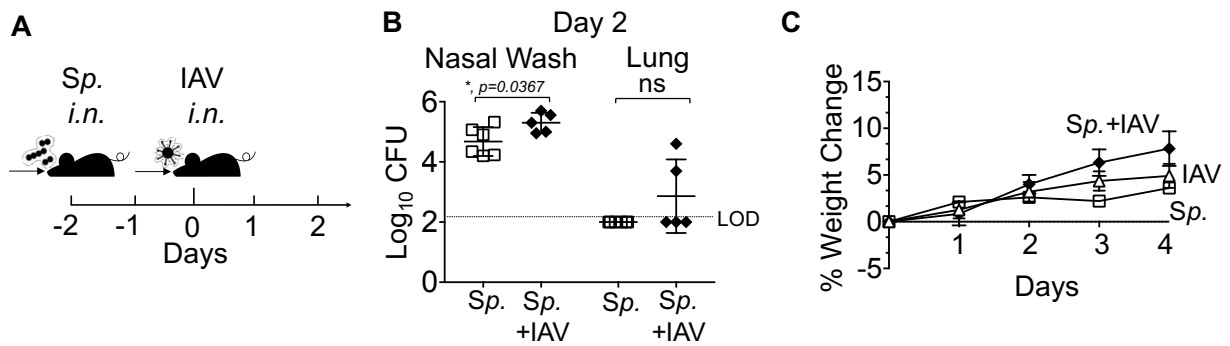


1 SUPPLEMENTAL MATERIAL



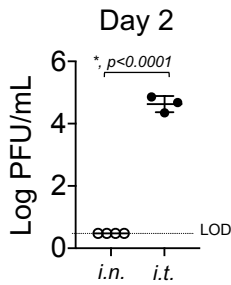
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3 **Figure S1: Course of disease following i.n. IAV inoculation.** (A) 8-10 weeks old  
4 C57BL/6 mice were pre-colonized i.n. with biofilm generated *S. pneumoniae* TIGR4  
5 (*Sp.*) at  $5 \times 10^6$  CFU, followed by i.n. co-infection with 500 PFU of Influenza A virus (PR8).  
6 (B) Two days post IAV-infection, mice were sacrificed and bacterial burdens in nasal  
7 lavage (left) and lung homogenates (right) were determined. Data were pooled from two  
8 separate experiments with  $n=6$  *Sp.* infected mice and  $n=5$  co-infected mice per group.  
9 (C) Mice were also monitored daily for weight change. Data were pooled from two  
10 separate experiments with  $n=9$  mice per group. Dotted lines represent the limit of  
11 detection. Asterisks (\*) represent statistical significance, and *ns* = not significant,  
12 determined using Student's t-test.

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17 **Figure S2. Intratracheal but not intranasal delivery of IAV results in viral infection**

18 **of the lungs.** 8-10 weeks old C57BL/6 mice were infected i.n. with 500 PFU of

19 Influenza A virus (PR8) or challenged i.t. with 20 PFU of PR8. Two days later, mice

20 were sacrificed and viral burdens in lung homogenates were determined. Data from

21 three mice per group are shown. Dotted lines represent the limit of detection. Asterisks

22 (\*) represent statistical significance, determined using Student's t-test.

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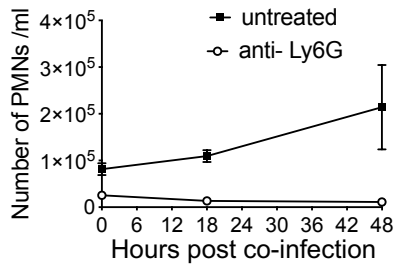
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33 **Figure S3. PMN numbers following depletion in co-infected mice.** 8-10 weeks old  
 34 C57BL/6 mice were intraperitoneally (i.p.) injected with anti-Ly6G (clone 1A8) antibodies  
 35 to deplete neutrophils or mock treated. The antibodies were given daily from day -3 to  
 36 day 1, and every other day from day 3 to the end of each experiment (with respect to  
 37 IAV-infection). At 0, 18 and 48 hours post IAV infection, blood was collected and the  
 38 number of circulating PMNs (RB6<sup>hi</sup>) was determined by flow cytometry. Data shown  
 39 represent the means +/- SEM and are representative from one of three separate  
 40 experiments with n=3 mice per group.

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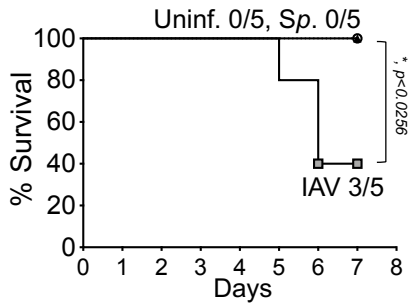
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49 **Figure S4. Course of survival following intranasal *S. pneumoniae* inoculation or**

50 **intranasal/intratracheal IAV inoculation of aged mice.** Aged (18-24 months)

51 C57BL/6 male mice were singly infected with *S. pneumoniae* TIGR4 i.n. (*Sp*) or

52 Influenza A virus PR8 i.n and i.t. (IAV) or mock-treated (uninfected) and survival was

53 assessed over time and compared to uninfected controls. Fractions denote survivors

54 over total number of mice.

