

## Supplemental information for Roach *et al.*

Synergy between the host immune system and bacteriophage is essential  
for successful phage therapy against an acute respiratory pathogen

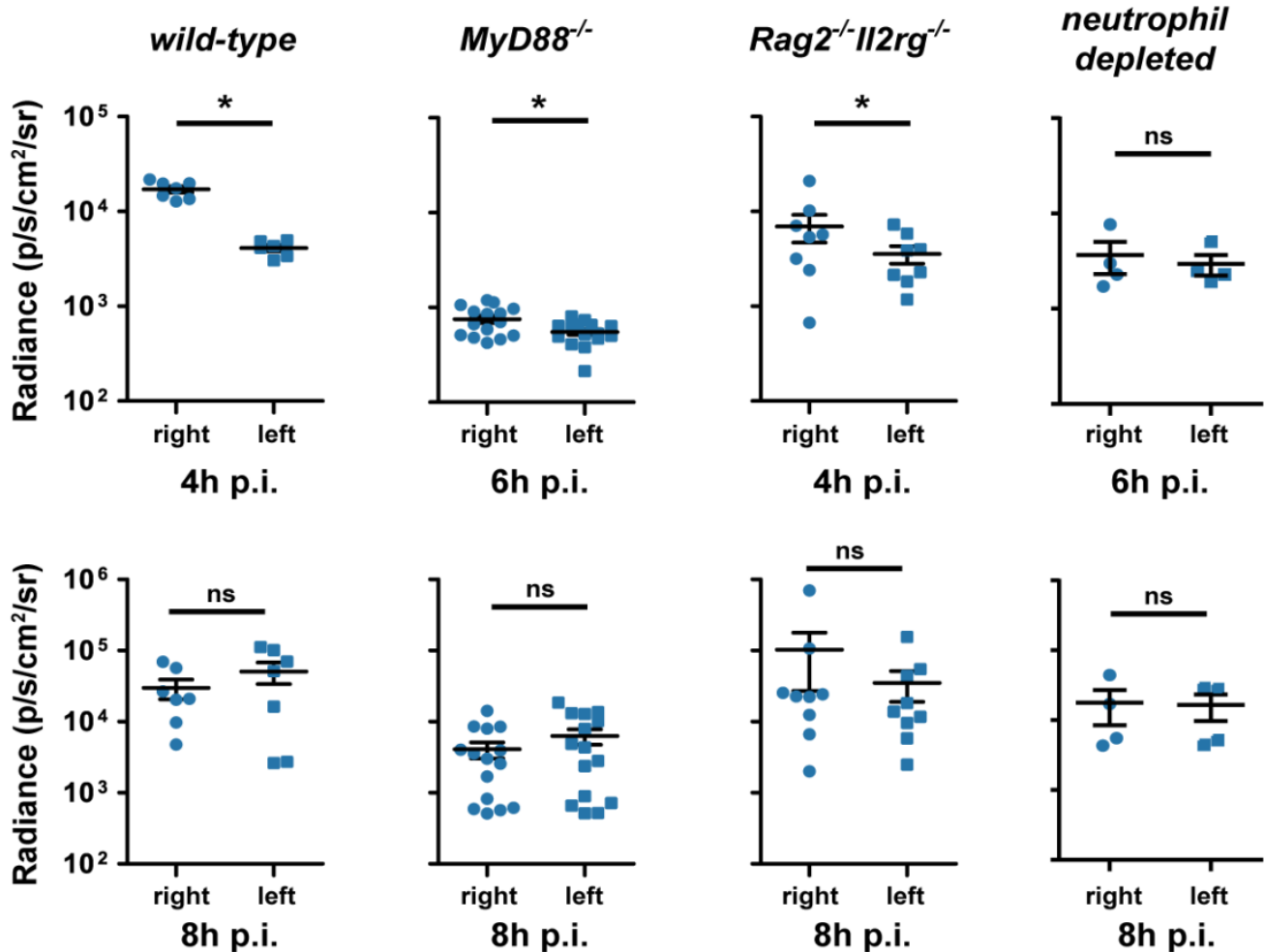
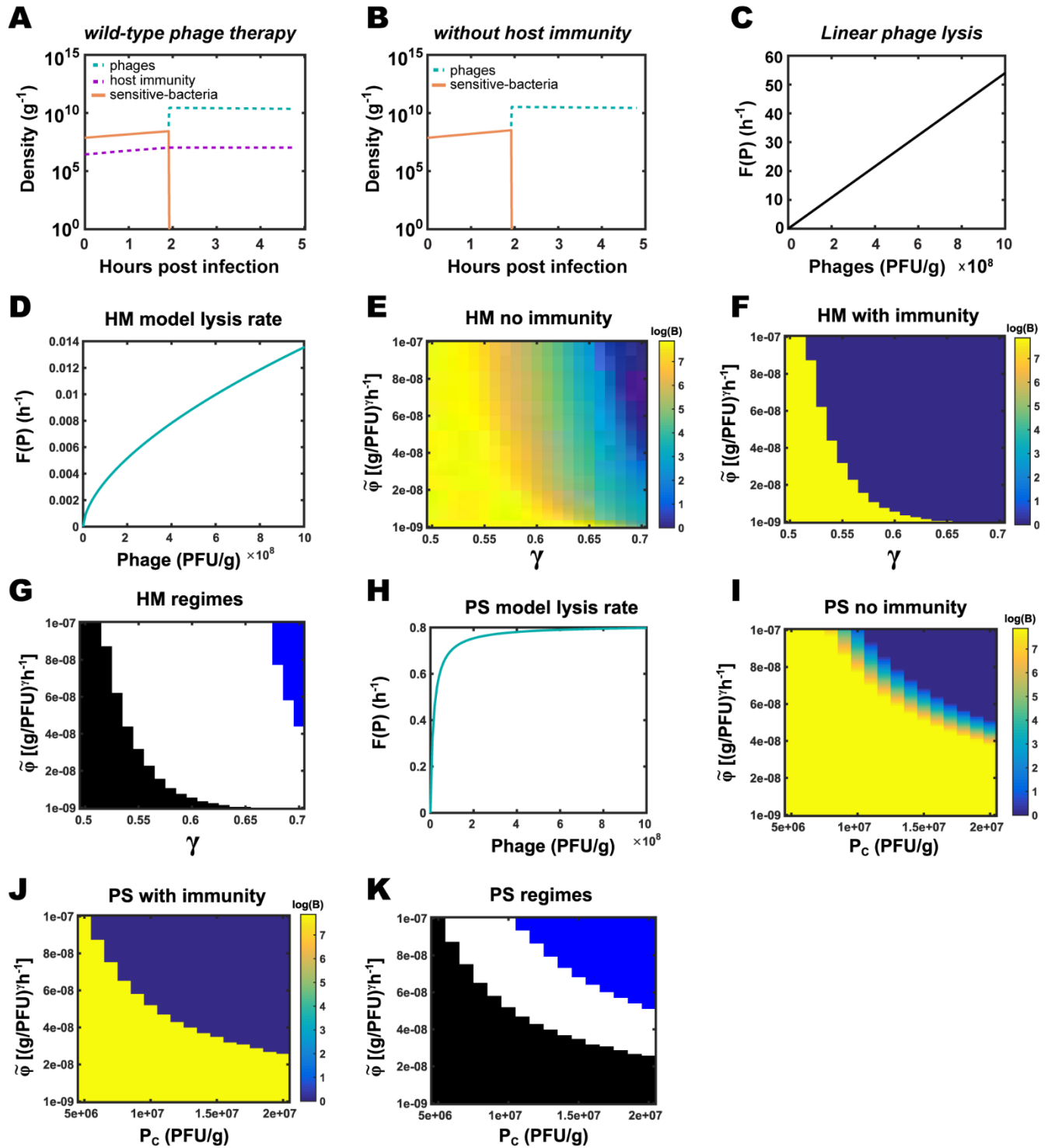


