Supplementary Tables:

Supplementary Table 1. Strains and plasmids used in this study. Antibiotic resistance is indicated by Gm (gentamycin), Cb (carbenicillin), and Kn (kanamycin).

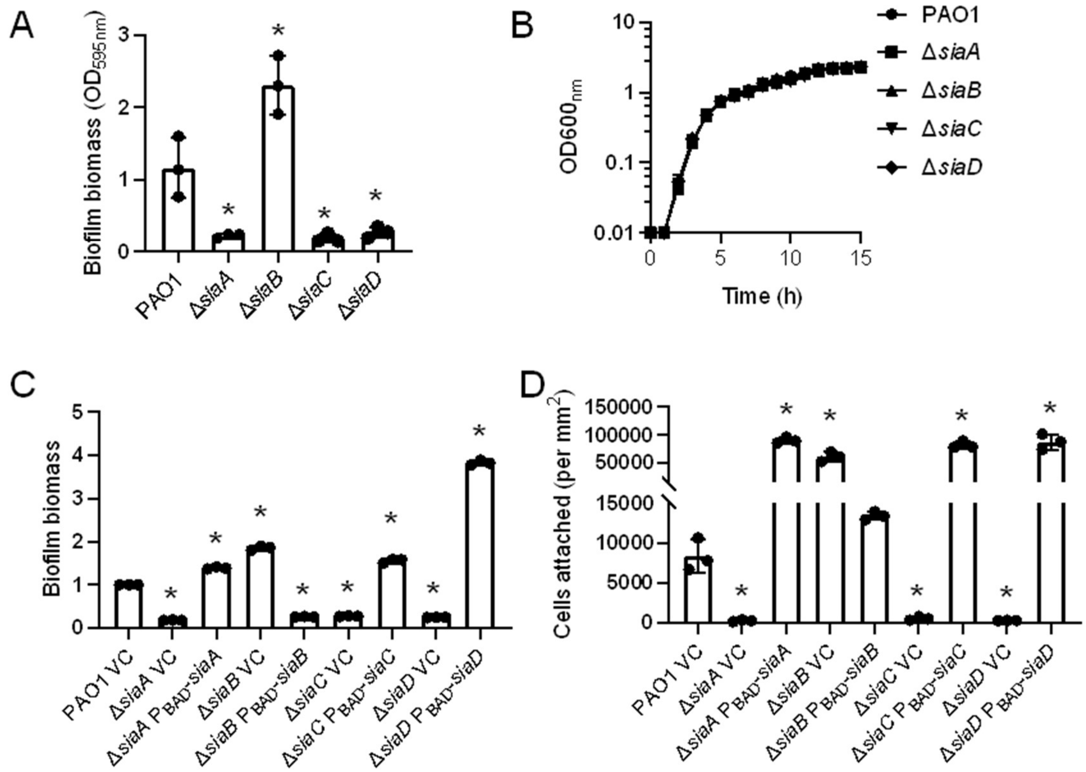
Strain name	Source	Antibiotic resistance
PAO1 (Parsek)	(53)	
PAO1 Δ*siaA*	(48)	
PAO1 Δ*siaB*	This work	
PAO1 Δ*siaC*	This work	
PAO1 Δ*siaD*	(48)	
PAO1 Δ*pslD*	(13)	
PAO1 pJN105	This work	Gm
PAO1 Δ*siaA* pJN105	This work	Gm
PAO1 Δ*siaB* pJN105	This work	Gm
PAO1 Δ*siaC* pJN105	This work	Gm
PAO1 Δ*siaD* pJN105	This work	Gm
PAO1 Δ*siaA* pJN105::P_{BAD}-*siaA*	This work	Gm
PAO1 Δ*siaB* pJN105::P_{BAD}-*siaB*	This work	Gm
PAO1 Δ*siaC* pJN105::P_{BAD}-*siaC*	This work	Gm
PAO1 Δ*siaD* pJN105::P_{BAD}-*siaD*	This work	Gm
PAO1 Δ*pelA*	(54)	
PAO1 Δ*cdrA*	(11)	
PAO1 miniTn7T2-PA1/04/03-GFP	This work	
PAO1 Δ*siaA* miniTn7T2-PA1/04/03-GFP	This work	
PAO1 Δ*siaB* miniTn7T2-PA1/04/03-GFP	This work	
PAO1 Δ*siaC* miniTn7T2-PA1/04/03-GFP	This work	
PAO1 Δ*siaD* miniTn7T2-PA1/04/03-GFP	This work	
PAO1 miniTn7T2-PA1/04/03-GFP, pJN105	This work	Gm
PAO1 Δ*siaA* miniTn7T2-PA1/04/03-GFP, pJN105	This work	Gm
PAO1 Δ*siaB* miniTn7T2-PA1/04/03-GFP, pJN105	This work	Gm
PAO1 Δ*siaC* miniTn7T2-PA1/04/03-GFP, pJN105	This work	Gm
PAO1 Δ*siaD* miniTn7T2-PA1/04/03-GFP, pJN105	This work	Gm
PAO1 Δ*siaA* miniTn7T2-PA1/04/03-GFP, pJN105::P_{BAD}-*siaA*	This work	Gm
PAO1 Δ*siaB* miniTn7T2-PA1/04/03-GFP, pJN105::P_{BAD}-*siaB*	This work	Gm
PAO1 Δ*siaC* miniTn7T2-PA1/04/03-GFP, pJN105::P_{BAD}-*siaC*	This work	Gm
PAO1 Δ*siaD* miniTn7T2-PA1/04/03-GFP, pJN105::P_{BAD}-*siaD*	This work	Gm
PAO1 Δ*pslD* miniTn7T2-PA1/04/03-GFP	This work	
PAO1 Δ*pelA* miniTn7T2-PA1/04/03-GFP	This work	
PAO1 Δ*cdrA* miniTn7T2-PA1/04/03-GFP	This work	
PAO1 Δ*pslD* pJN105	This work	Gm
PAO1 Δ*pslD* pJN105::P_{BAD}-*pslD*	This work	Gm
PAO1 Δ*pslD* miniTn7T2-PA1/04/03-GFP, pJN105	This work	Gm
PAO1 Δ*pslD* miniTn7T2-PA1/04/03-GFP, pJN105::P_{BAD}-*pslD*	This work	Gm
PAO1 Δ*wspR*	(25)	
PAO1 Δ*sadC*	(48)	
PAO1 *siaD*^{E142A}	This work	
PAO1 *siaD*^{E142A} pJN105	This work	Gm
PAO1 *siaD*^{E142A} pJN105::P_{BAD}-*siaD*	This work	Gm
PAO1 *siaD*^{E142A} miniTn7T2-PA1/04/03-GFP	This work	
PAO1 *siaD*^{E142A} miniTn7T2-PA1/04/03-GFP, pJN105	This work	Gm
PAO1 *siaD*^{E142A} miniTn7T2-PA1/04/03-GFP, pJN105::P_{BAD}-*siaD*	This work	Gm

