Supplementary Data

Visualization of ligand-induced *c-MYC* duplex–quadruplex transition and direct exploration of the altered *c-MYC* DNA-protein interactions in cells

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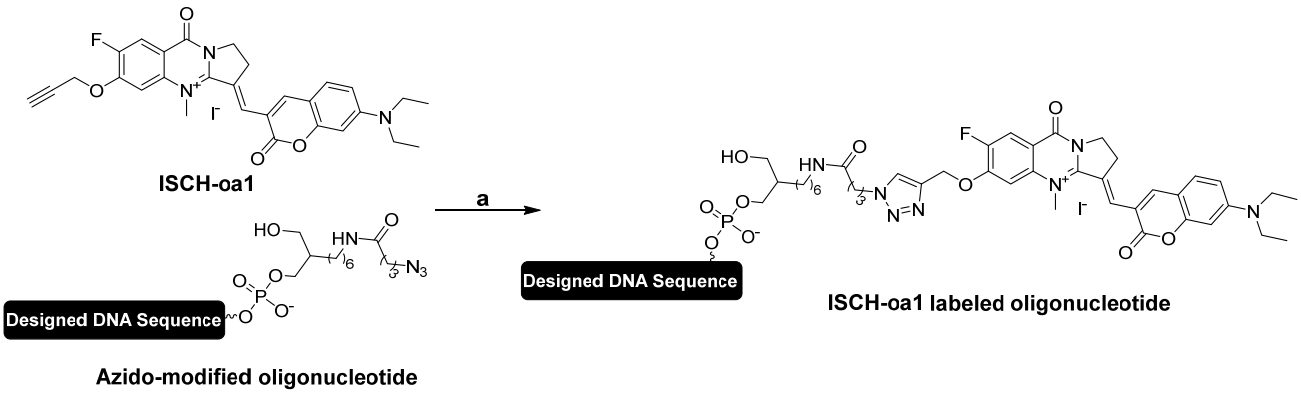
1. Experimental Section

1.1 Synthesis and characterization

All chemical reagents and solvents for synthesis and characterization were purchased from commercial suppliers (J&K Scientific Ltd., Sigma-Aldrich Chemical Co. and Shanghai Aladdin Bio-Chem Technology Co. Ltd.) unless otherwise specified and were used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Ascend^{TM} 400 or Ascend^{TM} 500 spectrometer. Mass spectra (MS) were recorded on a Shimadzu LCMS-2010A and a Thermo Scientific LTQ XL^{TM} linear ion trap instrument. Flash column chromatography was performed with silica gel (200-300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. The purity of synthesized compound was determined by using analytical HPLC.

Scheme S1. Synthesis of GTFH probes.

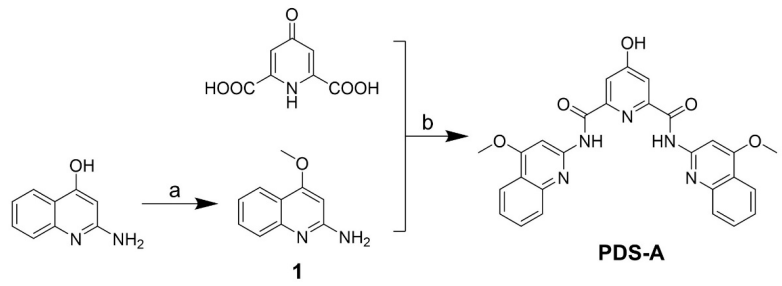


Reagents and conditions: (a) water, sodium ascorbate, copper sulfate, 37°C, 24 h.

