

Supplementary information for:

The social amoeba *Dictyostelium discoideum* shows reduced fitness on unpalatable bacteria but does not suffer social costs in chimeras

Additional file 1: Supplementary Figures S1-S4

Supplementary Figure Legends

Supplementary Figure 1. When starting with 2×10^7 cells, *D. discoideum* cells have a greater cell proliferation on *K. pneumoniae* and on palatable *P. vindicans* than that on unpalatable *P. vindicans*. A. The cell numbers per each 10 mm diameter agar piece suspended in 600 μ L KK2. B. The example proliferation slopes of $\log_{10}$ transformed cell numbers during log growth. The slopes are steeper for cells growing on *K. pneumoniae* and on palatable *P. vindicans* than the slope for cells growing on unpalatable *P. vindicans*.

Supplementary Figure 2. Growth comparison of *Klebsiella pneumoniae*, palatable *P. vindicans*, and unpalatable *P. vindicans* on SM/5 plates over a 120 h period. At each time point, a 10-mm-diameter agar plug was excised from a plate with bacterial growth and resuspended in buffer. The suspension was serially diluted (10-fold) 11 times, and 5 μ L of each dilution was spotted onto a fresh plate. After 12-24 h incubation at 30 °C, colony-forming units (CFU) were counted and used to estimate CFU per plate (scaled to the full plate area).

Supplementary Figure 3. 2×10^5 spores produced from *D. discoideum* previously fed on *K. pneumoniae* (blue), palatable *P. vindicans* (purple), and unpalatable *P. vindicans* (orange) were plated with *K. pneumoniae* (circle) or palatable *P. vindicans* (triangle). The percentage of hatched amoebae among all cell forms were counted under microscope at hours 5.5, 12, 17.5, and 15.5. The percentage of amoebae among total cells are shown on the y axis. Statistical analysis: To test whether bacterial feeding history and lawn type affected amoeba recovery, we performed a nonparametric aligned rank transform (ART) ANOVA using the ARTTool package in R (Wobbrock et al., 2011). The model included feeding history (*K. pneumoniae*, palatable *P. vindicans*, unpalatable *P. vindicans*) and lawn type (*K. pneumoniae*, palatable *P. vindicans*) as fixed factors, and the aligned-rank-transformed amoebae percentage as the response variable. Wald F-tests with Kenward-Roger degrees of freedom were used to assess significance. Pairwise comparisons were performed using estimated marginal means with Holm correction for multiple testing. ART ANOVA revealed a highly significant main effect of feeding history ($F_{2,63} = 281.16$, $p < 2.2 \times 10^{-16}$) and a significant main effect of lawn type ($F_{1,63} = 16.94$, $p = 0.00011$). Post hoc pairwise comparisons confirmed that amoebae previously fed on *K. pneumoniae* exhibited higher recovery on *K. pneumoniae* lawns than on palatable *P. vindicans* lawns (Holm-adjusted $p < 0.05$), while palatable and unpalatable *P. vindicans*-fed cells showed weaker or nonsignificant differences.

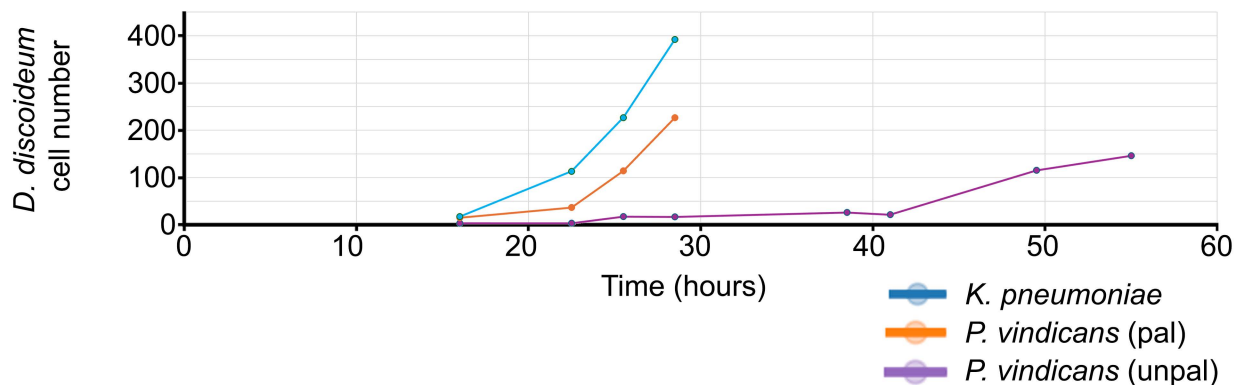
Supplementary Figure 4: The workflow of setting up the chimeric fruiting body experiments.

