Extended Data

Targeting MDR *Pseudomonas aeruginosa* biofilm with an evolutionary trained bacteriophage cocktail exploiting phage resistance trade-offs

Figures

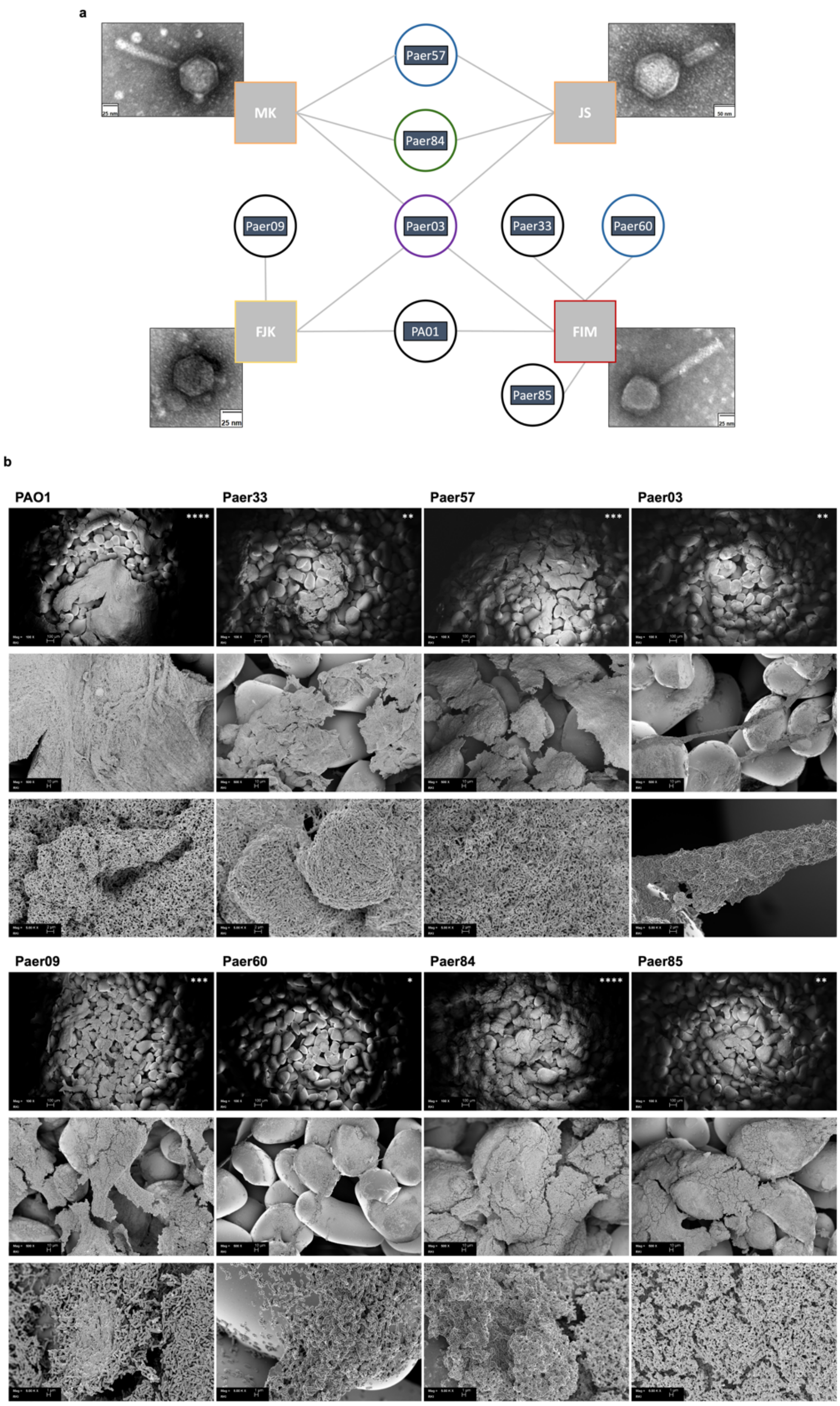

Fig. E1: Overview of the bacteriophages and bacterial strains included in the evolution assay.

a, Transmission electron microscopy images of the four ancestral bacteriophages (*Pakpunavirus*: MK/JS, orange square; *Bruynoghevirus*: FJK, yellow square and *Pbunavirus*: FIM, red square) and their host range (connecting lines) among the eight *P. aeruginosa* strains included in the *in-vitro* evolution and belonging to four genomic clusters (Cluster 1: PAO1/Paer09/Paer33/Paer85, black circle; Cluster 2: Paer57/Paer60, blue circle; Cluster 3: Paer03, violet circle; Cluster 4: Paer84, green circle). **b**, Scanning electron microscopy images of the pre-stablished 24-h-biofilm of each *P. aeruginosa* strain included in the evolution assay displayed at three different magnifications (top row – Mag 100 X; middle row – Mag 500 X; bottom row – Mag 5000 X). The number of stars in the top row indicate the biofilm density (more stars corresponding to higher biofilm density) for each strain in comparison to one another.