General Synthesis of GTFH probes.[1] ISCH-oa1 (1 mM) and azido-modified DNA oligonucleotide (0.05 mM) were mixed in water (200 μL) containing fresh sodium ascorbate (1.2 mM). Copper sulfate (0.6 mM) was then added and stirred for 24 h at 37°C. The crude products were loaded onto Sep-Pak C18 cartridges and washed with distilled water for multiple times, then eluted from cartridges with 20:80 (v/v) acetonitrile/water and concentrated with rotary evaporator. GTFH probes were later separated using reverse-phase HPLC (RP-HPLC) equipped with Elite SinoChrom ODS-BP C18-column (10.0 X 250 mm, 5 μm). Elution was performed with a linear gradient of buffer B (0%-50%) over 30 min at a flow rate of 3 mL/min (Buffer A: 0.1 M triethylammonium acetate in distilled water, pH 7.0; Buffer B: acetonitrile). The solution of collected fraction was concentrated to dryness with rotary evaporator. The final products were characterized by ESI mass spectrometry and HPLC analysis.

	Sequences	Target MW	Observed MW	Purity
ISCH-MYC	5'-d[GCTGCCCGGCTGAGTCTCCTCCCCA]-3'-ISCH-oa1	8352.6	8349.4	90.5%
ISCH-MUT	5'-d[ATCTACTACACTACGCTACACTCTT]-3'-ISCH-oa1	8316.7	8315.5	94.9%

Scheme S2. Synthesis of PDS-A.



Reagents and conditions: (a) Me_3SiCHN_2, toluene–MeOH, r.t., 1 h; (b) Ghosez's reagent, Et_3N, r.t., overnight.

Synthesis of 1. 2-Amino-4-quinolinol (0.64 g, 4.0 mmol) was dissolved in a 100 mL solution of toluene and methanol (3:2). (Trimethylsilyl)diazomethane (2M solution in hexanes, 4.0 ml, 8.0 mmol) was added dropwise, and the reaction left to stir for 1 h at room temperature. The solvent was removed under reduced pressure and the crude product was dissolved in 0.1M NaOH solution. The aqueous layer was extracted with EtOAc and the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by using flash column chromatography with PE/DCM (3:1) elution to afford a white solid (0.38 g, yield 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (dd, *J* = 8.2, 1.1 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.55 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H), 7.26 – 7.21 (m, 1H), 6.07 (s, 1H), 4.94 (bs, 2H), 3.98 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.68, 158.36, 148.32, 130.34, 125.45, 122.16, 121.85, 117.84, 89.58, 55.66. ESI-MS m/z: 175.1 $[M+H]^+$.

Synthesis of PDS-A.[2] 1-Chloro-N,N,2-trimethylpropenyl-amine (0.3 ml, 2.2 mmol) was added slowly to a solution of chelidamic acid hydrate (0.20 g, 1.0 mmol) in MeOH (4.0 mL) and the resulting mixture was stirred at room temperature under argon atmosphere for 3 h. Then, the mixture was cooled to 0 °C and dry triethylamine (0.3 ml, 2.2 mmol) was slowly added. This reaction mixture was stirred at room temperature for another 2 h. After that, compound 1 (0.35 g, 2.0 mmol) was added and the mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the crude was precipitated from hot acetonitrile to yield a white solid. The crude product was purified by using flash column chromatography with DCM/MeOH (50:1) elution to afford a white solid (0.39 g, yield 79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96 (s, 2H), 11.73 (bs, 1H), 8.14-8.10 (m, 4H), 7.94 (d, J = 8.3 Hz, 2H), 7.80 – 7.74 (m, 4H), 7.55 – 7.48 (m, 2H), 4.12 (s, 6H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.73, 163.42 (2C), 162.96 (2C), 152.51 (2C), 150.95 (2C), 146.95 (2C), 130.55 (2C), 126.88 (2C), 124.63 (2C), 121.63 (2C), 119.14 (2C), 113.15 (2C), 94.47 (2C), 56.08 (2C). ESI-MS m/z: 496.1 $[M+H]^+$.

