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Supplemental Data

Microbial Pathogen-Induced Necrotic Cell Death

Mediated by the Inflammasome Components

CIAS1/Cryopyin/NLRP3 and ASC

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Figure S1 - A) Adenoviral constructs promote similar expression of FLAG-tagged *CIAS1* variant and wildtype proteins in the 293T cell line as revealed by immunoblotting. B and C) Increased cell-permeability to two viability dyes indicates that disease-associated cryopyrin reduced cell viability. Cell-permeability following 48 hours of staurosporine treatment or infection with the indicated construct was determined by (B) trypan blue and (C) flow cytometry performed on Viaprobe stained cells.

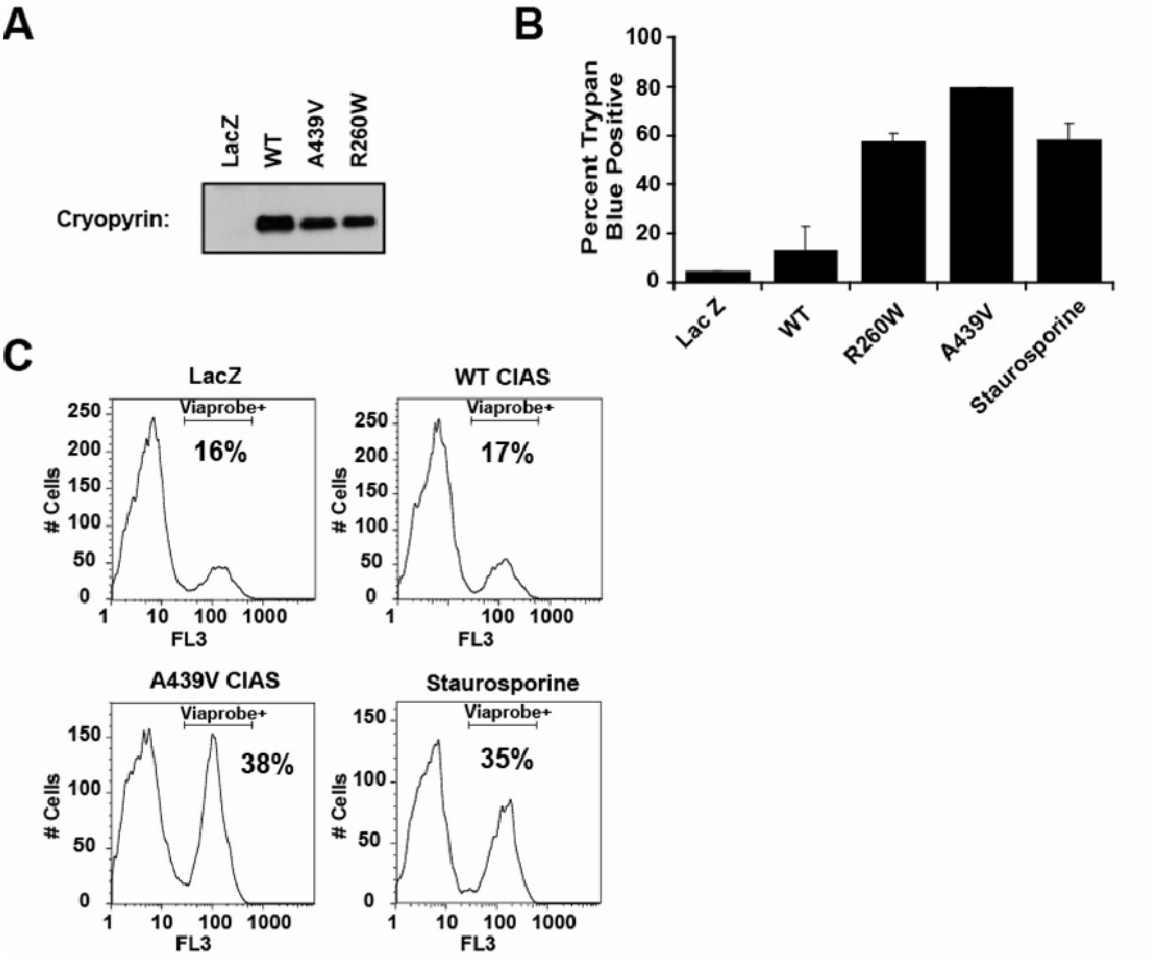

Figure S2 - A) Staurosporine-induced cell death causes DNA fragmentation that is sensitive to a pan-caspase inhibitor. One hour pretreatment with 100 μM zVAD-fmk blocks DNA fragmentation induced by 1 μM staurosporine. B) Cells expressing disease-associated cryopyrin do not undergo a mitochondrial membrane permeability transition. Cells were treated as indicated for 4 or 8 hours before staining with TMRE to measure membrane permeability. Results are presented along two axes, forward scatter (*y* axis) and TMRE (*x* axis). Healthy cells are positive along both axes (Gate A, see first panel). Cells which have lost both forward scatter and membrane potential are considered dead (Gate C). Cells which maintain forward scatter while losing membrane potential are undergoing a transition state (Gate B). Only cells treated with the pro-apoptotic agents etoposide and staurosporine show an accumulation in Gate B. However, cells expressing mutant cryopyrin or treated with pro-apoptotic agents show an accumulation in gate C, indicating that all three treatments result in cell death. C) HMGB1 is not released from THP1 at the 2 and 6 hr time points after treatment with 1 μM staurosporine, but it is released upon prolonged (24 hr) treatment, likely after secondary necrosis reported in the literature.

