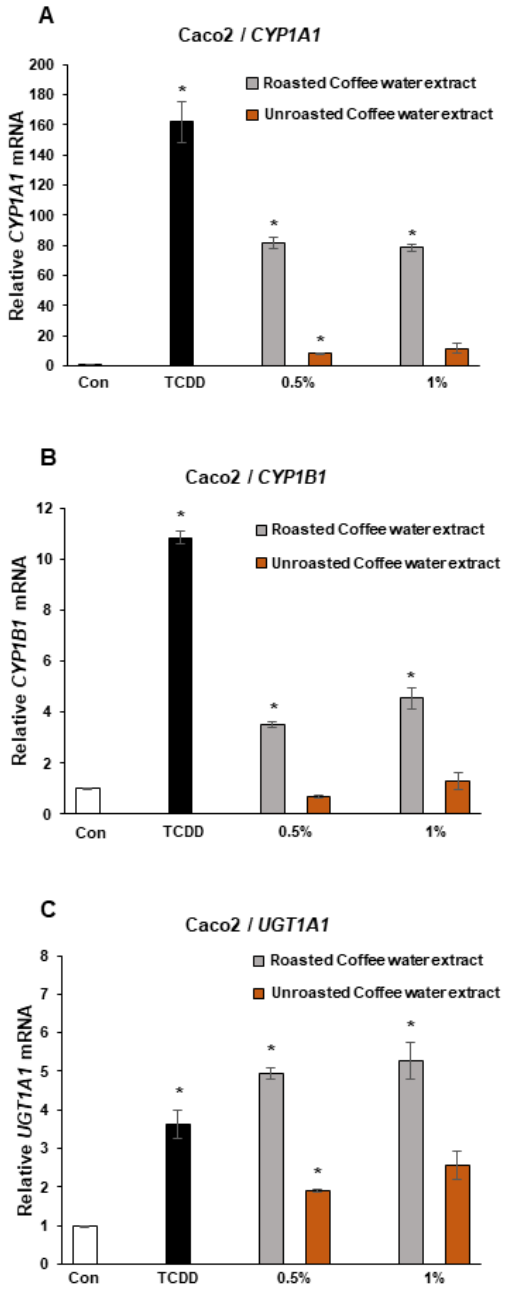
Supplemental Figure 1



SUPPLEMENTAL FIGURES CAPTIONS

Figure S1. AhR activity of roasted and unroasted coffee. Caco2 cells were treated with DMSO, 10 nM TCDD roasted and unroasted coffee extracts for 24 h and induction of (A) CYP1A1, (B) CYP1B1 and (C) UGT1A1 was determined by real time PCR as outlined in the Materials and

Methods. Results are expressed as means ± SE for 3 separate determinations for each treatment group and significant ($p<0.05$) induction is indicated (*).

