Supporting Information

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SI Materials and Methods

Reagents. DNA primers, including a biotinylated DNA primer, were custom-synthesized at Sigma. Streptavidin-conjugated agarose beads and the Colloidal Blue Staining Kit were purchased from Invitrogen. The TALON Metal Affinity Resin was purchased from Clontech. PfuUltra High-Fidelity DNA Polymerase and the QuikChange Site-Directed Mutagenesis Kit were purchased from Agilent Technologies. Taq DNA polymerase, T4 polynucleotide kinase, and DNA endorestriction enzymes were purchased from New England Biolabs. The 4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF) was purchased from Merck. Strep-Tactin Sepharose and D-desthiobiotin were purchased from IBA BioTAGnology. [α-^{32}P]CTP and [γ-^{32}P]ATP were purchased from MP Biochemicals. σ^{70}-Saturated *Escherichia coli* RNA polymerase (RNAP) holoenzyme (σ^{70}-RNAP) and *E. coli* RNAP core enzyme (eCore) was purchased from EPICENTRE. Polyclonal mouse anti-GrgA antibody was a generous gift from Guangming Zhong (University of Texas Health Sciences Center, San Antonio, TX). Mouse anti-His antibody and horseradish-conjugated mouse anti-His antibody were purchased from Sigma-Aldrich and Clontech, respectively. The TEN buffer contained 40 mM Tris·HCl (pH 7.5), 1 mM EDTA, and 150 mM NaCl. Buffer I contained 10 mM Tris·HCl (pH 8.0), 10 mM $MgCl_2$, 1 mM EDTA, 10 mM 2-mercaptoethanol, and 7.5% (vol/vol) glycerol. The RB lysis buffer contained 10 mM Tris·HCl (pH 8.0), 0.6 M NaCl, 10 mM $MgCl_2$, 1 mM EDTA, 7.5% (vol/vol) glycerol, 0.1% (vol/vol) Nonidet P-40, 0.3 mM DTT, 1 mM AEBSF, 10 mg/mL pepstatin, and 150 mg/mL lysozyme; DTT, AESBF, pepstatin A, and lysosome were added just before use. The chlamydial RNAP (cRNAP) storage buffer contained 10 mM Tris·HCl (pH 8.0), 10 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM DTT, 100 mM NaCl, and 30% (vol/vol) glycerol. The TNG buffer contained 25 mM Tris acetate (pH 8.0), 150 mM NaCl, and 10% (vol/vol) glycerol.

Strains and Culture Conditions. *Chlamydia trachomatis* serovar L2 (L2; strain 434/Bu) was purchased from ATCC. HeLa cells, grown as adherent cultures, were used for the preparation of elementary body (EB) stocks (1, 2). For the preparation of cRNAP, and purification of PDF promoter-binding proteins, mouse L929 cell suspension cultures were infected with EBs at an inoculating dosage of 3 inclusion-forming units per cell (2, 3). *E. coli* ArcticExpress was purchased from Agilent and cultured with the LB media.

Vectors. Plasmids for expressing His- or Strep-tagged GrgA and σ^{66} and their mutants are listed in Table S1. For the construction of wild-type expression vectors, DNA fragments were amplified using PfuUltra, digested with one or two endorestriction enzymes, and ligated to properly prepared plasmids. Most deletion mutants with truncation at either one or both termini were constructed through a similar strategy, but some were made using the QuikChange site-directed mutagenesis kit. Point mutations were constructed using the QuikChange kit.

Preparation of Biotinylated DNA. Biotinylated DNA fragments were produced by Taq polymerase using a nonbiotinylated 5′ primer and a biotinylated 3′ primer designated PDF-TF-R-(5′-biotin), which carried a biotin residue at the 5′ position. The PCR products were purified using Qiagen PCR purification columns.

Preparation of PDF Promoter-Bound Agarose Beads. The above-purified biotinylated DNA was diluted 1:5 in TEN buffer. Streptavidin-conjugated agarose beads were washed twice with TEN buffer and then mixed with biotinylated DNA on a nutator at 4 °C. At 30 min later, beads were washed three times with TEN buffer to remove unbound DNA.

Preparation of Partially Purified Chlamydiae from Bulk Culture. At 22 h after inoculation, L2-infected L929 cells were collected by centrifugation at 2,500 × *g* for 10 min at 4 °C. All subsequent steps were performed on ice or at 4 °C. The pellet was resuspended in 8 mL of cold PBS (pH 7.4) per liter culture. Cells were disrupted using a Daigger GEX130 Ultrasonic processor. The cell suspension was subjected to three 10-s cycles of sonication with the energy intensity level set at 30%. The lysate was centrifuged at 1,000 × *g* for 10 min, and the supernatant was collected. The pellet was resuspended in another 8 mL PBS, and subjected to an additional round of sonication and centrifugation as described above. The two supernatants were combined and centrifuged at 18,000 × *g* for 15 min. The resulting pellet contained partially purified chlamydiae.

Purification of PDF Promoter-Binding Proteins. All procedures were performed on ice or at 4 °C. Chlamydiae partially purified from 12 L of suspension culture were resuspended in 24 mL reticulate body (RB) lysis buffer, and disrupted by ten 10-s sonication at 45% energy intensity. The lysate was centrifuged at 14,000 × *g* for 30 min. The supernatant was further clarified by four additional rounds of 15-min centrifugation. The supernatant resulted from the final round of centrifugation was diluted with 3 vol of buffer I. One-half of the diluted lysate was mixed with PDF promoter DNA-coated beads, prepared as described above, on a nutator (Clay Adams). At 1 h later, the beads were packed into a column, which were then washed with 20 resin vol of buffer I supplemented with 200 mM NaCl. The bound proteins were eluted using 4 vol of buffer I containing 600 mM NaCl. The elution was concentrated to 20 μL using Amicon centrifugal filters with a cutoff size of 3 kDa (Millipore). As control, nonbiotinylated PDF promoter DNA generated using the 5′ primer, PDF P1-F, and 3′, PDF-TF-R were added to the other half of the lysate, which were then mixed with plain streptavidin beads prewashed with TEN buffer. The remaining procedures for handling mock pull-down were the same as real pull-down.

