

# Supplemental Figure 1

## A)

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## B)

Opa50 A<sup>EDGGRGPVQADLAYA</sup>EHITHDYP<sup>PTDPSK</sup>G<sup>KISTVSDYFRNIRTHSIHPRVSVGYDFGGWRIAADYARYRKW</sup>ND<sup>KYSV</sup>IK<sup>NMRVHKH</sup>---- 94  
Opa54 A<sup>EGNGRGPVQADLAYA</sup>EHITHDYP<sup>PTGPK</sup>K<sup>KISTVSDYFRNIRTHSIHPRVSVGYDFGGWRIAADYARYRKW</sup>N<sup>KYSVNIK</sup>L<sup>ERKNNKTS</sup>GG 99  
OpaD A<sup>EGNGRGPVQADLAYA</sup>ERITHDYP<sup>PTA</sup>PK<sup>KISTVSDYFRNIRTHSIHPRVSVGYDFGGWRIAADYARYRKW</sup>N<sup>KYSVNIK</sup>L<sup>ERKNNKTF</sup>GG 99  
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OpaF A<sup>EGNGRGPVQADLAYA</sup>ERITHDYP<sup>PTGAK</sup>K<sup>KISTVSDYFRNIRTHSIHPRVSVGYDFG</sup>WRIAADYARYRKW<sup>N</sup><sup>KYSVNIK</sup>V<sup>KENNG</sup>----S 96  
OpaI A<sup>EDHGRGPVQADLAYA</sup>EHITRDYP<sup>DAGANK</sup>G<sup>KISTVSDYFRNIRTHSIHPRVSVGYDFGGWRIAADYARYRKW</sup>N<sup>KYSVNIK</sup>L<sup>ERKNNKTF</sup>GG 99

Opa50 -NSNRKLNKTE<sup>QENGTFHAVSSLGLSAIYDF</sup>Q<sup>LNDRKFKPYIG</sup>RVAYGHRH<sup>I</sup>STKKI<sup>TGLT</sup>FSTPGIMSGVYKVL<sup>---</sup>RTPGA<sup>RES</sup>S<sup>RRV</sup> 188  
Opa54 DQLNLIK<sup>QKTE</sup>ENGTF<sup>HAVSSLGLSAIYDF</sup>D<sup>TGSRFKFKPYIG</sup>RVAYGHRH<sup>V</sup>SVQQ<sup>TIAVT</sup>TPYQNA<sup>ASSV</sup>---TTNAP<sup>IRKLP</sup>HES<sup>IS</sup>SS 195  
OpaD NQLNLIK<sup>QKTE</sup>ENGTF<sup>HAVSSLGLSAIYDF</sup>KL<sup>NDRFKFKPYIG</sup>RVAYGHRH<sup>I</sup>STKKI<sup>TGLT</sup>AYPSDA<sup>DAV</sup>--TVYPD<sup>GHPQRNTYQ</sup>SN<sup>S</sup>RR 197  
OpaA KINVT<sup>QYIK</sup>E<sup>QENGTFHAVSSLGLSAIYDF</sup>KL<sup>NDRFKFKPYIG</sup>RVYGHV<sup>RV</sup>SV<sup>EQ</sup>T<sup>TVT</sup>FYLSG<sup>KPSP</sup>---IVRGS<sup>TLKL</sup>PHES<sup>S</sup>RR 191  
OpaF KKKL<sup>QDLKTE</sup>ENGTF<sup>HAVSSLGLSAIYDF</sup>D<sup>TGSRFKFKPYIG</sup>RVYGHV<sup>RV</sup>SV<sup>EQ</sup>T<sup>DVI</sup>APPTSD<sup>GAP</sup>--TTYNAP<sup>QTNP</sup>HQS<sup>S</sup>RR 194  
OpaI NQLNLIK<sup>QKTE</sup>ENGTF<sup>HAVSSLGLSAIYDF</sup>RV<sup>NDRFKFKPYIG</sup>RVYGHV<sup>RV</sup>SV<sup>EQ</sup>T<sup>DVI</sup>AYHSAG<sup>KPYTYDD</sup>IDS<sup>GKQKNTY</sup>RQ<sup>NS</sup>RR 199

