Opa ₆₀ OpaD Opa ₅₀	1 MGSSHHHHHH MGSSHHHHHH MGSSHHHHHH	SSGLVPRGSH SSGLVPRGSH SSGLVPRGSH	MASEDGGRGP MASEGNGRGP MASEDGGRGP	YVQADLAYAY YVQADLAYAA YVQADLAYAY	EHITHDYPEP ERITHDYPEP EHITHDYPKP	TAPNKNKIST TAPGKNKIST TDPSKGKIST
Opa ₆₀ OpaD Opa ₅₀	61 VSDYFRNIRT VSDYFRNIRT VSDYFRNIRT	RSVHPRVSVG HSIHPRVSVG HSIHPRVSVG	YDFGGWRIAA YDFGGWRIAA YDFGGWRIAA	DYARYRKWNN DYARYRKWHN DYARYRKWSD	NKYSVNIENV NKYSVNIKEL NKYSVSIKNM	RIRKENGIRI ERKNNKTFGG RVHKHNSNRK
Opa ₆₀ OpaD Opa ₅₀	121 DRKTENQ NQLNIKYQKT NLKTENQ	ENGTFHA EHQENGTFHA ENGSFHA	VSSLGLSAIY VSSLGLSAVY VSSLGLSAIY	DFQINDKFKP DFKLNDKFKP DFQINDKFKP	YIGARVAYGH YIGARVAYGH YIGARVAYGH	VRHSIDSTKK VRHSIDSTKK VRHSIDSTKK
Opa ₆₀ OpaD Opa ₅₀	181 TIEVTTVPSN ITGTLTAYPS ITGLLTTSTP	APNGAVTTYN DADAAVTVYP GIMSGVYKVL	TDPKTQNDYQ DGHPQKNTYQ RTPGAHRE	-SNSIRRVGL KSNSSRRLGF -SDSIRRVGL	GVIAGVGFDI GAMAGVGIDV GVIAGVGFDI	TPKLTLDAGY APGLTLDAGY TPKLTLDAGY
Opa ₆₀ OpaD Opa ₅₀	241 RYHNWGRLEN RYHNWGRLEN	TRFKTHEASL TRFKTHEASL TRFKTHEASL	GVRYRF GMRYRF GVRYRF			

Figure S1. Sequence alignment of Opa_{60} from Gc strain MS11, OpaD from Gc strain FA1090, and Opa_{50} from Gc strain MS11. Semivariable regions are highlighted in yellow and hypervariable regions are highlighted in red. Black arrows indicate β -strands which form the membrane spanning barrel.

	40	50	60			70			80		90	
CEACAM1	QLTTESMPFNVAE	GKEVLLI	VHNLPQQL	FGY S	SWYK	GER	DGI	1RQ	IVGY	AIGT-	QQATPG	Ρ
CEACAM5	KLTIEST.FNV	E	VH.LPQHL	FG.S	5.Y.	.ER	7DGI	JR <mark>Q</mark>	.I	VIGT-	.QAT	Ρ
CEACAM3	KLTIESM.LSV	E	VH.LPQHL	FG.S	5.Y.	.ER	7DGI	IS <mark>L</mark>	.v	VIGT-	.QAT	А
CEACAM6	KLTIEST.FNV	E	AH.LPQNR	IG.S	5.Y.	.ER	7DGI	1SL	.v	VIGT-	.QAT	Ρ
CEACAM8	QLTIEAV.SNA	E	VH.LPQDP	RG.N	J.Y.	.ET	7DAN	JR <mark>R</mark>	.I	VISN-	.QIT	Р
CEACAM7	QTNIDVV.FNV	E	VH.ESQNL	YG.N	Ι.Υ.	.ER	/HAN	JY <mark>R</mark>	.I	VKNIS	.ENA	Ρ
CEACAM4	QFTIEAL.SSA	D	AC.ISETI	QA.Y	<mark>с.н</mark> .	.KT	AEG S	SPL	.A	ITDI-	.ANI	Α
	100	110	120			130			140			
CEACAM1	ANSGRETIYPNAS	SLLIQNVI	QNDTGFYT	LQV	KSD	LVNE	EAT	ſGQ	FHVY	Р		
CEACAM5	.YSGII.P.AS	IQ.II	QN.T.F	.HV.	.KSD	LVNE	CEA.	.G.	FR.Y	Р		
CEACAM3	.YSGTI.T.AS	SIQ.VI	QN.I.F	.QV.	/ <mark>.</mark> KSDLVNEEA.G.FH.YQ							
CEACAM6	.YSGTI.P.AS	SIQ.VI	CQN.T.F	.QV.	/ <mark>.</mark> KSDLVNEEA.G.FH.YP							
CEACAM8	.YSNTI.P.AS	SMR.VI	RN.T.S	.QV.	V <mark>.</mark> KLNLMSEEV.G.FS.HP							
CEACAM7	.HNGTI.P.GT	IQ.VI	HN.A.F	.HV.	V.KENLVNEEV.R.FY.FS							
CEACAM4	.YSGTV.P.GS	5FQ.I7	CLE.A.S	.RT.	[.NASYDSDQA.G.LH.HQ							

