Supporting Information

Capture of RNA G-quadruplex structure using L-RNA aptamer

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References

Materials and Methods

Oligonucleotides Preparation

All oligonucleotides used in this study were listed in Table S1. The FAM-labelled D-RNA, G4, and non-G4(s) used in this study were obtained through chemical synthesis from either Integrated DNA Technologies (IDT) or Genewiz (China). The 5'-Biotin-L-Apt.4-1c and 5'-Biotin-L-Apt.4-1c M9 (Biotin-L-mutant) were chemically synthesized from Bio-synthesis Inc. They were dissolved with nuclease-free distilled water (Invitrogen) to a concentration of 100 μM. The mass spectrometry data of all synthetic oligonucleotides were listed in Table S2. BioTASQ v.1 was dissolved in DMSO to a concentration of 1 mM. All oligonucleotides and ligand were stored at -20℃. Dynabeads™ MyOne™ Streptavidin C1 magnetic beads (Thermo) were used in the pulldown assay and were stored at 4℃.

Microscale Thermophoresis (MST) assay

MST assay was carried out by Monolith NT.115 (NanoTemper, Munich, Germany) to verify the binding affinity between biomolecules. The binding check mode with nano-blue excitation was set in NT. control software for FAM-labelled oligos at 25°C. Reaction of 10 μl consisted of 40 nM FAM-D-*hTERC* rG4 or D-*hTELO* dG4 and binding buffer (150 mM KCl, 1 mM $MgCl_2$, 25 mM Tris-HCl, pH 7.5) were prepared, denatured at 75°C for 3 minutes and cooled down at 4°C. Different concentrations of BioTASQ v.1 or Biotin-L-Apt.4-1c (0.15 – 5000 nM) were prepared through serial dilution. Then the mixture of BioTASQ v.1 or Biotin-L-Apt.4-1c and FAM-D-*hTERC* rG4 or D-*hTELO* dG4 were incubated at 37°C for 30 minutes and gradually cooled down to 4°C for 30 minutes.[1] Data were fitted and normalized by M.O. Affinity Analysis Software with a K_d value. Error bars represent standard deviation from three independent replicates. GraphPad Prism 9 was used to fit the curves with error bar and determine the K_d value.

Total RNA preparation

HEK293T cells were cultured in DMEM medium (Thermo Fisher Scientific) supplemented with 10% FBS (Thermo Fisher Scientific) and 1X antibiotic antimycotic (Thermo Fisher Scientific). Cells were seeded in 10 mm dish and collected for RNA isolation. Total RNA was extracted using RNeasy Plus Mini Kit (Qiagen) following manufacturer's instructions. The concentration of prepared total RNA was quantified by NanoDrop 1000 Spectrophotometer in ng/μL.

Cell lysate preparation

HEK293T cells were cultured in a 175 cm^3 cell culture flask, and collected about 4.2×10^6/mL. The collected cells were lysed at 70% confluence using 1 mL RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific, USA) supplemented with proteinase inhibitor (Thermo Fisher Scientific, USA) and RiboLock RNase Inhibitor (Thermo Fisher Scientific, USA). Cells were then centrifuged at 16,000 x g for 10 minutes at 4°C supernatant was collected in a new 2 mL tube. The concentration of prepared cell lysate was quantified by NanoDrop 1000 Spectrophotometer in ng/μL.

Preparation of rG4 pulldown assay

Streptavidin-coated magnetic beads (Thermo) were prepared; beads were vortexed and re-suspended for 1 minute, and desired volume of 170 μL were transferred into a 1.5 mL tube at a final concentration of 1.7 mg/100 μL. The beads were washed three times with at least 1 mL of wash buffer (containing 0.5 mM EDTA and 1 M NaCl, 5 mM Tris HCl, pH 7.5) then washed twice with at least 300 μL solution A (containing 0.1 M nuclease-free NaOH and 0.05 M nuclease-free NaCl) for two minutes. The beads were further washed once with 300 μL of

solution B (containing 0.1 M nuclease-free NaCl) for two minutes and then incubated in a total reaction volume of 100 µL consisting of final concentrations of 0.1 mg/mL yeast tRNA (Thermo Fisher) and annealing buffer (containing 1 mM $MgCl_2$, 150 mM KCl, 25 mM Tris-HCl, pH 7.5) at room temperature for an hour with shaking at 300 rpm. Note, after incubation with tRNA, the beads solution was divided into two and excess solution is removed prior to target incubation.

Pulldown assay

For non-competitive pulldown, 100 nM final concentration of either FAM labelled target or non-target (D-RNA, dG4, non-G4) and varying concentrations (0, 2.5, 120, 300, 800, and 1600 nM) of either Biotin-L-Apt.4-1c or Biotin-L-mutant or BioTASQ v.1 were separately denatured in annealing buffer (1 mM $MgCl_2$, 150 mM KCl, 25 mM Tris-HCl, pH 7.5) at 75ºC and cooled down at 0.1ºC/min. The 5'-FAM D-RNA target(s) and the Biotin-L-Apt.4-1c or or Biotin-L-mutant or BioTASQ v.1 were then mixed and incubated at 37ºC for an hour with shaking at 300 rpm, followed by the addition of 0.85 mg of pre-washed streptavidin-coated magnetic beads at 25ºC and further incubated for an additional 30 minutes at 37ºC with shaking at 300 rpm. The beads were placed on a magnet and the supernatant was transferred into a clean 1.5 mL tubes for first fluorescent intensity measurement (supernatant). While the bound target mixture contained in beads (residue) was washed once with 100 µL of annealing buffer (1 mM $MgCl_2$, 150 mM KCl, 25 mM Tris-HCl, pH 7.5) at room temperature for two minutes to ensure FAM-labelled unbound are removed. The remaining bound target mixture was recovered by re-suspending in in 100 µL of 10 M urea and incubated at 95ºC for 15 minutes with shaking at 300 rpm and cooled down for 5 minutes. The beads were then placed on a magnet for 5 minutes and the supernatant was transferred into a clean 1.5 mL tube for second fluorescent measurement (recovery). The quartz cuvette with 1cm path length was used for fluorescent measurements and was measured using the HORIBA FluoroMax-4. The sample was excited at 485 nm and the emission spectrum was monitored between 500 and 700 nm.

For competitive pulldown assay, similar procedures to non-competitive pulldown assay (as mentioned above) were followed except either *hTELO* dG4 (1X or 10X) or a mixture of poly rA, rU, and rC (1X each) or complex media (5 µg total RNA or 3.4 µg cell lysate) was added to the reaction mixture alongside FAM-D-*hTERC* rG4 or BioTASQ v.1 and denatured as indicated above.

Endogenous pulldown and RT-qPCR for total RNA and cell lysate

For endogenous pulldown, 1600 nM final concentration of Biotin-L-Apt.4-1c and Biotin-L-Apt.4-1c M9 (Biotin-L-mutant) were separately denatured in annealing buffer at 75ºC and cooled down at 0.1ºC/min. The extracted total RNA and cell lysate were separately incubated with Biotin-L-Apt.4-1c at 4ºC for an hour with shaking at 300 rpm, followed by the addition of 0.85 mg of pre-washed streptavidin-coated magnetic beads at 25ºC and further incubated for an additional 30 minutes at 4ºC with shaking at 300 rpm. The beads were placed on a magnet and the supernatant was discarded. While the bound target mixture contained in beads was washed once with 100 µL of annealing buffer (1 mM $MgCl_2$, 150 mM KCl, 25 mM Tris-HCl, pH 7.5) at room temperature for two minutes to ensure FAM-labelled unbound are removed. The remaining bound target mixture was recovered by re-suspending in in 50 µL of 10 M urea and incubated at 95ºC for 15 minutes with shaking at 300 rpm and cooled down for 5 minutes. The beads were then placed on a magnet for 5 minutes and the supernatant (elution) of total RNA was transferred into a clean 1.5 mL tube for RT-qPCR. For the input and elution of cell lysate, RNA needs to be isolated using TRIzol™ Reagent (Invitrogen) following the manufacturer's instructions.

