Supplementary Methods and Data

Plasma retinol and retinol-binding protein (RBP) analysis: Plasma samples were collected in early infancy at 6 and 15 wk of age. All samples were stored at -80° C until completion of the enrolment and were analyzed together to minimized batch effect. Plasma retinol from 15 wk was measured in 45 samples using reversed-phase HPLC (Waters Corp., Milford, MA) at the University of Wisconsin-Madison [29]. At 2 y, plasma retinol was measured in 75 sample by reverse phase HPLC (SHIMADZU, Tokyo, Japan) in the Nutritional Biochemistry Laboratory, icddr,b [30]. Plasma RBP was measured using a human RBP4 kit (MSD, Gaithersburg, MD, USA) at the WHNRC. A linear regression model was developed (R^2 = 0.369, p <0.001) with 15 wk plasma retinol and RBP4 to predict plasma retinol from 6 wk and 15 wk RBP data using the following equation: retinol [mmol/L] = (RBP4 [mg/L] x 0.00882 [SE = 0.00185]) + 0.381 [SE = 0.0589]). The same approach was used at 2 y using the 2-y retinol and RBP4 data (R^2 = 0.320, p <0.001) using the following equation: retinol [mmol/L] = (RBP4 [mg/L] x 0.0159 [SE = 0.00271]) + 0.432 [SE = 0.0869]).

16s rRNA gene analysis: Total stool DNA was extracted using the ZR Fecal DNA Miniprep kit (Zymo Research, Irvine, CA). Mixed template amplicon library of the hypervariable region V4 of the bacterial 16S rRNA gene was prepared using the primers set (515F and 806R) as previously described [1] with some modification. In brief, a master mix was prepared that consisted of 7.5µL of GoTaq Green Master Mix (Promega, Madison, WI), 0.6 µL of 25mM $MgCl_2$, 0.3 µL of 10 µM reverse primer 806R, and 3.6 µL of nuclease-free water. Then, 12 µL of this master mix was added to the 96-well plate, which was followed by 1.5 µL of unique barcoded forward primer 515F and 1.5 µL of stool DNA. Extraction control and PCR non-template control (NTC) were also added to check contamination and nonspecific DNA amplification during stool DNA extraction or PCR amplification. The plate was closed tightly and centrifuged for 2 min. PCR was then performed under the following conditions: denaturation (1 cycle) at 94° C for 3 min; amplification of 25 cycles at 94° C for 45 s, 50° C for 60 s, and 72° C for 90 s; and a final extension step cycle at 72° C for 10 min. DNA amplification was confirmed by 0.8% agarose gel. Amplicon DNA was multiplexed and purified using QIAquick PCR purification kit (Qiagen, Germantown, MD). The amplicon library was sequenced using the Illumina MiSEQ platform with 2x250bp paired-end sequencing. Two sequencing runs were performed to cover all 1099 samples (280 samples at each of the 6 wk and 11 wk, 262 samples at 15 wk, and 249 sample at the 2y time point), and raw data were combined for bioinformatics analysis. Matching paired-end sequences merging, de-multiplexing, quality-filtering, and OTU picking were carried out using the open-source software Quantitative Insights Into Microbial Ecology (QIIME) version 1.8 [2] as described in **Supplemental figure 1**. Matching paired-end sequences were merged using fastq-join [3] with a minimum overlap of 200 base pairs [4]. Average Phred quality scores of raw and merged sequences (**Supplemental figure 2**) were analyzed by FastQC [5]. A total of 15,047,801 sequences were survived with an average of 13,818 ± 4,976 (mean ± SD) sequence per sample after quality filtering and demultiplexing using QIIME default setting. Sequences were clustered into Operational taxonomic units (OTU) using the default pick open-reference OTU pipeline with the QIIME implementation of 64-bit UCLUST [6] using the Greengenes May 2013 reference database [7] at the threshold of 97% pairwise identity. Shannon diversity index on the alpha rarefaction curve (**Supplemental figure 3**) reached a plateau at about 1700 sequences per sample. We performed a single rarefaction at a sequence depth of 3000 sequences per sample. Out of total 1099 stool samples, only 20 samples (3 samples at 6 wk, 2 samples at 11 wk, 4 samples at 15 wk, and 1 sample at 2 y; <2% of all samples) failed to meet the minimum required sequence

