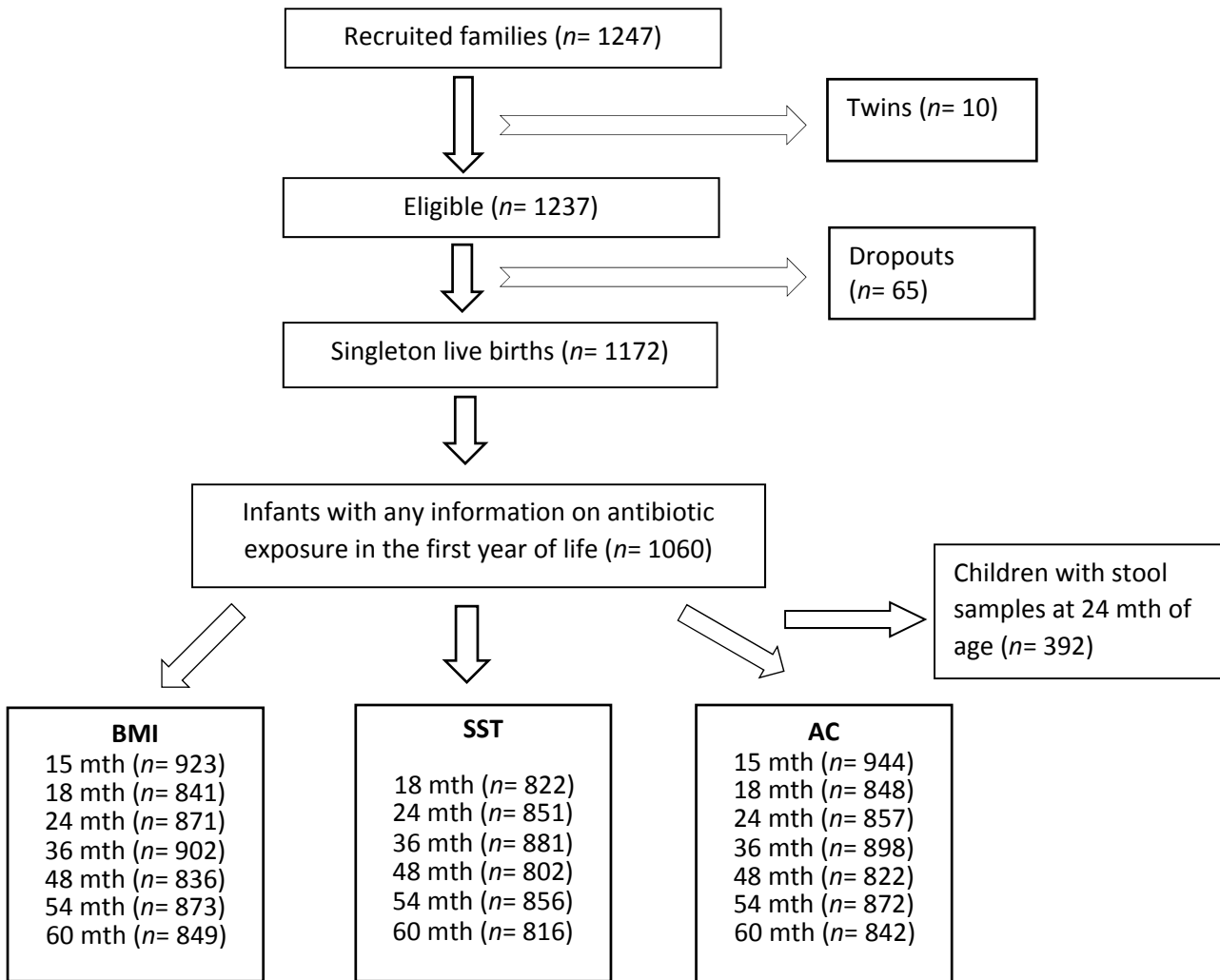


Supplementary Figure 1 Participant flow chart



Supplementary Text 1 Gut microbiota sequencing data analysis

Quality control and processing of sequencing data

QIIME 1.9.0 was used to process the raw reads for quality filtration, merging of paired-end reads and demultiplexing³⁹. Altogether, we obtained 9,868,285 high quality reads with an average of 25174 ± 9688 from 392 fecal samples. At the 97% similarity level, 1,445 operational taxonomic units (OTUs) were obtained by using USEARCH v 9.2.64⁴⁰. Rarefaction of sequence reads was performed to normalize the sequence depth across the samples. For this, the sequence reads from each sample were rarefied to 6,500 reads per sample for 1000 times for downstream analysis. OTUs with <0.1% relative abundance within each sample and <5% prevalence were removed³⁹, resulting in 312 OTUs for subsequent analysis. The taxonomic identity of the surviving OTUs was assigned against the SILVA 123 rRNA database.

