Supplemental Figure Legends

Fig S1. Nel kills Ngo clinical isolates D006 and D020 in mixed culture, Related to Fig 2.
CFUs of Ngo clinical isolates D006 and D0020 and lab strain MS11 cultured alone (~$5x10^7$ CFUs total CFUs) or in the presence of Nel (~$5x10^7$ total CFUs each strain). Time of first plating: 6 h post-inoculation. Level of detection: 10 CFUs.

Fig S2. CFU/mL of vaginal swab suspension in mice colonized by Ngo and Nel, Related to Fig 3.
Average number of (A) Ngo or (B) Nel CFUs recovered from 1 ml vaginal swab suspensions from mice inoculated vaginally with 10^6 CFU of Nel or Ngo alone or a mixture containing 10^6 CFU of each species. (n = 8-9 mice/group). *P*=0.0013 for the difference in recovery of Ngo from mice inoculated with Ngo alone or Ngo+Nel (repeated measures ANOVA). The number of (C, E) Nel or (D, F) Ngo CFU isolated from vaginal swab suspensions of individual mice inoculated with (C) Nel alone, (D) Ngo alone or (E, F) Ngo + Nel. Dotted line indicates level of detection.

Fig S3. CFU/ml from mice inoculated with WT or ComP-deficient Ngo, or either Ngo strain plus Nel, Related to Fig 6.
(A-D) Average number of Nel or Ngo CFUs recovered from 1 ml vaginal swab suspensions from mice inoculated vaginally with 10^6 CFU of Nel, WT Ngo or the NgoΔ*comP* mutant alone or a mixture containing 10^6 CFU of Nel and either Ngo strain. (n = 8-9 mice/group). *P*=0.007 for the difference in recovery of WT Ngo from mice inoculated with WT Ngo alone or WT Ngo+Nel (repeated measures ANOVA). The number of (E,H) Nel, (F,I) WT Ngo, or (G,J) NgoΔ*comP* CFU isolated from individual mice inoculated with (E) Nel alone, (F) WT Ngo alone, (G) Ngo Δ*comP* alone, (H,I) WT Ngo + Nel or (J) NgoΔ*comP* + Nel. Dotted line indicates level of detection.

Fig S4. Restriction digests of Nel DNA confirm methylation of GpC and CpG sequences, Related to Fig 7.
Purified Nel DNA was incubated with buffer or M.CviPI and/or M.SssI methyltransferases, and subsequently digested with HaeIII or BstUI, restriction enzymes which cleave only unmethylated GGCC and CGCG sequence, respectively.

Fig S5. *iga* DNA from *E. coli* but not Ngo i35A is fragmented by Ngo restriction enzymes, Related to Fig 7.

ADIDA DNA fragment derived from Ngo i35A (left) and *E. coli* K-12 (right) digested with HaeII or NlaIV (isoschizomers of NgoI and NgoV) and separated in a 0.7% agarose gel. (-) indicates incubation with buffer alone.

Fig S6. Nel is not killed by Nel or Ngo DNA, Related to Fig 7.

Nel and Ngo CFUs recovered after a 4 h incubation with chromosomal DNA purified from Nel and Ngo (5 µg/mL). Starting CFUs: $5x10^5$. n=3. Error bars represent SEM. LOD: 10 CFUs. (**P<0.01; Student's t-test).

Supplemental Tables

Table S1. Bacterial strains and primers used in this study, Related to STAR Methods**.**

Table S2. Identification of DNA as the toxic compound in *N. elongata* supernatant, Related to Fig 4**.**

Table S3. Resistance of *N. gonorrhoeae* DNA uptake mutants to killing by *N. elongata* DNA, Related to Fig 5**.**

Table S4. Toxicity of DNA from BACs and BAC subclones for *N. gonorrhoeae*, Related to Fig 7.

Table S5. Modifications in Nel 29315 (this study), Ngo FA1090 (Blow *et al.,* 2016, Srikhanta *et al.* 2009), Ngo MS11 (Stein *et al.,* 1992) and *E. coli* K-12 (Marinus and Løbner-Olesen, 2014) DNA, Related to Fig 7**.**

Table S6. Toxic DNAs in this study contain sequences recognized by Ngo MS11 R/M systems, Related to Fig 4 and Fig 7.

Table S7. Short regions of homology (9-20 bp) between *E. coli* plasmids and Ngo MS11 chromosome contain recognition sequences from Ngo MS11 restriction modification (R/M) systems, Related to Fig 7.

Table S8. Orthologs of *N*-acetylmuramyl-L-alanine-amidase AmiC and lytic transglycosylase LtgA in commensal *Neisseria*, Related to Fig 2 and Fig 3.

Fig S1. Nel kills Ngo clinical isolates D006 and D020 in mixed culture.

