Supplemental Tables 1 and 2

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Orientation of *Pseudomonas aeruginosa* ExsA monomers bound to promoter DNA and base-specific contacts with the P_{exoT} promoter

Table S1. Bacterial strains, reporters, and plasmids used in this study.

Bacterial strains	Relevant characteristics	Reference
PA1O3	wt strain, burn wound isolate	(3)
PA1O3 *exsA*::Ω	chromosomal interposon insertion in *exsA*	(2)

Reporter fusions	Integration Plasmid	Experiment	Primer Pair	Reference
$P_{exsD\text{-}lacZ}$	mini-CTX-$P_{exsD\text{-}lacZ}$ (-152 to + 16)	Fig. 2A	NA	(4)
$P_{exoT\text{-}lacZ}$	mini-CTX-$P_{exoT\text{-}lacZ}$ (-179 to + 17)	Fig. 3-6, S2	NA	(1)
EB127	mini-CTX-$P_{exoT\text{-}lacZ}$ (C45A)	Fig. 3C, 4B, 5, 7, S2	NA	(1)
EB134	mini-CTX-$P_{exoT\text{-}lacZ}$ (G47A)	Fig. 3C, 4B, 5, 7, S2	NA	(1)
EB150	mini-CTX-$P_{exoT\text{-}lacZ}$ (A34C)	Fig. 3D, 4B, 5, 7, S2	50405766-43613960	This study
EB126	mini-CTX-$P_{exoT\text{-}lacZ}$ (C50G)	Fig. 4, 5, 7, S2	NA	(1)
EB135	mini-CTX-$P_{exoT\text{-}lacZ}$ (T38A)	Fig. 4, 5, 7, S2	NA	(1)
EB136	mini-CTX-$P_{exoT\text{-}lacZ}$ (G37C)	Fig. 4, 5, 7, S2	NA	(1)
EB137	mini-CTX-$P_{exoT\text{-}lacZ}$ (A34T)	Fig. 4, 5, 7, S2	NA	(1)
JK121	mini-CTX-$P_{exoT\text{-}lacZ}$ (C39A)	Fig. 4, 5, 7, S2	79346690-43613960	This study
JK122	mini-CTX-$P_{exoT\text{-}lacZ}$ (A36C)	Fig. 4, 5, 7, S2	79346689-43613960	This study
JK123	mini-CTX-$P_{exoT\text{-}lacZ}$ (T35G)	Fig. 4, 5, 7, S2	79346688-43613960	This study
$P_{exsC\text{-}lacZ}$	mini-CTX-$P_{exsC\text{-}lacZ}$ (-186 to + 17)	Fig. 4B, 6A-B	NA	(4)

Expression Plasmids	Experiment	Primer or Primer Pair	Reference
pJN105	Fig. 2A, 3B-D, 4C, 6A-B	NA	(5)
pEB124 (wt *exsA* expression vector)	Fig. 2A	NA	(1)
pEB124 Δcys	Fig. 2A	multiple	This study
pEB124 Δcys E193C	Fig. 2A	38799024	This study
pEB124 Δcys M196C	Fig. 2A	38799023	This study
pEB124 Δcys G244C	Fig. 2A	38799022	This study
pEB124 Δcys S246C	Fig. 2A	38799021	This study
pET16b *exsA*	Fig. 2B, 8	NA	(1)
pET16b *exsA* Δcys M196C	Fig. 2B	NA	This study
pET16b *exsA* Δcys S246C	Fig. 2B	NA	This study
pEB124 K202R	Fig. 3B	Isolated from screen	This study

Table S1 (continued)

