

Supplementary Information

***Listeria* exploits IFITM3 to suppress antibacterial activity in phagocytes**

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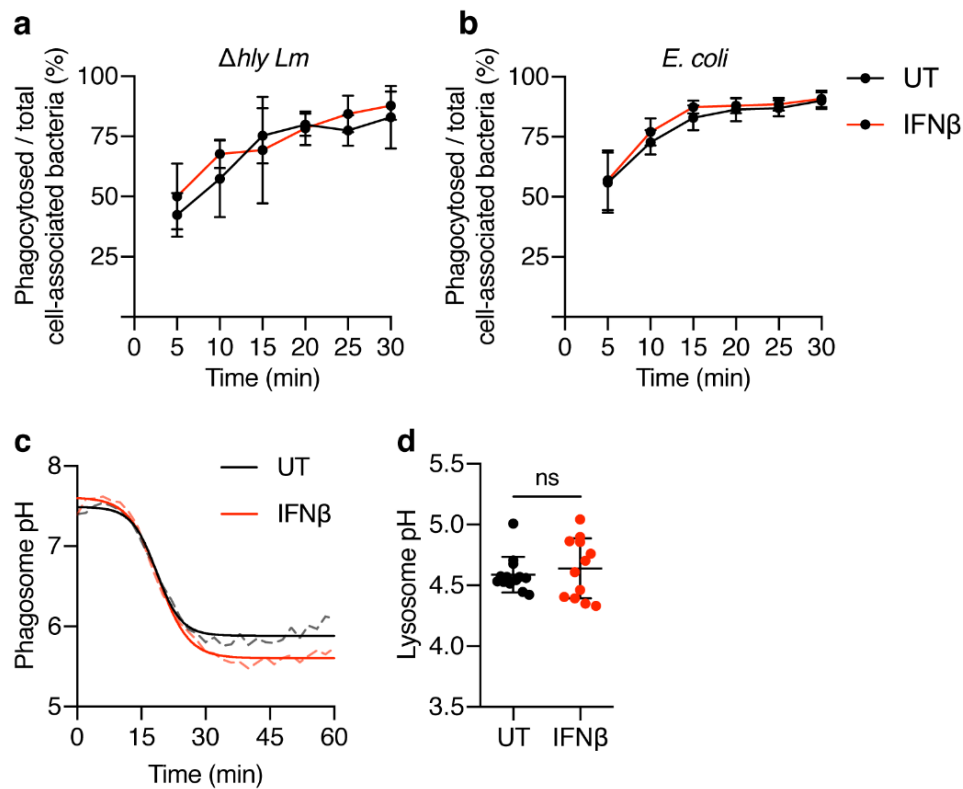
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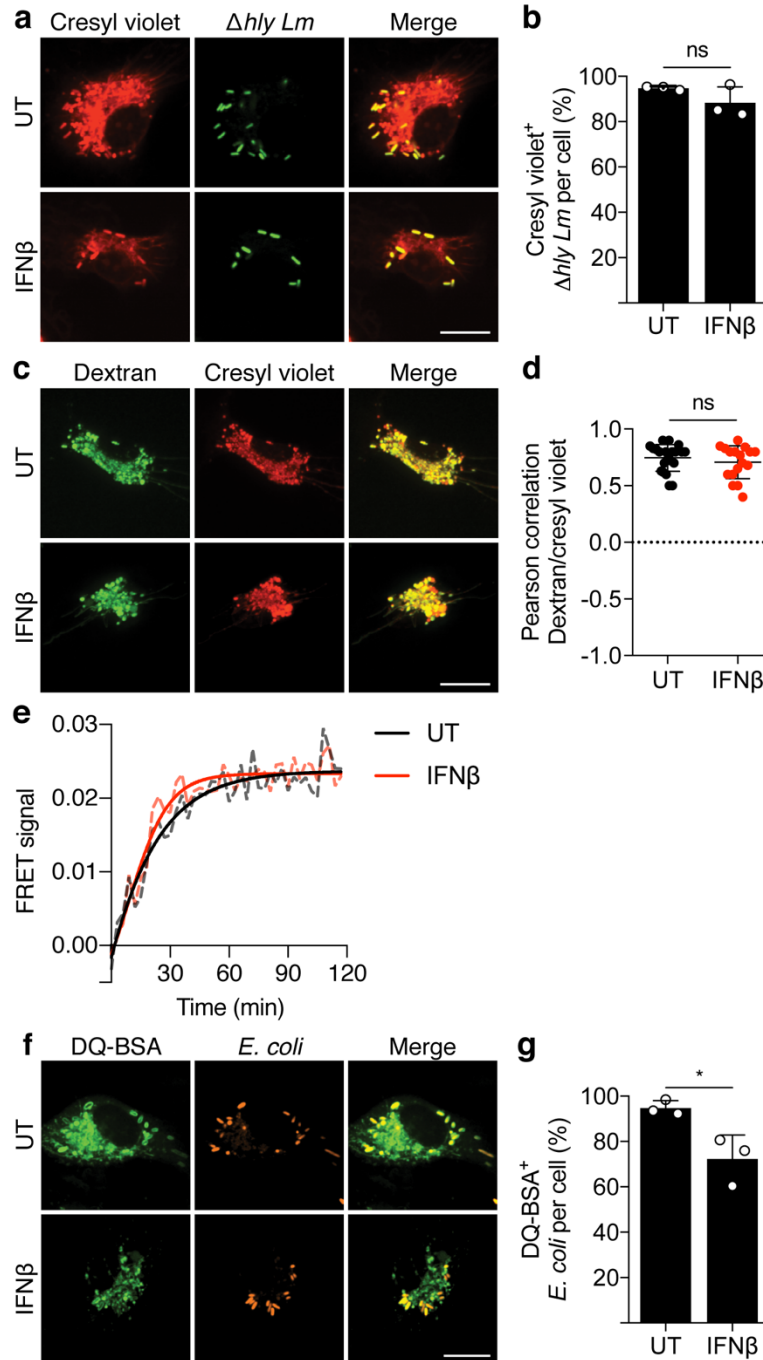
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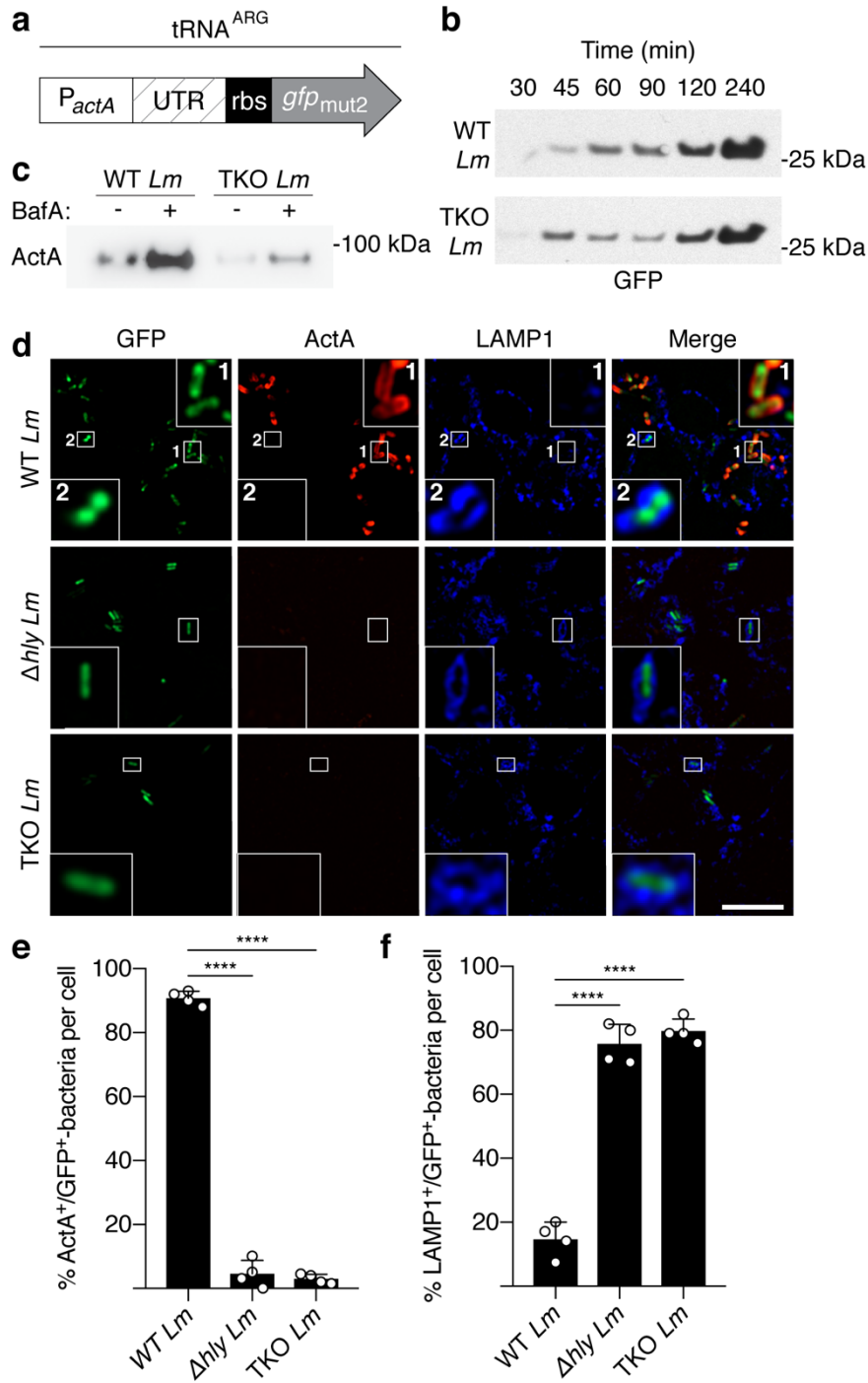
Supplementary Figures



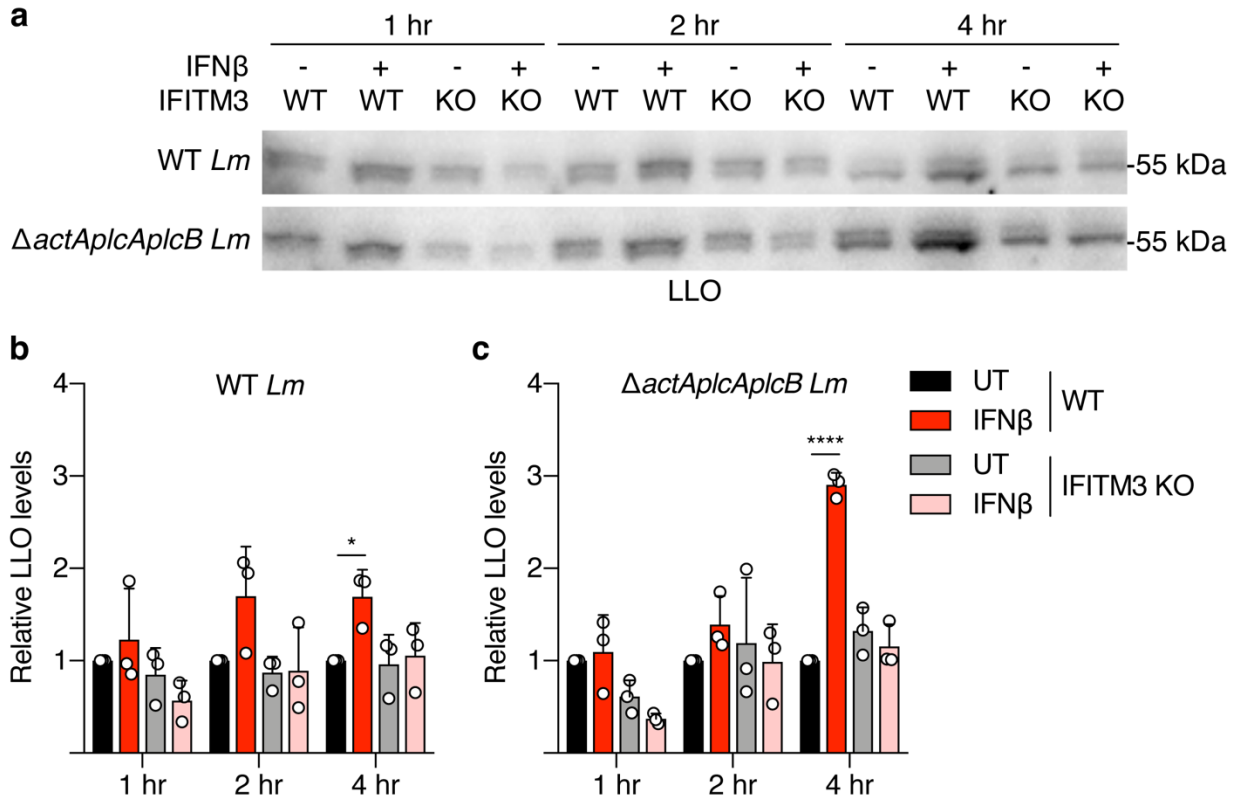
Supplementary Fig. 1 | Type I IFN does not affect phagocytosis or acidification. Number of internalized *Δhly Lm* (a) and *E. coli* (b) in BMDMs untreated (UT) or treated with IFN β were enumerated over a 30 min time course. Differential antibody staining was carried out to determine the population of intracellular versus extracellular bacteria. Phagosome (c) and lysosome (d) acidification in BMDMs treated with or without IFN β fed with carboxyfluorescein succinimidyl ester (CFSE)-particle (phagosome) or FITC-dextran (lysosome). Fluorescence ratiometric imaging was used to determine pH value of phagosomes (over 60 min) and lysosomes (at 60 min) calibrated against a standard curve. Solid lines represent nonlinear regression fit of the dashed lines. Data shown are means \pm standard deviation (s.d.) for n = 3 independent experiments. *P* value was calculated using two-tailed unpaired t-test in (d). ns, not significant, *P* = 0.5348.



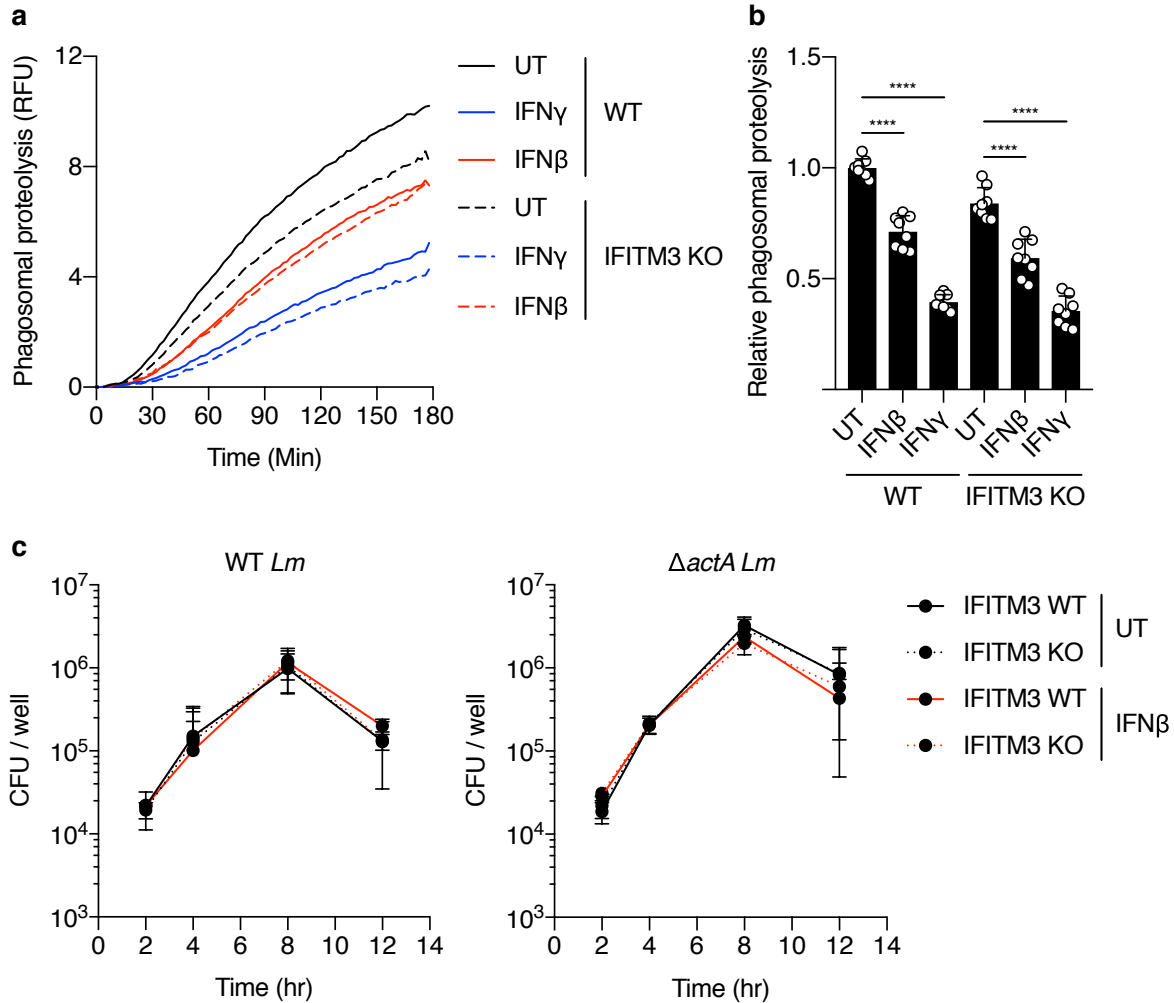
Supplementary Fig. 2 | Effect of type I IFN on early phagosome maturation events. **a, b**, Representative images (**a**) and quantification (**b**) of $\Delta hly Lm$ in lysosomes of BMDMs treated with or without IFN β 4 h post-infection (p.i.) using lysosomal marker cresyl violet after 30 min. **c, d**, Endolysosomal fusion measured in dextran-fed BMDMs treated with or without IFN β using lysosomal marker cresyl violet after 30 min. Representative images (**c**) and Pearson correlation (**d**) of colocalization between dextran and cresyl violet-positive compartments. **e**, Phagolysosomal fusion measured using FRET-based assay in BMDMs treated with or without IFN β using particles labeled with donor fluorophore (phagosomes) and acceptor fluorophore (lysosomes) over 2 h. Solid lines represent nonlinear regression fit of the dashed lines. **f, g**, Representative images (**f**) and quantification (**g**) of phagosomal proteolysis measured using self-quenched substrate DQ-BSA that fluoresces in the presence of proteases. *E. coli* residing in DQ-BSA-positive compartments in BMDMs treated with or without IFN β were enumerated after 4 h. Data shown are means \pm s.d. for at least $n = 3$ independent experiments. P value (**b, d, g**) was calculated using two-tailed unpaired t -test. * $P = 0.0252$. ns, not significant, $P > 0.1893$. Scale bars, 11 μm .



