PNAS
www.pnas.org

Supplementary Information for

Calpain drives pyroptotic vimentin cleavage, intermediate filament loss, and cell rupture that mediates immunostimulation

Michael A. Davis[1, 2], Marian R. Fairgrieve[1, 2], Andreas Den Hartigh[3], Olga Yakovenko[4], Bhargavi Duvvuri[5], Christian Lood[5], Wendy E. Thomas[4], Susan L. Fink[3], Michael Gale, Jr[1, 2]

[1] Department of Immunology, University of Washington, Seattle WA
[2] Center for Innate Immunity & Immune Diseases, University of Washington, Seattle WA
[3] Department of Laboratory Medicine, University of Washington, Seattle WA
[4] Department of Bioengineering, University of Washington, Seattle WA
[5] Division of Rheumatology, University of Washington, Seattle WA

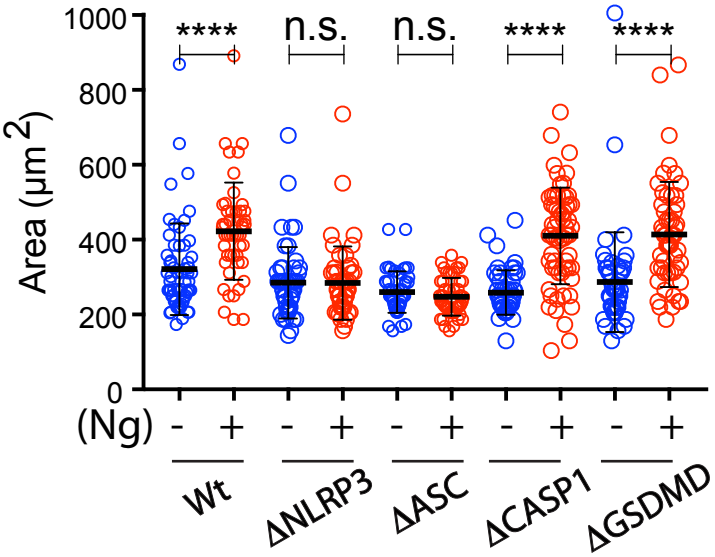
Corresponding author
Michael Gale, Jr.
Department of Immunology
University of Washington
Office E383, Box 358059
750 Republican St
Seattle WA, 98109
mgale@uw.edu
(206) 543-8514

This PDF file includes:

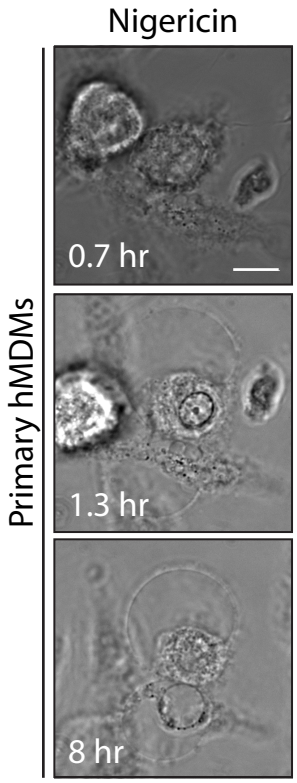
Figs. S1 to S9
Captions for movies S1 to S7

Other supplementary materials for this manuscript include the following:

