# SUPPLEMENTARY DATA

## DNA G-Quadruplex-Binding Protein Developed Using the RGG Domain of Translocated in Liposarcoma/Fused in Sarcoma Inhibits Transcription of *bcl-2*

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## Experimental Section

**Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)**: RGGF and GST-nucleolin RBDs-RGG were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 12% (RGGF) and 8% (GST-nucleolin RBDs-RGG) gel, respectively.

**Circular Dichroism Spectroscopy**: Circular dichroism (CD) spectra of oligo 5 was recorded on a model J-820 instrument (Jasco, Japan). The CD spectra of oligo 5 (0.2 mM base concentration) in 100 mM KCl and 50 mM Tris-HCl (pH 7.5) was recorded using a 0.2-cm pathlength cell at 20°C. The spectrum of the corresponding buffer was subtracted, and this data was not further processed (e.g., by smoothing).

**Melting temperature ($T_m$) measurement**: Melting profile of Oligo 5 (3 μM strand concentration) in 50 mM Tris-HCl at pH 7.5 and 100 mM KCl was recorded on a UV-1800 Spectrometer (Shimadzu, JP) equipped with a TMSPC-8 controller and a 1 cm path-length cell. The absorbance of samples was monitored at 295 nm from 30 °C to 90 °C with heating of 1 °C/min. $T_m$ value was calculated by median methods.

**Cell Viability Assay:** HeLa cells expressed RGGF were aliquoted in 24-well plates and treated in RPMI-1640 containing FBS (10%). We used the transiently transfection for the experiment after 48 h of incubation. Cell viability was assayed by the CellTiter-Glo cell viability assay (Promega, WI, USA) with Luminescencer PSN (model AB-2200-R; ATTO, Tokyo, JP), according to the manufacture's protocol.

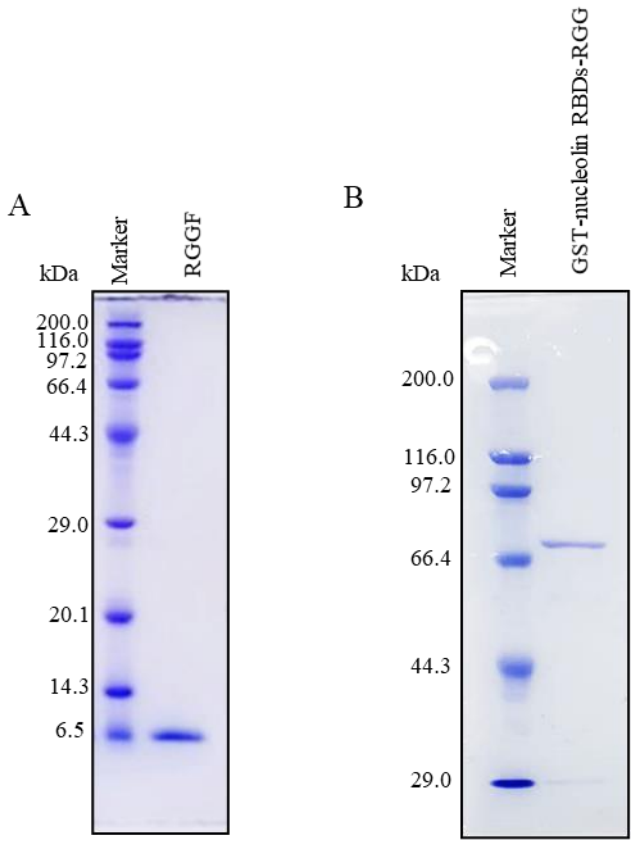



**Figure S1.** SDS-PAGE of RGGF and GST-nucleolin RBDs-RGG on 12 and 8 % polyacrylamide gel. *E. coli* strain BL21 (DE3) pLysS-competent cells were transformed with the vectors to express each proteins as GST-fused proteins. The proteins were purified by glutathione agarose and GST-tag of RGGF was digested by PreScission Protease. (A) Lane 1, molecular weight makers; Lane 2, RGGF (6.6 kDa). (B) Lane 1, molecular weight makers; Lane 2, GST-nucleolin RBDs-RGG (71 kDa). Molecular weight markers (kDa): pig myosin (200), *Escherichia coli* b-galactosidase (116), rabbit muscle phosphorylase B (97.2), bovine serum albumin (66.4), hen egg white ovalbumin (44.3), bovine carbonic anhydrase (29.0), soybean trypsin inhibitor (20.1), hen egg white lysozyme (14.3), bovine pancreas aprotinin (6.5).

