

**Supplemental information**

**Filamentous bacteriophage delays  
healing of Pseudomonas-infected wounds**

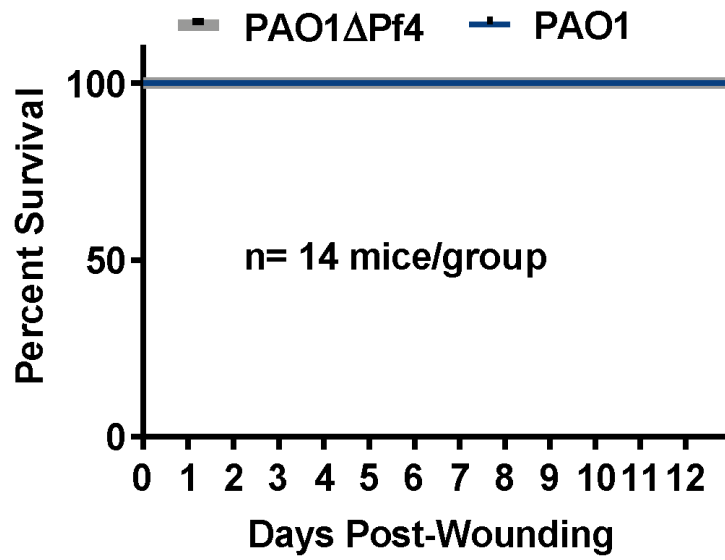
**Michelle S. Bach, Christiaan R. de Vries, Arya Khosravi, Johanna M. Sweere, Medeea C. Popescu, Qingquan Chen, Sally Demirdjian, Aviv Hargil, Jonas D. Van Belleghem, Gernot Kaber, Maryam Hajfathalian, Elizabeth B. Burgener, Dan Liu, Quynh-Lam Tran, Tejas Dharmaraj, Maria Birukova, Vivekananda Sunkari, Swathi Balaji, Nandini Ghosh, Shomita S. Mathew-Steiner, Mohamed S. El Masry, Sundeep G. Keswani, Niaz Banaei, Laurence Nedelec, Chandan K. Sen, Venita Chandra, Patrick R. Secor, Gina A. Suh, and Paul L. Bollyky**

	Pa(+)Pf(+)	Pa(+)Pf(-)	p-value
Samples (n, %)	25, 69%	11, 31%	-
<b>Age of Patients (years)</b> <b>Mean (SD)</b>	61.6 (14.7)	76.6 (14.5)	<u>0.008</u>
Gender (n)			
Male	9 (36%)	6 (55%)	0.465
Female	16 (64%)	6 (45%)	
Race/Ethnicity			
Caucasian	17 (68%)	5 (45%)	0.144
Asian	3 (12%)	1 (9%)	
Hispanic	3 (12%)	5 (45%)	
African-American	2 (8%)	0 (0%)	
Body Mass Index (kg/m <sup>2</sup> ) Mean (SD)	27.95 (11.9)	30.28 (6.9)	0.551
<b>Age of Wound (years)</b> <b>Median</b>	2.1	0.5	<u>0.046</u>
Recurrence of Infection	9 (43%)	3 (43%)	0.756
Co-Morbidities			
Diabetes Mellitus	12 (48%)	3 (27%)	0.295
Renal Disease	8 (32%)	1 (9%)	0.223