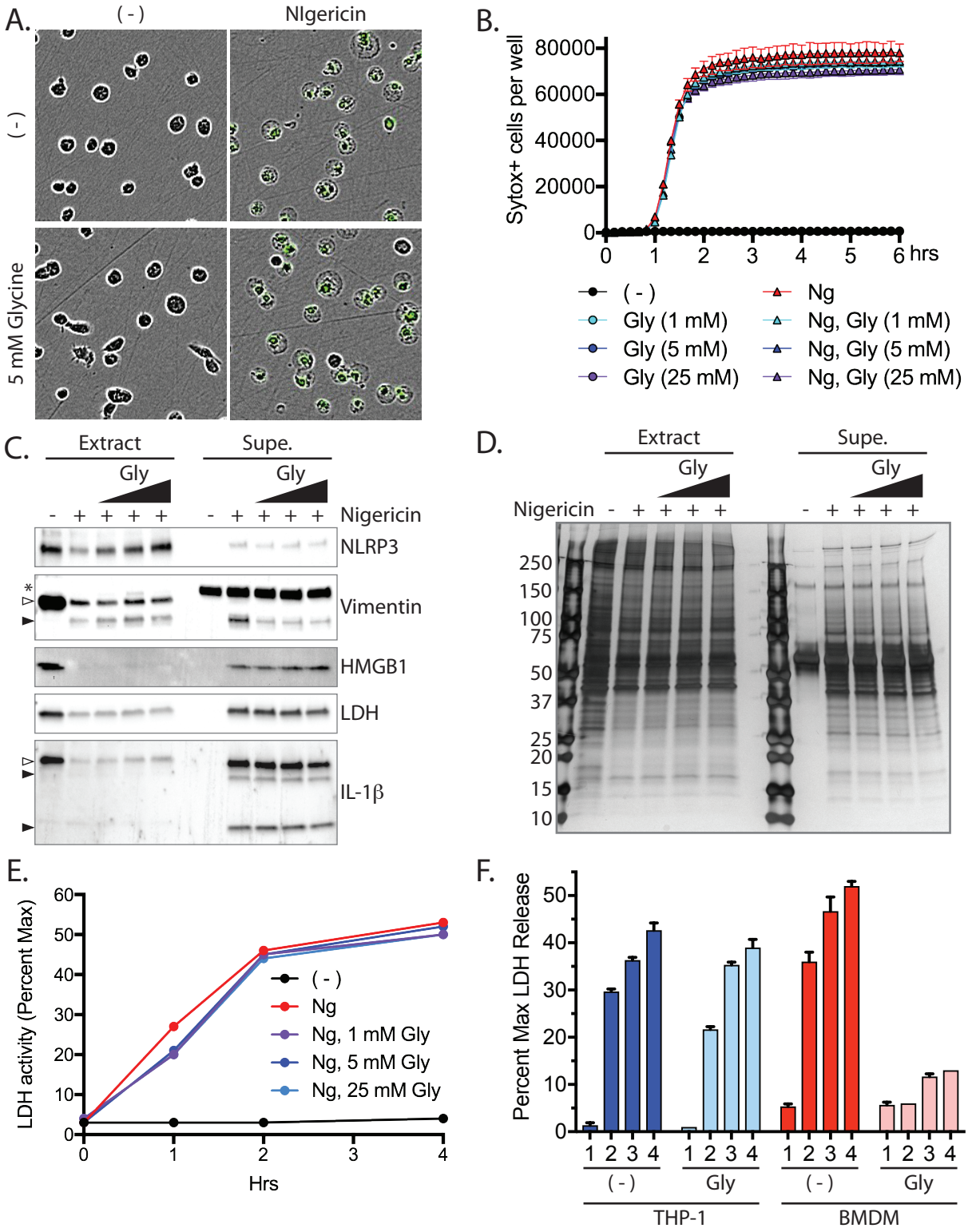
Movies S1 to S7



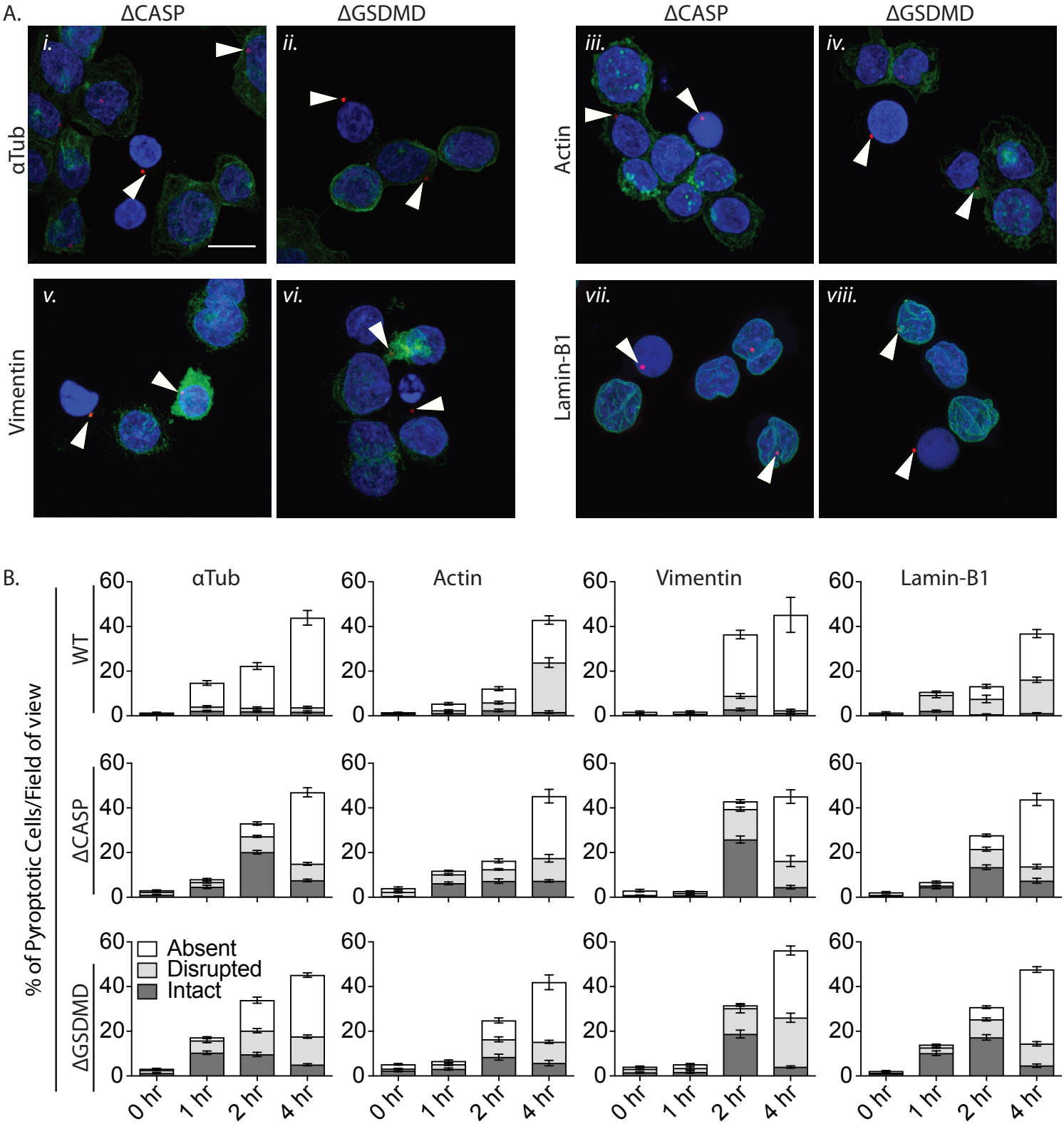
Supplemental Figure 1. Cell swelling 3 hr after Ng treatment was quantified from IncuCyte images using ImageJ/FIJI. At least 50 cells from a field of view were analyzed. Statistics were calculated via One-way ordinary ANOVA.



