**Microbial structure and function in infant and juvenile rhesus macaques are primarily affected by age, not vaccination status.**

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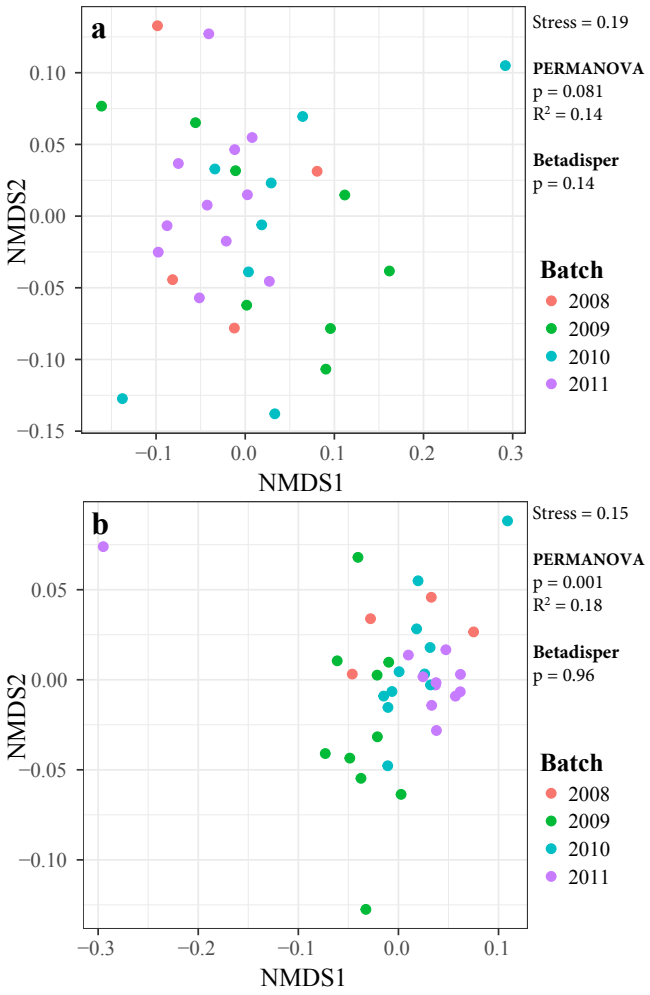
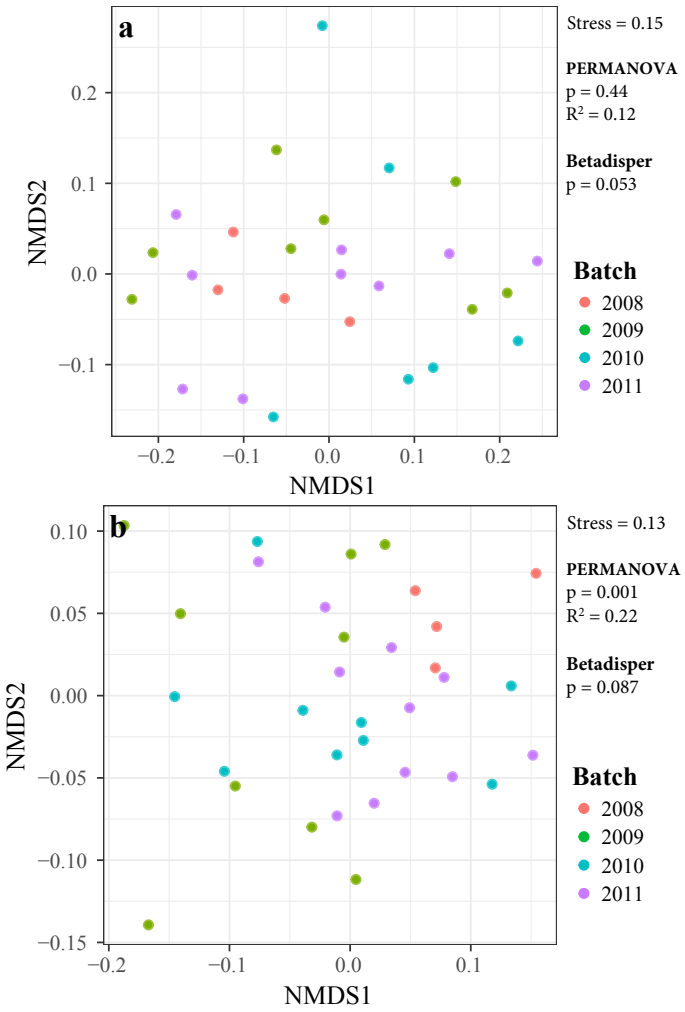
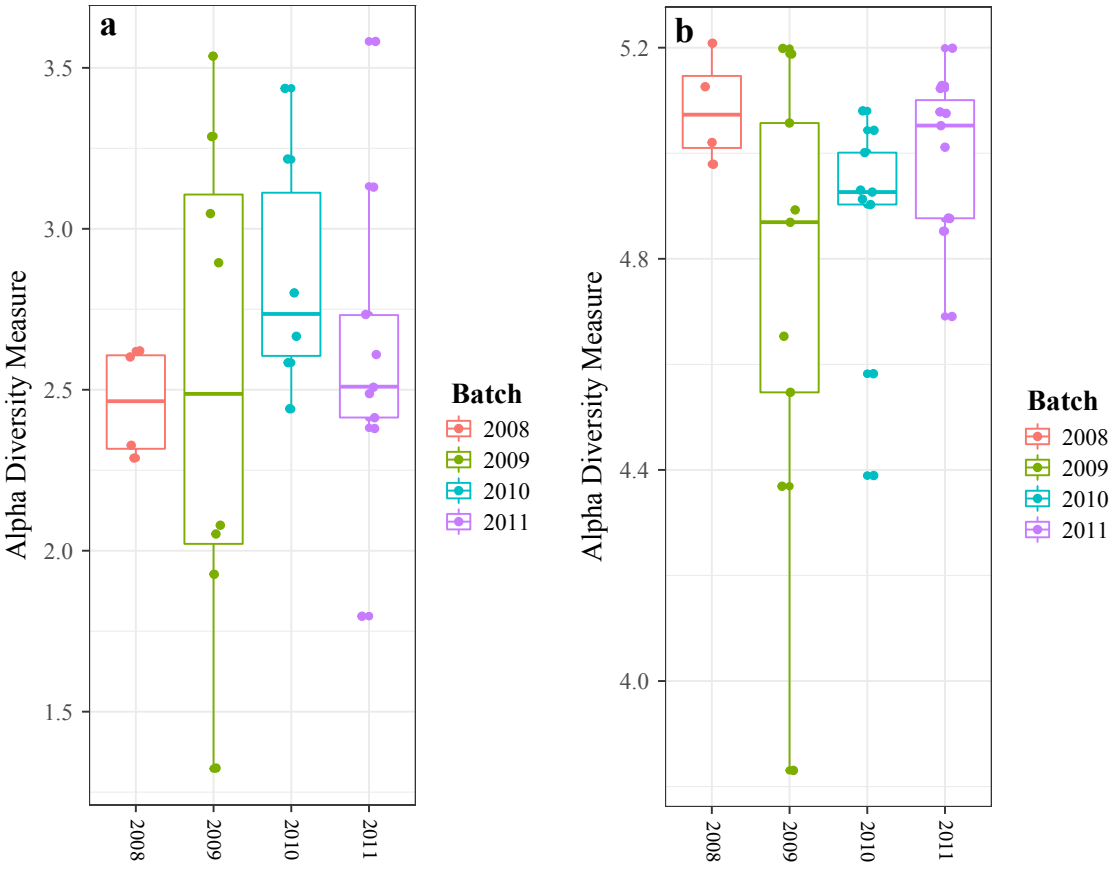


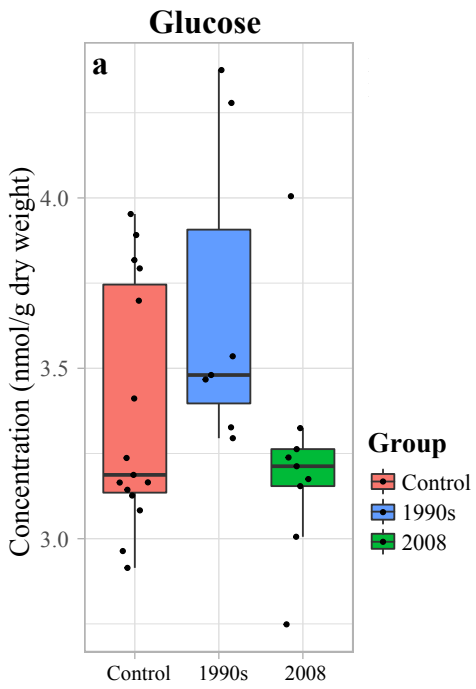

**Supplementary Figure S1.** NMDS plots showing the batch effect (birth year) on metabolome datasets at (a) Infant and (b) Juvenile time points. The stress value, as well as results from the PERMANOVA and betadisper analyses are included in each plot. 2008 (red), 2009 (green), 2010 (blue), and 2011 (purple).

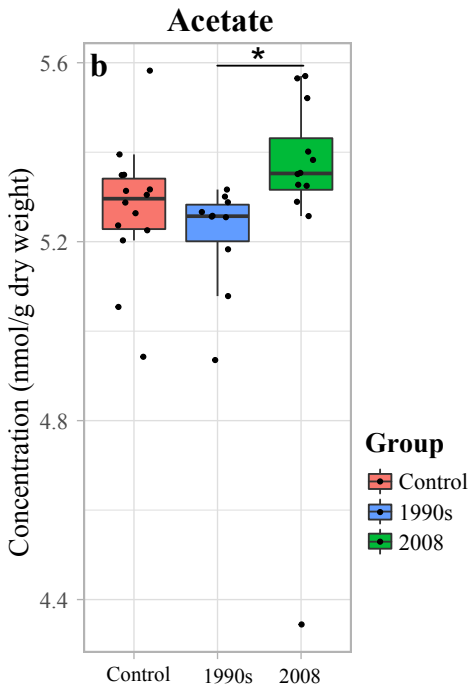


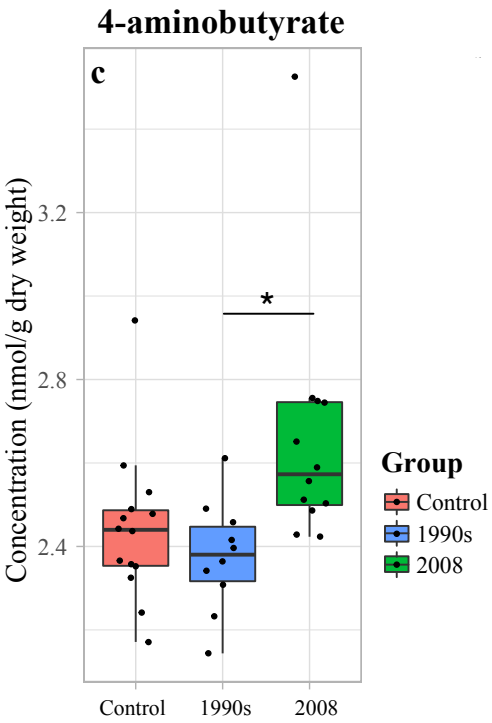

**Supplementary Figure S2.** NMDS plots showing the batch effect (birth year) on microbiota datasets at (a) Infant and (b) Juvenile time points. The stress value, as well as results from the PERMANOVA and betadisper analyses, are included in each plot. 2008 (red), 2009 (green), 2010 (blue), and 2011 (purple).

