FIG S1 Trophic forms induce the expression of genes encoding chemokines and complement proteins. The relative gene expression after 8hrs of treatment was calculated as the log2 value of the quotient of the treated expression value divided by the untreated expression value. The data in Fig 3A represent genes in the NanoString panel with a statistically significant (p < 0.01) four-fold or greater increase in expression between BMDCs treated with trophic forms compared to unstimulated BMDCs. The data represent all genes encoding regulators of inflammation (B) or immunity-related transcription factors (C) with a statistically significant (p < 0.01) two-fold or greater increase in expression between BMDCs treated with trophic forms compared to unstimulated BMDCs. Differences in expression between the groups were identified by log transformed two way ANOVA followed by pairwise comparisons using the Least Significant Difference Method. Five biological replicates were used per group. Genes with differences in expression with p < 0.01 between BMDCs treated with trophic forms vs unstimulated cells are denoted *, trophic forms vs mixed P. murina organisms are denoted †, and trophic forms vs curdlan are denoted ‡.
FIG S2 Cysts, but not trophic forms, stimulate production of the proinflammatory cytokines IL-1β and IL-6 by BMDCs in vitro. BMDCs from adult BALB/cJ mice were incubated with 3 X 10^4 trophic forms or 3 X 10^4 mixed P. murina organisms for 72hrs. Curdlan was included as a positive control for cytokine production. TNFα (A), IL-1β (B), and IL-6 (C) cytokine production was quantified by ELISA. Data represent the mean ±SD of 3 biological replicates per group and are representative of 2 separate experiments. One way ANOVA with Student-Newman-Keuls post-hoc test was used to compare supernatant cytokine concentration between the groups, **p ≤ 0.01, ***p ≤ 0.001, n.s., not statistically significant.
FIG S3 A ratio of 10 trophic forms to 1 BMDC is required for suppression of cytokine expression. 1 X 10^5 BMDCs were incubated with 10 µg/ml curdlan, and increasing numbers of trophic forms for 72 h. TNFα (A), IL-1β (B), and IL-6 (C) cytokine levels in the supernatant were quantified by ELISA. Data represent the mean ±SD of 3 biological replicates per group and are representative of 2 separate experiments. One way ANOVA with Student-Newman-Keuls post-hoc test was used to compare cytokine concentrations among the groups, *p ≤ 0.05, n.s., not statistically significant.
Trophic forms do not have a negative impact on the phagocytic capacity of dendritic cells. CFSE-stained BMDCs from BALB/cJ mice were incubated with DDAO-SE-stained trophic forms or mixed *P. murina* organisms for one hour in a chamber slide (A). Cells were fixed in neutral buffered formalin, and confocal microscopy was used to identify internalized trophic forms (A). The z-stacks demonstrate that BMDCs internalize DDAO-SE-labeled trophic forms (A). BMDCs were incubated with pHrodo Green zymosan bioparticles and/or DDAO-SE-stained trophic forms or mixed *P. murina* organisms for one hour in chamber slides (B-C) or FACS tubes (D-E). The chamber slides were fixed in neutral buffered formalin, and stained with DAPI (B-C). Confocal microscopy was used determine the percentage of BMDCs that had phagocytosed the pH-sensitive pHrodo Green zymosan bioparticles based on at least 100 events per group (B-C). Flow cytometry was used to determine the percentage of BMDCs that had phagocytosed the pHrodo Green zymosan bioparticles based on at least 1000 events per group (D). The geometric mean fluorescence intensity (MFI) in the FITC channel of the zymosan+ BMDCs was calculated for each group (E). Data represent the mean ±SD of 3 biological replicates per group and are representative of 2 separate experiments. One way ANOVA with Student-Newman-Keuls post-hoc test was used to compare supernatant cytokine concentration between the groups, n.s., not statistically significant (p > 0.05).