Supporting Information

Malott et al. 10.1073/pnas.1303738110

SI Materials and Methods

Cell Lines, Bacteria Strains, and Growth Conditions. Jurkat E6-1 (ATCC; TIB-152), Jurkat 1G5 (1) (a gift from M. Tremblay, Laval University, Quebec City, QC, Canada), and A3.01 [National Institutes of Health (NIH) AIDS Research and Reference Reagent Program (RRRP), Division of AIDS, NIAD, NIH] and ACH-2 (NIH AIDS RRRP) $CD4^+$ T cells lines were maintained in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% (vol/vol) heat-inactivated FBS (Invitrogen) and 1% (vol/vol) GlutaMax-1 (Invitrogen) at 37 °C with 5% CO_2. For selection, 4 μg/mL of puromycin was added to the medium. The InvivoGen HEK-Blue human Toll-like receptor 4 (hTLR4) reporter cell line (a gift from S. Turvey, University of British Columbia, Vancouver, BC, Canada) was grown in high-glucose DMEM (Invitrogen) with 10% heat-inactivated FBS, 1% GlutaMax-1, and selective antibiotics at 37 °C with 5% CO_2 according to the manufacturer's instructions. Culture density was monitored by Trypan Blue exclusion.

Bacteria strains used in this study are detailed in Table S1. Cultures typically were grown from frozen stocks at 37 °C in the presence of 5% CO_2 when required. *Neisseria gonorrhoeae* (Ng) strains were grown on GC agar base supplemented with 1% (vol/vol) IsoVitaleX enrichment (BD Biosciences); *Neisseria meningitidis* (Nm), *Neisseria lactamica*, *Moraxella catarrhalis*, and *Staphylococcus aureus* strains were grown on brain heart infusion (BHI) (Difco); *Haemophilus influenzae* strains were grown on chocolate agar; *Streptococcus pneumoniae* strains were grown on Columbia blood agar containing 5% (vol/vol) sheep blood; and *Escherichia coli* was grown on LB medium. Nm was grown in BHI with 1% (vol/vol) IsovitaleX for growth curves. For collection of spent culture supernatants, bacteria were grown in RPMI at 37 °C for 6 h (OD_{550} of ∼0.4). Bacteria were grown in prewarmed RPMI (no phenol red) (Invitrogen) with 1% (vol/vol) IsovitaleX, 0.042% (wt/vol) sodium bicarbonate at pH 7.2 at 37 °C with 100 rpm agitation to prepare conditioned medium or heptose-monophosphate (HMP). For *lac* promoter induction, 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to the culture medium. Bacterial overgrowth of cells was prevented by the addition of 50 μg/mL or 100 μg/mL of gentamicin to the culture medium after 2 or 3 h. During molecular manipulations, 60 μg/mL of kanamycin or 25 μg/mL erythromycin was added when necessary for positive selection.

Jurkat 1G5 Bioassay for HIV-1 LTR Expression. Jurkat 1G5 cells (1) were seeded at 1.0×10^6 cells/mL in RPMI medium supplemented with 1% (vol/vol) GlutaMax-1 (Invitrogen) and were infected with bacterial strains at a multiplicity of infection (MOI) = 2 or MOI = 10 bacteria per cell, treated with increasing concentrations of HMP, resuspended in conditioned medium, treated with 100 nM phorbol myristate acetate (PMA) as a positive control or were left untreated. Infections proceeded for 2 or 3 h before 50 or 100 μg/mL of gentamicin was added to prevent overgrowth; then cultures were incubated an additional 16 h at 37 °C. The cells were lysed, and luciferase activity determined using a luciferase assay kit (Promega). Counts of luminescence per second were recorded in a Wallac Victor 2 (Perkin-Elmer) or a Tecan Infinite M200 (Tecan) luminometer. The human TLR1-9 agonist kit (InvivoGen) was used to test the response of Jukat 1G5 cells to various microbe-inscribed molecular patterns (MAMPs). For Toll-like receptor 5 (TLR5) neutralization, Jurkat 1G5 cells were preincubated with anti-TLR5 or isotype control antibodies (InvivoGen) for 2 h at 37 °C. *Salmonella typhimurium* flagellin (InvivoGen) at 1 μg/mL was used as a positive control. Spent bacterial culture supernatants were centrifuged at 4,000 × *g* for 10 min, were filtered through a 0.22-μm filter, and were used as the growth medium. For proteosome inhibition, Jurkat 1G5 cells were pretreated with up to 250 nM of MG-132 (Calbiochem). For screening of the *N. meningiditis* Tn*5*-based transposon library, Jurkat 1G5 cells were seeded in a 96-well plate and were infected with 2 μL of a stationary-phase bacteria culture. For determination of bioactive HPLC fractions, each fraction was dried in a Thermo Scientific Savant SPD131DDA SpeedVac concentrator or under a stream of N_2 and was resuspended in RPMI. The reconstituted fractions were used as the assay medium for the Jurkat 1G5 cells.

