

Development of TEM-1 β -lactamase based protein translocation assay for identification of

***Anaplasma phagocytophilum* type IV secretion system effector proteins**

Jiafeng Zhu, Meiling He, Wenting Xu, Yuanyuan Li, Rui Huang, Shuyan Wu, Hua Niu*

Department of Microbiology, College of Medicine, Soochow University, Suzhou, Jiangsu
Province, 215123, China.

*Corresponding Author:

Hua Niu, PhD

Department of Microbiology, College of Medicine, Soochow University,
199 Ren Ai Road,
Suzhou, Jiangsu Province, 215123, China.

E-mail: hniu@suda.edu.cn, or niu_hua@126.com

Tel: +86-512-65880132

Supplementary Information

Table S1. Primers used for PCR amplification

Primer ID	Sequence (5'-3')	Note	Purpose
SM-F	CTT <u>CGGCCCGCCT</u> GCCTATCCACG GAATCT	NotI site is underlined	
SM-R	TCAC <u>CATATG</u> TATTGCGGACTACC TTGGTGATC	NdeI site is underlined	Replacement of ampicillin resistance
pMal-F	TCAC <u>CATATG</u> CTGTCAGACCAAGTT TACTCAT	NdeI site is underlined	gene <i>bla</i> with <i>Sm^R</i> in plasmid pTraG-VirD4
pMal-R	CTT <u>CGGCCCGCAAC</u> CGGACCGAAA GAGTTT	NotI site is underlined	
VirD4-F	TCC <u>GTCGACC</u> GACATGTTCTTATT GATTGGC	SalI site is underlined	Construction of pVirD4
VirD4-R	TCC <u>GTCGACC</u> CATAATCTATGGTCC TTGTTGG	SalI site is underlined	
TEM-1-F	TATGCGACTCCTGCATTAGGCATA ACG		Cloning of TEM-1 β -lactamase gene into plasmid pACYCDuet-1 to generate plasmid pTEM-1
TEM-1-R	TGCTTCTCAAATGCCTGAGGAGTT TGTAGAAAC		
pACYCDuet-F	CCTCAGGCATTGAGAAGCACA		
pACYCDuet-R	CCTAATGCAGGAGTCGCATAAGG		
TEM-1-Ats-1-F	GGCGGTGGAGGCAGCCATATGCT AATAAGAAGAATTCTGAC		Construction of plasmid pTEM-1-Ats-1
TEM-1-Ats-1-R	GCGGCCGCCCATGGACATATGTTA CCTCGTACCTTTACCAT		
TEM-1-APH0215-F	GGCGGTGGAGGCAGCCATATGGT CGATAACACCACGAT		Construction of plasmid pTEM-1-APH0215
TEM-1-APH0215-R	GCGGCCGCCCATGGACATATGTTA CGTTCTCGATCGGCC		
TEM-1-APH0756-F	GGCGGTGGAGGCAGCCATATGCG TCCTGATGGGCAAC		Construction of plasmid pTEM-1-APH0756
TEM-1-APH0756-R	GCGGCCGCCCATGGACATATGTTA TCGCGAGACACCACTTC		
VirD4-Expression-F	ATCC <u>GAATTC</u> AAGCTTGAAAATA CGACCTCAC	EcoRI site is underlined	Expression of truncated recombinant VirD4 protein
VirD4-Expression-R	CTT <u>CGGCCCGCCT</u> TTAGTCTTCCG TTACTGCTATC	NotI site is underlined	
215-Delta-C-R	TAG <u>GATCC</u> TTAATCGCAGTGTAT GTCATAAAAAGT	BamHI site is underlined	Construction of plasmid pTEM-1-APH0215 Δ C
215-Delta-C-F	ATC <u>GATCC</u> GAATCCCTGCA	BamHI site is underlined	

APH0215-GFP-F	GCACTCGAGGCCACCATGGTCGA TAACACCACGATAC	XhoI site is underlined	Construction of plasmid pAPH0215-GFP
APH0215-GFP-R	GGTGGATCCCGCGTTCTCGATCGG CCAATAG	BamHI site is underlined	

