Supporting Information for

A Cocktail Therapeutic Strategy Based on Clofarabine-Containing Aptamer-PROTAC for Enhanced Cancer Therapy

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Experimental section

General information and materials

Thin layer chromatography was performed on silica gel 60 F_{254} plates (250 μm) and column chromatography was conducted over silica gel (300-400 mesh). Visualization of the developed chromatogram was accomplished by a UV lamp. Unless noted otherwise, reagents and solvents were obtained from commercial suppliers and employed without further purification. Nuclear magnetic resonance (NMR) spectra were obtained by using a Bruker DRX-500 operated at 500/125/125 MHz for ^{1}H NMR ,^{13}C NMR and ^{31}P NMR respectively. High-resolution mass spectra (HRMS) were measured by using a mass spectrometer.

All HPLC-purified oligonucleotides used in this study were synthesized at Shanghai Sangon Biological Engineering Co., Ltd. (Shanghai, China). Streptavidin-2 biosensors were sourced from Fortebio (Menlo Park, CA, USA), while Cloud-clone Corp (Wuhan, China) provided recombinant Human Nucleolin (RPC242Hu01). Membrane/Cytosol/Nuclear Protein Extraction Kit (BB-3104) was sourced from Bestbio (Jiangsu,China). Shanghai Sangon Biological Engineering Co., Ltd. (Shanghai, China) supplied the high sensitive ECL luminescence reagent (C500044) and Cell Counting Kit-8 (CCK-8, E606335). We obtained anti-Nucleolin antibody (ab129200) and anti-Histone H3 antibody (ab32356) from Abcam (Shanghai, China), and β-Actin Rabbit mAb (8457S) from Cell Signaling Technology, Inc. (Shanghai,China). MLN4924 (S81085) was purchased from Shanghai yuanye Bio-Technology Co., Ltd (Shanghai, China), while pomalidomide-PEG4-azide (HY-141015) was sourced from MedChemExpress (Shanghai, China). To complete the experimental setup, we used Zeba™ Spin Desalting Columns (89892) from Thermo Fisher Scientific (USA). MG132(T2154) and Chloroquine(T8689) was obtained from TargetMOL (USA).Cyto Tracered fluorescent probe was purchased from Yeasen Biological Technology (Shanghai, China), while the Cell Cycle Detection Kit (KGA512) and FITC Annexin V Apoptosis Detection Kit (KG556547) were obtained

from KeyGEN BioTECH (Jiangsu, China).

Synthesis of 5'-O-DMT-N^6-(N,N-dimethylformamide)-2-Chloro-2'-Fluoro-2'-deoxyadenosine-3'-CE phosphoramidite, (CDDP). The synthesis route of CDDP is shown in Scheme S1. Clo-DMT was first prepared as follows: Clo (303 mg, 1 mmol) and DMT-Cl (372 mg, 1.1 mmol) were dissolved in anhydrous pyridine (10 mL) and stirred at room temperature for 5 h. The mixture was then concentrated under vacuum. The residue was washed by ethyl acetate (EtOAc, 3×10 mL) and saturated $NaHCO_3$ aqueous solution (5 mL) in a 25 mL separating funnel. The aqueous layer was extracted with EtOAc (20 mL) twice and the combined organic solvents were concentrated by a rotavap. The resulting solid was purified by crystallization from hexane-EtOAc (1:1) to give a light brown solid (484 mg, 80%). Then, Clo-DMT (303 mg, 0.5 mmol) was suspended in dry N, N-dimethylformamide (DMF). Then, DMF-DMA (112 μL, 0.8 mmol) was added and the mixture was stirred at 60°C for 2 h. Then, the reaction mixture was diluted with EtOAc (3×10 mL) and was washed with saturated $NaHCO_3$ and DI water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated by a rotary evaporator. The residue was purified by silica-gel column chromatography (dichloromethane/acetone=20:1) as eluent to obtain Clo-DMT-DMF (297 mg, 90%). Finally, Clo-DMT-DMF (198 mg, 0.3 mmol), 5-(Ethylthio)-1*H*-tetrazole (57 mg, 0.45 mmol), and 2-cyanoethyl-*N,N,N′,N′*-tetraisopropyl-phosphoramidite (136 mg, 0.45 mmol) were dissolved in anhydrous acetonitrile and reacted under argon atmosphere at room temperature for 2 h. It was further purified by silica gel flash column chromatography using methanol/ethyl acetate to obtain CDDP (168 mg, 65%).

Synthesis and mass characterization of ApTCs-3X. The HPLC-purified DBCO-AS1411-3X was synthesized at Shanghai Sangon Biological Engineering Co., Ltd. (Shanghai, China) by using the purified CDDP as one of the essential precursors. After confirming the purity and molecular weight using HPLC and mass spectroscopy, we conjugated it with pom-PEG4-azide to obtain ApTCs-3X through the copper-free click chemistry. Specifically, 140 μL of DBCO-AS1411-3X (100 μM) and 20 μL of POMA-PEG4-azide (10 mM) were mixed with 40 μL of a 5× click buffer, and then

reacted at 37°C for 24 hours. After that, we purified the product by performing desalting with a Zeba™ 7K MWCO spin column (Thermo Fisher) to remove any unreacted POMA-PEG4-azide. The final concentration of the purified ApTCs-3X was detected using a Nano6Drop One instrument (Thermo Fisher), and diluted to 100 μM for further use using nuclease-free water. The purity and molecular weight of DBCO-AS1411-3X and ApTCs-3X were verified using HPLC and MALDI-TOF mass spectrometry with an ABI-MDS SCIEX 4800 Plus MALDI TOF/TOF™ spectrometer."

Stability assay of ApTCs-3X in serum. To carry out the experiment, we incubated a mixture of 2 μL fresh fetal bovine serum, 2 μL ApTCs-3X (10 μM), and 16 μL enzyme-free water for pre-determined time points (0, 2, 4, 8, 12, and 24 h) at 37 °C. We subsequently analyzed the resulting mixture using native polyacrylamide gel electrophoresis (nPAGE) at 80 V and 0.5X TBE buffer for 80 min, with visualization and quantification of the bands completed using Image J. For comparison purposes, we also subjected ApTCs to the same protocol under identical experimental conditions.

The binding affinity of ApTCs-3X was tested using biolayer interferometry (BLI) technology. The experiments were conducted on an Octet K2 instrument (ForteBio) that was equipped with a high-precision streptavidin biosensor. All reactions were performed at 35 °C in PBS buffer (pH 7.0, 0.1 M) containing 0.02% Tween 20 (PBST). Firstly, Biotin-NCL was prepared using the EZ-Link Sulfo-NHS-LC-LC-Biotin kit according to the manufacturer's protocol (#21338, ThermoFisher Scientific). Briefly, 200 μL of 10 mg/mL NCL solution (pH 7.0, 0.1 M PBS buffer) was mixed with 30 μL of 10 mM freshly prepared biotin reagent (in ultrapure water), and then incubated at room temperature for 30 min. To remove un-conjugated biotin reagent, the solution of Biotin-NCL was purified by Amicon-3K for 8 times using PBS buffer. The concentration of purified Biotin-NCL was quantified using a NanoDrop Onec (Thermo Fisher), and the final stock solution of Biotin-NCL was 200 nM. Secondly, the typical BLI procedure include four steps: 1) the streptavidin sensors were washed in PBST buffer for 60 s to ensure a stable baseline; 2) the biotin-NCL (200 μL, 200

nM) was loaded onto streptavidin biosensors for 150 s, followed by washing in PBST for 60 s; 3) the sensor was then incubated with 200 μL of ApTCs-3X (200 nM) for 270 s; 4) the sensor was further incubated with 200 μL of cereblon (CRBN, 200 nM) for 300 s, followed by washing in PBST for 60 s. To evaluate the specific binding between ApTCs-3X and CRBN/NCL, biotin-AS1411 and biotin-Random DNA sequence was immobilized on the streptavidin biosensor, where AS1411 and CRBN alone was used as positive controls. Then, different concentrations of either NCL or CRBN were tested by following the above procedure with slightly modification on the incubation time.

Time-dependent localization of ApTCs-3X in cells. AS1411-3X labeled with 5'-DBCO and FAM-labeled azide were subjected to in vitro click chemistry, and AS1411-3X-FAM was purified using a rotating desalting column (Zeba ™ 7K MWCO, Thermo Fisher). HeLa cells were seeded in a confocal culture dish and grown until reaching 50% confluency, after which they were incubated with AS1411-3X-FAM (200 nM) for up to 240 min. To label the cytoplasm and nuclei, cells were fixed with 4% paraformaldehyde for 20 min, followed by staining with lysosome red fluorescent probe (250 μL, 1×) and Hoechst (250 μL, 12.5 μg/mL). Each incubation step was followed by three washes with DPBS buffer. Imaging was performed using a Leica SP8 laser scanning confocal microscope (Leica, Germany) with excitation wavelengths of 405, 488, and 577 nm for Hoechst, FAM, and CytoTrace, respectively. Image J software was used to analyze each cell group and calculate Pearson's R value.

Stability assay of ApTCs-3X in Dnase II. To carry out the experiment, we incubated a mixture of 10 μL ApTCs-3X (500 nM) and Dnase II (final concentration: 2 U/mL). It is then treated using 6x DNA buffer at the following time points (0, 1, 2, 3, 4, 5, 6, 7 and 8 h) at 37°C. We subsequently analyzed the resulting mixture using native polyacrylamide gel electrophoresis (nPAGE) at 80 V and 0.5X TBE buffer for 80 min, with visualization and quantification of the bands completed using Image J.