Co-abundant network construction

Co-abundant network was constructed by using the SparCC algorithm and PERMANOVA test. SparCC algorithm (bootstrap value, 100) was used to calculate the correlation coefficient between OTUs²⁸. The 312 OTUs were clustered into 34 co-abundant groups (CAGs) by using hierarchical Ward's linkage clustering and PERMANOVA (999 permutations, $P < 0.001$) test based on the SparCC correlation coefficient matrix in R version 3.3.1.

α - and β -diversity analysis of the gut microbiota

The abundance table of OTUs was used to calculate α -diversity in QIIME 1.9.0., including Shannon's index, Simpson's index, Faith's phylogenetic diversity whole tree, observed species, and Chao1. β -diversity including principal coordinate analysis (PCoA) based on Euclidean, Bray-Curtis, UniFrac, and Jaccard distances was also performed using QIIME 1.9.0.. Linear regression analysis was used to assess the effect of antibiotic exposure on α - and β -diversity of gut microbiota after accounting for covariates in MATLAB 2016b (The MathWorks, MA, USA).

-Supplemental Materials-

Supplementary Table 1 Characteristics of included GUSTO participants according to cumulative antibiotic exposure in the first year of life

	Not exposed (n= 660)	Exposed 1 time (n= 271)	Exposed 2 times (n= 92)	Exposed ≥ 3 times (n= 30)	P-value ¹
<i>Sex</i>					0.026
Male	329 (50%)	146 (54%)	55 (60%)	22 (73%)	
Female	331 (50%)	125 (46%)	37 (40%)	8 (27%)	
<i>Ethnicity</i>					0.007
Chinese	396 (60%)	147 (54%)	39 (42%)	19 (63%)	
Malay	153 (23%)	80 (30%)	25 (27%)	7 (23%)	
Indian	111 (17%)	44 (16%)	28 (30%)	4 (13%)	
<i>Birth order</i>					0.77
First-born	298 (45%)	126 (46%)	37 (40%)	14 (47%)	
Non-first-born	362 (55%)	145 (54%)	55 (60%)	16 (53%)	
<i>Education status</i>					0.79
Primary or secondary	191 (29%)	83 (31%)	25 (27%)	8 (27%)	
Post-secondary	228 (35%)	104 (38%)	35 (38%)	12 (40%)	
University	241 (37%)	84 (31%)	32 (35%)	10 (33%)	
<i>Maternal gestational diabetes</i>					0.55
No	539 (82%)	227 (84%)	78 (85%)	27 (90%)	
Yes	121 (18%)	44 (16%)	14 (15%)	3 (10%)	
<i>Preterm birth</i>					0.29
No	621 (94%)	251 (93%)	88 (96%)	26 (87%)	
Yes	39 (6%)	20 (7%)	4 (4%)	4 (13%)	
<i>Low birthweight</i>					0.70
No	614 (93%)	246 (91%)	85 (92%)	28 (93%)	
Yes	46 (7%)	25 (9%)	7 (8%)	2 (7%)	
<i>Mode of delivery</i>					0.48
Vaginal	460 (70%)	181 (67%)	69 (75%)	22 (73%)	
Non-vaginal	200 (30%)	90 (33%)	23 (25%)	8 (27%)	
<i>Maternal pre-pregnancy regular smoking</i>					0.025
No	591 (90%)	225 (83%)	78 (85%)	24 (80%)	
Yes	69 (10%)	46 (17%)	14 (15%)	6 (20%)	
<i>Breastfeeding duration</i>					0.46
<1 month	192 (29%)	80 (30%)	22 (24%)	5 (17%)	
1 to <3 months	125 (19%)	44 (16%)	21 (23%)	7 (23%)	
3 to <6 months	106 (16%)	47 (17%)	11 (12%)	5 (17%)	
6 to <12 months	102 (15%)	55 (20%)	15 (16%)	5 (17%)	
≥12 months	135 (20%)	45 (17%)	23 (25%)	8 (27%)	
Maternal age, y	31.1 ± 5.1	30.9 ± 5.1	29.4 ± 5.0	29.6 ± 4.8	0.016
Maternal height, cm	158.4 ± 5.7	158.0 ± 5.4	157.7 ± 5.8	159.9 ± 5.6	0.21
Maternal pre-pregnancy BMI, kg/m ²	22.4 ± 4.3	23.2 ± 4.4	23.4 ± 4.6	23.0 ± 5.1	0.037
Maternal pregnancy weight gain, kg	13.4 ± 5.1	13.4 ± 5.8	14.1 ± 5.7	13.3 ± 6.7	0.71

¹From one-way ANOVA (continuous variables) and chi-square test (categorical variables)

-Supplemental Materials-

Supplementary Table 2 Characteristics of included GUSTO participants according to any obesity between ages 15-60 months