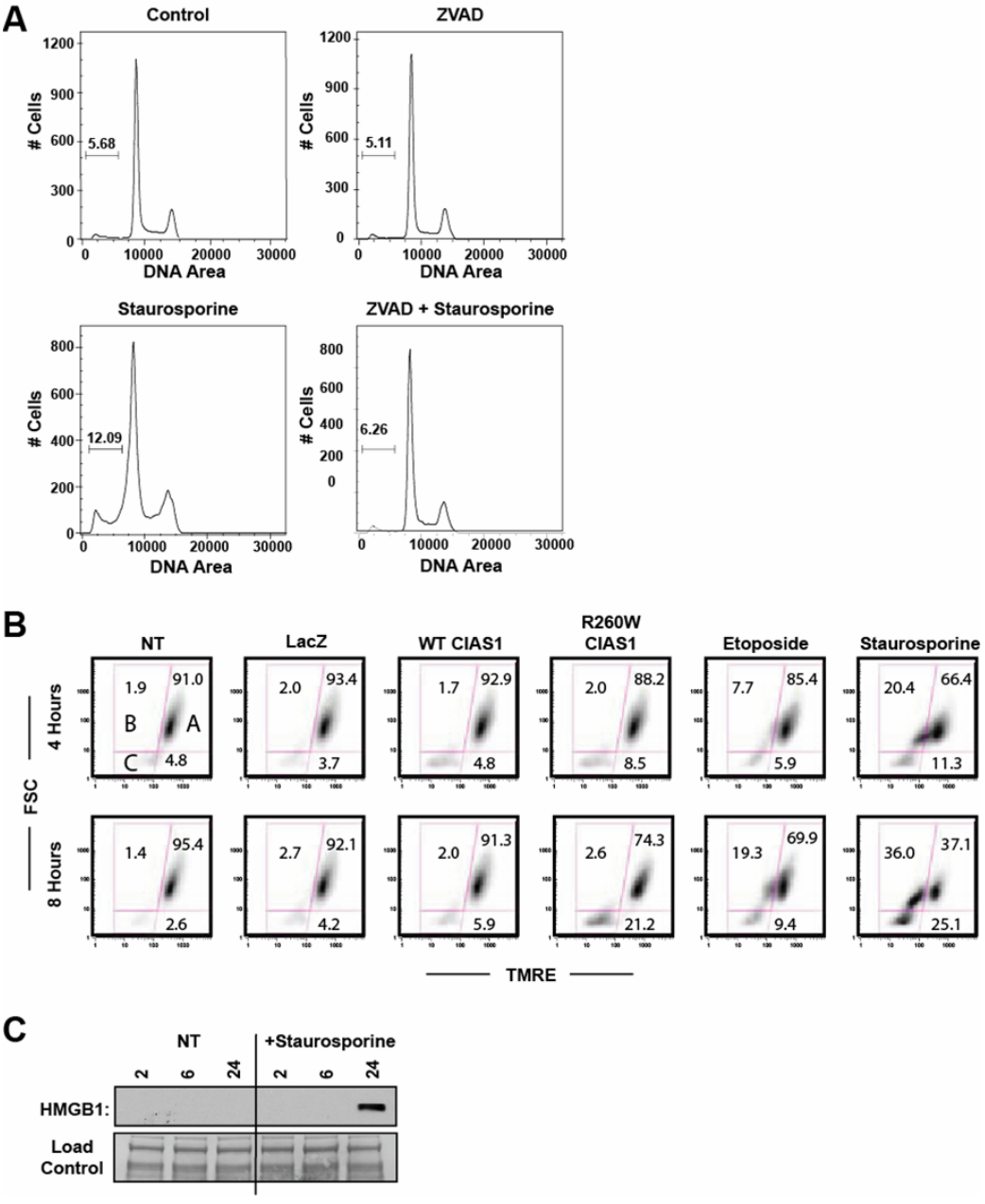

Figure S3 – Salmonella induced cell death does not require *CIAS1*. Bone marrow derived macrophages were infected with *Salmonella typhi* at a MOI of 50 for 2 hours. Cell death was determined by flow cytometry performed on 7-aad stained cells.

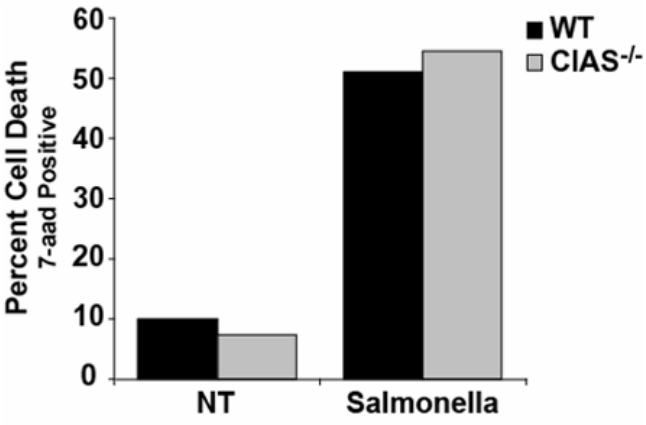

Figure S4 - Glycine fails to abrogate *S.flexneri* initiated cell death or Il-1β release.

A) Glycine did not reduce cryopyrin mediated cell death. THP-1 cells were pretreated with 10mM glycine for 1 hour before infection with *S. flexneri* at a MOI of 50 for the indicated time period.

B) Glycine does not affect *Shigella* induced IL-1β at the indicated time period.