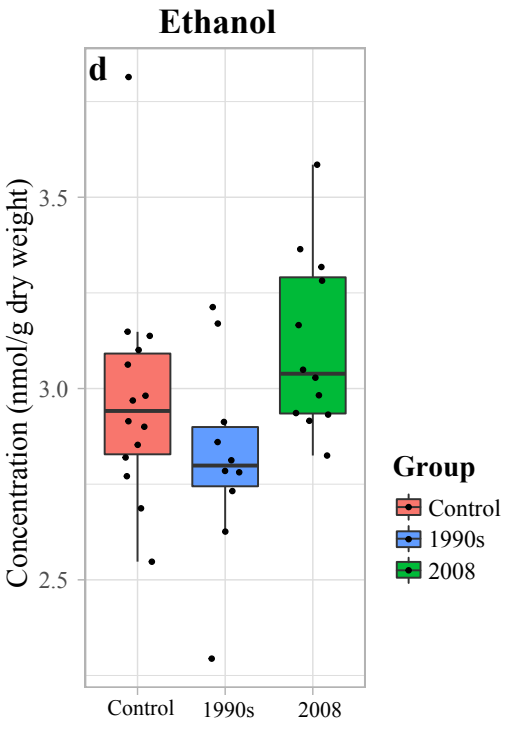


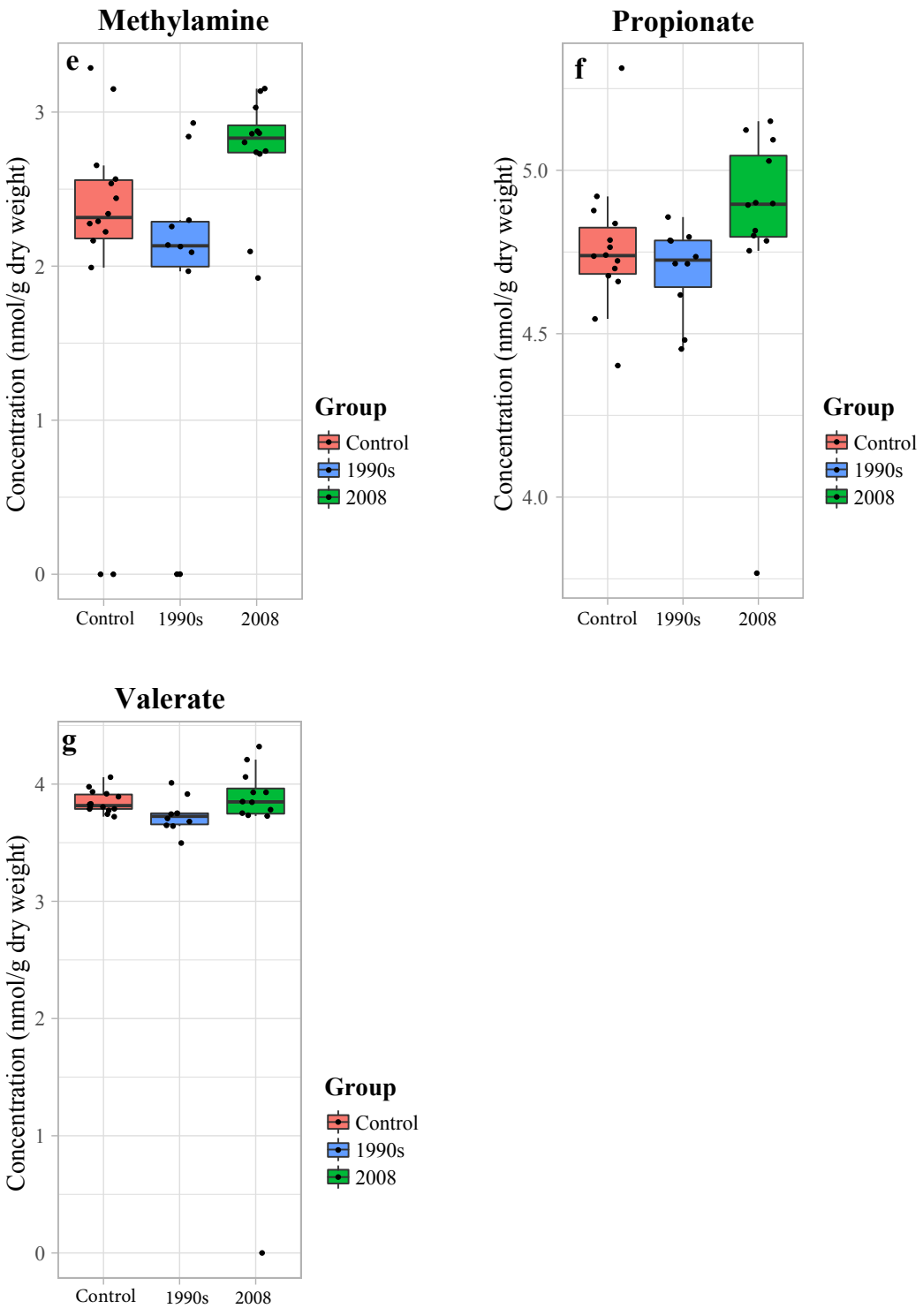

**Supplementary Figure S3.** Box plots for the alpha diversity values based on the Shannon method showing the batch effect (birth year) at the (a) Infant and (b) Juvenile time points. 2008 (red), 2009 (green), 2010 (blue), and 2011 (purple).


Glucose
a
Concentration (nmol/g dry weight)
4.0
3.5
3.0
Control
1990s
2008
Group
Control
1990s
2008


Acetate
b
*
Concentration (nmol/g dry weight)
5.6
5.2
4.8
4.4
Control
1990s
2008
Group
Control
1990s
2008

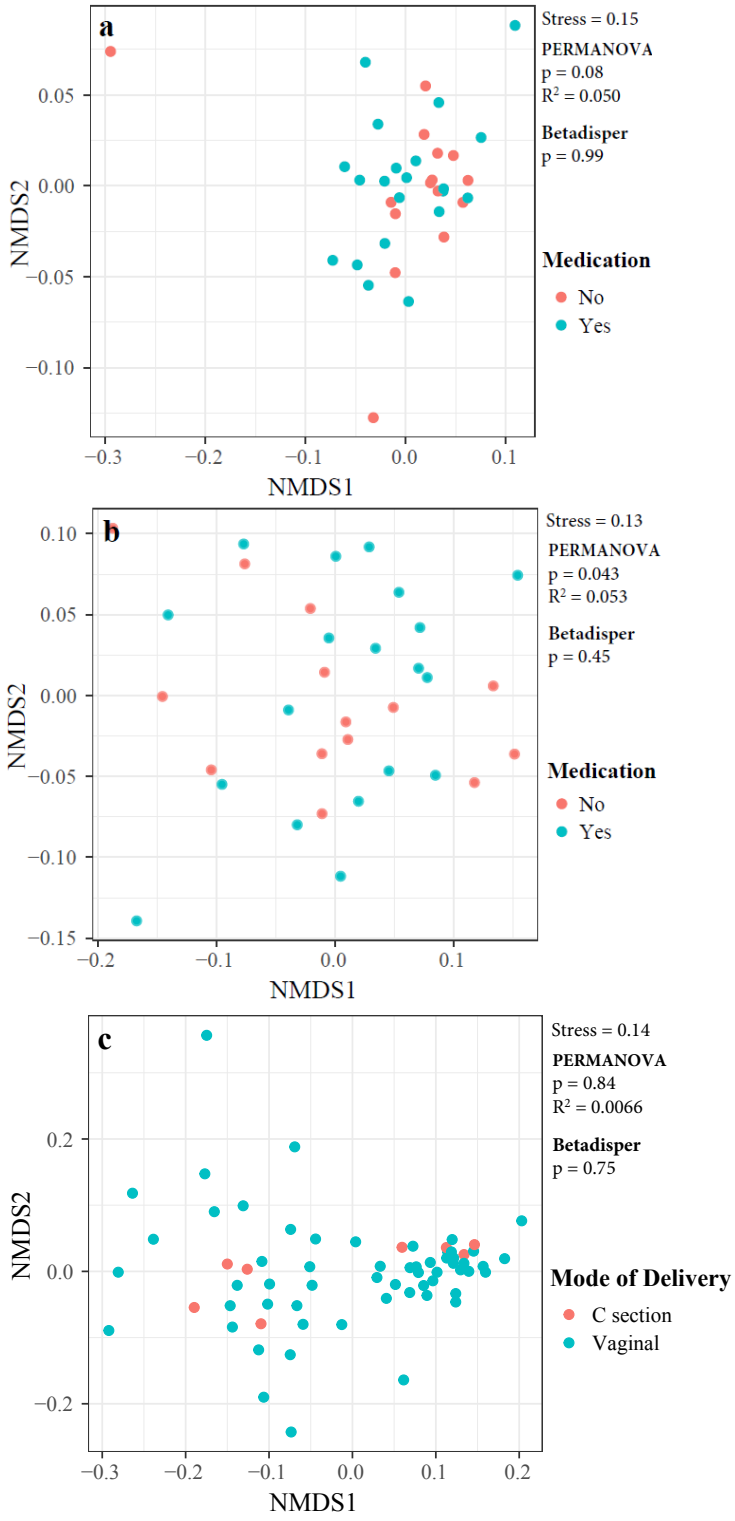

4-aminobutyrate
c
*
Concentration (nmol/g dry weight)
3.2
2.8
2.4
Control
1990s
2008
Group
Control
1990s
2008

