Arginine-rich small proteins with a domain of unknown function DUF1127 play a role in phosphate and carbon metabolism of *Agrobacterium tumefaciens*

- Supplementary file –

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Abbreviations

DP (DUF1127-containing protein), SDP (Short DP), LDP (Large DP), OD (optical density), RNA-seq (RNA sequencing)

Explanatory notes to RNA-seq results

sRNAs gene names are composed of the corresponding replicon (C1: circular chromosome, C2: linear chromosome, pAt: At-plasmid, pTi: Ti-plasmid), the corresponding position of the transcriptional start site and the orientation of the gene (F: encoded on 'plus' strand, R: encoded on 'minus' strand). The sRNA gene *C2_1831446F* corresponds to the sRNA L5 (as annotated by Wilms *et al.* (1)).

References

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A

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L4           MNVTRSFNNWRKYRQTINELGRMSARELHDLGIDRSQITSVARAAVGK
A0A0F4FNQ6   MNVTRSFNNWRKYRQTINELGRMSARELHDLGIDRSQITSVARAAVGK 100.0%
A0A1G8Y768   MNVTRSFNNWRKYRQTINELGRMSARELHDLGIDRSQITSVARAAVGK 100.0%
A0A1S7QGG0   MNVTRSFNNWRKYRQTINELGRMSTRELHDLGIDRSQITSVARAAVGK  97.9%
F5JAJ7       MNVTRSFNNWRKYRQTINELGRMSTRELHDLGIDRSQITSVARAAVGK  97.9%
A0A024IZE6   MNVARSFNNWRKYRQTINELGRMSTRELHDLGIDRSQITSVARAAVGK  95.8%
A0A1S7QC19   MNVARSFNNWRKYRQTINELGRMSSRELHDLGIDRSQITSVARAAVGK  95.8%
A0A1S7RCR0   MNVARSFNNWRKYRQTINELGRMSSRELHDLGIDRSQITSVARAAVGK  95.8%
A0A1S7RQW2   MNVARSFNNWRKYRQTINELGRMSTRELHDLGIDRSQITSVARAAVGK  95.8%
A0A1S7S6T7   MNVARSFNNWRKYRQTINELGRMSTRELHDLGIDRSQITSVARAAVGK  95.8%
A0A1S7U662   MNVARSFNNWRKYRQTINELGRMSTRELHDLGIDRSQITSVARAAVGK  95.8%
             ***:********************:***********************
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B

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Atu1766      MTMIHYLPATNRSSLRPSPSGWLDRLSSHFSARYAEWRRERMLRALEALPPETLKDIGWPTTDTTRIHAVRK
A0A1B9SY55   MTMIHYLPVTNRSTHRPSPIGWLHRLSDQVSARYAEWRRARMLRALEALPPETLKDIGWPTTDTTRIHTIRK 84.7%
A0A178HA42   MTMIHYLPAANRSTHRPSSGGWLHRLSSHVSAAYASWRRARMLRALEALPPETLKDIGWPTTDNNRISVIRK 79.2%
A0A2V5CI05   MTMIHYLPAANRSTHRPSSGGWLHRLSSHVSAAYASWRRARMLRALEALPPETLKDIGWPTTDNNRISLIRK 79.2%
A0A1S7P179   MTMIHYLPAANRSPHRPSSGGWLHRLSGHVSALYRSWRRARMLRALEALPPETLKDIGWPTTDNYRTNAIRK 76.4%
A0A0Q8FUT7   MTMIHYLPAANRSPHRPSSGGWLHRLSGHVSALYRSWRRARMLRALEALPPETLKDIGWPTTDNYRTNAIRK 76.4%
A0A0L6K5N0   MTMIHYLPAANRSPHRPSSGGWLHRLSGHVSALYRSWRRARMLRALEALPPETLKDIGWPTTDNYRTNAIRK 76.4%
A0A0D8KCQ5   MTMIHYLPAASRSSHRPSFGGWLHRLSAQVSALYAARQRARMLRALEALPPETLKDIGWPTTDSSRIRVIRK 73.6%
U4Q4J2       MTMIHYLPAANRSPHRPSSAGWLQRLSGNIASHYASWRRARMLRALEALPPETLKDIGWPTTDNYGRSVIRK 70.8%
K5D188       MTMIHYLPAANRSPHRPSSAGWLQRLSGNIASHYASWRRARMLRALEALPPETLKDIGWPTTDNYGRSVIRK 70.8%
A0A1S9EMK1   MTMIHYLPAANRSPHRPSSAGWLQRLSGNIASHYASWRRARMLRALEALPPETLKDIGWPTTDNYCRSVIRK 70.8%
             ********.:.**  ***  ***.*** :.:: *   :* ***********************.     :**
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C

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Atu1865      MQNDQPLVAYALPDAVDRLFVTFGVWKTLKAVVVAAMVPRAPPTDLADLPDRLLVDIGFVPSQVKRRRDWSVP-WAPRF
F0L6Y4       MQKNQPLVAYALPDAVDRLFVTFGVWKTLKAVLVAAMVPRPPPTDLSDLPERLLDDIGLEPSDRKMRREWAPPYWAPRF 80.8%
A0A285Y8X8   MQKNQPLVAYALPDAVDRLFVTFGVWKTLKAVLVAAMVPRPPPTDLSDLPERLLDDIGLEPSDRKMRREWAPPYWAPRF 80.8%
A0A1B9V6F4   MQKNQPLVAYALPDAVDRLFVTFGVWKTLKAVLVAAMVPRPPPTDLSDLPERLLDDIGLEPSDRKMRREWAPPYWAPRF 80.8%
H0HBL4       ------MVAYALPDAVDRLFVTFGVWKTLKAVLVAAMVPRPPPTDLSDLPERLLDDIGLEPSDRKKRREWAPPYWAPRF 79.5%
W8FGC7       MQKNQPLVAYALPDAVDRLFVTFGVWTTLKAVLVAAMVPRPPPTDLSDLPERLLDDIGLEPSDRKMRREWAPPYWAPRF 79.5%
A0A1V2AEM7   MQKNQPLVAYALPDAVDRLFVTFGVWTTLKAVLVAAMVPRPPPTDLSDLPERLLDDIGLEPSDRKMRREWAPPYWAPRF 79.5%
A0A1S7SV95   MQKNQPLVAYALPDAVDRLFVTFGVWTTLKAVLVAAMVPRPPPTDLSDLPERLLDDIGLEPSDRKMRREWAPPYWAPRF 79.5%
A0A1S7MPF6   MQKNQPLVAYALPDAVDRLFVTFGVWTTLKAVLVAAMVPRPPPTDLSDLPERLLDDIGLEPSDRKMRREWAPPYWAPRF 79.5%
A0A1S7MJE2   MQKNQPLVAYALPDAVDRLFVTFGVWTTLKAVLVAAMVPRPPPTDLSDLPERLLDDIGLEPSDRKMRREWAPPYWAPRF 79.5%
                   :*******************.*****:******* *****:***:*** ***: **: * **:*: * *****
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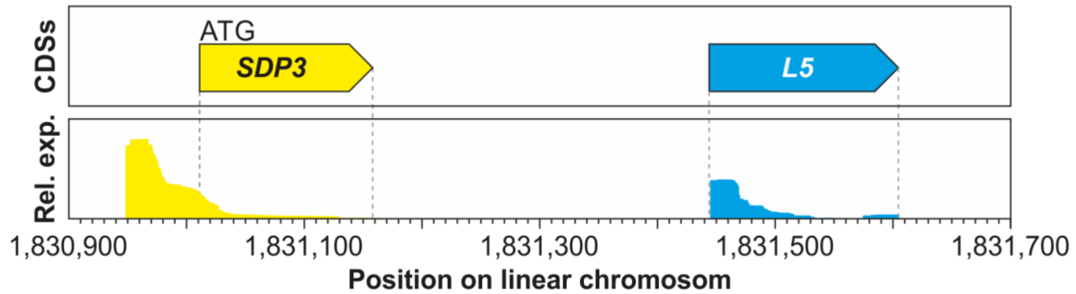
FIG S1. Alignments of newly discovered DPs with annotated DPs. The proteins most similar to L4 (SDP3), Atu1766 (LDP2) and Atu1865 (LDP4) are shown. The UniProtKB accession number is given on the left, the protein identity compared to the newly discovered DP is given on the right side. Identical aa are marked with an asterisk (*), highly similar ones with a colon (:) and those with minor similarity with a period (.). Arginine residues are highlighted in yellow.

