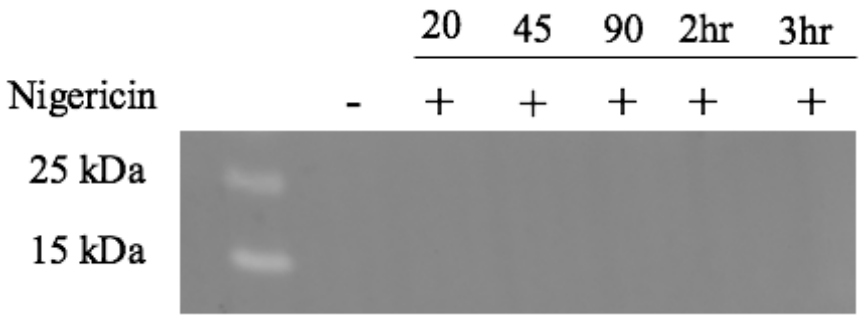
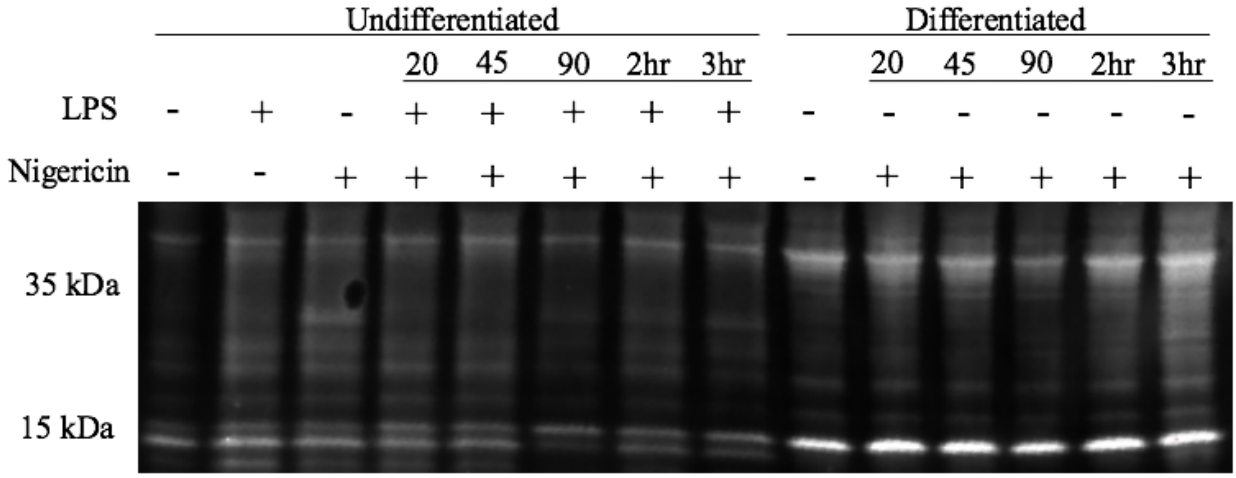
A

