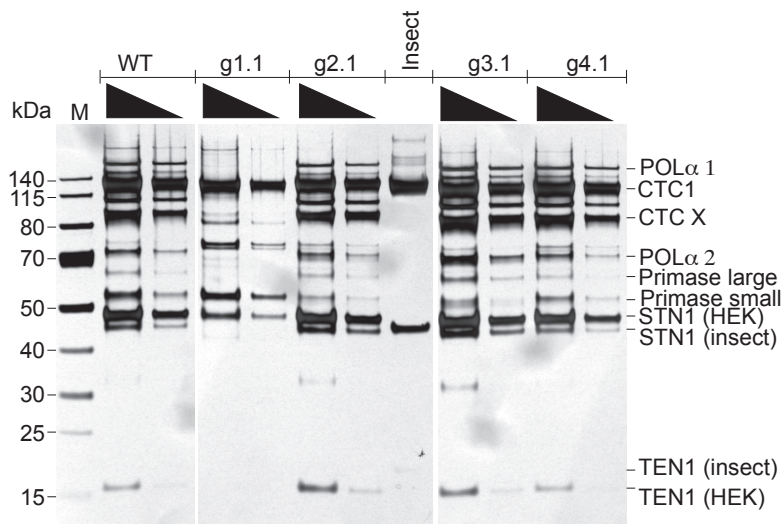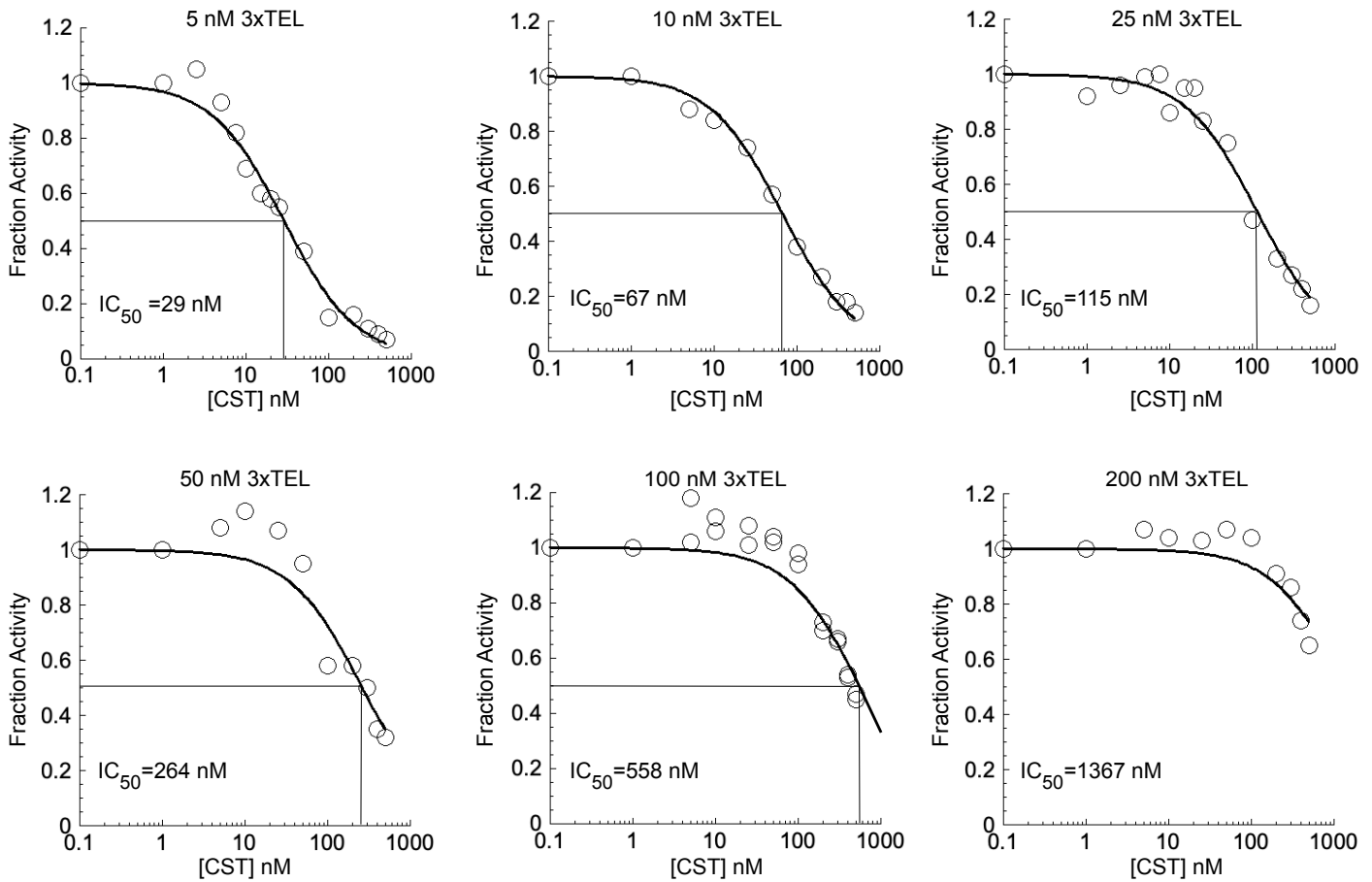Supplementary Information

CST does not evict elongating telomerase but prevents initiation by ssDNA binding

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Supplementary Figure S1. SDS-PAGE analysis of CST protein preparations. Wedges represent two-fold dilutions. Gels were silver-stained. CTC1 X is the CTC1 isoform described in the text. Western blot analysis confirmed that the band in g1.1 co-migrating with primase small is not primase small. Recombinant CST expressed in insect cells (22) gave STN1 and TEN1 with slightly different mobility than those expressed in HEK293T cells due to different N-terminal tags. TEN1 was close to the limit of detection, so its presence was evaluated by western blotting (Figure 1B).

Supplementary Figure S2. Inhibition of telomerase activity by pre-incubation with WT CST protein. Each point represents a lane on telomerase activity gel, with total radioactivity incorporated normalized to that obtained with no CST added. 3xTEL primer concentrations varied as indicated. Data points were fit with equation 3. The 200 nM 3xTEL data do not extend to 50% inhibition, so the IC_{50} is approximate. In this and all of the following telomerase inhibition experiments, CST concentration was determined using the anti-STN1 antibody (see Materials and Methods).