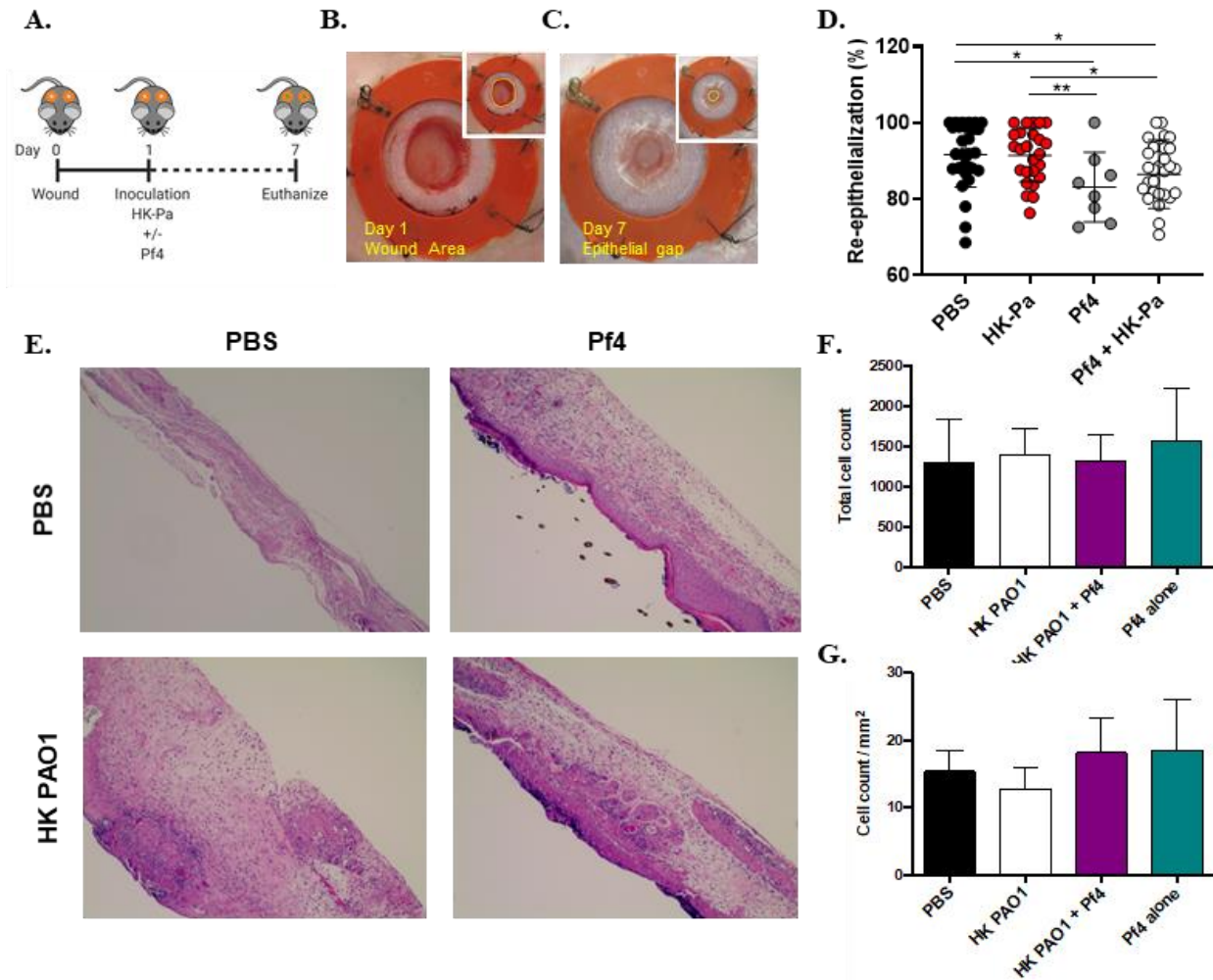
**Table S1. Patient Demographic Data.** Related to Figure 4. Clinical information on patients from the AWCC with culture-positive and qPCR-positive *Pa*-infected non-healing wounds who participated in the wound swab study. Renal Disease was defined as patients with Chronic Kidney Disease (CKD) or End Stage Renal Disease (ESRD). Statistical significance was measured using Fisher's Exact Test for the following parameters: Gender, Infection Recurrence, and Co-Morbidities. Statistical significance was measured using an Unpaired T-Test for Age and BMI, Chi-Square Test for Race/Ethnicity, and Unpaired Two-Tailed Mann-Whitney Test for Age of Wound because the data was nonparametric. 9 patients were excluded from the Recurrence of Infection analysis because they did not provide an answer on the patient Intake form provided by the AWCC.

