

Figure S1

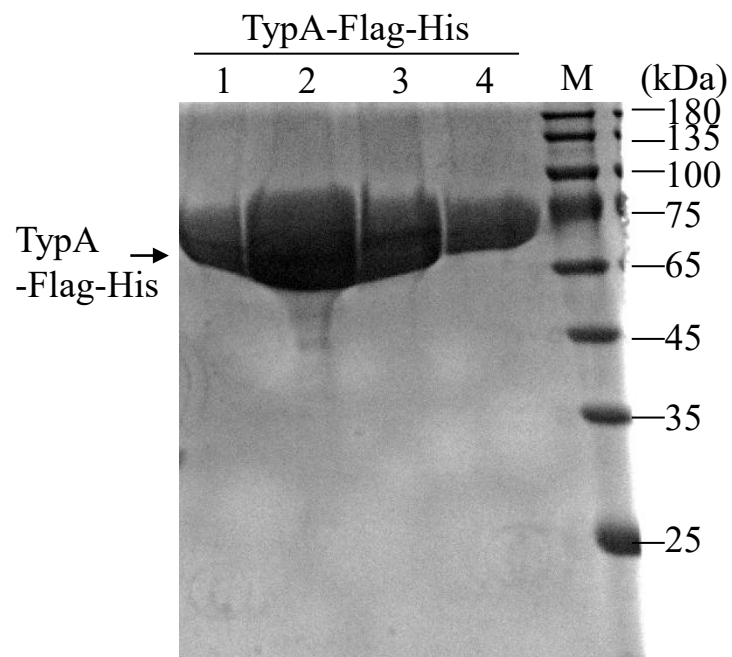


Figure S1. Coomassie brilliant blue staining of purified TypA-Flag-His proteins. Recombinant TypA-Flag-His protein was separated by SDS-PAGE gel and stained with Coomassie brilliant blue. Lanes 1-4, samples were eluted successively with 1 mL elution buffer; M, marker.

