Supplemental Table S1: Primers used in this study

Primers	**5'-3' Sequence**
lgtG_F	CTATCTGTACGACGTTTTGAAAATTGC
lgtG_R	CCCCGTATTTAAAGGATAAAGGCAAAA
lgtG530	CGCATTACCCTACCCCCTCACGCAC
lgtG1729	TCTGTACGACGTTTTGAAAATTGC
LaI-6	GCGACAGGGACGGGTTGTAGTTCAG
LaI-7	GGCACGGAACGCTACACATTGGAT
lgtA Fwd-Ext	AGGCAATTTCCAAACTGCTTTGTCCGA
lgtA Rev-Ext	GTTTGGCGGTATTCTAGGCTGTCGAAC
lgtC Fwd-Ext	TATTTGAGCGGAGTGGAAAAAGCCTGC
lgtC Rev-Ext	ATCCAAGTTGCGCCAAGTCTGATTCAC
lgtD Fwd-Ext	TCAAAATGTCCTGCGAATGGGTGGAAC
lgtD Rev-Ext	AGCCAAGCTGATAACGTGGTTTTGCAT
lgtA-On Fwd[A]	ATTGGCAAAGTCGGG**C**GG**A**GG**T**GGATATATTGCGCGC
lgtA-On Rev[A]	GCGCGCAATATATCC**A**CC**T**CC**G**CCCGACTTTGCCAAT
lgtC-On Fwd[A]	CGCCAATTTGCGGGG**C**GG**A**G*GT*AATATCCGCTTT
lgtC-On Rev[A]	AAAGCGGATATT*AC*C**T**CC**G**CCCCGCAAATTGGCG
lgtC-Off Fwd[A]	CCGCCAATTTGCGG**T**G**A**GGGGGGGGGTAATA
lgtC-Off Rev[A]	TATTACCCCCCCCC**T**C**A**CCGCAAATTGGCGG
lgtD-On Fwd[A]	GAATTGGCAAAGTCGGG**C**GG**A**GG**T***GA*ATATATTGCGCGCAC
lgtD-On Rev[A]	GTGCGCGCAATATAT*TC***A**CC**T**CC**G**CCCGACTTTGCCAATTC
lgtA Fwd-Int[B]	*AAAAGCGGGACAGCCGTATCAAAAT*CCCGGG**ATTGAGAAAATCGTGGGCGAGATGG**
lgtA Rev-Int[B]	*CCATCTCGCCCACGATTTTCTCAAT*CCCGGG**ATTTTGATACGGCTGTCCCGCTTTT**
lgtC Fwd-Int[B]	*GAGATGGACATCGTATTTGCGGCAGAC*CCCGGG**CCTTATGGGATACCGATTTGGGCGGTA**
lgtC Rev-Int[B]	*TACCGCCCAAATCGGTATCCCATAAGG*CCCGGG**GTCTGCCGCAAATACGATGTCCATCTC**
lgtD Fwd-Int[B]	*GTGAATCAGACTTGGCGCAACTTGGAT*CCCGGG**CATGAAGACATTGTCGCCGTTTTCCCT**
lgtD Rev-Int[B]	*AGGGAAAACGGCGACAATGTCTTCATG*CCCGGG**ATCCAAGTTGCGCCAAGTCTGATTCAC**

[A] Indicates primers for mutagenesis; Single base substitutions are **underlined in bold**, consecutive bases *underlined in italics* span short deletions that render the sequence in frame for full length protein translation

[B] SmaI site – CCCGGG; Primer regions corresponding to the indicated *lgt* are indicated in **bold**, overlap regions are indicated in *italics*

