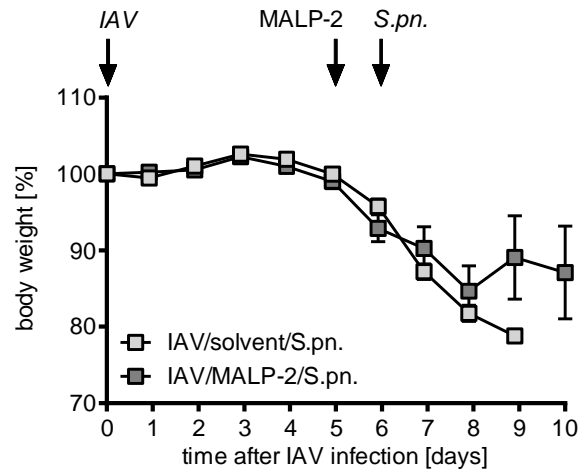


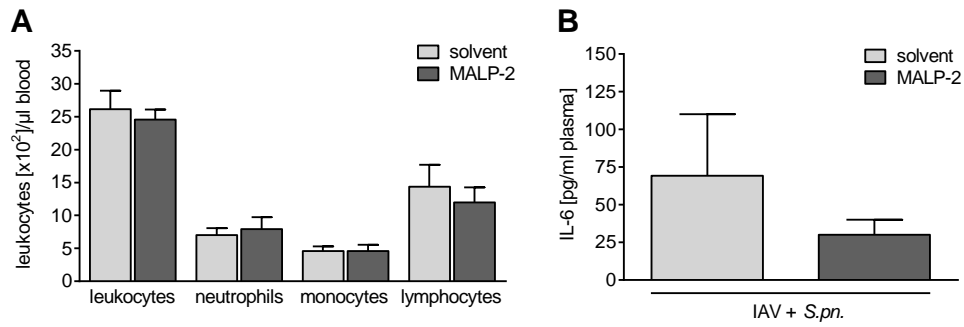
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2 **Supplemental Figure 1.** Pulmonary MALP-2 stimulation did not affect systemic interleukin-6  
3 (*IL-6*) levels or clinical parameters 6 d after IAV infection. Mice were treated with 0.5 µg  
4 MALP-2 or solvent 5 d post infection with 10<sup>2</sup> PFU  
5 Influenza virus A/H1N1/PR/8/34 (IAV) or sham infection with PBS. (A) Plasma IL-6 levels  
6 were quantified using cytokine singleplex assay (Bioplex®, BioRad, Hercules, CA). (B) Body  
7 weight and body temperature were measured. Values are given as mean + SEM (n=6-8 (A) or  
8 8 (B) each group). <sup>##</sup> *p*<0.01 vs. corresponding sham-infected group.



