Appendix A: Supplemental Methods, Data tables, and Figures.

A.1 Supplemental Methods

A.1.1 Broccoli Glucobrassicin Quantification

Levels of glucobrassicin in broccoli were analyzed according to previously published extraction and instrumental parameters(Mellon, Bennett et al. 2002). In brief triplicate samples of 20 mg of freeze-dried broccoli were homogenized in 1.5 mL of 70% methanol and agitated for 30 minutes at 70°C. The homogenate was centrifuged, supernatant was isolated, and the solvent evaporated. The dry extract was dissolved in an equal volume of HPLC-grade water and subjected to analysis by HPLC-MS. Samples (5ul) were separated by reverse phase HPLC using a Prominence 20 UFLCXR system (Shimadzu, Columbia MD) with a Waters (Milford, MA) BEH C18 column (100 mm x 2.1 mm, 1.7 μm particle size) maintained at 55°C and a 20-min aqueous acetonitrile gradient, at a flow rate of 250 μl/min. Solvent A was HPLC grade water with 0.1% formic acid and Solvent B was HPLC grade acetonitrile with 0.1% formic acid. The initial condition were 97% A and 3% B, increasing to 45% B at 10 min, 75% B at 12 min where it was held at 75% B until 17.5 min before returning to the initial conditions. The eluate was delivered into a 5600 (QTOF) TripleTOF using a Duospray™ ion source (all AB Sciex, Framingham, MA). The capillary voltage was set at 4.5 kV in negative ion mode, with a declustering potential of 80 V. The mass spectrometer was operated in IDA (Information Dependent Acquisition) mode with a 100 ms survey scan from 100 to 1200 m/z. Broccoli glucobrassicin (447.05 m/z) content was quantified via standard curve analysis with known sinigrin (358.027 m/z) standards. Peak (AUC) quantities were normalized to the internal standard, chloropropamide (1 μM).

A.1.2 Bacterial DNA isolation

Bacterial genomic DNA was isolated from 100 mg cecal contents using Powersoil DNA isolation kit (Mobio, Carlsbad, CA.) according to the manufacturer instructions. Following extraction, DNA concentration was normalized to 20 ng/µl and stored at −80 °C.

A.1.3 ^{1}H-NMR cecal metabolite analysis

^{1}H-NMR analyses were performed as previously described (Zhang, Nichols et al. 2015). In brief, the cecal contents (~50 mg) were subjected to three consecutive freeze-thaws and directly extracted by pre-cooled phosphate buffer with homogenization using the Precellys Tissue Homogenizer. Following extraction, 550 µL of each extract was centrifuged and the supernatant was transferred to 5 mm NMR tubes. Cecal extracts were analyzed at 298 K on a Bruker Avance III 600 MHz spectrometer (Bruker Biospin, Germany) equipped with a Bruker inverse cryogenic probe.

A.1.4 Dextran sodium sulfate colitis model-disease severity index

C57BL6/J-*Ahr*$^{b/b}$/*Ahr*$^{d/d}$ mice were weaned onto a standard animal chow diet. At eight weeks of age all mice were fed AIN-93G purified rodent chow for seven days. This was followed by continuation of the AIN-93G diet in control groups or administration of the 15% broccoli diet for 14 days prior to initiation of colitis. To investigate the functional interaction between the diet and *Ahr status*, mice were allocated to four groups: *Ahr* $^{b/b}$, control and broccoli diet groups (n=6 each), and *Ahr*$^{d/d}$, control and broccoli diet groups (*n*=6 and n=7 respectively). After the initial feeding period of 14 days, mice were given *ad libitum* access to (3.5%) dextran sodium sulfate (MP Biomedical, Santa Ana, CA) in drinking water for a period of 6 days to mediate induction of colitis. This was followed by an additional two days on normal drinking water, prior to euthanasia. During the eight day timeline, weight, food consumption, and markers of disease

activity were measured daily. The disease activity index were measured by analyses of the following parameters; Stool consistency (0 Normal, 1 soft but still formed, 2 very soft, 3 diarrhea), Blood in stool (0 no blood, 1 traces of blood, 2 noticeable blood, 3 obvious blood), Rectal bleeding (0 no blood, 1 traces of blood, 2 noticeable blood, 3 obvious blood), and general motility (0 normal activity, 1 less active, 2 no movement). Feces were collected daily for fecal lipocalin quantification. After 8 days mice were euthanized by carbon dioxide asphyxiation, tissues/serum were excised and immediately frozen in liquid nitrogen and stored at −80°C, spleen weight and colon length were also measured. RNA from the tissues of DSS treated animals was subjected to lithium chloride purification to remove excess DSS.

A.1.5 Fecal lipocalin quantification

Fecal extracts were prepared by homogenization of 50 mg feces per mL of phosphate buffered saline +BSA (1%). Homogenates were spun at 10,000 x g for 10 min and supernatant was collected for further analysis. Fecal lipocalin was quantified through use of the Mouse Lipocalin-2/NGAL Quantikine ELISA Kit (R&D Systems, Minneapolis, MN), according to manufacturer's instructions.

A.1.6 Flow cytometric analysis of T-cell populations

Splenocyte and mesenchymal lymph node single cell suspensions were generated by mechanical dissociation of tissue followed by filtration through a 70 μM cell strainer (BD Biosciences). Suspension of cells in a hypotonic buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.3) for 5 min at room temperature specifically lysed erythrocytes. Live cells were quantified using a hemacytometer, and suspended in 1% BSA/1x PBS (FCS Buffer) at a concentration of $1x10^7$ cells/mL. For cytokine staining, cells

were stimulated in RPMI 1640 media supplemented with 20 ng/mL phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich), 1μg/mL Ionomycin (Sigma-Aldrich), and 5 μg/mL brefeldin A (Biolegend) for 4.5 h at 37^{o}C 5% CO_2. Live/Dead distinction was conducted using LIVE/DEAD® Fixable Yellow Dead Cell Stain Kit (Molecular Probes by Life Technologies) at a concentration of 1x10^{6} cells/mL in 1x PBS. Prior to staining, all cells were blocked with α-CD16/CD32 (Biolegend) for 20 min at 4^{o}C, to prevent non-specific binding of fluorescently labeled antibodies. Immediately following CD16/CD32 blockade, fluorescently labeled antibodies were added, and cells were incubated for 30 min at 4^{o}C. Following staining, cells were fixed in 4% Paraformaldehyde (Electron Microscopy Sciences) overnight. For intracellular staining, cells were washed the following day in 0.2% Saponin/1%BSA/1xPBS (saponin buffer). To permeabilize cell membranes for intracellular staining, cells were suspended in saponin buffer at a concentration of 3.3 x10^{6} cells/mL and incubated at 4^{o}C for 20 minutes. Intracellular staining was conducted following permeabilization in saponin buffer at a concentration of 1x10^{7} cells/mL for 30 min at 4^{o}C. Cells were resuspended in 1x PBS for flow cytometric analysis using an LSRFortessa Cytometer (BD Biosciences) and FlowJo version 7.

