Supplementary Material to:

Mode of delivery modulates the intestinal microbiota and impacts the response to vaccination

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Supplementary Figures

Supplementary Figure 1. Sample overview
Fecal samples were collected for gut microbiota characterization from 120 healthy infants at days (d)0 and 1, weeks (w)1 and 2, and months (m)1, 2, 4, 6, 9 and 12. Fecal samples were excluded from the analysis if they had insufficient bacterial DNA available (n=104). Saliva was collected from 118 infants at the age of 12 months for measuring anti-pneumococcal immunoglobulin G (IgG) and from 78 infants at the age of 18 months for measuring anti-menningococcal IgG. Saliva samples were excluded if infants did not receive their vaccinations in time (n=8 at month 12, n=1 at month 18), or if the saliva sample did not have a sufficient volume for laboratory analysis (n=8 at month 12, n=11 at month 18). N=total number of infants from which samples were collected; n=total number of available samples. Source data are provided in the Source Data file.
Supplementary Figure 2. Dirichlet multinomial mixture model fit.
Dirichlet multinomial mixture model identified 3 compositionally distinct community state types (CST) as the best model fit at the week 1 timepoint. Model fit was based on the Laplace approximation to the negative log model where a lower value indicates a better model fit. Source data are provided in the Source Data file.
Supplementary Figure 3. Species-level composition of community state types at week 1 of age.
Relative abundances of the top 10 species in 20 week 1 samples, determined by whole genome shotgun sequencing. Samples were ordered by week 1 community state type (CST). Source data are provided in the Source Data file.
Supplementary Figure 4. Community state types at 12 months of age.

(A) Dirichlet multinomial mixture model identified two compositionally distinct community state types (CST) as the best model fit at the month 12 timepoint. Model fit was based on the Laplace approximation to the negative log model where a lower value indicates a better model fit. (B) Boxplot of relative abundances of the top 7 operational taxonomic units (OTUs) per community state type (CST) defined at 12 months of age. Boxes show medians with interquartile ranges. (C) Month 12 CSTs are plotted against anti-Ps6B IgG concentrations (left) and anti-MenC IgG concentrations (right). Dots are colored according to mode of delivery and feeding type from birth. Black dots and error bars represent geometric mean concentrations with 95% confidence intervals. Significance was assessed using two-sided ANOVA on log-transformed IgG concentrations, correcting for time between vaccination and IgG measurements. Source data are provided in the Source Data file.
Supplementary Tables