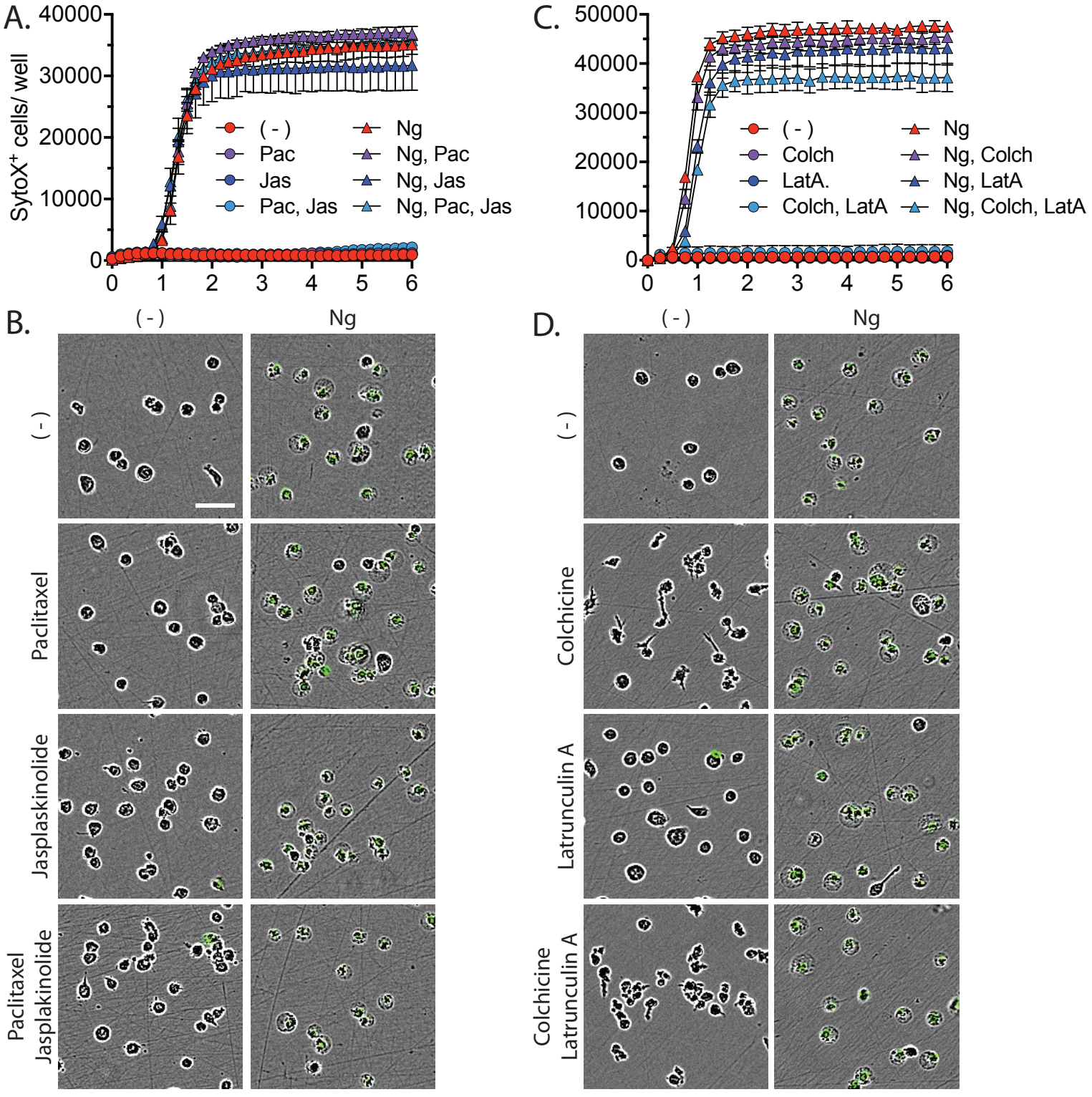
Supplemental Figure 2. Pyroptotic hMDMs do not rupture in vitro. Primary human monocyte-derived macrophages were primed with LPS and then treated with LPS/Nigericin and imaged every 30 seconds over a course of 8 hr. Scale bar is 10 μm.



