Depurination of colibactin-derived interstrand cross-links

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Biochemistry

Supporting Information

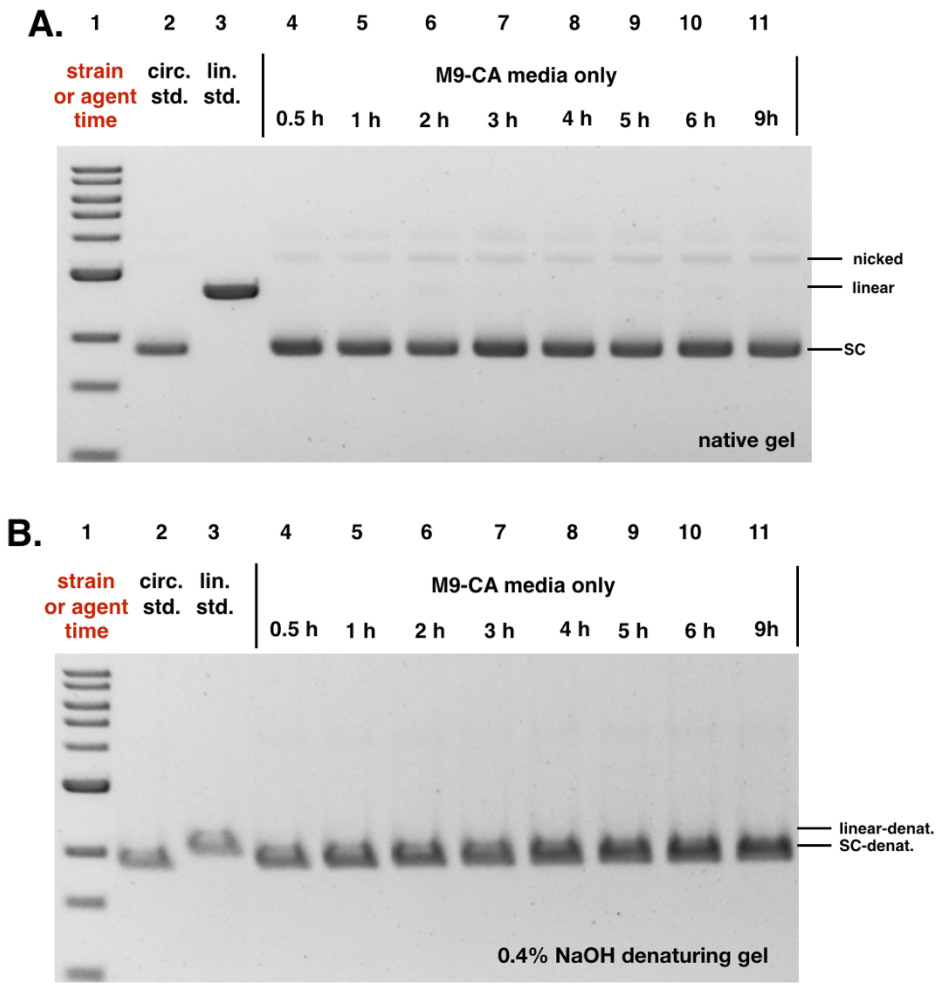

Fig. S1. DNA plasmid cleavage assay employing circular pUC19 DNA and M9-cas amino acid media (M9-CA media) only. **A. Native gel; B. 0.4% NaOH denaturing gel.** DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); linearized pUC19 DNA standard (Lane # 3); M9-CA media 0.5 h (Lane #4), 1 h (Lane #5), 2 h (Lane #6), 3 h (Lane #7) 4 h (Lane #8), 5 h (Lane #9), 6 h (Lane #10), and 9 h (Lane #11). Conditions (Lane #4–#11): circular pUC19 DNA (7.7 μM in base pairs), M9-CA media, 37 °C, reaction proceed for 0.5 h,1 h, 2 h, 3 h, 4 h, 5 h, 6 h, and 9 h. The DNA was isolated, purified, and analyzed either (**Fig. S1A**) by native agarose gel electrophoresis, or (**Fig. S1B**) by 0.4% NaOH denaturing agarose gel electrophoresis (90 V, 2 h). SC-denat. = supercoiled DNA in denaturing form, linear-denat. = linearized DNA in denaturing form.

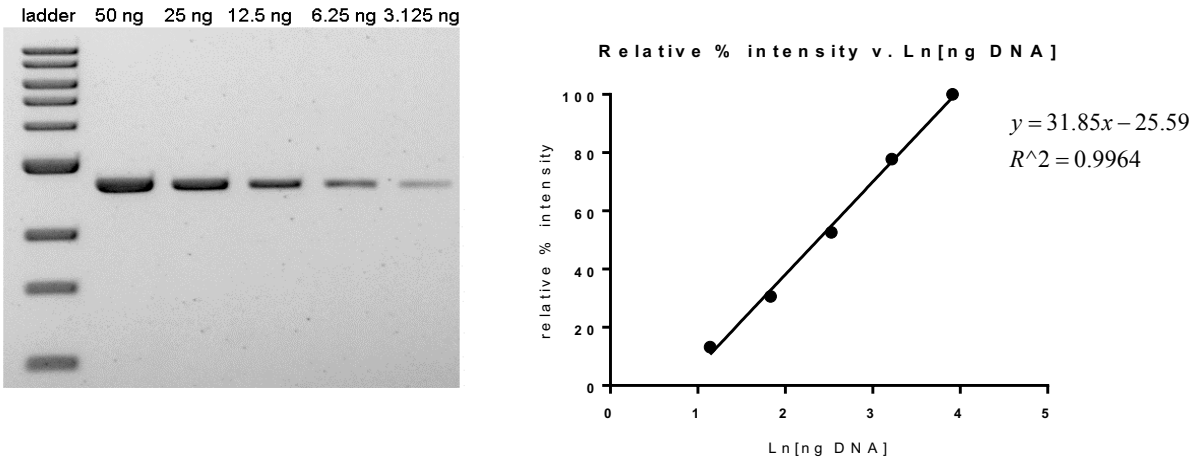

Fig. S2. Calibration curve correlating the intensity of the gel band with the amount of DNA present. DNA ladder (Lane #1); 50 ng DNA (Lane #2); 25 ng DNA (Lane #3); 12.5 ng DNA (Lane #3); 6.25 ng DNA (Lane #4); 3.125 ng DNA (Lane #5). Gel band intensity is expressed as a ratio vs. Lane #2. The data show that the gel band intensity increases as the ln (DNA quantity in ng) increases. $R^2 = 0.9964$.

