Supplementary Videos

Video S1. CLC12N2-Nec cells were treated with MDP (100 ng/ml). Starting 3 h after the MDP addition, images were captured every 7.5 s for 25 min and are displayed at 500 frames/ min.

Video S2. CLC12N2-Apo cells were treated with MDP (100 ng/ml). Starting 3 h after the MDP addition, images were captured every minute for 3 h and are displayed at 500 frames/min.

Supplementary Figures

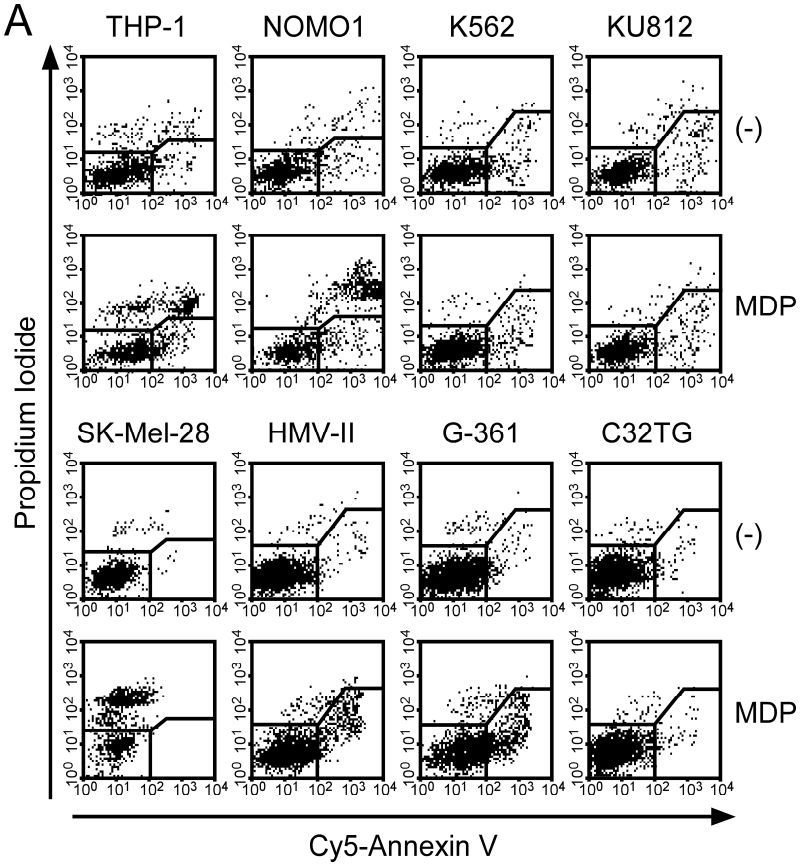
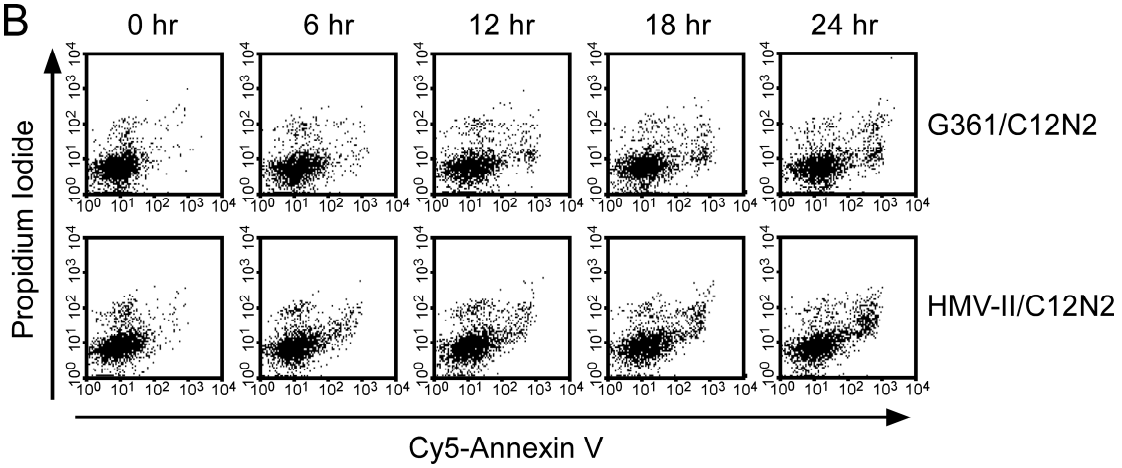


