Supporting Information

G-Quadruplex DNA- and RNA-Specific-Binding Proteins Engineered from the RGG Domain of TLS/FUS

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Primer		DNA sequense
RGGF	Δ468-484 forward	d(CGG GGC CGC GGC GGG GAC CG)
	Δ468-484 reverse	d(GTT ACC CCC CAT GTG AGA GCC ACC)
RGGY	Δ489-506 forward	d(GGC CCT GGC AAG ATG GAT TCC AGG)
	Δ489-506 reverse	d(GTC CCC GCC GCG GCC CCG G)
RGGF/A	F494A forward	d(GGA GGC GCC CGA GGG GGC CGG GG)
	F494A reverse	d(ACG GTC CCC GCC GCG GCC CCG G)
	F506A forward	d(GCT GGC CCT GGC AAG ATG GAT TCC)
	F506A reverse	d(GCC ACC TCT GTC CCC ACC ACC)
	Δ468-484 forward	d(CGG GGC CGC GGC GGG GAC CG)
	Δ468-484 reverse	d(GTT ACC CCC CAT GTG AGA GCC ACC)
RGGY/A	Y468A forward	d(GCT GAT CGA GGC TAC CGG G)
	Y468A reverse	d(GCC TCC TCT GCC ACC ACG ACG)
	Y479A, Y484A forward	d(GGG CCG CGG GGA CCG T)
	Y479A, Y484A reverse	d(CGG GCG CCG CCT CGA TCA GCG)
	Δ489-506 forward	d(GGC CCT GGC AAG ATG GAT TCC AGG)
	Δ489-506 reverse	d(GTC CCC GCC GCG GCC CCG G)
RGG3	Δ1-448 forward	d(GCC CCT AAA CCA GAT GGC CC)
	Δ1-448 reverse	d(GGA TCC ACT AGT CCA GTG TGG TGG)

Table S1. Primer sequences used in this study.

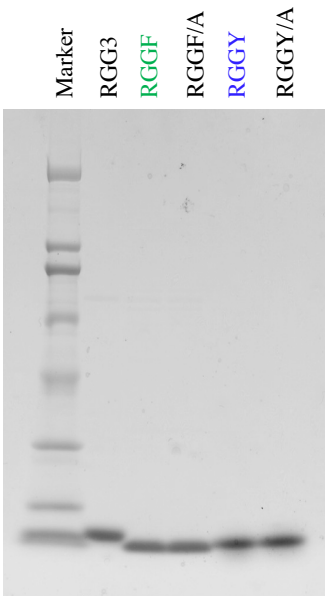

FIGURE S1. SDS-PAGE of mutated RGG3 in TLS on 5-20 % gradient polyacrylamide gel. *E. coli* strain BL21 (DE3) pLysS-competent cells were transformed with the vectors to express each proteins as GST-fused proteins. The proteins were purified by glutathione agarose and GST-tag was digested by precision protease. Lane 1, molecular weight makers ; Lane 2, RGG3 (8.5 kDa); Lane 3, RGGF (6.6 kDa); Lane 4, RGGF/A (6.5 kDa); Lane 5, RGGY (6.9 kDa); Lane 6, RGGY/A (6.7 kDa) each 10 μg with heating and in reducing conditions. Molecular weight markers (kDa): pig myosin (200), *Escherichia coli* β-galactosidase (116), rabbit muscle phosphorylase B (97.2), bovine serum albumin (66.4), hen egg white ovalbumin (44.3), bovine carbonic anhydrase (29), soybean trypsin inhibitor (20.1), hen egg white lysozyme (14.3), bovine pancreas aprotinin (6.5).

