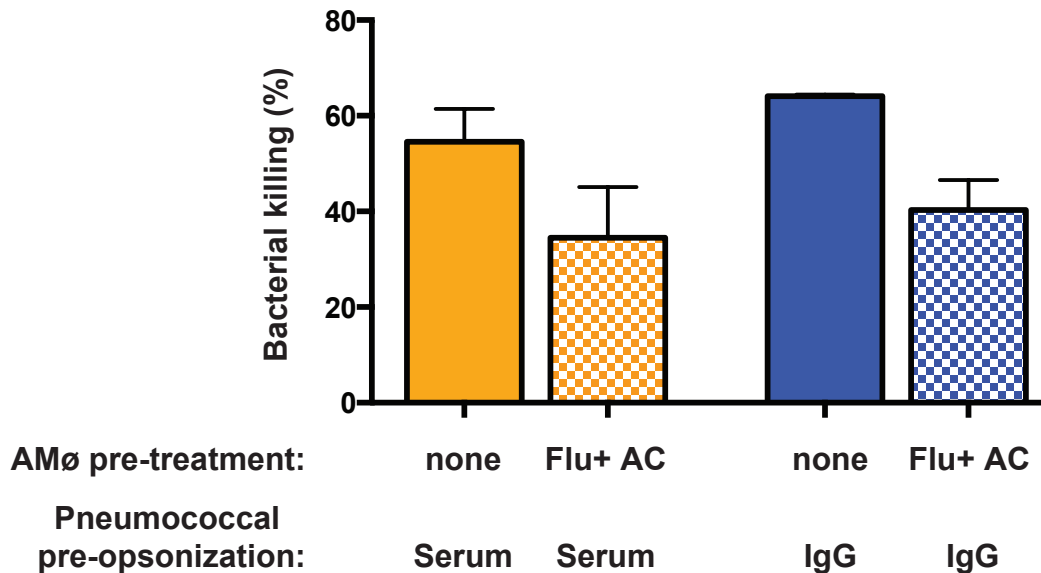


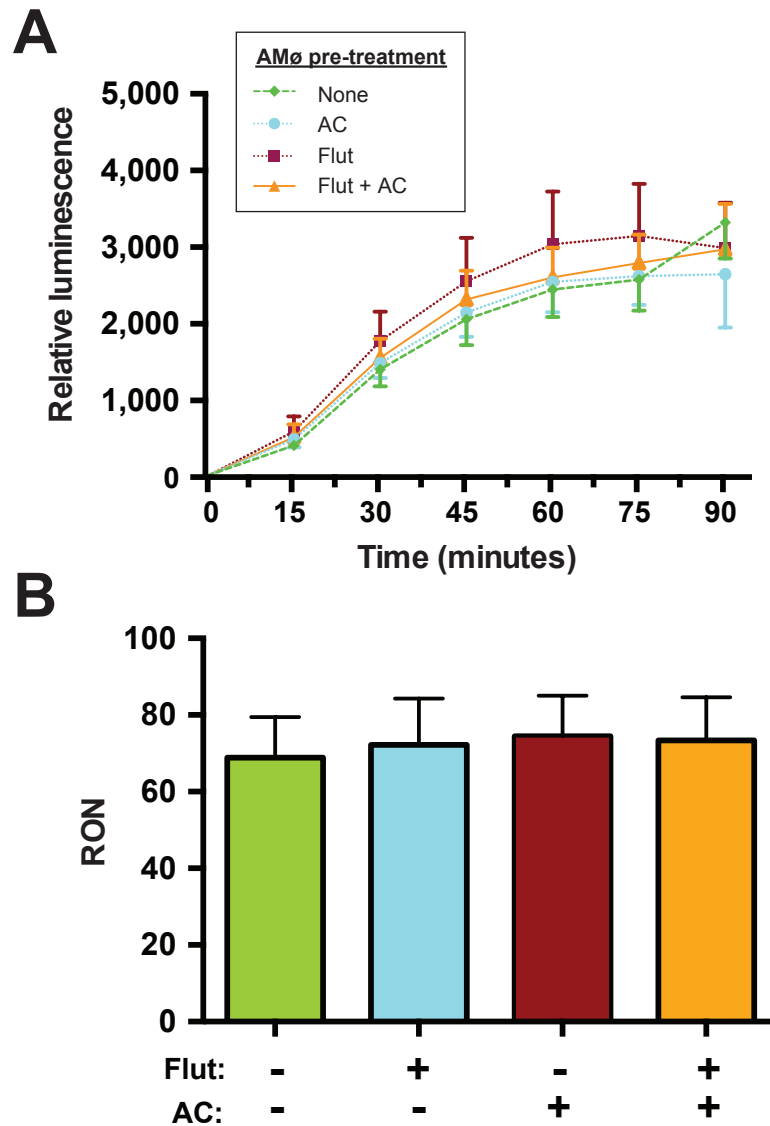
Supplemental Figure 1

Stolberg et al.



Supplemental Figure 1. The suppressive effect of GCAE on in vitro killing is seen whether pneumococci are opsonized using fresh rat serum or specific murine anti-IgG. Adherence-purified AMø from normal C57BL/6 mice were pre-treated with media alone (none) or 2 μ M fluticasone for 22 h followed by AC (at a ratio of 10:1 relative to AMø) for 2h (Flu + AC). Viable pneumococci strain 3 were opsonized by incubation for 1 h at 37°C with constant shaking, either with normal rat serum (10% in Hank's Balanced Salt Solution) that had not been heat-in-activated (as a source of complement and other innate opsonins) (serum), or with specific mouse anti-pneumococcal IgG at 20 μ g/ml (Abcam, Cambridge, MA) (IgG). Next, pneumococci were added (2×10^6 CFU/well), and bacterial killing was assayed as described in Material & Methods. Data are expressed as the percentage of bacterial killing. Results are mean \pm SEM of a single experiment using pooled AMø from four mice. There was no significant difference between serum-opsonization and Ig-opsonization by unpaired two-tailed t test.

Supplemental Figure 2 Stolberg et al.



Supplemental Figure 2. GCAE does not significantly impair in vitro murine AMØ production of reactive oxygen species (ROS) or reactive nitrogen species (RNS) in response to viable pneumococci. Adherence-purified AMØ from normal C57BL/6 mice were pre-treated with media alone (none), 2 µM fluticasone for 22 h (Flu), AC (at a ratio of 10 AC/AMØ) for 2 h (AC); or 2 µM fluticasone for 22 h followed by AC for 2h (Flu + AC) as described in the Supplemental Methods. Next, pneumococci were added (2×10^6 CFU/well). (A) ROS was assayed via fluorescent plate reader every 15 mins for a total of 90 mins and (B) RNS was assayed at 2 h. Data are expressed as the relative luminescence. Results are mean \pm SEM of two experiments for ROS assay and three experiments for RNS assay, each using pooled AMØ from four to five mice. There were no significant differences between the conditions.