

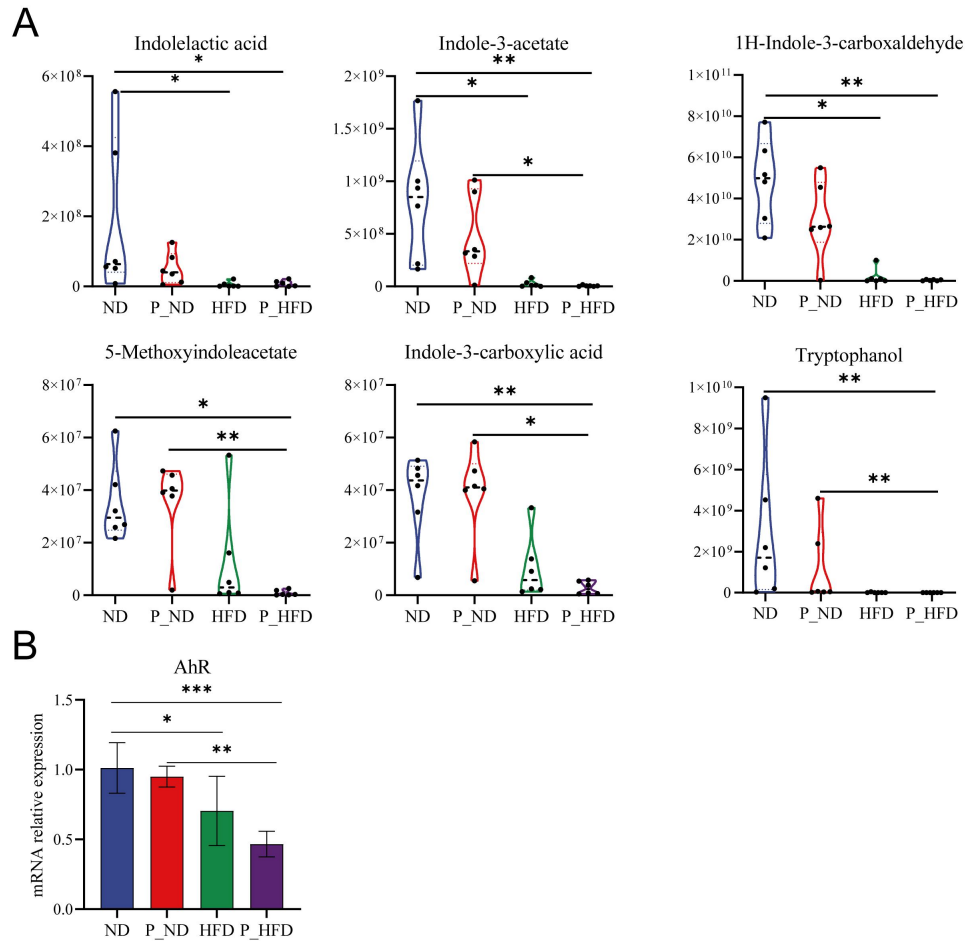
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## **Supplemental information**

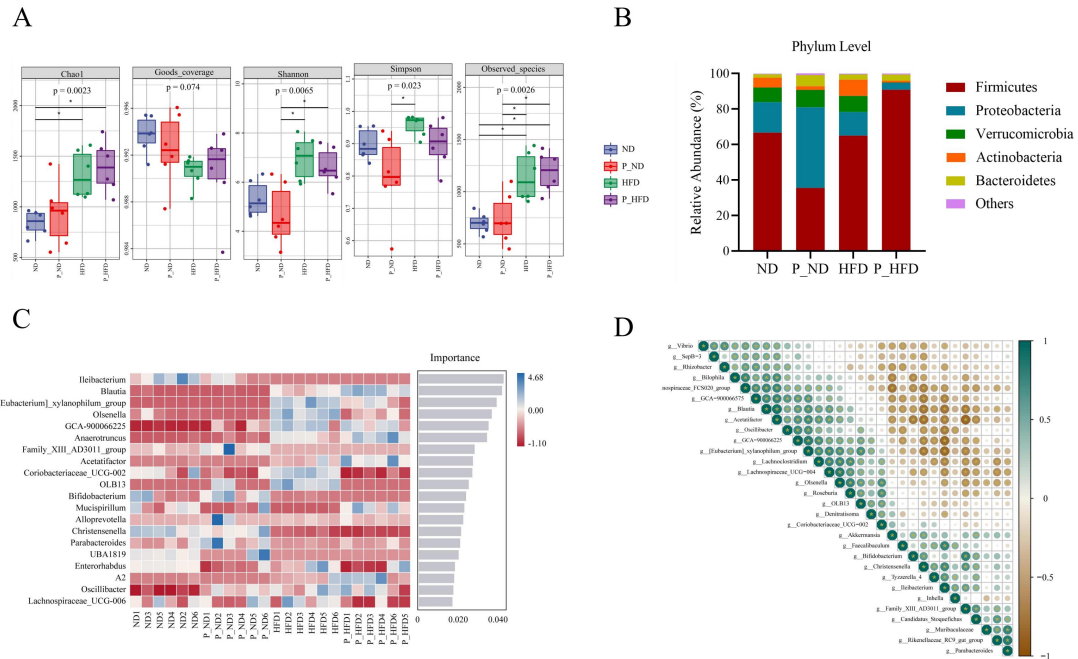
### **Periodontitis salivary microbiota exacerbates nonalcoholic fatty liver disease in high-fat diet-induced obese mice**

