Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information

Semi-automated and efficient parallel SELEX of aptamers for multiple targets

Meng Jiang[a†], Xiaona Fang[b †], Han Diao[c, b], Shaokang Lv[c, b], Zheng Zhang[b], Xiang Zhang[b], Zhiwei Chen[a, d, e*], Zhaofeng Luo[b*]

a. School of Life Sciences and Medicine, Shandong University of Technology, Zibo, 255049, China

b. The Key Laboratory of Zhejiang Province for Aptamers and Theranostics, Institute of Basic Medicine and Cancer (IBMC), Chinese Academy of Sciences, Hangzhou, 310022, China

c. College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou 310014, China.

d. Institute of Food and Nutrition Science, Shandong University of Technology, Zibo, 255049, P.R. China

e. School of Agriculture Engineering and Food Science, Shandong University of Technology, Zibo, 255049, P.R. China.

† Co-first authors

*Corresponding author e-mails:

biometrysdut@163.com

Contents

Chemical and reagents

The initial ssDNA library consists of a random region of 36 nucleotides flanked by two constant primer binding sequences. The ssDNA library used was purchased from Sangon Biotech (Shanghai, China) with a concentrated 36-nucleotide region representing random oligonucleotides (Table S1). Takara offers Taq DNA polymerase and dNTPs for polymerase chain reaction (PCR) amplification. GenScript (Nanjing, China) synthesized all primers used and 10×histidine peptide. The modified oligonucleotides were purified by HPLC. Genwiz prepared all aptamer sequences. Primers and libraries were diluted with double deionized water to prepare 100 μM stock solutions, which were then stored frozen at -20°C for aptamer SELEX and characterization. Phosphate-buffered saline (DPBS) solution (pH 7.4) used 2.68 mM KCl, 1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4 and 137 mM NaCl purchased from Sinopharm Chemical Reagent (Shanghai, China). Streptavidin-coated magnetic beads were purchased from Smart-Lifesciences Biotechnology (Changzhou, China). The experiments used double deionized water (ddH_2O) from a Cascade PALL (America). Except for TDP-43, which was purified in the laboratory, ROR1 was purchased from Sino Biological (Beijing, China), and the other proteins and R660-His antibody were purchased from PTM Bio (Hangzhou, China). The BSA and Tween 20 required in the binding buffer were purchased from Sangon Biotech (Shanghai, China), and HsDNA was purchased from Solarbio (Beijing, China). The binding buffer was composed of DPBS containing herring sperm DNA, BSA, tween-20 and 1.25mM Magnesium chloride, The wash buffer contains DPBS and Tween 20 (Concentration See supporting information Table S2), EDC of Activated Carboxyl Magnetic Beads purchased from Sigma-Aldrich(America), NHS purchased from J&K scientific(Beijing, China), q-PCR used Eva-green purchased from Biotium(America), Purification kit for library purchased from Sangon Biotech (Shanghai, China), semi-automatic instrument purchased from Aptamy (Hefei, China), The carboxyl magnetic beads required by SELEX are purchased from Zecen (Taizhou, China).

Experimental Procedures

Beads coupled with protein preparation for SELEX

The selection protein or the counter-selection protein was coupled to the magnetic beads by generating covalent bond between the -COOH modified beads and the primary amine on the protein. Briefly, 400 μL of magnetic beads (Zecen, 2.7 μm) was washed with 1 mL of 10 mM NaOH once and 1 mL of ddH_2O twice. Then the beads were activated in 800 μL of 0.4 M EDC and 0.1 M NHS (1:1, v/v) for 15 min. After activation, 80 μg protein diluted in sodium acetate was added to the beads and incubated for at least 2 h at room temperature. Supernatant was discarded and the beads were washed 3 times with 1 mL of DPBS. Then, the beads were blocked in 1 mL of 10 mM ethanolamine (PH8.0) and incubated for 15 min, washed for 3 times with 800 μL of 1× Wash buffer, resuspended in 400 μL antibody diluent buffer. Most of the proteins are his tagged, so the coupling efficiency were tested by flow cytometry using 200 μL of IR680 labeled anti-his antibody incubated with 1 μL of protein coupled beads.

Automated Aptamer SELEX procedure

Before starting the SELEX denaturation with different library to (95 ℃ for 10 min 4 ℃ 6 min 25 ℃ for 2 min), The libraries used by SELEX are random, and the reason for using different libraries is also to prevent aerosol contamination between parallel SELEX. The primers on the two ends of the libraries are different, so they cannot be amplified during PCR, which prevents contamination.

Generally, each round of counter-selection and selection are conducted by Auto-SELEX machine executing pre-defined program. The counterselection starts by washing counter selection beads by mixing the suspension of counterselection beads and 1×DPBS using an 8 tooth comb shell covered magnetic stick. Subsequently, the negative beads are transferred to the aptamer pool, incubated at 25°C while mixing. This step is followed by successive washing steps using 1× Wash buffer. Finally, the

counterselection beads are discarded in ddH_2O and the aptamer pool is then ready for positive selection.

The positive selection is performed using the same strategy as the counter-selection, but with different stringency regarding q-PCR results of each round. Detailed SELEX information is stated in the SELEX table (See Fig S1).

SELEX progress monitoring via q-PCR

The elution was done by boiling the counterselection beads or selection beads in 100 μL of ddH_2O at 95°C for 10 min. For each round of selection, the q-PCR was performed in 1× rTaq Reaction Buffer, 5 U/μl of rTaq DNA Polymerase, 4% Evagreen, 500 nM of forward primer S1, 500 nM of reverse primer A2 and 2 μL of the elution were added. The q-PCR was initiated by denaturing at 95°C for 2 min, following standard three steps amplification(95°C 30s 60°C 30s 72°C 30s) detection at annealing and a melting curve measurement at the end. The data was analyzed for determination of next round of SELEX.

ssDNA library preparation

DNA library for next round of SELEX was generated by PCR using rTaq DNA Polymerase , followed by strand separation by Auto-SELEX machine(For the reagents used, see Fig S2). Briefly, We use MIX configuration with S1-FAM and A2-biotin primers, and the ssDNA eluted in the positive SELEX was used as the template. Then the double-strand DNA obtained by PCR was immobilized on the streptavidin magnetic beads through the affinity relationship between streptavidin and biotin for 30 min, washed with wash buffer three times, treated with 100μL of 40 mM NaOH, Unwrap into single strands. The A2-Biotin strands left on streptavidin magnetic beads to be removed by magnetic suction. Neutralized with 4 μL of 1 M HCl, combined with an equal volume of 2× DPBS buffer. For each round of selection, the single-stranded DNA library was analyzed by urea denatured 8% PAGE gel to make sure there are no

byproducts that would possibly affect the SELEX progress.

Affinity test of enriched libraries

According to the results of real-time monitoring by q-PCR , when positive SELEX shows obvious enrichment, the enriched library needs to be affinity identified by flow cytometry or SPR.

After diluting the library to 300nM and 100μL, it will be divided into two groups, one group will add 2μL positive sieve magnetic beads, and the other group will add 2μL reverse Sieve magnetic beads. After incubation for 1h, wash with wash buffer, redissolve in binding buffer, and make a group with only magnetic beads added as blank control. All libraries are 5' FAM-modified libraries, so they were identified by FITC channel during cytometry. Generally, the binding of libraries can be detected by flow cytometry, but some libraries also have fluorescence quenching phenomenon or low affinity, so we will use SPR to identify some of the targets for their affinity.

NGS Sequencing of Enriched library

The sample preparation for NGS sequencing was performed using rTaq DNA Polymerase following standard PCR procedure to generate optimal amount of DNA oligos with an adapter on 5’ of each strand of the duplex. Polyclonal amplicons were purified by DNA purification kit (Sangon) following the protocol of the manufacture, quantified by QUBIT (Invitrogen). Then the sample was sent to NOVO GENE(Beijing, China) for sequencing. Data was analyzed by ClustalX2 software and aptamer candidates were picked regarding the sequencing results.

