A)

Opa50 gcaagtgaagacgg_ggccggggccgtatgtgcaggcggatttagcctacgcclacgaacacattacccacgattatccgaaaccgatcaccgat_ OpaD OpaA $\underline{gcaagtgaagacgg} \\ \underline{gcgcggcccgtatgtgcaggcggatttagcctacgccg} \\ \underline{cgaacgcattacccacgattatccggaaccaaccggtgcaa} \\ 100$ OpaF OpaI gcgggtgaagac atggjcgcggccgtatgtgcaggcggat tggc tacgcc tacgagcacattacccgcgattatcc at cagecggt caaa a 100 Opa50 aagg Opa54 <u>aag</u>a OpaD <u>aa</u>aa OpaA aaqq OpaF <u>aag</u>a OpaI Opa54 gatageggcagattatgecegttacagaaagtggaacaacaataatatteegtgaacataaaagagttggaaagaagaataataaaacttetggegge 297 OpaD c<u>atogcoggattatgcccgttacaggaaatggoacaacaataatattccgtoaacataaaagag</u>t<u>tgga</u>aag<u>aaagaa</u>laataaaatttttggcggc 297 OpaA g<u>atage</u>ggcagattatgcccgttacagaaagtgga<u>acaacagtaaatattecgt</u>a<u>aca</u>aaaaaggtgaa_gaaaacaagggggaaaag----- 288 OpaF gatagcqcagattatqcccqttacaqaaaqtqgaacaacaqtaaatattccqtcaacataaaaaqqqtgaaaqaaacaatqqcaqc---<u>at gc gcgggttatgcccgttacaggaatgg acaacaataatattccgt aacataaaagag tggaaagaaagaa</u>taataaacttttggcggc 297 OpaI -a<u>a</u>tagc**a**aga<u>aaaacc</u>tg<u>aagacggaa</u>a<u>atcaggaaaacggca</u>gc<u>ttccacgccgtttcttctctcggcttatccgc</u>ta<u>tttacgatttc</u>aaa 379 Opa50 Opa54 gaccagettaacataaaataccaaaagacggaacategggaaaacggcacattecacgecgctetteteteggettgteegeegtttaceatttegata 397 OpaD aaccagettaacataaaataccaaaagaeggaacateggaaaaeggcacattecaegeegtttettetteteteggettgteegeegtttaegattteaa</u>ac 397 OpaA ---ataaacgtg<u>a</u>cgc<u>aa</u>tat<u>c</u>tg<u>aaggcggaa</u>a<u>atcaggaaaacggtacgttccacgccgtttcttctctcggettgtccgccgtttacgatttc</u>a<u>a</u>ac 385 OpaF ggga**a**aaactg**a**cgc<mark>aagacc</mark>t<mark>gaagacggaaaatcagggaaaacggtacgttccacgccgtttcttcttctccggcttgtccgccgtttacgatttcga</mark>ta 388 aacc<u>ag</u>ettaac<u>a</u>taa<u>aatacc</u>aa<u>aagacggaacatcagggaaaacggcacattecaegecgtttetteteteeggettgte</u>aa<u>ccgtttacgattte</u>agag 397 OpaI Opa50 taaacgataaattcaaaccctatatcggcgcgcgcgccgcccacggcaccgtcagacacagcatcgattcgactaaaaaaataacagggcttcttaccac 479 Opa54 c<u>cggttcccg_ttcaaacectatategg_atgcgcgtcg_ctacggacacgtcagaca</u>t_aggttc<u>gttcggtt_aacaagaaac</u>attg<u>tgtttcccac</u> 497 OpaD t<u>c</u>aacgacaaa<u>ttcaaaccctatatcgg</u>tg<u>gcgcgtcgcctacggacacgtcagaca</u>cagca<u>t</u>cgattcgactaaaaaaat<u>aac</u>aggtac<u>tcttaccgc</u> 497 t<u>c</u>aacgacaaa<u>ttcaaaccctatatcgg</u>cat<u>gcgcgtcggctcggctacgggcacgtcagaca</u>tcag<u>gt</u>tcg<u>gttcggttgaacaagaaac</u>acgac<u>tgttaccac</u> 485 OpaA OpaF