Expression Plasmids	Experiment	Primer or Primer Pair	Reference
pEB124 T199S	Fig. 3C	47632614	This study
pEB124 T252S	Fig. 3D	51494557	This study
pEB124 K202A	Fig. 4C, 5	51222351-42148424	This study
pEB124 T199A	Fig. 4C, 5	51222353-42148424	This study
pEB124 T252A	Fig. 4C, 5	51494557-42148424	This study
pEB124 L198A	Fig. 6A, 7	51222354-42148424	This study
pEB124 T200A	Fig. 6A, S2	51222352-42148424	This study
pEB124 E203A	Fig. 6A	Isolated from screen	This study
pEB124 L204A	Fig. 6A, S2	51222350-42148424	This study
pEB124 G206A	Fig. 6A	51222349-42148424	This study
pEB124 S207A	Fig. 6A	51222348-42148424	This study
pEB124 V208A	Fig. 6A, S2	51222347-42148424	This study
pEB124 Q248A	Fig. 6B, 7	51222346-42148424	This study
pEB124 S249A	Fig. 6B, S2	43687091-42148424	This study
pEB124 Y250A	Fig. 6B, 7	51494559-42148424	This study
pEB124 Q253A	Fig. 6B	43687088-42148424	This study
pEB124 S254A	Fig. 6B	51222345-42148424	This study
pEB124 R256A	Fig. 6B, S2	51222344-42148424	This study
pEB124 R257A	Fig. 6B, 7	51222343-42148424	This study
pEB124 R258A	Fig. 6B, S2	51222342-42148424	This study
pET16b *exsA* L198A	Fig. 8, Table 1	42308574-80815243	This study
pET16b *exsA* T199A	Fig. 8, Table 1	42308574-80815243	This study
pET16b *exsA* K202A	Fig. 8, Table 1	42308574-80815243	This study
pET16b *exsA* Q248A	Fig. 8, Table 1	42308574-80815243	This study
pET16b *exsA* Y250A	Fig. 8, Table 1	42308574-80815243	This study
pET16b *exsA* T252A	Fig. 8, Table 1	42308574-80815243	This study
pET16b *exsA* R257A	Fig. 8, Table 1	42308574-80815243	This study

Table S2. Primers used in this study.

Primer ID	Name	Primer sequence
3242037	pD5'Hind10-15	5'-ACAGAAGCTTTTCCAGCCAGTCCTATTTCACC
22963127	P_{exsC} Hind for	5'-ACGCAAGCTTATGAAGGACGTCCTGCAGCTCATCC
28433493	UY133B	5'-TTTGAATTCGGTGTATTCGTTCATGTCGCCC
28433494	UY133A	5'-ATATAAGCTTCGGAGGCCACGCTCGATACGC
29590679	AinternalstartXba5'	5'-CTGCACTCTAGAGCGGGGCATTCAAGGTACGACGGGAAGTGTTGG
29690545	EB126	5'-CGATCTAAGCTTGATAAAAAAACGACGGCCAATCCTGATAGGCGGAGGGGCGCCTCG
31216337	EB127	5'-AAAAAAACCACGGACAATCCTGATAGGCGGAGGGGC
31216338	P_{exoT} Hind for	5'-CGAGTAAGCTTGCCAATATCCCATCGGGTTCTCCGCCCCGG
31590566	EB137	5'-AAAAAAACCACGGCCAATCCTGATTGGCGGAGGGGC
31590567	EB136	5'-AAAAAAACCACGGCCAATCCTCATAGGCGGAGGGGC
31590568	EB135	5'-AAAAAAACCACGGCCAATCCAGATAGGCGGAGGGGC
31590569	EB134	5'-AAAAAAACCACAGCCAATCCTGATAGGCGGAGGGGC
42148424	exsA3'SacI	5'-GTGCGAGCTCCTCGTCAGAGCCTGCAATTTGG
42308574	exsA5'Nde	5'-GTCACATATGCAAGGAGCCAATC
43613960	P_{exoT} EcoR1 rev	5'-CGACGAATTCGACGTCTCCTGATGTTTCCCCGCCAGTCTAGGAACG
49188918	pC3'EcoRI	5'-TGATGAATTCGCCTCCTAAAGCTCAGCGCATGC
49865141	pD3'Eco(2)	5'-CGACTCGAATTCTCTGCCTTGGCTTCCTCACTAC
50405765	MPpT(A26G)	5'-AAAAAAACCACGGCCAATCCTGATGGGCGGAGGGGC
79346688	minictxJK123	5'-AAAAAAACCACGGCCAATCCTGAGAGGCGGA
79346689	minictxJK122	5'-AAAAAAACCACGGCCAATCCTGCTAGGCGGA
79346690	minictxJK121	5'-AAAAAAACCACGGCCAATCATGATAGGCGGA
80815243	exsA3'BamIN	5'-GTGCGGATCCCTCGTCAGAGCCTGCAATTTG
Site-directed mutagenesis of exsA		
36672411	ExsA (C15A)	5'-TGGCCGAAAGCAGATAACGTCTGCTCATTGGAACATTCCAA
36672410	ExsA (C50A)	5'-CAGGACATCGATTCCACTTTTGCCCTGGCGCCTG
36672409	ExsA (C102A)	5'-GTTGAGTGAAGTCGAGCGTGCCGACGAGCCCG
36672408	ExsA (C121A)	5'-CCTCTGCTGGCCGGTGCCGTCAAGGGGTTG
36672407	ExsA (C139A)	5'-CGCCGATGCTCGCCGCCCTGAAGATCGAGG
36672406	ExsA (C261A)	5'-CCGCCGTTTCGGCGCCACACCGAGCCGC
36672405	ExsA (C271A)	5'-GCGGCAGGGGAAGGACGAAGCCCGGGCTAAA