The reverse transcription containing a final concentration of 150 ng cell lysate or total RNA, 1X PrimeScript RT Master Mix (Takara) in a 10 μL reaction was heated at 37ºC for 15 minutes, 85ºC for 5 seconds, and then cooled down to 4ºC. After that, the cDNA solutions were diluted to 12.5 ng/μL for the qPCR test. The qPCR reaction mixture contained 50 ng cDNA, 1 μL of primers (*hTERC* or *APP*), and 1X Ssoadvanced Universal SYBR Green Supermix (Biorad) in a 10 μL reaction. Amplification was carried out in a Bio-Rad CFX96 Touch^{TM} Real-Time PCR Detection System and included an initial denaturation step at 98ºC for 30 s; 40 cycles of 98ºC for 10 s and 60ºC for 30 s; and final extension steps at 65ºC for 5 s and 95ºC for 5 s. Three independent replicates were analyzed, and standard deviation was plotted as the error bar.

Data analysis for pulldown assay

The normalized FAM intensities are averages of three independent replicates as follows; fraction of FAM-target either pulled down or not pulled down by Biotin-L-Apt.4-1c is calculated as the FAM intensity of either supernatant (Measurement 1) or recovery (Measurement 2). Then, the fractions were divided by the summation of FAM intensities of supernatant (Measurement 1) or recovery (Measurement 2) and normalized to 100, i.e., [Measurement 1 or 2/ (Measurement 1 + Measurement 2)] x 100. Error bars indicate the standard deviation from three independent replicates. Data analysis was performed using Microsoft Excel, and the plot was generated using GraphPad Prism 9.

Table S1. Oligonucleotides used in this study.

Name	**Sequence (5' – 3')**
Biotin-L-Apt.4-1c	Biotin-GCCCUAAAGGUGGUGGUGGGAGGGC
Biotin-L-Apt.4-1c M9	Biotin-GCCCUAAAAGUGGUGGUGGGAGGGC
FAM-*hTERC* rG4	FAM-GGGUUGCGGAGGGUGGGCCU
FAM-*NRAS* rG4	FAM-GGGAGGGGCGGGUCUGGG
FAM-*APP* rG4	FAM-GGGGCGGGUGGGGAGGGG
FAM-RNA hairpin	FAM-CAGUACAGAUCUGUACUG
FAM-*hTERC* dG4	FAM-GGGTTGCGGAGGGTGGGCCT
FAM-*hTELO* dG4	FAM-TTAGGGTTAGGGTTAGGGTTAGGG
Poly rA	AAAAAAAAAAAAAAAAAA
Poly rU	UUUUUUUUUUUUUUUUU
Poly rC	CCCCCCCCCCCCCCCCCC
hTERC dG4	GGGTTGCGGAGGGTGGGCCT
hTELO dG4	TTAGGGTTAGGGTTAGGGTTAGGG
hTERC rG4 mutant	GAAUUGCGGAGAAUGAACCU
NRAS rG4 mutant	AGAAAGAGCAGAUCUAGA
APP rG4 mutant	GAAGCGAGUGAAGAGAAG
APP-FP	CAAGCAGTGCAAGACCCATC
APP-RP	AGAAGGGCATCACTTACAAACTC
hTERC-FP	GTGGTGGCCATTTTTTGTCTAAC
hTERC-RP	TGCTCTAGAATGAACGGTGGAA

Table S2. Mass spectrometry data of oligonucleotides used in this study.

Name	Measured molecular weight[4]	Calculated molecular weight[4]
Biotin-L-Apt.4-1c[1]	8593.1	8593.0
Biotin-L-Apt.4-1c M9[1]	8576.3	8577.0
FAM-*hTERC* rG4[2]	7087.4	7087.4
FAM-*NRAS* rG4[2]	6515.7	6515.1
FAM-*APP* rG4[2]	6594.1	6594.2
FAM-RNA hairpin[2]	6253.5	6253.9
FAM-*hTERC* dG4[3]	6824.1	6823.6
FAM-*hTELO* dG4[3]	8111.7	8112.4
Poly rA[2]	5683.2	5683.8
Poly rU[2]	5431.1	5431.3
Poly rC[2]	5448.4	5449.1
hTERC dG4[3]	6285.8	6286.1
hTELO dG4[3]	7574.5	7574.9
hTERC rG4 mutant[2]	6454.5	6454.0
NRAS rG4 mutant[2]	5850.2	5849.6
APP rG4 mutant[2]	5945.0	5944.7
APP-FP[3]	6081.0	6080.0
APP-RP[3]	7027.0	7025.6
hTERC-FP[3]	7052.5	7051.6
hTERC-RP[3]	6824.6	6823.5

Note:

[1] The measured and calculated molecular weights of the oligonucleotides were provided by Bio-synthesis Inc

[2] The measured and calculated molecular weight of the oligonucleotides were provided by Integrated DNA Technologies (IDT)

[3] The measured and calculated molecular weights of the oligonucleotides were provided by Genewiz (China).

[4] The measured and calculated molecular weights of all oligonucleotides used in this work are highly consistent, providing authentication of these reagents.

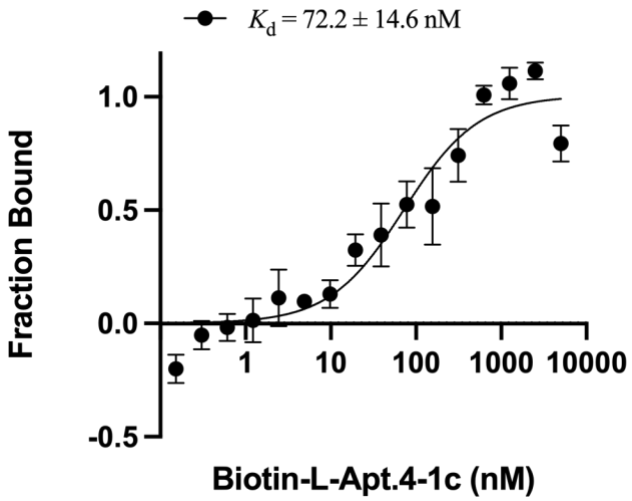

Figure S1. MST assay determines the binding affinity between Biotin-L-Apt.4-1c and FAM-*hTERC* rG4. The binding saturation plots of Biotin-L-Apt.4-1c-D-*hTERC* rG4 interaction. Reaction mixture contained 40 nM FAM-D-*hTERC* rG4 and the varying concentrations of Biotin-L-Apt.4-1c (0.15-5000 nM). The K_d was found to be 72.2 ± 14.6 nM.

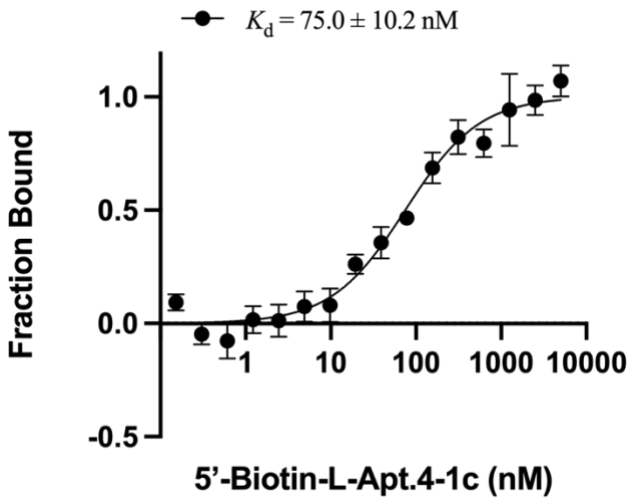

Figure S2. MST assay determines the binding affinity between Biotin-L-Apt.4-1c and FAM-*NRAS* rG4. The binding saturation plots of Biotin-L-Apt.4-1c-D-*NRAS* rG4 interaction. Reaction mixture contained 40 nM FAM-D-*NRAS* rG4 and the varying concentrations of Biotin-L-Apt.4-1c (0.15-5000 nM). The K_d was found to be 75.0 ± 10.2 nM.

