Supporting Information

An RNA/DNA hybrid origami-based nanoplatform for efficient gene therapy†

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Materials and methods

Materials and agents

All chemicals and solvents used were in analytic level. DNase-RNase-Free (DEPC) water purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China) was used to prepare all aqueous solutions. All oligonucleotides were purchased from Sangon Biotech (China). The unmodified DNA strands were purified by polyacrylamide gel electrophoresis. The modified DNA strands were purified by high-performance liquid chromatography. The concentrations of all the strands were normalized to 100 μM. Mini plasmid purification kit was purchased from Tiangen (China). The La-Taq DNA polymerase and gel extraction kit were purchased from TaKaRa (China). HiScribe T7 Quick High Yield RNA Synthesis Kit was purchased from New England Biolabs (USA). RNase H was provided by Thermo (USA). The Amicon Ultra Centrifugal Filters were purchased from Merck Millipore (Germany).

Construction of dsDNA template

50.0 ng plasmid (pEGFP-N1 for EGFP, PLK1-pcDNA3.1 for PLK1), 0.5 μM primer pair (listed in Table S1), 0.2 mM each dNTP, 1 U La-Taq DNA polymerase, 2.5 μl 10×PCR reaction buffer and DEPC H_2O were added into 25 μl PCR system. The PCR product was purified by gel extraction kit and directly employed for the following transcription process.

Preparation of RNA scaffold

HiScribe T7 Quick High Yield RNA Synthesis Kit was used to transcribe single-stranded RNA from dsDNA template, following the protocol provided with the kit. Briefly, 500 ng DNA template, 20 μl each rNTP, 2 μl T7 RNA Polymerase Mix and DEPC H_2O were added into 50 μl transcription system and incubated at 37 ℃ overnight. The transcription product was then treated by 2 μl DNase I at 37 ℃ for 30 min to digest DNA template. Following the incubation with 25 μl LiCl solution at -20 ℃ for 30 min, RNA was precipitated by centrifuging at 13000 rpm for 20 min at 4 ℃ to remove excess rNTPs. Finally, RNA solution was obtained by dissolving the precipitate with 100 μl DEPC H_2O.

Assembly of RNA/DNA origami[1]

The RNA/DNA hybrid origami was designed by caDNAno software. The sequences of the DNA staple strands are shown in Tables S2-S4. 10 nM RNA scaffold and 100 nM staple strands (10 eq) were mixed in 1×TAE/Mg^{2+} buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA and 12.5 mM magnesium acetate, pH=8.3) and annealed under the following cooling protocol: 10 mins each at 65, 50, 37 and 25 ℃. The additional staple strands were removed by centrifugation with 50 kDa MWCO filters for three times. The assembled RNA/DNA origami was characterized by 2.5% agarose gel at 4 ℃.

TEM characterization

10 μl sample was deposited onto a negative glow discharged carbon-coated grid for 10 min at room temperature. After removed the sample, 7 μl uranyl acetate solution (1%) was dropped onto the grid and cultured for 1 min. TEM imaging was performed by a HT7700 (Hitachi Limited), operated at 80 kV in the dark-field mode.

AFM characterization

25 μl sample was deposited onto freshly cleaved mica. After adsorbing for 1 min, the sample was subsequently washed with 1 ml DEPC H_2O and then dried at room temperature before imaging with a MultiMode 8 AFM (Bruker) in ScanAsyst mode in air at room temperature.

DLS and zeta potential characterization

Hydrodynamic diameter and zeta potential of the RNA/DNA origami were detected on Malvern Zetasizer Nano (U.K.) at 25 ℃.

RNase H cleavage[2]

RNA/DNA origami sample was incubated with 50 U/mL RNase H in the provided RNase H buffer at 37 ℃ for 30 min.

Serum stability

After the treatment with 10% fetal bovine serum (Hyclone, Thermo Scientific) at 37 ℃ for 0, 6, 12, and 24 h, respectively, the mRNA, staples, or RNA/DNA origami was characterized by 1% agarose gel at 4 ℃. Image J analysis was performed to quantify the nucleic acid residual.