	Not obese (n= 874)	Obese (n= 142)	P-value ¹
<i>Sex</i>			0.26
Male	454 (52%)	81 (57%)	
Female	420 (48%)	61 (43%)	
<i>Ethnicity</i>			<0.001
Chinese	530 (61%)	57 (40%)	
Malay	197 (23%)	50 (35%)	
Indian	147 (17%)	35 (25%)	
<i>Birth order</i>			0.57
First-born	390 (45%)	67 (47%)	
Non-first-born	484 (55%)	75 (53%)	
<i>Education status</i>			0.001
Primary or secondary	243 (28%)	48 (34%)	
Post-secondary	304 (35%)	63 (44%)	
University	327 (37%)	31 (22%)	
<i>Maternal gestational diabetes</i>			0.15
No	713 (82%)	123 (87%)	
Yes	161 (18%)	19 (13%)	
<i>Preterm birth</i>			0.44
No	816 (93%)	135 (95%)	
Yes	58 (7%)	7 (5%)	
<i>Low birthweight</i>			0.011
No	803 (92%)	139 (98%)	
Yes	71 (8%)	3 (2%)	
<i>Mode of delivery</i>			0.92
Vaginal	607 (69%)	98 (69%)	
Non-vaginal	267 (31%)	44 (31%)	
<i>Maternal pre-pregnancy regular smoking</i>			0.11
No	768 (88%)	118 (83%)	
Yes	106 (12%)	24 (17%)	
<i>Breastfeeding duration</i>			0.09
<1 month	230 (26%)	45 (32%)	
1 to <3 months	152 (17%)	33 (23%)	
3 to <6 months	143 (16%)	23 (16%)	
6 to <12 months	156 (18%)	21 (15%)	
≥12 months	193 (22%)	20 (14%)	
Maternal age, y	31.0 ± 5.0	30.5 ± 5.4	0.31
Maternal height, cm	158.3 ± 5.7	158.3 ± 5.4	0.98
Maternal pre-pregnancy BMI, kg/m ²	22.5 ± 4.2	24.4 ± 4.8	<0.001
Maternal pregnancy weight gain, kg	13.3 ± 5.3	14.3 ± 5.7	0.05

¹From independent sample t-test (continuous variables) and chi-square test (categorical variables)

-Supplemental Materials-

Supplementary Table 3 Associations between early life antibiotic exposure with later childhood obesity [age-and-sex-specific BMI (generated using WHO reference) > 95th percentile] (*n*= 1016)¹

	Unadjusted OR	Model 1 ²	Model 2 ³
<i>Any antibiotic in first year</i>			
Not exposed	Ref.	Ref.	Ref.
Exposed	1.49 (1.06, 2.09)*	1.37 (0.97, 1.93)	1.38 (0.97, 1.97)
<i>Number of antibiotic courses</i>			
Not exposed	Ref.	Ref.	Ref.
1	1.43 (0.97, 2.10)	1.32 (0.89, 1.96)	1.32 (0.88, 1.97)
2	1.25 (0.68, 2.27)	1.07 (0.58, 1.97)	1.07 (0.56, 2.02)
≥3	2.30 (0.99, 5.32)	2.29 (0.96, 5.44)	2.45 (1.01, 5.98)*
<i>Age at first antibiotic prescription</i>			
Not exposed	Ref.	Ref.	Ref.
<3 months	0.76 (0.34, 1.73)	0.69 (0.30, 1.58)	0.65 (0.28, 1.51)
3 to <6 months	1.39 (0.79, 2.47)	1.35 (0.75, 2.42)	1.29 (0.70, 2.36)
6 to 12 months	1.68 (1.14, 2.49)*	1.51 (1.01, 2.26)*	1.58 (1.04, 2.38)*

¹Values are odds ratio (95% CI) from logistic regressions. **P*< 0.05

²Model 1: adjusted for ethnicity and pre-pregnancy BMI

³Model 2: adjusted for ethnicity, maternal age, education station, height, pre-pregnancy BMI, weight gain during pregnancy, gestational diabetes, regular smoking, mode of delivery, child birth order, preterm birth, low birth weight, duration of any breastfeeding

-Supplemental Materials-

Supplementary Table 4 Associations between antibiotic exposures in the first year of life with later childhood obesity using cox proportional hazard regression ($n= 1016$)¹

	Unadjusted HR	Model 1 ²	Model 2 ³
<i>Any antibiotic in first year</i>			
Not exposed	Ref.	Ref.	Ref.
Exposed	1.54 (1.11, 2.13)*	1.43 (1.03, 1.99)*	1.40 (0.995, 1.98)
<i>Number of antibiotic courses</i>			
Not exposed	Ref.	Ref.	Ref.
1	1.45 (0.9994, 2.11)	1.37 (0.94, 2.00)	1.33 (0.90, 1.98)
2	1.32 (0.75, 2.33)	1.12 (0.64, 1.98)	1.10 (0.62, 1.94)
≥3	2.45 (1.18, 5.09)*	2.49 (1.22, 5.10)*	2.50 (1.23, 5.07)
<i>Age at first antibiotic prescription</i>			
Not exposed	Ref.	Ref.	Ref.
<3 months	1.03 (0.50, 2.11)	0.94 (0.46, 1.94)	0.86 (0.42, 1.77)
3 to <6 months	1.52 (0.88, 2.61)	1.49 (0.87, 2.55)	1.46 (0.86, 2.47)
6 to 12 months	1.63 (1.11, 2.38)*	1.47 (1.004, 2.16)*	1.47 (0.98, 2.19)

