1 **SUPPLEMENTARY MATERIAL**

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4 Mini-Tn7 insertion in an artificial *att*Tn7 site enables depletion of the essential master

5 regulator CtrA in the phytopathogen *Agrobacterium tumefaciens*

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13 Running Title: Depletion of an essential gene in *Agrobacterium tumefaciens*

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16 **1) SUPPLEMENTARY METHODS**

17 **2) SUPPLEMENTARY FIGURES AND LEGENDS, S1-S2**

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21 **SUPPLEMENTARY METHODS**

22 **Modification of complementation plasmids**. The backbones of pSRKKm and pUC18-
23 mini-Tn7T-GM-LAC were modified to share a common multiple cloning site (MCS) and
24 add additional promoters. To add a new MCS, pSRKKm was digested with NdeI/NheI,
25 backbone purified, and ligated with NdeI/NheI digested fragment from pRVGFPC-2 (x).
26 For sfGFP expression, the resulting plasmid was cut with SacI/NheI and double ligated
27 with PCR amplified *sfgfp* from pKC129 with primers sfGFP HinkIII F/ *sfgfp* NheI R and
28 annealed primers Linker SacIBamHI F/ Linker Hind R. The resulting plasmid was named
29 pSRKKm-Plac-*sfgfp*. To add a HA tag, pSRKKm-Plac-*sfgfp* was cut with BamHI/NheI
30 and ligated with the annealed primers HA BamHI /HA NheI to make pSRKKm-Plac-HA.
31 The promoter was changed by PCR amplifying the *tac* promoter and repressor from
32 pUC18-mini-Tn7T-GM-LAC with the primers pTAC BstBI F/ pTAC NdeI R. The
33 fragment was then ligated into BstBI / NdeI digested and purified pSRKKm-Plac-*sfgfp*
34 and pSRKKm-Plac-HA backbones. The resulting *tac* promoter plasmids were named
35 pSRKKm-Ptac-*sfgfp* and pSRKKm-Ptac-HA. Modification of pUC18-mini-Tn7T-GM-
36 LAC was done by PCR amplifying the backbone of the plasmid with primers
37 BBMiniTn7 NheI F / BBMiniTn7 NdeI R. The digested backbone was ligated with the
38 gene fragment, miniTn7 MCS, which was digested with AseI / NheI. The gentamycin
39 cassette and *lacIQ* gene from pUC18-mini-Tn7T-GM-LAC were PCR amplified using
40 the primers Gm SpeI F / Gm SalI R and LacR SalI F / LacR AgeI R. Each was separately
41 ligated into the plasmid with corresponding digestion sites. This plasmid was then
42 digested with AgeI / NheI and ligated with the gene fragment, lac promoter. A HA tag
43 was added as previously described and the construct was named pUC18-mini-Tn7T-Gm-

44 Plac-HA. To replace the HA tag with *sfgfp*, pSRKKm-Plac-*sfgfp* was digested with

45 BamHI / NheI to release *sfgfp* which was ligated into BamHI / NheI digested pUC18-

46 mini-Tn7T-Gm-Plac-HA, resulting in construct pUC18-mini-Tn7T-Gm-Plac-*sfgfp*. The

47 two plasmids pUC18-mini-Tn7T-Gm-Plac-*sfgfp* and pUC18-mini-Tn7T-Gm-Plac-HA

48 were both digested with SalI / NdeI and ligated with PCR amplified fragment from

49 pSRKKm-Ptac-*sfgfp* containing the *tac* promoter, using primers LacR SalI F / pTAC

50 NdeI R. These *tac* promoter plasmids were named pUC18-mini-Tn7T-Gm-Ptac-*sfgfp*

51 and pUC18-mini-Tn7T-Gm-Ptac-HA.

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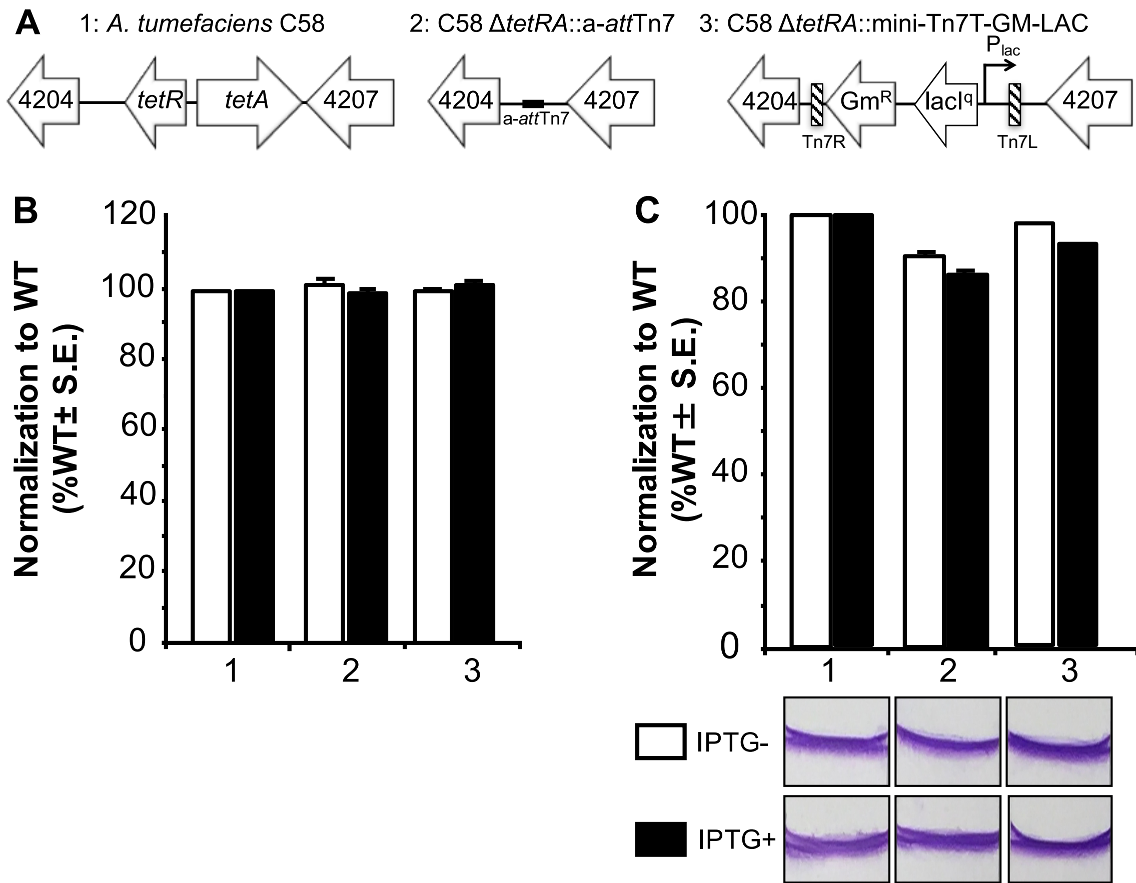
53 **SUPPLEMENTARY FIGURES**

