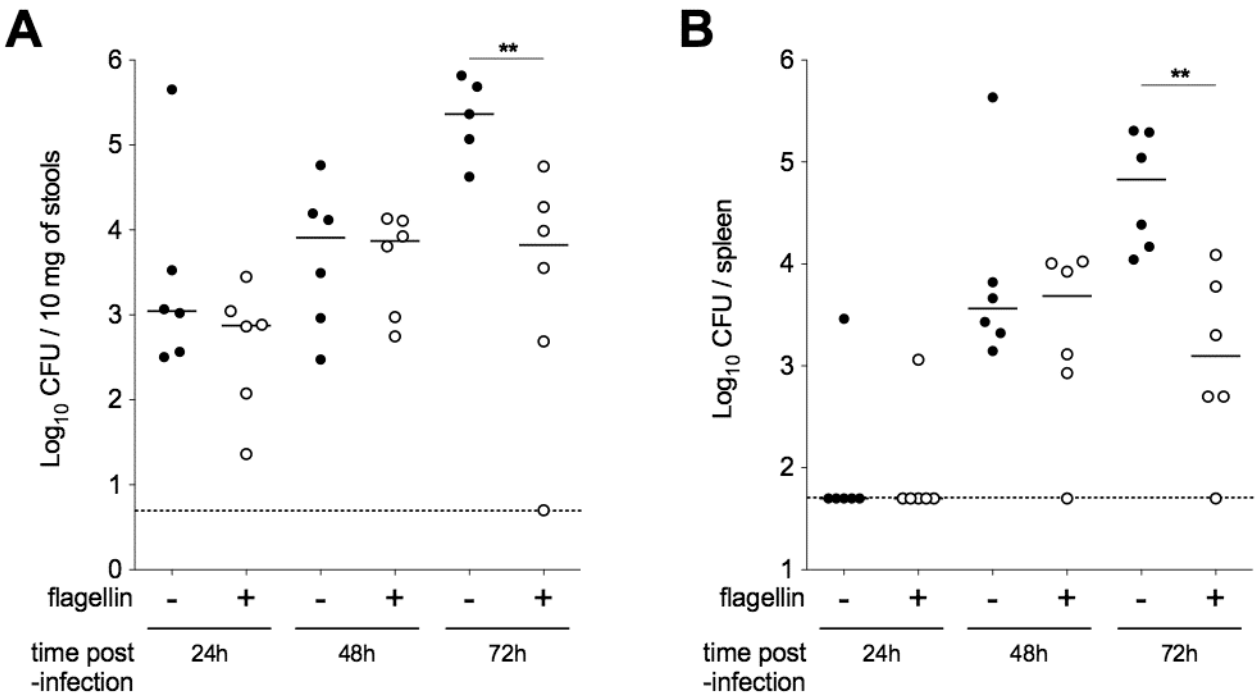

FIGURE S1: **Flagellin treatment is associated with low intestinal colonization and dissemination of *Y. pseudotuberculosis* three days after challenge**. Female C57BL/6 mice were treated intraperitoneally with PBS alone or flagellin (5 µg) in PBS 30 min before an intragastric challenge with 5×10^8 *Y. pseudotuberculosis*. Bacterial counts were determined in the stools (A) and the spleen (B). CFU counts for individual mice (n=5-6) at 24, 48 and 72 hours post-infection are shown. The solid line corresponds to the median value, and the dashed bar represents the detection threshold. Data from flagellin-treated and untreated mice were compared in a Mann-Whitney test (** P<0.01).

