Supporting Information

Miao et al. 10.1073/pnas.0913087107

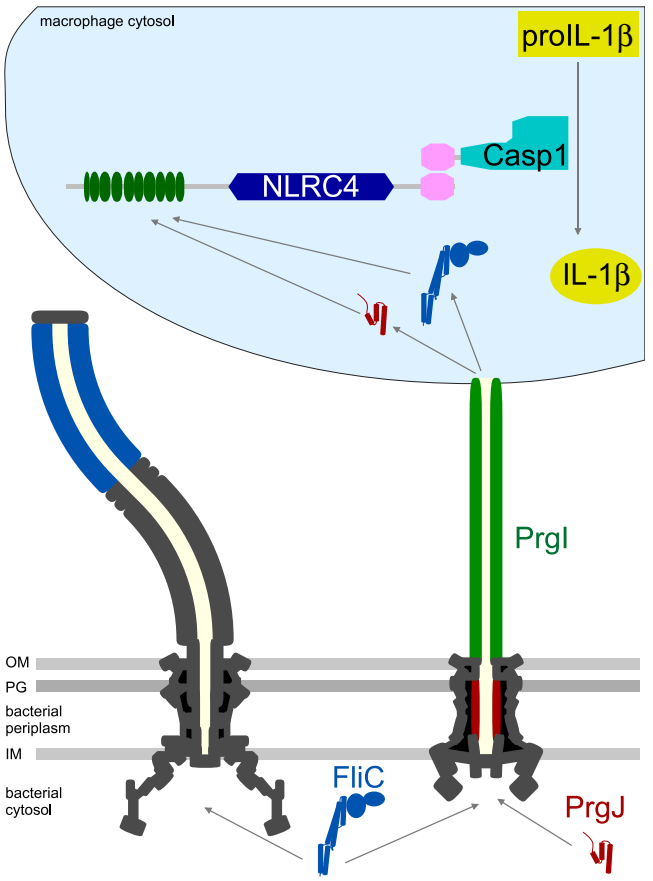


Fig. S1. Model for mechanism of PrgJ and Flagellin detection by NLRC4. Both the flagellar secretion system and the SPI1 secretion system are classified as T3SS, based on shared evolutionary origin and mechanism of secretion. Flagellin (FliC, blue) is exported through the hollow hook–basal body apparatus and polymerizes at the end of the hook to form a long whip-like filament. In the virulence-associated SPI1 T3SS, PrgI (green) and PrgJ (red) form the needle and rod component, respectively. Both FliC and PrgJ can be secreted by the SPI1 T3SS. The presence of FliC or PrgJ in the macrophage cytosol is detected by NLRC4, which promotes IL-1β processing and secretion by activating Caspase 1. IM, inner membrane; OM, outer membrane, PG, peptidoglycan.

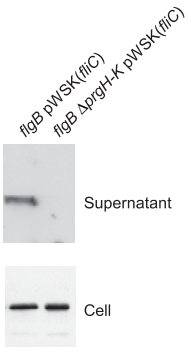


Fig. S2. SPI1 T3SS secretes flagellin. *S. typhimurium* were grown in LB broth for 3 h, and secretion of flagellin was determined by Western blot of TCA–precipitated supernatant proteins. *flgB* mutants were analyzed as they are deficient for the flagellar secretion apparatus. *flgB* mutants repress *fliC* transcription by retention of the anti-sigma factor protein FlgM, and thus *fliC* expression was restored in *flgB* mutants by expression on a low-copy plasmid (pWSK *fliC*). Secretion in *flgB* strains was observed and required the SPI1 T3SS apparatus proteins in the *prg* operon (Δ*prgHIJK*) (supernatant). Flagellin was expressed at equivalent levels by both *flgB* pWSK *fliC* and *flgB* Δ*prgHIJK* pWSK *fliC* (cell).

