SUPPLEMENTARY DATA

Supp. Data Fig. 1. FANCJ sequestration analyses with undamaged or thymine glycol forked duplex DNA competitor molecules. Sequestration assays with 9.6 nM FANCJ and the indicated concentrations (0 – 12.5 nM) of the specified forked duplex competitor DNA molecules were performed as in Fig. 6, but pre-incubated for 10 min with competitor DNA. 70% of the tracker DNA substrate was unwound in the absence of competitor DNA. Quantitative analyses of the helicase data (mean of at least three independent experiments with SD indicated by error bars) are shown. Filled circles, competitor forked duplex with thymine glycol in top (translocating) strand (TG-C); open squares, competitor forked duplex with thymine glycol in bottom (non-translocating) strand (TG-B), filled squares, competitor control undamaged forked duplex.

Supp. Data Fig. 2. RPA binding to forked duplex DNA substrate containing a single thymine glycol compared to undamaged DNA. *Panels A and B,* Increasing concentrations of RPA were incubated with either 5 fmol forked duplex (*Panel A*) or blunt duplex (*Panel B*) containing thymine glycol in the top strand (TG-C), bottom strand (TG-B), or not at all as indicated under standard gel-shift conditions as described in Materials and Methods. The DNA-protein complexes were resolved on native 6% polyacrylamide gels. Phosphorimages of typical gels are shown. *Panel C,* Quantitative analyses of RPA binding to the forked duplex and blunt duplex substrates are shown. Filled square, control undamaged forked duplex; open square, forked duplex with thymine glycol in bottom strand; filled circle, forked duplex with thymine glycol in top strand; filled triangle, control undamaged blunt duplex; open triangle, blunt duplex with thymine glycol in bottom strand; open circle, blunt duplex with thymine glycol in top strand. Experiments were repeated at least three times and means are shown with SD indicated by error bars.

Supp. Data Fig. 3. Secondary structure analysis of the thymine glycol-containing 66-mer. Shown is the predicted minimum free energy secondary structure for the 66-mer. The position of the thymine glycol is indicated by an arrow. For reference, specific nt positions are indicated.

Supp. Data Fig. 4. Effect of RPA on FANCJ helicase activity on forked duplex substrates with thymine glycol in the ssDNA arms of the DNA substrate. Helicase reaction mixtures contained 3 nM RPA, 1.2 nM FANCJ, and 0.5 nM forked DNA substrate with thymine glycol in the ssDNA arm of the bottom strand (TG26-B) or top strand (TG41-C) (Table I) under standard helicase assay conditions as described under Materials and Methods for the indicated times from 0-32 min. Quantitative analyses of FANCJ helicase data are shown (open squares, FANCJ; filled squares, FANCJ + RPA).