Figure S1. Colonization pattern of *Pseudomonas aeruginosa* in the lungs of different mouse strains. Related to Figures 1, 3, 4 and 5. *P. aeruginosa* strain PAKlumi luminescence post infection (p.i.) plotted as mean radiance (p/s/cm<sup>2</sup>/sr) in each lung of wild-type mice (related to Fig. 1), myeloid differentiation primary response gene 88 (*MyD88*<sup>-/-</sup>) deficient (related to Fig. 3), lymphocyte deficient (*Rag2*<sup>-/-</sup>*Il2rg*<sup>-/-</sup>) mice (related to Fig. 4), and anti-Gr1 neutrophil depleted wild-type mice (related to Fig.5). Error bars indicate SEM; \* p < 0.05; ns = no significance.

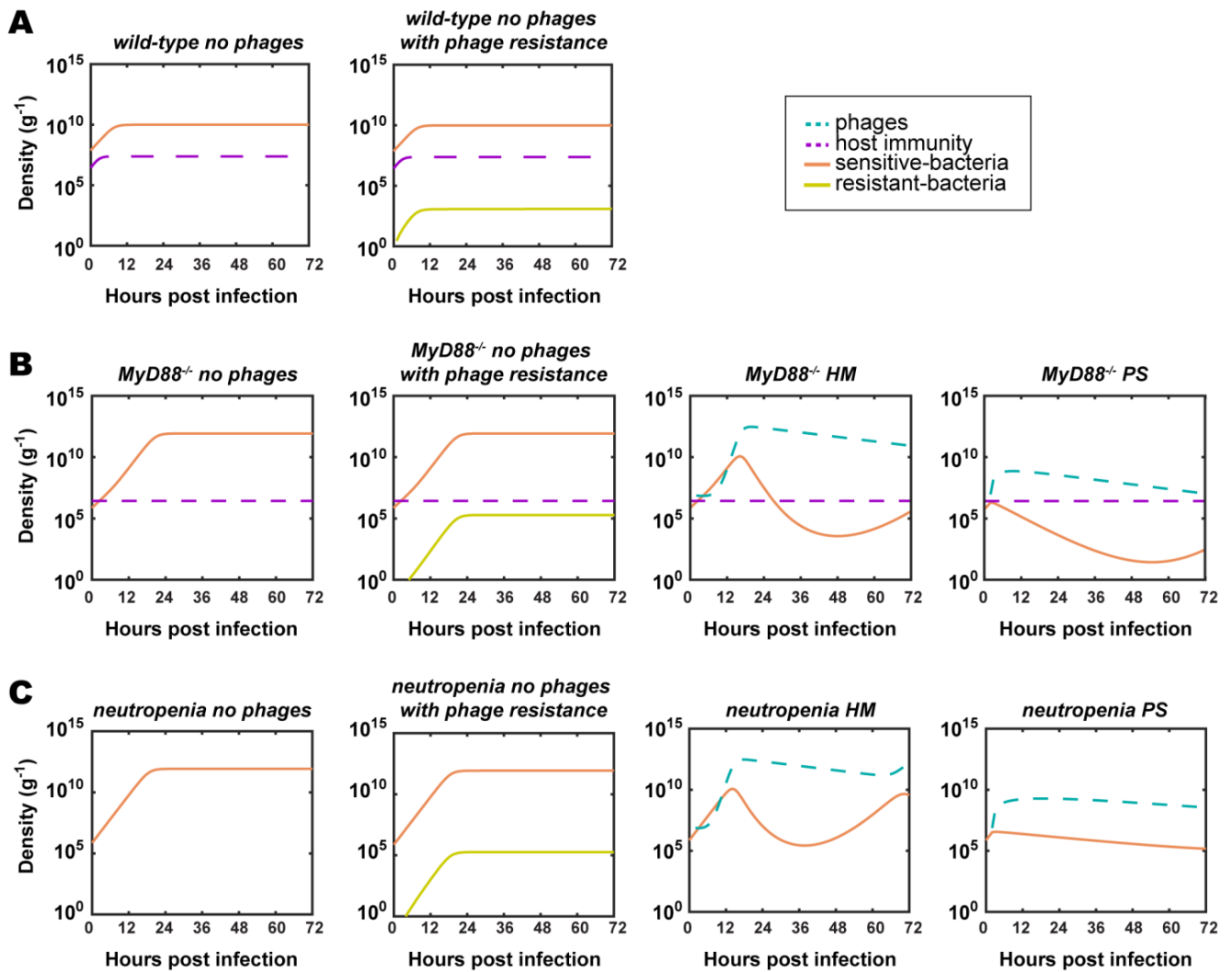


**Figure S2.** *In silico* simulations of phage and bacterial densities for the linear phage lysis rate model, and sensitivity analysis of the heterogeneous mixing (HM) and phage saturation (PS) models. Related to Figure 2. Effect of phage administration in (A) fully functioning host immunity, and (B) in the absence of host immunity from the linear phage lysis model. (C) The functional

