

1 **Expression of the aryl hydrocarbon receptor contributes to the establishment of**
2 **intestinal microbial community structure in mice.**

3 Iain A. Murray¹, Robert G. Nichols¹, Limin Zhang^{1,2}, Andrew D. Patterson¹ and Gary H.
4 Perdew^{1*}.

5

6 ¹Center for Molecular Toxicology & Carcinogenesis Department of Veterinary and
7 Biomedical Sciences, The Pennsylvania State University, University Park, PA-16802,
8 USA

9 ²Huck Institutes of the Life Sciences. The Pennsylvania State University, University
10 Park, PA-16802, USA.

11

12

13 **Supplementary information**

14 **Quantitative PCR oligonucleotide sequences**

Target	Forward (5'-3')	Reverse (5'-3')
<i>Saa1</i>	acaccaggatgaagctactacca	cccttggaagcctcgtgaacaaa
<i>Saa2</i>	agctggctggaaagatggagacaa	tgtcctctgccgaagaattcctga
<i>Saa3</i>	atgccagagaggctgttcagaagt	tatcttttaggcaggccagcaggt
<i>Cd14</i>	ttcagaatctaccgacctggagc	caattgaaagcgtggaccaa
<i>Il17a</i>	cagactacctcaaccgttcac	tccagtttccctccgcattga
<i>Lcn2</i>	atttccagagtgaactggc	aatgtcacctccatcctggt
<i>Rpl13a</i>	ttcggtgaagcctaccagaaagt	gcatcttggccttttccgtt
<i>16S Eubacteria</i>	actcctacgggaggcagcag	attaccgcggtgctgg
<i>16S Firmicutes</i>	gcagtagggaatcttccg	attaccgcggtgctgg
<i>16S Bacteroidetes</i>	gtactgagacacggacca	attaccgcggtgctgg
<i>16S SFB</i>	gacgctgcggcatgagagcat	gacggcacggattgtattca
<i>16S A. muciniphila</i>	cagcacgtgaaggtggggac	ccttgcggttggcttcagat

15

16 **Supplemental figures**

17 **Supplemental figure 1**

18 **Supplemental figure 1. Experimental schematic.** Illustration highlighting the overall
19 methodologies utilized in this study.

20

21 **Supplemental figure 2**

22 **Supplemental figure 2. Genotypic segregation of *Ahr*^{-/+} and *Ahr*^{-/-} littermates alters**
23 **the community structure of the cecal microbiota.** Abundance of individual microbiota
24 phyla within the cecal luminal contents of genotypically segregated littermates (blue *Ahr*^{-/+}
25 ^{+/+}, red *Ahr*^{-/-}) as determined through 16S rDNA gene illumina MiSeq sequencing. Data
26 represent min-max percentage abundance with median indicated by a horizontal bar ($n =$
27 $4/\text{genotype}$; $p < 0.05^*$, $p < 0.01^{**}$ Student's *t*-test).

28

29 **Supplemental figure 3**

30 **Supplemental figure 3. Quantitative analysis of cecal bacterial burden and phyla**
31 **abundance in genotypically segregated *Ahr*^{-/+} and *Ahr*^{-/-} littermates.** (a) Real time
32 PCR quantification of bacterial burden and relative phyla abundance normalized to
33 eubacteria using phyla-specific primers upon cecal microbial DNA from *Ahr*^{-/+} (blue) and
34 *Ahr*^{-/-} (red) mice. (b) Real time PCR quantification of bacterial burden and relative phyla
35 abundance normalized to eukaryotic *Rpl13a* using phyla-specific primers upon cDNA
36 generated from total RNA isolated from intact cecal tissue (ceca and luminal contents)
37 from *Ahr*^{-/+} (blue) and *Ahr*^{-/-} (red) mice. Data represent mean ($n = 4/\text{genotype}$) 16S

38 rDNA or rRNA gene abundance \pm SD. Analyses were performed using Student's *t*-test
39 $p < 0.05^*$, $p < 0.01^{**}$.

40

41 **Supplemental figure 4**

42 **Supplemental figure 4. Analysis of correlative taxa-level interactions between cecal**

43 **microbiota in genotypically segregated *Ahr*^{-/+} and *Ahr*^{-/-} mice.** Heat map

44 representations of correlative taxa-level interactions of cecal microbiota from

45 genotypically segregated *Ahr*^{-/+} and *Ahr*^{-/-} littermates. Data represent Pearson

46 correlation (*r*) coefficients with positive to negative correlation indicated by red to blue,

47 respectively ($n = 4/\text{genotype}$). Correlation coefficients and *p* values are detailed in

48 Supplementary file 1.

49

50 **Supplemental file 1**

51 Excel worksheets containing Pearson correlation coefficients and associated *p* values for

52 taxa-taxa correlations. *Ahr*^{-/+} genotype in column A (blue), *Ahr*^{-/-} genotype in column A

53 (red). Positive correlations are colored red, negative correlations are colored blue and

54 those correlations with a $p < 0.05$ are presented in red text.

