Supporting Information

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SI Materials and Methods

Bacterial Strains and Plasmids. For the in vitro assays, we used the previously described (1) *E. coli* strain DH10B hosting a BAC bearing the *pks* island (*E. coli pks*$^+$) that produces Colibactin, the isogenic *clbA* mutant (deficient in the phosphopantetheinyl transferase gene and thus impaired for Colibactin biosynthesis), or DH10B hosting the pBeloBAC11 vector only (*E. coli pks*$^-$). For the in vivo assay, the wild-type *E. coli* strain SP15 (1) was used. The isogenic SP15 *clbA* mutant was constructed using lambda red recombination and the primers IHAPJPN44/45 as previously described (1, 2).

Cloning of the *clbA* gene for complementation of both mutants was performed by high-fidelity PCR amplification (DeepVent; New England Biolabs) using primers IHAPJPN118 (5′-GGA TCC TAG ATT ATC CGT GGC GAT TC) and IHAPJPN119 (5′-GGA TCC TAA ATG GCA CAC CTA TCC GC) and cloning into pCR-Script (Stratagene). The resulting pMB808 (pclbA) plasmid was transformed into each *clbA* mutant.

To visualize SP15 strains in vivo, the bacteria were transformed with plasmid pFPV25.1 that encodes a *gfp* gene under control of an *rpsM* constitutive promoter (3). To construct the plasmid pJN871 that encodes the *gfp* gene under control of the *clbA* promoter/regulatory region, the PCR amplicon using primers IHAPJPN115 (5′-GAA TTC ATC ACC TTA TTA TCG GAT TTA TAG) and IHAPJPN117 (5′-GGA TCC TTA GAT AAT CTC ATT CCT GTT AGC) was cloned (BamHI/EcoRI) into pFPV25 (3).

For the infection assay, bacteria were cultured overnight in LB broth at 37 °C with shaking. The next day, this culture was diluted 1:100 in interaction medium (IM), consisting of DMEM or MEMα (Invitrogen) buffered with 25 mM Hepes and supplemented with 5% FCS (Eurobio). This preactivated culture was incubated at 37 °C with shaking until an optical density of 0.6 at 600 nm was obtained. Then the bacteria were added to the cells.

Cell Culture. Parental CHO AA8 cells from the ATCC, and CHO xrs-6 (Ku80-deficient) cells (a kind gift of M. Defais, Institute of Pharmacology and Structural Biology, Centre National de la Recherche Scientifique 205, Toulouse, France) were grown in MEMα with 10% FCS and 80 μg/mL gentamicin. Nontransformed rat intestinal epithelial cells (IEC-6; ATCC CRL-1592) were cultured in DMEM, 10% FCS, 80 μg/mL gentamicin, supplemented with 4 μg/mL insulin (Sigma). Human colon tumor cells (HCT-116) were cultured in McCoy's medium (Invitrogen) with 10% FCS, 80 μg/mL gentamicin, and 1% nonessential amino acids (Invitrogen). All cell lines were grown at 37 °C in a 5% CO_2 humidified atmosphere and split regularly to maintain exponential growth. A fresh culture was started from a liquid nitrogen stock every 20 passages. The cell lines were confirmed free from mycoplasma contamination by PCR.

In Vitro Infection Assay. Cells (~75% confluent) were washed four times with warm HBSS (Invitrogen) and incubated in IM based on DMEM for IEC-6 and HCT-116 cells or MEMα for CHO cells. The infection dose was calculated according to a multiplicity of infection (number of bacteria per cell at the onset of infection, MOI). After a 4-h infection at 37 °C in a 5% CO_2 atmosphere, cells were washed four to six times with warm HBSS and incubated in cell-culture medium supplemented with 200 μg/mL gentamicin. The culture medium was changed the next day, and thereafter, cells were split twice a week until analysis (up to 21 d).

Cell Cycle, Apoptosis, γH2AX, and Aneuploidy Flow Cytometry Analyses. Cells were collected by trypsin treatment.