	Pa(+)Pf(+)	Pa(+)Pf(-)	p-value
Pf Phage, copies/swab, mean (range)	2.16x10 <sup>7</sup> (3.55x10 <sup>3</sup> - 2.69x10 <sup>8</sup> )	0	-
<i>P. aeruginosa</i> , copies/swab, mean (range)	7.55x10 <sup>7</sup> (6.82x10 <sup>2</sup> – 9.2x10 <sup>8</sup> )	2.74x10 <sup>7</sup> (5.56x10 <sup>3</sup> – 1.79x10 <sup>8</sup> )	0.395
<b>Antimicrobials at Time of Swab</b>			
<b>Antimicrobials</b>	14 (56%)	2 (18%)	0.067
Anti-Pseudomonals	6 (24%)	2 (18%)	>0.999
<b>Antibiotic Resistance</b>			
Levofloxacin	8 (32%)	1 (9%)	0.223
Ciprofloxacin	5 (20%)	1 (9%)	0.643
Imipenem	4 (16%)	4 (36%)	0.214
Meropenem	4 (16%)	3 (27%)	0.650
Gentamicin	2 (8%)	1 (9%)	>0.999
Piperacillin	1 (4%)	1 (9%)	0.524
<b>Co-Infection</b>			
Presence of Co-Infection	19 (76%)	7 (64%)	0.454
<i>Staphylococcus aureus</i>	10 (40%)	4 (36%)	>0.999
Gram-Positive	8 (32%)	2 (18%)	0.690
Other Gram-Negative	11 (44%)	5 (45%)	>0.999
<i>Candida</i> Species	0 (0%)	1 (9%)	0.306

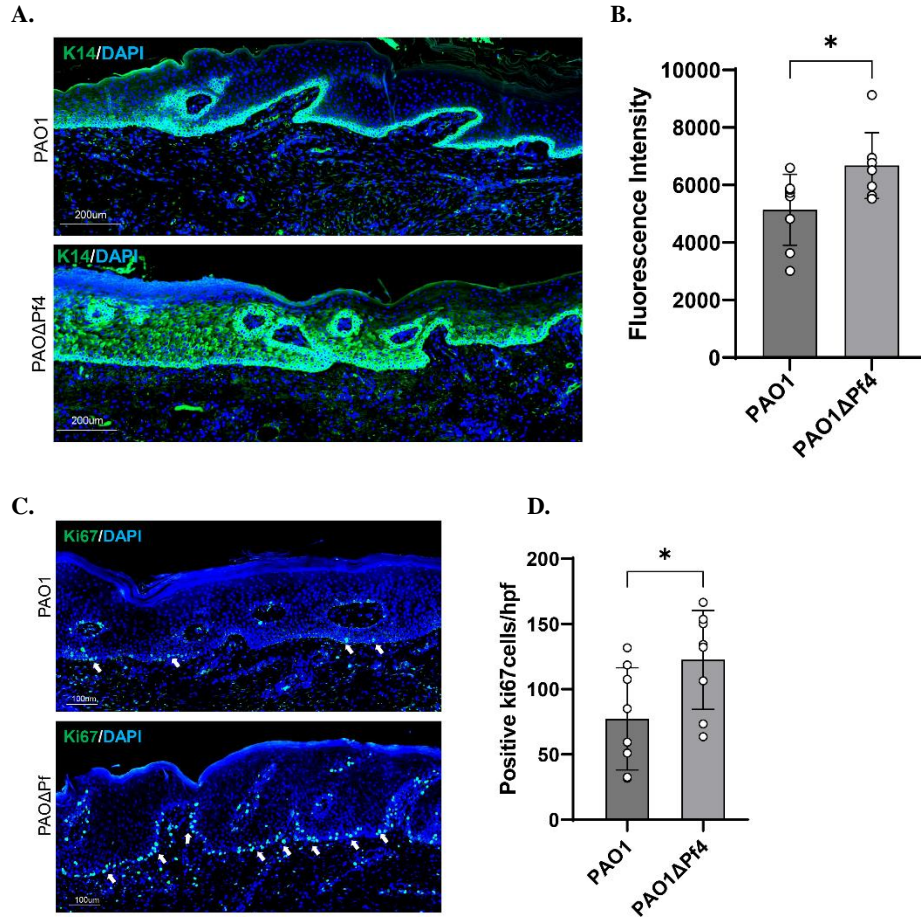
**Table S2. Human Wound Microbiology Data.** Related to Figure 4. Comparison of antibiotic use and co-infection among wounds infected with Pf(+) or Pf(-) strains of *Pa*. Statistical significance for *Pa* copies/swab was analyzed using an Unpaired T-Test. Statistical significance for Antimicrobials at Time of Swab, Antibiotic Resistance, and Co-Infection was analyzed using Fisher’s Exact Test. Anti-Pseudomonal antibiotics included in the analysis were ciprofloxacin and levofloxacin. 0 patients were resistant to Tobramycin. None of the Co-Infection parameters were significant, which strengthens the correlation between Pf phage and clinical outcomes.