ApTCs-3X degrades nucleolin in HeLa cells. The degradation of nucleolin in cell membrane, cytoplasm, and nucleus was measured by Western blotting after treatment with ApTCs-3X at different concentrations and time points. HeLa cells were seeded

in a 6-well plate and cultured until reaching 70% confluency. The cells were then treated with ApTCs-3X at various concentrations (diluted in 1000 μL DMEM medium) and cultured for 24 hours or collected at different time-points after treatment with ApTCs-3X (200 nM). Cell fractions were separated using the Bestbio BB-3104 Membrane/Cytosol/Nuclear Protein Extraction Kit, and protein concentration was determined and normalized using the Shanghai Sangon C503021 BCA Protein Quantitation Kit before performing Western blot analysis. β-actin antibody served as the control for the cell membrane and cytoplasm, while histone antibody was used as the control for the nucleus. Western blot images were obtained using the Tanon-4600 Chemiluminescent Imaging System (Tanon, China), and Image J was utilized for quantifying the Western blot bands.

The effect of ApTCs-3X treatment on nucleolin expression in HeLa cell membranes was analyzed by flow cytometry. HeLa cells were seeded in a 6-well plate and grown to 70% confluency before treatment with varying concentrations of ApTCs-3X (diluted in 1000 μL DMEM medium) for 24 hours, followed by washing with DPBS buffer. The cells were then digested using trypsin, incubated with 200 nM AS1411-FAM for 30 minutes, and washed with DPBS buffer prior to flow cytometric analysis. A CytoFlex flow cytometry system (Beckman Coulter Inc, USA) was used to analyze 5000 cells per sample, with FAM excitation at 488 nm and emission at 525 nm.

The ubiquitination pathway-mediated degradation of nucleolin induced by ApTCs-3X was confirmed using Western blot assay. HeLa cells were seeded in a 6-well plate and grown to 70% confluency, followed by washing with DPBS buffer. The cells were then treated with different concentrations of AS1411-3X (200 nM), ApTCs (200 nM), ApTCs-3X (200 nM), ApTCs-3X (200 nM) + MG132 (200 nM), and ApTCs-3X (200 nM) + Chloroquine (CQ) (50 μM) for 24 hours. Western blotting was employed to analyze the degradation of nucleolin in the cell membrane, cytoplasm, and nucleus. Cell fractions were separated using the Bestbio BB-3104 Membrane/Cytosol/Nuclear Protein Extraction Kit, quantifying protein concentration with the Shanghai Bioengineering Inc. C503021 BCA Protein Quantitation Kit. β-

actin antibody served as a control for the cell membrane and cytoplasm during Western blot analysis. Images were obtained using the Tanon-4600 Chemiluminescent Imaging System (Tanon, China), and quantification of the Western blot bands was achieved using ImageJ.

The degradation of nucleolin by ApTCs-3X via the ubiquitination pathway was demonstrated through co-localization analysis of HeLa cell membrane nucleolin after treatment with ApTCs-3X. HeLa cells were grown to 70% confluency in a 6-well plate and then treated with various compounds, including AS1411-3X (200 nM), ApTCs (200 nM), ApTCs-3X (200 nM), ApTCs-3X (200 nM) + MG132 (200 nM), and ApTCs-3X (200 nM) + Chloroquine (CQ) (50 μM). All compounds were diluted in 1000 μL DMEM medium and incubated with the cells for 24 hours. The cells were then washed with DPBS buffer and digested with 0.25% trypsin (1000 μL) at 37°C for 10 minutes before being collected. Next, the cells were incubated with 200 nM FAM-AS1411 in DPBS buffer for 30 minutes at 4 °C and stained with Hoechst for 15 minutes. After washing with DPBS buffer, the cells were resuspended in 500 μL DPBS buffer and imaged using the Leica SP8 confocal laser scanning microscope (Leica Microsystems, Germany). The excitation wavelengths for Hoechst and FAM were 405 nm and 488 nm, respectively. Similarly, the image acquisition method for the analysis of NCL expression in HeLa and HEK-293 cells were the same as described above except cells were not treated with any PROTAC compound.

The degradation of nucleolin by ApTCs-3X via the ubiquitination pathway was demonstrated through Flow cytometry analysis of HeLa cell membrane nucleolin after treatment with ApTCs-3X. HeLa cells were cultured in a 6-well plate until they reached 70% confluency, and then exposed to various compounds, including AS1411-3X (200 nM), ApTCs (200 nM), ApTCs-3X (200 nM), ApTCs-3X (200 nM) + MG132(200 nM), and ApTCs-3X (200 nM) + Chloroquine (CQ) (50 μM) . All compounds were diluted in 1000 μL DMEM medium and treated for 24 h. After washing with DPBS buffer, the cells were digested with 0.25% trypsin (1000 μL) at 37°C for 10 min and then collected again. The cells were incubated with 200 nM FAM-AS1411 in DPBS buffer at 4°C for 30 min. The analysis was performed using

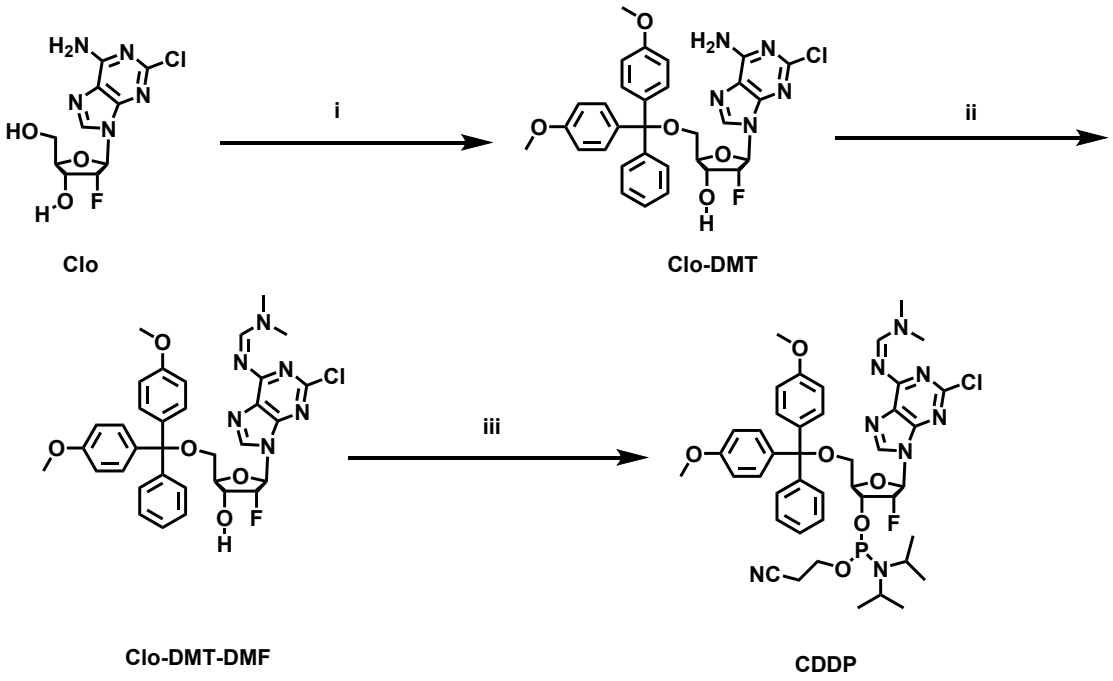
flow cytometry (Beckman Coulter Inc, USA) with 5000 cells per sample. Excitation wavelength for FAM was set at 488 nm and emission wavelength at 525 nm. Similarly, the flow cytometric analysis of the NCL expression in HeLa and HEK-293 cells were the same as described above except cells were not treated with any PROTAC compound.

The evaluation of cell viability was performed by CCK-8 assay under various treatment conditions. HeLa and HEK-293 Cells were seeded at a density of 10,000 cells per well in a 96-well plate and allowed to grow until they reached 70% confluency. After washing with DPBS buffer, the cells were incubated with various treatment conditions and diluted in 200 μL DMEM medium for 24 h. At the end of the predetermined incubation period, 100 μLCCK-8 reagent (final concentration: 5%) was added to each well and allowed to incubate at 37°C with 5% CO_2 for 1 h. The absorbance was measured at a wavelength of 450 nm using a microplate reader (Bio-Tek Instrument, Winooski, VT, USA).

Cell viability was evaluated using Annexin V-FITC/PI apoptosis detection assay under various treatment conditions. The Annexin V-FITC/PI Apoptosis Detection Kit I (KGA105) was employed as per the manufacturer's instructions to assess the apoptosis of membrane-bound protein V. HeLa cells were seeded in a 6-well plate and cultured until they reached 70% confluency. Afterwards, the cells were treated with different compounds, including AS1411 (200 nM), 3X (200 nM), AS1411-3X (200 nM), ApTCs (200 nM), ApTCs-3X (200 nM), ApTCs-3X (200 nM) + MG132 (200 nM), and ApTCs-3X (200 nM) + Chloroquine (CQ) (50 μM) , all of which were diluted in 1000 μL DMEM medium. After 24 hours of exposure, the cells were washed with DPBS buffer, digested with trypsin, washed again with DPBS, and resuspended in 1× binding buffer. Subsequently, the suspended cells were stained with propidium iodide (PI) and Annexin V conjugated with FITC, followed by flow cytometry analysis to determine the apoptotic cell count. Cells displaying positive fluorescence intensity signals for FITC and PI were considered as apoptotic cells.

Cell cycle was evaluated via flow cytometry analysis under various treatment conditions. For the cell cycle arrest experiment, the Cell Cycle Detection Kit

KGA511 was utilized in accordance with the manufacturer's protocol. HeLa cells were cultured in a 6-well plate until they reached 70% confluency. Subsequently, the cells were treated with different compounds, including ApTCs (200 nM), ApTCs-3X (200 nM), AS1411-3X (200 nM), all diluted in 1000 μL DMEM medium. After 24 hours of exposure, the cells were washed thrice with DPBS buffer, digested with trypsin, and washed again thrice with DPBS buffer. The fixed cells were suspended in cold 70% ethanol at 4°C for 4 hours, washed thrice with DPBS buffer to eliminate residual ethanol, and then resuspended in PBS supplemented with 1% Triton X-100, 5 mg mL−1 propidium iodide (PI), and 1 mg/mL RNAse. The samples were stained at 37°C for 30 minutes, and then analyzed using flow cytometry to determine the distribution of cells across the G0/G1, S, and G2/M phases. FlowJo software was employed for data analysis.



