

## Synthesis and Characterization of CD133 Targeted Aptamer-Drug Conjugate for Precision Therapy of Anaplastic Thyroid Cancer

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## Legend to Figures

### **FigureS1. Monitoring the CD133 Levels in CD133-Transfected HEK293T Cells at Different Round of Positive Selection**

Expression levels and localization of CD133 antigen in CD133 transfected HEK293T cells at different positive selection steps were confirmed by laser scanning confocal microscopy. The red and blue (DAPI) fluorescence indicate CD133 antigen and nucleus, respectively. Scale bar indicates 100 $\mu$ m.

### **FigureS2. Determination of ssDNA Pool Concentration in Each Negative Selection Step**

Changes in ssDNA concentration were determined after incubation of ssDNA pool with CD133 negative HEK293T (i.e., wild type) in each negative selection steps, as described in Materials and Methods.

### **FigureS3. Assessment for Internalization of Aptamer AP-1 into Anaplastic Thyroid Cancer FRO Cells and Optimization of Aptamer AP-1 by truncating**

(A) Binding of aptamer AP-1 to anaplastic thyroid cancer FRO cells at 37°C was determined by confocal laser microscopy. Green and blue (DAPI) fluorescence indicate aptamer AP-1 and nucleus, respectively. Moreover, aptamer AP-1 was divided into aptamer AP-1-L and AP-1-M by truncating technology (B), and their secondary structures were predicted by IDT (C). Scale bar indicates 100 $\mu$ m.

### **FigureS4. Characterization of Aptamer-Dox Conjugates by HPLC.**

Dox (final concentration was 100 $\mu$ M) was incubated with aptamer on ice at a ratio of 10:1. After incubation for 2h, aptamer alone, doxorubicin alone and mixture of aptamer with dox were analyzed by HPLC.

### **FigureS5. Stability of AP-1-M-Dox Conjugates in Fresh Serum and its Specificity**

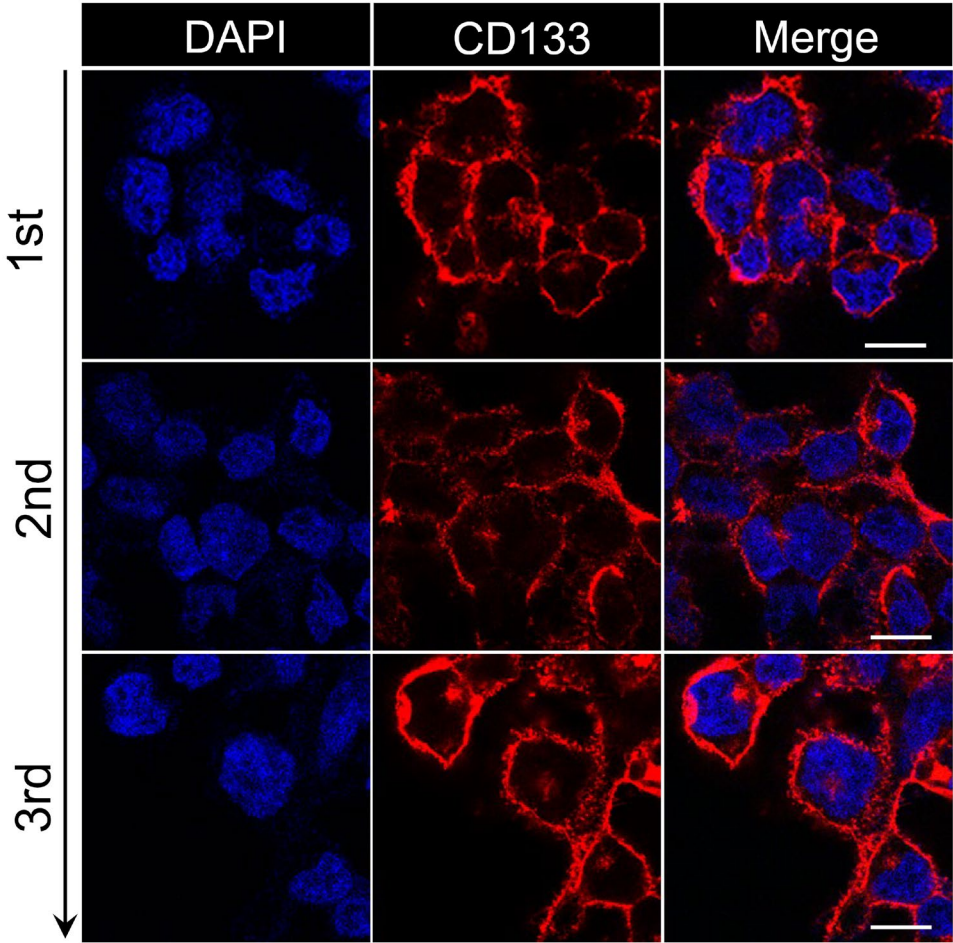
AP-1-M-Dox conjugates were incubated in 10% FBS at indicated time periods (0, 6, 12 and 24h). After incubation, degradation of AP-1-M-Dox conjugates in serum at different time periods was analyzed by 3% agarose gel electrophoresis and the release of free dox was detected by fluorescence spectroscopy (A-B). Moreover, CD133 negative Nthy-ori3-1 cells (left) were exposed to AP-1-M-Dox (5 $\mu$ M) and/or CD133 positive FRO cells (right) were exposed to Dox (5 $\mu$ M) at 37°C for 3h, and then washed twice with PBS and cultured in drug free fresh medium for up to 12h (C). Internalization and release of Dox into cells were confirmed by confocal microscopy.