Characterization of aptamer dissociation constant (KD) via SPR

Overall, the dissociation constant of aptamer was characterized by surface plasmon resonance (SPR) spectroscopy experiments on Biacore 8K (GE Healthcare). All SPR experiments were carried out at 24°C using 1× PBS containing 1.25mM Mg^{2+}

as running buffer. The experiments were initiated by activating of the sensor chip (CM5, Cytiva) by injecting a 50:50 (v/v) mixture of NHS (0.1 M) and EDC (0.4 M) for 5 min. Target protein (50 μg/mL) was diluted in sodium acetate (Cytiva), the pH of the solution was determined by the isoelectric point of target protein, injected to flow cell 2 at a flow rate of 10μL/min for 5 min. Next, the control protein (5 μg/mL) diluted in sodium acetate was injected to flow cell 1as a control channel. Finally, the sensor chip was blocked by ethanolamine-HCl (1 M, pH 8.5) for 2 min.

The aptamers were diluted to 500 nM using 1× PBS containing 1.25mM Magnesium chloride. Prior to each experiment, all buffers are filtered and degassed. The binding analysis was carried out with aptamers at different concentrations 30 μL/min flow rate, 3 min for association time and 3 min for dissociation while PBS and 1M NaCl were used as the running and the regeneration buffer, respectively. The recorded sensor grams represented the association /dissociation of protein-aptamer complex. Channel 1 was used for background subtraction (His). By varying the aptamer concentration, a series of sensor grams were obtained and the Cytiva evaluation software provided 1:1 Langmuir model which was employed to calculate the equilibrium dissociation constant K_d.

Supplemental Figures

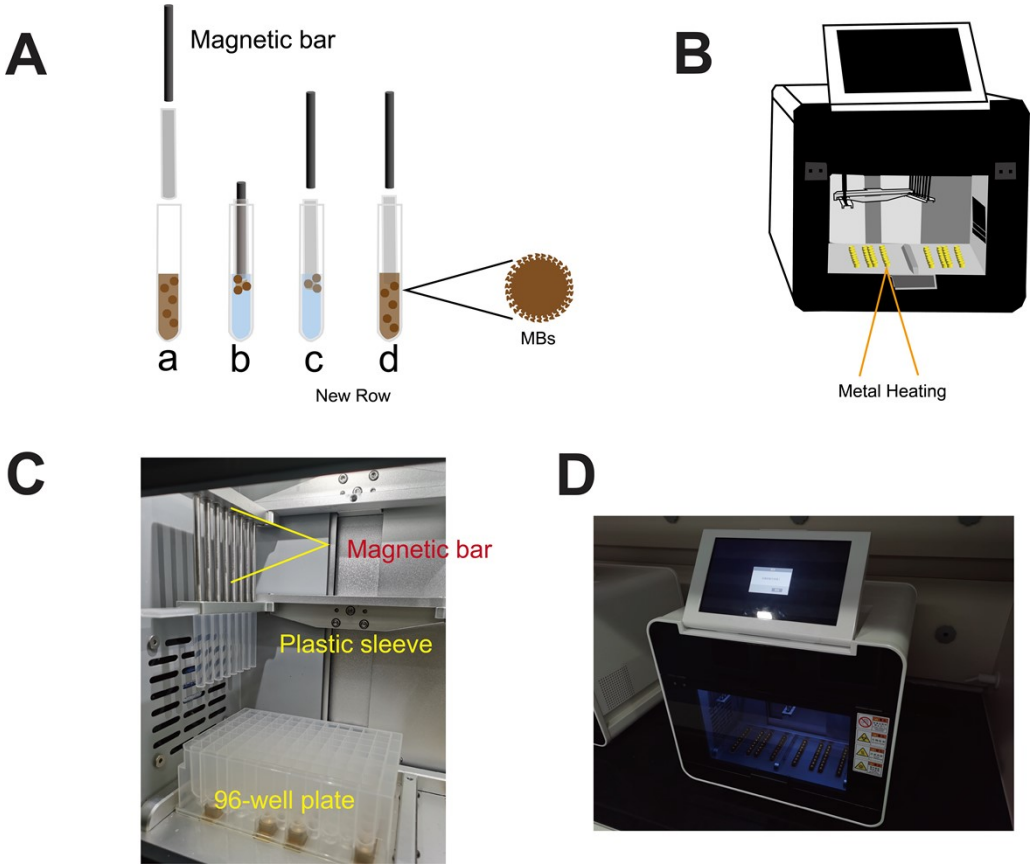


Figure S1 (A) Working principle of the magnetic rod. a. The magnet bar sinks into the wells of the 96-well plate. b. Magnetic attraction. c. Aspirate the beads to a new 96-well plate well. d. Mixing (B) Semi-automatic instrument (C) Interior drawing of semi-automatic instrument, including magnetic bar, plastic sleeve, 96-well plate (D) Actual photos of the semi-automatic instrument

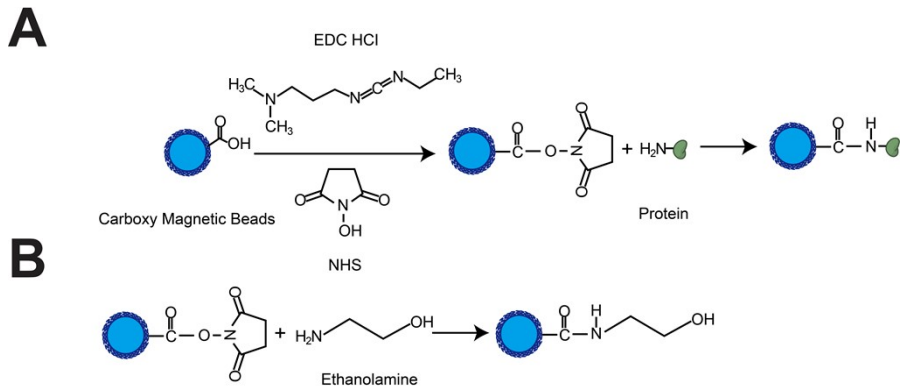


Figure S2 (A) Procedure for preparing protein beads(B) Ethanolamine terminates excess activated carboxyl

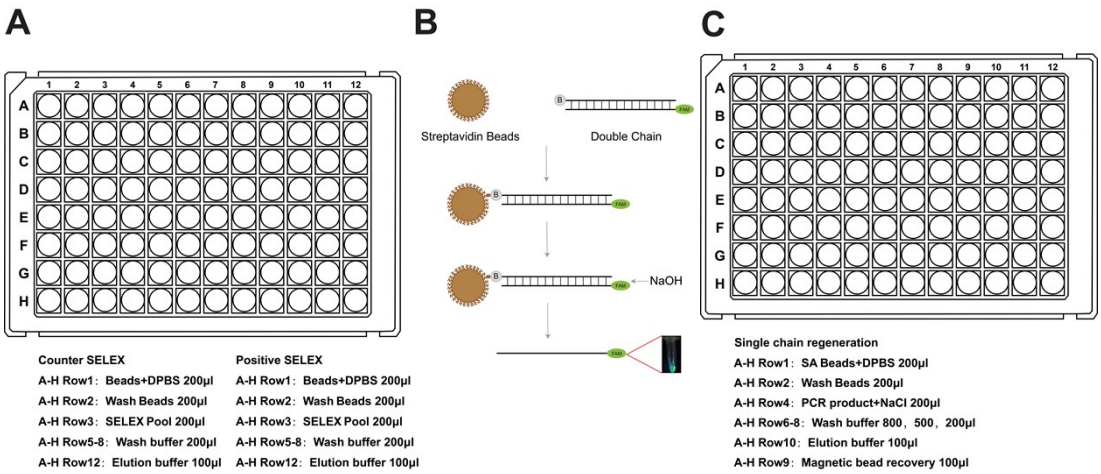


Figure S3: (A) Reagent information corresponding to the well position of the 96-well plate in SELEX(B) Principle of ssDNA regeneration(C) Corresponding reagents in wells during ssDNA regeneration

The pore position used for SELEX reagents:

Row 1: After add negative or positive magnetic beads and supplemented with without calcium and magnesium DPBS to 200μl.

Row 2: 200μl without calcium and magnesium DPBS contain 2.5‰ tween 20.

Row 3: The ssDNA library after renaturation was added to this well site waiting for incubation with positive and negative SELEX magnetic beads.

Row 5-8: 200μl with 1.25mM calcium and without magnesium DPBS contain 2.5‰ tween 20.

