**SUPPLEMENTAL MATERIAL**

# Effects of (p)ppGpp on the progression of the cell cycle of *Caulobacter crescentus*

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**Supplementary figures:**

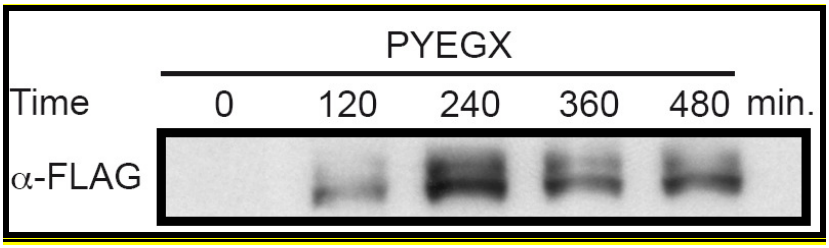


**Figure S1: RelA'-FLAG is expressed by strain JC820 for up to eight hours after the addition of xylose into rich medium.** Strain JC820 was cultivated in exponential phase in PXEG medium and 0.3% xylose was added at time 0 minutes. Cell extracts collected at times 0, 120, 240, 360 and 480 minutes were used to perform immunoblotting experiments using anti-FLAG antibodies.

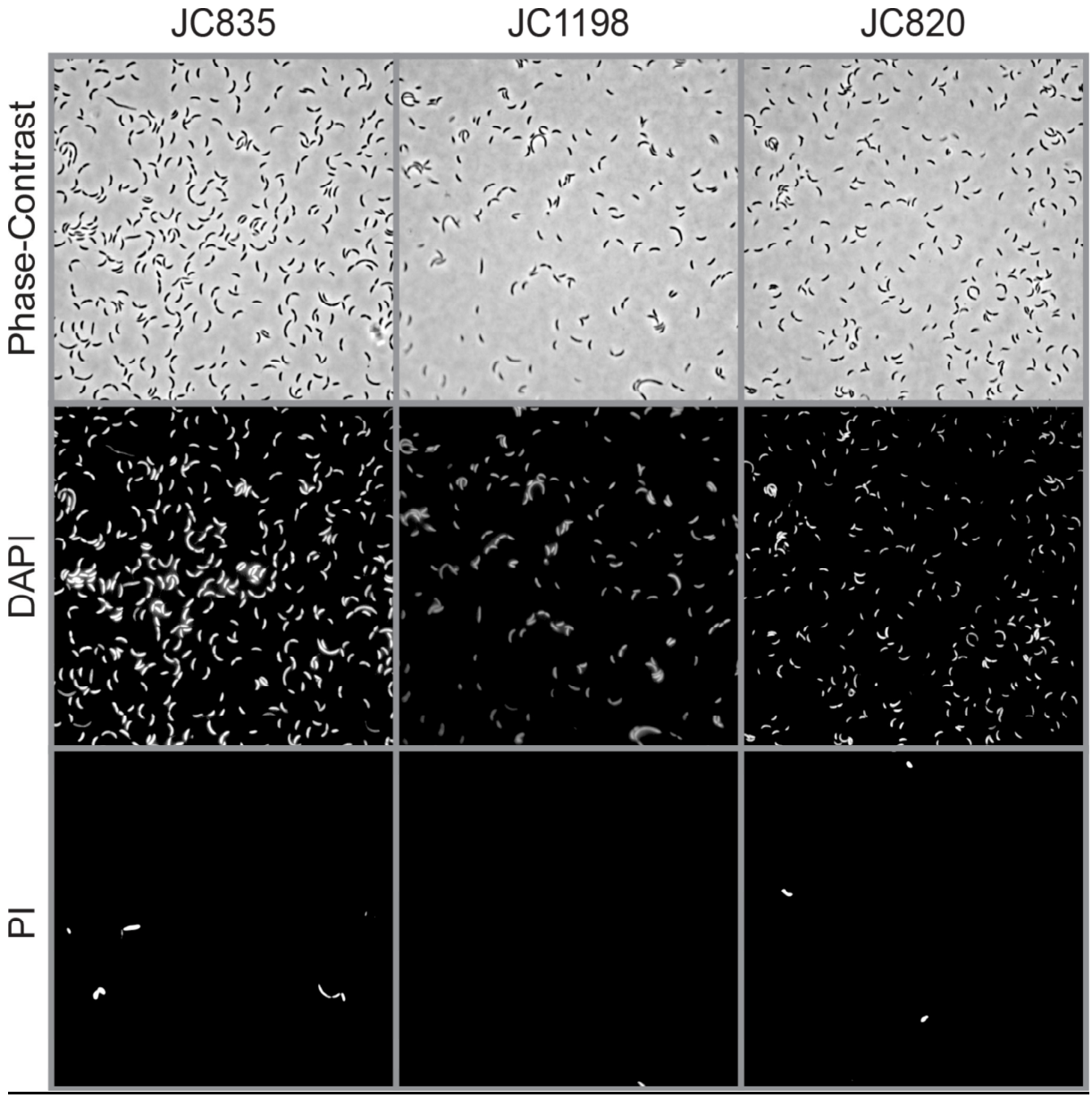


**Figure S2: Phase contrast and live/dead stained images of cells expressing RelA'-FLAG or control cells.** Strains JC835 (NA1000 pXTCYC-4), JC1198 (NA1000 pXTCYC-4-*relA'(E335Q)*-FLAG) and JC820 (NA1000 pXTCYC-4-*relA'*-FLAG) were cultivated in