Supplementary figure 5. A. The comparison of mortality rate after washing with KK2 buffer. *D. discoideum* cells were stained with propidium iodide (PI), which labels dead cells, right after being washed by buffer. The proportion of stained cells relative to total cells was calculated and compared between *D. discoideum* fed on palatable and unpalatable *P. vindicans*. More than 200 cells were imaged and counted. The data is analyzed by the Wilcoxon rank sum test with

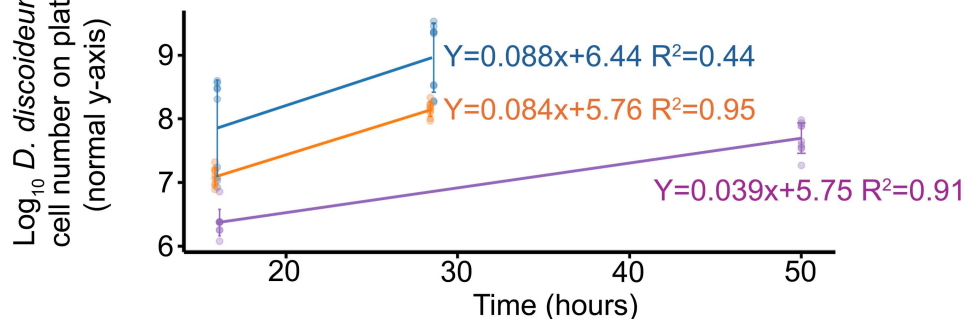
continuity correction and shows no significant difference between the two groups ($W = 5$, $p = 1.00$). B. The comparison of total spore production after washing with buffer, antibiotic, and G418. Statistics: The data residue is normally distributed to fit in LMM with day as a random factor. The effect of wash and the effect of feeding history on the total spore numbers are tested using Type III Analysis of Variance Table with Satterthwaite's method. The growth history on different bacteria has no significant effect on spore production within each washing method.

Supplementary Figure 1

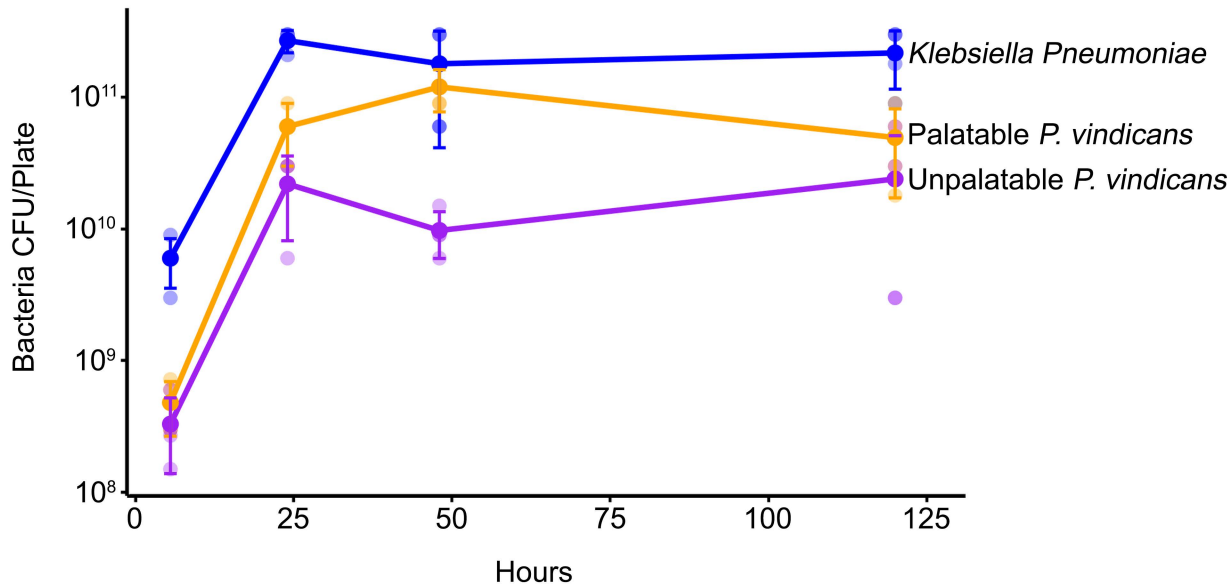
(A) Example of one day of three replicates average of *D. discoideum* cell number/agar disk on *K. pneumoniae*, palatable *P. vindicans*, and unpalatable *P. vindicans*



