# Supporting Information

# Nucleolin Targeted DNA Nanotube for Precise Cancer Therapy through FRET-Indicated Telomerase Responsiveness

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**Table S1. Oligonucleotide sequences for DNA nanosheet/nanotube.**

| Name | Sequence (5'to 3') |
|---|---|
| F1 | TGTGTGTGTGTGTTCCAGCGCTTACGGCGCCAGTGGCGCCTTGGTG ATGGCATGGATGCGACGAGATCACCTCCTTC |
| F2 | TGTGTGTGTGTGTATGTGAGGCCCGTATCTGATGCTAAAACCGTAC GAAGCGTGCAAATCCGTCCGAGAGTATGGAT |
| F3 | TTTTTTTTTTTATCGTGCTTCACGTTCGGCGATAATGTGATCCTAGC AACCTACTCTATCCACCACGGGTTGGAC |
| F4 | TGTGTGTGTGTGTAGGTATGGATGTCGGAATTATGGGTCCCTGAGC TTGCACTTTGTTCACAGGTGAAAGAAGATGT |
| F5 | TGTGTGTGTGTGTTTAGTTCCGGACCCTCCCGCATTCTAGGAAGTG AATTGACATTATAATCGTCACCCACACCGCC |
| F6 | TTTTTTTTTTTACACGGGTTCAAGAACCTGTCCTCCGCCCAAAGGA GTTCGTCTACTGATTAACTTCGACTACGG |
| F7 | ATCGCCGCCGGACGTAGAGCCTAATGTGGGCAGTGACTACGAACT GACCAGATGAGGGCGGTTG |
| F8 | CGGCGCTGTTAATATTCCATTTATCATTTCTTTGGGTAGCTTAGGA GTGTTCGCACAAGCACAC |
| F9 | CACACTACGGAGGCCCCATCTCATAATGGTTTATGGGATTTGTGTG AGTCTAACCTACCGAGGC |
| F10 | TTTTTTTTTTTACCAAGGCGCCCAAAGTGCAAGTT |
| F11 | ACTGGCGCCGTAAGCGCTGGATACGGTTTTAGAATGTCAATTCAC ATCTTCTTTCACCTGTGAA |
| F12 | CATCAGATACGGGCCTCACATGGATCACATTAAGACGAACTCCGG CGGTGTGGGTGACGATTAT |
| F13 | TTTTTTTTTTTCTCAGGGACCCTTCGTAGTCACTT |
| F14 | ATAATTCCGACATCCATACCTACTTCCTAGAATAAGCTACCCACAA CCGCCCTCATCTGGTCAG |

| | |
|---|---|
| F15 | TGCGGGAGGGTCCGGAACTAATTTGGGCGGAGACAAATCCCATGTGTGCTTGTGCGAACACTCC |
| F16 | TCCATGCCATCTT |
| F17 | TGCACGCTTCGGAAGGAGGTGATCTCGTCGCAAATCCGTCGAGCAGAGTT |
| F18 | GTAGGTTGCTAATCCATACTCTCGGACGGATTAATCCGTCGAGCAGAGTT |
| F19 | TTTGCCCACATTA |
| F20 | **Cy5**-TTAGGGTTAGGGGGCTCTACGTCCGGCGGCGATAAGAAATGATA |
| F21 | **Cy5**-TTAGGGTTAGGGAATGGAATATTAACAGCGCCGAAACCATTATG |
| F22 | TCGCCGAACGTGAAGCACGATTTTTTTTTTTTTTTTTTTTTCCGTAGTCGAAGTTAATCAGT |
| F23 | GACAGGTTCTTGAACCCGTGTTTTTTTTTTTTTTTTTTTTTTGCCTCGGTAGGTTAGACTCAC |
| F24 | TTTTTGTCCAACCCGTGGTGGATAGAAATCCGTCGAGCAGAGTT |
| F25 | **Cy5**-TTAGGGTTAGGGAGATGGGGCCTCCGTAGTGTGTTTTT |
| F26 | CCCTAACCCTAACCC (T-cy3) AACTCTGCTCGACGGATT |
| F27 | ACACACACACACATTTTTTTTTTT-SH |
| F28 | AAAAAAAAAAAGGTGGTGGTGGTTGTGGTGGTGGTGG |
| F281 | AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| F29 | GGTGGTGGTGGTTGTGGTGGTGGTGGTT-FAM |

