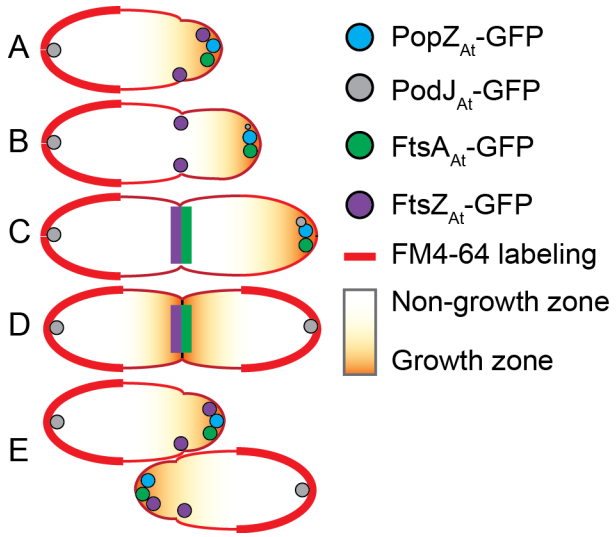
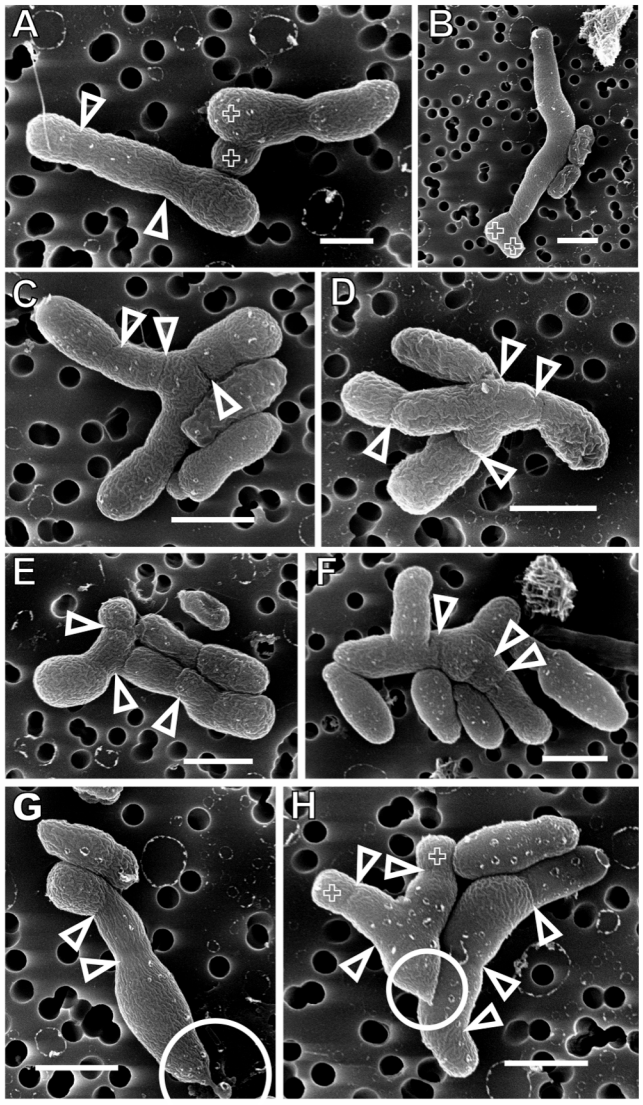
SUPPLEMENTARY INFORMATION