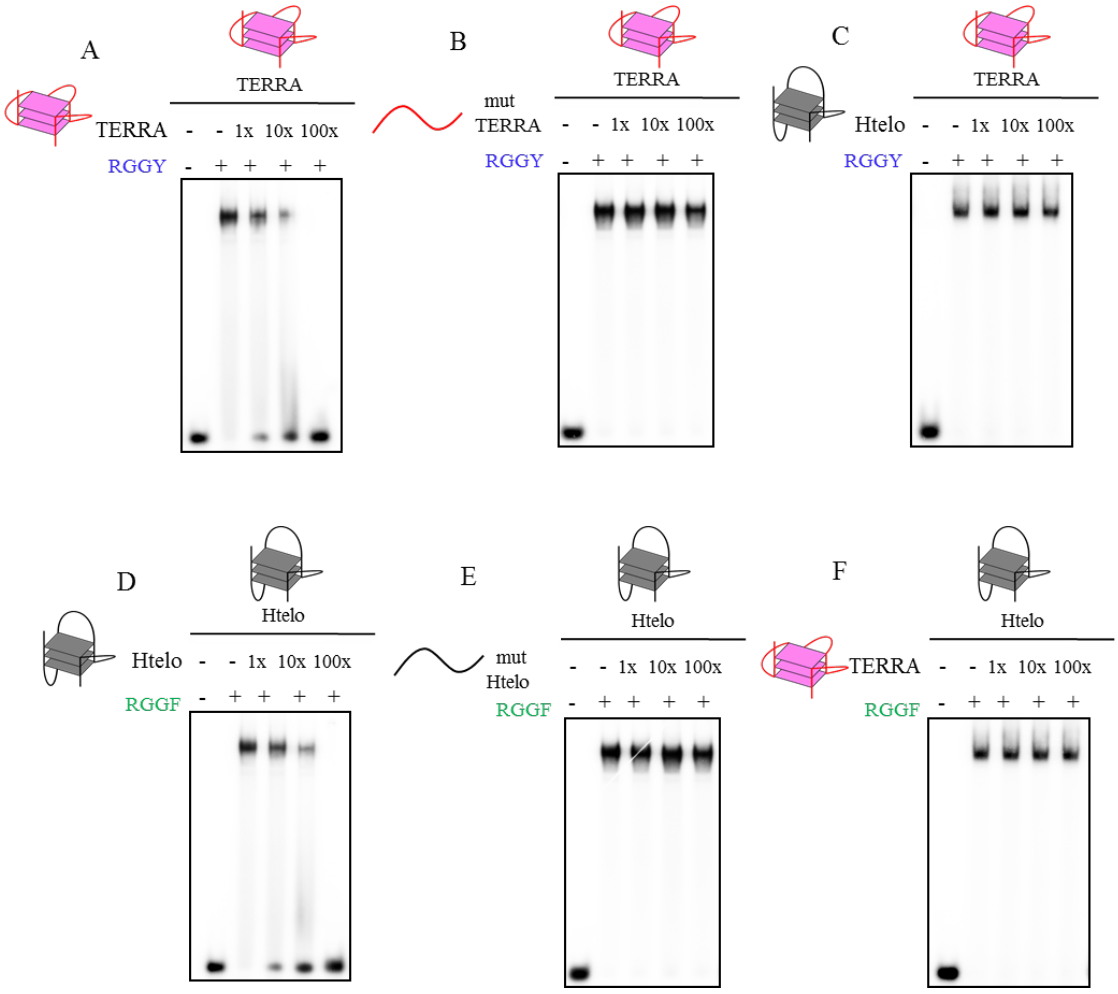

FIGURE S2. G-quadruplex DNA or RNA binding specificity of RGGY and RGGF. Binding competition assay, assaying binding of RGGY to ^{32}P-labeled TERRA in the presence of unlabeled TERRA (A), mutTERRA (B) or Htelo (C) or RGGF to ^{32}P-labeled Htelo in the presence of unlabeled Htelo (D), mutHtelo (E) or TERRA (F) at the indicated molar ratios of unlabeled/labeled nucleic acids. The DNA- or RNA-protein complexes were resolved by 6% polyacrylamide gel electrophoresis and visualized by autoradiography.