Scheme S1. Synthetic strategy of CDDP (i. anhydrous pyridine, DMT-Cl, rt, 5 h; ii. DMF, DMF-DMA, 60 ℃, 2 h; iii. anhydrous acetonitrile, 5-(Ethylthio)-1H-tetrazole/ 2-cyanoethyl-N, N, N′, N′-tetraisopropyl-phosphoramidite, rt, 2 h.)

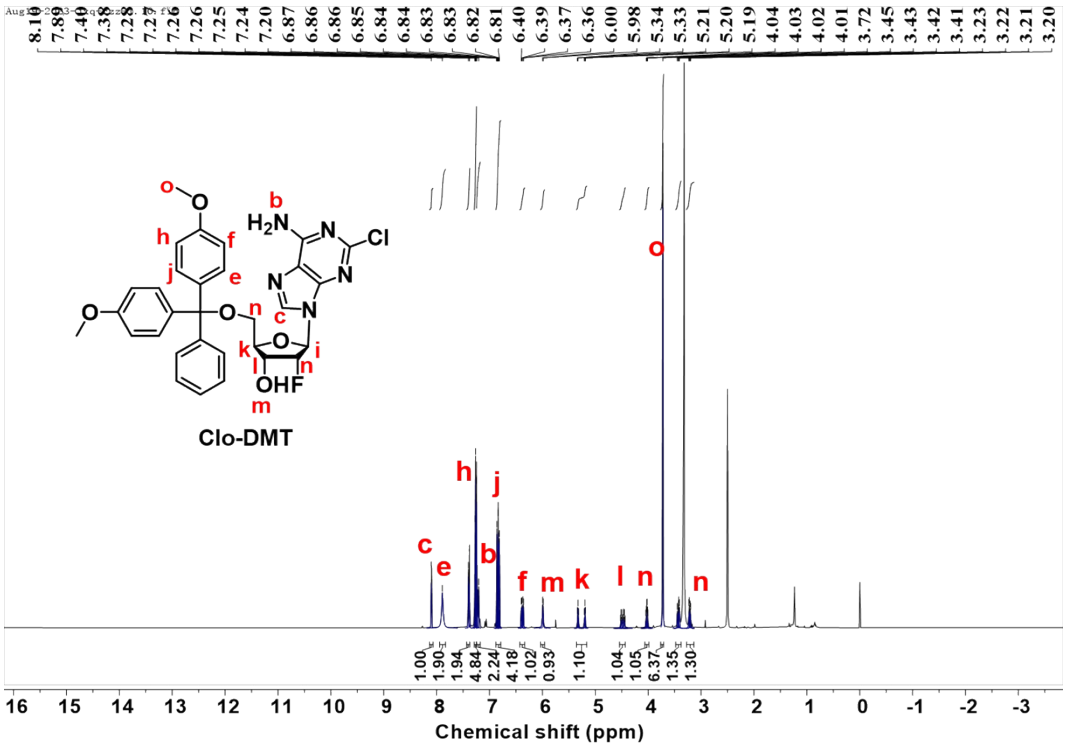


Figure S1. 1H NMR spectrum of Clo-DMT. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.20-3.23 (1H, m, F-CH), 3.41-3.45 (1H, m, -CH_2) 3.72 (6H, s, O-CH_3), 4.00-4.04 (1H, m, O-CH), 4.45-4.52 (1H, m, O-CH), 5.19-5.34 (1H, m, O-CH), 5.99 (1H, d, -OH), 6.36-6.40 (1H, dd, -OCHN-), 6.80-6.87 (4H, m, Ar-H), 7.20 (2H, s, -NH_2), 7.24-7.28 (5H, m, Ar-H), 7.39 (2H, d, Ar-H), 7.89 (2H, s, Ar-H), 8.10 (1H, s, -NCHN-).

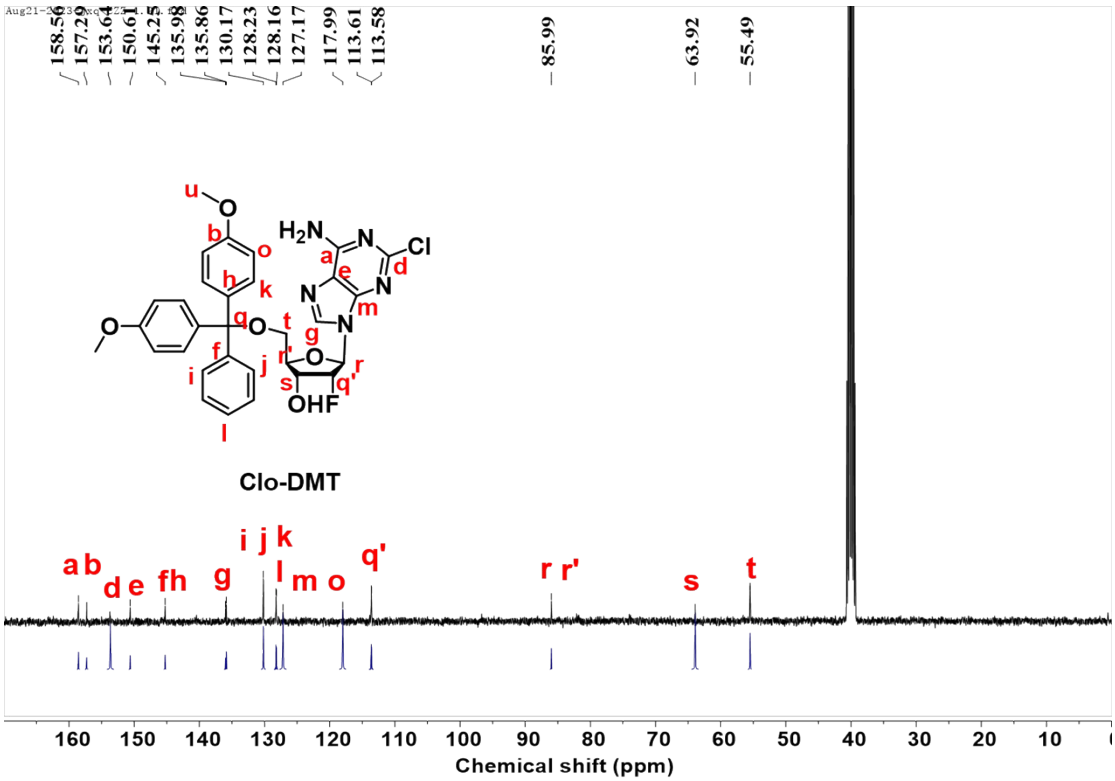


Figure S2. ^{13}C NMR spectrum of Clo-DMT. ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 158.56, 157.29, 153.64, 150.61, 145.25, 135.98, 135.86, 130.17, 128.23, 128.16, 127.17, 117.99, 113.61, 113.58, 85.99, 63.92, 55.49.

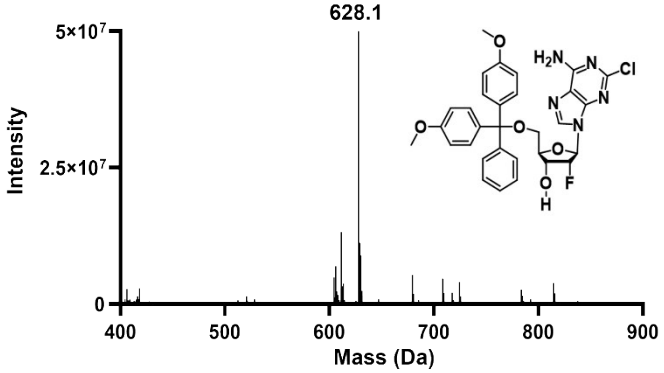


Figure S3. ESI-HRMS of Clo-DMT. $C_{31}H_{29}ClFN_5O_5$ calculated for $[M+Na]^+$: 628.1, found: 628.1.

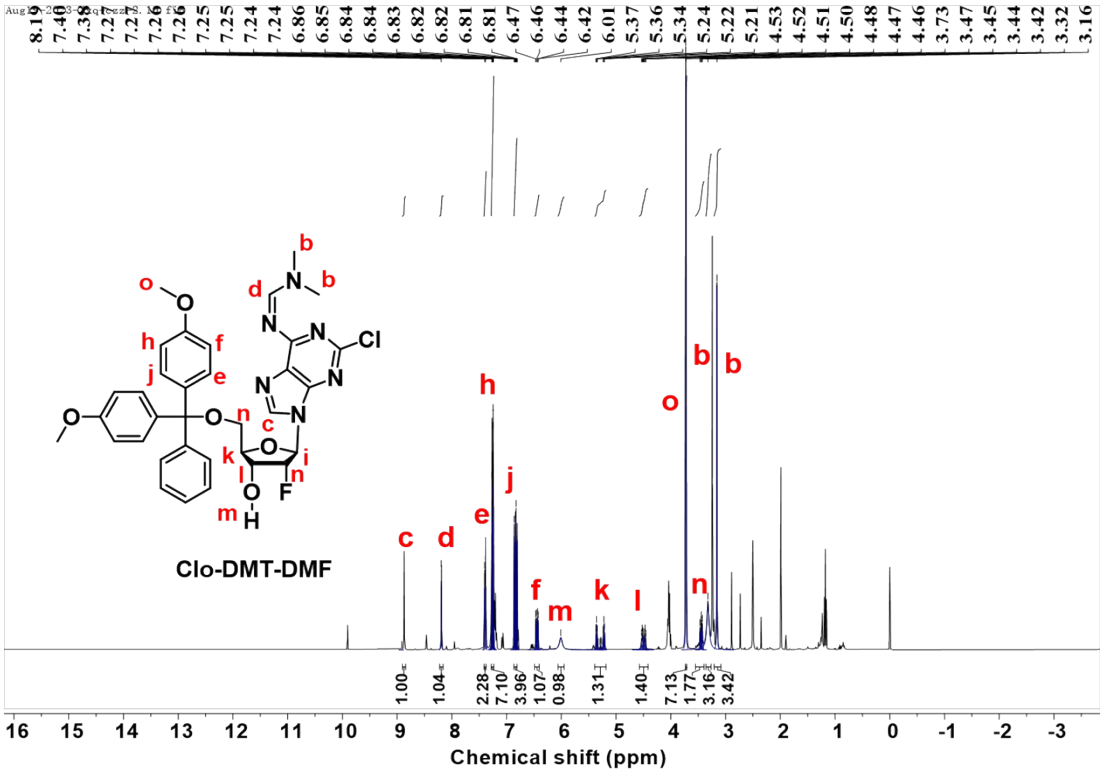


Figure S4. ^{1}H NMR spectrum of Clo-DMT-DMF. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.16 (3H, s, N-CH_3), 3.32 (3H, s, N-CH_3), 3.42-3.47 (2H, m,-CH_2), 3.73 (7H, s, O-CH_3, F-CH), 4.46-4.53 (1H, m, O-CH), 5.21-5.53 (1H, m, O-CH), 6.01 (1H, s, -OH), 6.42-6.47 (1H, m, -OCHN-), 6.81-6.86 (4H, m, Ar-H), 7.24-7.27 (7H, m, Ar-H), 7.39 (2H, d, Ar-H), 8.19 (1H, s, -NCHN-), 8.81 (1H, s, -NCHN-).