A.1.7 Histopathlogical assessment of intestinal damage:

Colon sections were fixed in 10% Zinc Buffered Formalin (NBF) and following overnight fixation, were moved to 70% ethanol for storage. Samples were transferred to the PSU Animal Diagnostic laboratory for paraffin embedding and Hematoxylin/eosin staining. Samples were blindly scored for visual manifestation of intestinal inflammation and mucosal damage by a trained veterinary pathologist. Scores were determined for the following parameters; Inflammatory Cell Infiltrates (ICI-extent, ICI-severity, and Intestinal mucosal damage (crypt loss)(Cooper, Murthy et al. 1993; Erben, Loddenkemper et al. 2014).

A.1.8 Nanostring nCounter hybridization gene expression analysis

C57BL6/J male mice subjected to chemically induced colitis as described above, were euthanized by carbon dioxide asphyxiation and quickly frozen by liquid nitrogen and stored at −80°C. Colonic tissue RNA was extracted as described above and subjected to further purification by an RNA Clean & Concentrator™ -25 kit (Zymo Research, Irvine, CA) according to the manufacturer instructions. The RNA samples were transferred to the Genomics Core Facility (The Pennsylvania State University) for NanoString®- Multiplexed Digital Quantification of Nucleic Acids without Sample Amplification on the nCounter® platform. A total of 33 genes were subject to quantification. Data was assessed according to manufacturer's instructions using the provided nSolver 3.0 software.

A.1.9 Cecal microbiota transfer

C57BL6/J male mice were fed control or broccoli supplemented diet as described above. Animals were euthanized by carbon dioxide asphyxiation and placed into an anaerobic chamber (Coy Lab Products, Grass Lake, MI), Hydrogen~3.3%, and oxygen <15 ppm. Cecal contents were pooled (control n=3, broccoli n=4) and extracted under anaerobic conditions into anaerobic liquid dental medium (Anaerobe Systems, Morgan Hill, CA.) at a ratio of 1 mg cecal contents to 1.8 μL media. Cecal content suspensions were filtered through a 70 micron cell strainer prior to transfer to the Pennsylvania State University germ free facility. Germ-free 129S6/SvEv-*Il10*$^{-/-}$ mice were gavaged with 100 μL of control or broccoli diet derived cecal content suspension, (control n=2 females/n=2 males, broccoli n=2 females/n=3 males). Mice were maintained within the germ free facility for a period of five days post-gavage with *ad libitum* access to sterile standard animal chow and water prior to euthanasia and sample collection.

A.1.10 Indole 3-carbinol feeding study

Conventional and germfree C57BL6/J-*Ahr*$^{b/b}$ were fed were weaned onto a standard animal chow diet. At eight weeks of age all mice were fed AIN-93G purified rodent chow for seven days. This was followed by continuation of the AIN-93G diet in control groups or administration of AIN-93G diet supplemented with indole-3-carbinol at 125 mg/kg for 7 days prior to euthanasia. To investigate the functional interaction between the diet and microbial status, mice were allocated to four groups: Germ free *Ahr* $^{b/b}$ mice, control and I3C diet groups (n=4, n=6 respectively), and Conventional *Ahr*$^{b/b}$ mice, control and I3C diet groups (*n*=5 and n=6 respectively). At the conclusion of the study, mice were euthanized by carbon dioxide asphyxiation, tissues/serum were excised and immediately frozen in liquid nitrogen and stored at −80°C.

A.2 Supplemental Data Tables

Data Table S1. Composition and caloric content of control (AIN-93G) and broccoli diet (15%).

Diet		AIN-93G		Modified AIN-93G+15% Broccoli	
Ingredient	**kcal./gm**	**grams/Kg**	**kcal./kg**	**grams/Kg**	**kcal./kg**
Casein, High Nitrogen	3.58	200	716	144.2	516
L-Cystine	4	3	12	3	12
Sucrose	4	100	400	80.95	324
Cornstarch	3.6	397.486	1430.9496	374.338	1347.6168
Dyetrose	3.8	132	501.6	124	471.2
Soybean Oil	9	70	630	59	531
t-Butylhydroquinone	0	0.014	0	0.012	0
Cellulose	0	50	0	17	0
Mineral Mix #210025	0.88	35	30.8	35	30.8
Vitamin Mix #310025	3.87	10	38.7	10	38.7
Choline Bitartrate	0	2.5	0	2.5	0
Freeze Dried Broccoli	3.37	0	0	150	506
	Total	**1000**	**3760.0496**	**1000**	**3777.3168**

Data Table S2. Primers used for 16S rDNA amplification and qPCR.

Gene	F/R	Sequence 5'--3'
16S rDNA V4-V4	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGCGGTAA
16S rDNA V4-V4	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT
Cyp1a1	F	CTC TTC CCT GGA TGC CTT CAA
Cyp1a1	R	GGA TGT GGC CCT TCT CAA ATG
Cyp1a2	F	GCC CCT GCC CTT CAG TGG TAC AG
Cyp1a2	R	AGG AGT GGA GCC GAT GCG GA
Ccl28	F	TCC GGA AAC ATG ATG AGA CA
Ccl28	R	ACT CAT GGC TGT GGC TGT GT
Il1b	F	AGC TTC CTT GTG CAA GTG TCT
Il1b	R	GAC AGC CCA GGT CAA AGG TT
Il10	F	GGC GCT GTC ATC GAT TTC TC
Il10	R	ATG GCC TTG TAG ACA CCT TGG
Il17a	F	CAG ACT ACC TCA ACC GTT CCA C
Il17a	R	TCC AGC TTT CCC TCC GCA TTG A
Il6	F	ATC CAG TTG CCT TCT TGG GAC TGA
Il6	R	TAA GCC TCC GAC TTG TGA AGT GGT
Lcn2	F	ATT TCC CAG AGT GAA CTG GC
Lcn2	R	AAT GTC ACC TCC ATC CTG GT
Rpl13a	F	TTC GGC TGA AGC CTA CCA GAA AGT
Rpl13a	R	GCA TCT TGG CCT TTT TCC GTT
Reg1	F	TGT TAG GAG ACC CAG TTG CC
Reg1	R	ACT GGA CTC CAT GAT CCC AA
Reg2	F	GTG GCC CAT GAC TTG AAG AG
Reg2	R	GCT TCC AAT GTC TGG ACT GG
Tnfa	F	TGG AGT CAT TGC TCT GTG AAG GGA
Tnfa	R	AGT CCT TGA TGG TGG TGC ATG AGA
Tff2	F	GAG AAA CCT TCC CCC TGT CG
Tff2	R	TTT CGA CTG GCA CAG TCC TC

Data Table S3. Alignment results for individual samples submitted for RNA-Sequencing analyses.

Samples	input	mapped	multiple alignment (of mapped)
purified_1	57613751	24721722	1510084
purified_2	56874356	24881710	1538250
purified_3	62578294	27093228	1547388
broccoli_1	59835295	23504726	1476545
broccoli_2	53386172	20934757	1523480
broccoli_3	59925412	23889540	1665978

Data Table S4. Summary of RNA-Seq results displaying genes that displayed the highest fold increase (red) and decrease (green) of mapped reads.

