Electronic Supplementary Material (ESI) for Food & Function.

Supplementary information

Multi-omics analysis detected multiple pathways by which the pomegranate punicalagin exerts its biological effects in modulating host-microbiota interactions in murine colitis models

Fang Liu, Allen D. Smith, Thomas T. Y. Wang, Quynhchi Pham, Haiyan Yang, Robert W. Li

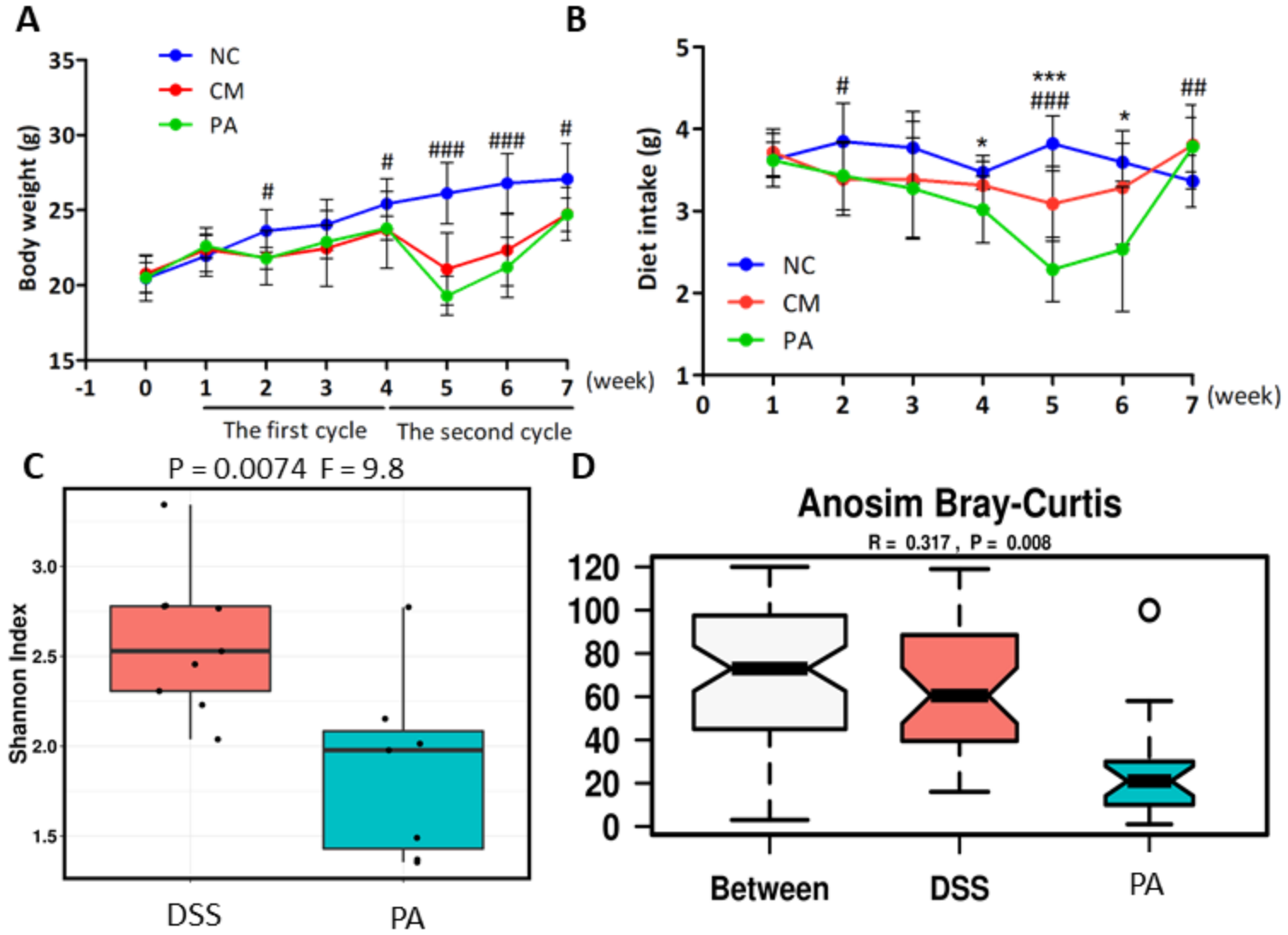

Figure S1. The effect of punicalagin ingestion on physiological and molecular indicators in dextran sodium sulfate (DSS) induced colitis mice. The changes in the bodyweight (A) and feed intake (B) during the experimental duration. NC: Healthy control mice without supplementation. CM: DSS induced colitis mice without supplementation. PA. DSS induced colitis mice with 50-day PA supplementation. NC vs CM: $^{###}P < 0.001$; $^{##}P < 0.01$; CM vs PA: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. (C). The Shannon α diversity index was significantly reduced by PA supplementation in DSS induced colitis mice ($P = 0.0074$). (D). Analysis of similarities (ANOSIM) at the genus level suggests a clear separation in the β diversity in the gut microbial communities with PA or without PA supplementation (DSS; $P = 0.008$).