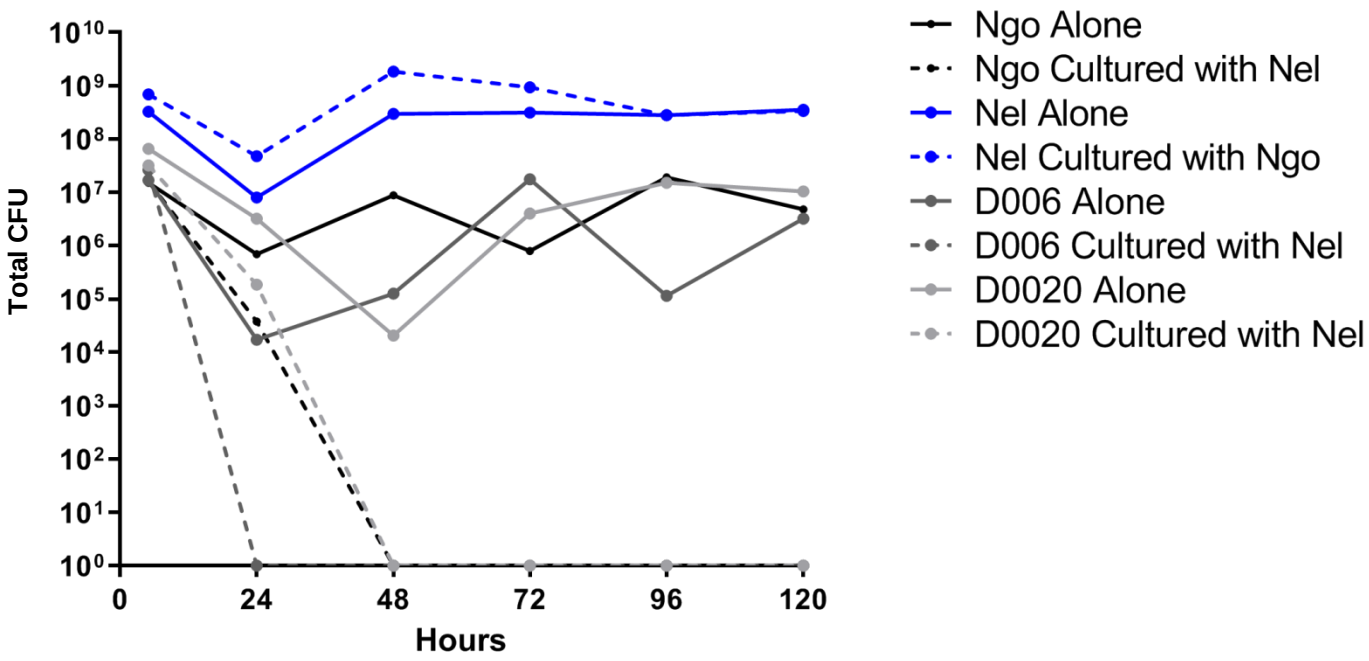


Fig S2. CFU/mL of vaginal swab suspension in mice colonized by Ngo and Nel.

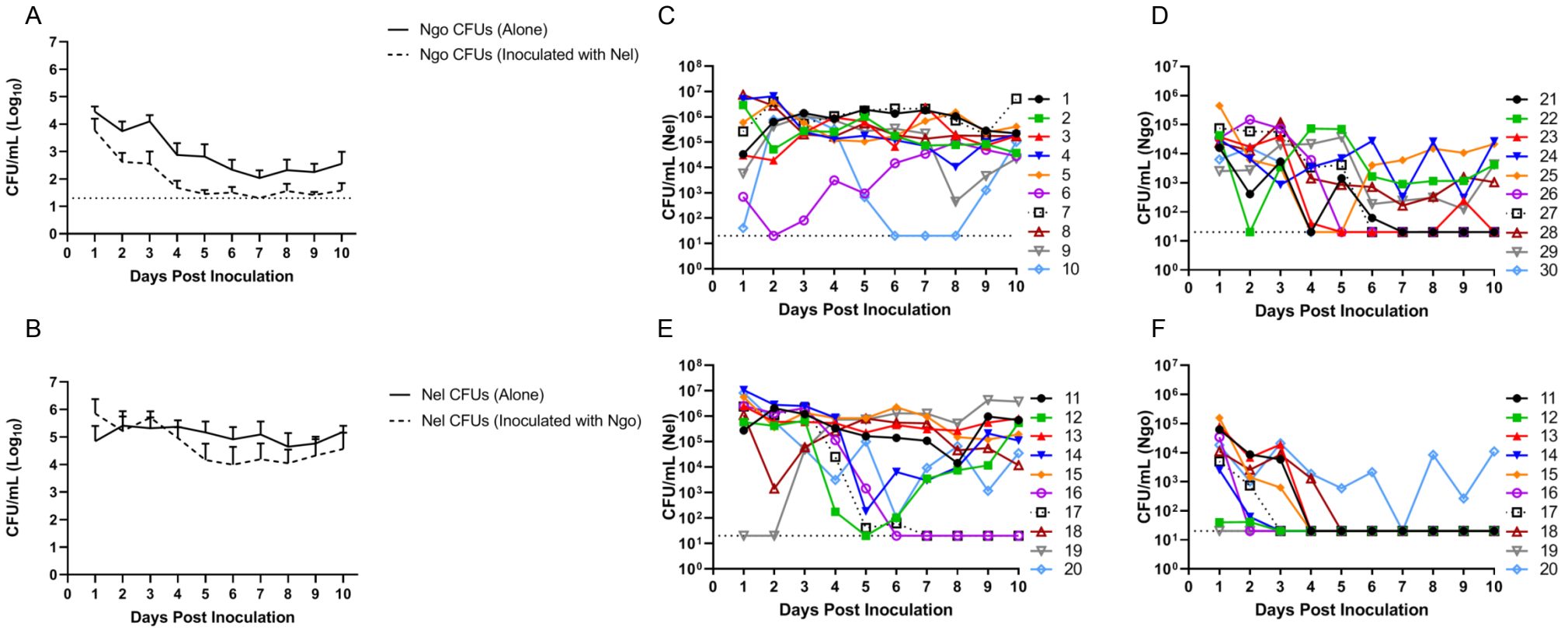


Fig S3. CFU/ml from mice inoculated with WT or ComP-deficient Ngo, or either Ngo strain plus Nel.