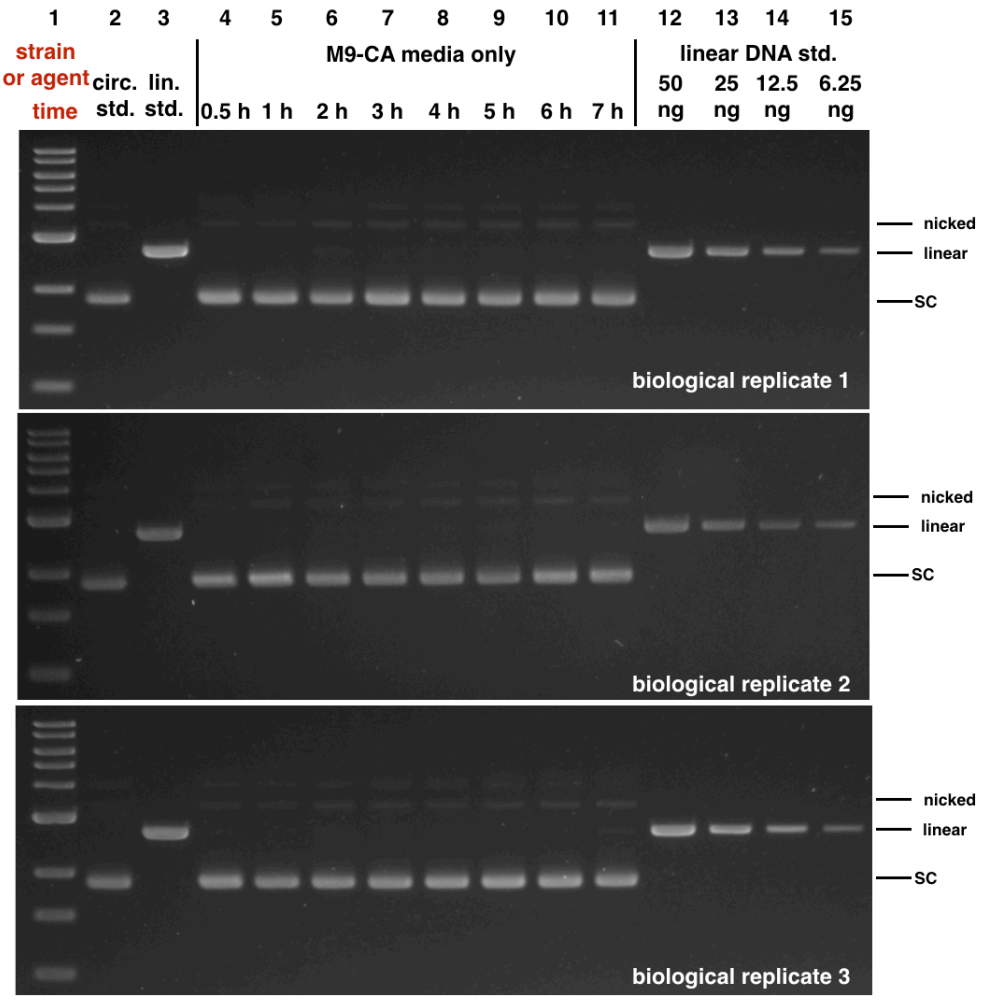

Fig. S3. DNA plasmid cleavage assay employing circular pUC19 DNA and M9-CA media. DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); linearized pUC19 DNA standard (Lane # 3); M9-CA media 0.5 h (Lane #4), 1 h (Lane #5), 2 h (Lane #6), 3 h (Lane #7), 4 h (Lane #8), 5 h (Lane #9), 6 h (Lane #10), and 9 h (Lane #11); linearized pUC19 DNA standard 50 ng (Lane #12), 25 ng (Lane #13), 12.5 ng (Lane #14), and 6.25 ng (Lane #15). Conditions (Lane #4–#11): circular pUC19 DNA (7.7 μM in base pairs), M9-CA media, 37 °C, reaction proceed for 0.5 h,1 h, 2 h, 3 h, 4 h, 5 h, 6 h, and 9 h. The DNA was isolated, purified, and analyzed by native agarose gel electrophoresis (90 V, 2 hr). The experiment was performed and analyzed in biological triplicates for quantification purpose. SC = supercoiled, nicked = SSB, linear = DSB.

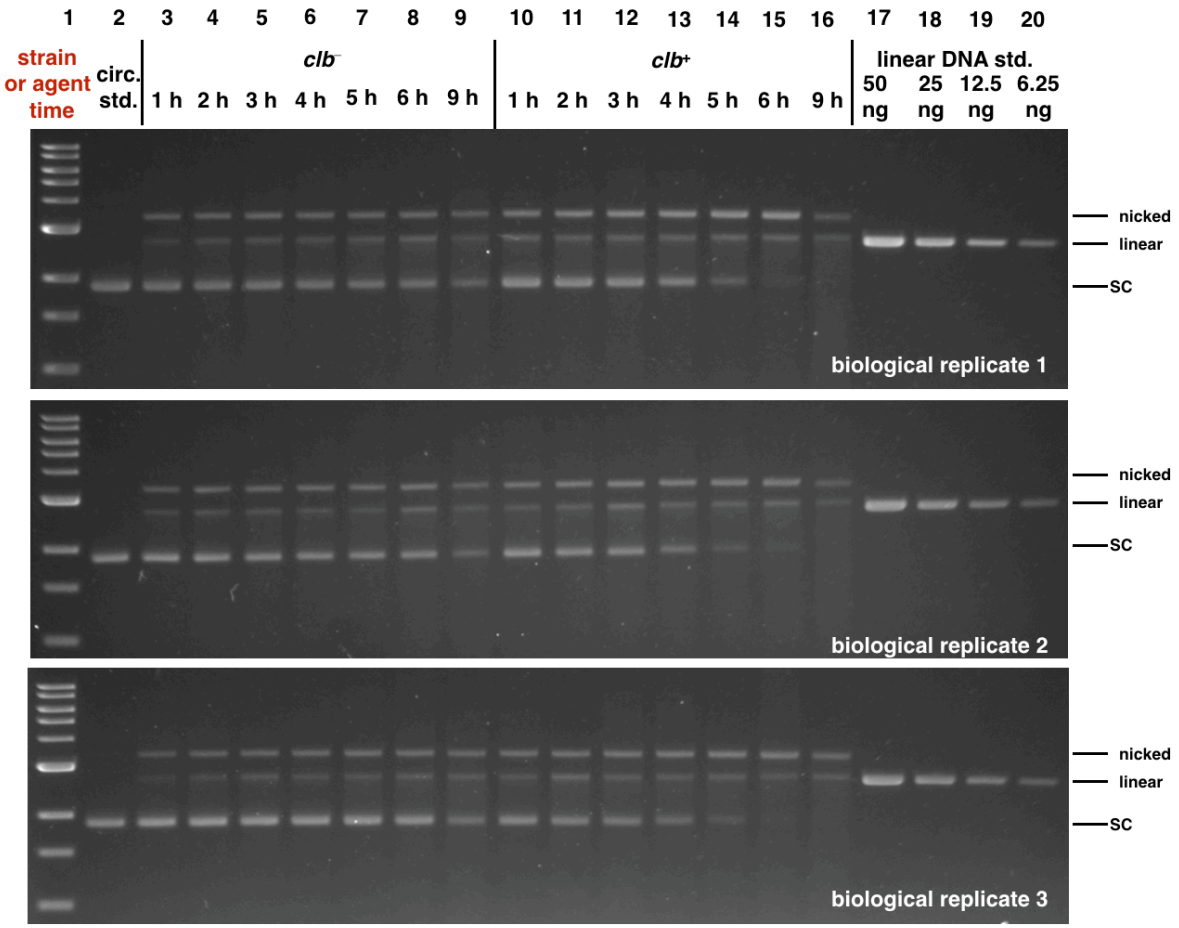

Fig. S4. DNA plasmid cleavage assay employing circular pUC19 DNA and *clb*⁻ (or *clb*⁺) BW25113 *E. coli*. DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); *clb*⁻ BW25113 *E. coli.* 1 h (Lane #3), 2 h (Lane #4), 3 h (Lane #5), 4 h (Lane #6), 5 h (Lane #7), 6 h (Lane #8), and 9 h (Lane #9); *clb*⁺ BW25113 *E. coli.* 1 h (Lane #10), 2 h (Lane #11), 3 h (Lane #12), 4 h (Lane #13), 5 h (Lane #14), 6 h (Lane #15), and 9 h (Lane #16); linearized pUC19 DNA standard 50 ng (Lane #17), 25 ng (Lane #18), 12.5 ng (Lane #19), and 6.25 ng (Lane #20). Conditions (Lane #3–#16): *clb*⁻ BW25113 *E. coli.* (Lane #3–#9) or *clb*⁺ BW25113 *E. coli.* (Lane #10–#16), circular pUC19 DNA (7.7 μM in base pairs), M9-CA media, 37 °C, reaction proceed for 1 h, 2 h, 4 h, 6 h, and 9 h. The DNA was isolated, purified, and analyzed by native agarose gel electrophoresis (90 V, 2 hr). The experiment was performed and analyzed in biological triplicates for quantification purpose. SC = supercoiled, nicked = SSB, linear = DSB.