Row 12: 100μl ddH_2O

The pore position used to prepare single-chain reagents:

Row 1: Usually add 80 μl(According to the loading capacity of the magnetic beads) streptavidin magnetic beads and 120μl without calcium and magnesium DPBS.

Row 2: 200μl without calcium and magnesium DPBS contain 5‰ tween 20.

Row 4: According to the volume of PCR product, add sodium chloride to 1M, Sodium chloride can increase the capture efficiency of biotin and streptavidin.

Row 6-8: 800, 500, 200μl without calcium and magnesium DPBS contain 5‰ tween 20.

Row 10: 40mM sodium hydroxide

Row 9: ddH_2O

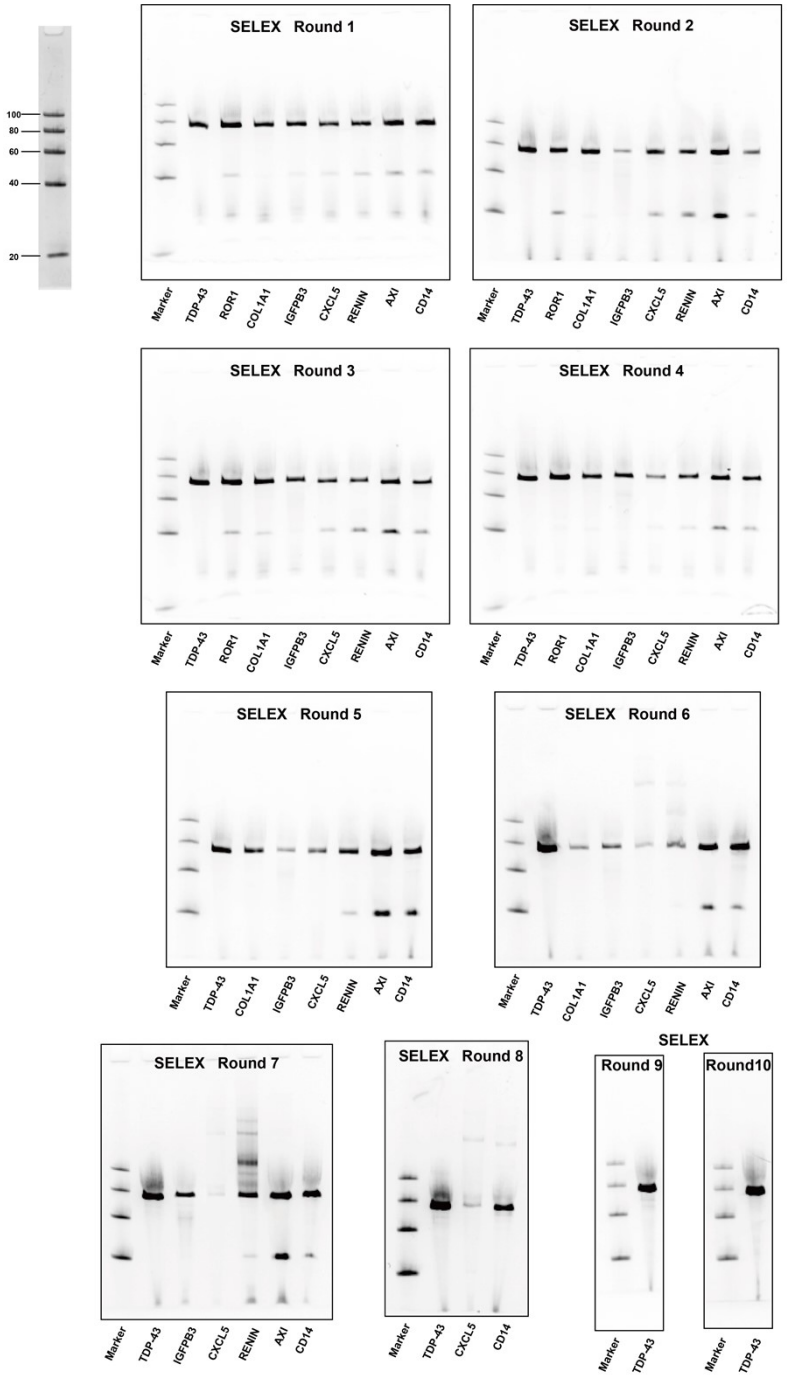


Figure S4 Monitoring of single-stranded DNA with urea denatured 8% PAGE gel

From the urea denatured 8% PAGE gel(Fig S3), the unsuccessful AXL and CD14 ssDNA have by-products as the number of SELEX rounds increases. Although some targets that successfully obtain aptamers also have by-products, but it will decrease with enrichment. After by-products appear during the experiment, it will be judged according to the number of by-products whether it is necessary to prepare ssDNA by using different primers to prepare the library for long (Table S2 A2-PolyA) short chain (Table S2 S1-FAM) method. For the next round of SELEX short chains are purified by urea denatured 8% PAGE gel.

Calculation formula obtained by qPCR

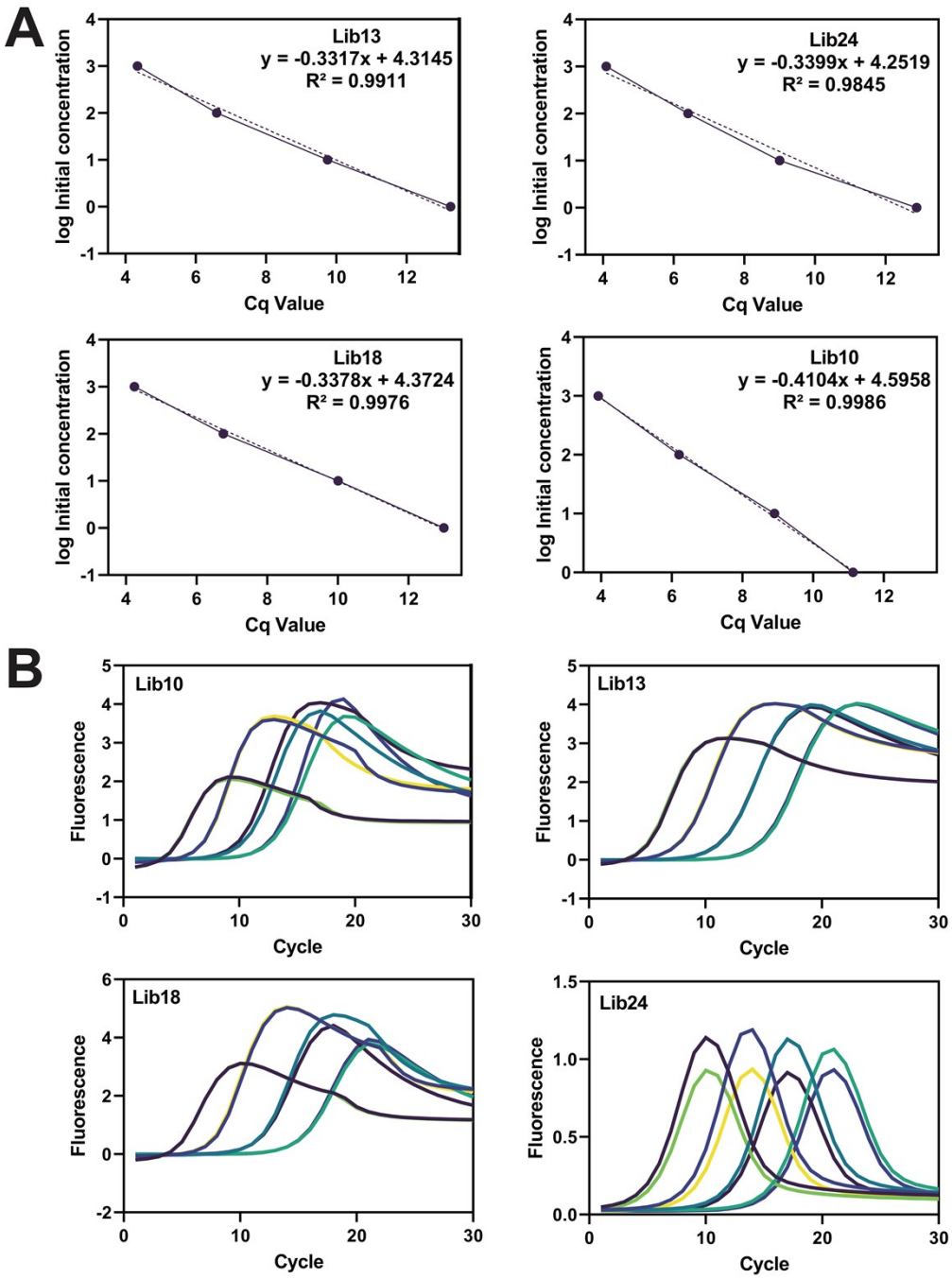


Figure S5 Calculation formula obtained by qPCR (A) The formula is obtained by logarithmic curve(B) Initial data obtained with a qPCR instrument (Roche LC96) diluted four concentration gradients of ssDNA

