Matrikines are key regulators in modulating the amplitude of lung inflammation in acute pulmonary infection

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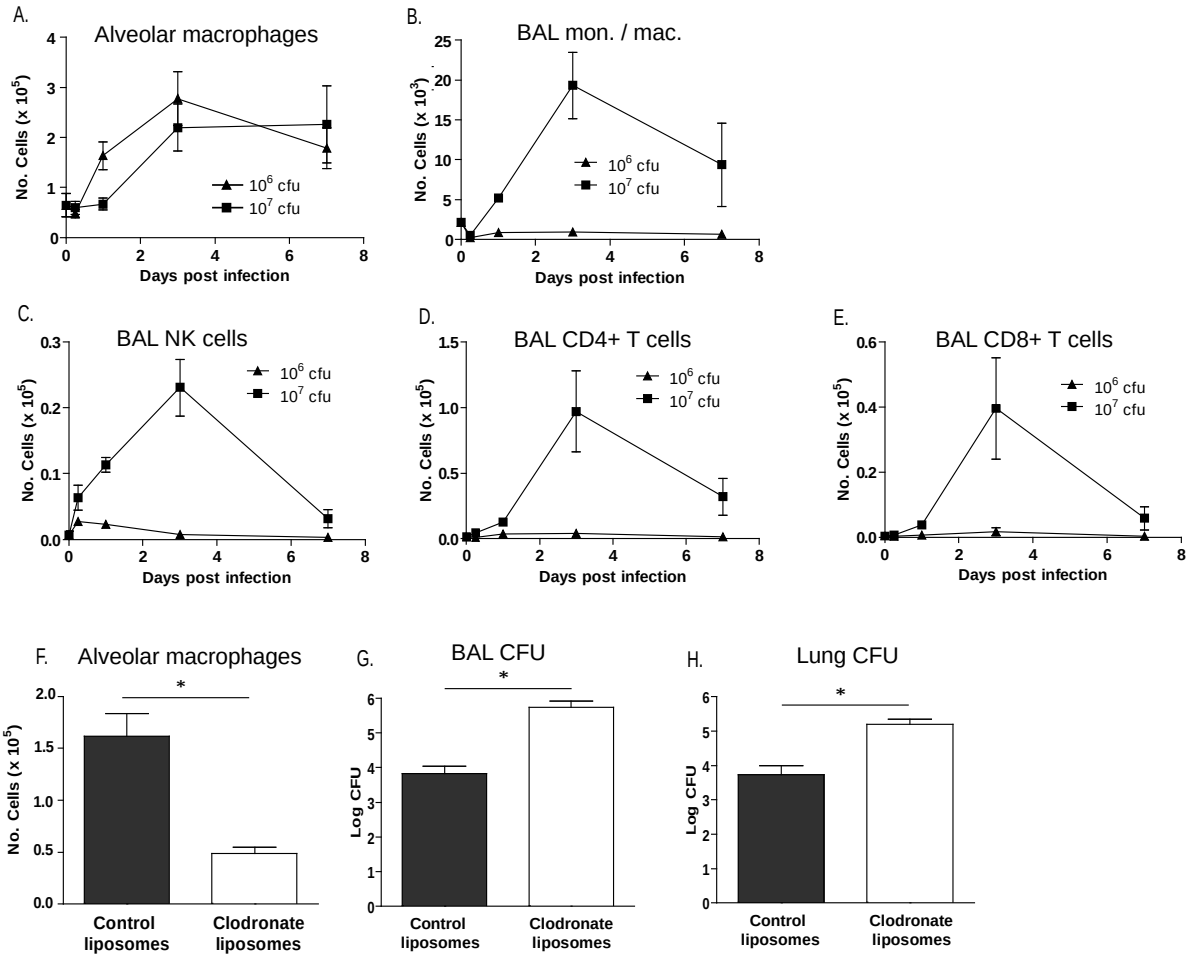
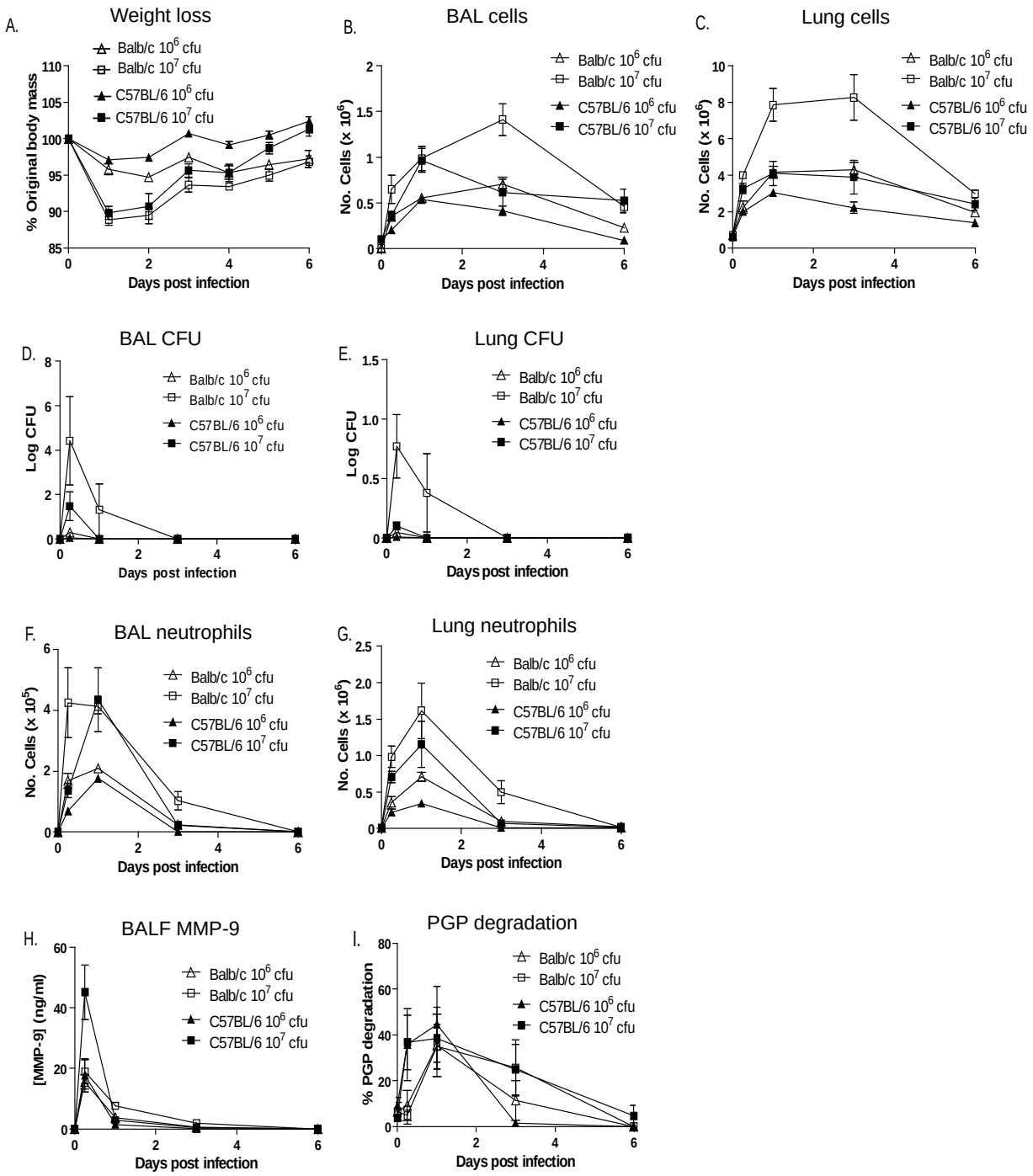
Supplementary Material.

