

**Table S1. Model statistics for study subject subgroups**

Dataset*	PCA					OPLS-DA					
	Model statistics	Delivery mode		Feeding method		Delivery mode			Feeding method		
		Unadjusted <i>P</i>	Adjusted† <i>P</i>	Unadjusted <i>P</i>	Adjusted† <i>P</i>	Model statistics	Unadjusted <i>P</i>	Adjusted† <i>P</i>	Model statistics	Unadjusted <i>P</i>	Adjusted† <i>P</i>
1. N=121	15 comp R <sup>2</sup> X=0.9 Q <sup>2</sup> =0.46	P1; p<0.001 P2; p<0.001	P1; p=0.04 P2; p<0.001	P1; p<0.001 P2; p=0.11	P1; p<0.001 P2; p=0.14	1+1 R <sup>2</sup> X= 0.55 R <sup>2</sup> Y=0.31 Q <sup>2</sup> =0.14	Pre p<0.001 Ort p=1.00	Pre p=0.02 Ort p<0.01	2+1 R <sup>2</sup> X= 0.58 R <sup>2</sup> Y=0.39 Q <sup>2</sup> =0.18	Pre p<0.001 Ort p=0.23	Pre p<0.001 Ort p=0.93
2. N=119	4 comp R <sup>2</sup> X= 0.66 Q <sup>2</sup> =0.46	P1; p=0.04 P2; p<0.001	P1; p=0.05 P2; p<0.01	P1; p<0.001 P2; p=0.03	P1; p<0.001 P2; p=0.04	1+1 R <sup>2</sup> X= 0.55 R <sup>2</sup> Y=0.31 Q <sup>2</sup> =0.11	Pre p<0.001 Ort p=1.00	Pre p<0.001 Ort p=1.00	2+1 R <sup>2</sup> X= 0.58 R <sup>2</sup> Y=0.39 Q <sup>2</sup> =0.13	Pre p<0.001 Ort p=0.93	Pre p<0.001 Ort 0.93
3. N=67	3 comp R <sup>2</sup> X= 0.63 Q <sup>2</sup> =0.42	P1; p=0.95 P2; p=0.96	P1; p=1.00 P2; p=0.99	P1; p<0.001 P2; p=0.71	P1; p<0.001 P2; p=0.85	1+1 R <sup>2</sup> X= 0.29 R <sup>2</sup> Y=0.16 Q <sup>2</sup> =-0.21	Pre p<0.001 Ort p=1.000	Pre p<0.001 Ort p=1.00	2+1 R <sup>2</sup> X= 0.52 R <sup>2</sup> Y=0.47 Q <sup>2</sup> =0.30	Pre p<0.001 Ort p=0.99	Pre p<0.001 Ort p=1.00
4. N=54	6 comp R <sup>2</sup> X= 0.71 Q <sup>2</sup> =0.54	P1; p=0.16 P2; p=0.67	P1; p=0.32 P2; p=0.56	P1; p<0.001 P2; p=0.21	P1; p<0.001 P2; p=0.31	1+1 R <sup>2</sup> X= 0.44 R <sup>2</sup> Y=0.43 Q <sup>2</sup> =-0.02	Pre p<0.001 Ort p=1.00	Pre p<0.001 Ort p=1.00	2+1 R <sup>2</sup> X= 0.54 R <sup>2</sup> Y=0.57 Q <sup>2</sup> =0.12	Pre p<0.001 Ort p=0.93	Pre p<0.001 Ort p=0.90
5.‡ N=34	4 comp R <sup>2</sup> X= 0.66 Q <sup>2</sup> =0.47	P1; p=0.49 P2; p=0.47	P1; p=0.53 P2; p=0.56	P1; p<0.001 P2; p=0.21	P1; p<0.001 P2; p=0.32	1+1 R <sup>2</sup> X= 0.64 R <sup>2</sup> Y=0.72 Q <sup>2</sup> =0.07	Pre p<0.001 Ort p<0.001	Pre p<0.001 Ort p<0.001	2+1 R <sup>2</sup> X= 0.54 R <sup>2</sup> Y=0.45 Q <sup>2</sup> =0.30	Pre p<0.001 Ort p=0.90	Pre p<0.001 Ort p=0.94

\***Dataset 1:** All subjects excluding infants born < 37 weeks (main analysis group); **2:** Dataset 1 excluding infants who received antibiotics (intrapartum and postpartum); **3:** Males of Dataset 1; **4:** Females of Dataset 1; **5:** Cesarean-born infants of Dataset 1 (see note below); comp: components; P1: first principal component; P2: second principal component; PCA: principal components analysis; OPLS-DA: orthogonal partial least squares discriminant analysis; Pre: predictive axis; Ort: orthogonal axis.