Table S2 (continued)

Primer ID	Name	Primer sequence
38799021	ExsA (S246C)	5’-ATGGAGGCGGGCTTTTGCAGCCAGTCCTATTTCACCC
38799022	ExsA (G244C)	5’-CGCCATGGAGGCGTGCTTTTCCAGCCAGTCC
38799023	ExsA (M196C)	5’-CCGCGAGTTCGGCTGCGGGCTGACCACC
38799024	ExsA (E193C)	5’-CGACTTCTCCCGCTGCTTCGGCATGGGGC
47632614	A-T199S-top	5’-CGGCATGGGGCTGAGCACCTTCAAGGAGC
51494557	ExsA_T252Stop	5’-CCAGCCAGTCCTATTTCAGCCAGAGCTATCGCCGCCGTTTCGGC
RH1 and RH2 directed mutagenesis		
42128175	Mp_exsA_S254	5’-CCAGCCAGTCCTATTTCACCCAGNNNTATCGCCGCCGTTTCGGC
42128176	Mp_exsA_Q253	5’-CCAGCCAGTCCTATTTCACCNNNAGCTATCGCCGCCGTTTCGGC
42128177	Mp_exsA_T252	5’-CCAGCCAGTCCTATTTCNNNCAGAGCTATCGCCGCCG
42128178	Mp_exsA_Y250	5’-GGAGGCGGGCTTTTCCAGCCAGTCCNNNTTCACCCAGAGCTATCGCC
42128179	Mp_exsA_S249	5’-GGAGGCGGGCTTTTCCAGCCAGNNNTATTTCACCCAGAGC
42128180	Mp_exsA_Q248	5’-GGAGGCGGGCTTTTCCAGCNNNTCCTATTTCACCCAGAGC
42128181	Mp_exsA_L204	5’-CGGCATGGGGCTGACCACCTTCAAGGAGNNNTTCGGCAGTGTCTACGGGG
42128182	Mp_exsA_E203	5’-CGGCATGGGGCTGACCACCTTCAAGNNNCTGTTCGGCAGTGTCTACGGGG
42128183	Mp_exsA_K202	5’-CGGCATGGGGCTGACCACCTTCNNNGAGCTGTTCGGCAGTGTCTACGGGG
42128184	Mp_exsA_T200	5’-CGGCATGGGGCTGACCNNNTTCAAGGAGCTGTTCGGC
42128185	Mp_exsA_T199	5’-CCCGCGAGTTCGGCATGGGGCTGNNNACCTTCAAGGAGCTGTTCGGC
42128186	Mp_exsA_L198	5’-CCCGCGAGTTCGGCATGGGGNNNACCACCTTCAAGGAGCTGTTCGGC
Alanine scanning mutagenesis of RH1 and RH2		
43687088	Mp_exsA_Q253	5’-CCAGCCAGTCCTATTTCACCGCCAGCTATCGCCGCCGTTTCGGC
43687089	Mp_exsA_T252	5’-CCAGCCAGTCCTATTTCGCCCAGAGCTATCGCCGCCG
43687090	Mp_exsA_Y250	5’-GGAGGCGGGCTTTTCCAGCCAGTCCGCCTTCACCCAGAGCTATCGCC
43687091	Mp_exsA_S249	5’-GGAGGCGGGCTTTTCCAGCCAGGCCTATTTCACCCAGAGC
51222346	Mp_exsA_Q248	5’-GGAGGCGGGCTTTTCCAGCGCCTCCTATTTCACCCAGAGC
51222347	Mp_exsA_V208	5’-CCACCTTCAAGGAGCTGTTCGGCAGTGCCTACGGGGTTTCGCCGCGCGCCTGG
51222348	Mp_exsA_S207	5’-CCACCTTCAAGGAGCTGTTCGGCGCCGTCTACGGGGTTTCGCCGCGCGCCTGG
51222349	Mp_exsA_G206	5’-CCACCTTCAAGGAGCTGTTCGCCAGTGTCTACGGGGTTTCGCCGCGCGCCTGG
51222350	Mp_exsA_L204	5’-CGGCATGGGGCTGACCACCTTCAAGGAGGCCTTCGGCAGTGTCTACGGGG

