Sulfamethoxazole drug stress upregulates antioxidant immunomodulatory metabolites in *Escherichia coli* 

Hyun Bong Park, Zheng Wei, Joonseok Oh, Hao Xu, Chung Sub Kim, Runrun Wang, Thomas P. Wyche, Grazia Piizzi, Richard A. Flavell and Jason M. Crawford

*Escherichia coli* is an important model organism in microbiology and a prominent member of the human microbiota. Environmental isolates readily colonize the gastrointestinal tract of humans and other animals, and they can serve diverse probiotic, commensal and pathogenic roles in the host. Although certain strains have been associated with the severity of inflammatory bowel disease (IBD), the diverse immunomodulatory phenotypes remain largely unexplored at the molecular level. Here, we decode a previously unknown *E. coli* metabolic pathway that produces a family of hybrid pterin-phenolpyruvate conjugates, which we named the colipterins. The metabolites are upregulated by subinhibitory levels of the antifolate sulfamethoxazole, which is used to treat infections including in patients with IBD. The genes *folX/M* and *aspC/tyrB* involved in monapterin biosynthesis and aromatic amino acid transamination, respectively, were required to initiate the colipterin pathway. We show that the colipterins are antioxidants, harbour diverse immunological activities in primary human tissues, activate anti-inflammatory interleukin-10 and improve colitis symptoms in a colitis mouse model. Our study identifies an antifolate stress response in *E. coli* and links its associated metabolites to a major immunomodulatory marker of IBD.

Antibiotics are widely used to treat infectious diseases, leading to dynamic alterations of bacterial metabolic responses involved in growth and survival. However, some of these drugs also lead to immunological activities in the host itself. While well known at the phenotypic level, the diverse mechanisms of antibiotic-mediated immune regulation at the molecular level remain largely unexplored. Three major models have been proposed to account for the phenotypic and clinical observations: (1) antibiotics can alter the microbiome community structure, leading to dysbiosis and inflammatory responses; (2) antibiotics can bind to host targets and directly modulate host immune responses, as supported in germ-free mouse model studies; and/or (3) antimicrobial molecules can be biotransformed by microbes or the host into other antibiotic-derived immunomodulatory metabolites. In the current study, we propose a fourth model and show that subgrowth-inhibitory levels of the antibiotic sulfamethoxazole (SMX) activate a cellular stress response in the common gut bacterium *Escherichia coli*, leading to the identification of previously unknown immunomodulatory metabolites.

*Escherichia coli* infections are commonly treated with SMX in combination with trimethoprim (TMP) due to their synergistic activities. These drugs inhibit folate metabolism, including key steps in DNA synthesis. While TMP inhibits dihydrofolate reductase responsible for tetrahydrofolate production and recycling from dihydrololate, SMX inhibits the upstream folate biosynthetic enzyme dihydropteroate synthase as a competitive inhibitor of its native substrate, para-aminobenzoate. Because folate is of the pteridine class of metabolites, we assessed potential pteridine metabolic rerouting when the early steps of folate biosynthesis were suppressed (that is, subinhibitory levels of SMX). Subinhibitory levels of antibiotics have been used to upregulate ‘cryptic’ bacterial metabolic pathways, including in *E. coli*.

We selected the human probiotic *E. coli* Nissle1917 as a representative *E. coli* strain for our antifolate metabolism studies. Although Nissle1917 is clinically prescribed in Europe to treat inflammatory bowel disease (IBD), it is also a producer of the genotoxin colibactin, which has been implicated in colitis-associated colorectal cancer in adherent invasive *E. coli* (AIEC) pathotypes. Using the broth dilution method, we first established the subinhibitory concentration range of SMX for Nissle1917 in Luria broth (LB) medium. We selected four subinhibitory conditions for further metabolite analysis compared to controls lacking SMX. While we saw no significant difference in growth over a 24-h cultivation period, using high-resolution electrospray ionization–quadrupole–time-of-flight–mass spectrometry (ESI–Q–TOF–MS) and photodiode array spectroscopy, we observed SMX-mediated upregulation of a family of eight metabolites in supernatants (Fig. 1e and Extended Data Fig. 1a). We identified these metabolites, which we named colipterins, by high-resolution electrospray ionization–quadrupole–time-of-flight–mass spectrometry (ESI–Q–TOF–MS) and photodiode array spectroscopy, we observed SMX-mediated upregulation of a family of eight metabolites in supernatants (Fig. 1e and Extended Data Fig. 1a). These colipterins, which we named colipterins 1–8, shared pteridine-like ultraviolet-visible spectra (Extended Data Fig. 1b). The ESI–Q–TOF–MS data allowed us to propose their molecular compositions (Supplementary Figs. 1 and 2). To establish the temporal dynamics of the major colipterins, we measured their production at 12, 24 and 48 h from culture supernatants. Colipterins 1 and 2 were detected at 12 and 24 h before being depleted at 48 h, while the levels of 3 and 4 increased over time (Extended Data Fig. 1c).
Fig. 1 | Colipterins upregulated by SMX antifolate drug stress in *E. coli* Nissle1917. **a.** Mechanism of action of SMX/TMP antibiotics. **b.** Chemical structure of SMX. **c.** Determination of subinhibitory growth ranges of SMX against *E. coli* Nissle1917 (yellow area). **d.** Optical density (OD<sub>600</sub>) of *E. coli* Nissle1917 in the presence of four subinhibitory concentrations of SMX used for metabolite analysis. **e.** Dose-dependent upregulation of colipterins 1–8 in supernatants in response to SMX stress. Ctrl (control) indicates no SMX treatment. The mean and s.d. (error bars) are derived from three biological replicates (*n* = 3). A two-tailed unpaired t-test was used to calculate *P* values; NS, not significant. Corresponding EIC chromatograms are shown in Extended Data Fig. 1a. **f.** Chemical structure of colipterins 1–8. Detailed NMR-based structure elucidation of the colipterins is described in the Supplementary Discussion.

Fig. 2a). In cell pellets, colipterins 3 and 4 were detected only at 24 and 48 h (Extended Data Fig. 2b). Quantification results of all compounds are given in Extended Data Fig. 2c. Finally, to address whether these metabolites were more broadly detected across *E. coli* strains, we also analysed the production of colipterins 1–8 in a representative pathogen (AIEC LF82) and a representative commensal, *E. coli* BW25113. While the subinhibitory antibiotic concentration range of SMX varied among the isolates, SMX supplementation enhanced the production of colipterins 1–5 and 8 (Extended Data Fig. 3); however, 6 and 7 were below the detection limits under these in vitro conditions. Collectively, our data suggest that select colipterins are upregulated in response to antifolate cell stress and are conserved among these probiotic, pathogenic and commensal *E. coli*.

