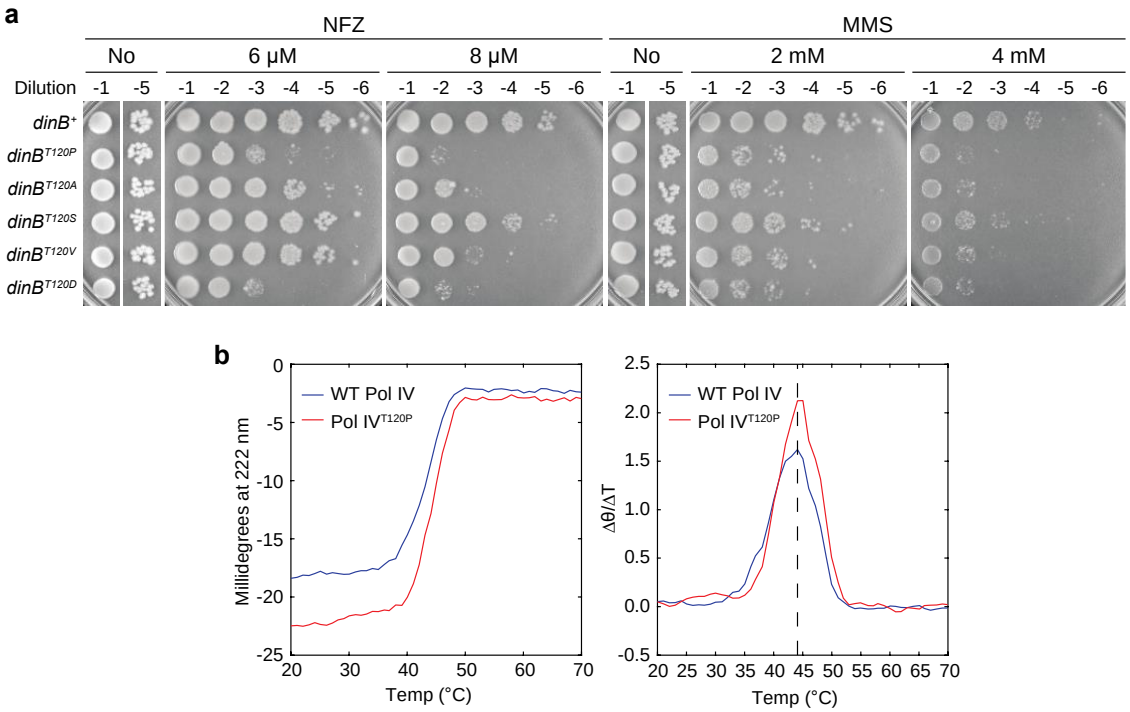
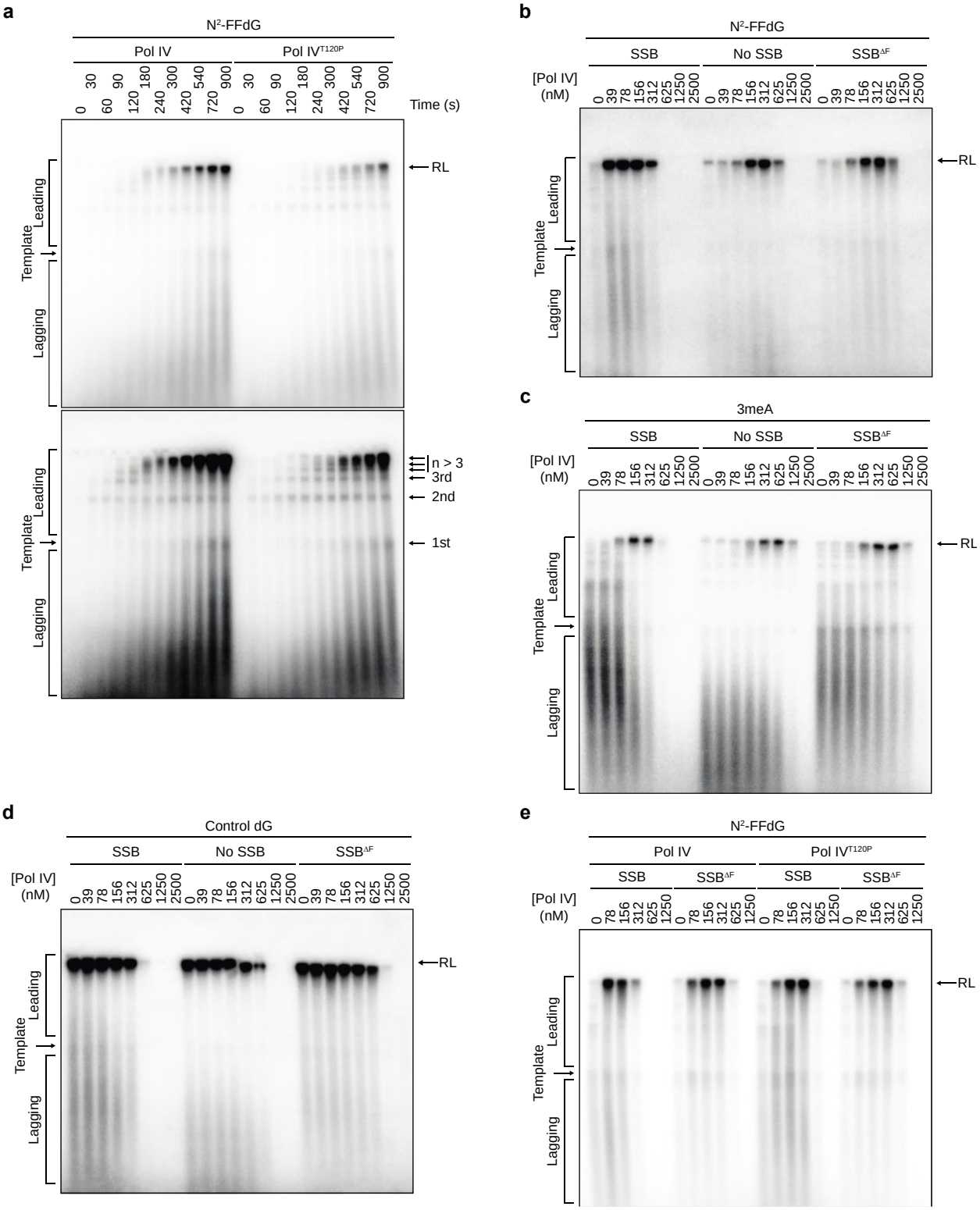