Gene	Fold-Increase	Gene	Fold-Decrease
Serpini2	68	*Slco1a4*	34
PNLIP	20	*Cpb2*	29
mir-143	20	*Cstad*	27
CPB1	20	*Trappc8*	18
CEL	18	*Igdcc3*	15
Stox2	17	*Spatc1l*	15
Terf2	17	*Cyp4a11*	14
Tbx15	14	*Mbd2*	13
Cela2a	14	*Dmnt1*	13
Dlg3	13	*Ugt1a6*	11

A.3 Supplemental Figures

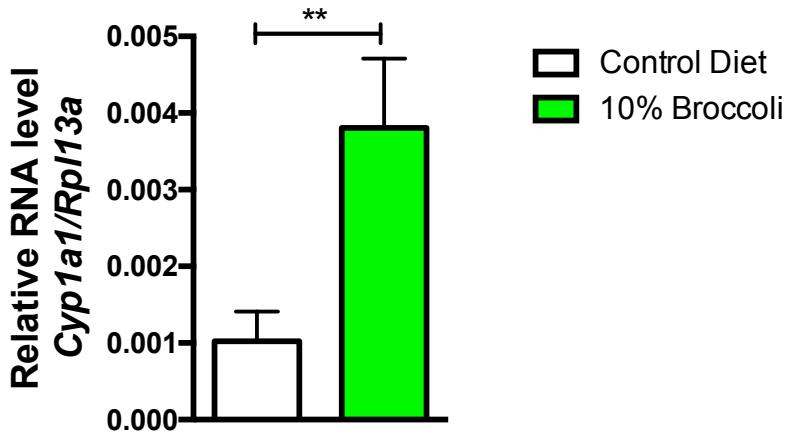

Fig. S1. Duodenal *Cyp1a1* expression normalized to eukaryotic *Rpl13a* from $Ahr^{b/b}$ mice fed for 7 days with ground AIN-93G (control) diet supplemented with 0% or 10% freeze dried broccoli. Data represent the mean gene expression (n=3 per diet group) ± standard error of mean (SEM).

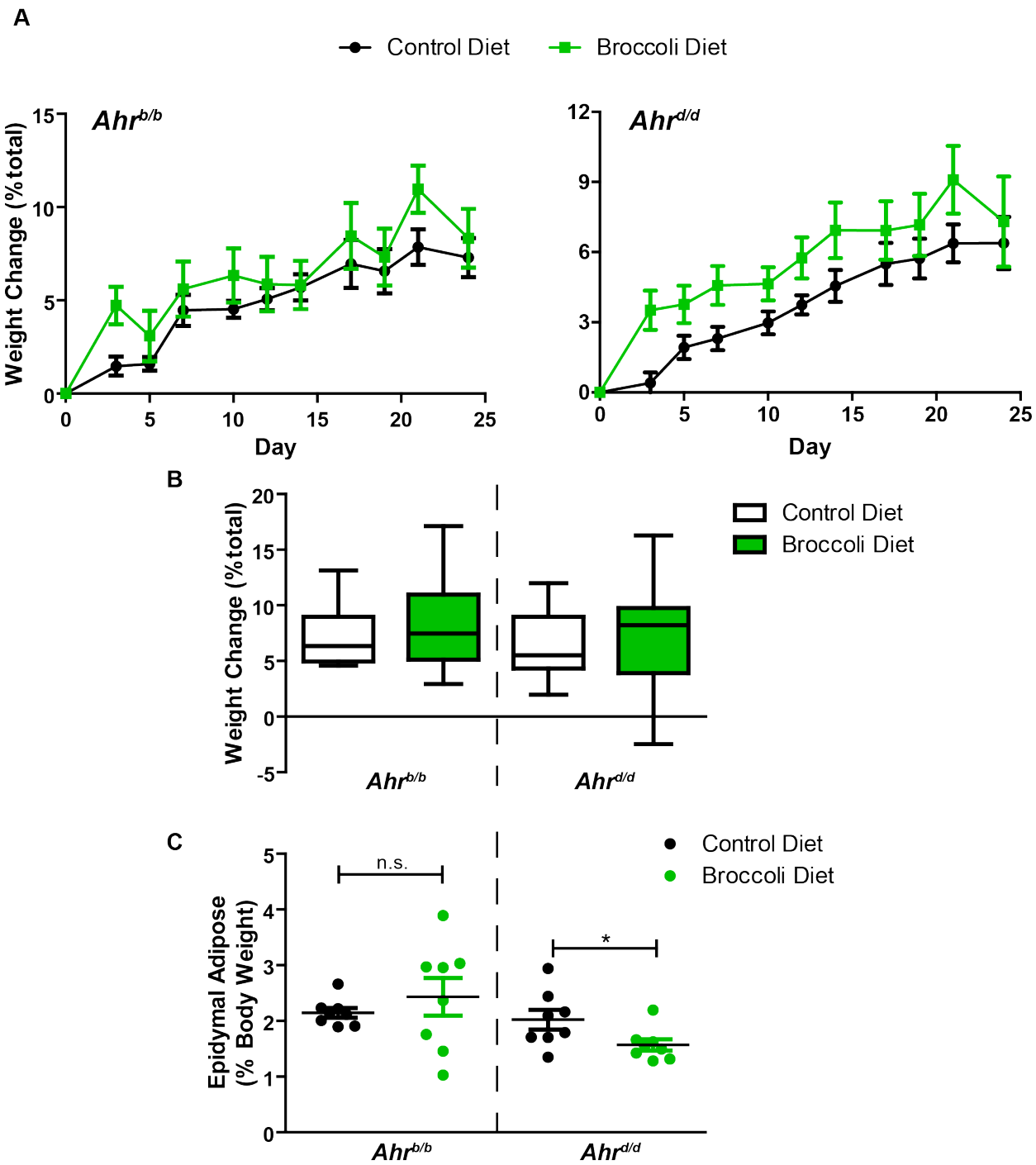

Fig. S2. Impact of 24 day broccoli diet feeding upon weight and epidymal adipose mass in *Ahr*$^{b/b}$ and *Ahr*$^{d/d}$ mice. (A) Time course analysis of percent weight change of mice fed broccoli diet for 24 days. (B) Overall weight change associated with broccoli consumption. (C) Epidymal adipose mass expressed as percent total body weight following 24 day feeding time course. (n=3 per diet group).