To identify the structures of colipterins 1–8, we isolated and characterized them by nuclear magnetic resonance (NMR), tandem MS, biomimetic chemical synthesis, Marfey’s configurational analysis and CP3 computational analysis (Fig. 1f, Supplementary Figs. 3–64 and Tables 1–5). Details are provided in the Supplementary Information. Although all of the compounds represent previously unknown metabolites, 3 and 4 have been described as synthetic products with no reported activities<sup>45</sup>. To provide genetic support for colipterin biosynthesis, we first evaluated non-essential *fol* genes involved in folate and monapterin metabolism<sup>46,47</sup>. While folate itself is required for bacterial growth, the role of monapterin in *E. coli* remains obscure<sup>48,49</sup>. Since colipterins are upregulated in antifolate SMX stress, we hypothesized that inhibition of the folate metabolism in *E. coli* could inversely lead
Data Fig. 4b), further supporting the premise that colipterins derive in trans with the expression of folX1–4 (Fig. 2b). These metabolic phenotypes could be complemented folX and ΔBW25113, strains

E. coli dominant products at this time point. Relative to the parent

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monapterin (THM) by 7,8-dihydromonapterin reductase (FolM) catalyses the conversion of 2,3-dihydroneopterin triphosphate (DHMTP) which is followed by dephosphorylation to yield 2,3-dihydromonapterin triphosphate (DHNTP) to 2,3-dihydmomonapterin (DHM) and reduction to tetrahydro-monapterin (THM) by 7,8-dihymonapterin reductase (FoM) (Extended Data Fig. 4a)10–12. We thus grew E. coli mutants of folX and folM for 24 h and analysed the production of colipterins 1–4, the dominant products at this time point. Relative to the parent E. coli BW25113, strains ΔfolX and ΔfolM led to complete abolishment of 1–4 (Fig. 2b). These metabolic phenotypes could be complemented in trans with the expression of folX and folM, respectively (Extended Data Fig. 4b), further supporting the premise that colipterins derive from the monapterin pathway. We next focused on the aromatic amino acid transaminases AspC and TryB and their potential role in colipterin biosynthesis. Notably, it has been documented that aspC, which is responsible for the conversion of aromatic amino acids to their α-keto acid forms, is required for drug stress responses in E. coli10–32. In our analysis of the single mutants ΔaspC and ΔtryB, we observed no significant differences in colipterin production compared to wild type (WT). However, we constructed two double mutants, ΔaspC-tryB, in both ΔaspC and ΔtryB backgrounds, and colipterins were completely abrogated (Fig. 2c). These data indicate that aromatic amino acid transamination is required for colipterin biosynthesis and that AspC and TryB are functionally redundant in this regard. Collectively, these studies demonstrate that monapterin biosynthesis and aromatic amino acid transamination

Fig. 2 | Genetic and biomimetic synthesis support for the colipterin pathway. a, Schematic representation of the folate and monapterin pathway. Chemical structures and enzyme annotations are given in Extended Data Fig. 4a. b, Abolition of major colipterins 1–4 in monapterin biosynthetic mutants ΔfolX and ΔfolM. c, Abolition of colipterins 1–4 in two distinct double mutants of aspC and tyrB in E. coli BW25113. d, Production of colipterins 3–8 by in vitro biomimetic synthesis. THP was used as a DHP substrate, which reacts with PP to yield colipterins 3–6 (middle) and with l-Phe to yield 7 and 8 (bottom).

e, Pre-incubated PP and l-Phe reactions with THP yielded colipterins 1 and 2. f, Proposed colipterin pathway in E. coli. The proposed monapterin recycling is boxed. Enzyme annotations (blue): FolE, GTP cyclohydrolase I; qDHM, quinoid dihydromonapterin. Solid blue and red arrows indicate coupling of PP and α-Phe reactions with THP yielded colipterins 1 and 2. f, Proposed colipterin pathway in E. coli. The proposed monapterin recycling is boxed. Enzyme annotations (blue): FolE, GTP cyclohydrolase I; qDHM, quinoid dihydromonapterin. Solid blue and red arrows indicate coupling of PP and α-Phe reactions with THP yielded colipterins 1 and 2.
converge in colipterin production. However, potential catalysts or cellular conditions that facilitate coupling of dihydropterin (DHP) and phenylpyruvate (PP)—and, to a lesser extent, phenylalanine (Phe)—remain undefined.

With the necessary colipterin substrate pathways defined, we turned to biomimetic synthesis studies to explain colipterin family production. We proposed that nucleophilic substitution reactions at the electrophilic C6 position of DHP could account for colipterins 1–8. To test this proposal, we prepared tetrahydropterin (THP) as previously described. Individual reactions (2-h anaerobic, 2-h aerobic) with THP and phenylpyruvate or Phe yielded two groups, colipterins 3–6 and 7–8, respectively (Fig. 2d); 3 and 4 were established as degradation products of 5 and 6, and auto-oxidation of 3 to 4, and of 7 to 8, was detected (Extended Data Fig. 5a–d). To facilitate the production of 1 and 2, we reacted Phe and phenylpyruvate (1:1 ratio) in dimethylformamide (DMF) before supplementation of THP. Only these specific sequential conditions led to the production of 1 and 2 (Fig. 2e), supporting the order of reactivity. Lastly, the oxidation state of the pterin reactant in these studies appeared to be free DHP, because pterin and THP (anaerobic) were not competitive substrates (Extended Data Fig. 5e,f). Importantly, these studies support a mechanistic model in which DHP could undergo ‘nucleophilic reduction’ to generate C6-functionalized THP conjugates in the absence of a biological reductant (for example, reduced nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate (NADPH)) (Fig. 2f).

With our understanding of colipterin origins from monapterin genetic studies and biomimetic synthesis efforts, we turned to the establishment of dominant DHP substrate origins in E. coli. Monapterin and folate are derived from guanosine triphosphate (GTP) through the key intermediate 2,3-dihydroneopterin triphosphate (DHNTP) by GTP cyclohydrolase I (GTPCH, folE) in E. coli. Previous studies have proposed a recycling pathway in mammalian cells for the hydroxyl analogue of monapterin, tetrahydrobipterin (BH4), that serves as an antioxidant and essential redox co-factor for aromatic amino acid hydroxylases and nitric oxide synthase. BH4 is oxidized to the unstable quinoid dihydrobipterin (qBH4), which isomerizes to dihydrobipterin (BH3), and dihydropterin reductase recycles qBH4 and BH3 back to BH4. Addition of samples of both IL-10+ and IL-10− mice colonized with WT E. coli BW25113 and its folM mutant strain, and dextran sulfate sodium (DSS, 2.5%) was administered. We then fed SMX to the mice compared to no-SMX controls (Fig. 4a). While there was no difference in E. coli colonization among the groups as expected, slight body weight loss in mice with ΔfolM was observed following SMX administration (Fig. 4b,c). Although DSS scores in IL-10-deficient mice were similar between WT and ΔfolM-colonized groups, IL-10− mice colonized by ΔfolM exhibited significantly higher DSS scores compared to those of mice colonized with WT E. coli, suggesting that folM-derived metabolites, such as colipterins, protect mice from DSS-induced colitis in an IL-10-dependent manner (Fig. 4b,c). We also observed significantly higher in vivo levels of major colipterins 3 and 6 in stool samples of both IL-10+/+ and IL-10− mice colonized with WT E. coli compared to ΔfolM-colonized groups (Extended Data Fig. 7a). Importantly, we also detected basal levels of colipterins 3 and 6 in ΔfolM E. coli systems, suggesting that other members of the microbiome also produce colipterins.