Supplemental Figure 2

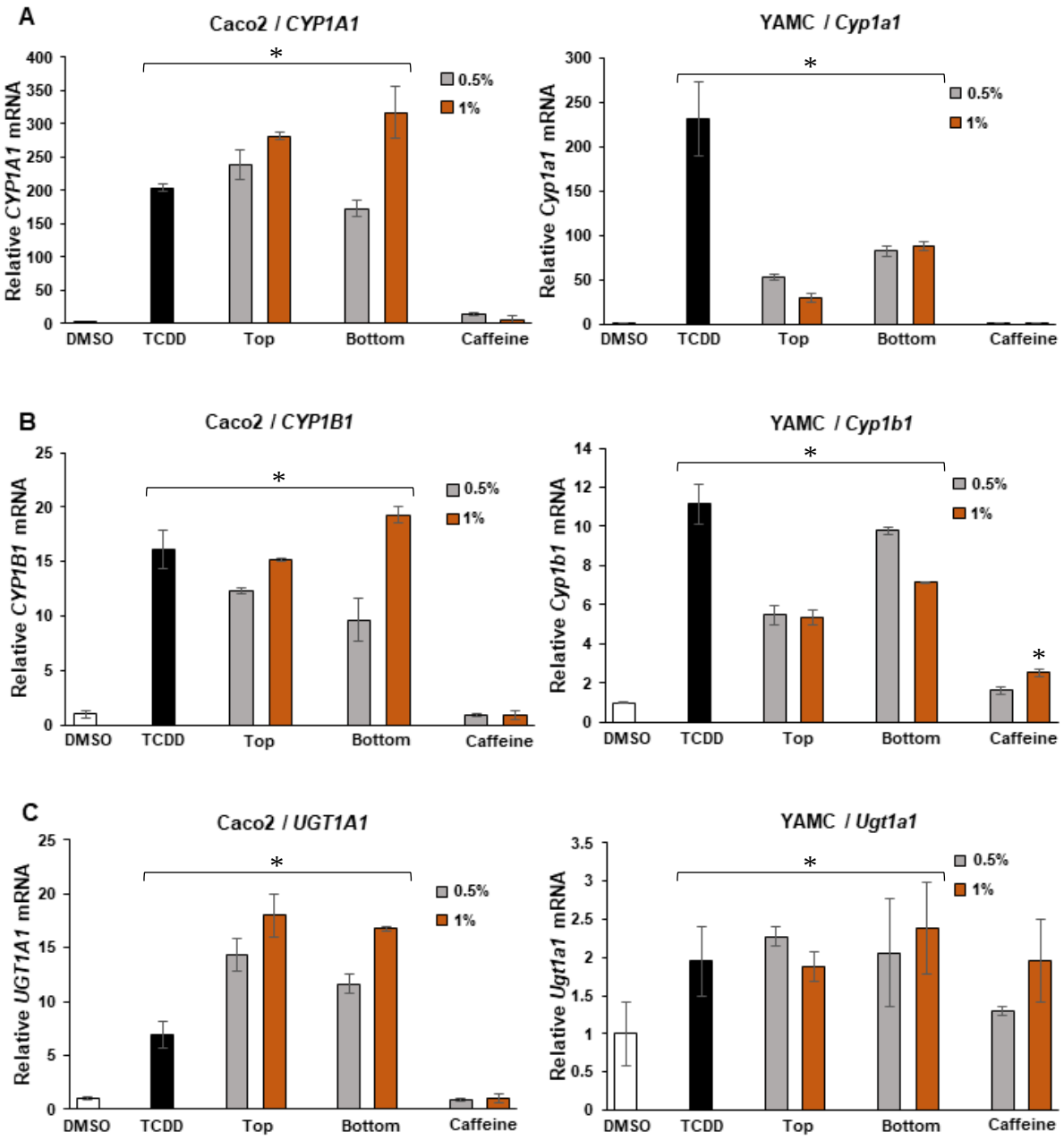


Figure S2. AhR activity of coffee extract ($CHCl_3$) TLC fractions. Caco2 and YAMC cells were treated with DMSO, 10 nM TCDD and TLC fractions top, bottom and middle (caffeine) and induction of (A) CYP1A1, (B) CYP1B1 and (C) UGT1A1 were determined by real time PCR as outlined in the Materials and Methods. Results are expressed as means ± SE for 3 separate experiments for each treatment group and significant ($p<0.05$) induction is indicated (*).