Cell culture

MCF-7 cells were cultured in DMEM complete medium (Hyclone, Thermo Scientific) supplemented with 10% fetal bovine serum (Hyclone, Thermo Scientific), 1% penicillin and streptomycin (GIBICO, Invitrogen) in an atmosphere of 5% CO_2 at 37 ℃.

Confocal imaging analysis

After seeding in 35 mm confocal dishes and culturing overnight, the MCF-7 cells were incubated with PBS, Cy5-labeled RDO with or without anti-MUC1 aptamer (the final concentration of origami is 1.2 nM) in 1 mL Extreme-mem medium for 6 h. After drug incubation, the cells were treated with Hoechst for 5 min to label nucleus and then washed with 1×PBS buffer for three times. The fluorescence signal of Cy5 was detected by confocal laser scanning microscope (Zeiss) with 633 nm excitation.

Flow cytometry analysis

After seeding in 6-well plates and culturing overnight, the MCF-7 cells were incubated with PBS, Cy5-labeled RDO with or without anti-MUC1 aptamer (the final concentration of origami is 1.2 nM) in 1 ml Extreme-mem medium for 6 h. After

incubation, the cells were trypsinized for 3 min to obtain the cell suspension and washed with 1×PBS buffer for three times. The fluorescence signal of Cy5 was detected by flow cytometry (ACEA Biosciences) with 633 nm excitation.

EGFP gene silencing

After seeding in 48-well plates and culturing overnight, the MCF-7 cells were incubated with PBS, staple strands (AS_E) transfected by lipofectamine 2000, or R_EDO (targeting EGFP) with aptamer (the final concentrations of staple strands and R_EDO are 600 nM) in 250 μL Extreme-mem medium for 12 h. Then, 250 ng plasmid pEGFP-N1 was transfected into cells by lipofectamine 2000. The medium was then replaced by fresh DMEM complete medium after 4 h. Following incubation for another 20 h, the cells were imaged by fluorescence microscope (Leica) with 488 nm excitation for EGFP. After imaging, the cells were trypsinized and analyzed by flow cytometry (ACEA Biosciences) with 488 nm excitation for measurement of the fluorescence intensities of EGFP.

Live/Dead staining assay

After seeding in 48-well plates and culturing overnight, MCF-7 cells were incubated with PBS or R_PDO (targeting PLK1) with aptamer (the final concentration of R_PDO is 600 nM) in 250 μL Extreme-mem medium for 30 min and then 28 μL fetal bovine serum was added followed by further incubation for 72 h. 600 nM staple strands (AS_p) were transfected into MCF-7 cells by lipofectamine 2000 for 4 h and then replaced with fresh complete medium for 68 h. The cells were stained by Calcein-AM/PI solution (Calcein-AM/PI Double Stain Kit, Yeasen, China) for 15 min at 37 ℃ before imaging with fluorescence microscope (Leica) with 488 nm excitation for Calcein and 545 nm excitation for PI-DNA.

Cell viability assay

After seeding in 96-well plates and culturing overnight, MCF-7 cells were incubated with PBS or R_PDO with aptamer (the final concentration of R_PDO is 600 nM) in 100 μL Extreme-mem medium for 30 min and then 12 μL fetal bovine serum was added followed by further incubation for 72 h. 600 nM staple strands (AS_p) were transfected into MCF-7 cells by lipofectamine 2000 for 4 h and then replaced with fresh complete medium for 68 h. The cells were cultured in fresh DMEM medium containing 10% cell counting kit-8 solution for 1 h before measuring the absorbance at 540 nm on a microplate reader (Multiskan FC, Thermo).

