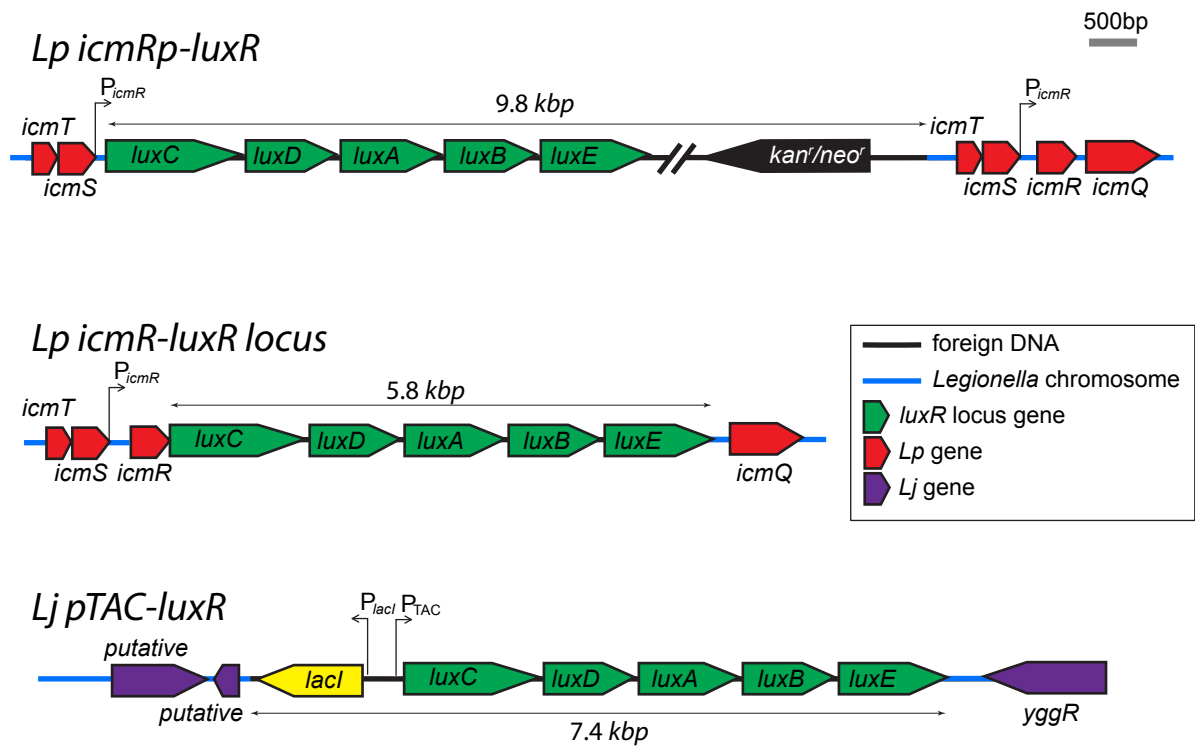
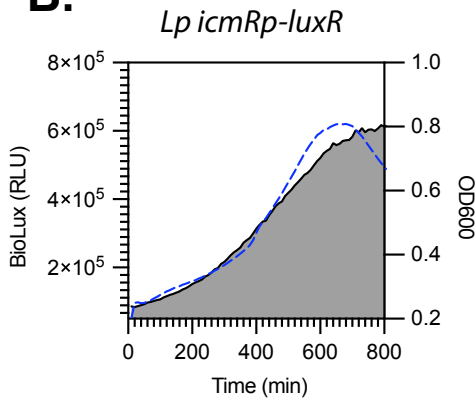


Supplementary Figure 1.

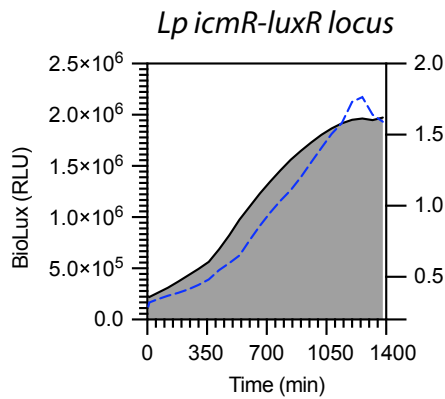
A.



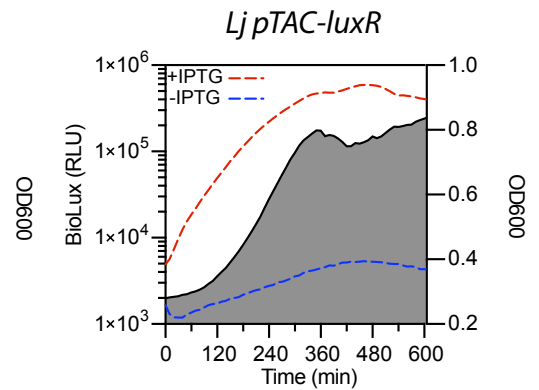
B.



C.