PAO1 Δ*siaD* pJN105::P_{BAD}-*siaD*-his	This work	Gm
PAO1 Δ*siaD* pJN105::P_{BAD}-*siaD*^{E142A}-his	This work	Gm
MPAO1 (Manoil PAO1)	(37, 38)	
MPAO1 (PW1288) *siaD* (PA0169)::ISphoA/hah	(37, 38)	
MPAO1 (PW1289) *siaD* (PA0169)::ISphoA/hah	(37, 38)	
MPAO1 (PW1627) PA0338::ISphoA/hah	(37, 38)	
MPAO1 (PW1626) PA0338::ISlacZ/hah	(37, 38)	
MPAO1 (PW2543) PA0847::ISphoA/hah	(37, 38)	
MPAO1 (PW2544) PA0847::ISphoA/hah	(37, 38)	
MPAO1 (PW2999) *roeA* (PA1107)::ISlacZ/hah	(37, 38)	
MPAO1 (PW3000) *roeA* (PA1107)::ISphoA/hah	(37, 38)	
MPAO1 (PW3023) *tpbB* (PA1120)::ISlacZ/hah	(37, 38)	
MPAO1 (PW3024) *tpbB* (PA1120)::ISphoA/hah	(37, 38)	
MPAO1 (PW4043) *mucR* (PA1727)::ISlacZ/hah	(37, 38)	
MPAO1 (PW4045) *mucR* (PA1727)::ISphoA/hah	(37, 38)	
MPAO1 (PW7263) *wspR* (PA3702)::ISlacZ/hah	(37, 38)	
MPAO1 (PW7264) *wspR* (PA3702)::ISphoA/hah	(37, 38)	
MPAO1 (PW8315) *sadC* (PA4332)::ISphoA/hah	(37, 38)	
MPAO1 (PW8314) *sadC* (PA4332)::ISlacZ/hah	(37, 38)	
MPAO1 (PW9146) *gcbA* (PA4843)::ISphoA/hah	(37, 38)	
MPAO1 (PW9145) *gcbA* (PA4843)::ISlacZ/hah	(37, 38)	
MPAO1 (PW9347) *fimX* (PA4959)::ISphoA/hah	(37, 38)	
MPAO1 (PW9346) *fimX* (PA4959)::ISlacZ/hah	(37, 38)	
MPAO1 (PW10280) *dgcH* (PA5487)::ISlacZ/hah	(37, 38)	
MPAO1 (PW10281) *dgcH* (PA5487)::ISphoA/hah	(37, 38)	
PAO1 Δ*siaD* pJN105::P_{BAD}-*ml1419c*	This work	Gm
PAO1 Δ*siaD* pJN105::P_{BAD}-*ml1419c* miniTn7T2-PA1/04/03-GFP	This work	Gm
PAO1 P_{BAD}-*pslABCDEFGHIJKL*	(20)	
PAO1 Δ*siaD* P_{BAD}-*pslABCDEFGHIJKL*	This work	
PAO1 P_{BAD}-*pslABCDEFGHIJKL* miniTn7T2-PA1/04/03-GFP	This work	
PAO1 Δ*siaD* P_{BAD}-*pslABCDEFGHIJKL* miniTn7T2-PA1/04/03-GFP	This work	
DH5α pRK2013	ATCC 37159	Kn
DH5α pDONRPEX18Gm::Δ*siaB*$_{26-128}$	This work	Gm
DH5α pDONRPEX18Gm::Δ*siaC*$_{7-121}$	This work	Gm
DH5α pDONRPEX18Gm::Δ*siaD*	(48)	Gm
DH5α pDONRPEX18Gm::*siaD*^{E142A}	This work	Gm
DH5α pJN105	(55)	Gm
DH5α pJN105::P_{BAD}-*pslD*	This work	Gm
DH5α pJN105::P_{BAD}-*siaA*	This work	Gm
DH5α pJN105::P_{BAD}-*siaB*	This work	Gm
DH5α pJN105::P_{BAD}-*siaC*	This work	Gm
DH5α pJN105::P_{BAD}-*siaD*	This work	Gm
DH5α pJN105::P_{BAD}-*siaD*-his	This work	Gm
DH5α pJN105::P_{BAD}-*siaD*^{E142A}-his	This work	Gm
DH5α pJN105::P_{BAD}-*ml1419c*	(43)	Gm
DH5α pBT270 (pUC18-miniTn7T2-PA1/04/03-GFP)	(56)	Gm
DH5α pTNS1	(57)	Cb

