Supplemental material

Zhu et al., https://doi.org/10.1084/jem.20181939

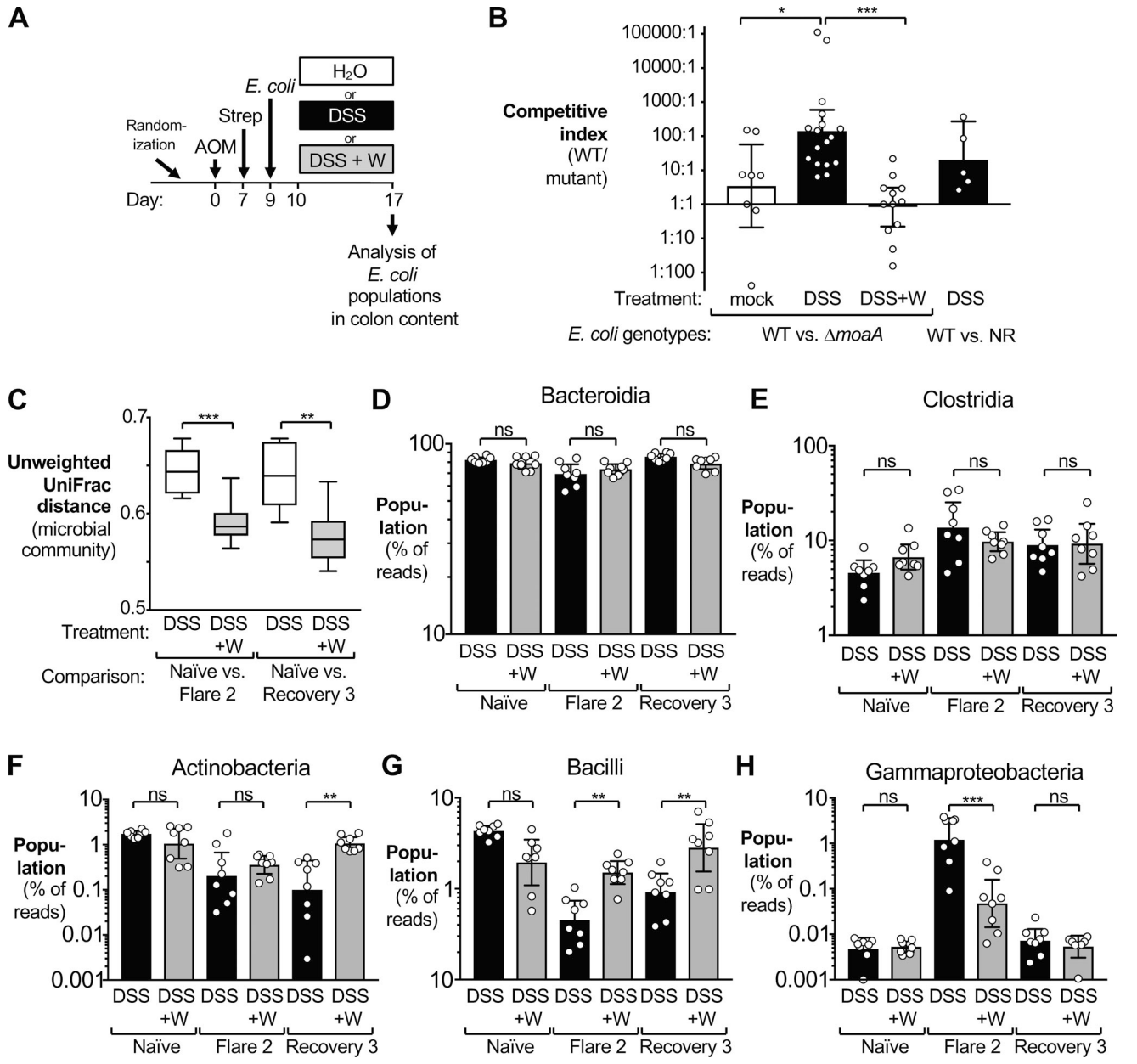

Figure S1. **Impact of tungstate treatment on fitness of *E. coli* strains and the dynamics of gut microbial communities in the AOM/DSS colitis model. (A and B)** Groups of C57BL/6 mice received an intraperitoneal injection with AOM and were treated with streptomycin in drinking water as described in Fig. 1. Mice were then intragastrically inoculated with an equal mixture of the *E. coli* Nissle 1917 wild-type strain and a Δ*moaA* mutant or the wild-type strain and a Δ*narZ* Δ*narG* Δ*napA* (NR) mutant. Animals were then treated with 2% DSS, 2% DSS and 0.2% sodium tungstate (W), or mock-treated. Data from two independent experiments are shown. **(A)** Schematic representation of the experimental design. **(B)** The colon content was analyzed 17 d after AOM treatment by plating on selective agar plates. The competitive index is the ratio of the number of wild-type bacteria to mutant bacteria in the colon content, corrected by the analogous ratio in the inoculum. Bars represent the geometric mean ± 95% confidence interval. **(C–H)** The gut microbiota of the mice shown in Figs. 1 and 2 (A–C), determined by 16S rDNA amplicon