1.2 Circular dichroism (CD) spectroscopy

CD studies were performed on a Chirascan circular dichroism spectrophotometer (Applied Photophysics). A quartz cuvette with a 1 cm path length was used for the recording of spectra over a wavelength range of 230–330 nm with a 1 nm bandwidth, 1 nm step size and time of 0.5 s per point. All oligonucleotides were annealed in relevant buffer by heating to 95 °C for 5 min, followed by gradual cooling to room temperature. CD melting was performed at a fixed concentration of different DNAs (2 μM), either with or without a fixed concentration (5 μM) of PDS or PDS-A in Tris-HCl buffer (10 mM, pH 7.2) with 100 mM KCl. The data was recorded at intervals of 2.5 °C over a range of 25–95 °C, with a heating rate of 1.0 °C/min. A buffer baseline was collected in the same cuvette and was subtracted from the sample spectra. Final analysis of the data was conducted using Origin 7.0 (OriginLab Corp.).

1.3 Thermal difference spectrum (TDS) studies

TDS studies were performed on a UV-2450 spectrophotometer (Shimadzu) using 1 cm path length quartz cuvette. All DNA oligonucleotides were diluted from stock to final concentration (1 μM) in Tris-HCl buffer (10 mM, pH 7.2) with 100 mM KCl. All samples were annealed by heating at 95 °C for 5 min, gradually cooled to room temperature and measured after 24 h. UV-Vis spectra were recorded at 25 and 95 °C. A 10 min equilibration period at each measurement was allowed to ensure homogeneous sample temperature. TDS spectra were calculated by subtracting the spectrum at 25 °C from the spectrum at 95 °C.

1.4 Electrophoretic mobility shift assay (EMSA) studies

Different oligonucleotides were loaded onto a 20% bisacrylamide gel in 1×TBE buffer containing 100 mM KCl and were electrophoresed at 4 °C. FAM-labeled oligonucleotides or SYBR Gold-stained oligonucleotides were visualized under UV light and photographed using AlphaImager EC (Protein Simple).

2. Supporting Spectra and Graphs

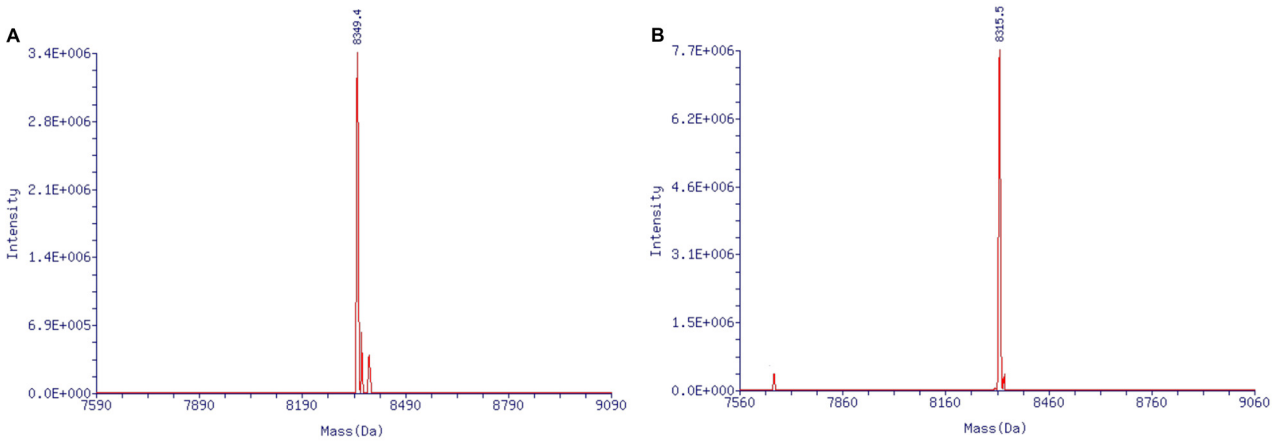


Figure S1. Mass spectra of **ISCH-MYC** (A) and ISCH-MUT (B).

