Supporting Information

Mismatch repair causes the dynamic release of an essential DNA polymerase from the replication fork

Andrew D. Klocko, Jeremy W. Schroeder, Brian W. Walsh, Justin S. Lenhart, Margery L. Evans and Lyle A. Simmons*

Department of Molecular, Cellular, and Developmental Biology
University of Michigan, Ann Arbor, MI 48109

*Corresponding Author
Mailing address: Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, Michigan 48109. Phone: (734) 647-2016. E-mail: lasimm@umich.edu
Fax: (734) 647-0884.
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Table S1. *Bacillus subtilis* strains used in this study.

Strain	Relevant Genotype	Source or Reference
PY79	Prototroph, SPβ^{o}	(Youngman *et al*., 1984)
LAS440	*mutS*::*mutS-gfp* (*spc*); *amyE*::P_{spac}*mutL* (*cat*)	(Smith *et al*., 2001)
LAS397	*mutL*::*mutL-gfp* (*spc*)	(Smith *et al*., 2001)
LAS257	*dnaN*::*dnaN-mgfpA206K* (*spc*)	(Simmons *et al*., 2008)
LAS385	*dnaX*::*dnaX-gfp* (*spc*)	(Smith *et al*., 2001)
AK151	*holB*::*holB-gfp* (*spc*)	(Lemon and Grossman, 1998)
LAS267	*lacA*::P_{rps} *ssb-gfp* (*tet*)	(Berkmen and Grossman, 2006)
LAS387	*polC*::*polC-gfp* (*spc*)	(Lemon and Grossman, 1998)
AK74	*amyE*::P_{xyl} *dnaE-gfp* (*spc*)	(Dervyn *et al*., 2001)
LAS38	*mutSL*::*kan*	(Simmons *et al*., 2008)
AK111	*amyE*::P_{xyl} *dnaE-gfp* (*spc*); *mutSL*::*kan*	This work
AK121	*mutL*::*kan*	This work
AK124	*amyE*::P_{xyl} *dnaE-gfp* (*spc*); *mutL*::*kan*	This work
LAS40	*recA*::*recA-mgfp (spc)*	(Simmons *et al*., 2009)
JWS68	*amyE::*P_{xyl}*dnaE-gfp (spc); mutSL*::*kan*; *lacA*::P_{spac}*mutL*$^{+}$ (*erm*)	This work
JSL203	*amyE::*P_{xyl}*dnaE-gfp (spc); mut-1*[*polC G430E,S621N*] *(cat)*	This work
BWW88	*dnaE::dnaE-gfp (spc), ytsJ*$^{-}$	This work
BWW96	*dnaE::dnaE-mgfp (spc), ytsJ*$^{-}$	This work

All strains used are derivates of PY79.

