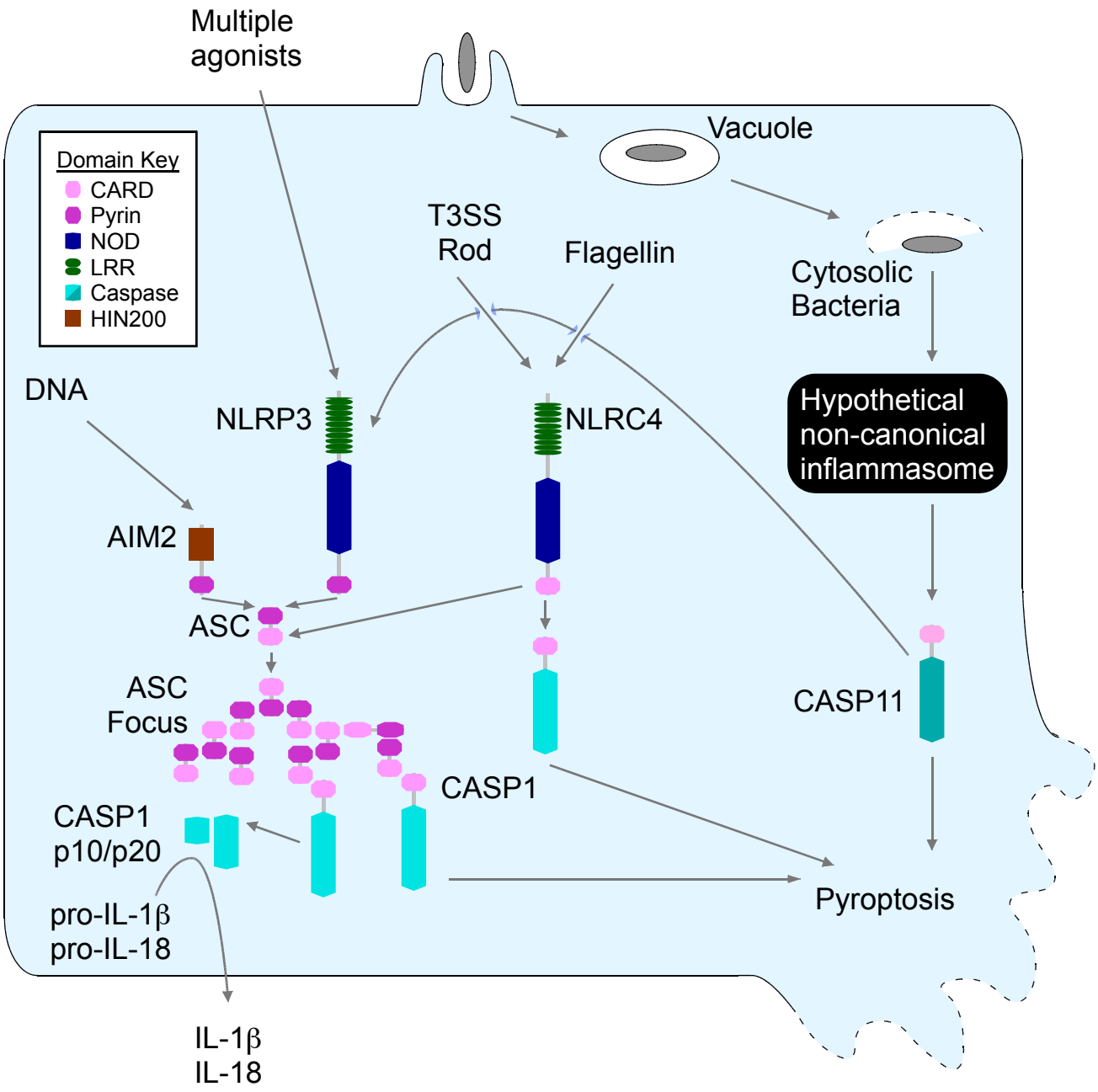

Figure S1. Schematic of inflammasome detection pathways. Canonical inflammasomes including NLRP3, AIM2, and NLRC4 activate caspase-1. NLRC4 contains a CARD domain that can bind to the CARD of caspase-1 directly through homotypic interaction, triggering pyroptosis. The NLRC4 also binds ASC through CARD homotypic interactions, resulting in recruitment of the entire complement of cellular ASC into a single ASC focus. The Pyrin domain of NLRP3 or AIM2 cannot bind directly to caspase-1, but triggers formation of the ASC focus via Pyrin-Pyrin homotypic interactions. The ASC focus recruits and activates caspase-1, resulting in its proteolytic maturation to the p10 and p20 fragments, and subsequent IL-1 and IL-18 cleavage and secretion. Therefore, cells that are deficient in both *Nlrc4* and *Asc* cannot signal through any known canonical inflammasome. The activating platform for