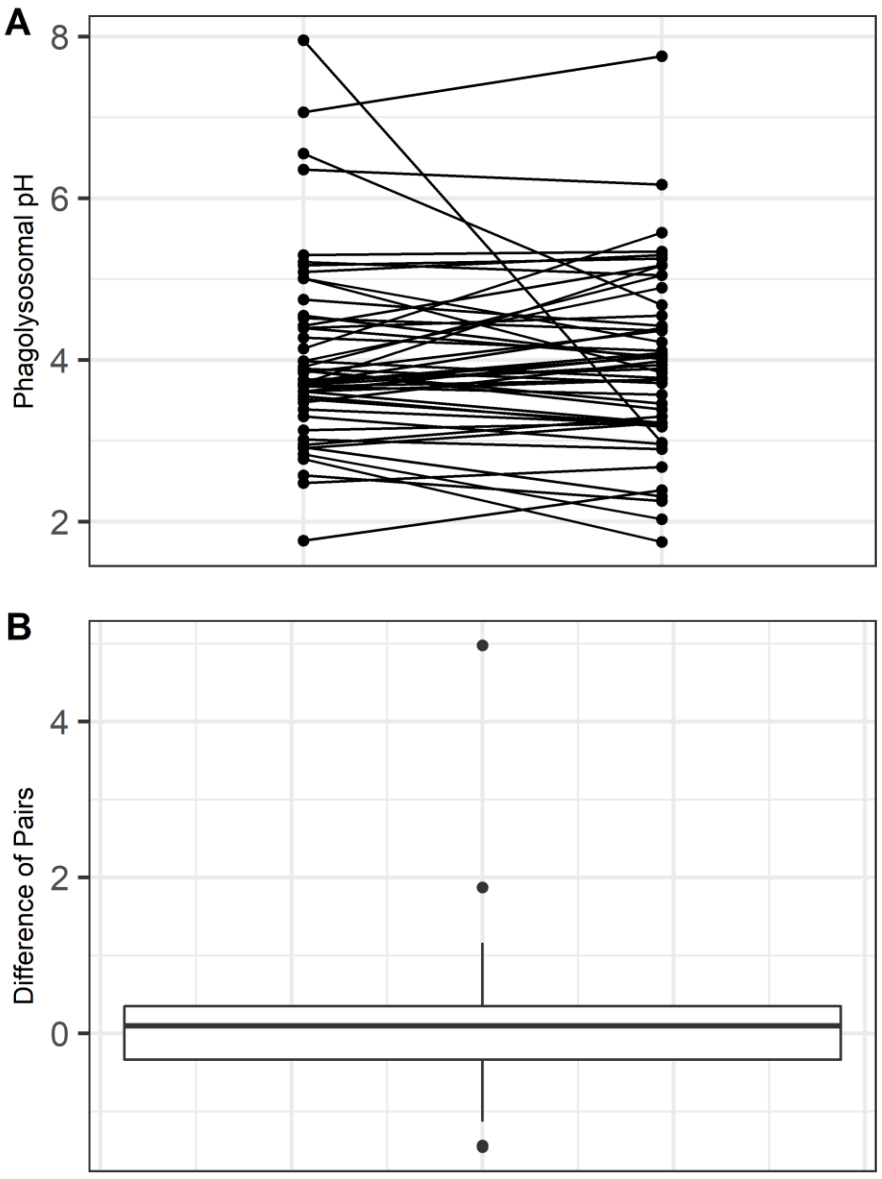
Supplemental Information

Supplemental Table-I: pH Susceptibility of Common Pathogens.