55

56 **Supplemental file 2**

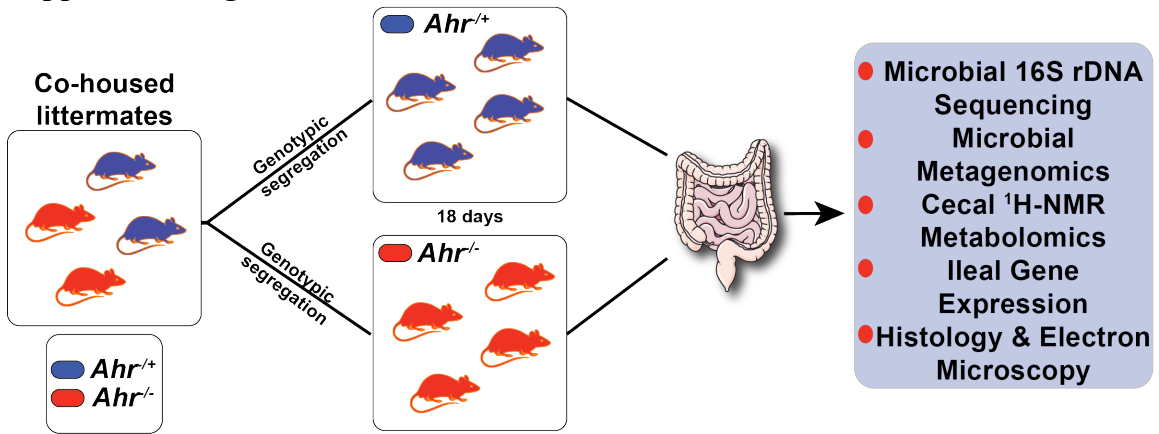
57 Excel worksheets containing Pearson correlation coefficients and associated *p* values for

58 taxa-cecal metabolite correlations. *Ahr*^{-/+} genotype in column A (blue), *Ahr*^{-/-} genotype

59 in column A (red). Positive correlations are colored red, negative correlations are colored

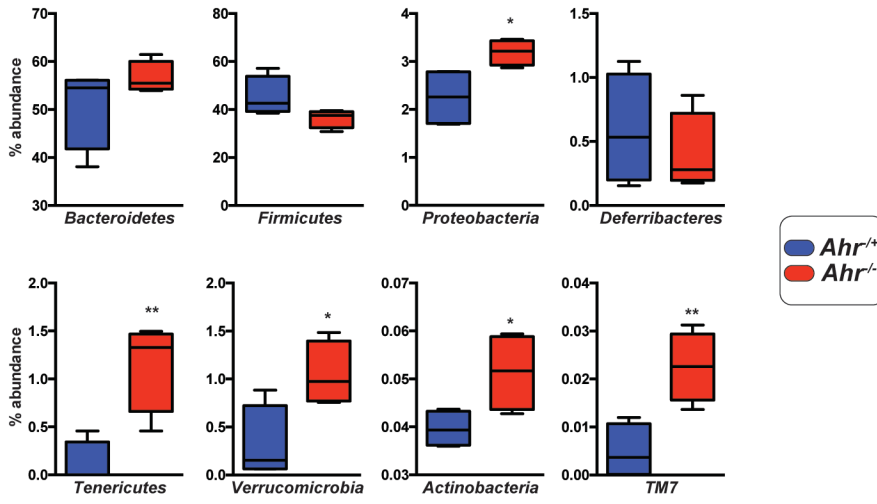
60 blue and those correlations with a $p < 0.05$ are presented in red text.

