Supplementary data for manuscript:

Phagocytosis and phagosome acidification are required for pathogen processing and MyD88-dependent responses to *Staphylococcus aureus*.

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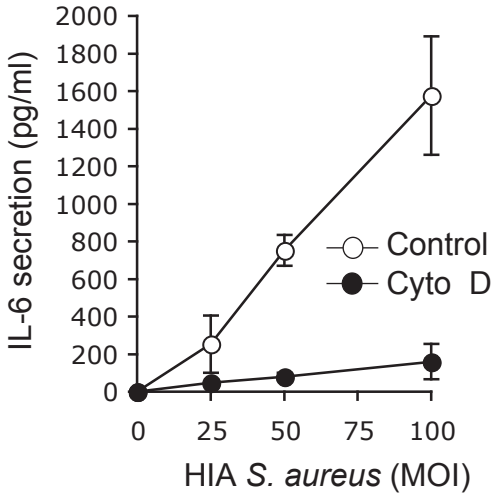
Supplementary Figure SF1. Internalization and phagosome formation is required for IL-6 response to Gram-positive but not Gram-negative pathogens.

Supplementary Figure SF2. Intracellular TNF-α production in macrophages with defined bacterial loads.

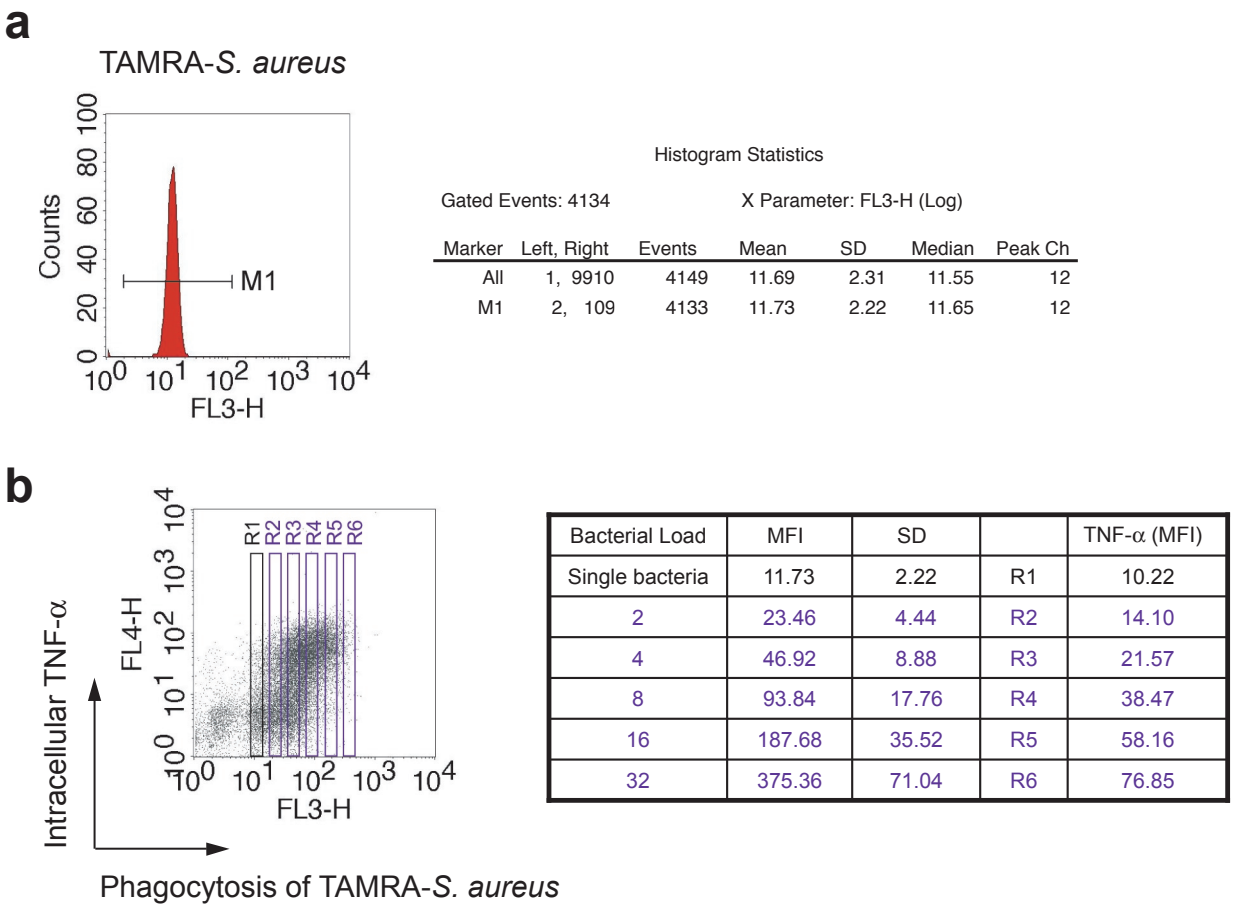
Supplementary Figure SF3. The nature of the ligand, and not the receptor, determines the need for internalization