|                  | Formula feeding vs breastfeeding | Vaginal birth vs C-section birth | Female vs male | AB vs no AB in first 3 months | Pets vs no pets in household | Formula feeding * vs vaginal birth |
|------------------|---------------------------------|---------------------------------|----------------|-------------------------------|-----------------------------|-----------------------------------|
| Ps               | \( \beta \) (95% CI)            | \( p \)-value                   | \( \beta \)    | \( p \)-value                 | \( \beta \)                  | \( p \)-value                      |
| 1                | 0.57 (-0.19-1.33)               | 0.142                           | 0.48 (-0.01-0.96) | 0.046                         | -0.09 (-0.52-0.34)           | 0.682                             | -0.26 (-0.91-0.39)               | 0.429 | -0.19 (-0.62-0.24) | 0.384 | -1.87 (-3.00-0.73) | 0.002 |
| 4                | -0.17 (-0.82-0.47)              | 0.596                           | 0.26 (-0.14-0.66) | 0.194                         | -0.01 (-0.37-0.36)           | 0.971                             | -0.29 (-0.83-0.26)               | 0.302 | -0.38 (-0.74-0.02) | 0.041 | -0.80 (-1.76-0.16) | 0.100 |
| 5                | 0.35 (-0.27-0.97)               | 0.263                           | 0.49 (-0.11-0.87) | 0.013                         | 0.06 (-0.29-0.40)            | 0.751                             | -0.25 (-0.78-0.27)               | 0.340 | -0.15 (-0.50-0.20) | 0.394 | -1.37 (-2.28-0.45) | 0.004 |
| 7F               | 0.35 (-0.29-1.00)               | 0.281                           | 0.41 (-0.01-0.81) | 0.045                         | 0.38 (-0.02-0.75)            | 0.040                             | -0.32 (-0.87-0.23)               | 0.250 | -0.25 (-0.62-0.11) | 0.171 | -1.46 (-2.42-0.50) | 0.003 |
| 9V               | 0.36 (-0.26-0.99)               | 0.246                           | 0.36 (-0.02-0.75) | 0.065                         | 0.03 (-0.32-0.38)            | 0.874                             | -0.40 (-0.92-0.13)               | 0.140 | -0.19 (-0.54-0.16) | 0.291 | -1.23 (-2.16-0.31) | 0.009 |
| 14               | 0.41 (-0.31-1.12)               | 0.262                           | 0.32 (-0.12-0.76) | 0.154                         | 0.16 (-0.24-0.57)            | 0.422                             | -0.23 (-0.84-0.37)               | 0.444 | -0.37 (-0.77-0.03) | 0.070 | -1.50 (-2.56-0.44) | 0.006 |
| 18C              | -0.49 (-1.23-0.25)              | 0.191                           | 0.05 (-0.40-0.51) | 0.820                         | 0.09 (-0.33-0.50)            | 0.674                             | -0.72 (-1.34-0.09)               | 0.025 | -0.52 (-0.94-0.11) | 0.014 | -0.28 (-1.38-0.81) | 0.610 |
| 19F              | 0.27 (-0.44-0.99)               | 0.452                           | 0.29 (-0.16-0.73) | 0.203                         | 0.16 (-0.24-0.56)            | 0.434                             | -0.53 (-1.14-0.08)               | 0.087 | -0.29 (-0.69-0.11) | 0.154 | -1.32 (-2.38-0.26) | 0.015 |
| 23F              | 0.10 (-0.60-0.80)               | 0.778                           | 0.39 (-0.04-0.83) | 0.076                         | -0.01 (-0.40-0.39)           | 0.978                             | -0.40 (-0.99-0.20)               | 0.187 | -0.28 (-0.66-0.13) | 0.186 | -0.79 (-1.83-0.25) | 0.135 |

Supplementary Table 1. Validation of associations between early-life characteristics and anti-pneumococcal IgG concentrations following vaccination. Model coefficients (\( \beta \)) with 95% CI and \( p \)-values were computed with two-sided multivariable linear regression on log-transformed anti-pneumococcal concentrations per serotype (Ps; \( n=101 \)). All analyses were corrected for time between vaccination and IgG measurement, but not for multiple comparisons. Abbreviations: C-section = caesarean section; AB = antibiotics.
| Serotype | Time Interval  | $\beta$ (95% CI) | Adjusted p-value |
|----------|---------------|------------------|------------------|
| 1        | d1-w1         | 1.20 (-0.03 - 2.42) | 0.065            |
|          | w1-w2         | 0.86 (-0.05 - 1.78) | 0.065            |
| 4        | d1-w1         | 1.07 (0.08 - 2.06)  | 0.055            |
|          | w1-w2         | 0.75 (-0.02 - 1.51) | 0.055            |
| 5        | d1-w1         | 1.00 (0.05 - 1.95)  | 0.040            |
|          | w1-w2         | 0.80 (0.09 - 1.51)  | 0.040            |
| 7F       | d1-w1         | 0.72 (-0.29 - 1.73) | 0.162            |
|          | w1-w2         | 0.96 (0.22 - 1.70)  | 0.024            |
| 9V       | d1-w1         | 1.22 (0.29 - 2.15)  | 0.011            |
|          | w1-w2         | 1.02 (0.33 - 1.72)  | 0.009            |
| 14       | d1-w1         | 1.25 (0.18 - 2.33)  | 0.023            |
|          | w1-w2         | 1.25 (0.43 - 2.08)  | 0.006            |
| 18C      | d1-w1         | 1.77 (0.72 - 2.82)  | 0.002            |
|          | w1-w2         | 0.77 (-0.10 - 1.65) | 0.083            |
| 19F      | d1-w1         | 1.14 (0.13 - 2.15)  | 0.056            |
|          | w1-w2         | 0.41 (-0.38 - 1.20) | 0.307            |
| 23F      | d1-w1         | 1.01 (-0.11 - 2.13) | 0.077            |
|          | w1-w2         | 1.15 (0.27 - 2.03)  | 0.023            |