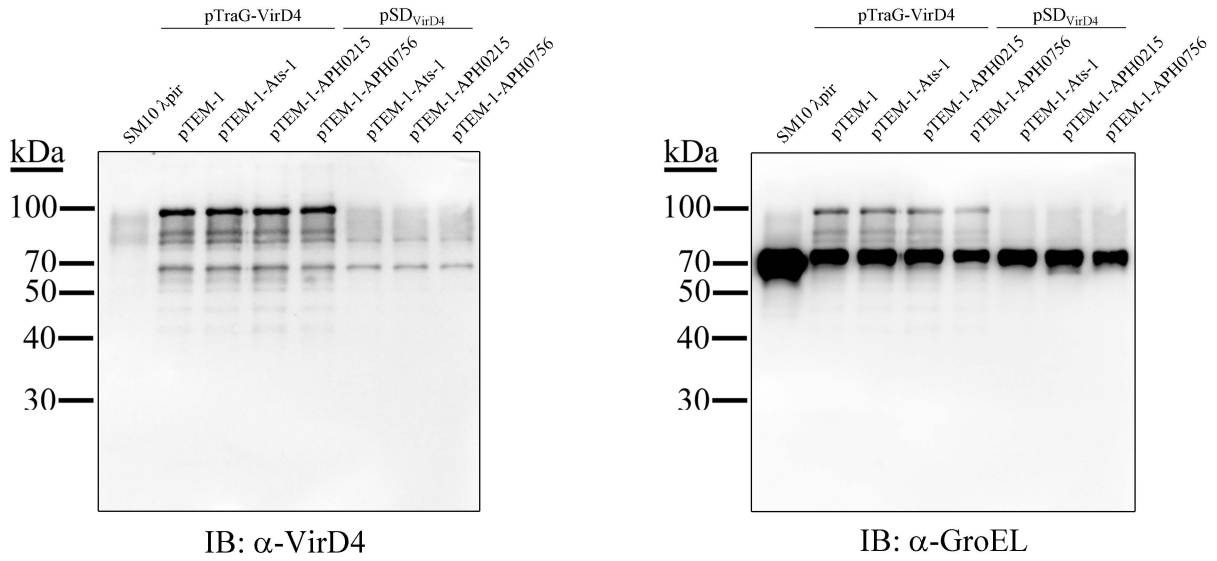
Supplemental Fig. S1

Full-length blots for Figure 2B and 2C.

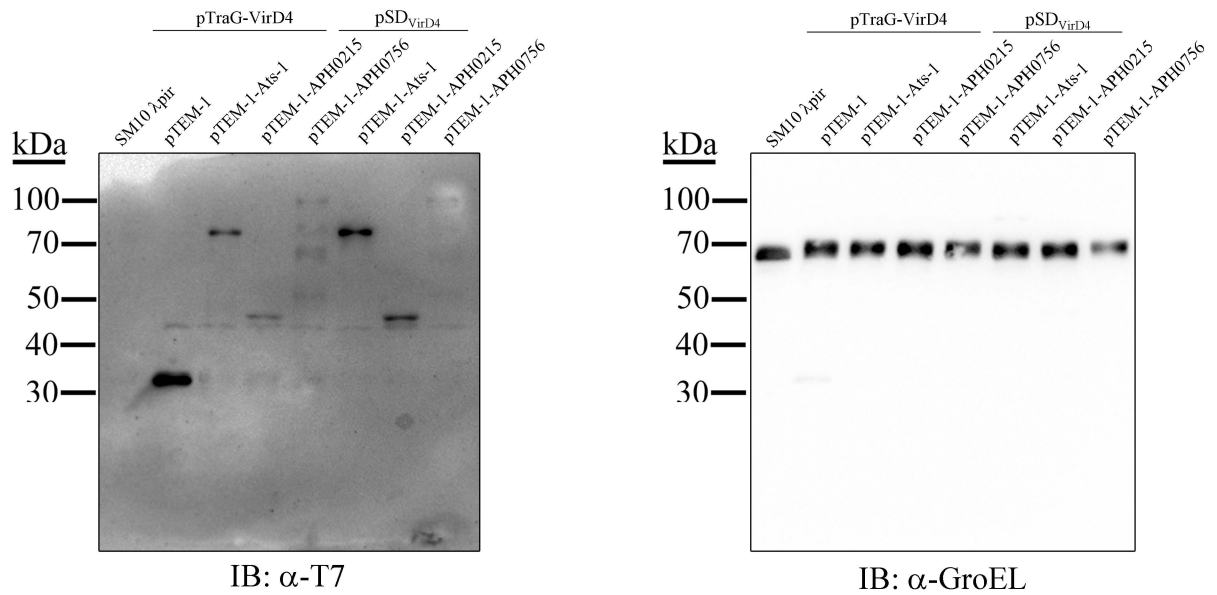
Supplemental Fig. S1

Zhu et al.