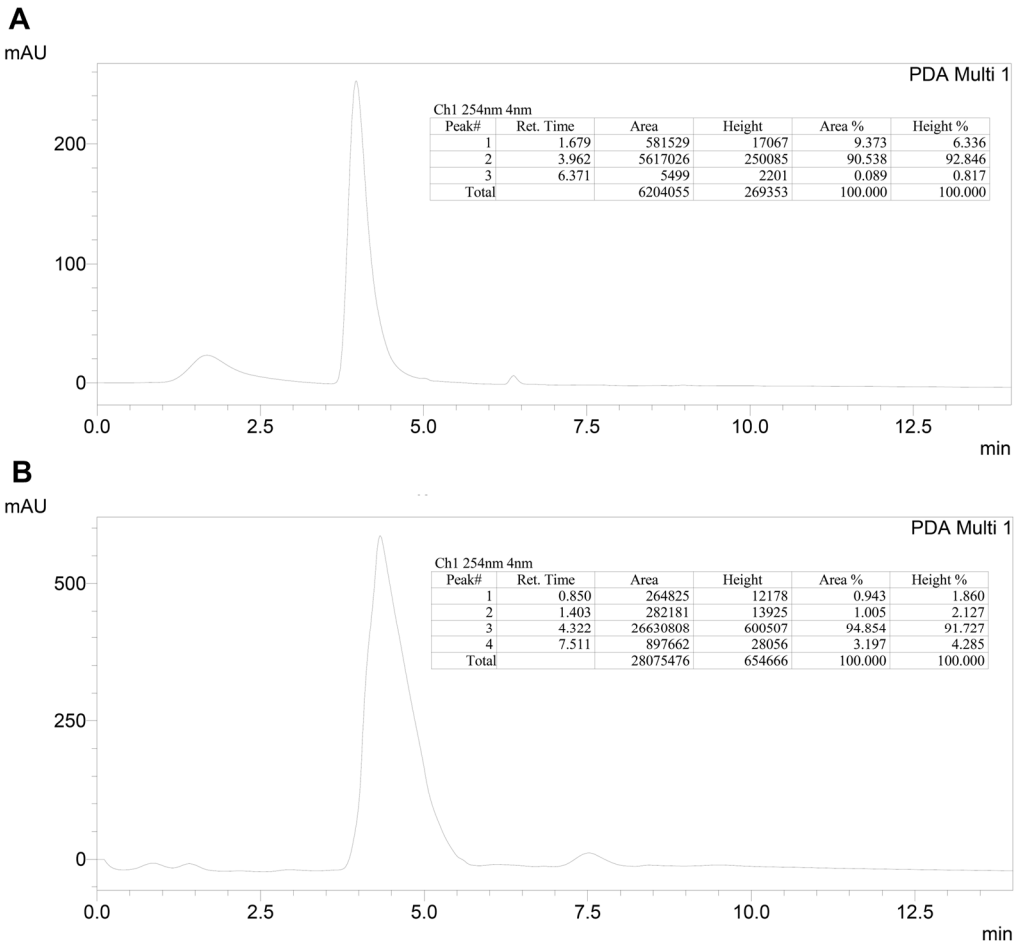


Figure S2. HPLC analysis of **ISCH-MYC** (A) and ISCH-MUT (B).

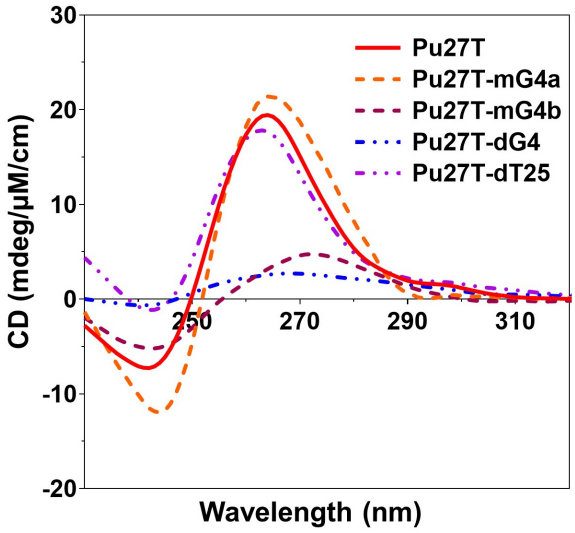


Figure S3. CD spectra of 1 μM DNAs in 10 mM Tris-HCl buffer, 100 mM KCl, pH 7.2. The CD profiles of the DNAs with G-rich sequence (Pu27T and Pu27T-dT25) showed a sharp positive peak at around 265 nm, and a negative peak at around 240 nm. These peaks were typical signatures of G-quadruplex structures.

