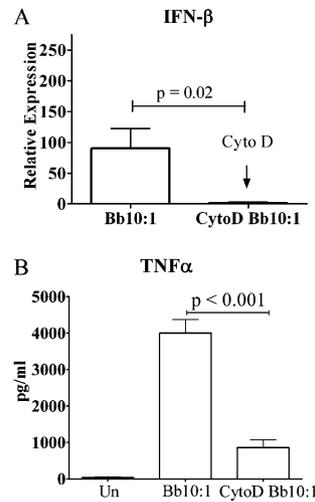
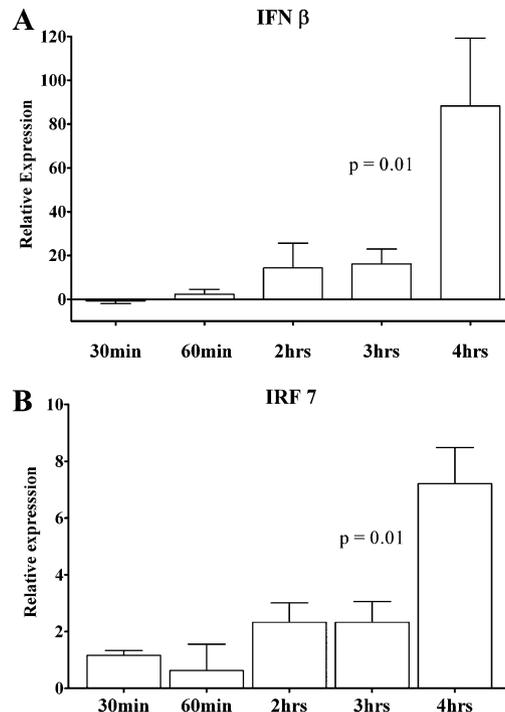


# Supporting Information

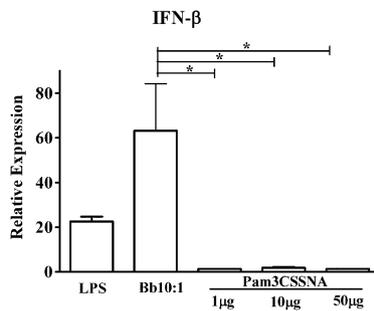
Cervantes et al. 10.1073/pnas.1013776108



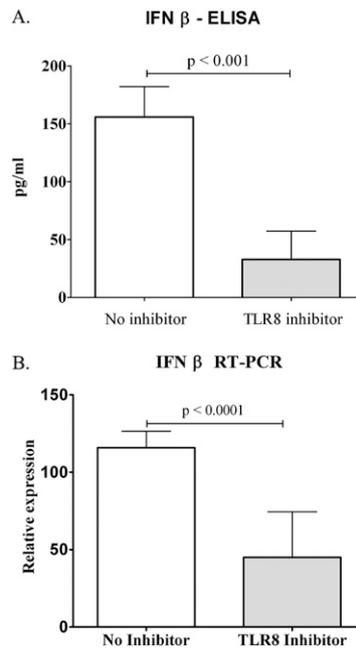
**Fig. S1.** Phagocytosis of Bb is necessary for transcription of IFN- $\beta$  and optimal production of TNF- $\alpha$  in human monocytes. Isolated human monocytes were stimulated for 4 h with Bb (10:1 multiplicity of infection), with and without 1  $\mu$ g/mL CytoD. (A) Transcription of IFN- $\beta$  is inhibited in the presence of CytoD. Relative expression refers to *ifnb* gene expression normalized to *gapdh*. (B) CytoD markedly diminished secretion of TNF- $\alpha$  in response to live Bb. *P* values shown correspond to paired analysis using the Mann-Whitney *U* test from a minimum of three independent experiments. CytoD, Cytochalasin-D; Un, uninfected.



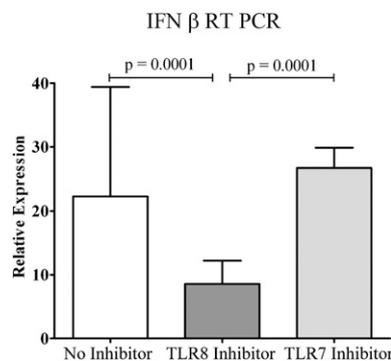
**Fig. S2.** Bb induces sequential expression of IFN- $\beta$  and IRF7. Time-chase experiment of monocytes inoculated with Bb multiplicity of infection (10:1) at several time points over a 4-h period showing transcription of IFN- $\beta$  (A) and IRF-7 (B). Relative expression refers to *ifnb* and *irf7* gene expression normalized to *gapdh*. *P* values shown correspond to trend analysis (Kruskal-Wallis test) from a minimum of three independent experiments.



