Supplementary Methods

Reagents and antibodies

All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified. Oxidized (ox) low-density lipoprotein (LDL) and acetylated LDL were purchased from Calbiochem (San Diego, CA, USA). The primary antibodies used included: rabbit antibodies against CD36, ATP-binding cassette transporters (ABCA1), and neutral cholesteryl ester hydrolase 1 (NCEH1) (Abcam, Cambridge, MA, USA) acyl coenzyme A: cholesterol acyltransferase-1 (ACAT1), and cholesterol 24-hydroxylase (CYP46A1) (Cell Signaling Technology Inc., Beverly, MA, USA); a rabbit antibody against ATP-binding cassette transporters G1 (ABCG1) (Novus Biologicals, Littleton, CO, USA); and mouse antibodies against macrophage scavenger receptor 1 (SR-A1), lysosomal acid lipase (LAL), and beta-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Infection of macrophages with periodontal pathogens

Before the infection with periodontal pathogens, THP-1 monocytes were attached and differentiated into macrophages by treatment with 2 ng/mL phorbol 12-myristate 13-acetate (PMA). Cells were infected with *P. gingivalis* or *F. nucleatum* at a multiplicity of infection (MOI) of 100 for 3 h. The cells were then washed thrice with phosphate-buffered saline (PBS), and fresh antibiotic-containing medium was added.

Quantitative real-time PCR

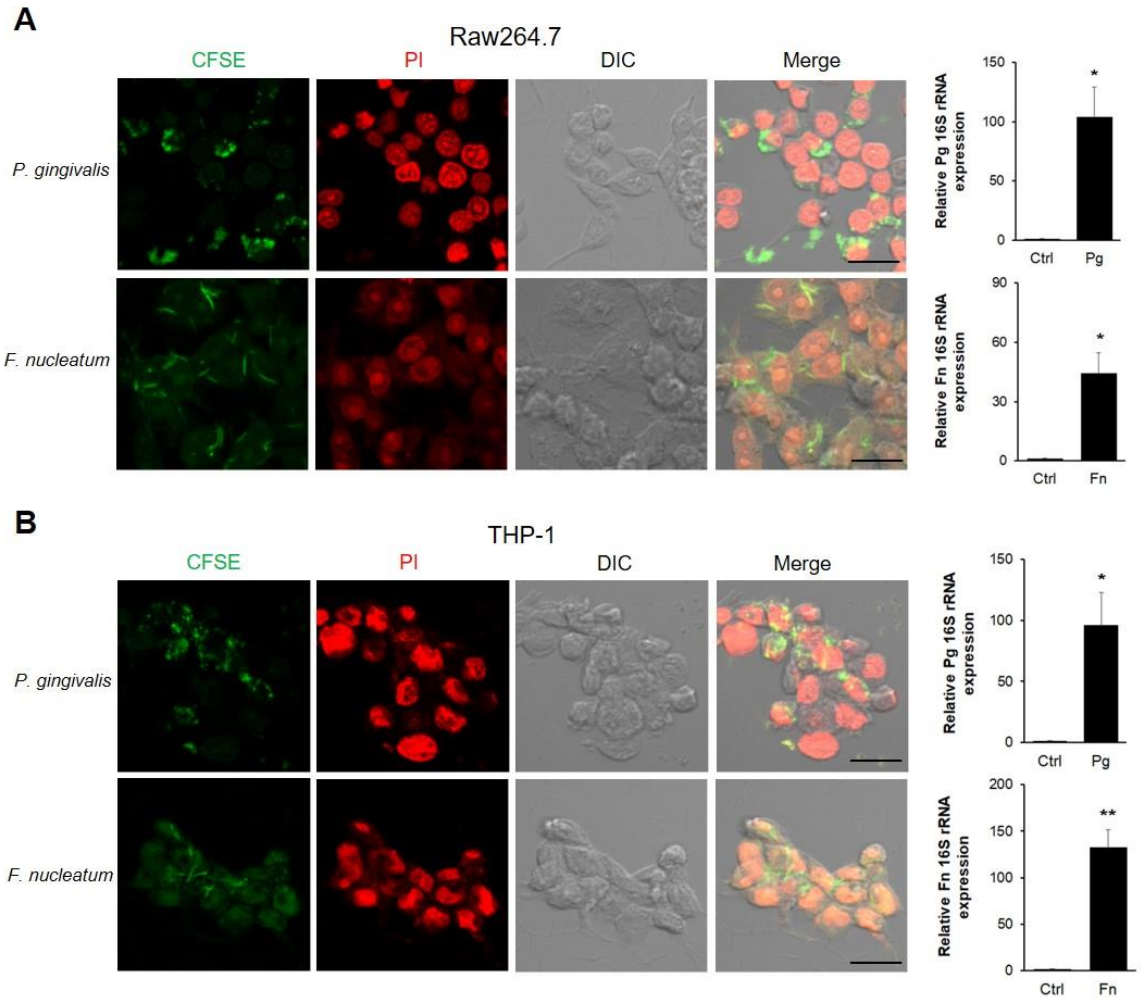
Total RNA was extracted using Trizol reagent (Invitrogen), and 1 μg of total RNA was reverse transcribed using the QuantiTect reverse transcription kit (Qiagen). Real-time PCR assays were performed with the TOPreal SYBR Green PCR Kit (Enzynomics, Daejeon, South Korea) in the ABI 7500 Real-Time PCR Detection System (Applied Biosystems, Carlsbad, CA, USA). The expression levels of the target genes in the samples were normalized to those observed in parallel reactions

