Fig. S1

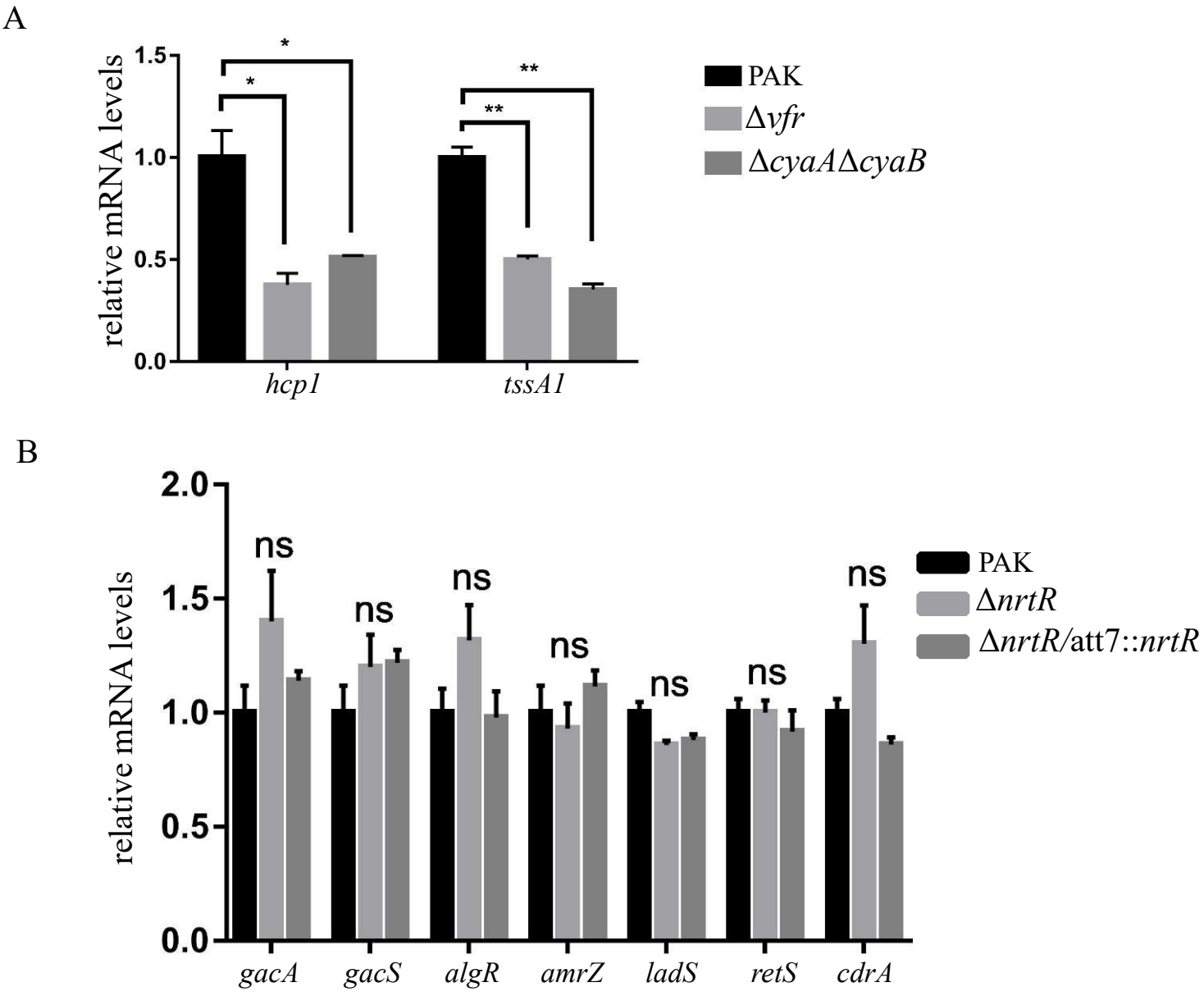


**Fig. S1.** The relative mRNA levels of the indicated genes in PAK, Δ*nrtR*, Δ*nrtR*/*att7*::*nrtR*, Δ*cyaA*Δ*cyaB* or Δ*vfr*. Total RNA was isolated from bacteria at an $OD_{600}$ of 1.0, and the mRNA levels were examined by real-time qPCR using *rpsL* as an internal control. ns, not significant, *, $P<0.05$, **, $P<0.01$, compared to PAK by Student's *t* test.