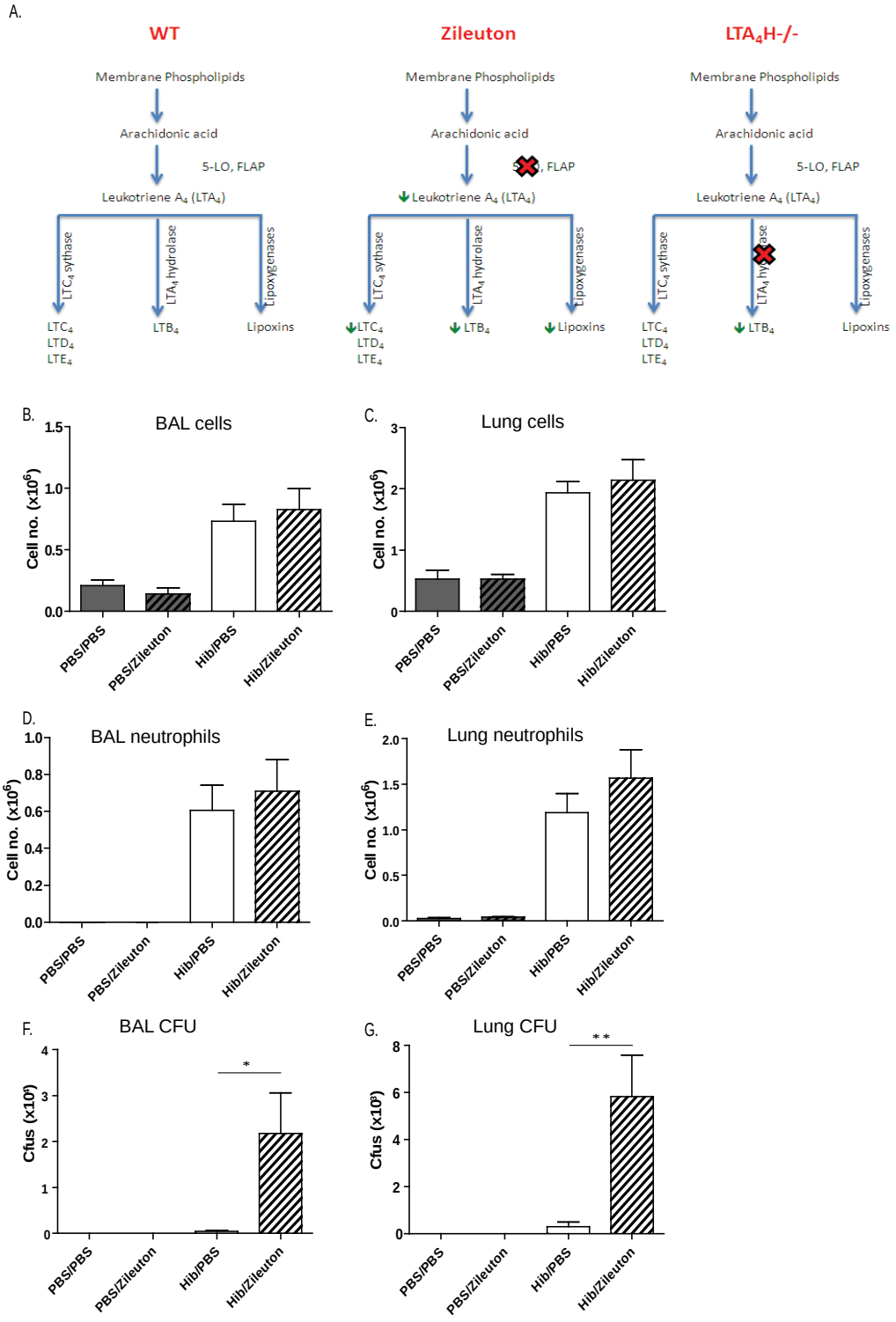


Supplementary figure 1. *Macrophage and lymphocyte response to Haemophilus influenzae b infection.*129/S6 mice were infected intranasally with 1x10^6 or 1 x10^7 *Haemophilus influenzae b* (Hib) and the numbers of alveolar macrophages (**A**), infiltrating monocytes / macrophages (**B**), NK cells (**C**), $CD4^+$ T cells (**D**) and $CD8^+$ T cells (**E**) in the airways of Hib infected mice was determined by flow cytometry. Hib-infected mice were administered control or clodronate liposomes and alveolar macrophage numbers (**F**) and CFU in the airways (**G**) and lung tissue (**H**) assessed at 24 hours post infection. Data (mean ± S.E.M.) are representative of at least 2 experiments with 5-6 mice per group. *, $P<0.05$; **, $P<0.01$ using Mann-Whitney.