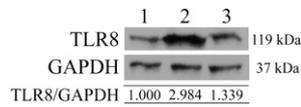
**Fig. S3.** TLR2 surface signaling is unable to induce IFN- $\beta$  transcription in isolated human monocytes. Human monocytes were stimulated for 4 h with LPS (0.2  $\mu$ g/mL), Bb (10:1 multiplicity of infection), or a synthetic TLR2 ligand (Pam<sub>3</sub>CSSNA) at three different concentrations. Pam<sub>3</sub>CSSNA was unable to induce transcription of IFN- $\beta$ . Relative expression refers to *ifnb* gene expression normalized to *gapdh*. *P* values correspond to paired analysis from a minimum of three independent experiments using the Mann-Whitney *U* test. \**P* < 0.01.



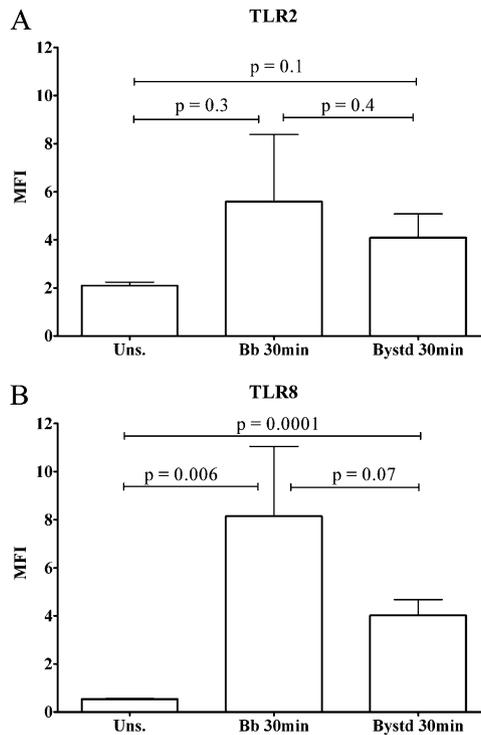
**Fig. S4.** TLR8 inhibition diminishes production of IFN- $\beta$  in R848-treated human monocytes. Human monocytes were pretreated with and without IRS-957 and stimulated with R848 (1  $\mu$ g/mL). (A) Secreted IFN- $\beta$  was determined by ELISA. (B) Transcription of *ifnb* was measured by RT-PCR. *P* values shown correspond to paired analysis using the Mann-Whitney *U* test from a minimum of six independent experiments.



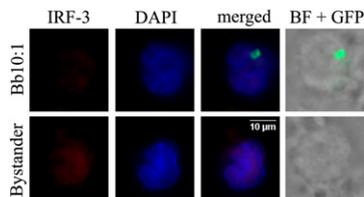
**Fig. S5.** IRS957 specifically inhibits TLR8-dependent transcription of IFN- $\beta$  in 3M-002-treated human monocytes. Stimulation of human monocytes with 5  $\mu$ M TLR8 ligand 3M-002 inhibits transcription of *ifnb* only when cells were pretreated with the TLR8 inhibitor (IRS-957) but not with the TLR7 inhibitor (IRS-661). Values are representative of two independent experiments.



**Fig. S6.** TLR8 expression is induced in Bb-infected human monocytes and is inhibited by IRS-957. TLR8 protein expression determined by Western blot analysis shows the extent of TLR8 overexpression in Bb-infected human monocytes (lane 2), which returns to baseline levels (lane 1) when cells were preincubated with the TLR8 inhibitor IRS-957 (lane 3). TLR8 is detected as a band of ~119 kDa, and housekeeping GAPDH is depicted as a band of 37 kDa. Densitometric values are shown beneath the corresponding bands. The analysis was performed using average pixel values for each band determined in an equally sized box. The background was automatically deducted from these pixel values, and the pixel values for TLR8 were divided by the pixel values for GAPDH. An uninfected sample was set as "1" by dividing all for comparison.