Table S2 (continued)

Primer ID	Name	Primer sequence
51222351	Mp_exsA_K202	5’-CGGCATGGGGCTGACCACCTTCGCCGAGCTGTTCGGCAGTGTCTACGGGG
51222352	Mp_exsA_T200	5’-CGGCATGGGGCTGACCGCCTTCAAGGAGCTGTTCGGC
51222353	Mp_exsA_T199	5’-CCCGCGAGTTCGGCATGGGGCTGGCCACCTTCAAGGAGCTGTTCGGC
51222354	Mp_exsA_L198	5’-CCCGCGAGTTCGGCATGGGGGCCACCACCTTCAAGGAGCTGTTCGGC
51222342	Mp_exsA_R258	5’-GCCAGTCCTATTTCACCCAGAGCTATCGCCGCGCCTTCGGCTGCACACCGAGCCGC
51222343	Mp_exsA_R257	5’-GCCAGTCCTATTTCACCCAGAGCTATCGCGCCCGTTTCGGCTGCACACCGAGCCGC
51222344	Mp_exsA_R256	5’-GCCAGTCCTATTTCACCCAGAGCTATGCCCGCCGTTTCGGCTGCACACCGAGCCGC
51222345	Mp_exsA_S254	5’-CCAGCCAGTCCTATTTCACCCAGGCCTATCGCCGCCGTTTCGGC

Table S3. Activity of alanine substitution mutants

ExsA	- Arabinose	+ Arabinose
None	25 ± 3	28 ± 4
wt	803 ± 52	37,849 ± 8,125
L198A	71 ± 11	8,524 ± 449
T199A	59 ± 3	5,215 ± 792
T200A	169 ± 38	9,835 ± 467
K202A	33 ± 2	877 ± 119
L204A	116 ± 9	5,194 ± 710
V208A	182 ± 10	12,477 ± 823
Q248A	35 ± 1	731 ± 14
S249A	124 ± 46	9,622 ± 717
Y250A	40 ± 2	743 ± 7
T252A	20 ± 1	83 ± 1
R256A	79 ± 3	8,263 ± 243
R257A	28 ± 3	123 ± 18
R258A	112 ± 4	8,070 ± 275

PA1O3 exsA::Ω carrying the wt $P_{exoT\text{-}lacZ}$ reporter and either a vector control (none) or vectors expressing wt ExsA or the indicated alanine-substitution mutants were cultured in TSB with EGTA in the absence or presence of 0.1% arabinose. The reported values are β-galactosidase activity in Miller units.

REFERENCES

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3. **Liu, P. V.** 1966. The roles of various fractions of Pseudomonas aeruginosa in its pathogenesis. 3. Identity of the lethal toxins produced in vitro and in vivo. The Journal of infectious diseases **116:**481-489.
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5. **Newman, J. R., and C. Fuqua.** 1999. Broad-host-range expression vectors that carry the L-arabinose-inducible Escherichia coli araBAD promoter and the araC regulator. Gene **227:**197-203.