sequencing, was further analyzed. Two experiments were performed and results from one experiment are shown. **(C)** Unweighted UniFrac distances of DSS and DSS+W–treated mice, comparing the naive state (day −1) with the state in flare 2 (day 38) and the naive state with the final recovery state (recovery 3; day 73). **(D–H)** Abundance of the five most abundant classes, i.e., Bacteroidia (D), Clostridia (E), Actinobacteria (F), Bacilli (G), and Gammaproteobacteria (H), as determined by the number of class-specific reads within all bacterial reads. Bars represent the geometric mean ± 95% confidence interval. P values were calculated by unpaired, two-tailed Student's *t* test on log-transformed data (B and D–H) or raw data (C). *, P < 0.05; **, P < 0.01; ***, P < 0.001; ns, not statistically significant.

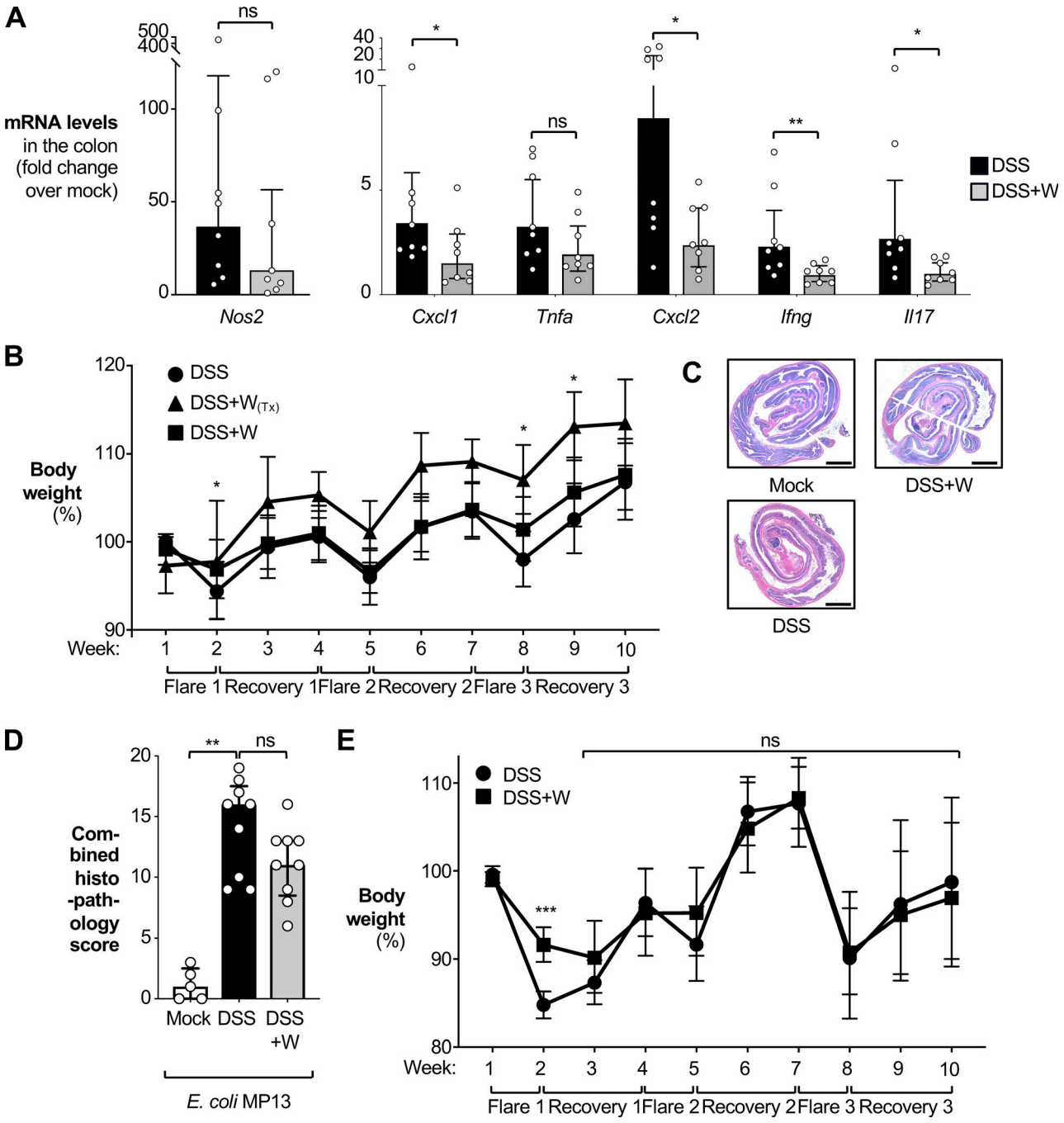

Figure S2. **Analysis of colitis markers in the AOM/DSS colitis model. (A–D)** Groups of C57BL/6 mice were treated as described in Fig. 1 A. Colonic tissue was harvested at 73 d after AOM treatment. Data from two independent experiments are shown. **(A)** Relative mRNA levels in distal colonic tissue for a panel of proinflammatory markers was determined by quantitative RT-PCR. Transcription