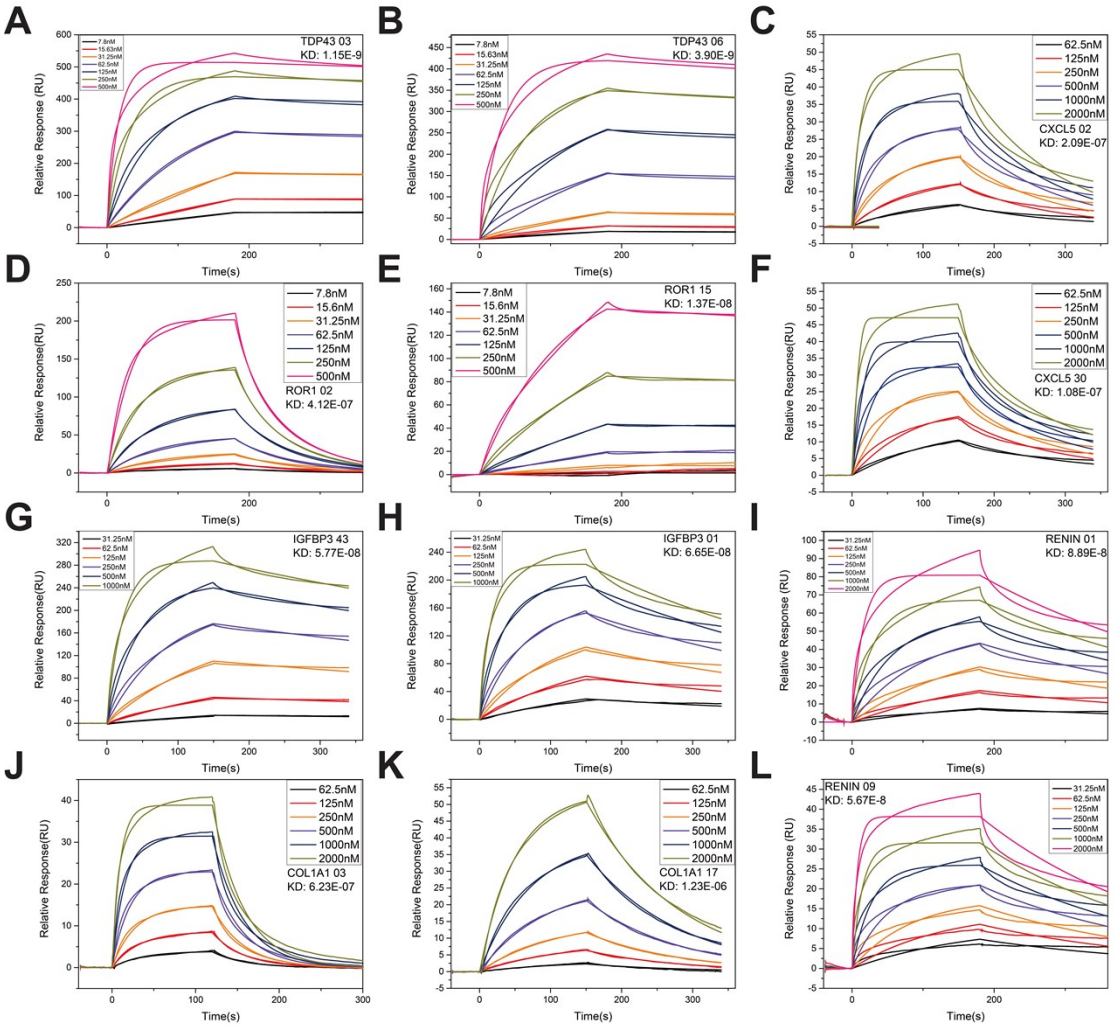
Equation from the chart(Fig S4 A) Bring the Cq value as X into further get the log initial concentration(Table S5) the resulting value is recorded as V_{in} ,Dilute multipe is recorded as D_m, V means volume(liter, L), NA means Avogadro's constant, The CQ value can be converted to the number of molecules by the following equation, Ratio of

the number of molecules remaining in SELEX to the number of starting molecules put into SELEX, the retention rate can be obtained.