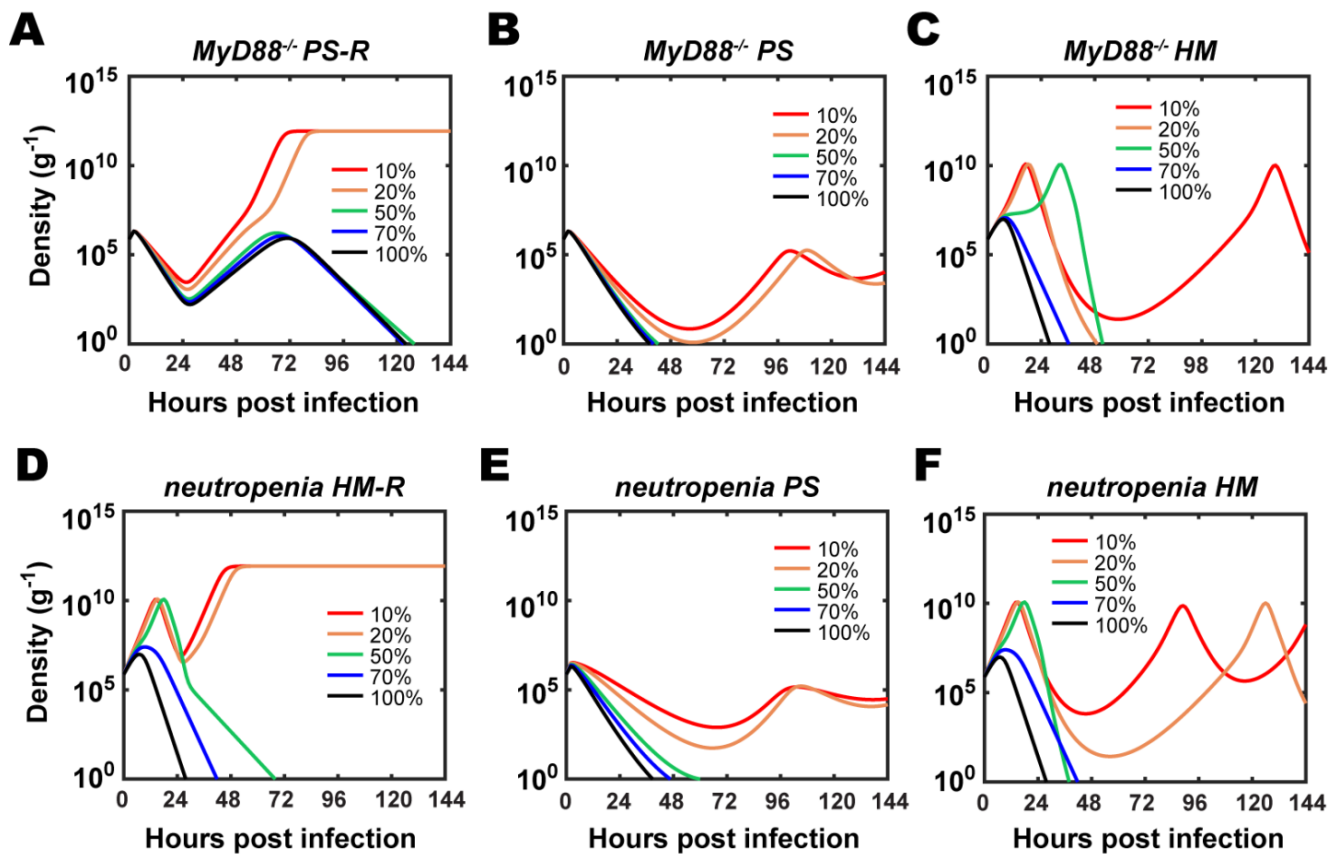
dependence of the per-capita phage lysis rate in the linear model  $F(P) = \phi P$  (see Eq. 1) on phage population density. The initial phage treatment dose and timing correspond to the experimental curative phage therapy (related to Fig. 1). Phage causes extremely rapid elimination of bacteria in this linear model, which is inconsistent with our experimental bacterial infection profile in mouse lungs (Fig. 1C).

