# Supporting Information

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### SI Materials and Methods

**Growth and Maintenance of HCT-8 Cells.** The human colon cancer cell line HCT-8 (ATCC), derived from enterocytes at the junction of the large and small bowel, was maintained in RPMI 1640 medium with 10% horse serum, 1 mM sodium pyruvate, 10 mM Hepes, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37°C in 5% $CO_2$, according to standard ATCC protocols (henceforth referred to as RPMI medium).

**RNA Isolation and Microarrays.** HCT-8 cells were grown in monolayers in standard tissue-culture T-25 flasks to 80% confluence. Cells were washed twice with sterile PBS (9 g/L NaCl, 0.795 g/L $Na_2HPO_4$, 0.144 g/L $KH_2PO_4$) and exposed to 1 mM indole (Acros Organics; stock solution of 250 mM in DMF) in triplicate for 4 h or 24 h. This concentration of indole was chosen based on detection of 30–130 μg indole/g fecal matter (1, 2) [or 250–1,100 μM, assuming a feces density of 1 g/cm$^3$ (3)]. Control cells were exposed to same volume of solvent, dimethyl formamide (DMF). At the end of exposure, cells were washed twice with PBS, detached from the flasks by using trypsin-EDTA, centrifuged, and stored at −80° C. RNA was isolated by using Qiagen's RNeasy Mini Kit, and the quality of RNA was assessed by using gel electrophoresis (gel images were observed for high- and low-molecular-weight smears, which indicate DNA and RNase contamination, respectively) and spectrophotometrically ($A_{260}/A_{280}$ ratio between 1.9 and 2.1). Reverse transcription, cDNA labeling, hybridization of arrays, and scanning were performed by using standard protocols at the Center for Environmental and Rural Health (CERH) Genomics core facility at Texas A&M University. Following quality assessment on an Agilent Bioanalyzer, 2 μg of total RNA was used to generate biotin-labeled cRNA via a modified Eberwine RNA amplification protocol using the CodeLink iExpress Kit (Applied Microarrays). Labeled cRNA was hybridized to CodeLink human genome arrays (catalog no. 300026) for 18 h followed by washing, staining, and scanning according to the standard CodeLink protocol. Array images were processed by using CodeLink system software, and global median normalization was used to generate normalized expression values. A total of nine arrays were performed with three arrays each for control and exposure to indole at 4 h and 24 h.

Pairwise comparison between control and indole-treated cells and identification of differentially expressed genes was carried out using a sequential three-step procedure. First, only those transcripts that were detected in all six arrays (three arrays each for control and indole) were selected for further analysis. Second, the data were filtered for quality and only those signals that were classified as "G" (good) by the CodeLink analysis software in all six arrays were processed further. Third, transcripts exhibiting a statistically significant change in expression in indole-treated arrays with respect to control were identified by using Student's $t$ test at a significance level of $P < 0.01$. Using this approach, 523 and 4,102 genes were differentially expressed upon indole exposure after 4 h and 24 h, respectively.

**In Vitro Colonization Assays.** Adhesion of EHEC to HCT-8 cells was performed by using a previously described protocol (4). Low-passage-number HCT-8 cells were cultured in standard 24-well tissue culture plates and grown at 37°C in 5% $CO_2$ until ∼80% confluence. Indole (1 mM final concentration) was added to some wells 24 h before the experiment. After the preincubation, HCT-8 cell monolayer was washed two times with PBS to remove traces of indole, and antibiotic-free RPMI with 10% heat-inactivated horse serum was added. Approximately $10^7$ cells of a freshly grown EHEC culture (turbidity of ∼0.8 at 600 nm) were added to each well along with 500 μM NE and incubated for 3 h at 37°C and 5% $CO_2$ as described in ref. 4. Loosely attached bacteria were removed by washing the wells twice with PBS, and the HCT-8 cells were lysed in the wells by using 0.1% Triton X-100 in PBS. The cell suspension in each well was vigorously vortexed, and serial dilutions of the bacteria were grown on LB-agar plates. Colonies were counted after a 24-h incubation at 37°C.