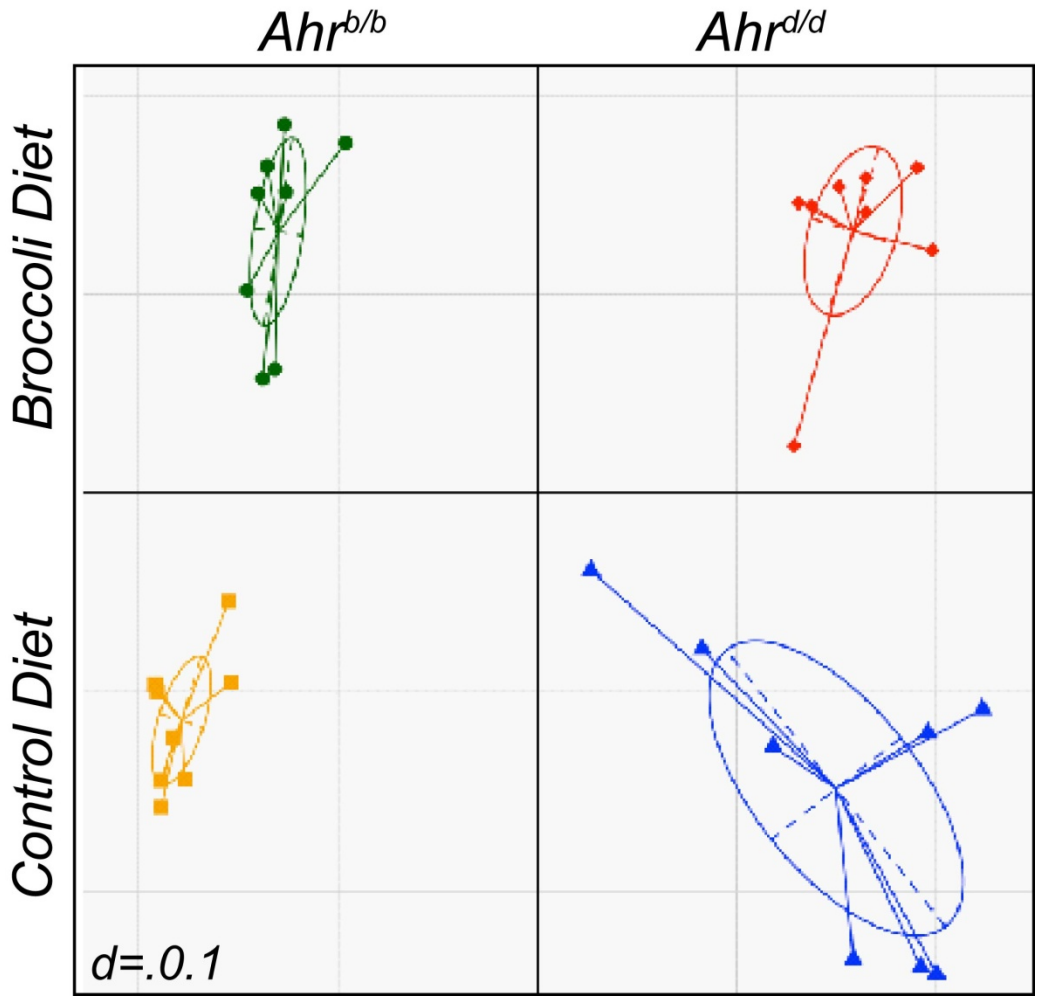

Fig. S3. Broccoli Diet and Ahr status contribute to the establishment of unique microbial community profiles. G-unifrac distance matrix analyses determine likeness of microbial community composition by assessing relative species (16S rDNA) abundance and the presence of rare species.

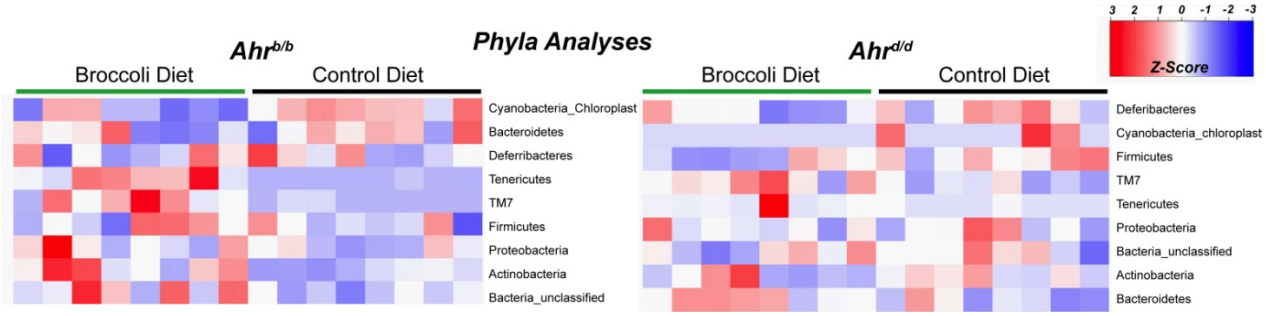

Fig. S4. Analysis of cecal microbial taxonomic phyla composition in $Ahr^{b/b}$ and $Ahr^{d/d}$ mice following consumption of broccoli. Heat map representation of relative individual phyla levels based upon 16S rDNA read abundances. Data indicates increased (red), decreased (blue), or mean (white) relative abundance of associated microbial phyla (n=8 per diet/genotype).

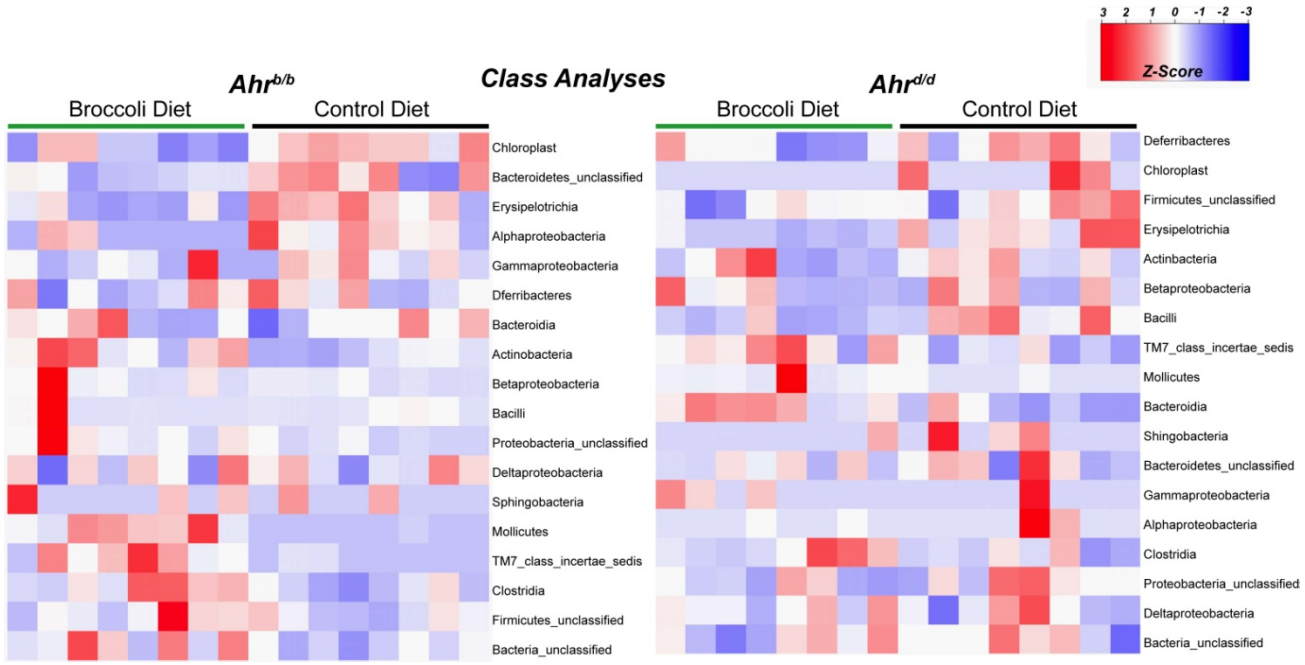

Fig. S5. Analysis of cecal microbial taxonomic class composition in *Ahr*$^{b/b}$ and *Ahr*$^{d/d}$ mice following consumption of broccoli. Heat map representation of relative individual class levels based upon 16S rDNA read abundances. Data indicates increased (red), decreased (blue), or mean (white) relative abundance of associated microbial class (n=8 per diet/genotype).