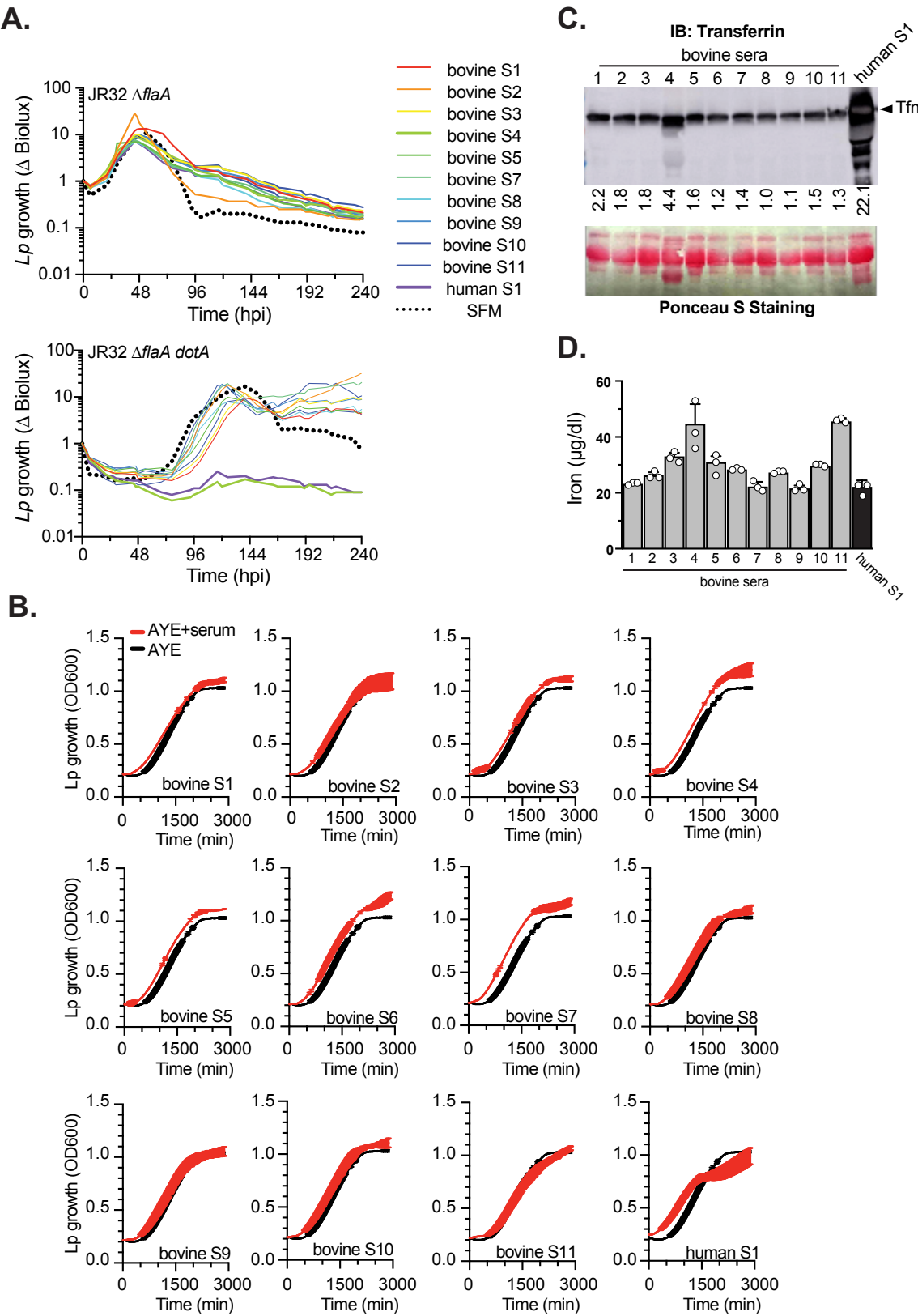


D.



Supplementary Figure 1. Genetics and characterization of the *Legionella* strains encoding the *luxR* operon. (A) Gene topology of the *luxR* operon incorporated on the chromosome in the *L. pneumophila* (Lp) and *L. jordanis* (Lj) reporter strains used in this study. The *luxR* operon under the control of the *icmR* promoter was inserted on the chromosome via insertion duplication mutagenesis (*Lp icmRp-luxR*) for the generation of all bioluminescent reporter strains on the JR32 background used in the study. The *luxR* operon was inserted immediately downstream of the *icmR* gene on the chromosome via allelic exchange (*Lp icmR-luxR* locus) for the generation of all bioluminescent reporter strains on the Lp01 background used in this study. The *luxR* operon under the pTAC IPTG-inducible promoter together with the *lacI* repressor were incorporated within an intergenic region on the Lj chromosome via allelic exchange for the generation of all Lj bioluminescent reporter strains used in this study. (B-D) Axenic growth curves of *Legionella* strains encoding the indicated *luxR* reporters. The grey area curve indicates the change in optical density (OD₆₀₀) over time. The dashed line indicates the changes in total bioluminescence of the bacterial culture over the indicated time period in the absence (blue) (B-D) or in the presence (red) (D) of IPTG. (B-D) Representative of three biological replicates for each experiment is shown.

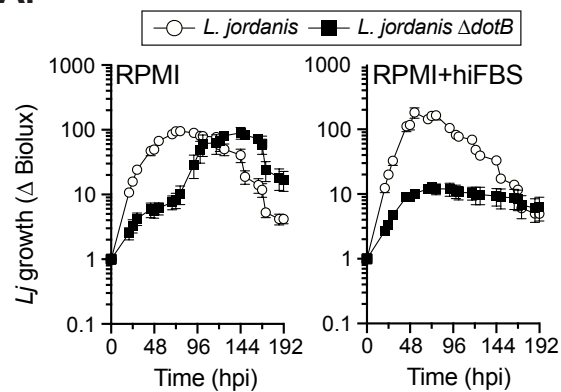
Supplementary Figure 2.



