

S1 Table. Strains and plasmids used in this study

Strain	Description	Source
<i>L. monocytogenes</i> 10403S	WT <i>L. monocytogenes</i>	D. Portnoy lab
$\Delta codY$	<i>codY</i> (LMRG_00730) deletion strain	[19]
$\Delta ilvC$	<i>ilvC</i> (LMRG_01134) deletion strain, a BCAA auxotroph	[19]
$\Delta rli60$	<i>rli60</i> complete deletion strain (220 nt deletion)	This study
$\Delta codY/\Delta rli60$	<i>codY</i> and <i>rli60</i> double deletion strain	This study
<i>codY-R61A</i>	Mutated in CodY BCAA-binding pocket, substitution of arginine-61 to alanine	[20]
<i>codY-R61A/\Delta rli60</i>	<i>codY-R61A</i> and <i>rli60</i> double mutant	This study
WT <i>ilvD-6his</i>	C terminus 6-his tagged <i>ilvD</i> (at the native locus)	This study
$\Delta codY$ <i>ilvD-6his</i>	<i>codY</i> deletion strain with 6-his tagged <i>ilvD</i>	This study
$\Delta rli60$ <i>ilvD-6his</i>	<i>rli60</i> deletion strain with 6-his tagged <i>ilvD</i>	This study
$\Delta codY/\Delta rli60$ <i>ilvD-6his</i>	<i>codY</i> and <i>rli60</i> double deletion strain with 6-his tagged <i>ilvD</i>	This study
WT pPL2 <i>rli60-peptide-EGFP</i>	WT harboring pPL2 <i>rli60-peptide-EGFP</i>	This study
$\Delta codY$ pPL2 <i>rli60-peptide-EGFP</i>	<i>codY</i> deletion strain harboring pPL2 <i>rli60-peptide-EGFP</i>	This study
<i>rli60-ter</i>	CAUUGGGGUGC→GAUUCGCGUCC substitution in the stem of the terminator (at the native locus)	This study
<i>rli60-atg</i>	AUG→AAC substitution in the start codon of the leader peptide (at the native locus)	This study
<i>rli60-rbs</i>	AAGGAGA→AACCACA substitution in the ribosome binding site of the leader peptide (at the native locus)	This study
WT pPL2 Spec	WT harboring pPL2 Spec	This study
WT pPL2 Km	WT harboring pPL2 Km	This study
$\Delta rli60$ pPL2 Spec	<i>rli60</i> deletion strain harboring pPL2 Spec	This study
$\Delta rli60$ pPL2 Km	<i>rli60</i> deletion strain harboring pPL2 Km	This study
<i>codY-R61A/\Delta rli60</i> pPL2 Spec	<i>codY-R61A</i> and <i>rli60</i> double mutant harboring pPL2 Spec	This study
<i>codY-R61A/\Delta rli60</i> pPL2 Km	<i>codY-R61A</i> and <i>rli60</i> double mutant harboring pPL2 Km	This study

<i>E. coli</i> XL-1 Blue	Plasmid propagation strain	Stratagene
<i>E. coli</i> SM-10	For plasmid conjugation to <i>L. monocytogenes</i>	[55]
<i>E. coli</i> K-12 <i>ilvC::Km</i>	Mutated in <i>ilvC</i> , BCAA auxotroph strain	Keio collection (obtained from U. Qimron lab)
<i>E. coli</i> K-12 <i>ilvC::Km pPL2 rli60-luxABCDE</i>	Mutated in <i>ilvC</i> , a BCAA auxotroph strain harboring pPL2 <i>rli60-luxABCDE</i>	This study
<i>E. coli</i> K-12 <i>ilvC::Km pPL2 Δrli60-luxABCDE</i>	Mutated in <i>ilvC</i> , a BCAA auxotroph strain harboring pPL2 <i>Δrli60-luxABCDE</i>	This study

Plasmid	Description	Source
pBHE261 (pKS7 oriT)	A conjugative plasmid for delivery to <i>L. monocytogenes</i> , used for generating clean deletion/substitution strains	[55]
pUC18	A plasmid used for sequencing of 5'RACE products	Thermo Fisher Scientific
pPL2	A chromosomally integrative plasmid of <i>L. monocytogenes</i>	[65]
pPL2 <i>luxABCDE</i>	A promoterless Luciferase reporter system cloned in pPL2	[66]
pPL2 <i>rli60-luxABCDE</i>	<i>rli60</i> locus fused to a luciferase reporter system in pPL2	This study
pPL2 <i>Δrli60-luxABCDE</i>	<i>Δrli60</i> locus fused to a luciferase reporter system in pPL2	This study
pPL2 <i>EGFP</i>	An enhanced green fluorescence protein cloned in pPL2	This study
pPL2 <i>rli60-peptide-EGFP</i>	An in-frame fusion of <i>rli60</i> leader peptide to EGFP cloned in pPL2	This study
pPL2 Spec	A Spectinomycin resistance cassette cloned in pPL2, for mice infections	This study
pPL2 Km	A Kanamycin resistance cassette cloned in pPL2, for mice infections	This study

Additional Supporting Information References

65. Construction, characterization, and use of two *Listeria monocytogenes* site-specific phage integration vectors. *J Bacteriol* 184(15):4177–4186.
66. Bron PA, Monk IR, Corr SC, Hill C, Gahan CGM (2006) Novel luciferase reporter system for in vitro and organ-specific monitoring of differential gene expression in *Listeria monocytogenes*. *Appl Environ Microbiol* 72(4):2876–2884.