A



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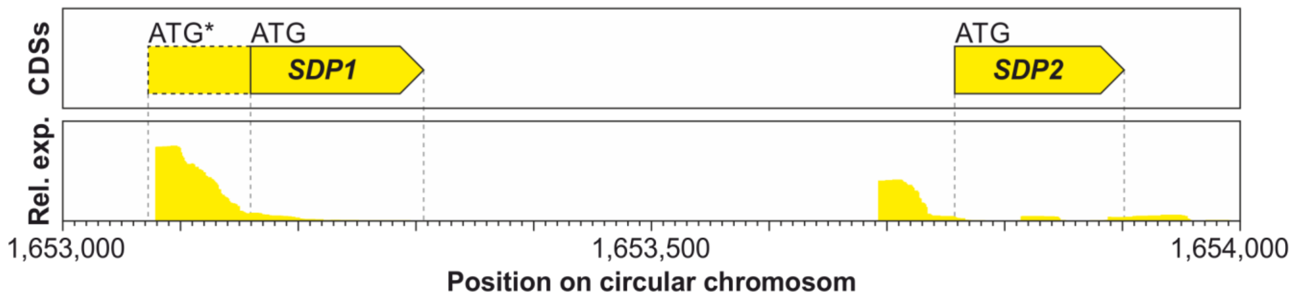
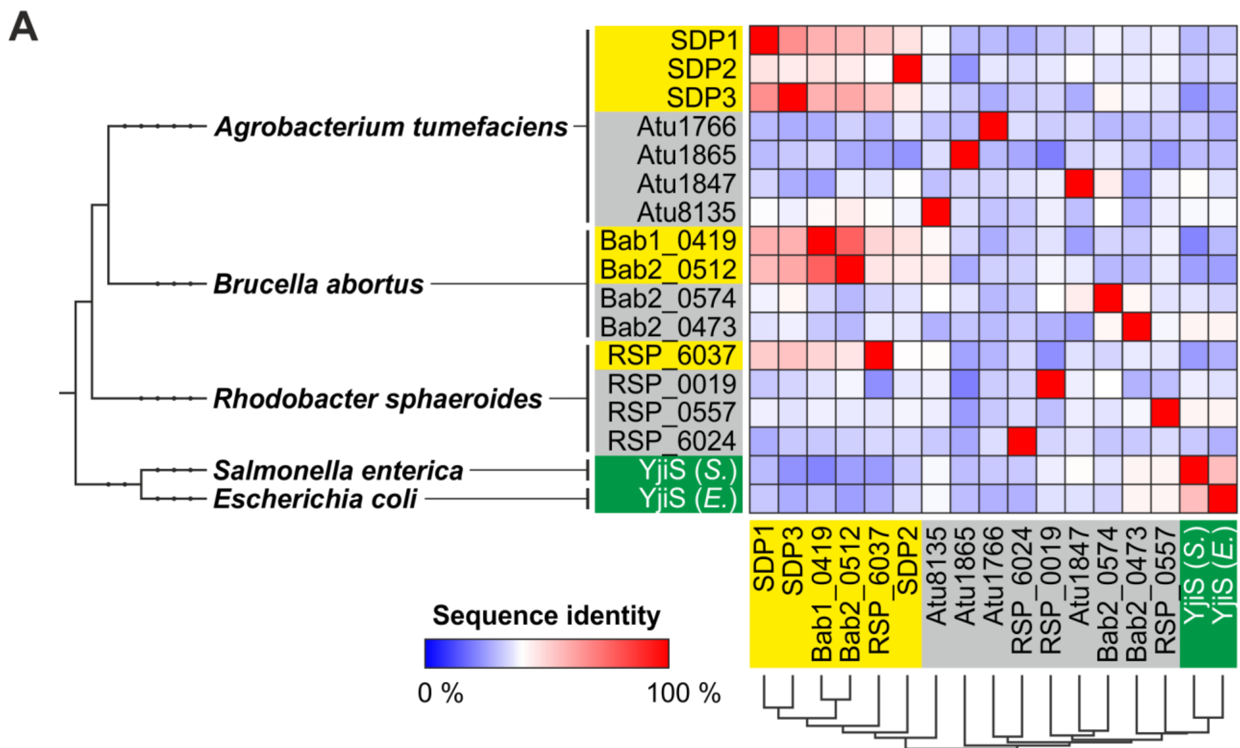


FIG S2. Transcriptomic data of SDP genes. In the upper panel relative positions of coding sequences (CDS) are shown. Start codons are marked with ATG. In the lower panel the relative expression (rel. exp.) is shown. Transcriptomic data were taken from Wilms *et al.* (1). SDP genes are marked in yellow and sRNAs in blue. **(A)** *SDP3* and *L5* lie in direct genomic neighborhood but have independent transcription-start sites. **(B)** The previous annotation of the *SDP1* ORF had been incorrect. The previously annotated ORF (*atu1667*, dashed lines) and the corrected ORF (*ATU_RS08170*, continuous line) are shown as well as the positions of the annotated start codon (ATG*) and the correct start codon (ATG). The annotated ORF starts at position 1,653,073 on the circular chromosome. The transcription start lies downstream of this annotation at position 1,653,160.