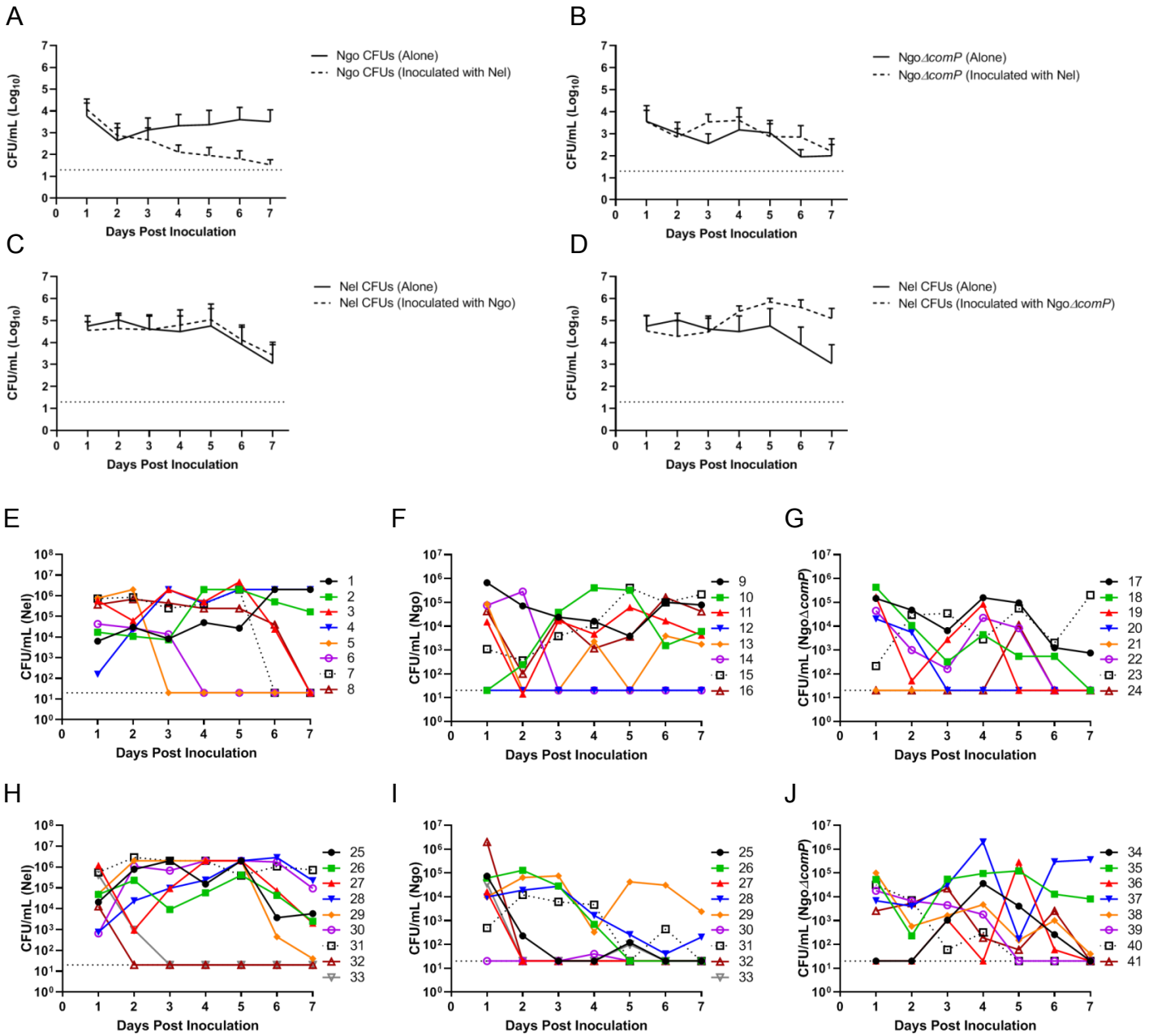
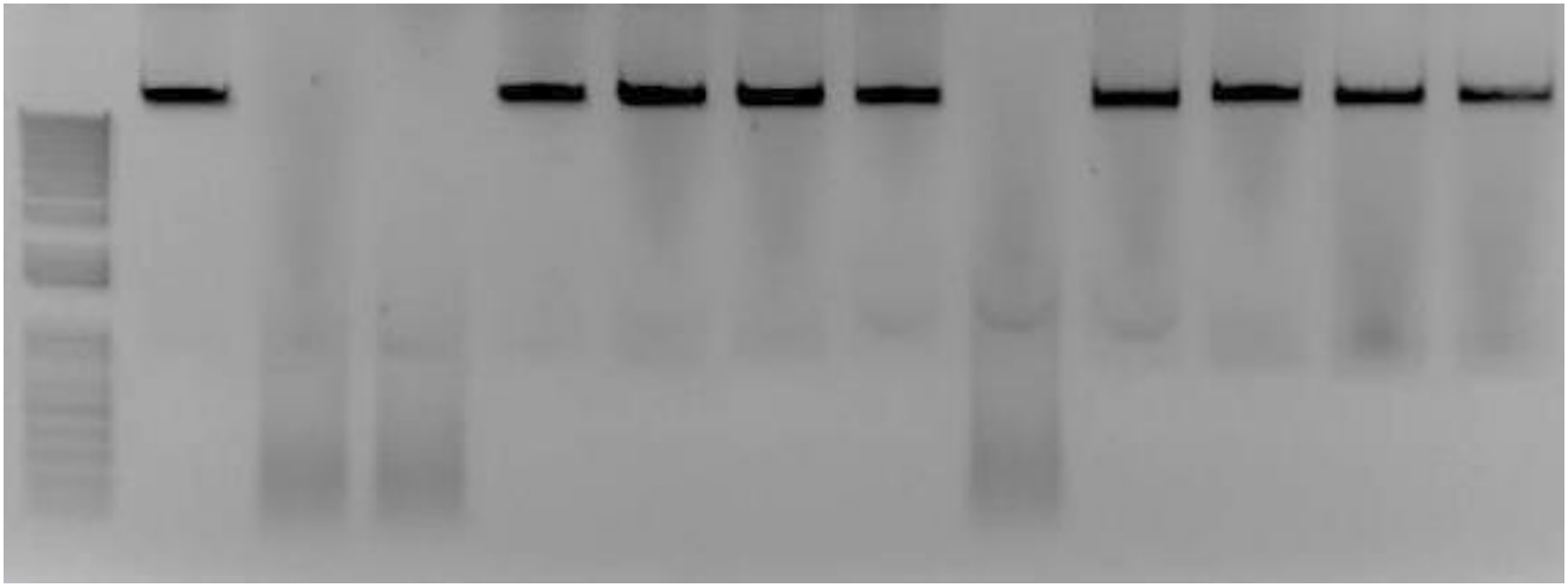