Fig. S1

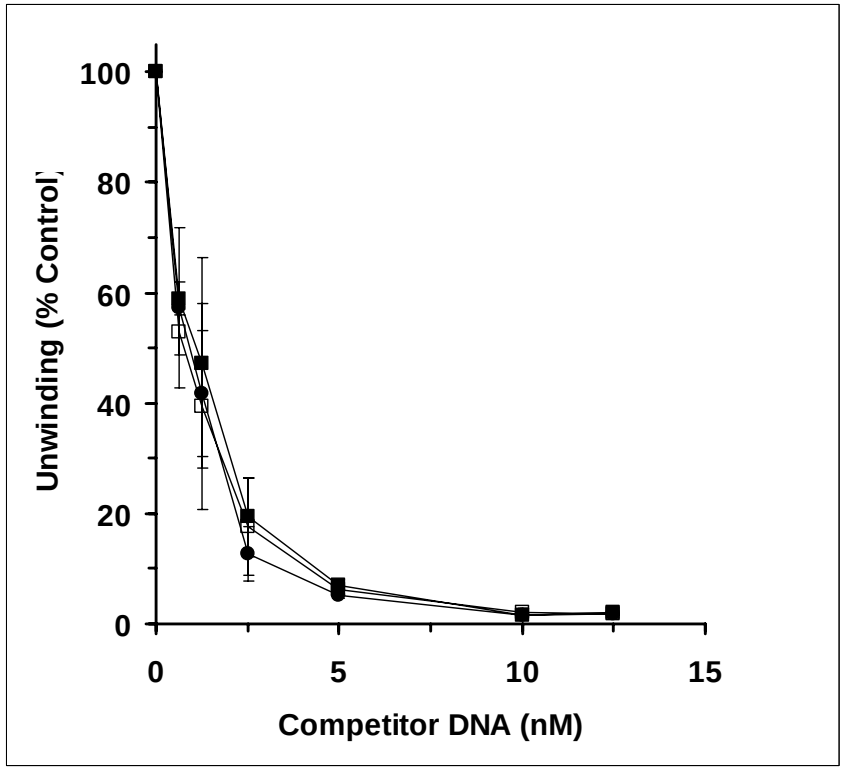


Fig. S2

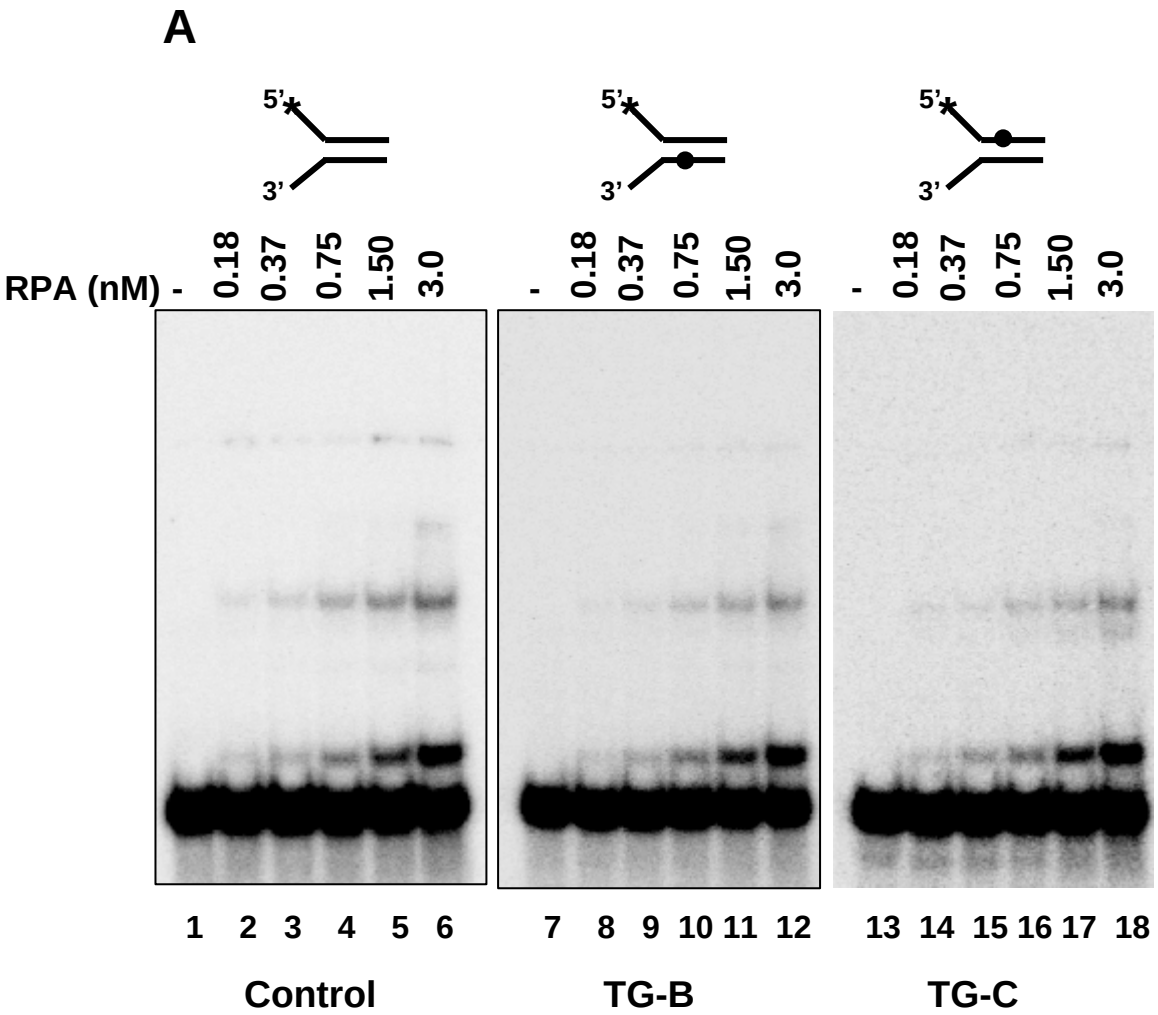

A
5'
3'
RPA (nM) - 0.18 0.37 0.75 1.50 3.0
1 2 3 4 5 6
7 8 9 10 11 12
13 14 15 16 17 18
Control
TG-B
TG-C