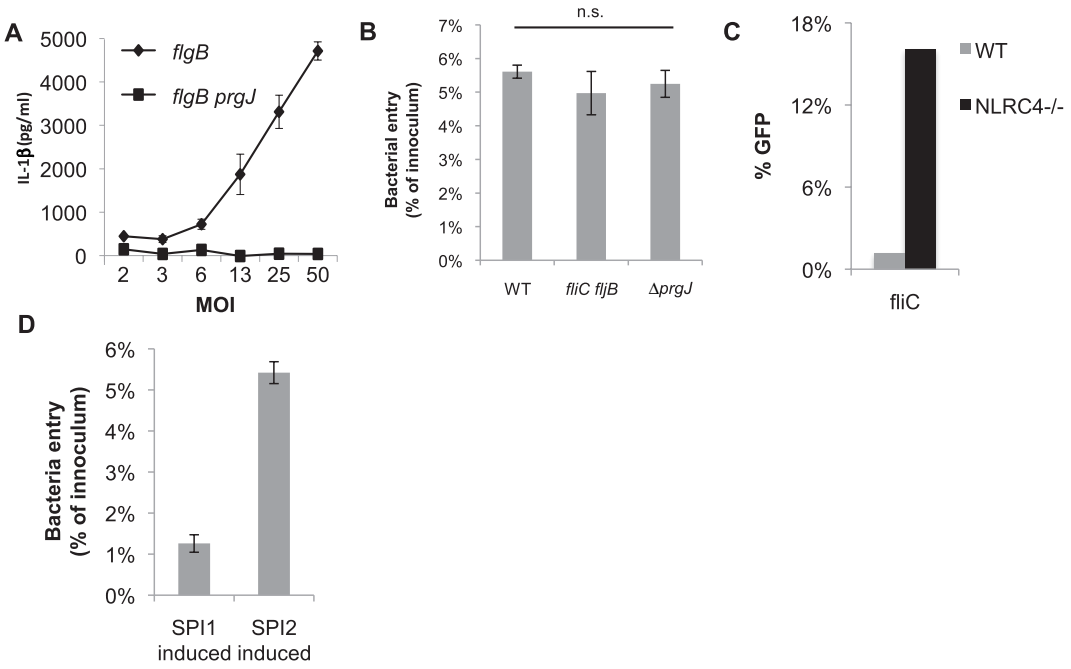


Fig. S3. Phagocytosis of SPI1 mutant *S. typhimurium* and analysis of flagellin detection by retroviral screen. (*A*) BMM were primed with LPS for 4 h, then infected with the flagellin mutant (*flgB*) or flagellin/SPI1 double mutant (*flgB prgJ*) *S. typhimurium* strain at the indicated multiplicity of infection (MOI) for 2 h and IL-1β secretion determined by ELISA. (*B*) LPS-primed BMMs were infected with SPI1 induced (microaerophilic) *S. typhimurium* at MOI 10 for 1 h, cells were washed and treated with media containing gentamicin to kill extracellular bacteria for 30 min, after which colony forming units were determined by dilutional plating. n.s., not significant; $P > 0.2$. *$P < 0.05$. (*C*) Retroviral lethality screen using *fliC*-IRES-GFP retrovirus was performed as in Fig. 1. (*D*) SPI1- or SPI2-induced *S. typhimurium* were used to infect BMM at MOI 10 for 1 h, after which bacterial entry was determined by gentamicin protection.

