Supporting Information (SI)

Polyphosphate granule biogenesis is temporally and functionally tied to cell cycle exit during starvation in *Pseudomonas aeruginosa*

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Supporting Information Appendix

SI METHODS

Strain construction

Strains, plasmids, and primers used in this study are listed in Tables S1-3 respectively.

Plasmids

All plasmids were generated using Gibson cloning (1). Plasmids pLREX9, pLREX21, pLREX23, pLREX24, pLREX25, pLREX27, pLREX35, and pLREX36 are derivatives of suicide vector pMQ30 (2), generated by amplifying 1 kb of sequence upstream and downstream of the target gene from *P. aeruginosa* genomic DNA. For pLREX24, 1kb of sequence upstream of *phaC1* and 1 kb of sequence downstream of *phaC2* were used, resulting in deletion of three intervening genes: *phaC1*, *phaD* and *phaC2*. For pLREX27, the last 13 bases of ppk1 were retained because of overlap with the c-terminus of the coding region of the exopolyphosphatase *ppx*, transcribed from the opposite strand. Plasmid pLREX15 is a derivative of pMQ72 (2), generated by amplifying the coding sequence for *ppk2B* (PA14_33240) from *P. aeruginosa* genomic DNA using primers that insert a 6His tag, followed by the Tev cleavage site on the 5' terminus of the protein. Plasmids pLREX60 and pLREX61 are derivatives of pMQ70 (2), generated by amplifying the coding sequence for *ppk2A* (PA14_01730) from *P. aeruginosa* genomic DNA using primers that insert a Flag tag, followed by a linker containing an 8 amino acid serine/glycine linker, on the 5' terminus of the protein. Plasmids pLREX38, pLREX49, pLREX62, pLREX63, pLREX64, pLREX65, and pLREX66 are derivatives of pUC18R6K- mini-Tn7T-Gm (3). pLREX38 was generated by amplifying the *ssb* coding sequence and 1kb upstream sequence from *P. aeruginosa* genomic DNA. pLREX49 was generated by amplifying the $ParS^{pMT1}$ sequence from pFHC3228 (4), the upstream sequence containing the *ssb* promoter from *P. aeruginosa* genomic DNA, and the GFP-$parB^{pMT1}$ chimera from pFHC2973 (4). pLREX62 was generated by amplifying the $ParS^{pMT1}$ sequence from pFHC3228 (4), the *ssb* coding sequence and 1kb upstream sequence from *P. aeruginosa* genomic DNA, and the GFP-$parB^{pMT1}$ chimera from pFHC2973 (4). pLREX63 was generated by amplifying the *ssb* coding sequence and 1kb upstream sequence from *P. aeruginosa* genomic DNA, and the GFP-$parB^{pMT1}$ chimera from pFHC2973 (4). pLREX64 was generated by amplifying a fragment containing *araC*, and P_{ara} *Flag-ppk2A* (PA14_01730) followed by the T1T2 transcriptional terminator from pLREX60, and a fragment containing $ParS^{pMT1}$ P_{ssb} *ssb*-mCherry GFP-$parB^{pMT1}$ from pLREX62. pLREX65 was generated by amplifying a fragment containing *araC*, and P_{ara} *Flag-ppk2A*^{D183A,R184A} (PA14_01730) followed by the T1T2 transcriptional terminator from pLREX61, and a fragment containing $ParS^{pMT1}$ P_{ssb} *ssb* - mCherry GFP-$parB^{pMT1}$ from pLREX62. pLREX66 was generated by amplifying a fragment containing araC, and P_{ara} *His-Tev-ppk2B* (PA14_33240) followed by the T1T2 transcriptional terminator from pLREX15, and a fragment containing $ParS^{pMT1}$ P_{ssb} *ssb*-mCherry GFP-$parB^{pMT1}$ from pLREX62.

Strains

All unmarked deletion strains, and strains in which the endogenous *ppk2A* is replaced by a *ppk2A*-mCherry chimera, were generated by triparental conjugation with *P. aeruginosa* UCBPP-PA14, and then merodiploids were selected as described by Choi and Schweizer (3) on VBMM medium (3 g/L trisodium citrate, 2 g/L citric acid, 10g/L K_2HO_4, 3.5 g/L

$NaNH_4PO_4$, 1mM $MgSO_4$, 100uM $CaCl_2$, pH 7) containing 100 ug/mL gentamicin. Counterselection for homologous recombination events removing the endogenous copy of the gene in question was then performed on LB plates without NaCl and containing 20mM sucrose, followed by PCR. All strains with insertions at the *att*Tn7 site were generated by tetraparental conjugation with *P. aeruginosa* UCBPP-PA14, and then exconjugants were selected as described by Choi and Schweizer (3) on VBMM medium, and verified by PCR.

Media and Growth conditions

For nitrogen starvation experiments, strains were grown at 37°C shaking in MOPS-buffered minimal media overnight (40mM sodium succinate, 22mM NH_4Cl, 43mM NaCl, 2.2mM KCl, 1.25mM NaH_2P0_4, 1mM $MgSO_4$, 0.1mM $CaCl_2$, 7.5µM $FeCl_2 \cdot 4H_2O$, 0.8µM $CoCl_2 \cdot 6H_2O$, 0.5µM $MnCl_2 \cdot 4H_2O$, 0.5µM ZnCl2, 0.2µM $Na_2MoO_4 \cdot 2H_2O$, 0.1µM $NiCl_2 \cdot 6H_2O$, 0.1µM H_3BO_3, and 0.01µM $CuCl_2 \cdot 2H_2O$, 50mM MOPS, pH 7.2). 10-25mL cultures at OD_{500} = 0.0125 to 0.025 were grown in 250mL Erlenmeyer flasks at 37°C shaking to OD_{500} = 0.4 to 0.6, then spun down at room temperature in 50mL conical tubes at 5000xG, and resuspended to OD_{500} = 0.4 in nitrogen-limited MOPS-buffered minimal media (Identical to MOPS-buffered media, but with 1mM NH_4Cl instead of 22mM) in clean Erlenmeyer flasks and grown at 37°C shaking. Time 0h = cells collected immediately before being spun down and shifted to nitrogen-limited medium.

RNA Extraction

Total RNA was extracted from ~10^9 cells using the RNeasy Mini Kit (Qiagen), including the optional on-column DNase treatment step. 5-10 µg per sample were subsequently treated with Turbo DNase Free (Ambion) according to the manufacturer's instructions. Absorbance at 260 nm was used to quantify purified RNA, and qPCR of the purified RNA was used to verify thorough removal of free of genomic DNA.

qRT-PCR

The iScript cDNA synthesis kit (BioRad) was used to convert 1 µg DNase-treated total RNA to cDNA. $1/100^{th}$ of the reaction mix (representing 10 ng of total RNA) was subsequently used as template for each qRT-PCR reaction using the iTaq SYBR Green reaction mix (BioRad) and 500 nM each of forward and reverse primers. Samples were run using a 40 cycle program with an annealing temperature of 60°C on a Real Time 7500 PCR Machine (Applied Biosystems). Samples were assayed in biological triplicate. Threshold cycle values of OprI were used as an endogenous control. Expression values are reported relative to the expression value of the same gene in exponential growth phase (0h). Primers were designed using Primer3 and are listed in Table S3.

Granule statistics (TEM)

For Figure 3, global analysis of granule volume and count are shown, combining all cells from multiple independent experiments for a given timepoint (n = 4 experiments for 1h, n = 3 for 1.5h, n = 2 for 2h, n = 4 for 3h, n = 3 for 6h, n = 2 for 24h). Average of means from individual data sets: Granules/cell: 11.7±2.5 at 1h; 7.1±1.8 at 1.5h, 5.4±0.3 at 2h, 3.7±1.9 at 3h, 5.6±1.7 at 6h, 6.7±0.9 at 24h. Granule volume: 0.0020±.0001µm^3 at 1h, 0.0048±.0015 µm^3 at 1.5h, 0.0096±.0057 µm^3 at 2h, 0.0129±,0049 µm^3 at 3h, 0.0137±0.0013 µm^3 at 6h, 0.0081±.0006 µm^3 at 24h.

Simulation of Granule Positioning

Model for the simulation (See Fig S6a): mEnd = user-defined constraint on the minimum distance (in microns) from cell ends. For the first granule, lowerBound1 and upperBound1 are defined by the sum of the radius of granule 1 (r1) and a user- defined minimum distance for granules from cell ends (mEnd). The allowed region for the origin of granule 1: between lowerBound and (cell Length minus upperBound). For the second granule, lowerBound2 and upperBound2 defined by the sum of the radius of granule 2 (r2) and the user-defined minimum distance for granules from cell ends. A user-defined minimum distance between granules (mGran) constrains the origins of granules 1 and 2 to be at least r1+mGran+r2 apart.

To determine the best mEnd and mGran parameters, we analyzed all combinations of mEnd and mGran from 0 to 0.6 μm in 0.1 μm increments, see heat map in Figure 4b, as well as Figure S6. For each combination of parameters, we modeled 229,000 cells. We then used the non-parametric Kolmogorov-Smirnov (KS) test to compare the experimentally observed distribution to the modeled distributions. For the KS testing, we combined the positions of both granules into a single bi-modal distribution. A heat map of the resulting KS test statistics, and the resulting p-values are consistent, indicating that a minimum distance from cell ends of 0.3 μm and between granules of 0.2 μm gives the best fit.

Simulation procedure: The matlab script 'peas2pod_simulation.m', included at the end of the supplement, takes the following input values: cells = number of cells to simulate; muLength = mean cell Length (μm), sigmaLength = standard deviation of distribution of cell lengths (μm), muDiameter = mean diameter of granules (μm), sigmaDiameter = standard deviation of distribution granule diameters (μm), mEnd = minimum distance of edge of granule from cell end (μm), mGran = minimum distance from edge of granule1 to edge of granule2 (μm).

This function generates a population of a specified number of cells with a normal distribution of cell lengths specified by input mean and standard deviation values, and also generates two populations of granules, each with a normal distribution of granule diameters using mean and standard deviation values from the EM data. Each cell is screened to make sure that both granules can fit into the cell length given the constraints of minimum distance from ends and between granules; if both granules do not fit, then the function randomly re-generates a new length and new granule diameters using the input mean and standard deviation values for cell length and granule diameter. For each cell, the function positions granule 1 randomly, but excluding it from the cell ends by input mEnd + radius of granule 1. The function then does the same for granule 2. The function then checks that the position of granule 2 satisfies the following condition: it must be at least radius granule1+mGran+radius granule2 away from granule 1. If it doesn't satisfy this condition, the function randomly re-positions one of the granules within the cell (which granule is determined by a coin toss) and again checks that this new pair of granules satisfies both mEnd and mGran. The function repeats this process until the mEnd and mGran constraints are satisfied, and then goes on to the next cell.