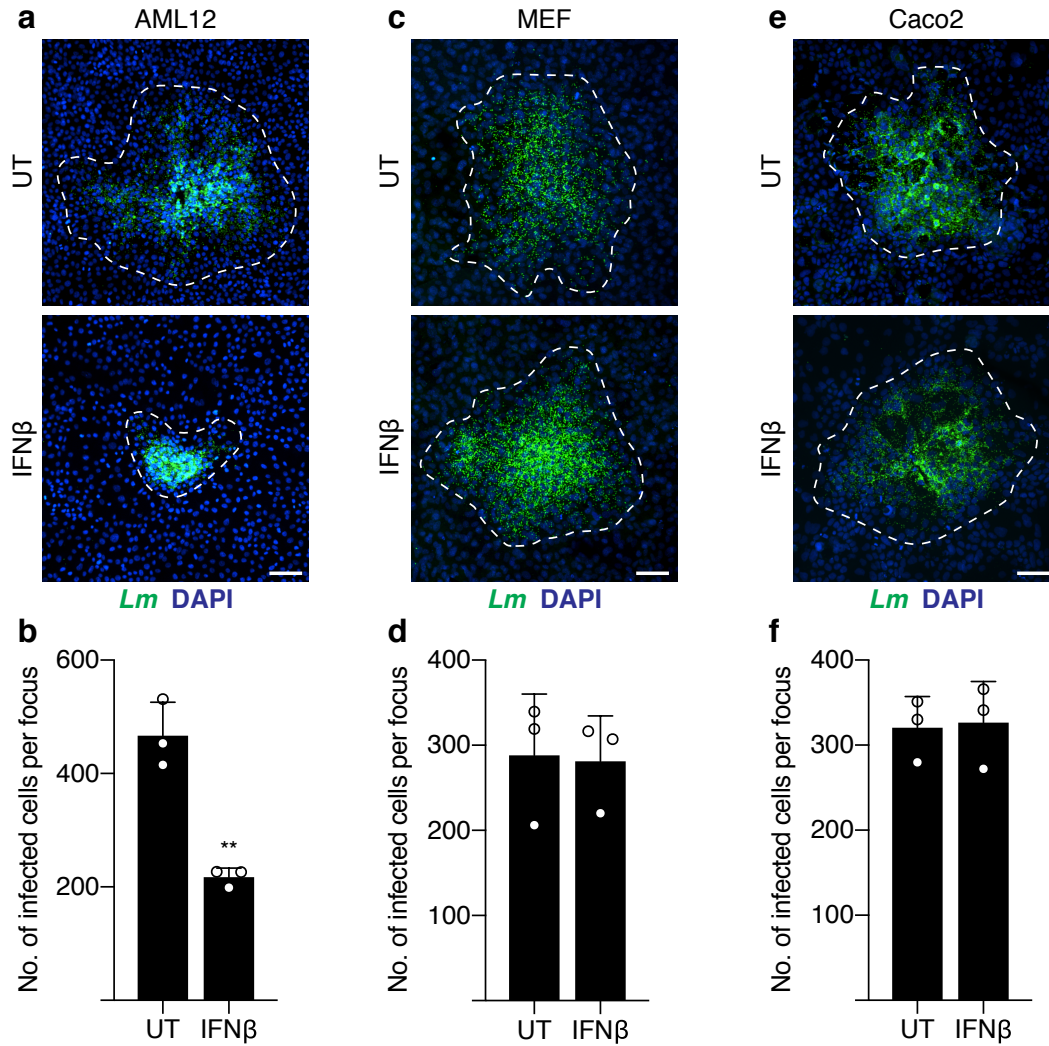
Supplementary Fig. 3 | *Lm* expresses ActA within phagosomes. **a**, Schematic of GFP reporter fusion construct separately integrated in single copy at the tRNA^{ARG} locus of WT *Lm* 10403S strain. *PactA* promoter with the full-length *actA* 5' untranslated region (UTR) and ribosome binding site (rbs). **b**, Representative images of the production of *actA*:GFP measured by Western blot in J774 macrophages infected with indicated GFP reporter strains over 4 h. **c**, Representative image of the production of ActA measured by Western blot in BMDMs treated with and without bafilomycin A infected with indicated strains 4 h p.i. **d-f**, Representative images (**d**) and quantification of ActA-positive bacteria expressing *actA*:GFP (**e**) or *actA*:GFP-positive bacteria in LAMP1-positive compartments (**f**) in RAW macrophages infected with indicated strains 4 h p.i. Data and images shown are means \pm s.d. for at least $n = 3$ independent experiments. *P* value was calculated using one-way ANOVA. **** $P < 0.0001$. Scale bar, 10 μ m. TKO, triple knockout mutant strain $\Delta hlyplcAplcB$ *Lm*. Source data are provided as a Source Data file.