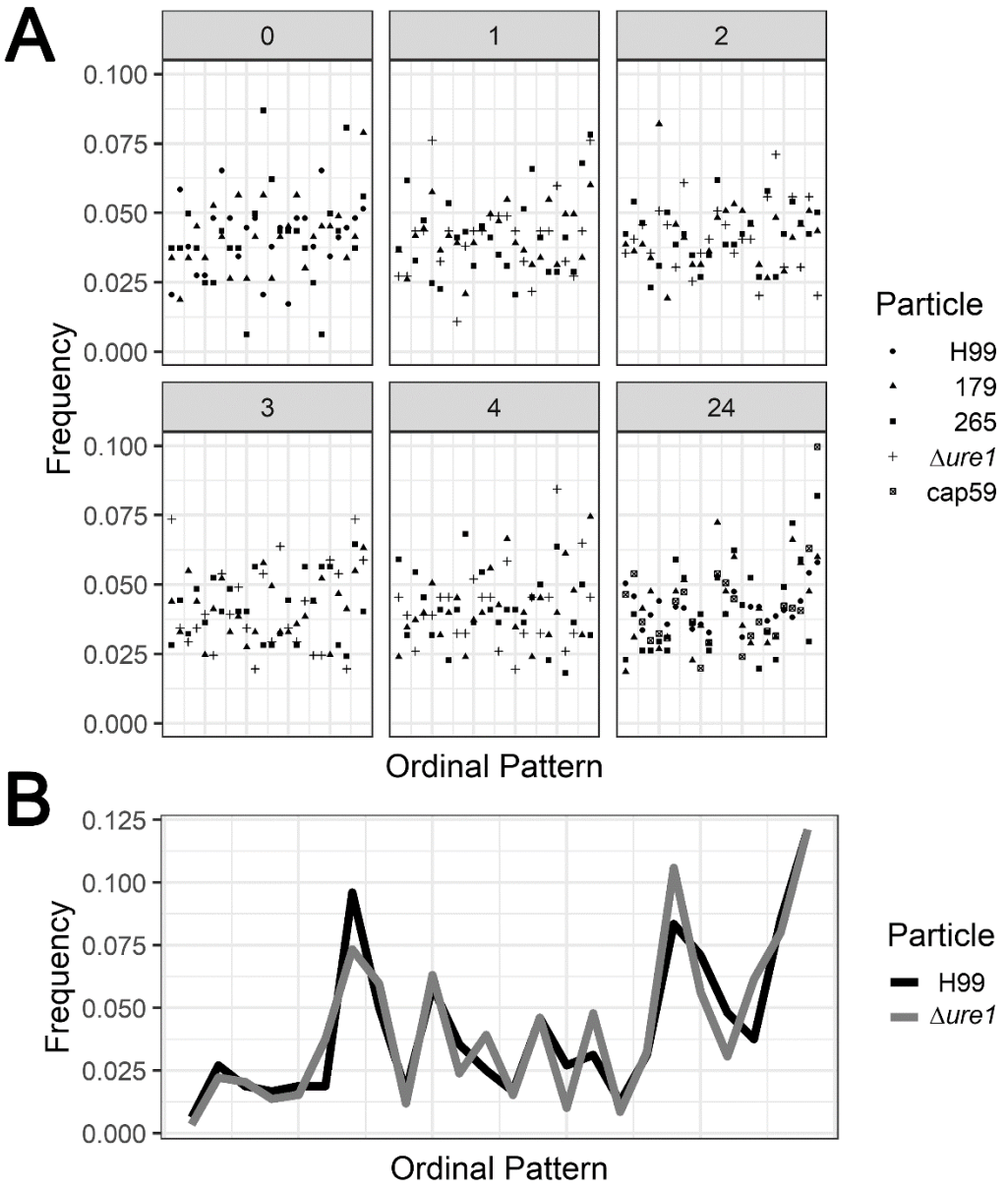
Microorganism	Inhibitory pH	Optimal Growth pH (range, average)	Reference
Acetinobacter baumannii	5	7	(1)
Bacillus cereus	4.3	6.75	(2)
Bartonella henselae	<6.6	6.8-7.2 (7.0)	(3)
Bacteroides fragilis	5	7-7.5 (7.25)	(4)
Bordetella pertusis	6	7-7.5 (7.25)	(5)
Brucella spp.	3.5	6.6-7.4 (7.0)	(6)
E. coli	~4	6-8 (7.0)	(7)
Campylobacter jujeni	4	6.5-7.5 (7.0)	(8)
Enterococcus spp.	4.5	7.5	(9)
Group A Streptococcus	5.5	7.5	(10, 11)
Group B Streptococcus	4.3	7	(12)
Histoplasma capsulatum	5	6-9 (7)	(13)
Legionella pneumophila	5	5.5-9.2 (7.35)	(14)
Listeria monocytogenes	3.3	5.6	(15, 16)
Mycobacterium tuberculosis	6.0	7.0	(17)
Mycobacterium avium	4.6	6	(18, 19)
Neisseria gonorrhea	5.8	6.7	(20, 21)
Neisseria menigitidis	6	7.2-9.0 (8.1)	(22)

Pseudomonas aeruginosa	5.4	6-7.5 (6.75)	(23)
Salmonella typhimurium	5	7	(24)
Staphylococcus aureus	4.3	6.5	(25)
Streptococcus pneumoniae	7.0	7.9	(26)
Serratia marcesens	3.0	9.0	(27, 28)
Shigella flexneri	5.5	7.5	(29)
Vibrio cholerae	5.0	8.5	(30, 31)
Yersinia enterocolitica	5.0		(32)
Yersinia pestis	5	7.6	(33, 34)

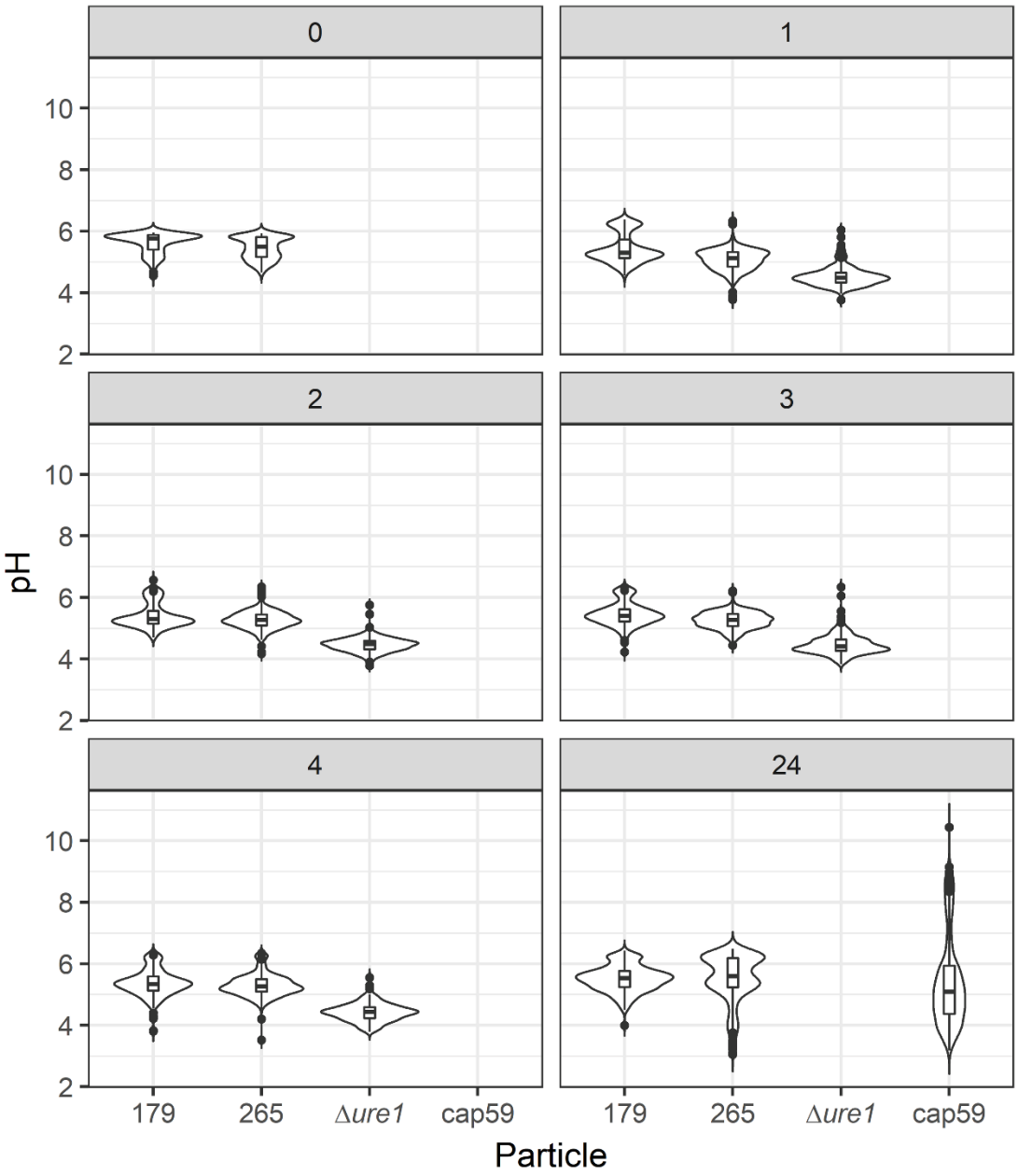
Supplemental Table-I. pH Susceptibility of Common Pathogens. pH tolerances of pathogens were found through literature search. In the case of a range of optimal pH, the average was used for simulation data. Species names are italicized.



