SUPPLEMENTAL MATERIAL

Data S1. Supplemental Methods

Study population and definitions of clinical presentation

Patients with stable angina pectoris (SAP) or acute coronary syndromes (ACS) who underwent cardiac catheterization were enrolled. Patients underwent both optical coherence tomography (OCT) and intravascular ultrasound (IVUS) prior to intervention. SAP was defined as chest pain on exertion without changes in frequency, intensity, and duration of symptoms in the previous 4 weeks and was judged to require coronary intervention by physicians [10, 37]. ACS included ST elevation myocardial infarction (STEMI) and non-ST-segment elevation acute coronary syndromes (NSTE-ACS) [38, 39]. STEMI was defined as continuous chest pain lasting >30 min, arrival at the hospital within 12 h from the onset of symptoms, ST-segment elevation >0.1 mV in ≥2 contiguous leads or new left bundle-branch block on 12-lead electrocardiography, and elevated cardiac marker levels (creatine kinase-MB or troponin). NSTE-ACS included non-ST-segment elevation myocardial infarction (NSTEMI) and unstable angina pectoris (UAP). NSTEMI was defined as ischemic symptoms in the absence of ST-segment elevation on electrocardiogram with elevated cardiac marker levels. UAP was defined as the presence of newly developed/accelerating chest symptoms on exertion or rest angina within 2 weeks of presentation without biomarker release [38, 39]. Culprit lesions were identified based on coronary angiographic findings, electrocardiographic changes, or wall motion abnormalities on ventriculogram or echocardiogram [40]. In patients with SAP with multiple lesions, the lesion with the most severe stenosis was selected as the culprit lesion [10, 37].

Patients with clinical manifestation caused by instent restenosis, with tortuous or heavily calcified vessel, with severe chronic kidney disease (estimated glomerular filtration rate < 30 mL/min per 1.73 m^2), or with cardiogenic shock were excluded.

Coronary angiography analysis

Quantitative coronary angiogram analysis was performed using Cardiovascular Angiography Analysis System 5.10.1 software (Pie Medical Imaging BV, Maastricht, the Netherlands). The culprit lesion length, minimal lumen diameter, reference lumen diameter, and percentage diameter stenosis were measured. Lesion complexity was evaluated according to the AHA/ACC classification [41].

OCT analysis

Macrophages were identified as signal-rich, distinct, or confluent punctuate regions with heterogeneous back shadow [8]. Microvessels were identified as the presence of signal-poor structures with vesicular or tubular shapes [8, 10]. Calcification was identified as heterogeneous areas of high and low reflectivity, with low signal attenuation and sharply demarcated border. Calcification index was calculated as the product of mean calcification arc and calcification length [40]. Plaque rupture was identified by the presence of fibrous cap discontinuity with a communication between the lumen and the inner core of plaque, or with a cavity formation within the plaque [11, 42]. Plaque erosion was identified by the presence of attached thrombus overlying an intact plaque or luminal surface

irregularity at the culprit lesion [43]. Minimal lumen area was the smallest lumen area within the length of the entire lesion. Reference lumen area was defined as the mean of the largest lumen area proximal and distal to the stenosis within 10 mm from the edge. Area stenosis was calculated as: (mean reference lumen area − minimal lumen area) /mean reference lumen area × 100. Representative OCT images are shown in Figure S2.

Blood biomarker analysis

The blood samples for biomarker analysis were collected from patients who participated in the biomarker sub-study during the pre-procedural period (within 12 hours prior to procedure, but only after informed consent was obtained). All patients who participated in the microbiome sub-study also participated in the biomarker sub-study. Biomarker analyses other than trimethylamine-*N*-oxide (TMAO) and short-chain fatty acids (SCFAs) were performed at an independent laboratory (SRL Inc, Tokyo, Japan). Plasminogen activator inhibitor-1 (PAI-1), fibrin monomer, amyloid A, von Willebrand factor, and lipoprotein (a) were analyzed using the latex agglutination method. Thrombin – anti-thrombin complex (TAT), interleukin-4 (IL-4), and interleukin-6 (IL-6) were analyzed using the chemiluminescent enzyme immunoassay (CLEIA) method. Prothrombin fragment F1+2 (F1+2), tumor necrosis factor α (TNFα), and transforming growth factor β (TGFβ) were analyzed using the enzyme-linked immunosorbent assay (ELISA) method. Plasminogen and Antithrombin III were analyzed using chromogenic substrate assay. Factor II was analyzed using the coagulation time method.

Total homocysteine was analyzed using liquid chromatography tandem mass spectrometry (LC/MS/MS). Renin, angiotensin II, aldosterone, and insulin like growth factor 1 (IGF-1) were analyzed using the radio immunoassay method. Bile acid was analyzed using the enzyme method. TMAO and SCFAs analyses were performed using validated LC-MS/MS methods by LSI Medience Corporation (Tokyo, Japan), a contract laboratory for biological analysis. Briefly, SCFAs were analyzed with the Shimadzu HPLC system (Nexera X2 LC0AD) and 8050 triple stage quadrupole mass spectrometry (Shimadzu, Kyoto, Japan) equipped with ACQUITY UPLC HSS T3 (50mm×2.1 mm I.D., 1.8 μm, Waters, Tokyo, Japan). TMAO was analyzed by LC-Q-TOF mass spectrometry (1260 Infinity and 6545 Q-TOF system, Agilent Technologies, Palo Alto, CA). The HPLC column, Atlantis HILIC Silica 2.1 mm ×100 mm, 3.0 μm (Waters, Milford MA) was used for the analysis.

Table S1. Biomarker analysis

Biomarker	N = 55
TMAO, µg/ml	0.411 (0.196 – 0.699)
SCFA	
Acetate, µg/ml	2.33 (1.57 – 3.81)
3-hydroxybutyric acid, µg/ml	2.74 (1.46 – 7.32)
Propionate, µg/ml	0.104 (0.067 – 0.138)
Butyrate, µg/ml	0.053 (0.036 – 0.089)
PAI-1, ng/ml	14.0 (11.0 – 20.0)
TAT, ng/ml	1.90 (1.30 – 2.70)
D-dimer, µg/ml	0.60 (0.39 – 1.30)
Fibrin monomer, µg/ml	3.0 (3.0 – 3.2)
Fibrinogen, mg/dl	290 ± 56
F1+2, pmol/l	257 (171 – 331)
Plasminogen, %	101 (94 – 110)
Factor II, %	84.3 ± 11.4
AT3, %	89.5 ± 14.4
TNFα, pg/ml	0.70 (0.57 – 0.92)
TGFβ, ng/ml	13.1 (7.1 – 18.3)
IL-4, pg/ml	5.1 (2.0 – 12.0)
IL-6, pg/ml	2.1 (1.7 – 3.2)
Homocysteine, nmol/ml	11.3 ± 3.3
Amyloid A, µg/ml	6.1 (3.6 – 9.2)
Renin, pg/ml	7.5 (4.4 – 17.0)
Angiotensin II, pg/ml	3.0 (2.9 – 6.0)
Aldosterone, pg/ml	73.2 (56.2 – 99.5)
IGF 1, ng/ml	78.0 (64.0 – 107.0)
Von Willebrand factor, %	155 ± 56
Lp(a), mg/dl	11.0 (7.0 – 28.0)
Bile acid, µmol/l	4.4 (2.9 – 7.4)

Values are median (interquartile range) or mean ± SD.