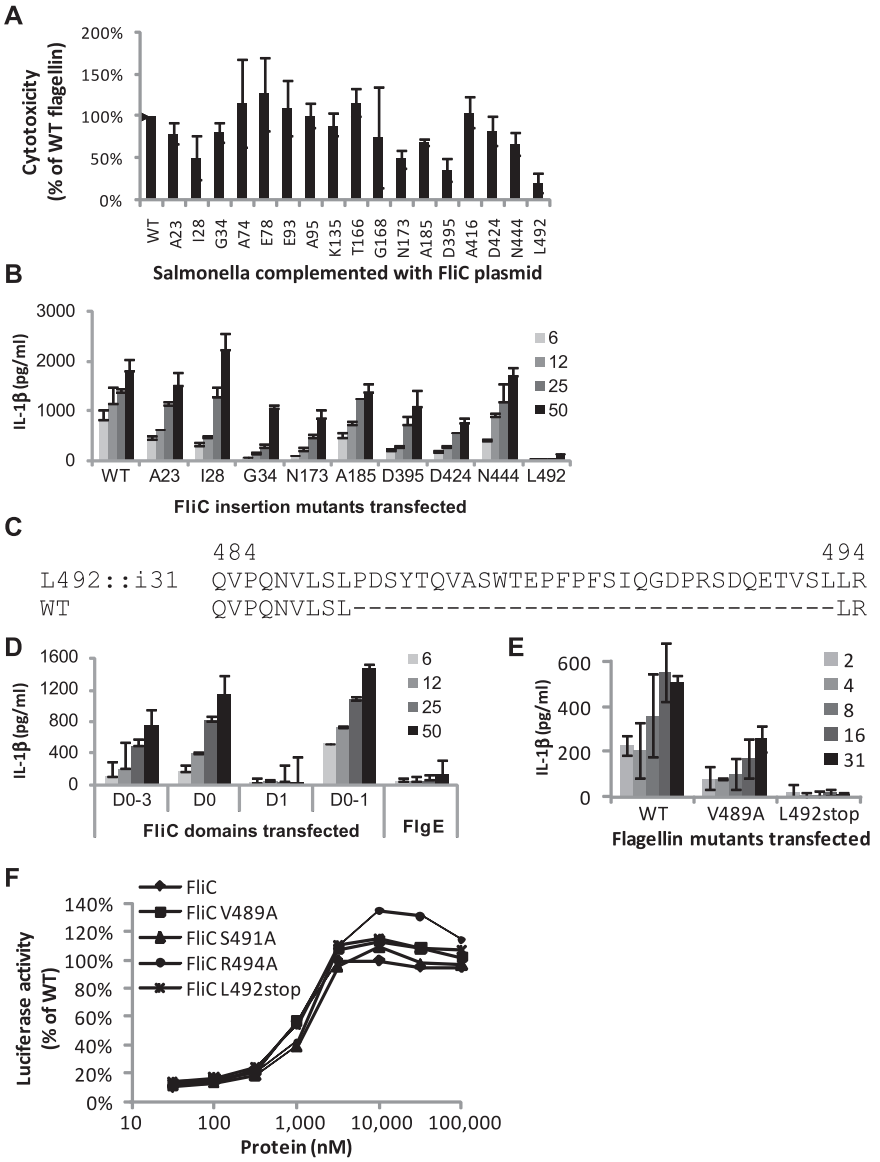


Fig. S4. Mapping FliC residues required for NLRC4 activation. (*A*) An unbiased screen was used to map the domain of FliC required for NLRC4 activation. An *S. typhimurium* flagellar mutant was complemented with WT flagellin or flagellin-containing insertions of 31 amino acids at indicated location. These strains were used to infect BMM for 1 h, and pyroptosis was determined by LDH release as a measure of caspase 1–dependent macrophage cell death. (*B*) Insertion mutations that affected pyroptosis were cloned into an N-terminal his tag vector, and purified protein was delivered to LPS primed BMM cytosol. IL-1β secretion was determined 1 h later by ELISA. Only L492, carrying an insertion two amino acid residues from the carboxy-terminus, was severely deficient for inducing IL-1β secretion. (*C*) Alignment of carboxy terminal domains of WT FliC and L492::i31 FliC. (*D* and *E*) Purified protein containing indicated domain of FliC or indicated mutation was delivered by protein transfection as in (*B*). Hook protein (FlgE) is used as negative control. (*F*) CHO cells expressing mTLR5 were stimulated with indicated protein, and NF-κB–driven luciferase activity was assessed after 4 h. Standard deviations are indicated.

