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Supplementary Materials for

Chemical disruption of the pyroptotic pore-forming protein gasdermin D inhibits inflammatory cell death and sepsis

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at immunology.sciencemag.org/cgi/content/full/3/26/eaat2738/DC1)

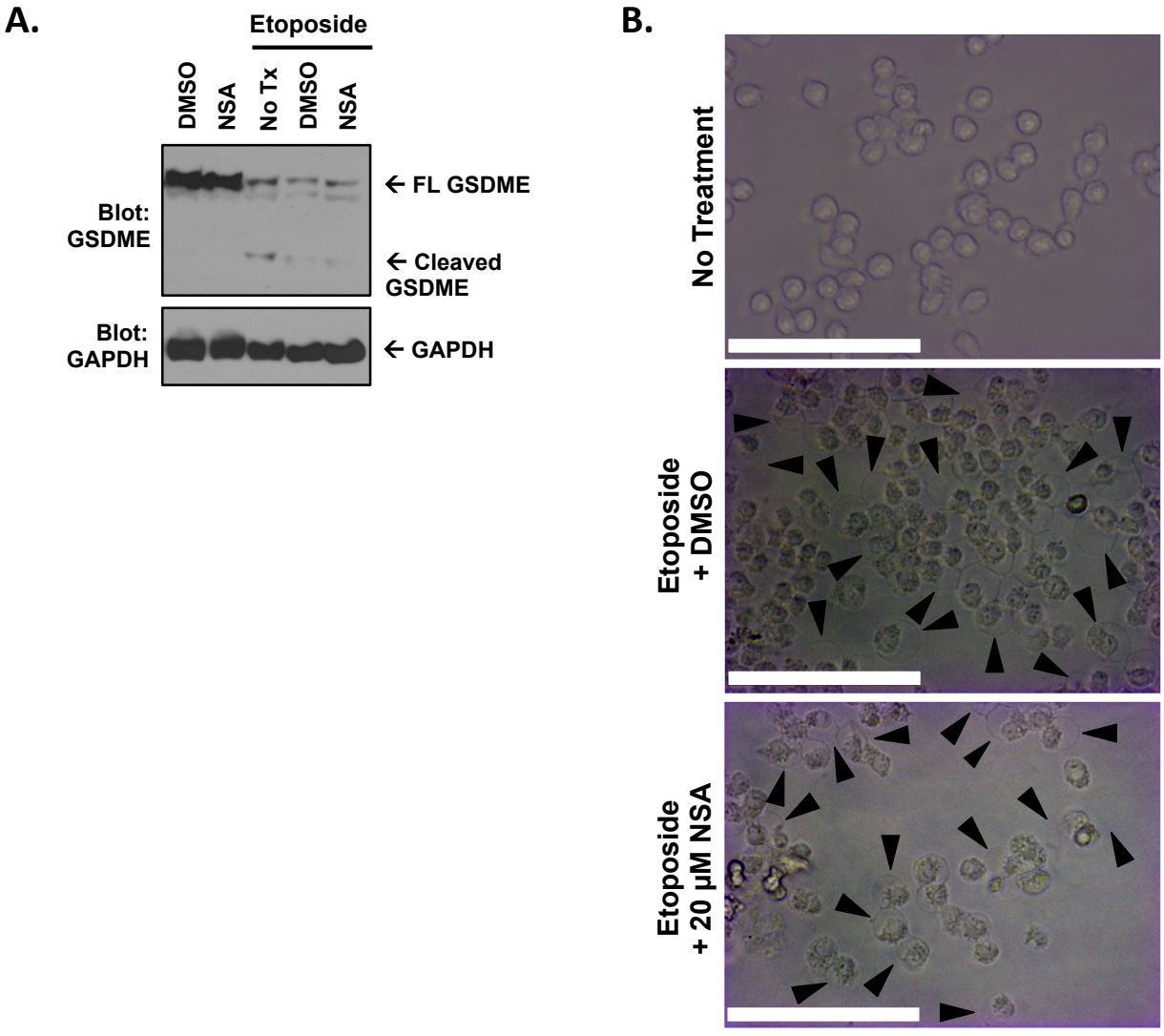

Fig. S1. Etoposide-induced cell death in macrophages is independent of NSA. (A) Cleavage assay of GSDME in murine macrophages treated with or without 100 μM etoposide for 8 hours alongside either DMSO or 20 μM NSA. (B) Bright-field microscopy of macrophages treated for 8 hours with etoposide in the presence of DMSO or NSA. Scale bar equals 100 μm.