**Min Wang, Lili Li, Jun Qian, Nannan Wang, Jun Bao, Jiangyue Lu, Faming Chen, Yanfen Li, Yangheng Zhang, and Fuhua Yan**

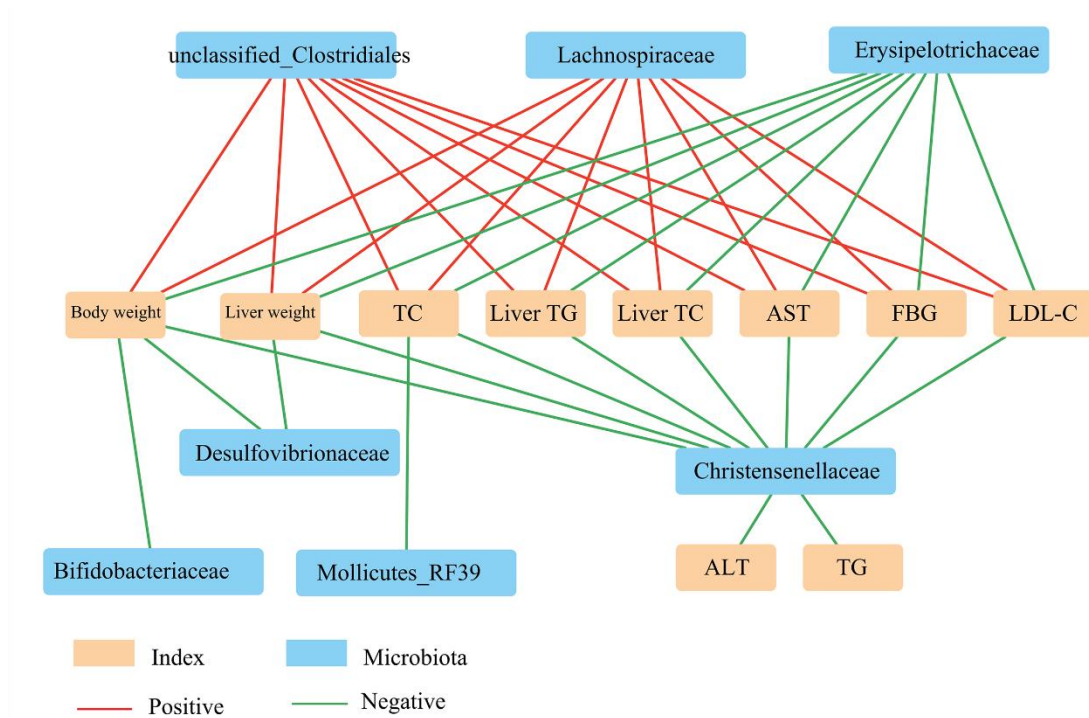
## Supporting information



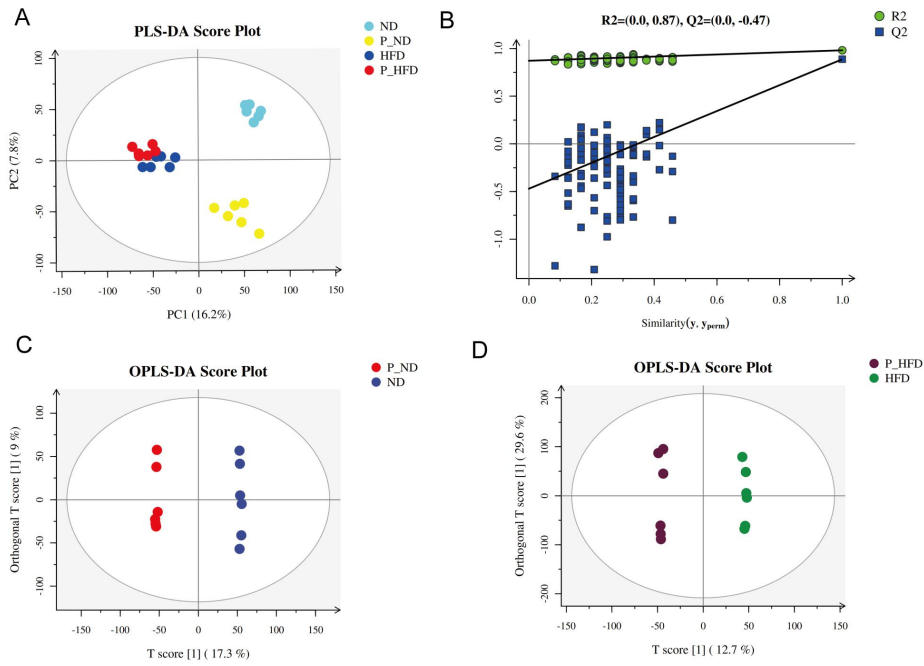
**Figure S1.** Periodontitis salivary microbiota downregulates the tryptophan-indole derivative-AhR axis in the intestine. Related to Figure 3. **(A)** The relative concentration of indole derivatives in cecal contents. **(B)** Relative mRNA levels of AhR in colon tissues. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .



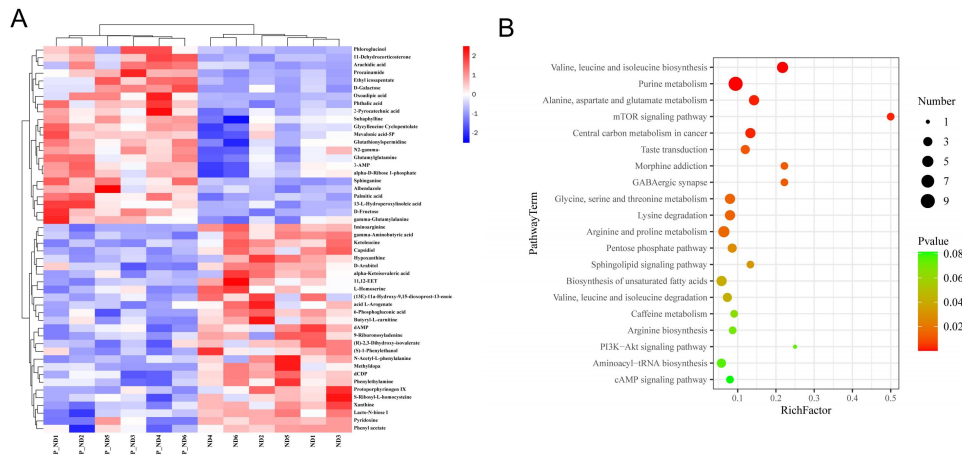
**Figure S2.** Periodontitis salivary microbiota aggravates gut microbiota dysbiosis in mice. Related to Figure 4. **(A)**  $\alpha$ -Diversity analysis based on Chao 1, Goods\_coverage, Shannon, Simpson, and Observed species. **(B)** Proportion of bacteria at the phylum level. **(C)** Random forest heat map showing the importance ranking of genera for classifier models. **(D)** Spearman's correlation analysis of the four groups. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .



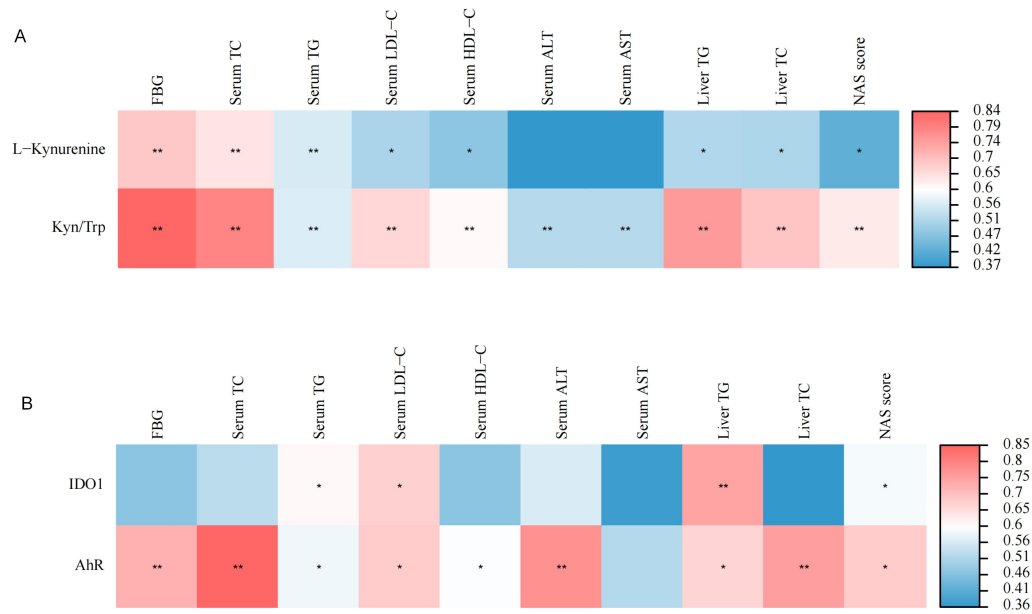
**Figure S3.** Spearman's correlation analysis between taxa at the family level and obesity-related parameters. Related to Figure 5. Significant associations with  $P < 0.05$  and  $|r| > 0.7$  are shown. Red lines indicates positive relationships between nodes, and green lines indicates negative correlations. The thickness of the lines indicates the strength of the correlation.



