Supplementary information

Supplementary methods

PCNA antibody and immunodepletion

Antibodies against PCNA were previously described [1]. To deplete PCNA from *Xenopus* egg extracts, one volume of protein A-sepharose Fast Flow (GE Healthcare, Piscataway, NJ, USA) was bound to 3 volumes of α-*xl*PCNA serum or pre-immune serum through an overnight incubation at 4°C. Beads were washed and six rounds of depletion were performed for 20 minutes at 22°C using one volume of pre-cleared HSS mixed with 0.2 volumes of the antibody-bound sepharose. Extracts were collected and immediately used for DNA replication experiments.

Preparation of ssDNA templates in the presence of TMPyP4 and TMPyP2 chemical compounds

G4 and non-G4 ssDNA plasmids were incubated with primer A in the presence of potassium as described previously. The indicated doses of the G4 ligand TMPyP4 tetrachloride (Frontier Scientific, Logan, UT, USA) and its related cationic porphyrin TMPyP2 tetrachloride (Frontier Scientific, Logan, UT, USA) were added at 50°C and incubated for 30 minutes after which the mix was slowly cooled down to 20°C.

Sequence analysis of G4 templates

Xenopus egg extracts were used to replicate the indicated ssDNA plasmid templates as previously described. After 120 minutes, replication samples were collected and phenol-chloroform extracted. After extraction, the recovered plasmids were sub-cloned into DH5α

bacterial cells. Single colonies were picked, plasmids were isolated and the G4 site was sequenced using primer B. Double stranded pBluescript plasmids containing the relevant G4 sequences were also directly transformed into DH5α cells after which individual clones were sequenced as control.

Purification of *Xenopus* FANCJ

A cDNA library from *Xenopus laevis* eggs was used to amplify FANCJ cDNA by PCR. Baculoviruses expressing FLAG-*xl*FANCJ were produced using the Baculodirect system following manufacturer instructions (Invitrogen). FANCJ was expressed in suspension Sf9 cell cultures for 65 hours. Cells from 300 mL culture were collected and resuspended in 10 mL lysis buffer (200 mM NaCl, 50 mM Tris pH8, 0.1% NP-40, 0.5 mM PMSF, 1 mM EDTA, 5 % glycerol and 1 tablet of EDTA-free protein inhibitor cocktail (Roche) per 50 mL of lysis buffer). Cells were lysed by sonication and soluble fraction was collected after centrifugation at 13000 rpm for 30 minutes at 4°C. The soluble fraction was incubated for 2 hours at 4°C with 350 μL of anti-FLAG M2 affinity resin (Sigma) that was prewashed twice with lysis buffer. Beads were collected and washed in 10 mL of lysis buffer and 30 mL of wash buffer (200 mM NaCl, 200 mM tris ph8, 10 μg/mL Aprotinin/leupeptin, 0.1% NP-40 and 5 % glycerol). The protein was eluted with wash buffer containing 100 μg/mL 3xFLAG peptide and the FANCJ-containing fractions were flash frozen and stored at -80°C.

Supplementary Figure Legends

Supplementary Figure S1. Primer extension block on G4 plasmid templates depends on the presence of potassium. (A) Schematic representation of primer positions on pBS-G4 plasmid templates indicating the sizes of products generated when replication starting from these primers is blocked at the G4 sequence. **(B**) Primer B was annealed to the non-G4^{G2N} and non-

$G4^{A20}$ plasmid templates and extended using the modified T7 DNA polymerase (sequenase). Extension was stopped at the indicated times, products were separated on 6 % urea-PAGE gels and visualized by autoradiography. **(C)** Primer extension on non-G4 and G4 plasmid templates as described in B. **(D)** The primer extension stalling products after 1 minute (from C) were quantified using ImageQuant software and the percentage of ~200 nt product versus total products that have arrived or bypassed the G4 site are depicted. (**E**) The $G4^{G5N}$ plasmid was subjected to primer extension in the presence or the absence of potassium. Extension was stopped at the indicated time points, reaction products were separated on 6 % urea-PAGE gels and visualized by autoradiography **(F)** The G4 stalling products in (E) were quantified as in (D).

Supplementary Figure S2. PCNA Depletion inhibits ssDNA replication in HSS (A) Mock-depleted and PCNA-depleted HSS were analyzed by Western blotting using *xl*PCNA antibody. A dilution series of undepleted extract was loaded on the same blot to determine the degree of depletion. A relative volume of 100 corresponds to 0.7 μL of HSS. A non-specific band cross-reacting with PCNA antibody is used as loading control ('Loading') **(B)** Mock-depleted and PCNA-depleted HSS were used to replicate $G4^{G3N}$ plasmids starting from primer A. Samples collected at the indicated times were separated on an agarose gel, and visualized by autoradiography.

Supplementary Figure S3. The G4 stalled band corresponds to a product of ~750nt. (A) Schematic representation of a G4 plasmid template showing ApaL1 and XbaI restriction sites. Products after single digestion with ApaL1 and double digestion with ApaL1 and XbaI are depicted on the bottom. **(B)** Non-$G4^{C17}$ and $G4^{endo}$ plasmid templates were replicated in HSS in the absence or the presence of 5 μM of Phen-DC_3 respectively. Samples were collected at the indicated time points, phenol-chloroform extracted and digested with the

indicated restriction enzymes. Samples were separated on 6 % urea-PAGE gels and visualized by autoradiography. The sizes of the digestion products are indicated on the right side. Notice that this gel can not resolve the two products of 1246 and 1740 nt.