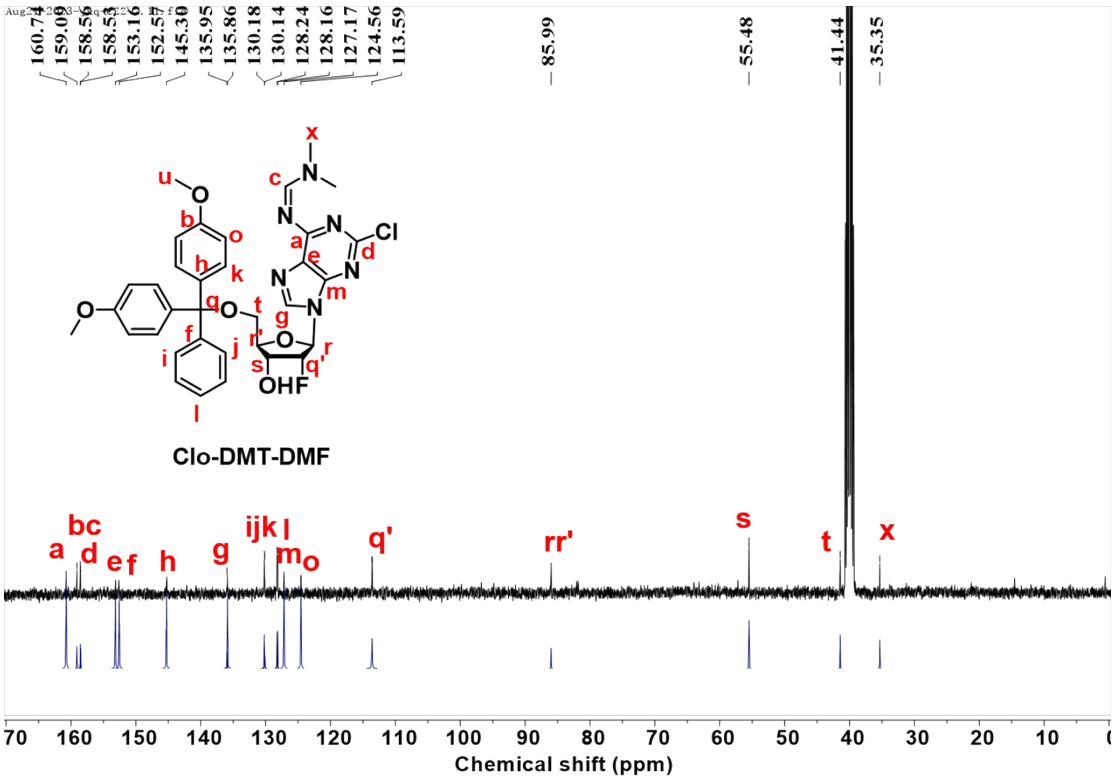


Figure S5. ^{13}C NMR spectrum of Clo-DMT-DMF. ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 160.74, 159.09, 158.56, 158.53, 153.16, 152.57, 145.30, 135.95, 135.86, 130.18, 130.14, 128.24, 128.16, 127.17, 124.56, 113.59, 85.99, 55.48, 41.44, 35.35.

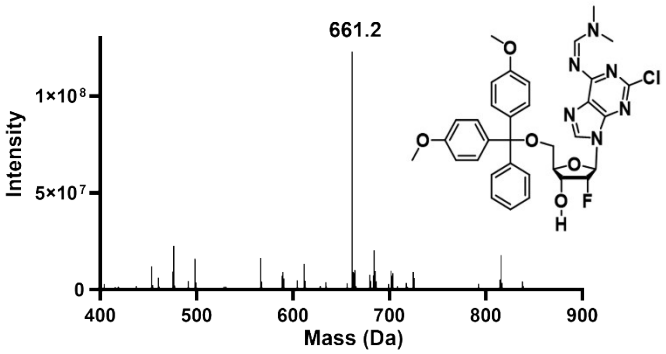


Figure S6. ESI-HRMS of Clo-DMT-DMF. $C_{34}H_{34}ClFN_6O_5$ calculated for $[M+H]^+$: 661.2, found: 661.2.

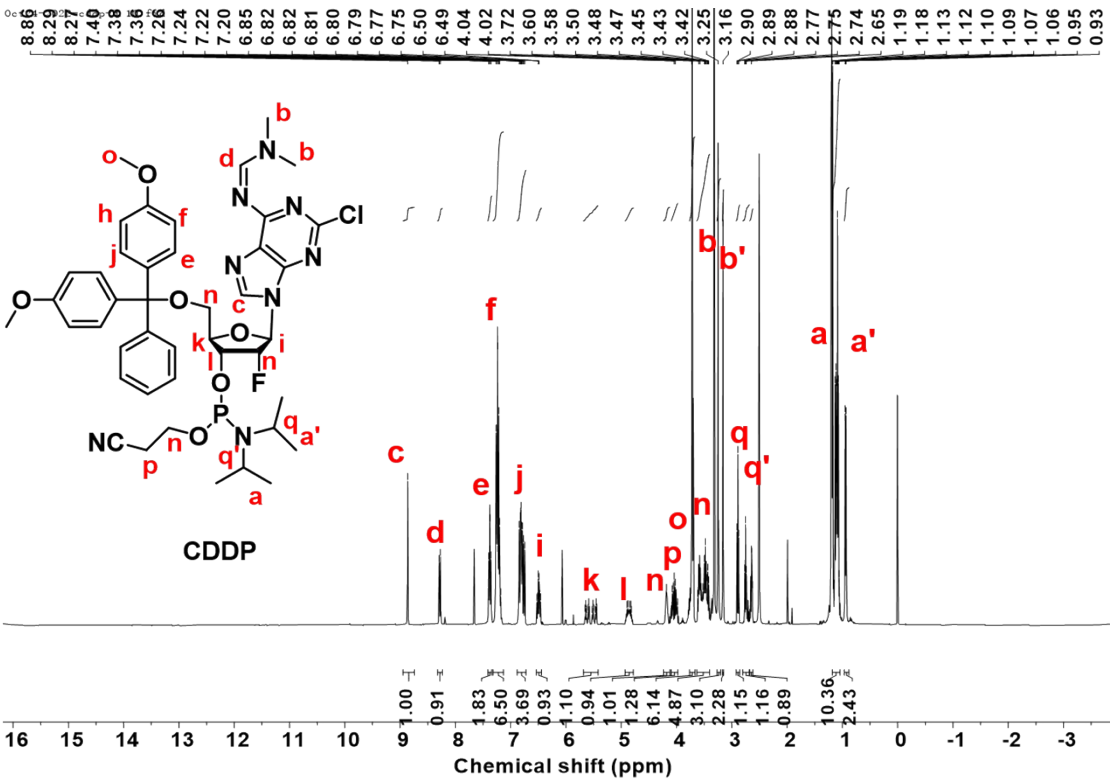


Figure S7. ^{1}H NMR spectrum of CDDP. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 0.93-1.19 (12H, m, isopropyl-CH_3), 2.65-2.77 (2H, m, isopropyl -CH), 2.88-3.25 (6H, m, N-CH_3), 3.43 (2H, t, -CH_2), 3.47-3.60 (4H, m, -CH_2), 3.72 (6H, s, O-CH_3), 4.02 (1H, s, F-CH), 4.04 (1H, s, O-CH), 4.82-4.89 (1H, m, O-CH), 6.49-6.50 (1H, m, -OCHN-), 6.75-6.79 (3H, m, Ar-H), 6.80-6.85 (1H, m, Ar-H), 7.20-7.26 (7H, m, Ar-H), 7.38 (2H, t, Ar-H), 8.29 (1H, d, -NCHN-), 8.86 (1H, s, -NCHN-).

