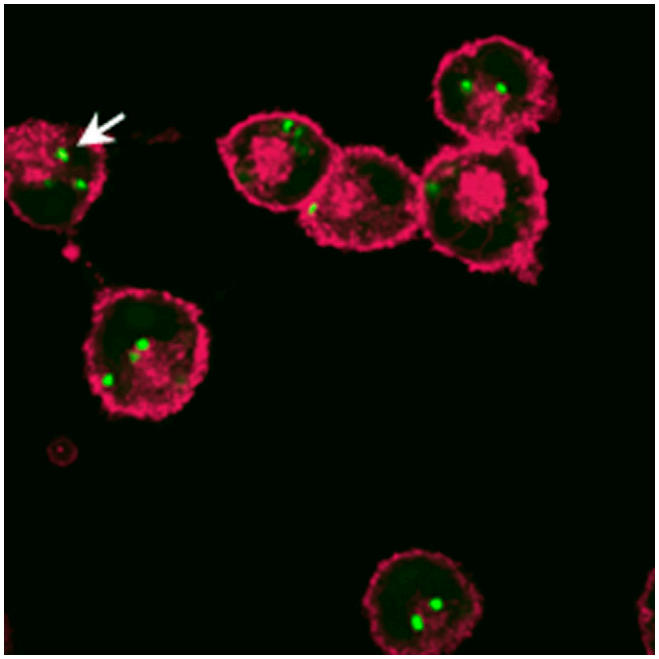
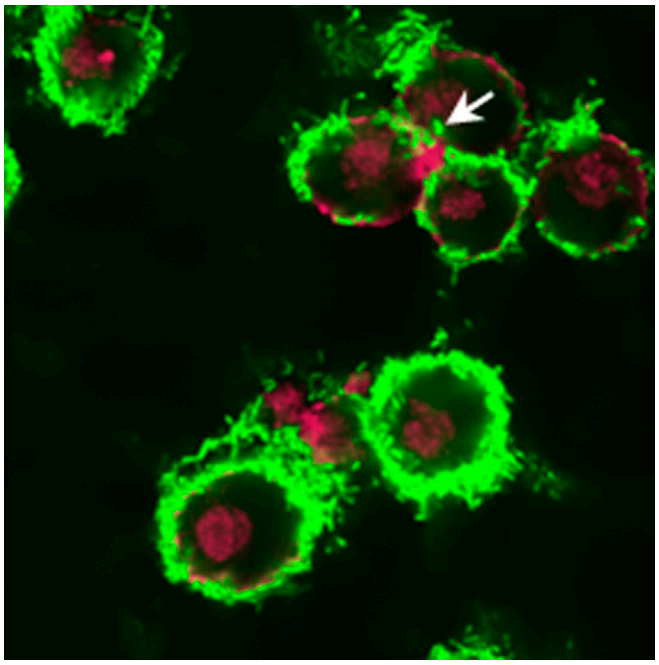
Supporting Information

Mahdavi et al. 10.1073/pnas.1301740111



Movie S1. Proteomic labeling of internalized *Yersinia* inside infected HeLa cells in the presence of gentamicin. *Y. enterocolitica* (T3SS-Wt) were diluted 1:25 from overnight cultures in LB and incubated at 26 °C with agitation until an $OD_{600} = 0.5$ was reached. Infection was initiated at a multiplicity of infection of 100 in Opti-MEM. After 1 h of infection, 80 μg/mL gentamicin was added. After 1 h, the medium was changed to fresh Opti-MEM, and 1 mM Anl was added to both samples. The medium was supplemented with 4 μg/mL gentamicin to maintain inhibition of protein synthesis by extracellular bacteria. After 3 h of labeling, cells were fixed with 3.7% formaldehyde for fluorescence confocal microscopy. Alexa Fluor 633 conjugated to wheat germ agglutinin (WGA) was used to label the membranes of HeLa cells; the associated fluorescence is shown in red. Anl-labeled proteins were treated with alkyne-functionalized Alexa Fluor 488; the associated fluorescence is shown in green. Confocal fluorescence microscopy was used to image a series of Z-sections through the infected HeLa cells. Z-stacks were taken from the top of the HeLa cells to the bottom of the glass surface. Internalized *Yersinia* cells are labeled inside infected HeLa cells. Extracellular bacteria are not labeled, because protein synthesis is inhibited in these cells.

Movie S1



Movie S2. Proteomic labeling of internalized and extracellular *Yersinia* in HeLa cell infections lacking gentamicin. Infections were performed under identical conditions as in Movie S1 but lacking gentamicin. Labeling times were identical to those for Movie S1. Alexa Fluor 633 conjugated to WGA was used to label the membranes of HeLa cells; the associated fluorescence is shown in red. Anl-labeled proteins were treated with alkyne-functionalized Alexa Fluor 488; the associated fluorescence is shown in green. Confocal fluorescence microscopy was used to image a series of Z-sections through the infected HeLa cells. Z-stacks are taken from the top of the HeLa cells to the bottom of the glass surface, showing labeled bacteria on the surface of the glass substrate. Anl was incorporated during protein synthesis by the internalized and extracellular *Y. enterocolitica*, and both populations are labeled in green.

Movie S2

Other Supporting Information Files

SI Appendix (PDF)