Supplementary Table 2. Validation of associations between Bray-Curtis similarity and anti-pneumococcal IgG levels.

Model coefficients ($\beta$) with 95% CI and adjusted p-values were computed with two-sided multivariable linear regression on log-transformed anti-pneumococcal concentrations per serotype (Ps; n=101). All analyses were corrected for time between vaccination and IgG measurement and for multiple comparisons.

Abbreviations: Ps = pneumococcal serotype; d1=day 1; w1=week 1; w2=week 2.
| CST | p-value | difference (adjusted p-value) | difference (adjusted p-value) | difference (adjusted p-value) | difference (adjusted p-value) |
|-----|---------|------------------------------|------------------------------|------------------------------|------------------------------|
| 1   | 3.2     | 0.043                        | 0.239                        | -0.765                       | -0.211                       | 0.808                        |
| 4   | 2.9     | 0.062                        | -0.450                       | 0.267                        | -0.595                       | 0.102                        | 0.145                        | 0.869                        |
| 5   | 3.6     | 0.031                        | -0.537                       | 0.114                        | -0.469                       | 0.188                        | 0.067                        | 0.965                        |
| 7F  | 2.5     | 0.084                        | 0.203                        | -0.382                       | 0.361                        | 0.098                        | 0.934                        |
| 9V  | 3.1     | 0.049                        | 0.188                        | -0.512                       | 0.135                        | -0.045                       | 0.985                        |
| 14  | 3.0     | 0.056                        | -0.540                       | 0.204                        | -0.596                       | 0.145                        | -0.056                       | 0.982                        |
| 18C | 3.1     | 0.050                        | -0.580                       | 0.179                        | -0.598                       | 0.161                        | -0.018                       | 0.998                        |
| 19F | 1.0     | 0.386                        | -0.297                       | 0.576                        | -0.300                       | 0.571                        | -0.002                       | 1.000                        |
| 23F | 3.0     | 0.056                        | -0.642                       | 0.139                        | -0.356                       | 0.538                        | 0.286                        | 0.670                        |

Supplementary Table 3. Validation of association between week 1 CST and IgG concentrations against pneumococcal serotypes.

All analyses were two-sided and corrected for time between vaccination and IgG measurement. Abbreviations: CST = community state type; Ps = pneumococcal serotype.
Supplementary Table 4. CST as a mediator between mode of birth and anti-Ps6B and anti-MenC IgG concentrations.

Model coefficients ($\beta$) with 95% CI and adjusted p-values were computed with two-sided multivariable linear regression on log-transformed anti-Ps6B (n=101) and anti-MenC concentrations. All analyses were corrected for time between vaccination and IgG measurement.