caspase-11 remains unknown; nevertheless, the hypothetical activator was named the non-canonical inflammasome. Our data indicate that cytosolic bacteria are detected through this hypothetical non-canonical inflammasome, resulting in caspase-11-dependent pyroptosis. Caspase-11 activation also triggers NLRP3 activation via an unknown mechanism (denoted by an arrow through tunnels).

Figure S2

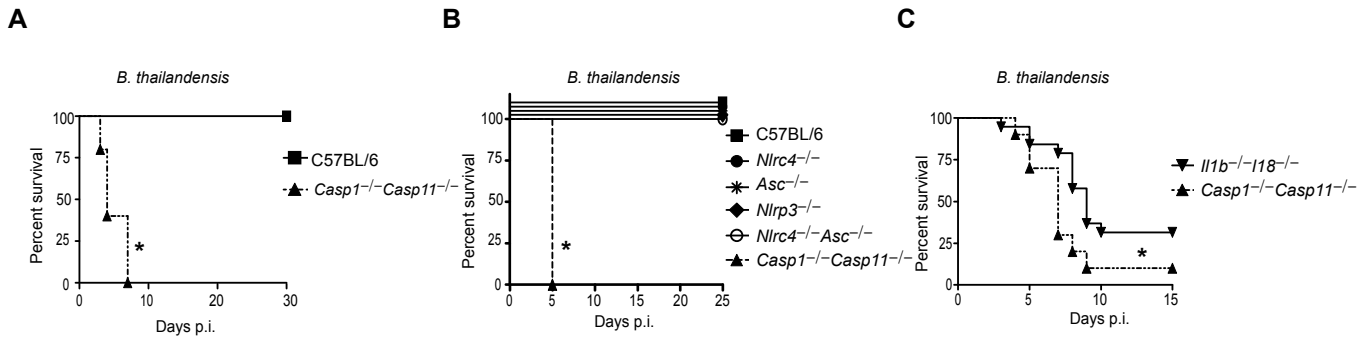

Fig. S2. Burkholderia protection conferred by Casp1/11 is independent of all known canonical inflammasomes (**A**-**C**) Wild type C57BL/6 or the indicated knockout mice were infected i.n. with *B. thailandensis* and survival was monitored. Data are representative of 4 (**A**), 1 (**B**) or pooled from 2 (**C**) experiments. For number of mice in each panel see Table S2. Statistically significant differences with respect to controls are indicated (log rank test for survival; * = $p \leq 0.05$).

