Supporting Material
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TABLE S1. Strains and plasmids used in this study

Strain or Plasmid	Relevant Feature(s)	Source or Reference
Strains		
Escherichia coli		
DH5α/λ*pir*	λ*pir*; cloning strain	(1)
Top10F'	Cloning strain	Invitrogen
S17-1/λ*pir*	λ*pir*; Tra^+, cloning strain	(2)
S17-1/λ*pir* (pFD1)	*Himar1* conjugal donor	(3)
A. tumefaciens		
C58	Nopaline type strain; pTiC58; pAtC58	(4)
Δ*ctpA*	ΔAtu0224 C58 derivative	This study
Δ*ctpB*	ΔAtu0223 C58 derivative	This study
Δ*ctpCD*	ΔAtu0222ΔAtu0221 C58 derivative	This study
Δ*ctpE*	ΔAtu0220 C58 derivative	This study
Δ*ctpF*	ΔAtu0219 C58 derivative	This study
Δ*ctpG*	ΔAtu0218 C58 derivative	This study
Δ*ctpH*	ΔAtu0217 C58 derivative	This study
Δ*ctpI*	ΔAtu0216 C58 derivative	This study
Δ*pilA*	ΔAtu3514 C58 derivative	This study
Δ*pilA*Δ*ctpA*	ΔAtu0224ΔAtu3514 C58 derivative	This study
Δ*ctpCD*Δ*ctpA*	ΔAtu0224ΔAtu0222ΔAtu0221 C58 derivative	This study

Δ*ctpE*Δ*ctpA*	ΔAtu0224ΔAtu0220 C58 derivative	This study
Δ*ctpF*Δ*ctpA*	ΔAtu0224ΔAtu0219 C58 derivative	This study
Δ*ctpH*Δ*ctpA*	ΔAtu0224ΔAtu0217 C58 derivative	This study
Δ*ctpI*Δ*ctpA*	ΔAtu0224ΔAtu0216 C58 derivative	This study
Δ*uppC*	ΔAtu1238 C58 derivative	From the lab
Δ*ctpCD*Δ*uppC*	ΔAtu1238ΔAtu0222ΔAtu0221 C58 derivative	This study
Δ*ctpE*Δ*uppC*	ΔAtu1238ΔAtu0220 C58 derivative	This study
Δ*ctpF*Δ*uppC*	ΔAtu1238ΔAtu0219 C58 derivative	This study
Δ*ctpH*Δ*uppC*	ΔAtu1238ΔAtu0217 C58 derivative	This study
Δ*ctpI*Δ*uppC*	ΔAtu1238ΔAtu0216 C58 derivative	This study
C58p-	Ti and At plasmid cured C58 derivative	From the lab
C58p-Δ*ctpCD*	ΔAtu0222ΔAtu0221 C58p-derivative	This study
C58p-Δ*ctpE*	ΔAtu0220 C58p- derivative	This study
C58p-Δ*ctpF*	ΔAtu0219 C58p- derivative	This study
C58p-Δ*ctpH*	ΔAtu0217 C58p- derivative	This study
C58p-Δ*ctpI*	ΔAtu0216 C58p- derivative	This study
Plasmids		
pGEM-T easy	PCR cloning vector; amp^R	Promega
pJZ383	P_{tac}-*gfpmut3*, Sp^R, pVS replicon	J. Zhu, (5)
pNPTS138	colE1 suicide plasmid; *sacB* (Suc^S); Kan^R	(6)
pSRKKm	Broad host range P_{lac} expression vector; *lacIQ*; Kan^R	(7)
pSRKGm	Broad host range P_{lac} expression vector; *lacIQ*; Gen^R	(7)