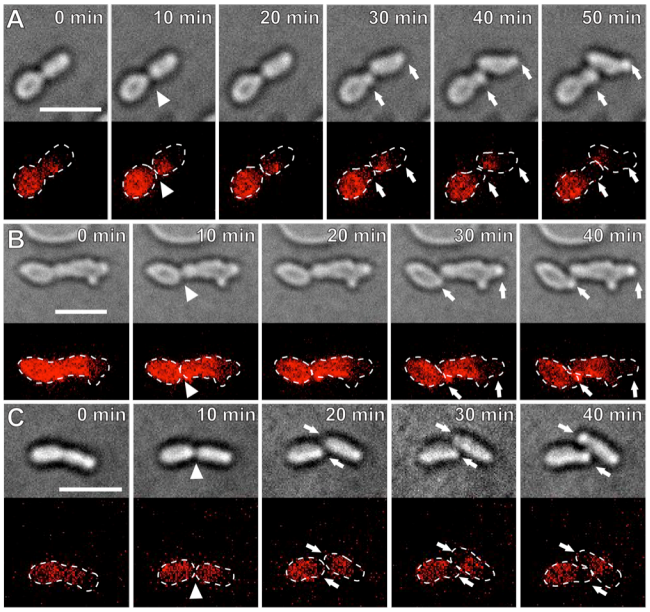
SUPPLEMENTARY FIGURE LEGENDS



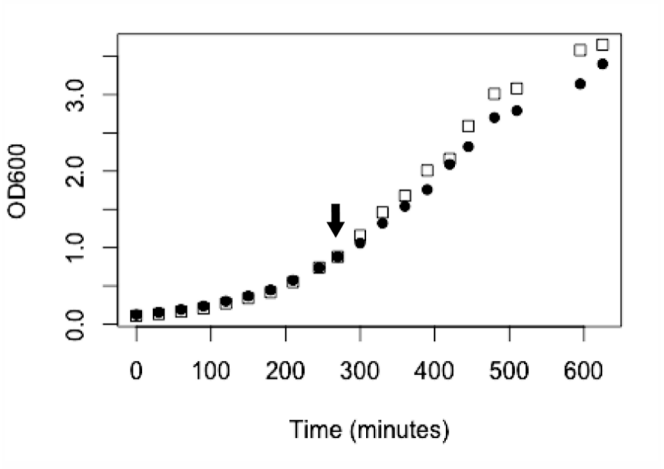
Supplementary Figure 1. Localization of cell division proteins, polar marker proteins, and membrane staining patterns in WT *A. tumefaciens.* A) newborn cell, B-C) elongating cell, D) dividing cell, and E) newborn sibling cell stages of the cell cycle. FM4-64 labeling patterns are shown, with thicker red lines indicating areas of increased fluorescence intensity (1).



Supplementary Figure 2. Gallery of scanning electron microscope images of *ΔpodJ*$_{At}$ cells. A) and B) show elongated cells with branches and constrictions. C)-F) show branched cells with multiple constrictions. G) and H) show cells with tapered poles, branches, and multiple constrictions. Arrowheads denote constrictions, + signs denote branch tips, and circles denote tapered poles. Scale bars, 3 µm.



Supplementary Figure 3. Pulse-chase labeling indicates growth abnormalities and that growing poles are smaller in diameter than non-growing poles in *ΔpodJ$_{At}$*. Live-cell imaging of *ΔpodJ$_{At}$* cells pulse-chase labeled with TRSE. Unlabeled areas indicate sites of new cell growth during chase period. A) After division (white arrowhead) the cell on the right continues to grow from the growth pole (white arrow). B) After division (white arrowhead), the cell on the right continues to grow from the growth pole (white arrow) and develops a branch morphology via ectopic polar growth. C) A WT cell undergoing normal polar growth (white arrows) from poles derived from a division event (white arrowhead). Top: brightfield images. Bottom: red fluorescence images. Scale bar, 3 µm.



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26 **Supplementary Figure 4. Growth of *ΔpodJ*$_{At}$ cells is similar to wild type.** OD600 growth curve for C58

27 (□) and *ΔpodJ*$_{At}$ (•). Arrow indicates time when CFU/mL were determined (see text).

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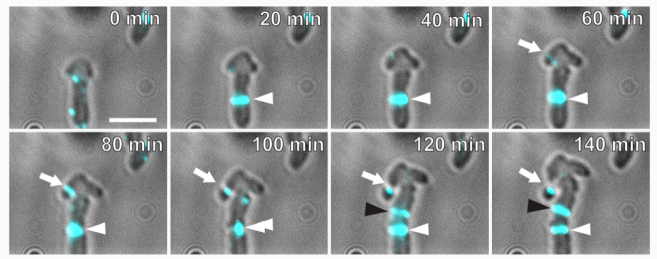
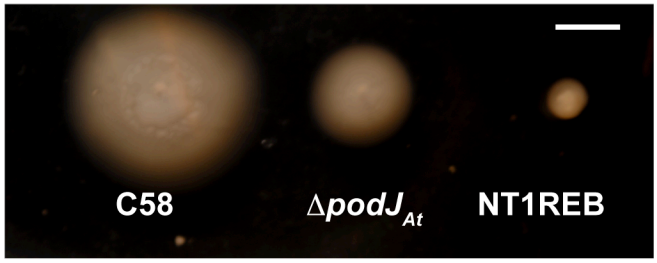
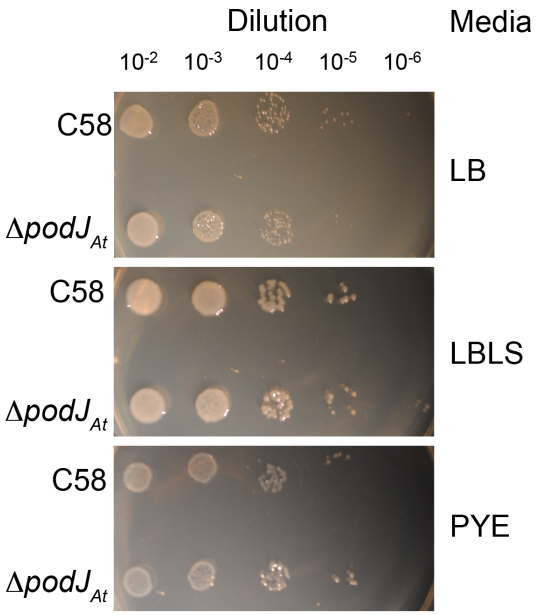