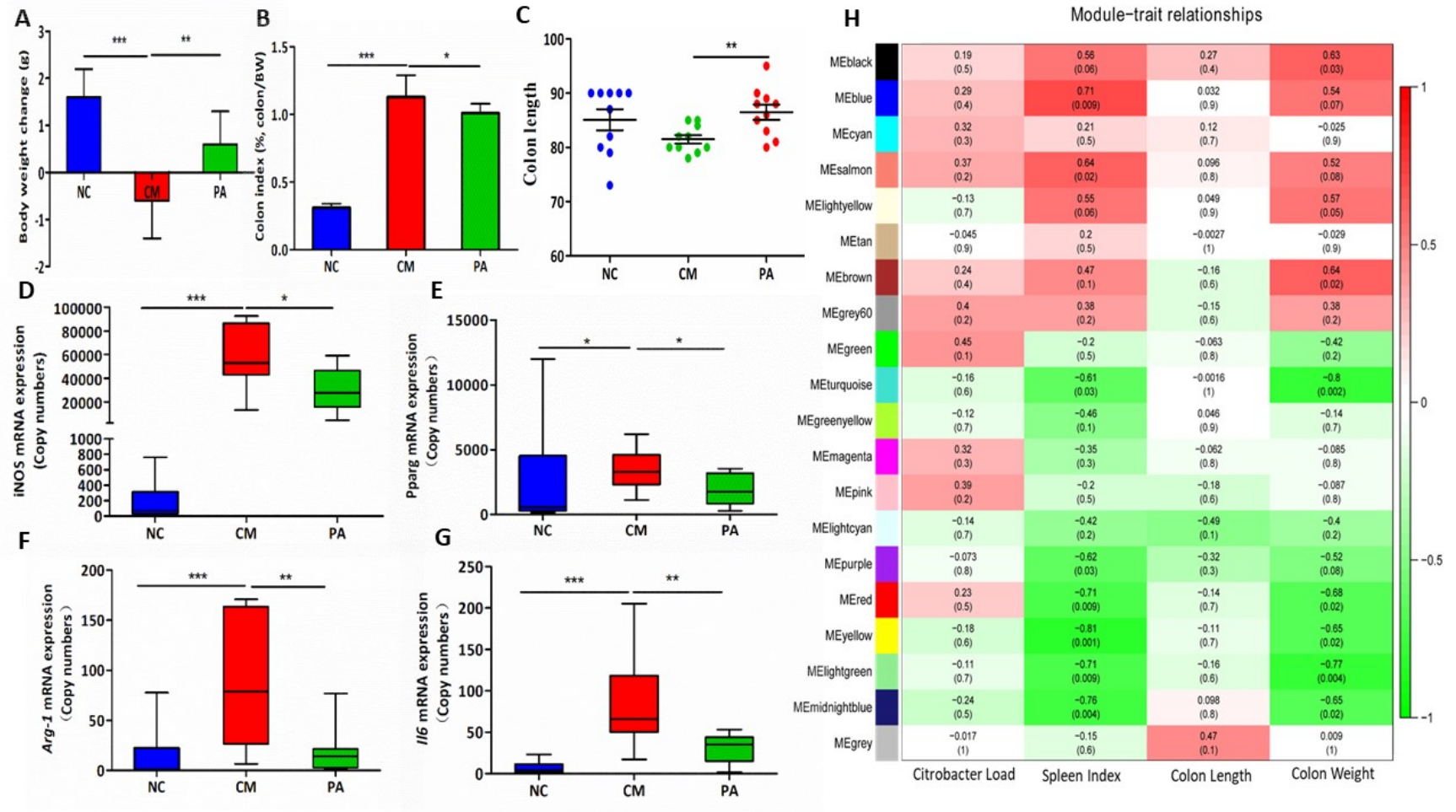

Figure S2. Punicalagin affected the colitis related phenotypes and induced changes in the colon transcriptome of *Citrobacter rodentium* infected mice. (A): Punicalagin (PA) supplementation improved body weight (BW) loss associated with the infection. (B): PA reduced the colon index. (C): Colon length (mm). PA reduced the mRNA expression of select genes in the mouse proximal colon tissue. (D): Nos2 (iNOS). (E): Pparg. (F): Arg1. (G): Il6. (H): The module-trait relationships detected in the network *C. rodentium* induced colitis mice (MD) using WGCNA. The number represents the correlation coefficient (and *P* value in parentheses). NC: Uninfected control mice without supplementation. CM: *C. rodentium* induced colitis mice without supplementation. PA: *C. rodentium* induced colitis mice with 17-day PA supplementation. $N = 10$ per group. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