Supplemental Figure 3

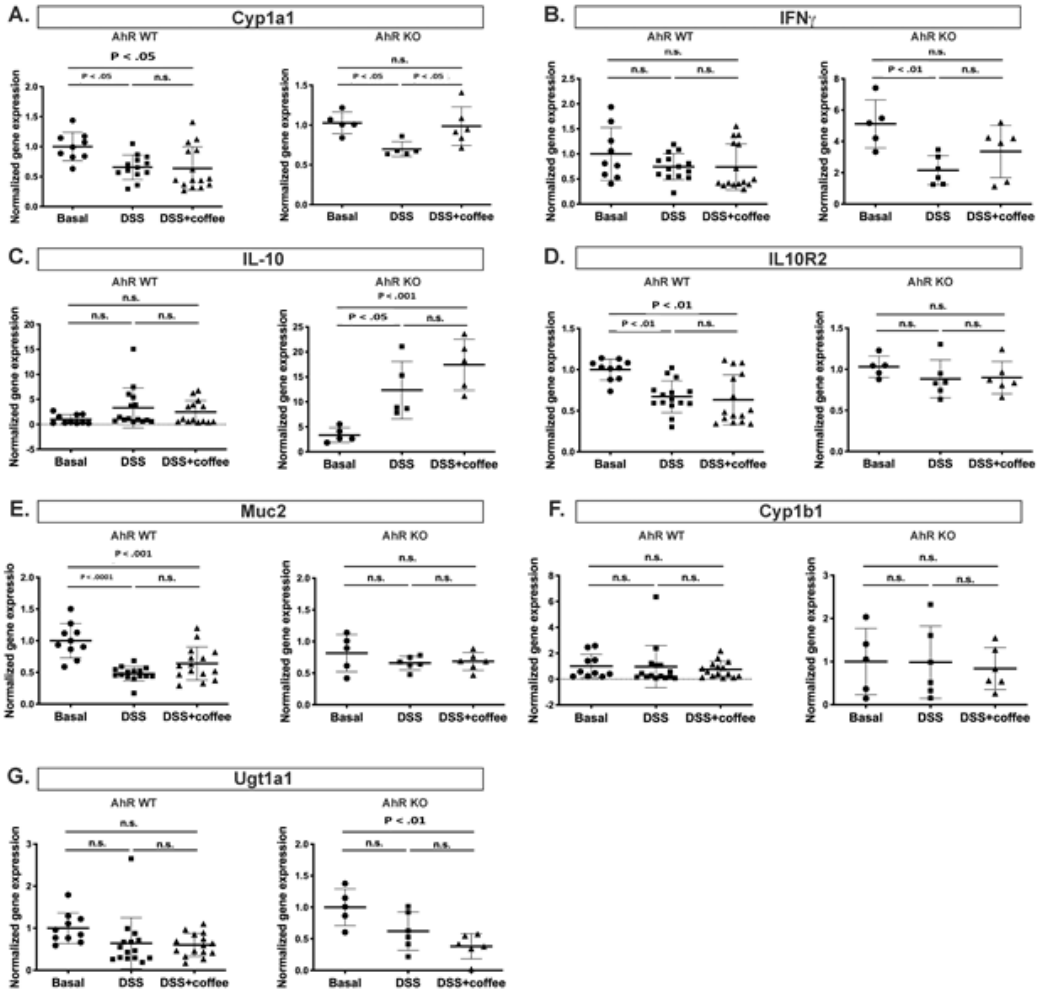


Figure S3. Effects of coffee extracts on DSS-induced mucosal gene expression. Coffee did not alter the DSS-induced effect on (A) Cyp1a1, (B) IFNγ, (C) IL-10, (D) IL10R2, (E) Muc2, (F) Cyp1b1 or (G) Ugt1a1. mRNA levels were determined as outlined in the Methods. Results are expressed as means ± SD for at least five determinations per treatment group and significance as indicated in the figure.

