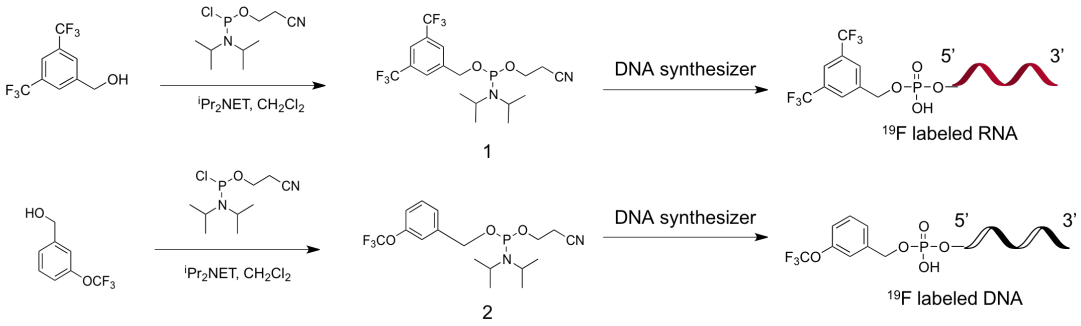
Supporting information



Supplementary Figure S1. Synthetic scheme of ^{19}F labeled DNA and RNA by using phosphoramidite chemistry. The detail synthesis procedure of compound **1** could refer our previous work (*Nucleic Acids Res.* 2019, **47**, 4940-4947).

Synthesis of compound 2:
1-*O*-[(2-cyanoethoxy)-(*N*,*N*-diisopropylamino)phosphinyl]-3-(trifluoromethoxy)benzene (2)

The 3-(trifluoromethoxy)benzyl alcohol (530 μg, 3.7 mmol) was dried three times with 5 mL anhydrous acetonitrile. The dried residues was treated with dry *N*,*N*-diisopropyl ethylamine (1.0 mL, 7.4 mmol) and 2-cyanoethyl-*N*,*N*- diisopropylchlorophosphoramidite (1 mL, 4.3 mmol) in dry dichloromethane (3 mL) and stirred at room temperature for 2 h. The reaction was stopped by adding 5% $NaHCO_3$ aqueous solution (50 ml). After addition of dichloromethane (50 ml), the aqueous layer was extracted three times with dichloromethane (50 ml). The combined organic layers were dried over by Na_2SO_4 and the solvent was evaporated *in vacuum*. The residue was purified via recycling preparative HPLC to give the compound **2** (1.2 g, 3.1 mmol, 83%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.38-7.11 (m, 4H), 4.73 (m, 2H), 3.86 (m, 2H), 3.66 (m, 2H), 2.64 (t, J = 6.4 Hz, 2H), 1.20 (m, 12H). ^{31}P-NMR (162 MHz, $CDCl_3$) δ −148.93. ESI-MS for $C_{17}H_{25}O_3N_2F_3P$ $[M+H]^+$: Calcd. 393.15; Found. 393.15.

Sample preparation

3,5-bis(trifluoromethyl)phenyl and 3-(trifluoromethoxy)benzyl moieties were incorporated into the RNA and DNA sequence on a 1.0 μmol scale by using an automatic solid-phase phosphoramidite chemistry and a DNA/RNA synthesizer. After synthesis, the oligomer was detached from the support, deprotected by using an AMA solution. The deprotected DNA and RNA were further purified by HPLC with a linear gradient of 50 mM ammonium formate in 1:1 acetonitrile/H_2O and 50 mM ammonium formate in H_2O. The oligomers were desalted by NAP 10 column (GE Healthcare) and identified by MALDI-TOF-MS on an autoflex III smart beam mass spectrometer (negative mode).

CD measurement

CD experiments were performed by using a Jasco model J-820 CD spectrophotometer. A 1 cm path length cell was used for spectra record. The oligonucleotides were heated at 90 °C for 3 min and gradually cooling to room temperature. A 0.3 mL sample was made at a concentration of 10 μM RNA in the presence of 100 mM KCl and 20 mM K-PO_4 buffer (pH 7.0).

