Material and methods

Mice

Animal experiments were performed according to the Institut Pasteur guidelines for laboratory animals' husbandry and in compliance with European regulation 2010/63 EU. All procedures were approved by the Animal Ethics Committee of Institut Pasteur, authorized by the French Ministry of Research and registered under #11995-201703115103592 and #14644-2018041116183944.

C57BL/6JRj and BALB/c mice were purchased from Janvier Labs (France) and bred at Institut Pasteur. KIE16P mice expressing humanized E16P E-cadherin[7], iFABP-hEcad mice[15], *Rag2*$^{-/-}$ mice[45], CD3ε$^{-/-}$ mice[46], muMt$^{-/-}$[47], *Ccr2*$^{-/-}$ mice[12], Rosa26CreER^{T2} [48] and Rosa26iDTR[49] were bred at Institut Pasteur. *Fas*$^{lpr\text{-}cg}$ carrying a spontaneous mutation at the Fas locus[50], and Prf1 KO mice deleted for the perforin gene[51] were obtained from Frederic Rieux-Laucat and Fernando Sepulveda, Institut Imagine, Paris, respectively. *Cflar*$^{flox/flox}$ (FLIP$^{f/f}$) mice were obtained from Richard M. Pope (Northwestern University, USA)[52], Met$^{flox/flox}$ from Alain Eychene (Institut Curie, Paris)[53] and *LysM*CreER^{T2} from Florian Greten (Georg Speyer Haus, Germany)[54].

All experiments were performed on mice between 7 and 10 weeks of age, randomly assigned to each different condition. Unless stated otherwise in the figure legends, mice refer to KIE16P mice.

Bacterial strains

All bacterial strains used in this study are presented in Extended Data Table S. To obtain growth curves, bacteria grown overnight in BHI (with chloramphenicol if needed), at 37°C and 200 rpm, were diluted 1:100 in BHI (with chloramphenicol if needed), incubated at 37°C and 200 rpm, and OD_{600} was measured every hour for 9 hours. Growth curves were fitted using Gompertz model to compare parameters between each strain.

Mutagenesis and plasmids

The oligonucleotide primers and plasmids used in this study are listed in Extended Data Table 2 and 3, respectively. Deletion mutants were constructed as described by Monk et *al*.[55]. The flanking regions of the target genes were PCR amplified. After purification, the fragments were stitched together by sequence overlap extension then cloned into the pMAD shuttle vector[56]. The vector was then electroporated into electrocompetent *Lm* cells. After plasmid integration and excision by sequence homology, gene deletion was verified by sequencing the PCR product of the target region.

For *Lm*Δ*inlA* mutants, to avoid alteration of InlB expression in the operon, the *inlA* gene with flanking regions was PCR amplified and cloned into pLR16-pheS plasmid[57] (kind gift from Prof. Anat Herskovits, Tel Aviv University) and a point mutation was introduced via PCR in the *inlA* gene resulting in a premature stop codon. The plasmid was then purified and electroporated into electrocompetent *Lm*Δ*inlA* bacteria. After plasmid integration and excision by sequence homology, allele replacement was verified by sequencing the PCR product of the target region.

The integrative pAD backbone[58] was used to allow expression of TdTomato and GFP. First an EcoRV restriction site was added in pAD between the 5'UTRhly and the ATG start codon, generating the plasmid pCMC12. TdTomato and GFP sequences were codon-optimized for expression in *Lm* using Optimizer (http://genomes.urv.es/OPTIMIZER/), synthesized by Eurofins with EcoRV and SalI flanking restriction sites and inserted in pCMC12 between EcoRV and SalI sites. *inlB* sequences were amplified from EGDe (MBHL0005), CC4 (MBHL0257) and *Listeria innocua* BUG1642 (MBHL0052, expressing a construct where InlB anchoring region has been replaced by the anchoring domain of *S. aureus* protein A[17]) genomic DNAs and all were inserted in pCMC12 between EcoRV and SalI sites. The β-lactamase construct was described in Quereda et *al*[59]. All constructs were electroporated into electrocompetent *Lm* cells. Integration was verified by PCR.

Infections and bacterial enumerations

Bacterial inocula were prepared by centrifugation of a bacterial culture grown in BHI, at 37°C and shaken at 200 rpm, until OD_{600} of 0.8 (8.10^8 viable bacteria per mL), washed in PBS and resuspended in PBS at the appropriate dilution. For oral inoculation, 0.2 mL of bacteria were mixed to 0.3 mL of PBS containing 50 $mg.mL^{-1}$ of $CaCO_3^-$ (Sigma) and injected intragastrically via a feeding needle (ECIMED) to isoflurane-anesthetized mice. For intravenous infection, 0.1 mL of bacteria were injected in the tail vein using a 25G needle. For intracerebral infections, mice were anesthetized in 3% isoflurane, then received 10 µl of bacterial suspension by intracranial injection using a 26G needle, inserted approximately 2 mm anterior to the bregma, 1 mm laterally and 1.5 mm ventrally.