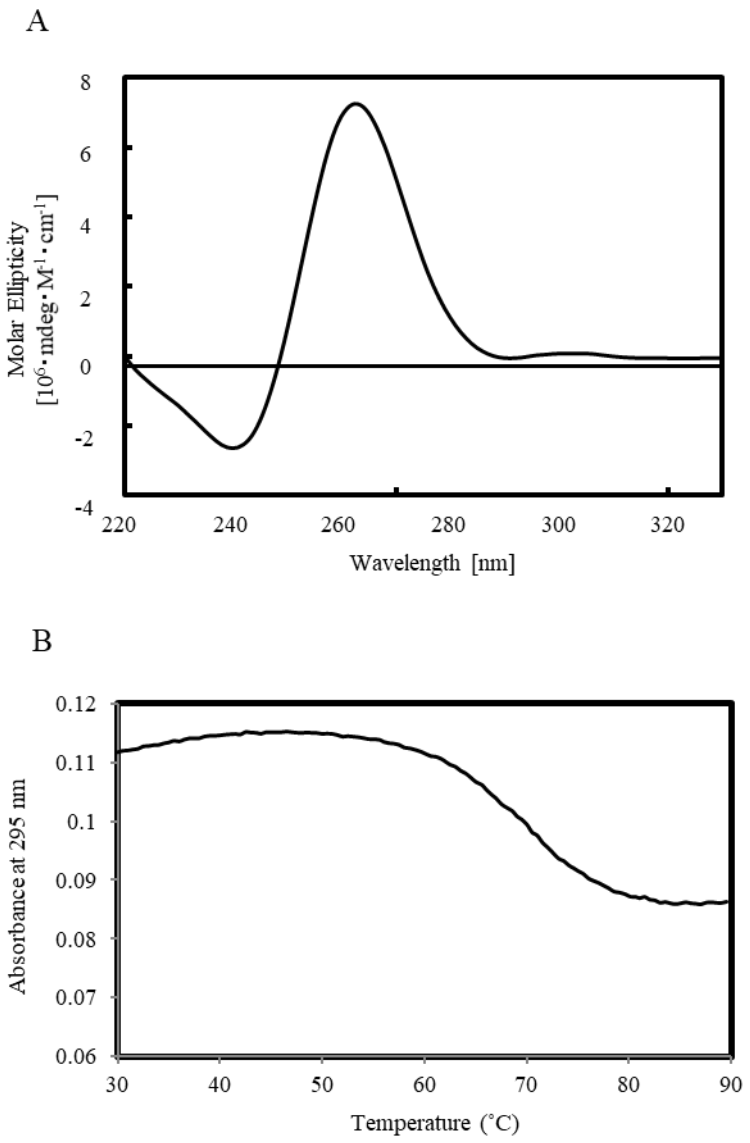
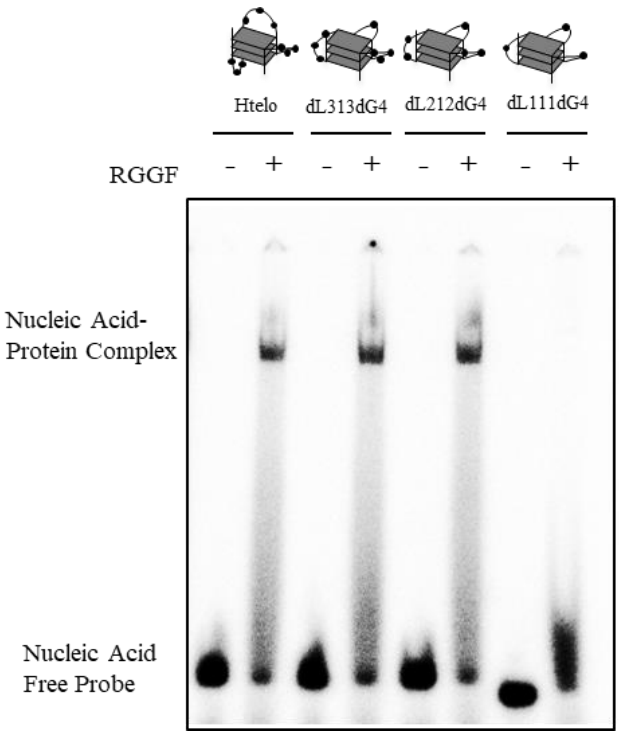



**Figure S2.** Circular dichroism (CD) spectrum and melting profile of Oligo 5. (A) CD spectrum of Oligo 5 in 100 mM KCl and 50 mM Tris-HCl (pH 7.5). The concentration of oligomer was 0.2 mM base concentration. (B) Melting profile obtained for Oligo 5 at 295 nm in 50 mM Tris-HCl at pH 7.5 and 100 mM KCl. The $T_m$ of Oligo 5 is 70.4 ˚C. The concentration of oligomer was 3 μM strand concentration.