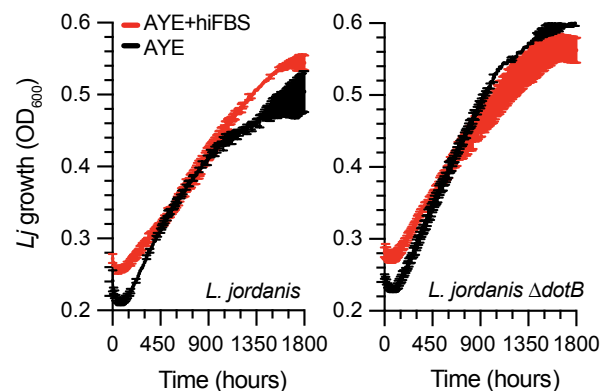
Supplementary Figure 2. The effect of bovine and human serum on Lp replication in macrophage infections and axenic growth cultures. (A) Growth kinetics of the indicated Lp strains with U937 macrophages in serum-free RPMI media (SFM) or RPMI supplemented with serum from different sources (10% v/v). Averages of three technical replicates are shown. One of two biological replicates is shown. (B) JR32 $\Delta flaA dotA$ growth kinetics in axenic AYE media or AYE supplemented with serum from different sources (10% v/v) as measured by changes in the culture optical density over time. Averages of three technical replicates $\pm$ StDev are shown. One of two biological replicates is shown. (C) Immunoblot analysis of the sera used in the experiments shown in (A) and (B). Transferrin band integrated intensity is shown for each lane on the blot and was normalized to the serum with the lowest Transferrin abundance. Total protein content for each serum was detected by Ponceau S staining as shown. (D) Total iron content of the sera used in the study as detected by ferrozine-based colorimetric assay. Averages of three technical replicates $\pm$ StDev are shown. Source data are provided as a Source Data file.

Supplementary Figure 3.

A.

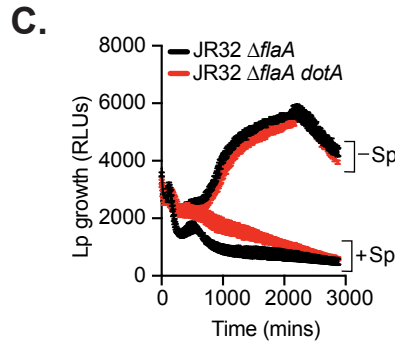
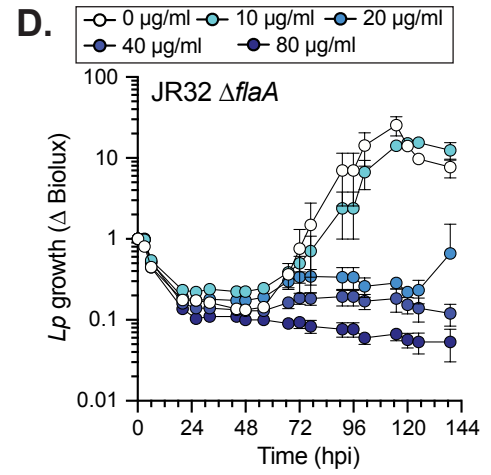
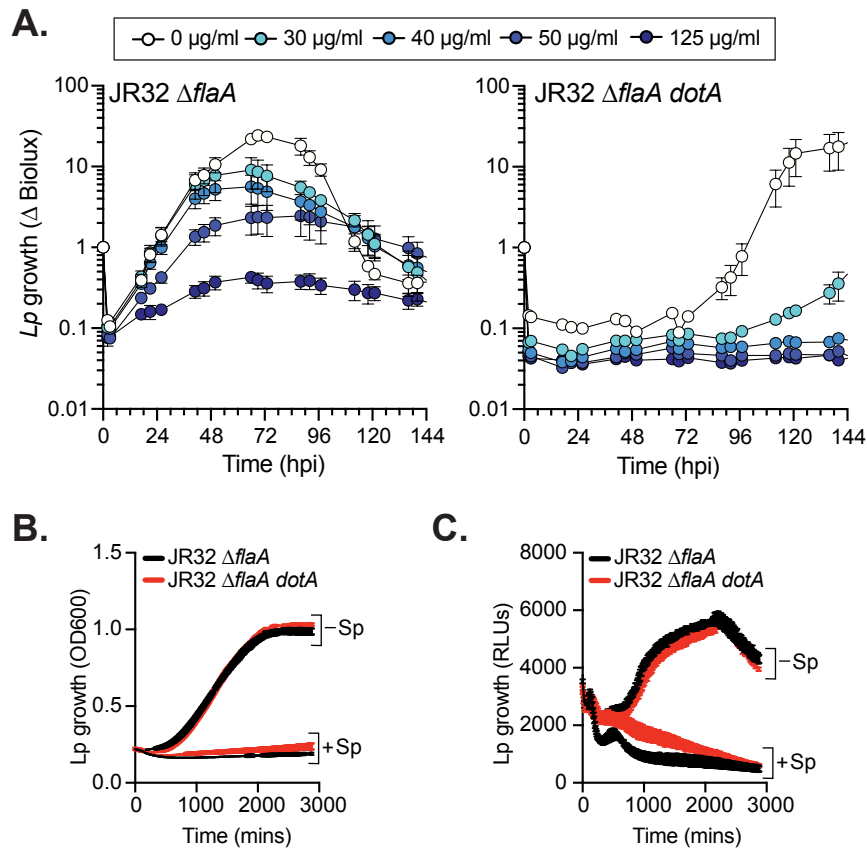


B.



