Supplemental Inventory

Figure S1 addresses the reviewer's concern about whether or not access to the cytosol is required for induction of pyroptosis. It is referenced with regard to figures 1 and 3. Figure S2 addresses multiple reviewer concerns about an alternative mechanism to show pyroptosis and it is referenced with regard to figure 1. Figure S3 addresses the reviewers question shows the controls that our cells respond properly to previously reported stimuli and is referenced in regard to figure 1. Figure S4 shows that AIM2 is involved in activating caspase-1 and that *L. monocytogenes* DNA is capable of stimulating pyroptosis, both in response to a reviewers' questions. It is referenced pertaining to figure 4.

Figure S1

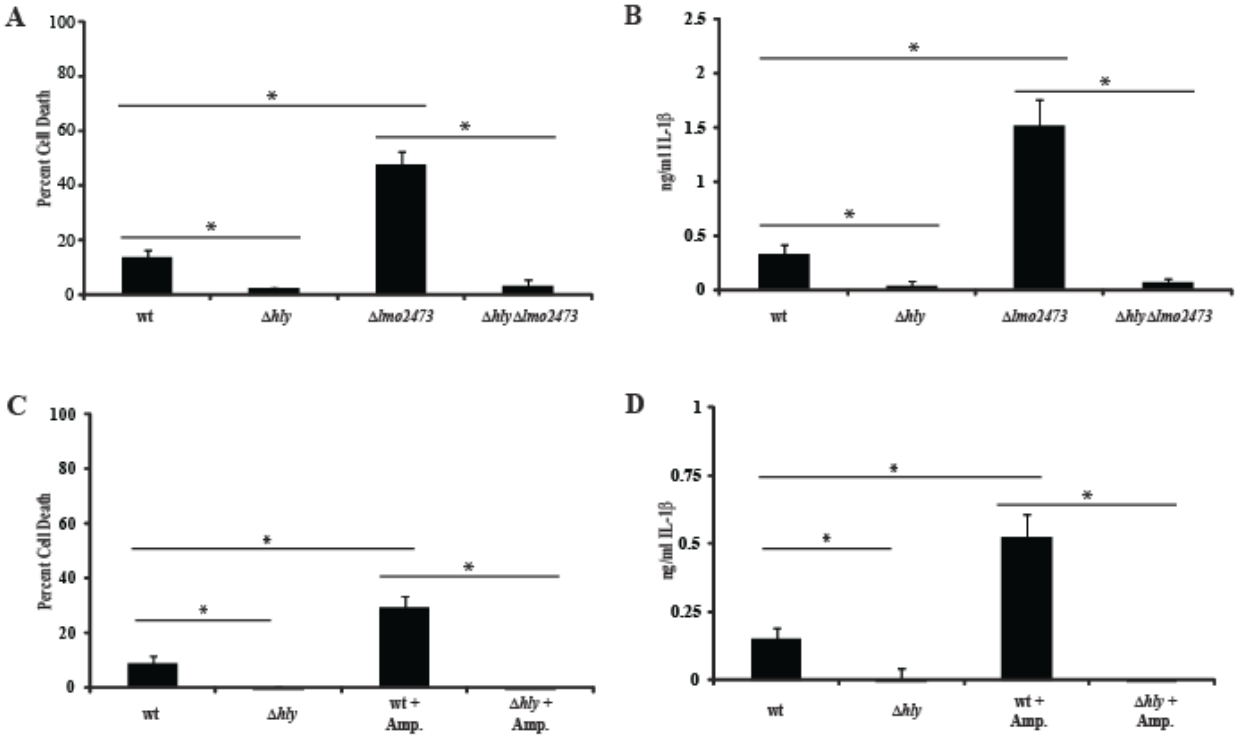


Figure S1

Access to the cytosol is required for induction of pyroptosis. Cell death (A, C) and IL-1β (B, D) were measured following 6 hour infection, at an MOI of 5, of wild type bone marrow-derived macrophages with indicated strains. Antibiotics were added, where indicated, 2 hours post infection for the duration of the infection. *indicates these values are statistically different with a *p* value <0.05 by the Students' t-test.