¹Values are hazard ratio (95% CI) from cox proportional hazard regression. * $P < 0.05$

²Model 1: adjusted for ethnicity and pre-pregnancy BMI

³Model 2: adjusted for ethnicity, maternal age, education station, height, pre-pregnancy BMI, weight gain during pregnancy, gestational diabetes, regular smoking, mode of delivery, child birth order, preterm birth, low birth weight, duration of any breastfeeding

-Supplemental Materials-

Supplementary Table 5 Associations between antibiotic exposures in the first year of life with later childhood obesity using generalized estimating equation ($n= 1016$)¹

	Unadjusted OR	Model 1 ²	Model 2 ³
<i>Any antibiotic in first year</i>			
Not exposed	Ref.	Ref.	Ref.
Exposed	1.49 (1.01, 2.20)*	1.31 (0.89, 1.92)	1.24 (0.86, 1.80)
<i>Number of antibiotic courses</i>			
Not exposed	Ref.	Ref.	Ref.
1	1.49 (0.96, 2.30)	1.33 (0.87, 2.04)	1.26 (0.83, 1.91)
2	0.96 (0.45, 2.05)	0.74 (0.35, 1.57)	0.69 (0.33, 1.44)
≥3	2.41 (1.01, 5.74)*	2.25 (0.94, 5.41)	2.04 (0.86, 4.85)
<i>Age at first antibiotic prescription</i>			
Not exposed	Ref.	Ref.	Ref.
<3 months	0.86 (0.34, 2.15)	0.75 (0.31, 1.86)	0.70 (0.30, 1.64)
3 to <6 months	1.58 (0.85, 2.94)	1.40 (0.75, 2.61)	1.26 (0.69, 2.32)
6 to 12 months	1.56 (0.996, 2.44)	1.33 (0.85, 2.08)	1.30 (0.84, 2.02)

¹Values are odds ratio (95% CI) from generalized estimating equation. * $P < 0.05$

²Model 1: adjusted for ethnicity and pre-pregnancy BMI

³Model 2: adjusted for ethnicity, maternal age, education station, height, pre-pregnancy BMI, weight gain during pregnancy, gestational diabetes, regular smoking, mode of delivery, child birth order, preterm birth, low birth weight, duration of any breastfeeding

-Supplemental Materials-

Supplementary Table 6 The relative abundances [%] and taxonomy of the key co-abundant groups (CAGs) related with the antibiotic exposure.

CAG ID	Representative genus	Trend with the antibiotics exposure	Number of antibiotics courses				P-value ¹	FDR corrected P-value ²
			0	1	2	≥3		
CAG1	<i>Eubacterium hallii</i> group	↓	1.78±18.52	0.81±3.61	0.01±2.47	0.01±0.01	0.0005	0.0009
CAG7	<i>Bacteroides</i>	↓	3.36±2.55	2.32±6.37	3.41±4.61	1.74±6.96	0.0695	0.0695
CAG13	<i>Gemella</i>	↑	0.15±0.08	0.19±0.15	0.23±0.18	0.16±0.18	0.0186	0.0248
CAG32	<i>Tyzzarella 4</i>	↑	0.63±0.31	0.64±1.52	1.63±1.18	0.89±1.95	0.0005	0.0009

Data are presented as mean ± standard deviation.

Arrows in red indicate the directionality of associations with antibiotic exposure for CAGs that were associated with accumulation of adiposity.

¹P-values from MaAsLin analysis after adjusting for covariates (*FUT2* secretor status, delivery mode, duration of breastfeeding and birth weight).

²FDR-corrected *P*-value using the Benjamini-Hochberg method.

-Supplemental Materials-

Supplementary Table 7 The relative abundances [%] and taxonomy of the key operational taxonomic units (OTUs) related to the antibiotic exposure.