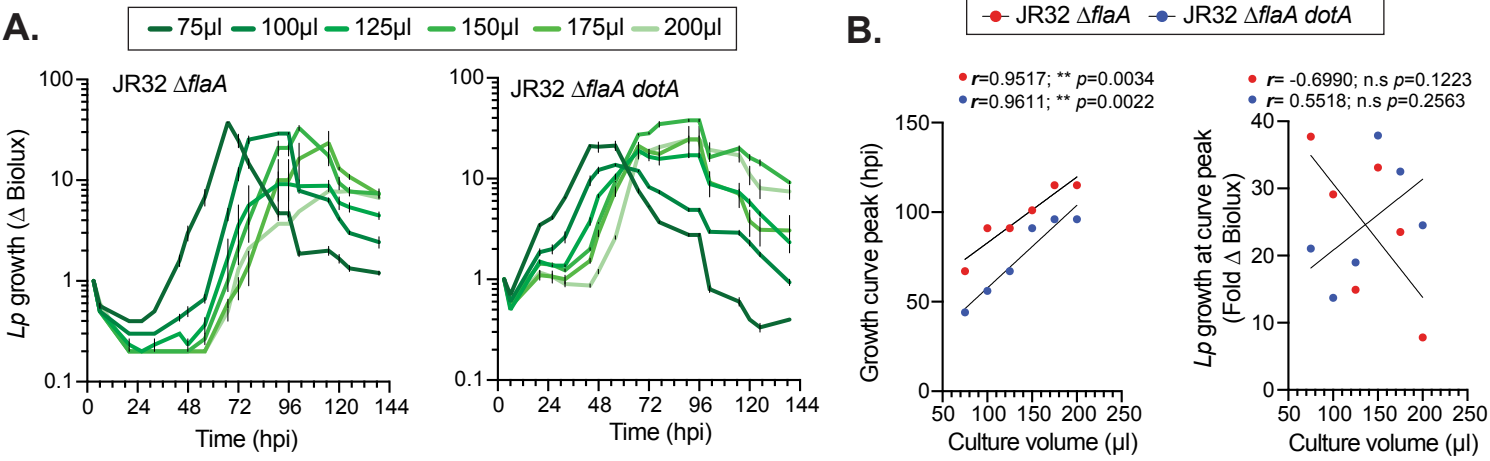
Supplementary Figure 3. *L. jordanis* replication in macrophage infections and axenic growth cultures. **(A)** Growth kinetics of the indicated *Lj* strains with U937 macrophages at MOI=5 in serum-free RPMI media (SFM) or RPMI supplemented with bovine S4 serum (10% v/v). **(B)** Growth kinetics of the indicated *Lj* strains in axenic AYE media or AYE supplemented with bovine S4 serum (10% v/v) as measured by changes in the culture optical density over time. **(A-B)** Averages of three technical replicates $\pm$ StDev are shown. One of two biological replicates is shown for each experiment. Source data are provided as a Source Data file.

Supplementary Figure 4.



Supplementary Figure 4. Lp growth kinetics in spectinomycin treated macrophages and monocytes. (A-D) Kinetics of Lp growth in the presence of spectinomycin (Sp). **(A and D)** Spectinomycin protection assay showing growth kinetics of the indicated Lp strains in U937 macrophages **(A)** and U937 monocytes **(D)** infections at MOI=5. **(B-C)** Axenic growth kinetics in AYE media of the indicated Lp strains in the absence or presence of 20 $\mu\text{g/ml}$ spectinomycin as measured by changes in optical density (OD₆₀₀) **(B)** or Bioluminescence (RLUs) **(C)**. **(A-D)** Averages of three technical replicates $\pm$ StDev are shown. One of three biological replicates is shown for each experiment. Source data are provided as a Source Data file.

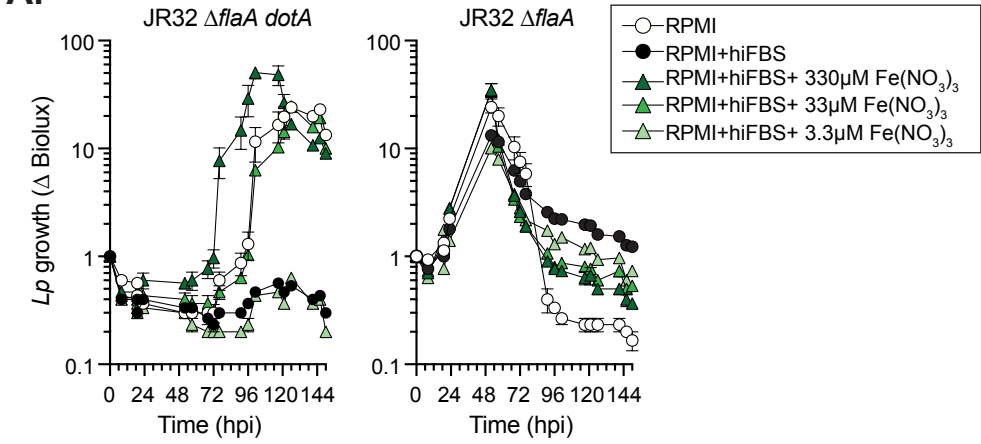
Supplementary Figure 5.