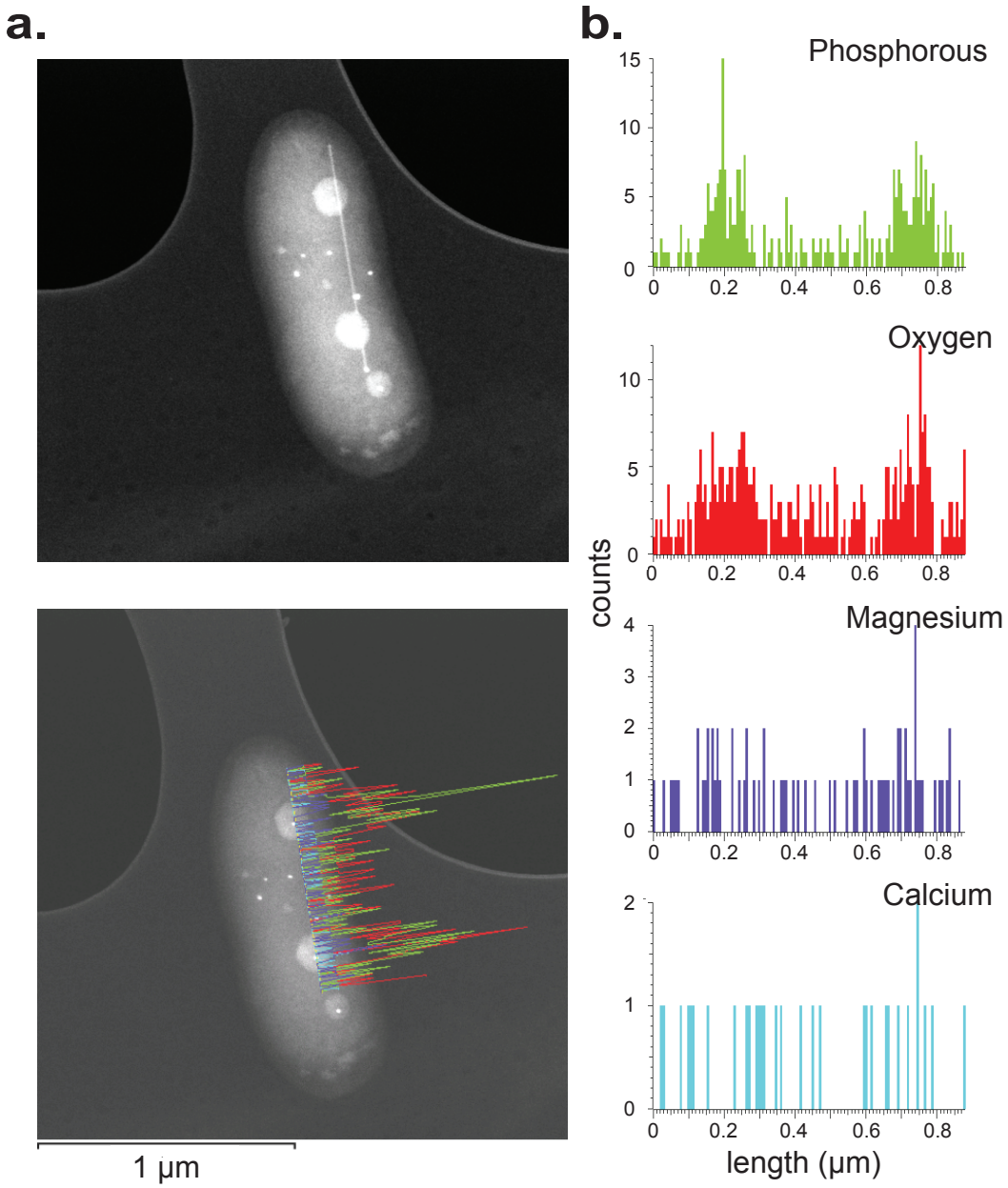

Figure S1. Elemental analysis of putative polyphosphate granules by Energy Dispersive X-ray Spectroscopy (EDS). A) Top Panel: Scanning emission EM image of cell after line analysis. Bottom Panel: Overlay of line analysis for phosphorous (green), oxygen (red), magnesium (blue), and calcium (cyan). B) Line analysis transecting two polyphosphate granules shows elevated counts of phosphorous and oxygen relative to cell background.

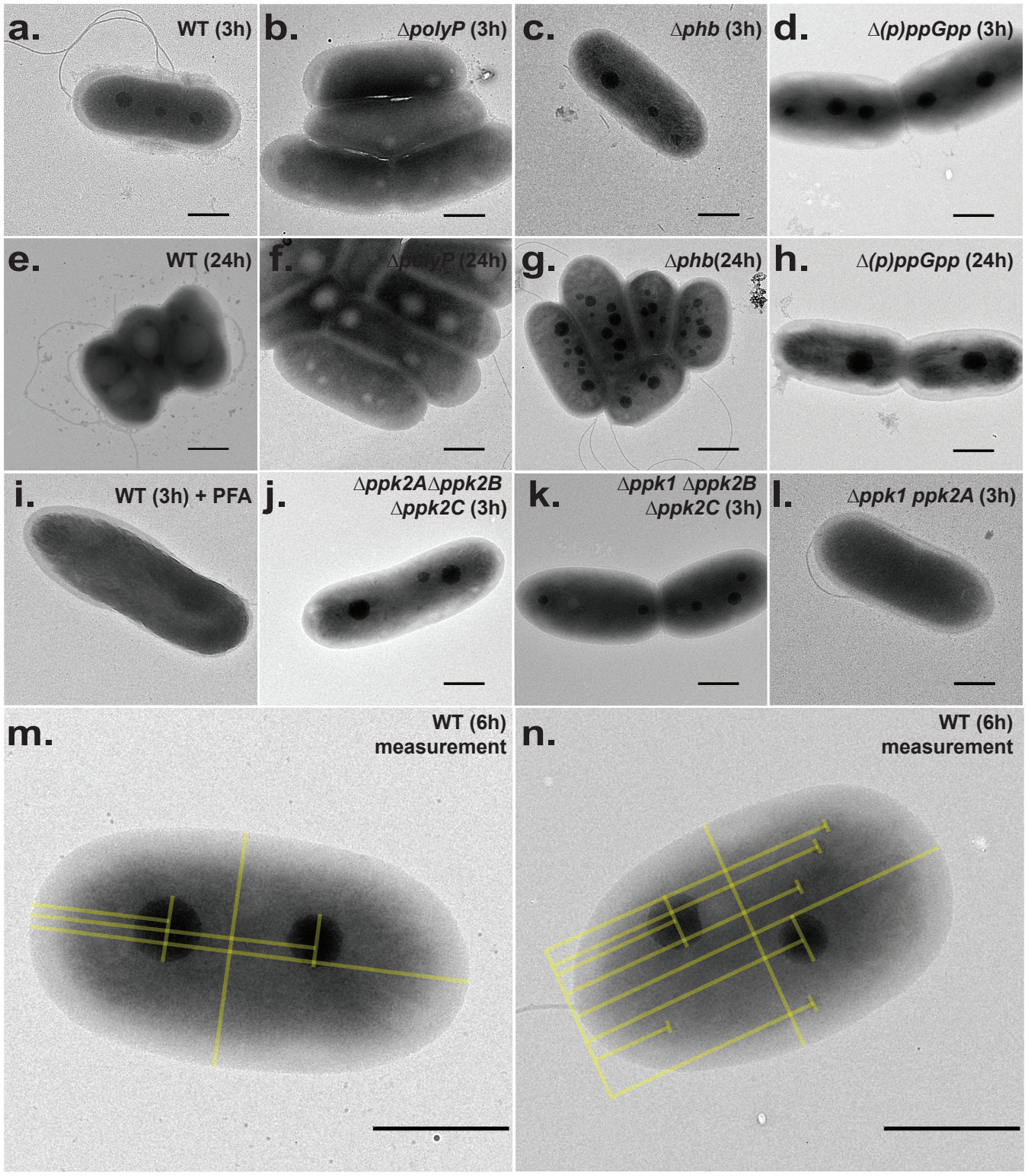

Figure S2. TEM images of polyphosphate mutants under nitrogen starvation. Scale bar = 0.5 μm A) 3h WT cell, B) 3h Δ*polyP cells* (Δ*ppk1*Δ*ppk2A*Δ*ppk2B*Δ*ppk2C*), C) 3h Δ*phb* cell lacking both polyhydroxyalkanoate synthases, Δ*(phaC1-phaC2)*. D) 3h Δ*(p)ppGpp* cell lacking both (p)ppGpp synthases, Δ*relA*Δ*spoT*. E) 24h WT cells. F) 24h, ΔpolyP cells. G) 24h, Δ*phb* cells. H) 24h, Δ*(p)ppGpp* cell. I) 3h WT cell fixed with paraformaldehyde before drying on EM grid. J) 3h triple mutant cell Δ*ppk2A*Δ*ppk2B*Δ*ppk2C*. K) 3h triple mutant cell Δ*ppk1*Δ*ppk2B*Δ*ppk2C*. L) 3h Δ*ppk1*Δ*ppk2A* double mutant cell. M) 6h WT cell showing measurement of cell and granule dimensions. N) 6h WT cell showing measurement with satellite polyP granules.