For immunization experiments, mice were first infected with WT or Δ*inlB* strains by oral gavage with 5.10^7 CFU. After 30 days, mice were then infected intragastrically for the second challenge with 2.10^9 WT bacteria for 3 days.

At indicated times, mice were euthanized and their organs aseptically harvested. Intestine, colon and cecum were incubated with gentamicin 100 µg/mL for 2 hours to eliminate extra-tissular bacteria. All organs were homogenized in PBS with a tissue homogenizer (UltraTurrax T-25 basic, IKA works). Serial dilutions of the homogenate were

plated onto BHI agar (or ALOA for feces and intestinal content) and CFUs enumerated. CFUs are expressed per mL for blood and per whole organ otherwise.

Competition index experiments were performed as described in Disson *et al*[60]. Briefly, a 1:1 ratio of wild-type bacteria, expressing TdTomato, and mutant bacteria, expressing GFP, was injected into mice, confirmed by CFUs enumeration of the inoculum onto BHI agar. WT and mutant bacteria CFUs were distinguished by colonies' color onto BHI agar. Competition index is calculated as the ratio of WT versus mutant CFUs in each organ. Except stated otherwise, competition indexes were performed 5 days post-inoculation, a time point late enough to have consistent CNS invasion and induction of adaptive immune responses but before humane endpoints were reached. Whenever the mouse strains were permissive to *Lm* oral infection, intragastric inoculation was used for competition index assays.

Drug treatments of mice

All drugs used in this study and their mode of delivery are presented in Extended Data Table 4.

RNA isolation and qRT-PCR

For *in vitro* analysis, bacteria were grown in BHI at 37°C and 200 rpm until OD_{600} 0.8. They were then centrifuged, lysed in resuspension buffer (10% glucose, 12.5 mM Tris, 10 mM EDTA in nuclease-free water), transferred into Precellys tube (Ozyme) containing 0.1 mm ceramic beads (Ozyme) and acid phenol (Sigma) and homogenized using a Precellys 24 apparatus (Ozyme). Aqueous phase was transferred into a new tube containing TRIzol (Invitrogen) and chloroform (Sigma), shaken and centrifuged at maximum speed at 4°C for 15 min. Aqueous phase was transferred into a new tube containing chloroform, shaken and centrifuged at maximum speed at 4°C for 15 min. Aqueous phase was transferred into a new tube containing 1 volume of isopropanol (Sigma) and 0.1 volume of 3M sodium acetate (Sigma), incubated for at least 20 min at -20°C.

For *in vivo* analysis, spleens were harvested aseptically and single cells suspensions were obtained by homogenizing through a 40 µm cell strainer. Cells were lysed in TRIzol and chloroform, shaken and centrifuged at maximum speed at 4°C for 15 min. Aqueous phase was transferred into a new tube containing 1 volume of isopropanol, incubated for at least 20 min at -20°C.

Both for *in vitro* and *in vivo* samples, RNAs were pelleted at maximum speed at 4°C for at least 20 min and washed three times in 80% ethanol (Sigma), air-dried and dissolved in RNase-free water. Total RNA was reverse-transcribed

using hexameric random primers (Invitrogen) and M-MLV reverse transcriptase (Invitrogen) following manufacturer's instructions. Quantification of gene expression was performed using Power SYBR™ Green PCR Master Mix, a Step-One Real-Time PCR apparatus (both from Applied Biosystems) and primers listed in Extended Data Table 2. Expression of *inlA* and *inlB* was normalized to that of *gyrB* and compared to EGDe using the ΔΔCt method.

Flow cytometry

Mice were euthanized at the indicated times post-infection. Functional characterization of $CD8^+$ T cells and infected monocytes were performed after iv inoculation, unless stated otherwise, which allows for less intra-animal variation and thus reduces the number of animals needed for experiments. Counting and characterization of infected cells were performed at 4 days post-inoculation (both orally and iv), when bacteremia is the highest. For orally infected mice, the bacteremia being very low even at 4 days post-inoculation, blood of 3 mice was pooled and analyzed as one sample. Spleens, mesenteric lymph nodes (MLN) and blood (by cardiac puncture in heparin-coated syringe) were harvested aseptically. Single cells suspensions were obtained from spleen and MLN by homogenizing through a 40 μm cell strainer. After red blood cells lysis using 1 X RBC lysis buffer (eBiosciences), cells were washed in Cell Staining Buffer (CSB) (BioLegend) before further processing. If required, cells were labeled using the LiveBLAzer™ FRET-B/G Loading Kit with CCF2-AM (Invitrogen), a fluorescent substrate of β-lactamase that can cross the plasma membrane. Presence of β-lactamase-expressing bacteria in cells induces a shift in the fluorescence emission of the CCF2-AM substrate from 518 nM (green) to 447 nM (blue) upon excitation with the 405 nM laser and thus allows for identification of infected cells. Cells were loaded with the CCF2-AM substrate following manufacturer's instructions for 2h30 at room temperature in CSB containing 1mM probenecid (Sigma). After washing, cells were blocked using CD16/32 (BioLegend) for 5 min at room temperature, washed in CSB, stained with the appropriate antibodies (listed in Extended Data Table 5) for 45 min at 4°C and washed in CSB. If no intracellular staining was required, cells were suspended in CSB containing CountCAL beads (Sony) for absolute counting of cells. For intracellular staining, cells were fixed for 20 min at room temperature in IC fixation buffer (eBiosciences), washed three times in 1X permeabilization buffer (eBiosciences), incubated with primary antibodies for 1 hour at room temperature, washed and incubated with secondary antibodies for 45 min at room temperature. After washing, cells were suspended in CSB containing CountCAL beads for absolute counting of cells. Cells were acquired on a Fortessa X-20 SORP apparatus