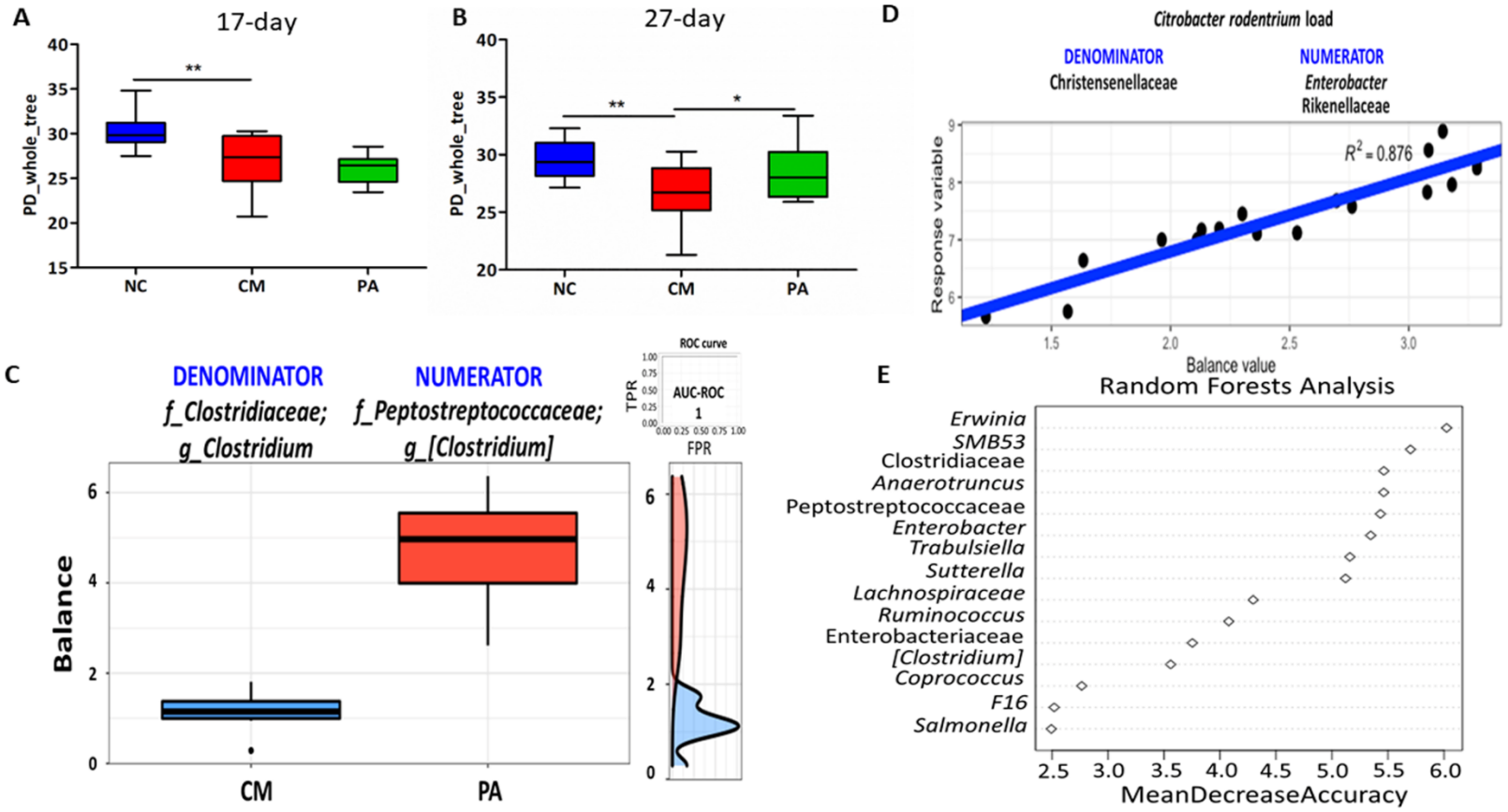

Figure S3. Important microbiome features and microbial signatures for colitis related parameters in the *Citrobacter rodentium* infection mouse model. A longer-term punicalagin (PA) supplementation (27 days) restored Phylogenetic diversity (PD) from the repressed level resulting from the infection. (A): 17-day PA supplementation did not improve PD. (B): 27-day PA supplementation. (C): A molecular signature or balance consisting of four taxa, including an unclassified genus in the family *Peptostreptococccceae* and *[Clostridium]* (Numerator) and an unclassified genus in the family *Clostridiaceae* and *Clostridium* (Denominator), had a high predictive accuracy in distinguishing the gut microbial communities with and without PA supplementation. (D): Microbial signatures or balances with high predictive accuracy for the *C. rodentium* bacterial load ($R^2 = 0.876$). (E): The Random Forests algorithm identified the 15 most important taxa that distinguished the *C. rodentium* infection status based on the scaled mean decrease accuracy. NC: Uninfected control mice without supplementation. CM: *C. rodentium* induced colitis mice without supplementation. PA: *C. rodentium* induced colitis mice with PA supplementation. ** $P < 0.01$; * $P < 0.05$.