### **FigureS6. Anticancer Activity and Toxic Effect of AP-1-M-Dox conjugates *in vivo***

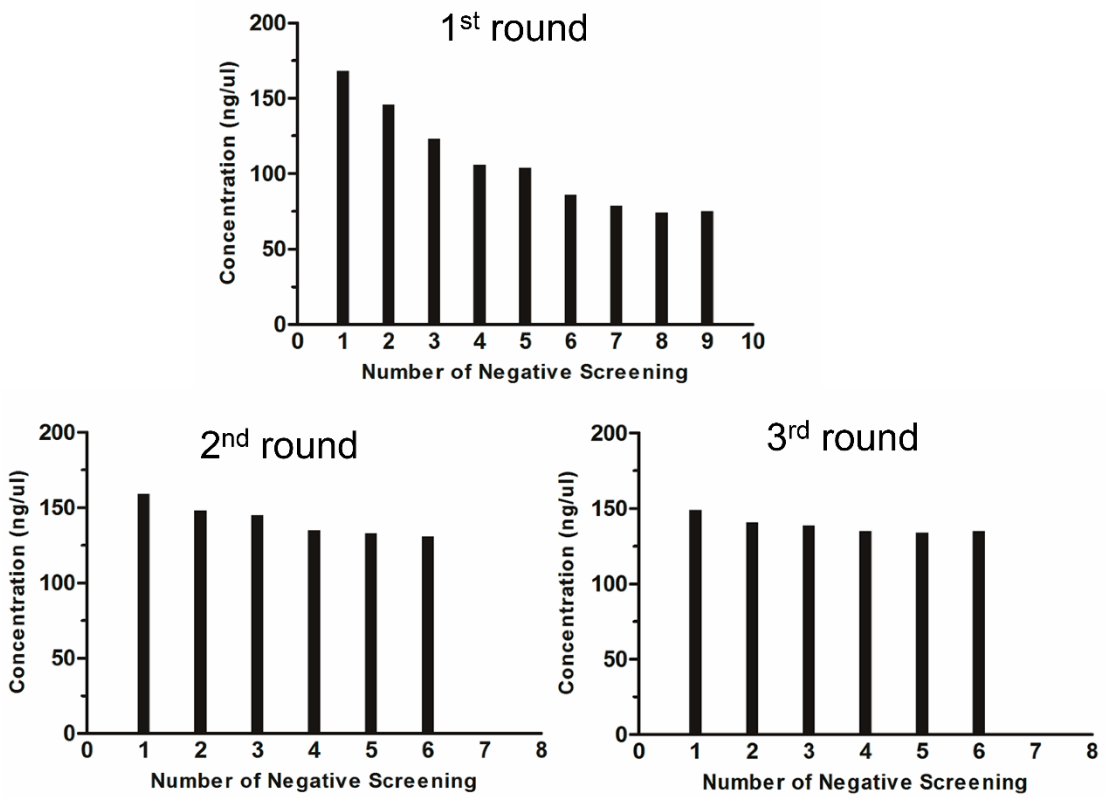
AP-1-M-dox conjugates (10mg/kg of doxorubicin payload), unconjugated free doxorubicin (10mg/kg), PBS and aptamer AP-1-M were intravenously injected into FRO cells xenograft immunodeficient mice (i.e., CD133-positive tumor) every 2 days