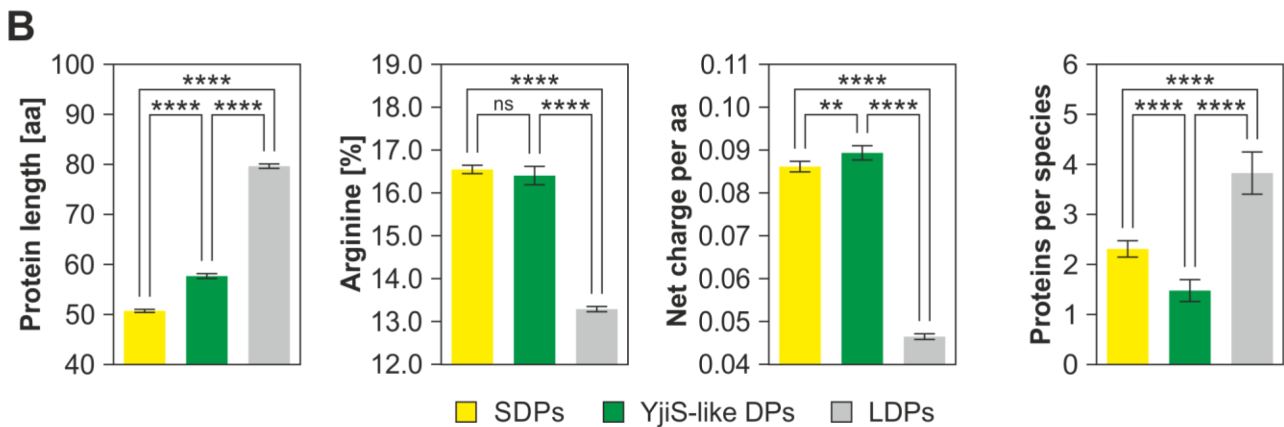
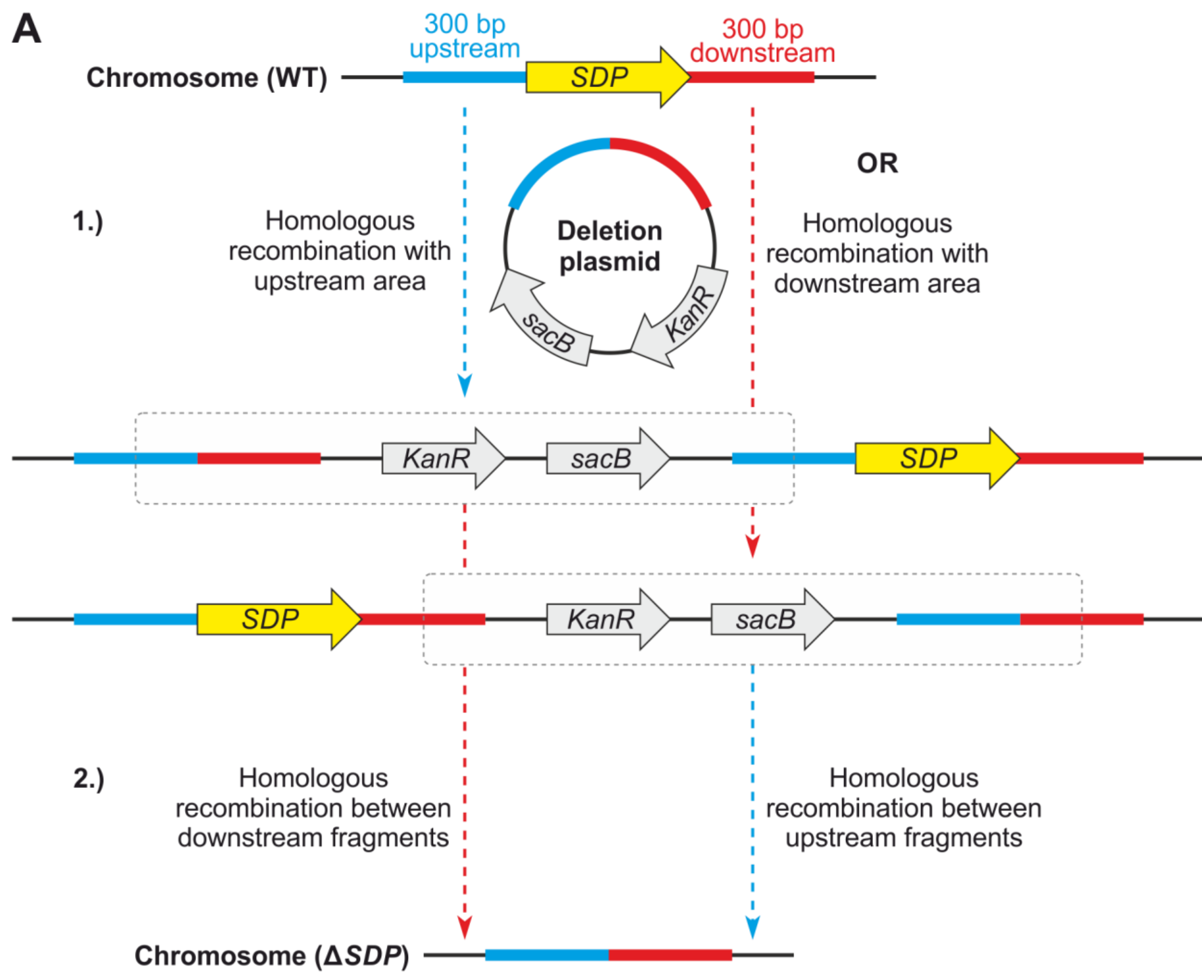


FIG S3. Further insights into DP-sub-division. **(A)** Sub-division of DPs that have been subject to previous studies. The proteins were clustered according to the sequence identity to each other via nearest-neighbor-clustering algorithm (2). **(B)** Statistical analyses of DP-sub-class properties concerning protein length, relative arginine content, protein net charge per aa and proteins per species. For each sub-class, means were plotted. Upper and lower border of a 95 % confidence interval are marked as horizontal lines. The corresponding p values were calculated by Welch's t-test (3) and are indicated by asterisks (****: p≤0.0001, **: p≤0.01, ns (not significant): p>0.05). Protein net charge was calculated using the Henderson-Hasselbalch equation for each N or C terminus and all side chains as described by Requião *et al.* (4). The aa pKa values were taken from "CRC handbook of chemistry and physics" (99th edition) (5).



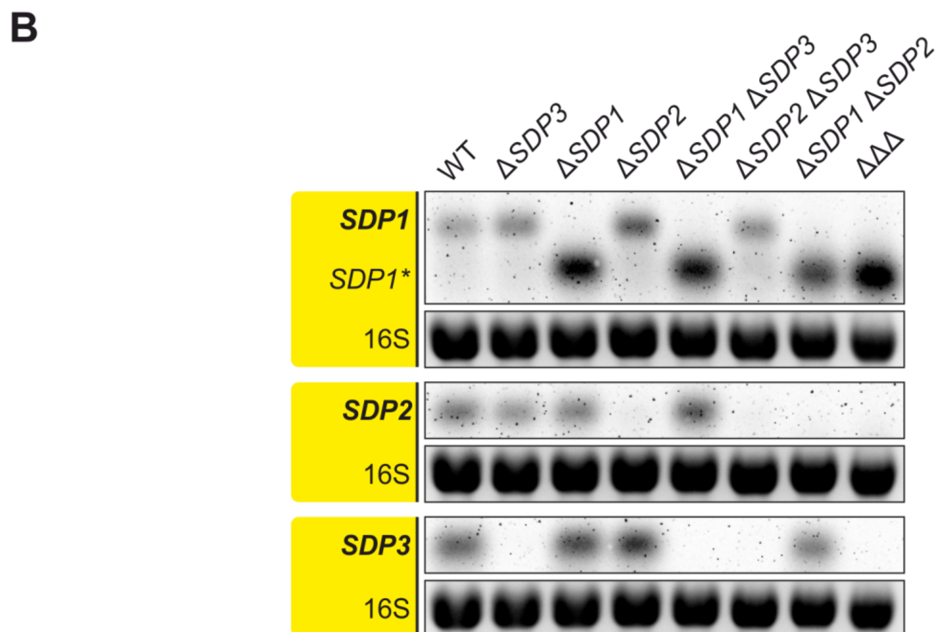


FIG S4. Deletion of SDP genes. **(A)** Construction of marker-less deletion mutants. Approximately 300 bp upstream and downstream of the target gene were amplified via PCR and cloned into pK19*mobsacB*. Via single-crossover-homologous recombination with the upstream (left arrow) or the downstream fragment (right arrow) the plasmid was integrated into the genome. Successful integration resulted in resistance against kanamycin (*KanR*). In the second step, cells were exposed to medium containing sucrose, which is