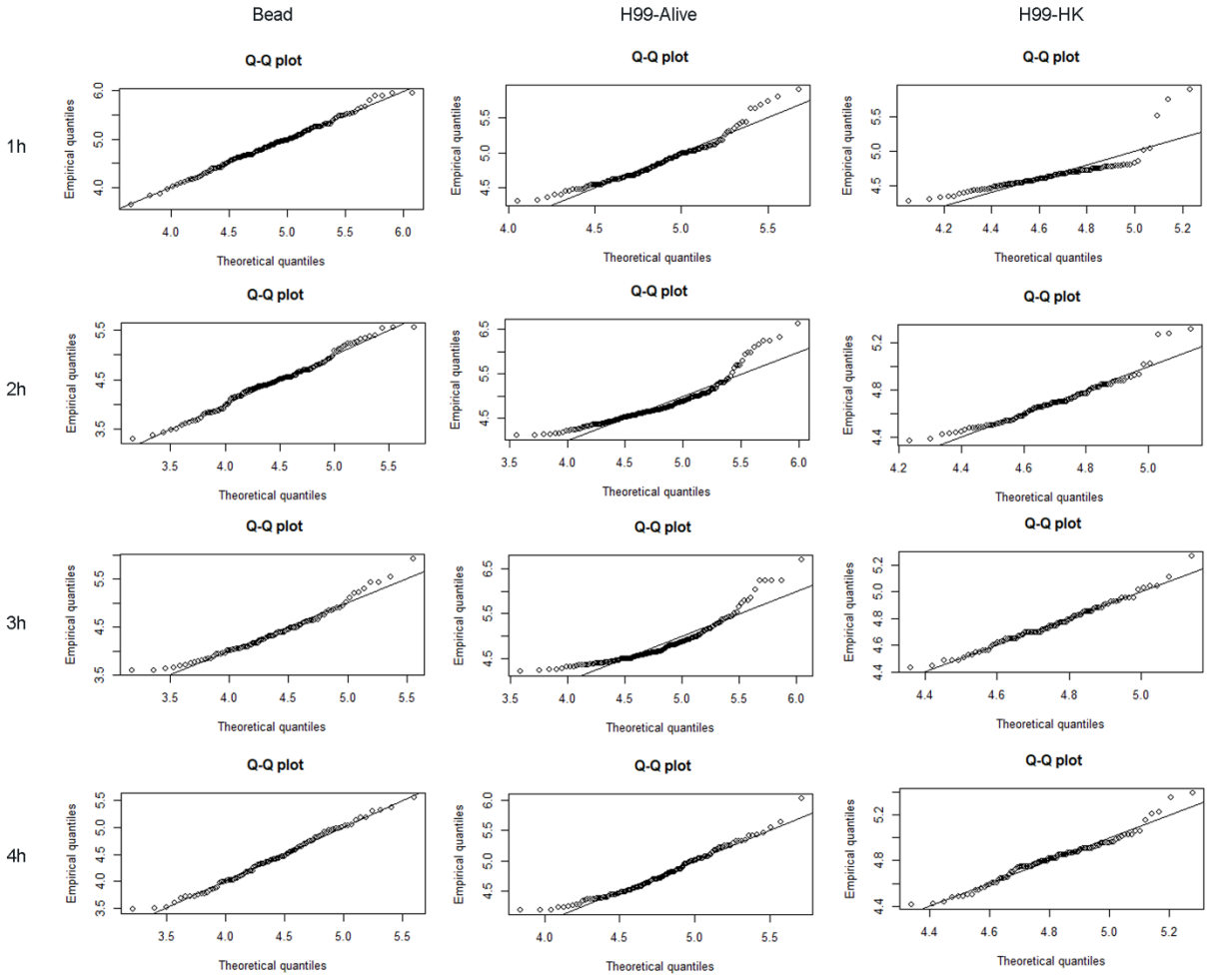
Supplemental Figure 1. Analysis of 80 pairs of phagolysosomes within single cells. **A.** Measured phagolysosomal pH of multiple beads located in the same macrophage but non-proximal phagolysosomes. Lines indicate particles within the same macrophage. Phagolysosomes were measured in no particular order. **B.** Differences of each phagolysosomal pH pair approximate a normal distribution centered around 0 with non-zero values despite being generated within the same macrophage at a synchronized starting time.