*Abbreviations: CST = community state type; NA = not applicable.*
| OTU                          | Interval number | Interval start | Interval end | Area | Association | p-value | Adjusted p-value | Validated for Ps*: |
|-----------------------------|-----------------|----------------|--------------|------|-------------|---------|------------------|------------------|
| *Bifidobacterium* (1)       | interval:1      | 0              | 5            | 7.2  | Above median | 0.013   | 0.027            | 1, 4, 5, 7F, 9V, 19F, 23F |
| *Escherichia coli* (2)      | interval:1      | 0              | 41           | 82.0 | Above median | 0.003   | 0.013            | 1, 4, 5, 7F, 9V, 18C, 23F |
| *Ruminococcus gnavus* (9)   | interval:1      | 0              | 16           | 20.5 | Above median | 0.016   | 0.031            | 7F               |
| *Pseudobutyroviolibrio* (13)| interval:1      | 0              | 20           | -14.3| Below median | 0.050   | 0.071            | 1, 4, 5, 7F, 9V, 18C, 23F |
| *Anaerostipes* (20)         | interval:1      | 0              | 17           | -8.6 | Below median | 0.064   | 0.084            | 5, 7F, 9V        |
| *Clostridium sensu stricto* 1 (21) | interval:1 | 0              | 31           | -46.0| Below median | 0.023   | 0.040            | 5, 7F, 23F       |
| *Prevotella* (25)           | interval:1      | 0              | 23           | -22.4| Below median | 0.001   | 0.010            | 1, 4, 5, 7F, 9V, 14, 18C, 19F, 23F |
| *Streptococcus pyogenes* (26) | interval:1     | 0              | 33           | -21.4| Below median | 0.013   | 0.027            | 1, 9V, 14        |
| *Dorea* (32)                | interval:1      | 0              | 4            | -5.2 | Below median | 0.070   | 0.089            | 4, 5, 7F, 9V     |
| *Bacteroides* (53)          | interval:1      | 0              | 48           | 42.7 | Above median | 0.002   | 0.010            | 1, 4, 5, 7F, 9V, 19F, 23F |
| *Streptococcus* (55)        | interval:1      | 0              | 43           | -50.3| Below median | 0.024   | 0.040            | 1, 4, 9V         |
| *Lactococcus lactis* (80)   | interval:1      | 0              | 33           | -35.7| Below median | 0.001   | 0.010            | 1, 4, 5, 7F, 9V, 14, 18C, 19F, 23F |
| *Bifidobacterium* (147)     | interval:1      | 0              | 19           | 18.0 | Above median | 0.002   | 0.010            | 1, 4, 5, 7F, 19F, 23F |
| *Escherichia/Shigella* (185)| interval:1      | 0              | 36           | 46.7 | Above median | 0.002   | 0.010            | 1, 7F, 18C       |
| *Pseudomonas fluorescens* (236) | interval:1 | 0              | 5            | -3.7 | Below median | 0.088   | 0.099            | 5, 7F, 9V        |
| *Bacillales* (255)          | interval:1      | 0              | 39           | -38.7| Below median | 0.001   | 0.010            | 4, 5, 7F, 9V, 19F, 23F |
| *Enterococcus* (256)        | interval:1      | 0              | 45           | -48.8| Below median | 0.002   | 0.010            | 1, 4, 5, 7F, 9V, 18C, 19F, 23F |
| *Enterobacteriaceae* (345)  | interval:1      | 0              | 15           | 7.4  | Above median | 0.020   | 0.038            |                  |
| *Staphylococaceae* (382)    | interval:1      | 0              | 58           | -52.1| Below median | 0.001   | 0.010            | 4, 5, 7F, 9V, 19F, 23F |
| *Streptococcus gallolyticus* (18) | interval:1 | 2              | 44           | 45.0 | Above median | 0.013   | 0.027            | 14               |
| *Bacteroides* (433)         | interval:1      | 3              | 8            | 2.0  | Above median | 0.023   | 0.040            | 4, 9V, 19F, 23F  |
| *Streptococcus* (502)       | interval:1      | 3              | 49           | -42.3| Below median | 0.002   | 0.010            | 1, 5, 7F, 9V, 23F |
| *Bacteroides* (19)          | interval:1      | 4              | 47           | 58.0 | Above median | 0.021   | 0.039            | 1, 4, 5, 7F, 9V, 18C, 19F, 23F |
