Supporting information

The *Pseudomonas aeruginosa* type III secretion translocator PopB assists the insertion of the PopD translocator into host cell membranes

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Content: Figures S1-S7

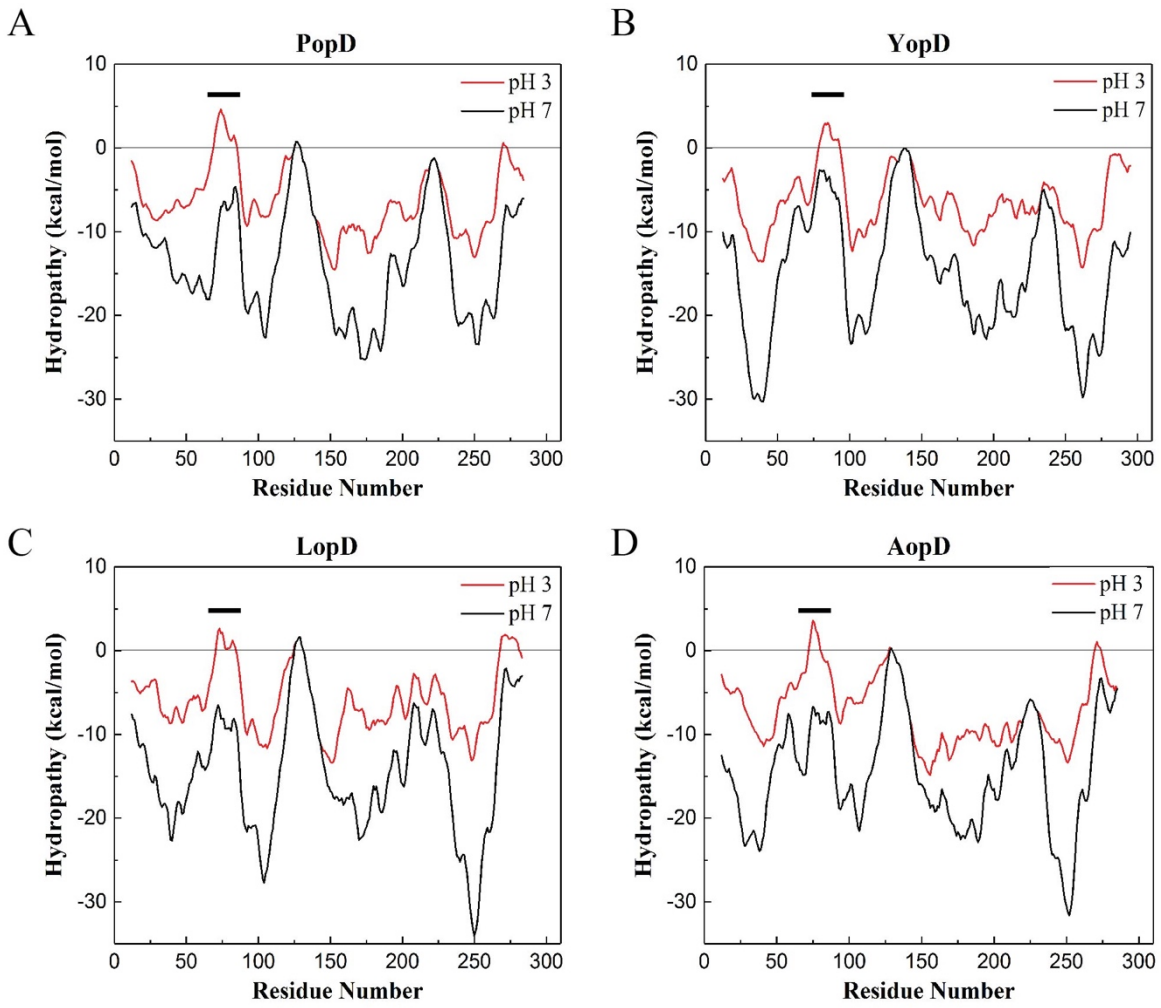

Figure S1. The pH-sensitive hydrophobicity of segment H2 is conserved among PopD homologues. Hydropathy plot of PopD (AAO91774) in *P. aeruginosa* PAK (**A**); YopD (ACX69982) in *Y. enterocolitica*, (**B**); LopD (AAO18056) in *Photorhabdus luminescens*, (**C**); AopD (AAV30236) in *Aeromonas hydrophila*, (**D**). The percentage of identity with PopD for LopD, AopD, and YopD was 45, 41, and 38, respectively (calculated using EMBOSS Needle with