performed using GAPDH-specific primers. The primers used in this study include: mouse GAPDH forward primer 5'-CGGGTTCCTATAAATACGGACTG, mouse GAPDH reverse primer 3"-TCTACGGGACGAGGCTGG, human GAPDH forward primer 5'- GAAGGTGAAGGTCGGAGTCA-AC, human GAPDH reverse primer 3'- CAGAGTTAAAAGCAGCCCTGGT, *P. gingivalis* 16S rRNA forward primer 5'-TCGGTAAGTCAGCGGTGAAAC, *P. gingivalis* 16S rRNA reverse primer 3'-GCAAGCTGCCTTCGCAAT, *F. nucleatum* 16S rRNA forward primer 5'-GGGCTCAACTCTGTATT-GCG, and *F. nucleatum* 16S reverse primer 3'-CTGTTTGCTACCCACGCTTT.

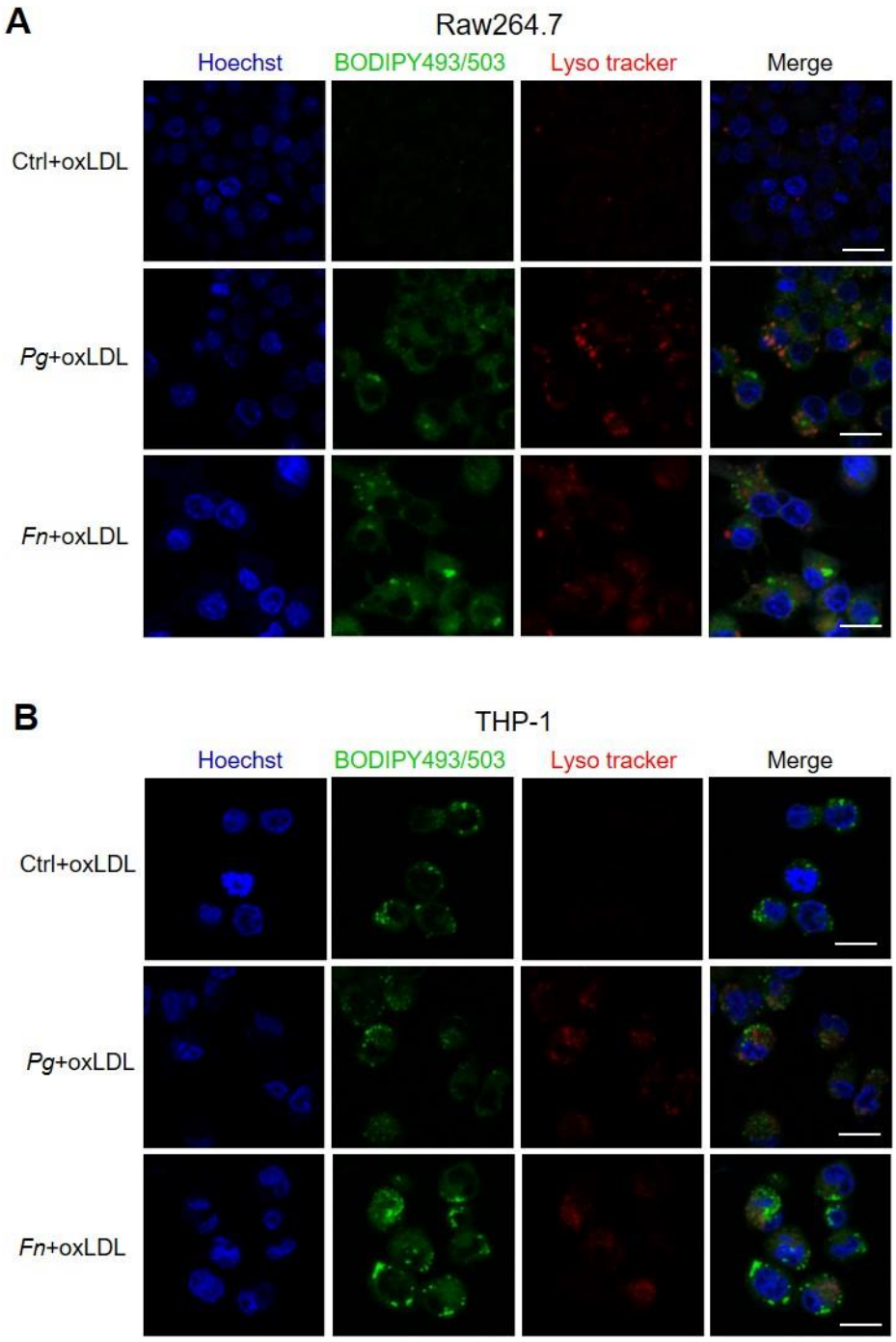
Western blotting

The cell lysates were separated on sodium dodecyl sulfate (SDS)-polyacrylamide gels and transferred to polyvinylidene fluoride membranes (Bio-Rad, Hercules, CA, USA). The membranes were then incubated with the appropriate primary antibodies, and then with the secondary antibodies. Antigen-antibody complexes were visualized with SuperSignal West Femto substrate (Pierce, Rockford, IL, USA). Immunoreactive bands were observed using a ChemiDoc gel documentation XRS system (Bio-Rad).