(FIG S4. Legend continued) metabolized into toxic levels of levan by SacB. This forces the cells to excise the plasmid via homologous recombination again. Since recombination between upstream fragments or downstream fragments are similarly likely, this can either result in restoration of the WT or in successful marker-less gene deletion. **(B)** Northern-blot analysis verified successful construction of all seven deletion strains. All single, double and triple-deletion mutants (ΔΔΔ) were created by the scheme presented in (A). A truncated transcript of the *SDP1* mRNA (*SDP1**) was detected, because only the protein-coding sequences of *SDP1* and *SDP2* were deleted, but not the UTRs. EtBr-stained 16S rRNA served as loading control.

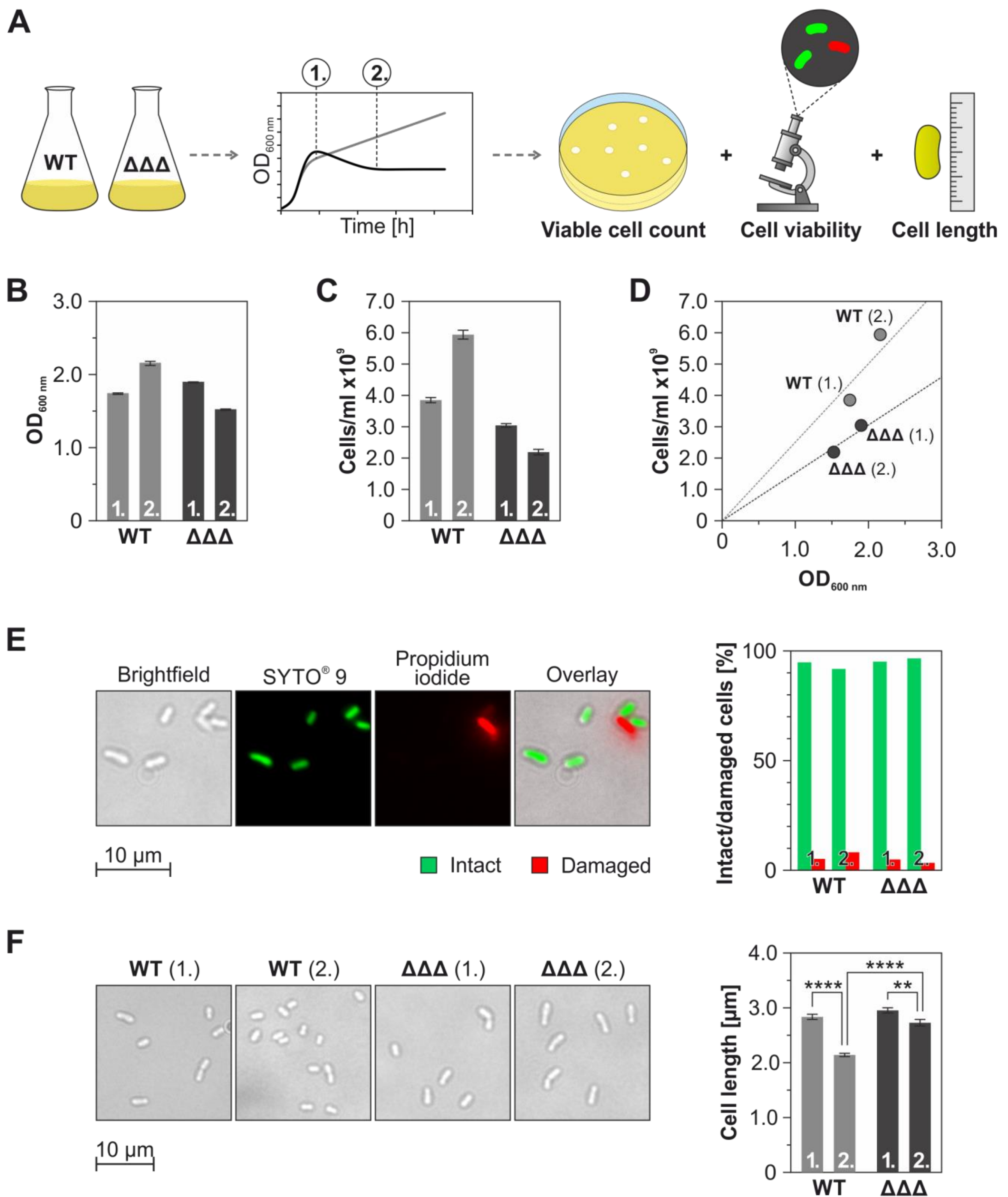


FIG S5. Viability and cell morphology of ΔΔΔ. **(A)** To investigate whether the declining $OD_{600\ nm}$ of ΔΔΔ correlates with the cell number several assays were performed at two points of time: after approximately 12 h of growth and after approximately 24 h. Viable cells were determined by plate counting. To determine the relative amount of dead cells a *LIVE/DEAD BacLight*™ bacterial viability assay was quantified by counting cells under the microscope. The cell length was measured under the microscope using the ImageJ software. **(B-D)** $OD_{600\ nm}$ and cell number correlate with each other. The $OD_{600\ nm}$ and the viable cell count of the WT

(FIG S5. Legend continued) increased over night, whereas $OD_{600\ nm}$ and cell number of ΔΔΔ decreased. An increased $OD_{600\ nm}$ went along with an increased number of cells. **(E)** ΔΔΔ and WT showed a similar amount of damaged cells. Via *LIVE/DEAD BacLight*™ assay, intact cells were stained with a green-fluorescent dye (SYTO® 9) and damaged cells were stained with a red-fluorescent dye (propidium iodide). Cells were counted via fluorescence microscopy. **(F)** Cell length of ΔΔΔ decreased to a minor extent on day two compared to the WT. The corresponding p values were calculated by Welch's *t*-test and are indicated by asterisks (****: $p \leq 0.0001$, **: $p \leq 0.01$).