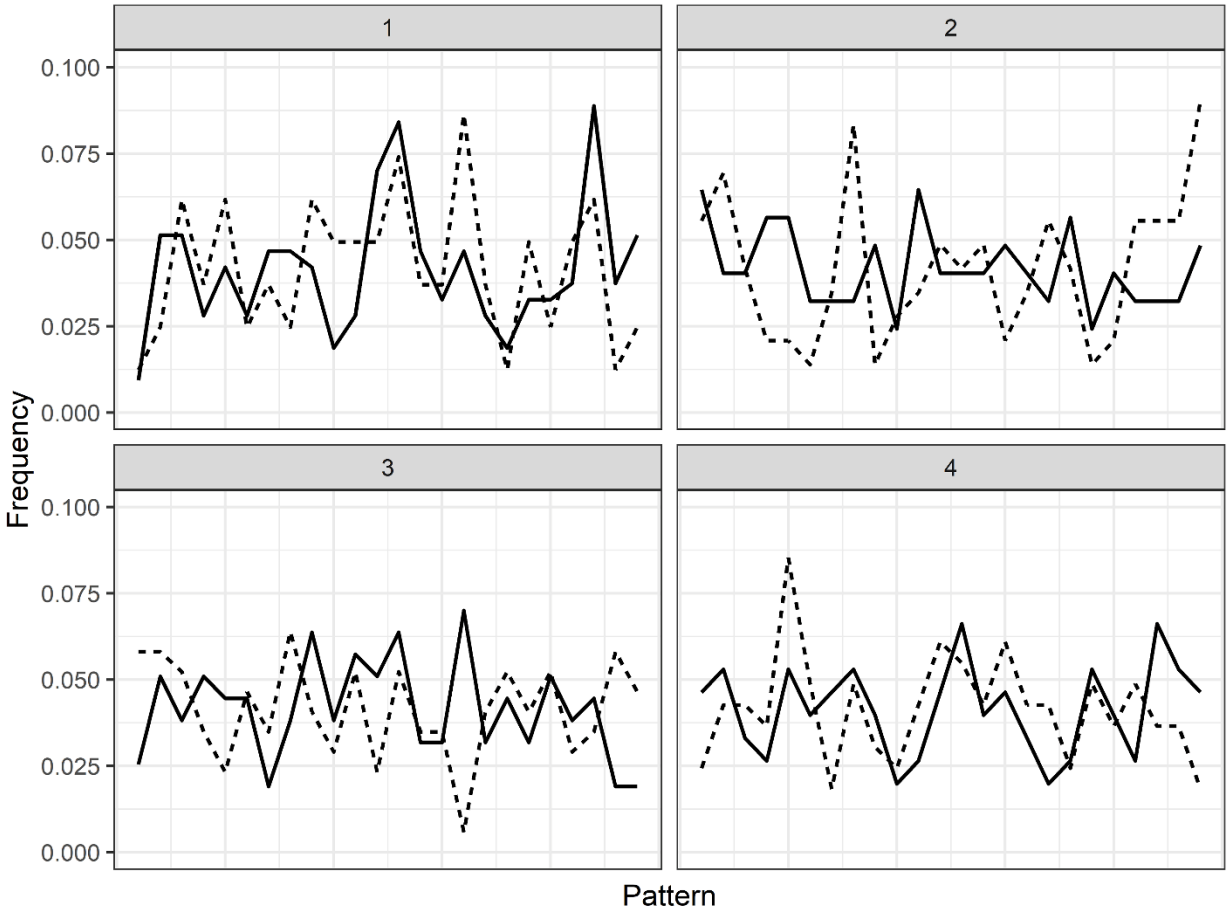
Supplemental Figure 2. Ordinal pattern analysis for expanded samples. **A.** Ordinal pattern frequencies for phagolysosomal acidification intervals in BMDMs infected with various particles at various hours post infection (gray box values). **B.** Ordinal pattern frequencies for time elapsed before initiation of budding for yeast strains ingested by BMDMs.



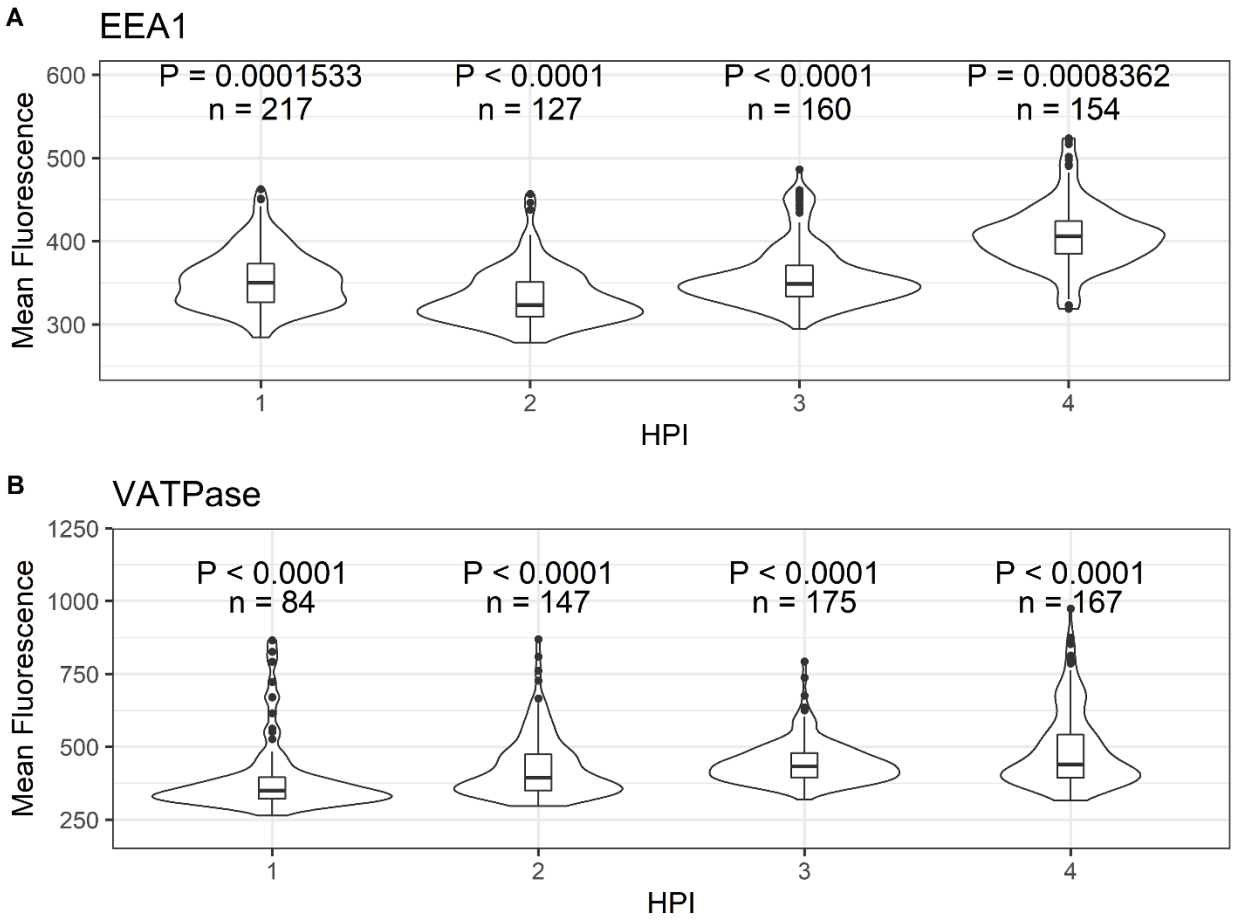
Supplemental Figure 3. Phagolysosomal pH distributions for expanded samples. Two strains of *C. gattii* (179 and 265), a urease deficient H99 mutant (*Δure1),* and a capsule deficient H99 mutant (cap59) were analyzed at various hours post infection (gray box value).