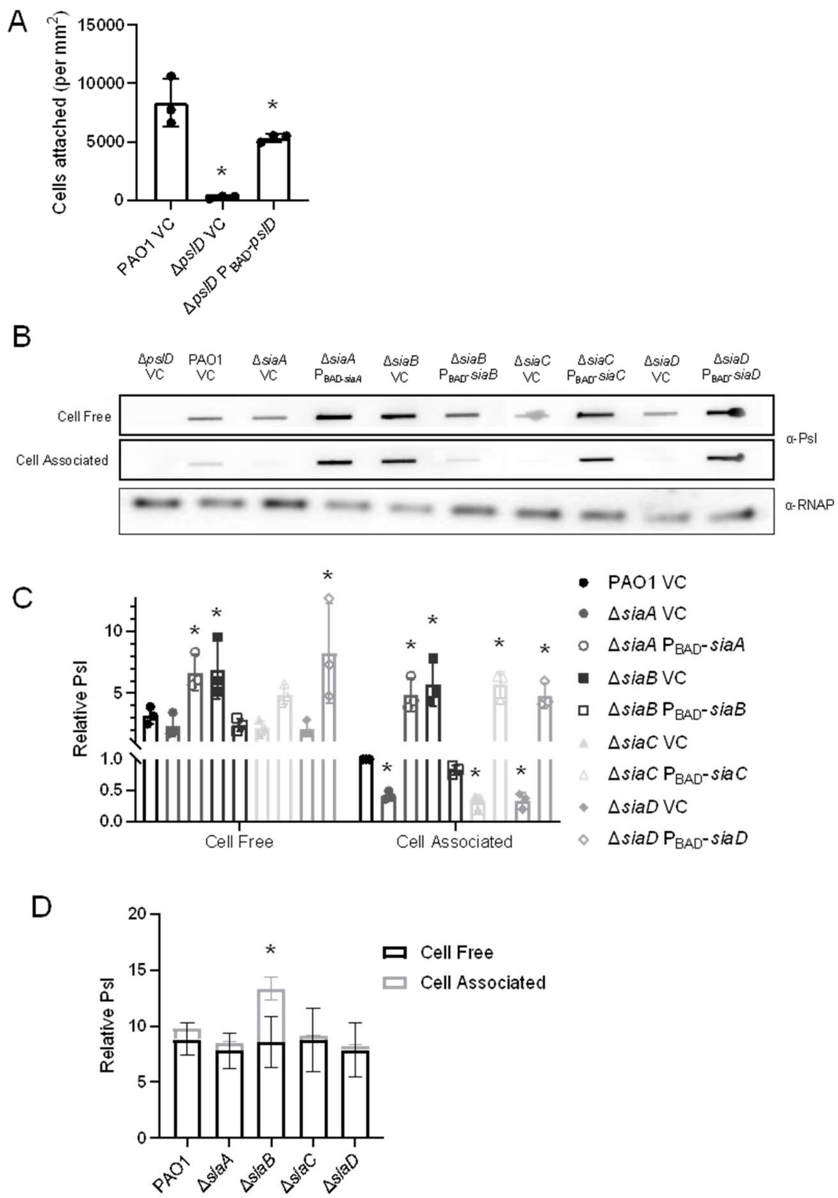
Supplementary Table 2. Primers used in this study.