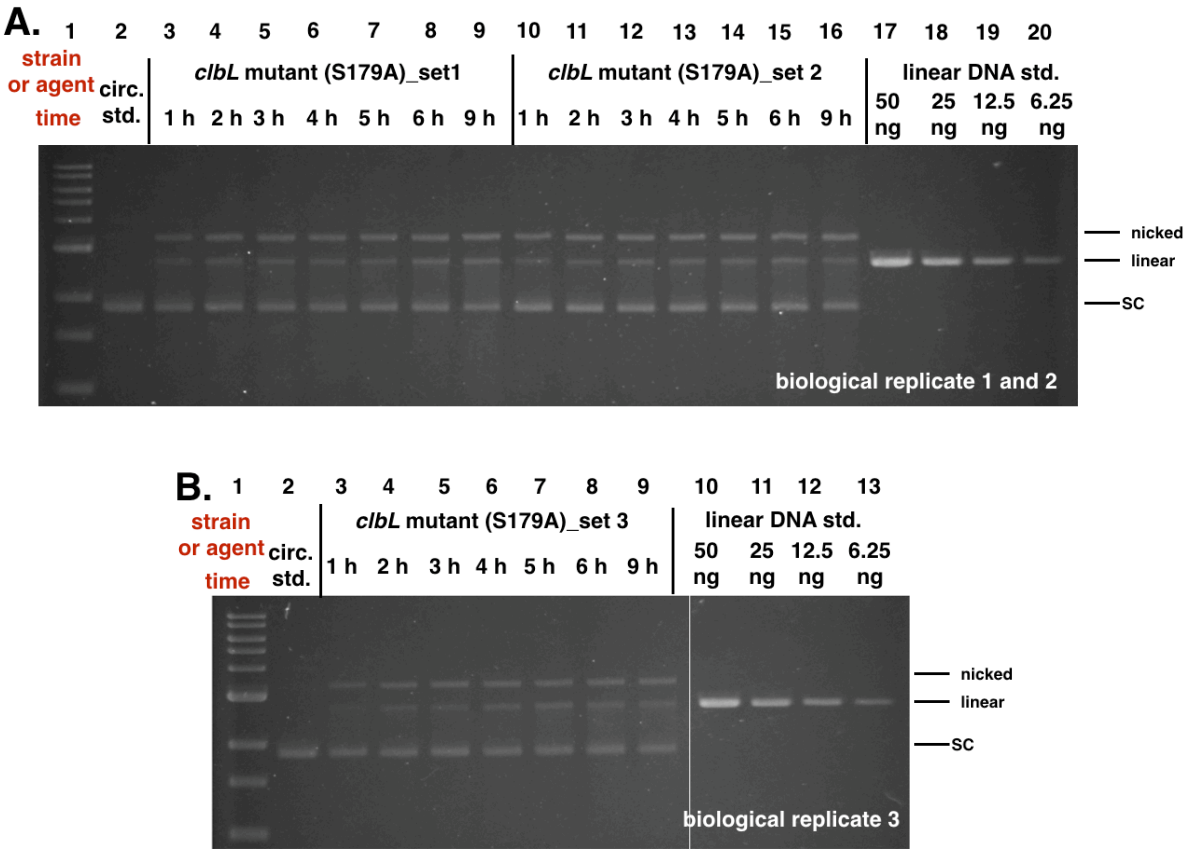

Fig. S5. DNA plasmid cleavage assay employing circular pUC19 DNA and *clbL* mutant (S179A) BW25113 *E. coli*. A. Biological replicate 1 and 2; B. Biological replicate 3. (Fig. S5A.) DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); *clbL* point mutant (S179A) BW25113 *E. coli* set #1, 1 h (Lane #3), 2 h (Lane #4), 3 h (Lane #5), 4 h (Lane #6), 5 h (Lane #7), 6 h (Lane #8), and 9 h (Lane #9); *clbL* point mutant (S179A) set #2, 1 h (Lane #10), 2 h (Lane #11), 3 h (Lane #12), 4 h (Lane #13), 5 h (Lane #14), 6 h (Lane #15), and 9 h (Lane #16); linearized pUC19 DNA standard 50 ng(Lane #17), 25 ng (Lane #18), 12.5 ng (Lane #19), and 6.25 ng (Lane #20). **(Fig. S5B.)** DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); *clbL* point mutant (S179A) BW25113 *E. coli* set #3, 1 h (Lane #3), 2 h (Lane #4), 3 h (Lane #5), 4 h (Lane #6), 5 h (Lane #7), 6 h (Lane #8), and 9 h (Lane #9); linearized pUC19 DNA standard 50 ng (Lane #10), 25 ng (Lane #11), 12.5 ng (Lane #12), and 6.25 ng (Lane #13). Conditions (**Fig. S5A.** Lane #3–#16, and **Fig. S5B.** Lane #3–#9): *clbL* point mutant (S179A) BW25113 *E. coli*, circular pUC19 DNA (7.7 µM in base pairs), M9-CA media, 37 °C, reaction proceed for 1 h, 2 h, 4 h, 6 h, and 9 h. The DNA was isolated, purified, and analyzed by native agarose gel electrophoresis (90 V, 2 hr). The experiment was performed and analyzed in biological triplicates for quantification purpose. SC = supercoiled, nicked = SSB, linear = DSB.

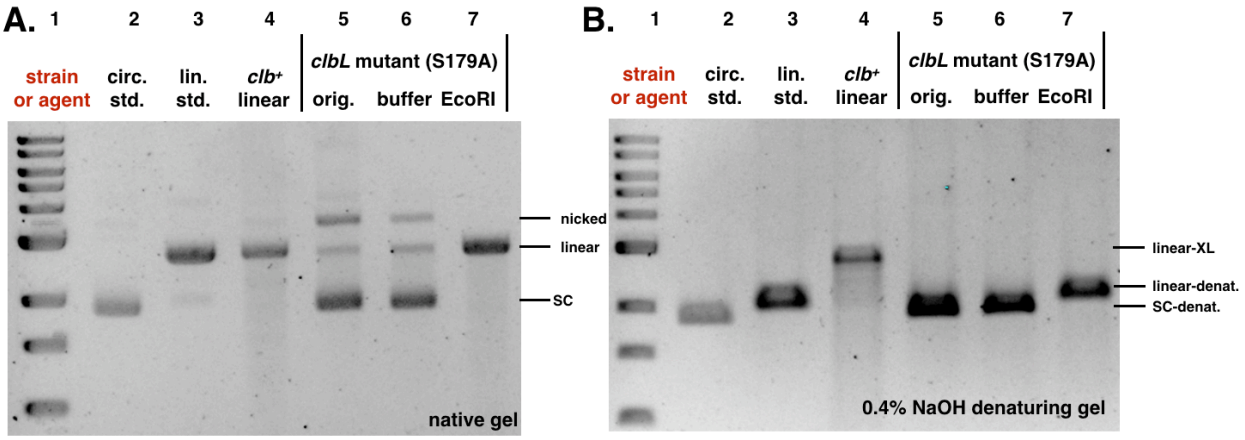

Fig. S6. Plasmid DNA linearization test employing circular pUC19 DNA isolated from co-culture with *clbL* point mutant (S179A) BW25113 *E. coli*. A. Native gel. B. 0.4% NaOH denaturing gel. The cross-linked linearized pUC19 isolated from co-culture with *clb*$^+$ BW25113 *E. coli* was used as a positive control. DNA ladder (Lane #1); circular pUC19 DNA (Lane #2); linearized pUC19 DNA (Lane #3); linearized pUC19 DNA co-cultured with *clb*$^+$ BW25113 *E. coli* (Lane #4); circular pUC19 DNA isolated from co-culture with *clbL* point mutant (S179A) BW25113 *E. coli* (Lane #5), reacted with buffer (Lane #6), reacted with EcoRI restriction enzyme (Lane #7). Conditions (Lane #4): linearized pUC19 DNA, *clb*$^+$ BW25113 *E. coli*, M9-CA media, 4 h at 37 °C. Conditions (Lanes #5–#7): circular pUC19 DNA isolated from co-culture with *clbL* point mutant (S179A) BW25113 *E. coli* in M9-CA media for 4 h at 37 °C (Lane # 5); the DNA (15.4 μM base pair) was reacted with CutSmart Buffer® (New England Biolabs®), pH 7.9, at 37 °C for 30 min (Lane #6); the DNA (15.4 μM base pair) was reacted with 20 units of EcoRI-HF restriction enzyme in CutSmart Buffer® (New England Biolabs®), pH 7.9, at 37 °C for 30 min (Lane #7). The DNA was isolated and analyzed by either (**Fig. S6A**) 0% NaOH native agarose gel electrophoresis, or (**Fig. S6B**) 0.4% NaOH denature agarose gel electrophoresis (90 V, 1.5 h). SC = supercoiled, nicked = SSB, linear = DSB, SC-denat. = supercoiled DNA in denaturing form, linear-denat. = DSB/linearized DNA in denaturing form, linear-XL = DSB/linearized DNA cross-linked by colibactin.