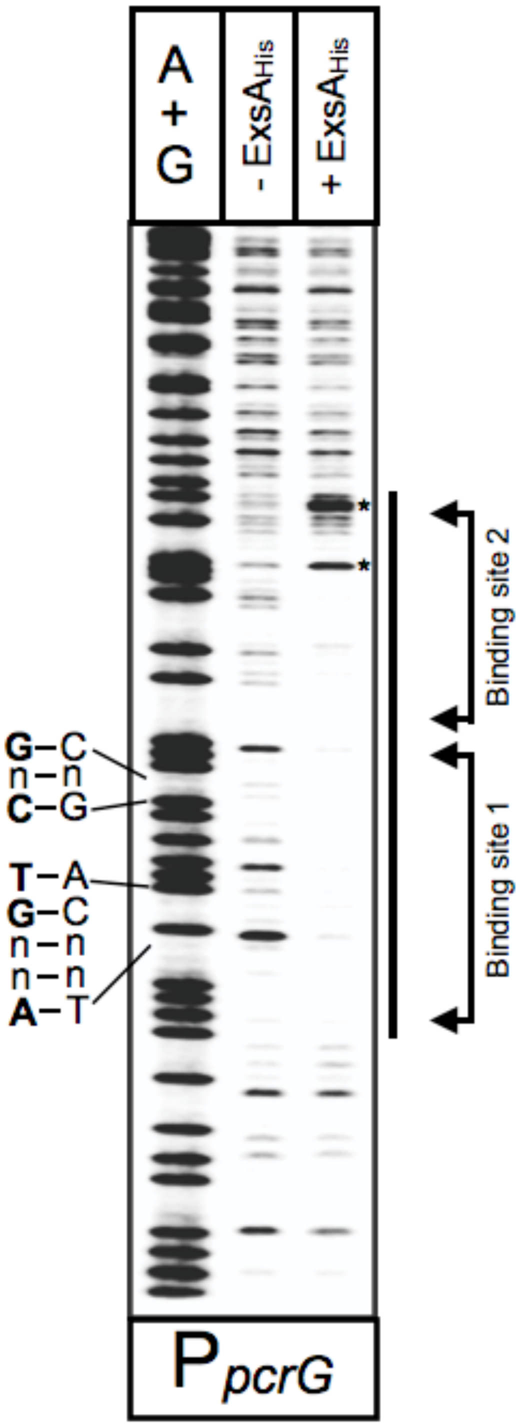

A + G
- ExsA$_{His}$
+ ExsA$_{His}$
G-C
n-n
C-G
T-A
G-C
n-n
n-n
A-T
*
*
Binding site 2
Binding site 1
P$_{pcrG}$