**Figure S4.** Overall metabolic profiles of liver tissues. Related to Figure 6. **(A)** PLS-DA score plot of cecal contents metabolism from the four groups. **(B)** Permutations plot for the PLS-DA analysis. **(C)** OPLS-DA score plot between P\_ND and ND groups. **(D)** OPLS-DA score plot between P\_HFD and HFD groups.



**Figure S5.** Differential metabolites and metabolic pathways between the P\_ND and ND groups. Related to Figure 6. **(A)** Heat map of the top 50 differential metabolites based on VIP-value. **(B)** KEGG pathway analysis of the top 20 enriched metabolic pathways.



**Figure S6.** Spearman's correlation analysis between the changes in Kynurenine-AhR axis and NAFLD parameters. Related to Figure 6. **(A-B)** The heat map showed the correlations between L-Kynurenine, Kyn/Trp, IDO1, AhR and NAFLD, glucolipid metabolism parameters. \*  $P < 0.05$ , \*\*  $P < 0.01$ .

**Table S1.** Primer sequences. Related to STAR Methods.

| Gene          | Forward primer (5'-3')  | Reverse primer (5'-3')  |
|---------------|-------------------------|-------------------------|
| ZO-1          | GGGCCATCTCAACTCCTGTA    | AGAAGGGCTGACGGGTAAT     |
| Occludin      | ACTATGCGGAAAGAGTTGACAG  | GTCATCCACACTCAAGGTCAG   |
| Claudin-1     | GAATTCTATGACCCCTTGACCC  | TGGTGTTGGGTAAGAGGTTG    |
| AhR           | CATCGACATAACGGACGAAATC  | CTGTTGCTGTTGCTCTAGTTG   |
| IDO1          | CGAGAACATGGACATTCTGTTC  | TTCCAATGCTTTCAGGTCTTG   |
| IL-1 $\beta$  | GGTGTGTGACGTTCCCATTA    | ATTGAGGTGGAGAGCTTTCAG   |
| TNF- $\alpha$ | CCCTCACACTCAGATCATCTTCT | GCTACGACGTGGGCTACAG     |
| TLR4          | CTGTTCTCCAGTCGGTCAG     | CGTCGCAGGAGGGAAGTTAG    |
| GAPDH         | CATCACTGCCACCCAGAAGACTG | ATGCCAGTGAGCTTCCCGTTCAG |

**Table S2.** Spearman's correlation analysis between taxa at the family level and obesity-related parameters. Related to Figure 5.

| Index        | Family                     | P-value     | r-value      |
|--------------|----------------------------|-------------|--------------|
| TC           | Christensenellaceae        | 2.50E-06    | -0.919130435 |
| Liver TG     | Christensenellaceae        | 2.90E-07    | -0.839653409 |
| TC           | Erysipelotrichaceae        | 2.09E-06    | -0.829565217 |
| FBG          | Christensenellaceae        | 5.59E-07    | -0.828982037 |
| Body weight  | Christensenellaceae        | 6.65E-07    | -0.826011387 |
| Liver TC     | Christensenellaceae        | 2.18E-06    | -0.825217391 |
| Liver TG     | Erysipelotrichaceae        | 8.69E-07    | -0.821352712 |
| FBG          | Erysipelotrichaceae        | 1.09E-06    | -0.817232685 |
| ALT          | Christensenellaceae        | 1.18E-06    | -0.815829547 |
| Liver TC     | Erysipelotrichaceae        | 3.19E-06    | -0.809565217 |
| TC           | Mollicutes_RF39            | 2.35E-06    | -0.802799325 |
| AST          | Erysipelotrichaceae        | 5.36E-06    | -0.796521739 |
| Body weight  | Erysipelotrichaceae        | 5.00E-06    | -0.7872989   |
| LDL-C        | Christensenellaceae        | 1.36E-05    | -0.775652174 |
| AST          | Christensenellaceae        | 1.70E-05    | -0.770434783 |
| Liver weight | Christensenellaceae        | 3.35E-05    | -0.753913043 |
| LDL-C        | Erysipelotrichaceae        | 3.35E-05    | -0.753913043 |
| Liver weight | Desulfovibrionaceae        | 4.37E-05    | -0.746956522 |
| Body weight  | Desulfovibrionaceae        | 4.35E-05    | -0.734667316 |
| Body weight  | Bifidobacteriaceae         | 5.78E-05    | -0.726679396 |
| Liver weight | Erysipelotrichaceae        | 0.000121561 | -0.717391304 |
| TG           | Christensenellaceae        | 0.000119279 | -0.705089236 |
| Body weight  | unclassified_Clostridiales | 9.24E-05    | 0.712918728  |
| LDL-C        | Lachnospiraceae            | 7.00E-05    | 0.733913043  |
| AST          | unclassified_Clostridiales | 6.59E-05    | 0.735652174  |
| TC           | unclassified_Clostridiales | 4.97E-05    | 0.743478261  |
| Liver weight | unclassified_Clostridiales | 3.70E-05    | 0.751304348  |
| TC           | Lachnospiraceae            | 3.46E-05    | 0.753043478  |
| Liver TG     | unclassified_Clostridiales | 1.51E-05    | 0.762093312  |
| Liver TC     | unclassified_Clostridiales | 1.83E-05    | 0.768695652  |
| LDL-C        | unclassified_Clostridiales | 1.21E-05    | 0.77826087   |
| FBG          | unclassified_Clostridiales | 3.24E-06    | 0.796344949  |
| Liver TC     | Lachnospiraceae            | 2.77E-06    | 0.813913043  |
| Liver weight | Lachnospiraceae            | 2.30E-06    | 0.82173913   |
| Liver TG     | Lachnospiraceae            | 8.07E-07    | 0.822659904  |
| Body weight  | Lachnospiraceae            | 6.32E-07    | 0.826881331  |
| FBG          | Lachnospiraceae            | 3.86E-07    | 0.835074294  |
| AST          | Lachnospiraceae            | 2.30E-06    | 0.856521739  |