per sample and were removed from the downstream microbiota analysis. α-diversity (Shannon diversity index and observed species) and β-diversity (unweighted UniFrac, weighted UniFrac, and Bray Curtis) were calculated from the unfiltered OTU table. The OTU table was filtered by removing any OTU present in fewer than 5 samples and with a relative abundance across all samples ≤0.005% to calculate relative abundance. There were no differences in alpha- and beta-diversity or in composition between the two MiSeq sequence batches, even though the initial raw sequence depth was higher in batch 2 compared to batch 1 (**Supplemental figure 4**).

Bifidobacterium species and subspecies analysis: *Bifidobacterium*-specific terminal restriction fragment length polymorphism (Bif-TRFLP) assay was used to find out the ratios of different Bifidobacteria species abundance present at 6 wk of age. Detailed methodology has been reported earlier [1]. Briefly, stool DNA was amplified by PCR using primers NBIF389 (5′-[HEX]-GCCTTCGGGTTGTAAAC) and NBIF1018 REV (5′-GACCATGCACCACCTGTG), purified and then digested with restriction enzymes AluI and Hae-III. The resulting fragments were analyzed on an ABI 3100 Capillary Electrophoresis Genetic Analyzer and the fragment length was analyzed with PeakScanner 2.0 software (Applied Biosystems, Carlsbad, CA) and compared against the published database [8] for species identification. Peak area was used to determine the relative proportion the *Bifidobacterium species* in the sample.

BLIR (*Bifidobacterium longum* subsp. *longum*-*Infantis* Ratio) assay was used to determine the ratio of the relative abundance of *Bifidobacterium* belonging to *Bifidobacetrium longum* subsp. *longum* and *Bifidobacetrium longum* subsp. *infantis* in each sample at 6 wk of age [1, 9]. In brief, a PCR reaction using three primers (FWD_BL_BI (5′-[HEX]-AAAACGTCCATCCATCACA), REV_BL (5′-ACGACCAGGTTCCACTTGAT), and REV_BI (5′-CGCCTCAGTTCTTTAATGT)) was completed to amplify the non-transcribed targeted genomic regions which produce a 145 bp amplified fragment from *Bifidobacterium longum* subsp. *longum* genome and a 114 bp amplified fragment from *Bifidobacterium longum* subsp. *infantis*. PCR products were analyzed by capillary electrophoresis on an ABI 3100 Capillary Electrophoresis Genetic Analyzer. Based on the fragment length and peak area we estimated the ratio of *Bifidobacterium longum* subsp. *infantis* and *Bifidobacterium longum* subsp. *longum* of each amplicon based using PeakScanner 2.0 software (Applied Biosystems, Carlsbad, CA) and determined the relative abundances in the sample.