Figure S1

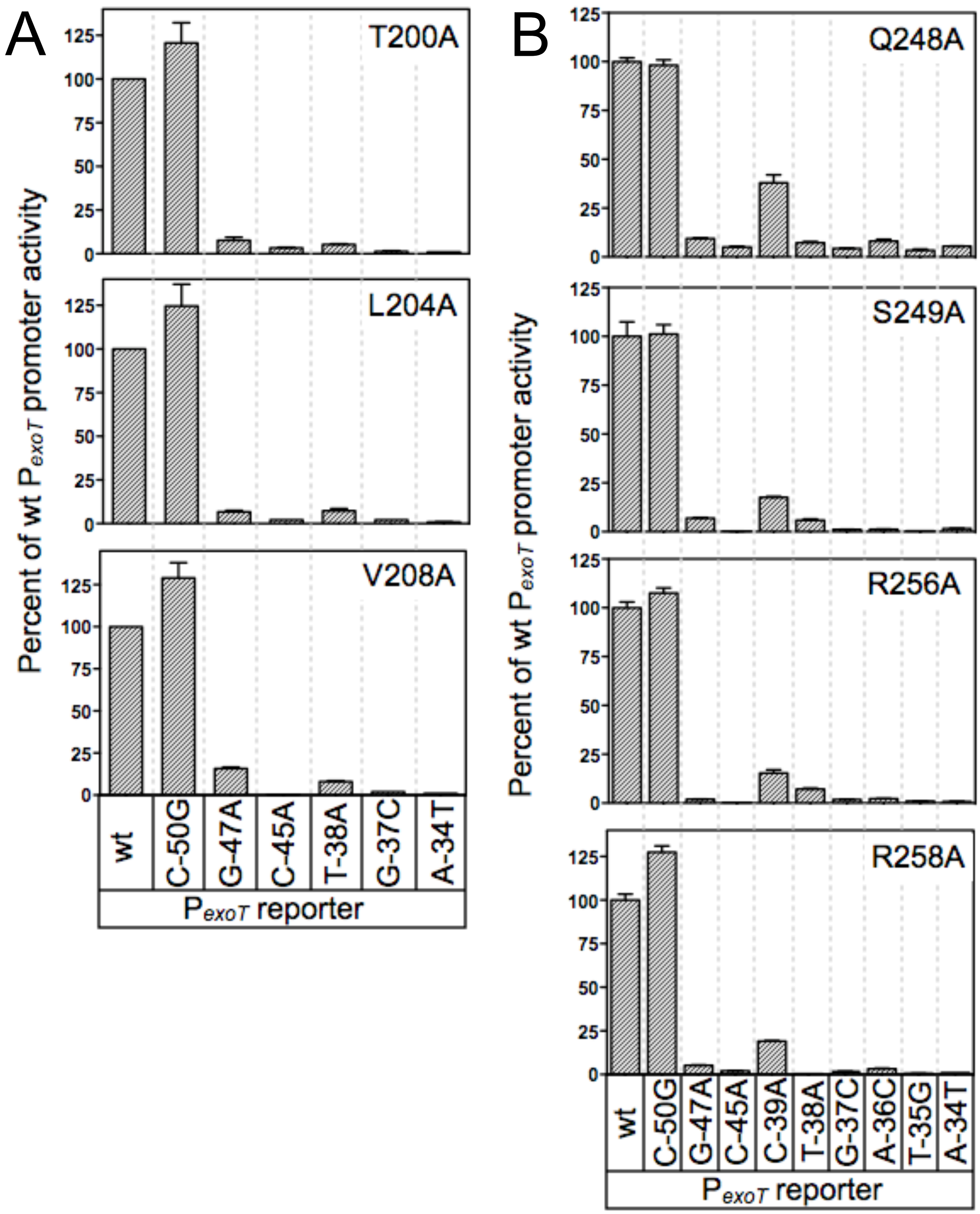
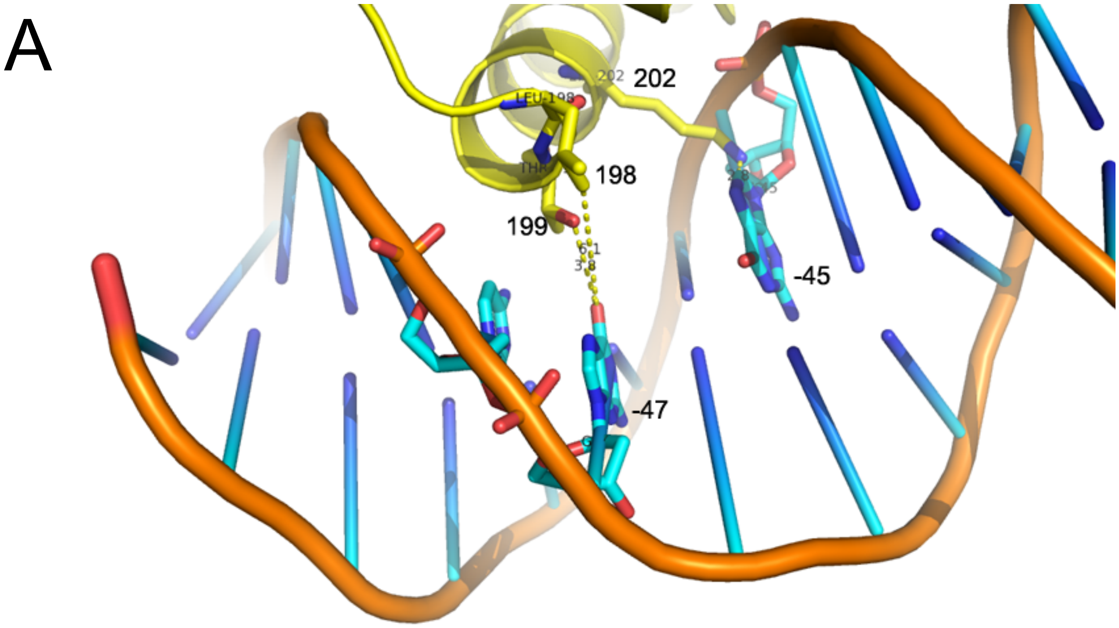
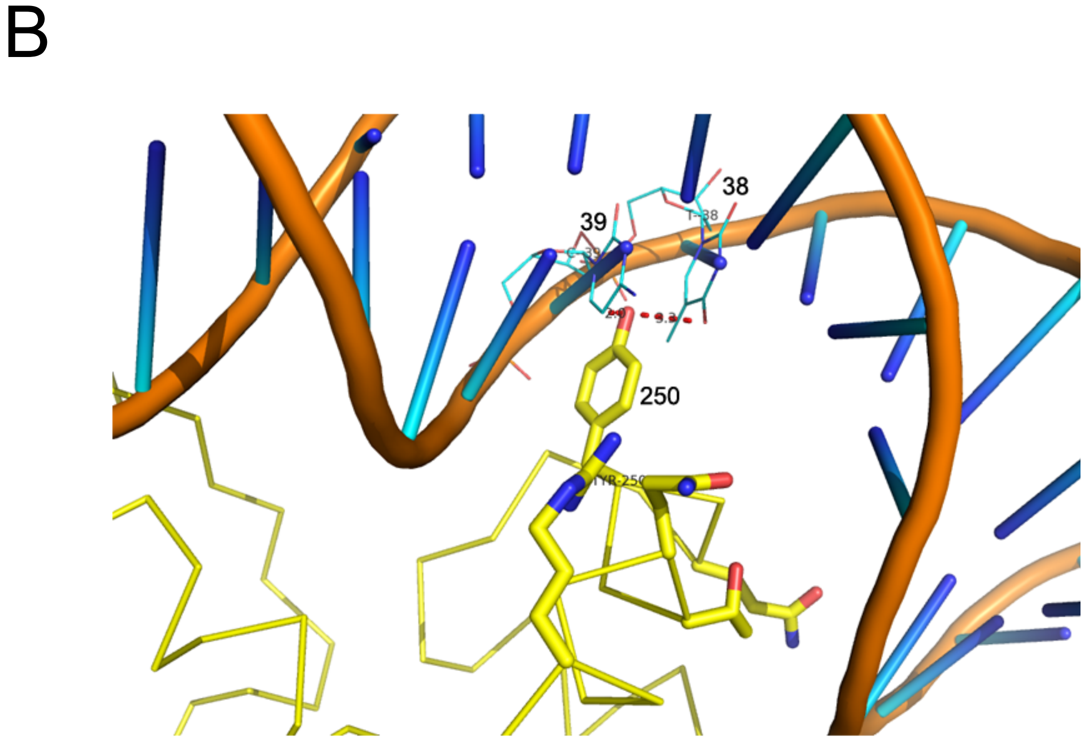