FIGURE S1. **ASC activation induces apoptosis or necrosis.** *A*, Two-dimensional (propidium iodide and Cy5-annexin V) staining profiles of the cells described in Fig. 1*C*. *B*, Time course of the staining profiles of apoptosis-type cells after MDP addition. *A* and *B*, MDP-treated THP-1, NOMO-1, and SK-Mel-28 cells exhibited increased PI staining preceding or occurring simultaneously with increased Cy5-Annexin V staining. Therefore, the PI(+) Cy5-Annexin V(+) cells were considered necrotic cells. On the other hand, MDP-treated HMV-II and G-361 cells exhibited an increase in Cy5-Annexin V staining that preceded PI staining. Thus, the PI(+) Cy5-Annexin V(+) cells were considered apoptotic cells. The morphology of the dying cells (Fig. 1*B*) and the fact that pretreatment with Z-IETD-FMK inhibited the development of PI(+) Cy5-Annexin V(+) cells from the latter cell lines but not from the former cell lines (Fig. 2) support this evaluation.



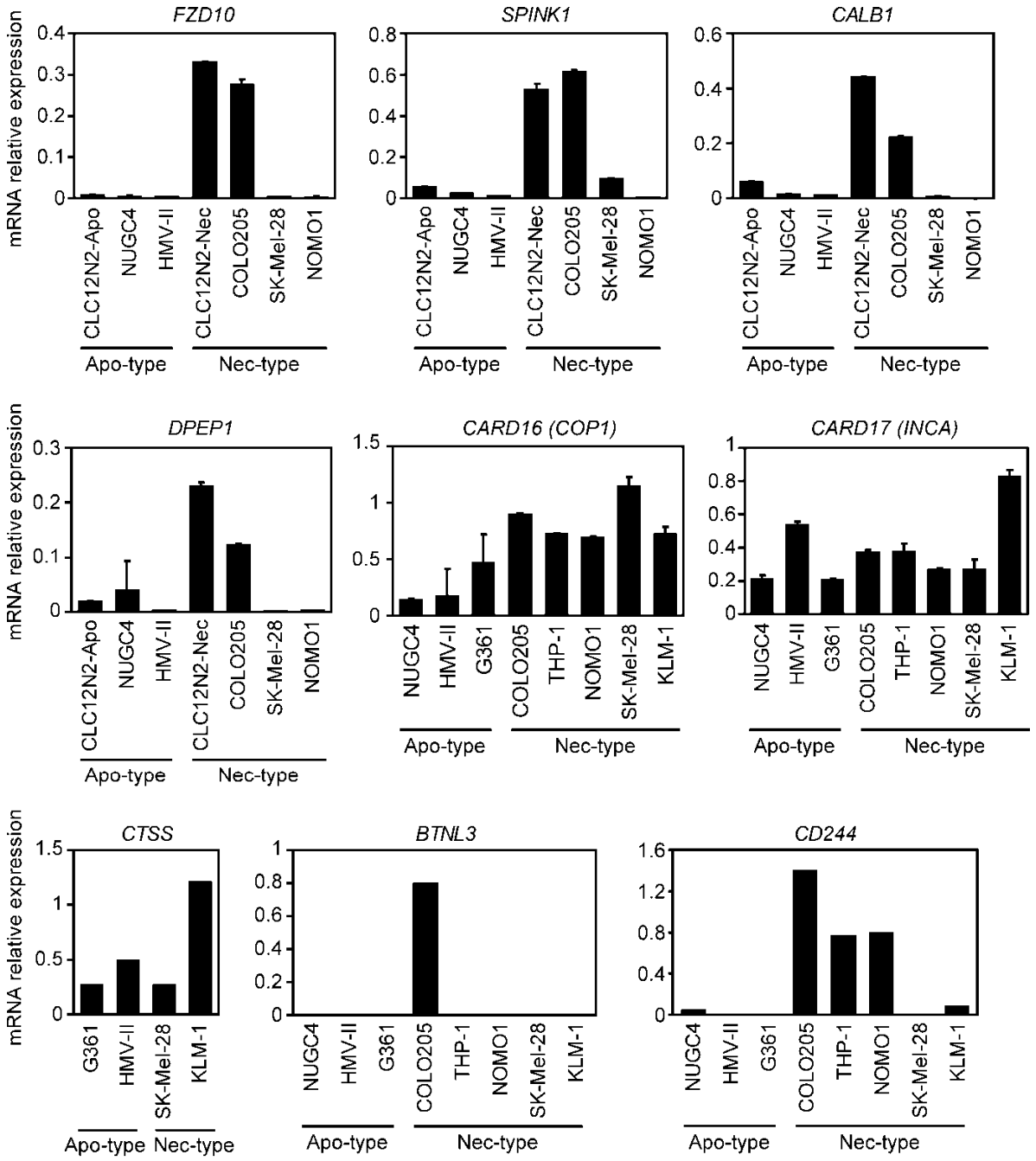


FIGURE S2. **The mRNA expression levels in apoptosis-type and necrosis-type cell lines of the genes listed in the upper panel of Fig. 1*C*.