Supplemental Materials

Molecular Biology of the Cell

Henry et al.

Supplementary Table and Figures

Table S1. Strains and Plasmids

Strain or plasmid	Genotype or Description	Reference(s) or source
Strain (FC #)		
2051	*Escherichia coli* K12	(1)
2053	K12 Δ*ppk1*	(1)
2055	K12 Δ*ppk1* pSRK-*venus*	This work
2054	K12 Δ*ppk1* pSRK-*venus-ppk1 (E. coli* Δ*ppk1 venus-ppk1*$^{++}$*)*	This work
2106	K12 Δ*ppk1* pBAD24-*mCherry-ppk1(H434A)* pSRK-*venus-ppk1(CC)*	This work
2107	K12 Δ*ppk1* pBAD24-mCherry-*ppk1(H434A)* pSRK-*venus-ppk1(EC)*	This work
82	*Asticcacaulis biprosthecum*	Yves Brun
163	*Rhodobacter capsulatus*	Carl Bauer
20	*Caulobacter crescentus* NA1000	(3, 6)
1460	NA1000 Δ*ppk1*	(2)
1454	NA1000 *ppk1(H434A)* (formerly annotated *H460A*)	(2)
1579	NA1000 *xylX*::pMT585 (WT *EV)*	(2)
1582	NA1000 *xylX*::pMT585-*ppk1 (WT ppk1*$^{++}$*)*	(2)
1581	Δ*ppk1 xylX*::pMT585 (Δ*ppk1 EV)*	(2)
1584	Δ*ppk1 xylX*::pMT585-*ppk1 (*Δ*ppk1 ppk1*$^{++}$*)*	(2)
2039	NA1000 *venus-ppk1*	This work
2050	NA1000 *venus-ppk1(H434A)*	This work
2037	Δ*ppk1 xylX::*pMT854-*ppk1 (*Δ*ppk1 venus-ppk1*$^{++}$*)*	This work
2059	NA1000 *xylX::*MT854-*ppk1(H434A) (venus-ppk1(H434A)*$^{++}$*)*	This work
2035	Δ*ppk1 xylX::*pMT854 *(*Δ*ppk1 EV)*	This work
2060	Δ*ppk1 xylX::*pMT854-*ppk1(H434A) (*Δ*ppk1 venus-ppk1(H434A)*$^{++}$*)*	This work
2062	NA1000 *xylX*::pMT854-*ppk1(1-696) (venus-ppk1(*Δ*CT16)*$^{++}$*)*	This work
2063	Δ*ppk1 xylX::*pMT854-*ppk1(1-696) (*Δ*ppk1 venus-ppk1(*Δ*CT16)*$^{++}$*)*	This work
2065	Δ*ppk1 xylX::*pMT854-*ppk1(109-712) (*Δ*ppk1 venus-ppk1 (*Δ*N)*$^{++}$*)*	This work
2067	Δ*ppk1 xylX::*pMT854-*ppk1(313-712) (*Δ*ppk1 venus-ppk1 (*Δ*NH)*$^{++}$*)*	This work
2069	Δ*ppk1 xylX::*pMT854-*ppk1(1-317) (*Δ*ppk1 venus-ppk1 (*Δ*C1C2)*$^{++}$*)*	This work
2071	Δ*ppk1 xylX::*pMT854-*ppk1(1-500) (*Δ*ppk1 venus-ppk1(*Δ*C2)*$^{++}$*)*	This work

1103	NA1000 *dnaC303(ts) (holB(ts))*	(7)
2046	NA1000 *xylX::*pMT590-*parA (parA-mCherry^{++})*	This work
2047	NA1000 *xylX::*pMT590-*parA(K20R) (parA(K20R)-mCherry^{++})*	This work
791	NA1000 *egfp-parB*	(9)
2048	NA1000 *egfp-parB xylX::*pMT590-*parA*	This work
2049	NA1000 *egfp-parB xylX::*pMT590-*parA(K20R)*	This work
2057	Δ*ppk1 xylX::*pMT697-*ppk1 (*Δ*ppk1 mCherry-ppk1^{++})*	This work
2105	Δ*ppk1* pMT854-*ppk1(EC)* (Δ*ppk1 venus-ppk1(EC)*$^{++}$)	This work
Plasmids		
pNPTS138	*sacB* counterselectable, for making allelic replacements	Dickon Alley
pSRK-Kn	IPTG-inducible expression	(5)
pMT585	xylose-inducible expression	(8)
pMT590	xylose-inducible expression of C-terminal mCherry fusion	(8)
pMT697	xylose-inducible expression of N-terminal mCherry fusion	(8)
pMT854	xylose-inducible expression of N-terminal Venus fusion	(8)
pBAD24	arabinose-inducible expression	(4)