For cell-cycle analysis, cells were fixed overnight in 70% ethanol at −20 °C, treated with PBS 0.1% Triton ×100 for 5 min, washed and resuspended in PBS containing 15 μg/mL propidium iodide (Sigma), and 100 μg/mL RNase (Fermentas).

For cell-death quantification, cells were stained (1 h at 37 °C, 5% CO_2) with carboxyfluorescein-labeled fluoromethyl ketone peptide inhibitor of caspases (FAM-VAD-FMK, FLICA CaspaTag; Chemicon) diluted in PBS.

For γH2AX quantification, cells were fixed in PBS 1% formaldehyde, then in 90% methanol. After treatment in PBS 0.5% BSA (Sigma), cells were incubated with mouse anti- γH2AX monoclonal antibodies (Upstate) diluted 1:400 in blocking solution, washed, and incubated with FITC-conjugated anti-mouse antibody (Zymed) 1:1,000 in blocking solution. Cells were washed and resuspended in PBS.

For aneuploidy evaluation, DNA-content analysis in mitotic cells was performed as described (4) with some modifications: Cells were fixed overnight at −20 °C in 70% ethanol, rehydrated with PBS 0.1% Tween, and incubated with anti-phosphorylated histone H3 (Ser10, pH3) antibody (CST) diluted 1:400 in PBS. After rinsing, the cells were incubated with FITC-conjugated anti-rabbit antibody (Zymed) at 1:1,000 and then rinsed and resuspended in PBS.

Flow cytometry analyses were done with a FACScalibur flow cytometer (Beckton Dickinson). Aggregates were removed from analysis by FL2W/FL2A gating during acquisition (4). Data were analyzed with the FlowJo software (Tree Star). At least 2×10^4 cells were acquired per sample, and every experiment was repeated at least three times.

γ-H2AX Foci and Anaphase Bridges Scoring. Cells were grown and infected on Lab-Tech slides (Falcon). For γ-H2AX and pH3 immunofluorescence, cells were fixed with PBS 4% formaldehyde for 12 min, permeabilized in PBS 0.25% Triton X-100 for 5 min, and blocked with PBS 0.1% Tween and 5% normal goat serum for 30 min. Primary antibodies [mouse anti-γH2AX (Upstate) and rabbit anti-pH3 (Ser10; CST)] were diluted 1:400 in blocking solution and incubated overnight at 4 °C. FITC- or TRITC-conjugated secondary antibodies (Zymed) were diluted 1:1,000 in blocking solution and incubated for 5 h. DNA was stained for 5 min with TO-PRO-3 (Molecular Probes) or with DAPI (Vector). Anaphase bridge quantification was done as described (5). Slides were mounted in VectaShield containing DAPI. Images were acquired with a Leica DMRB fluorescence microscope equipped with a DFC300FX digital camera or with an Olympus IX70 laser scanning confocal microscope, in sequential mode, with the Fluoview software FV500, the confocal aperture being set to achieve a z optical thickness of ~0.5 μm for foci quantification and ~0.26 μm for foci localization analysis. At least 300 cells per anaphase were counted by three different observers without knowledge of the cell treatment. We repeated the experiments three times.

Metaphasic Chromosome Spreading. Cells were treated with 1 μg/mL Colchicine (Sigma) for 24 h and then were collected by trypsin treatment and suspended in a hypotonic solution (FCS 1:6 in distillated H_2O) for 20 min at 37 °C. After overnight fixation at −20 °C in 75% methanol/25% acetic acid, cells were streamed on wet, cold microscopic slides and stained with Giemsa-R (RAL) or DAPI. Metaphases were analyzed with the Genus software (Applied Imaging). Slides were scored blindly as above. At least 100 metaphases were scored per group; the total quantification was obtained after three independent experiments.

Micronuclei and Nucleoplasmic Bridges Assay. The assay was performed as described (6). Briefly, cells were treated with 2 μg/mL cytochalasin-B (Sigma) for 24 h, washed, and incubated in culture medium for 20 min. Cells were collected by trypsin treatment and resuspended in hypotonic solution for 20 min at 37 °C, and were fixed overnight at −20 °C in methanol. Streams were done on dry microscopic slides and stained with Giemsa. Slides were scored blindly as above. At least 1,000 binucleate cells were counted per group, and the experiments were repeated three times.