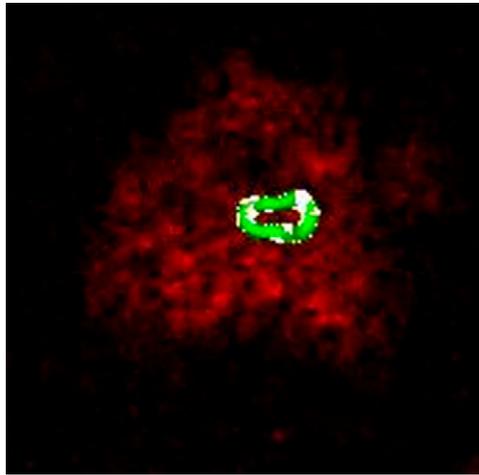


**Fig. S7.** TLR2 and TLR8 MFIs in Bb-infected and uninfected human monocytes. Baseline TLR2 expression (A) is higher than that of TLR8 (B) in Uns. cells. A statistically significant increase in TLR8 expression is observed within 30 min of Bb inoculation (10:1 multiplicity of infection) in both infected and Bystd uninfected cells. Individual MFI values were measured per selected cell surface area as described in *Materials and Methods*. *P* values shown correspond to paired analysis using the Mann–Whitney *U* test. Bystd, bystander; MFI, mean fluorescence intensity; Uns., unstimulated.



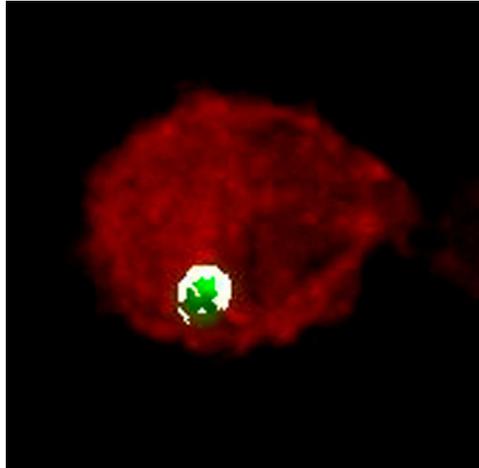
**Fig. S8.** Live Bb does not induce expression of IRF3 in human monocytes. Immunofluorescence assay for IRF3 (red) reveals a very low signal in a Bb-infected monocyte (green). An uninfected bystander monocyte is also shown. Nuclei stained with DAPI (blue). Translocation of IRF3 into the nucleus does not take place. BF, bright field.





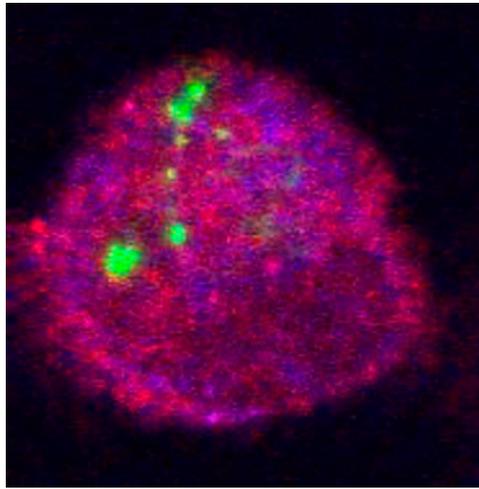
**Movie S2.** TLR2 interacts with phagocytosed GFP-expressing Bb (coil) inside the monocyte. Z-stack confocal sections show internalized coiled GFP-expressing Bb (green) colocalizing (white) with TLR2 (red) inside a monocyte.

[Movie S2](#)



**Movie S3.** TLR8 interacts with phagocytosed GFP-expressing Bb inside the monocyte. Z-stack confocal sections show internalized degraded GFP-expressing Bb (green) colocalizing (white) with TLR8 (red) inside a monocyte.

[Movie S3](#)



**Movie S4.** TLR2 and TLR8 colocalize with internalized degraded spirochetes. The movie is divided into three sections; the first shows Z-stack confocal sections of TLR2 (red) colocalizing (white) with GFP-expressing Bb (green), followed by TLR8 (red) colocalizing (white) with the same spirochete (green) in the same cell. The third concatenated file shows costaining of TLR2 (blue) and TLR8 (red) colocalization in the same cell.

[Movie S4](#)