| | |
|---|---|
| Htelo | AGGG**TTA**GGG**TTA**GGG**TTA**GGG |
| dL313dG4 | TGGG**TTT**GGG**T**GGG**TTT**GGGT |
| dL211dG4 | TGGG**TT**GGG**T**GGG**TT**GGGT |
| dL111dG4 | TGGG**T**GGG**T**GGG**T**GGGT |

**Figure S3.** Effect of G4 loop length on the binding affinity of RGGF. The EMSA was performed using RGGF (lanes 2, 4, 6, and 8) with $^{32}$P-labeled Htelo (lanes 1 and 2), dL313dG4 (lanes 3 and 4), dL212dG4 (lanes 5 and 6), or dL111dG4 (lanes 7 and 8). The DNA-protein complexes were resolved by 6% polyacrylamide gel electrophoresis and visualized by autoradiography. Black circles in the cartoons of each G4 represent a nucelotides residue in the loops. The sequences of oligonucleotides used in EMSA are presented. The bases of the loops are underlined.

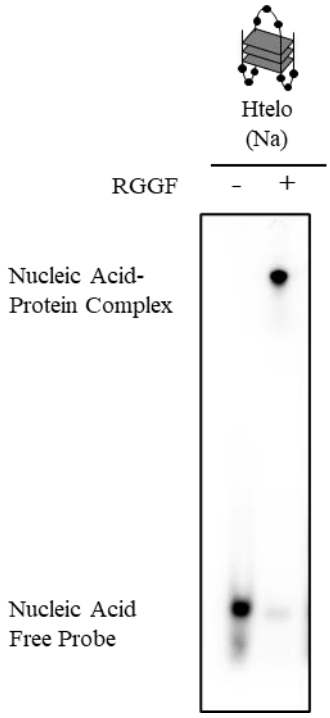



**Figure S4.** RGGF binding to Htelo fold in the presence of NaCl. EMSA was performed with RGGF (Lane 2) and $^{32}$P-labeled Htelo (1 and 2) fold in 50 mM Tris-HCl (pH 7.5) plus 100 mM NaCl. The DNA-protein complex was resolved by 6% polyacrylamide gel electrophoresis and visualized by autoradiography. Black circles in the cartoons of each G4 represent a nucelotides residue in the loops.