Supplemental Figure 4

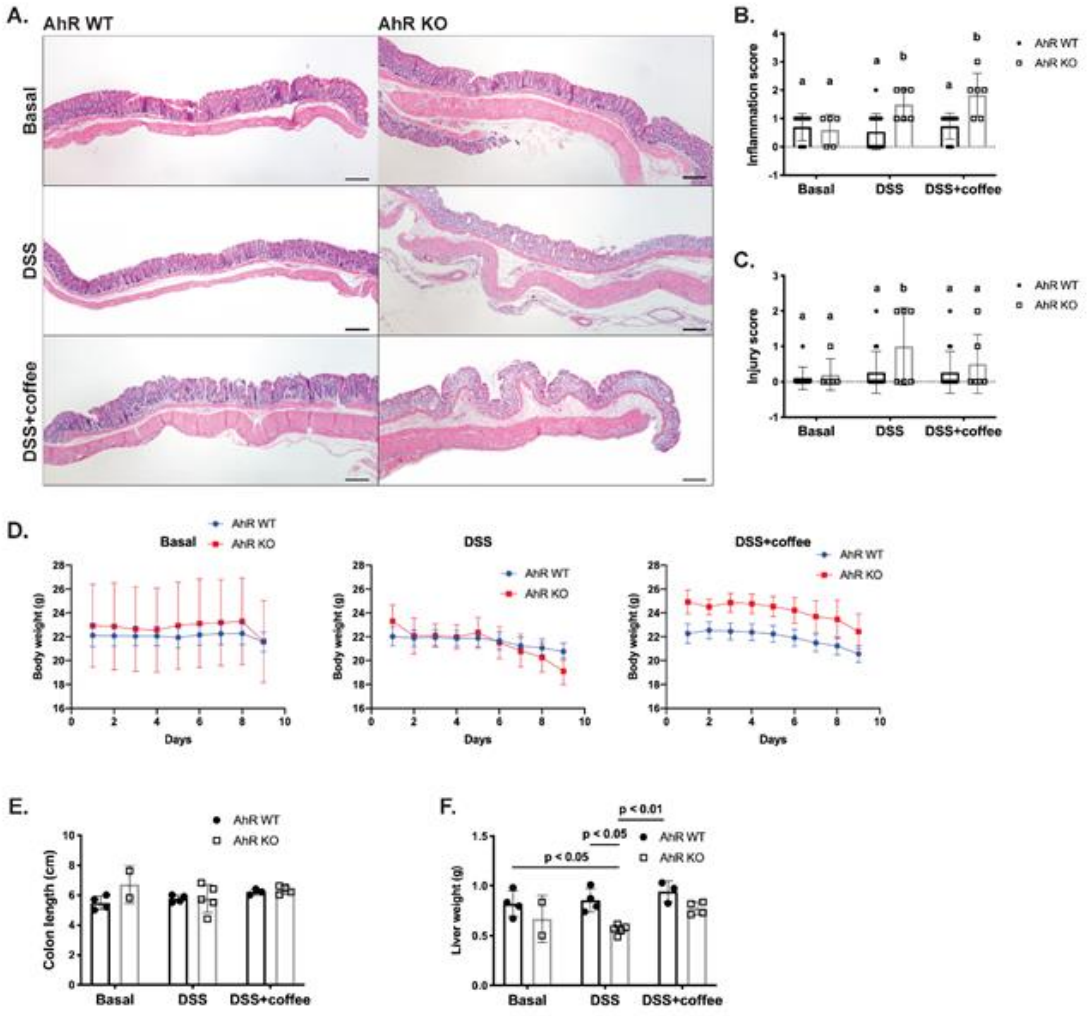


Figure S4. Lack of effect of coffee on clinical markers. (A) H&E stained sections of distal colon in AhR wild type and KO mice. (B) Inflammation and (C) injury scores were assessed by a board-certified pathologist. (D) Body weights through the study period were taken daily starting with DSS treatment. No differences in the rate of weight loss due to DSS were noted between WT and KO mice. (E) Colon length and (F) liver weight were not affected by coffee treatment.