Fig. S2

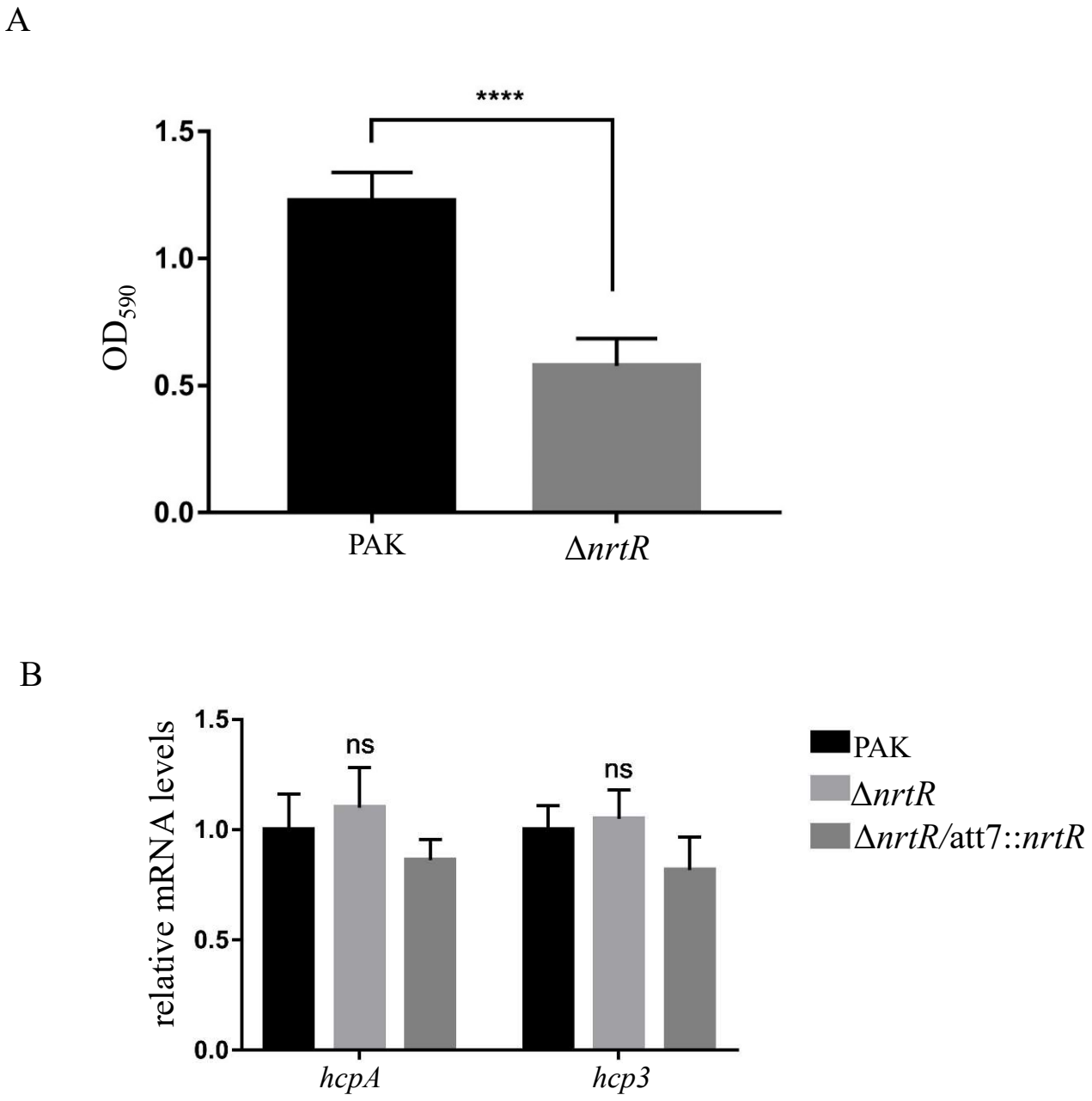


**Fig. S2. (A)** Biofilm formation by the PAK and Δ*nrtR* strains. PAK and Δ*nrtR* were grown in a 96-well plate for 24 h. The wells were washed with $H_2O$ and stained with 2.5% crystal violet. The crystal violet was dissolved in destaining solution and measured at a wavelength of 590 nm. ****, $P<0.0001$, compared to PAK by Student's *t* test. **(B)** The relative mRNA levels of the indicated genes in PAK, Δ*nrtR*, and Δ*nrtR*/*att7*::*nrtR*. Total RNA was isolated from bacteria at an $OD_{600}$ of 1.0, and the mRNA levels were examined by real-time qPCR using *rpsL* as an internal control. ns, not significant compared to PAK by Student's *t* test.

**Table S1.** Bacterial strains and plasmids used in this study.

| Strain or plasmid | Description | Source or reference |
|---|---|---|
| **strains** | | |
| *E. coli* strains | | |
| DH5α | F⁻ϕ 80d*lacZ*ΔM15 *endA1 recA1 hsdR17*($r_K^- m_K^+$) *supE44 thi-1 relA1* Δ(*lacZYA-argF*) *U169 gyrA96 deoR* | TransGen |
| S17-1 | RP4-2 Tc::Mu Km::Tn*7* $Tp^r$ $Sm^r$ Pro $Res^-$ $Mod^+$ | Dr. Ramphal |
| BL21(DE3) | $F^-$ *ompT hsdSB (rB⁻, mB⁻) gal dcm* (DE3) | invitrogen |
| ***P. aeruginosa* strains** | | |
| PAK | Wild type *P. aeruginosa* strain | (1) |
| Δ*nrtR* | PAK with *nrtR* deleted | (1) |
| Δ*nrtR*/att7::*hcp1-flag* | $P_{hcp1}$-*hcp1*-Flag integrated into the chromosome of Δ*nrtR* with mini-Tn7 | This study |
| PAK/att7::*hcp1-flag* | $P_{hcp1}$-*hcp1*-Flag integrated into chromosome of PAK with mini-Tn7 | This study |