**Reporter Lentivirus and NF-κB Activity Measurements.** HCT-8 cells were transduced with a NF-κB reporter lentivirus (kind gift of Stelios Andreadis, State University of New York at Buffalo). The third-generation lentiviral plasmid contains a fluorescent protein (ZsGreen-DR) that is under the control of an inducible phosphoglycerate kinase (PGK) promoter and tandem repeats of the NF-κB binding sequence (response element). Binding of NF-κB to its response element results in transcription of the ZsGreen-DR protein and can be used to monitor activation of NF-κB. In addition, the reporter plasmid also contains a DsRed2 fluorescent protein under control of a cytomegalovirus (CMV) constitutive promoter for monitoring transduction efficiency. Details on construction of the lentivirus reporter plasmid are described in Tian and Andreadis (5). Lentiviral particles were produced by co-transfection of the NF-κB reporter plasmid and three other plasmids (plasmid pMDL-g/p, plasmid pSRV-rev, and plasmid pMDG-VSVG) into 293T/17 cells. Virus particles in the supernatant were harvested after 24 h, pelleted by ultracentrifugation (50,000 × $g$ at 4°C for 2 h), and resuspended in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS.

For transduction, HCT-8 cells were seeded at low density (∼30,000 cells per well in a 24-well plate; ∼ 30% confluence) and incubated for 24 h at 37°C and 5% $CO_2$. Next day, the reporter lentivirus was diluted 10-fold in RPMI medium and 8 μg/mL Polybrene (hexadimethrine bromide) was added to lentivirus solution. To each well, 200 μl of the lentivirus-polybrene mixture was added, and the plate was incubated for 24 h at 37°C and 5% $CO_2$. The supernatant was removed, fresh RPMI medium was added, and the cells were maintained in culture for a further 3–5 days. The transduction efficiency (determined by using expression of the DsRed2 fluorescent protein) was ∼70%.

Reporter cells transduced with the lentivirus were then exposed to different bicyclic-aromatics for 4 h, followed by incubation with 40 ng/mL TNF-α to activate NF-κB. The different compounds and the concentrations used were: 1 mM indole and 250 μM each indole-2,3-dione, 2-hydroxyindole, 5-hydroxyindole, 7-hydroxyindole, and indole-3-acetic acid. These concentrations were chosen based on preliminary data from our laboratory on the toxicity of these compounds to HCT-8 cells. Expression of the NF-κB-driven ZsGreen-DR fluorescent protein was monitored by using a Zeiss Axiovert 200 fluorescence microscope. Positive control was cells exposed to TNF-α, whereas the negative control cells were exposed to neither TNF-α nor bicyclic aromatics. The cells were positioned on an automated stage on the microscope and enclosed in an incubated chamber at 37°C and 5% $CO_2$, and fluorescence and transmitted light images were then taken at different time points. For experiments with indole, images were taken every hour for 18 h, whereas for experiments with the other compounds images were obtained at a single time point after 18 h. For all experiments, images were taken at the identical spot at every time by using the mark-and-find feature of the microscope, thus eliminating any user bias. Fluorescent images were analyzed by using an in-house-developed image-analysis program developed in MatLab (The MathWorks) (6).

**Transepithelial Resistance (TER) Measurements.** Approximately $10^5$ HCT-8 cells were grown on 0.33-$cm^2$, 0.4-μm Transwell filter units (Costar) for TER measurement (7), with 100 μl and 600 μl of RPMI medium added to the apical side and basolateral sides, respectively. Polarization of HCT-8 cells was observed by using a volt-ohmmeter (World Precision Instruments). Cells were considered to be polarized when the TER was ~500 Ω.$cm^2$ (typically after 5–6 days in culture) (7). After verification of polarization, different bicyclic aromatics were added to both apical and basolateral chambers, and the TER was monitored. The different compounds and the concentrations used were: 1 mM indole and 250 μM each indole-2,3-dione, 2-hydroxyindole, 5-hydroxyindole, 7-hydroxyindole, and indole-3-acetic acid. For experiments with indole, TER was monitored at different time points, whereas the TER upon exposure to the other compounds was measured at a single time point after 24 h. The change in TER upon exposure to bicyclic aromatics was calculated relative to control cells (exposed to DMF only).