61 Supplemental figure 1



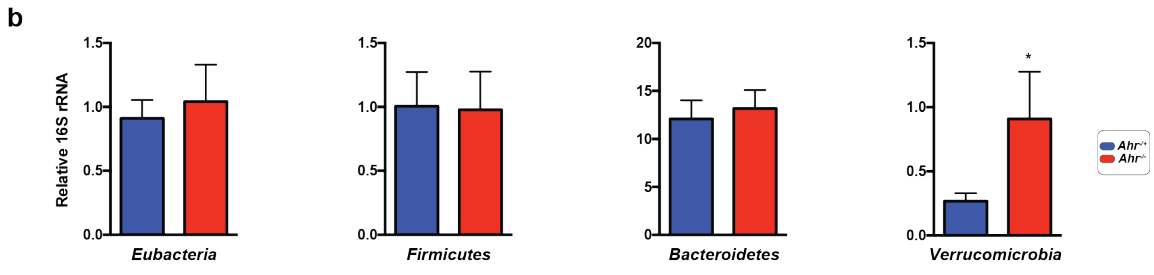
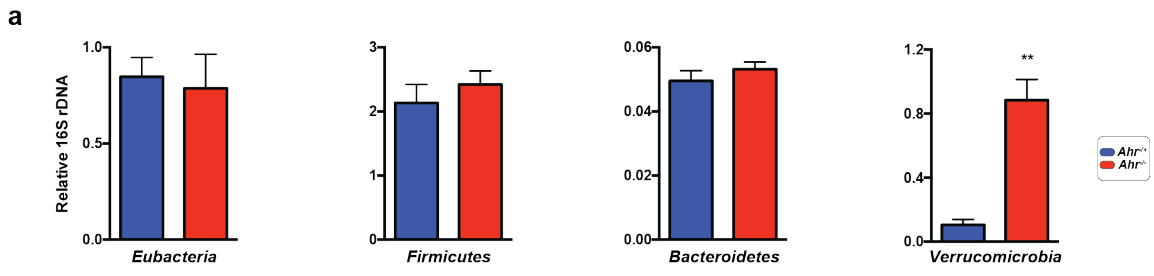
62