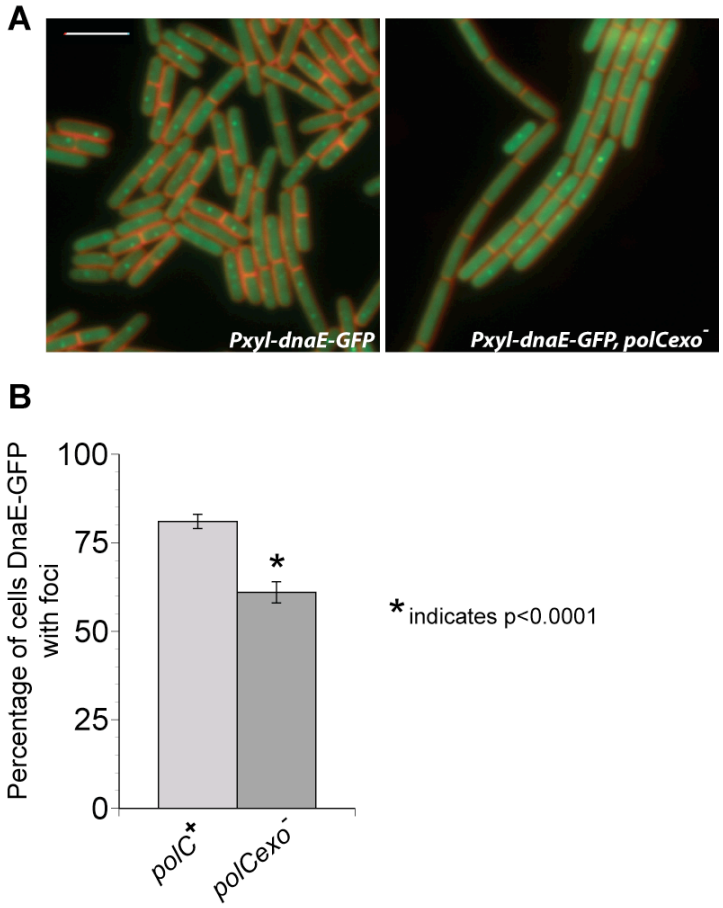

Figure S1. The percentage of cells with DnaE-GFP foci decrease in a strain bearing a proofreading deficient *polC* allele. The percentage of cells with DnaE-GFP foci were scored in an isogenic strain bearing the *polC mut-1* allele (Sanjanwala and Ganesan, 1991). This allele is defective in 3´ to 5´ exonuclease (Sanjanwala and Ganesan, 1991). **(A)** DnaE-GFP is shown in green while the membranes are pseudo-colored red and visualized with the vital membrane dye TMA-DPH. The wild type *polC* allele was used in the left panel, while the *polC mut-1* allele (*polCexo*$^-$) was used in the right panel to evaluate the effect on DnaE-GFP foci. Exposure length for imaging DnaE-GFP was 500 ms while the TMA-DPH was imaged at 65 ms. **(B)** Shows a quantification of the percentage of cells with DnaE-GFP foci under the indicated conditions. The number of cells scored for *polC*$^+$ was 1644 cells and for DnaE-GFP scored in the *polC mut-1* background we scored 1101 cells. The asterisk indicates that the results are significant with $p<0.0001$. The white bar indicates 4 μm.

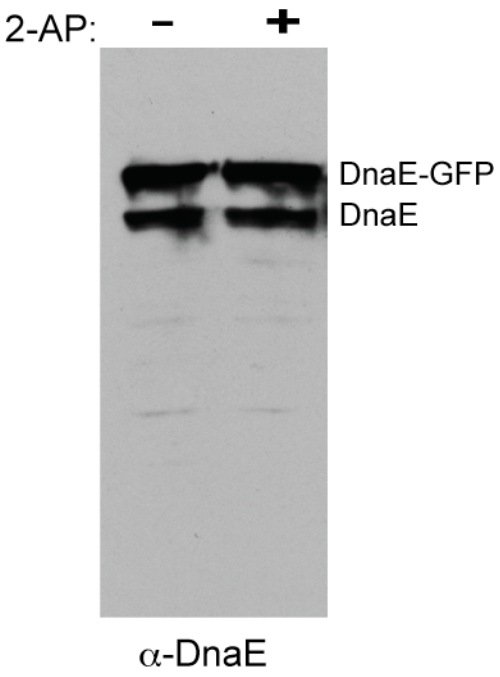

Figure S2. DnaE and DnaE-GFP levels are unchanged following 2-AP challenge.
Shown is an immunoblot of strain AK74 (relevant genotype *amyE*::P_{xyl} *dnaE*$^{+}$, *dnaE*$^{+}$), +/- 2-AP treatment, as indicated above. Cells were grown to mid exponential phase optical density (OD_{600}) of 0.4 in 0.125% D-xylose. The culture was split with one culture challenging with 600 μg/ml 2-AP for 1 hour while the control culture was grown in the absence of 2-AP challenge. Cells were harvested and processed as described (Rokop *et al*., 2004). Cell load was normalized to cell number as determined by optical density between the samples shown. The anti-DnaE antiserum (MI1185) was used in 1:5000, the HRP-conjugated secondary goat anti-rabbit was used with a 1:5000 dilution (Pierce) as described in "Experimental Procedures."

