Fig. S1. Bacterial growth, bacterial binding to H292 monolayers, and PMN transepithelial migration are unaffected by pan-PLA_2 or $cPLA_2\alpha$ inhibitors. (**A**) *S. pneumoniae* TIGR4 was grown as described in *Materials and Methods*, in the presence or absence of pan-PLA_2 inhibitors ACA and ONO-RS-082 or of the $cPLA_2\alpha$ inhibitor. Growth was measured using a spectrophotometer. Shown is a representative of two experiments. **(B)** H292 monolayers were treated with pan-PLA_2 inhibitors ACA (100μM) or ONO-RS-082 (20μM); or the $cPLA_2\alpha$ inhibitor (6μM) (please see *Materials and Methods*). Monolayers were washed and infected with *S. pneumoniae* TIGR4. The number of bacteria bound to monolayers was determined by viable counts. Shown is a representative of two experiments. **(C)** H292 monolayers were treated with pan-PLA_2 inhibitors ACA (100μM) or ONO-RS-082 (20μM); or the DAG lipase inhibitor RHC-80267 (labeled as RHC, 100μM); or the $cPLA_2\alpha$ inhibitor (6μM) (please see *Materials and Methods*). The monolayers were washed and PMN transepithelial migration in response to fMLP was quantified by myeloperoxidase (MPO) assay. Monolayers treated with DMSO and HBSS +Ca/Mg were used as the vehicle control and negative control, respectively. Shown is a representative of two experiments.

Figure S2. $cPLA_2\alpha$ promotes pulmonary inflammation in *S. pneumoniae*-infected mice. Mice ($cPLA_2\alpha^{-/-}$ or their WT littermates) were mock–infected with PBS or infected intratracheally with *S. pneumoniae* TIGR4, as detailed in *Materials and Methods*. Mice were sacrificed and bronchoalveolar lavage fluid (BALF) and lungs were collected. **(A)** Myeloperoxidase (MPO) in the homogenized lung samples and BALF was determined biochemically. Figures are representative of two independent experiments. $^{**}p < 0.005$, $^{***}p < 0.001$, using one-way

ANOVA. **(B)** Serial dilutions of the lung homogenate and the BALF were plated on blood agar plates and bacterial CFUs were counted, ns= not significant.