Figure S2

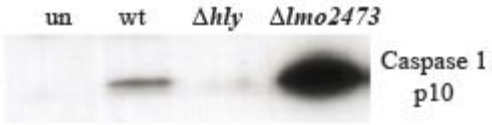


Figure S2

Caspase-1 processing in *L. monocytogenes* infection. Wild type bone marrow-derived macrophages were infected for 6 hours, at an MOI of 5, with the indicated strains at which time proteins were precipitated from macrophage supernatants. Samples were Western blotted then probed for the active p10 fragment of caspase-1.

Figure S3

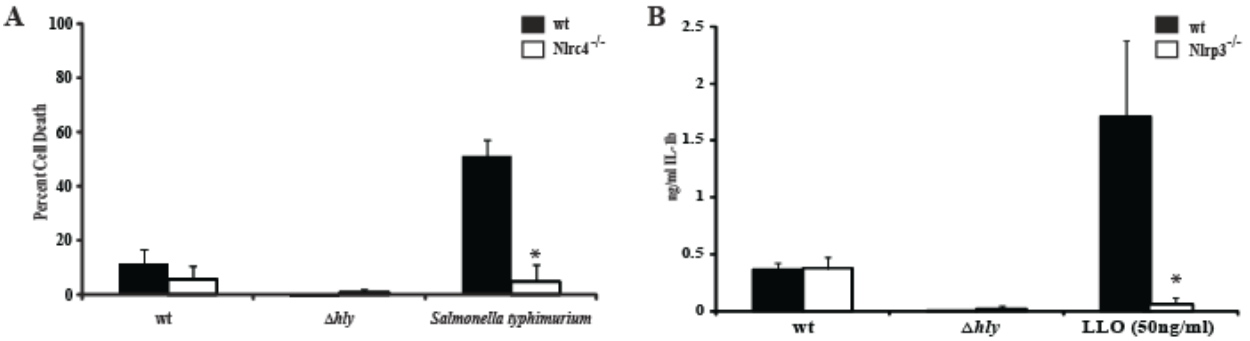


Figure S3

Macrophages deficient in Nlrc4 or Nlrp3 do not respond to *Salmonella typhimurium* and purified LLO, respectively. Cell death (A) was measured following 6 hour infection of wild type or Nlrc4$^{-/-}$ bone marrow-derived macrophages with the indicated strains, each at an MOI of 5. (B) IL-1β was measured following either 6 hour infection with the indicated strains at an MOI of 5 or 6 hour treatment with 50 ng/mL purified Listeriolysin O. *indicates these values are statistically different with a *p* value <0.05 by the Students' t-test.