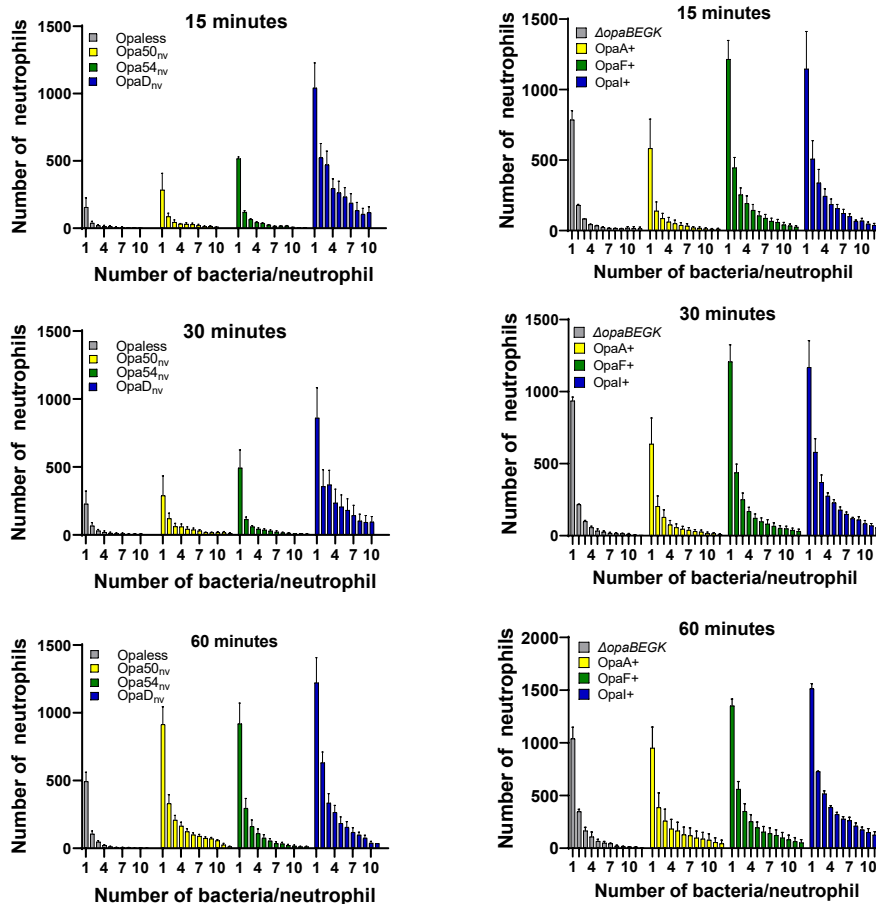
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OpaF G<sup>GV</sup>AGVG<sup>DI</sup>P<sup>L</sup>TLTD<sup>GYRYH</sup>WGRLENTREKTHEASLG<sup>RYR</sup> 241  
OpaI G<sup>GA</sup>AGVG<sup>DV</sup>P<sup>L</sup>TLTD<sup>GYRYH</sup>WGRLENTREKTHEASLG<sup>RYR</sup> 246

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.