63 Supplemental figure 2



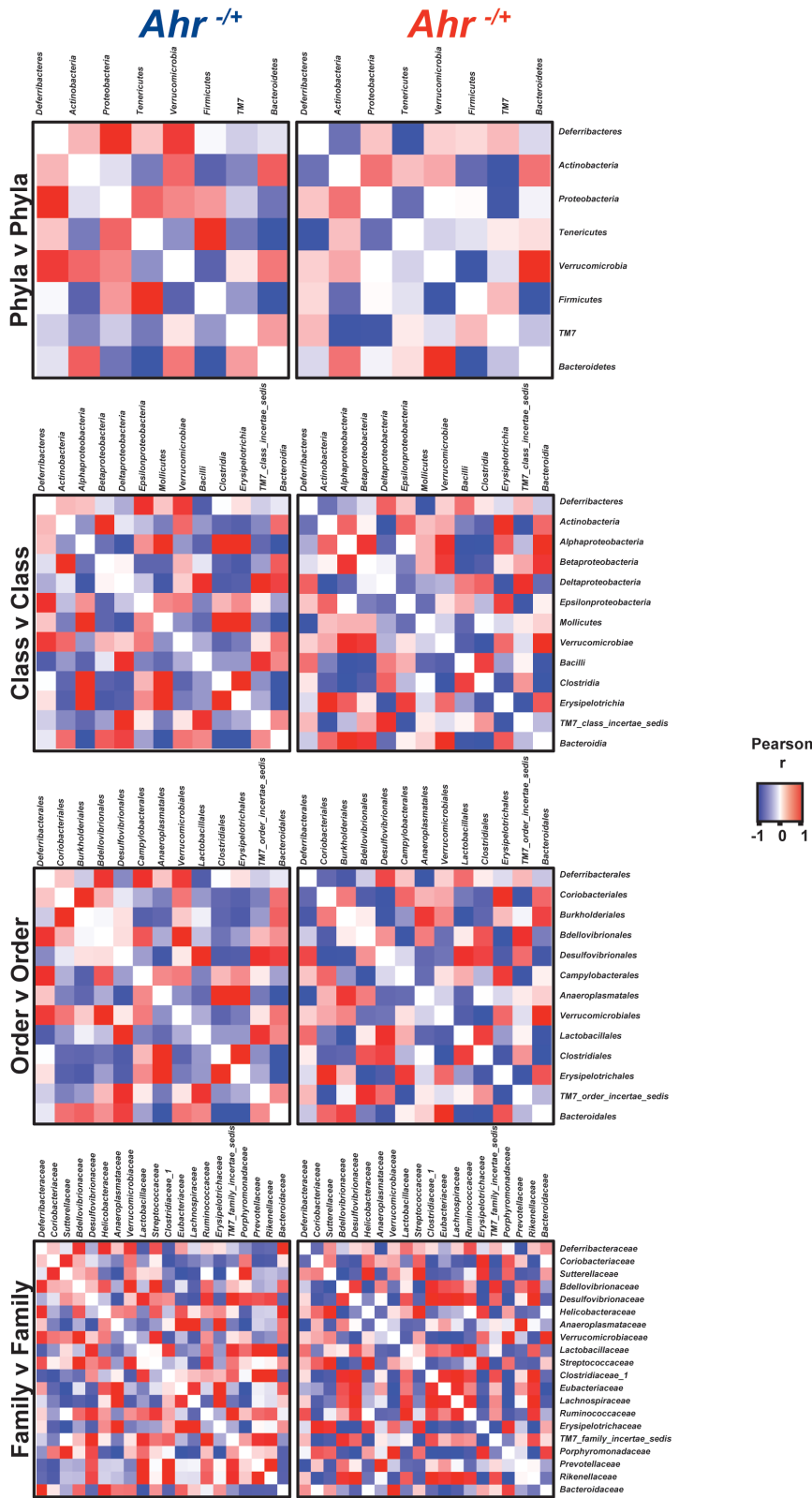
64
65

66 Supplemental figure 3



67

68



Ahr -/+								
Phyla v Phyla Pearson correlation r								
	"Deferribacteres"	"Actinobacteria"	"Proteobacteria"	"Tenericutes"	"Verrucomicrobia"	Firmicutes	TM7	"Bacteroidetes"
"Deferribacteres"		0.2787	0.8973	0.2273	0.7911	-0.02782	-0.196	-0.1136
"Actinobacteria"	0.2787		-0.1212	-0.4919	0.588	-0.6386	-0.453	0.6281
"Proteobacteria"	0.8973	-0.1212		0.5945	0.4601	0.3896	-0.205	-0.5206
"Tenericutes"	0.2273	-0.4919	0.5945		-0.4154	0.9639	-0.502	-0.9854
"Verrucomicrobia"	0.7911	0.588	0.4601	-0.4154		-0.6329	0.1103	0.5155
Firmicutes	-0.02782	-0.6386	0.3896	0.9639	-0.6329		-0.392	-0.9889
TM7	-0.1962	-0.4526	-0.2053	-0.5015	0.1103	-0.392		0.3755
"Bacteroidetes"	-0.1136	0.6281	-0.5206	-0.9854	0.5155	-0.9889	0.3755	

Ahr -/+								
Phyla v Phyla Pearson correlation p								
	"Deferribacteres"	"Actinobacteria"	"Proteobacteria"	"Tenericutes"	"Verrucomicrobia"	Firmicutes	TM7	"Bacteroidetes"
"Deferribacteres"		0.7213	0.1027	0.7727	0.2089	0.9722	0.8038	0.8864
"Actinobacteria"	0.7213		0.8788	0.5081	0.412	0.3614	0.5474	0.3719
"Proteobacteria"	0.1027	0.8788		0.4055	0.5399	0.6104	0.7947	0.4794
"Tenericutes"	0.7727	0.5081	0.4055		0.5846	0.0361	0.4985	0.0146
"Verrucomicrobia"	0.2089	0.412	0.5399	0.5846		0.3671	0.8897	0.4845
Firmicutes	0.9722	0.3614	0.6104	0.0361	0.3671		0.608	0.0111
TM7	0.8038	0.5474	0.7947	0.4985	0.8897	0.608		0.6245
"Bacteroidetes"	0.8864	0.3719	0.4794	0.0146	0.4845	0.0111	0.6245	

Ahr -/-								
Phyla v Phyla Pearson correlation r								
	"Deferribacteres"	"Actinobacteria"	"Proteobacteria"	"Tenericutes"	"Verrucomicrobia"	Firmicutes	TM7	"Bacteroidetes"
"Deferribacteres"		-0.5413	0.2258	-0.9361	0.1845	0.1543	0.2668	-0.1568
"Actinobacteria"	-0.5413		0.5513	0.2545	0.3398	-0.5689	-0.897	0.5442
"Proteobacteria"	0.2258	0.5513		-0.5533	-0.000918	0.01703	-0.855	-0.05405
"Tenericutes"	-0.9361	0.2545	-0.5533		-0.1768	-0.1185	0.0843	0.134
"Verrucomicrobia"	0.1845	0.3398	-0.000918	-0.1768		-0.9407	-0.121	0.9414
Firmicutes	0.1543	-0.5689	0.01703	-0.1185	-0.9407		0.2711	-0.9993
TM7	0.2668	-0.8969	-0.8549	0.08428	-0.1212	0.2711		-0.2365
"Bacteroidetes"	-0.1568	0.5442	-0.05405	0.134	0.9414	-0.9993	-0.237	