(BD biosciences) and analyzed using FlowJo software (TreeStar). B-cells were defined as $CD45^+$ $CD19^+$ $CD3^-$; $CD8^+$ T-cells as $CD45^+$ $CD3^+$ $CD8^+$ $CD19^-$; LLO-specific $CD8^+$ T-cells as $CD45^+$ $CD3^+$ $CD8^+$ LLO-pentamer$^+$ $CD19^-$; $CD4^+$ T-cells as $CD45^+$ $CD3^+$ $CD4^+$ $CD19^-$; granulocytes as $CD45^+$ $Ly6G^+$ $CD3^-$ $CD19^-$; patrolling monocytes in blood as $CD45^+$ $CD11b^+$ $CD11c^+$ $CD3^-$ $CD19^-$ $Ly6G^-$ $Ly6C^-$; inflammatory monocytes as $CD45^+$ $CD11b^+$ $Ly6C^{high}$ $CD3^-$ $CD19^-$ $Ly6G^-$ $CD11c^-$; macrophages in spleen as $CD45^+$ $CD11b^+$ $CD11c^+$ $CD3^-$ $CD19^-$ $Ly6G^-$ $Ly6C^-$; dendritic cells as $CD45^+$ $CD11c^+$ $CD3^-$ $CD19^-$ $Ly6G^-$ $Ly6C^-$; infected cells as CCF2-blue and non-infected cells as CFF2-green. Number of cells are expressed per mL of blood and per spleen.

CTL assays

Infected mice were euthanized 3 days post-infection, a time point where activated $CD8^+$ T cells are already present but prior to cell death induced in infected monocytes. Spleens were harvested aseptically and cells prepared as described above for flow cytometry, resuspended in CSB and sorted on a FACSAria III apparatus (BD Biosciences) into fetal calf serum-containing tubes. Activated $CD8^+$ T-cells were defined as $CD45^+$ $CD3^+$ $CD8^+$ $CD69^+$ $CD19^-$, infected inflammatory monocytes as $CD45^+$ $CD11b^+$ $Ly6C^{high}$ $CD3^-$ $CD19^-$ $Ly6G^-$ $CD11c^-$ CCF2-blue and non-infected inflammatory monocytes as $CD45^+$ $CD11b^+$ Ly-C^{high} $CD3^-$ $CD19^-$ $Ly6G^-$ $CD11c^-$ CCF2-green. For mock-treated mice, $CD8^+$ T-cells and inflammatory monocytes were sorted. After sorting, cells were washed and resuspended in RPMI medium (Invitrogen) containing 10% fetal calf serum. Activated $CD8^+$ T-cells and monocytes (infected and non-infected), isolated from independent mice, were co-incubated at the indicated ratio for 80 minutes at 37°C, washed and fixed with IC fixation buffer overnight at 4°C. After three washes in 1X permeabilization buffer, cells were stained with anti-cleaved caspase-3 antibody for 1 hour at room temperature, washed and stained with secondary antibody for 45 minutes at room temperature. After washing, cells were acquired on a X-20 Fortessa SORP apparatus and percentage of monocytes positive for cleaved caspase-3 signal analyzed using FlowJo software.

Half-life of infected monocytes

Mice were infected intravenously with 10^4 *Lm* CFUs. At day 2 post inoculation, single cells suspensions were obtained from spleens of non-infected mice as described in the flow cytometry section. Cells were then labelled with Vybrant DiD solution (Invitrogen™) for 20 minutes at 37°C, according to the manufacturer's instructions. After 3 washes in DMEM-F12 medium, cells were resuspended in PBS and immediately transferred into infected mice intravenously.

Mice were then euthanized at indicated time points post-infection and spleens and blood samples were harvested aseptically. Single cells suspensions were then prepared and loaded with the CCF2-AM substrate as described above. After washing, cells were acquired on a X-20 Fortessa SORP apparatus and the percentage of infected monocytes positive for Vybrant DiD signal was analyzed using FlowJo software.

Estimation of half-life times from exponential fits

Means and standard deviations of infected monocytes were estimated at each time point (0, 12, 24, 36 hour) assuming they are drawn from a normal distribution. At each time point, random values calculated from the normal distribution were drawn and a new decay curve was simulated. To estimate the half-life of the infected monocytes, 1000 curves were simulated and fitted with an exponential decay. Non-convergent fits or those with $R^2 < 0.97$ were discarded; half-life times were calculated from the remaining fits, and distributions are inferred from the [0 ; 95] percentiles. The differences between half-life were assessed with a non-parametric Mood test in which the null hypothesis assumes that the compared samples come from populations with the same median.