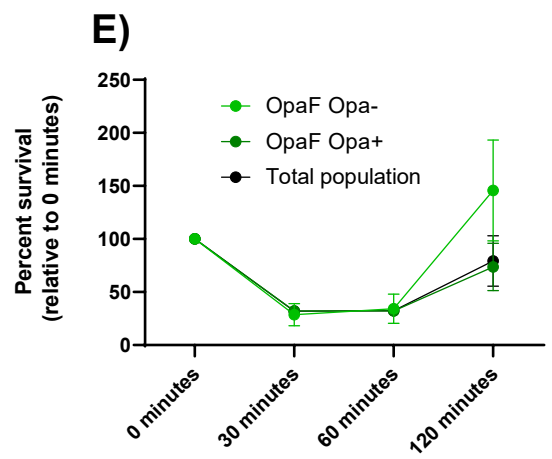
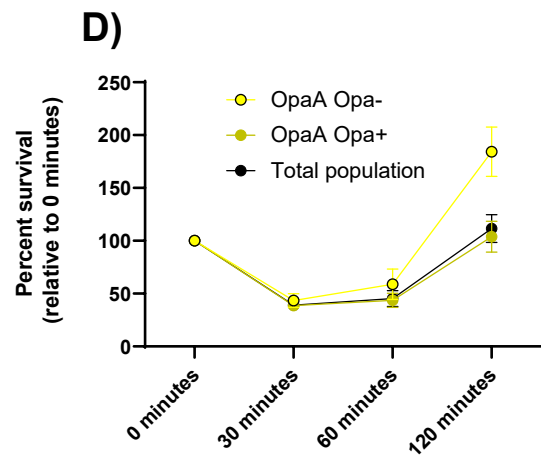
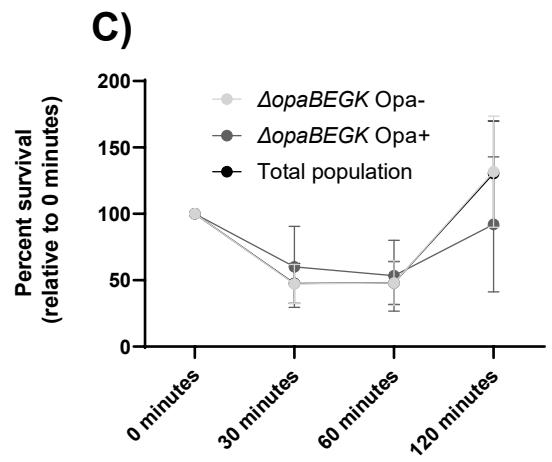
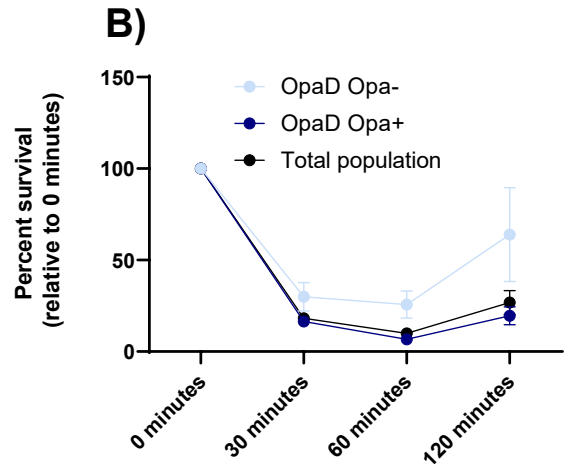
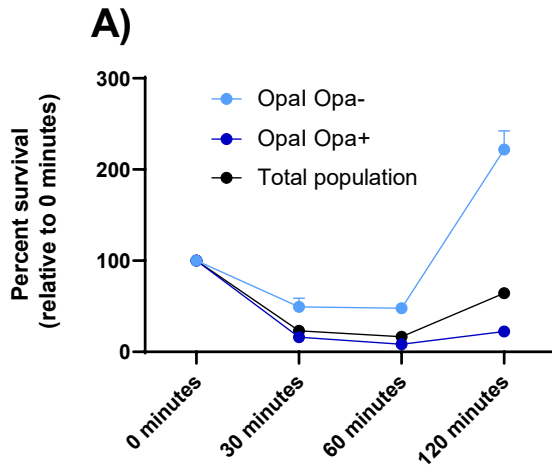
## Supplemental Figure 2



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<sup>®</sup> 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  $n \geq 3$  biological replicates. Colors are as in Figure 2.

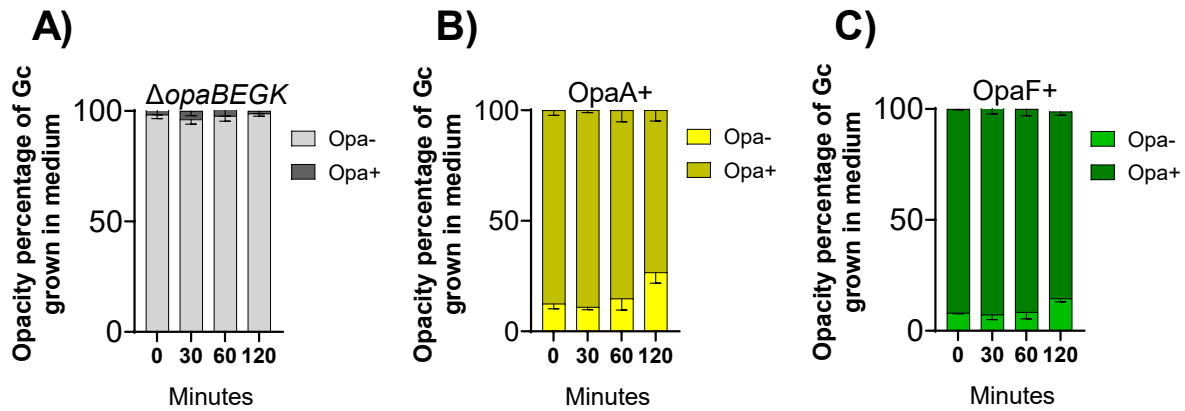
Supplemental Figure 3



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  $\Delta opaBEGK$  (A), OpaA+ (B), or OpaF+ (C) as determined by two-way ANOVA with post-hoc Tukey multiple comparisons test.