Primers	**Function**	**Sequence 5'-3'**
siaB-UP-F	Creation of pDONRPEX18Gm::Δ*siaB*$_{26\text{-}128}$	ATCCGGAAGCTTCTGCCAGTCGCCCTGGAT
siaB-UP-R	Creation of pDONRPEX18Gm::Δ*siaB*$_{26\text{-}128}$	TGCTTCAACGGACCGTACAAGGAGCAGCTACGCCG
siaB-DN-F	Creation of pDONRPEX18Gm::Δ*siaB*$_{26\text{-}128}$	TAGCTGCTCCTTGTACGGTCCGTTGAAGCAGAGC
siaB-DN-R	Creation of pDONRPEX18Gm::Δ*siaB*$_{26\text{-}128}$	ATCCGGCCCGGGACTGACGGTTTCCTCGACCA
siaC-UP-F	Creation of pDONRPEX18Gm::Δ*siaC*$_{7\text{-}121}$	ATCCGGCTGCAGGCCAATCTCAAGGGCTAC
siaC-UP-R	Creation of pDONRPEX18Gm::Δ*siaC*$_{7\text{-}121}$	CGGCTACTCGTCGTGGCCTGTATGTGCAGGTCACTCATG
siaC-DN-F	Creation of pDONRPEX18Gm::Δ*siaC*$_{7\text{-}121}$	CAGGCCCACGACGAGTCAGGCCCACGACGAGT
siaC-DN-R	Creation of pDONRPEX18Gm::Δ*siaC*$_{7\text{-}121}$	ATCCGGGAATCCCGCGGATCGAGGCTTC
P_{BAD} -*siaA*-F	Creation of pJN105::P_{BAD}-*siaA*	ATCCGGTCTAGACTAGTCGAATCGGAAGGACAGGATGG
P_{BAD} -*siaA*-R	Creation of pJN105::P_{BAD}-*siaA*	CCGGATCCCGGGGGATAGCCATGGCGGCGAAC
P_{BAD} -*siaB*-F	Creation of pJN105::P_{BAD}-*siaB*	ATCCGGTCTAGATCAGATCACGGCGCGCAG
P_{BAD} -*siaB*-R	Creation of pJN105::P_{BAD}-*siaB*	CCGGATCCCGGGGGATAGCCATGGAAACGCTAGACCTGCTGG
P_{BAD} -*siaC*-F	Creation of pJN105::P_{BAD}-*siaC*	ATCCGGTCTAGACTACTCGTCGTGGGCCTGGAT
P_{BAD} -*siaC*-R	Creation of pJN105::P_{BAD}-*siaC*	CCGGATCCCGGGGGAAGAACACCATCATGAGTGACCT
P_{BAD} -*siaD*-F	Creation of pJN105::P_{BAD}-*siaD*	ATCCGGGAGCTCTCAGCGCGCTGGAGCC
P_{BAD} -*siaD*-R	Creation of pJN105::P_{BAD}-*siaD*	CCGGATTCTAGAGAACGGGTCGCCGAGCTG
P_{BAD} -*siaD*-his-R	Creation of pJN105::P_{BAD}-*siaD*-his	ATCCGGGAGCTCTCAGTGATGATGATGATGATGATGATGATGATG GCGCGCTGGAGCCGG
P_{BAD} -*pslD*-F	Creation of pJN105::P_{BAD}-*pslD*	ATCCGGTCTAGAATCATGACCTACAGGAAGTGCT
P_{BAD} -*pslD*-R	Creation of pJN105::P_{BAD}-*pslD*	CCGGATGAGCTCTCAGCGATCATTGTTGACGGT
siaD^{E142A}-F	For sited directed mutagenesis	GGCGGCGAGGcATTCCTCCTG
siaD^{E142A}-R	For sited directed mutagenesis	CCAGCGGCCGCAGAGGTC
pslA RT-F	For qPCR	TGCACAAGATCAAGAAACGCGTGG
pslA RT-R	For qPCR	ACGGAACAGGATGTAGAGGTCGAA
rpoD RT-F	For qPCR	GAACAGGCGCAGGAAGTCGG
rpoD RT-R	For qPCR	GCCGAGCTGTTCATGCCGAT