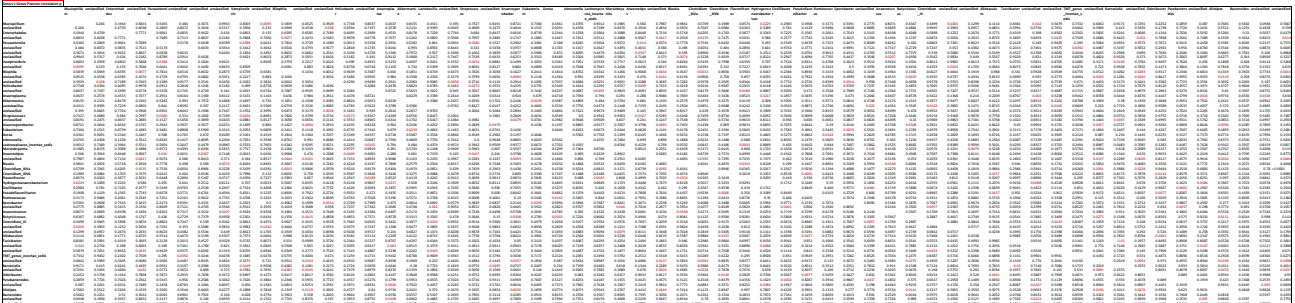
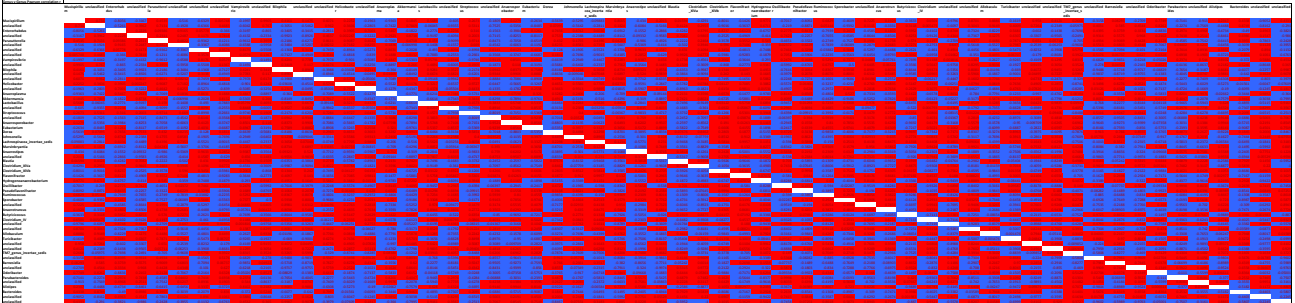
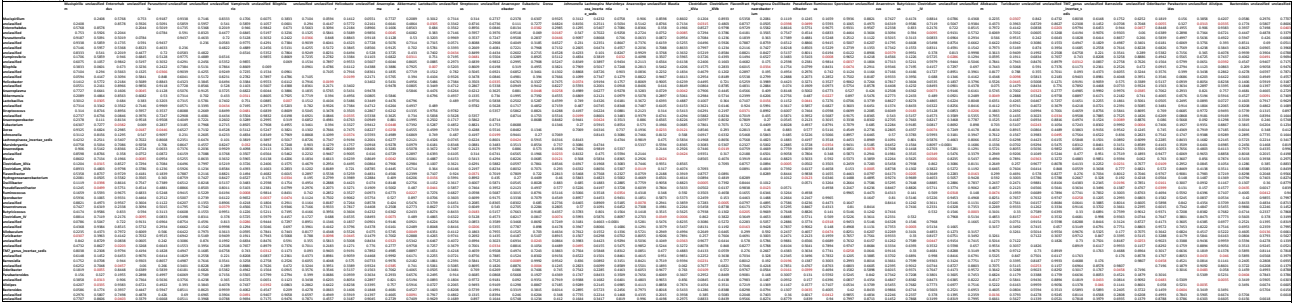
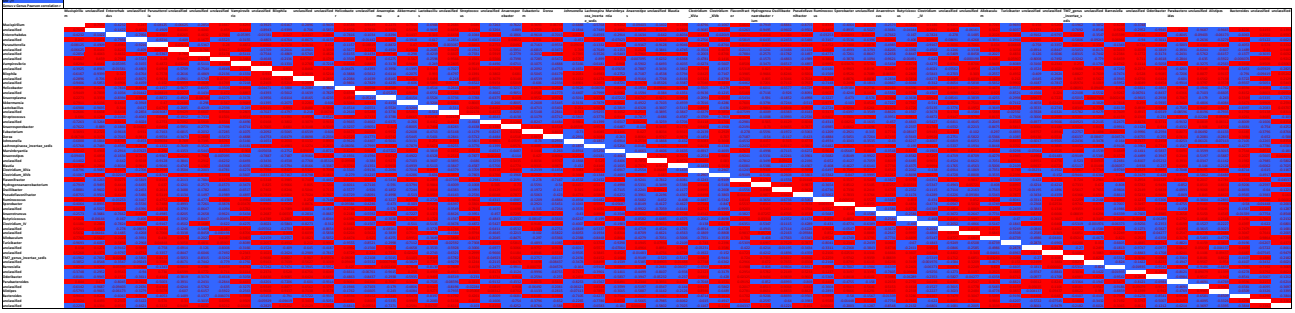
Ahr -/-								
Phyla v Phyla Pearson correlation p								
	"Deferribacteres"	"Actinobacteria"	"Proteobacteria"	"Tenericutes"	"Verrucomicrobia"	Firmicutes	TM7	"Bacteroidetes"
"Deferribacteres"		0.4587	0.7742	0.0639	0.8155	0.8457	0.7332	0.8432
"Actinobacteria"	0.4587		0.4487	0.7455	0.6602	0.4311	0.1031	0.4558
"Proteobacteria"	0.7742	0.4487		0.4467	0.9991	0.983	0.1451	0.946
"Tenericutes"	0.0639	0.7455	0.4467		0.8232	0.8815	0.9157	0.866
"Verrucomicrobia"	0.8155	0.6602	0.9991	0.8232		0.0593	0.8788	0.0586
Firmicutes	0.8457	0.4311	0.983	0.8815	0.0593		0.7289	0.0007
TM7	0.7332	0.1031	0.1451	0.9157	0.8788	0.7289		0.7635
"Bacteroidetes"	0.8432	0.4558	0.946	0.866	0.0586	0.0007	0.7635	

