

SUPPLEMENTARY MATERIALS

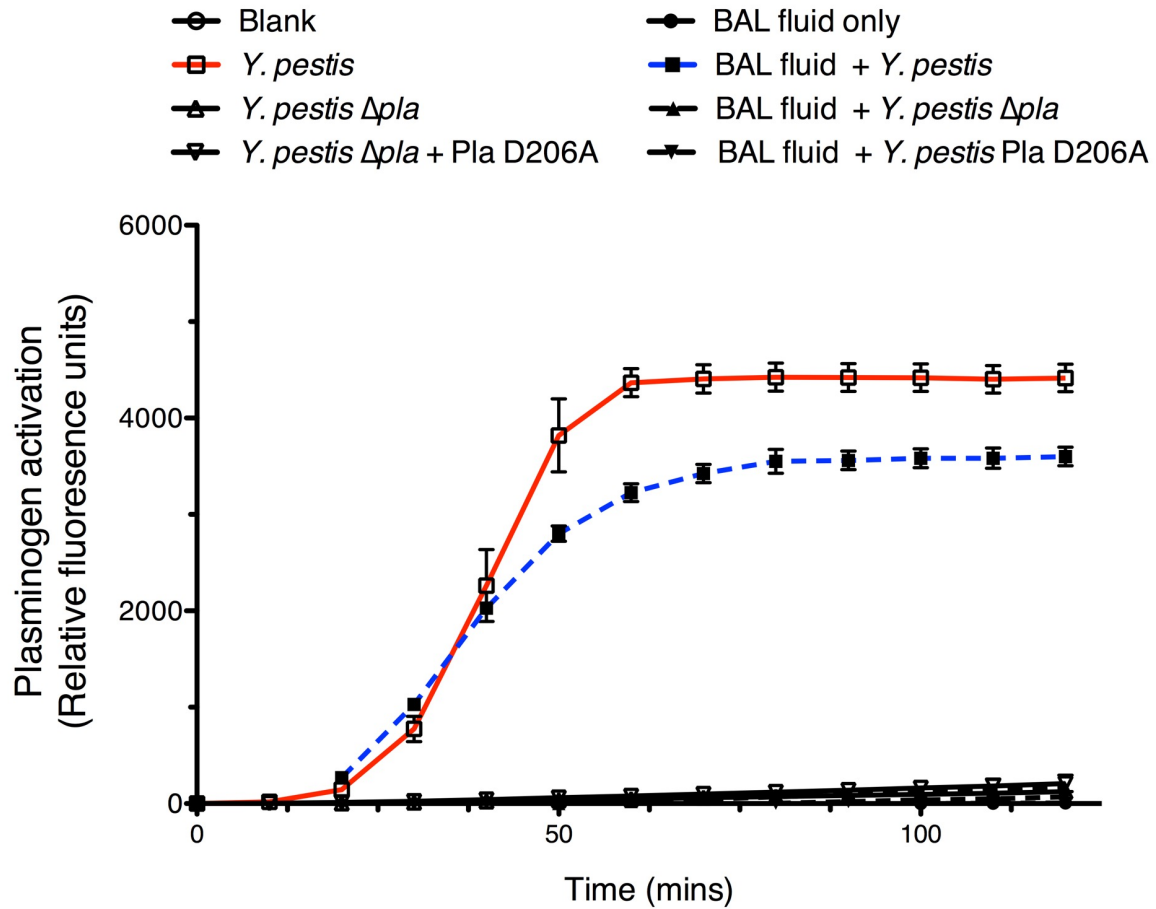


Fig. S1. Protease activity of Pla following *Y. pestis* incubation in BALF. *Y. pestis*, *Y. pestis* Δpla , and *Y. pestis* Pla D206A were incubated in BHI (solid lines) or BALF (dashed lines) for 6 hours at 37°C. Bacteria were separated from the media and the plg-activating ability of the strains was determined by incubating with purified human glu-plasminogen and a fluorescent substrate of plasmin. Data are representative of 3 independent experiments performed in triplicate; error bars represent the s.e.m.

