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Supplemental information

Longitudinal 16S rRNA gut microbiota data of infant triplets show partial susceptibility to host genetics

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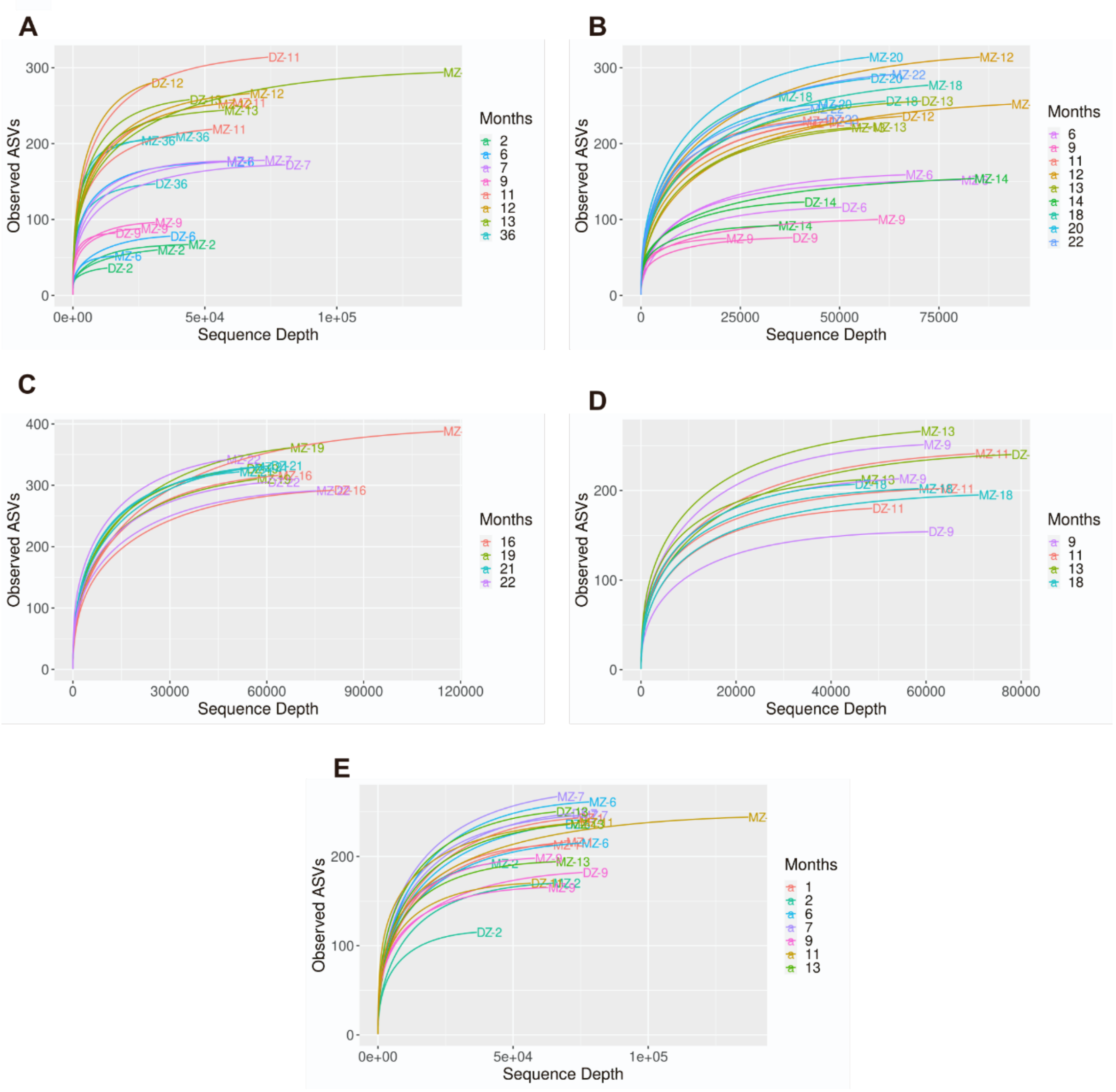

Figure S1. Rarefaction curves. (A) set A, (B) set B, (C) set C, (D) set D, (E), set E. Samples reached a plateau indicating sufficient sequence depth. Each curve is a sample that is color-coded by time point at the far right of each graph. MZ1 = monozygotic twin 1, MZ2 = monozygotic twin 2, DZ = dizygotic twin; numbers are the time points in months. Related to STAR Methods.

