

Airway *Prevotella* promote TLR2-dependent neutrophil activation and rapid clearance of *Streptococcus pneumoniae* from the lung

SUPPLEMENTARY INFORMATION

Supplementary Figures

Supplementary Figure 1

Supplementary Figure 2

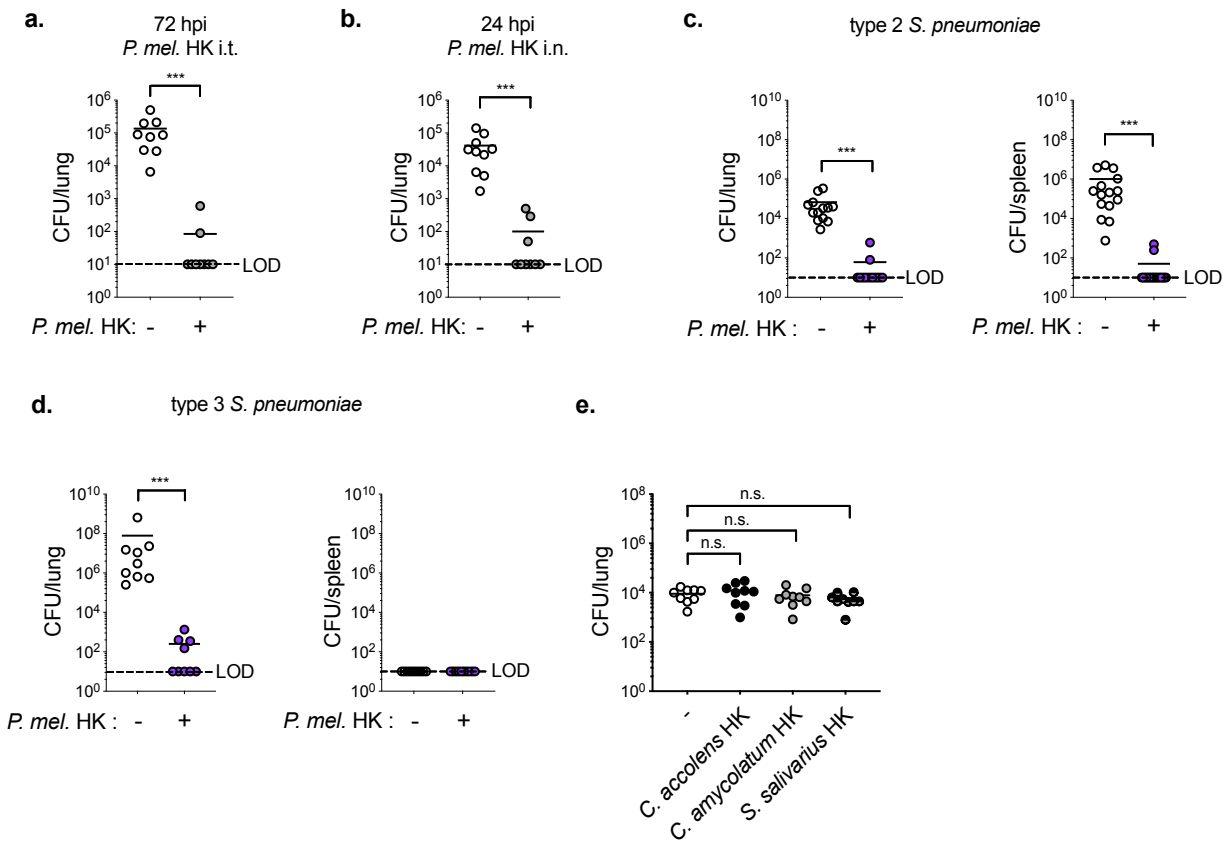
Supplementary Figure 3

Supplementary Figure 4

Supplementary Figure 5

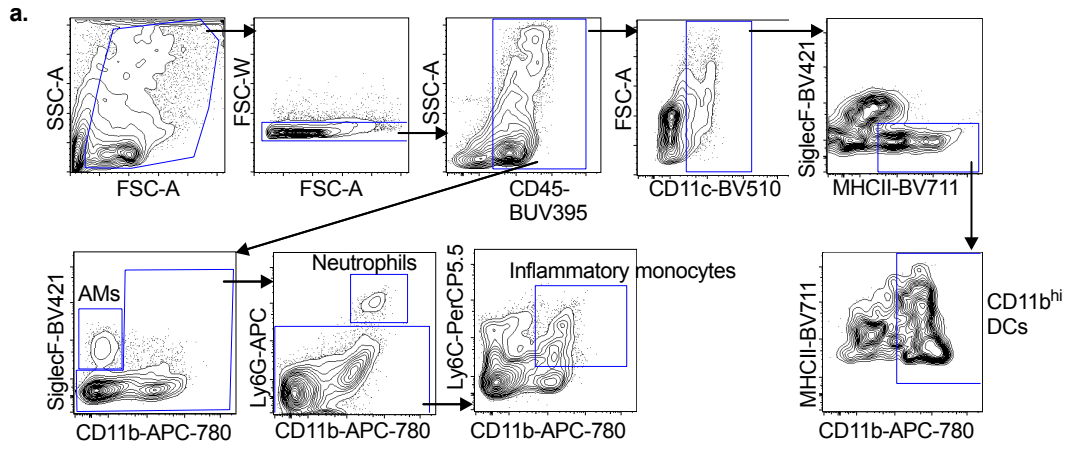
Supplementary Figure 6

Supplementary Figure 7

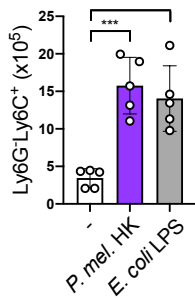


Supplementary Figure 1. Exposure to airway *P. melaninogenica* (*P. mel.*) heat-killed (HK) i.t. or i.n. induces clearance of *S. pneumoniae*. **a** Lung type 2 *S. pneumoniae* burdens in mice exposed to PBS (-) or *P. melaninogenica* (*P. mel.*) strain 25845 HK i.t. prior to 72 h *S. pneumoniae* infection, 5×10^6 CFU/mouse (n= 9 mice/group). **b** Lung *S. pneumoniae* burdens in mice exposed to PBS (-) or *P. mel.* HK i.n. prior to 24 h *S. pneumoniae* infection (n= 10 mice/group). **c - d** Tissue burdens of type 2 *S. pneumoniae* strain D39 (n= 13 mice/group) (**c**) or type 3 *S. pneumoniae* strain ATCC 6303 (n= 9 mice/group) (**d**) following exposure to PBS (-) or *P. mel.