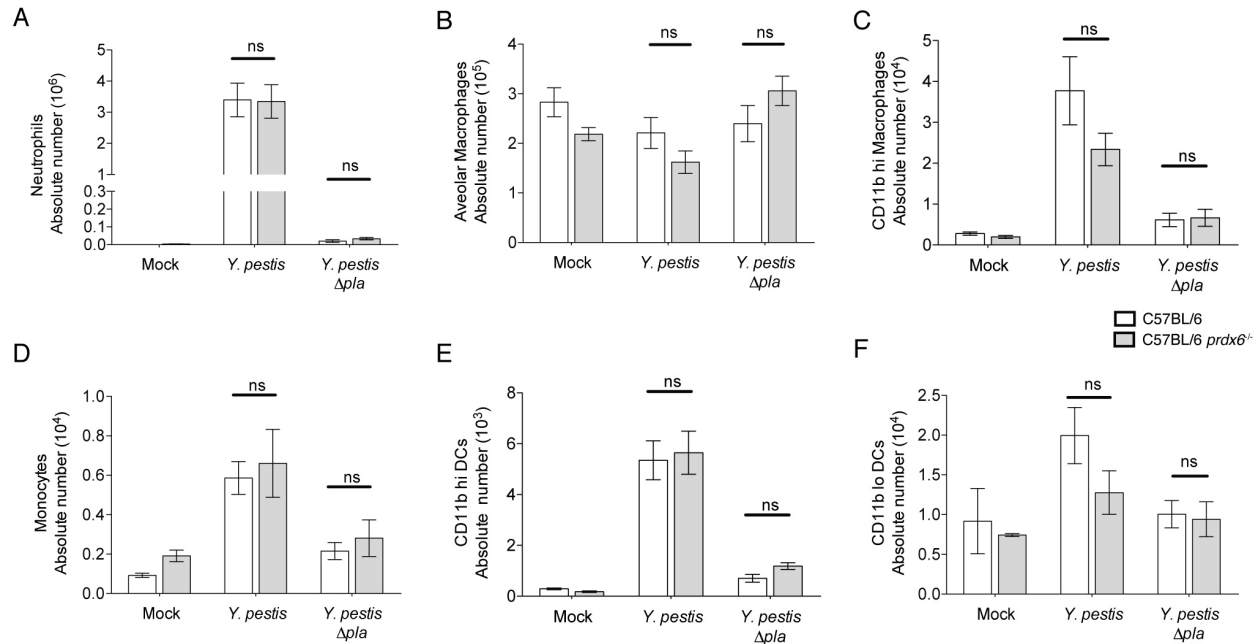


Fig. S2. Prdx6 does not affect immune cell populations in the airspace following *Y. pestis* infection. C57BL/6 or C57BL/6 *prdx6*^{-/-} mice were inoculated with PBS (mock), 10⁴ CFU of *Y. pestis*, or 10⁴ CFU *Y. pestis* Δpla , and after 48 hours BAL was performed to recover immune cells. Flow cytometry was performed to determine the percentages and absolute numbers of (A) neutrophils, (B) alveolar macrophages, (C) CD11b hi macrophages, (D) monocytes, (E) CD11b hi dendritic cells (DCs), and (F) CD11b lo DCs recruited to the lungs. Data are combined from 2 independent experiments ($n=10$ for each group); error bars represent the s.e.m. (One-way ANOVA with Bonferroni's Multiple Comparison test, ns = not significant).

Table S1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Designation	Relevant characteristics*	Source/reference
<i>Y. pestis</i> strains			
CO92	SAN2	pCD1 ⁺ , pMT1 ⁺ , pPCP1 ⁺ , pgm ⁺	(1); Lab stock
CO92 Δ <i>pla</i>	SAN6	Δ <i>pla</i> ; pCD1 ⁺ , pMT1 ⁺ , pPCP1 ⁺ , pgm ⁺	(2)
CO92 LCR ⁻	PAN259	pCD1 ⁻ , pMT1 ⁺ , pPCP1 ⁺ , pgm ⁺	(2); Lab stock
CO92 LCR ⁻ Δ <i>pla</i>	PAN314	Δ <i>pla</i> ; pCD1 ⁻ , pMT1 ⁺ , pPCP1 ⁺ , pgm ⁺	(2)
CO92 LCR ⁻ Pla D206A	PAN163	Δ <i>pla</i> + <i>pla</i> D206A; pCD1 ⁻ , pMT1 ⁺ , pPCP1 ⁺ , pgm ⁺	(2)
<i>E. coli</i> strains			
BL21	LAN211	pSE380; Ap ^R	(3)
BL21	LAN212	pMRK1; Ap ^R	(3)
BL21	LAN213	pMRK1206; Ap ^R	(3)
Plasmids			
pSE380	expression vector		Invitrogen
pMRKI	<i>pla</i> in pSE380		(4)
pMRK1206	<i>pla</i> D206A in pSE380		(4)

*Ap^R, ampicillin resistance

Table S2. *Ex vivo* effect of proteolytically active Pla on protein abundance in mouse BALF.

