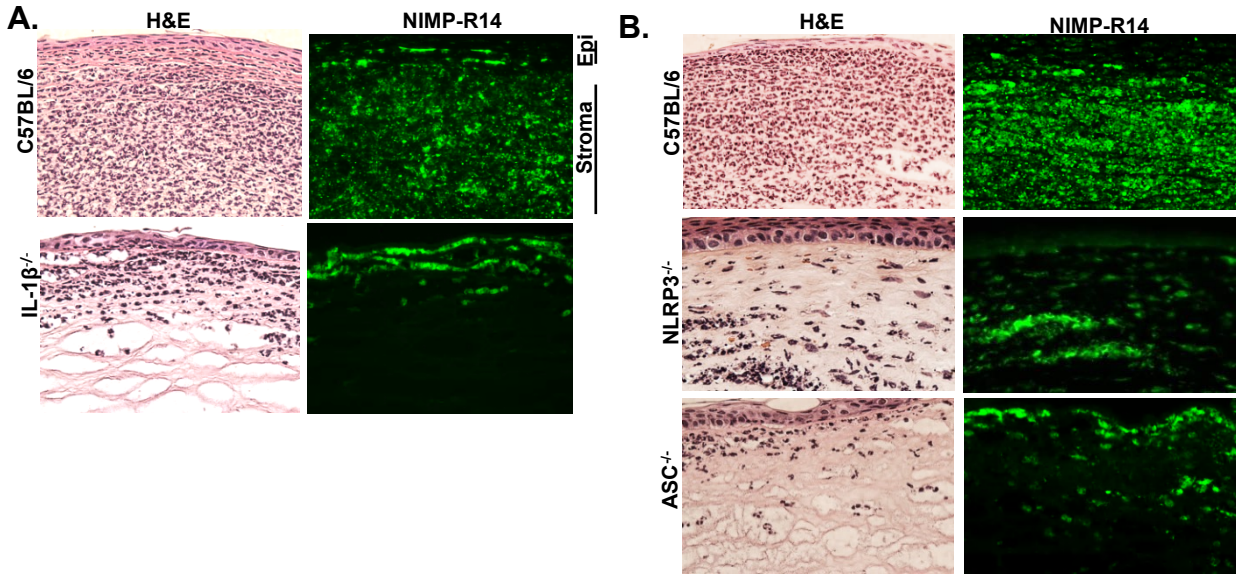
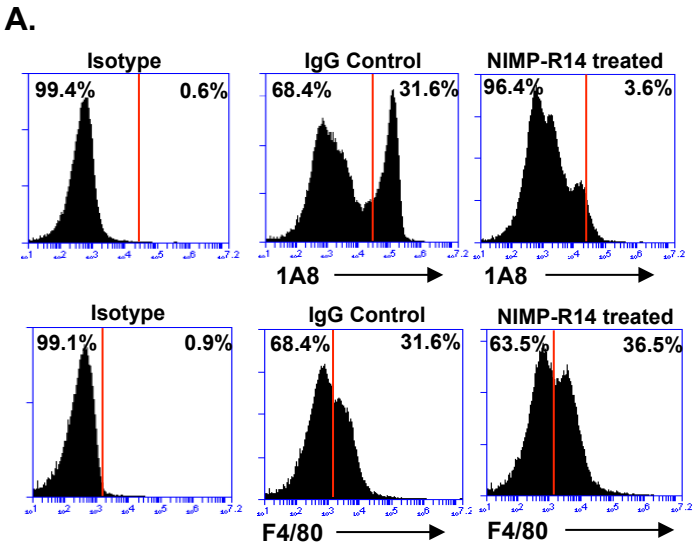
Supplementary Figure 1



Histology and Neutrophil staining of corneas infected with Sp. C57BL/6 and IL-1β$^{-/-}$ mice (**A**), and NLRP3$^{-/-}$ and ASC$^{-/-}$ mice were infected intrastromally with *S. pneumoniae.* After 24h, eyes were enucleated and 5µm corneal sections were stained with H&E to NIMP-R14 antibody to detect infiltrating neutrophils. Corneal epithelium (Epi) and stroma are noted.

Eyes were enucleated and fixed overnight in 10% formalin buffer (Fisher Scientific). 5µm section was cut from the center of the paraffin embedded cornea and stained with Gill's hematoxylin following deparaffinization. To stain for infiltrating neutrophils, fixed sections were stained with rat anti-mouse NIMP R14 and counter stained with Alexa-488 goat anti-rat (Invitrogen). Slides were mounted with DAPI containing mounting media (Vector Laboratories) and imaged by fluorescence microscopy.

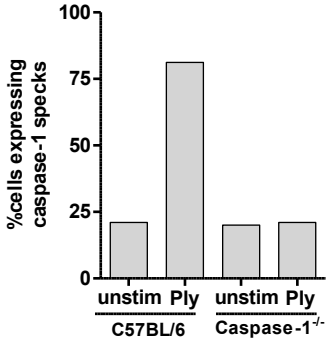
Supplementary Figure 2



S3. C57BL/6 mice were injected with 250µg of NIMP-R14 or IgG control antibody by ip injection 24h prior to infection with *S.pneumoniae.* **A**. Neutrophil depletion was confirmed by flow cytometry using 1A8 antibody (neutrophil specific marker).

Supplementary Figure 3

	Total no. of cells analyzed	No. of cells with 0 speck	No. of cells multiple specks
C57BL/6 unstim	5189 (100%)	4100 (79%)	1089 (21%)
C57BL/6 Ply (500ng/ml)	3576 (100%)	700 (18.6%)	3048 (81.2%)
Caspase1/11$^{-/-}$ unstim	5076 (100%)	4063 (80%)	1013 (20%)
Caspase1/11$^{-/-}$ Ply (500ng/ml)	4550 (100%)	3594 (79%)	956 (21%)



Pneumolysin does not induce speck formation in Caspase 1/11$^{-/-}$ neutrophils. Bone marrow neutrophils isolated from C57BL/6 and Caspase 1/11$^{-/-}$ mice were primed with hkSp and stimulated with active pneumolysin. 3576-5189 cells were counted, and the number of specks per cell was quantified using multispectral imaging flow cytometry as described in the Methods. Background specks in unstimulated neutrophils is likely due to necrotic cells in the population that release ATP and activate NLRP3 as described (Sutterwala, PNAS 2009).

Supplemental Table 1. Primer sequences for generation of Δ*ply* strain

SP_1923 F1	GTTAAAAAAGAGTAGGAGGTAGAGG
SP_1923 R1	ATACCGTCGACCTCGAGATC**TCCAAC**CTTCTACCTCCTAATAAGTTCCTG
SP_1923 F2	CTAATGACTGGCTTTTATAA**TCCAAC**GAGAGGAGAATGCTTGCGACAA
SP_1923 R2	ATCTGGATCACCTTTTTTAGCTG
cat F	GATCTCGAGGTCGACGGTA
cat R	TTATAAAAGCCAGTCATTAGGCC

Underlined sequence indicates homology to the *cat* cassette
Bold sequence indicates an MmeI restriction site