## Supplementary Figures

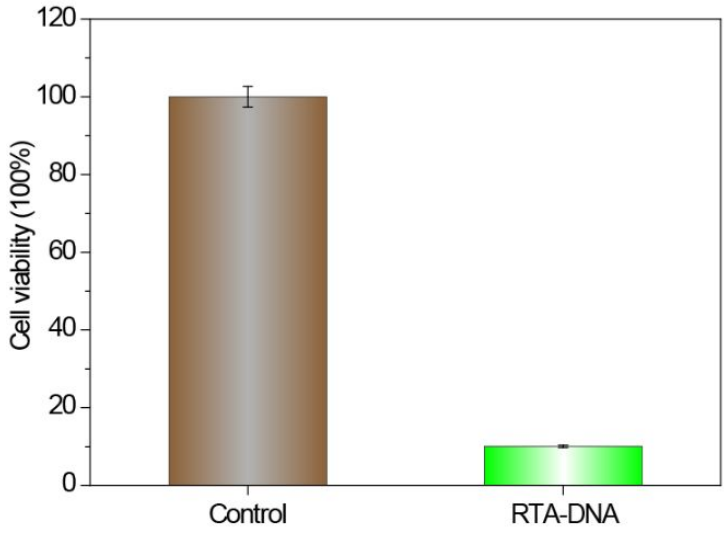


**Figure S1**. The cell viability of RTA-DNA conjugates. HeLa cells were seeded in a 96-well plate at a density of $1 \times 10^4$ cells per well and incubated for 24 h at 37 °C and 5% $CO_2$. After that 40 nM RTA-DNA conjugates was added and incubated for further 24h. The RTA-DNA conjugates exhibit excellent cell toxicity, indicating the feasibility of RTA for tumor therapy.

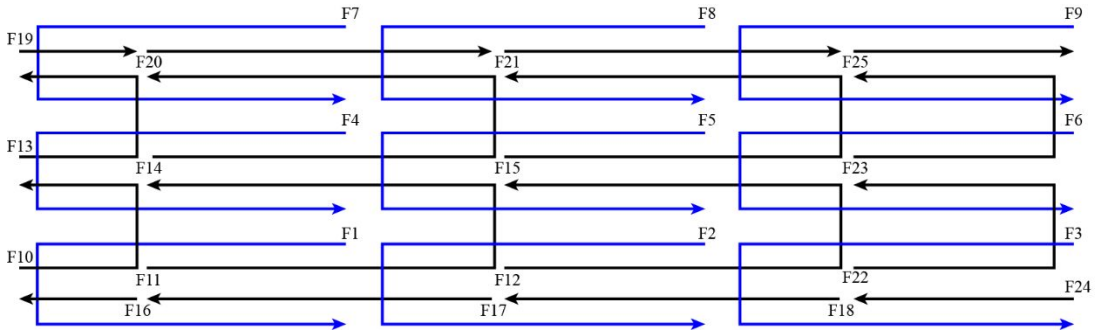


**Figure S2.** Detailed schematic diagrams of DNA nanosheet.

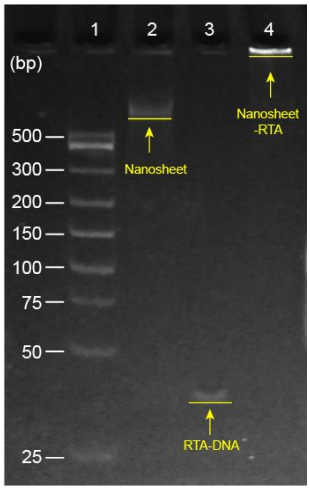


**Figure S3.** Gel characterization of DNA nanosheet-RTA. The band of DNA nanosheet-RTA was distinct retarded than that of pure DNA nanosheet on the 8% PAGE, indicating the successful loading of RTA on DNA nanosheet. Lane 1–4: DNA marker, DNA nanosheet, RTA-DNA conjugates and DNA nanosheet-RTA.