| Δ*nrtR*/att7::*nrtR* | Δ*nrtR* with *nrtR* inserted into the chromosome with mini-Tn7 insertion | (1) |
| Δ*rsmY*Δ*rsmZ* | PAK with *rsmY* and *rsmZ* gene deleted | (2) |
| Δ*nrtR*Δ*rsmY*Δ*rsmZ* | Δ*nrtR* with *rsmY* and *rsmZ* gene deleted | This study |
| Δ*vfr* | PAK with *vfr* gene deleted | (1) |
| Δ*cyaA*Δ*cyaB* | PAK with *cyaA* and *cyaB* gene deleted | (1) |
| **Plasmids** | | |
| pUCP20 | Shuttle vector between *E. coli* and *P. aeruginosa*; $Amp^r$ | (3) |
| pUCP24 | Shuttle vector between *E. coli* and *P. aeruginosa*; $Gm^r$ | (3) |
| pTNS3 | Helper plasmid encoding Tn7 site-specific transposition pathway; $Amp^r$ | (4) |
| pET28a | Expression vector, $Kan^r$ | Novagen |
| E1553-*hcp1-flag* | *hcp1* with its promoter cloned into promoterless pUCP20 | This study |
| pUCP20-*rsmA* | *rsmA* gene from PAK in pUCP20; $Amp^r$ | This study |
| pUCP20-*rsmN* | *rsmN* gene from PAK in pUCP20; $Amp^r$ | This study |
| pUCP24-*nrtR* | N terminal Flag-tagged *nrtR* from PAK in pUCP24; $Gm^r$ | This study |
| pEX18-*rsmY* | *rsmY* gene deletion on pEX18Tc; $Tc^r$ | (2) |
| pEX18-*rsmZ* | *rsmZ* gene deletion on pEX18Tc; $Tc^r$ | (2) |
| p19-EGFP | EGFP gene cloned into pDN19*lacZ*Ω which was cut with *Bam*HI-*Hin*dIII, $Tc^r$ | This study |
| $P_{rsmY}$-EGFP | *rsmY* promoter fused to P19-EGFP; $Tc^r$ | This study |
| $P_{rsmZ}$-EGFP | *rsmZ* promoter fused to P19-EGFP; $Tc^r$ | This study |
| $P_{tssA1}$-EGFP | *tssA1* promoter fused to P19-EGFP; $Tc^r$ | This study |