**Table S3.** Spearman's correlation analysis between the representative genera and obesity-related parameters. Related to Figure 5.

| Index        | Genera                       | P-value     | r-value      |
|--------------|------------------------------|-------------|--------------|
| Liver weight | Bifidobacterium              | 0.000100941 | -0.710241337 |
| Body weight  | Christensenella              | 6.14E-09    | -0.889517266 |
| TC           | Christensenella              | 2.69E-06    | -0.886086957 |
| Liver TC     | Christensenella              | 2.07E-06    | -0.840869565 |
| FBG          | Christensenella              | 4.41E-07    | -0.832898488 |
| Liver weight | Christensenella              | 2.16E-06    | -0.826086957 |
| TC           | Ileibacterium                | 2.19E-06    | -0.80417785  |
| LDL-C        | Christensenella              | 5.79E-06    | -0.794782609 |
| AST          | Christensenella              | 1.26E-05    | -0.777391304 |
| Liver TG     | Ileibacterium                | 8.24E-06    | -0.776276286 |
| AST          | Ileibacterium                | 1.11E-05    | -0.769364956 |
| Liver TG     | Christensenella              | 1.17E-05    | -0.768193544 |
| LDL-C        | Ileibacterium                | 1.24E-05    | -0.766753989 |
| ALT          | Ileibacterium                | 1.35E-05    | -0.76474445  |
| Body weight  | Ileibacterium                | 1.49E-05    | -0.762298723 |
| Liver TC     | Ileibacterium                | 1.54E-05    | -0.761532055 |
| FBG          | Ileibacterium                | 1.86E-05    | -0.756968641 |
| ALT          | Christensenella              | 2.97E-05    | -0.744944571 |
| TG           | Christensenella              | 6.30E-05    | -0.724227994 |
| Liver weight | Bifidobacterium              | 0.000100941 | -0.710241337 |
| Liver weight | Ileibacterium                | 0.000133803 | -0.701479813 |
| Liver weight | Lachnospiraceae_FCS020_group | 0.000134282 | 0.701366665  |
| Liver TC     | GCA-900066575                | 0.000133338 | 0.701589848  |
| Liver TC     | Lachnospiraceae_FCS020_group | 0.000115447 | 0.706105629  |
| AST          | Lachnospiraceae_FCS020_group | 0.00011197  | 0.707053422  |
| FBG          | GCA-900066575                | 0.000100619 | 0.710338811  |
| LDL-C        | GCA-900066575                | 9.16E-05    | 0.713205574  |
| Liver weight | Lachnospiraceae_UCG-004      | 0.000124983 | 0.716521739  |
| LDL-C        | Lachnospiraceae_FCS020_group | 7.44E-05    | 0.719374728  |
| FBG          | Lachnospiraceae_FCS020_group | 4.50E-05    | 0.73375605   |
| TG           | Acetatifactor                | 4.10E-05    | 0.736298555  |
| TC           | Lachnospiraceae_FCS020_group | 3.94E-05    | 0.737382792  |
| LDL-C        | Anaerotruncus                | 3.64E-05    | 0.739492779  |
| Liver TG     | GCA-900066575                | 3.47E-05    | 0.74083707   |
| Liver weight | Blautia                      | 4.23E-05    | 0.747826087  |
| TG           | Anaerotruncus                | 2.62E-05    | 0.748181457  |
| TG           | Blautia                      | 2.37E-05    | 0.750761272  |
| ALT          | Blautia                      | 2.05E-05    | 0.754511868  |
| TC           | GCA-900066575                | 1.35E-05    | 0.764779397  |
| LDL-C        | Acetatifactor                | 1.05E-05    | 0.770812283  |