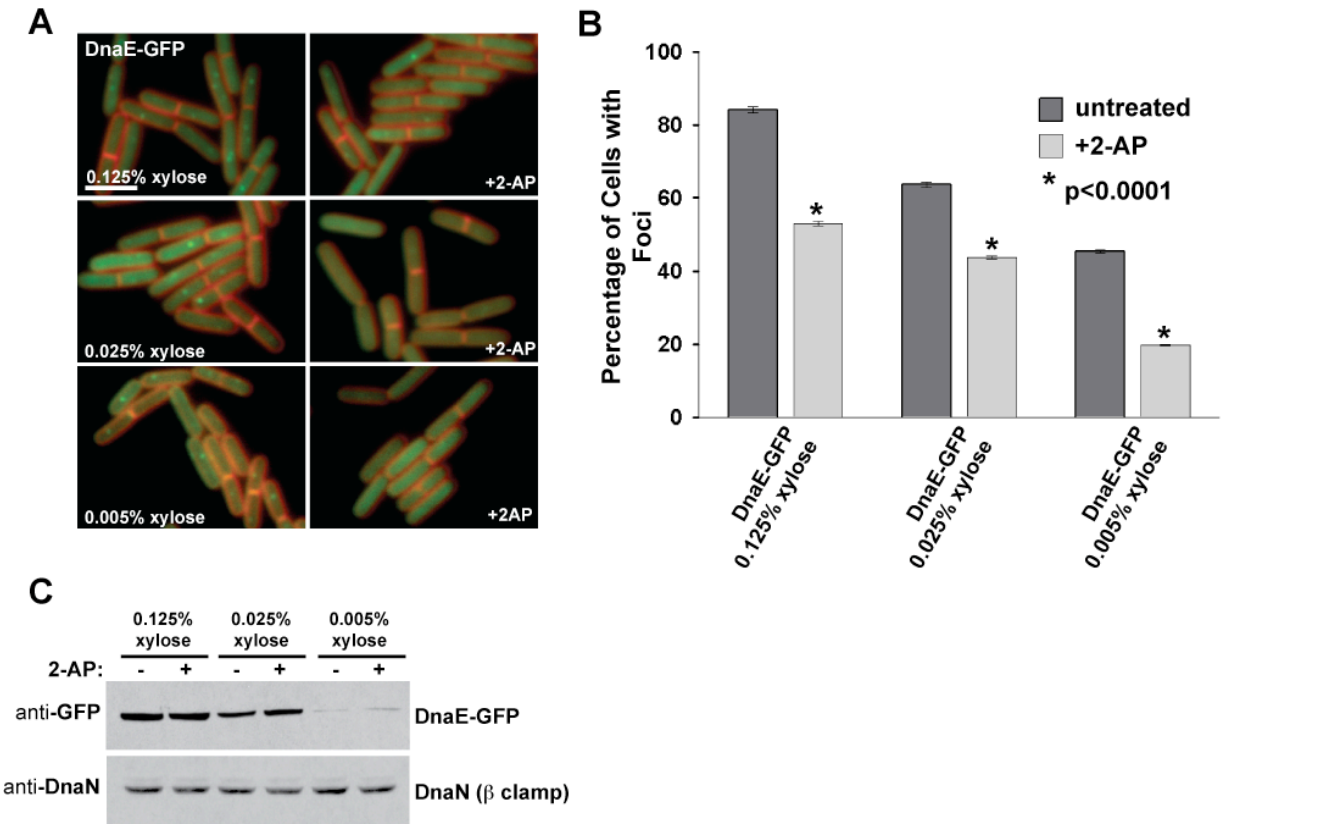

Figure S3. 2-AP mediated loss of DnaE-GFP foci is independent of DnaE levels *in vivo*. (A) DnaE-GFP with the indicated percentage of D-xylose added to the growth medium. The left panel is in the absence of 2-AP and the corresponding right panel is with 600 μg/ml 2-AP. The membrane is stained with the vital membrane stain FM4-64 and the white bar indicates 3 μm. The exposure time for DnaE-GFP in 0.125% xylose was 400 ms. We used longer exposures of 500 ms and 1000 ms to image the cells grown in 0.025% xylose and 0.005% xylose, respectively, for improved foci detection in these cells that contain lower levels of DnaE-GFP. **(B)** Bar graph of the percentage of cells with DnaE-GFP foci untreated (dark grey bars) and in the presence of 2-AP (light grey bars). The error bars reflect the 95% confidence interval. The asterisk indicates $p<0.0001$ between the untreated and 2-AP treated samples. The bar graph represents a summary of the complete data set shown in Table 3. **(C)** A representative immunoblot of DnaE-GFP and β clamp (DnaN) from cells with the amount of xylose and 2-AP indicated is shown.

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