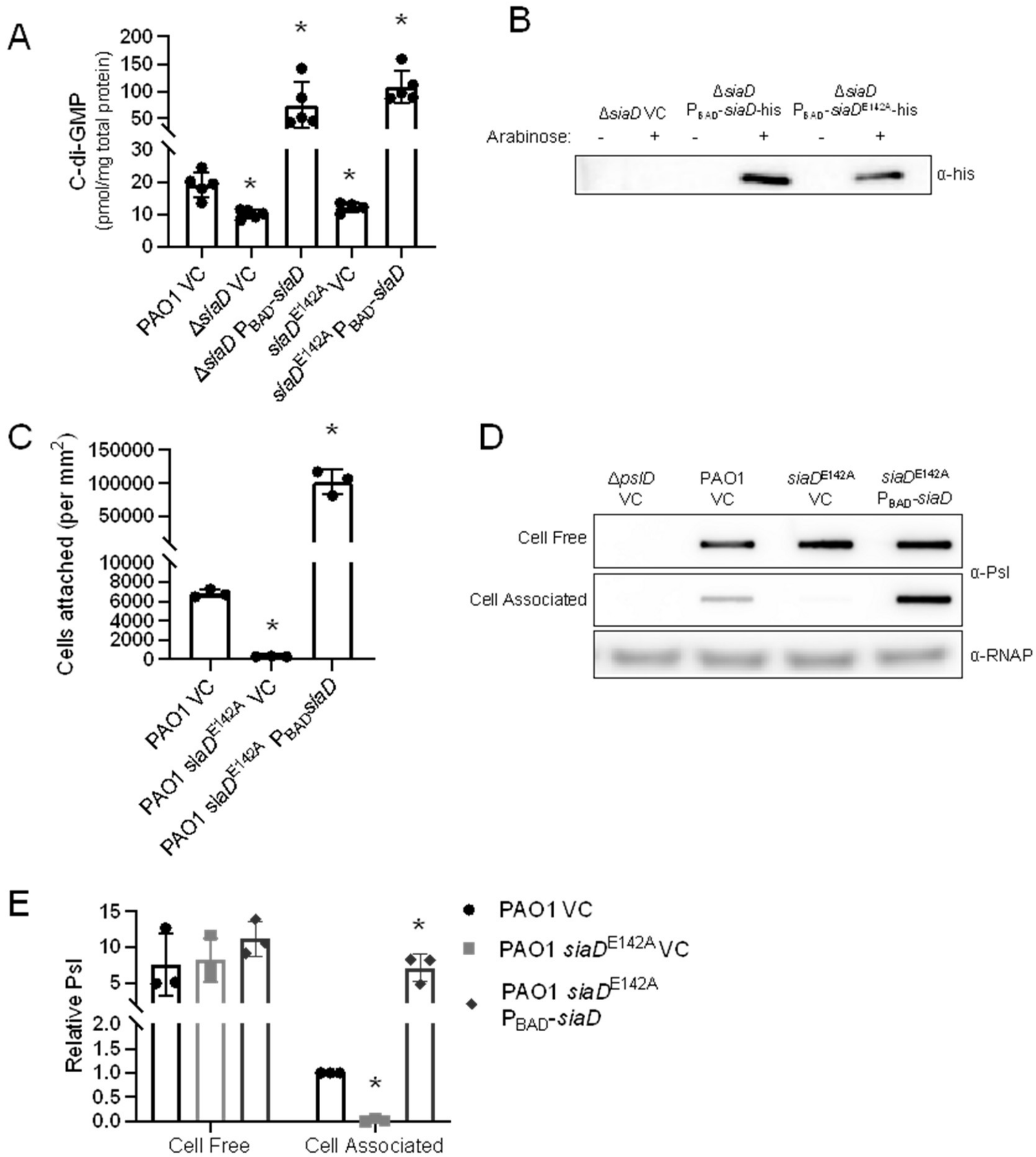
Supplementary Figures



Supplemental Figure 1: Attachment and biofilm formation of *sia* mutants and overexpression strains. (A) Raw values from the static biofilm formation assay shown in Figure 1B. Biofilm biomass produced by each strain was measured by crystal violet staining. Presented as mean and standard deviation. N = 3 biological replicates, *p<0.05. (B) Growth curve of *sia* mutants in Lennox Broth. (C) Static biofilm formation by *sia* overexpression strains Biofilm biomass produced by each strain was measured by crystal violet staining and normalized to the wild-type vector control (PAO1 VC). (D) Pair-wise statistical comparisons, p-values, of the initial attachment data presented in Fig 1C. (E) Adherence of *sia* overexpression strains. Cells were incubated on a glass coverslip, rinsed and attached cells were immediately quantified by microscopy. Normalized to the wild-type vector control (PAO1 VC), VC=vector control. Presented as mean and standard deviation. N = 3 biological replicates, *p<0.05.

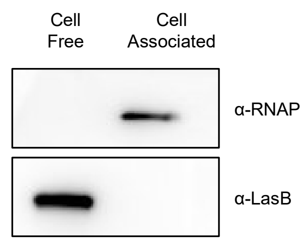


Supplemental Figure 2: Psl production of *sia* mutants and overexpression strains. (A) Adherence of the *pslD* complementation strain. Cells were incubated on a glass coverslip, rinsed and attached cells were immediately quantified by microscopy. (B) Representative immunoblot for Psl from *sia* mutant overexpression strains, extracted from mid-log planktonic cells (OD_{600nm}=0.5). RNAP served as a loading control. (C) Quantification of relative Psl production calculated using blots in S2B. Psl band intensity was normalized to RNAP levels and then compared to the wild-type vector control (PAO1 VC) cell-associated Psl. VC=vector control. (D) Quantification of Psl produced by *sia* mutants. Re-graphing of data presented in **Fig 2C**. Presented as mean and standard deviation. N = 3 biological replicates, *p<0.05.