Figure S3

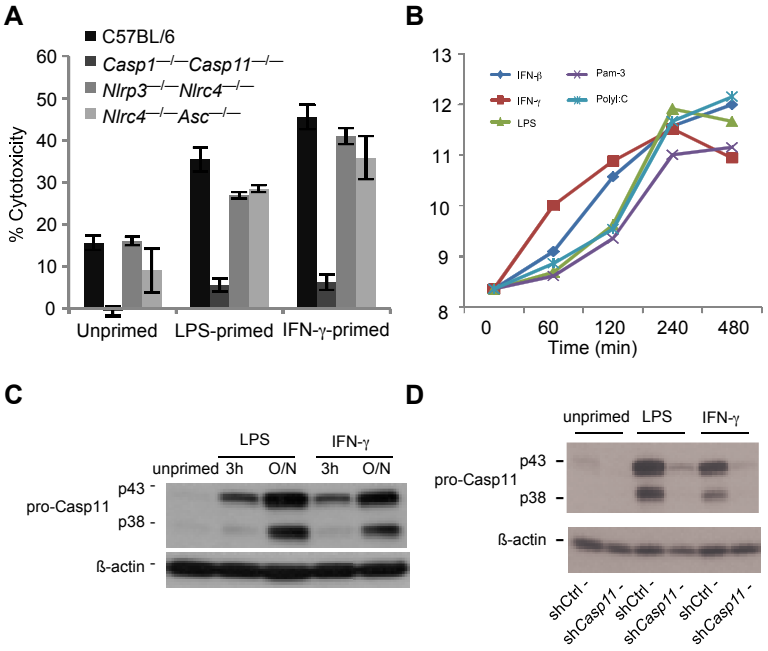

Fig. S3. TLR ligands and IFN-γ enhance *Casp11* expression and Caspase-11-dependent cell death. (**A**) Untreated, LPS-primed, or IFN-γ-primed BMMs were infected with *S. typhimurium* Δ*sifA* and cytotoxicity was determined. (**B**) Transcriptional upregulation of *Casp11* in C57BL/6 BMMs after priming with the indicated molecules was determined using Affymetrix GeneChip technology. (**C**) Caspase-11 expression in untreated, LPS-primed, and IFN-γ-primed C57BL/6 BMMs was determined by immunoblot. Blots were stripped and β-actin expression was determined as a loading control. (**D**) Caspase-11 expression in untreated, LPS-primed, and IFN-γ-primed control or *Casp11* shRNA-expressing *Nlrc4-Asc* iBMMs was determined by immunoblot. Loading controls were performed as in (**C**). Results are representative of more than 3 (**A**, **B**), 2 (**C**), or 1 (**D**) experiments. Statistically significant differences with respect to controls are indicated (Student's T-test; * = $p < 0.05$).

Figure S4

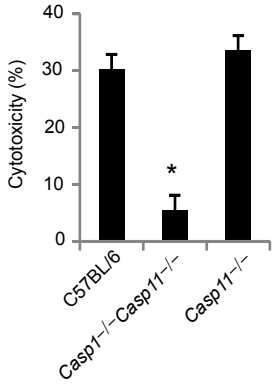

Fig. S4. Caspase-11 is not required for pyroptosis induced by flagellin expressing wild type *L. pneumophila*. Wild type *L. pneumophila* inadvertently translocate flagellin into the macrophage cytosol, resulting in detection through NLRC4, which activates Caspase-1. We investigated whether this response was altered in the absence of Caspase-11. C57BL/6, *Casp*1−/−*Casp*11−/−, and *Casp*11−/−BMM infected with *L. pneumophila* at an MOI of 1 and cytotoxicity was determined 4 hours later; C57BL/6 and *Casp*11−/− BMMs showed similar cytotoxicity, indicating that Caspase-11 is not required for NLRC4-induced pyroptosis. Data are representative of at least 3 independent experiments. Statistically significant differences with respect to controls are indicated (Student's T-test; * = $p \leq 0.05$).