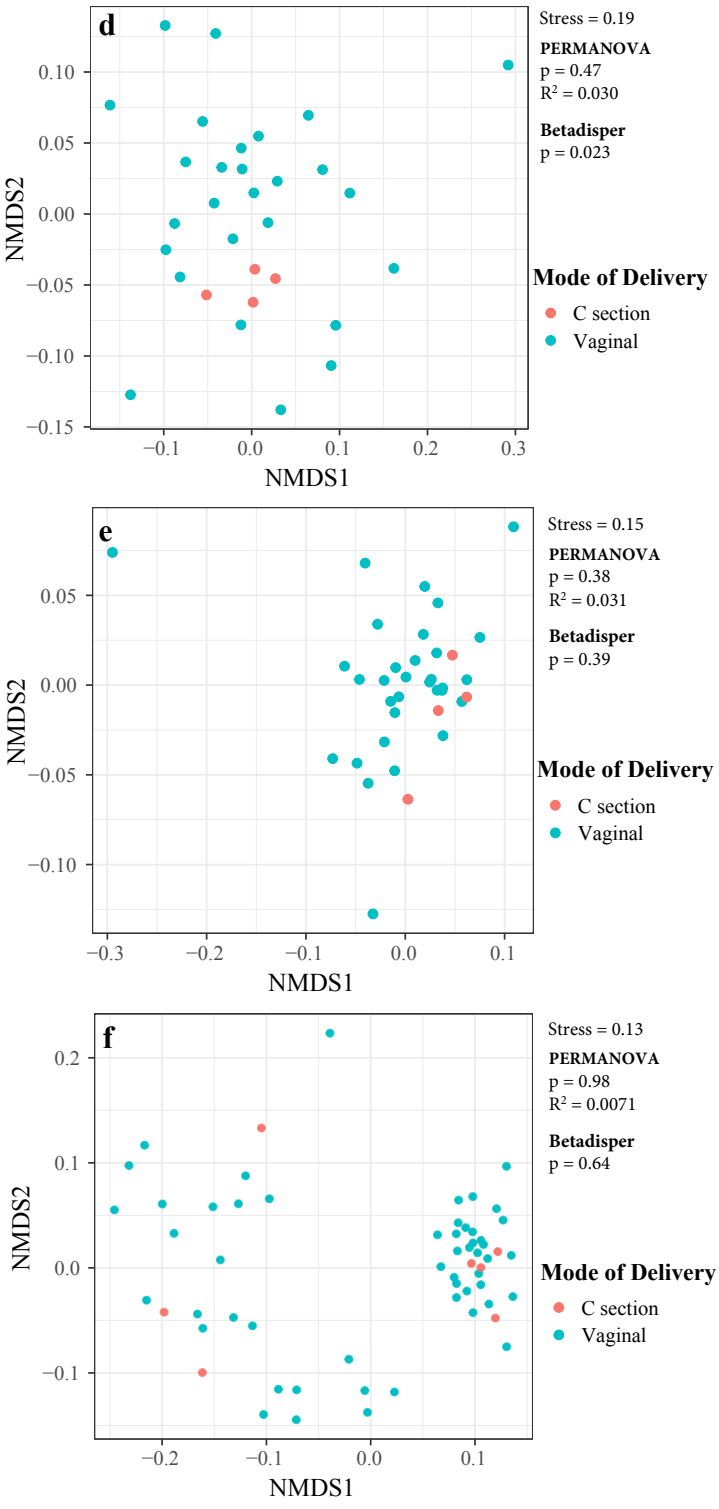

Ethanol
d
Concentration (nmol/g dry weight)
3.5
3.0
2.5
Control
1990s
2008
Group
Control
1990s
2008

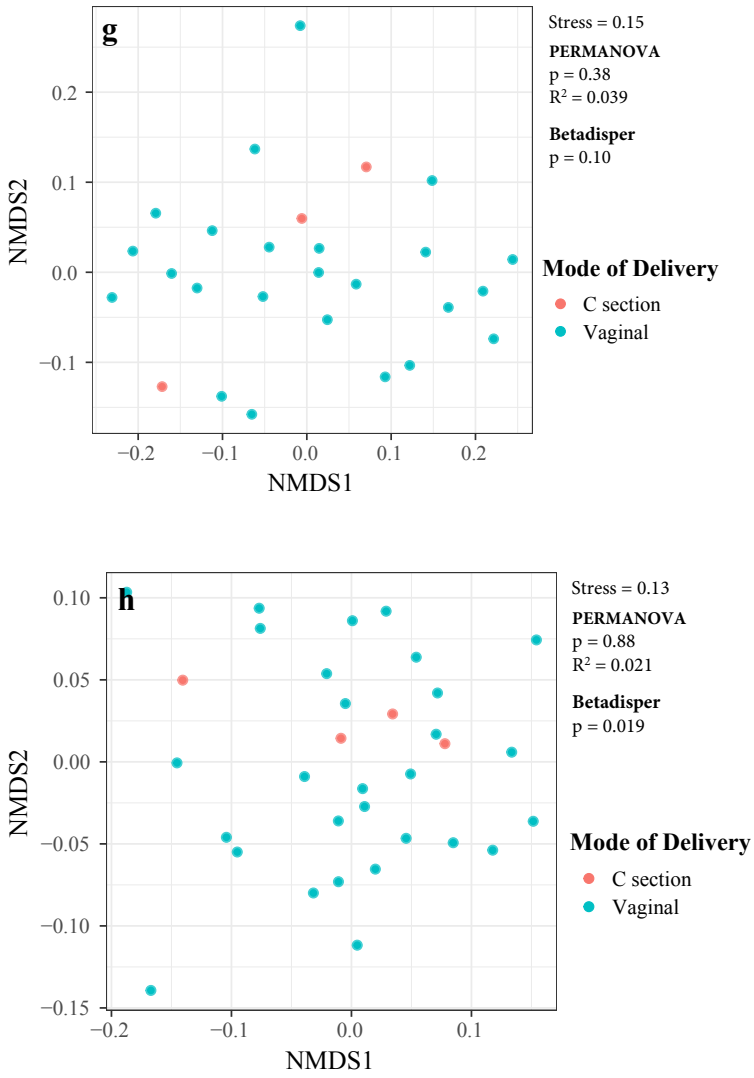



**Supplementary Figure S4.** Box plots showing the concentrations of metabolites with significant p-values by the KW test prior to the FDR correction. All plots were generated with the data collected at the Juvenile time point except for plot (a) for data collected at the Infant time point. Concentration of metabolites were log transformed. The Mann-Whitney U test was applied as a post hoc test. In order to avoid inflation of the false positive rate in the Mann-Whitney U test,

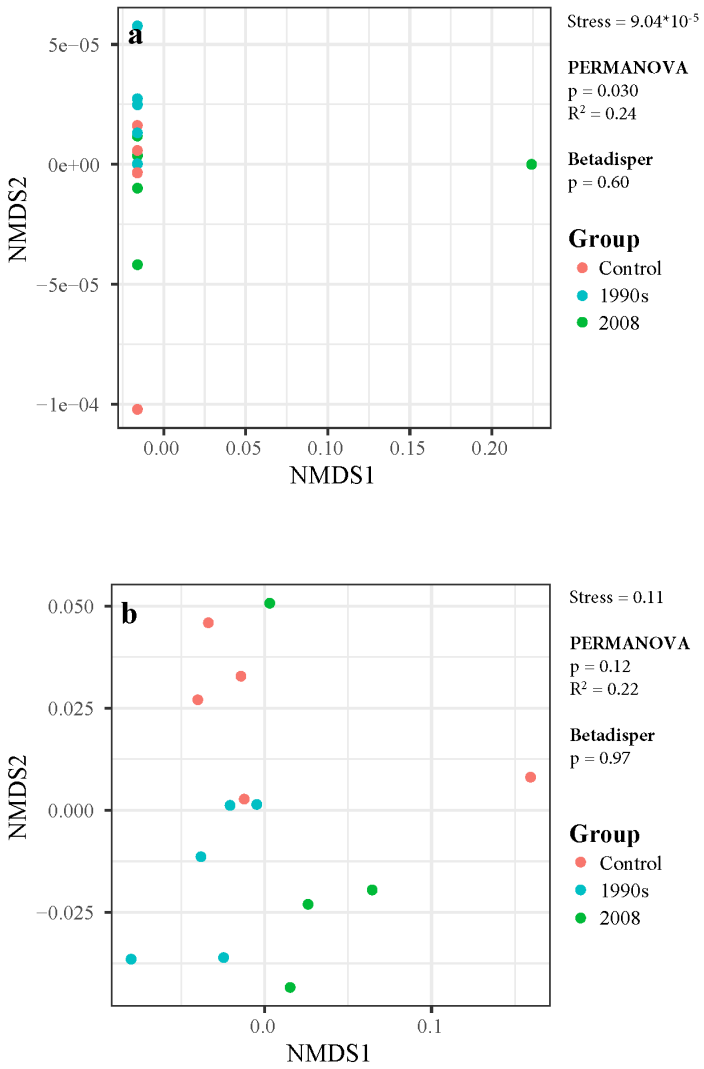
Bonferroni was used to obtain a new threshold, with significance assumed when p-values were less than 0.05/3=0.017. (*: $p<0.017$). Controls (red), 1990s (blue), and 2008 (green) groups.


a
Stress = 0.15
PERMANOVA
p = 0.08
R² = 0.050
Betadisper
p = 0.99
Medication
No
Yes
NMDS1
NMDS2
b
Stress = 0.13
PERMANOVA
p = 0.043
R² = 0.053
Betadisper
p = 0.45
Medication
No
Yes
NMDS1
NMDS2
c
Stress = 0.14
PERMANOVA
p = 0.84
R² = 0.0066
Betadisper
p = 0.75
Mode of Delivery
C section
Vaginal
NMDS1
NMDS2

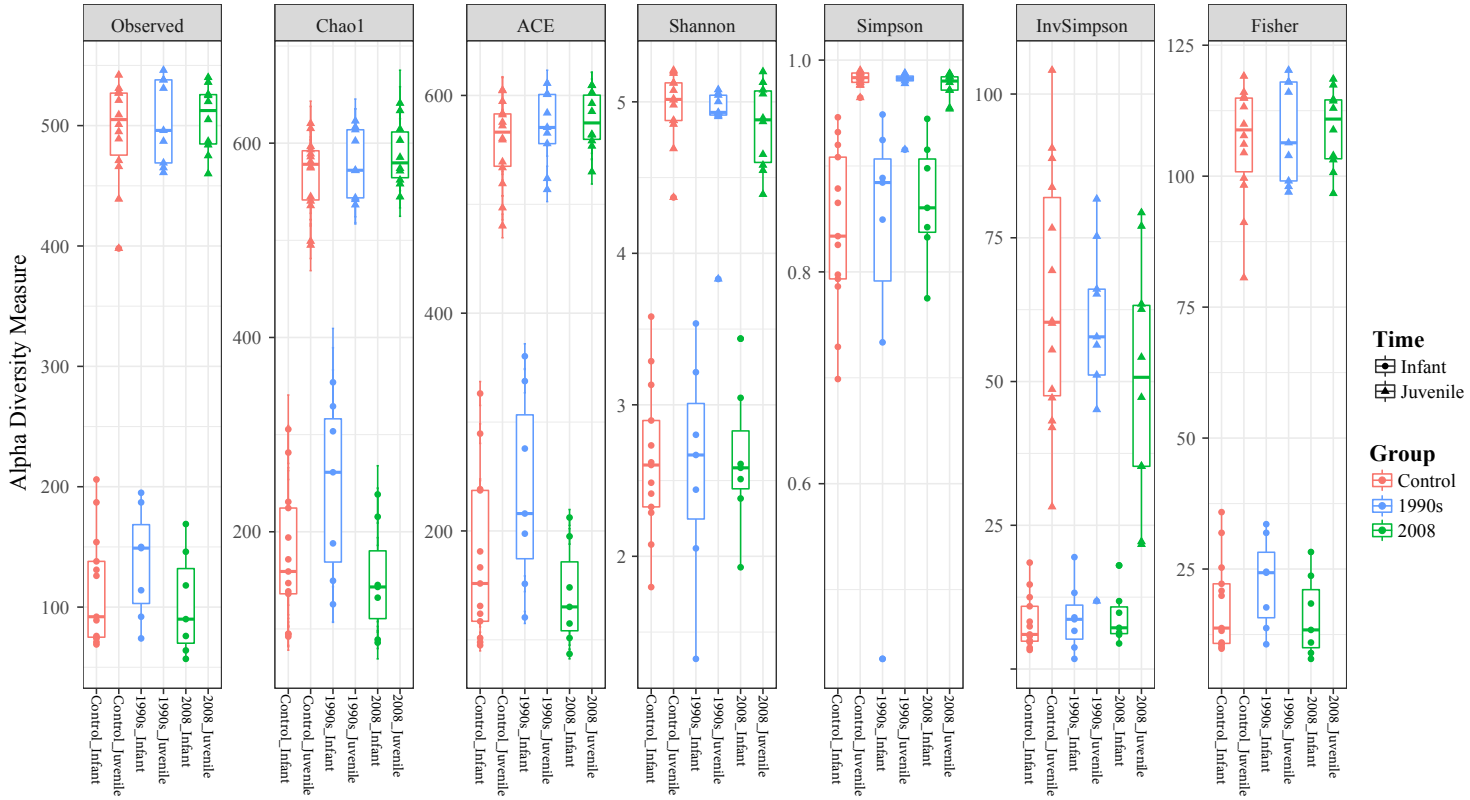

d
Stress = 0.19
PERMANOVA
p = 0.47
R² = 0.030
Betadisper
p = 0.023
Mode of Delivery
C section
Vaginal
NMDS2
NMDS1
e
Stress = 0.15
PERMANOVA
p = 0.38
R² = 0.031
Betadisper
p = 0.39
Mode of Delivery
C section
Vaginal
NMDS2
NMDS1
f
Stress = 0.13
PERMANOVA
p = 0.98
R² = 0.0071
Betadisper
p = 0.64
Mode of Delivery
C section
Vaginal
NMDS2
NMDS1