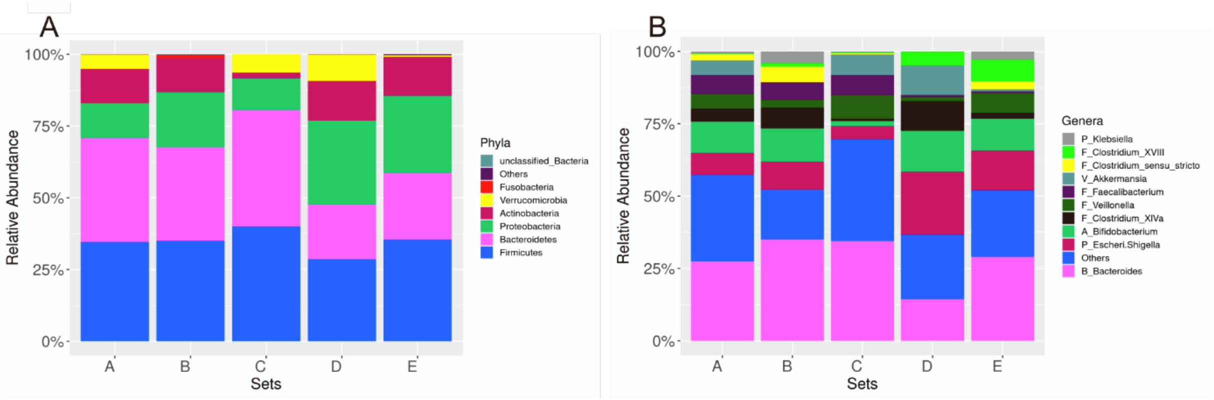

Figure S2. Firmicutes and Bacteroidetes presented the highest relative abundance in almost all sets. Relative abundance of Phyla (A) and Genera (B) per set. (A) Only the six most relatively abundant Phyla and unclassified bacteria are shown. The other remaining phyla are grouped in “Others”. (B) Only the 10 most relatively abundant genera are shown. The other remaining genera are grouped in “Others”. Related to Figure 1 and Table S3.