* HK i.t. prior to 24 h *S. pneumoniae* infection, 5×10^6 CFU/mouse. **e** Lung type 2 *S. pneumoniae* burdens in mice exposed to PBS (-), *C. accolens* HK, *C. amycolatum* HK, or *S. salivarius* HK i.t. (10^7 CFU equivalents/mouse each) i.t. prior to 24 h *S. pneumoniae* infection, 5×10^6 CFU/mouse (n= 9 mice/group). LOD = limit of detection. Data are pooled from 3

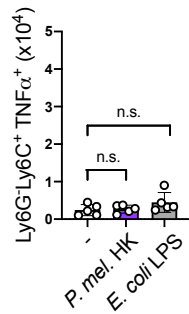
independent experiments, displayed as mean \pm SEM. For (a), $***p < .0001$, two-tailed Mann-Whitney U test, (b) $***p < .0001$, two-tailed Mann-Whitney U test, (c) $***p < .0001$, two-tailed Mann-Whitney U test, (d) $***p < .0001$, two-tailed Mann-Whitney U test, (e) from left to right $p > .9999$, $p > .9999$, $p = .5156$, Kruskal-Wallis with Dunn's *post-hoc* test. Source data are provided as a Source Data file.



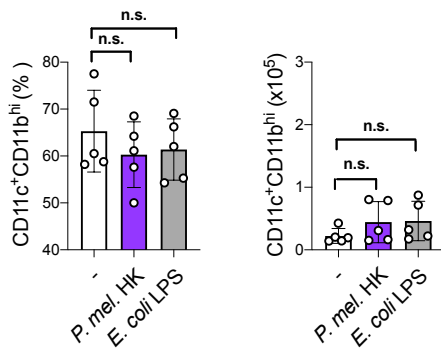
b. Inflammatory monocytes -uninfected mice-



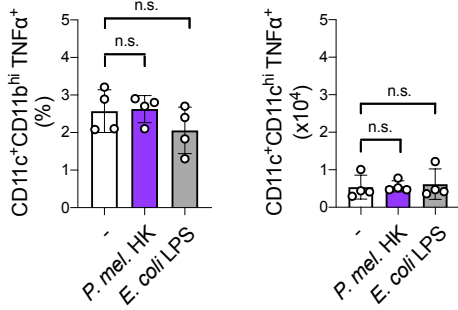
c. Inflammatory monocyte TNF α -uninfected mice-