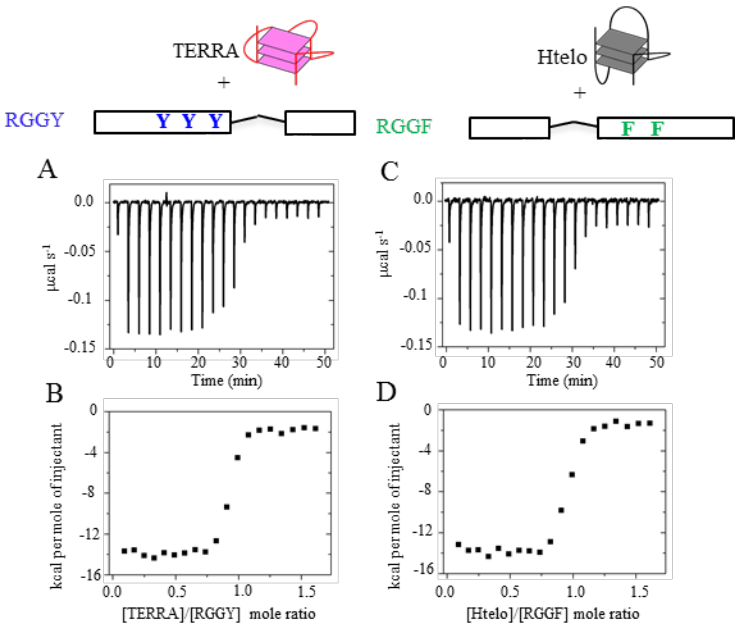

FIGURE S3. Binding affinities and stoichiometries of RGGY or RGGF and TERRA or Htelo measured by ITC. Raw calorimetric data for titration of RGGY (3 μM) (A) or RGGF (3 μM) (C) solution in potassium buffer (50 mM Tris-HCl [pH 7.5], 100 mM KCl) with serial injections of TERRA-(A) or Htelo-(C) containing solution (24 μM) in the potassium buffer at 25 °C. Titrations comprised 20 injections of nucleic acids (one 0.5-μL injection followed by nineteen 2-μL injections). Binding isotherms (B, D) resulting from the integration of raw calorimetric data corrected for the dilution heat of each nucleic acid were normalized as kilocalories per mole of the injected nucleic acids.

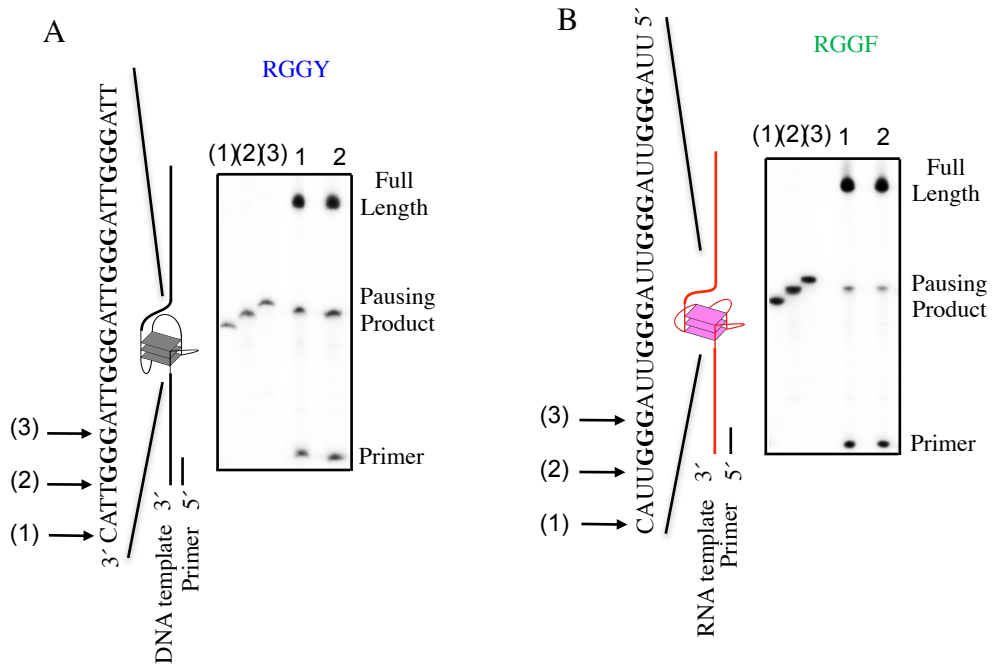

FIGURE S4. DNA polymerase stop assays with RGGY and reverse transcriptase assays with RGGF. (A) DNA polymerase stop assays in the presence of RGGY. (B) Reverse transcriptase assays in the presence of RGGF. The protein concentrations were 0 μM (lane 1) and 3 μM (lane 2). (1), (2), and (3) indicate DNA markers