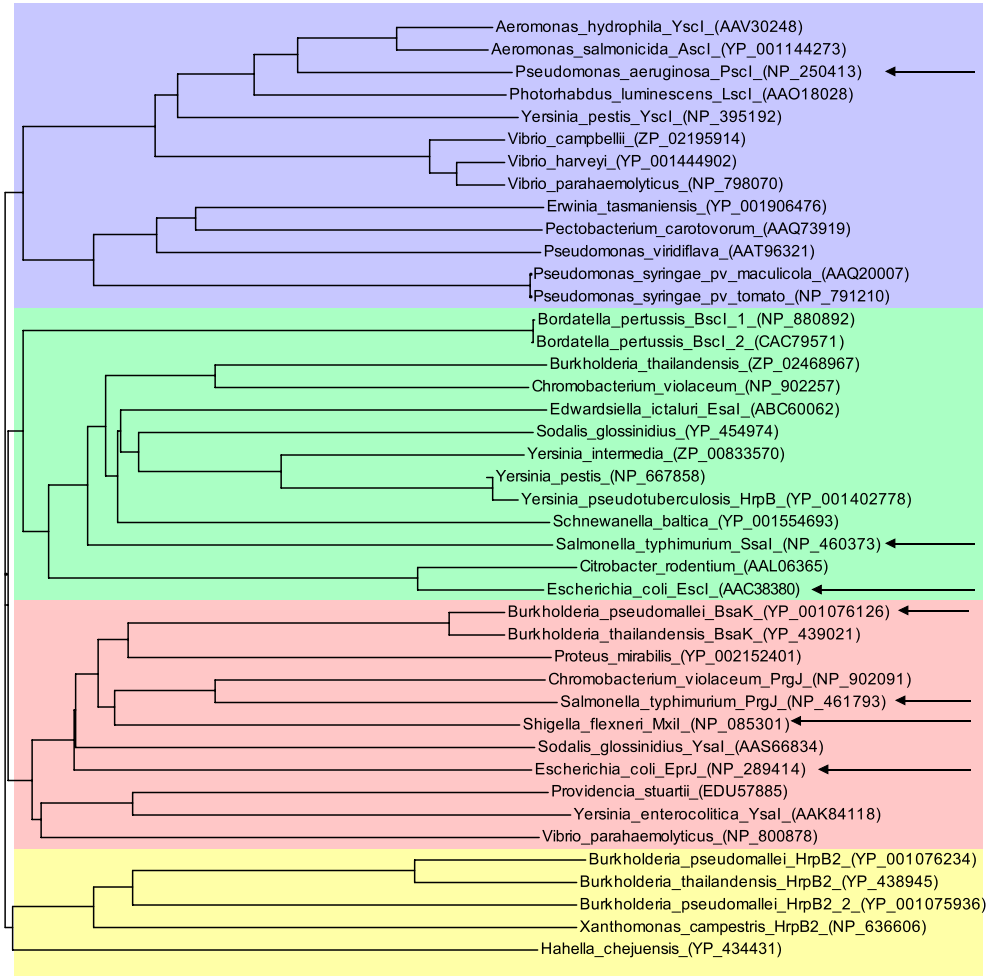
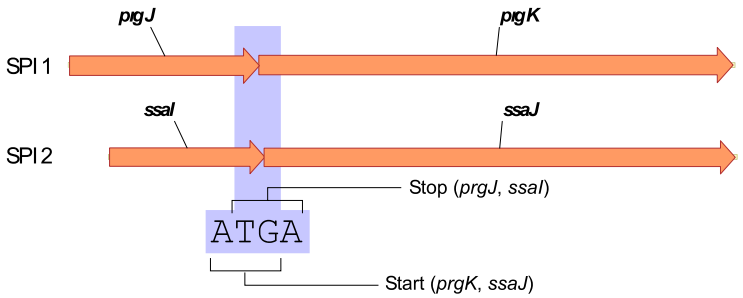


Fig. S5. Alignment of PrgJ homologs. A list of PrgJ homologs was assembled using PSI-BLAST (www.ncbi.nlm.nih.gov). Alignments were performing using Vector NTI 9.0 AlignX (Invitrogen). Proteins are listed as Genus_species_annotation_(accession number). Four large clades are denoted by color boxes. PrgJ, BsaK, EprJ, EscI, MxiI, PscI, and SsaI proteins investigated in this work are indicated with arrows.