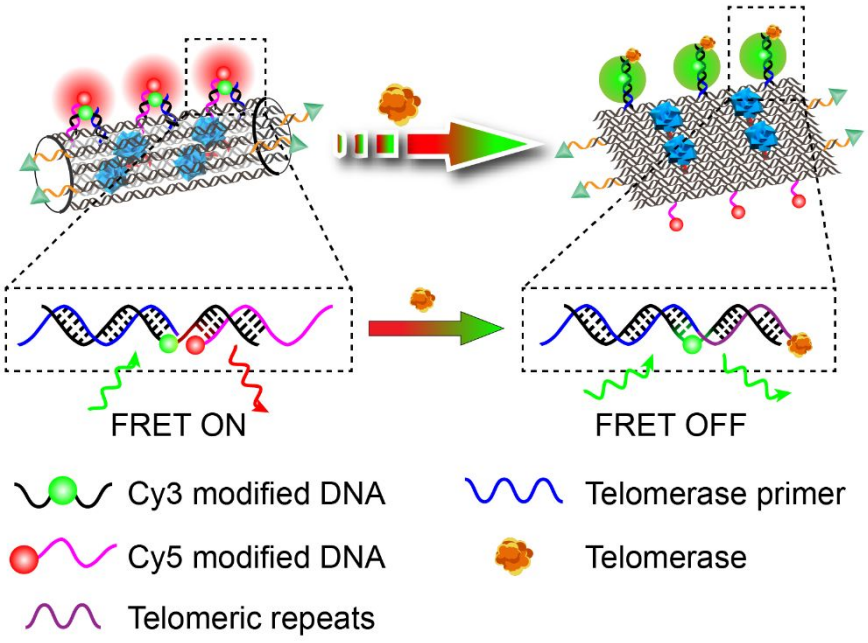


**Figure S4**. Schematic diagram of DNA nanotube in response to telomerase.

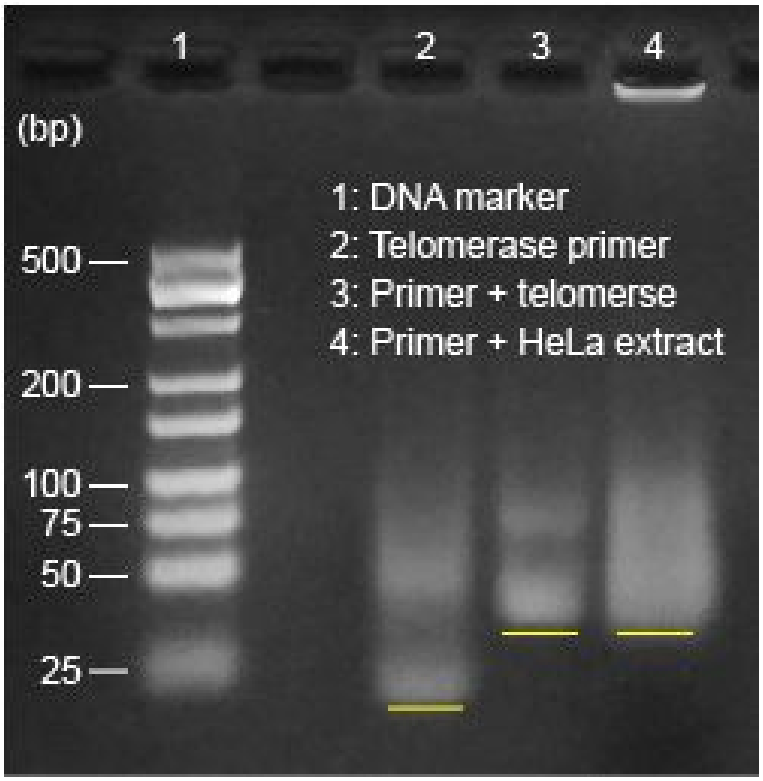


**Figure S5.** Verification of the telomerase activity. A 11 bp telomerase primer strand was reacted with pure telomerase, telomerase extraction from HeLa cells and incubated at 37 °C for 1 h. A distinct retarded band appeared in the case of pure telomerase and HeLa lysate in the 2% agarose gel electrophoresis compare to the telomerase primer strands alone, indicating that the telomerase was successfully extracted from HeLa cells. Lane 1–4: DNA marker, telomerase primer stand, telomerase primer incubated with telomerase, telomerase primer incubated with HeLa cells extract.