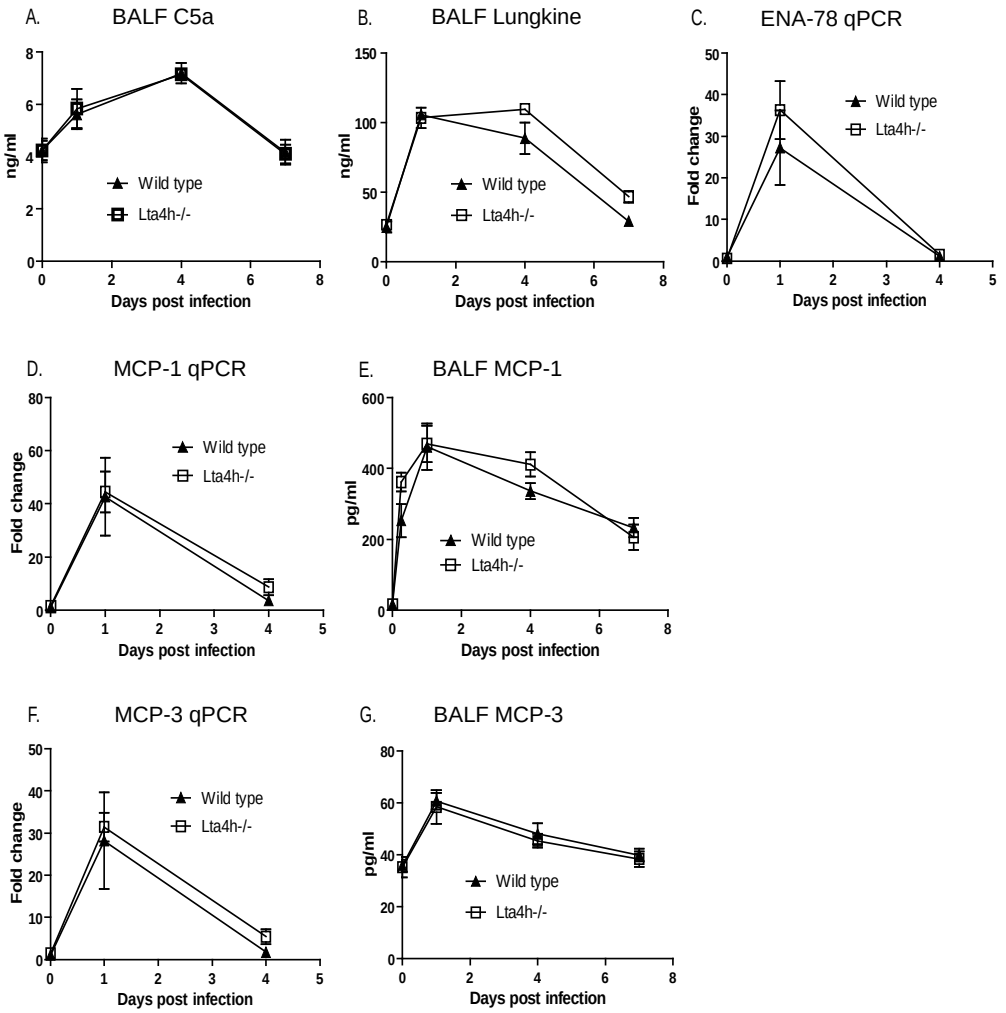
Supplementary figure 2. *Robust, acute pulmonary infiltrate controls Haemophilus influenzae infection in Balb/c and C57BL/6 mice.* Balb/c or C57BL/6 mice were infected intranasally with $1x10^6$ or $1 x10^7$ *Haemophilus influenzae b* (Hib) and weight loss assessed daily and expressed as a percentage of the original body mass (**A**). Total cell numbers in the airways (**B**) and lung tissue (**C**) of Hib-infected mice was enumerated by trypan blue exclusion. Bacterial burden was

assessed by performing serial dilutions of BALF (**D**) and lung homogenate (**E**) on Brain Heart Infusion (BHI) agar plates and counting colony forming units (CFU). The number of neutrophils recruited into the airways (**F**) and lung tissue (**G**) of Hib infected mice was determined by flow cytometry. Amounts of total MMP-9 were assessed