P values were derived from linear regression; †Adjusted for maternal smoking and BMI; ‡The column for delivery mode is not applicable here; we instead examine infants born via Cesarean section using epidural anesthesia vs. general anesthesia

**Table S2. Significant NMR bins and library matched metabolites that distinguish Caesarean-delivered vs. vaginally delivered infants (in order of increasing fold change; N=32 bins). These features represent signals that have p value of <0.1 and VIP score of  $\geq 1$  (no metabolites were significant at FDR p value (q) of <0.1).**

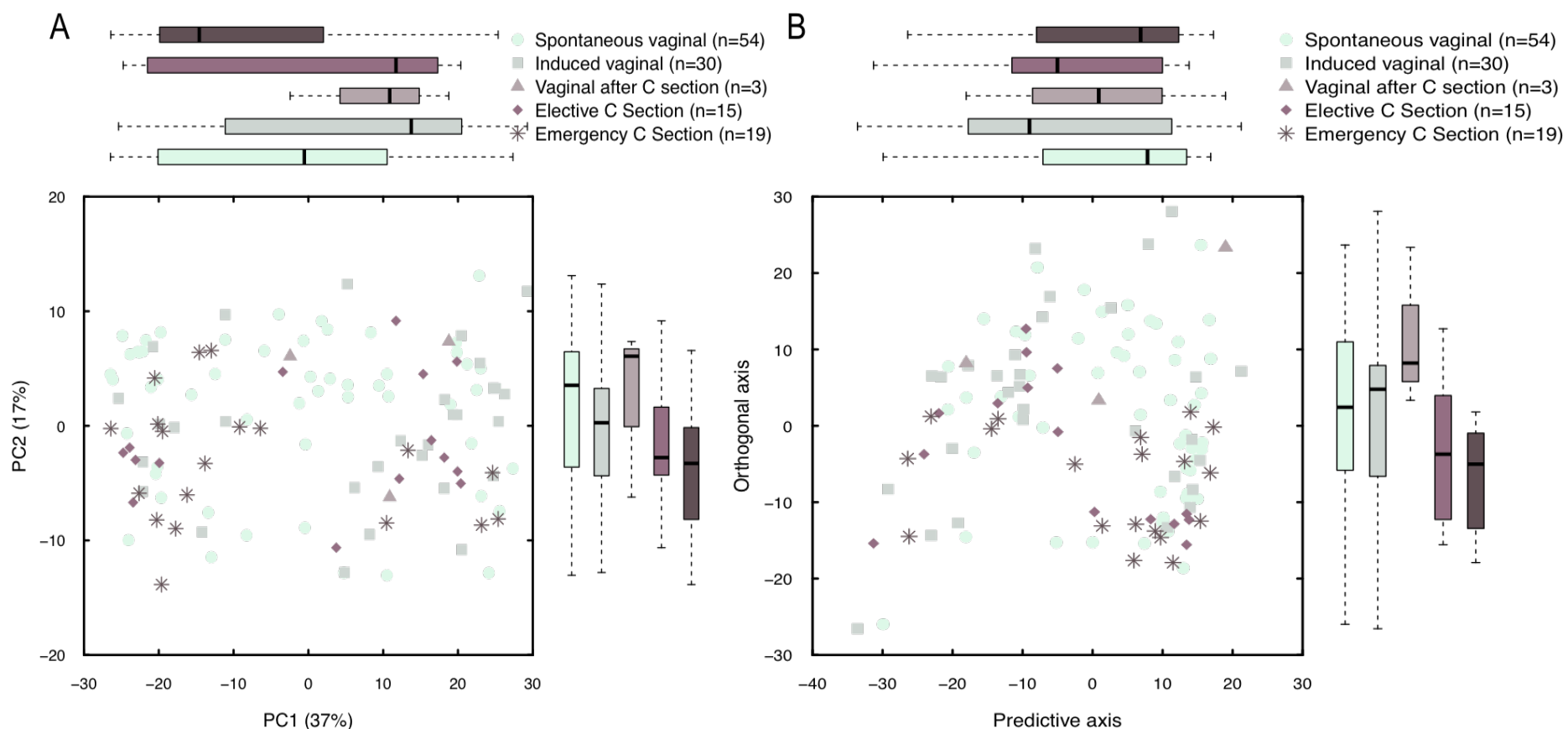
Bin (ppm)	Library-matched metabolite(s)	VIP score	Log <sub>2</sub> fold change	Unadjusted P value	FDR p value (q)
2.37 .. 2.43	Succinate	2.98	-0.606	0.008	0.12
2.49 .. 2.52	Overlapped	1.40	-0.452	0.013	0.13
2.32 .. 2.37	Glutamate	1.71	-0.395	0.006	0.10
3.39 .. 3.44	Glucose	1.56	-0.361	0.001	0.10
2.60 .. 2.65	Methionine	1.31	-0.358	0.005	0.10
1.44 .. 1.50	Alanine	2.39	-0.358	0.010	0.12
2.98 .. 3.04	Lysine   Creatinine   Creatine	1.86	-0.337	0.051	0.25
1.30 .. 1.36	Lactate   Threonine	3.03	-0.335	0.005	0.10
2.24 .. 2.29	Valine	1.49	-0.322	0.008	0.12
2.20 .. 2.24	Fatty acids	1.18	-0.305	0.058	0.26