Introduction of ^{19}F labeled DNA/RNA mixture into Hela cells by streptolysin O (SLO)

HeLa cells (2×10^7) grown in Dulbecco's Modified Eagle Medium (DMEM) medium containing 10% FBS under a 5% CO_2 atmosphere were collected and then washed twice with HBSS buffer. SLO (biologicalemia) was activated with 10 mM DTT and 0.05% BSA at 37 °C for 2 h. To form pores in the

plasma membrane, activated SLO was added to Hela cells at a final concentration of 0.1 μg / mL, followed by gentle rotation incubation at 4 ° C for 15 minutes. After washing three times with ice-cold HBSS buffer, cells were incubated with 1 mM ^{19}F labeled RNA with or without 3 mM native DNA were incubated in 500 μl HBSS buffer at 37°C for 30 min and then shaken. Cells were then resealed by adding ice-cold HBSS buffer (containing 1 mM $CaCl_2$). After 30 minutes incubation at 4 °C, the cells were washed twice with HBSS buffer containing 1 mM $CaCl_2$. The resealed cells were seeded in HBSS buffer containing 14% percoll (centrifuged at 2,000 × g for about 1 hour), and then centrifuged at 400 × g for 3 minutes. After centrifugation, the cell pellet (viable cells) was washed 3 times with HBSS buffer.

^{19}F NMR measurement

For ^{19}F NMR measurement, ^{19}F labeled DNA or RNA samples of 0.2 mM were dissolved in 150 μL of designed solvent which containing 100 mM or 200 mM KCl and 20 mM K-PO_4 buffer (pH 7.0) and 10% D_2O. The oligonucleotides were heated at 90 °C for 3 min and gradually cooling to room temperature. The samples were individually annealed for each ^{19}F NMR experiment. ^{19}F NMR spectra were measured on a Bruker AVANCE 400 MHz spectrometer at 376.05 MHz frequency and were referenced relative to internal standard CF_3COOH (−75.66 ppm). Experimental parameters were recorded as follows: spectral width 89.3 kHz, ^{19}F excitation pulse 15.0 μs, relaxation delay 1.5 s, acquisition time 0.73 s, number of scans 256 and line broad 3. For in-cell ^{19}F NMR measurement, HeLa cells with SLO treatment were collected and suspended in 200 μL of DMEM with 20 % D_2O. Number of scans for in-cell measurement is 2048. After the in-cell ^{19}F NMR measurement, the cell suspension was supplemented with 100 μL of DMEM, and the supernatant was collected after centrifugation for 3 min at 400 G. The ^{19}F NMR spectrum of the supernatant was measured with same numbers of scans as the in-cell NMR measurement.