Family Family Pearson correlation matrix showing relationships between various bacterial families. The matrix is upper triangular, with the diagonal representing self-correlations (all 1.000). The rows and columns are labeled with family names such as Deferrisphaerae, Coriobacteriales, and Bacillales. The cells contain Pearson correlation coefficients, with red shading indicating positive correlations and white shading indicating negative or zero correlations.

Family Family Pearson correlation matrix showing relationships between various bacterial families. This matrix is more densely populated with values than the first one, indicating a higher density of significant correlations. The layout and labels are consistent with the first matrix.

Family Family Pearson correlation matrix showing relationships between various bacterial families. This matrix shows a different set of correlations compared to the previous two, with many cells appearing white, suggesting a lower density of significant correlations.

Family Family Pearson correlation matrix showing relationships between various bacterial families. This matrix shows a high density of significant correlations, with many cells shaded in red, indicating strong positive relationships between many of the families.



Ahr -/+									
Phyla v Metabolite Pearson correlation r									
	"Deferribac teres"	"Actinobact eria"	"Proteobact eria"	"Tenericute s"	"Verrucomi crobia"	Firmicutes	TM7	unclassified	"Bacteroides"
cecal butyrate	-0.1276	0.7912	-0.5523	-0.9127	0.4636	-0.9331	0.1062	-0.4008	0.9595
cecal propionate	0.14	-0.8373	-0.3105	-0.8705	0.6856	-0.9554	0.1065	-0.4587	0.9413
cecal acetate	-0.969	-0.5069	-0.7771	-0.08862	-0.8543	0.1784	0.3047	-0.5754	-0.04925
cecal baa	0.3779	0.6764	-0.04206	-0.814	0.8652	-0.9358	0.3102	-0.2819	0.8749
cecal lactate	0.2768	0.7587	-0.1641	-0.8551	0.7997	-0.962	0.2275	-0.3633	0.9211
cecal alanine	0.5469	0.6907	0.1414	-0.6871	0.9439	-0.852	0.2131	-0.3615	0.7693
cecal lysine	0.4778	0.4477	0.1283	-0.7196	0.8923	-0.8426	0.495	-0.05885	0.7558
cecal glutamate	0.3016	0.5298	0.2687	0.4529	0.0188	0.3143	-0.9916	-0.8774	-0.3132
cecal tyrosine	0.6465	0.7805	0.4316	0.1283	0.5419	-0.09901	-0.7746	-0.9512	0.04202
cecal phenylalanine	0.7941	0.6869	0.4469	-0.3871	0.9887	-0.6168	-0.03095	-0.5173	0.5059
cecal glucose	-0.1598	-0.2971	0.1611	0.8613	-0.6811	0.8941	-0.7053	-0.1792	-0.8454
cecal oligosacc	-0.6017	-0.229	-0.6849	-0.7238	-0.1205	-0.5413	0.8521	0.6583	0.596