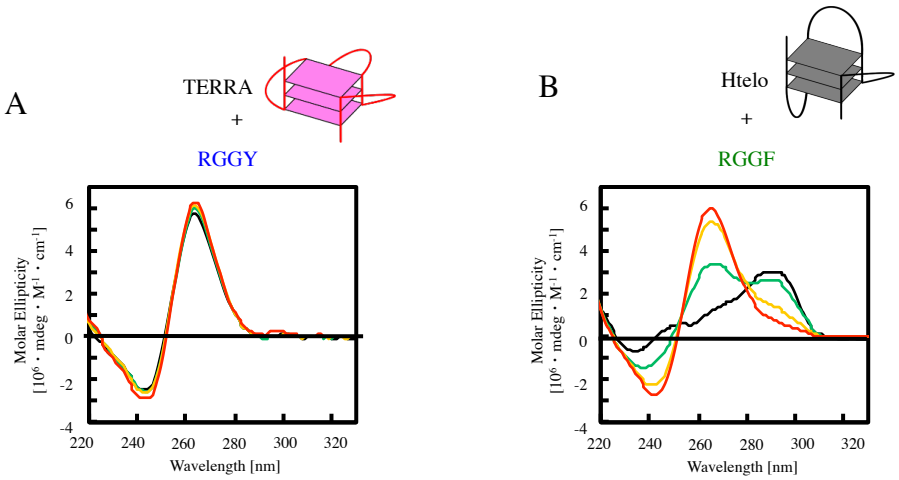

FIGURE S5. Circular dichroism spectra of TERRA or Htelo in the presence of various amounts of RGGY or RGGF. Titration of TERRA with RGGY (5, 2, 1, and 0 equiv RGGY) (A) or Htelo with RGGF (5, 2, 1, and 0 equiv RGGF) (B) in 10 mM KCl and 50 mM Tris-HCl (pH 7.5). The concentration of DNA was 0.2 mM base concentration. Line colors: black = 0 eq.; green = 1 eq.; yellow = 2 eq.; red = 5 eq.