AT3 = Antithrombin III; F1+2 = prothrombin fragment F1+2; Lp(a) = lipoprotein (a); IGF 1 = insulin like growth factor 1; IL = interleukin; PAI-1 = plasminogen activator inhibitor-1; SCFA = short-chain fatty acid;

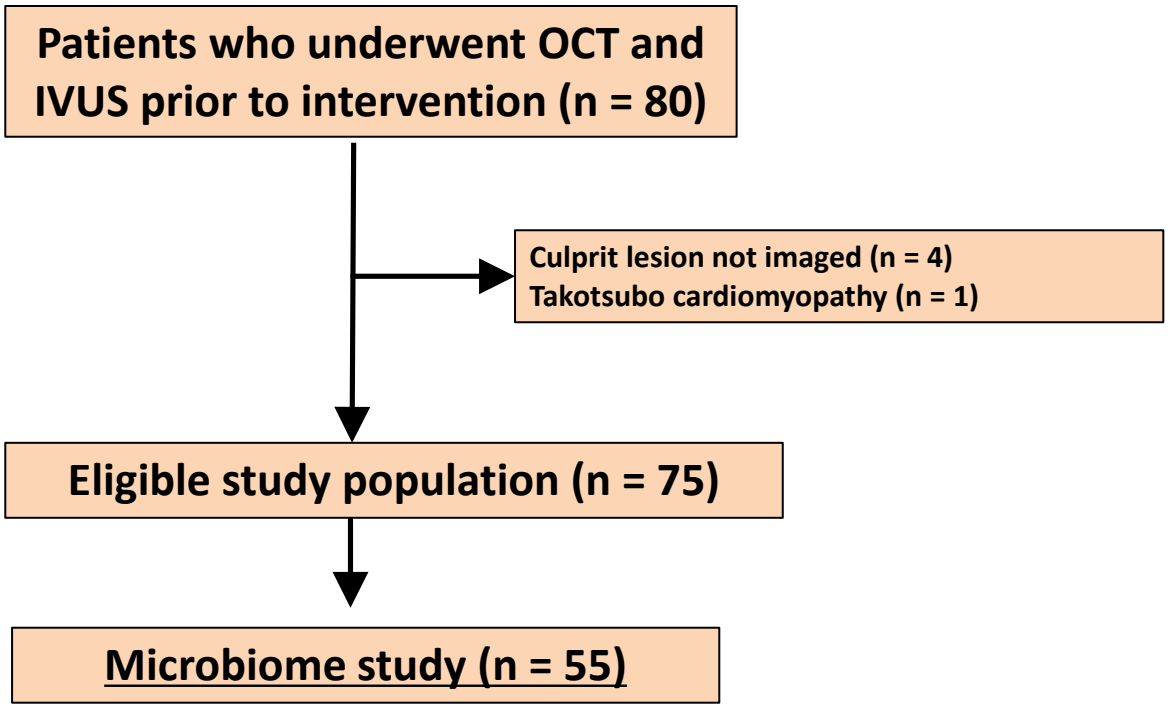
TAT = thrombin – anti-thrombin complex; TGFβ = transforming growth factor β; TMAO = trimethylamine N-oxide; TNFα = tumor necrosis factor α.

Table S2. Angiographic findings

Characteristic	N = 55
B2/C lesion, n (%)	36 (65.5)
Multivessel disease, n (%)	22 (40.0)
Quantitative coronary angiography analysis	
Minimal lumen diameter, mm	0.90 ± 0.44
Reference vessel diameter, mm	2.71 ± 0.63
Diameter stenosis, %	65.6 ± 15.6
Lesion length, mm	15.6 ± 7.2

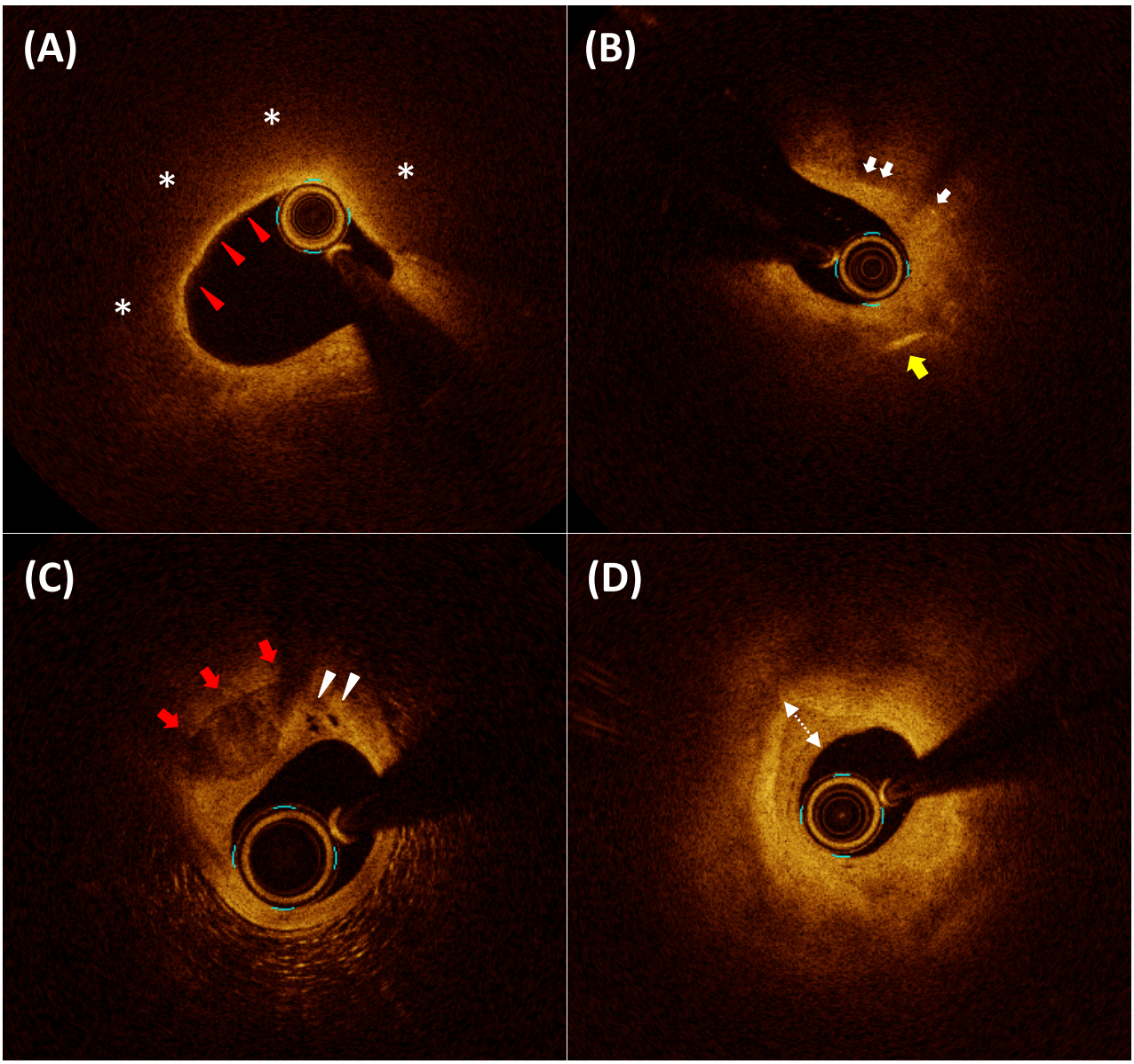
Values are n (%) or mean ± SD.