Fig. S2

B

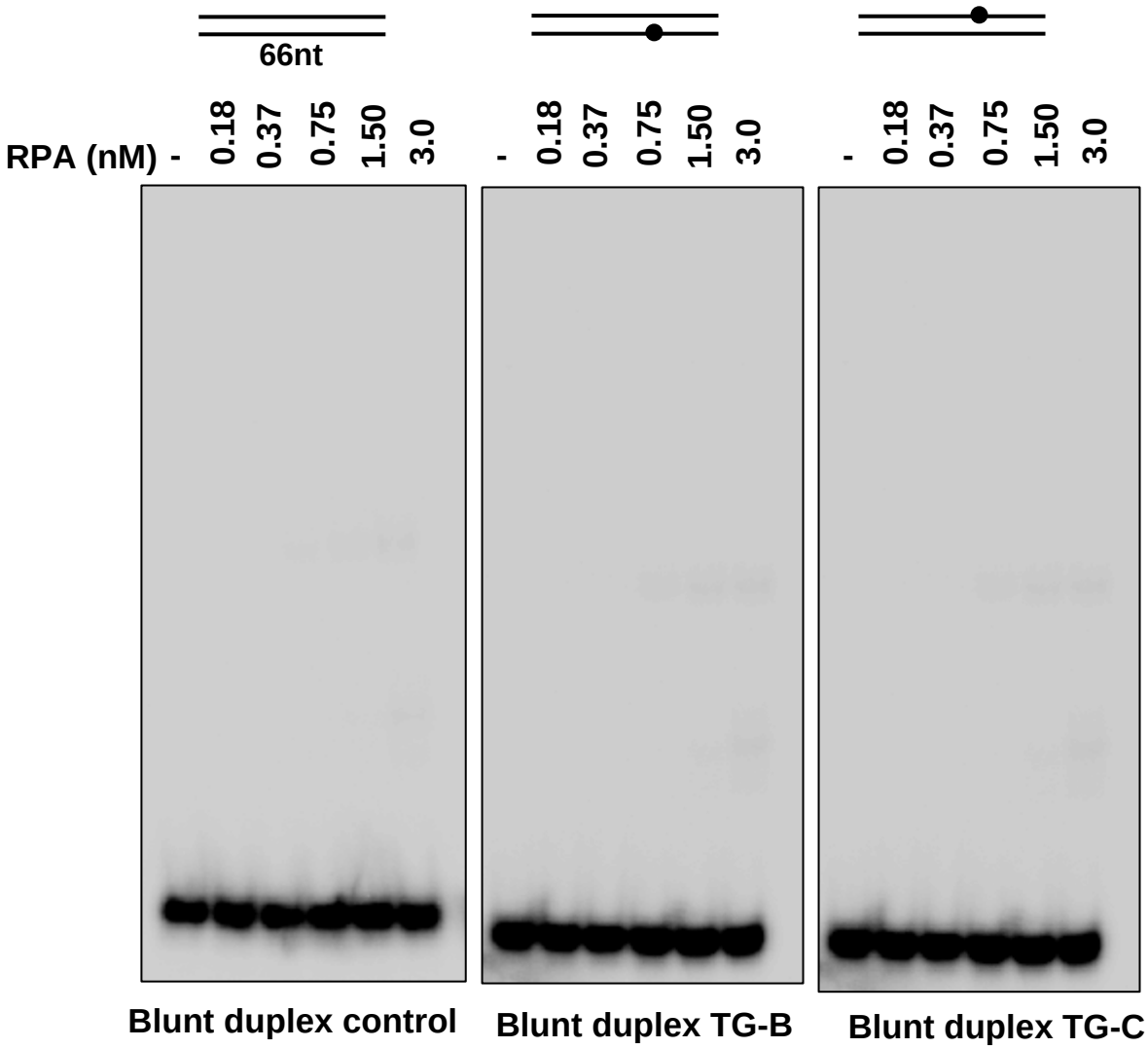

66nt
RPA (nM) - 0.18 0.37 0.75 1.50 3.0
- 0.18 0.37 0.75 1.50 3.0
- 0.18 0.37 0.75 1.50 3.0
Blunt duplex control
Blunt duplex TG-B
Blunt duplex TG-C