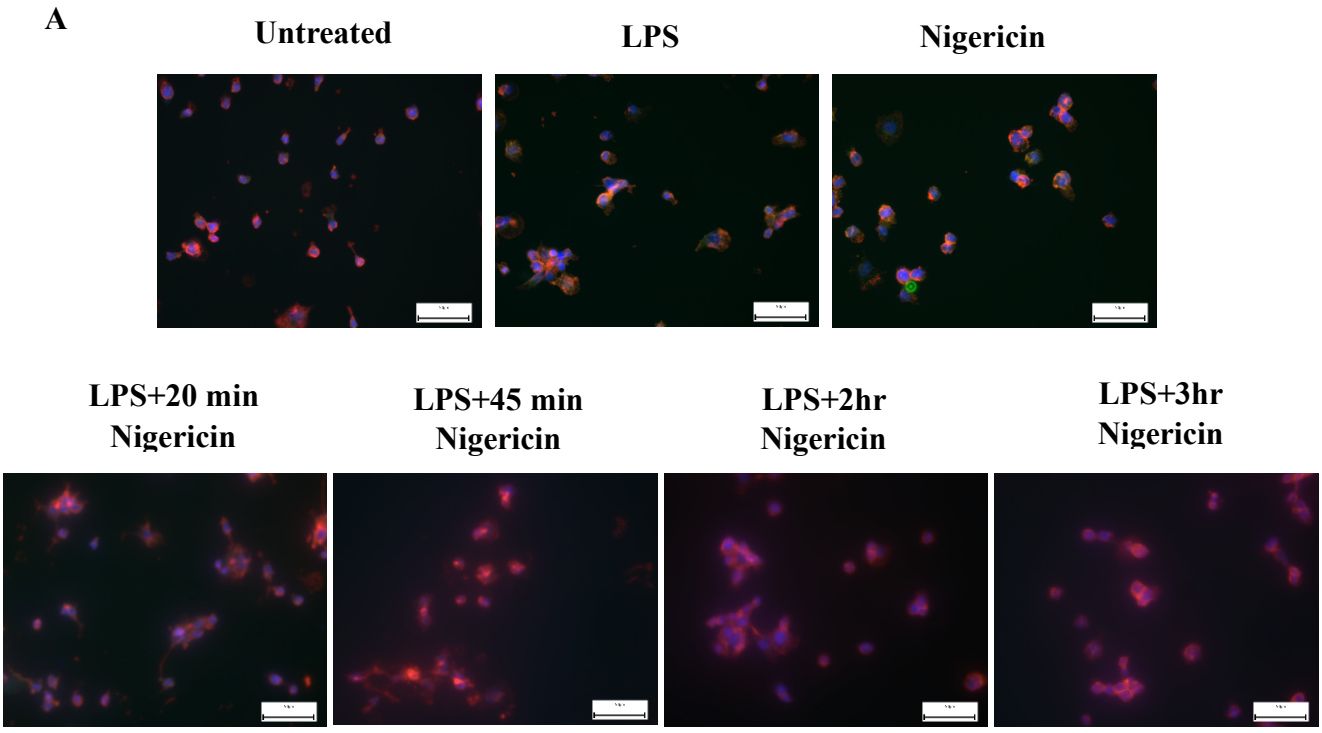


Supplementary figure S1: Absence of cleaved caspase-1 in PMA-differentiated THP-1 cells. PMA-differentiated (500 nM, 24 hrs) cells were treated with nigericin for 20 mins, 45 mins, 90 mins, 2 hrs and 3 hrs. **(A)** Western blot probing for cleaved caspase-1 in cell lysates. Expected band size at 20 kDa.

A



Supplementary figure S2: Inconclusive GSDMD^{Nterm} detection in THP-1 cells. LPS-primed (1 ug/mL, 4 hrs) undifferentiated and PMA-differentiated (500 nM, 24 hrs) cells were treated with nigericin for 20 mins, 45 mins, 90 mins, 2 hrs and 3 hrs. **(A)** Western blot probing for GSDMD^{Nterm} cell lysates. Expected band size at 31 kDa.



Supplementary figure S3: Actin reorganization in PMA-differentiated THP-1 cells immunostained for GSDMD^{Nterm}. PMA-differentiated (500 nM, 24 hrs) cells were treated with nigericin for 20 mins, 45 mins, 90 mins, 2 hrs and 3 hrs and immunostained using fluorescence conjugated antibodies (488 nm), Phalloidin (568 nm), and DAPI (358 nm). **(A)** Epifluorescence imaging displaying actin reorganization (red) in various LPS + nigericin treatment durations. GSDMD$^{N\text{-}term}$ signals are masked with background noise from phalloidin. Scale bar = 50 μm.

SUPPLEMENTARY METHODS

Western Blots

The membrane was blocked in 5% skim milk in TBS-T for 1 hr and incubated in either anti-cleaved caspase-1 rabbit monoclonal primary antibody (1:1000 dilution; Cell Signaling, Cat no. 4199) or anti-cleaved N-terminal GSDMD rabbit monoclonal primary antibody (1:1000 dilution; abcam, Cat no. ab215203) in 1% skim milk-TBS-T overnight at 4°C. Membrane was subsequently incubated for 1 hour in rabbit IgG HRP-conjugated secondary antibody (1:15000 dilution) in 1% skim milk-TBS-T.

Epifluorescence Microscopy

Differentiated THP-1 cells were seeded on glass slides in 12-well plates at $1.0x10^5$ cells/well, primed with 1 μg/mL LPS for 4 hrs, and treated with 10 μM nigericin. The glass coverslips were coated with Poly-L Lysine Solution (Sigma Aldrich, Cat no. RNBL0145) according to manufacturer's protocol and rinsed in deionized H_2O and 70% ethanol before use. Following cell stimulation, the cells were fixed in 4% paraformaldehyde, permeabilized in 0.1% triton X-100 detergent, and blocked in 5%BSA+1xPBS for 1 hour. The slides were incubated anti-cleaved N-terminal GSDMD primary antibody (1:100 dilution; Abcam, Cat no. ab215203) in 1%BSA+1xPBS overnight at 4°C followed by incubation in rabbit IgG Alexa Fluor 488 secondary antibody (1:1500 dilution; Invitrogen, Cat no. A11008) in 1%BSA+1xPBS for 60 minutes. Next, the cells were stained with phalloidin-Alexa 568 conjugate (1:100 dilution; Invitrogen, Cat no. A12380) in 1xPBS+1%BSA and incubated for 20 minutes in aluminum foil. The nuclear DNA was labeled with 1:1000 dilution of DAPI in 1xPBS+1%BSA for 2-3 minutes. The glass coverslips were placed onto ProLong gold mounting media on glass slides, sealed with nail polish and stored at 4°C. Images of the stained cells were observed and captured using an upright epi-fluorescence microscope (Zeiss Axioscope).