is shown as fold change compared with mock-treated animals. Bars represent the geometric mean ± 95% confidence interval. P values were calculated by unpaired, two-tailed Student's *t* test on log-transformed data. **(B)** Weekly average of animal body weight throughout the experiment. Week 2, DSS versus DSS+W, P = 0.037; week 7, DSS versus DSS+W, P = 0.026; week 8, DSS versus DSS+W, P = 0.037. Statistical analysis was calculated using two-way ANOVA followed by a Tukey's multiple comparisons test. Bars represent the geometric mean ± 95% confidence interval. **(C and D)** H&E-stained sections were scored by a veterinary pathologist for epithelial damage, polymorphonuclear neutrophil infiltration, submucosal edema and exudate in the lumen. **(C)** Representative images. Scale bars represent 250 µm. **(D)** Combined histopathology score bars represent the median, error bars the interquartile range. P values were calculated by two-tailed Mann–Whitney *U* test. DSS versus DSS+W, P = 0.08 (ns). **(E)** Groups of naive C57BL/6 mice (obtained from Charles River) were treated as described in Fig. 3. Weekly average of animal body weight throughout the experiment. Experiment was performed once. Statistical analysis was calculated using two-way ANOVA followed by a Tukey's multiple comparison test. Bars represent the geometric mean ± 95% confidence interval. ns, not significant. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

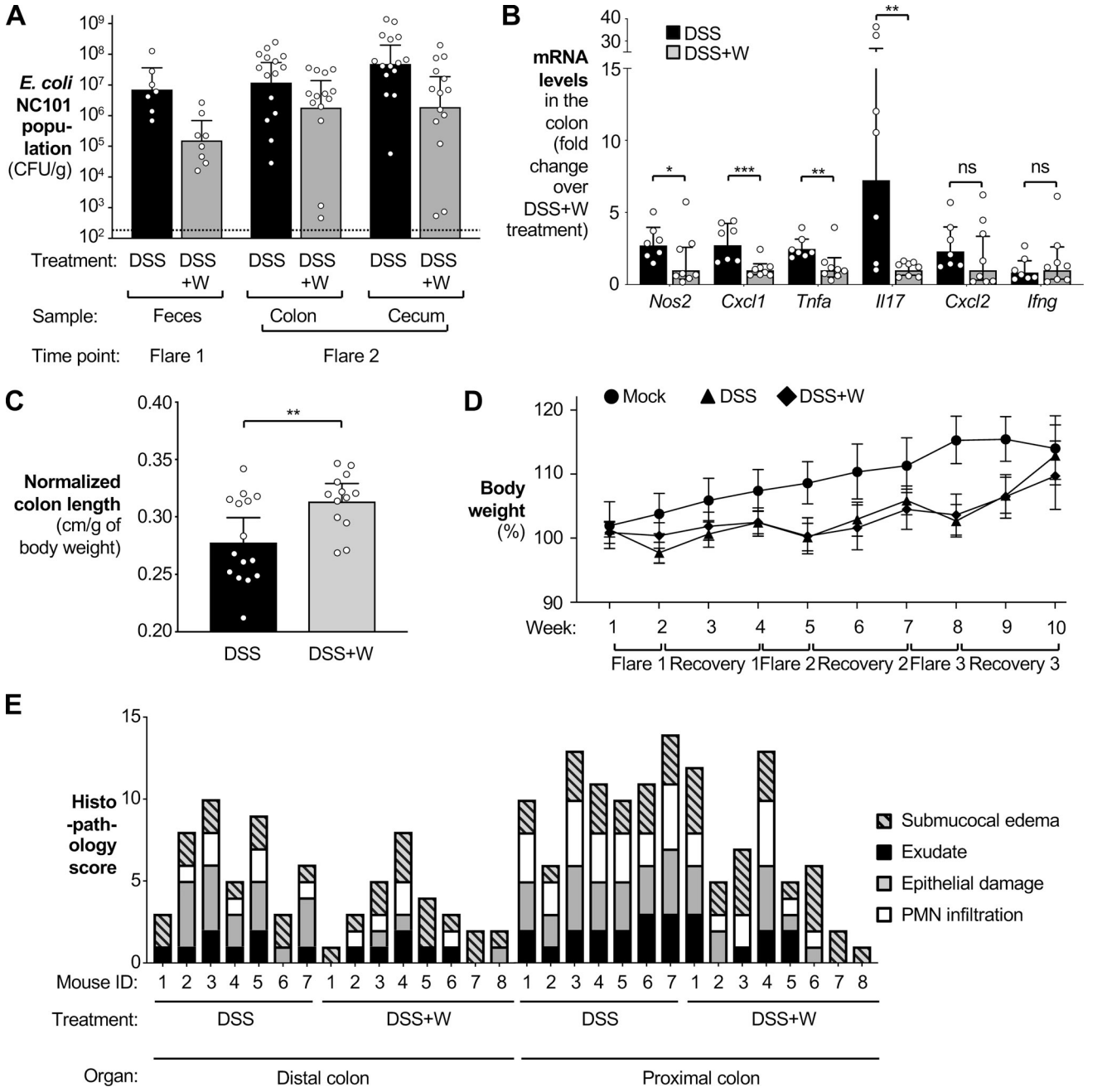

Figure S3. **Colonization of NC101 and inflammatory responses in a model of colibactin-driven tumorigenesis.** Samples of the animals described in Fig. 5 were further analyzed (day 38). **(A–C)** Data from two independent experiments are shown. For A–C, P values were calculated by unpaired, two-tailed Student's *t* test on log-transformed data. **(A)** Abundance of *E. coli* NC101 in the feces on day 17 (flare 1) and in the colon and cecum content at day 38 (flare 2). **(B)** mRNA levels of inflammatory markers in the distal colonic tissue at day 38. ns, not significant. **(C)** Colon length at day 38, normalized to total body weight. **(D)** Weekly average of animal body weight throughout the experiment. Statistical analysis was calculated using two-way ANOVA complemented with Tukey's multiple comparison test. Data from five independent experiments are shown. **(E)** H&E-stained sections were scored by a veterinary pathologist for epithelial damage, polymorphonuclear neutrophil infiltration, submucosal