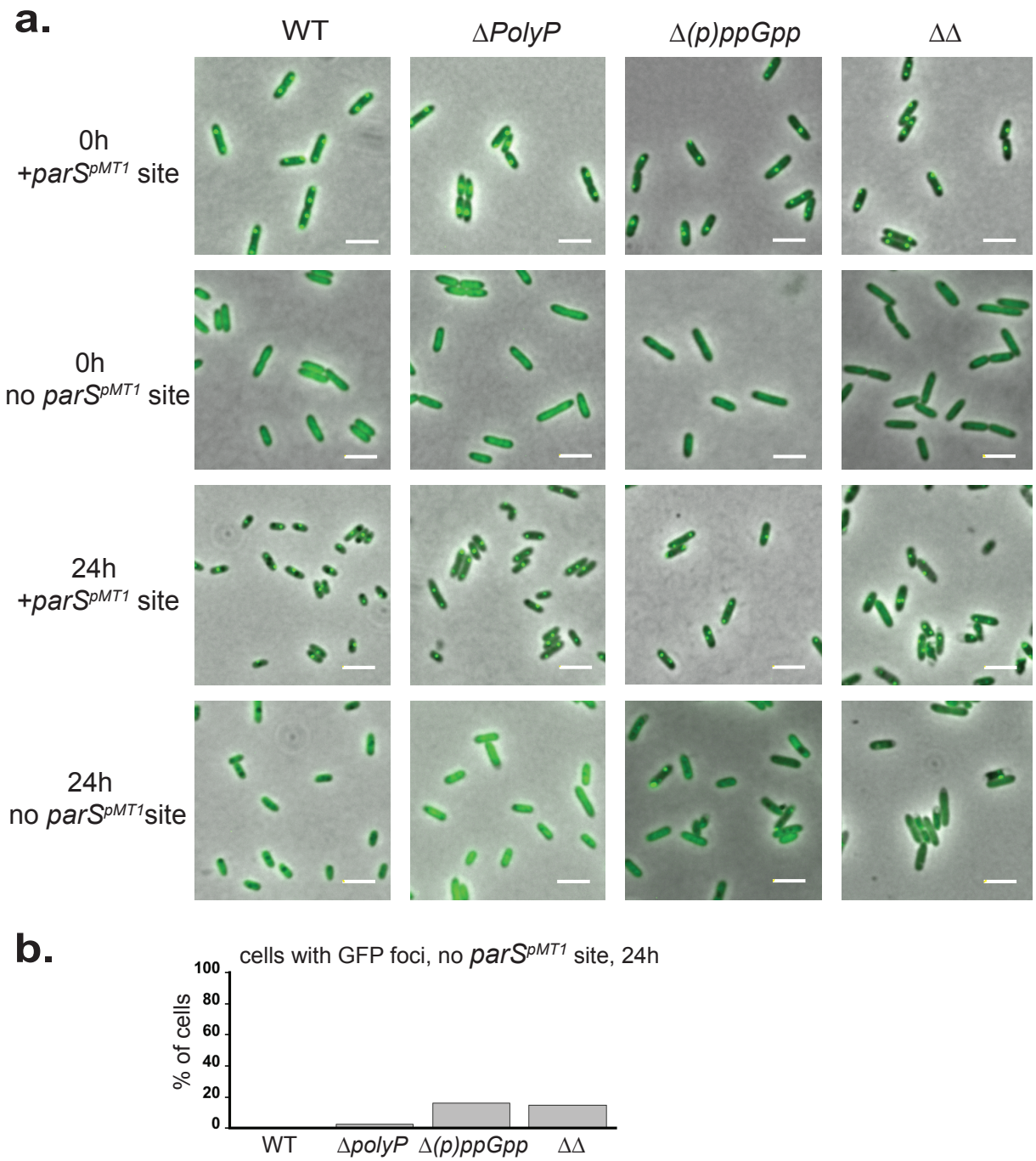

Figure S3. Control for specificity of GFP-ParB^{pMT1}-*parS*^{pMT1}-interaction. Scale bar = 2 μm A) Top Panel: All four strains carrying both GFP-ParB^{pMT1} under control of the SSB promoter and *parS*^{pMT1} at the *att*T7 site. Exponential phase cells grown in MOPS minimal media. 2nd Panel: All four strains carrying GFP-ParB^{pMT1} under control of the SSB promoter at the *att*T7 site, without a *parS*^{pMT1} site, exponential phase. 3rd Panel: All four strains carrying both GFP-ParB^{pMT1} under control of the SSB promoter and *parS*^{pMT1} at the *att*T7 site. 24h nitrogen-starved cells. Bottom Panel: All four strains carrying GFP-ParB^{pMT1} under control of the SSB promoter at the *att*T7 site, without a *parS*^{pMT1} site. 24h nitrgen-starved cells. B) Fraction of cells lacking the *parS*^{pMT1} site with GFP foci after 24h nitrogen starvation (As in 3rd panel of A).

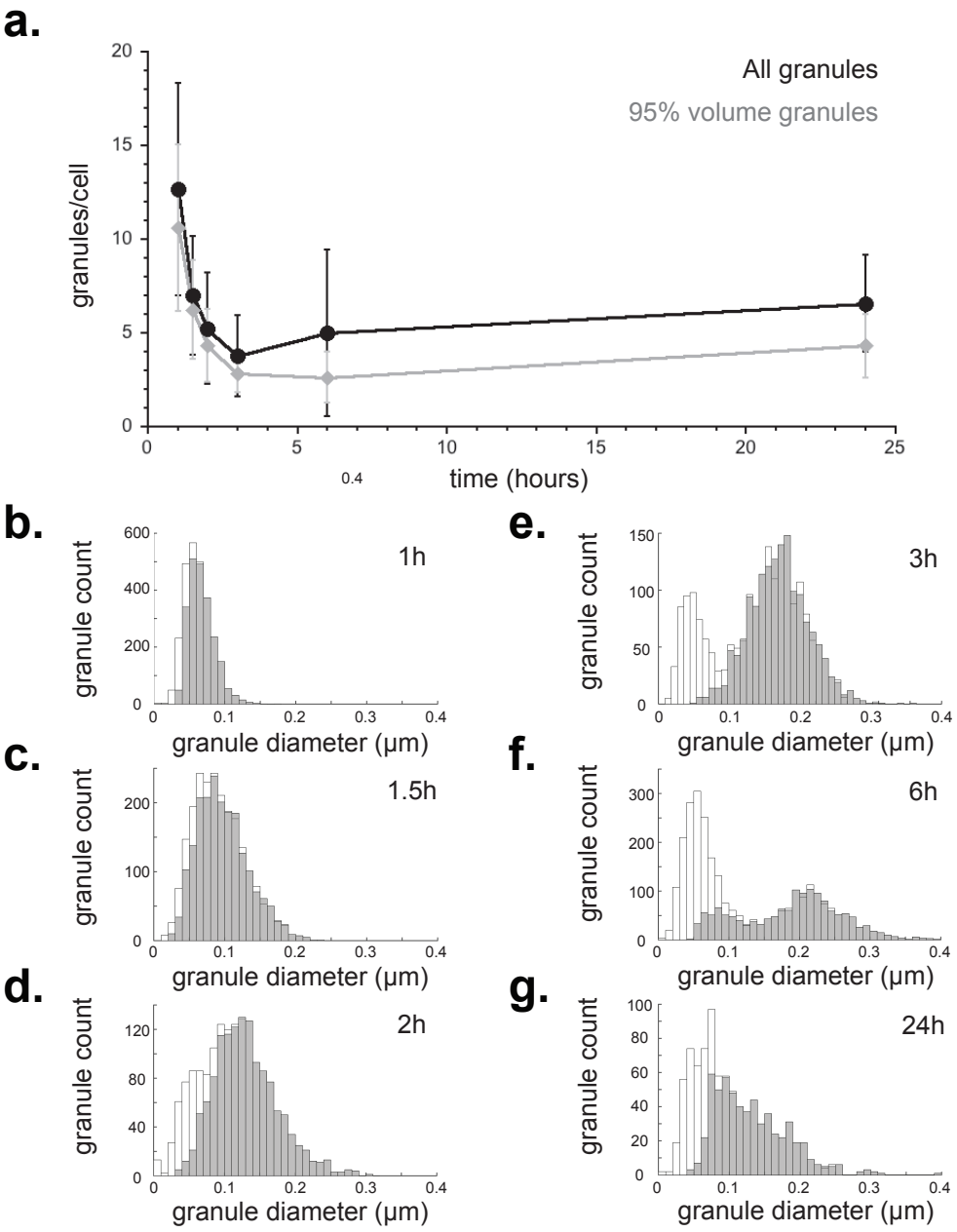

Figure S4. Changes in granule number and size. A) Total number of granules per cell. All granules shown in black circles, granules constituting 95% of total granular volume per cell in grey diamonds. Granule diameter for all granules (white bars) and granules constituting 95% of total granular volume (grey bars). B) 1h, mean granule diameter for all granules of 0.062± 0.02 µm. C) mean granule diameter for all granules of 1.5h, 0.092±.037 µm. D) 2h, mean granule diameter for all granules of 0.115± .052 µm. E) 3h, mean granule diameter for all granules, bimodal distribution, 0.052±.019 µ m and 0.188±.050 µm. F) 6h, mean granule diameter for all granules, bimodal distribution 0.056±.020 µm and 0.21±.060 µm; G) 24h, 0.104±.057 µm.