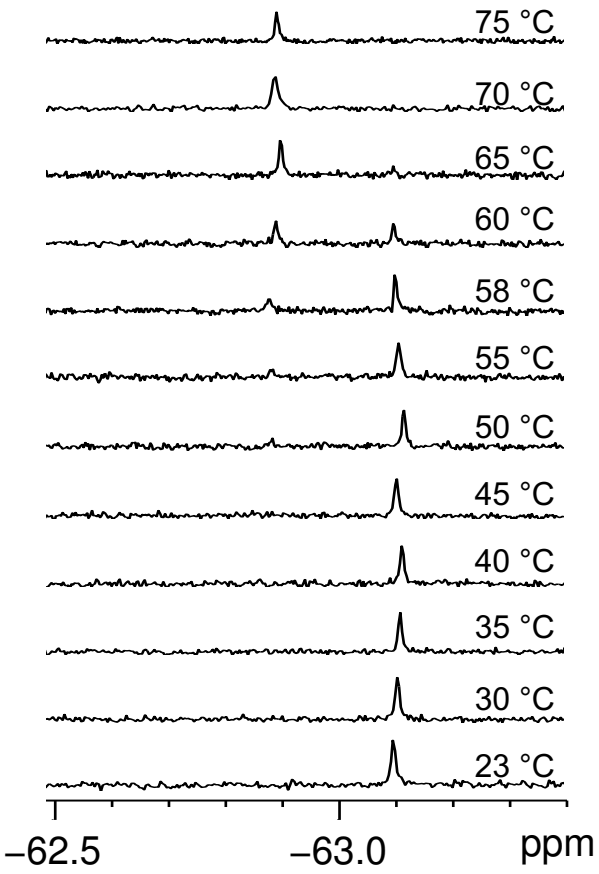
^{1}H NMR measurement

For ^{1}H NMR measurement, DNA or RNA samples of designed concentration were dissolved in 150 μL of designed solvent containing 10% D_2O, 100 mM or 200 mM KCl and 20 mM K-PO4 buffer (pH 7.0). The oligonucleotides were heated at 90 °C for 3 min and gradually cooling to room temperature.

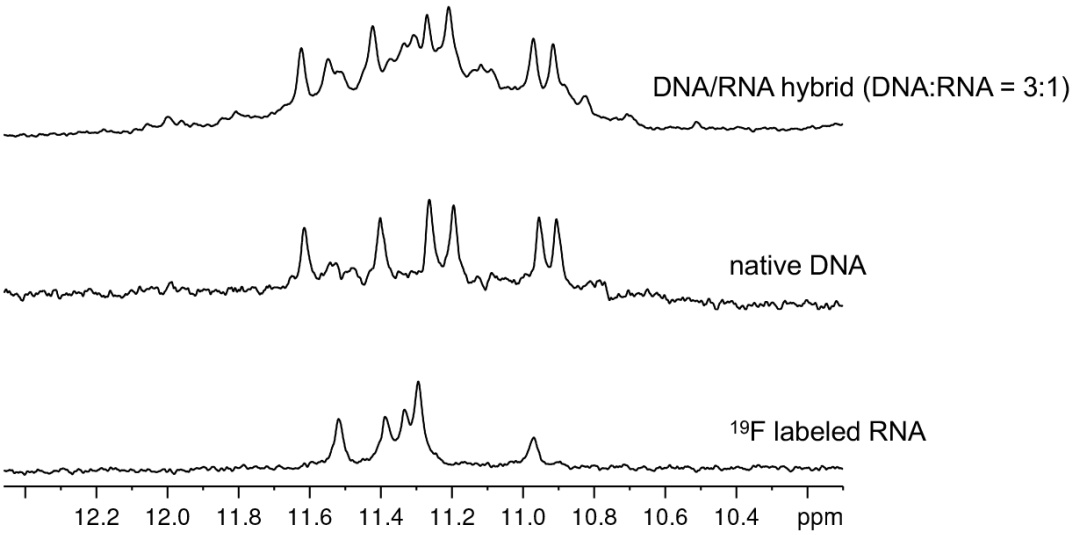
Table S1 Thermodynamic parameters of ^{19}F labeled 12-mer telomeric DNA and DNA-RNA hybrid G-quadruplex consisted by the ^{19}F labeled 12-mer telomeric DNA and native 12-mer RNA determined by ^{19}F NMR spectroscopy

G-quadruplex	$-\Delta H$ (kJ/mol)	$-\Delta S$ (J/mol K)	$-\Delta G_{298}$ (kJ/mol)	T_m (°C)
HQ	163.2	502.4	13.5	51.7
Parallel DQ	159.9	488.2	14.4	54.4
Antiparallel DQ	121.4	388.0	5.8	39.8