LC-MS/MS. The concentrated pull-down samples were mixed with SDS/PAGE sample buffer and run ~1 cm into a Bis-Tris 10% polyacrylamide gel (Novex Biotech). The entire band was excised, and proteins in the gel were reduced, carboxymethylated, and digested with trypsin using standard protocols. Peptides were extracted, solubilized in 0.1% trifluoroacetic acid, and analyzed by nanoLC-MS/MS using rapid-separation LC (Dionex) interfaced with a LTQ Orbitrap Velos (ThermoFisher). Samples were loaded onto a self-packed 100-μm × 2-cm trap packed with Magic C18AQ, 5 μm, 200 Å (Michrom Bioresources Inc.) and washed with buffer A (0.2% formic acid) for 5 min with a flow rate of 10 μL/min. The trap was brought in-line with the analytical column (Magic C18AQ, 3 μm, 200 Å, 75 μm × 50 cm) and peptides fractionated at 300 nL/min with a 30-min linear gradient of 2–45% buffer B [0.08% formic acid, 80% (vol/vol) acetonitrile]. MS data were acquired using a data-dependent acquisition procedure with a cyclic series of a full scan acquired in Orbitrap with resolution of 60,000 followed by MS/MS scans (acquired in linear ion trap) of 20 most-intense ions with a repeat count of two and the dynamic exclusion duration of 60 s.

The LC-MS/MS data were searched against the TrEMBL L2 using a local version of the Global Proteome Machine (GPM USB; Beavis Informatics Ltd.) with carbamidoethyl on cysteine as fixed modification and oxidation of methionine and tryptophan as variable modifications using a 10-ppm precursor ion tolerance and a 0.4-Da fragment ion tolerance.

MALDI-TOF/TOF Mass Spectrometry. Protein sample was diluted with 10 vol of matrix solution [10 mg/mL sinapinic acid in 50% (vol/vol) acetylnitrole/0.1% trifluoric acid]. A total of 1 μL of the mix was loaded onto an Opti-TOF 384-well plate and air-dried. Spectra were acquired with a 4800 MALDI TOF/TOF analyzer (AB Sciex) using positive linear high-mass mode from 15 K to 100 K. Each spectrum reported was the average of spectra generated from 2,000 lacer shots. BSA was used as external calibration.

Preparation of cRNAP. cRNAP was prepared using a published protocol (4) with modifications. A typical cRNAP purification experiment starts with 4 L of suspension culture. Chlamydial organisms were partially purified as described above, resuspended in 8 mL of freshly prepared RB lysis buffer with the omission of NaCl, and disrupted by sonication. Cell debris was removed by centrifugation as described for the preparation of lysate for the purification of promoter-binding proteins. To the final supernatant was added one-ninth vol of buffer I containing 1.5 M NaCl, and then 4 mL of the heparin-conjugated agarose beads. Following 2 h of mixing, each milliliter of the beads was packed into a column and washed with 20 mL of buffer I containing 200 mM NaCl. cRNAP was eluted with buffer I containing 1.5 M NaCl and collected as 250-μL fractions. Corresponding fractions collected from different columns were combined and dialyzed overnight against the cRNAP storage buffer. Small aliquots were made and stored at −80 °C. Typically, fraction 5 was used for experiments.

Purification and Refolding of GrgA, σ^{70}, σ^{28} from Denatured Cell Extracts. ArcticExpress *E. coli* cells expressing His-tagged proteins were resuspended and lysed in 6 M guanidine hydrochloride solution containing 50 mM Hepes (pH 7.4) and 300 mM NaCl. After the removal of cell debris by centrifugation, the supernatant was incubated with TALON metal affinity resin on a nutator for 1 h at room temperature. Resin was washed five times with the guanidine hydrochloride solution, packed into a column, and eluted with 45 mM Hepes (pH 7.4) containing 270 mM NaCl and 150 mM imidazole. To refold GrgA, the elution was dialyzed first overnight against a solution containing 2 M urea, 25 mM Hepes (pH 7.4), 300 mM NaCl, 0.5 mM AEBSF, 1 mM reduced glutathione, and 10% (vol/vol) glycerol, and then for additional 4 h against 25 mM Hepes (pH 7.4) containing 300 mM NaCl and 10% (vol/vol) glycerol. The dialyzed GrgA was concentrated and exchanged to the TNG buffer before it was aliquoted and stored at −80 °C. Refold of σ^{70} and σ^{28} was accomplished by following procedures described by Panaghie et al. (5) and Yu and Tan (6), respectively.

