

Supplemental Figure 1: Effect of preincubating Opa+ Gc with N-CEACAM1 on the PMN oxidative burst and Gc internalization. A. Prior to infection of PMNs, Opa+ Gc was incubated with indicated concentrations of N-CEACAM1 or left untreated. ROS production was measured as luminol-dependent chemiluminescence produced over 1 hr. B. PMNs were infected with Opa+ Gc or Opa+ Gc with N-CEACAM1 for 1 hr. Intracellular and extracellular Gc were discriminated from one another by accessibility to fluorescent antibodies before and after cell permeabilization, and the percent of internalized Gc is expressed relative to total cell-associated bacteria. Asterisks indicate  $P < 0.05$  by Student's two-tailed  $t$  test.

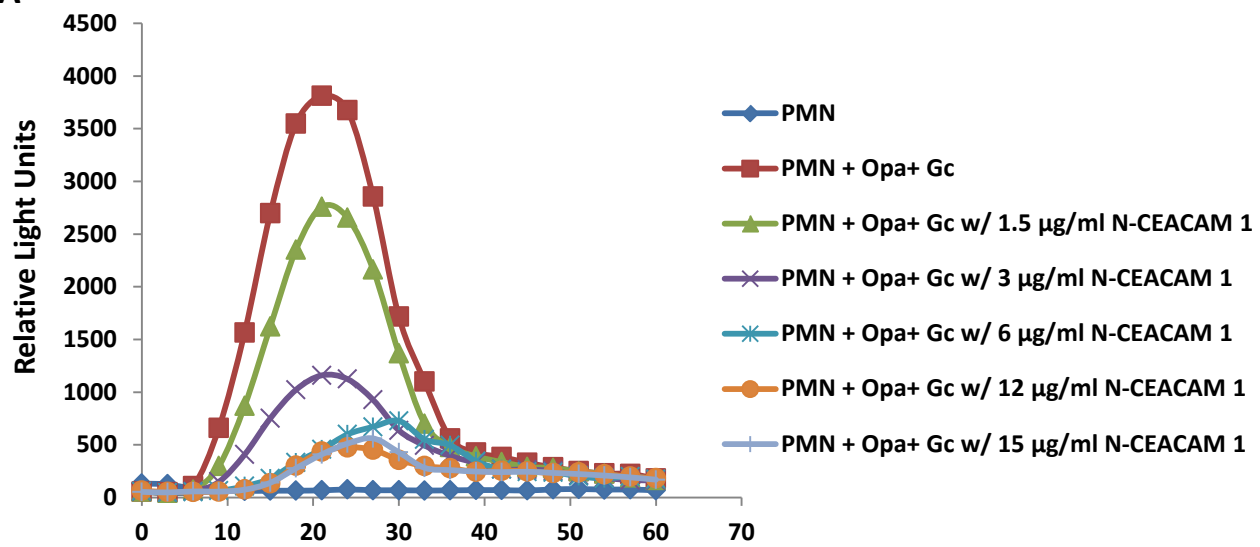
Supplemental Figure 2: Effect of inhibitors of signal transduction proteins downstream of CEACAM3 on the PMN oxidative burst and Gc internalization. A. PMNs were treated with piceatannol, LY 294002, EHT1864, or PP1 or left untreated, then infected with Opa+ Gc. ROS production was measured as luminol-dependent chemiluminescence produced over 1 hr. All inhibitors markedly inhibited the PMN oxidative burst in response to Opa+ Gc. B. PMNs were treated with piceatannol, LY 294002, EHT1864, or PP1 or left untreated, then infected with Opa+ Gc for 1 hr. Percent bacterial internalization was measured as in Supplemental Figure 1 legend. C. PMNs were treated with PP1 or left untreated, then infected with Opa- Gc for 1 hr, and percent bacterial internalization was measured as above. There were no significant differences in internalization of Gc by PMNs treated with these inhibitors.

Supplemental Figure 3: Effect of phosphoinositide-3-kinase inhibition on Gc phagosome maturation. PMNs were treated with LY294002 or not, then exposed to Opa- or Opa+ Gc for 1 hr. Phagosomal maturation was assessed using antibodies against lactoferrin