the following settings: Matrix BLOSUM62, Gap open 10, Gap extend 0.5). The short horizontal line indicates the location of the H2 segment.

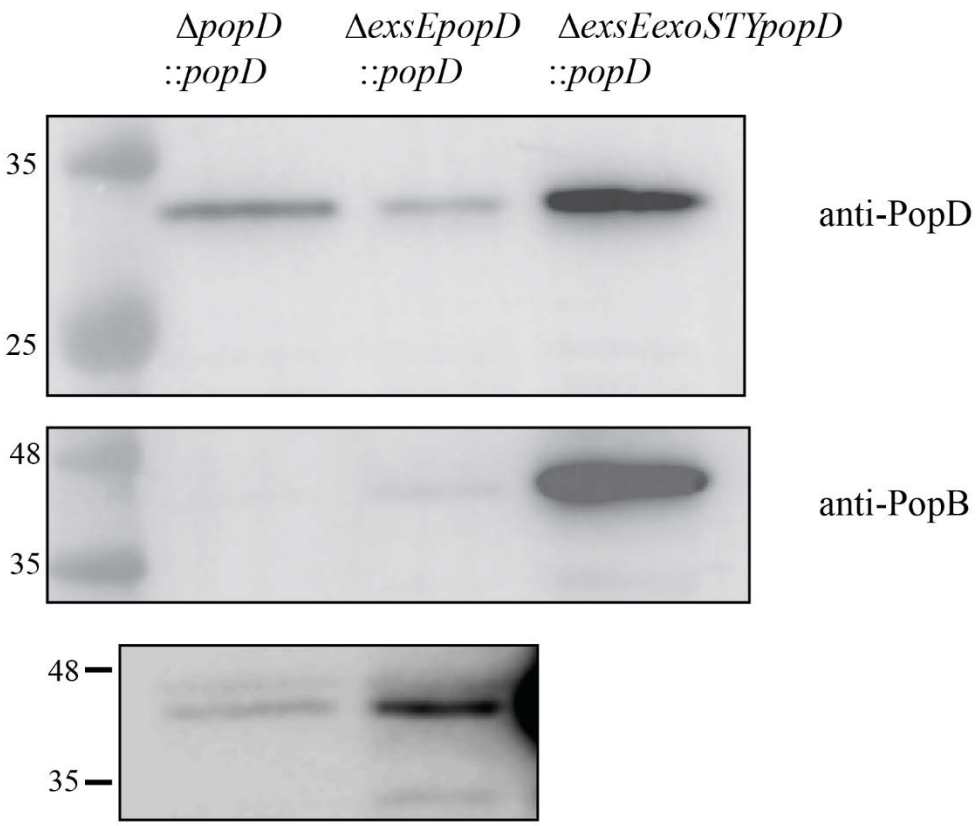

Figure S2. PAKΔ*exsE*Δ*exoSTY*Δ*popD* showed increased insertion of translocator PopB and PopD in HeLa cells. HeLa cells infected with indicated *P. aeruginosa* PAK strain were lysed with 0.1% Triton X-100. Cell lysate was collected as described in Experimental Procedures, and quantified using BCA assay. A total of 30 µg protein from each sample was analyzed for PopB and PopD by immunoblotting. The bottom image shows the overexposure for PopB detection for the first two lanes. Representative blots from two independent experiments are shown.