To further support colipterin-mediated upregulation of IL-10 in vivo, we individually fed colipterins 3, 4 and 6 and folic acid (each 10 mg kg−1) every other day to WT mice (IL-10+/+) using the DSS inflammation model (Fig. 4d). Colipterins 4 and 6 significantly reduced colitis severity, whereas folic acid showed no effect in our experiment. Furthermore, we individually fed colipterins 4 and 6 (10 mg kg−1 each) to IL-10−/− mice, isolated immune cells from the colon and quantified enhanced green fluorescent protein (eGFP) signals in CD45+CD3+CD11b−F4/80+ macrophages using fluorescence-activated cell sorting (FACS). Relative to DMSO control,
Fig. 3 | Immunomodulatory activities of the colipterins. a, BioMap phenotypic profiling assays of colipterins (single dose) against human primary cells. Cell types and stimuli used in each system are as follows: 3C (human umbilical vein endothelial cells (HUVEC) + (IL-1β, TNF-α and IFN-γ)); 4H (HUVEC + (IL-4 and histamine)); LPS (PBMC and HUVEC + LPS (TLR4 ligand)); SAg (PBMC and HUVEC + TCR ligands (1x)); BT (CD19+ B cells and PBMC + α-1[3]m and TCR ligands (0.001x)); BF4T (bronchial epithelial cells and HDFn + (TNF-α and IL-4)); BE3C (bursal epithelial cells + (IL-1β), TNF-α and IFN-γ)); CASM3C (coronary artery smooth muscle cells + (IL-1β, TNF-α and IFN-γ)); KF3CT (keratinocytes and HDFn + (IL-1β, TNF-α, IFN-γ, EGF, bFGF and PDGF-BB)); MyoF (differentiated lung myofibroblasts + (TNF-α and TGF-β)); and /Mphg (HUVEC and M1 macrophages + Zymosan (TLR2 ligand)). b, Dose–response (17, 1, 0.1 and 0.01 μM) analysis of IL-8 levels (pg ml−1) in macrophage-like cells differentiated from human THP-1 cells in the presence of colipterins 1–6. c, Error bars represent mean ± s.e.m., n = 3. P values between DMSO and other treatments were determined by unpaired, one-way ANOVA with Dunnett’s test. d, Stable DPPH radical scavenging activities of colipterins 1–6; 3a and 4a are chemically reduced tetrahydropterin variants of 3 and 4, respectively. THP and L-ascorbic acid were used as positive controls. Data are presented as mean ± s.d. (n = 3). e, Disk diffusion test of colipterins 3, 4 and 6 and THP against E. coli Nissle1917 in the presence of hydrogen peroxide. Compounds (100 μg per disk) were treated, incubated for 3 h at 37 °C and 1 μl of 30% H2O2 was subsequently added to the disk. The P value was determined by two-tailed unpaired t-test. Mean and s.d. are presented from three biological replicates (n = 3).

Colipterins 4 and 6 significantly upregulated IL-10 production in macrophages (Fig. 4e). Finally, we examined the effects of colipterins 3, 4 and 6 and folic acid on colitis severity in IL-10−/− mice that spontaneously developed colitis at 10 weeks of age. None of the compounds were protective in IL-10−/− mice (Fig. 4g), further suggesting that colipterins ameliorate colitis in an IL-10-dependent manner. Consistent with these findings, IL-10−/− spontaneous-colitis mice colonized with E. coli (WT BW25113 versus ΔfolM) in the presence of SMX and absence of DSS similarly showed no protective effects (Extended Data Fig. 7b). Collectively, these data link folM-derived
colipterins to anti-inflammatory IL-10 responses in vivo, leading to the improvement of colitis symptoms in an IL-10-dependent manner.

**Discussion**

Modern analytical technologies have led to the realization that the vast majority of metabolites found in any microbe or animal, including humans, remain unknown. Some of the metabolites in this ‘dark matter’ of the metabolome will undoubtedly have profound functional consequences. Not only will they affect microbial compositions and activities, but they will also regulate host phenotypes such as immunomodulatory responses. Further complicating the observed chemical smorgasbord of metabolites in the intestinal tract, antibiotics dramatically impact microbial metabolism through a variety of mechanisms. However, we are only beginning to understand how the microbiome regulates the immune system at the detailed molecular level and how antibiotics participate directly or indirectly in these molecular responses. Subinhibitory levels of antibiotics provide a means to...
study the stress responses of selected microbiome members such as E. coli without significantly impacting their cell growth in vitro or intestinal colonization in vivo. In our study, application of the anti-folate antibiotic SMX to intestinal E. coli strains led to the upregulation of a previously undescribed family of pteridines termed the colipterins. In addition to harbouring antioxidant roles for E. coli, these metabolites exhibit anti-inflammatory activities in primary human tissues, in laboratory tissue cultures and in mouse models of intestinal inflammation. These chemical biological studies thus provide an example that could support a broader model in which antibiotics indirectly regulate immune responses through the activation of bacterial stress responses.

Escherichia coli strains have been linked to the severity of IBD, including Crohn’s disease and ulcerative colitis, but the mechanisms similarly remain largely undefined at the molecular level. For example, AIEC LF82 has been implicated in the pathogenesis of Crohn’s disease whereas human probiotic E. coli 1917 supported the remission of ulcerative colitis in clinical trials. SMX is used to treat E. coli infections, including those in patients with IBD. Strikingly, we show that SMX antifolate stress upregulates colipterin production in E. coli and hypothesize that our nucleophilic DHP-reduction model, which accounts for the pathogenesis of Crohn’s disease whereas human probiotic E. coli strain Nissle1917 were individually inoculated into 5-ml LB cultures and incubated at 37°C with agitation (250 r.p.m). Overnight cultures were subcultured (5 ml) into fresh 5-ml LB aliquots with or without SMX (200 μg ml−1) and further incubated at 37°C overnight. Cell pellets were harvested at three different time points (12, 24 and 48 h) and washed three times in sterile PBS (2 ml). Next, the cell pellets were extracted with 2 ml of an organic solvent mixture containing methanol/acetone/water (1/1/0.5, v/v/v) and sonicated for 10 min. The extracts were dried under reduced pressure on a Genevac HT-4X system for 3h. The samples were dissolved in 100 μl of methanol and a 2-μl sample was injected for HR-ESI–TOF–MS analysis. The samples were analysed as described in Metabolome analysis of E. coli in response to SMX stress.

