

## **Supporting information**

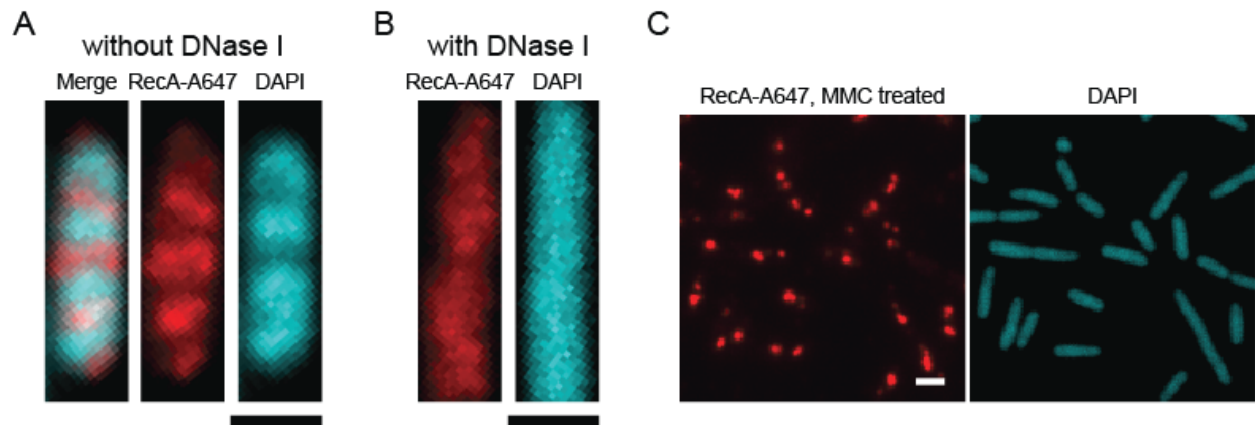
### **An improved method for bacterial immunofluorescence staining to eliminate antibody exclusion from the fixed nucleoid**