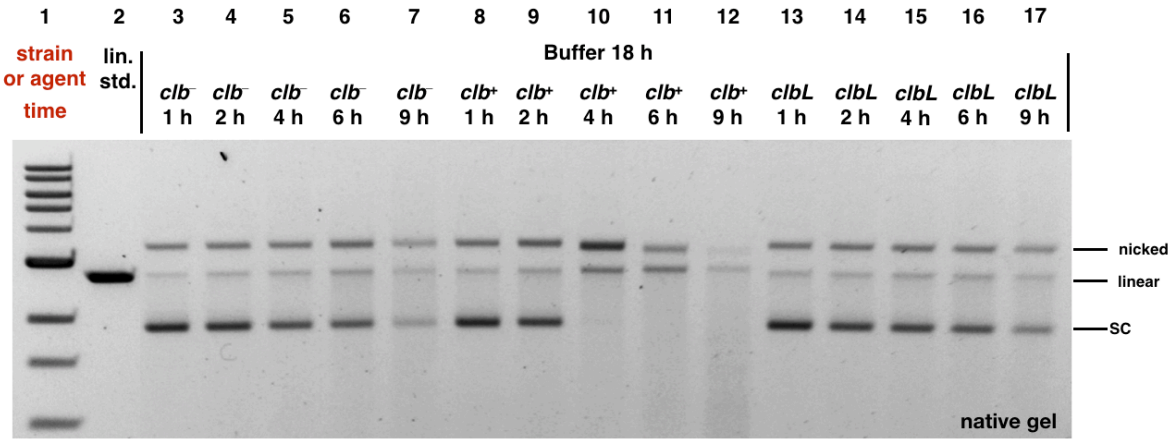

Fig. S7. Plasmid DNA stability test employing circular pUC19 DNA isolated from co-culture with *clb*⍰, *clb*$^+$, or *clbL* mutant (S179A) BW25113 *E. coli.* DNA ladder (Lane #1); linearized pUC19 DNA standard (Lane # 2); post buffer-reacted with DNA isolated from *clb*$^-$ BW25113 *E. coli* 1 h (Lane #3), 2 h (Lane #4), 4 h (Lane #5), 6 h (Lane #6), and 9 h (Lane #7); post buffer-reacted with DNA isolated from *clb*$^+$ BW25113 *E. coli* 1 h (Lane #8), 2 h (Lane #9), 4 h (Lane #10), 6 h (Lane #11), and 9 h (Lane #12); post buffer-reacted with DNA isolated from *clbL* point mutant (S179A) BW25113 *E. coli* 1 h (Lane #13), 2 h (Lane #14), 4 h (Lane #15), 6 h (Lane #16), and 9 h (Lane #17). Conditions (Lane #3–#17): NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 18 h, 50 µg of DNA isolated from reacting with *clb*$^-$ BW25113 *E. coli* for 1 to 9 hours (Lane #3–#7), reacting with *clb*$^+$ BW25113 *E. coli* for 1 to 9 hours (Lane #8–#12), and reacting with *clbL* point mutant (S179A) BW25113 *E. coli* for 1 to 9 hours (Lane #13–#17). The DNA was not further purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, nicked = SSB, linear = DSB.

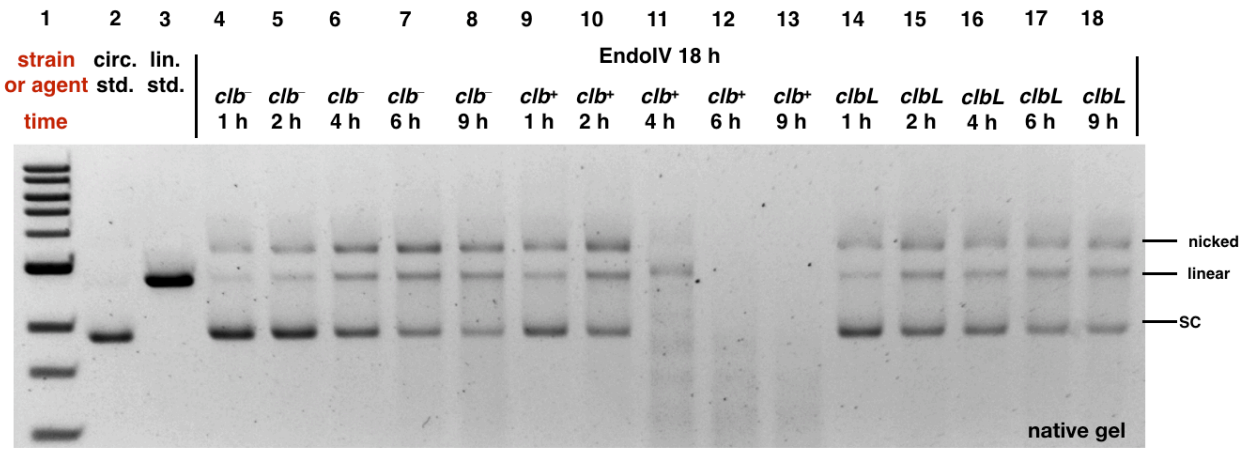

Fig. S8. Plasmid DNA Endonuclease IV stability test employing circular pUC19 DNA isolated from co-culture with *clb*⍰, *clb*⁺, or *clbL* mutant (S179A) BW25113 *E. coli*. The Endonuclease IV selectively cleaves DNA 5' apurinic sites. DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); linearized pUC19 DNA standard (Lane # 3); EndoIV-reacted with DNA isolated from *clb*⁻ BW25113 *E. coli* 1 h (Lane #4), 2 h (Lane #5), 4 h (Lane #6), 6 h (Lane #7), and 9 h (Lane #8); EndoIV-reacted with DNA isolated from *clb*⁺ BW25113 *E. coli* 1 h (Lane #9), 2 h (Lane #10), 4 h (Lane #11), 6 h (Lane #12), and 9 h (Lane #13); post buffer-reacted with DNA isolated from *clbL* point mutant (S179A) BW25113 *E. coli* 1 h (Lane #14), 2 h (Lane #15), 4 h (Lane #16), 6 h (Lane #17), and 9 h (Lane #18). Conditions (Lane #4–#18): 20 units of Endonuclease IV (New England Biolabs®), NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 18 h, 50 µg of DNA isolated from reacting with *clb*⁻ BW25113 *E. coli* for 1 to 9 hours (Lane #4–#8), reacting with *clb*⁺ BW25113 *E. coli* from 1 to 9 hours (Lane #9–#13), and reacting with *clbL* point mutant (S179A) BW25113 *E. coli* for 1 to 9 hours (Lane #14–#18). The DNA was not further purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, nicked = SSB, linear = DSB.