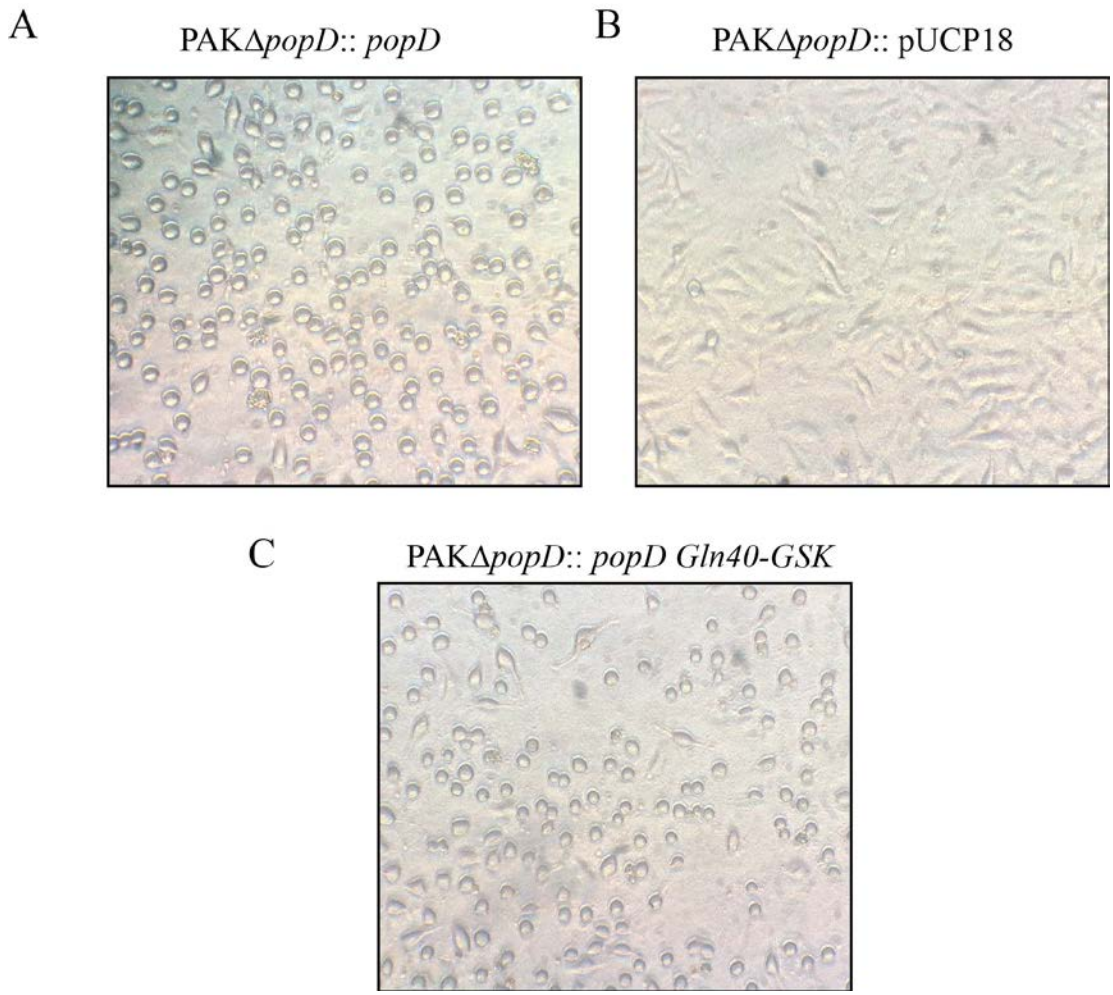

Figure S3. GSK insertion after residue Gln40 did not affect the function of PopD. To test the functionality of PopD-GSK derivative, PAKΔ*popD* complemented with a plasmid containing the gene encoding for PopD WT (**A**), the empty vector (**B**) or a plasmid containing the gene encoding for PopD Gln40-GSK (**C**) were incubated with HeLa cells for 2 hrs. HeLa cell rounding due to disrupted actin cytoskeleton caused by effector translocation was assessed. It is clear that insertion of the GSK-tag does not impair the translocation function of PopD.