exponential phase in PYEG+0.3% xylose for six hours. Upper panels: phase contrast microscopy images of fixed cells. Lower two panels: Fluorescence microscopy images of live/dead stained cells. Cells giving a signal for DAPI (4′,6-diamidino-2-phenylindole) and not for PI (propidium iodide) can be considered alive; cells giving a signal for PI can be considered permeable or dead.

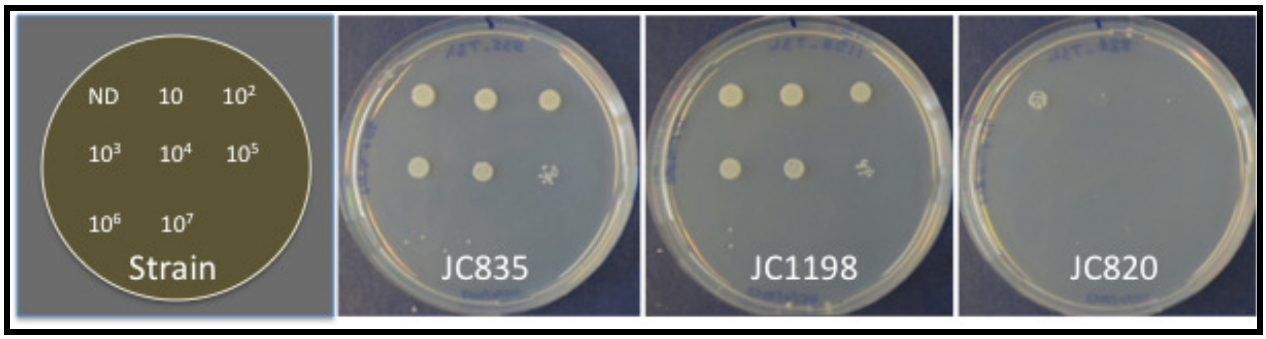


**Figure S3: Very few cells accumulating excess (p)ppGpp can form visible colonies on solid medium.** Strains JC835 (NA1000 pXTCYC-4), JC1198 (NA1000 pXTCYC-4-*relA'(E335Q)*-FLAG) and JC820 (NA1000 pXTCYC-4-*relA'*-FLAG) were cultivated in exponential phase in PYEG+0.3% xylose for three hours. 5µL of non-diluted (ND) cultures ($OD_{660nm}$ between 0.15 and 0.35) and serial 10-fold dilutions were spotted onto PYEGA plates. Plates were then incubated for three days at 28°C.