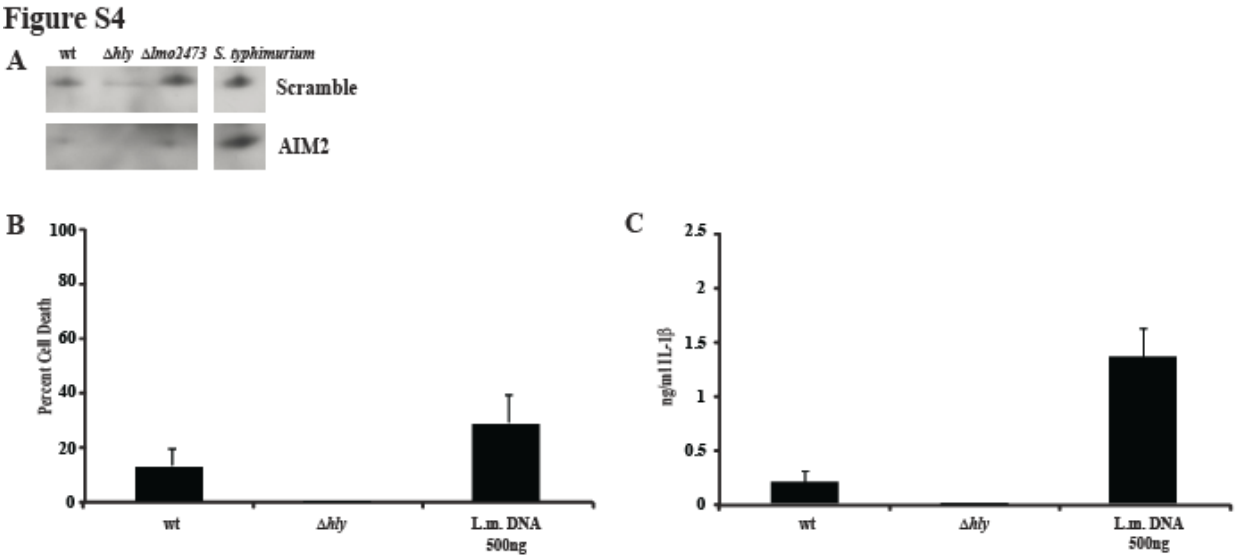


Figure S4

AIM2 activates caspase-1 in response to *L. monocytogenes* infection or purified DNA. Immortalized bone marrow-derived macrophages were transduced with either scramble shRNA (top) or AIM2 shRNA (bottom) and and infected for 6 hours at an MOI of 5. Supernatants were collected and analyzed for the presence of the active p10 subunit of caspase-1 by Western blotting. Cell death (B) and IL-1β (C) were measured following 6 hour infection with the indicated strains or 6 hour treatment with 500 ng bacterial genomic DNA. *indicates these values are statistically different with a *p* value <0.05 by the Students' t-test.

Supplemental Methods

Caspase-1 Western blot analysis

Macrophages were pretreated for 12-16 hours with 100 ng/ml Pam3CSK4 (Invivogen, San Diego, CA) prior to infection. $1x10^6$ bone marrow-derived or immortalized bone marrow-derived macrophages were infected at a multiplicity of infection of 5 bacteria per cell in 6-well plates for 30 minutes. At 30 minutes post infection media was remove and replaced with media containing 50 □g/ml gentamicin and 100 ng/ml Pam3CSK4. Six hours post infection, supernatants were collected and precipitated with 10% Trichloroacetic acid (EMD Biosciences, La Jolla, CA). Samples were separated by SDS-PAGE (Invitrogen, Grand Island, CA) and transferred to polyvinylidene membrane (Millipore, Billerica, MA). Membranes were probed for caspase-1 (Santa Cruz Biotechnology, Santa Cruz, CA) followed by HRP-conjugated secondary antibody (GE Healthcare, Buckinghamshire, UK).

Listeriolysin O inflammasome activation

Listeriolysin O protein was purified as in Zemansky et al. Macrophages were pretreated for 12-16 hours with 100 ng/ml Pam3CSK4 (Invivogen, San Diego, CA) prior to infection. $5x10^5$ bone

