The aryl hydrocarbon receptor (AHR) recognizes xenobiotics as well as natural compounds such as tryptophan metabolites, dietary components and microbiota-derived factors\textsuperscript{1-4}, and it is important for maintenance of homeostasis at mucosal surfaces. AHR activation induces cytochrome P4501 (CYP1) enzymes, which oxygenate AHR ligands, leading to their metabolic clearance and detoxification\textsuperscript{5}. Thus, CYP1 enzymes have an important feedback role that curtails the duration of AHR signalling\textsuperscript{6}, but it remains unclear whether they also regulate AHR ligand availability in vivo. Here we show that dysregulated expression of \textit{Cyp1a1} in mice depletes the reservoir of natural AHR ligands, generating a quasi AHR-deficient state. Constitutive expression of \textit{Cyp1a1} throughout the body or restricted specifically to intestinal epithelial cells resulted in loss of AHR-dependent type 3 innate lymphoid cells and T helper 17 cells and increased susceptibility to enteric infection. The deleterious effects of excessive AHR ligand degradation on intestinal immune functions could be counter-balanced by increasing the intake of AHR ligands in the diet. Thus, our data indicate that intestinal epithelial cells serve as gatekeepers for the supply of AHR ligands to the host and emphasize the importance of feedback control in modulating AHR pathway activation.

It is increasingly understood that AHR signalling needs to be tightly controlled, as prolonged activation either by ligands that resist metabolic clearance or by constitutively active AHR has deleterious effects\textsuperscript{7-10}. Enzymes of the CYP1A and CYP1B sub-families (CYP1) control AHR signalling owing to their capacity for metabolizing ligands and thereby terminating AHR activation.

We hypothesized that excessive CYP1A1-mediated metabolic clearance of natural AHR ligands would affect the intestinal immune system, in which several immune cell types are dependent on AHR signalling for their survival. To investigate this, we generated a mouse model with constitutive \textit{Cyp1a1} expression owing to its placement under control of the \textit{Rosa26} promoter (mice denoted hereafter as \textit{R26\textsuperscript{Cyp1a1}}) (Extended Data Fig. 1). As proof of principle, we investigated the effect of constitutive \textit{Cyp1a1} expression on T helper 17 (\textit{T\textsubscript{H}17}) cells, which express AHR and produce interleukin 22 (IL-22) in an AHR-dependent manner\textsuperscript{11}. \textit{T\textsubscript{H}17} cells generated from \textit{R26\textsuperscript{Cyp1a1}} mice had increased CYP1A1 enzymatic activity compared with wild-type B6 or \textit{Ahr}\textsuperscript{-deficient} \textit{T\textsubscript{H}17} cells (Fig. 1a). To assess metabolic clearance of AHR ligands, CD4\textsuperscript{+} T cells from wild-type, \textit{Ahr}\textsuperscript{-deficient} or \textit{R26\textsuperscript{Cyp1a1}} mice were cultured under \textit{T\textsubscript{H}17}-cell-inducing conditions in the presence of the endogenous, tryptophan-derived AHR ligand 6-formylindolo[3,2-b]carbazole (FICZ) and residual cellular FICZ levels determined at various time points. FICZ did not decay in cultures of \textit{Ahr}\textsuperscript{-deficient} \textit{T\textsubscript{H}17} cells owing to the absence of CYP1A1 induction, but cultures of \textit{R26\textsuperscript{Cyp1a1}} \textit{T\textsubscript{H}17} cells showed accelerated clearance of FICZ compared with those from wild-type \textit{T\textsubscript{H}17} cells (Fig. 1b). Furthermore, \textit{T\textsubscript{H}17} cells generated from \textit{R26\textsuperscript{Cyp1a1}} mice produced less IL-22 in response to baseline AHR ligands present in culture medium\textsuperscript{12} and to low, but not higher, FICZ concentrations (Fig. 1c, Extended Data Fig. 2). \textit{Ahr}\textsuperscript{-deficient} \textit{T\textsubscript{H}17} cells did not produce IL-22 under any condition (Fig. 1c). Thus, constitutive CYP1A1 activity decreased AHR ligands and compromised IL-22 production.