pRA301	Broad host range; promoterless *lacZ*; Spc^R	(8)
pCTPA101	pNPTS138 carrying Atu0224 SOE deletion fragment	This study
pCTPB101	pNPTS138 carrying Atu0223 SOE deletion fragment	This study
pCTPCD101	pNPTS138 carrying Atu0222 and Atu0221 SOE deletion fragment	This study
pCTPE101	pNPTS138 carrying Atu0220 SOE deletion fragment	This study
pCTPF101	pNPTS138 carrying Atu0219 SOE deletion fragment	This study
pCTPG101	pNPTS138 carrying Atu0218 SOE deletion fragment	This study
pCTPH101	pNPTS138 carrying Atu0217 SOE deletion fragment	This study
pCTPI101	pNPTS138 carrying Atu0216 SOE deletion fragment	This study
pPilA101	pNPTS138 carrying Atu3514 SOE deletion fragment	This study
pCTPA201	pSRKKm P_{lac}::Atu0224	This study
pCTPB201	pSRKKm P_{lac}::Atu0223	This study
pCTPE201	pSRKGm P_{lac}::Atu0220	This study
pCTPG201	pSRKKm P_{lac}::Atu0218	This study
pPilA201	pSRKKm P_{lac}::Atu3514	This study
pCTPA301	*ctpA* promoter fragment in-frame fused to *lacZ* in pRA301	This study
pCTPB301	Intergenic fragment between *ctpA* and *ctpB* in-frame fused to *lacZ* in pRA301	This study

pCTPC301	Intergenic fragment between *ctpB* and *ctpC* in-frame fused to *lacZ* in pRA301	This study
pCTPE301	Intergenic fragment between *ctpD* and *ctpE* in-frame fused to *lacZ* in pRA301	This study
pPilA301	*pilA* promoter fragment in-frame fused to *lacZ* in pRA301	This study

Table S2. Primer Sequences

Primer	Restriction Site[a]	Sequence[b]
ctpA1	*SpeI*	actagtATGATTTCCTGTGACCGA
ctpA2	NE	aagcttggtaccgaattcTGATCATTGAAAAGCATCGTGC
ctpA3	NE	gaattcggtaccaagcttCATGAGGTCTCTCCTAAATCTT
ctpA4	*SphI*	gcatgcCGGATGATCGTCACGTGT
ctpB1	*SpeI*	actagtGTTATGAAACTGTCGACCAC
ctpB2	NE	aagcttggtaccgaattcGCCAGTATTTTATAATTCCG
ctpB3	NE	gaattcggtaccaagcttCATAAATAGCCTACCTTCGT
ctpB4	*SphI*	gcatgcAACTCATCCTTCCGTTTACT
ctpC1	*SpeI*	actagtCATCTGAATGTTGACGATAC
ctpC2	NE	aagcttggtaccgaattcGGGTTCATCTACAAATGACG
ctpD3	NE	gaattcggtaccaagcttAACGGAAAGAATGACGATAC
ctpD4	*SphI*	gcatgcGTCTTTCGCTTTTATCGCTC
ctpE1	*SpeI*	actagtGATAAAGCGTGATGTCGTTG
ctpE2	NE	aagcttggtaccgaattcTGATGCAGGTCCACCATTTC
ctpE3	NE	gaattcggtaccaagcttTTCCGACAAGGTGATAGGGT
ctpE4	*SphI*	gcatgcGCAACGAAACGGAACTTGTC
ctpF1	*SpeI*	actagt GGCGAAATTTTTGATCGTGA
ctpF2	NE	aagcttggtaccgaattcCTGAAGAATATTCTCAAGCG
ctpF3	NE	gaattcggtaccaagcttGTATTCTACAGCGCTCATCG
ctpF4	*SphI*	gcatgcGTCAACATTCAGATGCCTTC
ctpG1	*SpeI*	actagtGGTCAACAAGGACAGACCAT