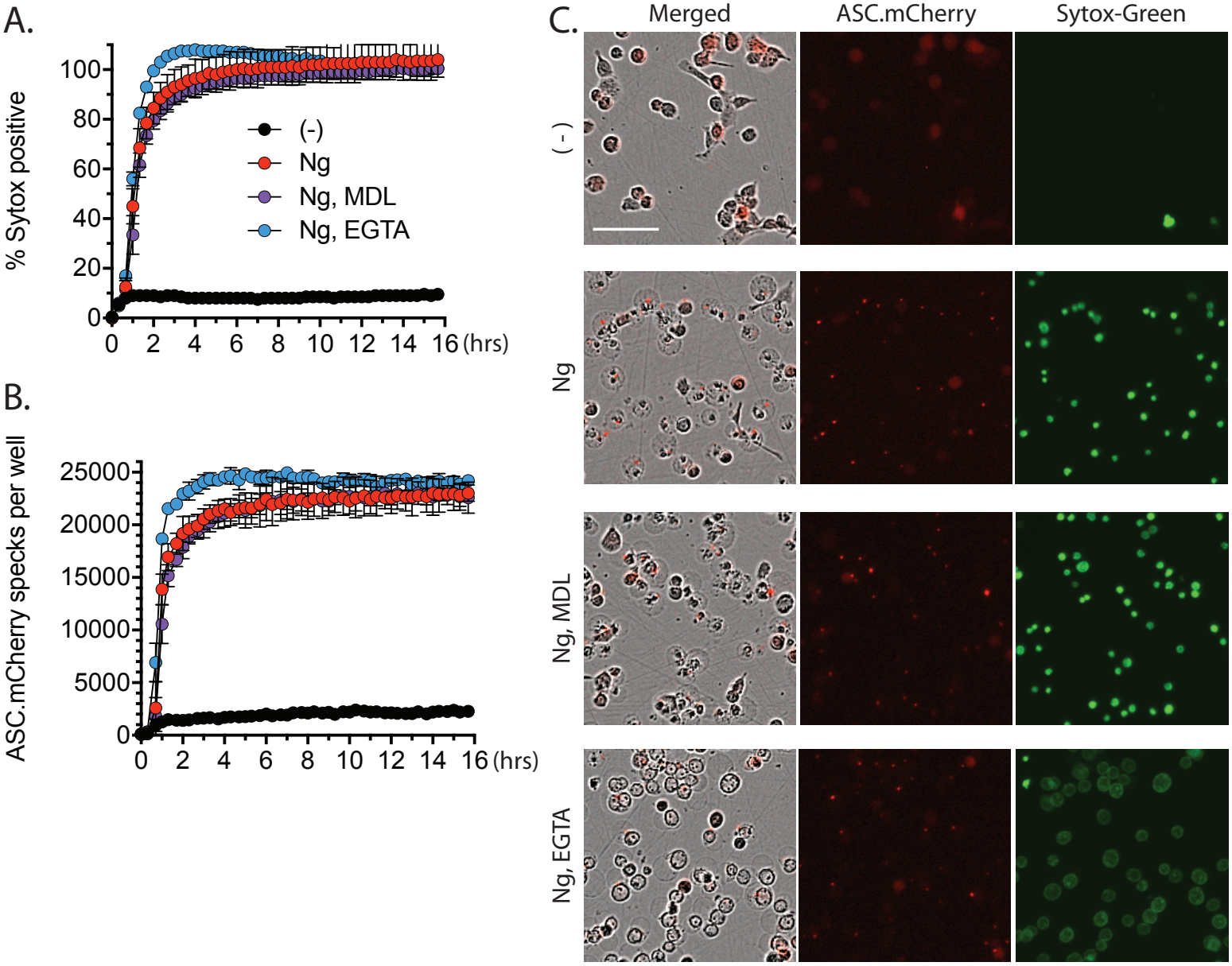
Supplemental Figure 3. The effects of glycine on pyroptotic cells are protein and cell specific. (A) THP-1 cells were treated with Sytox and 0, 1, 5, or 25 mM Glycine in the presence or absence of Ng. Cells were imaged every 10 minutes for 6 hours via IncuCyte at 20x. Images show merged images of phase and green channels for samples treated with or without 5 mM Glycine at 3 hr post Ng treatment. (B) Quantitation of Sytox-positive cells from A. (C) THP-1 cells were treated with Ng plus 0, 1, 5, or 25 mM Glycine or left untreated for 3 hrs in serum-free medium. Cell extracts and supernatants were analyzed by immunoblot for the indicated proteins. Open and closed arrowheads indicate full length and cleaved proteins, respectively. Asterisk indicates non-specific band. (D) THP-1 cells were treated as in C and analyzed by silver stain. (E) THP-1 cells were treated as in C and supernatants were analyzed for LDH activity. (F) THP-1 cells and BMDMs were treated as in E.