Figure S1. Study flowchart



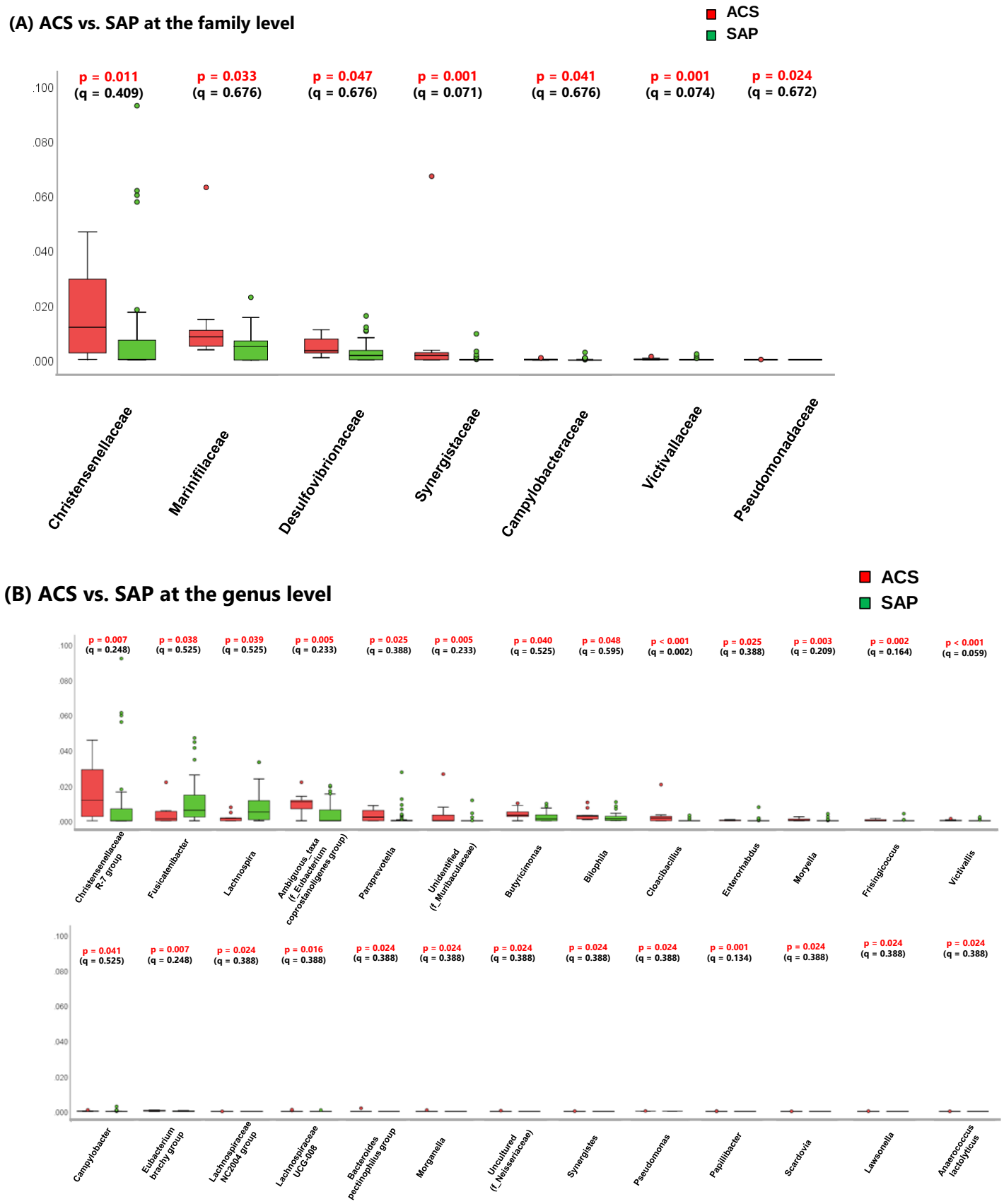
Patients who underwent both OCT and IVUS prior to intervention were included in this study (n = 80). Among them, 4 patients without images at culprit lesion and 1 patient diagnosed Takotsubo cardiomyopathy were excluded. Out of 75 patients, 55 subjects whose stool specimens were successfully collected were included in the final analysis. All these 55 patients also participated in the biomarker sub-study.

Figure S2. Representative OCT images



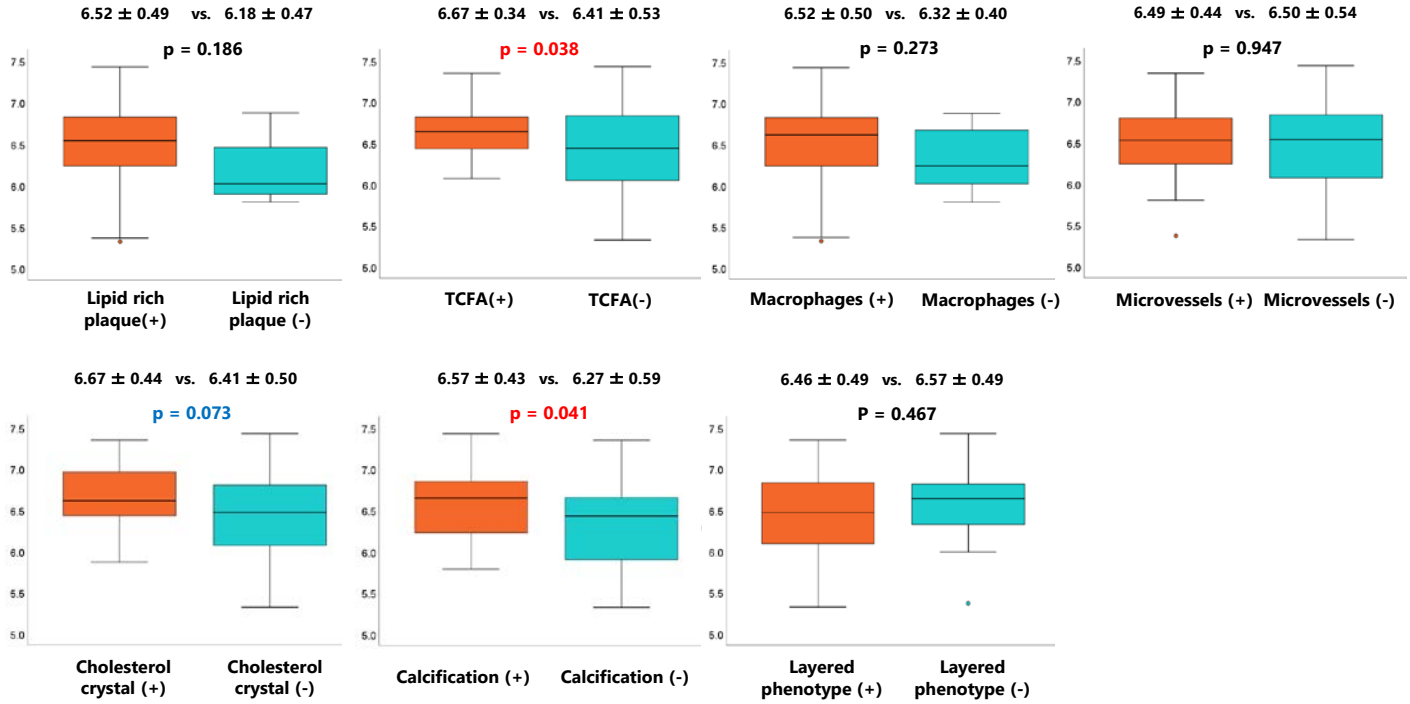
Representative OCT images. (A) lipid rich plaque (asterisk) and TCFA (red arrowhead). (B) macrophages (white arrows) and cholesterol crystal (yellow arrow). (C) microvessels (white arrowheads) and calcification (red arrows). (D) plaque with layered phenotype (double dotted arrow).

Figure S3. Differences in gut microbial composition between patients with ACS versus SAP at the family level and at the genus level



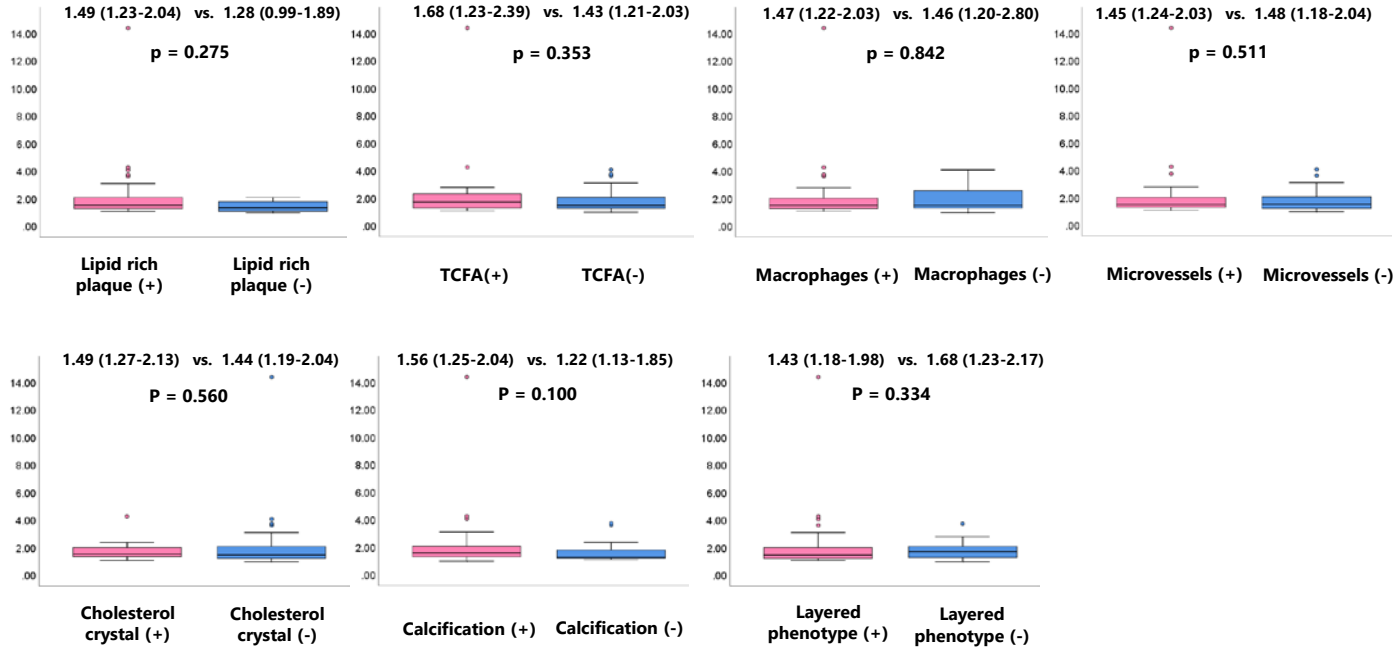
The differences in gut microbial composition between patients with ACS and those with SAP at the family level (A) and at the genus level (B) are shown. Mann–Whitney *U* test and Benjamin-Hochberg multiple testing were applied to obtain p-values and q-value, respectively.