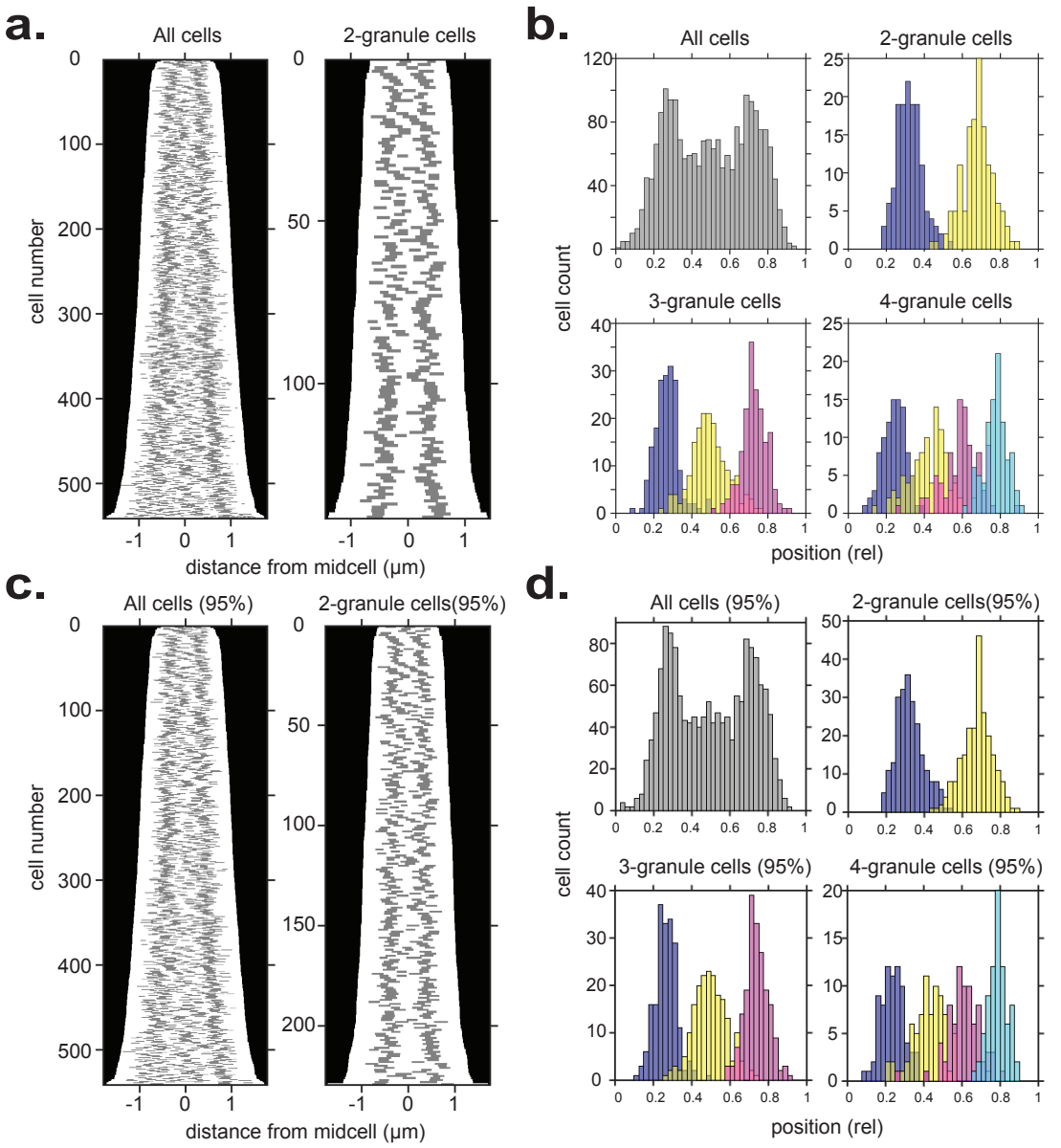

Figure S5. Granule spacing on long axis of the cell. A) Left: Demograph of all granules in 3h-starved cells imaged by TEM. Right: Demograph of all 2-granule cells in 3h-starved cells. B) Histogram of relative position of granules along long axis of cells imaged by TEM as in (A). Top Left: All cells, Top Right: 2-granule cells, 1st granule 0.32±0.07, 2nd granule 0.68±0.08, Bottom Left: 3-granule cells, 1st granule 0.27±0.06, 2nd granule 0.49±0.10, 3rd granule 0.73±0.06. Bottom Right: 4-granule cells, 1st granule 0.24±0.1, 2nd granule 0.42±0.10, 3rd granule 0.60±0.10, 4th 0.78±0.06 C) As in (A), but imaging only size-ranked granules contributing to 95% or more of total granular volume per cell. D) As in (B), but for size-ranked granules contributing to 95% or more of total granular volume per cell. Top Left: All cells. Top Right: 2-granule cells, 1st granule 0.24±0.06, 2nd granule 0.68±0.08, Bottom Left: 3-granule cells, 1st granule 0.26±0.06, 2nd granule 0.50±0.09, 3rd granule 0.74±0.06, Bottom Right: 4-granule cells, 1st granule 0.24±0.06, 2nd granule 0.43±0.09, 3rd granule 0.61±0.08, 4th granule 0.78±0.05.

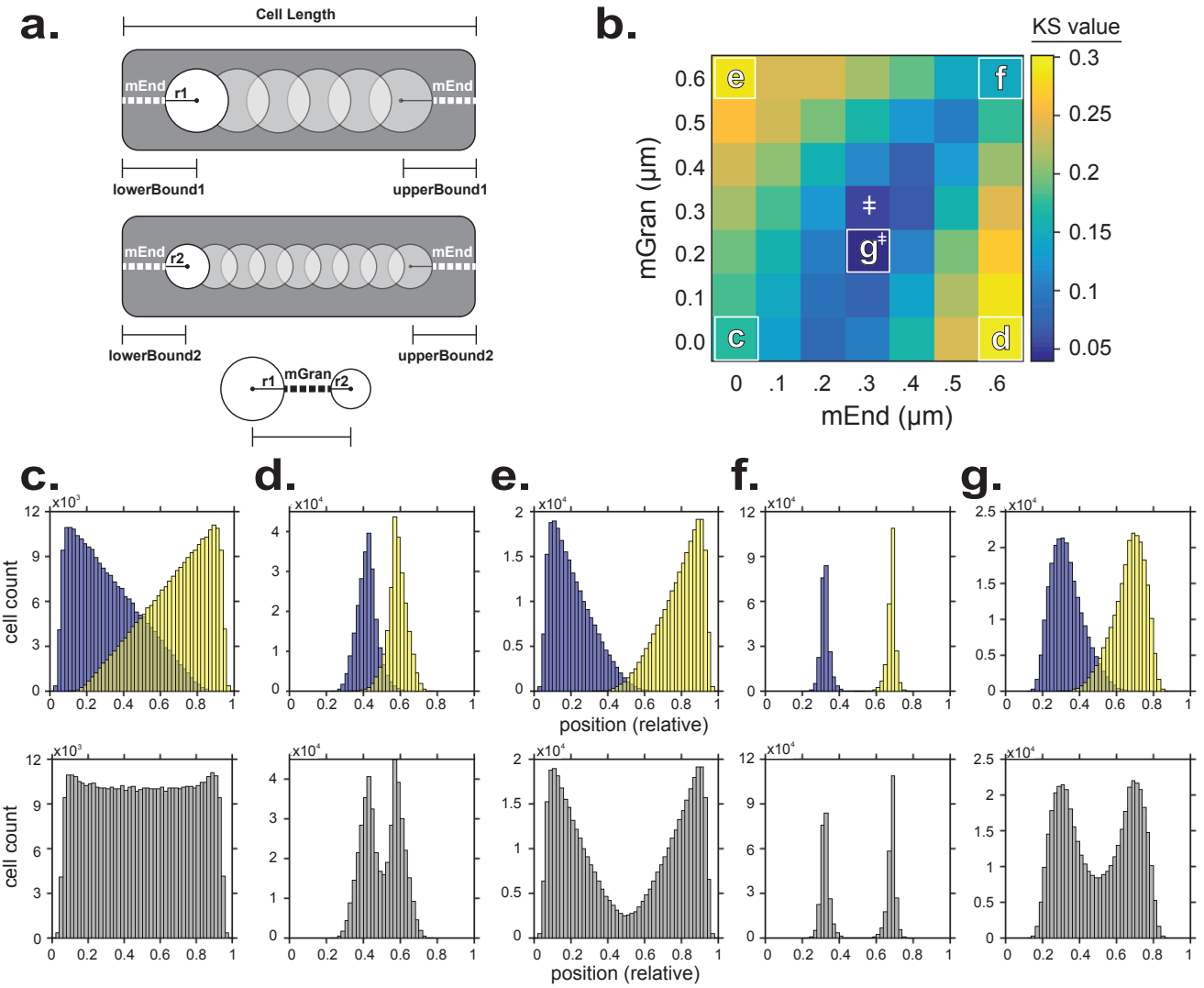

Figure S6. Simulation of polyP granule positioning on long axis of the cell. A) Model for the simulation. mEnd = user-defined constraint on the minimum distance (in μm) from cell ends. For the first granule, lowerBound1 and upperBound1 defined by the sum of the radius of granule 1 (r1) and a user-defined minimum distance for granules from cell ends (mEnd). The allowed region for the origin of granule 1: between lowerBound and (cell Length minus upperBound). For the second granule, lowerBound2 and upperBound2 defined by the sum of the radius of granule 2 (r2) and the user-defined minimum distance for granules from cell ends. A user-defined minimum distance between granules (mGran) constrains the origins of granules 1 and 2 to be at least r1+mGran+r2 apart. B) Heat map of KS-test statistic from comparing the experimentally observed distribution of granules in 2-granule cells to a simulation of randomly positioning granules along the long axis of the cell, with two added constraints: First, a minimum distance between granules and cell ends (mEnd) and second, a minimum distance between 1st and 2nd granule (mGran). Symbol ǂ indicates parameter space where the model is statistically indistinguishable from the data. C) Top panel: Histogram of relative granule positions (blue for granule 1, yellow for granule 2) along long axis of cells from a simulation of 229,000 cells in which the only constraint is that granules cannot overlap along long axis of the cell (mEnd = 0 μm, mGran = 0 μm). Bottom panel: Histogram of combined granule 1 and granule 2 relative granule relative positions, this combined distribution was used for KS-testing. D) As in (C), but mEnd = 0.6 μm, mGran = 0 μm, E) As in (C), but mEnd = 0 μm, mGran = 0.6 μm. F) As in (C), but mEnd = 0.6 μm, mGran = 0.6 μm. G) As in (C), but mEnd = 0.3 μm, mGran = 0.2 μm.