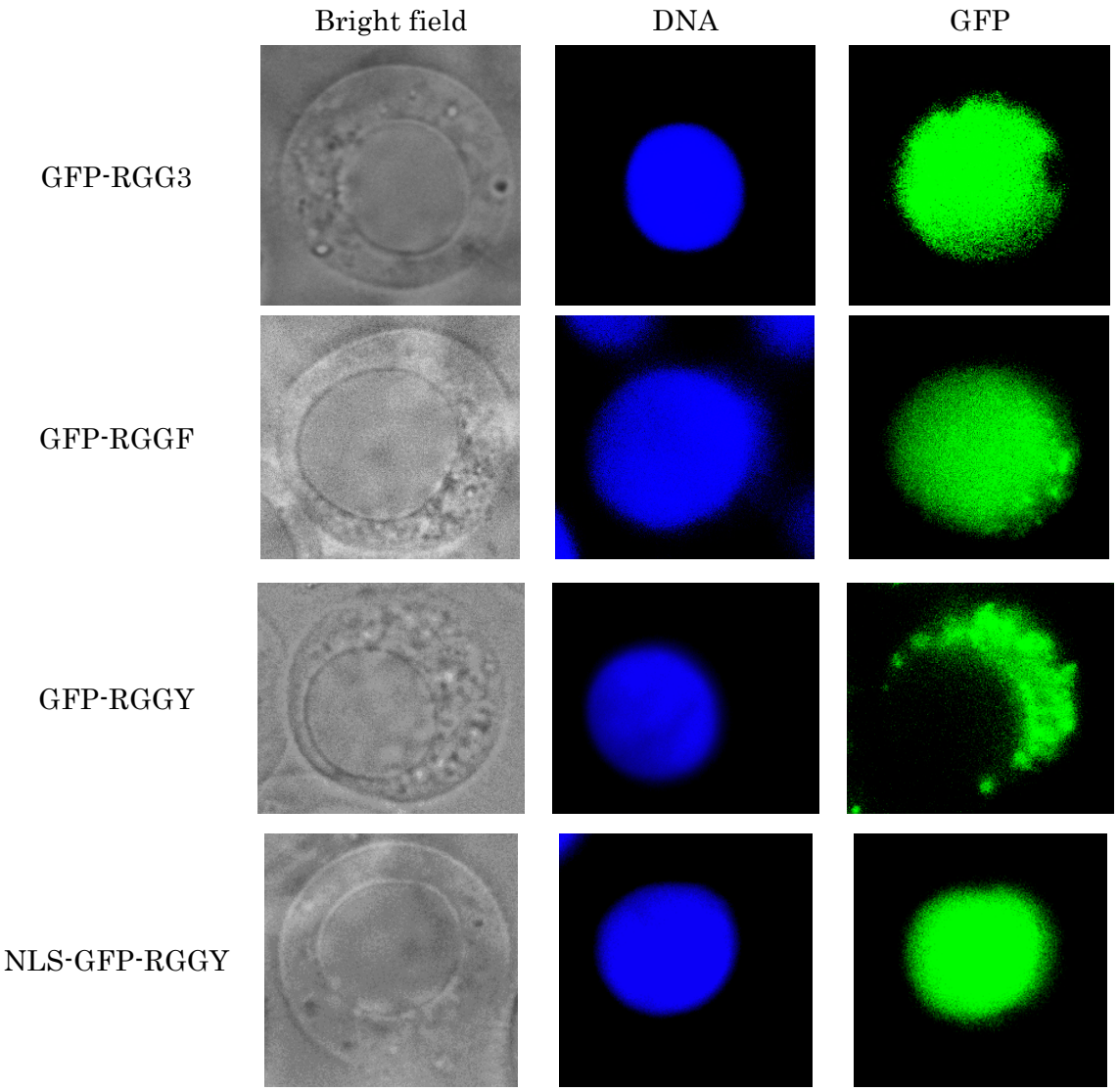

FIGURE S6. Identification of a localization about each proteins in HeLa cells. HeLa cells were transiently transfected with GFP-RGG3, GFP-RGGF, GFP-RGGY and NLS-GFP-RGGY. Nuclei were counterstained with hoechst (blue).

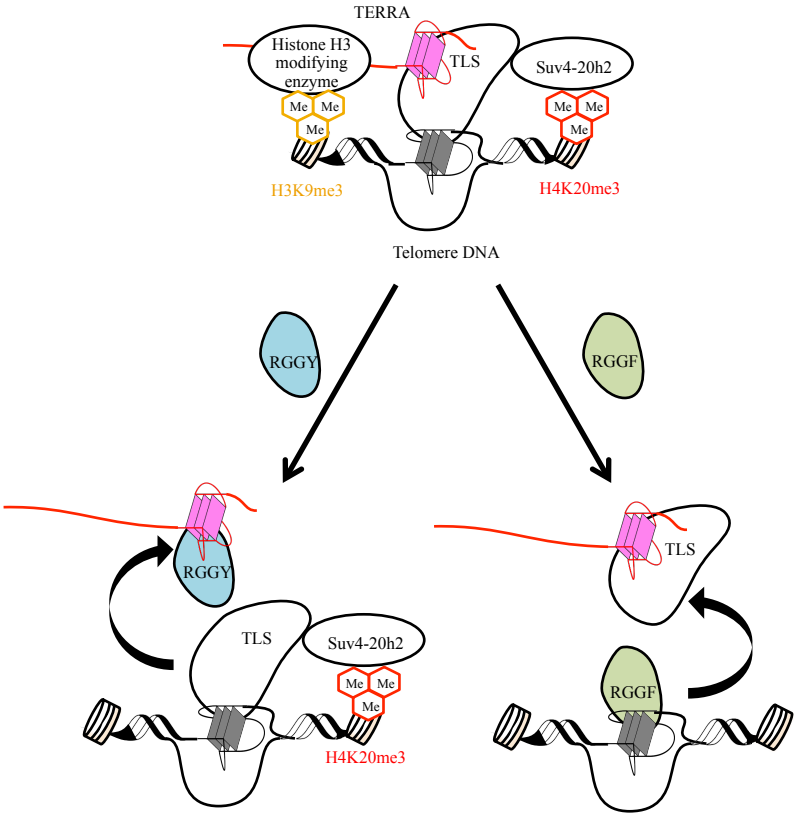

FIGURE S7. Models of the proposed role of RGGF and RGGY in the telomere regions. RGGY might inhibit TERRA from binding TLS, but not TLS binding to Htelo, which results in H4K20me3, while RGGF might inhibit TLS from binding telomeres and methylating H3K9 and H4K20.