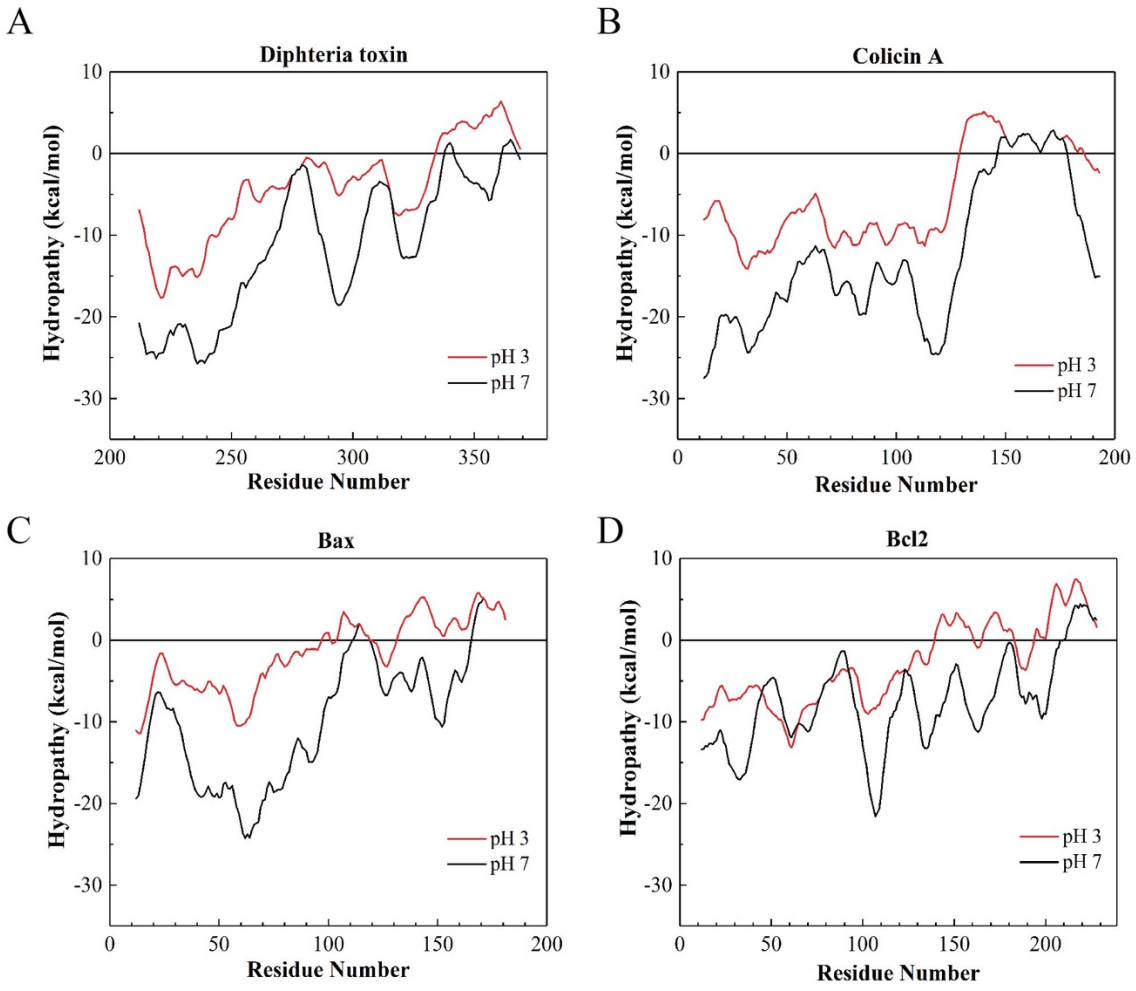

Figure S4. The isolated pH-sensitive hydrophobic segment is not found in other pore forming proteins. Hydropathy plot of the T domain of Diphtheria toxin (AAV70486) in *Corynebacterium diphtheriae* (**A**); Colicin A (Q47108) in *Escherichia coli*, (**B**); apoptosis regulator Bax (Q07812) in *Homo sapiens*, (**C**); apoptosis regulator Bcl-2 (P10415) in *Homo sapiens*, (**D**).