ctpG2	NE	aagcttggtaccgaattcAAGTCATCCTAAGGAAAGCG
ctpG3	NE	gaattcggtaccaagcttCATCGGCTTTTCCGTCTAAG
ctpG4	*SphI*	gcatgcGTTGCTGTTCTCGATCTTCC
ctpH1	*SpeI*	actagtCGATATAGCCGAAAACAAGC
ctpH2	NE	aagcttggtaccgaattcGATATTTGAGAGGCGACGAT
ctpH3	NE	gaattcggtaccaagcttTAACACTATTGTCGGGTCCA
ctpH4	*SphI*	gcatgcTCAAGAACTGCCTTCGTATG
ctpI1	*SpeI*	actagtTGACCTCAAGCCGAATGATC
ctpI2	NE	aagcttggtaccgaattcTTCTGACGTTTACGCACAGC
ctpI3	NE	gaattcggtaccaagcttCATCGTCGCCTCTCAAATAT
ctpI4	*SphI*	gcatgcGGTATTGGCATTTCTCCTCA
pilA1	*SpeI*	actagtAAGCCCGATGGTCTGTTTCT
pilA2	NE	aagcttggtaccgaattcTTTCCCAAGGGTTGAAAGCT
pilA3	NE	gaattcggtaccaagcttCACACGCCCTGTTGAAATTC
pilA4	*SphI*	gcatgcACGAGGATTTCTGGAAGACG
ctpA7	*NdeI*	GCATcatATGACCAAGATTTTCGCTCG
ctpA8	*HindIII*	GCATaagcttCTTTTCAATGATCAGACGCC
ctpB5	*NdeI*	GACTcatATGATTGTCGCGGCAATT
ctpB6	*HindIII*	GACTaagcttTTATAAAATACTGGCGAT
ctpE5	*NdeI*	GACTcatatgACCACCAACGCCATTCC
ctpE6	*HindIII*	AGTCaagcttCATCAGTTTGTCGGTGCCGT
ctpG5	*NdeI*	GACTcatATGTTCGGAAAACGCGGG
ctpG6	*HindIII*	ACTaagcttTTAGGATGACTTTTCCATCT
PilA5	*NdeI*	GACTcatATGCCGGTTGTCGAAACGCA

PilA6	*HindIII*	GACTaagcttTCAACCCTTGGGAAAAGCGT
CtpAP1	*EcoRI*	gaattcATGATTTTCGCCAGTTTTCCG
CtpAP2	*PstI*	ctgcagCATGAGGTCTCTCCTAAATCT
CtpBP1	*EcoRI*	gaattcGATCATTGAAAAGCATCGTGC
CtpBP2	*PstI*	ctgcagCATAAATAGCCTACCTTCGTT
CtpCP1	*BamHI*	ggatccAAGCTCCTCTCGTCAAAGCAG
CtpCP2	*HindIII*	aagcttCATGCTCGATCCTTGAGGGAA
CtpEP1	*BamHI*	ggatccGGATCAGTGGGGTTCATC
CtpEP2	*HindIII*	aagcttGTTGGTGGTCATTGGGTC
PilAP1	*BamHI*	ggatccTGATCATTTACCATCAACGG
PilAP2	*HindIII*	aagcttCACACGCCCTGTTGAAATTC

Upper case sequence anneals to target sequences; lower case residues are engineered into oligonucleotides but do not anneal with target sequences

Supporting Information References

1. **Chiang SL, and Rubin EJ.** 2002. Construction of a mariner-based transposon for epitope-tagging and genomic targeting. Gene **296:**179-185.
2. **Kalogeraki VS, Winans SC.** 1997. Suicide plasmids containing promoterless reporter genes can simultaneously disrupt and create fusions to target genes of diverse bacteria. Gene **188:**69-75.
3. **Lampe DJ, Akerley BJ, Rubin EJ, Mekalanos JJ, Robertson HM.** 1999. Hyperactive transposase mutants of the Himar1 mariner transposon. Proceedings of the National Academy of Sciences, USA **96:**11428-11433.

4. **Watson B, Currier TC, Gordon MP, Chilton MD, and Nester EW.** 1975. Plasmid Required for Virulence of *Agrobacterium tumefaciens*. J. Bacteriol. **123:**255-264.