| | | |
|---|---|---|
| pUC18T-mini-Tn7T-Gm-*hcp1-flag* | Flag tagged *hcp1* on pUC18T-mini-Tn7T driven by *hcp1* promotor; $Gm^r$ | (2) |
| pET28a-*nrtR* | *nrtR* of PAK cloned into pET28a, $Kan^r$ | This study |


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2. Li K, Xu C, Jin Y, Sun Z, Liu C, Shi J, Chen G, Chen R, Jin S, Wu W. 2013. SuhB is a regulator of multiple virulence genes and essential for pathogenesis of *Pseudomonas aeruginosa*. mBio 4:e00419-13.
3. West SE, Schweizer HP, Dall C, Sample AK, Runyen-Janecky LJ. 1994. Construction of improved *Escherichia-Pseudomonas* shuttle vectors derived from pUC18/19 and sequence of the region required for their replication in *Pseudomonas aeruginosa*. Gene 148:81-6.
4. Choi KH, Schweizer HP. 2006. mini-Tn7 insertion in bacteria with single attTn7 sites: example *Pseudomonas aeruginosa*. Nat Protoc 1:153-61.

**Table S2.** Primers used in this study.

| Primer[a] | Sequence 5'-3'[b] |
|---|---|
| pUCP-*rsmN*F | GGAATTCTGACTGGCAAGGACGCGGAACATGG |
| pUCP-*rsmN*R | CCCAAGCTTTCAGTGGTGGTGGTGGTGGTGGCCTTTCGGTGCCGTTTTCAAC |
| pUCP-*rsmA*F | GGAATTCTTCGCGTGAGGAGAAAGGAATGCTG |
| pUCP-*rsmA*R | CCCAAGCTTTTAGTGGTGGTGGTGGTGGTGATGGTTTGGCTCTTGATCTTTCTC |
| E1553-*hcp1-flag*F | GCTCTAGAACACCGACTTCGCCGCCTTCATCGG |
| E1553-*hcp1-flag*R | CCCAAGCTTTCACTTGTCGTCATCGTCCTTGTAGTCGGCCTGCACGTTCTGGCGGATGTTCC |
| *nrtR-flag*F | CGGGATCCGATTACAAGGACGACGATGACAAGAGTTCAGCGGAAGTATTGGCC |
| *nrtR-flag*R | CCCAAGCTTTCAGGACGCCAGCAGGCTGCG |
| pET- *nrtR*F | CCCATATGAGTTCAGCGGAAGTATTGGCC |
| pET- *nrtR*R | CCCTCGAGTCAGGACGCCAGCAGGCTG |
| $P_{Tn7R}$ | CACAGCATAACTGGACTGATTTC |
| $P_{glmS}$-down | GCACATCGGCGACGTGCTCTC |
| $P_{rsmY}$F | ATCGGTCTCGAATTCGGTGGCCACGTAGTTCGG |
| $P_{rsmY}$R | ATCGGTCTCGGATCCGGTTTGAAGATTACGCATCTC |
| $P_{rsmZ}$F | GGAATTCCGAGCTGCTGCAGGATGAC |
| $P_{rsmZ}$R | CGGGATCC CAGGAGT GATATTAGCGATTC |
| $P_{tssA1}$F | GGAATTCGCTCCGGGTCCGGCAGGAC |
| $P_{tssA1}$R | CGGGATCCGGTGACGATCTCCCTATCATC |
| Deletion test primer | |
| *rsmY*TF | CGGCGAGCGGAACTATTACA |
| *rsmY*TR | TGCTGGAAGGCGTGGTCTGA |
| *rsmZ*TF | AGCATCTGGAGCGCTGATAC |
| *rsmZ*TR | GCCAAAAACGCTCGGTGAAT |
| EMSA primer | |
| *tssA1*F | TGCTACTCCTTGCATTGCCAG |