Molecular Manipulations. Standard molecular biology techniques were performed according to methods described by Sambrook et al. (2). Molecular manipulations in *Neisseria* spp. were carried out as previously described (3). Plasmid DNA and RNA was extracted with the QIAprep Spin Miniprep and RNeasy kits, respectively (Qiagen). Restriction endonucleases, DNA polymerases, T4 DNA polymerase, and T4 DNA ligase were purchased from New England Biolabs, Turbo DNase was purchased from Ambion, and all were used according to the manufacturer's instructions. DNA fragments used in cloning procedures were purified with a QIAquick gel extraction kit (Qiagen). Oligonucleotide primers were purchased from Integrated DNA Technologies. The published *N. meningiditis* MC58 genome sequence (4) was obtained via GenBank [National Center for Biotechnology Information (NCBI)]. Sequence analysis was performed using DNAMAN sequence analysis software (Lynnon Biosoft). Sequence similarity searches were performed using the NCBI-BLAST (5).

Nm B16B6 Tn*5* mutants were generated by in vitro transposition using the EZ::Tn*5* transposition system (Epicentre Technologies) as previously described (6). Transposon insertions were identified by PCR amplification and sequencing with the transposon specific primers KANFOR (5′-TTGATGCTCGATGAGTTTTTCTAA-3′) and KANREV (5′-GTTTCCCGTTGCCTATGGCTCATA-3′). Mutated alleles were confirmed by PCR with primers internal to ORF NMB0825, NMB825F (5′-CAAAGGCGACGATTAC-GAA-3′) and NMB825R (5′-CCGCAGTATTGGCAAGGTA-3′). To construct the NMB0825 complementation vector pRM505, a 1,247-bp fragment containing NMB0825 was amplified with primers 825PacI (5′-GGCATTAATTAAGACCAGCGCCG-3′; PacI site underlined) and 825FseI (5′-GTGGCCGGCCCATTGTCG-3′; FseI site underlined) and cloned into the PacI/FseI site in the *Neisseria* spp.-targeted chromosomal complementation vector, pGCC4 (3) (a gift from Hank Seifert, Northwestern University, Chicago, IL), downstream of the *lac* promoter. Integration of the ORF was confirmed by acquisition of erythromycin resistance and by PCR with primers GCC4F (5′-TCGCGCCGGATGCGTCT-TA-3′) and GCC4R (5′-CCGGCCTGTCCGTTACCCTG-3′) that bind external to the targeted integration site. The Ng N302 *hldA::*Tn*5* mutant was constructed by introducing the 1,556-bp *hldA::*Tn*5* fragment amplified with NMB825F and NMB825R primers from the Nm *hldA::*Tn*5* mutant genomic DNA into Ng by electroporation (3). Mutants were selected by kanamycin resistance and confirmed by PCR with NMB825F and NMB825R primers.

For quantitative RT-PCR (qRT-PCR), cDNA was generated with iScript RT-supermix (Bio-Rad). PCR was preformed using SsoAdvanced Sybr Green Supermix (Bio-Rad) using a C1000 thermal cycler (Bio-Rad) with the following primers: reticuloendotheliosis viral oncogene homolog (*RelA*): RelAa (5′-CCC-ACGAGCTTGTAGGAAAGG-3′) and RelAb (5′-GGATT-CCCAGGTTCTGGAAAC-3′); *NFKBF1*: NFKB1a (5′-GAAG-CACGAATGACAGAGGC-3′) and NFKB1b (5′-GCTTGGC-

GGATTAGCTCTTTT-3′); *GAPDH*: GAPDHa (5′-TTGAGGTCAATGAAGGGGTC-3′) and GAPDHb (5′-GAAGGTGAAGGTCGGAGTCA-3′); and *ACTB*: ACTBa (5′-CCTTGCACATGCCGGAG-3′) and ACTBb (5′-GCACAGAGCCTCGCCTT-3′). Levels of the target gene were normalized to GAPDH as an endogenous reference, and relative gene expression compared with RNA harvested from nontransduced, untreated cells was calculated using the $2^{-\Delta\Delta C_T}$ method (7).