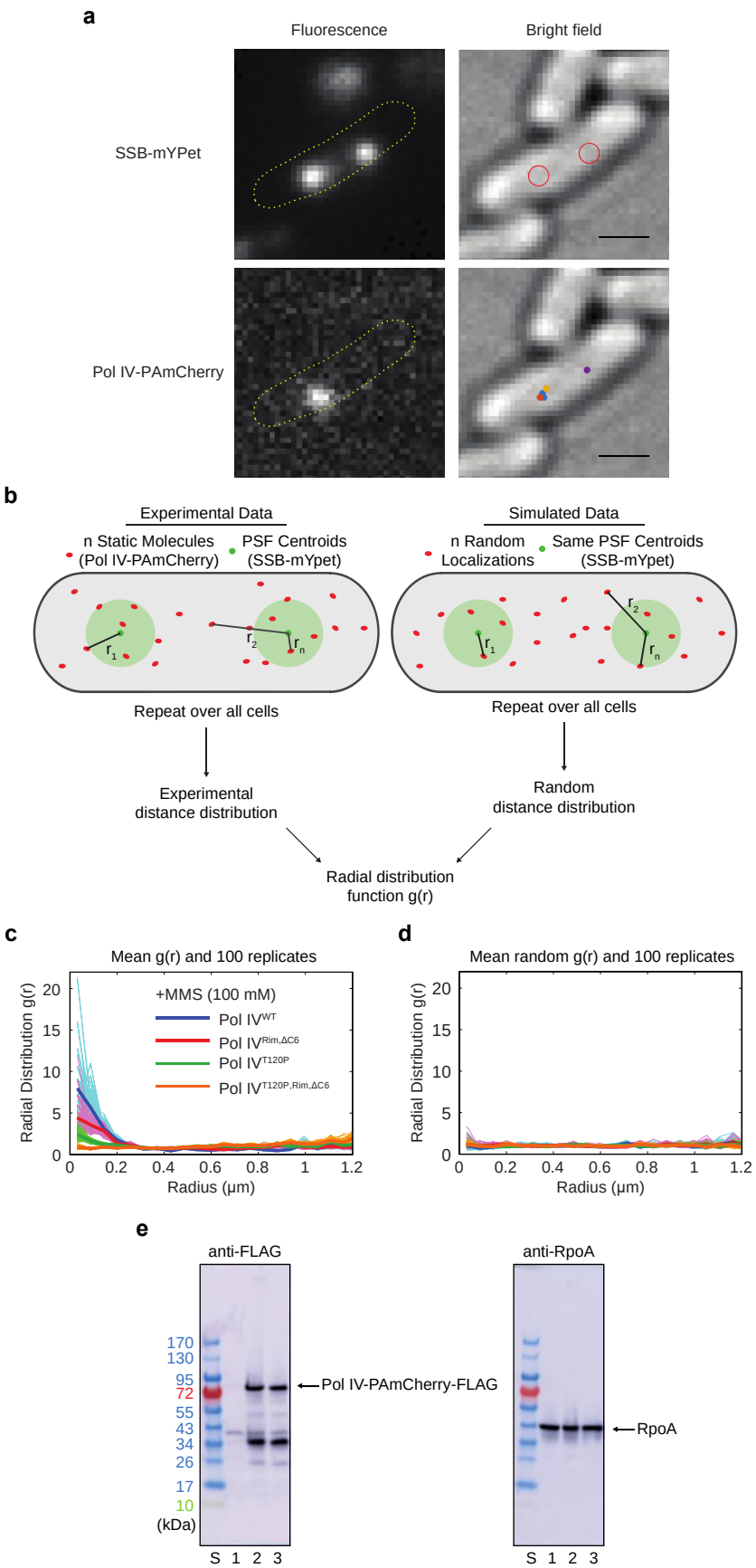