Figure S2

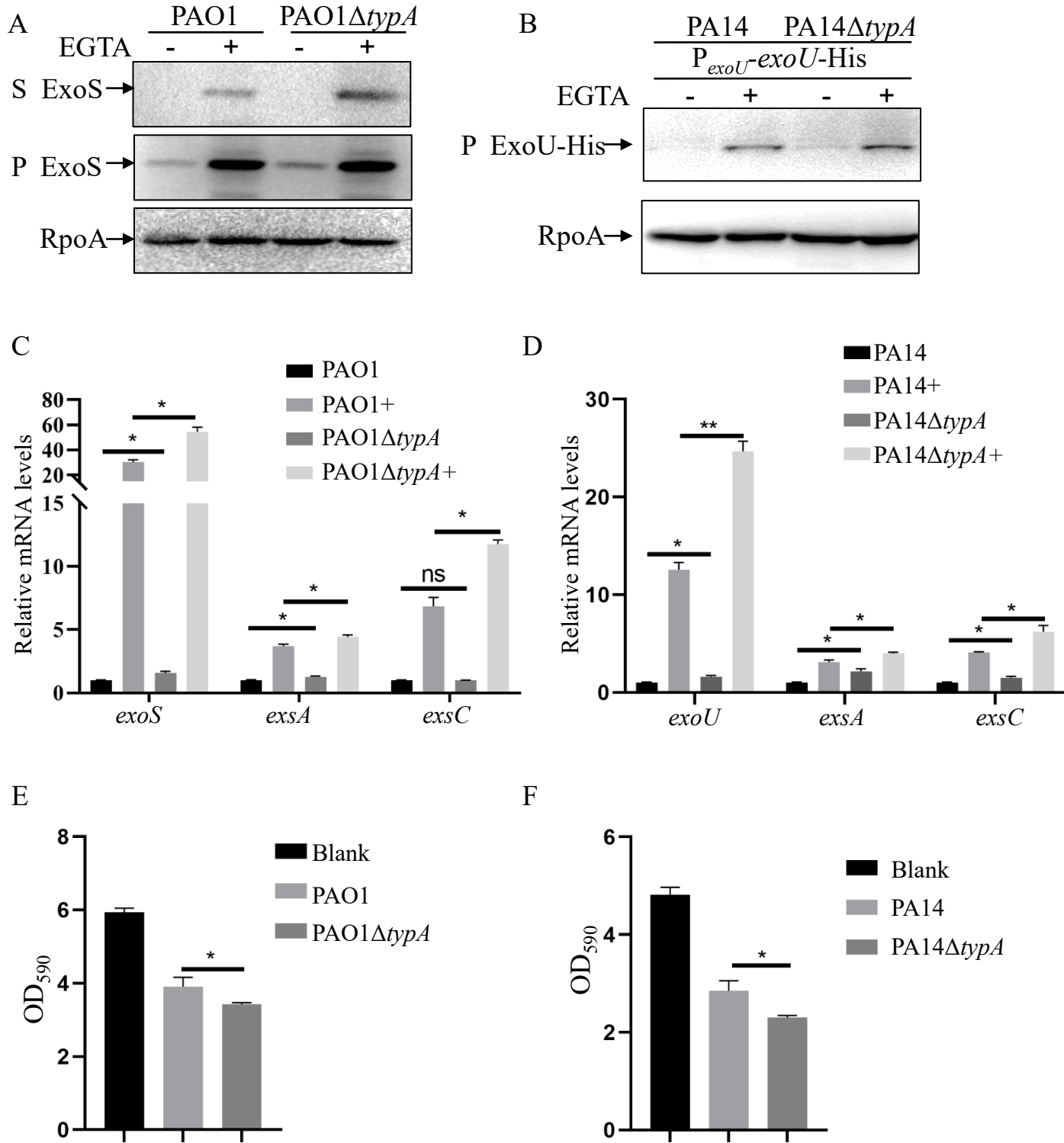


Figure S2. *typA* represses T3SS in PA14 and PAO1 strains. (A,B) Expression of ExoS and ExoU or secretion of ExoS in the indicated strains. (C,D) Relative mRNA levels of the indicated genes in the indicated strains. Total RNA was purified from bacteria at an OD₆₀₀ of 1.0, and mRNA levels were determined by real-time qPCR using the ribosomal encoding gene *rpsL* as an internal control. (E,F) Cytotoxicity of indicated strains by crystal violet assay. ns, not significant, *, $p < 0.05$; **, $p < 0.01$; by Student's *t*-test.

Figure S3

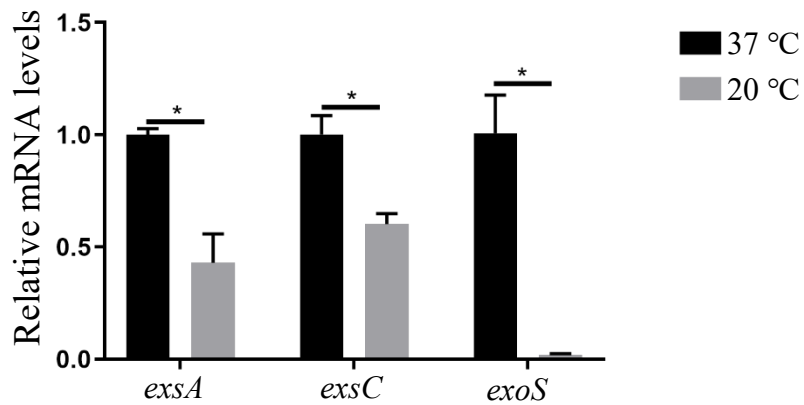


Figure S3. Relative mRNA levels of the indicated genes in PAK cultured at 37 and 20 °C.

Total RNA was purified from bacteria at an OD₆₀₀ of 1.0 cultured at 37 or 20 °C, and mRNA levels were determined by real-time qPCR using the ribosomal encoding gene *rpsL* as an internal control.

*, $p < 0.05$ by Student's *t*-test.

Table S2. Bacterial strains and plasmids used in this study [19,24,40–44].

