SUPPLEMENTAL INFORMATION

Figure S1. Caspase-11 is cleaved after Gram-positive bacteria infection, Related to Figure 1. (A−F, H, I) Primary BMDMs or (G) THP-1 cells were left uninfected or infected with (A−G) *Listeria*, (B and C) *Salmonella* or (H and I) *Staphylococcus aureus* for 12 h or indicated hours. (A, B, F, H) The cell lysates (Lysate) and supernatants (Sup) were subjected to immunoblotting. (C−E, G) The supernatants were subjected to LDH assay or ELISA. (I) The cell lysates were subjected to caspase substrate cleavage assay. Blots of caspase-11 were cropped to reveal protein bands at different exposures. Results are representative of at least (B−F) three or (A, G−I) two independent experiments, and error bars denote s.d. of triplicate wells. $*P < 0.05$, $****P < 0.0001$.

Figure S2. Caspase-11 does not affect cleavage of caspase-8 or -3 substrate and IL-1β secretion in response to nigericin, poly(dA:dT), *Francisella* and *Salmonella*, Related to Figure 1 and 2. (A, B, E, I, M) Primary BMDMs were infected with *Listeria* or *Francisella* for 12 h or indicated times. (A and B) Poly(I:C)-primed BMDMs were transfected with LTA for 4 h. (C, D, F−H, J−L, N) LPS-primed BMDMs were incubated with indicated stimuli for indicated times. (A, B, G−J) The cell lysates were subjected to

caspase substrate cleavage assay. (C−F) The cell lysates (Lysate) and supernatants (Sup) were subjected to immunoblotting. (K−N) The supernatants were subjected to ELISA. Blots of caspase-11 were cropped to reveal protein bands at different exposures. Results are representative of (A and B) three or (C−N) at least two independent experiments, and error bars denote s.d. of triplicate wells. NS, no significant difference.

Figure S3. Cytosolic LTA induces caspase-11 cleavage and IL-1β maturation in a NLRP6-dependent manner, Related to Figure 2. (A, B, D−J) Poly(I:C)-primed BMDMs were transfected with indicated ligands for 4 h or indicated time. (A, D−G, I) The cell lysates (lysate) were subjected to immunoblotting or caspase substrate cleavage assay. (B, H, J) The supernatants were subjected to LDH assay or ELISA. Blots of caspase-11 were cropped to reveal protein bands at different exposures. (C) Schematic representation of sLTA and sLTA ΔGPR structure. Results are representative of at least (A, D−H) three or (B, I, J) two independent experiments, and error bars denote s.d. of triplicate wells. sLTA, synthetic LTA; GPR, glycerophosphate repeat. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Figure S4. LTA interacts with NLRP6 ΔPYD, Related to Figure 3. (A) S-tagged indicated molecules were expressed in HEK293T cells. Cell lysates were incubated with LTA, immunoprecipitated (IP) and analyzed by immunoblotting (IB). (B) Purified protein was subjected to SDS-PAGE followed by silver staining and immunoblotting. (C−G) BLI analysis of the interaction between indicated ligands and chip-immobilized purified S-NLRP6 ΔPYD, human NOD1, or rat IgG in PBS (D) with or (C, E−G) without 0.001% Tween 20. (H) An amino acid alignment analysis of PYDs. (I) Immortalized *Nlrp6*$^{-/-}$ BMDMs were reconstituted with WT NLRP6 or chimeric NLRP6 with NLRP3 PYD. After priming with poly(I:C), the cells were transfected with LTA for 2.5 h. (J and K) Immortalized *Nlrp3*$^{-/-}$ BMDMs were reconstituted with chimeric NLRP3 with NLRP6 PYD or NLRP6 with NLRP3 LRR. After priming with LPS, the cells were stimulated with nigericin for indicated times. The cell lysates (Lysate) were subjected to immunoblotting. Blots of caspase-11 were cropped to reveal protein bands at different exposures. (L) LPS-primed macrophages were transfected with poly(dA:dT) for 2 h. Results are representative of (A, C, F, G, J−L) three or (B, D, E, I) at least two independent experiments.

Figure S5. Caspase-11 is recruited to NLRP6 inflammasome, Related to Figure 4 and 5. (A) Unstimulated WT and *Pycard*$^{-/-}$ immortalized BMDMs reconstituted with tagged ASC were subjected to immunoblotting. (B) LPS-primed *Casp11*$^{-/-}$ immortalized BMDMs reconstituted with tagged caspase-11 were incubated with indicated stimuli. Poly(I:C)-primed cells were transfected with LPS or LTA. (C and D) *Casp11*$^{-/-}$ immortalized BMDMs reconstituted with tagged caspase-11 and *Nlrp6*^{3xFlag} immortalized BMDMs were infected with *Listeria* for 12 h or transfected with LTA for 4 h. (C) z-VAD-FMK (40 μM) was added 1 h post infection. (B−D) The cells were fixed to stain inflammasome specks (arrowheads). Caspase-1 or ASC, green; caspase-11 (Flag) or NLRP6 (Flag), red; and nuclei, blue. Scale bars, 10 μm. (E) Indicated molecules were expressed in HEK293T cells. The cells were left untransfected or transfected with LTA for 4 h, lysed, and immunoprecipitated. Whole cell lysates are shown as the input. (F) Band intensity of caspase-11 after IP in E was quantified using ImageJ. (G−I) Immortalized *Casp11*$^{-/-}$ BMDMs were reconstituted with WT or mutants of caspase-11. (G, I) Poly(I:C)-primed cells were transfected with LTA for 4h. (G) The supernatants were subjected to LDH assay. (H) The cells were infected with *Listeria* for 12 h. The cell lysates (Lysate) were subjected to immunoblotting. Blots of caspase-11 were cropped to reveal

protein bands at different exposures. Results are representative of at least (A, E−G) three or (B−D, H, I) two independent experiments.

Figure S6. *Casp11*$^{-/-}$ mice are more resistant to WT *Listeria* and *Staphylococcus aureus*, but not to *Listeria* Δ*lmo0927* or *Salmonella*, Related to Figure 7. Mice were infected with 10^4 cfu of (A, G, H, K) *Listeria* EGD, (B) *Listeria* LO28, (C) *Listeria* 10403S, (F) *Salmonella*, (D) 10^6 cfu of *Listeria* Δ*lmo0927* or (I and J) 2×10^8 cfu of *Staphylococcus aureus* intravenously. (E) Mice were infected with 10^4 cfu of *Salmonella* intraperitoneally. The spleens and/or livers were removed on (A) day 2, (E and F) day 3, (B−D, G, H, K) day 4 or (J) 8 h post infection for cfu counting. (I) Mouse survival was monitored for 50 h. Results are (A−F, H, K) representative of at least two independent experiments or (G, I, J) pooled from two independent experiments. NS, no significant difference. **P* < 0.05, ***P* < 0.01.

Figure S7. NLRP6 and caspase-11 are required for IL-18 production in mice infected with WT *Listeria* or *Staphylococcus aureus*, Related to Figure 7. Mice were infected with (A and B) 10^4 or (C) 10^6 cfu of WT *Listeria*, (D) 10^6 cfu of *Listeria* Δ*lmo0927*, (E) 2

$\times 10^8$ cfu of *Staphylococcus aureus* intravenously. The sera were collected on (A and D) day 4, (B and C) day 2, or (E) 8 h post infection and subjected to ELISA. Results are representative of at least (A and E) three or (B−D) two independent experiments, and error bars denote s.d. of each group. NS, no significant difference; ND, not detected. $^{**}P < 0.01$, $^{****}P < 0.0001$.