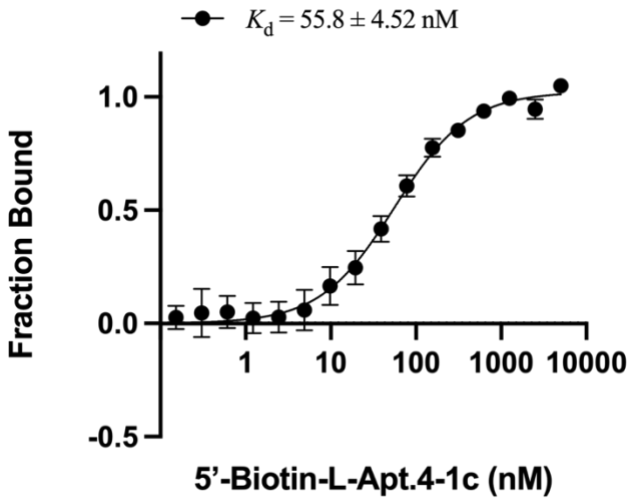

Figure S3. MST assay determines the binding affinity between Biotin-L-Apt.4-1c and FAM-*APP* rG4. The binding saturation plots of Biotin-L-Apt.4-1c-D-*APP* rG4 interaction. Reaction mixture contained 40 nM FAM-D-*APP* rG4 and the varying concentrations of Biotin-L-Apt.4-1c (0.15-5000 nM). The K_d was found to be 55.8 ± 4.52 nM.

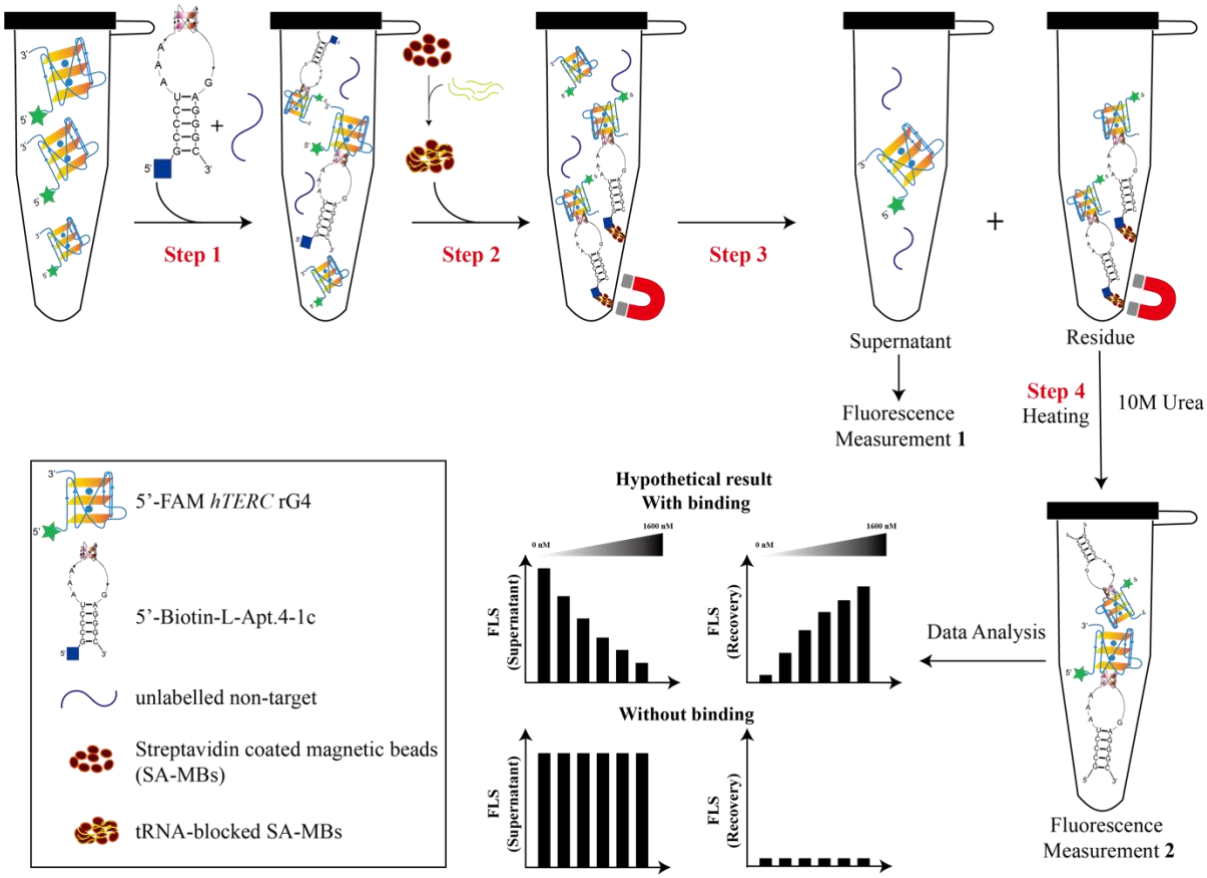

Figure S4. Schematic illustration of competitive pulldown assay. Similar procedures were followed except for step 1. For competitive pulldown approach, the 5'-Biotin-L-Apt.4-1c was incubated with 5'-FAM-*hTERC* rG4 in the presence of unlabelled non-target competitor, including *hTERC* dG4 (1X or 10X), *hTELO* dG4 (1X or 10X), a mixture of poly rA, rC, & rU (1X each), and complex media (total RNA or cell lysate).

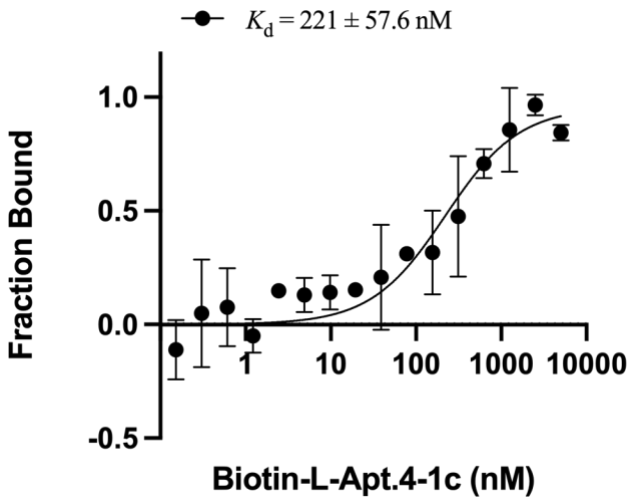

Figure S5. MST assay determines the binding affinity between Biotin-L-Apt.4-1c and FAM-*hTERC* dG4. The binding saturation plots of Biotin-L-Apt.4-1c-D-*hTERC* dG4 interaction. Reaction mixture contained 40 nM FAM-D-*hTERC* dG4 and the varying concentrations of Biotin-L-Apt.4-1c (0.15-5000 nM). The K_d was found to be 221 ± 57.6 nM.