Supplemental Figure 5

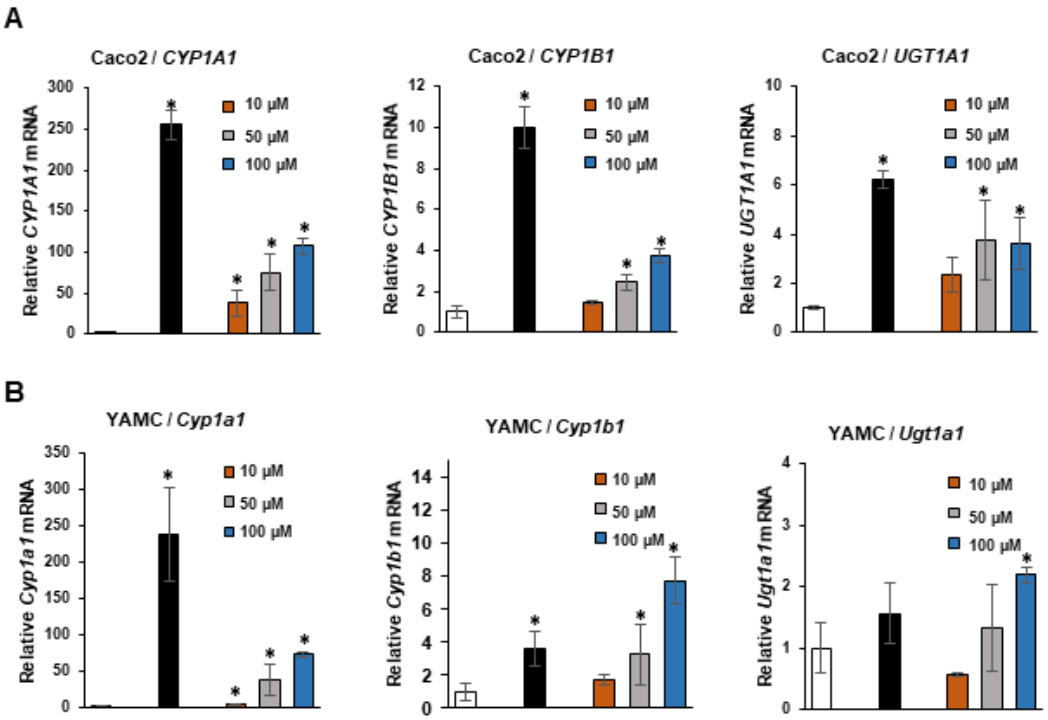


Figure S5. AhR activity of indole-3-pyruvic acid. (A) Caco2 and (B) YAMC cells were treated with 10 nM TCDD, 10, 50 or 100 µM indole-3-pyruvic acid for 24 h and induction of CYP1A1, CYP1B1 and UGT1A1 were determined as outlined in the Methods. Results are expressed as means ± SE (3 determinations) and significant ($p<0.05$) induction is indicated (*).

SUPPLEMENTAL TABLES

Supplemental Table S1. Primers sequences and/or sources used in this study

Gene	Sequence/Source
Mouse IL-10	Mm01288386_m1 (Taqman assay from Life Technologies)
Mouse IL-6	Mm00446190_m1 (Taqman assay from Life Technologies)
Mouse Muc2	Mm00446190_m1 (Taqman assay from Life Technologies)
Mouse TBP	Mm01277042_m1 (Taqman assay from Life Technologies)
Mouse AhR	Forward: GCCCTTCCCGCAAGATGTTAT Reverse: GCTGACGCTGAGCCTAAGAAC
Mouse Cyp1a1	Forward: CAATGAGTTTGGGGAGGTTACTG Reverse: CCCTTCTCAAATGTCCTGTAGTG
Mouse Cyp1b1	Forward: CCACCAGCCTTAGTGCAGAC Reverse: GGCCAGGACGGAGAAGAGT
Mouse Ugt1a1	Forward: ATGGCTTTCTTCTCCGGAAT Reverse: TCAGAAAAAGCCCCTATCCC
Mouse Tgf- β1	Forward: CTGCTGACCCCCACTGATAC Reverse: AGCCCTGTATTCCGTCTCCT
Mouse IFNγ	Forward: TCAAGTGGCATAGATGTGGAAGAA Reverse: TGGCTCTGCAGGATTTTCATG
Mouse IL-1β	F: GGTCAAAGGTTTGGAAGCAG R: TGTGAAATGCCACCTTTTGA
Mouse IL-10R1	Forward: CCCATTCCTCGTCACGATCTC Reverse: TCAGACTGGTTTGGGATAGGTTT
Mouse IL-10R2	Forward: CGGACAGGCAATGACGAAAT Reverse: GACCACCAGGACGGAGACTA
Mouse Gapdh	Forward: AGGTCGGTGTGAACGGATTTG Reverse: GGGGTCGTTGATGGCAACA
Human Cyp1a1	Forward: GAGGCCAGAAGAAACTCCGT Reverse: CCCAGCTCAGCTCAGTACCT
Human Cyp1b1	Forward: ACCTGATCCAATTCTGCCTG Reverse: TATCACTGACATCTTCGGCG
Human Ugt1a1	Forward: GAATCAACTGCCTTCACCAAAAT Reverse: AGAGAAAACCACAATTCCATGTTCT
Human TBP	Forward: TTCTGAATAGGCTGTGGGGT Reverse: GATCAGAACAACAGCCTGCC