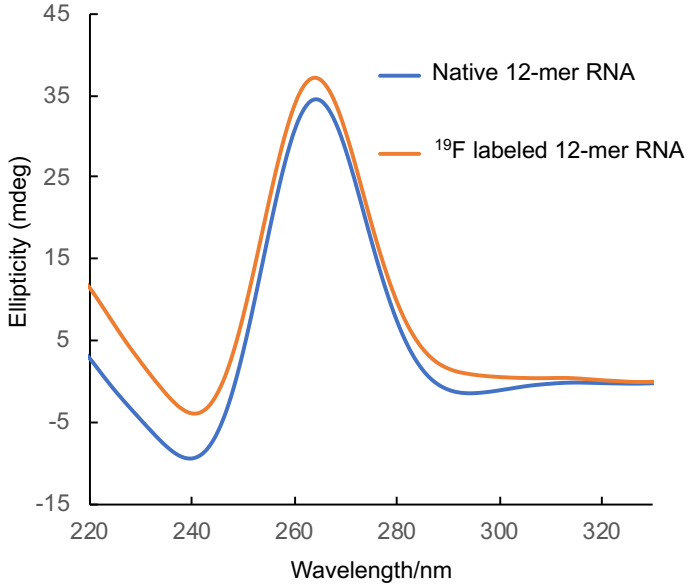
The thermodynamic parameters were determined from van't Hoff plots. Detail procedure was reported in our previous reference (*Nat. Protoc.* 2018, **13**, 652-665). The experimental errors for enthalpy (ΔH) and entropy (ΔS) were ± 5 kJ/mol and ± 10 J/mol K, respectively.



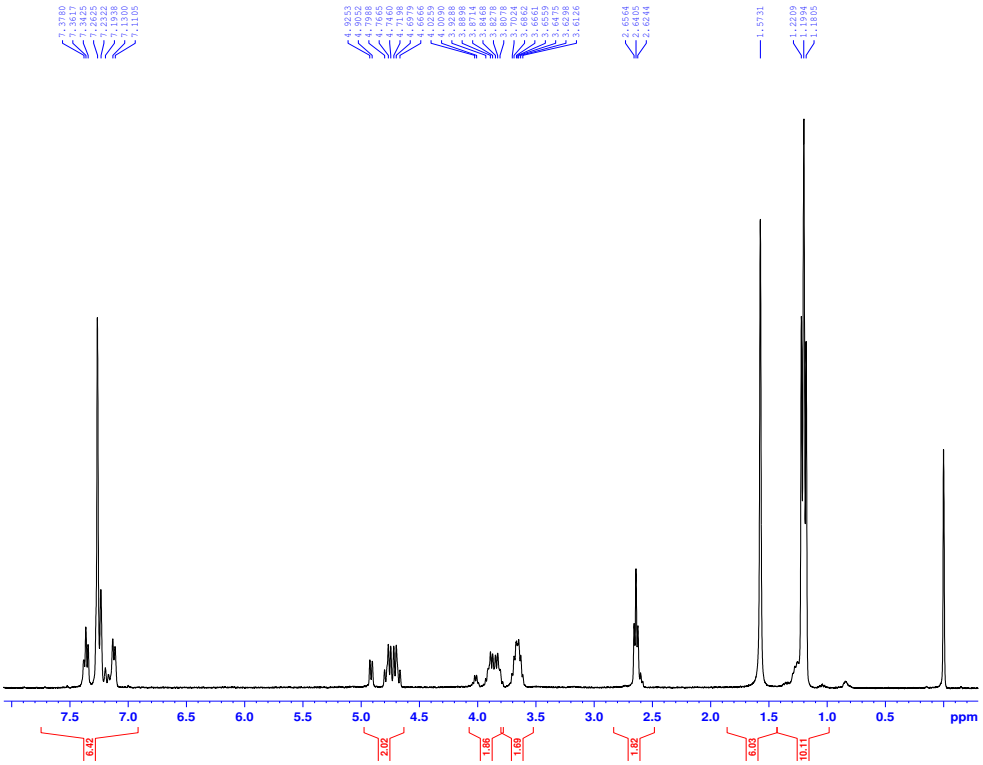
Supplementary Figure S2. ^{19}F NMR spectra of ^{19}F labeled 12-mer RNA at different temperature. Condition: 100 μM RNA in 100 mM KCl and 20 mM K-PO_4 buffer (pH 7.0).