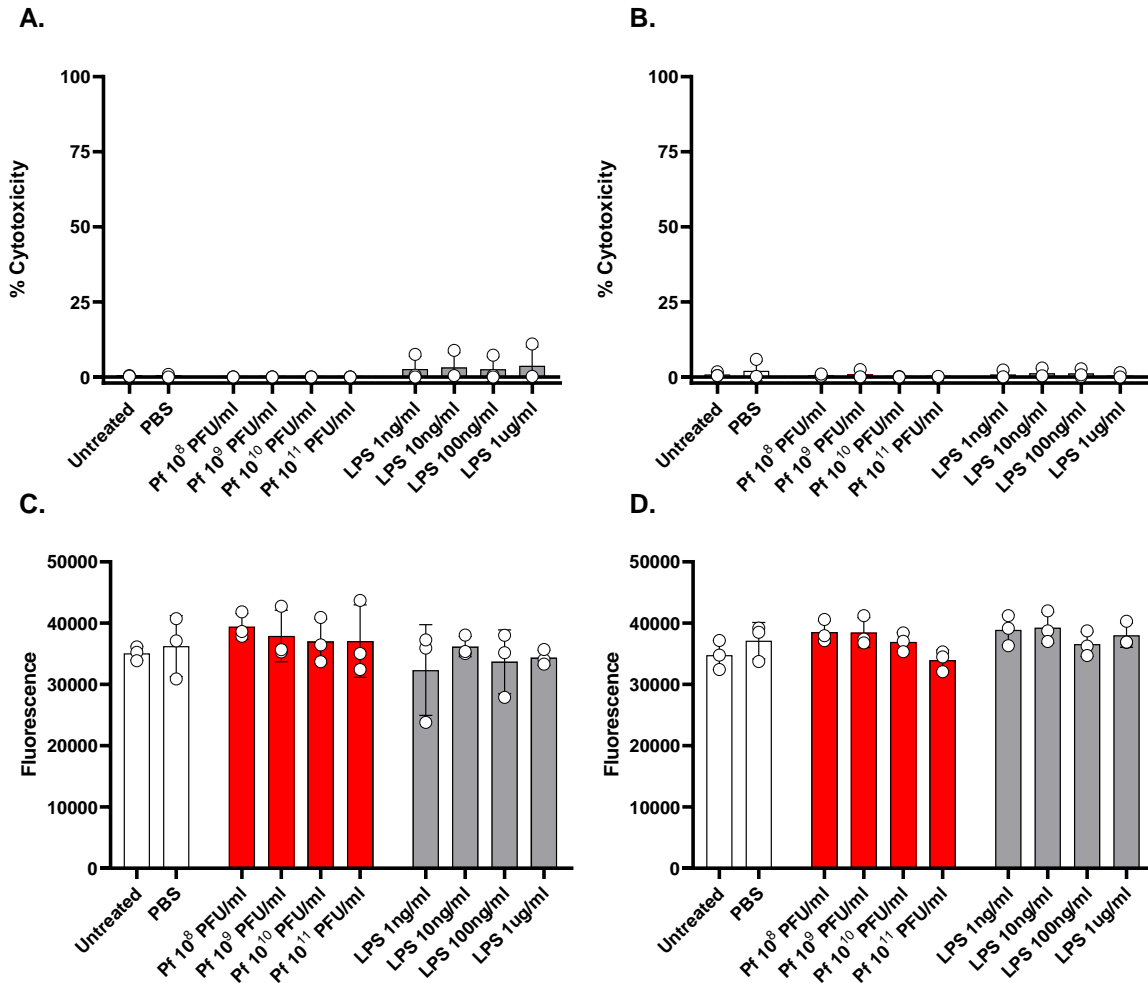
**Supplemental Figure S1. Percent Survival of Mice.** Related to Figure 1. Kaplan-Meier plot of survival of mice infected with either PAO1 or PAO1ΔPf4 using the chronic *Pa* wound infection model. All mice survived until Day 13 of the experiment, when they were euthanized.