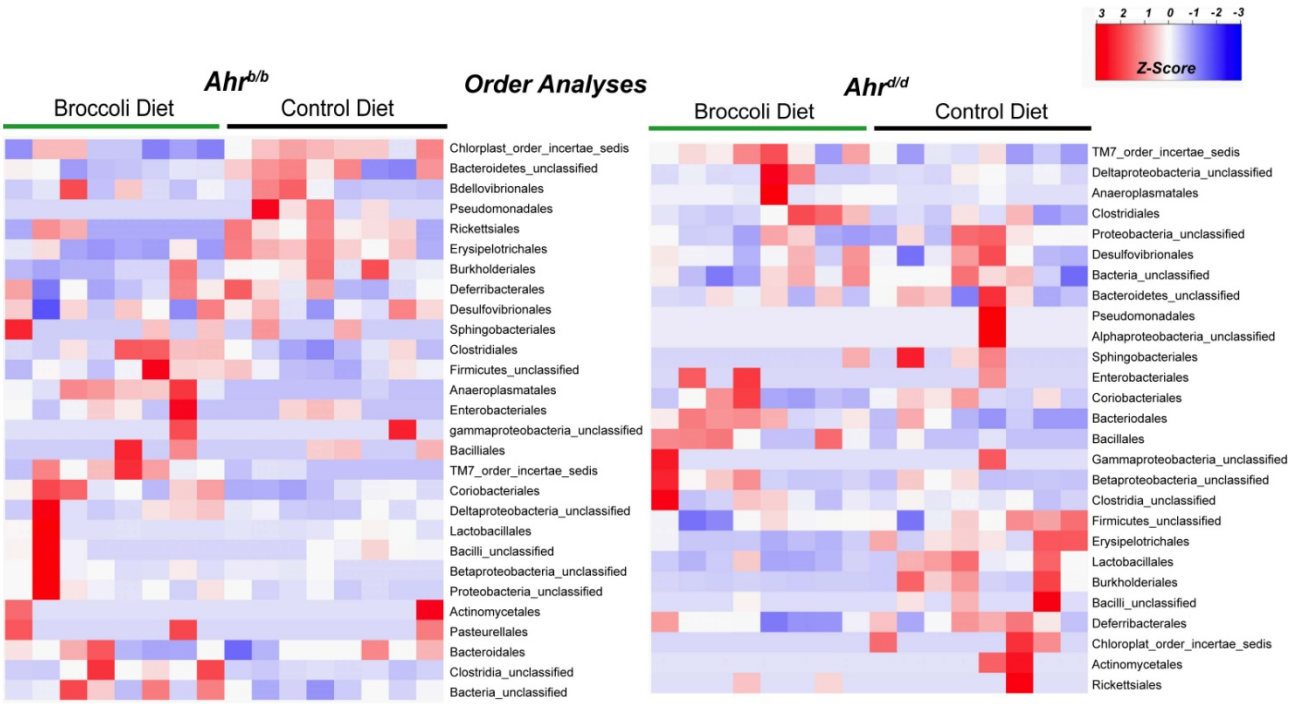

Fig. S6. Analysis of cecal microbial taxonomic order composition in *Ahr*$^{b/b}$ and *Ahr*$^{d/d}$ mice following consumption of broccoli. Heat map representation of relative individual order levels based upon 16S rDNA read abundances. Data indicates increased (red), decreased (blue), or mean (white) relative abundance of associated microbial order (n=8 per diet/genotype).

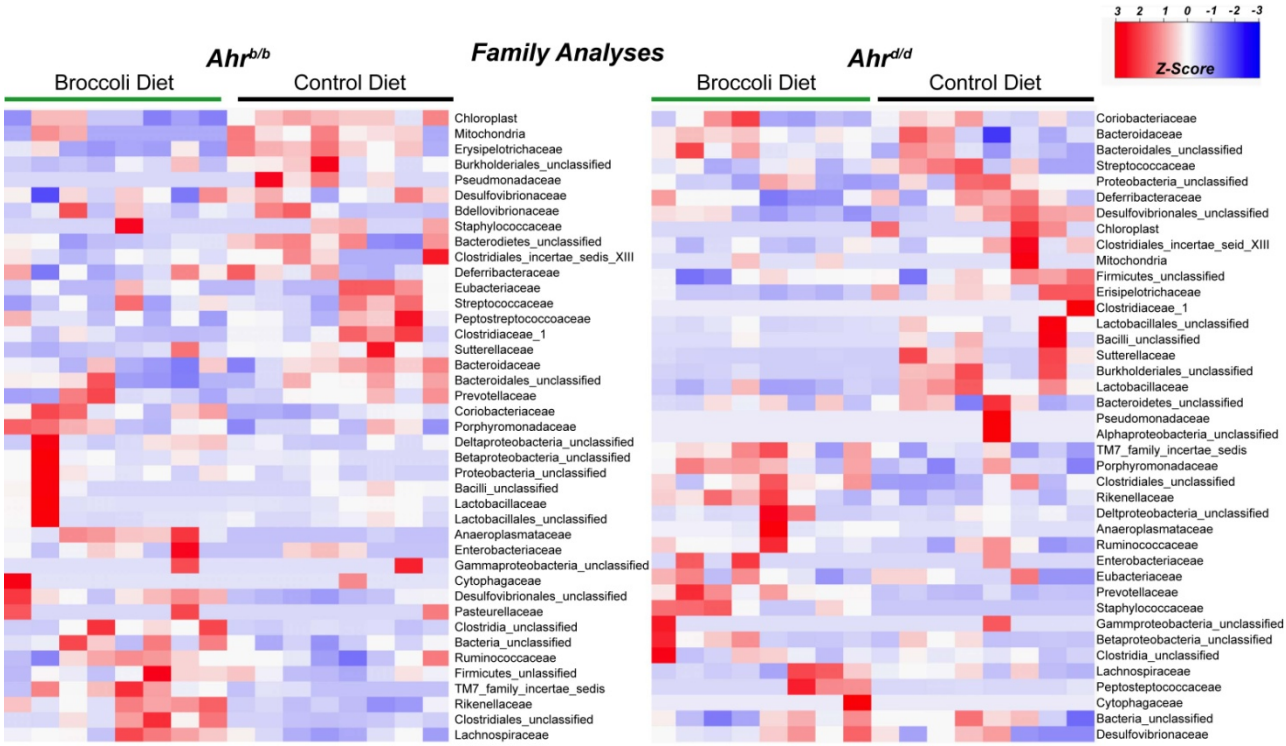

Fig. S7. Analysis of cecal microbial taxonomic family composition in *Ahr*$^{b/b}$ and *Ahr*$^{d/d}$ mice following consumption of broccoli. Heat map representation of relative individual family levels based upon 16S rDNA read abundances. Data indicates increased (red), decreased (blue), or mean (white) relative abundance of associated microbial family (n=8 per diet/genotype).