Fig. S2

C

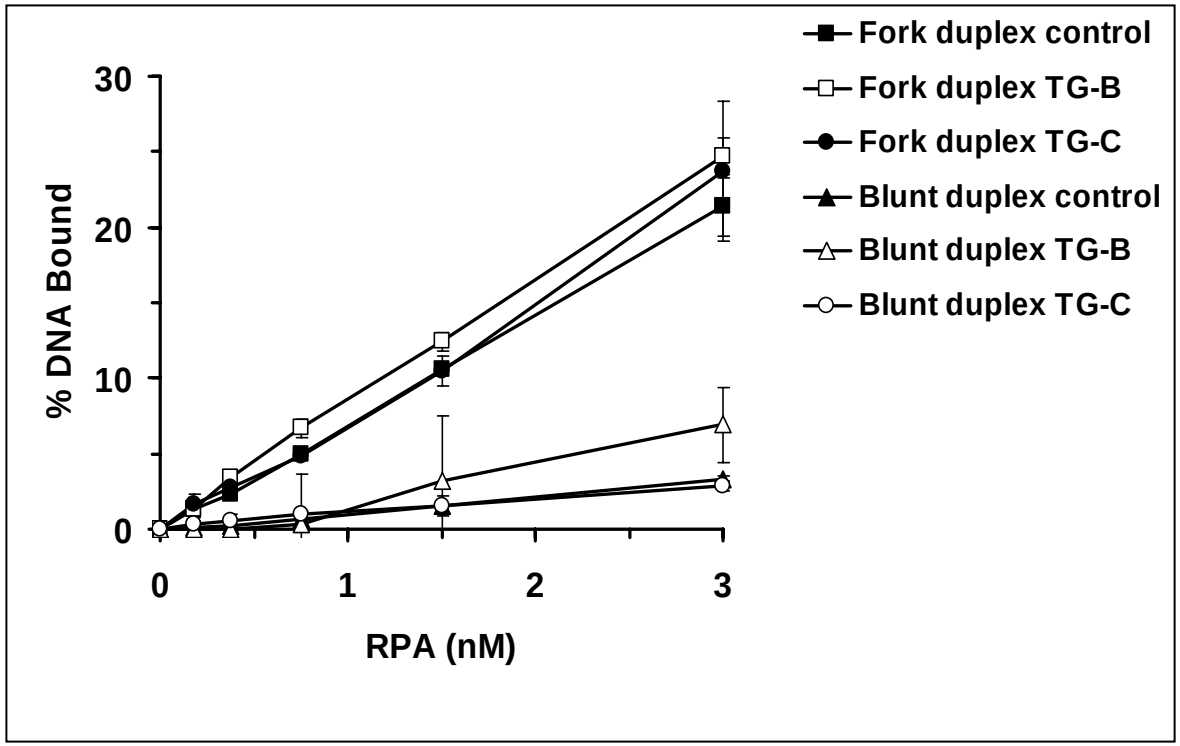

Fork duplex control
Fork duplex TG-B
Fork duplex TG-C
Blunt duplex control
Blunt duplex TG-B
Blunt duplex TG-C
% DNA Bound
30
20
10
0
0
1
2
3
RPA (nM)