Supplementary Figure 1.
a. Sensitivity of various Thr^{120} mutants of *dinB* to NFZ and MMS.
b. (Left) Changes in the ellipticity of Pol IV or Pol IV^{T120P} at 222 nm upon increasing temperature. (Right) 1st derivatives of the unfolding curves shown in the left panel. The maxima correspond to the midpoints (T_Ms) of the unfolding curve. Similar results were reproduced twice.



Supplementary Figure 2.
a. (Top) The entire gel for the replication products at RL shown in Fig. 2c. (Bottom) The same gel shown in the top was log-transformed to clearly show stalled replication. Numbers, stalling/collapse of replisomes through multiple passages through N^2-FFdG during rolling circle replication.

b, c. The entire gels for the replication products at RL shown in Fig. 2d.
d. SSB weakly potentiates replication inhibitory activity of Pol IV through SSB-Ct. A lesion-free control substrate was replicated with SSB or $SSB^{\Delta F}$ or without SSB. As previously reported[1,2], replication of both leading and lagging strands was reduced in the absence of SSB or presence of $SSB^{\Delta F}$ with lagging strands products becoming shorter. The effects of $SSB^{\Delta F}$ on replication were less compared with omitting SSB. The reductions in the amount of replication products from lesion-containing templates in the absence of SSB or the presence of $SSB^{\Delta F}$ are attributable to this general reduction in processive replication. Similar results were reproduced twice.
e. The entire gel for the replication products at RL shown in Fig. 2e.


a
Fluorescence
Bright field
SSB-mYPet
Pol IV-PAmCherry
b
Experimental Data
Simulated Data
n Static Molecules
(Pol IV-PAmCherry)
PSF Centroids
(SSB-mYpet)
n Random
Localizations
Same PSF Centroids
(SSB-mYpet)
Repeat over all cells
Repeat over all cells
Experimental
distance distribution
Random
distance distribution
Radial distribution
function g(r)
c
Mean g(r) and 100 replicates
+MMS (100 mM)
Pol IV^WT
Pol IV^Rim,ΔC6
Pol IV^T120P
Pol IV^T120P,Rim,ΔC6
Radial Distribution g(r)
Radius (μm)
d
Mean random g(r) and 100 replicates
Radial Distribution g(r)
Radius (μm)
e
anti-FLAG
anti-RpoA
170
130
95
72
55
43
34
26
17
10
(kDa)
Pol IV-PAmCherry-FLAG
RpoA
S 1 2 3
S 1 2 3

Supplementary Figure 3.