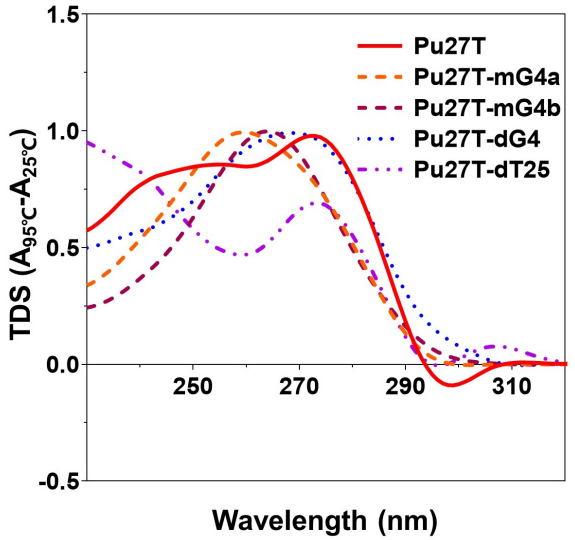


Figure S4. TDS studies of 1 μM DNAs in 10 mM Tris-HCl buffer, 100 mM KCl, pH 7.2. The TDS profiles of the DNAs with G-rich sequence (Pu27T and Pu27T-dT25) showed a positive peak at around 273 nm, and a negative peak at round 295 nm. These peaks were typical signature of G-quadruplex structures.

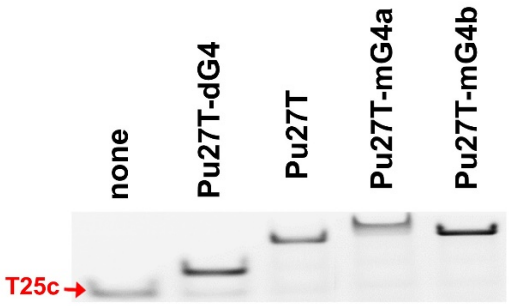


Figure S5. Electrophoresis of T25c with or without DNAs stained by SYBR Gold.

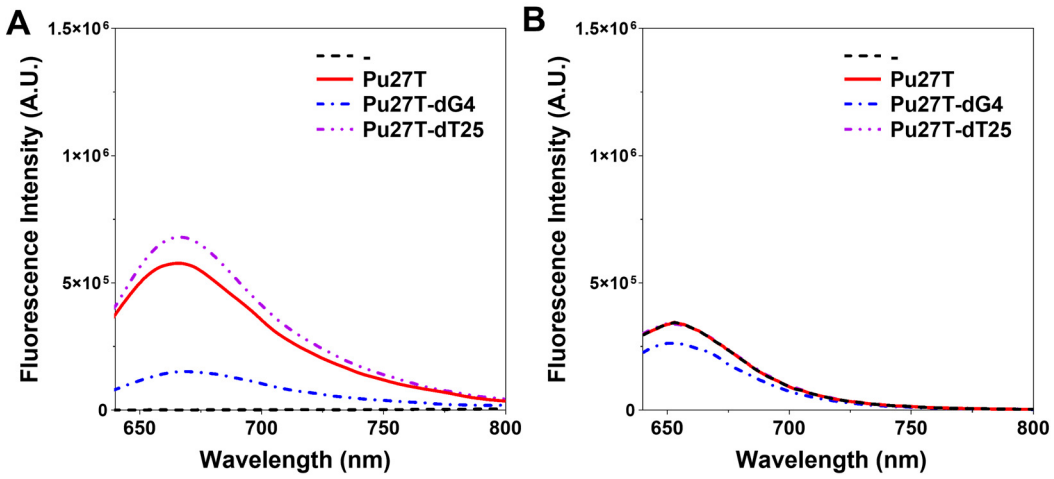


Figure S6. Fluorescence spectra of 1 μM ISCH-oa1 (A) or ISCH-MUT (B) with or without 2 μM DNAs in 10 mM Tris-HCl buffer, 100 mM KCl, pH 7.2.