** The mRNA levels of the indicated genes were analyzed by real-time PCR. Relative mRNA expression levels normalized to that of *ACTB* (β-Actin) are shown. The primer sequences are shown in supplementary Table S1. *FZD10*, frizzled homolog 10; *SPINK1*, serine peptidase inhibitor, Kazal type 1; *CALB1*, calbindin 1; *DPEP1*, dipeptidase 1; *CARD16*, caspase recruitment domain family, member 16 (also known as COP1); *CARD17*, caspase recruitment domain family, member 17 (also known as INCA); *CTSS*, cathepsin S; BTNL3, butyrophilin-like 3; *CD244*, CD244 molecule, natural killer cell receptor 2B4.

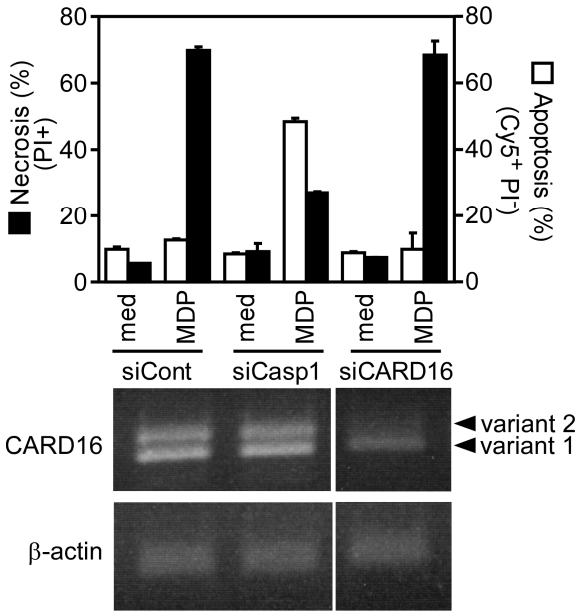


FIGURE S3. **CARD16 knockdown in CLC12N2-Nec cells does not convert the cell death mode.** CLC12N2-Nec cells were transfected with control, caspase-1-targeting, or *CARD16*-targeting siRNA (Invitrogen, HSS174333) on days 0, 3, and 6. On day 9, the cells were treated with MDP (100 ng/ml) for 12 h, and the proportions of apoptotic and necrotic cells were determined by flow cytometry as described in Fig. 1*B*. The expression levels of *CARD16* and *ACTB* (β-actin) on day 8 were examined by RT-PCR (lower panels). The following primers were used for PCR: *CARD16*, 5'-GCCATGGCCGACAAGGT-3' and 5'-ACCTAGGAAGGAAGTACTATTTGAG-3'; β-actin, 5'-TCCCTGGAGAAGAGCTACGA-3' and 5'-AAAGCCATGCCAATCTCATC-3'.