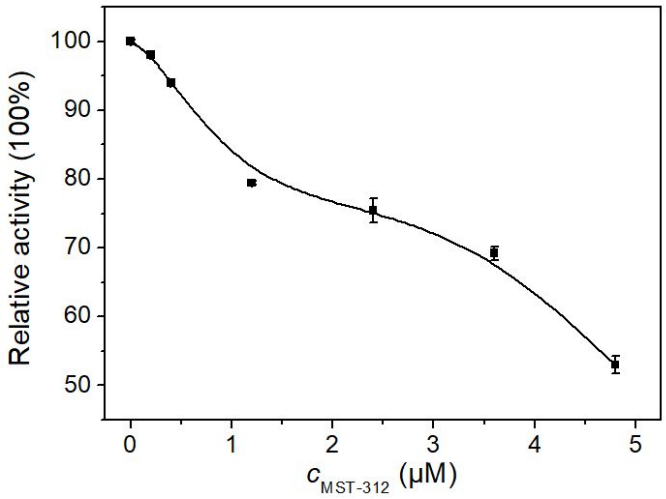


**Figure S6.** Dose-dependent inhibition of telomerase activity by telomerase inhibitor in HeLa cells. The telomerase inhibitor, N, N'-1, 3-phenylenebis-[2,3-dihydroxy-benzamide] (MST-312) was used to explore the inhibition effect upon telomerase activity. After incubation with 0 – 4.8 μM MST-312 for 62 h, the HeLa cells were collected and telomerase was extracted to measure its activity. The relative activity of telomerase decreases with the increase of MST-312 concentration, indicating that the DNA nanotube could applied for the recognition and response of telomerase.

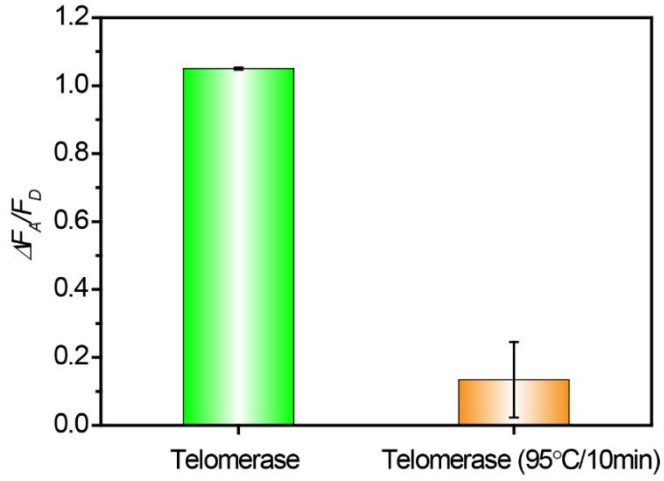


**Figure S7.** Verification of telomerase extracts response to DNA nanotube. The DNA nanotube was treated with HeLa cells lysate or heat-inactive Hela lysate. Experimental Conditions: DNA nanotube, 50 nM; dNTP, 200 μM; HeLa cells, 2400

cells; incubation at 37 °C for 1h.