Supplemental References

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3. Aronesty E: **ea-utils: Command-line tools for processing biological sequencing data.** *Durham, NC: Expression Analysis* 2011.
4. Goodrich JK, Davenport ER, Beaumont M, Jackson MA, Knight R, Ober C, Spector TD, Bell JT, Clark AG, Ley RE: **Genetic Determinants of the Gut Microbiome in UK Twins.** *Cell Host Microbe* 2016, **19:**731-743.
5. Andrews S: **FastQC: A quality control tool for high throughput sequence data.** *Reference Source* 2010.
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7. McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P: **An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea.** *ISME J* 2012, **6:**610-618.
8. Lewis ZT, Bokulich NA, Kalanetra KM, Ruiz-Moyano S, Underwood MA, Mills DA: **Use of bifidobacterial specific terminal restriction fragment length polymorphisms to complement next generation sequence profiling of infant gut communities.** *Anaerobe* 2013, **19:**62-69.
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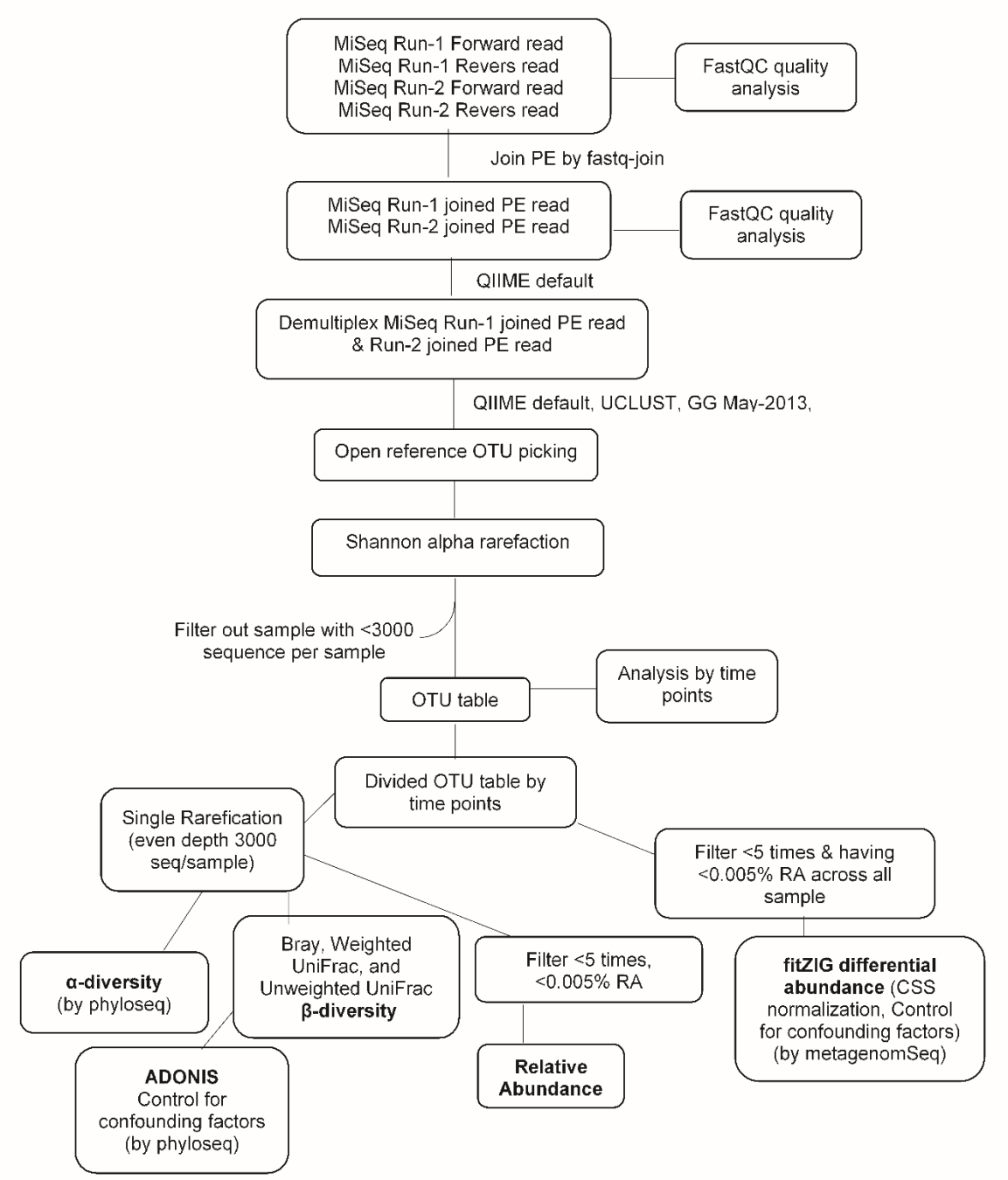
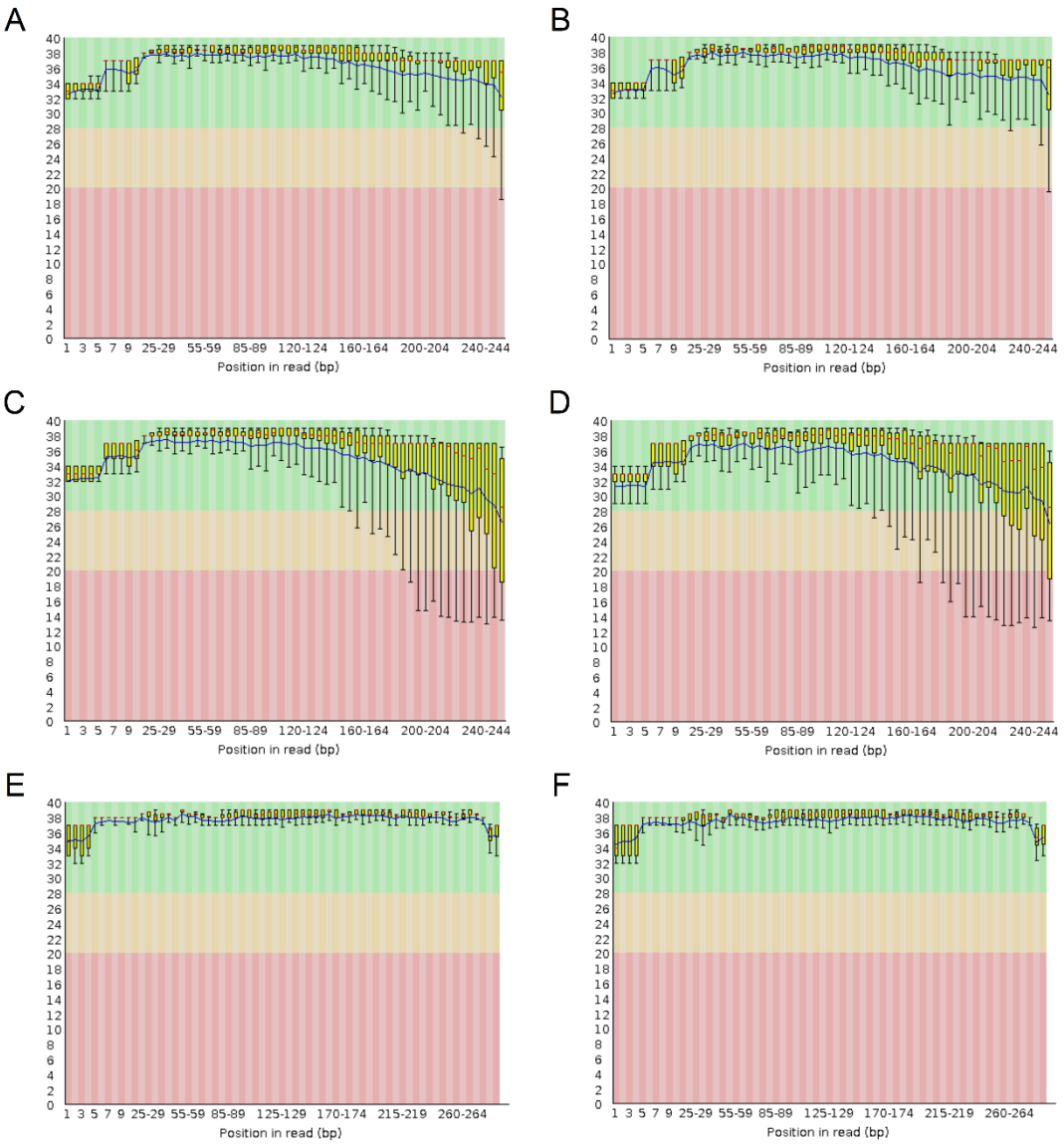
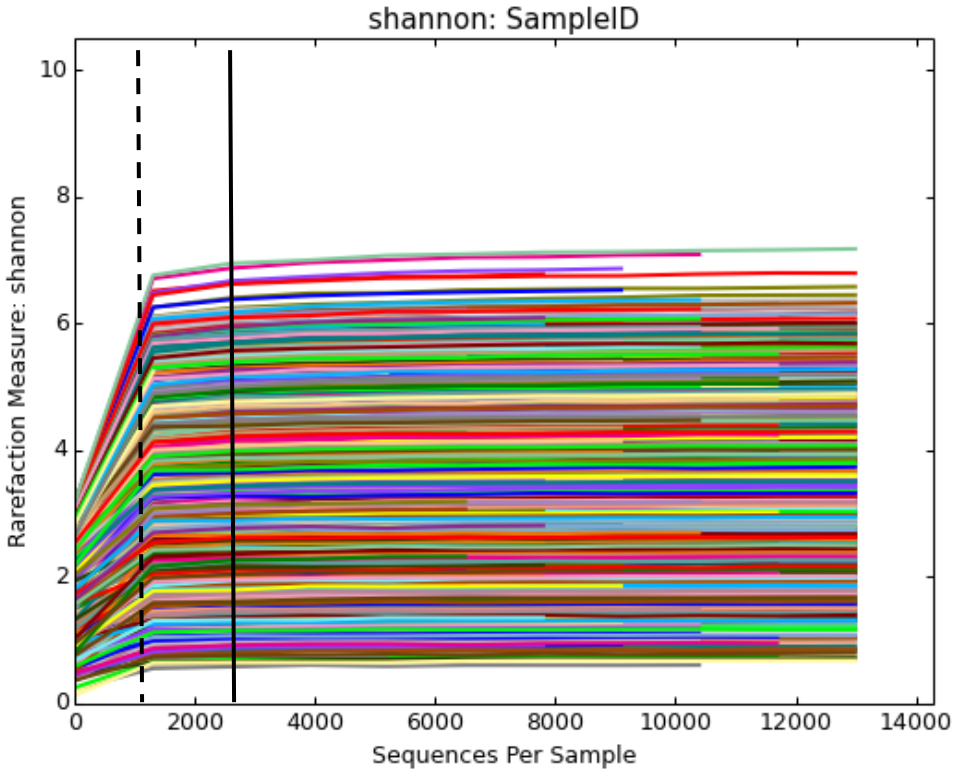