\textit{Ahr} deficiency in hematopoietic cells causes the disappearance of intraepithelial lymphocytes\textsuperscript{13} and type 3 innate lymphoid cells (ILC3) in the intestinal immune system\textsuperscript{14-16}. \textit{R26\textsuperscript{Cyp1a1}} mice exhibited enhanced intestinal CYP1A1 enzyme activity (Extended Data Fig. 3a) and loss of ILC3 in colon and small intestine similar to \textit{Ahr}\textsuperscript{-deficient} mice (Fig. 1d, e, Extended Data Fig. 3b), suggesting that continuous metabolic clearance of natural AHR ligands mimics an \textit{Ahr}\textsuperscript{-deficient} state. In addition, \textit{R26\textsuperscript{Cyp1a1}} mice displayed similar developmental abnormalities to \textit{Ahr}\textsuperscript{-deficient} mice, such as decreased liver weight indicative of patent ductus venosus\textsuperscript{17} (Extended Data Fig. 4).

We next investigated the response of \textit{R26\textsuperscript{Cyp1a1}} mice to infection with the intestinal pathogen \textit{Citrobacter rodentium}, which causes severe pathology in \textit{Ahr}\textsuperscript{-deficient} mice\textsuperscript{14-16}, compared with wild-type mice. \textit{R26\textsuperscript{Cyp1a1}} mice also had increased pathology, albeit not as extensive as that seen in \textit{Ahr}\textsuperscript{-deficient} mice (Fig. 2a,b). Whereas all wild-type mice survived the infection, \textit{Ahr}\textsuperscript{-deficient} mice reached end-point by day 9 after infection and \textit{R26\textsuperscript{Cyp1a1}} mice survived only a few days longer (Fig. 2c). \textit{C. rodentium} penetrated deeply into the base of the crypts in \textit{Ahr}\textsuperscript{-deficient} and \textit{R26\textsuperscript{Cyp1a1}} mice, but mainly attached to the luminal surface of the epithelium in wild-type mice (Fig. 2d). The bacterial burden in the colon of \textit{Ahr}\textsuperscript{-deficient} and \textit{R26\textsuperscript{Cyp1a1}} mice was higher and failure to clear the infection resulted in dissemination of bacteria to liver and spleen (Fig. 2e). IL-22 is essential in the defence against \textit{C. rodentium}\textsuperscript{18} and the marked susceptibility of \textit{Ahr}\textsuperscript{-deficient} mice can be attributed to their lack of ILC3 as well as failure to mount a \textit{T\textsubscript{H}17}-cell IL-22 response. This deficiency in IL-22 as well as \textit{T\textsubscript{H}17} cells and ILC3 was mirrored in \textit{R26\textsuperscript{Cyp1a1}} mice (Fig. 2f, g). Administration of recombinant IL-22–Fc fusion protein significantly reduced bacterial burden and crypt invasion and prolonged the survival of \textit{R26\textsuperscript{Cyp1a1}} mice (Extended Data Fig. 5). Thus, constitutive \textit{Cyp1a1} expression severely impairs the intestinal immune response against an enteric pathogen.

Given that CYP1A1-mediated clearance of natural ligands produces a quasi \textit{Ahr}\textsuperscript{-deficient} state, the absence of metabolic clearance should increase ligand availability. To test this, we investigated mice with deletion of the three AHR-controlled CYP enzymes CYP1A1, CYP1A2 and CYP1B1, termed Cyp1-knockout mice\textsuperscript{19}. \textit{T\textsubscript{H}17} cells from Cyp1-knockout mice failed to metabolize FICZ and showed increased \textit{Cyp1a1} expression and enhanced IL-22 production in the absence of added AHR ligands as well as reduced histopathology scores and bacterial burdens upon infection (Extended Data Fig. 6).

Increased ligand availability in Cyp1-deficient mice was visualized in a \textit{Cyp1a1} fate-reporter strain that reports AHR activity through induction of an enhanced yellow fluorescent protein (eYFP) reporter activated through targeted insertion of Cre recombinase in the mouse \textit{Cyp1a1} locus\textsuperscript{20}. Although Cyp1-deficient mice failed to produce

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this enzyme, they still reported AHR stimulation with induction of Cre-mediated eYFP expression. In the intestine of wild-type Cyp1a1 reporter mice, eYFP expression was scarce, which reflected the transient nature of AHR signalling that limits Cre induction (Fig. 3a, left). However, Cyp1-deficient mice showed enhanced eYFP expression primarily in EpCAM\(^+\) intestinal epithelial cells (IECs) (Fig. 3a left). Dietary supplementation with indole-3-carbinol (I3C), a tryptophan-derived phytochemical that is converted to high-affinity AHR ligands by exposure to stomach acid\(^1\), further enhanced the eYFP signal in EpCAM\(^+\) IECs of Cyp1-deficient mice (Fig. 3a, b).