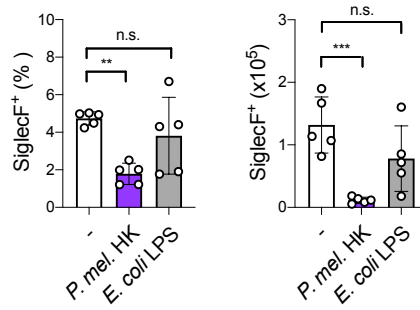
d. CD11b^{hi} DCs -uninfected mice-



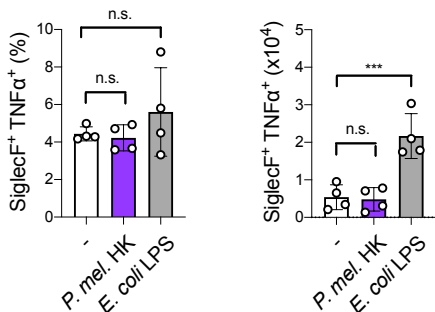
e. CD11b^{hi} DC TNF α -uninfected mice-



f. AMs -uninfected mice-



g. AM TNF α -uninfected mice-

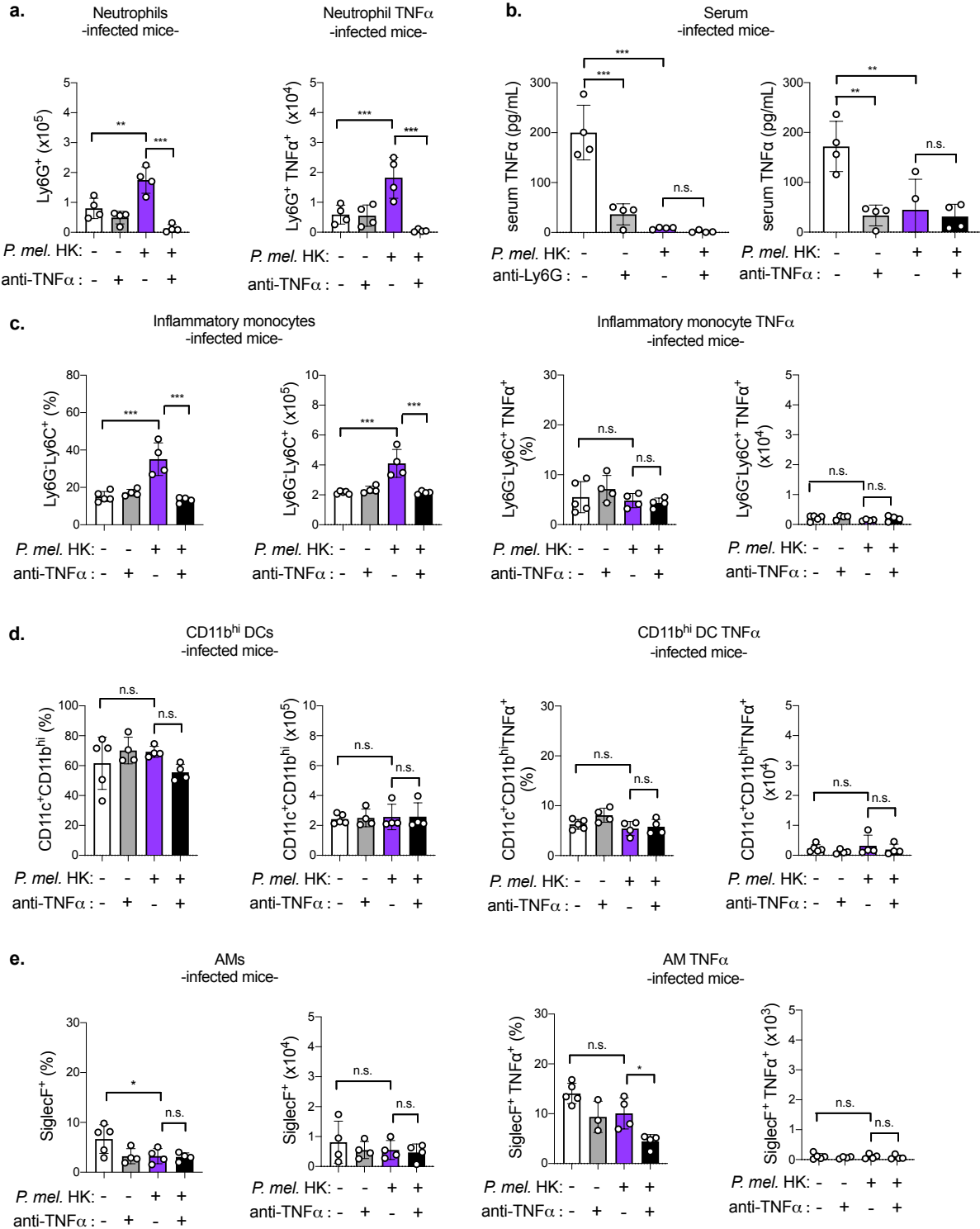


h.

BAL TNF α (pg/mL)

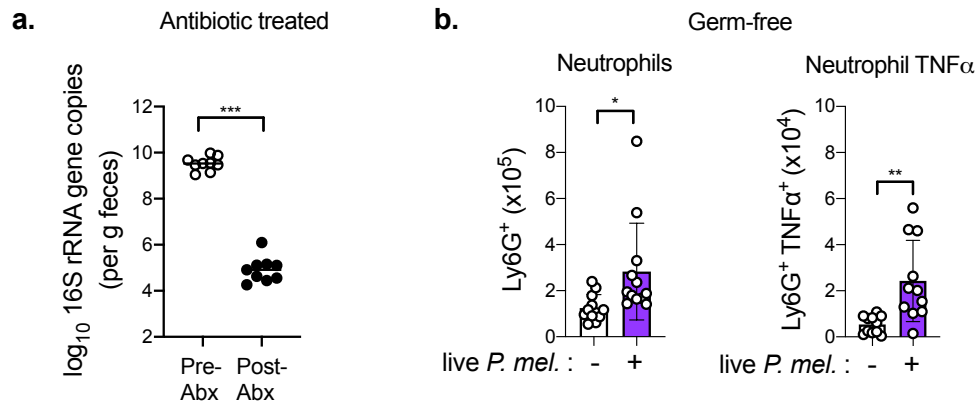
P. mel. HK: + + -
anti-Ly6G: - + -

Supplementary Figure 2. Lung myeloid cell responses to *P. melaninogenica* (*P. mel.*) lung exposure. **a** Flow cytometry gating strategy defining neutrophils, alveolar macrophages (AMs), inflammatory monocytes, and CD11b^{hi} dendritic cells (DCs) from single cell lung populations. This gating strategy was used for all flow cytometry analysis. **b - g** Total cell number of inflammatory monocytes (**b**), inflammatory monocyte TNF α (**c**), percentage and total cell number of CD11b^{hi} DCs (**d**), CD11b^{hi} DC TNF α (**e**), AMs (**f**), and AM TNF α (**g**) detected by intracellular flow cytometry in mice treated with PBS (-), *P. mel.* strain 25845 heat-killed (HK), or *E. coli* lipopolysaccharide (LPS) i.t. for 24 h. **h** Bronchoalveolar lavage (BAL) TNF α detected in mice with or without exposure to *P. mel.