**Extracellular Indole Assay.** Extracellular indole assay was performed as described in ref. 8. Briefly, HCT-8 cells were grown in 24-well plates until ~80% confluence. To cells in three wells, 1 mM indole was added, and to cells in three wells, no indole was added (negative control). Also, to three empty wells, 1 mM indole was added to RPMI medium alone, as positive control. The plate was then incubated for 24 h and the supernatants from all of the wells were collected. Extracellular indole was measured by mixing 500 μl of supernatant with 200 μl of Kovac's reagent. The pink-colored solution, arising from the reaction of *p*-dimethylaminobenzaldehyde with indole, was diluted in HCl-amyl alcohol mixture, and the absorbance was measured at 540 nm. Indole concentrations were determined according to standard concentration curves.

**HCT-8 Viability Assay.** HCT-8 cells were grown in 24-well plates until ~80% confluence and washed once with PBS before the experiment. Indole (1 mM) in fresh RPMI medium was added to triplicate wells while the same volume of DMF was added to the negative control wells. The plate was incubated at 37°C and 5% $CO_2$ for 24 h. The next day, the cells were washed twice with PBS and detached from the surface by using 100 μl of trypsin-EDTA. The cells were then resuspended in 500 μl of RPMI medium, and an equal volume of 0.4% trypan blue solution (Mediatech) was added to cells. The cells were then incubated at room temperature for 2 min, and the number of live (transparent) and dead (blue) cells in 10 μl of this solution was counted by using a hemocytometer (Hausser Scientific) at 25× magnification. For each sample, cells present in five 1 mm × 1 mm squares were counted and used for calculating the cell density.

**Quantitative RT-PCR.** DNA microarray data were corroborated by using qRT-PCR (4). The primers were designed by using PrimerQuest online software (Table S3). qRT-PCR was performed by using iScript one-step RT-PCR kit with SYBR green (Bio-Rad Laboratories) on a MyiQ single-color real-time PCR detection system (Bio-Rad Laboratories). The threshold cycles, as calculated by the MyiQ optical system software (Bio-Rad Laboratories), were used to determine the relative changes between the samples. The experiments were run in triplicate in 20-μl reactions, and 50 ng of total RNA was used for each reaction, with the final forward and reverse primer concentrations at 0.15 μM each. After amplification, template specificity was ensured through melting curve analysis. 18S RNA was used as the housekeeping gene for normalizing the data.

**IL-8 Enzyme-Linked Immunosorbent Assay (ELISA).** IL-8 concentration in the HCT-8 cell supernatants was determined by using a commercially available human IL-8 ELISA kit (Pierce Biotechnology) according to the manufacturer's instructions. HCT-8 cells were exposed to solvent (control) or 1 mM indole for either 4 h or 24 h, and the supernatants were collected. To some wells, 1 mM indole was added for 4 h and then 20 ng/mL TNF-α was added to stimulate IL-8 production. Four hours later, supernatants from those cells were collected. IL-8 standard supplied by the manufacturer was diluted serially at 1,000, 400, 160, 64, 25.6, and 0 pg/mL, and was used to determine the concentration of IL-8 released by cells. The assay range is 25.6–1,000 pg/mL and the sensitivity is <2 pg/mL The assay was performed with three biological replicates per condition. The samples were diluted as required to maintain IL-8 concentration in the assay range.

**Flow Cytometry and Intracellular Cytokine Staining.** Changes in IL-10 production in response to indole or DMF (solvent control) were determined by using intracellular cytokine staining as described in ref. 9. Briefly, confluent monolayers of HCT-8 cells in a 24-well plate were exposed to 1 mM indole or solvent for 24 h. BD GolgiPlug (BD Biosciences), a protein transport inhibitor containing brefeldin A, was added to the cells for the final 6 h of incubation, at a 1,000-fold final dilution. Cells were washed twice with PBS, detached from the surface by using trypsin-EDTA, resuspended in RPMI, and added to 96-well round-bottom plates. After two washes in PBS supplemented with 0.5% BSA, cells were fixed on ice with 4% paraformaldehyde, permeabilized with Perm/Wash buffer (BD Biosciences), and stained with phycoerythrin (PE)-labeled anti-human IL-10 antibody (eBioscience) for 2 h. Cells were resuspended in PBSA and stored in the dark at 4°C until analysis. Unstained cells were used as negative control. Data (8,000 events per sample) were acquired on a BD FACSAria II cell sorter system (BD Biosciences) and analyzed with FlowJo software (Tree Star).