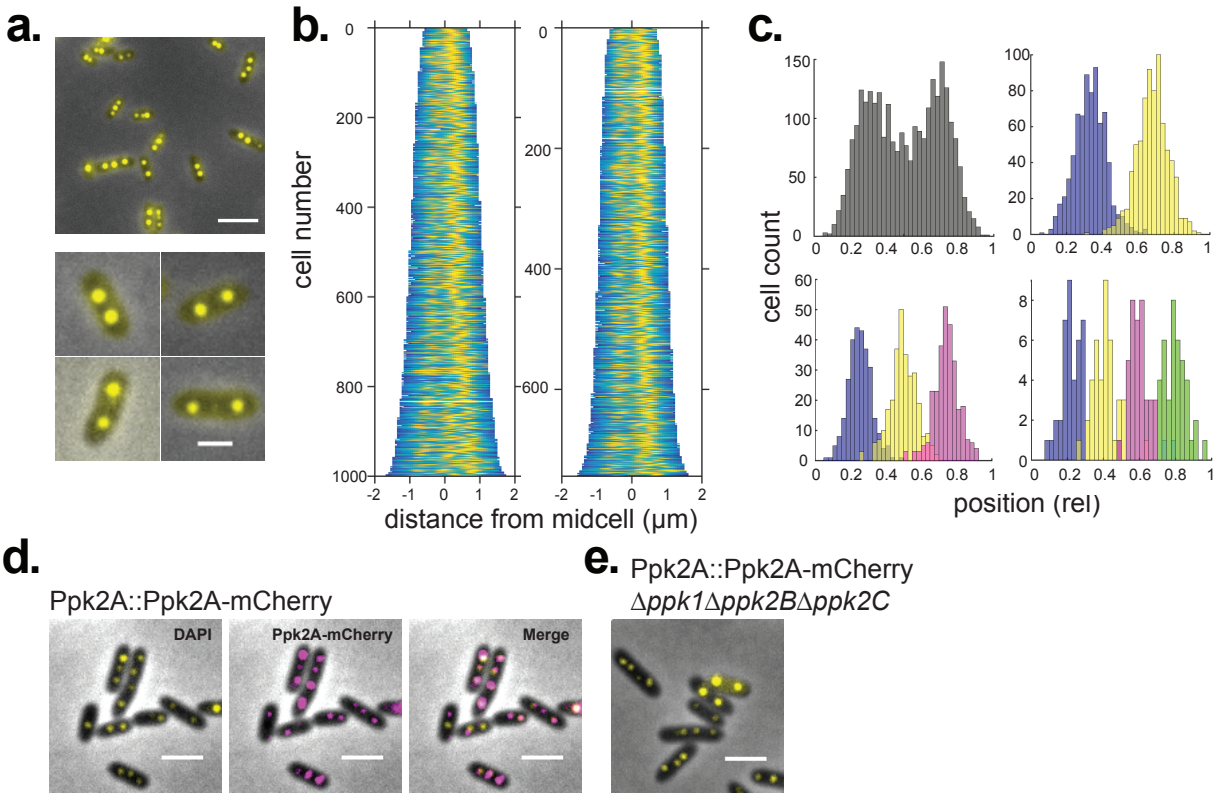

Figure S7. Granule spatial organization imaged by DAPI fluorescence. A) Top panel: Granules stained with DAPI in 3h-starved cells (scale bar 3 μm). Bottom panel: Example cells with 2 granules (scale bar 2 μm). B) Left panel: Demograph of DAPI fluorescence in 3h-starved cells; Right panel: Demograph of DAPI fluorescence in 3h-starved 2-granule cells, C) Histograms of relative position of granules along the long axis of cells imaged by DAPI in 3h-starved cells. Top left panel: all granules, Top right panel: 2-granule cells, 1st granule 0.32±.08 (blue), 2nd granule 0.67±.09 (yellow); Bottom left panel: 3-granule cells, 1st granule 0.25±.07 (blue), 2nd granule 0.50±.08 (yellow), 3rd granule 0.74±.08 magenta; Bottom right panel: 4-granule cells, 1st granule 0.23±.07 (blue), 2nd granule 0.41±.07 (yellow), 3rd granule 0.60±.06 (magenta), 4th granule 0.80±.06 (green). D) Granules imaged with DAPI (yellow) and co-localization with Ppk2A-mCherry chimera (magenta). Scale bar 2 μm. E) Ppk2A::Ppk2A-mCherry imaging in cells lacking the other three known polyphosphate kinases (Δ*ppk1* Δ*ppk2B*Δ*ppk2C*). Scale bar 2 μm.

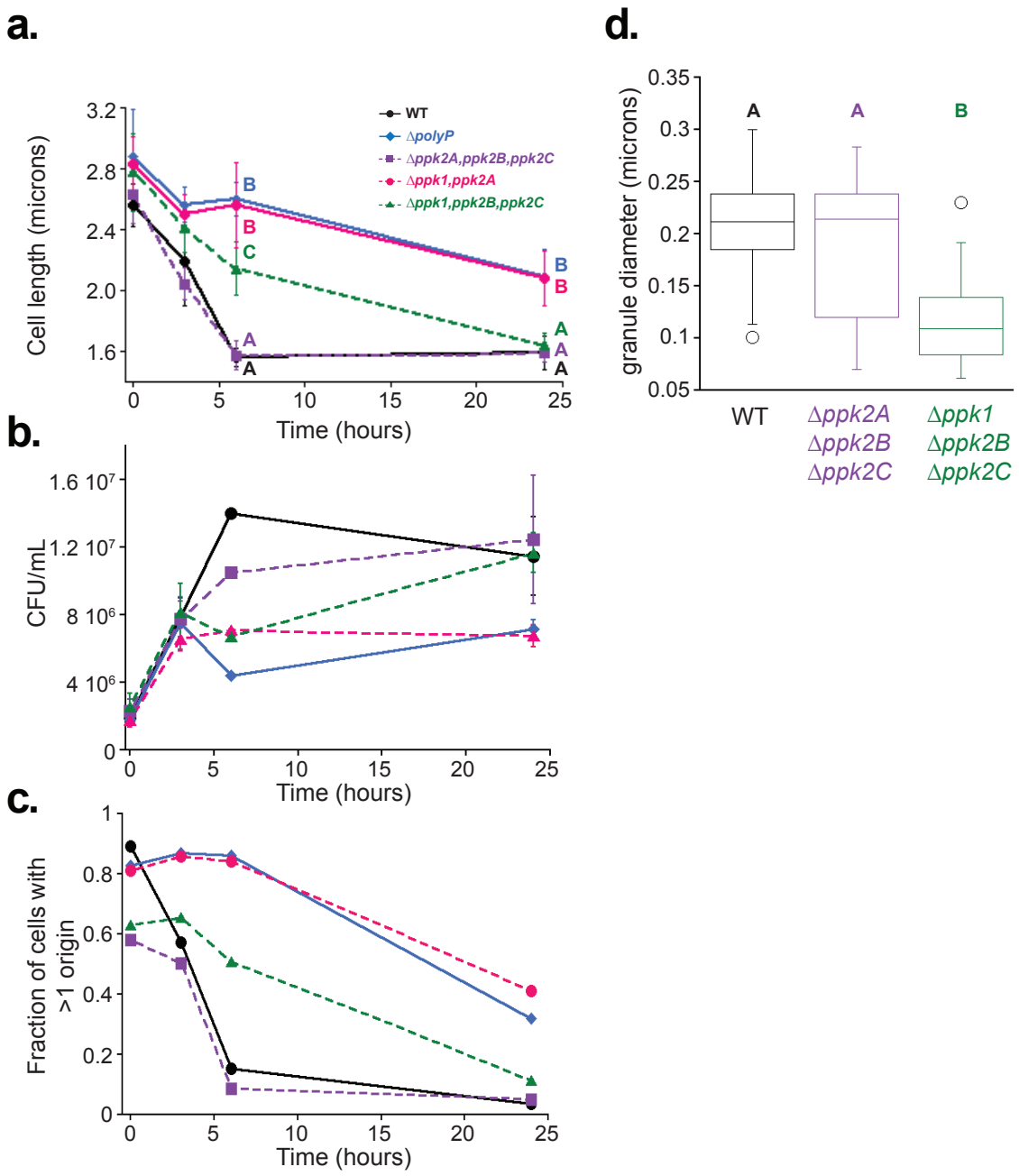

Figure S8. Effects of individual polyphosphate kinases on cell cycle exit. WT black circles; Δ*polyP* (Δ*ppk1*Δ*ppk2A*Δ*ppk2B*Δ*ppk2C*) blue diamonds; Δ*ppk2A*Δ*ppk2B*Δ*ppk2C* purple squares/-dashed line; Δ*ppk1*Δ*ppk2A* magenta circles/dashed line; Δ*ppk1*Δ*ppk2B*Δ*ppk2C* green triangles/-dashed line. A). Average cell length as a function of time after induction of nitrogen starvation. Mean and SD from at least 3 independent experiments, variance analyzed using a one-way ANOVA. Significant differences between strains at the same timepoint are marked with upper case letters based on a post hoc Tukey test. Strains at the same timepoint marked with different letters have significantly different means, $P < 0.05$. B) Cell counts (CFU) after the shift to nitrogen-limited medium (note that 6h timepoint does not have error bars because it was not done in triplicate). C) Fraction of cells with >1 GFP-ParB^{pMT1} focus as a function of time after induction of nitrogen starvation. D) Granule diameter at 3h nitrogen-starved cells. Box represents two middle quartiles separated by the median. Means: WT 0.207±.043 μm, Δ*ppk2A*Δ*ppk2B*Δ*ppk2C* 0.185±.069, Δ*ppk1*Δ*ppk2B*Δ*ppk2C* 0.117±0.041. Variance analyzed using a one-way ANOVA, significant differences between strains are marked with upper case letters based on a post hoc Tukey test. Strains marked with different letters have significantly different means, $P < 0.05$.

Table S1: Strains

Name	Genotype	Source
DKN263	*P. aeruginosa* UCBPP-PA14	
DKN303	*E. coli* DH5α (F− ***Δ****(argF-lac)169* ***Φ****80dlacZ58(****Δ****M15) glnV44(AS)* ***λ****− rfbC1 gyrA96(NalR) recA1 endA1 spoT1 thi-1 hsdR17 deoR)*, pMQ30	(2)
DKN546	*E. coli* DH5α (F− ***Δ****(argF-lac)169* ***Φ****80dlacZ58(****Δ****M15) glnV44(AS)* ***λ****− rfbC1 gyrA96(NalR) recA1 endA1 spoT1 thi-1 hsdR17 deoR*), pMQ70	(2)
DKN548	*E. coli* DH5α (F− ***Δ****(argF-lac)169* ***Φ****80dlacZ58(****Δ****M15) glnV44(AS)* ***λ****− rfbC1 gyrA96(NalR) recA1 endA1 spoT1 thi-1 hsdR17 deoR*), pMQ72	(2)
DKN1297	*E. coli* DH5α (F− ***Δ****(argF-lac)169* ***Φ****80dlacZ58(****Δ****M15) glnV44(AS)* ***λ****− rfbC1 gyrA96(NalR) recA1 endA1 spoT1 thi-1 hsdR17 deoR*), pUC18R6K-mini-Tn7T-Gm	(3)
DKN1298	SM10, pTNS1	(3)
DKN1299	HB101 (F− ***λ****− ****Δ****(gpt-proA)62 leuB6 glnV44(AS) araC14 galK2(Oc) lacY1* ***Δ****(mcrC-mrr) rpsL20(StrR) xylA5 mtl-1 recA13 hsdS20*), pRK2013 pRK2013 has a *ColE1* replicon and carries the *RK2 tra* genes and Tn903 (which is *KanR*)	(3)
LR51;DKN1723	*P. aeruginosa* UCBPP-PA14 Δ*(phaC1-* Δ*phaC2)*; deletion of PA14_66820, PA14_66830, PA14_66840 using pLREX24	This study
LR60;DKN1724	*P. aeruginosa* UCBPP-PA14 Δ*ppk2A*; deletion of PA14_01730 in DKN263 using pLREX25	This study
LR49;DKN1725	*P. aeruginosa* UCBPP-PA14 Δ*ppk2B*; deletion of PA14_33240 in DKN263 using pLREX21	This study
LR50;DKN1726	*P. aeruginosa* UCBPP-PA14 Δ*ppk2C*; deletion of PA14_19410 in DKN263 using pLREX23	This study
LR105;DKN1727	*P. aeruginosa* UCBPP-PA14 Δ*ppk1*; deletion of PA14_69230 in DKN263	This study