Figure S2. Sequence alignment of human NCCMs 1, 3, 4, 5, 6, 7, and 8. Essential residues for binding all Opa proteins are highlighted with red boxes and residues that are important to some Opa protein interactions with orange boxes (Figure 1A and 5). Residue numbering corresponds to CEACAM1 sequence, UniProt ID P13688-1.



Figure S3. SEC chromatogram of molecular weight standards (top) and NCCM1 (bottom). Following GST tag cleavage, the NCCM1 was purified using SEC. Species were present with molecular weights corresponding to ~25 kDa (approximate MW of GST, TEV, and NCCM dimer), and ~12 kDa (approximate MW of NCCM monomer). A second passage through the SEC column was conducted immediately following (bottom), and fractions containing the monomeric NCCM (MW ~ 12 kDa) as assessed by SDS-PAGE were pooled and concentrated (orange box). Absorbance is at 280 nm.



Figure S4. Ribbon representation of NCCM1 H139. The cysteine mutant (H139C), is located on the opposite side of the Opa-binding face. Opa-binding residues are colored as in Figure S2, H139 is colored green.





Figure S5. MALDI spectra of fluoresently labled (Fl-) NCCM1 (A) and NCCM3 (B). These spectra indicate that there is >95 % labeling efficiency of the AMS fluorophore (MW = 536.4 Da) with both NCCM1 and NCCM3.



Figure S6. SDS-PAGE of Opa proteins. Folded Opa proteins (lanes labeled lipid) migrate at an apparent lower molecular weight than unfolded Opa proteins (8 M Urea) allowing folding to be assessed using SDS-PAGE. Fully folded samples were used for all experiments in this manuscript.



Figure S7. SDS-PAGE of trypsin cleavage of Opa_{60} in liposomes. Lane 1 contains folded Opa_{60} protein in liposomes, before the addition of trypsin (Opa_{60}). Lane 2 is the sample after the addition of trypsin and incubation (~ 4 h), before the trypsin was removed (Opa_{Trp} Pre). Lane 3 contains the flow through after passing over the benzamidine column (Opa_{Trp} Post) and lane 4 contains the molecular weight marker (MWM).



Figure S8. Immunoblot of pull-down assay using Opa expressing Gc and NCCM1that has been cleaved and purified from the GST tag. Bacteria expressing OpaD, Opa₅₀, or Opa₆₀, or Opa⁻Gc, were incubated with NCCM1 (3 mg/mL), and samples were centrifuged for pellet (P) and supernatant (S) immunoblot assessment. Opa₆₀ and OpaD Gc interacted with cleaved NCCM1 similarly to GST-NCCM1 (compare to Figure 2) indicating the GST tag does not interfere with NCCM binding to Opa proteins. There is less GST-NCCM1 (Figure 2) present in the supernantant samples compared to the cleaved NCCM1, which indicates there may be some aggregation of the cleaved NCCM1. Folded Opa proteins migrate at a lower apparent molecular weight than unfolded Opa proteins. As such, the two bands correspond to folded (lower band) and unfolded (upper band) protein in the Opa immunoblots. *Higher molecular weight bands in the Gc pellet samples of the CEACAM blots indicate nonspecific CEACAM antibody reactivity with antigens on the surface of Gc



Figure S9. Competition FP assays with Opa_{60} . FP experiments were carried out as previously described with Opa_{60} proteoliposomes, incorporating half fl-labeled and half unlabeled NCCM1 (A) or NCCM3 (B). The total [NCCM] remained 5 nM. In both cases, the K_d values approximately doubled, indicating that the interaction is specific.