Supplementary Figures:

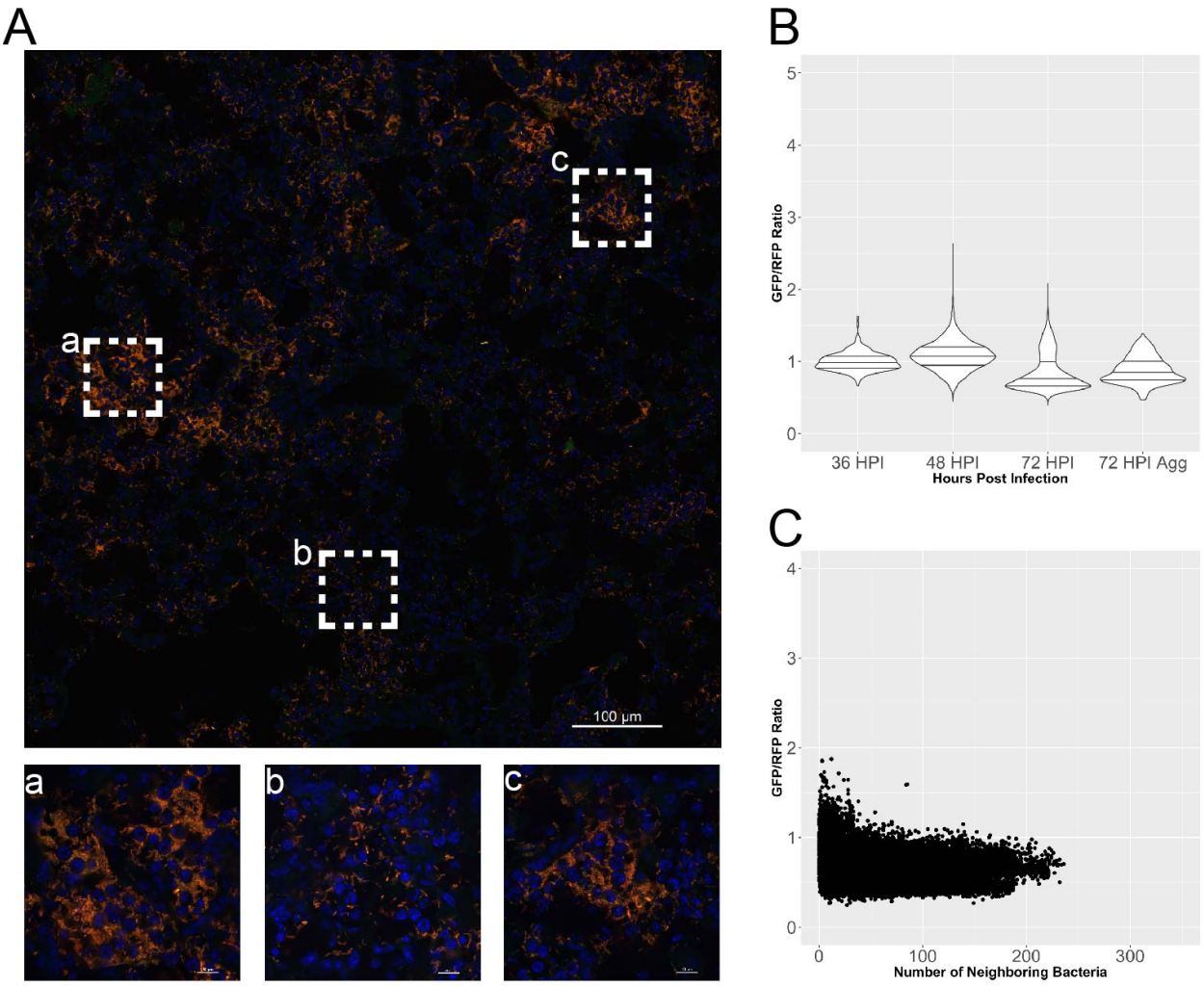


Figure S1. No increased spatiotemporal expression of *gfp* driven from the constitutive *tetO* promoter.

Cross sections of lungs from mice infected with CO92 with the P*tetO*-GFP reporter (green) and pGEN-RFP plasmid (red) were stained with DAPI (blue) and imaged by confocal microscopy. (A) Large images were generated with the tile feature in NIS elements acquisition software to cover *Y. pestis* containing lesions within the lung space. (a-c) Magnified images from selected regions of the bigger lesion in (A). (B) Violin plots displaying the relative expression of *tetO* as a ratio of GFP/RFP quantified in individual cells combined from the lung lesions of at

least three mice at each time-point, across two independent infection experiments at 36, 48, and 72 hpi. Horizontal lines within the violins represent the 25th percentile, median, and 75th percentile. Aggregates were also imaged and analyzed separately. (C) Expression of *tetO* in individual cells as a function of neighboring bacterial cell density from all images and time points post infection.