	using pLREX27	
LR110;DKN1728	*P. aeruginosa* UCBPP-PA14 Δ*ppk1* Δ*ppk2A*; deletion of PA14_69230 and PA14_1730 in DKN263	This study
LR135;DKN1729	*P. aeruginosa* UCBPP-PA14 Δ*ppk1* Δ*ppk2B* Δ*ppk2C*; deletion of PA14_69230, PA14_33240, and PA14_19410 in DKN263	This study
LR79;DKN1730	*P. aeruginosa* UCBPP-PA14 Δ*ppk2A* Δ*ppk2B* Δ*ppk2C*; deletion of PA14_01730, PA14_33240, and PA14_19410 in DKN263	This study
LR119;DKN1731	*P. aeruginosa* UCBPP-PA14 Δ*ppk1* Δ*ppk2A* Δ*ppk2B* Δ*ppk2C*; deletion of PA14_69230, PA14_01730, PA14_33240, and PA14_19410 in DKN263	This study
LR171;DKN1732	*P. aeruginosa* UCBPP-PA14 Δ*relA*	This study
LR178;DKN1733	*P. aeruginosa* UCBPP-PA14 Δ*relA* Δ*spoT*	This study
LR193;DKN1734	*P. aeruginosa* UCBPP-PA14 Δ*ppk1* Δ*ppk2A* Δ*ppk2B* Δ*pp2C* Δ*relA* Δ*spoT*	This study
LR176;DKN1735	*P. aeruginosa* UCBPP-PA14 *att*Tn7:: mini-Tn7T-Gm^{R} P_{ssb}*:ssb*-mCherry	This study
LR177;DKN1736	*P. aeruginosa* UCBPP-PA14 Δ*ppk1* Δ*ppk2A* Δ*ppk2B* Δ*ppk2C att*Tn7:: mini-Tn7T-Gm^{R} P_{ssb}*:ssb*-mCherry	This study
LR190;DKN1737	*P. aeruginosa* UCBPP-PA14 Δ*relA* Δ*spoT* attTn7:: mini-Tn7T-Gm^{R} P_{ssb}*:ssb*-mCherry	This study
LR207;DKN1738	*P. aeruginosa* UCBPP-PA14 Δ*ppk1* Δ*ppk2a* Δ*ppk2b* Δ*ppk2c* Δ*relA* Δ*spoT* *att*Tn7:: mini-Tn7T-Gm^{R} P_{ssb}*:ssb*-mCherry	This study
	P. aeruginosa UCBPP-PA14 *att*Tn7:: mini-Tn7T-Gm^{R} P_{ssb}*:*GFP-$parB^{pMT1}$	This study
LR209;DKN1739	*P. aeruginosa* UCBPP-PA14 attTn7:: mini-Tn7T-Gm^{R} $parS^{pMT1}$ P_{ssb}*:* GFP-$parB^{pMT1}$	This study
	P. aeruginosa UCBPP-PA14 Δ*ppk1* Δ*ppk2A* Δ*ppk2B* Δ*ppk2C att*Tn7:: mini-Tn7T-Gm^{R} P_{ssb}*:*GFP-$parB^{pMT1}$	This study
LR210;DKN1740	*P. aeruginosa* UCBPP-PA14 Δ*ppk1* Δ*ppk2A* Δ*ppk2B* Δ*ppk2C att*Tn7:: mini-Tn7T-Gm^{R} $parS^{pMT1}$ P_{ssb}*:* GFP-$parB^{pMT1}$	This study

	P. aeruginosa UCBPP-PA14 Δ*relA* Δ*spoT att*Tn7:: mini-Tn7T-Gm^{R} P_{ssb}:GFP-*parB*^{pMT1}	This study
LR213;DKN1741	*P. aeruginosa* UCBPP-PA14 Δ*relA* Δ*spoT* attTn7:: mini-Tn7T-Gm^{R} *parS*^{pMT1} P_{ssb}: GFP-*parB*^{pMT}	This study
	P. aeruginosa UCBPP-PA14 Δ*ppk1* Δ*ppk2A* Δ*ppk2B* Δ*pp2C* Δ*relA* Δ*spoT* attTn7:: mini-Tn7T-Gm^{R} P_{ssb}: GFP-*parB*^{pMT1}	This study
LR214;DKN1742	*P. aeruginosa* UCBPP-PA14 Δ*ppk1* Δ*ppk2A* Δ*ppk2B* Δ*pp2C* Δ*relA* Δ*spoT* attTn7:: mini-Tn7T-Gm^{R} *parS*^{pMT1} P_{ssb}: GFP-*parB*^{pMT1}	This study
LR116;DKN1743	*P. aeruginosa* UCBPP-PA14 *ppk2A::ppk2A*-mCherry	This study
LR151;DKN1744	*P. aeruginosa* UCBPP-PA14 *ppk2A::ppk2A*-mCherry Δ*ppk1* Δ*ppk2B* Δ*ppk2C*	This study
LR216;DKN1745	*P. aeruginosa* UCBPP-PA14 *ppk2A::ppk2A*-mCherry *att*Tn7:: mini-Tn7T-Gm^{R} *parS*^{pMT1} P_{ssb}: GFP-*parB*^{pMT}	This study
LR263;DKN1746	*P. aeruginosa* UCBPP-PA14 *att*Tn7:: mini-Tn7T-Gm^{R} P_{bad} Flag-*ppK2A* *parS*^{pMT1} P_{ssb}:*ssb*-mCherry GFP-*parB*^{pMT1}	This study
LR258;DKN1747	*P. aeruginosa* UCBPP-PA14 *att*Tn7:: mini-Tn7T-Gm^{R} P_{bad} Flag-*ppk2A*$^{D183A\ R194A}$ *parS*^{pMT1} P_{ssb}:*ssb*-mCherry GFP-*parB*^{pMT1}	This study
LR262;DKN1748	*P. aeruginosa* UCBPP-PA14 *att*Tn7:: mini-Tn7T-Gm^{R} P_{bad} His-TEV-*ppk2B parS*^{pMT1} P_{ssb}:*ssb*-mCherry GFP-*parB*^{pMT1}	This study
LR107;DKN1749	*E. coli* TOP10(F– *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX9	This study
LR42;DKN1750	*E. coli* TOP10(F– *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX15	This study
LR48;DKN1751	*E. coli* TOP10(F– *mcr*A Δ(*mrr-*	This study

	*hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX21	
LR55;DKN1752	*E. coli* TOP10(F– *mcr*A Δ(*mrr*-*hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX23	This study
LR53;DKN1753	*E. coli* TOP10(F– *mcr*A Δ(*mrr*-*hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX24	This study
LR52;DKN1754	*E. coli* TOP10(F– *mcr*A Δ(*mrr*-*hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX25	This study
LR86;DKN1755	*E. coli* TOP10(F– *mcr*A Δ(*mrr*-*hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX27	This study
LR169;DKN1756	*E. coli* TOP10(F– *mcr*A Δ(*mrr*-*hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX35	This study
LR170;DKN1757	*E. coli* TOP10(F– *mcr*A Δ(*mrr*-*hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX36	This study
LR173;DKN1758	*E. coli* TOP10(F– *mcr*A Δ(*mrr*-*hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX38	This study
LR211;DKN1759	*E. coli* TOP10(F– *mcr*A Δ(*mrr*-*hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX49	This study
LR227;DKN1760	*E. coli* TOP10(F– *mcr*A Δ(*mrr*-*hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15	This study

	Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX60	
LR228;DKN1761	*E. coli* TOP10(F– *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX61	This study
LR229;DKN1762	*E. coli* TOP10(F– *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX62	This study
LR245;DKN1763	*E. coli* TOP10(F– *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX63	This study
LR282;DKN1764	*E. coli* TOP10(F– *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX64	This study
LR283;DKN1765	*E. coli* TOP10(F– *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX65	This study
LR284;DKN1766	*E. coli* TOP10(F– *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pLREX66	This study
LR285; DKN1767	*E. coli* TOP10(F– *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pFHC3228	(4)
LR286; DKN1768	*E. coli* TOP10(F– *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G), pFHC2973	(4)

Table S2: Plasmids

Name	Genotype/Purpose		Source
pMQ30	Allelic replacement vector	Gm^R	(2)
pMQ70	Expression vector with P_{ara} and *araC*	Amp^R	(2)
pMQ72	Expression vector with P_{ara} and *araC*	Gm^R	(2)
pUC18R6K-mini-Tn7T-Gm	Mobilizable mini-Tn7 base vector with MCS	Gm^R	(3)
pFHC3228	Source of $parS^{pMT1}$ DNA binding sequence	Amp^R	(4)
pFHC2973	Source of GFP-$ParB^{pMT1}$	Amp^R	(4)
pLREX9	*ppk2A(*PA14_01730)*::ppk2A*-mCherry; pMQ30 derivative	Gm^R	This study
pLREX15	P_{ara} His-TEV-*ppK2B* (PA14_33240) expression vector; pMQ72 derivative	Gm^R	This study
pLREX21	*ppk2B (*PA14_33240) deletion vector; pMQ30 derivative	Gent	This study
pLREX23	*ppk2C* (PA14_19410) deletion vector; pMQ30 derivative	Gent	This study
pLREX24	*(phaC1-phaC2)* (PA14_66820, PA14_66830, PA14_66840) deletion vector; pMQ30 derivative	Gent	This study
pLREX25	*ppk2A* (PA14_01730)deletion vector; pMQ30 derivative	Gent	This study
pLREX27	*ppk1* (PA14_69230) deletion vector; pMQ30 derivative	Gent	This study
pLREX35	*spoT* deletion vector; pMQ30 derivative	Gm^R	This study
pLREX36	*relA* deletion vector; pMQ30 derivative	Gm^R	This study
pLREX38	pUC18T-mini-Tn7T-G^R P_{ssb} *ssb*-mCherry*;* pUC18R6K-mini-Tn7T-Gm derivative	Gm^R	This study
pLREX49	pUC18T-mini-Tn7T-G^R $parS^{pMT1}$ P_{ssb} GFP-$parB^{pMT1}$*;*	Gm^R	This study
pLREX60	P_{ara} Flag-*ppk2A* (PA14_01730) expression vector; pMQ70 derivative	Amp^R	This study
pLREX61	P_{ara} Flag-$ppk2A^{D183A,R184A}$ *(*PA14_01730) expression vector; pMQ70 derivative	Amp^R	This study
pLREX62	pUC18T-mini-Tn7T-G^R $parS^{pMT1}$ P_{ssb} *ssb*-mCherry GFP-$parB^{pMT1}$*;*	Gm^R	This study