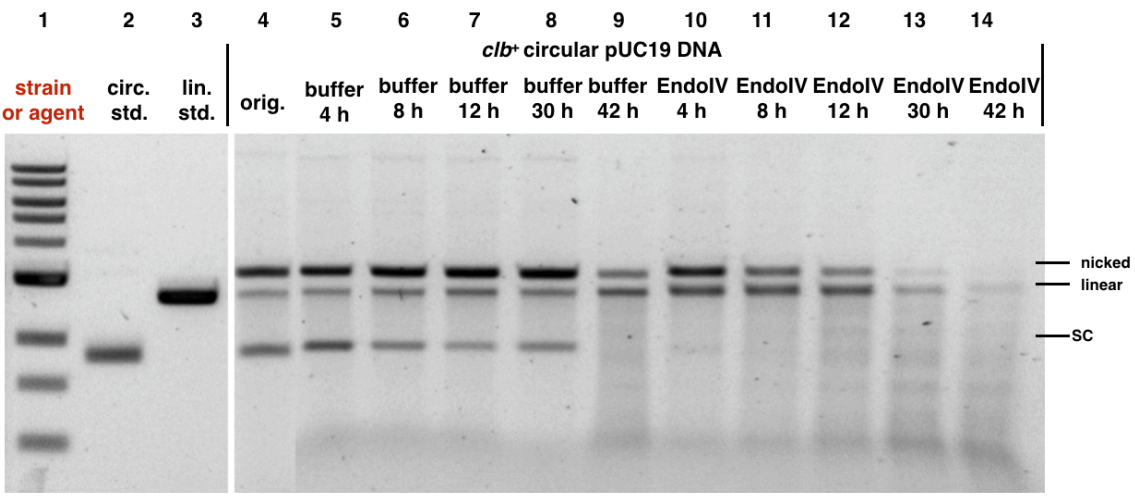

Fig. S9. Plasmid DNA Endonuclease IV stability test employing circular pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli*. Endonuclease IV selectively cleaves DNA 5′ to apurinic sites. DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); linearized pUC19 DNA standard (Lane #3); circular pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli* (Lane #4), reacted with buffer only for 4 h (Lane #5), 8 h (Lane #6), 12 h (Lane #7), 30 h (Lane #8), and 42 h (Lane #9); circular pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli* reacted with Endonuclease IV for 4 h (Lane #10), 8 h (Lane #11), 12 h (Lane #12), 30 h (Lane #13), and 42 h (Lane #14). Conditions (Lanes #4–#14): circular pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli* in M9-CA media for 4 h at 37 °C (Lane #4); the DNA (3.9 µM base pair) was reacted with NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #5–#9); the DNA (3.9 µM base pair) was reacted with 20 units of Endonuclease IV in NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #10–#14). The DNA was not re-purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, nicked = SSB, linear = DSB.

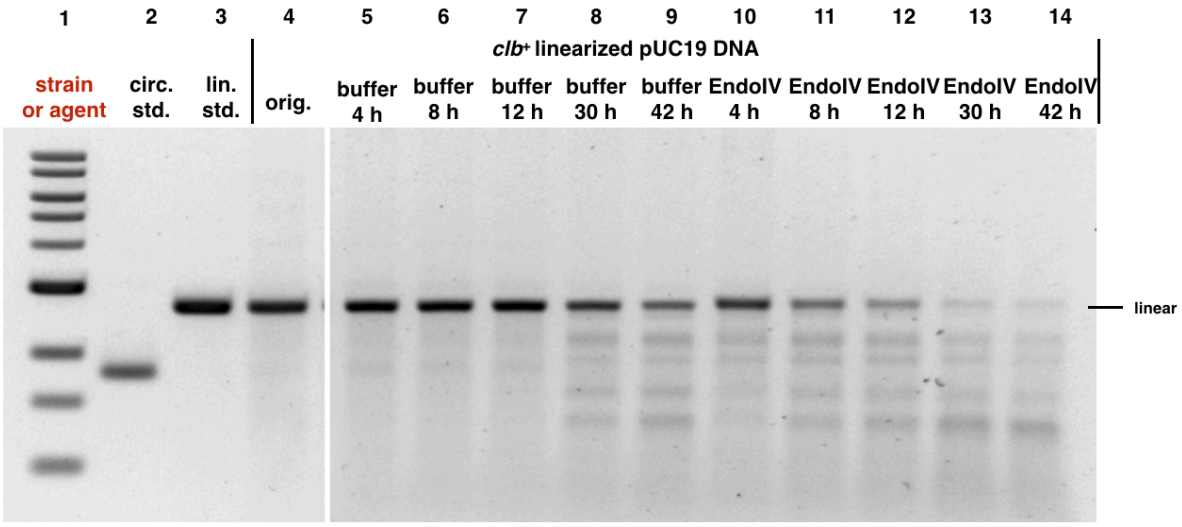

Fig. S10. Plasmid DNA Endonuclease IV stability test employing linearized pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli*. Endonuclease IV selectively cleaves DNA 5′ to apurinic sites. DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); linearized pUC19 DNA standard (Lane #3); linearized pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli* (Lane #4), reacted with buffer only for 4 h (Lane #5), 8 h (Lane #6), 12 h (Lane #7), 30 h (Lane #8), and 42 h (Lane #9); linearized pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli* reacted with Endonuclease IV for 4 h (Lane #10), 8 h (Lane #11), 12 h (Lane #12), 30 h (Lane #13), and 42 h (Lane #14). Conditions (Lanes #4–#14): linearized pUC19 DNA isolated from co-culture with *clb*$^+$ BW25113 *E. coli* in M9-CA media for 4 h at 37 °C (Lane #4); the DNA (3.9 µM base pair) was reacted with NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #5–#9); the DNA (3.9 µM base pair) was reacted with 20 units of Endonuclease IV in NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #10–#14). The DNA was not re-purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). Linear = linearized DNA cross-linked by colibactin

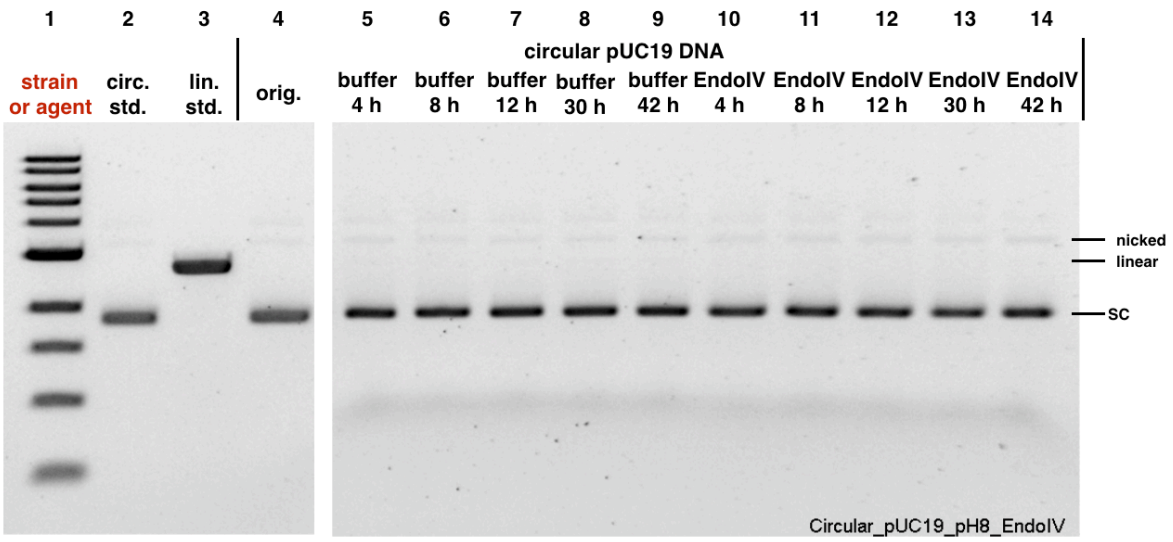

Fig. S11. Plasmid DNA Endonuclease IV stability test employing circular pUC19 DNA. Endonuclease IV selectively cleaves DNA 5′ to apurinic sites. DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); linearized pUC19 DNA standard (Lane #3); circular pUC19 DNA original sample (Lane #4), reacted with buffer only for 4 h (Lane #5), 8 h (Lane #6), 12 h (Lane #7), 30 h (Lane #8), and 42 h (Lane #9); circular pUC19 DNA reacted with Endonuclease IV for 4 h (Lane #10), 8 h (Lane #11), 12 h (Lane #12), 30 h (Lane #13), and 42 h (Lane #14). Conditions (Lanes #5–#14): circular pUC19 DNA (3.9 µM base pair) was reacted with NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #5–#9); the DNA (3.9 µM base pair) was reacted with 20 units of Endonuclease IV in NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #10–#14). The DNA was not re-purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, nicked = SSB, linear = DSB.

