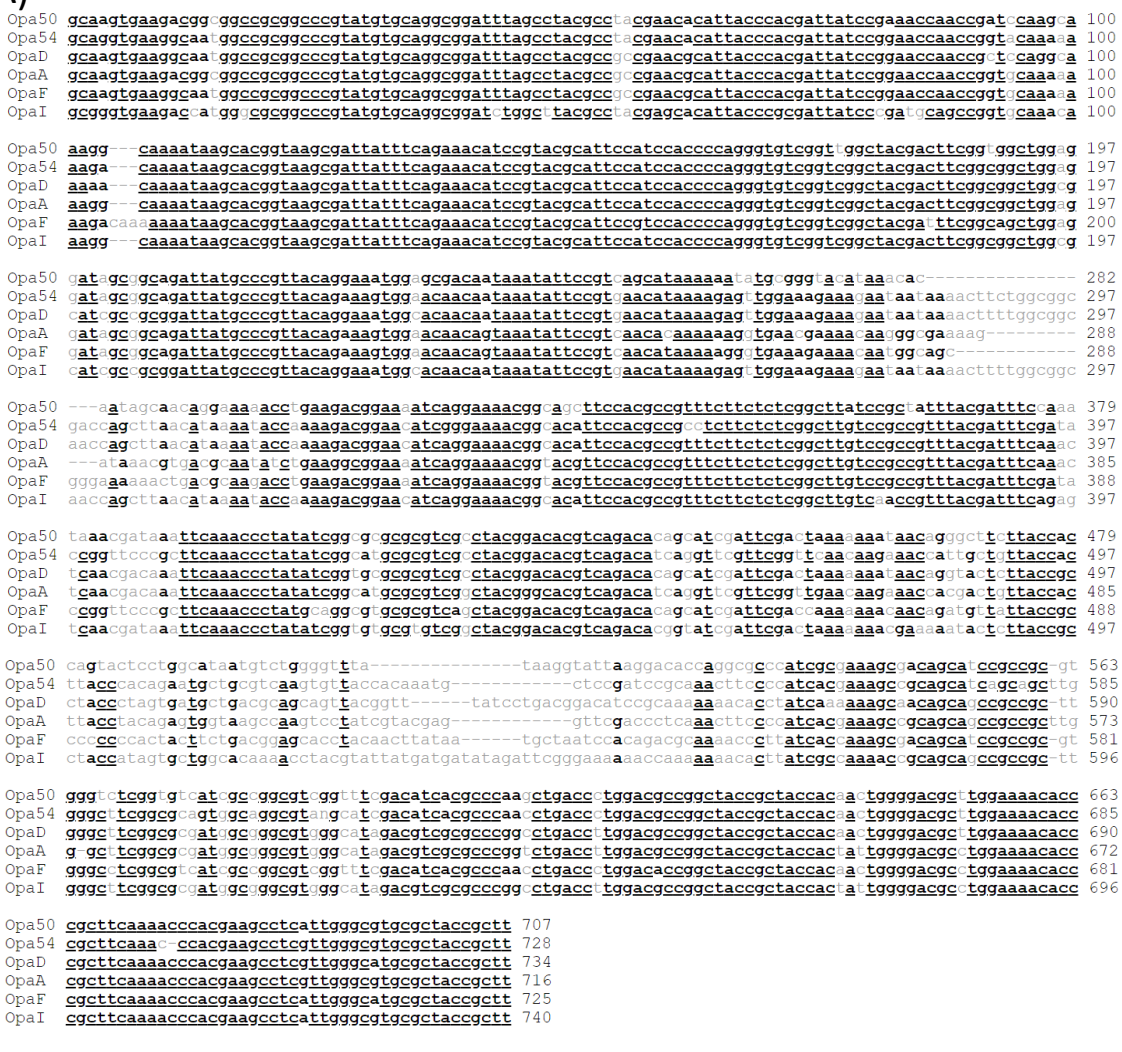
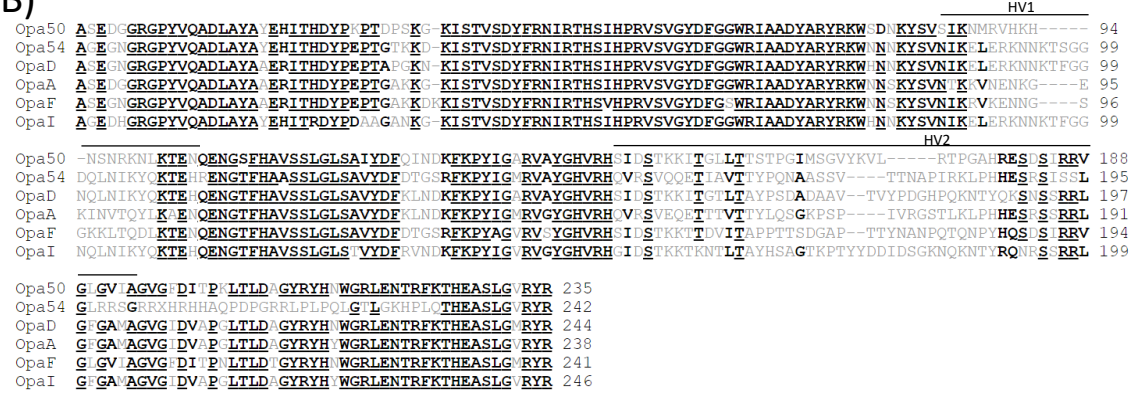
Supplemental Figure 1