It has been shown previously that IEC-specific deletion of the AHR partner ARNT (AHR nuclear translocator) causes systemic upregulation of Cyp1a1 (ref. 22). Thus, we next tested Vill-cre-R26\(^{SL-Cyp1a1}\) mice (termed IEC\(^{Cyp1a1}\)), in which Cyp1a1 expression was restricted to IECs (Extended Data Fig. 1b). Notably, IEC\(^{Cyp1a1}\) mice had markedly reduced numbers of ILC3, similar to Ahr-deficient mice and mice with constitutive Cyp1a1 expression throughout the body (Fig. 3c). In contrast, mice with constitutive Cyp1a1 expression restricted to adaptive immune cells through Rag1-cre (Rag1\(^{Cyp1a1}\)), had normal numbers of ILC3 under steady state conditions (Fig. 3c, Extended Data Fig. 1b). This suggests that constitutive CYP1A1 activity in IECs, but not in adaptive immune cells, restricts the availability of AHR ligands to cells in the intestinal lamina propria, resulting in loss of AHR ligand-dependent ILC3.

Upon infection with C. rodentium, IEC\(^{Cyp1a1}\) mice rapidly succumbed (Fig. 3d), with increased bacterial crypt invasion (Fig. 3e) and intestinal pathology (Fig. 3f). C. rodentium disseminated into the liver and spleen of IEC\(^{Cyp1a1}\) mice (Fig. 3g), and the reduction of ILC3 and T\(_{H17}\) cells collectively affected the level of IL-22 that was detectable in colon tissue (Fig. 3h). In contrast, Rag1\(^{Cyp1a1}\) mice infected with C. rodentium had survival rates similar to wild-type mice over 14 days after infection and had no significant differences in bacterial load, pathology score or dissemination of bacteria (Extended Data Fig. 7a–c). Whereas ILC3 were not affected, T\(_{H17}\) cells and their IL-22 production were strongly reduced (Extended Data Fig. 7d). Thus, enhanced AHR ligand degradation in T cells compromises T-cell-derived IL-22 responses, but spares ILC3. In contrast, enhanced AHR ligand degradation in IECs deprives ILC3 as well as T cells of AHR stimulation, thereby causing a more severe deficiency in immune defence against C. rodentium.

We next investigated whether lack of AHR ligand metabolism in non-haematopoietic cells affects the immune response to C. rodentium infection. We generated bone marrow chimaeras by transferring wild-type donor bone marrow into irradiated wild-type (WT→WT) or Cyp1-deficient (WT→Cyp1\(^{−/−}\)) recipients, followed by infection with C. rodentium. WT→Cyp1\(^{−/−}\) chimaeras showed significantly reduced pathology (Fig. 3i), as well as bacterial loads (Fig. 3j), compared with wild-type recipients (WT→WT). Furthermore, Cyp1\(^{−/−}\) recipients had increased numbers of wild-type donor-derived ILC3 and T\(_{H17}\) cells and markedly higher IL-22 levels (Fig. 3k). Thus, absence of CYP1A1-mediated AHR ligand metabolism in IECs results in increased ligand availability to intestinal immune cells and promotes resistance to enteric infection. These data are consistent with a crucial role for IECs as gatekeepers for the availability of AHR ligands in the intestinal immune system.