Lipid A Isolation and MALDI-TOF/TOF Analysis. After one washing with PBS buffer and three washings with deionized water, heat-killed bacteria were suspended in 100 μL of 50 mM sodium acetate buffer (pH 4.5), and the suspension was heated at 100 °C for 1 h. The mixture was centrifuged at 10,000 × *g* for 5 min, and the pellets were washed twice with methanol. Lipids were extracted from the pellets with a 100-μL mixture of chloroform, methanol, and 1 M ammonium acetate (4:4:1, vol/vol). After centrifugation at 8,000 × *g* for 5 min, the supernatant was dried under a stream of nitrogen. Lipids were analyzed using a 4800 MALDI-TOF/TOF (Applied Biosystems) in the negative ion mode. 5-Chloro-2-mercaptobenzothiazole (CMBT) at 20 mg/mL in a mixture of chloroform-methanol-water (4:4:1, vol/vol) containing 20 mM EDTA ammonium salt was used as a matrix. Lipids and lipid A samples were dissolved in a mixture of chloroform-methanol-water (4:4:1, vol/vol) and then were mixed with an equal volume of the matrix, from which 0.2-μL samples were loaded onto the MALDI target. MS data were acquired in reflection mode. Four hundred shots were accumulated for each MS spectrum.

Purification of HMP from Ng. A 20-mL culture of 1×10^8 bacteria/mL was grown to midlog phase at 37 °C at 100 rpm. A 1/10 subculture into 200 mL of fresh medium was grown for an additional 6 h (OD_{550} of ∼0.4). The cell-free supernatant was collected by centrifugation at 4,000 × *g* for 10 min and was filtered through a 0.22-μm filter. The supernatant was ultracentrifuged at 120,000 × *g* for 2.25 h at 4 °C, treated with 10 μg/mL RNase and DNase at 37 °C for 1 h, treated with 200 μg/mL Proteinase K at 56 °C for 3 h, and then heat-inactivated at 95 °C for 20 min. Debris was removed by centrifugation at 4,000 × *g* for 10 min. The supernatant was extracted twice with an equal volume of phenol (pH 6.6), and the phases were separated by centrifugation at 4000 × *g* for 10 min. The remaining aqueous phase was extracted twice with chloroform:methanol (2:1 vol/vol). The aqueous phase was dialyzed against distilled H_2O in 3-kDa molecular weight cutoff tubing to remove organic solvents. The contents of dialysis tube were frozen at −80 °C and lyophilized until dry. The lyophilized product was resuspended in dH_20 10 mg/mL and treated for endotoxin removal with the UltraClean endotoxin removal kit (MO BIO Laboratories, Inc.) according to the manufacturer.

TLR4 Reporter Assay. HEK-Blue hTLR4 cells (Invivogen) were seeded at 2.5×10^4 cells per well in a 96-well plate according to the manufacturer's instructions. Cells were stimulated with increasing concentrations of *E. coli* lipopolysaccharide (Sigma) and were infected with live or heat-killed bacteria at MOI = 1 or were treated with HMP. *S. pneumoniae* was used as a negative control. At 2 h postinfection, 100 μg/mL of gentamicin was added to stop the live infections, and the cultures were incubated for an additional 22 h. Secreted embryonic alkaline phosphatase reporter activity was measured in the culture supernatant with QuantiBlue substrate (InvivoGen) according to the manufacturer's instructions, and colormetrics were monitored at an optical density of 650 nm with a Tecan Infinite M200 (Tecan).