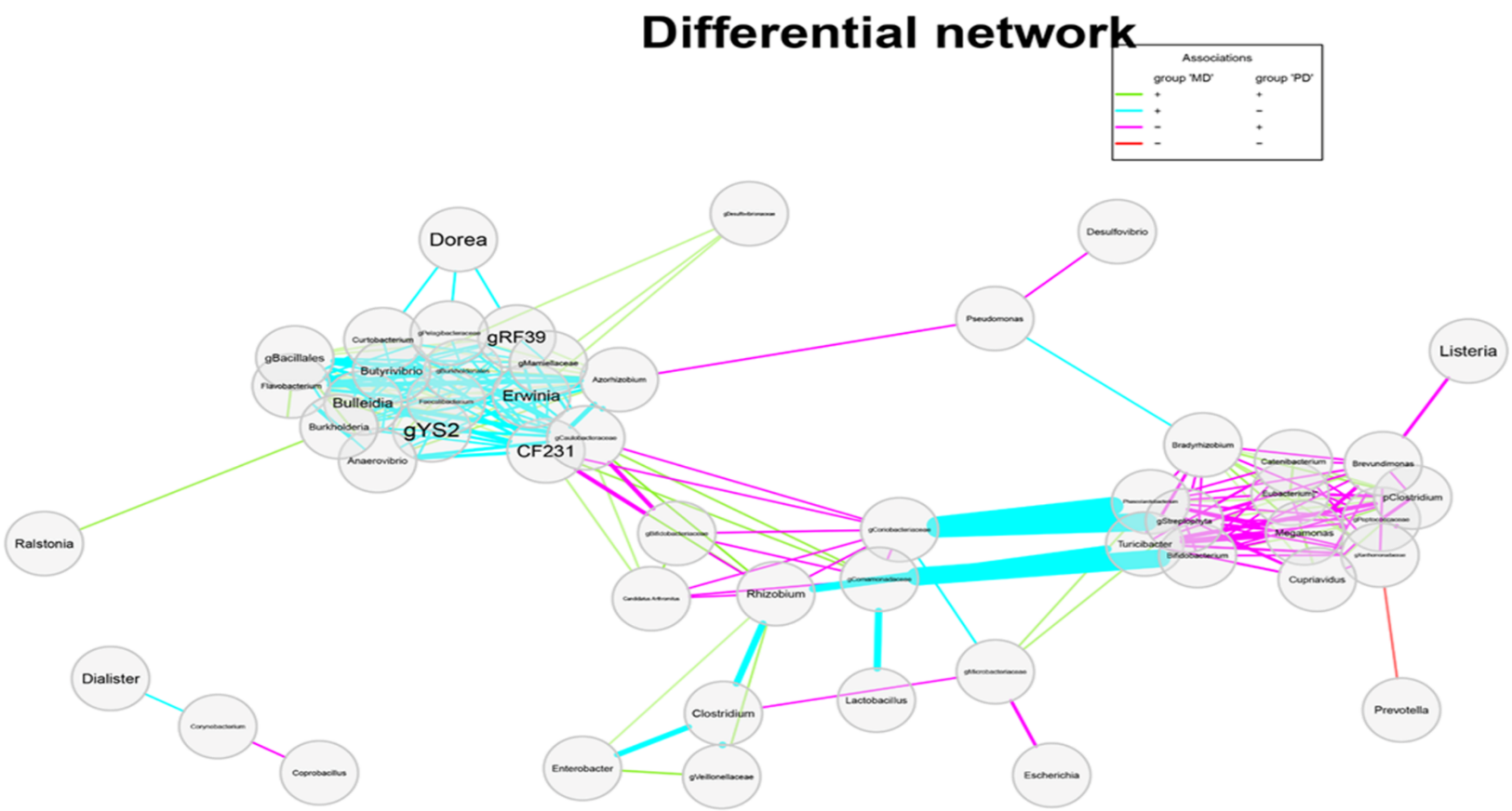

Figure S4. Differential networks constructed and compared using the NetCoMi algorithm. The color represents the differential association in the networks with and without 27-day punicalagin (PA) supplementation. MD: *Citrobacter rodentium* infected mice without PA supplementation. PA: *C. rodentium* infected mice without PA supplementation.

Table S1: The dietary formulations used in the study.

Formulation	**D10012M (AIN-93M)**		**D10012X (AIN-93X) (PA)**	
	gm%	***kcal%***	**gm%**	***kcal%***
Protein	14	*15*	14	*15*
Carbohydrate	73	*76*	73	*76*
Fat	4	9	4	9
Total		*100*		*100*
kcal/gm	3.85		3.85	
Ingredient	**gm**	***kcal***	**gm**	***kcal***
Casein	140	*560*	140	*560*
L-Cystine	1.8	*7*	1.8	*7*
Corn Starch	495.692	*1983*	495.692	*1983*
Maltodextrin 10	125	*500*	125	*500*
Sucrose	100	*400*	100	*400*
Cellulose, BW200	50	*0*	49.758	*0*
Soybean Oil	40	*360*	40	*360*
Palm Oil	0	*0*	0	*0*
Safflower Oil, High Oleic	0	*0*	0	*0*
Safflower Oil	0	*0*	0	*0*
t-Butylhydroquinone	0.008	*0*	0.008	*0*
Mineral Mix S10022M	35	*0*	35	*0*
Vitamin Mix V10037	10	*40*	10	*40*
Choline Bitartrate	2.5	*0*	2.5	*0*
Punicalagin (PA)	0	*0*	**0.242**	*0*
FD&C Yellow Dye #5	0	*0*	0	*0*
FD&C Red Dye #40	0	*0*	0	*0*
FD&C Blue Dye #1	0	*0*	0	*0*
Total	**1000.00**	***3850***	**1000.00**	***3850***
Test compounds:				
Punicalagin (µg/gm diet)	0		**242**	

Table S2. The primers used for real-time PCR in the study.