Supplemental Table S2

Gene Expression p-values
Villin specific AhR KO

HVG						
Gene	AhR WT			AhR KO		
	Basal v. DSS	Basal v. D+C	DSS v. D+C	Basal v. DSS	Basal v. D+C	DSS v. D+C
IL-10	0.0004	<0.0001	0.7138	0.0001	<0.0001	0.1912
IL-6	0.0162	0.2278	0.3304	<0.0001	0.0057	0.0049
TGFb1	0.0009	0.8883	0.0017	0.0004	<0.0001	0.0312
AhR	<0.0001	<0.0001	0.9099	0.9407	0.8675	0.7994
Muc2	<0.0001	0.0009	0.0507	0.0005	0.0367	0.1033
IL1b	0.0143	0.2745	0.2412	<0.0001	<0.0001	0.9514
IL-10R1	<0.0001	0.177	0.0023	<0.0001	0.0033	0.0003
IL-10R2	<0.0001	<0.0001	0.7445	<0.0001	0.0005	0.0596
IFNgamma	0.9495	0.9232	0.9748	0.0005	0.0868	0.0463
Cyp1a1 (SYBR)	0.6582	0.0069	0.0208	0.0998	0.3905	0.3945
Cyp1a1 (Taqman)	0.6539	0.3801	0.6512	0.6982	0.3221	0.1447
Cyp1b1 (SYBR)	0.2495	0.1362	0.6661	0.5524	0.3312	0.676
Cyp1b1 (Taqman)	0.0008	0.0426	0.2004	0.0016	0.3092	0.0213
Ugt1a1 (SYBR)	<0.0001	<0.0001	0.6664	<0.0001	<0.0001	0.2872
Ugt1a1 (Taqman)	0.2955	0.7241	0.5173	0.1181	0.867	0.0698
TFF3	0.0059	0.0618	0.4475	0.0928	0.8293	0.1234
Occludin	<0.0001	<0.0001	0.8602	<0.0001	<0.0001	0.1859
*Note: "D+C" refers to DSS+coffee						

Supplemental Table S3: Indole compounds identified in fecal and coffee extracts

Indole-3-methyl acetate a
Indole-3-propionic acid a
Methyl 1-methoxy-1H-indole-3-carboxylate a
Methyl 2,3-dihydro-3,5-dihydroxy-2-oxo-3-indoleacetic acid a
Methyl 5-hydroxyoxindole-3-acetate a
xi-2,3-Dihydro-2-oxo-1H-indole-3-acetic acid a
3-Carboxy-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-propanoic acid b
3-Indolehydracrylic acid b
3-Methyldioxyindole b
5-Hydroxyindoleacetaldehyde b
5-Hydroxyindoleacetic acid b
Indole 3 lactic acid b
Indoleacetic acid b
Indoleacrylic acid b
Indolepyruvate b

[a] identified in fecal extracts but not in coffee extracts

[b] identified in coffee extracts

SUPPLEMENTAL METHODS

For genotyping analysis of whole body knock out ($Ahr^{-/-}$) mice, DNA was extracted from tails using DNeasy Blood and Tissue Kit (Qiagen; 69506) and PCR was performed using the following primers: (F: 5'-CAGTGGGAATAAGGCAAGAGTGA-3', R: 5'-AGGGAGATGAAGTATGTGTATGTA-3') resulting in a Wt 300 bp product compared to 260 bp in the AhR null mouse as previously described (27). $AhR^{-/-}$ whole body KO breeder mice were kindly provided by Dr. Bhagavatula Moorthy, Baylor College of Medicine. For villin Cre intestinal-specific AhR KO mouse genotyping, AhR primers were designed to amplify exon 2 of the AhR genome which was deleted as previously described (PMID: 32915464). Specifically, F: GGTACAAGTGCACATGCCTGC, Fw2: GTCACTCAGCATTACACTTTCTA, R: CAGTGGGAATAAGGCAAGAGTGA, primers generated 100 bp in Wt, 140 bp in heterozygous Floxed, and 180 bp in homozygous deleted mice.

For further confirmation of successful deletion, protein levels of AhR were measured using isolated colonic crypts via Western blot and rabbit anti-mouse AhR (1:2,000 dilution; Enzo Life Sciences, cat. no. BML-SA210) (27). mRNA levels of AhR were also measured using iTaq Universal SYBR Green 1-Step Kit according to the manufacturer's protocol with the CFX384 real-time PCR System (Bio-Rad Laboratories, Hercules, CA). The comparative CT method was used for relative quantitation of samples. Values for each gene were normalized to expression levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Primer sequences used for real-time PCR were as follows: (Mouse AhR-F: 5'-GCCCTTCCCGCAAGATGTTAT-3' and Mouse AhR-R: 5'-GCTGACGCTGAGCCTAAGAAC-3', Mouse GAPDH-F: 5'-AGGTCGGTGTGAACGGATTTG-3' and Mouse GAPDH-R: 5'-GGGGTCGTTGATGGCAACA-3').