Supplementary Table ST1.Protease inhibitor targets.

a

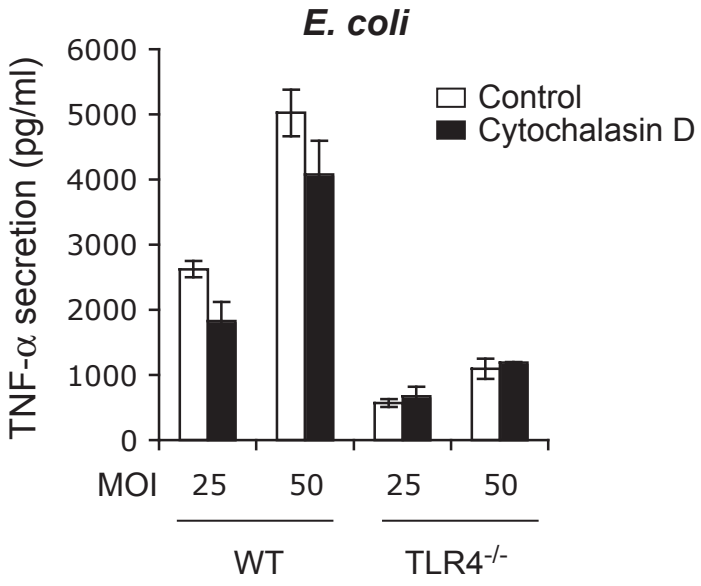


Supplementary Figure 1. Internalization and phagosome formation is required for IL-6 response to Gram-positive but not Gram-negative pathogens.
a) IL-6 production by control (white circles) and cytochalasin D treated macrophages (black circles) exposed to increasing MOI of HIA S. aureus.



Bacterial Load	MFI	SD		TNF-α (MFI)
Single bacteria	11.73	2.22	R1	10.22
2	23.46	4.44	R2	14.10
4	46.92	8.88	R3	21.57
8	93.84	17.76	R4	38.47
16	187.68	35.52	R5	58.16
32	375.36	71.04	R6	76.85

Figure SF2. **Intracellular TNF-α production in macrophages with defined bacterial loads.** (**a**) Mean of fluorescence intensity (MFI) of TAMRA-labeled *S. aureus* at the single-bacteria level was measured by flow cytometry. (**b**) Macrophages were fed with the TAMRA-*S. aureus* for 2 h. Phagocytosis and intracellular TNF-α were measured by flow cytometry. Total MFI ± SD (at FL3-H) for one- or twofold increasing bacterial loads were predicted (table; right) based on the MFI ± SD of a single bacteria observed in (a), which allows to define the regions (R1 - 6) of cells that had engulfed one- or twofold increasing numbers of bacteria (density plot; left). Intracellular TNF-α production (MFI at FL4-H) from the corresponding regions were then determined by the gating analysis (table; left), and these data are plotted against the number of bacteria engulfed as shown in Figure 1d (left).



Supplementary Figure 3. The nature of the ligand, and not the receptor, determines the need for internalization.

The requirement for internalization on TLR4-dependent and TLR4-independent r esponses to E. coli was determined by monitoring the effect of blocking phagocytosis on cytokine production triggered in WT and Tlr4-/- cells stimulated with E. coli

Table ST1.Protease inhibitor targets.

Protease inhibitor	Targets*
AEBSF	serine protease
EACA	chymotrypsin, Factor VIIa, lysine carboxypeptidase, plasmin, plasminogen activator
Antipain	serine protease, cysteine protease, some trypsin-like serine proteases
Aprotinin	serine protease
Benzamidine HCl	trypsin, trypsin-like enzymes, and serine proteases
Bestatin	leucine aminopeptidase, aminopeptidase B, triamino peptidase
Chymostatin	chymotrypsin, chymotrypsin-like serine proteinases, chymases, lysosomal cysteine proteinases
E-64	cystein proteases
EDTA	Zinc-dependent metalloproteinases
N-Ethylmaleimide	cystein proteases
Leupeptin	serine protease, thiol protease
Pepstatin	acid protease (aspartyl peptidases): pepsin, renin, cathepsin D, bovine chymosin, protease B
Phosphoramidon	bacterial metalloendoproteinases, thermolysin, elastase (weak for collagenase)
Trypsin inhibitor	trypsin

Table ST1b. **Specific targets of the protease inhibitor panel.**
*summerized from the manufacturer's product information (Sigma).