Cell apoptosis assay

After seeding in 48-well plates and culturing overnight, MCF-7 cells were incubated with PBS or R_PDO with aptamer (the final concentration of R_PDO is 600 nM) in 250 μL Extreme-mem medium for 30 min and then 28 μL fetal bovine serum was added followed by further incubation for 48 h. 600 nM staple strands (AS_p) were transfected into MCF-7 cells by lipofectamine 2000 for 4 h and then replaced with fresh complete medium for 44 h. The cells were stained with Annexin V-FITC/PI solution (Annexin

V-FITC/PI apoptosis detection kit, BD Bioscience, USA) for 15 min at room temperature in darkness. Flow cytometry (ACEA Biosciences) was performed to detect cell apoptosis.

Western blot analysis

After seeding in 12-well plates and culturing overnight, the MCF-7 cells were incubated with PBS or R_PDO with aptamer (the final concentration of R_PDO is 600 nM) in 500 μL Extreme-mem medium for 30 min and then 60 μL fetal bovine serum was added followed by further incubation for 72 h. 600 nM staple strands (AS_p) were transfected into MCF-7 cells by lipofectamine 2000 for 4 h and then replaced with fresh complete medium for 68 h. The cells were washed with cold 1×PBS buffer for three times before extracting the total cell protein by RIPA lysis buffer (Solarbio, China). Proteins were separated by SDS-PAGE and then transferred to polyvinylidene fluoride (PVDF) membrane using a wet transfer cell (Bio-rad). Blocked by 1% BSA (dissolved in 1×TBST buffer: 1.5 M NaCl, 20 mM Tris-HCl, 0.05% Tween-20) for 3 h and washed with 1×TBST buffer, the PVDF membrane was incubated with Anti-PLK1 mouse monoclonal antibody (Sangon, China) overnight at 4 ℃. Washed three times with 1×TBST buffer, the membrane was incubated with the HRP-labeled secondary antibody (Santa Cruz Biotechnology, USA) for 1 h at room temperature. Finally, the PLK1 protein was detected with a SuperSignal West Pico Trial Kit (Thermo Scientific, USA).

qRT-PCR analysis

MCF-7 cells were treated as same as the western blot assay for 72 h. The total RNA was obtained using Trizol reagent (Solarbio, China). The cDNA was synthesized from each sample by a Hifair III 1st Strand cDNA Synthesis kit with gDNA digester (Yeasen, China) and employed as a template for qPCR amplification following the protocol of NovoStart®SYBR qPCR SuperMix plus (Novoprotein, China). The transcription of PLK1 gene was normalized to the β-actin.

Statistical analysis

One-way ANOVA with the Tukey's multiple comparisons was conducted to determine the levels of data differences between the groups. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ were considered statistically significant. GraphPad Prism software (version 8.0.2) was used for statistical analysis.

Additional figures

Fig. S1 Illustration of structure and sequence of R_EDO (targeting EGFP).