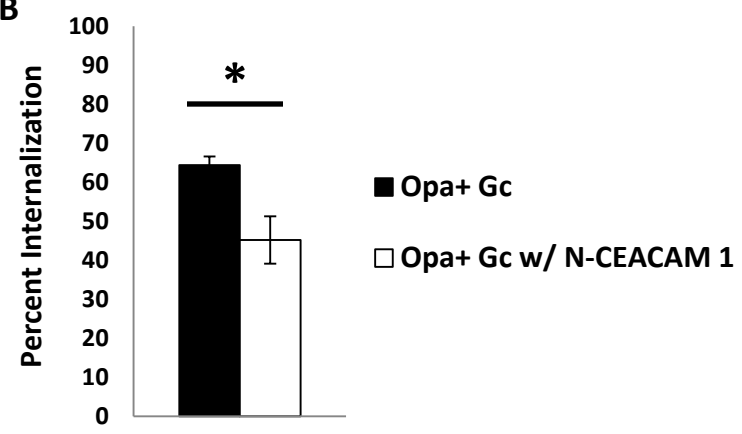
(A) or neutrophil elastase (B), which appear green. External Gc appear red/blue and internal Gc appear blue. Arrowheads point to bacterial phagosomes positive for the granule protein, while arrows point to phagosomes lacking the protein. The percent of phagosomes positive for lactoferrin and neutrophil elastase are reported in C and D, respectively. Asterisk indicates  $P < 0.05$  by Student's two-tailed  $t$  test.

# Supplemental Figure 1

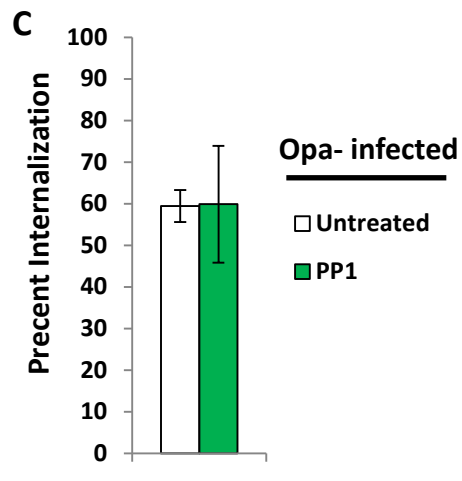
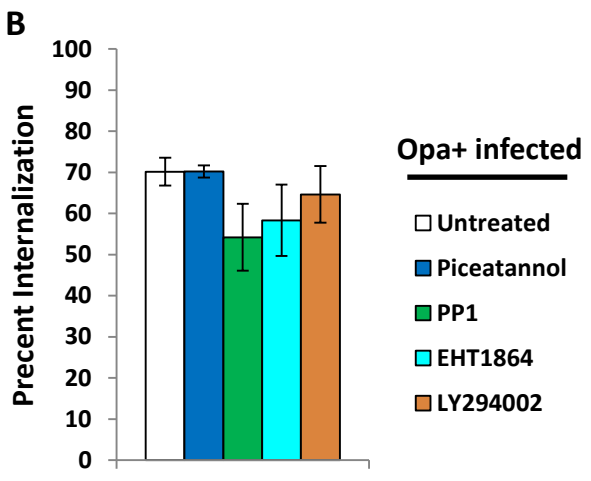
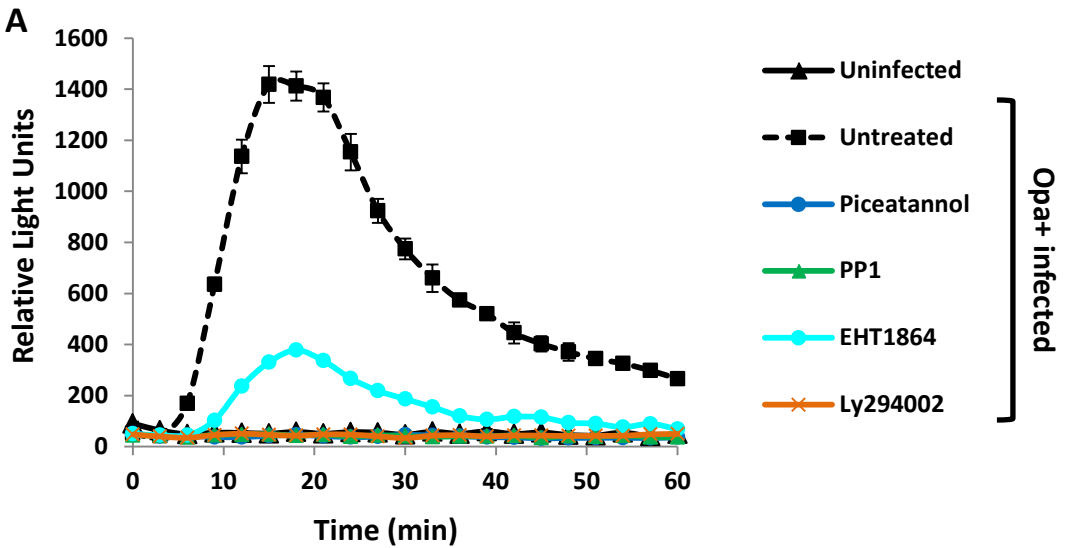
**A**



**B**



# Supplemental Figure 2



# Supplemental Figure 3