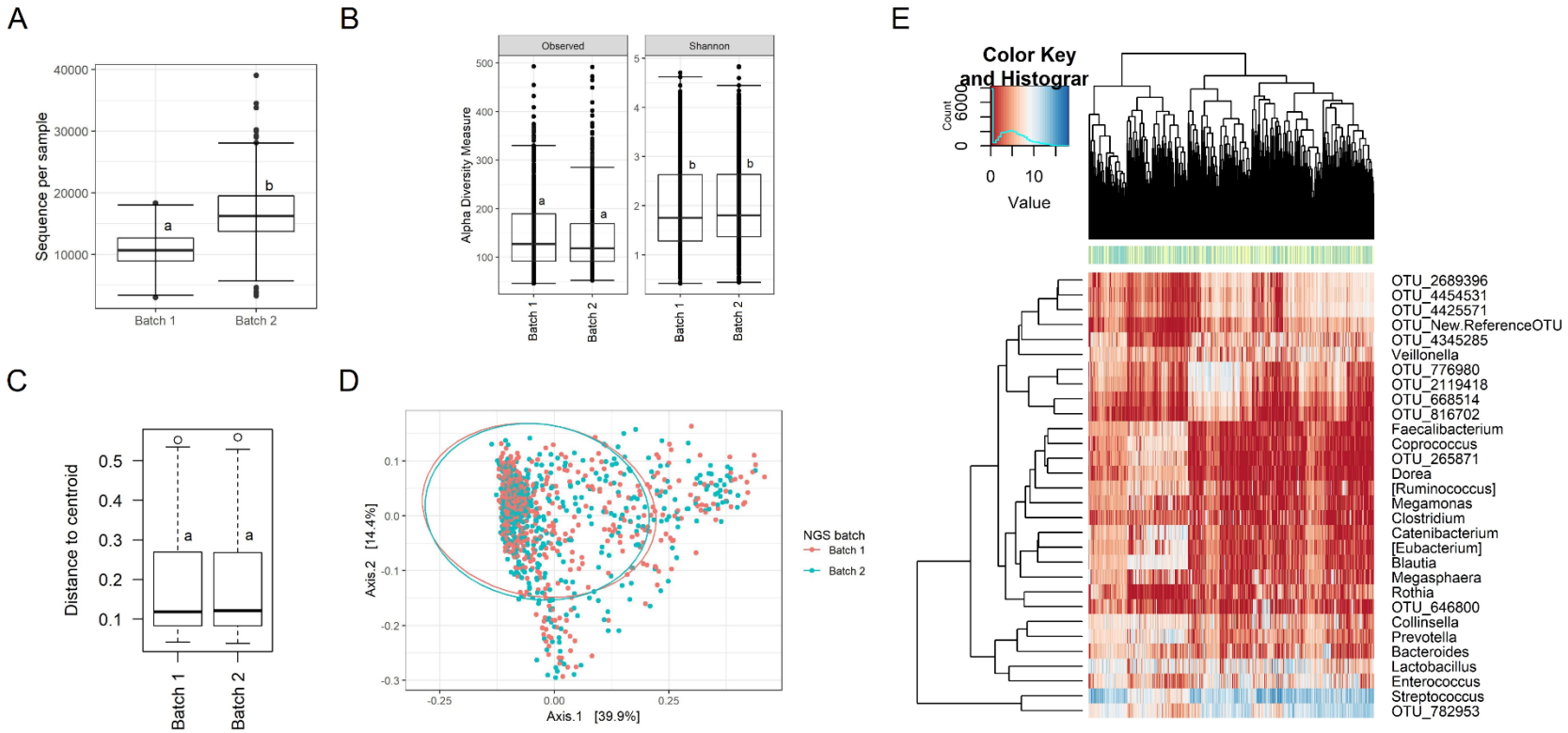
Supplemental Figure 1: Flow diagram of bioinformatics (QIIME) and downstream statistical analysis.