by ELISA in the BALF at different times post infection (**H**). BALF from different time points post-Hib infection, was incubated with 4mM PGP and degradation assessed after 2 hours by mass spectrometry (**I**). Data (mean ± S.E.M.) are representative of at least 2 experiments with 5-6 mice per group. *, $P<0.05$; **, $P<0.01$ using Mann-Whitney.

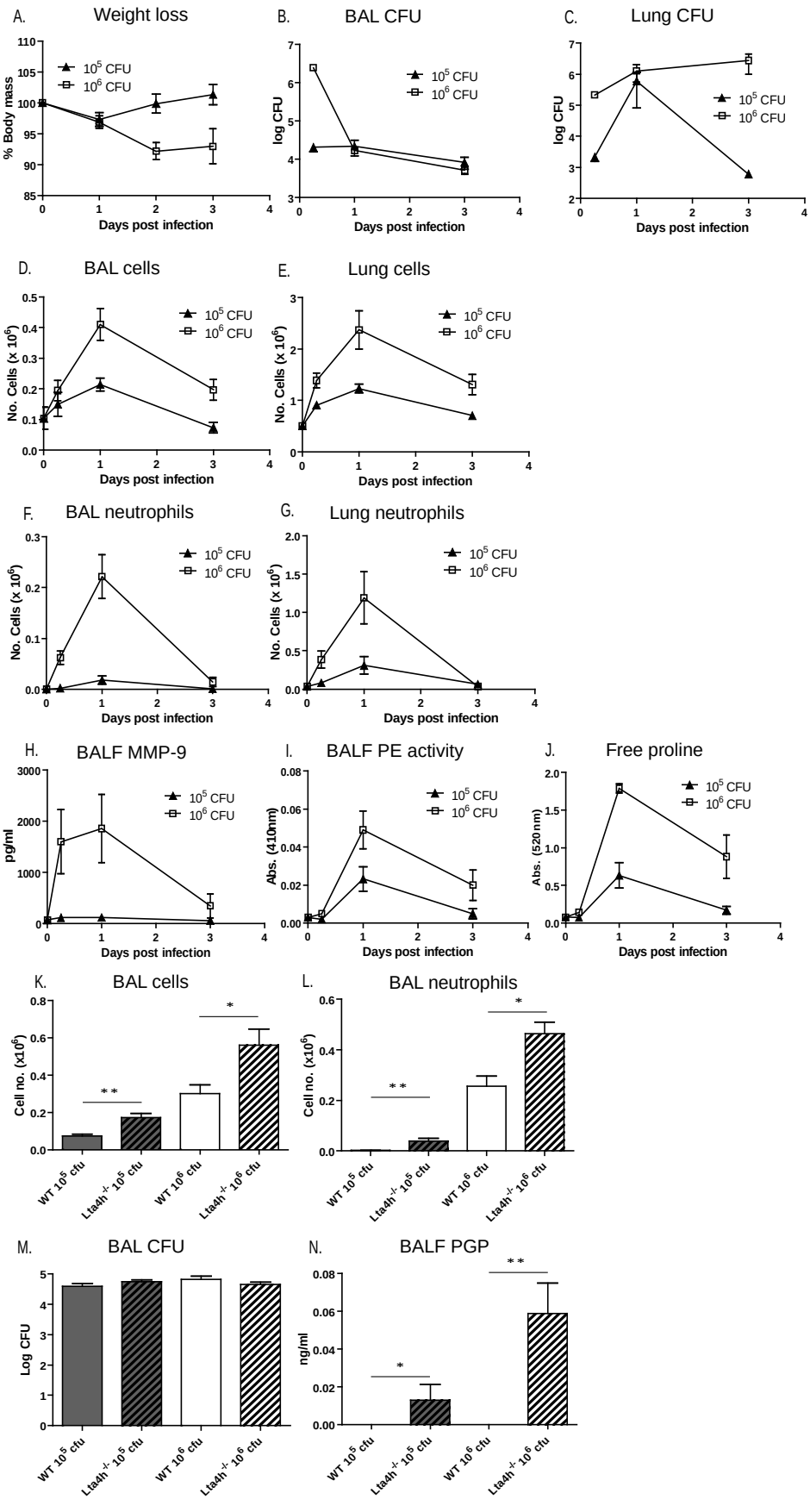


Supplementary figure 3. *5-lipoxygenase inhibition does not alter pulmonary inflammation but compromises bacterial clearance.* (**A**) Arachidonic acid, derived from membrane phospholipids, is converted to leukotriene A_4 (LTA_4) through the action of 5-lipoxygenase (5-LO) and 5-lipoxygenase-activating protein (FLAP). LTA_4 can subsequently be converted to cysteinyl

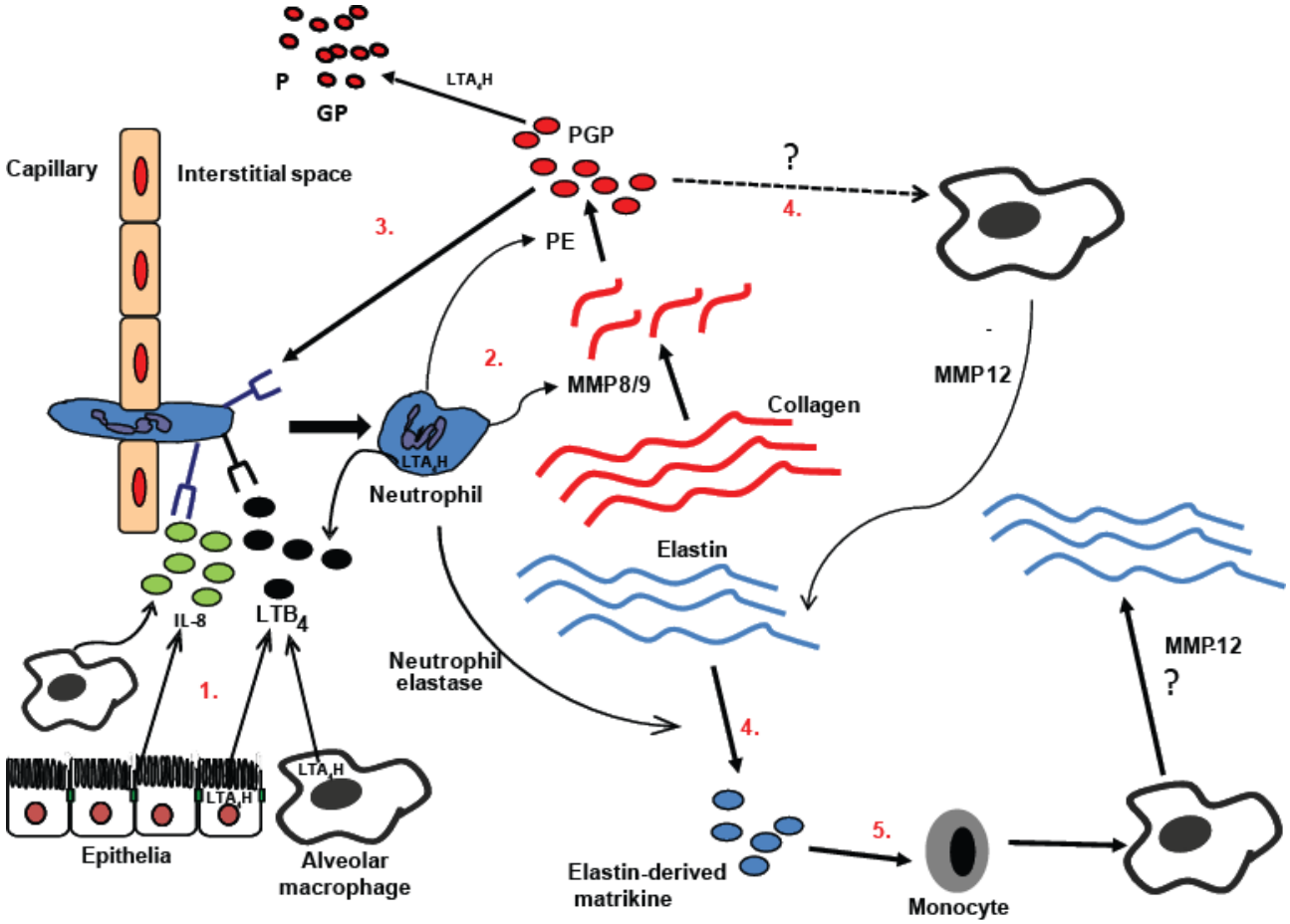