ccggttccogcttcaaaccctatgcaggcgtcgcgtcagctacggcaccgtcagacacagcatcgattcgaccaaaaaaacaacagatgttattaccgc 488 Opal t<u>c</u>aacgataaa<u>ttcaaaccetatategg</u>tgt<u>gegtgteggetaeggacaegteagaca</u>eggta<u>t</u>cga<u>ttega</u><u>taa</u>aa<u>aa</u>aaa<u>a</u>aaaa<u>ta</u><u>tettaeege</u> 497 Opa50 cagtacteetggcataatgtetggggt<u>t</u>ta-----taaggtattaaggacaec<u>aggege</u>ec<u>ategegaaage</u>ga<u>cageategege</u>gt 563 Opa54 tta<u>ee</u>caeagaa<u>tg</u>etgegtegagtgt<u>t</u>aecaeaatg------teegateegea<u>a</u>ette<u>e</u>cea<u>teaegaaage</u>g<u>eage</u>tg 585 OpaD eta<u>ee</u>ctagtga<u>tg</u>etgaegeageagt<u>t</u>aeggtt-----tateetgaeggaeateegeaaaaeeet<u>ate</u>aaa<u>aaage</u>a<u>eageageege</u>tt 590 tta<u>cc</u>tacagag<u>t</u>ggtaagcc<u>a</u>agtoc<u>t</u>acgtacgag------gttcgaccotca<u>aa</u>cttc<u>c</u>cc<u>atcacgaaagccgcagccgccgc</u>ttg 573 cccccccactacttctgacguagcacctacaacttataa-----tgctaatccacagacgcaaaacccttactcaccaaagcuacagcaiccgccgc_dt 581 OpaA OpaF cta<u>cc</u>atagtgc<u>t</u>gccacaaa<u>a</u>cctacgtattatgatgatatagattcgggaaaaaaccaaa<u>a</u>aaca<u>c</u>tt<u>atcgc</u>a<u>aaac</u>g<u>cagccgccgc</u>+t 596 OpaI Opa50 gggtctcggtgtcatcgccgcgcgtcggtttcgacatcacgcccaaactgaccctggacgccggctaccgctaccacaactggggacgcttggaaaacacc 663 Opa54 gggcttcggcgcagtggcaggcgtangcatcgcccaactgaccctgaccctggcccggctaccgctaccacaatggggacgcttggaaaacacc 685 OpaD ggggtltcggcgsqatugguggcgtuggsatuggcgccggsccggsccggsccggcccggcccggctaccgctaccasastgggggacgstuggggacgstuggggacgstuggggstuggsatuggggsgtuggsatuggggsgtuggsatuggggsgtuggsatuggsgcgsggtotgsaccsssacs 672 OpaA <u>gggc=tcggcgt=at=gc=ggcgt=ggt=t_cgacatcacgcccaa=ctgacc=tggacaccggctaccgctaccac=a_tggggacgc=tggaaaacacc</u> 681 OpaF OpaI gggetteggegegatggegtgggeatggegetggeetggeetggeetggeetggeetggeetggetacegetacegetaceatattgggggaegeetggaaaacace 696 $\texttt{Opa50} \quad \underline{\texttt{cgcttcaaaacccacgaagcctc}} \texttt{attgggcgtgcgctaccgctt}$ Opa54 cgcttcaaac-ccacgaagcctcgttgggcgtgcgctaccgctt 728 OpaD cgcttcaaaacccacgaagcctcgttgggcatgcgctaccgctt 734 OpaA cgcttcaaaacccacgaagcctcgttgggcgtgcgctaccgctt 716 OpaF cgcttcaaaacccacgaagcctcattgggcatgcgctaccgctt OpaI cgcttcaaaacccacgaagcctcattgggcgtgcgctaccgctt 740

| ח) | | | |
|---------------|--|---|-----|
| B) | | HV1 | |
| Opa50 |) <u>ASEDGGRGPYVQADLAYAYEHITHDYPKPTDPSK</u> G- <u>KISTVSDYFRNIRTHSIHPRVSVGYDFGGWRIAADYARYRKW</u> SDN <u>KYSV</u> | S IK NMRVHKH 9 | 94 |
| Opa54 | 4 AGEGN <u>GRGPYVQADLAYAYEHITHDYPEPTGTKKD-KISTVSDYFRNIRTHSIHPRVSVGYDFGGWRIAADYARYRKW</u> N <u>NKYSV</u> | NIKELERKNNKTSGG | 99 |