Fig. S3

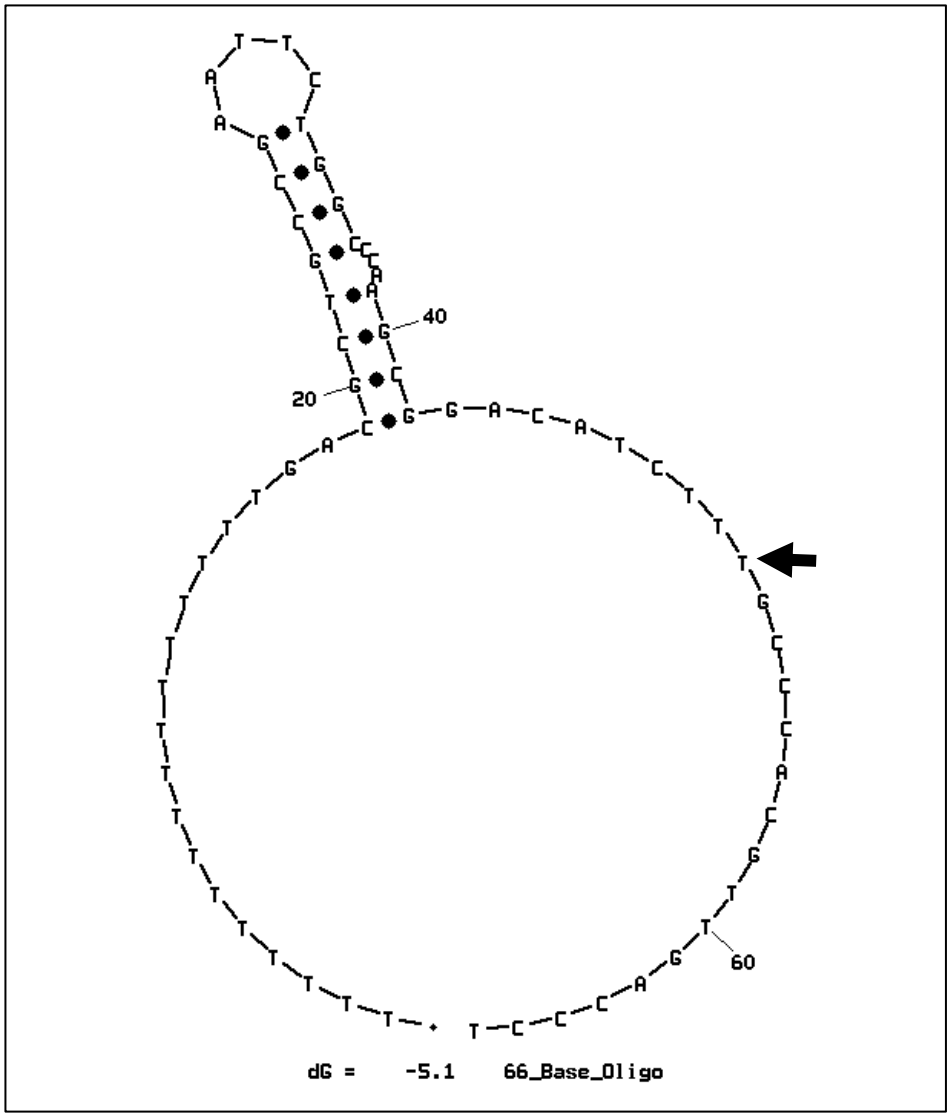
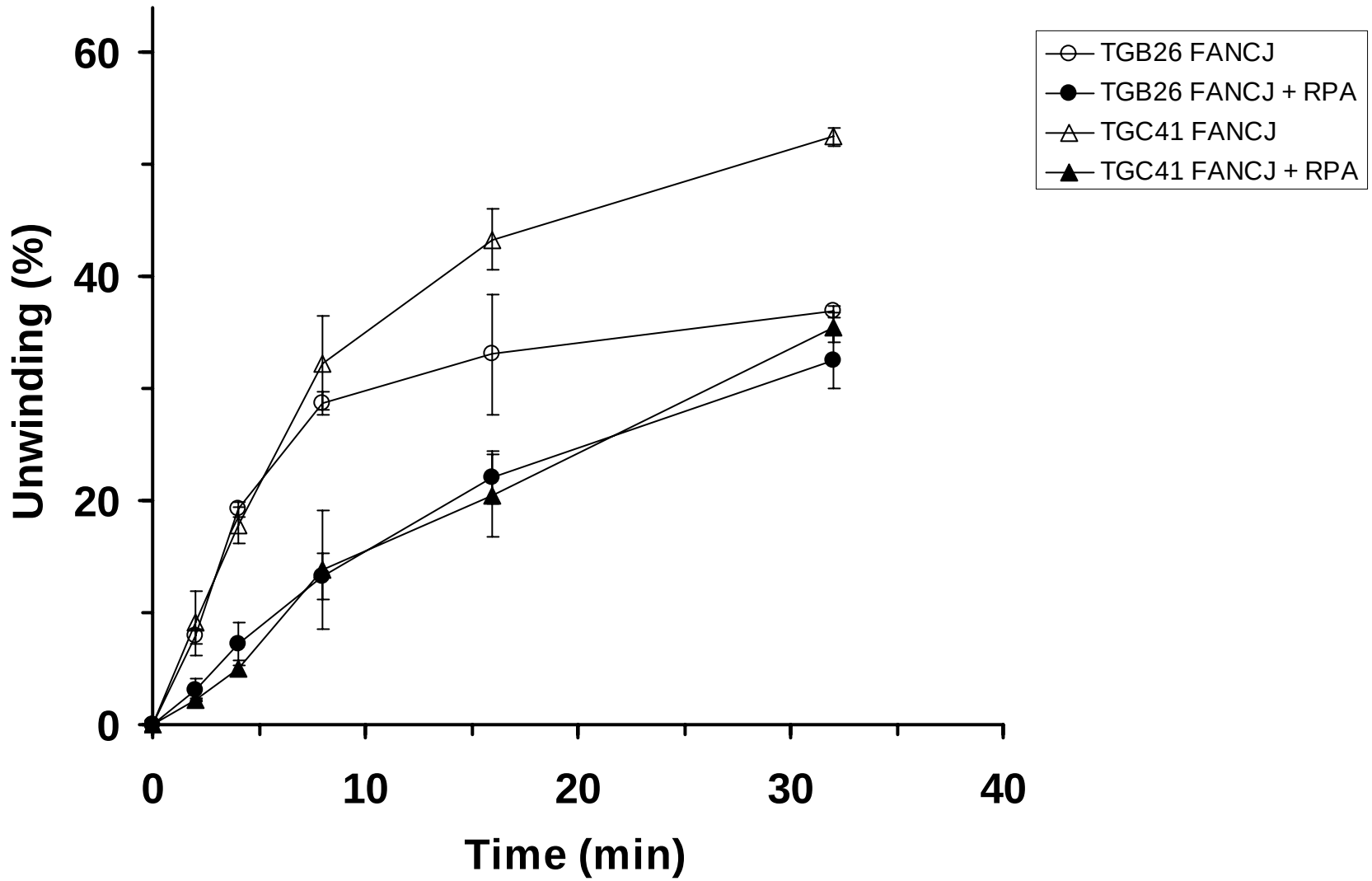


Fig. S4


Unwinding (%)
60
40
20
0
0
10
20
30
40
Time (min)
TGB26 FANCJ
TGB26 FANCJ + RPA
TGC41 FANCJ
TGC41 FANCJ + RPA