**(D)** The power-law dependence of per-capita phage lysis rate  $F(P)$  in the *HM* model (see Eq. 4). Dependence of minimum bacterial density from the *HM* model within 72h post infection on sublinear adsorption rate  $\tilde{\phi}$  and power-law exponent  $\gamma$  in **(E)** the absence of innate immunity and **(F)** immunocompetent host. The bacterial population is assumed to be eliminated when the density drops below 1 CFU/g. **(G)** Phase diagram showing the phage sufficient (blue), phage-immune synergistic (white) and phage-immune insufficient (black) regimes. **(H-K)** Same as (D-G) but for the *PS* model.

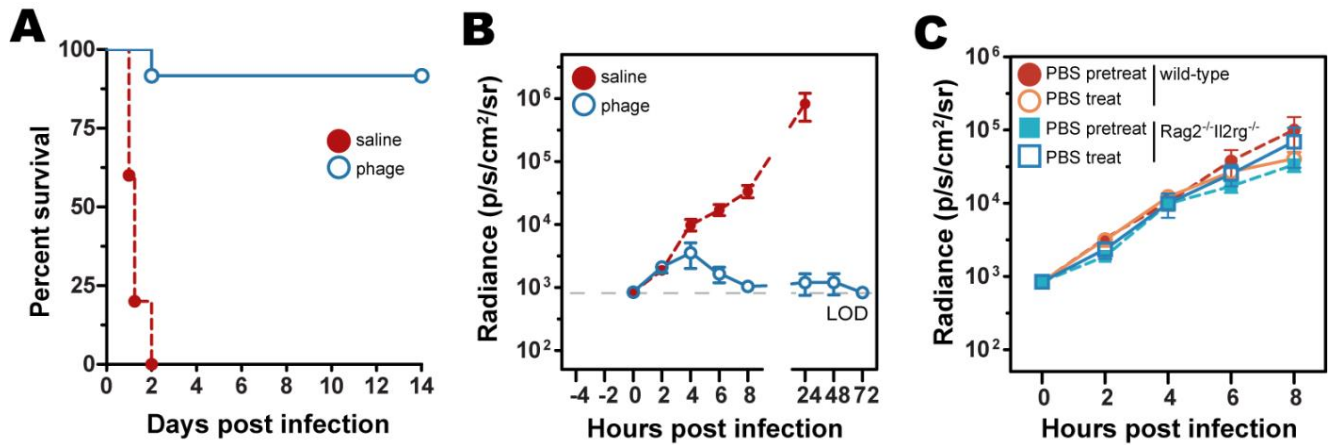
**(H)** The per-capita phage lysis rate  $F(P)$  is given by a saturating Hill function in the *PS* model (see Eq. 5). Phage-immune synergism is robust to changes in lysis rate parameters for both models. In the simulations, the carrying capacity of bacteria ( $K_C$ ) were fixed at  $8.5 \times 10^{11}$  CFU/g in both immunocompetent and immunodeficient hosts for comparative purposes. See Table S1 for *in silico* simulation parameters.