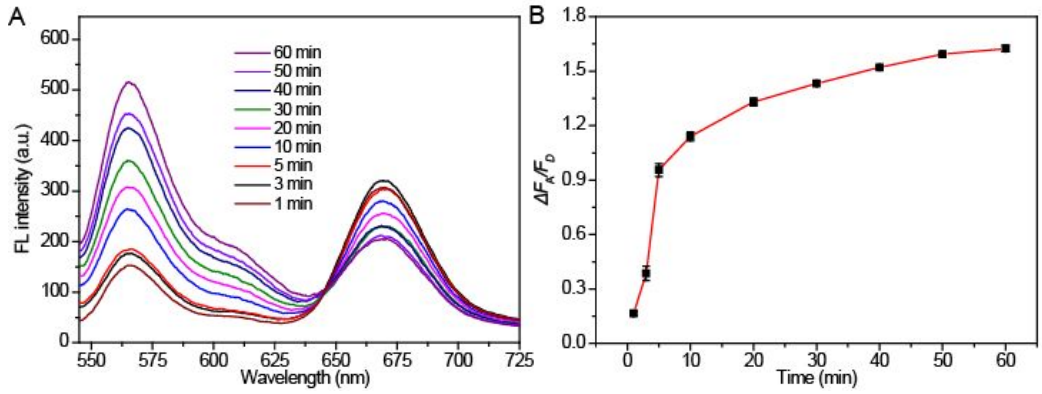


**Figure S8.** Time optimization of DNA nanotube in response of telomerase. (A) Fluorescence spectra of 50 nM DNA nanotube after incubating with telomerase from HeLa cells at different time ($\lambda_{ex}$ = 525 nm, $\lambda_{em}$ = 570 nm and 670 nm). (B) Relationship between the relative acceptor/donor ratio ($\Delta F_A/F_D$) and different incubation time.

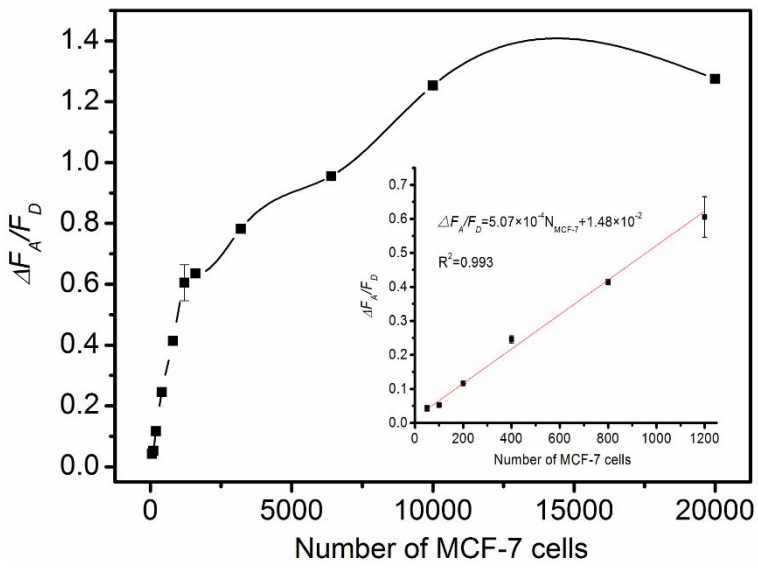


**Figure S9.** The relationship between $\Delta F_A/F_D$ and the number of MCF-7 cells. The results demonstrated the positive correlation between $\Delta F_A/F_D$ and the number of MCF-7 cells, the limit of detection (LOD, 3σ) was calculated to be 64 MCF-7 cells with the linear relationship ranges from 100-2400 cells.