5. **Cormack BP, Valdivia RH, and Falkow S.** 1996. FACS-optimized mutants of the green fluorescent protein (GFP). Gene **173:**33-38.

6. **Hibbing ME, Fuqua C.** 2011. Antiparallel and interlinked control of cellular iron levels by the Irr and RirA regulators of *Agrobacterium tumefaciens*. J. Bacteriol. **193:**3461-3472.

7. **Khan SR, Gaines J, Roop RM, Farrand SK.** 2008. Broad-host-range expression vectors with tightly regulated promoters and their use to examine the influence of TraR and TraM expression on Ti plasmid quorum sensing. . Appl. Environ. Microbiol. **74:**5053-5062.

8. **Akakura R, Winans SC.** 2002. Constitutive mutations of the OccR regulatory protein affect DNA bending in response to metabolites released from plant tumors. *J Biol Chem* **277:**5866-5874.

Supplemental Figure Legends

Supplemental Figure 1. Time courses for static biofilm formation of *A. tumefaciens*. (A) Wild type C58 and Class I Ctp mutants (B) Wild type C58 and Class II mutants. Quantification of acetic acid solubilized crystal violet for coverslip biofilms at 12, 24, 36, 48, 60, and 72 h post-inoculation. Adherent biomass was normalized by growth (A_{600}/OD_{600}), and error bars are standard deviations for assays performed in triplicate.

Supplemental Figure 2. Ectopic expression of *pilA* and *ctpA* rescues the Δ*ctpA* (A) but not the Δ*ctpB* (B) biofilm deficiency. Quantification of acetic acid solubilized crystal violet for 72 h coverslip biofilms from *A. tumefaciens* derivatives, some of which harbor P_{lac}-*ctpA* and P_{lac}-*pilA* expression plasmids grown in the presence of 500 μM IPTG. Adherent biomass was normalized by growth (A_{600}/OD_{600}), and error bars are standard deviation for assays performed in triplicate.

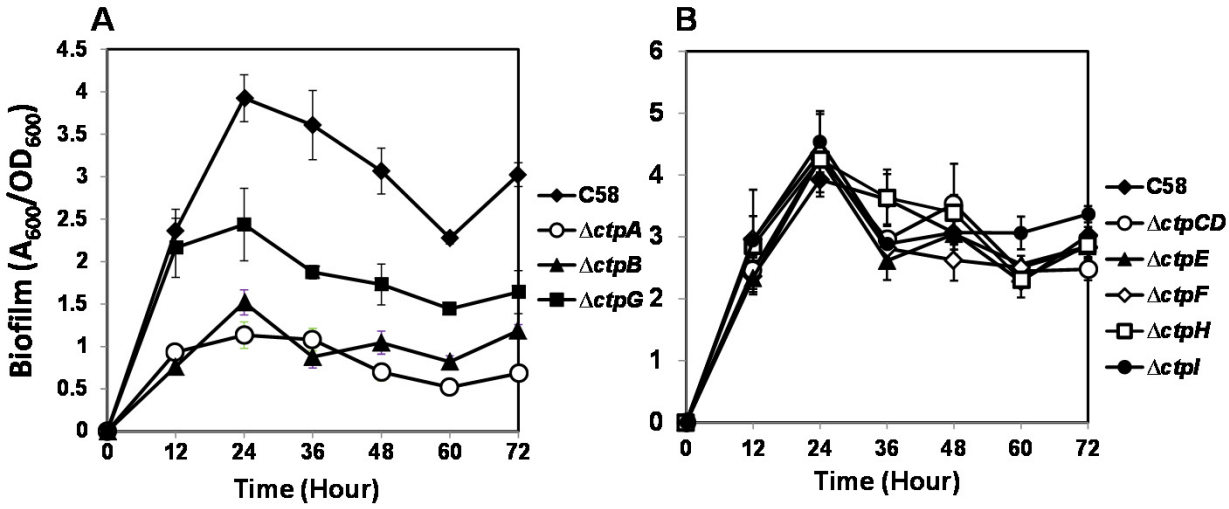
Supplemental Figure 3. Biofilm accumulation of wild type and Class I Ctp mutants. Linear regressions of time-courses for coverslip biofilm formation data for *A. tumefaciens* C58 wild type and Class I mutants: The least square linear regressions of adherent biomass (A_{600}) versus days was performed and the fitted lines show good fits for C58 and Class I mutants. Slopes reflect the estimated increased rate of biofilm accumulation.