HMP Separation and Mass Spectrometry. Isolation of the active component in the HMP preparation was performed initially by HPLC with a C_{18} reversed-phase Agilent Zorbax Eclipse AAA column, 2.1 × 150 mm, with 3.5-μm particles at a flow rate of 0.45 mL/min. The bioactive, nonretained first-eluting injection peak subsequently was separated on an Agilent 1200 SL HPLC System with a normal-phase Agilent ZORBAX RX-SIL, 5 μm, 2.1 × 150 mm column. The flow rate was 0.45 mL/min with an acetonitrile/water gradient. Eluent was directly fed into the electrospray ionization (ESI) source of an Agilent 6220 orthogonal acceleration TOF instrument, with a regular electrospray source, operated in negative mode. MS was performed at the Department of Chemistry, University of Alberta, Edmonton, AB, Canada. The raw data were imported into IGOR Pro version 6.0 (Wavemetrics), and the spectrum was normalized to the highest signal.

Replication of HIV-1 LAV from Latently Infected A3.01 CD4$^+$ T Cells. ACH-2 cells seeded at 2.0×10^5 cells/mL were infected with Ng at the indicated MOI or were exposed to either increasing concentrations of HMP or 100 nM PMA as a positive control. The infection was stopped after 3 h with gentamicin, and the cells were incubated for an additional 4 d. Levels of protein 24 (p24) in the spent culture medium were quantified by ELISA.

Replication of NL4-3 from Acutely Infected CD4$^+$ T Cells. Generation of replication-competent HIV-1 X4 virus NL4-3 and spinoculation of Jurkat E6-1 cells were done as previously described (8). Virus-infected cells were coinfected with Ng at MOI = 2 or MOI = 10 or were treated with increasing concentrations of HMP. Cultures were maintained for 5 d with half of the medium changed after 3 d. Levels of p24 in the spent culture medium were quantified by ELISA.

HIV-1 p24 Antigen ELISA. The levels of HIV-1 present in culture supernatants were quantified by ELISA for p24 antigen using kits purchased from ZeptoMetrix or from Biological Products Laboratory as previously described (8).

Flow Cytometric Analysis of Surface CD69 Expression. A3.01 cells seeded at 1.0×10^6 cells/mL were infected at an MOI = 2 or MOI = 10 and were exposed to either increasing concentrations of HMP or 100 nM PMA as a positive control. The infection was stopped with gentamicin after 3 h. The proportion of the A3.01 cell population that expressed the cluster of differentiation 69 (CD69) early activation marker was determined 6 h postinfection by staining live with CD69-allophycocyanin (APC)–conjugated antibodies or isotype control (BD Biosciences) in HBSS (Invitrogen) containing 2% FBS (vol/vol) according to the manufacturer's directions. Cells were fixed with 4% (wt/vol) paraformaldehyde and analyzed by flow cytometry using a FACSCalibur with CellQuest software (Becton Dickinson) A minimum of 2.5×10^4 cells were analyzed for each sample. Further analysis was performed using FlowJo software (TreeStar).

Analysis of Cytokines by Multiplex ELISA. A3.01 cells seeded at 1.0×10^6 cells/mL were infected at an MOI = 2 or MOI = 10 or exposed to 20 μg/mL of HMP. After 3 h, infections were stopped with gentamycin. Culture supernatants were collected at 6 and 24 h, and the cytokines present were determined by multiplex ELISA using the 10-plex human Th_1/Th_2 plate (Meso Scale Discovery) according to the manufacturer's instructions and were read in a SECTOR Imager 2400 (Meso Scale Discovery).

Western Blot Analysis. A3.01 CD4$^+$ T cells were exposed to 10 ng/mL TNF-α or 20 μg/mL HMP or were infected with an MOI = 10 Ng per cell for the indicated periods of time. Whole-cell lysates were immunoblotted with antibodies (Cell Signaling Technology) directed against IκBα and its phosphorylated form, with anti-α/β tubulin antibodies used as a loading control.

Lentiviral Packaging and Jurkat 1G5 Lymphocyte Transfection. Lentiviral particles were containing *NFKB, RelA*, or scramble shRNAs were produced with a pLKO-based three-vector system (a gift

from J. Moffat, University of Toronto, Toronto, ON, Canada) in 293T cells as described (9) with the TransIT-LTI (Mirus Bio) transfection reagent according to the manufacturer's instructions. Virus-containing supernatants were collected 48 h after transfection. Jurkat 1G5 cells were treated with lentiviral supernatants in the presence of Polybrene (hexadimethrine bromide) (Sigma). Medium was changed 24 h post-transduction, and cells were maintained under puromycin selection for 72 h before used to assay for HIV-1 LTR expression. RNAi was confirmed by qRT-PCR.