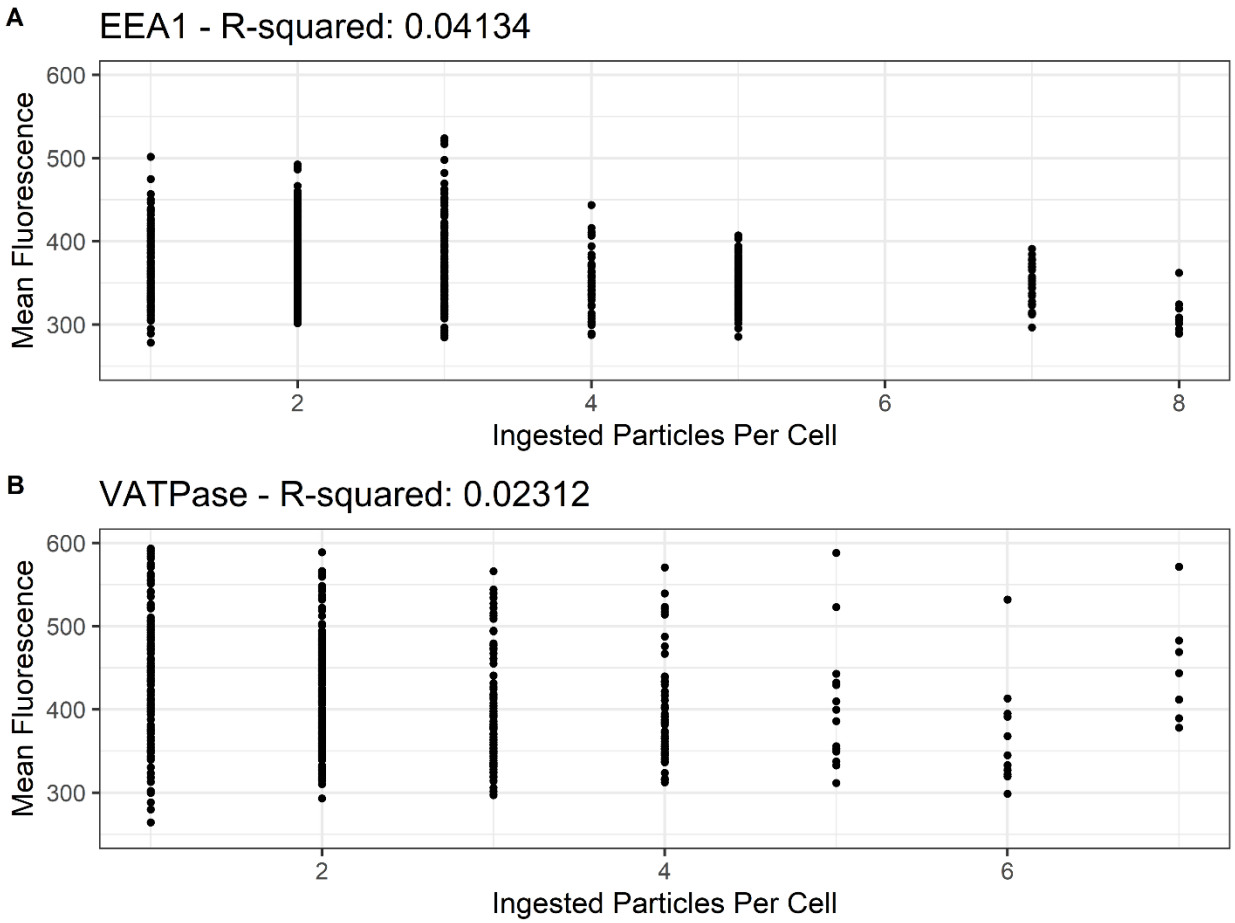
Supplemental Figure 4. Q-Q plots of macrophages 1-4 HPI after ingesting various particles. Note that bead containing phagolysosomes closely approximate a normal distribution, while *C. neoformans* containing phagolysosomes have heavy tails, regardless if the particle is live or dead.