$$C(pM)=10\text{^ } V_{in}$$

$$N = n*NA$$

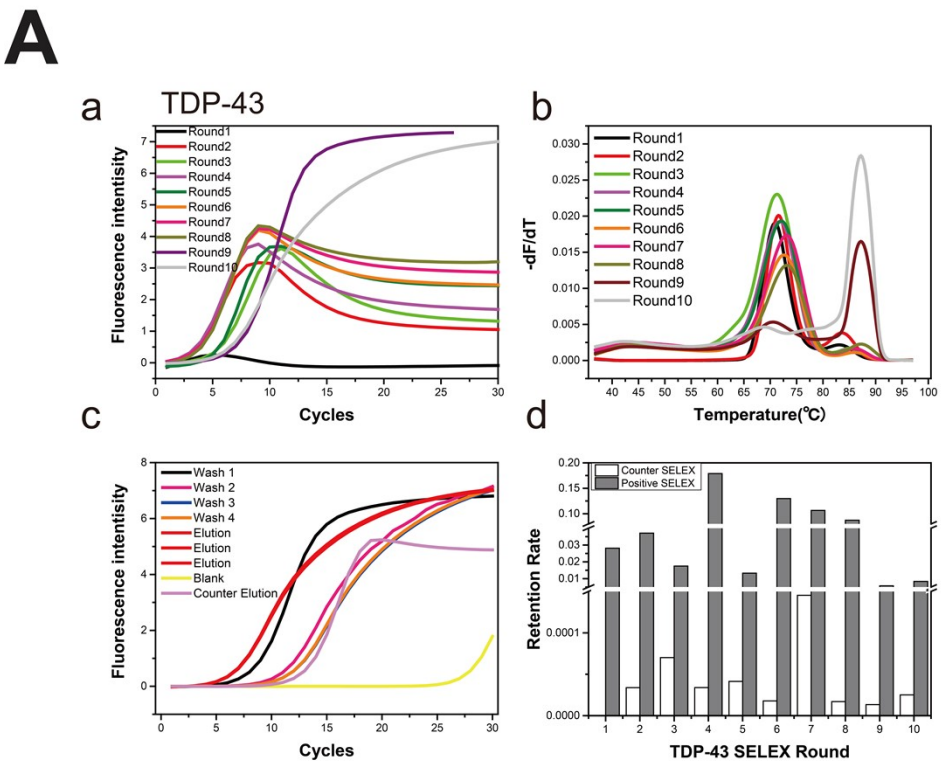
$$=C*10^{-12}* D_m*V*6.02*10^{23}$$

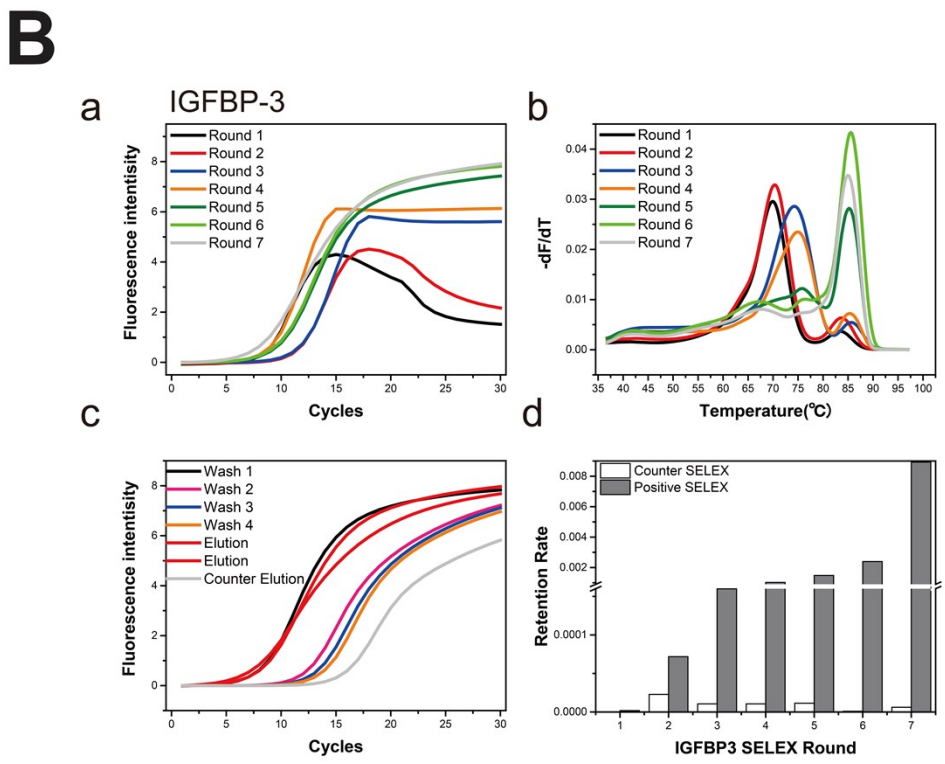


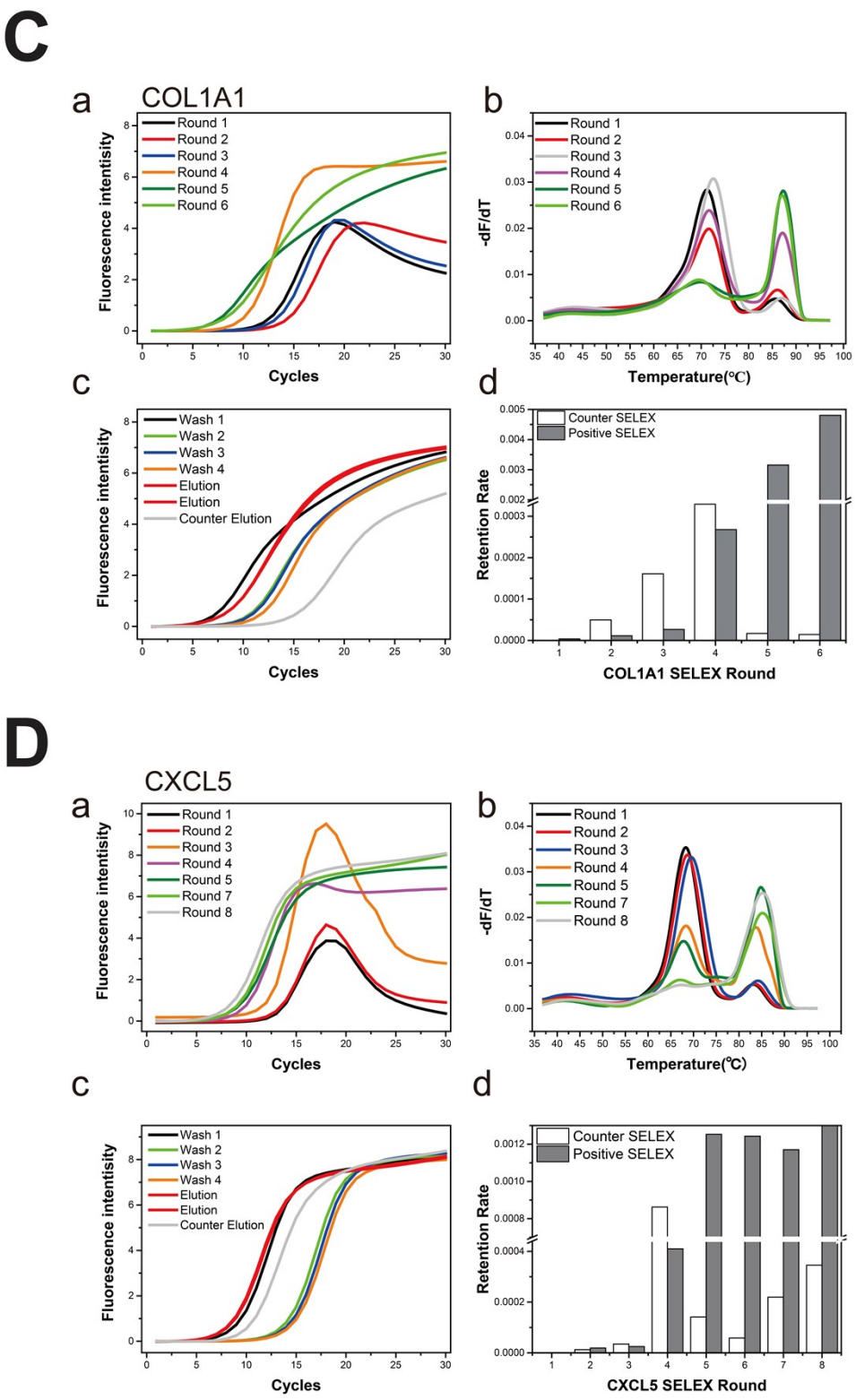
Response curves for characterization of aptamer affinity by SPR

Figure S6. Response curves for characterization of aptamer affinity by SPR (A) TDP43 03 aptamer concentration from 500nM to 7.8nM (B) TDP43 06 aptamer concentration from 500nM to 7.8nM (C) CXCL5 02 aptamer concentration from 2000nM to 62.5nM (D) ROR1 02 aptamer concentration from 500nM to 7.8nM (E) ROR1 15 aptamer concentration from 500nM to 7.8nM (F) CXCL5 30 aptamer concentration from 2000nM to 62.5nM (G) IGFBP3 43 aptamer concentration from 1000nM to 31.25nM (H) IGFBP3 01 aptamer concentration from 1000nM to 31.25nM (I) RENIN 01 aptamer concentration from 2000nM to 31.25Nm (J) COL1A1 03 aptamer concentration from 2000nM to 62.5nM (K) COL1A1 17 aptamer concentration from 2000nM to 62.5nM (L) RENIN 09 aptamer concentration from 2000nM to 31.25nM

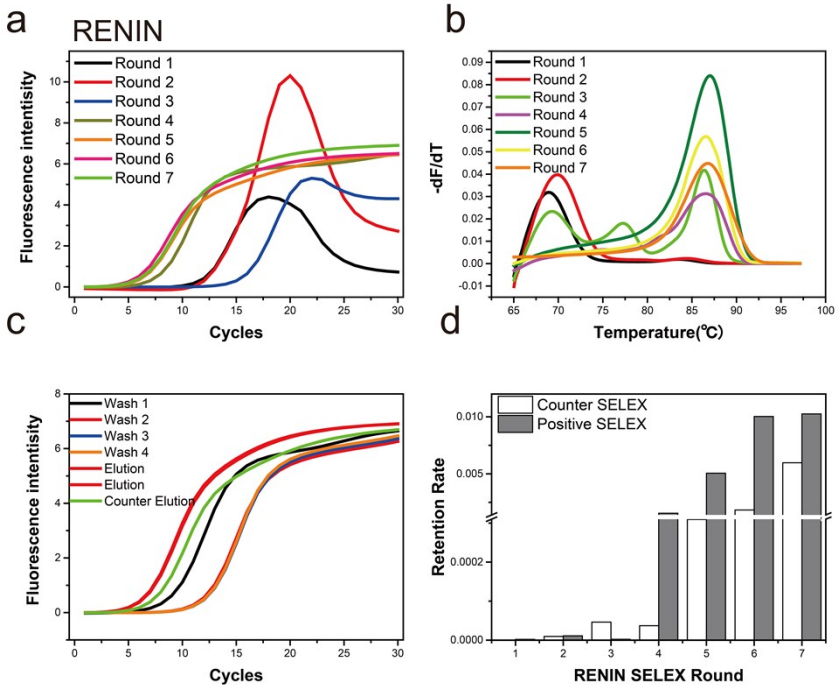
Other target protein qPCR data to monitor SELEX progress