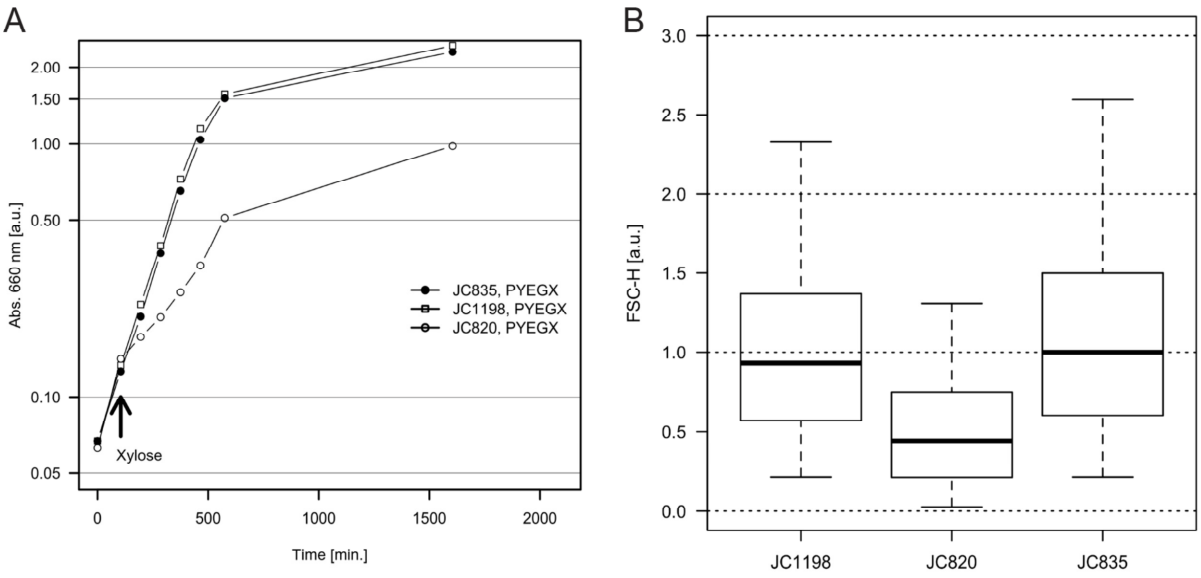


**Figure S4: Cells expressing an inactive RelA'(E335Q)-FLAG protein have the same growth rate and size as control cells, unlike cells expressing an active RelA'-FLAG protein.** Strains JC835 (NA1000 pXTCYC-4), JC1198 (NA1000 pXTCYC-4-*relA'(E335Q)*-FLAG) and JC820 (NA1000 pXTCYC-4-*relA'*-FLAG) were cultivated in exponential phase in PYEG. 0.3% xylose was added (PYEGX) at time 105 minutes. **(A)** The absorbance at 660 nm was measured as a function of time to compare growth. **(B)** The forward scattering (FSC) of 20'000 individual cells for each population was measured using a flow cytometer three hours after xylose addition, to estimate cell size. Plotted values are the average median FSC value for each strain and condition normalized by the average

median FSC value of JC835 in PYEG at the time of xylose addition; error bars refer to the standard deviations.