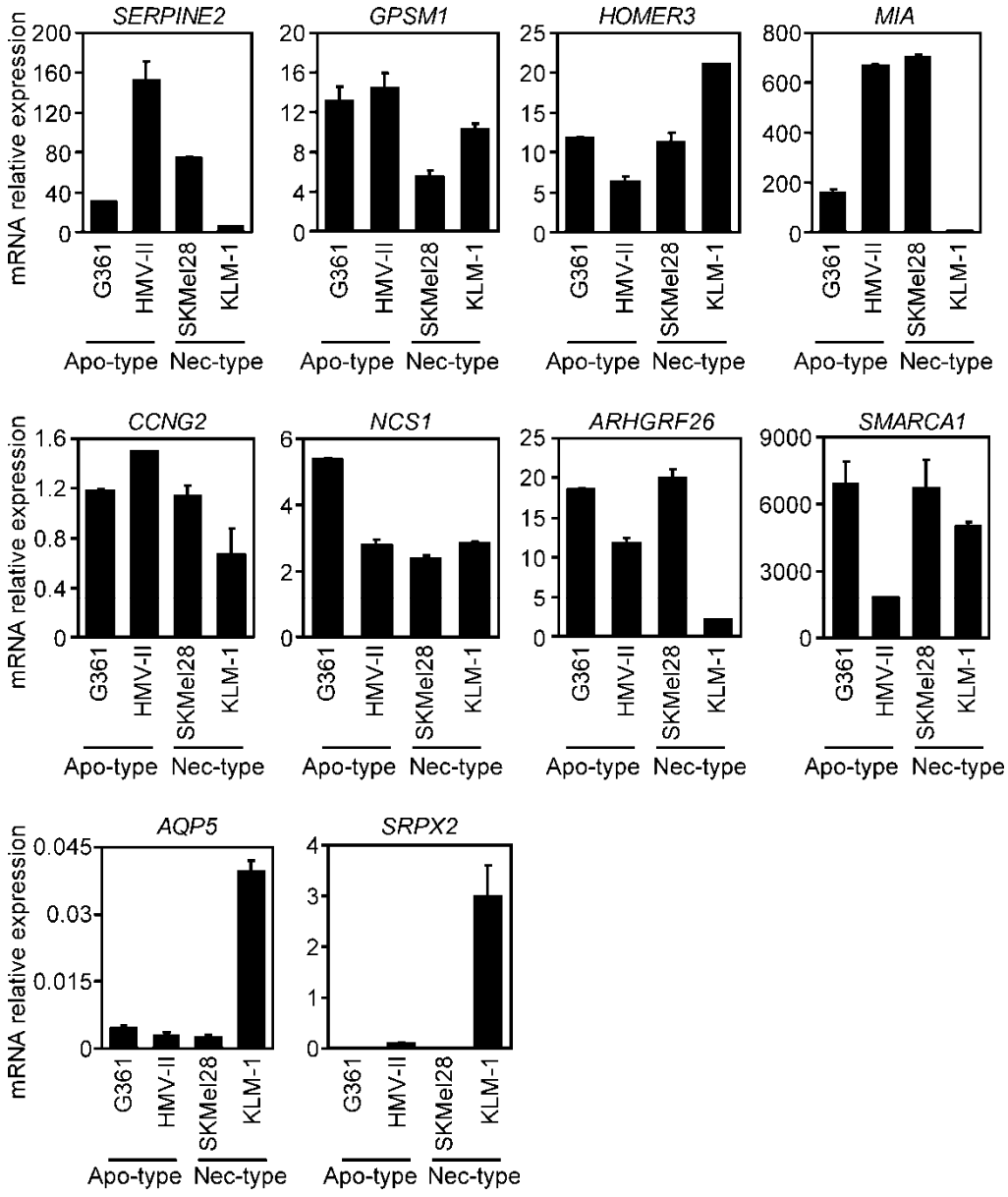


FIGURE S4. **The mRNA expression levels in apoptosis-type and necrosis-type cell lines of the genes listed in the lower panel of Fig. 1*C*.** The mRNA levels of the indicated genes were analyzed by real-time PCR. Relative mRNA expression levels normalized to that of *ACTB* (β-Actin) are shown. The primer sequences are shown in supplementary Table S1. *SERPINE2*, serpin peptidase inhibitor, clade E, member 2; *GPSM1*, G-protein signaling modulator 1; *HOMER3*, homer homolog 3; *MIA*, melanoma inhibitory activity; *CCNG2*, cyclin G2; *NCS1*, neuronal calcium sensor 1; *ARHGEF26*, Rho guanine nucleotide exchange factor 26; *SMARCA1*, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1; *AQP5*, aquaporin 5; *SRPX2*, sushi-repeat-containing protein, X-linked 2.