In Vitro Transcription Assay. The assay in a total volume of 30 μL contained 400 ng supercoiled plasmid DNA, 1 mM potassium acetate, 8.1 mM magnesium acetate, 50 mM Tris acetate (pH 8.0), 27 mM ammonium acetate, 1 mM DTT, 3.5% (wt/vol) polyethylene glycol (average molecular weight, 8,000), 330 μM ATP, 330 μM UTP, 1 μM CTP, 0.2 μM [α-^{32}P]CTP (3,000 Ci/mmol), 100 μM 3′-*O*-methyl-GTP, 36 units of RNasin, RNAP, and indicated amount of GrgA or GrgA mutant, purified by procedures involving denaturing and refolding as described above. For reactions using cRNAP, the amount of cRNAP was 3.0 μL/reaction. For reactions using eCore and σ^{66}, their concentrations were 20 nM and 100 nM, respectively. When different amounts of GrgA were used, the amount of GrgA storage buffer (i.e., the TNG buffer) remained constant. All control reactions involving GrgA received equal volume of TNG buffer. The reaction was allowed to pursue at 37 °C for 30 min and terminated by the addition of 70 μL of 2.86 M ammonium acetate containing 4 mg of glycogen. After ethanol precipitation, ^{32}P-labeled RNA was resolved by urea–polyacrylamide gel electrophoresis, and visualized on a phosphorimager, and the intensities of the 157 base transcript bands were determined by ImageQuant software. Relative amounts of transcript were presented with that of the control reaction set as 1 unit. Data shown in bar graphs represent averages ± SDs from three or more independent experiments. Pairwise, two-tailed Student *t* tests were used to compare data from groups. Single and double asterisks indicate $P \leq 0.05$ and $P \leq 0.01$, respectively.

EMSA. EMSA was performed following a standard protocol (7). A 5′ primer designated PDF P1-F of the PDF promoter, was reacted with γ-[^{32}P]ATP in the presence of the T4 polynucleotide kinase. The resulting ^{32}P-labeled primer was used in conjunction with an unlabeled 3′ primer designated PDF-TF-R to amplify a DNA fragment containing the PDF promoter. The PCR-amplified fragment was purified with a Qiagen column. The GrgA-DNA binding reaction contained, in a total volume of 10 μL, 10 nM promoter fragment, an indicated amount of NH·GrgA, 1 mM potassium acetate, 8.1 mM magnesium acetate, 50 mM Tris acetate (pH 8.0), 27 mM ammonium acetate, 1 mM DTT, and 3.5% (wt/vol) polyethylene glycol (average molecular weight, 8,000), with or without 0.5 μg poly(dI-dC). After mixing for 1 h at 4 °C, the binding mixture was loaded onto 6% (wt/vol) nondenaturing polyacrylamide gel. Free and GrgA-bound DNA fragments were visualized on a Storm Phosphorimager (Molecular Dynamics).

DNA Pull-Down of GrgA. The 50-μL streptavidin-conjugated agarose beads were washed twice with buffer TEN and mixed with 40 pmol of a biotinylated DNA fragment on a nutator for 30 min at 4 °C. Beads were washed three times with buffer TEN to remove unbound DNA, and twice with transcription buffer. A 5-μg native purified NH·GrgA (full-length or deletion mutant) was added to the beads. The mixtures were incubated on a nutator for 1 h at 4 °C. After two washes with transcription buffer and three times with PBS containing 1% (vol/vol) Triton X-100 (PBST), GrgA was resolved by SDS/PAGE and detected by colloidal blue stain or Western blotting using HRP-conjugated anti-His.

GrgA Pull-Down of DNA. DNA fragments corresponding to different portions of the PDF gene were produced by PCR. Primers were removed by PCR purification columns (Qiagen). DNA were eluted with water and diluted in transcription buffer. A total of 2 μg NH·GrgA were incubated on a nutator with 0.5 μL mouse anti-His (Sigma) or 1.0 μL control normal mouse serum for 1 h at 4 °C, and then with 20 μL of protein A/G agarose (Sigma) for 2 h at 4 °C. The beads were washed four times with PBST, resuspended in the transcription buffer, and mixed with a DNA fragment (40 pmol) on a nutator for 2 h at 4 °C. After four washes with PBST, the reaction was heated at 95 °C for 5 min to dissociate DNA from GrgA. DNA was visualized after electrophoresis in a 1.5% (wt/vol) agarose gel containing ethidium bromide.

Preparation of Highly Purified EBs and RBs. HeLa cells were infected with L2. EBs and RBs were released from infected cells 36 h later by sonication. Host DNA and RNA were removed by incubating the lysates at 37 °C for 30 min after the addition of DNase (final concentration: 10 μg/mL) and RNase (100 μg/mL). The lysates were layered over 8 mL of 35% (vol/vol) RenoCal and centrifuged at 43,000 × *g* for 60 min in an SW28 rotor (Beckman Coulter). The EBs and RBs in the pellet were further purified by centrifugation through a RenoCal gradient (13 mL of 40%, 8 mL of 44%, and 5 mL of 52%) at 43,000 × *g* for 90 min in an SW28 rotor (8).

1. Balakrishnan A, et al. (2006) Metalloprotease inhibitors GM6001 and TAPI-0 inhibit the obligate intracellular human pathogen *Chlamydia trachomatis* by targeting peptide deformylase of the bacterium. *J Biol Chem* 281:16691–16699.
2. Bao X, et al. (2011) Non-coding nucleotides and amino acids near the active site regulate peptide deformylase expression and inhibitor susceptibility in *Chlamydia trachomatis*. *Microbiology* 157:2569–2581.
3. Fan H, Brunham RC, McClarty G (1992) Acquisition and synthesis of folates by obligate intracellular bacteria of the genus Chlamydia. *J Clin Invest* 90:1803–1811.
4. Tan M, Engel JN (1996) Identification of sequences necessary for transcription in vitro from the *Chlamydia trachomatis* rRNA P1 promoter. *J Bacteriol* 178: 6975–6982.
5. Panaghie G, Aiyar SE, Bobb KL, Hayward RS, de Haseth PL (2000) Aromatic amino acids in region 2.3 of *Escherichia coli* sigma 70 participate collectively in the formation of an RNA polymerase-promoter open complex. *J Mol Biol* 299:1217–1230.
6. Yu HH, Tan M (2003) Sigma28 RNA polymerase regulates hctB, a late developmental gene in Chlamydia. *Mol Microbiol* 50:577–584.
7. Galas DJ, Schmitz A (1978) DNAse footprinting: A simple method for the detection of protein-DNA binding specificity. *Nucleic Acids Res* 5:3157–3170.
8. Caldwell HD, Kromhout J, Schachter J (1981) Purification and partial characterization of the major outer membrane protein of *Chlamydia trachomatis*. *Infect Immun* 31:1161–1176.