To further substantiate the role of CYP1A1 in controlling ligand availability, we investigated whether increasing AHR ligands by administration of I3C in the diet might mitigate the loss of AHR signalling in R26\(^{Cyp1a1}\) mice. R26\(^{Cyp1a1}\) mice that were fed on control diet and infected with C. rodentium succumbed to infection, whereas mice on the I3C-supplemented diet survived and had cleared the infection by day 14 (Fig. 4a, b). Penetration of C. rodentium to the crypts was abrogated (Fig. 4c) and mice on the I3C diet had markedly
Figure 4 | Dietary AHR ligands restore immunity to C. rodentium.
a, Survival plot of R26\textsuperscript{Cyp1a1}\textsuperscript{mice infected with C. rodentium, fed control or I3C diet (n = 6 per group). b, C. rodentium burdens in faeces (control, n = 5; I3C, n = 6). c, Colon sections of mice infected with C. rodentium stained for E-cadherin (green), C. rodentium (red) and DAPI (blue). d, Representative photomicrographs of I\&E-stained colon sections. Scale bars, 100 μm (c, d). e, Pathology scores. Bars show the mean, squares represent individual mice. f, Absolute numbers of cytokine-producing TCR\textsuperscript{+}CD4\textsuperscript{+} T cells in the colon of mice infected with C. rodentium (n = 4 per group). Error bars, mean ± s.e.m. g, IL-17A and IL-22 expression in TCR\textsuperscript{+}CD4\textsuperscript{+} T cells from the colon of mice infected with C. rodentium. h, Survival plot of R26\textsuperscript{Cyp1a1}\textsuperscript{mice fed I3C diet treated with anti-IL-22 blocking antibody (n = 6) or isotype control (n = 5). i, Concentration-dependent inhibition of human recombinant CYP1A1 enzyme activity by I3C, DIM and FICZ. Results are representative of three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001, as calculated by Student’s t-test.

The AHR pathway is of crucial importance in the intestinal immune system and genome-wide association studies have identified AHR as a susceptibility locus in inflammatory bowel disease\textsuperscript{24}. Although the main focus has been on AHR itself or on its ligands\textsuperscript{2,4,25}, we show here that feedback regulation of the AHR pathway by CYP1 enzymes controls ligand availability and thereby activation of the AHR pathway, with a critical role for IECs in controlling availability of ligands to the intestinal immune system. Thus, constitutive CYP1A1 activity in IECs reduced the availability of AHR ligands in the body, causing the decay of intestinal immune cells, such as ILC3, that depend on AHR signals for survival and impairment of the adaptive IL-22 response upon infection with intestinal pathogens. Conversely, lack of CYP1A1-mediated metabolism confined to IECs had the opposite effect, increasing protection against intestinal infection.

As previously shown in an in vitro setting, inhibition of CYP1A1 results in a boost to AHR pathway activation owing to increased availability of the AHR ligand FICZ\textsuperscript{26}. Here we demonstrate that genetic deletion of CYP1 enzymes delays ligand metabolism, resulting in increased AHR signals in vivo. Sources of AHR ligands are abundant and include dietary compounds\textsuperscript{31}, microbial virulence factors\textsuperscript{4} and AHR ligands derived through microbiota- or host-cell-mediated tryptophan metabolism\textsuperscript{2}. Given that environmental factors such as oxidative stress, chemical pollutants and dietary components modulate CYP1 enzyme activity\textsuperscript{30}, interference with CYP1-mediated AHR ligand degradation has potential consequences for AHR pathway activation and intestinal homeostasis.
Our finding that the intestinal pathology resulting from constitutive CYP1A1 activity could be counter-balanced by increasing the intake of AHR ligands in the diet suggests that dysregulated CYP1A1 is potentially amenable to therapeutic manipulation through dietary supplementation.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions C.S. designed, performed and analysed most of the experiments with input from A.M.A., Y.L., S.O. and E.W. performed the metabolic studies. A.P. assisted in designing the construct for R26<sup>R26Cyp1a1</sup> mice, C.J.H. and C.R.W. provided Cyp1a1 reporter mice, and D.W.N. provided Cyp1a1-knockout mice. B.S. conceived the project and wrote the manuscript together with C.S.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to B.S. (Brigitta.Stockinger@crick.ac.uk).