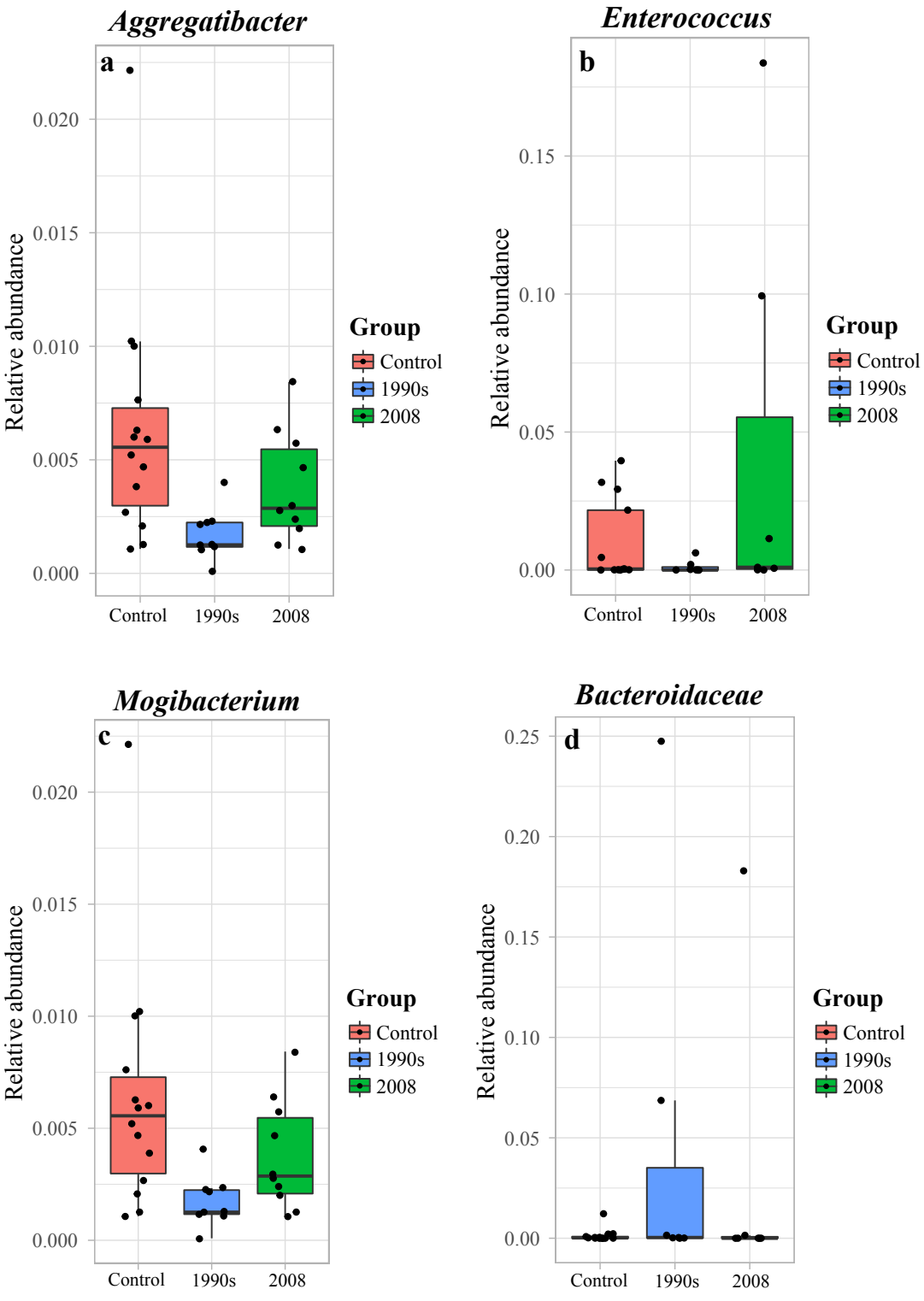

**Supplementary Figure S5.** The effect of potential confounding factors (medications or mode of birth) on metabolome and microbiota data. NMDS plots for analysis of medications for (a) metabolome, and (b) microbiota datasets at the Juvenile time point. NMDS plots for mode of birth for metabolome analysis (c) overall, (d) Infant, and (e) Juvenile time points, and for microbiota analysis (f) overall, (g) Infant, and (h) Juvenile time points. The stress value, as well as results from the PERMANOVA and betadisper analyses, are included in each plot.

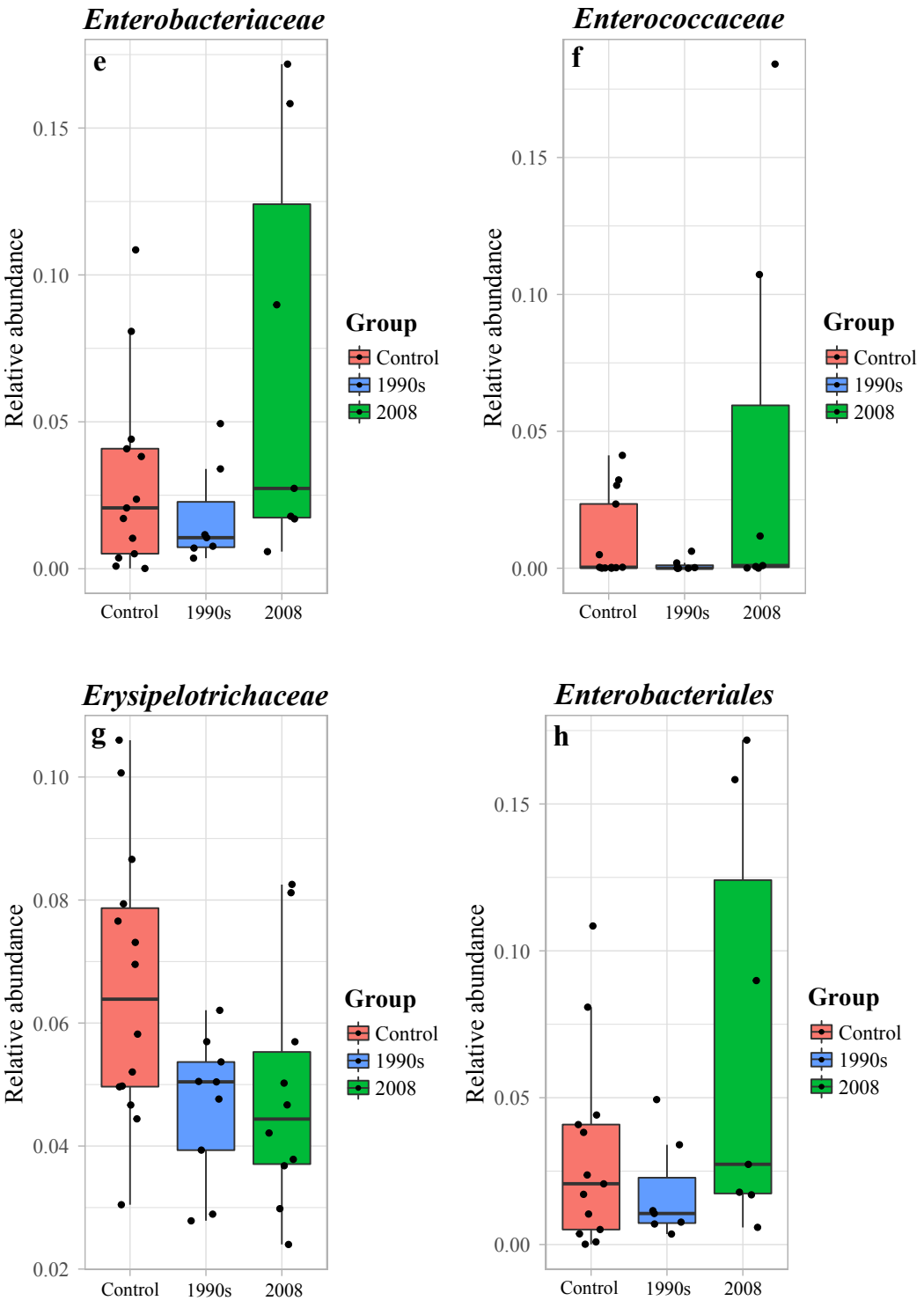


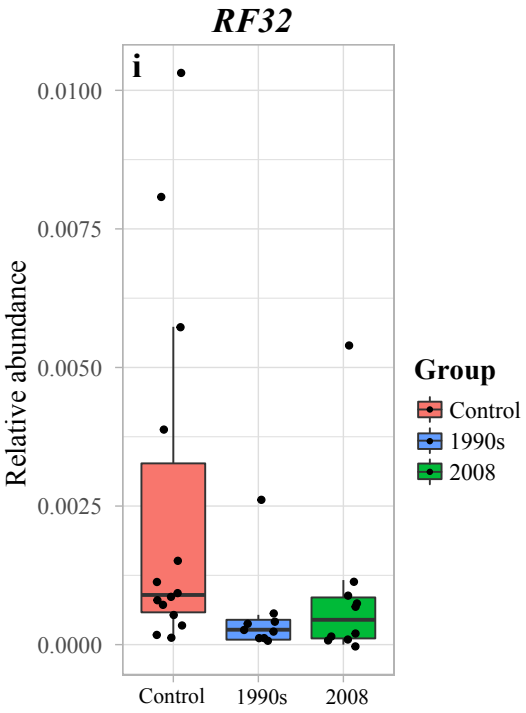

**Supplementary Figure S6.** NMDS plots on the metabolome dataset generated for animals reciving no medications. The outlier-like datapoint that made it difficult to observe the data distribution in plot (a) was removed to generate plot (b). (b) Although there seemed to be a cluster, PERMANOVA and betadisper tests did not show significant p-values (p=0.12 by PERMANOVA and p=0.97 by betadisper tests). Controls (red), 1990s (blue), and 2008 (green) groups.