Figure S4. Differences in Shannon index between patients with and without specific OCT features



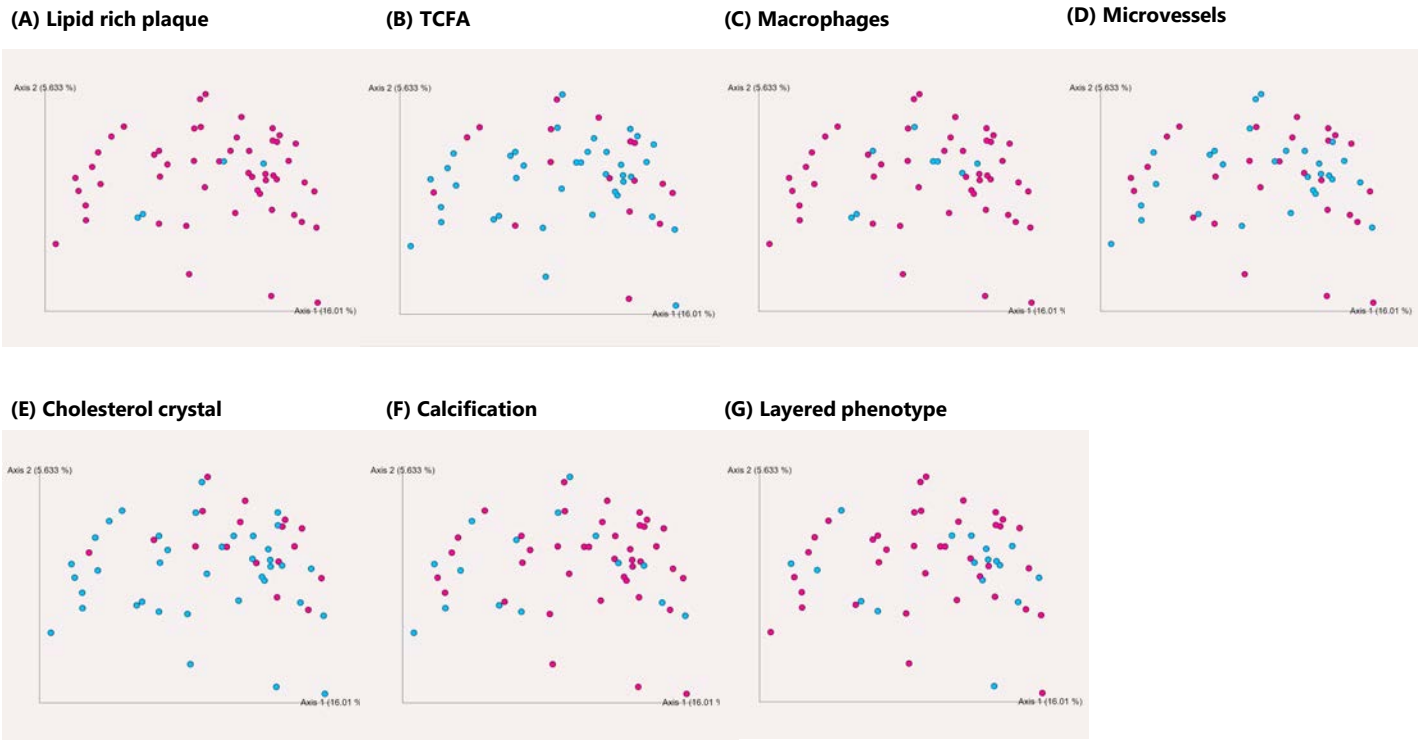
Patients with TCFA and calcification had higher Shannon index, whereas those with other qualitative OCT features did not show difference in Shannon index.

Figure S5. Differences in Firmicutes / Bacteroidetes ratio between patients with and without specific OCT features



Differences in Firmicutes / Bacteroidetes ratio between patients with and without specific OCT features. None of the features shows statistically significant difference.

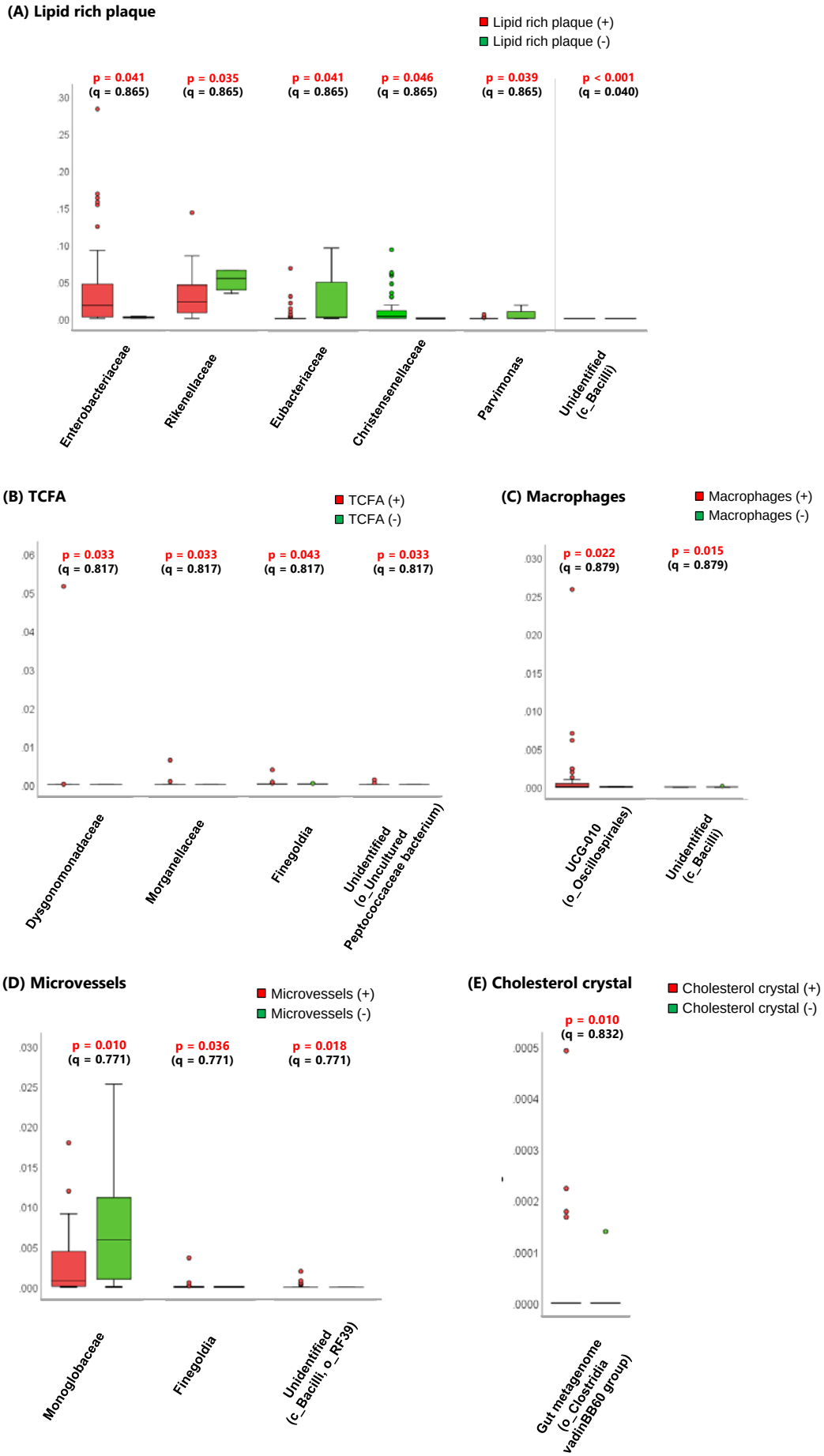
Figure S6. Principal coordinate analyses in patients with and without specific OCT features