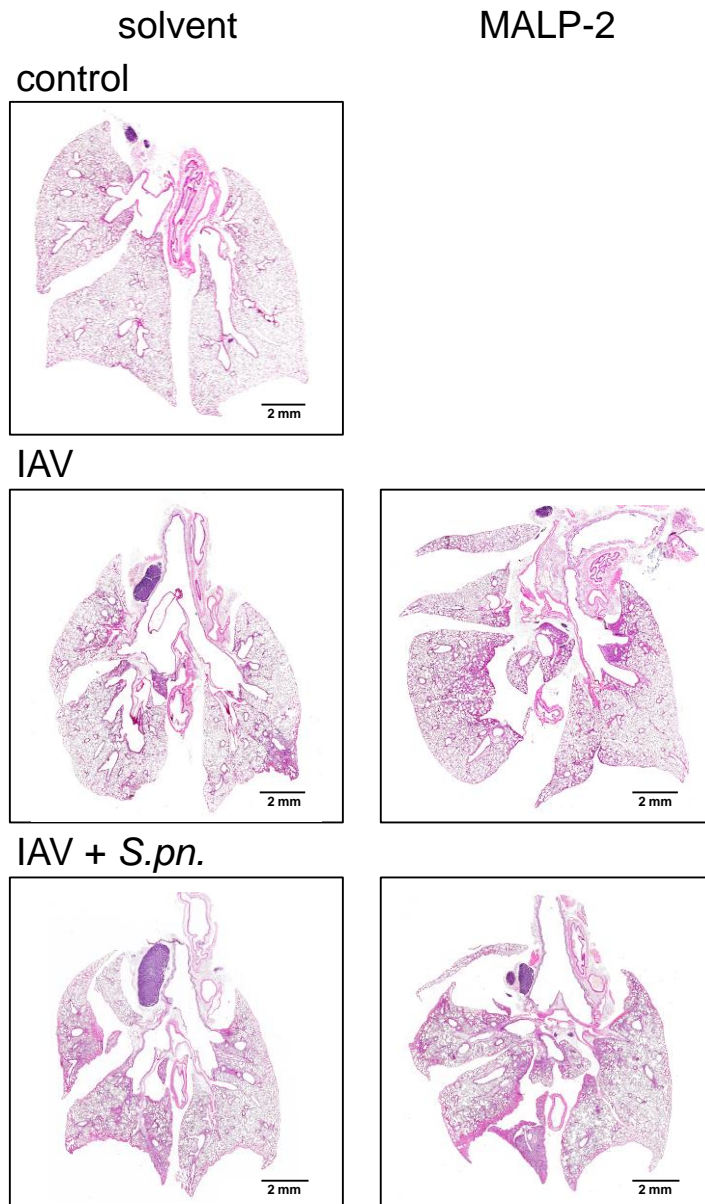
9

10 **Supplemental Figure 2.** Pulmonary MALP-2 treatment of IAV-infected mice did not affect  
 11 the course of body weight after pneumococcal superinfection. Mice infected with  $10^2$  PFU  
 12 IAV were treated with 0.5  $\mu$ g MALP-2 or solvent on day 5. Secondary infection with  $10^3$   
 13 CFU *S. pneumoniae* (S.pn) was performed on day 6. Body weight was monitored for 10 d after  
 14 IAV infection. Values are given as mean + SEM (n=10 (MALP-2) or 9 (solvent)).



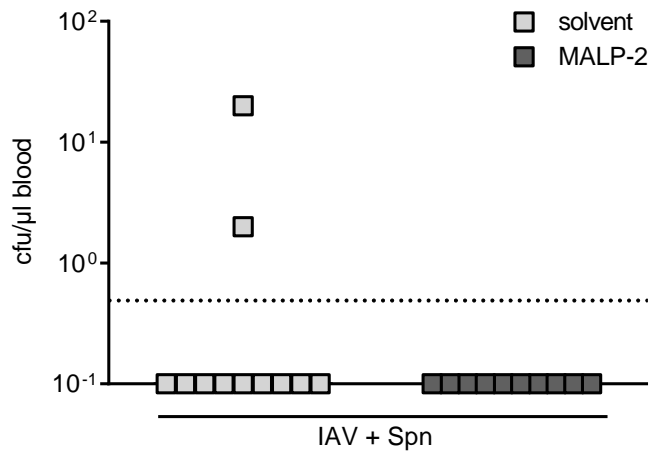
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16 **Supplemental Figure 3.** Pulmonary MALP-2 stimulation did not affect the systemic  
 17 inflammatory response after secondary pneumococcal infection. Mice infected with  $10^2$  PFU  
 18 IAV were treated with 0.5  $\mu$ g MALP-2 or solvent on day 5 and challenged with  $10^3$  CFU *S.*  
 19 *pneumoniae* (Spn) on day 6. (A) Blood leukocytes were quantified by FACS on day 7. Values  
 20 are given as mean + SEM (n=7-8 each group). (B) Plasma interleukin-6 (IL-6) levels were  
 21 measured using cytokine singleplex assay. Values are given as mean + SEM (n=7-8 each  
 22 group).