| *Streptococcus* (189)       | interval:1      | 4              | 26           | -26.4| Below median | 0.007   | 0.019            | 1, 4, 5, 9V      |
| *Gardnerella* (333)         | interval:1      | 11             | 60           | -51.8| Below median | 0.004   | 0.014            | 1, 5, 9V, 14, 18C |
| *Veillonella* (366)         | interval:1      | 11             | 62           | -44.3| Below median | 0.004   | 0.014            | 1, 5, 7F, 9V, 14, 23F |
| *Bacteroides* (35)          | interval:1      | 12             | 47           | 42.3 | Above median | 0.009   | 0.022            | 1, 4, 5, 18C, 19F, 23F |
| *Bilophila wadsworthia* (136)| interval:1     | 14             | 70           | 52.7 | Above median | 0.006   | 0.018            | 1, 4, 5, 9V, 14, 23F |
| Species/Genus                | Interval | Above Median | Below Median | Additional Information |
|-----------------------------|----------|--------------|--------------|------------------------|
| **Subdoligranulum**         | 14       | 15-30        | 13.4         | 0.006-0.018             | 1, 4, 14                |
| **Bifidobacterium bifidum** | 14       | 16-70        | -101.4       | 0.026-0.041             | 9V, 14, 18C, 19F        |
| **Rothia**                  | 14       | 17-29        | -9.1         | 0.062-0.084             | 4, 9V, 23F              |
| **Peptostreptococcus**      | 14       | 17-70        | 50.8         | 0.081-0.095             | 9V, 23F                 |
| **Bacteroides**             | 14       | 17-70        | 39.4         | 0.002-0.010             | 4, 5, 7F, 9V, 19F, 23F  |
| **Streptococcus**           | 14       | 18-55        | -35.0        | 0.074-0.090             |                         |
| **Lactobacillus fermentum** | 14       | 18-30        | -6.0         | 0.074-0.090             | 4                       |
| **Veillonella**             | 14       | 19-32        | -7.3         | 0.029-0.045             | 1, 4, 5, 7F, 9V, 19F    |
| **Enterococcus faecium**    | 14       | 20-23        | -3.8         | 0.046-0.067             | 5, 7F, 23F              |
| **Bifidobacteriaceae**      | 14       | 21-48        | -14.5        | 0.089-0.099             | 4, 7F, 9V, 18C, 23F     |
| **Bifidobacterium animalis**| 14       | 22-64        | -54.7        | 0.015-0.031             | 1, 5, 9V, 14, 18C, 19F, 23F |
| **Bacteroides**             | 14       | 24-41        | 14.6         | 0.065-0.084             | 14, 23F                 |
| **Bacteroides**             | 14       | 26-70        | 81.1         | 0.007-0.019             | 7F, 23F                 |
| **Bifidobacterium**         | 14       | 29-70        | -40.3        | 0.005-0.017             | 9V, 14, 18C, 19F        |
| **Blautia**                 | 14       | 33-59        | 140.0        | 0.003-0.013             | 1, 4, 19F, 23F          |
| **Enterobacteriaceae**      | 14       | 33-70        | -42.4        | 0.002-0.010             | 9V, 18C, 19F, 23F       |
| **Klebsiella**              | 14       | 34-70        | -80.7        | 0.004-0.014             |                         |
| **Carnobacteriaceae**       | 14       | 38-70        | 22.6         | 0.061-0.084             | 14, 19F                 |
| **Bifidobacterium animalis**| 14       | 39-70        | -27.0        | 0.082-0.095             | 14                      |
| **Citrobacter sedlakii**    | 14       | 46-70        | -23.1        | 0.026-0.041             | 9V                      |
| **Finegoldia**              | 14       | 50-70        | -20.0        | 0.034-0.051             |                         |
| **Blautia**                 | 14       | 57-70        | -23.5        | 0.012-0.027             | 4, 5, 7F, 9V, 18C       |
| **Corynebacterium propinquum** | 14       | 66-70        | -11.6        | 0.009-0.022             |                         |

