Supporting Information

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SI Materials and Methods

Cell Death Infections. *Listeria monocytogenes* 10403s and the isogenic *Δhly* mutant were grown overnight in brain heart infusion (BHI) at 30 °C stationary. *Legionella pneumophila* Lp02 were grown in Aces-buffered Yeast Extract Broth (AYE) before infection (1). *L. pneumophila* cultures were subcultured in AYE at 37 °C shaking overnight until bacteria reached stationary phase (OD_{600} ~4.0) before infection.

Strain Construction. For construction of the L.p.FlaA construct, the *flaA* coding sequence was PCR amplified from *L. pneumophila* LP02 genomic DNA with the primers (AAAAGGATCCGCTCAAGTAATCAACACTAATGTG) and (AAAAGCGGCCGCAGAAATCGAAGTGCAGTTGTT) (underlines signify restriction digest sites built into the primers). The 1-490 bp PCR product was digested with BamHI and EagI and cloned downstream of the *actA* promoter and in-frame with the amino terminal 300 bp of the *actA* gene in the site-specific integration vector pPL2. The construct was integrated at the $tRNA^{Arg}$ locus of the *L. monocytogenes* chromosome in 10403s background, as previously described (2).

The Δ*actA*/Δ*inlB* background was previously described (3). For construction of the ovalbumen (OVA)-B8R construct, the immunodominant H-$2K^b$ restricted vaccinia $B8R_{20–27}$ epitope (TSYKFESV) was inserted at the junction of an ActA-OVA fusion construct by oligonucleotide directed PCR as previously described (4). This construct was subcloned using KpnI and SacI into an erythromycin resistant version of pPL2 and integrated on the chromosome at the $tRNA^{Arg}$ locus of the Δ*actA*/Δ*inlB* strain as previously described (2). For strains expressing both OVA/B8R and L.p.FlaA, the L.p.FlaA construct described above was subcloned into pPL1 using KpnI and EagI and integrated into the chromosome at the *comK* locus as previously described (2).

Tetramer Staining. Tetramer staining was performed using K^b-SIINFEKL-phycoerythrin tetramer, a kind gift from Mark Davis (Stanford University, Stanford, CA). Tetramer was added along with surface antibodies and incubated for 1 h at room temperature.

Dendritic Cell Quantification. Mice were infected intravenously with 0.1 LD50 (1×10^7) of either Δ*actA*/*inlB*, Δ*actA*/*inlB L. monocytogenes* - L.p.FlaA or PBS as a control. Six, 24, or 48 h post-infection spleens were harvested and dissociated and red blood cells removed using red blood cell lysing buffer (Sigma). Next, 1.4×10^6 splenocytes were stained and dendritic cells were characterized as $CD11b^+$ (Clone M1/70; eBiosciences) and $CD11c^+$ (Clone N418; eBiosciences). Dead dendritic cells were scored based on 7AAD-positivity (eBiosciences).

1. Molofsky AB, et al. (2006) Cytosolic recognition of flagellin by mouse macrophages restricts Legionella pneumophila infection. *J Exp Med* 203(4):1093–1104.
2. Lauer P, Chow MY, Loessner MJ, Portnoy DA, Calendar R (2002) Construction, characterization, and use of two Listeria monocytogenes site-specific phage integration vectors. *J Bacteriol* 184(15):4177–4186.
3. Brockstedt DG, et al. (2004) Listeria-based cancer vaccines that segregate immunogenicity from toxicity. *Proc Natl Acad Sci USA* 101:13832–13837.
4. Sinnathamby G, et al. (2009) Priming and activation of human ovarian and breast cancer-specific CD8+ T cells by polyvalent Listeria monocytogenes-based vaccines. *Immunother* 32(8):856–869.