Fig S4. Restriction digests of Nel DNA confirm methylation of GpC and CpG sequences.

Motifs methylated	-	-	-	Gp^{m}C	Gp^{m}C	Gp^{m}C	^{m}CpG	^{m}CpG	^{m}CpG	Both	Both	Both
Restriction enzyme	-	HaeIII	BstUI	-	HaeIII	BstUI	-	HaeIII	BstUI	-	HaeIII	BstUI



HaeIII recognition sequence: GGCC
BstUI recognition sequence: CGCG

Fig S5. *iga* DNA from *E. coli* but not from Ngo i35A is fragmented by Ngo restriction enzymes.

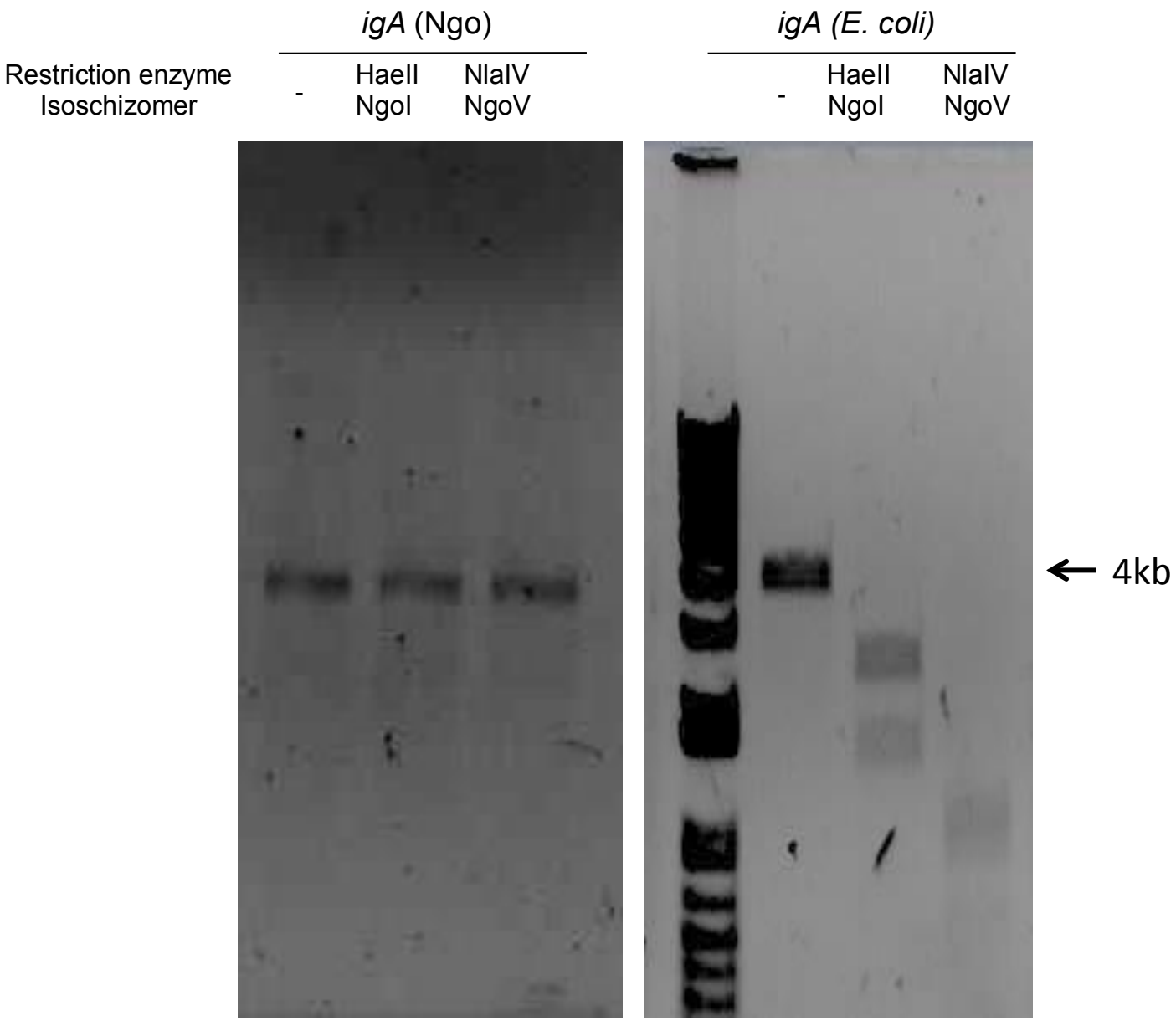


Fig S6. Nel is not killed by Nel or Ngo DNA.

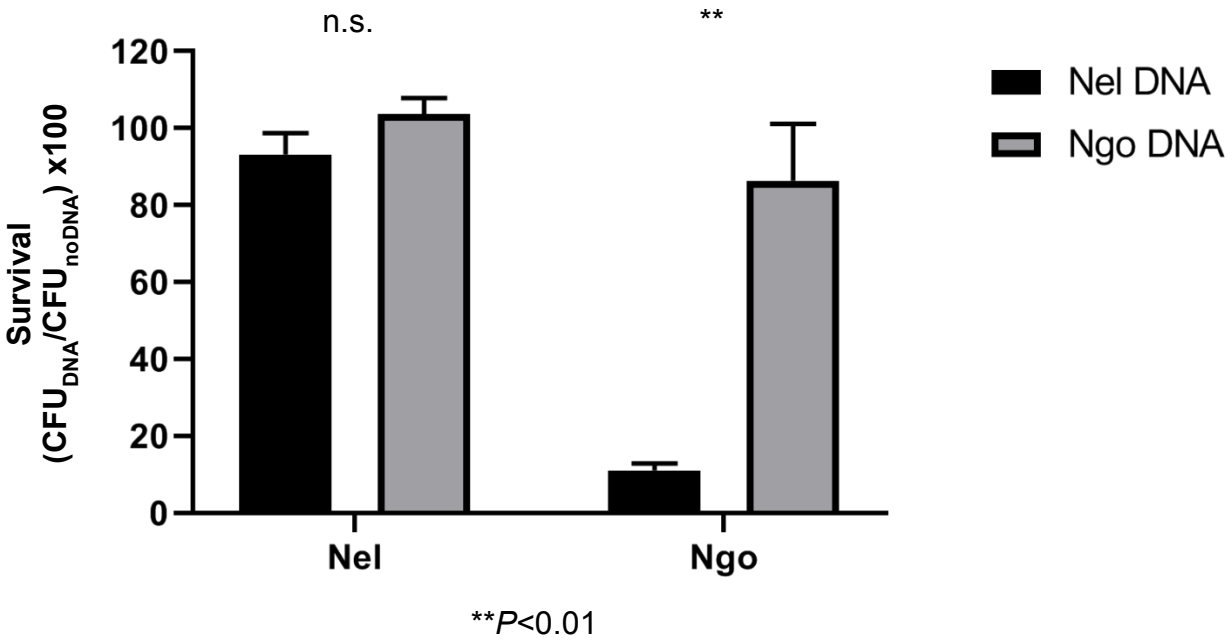


Table S1. Bacterial strains and primers used in this study.