leukotrienes (LTC_4, D_4, E_4) by the action of LTC_4 synthase, to LTB_4 by the action of leukotriene A_4 hydrolase (LTA_4H) or to lipoxins via the action of lipoxygenases. Zileuton inhibits 5-LO, preventing LTA_4 formation and thus the ensuing generation of cysteinyl leukotrienes, LTB_4 and lipoxins. LTA_4H-/- mice selectively lose the capacity to convert LTA_4 to LTB_4. 129/S6 mice were infected intranasally with 1 $x10^7$ Hib and administered vehicle or zileuton. Total cell numbers in the airways (**B**) and lung tissue (**C**) were enumerated at 24 hours post infection. The number of neutrophils recruited into the airways (**D**) and lung tissue (**E**) of Hib infected mice was determined by flow cytometry. Bacterial burden at this time point was assessed by performing serial dilutions of BALF (**F**) and lung homogenate (**G**) on Brain Heart Infusion (BHI) agar plates. Data (mean ± S.E.M.) are from 5-6 mice per group.*, $P<0.05$; **, $P<0.01$ using Mann-Whitney.



Supplementary figure 4. *Hib-infected lta4h-/- mice display comparable levels of classical neutrophil and monocyte chemoattractants.* Lta4h-/- mice and littermate controls were infected intranasally with $1x10^7$ Hib and levels of C5a (**A**) and lungkine/CXCL15 (**B**) in the BALF were assessed by ELISA. Levels of ENA-78/CXCL5 mRNA were assessed in lung tissue by real time PCR at different times post Hib infection (**C**). Lung mRNA and BALF protein levels of MCP-1/CCL2 (**D** and **E,** respectively) and MCP-3/CCL7 (**F** and **G,** respectively) were determined at different times post Hib infection. Data (mean ± S.E.M.) are representative of at least 2 experiments with 5-6 mice per group (A, B, E and G) or are from a single experiment with 5 mice per group (C, D and F). *, $P<0.05$; **, $P<0.01$ using Mann-Whitney.