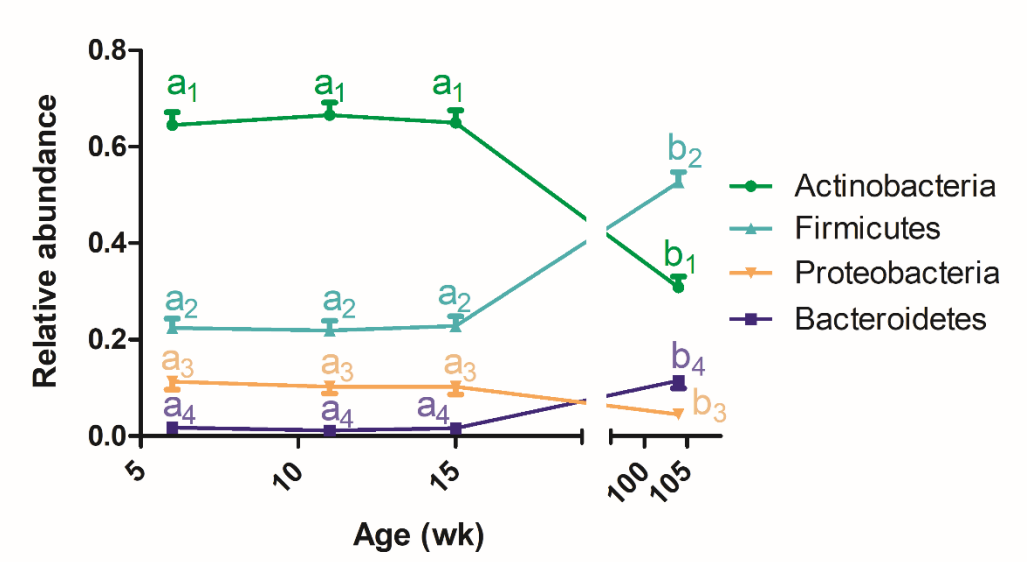
Supplemental Figure 2: Average per base phread quality scores of forward (A & B), reverse (C & D), and joined peered end (E & F) sequence reads of MiSeq sequencing batch 1 (n = 535, left panels) and 2 (n = 554, right panels). Each box and the central line in the box represent the inter-quartile range and the median phread quality scores, respectively. The lower and upper whiskers represent the 10th and 90th percentile, respectively. The blue line represents the mean quality score.