Quantification of colipterins from E. coli Nissle1917 culture. Three colonies of E. coli Nissle1917 were individually inoculated into 5-ml LB cultures and incubated overnight at 37°C with agitation (250 r.p.m). Overnight cultures (5 ml) were dispensed into 5-ml fresh LB cultures and further cultivated for 24 h, the cell pellets were harvested at three different time points (12, 24 and 48 h) and washed three times in sterile PBS (2 ml). Next, the cell pellets were extracted with 2 ml of an organic solvent mixture containing methanol/acetone/water (1/1/0.5, v/v/v) and sonicated for 10 min. The extracts were dried under reduced pressure on a Genevac HT-4X system for 3h. Whole extracts were dissolved in 200 μl of methanol. Stock solutions of individual colipterin standards were prepared at a concentration of 10 mM in methanol. Six different working solutions of all colipterin standards were individually prepared in 200 μl of methanol to establish a calibration curve, then 2 μl of each sample was analysed by HR-ESI–TOF–MS with a reverse-phase HPLC column (Phenomenex Kinetix C18 (100 Å) 5 μm (4.6 × 250 mm)); flow rate, 0.7 ml min−1; mobile phase, a H2O/acetone/trifluoroacetic acid (0.1% v/v) gradient: 0–30 min, 100–10% acetone).

Isolation of metabolites. Liquid chromatography (LC) for colipterins 1–4: n-butanol extracts (3.2 g) were subjected to medium-pressure liquid chromatography (Biotage, SNAP cartridge KP-C18-HS, 120 g) and fractionated with a linear gradient elution of methanol/water (flow rate, 35 ml min−1; 0–20 min, 10/90%; 20–60 min, 10/90%–100/0%) to give 15 fractions (1–15). Fraction 10 was found to contain two mass peaks of interest (that is, M+H+ at m/z 459) following LC–MS analysis. Thus, fraction 10 (~400 mg) was further separated into 60 HPLC fractions using an Agilent Polaris C18 column (21.2 × 250 mm) with a gradient of 0.01% trifluoroacetic acid (TFA) in methanol/water (flow rate, 10 ml min−1; 0–60 min, 10/90%–100/0%, 1 min fraction collection). HPLC fraction 23 (12 mg) was subjected twice to subsequent reverse-phase HPLC column (Phenomenex Luna C18 (100 Å) 10 μm (21.2 × 250 mm)); flow rate, 0.1 ml min−1; mobile phase, a H2O/acetone/trifluoroacetic acid (0.1% v/v) gradient: 0–30 min, 100–10% acetone).

Methods General chemical analysis. Analytical high-performance liquid chromatography (HPLC)–MS spectra of metabolites were collected using an Agilent 1260 Infinity HPLC with an Agilent 6120 Quadrupole low-resolution electrospray ionization (ESI) mass spectrometer (Agilent). Liquid column chromatography was carried out using either a LiChroprep RP-18 (40–63 μm) (Merck Millipore) or a Sep-Pak C18 cartridge (Waters). Preparative HPLC for the isolation of the pathogenesis of Crohn’s disease whereas human probiotic E. coli strains (probiotic Nissle1917, pathogenic LF82 and commensal BW25113) were cultured using either a LiChroprep RP-18 (40–63 μm) (Merck Millipore) or a Sep-Pak C18 cartridge (Waters). Preparative HPLC for the isolation of metabolites from an Agilent Preparative HPLC system with the columns Agilent PolarLich C8 5 μm (21.2 × 250 mm), Phenomenex Luna C8 (2) or C2 (2) (100 Å) 10 μm (10.0 × 250 mm) (Phenomenex) and Agilent Phenyl-Hexyl 5 μm (9.4 × 250 mm). High-resolution mass spectra were obtained using an Agilent 6530 Q–TOF–MS instrument fitted with an ESI source coupled to an Agilent 1290 Infinity HPLC system.

Determination of subinhibitory levels of SMX against E. coli. Escherichia coli strains (probiotic Nissle1917, pathogenic LF82 and commensal BW25113) were grown on LB agar plates (1% tryptone (w/v), 0.5% yeast extract (w/v), 1% sodium chloride (w/v) and 1.5% agar (w/v)) at 37°C for 18 h. Three well-defined colonies from each strain were inoculated into 3 × 5 ml of LB and incubated at 37°C with agitation (250 r.p.m). Overnight E. coli cultures (5 μl) were seeded into 5 ml LB and were further grown for 18 h. Sulfamethoxazole (SMX, TCI), when needed, was prepared in fresh LB medium at a maximal concentration of 1.6 mg ml−1, which was then serially diluted. The 5 μl of SMX containing medium was dispensed into 14 ml sterile culture tubes and 5 μl of E. coli culture was inoculated and grown at 37°C for 24 h at 250 r.p.m. The optical density (OD600) of cultures was measured, and growth curves were generated to determine subinhibitory levels of SMX against E. coli.

Metabolome analysis of E. coli in response to SMX stress. Escherichia coli cultures (5 μl, 3 × 5 ml in LB medium) were inoculated into 5 ml of LB in the presence of a subinhibitory range of SMX, and incubated at 37°C and 250 r.p.m. for 24 and 48 h, and then further purifed using a Phenomenex Luna C18 (100 Å) 10 μm LC–MS analysis. The samples were analysed as described in Metabolome analysis of E. coli in response to SMX stress.
(10.0 x 250 mm²) column with a gradient of 10–100% acetonitrile in water over 60 min and a flow rate of 4 ml of min⁻¹, yielding colipterins 5 (t₁ = 11.3 min, 1.5 mg) and 6 (t₁ = 13.7 min, 2.4 mg), respectively. The 40% methanol fraction (400 ml) from the Sep-Pak column and colipterins 7 and 8, which were purged using reverse-phase HPLC over an Agilent Polaris C₁₈ (5 μm) 21.2 x 250 mm column (flow rate, 10 ml min⁻¹; 20–20 min, 5% acetonitrile in water; 20–80 min, 5–60% acetonitrile in water). Colipterins 7 and 8 were eluted in fractions 47 and 56, respectively. Fractions 47 (9 mg) and 56 (7 mg) were further individually fractionated using a Phenomenex Luna C₁₈ (100 Å) 10 μm (10.0 x 250 mm) column with a gradient of 5–100% acetonitrile in water over 60 min and a flow rate of 4 ml of min⁻¹. Finally, purification of colipterins 7 (t₁ = 11.8 min, 4 mg) and 8 (t₁ = 16.6 min, 2.5 mg) was achieved using a Phenomenex Luna C₁₈ (100 Å) 10 μm (10.0 x 250 mm) column, eluting with a 5–100% acetonitrile gradient in water for 60 min at a flow rate of 4 ml of min⁻¹.

NMR-based structure elucidation of metabolites. NMR spectra were recorded on an Agilent 600-, 500- and 400-MHz NMR spectrometers. ¹H and ¹³C chemical shifts (given in ppm (δ) and coupling constants (J) in Hz) were referenced to the solvent residual peaks of DMSO-d₆. Detailed structural elucidation efforts are described in Supplementary Discussions.