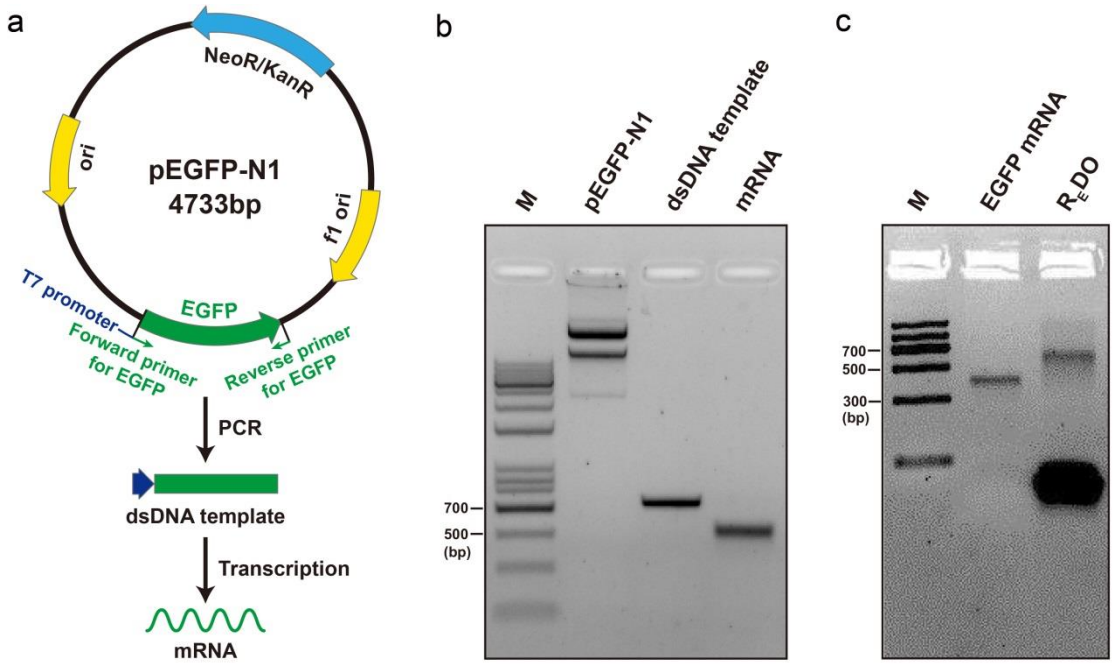

Fig. S2 (a) Preparation method of EGFP mRNA (scaffold of R_EDO). (b) 1% agarose gel electrophoresis analysis of plasmid, dsDNA template and mRNA for EGFP. (c) 2.5% agarose gel electrophoresis analysis of R_EDO.

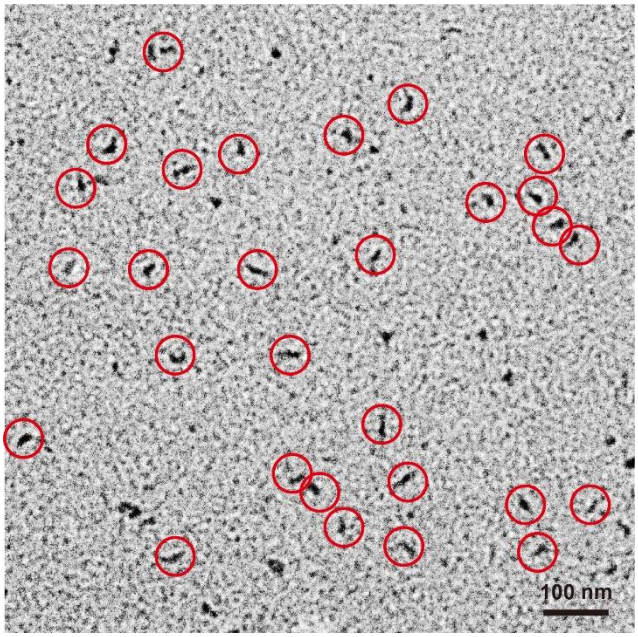

Fig. S3 A representative large-scale TEM image of R_EDO, scale bar: 100 nm.

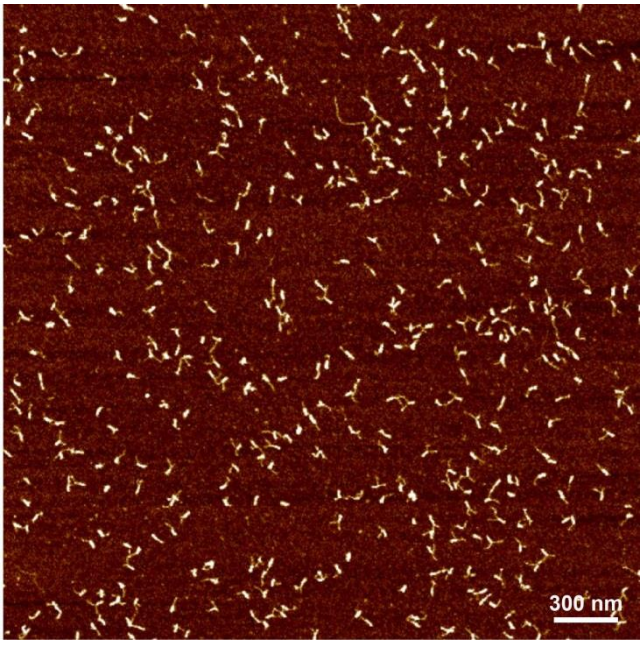

Fig. S4 A representative large-scale AFM image of R_EDO, scale bar: 300 nm.