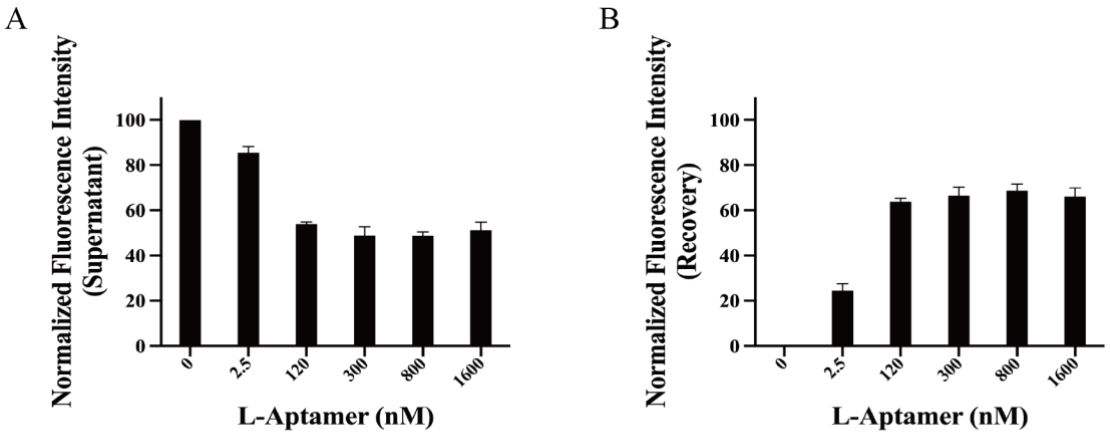

Figure S6. Pulldown efficiency for FAM-*NRAS* rG4 by Biotin-L-Apt.4-1c. The pulldown of FAM-D-targets (100 nM) by Biotin-L-Apt.4-1c (0, 2.5, 120, 300, 800, 1600 nM) were monitored by the fluorescence intensities and normalized at 100%. (A) Supernatant. (B) Recovery. Left panel represent the fluorescence intensity of supernatant, while right panel represent the fluorescence intensity of recovery. Pulldown efficiency of L-aptamer for FAM-*NRAS* rG4 are similar as for FAM-*hTERC* rG4. Error bars indicate the standard deviation from three independent replicates.

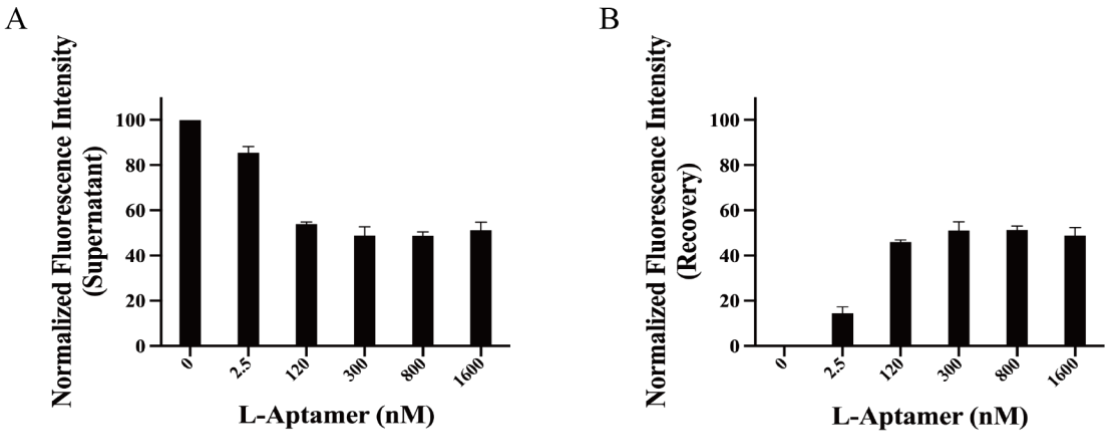

Figure S7. Pulldown efficiency for FAM-*APP* rG4 by Biotin-L-Apt.4-1c. The pulldown of FAM-D-targets (100 nM) by Biotin-L-Apt.4-1c (0, 2.5, 120, 300, 800, 1600 nM) were monitored by the fluorescence intensities and normalized at 100%. (A) Supernatant. (B) Recovery. Left panel represent the fluorescence intensity of supernatant, while right panel represent the fluorescence intensity of recovery. Pulldown efficiency of L-aptamer for FAM-*APP* rG4 are similar as for FAM-*hTERC* rG4. Error bars indicate the standard deviation from three independent replicates.

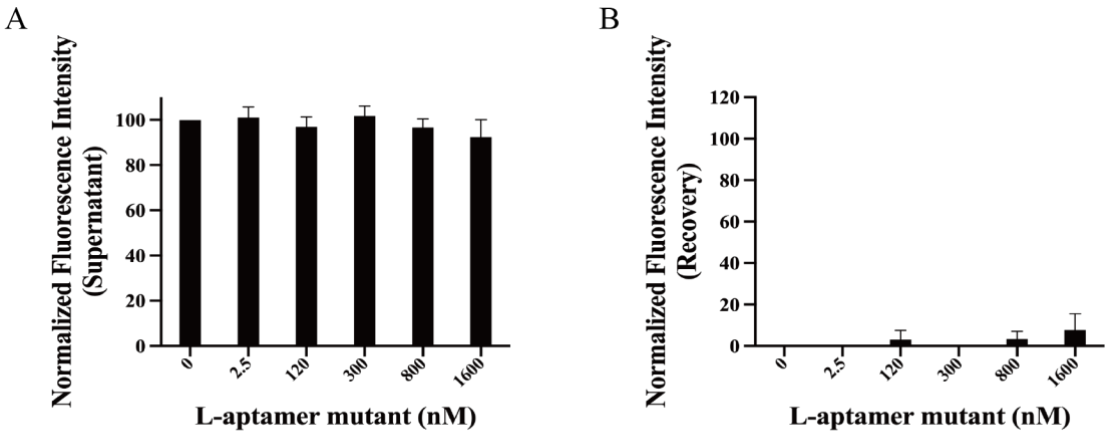

Figure S8. Pulldown efficiency for FAM-*hTERC* rG4 by Biotin-L-Apt.4-1c M9 (L-aptamer mutant). The pulldown of FAM-*hTERC* rG4 (100 nM) by Biotin-L-aptamer mutant (0, 2.5, 120, 300, 800, 1600 nM) were monitored by the fluorescence intensities and normalized at 100%. (A) Supernatant. (B) Recovery. Left panels represent the fluorescence intensity of supernatant, while right panels represent the fluorescence intensity of recovery. Biotin-L-aptamer mutant is not able to pulldown *hTERC* rG4. Error bars indicate the standard deviation from three independent replicates.

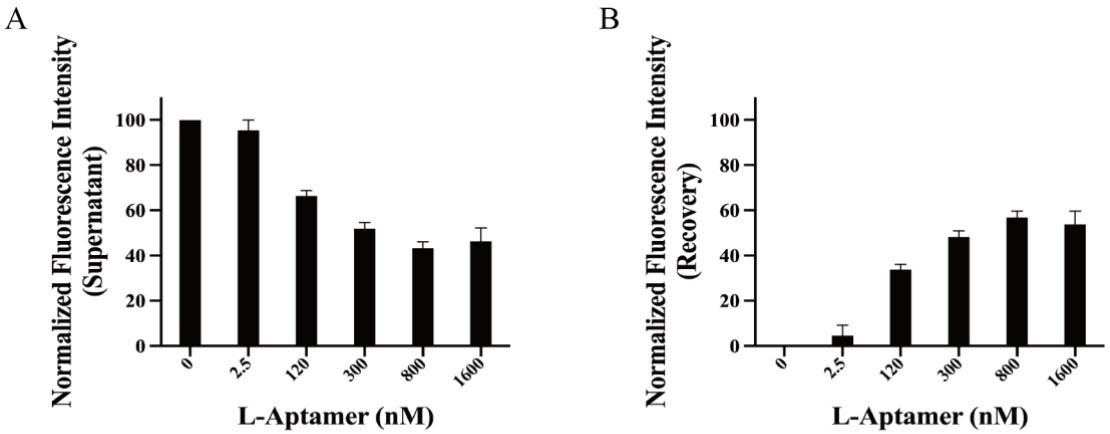

Figure S9. Pulldown efficiency for FAM-*hTERC* rG4 by Biotin-L-Apt.4-1c in the presence of 1X unlabelled *hTERC* rG4 mutant. The pulldown of FAM-D-targets (100 nM) by Biotin-L-Apt.4-1c (0, 2.5, 120, 300, 800, 1600 nM) in the presence of unlabelled *hTERC* rG4 mutant (final concentration at 100 nM) were monitored by the fluorescence intensities and normalized at 100%. (A) Supernatant. (B) Recovery. Left panel represent the fluorescence intensity of supernatant, while right panel represent the fluorescence intensity of recovery. Pulldown efficiency of L-aptamer for FAM-*hTERC* rG4 with or without the rG4 mutant are similar. Error bars indicate the standard deviation from three independent replicates.