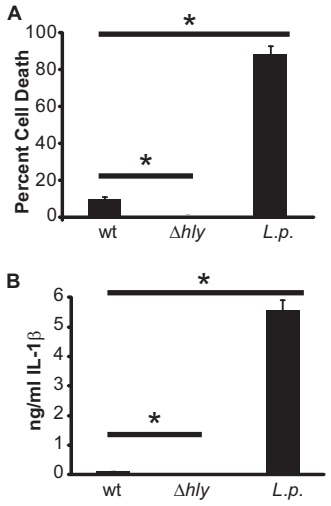

Fig. S1. Inflammasome activation by *L. monocytogenes* and *L. pneumophila.* Cell death (*A*) and IL-1β (*B*) secretion were measured 6 h after infection of bone marrow-derived macrophages with a multiplicity of infection (MOI) of five bacteria per cell. Data are representative of at least three independent experiments. *$P < 0.05$ by Student's *t*-test.

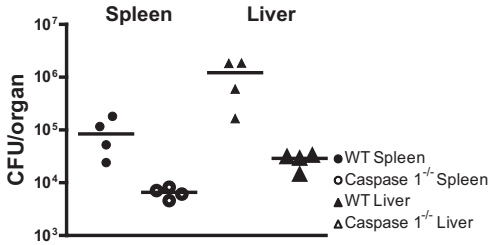

Fig. S2. Caspase-1 is not required for control of *L. monocytogenes* infection. Wild-type (closed symbols) or Caspase-$1^{-/-}$ (open symbols) mice were infected with 1×10^4 wild-type *L. monocytogenes* and CFU per organ were determined 5 d postinfection. Data are representative of at least two independent experiments.

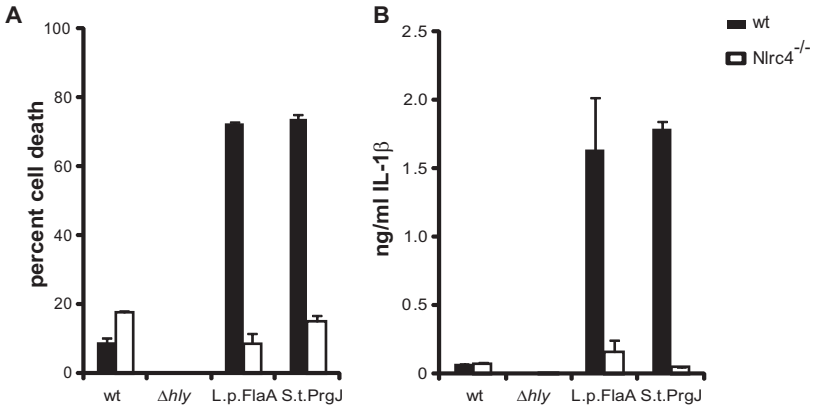

Fig. S3. Induction of host cell death and IL-1β by *L. monocytogenes* strains that ectopically secrete type III secretion rod protein PrgJ. Cell death (*A*) and IL-1β (*B*) secretion were measured following 6 h of infection at an MOI of 5 in wild-type or Nlrc4$^{-/-}$ bone marrow-derived macrophages. Data are representative of at least three independent experiments.

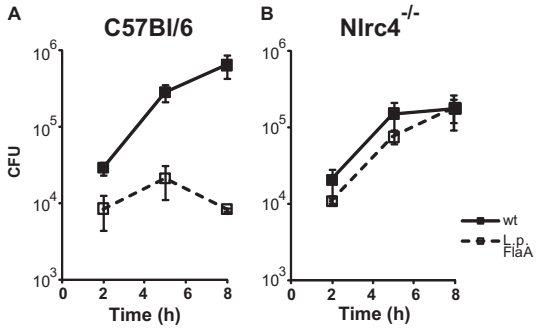

Fig. S4. Intracellular growth of wild-type and L.p.FlaA *L. monocytogenes.* Wild-type (solid line) or *L. monocytogenes*-L.p.FlaA (dashed line) were grown in wild-type (*A*) or Nlrc4$^{-/-}$ (*B*) bone marrow-derived macrophages and CFU enumerated at indicated times. Growth curves are representative of at least three independent experiments.