A)



B)



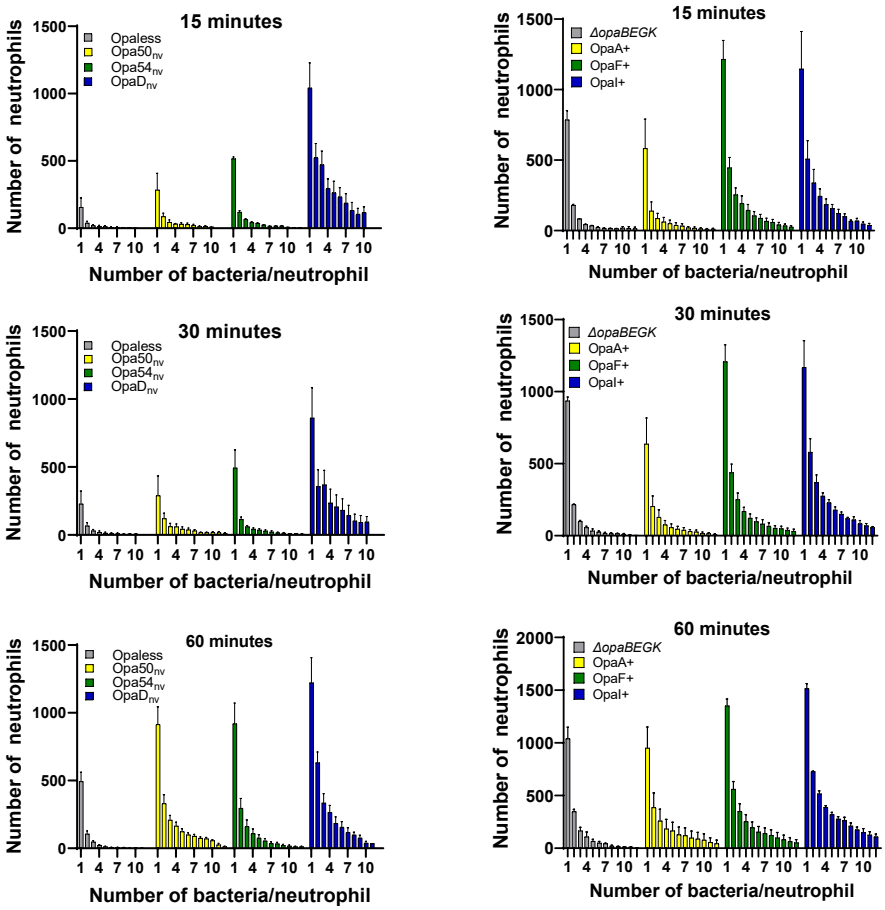
Supplemental Figure 1: Opa nucleotide and amino acid sequences used in this study.