Fas ligand treatment

Infected mice were euthanized 3 days post-infection and spleens were harvested aseptically. Cells were prepared and sorted in the same conditions as for CTL assays. Infected and non-infected inflammatory monocytes were sorted into fetal calf serum-containing tubes. After sorting, cells were washed and resuspended in RPMI medium containing 10% fetal calf serum. Cells were then treated with either HA antibody as control (Cell Signaling) or with HA antibody plus recombinant mouse Fas ligand/TNFSF6 (R&D systems) for 80 minutes at 37°C. After washing, cells were fixed overnight in IC fixation buffer. After three washes in 1X permeabilization buffer, cells were stained with anti-cleaved caspase-3 antibody for 1 hour at room temperature, washed and stained with secondary antibody for 45 minutes at room temperature. After washing, cells were acquired on a X-20 Fortessa SORP apparatus and percentage of monocytes positive for cleaved caspase-3 signal analyzed using FlowJo software.

Caspase 8 activity assay

Mice were inoculated intravenously, treated with either BYL-719 or Capmatinib and euthanized at 3 days post-infection. Single cells suspensions were prepared from spleen as described in the flow cytometry section. After CCF2-AM loading, blocking with CD16/32 and staining with the appropriate antibodies, cells were incubated for 30 min at 37°C with Red-IETD-FMK from CaspGLOW^{TM} Red Active Caspase-8 Staining Kit (Clinisciences), washed and suspended in Wash buffer containing CountCAL beads for absolute counting of cells. Cells were immediately acquired on a Fortessa X-20 SORP apparatus and analyzed using FlowJo software.

Transfer of infected monocytes

Infected KIE16P mice were euthanized 3 days post-infection and spleens were harvested aseptically. Cells were prepared as described above and sorted in the same conditions as for CTL assays. Infected monocytes from 6 mice were sorted into fetal calf serum-containing tubes, pooled, washed and injected into a naïve mouse treated with gentamicin, corresponding to an inoculum of 10^4 live CFUs. Two days post-injection, the mouse was euthanized, organs harvested aseptically and bacterial enumerated as described above.

LysM-CreER$^{T2+/-}$ × *Rosa26*-iDTR$^{+/-}$ and their littermates were infected intravenously with CC4-*Lm*, treated with tamoxifen to allow for Cre expression, euthanized 4 days after infection and spleens harvested aseptically. Cells were prepared as described above and sorted in the same conditions as for CTL assays. Infected monocytes from 3 mice were sorted into fetal calf-serum containing tubes, pooled (corresponding to an inoculum of $2x10^4$ live CFUs), washed and injected into a naïve recipient mouse (same genotype as donor mice) treated with both diphtheria toxin and gentamicin, to kill Cre-expressing monocytes and extracellular bacteria. Four days post-injection, mice were euthanized, organs harvested aseptically and bacteria enumerated as described above.

Bacterial enumeration in infected monocytes

Infected mice were euthanized 4 days post-infection, when the bacteremia is the highest, and spleens and blood were harvested aseptically. Cells were prepared as described above and sorted in the same conditions as described for CTL assays. Monocytes were collected into fetal calf serum-containing tubes, washed and resuspended into 0.1% triton, serially diluted in PBS and plated on BHI agar.

Immunofluorescence labelling for microscopy

For brain sections microscopy, mice were infected with a higher inoculum, 5.10^5 CFUs, and euthanized 2 days post-iv inoculation, a time point with enough crossing events to allow analysis but without damages to the blood-brain barrier. Brain hemispheres were fixed in 4% paraformaldehyde in PBS overnight at 4°C then washed in PBS, embedded in 4% agarose and sectioned into 40 µm-thick slices using a vibratome (ThermoScientific, HM 650V). Slices were washed in PBS then incubated for 2 hours in blocking-permeabilization solution (10% goat serum, 4% fetal calf serum and 0.4% Triton X-100 in PBS). Tissues were then labeled with the appropriate primary antibodies (listed in Table S5) overnight at 4°C in mild blocking conditions (4% goat serum, 4% fetal calf serum and 0.4% Triton X100 in PBS), washed in PBS, then incubated with secondary antibodies (listed in Table S5), Hoechst-3342 and Phalloidin-Alexa 647 for 2 hours at room temperature. Tissues were washed in PBS and then mounted on glass slides under coverslips in mounting medium (Invitrogen). The slides are let in obscurity overnight before observation under a Zeiss LM710 or LM700 microscope and acquisition with the ZEN software.