	pUC18R6K-mini-Tn7T-Gm derivative		
pLREX63	pUC18T-mini-Tn7T-G^R P_{ssb} *ssb*-mCherry GFP-*parB* ^{pMT1}; pUC18R6K-mini-Tn7T-Gm derivative	Gm^R	This study
pLREX64	pUC18T-mini-Tn7T-G^R P_{ara} Flag-*ppk2A*(PA14_01730) $ParS^{pMT1}$ P_{ssb} *ssb*-mCherry GFP-*parB* ^{pMT1}; pUC18R6K-mini-Tn7T-Gm derivative	Gm^R	This study
pLREX65	pUC18T-mini-Tn7T-G^R P_{ara} Flag-*ppk2A*^{D183A,R184A} (PA14_01730) *parS*^{pMT1} P_{ssb} *ssb*-mCherry GFP-*parB* ^{pMT1}; pUC18R6K-mini-Tn7T-Gm derivative	Gm^R	This study
pLREX66	pUC18T-mini-Tn7T-G^R P_{ara} Flag-*ppk2B* (PA14_33240) *parS*^{pMT1} P_{ssb} *ssb*-mCherry GFP-*parB* ^{pMT1}; pUC18R6K-mini-Tn7T-Gm derivative	Gm^R	This study

Table S3: Primers

Name	Purpose	Sequence
LRPR20	Construction of pLREX27, *ppk1* deletion vector	GTAAAACGACGGCCAGTGCCAAGCTTcctacacccgcctgcggcgcaat
LRPR23	Construction of pLREX27, *ppk1* deletion vector	ATGATTACGAATTCGAGCTCGGTACCagcctgcagatgggttgcgtcag
LRPR44	Construction of pLREX25, *ppk2A* deletion vector	GTAAAACGACGGCCAGTGCCAAGCTTgctgtcggcgaggatggtct
LRPR45	Construction of pLREX21, *ppk2A* deletion vector	ATGATTACGAATTCGAGCTCGGTACCatggccatcgagaacttcat
LRPR54	Construction of pLREX21, *ppk2B* deletion vector	GTAAAACGACGGCCAGTGCCAAGCTTgtcagcggctacgtgaccaatct
LRPR55	Construction of pLREX21, *ppk2B* deletion vector	ATGATTACGAATTCGAGCTCGGTACCtgcgttcttcaggctccagt
LRPR56	Construction of pLREX21, *ppk2B* deletion vector	agcccggcgtccggccgaacggccagcgctgctctccacgtcatgccgaaat
LRPR57	Construction of pLREX23, *ppk2B* deletion vector	atttcggcatgacgtggagagcagcgctggccgttcggccggacgccgggct
LRPR64	Construction of pLREX23, *ppk2C* deletion vector	GTAAAACGACGGCCAGTGCCAAGCTTcgttcgatcagggtcacctg
LRPR65	Construction of pLREX23, *ppk2C* deletion vector	ATGATTACGAATTCGAGCTCGGTACCccggccacggtgaagccacggaa gaa
LRPR66	Construction of pLREX23, *ppk2C* deletion vector	gacggcgccgcattccggcaggccggaggtctcccttaacctgagt
LRPR67	Construction of pLREX23, *ppk2C* deletion vector	actcaggttaagggagacctccggcctgccggaatgcggcgccgtc
LRPR82	Construction of pLREX60, P_{ara} Flag-*ppk2A*	GGCTGAAAATCTTCTCTCATCCGCCAAAACAGCCAAGCTTT CAGGCCGGGATATCCAGGT
LRPR83	Construction of pLREX15, P_{ara} Flag-*ppk2B*	CATCACCATCACCATCACGAAAATTTATATTTTCAGGGTAT GGACTCCTATGGCGATAC
LRPR84	Construction of pLREX15, P_{ara} *Flag-ppk2b*	GGCTGAAAATCTTCTCTCATCCGCCAAAACAGCCAAGCTTT CAATAGACTTCCGGCACGA
LRPR227	Construction of pLREX25, *ppk2A* deletion vector	GAAAACGTAGAACGGAAGGAGCCCCGCGGCGGGCGGTCGC GCCAACGAAAACGC
LRPR228	Construction of pLREX25, *ppk2A* deletion vector	GCGTTTTCGTTGGCGCGACCGCCCGCCGCGGGGCTCCTTCC GTTCTACGTTTTC
LRPR234	Construction of pLREX24, *(phaC1-phaC2)* deletion vector	GTAAAACGACGGCCAGTGCCAAGCTTCAGCCGACCTGGCC AGCGAGCATAG
LRPR235	Construction of pLREX24, *(phaC1-phaC2)* deletion vector	CGCCTCGAACAATGGAGCGTTGCCGAAGACCCGGCCCGGG CGCCTGGAGC
LRPR236	Construction of pLREX24, *(phaC1-phaC2)* deletion vector	GCTCCAGGCGCCCGGGCCGGGTCTTCGGCAACGCTCCATTG TTCGAGGCG
LRPR237	Construction of pLREX24, *(phaC1-phaC2)* deletion vector	ATGATTACGAATTCGAGCTCGGTACCCAGCCAAGGCTGCGG CGAAGCCTGC
LRPR238	Construction of pLREX60, P_{ara} Flag-*ppk2A*	GGCTAGCGAATTCGAGCTCGGTACCAGGAGGATATACATAT GATGGACTACAAGGACGAT
LRPR271	Construction of pLREX27, *ppk1* deletion vector	TtgacccctcgggaagatgaGTAACCAGAACCCCCGCAATACCCAGG
LRPR272	Construction of pLREX27, *ppk1* deletion vector	CCTGGGTATTGCGGGGGTTCTGGTTACtcatcttcccgaggggtcaA
LRPR292	Construction of pLREX61, P_{ara} Flag- $ppk2A^{D183A,R184A}$	GAGATGGTCTTCTTCGCCGCCTCCTGGTACAACCGC
LRPR293	Construction of pLREX61, P_{ara} Flag- $ppk2A^{D183A,R184A}$	GCGGTTGTACCAGGAGGCGGCGAAGAAGACCATCTC

LRPR349	Construction of pLREX36, *relA* deletion vector	CTACGCGCACCGTGGTAAAGGGTAGGCAAGGGCGAGGCGGAAACAGGCCACGGGCGCTCG
LRPR350	Construction of pLREX36, *relA* deletion vector	CGAGCGCCCGTGGCCTGTTTCCGCCTCGCCCTTGCCTACCCTTTACCACGGTGCGCGTAG
LRPR351	Construction of pLREX36, *relA* deletion vector	GTAAAACGACGGCCAGTGCCAAGCTTCCGCGCTCGGATCGCCGTGCGCTGGG
LRPR352	Construction of pLREX36, *relA* deletion vector	GAATCAGCCGAAGATCCAACGATAGAGGTACCGAGCTCGAATTCGTAATCATG
LRPR353	Construction of pLREX35, *spoT* deletion vector	GGGTGAACCCTTGCCGGGCATACGCAGCTGACCCGCTTTTTCCTGTGTCATC
LRPR354	Construction of pLREX35, *spoT* deletion vector	GATGACACAGGAAAAAGCGGGTCAGCTGCGTATGCCCGGCAAGGGTTCACCC
LRPR355	Construction of pLREX35, *spoT* deletion vector	GTTGTAAAACGACGGCCAGTGCCAAGCTTCTCCGGCGCCGGCAAGACCAGCCTG
LRPR356	Construction of pLREX35, *spoT* deletion vector	ATGATTACGAATTCGAGCTCGGTACCGACCAGGCCATTGGCCTGGAACTC
LRPR389	Construction of pLREX38, *att*Tn7:: mini-Tn7T-Gm^{R} P_{ssb} *ssb*-mCherry	AGCTAATTCGATCATGCATGAGCTCACTAGTTACTTGTACAGCTCGTCCATGCCGCCGG
LRPR390	Construction of pLREX38, *att*Tn7:: mini-Tn7T-Gm^{R} P_{ssb} *ssb*-mCherry	AGGCCTTCGCGAGGTACCGGGCCCAAGCTTTGTGGGTACGCGCCCCACGAATCAGG
LRPR391	Construction of pLREX38, *att*Tn7:: mini-Tn7T-Gm^{R} P_{ssb} *ssb*-mCherry	CTTGCTCACGCCCGAGCCCCCGCCGCTGCCGCCGAACGGAATGTCGTCGTCGAAGCTGTC
LRPR392	Construction of pLREX38, *att*Tn7:: mini-Tn7T-Gm^{R} P_{ssb} *ssb*-mCherry	CATTCCGTTCGGCGGCAGCGGCGGGGGCTCGGGCGTGAGCAAGGGCGAGGAGGATAAC
LRPR435	Construction of pLREX62, *att*Tn7:: mini-Tn7T-Gm^{R} $parS^{pMT1}$ P_{ssb} *ssb*-mCherry GFP-$parB^{pMT1}$	CGGCATGGACGAGCTGTACAAGTAAAGGAGGATATACATATGTCTAAAGGTGAAGAACTG
LRPR436	Construction of pLREX62, *att*Tn7:: mini-Tn7T-Gm^{R} $parS^{pMT1}$ P_{ssb} *ssb*-mCherry GFP-$parB^{pMT1}$	CAGTTCTTCACCTTTAGACATATGTATATCCTCCTTTACTTGTACAGCTCGTCCATGCCG
LRPR437	Construction of pLREX62, *att*Tn7:: mini-Tn7T-Gm^{R} $parS^{pMT1}$ P_{ssb} *ssb*-mCherry GFP-$parB^{pMT1}$	GAGCGCTTTTGAAGCTAATTCGATCATGCATGAGCTCACTAGTTACTCACCTGATTCTGG
LRPR442	Construction of pLREX49, *att*Tn7:: mini-Tn7T-Gm^{R} $parS^{pMT1}$ P_{ssb} GFP-$ParB^{pMT1}$	GGCCTTCGCGAGGTACCGGGCCCAAGCTTGATCCAGGATGCCGAAGAGCATCCTTTTTTG
LRPR443	Construction of pLREX49, attTn7:: mini-Tn7T-Gm^{R} $parS^{pMT1}$ P_{ssb} GFP-$parB^{pMT1}$	GATCCTGATTCGTGGGGCGCGTACCCACAGCGCGAAATTATGAGTCACGAAGAGGTTG
LRPR444	Construction of pLREX49, attTn7:: mini-Tn7T-Gm^{R} $parS^{pMT1}$ P_{ssb} GFP-$parB^{pMT1}$	CAACCTCTTCGTGACTCATAATTTCGCGCTGTGGGTACGCGCCCCACGAATCAGGATC
LRPR561	oprI qPCR F	AGCAGCCACTCCAAAGAAAC
LRPR562	oprI qPCR R	CAGAGCTTCGTCAGCCTTG
LRPR555	recA qPCR F	GCCCTGGAAATCACCGACAT
LRPR556	recA qPCR R	TTCGATCTCGGCCTTGGGTA
LRPR553	lexA qPCR F	CCAGGAACTCGGCTTCAAGT
LRPR554	lexA qPCR R	TGTTCTGTTCGGCGAGGATC
LRPR611	Construction of pLREX64,	CAGGACGCCCGCCATAAACTGCCAGGCATGATCCAGGATG