| OpaD | <u>ASEGNGRGPYVQADLAYAAERITHDYPEPT</u> APG <u>K</u> N- <u>KISTVSDYFRNIRTHSIHPRVSVGYDFGGWRIAADYARYRKWHNNKYSV</u> | NIKELERKNNKTFGG | 99 |
| OpaA | <u>ASEDGGRGPYVQADLAYAAERITHDYPEPTGAKKG-KISTVSDYFRNIRTHSIHPRVSVGYDFGGWRIAADYARYRKWNSKYSV</u> | <u>n</u> t <u>k</u> kvnenkge 9 | 95 |
| OpaF | <u>ASEGNGRGPYVQADLAYAAERITHDYPEPTGAKK</u> DK <u>KISTVSDYFRNIRTHS</u> V <u>HPRVSVGYDFGSWRIAADYARYRKWNSKYSV</u> | <u>NIK</u> RVKENNGS 9 | 96 |
| OpaI | AGEDH <u>GRGPYVQADLAYAYEHITRDYP</u> DAAGAN <u>K</u> G- <u>KISTVSDYFRNIRTHSIHPRVSVGYDFGGWRIAADYARYRKWHNNKYSV</u> | <u>NIK</u> ELERKNNKTFGG 9 | 99 |
| | HH | √2 | |
| Opa50 |) -NSNRKNL <u>KTE</u> NQ <u>ENG</u> SFHAVSSLGLSAIYDFQINDKFKPYIGA RVAYGHVRH SIDSTKKI <u>T</u> GLLTTSTPGIMSGVYKVL | RTPGAHRESDSIRRV 1 | 188 |
| Opa54 | ŧ dolnikyo kte hr engtfhaasslglsavydf dtgs r<u>fkpyig</u>mrvayghvrhovrsvooe<u>t</u>iav<u>t</u>typonaassvttnap | IRKLPH HE<u>s</u>r<u>s</u>issl 1 | 195 |
| OpaD | NQLNIKYQ KTE HQ ENGTFHAVSSLGLSAVYDF KLNDK <u>FKPYIG</u> A RVAYGHVRH SIDSTKKI <u>T</u> GTL T AYPSDADAAVTVYPDGH | PQKNTYQK <u>S</u> N <u>S</u> S <u>RR</u> L 1 | 197 |
| OpaA | RINVTQYLKAENQENGTFHAVSSLGLSAVYDFKLNDKEKPYIGMEVGYGHVRHQVRSVEQETTVVTPLQSGRPSPIVRGS | TLKLPH HE<u>s</u>r<u>s</u>s<u>rrl</u> 1 | 191 |
| Opar | GRKLTQDLKTENQENGTFHAVSSLGLSAVYDFDTGSREKPYAGVRVSYGHVRHSIDSTKKTTDVITAPPTTSDGAPTTYNANP | QTQNPYHQSDSIRRV 1 | 194 |
| Opal | nqlnikyq ktehqengtfhavsslgls t yydf rvnuk <u>fkpyig</u> v gyghvrh giu <u>s</u> tkktkntl t ayhsagtkptyyddidsgr | NŐKNI.J KÖ NK Z Z KK T 1 | 199 |
| OreEO | | | |
| Opasu | GLOVIGCORVINIAODOCINIA DOLOTICI CIUDICULA (UNDI | | |
| Opa04 OpaD | GEARSGRAAMMARGPDFORMEDEUGILGANELQIIIGANELQIIIIIGANELQIIIIIGANELQIIIIGANELQIIIIGANELQIIIIGANELQIIIIGANELQIIIIIGANELQIIIIIIIGANELQIIIIIGANELQIIIIIIIIIGANELQIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII | | |
| OpaD | GEGANAGVGIDVADGITIDAGVDVUVWGDLENIKERIABAGUGVUZ 244 | | |
| OpaR | GE GRUNDYGE 12 MECHINDRESTATI I TOAMBATKENTINGADU VIIK 230 | | |
| Opar | GECKAGVGEDIIF WITHD GERTHINGGEDENTERTENTERTENTER Z-11 | | |
| opar | Gronowy to a for the other than the state of | | |

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: 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 $n\geq 3$ biological replicates. Colors are as in Figure 2.



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: 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.