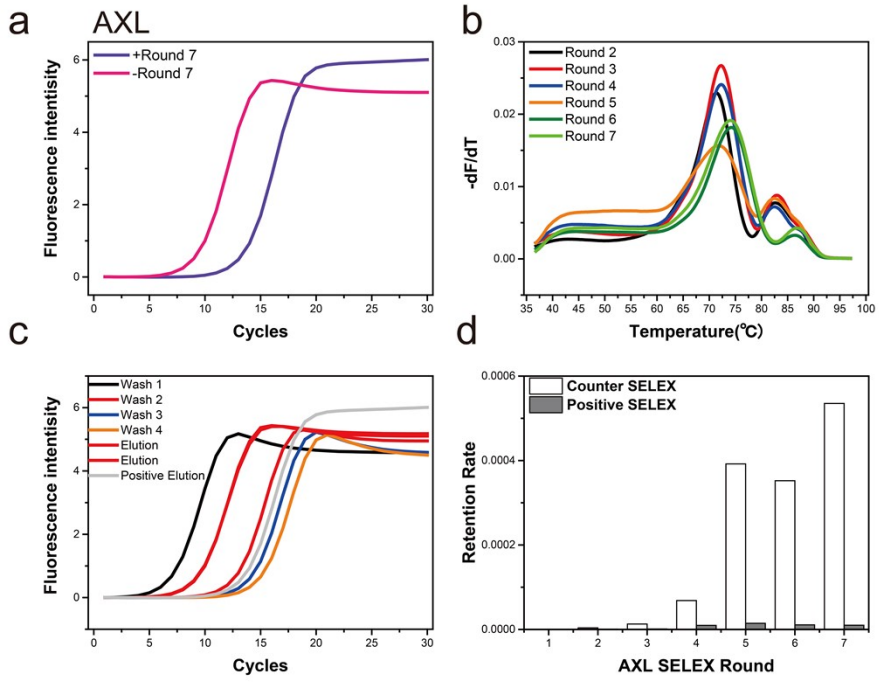



C
a
COL1A1
Round 1
Round 2
Round 3
Round 4
Round 5
Round 6
Fluorescence intentisity
Cycles
b
-dF/dT
Temperature(℃)
c
Wash 1
Wash 2
Wash 3
Wash 4
Elution
Elution
Counter Elution
d
Counter SELEX
Positive SELEX
Retention Rate
COL1A1 SELEX Round
D
CXCL5
Round 7
Round 8
CXCL5 SELEX Round

E


a
RENIN
Round 1
Round 2
Round 3
Round 4
Round 5
Round 6
Round 7
Fluorescence intentisity
Cycles
b
-dF/dT
Temperature(℃)
c
Wash 1
Wash 2
Wash 3
Wash 4
Elution
Elution
Counter Elution
d
Counter SELEX
Positive SELEX
Retention Rate
RENIN SELEX Round

F


a
AXL
+Round 7
-Round 7
Fluorescence intentisity
Cycles
b
Round 2
Round 3
Round 4
Round 5
Round 6
Round 7
-dF/dT
Temperature(℃)
c
Wash 1
Wash 2
Wash 3
Wash 4
Elution
Elution
Positive Elution
d
Counter SELEX
Positive SELEX
Retention Rate
AXL SELEX Round

G

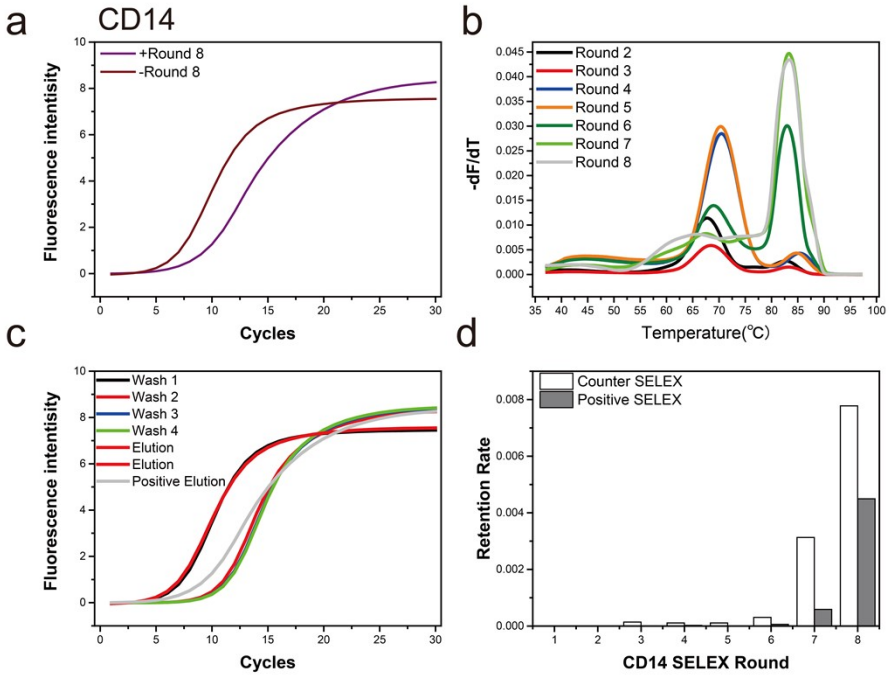
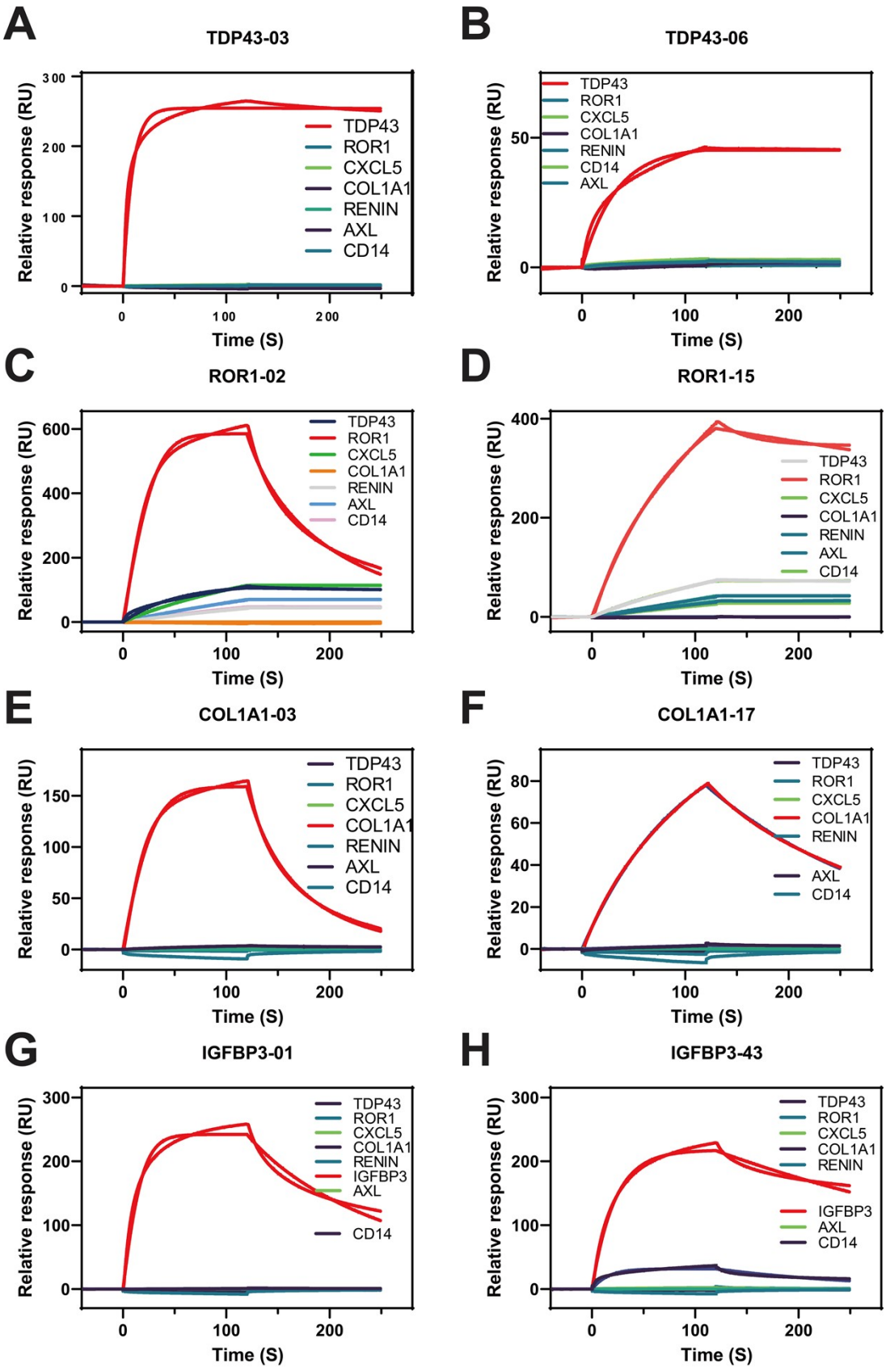


