Table S1. Primers used in this study

#	Sequence (5' to 3')	Construct	Vector Template	Insert Template	Pair
1	ctttaagaaggagatatacatatggttagtaaaggtgaagaagat	mCherry-LLO-His_6	pET29b-hly	pHpL3-mCherry	2
2	gaatgcagatgcatccttcatatgtttatataattcatccataccacc	mCherry-LLO-His_6	pET29b-hly	pHpL3-mCherry	1
3	ctttaagaaggagatatacatatggttagtaaaggtgaagaagat	mCherry-C484A-His_6	pET29b-hly^{C484A}	pHpL3-mCherry	4
4	gaatgcagatgcatccttcatatgtttatataattcatccataccacc	mCherry-C484A-His_6	pET29b-hly^{C484A}	pHpL3-mCherry	3

Table S2. Bacterial strains used in this study

Strain Code	Parent Strain	Description	Reference
10403S	*L.monocytogenes*	Wild-type	(1)
DP-L2261	*L.monocytogenes*	Δhly	(2)
NF-L1177	*L.monocytogenes*	PrfA* (G145S)	(3)
DP-L6188	*L.monocytogenes*	ΔgshF	(4)
DP-L4351	*L.monocytogenes*	LLO^{C484A}	This Study
DP-L3903	*L.monocytogenes*	Wild-type (ErmR)	(5)
DP-E3570	*E.coli*	pET29b_hly	(6)
DP-E6560	*E.coli*	pET29b_hly^{C484A}	This Study
DP-E6502	*E.coli*	pET29b_hly_mCherry	This Study
DP-E6504	*E.coli*	pET29b_hly^{C484A}_mCherry	This Study

Figure S1.

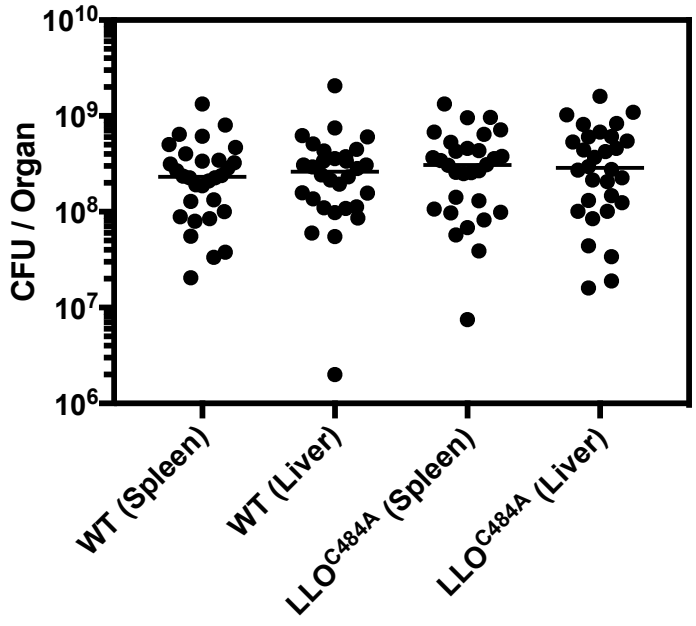
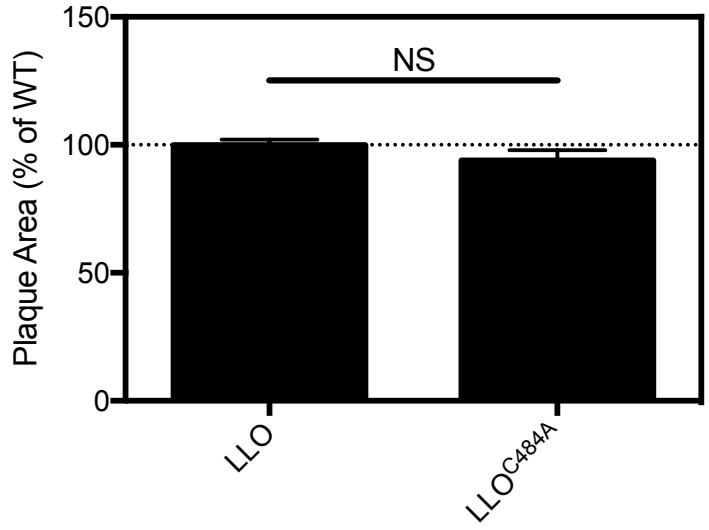
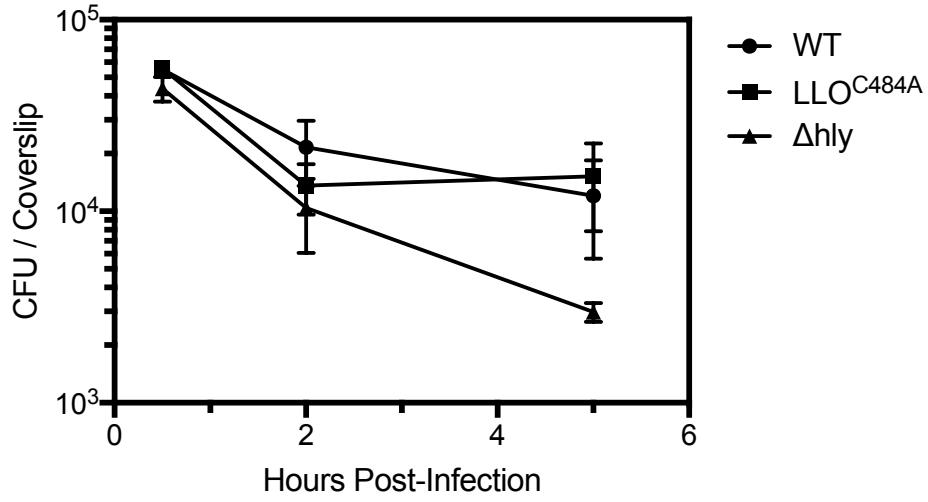


