SUPPLEMENTARY MATERIAL

Spermidine Inversely Influences Surface Interactions and Planktonic Growth in *Agrobacterium tumefaciens*

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Running title: Polyamines and *Agrobacterium* surface adherence

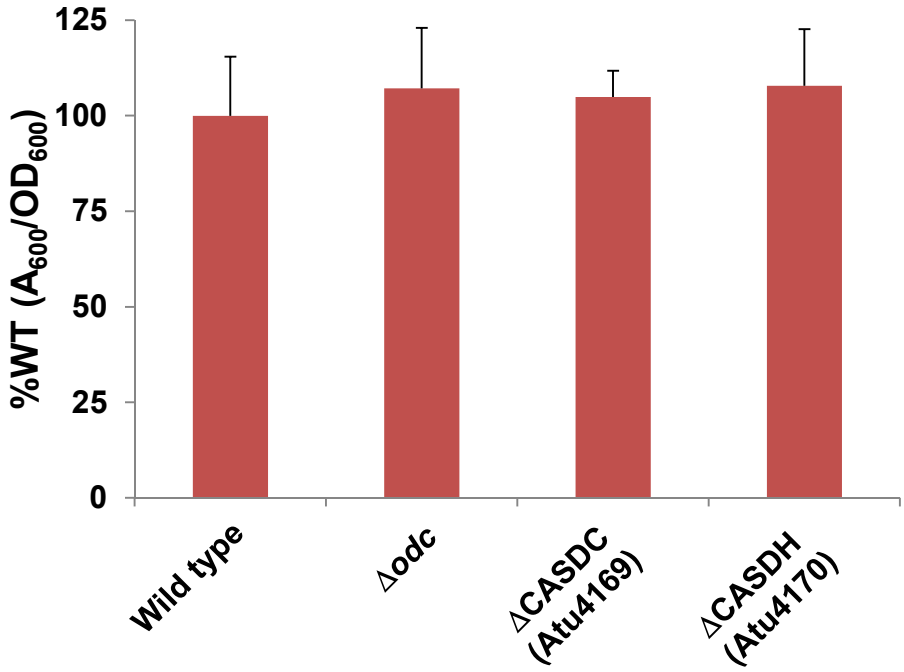

Figure S1. Biofilm formation of *A. tumefaciens* polyamine mutants in rich medium. Biofilm assays on coverslips in LB medium. *A. tumefaciens* C58 and derivatives incubated in 12-well plates with PVC coverslips for 96 h at 28°C. After rinsing, coverslips were stained with 1% crystal violet and adherent biomass measured as absorbance of acetic acid-solubilized crystal violet, normalized for planktonic culture growth (A_{600}/OD_{600}). Error bars show the standard deviation of a minimum of three biological replicates.