Supplementary Table S2: Plasmids used in this study

Plasmids	Relevant characteristics and construction details	Ref.
pFLOB4270[2]	Two-step mutagenesis system used to construct unmarked deletions or mutations. Contains an Erm^R-Sm^S cassette that encodes resistance to Erm and sensitivity to Sm (*rpsL*$_{F62}$) Used to construct unmarked mutations in *lgtG, C, A and D*	(1)
lgtG constructs		
pRYGW2	Contains wildtype *lgtG* from Ng strain 398079 amplified with primers lgtG530 and lgtG1729 in pCR2.1-TOPO (Invitrogen, Carlsbad, CA). Analogous to pRYGW1, described previously (2) Used to create pRYGW2ES1	This study
pRYGW2ES1	pRYGW2 with the Erm^R-Sm^S cassette that encodes resistance to Erm (*ErmC*) and sensitivity to Sm (*rpsL*$_{F62}$) from pFLOB4300[2]. The Erm^R-Sm^S cassette was liberated with *PvuII* and cloned into the *StuI* site of pRYGW2. Used to construct MS11 4/3/1 lgtG locked ON	This study
plgtG+	Contains lgtG locked ON (C_{11} →CCCCTCCGCCA); *lgtG*+ was amplified from Ng F62 dlgtA lgtG+ (3) with primers lal6 and lal7 and cloned into in to pCR2.1 TOPO (Invitrogen). Used to create lgtG locked ON	This study
lgtA constructs		
plgtA	Contains wildtype MS11 4/3/1 lgtA amplified with lgtA Fwd-Ext and lgtA Rev-Ext and cloned into pCR2.1 TOPO TOPO (Life Technologies, USA) Used as a template to construct lgtA locked on by site directed mutagenesis with primers lgtA-ON FWD and lgtA-ON REV	This study
plgtA-ES	Contains lgtA with Erm^R-Sm^S from pFLOB4300 inserted into an engineered *Sma*I site in the homopolymeric tract Used to construct unmarked lgtA mutations (ON and OFF)	This study
plgtA-ON	Contains lgtA with homopolymeric tract locked ON (G_{12} → GGGCGGAGGTGG) by SDM (Quick change Lightening Multi SDM kit; Agilent Tech) with primers lgtA-ON FWD and lgtA-ON REV Used to generate lgtA locked ON	This study
plgtA-del	Contains WT lgtA with 417 bp deletion corresponding to bp 50-467 of the coding sequence. The deletion was created by double digesting plgtA with BbsI and SspI and ligating the larger fragment. Used to generate lgtA locked OFF (deletion)	This study
lgtC constructs		
plgtC	Contains wildtype MS11 4/3/1 lgtC amplified with lgtC Fwd-Ext and lgtC Rev-Ext and cloned into pCR2.1 TOPO TOPO (Life Technologies, USA). Used to construct plgtC-ON and plgtC-OFF	This study
plgtC-ES	Contains lgtC with ErmR-SmS from pFLOB4300 inserted into an engineered SmaI site in the homopolymeric tract Used to construct unmarked lgtC mutations (ON and OFF)	This study
plgtC-ON	Contains lgtC with homopolymeric tract locked ON (G_{14} → GGGGCGGAGG) (by SDM (Quick change Lightening Multi SDM kit; Agilent Tech) with primers lgtC-ON FWD and lgtC-ON REV Used to generate lgtC locked ON	This study
plgtC-OFF	Contains lgtC with homopolymeric tract locked OFF (G_{14} → GGTGAGGGGGGGGG) by SDM (Quick change Lightening Multi SDM kit; Agilent Tech) with primers lgtC-OFF FWD and lgtC-OFF REV Used to generate lgtC locked OFF	This study
lgtD constructs		
plgtD	Contains wildtype MS11 4/3/1 lgtD amplified with lgtD Fwd-Ext and lgtD Rev-Ext and cloned into pCR2.1 TOPO (Life Technologies, USA) Used to construct plgtD-del and plgtD-ON	This study
plgtD-ES	Contains lgtD with ErmR-SmS from pFLOB4300 inserted into an engineered SmaI site in the homopolymeric tract Used to construct unmarked lgtD mutations (ON and OFF)	This study
plgtD-ON	Contains lgtD with homopolymeric tract locked ON (G_{13} → GGGCGGAGGTG) by SDM (Quick change Lightening Multi SDM kit; Agilent Tech) with primers lgtD-ON FWD and lgtD-ON REV Used to generate lgtD locked ON	This study
plgtD-del	Contains lgtD with 744 bp deletion corresponding to bp 64-808 of the coding sequence. The deletion was created by digesting plgtD with *Pfl*MI and *Nde*I and ligating the larger fragment. Used to generate lgtD locked OFF (deletion)	This study