Supplementary Figure 5. FtsZ-GFP localizes in multiple rings in *ΔpodJ*$_{At}$. FtsZ-GFP (cyan) localizes in multiple "Z-rings" (white arrowhead, black arrowhead). FtsZ-GFP also localizes to an ectopic division site producing a small spherical cell (white arrow).



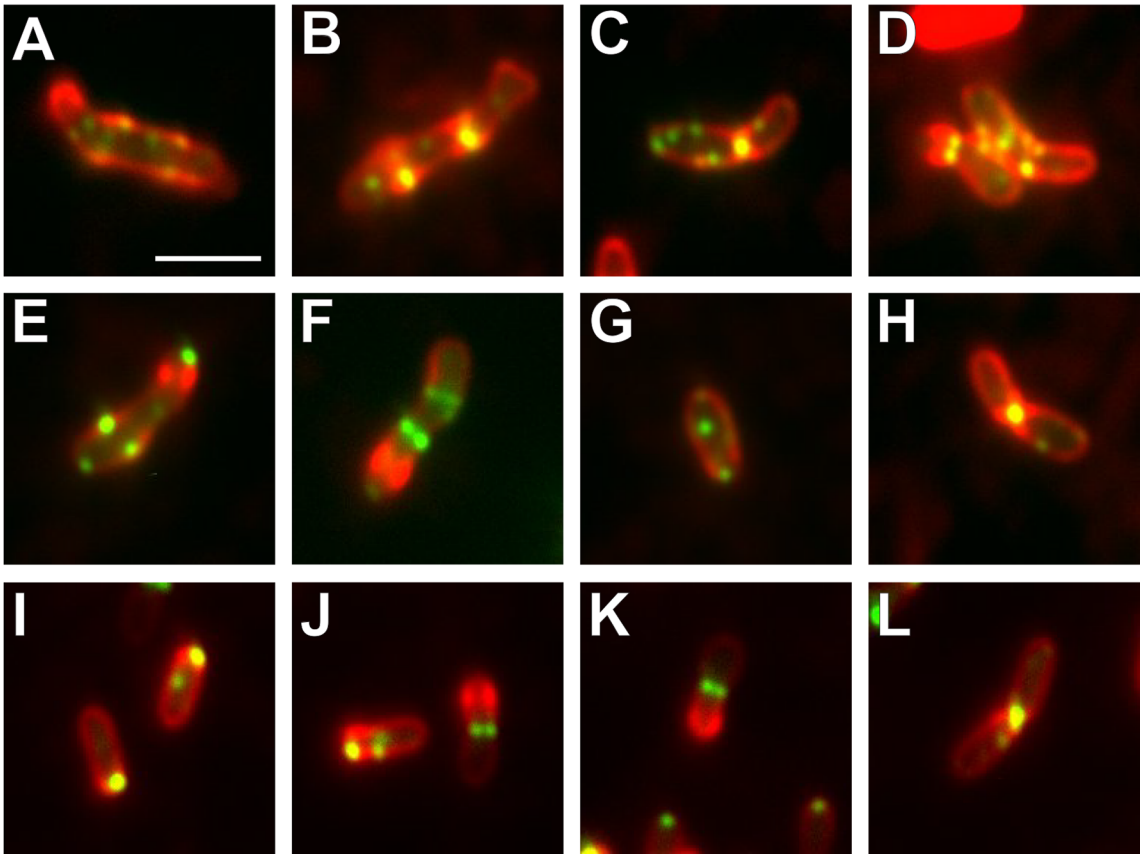
Supplementary Figure 6. Motility of *ΔpodJ*$_{At}$ is intermediate between WT and flagella minus strains.

Soft agar motility assay of *ΔpodJ*$_{At}$, WT, and NT1REB (bald/flagella minus strain). Scale bar, 0.5 cm.