Gene Mutation Assay. 6-Thioguanine (6-TG) (Sigma) was dissolved in DMEM at a concentration of 2 mg/mL. Trifluorothymidine (TFT) (Sigma) was dissolved in sterile DMSO and diluted in culture medium to 2 μg/mL solution, then stored at −20 °C. CHO and HCT-116 cells were treated with culture medium supplemented with 10 mM deoxycytidine, 200 mM hypoxanthine, 0.2 mM aminoprotein, and 17.5 mM thymidine for 1 wk to eliminate preexisting *hprt* or *tk* mutants (7). After infection, the cells were cultured 1 wk for the 6-TG (*hprt*) test, and 3 d for the TFT (*tk*) test. Then 10-cm^2 Petri dishes were seeded with 2×10^5 cells, using culture medium containing 400 μg/mL 6-TG or 2 μg/mL TFT. Cells also were plated without 6-TG or TFT to determine plating efficiency. The culture medium was changed twice a week for 21 d for the 6-TG test and for 18 d for the TFT test (8). Then plates were fixed with 4% formaldehyde and stained with Giemsa. Four repeats were done per group per test, and each test was done at least three times.

Clonogenicity in Soft Agar. After infection, the cells were cultured for 3 d. Then 10,000 cells were seeded in culture medium with 0.3% soft agar and plated on 3.5-cm^2 dishes. After incubation for 7 d and staining with 0.005% crystal violet (RAL) for 1 h, colonies (>50 cells) were counted using a stereomicroscope (9).

In Vivo Assays. Animal experiments were carried out in accordance with European Guidelines for the Care and Use of Animals for Research Purposes.

For the ligated colon loop assay, 6-wk-old BALB/cJ Rj (Janvier) female mice were starved for 24 h before anesthesia with a mix of ketamine and xylazine. After a midline incision was made, the colon was exteriorized, a ligature was placed on the proximal and distal ends of the colon, and 300 μL of a bacterial concentrate (3×10^9 bacteria) grown in IM was injected directly into the lumen. Because the rodent colon contains, on average, 295×10^3 cells/mm^2 (10), and that the ligated colon measured, on average, 2.5 cm^2, the calculated infectious dose was four bacteria per enterocyte. The midline incision was sutured, and the mice were left in a dark, warm environment to recover. Mice were killed by neck dislocation 6 h later, and the colon was recovered immediately. The experiment was repeated three times.

For the antibiotic treatment assay, 3-wk-old BALB/cj Rj (Janvier) female mice were treated for 5 d with a mix of streptomycin (2 g/L; Sigma), bacitracin (2 g/L; Sigma), and neomycin (1 g/L; Sigma) in the drinking water (11); antibiotics were withdrawn 24 h before bacterial inoculation. Mice were inoculated with 10^9 bacteria by intragastric gavage twice at 24-h intervals. Mice were killed by neck dislocation 12 h after the second inoculation, and the colon was recovered immediately.

Irradiated mice were used as positive control. Mice received either 0.5- or 2-Gy gamma ray irradiation from a ^{137}Cs radiation source (630 rad/min). Mice were killed 30 min later by neck dislocation, and the colon was recovered immediately.

Colonocyte Western Blot Analyses. The colon was placed in ice-cold PBS and opened through its mesenteric band. Mucus and feces were washed out. Then, for enterocyte collection, the colon was placed in a solution composed of 67.5 mM NaCl, 1.5 mM KCl, 0.96 mM NaH_2PO_4, 26.19 mM $NaHCO_3$, 27 mM NaCl, 1.5 mM EDTA, 0.5 mM DTT, pH 7.4, for 10 min, at 37 °C, under agitation (12). The remnants of soft tissue were discarded, and the cell solution was centrifuged for 5 min at $300 \times g$. The cell pellet was rinsed with PBS plus DTT 0.5 mM, and resuspended in 500 μL of Laemmli sample buffer. Samples were sonicated twice for 2 s incubated at 100 °C for 5 min, and stored at −20 °C. Proteins were separated on NuPage gels (Invitrogen), transferred to nitrocellulose membranes, blocked with 5% skimmed milk buffer, and probed with mouse anti-γH2AX (Upstate), mouse anti-histone H3 (Upstate), and mouse anti-Actin (Sigma). Secondary antibodies used were HRP goat anti-mouse (Zymed) and HRP goat anti-rabbit (Zymed). Proteins were detected by chemiluminescence with the ChemiDoc XRS system (Bio-Rad). Densitometric analyses were done using QuantityOne 4.6.5 software (Bio-Rad).