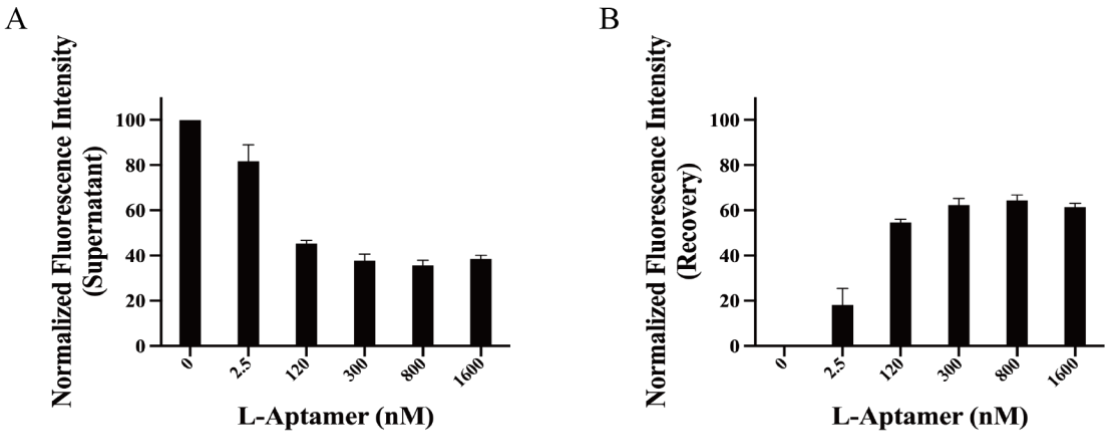

Figure S10. Pulldown efficiency for FAM-*NRAS* rG4 by Biotin-L-Apt.4-1c in the presence of 1X unlabelled *NRAS* rG4 mutant. The pulldown of FAM-D-targets (100 nM) by Biotin-L-Apt.4-1c (0, 2.5, 120, 300, 800, 1600 nM) in the presence of unlabelled *NRAS* rG4 mutant (final concentration at 100 nM) were monitored by the fluorescence intensities and normalized at 100%. (A) Supernatant. (B) Recovery. Left panel represent the fluorescence intensity of supernatant, while right panel represent the fluorescence intensity of recovery. Pulldown efficiency of L-aptamer for FAM-*NRAS* rG4 with or without the rG4 mutant are similar. Error bars indicate the standard deviation from three independent replicates.

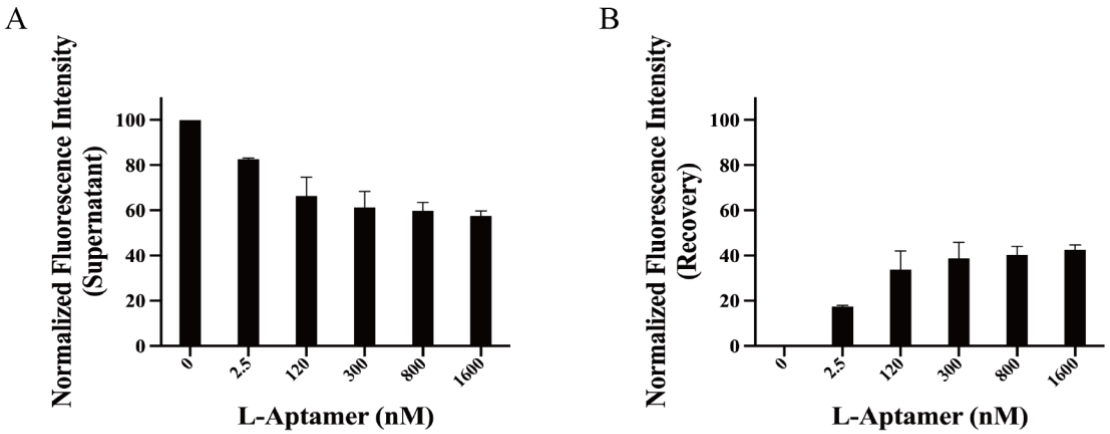

Figure S11. Pulldown efficiency for FAM-*APP* rG4 by Biotin-L-Apt.4-1c in the presence of 1X unlabelled *APP* rG4 mutant. The pulldown of FAM-D-targets (100 nM) by Biotin-L-Apt.4-1c (0, 2.5, 120, 300, 800, 1600 nM) in the presence of unlabelled *APP* rG4 mutant (final concentration at 100 nM) were monitored by the fluorescence intensities and normalized at 100%. (A) Supernatant. (B) Recovery. Left panel represent the fluorescence intensity of supernatant, while right panel represent the fluorescence intensity of recovery. Pulldown efficiency of L-aptamer for FAM-*APP* rG4 with or without the rG4 mutant are similar. Error bars indicate the standard deviation from three independent replicates.