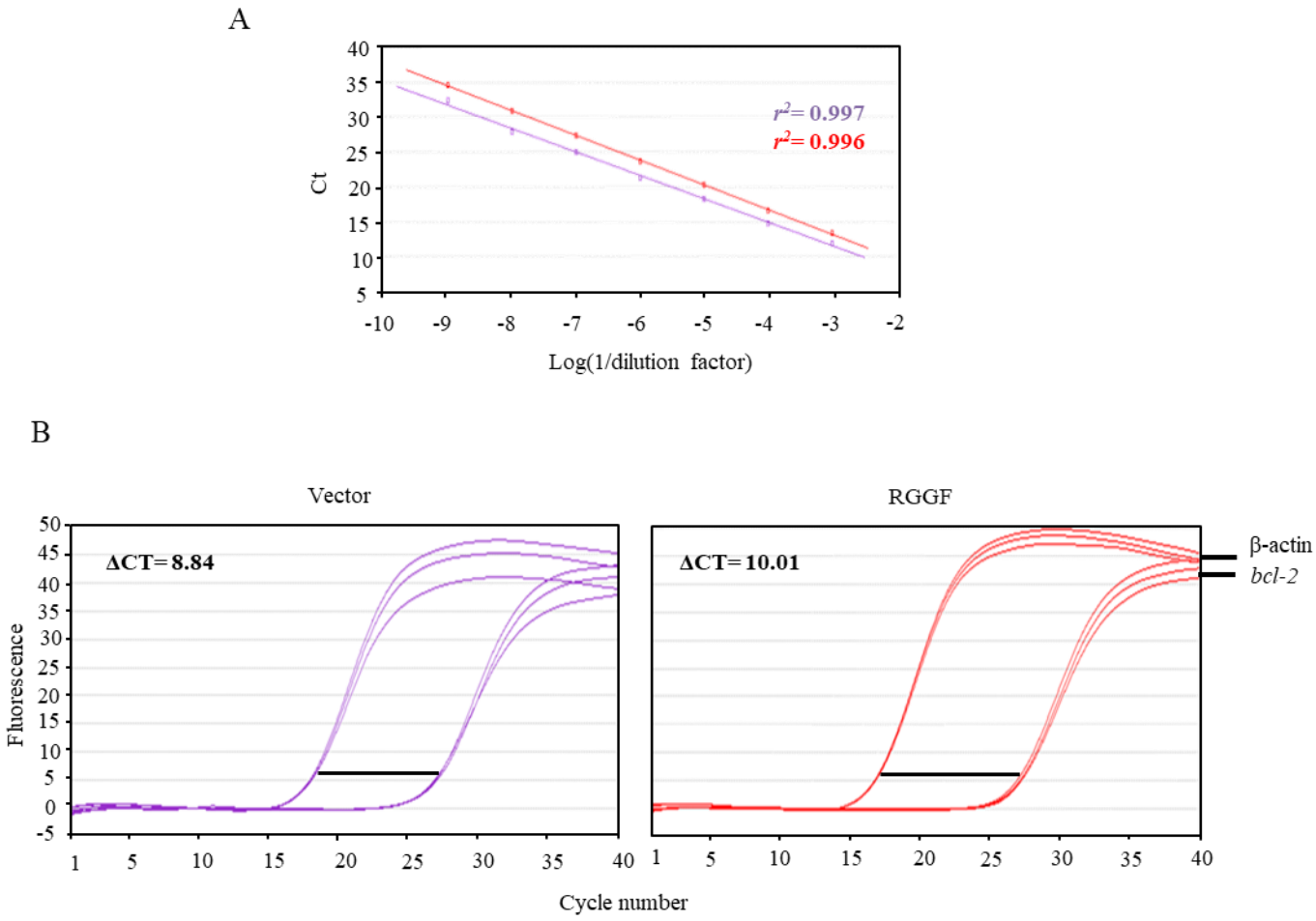



**Figure S5.** RT-qPCR amplification plots of β-actin and *bcl-2* from the vector or RGGF-overexpressing cells. (A) Standard curves of *bcl-2* (purple line) and β-actin (red line) based on the sample dilution factors ($10^3$, $10^4$, $10^5$, $10^6$, $10^7$, $10^8$, and $10^9$). The PCR efficiency was estimated through the linier regression of standard curve (*bcl-2*: $r^2 = 0.997$; β-actin*:* $r^2 = 0.996$). (B) Representative RT-qPCR amplification plots from the RNA samples of vector and RGGF-overexpressing HeLa cells. The purple line is vector-transfected HeLa cells, and the red line is RGGF-overexpressing HeLa cells. **Δ**CT, average differences of CT values.

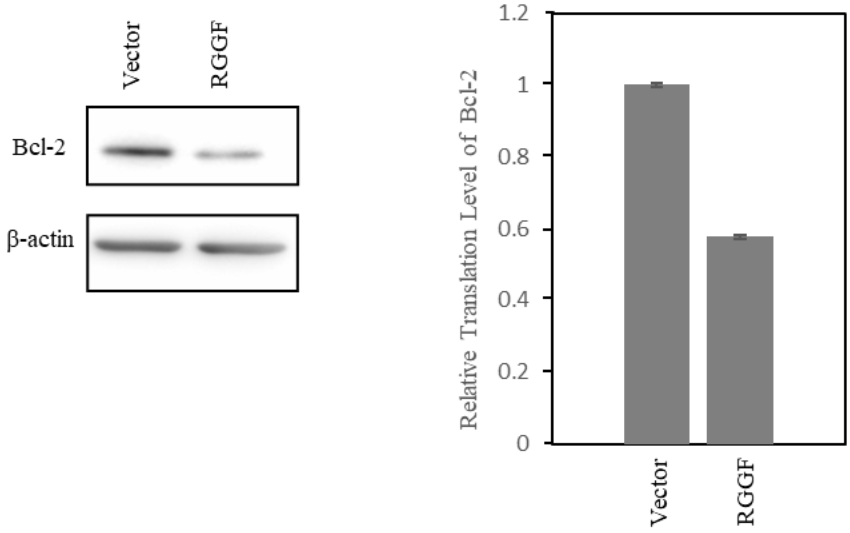



**Figure S6.** Translation level changes of Bcl-2 in RGGF-overexpressing HeLa cells. Relative translation level of Bcl-2 in RGGF-overexpressing HeLa cells was analyzed by Western blot with a Bcl-2 antibody. The translation levels of Bcl-2 and β-actin were analyzed with quantified. Bars represent mean values (± errors) obtained from 3 independent experiments.

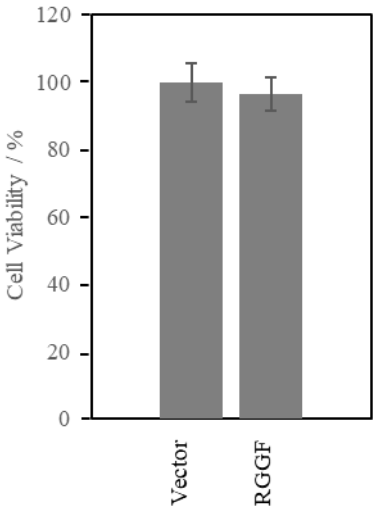



**Figure S7.** Cell viability assay. We used the transiently transfection of vector or RGGF for the experiment after 48 h of incubation. Cell viability was measured with a CellTiter-Glo luminescent kit. Bars represent mean values (± errors) obtained from three independent experiments.