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METHODS

Mice. For the generation of a constitutively active as well as an inducible Cyp1a1 allele the coding sequence (CDS) of Cyp1a1 was linked to the CDS of rat Thy1 using a 2A sequence. In order to stabilize the transcriptional activity of the inserted genes, a Woodchuck-hepatitis-virus-derived regulatory element in conjunction with a bovine growth hormone polyadenylation site was inserted 3′ end of the CDSs. This module was inserted downstream of a cassette containing an FRT-flanked neomycin and loxP-flanked transcriptional stop element. The targeting vector did not contain any artificial promoter for the expression of both Cyp1a1 and rat Thy1 to avoid toxicity caused by CYP1A1 overexpression (details of the gene targeting and Rosa26-targeting strategy, Extended Data Fig. 1). The targeting vector was generated by Biogeny and used to establish R26<sup>Cre</sup>−loxPflox<sup>−</sup>neo<sup>−</sup> mice by homologous recombination in B6Gcen<sup>−</sup>6 (C57BL/6) embryonic stem cells. R26<sup>Cre</sup>−loxPflox<sup>−</sup>neo<sup>−</sup> mice were generated after successful germ-line transmission and backcrossed to C57BL/6<sup>−</sup>. This strain was further bred with mice expressing Cre recombinase under the control of the mouse protamine (P<sub>nr1</sub>) promoter to obtain constitutive Cyp1a1 expression in all cell types (referred to as R26<sup>Cre</sup>−loxPflox<sup>−</sup> mice) or with ACTB–FLPe mice to obtain mice with a conditional floxed Cyp1a1 allele (referred to as R26<sup>SIL−</sup>Cre<sup>−</sup>). These were crossed with V<sub>H1</sub>−cre mice to generate the E<sub>Ico</sub>gp<sup>−</sup> striatum (constitutive Cyp1a1 expression specific to intestinal epithelial cells) and Rbg1−cre mice to generate the Rag1<sup>−</sup> striatum (constitutive Cyp1a1 expression specific to T and B cells).

To generate the Cyp1a1 reporter strain Cyp1a1−cre mice<sup>26</sup> (with targeted insertion of Cre recombinase in the mouse Cyp1a1 locus) were crossed with R26<sup>−</sup>eYFP mice<sup>27</sup>, expressing eYFP from the ubiquitous Rosa26 promoter downstream of a loxP-flanked transcriptional stop element. This strain was further bred with Cyp1<sup>−</sup> mice<sup>28</sup> to obtain Cyp1<sup>−</sup>−Cre<sup>−</sup> mouse reporter mice. All mice used in this study were either generated in C57BL/6 embryonic stem cells or backcrossed to C57BL/6 mice for at least 10 generations. All mice were bred in the Francis Crick Institute animal facility under specified pathogen-free conditions.

All animal procedures were conducted under a Project Licence granted by the UK Home Office. Mice were age- and sex-matched and more than 6 weeks old when first used. Both female and male mice were used in experiments. Generally, each mouse of the different experimental groups is reported. Exclusion criteria such as inadequate staining or low cell yield owing to technical problems were pre-determined. Animals were assigned randomly to experimental groups.

In vitro T-cell differentiation. CD<sup>4+</sup> T cells were isolated using EasySep mouse CD<sup>4+</sup> T cell isolation kit (StemCell Technologies) with the addition of biotinylated mouse anti-E-cadherin (BD, 560062) and visualized using a Leica Confocal SP5-invert microscope. For staining of eYFP, tissue sections were fixed in 4% paraformaldehyde at 4 °C for 16 h followed by incubation in 610181 and rabbit anti-C. rodentium antiserum, followed by staining with Alexa 488 and Alexa 594 conjugated secondary antibodies (AF555-conjugated goat anti-rabbit and AF488-conjugated goat anti-mouse from ThermoFisher). Slides were further stained with DAPI (Sigma) and mounted in Fluoromount-G (SouthernBiotech) and visualized using a Leica Confocal SP5-Invert microscope. For staining of eYFP, tissue sections were fixed in 4% paraformaldehyde solution for 4 h for 4 h followed by incubation in 30% sucrose for 24 h. Tissues were embedded in OCT compound coupled to a reverse-phase C18 analytical column, was performed as previously described<sup>26</sup>. FICZ quantity was determined according to a standard curve of FICZ and the results were normalized to total protein contents determined by Pierce Coomassie protein assay kit according to the manufacturer’s instructions.