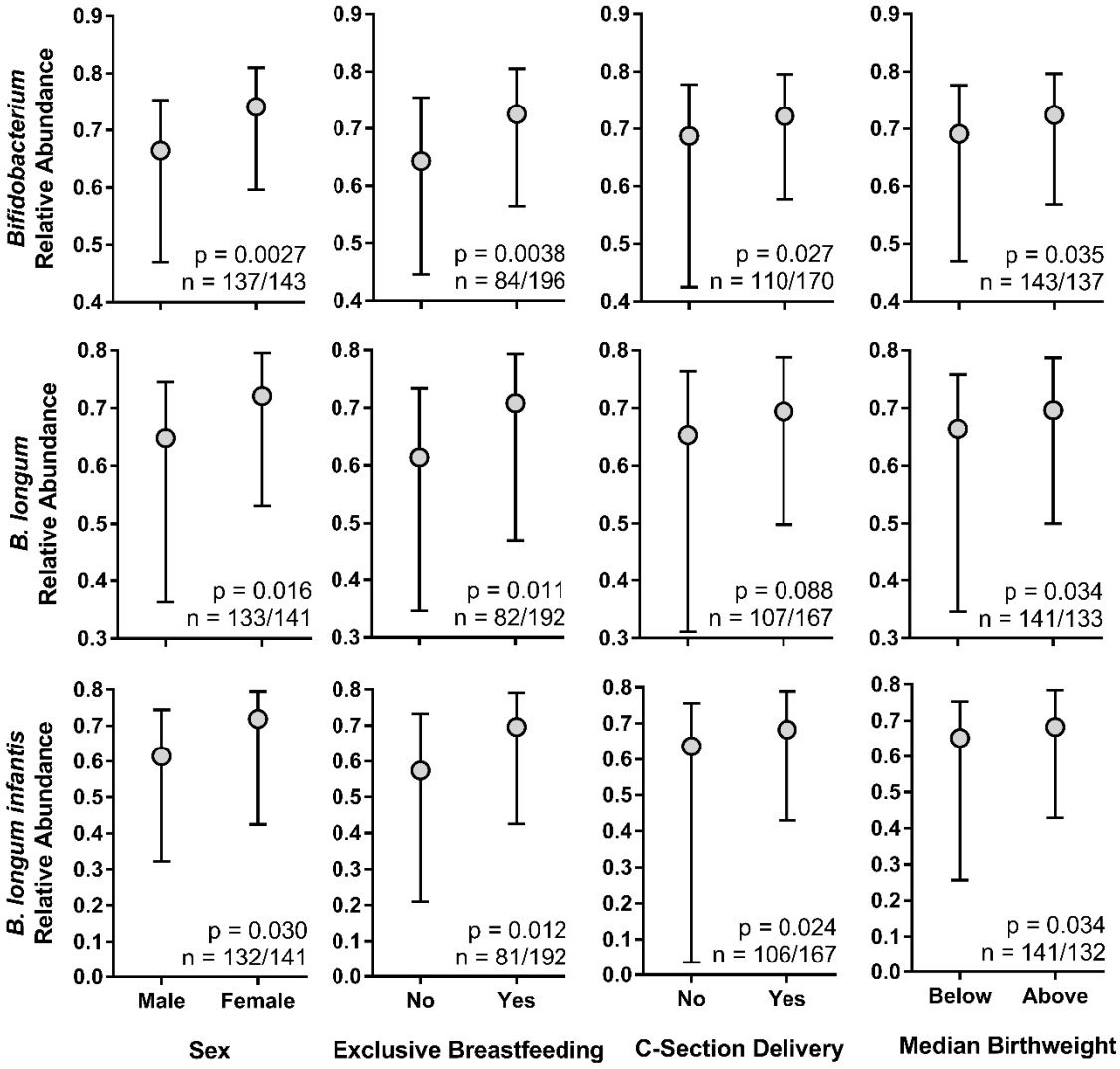
Supplemental Figure 3: Shannon diversity index rarefied by sequence per sample. Dotted vertical line represents the minimum sequence per sample at which Shannon diversity index reached a plateau. The solid line represents the sequence depth (3000 sequences per sample) selected for the single rarefaction.