Identification of phenylalanine motif stereochemistry. Standard d/-l-Phe and N₆-(2,4-dinitro-5-fluorophenyl)-l-alaninamide (FDAA) were purchased from Millipore Sigma. To liberate free phenylalanine, metabolites 1, 2, 7 and 8 (4-6 mg) were hydrolysed using 6 N hydrochloric acid (2 ml) at 100 °C for 1 h, and the resulting hydrolysates were dried in a nitrogen stream. The residues were dissolved in water and completely dried for 24 h in a Genevac HT-4X system to remove residual acid. Identification of phenylalanine was performed via standard Marley’s derivatization. The hydrolysed materials and standard amino acids were derivatized following the addition of 50 μl of a solution of FDA (10 mg in acetonitrile) in acetone followed by the addition of 100 μl of 1 N sodim bicarbonate. The reactions were incubated for 3 min at 80 °C and quenched by the addition of 50 μl of 2 N hydrochloric acid. The derivatives were diluted in 300 μl of 50% aqueous acetonitrile, and 10 μl of the samples was analysed by LC–MS.

Computational analysis for absolute structure elucidation of 1 and 2. Conformational searches were conducted using the Macromodel (v.2015.2, Schrödinger) programme with ‘mixed torsional/low mode sampling’ in the MMFF94 force field. The searches were implemented in the gas phase with a 50-kl mol⁻¹ energy window limit and 10,000 maximum number of steps, to fully explore low-energy conformers. The Polak–Ribière conjugate gradient method was used for minimization processes, with 10,000 maximum iterations and a 0.001-kj mol⁻¹ (mol Å⁻¹) convergence threshold on the rms gradient. Conformers within 10 kJ mol⁻¹ of each global minimum of plausible epimers addressed in this study were provided for the gauge-independent atomic orbital sheliding constant calculations without geometry optimization using the Gaussian 09 package (Gaussian, Inc.) on the BLYP/6-31 G(d) level in the gas phase. These calculated chemical shift values were averaged based on their Boltzmann populations (Supplementary Table 2) and used for CP3 analysis, facilitated by the applets available at http://www.img.jc.cam.ac.uk/tools/nmr/CP3.html. In particular, calculated and experimental chemical shift values of diastereotopic protons were compared with their closest matches based on the original author’s recommendation.

Chemical synthesis of colipterins 3 and 4. Cinnamaldehyde and 2,4,5-tri-aminoo-6-oxo-dihydroprymidine dihydrochloride were purchased from Millipore Sigma and Santa Cruz Biotechnology, respectively. 2-Bromo-3-phenyl-propionaldehyde was re-dissolved in ethanol (50 ml) followed by the addition of 2-Bromo-3-phenyl-propionaldehyde and Santa Cruz Biotechnology, respectively. 2-Bromo-3-phenyl-propionaldehyde and Santa Cruz Biotechnology, respectively. The hydrolysed materials and standard amino acids were derivatized following the addition of 50 μl of a solution of FDA (10 mg in acetonitrile) in acetone followed by the addition of 100 μl of 1 N sodim bicarbonate. The reactions were incubated for 3 min at 80 °C and quenched by the addition of 50 μl of 2 N hydrochloric acid. The derivatives were diluted in 300 μl of 50% aqueous acetonitrile, and 10 μl of the samples was analysed by LC–MS.

preparation and HR–ESI–Q–TOF–MS analysis were performed as described in Metabolome analysis of E. coli in response to SMX stress.

Genetic complementation of mutants fdólX and fdólM. The genes fdólX and fdólM were PCR amplified from E. coli BW25113. The primers used for amplification are shown in Supplementary Table 6. The purified PCR products and pBAD18 vector were independently digested using SacI and KpnI and were ligated using T4 DNA ligase (NEB) according to the manufacturer’s protocol. These products—pBAD-empty vector, pBAD-fdólX and pBAD-fdólM—were then transformed by heat shock into chemically competent E. coli DH5α followed by sequence validation. Electrocompetent E. coli strains BW25113, ΔfdólX and ΔfdólM were generated, and the empty vector, pBAD-fdólX or pBAD-fdólM was electroporated into the corresponding electrocompetent strains. Cells were grown on LB agar plates supplemented with ampicillin at 100 μg ml⁻¹. Overnight cultures (5 ml) in LB in a sterile tube were subcultured into a 10 ml (1:1000) in LB supplemented with 100 μg ml⁻¹ ampicillin and grown at 37 °C to OD₆₅₀= 0.5–0.5. Cultures were induced with 0.1% 1- arabinose and further incubated at 37 °C with agitation (250 r.p.m.) for 48 h. After centrifugation, the supernatants were extracted with 6 ml of n-butanol and the extracts were dried under reduced pressure on an HT-4X evaporation system. The samples were analysed as described in Metabolome analysis of E. coli in response to SMX stress.

Construction of double mutants aspC and tyrB. Escherichia coli BW25113 double-mutant strains Δ/ΔaspCΔ/ΔaspB were constructed using the Red recombination system for back-bone deletion. The Flippase recognition target (FRT) pair comprised of the monomer resistance cassette of pM78 was amplified (primers are given in Supplementary Table 6) with short sequence extensions homologous to the flanking regions of aspC or tyrB. Purified PCR products were desalted and respectively transformed into E. coli JW4014-2 (Δ/Δrbp74-kan) or JW9411-1 (Δaspc745-kan) carrying plasmid pKD46. Transformants were selected on LB agar supplemented with spectinomycin (50 μg ml⁻¹). Colonies were analysed with overspanning PCR, and the resulting products were sequence validated and cured of pKD46.

Biomimetic synthesis. Pterin, l-Phe, PP, and phenylacetic acid (PAA) were purchased from Millipore Sigma. To prepare THP, pterin (500 mg, 3.0 mmol) was suspended in anhydrous methanol (200 ml) following the addition of platinum oxide (50 mg, 0.22 mmol) as catalyst. Hydrogenation of pterin was initiated in the presence of hydrogen gas. After overnight reaction at room temperature, the resulting materials were filtered through Celite 545 and the filtrates dried under nitrogen gas purge in a glove bag to yield THP (260 mg, 38%). The three free

Radical scavenging assays were performed as previously described, in triplicate. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Radical scavenging assays were performed as previously described, in triplicate.

Cellular protection assay. Escherichia coli Nissle1917 was streaked onto an LB agar plate. Single colonies were selected and inoculated into 5 ml LB and incubated at 37 °C with agitation (250 r.p.m.) overnight. Cell densities were adjusted to OD₆₅₀ = 0.05, and 100 μl of diluted cells was spread onto fresh LB agar plates. Compounds were prepared in DMSO (10 mg ml⁻¹) and 10 μl of solution was soaked onto a paper disk, with DMSO as control. The disk was placed on the agar plate containing E. coli and incubated at 37 °C for 3 h, then 1 μl of 30% hydrogen peroxide was added to the control and further incubated overnight. Zones of inhibition were measured after overnight incubation. This assay was performed in biological triplicate.