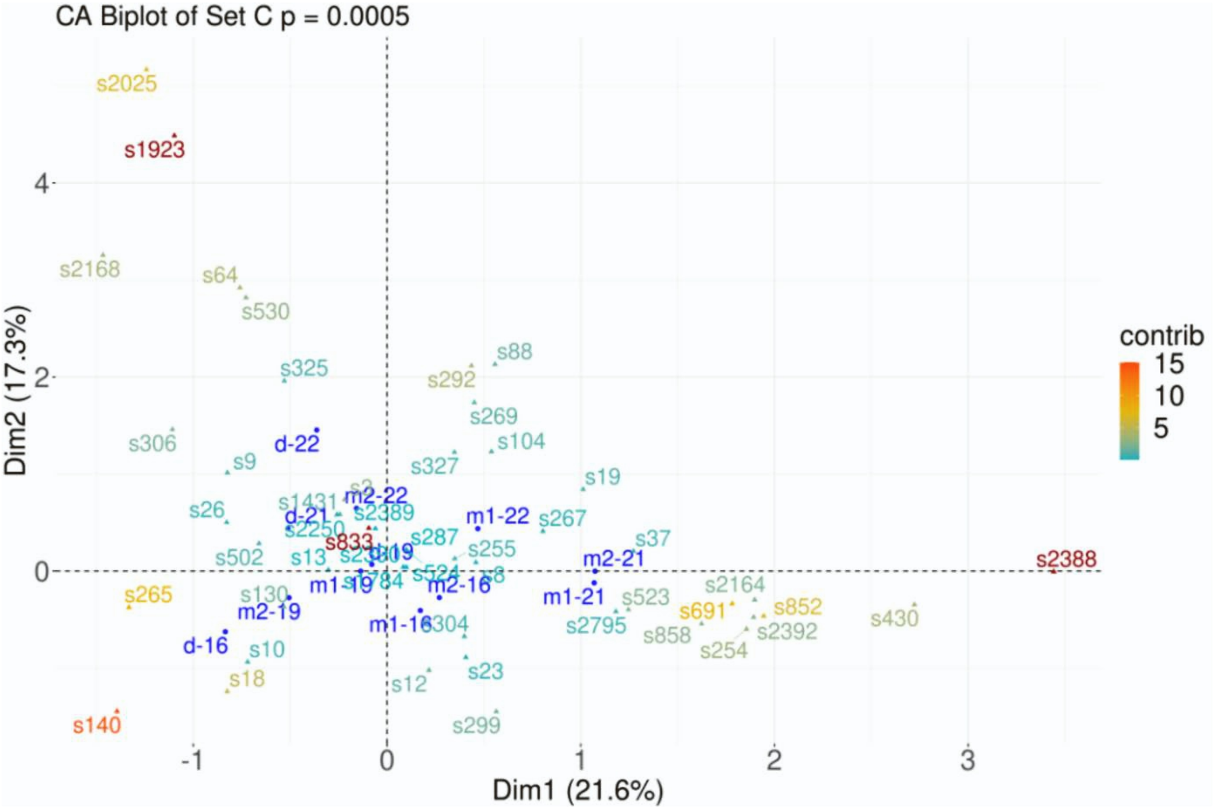

Figure S3. MZs clustered in two out of the four time points analyzed. Correspondence analysis on set C presenting clusterization of MZs at 16 and 21 months. Samples are in blue, and ASVs, denoted by s#, are colored by contribution to the coordinates. ASVs in color wine are outliers. m1 = monozygotic twin 1; m2 = monozygotic twin 2; and d = dizygotic twin. Related to Figure 4 and Figure S4.