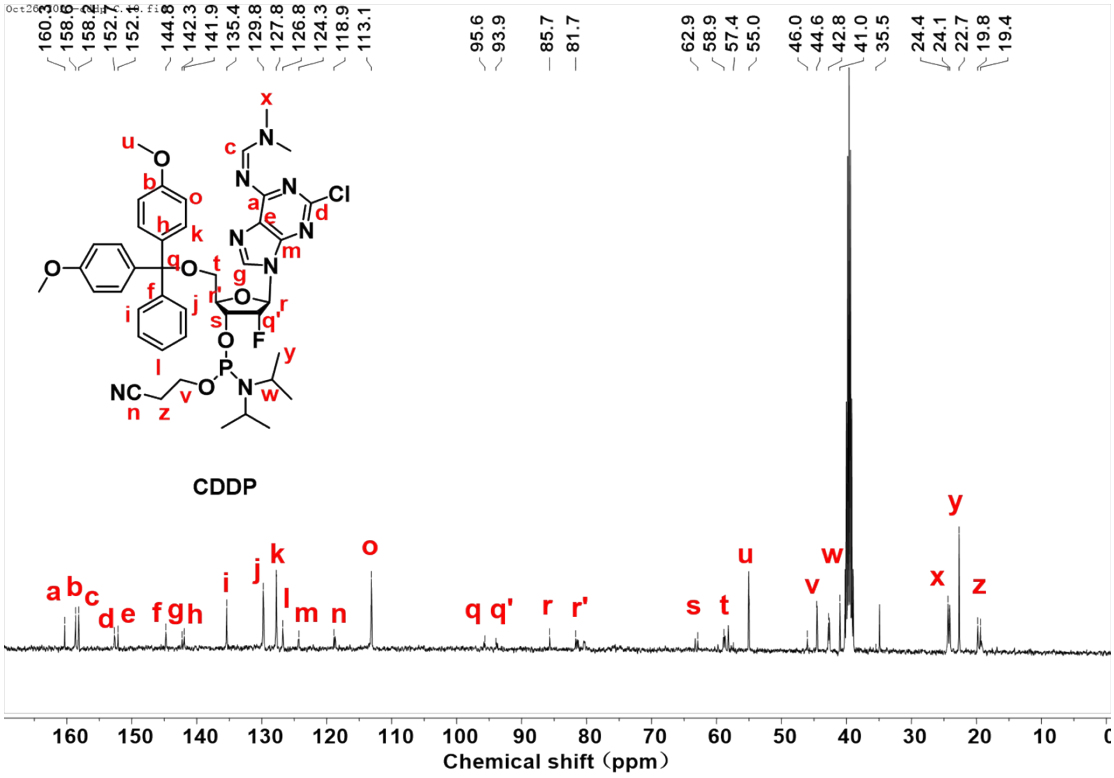


Figure S8. ^{13}C NMR spectrum of CDDP. ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 160.3, 158.6, 158.1, 152.6, 152.1, 144.8, 142.3, 141.9, 135.4, 129.8, 127.8, 126.8, 124.3, 118.9, 113.1, 95.6, 93.9, 85.7, 81.7, 62.9, 58.9, 57.4, 55.0, 46.0, 44.6, 42.8, 41.0, 35.5, 24.4, 24.1, 22.7, 19.8, 19.4.

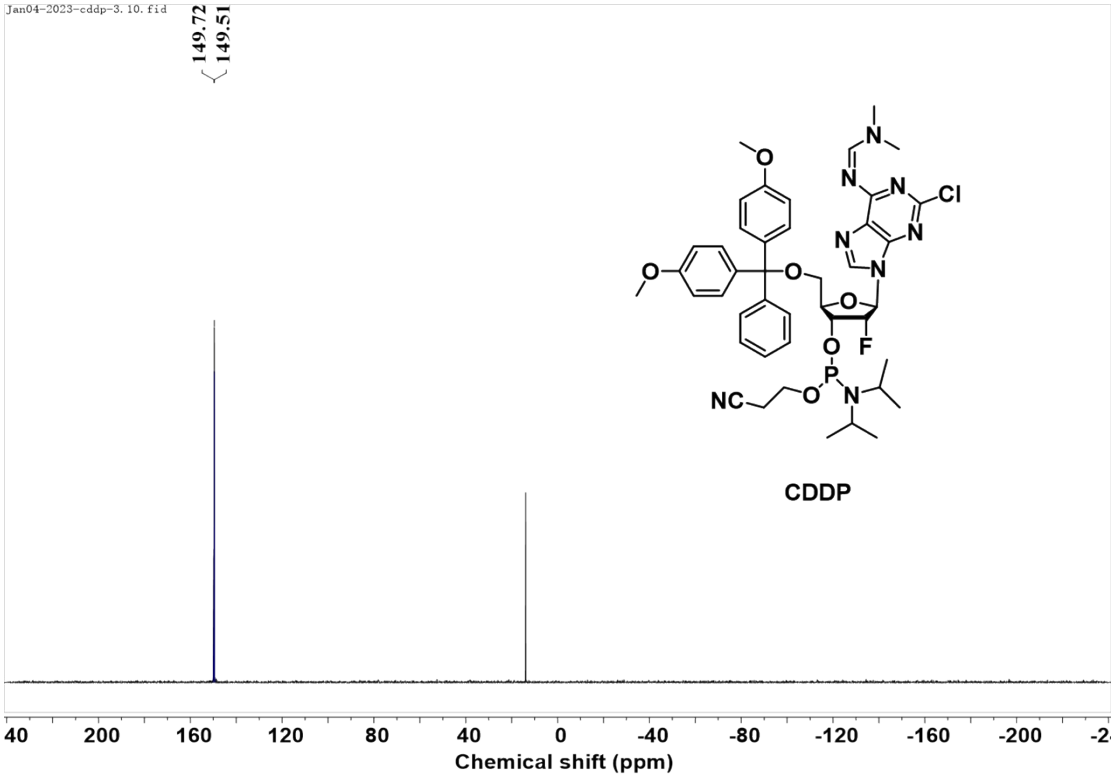


Figure S9. ^{31}P NMR spectrum of CDDP. ^{31}P NMR (125 MHz, DMSO-d_6) δ (ppm): 149.72, 149.51.

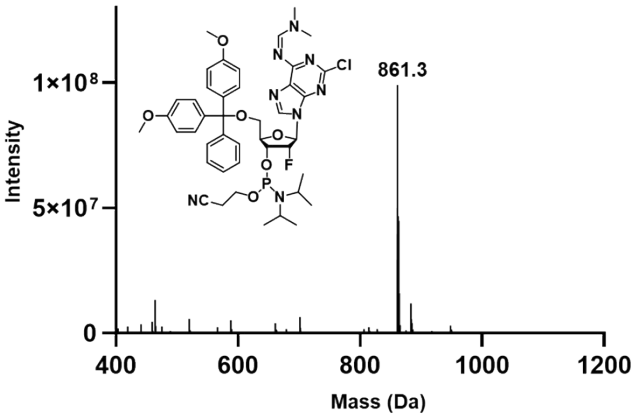


Figure S10. ESI-HRMS of CDDP. $C_{43}H_{51}ClFN_8O_6P$ calculated for $[M]^+$: 861.3403, found: 861.3397.

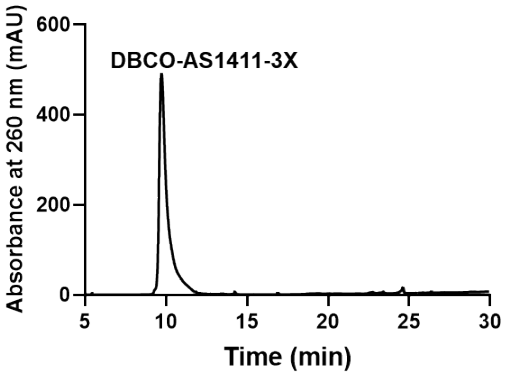


Figure S11. HPLC analysis showed the purity of DBCO-AS1411-3X is 99.9%. Purity was calculated as the percent area of integrated peaks in the HPLC chromatogram.

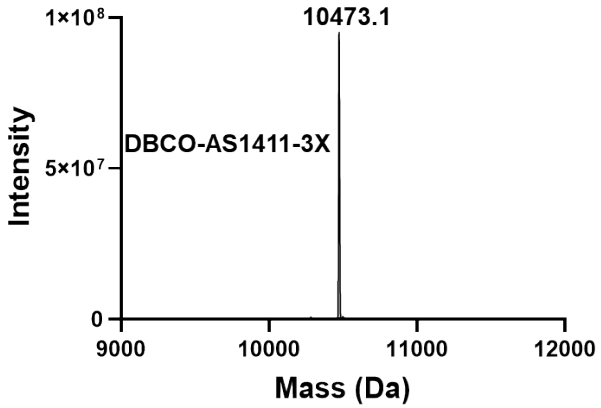


Figure S12. Mass analysis of DBCO-AS1411-3X. DBCO-AS1411-3X calculated molecular weight:10580.1, Found: 10473.1.

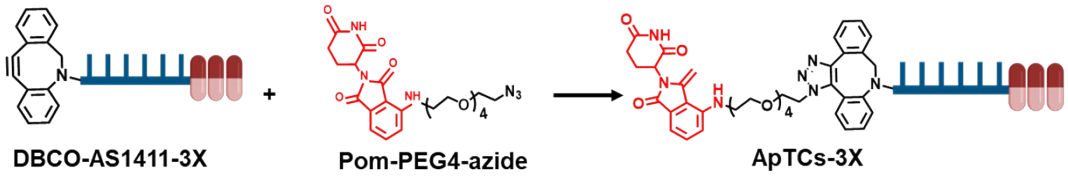


Figure S13. DBCO-AS1411-3X was conjugated with Pom-PEG4-azide to form Poly (Clo)-tailed Aptamer-PROTAC (ApTCs-3X).

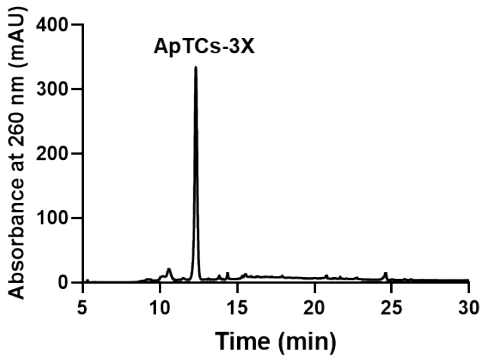


Figure S14. HPLC analysis showed the purity of ApTCs-3X is 99.3%. Purity was calculated as the percent area of integrated peaks in the HPLC chromatogram.