Strains or plasmids	Description	Source or reference
strains		
DH5 α	F ⁻ ϕ 80 <i>lacZ</i> Δ M15 <i>endA1 recA1 hsdR17</i> (r κ ⁻ m κ ⁺) <i>supE44 thi-1 relA1</i> Δ (<i>lacZYA-argF</i>) <i>U169 gyrA96 deoR</i>	TransGen
S17-1	RP4-2 Tc ^r ::Mu Km ^r ::Tn7 Tp ^r Sm ^r Pro Re ^s Mod ⁺	Stratagene
BL21 (DE3)	F ⁻ <i>ompT hsdSB (rB-, mB-) gal dcm</i> (DE3)	invitrogen
RS	Δ (<i>mcrA</i>)183 Δ (<i>mcrCB-hsdSMR-mrr</i>)173 <i>endA1 supE44 thi-1 recA1 gyrA96 relA1 lac</i> [F' <i>laqI^a bla lacZ Kan^r</i>]	Stratagene
PAK	Wild type <i>P. aeruginosa</i> strain	David Bradley
PAK Δ <i>exsA</i>	PAK with <i>exsA</i> gene deleted	[40]
PAK Δ <i>typA</i>	PAK with <i>typA</i> gene deleted	This study
PA14	Wild type <i>P. aeruginosa</i> strain	Liberati, N.T.,2006
PA14 Δ <i>typA</i>	PA14 with <i>typA</i> gene deleted	This study
PAO1	Wild type <i>P. aeruginosa</i> strain	[41]
PAO1 Δ <i>typA</i>	PAO1 with <i>typA</i> gene deleted	This study
Plasmids		
pUCP21	Shuttle vector between <i>E. coli</i> and <i>P. aeruginosa</i> ; Amp ^r	[42]
pUCP21- <i>exsA</i> -Flag-His	Flag-His-tagged <i>exsA</i> gene from PAK in pUCP21; Amp ^r	This study
pUCP24	Shuttle vector between <i>E. coli</i> and <i>P. aeruginosa</i> ; Gm ^r	[42]
pUCP24- <i>typA</i> -Flag	Flag-tagged <i>typA</i> gene from PAK in pUCP24; Gm ^r	This study
pUC18T-mini-Tn7T- <i>typA</i> -Flag	Flag-tagged <i>typA</i> gene from PAK in pUC18T-mini-Tn7T; Gm ^r	This study
pUCP19-GFP-His	His-tagged <i>gfp</i> gene in pUCP19; Amp ^r	This study
pMMB67EH	Shuttle vector between <i>E. coli</i> and <i>P. aeruginosa</i> ; Amp ^r	[43]
pMMB67EH- <i>exsA</i> -His	His-tagged <i>exsA</i> gene from PAK in pMMB67EH; Amp ^r	This study
pMMB67EH-GST- <i>exsA</i> -His	GST-His-tagged <i>exsA</i> gene from PAK in pMMB67EH; Amp ^r	This study
pEX18Tc	Gene knockout vector; Tc ^r	[19]
pEX18Tc- <i>typA</i>	<i>typA</i> gene deletion on pEX18Tc; Tc ^r	This study
pDN19-P _{<i>exsC</i>} - <i>lacZ</i>	<i>exsC</i> promoter fused to promoterless <i>lacZ</i> on pDN19 <i>lacZ</i> Ω ; Sp ^r , Sm ^r , Tc ^r	[44]

pET41a	Expression vector, Kan ^r	Novagen
pET41a-GST-His	GST gene cloned into pET41a expression vector, Kan ^r	This study
pET28a	expression vector, Kan ^r	Novagen
pET28a- <i>exsA</i> -His	<i>exsA</i> gene of PAK cloned into pET28a expression vector, Kan ^r	[24]
pET28a-GST- <i>exsA</i> -His	<i>exsA</i> gene of PAK cloned into pET28a expression vector, Kan ^r	This study
pET28a- <i>typA</i> -Flag-His	Flag-tagged <i>typA</i> gene of PAK cloned into pET28a expression vector, Kan ^r	This study
pBT	Vector for bacteria two-hybrid, Chl ^r	Stratagene
pBT- <i>exsA</i>	<i>exsA</i> from PAK cloned into pBT, Chl ^r	This study
pTRG	Vector for bacteria two-hybrid, Tc ^r	Stratagene
pTRG- <i>typA</i>	<i>typA</i> from PAK cloned into pTRG, Tc ^r	This study
pTRG- <i>exsD</i>	<i>exsD</i> from PAK cloned into pTRG, Tc ^r	This study

Table S3. Primers used in this study.