|              |                              |          |             |
|--------------|------------------------------|----------|-------------|
| Liver weight | Anaerotruncus                | 8.83E-06 | 0.774706721 |
| TC           | Acetatifactor                | 8.28E-06 | 0.776171349 |
| Liver TG     | Anaerotruncus                | 7.73E-06 | 0.77771978  |
| ALT          | Anaerotruncus                | 5.91E-06 | 0.78368059  |
| Liver TC     | Blautia                      | 8.88E-06 | 0.785217391 |
| Liver weight | Acetatifactor                | 4.49E-06 | 0.789569013 |
| Body weight  | Acetatifactor                | 3.89E-06 | 0.792593226 |
| Body weight  | Lachnospiraceae_FCS020_group | 3.78E-06 | 0.793173603 |
| FBG          | Acetatifactor                | 3.74E-06 | 0.793385335 |
| FBG          | Anaerotruncus                | 3.14E-06 | 0.796968578 |
| AST          | Acetatifactor                | 2.92E-06 | 0.798500789 |
| LDL-C        | Blautia                      | 4.01E-06 | 0.803478261 |
| FBG          | Blautia                      | 2.19E-06 | 0.80417785  |
| TC           | Blautia                      | 3.19E-06 | 0.809565217 |
| Liver TC     | Anaerotruncus                | 1.06E-06 | 0.817843799 |
| Liver TC     | Acetatifactor                | 8.09E-07 | 0.822616585 |
| Liver TG     | Acetatifactor                | 7.30E-07 | 0.824410734 |
| Body weight  | Blautia                      | 6.32E-07 | 0.826881331 |
| AST          | Blautia                      | 2.06E-06 | 0.833043478 |
| Body weight  | Anaerotruncus                | 4.34E-07 | 0.833172052 |
| AST          | Anaerotruncus                | 3.98E-07 | 0.834570422 |
| Liver TG     | Blautia                      | 2.67E-07 | 0.840960601 |
| TC           | Anaerotruncus                | 2.75E-08 | 0.872425409 |

**Table S4.** Correlation analysis of representative genera, indole derivatives, and serum LPS. Related to Figure 5.

| Category1                  | Category2                    | P-value     | r-value      |
|----------------------------|------------------------------|-------------|--------------|
| LPS                        | Christensenella              | 8.53E-05    | -0.764040704 |
| Tryptophanol               | Blautia                      | 0.000164902 | -0.744347826 |
| Indole-3-carboxylic acid   | Blautia                      | 0.000208758 | -0.736521739 |
| Indole-3-acetate           | Blautia                      | 0.000249648 | -0.728695652 |
| Indole-3-acetate           | Acetatifactor                | 0.000274945 | -0.725260225 |
| Tryptophanol               | Anaerotruncus                | 0.000314137 | -0.717484065 |
| Tryptophanol               | Acetatifactor                | 0.000342813 | -0.713648916 |
| 1H-Indole-3-carboxaldehyde | Blautia                      | 0.002210214 | -0.702608696 |
| Tryptophanol               | GCA-900066575                | 0.000452398 | -0.701589848 |
| LPS                        | Tryptophanol                 | 0.000625965 | -0.687854309 |
| Indole-3-carboxylic acid   | Acetatifactor                | 0.000878122 | -0.673455923 |
| 5-Methoxyindoleacetate     | Blautia                      | 0.000897182 | -0.672173913 |
| Indole-3-acetate           | Anaerotruncus                | 0.000905285 | -0.670825592 |
| 1H-Indole-3-carboxaldehyde | Acetatifactor                | 0.001047476 | -0.664524147 |
| LPS                        | Ileibacterium                | 0.001837863 | -0.638780017 |
| 5-Methoxyindoleacetate     | Acetatifactor                | 0.001837863 | -0.638621996 |
| LPS                        | Indole-3-acetate             | 0.001891878 | -0.636482911 |
| Indole-3-carboxylic acid   | GCA-900066575                | 0.00193227  | -0.635147896 |
| Indole-3-acetate           | GCA-900066575                | 0.002044543 | -0.631895492 |
| Indolelactic acid          | Blautia                      | 0.002131399 | -0.629565217 |
| 1H-Indole-3-carboxaldehyde | Anaerotruncus                | 0.002801666 | -0.626808165 |
| Indole-3-carboxylic acid   | Anaerotruncus                | 0.002801666 | -0.615363634 |
| LPS                        | Indolelactic acid            | 0.002881497 | -0.613409317 |
| Tryptophanol               | Lachnospiraceae_FCS020_group | 0.002881497 | -0.613221936 |
| 1H-Indole-3-carboxaldehyde | GCA-900066575                | 0.003001118 | -0.610987186 |
| LPS                        | 1H-Indole-3-carboxaldehyde   | 0.003259798 | -0.606879055 |
| Indolelactic acid          | Anaerotruncus                | 0.004258158 | -0.59335492  |
| Indolelactic acid          | Acetatifactor                | 0.005612521 | -0.577885918 |
| Indole-3-acetate           | Lachnospiraceae_FCS020_group | 0.005693998 | -0.576258017 |
| 5-Methoxyindoleacetate     | Anaerotruncus                | 0.006454423 | -0.569585509 |
| 5-Methoxyindoleacetate     | GCA-900066575                | 0.009154698 | -0.549191523 |
| LPS                        | Indole-3-carboxylic acid     | 0.009710053 | -0.544623886 |
| LPS                        | 5-Methoxyindoleacetate       | 0.014747801 | -0.52024424  |
| Indole-3-carboxylic acid   | Lachnospiraceae_FCS020_group | 0.014840021 | -0.51939045  |
| 5-Methoxyindoleacetate     | Lachnospiraceae_FCS020_group | 0.030829371 | -0.470105224 |
| 1H-Indole-3-carboxaldehyde | Lachnospiraceae_FCS020_group | 0.031904369 | -0.467261846 |
| Tryptophanol               | Bifidobacterium              | 0.046341527 | 0.41045849   |
| Tryptophanol               | Akkermansia                  | 0.043530779 | 0.417391304  |
| LPS                        | GCA-900066575                | 0.015546096 | 0.516179636  |
| Indolelactic acid          | Christensenella              | 0.009309343 | 0.547826087  |