1.02 .. 1.07	Propionate   Valine	1.74	-0.300	0.059	0.26
0.97 .. 1.02	Valine   Isoleucine	1.77	-0.259	0.023	0.16
1.95 .. 1.99	Fatty acids	1.30	-0.253	0.027	0.17
1.68 .. 1.74	Leucine   Lysine	1.84	-0.232	0.095	0.33
4.02 .. 4.07	Overlapped	1.01	-0.205	0.004	0.10
2.08 .. 2.14	Butyrate   Glutamate   Glutamine   Methionine	1.43	-0.167	0.069	0.27
4.07 .. 4.13	Lactate   Proline	1.01	-0.097	0.014	0.13
3.81 .. 3.86	Overlapped	3.34	0.163	0.051	0.25
3.71 .. 3.77	Glutamate   Leucine   Lysine   Fucose   Glucose	3.32	0.170	0.045	0.24
3.91 .. 3.96	Overlapped	2.37	0.176	0.072	0.28
4.22 .. 4.27	Threonine   Sugars	1.66	0.190	0.045	0.24
1.25 .. 1.30	Fatty acids	2.12	0.225	0.065	0.26
3.66 .. 3.68	Glycerol   isoleucine	2.52	0.252	0.001	0.10
3.77 .. 3.81	Alanine	3.76	0.257	0.032	0.19
3.86 .. 3.91	Overlapped	3.22	0.258	0.017	0.13
4.39 .. 4.45	Overlapped	1.45	0.288	0.054	0.26
3.68 .. 3.71	Unknown   Glucose	3.73	0.298	0.010	0.12
3.57 .. 3.59	Threonine   Sugars	1.97	0.305	0.031	0.19
4.49 .. 4.55	Arabinose   Fucose	1.13	0.324	0.010	0.12
4.61 .. 4.67	Glucose	1.47	0.334	0.041	0.23
5.26 .. 5.31	Galactose   Arabinose	1.50	0.338	0.073	0.28
1.20 .. 1.22	Fucose   Fatty acids	1.90	0.489	0.003	0.10