* HK i.t. or anti-Ly6G antibodies (200 μ g/mouse) i.p. for 24 h. Data are representative from one of four independent experiments with n= 5 mice/group (**b - g**) or pooled from two independent experiments with n= 3 mice/group (replicate 1) or n= 4 mice/group (replicate 2) (**h**), displayed as mean \pm SEM. For (**b**) from left to right *** p =.0002, *** p =.0007, one-way ANOVA with Dunnett's *post-hoc* test, (**c**) from left to right p =.9681, p =.1850, one-way ANOVA with Dunnett's *post-hoc* test, (**d**) from left to right p =.4835, p =.6292, p =.3598, p =.3099, one-way ANOVA with Dunnett's *post-hoc* test, (**e**) from left to right p =.9842, p =.3316, p =.3396, p >.9999, one-way ANOVA with Dunnett's *post-hoc* test, (**f**) from left to right ** p =.0052, p =.4202, *** p =.0009, p =.0968, one-way ANOVA with Dunnett's *post-hoc* test, (**g**) from left to right p =.9676, p =.4429, p =.0968, *** p =.0009, one-way ANOVA with Dunnett's *post-hoc* test, (**h**) *** p <.0001, one-way ANOVA with Dunnett's *post-hoc* test. Source data are provided as a Source Data file.