Supplemental Figure 4. Surface attachment to plant roots (A) and tumor formation on potato disks (B). (A) Qualitative attachment assays were performed using cuttings of *Arabidopsis thaliana* roots with the wild type C58 and the Δ*ctpA* mutant expressing GFP, incubated two days. Roots were viewed using a Nikon E800 at a 100Xmagnification. Green fluorescent foci are *A. tumefaciens cells,* plant root was non-fluorescent. (B) Tumor formations of the wild type C58, the Δ*ctpA* mutant, and the Δ*ctpCD* mutant were examined on organic red potato disks for 4 weeks incubation at

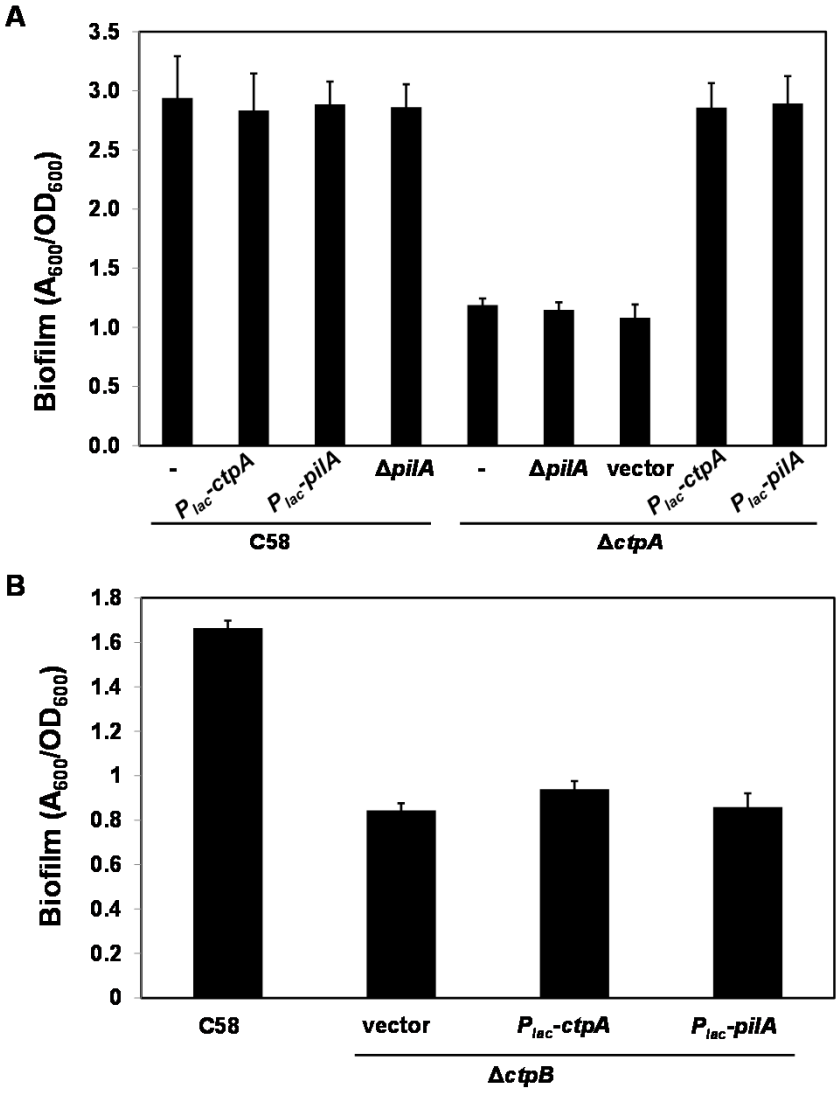
room temperature. Each strain was tested in three independent experiments containing five technical replicates per experiment per inoculum.

