Supporting Information

PPARα exacerbates necroptosis leading to increased mortality in post-influenza bacterial super-infection.

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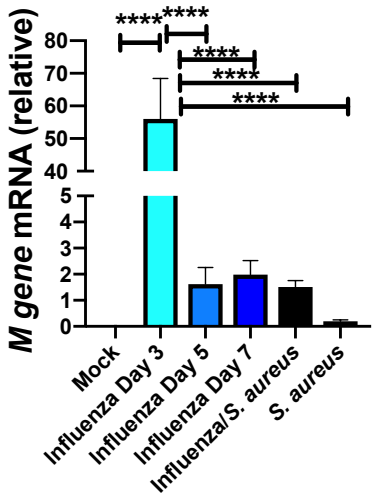

Figure S1. Viral load in influenza-infected mice. Bar graph depicts transcript levels of the influenza M-gene relative to EF1α as measured by RT-PCR from total BAL cells isolated from wild-type C57BL/6 mice that were mock infected, influenza infected, influenza/*S. aureus* infected (measured 4 hours after *S. aureus* infection), or *S. aureus* infected (measured 4 hours after *S. aureus* infection). Statistical significance was measured by One-way ANOVA (**** $p < 0.0001$).

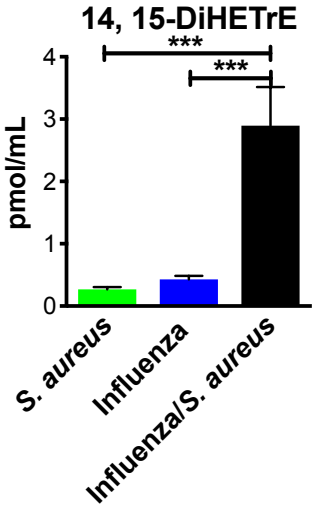

Figure S2. Increased production of 14,15-DiHETrE during *S. aureus* and influenza/*S. aureus* super-infection. Mice were left uninfected or infected with influenza for 7 days then infected with *S. aureus.* BAL was extracted 4 hours after *S. aureus* infection (or 7 days following influenza infection alone) and analyzed by LC/MS. Bar graph depicts the mean with SEM as error bars. Statistical significance was determined by unpaired Student's T-test (*** $p < 0.001$) Data are representative of two independent experiments with 7-10 mice/condition.

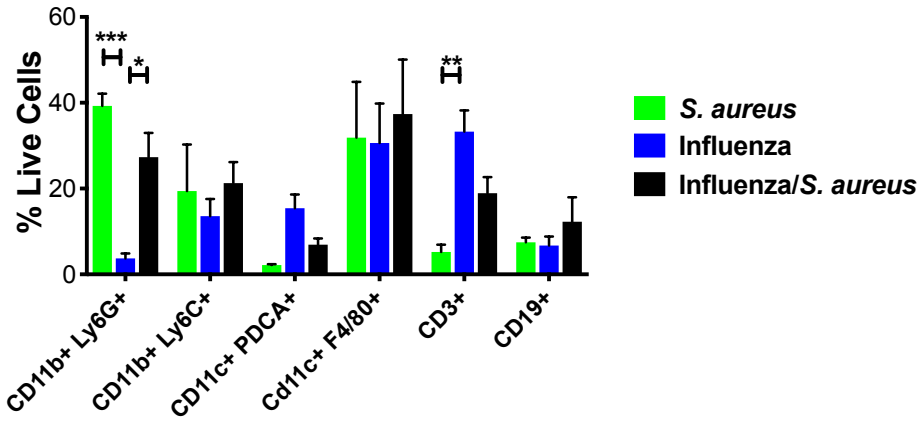

Figure S3. Cellularity in BAL during *S. aureus* infection, influenza infection or influenza/*S. aureus* super-infection. Neutrophils (CD11b+ Ly6G+), inflammatory monocytes (CD11b+, Ly6C+), DC (CD11c+ PDCA+), alveolar macrophages (CD11c+ F4.80+), T cells (CD3+), and B cells (CD19+) were enumerated by cytometry. 2-way ANOVA was performed to determine statistical significance (* P<0.05; ** P<0.01; *** P<0.001). Data are representative of two independent experiments with three mice/condition.

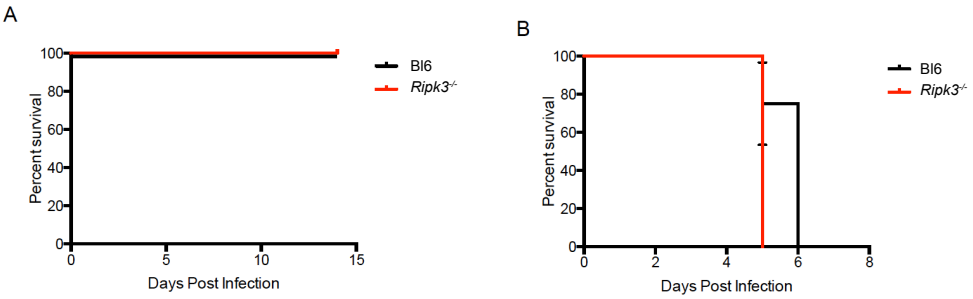

Figure S4. Mortality from influenza infection in wild-type and *Ripk3*$^{-/-}$ mice. (A) Survival curve of wild-type C57BL/6 (Black) or *Ripk3*$^{-/-}$ mice (Red) infected with 100 PFU of influenza (PR8). (B) Survival curve of wild-type C57BL/6 (Black) or *Ripk3*$^{-/-}$ mice (Red) infected with 2x10^5 PFU of influenza (PR8).

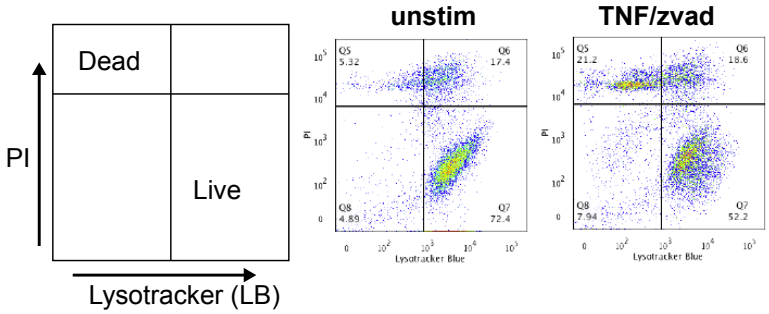

Figure S5. Assessment of the dead/live cell ratio by flow cytometry. Hox-derived macrophages from C57BL/6 mice were mock treated or treated with TNF and zvad for 16 hours, stained with propidium iodide (PI) and lysotracker blue (LB), and analyzed by flow cytometry. Dead cells are PI+ LB- and live cells are PI- LB+.