**Supplementary Table 5. Differentially abundant OTUs in the first 2 months of life between infants with above vs. below median anti-Ps6B IgG levels**

Differential abundance analysis was performed using two-sided smoothing spline ANOVA (ss-ANOVA). All analyses were corrected for time between vaccination and IgG measurements and for multiple comparisons.

* Validation for other pneumococcal vaccine serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19F and 23F was performed using the same method. OTUs were considered validated for a given serotype if they were significantly differentially abundant (adjusted p<0.100) and were associated with the same response category (above or below median).

* Abbreviations: Ps = pneumococcal serotype
| OTU                                     | Interval number | Interval start | Interval end | Area  | Association     | p-value | Adjusted p-value |
|-----------------------------------------|-----------------|----------------|--------------|-------|-----------------|---------|------------------|
| Escherichia coli (2)                    | interval:1      | 0              | 13           | 23.2  | Above median    | 0.062   | 0.072            |
| Veillonella (8)                         | interval:1      | 0              | 7            | -9.2  | Below median    | 0.037   | 0.055            |
| Peptostreptococcaceae (46)              | interval:1      | 0              | 7            | 9.4   | Above median    | 0.021   | 0.054            |
| Bacteroides (65)                        | interval:1      | 0              | 26           | 25.8  | Above median    | 0.014   | 0.054            |
| Streptococcus (69)                      | interval:1      | 0              | 14           | -18.5 | Below median    | 0.023   | 0.054            |
| Rothia (113)                            | interval:1      | 0              | 43           | -52.8 | Below median    | 0.027   | 0.054            |
| Bifidobacteriaceae (299)                | interval:1      | 0              | 16           | -7.9  | Below median    | 0.005   | 0.036            |
| Lachnospiraceae (96)                    | interval:1      | 1              | 51           | 47.4  | Above median    | 0.003   | 0.029            |
| Collinsella (16)                        | interval:1      | 3              | 20           | 23.7  | Above median    | 0.037   | 0.055            |
| Veillonella (85)                        | interval:1      | 3              | 16           | -20.9 | Below median    | 0.002   | 0.029            |
| Klebsiella (252)                        | interval:1      | 4              | 12           | -10.8 | Below median    | 0.001   | 0.029            |
| Veillonella (368)                       | interval:1      | 5              | 14           | -7.7  | Below median    | 0.013   | 0.054            |
| Bifidobacterium (218)                   | interval:1      | 6              | 42           | -25.1 | Below median    | 0.025   | 0.054            |
| Clostridium sensu stricto 1 (24)        | interval:1      | 7              | 20           | -19.7 | Below median    | 0.017   | 0.054            |
| Escherichia Shigella (185)              | interval:1      | 10             | 24           | 12.3  | Above median    | 0.043   | 0.059            |
| Bifidobacteriaceae (309)                | interval:1      | 11             | 53           | 34.6  | Above median    | 0.060   | 0.072            |
| Enterococcaceae (251)                   | interval:1      | 13             | 39           | -28.1 | Below median    | 0.012   | 0.054            |
| Veillonella (160)                       | interval:1      | 15             | 41           | -29.0 | Below median    | 0.034   | 0.055            |
| Lactobacillus (49)                      | interval:1      | 21             | 55           | -47.0 | Below median    | 0.062   | 0.072            |
| Bilophila wadsworthia (136)             | interval:1      | 21             | 69           | -54.8 | Below median    | 0.022   | 0.054            |
| bacterium NLAe zl C558 (45)             | interval:1      | 26             | 69           | 54.9  | Above median    | 0.068   | 0.076            |
| Lactobacillus fermentum (75)            | interval:1      | 33             | 69           | -49.0 | Below median    | 0.037   | 0.055            |
| Lachnospiraceae (30)                    | interval:1      | 35             | 69           | -39.3 | Below median    | 0.023   | 0.054            |
| Bifidobacterium breve (261)             | interval:1      | 38             | 69           | 30.6  | Above median    | 0.072   | 0.077            |
| Streptococcus salivarius (6)            | interval:1      | 40             | 69           | -42.4 | Below median    | 0.028   | 0.054            |
| Enterobacteriaceae (281)                | interval:1      | 45             | 62           | -11.9 | Below median    | 0.054   | 0.071            |
| Clostridium butyricum (33)              | interval:1      | 62             | 69           | 26.2  | Above median    | 0.038   | 0.055            |
Supplementary table 6. Differentially abundant OTUs in the first 2 months of life between infants with above vs. below median anti-MenC IgG levels