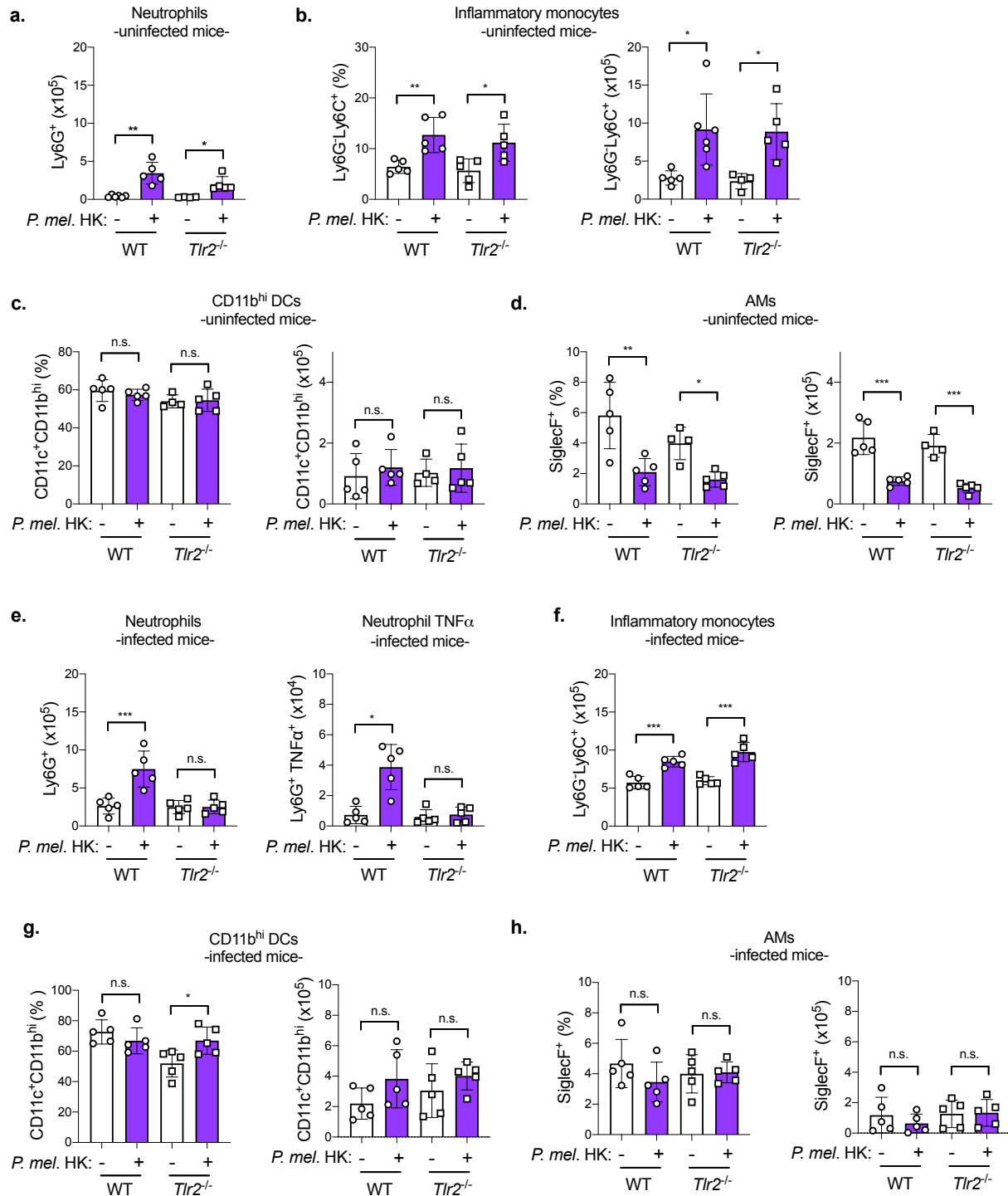


Supplementary Figure 3. Systemic and lung myeloid cell responses in neutrophil and TNF α depleted mice exposed to *P. melaninogenica* (*P. mel.*) prior to *S. pneumoniae* infection. **a** Total