Supplemental Figure 5. Analysis of *A. tumefaciens* C58 cured of its megaplasmids. Quantification of acetic acid solubilized crystal violet for 72 h coverslip biofilms from *A. tumefaciens* derivatives. Adherent biomass was normalized by growth (A_{600}/OD_{600}), and error bars are standard deviation for assays performed in triplicate.

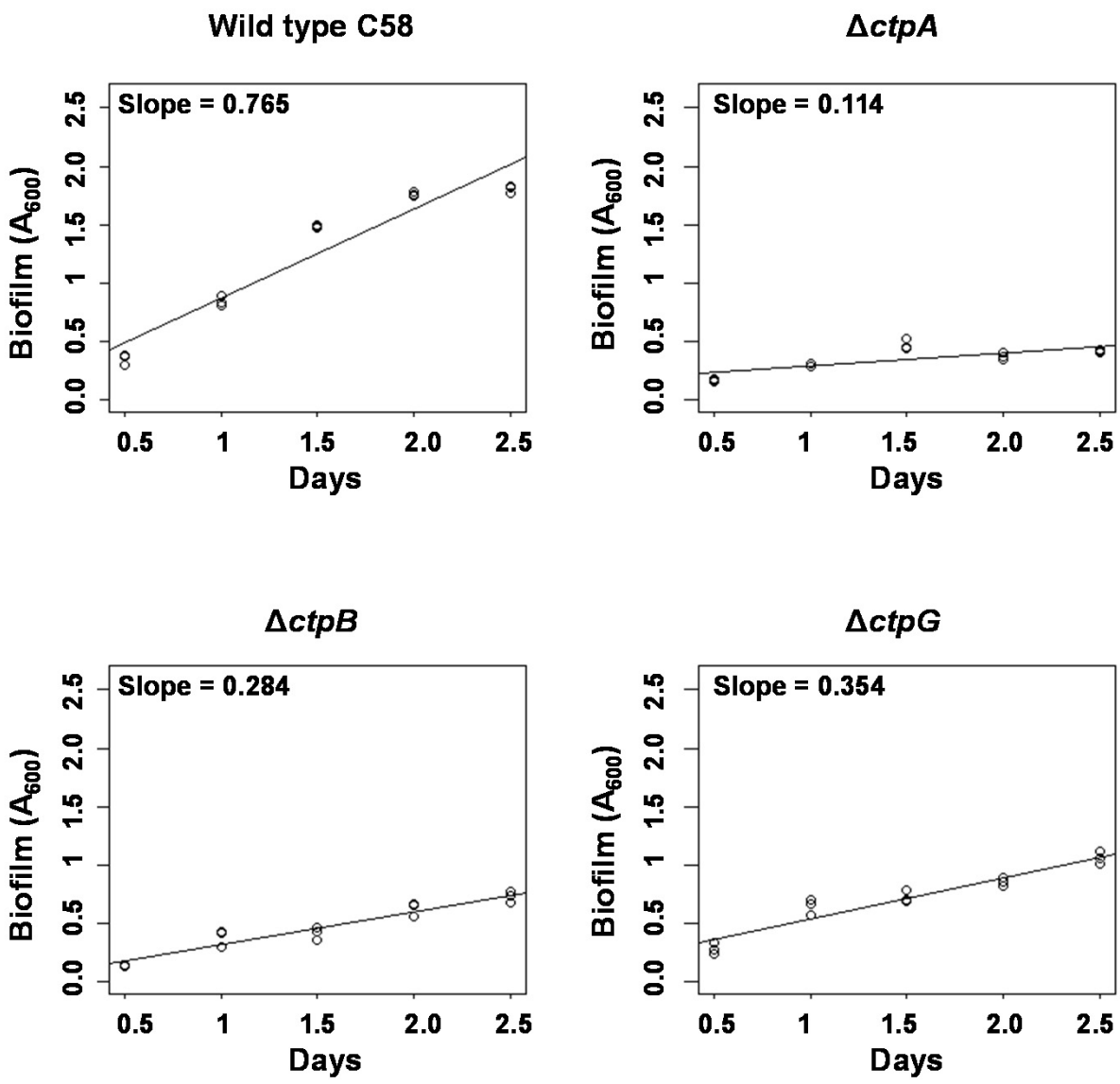
Supplemental Figure 6. Comparison of reversible and stable binding for *A. tumefaciens.* Attachment to coverslips by *A. tumefaciens* derivatives after 1 hour incubation at an inoculum of OD_{600} = 0.8, was evaluated using a Nikon E800 in bright field mode at a 100X magnification. (A) Coverslips viewed after minimal rinsing; (B): Coverslips viewed after vigorous rinsing.