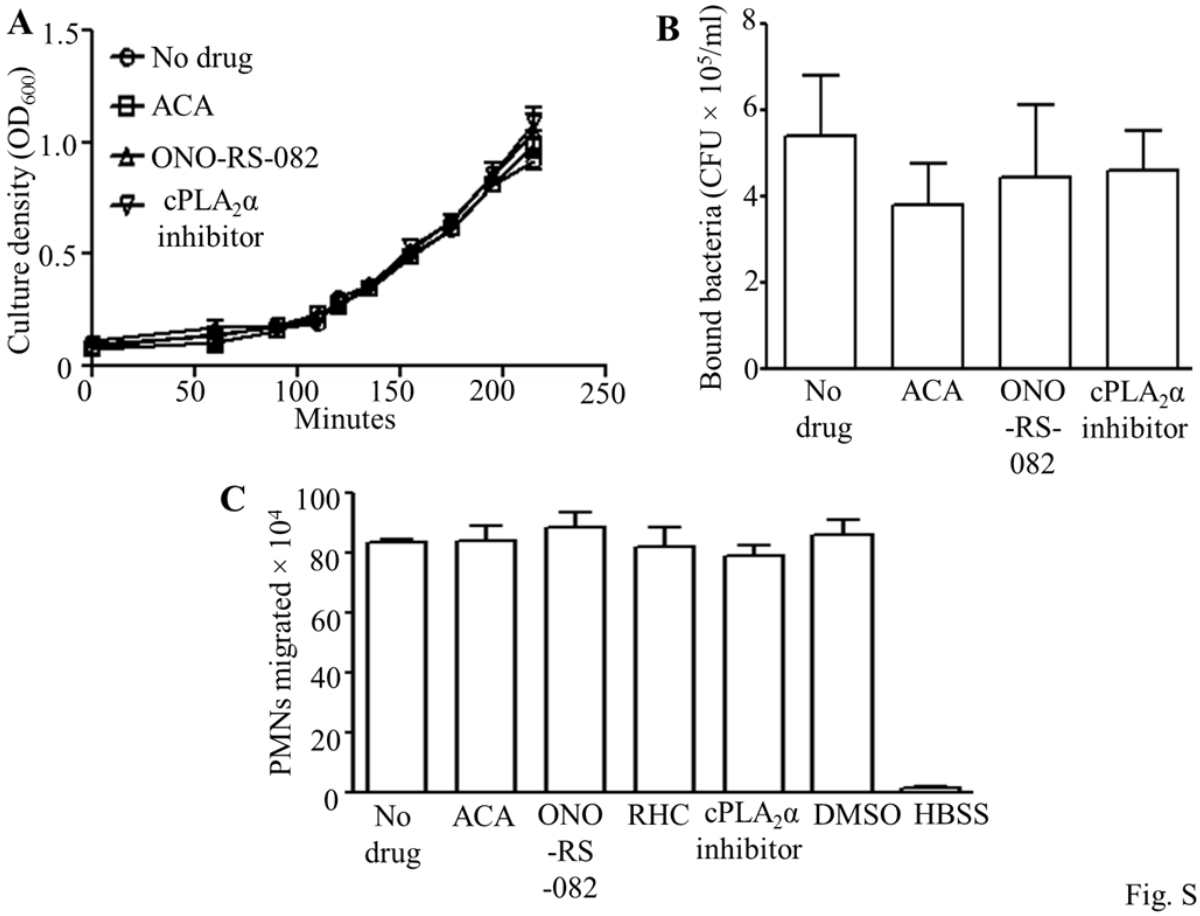

Fig. S1

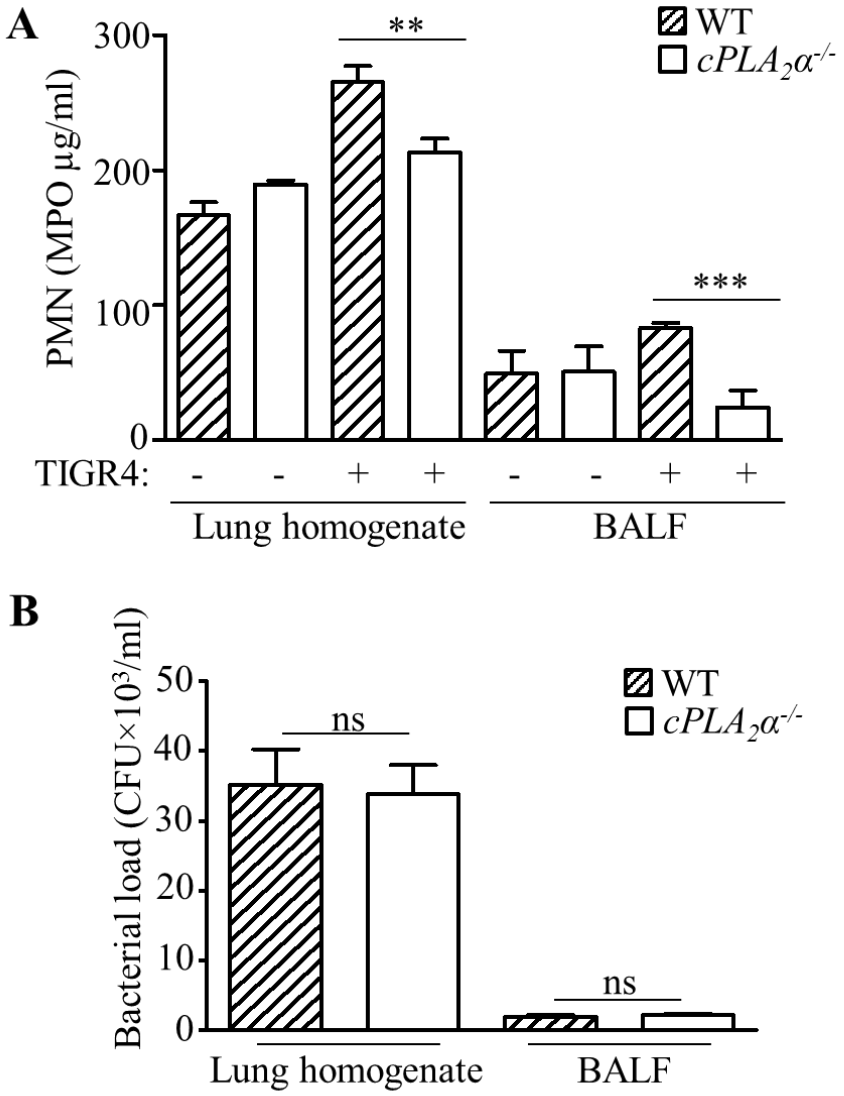
A
300
200
100
0
PMN (MPO μg/ml)
**
***
WT
cPLA2α-/-
TIGR4: - - + + - - + +
Lung homogenate
BALF
B
50
40
30
20
10
0
Bacterial load (CFU×10³/ml)
ns
ns
WT
cPLA2α-/-
Lung homogenate
BALF