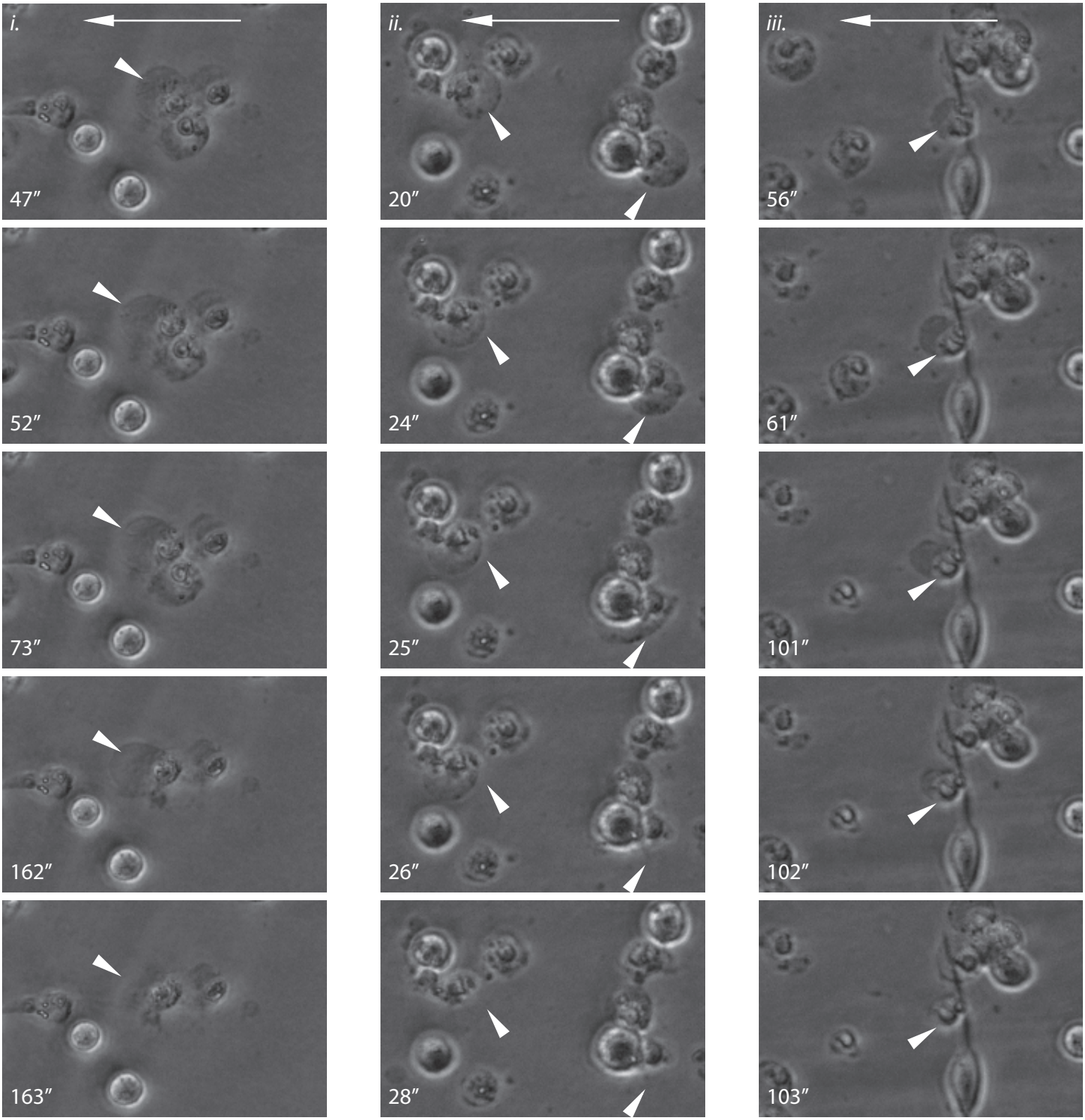
Supplemental Figure 4. Loss of Casp1 or GSDMD delays but does not prevent pyroptotic loss of the cytoskeleton. Wild type, ΔCasp1, or ΔGSDMD THP-1 cells were treated with Nigericin for 0, 1, 2, or 4 hr to induce NLRP3-mediated pyroptosis. Cells were stained for ASC (red) to mark the inflammasome and DAPI to mark the nuclei. Cells were co-stained with α-Tubulin, Phalloidin, Vimentin, or Lamin B1 to mark microtubules, actin filaments, intermediate filaments, and nuclear lamina, respectively. (A) 60x images; scale bar is 10 μm. Arrowheads mark inflammasome. (B) For quantitation of cytoskeletal loss, 5 – 10 random 40x images at each time point were used to score cells for absent, disrupted, or intact cytoskeletal features. Images and graphs are representative of at least two independent experiments. Error bars represent standard deviation.