For monocytes microscopy, sorted monocytes were seeded on poly-D-lysine-coated 96 wells-plates, fixed in 4% paraformaldehyde in PBS overnight at 4°C and then washed in PBS. Cells were then permeabilized in 0.1% Triton X-100 for 10 minutes, incubated with blocking solution (5% BSA in PBS) for 30 minutes at room temperature, then labeled with anti-*Lm* antibody for 1 hour at room temperature in PBS-BSA, washed in PBS, and then incubated with anti-rabbit secondary antibody, Hoechst-3342 (ThermoFisher) and Phalloidin-Alexa 647 (ThermoFisher) for 1 hour at room temperature. For c-Met staining, cells were incubated with anti-c-Met in mild blocking conditions (2.5% BSA, 0.2% Triton X-100 in PBS) overnight at 4°C, washed in PBS and incubated with anti-*Lm* overnight at 4°C. For p-Akt and LAMP-1 staining, cells were labelled with primary antibodies solution (anti p-Akt or anti LAMP-1 and anti-*Lm*) in mild blocking conditions (2.5% BSA, 0.2% Triton X-100 in PBS) overnight at 4°C then washed in PBS. Cells were then incubated with appropriate secondary antibodies solution (see Extended Data Table 5) and Hoechst-3342 for 2h at room temperature, washed in PBS and left in PBS at 4°C before observation under a Zeiss LM710 microscope and acquisition with the ZEN software. For microscopy of BHI grown-bacteria, 50 µL of the overnight culture were diluted in 1 mL PBS, spun down in a microfuge for 2 minutes, fixed in 4% paraformaldehyde in PBS for 15 minutes at room temperature then washed in PBS. Bacteria were then permeabilized using 0.5% Triton X100 for 10 min, washed in PBS, then incubated with blocking solution (1% BSA in PBS) for 30 minutes. Next, bacteria were labelled with anti-InlB primary antibody in blocking solution (1% BSA in PBS) for 1 hour at room temperature, washed 3 times in PBS, then incubated with anti-rabbit secondary antibody for 1h at room temperature. After 3 washes in PBS, bacteria were

resupended in Hoechst solution (dilution 1/5000 in PBS) for 15 minutes at room temperature, washed twice with PBS then resuspended in 4 µL PBS. Finally, the bacterial suspension was loaded onto a glass slide coated with 1% agarose gel and a coverslip.

Immunoblotting

To assess InlB expression level, bacterial cultures at OD_{600} of 0.8 were centrifuged at 3000 g for 10 minutes. Pellets were then incubated with B-PER™ Complete Bacterial Protein Extraction Reagent (ThermoFisher Scientific) for 15 minutes at room temperature and centrifuged at 16,000 g for 20 minutes to obtain lysates.

Lysates were mixed with reducing sample buffer (NuPAGE, Invitrogen) for electrophoresis and subsequently transferred onto a nitrocellulose membrane. Membranes were then blocked with 5% non fat milk diluted in PBS Tween 0.1 % for 1 hour and incubated with primary antibodies in blocking solution for 2 hours at room temperature. After 1 hour of secondary antibody incubation, immunodetection was performed by using a chemiluminescence kit (Amersham™ ECL™ Prime, GE Healthcare), and bands were revealed using the PXi imaging system (SYNGENE).

***In vitro* monocytes invasion assays**

C57BL/6JRj mice were euthanized and bone marrow was collected aseptically. Cells were washed in PBS, red blood cells lysed as described in the flow cytometry section and monocytes isolated using the mouse Monocyte Isolation Kit (Miltenyi Biotec) following manufacturer's instructions. Cells were incubated overnight at 37°C in RPMI + 10% fetal calf serum and penicillin/streptomycin, washed in RPMI, plated in 96 wells-plate, infected with GFP-expressing *Lm* at MOI 5 for 1 hour at 37°C and treated with 50 µg/mL gentamicin for 1 hour at 37°C. For bacterial enumeration, cells were washed in PBS, lysed in 0.1% triton, serially diluted in PBS and plated onto BHI agar. For flow cytometry analysis, cells were washed, fixed in IC fixation buffer in presence of CountCAL beads, acquired on a X-20 Fortessa SORP apparatus and analyzed using FlowJo software.

***In vitro* Vero cells infection**

Vero cells were seeded on poly-D-lysine-coated 96 wells-plates in DMEM (Invitrogen) + 5% fetal calf serum and penicillin/streptomycin 24 hours prior to infection. On the day of infection, cells were washed three times in DMEM + 0.2% fetal calf serum and incubated in this medium for four hours. Bacteria grown in BHI at 37° and 200 rpm until

OD_{600} 0.8 were centrifuged, washed in PBS, suspended in DMEM and added to the cells at a MOI of 50. After 1 min centrifugation at 200 g, cells were incubated at 37° for the indicated times, fixed for 15 min in 4% PFA and washed three times in PBS. Staining for microscopy was performed as for sorted monocytes in the above section.

Statistical analysis

Statistical details and number of replicates are found in the corresponding Figure legends. All statistical tests were two-sided. Analyses were performed using GraphPad Prism 8 Software.