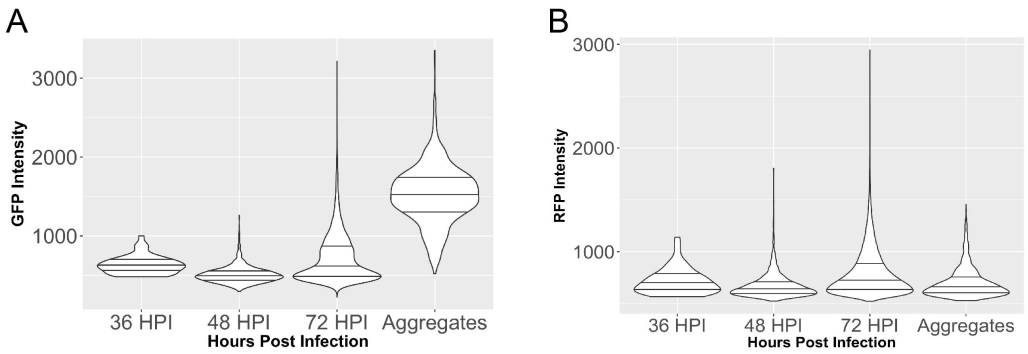


Figure S2. Separated GFP and RFP channels for P_{crp}-GFP infections.

(A) GFP and (B) RFP intensities alone measured from FIJI analysis at 36, 48, and 72 hpi and within aggregates of cells in the lungs. Horizontal lines within the violins represent the 25th percentile, median, and 75th percentile.