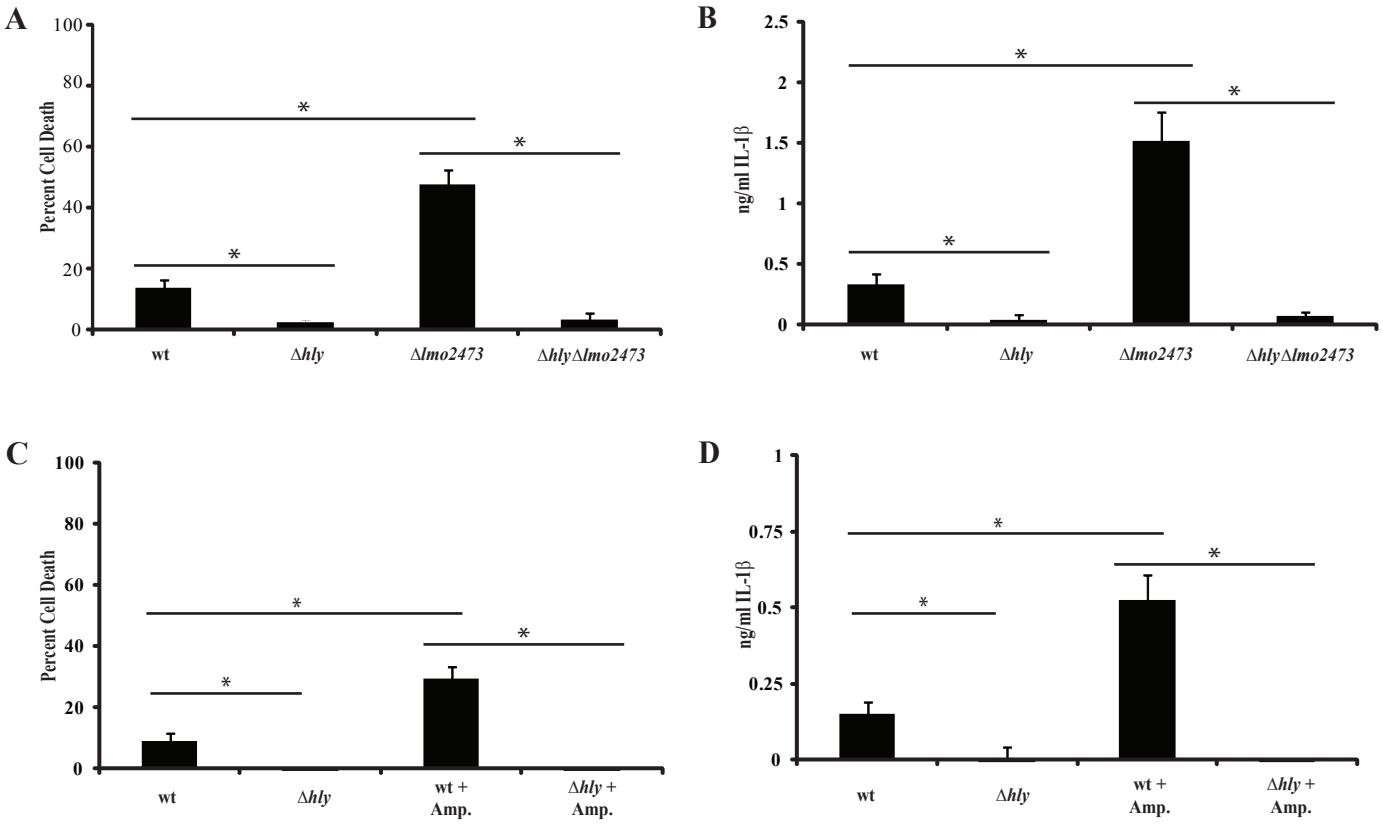
marrow-derived macrophages per well in 24-well plates were treated with 50 ng/ml purified Listeriolysin O for 6 hours. Supernatants were assayed for IL-1β secretion by ELISA.

Preparation of *L. monocytogenes* genomic DNA

L. monocytogenes genomic DNA was isolated using the MasterPure Gram Positive DNA Purification Kit (Epicentre Biotechnologies, Madison, WI) then digested to completion with EcoRI and BamHI (New England Biolabs, Ipswich, MA) and purified using the PCR cleanup kit (Qiagen, Valencia, CA). $5x10^5$ bone marrow-derived macrophages per well in 24-well plates were transfected with Lipofectamine2000 (Invitrogen) and 500 ng/ml prepared genomic DNA.