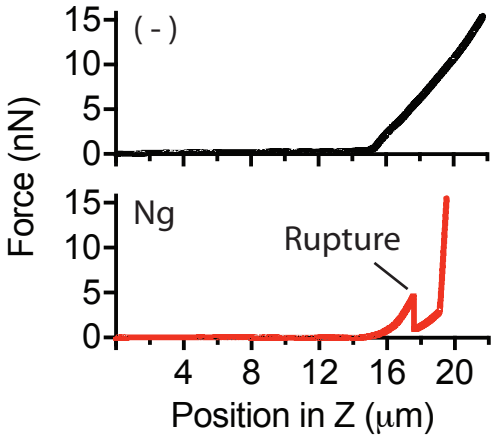
Supplemental Figure 5. Microtubule and/or actin dynamics do not drive cell swelling of Nigericin-treated THP-1 cells. (A-B) THP-1 cells were treated with Ng alone or with Paclitaxel (Pac), Jasplakinolide (Jas), or Paclitaxel plus Jasplakinolide to prevent microtubule and actin filament depolymerization. (A) Cells were co-treated with Sytox-green to visualize permeabilized cells and Sytox-green-positive cells were quantified over time via IncuCyte analysis. Error bars represent standard deviation from triplicate wells of a single experiment. (B) Images from A. Scale bar is 50 μm. (C-D) THP-1 cells were treated and analyzed as in A-B but treated with Ng alone or with Colchicine (Colch), Latrunculin A (LatA), or Colchicine plus Latrunculin A to depolymerize actin filaments and microtubules.



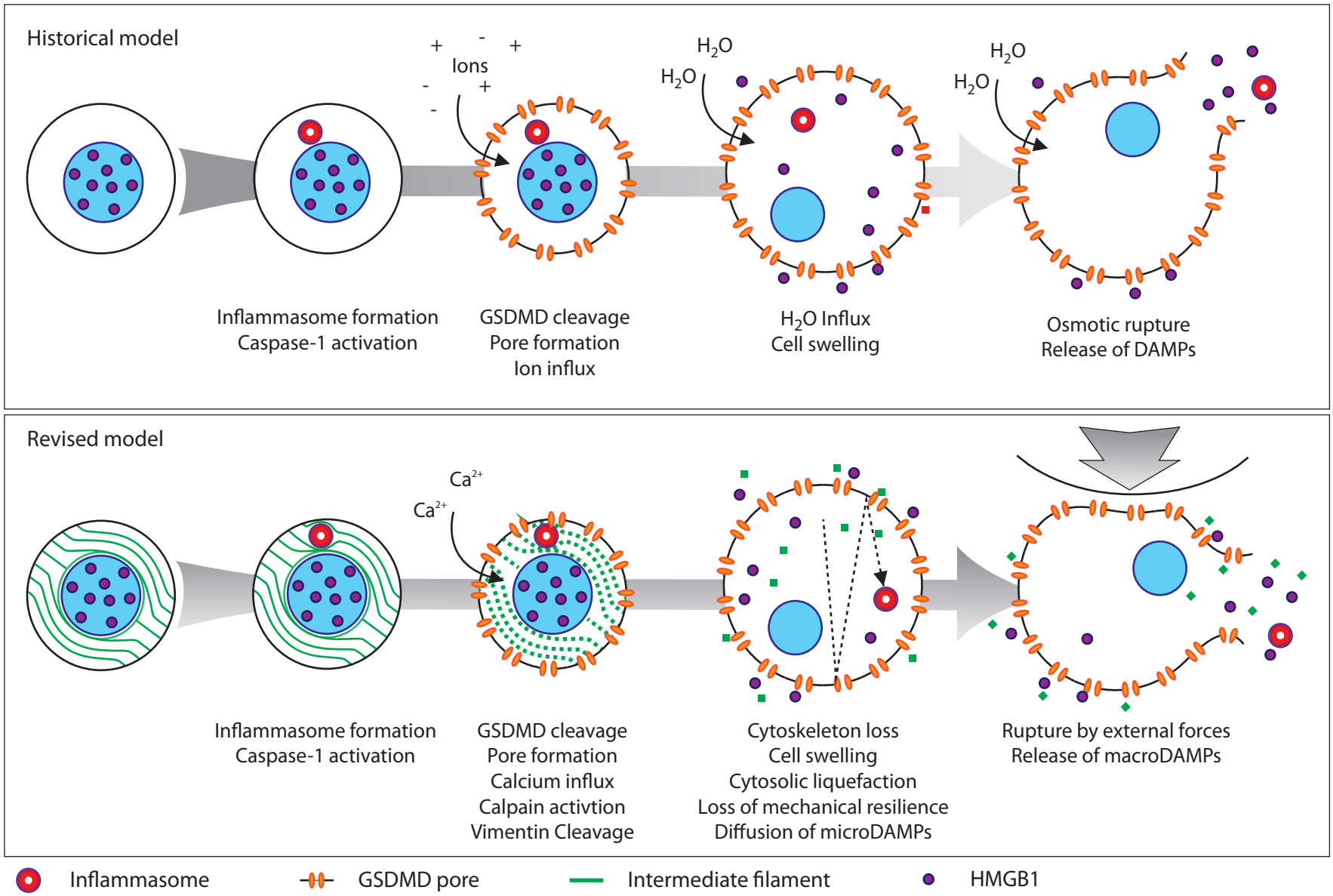
Supplemental Figure 6. Neither calpain inhibition nor EGTA treatment prevent inflammasome formation or pyroptotic permeabilization of Nigericin-treated THP-1 cells. THP-1 cells expressing mCherry-tagged ASC were treated as indicated. Formation of inflammasomes as well as uptake of Sytox were visualized over time at 20x and quantified by IncuCyte analysis. Scale bar is 50 μm.