Statistical Analysis. Multiple group comparisons were made using one-way or two-way (ANOVA) followed by Dunnett's, Newman–Keuls or Bonferroni post tests where appropriate, using Prism v5.0 (GraphPad Software). In all cases, a P value <0.05 was considered significant.

1. Aguilar-Cordova E, Chinen J, Donehower L, Lewis DE, Belmont JW (1994) A sensitive reporter cell line for HIV-1 tat activity, HIV-1 inhibitors, and T cell activation effects. *AIDS Res Hum Retroviruses* 10(3):295–301.
2. Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).
3. Dillard JP (2005) Genetic manipulation of *Neisseria gonorrhoeae*. *Current Protocols in Microbiology*, (John Wiley & Sons, Inc., New York), pp 4A.2.1–4A.2.19.
4. Tettelin H, et al. (2000) Complete genome sequence of *Neisseria meningitidis* serogroup B strain MC58. *Science* 287(5459):1809–1815.
5. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215(3):403–410.
6. Sechman EV, Rohrer MS, Seifert HS (2005) A genetic screen identifies genes and sites involved in pilin antigenic variation in *Neisseria gonorrhoeae*. *Mol Microbiol* 57(2): 468–483.
7. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25(4): 402–408.
8. Dobson-Belaire WN, Cochrane A, Ostrowski MA, Gray-Owen SD (2011) Differential response of primary and immortalized CD4+ T cells to *Neisseria gonorrhoeae*-induced cytokines determines the effect on HIV-1 replication. *PLoS ONE* 6(4):e18133.
9. Moffat J, et al. (2006) A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen. *Cell* 124(6):1283–1298.

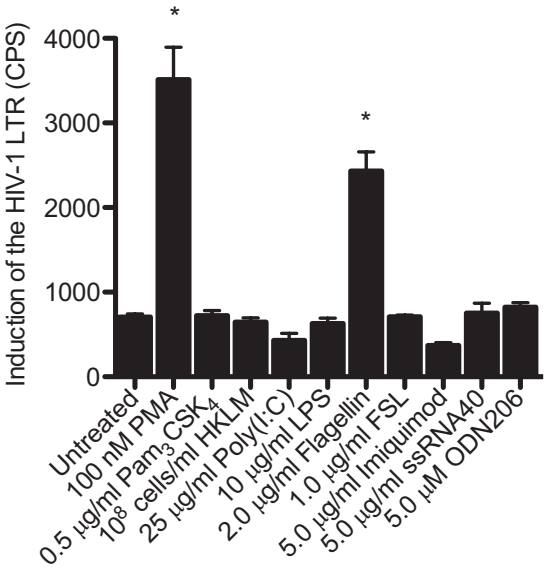

Fig. S1. The TLR5 agonist flagellin potently stimulates HIV-1 LTR expression in the Jurkat 1G5 model. HIV-1 LTR expression in response to a commercially prepared panel of innate immune agonists (Invivogen) was quantified by luciferase assay. PMA was used as a positive control. Data shown are the counts per second of luminesce as a function of HIV-1 LTR expression. Data are representative of three independent experiments and are reported as the average of triplicate samples ± SD. $^{**}P < 0.05$ versus untreated; ANOVA, Dunnett.