Figure S7 Other target protein qPCR data to monitor SELEX progress (A-E a) The number of molecules eluted in each round of positive SELEX (A-E b) Melting curves for each round of positive SELEX (A-E c) The Cq value of the last round of four positive SELEX washes and the positive and counter SELEX elution (A-E d) Retention rate calculated by Cq value for positive and counter SELEX (F&G a) The number of molecules eluted in last round of SELEX, +Round means positive SELEX, -Round means counter SELEX (F&G b) Melting curves for each round of counter SELEX (F&G c) The Cq value of the last round of four counter SELEX washes and the positive and counter SELEX elution (F&G d) Retention rate calculated by Cq value for positive and counter SELEX

Characterization of aptamer specificity by SPR



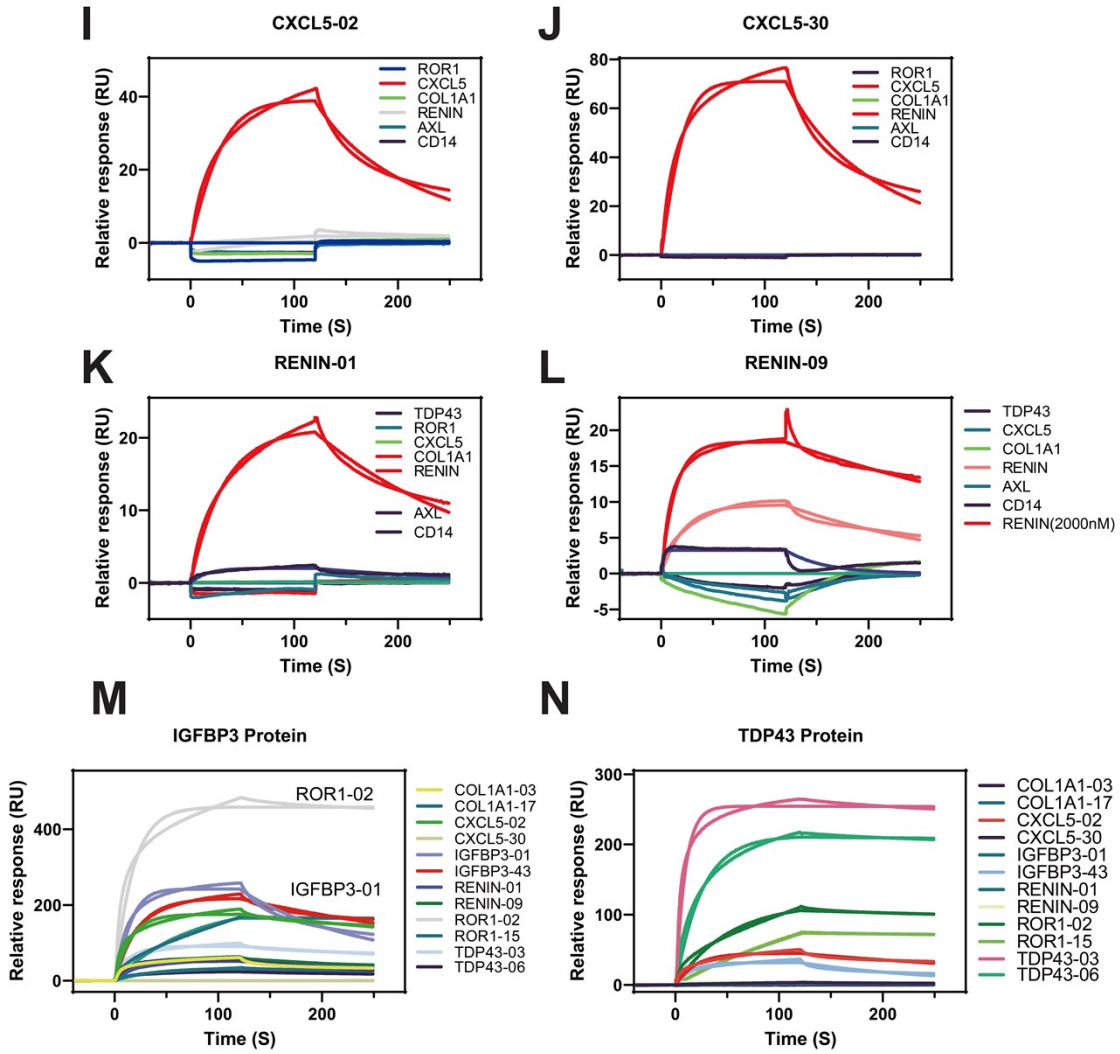


Figure S8 Characterization of aptamer specificity by SPR (A) The aptamer TDP43-03 binds to seven proteins at the same concentration (B) The aptamer TDP43-06 binds to seven proteins at the same concentration (C) The aptamer ROR1-02 binds to seven proteins at the same concentration (D) The aptamer ROR1-15 binds to seven proteins at the same concentration (E) The aptamer COL1A1-03 binds to seven proteins at the same concentration (F) The aptamer COL1A1-17 binds to seven proteins at the same concentration (G) The aptamer IGFBP3-01 binds to eight proteins at the same concentration (H) The aptamer IGFBP3-43 binds to eight proteins at the same concentration (I) The aptamer CXCL5-02 binds to six proteins at the same concentration (J) The aptamer CXCL5-30 binds to six proteins at the same concentration (K) The aptamer RENIN-01 binds to eight proteins at the same concentration (L) The aptamer RENIN-09 binds to eight proteins at the same concentration (M) Adsorption of IGFBP3 protein to each aptamer (N) Adsorption of TDP43 protein to each aptamer

Supplemental Tables

Table S1. Semi-automatic SELEX adjusts the stringency each round

SELEX Round ↓

ROR1 (lib10)	Library input concentration (pM)	HsDNA concentration (mg/ml)	BSA concentration (mg/ml)	His concentration (mg/ml)	Positive Beads(μl)	Counter Beads(μl)	Counter SELEX Time	Positive SELEX Time	Tween concentration
1	1400	0	0	0.05	50	50		1h	2.5‰
2	100	0	0	0.05	50	50	1h	1h	2.5‰
3	80	0.1	0.1	0.1	30	50	1h	1h	2.5‰
4	80	0.1	0.1	0.1	30	50	1h	50min	2.5‰

RENIN (lib24)	Library input concentration (pM)	HsDNA concentration (mg/ml)	BSA concentration (mg/ml)	His concentration (mg/ml)	Positive Beads(μl)	Counter Beads(μl)	Counter SELEX Time	Positive SELEX Time	Tween concentration
1	1400	0.05	0.05	0.05	80	0		1h	2.5‰
2	100	0.05	0.05	0.1	50	50	1	1	2.5‰
3	100	0.1	0.1	0.2	40	60	1	1	2.5‰
4	80	0.1	0.1	0.2	40	50	1	50	2.5‰
5	50	0.5	0.5	0.5	25	60	1	50	2.5‰
6	40	0.5	0.5	0.5	20	50	1	40	2.5‰
7	30	0.5	0.5	0.5	15	50	1	40	2.5‰