Primer ^a	Sequence 5'-3' ^b	Use
<i>typA</i> -UF	CGGAATTCGCCACTACCTGTGGGAGAA	for <i>typA</i> deletion
<i>typA</i> -UR	CGGGATCCAAGTCTTGCTCGTTTAACG	
<i>typA</i> -DF	CGGGATCCACCGTTGCCGTACCGGCGCG	
<i>typA</i> -DR	CCAAGCTTCGCGGCTCACCAGCAT	
<i>exsA</i> -Flag-His-F	GCGAGCTCGTACGACGGGAAGTG	pUCP21- <i>exsA</i> -Flag-His
<i>exsA</i> -Flag-His-R	CGGAATTCCTCAATGGTGATGGTGATGATGCTTGTCATCGTCGTCCTTGTAATCGTTATTTTGTAGCCCGGCATT	
<i>exsA</i> -His-F	CGGAATTCATGCAAGGAGCCAAATC	pMMB67EH- <i>exsA</i> -His
<i>exsA</i> -His-R	CCAAGCTTCTCAATGGTGATGGTGATGATGGTTATTTTGTAGCCCG	
<i>typA</i> -Flag-His-F	TTAAGAAGGAGATATACCATGGTGATCGAAAATCTGCGCAACA	pET28a- <i>typA</i> -Flag-His
<i>typA</i> -Flag-His-R	GGTGGTGGTGGTGGTGCTCGAGCTTGTCATCGTCGTCCTTGTAATC	
<i>typA</i> -Flag-F	CGGAATTCGGTGATCGAAAATCTGC	pUCP24- <i>typA</i> -Flag
<i>typA</i> -Flag-R	CCAAGCTTTTACTTGTCATCGTCGTCCTTGTAATCGACCTTGCTGCGCTC	
mini-P _{<i>typA</i>} - <i>typA</i> -F	CGGGATCCACGAACCTCGGCCAGGAC	pUC18T-mini-Tn7T- <i>typA</i> -Flag
<i>typA</i> -Flag-R	CCAAGCTTTTACTTGTCATCGTCGTCCTTGTAATCGACCTTGCTGCGCTC	
pBT- <i>exsA</i> -F	CGGAATTCATGCAAGGAGCCAAATC	pBT- <i>exsA</i>
pBT- <i>exsA</i> -R	CGGGATCCCTCAGTTATTTTGTAGCC	
pTRG- <i>typA</i> -F	CGGGATCCGTGATCGAAAATCTGC	pTRG- <i>typA</i>
pTRG- <i>typA</i> -R	CGGAATTCCTTAGACCTTGCTGCGCTC	
pTRG- <i>exsD</i> -F	CGGGATCCATGGAGCAGGAAGACGA	pTRG- <i>exsD</i>
pTRG- <i>exsD</i> -R	CGGAATTCCTCAGCTCTGCCAGTAGAA	
GST- <i>exsA</i> -F	CGGGATCCCAAGGAGCCAAATCTCTT	pMMB67EH-GST- <i>exsA</i> -His
GST- <i>exsA</i> -R	CCCAAGCTTCTCAATGGTGATGGTGATGATGGTTATTTGTAGCCCGGC	
GST-A-F	TTAAGAAGGAGATATACCATGGTGTCCTTATACTAGGTTATTGGA	pET28a-GST- <i>exsA</i> -His
GST-A-R	GGTGGTGGTGGTGGTGCTCGAGGTTATTTTGTAGCCCGGCATTC	
P _{<i>exsC</i>} -F	CGCCTCCTAAAGCTCAGCGCATGC	for EMSA
P _{<i>exsC</i>} -R	ATGAAGGACGTCCTGCAGCTCATCC	
<i>algD</i> -F	CAGGGGTGTCGGAGGGACGAACGGTA	for EMSA
<i>algD</i> -R	ACGGCTATTACTTCAGCGCCGAGCAATC	
qPCR primer		
q- <i>exoS</i> -F	GCATATTCAATCGCTTCAG	RT-qPCR of <i>exoS</i>
q- <i>exoS</i> -R	CCTCAATCTGTCCCAAAC	
q- <i>exsA</i> -F	GCTATGTCGTAAGTACCA	RT-qPCR of <i>exsA</i>
q- <i>exsA</i> -R	GAAGCCTTGTAGAAACTG	
q- <i>exsC</i> -F	ATGGATTAAACGAGCAAGGTCAA	RT-qPCR of <i>exsC</i>

q- <i>exsC</i> -R	GAGGGACAGGGAAGGCAAA	
q- <i>rpsL</i> -F	CAAAACTGCCCCGCAACGT	RT-qPCR of <i>rpsL</i>
q- <i>rpsL</i> -R	TTTCGGCGTGGTGGTGTAT	
q- <i>typA</i> -F	CGACTTCCCGATCGTCTACG	RT-qPCR of <i>typA</i>
q- <i>typA</i> -R	GTGGTGGCCCATGATCTTCA	
q- <i>capB</i> -F	ATGTCGAATCGTCAGAAC	RT-qPCR of <i>capB</i>
q- <i>capB</i> -R	GATGGAACGGAAGTGAAC	

a: F: forward; R, reverse; U, upstream of specific gene; D, downstream of specific gene; b: The underlines are the sites of restriction enzymes.