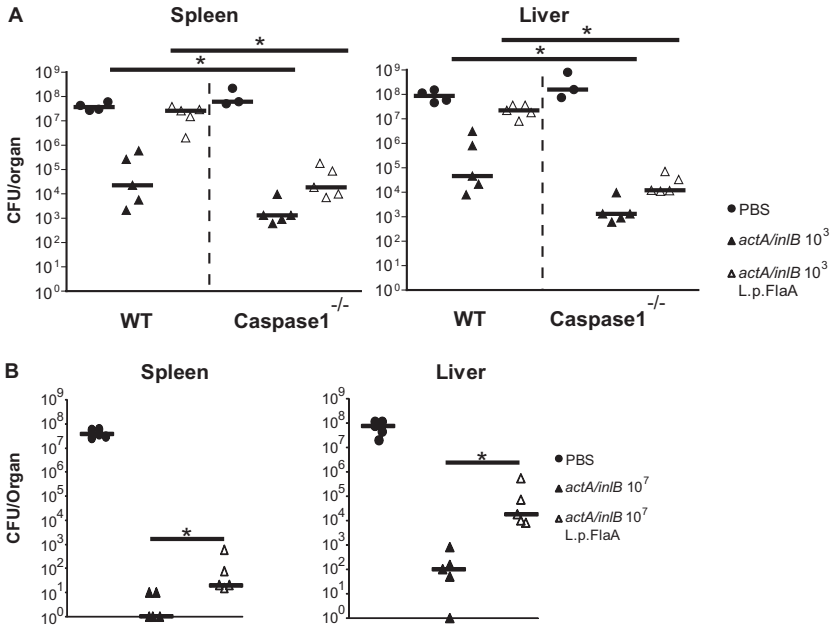

Fig. S5. Protective immunity in Caspase-1$^{-/-}$ mice 30 d postimmunization and in wild-type mice 90 d postimmunization. (*A*) Wild-type or Caspase-1$^{-/-}$ mice were immunized with 10^3 Δ*actA*/Δ*inlB* (closed symbols) or Δ*actA*/Δ*inlB L. monocytogenes*-L.p.FlaA (open symbols). Thirty days postimmunization, mice were rechallenged with 2×10^5 wild-type bacteria and CFU per organ were analyzed 68 to 72 h postchallenge. (*B*) Wild-type mice were immunized with1×10^7 Δ*actA/inlB* (closed symbols) or Δ*actA/inlB L. monocytogenes*-L.p.FlaA (open symbols) bacteria. Ninety days postimmunization, mice were challenged with 2×10^5 wild-type bacteria and CFU per organ were analyzed 68 to 72 h postchallenge. Data are representative of at least two independent experiments. *$P < 0.05$ by Mann-Whitney test.

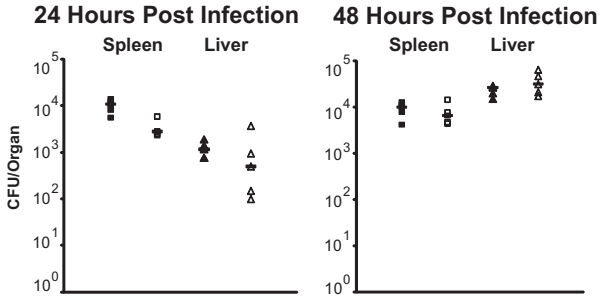

Fig. S6. Clearance of Δ*actA*/Δ*inlB* versus Δ*actA*/Δ*inlB L. monocytogenes*-L.p.FlaA following immunization. Wild-type mice were infected with 1×10^3 Δ*actA*/Δ*inlB* (closed symbols) or Δ*actA*/Δ*inlB L. monocytogenes*-L.p.FlaA (open symbols) and CFU was determined 24 or 48 h postinfection in the spleen (squares) and liver (triangles). Data are representative of at least two independent experiments.