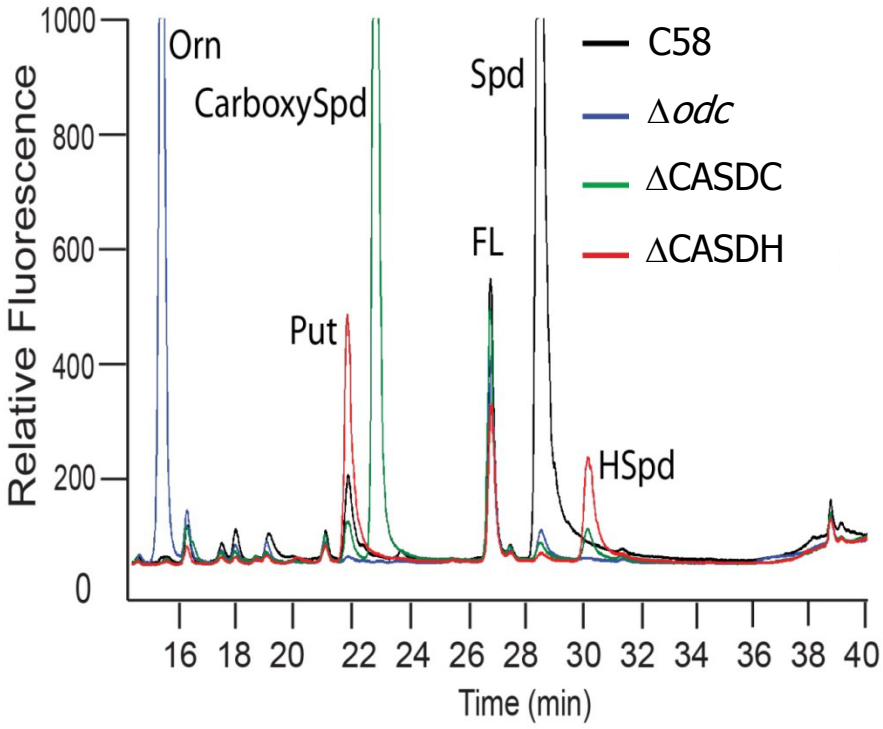

Figure S2. *A. tumefaciens* CASDH- mutant synthesizes homospermidine via the *hss* gene product. Polyamines were prepared from whole cell extracts, labeled with the AccQ-fluor, fractionated over a hydrolysate amino acid analysis HPLC column with an acetonitrile gradient. Polyamine and ornithine peaks were detected using an in-line fluorescence detector (excitation, 248 nm; emission, 398 nm). Orn, ornithine; Put, putrescine; FL, unconjugated AccQ fluor; SPD, spermidine; Hspd, homospermidine.