[1] All primer sequences are provided in Supplementary Table S2
[2] pFLOB4300 kindly provided by Dr. Janne Cannon, University of North Carolina, Chapel Hill

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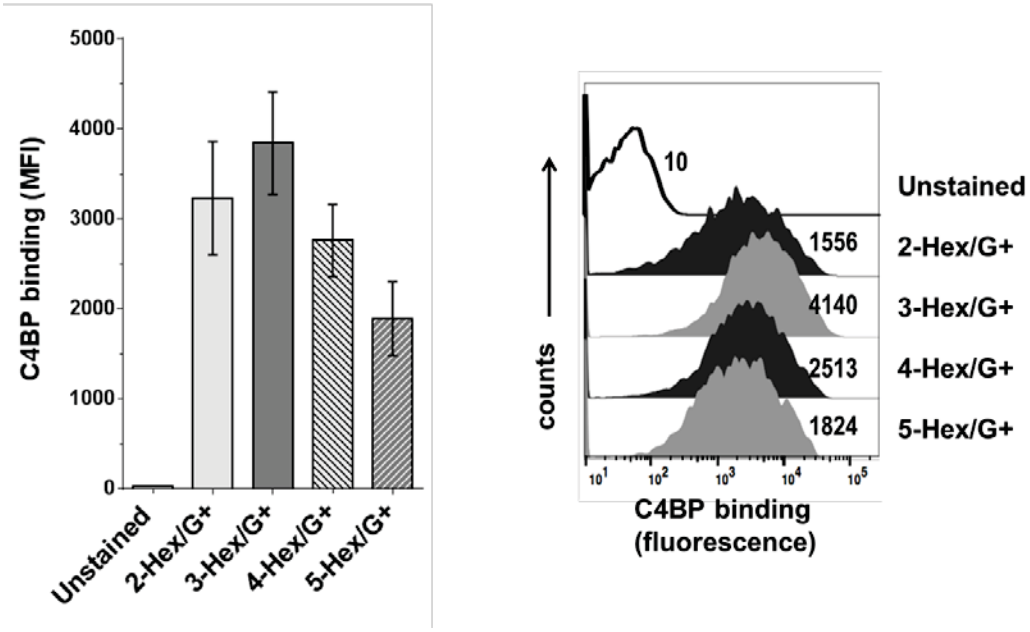
1. Johnston, D. M., and J. G. Cannon. 1999. Construction of mutant strains of Neisseria gonorrhoeae lacking new antibiotic resistance markers using a two gene cassette with positive and negative selection. *Gene* 236: 179-184.
2. Gulati, S., B. Zheng, G. W. Reed, X. Su, A. D. Cox, F. St Michael, J. Stupak, L. A. Lewis, S. Ram, and P. A. Rice. 2013. Immunization against a Saccharide Epitope Accelerates Clearance of Experimental Gonococcal Infection. *PLoS Pathog* 9: e1003559.
3. Tong, Y., V. Reinhold, B. Reinhold, B. Brandt, and D. C. Stein. 2001. Structural and immunochemical characterization of the lipooligosaccharides expressed by Neisseria subflava 44. *J Bacteriol* 183: 942-950.

Supplemental Table S3: Negative ion MS data and proposed compositions of *O*-deacylated LPS from *N. gonorrhea* strains. Average mass units were used for calculation of molecular weight based on proposed composition as follows: Hex, 162.15; Hep, 192.17; HexNAc, 203.19; PEtn, 123.05; Kdo, 220.18; Lipid A-OH, 952.00.
[1]The relative intensity is expressed as relative to the most populous glycoform / phosphoform.