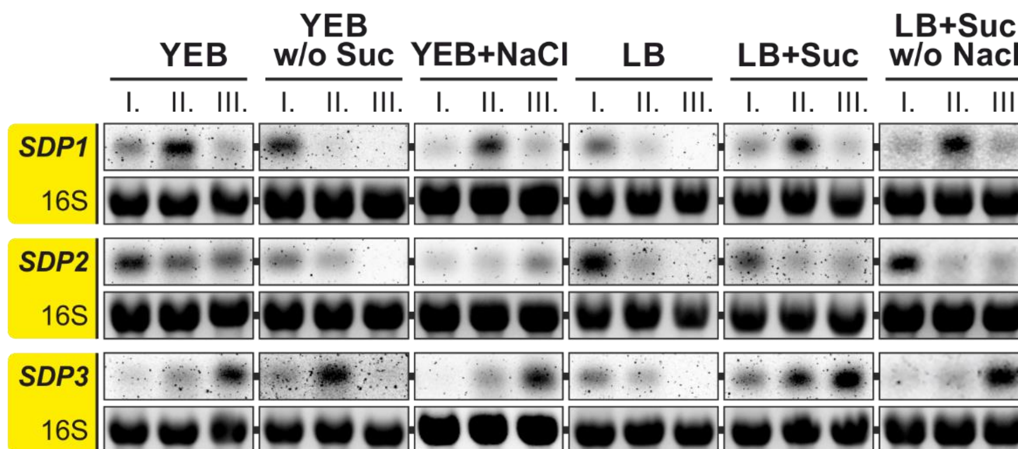
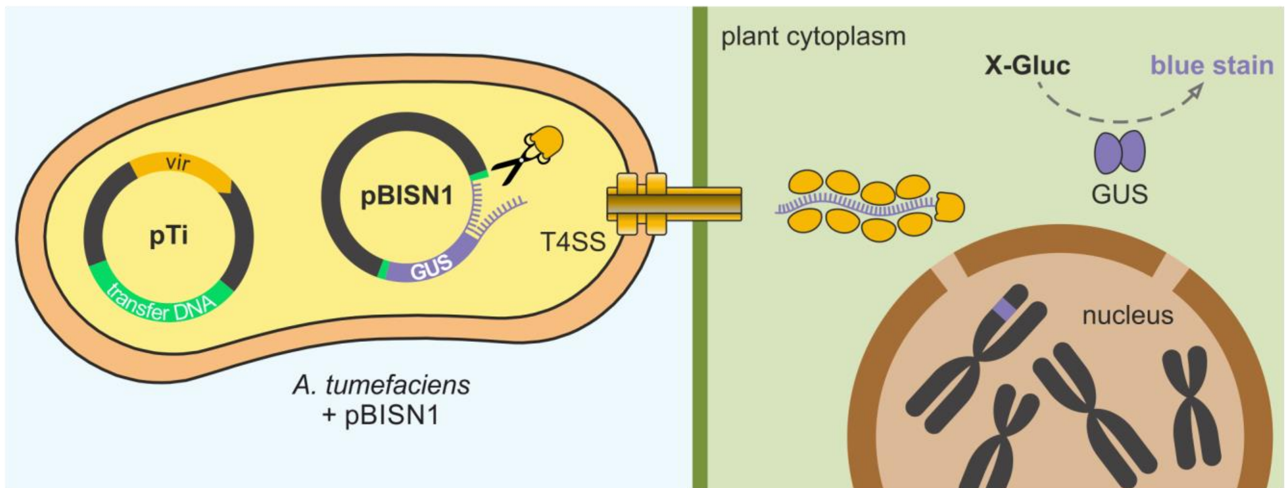


FIG S6. Sucrose and osmolarity-dependent expression of SDP genes. Samples for Northern blot analyses were taken at the indicated time points (see Fig. 8). EtBr-stained 16S rRNA served as loading control

A



B

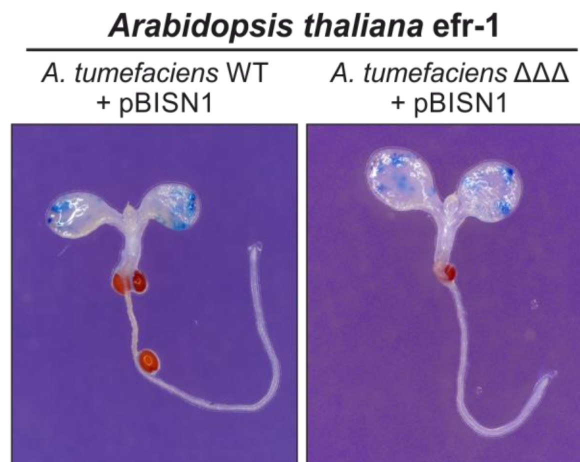


FIG S7. Seedling-infection assay. **(A)** *A. tumefaciens* is transformed with pBISN1 which contains a GUS gene under control of a so-called super-promoter between the two T-DNA borders. A single strand of the GUS gene is cleaved and transported into the plant host. It is translocated into the nucleus and integrated into the genome at a random position. GUS converts X-Gluc into a blue stain, which indicates a successful infection by *A. tumefaciens*. **(B)** ΔΔΔ is able to infect the *A. thaliana* efr-1 mutant. Blue stained spots indicate infection by *A. tumefaciens* transformed with pBISN1.

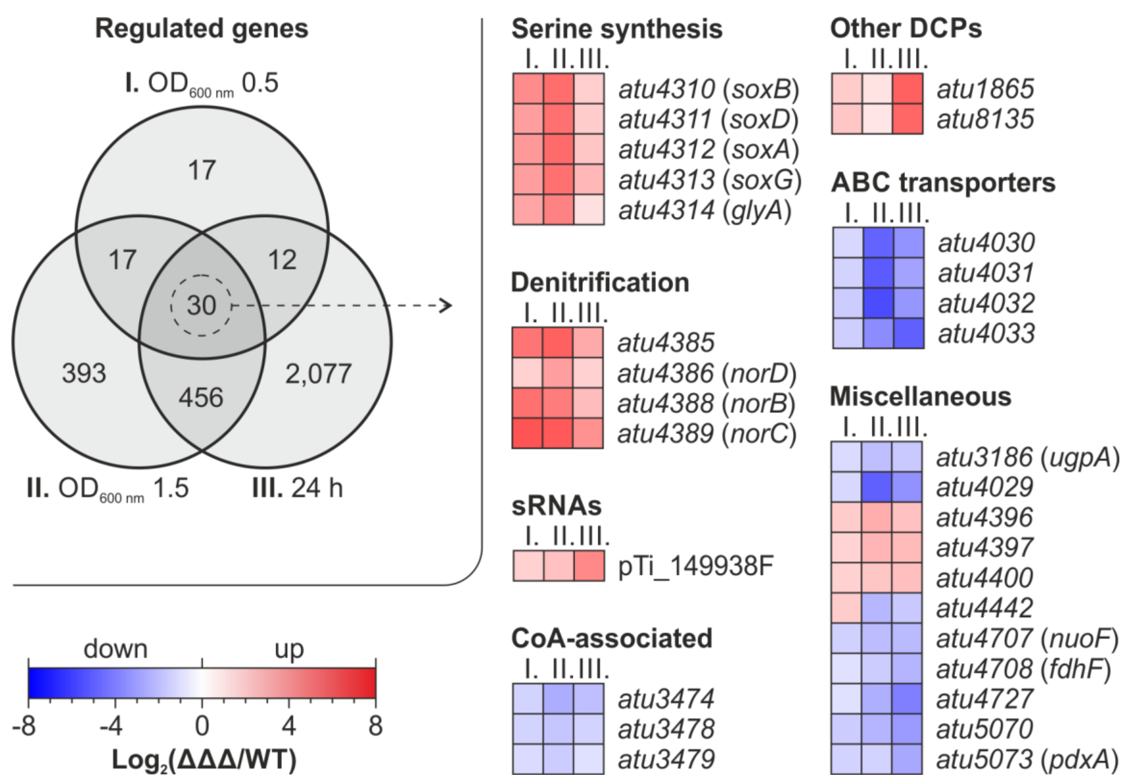


FIG S8. Differentially expressed genes in ΔΔΔ. The transcriptome of ΔΔΔ was compared with the WT via RNA-seq at $OD_{600\ nm}$ 0.5, at 1.5 and after 24 h of growth. The 30 genes that were differentially expressed at all three points of time are listed on the right. The color code represents the binary logarithm of the fold change in ΔΔΔ compared to the WT.