**Table S3. Significant NMR bins and library matched metabolites that distinguish formula-fed vs. exclusively breast-fed infants (in order of increasing fold change; N=49 bins). These features represent signals that have q value of <0.1 and VIP score of  $\geq 1$ .**

Bin (ppm)	Library-matched metabolite(s)	VIP score	Log <sub>2</sub> fold change	Unadjusted P value	FDR p value (q)
3.09 .. 3.15	Malonate   Phenylalanine	1.584	-1.238	<0.0001	4.48E-19
1.50 .. 1.56	Butyrate	2.121	-1.236	<0.0001	6.51E-20
0.86 .. 0.91	Butyrate	2.454	-1.207	<0.0001	1.58E-17
2.14 .. 2.20	Butyrate   Propionate	2.196	-1.094	<0.0001	6.95E-15
2.98 .. 3.04	Lysine   Creatinine   Creatine	2.483	-1.086	<0.0001	1.61E-14
1.74 .. 1.79	Overlapped	1.781	-1.055	<0.0001	7.12E-17
1.42 .. 1.44	Lysine   Isoleucine	1.111	-1.027	<0.0001	6.51E-20
1.58 .. 1.63	Overlapped	1.520	-1.020	<0.0001	7.86E-15
1.02 .. 1.07	Propionate   Valine	2.392	-1.018	<0.0001	2.37E-12
1.63 .. 1.68	Leucine   Lysine	1.790	-0.964	<0.0001	1.82E-14
1.38 .. 1.42	Overlapped	1.308	-0.910	<0.0001	2.01E-14
1.84 .. 1.86	Leucine   Lysine	1.112	-0.905	<0.0001	1.63E-15
1.68 .. 1.74	Leucine   Lysine	2.602	-0.899	<0.0001	3.61E-14
2.20 .. 2.24	Fatty acids	1.304	-0.867	<0.0001	1.61E-11
3.04 .. 3.09	Tyrosine	1.163	-0.847	<0.0001	5.83E-13
1.92 .. 1.95	Overlapped	1.112	-0.842	<0.0001	3.45E-17
2.32 .. 2.37	Glutamate	1.809	-0.799	<0.0001	1.52E-10
1.44 .. 1.50	Alanine	2.548	-0.778	<0.0001	4.23E-12
2.43 .. 2.49	Overlapped	1.015	-0.765	<0.0001	1.30E-09
0.91 .. 0.97	Isoleucine   Leucine	2.899	-0.705	<0.0001	5.83E-13
1.86 .. 1.92	Acetate   Lysine   Arginine	3.209	-0.681	<0.0001	3.70E-08
1.95 .. 1.99	Isoleucine   Proline	1.479	-0.644	<0.0001	3.66E-10
2.24 .. 2.29	Valine	1.301	-0.633	<0.0001	2.71E-10
0.97 .. 1.02	Valine   Isoleucine	1.781	-0.571	<0.0001	7.98E-09
2.08 .. 2.14	Butyrate   Glutamate   Glutamine   Methionine	1.748	-0.524	<0.0001	1.17E-10
3.15 .. 3.21	Choline   O-Acetylcholine   O-Acetylcarnitine   Tyrosine	1.020	-0.504	<0.0001	4.93E-08
2.37 .. 2.43	Succinate	1.734	-0.422	0.048	0.0613
4.22 .. 4.27	Threonine   Sugars	1.093	-0.369	<0.0001	9.21E-05
1.30 .. 1.36	Lactate   Threonine	1.144	-0.352	0.001	0.002
3.66 .. 3.68	Glycerol   isoleucine	1.245	0.193	0.006	0.008
3.53 .. 3.57	Glycine   Glycerol	1.607	0.379	<0.0001	3.52E-11
3.68 .. 3.71	Unknown   Glucose	1.181	0.388	<0.001	5.49E-04
3.81 .. 3.86	Overlapped	2.997	0.414	<0.0001	1.41E-07
3.44 .. 3.48	Glucose	1.720	0.488	<0.0001	3.61E-08
3.50 .. 3.53	Glucose	1.472	0.514	<0.0001	1.03E-11
3.62 .. 3.66	Fucose   Glycerol	2.692	0.523	<0.0001	1.11E-10

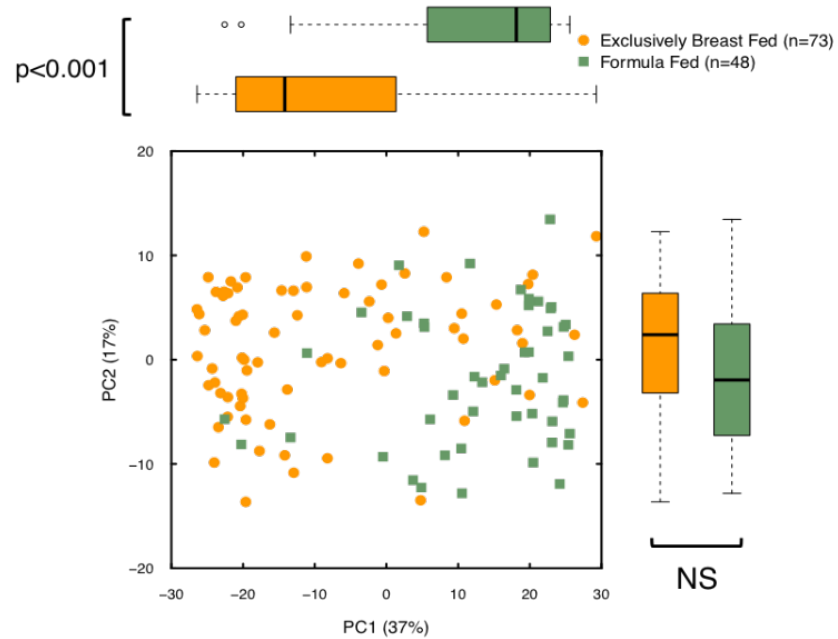
3.71 .. 3.77	Glutamate   Leucine   Lysine   Fucose   Glucose	4.091	0.535	<0.0001	4.23E-12
3.91 .. 3.96	Overlapped	2.819	0.561	<0.0001	1.11E-10
3.86 .. 3.91	Overlapped	3.291	0.579	<0.0001	9.63E-10
3.48 .. 3.50	Glucose	1.748	0.647	<0.0001	9.87E-11
2.01 .. 2.06	Glutamate   Proline   N-Acetyl amino acids	3.359	0.661	<0.0001	5.83E-13
3.77 .. 3.81	Alanine	3.663	0.682	<0.0001	5.10E-12
3.59 .. 3.62	Valine   Sugars	2.277	0.683	<0.0001	2.05E-14
3.57 .. 3.59	Threonine   Sugars	2.067	0.789	<0.0001	4.23E-12
1.22 .. 1.25	Fucose   Fatty acids	1.408	0.806	<0.0001	1.88E-07
4.39 .. 4.45	Overlapped	1.677	0.852	<0.0001	9.87E-11
4.61 .. 4.67	Glucose	1.069	0.869	<0.0001	2.84E-09
1.18 .. 1.20	Fatty acids	1.699	0.909	<0.0001	3.68E-12
1.12 .. 1.18	Propylene glycol   fatty acids	2.176	0.944	<0.0001	3.61E-14