Supplemental Figure 4: (A) MiSeq sequencing batch 1 (n = 535) had significantly lower sequence depth compared to batch 2 (n = 554). However, they did not have different (B) α-diversity indices, (C and D) weighted UniFrac β-diversity. Different letters on the box represent significant difference and common letters represent no differences. Ellipse on the PCoA plot indicates 95% confidence interval of the clusters by sequencing batch numbers. (E) Heatmap of to 30 OTU abundances. Top bar represent batch 1 (blue) and batch 2 (yellow).



Supplemental Figure 5: Mean relative abundance of major bacterial phylum by age. Bar represent 95% CI. Data points with different letter represent significantly different relative abundance.



Supplemental Figure 6: Median relative abundance (error bars indicate 25th/75th percentiles) of *Bifidobacterium*, *B. longum* and *B. longum* subsp. *infantis* at 6 wk of age compared by sex, breastfeeding status, delivery type and median birthweight. P-values are derived from a generalized linear model analysis (shown in **Supplemental Table 2**) including treatment group, sex, delivery type and median birthweight, with all possible interactions among these four variables per our original protocol, as well as breastfeeding status without interactions.

Supplemental Table 1: Number and percentage of infants receiving none, 1 2 or 3 high-dose vitamin A supplements from public health programs between the 15 wk and 2 y study visits [a]

Group		Number of vitamin A supplements				Total
		0	1	2	3	
Placebo	N	23	40	44	21	128
	%	18.0	31.3	34.4	16.4	100
Vitamin A	N	19	38	44	20	121
	%	15.7	31.4	36.4	16.5	100
Total	N	42	78	88	41	249
	%	16.9	31.3	35.3	16.5	100

[a] Frequencies did not differ between the groups (Chi-square = 0.260; p = 0.97).

Supplemental Table 2: Results of generalized linear regression analysis (F statistic and P value; described in Methods) assessing effect of vitamin A treatment group on *Bifidobacterium* genus, species (*B. longum*) and subspecies (*B. longum* subsp. *infantis*) relative abundance measured at 6 wk of age.

Effect [a]	*Bifidobacterium*		*B. longum*		*B. longum* subsp. *infantis*	
	F Value	*P*	F Value	*P*	F Value	*P*
Group	0.34	0.563	0.00	0.944	0.03	0.873
Sex	9.17	*0.003	5.83	*0.016	4.76	*0.030
BWM	4.51	*0.035	4.56	*0.034	4.54	*0.034
Type	4.94	*0.027	2.93	*0.088	5.13	*0.024
BF	8.53	*0.004	6.50	*0.011	6.45	*0.012
group x sex	1.19	0.276	0.08	0.783	0.28	0.598
group x BWM	0.05	0.829	1.06	0.304	2.66	0.104
group x type	1.71	0.192	0.03	0.870	0.14	0.706
sex x BWM	0.00	0.992	0.44	0.507	0.14	0.711
sex x type	0.51	0.475	0.68	0.410	0.62	0.430
BWM x type	0.87	0.352	0.82	0.366	1.06	0.305
group x sex x BWM	0.92	0.339	0.22	0.638	0.31	0.581
group x sex x type	1.08	0.300	2.17	0.142	2.47	0.118
group x BWM x type	0.12	0.730	0.27	0.602	0.41	0.522
group x sex x BWM x type	0.36	0.700	0.05	0.956	0.01	0.990

[a]P-values indicated by an asterisk (*) are <0.10. Abbreviations: Group, vitamin A or placebo treatment groups; BWM, birthweight median category (above/below); type, type of delivery (C-section/vaginal); and BF, breastfeeding status (1 = exclusive/0 = non-exclusive) at 6 wk of age).