Fig. S2

Table S1

	Cytotoxicity
HBSS, 2.5h	1.4 (0.4)
S. pneumoniae TIGR4, 2.5h	1.84 (0.4)
S. pneumoniae TIGR4, 5h	2.82 (0.8)
2% Triton X-100, 2.5h	68 (13.7)

Cytotoxicity in H292 monolayers in response to *S. pneumoniae* TIGR4 infection was assessed using the lactate dehydrogenase (LDH) assay [1]. Infection or treatment time is denoted in the table. Cytotoxicity was calculated as the amount of LDH in the supernatant as a percentage of total LDH (LDH inside the cell as well as released into the supernatant). Monolayers treated with Triton X-100 and HBSS (+Ca/Mg) were used as positive and negative controls, respectively. Shown is a representative of three experiments. Standard deviations are reported in parentheses.

Table S2

	HRP (ng/ml)
HBSS, 3.5h	7.4 (0.3)
S. pneumoniae TIGR4, 1.5h	11.67 (3)
S. pneumoniae TIGR4, 2.5h	8.216 (0.77)
S. pneumoniae TIGR4, 3.5h	12.73 (0.7)
Trypsin-EDTA, 2.5h	57.42 (2)

Effect of *S. pneumoniae* infection on the barrier integrity of H292 monolayers was assessed by horseradish peroxidase (HRP) assay [1]. Barrier integrity was measured as the amount of HRP (ng/ml) that could breach the H292 monolayer following infection. Monolayers treated with trypsin-EDTA and HBSS (+Ca/Mg) were used as positive and negative controls, respectively. Shown is a representative of two experiments. Standard deviations are reported in parentheses.

Table S3

	Cytotoxicity
HBSS	1.5 (0.4)
ACA (100µM)	2.7 (0.9)
ONO-RS-082 (20µM)	2.1 (0.8)
RHC-80267 (100µM)	1.74 (0.9)
$cPLA_2$-α inhibitor (6µM)	2.7 (1.3)
2% Triton X-100	66.37 (13.5)

Cytotoxicity of H292 monolayers in response to pharmacological inhibitors used in this study was assessed using LDH assay [1]. Inhibitor doses are denoted in the table. Cytotoxicity is calculated as the amount of LDH in the supernatant as a percentage of total LDH (LDH inside the cell as well as released into the supernatant). Monolayers treated with Triton X-100 and HBSS (+Ca/Mg) were used as positive and negative controls, respectively. Shown is a representative of two experiments. Standard deviations are reported in parentheses.

Table S4

	HRP (ng/ml)
HBSS	10.6 (2.5)
ACA (100μM)	13.07 (3.8)
ONO-RS-082(20μM)	10.48 (3.4)
RHC-80267 (100μM)	17.74 (6.2)
$cPLA_2$-α inhibitor (6μM)	12.43 (4.5)
Trypsin-EDTA	59.8 (9.8)

Effect of the pharmacological inhibitors on the barrier integrity of H292 monolayers was assessed by horseradish peroxidase (HRP) assay [1]. Inhibitor doses are denoted in the table. Barrier integrity was measured as the amount of HRP (ng/ml) that could breach the H292 monolayer in following the inhibitor treatment. Monolayers treated with trypsin-EDTA and HBSS (+Ca/Mg) were used as positive and negative controls, respectively. Shown is a representative of two experiments. Standard deviations are reported in parentheses.

1. Hurley, B.P., Siccardi, D., Mrsny, R.J., McCormick, B.A. , *Polymorphonuclear cell transmigration induced by Pseudomonas aeruginosa requires the eicosanoid hepoxilin A3.* J Immunol, 2004. **173**(9): p. 5712-20.