Table S1. Strains used in this study.

Strain	Description	Source
A. tumefaciens C58	WT strain (alias *Agrobacterium fabrum* C58).	C. Baron, Montréal, Canada
A. tumefaciens C58 Δ*L4*	Marker-less deletion of *L4* with pDel_*L4* in *A. tumefaciens* C58.	This study
A. tumefaciens C58 Δ*atu1667*	Marker-less deletion of *atu1667* with pDel_*atu1667* in *A. tumefaciens* C58.	This study
A. tumefaciens C58 Δ*atu8161*	Marker-less deletion of *atu8161* with pDel_ *atu8161* in *A. tumefaciens* C58.	This study
A. tumefaciens C58 Δ*L4* Δ*atu1667*	Marker-less deletion of *atu1667* with pDel_*atu1667* in *A. tumefaciens* Δ*L4*.	This study
A. tumefaciens C58 Δ*L4* Δ*atu8161*	Marker-less deletion of *L4* with pDel_*L4* in *A. tumefaciens* Δ*atu8161*.	This study
A. tumefaciens C58 Δ*atu1667* Δ*atu8161*	Marker-less deletion of *atu8161* with pDel_*atu8161* in *A. tumefaciens* Δ*atu1667*.	This study
A. tumefaciens C58 Δ*L4* Δ*atu1667* Δ*atu8161* (ΔΔΔ)	Marker-less deletion of *atu1667* with pDel_*atu1667* in *A. tumefaciens* Δ*L4* Δ*atu8161*.	This study
A. tumefaciens C58 Δ*lsrB*	Knock-out of *atu2186* (*lsrB*) in *A. tumefaciens* C58 with pKO_*lsrB*. Gentamycin resistant.	This study
A. tumefaciens C58 + EV	*A. tumefaciens* C58 with chromosomal integration of pInt_EV. Kanamycin resistant.	This study
A. tumefaciens C58 ΔΔΔ + EV	*A. tumefaciens* ΔΔΔ with chromosomal integration of pInt_EV. Kanamycin resistant.	This study
A. tumefaciens C58 ΔΔΔ + *L4*	*A. tumefaciens* ΔΔΔ with chromosomal integration of pInt_*L4*. Kanamycin resistant.	This study
A. tumefaciens C58 + $L4^{3xFLAG}$	Chromosomal integration of $L4^{3xFLAG}$ under control of native promoter. Cloned via recombination with pInt_$L4^{3xFLAG}$. Kanamycin resistant.	This study
A. tumefaciens C58 + $atu1667^{3xFLAG}$	Chromosomal integration of $atu1667^{3xFLAG}$ under control of native promoter. Cloned via recombination with pInt_ $atu1667^{3xFLAG}$. Kanamycin resistant.	This study
A. tumefaciens C58 + $atu8161^{3xFLAG}$	Chromosomal integration of $atu8161^{3xFLAG}$ under control of native promoter. Cloned via recombination with pInt_ $atu8161^{3xFLAG}$. Kanamycin resistant.	This study
A. tumefaciens C58 + $atu1766^{3xFLAG}$	Chromosomal integration of $atu1766^{3xFLAG}$ under control of native promoter. Cloned via recombination with pInt_ $atu1766^{3xFLAG}$. Kanamycin resistant.	This study
A. tumefaciens C58 + $atu1865^{3xFLAG}$	Chromosomal integration of $atu1865^{3xFLAG}$ under control of native promoter. Cloned via recombination with pInt_ $atu1865^{3xFLAG}$. Kanamycin resistant.	This study
A. tumefaciens C58 + $atu1847^{3xFLAG}$	Chromosomal integration of $atu1847^{3xFLAG}$ under control of native promoter. Cloned via recombination with pInt_ $atu1847^{3xFLAG}$. Kanamycin resistant.	This study
A. tumefaciens C58 + $atu8135^{3xFLAG}$	Chromosomal integration of $atu8135^{3xFLAG}$ under control of native promoter. Cloned via recombination with pInt_ $atu8135^{3xFLAG}$. Kanamycin resistant.	This study

Table S1. (Continued)

Strain	Description	Source
E. coli JM83	Cloning strain. *rpsL*, *ara*, Δ(*lac-proAB*), Φ80, d*lacZ*Δ*M15*.	(6)

Table S2. Plasmids used in this study. A detailed description on how plasmids were constructed is given in Tab. S3.

Plasmid	Description	Source
pK19*mobsacB*	Cloning vector.	(8)
pDel_*L4*	For marker-less deletion of *L4*. Constructed from pk19*mobsacB.*	This study
pDel_*atu1667*	For marker-less deletion of *atu1667*. Constructed from pk19*mobsacB.*	This study
pDel_*atu8161*	For marker-less deletion of *atu8161*. Constructed from pk19*mobsacB.*	This study
pYP240I	Cloning vector.	Masepohl, unpublished
pYP4II	Cloning vector.	Masepohl, unpublished
pKO_*lsrB*	For knock-out of *atu2186* (*lsrB*).	This study
pK18	Cloning vector.	(10)
pInt_EV	Empty vector control for integration via single-crossover. Contains *L4*-UTRs. Constructed from pK18.	This study
pInt_*L4*	For reintegration of *L4* in ΔΔΔ via single-crossover. Constructed from pK18.	This study
pYP247	Cloning vector.	(7)
pInt_$L4^{3xFLAG}$	For chromosomal $L4^{3xFLAG}$ via single-crossover. Constructed from pK18.	This study
pInt_$atu1667^{3xFLAG}$	For chromosomal $atu1667^{3xFLAG}$ via single-crossover. Constructed from pK18.	This study
pInt_ $atu8161^{3xFLAG}$	For chromosomal $atu8161^{3xFLAG}$ via single-crossover. Constructed from pK18.	This study
pInt_$atu1766^{3xFLAG}$	For chromosomal $atu1766^{3xFLAG}$ via single-crossover. Constructed from pK18.	This study
pInt_$atu1865^{3xFLAG}$	For chromosomal $atu1865^{3xFLAG}$ via single-crossover. Constructed from pK18.	This study
pInt_$atu1847^{3xFLAG}$	For chromosomal $atu1847^{3xFLAG}$ via single-crossover. Constructed from pK18.	This study
pInt_$atu8135^{3xFLAG}$	For chromosomal $atu8135^{3xFLAG}$ via single-crossover. Constructed from pK18.	This study
pBISN1	For seedling infection assays.	(9)

Table S3. Plasmid construction. The plasmids that were constructed in this study are listed with a detailed cloning scheme. For each PCR the primer numbers are given (see Tab. S4). The template is given in parentheses whenever it was not *A. tumefaciens* DNA. The nucleases in parentheses indicate that a DNA fragment was digested prior to ligation.