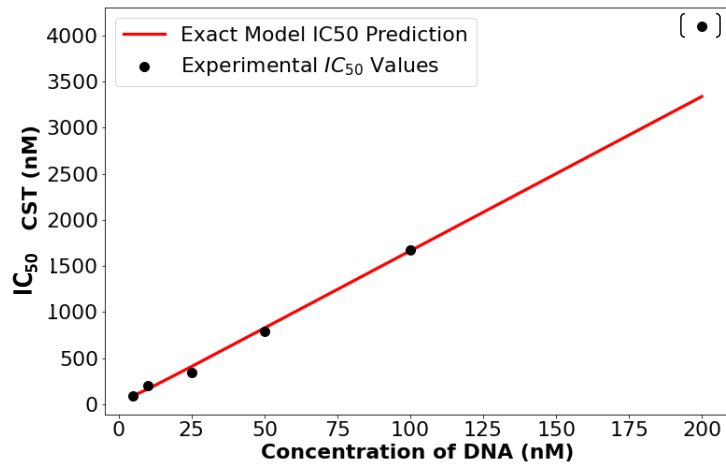
Supplementary Figure S3. Fitting of experimentally determined IC$_{50}$ values. IC$_{50}$ values obtained from telomerase assays with varying concentrations of DNA (black points) plotted with IC$_{50}$ curve predicted by competitive binding simulation. IC$_{50}$ values were predicted with the exact competitive binding equation for the six DNA concentrations used in telomerase-CST inhibition experiments (5 nM, 10 nM, 25 nM, 50 nM, 100 nM, and 200 nM) using a K_{d_A} of 2.20 nM (as determined by FA), a K_{d_B} of 0.324 nM (as previously optimized) and gamma of 0.465 (as previously optimized). Fraction bound values were calculated for each element of an array of 10,000 CST concentrations linearly spaced between 10 nM and 10,000 nM. The concentration of CST that resulted in a fraction bound value closest to 0.5 was returned as the (CST) IC$_{50}$ value for each concentration of DNA. Due to the linear spacing of CST concentrations, the predicted IC$_{50}$ values are within 1 nM of exact predictions. Parentheses on last data point indicate that it is underdetermined, because only partial inhibition was achieved at the highest CST concentration. Experimental IC$_{50}$ values were corrected by a factor of 3 relative to those reported in Supplementary Figure S2 to be consistent with K_d values (see Material and Methods).