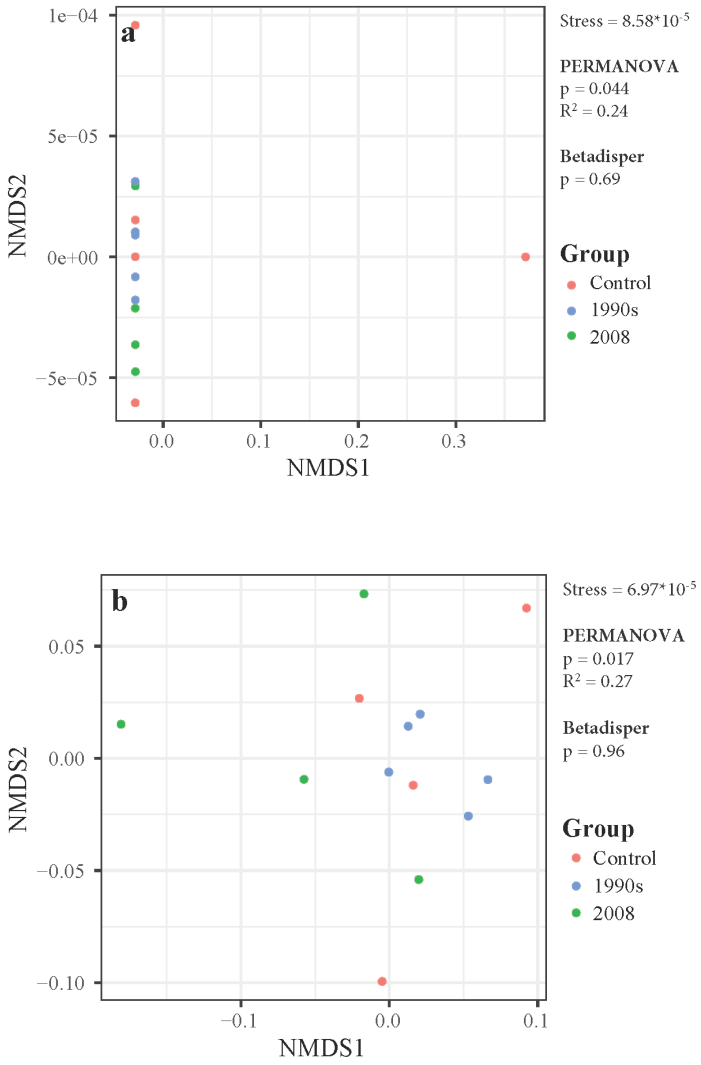

**Supplementary Figure S7.** Box plots showing the alpha diversity values based on the different methods. Circle represents data at Infant, triangle represents data at Juvenile time points. Controls (red), 1990s (blue), and 2008 (green) groups.


Aggregatibacter
a
Relative abundance
0.020
0.015
0.010
0.005
0.000
Control
1990s
2008
Group
Control
1990s
2008
Enterococcus
b
Relative abundance
0.15
0.10
0.05
0.00
Control
1990s
2008
Group
Control
1990s
2008
Mogibacterium
c
Relative abundance
0.020
0.015
0.010
0.005
0.000
Control
1990s
2008
Group
Control
1990s
2008
Bacteroidaceae
d
Relative abundance
0.25
0.20
0.15
0.10
0.05
0.00
Control
1990s
2008
Group
Control
1990s
2008

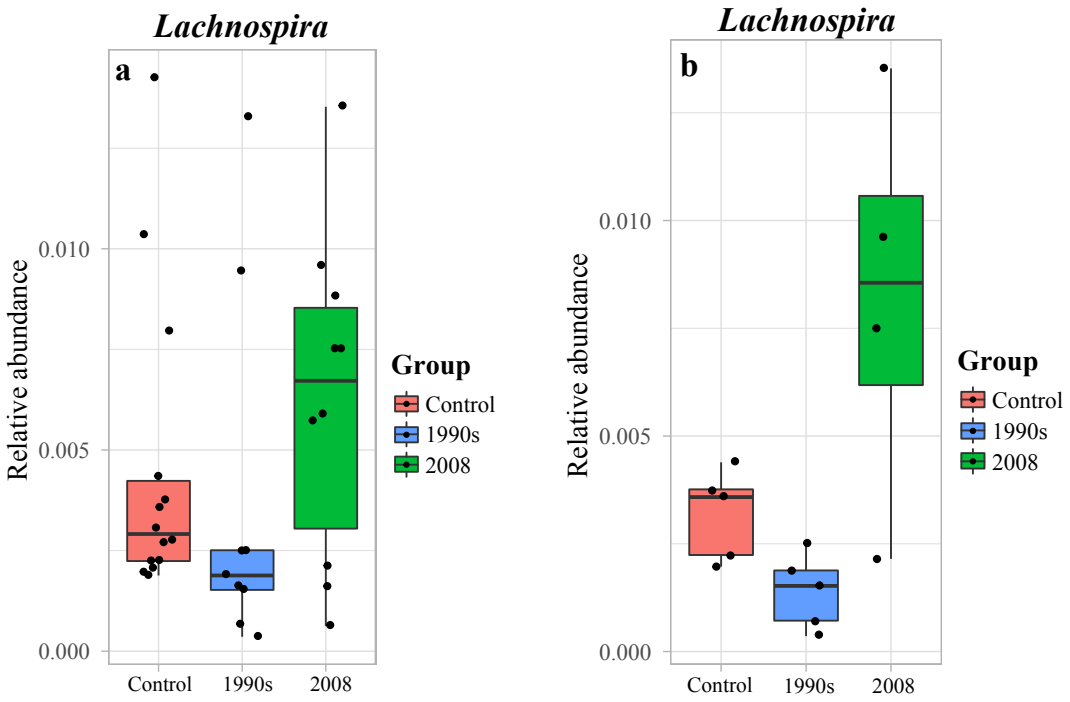

Enterobacteriaceae
e
Relative abundance
0.15
0.10
0.05
0.00
Control
1990s
2008
Group
Control
1990s
2008
Enterococcaceae
f
Relative abundance
0.15
0.10
0.05
0.00
Control
1990s
2008
Group
Control
1990s
2008
Erysipelotrichaceae
g
Relative abundance
0.10
0.08
0.06
0.04
0.02
Control
1990s
2008
Group
Control
1990s
2008
Enterobacteriales
h
Relative abundance
0.15
0.10
0.05
0.00
Control
1990s
2008
Group
Control
1990s
2008



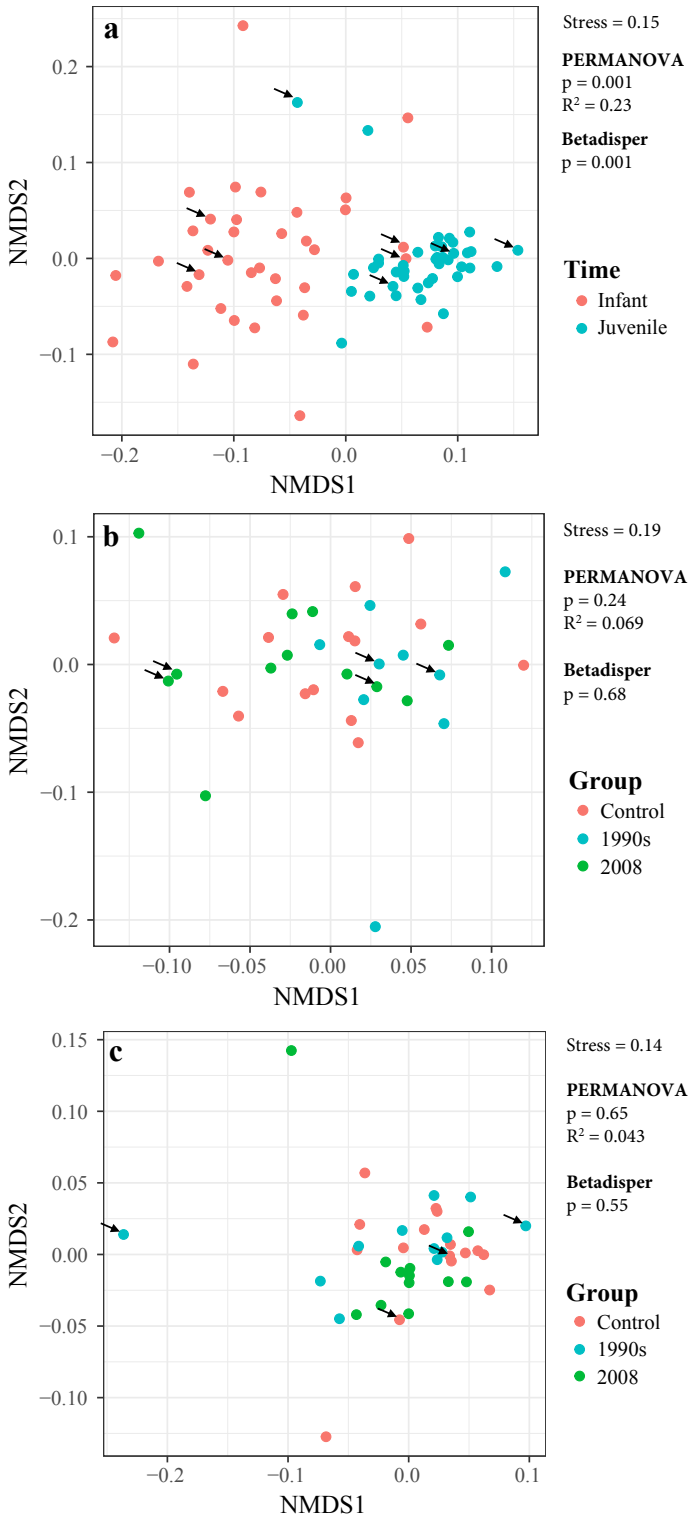

**Supplementary Figure S8.** Boxplots showing the relative abundance of bacteria with p-value of less than 0.05 prior to the FDR correction by the likelihood ratio test. At the genus level: (a) and (b) at the Infant and (c) at the Juvenile time point; at the family level: (d), (e) and (f) at the Infant and (g) at the Juvenile time point; at the order level: (h) at the Infant and (i) at the Juvenile time point. Controls (red), 1990s (blue), and 2008 (green) groups.