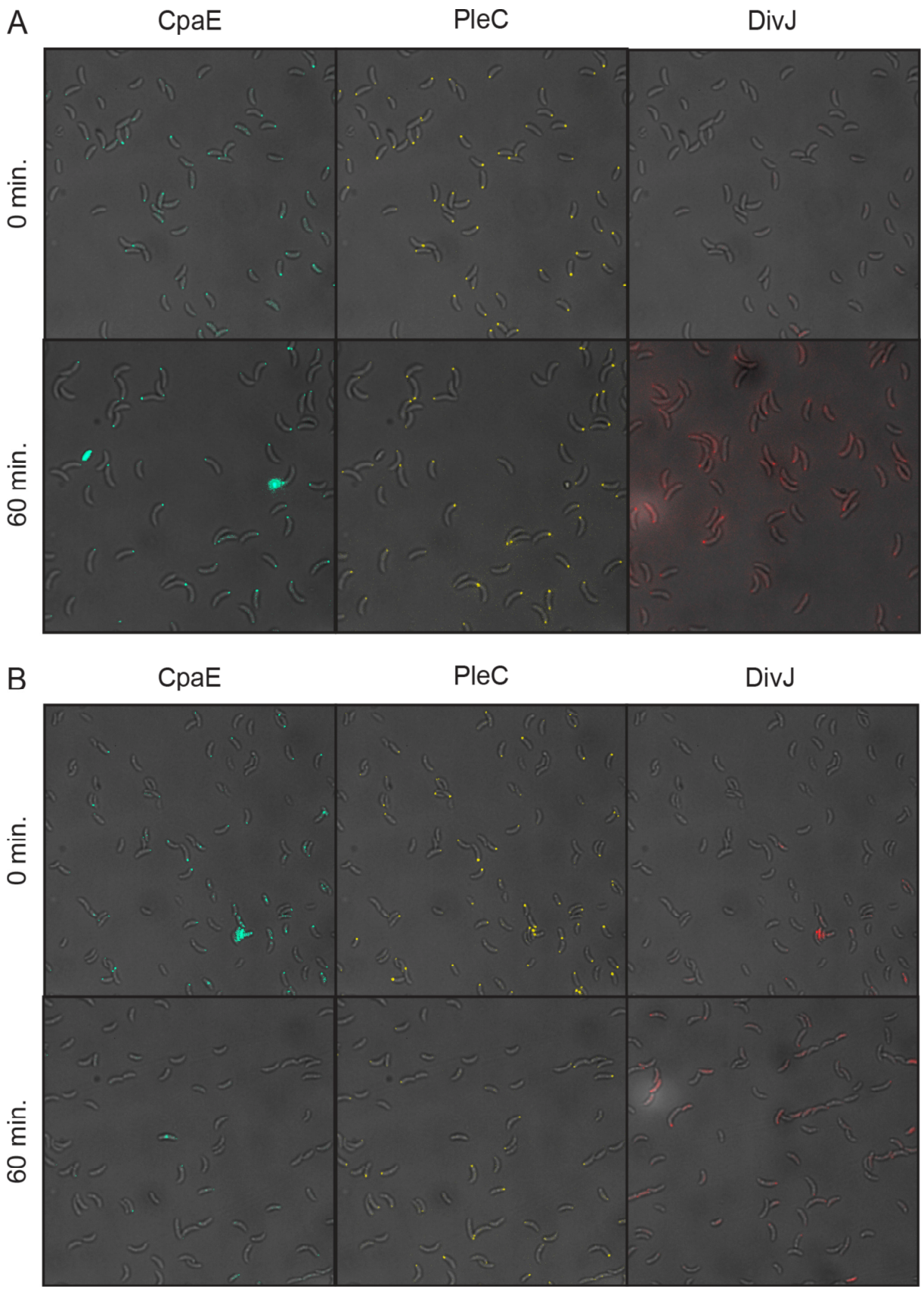

**Figure S5: Typical fluorescence microscopy images of JC845 cells expressing or not RelA'-FLAG.** Fluorescence microscopy experiments were performed on isolated swarmer cells from strain JC845 (NA1000 *pleC*::*pleC*-YFP *divJ*::*divJ*-RFP *cpaE*::*cpaE*-CFP pXTCYC-4-*relA*'-FLAG) grown in PYEG**. (A)** JC845 swarmer cells were resuspended into PYEGX and visualized by fluorescence microscopy at times 0 and 60 minutes. (B) JC845 swarmer cells were resuspended into PYEG and visualized by fluorescence microscopy at times 0 (swarmer cells) and 60 minutes (stalked cells).