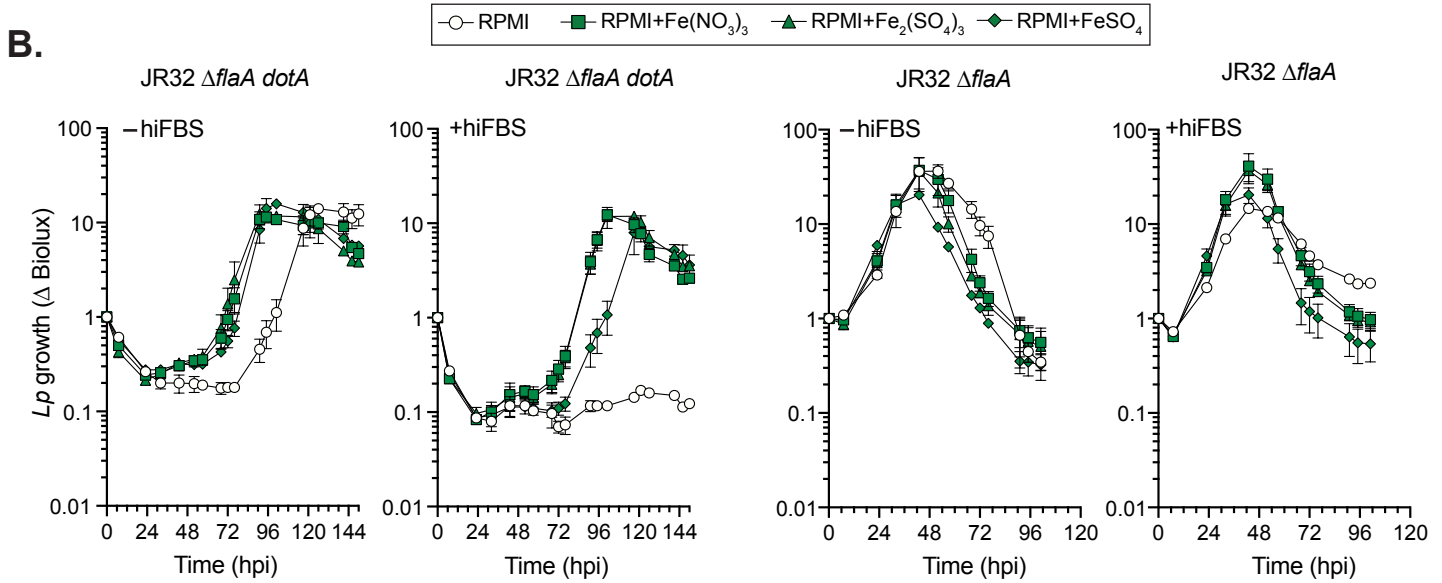
Supplementary Figure 5. Lp growth kinetics dependency on the cell culture volume. (A-B) Positive correlation between the cell culture volume and the kinetics of the peak Lp extracellular replication in U937 monocyte infections. Lp growth kinetics of the indicated strains in U937 monocytes tissue culture infections at MOI=5 with different media volumes. Averages of three technical replicates +/- StDev are shown. One of three biological replicates is shown for each experiment. **(B)** Correlation between cell culture volume during infection and (1) the time Lp growth peaks (left panel) and (2) the magnitude of Lp growth (right panel) in U937 monocyte infections for the indicated Lp strains. The Pearson r correlation coefficient (r) and p value is shown for each Lp strain. (two-tailed test, ** $p < 0.004$; 'n.s' – not significant). Source data are provided as a Source Data file.

Supplementary Figure 6.

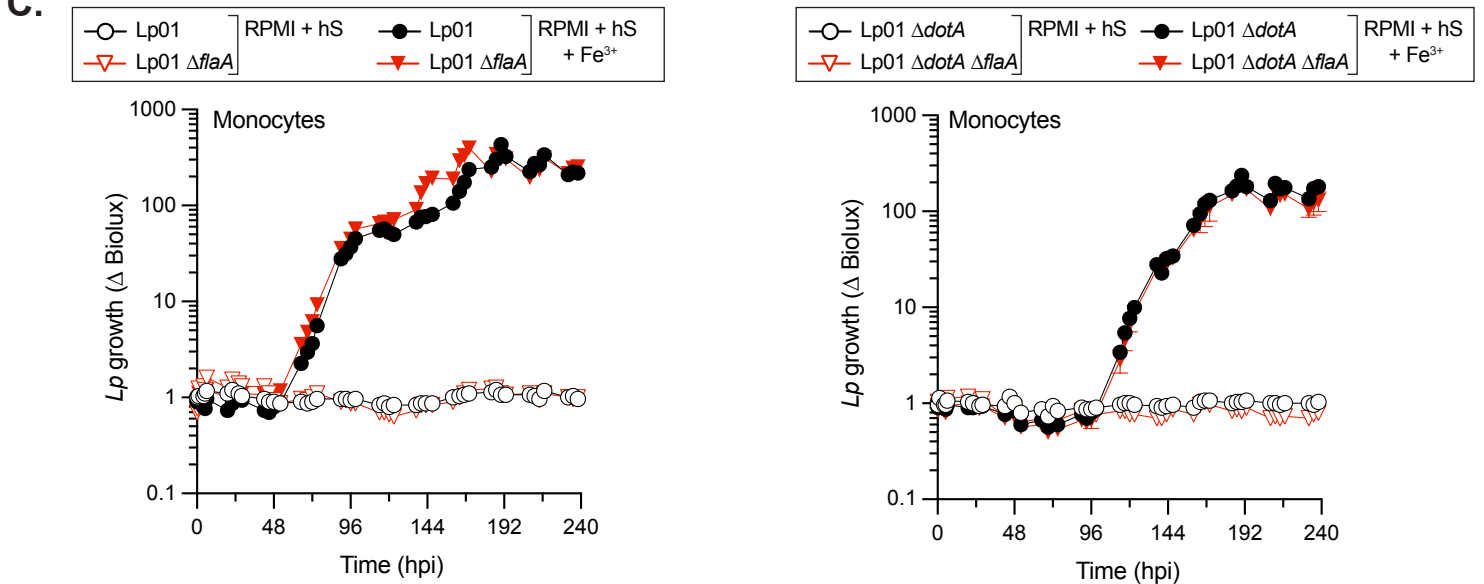
A.