Strain	Observed Ions (*m/z*) $(M-3H)^{3-}$	Observed Ions (*m/z*) $(M-2H)^{2-}$	Molecular Mass (Da) Observed	Calculated	Rel. Int.[1]	Proposed Composition
5-Hex/G+	997.2	1497.6	2995.9	2993.8	1.0	5Hex, 2HexNAc, 2Hep, 2Kdo, Lipid A-OH
	1065.0	-	3198.0	3197.0	0.4	5Hex, 3HexNAc, 2Hep, 2Kdo, Lipid A-OH
5-Hex/G-	888.9	1333.8	2669.7	2669.6	0.1	3Hex, 2HexNAc, 2Hep, 2Kdo, Lipid A-OH
	930.0	1395.3	2792.8	2792.6	1.0	3Hex, 2HexNAc, 2Hep, 2Kdo, PEtn, Lipid A-OH
	970.8	-	2915.4	2915.6	0.3	3Hex, 2HexNAc, 2Hep, 2Kdo, 2PEtn, Lipid A-OH
	997.8	1496.7	2995.9	2995.7	0.7	3Hex, 3HexNAc, 2Hep, 2Kdo, PEtn, Lipid A-OH
	1039.2	1558.5	3119.8	3118.8	0.2	3Hex, 3HexNAc, 2Hep, 2Kdo, 2PEtn, Lipid A-OH
4-Hex/G+	997.2	1495.8	2994.1	2993.8	1.0	5Hex, 2HexNAc, 2Hep, 2Kdo, Lipid A-OH
4-Hex/G-	888.9	1333.8	2669.7	2669.6	0.1	3Hex, 2HexNAc, 2Hep, 2Kdo, Lipid A-OH
	930.0	1395.3	2792.8	2792.6	1.0	3Hex, 2HexNAc, 2Hep, 2Kdo, PEtn, Lipid A-OH
3-Hex/G+	875.5	1313.5	2629.3	2630.5	0.4	4Hex, HexNAc, 2Hep, 2Kdo, Lipid A-OH
	930.0	1394.7	2792.2	2792.6	1.0	5Hex, HexNAc, 2Hep, 2Kdo, Lipid A-OH
	983.7	1475.7	2953.8	2954.8	1.0	6Hex, HexNAc, 2Hep, 2Kdo, Lipid A-OH
		1496.5	2995.0	2995.8	0.2	5Hex, 2HexNAc, 2Hep, 2Kdo, Lipid A-OH
	1051.5		3157.5	3157.9	0.1	6Hex, 2HexNAc, 2Hep, 2Kdo, Lipid A-OH
3-Hex/G-	-	1233.6	2469.2	2468.3	0.5	3Hex, HexNAc, 2Hep, 2Kdo, Lipid A-OH
	864.3	1295.4	2594.4	2591.3	1.0	3Hex, HexNAc, 2Hep, 2Kdo, PEtn, Lipid A-OH
2-Hex/G+	874.8	1313.4	2628.1	2630.4	1.0	4Hex, HexNAc, 2Hep, 2Kdo, Lipid A-OH
2-Hex/G-	808.4	1212.8	2427.9	2429.1	1.0	2Hex, HexNAc, 2Hep, 2Kdo, PEtn, Lipid A-OH

Supplemental Figure S1



Supplemental Figure S1. C4BP binding to the MS11 lgt 'ON' (G+) mutants. Bacteria were incubated with heat inactivated human complement for 30 min at 37 °C and bound C4BP was detected with anti-human C4BP mAb 67, followed by the biotinylated goat anti-mouse IgG (Molecular Probes), followed by Neuravidin conjugated DyLight 633 (ThermoFisher Scientific). Fluorescence was measured using a BD LSRII. Representative histograms from one of five separate experiments are shown. The number alongside each histogram indicates the median fluorescence intensity of the entire bacterial population.