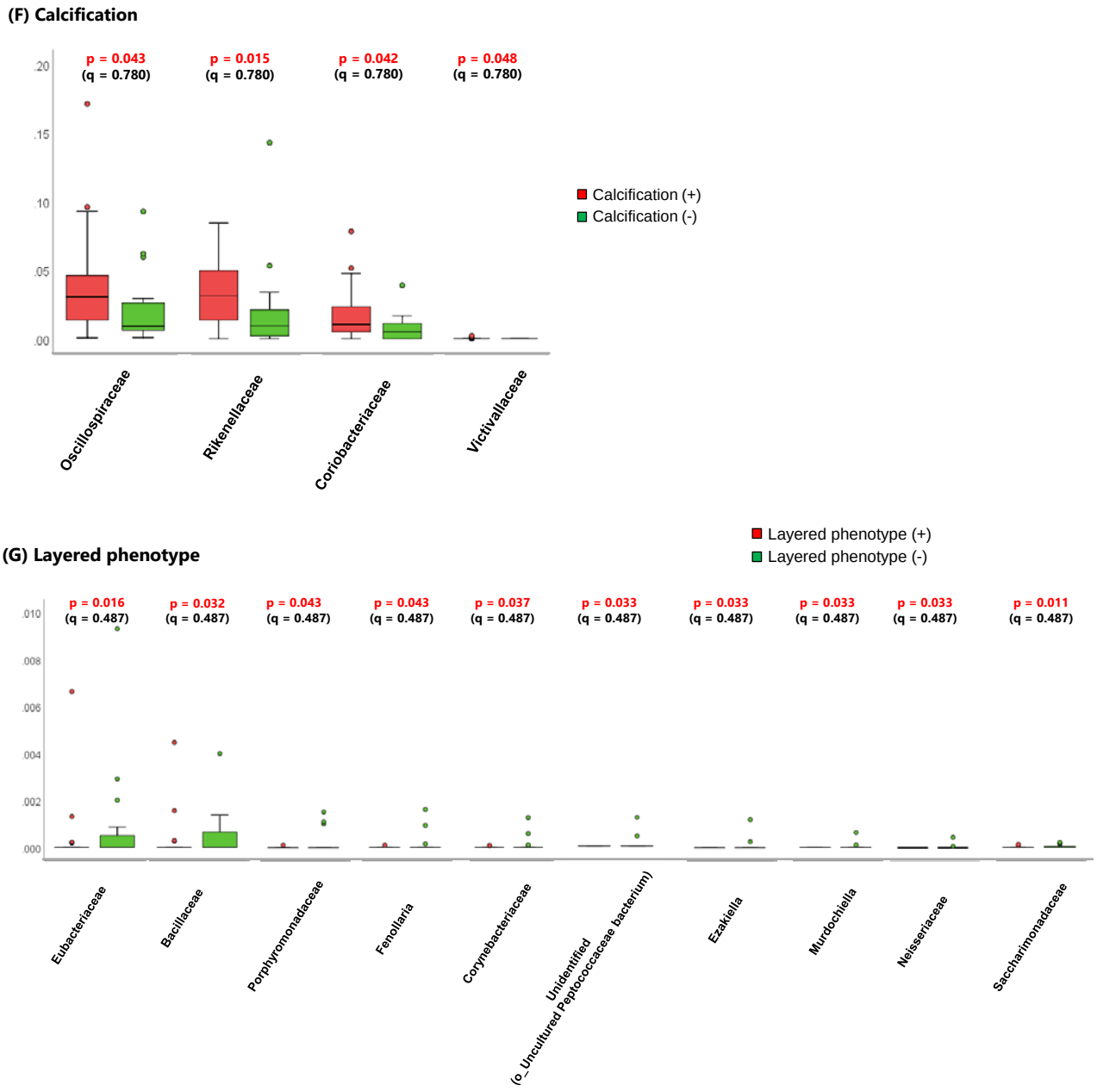


Principal coordinate analyses in patients with and without specific OCT features. None of the features show a clear trend.

Red dot: present, blue dot: absent.

Figure S7. Difference in gut microbial composition between patients with and without specific OCT features at the family level