GFP Imaging and γH2AX Immunohistology. For GFP-expressing bacteria imaging, after mice had been killed, 0.5-cm sections of colon were placed immediately on optimum cutting temperature compound (O.C.T.; Tissue-Tek) and frozen in liquid nitrogen. Frozen sections (8 μm) were placed on microscope slides and fixed in 4% formaldehyde. DNA was stained with TO-PRO-3 (Molecular Probes) diluted 1:1,000 in PBS, and actin was stained with rhodamine-phalloidin (Invitrogen) diluted 1:1,000 in PBS. Images were acquired with an Olympus IX70 laser scanning confocal microscope with the confocal aperture set to achieve a *z* optical thickness of ∼0.5 μm.

For γH2AX immunohistochemistry, the entire colon was recovered immediately after mice had been killed, was placed in 10% formalin for 24 h, and then was embedded in paraffin according to standard procedures. Slides of 8-μm sections were washed in xylene for 10 min, then washed with 100% ethanol, and finally rinsed with distillated water. For antigen unmasking, slides were incubated in boiling 10 mM Na-Citrate for 10 min. Samples were stained and developed using the Vectastain ABC and DAB kits (Vector). The primary antibody was rabbit anti- γH2AX (Cell Signaling Technology); the secondary antibody was the biotinylated anti-rabbit (Vector). Counterstaining was done using nuclear Hematoxylin QS (Vector). Slides were mounted with Vectamount (Vector). Gamma foci were counted in at least 1,000 cells per group by three different observers without knowledge of the colon treatment.

Statistical Analyses. SD and SEM were calculated as previously suggested (13). The exact Fisher test was used to calculate significant variances between cellular populations in the aneuploidy test. The mutation frequency for mammalian cell gene mutation assays was calculated following Arlett et al. (14), and the x^2 McNemar test was used to calculate the *P* value. For γH2AX foci quantification in mice colons, the ANOVA test with an exact Fisher posttest was used. For the soft agar clonogenicity test, an ANOVA with a Bonferroni posttest was used. The software GraphPad-Prism 5 was used for statistical testing. A *P* value was considered significant if $P < 0.05$, $P < 0.01$, or $P < 0.001$.

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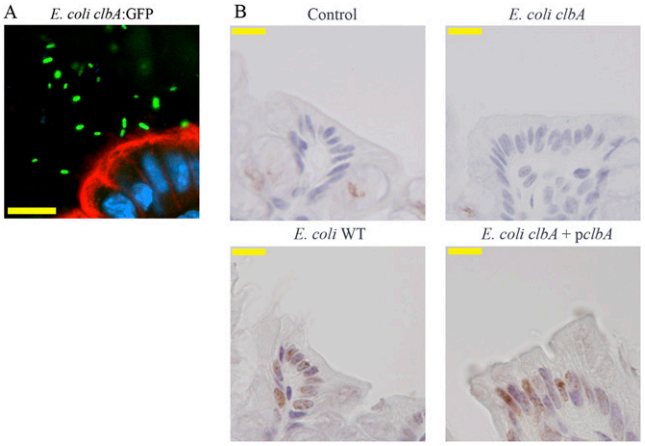