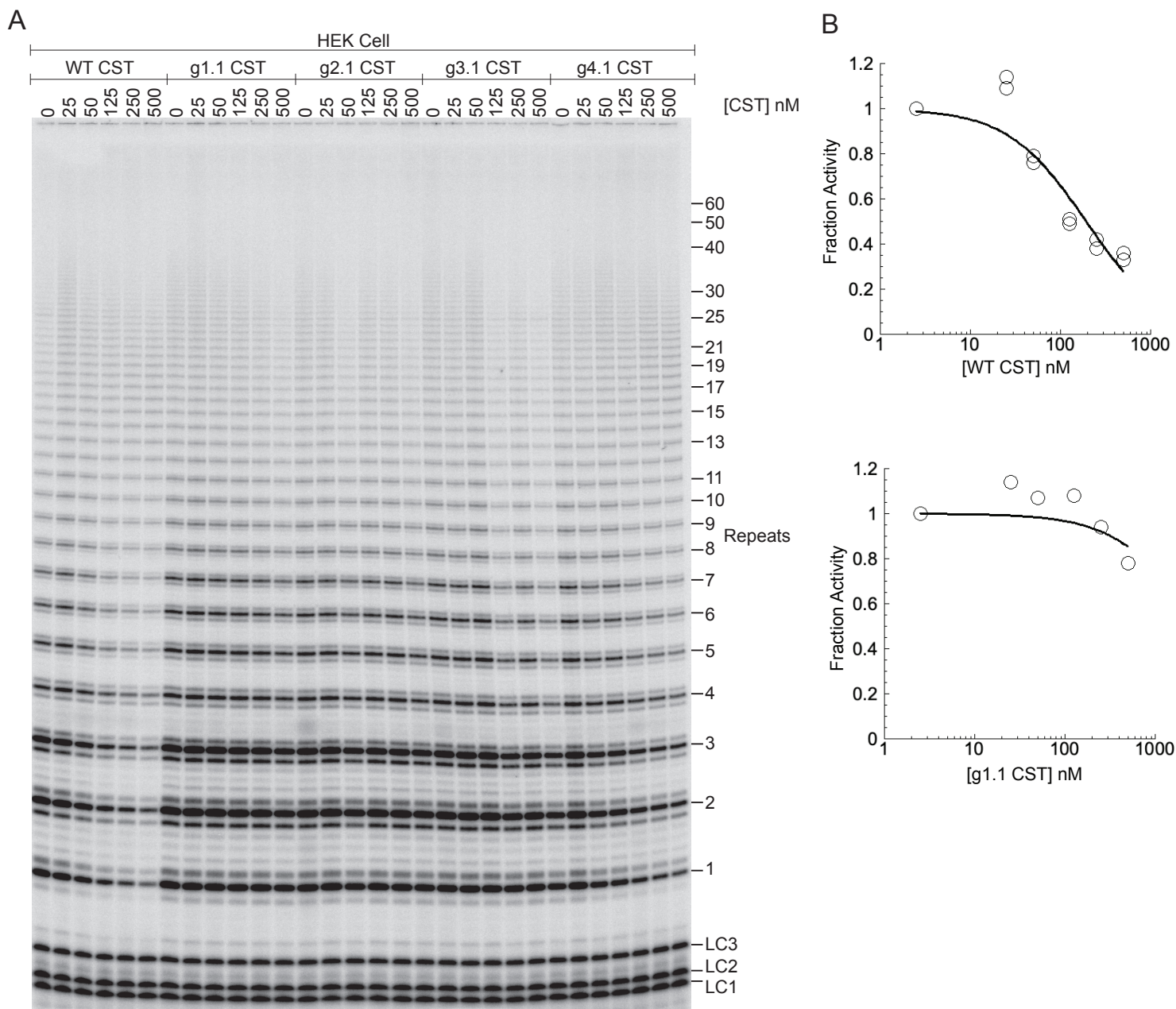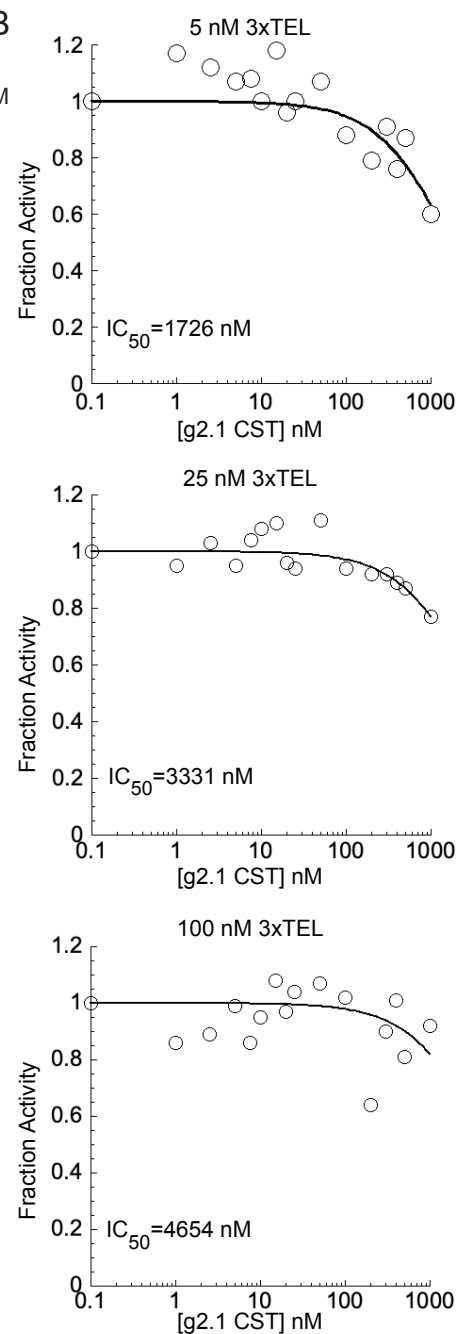
Supplementary Figure S4. Survey of telomerase inhibition by WT and mutant CST proteins. (**A**) Telomerase reactions with standard 10 nM 3xTEL primer for 60 min. (**B**) Quantification of WT and g1.1 inhibition, fit with equation 3; the other