A) The nucleotide sequence of each of the *opa* genes in this study, starting with the first codon after the phase-variable signal sequence. Black indicates 100% conservation of the nucleotide across the alignment, grey represents 70% similarity.

B) Amino acid sequence of each of the Opa proteins in this study, starting with the first amino acid of the mature protein (post signal sequence cleavage). Black indicates 100% conservation of the amino acid, grey represents 70% similarity. The hypervariable regions are marked with lines above the amino acids.

Opa50 and Opa54 are from strain MS11; OpaA, OpaD, OpaF, and OpaI from strain FA1090.

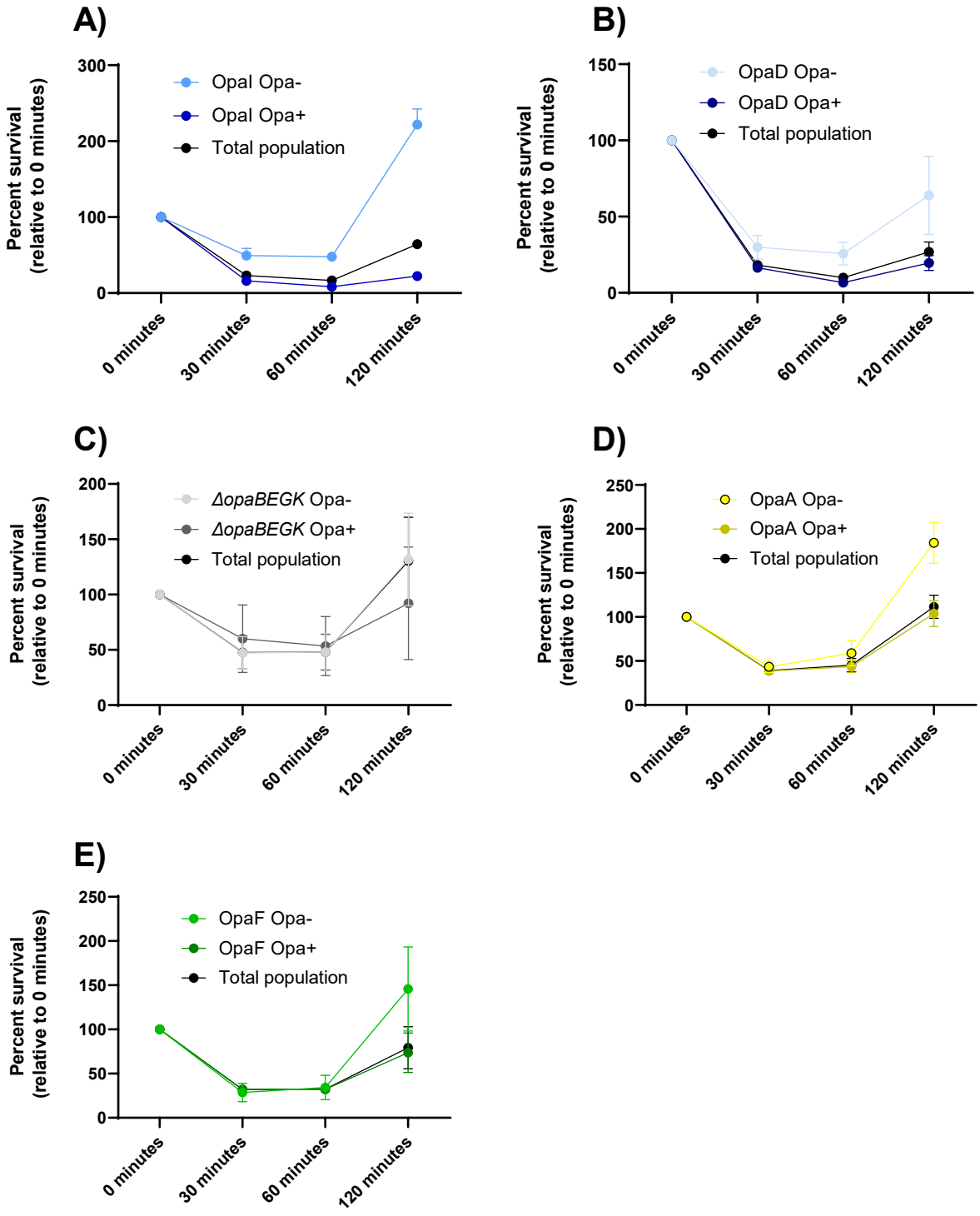
Supplemental Figure 2



Supplemental Figure 2: Histograms of Gc of different Opa expression states associating with neutrophils over time.

Adherent, IL-8 treated neutrophils were exposed to Tag-IT© Violet-labeled locked (A, C, E) or phase-variable (B, D, F) Gc of the indicated Opa profiles. Neutrophils were fixed at 15 (A-B), 30 (C-D), or 60 (E-F) min, stained for extracellular Gc with Dylight 650-labeled anti-Gc antibody, and processed for imaging flow cytometry as in Fig. 2. From the data generated with a spot count algorithm in Fig. 2, the number of neutrophils with the indicated number of cell-associated Gc was quantified. Neutrophils with zero Gc are not reported. Data are the average of n≥3 biological replicates. Colors are as in Figure 2.