Ahr -/+									
Phyla v Metabolite Pearson correlation p									
	"Deferribac teres"	"Actinobact eria"	"Proteobact eria"	"Tenericute s"	"Verrucomi crobia"	Firmicutes	TM7	unclassified	"Bacteroides"
cecal butyrate	0.8724	0.2088	0.4477	0.0873	0.5364	0.0669	0.8938	0.5992	0.0405
cecal propionate	0.86	0.1628	0.6895	0.1295	0.3144	0.0446	0.8935	0.5413	0.0587
cecal acetate	0.031	0.4931	0.2229	0.9114	0.1457	0.8216	0.6953	0.4246	0.9507
cecal baa	0.6221	0.3236	0.9579	0.186	0.1348	0.0642	0.6898	0.7181	0.1251
cecal lactate	0.7232	0.2413	0.8359	0.1449	0.2003	0.038	0.7725	0.6367	0.0789
cecal alanine	0.4531	0.3093	0.8586	0.3129	0.0561	0.148	0.7869	0.6385	0.2307
cecal lysine	0.5222	0.5523	0.8717	0.2804	0.1077	0.1574	0.505	0.9412	0.2442
cecal glutamate	0.6984	0.4702	0.7313	0.5471	0.9812	0.6857	0.0084	0.1226	0.6868
cecal tyrosine	0.3535	0.2195	0.5684	0.8717	0.4581	0.901	0.2254	0.0488	0.958
cecal phenylalanine	0.2059	0.3131	0.5531	0.6129	0.0113	0.3832	0.969	0.4827	0.4941
cecal glucose	0.8402	0.7029	0.8389	0.1387	0.3189	0.1059	0.2947	0.8208	0.1546
cecal oligosacc	0.3983	0.771	0.3151	0.2762	0.8795	0.4587	0.1479	0.3417	0.404

Ahr -/-									
Phyla v Metabolite Pearson correlation r									
	"Deferribac teres"	"Actinobact eria"	"Proteobact eria"	"Tenericute s"	"Verrucomi crobia"	Firmicutes	TM7	unclassified	"Bacteroides"
cecal butyrate	-0.7403	-0.3043	-0.6262	0.8489	0.3548	-0.5793	0.1419	0.5085	0.5986
cecal propionate	0.8501	-0.2019	0.6751	-0.9665	-0.0385	0.2969	-0.2009	-0.2654	-0.3175
cecal acetate	0.5602	0.25	0.9325	-0.8141	0.02859	0.1102	-0.6193	0.05626	-0.1425
cecal baa	0.1826	0.6983	0.945	-0.5031	0.3127	-0.3103	-0.8929	0.5333	0.275
cecal lactate	0.7258	-0.1464	0.05366	-0.6532	0.8051	-0.5562	0.1538	0.3392	0.5583
cecal alanine	0.2855	0.6212	0.6592	-0.4962	0.7513	-0.6969	-0.6565	0.7666	0.673
cecal lysine	-0.1823	0.924	0.7121	-0.1128	0.5288	-0.6422	-0.9058	0.8498	0.6135
cecal glutamate	-0.2412	-0.6549	-0.9486	0.5543	-0.3139	0.2909	0.8657	-0.5025	-0.2559
cecal tyrosine	0.5151	0.128	0.8961	-0.7572	-0.2407	0.3675	-0.5485	-0.1764	-0.3986
cecal phenylalanine	0.3202	-0.1557	0.8641	-0.5755	-0.4542	0.5153	-0.5733	-0.2715	-0.5463
cecal glucose	0.734	-0.9028	-0.1407	-0.5648	-0.4417	0.7135	0.621	-0.8704	-0.7034
cecal oligosacc	0.5201	-0.9876	-0.4686	-0.2637	-0.473	0.6902	0.8345	-0.8999	-0.6687

Ahr -/-									
Phyla v Metabolite Pearson correlation p									
	"Deferribac teres"	"Actinobact eria"	"Proteobact eria"	"Tenericute s"	"Verrucomi crobia"	Firmicutes	TM7	unclassified	"Bacteroides"
cecal butyrate	0.2597	0.6957	0.3738	0.1511	0.6452	0.4207	0.8581	0.4915	0.4014
cecal propionate	0.1499	0.7981	0.3249	0.0335	0.9615	0.7031	0.7991	0.7346	0.6825
cecal acetate	0.4398	0.75	0.0675	0.1859	0.9714	0.8898	0.3807	0.9437	0.8575
cecal baa	0.8174	0.3017	0.055	0.4969	0.6873	0.6897	0.1071	0.4667	0.725
cecal lactate	0.2742	0.8536	0.9463	0.3468	0.1949	0.4438	0.8462	0.6608	0.4417
cecal alanine	0.7145	0.3788	0.3408	0.5038	0.2487	0.3031	0.3435	0.2334	0.327
cecal lysine	0.8177	0.076	0.2879	0.8872	0.4712	0.3578	0.0942	0.1502	0.3865
cecal glutamate	0.7588	0.3451	0.0514	0.4457	0.6861	0.7091	0.1343	0.4975	0.7441
cecal tyrosine	0.4849	0.872	0.1039	0.2428	0.7593	0.6325	0.4515	0.8236	0.6014
cecal phenylalanine	0.6798	0.8443	0.1359	0.4245	0.5458	0.4847	0.4267	0.7285	0.4537
cecal glucose	0.266	0.0972	0.8593	0.4352	0.5583	0.2865	0.379	0.1296	0.2966
cecal oligosacc	0.4799	0.0124	0.5314	0.7363	0.527	0.3098	0.1655	0.1001	0.3313

Ahr -/+

Table with 15 rows and 25 columns showing Pearson correlation coefficients for Ahr -/+ across various bacterial genera.

