

Supplemental Fig. 1. White blood cell (WBC) counts in the BAL samples of mice infected with PA or SP following 3 days (A) or 7 days (B) post-MHV infection. Cell differentials were performed by staining the cells with Hema stain to visualize the cells. The number of macrophages, neutrophils and lymphocytes were counted in the BAL on mice infected with either PA or SP on day 3 (C) or day 7 (D). Levels of neutrophil chemoattractant KC and MIP-2 on day 3 (E) and day 7 (F) post-MHV infection in PA infection. Representative images of TUNEL stain in green and nuclear stain in blue with Hoechst 33342 in mice infected with bacterial infection post-MHV infection on either day 3 (G) or on day 7 (H). Mice were infected with MHV for 3 days, and then administered 5 μ g of LPS by the intratracheal route and euthanized at 18 hours post LPS to measure BAL total protein content (I), WBC counts (J), and IL-1 β levels (K). Data are pooled from two or three independent experiments or shown from one of the representative experiments. *, P < 0.05, **, P < 0.01, ***, P < 0.001, using either t-test or one-way ANOVA followed by Dunnett's multiple comparisons test, as appropriate.



Supplemental Fig. 2. (A) RAW 264.7 cells were infected with MHV for 20 hours and then infected with PA for 6 hours. Surviving bacterial numbers were counted by plating them on minimal agar. (B) Phagocytotic ability of peritoneal macrophages infected with MHV or mock-infected for 20 hours followed by incubation with the beads for 1 hour. The number of beads per 100 cells is reported. *, P < 0.05, **, P < 0.01 using t-test.



Supplemental Fig. 3. Peritoneal macrophages were infected with MHV for 20 hours and then stimulated with *Pseudomonal* LPS for 6 hours. Cell death was measured using PI staining, elevated LDH and IL-1 β levels in the cell supernatants (A-C). Peritoneal macrophages were infected with the H3N2 strain of influenza (MOI of 2.5) for 20 hours and then infected with PA (MOI of 20) to determine the cell death using PI staining (D) and LDH levels in cell supernatants (E). ****, P < 0.0001, using one-way ANOVA followed by Dunnett's multiple comparisons test, as appropriate.



Supplemental Fig. 4. Peritoneal macrophages were infected with MHV followed by a bacterial infection in the presence or absence of caspase-1 inhibitor Ac-YVAD-CMK. Cell death was measured using PI staining (A) and LDH in cell supernatants(B). Cathepsin B activity was measured in the cell culture supernatants using cathepsin B substrate Z-Arg-Arg-pNA at final concentration of 200 μ M and the reactivity OD was measured at 405 nm. The activity is represented as OD values after subtracting the OD values of blank samples (C). ****, P < 0.0001 using t-test (B) or one-way ANOVA (C).