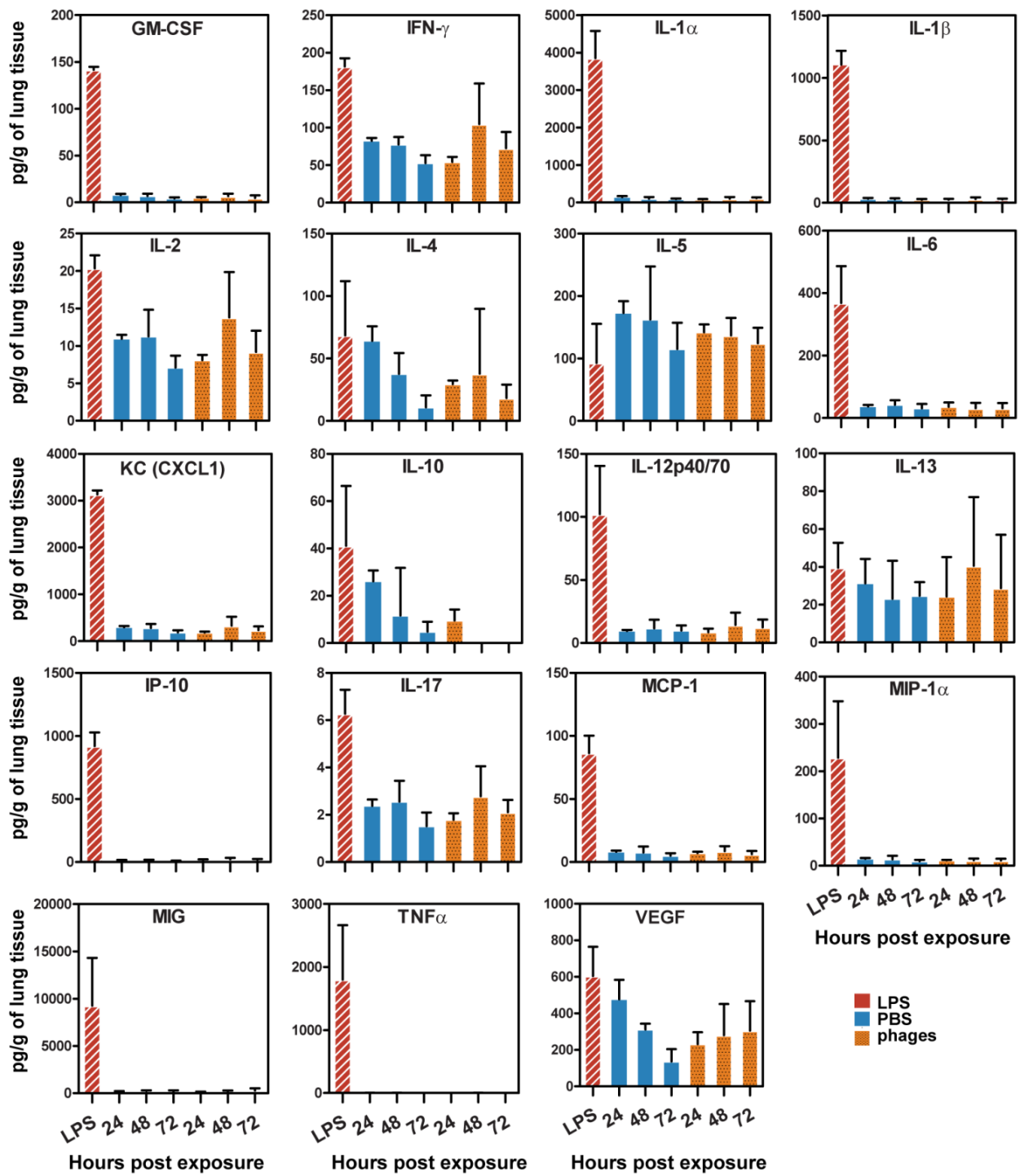
**Figure S3. Control simulations of the mathematical models. Related to Figure 1.** (A) Predicted *P. aeruginosa* population densities in the lungs of immunocompetent mice in the absence of phage therapy and without phage-resistant, or with phage-resistant bacteria emergence and outgrowth (related to Fig. 1). (B) Predicted bacterial densities in Myd88 deficient hosts without phage resistance, or with resistance. Phage therapy in Myd88 deficient hosts simulated using the heterogeneous mixing (*HM*) and the phage saturation (*PS*) models without phage resistance development (related to Fig. 3). (C) Predicted bacterial densities in neutropenic hosts without phage resistance, or with resistance. Phage therapy in neutropenic hosts simulated using the *HM* and *PS* models without phage resistance development (related to Fig. 5). See Table S1 for *in silico* simulation parameters.



**Figure S4. Simulated time series of restoration of phage therapy efficacy in hosts with partial Myd88 deficiency and reduced neutrophil activity. Related to Figure 3 and 5.** Predicted time series of total bacterial densities in hosts with different levels of innate immune activation (i.e. Myd88 deficiency) accounting for (A) the phage saturation with phage resistance (*PS-R*), (B) phage saturation (*PS*) without phage resistance, and (C) heterogeneous mixing (*HM*) without phage resistance. (D-F) Time series of total bacterial densities accounting for different lung neutrophil levels to simulate mild to severe neutropenia in mice (related to Fig.3). (D), (E) and (F) correspond to simulations of *HM-R*, *PS* and *HM* models, respectively (related to Fig.5). See Table S1 for *in silico* simulation parameters for immunodeficient hosts.