Plasmid	Cloning scheme
pDel_*L4*	- PCR_1: 1 + 2 - PCR_2: 3 + 4 - $Ligation_1$: PCR_1 (BamHI) + PCR_2 (BamHI) - $Ligation_2$: pK19*mobsacB* (PstI, HindIII) + $Ligation_1$ (PstI, HindIII)
pDel_*atu1667*	- PCR_1: 5 + 6 - PCR_2: 7 + 8 - $Ligation_1$: PCR_1 (BsaI) + PCR_2 (BsaI) - $Ligation_2$: pK19*mobsacB* (PstI) + $Ligation_1$ (PstI)
pDel_*atu8161*	- PCR_1: 9 + 10 - PCR_2: 11 + 12 - $Ligation_1$: PCR_1 (BsaI) + PCR_2 (BsaI) - $Ligation_2$: pK19*mobsacB* (PstI) + $Ligation_1$ (PstI)
pKO_*lsrB*	- PCR_1: 13 + 14 - $Ligation_1$: pYP240I (KpnI, SalI) + PCR_1 (KpnI, SalI) - $Restriction_1$: pYP4II (EcoRI, PstI) → Gm^R cassette - $Ligation_2$: $Ligation_1$ (EcoRI, PstI) + $Restriction_1$
pInt_EV	- PCR_1: 15 + 16 - PCR_2: 17 + 18 - $Ligation_1$: PCR_1 (XbaI) + PCR_2 (XbaI) - $Ligation_2$: pK18 (HindIII, PstI) + $Ligation_1$ (HindIII, PstI)
pInt_*L4*	- PCR: 19 + 20 - Ligation: pK18 (PstI, SmaI) + PCR (PstI, SmaI)
pInt_*L4*^{3xFLAG}	- PCR_1: 21 + 22 - PCR_2: 35 + 36 (pYP247) - $Ligation_1$: PCR_1 (SalI) + PCR_2 (SalI) - $Ligation_2$: pK18 (Acc65I, PstI) + $Ligation_1$ (Acc65I, PstI)
pInt_*atu1667*^{3xFLAG}	- PCR_1: 23 + 24 - PCR_2: 35 + 36 (pYP247) - $Ligation_1$: PCR_1 (SalI) + PCR_2 (SalI) - $Ligation_2$: pK18 (Acc65I, PstI) + $Ligation_1$ (Acc65I, PstI)
pInt_ *atu8161*^{3xFLAG}	- PCR_1: 25 + 26 - PCR_2: 35 + 36 (pYP247) - $Ligation_1$: PCR_1 (SalI) + PCR_2 (SalI) - $Ligation_2$: pK18 (Acc65I, PstI) + $Ligation_1$ (Acc65I, PstI)
pInt_*atu1766*^{3xFLAG}	- PCR: 27 + 28 - Ligation: pInt_*L4*^{3xFLAG} (EcoRI, PstI) + PCR (EcoRI, PstI)
pInt_*atu1865*^{3xFLAG}	- PCR: 29 + 30 - Ligation: pInt_*L4*^{3xFLAG} (EcoRI, PstI) + PCR (EcoRI, PstI)
pInt_*atu1847*^{3xFLAG}	- PCR_1: 31 + 32 - PCR_2: 35 + 36 (pYP247) - $Liation_1$: PCR_1 (SalI) + PCR_2 (SalI) - $Ligation_2$: pK18 (Acc65I, PstI) + $Ligation_1$ (Acc65I, PstI)
pInt_*atu8135*^{3xFLAG}	- PCR_1: 33 + 34 - PCR_2: 35 + 36 (pYP247) - $Liation_1$: PCR_1 (SalI) + PCR_2 (SalI) - $Ligation_2$: pK18 (Acc65I, PstI) + $Ligation_1$ (Acc65I, PstI)

Table S4. Oligonucleotides used in this study.