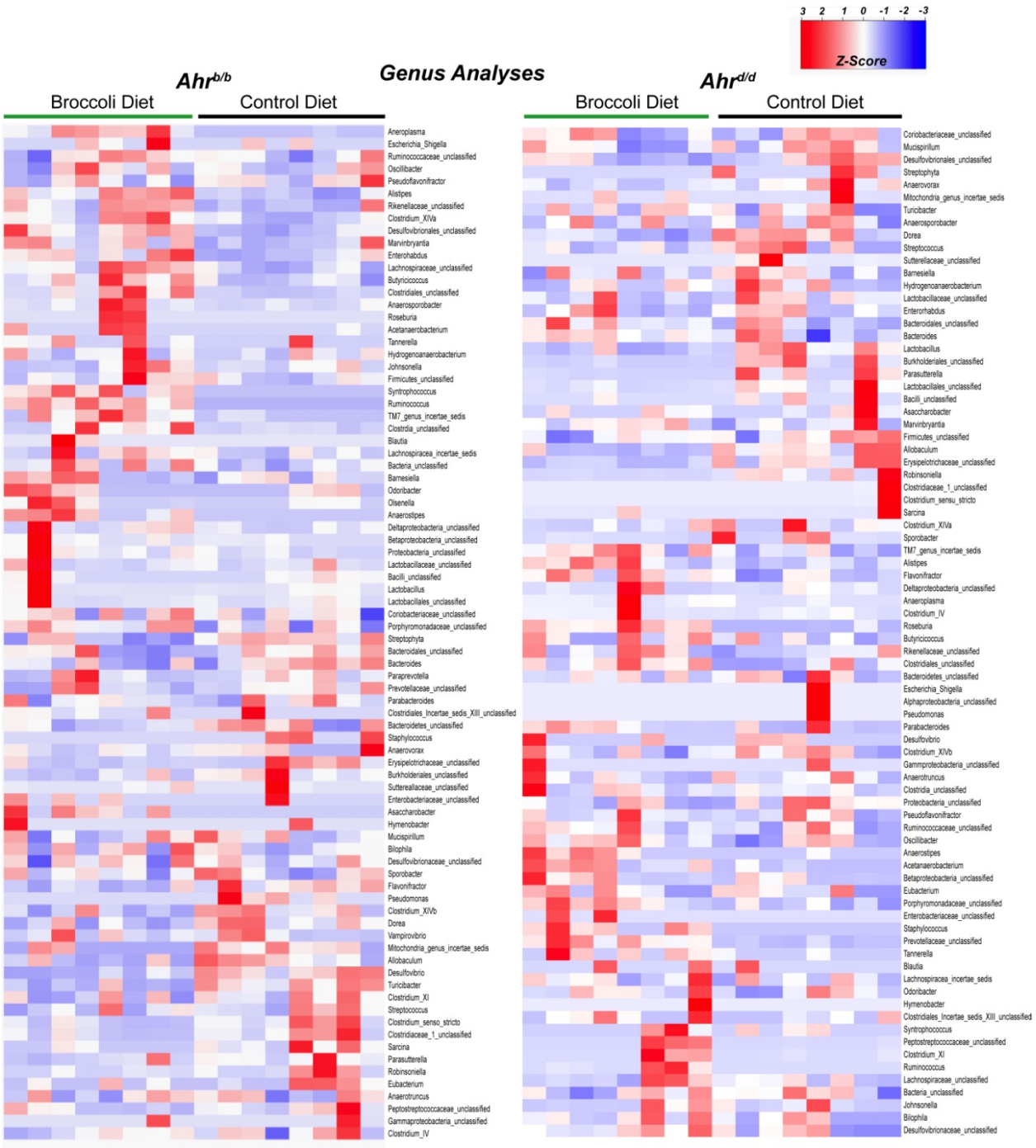

Fig. S8. Analysis of cecal microbial taxonomic genus composition in *Ahr*$^{b/b}$ and *Ahr*$^{d/d}$ mice following consumption of broccoli. Heat map representation of relative individual genus levels based upon 16S rDNA read abundances. Data indicates increased (red), decreased (blue), or mean (white) relative abundance of associated microbial genus (n=8 per diet/genotype).