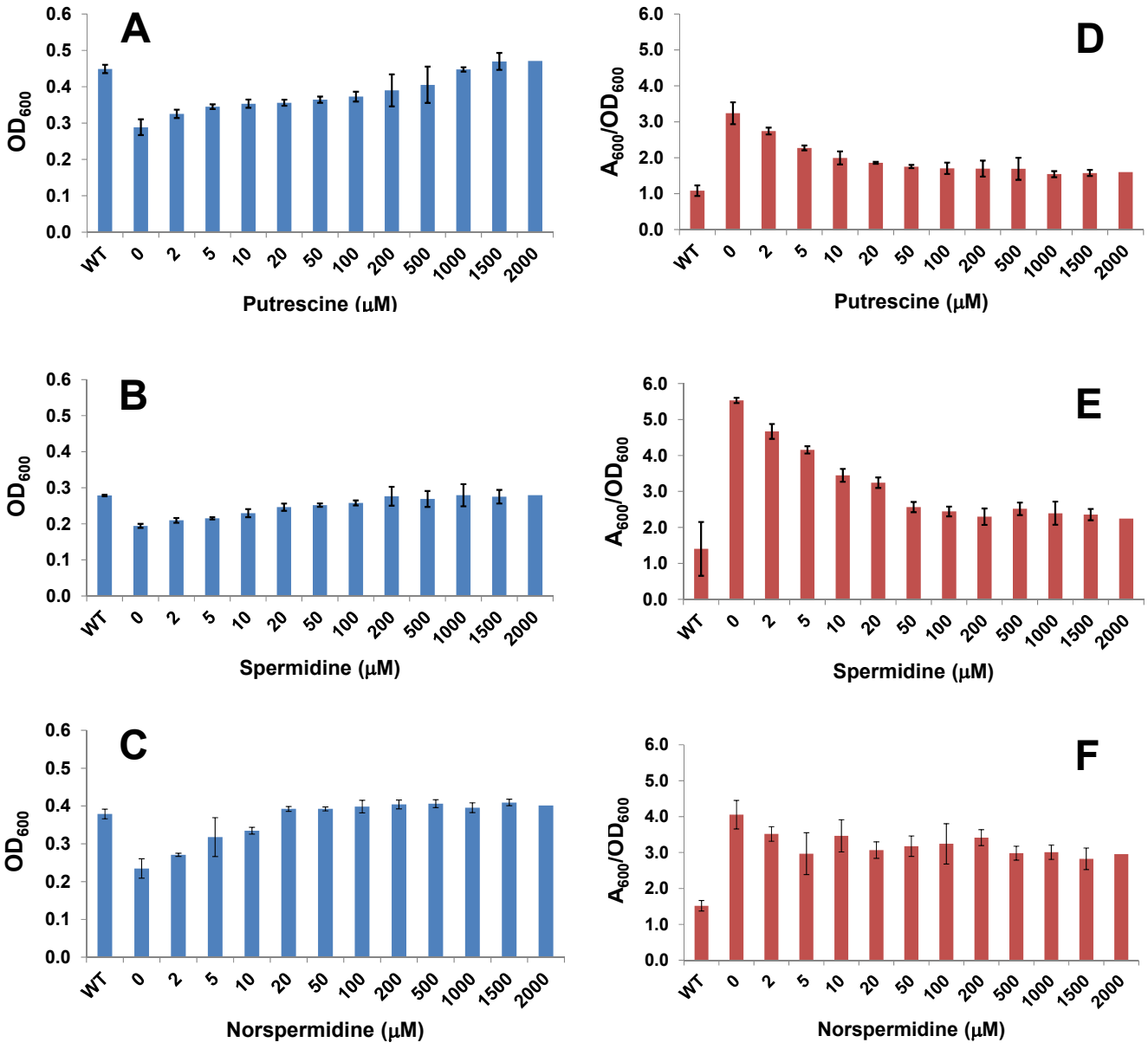

Figure S3. Rescue of growth and reversal of increased biofilm formation in the Δ*odc* mutant by exogenous polyamines. Exogenous putrescine (A and D), spermidine (B and E) and norspermidine (C and F) were tested across the indicated concentration range for their impact on 48 h biofilm cultures of the D*odc* mutant in 12-well plates for planktonic growth (OD_{600}, A, B,C) and biofilm formation on PVC coverslips with solubilized CV normalized to the planktonic culture density (A_{600}/OD_{600}). Error bars show the standard deviation of a minimum of three biological replicates.