Supplementary Figure S4. Effect of G4 stabilizing agents, Phen-DC_3 and TMPyP4, on primer extension with T7 polymerase and on replication in *Xenopus* egg extract. (A) $G4^{G3N}$ and non-$G4^{G2N}$ plasmid templates were extended by the T7 DNA polymerase in the presence or the absence of the indicated doses of the chemical compound Phen-DC_3. Extension was stopped at the indicated time points, products were separated on 6 % urea-PAGE gels and visualized by autoradiography. **(B)** $G4^{G3N}$ plasmid template was replicated in HSS in the presence of various concentrations TMPyP4. Samples were taken at the indicated times points, separated on 6 % urea-PAGE gels and visualized by autoradiography. Products stalled at the G4 ('stalled'), linear molecules resulting from denatured nicked products ('linear'), and closed supercoiled products ('supercoiled'), are indicated. **(C)** Non-$G4^{C3p}$, $G4^{G15}$ and $G4^{G5N}$ plasmids were replicated in HSS in the presence of 8 μM of TMPyP4. Samples were taken at the indicated times points, separated on 6 % urea-PAGE gels and visualized by autoradiography. **(D)** $G4^{G3N}$ plasmid template was replicated in HSS in the presence of the various concentrations of TMPyP2. Samples were taken at the indicated times points, separated on 6 % urea-PAGE gels and visualized by autoradiography.

Supplementary Figure S5. Sequence analysis of G4 plasmids replicated in HSS. Mock-depleted and FANCJ-depleted HSS were used to replicate the $G4^{G3N}$, $G4^{G5N}$ and $G4^{G23}$ plasmid templates. Replicated plasmids were isolated and sequenced. Double stranded pBluescript plasmids containing the $G4^{G3N}$, $G4^{G5N}$ and $G4^{G23}$ sequences were also sequenced (CNT). The total number (after dash) and the number of non-mutated (before dash) sequenced clones are indicated. Asterisk indicates that these clones were sequenced at least three times. Of

note, $G4^{G23}$ plasmid was difficult to sequence suggesting that the sequencing polymerase had problems reading through the G4 sequence.

Supplementary Figure S6. The chemical compound Phen-DC_3 does not induce stalling of the non-G4 plasmid templates in HSS. (A and B) Complete urea-PAGE high-resolution sequencing gels from main Figure 4C and 4D are depicted. **(C)** HSS was used to replicate the $G4^{G3N}$ and the non-$G4^{C3p}$ templates in the presence or the absence of 5μM of Phen-DC_3. Samples were taken at the indicated times and were phenol-chloroform extracted. Replication products were digested with AseI, separated on a high-resolution urea-PAGE sequencing gel and visualized by autoradiography. Sequencing ladder generated by extension of primer A on pBluescript allows the mapping of the replication products. Size markers are indicated on the left of the sequencing ladder. Of note, primer A without the exonuclease-resistant phosphorothioate bonds was used to initiate replication, therefore, the 465 nt product is subject to degradation.

Supplementary Figure S7. Depletion of FANCJ enhances stalling at G-quadruplex structures **(A)** Quantification of FANCJ protein in HSS extract. A known volume of extract was analyzed by western blot alongside a dilution series of recombinant *x*lFANCJ protein. The concentration of FANCJ in HSS was determined by quantification of the bands using ImageQuant Software. **(B)** Mock-depleted and FANCJ-depleted HSS were used to replicate the non-$G4^{C23}$ and the non-$G4^{C5p}$ plasmid template starting from primer A. Replication products were extracted, separated on 6 % urea-PAGE gels and visualized by autoradiography. Linear molecules resulting from denatured nicked products ('linear'), and closed supercoiled products ('supercoiled'), are indicated. **(C)** $G4^{G3N}$, $G4^{G5N}$ and non-$G4^{C5p}$ (from (B)) plasmids were replicated in mock-depleted and FANCJ-depleted HSS. Samples collected at the indicated times were extracted, digested with AseI, separated on a high-

resolution urea-PAGE sequencing gel, and visualized by autoradiography. The sections of the sequencing gels containing the stalled replication products are depicted. Replication stalling sites were mapped and depicted on the relevant G4 sequences below the gels. **(D)** Overexpression and purification of FLAG-*xl*FANCJ from Sf9 insect cells. Coomassie Brilliant Blue stained SDS-PAGE gel showing total Sf9 cell lysate before (-) and after (+) infection of the cells with a virus expressing *xl*FANCJ. Cells were harvested 72 hours after infection. Cell soluble (sol) and insoluble (in) fractions were collected. Soluble fraction contained recombinant *xl*FANCJ. Recombinant FANCJ was bound to anti-FLAG beads (beads) and eluted with 3xFLAG peptide (final). **(E)** Mock-depleted and FANCJ-depleted HSS were used to replicate $G4^{G23}$ and $G4^{G5N}$ starting from primer A. Replication products were extracted, separated on 6 % urea-PAGE gels and visualized by autoradiography. Products stalled at the G4 ('stalled'), linear molecules resulting from denatured nicked products ('linear'), and closed supercoiled products ('supercoiled'), are indicated.

Supplementary References

1. Kochaniak, A.B., et al., *Proliferating cell nuclear antigen uses two distinct modes to move along DNA.* J Biol Chem, 2009. **284**(26): p. 17700-10.