Supplementary Fig. 4 | IFITM3 suppresses LLO degradation in phagosomes. a-c, Representative images (a) and quantification of LLO production measured by Western blot in IFITM3 WT and KO BMDMs infected with (b) WT *Lm* or (c) *actAplcAplcB Lm* over 4 h. Data shown are means \pm standard deviation (s.d.) for n = 3 independent experiments. *P* value was calculated using two-way ANOVA. **P* = 0.0164, *****P* < 0.0001. Source data are provided as a Source Data file.



Supplementary Fig. 5 | Effect of IFITM3 on bulk proteolysis and *Lm* growth in the cytosol. **a, b**, Bulk proteolysis of DQ-BSA-labelled particles in phagosomes treated with IFN β or IFN γ , in IFITM3 WT and KO BMDMs. Slope of the curve's linear portion in **(a)** is displayed in **(b)**. **c**, Growth curve of IFITM3 WT and KO BMDMs infected with WT or $\Delta actA$ *Lm* over 12 h. Cells were seeded at sub-confluence density and infected with an MOI of 1. Data shown are means \pm standard deviation (s.d.) for at least $n = 3$ independent experiments. P value was calculated using one-way ANOVA. **** $P < 0.0001$.



Supplementary Fig. 6 | Type I IFN does not promote *Lm* cell-to-cell spread in AML12, MEF or Caco2 cells. a-f, Representative images (**a**, **c**, **e**) and quantification (**b**, **d**, **f**) of an infection focus assay to measure WT *Lm* cell-to-cell spread. Indicated cell types were treated with or without IFN β and infected for 18 h. Dotted lines delineate edge of the infection foci. Scale bars, 70 μ m. Data shown are means \pm s.d. for n = 3 independent experiments. *P* value was calculated using two-way ANOVA. ***P* = 0.0021.

Supplementary Table

Primer	Sequence
221	AAGTCGACTTATTTGTATAGTTCATCCATGCCATG
432	CGCGGCCGGTGGTATCCCGAATAAAGCAGCC
434	GAAAAGTTCTTCTCCTTTACTCATTTATACTCCCTCCTCGTGATAC
435	GTATCACGAGGAGGGAGTATAAATGAGTAAAGGAGAAGAAGACTTTTC
<i>Ifnβ</i>	F-CCCTATGGAGATGACGGAGA R-CTGTCTGCTGGTGGAGTTCA
<i>Ifnγ1</i>	F-GTAGCCTCACCGCCTATCAC R-GGCCTCTCCTGTGAGTCTA
<i>Tnfα</i>	F-CCCATGGATGTCCCATTTAG R-CAATCAGGAGGGTGTGTGTG
<i>Il4</i>	F-GCCTGCTTTTTCACATGAGGT R-AAATATGCGAAGCACCTTGG
<i>Il10</i>	F-CCAAGCCTTATCGGAAATGA R-TTTTCACAGGGGAGAAATCG
<i>Il12</i>	F-AGCATAAGAGACGCCCTCAA R-GGTGTTACAGGCCCAAAGAA

Supplementary Table 1 | Summary of primers used to generate *Lm* strains, and primers used for qPCR.