Organism (strain)	Genotype	Source
E. coli (DH5α)	*dam*$^+$, *dcm*$^+$	Lab collection
N. gonorrhoeae (MS11)	WT, P+, Opa-nonexpressing	(Segal *et al.,* 1985)
Δ*pilT*	Δ*pilT*	(Dietrich *et al*., 2009)
Δ*pilT/ipilT*	Δ*pilT* containing IPTG-inducible $pilT_{WT}$ inserted between *aspC* and *lctP*	This study
Δ*comP*	Δ*comP*	This study
Δ*comP/*$comP_{WT}$	$comP_{WT}$ inserted between *iga* and *trpB*	This study
TD3	Δ*ngoII*, Δ*ngoIV*, Δ*ngoV* R/M loci deleted	This study
N400	IPTG-inducible *recA*	(Tonjum *et. al.,* 1995)
i35A	AsiSI-DUS-*iga*-DUS-AsiSI (ADIDA)	This study
N. elongata	ATCC 29315	(Marri *et al.,* 2010)
N. lactamica	ATCC 23970	(Marri *et al.,* 2010)
N. cinerea	ATCC 14685	(Marri *et al.,* 2010)
N. mucosa	ATCC 25996	(Marri *et al.,* 2010)
N. sicca	ATCC 29256	(Marri *et al.,* 2010)
N. polysaccharea	ATCC 32768	(Marri *et al.,* 2010)
N. meningitidis	8013	(Nassif *et al.,* 1993)
Primer	**Sequence (5'-3')**	**Use**
comP_MS11_F	CGCCCGCACCAAAAGGCCCCGCCAAACCGGTGCTGCCTGCGGTTAAAAAATAGAGTGGGAAATATGCATACTGCTGAATGGGATAGTAAGTCTGTTCAAAGAAATATGTTGAATAATCTGTTCTTATTGGAAGTAAAGTAATGACTGATAATCGGGGGTTTCTCGAGGGCTTGACACTTTATG	Deletion of *com*P
comP_MS11_R	GATACGGATCACGGGTCATAACTATAGGCTTAATATTACACGATTCTCATTCCATCAAGGCGGAAAACCGCACAAATACTGAAACACTATCGATCGATTTGTAAACAAGCCTACTTAAGTAACTTGCAGTCATCGATGTTTAAACTTCAGACGGC	Deletion of *comP*
MR392	CCGTCCATTTCGGTATTCAC	Confirmation of *comP* deletion
MR395	TTTTCGATTTCTTCGCTGTG	Confirmation of *comP* deletion
MR396	GTAAACATCAATGCGGCTTC	Confirmation of *comP* insertion
165F	CTCGAAGCCTTTGACTTGCT	Confirmation of *comP* insertion
NgoII_FC_F	CAAAATGCGCCAAATCAAC	Deletion of *ngoII;* confirmation of *ngoII* deletion
NgoII_FC_R	GGGTTCAGTCCCAAGTTTGA	Delete *ngoII;* confirmation of *ngoII* deletion
NgoIV_FC_F	GAAATCGCCGAACACGTTAT	Deletion of *ngoIV;*

		confirmation of *ngoIV* deletion
NgoIV_FC_R	CCAATACGCCGACATAATCC	Deletion of *ngoIV;* confirmation of *ngoIV* deletion
NgoV_FC_F	TCGCGCACAATCAAAATATC	Deletion of *ngoV;* confirmation of *ngoV* deletion
NgoV_FC_R	ACGCGTAAAAACTTCGGTTG	Delete *ngoV;* confirmation of *ngoV* deletion
DUS_pCR-Blunt_F	ATGCCGTCTGAAGTACGGCA GTTTAAGGTTTACACC	Addition of DUS to pCR-Blunt; PCR amplification of pCR-Blunt
pCR-Blunt_R	GTATAGGCTGCGCAACTGTT	PCR amplification of pCR-Blunt
M13_F	TGTAAAACGACGGCCAGT	Sequencing of pCR-Blunt and BAC clones/ subclones
M13_R	CAGGAAACAGCTATGAC	Sequencing of pCR-Blunt and BAC clones/ subclones
Iga5_F	GCAGCTATCTGATGCAGGAC	Sequencing of i35A; PCR amplification of ADIDA
Iga5_R	AGCTTGAGAAGCCGGTTACA	Sequencing of i35A
Iga3_F	TTCCATTCTTGCCATGATTTT	Sequencing of i35A
Iga3_R	TTCTTGCCTATGCCCTTACG	Sequencing of i35A; PCR amplification of ADIDA
MR616	ATACGTTTCCTTAATTAACAGAGCCGCATTATGCAGATTA	*pilT* amplification to clone into pKH37
MR617	ATACGTTTCCATCGATGGTTGTGTGTCAGAAACTCATAC	*pilT* amplification to clone into pKH37
MR618	ATGCACTTCTTCGCAGGTTT	Confirmation of *pilT* insertion between *aspC* and *lctP*
Iga5AD	CGGTTTGGGCGTGGATACGCCTGACCACGCCGCGCCGATTACTTCGGG CGCACCGATTGGCGTACTGCACGGCTTCCGCAGCTATCTGATGCAGGA CGAAAACGGCCAAGTCTTGGGGACGCACTCTGTTTCCGCAGGCTTGGA TTACCCCGGCATCGGCCCGGAACACAGCCATCTGCACGACATCAAGCG CGTCGAATACACCGTTGCCAAAGATGACGAAGCACTCGAAGCCTTTGAC TTGCTCTGCCGATTCGAGGGCATCATCCCCGCGCTCGAATCCAGCCAC GCCGTTGCTTGGGCGGTGAAAAACGCGCCGAAAATGGGTAAAGACCAA GTGATTTTGGTCAACCTGTCGGGTCGCGGCGACAAAGACATCAATACCG TGGCGAAACTCAAAGGCATTGAGCTGTAGCTTTGTTAGTCTGATAAAAAT GCCGTCCGAAGCTTGAGTTCAGACGGCATTTTATTTTGCTATGAATTTGG TATT**GCGATCGC**ATGCCGTCTGAATTAGAAACGAATCTGTATTTTAATTT GTCCGGATTTTTGTTTTTCCAATTGTTTTCCTTTTGTAATACTGCCATTTA CGTTTAATGTAACATTACGGTACAGTAACGCGGCGCCTGCTGAATATTG	Introduction of **AsiSI** restriction site and DUS12 to 5’ end of *iga*