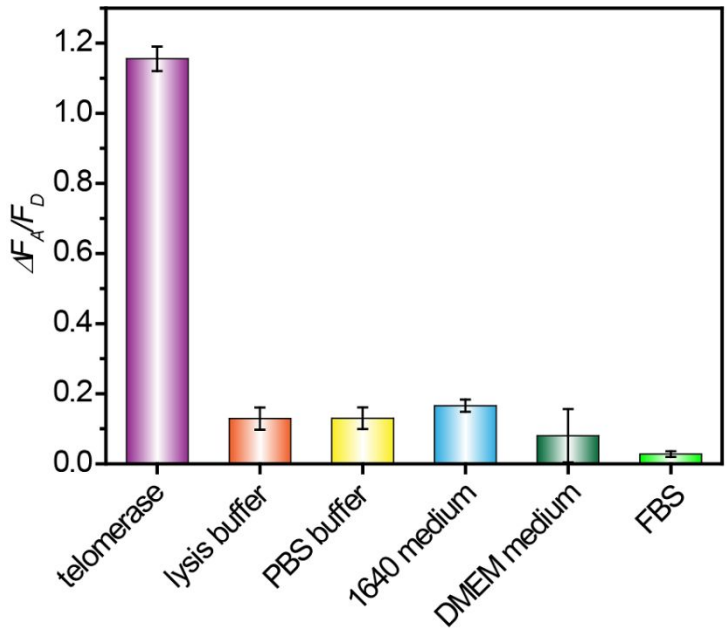


**Figure S10.** Selectivity of telomerase detection in the presence of various interfering substances. Equal amount of DNA nanotube was co-incubated with different media including lysis buffer, PBS buffer, DMEM medium, 1640 medium and FBS at 37 ºC for 1h.

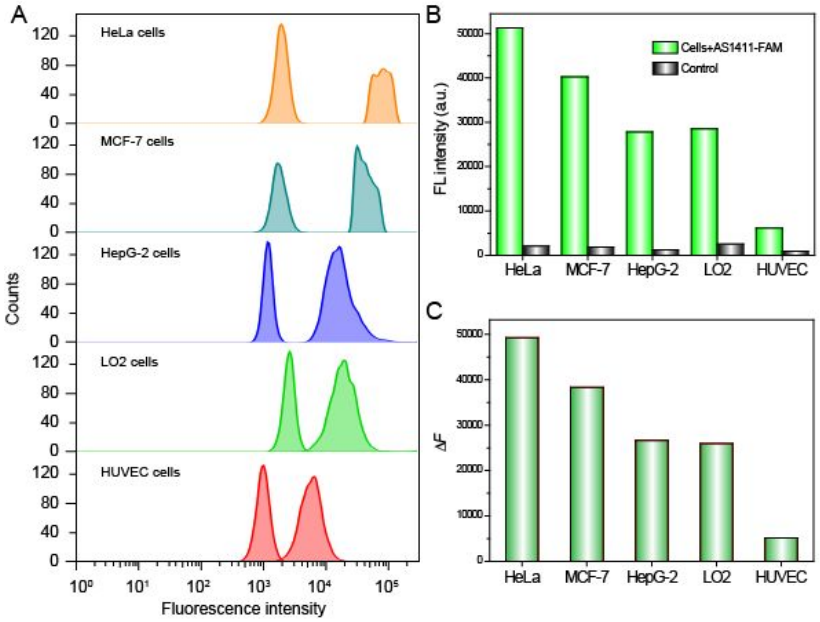


**Figure S11.** Verification of nucleolin expression level on various tumor cells and normal cells. (A) Flow cytometric histograms of various cell lines after incubated with or without FAM-labeled AS1411 (AS1411-FAM). The left line in each histogram correspond to the control groups of HeLa, MCF-7, HepG-2, LO2 and HUVEC cells, respectively. (B) The quantified profiles of mean fluorescence intensity in various cell lines treated with or without AS1411-FAM by flow cytometry. (C) The relative fluorescence intensity related to nucleolin expression in various cells.

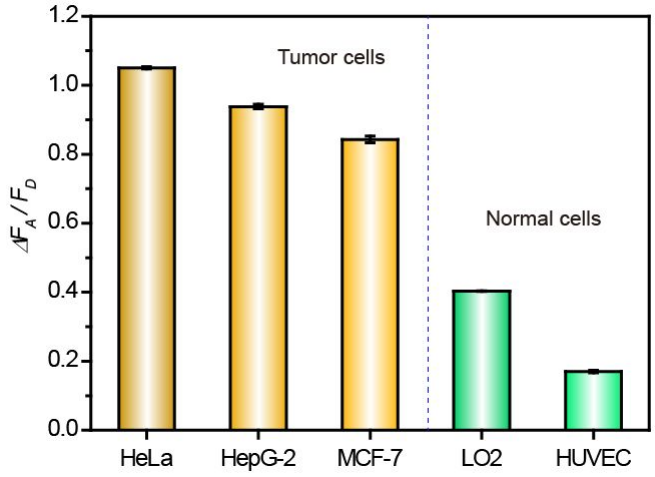


**Figure S12.** The specificity of telomerase response in different cells. Experimental conditions: DNA nanotube, 50 nM; dNTPs, 200 μM; HeLa, HepG-2, MCF-7, LO2, HUVEC cells, 2400 cells.