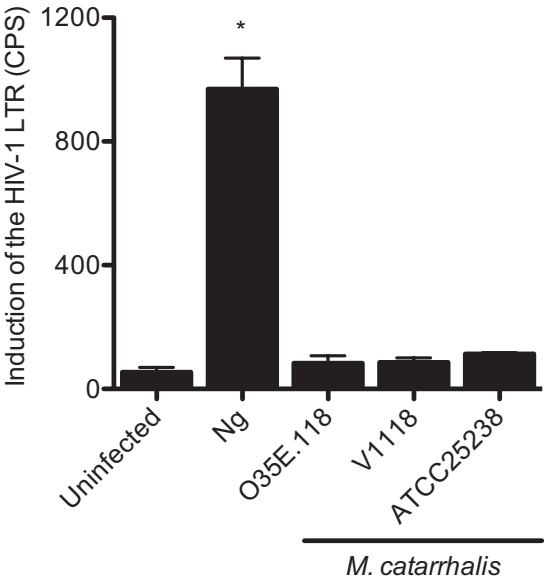

Fig. S2. *M. catarrhalis* strains do not induce HIV-1 LTR expression. HIV-1 LTR expression in Jurkat 1G5 $CD4^+$ T cells in response to infection with a MOI = 10 of different *M. catarrhalis* strains was quantified by luciferase assay. Ng at MOI = 10 was used as a positive control. Data shown are the counts per second of luminesce as a function of HIV-1 LTR expression. The average of triplicate samples is reported ± SD, representative of three independent experiments. *$P < 0.05$ versus untreated; ANOVA, Dunnett.

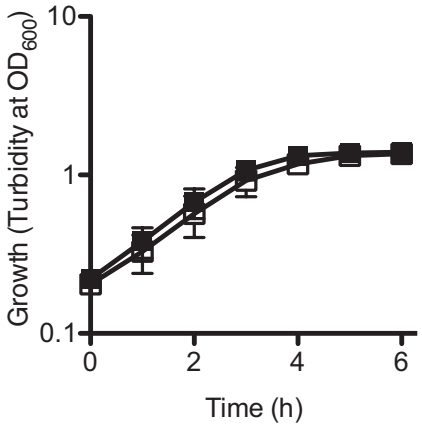

Fig. S3. Growth of *hldA* mutants. The growth of the parent and mutant strains was monitored by culture turbidity at an optical density at 550 nm in brain heart infusion broth supplemented with 1% IsoVitaleX at 37 °C. ■, Nm; □, Nm *hldA*::Tn*5*. Data shown are the mean of three individual experiments ± SEM.

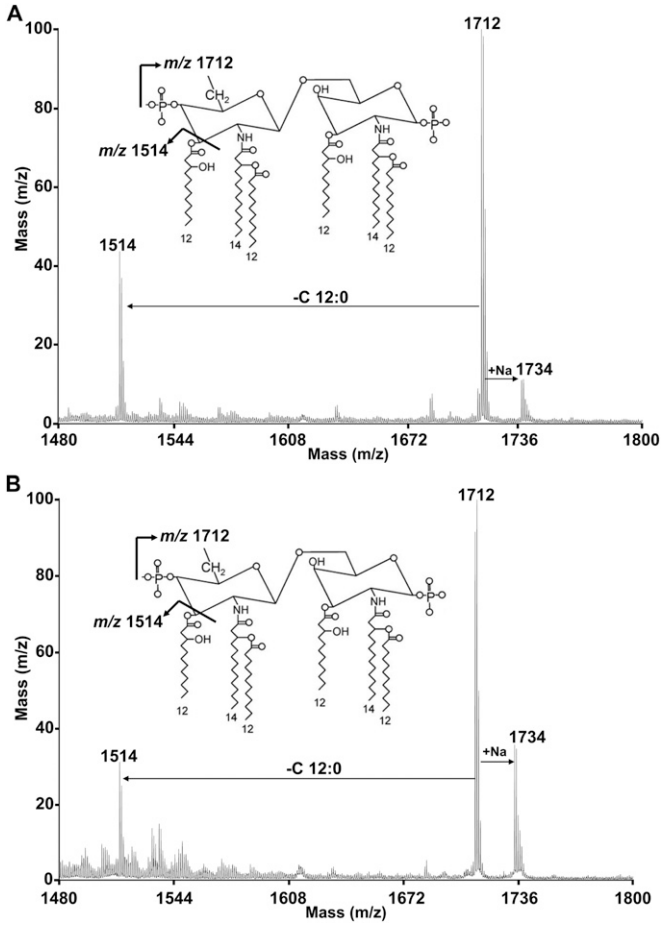

Fig. S4. The Nm *hldA*::Tn*5* mutant retains complete lipid A. (*A* and *B*) MALDI-MS of (*A*) Nm wild-type and (*B*) *hldA*::Tn*5* mutant lipid A moieties, isolated as described from heat-killed cells. The peak at 1,712 *m/z* reflecting lipid A and the fragmentation pattern indicating loss of a C12:0 fatty acid are illustrated.