mutants are analyzed in more detail in Supplementary Figures S5, S6, and S7.
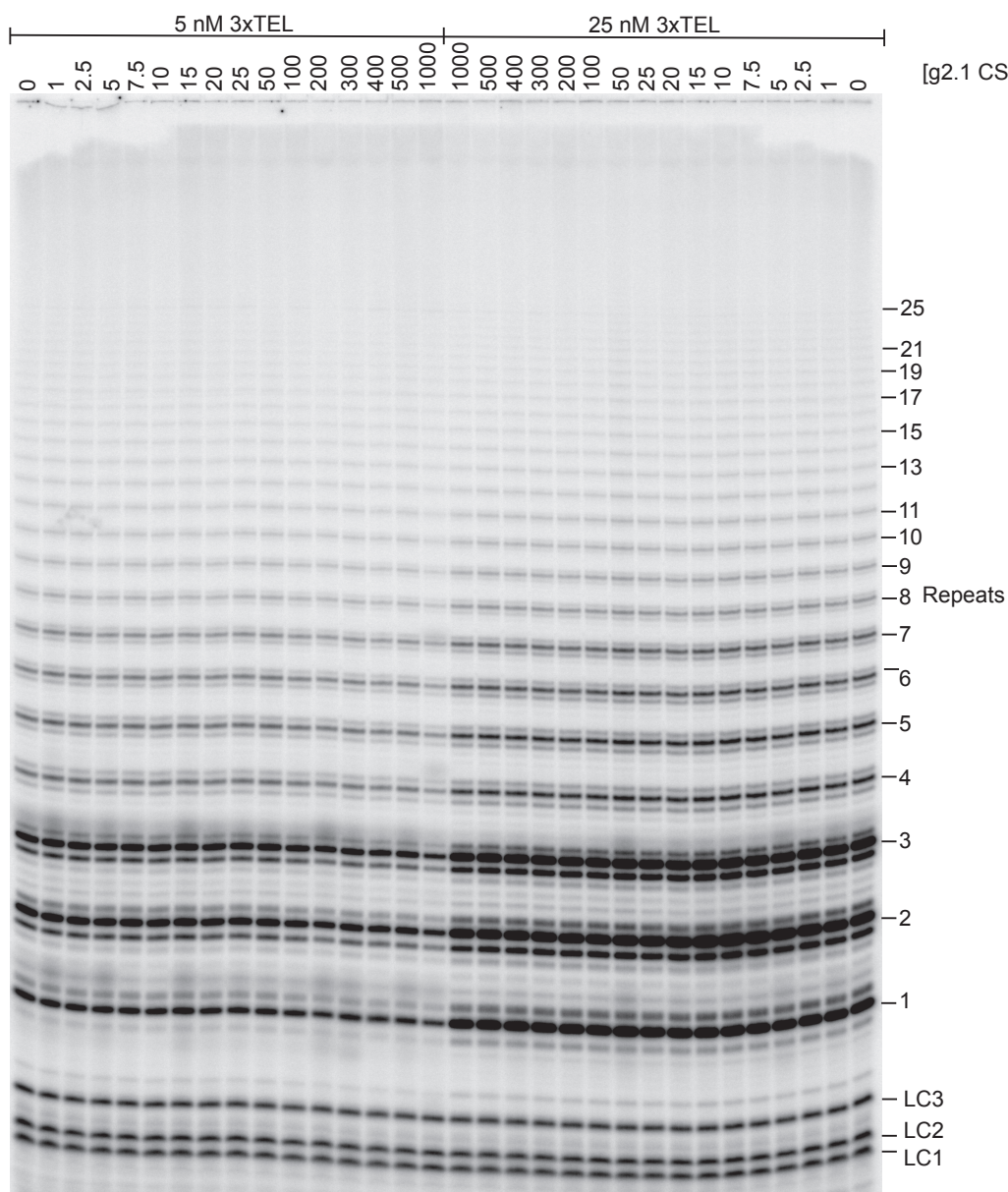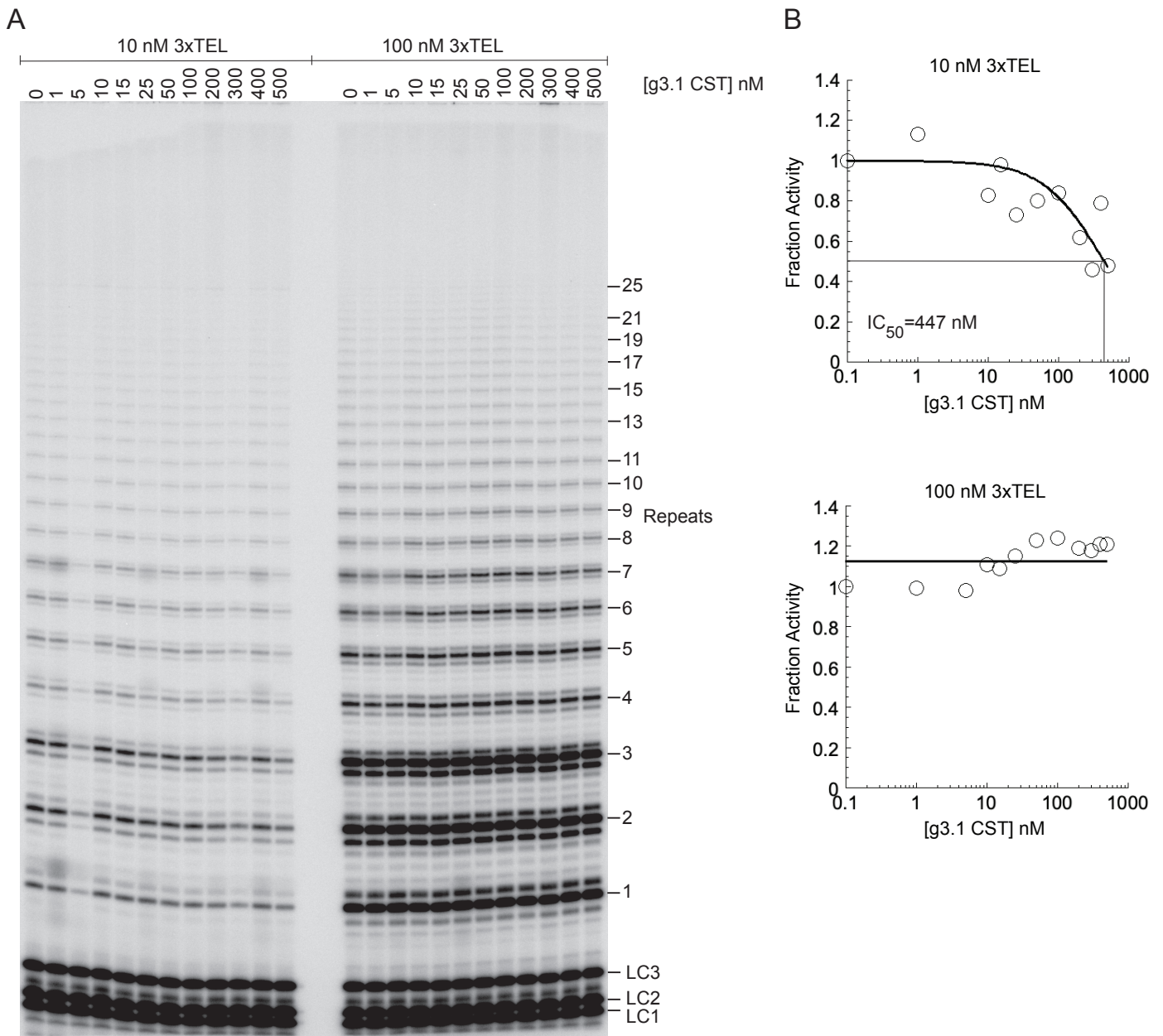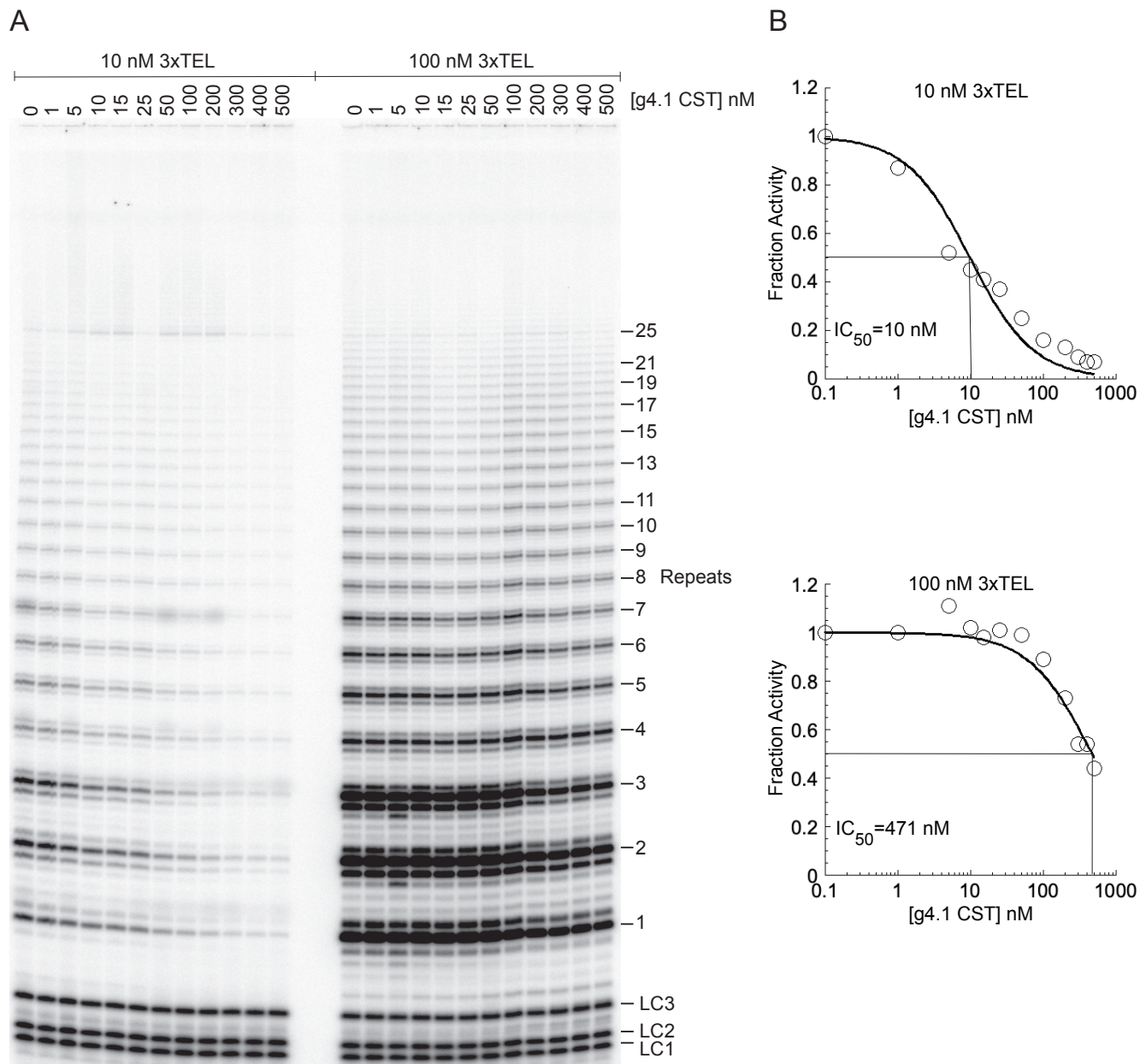
Supplementary Figure S5. Inhibition of telomerase activity by g2.1 mutant CST protein.