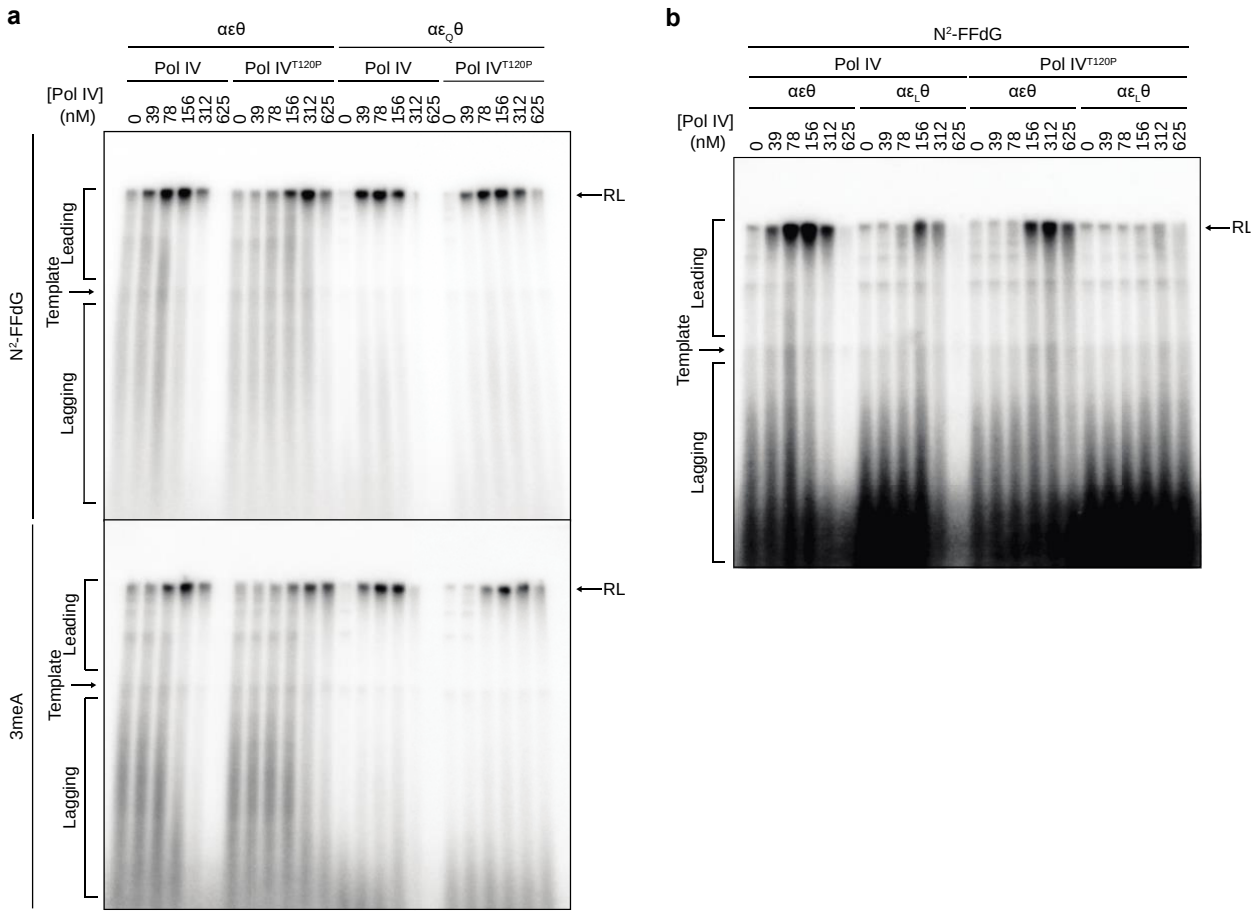
a. (Left) Representative fluorescence micrographs of a cell containing two SSB-mYPet foci (top) and a single photoactivated Pol IV-PAmCherry molecule (bottom) with overlays showing the cell outlines. (Right) Corresponding brightfield micrographs with overlays showing SSB foci (red circle, top) or all detected Pol IV tracks (colored dots, bottom) (Scale bars: 1 μM).

b. Cartoon of radial distribution function analysis. (Left) Experimental distances between static Pol IV-PAmCherry molecules and the nearest SSB-mYPet foci are calculated. (Right) For each cell, the same number of random localizations are generated and the distances to the same SSB-mYPet foci are determined. This calculation is performed over all cells and the resulting experimental distribution is normalized by the random distribution to give the radial distribution function *g(r)*. This procedure is repeated 100 times to generate 100 *g(r)* curves, which are averaged to give the final mean *g(r)* curve.