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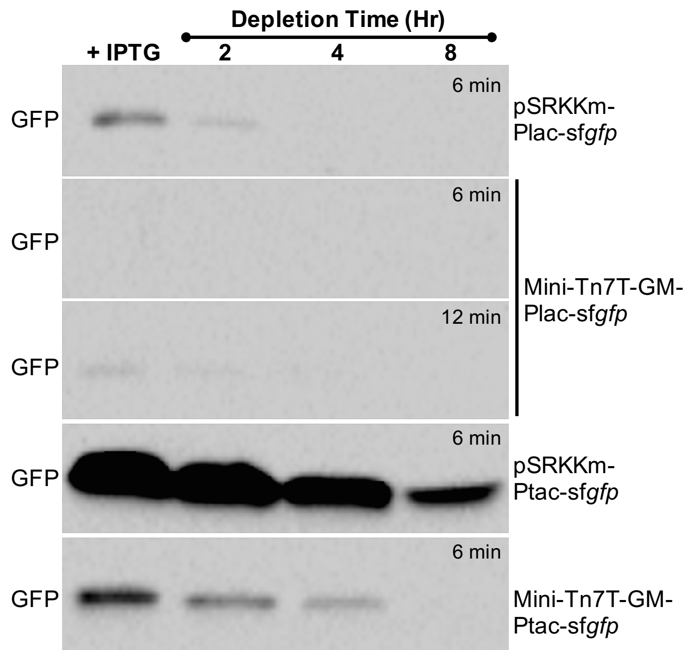
56 **Supplemental Figure 1.** Replacement of the *tetRA* locus with a-*att*Tn7 and subsequent
57 integration of a Tn7 cassette do not impact motility or biofilm formation of *A.*
58 *tumefaciens.* A. Schematics of strains used to determine impact of a-attTn7 and Tn7
59 insertion on motility and biofilm formation. B. Strains 1 – 3 were assayed for swimming
60 motility on ATGN soft agar plates in the presence (black bars) or absence (white bars) of
61 the inducer, IPTG. Data are normalized to results obtained in the wild-type strain (strain
62 1) and are shown as the %WT ± standard error of the mean (S.E). Swim ring diameters
63 were measured after 5 days of incubation at room temperature. Data shown are the mean
64 of three independent experiments completed in triplicate. Data for strains 1 and 2 are
65 also shown in Figure 2. C. Strains 1 – 3 were assayed for biofilm formation on vertical
66 plastic coverslips immersed in ATGN media in the presence (black bars) or absence
67 (white bars) of the inducer, IPTG. Data are normalized to results obtained in the wild-
68 type strain (strain 1) and are shown as the %WT ± S.E. Coverslips were removed after 48
69 hours of incubation at room temperature and rinsed to remove any loosely associated
70 cells. Adherent biomass was determined as the absorbance of solubilized crystal violet
71 ($A_{600}$) and the optical density of the planktonic culture ($OD_{600}$) was measured. Biofilm
72 scores were calculated as the ratio of $A_{600}/OD_{600}$ and data was normalized. Data shown
73 are the mean of three independent experiments completed in triplicate. Representative
74 coverslips prior to crystal violet solubilization are shown for each strain. Data for strains
75 1 and 2 are also shown in Figure 3.

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78 **Supplemental Figure 2.** Comparison of plasmid and chromosome based expression of
79 sf*gfp* from $P_{tac}$ and $P_{lac}$ promoters. Western blots illustrate the depletion of GFP
80 following the removal of the inducer (IPTG) in each case at 2, 4, and 8 hours post-
81 depletion. Exposure times are shown on the top right corner of each blot. Doubling the
82 exposure time allowed detection of GFP when expressed from the chromosomal $P_{lac}$
83 promoter.
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