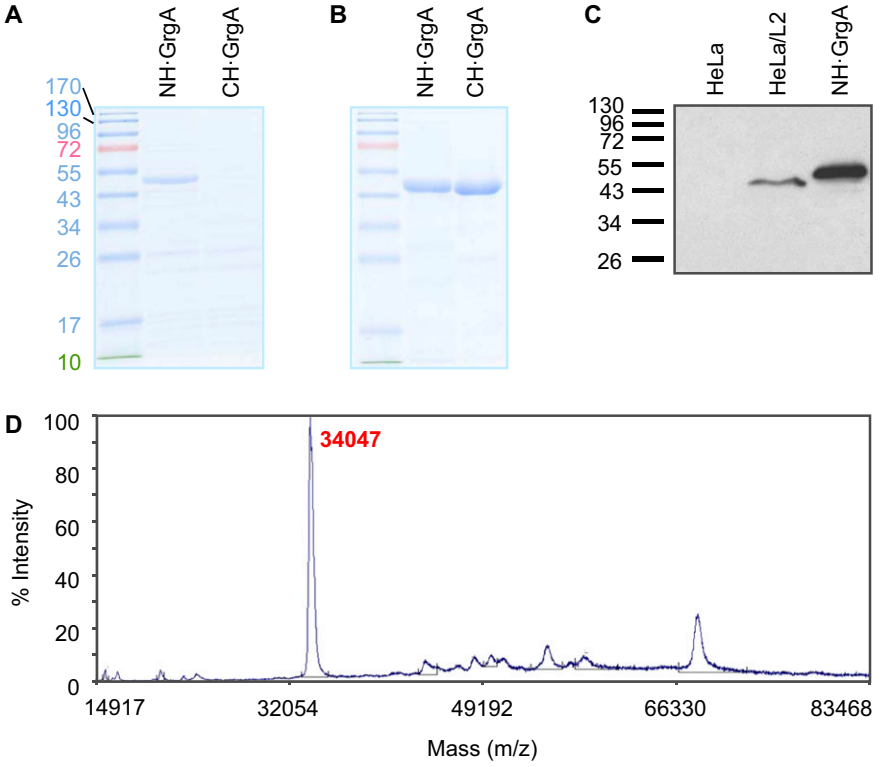

Fig. S1. Expression, purification, and molecular weight confirmation of GrgA. (*A*) Coomassie blue stain shows that the 34-kDa N-terminally His-tagged GrgA (NH·GrgA) purified from nondenatured cell extract migrated as a 47-kDa protein in SDS/PAGE, whereas no major protein band was observed for C-terminally His-tagged GrgA (CH·GrgA) using the same purification procedures. (*B*) NH·GrgA and CH·GrgA, purified from guanidine chloride-denatured cell extracts, migrated as a 47-kDa protein and a 46-kDa protein, respectively. Note that in NH·GrgA there is a 10-aa linker between the His-tag and GrgA, whereas no linker sequence existed between GrgA and the tag in CH·GrgA. (*C*) Western blotting showing that endogenous GrgA migrated as a 45-kDa protein. (*D*) MALDI-TOF/TOF mass spectrometry reveals that the major component in the NH·GrgA preparation had an expected 34,047-da molecular weight.

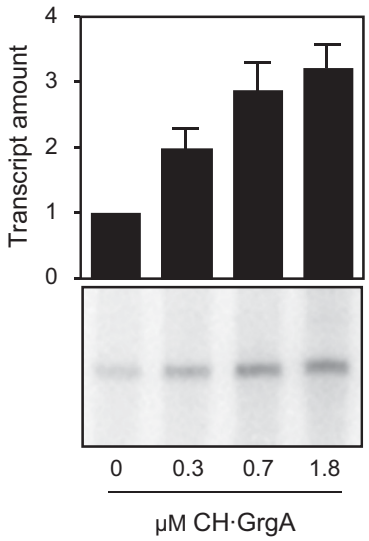

Fig. S2. GrgA with a C-terminal His-tag stimulates transcription from the *def*A promoter. Shown are in vitro transcription assays performed using cRNAP, a DNA template carrying the GR10 *def*A promoter variant, and the indicated concentration of C-terminally His-tagged GrgA (CH·GrgA). Graphs show the averages and SDs for three independent measurements.