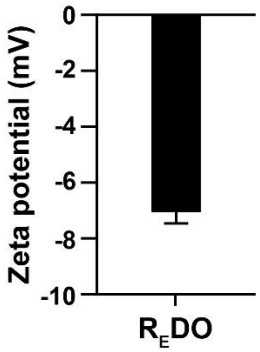

Fig. S5 Zeta potential of R_EDO, -7.14±0.40 mV.

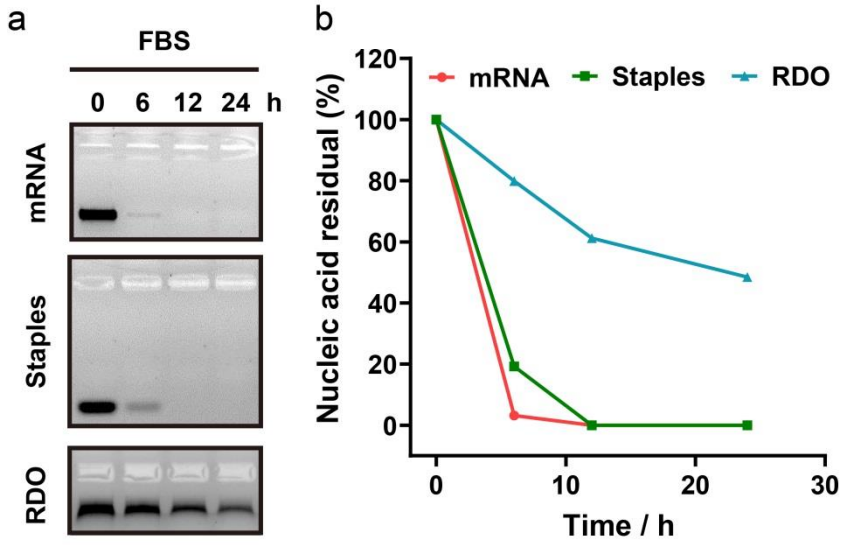

Fig. S6 (a) 1% agarose gel electrophoresis analysis of serum stability of mRNA, staples and RDO with the incubation of 10% FBS at 37 ℃. (b) Quantitative results of serum stability of mRNA, staples and RDO by Image J analysis.

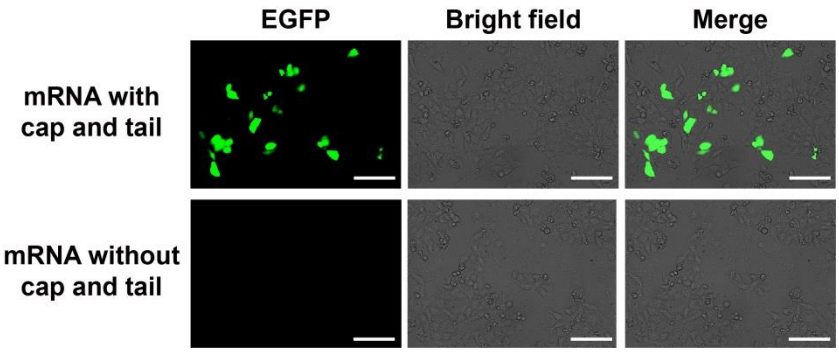

Fig. S7 Fluorescence microscope images of MCF-7 cells after incubation with EGFP mRNA with or without cap and tail (transfected by lipofectamine 2000) for 24h, (488 nm for EGFP, pseudocolor green; scale bars: 100 μm). The mRNA scaffold of RNA/DNA origami was mRNA without cap and tail.

Fig. S8 Illustration of structure and sequence of R_pDO (targeting PLK1).