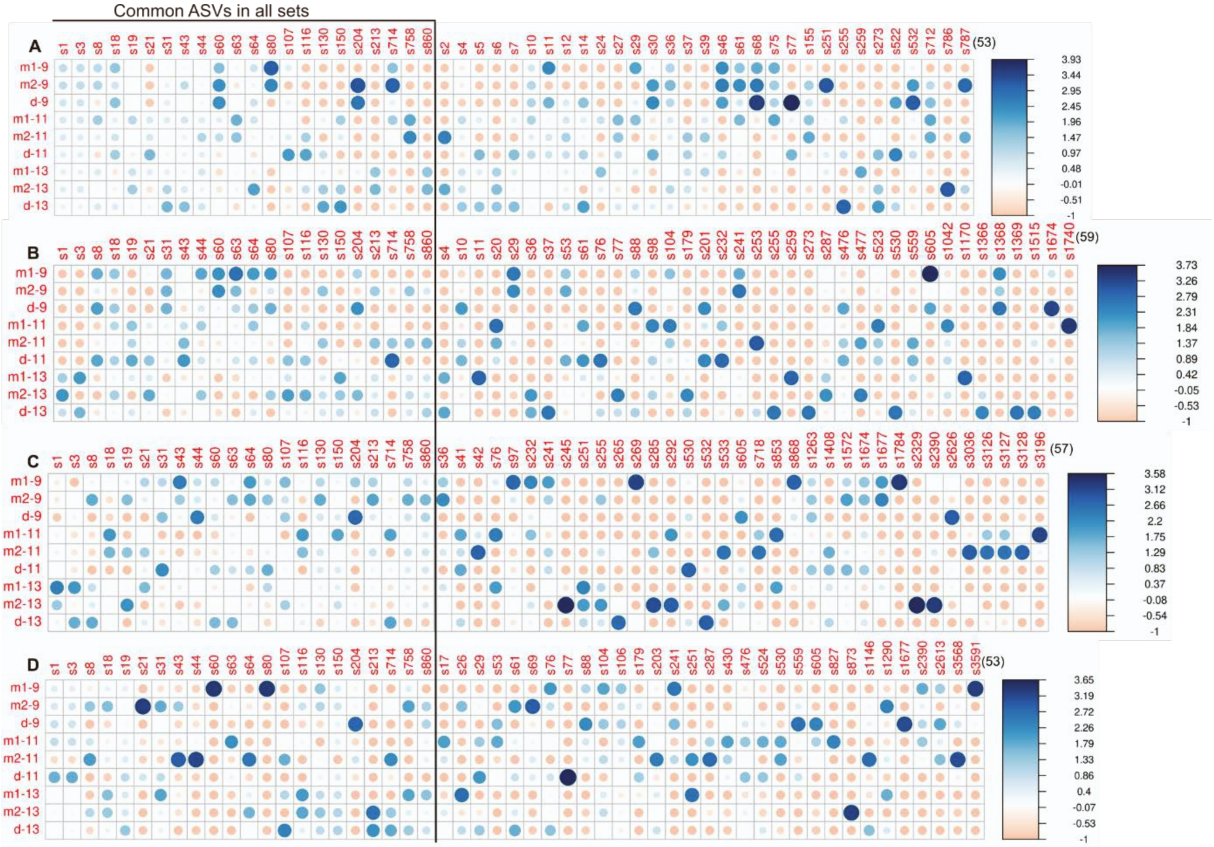

Figure S4. Sample-ASV Associations. Log2 of the likelihood ratio (observed value/expected value) as index of association between samples and ASVs, here identified as s#. (A) set A; (B) set B; (C) set D; and (D) set E. m1 is the monozygotic twin 1, m2 is the monozygotic twin 2, and d is the dizygotic twin, at time points 9, 11, and 13 months. Associations are graded by colors. Blue color represents association of attraction, red color represents association of repulsion. Numbers next to the color gradient bar are the values of the log2 of likelihood ratios. Values next to zero indicate no association, positive values indicate association of attraction, and negative values indicate association of repulsion. Numbers between parentheses next to the ASV IDs are the total number of ASVs. Related to Figure 4 and Figure S3.

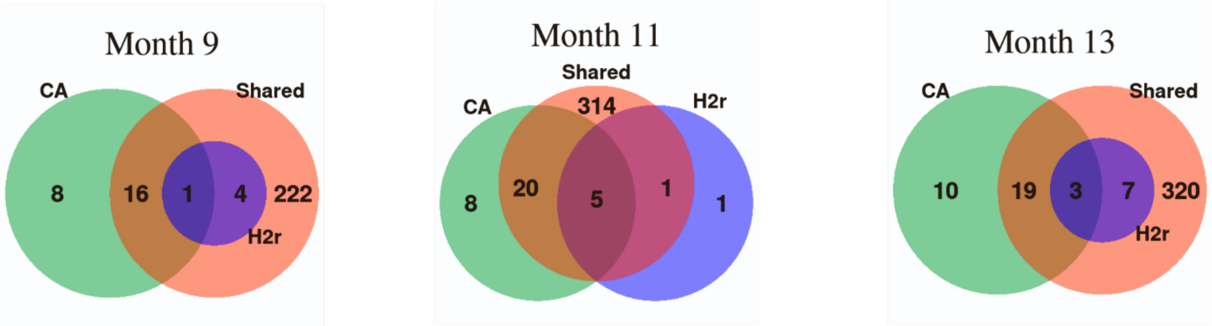

Figure S5. Comparison of results obtained in three separate analyses. (1) correspondence analysis where MZs were more similar, denoted by CA; (2) non exclusively shared ASVs between the MZs. denoted by the word ‘shared’; and (3) heritability (H2r) per time point. 251, 349, and 359 ASVs were analyzed at time points 9, 11 and 13 months, respectively. Related to Figures 3, 4, 5 and Tables S5 and S6.

Table S1. Clinical data of the study subjects. Related to STAR Methods.

Triplets set[a]	Infant-zygosity	Sex	In Vitro Fertilization (IVF)	Gestation (weeks)	Birth Weight (g)	Mode of feeding	Neonatal ICU Admission (days)	Antibiotic Use in ICU	Life Months with Antibiotic Use	Valid Sample Months (total # of samples)
A	MZ1	F	Yes	33	1775	Formula	21	No	None	2,6,7,9,11,12, 13,36 (24)
A	MZ2	F	Yes	33	2060	Formula	21	No	None	
A	DZ	M	Yes	33	2200	Formula	20	No	37	
B	MZ1	M	Yes	28	815	EBM + Formula	89	Yes	None	6,9,11,12,13, 14,18,20,21,22, (30)
B	MZ2	M	Yes	28	750	EBM + Formula	96	Yes	None	
B	DZ	F	Yes	28	1100	EBM + Formula	80	Yes	None	
C	MZ1	M	Yes	32	1870	Formula	45	No	20	16,19,21,22 (12)
C	MZ2	M	Yes	32	1710	Formula	46	No	20	
C	DZ	M	Yes	32	1915	Formula	47	No	20	
D	MZ1	M	Yes	30	1235	EBM + Formula	81	Yes	16	9,11,13,18 (12)
D	MZ2	M	Yes	30	1430	EBM +Formula	70	Yes	16	
D	DZ	F	Yes	30	1135	EBM + Formula	70	Yes	16	
E	MZ1	F	No[b]	34	1895	EBM + Formula	0	No	None	1,2,6,7,9,11,13 (21)
E	MZ2	F	No[b]	34	1755	EBM + Formula	0	No	None	
E	DZ	M	No[b]	34	2175	EBM + Formula	0	No	12	