Differential abundance analysis was performed using two-sided smoothing spline ANOVA (ss-ANOVA). All analyses were corrected for time between vaccination and IgG measurements and for multiple comparisons.
**Supplementary Table 7.** Differentially abundant OTUs of the Lachnospiraceae family in the first 12 months of life between infants with above vs. below median anti-MenC IgG levels

*Differential abundance analysis was performed using two-sided smoothing spline ANOVA (ss-ANOVA). All analyses were corrected for time between vaccination and IgG measurements and for multiple comparisons.*

| OTU                        | Interval number | Interval start | Interval end | Area  | Association     | p-value | Adjusted p-value |
|----------------------------|-----------------|----------------|--------------|-------|-----------------|---------|------------------|
| *Lachnospira* (89)         | interval:1      | 32             | 169          | 60.8  | Above median    | 0.013   | 0.052            |
| *Lachnospiraceae* (30)     | interval:1      | 82             | 134          | -49.9 | Below median    | 0.013   | 0.052            |
| *Pseudobutyribrio* (132)   | interval:1      | 90             | 381          | 144.2 | Above median    | 0.030   | 0.066            |
| *Blautia* (67)             | interval:1      | 100            | 381          | 313.6 | Above median    | 0.020   | 0.053            |
| *Fusicatenibacter saccharivorans* (15) | interval:1 | 101            | 381          | 439.7 | Above median    | 0.050   | 0.080            |
| *Pseudobutyribrio* (13)    | interval:1      | 125            | 381          | 353.8 | Above median    | 0.004   | 0.036            |
| *Lachnospiraceae* (306)    | interval:1      | 130            | 381          | 130.4 | Above median    | 0.023   | 0.054            |
| *Roseburia* (77)           | interval:1      | 155            | 381          | 284.2 | Above median    | 0.019   | 0.053            |
| *Dorea* (54)               | interval:1      | 156            | 381          | 185.8 | Above median    | 0.075   | 0.094            |
| *Blautia* (28)             | interval:1      | 204            | 286          | 230.8 | Above median    | 0.027   | 0.062            |
| *Lachnospira* (117)        | interval:1      | 207            | 357          | 242.1 | Above median    | 0.002   | 0.021            |
| *Roseburia* (133)          | interval:1      | 257            | 381          | 150.6 | Above median    | 0.002   | 0.021            |
| *Blautia* (47)             | interval:1      | 277            | 381          | 198.6 | Above median    | 0.023   | 0.054            |
| *Moryella* (71)            | interval:1      | 317            | 381          | 85.6  | Above median    | 0.065   | 0.092            |
| *Blautia* (28)             | interval:2      | 362            | 381          | 87.5  | Above median    | 0.015   | 0.053            |
|                               | Anti-Ps6B IgG response |             | Anti-MenC IgG response |             |
|-------------------------------|------------------------|-------------|------------------------|-------------|
|                               | High       | Low   | p-value    | High      | Low  | p-value |
| **n**                         | 49         | 50    |            | 33        | 31   |         |
| *Escherichia coli* presence (%) | 34 (69.4) | 25 (50.0) | 0.078     | 23 (69.7) | 19 (61.3) | 0.657 |
| *Klebsiella* spp. presence (%) | 23 (46.9) | 21 (42.0) | 0.770     | 16 (48.5) | 13 (41.9) | 0.783 |
| *Enterococcus* spp. presence (%) | 29 (59.2) | 37 (74.0) | 0.177     | 22 (66.7) | 19 (61.3) | 0.851 |

**Supplementary Table 8. Validation of 16S rRNA gene sequencing-based results by targeted qPCR**

Presence of *E. coli*, *Enterococcus* spp. and *Klebsiella* spp. identified by quantitative polymerase chain reaction (qPCR) on all week 1 samples, for infants with high and low anti-Ps6B and anti-MenC IgG responses. *p*-values were calculated with two-sided chi-square tests.