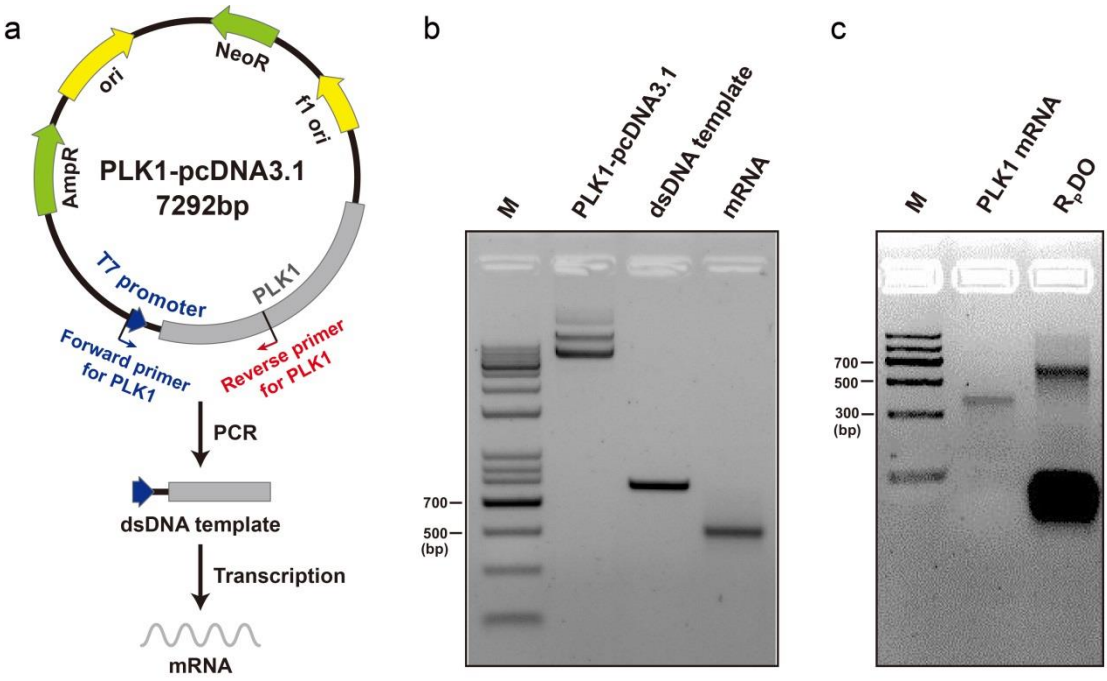

Fig. S9 (a) Preparation method of PLK1 mRNA (scaffold of R_PDO). (b) 1% agarose gel electrophoresis analysis of plasmid, dsDNA template and mRNA for PLK1. (c) 2.5% agarose gel electrophoresis analysis of R_PDO.

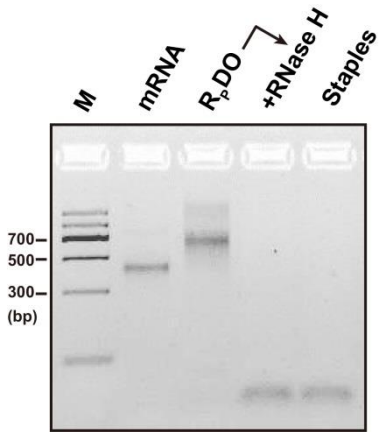

Fig. S10 2.5% agarose gel electrophoresis analysis of R_PDO treated with RNase H.