**Figure S1. Comparison of stool metabolome between specific delivery type groups for N=121 subjects.** **A.** Scores plot of PCA of binned NMR data colored by specific delivery type. Number of Components = 15, Model statistics:  $R^2Y = 0.82$ ,  $R^2Y = 0.92$ ,  $Q^2$  (cum) = 0.55; **B.** Scores plot of OPLS-DA of binned NMR data colored by specific delivery type. Model statistics: Components = 4 predictive, 1 orthogonal,  $R^2X = 0.62$ ,  $R^2Y = 0.57$ ,  $Q^2$  (cum) = -0.0003. Individual subjects are represented by points marked according to specific delivery type. Box plots compare groups along each axis with heavy black line indicating the group's median value, box representing the interquartile range, and whiskers extend to the most extreme point which is no more than 1.5X the interquartile range from the end of the box.

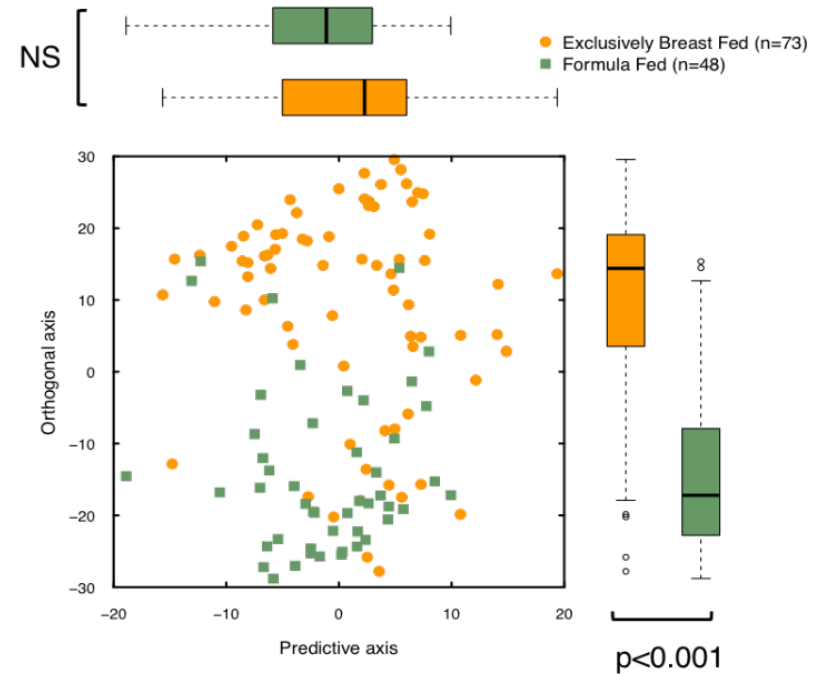


**Figure S2. Comparison of stool metabolome between feeding method groups collapsed into exclusively breast fed vs. formula fed (any formula) for N=121 subjects.** **A.** Scores plot of PCA of binned NMR data colored by broad feeding type. Number of components = 15, Model statistics:  $R^2X = 0.92$ ,  $Q^2$  (cum) = 0.52; **B.** Scores plot of OPLS-DA of binned data by broad feeding type. Number of components = 2 (1 predictive, 1 orthogonal), Model statistics:  $R^2X = 0.55$ ,  $R^2Y = 0.52$ ,  $Q^2$  (cum) = 0.38. Individual subjects are represented by points marked according to feeding method. Box plots compare groups along each axis with heavy black line indicating the group's median value, box representing the interquartile range, and whiskers extend to the most extreme point which is no more than 1.5X the interquartile range from the end of the box. P-values indicate significant differences between groups along individual axes according to Student's t-test (NS indicates not significant).