Supplementary Figure Legends

Supplemental Movie 1. Time-lapse microscopy of *venus-ppk1* cells on nutrient agarose pads. Micrographs of increasing exposure times were taken every 30 seconds for ten minutes.

Figure S1. Representative images of *venus-ppk1* truncations. A) Schematic representing the boundary residues of the truncated alleles used in this work. B) Representative micrographs from truncated alleles of *ppk1*. Each of the Venus and DAPI (polyP) images were captured with the same exposure and camera settings and scaled equally for comparison. In the merged image, Venus is depicted in the red channel, DAPI (polyP) is depicted in the green channel.

Figure S2. *mCherry-ppk1* representative micrographs and *E. coli* controls. A) Representative micrographs of Δ*ppk1 mCherry-ppk1* grown in M2G. mCherry-fluorescent foci were present and granule production was complemented without the addition of xylose. Induction with xylose significantly increased background fluorescence, so we relied on leaky expression from the xylose promoter, which was sufficient to drive production of DAPI-staining granules. In the merged image, mCherry is depicted in the red channel, DAPI (polyP) is depicted in the green channel. B) DAPI-stained *E. coli* K12 or K12 Δ*ppk1* expressing *venus* alone, demonstrating that neither strain produces DAPI-fluorescent granules, and than *venus* alone does not give fluorescent foci. In the merged image, Venus is depicted in the red channel, DAPI (polyP) is depicted in the green channel. C) *E. coli* K12 Δ*ppk1* expressing *mCherry-ppk1(H434A)* (from *C. crescentus*) and either *venus-ppk1(CC)* (from *C. crescentus*) or *venus-ppk1(EC)* (from *E. coli*). mCherry is depicted in the red channel, Venus is

depicted in the green channel. D) DAPI-stained *C. crescentus* Δ*ppk1* expressing *venus-ppk1(EC)*. Induction with xylose was necessary for granule production. Venus is depicted in the red channel, DAPI (polyP) is depicted in the green channel.

Figure S3. Representative micrographs depicting effects of *parA(K20R)* expression on ParB and polyP granule localization. A) Representative micrographs of *gfp-parB* expressing *parA-* or *parA(K20R)-mCherry*, confirming that *parA(K20R)-mCherry* expression blocks segregation of parB foci, a marker of chromosome origins. In the merged image, mCherry is depicted in the red channel, GFP is depicted in the green channel. B) Example micrographs from Figure 5D, showing impaired granule synthesis and abnormal positioning following *parA(K20R)-mCherry* expression. In the merged image, mCherry is depicted in the red channel, DAPI (polyP) is depicted in the green channel. C) Representative micrographs of *venus-ppk1* expressing *parA-* or *parA(K20R)-mCherry*, demonstrating the co-localization of Venus-Ppk1 with polyP granules under both conditions. In the merged image, Venus is depicted in the red channel, DAPI (polyP) is depicted in the green channel.