A.
Weight loss
% Body mass
Days post infection
B.
BAL CFU
log CFU
Days post infection
C.
Lung CFU
log CFU
Days post infection
D.
BAL cells
No. Cells (x 10^6)
Days post infection
E.
Lung cells
No. Cells (x 10^6)
Days post infection
F.
BAL neutrophils
No. Cells (x 10^6)
Days post infection
G.
Lung neutrophils
No. Cells (x 10^6)
Days post infection
H.
BALF MMP-9
pg/ml
Days post infection
I.
BALF PE activity
Abs. (410nm)
Days post infection
J.
Free proline
Abs. (520nm)
Days post infection
10^5 CFU
10^6 CFU
K.
BAL cells
Cell no. (x10^6)
L.
BAL neutrophils
Cell no. (x10^6)
M.
BAL CFU
Log CFU
N.
BALF PGP
ng/ml
WT 10^5 cfu
Lta4h^-/- 10^5 cfu
WT 10^6 cfu
Lta4h^-/- 10^6 cfu

Supplementary figure 5. *Streptococcus pneumoniae infected lta4h-/- mice display augmented pulmonary inflammation.* 129/S6 mice were infected intranasally with $1x10^5$ or $1x10^6$ *S. pneumoniae* and weight loss assessed daily and expressed as a percentage of the original body mass (**A**). Bacterial burden was assessed by performing serial dilutions of BALF (**B**) and lung homogenate (**C**) on Columbia blood agar. Total cell numbers in the airways (**D**) and lung tissue (**E**) of *S. pneumoniae* infected mice was assessed. The number of neutrophils recruited into the airways (**F**) and lung tissue (**G**) of *S. pneumoniae* infected mice was determined by flow cytometry. Amounts of total MMP-9 in BALF were assessed by ELISA (**H**) at different times post infection. (**I**) PE activity in BALF at different times after *S. pneumonia* infection was determined by change in absorbance at 410 nm following cleavage of p-nitroaniline from PE specific substrate ZGP-pNA. BALF from different time points post-Hib infection, was incubated with PGP and degradation assessed after 2 hours by release of free proline (**J**). Lta4h-/- mice and littermate controls were infected intranasally with $1x10^5$ or $1\ x10^6$ *S. pneumoniae* and total cell numbers in the airways enumerated at 24 hours post infection (**K**). The number of neutrophils recruited into the airways of *S. pneumoniae* infected mice was determined by flow cytometry (**L**). Bacterial burden was assessed by performing serial dilutions of BALF on Columbia blood agar plates (**M**). The concentration of PGP in BALF was determined by ESI-LC/MS/MS (**N**). Data (mean ± S.E.M.) are from 5-6 mice per group. *, $P<0.05$; **, $P<0.01$ using Mann-Whitney.



Supplementary figure 6. *A failure to degrade PGP leads to a protease-matrikine discord that exacerbates inflammation.* **1.** In response to infection or injury, pulmonary cells release classical neutrophil chemoattractants, such as IL-8 and LTB_4, leading to the recruitment of neutrophils from the vasculature and into the lung tissue. **2.** Recruited neutrophils release an array of proteases and the concerted actions of MMP-8/9 and PE target extracellular matrix collagen to yield the matrikine PGP. **3.** A failure to degrade PGP by LTA_4H enables it to promote further neutrophil recruitment and release of MMPs/PE with ensuing further generation of PGP – a feed forward mechanism to promote a vicious circle of neutrophilia. **4.** PGP also elicits the release of NE from neutrophils and, indirectly, the release of MMP-12 from alveolar macrophages. These elastases target extracellular matrix elastin to yield elastin-based matrikines that are chemotactic for monocytes. **5.** These chemotactic elastin fragments promote monocyte recruitment. Monocytes mature into tissue macrophages that may produce more MMP-12 to target elastin and generate further matrikines.