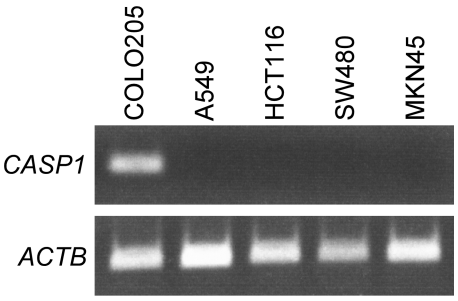


FIGURE S5. **Apoptosis-type cells do not express caspase-1.** RT-PCR was used to examine caspase-1 and β-actin mRNA expression in four human cancer cell lines (A549, HCT116, SW480, MKN45) that exhibit apoptosis in response to ASC activation by NLRC4-mimicry.

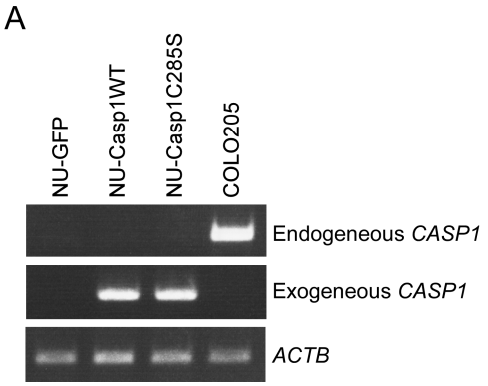


FIGURE S6. **The catalytic activity of caspase-1 is not required for ASC-mediated necrosis.** *A*, Total RNA was isolated from the indicated cell lines, and the mRNA levels of endogenous and exogenous caspse-1 and β-actin were detected by RT-PCR. *B*, NU-GFP, NU-Casp1WT, and NU-Casp1C285S cells were cultured with MDP (1000 ng/ml) for 6 h. Cytotoxicity was assessed by LDH release assay.