	*att*Tn7:: mini-Tn7T-Gm^{R} P_{ara} Flag-*ppk2A* P_{ssb} *ssb*-mCherry GFP-*parB*^{pMT1}	CCGAAGAGCATCCTTTTTTG
LRPR612	Construction of pLREX64, *att*Tn7:: mini-Tn7T-Gm^{R} P_{ara} Flag-*ppk2A* P_{ssb} *ssb*-mCherry GFP-*parB*^{pMT1}	CAAAAAAGGATGCTCTTCGGCATCCTGGATCATGCCTGGCA GTTTATGGCGGGCGTCCTG
LRPR621	Construction of pLREX64, *att*Tn7:: mini-Tn7T-Gm^{R} P_{ara} Flag-*ppk2A* P_{ssb} *ssb*-mCherry GFP-*parB*^{pMT1}	AGGCCTTCGCGAGGTACCGGGCCCAAGCTTTTATGACAACT TGACGGCTACATCATTCAC
LRPR637	Construction of pLREX49, *att*Tn7:: mini-Tn7T-Gm^{R} *parS*^{pMT1} P_{ssb} GFP-*parB*^{pMT1}	GGATGCTGAACCTTAGGAGAACGTCAGGAGGATATACATA TGTCTAAAGGTGAAGAACTG
LRPR638	Construction of pLREX49, *att*Tn7:: mini-Tn7T-Gm^{R} *parS*^{pMT1} P_{ssb} GFP-*parB*^{pMT1}	CAGTTCTTCACCTTTAGACATATGTATATCCTCCTGACGTTC TCCTAAGGTTCAGCATCC

```
function [twopeas,twopeasDemograph] =
peas2pod_simulation(cells,muLength,sigmaLength,muDiameter,sigmaDiameter
,mEnd,mGran)
%cells = number of cells to simulate
%muLength = mean cell Length
%sigmaLength = standard deviation of distribution of cell lengths
%muDiameter = mean diameter of granules
%sigmaDiameter = standard deviation of distribution granule diameters
% mEnd = minimum distince of edge of granule from cell end
% mGran = minimum distince from edge of granule1 to edge of granule2

%this function generates a population of a specified number of cells  with
%a normal distribution of cell lengths specified by input mean and  standard
%deviation vaules generates two population of granules, each with a  normal
%distribution of granule diameters using mu and sigma from EM data
%for each cell, positions granule 1 randomly, excluding from the ends
%by input mEnd + radius of granule 1
%do the same for granule 2, then check that the position of granule 2
%satisifes the following condition:   it must be at least
%radius granule 1+mEnd+radius granule2 away from G1
%if it doesn't satisfy this condition, roll the dice again until it  does,
%then go on to the next cell

%twopeas(i,1) = cell number
%twopeas(i,2) = cell length
%twopeas(i,3) = diameter granule 1
%twopeas(i,4) = diameter granule 2
%twopeas(i,5) = P1, position of center of granule 1
%twopeas(i,6) = P2, position of center of granule 2
%twopeas(i,7) = position of center of granule nearest to near end of  cell
%twopeas(i,8) = position of center of granule furthest from near end of
%cell
%twopeas(i,9) = normalized position of center of near granule
%twopeas(i,10) = normalized position of center of far granule
twopeas = zeros(cells,12);

%generate a cell with two granules specified by distribution of lengths
%and granule diameter specified by input mu and sigma
%but pre-screen to make sure that the granules can fit into the cell  length
%given the constraints of minimum distance from ends and between  granules
%if it doesn't, then re-generate new granule diameters and length
%this method will start to skew towards longer cells and smaller  granules
%as the constraints of minimum distance from cell ends and between  granules
%increases
```

```
for i=1:cells  m = i;

    while m == i;
    lengths = normrnd(muLength,sigmaLength);  granule1 =
    normrnd(muDiameter,sigmaDiameter);  granule2 =
    normrnd(muDiameter,sigmaDiameter);

              if   (granule1+granule2+mEnd+mGran)<lengths  m =
              m+1;
              else clear lengths granule1 granule2  end

    end


    twopeas(i,1) = i;  twopeas(i,2) =
    lengths;  twopeas(i,3) = granule1;
    twopeas(i,4) = granule2;
%r1, r2 = radiii of granules 1 and 2  r1 =
    granule1/2;
    r2 = granule2/2;
%specifcy a region1 in which center of granule 1 can reside, by  defining
%the lower and upper bounds using specified minimum distance from cell  ends
%the radius of the granule, and cell length  lowerBound1
    = mEnd+r1;
    upperBound1 = lengths-(mEnd+r1);  region1 =
    upperBound1 -lowerBound1;
%randomly position center position of granule 1 (P1) in the specified  allowed
%region1
    initialP1 = rand * region1;
%repeat the process of defining the region1 and randomly positioning  granule 2
%using radius of granule 2  lowerBound2 =
    mEnd+r2;  upperBound2 = lengths-(mEnd+r2);
    region2 = upperBound2 -lowerBound2;  initialP2 =
    rand * region2;

%now determine if positions P1 and P2 satisfy the criteria that they  are
%at least the minimum distance specified by the user (mGran) apart
%if they are not, then randomly re-position one of the granules (which  one
%determined by a coin toss), and re-test if conditions satisfied  j = i;

    while j == i;

        if initialP2 > initialP1...
            && (initialP2-r2)-(initialP1+r1) > mGran  P1 =
            lowerBound1+initialP1;
            P2 = lowerBound2+initialP2;  twopeas(i,5) = P1;
```

```
            twopeas(i,6) = P2;  j = j+1;
        elseif initialP1 > initialP2...
            && (initialP1-r1)-(initialP2+r2) > mGran  P1 =
            lowerBound1+initialP1;
            P2 = lowerBound2+initialP2;  twopeas(i,5)
            = P1;  twopeas(i,6) = P2;
            j = j+1; else
            flipcoin = randi([0,1]);  if
                flipcoin == 0 ...
                    initialP1 = rand * region1; elseif
                flipcoin == 1 ...
                    initialP2 = rand * region2;
                end

        end


         end
end
%sort the granules such that granule 1 is always closest to the near  end of
%the cell
for k = 1:cells
    if twopeas(k,5) > twopeas(k,6)  twopeas(k,7) =
        twopeas(k,6);  twopeas(k,8) = twopeas(k,5);
        twopeas(k,9) = twopeas(k,4);  twopeas(k,10) =
        twopeas(k,3);
    else
        twopeas(k,7) = twopeas(k,5);  twopeas(k,8) =
        twopeas(k,6);  twopeas(k,9) = twopeas(k,3);
        twopeas(k,10) = twopeas(k,4);
         end
         k = k+1;
end

% normalize to generate relative positions as well  for l =
1:cells
    twopeas(l,11) = (twopeas(l,7)/twopeas(l,2));
    twopeas(l,12) = (twopeas(l,8)/twopeas(l,2));
end

% convert data into format for running demograph program (TEM version)

twopeasDemograph = zeros((2*cells),5);
j = 1;
for i=1:cells
    twopeasDemograph(j,1) = i;  twopeasDemograph(j,2) = 2;
    twopeasDemograph(j,3) = twopeas(i,2);
    twopeasDemograph(j,4) = twopeas(i,7);
    twopeasDemograph(j,5) = twopeas(i,9);
    twopeasDemograph((j+1),1) = 0;
    twopeasDemograph((j+1),1) = 0;
```

```
            twopeasDemograph((j+1),3) = 0;
            twopeasDemograph((j+1),4) = twopeas(i,8);
            twopeasDemograph((j+1),5) = twopeas(i,10);  i =
            i+1;
            j = j+2;
end

figure
h = histogram(twopeas(:,11),'FaceAlpha',0.5,'FaceColor',[0 0 1]);

hold on
h = histogram(twopeas(:,12),'FaceAlpha',0.5,'FaceColor',[1 1 0]);  end
```

SI REFERENCES

1. Gibson DG, et al. (2009) Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat Meth* 6(5):343–345.

2. Shanks RMQ, Caiazza NC, Hinsa SM, Toutain CM, O'Toole GA (2006) Saccharomyces cerevisiae-Based Molecular Tool Kit for Manipulation of Genes from Gram-Negative Bacteria. *Applied and Environmental Microbiology* 72(7):5027–5036.

3. Choi K-H, Schweizer HP (2006) mini-Tn7 insertion in bacteria with single attTn7 sites: example Pseudomonas aeruginosa. *Nature Protocols* 1(1):153–161.

4. Nielsen HJ, Ottesen JR, Youngren B, Austin SJ, Hansen FG (2006) The Escherichia colichromosome is organized with the left and right chromosome arms in separate cell halves. *Molecular Microbiology* 62(2):331–338.