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|                            |                              |             |             |
|----------------------------|------------------------------|-------------|-------------|
| LPS                        | Lachnospiraceae_FCS020_group | 0.006541041 | 0.567995333 |
| LPS                        | Acetatifactor                | 0.004134379 | 0.595186541 |
| 5-Methoxyindoleacetate     | Ileibacterium                | 0.002131399 | 0.629243058 |
| LPS                        | Blautia                      | 0.001970691 | 0.633870806 |
| Indole-3-carboxylic acid   | Ileibacterium                | 0.001837863 | 0.638816604 |
| 5-Methoxyindoleacetate     | Christensenella              | 0.001415737 | 0.651304348 |
| Indolelactic acid          | Ileibacterium                | 0.001047476 | 0.664055952 |
| Indole-3-acetate           | Ileibacterium                | 0.000932087 | 0.669277886 |
| 1H-Indole-3-carboxaldehyde | Ileibacterium                | 0.000363385 | 0.711053359 |
| Tryptophanol               | Ileibacterium                | 0.000300939 | 0.720626905 |
| 1H-Indole-3-carboxaldehyde | Christensenella              | 8.37E-05    | 0.766086957 |
| Indole-3-carboxylic acid   | Christensenella              | 7.48E-05    | 0.771304348 |
| LPS                        | Anaerotruncus                | 7.48E-05    | 0.773956191 |
| Indole-3-acetate           | Christensenella              | 1.66E-05    | 0.809565217 |
| Tryptophanol               | Christensenella              | 8.23E-07    | 0.864347826 |

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**Table S5.** The description of periodontitis' oral status. Related to STAR Methods.

| Number | Sex    | Age | Diagnosis                          | DI | CI | PLI | GI |
|--------|--------|-----|------------------------------------|----|----|-----|----|
| P1     | Male   | 46  | Periodontitis (stage III, grade B) | 2  | 2  | 3   | 2  |
| P2     | Male   | 47  | Periodontitis (stage IV, grade B)  | 2  | 2  | 2   | 2  |
| P3     | Female | 34  | Periodontitis (stage IV, grade C)  | 2  | 2  | 2   | 2  |
| P4     | Male   | 43  | Periodontitis (stage III, grade B) | 3  | 2  | 2   | 2  |
| P5     | Female | 36  | Periodontitis (stage IV, grade C)  | 2  | 2  | 3   | 2  |
| P6     | Male   | 41  | Periodontitis (stage IV, grade C)  | 2  | 2  | 3   | 2  |
| P7     | Male   | 49  | Periodontitis (stage IV, grade C)  | 2  | 2  | 3   | 2  |
| P8     | Male   | 39  | Periodontitis (stage IV, grade C)  | 2  | 3  | 3   | 2  |
| P9     | Male   | 31  | Periodontitis (stage IV, grade B)  | 2  | 2  | 2   | 2  |
| P10    | Female | 46  | Periodontitis (stage IV, grade B)  | 2  | 2  | 2   | 2  |
| P11    | Female | 27  | Periodontitis (stage IV, grade C)  | 2  | 2  | 3   | 2  |
| P12    | Male   | 27  | Periodontitis (stage III, grade C) | 2  | 3  | 2   | 2  |
| P13    | Female | 32  | Periodontitis (stage IV, grade C)  | 2  | 3  | 3   | 2  |
| P14    | Female | 49  | Periodontitis (stage IV, grade C)  | 2  | 2  | 3   | 2  |
| P15    | Male   | 62  | Periodontitis (stage IV, grade B)  | 3  | 2  | 3   | 2  |
| P16    | Female | 38  | Periodontitis (stage IV, grade C)  | 2  | 2  | 2   | 2  |
| P17    | Female | 36  | Periodontitis (stage IV, grade B)  | 2  | 2  | 3   | 2  |
| P18    | Male   | 31  | Periodontitis (stage IV, grade C)  | 2  | 2  | 3   | 2  |
| P19    | Male   | 47  | Periodontitis (stage IV, grade B)  | 2  | 2  | 2   | 2  |
| P20    | Male   | 44  | Periodontitis (stage IV, grade C)  | 2  | 2  | 3   | 2  |