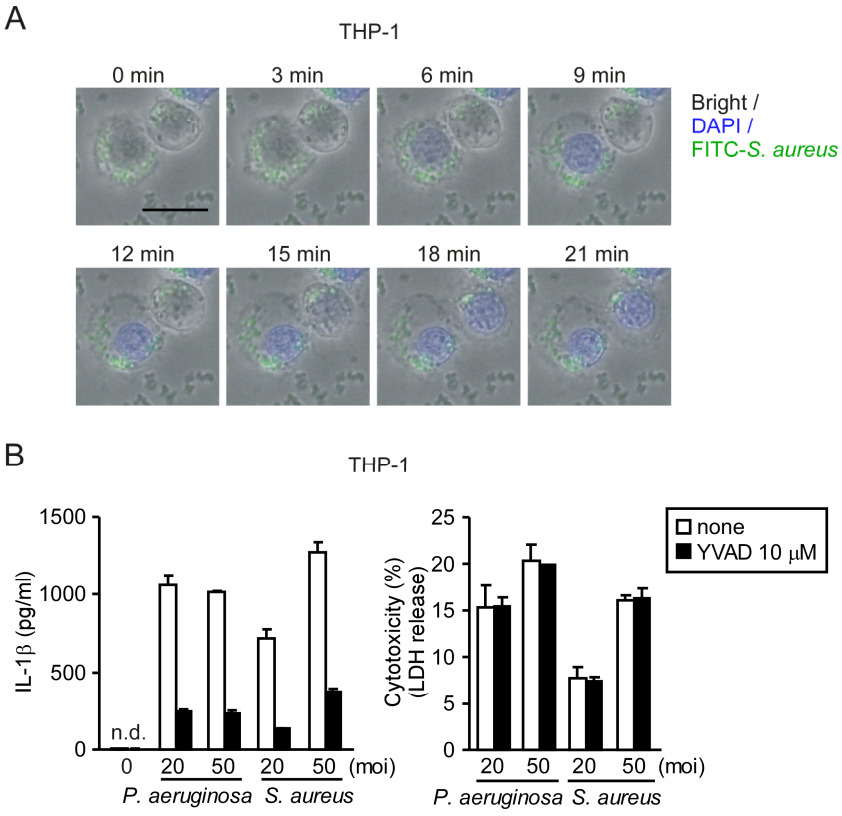


FIGURE S7. **Ac-YVAD-CMK inhibits the IL-1β release but not the necrosis induced by bacterial infection in THP-1 cells.** *A*, THP-1 cells were treated with PMA (10 ng/ml) for 12 h to allow cells to adhere to the culture plate, and the cells were then infected with FITC-labeled *S. aureus* (moi 50) in the presence of 4',6-diamidino-2-phenylindole (DAPI). Time-lapse images were recorded every 3 min beginning 3 h after infection. Scale bar, 20 μm. Nuclear staining with DAPI and the swelling of *S. aureus*-infected cells indicates that *S. aureus* induced necrosis in THP-1 cells. *B*, THP-1 cells were pretreated with Ac-YVAD-CMK (10 μM) for 1 h, and then infected with *S. aureus* for 2 h or with *P. aeruginosa* for 4 h. The culture supernatants were examined for LDH and IL-1β release.

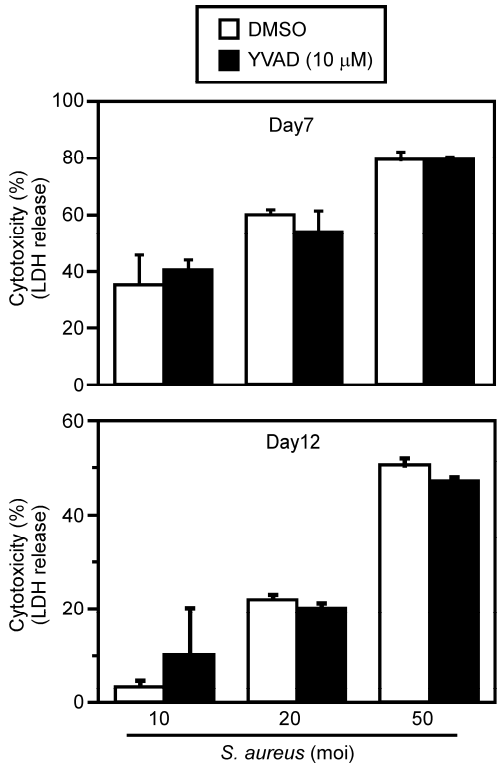


FIGURE S8. **Long-term inhibition of caspase-1 activity does not supress *S. aureus*-induced necrosis of NOMO-1 cells.** NOMO-1 cells were treated with Ac-YVAD-CMK for 7 or 12 days. Cells were then infected with *S. aureus* at the indicated moi in the presence of Ac-YVAD-CMK for 4 h. Cytotoxicity was assessed by LDH release assays.