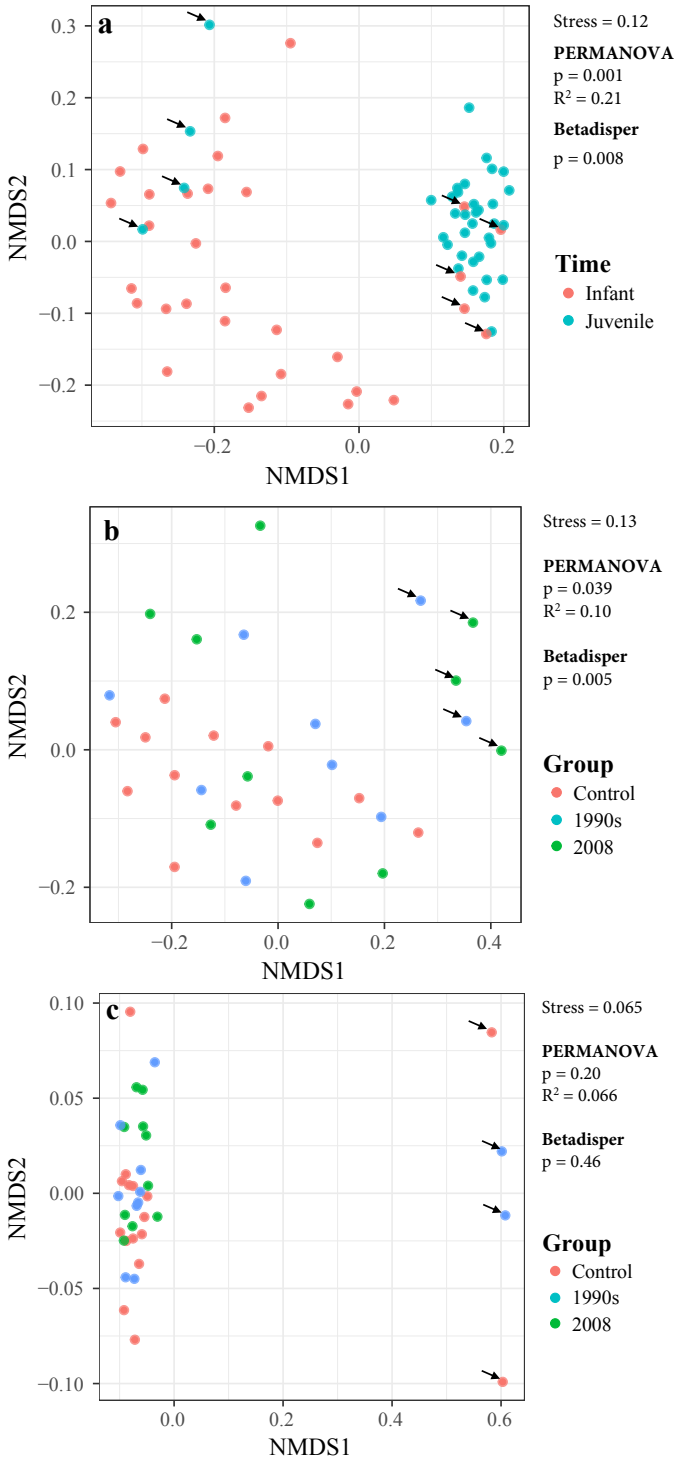

**Supplementary Figure S9.** NMDS plots on the microbiota dataset generated for animals receiving no medications (Juvenile time point). The outlier-like datapoint that made it difficult to observe the data distribution in plot (a) was removed to generate plot (b). (b) Although PERMANOVA test resulted in a significant p-values (p=0. 017), the $R^2$ value slows that relatively small portion of variation (27 %) could be explained by Group. Betadisper test showed insignificant p-value (p=0.96). Controls (red), 1990s (blue), and 2008 (green) groups.



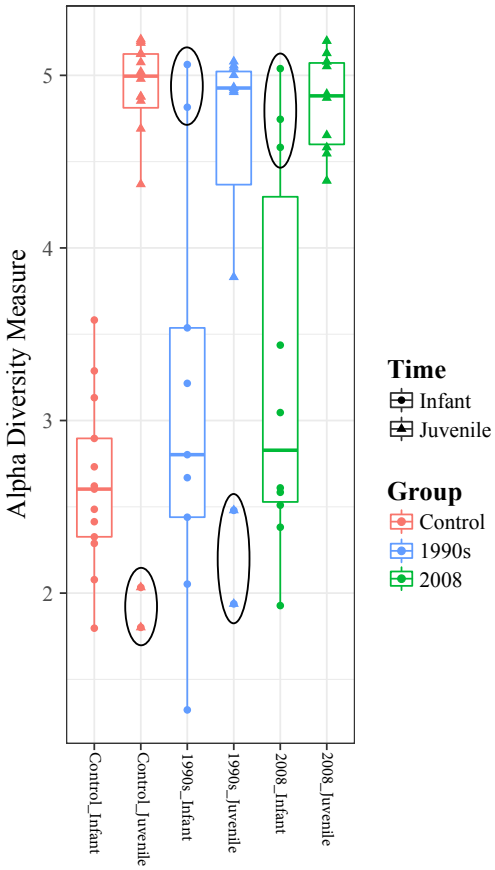

**Supplementary Figure S10.** Boxplots showing the relative abundance of *Lachnospira* (genus) with significant p-value with FDR correction by the likelihood ratio test. (a) was generated with all the animals available for the analysis, whereas (b) was generated for animals that did not receive any medications. Controls (red), 1990s (blue), and 2008 (green) groups.




**Supplementary Figure S11.** NMDS plots for metabolomics analysis including outliers. (a) Overall profile of NMDS plots including data both at Infant (red) and Juvenile (blue) time points. NMDS plots for data collected from controls (red), 1990s (blue), and 2008 (green) at the (b) Infant and (c) Juvenile time points. Outliers are indicated with arrows.




**Supplementary Figure S12.** NMDS plots for microbiota analysis including outliers, based on weighted UniFrac distance. (a) Overall NMDS plot including data from Infant (red) and Juvenile (blue) time points. NMDS plots for data collected from controls (red), 1990s (blue), and 2008 (green) groups at both (b) Infant and (c) Juvenile time points. Outliers are indicated with arrows.




**Supplementary Figure S13.** Box plot showing the alpha diversity values based on Shannon analysis method including outliers. Circles represent data at the Infant time point, triangles represent data at the Juvenile time point. Outliers are indicated with circles. The middle line in the box represents median of the data, and the box represents lower and higher quantiles. The edges of wiskers represent the lowest and highest values of the dataset. Controls (red), 1990s (blue), and 2008 (green) groups.

**Supplementary Table S1.** Mode of delivery, year of birth, age at collection, sample availability, and study outliers for samples analyzed. Abbrev: N/A, sample not available.

| Group | Animal ID | Mode of Delivery | Infant | | | Juvenile | | |
|---|---|---|---|---|---|---|---|---|
| | | | Sample year | Animal age (days) | Outlier | Sample year | Animal age (days) | Outlier |
| Control | 1 | Vaginal | 2008 | 5 | | 2010 | 604 | |
| | 2 | Vaginal | 2008 | 7 | | 2010 | 595 | |
| | 3 | Vaginal | 2008 | 9 | | 2010 | 615 | |
| | 4 | Vaginal | 2008 | 7 | | 2010 | 613 | |
| | 5 | Vaginal | 2009 | 8 | | 2010 | 578 | ✓ |
| | 6 | Vaginal | 2009 | 7 | | 2010 | 561 | |
| | 7 | Vaginal | N/A | N/A | | 2010 | 559 | |
| | 8 | Vaginal | 2009 | 7 | | 2010 | 548 | |
| | 9 | Vaginal | 2011 | 7 | | 2012 | 571 | |
| | 10 | Vaginal | 2011 | 7 | | 2012 | 564 | |
| | 11 | Vaginal | 2011 | 8 | | 2012 | 563 | ✓ |
| | 12 | Vaginal | 2011 | 7 | | 2012 | 549 | |
| | 13 | C-section | 2011 | 7 | | 2013 | 563 | |
| | 14 | Vaginal | 2011 | 8 | | 2013 | 563 | |
| | 15 | Vaginal | 2011 | 7 | | 2013 | 551 | |
| | 16 | Vaginal | 2011 | 7 | | 2013 | 543 | |
| 1990s | 1 | Vaginal | 2009 | 9 | | 2011 | 620 | |
| | 2 | Vaginal | 2009 | 7 | | 2011 | 610 | |
| | 3 | Vaginal | 2009 | 6 | | 2011 | 607 | |
| | 4 | C-section | 2009 | 8 | ✓ | 2011 | 595 | ✓ |
| | 5 | Vaginal | N/A | N/A | | 2011 | 540 | |
| | 6 | Vaginal | 2010 | 7 | ✓ | 2011 | 538 | |
| | 7 | Vaginal | 2010 | 7 | | 2011 | 537 | |
| | 8 | Vaginal | 2010 | 7 | | 2011 | 530 | |
| | 9 | C-section | 2010 | 7 | | 2012 | 563 | ✓ |
| | 10 | Vaginal | 2010 | 9 | | 2012 | 557 | |
| | 11 | Vaginal | 2010 | 8 | | 2012 | 556 | |
| | 12 | Vaginal | N/A | N/A | | 2012 | 554 | |
| 2008 | 1 | Vaginal | 2009 | 7 | ✓ | 2010 | 562 | |
| | 2 | Vaginal | 2009 | 8 | | 2010 | 554 | |
| | 3 | Vaginal | 2009 | 6 | | 2010 | 545 | |
| | 4 | C-section | 2009 | 7 | | 2010 | 543 | |
| | 5 | Vaginal | 2010 | 7 | ✓ | 2011 | 550 | |
| | 6 | Vaginal | 2010 | 7 | | 2011 | 542 | |
| | 7 | Vaginal | 2010 | 6 | | 2011 | 546 | |
| | 8 | Vaginal | 2010 | 7 | | 2011 | 539 | |
| | 9 | Vaginal | 2011 | 7 | | 2013 | 582 | |
| | 10 | Vaginal | 2011 | 7 | | 2013 | 577 | |
| | 11 | C-section | 2011 | 7 | | 2013 | 576 | |
| | 12 | C-section | 2011 | 7 | ✓ | 2013 | 576 | |

**Supplementary Table S2.** Summary of the result of the KW test on the metabolome dataset. Metabolites with significant p-values prior to FDR correction are listed. KW chi-squared value ($\chi^2$) and statistical power, as well as the p-value and the effect size (r) of the Mann-Whitney U test as a post hoc test are included. From the effect size measurement, the magnitude of difference can be considered to be small when r<0.3, medium when 0.3<r<0.5, and large when 0.5<r. Median and 95% CI are also shown. In order to avoid inflation of the false positive rate in the Mann-Whitney U test, Bonferroni was used to obtain a new threshold, with significance assumed when p-values were less than 0.05/3=0.017.