BioMAP phenotypic profiling assay. BioMAP Diversity PLUS assays were performed with Eurofins DiscoverX. Human primary cells in BioMAP systems were used at early passage (passage 4 or earlier), to ensure adaptation to cell culture conditions and preserve physiological signalling responses. All cells were obtained from a pool of multiple donors (n = 2–6), commercially purchased and handled according to the recommendations of the manufacturers. The following vendors supply Eurofins DiscoverX with primary human cells: Cell Applications, CellDirect, Celsi-IVT, Lee Lab, Life Technologies, Lonza, ScienCell, Sunriscare Corporation, Stemcell Technologies, AllCells, Physician’s Plasma Alliance, Lifeline Cell Technologies and Zen-Bio. Human blood-derived CD14+ monocytes were differentiated into macrophages in vitro before being added to the Mphp system. Abbreviations used are as follows: human umbilical vein endothelial cells, HUVEC; peripheral blood mononuclear cells, PBMC; human neonatal dermal fibroblasts,
HD Fn; B-cell receptor, BCR; T-cell receptor, TCR; Toll-like receptor,TLR. Cell types and stimuli used in each system are as follows: 3C system (HUVEC + (IL-1β), TNF-α and IFN-γ); 4H system (HUVEC + (IL-4 and histamine)); LPS system (HUVEC and LPS (20 ng ml−1); HUVEC + LPS (10 ng ml−1)); HUVEC + HVC and LPS (10 ng ml−1). The system consists of CD19+ B cells and PBMC + (or-IgM and TCR ligands (0.01 μM)); BFT system (bromochial epithelial cells and HD Fn + (TNF-α and IL-4)); BESC system (bromochial epithelial cells + (IL-1β), TNF-α and IFN-γ); CASM3 system (coronary artery smooth muscle cells + (IL-1β), TNF-α and IFN-γ); HDF3CGF system (HDFn + (IL-1β), TNF-α, IFN-γ, EGF; bFGF and PDGF-BB); KF3CT system (keratinocytes and HD Fn + (IL-1β), TNF-α and IFN-γ)); MyoF system (differentiated lung myofibroblasts + (TNF-α and TGF-β)); and /Mphg system (HUVEC and M1 macrophages + Zymosan (TLR2 ligand)). Systems are derived from either single cell types or co-culture systems. Adherent cell types were cultured in 96- or 384-well plates until confluence, followed by the addition of PBMC (Sag and LPS systems). The system consists of CD19+ B cells co-cultured with PBMC and stimulated with a BCR activator and low levels of TCR. Compounds 1–6 were prepared in DMSO (final concentration, 0.1%) and added at a final concentration of 17 μM, 1 h before stimulation and remained in culture for 24 h or as otherwise indicated (MyoF system, 48 h; BT system 72 h (soluble readouts) or 16 h (secreted IgG)). Each plate contained drug controls, negative controls (for example, non-stimulated conditions) and vehicle controls (for example, 0.1% DMSO) appropriate for each system. Direct enzyme-linked immunosorbent assay (ELISA) was used to measure biomarker levels of cell-associated and cell membrane targets. Soluble factors from supernatants were quantified using either 96-well plate detection, bead-based multiplex immunoassay or capture ELISA. Overt adverse effects of test agents on cell proliferation and viability (cytotoxicity) were detected by sulforhodamine B (SRB) staining (for adherent cells) or alamarBlue reduction (for cells in suspension). For proliferation assays, individual cell types were cultured at subconfluence and measured at time points optimized for each system (3C and CASM3 systems, 48 h; BT system and HDF3CGF systems, 72 h; SAg system, 96 h). Cytotoxicity assay for adherent cells was measured by SRB (3C, 4H, LPS, Sag, BFT, BESC, CSMC3, HDF3CGF, KF3CT and /Mphg, 24 h; MyoF system, 48 h), or by alamarBlue staining for cells in suspension (Sag system, 24 h), BT system, 42 h) at the time points indicated.

Mouse studies. C57BL/6 and IL-10−/− mice were used in this study.1 IL-10−/− mice (002251-B6.129P2-IIdmlCgn/J, Jackson Laboratory) were a gift from S. Morse. Experimental groups of WT and IL-10−/− mice were generated from heterozygote × heterozygote breeding. Sample sizes were chosen in line with previous experimental experience and consistent with the broader literature. Eight- to ten-week-old male and female mice were used in equal quantities unless otherwise specified. All experiments were performed under co-housed mouse littermate controls. Iso-sexual male or female littermates were co-housed at 21−24°C and 40−60% humidity. A 12:12 light/dark cycle was used. All mouse studies were performed in compliance with Yale Institutional Animal Care and Use Committee protocols. No formal blinding or randomization was conducted; however, control and treated groups were chosen arbitrarily for each experiment. Mouse weights and colony-forming units (cfu) were measured in a blinded manner.

In vitro IL-8 ELISA assay. Human THP-1 (ATCC TIB-202) cells were authenticated and purchased from ATCC, and no mycoplasma contamination was detected. Cells were cultured in RPMI medium (Gibco, no. 11879093) with 10% fetal bovine serum (FBS; Sigma, no. F8192-500ML) and 1% penicillin/streptomycin (Gibco, no. 15007063) and were differentiated into macrophage-like cells by incubation in the presence of 50 nM PMA (Sigma, no. 11875093) for 16 h. Culture supernatants were collected and analyzed for IL-8 content according to the manufacturer's protocol.

E. coli mouse colonization. Before colonization, 8–10-week-old mice were fasted for 4 h followed by gavage with kanamycin (20 mg). After 20 h, mice were fasted again for 4 h and administered 1×106 kanamycin-resistant E. coli (BW25113 WT) with or without a control. To calculate cfu, faecal pellets were resuspended in PBS. Serial dilutions were conducted, and bacteria were plated in triplicate on LB + kanamycin (50 μg/ml) plates. As previously described,3 we incubated cultures for 24 h. The colony-forming unit (cfu) was counted, and the colony-forming unit/ml was calculated.
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### Acknowledgements

This work was supported by the National Institutes of Health (nos. 1DP2-CA186575 and R00-GM097096 to J.M.C.), the Burroughs Wellcome Fund (no. 1016720 to J.M.C.), the Camille & Henry Dreyfus Foundation (no. TC-17-011 to J.M.C.), the Howard Hughes Medical Institute (to R.A.F) and the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (no. 2019R1A6A3A12033304 to C.S.K.). Z.W. was supported by the China Scholarship Council.

### Author contributions

H.B.P. and J.M.C. conceived the study and designed the metabolism experiments. H.B.P. characterized the colipterin pathway from *E. coli* by drug stress, metabolome analysis, isolation, structure characterization, synthesis, genetic complementation and antioxidant assays. Z.W. performed ELISA assays for IL-8/-10 and all mouse studies. J.O. performed CP3 computational analysis on colipterins 1/2 and contributed to colipterin accumulation and NMR measurements. H.X. maintained the IL-10*Cre* mice, isolated immune cells from the small intestine and analysed FACS data for IL-10 expression. C.S.K. constructed double-mutant strains of *aspC* and *tyrB*. R.W., T.P.W. and G.P. contributed to BioMap analysis of colipterins. R.A.F conceived and supervised in vivo mouse studies. H.B.P. and J.M.C. wrote the manuscript with input from all authors. All authors reviewed and edited the manuscript.