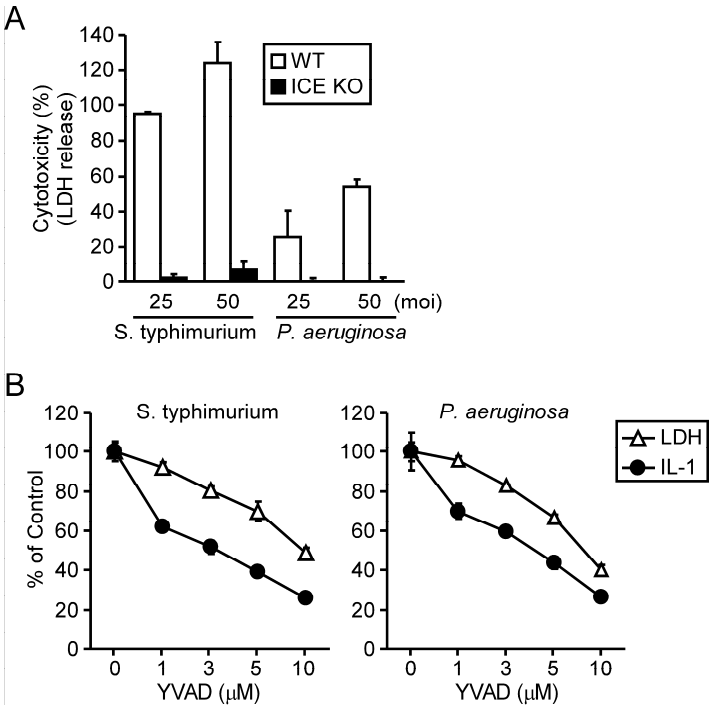


FIGURE S9. **Caspase-1 catalytic activity seems to be required for pyroptosis of mouse macrophages.** *A*, Thioglycollate-induced peritoneal macrophages were prepared from wild-type (WT, Caspase-$1^{+/+}$) or ICE KO (Caspase-$1^{-/-}$) mice. The macrophages were primed with LPS (100 ng/ml) for 12 h, and then infected with *Salmonella* (*S.*) *typhimurium* (ATCC 14028s) or *P. aeruginosa* for 2 h. *B*, Thioglycollate-induced peritoneal macrophages from wild-type mice were primed with LPS (100 ng/ml) for 4 h. The LPS-primed macrophages were pre-treated with the indicated concentrations of Ac-YVAD-CMK for 1 h, and then infected with *S. typhimurium* or *P. aeruginosa* for 1 h. LDH release and IL-1β production were analyzed as described in Fig. 7.