Protein name	Biological Function/Process ^a	Accession number	Size (AA)	<i>Y. pestis</i> /BAL ^a	pval ^c	<i>Y. pestis</i> Pla D206A /BAL ^a	pval ^c	<i>Y. pestis</i> / <i>Y. pestis</i> Pla D206A ^a	pval ^c
Glutathione S-transferase A3	immune system process; ROS metabolic process	spiP30115	220	0.395	0.022	0.751	0.019	0.610	0.031
Glutathione peroxidase 3	immune system process; response to toxin	spiP46412	226	0.386	0.001	0.804	0.044	0.526	0.002
Tubulin polymerization-promoting protein family	cell component; structure	spiQ9CRB6	176	0.464	0.000	0.892	0.734	0.619	0.000
Pigment epithelium-derived factor	protein binding; proteolysis	spiP97298	417	0.449	0.039	0.972	0.829	0.385	0.010
Alpha-2-HS-glycoprotein	protein binding; immune system process; proteolysis	spiP29699	345	0.561	0.000	0.888	0.002	0.642	0.000
Glutathione S-transferase Mu 1	immune system process; transferase activity	spiP10649	218	0.621	0.000	0.803	0.022	0.797	0.012
BPI fold-containing family A member 1 (sPlunc)	immune system response	spiP97361	278	0.653	0.007	0.772	0.072	0.906	0.397
Carboxypeptidase N subunit 2	immune system process; cytokine-mediated signaling	spiQ9DBB9	547	0.655	0.000	0.974	0.432	0.655	0.000
Sulfated glycoprotein 1	protein binding; lipid transport	spiQ61207	557	0.659	0.001	0.959	0.342	0.691	0.000
BPI fold-containing family B member 1 (Lplunc1)	MAC activation, response to stress	spiQ61114	474	0.669	0.057	0.832	0.175	0.817	0.069
Complement C3	cytokine activity; complement activation	spiP01027	1663	0.670	0.000	0.906	0.000	0.754	0.000
Vinculin	actin binding; cell adhesion	spiQ64727	1065	0.688	0.001	1.916	0.934	0.232	0.000
Plasminogen	serine-type peptidase activity; proteolysis	spiP20918	812	0.714	0.000	0.970	0.317	0.747	0.000
Actin Gamma	cell component; structure	spiP63260	375	0.724	0.000	0.819	0.000	0.893	0.031
Plastin-2	structure; actin binding	spiQ61233	626	0.739	0.001	0.823	0.003	0.919	0.169
Lipoprotein lipase	lipase activity; lipid transport	spiP11152	474	0.743	0.000	0.942	0.892	0.777	0.000
Peroxiredoxin 6	immune system process; ROS metabolic process	triQ6GT24	223	0.746	0.000	1.012	0.958	0.782	0.071
Phosphoglycerate mutase 1	glycolysis	spiQ9DBJ1	253	0.756	0.001	0.804	0.532	0.917	0.000
Complement C4-B	complement activation; signal transduction	spiP01029	1738	0.775	0.069	0.873	0.313	0.939	0.244
Hypoxanthine-guanine phosphoribosyltransferase	monosaccharide metabolic process	spiP00493	219	0.798	0.000	0.914	0.234	1.019	0.000
Calmodulin	Ca ²⁺ binding	spiP62304	149	0.830	0.080	0.749	0.014	1.179	0.032
Apolipoprotein A-IV	lipid transporter activity; blood circulation	spiP06728	395	0.842	0.000	0.973	0.000	0.885	0.014
Alpha-2-antiplasmin	proteolysis	spiQ61247	491	0.847	0.022	0.972	0.523	0.881	0.034

- Biological function are based on Uniprot (<http://www.uniprot.org>).
- Average relative protein expression level ratio in sample and control, quantified by Protein Pilot 4.0 software (ABSciex).
- Determined by Student's *t* test.

References

1. **Parkhill, J., B. W. Wren, N. R. Thomson, R. W. Titball, M. T. G. Holden, M. B. Prentice, M. Sebahia, K. D. James, C. Churcher, K. L. Mungall, S. Baker, D. Basham, S. D. Bentley, K. Brooks, A. M. Cerdeno-Tarraga, T. Chillingworth, A. Cronin, R. M. Davies, P. Davis, G. Dougan, T. Feltwell, N. Hamlin, S. Holroyd, K. Jagels, S. Leather, A. V. Karlyshev, S. Moule, P. C. F. Oyston, M. Quail, K. Rutherford, M. Simmonds, J. Skelton, K. Stevens, S. Whitehead, and B. G. Barrell.** 2001. Genome sequence of *Yersinia pestis*, the causative agent of plague. *Nature* **413**:523-527.
2. **Lathem, W. W., P. A. Price, V. L. Miller, and W. E. Goldman.** 2007. A plasminogen-activating protease specifically controls the development of primary pneumonic plague. *Science* **315**:509-513.
3. **Caulfield, A. J., M. E. Walker, L. M. Giolda, and W. W. Lathem.** 2014. The Pla protease of *Yersinia pestis* degrades fas ligand to manipulate host cell death and inflammation. *Cell Host Microbe* **15**:424-434.
4. **Kukkonen, M., K. Lahteenmaki, M. Suomalainen, N. Kalkkinen, L. Emody, H. Lang, and T. K. Korhonen.** 2001. Protein regions important for plasminogen activation and inactivation of alpha2-antiplasmin in the surface protease Pla of *Yersinia pestis*. *Mol Microbiol* **40**:1097-1111.