cell number of neutrophils and neutrophil TNF α in mice treated with isotype control antibody or anti-TNF α i.p. (200 μ g/mouse) together with either PBS (-) or *P. mel.* strain 25845 heat-killed (HK) i.t. prior to 24 h type 2 *S. pneumoniae* infection, 5x10⁶ CFU/mouse (n= 4 mice/group). **b** Serum TNF α detected in mice treated with isotype control antibody, anti-Ly6G or anti-TNF α i.p. (200 μ g/mouse) together with either PBS (-) or *P. mel.* HK i.t. prior to 24 h *S. pneumoniae* infection (n= 4 mice/group). **c - e** Percentage and total cell number of inflammatory monocytes and inflammatory monocyte TNF α (**c**), percentage and total cell number of CD11b^{hi} DCs and CD11b^{hi} DC TNF α (**d**), and percentage and total cell number of alveolar macrophages (AMs) and AM TNF α (**e**), detected by intracellular flow cytometry in mice treated with isotype control antibody or anti-TNF α i.p. together with either PBS (-) or *P. mel.* HK i.t. prior to 24 h *S. pneumoniae* infection (n= 5 mice/group). Data are representative from one of four independent experiments, displayed as mean \pm SEM. For (**a**) from left to right ** p =.0018, *** p <.0001, *** p <.0009, *** p <.0001, one-way ANOVA with Sidak's *post-hoc* test, (**b**) from left to right *** p <.0001, *** p <.0001, p =.9882, ** p =.0031, ** p =.0059, p =.9691, one-way ANOVA with Tukey's *post-hoc* test, (**c**) from left to right *** p <.0001, *** p <.0001, *** p <.0001, *** p =.0001, p =.8872, p =.9479, p =.3253, p =.5295, one-way ANOVA with Sidak's *post-hoc* test, (**d**) from left to right p =.5388, p =.1945, p =.9421, p =.9989, p =.5829, p =.9088, p =.7734, p =.6495, one-way ANOVA with Sidak's *post-hoc* test, (**e**) from left to right * p =.0414, p =.9898, p =.6551, p =.9580, p =.0617, * p =.0137, p =.9887, p =.9038, one-way ANOVA with Sidak's *post-hoc* test. Source data are provided as a Source Data file.

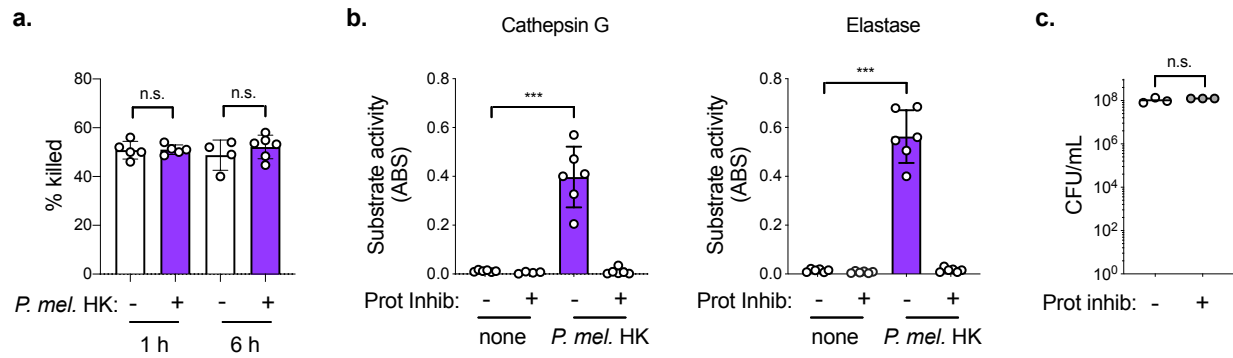


Supplementary Figure 4. Antibiotic depletion of the microbiome and *P. melaninogenica* (*P. mel.*)-induced neutrophil recruitment and activation in Germ-free mice. **a** 16S rRNA gene copies per gram of feces from naïve mice (pre-Abx) and following 7 days on water containing an antibiotic cocktail (post-Abx) detected by qPCR (n= 9 mice/group). **b** Total cell number of neutrophils and neutrophil TNF α detected by intracellular flow cytometry in Germ-free mice treated with either PBS (-) or live *P. mel.* strain 25845 i.t. prior to 24 h type 2 *S. pneumoniae* infection, 10⁶ CFU/mouse (n= 11 mice/group). Data are pooled three independent experiments, displayed as mean \pm SEM. For **(a)** *** p =.0004, two-tailed t test, **(b)** from left to right * p =.0197, ** p =.0024, two-tailed t test. Source data are provided as a Source Data file.