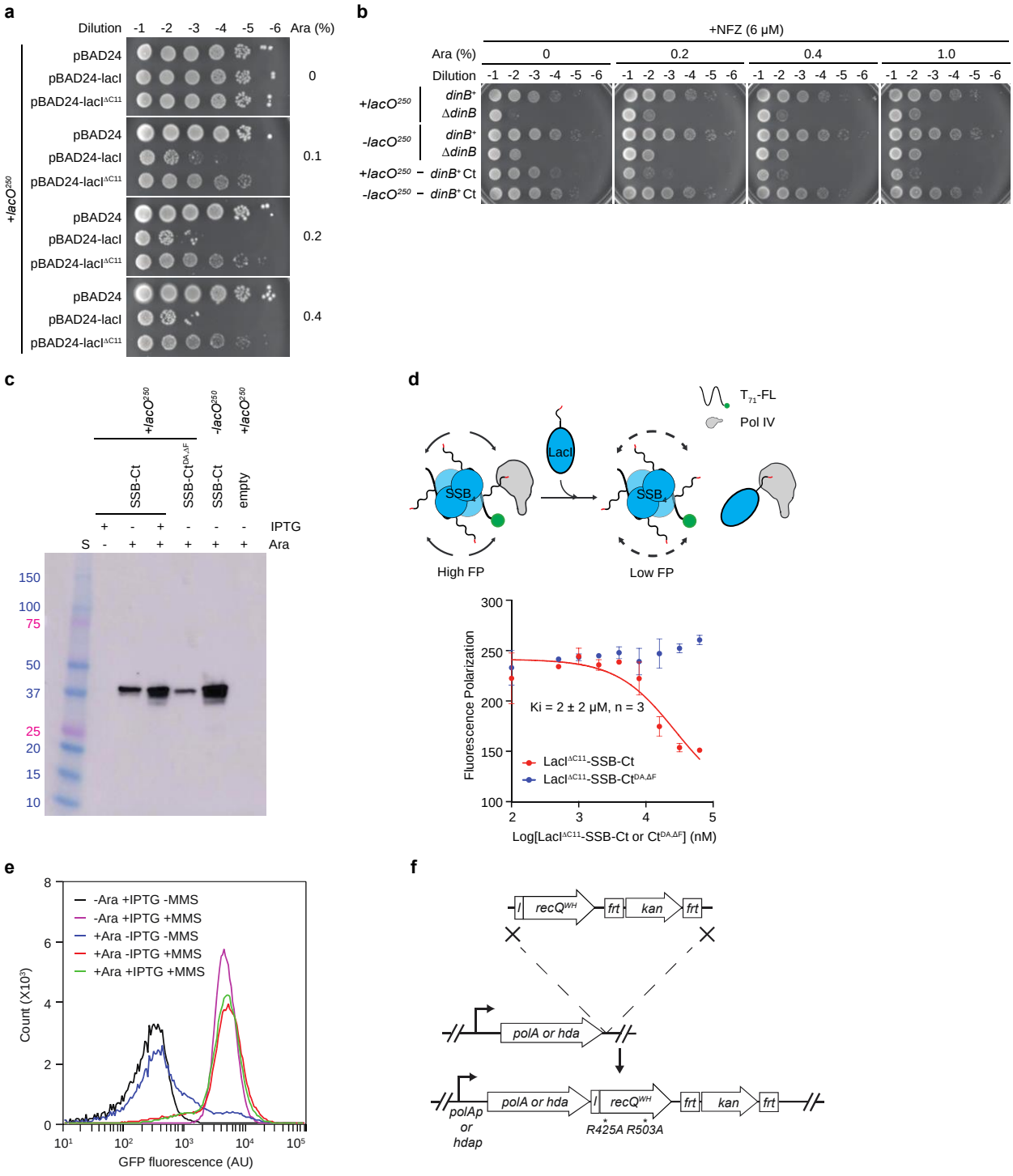
c. Variability of calculated Pol IV-SSB radial distribution function curves for Pol IV and mutants in MMS-treated cells. The 100 *g(r)* curves (thin lines) and mean *g(r)* curves (thick lines) are plotted for each data set.

d. As in B, but for the random *g(r)* curves.

e. Comparable expression of Pol IV-PAmCherry-FLAG and Pol IV^{T120P}-PAmCherry-FLAG in the imaging strains. FLAG-tagged proteins were detected by Western blotting with an anti-FLAG antibody. S. protein size markers; 1, the parent imaging strain; 2, an imaging strain expressing Pol IV-PAmCherry-FLAG; 3, an imaging strain expressing Pol IV^{T120P}-PAmCherry-FLAG. RpoA, a loading control, was probed by Western blotting with an anti-RpoA antibody. Similar results were reproduced twice.