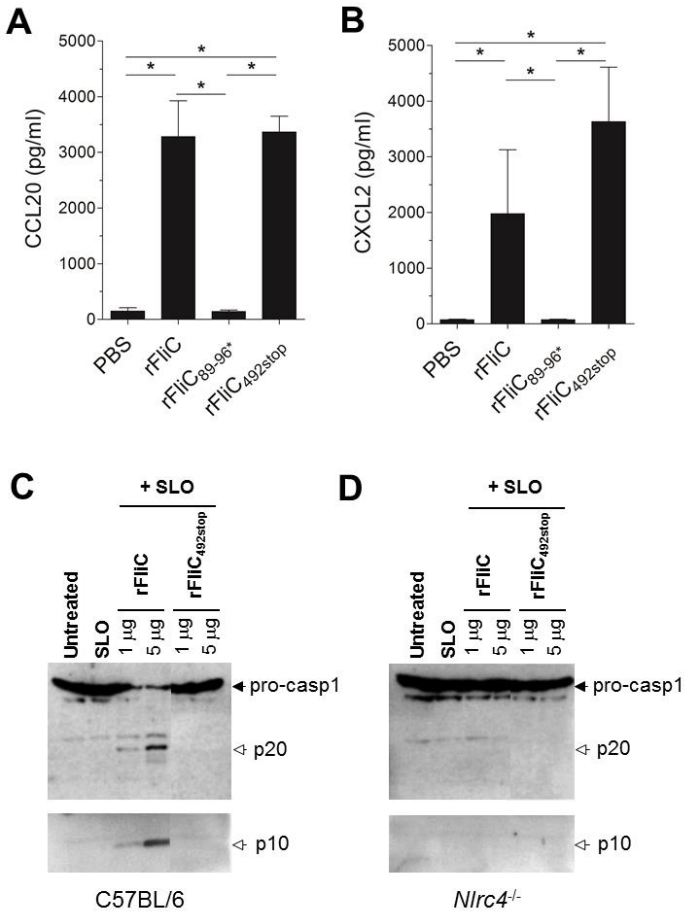

FIGURE S2: **Biological activity of recombinant flagellins**. (A-B) Female C57BL/6 mice (n=3-4) were injected intravenously with 5 µg of flagellin (either histidine-tagged flagellin rFliC, histidine-tagged mutant flagellin $rFliC_{89/96*}$ or histidine-tagged mutant flagellin $rFliC_{492stop}$). Two hours post-injection, the sera were assayed for CCL20 and CXCL2 production (using an ELISA). The data are quoted as the mean ± SD. Intergroup differences were analyzed in a Mann-Whitney test (* P<0.05). (C-D) Flagellin-specific caspase-1 activation. Bone marrow-derived macrophages from C57BL/6 (C) and *Nlrc4*$^{-/-}$ (D) mice were left untreated or incubated with streptolysin O (SLO, 25 µg/ml) in the presence or absence of rFliC or $rFliC_{492stop}$ (1 or 5 µg/ml). The macrophages were washed extensively and incubated for 2 h. Cell extracts were then prepared and immunoblotted for caspase-1. Arrows indicate procaspase-1 (p45) and its processed subunits (p10 and p20).