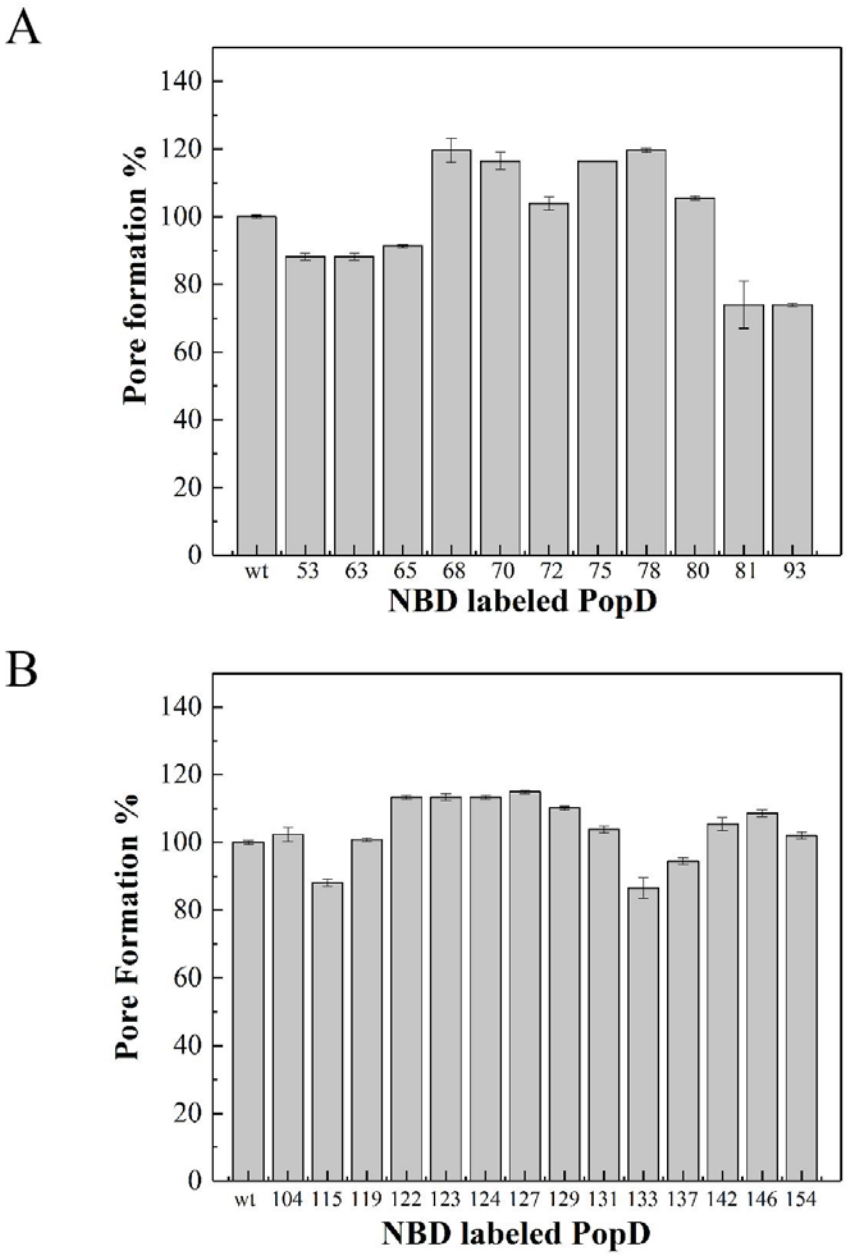

Figure S5. Pore formation of NBD-labeled PopD derivatives determined by percentage of liposome encapsulated $Tb(DPA)_3^{3-}$ quenched by the externally added EDTA as described previously (1). PopD was incubated with liposomes at 20-23˚C for 20 min. Protein: lipid ratio was 1:6000. The residues modified by NBD are indicated. Pore formation of NBD-labeled PopD was normalized to that of PopD WT. Data shown are an average of two measurements and error bars correspond to the range.