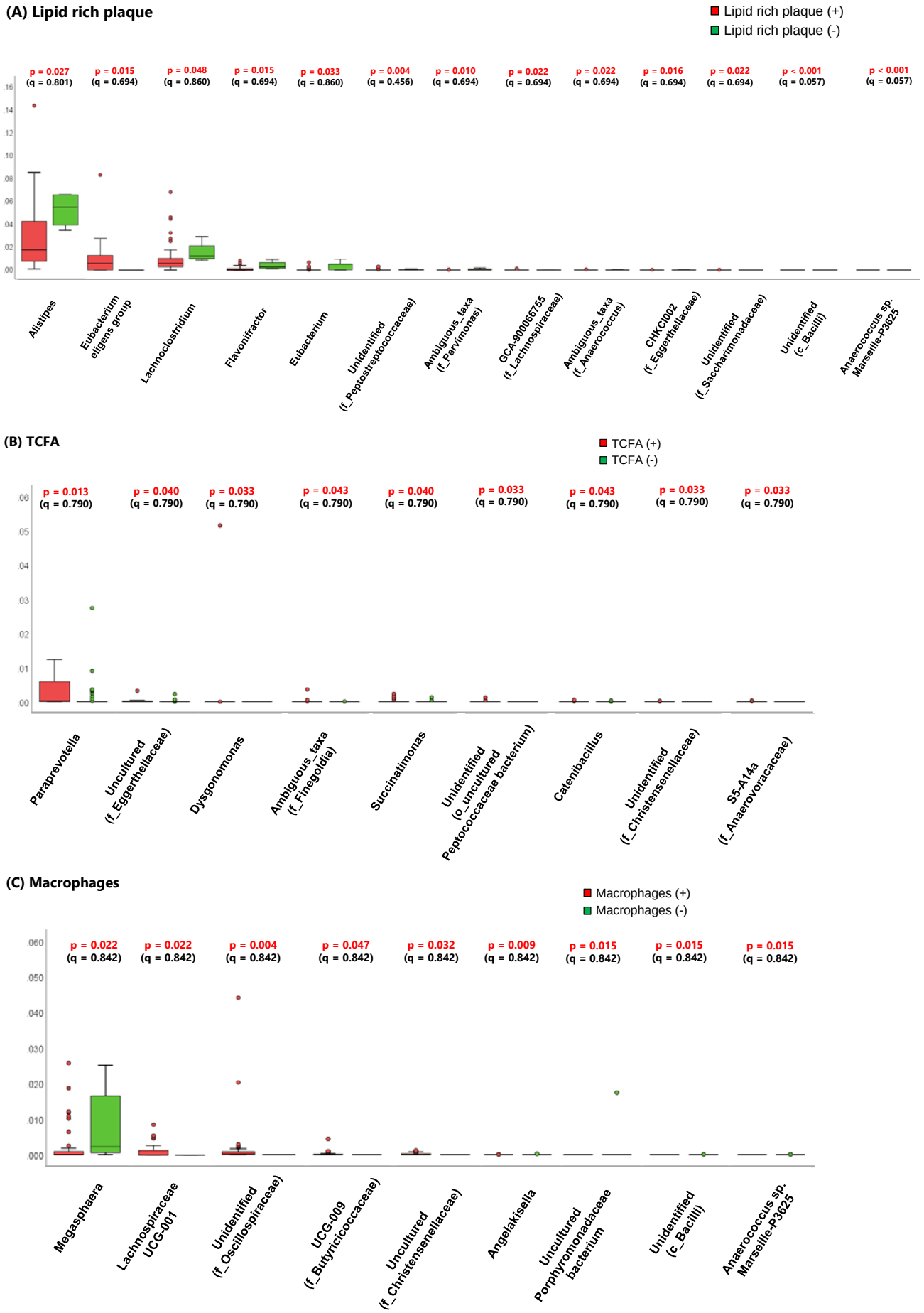




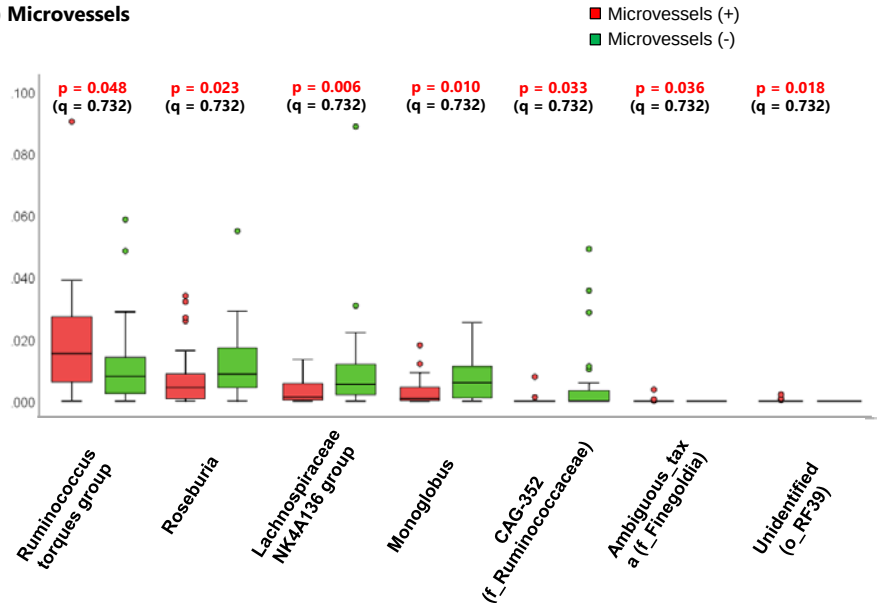
The results of difference in gut microbial composition between patients with and without specific OCT features at the family level. All the bacteria with significant differences by Mann–Whitney *U* test in specific OCT features are shown.

Mann–Whitney *U* test and Benjamin-Hochberg multiple testing were applied to obtain p-values and q-value, respectively.

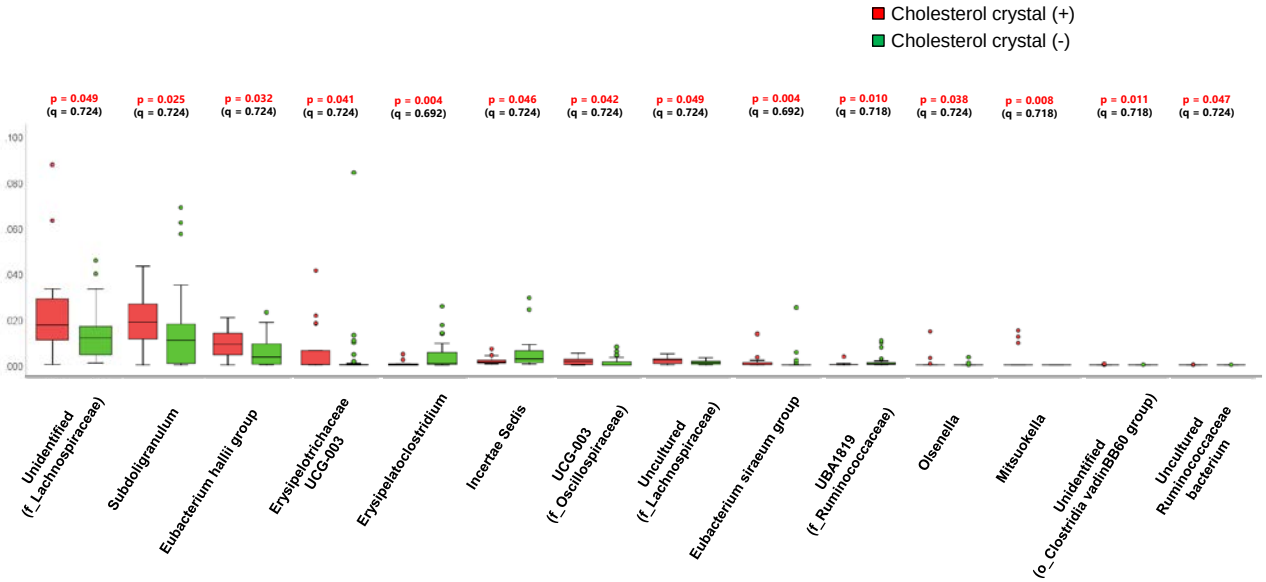
Figure S8. Difference in gut microbial composition between patients with and without specific OCT features at the genus level