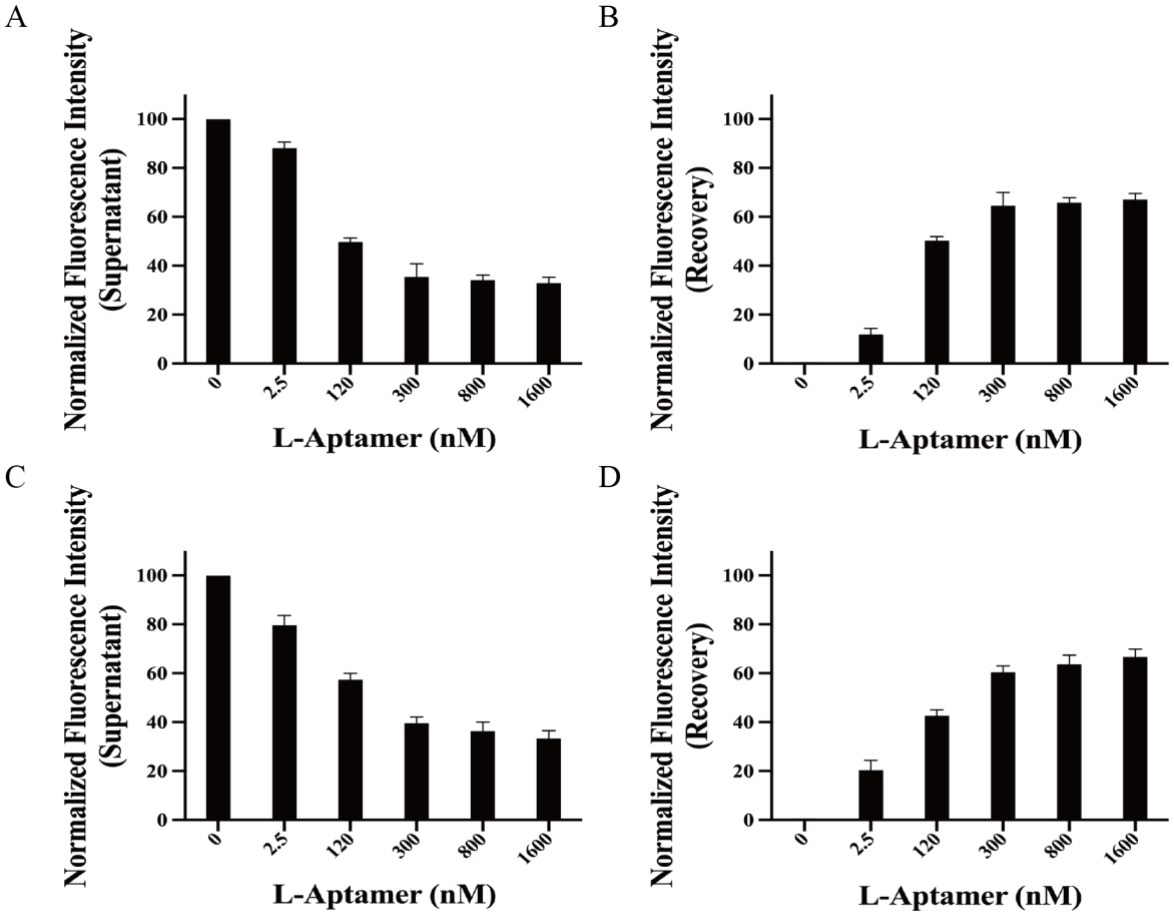

Figure S12. Pulldown efficiency for FAM-*hTERC* rG4 by Biotin-L-Apt.4-1c in the presence of 1X and 10X unlabelled *hTERC* dG4. The pulldown of FAM-*hTERC* rG4 (100 nM) by Biotin-L-Apt.4-1c (0, 2.5, 120, 300, 800, 1600 nM) in the presence of 1X (100 nM) and 10X (1 μM) unlabelled *hTERC* dG4 were monitored by the fluorescence intensities and normalized at 100%. (A-B) Pulldown was performed in the presence of 1X unlabelled *hTERC* dG4. (C-D) Similar set up as (A-B) except 10X unlabelled *hTERC* dG4 was used. Left panels represent the fluorescence intensity of supernatant, while right panels represent the fluorescence intensity of recovery. Despite binding between *hTERC* dG4 and L-aptamer, the pulldown efficiency of L-aptamer for FAM-*hTERC* rG4 remains similar, regardless of the presence of unlabelled D-*hTERC* dG4 (1X and 10X) competitor. Error bars indicate the standard deviation from three independent replicates.

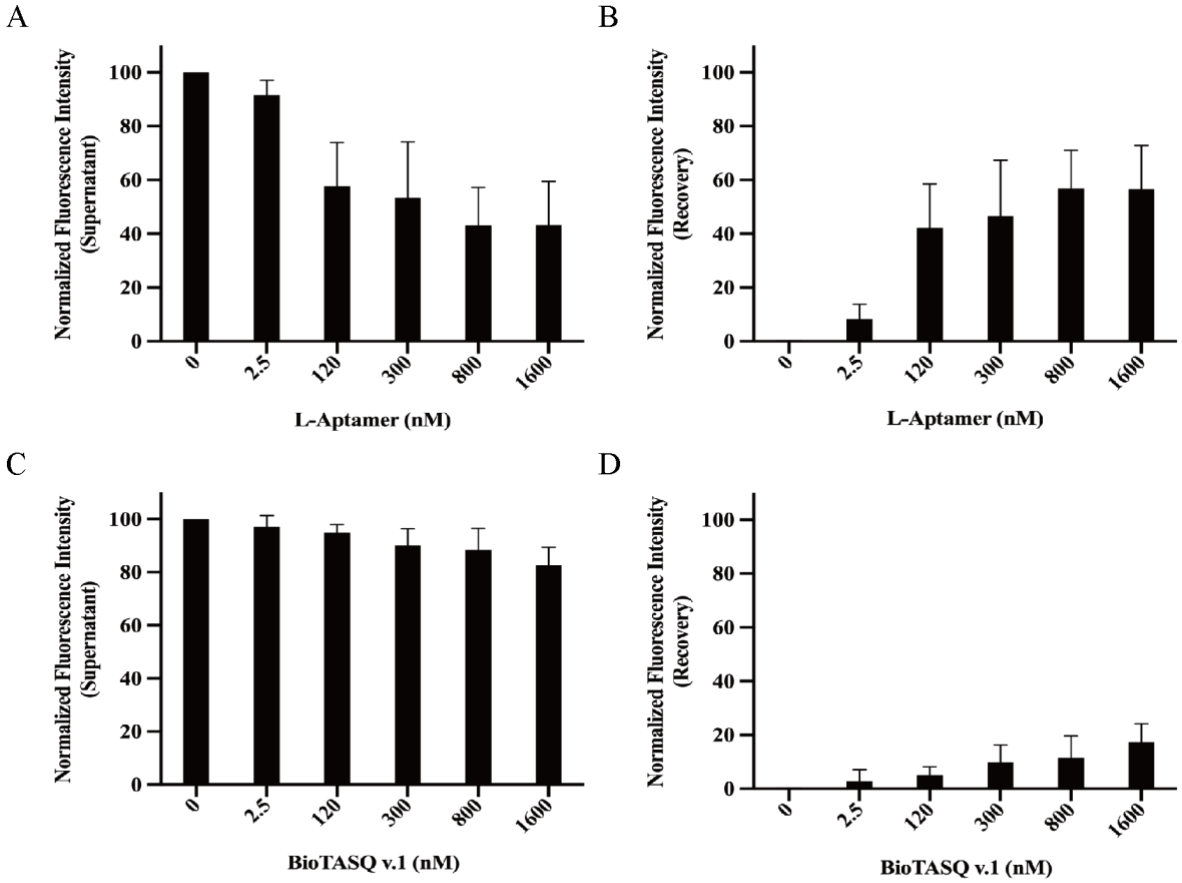

Figure S13. Pulldown efficiency for FAM-*hTERC* rG4 by Biotin-L-Apt.4-1c and BioTASQ v.1 in the presence of 10X unlabelled *hTELO* dG4. The pulldown of FAM-*hTERC* dG4 (100 nM) by BioTASQ v.1 (0, 2.5, 120, 300, 800, 1600 nM) in the presence of 10X unlabelled *hTELO* dG4 (1 μM) were monitored by the fluorescence intensities and normalized at 100%. (A-B) Pulldown was performed by Biotin-L-Apt.4-1c. (C-D) Similar set up as (A-B) except BioTASQ v.1 was used. Left panels represent the fluorescence intensity of supernatant, while right panels represent the fluorescence intensity of recovery. Pulldown efficiency of L-aptamer for FAM-*hTERC* rG4 with or without the presence of 10X unlabelled D-*hTELO* dG4 competitor are similar. However, BioTASQ v.1 binds to both D-*hTELO* dG4 and *hTERC* rG4, which greatly affected the pulldown efficiency for FAM-*hTERC* rG4 in the presence of unlabelled competitor. Error bars indicate the standard deviation from three independent replicates.

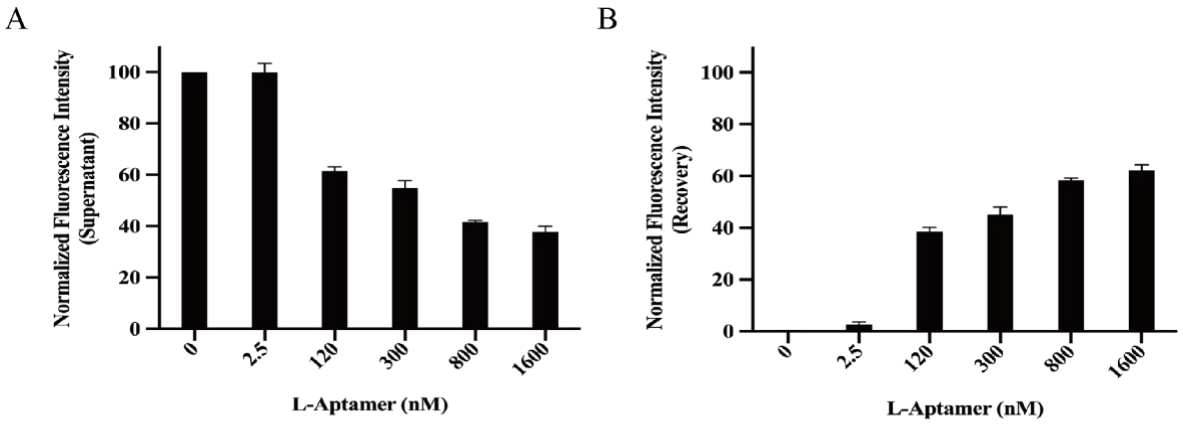

Figure S14. Biotin-L-Apt.4-1c specifically pulldown FAM-*hTERC* rG4 in presence of a mixture of unlabelled poly rA, rC and rU (1X each). The pulldown of FAM-D-targets (100 nM) by Biotin-L-Apt.4-1c (0, 2.5, 120, 300, 800, 1600 nM) in the presence of unlabelled poly rA, rU, and rC (final concentration at 100 nM each) were monitored by the fluorescence intensities and normalized at 100%. (A) Supernatant. (B) Recovery. Left panel represent the fluorescence intensity of supernatant, while right panel represent the fluorescence intensity of recovery. Pulldown efficiency of L-aptamer for FAM-*hTERC* rG4 with or without the presence of the mixture of unlabelled poly rA, rC and rU (1X each) are similar. Error bars indicate the standard deviation from three independent replicates.