B.

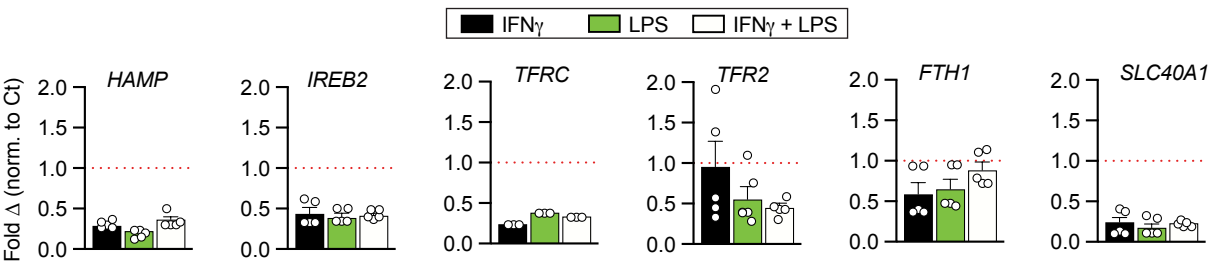


C.



Supplementary Figure 6. The effect of iron abundance on Lp growth with macrophages and monocytes. (A) Ferric Iron supplementation overcomes serum restriction of Lp extracellular replication in a dose-dependent manner. Growth kinetics of the indicated Lp strains with U937 macrophages at MOI=5 in the presence of hiFBS and different concentrations of Fe(NO₃)₃. **(B)** Ferric and ferrous salts remove the nutritional blockade imposed by hiFBS. Growth kinetics of the indicated Lp strains with U937 macrophages at MOI=5 in the presence/absence of hiFBS and different iron salts (330 μ M) – ferric nitrate, ferric sulfate and ferrous sulfate. **(C)** Lp extracellular replication is independent of flagellin expression. Bacterial growth curves of the indicated strains with U937 monocytes at MOI=5 in the presense of human serum supplemented or not with 33 μ M Fe(NO₃)₃ are shown. **(A-C)** Averages of three technical replicates $\pm$ StDev are shown. One of three biological replicates is shown for each experiment. Source data are provided as a Source Data file.

Supplementary Figure 7.



Supplementary Figure 7. Effects IFN γ and LPS stimulation on expression of iron homeostasis regulatory genes. LPS and IFN γ stimulation reduced the expression of iron import and export regulatory genes in macrophages. qPCR analysis of U937 macrophages stimulated as indicated with either IFN γ (2 μ g/ml), *E.coli* LPS (100ng/ml) or both for 16hrs. Data for each gene is normalized to gene expression data from unstimulated macrophages. Averages of at least three biological replicates +/- SEM are shown. *HAMP* – Hepcidin; *IREB2* - Iron Responsive Element Binding Protein 2; *TFRC* - Transferrin Receptor; *TFR2* - Transferrin Receptor 2; *FTH1* - Ferritin Heavy Chain 1; *SLC40A1* - Solute Carrier Family 40 Member 1 aka Ferroportin-1. Source data are provided as a Source Data file.

Supplementary Table 1. Strains used in this study

Strains	Genotype	Properties	Reference
CR19	<i>E. coli</i> DH5 α λ pir <i>endA1 hsdR17 glnV44</i> (= <i>supE44</i>) <i>thi-1 recA1 gyrA96 relA1</i> ϕ 80dlac Δ (<i>lacZ</i>)M15 Δ (<i>lacZYA-argF</i>)U169 <i>zdg-232::Tn10 uidA::pir</i> ⁺	conjugation strain helper	¹²⁵
Lp01 LuxR	<i>L. pneumophila</i> Lp01, <i>icmR</i> -LuxR locus	constitutive bioluminescence	This study
Lp01 Δ <i>flaA</i> LuxR	<i>L. pneumophila</i> Lp01 Δ <i>flaA</i> , <i>icmR</i> -LuxR locus	constitutive bioluminescence	This study
Lp01 Δ <i>dotA</i> LuxR	<i>L. pneumophila</i> Lp01 Δ <i>dotA</i> , <i>icmR</i> -LuxR locus	constitutive bioluminescence	This study
Lp01 Δ <i>dotA</i> Δ <i>flaA</i> LuxR	<i>L. pneumophila</i> Lp01 Δ <i>dotA</i> Δ <i>flaA</i> , <i>icmR</i> -LuxR locus	constitutive bioluminescence	This study
Lp01 Δ <i>sdhA</i> LuxR	<i>L. pneumophila</i> Lp01 Δ <i>sdhA</i> , <i>icmR</i> -LuxR locus	constitutive bioluminescence	This study
Lp01 Δ <i>flaA</i> <i>mavN::Kan</i> ^R LuxR	<i>L. pneumophila</i> Lp01 Δ <i>flaA</i> , <i>mavN::Kan</i> ^R <i>icmR</i> -LuxR locus	constitutive bioluminescence	This study
Lp01 <i>dotB::Kan</i> ^R LuxR	<i>L. pneumophila</i> Lp01 <i>dotB::Kan</i> ^R <i>icmR</i> -LuxR locus	constitutive bioluminescence	This study
Lp01 <i>dotH::Kan</i> ^R LuxR	<i>L. pneumophila</i> Lp01 <i>dotH::Kan</i> ^R <i>icmR</i> -LuxR locus	constitutive bioluminescence	This study
JR32 Δ <i>flaA</i> LuxR	<i>L. pneumophila</i> JR32 Δ <i>flaA</i> , <i>icmR_P</i> -LuxR	constitutive bioluminescence	This study
JR32 Δ <i>flaA</i> Δ <i>dotA</i> LuxR	<i>L. pneumophila</i> JR32 Δ <i>flaA</i> Δ <i>dotA</i> , <i>icmR_P</i> -LuxR	constitutive bioluminescence	This study
Lp01 Δ <i>flaA</i> GFP+	<i>L. pneumophila</i> LP01 Δ <i>flaA</i> Ptac-GFP	inducible GFP expression, <i>plasmid-borne</i> pAM239	¹²⁶
Lp01 Δ <i>dotA</i> GFP+	<i>L. pneumophila</i> LP01 Δ <i>dotA</i> Ptac-GFP	inducible GFP expression, <i>plasmid-borne</i> pAM239	¹²⁶
Lj LuxR	<i>L. jordanis</i> BL-540, Strep ^R Ptac-LuxR	inducible bioluminescence, <i>chromosome-borne</i>	⁸⁰
Lj Δ <i>dotB</i> LuxR	<i>L. jordanis</i> BL-540, Strep ^R Δ <i>dotB</i> Ptac-LuxR	inducible bioluminescence, <i>chromosome-borne</i>	This study