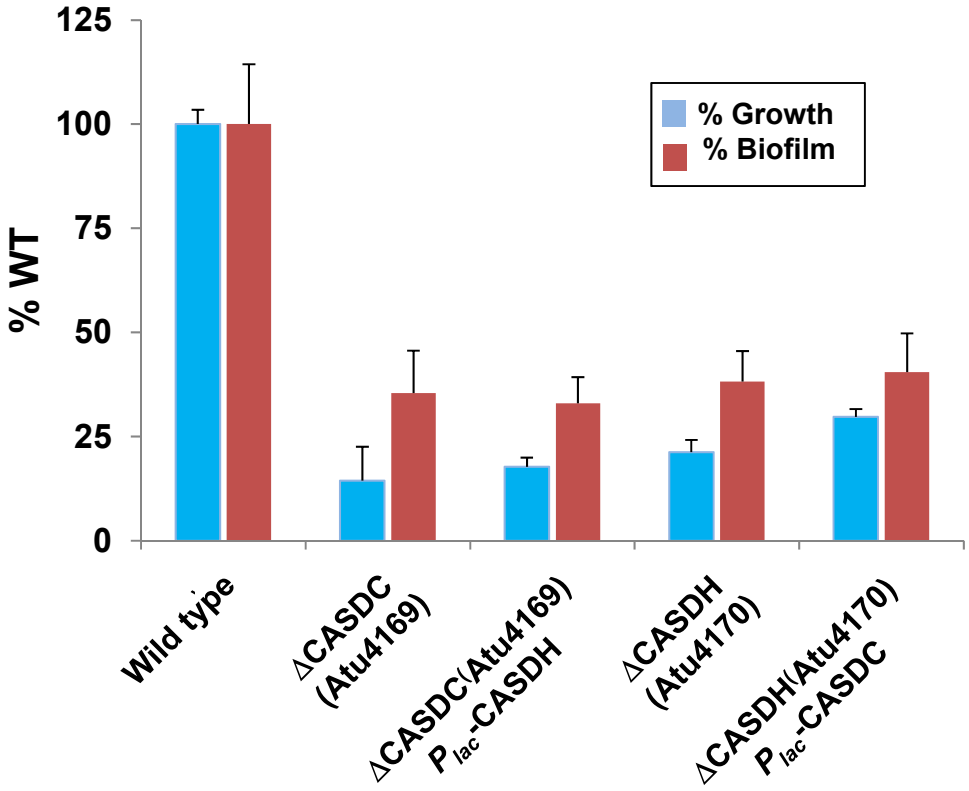

Figure S4. Plasmid-borne copies of CASDH and CASDC do not rescue each other's null mutations. Growth measured by OD_{600} (blue bars) and biofilm formation as A_{600}/OD_{600} (red bars), assayed as in Fig. S1 for *A. tumefaciens* C58 and derivatives. Plasmid harboring strains were induced with 500 µM IPTG. Values expressed as percent wild type. Error bars show the standard deviation of a minimum of three biological replicates.