Figure S5

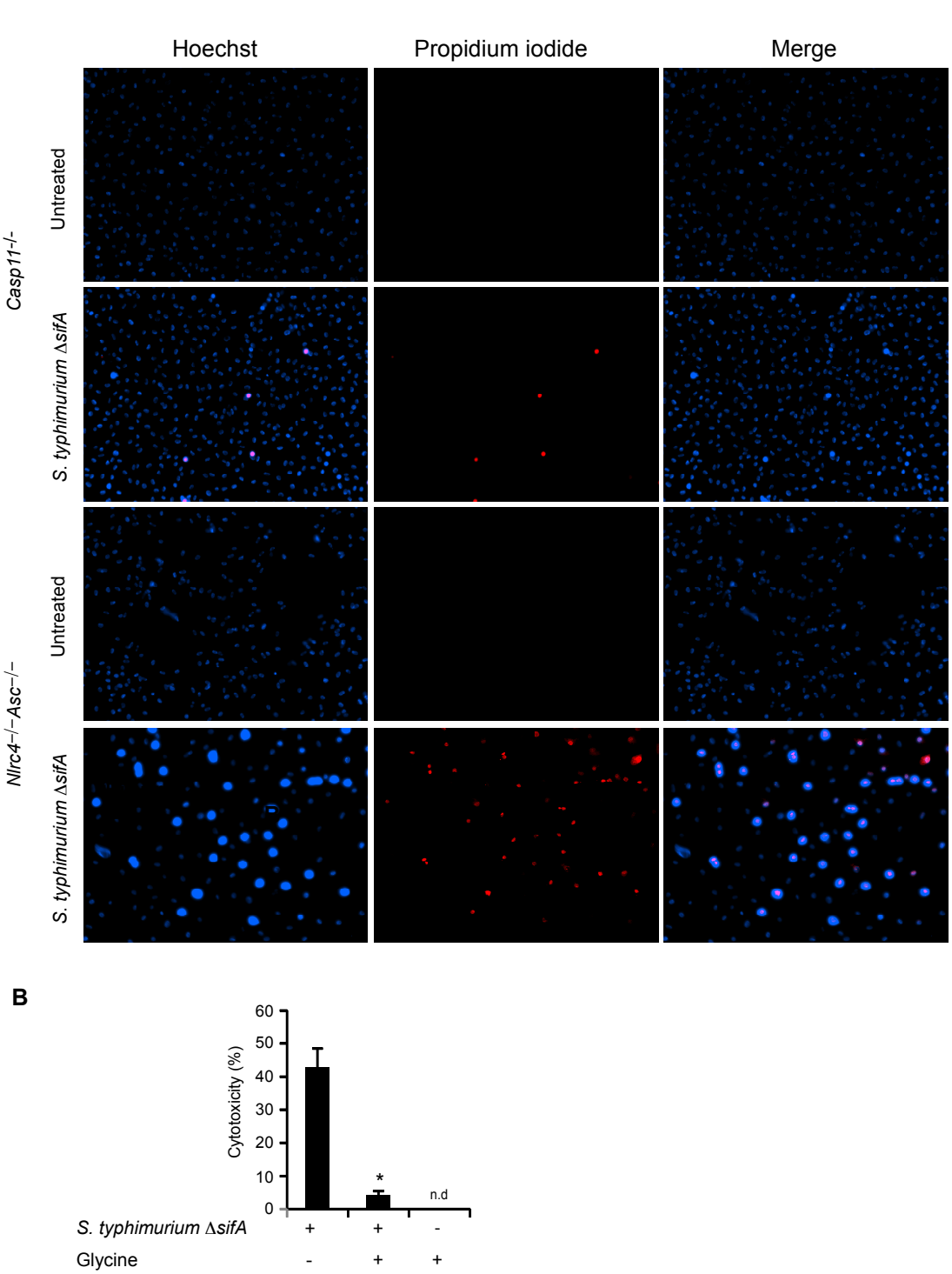

Fig. 5S. Morphology of *S. typhimurium* Δ*sifA*-induced pyroptosis. IFN-γ-primed *Nlrc*4$^{-/-}$ *Asc*$^{-/-}$ or *Casp11*$^{-/-}$ BMMs were infected for 8h with *S. typhimurium* Δ*sifA* (MOI 50) (**A**) Representative fluorescence microscopy images of BMM stained with membrane permeant Hoechst and membrane impermeant propidium iodide (PI) 8 hours post infection as a measure of cell death in addition to LDH release. Although Hoechst is a membrane permeant dye, its staining intensity significantly increased in pyroptotic cell due afer membrane rupture; in order to visualize both intact and pyroptotic cells the image is over-exposed for lysed cells, making their nucleus appear larger in the Hoechst channel. (**B**) Caspase-1-dependent pyroptotic cell death is known to be inhibited by addition of glycine to the media. In order to determine if Caspase-11-dependent cell death was occurring through a morphologically similar pathway, we added 20mM glycine at 4h post *S. typhimurium* Δ*sifA* infection. LDH release was determined 4h later (total of 8h infection).