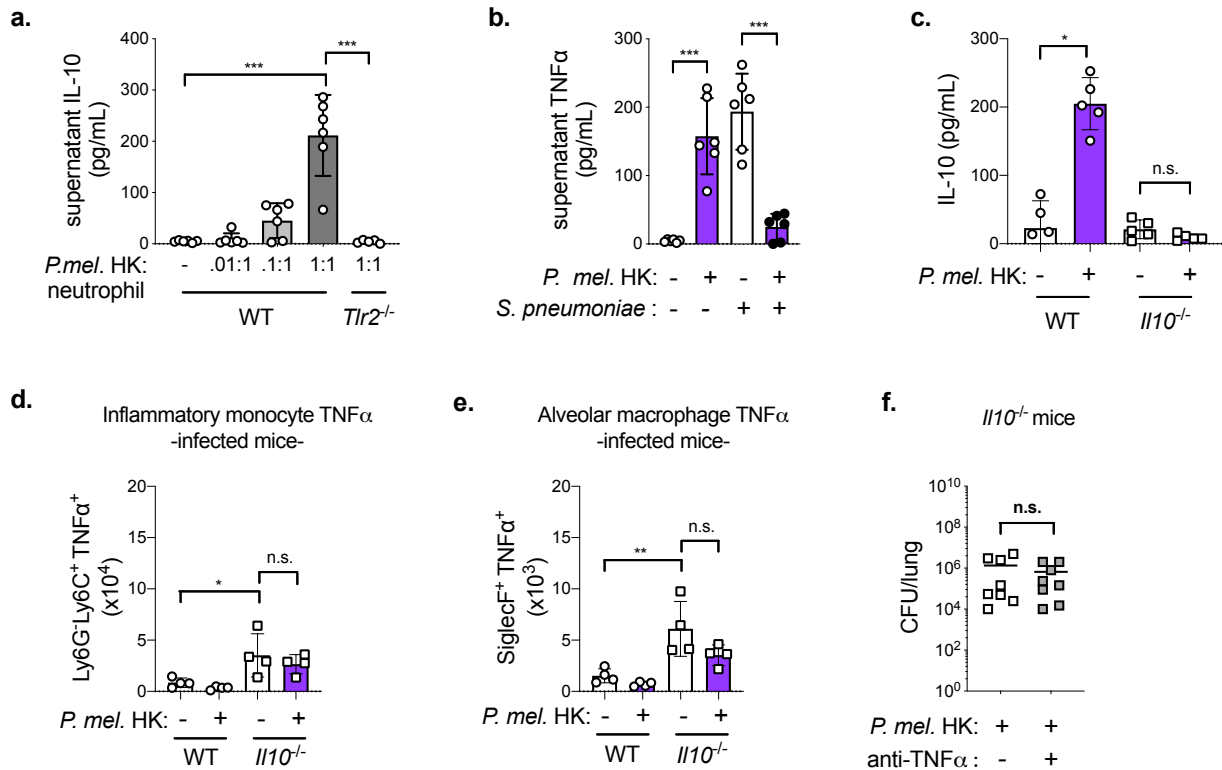


Supplementary Figure 5. The impact of TLR2 deficiency on the lung myeloid cell response to *P. melaninogenica* (*P. mel.*) with and without *S. pneumoniae* infection. **a - d** Total cell number of neutrophils (**a**), percentage and total cell number of inflammatory monocytes (**b**), percentage

and total cell number of CD11b^{hi} DCs (c), and percentage and total cell number of alveolar macrophages (AMs) (d) in WT or *Tlr2*^{-/-} mice treated with either PBS (-) or *P. mel.* strain 25845 heat-killed (HK) i.t. for 24 h (n= 5 mice/group for all except *Tlr2*^{-/-} mice treated with PBS, n= 4 mice/group). e - h Total cell number of neutrophils and neutrophil TNF α (e), total cell number of inflammatory monocytes (f), percentage and total cell number of CD11b^{hi} DCs (g), and percentage and total cell number of AMs (h) in WT or *Tlr2*^{-/-} mice treated with either PBS (-) or *P. mel.* HK i.t. prior to 24 h type 2 *S. pneumoniae* infection, 5x10⁶ CFU/mouse (n= 5 mice/group). Data are representative from one of four independent experiments, displayed as mean \pm SEM. For (a) **p=.0040 (WT), *p=.0368 (*Tlr2*^{-/-}), Kruskal-Wallis with Dunn's *post-hoc* test, (b) from left to right **p=.0059, *p=.0145, *p=.0106, *p=.0177, one-way ANOVA with Sidak's *post-hoc* test, (c) from left to right p=.7207, p=.9819, p=.7592, p=.9317, one-way ANOVA with Sidak's *post-hoc* test, (d) from left to right **p=.0010, *p=.0358, ***p<.0001, ***p<.0001, one-way ANOVA with Sidak's *post-hoc* test, (e) from left to right ***p=.0001, p=.9990, one-way ANOVA with Sidak's *post-hoc* test, *p=.0279, p>.9999, Kruskal-Wallis with Dunn's *post-hoc* test, (f) ***p=.0002 (WT), ***p<.0001 (*Tlr2*^{-/-}), one-way ANOVA with Sidak's *post-hoc* test, (g) from left to right p=.5095, *p=.0273, p=.1896, p=.5296, one-way ANOVA with Sidak's *post-hoc* test, (h) from left to right p=.2665, p=.9912, p=.5916, p=.9922, one-way ANOVA with Sidak's *post-hoc* test. Source data are provided as a Source Data file.