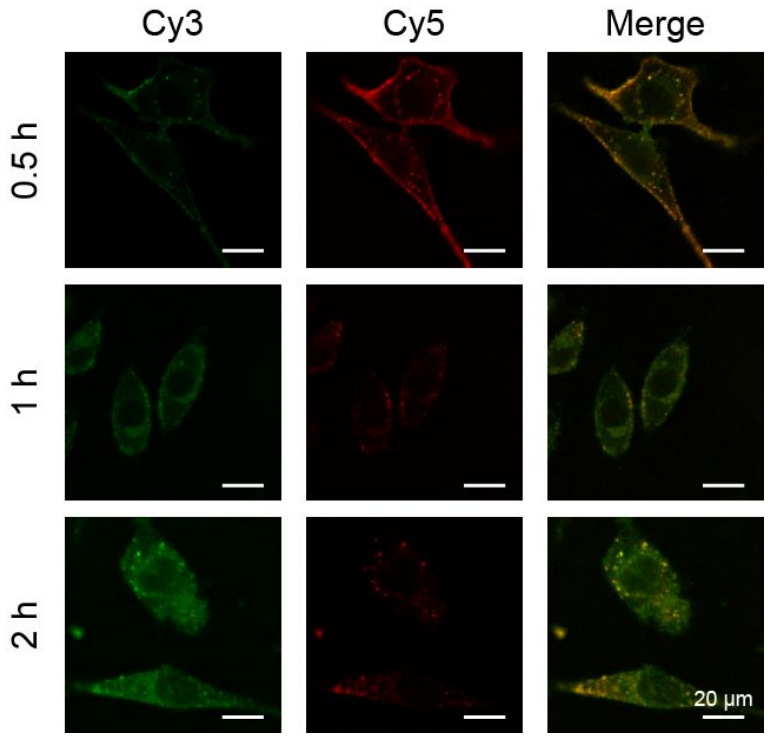


**Figure S13** Optimization of incubation time of DNA nanotube in cells. Fluorescent confocal microscopy images of HeLa cells after incubation with AS1411/nanotube (20 nM) for different time (0.5, 1, 2 h).

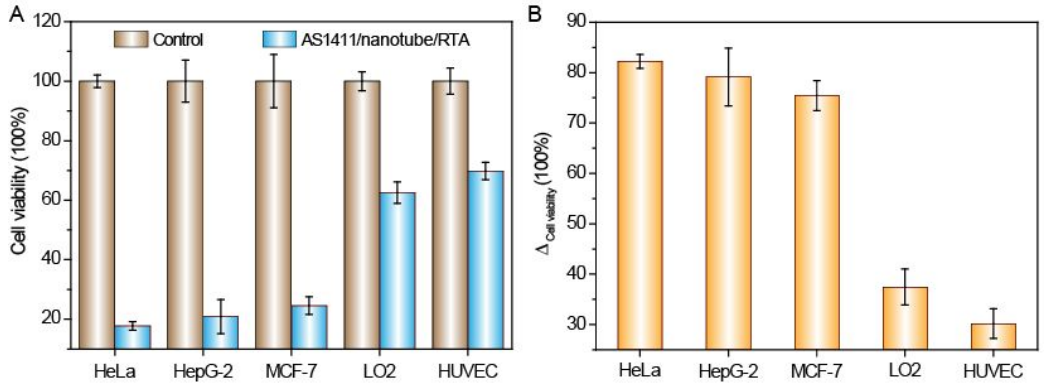


**Figure S14.** Cytotoxicity comparison of AS1411/nanotube/RTA in various cell lines. (A-B) The absolute and relative cytotoxicity of AS1411/nanotube/RTA on HepG-2, MCF-7, HeLa, LO2, and HUVEC cells.