Supplementary Figure 4.
a. The entire gels for the replication products at RL shown in Fig. 4a.
b. The entire gel for the replication products at RL shown in Fig. 4b.



Supplementary Figure 5.

a. Deleting the 11 C-terminal residues of LacI (LacI$^{\Delta C11}$) eliminates the cell-killing activity of over-expressed LacI in the *+lacO^{250}* strain. Over-expressing LacI$^{\Delta C11}$ slightly slowed down cell growth presumably because the LacI$^{\Delta C11}$ cluster temporarily attenuates the movement of replisomes. Similar results were reproduced twice.

b. Expression of LacI$^{\Delta C11}$-SSB-Ct in the absence of the genomic *lacO^{250}* array does not sensitize cells to NFZ. Assay strains bear *pBAD24* or *pBAD24-lacI$^{\Delta C11}$-ssb-Ct* (Ct) where indicated; + or -*lacO^{250}*, presence or absence of the genomic *lacO^{250}* array.
c. Expression of LacI$^{\Delta C11}$-SSB-Ct was not reduced by either addition of IPTG or the lack of the *lacO^{250}* array. Expression of LacI$^{\Delta C11}$ with indicated SSB-Ct appended to the C-terminal end was probed by Western blotting with an anti-LacI antibody. S, size markers; empty, insert-free pBAD24. Similar results were reproduced twice.
d. SSB-Ct in LacI$^{\Delta C11}$-SSB-Ct retains wild-type binding to Pol IV. Interaction between Pol IV and LacI$^{\Delta C11}$-SSB-Ct or SSB-Ct$^{DA,\Delta F}$ was determined by measuring FP. (Top) A competition binding assay scheme. (Bottom) FP changes in the presence of indicated concentrations of His$_6$-LacI$^{\Delta C11}$-SSB-Ct or Ct$^{DA,\Delta F}$. His$_6$-LacI$^{\Delta C11}$-SSB-Ct competitively inhibits the Pol IV-SSB interaction whereas His$_6$-LacI$^{\Delta C11}$-SSB-Ct$^{DA,\Delta F}$ does not. Means ± ranges of three duplicate measurements.
e. Nearly wild-type SOS responses were induced upon MMS treatment in assay strains. The +*lacO^{250}* strain bearing pBAD24-lacIΔC11-ssb-Ct was treated with indicated agents, and the MMS-induced SOS response was measured 90 minutes after the addition of MMS. To eliminate the delay in the expression of LacI$^{\Delta C11}$-SSB-Ct, cells were pre-treated with arabinose overnight and the same concentration of arabinose was present throughout the experiment. Cells were also treated with IPTG in the same way. In subpopulations of cells, induction of LacI$^{\Delta C11}$-SSB-Ct induced varying levels of the SOS response in the absence of MMS. This induction is presumably due to the slowing of replisomes upon encountering the LacI$^{\Delta C11}$-SSB-Ct cluster. In a small fraction of cells (~10%) expressing LacI$^{\Delta C11}$-SSB-Ct (+Ara), the MMS-induced SOS response was only weakly induced. Notably, there is no difference in the MMS-induced SOS response between IPTG-untreated and treated cells. Therefore, IPTG does not eliminate the increased sensitivity to damaging agents of the +*lacO^{250}* strains by influencing the damage-induced SOS response. Similar results were reproduced twice.
f. Addition of the coding sequence for wild-type (RecQ^{WH}) or SSB-binding defective RecQ^{WH} (RecQ$^{WH(R425A,R503A)}$) to the 3' end of native *polA* or *hda* gene. *l*, linker; *polAp* or *hdap*, promoter of *polA* or *hda*; *frt*, flippase recognition site; *kan*, kanamycin resistance gene.