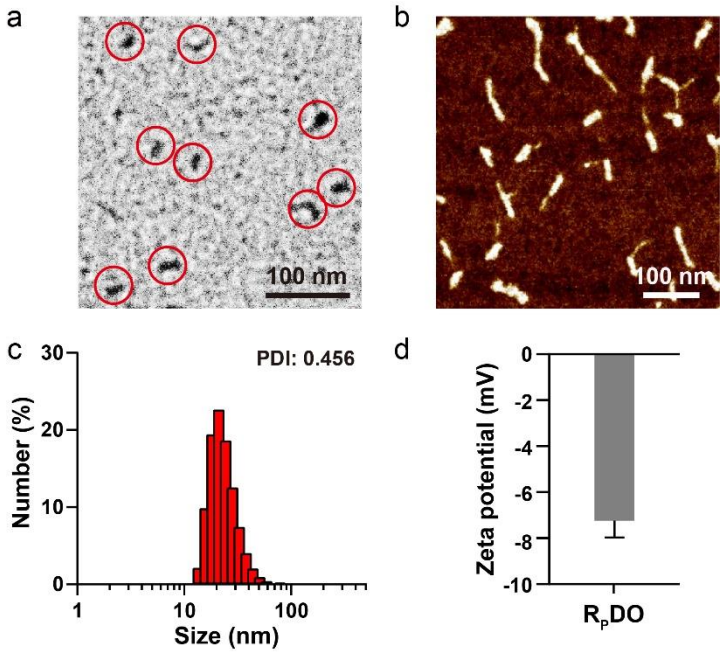

Fig. S11 Characterization of R_PDO. (a) TEM image, scale bar: 100 nm. (b) AFM image, scale bar: 100 nm. (c) DLS characterization, PDI: 0.456. (d) Zeta potential, -7.24±0.74 mV.

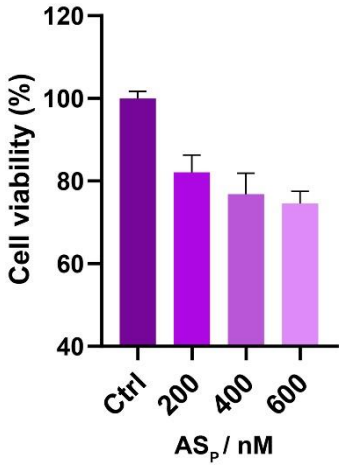

Fig. S12 Cell viability of MCF-7 cells after incubation with different concentrations (200, 400, or 600 nM) of AS_P transfected by lipofectamine 2000 for 72 h.

Table S1 DNA sequences of PCR primers

Name	Sequence (5’-3’)
Forward primer for EGFP	TAATACGACTCACTATAGGGGCCACCATGGTGAGCAAGGGCGAGGA
Reverse primer for EGFP	TTTTTTTTTACTTGTACAGCTCGTCCA
Forward primer for PLK1	TAATACGACTCACTATAGGG
Reverse primer for PLK1	AGGTCTCTTTTAGGCAAGAA

Table S2 DNA sequences of the staple strands for R_EDO (targeting EGFP)

Anti-MUC1 aptamer: <u>GCAGTTGATCCTTTGGATACCCTGG</u>

Name	Sequence (5'-3')
E-1	GACGTAGCCTTCGGGTCGGCCGTTGTGG
E-2	GACCAGGATGGGCACCACCCCGTGATCCCGGCGGCCCTCGAT
E-3	GGCAGCAGCACGGGTCCTTGAAGTCGATCGCCCTCAGATGAA
E-4	GAACTTCACCTCGGGCCGTCCGCCGTCGCCGATGGGGGTGTT
E-5	GAAGAAGATGGTGCGTGGCTGCTGCACGCTGCCGTGGTC
E-6	TTGTAGTTGTACTCCGTCCTTTCACGAGGGTGGGCCAGGGCA
E-7	CTTGTAGTTGCCGTCAGCTTGCTGCTGGTAGTGGTCGGCGAG
E-8	TTGCTCAGGGCGGACTGGGTGGGCATCGCCCTCGCGTGGTGC
E-9	ATGATATAGACGTTGCTCCTGGCACGCCGTAGGTCAGGGTGG
E-10	TGCCCCAGGATGTTCGCGGGTCGGGCAGCTTGCCGCCTCG
E-apt-1	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT ACAGCTCCTCGCCCTTGCTCACC
E-apt-2	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT TTGTACAGCTCGTCCATGCCGAGA
E-apt-3	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT CCGTTTACGTCGCCGTCCAGCTC
E-apt-4	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT ACGAACTCCAGCAGGACCATG
E-apt-5	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT CCGGACACGCTGAACTTGTGG
E-apt-6	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT TGATCGCGCTTCTCGTTGGGGTCT