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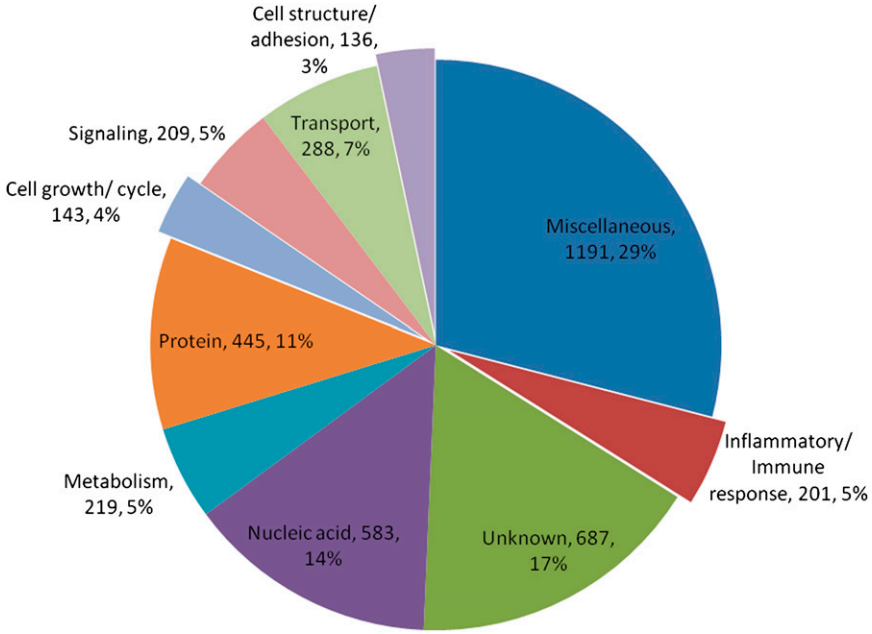


**Fig. S1.** Classification of genes differentially expressed upon indole exposure. Differentially expressed genes were identified from the microarray data (control and 24-h indole exposure) as described in *SI Materials and Methods*, and classified into various functional categories. The three entries in each data label represent, in order, functional category, number of genes differentially expressed, and % of total differentially expressed genes.

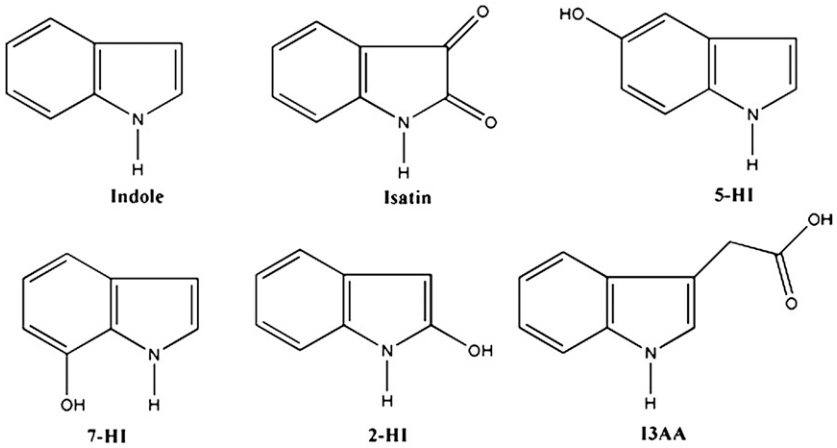


**Fig. S2.** Chemical structure of indole-like compounds used in the study—indole, isatin, 7-hydroxyindole (7-HI), 5-hydroxyindole (5-HI), 2-hydroxyindole (2-HI), and indole-3-acetic acid (I3AA).