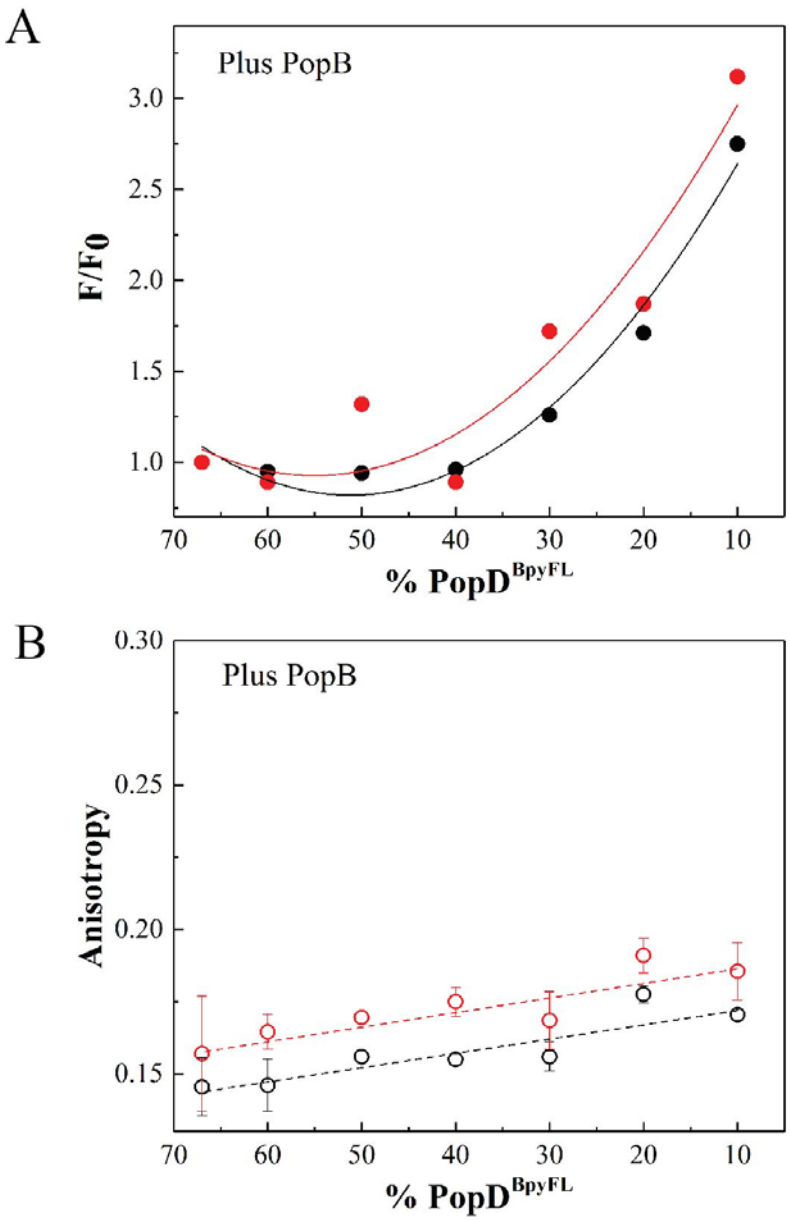

Figure S6. Replacing POPC with PSPC does not affect PopB and PopD oligomerization in membranes. Fluorescence intensity and anisotropy changes observed upon dilution of $PopD^{BpyFL}$ with increasing amounts of PopB WT, as described in Romano et al. (2). **A**, steady-state fluorescence intensity of $PopD^{BpyFL}$ measured at the indicated percentage of labeled protein when diluted with PopB WT reconstituted in liposomes containing 65% POPC (*Black circles*) or 40% PSPC (*Red circles*). **B,** anisotropy of $PopD^{BpyFL}$ when diluted with PopB WT reconstituted in liposomes containing 65% POPC (*Black empty circles*) or 40% PSPC (*Red empty circles*), was measured as described previously (2). Error bars represent the data range of two independent anisotropy determinations. Average lines are shown as a guide for the reader.