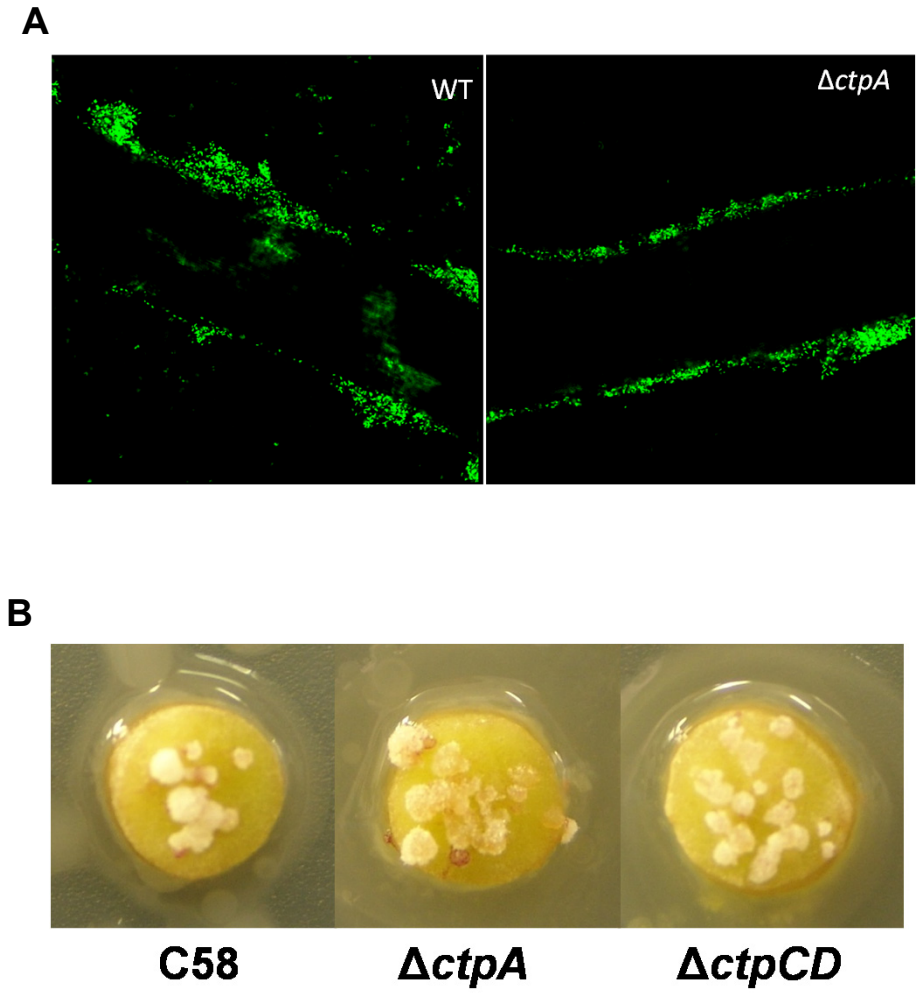
Supplementary Figure 1 – Wang et al.