it – bacterial species and gene names are italicized

Supplementary Table 2. Primers used in this study

Designation	Primer sequence (5' → 3')	RE site
F_BamHI-lpg2815- <i>mavN</i>	CGGGATCCCTGTCTGGAGCAAGCCTGATACTTGGC	BamHI
R_XbaI-lpg2815- <i>mavN</i>	GCTCTAGACATATCCAGTGATTGAGCCGT	XbaI
SP_F_lpg2815- <i>mavN</i>	GTTCTGCATTACGCATCAATG	
F_BamHI-lpg2676- <i>dotB</i>	GCGGATCCGGCGAACCCATTTTTGCAGAGGTT	BamHI
R_XbaI-lpg2676- <i>dotB</i>	GCTCTAGAGGTTGGGACCAGTTTTTGCCAAATG	XbaI
SP_F_lpg2676- <i>dotB</i>	GCAACCTATTATAGCACCAG	
F_BamHI-lpg0450- <i>dotH</i>	CGGGATCCCTGCGAATGCTACAACAAAATTATC	BamHI
R_XbaI-lpg0450- <i>dotH</i>	CGTCTAGACATTGGCTGATGGAGGAATGCCTTC	XbaI
SP_F_lpg0450- <i>dotH</i>	GTCATGCAGTATCTATGCGG	
UpF_Lj_ <i>dotB</i>	GTGGATCCAAATCACGAAGC	BamHI
UpR_Lj_ <i>dotB</i>	GGAATTCCATAAATACCGGGGTAAAACG	EcoRI
DownF_Lj_ <i>dotB</i>	GGAATTCAGTACCTGATGCCGATAGC	EcoRI
DownR_Lj_ <i>dotB</i>	ATCGAGCTCCTAAACTTCCAGTTTCCTGC	SacI
SP1_Lj_ <i>dotB</i>	CATGGAAAAATTGGCACAAAGC	
SP2_Lj_ <i>dotB</i>	AAAATCTCACCATCCATGCC	
SP3_Lj_ <i>dotB</i>	AGTTAATCAGATCGCCAAGC	
F_SLC40A1	CCTTCTCTACCTTGGTCATTCTC	
R_SLC40A1	AAAGGAGGCTGTTTCCATAGAG	
F_HAMP	GGATGCCCATGTTCCAGAG	
R_HAMP	AGCACATCCCACACTTTGAT	
F_FTH1	CGTTTACCTGTCCATGTCTTACT	
R_FTH1	TTTCTCAGCATGTTCCCTCTC	
F_TFRC	GACAATGCTGCTTCCCTTTC	
R_TFRC	TCCATGGTGGTACCCAAATAAG	
F_TFR2	CTATGAGCCTGACCTGGATTTC	
R_TFR2	GTTTGCCTGATGGTGTCTT	
F_IREB2	GAGAATACACCCATCCTGTGTC	
R_IREB2	AACTGAAGCCTCTCTCGATTTC	
F_IRF1	CTCTCACCAAGAACCAGAGAAA	
R_IRF1	GAAGGTATCAGGGCTGGAATC	
F_IRF8	AGCATGTATCCAGGACTGATTT	
R_IRF8	GAGGCATCCACTTCCTGATTAT	
F_IDO1	TGGAGAAAGCCCTTCAAGTG	
R_IDO1	CCAGAACCCTTCATACACCAG	
F_IL6	GATGAGTACAAAAGTCCTGATCCA	
R_IL6	CTGCAGCCACTGGTTCTGT	
F_GAPDH	AGCCACATCGCTCAGACAC	
R_GAPDH	GCCCAATACGACCAAATCC	

* Relevant restriction enzyme sites within DNA sequences are underlined
it – bacterial gene names are italicized