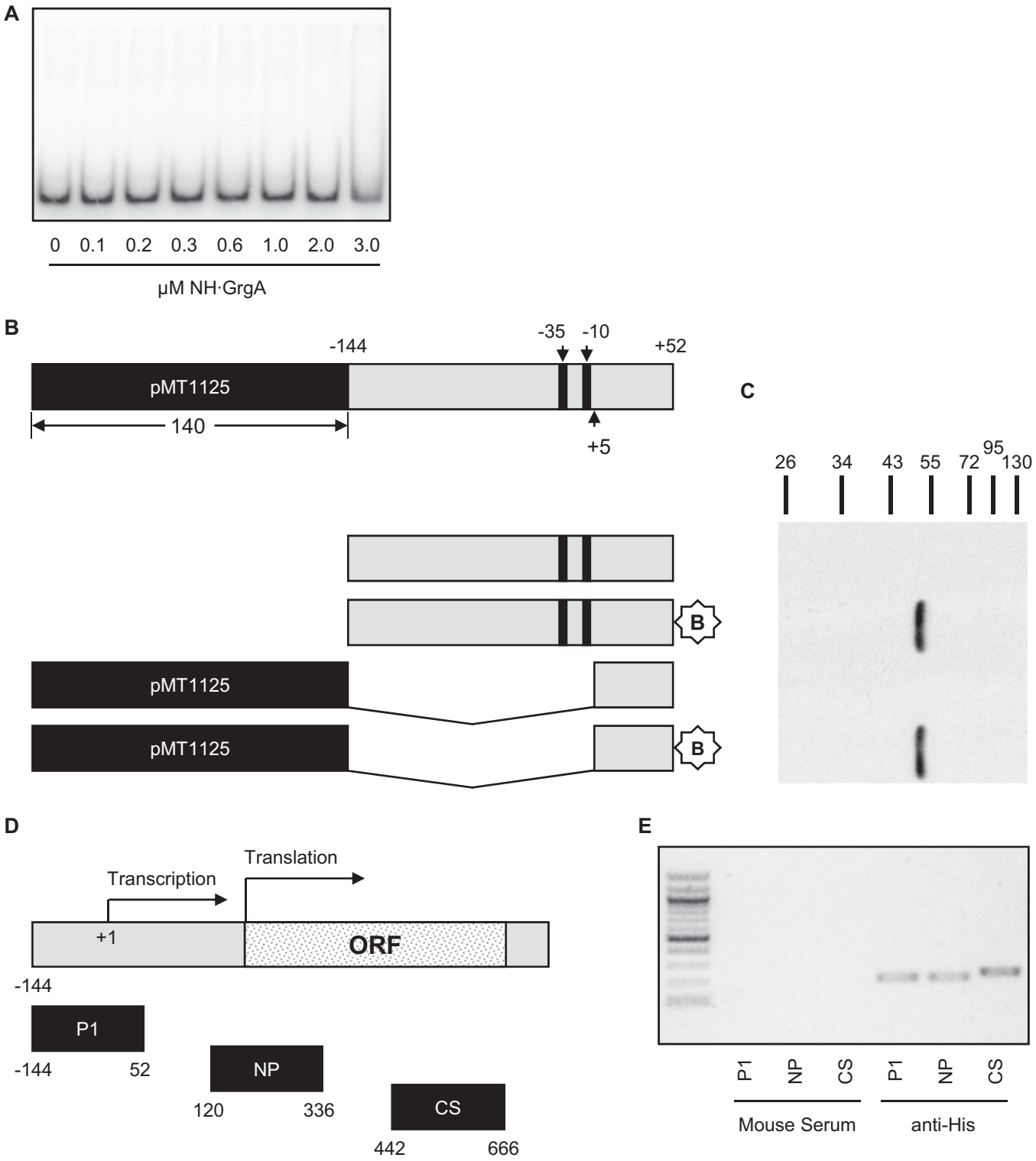

Fig. S3. Promoter-independent binding of DNA by GrgA. (*A*) Electrophoresis mobility shift of PDF promoter fragment by NH·GrgA in the presence of poly (deoxyinosinic-deoxycytidylic). (*B*) Schematic of the DNA fragments generated from transcriptional reporter plasmid vector pMT1125 that were used to precipitate NH·GrgA (star indicates the presence of a 3′ biotin moiety). (*C*) Western blot analysis of the amount of NH·GrgA precipitated by the corresponding DNA fragments in *B*. (*D*) Location of DNA fragments P1, NP, and CS with respect to the *def*A gene. (*E*) Precipitation of the P1, NP, and CS DNA fragments by anti-His protein A/G agarose-immobilized NH·GrgA. Substitution of anti-His with normal mouse serum resulted in loss of DNA precipitation. Left lane is the 100-bp molecular ladder.