CYP1A1-mediated metabolism of DIM, ICZ and FICZ. Time-dependent metabolism of DIM, ICZ and FICZ was studied in the presence of human recombinant CYP1A1 (3.5 nM) and the co-factor NADPH (1.0 mM). Each compound (0.1 μM) was incubated with CYP1A1 and NADPH in Tris-HCI (0.1 M, pH 7.4) with EDTA (1 mM) at 37 °C. At various time-points, samples were collected and acetonitrile was added to each sample at a final concentration of 20%, followed by vortexing (30 s) and centrifugation (10 min at 12 000 r.p.m. and 4 °C). Chemical analysis of the supernatants was performed using the same HPLC equipment as for the cellular clearance measurements. Separation of respective compound was achieved using a reverse-phase C18-AR column (ACE, 4.6 × 150 mm, 5 μm particle size) with a mobile phase consisting of water (A) and acetonitrile (B), both containing 1 mM formic acid. Initially, the solvent contained 30% B, with a linear increase to 100% B during a period of 20 min, at a flow rate of 0.8 ml min<sup>−1</sup>. DIM, ICZ and FICZ was detected using excitation/emission wavelengths of 230/460 (DIM and ICZ) or 390/525 nm (FICZ). All compounds were quantified according to standard curves. To determine non-enzymatic degradation of each compound parallel incubations were performed where phosphate buffer was substituted for NADPH.

Ethoxyresorufin-O-deethylase (EROD) assay. Intestinal tissue homogenates or CD<sup>4+</sup> T cells cultured for 48 h under T<sub>H1</sub>-cell-inducing conditions were washed in PBS followed by incubation with 2 μM 7-ethoxyresorufin in sodium phosphate buffer (50 mM, pH 8.0) at 37 °C for 30 min. The reaction was terminated by adding fluorescein (Sigma) dissolved in acetonitrile. Formation of resorufin (excitation/emission of 535/590 nm) and fluorescein (excitation/emission of 390/485 nm) was quantified using a plate reader.

Inhibition of CYP1A1 activity by IC3, DIM and ICZ. Effects on the EROD activity of human recombinant CYP1A1 (2.5 nM) was assayed by first pre-incubating the enzyme with the compound to be tested for 5 min in Tris-HCl (0.1 M, pH 7.4) with EDTA (1 mM) at 37 °C followed by addition of ethoxyresorufin (0.1 μM) and NADPH (0.4 mM). Formation of resorufin was quantified using a microplate reader with the excitation/emission wavelengths of 535/590 nm and activity of the enzyme was determined by the rate of resorufin formation. ICZ and FICZ were obtained from Systantic AB. DIM, ICZ, 7-ethoxyresorufin, 3-Nicotinamide adenine dinucleotide 2-phosphate (NADP, N705) and human recombinant cytochrome P4501A1 with P450 reductase (C3753) was purchased from Sigma-Aldrich.

Immunofluorescence microscopy. Tissues were fixed in 4% phosphate-buffered formaldehyde solution (Fisher Scientific) for 24 h. Fixed tissue sections were de-paraffinized and antigen retrieval performed in 0.01 M sodium citrate buffer. Slides were blocked with goat serum, stained with mouse anti-E-cadherin (BD, 610181) and rabbit anti-C. rodentium antiserum, followed by staining with secondary antibodies (AF555-conjugated goat anti-rabbit and AF488-conjugated goat anti-mouse from ThermoFisher). Slides were further stained with DAPI (Sigma) and mounted in Fluoromount-G (SouthernBiotech) and visualized using a Leica Confocal SP5-Invert microscope. For staining of eYFP, tissue sections were fixed in 4% parafomaldehyde at 4 °C for 16 h followed by incubation in 30% sucrose for 24 h. Tissues were embedded in OCT compound (WWR) followed by cryosectioning. Slides were blocked with rabbit serum and stained with AF488-conjugated rabbit anti-GFP (ThermoFisher, A21311) and AF647-conjugated mouse anti-E-cadherin (BD, 560062) and visualized using a Leica Confocal SP5-Invert microscope. Infection with Citrobacter rodentium. For C. rodentium infection, a single colony of strain DBS100 (ATCC 51459; American Type Culture Collection) was transferred to Luria–Bertani (LB) broth and grown to log phase, followed by centrifugation and resuspension in PBS. Mice were orally gavaged with 200 μl of PBS containing 2 × 10<sup>8</sup> C. rodentium CFU. To determine bacterial load, intestinal tissue pieces or fecal pellets were weighed and homogenized in sterile PBS and
serial dilutions were plated onto Brilliance \textit{E. coli}/coliform Selective Agar (Fisher Scientific) or LB agar plates (liver and spleen) for measurement of colony-forming units (CFU). For neutralization of IL-22, mice were injected intraperitoneally (i.p.) three times per week with 150\(\mu\)g per mouse per dose monoclonal anti-IL-22 (clone 8E11, Genentech) or mouse IgG1 isotype control (BioXCell/2BScientific). Where indicated, mice were injected i.p. three times per week with 125\(\mu\)g ml\(^{-1}\) per dose IL-22–Fc (PRO312045, Genentech) or mouse IgG2a isotype control (BioXCell/2BScientific).