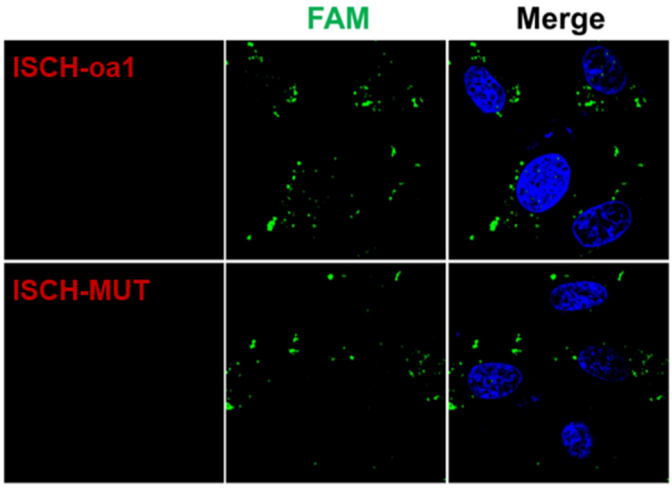


Figure S7. Confocal imaging of FAM-labeled Pu27T transfected HeLa cells stained by ISCH-oa1 or ISCH-MUT.

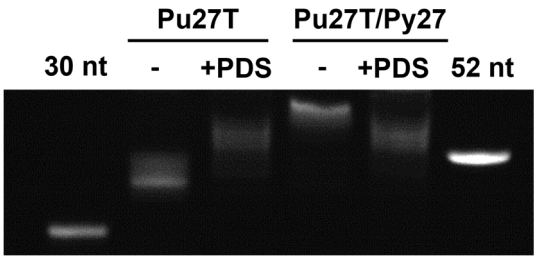


Figure S8. Electrophoresis of 5 μM FAM-labeled Pu27T and its complex with Py27 (Pu27T/Py27) in the absence/presence of 20 μM PDS. A 30 nt (5'-TTTTTTTTTTCGTACCCGATGTGTTCGTTC-3') and a 52 nt (5'-TACATACACTAGATATACTCGACAACGTGGGGAGGAGACTCAGCCGGGCAGC-3') FAM-labeled single-stranded DNAs were employed as the markers.

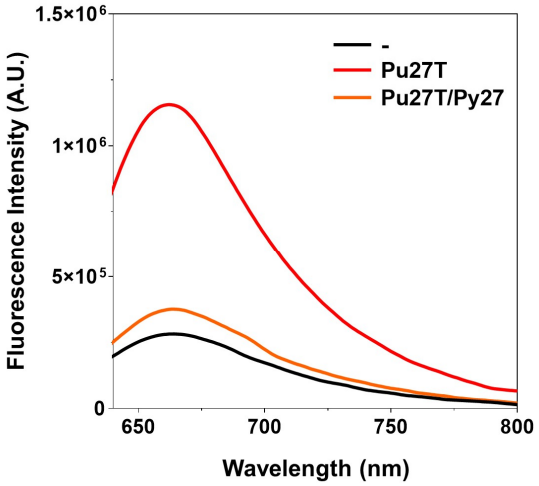


Figure S9. Fluorescence spectra of 1 μM **ISCH-MYC** with or without 2 μM Pu27T or Pu27T/Py27 in 10 mM Tris-HCl buffer, 100 mM KCl, pH 7.2.