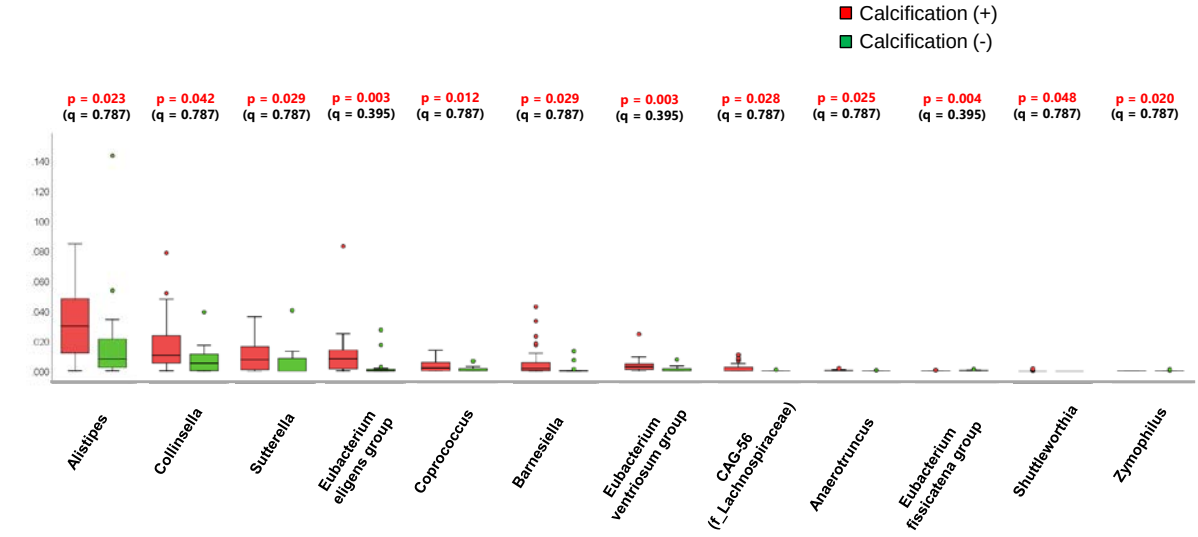
(D) Microvessels

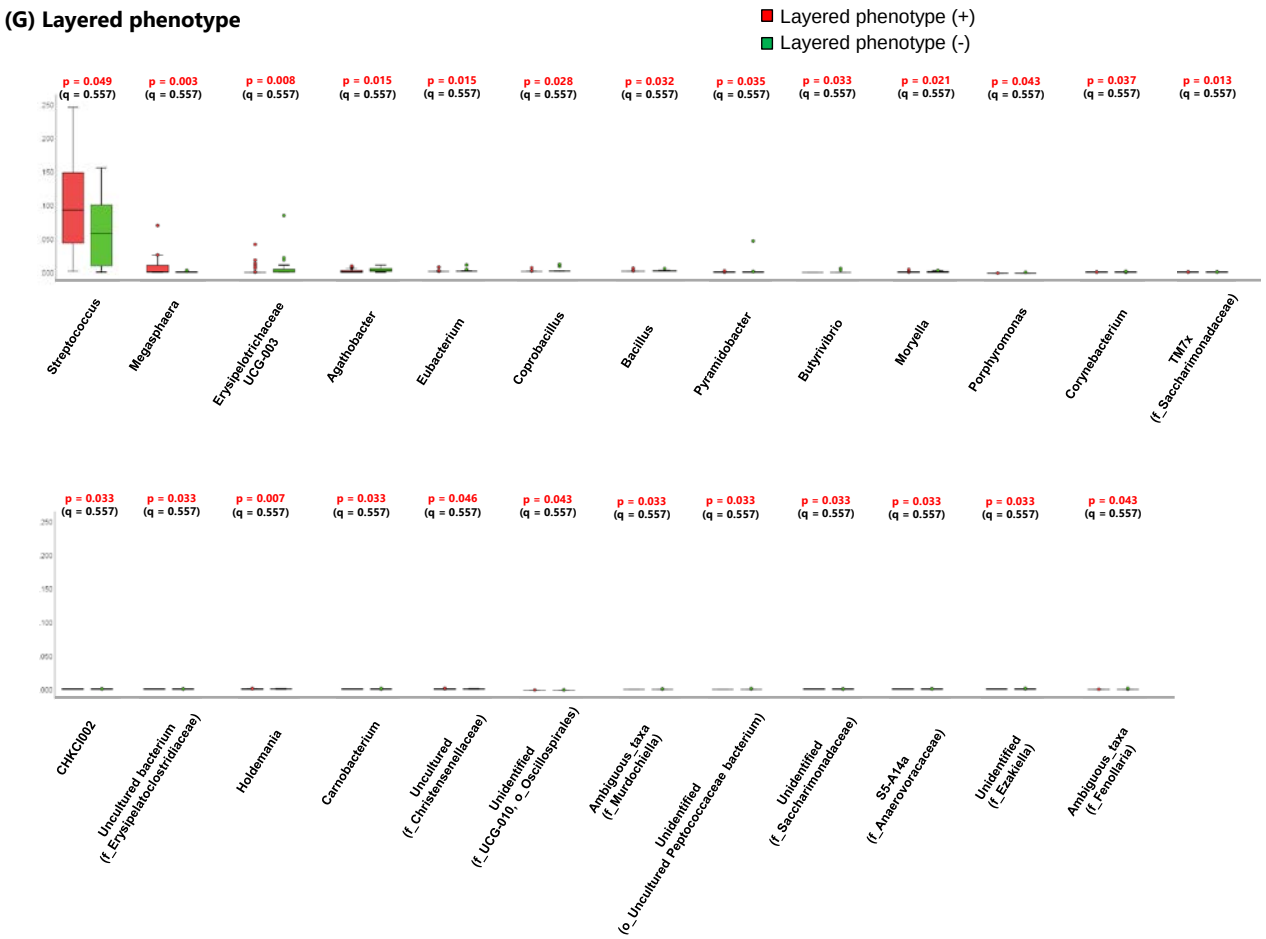


(E) Cholesterol crystal



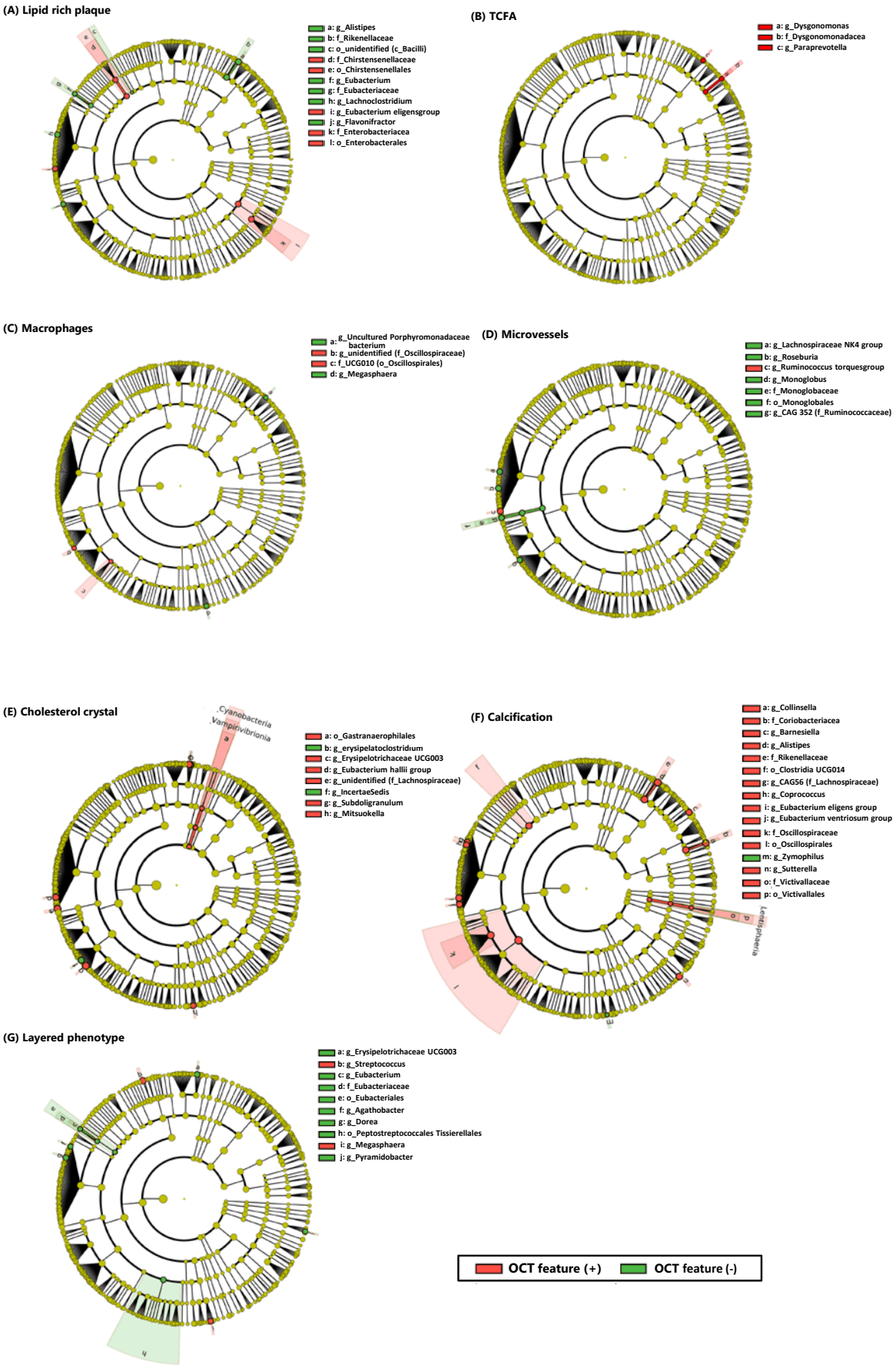
(F) Calcification





Difference in gut microbial composition between patients with and without specific OCT features at the genus level. All the bacteria with significant differences by Mann–Whitney *U* test in specific OCT features are shown. Mann–Whitney *U* test and Benjamin-Hochberg multiple testing were applied to obtain p-values and q-value, respectively.