[a] All triplets were born from C-section

[b] Use of ovulatory stimulant

EBM = Expressed Breast Milk; MZ1 = Monozygotic 1; MZ2 = Monozygotic 2; DZ = Dizygotic; g = grams; ICU = intensive care unit; # = number

Table S2. Description of processing of 16S sequences from fecal samples in each triplet set. Related to STAR Methods.

Triplet sets	Num. of samples	Total num. raw reads	Num. of ASVs	Total num. of processed reads	Mean reads per sample	Mean reads per ASVs	Highest num. reads in a sample	Lowest num. reads in a sample
A	27	3.982.550	1.364	1.251.106	46.337	917	140.443	13.109
B	30	5.813.571	1.343	1.758.163	58.605	1.309	93.279	21.489
C	15	3.713.794	1.178	964.244	64.283	819	114.698	46.084
D	15	3.442.790	992	1.012.249	67.483	1.020	130.203	44.995
E	24	5.149.164	1.285	1.686.289	70.262	1.312	137.098	36.550
Total	111[a]	22.101.869	4.168	6.672.051	NA	NA	NA	NA
Mean	22	4.420.374	1.232	1.334.410	61.394	1.075	123.144	32.445

[a] Twelve samples were removed from further analyses due to antibiotics intake

Num. = Number

Table S3. Summary of ASV classifications and absolute abundance. Related to STAR Methods.

Sets		Most abundant	Absolute abundance	Number of ASVs	Least abundant	Absolute abundance	Number of ASVs
A	Phylum	Bacteroidetes	457.776	181	Fusobacteria	19	3
	Genus	Bacteroides	343.631	98	Hydrogenoanaerobacterium	4	2
B	Phylum	Bacteroides	571.144	139	Enhydrobacter	4	1
	Genus	Firmicutes	614.935	703	Verrucomicrobia	133	2
C	Phylum	Bacteroides	288.147	149	Clostridium_XI	4	1
	Genus	Firmicutes	378.444	637	Fusobacteria	109	9
D	Phylum	Escherichia/Shigella	190.566	32	Novosphingobium	4	1
	Genus	Proteobacteria	287.662	211	Fusobacteria	4	1
E	Phylum	Bacteroides	457.626	107	unclassified_Oxalobacteraceae	4	1
	Genus	Firmicutes	557.435	693	Planctomycetes	12	1
Total number of ASVs in all sets			4,168				
Total number of distinct Phyla			13				

Table S5. Taxa whose ASVs are shared only between the MZs throughout the sets. Related to Figure 3.