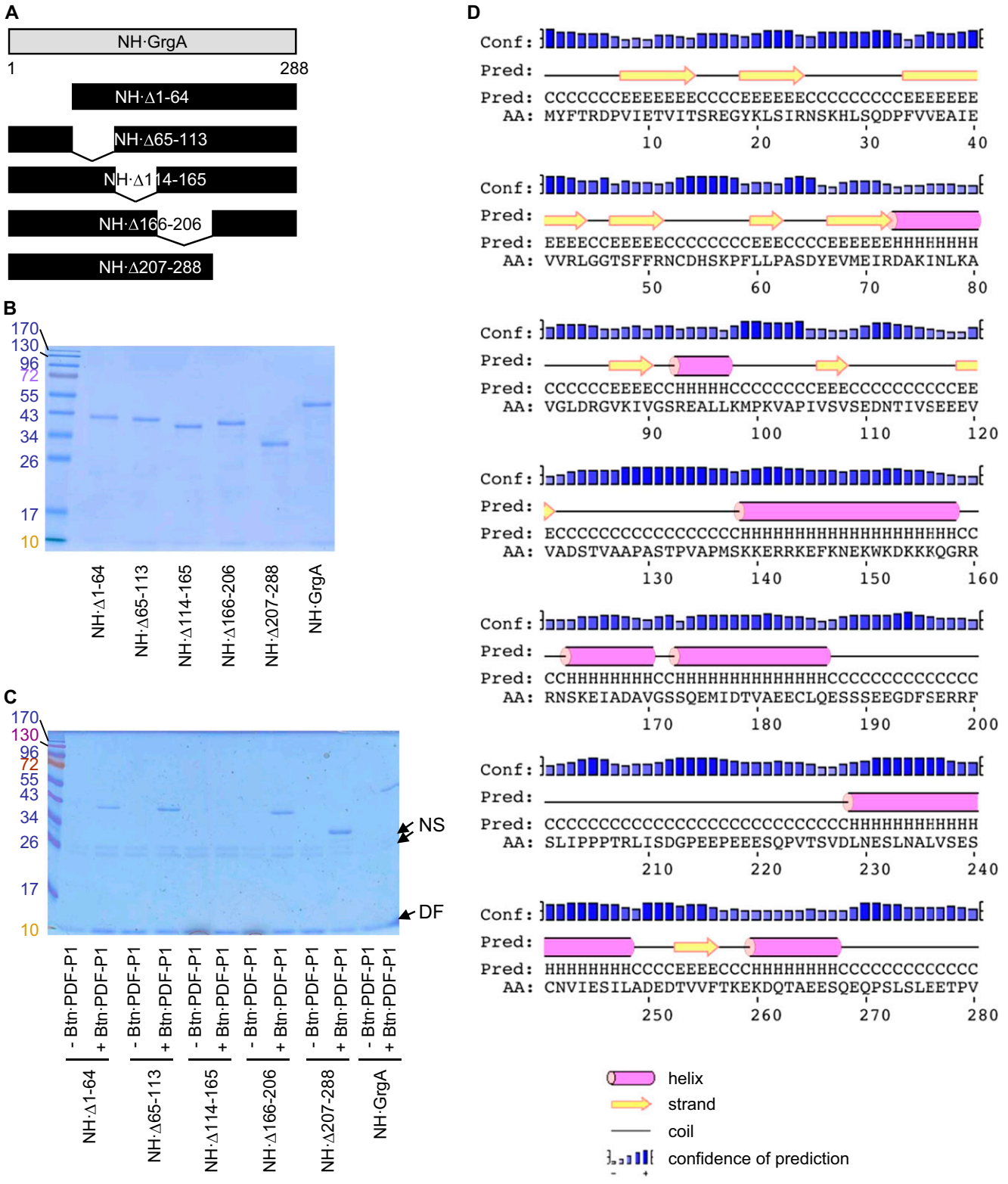

Fig. S4. Amino acids 114–165 in GrgA are required for DNA binding. (*A*) Schematic of the GrgA mutants lacking the indicated regions. (*B*) Coomassie blue stain of purified GrgA deletion constructs. (*C*) Deletion of amino acids 114–165 from NH·GrgA resulted in loss of precipitation by the PDF promoter fragment. Precipitated proteins were visualized by colloidal blue. DF, dye front; SN, nonspecific bands. (*D*) Prediction of GrgA as a potential helix-turn-helix protein by PSIPRED (1). Note that the predicted helix ranging from residues 139–158 is rich in positively charged lysine and arginine residues.

1. Jones DT (1999) Protein secondary structure prediction based on position-specific scoring matrices. *J Mol Biol* 292:195–202.

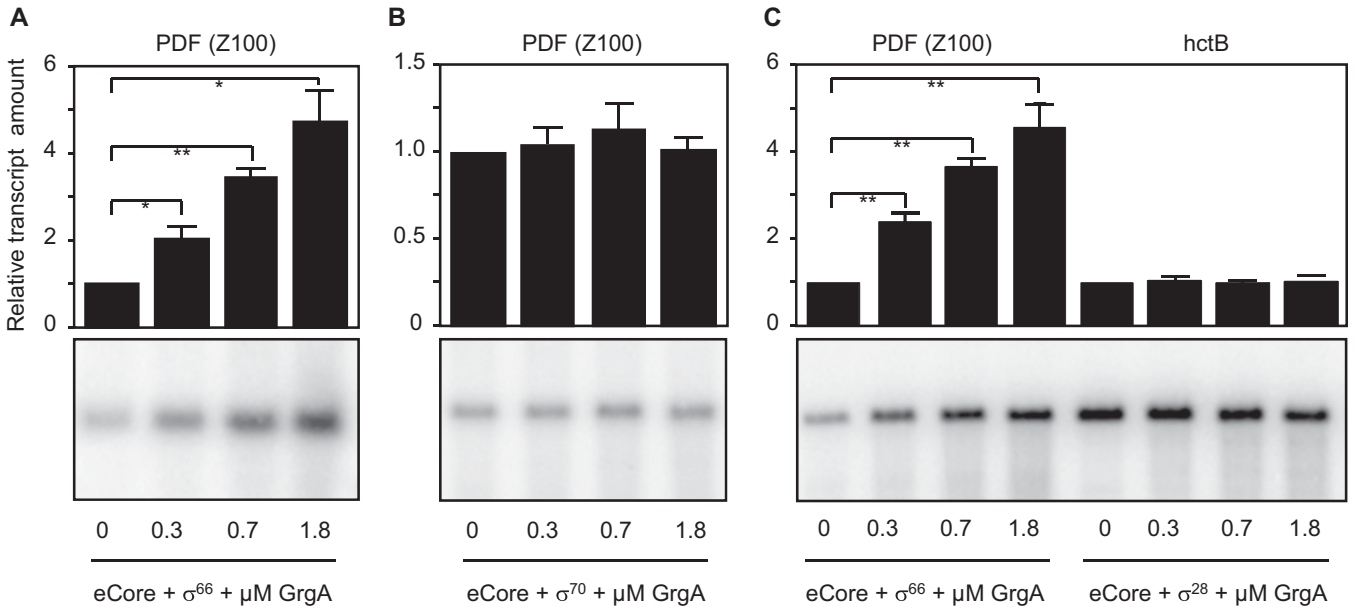

Fig. S5. Activation of PDF gene transcription by GrgA is σ^{66}-dependent. In vitro transcription assays performed using a hybrid holoenzyme consisting of *E. coli* core RNAP reconstituted with *C. trachomatis* σ^{66} (*A*), *E. coli* core RNAP reconstituted with *E. coli* σ^{70} (*B*), or *E. coli* core RNAP reconstituted with *C. trachomatis* σ^{28} (*C*). Reactions were done using a DNA template carrying the Z100 *def*A promoter variant (*A* and *B*, and *C Left*) or the hctB promoter (1) (*C Right*) in the presence of the indicated concentration of NH·GrgA. Graphs show the averages and SDs for three independent measurements.