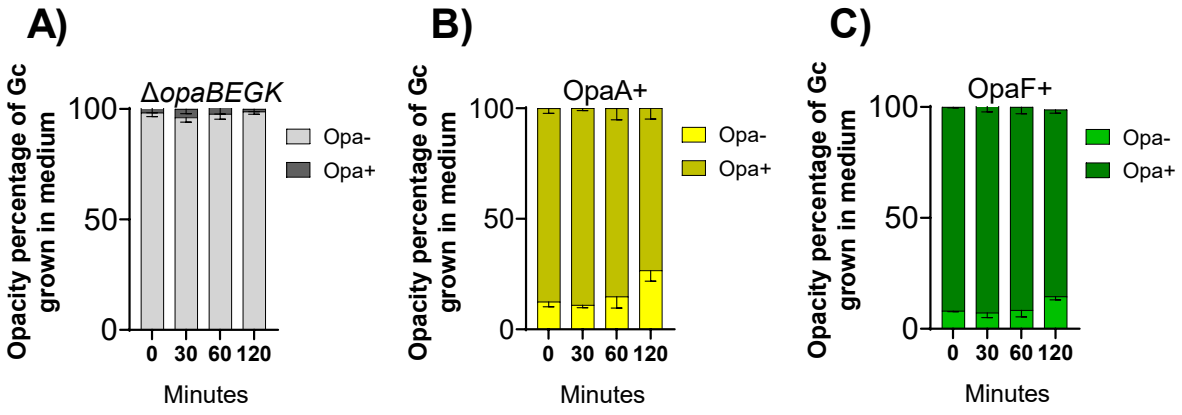
Supplemental Figure 3



Supplemental Figure 3: Survival of Opa phase ON and phase OFF Gc within a variable Opa-expressing population of Gc after neutrophil challenge.

The results from Fig. 5 for bacteria of the indicated starting Opa profile were plotted as the percent survival of the Opa+ and Opa- populations within each culture after exposure to primary human neutrophils. Percent survival is the CFU enumerated at the indicated time point, divided by the CFU enumerated at time = 0 min x 100%. The percent survival of all Gc in the population is also included on each graph.

Supplemental Figure 4



Supplemental Figure 4: Opa phase-variable Gc grown in medium without neutrophils maintains the starting Opa phenotype

The same cultures of Gc of the indicated Opa variable expression states used in Fig. 5 were inoculated into RPMI with 10% FBS. At the indicated time points, Gc CFU were enumerated based on opacity profile. There were no significant changes in Opa expression for Δ*opaBEGK* (A), OpaA+ (B), or OpaF+ (C) as determined by two-way ANOVA with post-hoc Tukey multiple comparisons test.