Figure S2.

A.



B.



C.

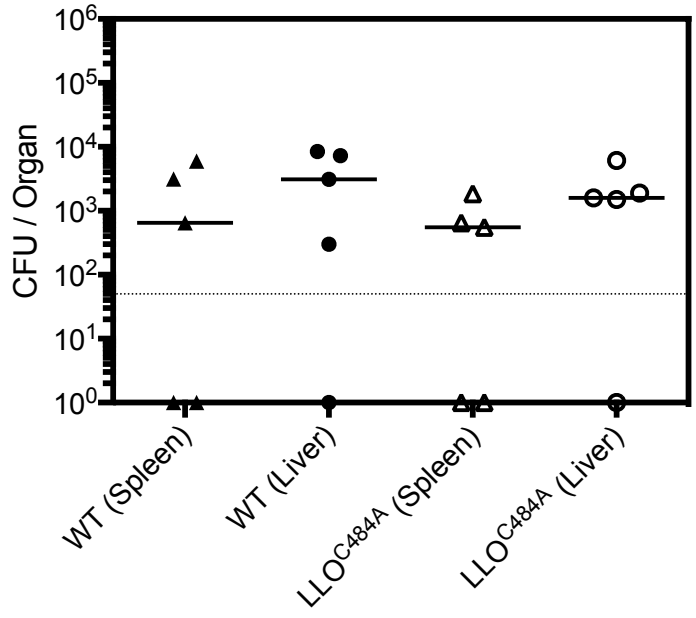
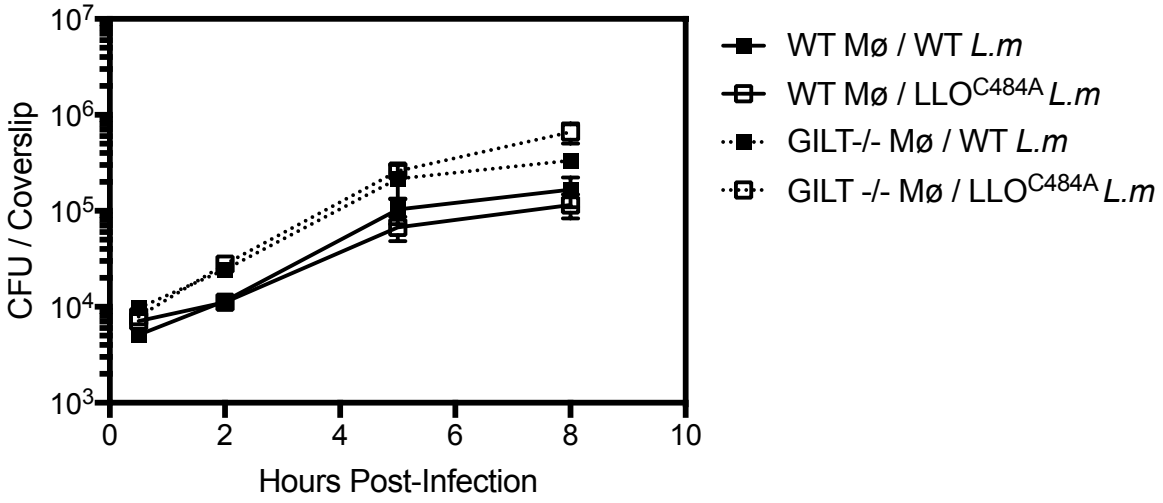