edema, and exudate in the lumen. Individual scores for each animal are shown. The experiment was performed twice; the analysis shown in A, B, and E was only performed in the second experiment. Each dot represents data from one individual animal. Bars represent the geometric mean ± 95% confidence interval. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

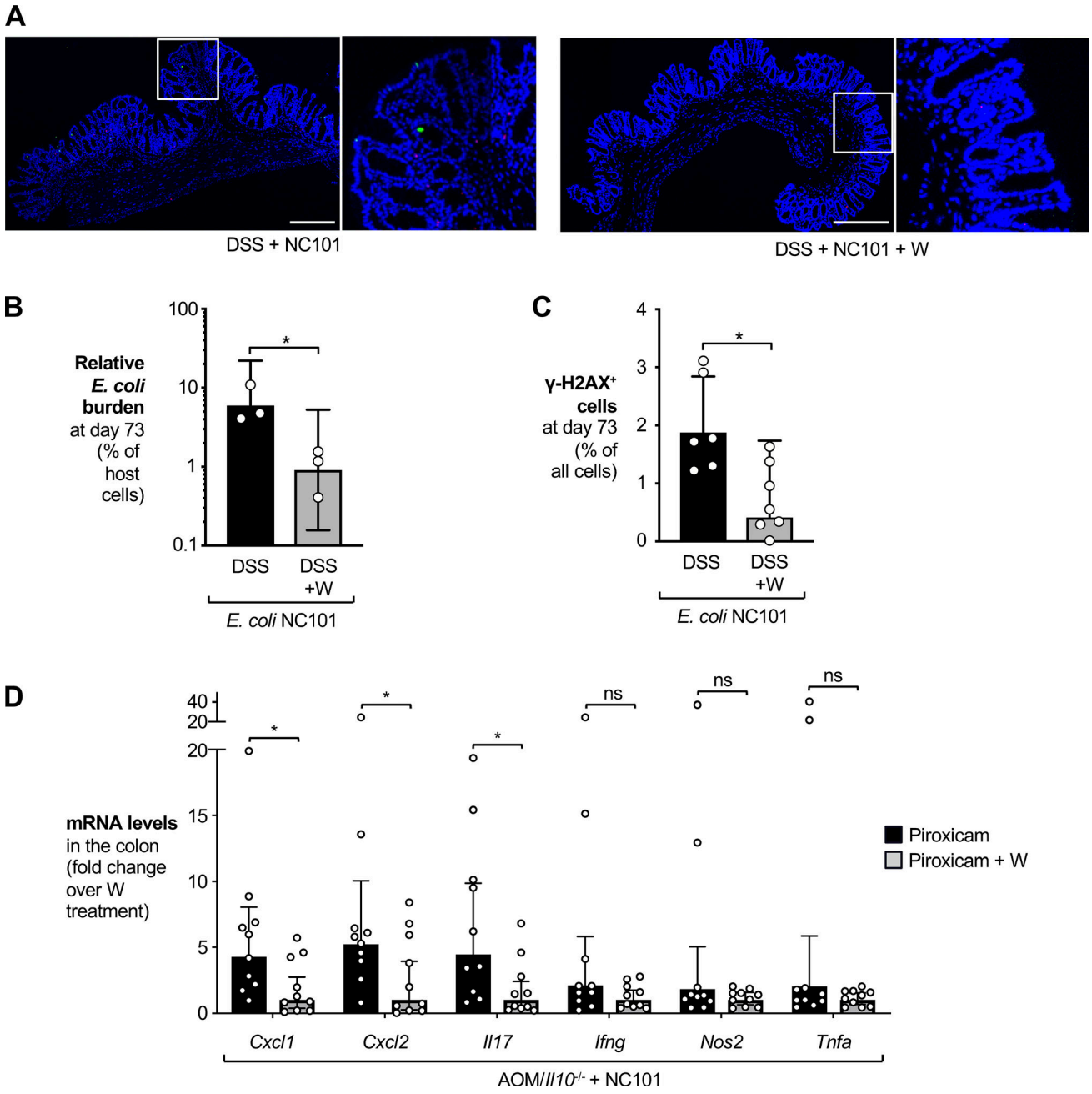

Figure S4. **Host DNA damage and inflammatory responses. (A–C)** Representative samples of the animals described in Fig. 4 (AOM/DSS model) were further analyzed (day 73). Transverse sections of colonic tissues were stained for tissue-associated *E. coli* (red), DNA damage using γ-H2AX foci as marker (green), and cell nuclei (blue). Individual image tiles were assembled to quantify *E. coli* burden and number of γ-H2AX–positive cells and total mammalian cells. Five experiments were performed and representative results of two experiments were shown. **(A)** Representative images. Scale bars represent 300 µm. **(B)** Number of *E. coli* bacteria as a fraction of the number of mammalian cells obtained from three randomly chosen mice in each treatment group. **(C)** Abundance of γ-H2AX–positive cells normalized to the number of all mammalian