Relative to Fig. 2B



Relative to Fig. 2C



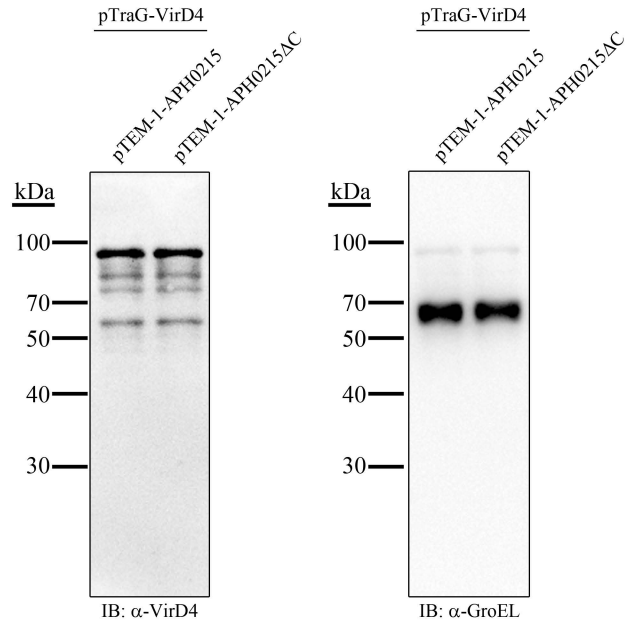
Supplemental Fig. S2

Full-length blots for Figure 3A and 3B.

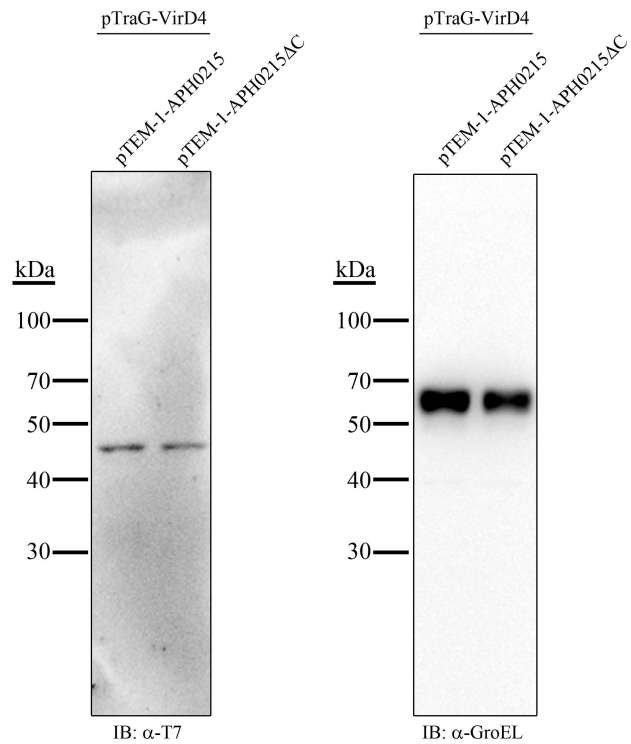
Supplemental Fig. S2

Zhu et al.

Relative to Fig. 3A



Relative to Fig. 3B



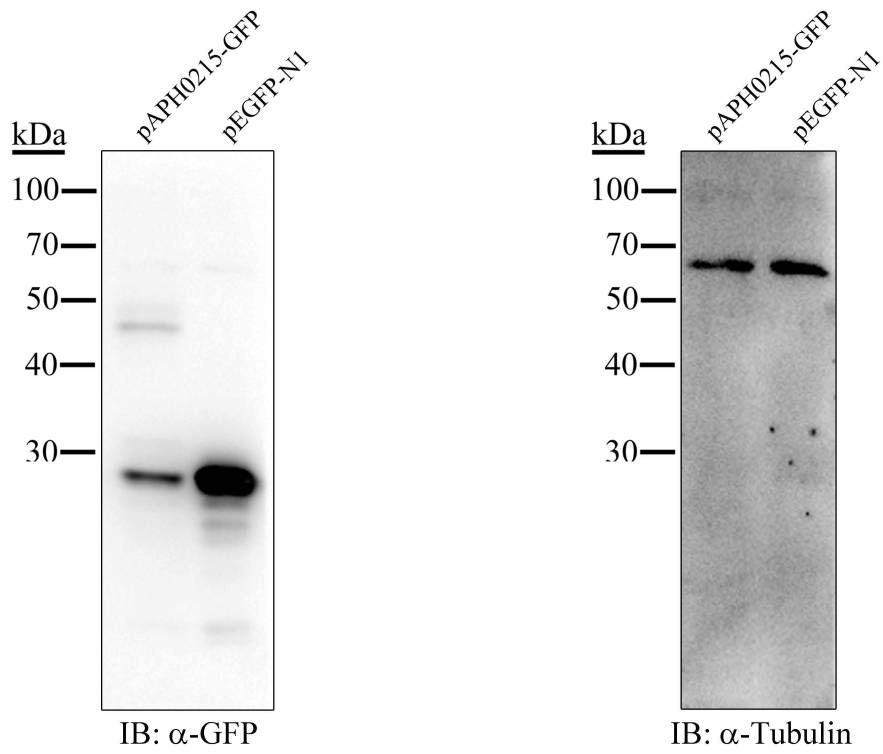
Supplemental Fig. S3

Full-length blots for Figure 4B and 4C.

Supplemental Fig. S3

Zhu et al.

Relative to Fig. 4B



Relative to Fig. 4C