No.	Primer name	Sequence	Designated use
1	DelUp_*L4*_fwd	TTTTCTGCAGGTTCGGCAAGGGTTTTAC	Construction of pDel_*L4*
2	DelUp_*L4*_rev	TTTTGGATCCGTGCAATGGTTCCAATTTACC	
3	DelDown_*L4*_fwd	TTTTGGATCCAACAATTGGCGCAAATATCG	
4	DelDown_*L4*_rev	TTTTAAGCTTGGAAGATAGCATTTTTCTGCG	
5	DelUp_*atu1667*_fwd	TTTTCTGCAGCCCGCTGTCAAACCCCGC	Construction of pDel_*atu1667*
6	DelUp_*atu1667*_rev	TTTTGGTCTCATTTCATCTTCCTCTTCGTGCTGTTC	
7	DelDown_*atu1667*_fwd	TTTTGGTCTCAGAAATAATAAAACGCGCCGCCGC	
8	DelDown_*atu1667*_rev	TTTTCTGCAGATGCATTTGTGCACTATGTCGGC	
9	DelUp_*atu8161*_fwd	TTTTCTGCAGCAAAACGCATGCGTCAAAGCAGC	Construction of pDel_*atu816.*
10	DelUp_*atu8161*_rev	TTTTGGTCTCAGATCGTTTCCTCAATCTTTCTTTGATTTC	
11	DelDown_*atu8161*_fwd	TTTTGGTCTCAGATCGGCGTAAAAGATCGTCCCGC	
12	DelDown_*atu8161*_rev	TTTTCTGCAGCTCGGGGACGCGGCATTGTTG	
13	KO_*lsrB*_fwd	TTTTGTCGACAAGGCAGGATAGGCATGGCA	Construction of pKO_*lsrB*
14	KO_*lsrB*_rev	TTTTGGTACCGATTATCTTTCGACGTCGATTAT	
15	IntUp_EV_fwd	TTTTAAGCTTCATGTGGCTGAATTTTCAGCGGTCAC	Construction of pInt_EV
16	IntUp_EV_rev	TTTTTCTAGAGGTCTCACATGACATGACCCTTTCTGAGGTTTAGG	
17	IntDown_EV_fwd	TTTTTCTAGAAGGTCTCATAAATGCAGGCGATATCGCCACTTATAAATA C	
18	IntDown_EV_rev	TTTTCTGCAGGAGAGCCATGGAACAAAGGGC	
19	Int_*L4*_fwd	TTTTGGTACCCATGTGGCTGAATTTTCAGCGGTCAC	Construction of pInt_*L4*
20	Int_*L4*_rev	TTTTCTGCAGGAGAGCCATGGAACAAAGGGC	
21	Int_*L4*^{3xFLAG}_fwd	TTTTGGTACCATCAAAGGACGCCGG	Construction of pInt_*L4*^{3xFLAG}
22	Int_*L4*^{3xFLAG}_rev	TTTTGTCGACCTTGCCGACGGCGG	
23	Int_*atu1667*^{3xFLAG}_fwd	TTTTGGTACCGGGTCAATTTACCGGC	Construction of pInt_*atu1667*^{3xFLAG}
24	Int_*atu1667*^{3xFLAG}_rev	TTTTGTCGACGAAGCCGACGGCGG	
25	Int_*atu8161*^{3xFLAG}_fwd	TTTTGGTACCTCAACAGCCGAGAGC	Construction of pInt_*atu8161*^{3xFLAG}
26	Int_*atu8161*^{3xFLAG}_rev	TTTTGTCGACGCGGAACGAACGGGAAG	
27	Int_*atu1766*^{3xFLAG}_fwd	TTTTGAATTCGGTACCATGATACATTATCTGCCGGCGAC	Construction of pInt_*atu1766*^{3xFLAG}
28	Int_*atu1766*^{3xFLAG}_rev	TTTTGTCGACTTTGCGGACGGCGTGTATGCGG	
29	Int_*atu1865*^{3xFLAG}_fwd	TTTTGAATTCGGTACCATTGGTAGCATATGCGCTGC	Construction of pInt_*atu1865*^{3xFLAG}
30	Int_*atu1865*^{3xFLAG}_rev	TTTTGTCGACAAACCGCGGCGCCCACGGTAC	
31	Int_*atu1847*^{3xFLAG}_fwd	TTTTGGTACCGCAAGATGGATCAATACCTTTCGAC	Construction of pInt_*atu1847*^{3xFLAG}
32	Int_*atu1847*^{3xFLAG}_rev	TTTTGTCGACGAGGGACCGCGTCGGCCAG	
33	Int_*atu8135*^{3xFLAG}_fwd	TTTTGGTACCATGCGCACGGCAGAACGGAG	Construction of pInt_*atu8135*^{3xFLAG}
34	Int_*atu8135*^{3xFLAG}_rev	TTTTGTCGACAAGCGTCTTGAAACGCGTGAC	
35	*3xFLAG*_fwd	TTTTGTCGACTACAAAGACCATGACG	C-terminal *3xFLAG*-tag fusions
36	*3xFLAG*_rev	TTTTCTGCAGGGATCTTATCATTTATCGTC	
37	TestDel_*L4*_fwd	CACGCCGTCCATGTGGCTG	Check deletion of *L4*
38	TestDel_*L4*_rev	GTGCAGTTCACGGGCGCTC	
39	TestDel_*atu1667*_fwd	GGTCAATTTACCGGCTTGCCTTGC	Check deletion of *atu1667*
40	TestDel_*atu1667*_rev	GCCAGAGACGGGAGCCTCAGG	
41	TestDel_*atu8161*_fwd	CCGCAAATTTAGCCGACATAGTGCAC	Check deletion of *atu8161*
42	TestDel_*atu8161*_rev	CGCTTTTCCAAAGGCTGGTTTCTCC	
43	TestKO_*lsrB*_fwd	TCCTGCAGGAACGGCTGTTT	Check knock-out of *lsrB*
44	TestKO_*lsrB*_rev	GTCACGTCTCTGTGAGCATC	
45	TestInt_*L4*_fwd	GCGCGACGAACGCTAACGTTTTCGGC	Check *A. tumefaciens* ΔΔΔ + *L4*
46	TestInt_*L4*_rev	GGTTTAGGTGACGTCAGCAACGTTTTCGTTC	
47	TestInt_*L4*_rev2	GTCACGACGTTGTAAAACGACGGCCAGTG	
48	TestInt_*L4*^{3xFLAG}_fwd	GGCATGATAAAGCGCATATAGAGGG	
49	TestInt_*atu1667*^{3xFLAG}_fwd	GCGTTTCGTGCATAGGTGACATC	Check integration of *3xFLAG*
50	TestInt_*atu8161*^{3xFLAG}_fwd	CACTGCACAACATGGCCTAATGTC	
51	TestInt_*atu1766*^{3xFLAG}_fwd	GGATCGTGGTTCACGAAAGGATC	
52	TestInt_*atu1865*^{3xFLAG}_fwd	GGGATGGATTTCCCATGCAAAATGATC	
53	TestInt_*atu1847*^{3xFLAG}_fwd	GCATTGAATGACATCAAACACACGG	
54	TestInt_*atu8135*^{3xFLAG}_fwd	CCAAGCAAATCAGTATCAACCTGG	
55	Test_*3xFLAG*_rev	CACCGTCATGGTCTTTGTAGTCG	
56	Probe_*L4*_fwd	ATCAAAGGACGCCGGAACG	PCR for RNA probe against *L4*
57	Probe_*L4*_rev	GAAATTAATACGACTCACTATAGGGCATGACATGACCCTTTCTG	

Table S4. (Continued)

No.	Primer name	Sequence	Designated use
58	Probe_*atu1667*_fwd	ATACAGAGATCAGTTCAGCCC	PCR for RNA probe against *atu1667*
59	Probe_*atu1667*_rev	GAAATTAATACGACTCACTATAGGGCATTTTCATCTTCCTCTTCG	
60	Probe_*atu8161*_fwd	CCTCAACAGCCGAGAGCACAG	PCR for RNA probe against *atu8161*
61	Probe_*atu8161*_rev	GAAATTAATACGACTCACTATAGGGTTCATGATCGTTTCCTC	
62	Probe_*atu1766*_fwd	GGATCAAAGACATGACCATGATACATTATC	PCR for RNA probe against *atu1766*
63	Probe_*atu1766*_rev	GAAATTAATACGACTCACTATAGGGTCGGCCATCATTTGCGGAC	
64	Probe_*atu1865*_fwd	GGATTTCCCATGCAAAATGATCAACCATTG	PCR for RNA probe against *atu1865*
65	Probe_*atu1865*_rev	GAAATTAATACGACTCACTATAGGGCCGCCACTGCTCAAAACCG	
66	Probe_*atu1847*_fwd	GCGCAAGATGGATCAATACCTTTCGACAG	PCR for RNA probe against *atu1847*
67	Probe_*atu1847*_rev	GAAATTAATACGACTCACTATAGGGCCAGGAGAAAAGCTGAGATTTCG	
68	Probe_*atu8135*_fwd	CGGAAGGATAAGTATCATGCGCACGG	PCR for RNA probe against *atu8135*
69	Probe_*atu8135*_rev	GAAATTAATACGACTCACTATAGGGACGTCGGATCTTCCAACAGG	
70	Probe_*pstS*_fwd	CGGCTGCGTCCACAAACAAGCCCACG	PCR for RNA probe against *pstS* (*atu0420*)
71	Probe_*pstS*_rev	GAAATTAATACGACTCACTATAGGGCGATGTCGATGGTGCCTTCGCC	
72	Probe_*adh*_fwd	CGACGTCGTCATCGATATCAAATATGCCGG	PCR for RNA probe against *adh* (*atu2022*)
73	Probe_*adh*_rev	GAAATTAATACGACTCACTATAGGGTGGCGCAGCCAACGCAGGAATC	