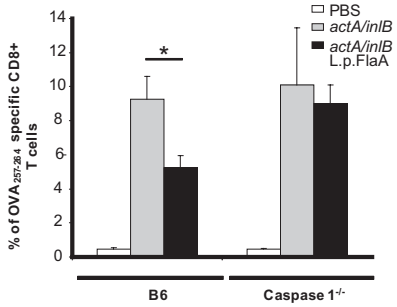

Fig. S7. Tetramer-positive antigen-specific CD8$^+$ T cells. Proportion of $OVA_{257–264}$-specific T cells within a CD8$^+$ cell gate, 7 d postinjection of 1×10^7 CFU of Δ*actA/inlB*, Δ*actA/inlB L. monocytogenes*-L.p.FlaA expressing OVA and B8R or PBS to C57BL/6 and caspase-1$^{-/-}$ mice, determined using K^b-$OVA_{257–264}$ tetramers. One representative experiment out of two is shown. *$P < 0.05$ by Mann-Whitney test.

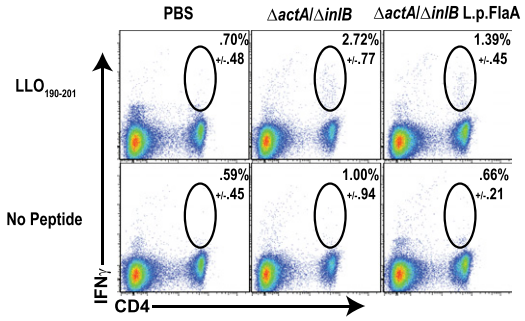

Fig. S8. Induction of $LLO_{190–201}$-specific $CD4^+$ T cells. C57BL/6 mice were injected with 1×10^7 CFU of Δ*actA/inlB*, Δ*actA/inlB L. monocytogenes*-L.p.FlaA expressing OVA and B8R epitopes or PBS. Seven days postimmunization, the percentage of antigen-specific IFN-γ^+ $CD4^+$ T cells was determined using intracellular cytokine staining after in vitro restimulation with the $LLO_{190–201}$. Values in each plot represent the mean ± SD of antigen-specific cells within a $CD4^+$ cell gate among splenocytes from four to five animals per group. One representative experiment of two to four is shown.