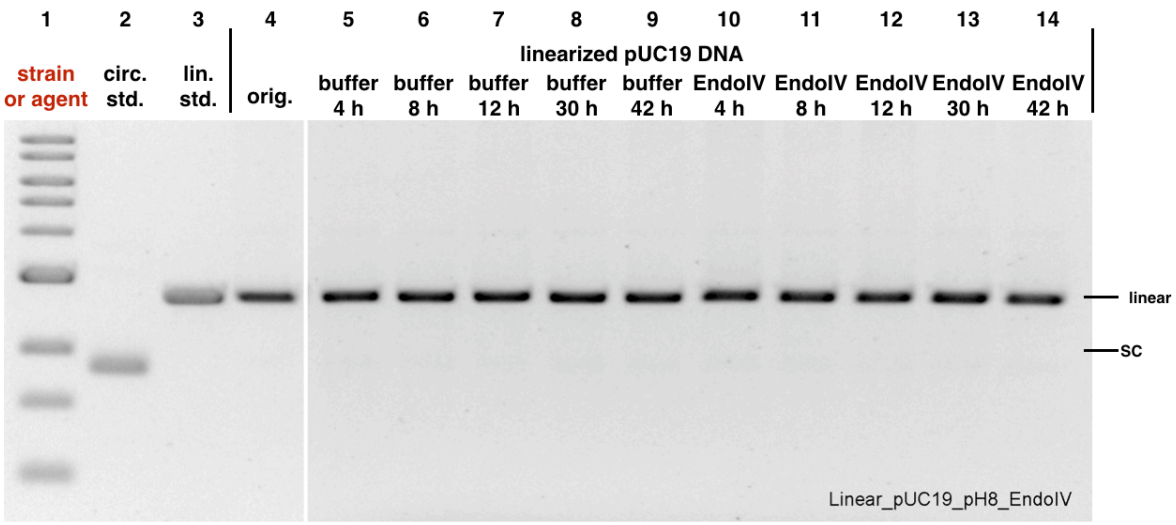

Fig. S12. Plasmid DNA Endonuclease IV stability test employing linearized pUC19 DNA. Endonuclease IV selectively cleaves DNA 5′ to apurinic sites. DNA ladder (Lane #1); circular pUC19 DNA standard (Lane #2); linearized pUC19 DNA standard (Lane # 3); linearized pUC19 DNA control (Lane #4), reacted with buffer only for 4 h (Lane #5), 8 h (Lane #6), 12 h (Lane #7), 30 h (Lane #8), and 42 h (Lane #9); circular pUC19 DNA reacted with Endonuclease IV for 4 h (Lane #10), 8 h (Lane #11), 12 h (Lane #12), 30 h (Lane #13), and 42 h (Lane #14). Conditions (Lanes #5–#14): linearized pUC19 DNA (3.9 µM base pair) was reacted with NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #5–#9); the DNA (3.9 µM base pair) was reacted with 20 units of Endonuclease IV in NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #10–#14). The DNA was not re-purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, linear = linearized DNA.

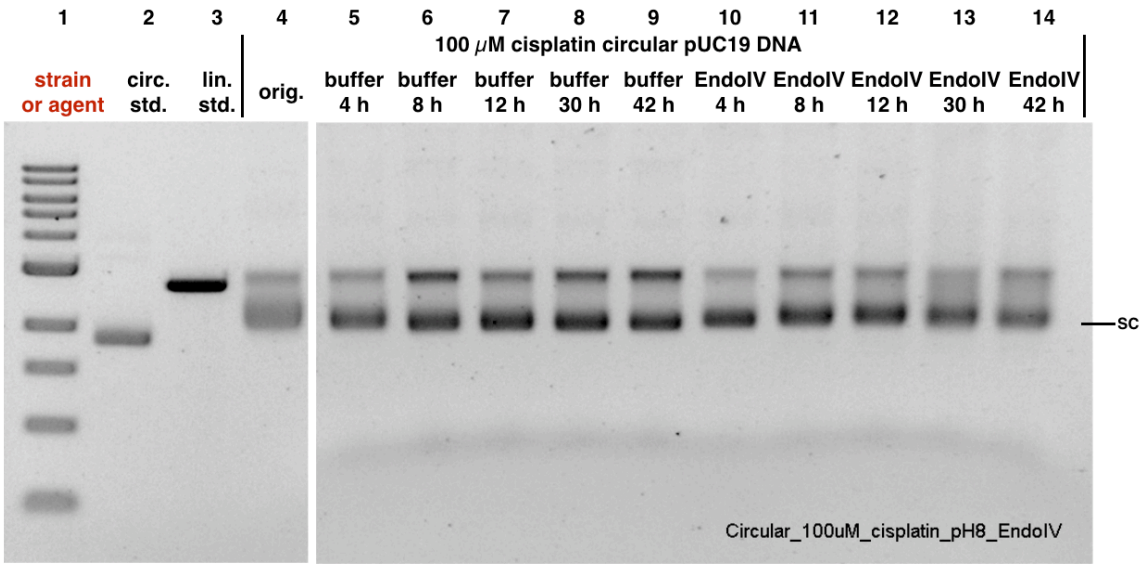

Fig. S13. Plasmid DNA Endonuclease IV stability test employing circular pUC19 DNA thathas been previously treated with 100 μM cisplatin. Endonuclease IV selectively cleaves DNA 5′ to apurinic sites. DNA ladder (Lane #1); circular pUC19 DNA control (Lane #2); linearized pUC19 DNA standard (Lane #3); circular pUC19 DNA 100 μM cisplatin, pH 5.0, 3 h (Lane #4), 100 μM cisplatin-treated circular pUC10 DNA reacted with buffer only for 4 h (Lane #5), 8 h (Lane #6), 12 h (Lane #7), 30 h (Lane #8), and 42 h (Lane #9); circular pUC19 DNA reacted with Endonuclease IV for 4 h (Lane #10), 8 h (Lane #11), 12 h (Lane #12), 30 h (Lane #13), and 42 h (Lane #14). Conditions (Lanes #4–#14): circular pUC19 DNA (15.4 μM base pair) that has been reacted with 100 μM cisplatin in 10 mM sodium citrate buffer, pH 5.0 for 4 h at 37 °C (Lane #4); the DNA (3.9 μM base pair) was further reacted with NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #5–#9); the DNA (3.9 μM base pair) was further reacted with 20 units of Endonuclease IV in NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #10–#14). The DNA was not re-purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled DNA cross-linked by cisplatin.