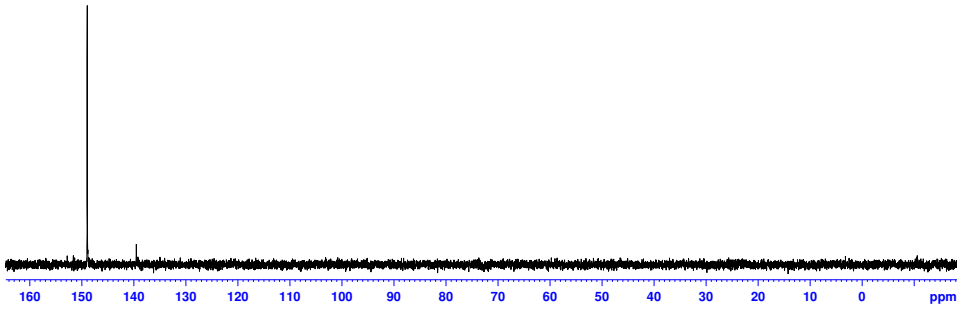
Supplementary Figure S3. Imino proton spectra of ^{19}F labeled 12-mer RNA, native 12-mer DNA and DNA-RNA (3:1) mixture.



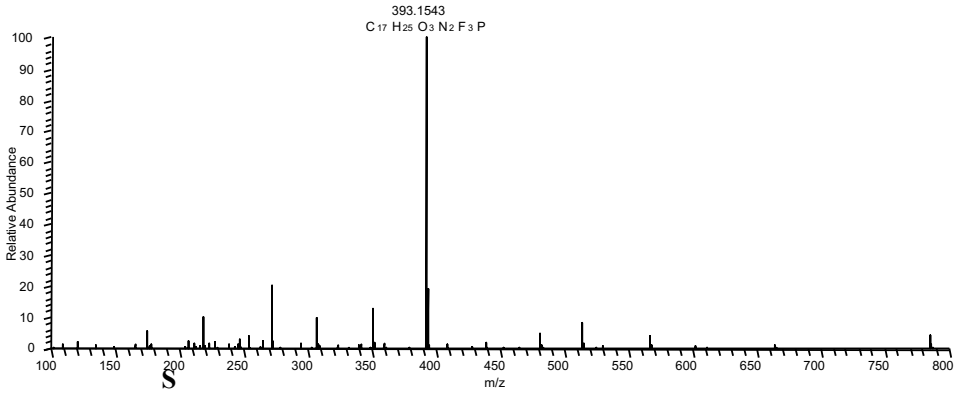
Supplementary Figure S4. CD spectra of ^{19}F labeled 12-mer RNA and unmodified 12-mer RNA. Condition: 10 μM RNA in 100 mM KCl and 20 mM K-PO_4 buffer (pH 7.0).



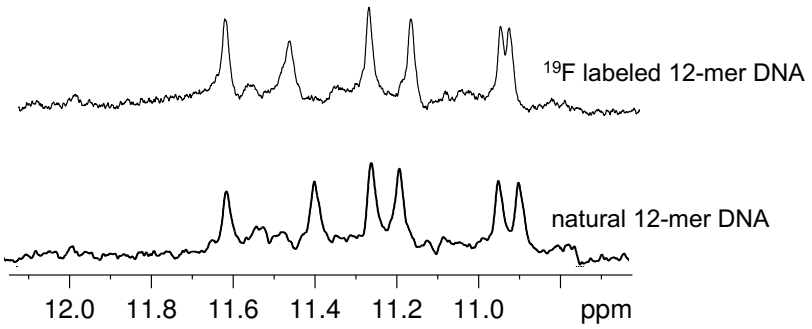
Supplementary Figure S5. ^{1}H NMR spectrum of compound 2.