| Time point | KW test | | | | | Post-hoc test | | | Median; 95% CI [lower limit, upper limit] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Metabolite | p-value | Adjusted p-value | KW chi-squared value | Estimated power (%) | Control vs 1990s | Control vs 2008 | 1990s vs 2008 | Control | 1990s | 2008 |
| Infant | Glucose | 0.049 | 0.91 | 6.02 | 66~97 | p=0.23, r=0.38 | p=1.00, r=0.042 | p=0.024, r=0.67 | 3.19; [3.13, 3.79] | 3.48; [3.33, 4.28] | 3.21; [3.01, 3.32] |
| Juvenile | Acetate | 0.013 | 0.27 | 8.62 | 75~89 | p=0.62, r=0.26 | p=0.20, r=0.36 | **p=0.015, r=0.60** | 5.30; [5.23, 5.35] | 5.26; [5.17, 5.29] | 5.35; [5.31, 5.46] |
| | 4-aminobutyrate | 0.0031 | 0.18 | 11.57 | 92~99 | p=1.00, r=0.18 | p=0.033, r=0.50 | **p=0.0052, r=0.67** | 2.44; [2.35, 2.49] | 2.38; [2.29, 2.46] | 2.57; [2.49, 2.75] |
| | Ethanol | 0.019 | 0.27 | 8.06 | 73~88 | p=0.69, r=0.25 | p=0.28, r=0.33 | p=0.019, r=0.59 | 2.94; [2.82, 3.10] | 2.80; [2.70, 3.01] | 3.04; [2.93, 3.30] |
| | Mythylamine | 0.023 | 0.27 | 7.89 | 65~81 | p=0.69, r=0.25 | p=0.098, r=0.42 | p=0.069, r=0.49 | 2.32; [2.17, 2.57] | 2.13; [1.04, 2.55] | 2.83; [2.73, 2.95] |
| | Propionate | 0.018 | 0.27 | 7.57 | 70~86 | p=1.00, r=0.13 | p=0.11, r=0.41 | p=0.023, r=0.58 | 4.74; [4.68, 4.83] | 4.73; [4.60, 4.79] | 4.90; [4.79, 5.06] |
| | Valerate | 0.043 | 0.28 | 6.30 | 58~75 | p=0.053, r=0.49 | p=1.00, r=0.050 | p=0.16, r=0.42 | 3.82; [3.79, 3.91] | 3.73; [3.65, 3.83] | 3.85; [3.74, 4.00] |

**Supplementary Table S3.** Clinical indication for treatment by group, medications administered, length of treatment, and the number of days post treatment before juvenile stool samples were collected. *The number of days post treatment represents the length of time (days) that passed after treatment was completed until the juvenile stool sample was collected. For all but one animal (a control), at least 60 days passed. None of the animals had received any medications prior to the collection of the Infant sample.

| Group | Animal ID | Clinical Indication | Medication | Length of Tx (days) | Number of days post Tx* |
|---|---|---|---|---|---|
| Control | 1 | Giardia - prophylactic | Metronidazole | 14 | 83 |
| | 2 | Giardia - prophylactic | Metronidazole | 14 | 83 |
| | 3 | Other bacterial infection | Amoxicillin clavulanate | 6 | 542 |
| | 3 | Giardia - prophylactic | Metronidazole | 14 | 101 |
| | 4 | Giardia - prophylactic | Metronidazole | 14 | 101 |
| | 5 | Giardia - prophylactic | Metronidazole | 14 | 317 |
| | 6 | Giardia - prophylactic | Metronidazole | 14 | 317 |
| | 7 | Other bacterial infection | Amoxicillin clavulanate | 8 | 498 |
| | 7 | Giardia - prophylactic | Metronidazole | 14 | 318 |
| | 7 | Diarrhea | Lactobacillus | 13 | 498 |
| | 8 | Giardia - prophylactic | Metronidazole | 14 | 318 |
| | 8 | Giardia | Metronidazole | 14 | 337 |
| | 8 | Diarrhea | Lactobacillus | 5 | 346 |
| | 10 | Other bacterial infection | Procaine penicillin G | 4 | 25 |
| | 12 | Other bacterial infection | Amoxicillin clavulanate | 8 | 553 |
| 1990s | 1 | Giardia - prophylactic | Metronidazole | 14 | 407 |
| | 2 | Other bacterial infection | Enrofloxacin | 10 | 377 |
| | 2 | Giardia - prophylactic | Metronidazole | 14 | 407 |
| | 3 | Other bacterial infection | Amoxicillin clavulanate | 14 | 538 |
| | 3 | Giardia - prophylactic | Metronidazole | 14 | 408 |
| | 4 | Giardia - prophylactic | Metronidazole | 14 | 408 |
| | 11 | Giardia | Metronidazole | 14 | 515 |
| 2008 | 1 | Giardia - prophylactic | Metronidazole | 14 | 254 |
| | 2 | Giardia - prophylactic | Metronidazole | 14 | 254 |
| | 3 | Giardia - prophylactic | Metronidazole | 14 | 255 |
| | 3 | Diarrhea | Lactobacillus | 7 | 479 |
| | 4 | Other bacterial infection | Amoxicillin clavulanate | 12 | 378 |
| | 4 | Giardia - prophylactic | Metronidazole | 14 | 255 |
| | 6 | Other bacterial infection | Amoxicillin clavulanate | 8 | 60 |
| | 9 | Other bacterial infection | Amoxicillin clavulanate | 8 | 511 |

**Supplementary Table S4.** The effect of potential confounding factors on the metabolome data analyzed by non-parametric ANCOVA. Age was included as the confounding factor for the Infant time point, whereas age and drug administration were added for the Juvenile time point. Metabolites that showed significant p-values prior to the FDR correction are included.

| Time point | Metabolite | p-value | Adjusted p-value |
|---|---|---|---|
| Infant | Isovalerate | 0.027 | 0.35 |
| | Trimethylamine | 0.036 | 0.35 |
| Juvenile | 4 Hydroxyphenylacetate | 0.045 | 0.43 |
| | Formate | 0.036 | 0.43 |
| | Fucose | 0.025 | 0.43 |
| | Taurine | 0.039 | 0.43 |
| | Uridine | 0.017 | 0.43 |

**Supplementary Table S5.** Summary of the results of the KW test on the metabolome data for animals receiving no medications at the Juvenile time point (Sample size: control=5, 1990s=5, 2008=4). Metabolites with significant p-values prior to FDR correction are listed. Outliers found in Supplementary Figure S6 were removed. The KW chi-squared value ($\chi^2$) and statistical power, as well as the p-value and the effect size (r) of the Mann-Whitney U test as a post hoc test are included. From the effect size measurement, the magnitude of difference can be considered to be small when $r<0.3$, medium when $0.3<r<0.5$, and large when $0.5<r$. Furthermore, median and the 95% CI are available. In order to avoid inflation of the false positive rate in the Mann-Whitney U test, Bonferroni was used to obtain the new threshold, with significance assumed when p-values were less than 0.05/3=0.017.

| Time point | Metabolites | KW test | | | | Post-hoc test | | | Median; 95% CI [lower limit, upper limit] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | p-value | Adjusted p-value | KW $\chi$2 value | Estimated power (%) | Control vs 1990s | Control vs 2008 | 1990s vs 2008 | Control | 1990s | 2008 |
| Juvenile | 4_Aminobutyrate | 0.030 | 0.29 | 7.05 | 71~93 | p=0.18, r=0.63 | p=1.00, r=0.16 | p=0.060, r=0.82 | 2.47; [2.32, 2.94] | 2.36; [2.14, 2.42] | 2.55; [2.43, 2.75] |
| | Acetate | 0.030 | 0.29 | 7.05 | 71~94 | p=0.18, r=0.63 | p=1.00, r=0.16 | p=0.060, r=0.82 | 5.35; [5.23, 5.58] | 5.25; [5.08, 5.30] | 5.35; [5.33, 5.57] |
| | Butyrate | 0.027 | 0.29 | 7.24 | 75~96 | p=0.18, r=0.63 | p=1.00, r=0.24 | p=0.060, r=0.82 | 4.50; [4.35, 4.88] | 4.41; [4.15, 4.46] | 4.57; [4.52, 4.85] |
| | Hypoxanthine | 0.0065 | 0.29 | 10.06 | 100 | p=0.18, r=0.63 | p=0.060, r=0.82 | p=0.060, r=0.82 | 3.11; [2.97, 3.15] | 3.02; [2.89, 3.06] | 3.32; [3.17, 3.30] |
| | Methylamine | 0.034 | 0.29 | 6.76 | 53~80 | p=0.037, r=0.83 | p=1.00, r=0.16 | p=0.33, r=0.57 | 2.54; [2.34, 3.29] | 2.13; [0, 2.26] | 2.80; [2.09, 3.15] |
| | Nicotinate | 0.031 | 0.29 | 6.94 | 74~94 | p=0.28, r=0.56 | p=1.00, r=0.33 | p=0.060, r=0.82 | 2.83; [2.72, 2.95] | 2.74; [2.40, 2.82] | 2.90; [2.86, 3.06] |
| | Propylene glycol | 0.022 | 0.29 | 7.60 | 85~98 | p=0.28, r=0.56 | p=0.054, r=0.83 | p=0.68, r=0.45 | 2.01; [1.90, 2.87] | 1.84; [0, 2.03] | 0; [0, 1.54] |

**Supplementary Table S6.** The batch effect on the metabolome data analyzed by non-parametric ANCOVA. Metabolites that showed significant p-values prior to the FDR correction at the Juvenile time point are included. At the Infant time point, no metabolites showed significant p-value even prior to FDR correction (data not shown).

| Compound | p-value | Adjusted p-value |
|---|---|---|
| 4-Aminobutyrate | 0.029 | 0.14 |
| Aspartate | 0.039 | 0.14 |
| Formate | 0.027 | 0.14 |
| Glutamate | 0.030 | 0.14 |
| Glycine | 0.040 | 0.14 |
| Hypoxanthine | 0.036 | 0.14 |
| Isoleucine | 0.023 | 0.14 |
| Isopropanol | 0.034 | 0.14 |
| Leucine | 0.029 | 0.14 |
| Methionine | 0.047 | 0.15 |
| myo-Inositol | 0.050 | 0.15 |
| Phenylalanine | 0.027 | 0.14 |
| Ribose | 0.035 | 0.14 |
| Serine | 0.023 | 0.14 |
| Succinate | 0.030 | 0.14 |
| Tyrosine | 0.031 | 0.14 |
| Uracil | 0.031 | 0.14 |
| Valerate | 0.026 | 0.14 |
| Valine | 0.048 | 0.15 |
| Xylose | 0.032 | 0.14 |

**Supplementary Table S7.** The results of the KW test on the different alpha diversity measurement methods on the microbiota data at both time points.

| Statistical Test | Infant P value | Juvenile P value |
|---|---|---|
| Chao1 | 0.88 | 0.63 |
| ACE | 0.40 | 0.54 |
| Shannon | 0.99 | 0.54 |
| Simpson | 0.82 | 0.39 |
| InvSimpson | 0.77 | 0.66 |
| Fisher | 0.62 | 0.15 |