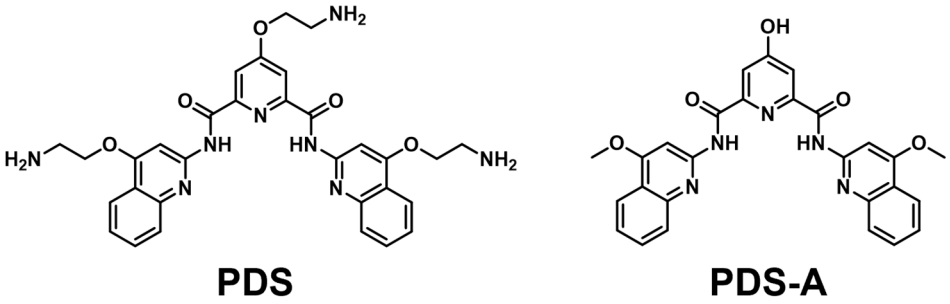


Figure S10. Chemical structures of PDS and PDS-A.

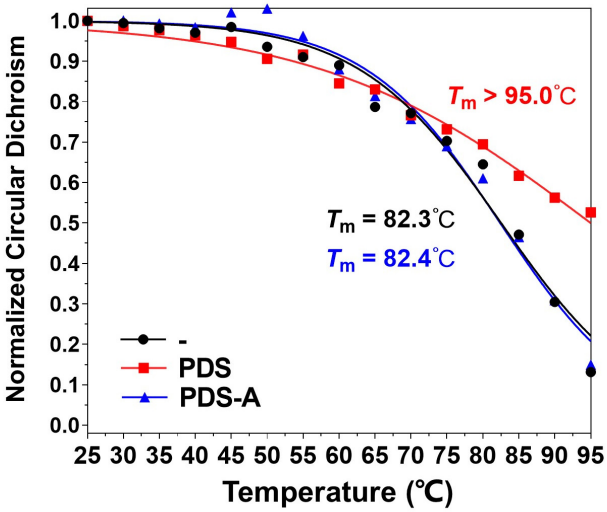


Figure S11. CD melting studies of 2 μM Pu27T with or without 5 μM PDS or PDS-A in 10 mM Tris-HCl buffer, 100 mM KCl, pH 7.2. CD signals were normalized by the positive peak at 265 nm.

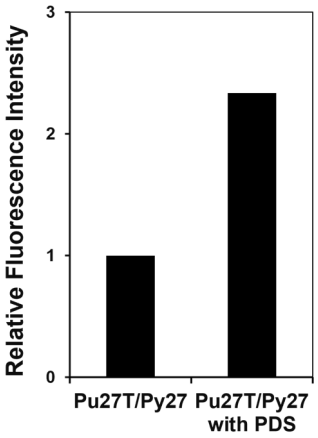


Figure S12. Fluorescence change of 1 μM **ISCH-MYC** with 2 μM Pu27T/Py27 in the absence/presence of 8 μM PDS at 655 nm in 10 mM Tris-HCl buffer, 100 mM KCl, pH 7.2. The relative fluorescence intensity is calculated as ratios to Pu27T/Py27.

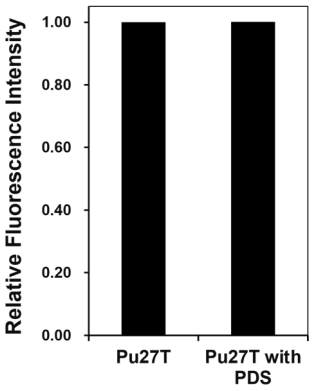


Figure S13. Fluorescence change of 1 μM **ISCH-MYC** with 2 μM Pu27T in the absence/presence of 8 μM PDS at 655 nm in 10 mM Tris-HCl buffer, 100 mM KCl, pH 7.2. The relative fluorescence intensity is calculated as ratios to Pu27T.

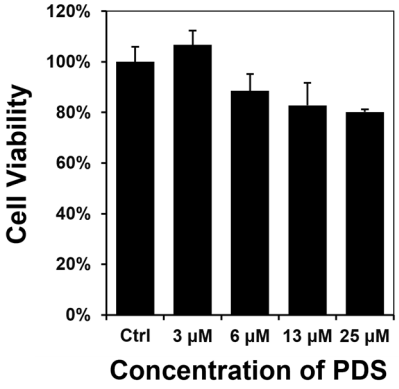


Figure S14. Cytotoxicity of PDS on HeLa cells after 24 h treatment.