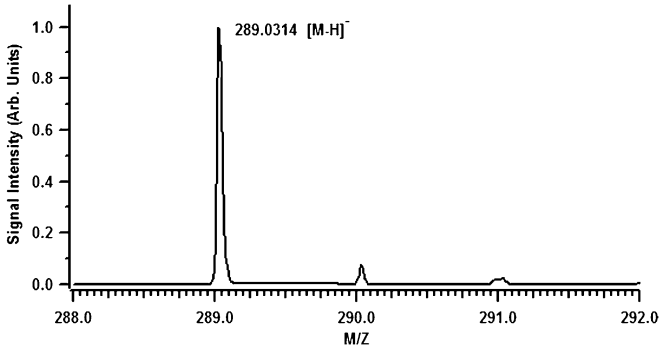

Fig. S5. MS analysis of HMP. Negative ESI-MS signal of a component consistent with HMP was observed in the active fraction of a normal-phase HPLC LC-MS separation. The raw data were imported into IGOR Pro (Wavemetrics), and the spectrum was normalized to the highest signal. Observed signal: *m/z* 289.0314; theoretical expected value for the negatively-charded, deprotonated molecular ion $(M\text{-}H)^-$ of HMP (formula: $C_7H_{15}O_{10}P$): 289.0324; mass error: 3.7 ppm.

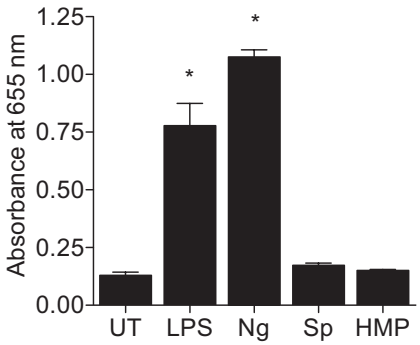

Fig. S6. HMP preparation does not contain bioactive endotoxin. HEK-Blue hTLR4 cells containing TLR4-induced secreted embryonic alkaline phosphatase reporter fusions were exposed to 0.1 ng/mL LPS, Ng, or *S. pneumoniae* (Sp, as a negative control), or 25 μg/mL HMP. The culture supernatants were mixed with QUANTI-Blue, and the colorimetric change, indicating TLR4 stimulation, was recorded at an optical density of 655 nm. Data shown are the mean of three replicates ± SD and are representative of three experiments. *$P < 0.05$ versus untreated (UT) cells; ANOVA, Dunnett).

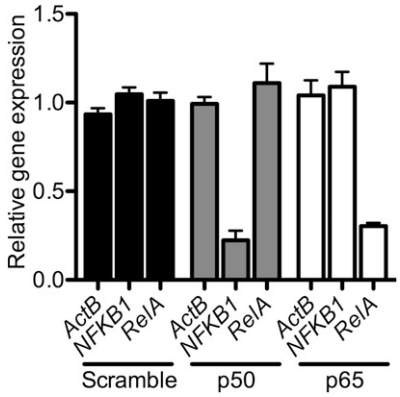

Fig. S7. Efficacy of shRNA-mediated knockdown of NF-κB subunits protein 50 (p50) and protein 65 (p65). Transcript levels were measured at 72 h post-transfection by qRT-PCR. Relative expressions of *ActB* as a housekeeping control, *NFKB1,* and *RelA* in each of the knocked-down cells were calculated using the $2^{-\Delta\Delta C_T}$ method (7), normalized to *GAPDH* as an endogenous reference gene, and compared with control 1G5 cells. Data represent the mean of three individual experiments, performed in triplicate, ± SEM.