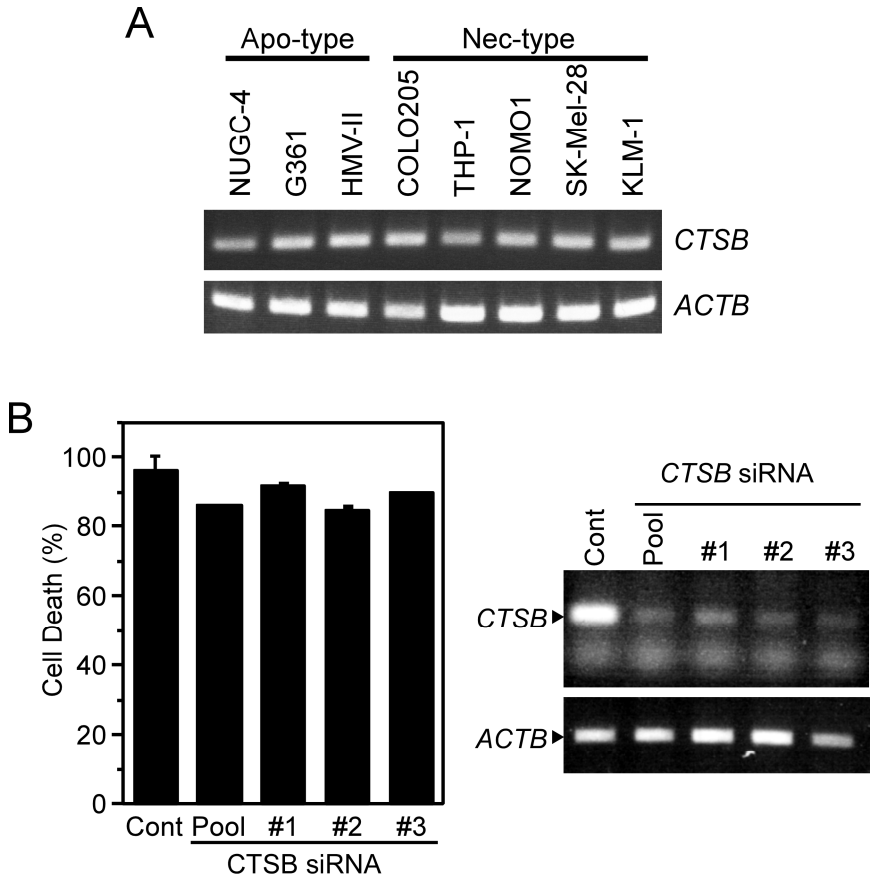


FIGURE S10. Cathepsin B may not be the target of CA-074Me in the inhibition of ASC-mediated necrosis. *A*, Total RNA was extracted from the indicated cell lines, and the expression of cathepsin B mRNA (*CTSB*) was examined by RT-PCR analysis. *B*, NOMO1-C12N2 cells were transfected with 20 nM of a control siRNA, a pool of four *CTSB* siRNAs (Dharmacon, L-004266-00), or three individual *CTSB* siRNAs (invitrogen, HSS102475-7). Forty eight hours later, cells were treated with MDP (300 ng/ml) for 6 h. Cell death was assessed by WST-1 assays (left panel). Knockdown of cathepsin B mRNA was confirmed by RT-PCR analyses (right panels).

Supplementary table

Table S1. The primer sequences[1)] used in Fig. S2 and S4.

Gene	Sense	Antisense
FZD10	CGGTGAAGACCATCCTGATCC	CAGCTTGTCCGTGTTCTCG
CD244	AAGCCACACCCTGAATCTCAC	CCAAAAACGGCCAAAATCTGAA
SPINK1	AGTCTATCTGGTAACACTGGAGC	ACACGCATTCATTGGGATAAGT
CALB1	AGGGAATCAAAATGTGTGGGAAA	TCCTTCAGTAAAGCATCCAGTTC
DPEP1	AGAGCCCCGGTCATCTTCA	CCTTGTTGGTGCAGGAAATGTA
CARD16	TGCAGAGGTGCCATGTTCAG	TTTATGCAAGGGGAGCAGCAGAAG
CARD17	AATGGCTTACTGGGTGAATTATTGG	TGTGATGCAAATTTGGCATGCTGGA
CTSS	ATGAAACGGCTGGTTTGTGTG	TGCTCCAGGTTGTGAAGCATC
BTNL3	GGGGCGTGTCTCTCTAAGG	CGTCAACATATCCCACGATGGA
SERPINE2	ACGCCGTGTTTGTTAAGAATGC	CGTTGACGAGGACCAGTCT
GPSM1	GGAGCCGGGCCTATCTCTAAA	CTCTTGCTGCCAGTAAGCATC
HOMER3	GGCGAGGAAAAACTGTTCCG	ACAACATCTTCTTTAGCCGCTC
MIA	GTCAGGGGTGGTCCTATGC	GGTCAGGAATCGGCAGTCG
CCNG2	TGCCTAGCCGAGTATTCTTCT	TGTTTGTGCCACTTTGAAGTTG
NCS1	TTCAAGCTCTACGACTTGGACA	GCTCCACGGTATTCCCCAC
ARHGEF26	CCGTGGTTTTGAGTACAAACAGC	CGCACCTTGAGGAGTCTCTTG
SMARCA1	GATGCGACCGCCACTATCG	AGATTTAGGCGCTTTAGCAGC
AQP5	CTGTCCATTGGCCTGTCTGTC	GGCTCATACGTGCCTTTGATG
SRPX2	GATGAGATGCCACGCACTACC	TCTTCCATGCAGATTCGGCTG

1) These primer sequences, with the exception of those for *CARD16* and *CARD17,* were obtained from PrimerBank (http://pga.mgh.harvard.edu/primerbank/).