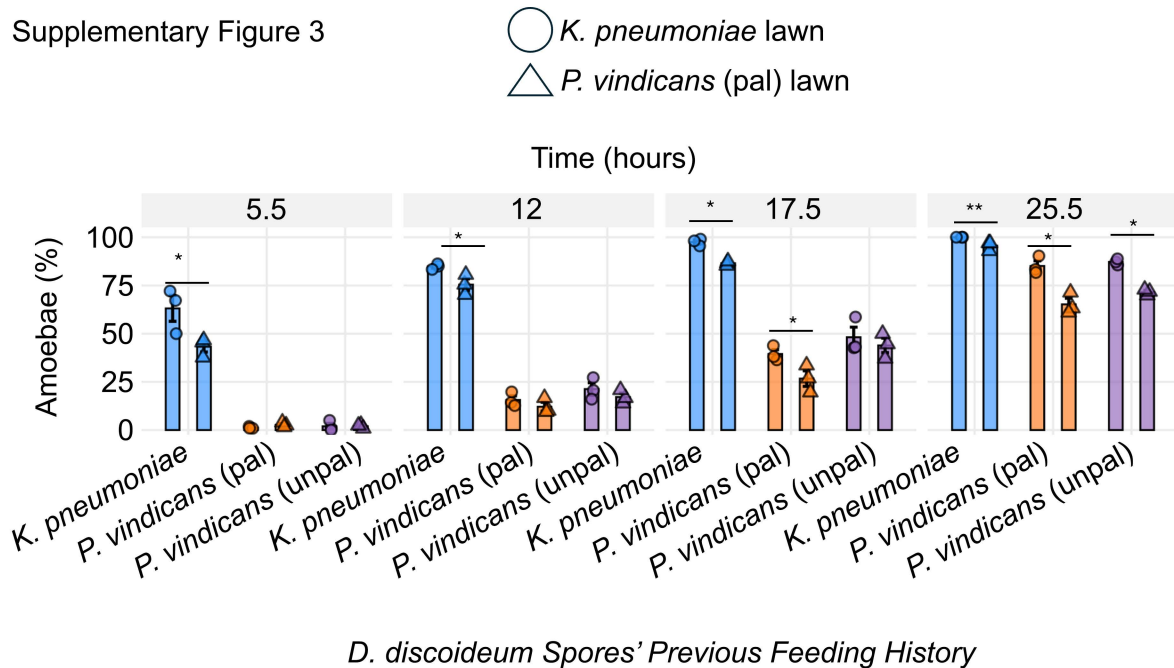
(B) Example slopes of $\log_{10}$ cell number representing the proliferation rates