Taxa(Phylum_Genus)	Amount of ASVs in each set					Total
	A	B	C	D	E	
Actinobacteria_Bifidobacterium	10	6	2	29	13	60
Bacteroidetes_Bacteroides	6	17	14	7	8	52
Firmicutes_Veillonella	10	18	5	8	9	50
Proteobacteria_unclassified_Enterobacteriaceae	5	4	9	10	16	44
Firmicutes_unclassified_Lachnospiraceae	15	14	4	5	4	42
Bacteroidetes_Prevotella	1	2	36	0	1	40
Firmicutes_unclassified_Ruminococcaceae	13	3	11	2	7	36
Firmicutes_Faecalibacterium	22	1	7	6	0	36
Proteobacteria_Haemophilus	6	17	5	0	2	30
Firmicutes_Clostridium_sensu_stricto	2	6	12	3	6	29
Firmicutes_Clostridium_XlVa	6	5	3	2	3	19
Firmicutes_Streptococcus	1	4	3	2	7	17
Proteobacteria_Klebsiella	2	12	2	1	0	17
Firmicutes_Blautia	4	1	3	3	4	15
Firmicutes_unclassified_Peptostreptococcaceae	3	4	0	1	7	15
Firmicutes_Dialister	0	0	13	0	0	13
Firmicutes_unclassified_Clostridiales	4	1	5	2	0	12
Actinobacteria_Actinomyces	2	1	0	4	4	11
Verrucomicrobia_Akkermansia	3	0	1	2	5	11
Firmicutes_Clostridium_IV	4	4	0	1	1	10
Firmicutes_Clostridium_XVIII	0	2	2	2	4	10
Proteobacteria_Escherichia/Shigella	3	1	1	2	2	9
Firmicutes_Ruminococcus2	4	1	3	0	1	9
Firmicutes_unclassified_Clostridiaceae_1	0	7	1	0	1	9
Firmicutes_Flavonifractor	3	2	1	2	0	8
Firmicutes_Roseburia	2	2	0	2	2	8
Firmicutes_Erysipelotrichaceae_incertae_sedis	2	0	1	3	2	8
Firmicutes_Lachnospiracea_incertae_sedis	6	1	0	0	0	7
Firmicutes_Gemmiger	1	1	1	2	1	6
Actinobacteria_Eggerthella	0	0	2	1	3	6
Firmicutes_Enterococcus	1	1	0	3	1	6
unclassified_Bacteria_unclassified_Bacteria	5	1	0	0	0	6
Firmicutes_Clostridium_XI	1	2	0	1	2	6
Firmicutes_Oscillibacter	2	0	0	2	2	6
Firmicutes_Ruminococcus	1	0	0	1	4	6
Bacteroidetes_Dysgonomonas	5	0	0	0	1	6
Firmicutes_Butyricicoccus	1	0	4	0	0	5
Fusobacteria_Fusobacterium	0	1	1	0	2	4
Firmicutes_Granulicatella	1	1	0	1	1	4

Actinobacteria_unclassified_Bifidobacteriaceae	2	0	0	0	2	4
Bacteroidetes_Parabacteroides	1	1	0	0	2	4
Firmicutes_Abiotrophia	0	1	2	0	1	4
Candidatus_Saccharibacteria_	0	0	1	0	3	4
Firmicutes_Anaerostipes	2	0	1	1	0	4
Bacteroidetes_Alistipes	2	0	1	1	0	4
Actinobacteria_Collinsella	0	0	0	0	4	4
Firmicutes_Lactobacillus	2	0	0	0	2	4
Proteobacteria_Proteus	1	0	0	2	0	3
Actinobacteria_Olsenella	3	0	0	0	0	3
Firmicutes_Megasphaera	1	0	0	1	1	3
Cyanobacteria/Chloroplast_Streptophyta	0	2	0	1	0	3
Actinobacteria_unclassified_Actinomycetales	1	1	0	0	1	3
Proteobacteria_Providencia	0	0	0	3	0	3
Firmicutes_unclassified_Peptoniphilaceae	0	0	0	0	3	3
Firmicutes_Turicibacter	0	1	0	0	1	2
Firmicutes_Lachnospira	0	2	0	0	0	2
Firmicutes_Phascolarctobacterium	0	1	0	1	0	2
Firmicutes_unclassified_Clostridia	1	1	0	0	0	2
Firmicutes_Romboutsia	0	1	1	0	0	2
Proteobacteria_Neisseria	0	1	1	0	0	2
Proteobacteria_Mesorhizobium	0	0	2	0	0	2
Proteobacteria_Cupriavidus	0	0	2	0	0	2
Proteobacteria_Rhizobium	0	0	1	1	0	2
Bacteroidetes_unclassified_Bacteroidales	1	0	1	0	0	2
Firmicutes_Gemella	1	0	1	0	0	2
Firmicutes_Anaerotruncus	0	0	0	2	0	2
Proteobacteria_Acinetobacter	0	0	0	1	1	2
Actinobacteria_Corynebacterium	0	0	0	1	1	2
Firmicutes_Paenibacillus	0	0	0	0	2	2
Proteobacteria_Citrobacter	2	0	0	0	0	2
Firmicutes_Dorea	0	0	0	0	1	1
Actinobacteria_Propionibacterium	0	0	1	0	0	1
Actinobacteria_Varibaculum	0	0	0	0	1	1
Firmicutes_Peptostreptococcus	0	1	0	0	0	1
Proteobacteria_unclassified_Pasteurellaceae	0	1	0	0	0	1
Fusobacteria_Leptotrichia	0	1	0	0	0	1
Actinobacteria_Atopobium	0	1	0	0	0	1
Proteobacteria_unclassified_Neisseriaceae	0	1	0	0	0	1
Proteobacteria_Caulobacter	0	0	1	0	0	1
Actinobacteria_Leifsonia	0	0	1	0	0	1
Bacteroidetes_Heliimonas	0	0	1	0	0	1
Proteobacteria_Cardiobacterium	0	0	1	0	0	1