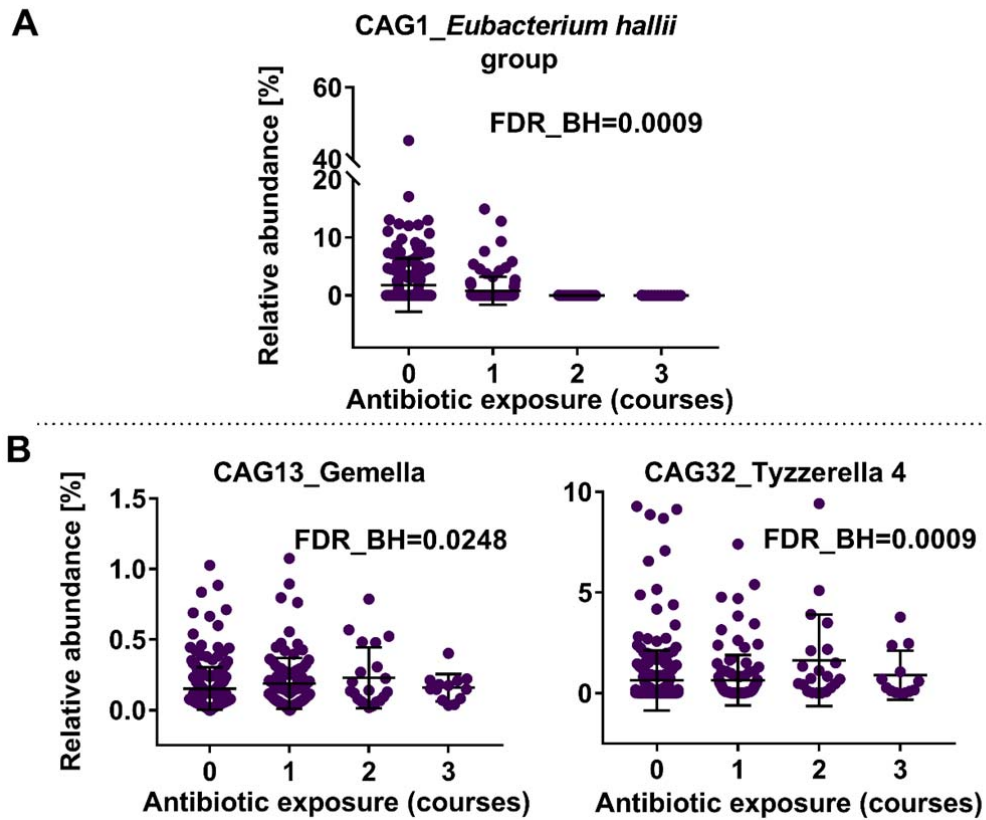
OTU ID	Phylum	Family	Genus	Trend with the antibiotics exposure	Antibiotics exposure (courses)				P-value ¹	FDR corrected P-value ²
					0	1	2	≥3		
OTU24	Firmicutes	Lachnospiraceae	<i>Eubacterium hallii</i> group	↓	1.74±4.51	0.79±2.36	0.01±0.01	0.01±0.01	0.0004	0.0022
OTU567	Firmicutes	Lachnospiraceae	<i>Eubacterium hallii</i> group	↓	0.03±0.07	0.01±0.04	0	0	0.0004	0.0022
OTU1466	Bacteroidetes	Bacteroidaceae	<i>Bacteroides</i>	↓	0.18±0.75	0.07±0.30	0.01±0.03	0.01±0.02	0.4613	0.4613
OTU82	Bacteroidetes	Bacteroidaceae	<i>Bacteroides</i>	↓	0.24±1.00	0.20±0.84	0.08±0.18	0.00±0.00	0.0529	0.0754
OTU99	Bacteroidetes	Porphyromonadaceae	<i>Dysgonomonas</i>	↓	0.06±0.55	0.01±0.06	0.00±0.00	0	0.1150	0.1341
OTU18	Firmicutes	Lachnospiraceae	<i>Tyzzarella 4</i>	↑	0.63±1.49	0.64±1.25	1.63±2.27	0.89±1.21	0.0005	0.0022
OTU4	Firmicutes	Lachnospiraceae	<i>Lachnoclostridium</i>	↑	4.83±6.69	6.52±9.67	7.82±6.81	5.75±6.06	0.0349	0.0610
OTU54	Firmicutes	Erysipelotrichaceae	<i>Faecalitalea</i>	↑	0.09±0.43	0.29±1.60	0.18±0.49	0.51±1.21	0.0104	0.0242
OTU101	Firmicutes	Erysipelotrichaceae	<i>Faecalitalea</i>	↑	0.03±0.11	0.05±0.18	0.11±0.19	0.03±0.07	0.1346	0.1449
OTU125	Firmicutes	Ruminococcaceae	<i>Ruminiclostridium 5</i>	↑	0.02±0.11	0.04±0.17	0.10±0.39	0.05±0.11	0.0180	0.0360
OTU154	Firmicutes	Lactobacillaceae	<i>Lactobacillus</i>	↑	0.00±0.01	0.00±0.00	0.00±0.00	0.79±2.84	0.0008	0.0027
OTU8	Firmicutes	Streptococcaceae	<i>Streptococcus</i>	↑	2.52±3.64	2.76±4.01	3.96±5.01	4.12±5.20	0.0935	0.1190
OTU9	Firmicutes	Peptostreptococcaceae	<i>Intestinibacter</i>	↑	2.06±3.64	2.15±3.38	4.10±5.45	2.67±3.18	0.0538	0.0754
OTU167	Actinobacteria	Actinomycetaceae	<i>Actinomyces</i>	↑	0.03±0.03	0.03±0.04	0.04±0.04	0.06±0.08	0.0069	0.0193

Data are presented as mean ± standard deviation.