Fig. S1. Antibiotic-treated mice enterocytes show γH2AX foci after gavage with *pks*$^{+}$ *E. coli*. BALB/c mice were treated for 5 d with a mix of streptomycin-neomycin-bacitracin and then inoculated by gastric gavage with 10^{9} bacteria or PBS as a control. (*A*) Frozen colon sections were stained for DNA (blue) and F-actin (red) and then were examined by confocal microcopy. *pks*$^{+}$ *E. coli* expressing GFP under control of the *clbA* promoter (clbA:GFP) were detected in the green channel. (Scale bars, 10 μm.) (*B*) Twelve hours after inoculation with wild-type *pks*$^{+}$ *E. coli* (WT), *clbA* mutant, or complemented mutant *(clbA* + p*clbA)*, paraffin colon sections were stained for γH2AX (brown) and counterstained with hematoxylin.(Scale bars, 10 μm.) γH2AX foci were found in enterocytes from mice inoculated with the wild-type *pks*$^{+}$ *E. coli* or the complemented mutant (8.25 and 7%, respectively) but not in the other groups.

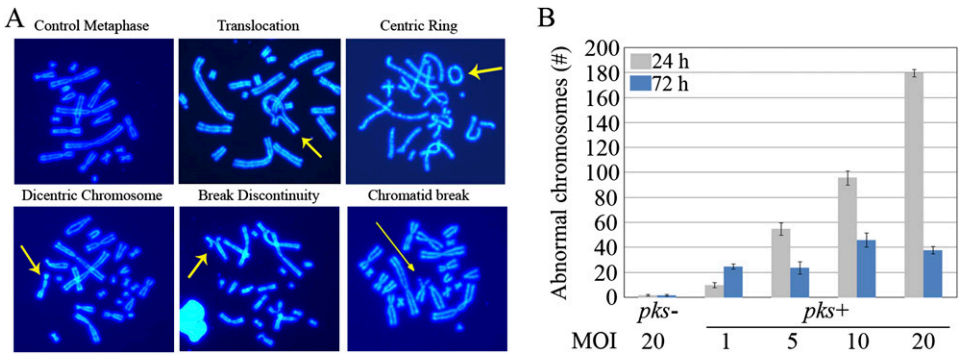

Fig. S2. Chromosome aberrations in metaphasic cells after infection with *pks*$^{+}$ *E. coli*. (*A*) Metaphasic chromosomes of CHO cells 24 h after infection with *pks*$^{+}$ *E. coli*. The chromosomes were labeled with DAPI after metaphase spreading. The most frequent chromosome rearrangements are shown. (*B*) Abnormal chromosomes were counted in 100 metaphases per group, 24 and 72 h after exposure to *pks*$^{+}$ or *pks*$^{-}$ *E. coli*. Error bars represent the SEM from three independent experiments.

Table S1. Infection with pks$^+$ E. coli induces aneuploidy and tetraploidy in CHO, IEC-6, and HCT-116 cells

Infection	MI (%)	Distribution of mitotic population (%)			
		Hypodiploid	Normal	Hyperdiploid	Polyploid
CHO					
Control	3.69	0.76	96.57	1.44	1.23
E. coli pks$^-$	3.36	0.72	96.01	1.76	1.52
E. coli pks$^+$	3.56	1.4***	90.45	3.23***	4.92***
E. coli clbA	3.72	0.71	96.76	1.47	1.24
E. coli clbA$^+$ p*clbA*	3.53	1.43***	90.86	3.20***	4.41***
IEC-6					
Control	6.7	0.65	96.29	1.28	1.78
E. coli pks$^-$	6.93	0.52	96.19	1.41	1.87
E. coli pks$^+$	6.22	1.88***	91.4	2.23***	4.49***
HCT-116					
Control	9.01	0.89	95.09	1.31	2.72
E. coli pks$^-$	9.17	0.97	94.71	1.5	2.83
E. coli pks$^+$	10.68	1	93.15	2.12**	3.73**

The distribution of mitotic cells according to their DNA content 3 d after infection with *pks*$^-$, *pks*$^+$, *clbA* mutant, or complemented mutant (p*clbA*) *E. coli*, was assessed by flow cytometry as in Fig. 3*B*.
**$P < 0.01$ and ***$P < 0.001$ in an exact Fisher test compared with controls. Mitotic indexes were not statistically different between treatments.