A
T200A
L204A
V208A
Percent of wt P_{exoT} promoter activity
0
25
50
75
100
125
wt
C-50G
G-47A
C-45A
T-38A
G-37C
A-34T
P_{exoT} reporter
B
Q248A
S249A
R256A
R258A
Percent of wt P_{exoT} promoter activity
wt
C-50G
G-47A
C-45A
C-39A
T-38A
G-37C
A-36C
T-35G
A-34T
P_{exoT} reporter

Figure S2



L198, T199, K202



Y250

Figure S3

Figure S1. DNase I footprint of the P_{pcrG} promoter region. The P_{pcrG} promoter probe was end-labeled with ^{32}P on the reverse strand, incubated in the absence (-) or presence of $ExsA_{His}$, and treated with DNase I as previously described (6). The reactions were separated by denaturing polyacrylamide gel electrophoresis and visualized by phosphorimaging. Maxam-Gilbert sequencing ladders (A+G) were included for orientation and the positions of the GnC and TGnnA sequences in ExsA binding site 1 are indicated. The promoter region protected by $ExsA_{His}$ from DNase I cleavage is indicated with a solid on the right side of the phophorimage. Nucleotides demonstrating hypersensitivity to DNase I cleavage are indicated with an asterisk.

Figure S2. Residues T200A, L204A, V208A, Q248A, S249A, R256A, and R258A show no loss-of-contact phenotype. PA1O3 *exsA*::Ω expressing the indicated ExsA mutants were assayed for reporter activity using the panel of mutant $P_{exoT-lacZ}$ reporter described in Fig. 4A. The strains were cultured in the presence of EGTA and 0.1% arabinose, and assayed for β-galactosidase activity. The reported values are the percent activity at the mutant promoter compared to the activity at the wt $P_{exoT-lacZ}$ reporter.

Figure S3. Molecular modeling the interaction of ExsA with the P_{exoT} promoter.