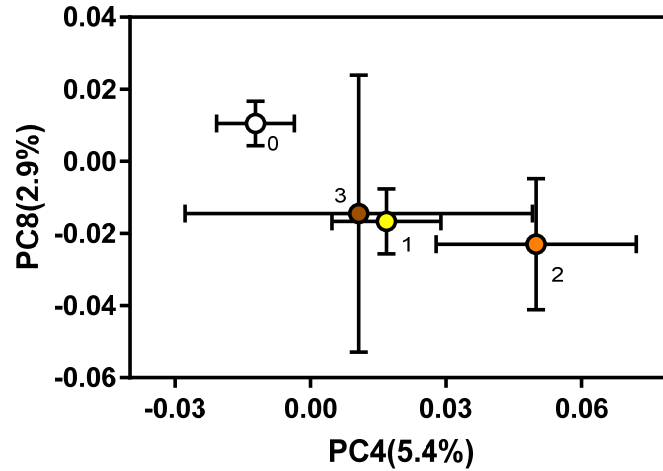
Arrows in red indicate the directionality of significant association with antibiotic exposure for CAGs that were significantly associated with accumulation of adiposity.

¹P-values from MaAsLin analysis after adjusting for covariates (*FUT2* secretor status, delivery mode, duration of breastfeeding and birth weight).

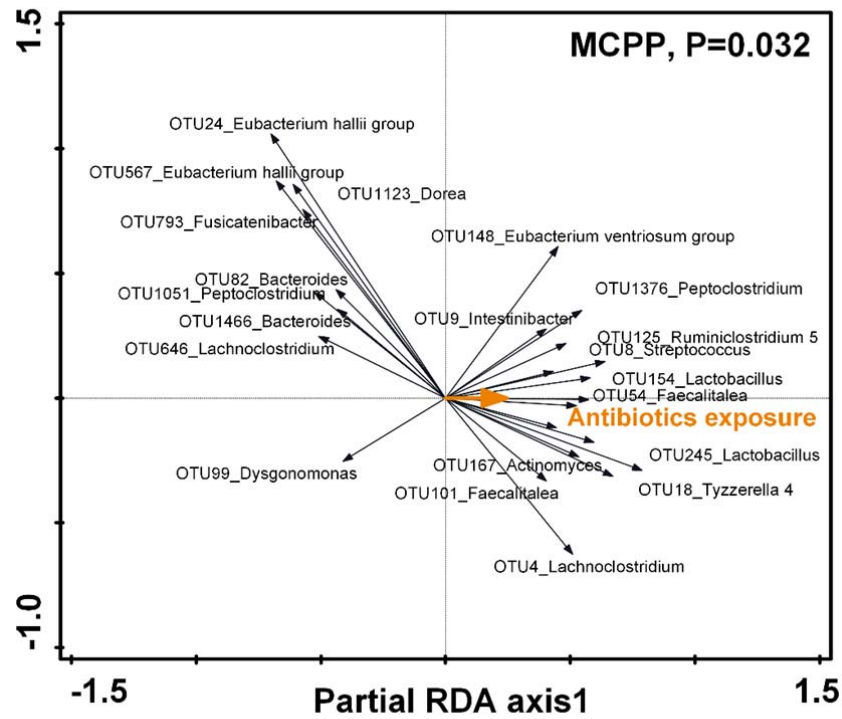
²FDR-corrected *P*-value using the Benjamini-Hochberg method.



Supplementary Figure 2 The relative abundances of 3 key CAGs associated with antibiotic exposure and adiposity accumulation. **A)** CAG1 abundances reduced with repeated antibiotic exposure; **B)** CAGs enriched by repeated antibiotic exposure. FDR_BHs represents the FDR corrected *P*-values determined by Benjamini-Hochberg method in MaAsLin analysis.