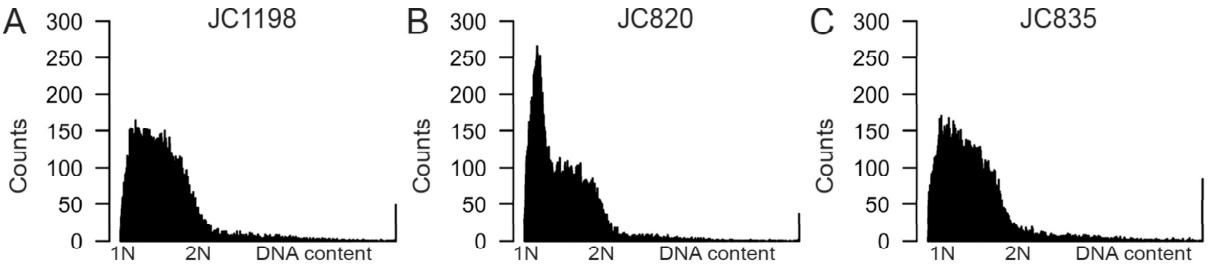


**Figure S6 : Expression of an inactive RelA'(E335Q)-FLAG protein does not increase the proportion of cells with one chromosome. (A)** Histograms of the DNA content of JC1198 (NA1000 pXTCYC-4-*relA'(E335Q)*-FLAG), JC820 (NA1000 pXTCYC-4-*relA'*-FLAG) and JC835 (NA1000 pXTCYC-4) cells cultivated for three hours in PYEG + 0.3% xylose. Cells were fixed and stained prior to flow cytometry analysis. The horizontal axis indicates the fluorescence intensity of individual cells and the number N of complete chromosomes. The vertical axis indicates the number of cells.