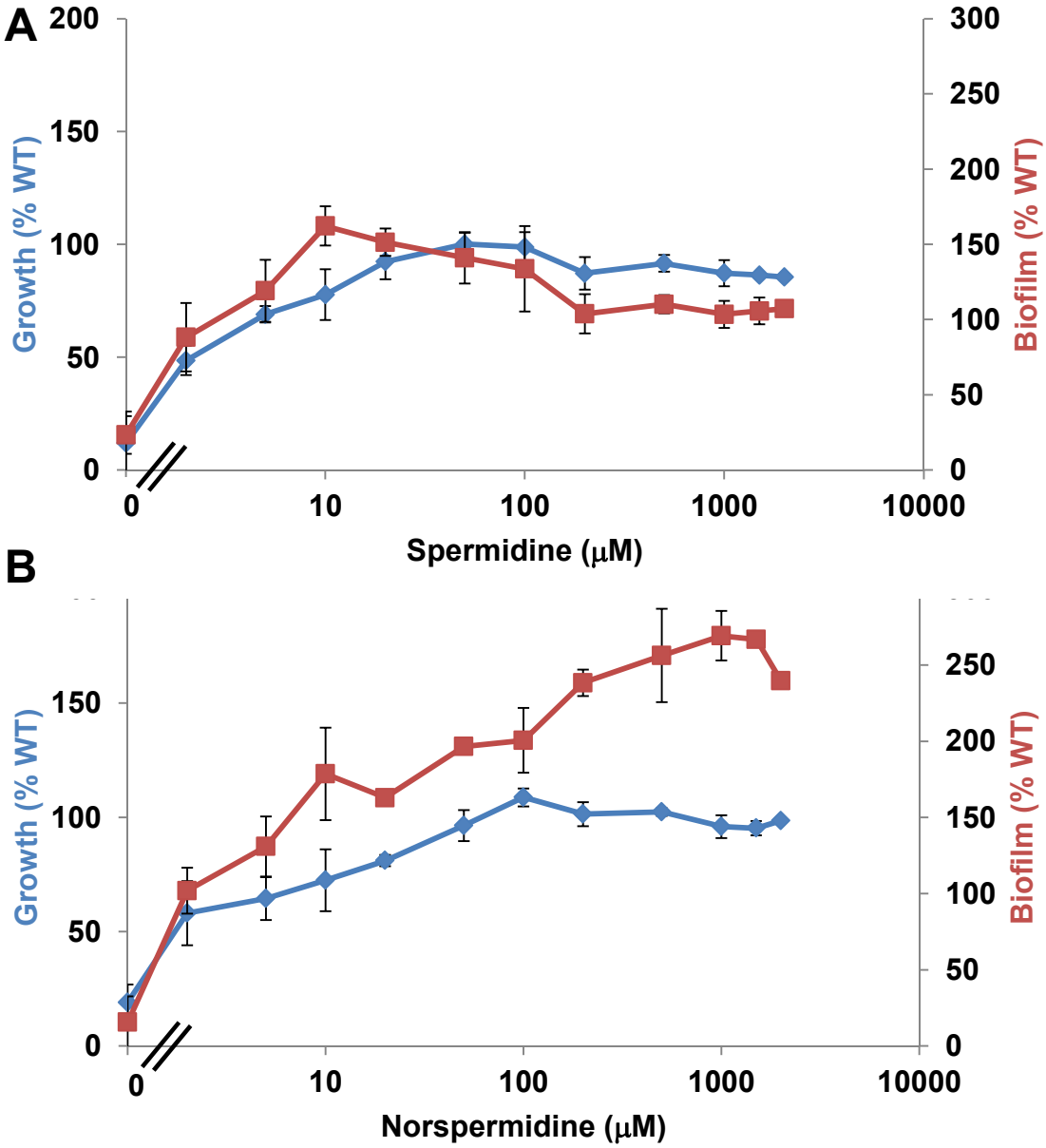

Figure S5. Growth rescue and biofilm control of the CASDH mutant by spermidine and norspermidine. Growth of the DCASDH mutant with spermidine (A) and norspermidine (B) measured by OD_{600} and biofilm formation as assayed as in Fig. S1. All values expressed as %WT and error bars show the standard deviation of a minimum of three biological replicates.. For the samples with no supplemental polyamines A_{600} readings were used instead of A_{600}/OD_{600} to avoid the very low growth due to polyamine auxotrophy from artificially inflating the reading.

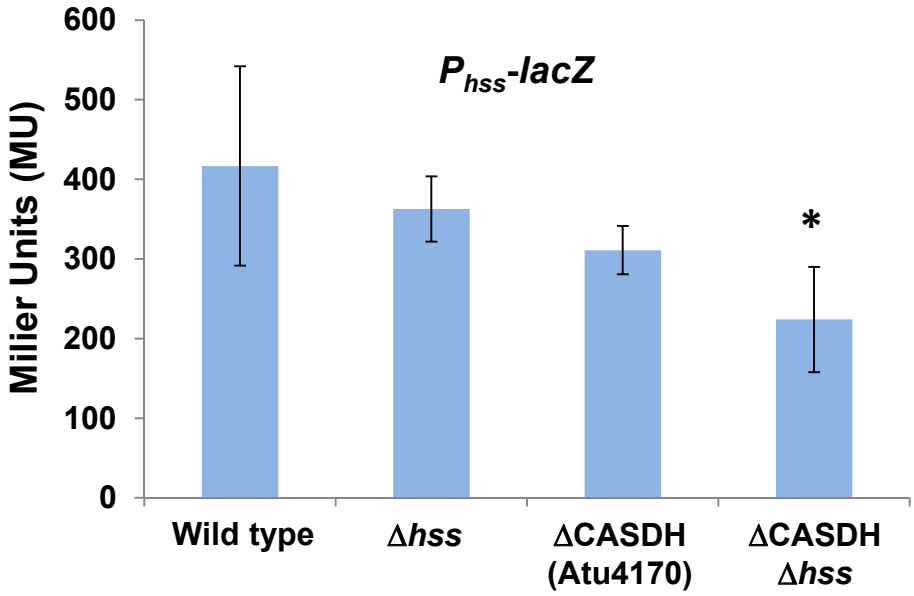

Figure S6. Expression of *hss* gene is not activated under conditions that cause elevated Hspd levels. b-galactosidase specific activity presented in Miller Units from ATGN-grown cultures in *A. tumefaciens* C58 and derivatives harboring a plasmid-borne P_{hss}-*lacZ* fusion. Error bars show the standard deviation of a minimum of three biological replicates. P value, *, < 0.06)

Supplementary Figure Legends

Figure S1. Biofilm formation of *A. tumefaciens* polyamine mutants in rich medium. Biofilm assays on coverslips in LB medium. *A. tumefaciens* C58 and derivatives incubated in 12-well plates with PVC coverslips for 96 h at 28°C. After rinsing, coverslips were stained with 1% crystal violet and adherent biomass measured as absorbance of acetic acid-solubilized crystal violet, normalized for planktonic culture growth (A_{600}/OD_{600}). Error bars show the standard deviation of a minimum of three biological replicates.