1. Yu HH, Tan M (2003) Sigma28 RNA polymerase regulates hctB, a late developmental gene in Chlamydia. *Mol Microbiol* 50:577–584.

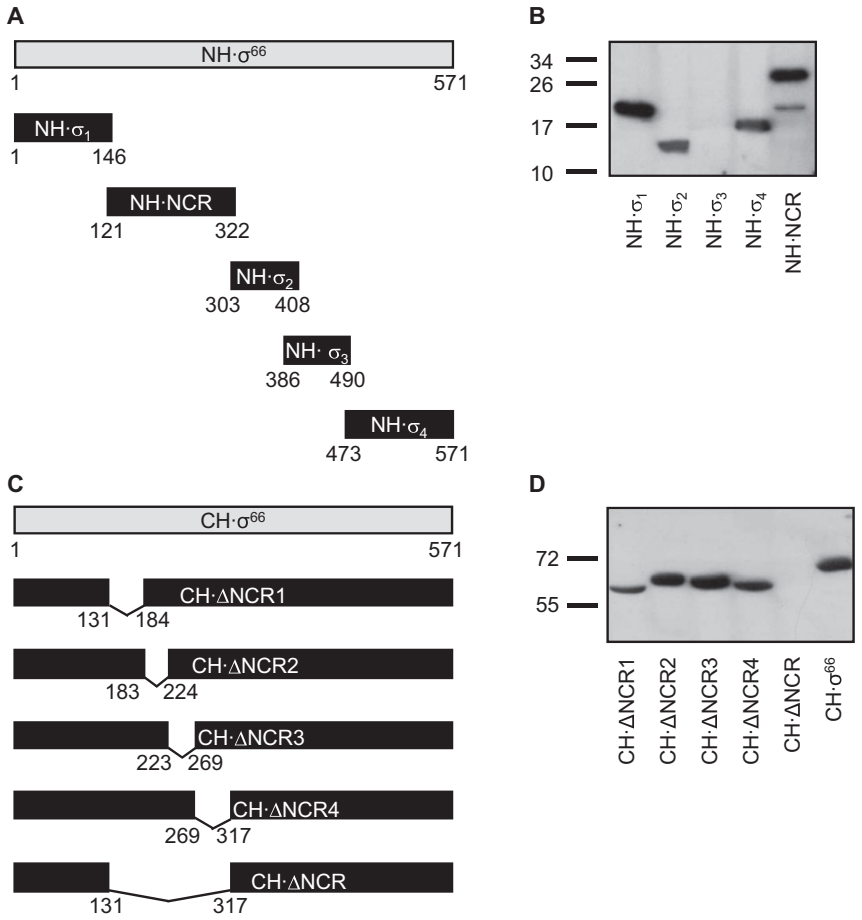

Fig. S6. Expression analyses of σ^{66} mutants in *E. coli*. (*A*) Schematic of the N-terminally His-tagged σ^{66} fragments constructed on the basis of the functional domains of the *E. coli* homolog σ^{70}. (*B*) Western blot analysis of the σ^{66} fragments indicate that all individual domains but σ_3 were successfully expressed. Shown are Western blots of crude *E. coli* extracts detected with an anti-His antibody. (*C*) Schematic of the σ^{66} mutants with nonconserved region (NCR) deletions. (*D*) Western blot analysis of the σ^{66} variants with subregion deletions indicate that only the variant lacking the entire NCR could not be expressed. Shown are Western blots of crude *E. coli* extracts detected with an anti-His antibody.

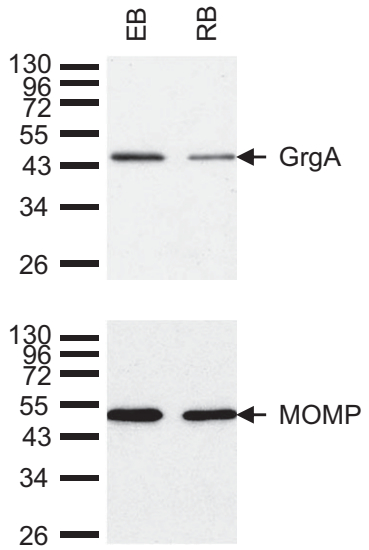

Fig. S7. Detection of GrgA in EBs and RBs. The two chlamydial cellular forms were highly purified by two rounds of RenoCal gradient ultracentrifugation. Western blot analysis was done using an antibody against GrgA or an antibody against the chlamydial major outer membrane protein (MOMP).

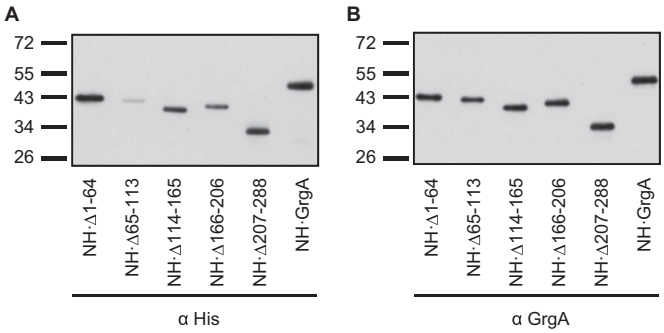

Fig. S8. His-tagged GrgA deletion constructs are recognized by the anti-His antibody with varying efficiencies. Western blot analysis of indicated His-tagged GrgA derivative using an anti-His antibody (*A*) or a polyclonal mouse anti-GrgA antibody (*B*). A total of 20 ng of each recombinant protein was loaded.