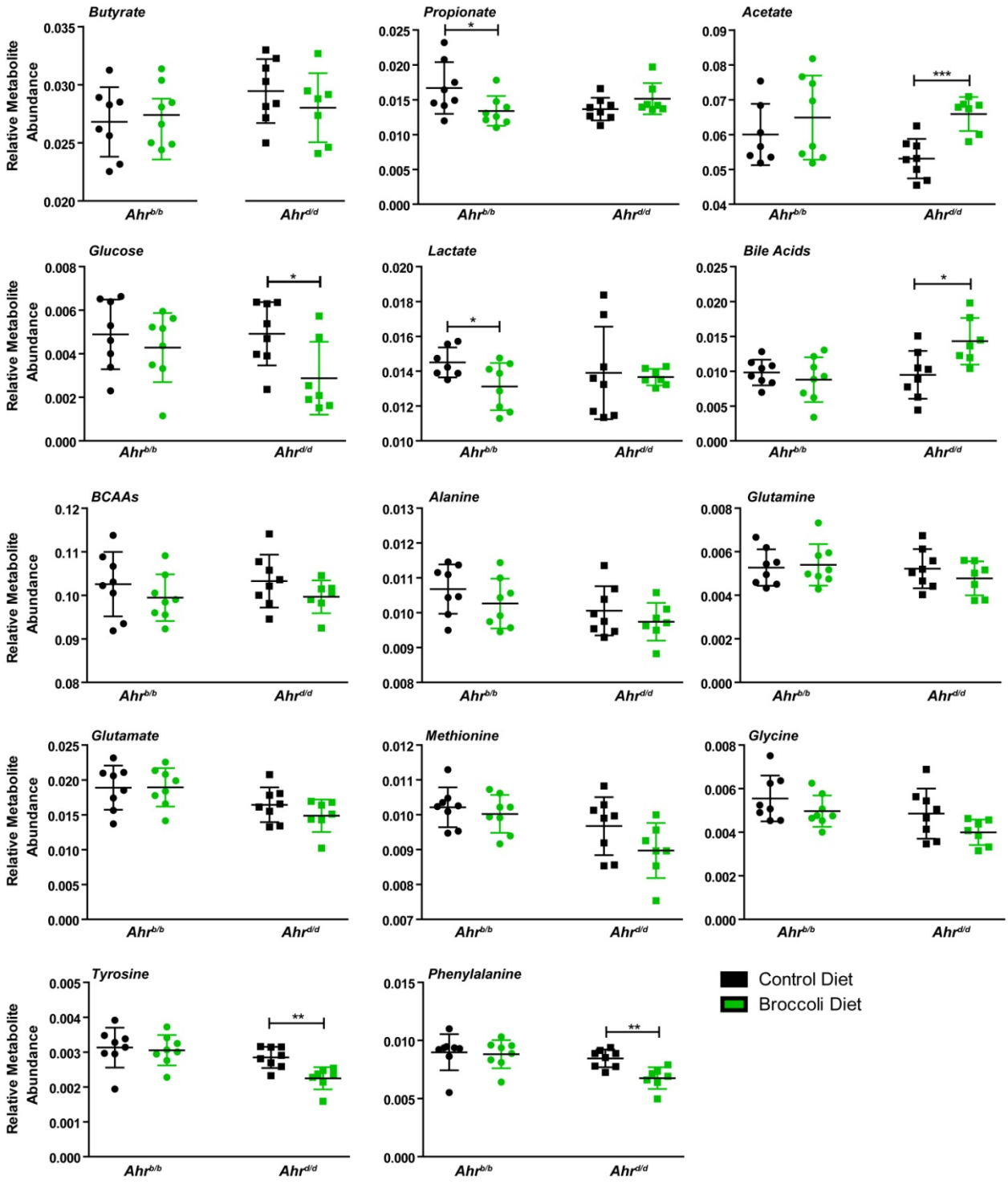

Fig. S9. Dietary broccoli and *Ahr* status influence cecal metabolite levels. Relative quantities of indicated metabolites were determined by ^{1}H-NMR analyses of cecal content extracts from $Ahr^{b/b}$ and $Ahr^{d/d}$ mice following 24 days of broccoli consumption.

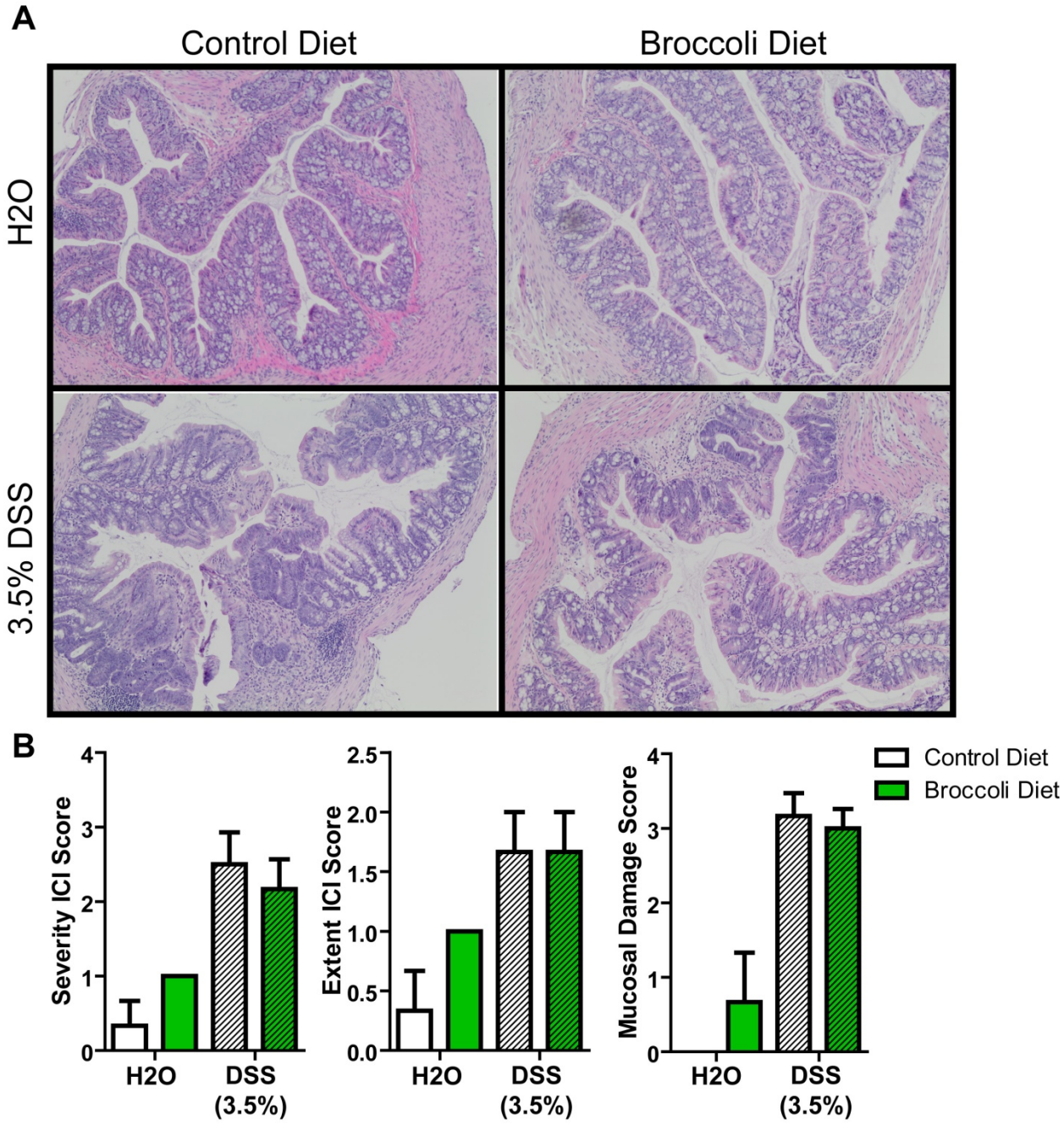

Fig. S10. Broccoli does not decrease incidence of histopathological evidence of intestinal inflammation associated with DSS-colitis. (A) Hematoxylin and Eosin stained colonic sections from *Ahr*$^{b/b}$ mice were visualized at 40x magnification. (B) Histological scoring indicators of intestinal inflammation, such as the severity and extent of inflammatory cell Infiltration (ICI) and mucosal damage. Data are representative of image/scoring from multiple mice. (n=3 per H2O group, n=6 per DSS group).

