Supplemental Methods

Supplementary figure 1. To make the chimeric protein G-ExoT/A+ExoS, the ADPRT domain of ExoS was amplified using primers PB18 (5'-aaagctagcTCGGCCGACAAGGCGCTG -3') and PB25 (5'-aaaaagcttCGAGCTCGGTACCCG -3'). Vector pUCP20 containing ExoT-GAP (R149K) was amplified using primers PB20 (5' –aaagctagcCTCGCCCTTTACCTCGCT –3') and LKG21 (5'-ttggaTCcGGCAAGCCCCAGGAACAG- 3'). The PCR products were digested with *Nhe*I and *Hind*III, ligated and transformed into *Escherichia coli* strain XL-2 Blue (Invitrogen). The plasmid was introduced into PA103Δ*exoU*Δ*exoT* by electroporation (36). The production and secretion of the chimeric protein was confirmed by standard assay protocols (24).

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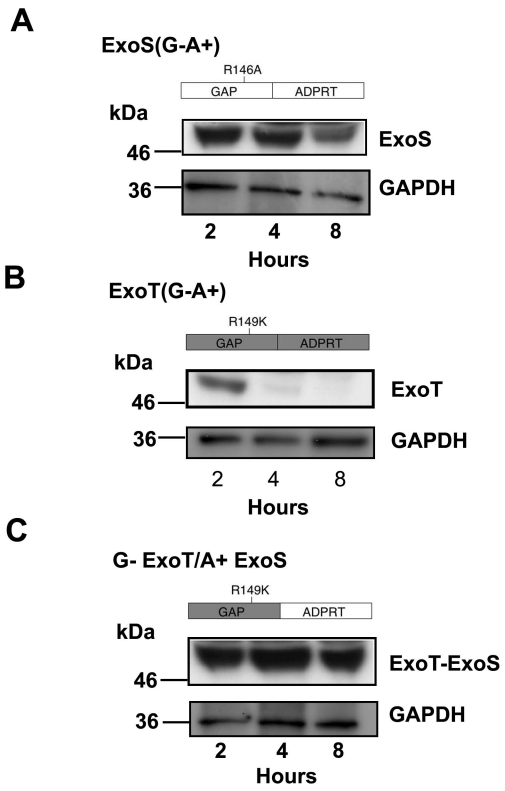
Supplemental Figure 1



Supplemental Figure Legends

Supplemental Figure 1. The ADPRT domain of ExoT is necessary and sufficient to alter its stability. Hela cells were co-cultivated for 1.5 h with (A) PA103Δ*exoU*Δ*exoT* + pUCP20ExoS(G-A+), (B) PA103Δ*exoU*/ExoT(G-A+) or (C) PA103Δ*exoU*Δ*exoT*-pUCP20 G-(ExoT)-A+ExoS. Translocation assays were performed as described in Figure 1. Cytoplasmic extracts were prepared at the indicated times and translocated proteins were immunoblotted with anti-ExoT (upper panels). As a control, the blots were also probed with anti-GAPDH antibody (lower panels).

Supplemental Table 1. *P. aeruginosa* strains, and plasmids used in this study.

Strain or Plasmid	Characteristics	References
PA103 Δ*exoU*	PA103 with an in-frame deletion of *exoU*	(11)
PA103 Δ*exoU*Δ*exoT*	PA103 with in-frame deletions in *exoU* and *exoT*	(11)
PA103 Δ*exoU*/*exoT*(G-A+)	PA103 Δ*exoU* with a point mutation in ExoT (R149K)	(24)
PA103 Δ*exoU* /*exoT*(G-A-)	PA103 Δ*exoU* with point mutations in ExoT (R149K and EQE383-385AAA)	(24)
PA103 Δ*exoU*Δ*exoT* -pUCP20 ExoS(G-A+)	PA103 Δ*exoU*Δ*exoT* expressing pUCP20-ExoS with a point mutation (R146A)	(37)
PA103 Δ*exoU*Δ*exoT* -pUCP20 ExoS(G-A-)	PA103 Δ*exoU*Δ*exoT* expressing pUCP20-ExoS with point mutations (R146A and E379A/E381A)	(37)
PA103 Δ*exoU*Δ*exoT* -pUCP20 G-(ExoT)A+(ExoS)	PA103 Δ*exoU*Δ*exoT* expressing pUCP20 containing GAP(R149K) of ExoT fused to the ADPRT domain of ExoS	This study
pIRESKII-EGFP	pIRESKII-EGFP (Clontech) with bases 1870-1910 removed	(24)
pIRESKII-EGFP/ExoT(G+A+)	pIRESKII-EGFP with ExoT cloned into the *EcoRI* site	(24)
pIRESKII-EGFP/ExoT(G-A+)	pIRESKII-EGFP/ExoT with the R149K mutation	(24)
pIRESKII-EGFP/ExoT(G+A-)	pIRESKII-EGFP/ExoT with the EQE383-385AAA mutation	(24)
pIRESKII-EGFP/ExoT(G-A-)	pIRESKII-EGFP/ExoT with the R149K and EQE383-385AAA mutations	(24)
pCruz-MycB	Mammalian expression vector containing multiple cloning site 3' to Myc tag	Santa Cruz Biotechnology
pCruz-MycB/ExoT(G-A+)	pCruz Myc-B carrying ExoT(R149K) cloned in frame to amino-terminal Myc epitope tag	(7)
pCruz-MycB/ Rev-ExoT(G-A+)	pCruz Myc-B carrying ExoT(R149K) cloned in reverse orientation to amino-terminal Myc epitope tag	(7)
pcDNA3	Mammalian expression vector	(38)
pcDNA3-(HA-Ub)^4	pcDNA3 containing four tandem repeats of Ub-HA cloned into the multiple cloning site	(38)
pCEFL	Mammalian expression vector	(27)
pCEFL-Cblb-HA	pCEFL containing full length Cbl-b with a C-terminal HA tag	(27)

pCEFL-Cblb(C373A)-HA	pCEFL containing RING finger mutant Cbl-b (C373A) with a C-terminal HA tag	(27)
pEBB	Mammalian expression vector	(39)
pEBB-CrkII	pEBB containing full length CrkII	(39)