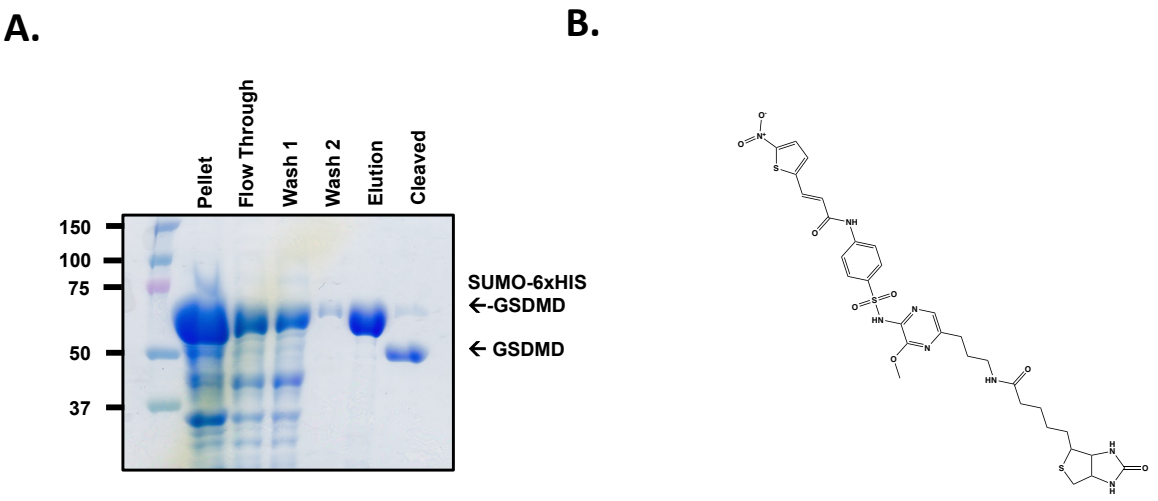

Fig. S2. Purification of human GSDMD. (A) Recombinant human GSDMD was purified using 6xHis-SUMO-GSDMD expressed in *Escherichia coli*. Protein was purified using a nickel column and verified with Coomassie Brilliant Blue staining**.** (B) The chemical structure of necrosulfonamide-biotin.

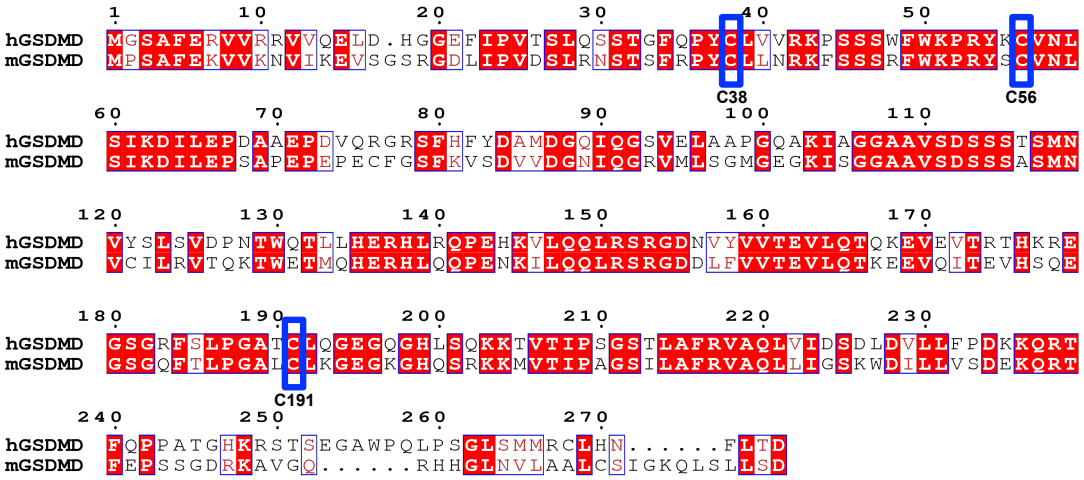

Fig. S3. Conserved cysteine residues between human and murine GSDMD in the p30-GSDMD fragment. Alignment of human and murine GSDMD was conducted using Clustal Omega and ESPript3.0.