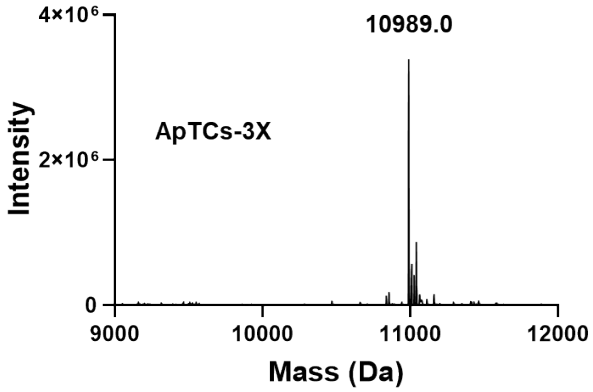


Figure S15. Mass analysis of ApTCs-3X. ApTCs-3X caculated molecular weight: 11098.1, Found:10989.0.

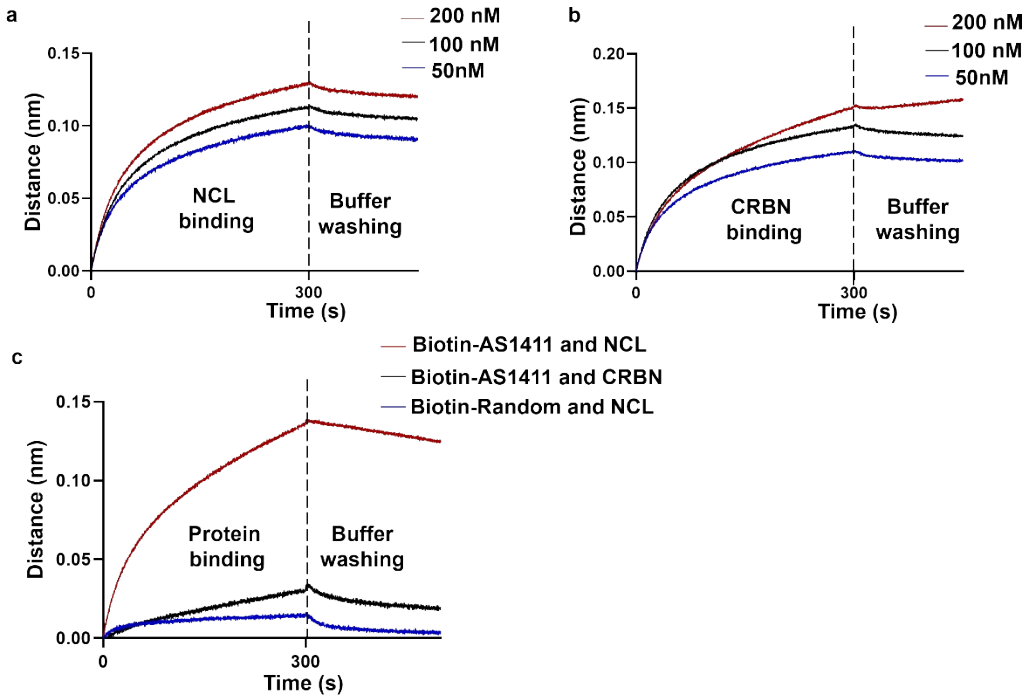


Figure S16. Dose-dependent BLI analysis of (a) AS1411 binding to NCL, and (b) CRBN binding to pomalidomide. (c) Specificity test.

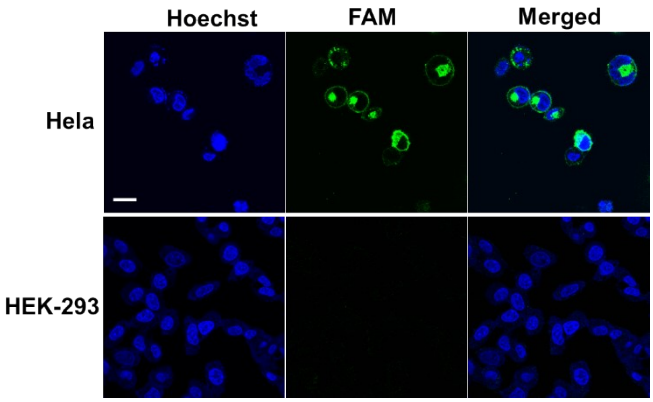


Figure S17. NCL of HeLa and HEK-293 cells were labeled with FAM-AS1411 (green) 30min. Scale bar: 20 μm.

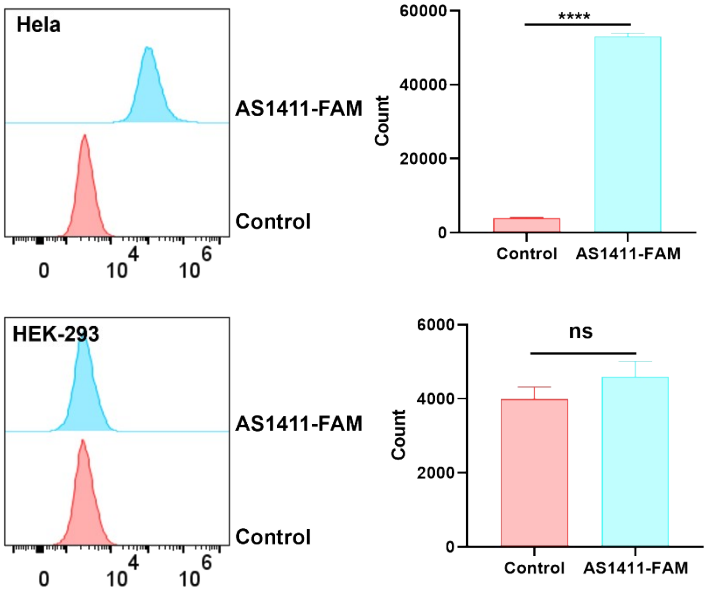


Figure S18. NCL of HeLa and HEK-293 cells were stained 30min with **AS1411-FAM at low temperature and detected by flow cytometry.** All data were presented as the mean ± SD (n = 3).

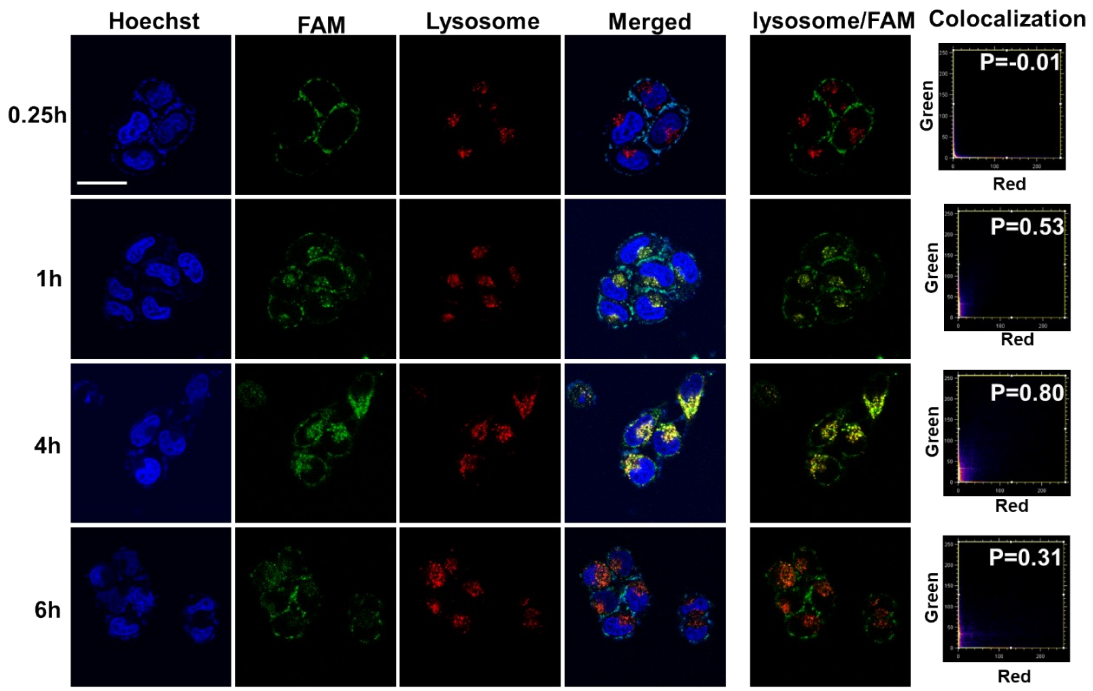


Figure S19. Confocal microscopy was used to image HeLa cells incubated with AS1411-3X-FAM at different time points. Cells were stained with lysosome (red fluorescence) and Hoechst 33342 (blue fluorescence) to localize the cytoplasm and nucleus, respectively. Green, blue and red colors indicate AS1411-3X-FAM, nucleus and Lysosome. Scale bar: 20 μm.

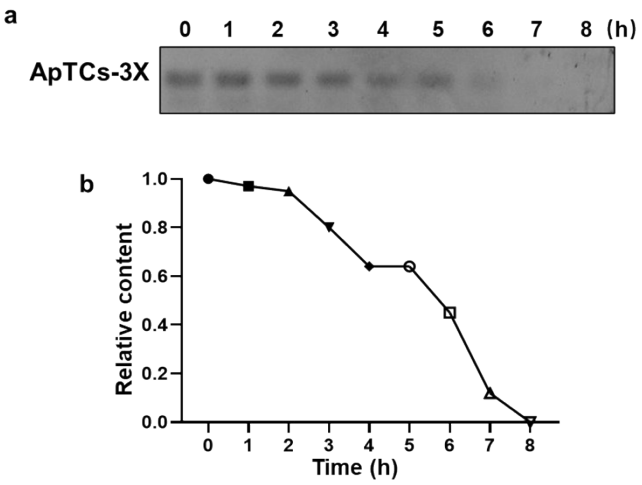


Figure S20. (a)Native PAGE analysis shows ApTCs-3X after incubation with DNase II and (b)its quantitative data. (DNase II concentration: 2 U/mL)

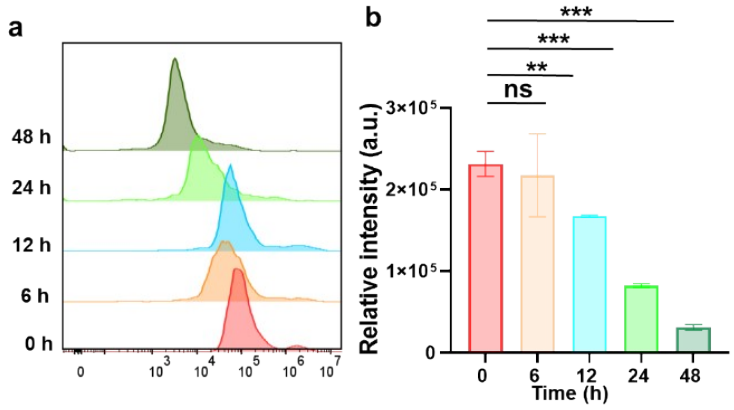


Figure S21. The HeLa cells after treatments with 200 nM of ApTCs-3X for different time were **stained with AS1411-FAM at low temperature and detected by flow cytometry.** All data were presented as the mean ± SD (n = 3).