### Competing interests

R.A.F. is a recipient of a grant from AbbVie, Inc. R.W., T.P.W. and G.P. are employees of Merck Exploratory Science Center, Merck & Co., Inc., Kenilworth, NJ, USA. Employees may hold stocks and/or stock options in Merck & Co., Inc., Kenilworth, NJ, USA.

The remaining authors declare no competing interests.

### Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41564-020-0763-4.

Supplementary information is available for this paper at https://doi.org/10.1038/s41564-020-0763-4.

Correspondence and requests for materials should be addressed to R.A.F or J.M.C.

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Extended Data Fig. 1 | UV-LC-MS detection of colipterins 1-8 produced by the probiotic *E. coli* Nissle1917. **a**, Dose-responses of 1-8 from the probiotic *E. coli* Nissle1917 exposed to the sub-lethal levels of sulfamethoxazole (SMX). Intensity was determined by extracted ion counts (EICs) with m/z corresponding to each colipterin within a 10 ppm error window. High-resolution ESI-QTOF-LC-MS spectra of samples were analyzed using Phenomenex Kinetex C18 (100 Å) 5 μm (250 × 4.6 mm) column with a gradient from 10%-100% aqueous acetonitrile in 0.1% formic acid over 30 min and with a 0.7 ml min⁻¹ flow rate. Data were collected in biological triplicate, and representative EIC chromatograms are shown. **b**, UV-Vis spectra of colipterins 1-8 and pterin.
Extended Data Fig. 2 | Production profiles of colipterins from E. coli Nissle1917 cultures. a, Time-course analyses of major colipterins 1-4 from E. coli Nissle1917 cultures. Peaks were extracted using the EIC method corresponding to the m/z of 1-4 within a 10 ppm window. The mean and s.d. (error bars) from three biological experiments (n = 3) are shown. Statistical significance (two-tailed unpaired t-test) is compared to 12 h; nd, not detected. b, Detection of col253 (3) and col267 (4) from the cell pellet extracts. SMX was supplemented into E. coli cultures with a sub-lethal concentration of 200 μg ml⁻¹. Representative data from 24 h cultures are given from three biological replicates (n = 3). The mean and s.d. (error bars) are presented. A two-tailed unpaired t-test was used to calculate P values. Concentrations of 3 and 4 in cell pellet extracts without (and with SMX 200 μg ml⁻¹) drug stress are as follows: 3, ~ 0.7 (~ 4.0) μM; 4, ~ 0.3 (~ 1.5) μM. c, Calibration curves for the colipterin quantification. Concentrations of 1-8 in culture without (and with SMX 400 μg ml⁻¹) drug stress are as follows: 1, ~ 0.2 (~ 1.0) μM; 2, ~ 0.3 (~ 1.5) μM; 3, ~ 2.0 (~ 7.1) μM; 4, ~ 1.3 (~ 5.3) μM; 5, ~ 0.06 (~ 0.2) μM; 6, nd (~ 0.6) μM; 7, ~ 0.07 (~ 0.2) μM. 7 oxidized to 8 in the methanol solution during the acquisition of Q-TOF-MS experiments. Data are shown as the mean ± s.d., n = 3 from 3 technical triplicates.
Extended Data Fig. 3 | Sulfamethoxazole (SMX) drug stress responses of colipterins 1–8 in the pathogenic *E. coli* LF82 and commensalistic *E. coli* BW25113. Yellow area for optical density (OD\textsubscript{600}) measurement represents a range of sub-lethal concentrations of SMX against both *E. coli* LF82 (a) and BW25113 (b) strains. Peaks were extracted using EIC method corresponding to the \textit{m/z} of colipterins 1–8 within a 10 ppm window. Red and black lines indicate Ctrl (Control, no SMX treatment) and SMX, respectively, in the MS chromatogram. Intensities in the bar graphs indicate integration value of EIC peak area. The mean and s.d. (error bars) from three biological experiments (\(n = 3\)) are shown. \(P\) values were analyzed by a two-tailed unpaired \(t\)-test; ns, not significant.
Extended Data Fig. 4 | Functional characterization of FolX and FolM involved in colipterin production. a, Folate and monapterin pathway are shown. b, Genetic complementation of folX and folM. Note that the production of colipterins is abolished in ΔfolX and ΔfolM strains. While the colipterins were not detected in both ΔfolX and ΔfolM carrying pBAD empty vector, complementation of folX and folM in pBAD into ΔfolX and ΔfolM strains, respectively, resulted in the recovery of major colipterins 3 and 4 production. 24 h E. coli cultures were analyzed by high-resolution ESI-QTOF-LC-MS using Phenomenex Kinetex C18 (100 Å) 5 μm (250 × 4.6 mm) column with a gradient from 10%-100% aqueous acetonitrile in 0.1% formic acid over 30 min and with a 0.7 ml min⁻¹ flow rate. Data are mean ± s.d. from three biological replicates (n = 3); nd, not detected.
Extended Data Fig. 5 | UV-LC-MS profiles of biomimetic synthesis of colipterins in various conditions. a, Differential production of 5 and 6 from the tetrahydropterin (THP)-phenylpyruvate (PP) coupling reaction. 5 and 6 were observed to be major products from the reaction in pH 5.0 and pH 6.0 in water, respectively. b, 3 (m/z 254.1042) and 4 (m/z 268.0834) from the reaction with THP in the presence of initially expected substrate, phenylacetic acid (PA), were not observed. c, Individual incubation of 3, 4, 5, and 6 in methanol at room temperature for 12 h was monitored by HPLC. While 4 was most stable, 5 and 6 were interconvertible and irreversibly degraded to 3 and 4 as anticipated. Image for 4 is not shown. d, 7 (~0.5 mg) in 2 ml methanol was incubated at room temperature for 96 h and analyzed at five different time-points (6, 24, 48, 72, and 96 h). Auto-oxidation of 7 to 8 was observed over time. Image for the color change of 7 (colorless) to 8 (yellow) is shown in inset. e, Demonstration of the oxidation state of the pterin reactant. Independent reactions with pterin-PP and THP-PP were performed. While colipterins were not detected in reactions with pterin as expected, a reaction with THP in the presence of oxygen led to the colipterins. Representatively, 3 and 4 are shown. f, Reaction with THP under anaerobic conditions in vitro yielded either no detectable levels or basal levels of production. The reaction materials were analyzed by LC-MS with Phenomenex Kinetex C18 (100 Å) 5 μm (250 x 4.6 mm) column with a gradient from 10%-100% acetonitrile in water containing 0.1% formic acid for 30 min and with a 0.7 ml min⁻¹ flow rate. All reaction was analyzed by UV (254 and 310 nm) and MS using EIC method.
Extended Data Fig. 6 | Pterin profile for the demonstration of DHP substrate origins. a, Previously described recycling and side-chain cleavage mechanism of bioppterin in mammalian cells. Pterin and 7,8-dihydroxanthopterin (XPH₂) are known as break-down products of bioppterin. Similarly, we observed pterin (m/z 164.0572) and XPH₂ (m/z 182.0678) in the BH₄ in vitro chemical reaction via hydrogenation of commercial BH₂ followed by aerobic incubation in water, but not in the BH₂ solution. Additionally, colipterins were detected in BH₄ solution supplemented with PP. Representatively, col327 (m/z 328.1046) is shown. b, Upregulation of pterin and XPH₂ in E. coli Nissle1917 in response to the sub-lethal levels of SMX. Dose-responses of pterin and XPH₂ in antifolate SMX stress. The mean and s.d. (error bars) from three biological experiments (n = 3) are shown. Statistical significance was accessed using a two-tailed unpaired t-test. MS intensity of EIC chromatogram was determined by ion counts corresponding to pterin ([M+H]⁺ m/z 164.0572) and XPH₂ ([M+H]⁺ m/z 182.0678) within a 10 ppm error window. High-resolution ESI-QTOF-LC-MS spectra of samples were analyzed using Phenomenex Kinetex C₁₈ (100 Å) 5 μm (250 × 4.6 mm) column with a gradient from 10%-100% aqueous acetonitrile in 0.1% formic acid over 30 min and with a 0.7 ml min⁻¹ flow rate.
**Extended Data Fig. 7** | *E. coli* BW25113 exposed to SMX drug stress reduce colitis severity in an IL-10-dependent manner. a, Primary evaluation on the reduction of colitis severity in SMX-treated colitis mice (IL-10+/+ and IL-10−/−) that were colonized with *E. coli* (wild-type *E. coli* BW25113 and its folM mutant strain). CFUs, body weight, and clinical scores were obtained as the same procedure that we described in Fig. 4. HR-ESI-QTOF-MS quantification of colipterins 3 and 6 in stool samples. *P* values were determined by a two-tailed unpaired t-test. Error bars represent mean ± s.e.m.; ns, not significant. b, Effects of SMX-treated *E. coli* (wild-type *E. coli* BW25113 and its folM mutant strain) in the IL-10−/− male and female mice that spontaneously develop colitis. IL-10+/+ male and female mice that do not develop spontaneous colitis were used as control groups. *E. coli* was colonized at week-3. CFUs were measured at 3 weeks after colonization. Body weight was monitored weekly. SMX was mixed in the drinking water at 0.3 mg ml−1 after the *E. coli* colonization. At week-10, endoscopy was performed and clinical scores were used as a metric of colitis severity. Data are presented by mean ± s.e.m. (error bars).
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Software and code