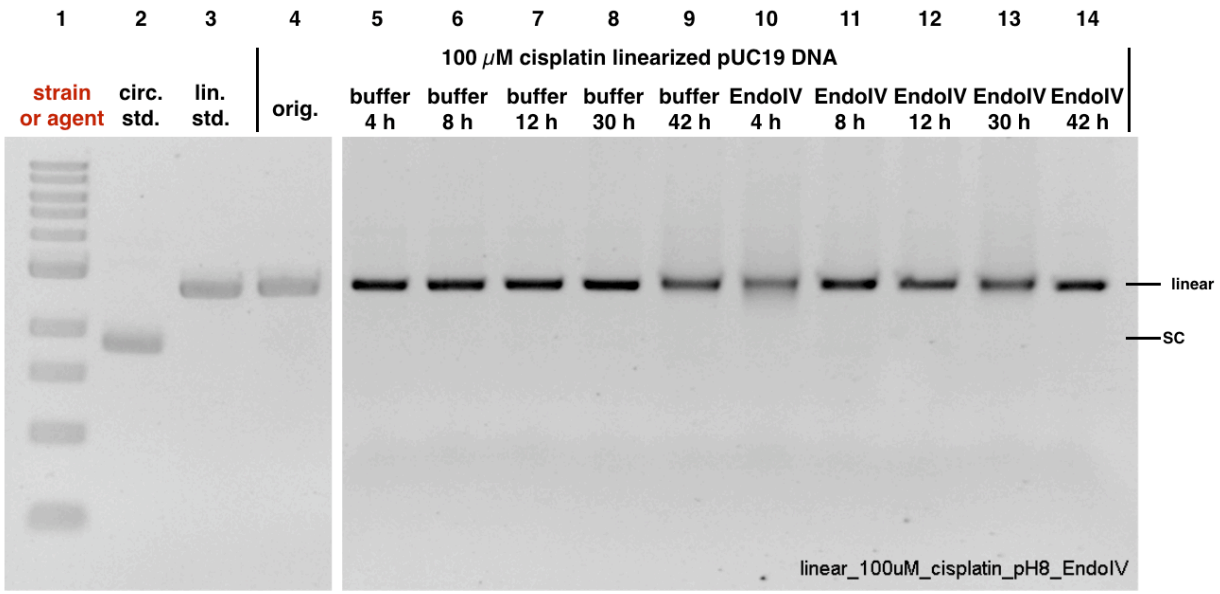

Fig. S14. Plasmid DNA Endonuclease IV stability test employing linearized pUC19 DNA which has been previously treated with 100 µM cisplatin. Endonuclease IV selectively cleaves DNA 5′ to apurinic sites. DNA ladder (Lane #1); circular pUC19 DNA control (Lane #2); linearized pUC19 DNA standard (Lane # 3); linearized pUC19 DNA 100 µM cisplatin, pH 5.0, 3 h (Lane #4), 100 µM cisplatin-treated linearized pUC10 DNA reacted with buffer only for 4 h (Lane #5), 8 h (Lane #6), 12 h (Lane #7), 30 h (Lane #8), and 42 h (Lane #9); linearized pUC19 DNA reacted with Endonuclease IV for 4 h (Lane #10), 8 h (Lane #11), 12 h (Lane #12), 30 h (Lane #13), and 42 h (Lane #14). Conditions (Lanes #4–#14): linearized pUC19 DNA (15.4 µM base pair) that has been reacted with 100 µM cisplatin in 10 mM sodium citrate buffer, pH 5.0 for 4 hour at 37 °C (Lane #4); the DNA (3.9 µM base pair) was further reacted with NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #5–#9); the DNA (3.9 µM base pair) was further reacted with 20 units of Endonuclease IV in NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 4 h, 8 h, 12 h, 30 h, or 42 h (Lanes #10–#14). The DNA was not re-purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, linear = linearized DNA cross-linked by cisplatin.

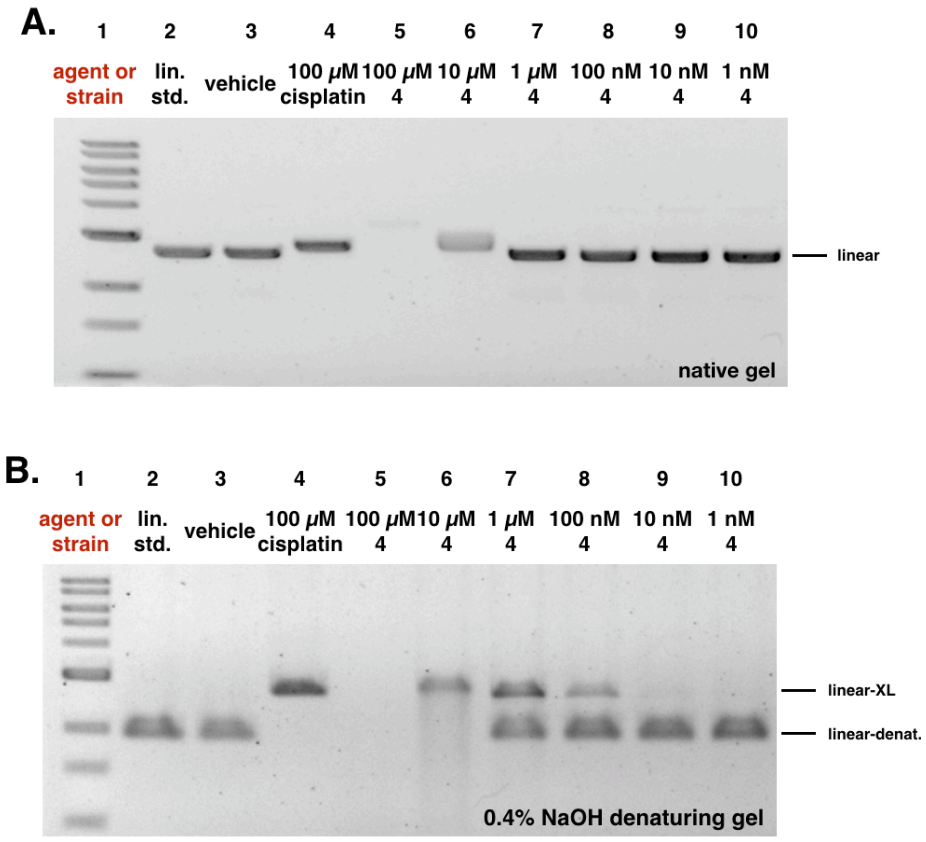

Fig. S15. DNA cross-linking assay employing linear pUC19 DNA and synthetic colibactin linear precursor 4. A. Native gel. B. 0.4% NaOH denaturing gel. 5% DMSO was used as vehicle (negative control), and 100 μM cisplatin was used as positive control. DNA ladder (Lane #1); linearized pUC19 DNA standard (Lane #2); 5% DMSO (Lane #3); 100 μM cisplatin (Lane #4); 100 μM **4** (Lane #5); 10 μM **4** (Lane #6); 1 μM **4** (Lane #7); 100 nM **4** (Lane #8); 10 nM **4** (Lane #9); 1 nM **4** (Lane #10). Conditions (Lane #3): linearized pUC19 DNA (15.4 μM in base pairs), 5% DMSO (vehicle), 10 mM citric buffer, pH 5.0, 4 h, 37 ºC. Conditions (Lane #4): linearized pUC19 DNA (15.4 μM in base pairs), 5% DMSO (vehicle), 100 μM cisplatin, 10 mM citric buffer, pH 5.0, 4 h, 37 ºC. Conditions (Lanes #5–#10): circular pUC19 DNA (15.4 μM in base pairs), **4** (100 μM–1 nM), 5% DMSO, 10 mM citric buffer, pH 5.0, 4 h, 37 ºC. The DNA was analyzed either (**Fig. S15A**) by native agarose gel electrophoresis, or (**Fig. S15B**) by 0.4% NaOH denaturing agarose gel electrophoresis (90 V, 1.5 h). Linear = linearized DNA, Linear-denat. = DSB/linearized DNA in denaturing form, linear-XL = DSB/linearized DNA cross-linked by colibactin or cisplatin.