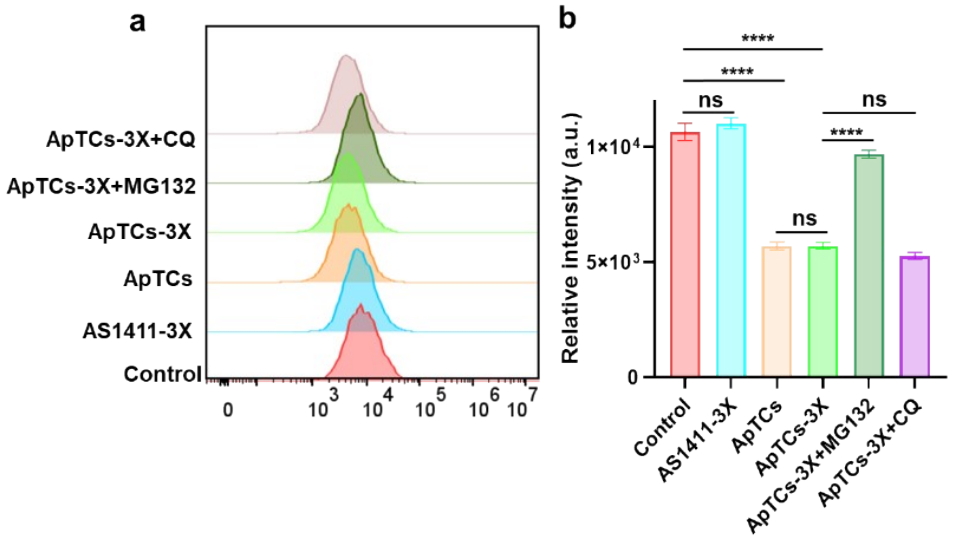


Figure S22. The HeLa cells treated with AS1411-3X (200 nM), ApTCs (200 nM), ApTCs-3X (200 nM), ApTCs-3X (200 nM) + MG132 (200 nM), and ApTCs-3X (200 nM) + Chloroquine (CQ) (50 μM) were stained with **AS1411-FAM at low temperature and detected by flow cytometry.** All data were presented as the mean ± SD (n = 3).

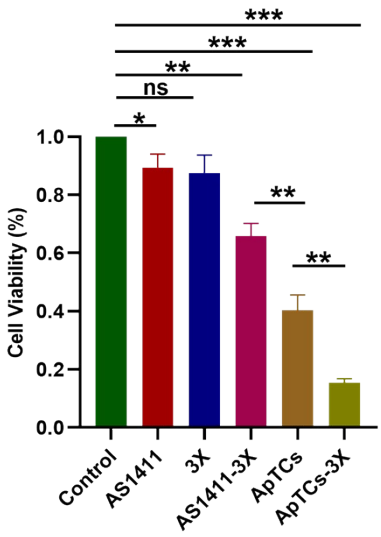


Figure S23. Cell proliferation assay of HeLa cells treated with AS1411 (200 nM), 3X (200 nM), AS1411-3X (200 nM), ApTCs (200 nM), ApTCs-3X (200 nM). All data were presented as the mean ± SD (n = 3).

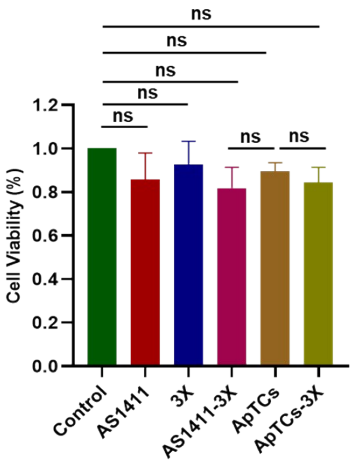


Figure S24. Cell proliferation assay of HEK-293 cells treated with AS1411 (200 nM)，3X (200 nM), AS1411-3X (200 nM), ApTCs (200 nM), ApTCs-3X (200 nM). All data were presented as the mean ± SD (n = 3).

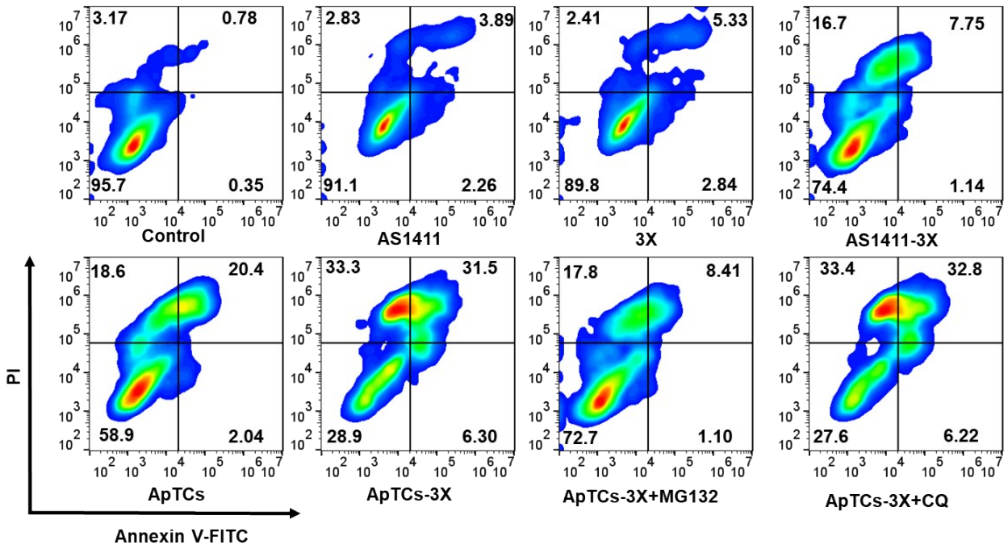


Figure S25. The HeLa cells treated with AS1411 (200 nM)，3X (200 nM), AS1411-3X (200 nM), ApTCs (200 nM), ApTCs-3X (200 nM), ApTCs-3X (200 nM) + MG132 (200 nM), and ApTCs-3X (200 nM) + Chloroquine (CQ) (50 μM) for 24 h were analyzed using flow cytometry with FITC-Annexin V/propidium iodide (PI) staining. All data were presented as the mean ± SD (n = 3).

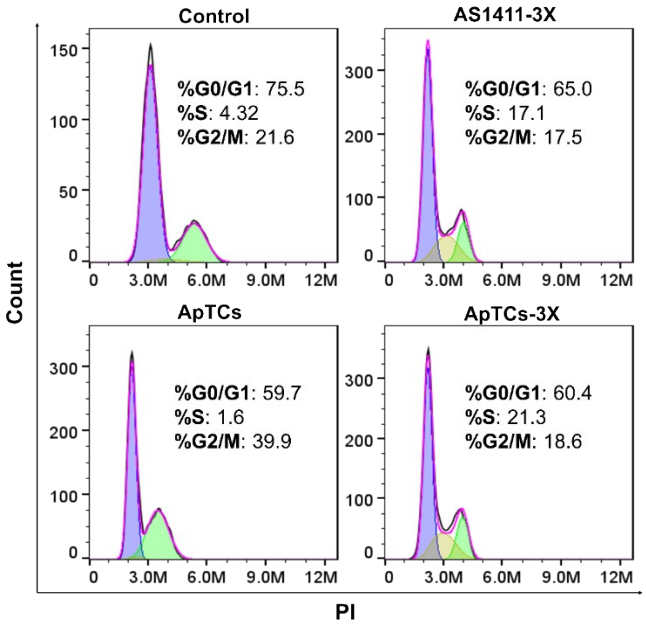
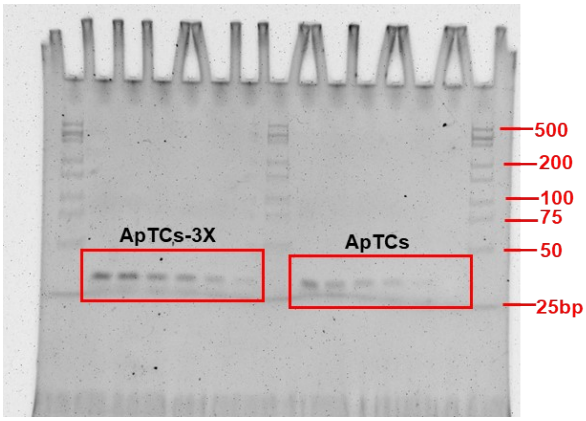


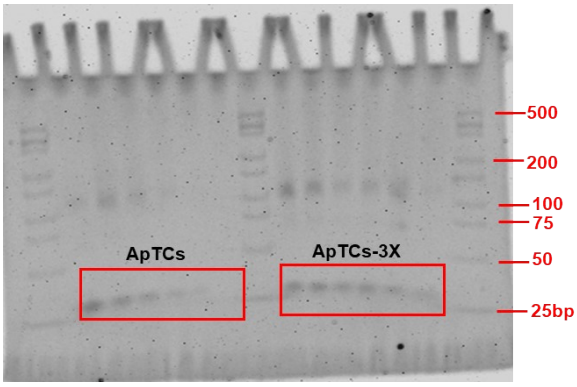
Figure S26. The HeLa cells treated with AS1411-3X (200 nM), ApTCs (200 nM), ApTCs-3X (200 nM) were used flow cytometry analysis of cell cycle. All data were presented as the mean ± SD (n = 3).