Table S1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Source
Strain		
N. gonorrhoeae		
N302	*opaC::cat* derivative of MS11, pili^{-}, S-pilin^{+}, CHL^{r}	(1)
*hldA::*Tn*5*	*hldA::*Tn*5* derivative of N302, KAN^{r}	This study
N. meningitidis		
MC58	Invasive meningitis isolate	ATCC
B16B6	Meningitis isolate	ATCC
NRCC6274	*lsi-1::nptII* derivative of MC58, KAN^{r}	R. Moxon (University of Oxford, Oxford, UK)
*hldA::*Tn*5*	*hldA::*Tn*5* derivative of B16B6, KAN^{r}	This study
*hldA::*Tn*5::lac*	*hldA::*Tn*5* derivative of B16B6 with the region between *lctP* and *aspC* from pGCC4 integrated into the chromosome, CHL^{r}, KAN^{r}	This study
*hldA::*Tn*5::hldA*	*hldA::*Tn*5* derivative of B16B6, with a functional copy of *hldA,* under *lac* control, inserted back into the chromosome between *lctP* and *aspC,* CHL^{r}, KAN^{r}	This study
M. catarrhalis		
O12E	Otitis media isolate	(2)
O35E.118	Otitis media isolate	(3)
V1118	Nasopharynx isolate from a healthy child	(4)
ATCC25238	Type strain	ATCC
E. coli		
DH5α	Laboratory adapted derivative of K-12	Invitrogen
TOP10	Common cloning derivative of K-12	Invitrogen
N. lactamica 020–06	Nasopharynx isolate	(5)
S. pneumoniae ATCC 49619	Sputum isolate	ATCC, D. Pillai (University of Calgary, Calgary, Alberta, Canada)
H. influenzae 1128	Middle ear isolate	(6)
S. aureus ATCC 29213	Skin wound isolate	ATCC, D. Pillai
Plasmid		
pGCC4	For complementation in *Neisseria* spp. chromosome between *lctP* and *aspC*; *lac* promoter/operator; *lacI,* CHL^{r}, KAN^{r}	7)
pRM505	pGCC4 with a 1247 bp fragment containing NBM0825 for expressing *hldA* under *lac* control; *lac* promoter/operator; *lacI,* CHL^{r}, KAN^{r}	This study
pLKO.1	Series of vectors containing shRNA hairpins for RNAi. *NFKB1* hairpin: GCCTGAACAAATGTTTCATTT (TRCN0000006519), *relA* hairpin: GCCTTAATAGTAGGGTAAGTT (TRCN0000014683), and scramble hairpin: CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG, AMP^{r}, PURO^{r}	(8, 9)
psPAX2	Lentiviral packaging vector, AMP^{r}	Addgene
pMD2.G	Vesicular stomatitis virus-g expressing viral envelope vector, AMP^{r}	Addgene

AMP, ampicillin; ATCC, American Type Culture Collection; CHL, chloramphenicol; KAN, kanamycin; PURO, puromycin; ^{r}, resistance; RIF, rifampin.

1. Kupsch EM, Knepper B, Kuroki T, Heuer I, Meyer TF (1993) Variable opacity (Opa) outer membrane proteins account for the cell tropisms displayed by *Neisseria gonorrhoeae* for human leukocytes and epithelial cells. *EMBO J* 12(2):641–650.
2. Lafontaine ER, et al. (2000) The UspA1 protein and a second type of UspA2 protein mediate adherence of *Moraxella catarrhalis* to human epithelial cells in vitro. *J Bacteriol* 182(5): 1364–1373.
3. Lafontaine ER, Wagner NJ, Hansen EJ (2001) Expression of the Moraxella catarrhalis UspA1 protein undergoes phase variation and is regulated at the transcriptional level. *J Bacteriol* 183(5):1540–1551.
4. Attia AS, Ram S, Rice PA, Hansen EJ (2006) Binding of vitronectin by the *Moraxella catarrhalis* UspA2 protein interferes with late stages of the complement cascade. *Infect Immun* 74(3): 1597–1611.
5. Bennett JS, et al. (2010) Independent evolution of the core and accessory gene sets in the genus Neisseria: Insights gained from the genome of *Neisseria lactamica* isolate 020-06. *BMC Genomics* 11:652.
6. Sirakova T, et al. (1994) Role of fimbriae expressed by nontypeable *Haemophilus influenzae* in pathogenesis of and protection against otitis media and relatedness of the fimbrin subunit to outer membrane protein A. *Infect Immun* 62(5):2002–2020.
7. Dillard JP (2005) Genetic manipulation of *Neisseria gonorrhoeae. Current Protocols in Microbiology*, (John Wiley & Sons, Inc., New York), pp 4A.2.1–4A.2.19.
8. Moffat J, et al. (2006) A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen. *Cell* 124(6):1283–1298.
9. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307(5712):1098–1101.