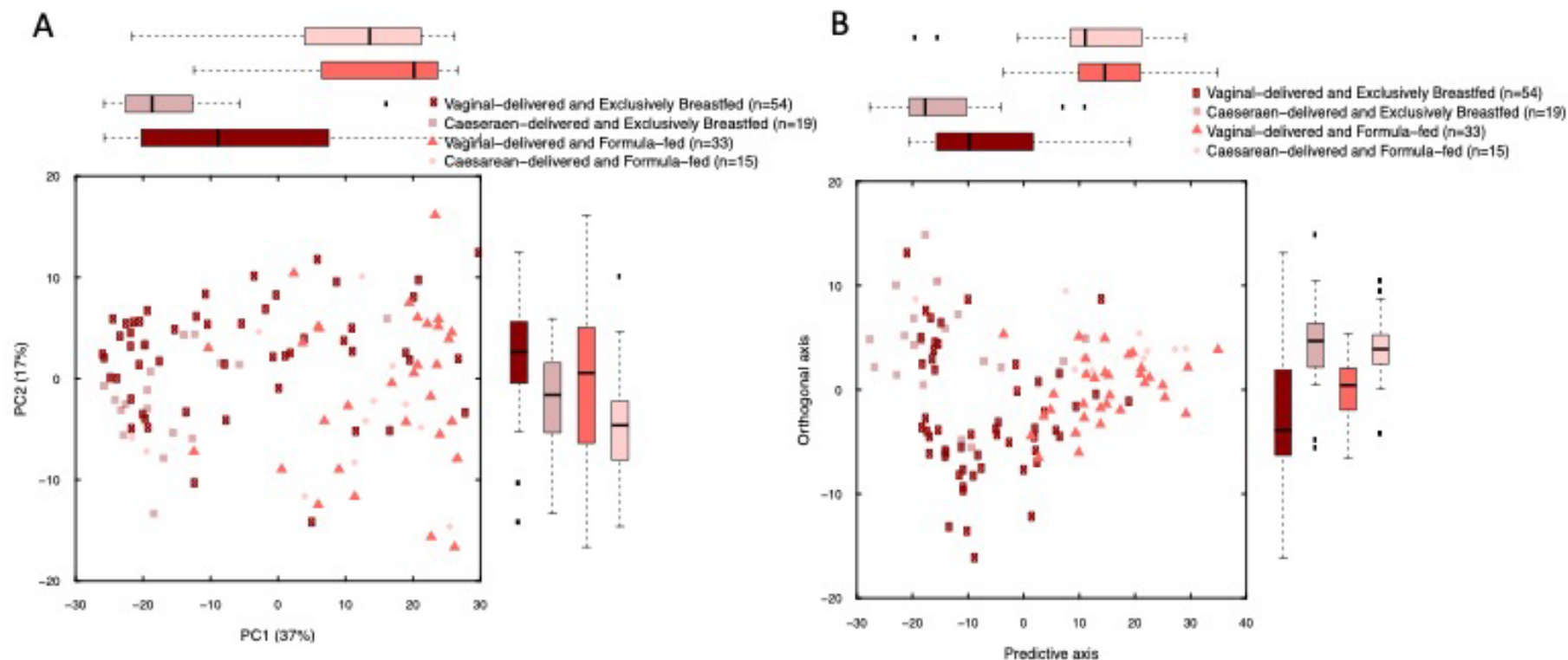
**A**



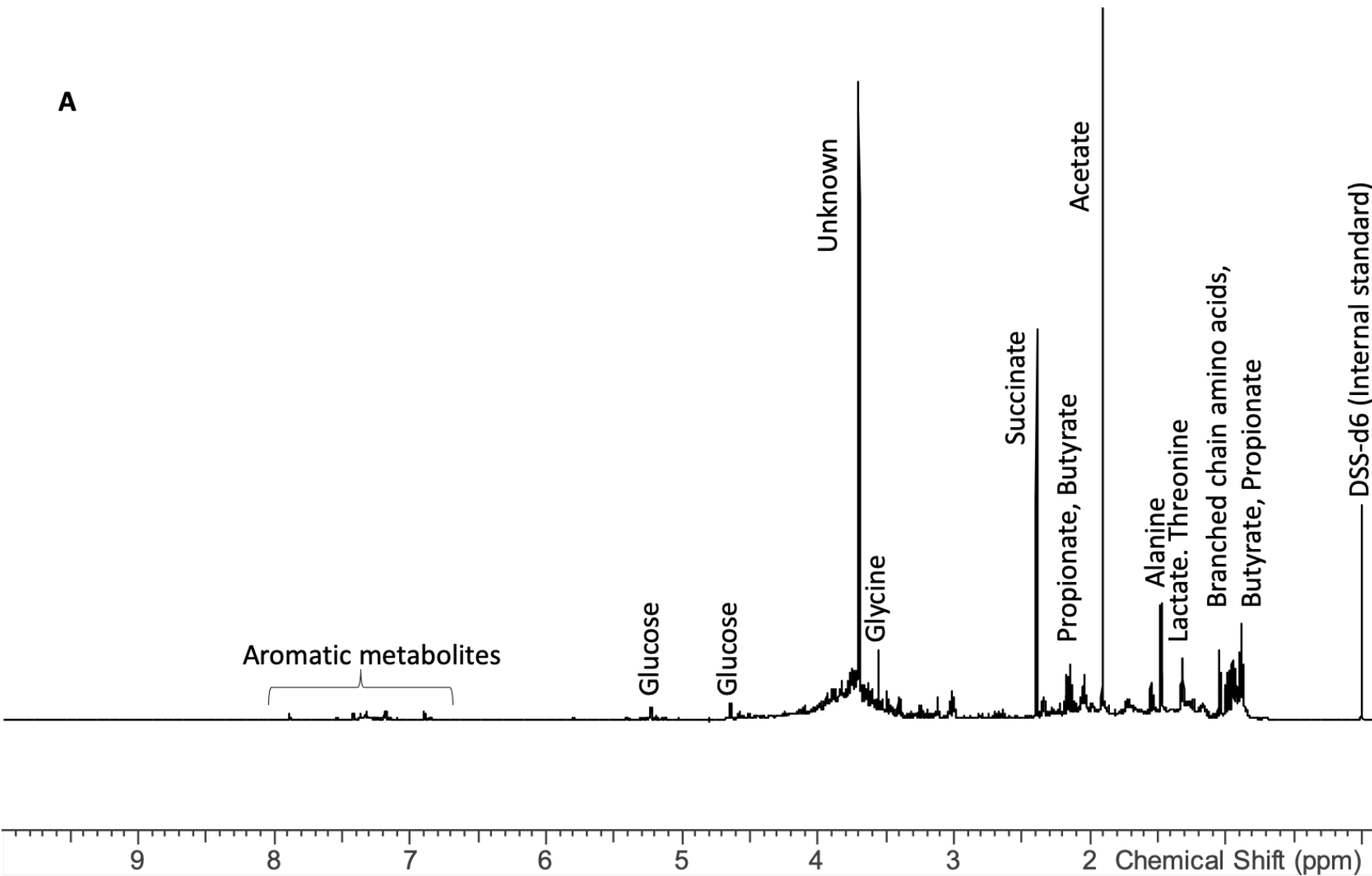
**B**



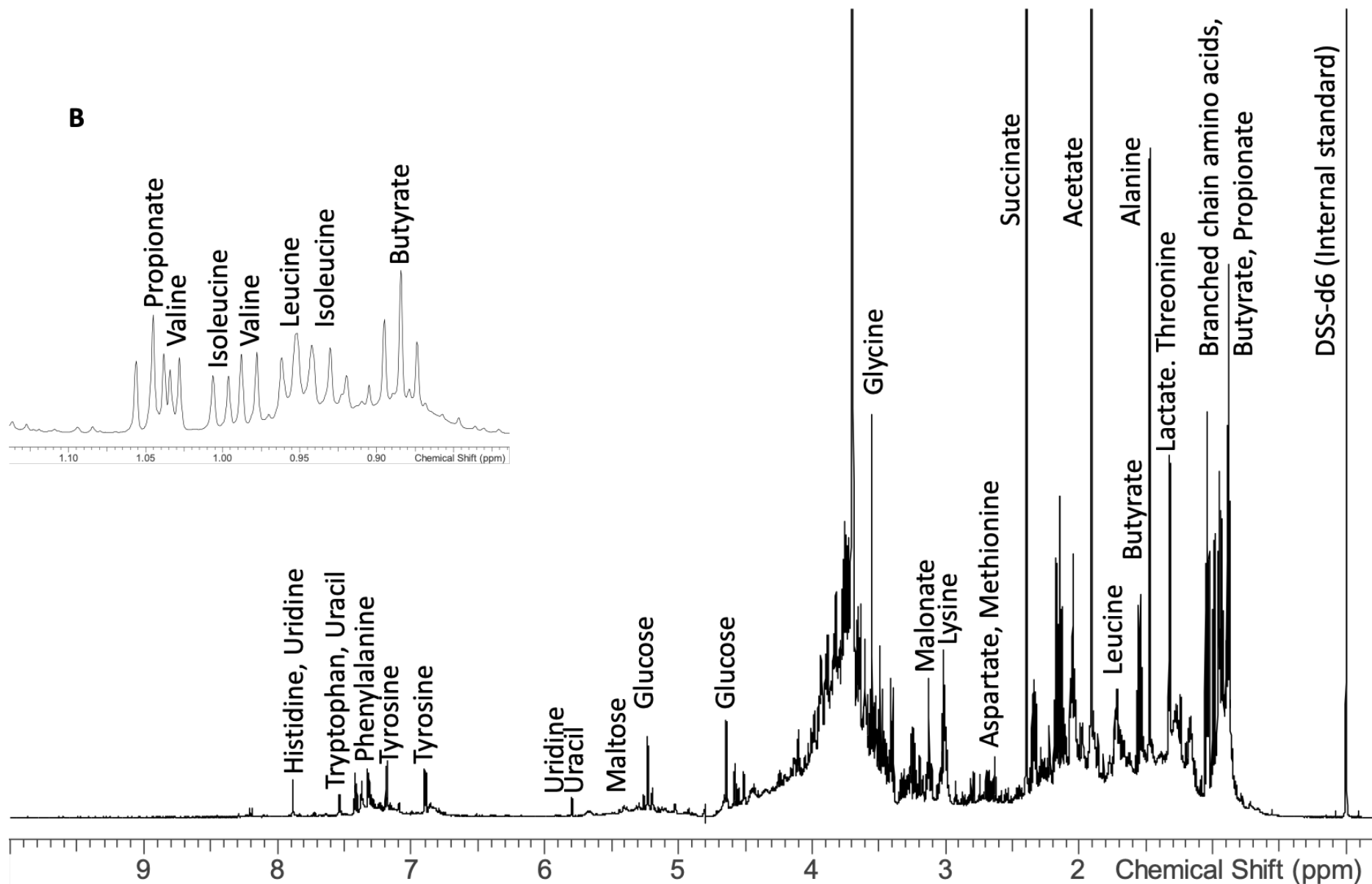
**Figure S3. Comparison of stool metabolome between groups based on joint analysis of delivery mode and feeding method for N=121 subjects.** **A.** Scores plot of PCA of binned NMR data colored by the 4 delivery and feeding groups. Number of components = 15, Model statistics:  $R^2X = 0.92$ ,  $Q^2$  (cum) = 0.52; **B.** Scores plot of OPLS-DA of binned data by the combined delivery and feeding groups. Number of components = 2 (3 predictive, 1 orthogonal), Model statistics:  $R^2X = 0.60$ ,  $R^2Y = 0.27$ ,  $Q^2$  (cum) = 0.12. Individual subjects are represented by points marked according to the 4 combined groups. Box plots compare groups along each axis with heavy black line indicating