Supplementary Information

Additional Materials and Methods Used for the Supplementary Data

Reagents
Anti-caspase-1 polyclonal antibody (Cell Signaling, Beverly, MA) and anti-GAPDH mAb (clone MAB374, Millipore, Billerica, MA) were purchased.

Human cell lines
The human monocytic leukemia NOMO-1 cell line was obtained from the Health Science Research Resources Bank (Osaka, Japan). To generate THP-1-shCont and THP-1-shCasp1 stable cell lines, THP-1 cells were transduced with a pLKO.1 lentiviral vector expressing control shRNA or caspase-1-targeting shRNA. The transduced cells were selected under puromycin treatment. The sequences of the control and caspase-1-targeting shRNAs are provided in the legend to Supplementary Figure S2.

Measurement of IL-1β
The amount of human IL-1β in culture supernatants was determined using the OptEIA ELISA kit (BD Pharmingen), according to the manufacturer’s protocol.

Supplementary movies

Movie S1. THP-1 cells killed by infection with FITC-labeled S. aureus showed necrotic morphology.
**Supplementary Figures**

**Fig. S1.** THP-1 cells killed by infection with *S. aureus* or *P. aeruginosa* showed a necrotic morphology. (A) THP-1 cells that were uninfected or infected with *S. aureus* (MOI 25) or *P. aeruginosa* (MOI 25) for 2.5 h were then stained with Hoechst33342 (Hoechst) and PI, and examined under a fluorescence microscope. Scale bar, 20 µm. (B) THP-1 cells were uninfected or infected with *S. aureus* (MOI 25) or *P. aeruginosa* (MOI 50) for 2.5 h. Proportion of cell death was determined by LDH release assay. Error bars and asterisks indicate standard deviation and statistical significance, respectively. ***, P<0.001** (C and D) THP-1 cells were treated as described in Figure 6. (C) Cells were then stained and examined as in A. Scale bar, 20 µm. (D) Cells were stained with Cy5-annexin V and propidium iodide, and analyzed by flow cytometry. THP-1 cells killed by *S. aureus* or *P. aeruginosa* infection were swollen and ruptured as indicated by nuclear staining by PI, a non-permeable fluorescent dye, just like heat-killed THP-1 cells. In contrast, apoptotic THP-1 cells killed by UVC or staurosporine exhibited pyknotic nuclei without PI-staining.
**Fig. S2.** Caspase-1 is involved in both cell death and IL-1β release induced by *S. aureus* and *P. aeruginosa* infection. (A) THP-1 cells were transduced with a lentiviral vector expressing a puromycin resistant gene and a control shRNA (Cont, CCTAAGGTTAAGTCGCCCTCGCTCTAGCGAGGGCGACTTAACCTTAGG), caspase-1-targeting shRNA #1 (Casp1#1, CACACGTCTTGCTCTCATTATCTCGAGATAATGAGAGCAAGACGTGTG) and #2 (Casp1#2, CTACAACTCAATGCAATCTTTCTCGAGAAAGATTGCATTGAGTTGTAG). Stable transfectants were established by selecting puromycin resistant cells. The expression of caspase-1 and GAPDH was assessed by Western blotting. GAPDH serves as a loading control. Casp1#2-expressing cells exhibited more complete knockdown of caspase-1 expression than Casp1#1-expressing cells. (B) THP-1-derived transfectants established in A were infected with *S. aureus* or *P. aeruginosa* at an indicated moi for 2 hr. Culture supernatants were assessed for LDH release and IL-1β concentration. Error bars and asterisks indicate standard deviation and statistical significance, respectively. *, *P*<0.05; **, *P*<0.01; ***, *P*<0.001.
Fig. S3. MDP stimulation induces apoptosis and pyroptosis in CLC12N2-Apo and CLC12N2-Pyr cells, respectively. (A and B) CLC12N2-Apo or CLC12N2-Pyr cells were stimulated with or without MDP (1 µg/ml) for 3 h. (A) Cells were then stained with Hoechst33342 (Hoechst) and propidium iodide (PI), and examined under a fluorescence microscope. Scale bar, 20 µm. (B) The cell viability was determined by WST-1 assay. Error bars and asterisks indicate standard deviation and statistical significance, respectively. ***, P<0.001. (C and D) CLC12N2-Pyr cells were treated as described in Figure 6. (C) Cells were then stained and examined as in A. Scale bar, 20 µm. (D) Cells were stained with Cy5–annexin V and propidium iodide, and analyzed by flow cytometry. We previously demonstrated that MDP-induced necrotic cell death of CLC12N2-Pyr cells was caspase-1-dependent (8). When pro-IL-1β was exogenously expressed in CLC12N2-Pyr cells, MDP stimulation induced caspase-1-dependent IL-1β secretion (8). Therefore, MDP-induced necrotic cell death of CLC12N2-Pyr cells is considered to be pyroptosis.
Fig. S4. *S. aureus-* or *P. aeruginosa*-infected NOMO-1 cells were engulfed by PMA-induced NOMO-1-derived macrophages in a PS-dependent manner. We previously demonstrated that another human monocytic cell line, NOMO-1 infected by *S. aureus* or *P. aeruginosa* are killed by typical pyroptosis which is caspase-1 dependent and characterized by rapid rupture of cells without marked change of nuclear morphology (8), just like THP-1 cells infected with the same bacteria. (A-C) PKH26-labeled NOMO-1 cells (target) were infected with *S. aureus* (*S.a.*; MOI 25) or *P. aeruginosa* (*P.a.*; MOI 50) for 2.5 h or were not infected (None). In C, PKH26-labeled target cells were collected and incubated with culture supernatant from mock-transfected (Control) or MFG-E8-D89E-expressing HEK293T cells for 30 min. The target cells were added to PKH67-labeled and PMA-induced NOMO-1-derived macrophages in a target : macrophage ratio of 1:2 and incubated for 2h. Phagocytosis was analyzed by flow cytometry. Percentages in A-C indicate percent phagocytosis calculated as described in Materials and Methods. Error bars and asterisks indicate standard deviation and statistical significance, respectively. ***, *P*<0.001.
Fig. S5. Apoptotic, pyroptotic, and heat-killed necrotic cells were stained by Tim4-Fc. COLO205 cells were untreated (COLO205) or heat-treated (COLO205-Heat) at 90°C for 3 min and then cultured for 3 h. CLC12N2-Apo and CLC12N2-Pyr cells were treated with MDP (1000 ng/ml) for 5 and 3 h, respectively. Cells were stained with Tim4-Fc followed by FITC-anti-human IgG Ab, and analyzed by flow cytometry.
Fig. S6. Pyroptosis and accidental necrosis induce greater ATP release than apoptosis in NOMO-1 cells. To induce apoptosis, NOMO-1 cells were irradiated with UVC (UV; 100 mJ/cm²) and then cultured for 5 h or cultured with staurosporine (Stauro; 1 µM) for 5 h. To induce pyroptosis, NOMO-1 cells were infected with *S. aureus* (*S.a.*; MOI 25) for 2.5 h. To induce accidental necrosis, the cells were heated (Heat; 90°C, 3 min) and then cultured for 4 h. The cell viability and the chemotactic activity of the culture supernatants for THP-1 cells were determined as described in the legend to Figure 6. Error bars and asterisks indicate standard deviation and statistical significance, respectively. *, *P*<0.05; **, *P*<0.01; ***, *P*<0.001.