\textbf{Diet studies.} For diet studies mice were fed purified diet AIN-93M (TestDiet-IPS) or AIN-93M supplemented with 200 p.p.m. indole-3-carbinol (Sigma). Mice were put on purified diets shortly after weaning for at least 4 weeks and maintained on the purified diets throughout experiments.

\textbf{Generation of bone marrow chimaeric mice.} Bone marrow was injected intravenously (2.5 \(\times\) 10\(^6\) cells per mouse) into recipient mice irradiated with two doses of 5 Gy using a \(^{137}\)Cs source. Donor bone marrow and recipient mice were distinguished on the basis of congenic markers. Chimaeric mice were used in experiments 6–8 weeks following reconstitution.

\textbf{Colon explant cultures.} Intestinal tissue pieces (0.5–1 cm length) were cultured for 24 h in complete IMDM medium. IL-22 cytokine levels in the supernatants were determined by ELISA (eBioscience) and concentrations were normalized to the weight of the explants.

\textbf{Histological assessment.} Tissues from distal colon and caecum were fixed in 4% phosphate-buffered formaldehyde solution (Fisher Scientific) for 24 h, cut and stained with haematoxylin and eosin. Slides were blinded and scored (0–15) for parameters of inflammation and tissue damage, as described in ref. 28.

\textbf{Statistical analysis.} For comparisons between two groups unpaired, two-tailed Student’s \(t\)-test was used or, when appropriate, a two-way ANOVA with Dunnett’s post-test. For the comparison of three groups, a one-way ANOVA followed by Tukey multiple comparison test was performed. All statistical analysis was calculated in Prism (GraphPad 6). No statistical methods were used to predetermine sample size.

\textbf{Data availability statement.} The authors declare that the source data supporting the findings of this study are available within the paper and its extended data files.

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Extended Data Figure 1 | Generation of R26Cyp1a1 allele. a, The endogenous Rosa26 locus, the gene-targeting vector, the targeted Rosa26 allele including the Neo resistance gene cassette (R26\textsubscript{Cyp1a1-neoR}), the targeted allele (R26\textsubscript{LSL-Cyp1a1}) after FlPe-mediated recombination and the ubiquitously expressed R26\textsubscript{Cyp1a1} are schematically depicted to scale. A minigene composed of the coding sequences of mouse Cyp1a1 and rat Thy1 connected by a 2A sequence followed by the Woodchuck hepatitis virus derived regulatory element (WPRE) and a bovine growth hormone polyadenylation site (bGH pA). b, Expression of rat THY1 in indicated cell types in the colon of R26\textsubscript{Cyp1a1}, IEC\textsubscript{Cyp1a1} (Vil1-cre-R26\textsubscript{LSL-Cyp1a1}), Rag1\textsuperscript{Cyp1a1} (Rag1-cre-R26\textsubscript{LSL-Cyp1a1}) strains.

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Extended Data Figure 2 | Altered AHR ligand availability affects IL-22 production. Flow cytometry analysis of IL-17A and IL-22 expression in in vitro differentiated T\(_{\text{h}17}\) cells from indicated genotypes (day 4) exposed to DMSO, 0.01 nM FICZ or 1 nM FICZ from the start of culture.
Extended Data Figure 3 | Altered AHR ligand availability affects intestinal AHR-dependent ILC populations. 