Supplementary Figure 7. *ΔpodJ*$_{At}$ grows equally as well as WT on LB, LBLS, and PYE solid media.

Growth on LB, LBLS, and PYE plates for the indicated dilutions is compared for the WT and *ΔpodJ*$_{At}$ strain.



Supplementary Figure 8. *ΔpodJ*$_{At}$ cells grown in PYE display abnormal FtsZ-GFP localization, whereas C58 FtsZ-GFP localization is normal. In *ΔpodJ*$_{At}$ cells grown in PYE, FtsZ-GFP localizes in multiple peripheral foci (A-E), multiple rings (F), and polar (G) and midcell foci (H). In WT cells grown in PYE, FtsZ-GFP localizes to the poles (I), the poles and midcell (J), and at the midcell (K-L). Scale bar indicates 3 μm.

SUPPLEMENTARY VIDEO LEGENDS

Supplementary Video 1. Morphologically normal-looking *ΔpodJ*$_{At}$ give rise to cells with defects in polar growth. Time lapse video of *ΔpodJ*$_{At}$ cells. Circles denote cell division events, white dots denote normally-localized growth poles (with respect to the previous division event), red dots indicate ectopic growth poles, and red asterisks denote morphologically abnormal cells. At the end

of the video numerous morphological alterations have arisen. One second of video corresponds to 20 minutes.

Supplementary Video 2. Morphologically normal-looking *ΔpodJ*$_{At}$ give rise to cells with defects in polar growth and small, non-growing cells. Time lapse video of *ΔpodJ*$_{At}$ cells. Circles denote cell division events, white dots denote normally-localized growth poles (with respect to the previous division event), red dots indicate ectopic growth poles, and red asterisks denote morphologically abnormal cells. At the end of the video numerous morphological alterations have arisen. One second of video corresponds to 20 minutes.

Suppemental Video 3. *ΔpodJ*$_{At}$ cells undergo ectopic polar growth that continues from a growth pole after division. Selected still images from this time-lapse video are summarized in Figure 2E. One second of video corresponds to 20 minutes.

Supplementary Video 4. *ΔpodJ*$_{At}$ cells expressing GFP-PodJ$_{At}$ undergo growth and division resembling WT cells, with GFP-PodJ$_{At}$ localization similar to WT. Selected still images from this time-lapse video are summarized in Figure 4. One second of video corresponds to 20 minutes.

Supplementary Video 5. *ΔpodJ*$_{At}$ cells expressing PopZ$_{At}$-GFP undergo ectopic polar growth that continues from a growth pole after division. See PopZ$_{At}$-GFP in top cell. Selected images from this time-lapse video are summarized in Figure 5A. One second of video corresponds to 20 minutes.

Supplementary Video 6. *ΔpodJ*$_{At}$ cells expressing PopZ$_{At}$-GFP undergo ectopic polar growth when one growth pole splits into two adjacent growth poles. Selected images from this time-lapse video are summarized in Figure 5B. One second of video corresponds to 20 minutes.

Supplementary Video 7. *ΔpodJ*$_{At}$ cells expressing PopZ$_{At}$-GFP undergo ectopic polar growth when a growth pole extends from a point along the sidewall of the cell. Selected images from this time-lapse video are summarized in Figure 5C. One second of video corresponds to 20 minutes.

Supplementary Video 8. FtsZ-GFP localize in multiple rings in *ΔpodJ*$_{At}$. Selected images from this time-lapse video summarized in Supplementary Figure 6. One second of video corresponds to 20 minutes.

Supplementary Video 9. *ΔpodJ*$_{At}$ produces cells which do not grow and divide and a fraction of *ΔpodJ*$_{At}$ cells are small and anucleate. Selected images from this time-lapse video summarized in Figure 8. One second of video corresponds to 20 minutes.

92 **Supplementary Table 1.** Strains and plasmids used in this study.

Strains	Relevant genotype	Source
XL1 Blue	E. coli cloning strain, *endA1 gyrA96*(nal^R) thi-1 *recA1 relA1* lac glnV44 F'[::Tn10 *proAB*+ *lacIq* Δ(*lacZ*)M15] hsdR17(rK-mK+), Tet^R.	Lab stock
C58	wild-type A. tumefaciens strain C58	Lab stock
A164	C58, *ΔpodJ$_{At}$* using pJZ237	This work
NT1REB	Rm^R Em^R pTi$^-$ mot$^-$ *ΔflaABC* (bald strain)	(2)