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(A_{600}/OD_{600}). Error bars show the standard deviation of a minimum of three biological replicates.

Figure S4. Plasmid-borne copies of CASDH and CASDC do not rescue each other's null mutations. Growth measured by OD_{600} (blue bars) and biofilm formation as A_{600}/OD_{600} (red bars), assayed as in Fig. S1 for *A. tumefaciens* C58 and derivatives. Plasmid harboring strains were induced with 500 μM IPTG. Values expressed as percent wild type. Error bars show the standard deviation of a minimum of three biological replicates.

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Figure S6. Expression of *hss* gene is not activated under conditions that cause elevated Hspd levels. β-galactosidase specific activity presented in Miller Units from ATGN-grown cultures in *A. tumefaciens* C58 and derivatives harboring a plasmid-borne

P_{hss}-*lacZ* fusion. Error bars show the standard deviation of a minimum of three biological replicates. P value, *, < 0.06)

Table S1. Strains and plasmids		
Strain or plasmid	Relevant characteristic(s)[a]	Reference or source
A. tumefaciens		
C58	Nopaline type strain; pTiC58; pAtC58	(1)
C58-YW1	C58Δ*cel*Δ*exoA*Δ*chvAB*Δ*crdS*	This lab
C58-YW2	C58Δ*upp*Δ*exoA*Δ*chvAB*Δ*crdS*	This lab
C58Δ*odc* (Δ*Atu3196*)	In-frame deletion of *odc* (*Atu3196*) in C58 background	This study
C58-YW1-Δ*odc*	In-frame deletion of *odc* (*Atu3196*) in C58-YW1 background	This study
C58-YW2-Δ*odc*	In-frame deletion of *odc* (*Atu3196*) in C58-YW2 background	This study
C58ΔCASDH (ΔAtu4170)	In-frame deletion of CADSH (Atu4170) in C58 background	This study
C58Δ*CASDC* (ΔAtu4169)	In-frame deletion of CASDC (Atu4169) in C58 background	This study
C58Δ*hss* (Δ*Atu3768*)	In-frame deletion of *hss* (Atu3768) in C58 background	This study
***E. coli* strains**		
DH5α/λ*pir*	λ*pir*; cloning strain	(2)
S17-1/λ*pir*	λ*pir*; Tra+, cloning strain	(3)
TOP10F'	Cloning strain	Invitrogen
Plasmids		
pGEM-T easy	PCR cloning vector; amp^R	Promega
pNPTS138	colE1 origin; *sacB* (Suc^S); Kan^R	MRK Alley (unpublished)
pRA301	Broad host range, promoterless *lacZ*, pVS1, Sp^R	(4)
pAtu3196-de	pNPTS138 carrying *Atu3196* SOE deletion	This study

	fragment	
pAtu4169-de	pNPTS138 carrying *Atu4169* SOE deletion fragment	This study
pAtu4170-de	pNPTS138 carrying *Atu4170* SOE deletion fragment	This study
pspeB-de	pNPTS138 carrying *speB* SOE deletion fragment	This study
phss-de	pNPTS138 carrying *Atu3768* SOE deletion fragment	This study
pSRK-Km	Broad host range P_{lac} expression vector; *lacIQ*; Kan^R	(5)
P_{lac}-*odc*	P_{lac}*::Atu3196* in pSRKKm	This study
P_{lac}-CASDH (Atu4170)	P_{lac}*::CASDH* (Atu4170)in pSRK-Km	This study
P_{lac}-CASDC *Atu4169*	P_{lac}*::*CASDC (Atu4170) in pSRK-Km	This study
P_{lac}-*dcpA*	P_{lac}*::dcpA* in pSRK-Km	This study
P_{hss}-*lacZ*	Upstream region of *hss* (Atu3768) translationally fused to *lacZ* in pRA301	This study