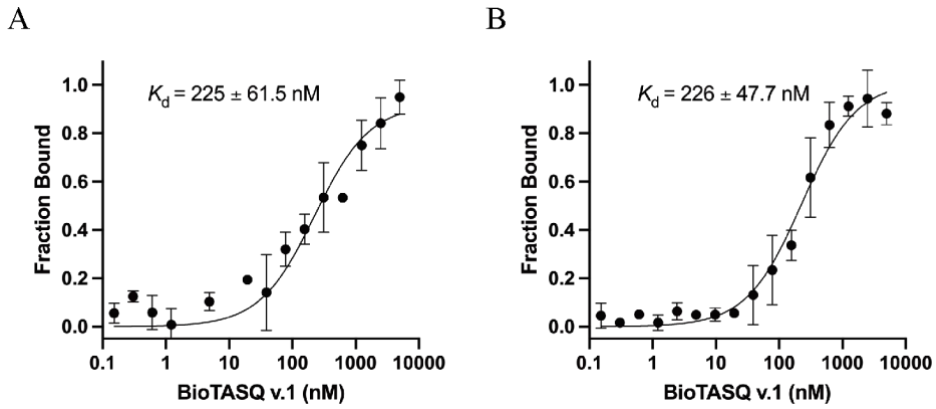

Figure S15. MST assay determines the binding affinity between BioTASQ v.1 and FAM-*hTERC* rG4 or FAM-*hTELO* dG4 respectively. (A) The binding saturation plots of BioTASQ v.1-D-*hTERC* rG4 interaction. Reaction mixture contained 40 nM FAM-D-*hTERC* rG4 and the varying concentrations of BioTASQ v.1 (0.15-5000 nM). The K_d was found to be 225 ± 61.5 nM. (B) Same experimental set up as (A) except 5'-FAM- *hTELO* dG4 is used. The K_d was found to be 226 ± 47.7 nM. Error bars represent standard deviation from three independent replicates.

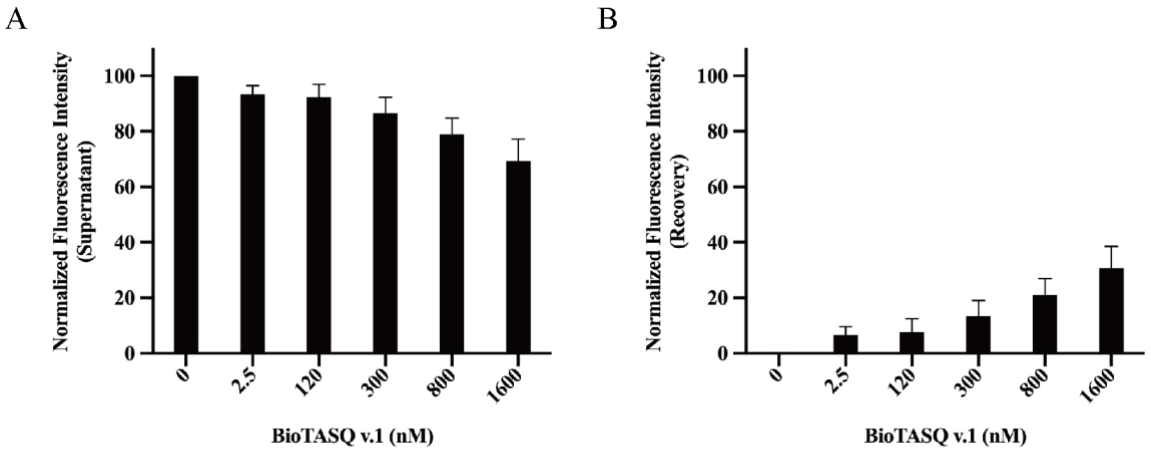

Figure S16. Pulldown efficiency for FAM-*hTERC* rG4 by BioTASQ v.1. The pulldown of FAM-*hTERC* rG4 (100 nM) by BioTASQ v.1 (0, 2.5, 120, 300, 800, 1600 nM) were monitored by the fluorescence intensities and normalized at 100%. (A) Supernatant. (B) Recovery. Left panels represent the fluorescence intensity of supernatant, while right panels represent the fluorescence intensity of recovery. Pulldown efficiency for FAM-*hTERC* rG4 by BioTASQ v.1 are poorer than that of L-aptamer. Error bars indicate the standard deviation from three independent replicates.

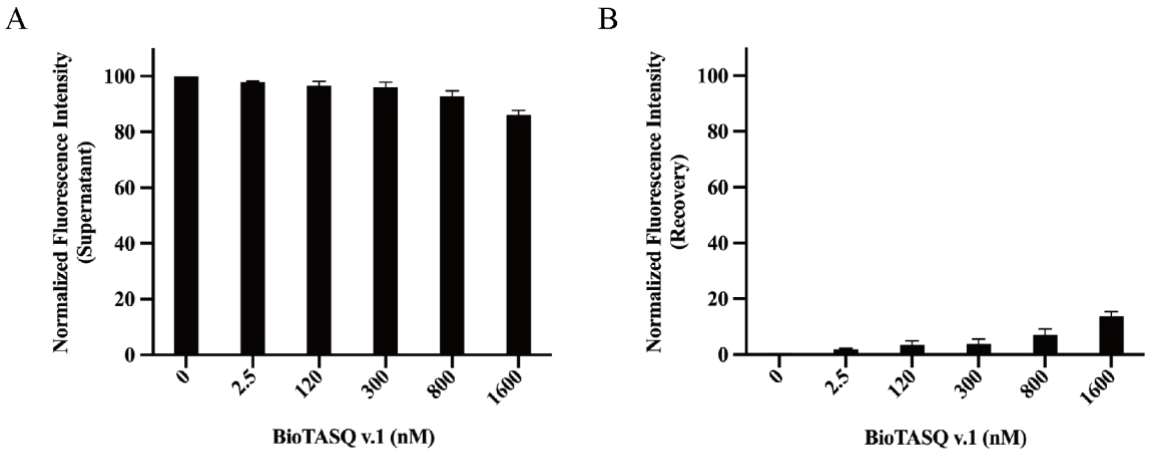

Figure S17. Pulldown efficiency for FAM-*hTELO* dG4 by BioTASQ v.1. The pulldown of FAM-*hTELO* dG4 (100 nM) by BioTASQ v.1 (0, 2.5, 120, 300, 800, 1600 nM) were monitored by the fluorescence intensities and normalized at 100%. (A) Supernatant. (B) Recovery. Left panels represent the fluorescence intensity of supernatant, while right panels represent the fluorescence intensity of recovery. Pulldown efficiency of BioTASQ v.1 for FAM-*hTELO* dG4 are much poorer than that of FAM-*hTERC* rG4. Error bars indicate the standard deviation from three independent replicates.