Ahr -/+

Table with 15 rows and 25 columns showing Pearson correlation coefficients for Ahr -/+ across various bacterial genera.

Ahr -/

Table with 15 rows and 25 columns showing Pearson correlation coefficients for Ahr -/ across various bacterial genera.

Ahr -/

Table with 15 rows and 25 columns showing Pearson correlation coefficients for Ahr -/ across various bacterial genera.

Ahr -/

Table with 15 rows and 25 columns showing Pearson correlation coefficients for Ahr -/ across various bacterial genera.

Ahr +/

Table with 15 rows and 25 columns showing Pearson correlation coefficients for Ahr +/- across various bacterial genera.

Ahr +/-

Table with 15 rows and 25 columns showing Pearson correlation coefficients for Ahr +/- across various bacterial genera.

Ahr +/-

Table with 15 rows and 25 columns showing Pearson correlation coefficients for Ahr +/- across various bacterial genera.

Family + Metabolic Pearson correlation r		Defensin	Combustion	unclassified	Sulfonamide	unclassified	unclassified	Betalactam	unclassified	Desulfonin	unclassified	Holistic	unclassified	Anesthetics	Vercurin	Lactobacilli	Streptococcus	unclassified	Clindamycin	Eubacterin	Lactinon	Romuncoc	unclassified	unclassified	Ergosterol	unclassified	TM7_family	unclassified	Perforin	Prevalence	Bacterin	unclassified	unclassified
rescue	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	
local hydrolyte	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757	0.8757

Family + Metabolic Pearson correlation p		Defensin	Combustion	unclassified	Sulfonamide	unclassified	unclassified	Betalactam	unclassified	Desulfonin	unclassified	Holistic	unclassified	Anesthetics	Vercurin	Lactobacilli	Streptococcus	unclassified	Clindamycin	Eubacterin	Lactinon	Romuncoc	unclassified	unclassified	Ergosterol	unclassified	TM7_family	unclassified	Perforin	Prevalence	Bacterin	unclassified	unclassified
rescue	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case
local hydrolyte	0.8724	0.21	0.1051	0.0054	0.727	0.1167	0.7157	0.0897	0.4991	0.1795	0.473	0.8002	0.0873	0.1564	0.7678	0.7803	0.7271	0.6058	0.0404	0.1883	0.9074	0.9405	0.85	0.0212	0.138	0.8938	0.9992	0.0099	0.7997	0.8549	0.6174	0.4602	0.0862

Family + Metabolic Pearson correlation r		Defensin	Combustion	unclassified	Sulfonamide	unclassified	unclassified	Betalactam	unclassified	Desulfonin	unclassified	Holistic	unclassified	Anesthetics	Vercurin	Lactobacilli	Streptococcus	unclassified	Clindamycin	Eubacterin	Lactinon	Romuncoc	unclassified	unclassified	Ergosterol	unclassified	TM7_family	unclassified	Perforin	Prevalence	Bacterin	unclassified	unclassified
rescue	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case
local hydrolyte	0.8724	0.21	0.1051	0.0054	0.727	0.1167	0.7157	0.0897	0.4991	0.1795	0.473	0.8002	0.0873	0.1564	0.7678	0.7803	0.7271	0.6058	0.0404	0.1883	0.9074	0.9405	0.85	0.0212	0.138	0.8938	0.9992	0.0099	0.7997	0.8549	0.6174	0.4602	0.0862

Family + Metabolic Pearson correlation p		Defensin	Combustion	unclassified	Sulfonamide	unclassified	unclassified	Betalactam	unclassified	Desulfonin	unclassified	Holistic	unclassified	Anesthetics	Vercurin	Lactobacilli	Streptococcus	unclassified	Clindamycin	Eubacterin	Lactinon	Romuncoc	unclassified	unclassified	Ergosterol	unclassified	TM7_family	unclassified	Perforin	Prevalence	Bacterin	unclassified	unclassified
rescue	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case	as	case
local hydrolyte	0.2597	0.6791	0.2814	0.512	0.2235	0.318	0.725	0.3579	0.64	0.7204	0.4154	0.5414	0.1154	0.6452	0.0784	0.5407	0.7972	0.7376	0.8468	0.5009	0.1792	0.2779	0.1494	0.9413	0.1391	0.8581	0.4615	0.3377	0.142	0.6607	0.7965	0.219	0.2802