| | |
|---|---|
| *tssA1*R | GGTGACGATCTCCCTATCATC |
| *rsmZ*F | CAGGAGTGATATTAGCGATTCCCAG |
| *rsmZ*R | CGGAAAACCTTAGACCCACTGAAG |
| *rsmY*F | GCTGGGAAGGCTCGCGATGATGAG |
| *rsmY*R | CCGTATTGTCTTTGGCGCTTCCTGC |
| *nadD2*F | AGAAAATACCTTCCACTGCGAA |
| *nadD2*R | GGAAGCCTCCGCTTGAC |
| *nrtR*F | TGTTGCTGATCCGCCGCGCCCAGGC |
| *nrtR*R | CCGCGTTGCCGACCGTGGCCACCTG |
| *tssA1up* | GTTGCATGACACCGCAGATC |
| *tssA1down* | CCTTCCTTGACAAGCCTTGC |
| qPCR primer | |
| *hcp1*F | AGGACCTGTCGTTCACCAA |
| *hcp1*R | ATAGTGCTTGCCGCTGGA |
| *gacS*F | GAGGAAATGCAGCACAAC |
| *gacS*R | GTTCTGGATCTCGATGGT |
| *gacA*F | CCTGATGATCGCCAACTG |
| *gacA*R | ATAGGTATTCACGGTCTTCG |
| *algR*F | CCAGCAATGGCGAAGAAGC |
| *algR*R | TCATGGGCCGTGCAGAAG |
| *amrZ*F | GGCGCTGCAAGACAATCT |
| *amrZ*R | GTTCTGCTGACGGTGGGT |
| *rpsL*F | GTAAGGTATGCCGTGTACG |
| *rpsL*R | CACTACGCTGTGCTCTTG |
| *ladS*F | ATCTACAACCTGTTCATCTT |
| *ladS*R | CCGAAGGAAGCGATATAG |
| *retS*F | GATACTCGACATCTCCAA |
| *retS*R | GAAGATATCCAGGCAGTC |
| *cdrA*F | ATGTGAATCCGACTCTGA |

| | |
|---|---|
| *cdrA*R | CGTTGAACTGACTGTTGA |
| *hcpA*F | CAAGGTCGAGATCCAGTGGT |
| *hcpA*R | TCCAGGTGATCTTGCGGTAG |
| *hcp3*F | ATGGATGCGATCATTCTC |
| *hcp3*R | GTTGTGGCTGTAGGACAT |

a: F: forward; R, reverse; T: test; b: The underlines are the sites of restriction enzymes.

**Table S3.** Transcriptome analysis: differentially expressed genes via RNAseq without those listed in Table 1.

**Table S4.** Potential NrtR binding sites identified via ChIP-Seq analysis without those listed in Table 2.