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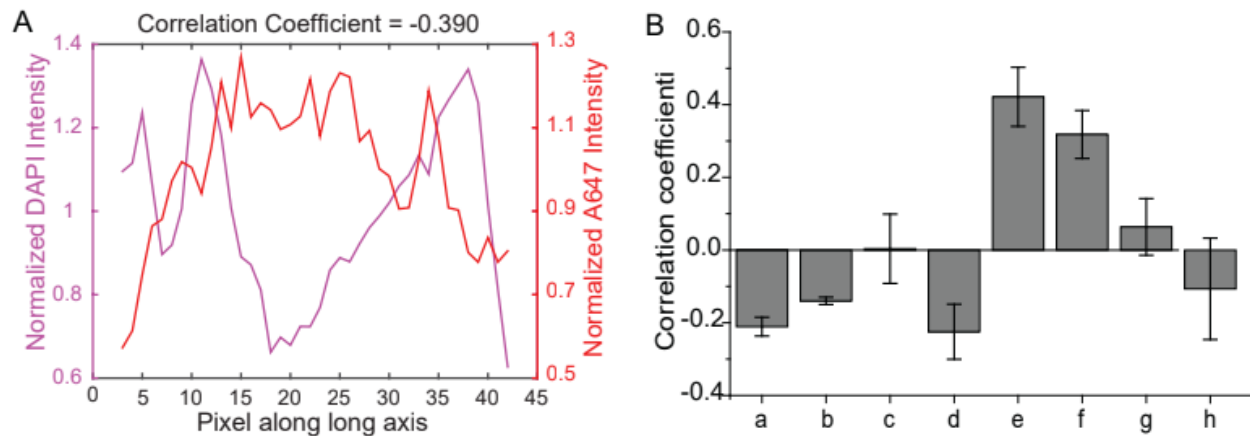
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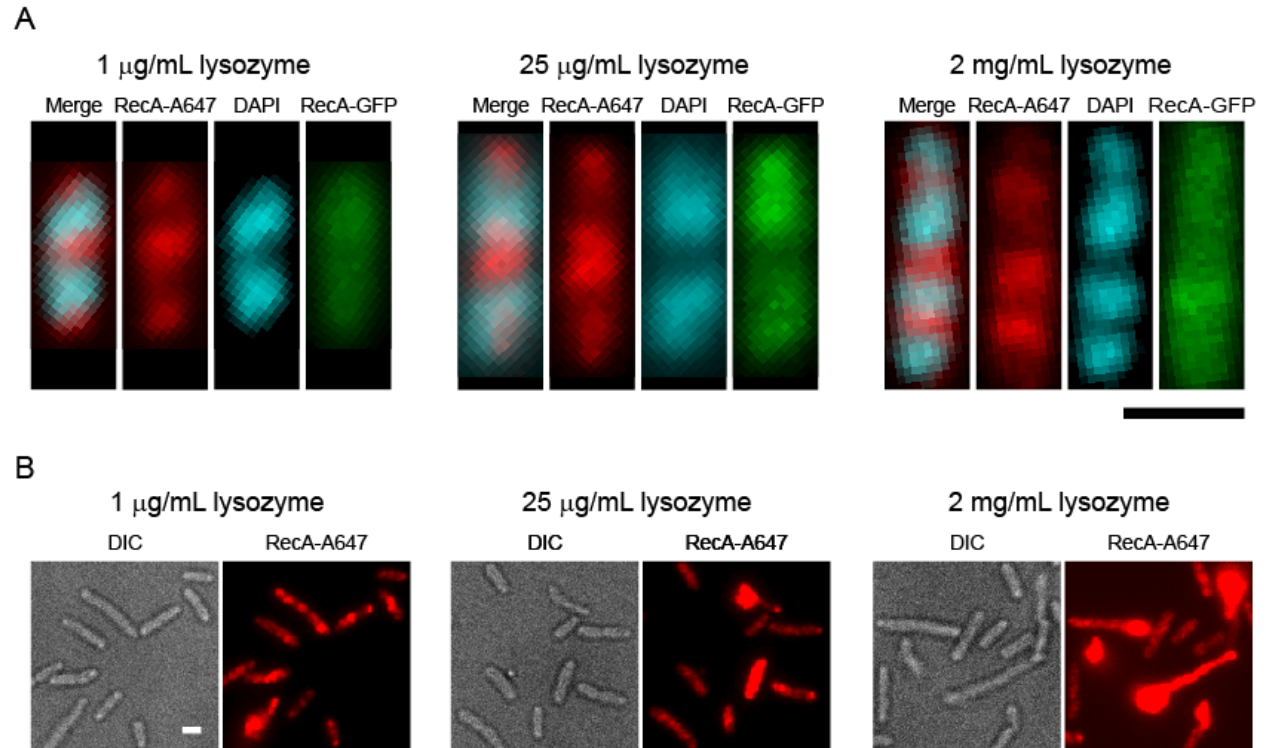
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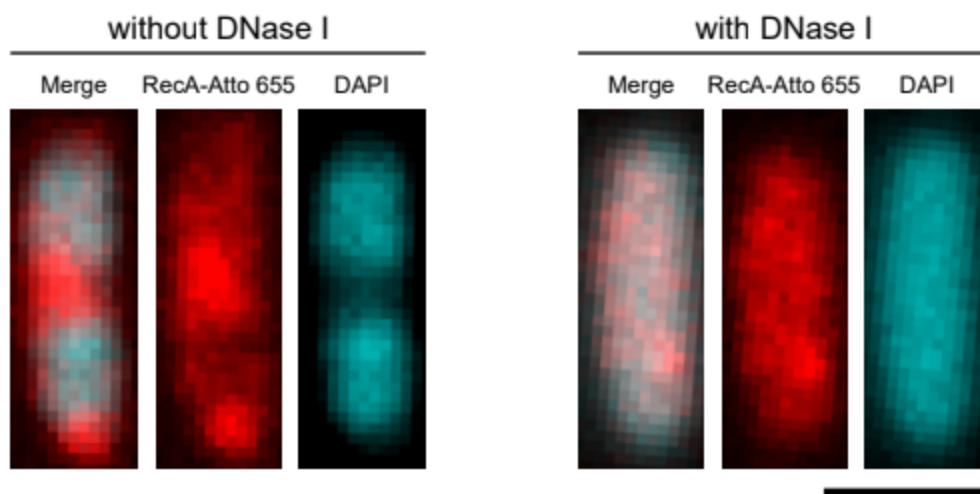
**Figure S1.** IF images of RecA in MG1655 cells. **(A)** A single MG1655 cell without any treatment before fixation. Permeabilization was performed with 70% ethanol and 25  $\mu$ g/mL lysozyme. **(B)** Same as in (A) but with 100 U/mL DNase I treatment. **(C)** IF image of RecA in MG1655 cells with the MMC treatment. IF sample was prepared in the same way as (B). The scale bars represent 2  $\mu$ m.