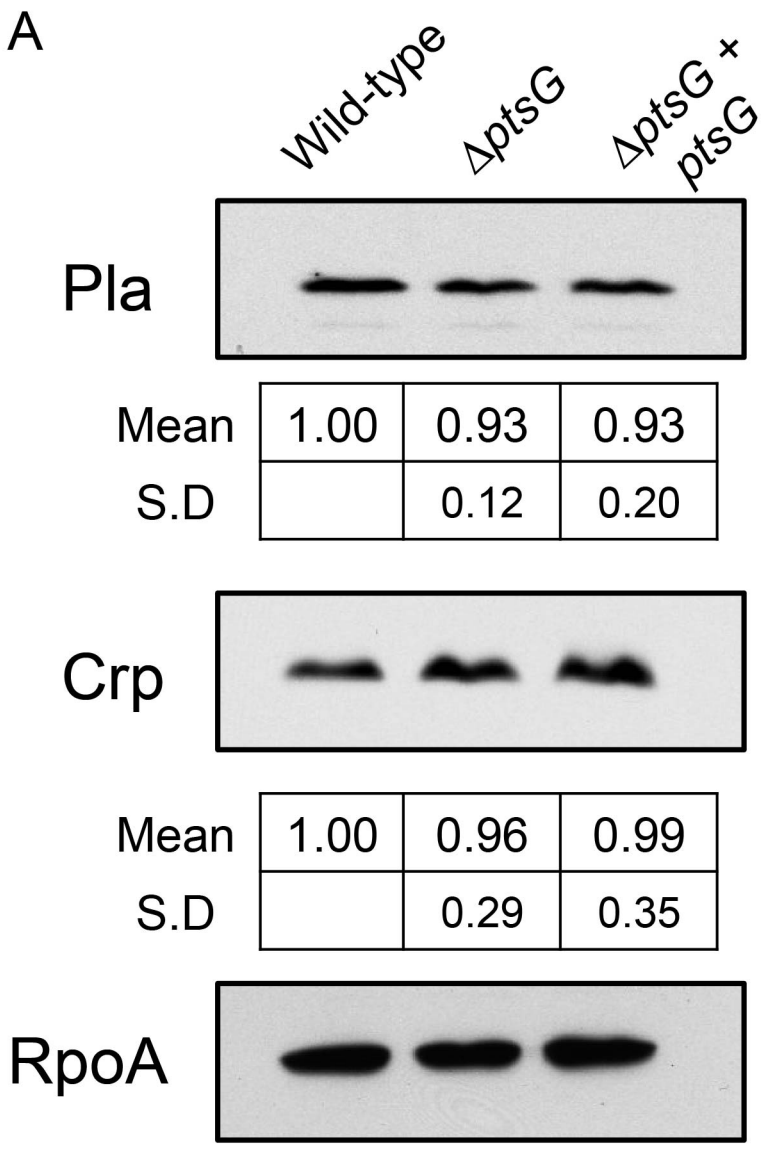
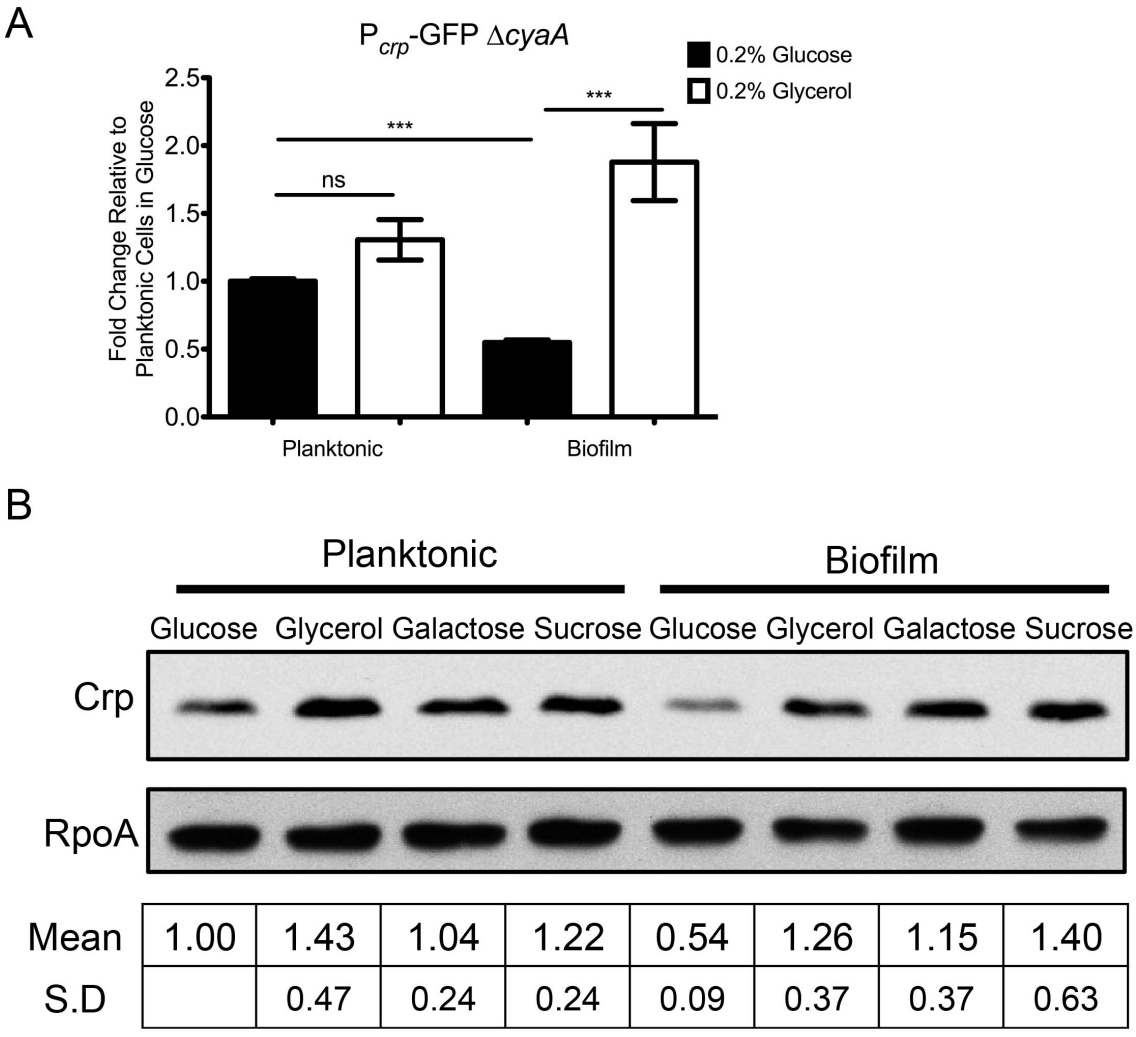


Figure S3. Deletion of *ptsG* does not alter levels of Crp and Pla protein levels *in vitro.*

(A) Representative blots of Crp and Pla protein levels of *Y. pestis* LCR-, *Y. pestis* LCR- Δ*ptsG,* and *Y. pestis* LCR- Δ*ptsG* + *ptsG* grown in BHI for 6 hours at 37°C. Quantification of Pla/RpoA

and Crp/RpoA ratios were measured in FIJI and standardized to wild-type *Y. pestis* from three independent experiments.