(**A**) Gel electrophoretic analysis of telomerase activity at two 3xTEL DNA primer concentrations.
(**B**) Quantification of counts in each lane of panel (A) and an additional experiment at 100 nM primer, fit with equation 3. The calculated IC_{50} values at 25 and 100 nM 3xTEL are included for completeness, but they are not expected to be accurate because so little inhibition was observed at the highest CST concentrations tested.

Supplementary Figure S6. Inhibition of telomerase activity by g3.1 mutant CST protein.
(**A**) Gel electrophoretic analysis of telomerase activity at two 3xTEL DNA primer concentrations.
(**B**) Quantification of counts in each lane of panel (A) fit with equation 3.
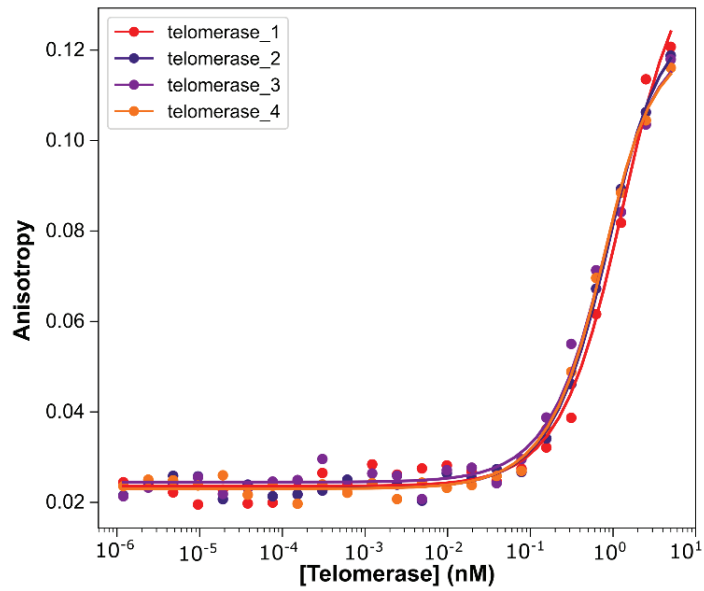
Supplementary Figure S7. Inhibition of telomerase activity by g4.1 mutant CST protein.(**A**) Gel electrophoretic analysis of telomerase activity at two 3xTEL DNA primer concentrations. (**B**) Quantification of counts in each lane of panel (A) fit with equation 3.