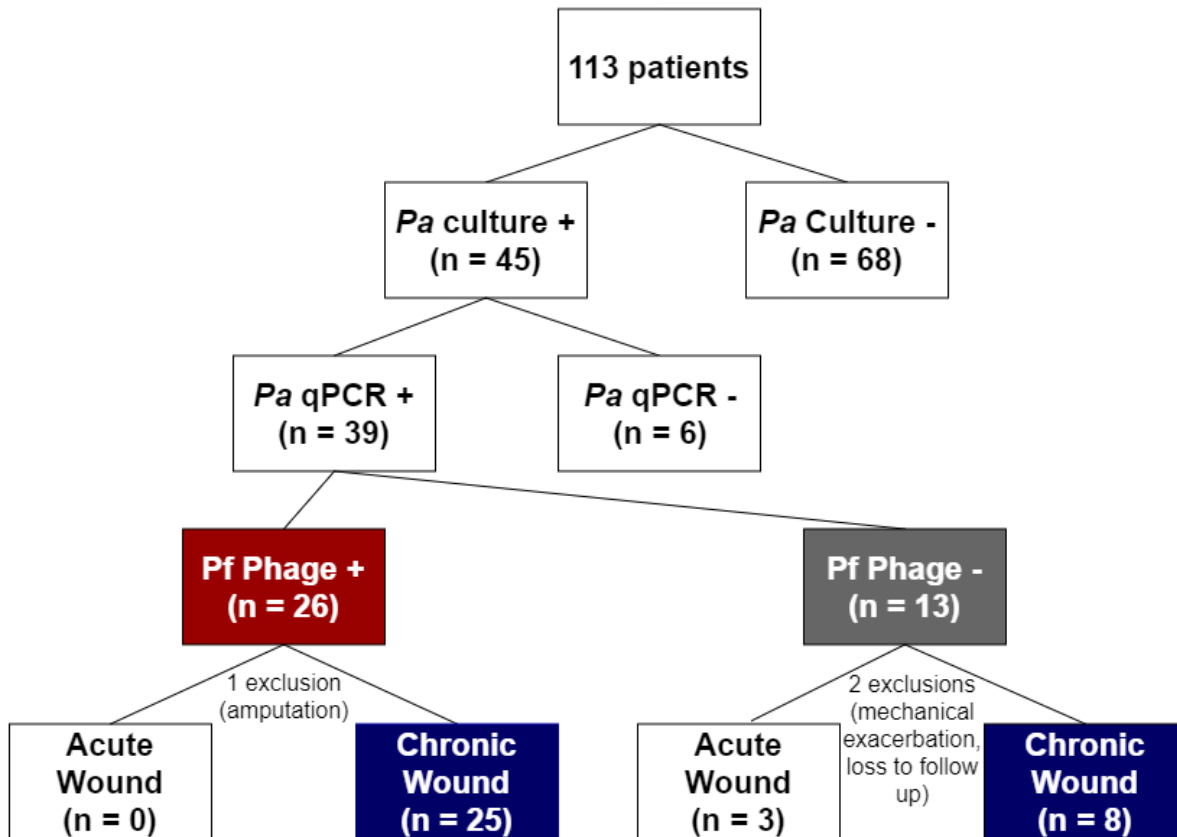
**Supplemental Figure S2. Pf phage delay re-epithelialization in the absence of bacterial infection in murine models.** Related to Figure 1. A) Schematic of full-thickness chronic *Pa* wound infection murine model using ring system. B) Image of a representative wound area inoculated with heat-killed *Pa* on Day 1. C) Image of the same wound area inoculated with heat-killed *Pa* on Day 7. D) Epithelial gap on Day 7 normalized to Day 1 wound area. Combined results from multiple experiments with 8-30 wounds in total per group. Statistics by unpaired Student's T-test. \* p<0.05, \*\*p<0.01. E) Representative H&E stains of formalin-fixed, paraffin-embedded samples from mouse wounds inoculated with heat-killed *Pa* (HK-PAO1) and/or Pf4. F) Total cell count based on H&E staining. G) Cell count/mm<sup>2</sup> based on H&E staining.