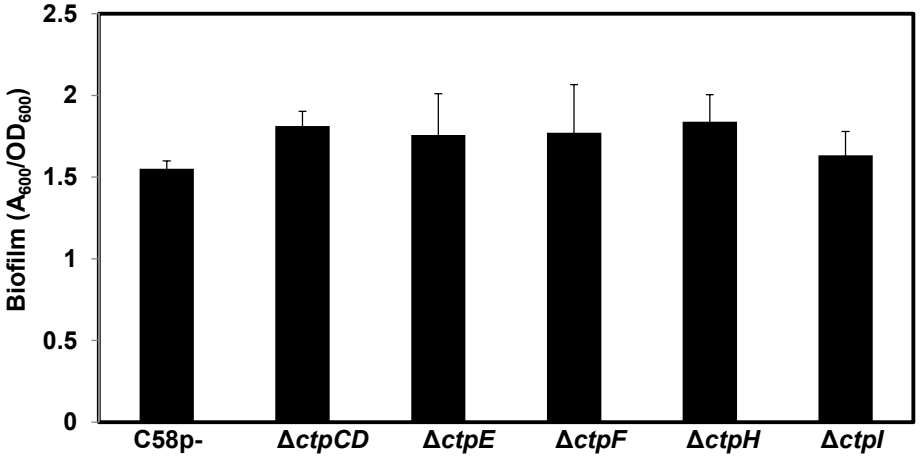
Supplemental Figure 2– Wang et al.



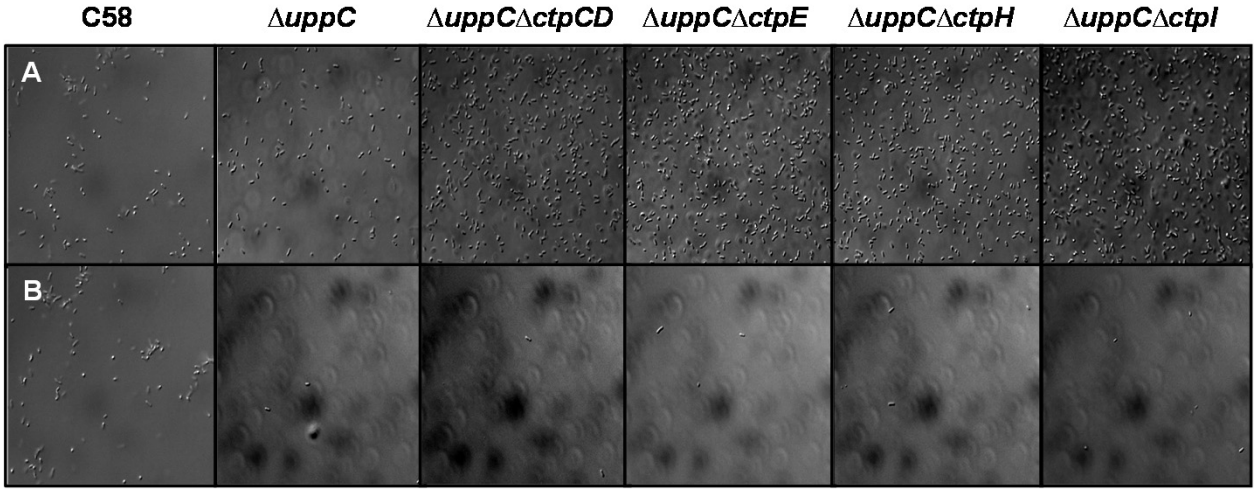
Supplemental Figure 3– Wang et al.



Supplemental Figure 4– Wang et al.



Supplementary Figure 5 – Wang et al.



Supplementary Figure 6 – Wang et al.