Supplemental Figure 7. Low shear stress is sufficient to rupture pyroptotic THP-1 cells. Cells were treated with Nigericin for 2 hr. Cells were then placed in a flow cell chamber and subjected to a flow force of 1.5 dynes/cm2 and imaged over time at 1 frame/sec. Images show seconds post start of flow, and arrow shows direction of flow. Arrowheads point to cells ruptured by shear force. Note that in the final frames, the plasma membrane of the marked cells is gone.



Supplemental Figure 8. Pyroptotic cells are ruptured by low compressive forces. Untreated (upper panel) or Nigericin-treated (lower panel) THP-1 cells were subjected to 15 nN of compressive force by atomic force microscopy (AFM). Rupture is visualized by a sudden loss of resistance as indicated in lower panel. Traces are representative of ~30 traces per condition over two independent experiments.



Supplemental Figure 9. (Top panel) Historical model of pyroptotic rupture through osmotic lysis. Inflammasome formation leads to auto-activation of casp1. Casp1 then cleaves GSDMD, which forms pores in the plasma membrane and permeabilizes the cell. Permeabilization allows and influx of ions, increasing cellular osmolarity and driving an influx of water. Water is thought to swell the cell past its breaking point, causing rupture and release of cytosolic content. (Bottom panel) Revised model of pyroptotic rupture. GSDMD-mediated permeabilization permits an influx of calcium that activates calpain. Calpain cleaves vimentin, leading to the disassembly of intermediate filaments. Loss of intermediate filaments uncages cytosolic structures, including the inflammasome, and decreases the cell's mechanical resilience. As a result, the cell becomes susceptible to rupturing forces from shear stress or compression in vivo. While microDAMPs—such as IL-1β and HMGB1—can be released by diffusion through GSDMD pores, release of macroDAMPs—such as inflammasomes, mitochondria, nuclei, and bacteria—requires rupture.

Supplemental Video legends

Supplemental Video 1

Pyroptotic swelling of THP-1 cells without bursting. THP-1 cells were treated with Ng and imaged at 60x every 5 seconds for 4 hrs and then every 1 minute for and additional 8 hrs. Scale bar is 10 μm.

Supplemental Video 2

Osmotic lysis of THP-1 cells. THP-1 cells were treated with water and imaged every 5 seconds at 60x for 30 minutes.

Supplemental Video 3

Cell swelling of THP-1 cells is coincident with uptake of Sytox and loss of mCherry. mCherry-expressing THP-1 cells were treated with Ng and imaged every 30 seconds in the presence of Sytox. Scale bar is 10 μm.

Supplemental Video 4

The ASC speck is highly mobile within pyroptotic cells. ASC.mCherry expressing THP-1 cells were treated with Ng and imaged every 30 seconds for 4 hrs.

Supplemental Video 5

Pyroptotic cells are ruptured by low shear stress. THP-1 cells were treated with Ng for 2 hrs and then subjected to shear stress at 1.5 dynes/cm^2.

Supplemental Video 6

Calpain-inhibition blocks inflammasome mobilization during pyroptosis. ASC.mCherry expressing THP-1 cells were treated with Ng plus MDL28170 and imaged every 30" for 4 hrs.

Supplemental Video 7

Rupture of pyroptotic THP-1 cells under shear stress releases mCherry-tagged inflammasomes. ASC.mCherry expressing THP-1 cells were treated with Ng for 2 hr. Cells were then subjected to shear stress in a flow cell chamber and imaged every 2 seconds.