Plasmids	Relevant genes and construction information	Source
pSRK-Km	Broad host-range, lac inducible, Kan^R	(3)
pSRK-Gm	Broad host-range, lac inducible, Gent^R	(3)
pBluescript II SK	Phagemid, Carb^R	Stratagene
pJZ156	pBSKII+ with *sacB*, Carb^R	This work
pJZ207	pSRK-Km with *ftsZ-GFP*, lac-inducible, Kan^R	(1)
pJZ208	pSRK-Km with *ftsA-GFP*, lac-inducible, Kan^R	(1)

pJZ232	pSRK-Gm with *gfp-podJ*$_{At}$, lac-inducible, Gent^{R}	(4)
pJZ237	pJZ156 with 1kb sequences homologous to C58 genomic DNA flanking Atu0499 (*podJ*$_{At}$) and Atu0500, Carb^{R}	This work
pJZ255	pSRK-Gm with *podJ*$_{At}$, lac-inducible, Gent^{R}	This work
pRG001	pSRK-Gm with *popZ*$_{At}$*-gfp*, lac-inducible, Gent^{R}	(4)

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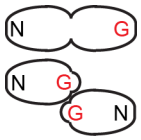
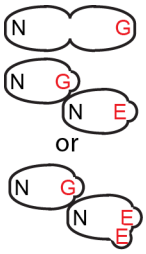
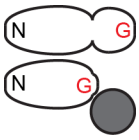
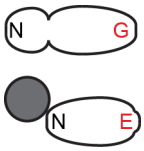
Supplementary Table 2. Percentages of WT, *ΔpodJ*$_{At}$, and *ΔpodJ*$_{At}$ + PodJ$_{At}$ division events producing normal polar growing, ectopic polar growing, and non-growing cells. Numbered schematics representing types of division events are shown, with the pre-divisional cell above and resulting sibling cells below. G, normal growth poles. E, ectopic growth poles. N, non-growing poles. Filled cells represent non-growing cells. These data are derived from time-lapse imaging of individual cells (n).

Types of division events	**WT** **(n=148)**	***ΔpodJ*$_{At}$** **(n=151)**	***ΔpodJ*$_{At}$ + PodJ$_{At}$** **(n=142)**
1) Producing two normal polar-growing cells	100%	53%	95%
2) Producing one normal polar-growing cell and one ectopic polar-growing cell	0%	37%	3%
3) Producing one normal polar-growing cell and one non-growing cell	**0%**	**3%**	**1%**
4) Producing one ectopic polar-growing cell and one non-growing cell	0%	7%	1%
1)  2)  3)  4) 			

Supplementary Table 3. Median cell lengths of WT and $\Delta podJ_{At}$ cells displaying the specified FtsA-GFP localization. Values for WT are from Zupan *et al.* (12).

		Unipolar	**Unipolar + midcell**	**Midcell**	**Aberrant polar**	**Aberrant midcell**
WT	Median cell length (μm)	1.9	2.7	3.2	NA	NA
	Percentage of cells	78	13	9	NA	NA
***ΔpodJ*$_{At}$**	Median cell length (μm)	2.4	3.3	3.5	2.7	4.3
	Percentage of cells	36	24	3	22	15

Supplementary Table 4. Growth in PYE partially complements the *ΔpodJ*$_{At}$ morphology phenotype.

Frequency of *ΔpodJ*$_{At}$ and WT cells grown in LB and PYE displaying different morphologies are compared for LB and PYE.

	LB		PYE	
	WT	*ΔpodJ*$_{At}$	WT	*ΔpodJ*$_{At}$
Rod-shaped	96 %	60 %	97 %	78 %
Multiple constriction/bent	4 %	29 %	3 %	17 %
Branched/ swollen	0 %	8 %	0 %	4 %
Minicell	0 %	3 %	0 %	1 %
Number of cells counted	283	262	295	340

SUPPLEMENTARY REFERENCES

1. **Zupan JR**, **Cameron TA**, **Anderson-Furgeson J**, **Zambryski PC**. 2013. Dynamic FtsA and FtsZ localization and outer membrane alterations during polar growth and cell division in *Agrobacterium tumefaciens.* Proc Natl Acad Sci USA **110**:9060–9065.

2. **Chesnokova O**, **Coutinho JB**, **Khan IH**, **Mikhail MS**, **Kado CI**. 1997. Characterization of flagella genes of *Agrobacterium tumefaciens*, and the effect of a bald strain on virulence. Mol Microbiol **23**:579–590.

3. **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.

4. **Grangeon R**, **Zupan JR**, **Anderson-Furgeson J**, **Zambryski PC**. 2015. PopZ identifies the new pole, and PodJ identifies the old pole during polar growth in *Agrobacterium tumefaciens*. Proc Natl Acad Sci USA **112**:11666–11671.