Supplemental Figure 3: C-di-GMP, attachment, and Psl production of *siaD* and *siaD*^{E142A} overexpression strains. (A) C-di-GMP levels of Δ*siaD* and *siaD*^{E142A} when SiaD is overexpressed. C-di-GMP was extracted from mid-log planktonic cells (OD_{600nm}=0.5). Presented as mean and standard deviation. N = 5 biological replicates, *p<0.05. (B) Representative α-his immunoblot for SiaD-his and SiaD^{E142A}-his to confirm protein expression and stability. (C) Adherence for *siaD*^{E142A} when SiaD is overexpressed. Cells were incubated on a glass coverslip, rinsed, and attached cells were immediately quantified by microscopy. (D) Representative immunoblot for Psl from mid-log, planktonic cells

(OD_{600nm}=0.5) for the *siaD*^{E142A} when SiaD is overexpressed. RNAP served as a loading control. (E) Quantification of relative Psl production calculated using blots in S3D. Psl band intensity was normalized to RNAP levels and then compared to the wild-type vector control (PAO1 VC) cell-associated Psl. Presented as mean and standard deviation. N = 3 biological replicates, *$p<0.05$. VC=vector control.



Supplemental Figure 4: Separation of cell-free and cell-associated fractions.

Representative immunoblot for RNAP and LasB after separation into cell-free and cell-associated fractions by centrifugation. RNAP is only found in the cell-associated fraction, and the cell-free protease LasB is only found in the cell-free fraction. N = 3 biological replicates.