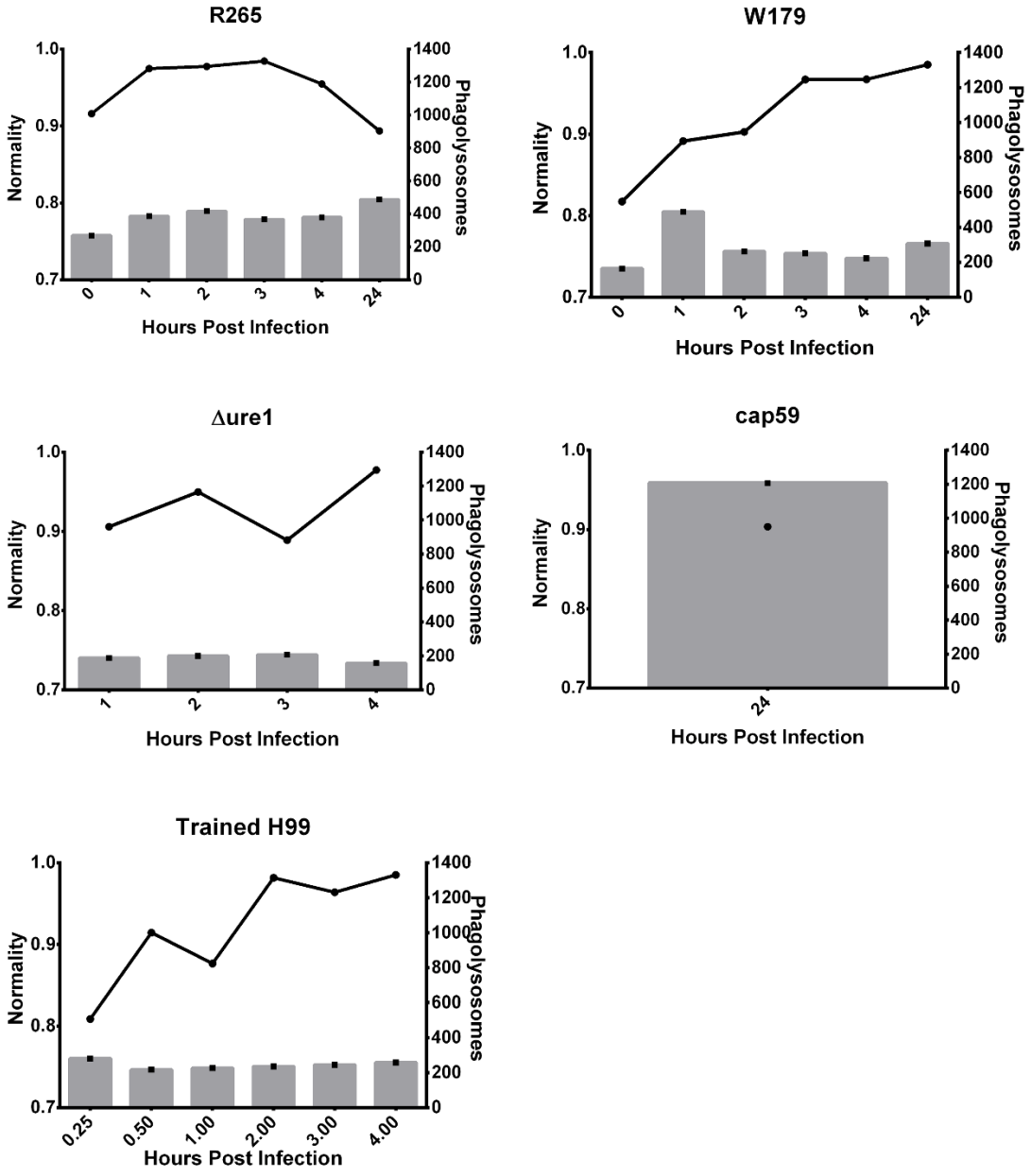
Supplemental Figure 5. Ordinal pattern analysis for EEA1 (solid) and VATPase (dashed) immunofluorescent staining for bead containing macrophage phagolysosomes at various hours post ingestion (gray box values). No forbidden ordinal patterns were detected for any samples, suggesting phagolysosomal maturation marker acquisition is a stochastic process.



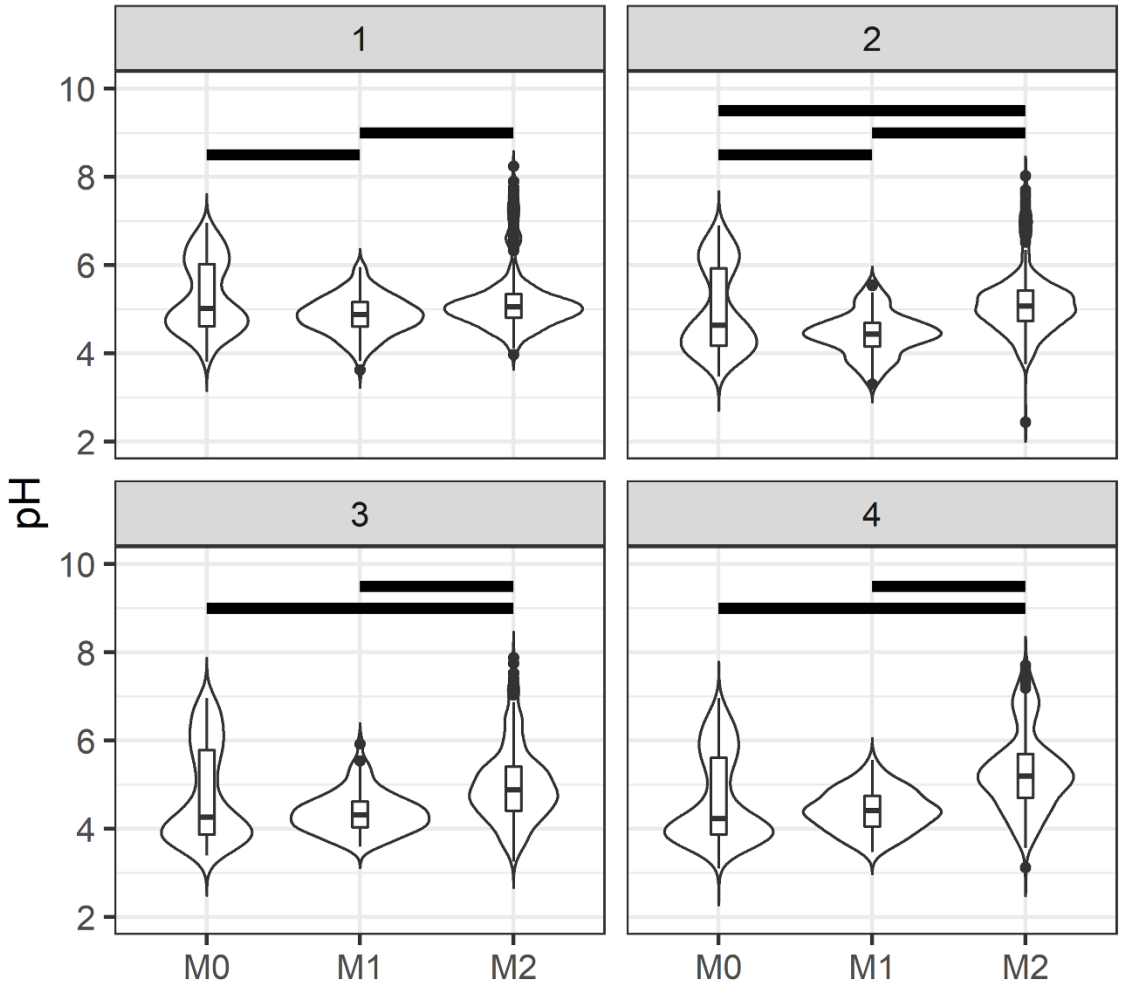
Supplemental Figure 6. Mean fluorescence intensity values for EEA1 and VATPase immunofluorescent staining. The acquisition of these phagolysosomal maturation markers does not resemble that of bead containing phagolysosomal pH, as no samples here are normally distributed. P values were determined via Shapiro-Wilk normality test.