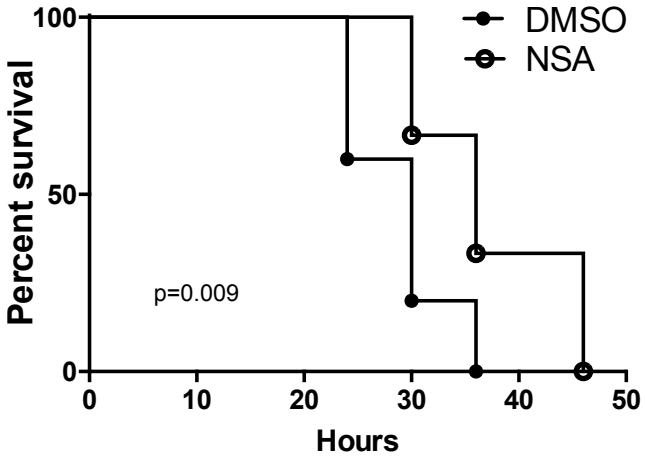

Fig. S4. Single-dose NSA prolongs survival in the LPS model of sepsis. C57BL/6J mice (n=10 in the DMSO treatment group and n=9 in the NSA treatment group) were injected with 25 mg kg^{-1} of LPS together with 20 mg kg^{-1} NSA or DMSO. Total volume of injected NSA or DSMO was approximately 25 µL per mouse. Survival curves were analyzed by Log-rank (Mantel-Cox) test demonstrating a median survival increase of 6 hours in the NSA treated group (p=0.009).

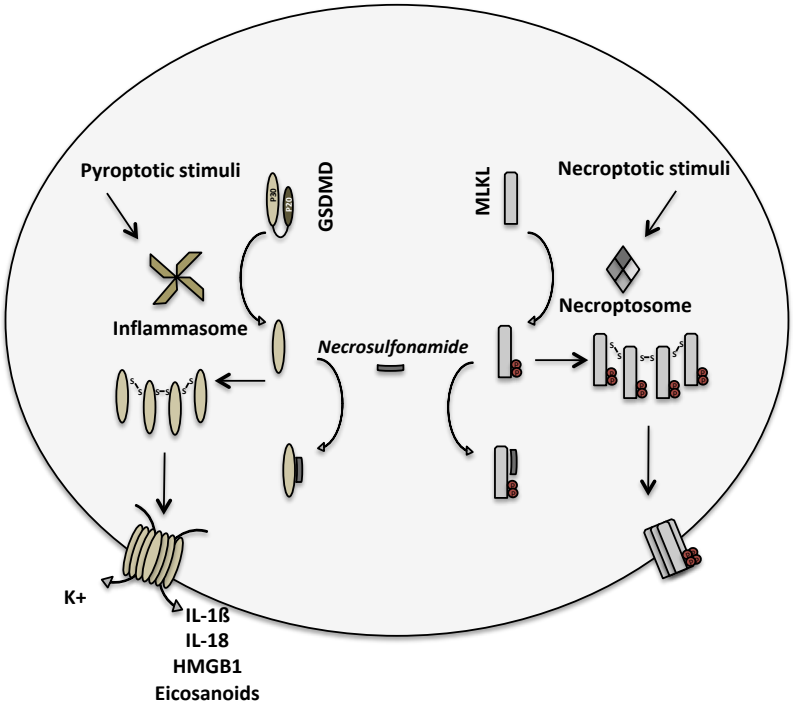

Fig. S5. NSA inhibits p30-GSDMD pore formation and cell death. Overview of NSA inhibition of MLKL and GSDMD leading to loss of pyroptosis and necroptosis. NSA disrupts oligomerization of GSDMD or MLKL pore forming units leading to loss of pyroptotic and necroptotic cell death.