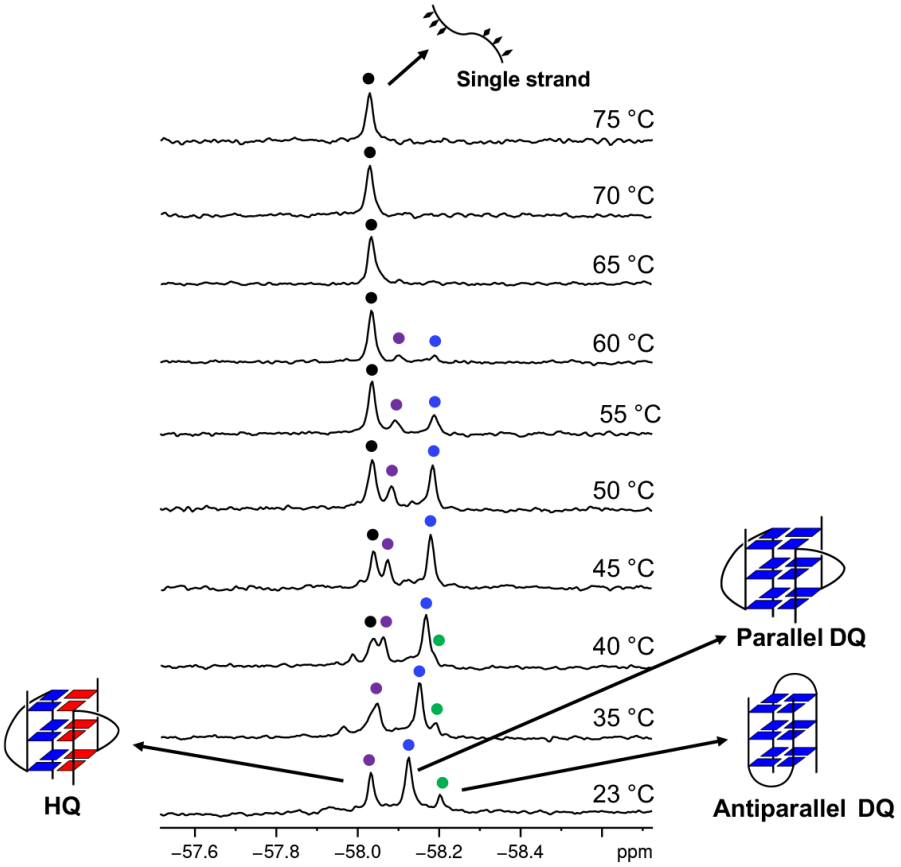
Supplementary Figure S6. ^{31}P NMR spectrum of compound 2.



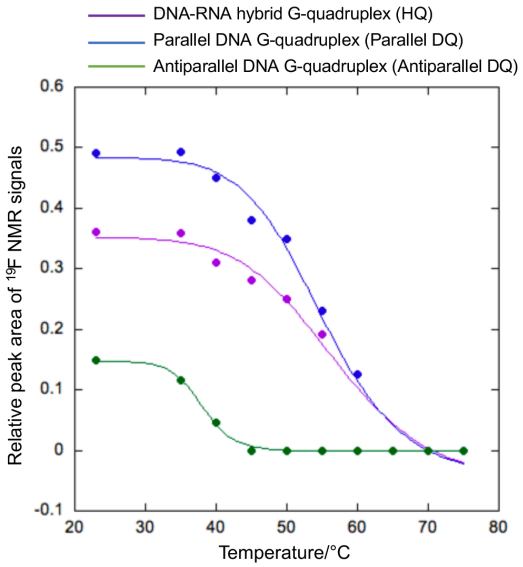
Supplementary Figure S7. ESI-MS spectrum of compound 2.



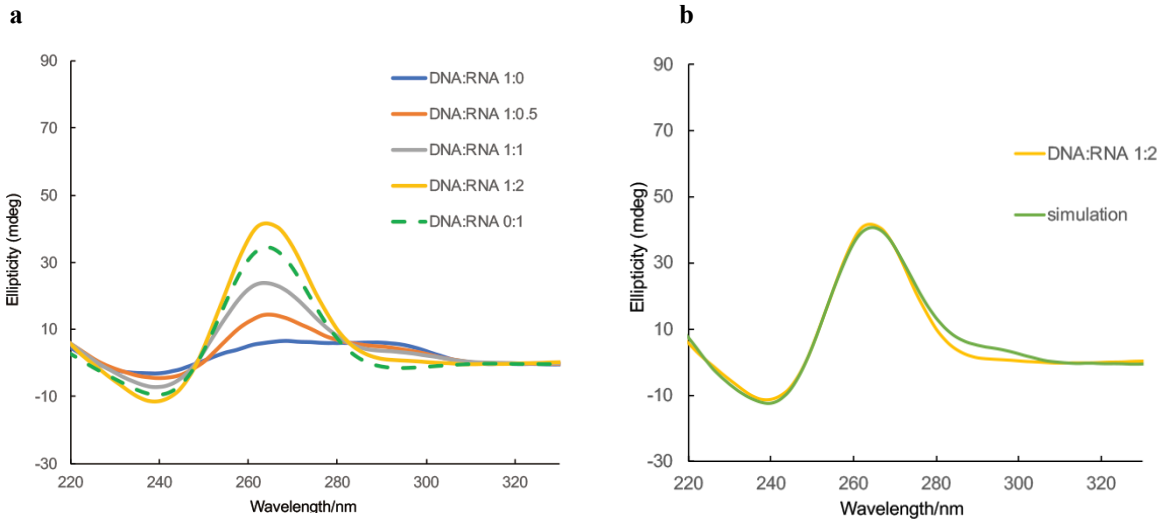
Supplementary Figure S8. ^{1}H NMR spectra of natural and ^{19}F labeled 12-mer DNA in 200 mM KCl and 20 mM K-PO4 buffer (pH 7.0).



