Supplementary Material

Use of a novel DNA-loaded alginate-calcium carbonate biopolymer surrogate to study engulfment of *Legionella pneumophila* by *Acanthamoeba polyphaga*

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Figure S1. CLSM bright-field (1A-5A) and composite (1B-5B) time-lapse images of *A. polyphaga* acanthopodia formation and surrogate engulfment immediately after co-culture establishment at 100x. Blue arrows point to *A. polyphaga* acanthopodia.
Figure S2. CLSM  bright-field (A), fluorescent (B), and composite (C) images of surrogate-A. polyphaga co-cultures at 12 h at 45x. Blue arrows point to rounded A. polyphaga trophozoites with retracted acanthopodia.

Figure S3. Size distribution of surrogate clumps released by A. polyphaga trophozoites at 24 h and 48 h following co-incubation at 30 °C.
**Figure S4.** CLSM 60× bright-field (A), fluorescent (B), and composite (C) images of surrogate-A. *polyphaga* co-cultures at 48 h. White and blue arrows point at lysed trophozoites and round trophozoites with retracted acanthopodia, respectively. Magnification 45×.
| hours after inoculation of surrogates | number of trophozoites w/ acanthopodia per field | number of round trophozoites per field | number of lysed trophozoites per field | active to round trophozoite ratio per field | average | std dev | T.test |
|-------------------------------------|-----------------------------------------------|--------------------------------------|--------------------------------------|------------------------------------------|--------|---------|--------|
| 12                                  | 17                                            | 4                                    | 0                                    | 4.3                                      | 5.0    | 1.6     |        |
| 12                                  | 21                                            | 6                                    | 0                                    | 3.5                                      |        |         |        |
| 12                                  | 19                                            | 3                                    | 0                                    | 6.3                                      |        |         |        |
| 12                                  | 22                                            | 3                                    | 0                                    | 7.3                                      |        |         |        |
| 12                                  | 14                                            | 3                                    | 0                                    | 4.7                                      |        |         |        |
| 12                                  | 16                                            | 4                                    | 0                                    | 4.0                                      |        |         |        |
| 12                                  | 22                                            | 7                                    | 0                                    | 3.1                                      |        |         |        |
| 12                                  | 20                                            | 3                                    | 0                                    | 6.7                                      |        |         |        |
| 48                                  | 15                                            | 1                                    | 3                                    | 15                                       | 13.3   | 3.6     | 0.601  |
| 48                                  | 16                                            | 1                                    | 2                                    | 16                                       |        |         |        |
| 48                                  | 13                                            | 0                                    | 4                                    | 0                                        |        |         |        |
| 48                                  | 13                                            | 1                                    | 4                                    | 0                                        |        |         |        |
| 48                                  | 15                                            | 0                                    | 2                                    | 0                                        |        |         |        |
| 48                                  | 14                                            | 0                                    | 0                                    | 0                                        |        |         |        |
| 48                                  | 14                                            | 1                                    | 3                                    | 14                                       |        |         |        |
| 48                                  | 16                                            | 2                                    | 3                                    | 8                                        |        |         |        |