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Table S1

Strains	CLIP number	Collection number	Plasmid	Antibiotic resistance	Comment	Reference
EGDe		MBHL0005				*(6)*
10403S		MBHL0308				(6)
CC1	2007/00596	MBHL 0237				(6)
CC4	2009/00558	MBHL 0257				(6)
CC6	2009/01092	MBHL 0255				(6)
EGDe β-lactamase		MBHL396	pCMC11	Chloramphenicol		This study
CC1 β-lactamase	2007/00596	MBHL398	pCMC11	Chloramphenicol		This study
CC4 β-lactamase	2009/00558	MBHL400	pCMC11	Chloramphenicol		This study
CC6 β-lactamase	2009/01092	MBHL425	pCMC11	Chloramphenicol		This study
EGDe TdTomato		MBHL418	pCMC34	Chloramphenicol		This study
CC1 TdTomato	2007/00596	MBHL416	pCMC34	Chloramphenicol		This study
CC4 TdTomato	2009/00558	MBHL412	pCMC34	Chloramphenicol		This study
CC6 TdTomato	2009/01092	MBHL420	pCMC34	Chloramphenicol		This study
EGDe ΔInlB		MBHL 0489				This study
CC1 ΔInlB	2007/00596	MBHL 0490				This study
CC4 ΔInlB	2009/00558	MBHL 0491				This study
CC6 ΔInlB	2009/01092	MBHL 0492				This study
CC1 ΔInlA	2007/00596	MBHL 0493				This study
CC4 ΔInlA	2009/00558	MBHL 0494				This study
CC6 ΔInlA	2009/01092	MBHL 0495				This study
CC4 ΔInlB β-lactamase	2009/00558	MBHL439	pCMC11	Chloramphenicol		This study
CC4 ΔInlB TdTomato	2009/00558	MBHL475	pCMC34	Chloramphenicol		This study
EGDe ΔInlB GFP		MBHL449	pCMC44	Chloramphenicol		This study
CC1 ΔInlB GFP	2007/00596	MBHL451	pCMC44	Chloramphenicol		This study
CC4 ΔInlB GFP	2009/00558	MBHL453	pCMC44	Chloramphenicol		This study
CC6 ΔInlB GFP	2009/01092	MBHL455	pCMC44	Chloramphenicol		This study
EGDe ΔInlB + InlB full length from CC4		MBHL457	pCMC58	Chloramphenicol		This study
CC4 ΔInlB + InlB full length from CC4	2009/00558	MBHL469	pCMC58	Chloramphenicol		This study
CC4 ΔInlB + released InlB from CC4	2009/00558	MBHL471	pCMC59	Chloramphenicol		This study
CC4 ΔInlB + anchored InlB from CC4	2009/00558	MBHL473	pCMC60	Chloramphenicol		This study
EGDe ΔInlB + InlB full length from EGDe		MBHL487	pCMC61	Chloramphenicol		This study
CC4 ΔInlB + InlB full length from EGDe	2009/00558	MBHL488	pCMC61	Chloramphenicol		This study
CC4 ΔActA β-lactamase	2009/00558	MBHL0497	pCMC11	Chloramphenicol		This study
Listeria Innocua InlB anchored		MBHL 0052 (BUG1642)	p1B1			(*17*)
CC4 ΔInlAΔInlB	2009/00558	MBHL0496				This study
CC1	2015/00085				Clinical strain	This study
CC1	2016/01406				Clinical strain	This study
CC1	2016/01398				Clinical strain	This study
CC1	2015/00918				Clinical strain	This study
CC1	2016/00717				Clinical strain	This study
CC1	2011/00412				Clinical strain	This study
CC1	2016/01677				Clinical strain	This study
CC1	2015/01910				Clinical strain	This study
CC1	2005/00008				Clinical strain	This study
CC4	2015/00091				Clinical strain	This study
CC4	2015/00895				Clinical strain	This study
CC4	2016/01178				Clinical strain	This study
CC4	2016/01062				Clinical strain	This study
CC4	2015/01543				Clinical strain	This study
CC4	2005/00190				Clinical strain	This study
CC4	2009/00994	MBHL 0353			Clinical strain	This study
CC4	2012/01728	MBHL 0352			Clinical strain	This study
CC4	2013/00255	MBHL 0350			Clinical strain	This study
CC4	2013/00344	MBHL 0354			Clinical strain	This study
CC6	2016/00566				Clinical strain	This study
CC6	2016/00074				Clinical strain	This study
CC6	2016/00783				Clinical strain	This study
CC6	2015/01823				Clinical strain	This study
CC6	2015/00880				Clinical strain	This study
CC6	2015/00001				Clinical strain	This study
CC6	2016/00551				Clinical strain	This study
CC6	2016/01114				Clinical strain	This study
CC6	2015/01823				Clinical strain	This study