**a**. CYP1A1 enzyme activity, measured by EROD assay, in intestinal tissue homogenates of steady-state mice. 

**b.** Flow cytometry analysis of NKp46 and RORγt expression in CD45+ lineage negative (TCRβ–CD3–TCRγδ–CD19–CD11b–Gr1–) Thy1+ live cells in the small intestine (upper panel) and phenotypic analysis of RORγt+ NKp46– innate lymphoid cells (lower panel). Results are representative of three independent experiments (n = 3). Error bars, mean ± s.e.m. ***P < 0.001, as calculated by one-way ANOVA with Tukey post-test.
Extended Data Figure 4 | Effects of Cyp1a1 overexpression on liver development. a, Liver weight represented as percentage of body weight. b, Liver (left lobe) weight represented as percentage of body weight. Results are representative of two independent experiments. Bars are the mean, each symbol represents an individual mouse. *P < 0.05, **P < 0.01, ***P < 0.001, as calculated by one-way ANOVA with Tukey post-test.
Extended Data Figure 5 | IL-22–Fc improves immunity to C. rodentium infection in R26Cyp1a1 mice. Mice of indicated genotypes were infected orally with ~2 × 10⁹ C. rodentium CFU and killed 7 days after infection or monitored for survival. a, C. rodentium burdens in the colon and caecum. Bars are the median, each symbol represents an individual mouse. b, Colon sections stained for E-cadherin (green), C. rodentium (red) and DAPI (blue). Scale bars, 100 μm. c, Survival plot (WT, n = 4; R26Cyp1a1 + control Ig, n = 6; R26Cyp1a1 + IL-22–Fc, n = 5). Results are representative of three independent experiments. NS, not significant. **P < 0.01, ***P < 0.001, as calculated by one-way ANOVA with Tukey post-test.
Extended Data Figure 6 | Cyp1 deficiency enhances AHR pathway activation. a, CD4+ T cells from indicated genotypes were cultured under Th17-cell-inducing conditions and exposed to FICZ from the start of culture. Intracellular levels of FICZ were determined by HPLC and normalized to total protein content at the indicated time points (n = 3 per time point). b, Frequencies of IL-22-producing cells after 4 days of culture under Th17-cell-inducing conditions in presence of indicated concentrations of FICZ. Results are representative of three independent experiments. Error bars, mean ± s.e.m. **P < 0.01, ***P < 0.001, as calculated by two-way ANOVA with Dunnett's post-test (a) or one-way ANOVA with Tukey post-test (b). c, Flow cytometry analysis of Cyp1a1 (eYFP) expression by Th17 cells differentiated from indicated genotypes. Plots are gated on IL-17A+ cells and numbers indicate frequencies. d, Flow cytometry plots of IL-17A and IL-22 expression in in vitro differentiated Th17 cells from indicated genotypes (day 4) exposed to DMSO, 0.01 nM FICZ or 1 nM FICZ from the start of culture. e, Mice of indicated genotypes were infected orally with ~2 × 10⁹ C. rodentium CFU and bacterial burdens measured in the faeces at various time points. f, Pathology scores of distal colon. Bars are the mean, symbol represents an individual mouse. Results are representative of at least two independent experiments. NS, not significant. **P < 0.01, ***P < 0.001, as calculated by Student's t-test.
Extended Data Figure 7 | Effects of Rag1-cre mediated Cyp1a1 expression on immunity to *C. rodentium*. Mice of indicated genotypes were infected orally with ~2 × 10^9* C. rodentium* CFU and killed 14 days after infection or monitored for survival. a, Survival plot. b, *C. rodentium* burdens in the colon, caecum, liver and spleen. c, Pathology scores of distal colon and caecum. Bars are the mean, each symbol represents an individual mouse (b, c). d, Absolute numbers of cytokine-producing TCRβ^+^CD4^+^ T cells in the colon of mice infected with *C. rodentium*. Error bars, mean ± s.e.m. Results are representative of two independent experiments (n = 5 per group). NS, not significant. **P < 0.01, as calculated by Student’s t-test.
Extended Data Figure 8 | CYP1A1-mediated metabolism of DIM, ICZ and FICZ. a–c, CYP1A1-mediated metabolism of DIM (a), ICZ (b) and FICZ (c) was studied over time in the presence of human recombinant CYP1A1 (3.5 nM) and the co-factor NADPH (1.0 mM). At indicated time points, samples from respective incubations were extracted and analysed by means of HPLC. All chemicals were quantified according to separate standard curves. Left panels show relative amount of compound remaining at each given time point compared to parallel incubations without co-factor present. Right panels show HPLC chromatograms at 40 min enzyme-incubation, with and without co-factor present. All three compounds were detected on the basis of their fluorescence properties (fluorescence units, FLU). Results are representative of two independent experiments with two biological replicates at each experiment. Error bars, mean ± s.d.