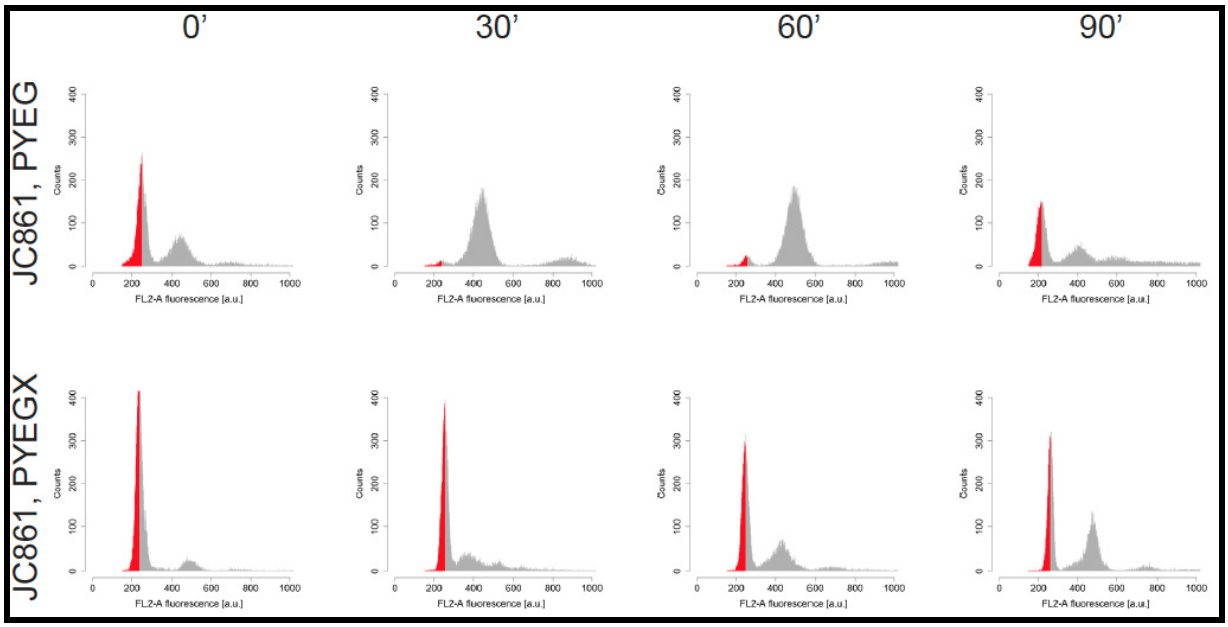


**Figure S7: Typical histograms of the DNA content of JC861 swarmer cells expressing or not RelA'-FLAG for 30 minutes and treated with rifampicin.** Isolated swarmer cells from strain JC861 (NA1000 *dnaN*::*dnaN*-RFP pXTCYC-4-*relA'*-FLAG) were grown in PYEG or PYEGX for 0, 30, 60 and 90 minutes prior to treatment with rifampicin, fixation and staining for flow cytometry analysis. Shown in red is the surface corresponding to cells whose DNA content is below the 1N maximum. The proportion of 1N cells was estimated as the double of the red surface divided by the total number of cells to create Fig.6.