Supplementary Figure 6. *P. melaninogenica* (*P. mel.*) enhances serine protease-mediated killing of *S. pneumoniae* by lung neutrophils. **a** Percent of type 2 *S. pneumoniae* killed by bone marrow (BM) neutrophils from naïve mice exposed to either PBS (-) or *P. melaninogenica* (*P. mel.*) HK at a 1:1 ratio *in vitro* for 1 h or 6 h prior to incubation with *S. pneumoniae* opsonized by 3% fresh mouse serum (n= 3 independent experiments/group). **b** Serine protease activity for cathepsin G and elastase +/- protease inhibitor cocktail (Prot Inhib) detected by substrate cleavage for lung neutrophils purified from WT mice exposed to either PBS (none) or *P. mel.* heat-killed (HK) i.t. for 24 h (n= cells isolated from 6 mice/group). **c** Burden of *S. pneumoniae* detected 24 h following 1 h incubation with protease inhibitor cocktail (n= 3 independent experiments/group). Data are pooled from three independent experiments, displayed as mean \pm SEM. For (a) $p=$.9967 (1 h), $p=$.4267 (6 h), one-way ANOVA with Sidak's *post-hoc* test, (b) *** p <.0001 (Cathepsin G), *** p <.0001 (Elastase), two-tailed t test, (c) $p=$.2573, two-tailed t test. Source data are provided as a Source Data file.



Supplementary Figure 7. *P. melaninogenica* (*P. mel.*) induces neutrophil IL-10 and co-infection limits neutrophil TNF α secretion. **a** Supernatant IL-10 detected 24 h following incubation of bone marrow (BM) neutrophils purified from naïve WT or *Tlr2*^{-/-} mice with *P. mel.* strain 25845 heat-killed (HK) at the indicated ratios (n= 3 independent experiments/group). **b** Supernatant TNF α detected 24 h following incubation of BM neutrophils with either PBS (-), *P. mel.* HK, or infected with type 2 *S. pneumoniae* for 1 h (n= 3 independent experiments/group). **c - e** Serum IL-10 (**c**), total cell numbers of inflammatory monocyte TNF α (**d**), and total cell numbers of alveolar macrophage (AM) TNF α (**e**) detected by intracellular flow cytometry in WT or *Il10*^{-/-} mice exposed to PBS (-, n= 4 mice/group) or *P. mel.* HK i.t. (n= 5 mice/group) prior to 24 h *S. pneumoniae* infection, 5x10⁶ CFU/mouse. **f** Lung burdens of *S. pneumoniae* in *Il10*^{-/-} mice treated with isotype control antibody or anti-TNF α (200 μ g/mouse) i.p. together with *P. mel.* HK i.t. prior to 24 h *S. pneumoniae* infection (n= 8 mice/group). Data are pooled from three

independent experiments (**a - b**), representative from one of three independent experiments (**c - e**), or pooled from two independent experiments (**f**). Data are displayed as mean \pm SEM. For (**a**) from left to right $***p < .0001$, $***p < .0001$, one-way ANOVA with Tukey's *post-hoc* test, (**b**) $***p < .0001$, one-way ANOVA with Tukey's *post-hoc* test, (**c**) $*p = .0431$ (WT), $p = .6196$ (*Il10^{-/-}*), Kruskal-Wallis with Dunn's *post-hoc* test, (**d**) from left to right $*p = .0151$, $p = .5387$, one-way ANOVA with Sidak's *post-hoc* test, (**e**) from left to right $**p = .0018$, $p = .0600$, one-way ANOVA with Sidak's *post-hoc* test, (**f**) $p = .8581$, two-tailed Mann-Whitney U test. Source data are provided as a Source Data file.