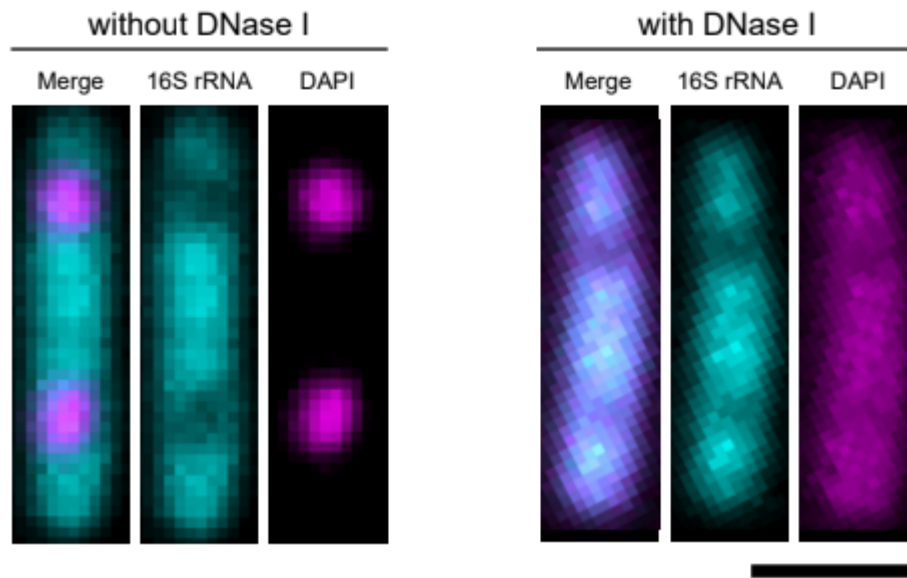
**Figure S2.** Correlation analysis between RecA-A647 and DAPI signals. Cells are first segmented based on the DIC image, and the two fluorescent channels are manually aligned. An intensity profile for each fluorescent channel is determined by scanning across that long axis of each cell. The correlation coefficient between the two intensity profiles is calculated for each cell. **(A)** A647 and DAPI intensity profiles from a representative cell from the 70% ethanol + 25  $\mu\text{g/mL}$  lysozyme treatment condition. **(B)** Average correlation coefficient between A647 and DAPI signals under various permeabilization conditions. (a) 70% ethanol + 25  $\mu\text{g/mL}$  lysozyme; (b) 1  $\mu\text{g/mL}$  lysozyme; (c) 25  $\mu\text{g/mL}$  lysozyme; (d) 70% ethanol + 25  $\mu\text{g/mL}$  lysozyme, with Atto 655 labeled secondary antibody (e) 70% ethanol + 25  $\mu\text{g/mL}$  lysozyme + 100 U/mL DNase I; (f) 70% ethanol + 25  $\mu\text{g/mL}$  lysozyme + 0.8 U/mL DNase I; (g) 70% ethanol + 25  $\mu\text{g/mL}$  lysozyme + 0.16 U/mL DNase I; (h) 70% ethanol + 25  $\mu\text{g/mL}$  lysozyme + 0.032 U/mL DNase I. Except for (d), all other conditions were prepared with A647 labeled secondary antibody. Error bars represent mean and standard deviations of 3-8 images, with each image containing 70-300 cells.