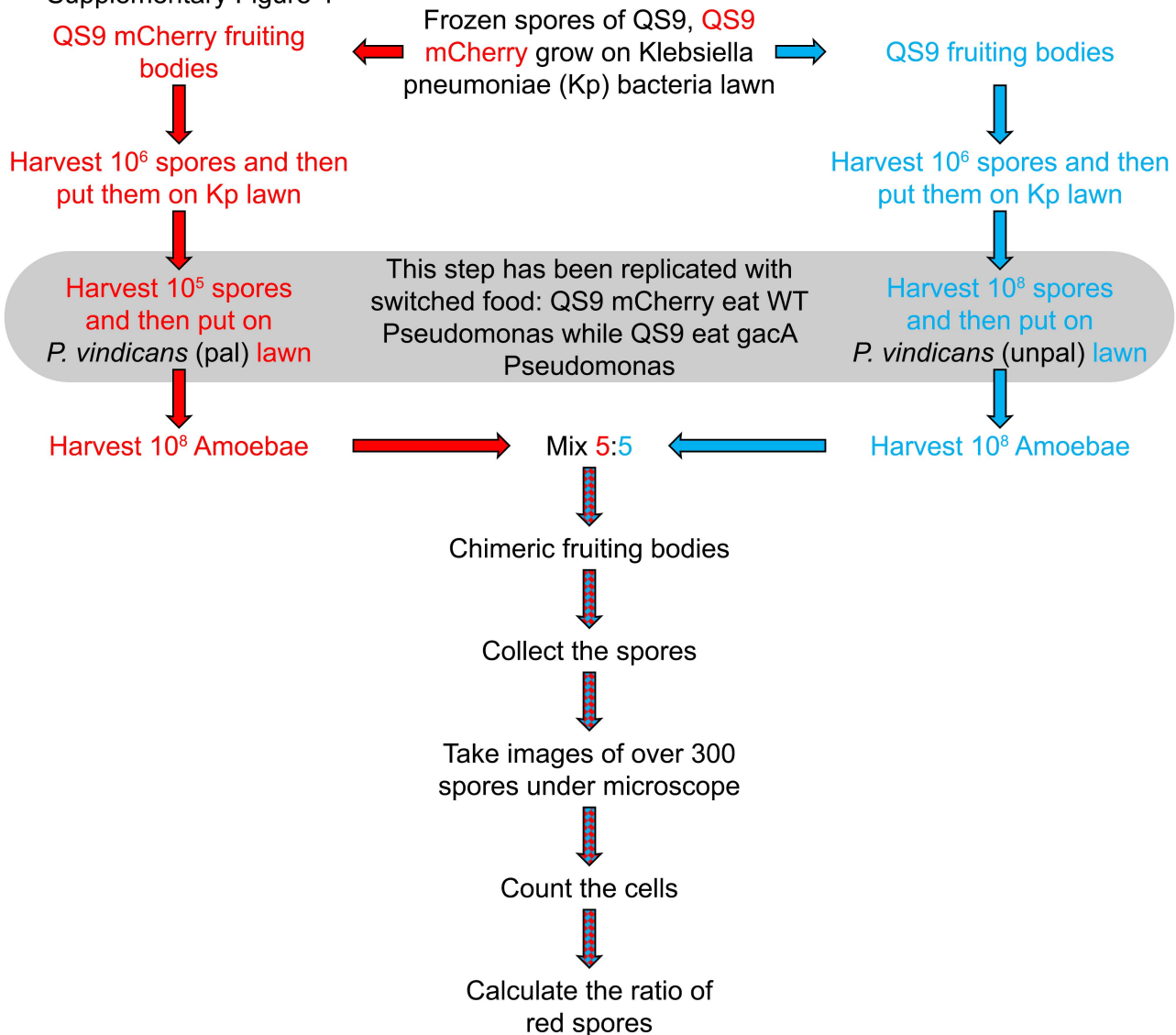
Supplementary Figure 2



Supplementary Figure 3

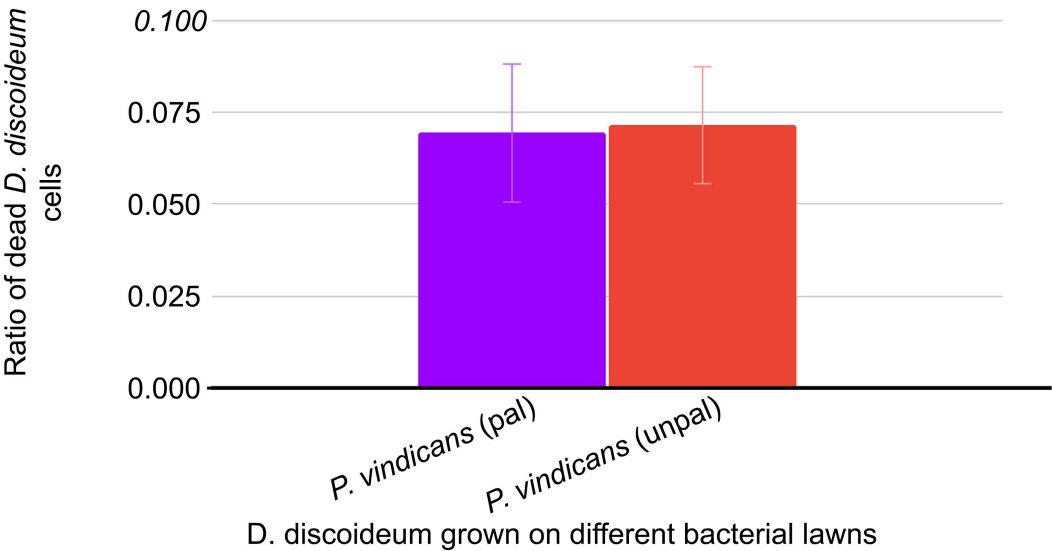


Supplementary Figure 4



Supplementary Figure 5

(A)



(B)