the group's median value, box representing the interquartile range, and whiskers extend to the most extreme point which is no more than 1.5X the interquartile range from the end of the box.



**Figure S4. A representative 700 MHz <sup>1</sup>H NMR spectrum of feces extract with some metabolite identifications.** These metabolites belong to alcohols, amino acids and derivatives, carbohydrates, energy metabolites, carboxylic acids including short chain fatty acids, and purine and pyrimidine metabolites consistent with other published NMR studies involving stool metabolome [1,2]. **A.** Metabolites that are with relatively high abundance in stool samples. The NMR signal labeled as "Unknown" at ~3.7 ppm could not be matched to any metabolite in the Chenomx metabolite library (or HMDB reference library). **B.** Vertically scaled spectrum (in **A**) to show additional identities of metabolites in low abundance in stool samples. Upper panel shows an expanded region (0.82 - 1.14 ppm) showing NMR peaks of short chain fatty acids, butyrate and propionate, and branched chain amino acids, isoleucine, leucine, and valine.







1. Bervoets, L.; Ippel, J.H.; Smolinska, A.; van Best, N.; Savelkoul, P.H.M.; Mommers, M.A.H.; Penders, J. Practical and Robust NMR-Based Metabolic Phenotyping of Gut Health in Early Life. *J Proteome Res* **2021**, doi:10.1021/acs.jproteome.1c00617.
2. Del Chierico, F.; Vernocchi, P.; Petrucca, A.; Paci, P.; Fuentes, S.; Pratico, G.; Capuani, G.; Masotti, A.; Reddel, S.; Russo, A.; et al. Phylogenetic and Metabolic Tracking of Gut Microbiota during Perinatal Development. *PLoS One* **2015**, *10*, e0137347, doi:10.1371/journal.pone.0137347.