Table S1. Vector information

Type/designation	Description	Source
Transcriptional reporter		
pMT1125	Plasmid harboring a promoterless guanine (G)-less cassette	(1)
pMT1125-WT	pMT1125 with wild-type cPDF promoter leading the G-less cassette	(2)
pMT1125-GR10	Same as pMT1125-WT except it contains a C→A UP element mutation	(2)
pMT1125-Z100	Same as pMT1125-WT except it contains a C→A −35 element mutation	(2)
pMT1125-rRNA-P1	pMT1125 with chlamydial rRNA P1 promoter leading the G-less cassette	This study
pMT1187	pMT1125 with ompA promoter leading the G-less cassette	(3)
pMT1185	pMT1125 with chlamydial hctA promoter leading the G-less cassette	(3)
pMT1125-WT-Δ(−144 through −105)	pMT1125-WT derivative with deletion of −144 through −105 from cPDF promoter	This study
pMT1125-WT-Δ(−144 through −65)	pMT1125-WT derivative with deletion of −144 through −65 from cPDF promoter	This study
pMT1125-WT-Δ(−144 through −29)	pMT1125-WT derivative with deletion of −144 through −29 from cPDF promoter	This study
pMT1212	Transcriptional reporter plasmid for the chlamydial hctB promoter recognized by s28	(4)
Wild-type GrgA and mutant expression		
pET28a	For expression of N-terminally His-tagged proteins in *E. coli*	Novogen, Ltd.
pET28a-GrgA	GrgA ORF inserted between NdeI and XhoI sites of pET28a for expression of N-(His)6-tagged GrgA (NH·GrgA)	This study
pET28a-GrgAΔ(1–64)	For expression of NH·Δ1–64; derived by deleting residues 1–64 from NH·GrgA in pET28a-GrgA	This study
pET28a-GrgAΔ(65–113)	For expression of NH·Δ65–113; derived by deleting residues 65–113 from NH·GrgA in pET28a-GrgA	This study
pET28a-GrgAΔ(114–165)	For expression of NH·Δ114–165; derived by deleting residues 114–165 from NH·GrgA in pET28a-GrgA	This study
pET28a-GrgAΔ(166–206)	For expression of NH·Δ166–206; derived by deleting residues 106–206 from NH·GrgA in pET28a-GrgA	This study
pET28a-GrgAΔ(207–288)	For expression of NH·Δ207–288; derived by deleting residues 207–288 from NH·GrgA in pET28a-GrgA	This study
pET21c	For expression of C-terminally His-tagged proteins in *E. coli*	Novogen, Ltd.
pET21c-GrgA	For expression of CH·GrgA; GrgA ORF inserted between NdeI and XhoI sites of pET21c	This study
pNS·GrgA	For expression of NS·GrgA; derived by inserting a strep tag to the BamHI site of pET21c-GrgA and deleting its C-terminal His-tag	This study
Expression of σ factors		
pCS·σ66	For expression of CS·σ66 using NdeI and BamHI site in pET21c with deleted His-tag	This study
pCOLADuet	For expression His-tagged and/or untagged proteins in *E. coli*	Novogen, Ltd.
pCOLADuet-σ66R1	For expression of region 1 (residues 1–146) of σ66 (NH·R1) using BamHI and NotI sites of pCOLADuet	This study
pCOLADuet-σ66R2	For expression of region 2 (residues 308–408) of σ66 (NH·R2) using BamHI and NotI sites of pCOLADuet	This study
pCOLADuet-σ66R3	For expression of region 3 (residues 386–490) of σ66 (NH·R3) using BamHI and NotI sites of pCOLADuet	This study
pCOLADuet-σ66R4	For expression of region 4 (residues 473–571) of σ66 (NH·R4) using BamHI and NotI sites of pCOLADuet	Novogen, Ltd.
pCOLADuet-σ66NCR	For expression of nonconserved region (residues 121–322) of σ66 (NH·NCR) using BamHI and NotI sites of pCOLADuet	This study
pET21c-σ66	For expression of C-terminal His-tagged σ66 (CH·σ66); σ66 ORF was inserted between NdeI and XhoI sites of pET21c	This study
pET21c-σ66-ΔNCR	For expression of CH·ΔNCR, σ66 lacking NCR (residues 132–316); derived from pET21c-σ66	This study
pET21c-σ66-ΔNCR1	For expression of CH·ΔNCR1, σ66 lacking residues 132–183 within the NCR; derived from pET21c-σ66	This study
pET21c-σ66-ΔNCR2	For expression of CH·ΔNCR1, σ66 lacking residues 184–223 within the NCR; derived from pET21c-σ66	This study
pET21c-σ66-ΔNCR3	For expression of CH·ΔNCR3, σ66 lacking residues 224–268 within the NCR; derived from pET21c-σ66	This study
pET21c-σ66-ΔNCR4	For expression of CH·ΔNCR4, σ66 lacking residues 269–316 within the NCR; derived from pET21c-σ66	This study
pLHN12-N-His-σ^{70}	For expression of N-terminally His-tagged *E. coli* σ70	(5)
pET28a-σ28	For expression of N-terminally His-tagged chlamydial σ28	This study

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