	AAGGGTAATCTCCGCGTCTT
qcc3236 _R	GCTTCGAGATTGGTCAGGAT
qcc1087_F	GAAGAAGGCGATAAGCAACG
qcc1087_R	GCTTTCCGGACCATAGACCT
qcc2590_F	ACAGGCAACAACCTGAAAGG
qcc2590_R	TCGATGACCTTGTCGAAGTG
att0627F	GGGGACAACTTTGTACAAAAAAGTTGGCATGGGGCTTGGTATGTCC
att0627_R_stop	GGGGACAACTTTGTACAAGAAAGTTGGGTCATCGCGACCAGCAGGCGACG
att0627Flag_R	GGGGACAACTTTGTACAAGAAAGTTGGGTCACTTGTCGTCGTCGTCCTTGTAGTCTCGCGACCAGCAGGCGACGC
att0627His_R	GGGGACAACTTTGTACAAGAAAGTTGGGTCAGTGGTGATGGTGATGATGTCGCGACCAGCAGGCGACG
pET0627_F	GGGAATTCCAGGAGGAAACGATGGGGCTTGGTATG
pET0627_F	GGGAATTCCAGGAGGAAACGATGGGGCTTGGTATG
3′R0627HIS_xhoI	CCGCCTCGAGTCGCGACCAGCAGGC