	CTGTTGATTATCTGCTTTATAGGCGAAGGATTTACCGCCCACATTCACGC CGCCTTTGCCATAATTGGCAAAGTAAGCTGCAGATAACAAGGGTTTTAC GGTAAGGTTGCCGACTTTAAACCGATAAGCAAAATCCAGTCCGGCCGTT AGTGTTTTCACTGCCATAGAACTTACTTTAACACTGTCGTCACCCAACTT GTAATCTGCAGATGACAGGCGGCTGTAACGGATACCCGCACTAGGGAC AATCTCGAATTGATTGATTTTCAGCGTATTGCCCAAAGTAAGGCCGGTTT GGATGCTTGTTCGGTTAAAGTTTGCTTTTTGCTGCGTTTGTAACCGGCTT CTCAAGCTGCCCGCACCA	
DAIga3	TCGTGTTGTTTGATTTTATCTGCAAATTCTTTTTTATAGATATTCCATTCTT GCCATGATTTTTTTCCGTAACCTGCCCAATAATCGTAAGTTCCCAAAAAG ACCCATTGATTTTTTTGTTTGTCAAAAGCAAATAGTGGAGAGCCGCTATC GCCCAACACGCCGTAATTTGTTAACGCATCTTGCGAAAGTGCTTGTTTAA GTTCTTCTGCCGAATACTGTTTATTATGATTACCAAAGCCGATCAAACCT TCGGTATTCATTGTTTGGTCAATATTAATATCTTTATAAGGCGTACCTGCA ATGGCATAACGATAGGCTTGTGAAAGATCGCGCAAATCGTAGCCTTTTT CATTTCCTTCTTGATGATAAACCCCTTTTTCATAAACTAATTGCCTGCCCG CACCGATTCTGACAAAAGAGGAAAAACGGTTTTTATCTTTGTAGGTATCC AATCCGCCGCCGGCATCAGTTGGTGCAATCGGTGCCAATGCCGTCTGA A**GCGATCGC**CTTCGGTTACAAACTTATTAAAACGCGCCATATTATAATCT TCGAGGCGGCCTAAATTGCTCGCACCCCAAGCTTTATGGGGTTCATAGT TATTTTGTTCGACAACGCGGTATTCATTTTCTTTGTCGGCTACATCATTAT GACCGTTGTATTGGCCGTAATAAAAAGTATGGACTTCTGCTTTGGCGTGT TTGACGCTGACGGCATATTGGGGATCGACTACCGTTGCTATGCGTTTGT TGACATCTGCAACGCTAAAATCAATCATCGGTACGTTGGATAATGCGTTG CCGATGTTTTGACCTCGTTTGTTTTTCACTGATAAATCGGTTGCGCCGAC AAAAAATTTGCCTTTGTTTTCTGCAAAGTCACGGAATATTTGATAATCGAC ATCGTCTCTCACCAATGCCGCTTCTGAGTATGGCGTAAGGGCATAGGCA AGAAAGATGGATAAGGATATGGCGTTAATTTTAAAACGTTTGGCTTTCAT	Introduction of DUS12 and **AsiSI** restriction site to 3' end of *iga*

Table S2. Identification of DNA as the toxic compound in *N. elongata* supernatant.

Supernatant treatment	Clearance zone
DNAse I	No
DNAse I, boiled	Yes
Proteinase K	Yes
Proteinase K, boiled	Yes
RNAse I	Yes

Table S3. Resistance of *N. gonorrhoeae* DNA uptake mutants to killing by *N. elongata* DNA.

Strain	Clearance zone
MS11 WT	Yes
Δ*pilE*	No
Δ*comP*	No
Δ*comP*/*comP*$_{WT}$	Yes

Table S4. Toxicity of DNA from BACs and BAC subclones for *N. gonorrhoeae*.

BAC clones	Insert start position	Insert end position	Insert length (bp)	Clearance zone
pBeloBAC11 vector (7.5kb)	-	-	0	No
6.1	1021119	1044807	23688	Yes
6.2	2097448	2126542	29094	Yes
6.3	1193060	1242918	49858	Yes
6.4	1030576	1081623	51047	Yes
6.5	2097448	2126542	28358	Yes
BAC 6.1 subclones				
pUC19 vector (2.7 kb)	-	-	0	No
6.1.3	1024777	1029727	4950	Yes
6.1.11	1033760	1038767	5007	Yes
6.1.13	1042473	1044193	1720	Yes
6.1.20	1021144	1024413	3269	Yes
6.1.24	1039200	1044555	5355	Yes
6.1.26	1031310	1038748	7438	Yes

Table S5. Modifications in Nel 29315 (this study), Ngo FA1090 (Blow *et al.,* 2016, Srikhanta *et al.* 2009), Ngo MS11 (Stein *et al.,* 1992) and *E. coli* K-12 (Marinus and Løbner-Olesen, 2014) DNA.