Literature Cited

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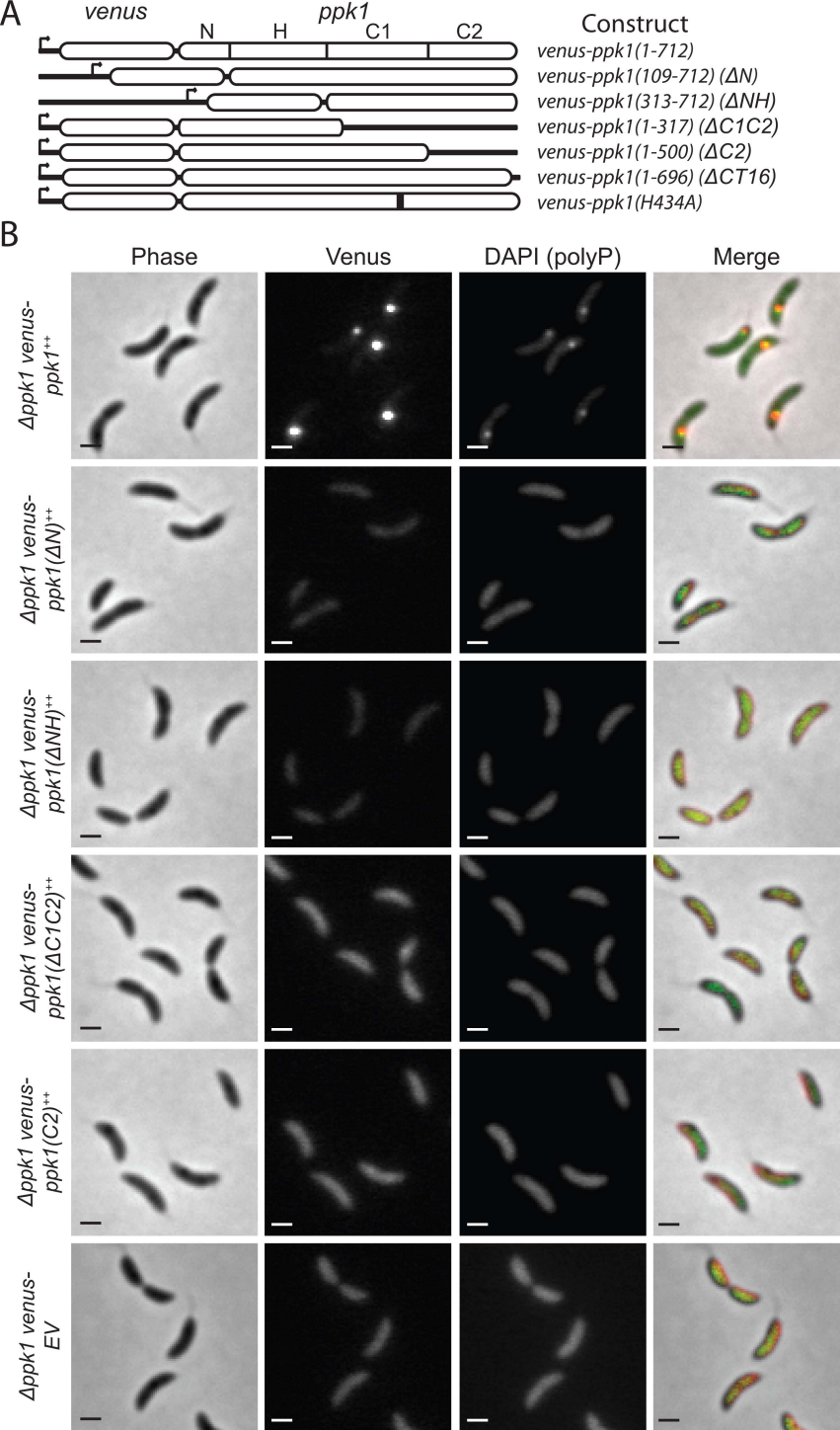