**Figure S5. Phage prophylaxis in the innate and adaptive lymphocyte deficient host. Related to Figure 6.** (A) *Rag2<sup>-/-</sup>Il2rg<sup>-/-</sup>* mice void of all innate lymphoid cells, B cells and T cells received a single inhaled monophage dose ( $10^9$  PFU) which gave prophylaxis for 4d against *P. aeruginosa* pneumonia ( $10^7$  CFU; n=12 per group). (B) Bacterial luminescence plotted as mean radiance (p/s<sup>2</sup>/cm<sup>2</sup>/sr) in the lungs of animals over time demonstrates phages maintain antibacterial activity after residing in the mouse airways for 4d. (C) Comparison between PBS mock-prophylactic and mock-curative treatment indicates no change in bacterial colonization pattern due to treatment intranasal instillation occurring before intranasal bacterial challenge (related to Fig. 4). Arrow indicates treatment point; *in vivo* radiance limit of detection (LOD); error bars indicate SEM.



**Figure S6. Cytokine, chemokine and growth factor levels in immunocompetent mouse lungs after exposure to LPS, saline or phages. Related to Figure 6.** Differential expression of analytes was quantified in lung tissue homogenate using a Luminex multi-analyte magnetic microspheres immunoassay after mice were intranasal instilled with 10  $\mu$ g LPS (hatched bars), phosphate buffered saline (solid bars), or pseudomonas phage PAK\_P1 ( $10^9$  PFU) (dotted bars). Related to Fig. 6E. (n=3 per time point); error bars indicate SEM.

**Table S1. Estimated values of parameters for *in silico* models, Related to STAR methods.**  
Concentrations are calculated based on mice lung mass of 0.135 g.

Parameters	Value	Estimated from
<b>Common between <i>HM</i> and <i>PS</i> models</b>		
$r$ , maximum growth rate of bacteria	0.75 h <sup>-1</sup>	<i>P. aeruginosa</i> murine pneumonia model (Drusano et al., 2011)
$K_C$ , carrying capacity of bacteria (wild-type)	1.0 x 10 <sup>10</sup> CFU/g	Assuming ~4 times above the typical bacterial density (2.4x10 <sup>9</sup> CFU/g) in wild-type mice 24h post infection
$K_C$ , carrying capacity of bacteria ( <i>Myd88</i> <sup>-/-</sup> and neutropenia)	8.5 x 10 <sup>11</sup> CFU/g	<i>P. aeruginosa</i> pneumonia model (Drusano et al., 2011)
$\beta$ , burst size of phage	100	this study
$\omega$ , decay rate of phage	0.07 h <sup>-1</sup>	this study, see Table S2
$\varepsilon$ , killing rate parameter of immune response	8.2 x 10 <sup>-8</sup> g/(h cell)	Set such that $\varepsilon K_I$ gives maximum granulocyte killing rate (Drusano et al., 2011)
$\alpha$ , maximum growth rate of immune response	0.97 h <sup>-1</sup>	Fitting of neutrophil recruitment data (Reutershan et al., 2005)
$K_I$ , maximum capacity of immune response	2.4 x 10 <sup>7</sup> cell/g	Fitting of neutrophil recruitment data (Reutershan et al., 2005)
$K_I$ , maximum capacity of immune response ( <i>Myd88</i> <sup>-/-</sup> )	Same as $I_0$	No innate immune activation
$K_D$ , bacterial concentration at which immune response is half as effective	4.1 x 10 <sup>7</sup> CFU/g	Corresponds to lethal dose of about 5.5 x 10 <sup>6</sup> CFU/lungs
$K_N$ , bacterial concentration when immune response growth rate is half its maximum	1.0 x 10 <sup>7</sup> CFU/g	<i>In vitro</i> data of TLR5 response to PAK strain (Zhang et al., 2005)
$B_0$ , initial bacterial density (curative and prophylaxis)	7.4 x 10 <sup>7</sup> CFU/g	Total inoculum of 10 <sup>7</sup> CFU
$B_0$ , initial bacterial density ( <i>Myd88</i> <sup>-/-</sup> and neutropenia)	7.4 x 10 <sup>5</sup> CFU/g	Total inoculum of 10 <sup>5</sup> CFU
$P_0$ , initial phage dose (curative)	7.4 x 10 <sup>8</sup> PFU/g	Total phage dose of 10 <sup>8</sup> PFU