Table S3 DNA sequences of the fluorescence capture strands for imaging

Cy5-labeled strand: TAAACTCTTTGCGCAC-Cy5

Fluorescence capture strand: GTGCGCAAAGAGTTTA for Cy5

Name	Sequence (5'-3')
cap-1	GTGCGCAAAGAGTTTA TTTTT TGCTTCATGTGGTCGGGGTA
cap-2	GTGCGCAAAGAGTTTA TTTTT ATGGCGGACTTGAAGAAG
cap-3	GTGCGCAAAGAGTTTA TTTTT ATGCCGTTCTTCTGCTT
cap-4	GTGCGCAAAGAGTTTA TTTTT CGGATCTTGAAGTTCAC

Table S4 DNA sequences of the staple strands for R_PDO (targeting PLK1)

Anti-MUC1 aptamer: <u>GCAGTTGATCCTTTGGATACCCTGG</u>

Name	Sequence (5'-3')
P-1	CTCCCTCTGGTGCGCCGTAGGAATAACT
P-2	CACAGGGTCTTCTTCCTCTCCGATCTCTTTCGCCGTAGCGCC
P-3	GGATATTTCCATGGAGGCTCAGCCCAGCTTGAGGTGTGC
P-4	GTGGGCGAGGCTGCCCTCTTGATCTTCATTCAGGAAAAGGTT
P-5	GTTGCCAGTCCAAACGGCGGCAGAGCTCAAAAGCCGCCGGCT
P-6	TTTCCCAGGGTCGGCCGGTGCTCCACCTCGAAACTCTCGATG
P-7	TAGTATCGGGCCTCACATCTTCCGAGATCTCGAAGCACTTGG
P-8	GTCAGGGCTTTCCTGGTGAATCAAAGCCGCCCTTGCCCAAAA
P-9	GTGGAATCCTACGAAGACCTCATCCCCTATTTTCACCTCCAG
P-10	TGCAGCTCCAGGAGCGTGCTGAGCGGCCCCGCACAGTGGA
P-apt-1	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT CCAGCTTCCCTGCAGTCACTGCA
P-apt-2	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT ATCTTGCCCGCGAACACCTC
P-apt-3	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT TTGAGCAGCAGAGACTTA
P-apt-4	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT CAGCCAAGCACAATTTG
P-apt-5	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT CGGTTTCGGTGCAGGTA
P-apt-6	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT ATGATACACCCAATGGACCACACA
P-apt-7	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT GCTGCAACTCCGGGGACCCCGGC
P-apt-8	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT CCTTTCTTGCTCAGCACCTCG
P-apt-9	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT GCCGCCGCCGGAGCTCCGGGA
P-apt-10	<u>GCAGTTGATCCTTTGGATACCCTGG</u>TTTTT GGAGCTATGTAATTAGGAGTCCCA

Table S5 DNA sequences of primers for qRT-PCR

Name	Sequence (5’-3’)
PLK1-Forward	GGCAACCTTTTCCTGAATGA
PLK1-Reverse	AATGGACCACACATCCACCT
β-actin-Forward	TCTGGCACCACACCTTCTACAATG
β-actin-Reverse	GGATAGCACAGCCTGGATAGCAA

References

[1] P. Wang, S. H. Ko, C. Tian, C. Hao and C. Mao, *Chem. Commun.*, 2013, **49**, 5462.

[2] F. Ding, Q. Mou, Y. Ma, G. Pan, Y. Guo, G. Tong, C. H. J. Choi, X. Zhu and C. Zhang, *Angew. Chem. Int. Ed.*, 2018, **57**, 3064.