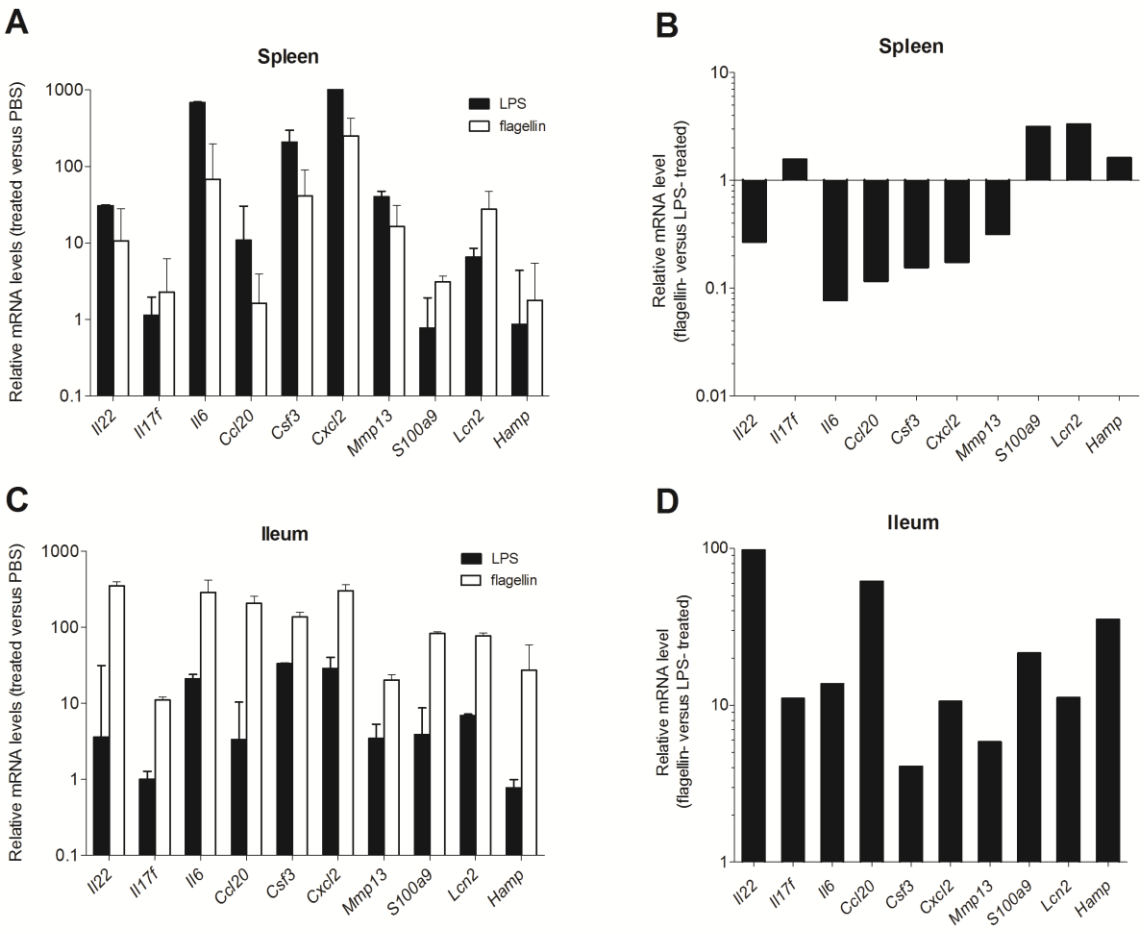

FIGURE S3: **Flagellin (but not LPS) mediates a strong transcriptional response in the intestine.** BALB/c mice were treated i.p. with PBS alone (n=2) or with flagellin (5 µg) or LPS (5 µg) in PBS (n=4). The transcriptional responses in the spleen (A-B) and the ileum (C-D) were analyzed by TaqMan Low Density Arrays 2 h after treatment. (A-C) mRNA levels are expressed relative to PBS-treated animals and are shown as the mean ± SD. (B-D) Expression of the results as the mean of mRNA level in flagellin-treated mice relative to that in LPS-treated animals.

Table S1: List of real time RT-qPCR primers

Target gene	forward primer	reverse primer
Actb	CGTCATCCATGGCGAACTG	GCTTCTTTGCAGCTCCTTCGT
Cxcl2	CCCTCAACGGAAGAACCAAA	CACATCAGGTACGATCCAGGC
Hamp	GATGGCACTCAGCACTCG	CTGCAGCTCTGTAGTCTGTCTCA
Il22	TTTCCTGACCAAACTCAGCA	TCTGGATGTTCTGGTCGTCA
Il6	GTTCTCTGGGAAATCGTGGAAA	AAGTGCATCATCGTTGTTCATACA
Reg3g	GGCATCTTTCTTGGCAACTT	ACCATCACCATCATGTCCTG
S100a9	CACCCTGAGCAAGAAGGAAT	TGTCATTTATGAGGGCTTCATTT

Table S2: References of the probes used in the Taqman Low Density Arrays

Gene Symbol	Assay ID (Applied Biosystems)
Actb	Mm00607939_s1
B2m	Mm00437762_m1
Ccl20	Mm00444228_m1
Csf3	Mm00438334_m1
Cxcl10	Mm99999072_m1
Cxcl2	Mm00436450_m1
Hamp	Mm00519025_m1
Il17c	Mm00521397_m1
Il17f	Mm00521423_m1
Il1b	Mm01336189_m1
Il22	Mm00444241_m1
Il23a	Mm00518984_m1
Il6	Mm99999064_m1
Lcn2	Mm01324470_m1
Mmp13	Mm00439491_m1
Reg3b	Mm00440616_g1
Reg3g	Mm00441127_m1
S100a9	Mm00656925_m1