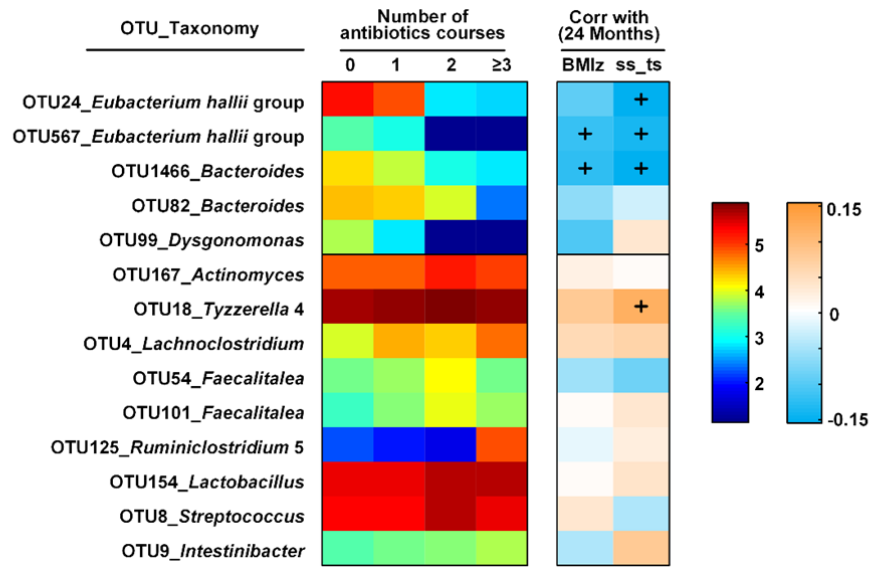


Supplementary Figure 3 Bray-Curtis PCoA of gut microbiota based on the OTU data. Each point represents the mean of principal coordinate (PC) score of samples in each group, and the error bar represents the standard error of the mean. The number labeling beside each point means grouping, namely child receiving none, one, two, and at least three courses of antibiotic exposure in the first year. The sample size (n) for each group is 243, 109, 21, and 13. PC4 and PC8 correlated with antibiotic exposures (P -value= 0.05 and 0.04) by using linear regression analysis after accounting for covariates.

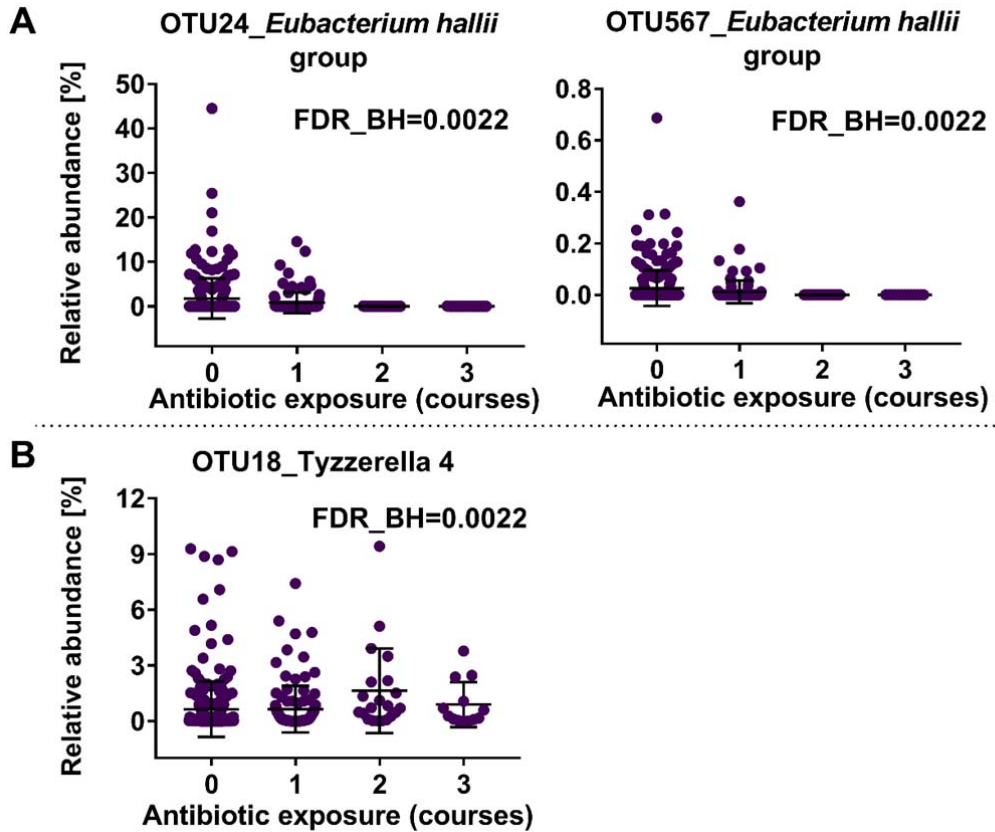


Supplementary Figure 4 Identification of operational taxonomic units (OTUs) associated with antibiotic exposure by partial redundancy analysis. The number of antibiotic courses infants received in the first year of life was used as the environmental variable. *FUT2* secretor status, delivery mode, duration of breastfeeding and birth weight were used as covariates. OTUs that explained $\geq 1\%$ inter-individual variability on the first axis are indicated by arrows. The statistical significance of the effects of the antibiotic exposure were tested using the Monte-Carlo Permutation Procedure (MCP).