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Data collection
- Agilent OpenLAB CDS ChemStation (Version C.01.04) and MassHunter Workstation Data Acquisition (Version B.05.01) were used for low-resolution UV/ESI-MS and high-resolution ESI-QTOF-MS data collection, respectively.

Data analysis
- All graphical and statistical analysis was performed using GraphPad Prism 8.
- MassHunter Qualitative Analysis (Version B.06.00) was used for the analysis of high-resolution ESI-QTOF-MS data.
- Conformational searches of compound were conducted using the Macromodel (version 2015-2, Schrödinger LLC) program.
- Gaussian 09 package (Gaussian Inc) was used for Geometry optimization of compound.
- BD FACSDiva Software (V8.0.1) was used to collect raw data files from all flow cytometry experiments. All resultant data files were analyzed using FlowJo version 10 or newer.

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High-resolution ESI-QTOF-MS/MS data sets for colipterins are available from GNPS public MassIVE under accession number MSV000085621. CP3 analysis was facilitated by the applets provided by the Goodman Group (http://www-jmg.ch.cam.ac.uk/tools/nmr/CP3.html). Supplementary information and source data are provided with this letter. Additional data that support the findings of this study are available from the corresponding author upon reasonable request.
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Life sciences study design

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- **Sample size**: No statistical models were utilized to predetermine sample size. Considering the minimal experimental variation in cell lines and BMDMs using inbred mouse strains as a cell source, a minimum of three biological replicates were used per group. In vivo experiments were performed with samples sizes consistent with the known literature (Nowarski R, et al. 2015).

- **Data exclusions**: No biological replicates were excluded from presented data.

- **Replication**: With the parameters described above, all experiments in the manuscript were reliably reproducible.

- **Randomization**: Because all cells were from the same inbred strain of mouse or the same cell strain, no randomization was performed. Mice were randomly assigned to non-treated and treated groups where applicable.

- **Blinding**: Bacterial CFUs and clinical scores were blinded.

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| □ | □ | Clinical data | |
| □ | X | ChIP-seq | |
| □ | X | Flow cytometry | |
| □ | X | MRI-based neuroimaging | |

**Antibodies**

- **Antibodies used**: CD45 (Biolegend, Clone# 30-F11, Cat#: 103128) (1:200)
- **CD3 (Biolegend, Clone# 145-2C11, Cat#: 100308) (1:200)**
- **CD11b (Biolegend, Clone# M1/70, Cat#: 101224) (1:200)**
- **F4/80 (Biolegend, Clone# BM8, Cat#: 123114) (1:200)**

- **Validation**: All antibodies listed were broadly used by many laboratories including our own publications. Each antibody is validated by the commercial company (available on www.biolegend.com), and dilution is experimentally determined for each clone individually.

**Eukaryotic cell lines**

- **Policy information about cell lines**

- **Cell line source(s)**: Human THP-1 (ATCC TIB-202)
- **Cell line was purchased from ATCC.**
- **Mycoplasma contamination**: No mycoplasma contamination was detected according to manufacturer.
- **Commonly misidentified lines** (See ICLAC register): The cell line used is not listed in the ICLAC database.
Animals and other organisms

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**Laboratory animals**
- C57BL/6, IL-10eGFP (in reference), and IL10-/- (002251-B6.129P2-Il10tm1Cgn/J, Jackson Laboratory, Bar Harbor, ME). Sex-matched 8-10 weeks old male and female were used in every in vivo experiment except IL10 deficiency induced spontaneous colitis experiments in which sex-matched weaning male and female were used at the beginning of the experiments.

**Wild animals**
- This study did not involve wild animals.

**Field-collected samples**
- This study did not involve filed-collected samples.

**Ethics oversight**
- All mice required for this study were housed and maintained under specific-pathogen-free conditions in the animal facility of the Yale University School of Medicine, and all corresponding animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Yale University. This study was conducted in compliance with all relevant ethical regulations. Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

**Plots**
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

**Methodology**

**Sample preparation**
- Intestinal tissues were first incubated with 1 mM DTE at 37 °C for 30 min and then further digested with collagenase from Clostridium histolyticum (Sigma, #2139) and DNase I at 37 °C for another 1 h. Cells from both fractions were pooled and further separated with Percoll gradient.

**Instrument**
- BD LSRII custom order product

**Software**
- BD FACSDiva Software (V8.0.1) was used to collect raw data files from all flow cytometry experiments. All resultant data files were analyzed using FlowJo version 10 or newer.

**Cell population abundance**
- No sorting used in this study. All population were clearly displayed in the dot plot.

**Gating strategy**
- Macrophages were gated on CD45pos, CD3neg, CD11bpos and F4/80pos.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.