**Debris index (DI)**

0= no debris or pigment on the tooth surface.

1= the area covered by debris or pigment is less than 1/3 of the tooth surface.

2= the debris covers the tooth surface 1/3 -2/3.

3= the debris covers more than 2/3 of the tooth surface.

**Calculus index (CI)**

0= no supragingival or subgingival calculus.

1= the area covered by supragingival calculus is less than 1/3 of the tooth surface.

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2= supragingival calculus covering 1/3 - 2/3 of the tooth surface, or there is scattered subgingival calculus in the tooth neck.

3= subgingival calculus covers more than 2/3 of the tooth surface, or there is thick subgingival calculus in the tooth neck.

**Plaque index (PLI)**

0= no plaque at gingival margin

1= thin plaque at gingival margin isn't visible visually, but can be seen by scraping with probe

2= moderate amount of plaque on gingival margin or adjacent surface

3= a large number of plaques can be seen in gingival sulcus, gingival margin or adjacent surface.

**Gingival index (GI)**

0= healthy

1= Gingiva has slight inflammation or mild edema, but without probing bleeding.

2= moderate inflammation of the gingiva with bright edema and probing bleeding.

3= severe inflammation of the gingiva with swelling gingiva or ulcer and the tendency of spontaneous bleeding.

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**Table S6.** The ingredients of ND and HFD. Related to STAR Methods.

|                                       | Normal diet<br>D12450J |             | High fat diet<br>D12492 |             |
|---------------------------------------|------------------------|-------------|-------------------------|-------------|
|                                       | gm%                    | Kcal%       | gm%                     | Kcal%       |
| <b>Macronutrient</b>                  |                        |             |                         |             |
| Protein                               | 19.2                   | 20.0        | 26.0                    | 20.0        |
| Carbohydrate                          | 67.3                   | 70.0        | 26.0                    | 20.0        |
| Fat                                   | 4.3                    | 10.0        | 35.0                    | 60.0        |
| Total                                 |                        | 100.0       |                         | 100.0       |
| <b>Ingredient</b>                     | <b>gm</b>              | <b>Kcal</b> | <b>gm</b>               | <b>Kcal</b> |
| Casein                                | 200                    | 800         | 200                     | 800         |
| L-Cystine                             | 3                      | 12          | 3                       | 12          |
| Corn starch                           | 506.2                  | 2024.8      | 0                       | 0           |
| Maltodextrin                          | 125                    | 500         | 125                     | 500         |
| Sucrose                               | 68.8                   | 275         | 68.8                    | 275         |
| Cellulose, BW200                      | 50                     | 0           | 50                      | 0           |
| Soybean oil                           | 25                     | 225         | 25                      | 225         |
| Lard                                  | 20                     | 180         | 245                     | 2205        |
| Mineral Mix S10026                    | 10                     | 0           | 10                      | 0           |
| Dicalcium phosphate                   | 13                     | 0           | 13                      | 0           |
| Calcium carbonate                     | 5.5                    | 0           | 5.5                     | 0           |
| Potassium citrate, 1 H <sub>2</sub> O | 16.5                   | 0           | 16.5                    | 0           |
| Vitamin Mix V10001                    | 10                     | 40          | 10                      | 40          |
| Choline Bitartrate                    | 2                      | 0           | 2                       | 0           |
| FD&C Yellow Dye #5                    | 0.4                    | 0           | 0                       | 0           |
| FD&C Red Dye #40                      | 0                      | 0           | 0                       | 0           |
| FD&C Blue Dye #1                      | 0.01                   | 0           | 0.05                    | 0           |