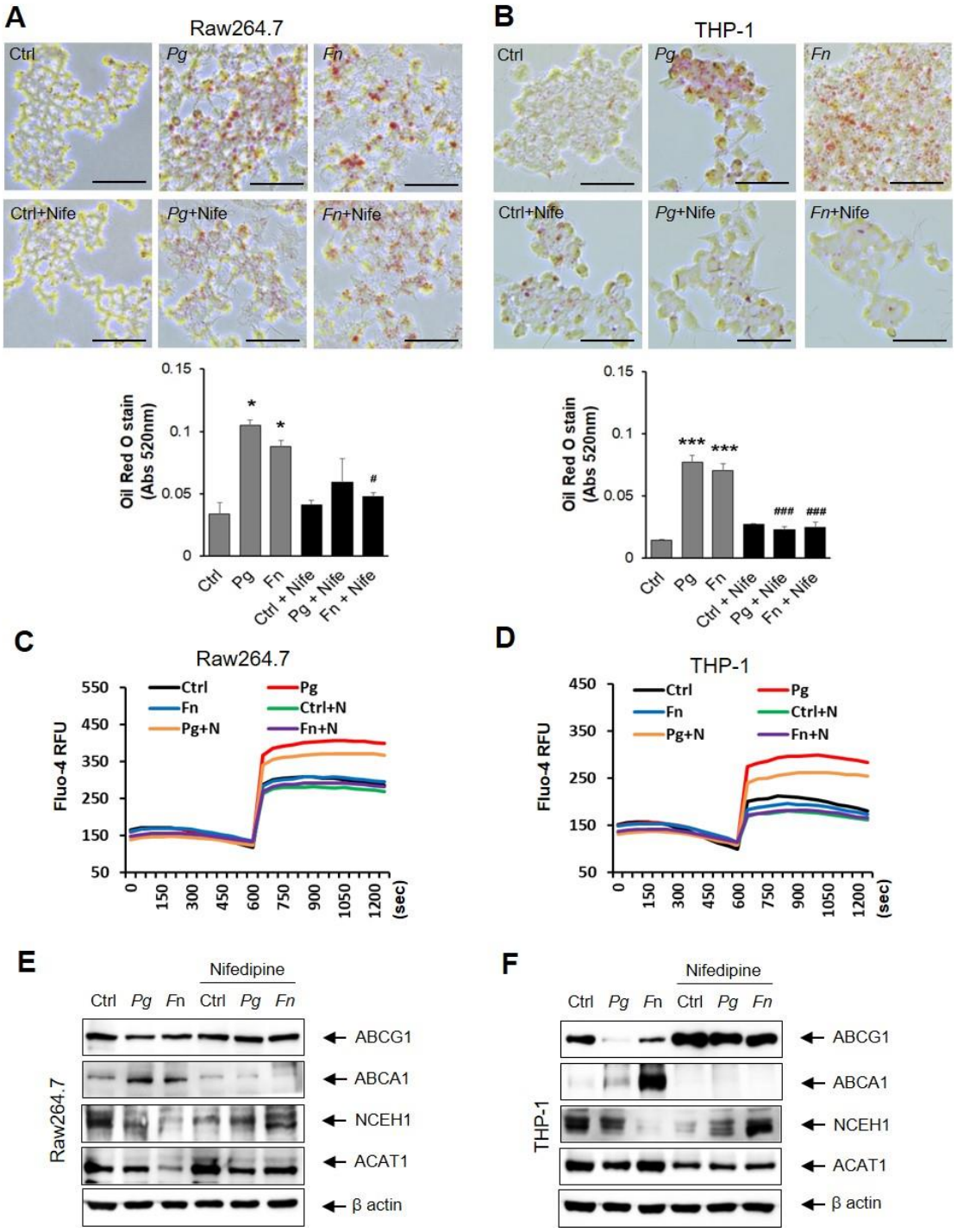
Supplementary Figures



Supplementary Figure 1. Representative fluorescent images of intracellular periodontal pathogens within macrophages. Raw264.7 (**A**) and THP-1 (**B**) cells were infected with carboxyfluorescein succinimidyl ester (CFSE)-prestained periodontal pathogens at an MOI of 100 for 3 h, and the cells were observed using confocal microscopy. Scale bars = 20 μm. Besides, the presence of periodontal pathogens was confirmed by measuring the 16S rRNA expression levels of the pathogens using real-time PCR (right panel). Data are presented as the mean ± SEM. **P*<0.05, ***P*<0.01 vs Ctrl.



Supplementary Figure 2. Confocal microscopic images of periodontal pathogen-infected macrophages in the presence of oxLDL. Raw264.7 (**A**) and THP-1 (**B**) cells were infected with *P. gingivalis* or *F. nucleatum* for 3 h and incubated in the presence of oxLDL for 24 h. The cells were stained with BODIPY 493/503 (green), lysotracker (red), and Hoechst 33342 (blue), and the association between lipid droplets and lysosomes was investigated using confocal microscopy. Scale bars = 20 μm. Pg, *P. gingivalis*; Fn, *F. nucleatum*; oxLDL, oxidized LDL.



Supplementary Figure 3. Nifedipine significantly abolished lipid droplet formation by reducing the calcium influx into periodontal pathogen-infected macrophages in the absence of exogenous lipids. The cells were treated with 10 μM nifedipine for 1 h and then infected with the periodontal pathogens for 3 h. **A** and **B**, Representative photographs of Oil Red O-stained Raw264.7 (**A**) and THP-1 (**B**) cells. Scale bars = 50 μm. Data are presented as the mean ± SEM. **P*<0.05, ****P*<0.001; #*P*<0.05,

###*P*<0.001 vs Nifedipine untreated group. **C** and **D**, Graphs showing the level of calcium influx into Raw264.7 (**C**) and THP-1 (**D**) cells. **E** and **F**, Western blotting analysis showing the protein expression of lipid metabolism- and cholesterol efflux-related factors in Raw264.7 (**E**) and THP-1 (**F**) cells.