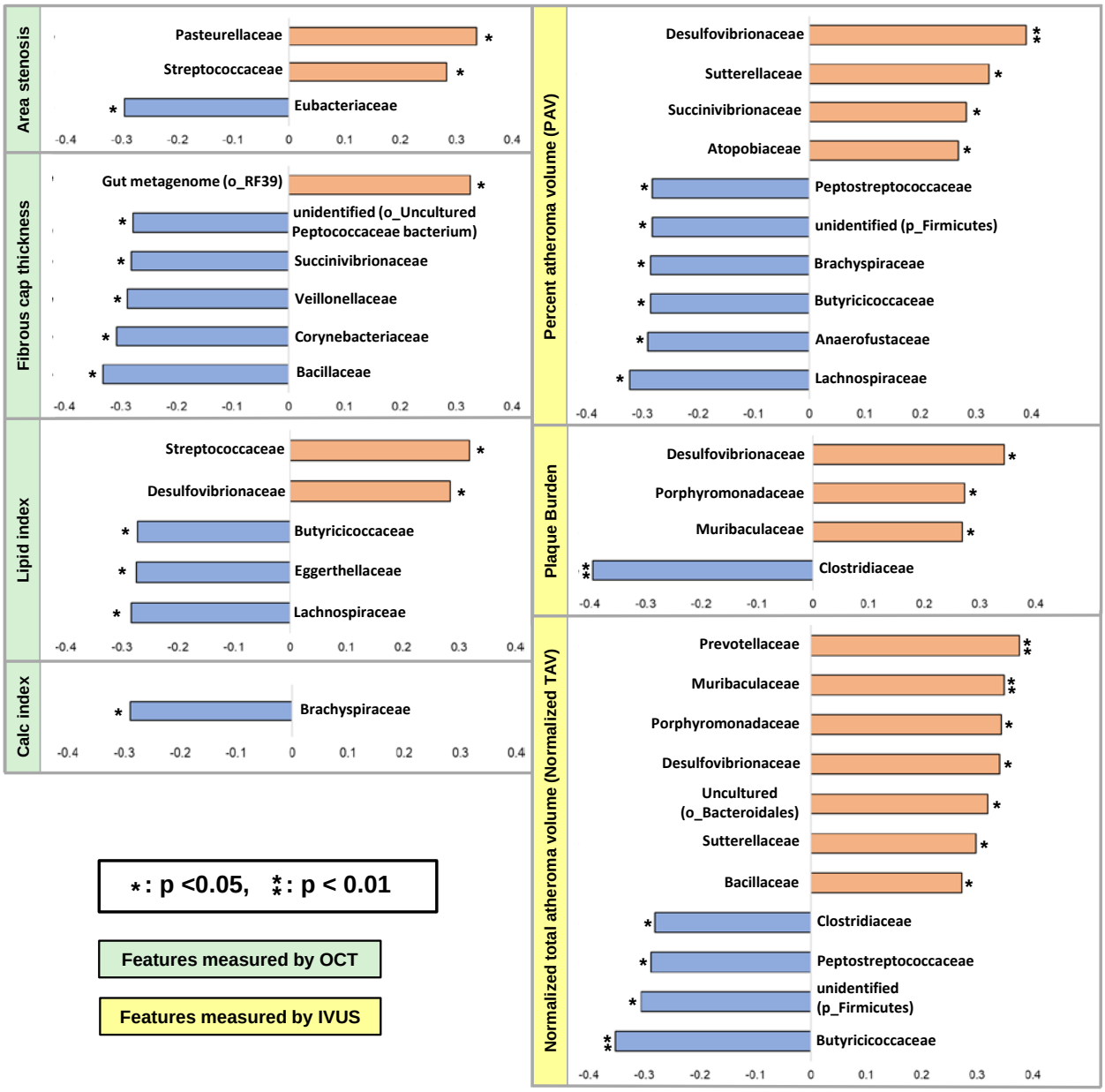
Figure S9. Cladogram that shows the bacteria which are associated with specific OCT features



Cladograms show the lineages of bacteria which are associated with specific OCT features. Two lineages (*Chirstensenellales – Chirstensenellaceae*, and *Enterobacterales - Enterobacteriacea*) were associated with the presence of lipid rich plaque (A). One lineage (*Dysgonomonadacea - Dysgonomonas*) was associated with

the presence of TCFA (B). No lineage was associated with macrophages (C). One lineage (*Monoglobales – Monoglobaceae - Monoglobus*) was associated with the absence of Microvessels (D). One lineage (*Cyanobacteria – Vampirivibrionia - Gastranaerophilales*) was associated with the presence cholesterol crystal (E). Four lineages (*Lentisphaeria – Victivallales – Victivallaceae*, *Coriobacteriacea – Collinsella*, *Rikenellaceae – Alistipes*, and *Oscillospirales - Oscillospiraceae*) were associated with the presence of calcification (F). One lineage (*Eubacteriales – Eubacteriaceae - Eubacterium*) was associated with the absence of layered phenotype (G).

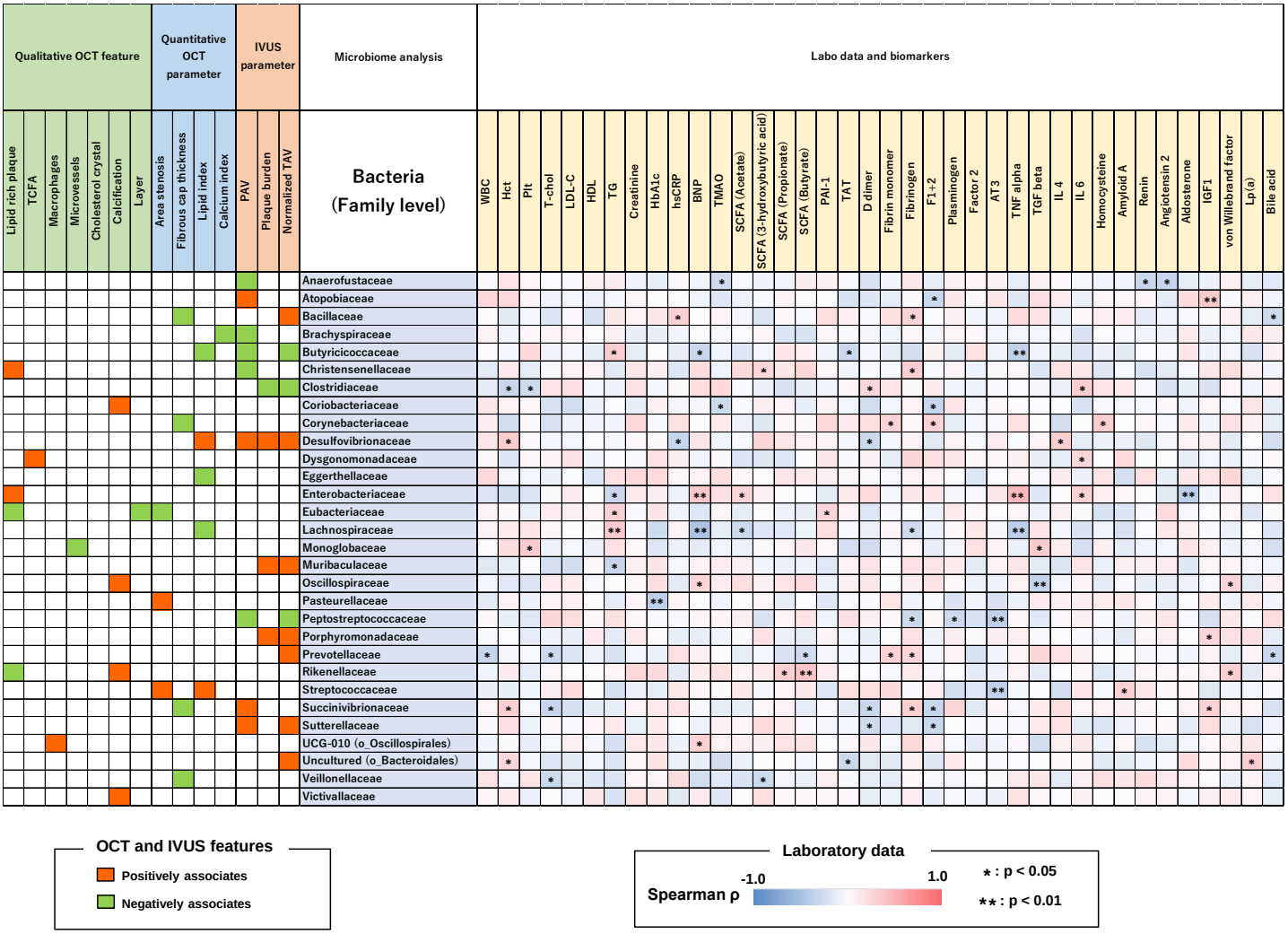
Figure S10. The correlations between gut bacteria and quantitative OCT/IVUS features at the family level



The figure shows the correlations between gut bacteria and quantitative OCT/IVUS features at the family level. Two bacteria and 2 bacteria were associated with positive correlations with area stenosis and lipid index, respectively, and 5 bacteria were associated with negative correlations with FCT measured by OCT. Four bacteria, 3 bacteria, and 7 bacteria were associated with positive correlations with PAV, PB, and $TAV_{normalized}$ measured by IVUS, respectively.

IVUS = intravascular ultrasound; OCT = optical coherence tomography.

Figure S11. Relationships between blood biomarkers and gut bacteria which associate with specific OCT/IVUS features at the family level



Relationships between gut bacteria and laboratory biomarkers which are associated with specific OCT/IVUS features at the family level.