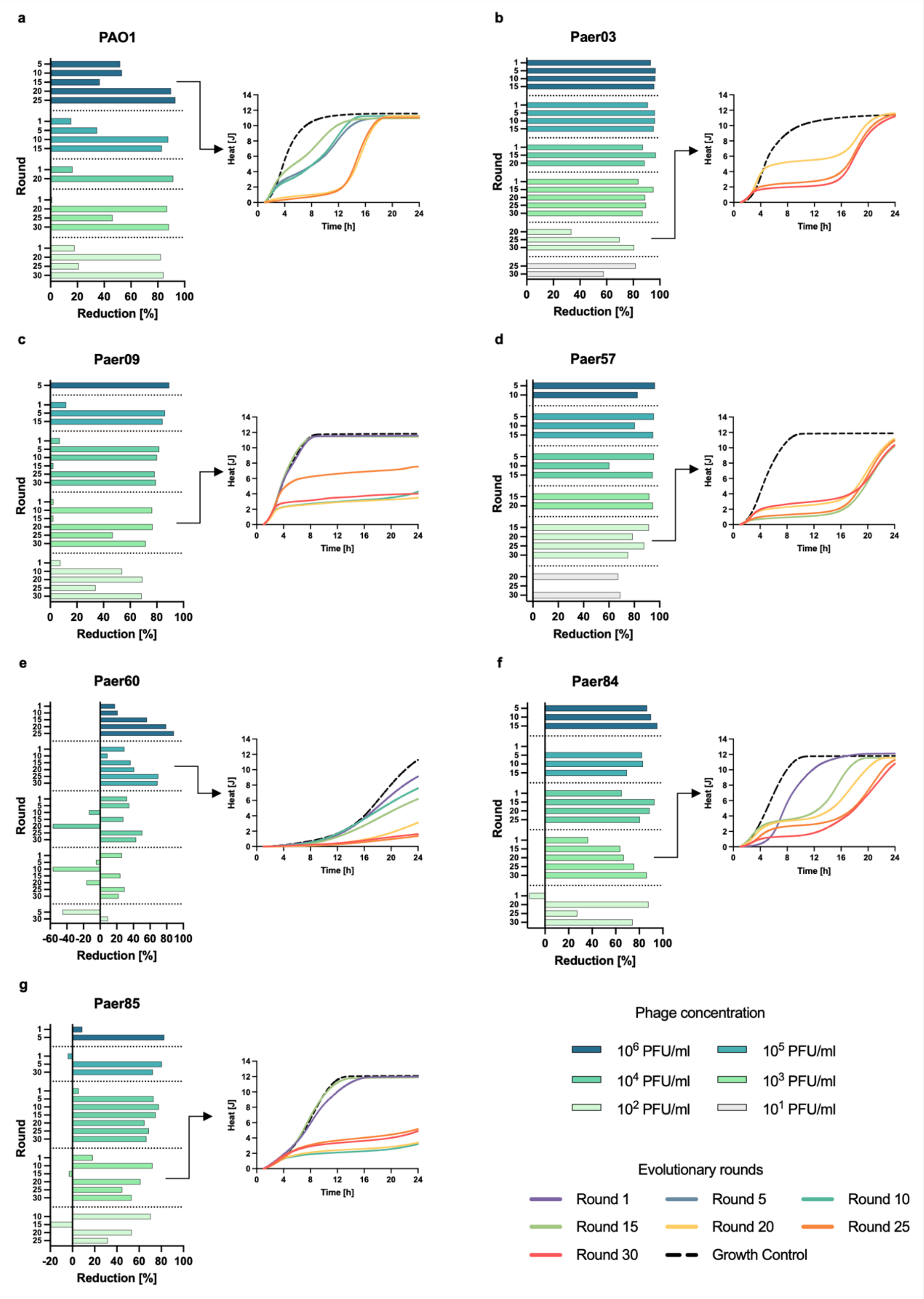

Fig. E2: Bacteriophage activity analysis during the evolution assay.

a, Heat reduction (%) plot of PAO1 exposed to the phage mixture at different concentrations (PFU/ml) compared to the growth control sample in different rounds of the evolution after 8 h of monitoring. Calorimetric heat (J) curves measured during a 24-h exposure of PAO1 to phages at the same concentration within different evolutionary rounds. **b**, Identical setup for the tested strain Paer03. **c**, Identical setup for the tested strain Paer09. **d**, Identical setup for the tested strain Paer57. No phage plaques could be identified when spotting the initial phage solution (round 0) on Paer57. **e**, Identical setup for the tested strain Paer60. **f**, Identical setup for the tested strain Paer84. **g**, Identical setup for the tested strain Paer85.