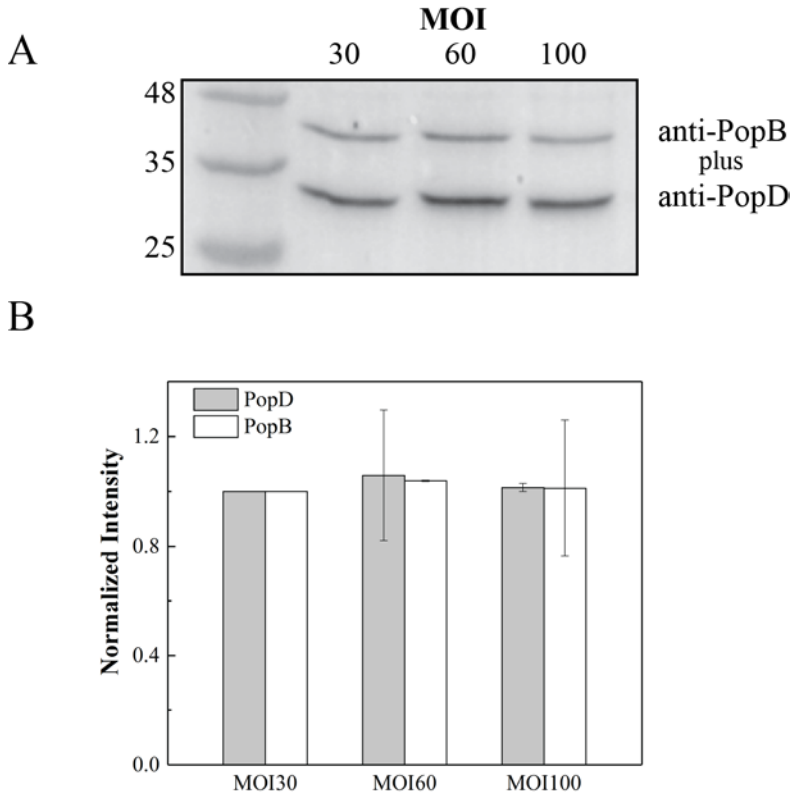

Figure S7. Comparison for PopD and PopB detected on HeLa cells when incubated with *P. aeruginosa* using different MOI. **A,** PAKΔ*exsE*Δ*exoSTY*Δ*popD::popD* was incubated with HeLa cells with an MOI of 30, 60 or 100 in FBS-free DMEM for 1 hr. Cell lysates were collected by Triton solubilization and quantified using BCA assay. A total of 30 µg protein from each sample was analyzed for PopB and PopD by immunoblotting. Representative blots from two independent experiments are shown. **B,** Quantification of band intensities of blots. Band intensities were quantified using ImageJ. To compare results from different lanes, band intensities of PopB and PopD were normalized to the one observed for MOI 30.

References

1. Romano, F. B., Rossi, K. C., Savva, C. G., Holzenburg, A., Clerico, E. M., and Heuck, A. P. (2011) Efficient isolation of Pseudomonas aeruginosa type III secretion translocators and assembly of heteromeric transmembrane pores in model membranes. *Biochemistry* **50**, 7117-7131

2. Romano, F. B., Tang, Y., Rossi, K. C., Monopoli, K. R., Ross, J. L., and Heuck, A. P. (2016) Type 3 Secretion Translocators Spontaneously Assemble a Hexadecameric Transmembrane Complex. *J Biol Chem* **291**, 6304-6315