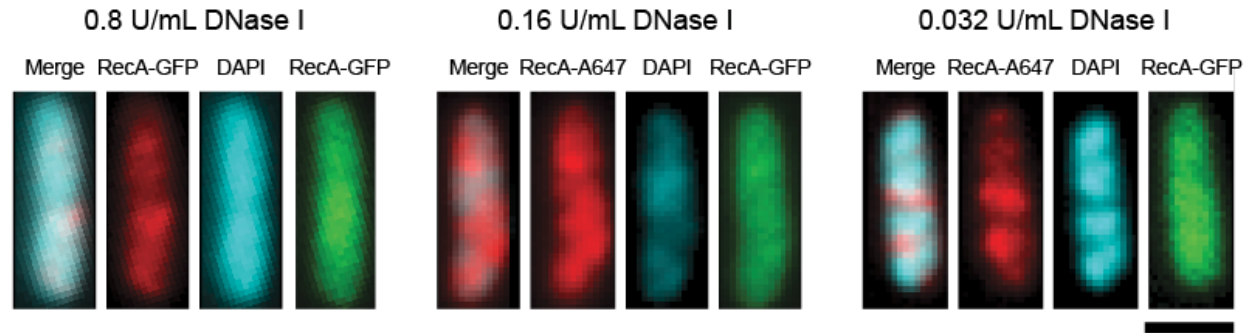
**Figure S3.** Antibody exclusion effect remains with methanol fixation. 1 mL of *recA-GFP* culture was fixed with 80% methanol and incubated on a nutator for 1 hour. The fixed cells were washed three times, and immobilized in chambered coverglass for the lysozyme treatment. **(A)** Single cell images with three different permeabilization conditions are shown: 1  $\mu\text{g/mL}$  lysozyme (left), 25  $\mu\text{g/mL}$  lysozyme (middle), and 2 mg/mL lysozyme (right). **(B)** Same order as in (A), with multiple cells shown per each case. Higher lysozyme concentration leads to more cell rupture. The scale bars represent 2  $\mu\text{m}$ .



**Figure S4.** Antibody exclusion effect remains with the antibody labeled with the neutrally charged dye. Secondary antibody was labeled with Atto 655. MG1655 cells were fixed with 4% formaldehyde and permeabilized with 70% ethanol and 25  $\mu\text{g/mL}$  lysozyme. The antibody exclusion effect is observed without the DNase I treatment (left), but eliminated with the DNase I (at 100 U/mL) treatment (right). The scale bar represents 2  $\mu\text{m}$ .



**Figure S5.** Nucleoid regions are preserved after the DNase I treatment. (Left). MG1655 cells were fixed with 4% formaldehyde and permeabilized with 70% ethanol and 25  $\mu\text{g/mL}$  lysozyme. 16S rRNA was stained by Alexa Fluor 488 NHS Ester (Invitrogen A20000) labeled FISH probes. 16S rRNA was depleted from the nucleoid region due to the DNA compaction under high salt treatment (500 mM NaCl for 20 minutes), which was maintained without (left) and with (right) the DNase I treatment (at 100 U/mL). The scale bar represents 2  $\mu\text{m}$ .



**Figure S6:** Immunofluorescence images of RecA-GFP in *recA-gfp* cells. Fixation was performed by 4% formaldehyde, and permeabilization was performed with 70% ethanol, 25  $\mu\text{g/mL}$  lysozyme, and various concentrations of DNase I. The scale bar represents 2  $\mu\text{m}$ .