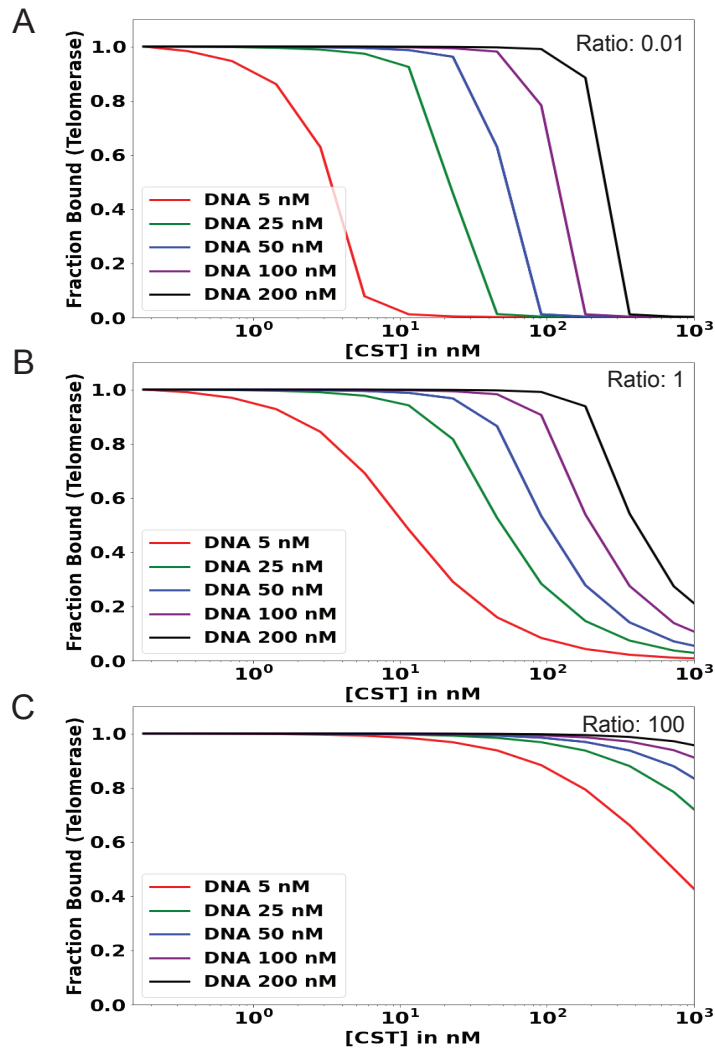
Supplementary Figure S8. Validation of the simulation of telomerase inhibition by CST. Simulations were performed under the following conditions: γ = 1.0 (100% active CST), concentration of telomerase = 2.0 nM, and a concentration range of WT CST from 0.0 to 1,000 nM. Predicted fraction bound curves for telomerase to DNA were plotted with initial DNA concentrations of 5 nM, 25 nM, 50 nM and 100 nM. This was done for three different values of CST-DNA, K_{dB}, and telomerase-DNA, K_{dA}: 0.01, 1, and 100 nM. The python Matplotlib graphics package (25) was used to plot the simulated data.

	telomerase_1	telomerase_2	telomerase_3	telomerase_4
Kd (nM)	0.9	0.48	0.39	0.37
S	0.121	0.1056	0.0989	0.0995
O	0.0236	0.0231	0.0245	0.023
R^2	0.988	0.9962	0.9888	0.9964

Supplementary Figure S9. Equilibrium binding constant of telomerase to 3xTEL DNA determined by FP.