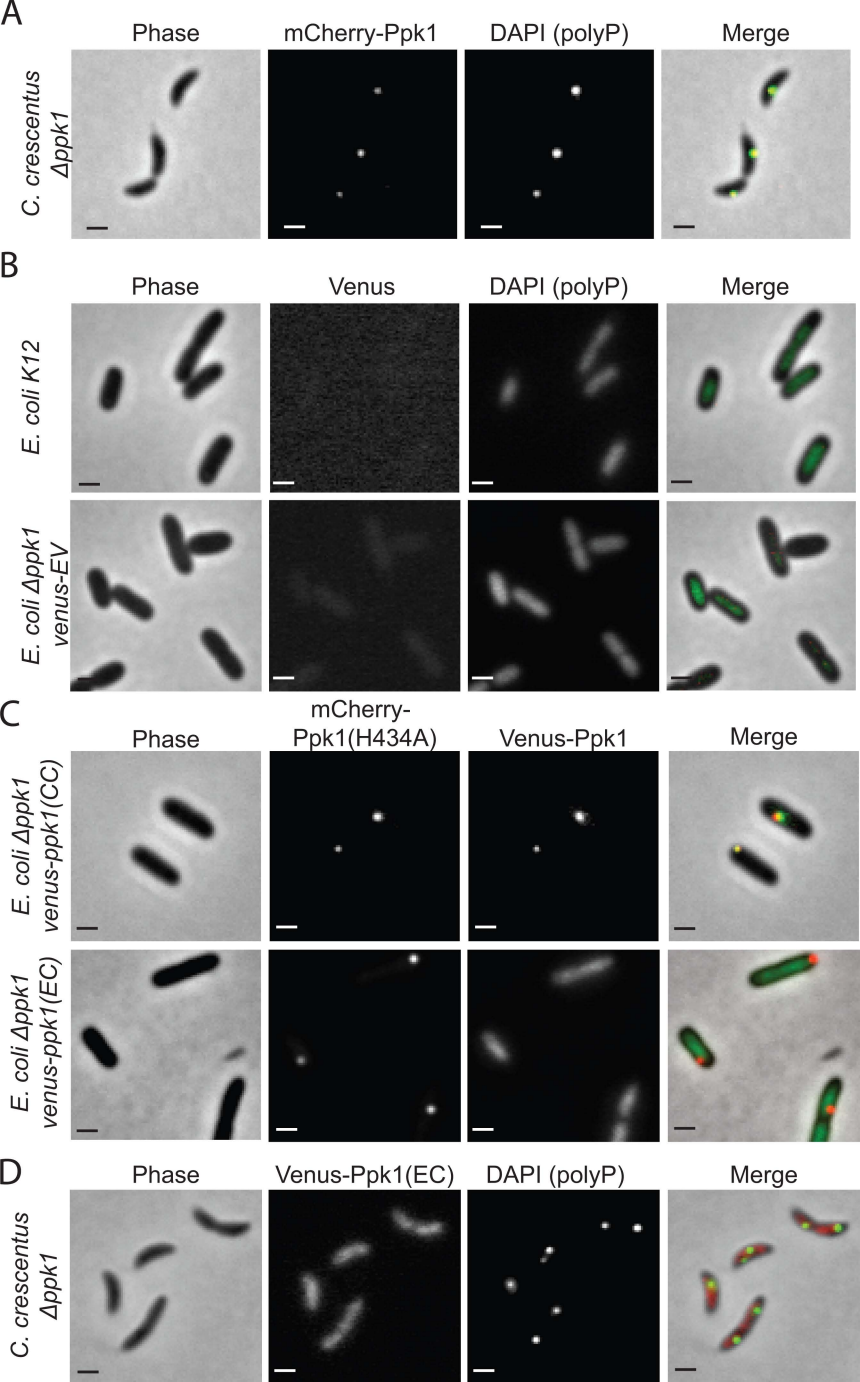
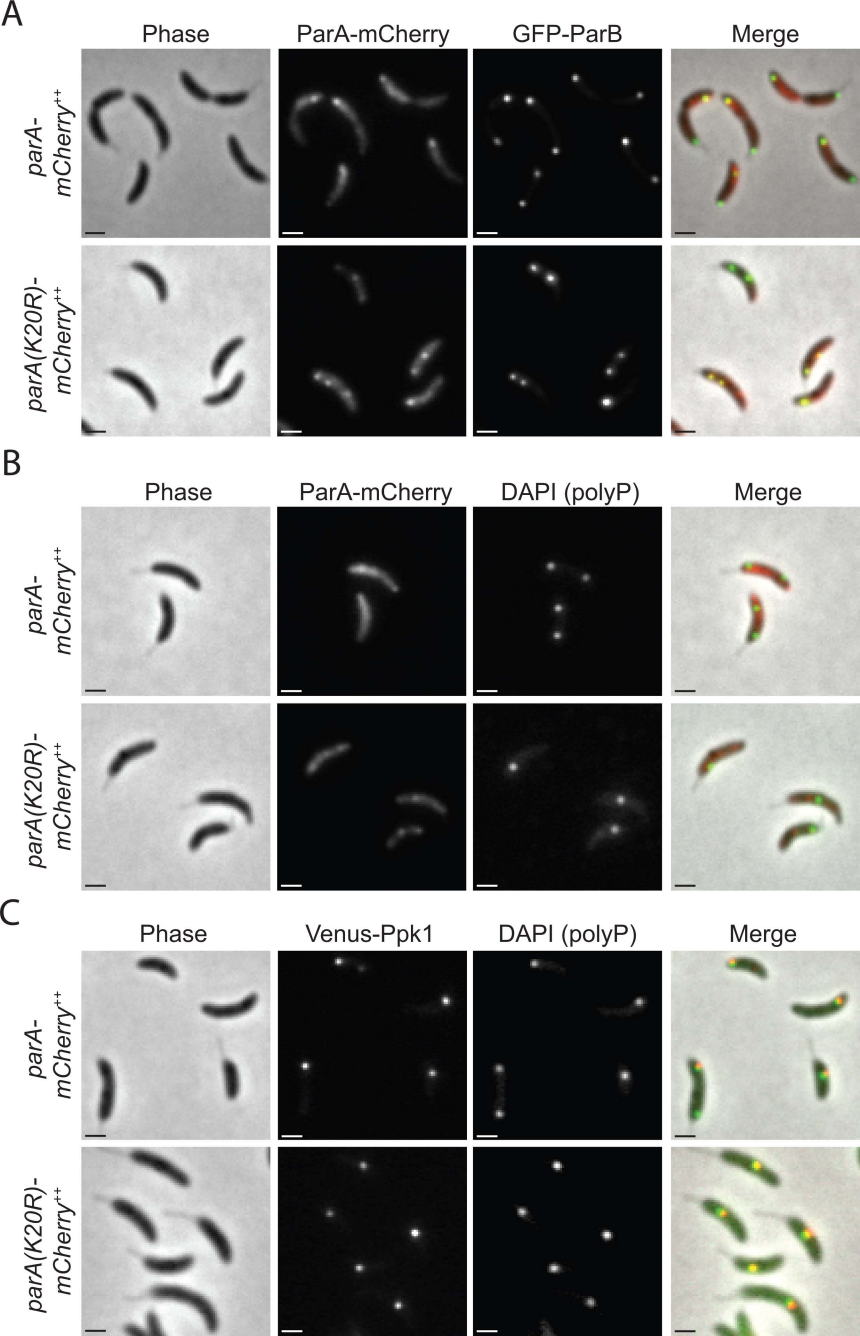