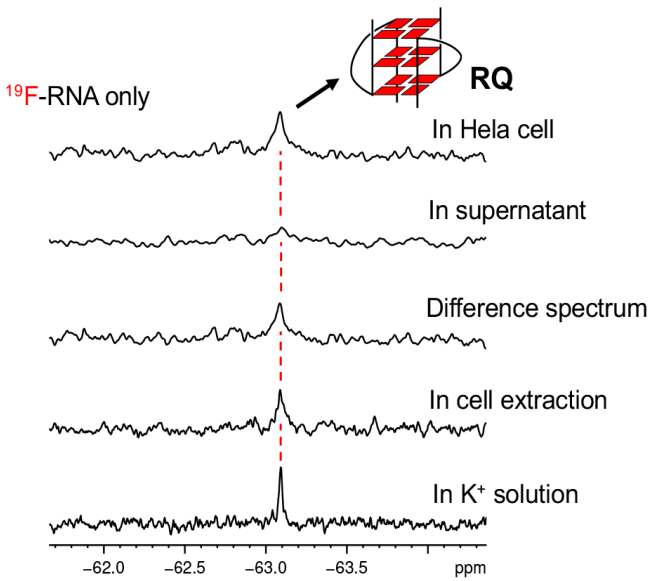
Supplementary Figure S9. ^{19}F NMR spectra of ^{19}F labeled 12-mer DNA and natural 12-mer RNA at different temperature. Condition: 0.2 mM DNA and 0.6 mM RNA in 200 mM KCl and 20 mM K-PO_4 buffer (pH 7.0). Temperature indicated on the right. Green and blue spots indicated antiparallel and parallel DNA G-quadruplex, respectively. Purple and black spots indicated DNA-RNA hybrid G-quadruplex and single strand.



Supplementary Figure S10. Profiles of the relative peak areas of the ^{19}F resonance signals versus temperature.



Supplementary Figure S11. a, CD spectra of the unmodified 12-mer DNA in the presence of different ratios of unmodified 12-mer RNA. Condition: 5 μM DNA in 100 mM KCl and 20 mM K-PO_4 buffer (pH 7.0). CD curve of unmodified 12-mer RNA was shown as a green dash line (10 μM RNA in 100 mM KCl and 20 mM K-PO_4 buffer (pH 7.0)). **b**, CD spectra of DNA:RNA at 1:2 ratio (yellow) and a simulation curve obtained by superposition of CD spectra for 5 μM DNA (blue line at Figure S11a,) and 10 μM RNA (blue line at Figure S4, blue). There is no obvious difference between the simulated curve and the actual measured curve, suggested that CD experiment is not enough to distinguish the DNA-RNA hybrid G-quadruplex from the DNA and RNA G-quadruplex.



Supplementary Figure S12. Comparison of ^{19}F NMR spectra of ^{19}F labeled 12-mer telomeric RNA in K^+ solution, in HeLa cell extraction, in Hela cells, in supernatant and difference spectrum between HeLa cell and supernatant.