for 7 times, respectively. **(A)** HE staining of resected tumor sections of xenograft mice in each groups (200X). **(B)** Blood parameter such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) activity, blood urea nitrogen (BUN) and creatinine (Cr) Levels were also analyzed, as described in Materials and Methods.

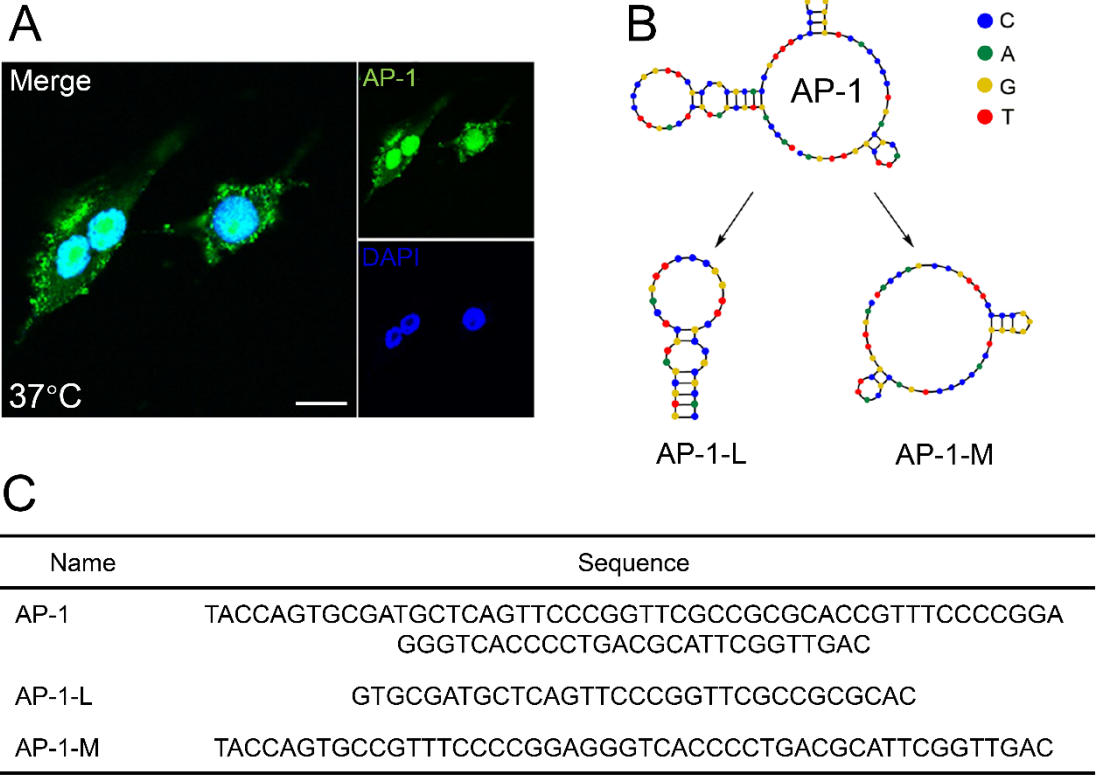
Supplementary Fig.1



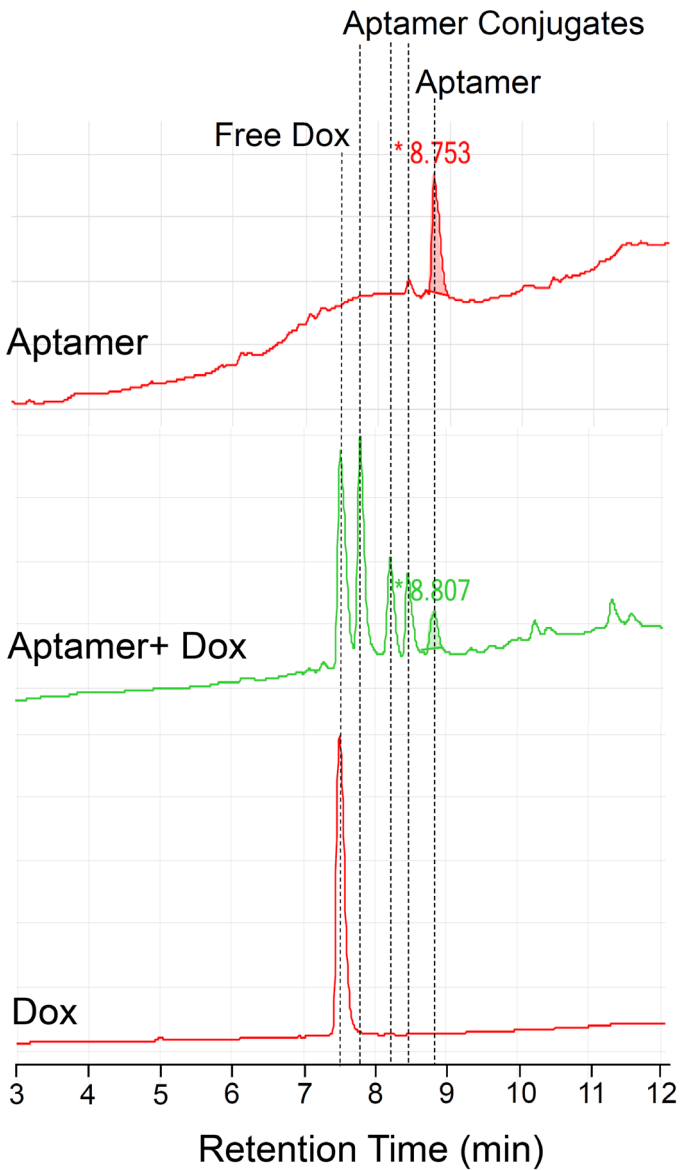
# Supplementary Fig.2



Supplementary Fig.3

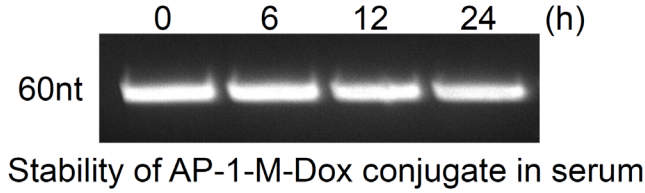


Supplementary Fig.4

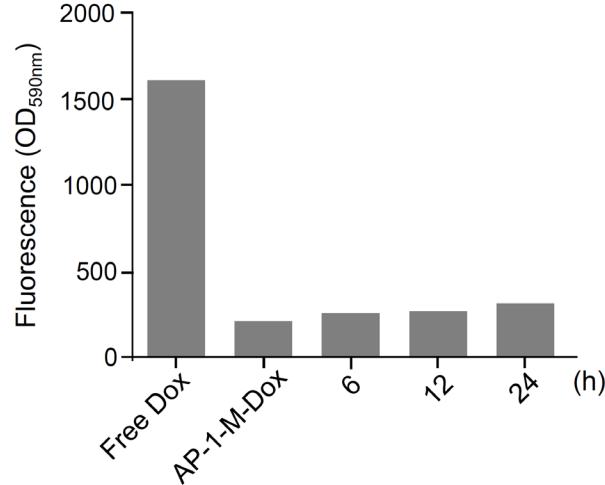


Supplementary Fig.5

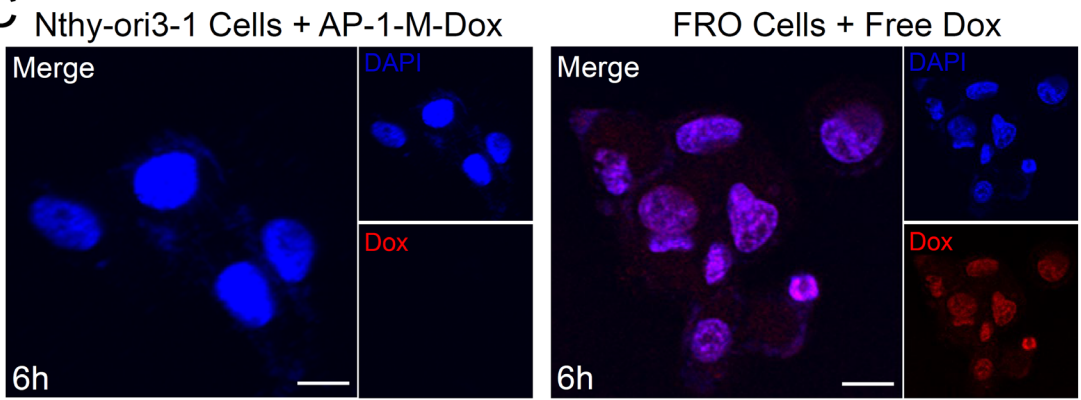
A



B



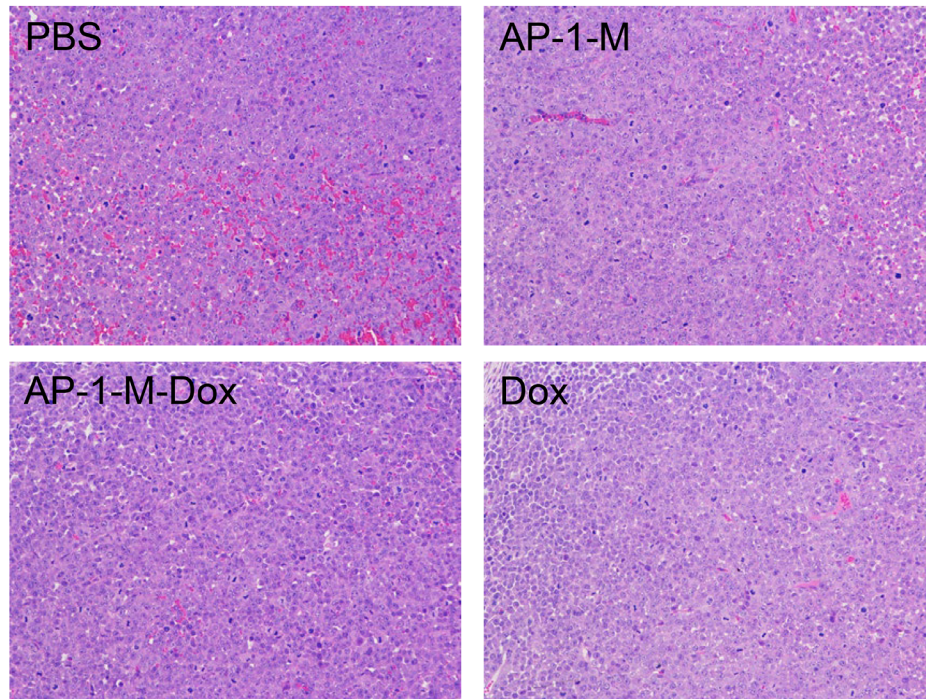
C





## Supplementary Fig.6

A



B

	AST(U/L)	ALT(U/L)	BUN(U/L)	Cr(mmol/L)
PBS	150.87 ± 19.35	66.93 ± 7.41	4.83 ± 0.53	69.03 ± 7.22
AP-1-M	162.33 ± 21.31	72.83 ± 8.29	5.02 ± 0.71	73.43 ± 8.83
AP-1-M-Dox	197.93 ± 24.38*	91.80 ± 12.60*	5.88 ± 0.82	90.93 ± 10.29*
Dox	249.33 ± 28.07**	129.07 ± 16.93**	6.42 ± 0.73*	98.97 ± 12.86*