A
venus
ppk1
N
H
C1
C2
Construct
venus-ppk1(1-712)
venus-ppk1(109-712) (ΔN)
venus-ppk1(313-712) (ΔNH)
venus-ppk1(1-317) (ΔC1C2)
venus-ppk1(1-500) (ΔC2)
venus-ppk1(1-696) (ΔCT16)
venus-ppk1(H434A)
B
Phase
Venus
DAPI (polyP)
Merge
Δppk1 venus-ppk1++
Δppk1 venus-ppk1(ΔN)++
Δppk1 venus-ppk1(ΔNH)++
Δppk1 venus-ppk1(ΔC1C2)++
Δppk1 venus-ppk1(C2)++
Δppk1 venus-EV


A
Phase
mCherry-Ppk1
DAPI (polyP)
Merge
C. crescentus Δppk1
B
Phase
Venus
DAPI (polyP)
Merge
E. coli K12
E. coli Δppk1 venus-EV
C
Phase
mCherry-Ppk1(H434A)
Venus-Ppk1
Merge
E. coli Δppk1 venus-ppk1(CC)
E. coli Δppk1 venus-ppk1(EC)
D
Phase
Venus-Ppk1(EC)
DAPI (polyP)
Merge
C. crescentus Δppk1


A
Phase
ParA-mCherry
GFP-ParB
Merge
parA-mCherry++
parA(K20R)-mCherry++
B
Phase
ParA-mCherry
DAPI (polyP)
Merge
parA-mCherry++
parA(K20R)-mCherry++
C
Phase
Venus-Ppk1
DAPI (polyP)
Merge
parA-mCherry++
parA(K20R)-mCherry++