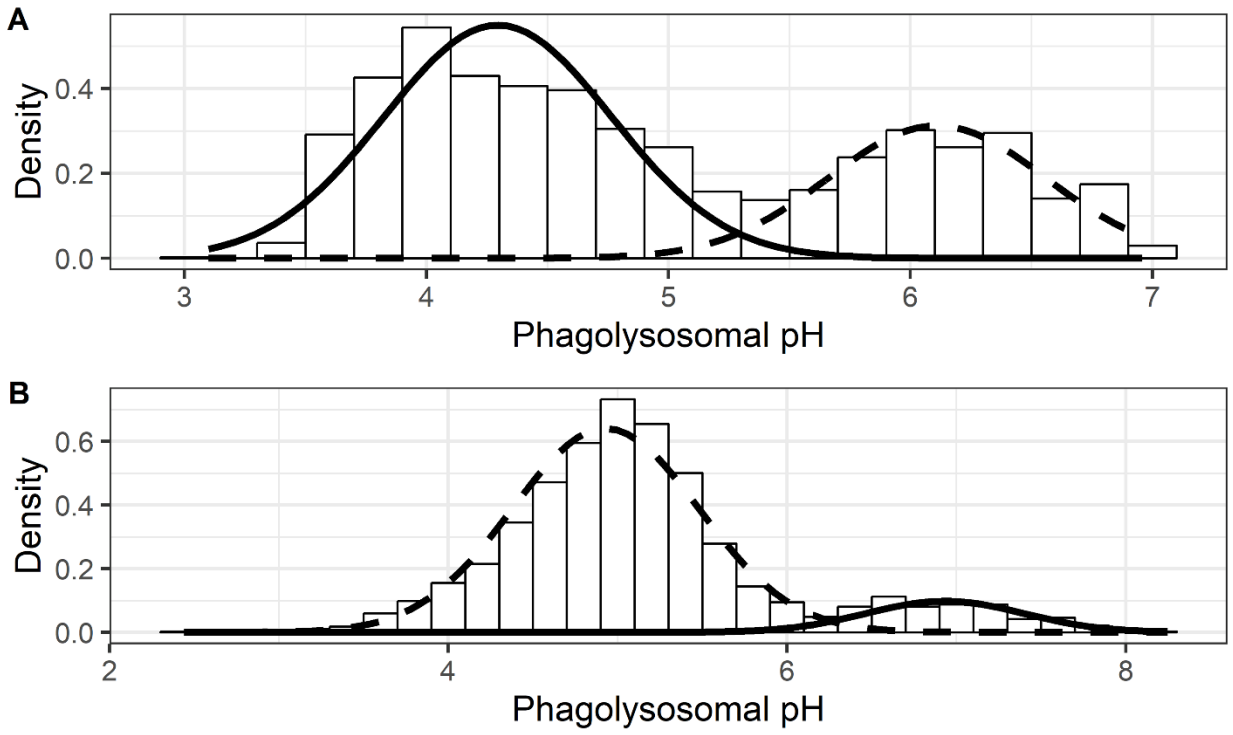
Supplemental Figure 7. Mean fluorescent intensity of macrophage ingested beads stained for EEA1 and VATPase, sorted according to total ingested particles per cell. Linear regressions were calculated for each sample set but clearly there is no significant correlation between total ingested particles and number of EEA1 or VATPase molecules as measured by fluorescent intensity.