Organism	R/M System	Recognition Sequence	Nucleotide	Detection Method
Nel 29315	Unknown	GGA^{m}AC	Adenine	PacBio SMRT
		CRT^{m}ANNNNNNTGC	Adenine	
		TA^{m}ATNNNNNNRTAY	Adenine	
Ngo FA1090	NgoI	RG^{m}CGCY	Cytosine	PacBio SMRT
	NgoII	GG^{m}CC	Cytosine	
	NgoIII	^{m}CCGCGG	Cytosine	
	NgoIV	G^{m}CCGGC	Cytosine	
	NgoV	GGNN^{m}CC	Cytosine	
	NgoAV	GC^{m}ANNNNNNNNTGC	Adenine	
	NgoAXVII	G^{m}AGNNNNNTAC	Adenine	
	NgoAXVI	GGTG^{m}A	Adenine	
	NgoAX	CC^{m}ACC	Adenine	
	NgoAXII	AGAA^{m}A	Adenine	Restriction analysis
Ngo MS11	NgoI	RG^{m}CGCY	Cytosine	Restriction analysis
	NgoII	GG^{m}CC	Cytosine	
	NgoIII	^{m}CCGCGG	Cytosine	
	NgoIV	G^{m}CCGGC	Cytosine	
	NgoV	GGNN^{m}CC	Cytosine	
	NgoVIII	T^{m}CACC	Cytosine	
	NgoAXVI	GGTG^{m}A	Adenine	PacBio SMRT
***E. coli* K-12**	EcoKI	A^{m}ACNNNNNNGTCG	Adenine	Review of previous studies
	Dam	G^{m}ATC	Adenine	
	Dcm	C^{m}CWGG	Cytosine	

R: A or G; **Y**: C or T; **N:** any base.

Table S6. Toxic DNAs in this study contain sequences recognized by Ngo MS11 R/M systems.

R/M system	Recognition sequence	# Occurrences		
		Nel DNA	pCR-Blunt	ADIDA
NgoI	RG^{m}CGCY	514	3	2
NgoII	GG^{m}CC	16392	24	9
NgoIII	^{m}CCGCGG	420	0	0
NgoIV	G^{m}CCGGC	1592	3	4
NgoV	GGNN^{m}CC	2710	9	7
NgoVIII	T^{m}CACC	1582	3	2
NgoAXVI	GGTG^{m}A	1656	3	2

R: A or G; **Y**: C or T; **N:** any base.

Table S7. Short regions of homology (9-20 bp) between *E. coli* plasmids and Ngo MS11 chromosome contain recognition sequences from Ngo MS11 restriction modification (R/M) systems.

Plasmid	R/M system	Recognition sequence	# of occurrences in Ngo chromosome	Homology length (minimum)	Homology length (maximum)
pCR-Blunt	NgoI	RG^{m}CGCY	11	10	12
	NgoII	GG^{m}CC	414	10	17
	NgoIII	^{m}CCGCGG	0	-	-
	NgoIV	G^{m}CCGGC	94	10	17
	NgoV	GGNN^{m}CC	36	10	15
	NgoVIII	T^{m}CACC	14	10	15
	NgoAXVI	GGTG^{m}A	21	10	13
pBeloBAC11	NgoI	RG^{m}CGCY	6	10	13
	NgoII	GG^{m}CC	205	10	18
	NgoIII	^{m}CCGCGG	0	-	-
	NgoIV	G^{m}CCGGC	104	10	17
	NgoV	GGNN^{m}CC	30	10	17
	NgoVIII	T^{m}CACC	51	10	15
	NgoAXVI	GGTG^{m}A	48	10	16
pUC19	NgoI	RG^{m}CGCY	20	9	15
	NgoII	GG^{m}CC	178	9	15
	NgoIII	^{m}CCGCGG	0	-	-
	NgoIV	G^{m}CCGGC	1	20	20
	NgoV	GGNN^{m}CC	47	9	15
	NgoVIII	T^{m}CACC	65	9	15
	NgoAXVI	GGTG^{m}A	64	9	13

R: A or G; **Y**: C or T; **N:** any base.

Table S8. Orthologs of *N*-acetylmuramyl-L-alanine-amidase AmiC and lytic transglycosylase LtgA in commensal *Neisseria*.

	AmiC		LtgA	
***Neisseria* species (strain)**	**Coverage (%)**	**Identity (%)**	**Coverage (%)**	**Identity (%)**
N. elongata (ATCC 29315)	99	56	99	61
N. sicca (ATCC 29256)	99	76	98	69
N. mucosa (ATCC 25996)	99	76	99	68
N. subflava (NJ9703)	100	75	98	71
N. flavescens (NRL30031)	86	79	98	71
*N. cinerea (*ATCC 14685)	100	94	99	88
N. polysaccharea (ATCC 43768)	100	95	100	96
N. lactamica (ATCC 23970)	100	97	100	97

Coverage and identity values were obtained by querying AmiC and LtgA amino acid sequences against the genome database using blastp at ncbi. (http://blast.ncbi.nnlm.nih.gov/Blast.cgi).