Supplementary Table 3. Plasmids used in this study

Plasmids	Important properties	Marker	Reference
pSR47	<i>oriT</i> mobRP4 R6K <i>ori</i> , Kan ^R - suicide plasmid for insertion mutagenesis	Kan	125
pSR47s	pSR47, <i>sacB</i> Kan ^R - gene replacement vector	Kan	125
pSR47-icmRp-LuxR	Plasmid encoding the <i>LuxR</i> operon under control of the Lp <i>icmR</i> promoter	Kan	79
pSR47s-icmR-LuxR locus	Replacement chromosomal region containing the <i>LuxR</i> operon downstream of the Lp <i>icmR</i> gene	Kan	This study
pSR47- <i>mavN</i>	pSR47 containing a 995nt <i>mavN</i> intragenic region (137→1,131nt)	Kan	This study
pSR47- <i>dotB</i>	pSR47 containing a 786nt <i>dotB</i> intragenic region (151→936 nt)	Kan	This study
pSR47- <i>dotH</i>	pSR47 containing a 709nt <i>dotH</i> intragenic region (121→830 nt)	Kan	This study
pSR47s-CD <i>dotB_{Lj}</i>	pSR47s containing the Δ <i>dotB</i> clean deletion allele		This study
pSR47s-CD0376	pSR47s containing the Δ <i>sdhA</i> clean deletion allele		126
pSR47s-CD1340	pSR47s containing the Δ <i>flaA</i> clean deletion allele		126

it – bacterial gene names are italicized

Supplementary Table 4. Reagents used in this study

Reagent	Catalog #	Lot #	Supplier
Heat-inactivated Fetal Bovine Serum (FBS 1)	512-048	16000-044	Gibco
Heat-inactivated Fetal Bovine Serum (FBS 2)	826-061	16000-044	Gibco
Heat-inactivated Fetal Bovine Serum (FBS 3)	APJ 27610	30070.03	Hyclone
Heat-inactivated Fetal Bovine Serum (FBS 4)	ABC 215874	SH 30087.03	Hyclone
Heat-inactivated Fetal Bovine Serum (FBS 5)	AC10335409	SH 30910.03	Hyclone
Heat-inactivated Fetal Bovine Serum (FBS 6)	1983447	26140-079	Gibco
Heat-inactivated Fetal Bovine Serum (FBS 7)	30407	S11150	Atlanta Biologicals
Heat-inactivated Fetal Bovine Serum (FBS 8)	30407	E18055	Atlanta Biologicals
Heat-inactivated Fetal Bovine Serum (FBS 9)	B21019	S11150H	R&D Bovine Serum
Heat-inactivated Fetal Bovine Serum (FBS 10)	F-0500-D	F25E21D1	Atlas Biologicals
Heat-inactivated Fetal Bovine Serum (FBS 11)	A 120030	900-208	Gemeni Bioproducts
Heat-inactivated human serum (hS1)	100-110	H86W03J	Gemeni Bioproducts
Cytochalasin D	11330		Cayman Chemical
Deferoxamine	14595	0618885-11	Cayman Chemical
Purified Apo Transferrin from bovine plasma	J61626.MC	S071053	Thermo Scientific
Human recombinant IFN γ	570206	B336905	BioLegend
<i>E. coli</i> LPS	tlrl-eklps		Invivogen
RPMI-1640	25-506	MS01GJ	Genesee
DMEM	25-500	MS0114L	Genesee
N-(2-acetamido)-2-aminoethanesulfonic acid	A11553-36	10239672	Thermo Scientific
Yeast extract	20-269		APEX Bioresearch
Agar granulated	20-248	AB-2210138	APEX Bioresearch
Activated charcoal	C9157-500G	SLBJ6388V	Sigma-Aldrich
Potassium hydroxide	0489-1kg	2746C296	AMRESCO
RNeasy Mini kit (250)	74106		QIAGEN
DNaseI	M0303L		New England Biolabs
High-Capacity cDNA Reverse Transcription Kit	4374966		Thermo Fisher
Phorbol 12-myristic 13-acetate (PMA)	AG-CN2-0010-M001		Adipogen
L-cysteine	A10435	Q08H112	Alfa Aesar
Fe(NO $_3$) $_3$	33315	W16A02	Alfa Aesar
ZnSO $_4$	19080	N23069	Research Organic
MnCl $_2$	155334	066J	MP Biomedicals
NiCl $_2$	N-5756	80K3489	Sigma
Native Human Lactoferrin protein	AB285896	1004797-2	ABCAN
Fe $_2$ SO $_4$	F1135	11KO171	Sigma
FeSO $_4$	F7002	50HO833	Sigma
CuSO $_4$	0330-500G	3586C030	AMRESCO
Kanamycin	BP906-5	14446	Fisher Scientific
Streptomycin	8610	3551b38	EM Science
Spectinomycin	14324	056 1689-24	Cayman Chemical
Chloramphenicol	0230-100G	1744CO11	AMRESCO
Plasmid Mini Kit	0230-100G	FH27305	IBI Scientific
L-Ascorbic acid	14656	0569615-46	Cayman Chemical
Ferrozine	32465	0648660-4	Cayman Chemical
Neocuproine	20874	0555221-5	Cayman Chemical
Ammonium acetate	09689	2111629	Fluka Biochemika
Sodium hydroxide	221465	MKBS2033V	Sigma-Aldrich
Hydrochloric acid	HX0603-4	62262	Sigma-Aldrich
Hoechst 33342	H3570		Life Technologies
Phalloidin-iFluor TM 594 Conjugate	20553		Cayman Chemical
goat α -chicken IgY-Alexa647	A-21449		Thermo Fisher

Monocyte Attachment Media	C-28051	Sigma
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