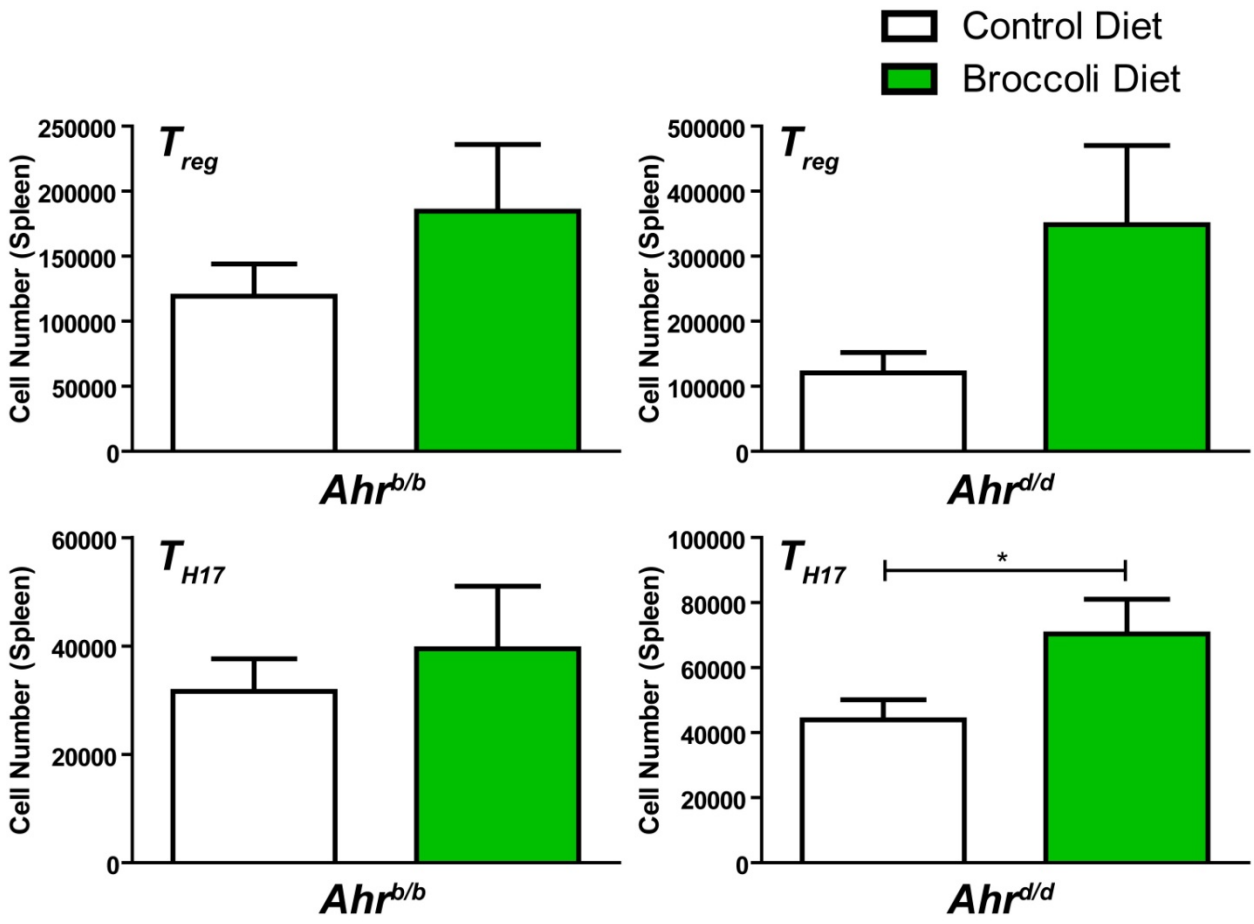

Fig. S11. Flow cytometric analysis of the broccoli associated changes in splenic T_{H17} and T_{reg} populations. Conventional $Ahr^{b/b}$ and $Ahr^{d/d}$ mice were fed the indicated diet for 24 days followed by isolation and flow cytometric analyses of splenic T-cell populations. Data represents the mean cell number (n=8 per diet/genotype group) ± standard error of mean (SEM).

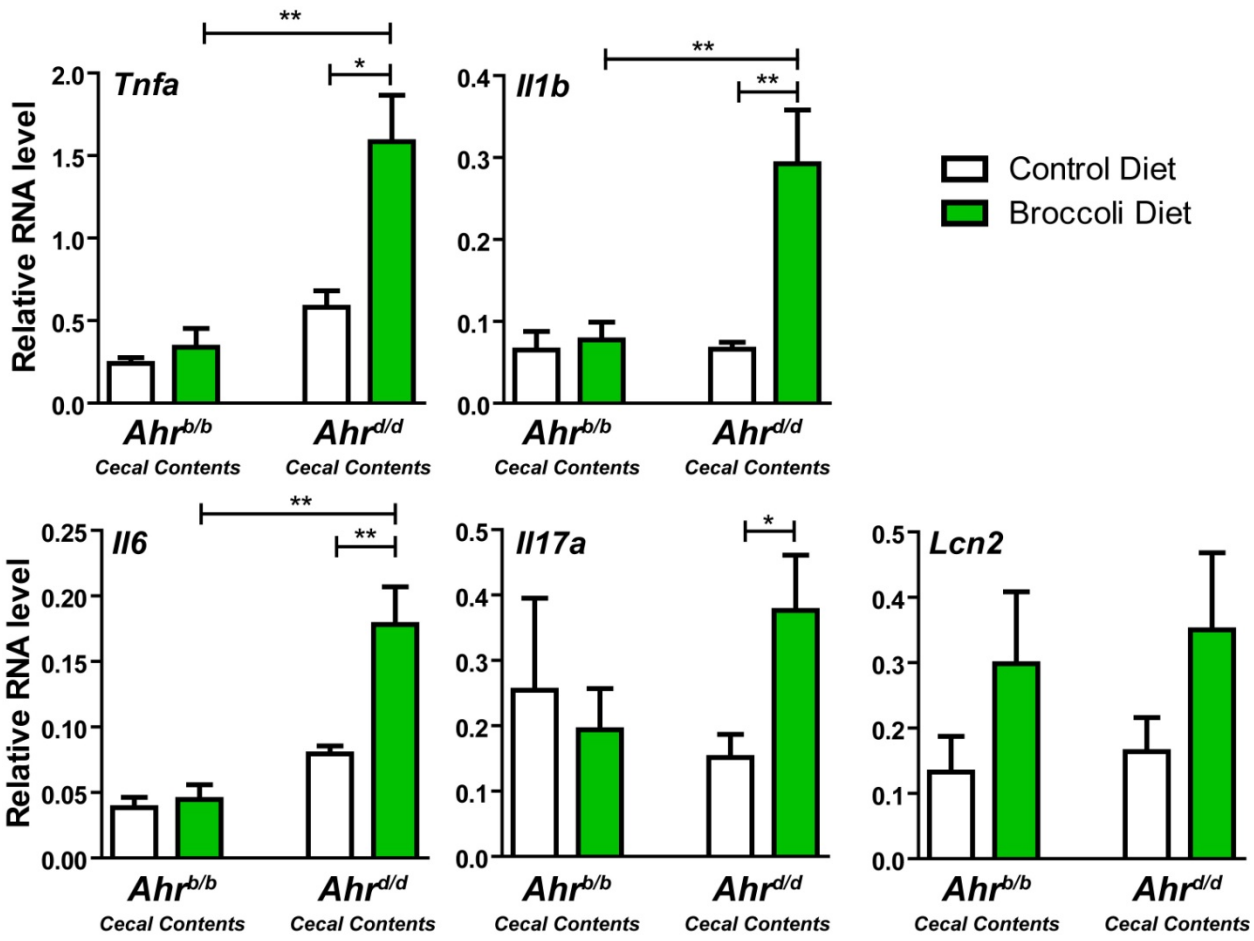

Fig. S12. Broccoli derived microbial communities of *Ahr*$^{d/d}$, but not *Ahr*$^{b/b}$ mice, produce an enhanced intestinal inflammatory tone. Colonic expression of inflammatory cytokines was assessed in 129S6/SvEv-*Il10*$^{-/-}$ mice five days after being orally gavaged with cecal content homogenates derived from *Ahr*$^{b/b}$ or *Ahr*$^{d/d}$ mice fed broccoli for 24 days. Data represent the mean gene expression normalized to eukaryotic *Rpl13a* (n=4-5 per diet/genotype group) ± standard error of mean (SEM).

Supplementary References

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