**Supplementary Table S8.** List of bacteria that showed the highest average relative abundances in each group.

| Time point | Rank | Infant | | Juvenile | |
|---|---|---|---|---|---|
| | | **Name** | **Abundance (%)** | **Name** | **Abundance (%)** |
| Control | 1 | *Bifidobacterium* | 30.45 | *Prevotella* | 7.72 |
| | 2 | *Blautia* | 8.79 | *Lactobacillus* | 7.27 |
| | 3 | *[Eubacterium]* | 8.63 | *Ruminococcus* | 7.17 |
| | 4 | *Catenibacterium* | 6.57 | *Blautia* | 3.64 |
| | 5 | *Collinsella* | 5.92 | *Coprococcus* | 3.19 |
| | 6 | *Streptococcus* | 5.17 | *[Eubacterium]* | 2.80 |
| | 7 | *Prevotella* | 3.17 | *Catenibacterium* | 2.18 |
| | 8 | *Lactobacillus* | 1.92 | *Dialister* | 1.94 |
| | 9 | *Bulleidia* | 1.56 | *Clostridium* | 1.47 |
| | 10 | *Dorea* | 1.53 | *Treponema* | 1.44 |
| 1990s | 1 | *Bifidobacterium* | 32.11 | *Lactobacillus* | 14.27 |
| | 2 | *[Eubacterium]* | 11.60 | *Prevotella* | 7.41 |
| | 3 | *Prevotella* | 7.04 | *Ruminococcus* | 6.35 |
| | 4 | *Catenibacterium* | 5.78 | *Coprococcus* | 3.79 |
| | 5 | *Blautia* | 5.65 | *Blautia* | 3.02 |
| | 6 | *Bacteroides* | 4.55 | *Treponema* | 2.30 |
| | 7 | *Streptococcus* | 3.65 | *Dialister* | 2.26 |
| | 8 | *[Prevotella]* | 3.59 | *[Eubacterium]* | 1.80 |
| | 9 | *[Ruminococcus]* | 2.35 | *Catenibacterium* | 1.60 |
| | 10 | *Lactobacillus* | 2.10 | *Faecalibacterium* | 1.40 |
| 2008 | 1 | *Bifidobacterium* | 20.10 | *Lactobacillus* | 14.04 |
| | 2 | *[Eubacterium]* | 11.30 | *Prevotella* | 9.34 |
| | 3 | *Blautia* | 8.99 | *Ruminococcus* | 4.34 |
| | 4 | *Catenibacterium* | 5.53 | *Blautia* | 4.12 |
| | 5 | *Enterococcus* | 4.23 | *Coprococcus* | 3.43 |
| | 6 | *Lactobacillus* | 3.75 | *Dialister* | 2.58 |
| | 7 | *Streptococcus* | 3.19 | *[Eubacterium]* | 1.90 |
| | 8 | *Bacteroides* | 2.63 | *Faecalibacterium* | 1.85 |
| | 9 | *Clostridium* | 2.36 | *Treponema* | 1.74 |
| | 10 | *[Ruminococcus]* | 2.33 | *Dorea* | 1.36 |

**Supplementary Table S9.** Summary of the results of likelihood ratio test on the bacterial abundance at the genus level. Bacteria with p-values less than 0.05 prior to FDR correction are included. The average of the normalized count values (baseMean), p-value, adjusted p-value, and the estimated power (%) are included. Also, the estimated fold difference in the bacterial abundance between the two group (log2FoldChange) is listed as the effect size measurement, as well as median and the 95% CI.

| Time point | Bacteria taxonomy | baseMean | p-value | Adjusted p-value | Estimated power (%) | log2FoldChange | | | Median; 95% CI [lower limit, upper limit] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Control/1990s | Control/2008 | 1990s/2008 | Control | 1990s | 2008 |
| Infant | *Aggregatibacter* | 60.07 | 0.047 | 0.80 | 41~46 | 0.97 | -3.13 | -4.10 | 1; [0, 25] | 3; [0, 14] | 18; [2, 308] |
| | *Enterococcus* | 202.44 | 0.0034 | 0.12 | 48~52 | 3.27 | -3.59 | -6.85 | 4; [1, 242] | 0; [0, 22] | 11; [1, 1110] |
| Juvenile | *Mogibacterium* | 46.60 | 0.0023 | 0.10 | 92~99 | 1.72 | 0.87 | -0.85 | 62; [30, 85] | 14; [12, 26] | 32; [21, 64] |

**Supplementary Table S10.** Summary of the results of likelihood ratio test on the bacterial abundance at the family level. Bacteria with p-values less than 0.05 prior to the FDR correction are included. The average of the normalized count values (baseMean), p-value, adjusted p-value, and the estimated power (%) are included. Also, the estimated fold difference in the bacterial abundance between the two group (log2FoldChange) is listed as the effect size measurement, as well as median and the 95% CI are available.

| Time point | Bacteria taxonomy | baseMean | p-value | Adjusted p-value | Estimated power (%) | log2FoldChange | | | Median; 95% CI [lower limit, upper limit] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Control/ 1990s | Control/ 2008 | 1990s/2008 | Control | 1990s | 2008 |
| Infant | *Bacteroidaceae* | 325.01 | 0.027 | 0.41 | 42~47 | -5.61 | -6.18 | -0.56 | 2; [0, 9] | 4; [1, 766] | 0; [0, 16] |
| | *Enterobacteriaceae* | 236.38 | 0.046 | 0.41 | 59~82 | 0.16 | -1.45 | -1.61 | 231; [57, 456] | 118; [78, 379] | 305; [189, 1770] |
| | *Enterococcaceae* | 121.44 | 0.046 | 0.41 | 54~72 | 1.08 | -2.94 | -4.02 | 4; [1, 262] | 0; [0, 22] | 11; [1, 1200] |
| Juvenile | *Erysipelotrichaceae* | 577.11 | 0.037 | 0.86 | 51~67 | 0.39 | 0.46 | 0.072 | 713; [554, 886] | 563; [323, 636] | 496; [378, 734] |

**Supplementary Table S11.** Summary of the results of likelihood ratio test on the bacterial abundance at order level. Bacteria with p-values less than 0.05 prior to the FDR correction are included. The average of the normalized count values (baseMean), p-value, adjusted p-value, and the estimated power (%) are included. Also, the estimated fold difference in the bacterial abundance between the two group (log2FoldChange) is listed as the effect size measurement, as well as median and the 95% CI.

| Time point | Bacteria taxonomy | baseMean | p-value | Adjusted p-value | Estimated power (%) | log2FoldChange | | | Median; 95% CI [lower limit, upper limit] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Control/1990s | Control/2008 | 1990s/2008 | Control | 1990s | 2008 |
| Infant | *Enterobacteriales* | 325.59 | 0.035 | 0.58 | 66~84 | 0.39 | -1.65 | -2.04 | 231; [57, 456] | 118; [78, 379] | 305; [189, 1770] |
| Juvenile | *RF32* | 15.76 | 0.031 | 0.56 | 63~80 | 2.11 | 1.20 | -0.91 | 10; [6, 41] | 3; [1, 6] | 5; [1, 11] |

**Supplementary Table S12.** The effect of potential confounding factors (age and/or administration of medications) on the microbiota data at the genus level was analyzed by the likelihood ratio test in DESeq2. Bacteria with p-values less than 0.05 prior to the FDR correction are included. The average of the normalized count values (baseMean), p-value, adjusted p-value, and log2FoldChange for each pair of groups are included.

| Time point | Bacteria taxonomy | baseMean | p-value | Adjusted p-value | log2FoldChange | | |
|---|---|---|---|---|---|---|---|
| | | | | | Control/1990s | Control/2008 | 1990s/2008 |
| Infant | *Enterococcus* | 202.44 | 0.0013 | 0.045 | 3.27 | -3.59 | -6.85 |
| Juvenile | *Catenibacterium* | 179.05 | 0.015 | 0.23 | 0.31 | 1.01 | 0.70 |
| | *Clostridium* | 130.89 | 0.030 | 0.35 | 1.13 | 0.51 | -0.62 |
| | *[Eubacterium]* | 236.53 | 0.0068 | 0.16 | 0.48 | 0.67 | 0.19 |
| | *Mogibacterium* | 46.60 | 0.0015 | 0.069 | 1.72 | 0.87 | -0.85 |

**Supplementary Table S13.** The batch effect on the microbiota data at the genus level was analyzed by the likelihood ratio test in DESeq2. Bacteria with p-values less than 0.05 prior to the FDR correction are included. The average of the normalized count values (baseMean), p-value, adjusted p-value, and log2FoldChange for each pair of groups are included.

| Time point | Bacteria taxonomy | baseMean | p-value | Adjusted p-value | log2FoldChange | | |
|---|---|---|---|---|---|---|---|
| | | | | | Control/1990s | Control/2008 | 1990s/2008 |
| Infant | *Aggregatibacter* | 60.07 | 0.038 | 0.65 | 0.97 | -3.13 | -4.10 |
| | *Enterococcus* | 202.44 | 0.0015 | 0.053 | 3.27 | -3.59 | -6.85 |
| Juvenile | *Catenibacterium* | 179.05 | 0.0079 | 0.12 | 0.31 | 1.01 | 0.70 |
| | *Clostridium* | 130.89 | 0.032 | 0.30 | 1.13 | 0.51 | -0.62 |
| | *[Eubacterium]* | 236.53 | 0.0040 | 0.092 | 0.48 | 0.67 | 0.19 |
| | *Mogibacterium* | 46.60 | 0.0026 | 0.092 | 1.72 | 0.87 | -0.85 |

**Supplementary Table S14.** Likelihood ratio test in DESeq2 was applied to the animals receiving no medications at the Juvenile time point. The outlier found in Supplementary Figure S9 was removed in this analysis. No animals at the Infant time point had received any medications. Bacteria with p-values less than 0.05 prior to the FDR correction are included. The average of the normalized count values (baseMean), p-value, adjusted p-value, and log2FoldChange for each pair of groups are included.

| Time point | Bacteria taxonomy | baseMean | p-value | Adjusted p-value | log2FoldChange | | |
|---|---|---|---|---|---|---|---|
| | | | | | Control/1990s | Control/2008 | 1990s/2008 |
| Juvenile | *Lachnospira* | 43.71 | 0.00013 | 0.0058 | 1.23 | -1.32 | -2.56 |

**Supplementary Table S15.** Vaccine source, ethylmercury (EtHg) content for thimersoal-containing vaccines created specifically for the study, and route of vaccine administration. Abbr: IM, intramuscular; sub Q, subcutaneous. [a] These vaccines were thimerosal-free for the 2008 schedule. [b] For pregnant dams only. Modified with permission[11].

| Vaccine | Trade name (Manufacture) NDC # | EtHg content (µg/0.5 ml dose) | Route of Administration |
|---|---|---|---|
| Hepatitis B | Recombivax HB (Merck) 0006-4981-00 | 1.98[a] | IM |
| Diphtheria, Tetanus, acellular Pertussis | Infanrix (GlaxoSmithKline) 58160-810-46 | 3.97[a] | IM |
| Haemophilus Influenza B | ActHIB (Sanofi Pasteur) 49281-545-05 | 3.96[a] | IM |
| Measles Mumps Rubella | MMR-II (Merck) 0006-4682-00 | N/A | sub Q |
| Inactivated Polio vaccine | IPOL (Sanofi Pasteur) 49281-860-10 | N/A | IM |
| Rotavirus | Rotateq (Merck) 0006-4047-41 | N/A | Oral gavage |
| Pneumococcal 7- valent Conjugate Vaccine | Prevnar (Wyeth) 0005-1970-67 | N/A | IM |
| Hepatitis A | VAQTA (Merck) 0006-4831-41 | N/A | IM |
| Varicellar | Varivax (Merck) 0006-4827-00 | N/A | sub Q |
| Meningococcal Polysaccharide Vaccine | Menomune (Sanofi Pasteur) 49281-489-05 | 3.96 | sub Q |
| Influenza | Fluzone (Sanofi Pasteur) 49281-009-50 | 3.96 | IM |
| Influenza | Fluzone (Sanofi Pasteur) 49281-382-15 | 25[b] | IM |