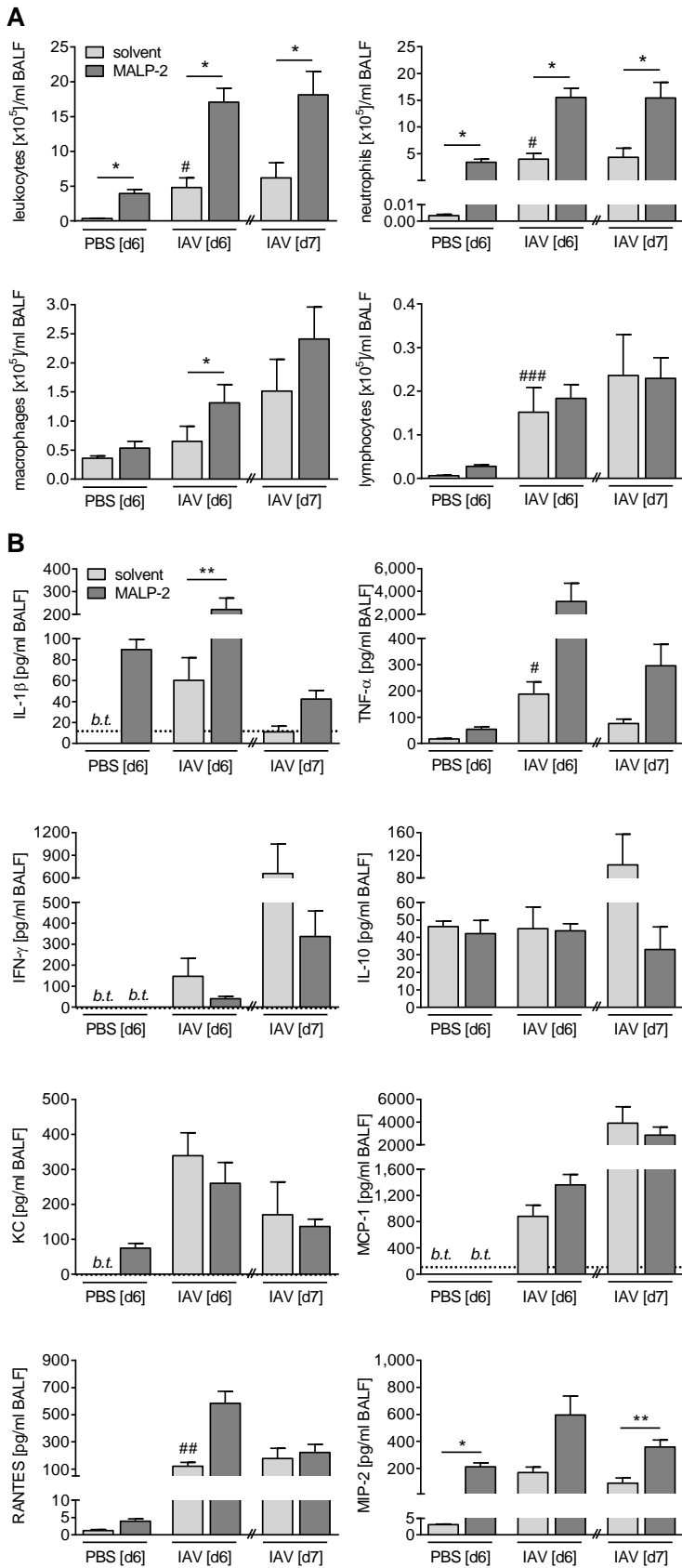
23

24 **Supplemental Figure 4.** MALP-2 did not alter the distribution of lung lesions after IAV  
 25 infection and pneumococcal superinfection. Mice infected with  $10^2$  PFU IAV were treated  
 26 with  $0.5 \mu\text{g}$  MALP-2 or solvent on day 5 and challenged with  $10^3$  CFU *S. pneumoniae* or  
 27 sham-infected with PBS on day 6. Lungs were harvested on day 7 and formalin-fixed and  
 28 paraffin-embedded sections were prepared and stained with hematoxylin and eosin for  
 29 histopathological analyses. Total lung area affected by inflammation was determined by  
 30 microscopic evaluation. Lung sections of sham-infected and solvent-treated mice served as  
 31 negative control. Representative images are shown (n=3-4 each group).



32

33 **Supplemental Figure 5.** *Blood bacterial load after secondary pneumococcal infection.* Mice  
 34 infected with 10<sup>2</sup> PFU IAV were treated with 0.5 μg MALP-2 or solvent on day 5 and  
 35 challenged with 10<sup>3</sup> CFU *S. pneumoniae* (Spn) on day 6. Blood bacterial load was determined  
 36 on day 7. Values are given as individual data (n=10-11 each group). The *dotted line* indicates  
 37 the lower detection limit.



38

39 **Supplemental Figure 6.**

40 *Overview of leukocyte recruitment and cytokine release in influenza A virus (IAV)-infected*  
41 *lungs on day 6 and 7 summarizing data from Figure 1 and 4 of the main article. Mice*  
42 *infected with 10<sup>2</sup> PFU IAV or sham-infected with PBS were treated with 0.5 µg MALP-2 or*  
43 *solvent on day 5 (PBS and IAV [d6]). Secondary sham infection with PBS was performed on*  
44 *day 6 (IAV [d7]). BAL was performed and BALF leukocytes (A) and cytokines (B) were*  
45 *quantified six and seven days post infection. Dotted lines indicate the lower limit of the*  
46 *cytokine assay working range and are missing if all values were within the working range. b.t.*  
47 *= values below threshold. Values are given as mean + SEM (n=5-8 (A) or 3-8 (B) each*  
48 *group). \*  $p < 0.05$ , \*\*  $p < 0.01$  as indicated; #  $p < 0.05$ , ##  $p < 0.01$ , ###  $p < 0.001$  vs.*  
49 *corresponding sham-infected group.*