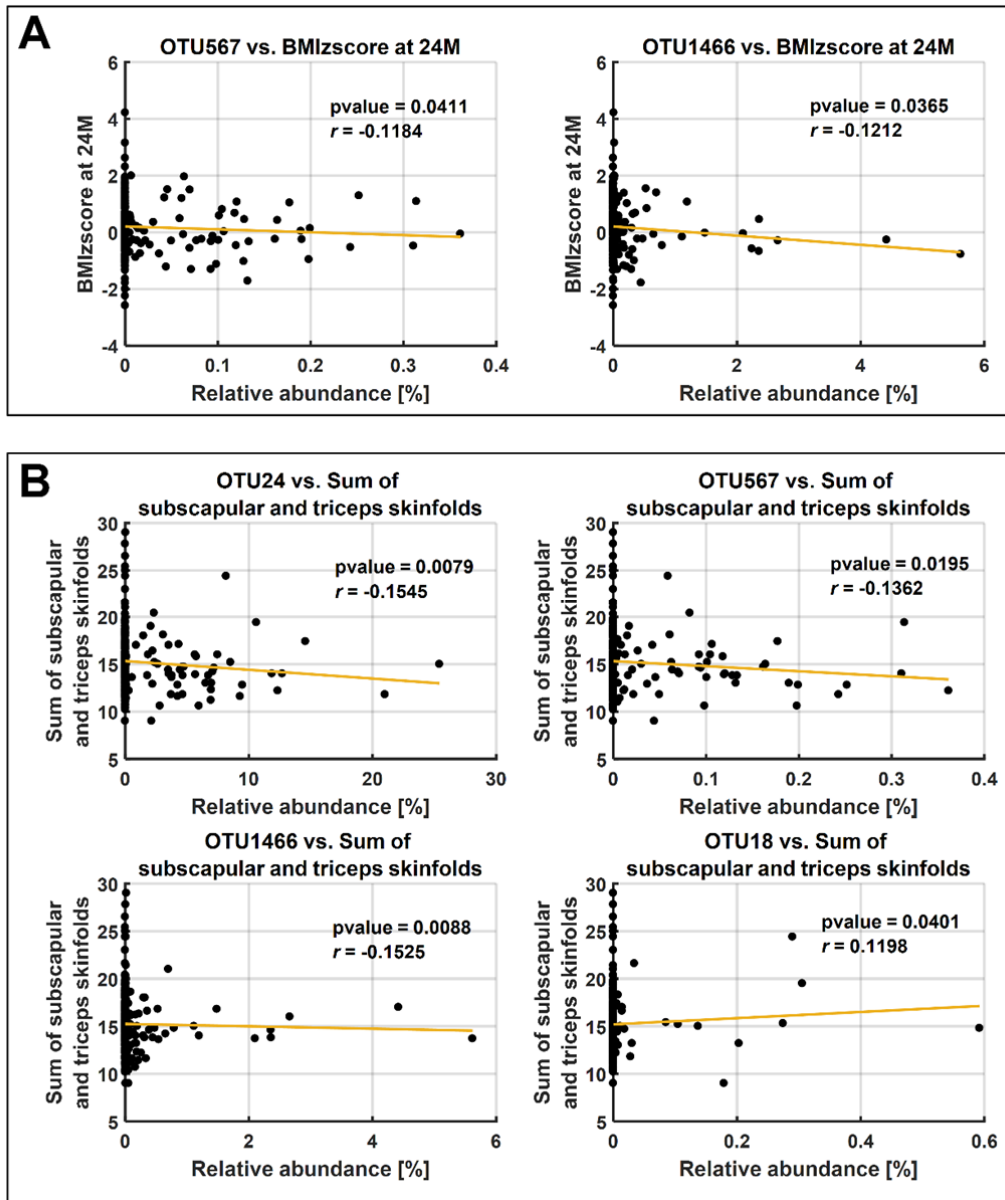
-Supplemental Materials-



Supplementary Figure 5 Heatmap of RDA-identified operational taxonomic units (OTUs) associated with antibiotic exposure, and Spearman’s correlation between the identified OTUs and the accumulation of adiposity at 24 months. The heatmap panel on the extreme left indicates the mean relative abundance (log-transformed) of the OTUs in each antibiotic exposure group, as per the color scale on right; brown represents the highest and dark blue represents the lowest abundance. The second panel from left represents R-value of Spearman’s correlation between the OTU and the obesity-related measurements, i.e. BMI z-score (BMIz) and the sum of subscapular and triceps skinfolds (ss_ts) at 24 months. ‘+’ indicates the associations that passed $P < 0.05$.



Supplementary Figure 6 The relative abundances of 3 key OTUs associated with antibiotic exposure and adiposity accumulation. **A)** OTU24 and 567 were reduced upon repeated antibiotic exposure; **B)** OTU18 was significantly enriched upon repeated antibiotic exposure. FDR_BHs represents the FDR corrected *P*-values determined by Benjamini-Hochberg method in MaAsLin analysis.



Supplementary Figure 7 Scatter plots between the key operational taxonomic units (OTUs) and obesity-related measurements (A) BMI z-score and (B) sum of subscapular and triceps skinfolds at 24 month.