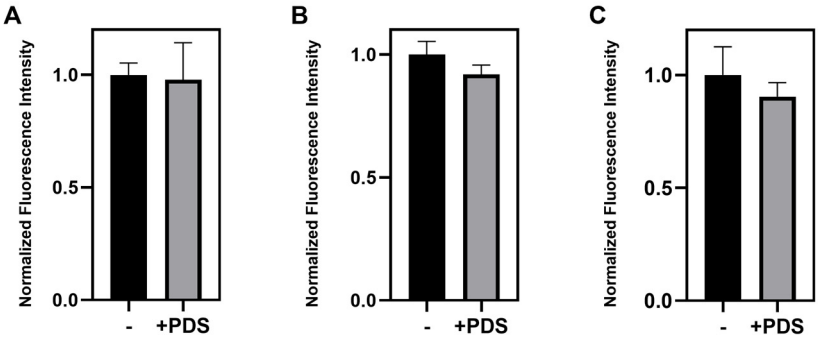


Figure S15. Quantification of transfected FAM-labeled DNAs before and after the treatment of 10 μM PDS. The mean FAM intensity of DNA alone is normalized to 1. (A) Pu27T is delivered into cytoplasm by Lipofectamine 3000. (B) Pu27T/Py27 is delivered into cytoplasm by Lipofectamine 3000. (C) Pu27T/Py27 is delivered into nucleus by Streptolysin O.

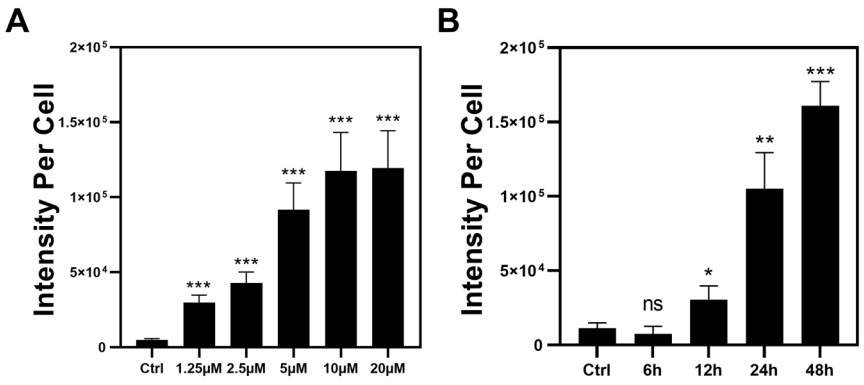


Figure S16. (A) Quantification of fluorescence intensity in double-stranded Pu27T/Py27 transfected cells treated with PDS in different concentrations for 24 h then stained by **ISCH-MYC**. (B) Quantification of fluorescence intensity in double-stranded Pu27T/Py27 transfected cells treated with PDS for a different duration then stained by **ISCH-MYC**. Quantification data are expressed as the mean ± SEM (standard error of mean). For each sample, approximately 300 cells were measured. The standard error was calculated from a set of three replicate experiments. Statistical significance was determined by the *t* test as (ns) not significant, (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$.

3. Reference

1. Chen, S.-B., Hu, M.-H., Liu, G.-C., Wang, J., Ou, T.-M., Gu, L.-Q., Huang, Z.-S. and Tan, J.-H. (2016) Visualization of NRAS RNA G-quadruplex structures in cells with an engineered fluorogenic hybridization probe. *J. Am. Chem. Soc.*, **138**, 10382-10385.

2. Mueller, S., Sanders, D.A., Di Antonio, M., Matsis, S., Riou, J.-F., Rodriguez, R. and Balasubramanian, S. (2012) Pyridostatin analogues promote telomere dysfunction and long-term growth inhibition in human cancer cells. *Org. Biomol. Chem.*, **10**, 6537-6546.