$P_0$ , initial phage dose (prophylaxis)	$7.4 \times 10^9$ PFU/g	Total phage dose of $10^9$ PFU
$P_0$ , initial phage dose ( <i>Myd88</i> <sup>-/-</sup> and neutropenia)	$7.4 \times 10^6$ PFU/g	Total phage dose of $10^6$ PFU
$I_0$ , initial immune response	$2.7 \times 10^6$ cell/g	Fitting of neutrophil recruitment data (Reutershan et al., 2005)
$I_0$ , initial immune response (neutropenia)	0 cell/g	Assuming no primary innate immunity
<b>HM model parameters</b>		
$\tilde{\phi}$ , nonlinear phage adsorption rate	$5.4 \times 10^{-8}$ (g/PFU) <sup><math>\gamma</math></sup> h <sup>-1</sup>	this study, see Methods
$\gamma$ , power law exponent in phage infection rate	0.6	this study, see Methods
<b>PS model parameters</b>		
$\phi$ , adsorption rate of phage	$5.4 \times 10^{-8}$ g/(h PFU)	this study, see Methods
$P_C$ , phage density at half saturation	$1.5 \times 10^7$ PFU/g	this study, see Methods
<b>Phage resistance model parameters</b>		
$\mu$ , probability of emergence of phage-resistant mutant per cellular division	$2.85 \times 10^{-8}$	Estimated from experimental measurements (Luria and Delbruck, 1943)
$r'$ , maximum growth rate of phage-resistant bacteria	0.675 h <sup>-1</sup>	10% trade-off between phage resistance and growth rate (Bohannon et al., 2002)

**Table S2. Pseudomonas phage PAK\_P1 one phase decay and calculated half-life ( $t_{1/2}$ ) in mouse lungs, Related to Figure 6.** Phage titers were determined from lung homogenates of wild-type, lymphocyte deficient ( $Rag2^{-/-}Il2rg^{-/-}$ ), and innate immune signaling deficient ( $Myd88^{-/-}$ ) mice at indicated time points after intranasal instillation of only phages ( $10^9$  PFU).

Mouse strain	Lungs $t_{1/2}$ (h)	PFU/g lung tissues <sup>a</sup>		
		1 d	4 d	5 d
Wild-type <sup>b</sup>	10.4	$2.5 \times 10^7 \pm 4.4 \times 10^6$	$6.4 \times 10^4 \pm 9.9 \times 10^3$	$1.1 \times 10^4 \pm 2.6 \times 10^3$
$Rag2^{-/-}Il2rg^{-/-}$	10.4	$2.0 \times 10^7 \pm 1.5 \times 10^6$	$3.0 \times 10^5 \pm 3.5 \times 10^4$	$3.5 \times 10^4 \pm 9.7 \times 10^3$
		ns	$p < 0.001$	$p < 0.001$
Wild-type <sup>c</sup>	7.7	$1.6 \times 10^8 \pm 6.1 \times 10^6$	$1.1 \times 10^6 \pm 4.5 \times 10^6$	$2.6 \times 10^5 \pm 8.6 \times 10^4$
$MyD88^{-/-}$	11.1	$3.1 \times 10^8 \pm 6.6 \times 10^6$	$3.3 \times 10^6 \pm 1.1 \times 10^6$	$1.2 \times 10^6 \pm 1.7 \times 10^5$
		$p < 0.001$	$p < 0.001$	$p < 0.001$

<sup>a</sup> mean  $\pm$  SEM (n=3); determined by spot titre method

<sup>b</sup> Balb/c background of  $Rag2^{-/-}Il2rg^{-/-}$  mice

<sup>c</sup> C57Bl6 background of  $MyD88^{-/-}$  mice

Differences were calculated by Mann Whitney; ns = not significant