Mean	1.00	1.43	1.04	1.22	0.54	1.26	1.15	1.40
S.D		0.47	0.24	0.24	0.09	0.37	0.37	0.63

Figure S4. Repression of *crp* expression in biofilms does not require *cyaA* and is specific to glucose.

(A) *Y. pestis* Δ*cyaA* with the P_{crp}-GFP reporter was grown in TMH supplemented with 0.2% glucose (black bars) or 0.2% glycerol (white bars) overnight. Planktonic and biofilm cells were separated and GFP intensity was standardized to planktonic cells grown in 0.2% glucose. (B) Representative blot measuring Crp protein levels in *Y. pestis* LCR- grown in TMH

supplemented with either 0.2% glucose, 0.2% glycerol, 0.2% galactose, or 0.2% sucrose overnight. Quantification of Crp/RpoA ratio was measured in FIJI and was standardized to planktonic cells in 0.2% glucose from three independent experiments.

Supplemental Information

Table S1. Bacterial strains used in this study

Y. pestis strains	Designation	Genotype/Characteristics	Source
CO92	SAN3	wild-type, pCD1+, pMT+, pPCP1+, pgm+	Lab Stock
CO92 Δ*ptsG*	SAN321	Δ*ptsG*, pCD1+, pMT+, pPCP1+, pgm+	This work
CO92 Δ*ptsG* + *ptsG*	SAN323	Δ*ptsG*, pCD1+, pMT+, pPCP1+, pgm+, *att*Tn7: *ptsG* complement	This work
CO92 P*crp*-GFP RFP+	SAN237	pCD1+, pMT+, pPCP1+, pgm+, pGEN-RFP, *att*Tn7: P*crp*-GFP	This work
CO92 P*pla*-GFP RFP+	SAN235	pCD1+, pMT+, pPCP1+, pgm+, pGEN-RFP, *att*Tn7: P*pla*-GFP	This work
CO92 P*tetO*-GFP RFP+	SAN289	pCD1+, pMT+, pPCP1+, pgm+, pGEN-RFP, *att*Tn7: P*tetO*-GFP	This work
CO92 P*crp-tetO* 5'UTR-GFP RFP+	SAN239	pCD1+, pMT+, pPCP1+, pgm+, pGEN-RFP, *att*Tn7: P*crp-tetO* 5'UTR-GFP	This work
CO92 Δ*ptsG* P*crp*-GFP RFP+ Δ*ptsG*	SAN327	Δ*ptsG,* pCD1+, pMT+, pPCP1+, pgm+, pGEN-RFP, *att*Tn7: P*crp*-GFP	This work
CO92 Δ*ptsG* P*pla*-GFP RFP+	SAN333	Δ*ptsG*, pCD1+, pMT+, pPCP1+, pgm+, pGEN-RFP, *att*Tn7: P*pla*-GFP	This work
CO92 Δ*ptsG* P*tetO*-GFP RFP+	SAN335	Δ*ptsG*, pCD1+, pMT+, pPCP1+, pgm+, pGEN-RFP, *att*Tn7: P*tetO*-GFP	This work
CO92 LCR-	PAN259	pCD1-, pMT+, pPCP1+, pgm+	Lab Stock
CO92 LCR- Δ*ptsG*	PAN1020	Δ*ptsG*, pCD1-, pMT+, pPCP1+, pgm+	This work
CO92 LCR- Δ*ptsG* + *ptsG*	PAN1024	Δ*ptsG*, pCD1-, pMT+, pPCP1+, pgm+, *att*Tn7: *ptsG* complement	This work
CO92 LCR- Promoterless GFP	PAN494	pCD1-, pMT+, pPCP1+, pgm+, *att*Tn7: GFP	(1)
CO92 LCR- P*crp*-GFP	PAN578	pCD1-, pMT+, pPCP1+, pgm+, *att*Tn7: P*crp*-GFP	(1)
CO92 LCR- P*pla*-GFP	PAN607	pCD1-, pMT+, pPCP1+, pgm+, *att*Tn7: Ppla-GFP	(1)
CO92 LCR- P*crp-tetO* 5'UTR-GFP	PAN828	pCD1-, pMT+, pPCP1+, pgm+, *att*Tn7: P*crp-tetO* 5'UTR-GFP	This work
CO92 LCR- Δ*cyaA* Promoterless GFP	PAN749	pCD1-, pMT+, pPCP1+, pgm+, *att*Tn7: GFP Δ*cyaA*	This work

CO92 LCR- Δ*cyaA* P*crp*-GFP	PAN857	pCD1-, pMT+, pPCP1+, pgm+, *att*Tn7: P*crp*-GFP Δ*cyaA*	This work

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Table S2. Plasmids used in this study.