A

	size (bp)	size (amino acids)	PrgJ Percent Similarity	PrgJ Percent Identity	PrgI Percent Similarity	PrgI Percent Identity
PrgI	240	80	23.3	15.5	100	100
PrgJ	303	101	100	100	23.3	15.5
SsaB	399	133	21.1	10.5	15.8	7.5
SsaE	240	80	25.2	9.7	20.7	9.8
SsaG	213	71	22.5	11.8	32.1	18.5
SsaH	225	75	16.8	6.9	19.8	11.1
SsaI	246	82	33.7	13.9	26.8	7.3
SsaM	366	122	22.1	12.3	18.7	10.6
SsaO	429	143	23.8	10.5	15.3	8.3
SsaP	372	124	23.2	12	25	12.9
SsaS	264	88	26.7	12.9	23.1	12.1

B



C

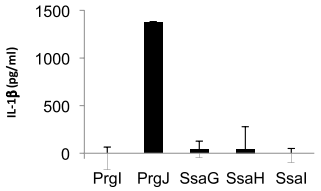


Fig. S6. SsaI is PrgJ homolog encoded by SPI2. (*A*) All putative apparatus components from SPI2 that contain less than 200 codons were analyzed for their percent identity and similarity to PrgI and PrgJ with Vector NTI 9.0 AlignX (Invitrogen). Closest homologs to PrgI and PrgJ are highlighted in yellow. SsaI is the only SPI2 protein that is found using PSI-BLAST with PrgJ (http://blast.ncbi.nlm.nih.gov). SsaG is most similar to PrgI and thus is predicted to be the SPI2 needle protein. (*B*) PrgJ homologs are most commonly are encoded directly upstream of the conserved inner membrane ring encoding gene; in SPI1, *prgJ* is encoded upstream of *prgK*, and the *prgJ* stop codon overlaps the *prgK* start codon. Similarly, the *prgJ* homolog *ssaI* stop codon overlaps the *ssaJ* start codon. PrgK and SsaJ encode homologous proteins (36.7% similar and 23.6% identical to each other) thought to form the inner membrane ring of the T3SS apparatus. (*C*) Purified PrgI, PrgJ, SsaG, SsaH, or SsaI protein was transfected into LPS primed macropahges as in Fig. 4*B*.

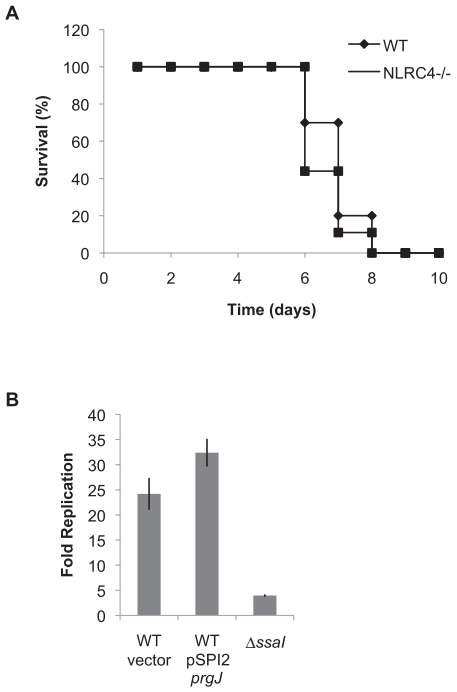


Fig. S7. NLRC4 does not contribute to clearance of WT *S. typhimurium*. (*A*) WT or NLRC4 null mice were infected with 100 cfu of WT *S. typhimurium* intraperitoneally, and survival was monitored. (*B*) HeLa cells were infected with *S. typhimurium* at MOI 10 for 1 h, followed by gentamicin treatment for 19 h. CFU were enumerated at 1.5 or 20 h postinfection and fold replication determined.