Figure S3.



Supplemental Figure Legends

Figure S1. Non-competitive infection masks the virulence defect of LLO^{C484A} *in vivo.*
Mice were infected intravenously with 1 x 10^5 CFU of either wild-type or LLO^{C484A} mutant bacteria for 48hrs and the final CFU per liver and spleen were enumerated as described in Materials and Methods. Using this model, there is no statistically significant defect of the LLO^{C484A} mutant *in vivo,* highlighting the sensitivity of the competitive model and the mild virulence defect of the LLO^{C484A} strain.

Figure S2. Glutathionylation of LLO is largely dispensable in alternative infection models.
Several infection models were tested for their potential to accentuate the virulence defect of the LLO^{C484A} mutant. **(A)** *In vitro* plaque assays were performed to monitor cell-to-cell spread of *L.monocytogenes* through a monolayer of immobilized L2 fibroblasts. **(B)** Intracellular growth in naturally restrictive resident peritoneal macrophages was assessed as described in supplemental Materials and Methods. **(C)** An *in vivo* oral infection model using contaminated bread was performed as described in supplemental Materials and Methods. No statistically significant defects were observed compared to wild-type bacteria in these models.

Figure S3. GILT is not required for efficient escape and replication of *L.monocytogenes* in bone-marrow derived macrophages.
Wild-type and LLO^{C484A} *L.monocytogenes* were used to infect either wild-type or GILT$^{-/-}$ bone-marrow derived macrophages *in vitro*. CFU per coverslip (~1.2 x 10^5 cells / coverslip) are reported at various time points after infection and representative of intracellular growth. Both strains of bacteria were able to efficiently replicate in GILT$^{-/-}$ BMMs.

Supplemental Materials and Methods

Plaque assay
Plaque assays in L2 murine fibroblasts were performed as previously described (7). Briefly, bacterial cultures were grown overnight at 30 °C, then washed and diluted 1:10 in sterile PBS. Six-well dishes containing 1.2 × 10^6 L2 cells per well were infected with *L. monocytogenes* for 1 h, then washed and overlaid with 3 ml of media containing 0.7% agarose and gentamicin (10 μg ml^{-1}) to prevent extracellular growth. After 3 days at 37 °C, an overlay containing gentamicin and neutral red dye (Sigma) was added and stained overnight. The plates were then scanned and analyzed with ImageJ software (8).

Virulence experiments
Six-to-eight-week-old female CD-1 mice (The Jackson Laboratory) were either infected intravenously with 1 × 10^5 colony-forming units (CFU) in 200ul of PBS or orally with 1 × 10^9 CFU in contaminated bread, (as described previously (9)). Forty-eight hours post-infection the mice were euthanized and spleens and livers

were harvested, homogenized in 5mls or 10mls 0.1% NP-40, respectively, and plated for enumeration of bacterial burdens. All animal work was done in accordance with university regulations. Protocols were reviewed and approved by the Animal Care and Use Committee at the University of California, Berkeley (MAUP# R235-0815B).

Intracellular Growth Curve of Peritoneal Macrophages

Resident peritoneal cavity cells were harvested from six-to-eight-week-old female C57BL/6 mice as described previously (10). The peritoneal exudates were then plated for 3hrs and peritoneal macrophages were enriched for by discarding non-adherent cells. These cells were then collected using cell-scrapers and growth curves were performed as described previously (11).

Supplemental References

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