E. coli Strain	Strain	Name	Description	Source
LAN24	CC118 λ-pir	puc18R6K-mini-tn7-km	Plasmid backbone used for Tn7 integration	Lab stock
LAN25	DH5aF' λ-pir	pTNS2	Tn7 transposase helper plasmid	Lab Stock
LAN29	DH5a	pSkippy	IPTG-inducible FLP recombinase plasmid	Lab Stock
LAN308	S17 λ-pir	pLB30	Contains entire CDS of gfp cloned into pUC18R6K-mini-Tn7-km	(1)
LAN357	S17 λ-pir	pLB35	Contains 496 bp upstream through first 27 nt of *crp* CDS fused to CDS of GFP cloned into BamHI/PstI site of puc18R6K-mini-tn7-km	(1)
LAN375	S17 λ-pir	pLB38	Contains 500 bp upstream through first 27 nt of *pla* CDS fused to CDS of GFP cloned into BamHI/PstI site of puc18R6K-mini-tn7-km	(1)
LAN450	EC100D λ-pir	pJR04	Contains P*crp-tetO* 5'UTR-GFP construct cloned into BamHI/PstI site of puc18R6K-mini-Tn7-km	This work
LAN468	Top10	pGEN-RFP	Constitutive dsredT3 expression under control of em7 promoter	(2)
LAN501	S17 λ-pir	pJR25	Contains P*tetO*-GFP construct cloned into BamHI/PstI site of puc18R6K-mini-Tn7-km	This work

Table S3. Primers used in this study.

Name	Sequence	Purpose
P63	GTGTAGGCTGGAGCTGCTTC	Amplifcation of Kan cassette for Lambda Red
P64	ATTCCGGGGATCCGTCGACC	Amplifcation of Kan cassette for Lambda Red
P345	AACTGCAGCTTTCACCAGCGTTTCTGGGTG	5' amplification of *tetO* promoter with PstI
P346	GGGTACCTTTCTCCTCTTTAATG	3' amplification of *tetO* promoter
P1089	CGGGATCCTTATTTGTATAGTTCATCCATG CCATGTG	3' amplification of *gfp* into BamHI Tn7
P1554	CTGCAGGACACGACATCAATGGCGCTACA CCCCCCCGC	5' amplification 496bp upstream of *crp* with PstI
P1772	ATCAGCAGGACGCACTGACCGAATTCATT AAAGAGGAGAAAGGTACCCATGAGTAAAG GAGAAGAAC	5' amplification of *gfp* with *tetO* 5'UTR overhang
P1773	GGGTACCTTTCTCCTCTTTAATGAATTCGG TCAGTGCGTCCTGCTGATCTTTCTTTTTAGC ATATTAAC	3' amplification of P*crp* with *tetO* 5'UTR overhang
P1993	ATAGGCTGTTGGTGGAAACAG	Deletion of *ptsG* by Lambda Red Recombination
P1994	GAAGCAGCTCCAGCCTACACAGTTGAGCG TGCTCCTGAGTAATAG	Deletion of *ptsG* by Lambda Red Recombination
P1995	GGTCGACGGATCCCCGGAATTTCAGGTAG GGGAGAGCAAAAG	Deletion of *ptsG* by Lambda Red Recombination
P1996	TCAACGTAATACCCTCGACACC	Deletion of *ptsG* by Lambda Red Recombination
P1997	GGGGGATCCATAGGCTGTTGGTGGAAACA G	Complementation of *ptsG* into BamHI/PstI Tn7
P1998	GGGCTGCAGTCAACGTAATACCCTCGACA CC	Complementation of *ptsG* into BamHI/PstI Tn7

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2. Price PA, Jin J, Goldman WE. 2012. Pulmonary infection by *Yersinia pestis* rapidly establishes a permissive environment for microbial proliferation. Proc Natl Acad Sci U S A 109:3083-8.