Table S2

Oligos	sequence	Comment
CMC15	GGTTAAAAAATGTAGAAGGAGAGTGATATCCATGAGTAAAGGAGAAGAACTTTTCACTGG	Addition of EcoRV site in pAD to obtain pCMC12-*Forward*
CMC16	CCAGTGAAAAGTTCTTCTCCTTTACTCATGGATATCACTCTCCTTCTACATTTTTTAACC	Addition of EcoRV site in pAD to obtain pCMC12-*Reverse*
CMC37	GCGCCGATATCCATGGTTTCTAAAGGTGAAGAAGTTATTAAAGAATTC	TdTomato (codon-optimized) cloning in pCMC12-*Forward*
CMC38	GCGCCGTCGACTTATTTATATAATTCATCCATACCATATAAGAATAAATGATGAC	TdTomato (codon optimized) cloning in pCMC12-*Reverse*
CMC62	GCGCCGATATCCATGTCTAAAGGTGAAGAATTATTC	GFP (codon-optimized) cloning in pCMC12-*Forward*
CMC63	GCGCCGTCGACTTATTTATATAATTCATCCATACC	GFP (codon-optimized) cloning in pCMC12-*Reverse*
CMC195	GCGCGGATATCCATGAAAGAAAAGCACAACCC	InlB cloning in pCMC12-*Forward*
CMC196	GCGCGGTCGACTTATTTCTGTGCCCTTAAATTAG	InlB cloning in pCMC12-*Reverse*
CMC197	GCGCGGTCGACTTACGTCCCTGCTTCTACTTTTG	InlB without anchoring region cloning in pCMC12-*Reverse*
CMC198	GCGCGGTCGACTTATAGTTCGCGACGACG	InlB with anchoring region of spA cloning in pCMC12-*Reverse*
inlA1F	ACGTGTCGACAGATAACACAATCACACCGTGT	inlA1: upstream flanking region of inlA
inlA1R	TACACTACTTTATATACACTCCTTTTTCAATAGTTAGAAACA	
inlA2F	AGTGTATATAAAGTAGTGTAAAGAGCTAGATGTGGT	inlA2: downstream flanking region of inlA
inlA2R	ACGTGGATCCTTTTCAAGTTTGCTAAGGGCTTT	
inlB1F	ACGTGTCGACACAACGACTCAAGCAGTAGACT	inlB1: upstream flanking region of inlB
inlB1R	TTTTCGTAGGACTATCCTCTCCTTGATTCTAGTTAT	
inlB2F	AGAGGATAGTCCTACGAAAAGCTATTTCTAAA	inlB2: downstream flanking region of inlB
inlB2R	ACGTGGATCCATGCTATCCACATTTTGGCT	
inlAB2R	TTTTCGTAGGTATATACACTCCTTTTTCAATAGTTAGAAACA	inlA1pB2: upstream flanking region of InlA containing overlap with inlB2
inlAB2F	AGTGTATATACCTACGAAAAGCTATTTCTAAA	inlB2pA1: downstream flanking region of InlB containing overlap with inlA1
actA1F	GGGGTCGACTAAAGGTCCACGTCACACCG	actA1: upstream flanking region of actA
actA1R	AACCGCTCCTACTACCATCATCGCACGCAT	
actA2F	TGATGGTAGTAGGAGCGGTTATCAAAATCATTCA	actA2: downstream flanking region of actA
actA2R	TGATGGTAGTAGGAGCGGTTATCAAAATCATTCA	
gyrB_F	AAGTATCTGGCGGACTTCACG	qPCR primer pair for gyrB
gyrB_R	TCACCACGTTCAAAGCGTTG	
inlA_F	CGAAAAATCCTGTGGCACCA	qPCR primer pair for inlA
inlA_R	TTTGCGGAAGGTGGTGTAGT	
inlB_F	AAGCACAACCCAAGAAGGAA	qPCR primer pair for inlB
inlB_R	CGGTGATAGTCTCCGCTTGT	

Table S3

Plasmids	Comment	Antibiotic resistance	Reference
pAD	chromosomally integrative plasmid	Chloramphenicol	(*58*)
pCMC11	pAD encoding for codon-optimized beta-lactamase anchored to Listeria cell-wall	Chloramphenicol	(*59*)
pCMC12	pAD modified to add an EcoRV restriction site between UTRhly and starting codon	Chloramphenicol	this study
pCMC34	pCMC12 encoding for codon-optimized TdTomato	Chloramphenicol	this study
pCMC44	pCMC12 encoding for codon-optimized GFP	Chloramphenicol	this study
pCMC58	pCMC12 encoding for full length InlB from CC4	Chloramphenicol	this study
pCMC59	pCMC12 encoding for InlB from CC4 truncated of its anchoring region	Chloramphenicol	this study
pCMC60	pCMC12 encoding for InlB from CC4 with the anchoring region of SpA	Chloramphenicol	this study
pCMC61	pCMC12 encoding for full length InlB from EGDe	Chloramphenicol	this study
pMAD		Erythromicin	(*56*)
pMAD-delta-inlA	pMAD+inlA1+inlA2, inlA deletion	Erythromicin	this study
pMAD-delta-inlB	pMAD+inlB1+inlB2, inlB deletion	Erythromicin	this study
pMAD-delta-inlAB	pMAD+inlA1+inlB2, inlAB deletion	Erythromicin	this study
pMAD-delta-actA	pMAD+actA1+actA2, actA deletion	Erythromicin	this study
pLR16		Chloramphenicol	(*57*)
pLR16-inlA-C1474T	introduce premature stop codon in inlA	Chloramphenicol	this study