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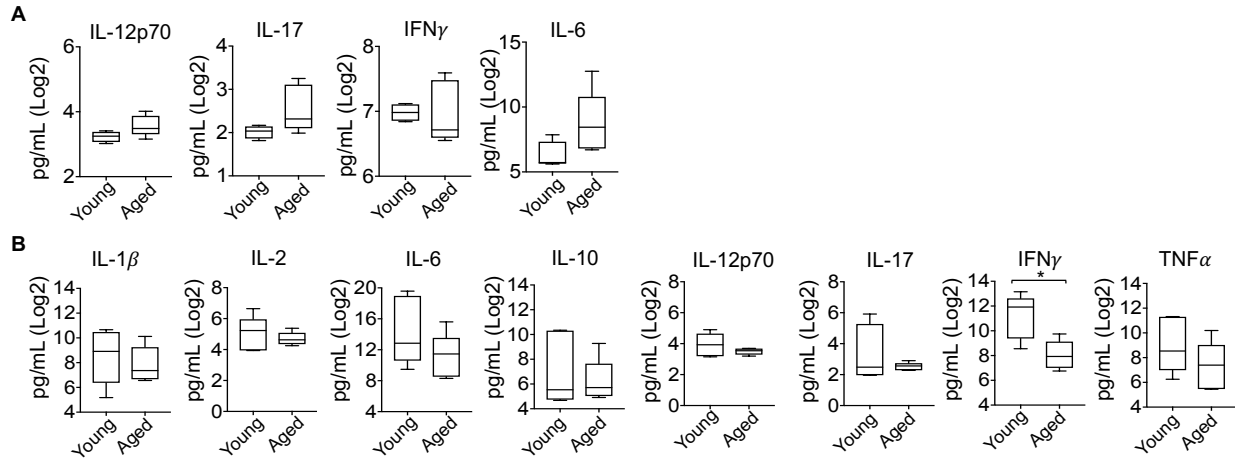
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63 **Figure S5: IAV/*S. pneumoniae* co-infection results in no significant age-**  
 64 **dependent differences in TH1/TH17 cytokines.** Young and aged C57BL/6 male mice  
 65 were co-infected with *S. pneumoniae* and Influenza A virus PR8. Cytokines in the  
 66 supernatants of lung homogenates of young (n=4) or aged (n=5) mice at (A) 18 hours  
 67 and (B) at 48 hours following co-infection were measured. Asterisks (\*) represent  
 68 statistical significance as determined by Student's t-test.

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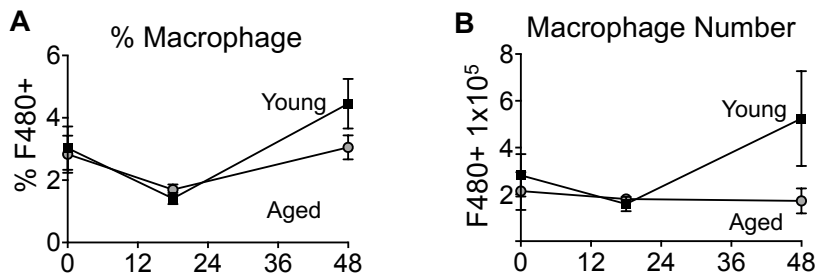
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76 **Figure S6: IAV/S. pneumoniae co-infection results in no significant age-**

77 **dependent differences in pulmonary macrophage influx.** The percentage (A) and

78 total number (B) of pulmonary macrophages of co-infected young or aged mice were

79 identified by flow cytometry using the F480 marker at the indicated times. The mean +/-

80 SEM pooled from three separate experiments are shown where data are pooled from

81 twelve mice per age group, except for 48 hours post-co-infection, where due to the

82 kinetics of disease data from only 7 surviving old mice are shown. No significant

83 differences were detected using Student's t-test.

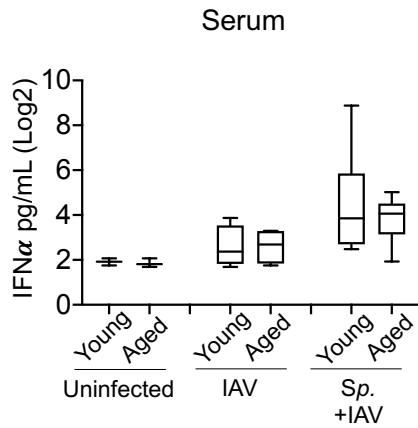
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90 **Figure S7. IFN- $\alpha$  levels in the sera following infection.** Young and aged C57BL/6 male  
 91 mice were either co-infected with *S. pneumoniae* and Influenza A virus PR8, challenged  
 92 with virus alone or mock challenged with PBS. 48 hours following IAV-infection (see  
 93 experimental design in Fig. 1A), blood was collected and serum levels of IFN- $\alpha$  were  
 94 measured by ELISA. Pooled data from two separate experiments with n=4 mice per group  
 95 are shown.

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