**Supplemental Figure S3. Pf4-infected wounds demonstrate decreased keratin 14 expression and cellular proliferation.** Related to Figure 2-3. A) Representative images of burn wounds on day 56 following infection with PAO1 or PAO1ΔPf4. Sections are stained with DAPI and K14. Scale bar = 200μm. B) Quantification of K14 staining intensity. C) Representative images of pig burn wounds on day 56 following infection with PAO1 or PAO1ΔPf4. Sections are stained with DAPI and Ki67. Arrows indicate Ki67 positive cells. Scale bar = 100μm. D) Quantification of Ki67 per high powered field. n = 8 per group, \*p<0.05 by unpaired Student's T test.



**Supplemental Figure S4. Pf phage treatment is not associated with cellular cytotoxicity.** Related to Figure 3. HaCaT human keratinocytes (A) and hTERT human lung fibroblasts (B) were treated for 24 hours with Pf or LPS, at indicated concentrations, and cellular cytotoxicity was measured by lactate dehydrogenase (LDH) release. Cell viability as determine by resorufin fluorescence from HaCaT (C) and hTERT cells (D) following treatment with Pf or LPS at indicated concentrations. Resazurin was added to the cells 24 hours after Pf or LPS treatment and incubated for 1 hour before fluorescence measurement. Results are derived from 3 independent experiments, with 3 technical replicates each. Statistics by one-way ANOVA with Dunnett's multiple comparison test (all non-significant).



**Supplemental Figure S5. Epidemiological flow chart.** Related to Figure 4. A total of 113 patients from the Stanford Advanced Wound Care Center (AWCC) were enrolled in a prospective cohort study. 39 patients were classified as Pa-positive based on culture and qPCR results. 26 patients were classified as Pf phage-positive based on qPCR results. 1 Pf phage-positive patient was excluded from the study due to an amputation (see Figure S5A). 13 patients were classified as Pf phage-negative based on qPCR results. 1 Pf phage-negative patient was excluded from the study due to a mechanical exacerbation (see Figure S5B). 1 Pf phage-negative patient was excluded from the study due to loss to follow up. Acute wounds are defined as equal to or younger than 3 months of age. Chronic wounds are defined as older than 3 months of age.