Table S4

Drug	Final concentration	Delivery mode	Frequence of delivery	Vehicule	Reference	Supplier	Comments
Ciclosporin A	40 mg/kg	Intraperitoneal	Every day, starting the day prior to infection	Olive oil	Sandimmun	Novartis Pharma	
Anti-CD8 antibody	0.3 mg/mice	Intraperitoneal	3 days and 1 day prior to infection, day of infection and day 4 post-infection	PBS	BX-BE0061	Euromedex	Clone 2.43
Isotype control	0.3 mg/mice	Intraperitoneal	3 days and 1 day prior to infection, day of infection and day 4 post-infection	PBS	BX-BE0090	Euromedex	Clone LTF-2
Z-IETD-FMK	5 mg/kg	Intraperitoneal	Every 2 days, starting the day of infection	DMSO/PBS (1/20)	M3136	Euromedex	Caspase 8 inhibitor
Capmatinib	3 mg/kg	Oral	Every day, starting the day of infection	DMSO/PEG-400 (1/9)	HY-13404	CliniSciences	c-Met inhibitor
Wortmannin	1 mg/kg	Intraperitoneal	Every day, starting the day of infection	DMSO/PBS (1/4)	HY-10197	CliniSciences	Pan PI3K inhibitor
BYL-719	50 mg/kg	Oral	Every day, starting the day of infection	PEG-400	HY-15244	CliniSciences	PI3Ka inhibitor
IC87114	30 mg/kg	Oral	Twice every day, starting the day of infection	PEG-400	HY-10110	CliniSciences	PI3Kd inhibitor
Gentamicin	30 mg/kg	Intraperitoneal or intravenous	Every day, starting the day after infection	PBS	G1272	Sigma	
Tamoxifen	1 mg/kg	intraperitoneal	Every day, staring 5 days prior to infection	Corn oil	T5648	Sigma	
Diphteria toxin	25ng/g	Intraperitoneal and intravenous	Intravenous morning of transfer and then IP at time of transfer and 2 days later	PBS	D0564	Sigma	

Table S5

Antibody target	Fluorophore	Reference	Supplier	Use
CD45	BV605	BLE103139	Ozyme	Flow cytometry
CD3	BV711 or FITC	BLE100241 or BLE100204	Ozyme	Flow cytometry
CD19	PE-Cy5	BLE115510	Ozyme	Flow cytometry
CD11b	BUV737	564443	BD Biosciences	Flow cytometry
CD11c	BV785	BLE117336	Ozyme	Flow cytometry
LY-6G	PE-Cy7 or APC-Cy7	BLE127618 or BLE 127624	Ozyme	Flow cytometry
LY-6C	PE or APC-Cy7	BLE128008 or BLE128026	Ozyme	Flow cytometry
CD8a	BUV395 or PE-Dazzle594	563786 or BLE100762	BD Biosciences or Ozyme	Flow cytometry
CD69	PE-Cy7	BLE104512	Ozyme	Flow cytometry
CD4	PerCP-Cy5-5	BLE100539	Ozyme	Flow cytometry
CD350 (NKp46)	BV650	740627	BD Biosciences	Flow cytometry
Fas/CD95	APC	BLE152604	Ozyme	Flow cytometry
LLO pentamer GYKDGNEYI	PE	178	ProImmune	Flow cytometry
FLIP	-	13250269	ThermoFisher	Flow cytometry
Perforin	PE	BLE154306	Ozyme	Flow cytometry
Granzyme B	Pacific blue	BLE372218	Ozyme	Flow cytometry
CD127	BV421	BLE135023	Ozyme	Flow cytometry
IFNγ	BV711	BLE505835	Ozyme	Flow cytometry
KLRG1	PE	BLE138408	Ozyme	Flow cytometry
Cleaved caspase-3 biotinylated	-	550557	BD Biosciences	Flow cytometry
Streptavidin	APC	BLE405243	Ozyme	Flow cytometry
CD11b	-	550282 or 13-0112	BD Biosciences	Microscopy
LY-6G	-	551459	BD Biosciences	Microscopy
LY-6C	-	Ab54223	Abcam	Microscopy
Listeria	-	294562	Denka Seiken	Microscopy
c-Met	-	AF527	R&D	Microscopy
Phospho-Akt	-	05-1003	Millipore	Microscopy
IgG Rat-Alexa Fluor A488	AF488	A11006	ThermoFisher	Microscopy
IgG Rabbit-Alexa Fluor A546	AF546	A11035	ThermoFisher	Microscopy
LAMP-1	-	553792	BD Pharmigen	Microscopy
InlB	-	(*82*)		Western blot
Ef-tu	-	(*83*)		Western blot
IgG Rabbit-Peroxidase	-	A6154	Sigma	Western blot