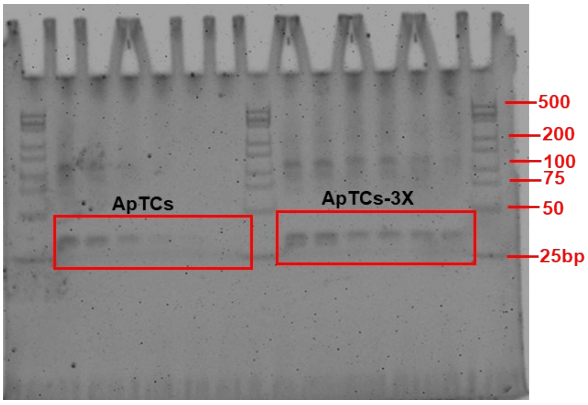
Table S1. The DNA oligonucleotide sequences utilized in this study.

Name	Sequence (5'-3')
AS1411	TTGGTGGTGGTGGTTGTGGTGGTGGTGG
DBCO- AS1411	DBCO-TTGGTGGTGGTGGTTGTGGTGGTGGTGG
DBCO-AS1411-3X	DBCO-TTGGTGGTGGTGGTTGTGGTGGTGGTGG-CDDP-CDDP-CDDP
FAM-AS1411	FAM-TTGGTGGTGGTGGTTGTGGTGGTGGTGG
FAM-AS1411-3X	FAM-azide-DBCO-TTGGTGGTGGTGGTTGTGGTGGTGGTGG-CDDP-CDDP-CDDP

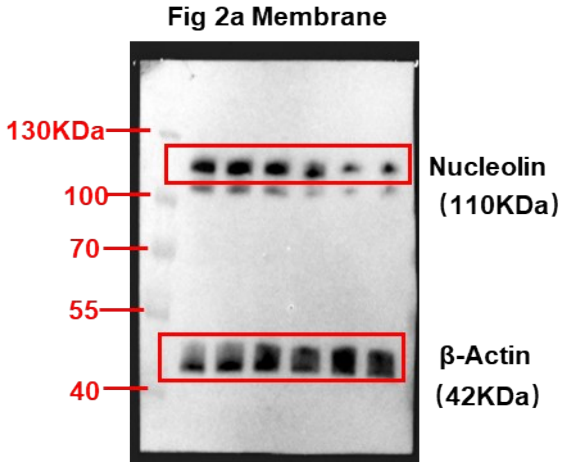
Original Gels of Fig. 1a







Original Blots of Fig. 2



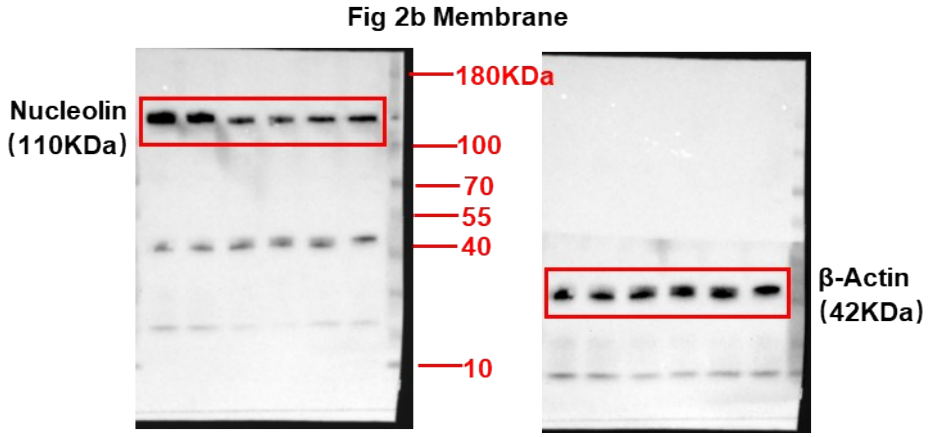


Fig 2a Cytoplasm

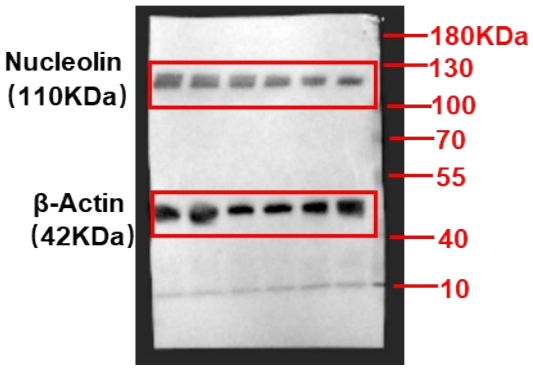


Fig 2b Cytoplasm

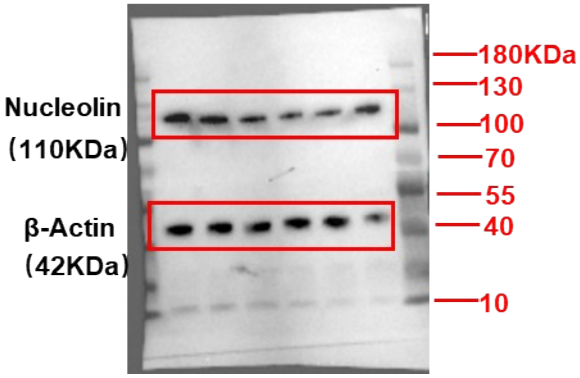
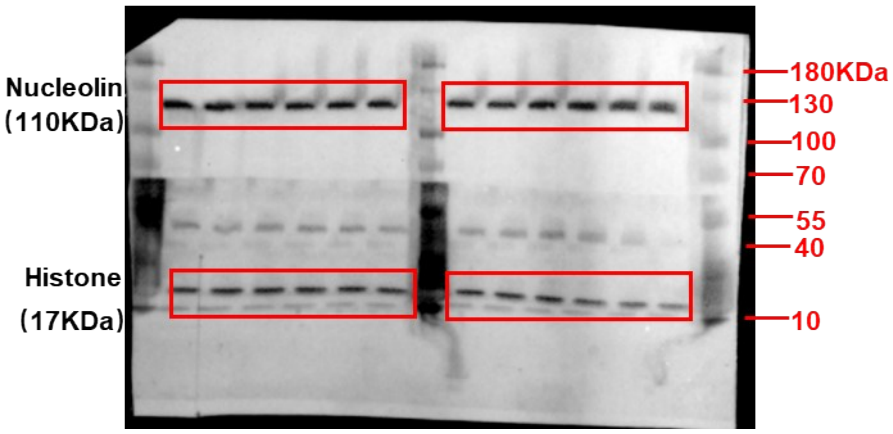
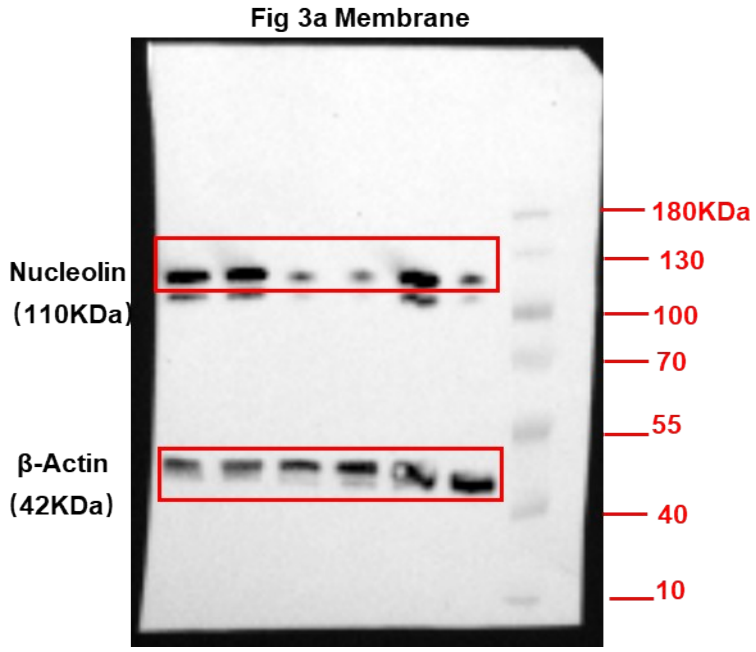
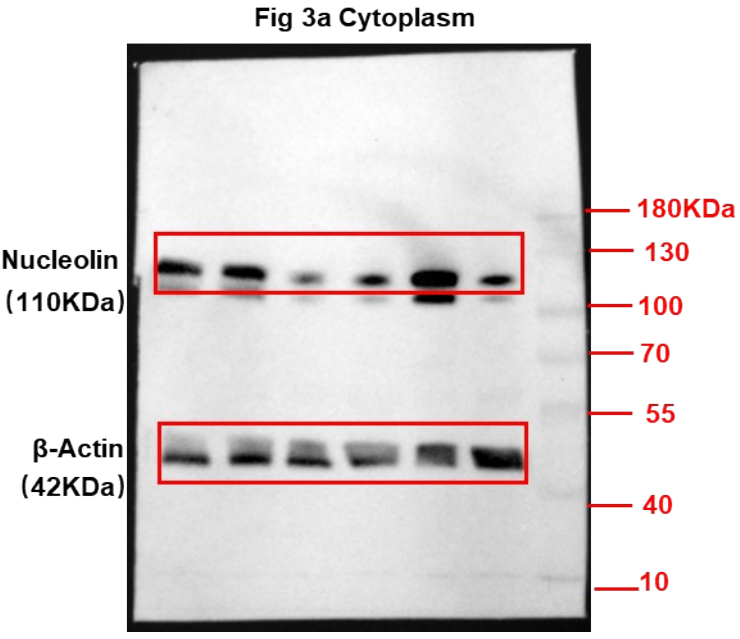


Fig 2b、a Nucleus



Original Blots of Fig. 3a





Original Gels of Figure S20