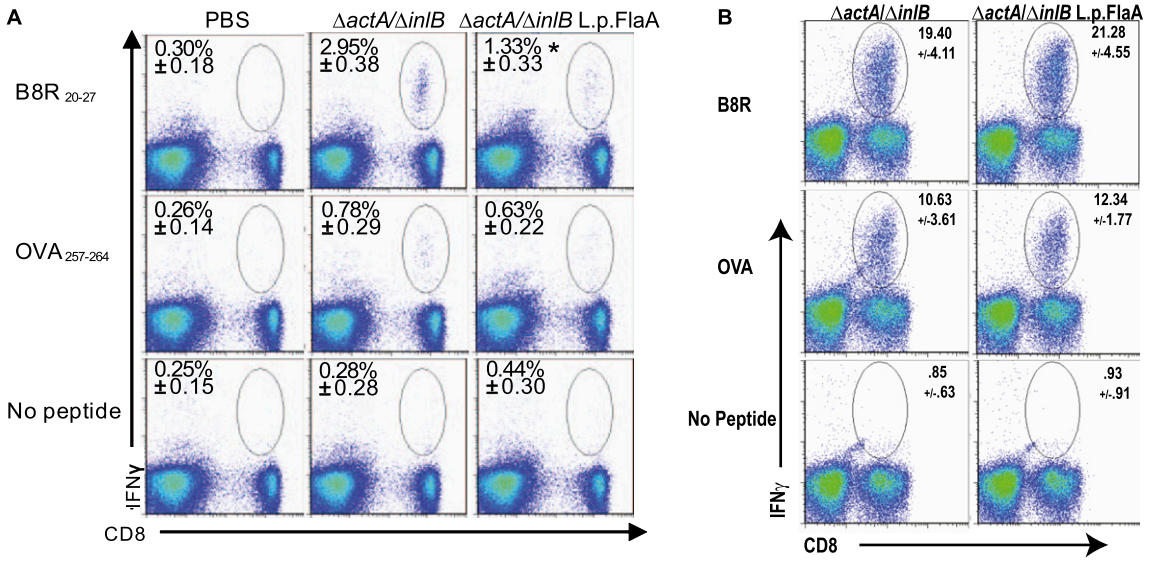

Fig. S9. $CD8^+$ memory responses following immunization (*A*) C57BL/6 mice were injected with 1×10^7 CFU of Δ*actA/inlB* or Δ*actA/inlB L. monocytogenes*-L.p.FlaA–expressing OVA and B8R epitope. Thirty-five days postimmunization, the percentage of antigen-specific IFN-γ^+ $CD8^+$ T cells was determined using intracellular cytokine staining after in vitro restimulation with the indicated peptide. Values in each plot represent the mean ± SD of antigen-specific cells within a $CD8^+$ cell gate among splenocytes from four to five animals per group. (*B*) C57BL/6 mice immunized with Δ*actA/inlB* or Δ*actA/inlB L. monocytogenes*-L.p.FlaA (10^7 CFU) expressing OVA and B8R were challenged with 2×10^5 *L. monocytogenes*-expressing OVA and B8R at 30 d postinfection. Splenocytes were harvested 5 d later and the percentage of antigen-specific IFN-γ^+ $CD8^+$ T cells was determined using intracellular cytokine staining after in vitro restimulation with the indicated peptide. Values in each plot represent the mean ± SD of antigen-specific cells within a $CD8^+$ cell gate among splenocytes from five animals per group. One representative experiment of two to four is shown.

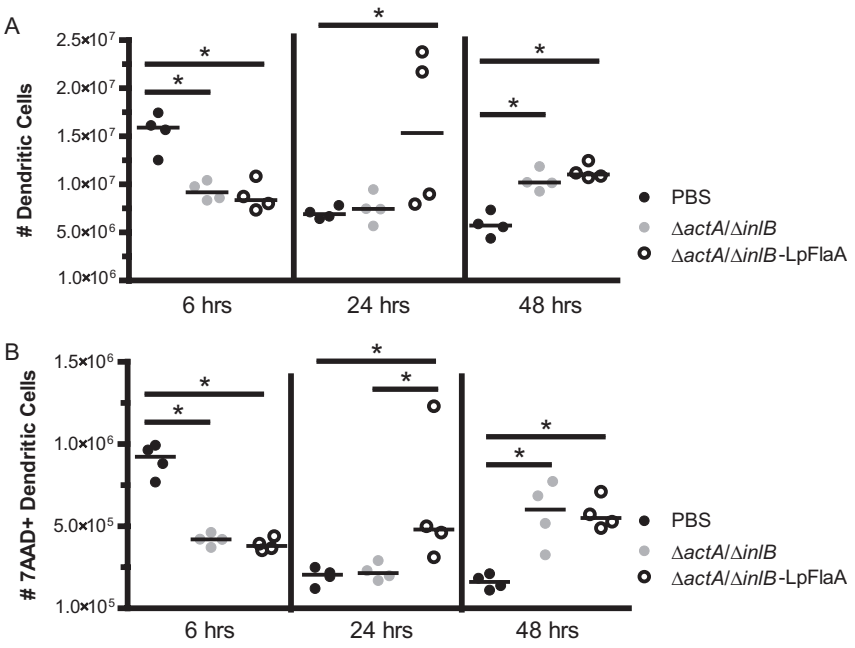

Fig. S10. Dendritic cell survival postimmunization. (*A*) Total and (*B*) dead dendritic cells were quantified 6, 24, and 48 h post immunization with 0.1 LD50 of Δ*actA*/Δ*inlB* or Δ*actA*/Δ*inlB L. monocytogenes*-L.p.FlaA. **P* < 0.05 by Mann-Whitney test.