cells. **(D)** mRNA levels of inflammatory markers in the distal colonic tissue of the mice shown in Fig. 7 (*Il10*/AOM model) were determined by quantitative RT-PCR. Data from two independent experiments are shown. Bars represent the geometric mean ± 95% confidence interval. P values were calculated by unpaired, two-tailed Student's *t* test on log-transformed data. *, $P < 0.05$.

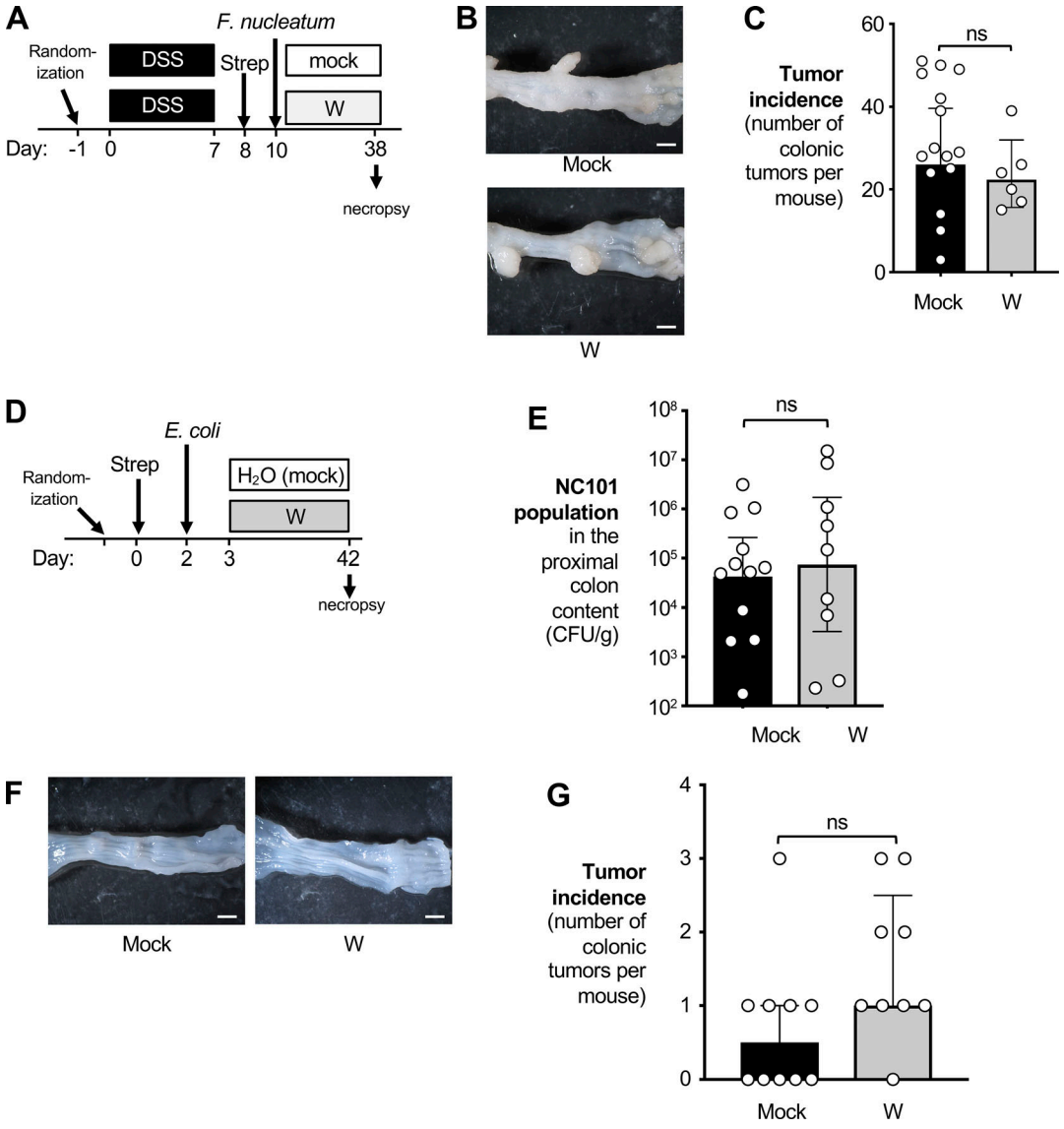

Figure S5. ***Apc*$^{Min+/-}$ models of tumorigenesis in the large intestine. (A–C)** Groups of *Apc*$^{Min+/-}$ mice received 2% DSS in the drinking water for 1 wk. Engraftment of *F. nucleatum* strain ATCC 23726 (10^9 CFU) was enhanced by treating mice with 2 mg/ml streptomycin in the drinking water for 2 d. A subset of mice was treated with 0.02% sodium tungstate in the drinking water. Samples were obtained 38 d after the start of the DSS treatment. Data from two independent experiments are shown. **(A)** Schematic representation of the experiment. **(B)** Representative images of the gross pathology of the distal large intestine. Scale bars represent 1 mm. **(C)** Number of tumors in the large intestine. Bars represent the median ± interquartile range. P values were calculated by two-tailed Mann–Whitney *U* test. **(D–G)** Groups of *Apc*$^{Min+/-}$ mice were treated 2 mg/ml streptomycin in the drinking water for 2 d. 1 d later, animals received 10^9 CFU of the *E. coli* NC101 strain. A subset of animals received 0.02% of sodium tungstate in the drinking water for a total of 38 d while the other subset received normal drinking water. Data from three independent experiments are shown. **(D)** Schematic representation of the experiment. **(E)** The abundance of NC101 was determined by plating colonic content on selective agar plates. Bars represent the geometric mean ± 95% confidence interval. P values were calculated by an unpaired, two-tailed Student's *t* test on log-transformed data. **(F)** Representative images of the gross pathology of the distal large intestine. Scale bars represent 1 mm. **(G)** Number of tumors in the large intestine. Bars represent the median ± interquartile range. P values were calculated by two-tailed Mann–Whitney *U* test. ns, not statistically significant.

Tables S1–S3 are provided online as separate Excel files. Table S1 lists scores of individual samples on the intestinal inflammation and tumor histology. Table S2 lists strains and plasmids used in this study. Table S3 lists all primers used in this study.