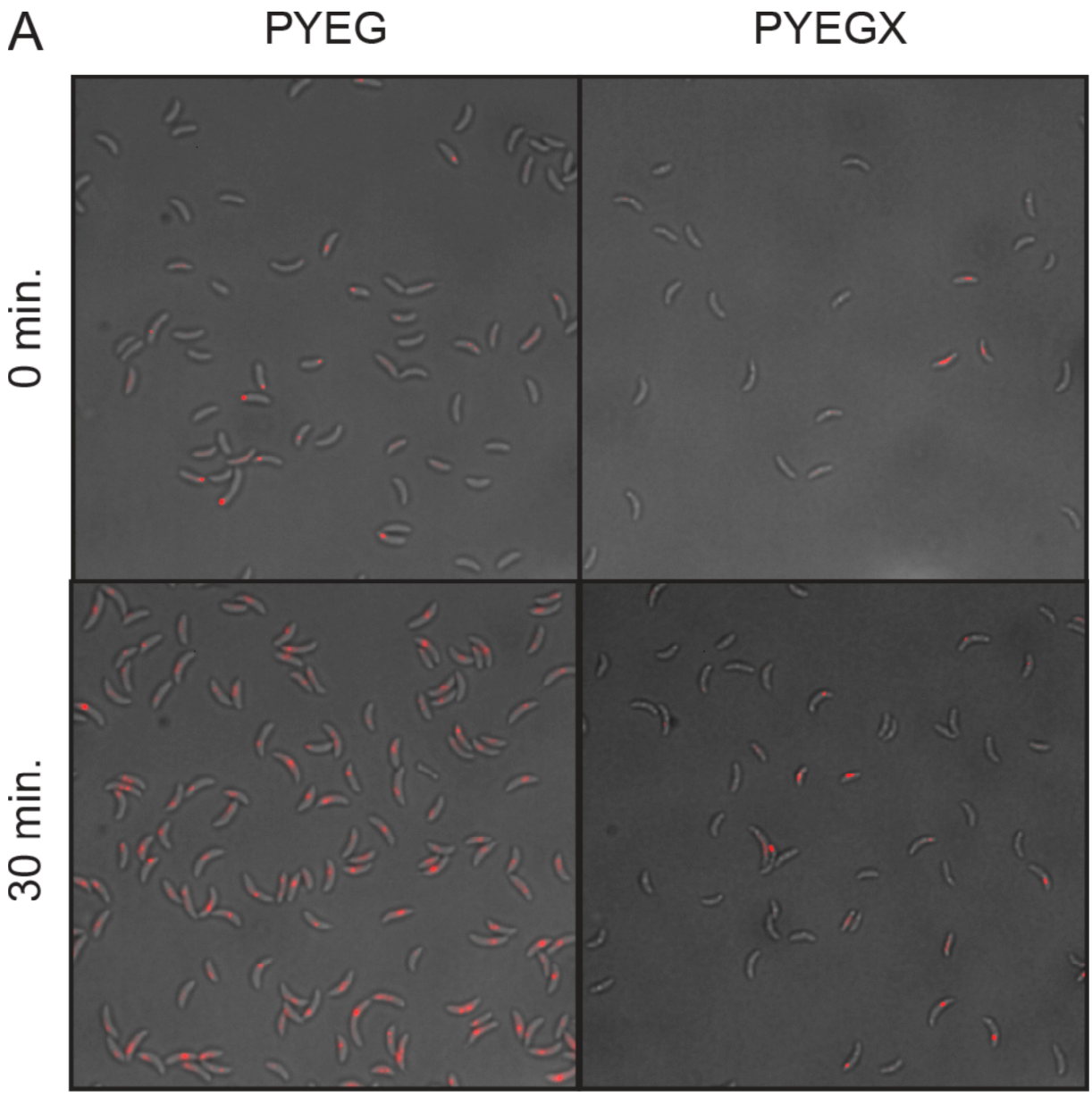


**Figure S8: Typical fluorescence microscopy images of JC861 cells expressing or not RelA'-FLAG.** Fluorescence microscopy experiments were performed on isolated swarmer cells from strain JC861 (NA1000 *dnaN*::*dnaN*-RFP pXTCYC-4-*relA*'-FLAG) grown in PYEG**.** Left panels: JC861 swarmer cells were resuspended into PYEG and visualized by fluorescence microscopy at times 0 (swarmer cells) and 30 minutes (swarmer-to-stalked cell transition). Right panels: JC861 swarmer cells were resuspended into PYEGX and visualized by fluorescence microscopy at times 0 and 30 minutes.

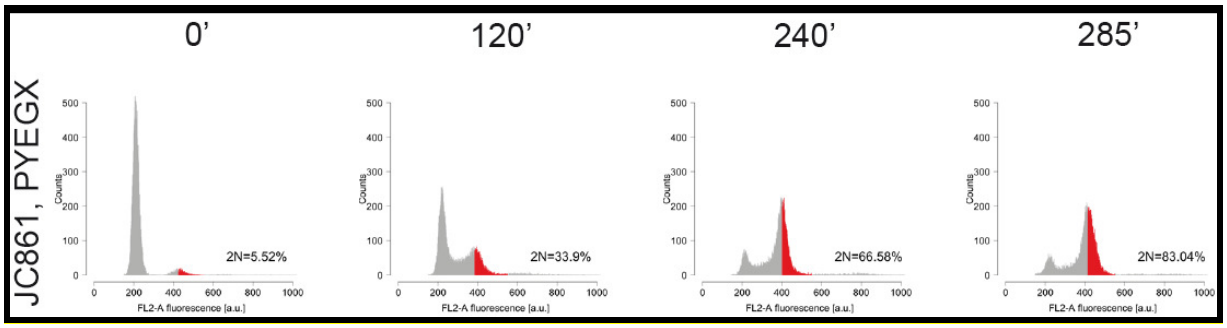


**Figure S9: Most (p)ppGpp-accumulating swarmer cells will eventually initiate chromosome replication.** Flow cytometry experiments using isolated swarmer cells from strain JC861 (NA1000 *dnaN*::*dnaN*-RFP pXTCYC-4-*relA'*-FLAG) grown in PYEGX for three hours. Cells were treated with penicillin to block cell division, sampled at the indicated times and then fixed and stained prior to flow cytometry analysis. Shown in red is the surface corresponding to cells whose DNA content is beyond the 2N maximum. The proportion of 2N cells was estimated as the double of the red surface divided by the total number of cells.