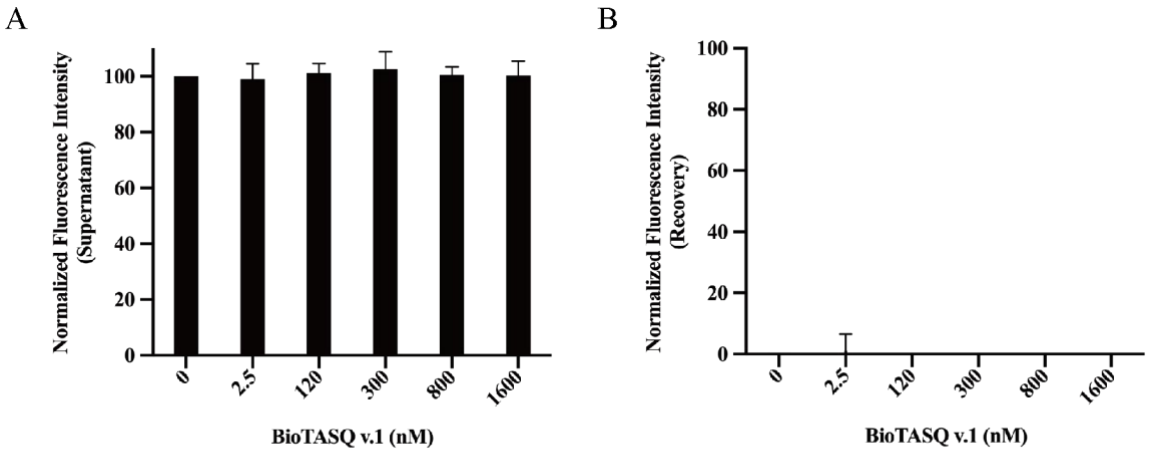

Figure S18. Pulldown efficiency for FAM-RNA hairpin by BioTASQ v.1. The pulldown of FAM-RNA hairpin (100 nM) by BioTASQ v.1 (0, 2.5, 120, 300, 800, 1600 nM) were monitored by the fluorescence intensities and normalized at 100%. (A) Supernatant. (B) Recovery. Left panels represent the fluorescence intensity of supernatant, while right panels represent the fluorescence intensity of recovery. BioTASQ v.1 is not able to pulldown RNA hairpin (non-G4 structure). Error bars indicate the standard deviation from three independent replicates.

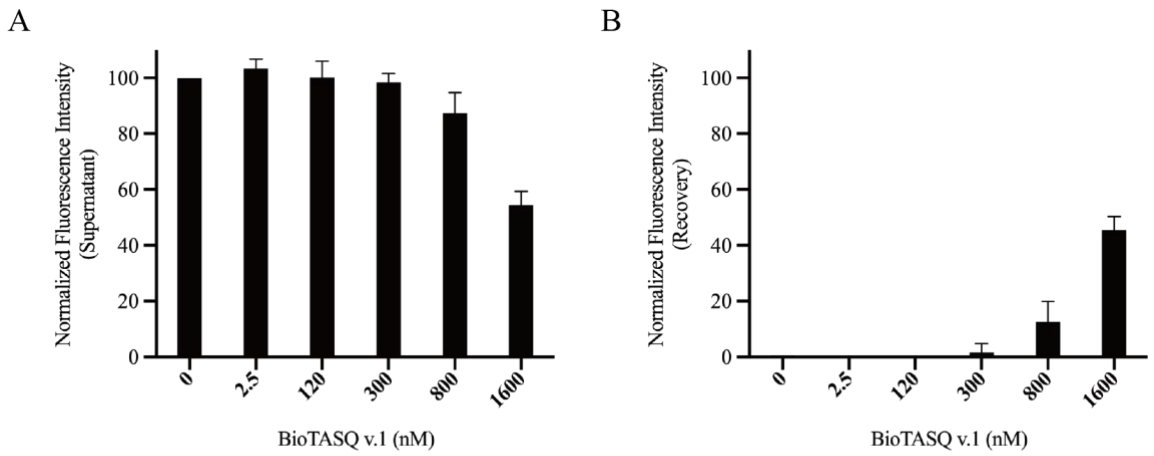

Figure S19. Pulldown efficiency for FAM-*hTERC* rG4 by BioTASQ v.1 in presence of a mixture of unlabelled poly rA, rC and rU (1X each). The pulldown of FAM-*hTERC* rG4 (100 nM) by Biotin-L-Apt.4-1c (0, 2.5, 120, 300, 800, 1600 nM) in the presence of unlabelled poly rA, rU, and rC (final concentration at 100 nM each) were monitored by the fluorescence intensities and normalized at 100%. (A) Supernatant. (B) Recovery. Left panels represent the fluorescence intensity of supernatant, while right panels represent the fluorescence intensity of recovery. Pulldown efficiency of BioTASQ v.1 for FAM- *hTERC* rG4 with or without the presence of the mixture of unlabelled poly rA, rC and rU (1X each) are similar. Error bars indicate the standard deviation from three independent replicates.

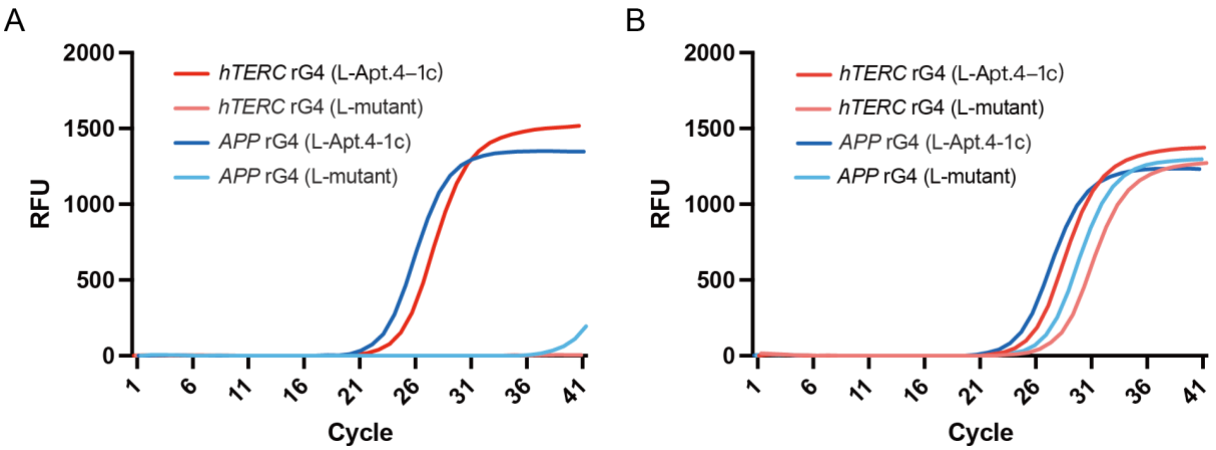

Figure S20. Endogenous *hTERC* rG4 and *APP* rG4 enrichment in complex media using biotinylated probes. The final concentration of Biotin-L-Apt.4-1c and Biotin-L-mutant at 1600 nM were used to pulldown target rG4s in total RNA and cell lysate, respectively. (A) The qPCR curves show great *hTERC* rG4 (dark red: 25.3 cycles) and *APP* rG4 (dark blue: 23.4 cycles) enrichment after total RNA endogenous pulldown using L-Apt.4-1c, while L-mutant cannot pull down either *hTERC* rG4 (light orange-red: >40 cycles) or *APP* rG4 (light blue: 38.7 cycles) efficiently. (B) The qPCR curves show greater *hTERC* rG4 (dark red: 26.3 cycles) and *APP* rG4 (dark blue: 25.3 cycles) enrichment after total RNA endogenous pulldown using L-Apt.4-1c) than using L-mutant (light orange-red: 28.6 cycles; light blue: 28.0 cycles). Three independent replicates are performed.

References

1. M. I. Umar and C. K. Kwok, *Nucleic Acids Res.*, 2020, **48**, 10125-10141.