Supplemental Text and Figures

Figure S1



Supplemental Text and Figures

Figure S2

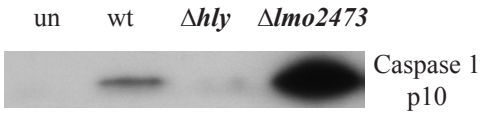
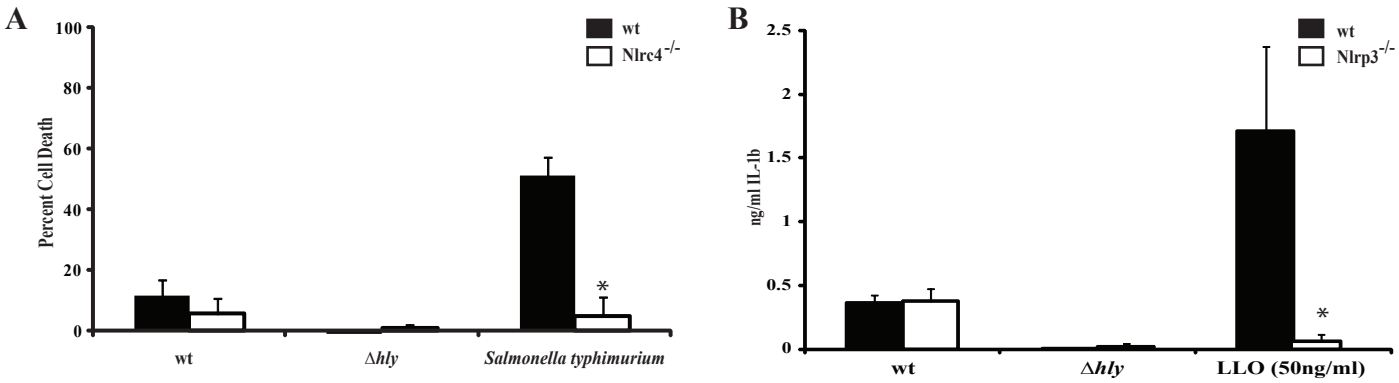


Figure S3


A
100
80
60
40
20
0
Percent Cell Death
wt
Nlrc4-/-
*
wt
Δhly
Salmonella typhimurium
B
2.5
2
1.5
1
0.5
0
ng/ml IL-1b
wt
Nlrp3-/-
*
wt
Δhly
LLO (50ng/ml)

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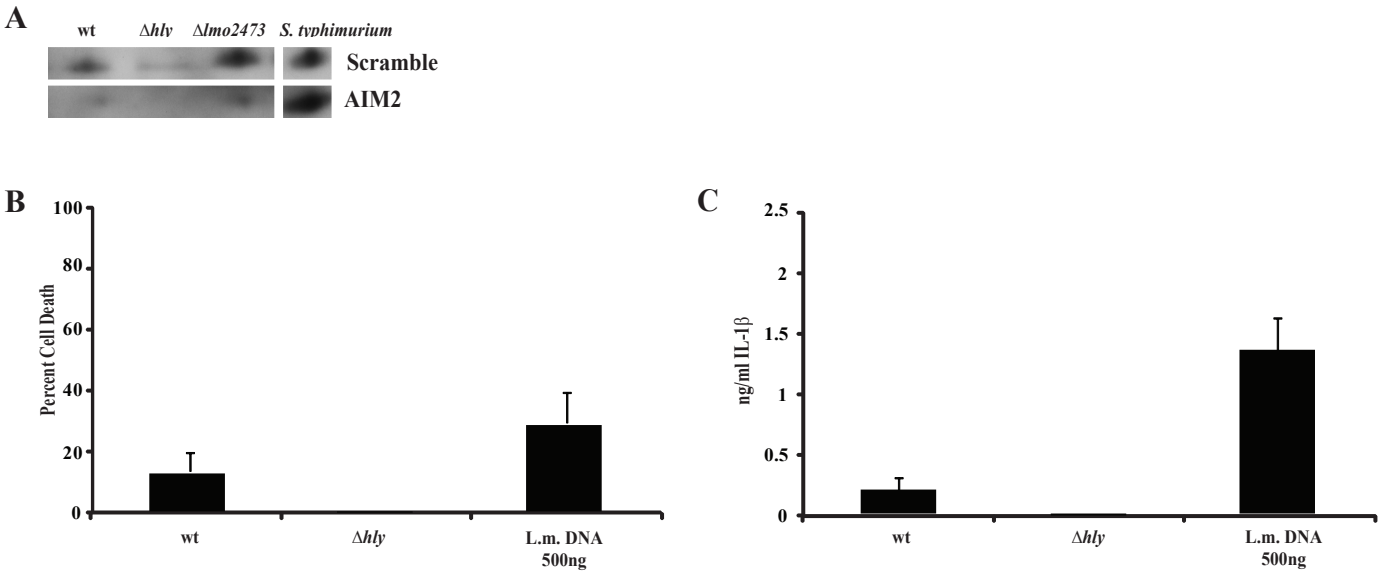

Supplemental Figure Legends

Supplemental Figure 1

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