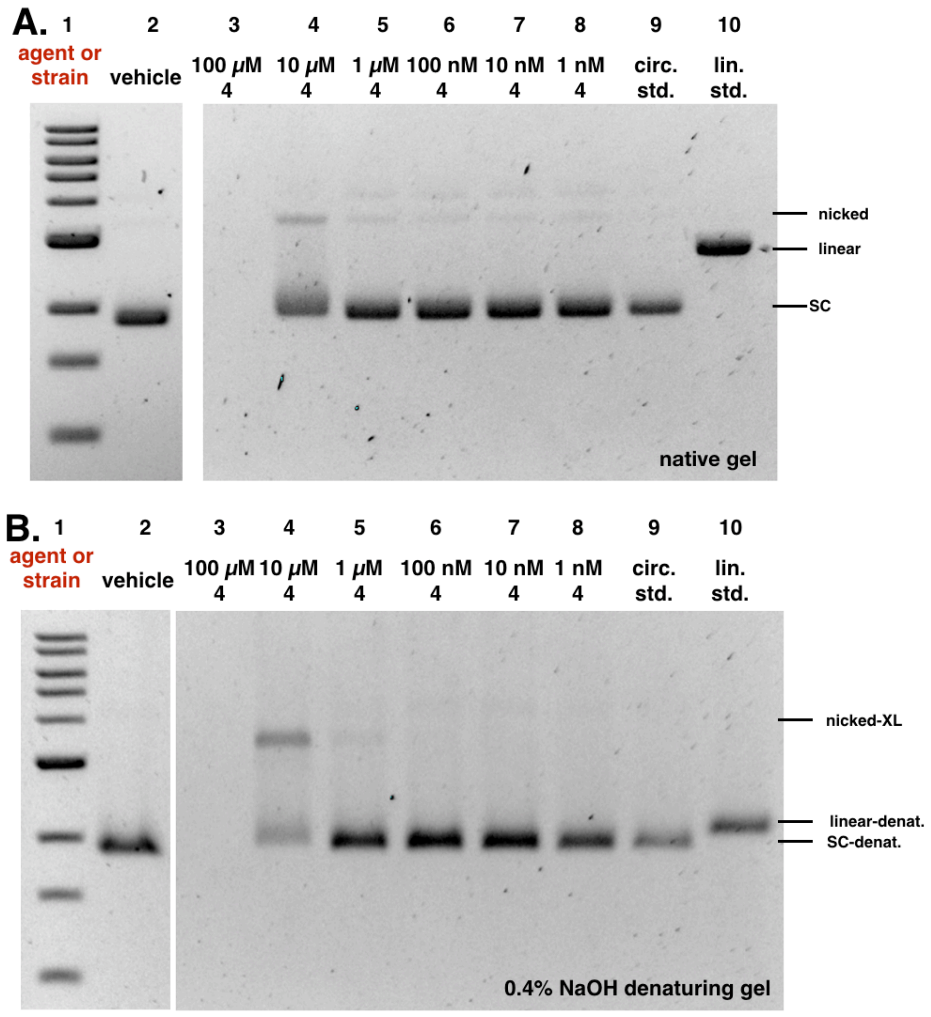

Fig. S16. DNA plasmid cleavage assay employing circular pUC19 DNA and synthetic colibactin linear precursor 4. **A. Native gel; B. 0.4% NaOH denaturing gel.** 5% DMSO was used as vehicle. DNA ladder (Lane #1); 5% DMSO (Lane #2); 100 μM **4** (Lane #3); 10 μM **4** (Lane #4); 1 μM **4** (Lane #5); 100 nM **4** (Lane #6); 10 nM **4** (Lane #7); 1 nM **4** (Lane #8); circular pUC19 plasmid standard (Lane #9); linearized pUC19 plasmid standard (Lane #10). Conditions (Lane #2): circular pUC19 DNA (15.4 μM in base pairs), 5% DMSO (vehicle), 10 mM citric buffer, pH 5.0, 4 h, 37 ºC. Conditions (Lanes #3–#8): circular pUC19 DNA (15.4 μM in base pairs), **4** (100 μM–1 nM), 5% DMSO, 10 mM citric buffer, pH 5.0, 3 h, 37 ºC. The DNA was analyzed either (**Fig. S16A**) by native agarose gel electrophoresis, or (**Fig. S16B**) by 0.4% NaOH denaturing agarose gel electrophoresis (90 V, 1.5 h). SC = supercoiled, nicked = SSB, linear = DSB, Linear-denat. = DSB/linearized DNA in denaturing form, linear-XL = DSB/linearized DNA cross-linked by colibactin or cisplatin.

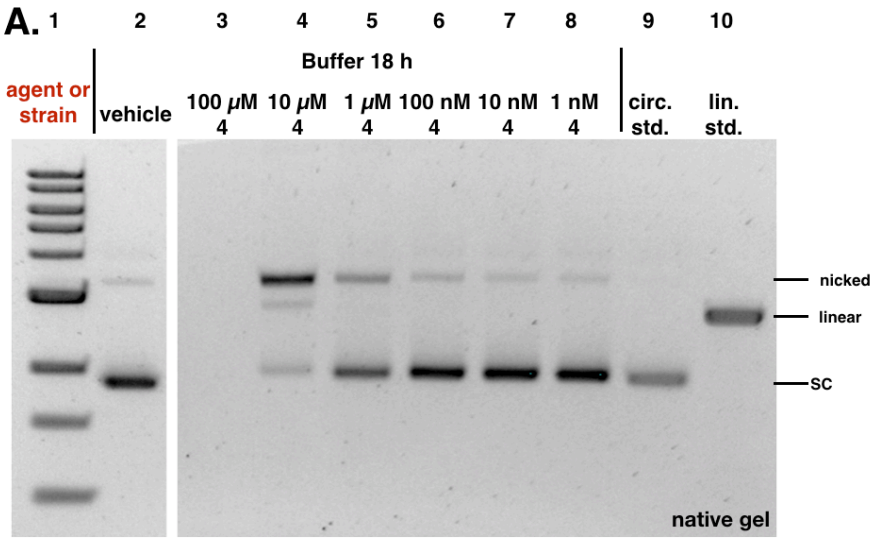

Fig. S17A. Plasmid DNA buffer stability test employing circular pUC19 DNA that had been previously treated with synthetic colibactin linear precursor 4 at different concentrations. 5% DMSO was used as vehicle when preparing the DNA samples for testing. DNA ladder (Lane #1); post buffer-reacted after 5% DMSO (Lane #2); post buffer-reacted after 100 μM **4** (Lane #3); post buffer-reacted after 10 μM **4** (Lane #4); post buffer-reacted after 1 μM **4** (Lane #5); post buffer-reacted after 100 nM **4** (Lane #6); post buffer-reacted after 10 nM **4** (Lane #7); post buffer-reacted after 1 nM **4** (Lane #8); circular pUC19 plasmid standard (Lane #9); linearized pUC19 plasmid standard (Lane #10). Conditions (Lane #2): the 5% DMSO-treated circular pUC19 DNA (3.9 μM in base pairs), NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 18 h. Conditions (Lanes #3–#8): **4** (100 μM–1 nM)-treated circular pUC19 DNA (3.9 μM in base pairs), NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 18 h. The DNA was not further purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, nicked = SSB, linear = DSB.

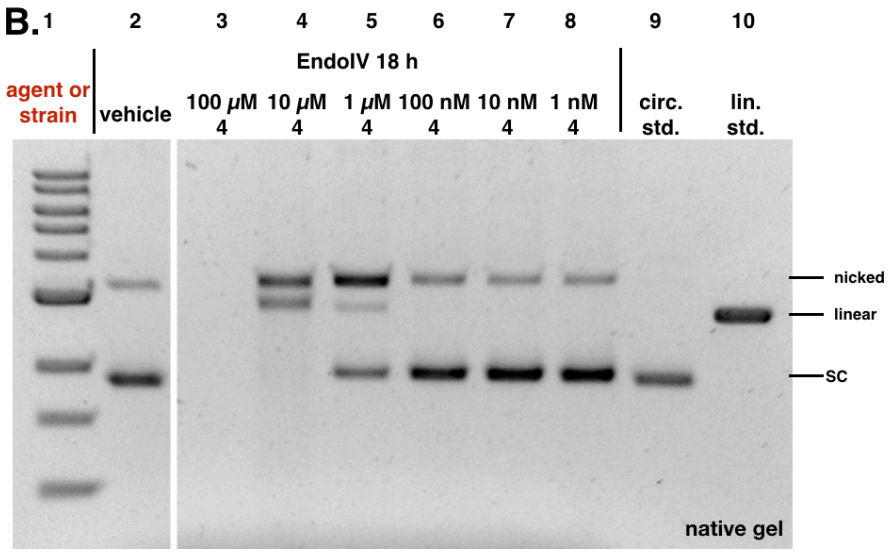

Fig. S17B. Plasmid DNA Endonuclease IV stability test employing circular pUC19 DNA that had been previously treated with synthetic colibactin linear precursor 4 at different concentrations. Endonuclease IV selectively cleaves DNA 5′ to apurinic sites. 5% DMSO was used as vehicle when preparing the DNA samples for testing. DNA ladder (Lane #1); post EndoIV-reacted after 5% DMSO (Lane #2); post EndoIV-reacted after 100 μM **4** (Lane #3); post EndoIV-reacted after 10 μM **4** (Lane #4); post EndoIV-reacted after 1 μM **4** (Lane #5); post EndoIV-reacted after 100 nM **4** (Lane #6); post EndoIV-reacted after 10 nM **4** (Lane #7); post EndoIV-reacted after 1 nM **4** (Lane #8); circular pUC19 plasmid standard (Lane #9); linearized pUC19 plasmid standard (Lane #10). Conditions (Lane #2): the 5% DMSO-treated circular pUC19 DNA (3.9 μM in base pairs), 20 units of Endonuclease IV (New England Biolabs®), NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 18 h. Conditions (Lanes #3–#8): **4** (100 μM–1 nM)-treated circular pUC19 DNA (3.9 μM in base pairs), 20 units of Endonuclease IV (New England Biolabs®), NEBuffer 3.1® (New England Biolabs®), pH 7.9, at 37 °C for 18 h. The DNA was not further purified and was directly analyzed by native agarose gel electrophoresis (90 V, 2 h). SC = supercoiled, nicked = SSB, linear = DSB.