Table S2. Primer Sequences		
Primer	Restriction Site[a]	Sequence[b]
De3196-1	SpeI	actagtCGGTCCGATGTATTATCTCA
De3196-2	NE	aagcttggtaccgaattcCTTCATGCACTTTCCCATAG
De3196-3	NE	gaattcggtaccaagcttTGAGCCCACGATGGTAGG
De3196-4	SphI	gcatgcAACCGTGTTCTGCTATACCG
De4169-1	SpeI	actagtTCAGGAGAGCAAGAACGAAAC
De4169-2	NE	aagcttggtaccgaattcCAGCATGTCGTTTCACATATT
De4169-3	NE	gaattcggtaccaagcttTAAAACCCTGTTTCAACATCC
De4169-4	SphI	gcatgcTTCCACTCGTAGTTTCCGTAC
De4170-1	SpeI	actagtAAATATGGCGTGCAGGTCTAT
De4170-2	NE	aagcttggtaccgaattcCTTCATTTCAGGTGATGCCTC
De4170-3	NE	gaattcggtaccaagcttGAGTTCTAAGCGCAGCATTCT
De4170-4	SphI	gcatgcAAGGACATGATGCTCGAAAAG
DeSpeB-1	SpeI	actagtCACGTCCGCAAGGTTGATAC
DeSpeB-2	NE	aagcttggtaccgaattcGATTGTTTTGGCCGGCATAT
DeSpeB-3	NE	gaattcggtaccaagcttGGCTGACGAATAAAACGGCA
DeSpeB-4	SphI	gcatgcGCATTGACACTGACCGGATA
Com3196_up	NdeI	CTGAcatATGACGACTGCACGCATTCTC
Com3196_down	HindIII	ACTGaagcttCCTAGATGACGTAAGCCTTCA
Com4169-up	BamHI	GTCAggatccCCGACCTTGACTTCAATCAA
Com4169-down	HindIII	AGTCaagcttAGGGATAAGCTTCGTTGGAT
Com4170-up	NdeI	GCATcatATGAAGAAGAACGTTCTGATC
Com4170-down	KpnI	GCATggtaccTGTCTCTATCCGTCAAGAATG
DeHss-1	SpeI	ACTAGTgccgggactgtgccgagagca
DeHss-2	NE	AAGCTTGGTACCGAATTCtcaattttccttttcagccaat
DeHss-3	NE	GAATTCGGTACCAAGCTTcggatcggcgtctcatatttg
DeHss-4	NheI	GCTAGCctggctgttcgcgtccgt
Phss-lacZ-up	EcoRI	GAATTCtcaacaggtagctcc
Phss-lacZ-down	XbaI	TCTAGACATtcgtcaattttcctt
Pplac-5'		CTTCCGGCTCGTATGTTGTGTGG
MarRSeq		CGGGTATCGCTCTTGAAGGGA
MarTDL2		GACACGGGCCTCGANGNNNCNTNGG
MarLSeq		GGGAATCATTTGAAGGTTGGT
MarTDR1		CAACCGTGGCGGGGNTNCNNGNCNCG
M13-forward		CGCCAGGGTTTTCCCAGTCACGAC
M13-reverse		TCACACAGGAAACAGCTATGAC

[a]NE; No site was engineered

[b]Upper case sequence anneals to target; lower case are engineered into oligonucleotides but do not anneal with target sequences in the genome.

Supplemental References

1. **Watson B, Currier TC, Gordon MP, Chilton MD, Nester EW. .** 1975. Plasmid Required for Virulence of *Agrobacterium tumefaciens*. . J. Bacteriol. **123:**255-264.
2. **Chiang SL, and Rubin EJ.** 2002. Construction of a mariner-based transposon for epitope-tagging and genomic targeting. Gene **296:**179-185.
3. **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.
4. **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.
5. **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.