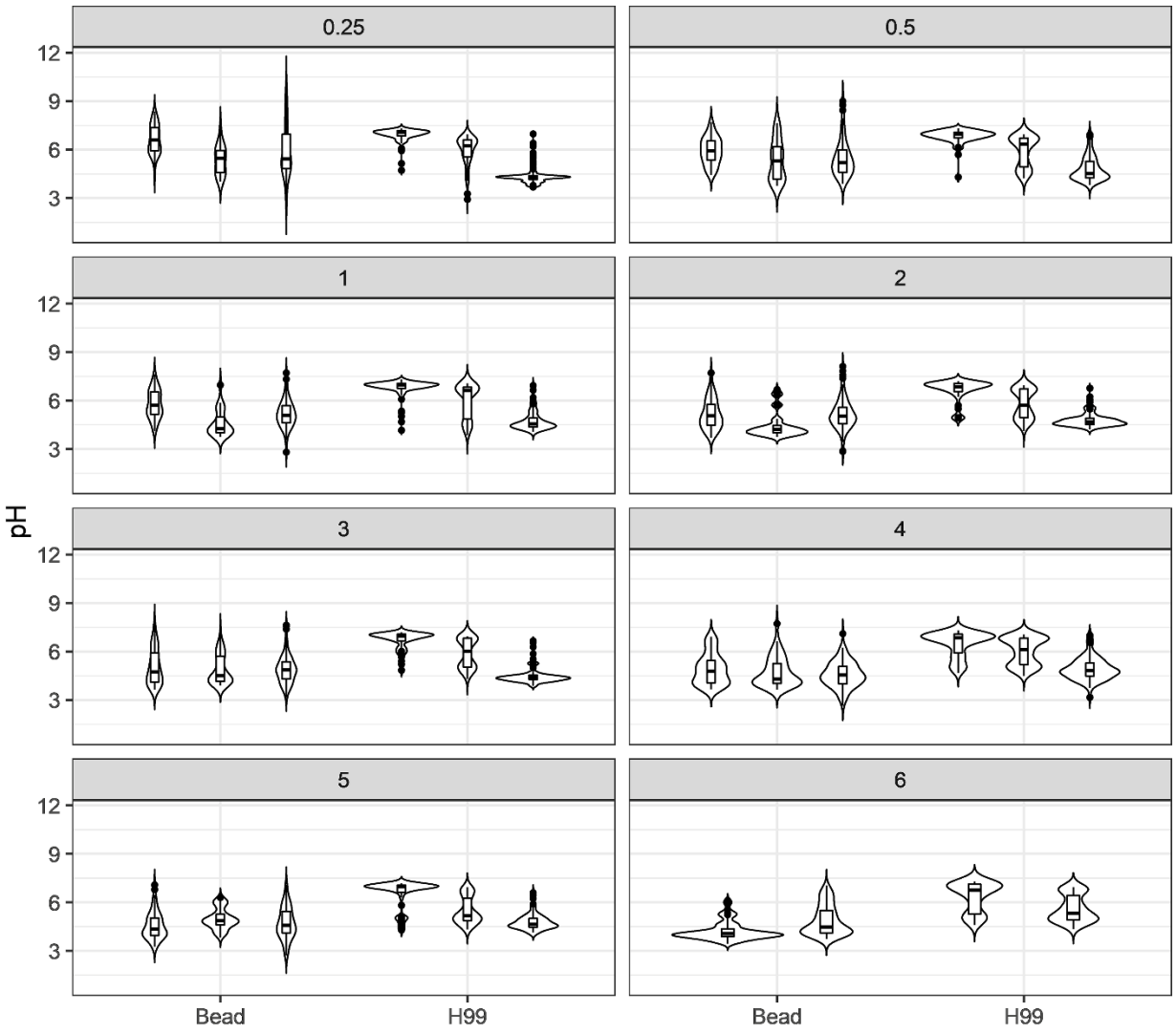
Supplemental Figure 8. Normality analysis for expanded samples. Shapiro-Wilk normality values (lines) alongside total phagolysosome sample sizes (bars) for two strains of *C. gattii* (179 and 265), a urease deficient H99 mutant (*Δure1),* a capsule deficient H99 mutant (cap59), and trained BMDMs on reinfection.



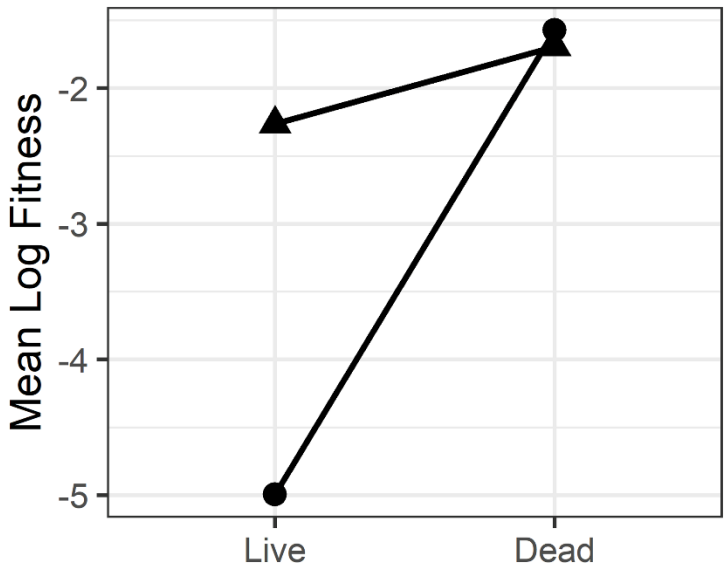
Supplemental Figure 9. Phagolysosomal pH distributions for bead containing phagolysosomes of differently polarized BMDMs at each measured time post infection (gray box values). Black bars represent $P < 0.0001$ via Kruskal-Wallis test with Wilcox rank pairing test.



Supplemental Figure 10. Bimodal models fitted to the observed data for **A.** M0 and **B.** M2 skewed macrophages. Models are attempted fits of two mixed Gaussian distributions. Histogram bars visualize the observed data while solid and dashed lines depict the relative contributions of the two hypothetical Gaussian distributions.



Supplemental Figure 11. Phagolysosomal pH distributions for human macrophages infected with H99 separated by individual donor. Human macrophages were analyzed after ingesting either H99 or inert beads at various hours post infection (gray box value).



Supplemental Figure 12. Estimated mean log fitness of macrophages containing live or killed *M. avium*. Phagolysosomal pH data was gathered from literature using either video microscopy (triangles) or confocal microscopy (circles).

References

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