Firmicutes_Oribacterium	0	0	1	0	0	1
Firmicutes_unclassified_Firmicutes	0	0	1	0	0	1
Proteobacteria_Buttiauxella	0	0	0	1	0	1
Proteobacteria_Delftia	0	0	0	1	0	1
Proteobacteria_Salmonella	0	0	0	1	0	1
Firmicutes_unclassified_Lactobacillaceae	0	0	0	1	0	1
Firmicutes_Coprobacillus	0	0	0	1	0	1
Proteobacteria_Cronobacter	0	0	0	1	0	1
Proteobacteria_Morganella	0	0	0	1	0	1
Firmicutes_Staphylococcus	0	0	0	0	1	1
Proteobacteria_Stenotrophomonas	0	0	0	0	1	1
Proteobacteria_Bosea	0	0	0	0	1	1
Proteobacteria_Methylobacterium	0	0	0	0	1	1
Proteobacteria_Sphingomonas	1	0	0	0	0	1
Proteobacteria_Serratia	1	0	0	0	0	1
Bacteroidetes_Barnesiella	1	0	0	0	0	1
Euryarchaeota_Methanobrevibacter	1	0	0	0	0	1
Firmicutes_Intestinimonas	1	0	0	0	0	1
Total	182	160	173	132	156	803

Table S6. Heritability (Hr2) test on ASV relative abundance by SOLAR-Eclipse. Related to Figure 5.

Time Points	ASVs	Heritability (Hr2)	Taxa	p-value	Sex covariate (beta values)	standard error of Hr2 estimate	N (families)[a]
9	ASV_1	0,81	Bacteroidetes_Bacteroides	0,0134	0,432	0,167	3
	ASV_44	0,72	Firmicutes_Clostridium_XVIII	0,0133	0,301	0,195	4
	ASV_714	0,72	Proteobacteria_unclassified_Enterobacteriaceae	0,0107	0,000	0,189	4
	ASV_43	0,69	Firmicutes_Erysipelotrichaceae_incertae_sedis	0,0243	0,023	0,225	4
	ASV_63	0,60	Firmicutes_Veillonella	0,0357	0,004	0,251	4
11	ASV-31	0,90	Firmicutes_Clostridium_XlVa	0,0025	0,083	0,082	4
	ASV-21	0,87	Firmicutes_Clostridium_XlVa	0,0048	0,051	0,113	4
	ASV-80	0,82	Firmicutes_Clostridium_sensu_stricto	0,0281	0,034	0,181	3
	ASV-3	0,78	Bacteroidetes_Bacteroides	0,0060	NE[b]	0,150	4
	ASV-60	0,73	Firmicutes_Blautia	0,0320	0,400	0,217	3
	ASV-63	0,68	Firmicutes_Veillonella	0,0209	0,107	0,208	4
	ASV-1	0,57	Bacteroidetes_Bacteroides	0,0476	0,040	0,251	4
13	ASV-1	0,90	Bacteroidetes_Bacteroides	0,0007	0,003	0,079	4
	ASV-130	0,86	Bacteroidetes_Bacteroides	0,0073	0,000	0,115	4
	ASV-860	0,86	Bacteroidetes_Bacteroides	0,0265	0,003	0,133	4
	ASV-213	0,79	Bacteroidetes_Bacteroides	0,0201	NE[b]	0,182	3
	ASV-18	0,77	Proteobacteria_Escherichia/Shigella	0,0053	0,018	0,156	4
	ASV-150	0,76	Bacteroidetes_Bacteroides	0,0049	0,368	0,158	4
	ASV-116	0,74	Proteobacteria_Escherichia/Shigella	0,0120	0,025	0,180	4
	ASV-36	0,73	Actinobacteria_Bifidobacterium	0,0184	0,001	0,206	4
	ASV-64	0,63	Firmicutes_unclassified_Peptostreptococcaceae	0,0200	0,147	0,215	4
	ASV-8	0,58	Firmicutes_Veillonella	0,0466	0,014	0,268	4

[a] Number of sets analyzed

[b] NE: not estimated due to instability