Symbol	GeneID	Forward primer	Reverse Primer	Species
Il10	NM_010548.2	GGTTGCCAAGCCTTATCGGA	ACCTGCTCCACTGCCTTGCT	*Mus musculus*
Il13	NM_008355.3	AGACCAGACTCCCCTGTGCA	TGGGTCCTGTAGATGGCATTG	*Mus musculus*
Il17a	NM_010552.3	ATCCCTCAAAGCTCAGCGTGTC	GGGTCTTCATTGCGGTGGAGAG	*Mus musculus*
Il1b	NM_008361.4	CCAGCTTCAAATCTCACAGCAG	CTTCTTTGGGTATTGCTTGGGATC	*Mus musculus*
Il4	NM_021283.2	ACAGGAGAAGGGACGCCAT	GAAGCCCTACAGACGAGCTCA	*Mus musculus*
Il5	NM_010558.1	AGCACAGTGGTGAAAGAGACCTT	TCCAATGCATAGCTGGTGATTT	*Mus musculus*
Il6	NM_031168.2	TCCAGTTGCCTTCTTGGGAC	GTACTCCAGAAGACCAGAGG	*Mus musculus*
Nod2	NM_145857.2	CCGCTTTCTACTTGGCTGTC	GTGATTTGCAGGTTGTGTGG	*Mus musculus*
Nos2	NM_010927.4	CGAAACGCTTCACTTCCAA	TGAGCCTATATTGCTGTGGCT	*Mus musculus*
Tlr4	NM_021297.3	GCCTTTCAGGGAATTAAGCTCC	AGATCAACCGATGGACGTGTAA	*Mus musculus*
Tlr9	NM_031178.2	ACTCCGACTTCGTCCACCT	GGCTCAATGGTCATGTGGCA	*Mus musculus*
Tnf	NM_013693.3	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG	*Mus musculus*

Table S3. Taxa significant impacted by PA supplementation in the *Citrobacter rodentium* induced colitis model. The significant taxa were identified using LEfSe and independently validated using ALDEx2 and ANCOM algorithms. The number under Relative abundance represented Mean ± SD. Both linear discriminant analysis scores (LDA) and *P* values were calculated using LEfSe. NC: Uninfected control mice without PA supplementation; CM: *Citrobacter rodentium* infected colitis mice without PA supplementation. PA: *Citrobacter rodentium* infected colitis mice with PA supplementation. The data were derived from the ASV table generated using QIIME2. The genus *Akkermansia* is the sole representative of the phylum *Verrucomicrobia*.

Taxon	**Rank**	**LDA**	***P* value**	**Relative abundance**			**Validated**
				NC	CM	PA	
TM7	Phylum	3.51	0.0104	0.0280±0.0234	0.0007±0.0022	0.0081±0.0098	
Verrucomicrobia	Phylum	3.51	0.0469	0.2379±0.3639	0.7718±1.4781	1.2481±0.9326	ALDEx2
Akkermansia	Genus	3.43	0.0469	0.2379±0.3639	0.7718±1.4781	1.2481±0.9326	ALDEx2 & ANCOM
Clostridium	Genus	2.69	0.0042	0.0001±0.0002	0.0735±0.1207	0.0001±0.0002	ALDEx2 & ANCOM
Coprococcus	Genus	3.17	0.0193	0.8043±0.6661	0.1667±0.1145	0.4562±0.2985	ALDEx2 & ANCOM
Erwinia	Genus	2.97	0.0054	0.0000±0.0000	0.0020±0.0017	0.0095±0.0072	ANCOM
{Eubacterium} [F16]	Genus	3.57	0.0042	0.0005±0.0014	0.6842±1.6496	0.0001±0.0002	ALDEx2
	Genus	3.50	0.0104	0.0464±0.0356	0.0015±0.0027	0.0000±0.0001	ANCOM