COL1A1 (lib13)	Library input concentration (pM)	HsDNA concentration (mg/ml)	BSA concentration (mg/ml)	His concentration (mg/ml)	Positive Beads(μl)	Counter Beads(μl)	Counter SELEX Time	Positive SELEX Time	Tween concentration	Add serum
1	1400	0	0	0.05	80	0		1h	2.5‰	
2	91	0.05	0.05	0.075	40	50	1h	1h	2.5‰	
3	100	0.1	0.1	0.1	40	50	1h	1h	2.5‰	
4	70	0.1	0.1	0.1	30	50	1h	50min	2.5‰	
5	60	0.1	0.1	0.1	30	50	1h	50min	2.5‰	
6	50	0.1	0.1	0.1	30	50	1h	50min	2.5‰	1%

CXCL5 (lib24)	Library input concentration (pM)	HsDNA concentration (mg/ml)	BSA concentration (mg/ml)	His concentration (mg/ml)	Positive Beads(μl)	Counter Beads(μl)	Counter SELEX Time	Positive SELEX Time	Tween concentration	Add serum
1	1400	0	0	0.05	80	0		1h	2.5‰	
2	75	0.05	0.05	0.075	40	50	1h	1h	2.5‰	
3	100	0.1	0.1	0.1	40	50	1h	1h	2.5‰	
4	70	0.1	0.1	0.1	30	50	1h	50min	2.5‰	
5	60	0.1	0.1	0.1	30	50	1h	50min	2.5‰	
6	60	0.1	0.1	0.2	30	50	1h	50min	2.5‰	
7	50	0.1	0.1	0.2	30	50	1h	50min	2.5‰	
8	50	0.1	0.1	0.2	30	80	1h	50min	1‰	1%

IGFBP3 (lib18)	Library input concentration (pM)	HsDNA concentration (mg/ml)	BSA concentration (mg/ml)	His concentration (mg/ml)	Positive Beads(μl)	Counter Beads(μl)	Counter SELEX Time	Positive SELEX Time	Tween concentration	Add serum
1	1400	0	0	0.05	80	0		1h	2.5‰	
2	100	0.05	0.05	0.075	40	50	1h	1h	2.5‰	
3	78	0.1	0.1	0.1	40	50	1h	1h	2.5‰	
4	70	0.1	0.1	0.1	30	50	1h	50min	2.5‰	
5	60	0.1	0.1	0.1	30	50	1h	50min	2.5‰	
6	50	0.1	0.1	0.1	30	50	1h	50min	2.5‰	
7	50	0.1	0.1	0.1	30	50	1h	50min	1‰	1%

TDP-43 (lib10)	Library input concentration (pM)	HsDNA concentration (mg/ml)	Na^+ concentration (mM)	BSA concentration (mg/ml)	His concentration (mg/ml)	Positive Beads(μl)	Counter Beads(μl)	Counter SELEX Time	Positive SELEX Time	Tween concentration
1	1400	0.1	0	0	0.05	100			1h	2.5‰
2	100	0.1	0	0.05	0.1	50	50	1h	1h	2.5‰
3	50	0.1	0	0.1	0.2	20	50	1h	1h	2.5‰
4	30	0.1	200	0.1	0.2	20	50	1h	50min	2.5‰
5	30	0.1	200	0.1	0.4	10	100	1h	50min	2.5‰
6	30	0.2	200	0.1	0.4	10	50	1h	50min	2.5‰
7	30	0.1	200	0.2	0.4	10	50	1h	50min	2.5‰
8	30	0.2	200	0.2	0.4	10	50	1h	50min	1‰

Table S2. Sequences of the library and primers

Name	Library and Primers (5' to 3')
library10	ATTGGCACTCCACGCATAGG-$(N)_{36}$-CCTATGCGTGCTACCGTGAA
library13	TTCAGCACTCCACGCATAGC-$(N)_{36}$-CCTATGCGTGCTACCGTGAA
library18	TCCAGCACTCCACGCATAAC-$(N)_{36}$-GTTATGCGTGCTACCGTGAA
library24	GCACGGACACAAGAACAAAG-$(N)_{36}$-CTTGTGCTGCCTTTGTTCTG
L10-S1	ATTGGCACTCCACGCATAGG
L10-A2	TTCACGGTAGCACGCATAGG
L10-FAM-S1	FAM-ATTGGCACTCCACGCATAGG
L10-Biotin-A2	Biotin-TTCACGGTAGCACGCATAGG
L10-polyA-A2	AAAAAAAAAAAAAAAAAAA-spacer18-TTCACGGTAGCACGCATAGG
L13-S1	TTCAGCACTCCACGCATAGC
L13-A2	TTCACGGTAGCACGCATAGG
L13-FAM-S1	FAM-TTCAGCACTCCACGCATAGC
L13-Biotin-A2	Biotin-TTCACGGTAGCACGCATAGG
L13-polyA-A2	AAAAAAAAAAAAAAAAAAA-spacer18-TTCACGGTAGCACGCATAGG
L18-S1	TCCAGCACTCCACGCATAAC
L18-A2	TTCACGGTAGCACGCATAAC
L18-FAM-S1	FAM-TCCAGCACTCCACGCATAAC
L18-Biotin-A2	Biotin-TTCACGGTAGCACGCATAAC
L18-polyA-A2	AAAAAAAAAAAAAAAAAAA-spacer18-TTCACGGTAGCACGCATAAC
L24-S1	GCACGGACACAAGAACAAAG
L24-A2	CAGAACAAAGGCAGCACAAG
L24-FAM-S1	FAM-GCACGGACACAAGAACAAAG
L24-Biotin-A2	Biotin-CAGAACAAAGGCAGCACAAG
L24-polyA-A2	AAAAAAAAAAAAAAAAAAA-spacer18-CAGAACAAAGGCAGCACAAG

Table S3. Target protein information used by SELEX

Protein name	Uniport	Isoelectric point predict	Molecular weight(kDa) predict	Source
TDP-43	Q13148	6.38	17.3	laboratory
ROR1	Q01973	6.42	43.5	Sino Biological
CXCL5	P42830	8.81	8.4	PTM Bio
COL1A1	P02452	4.19	14.2	PTM Bio
RENIN	P00797	6.13	42.3	PTM Bio
IGFBP3	P17936	8.76	28.8	PTM Bio
AXL	P30530	4.54	20.3	PTM Bio
CD14	P08571	5.44	35.8	PTM Bio

A variety of tumor or disease-related proteins were selected for SELEX, including TDP-43, ROR1, CXCL5, COL1A1, RENIN, IGFBP3, AXL, and CD14. TDP-43 protein is related to ALS [1], and ROR1 has not only been studied in ADC-related work in recent years, but also can mediate non-canonical Wnt signaling pathways (non-canonical Wnt pathways) signal transmission, It plays an important role in various physiological processes, especially Wnt5a[2, 3]. Both COL1A1 and CXCL5 are associated with Metastasis of cancer cells[4-7]. The level of IGFBP-3 was negatively correlated with the occurrence of cancer[8]. The study found that the tyrosine protein kinase receptor AXL can bind to the N-terminal domain (NTD) of the SARS-CoV-2 S protein, and overexpression of AXL in HEK293T species can enhance the ability of the virus to infect cells[9-11]. CD14 is a granulocyte differentiation antigen preferentially expressed on monocytes, macrophages and activated granulocytes[12]. Renin, also known as REN and angiotensinogenase, is a circulating enzyme involved in the human renin-angiotensin system (RAS) and plays an important role in elevated arterial blood pressure and increased renal sodium retention[13-16].

Table S4. Affinity of aptamers obtained by SPR characterization

Name	Sequence（5‘ to 3’）	Length（nt）	KD（M）
TDP-43 03	ATTGGCACTCCACGCATAGGCCAGTGTGCGGATAGTCGCCCGGCCGTCTGAGGGAACCTATGCGTGCTACCGTGAA	76	1.15E-09
TDP-43 06	ATTGGCACTCCACGCATAGGCTAGCACCCGAGAAGTGGTTCAGACGTCTGAGTGGACCTATGCGTGCTACCGTGAA	76	3.90E-9
ROR1 02	ATTGGCACTCCACGCATAGGAGGGCTCGTGGTTTACAGGGGGGAGAGGCGGAGGGGCCTATGCGTGCTACCGTGAA	76	4.12E-07
ROR1 15	ATTGGCACTCCACGCATAGGACGGGCGGAGGGGATTTACGGCTGCTCGGGTTGGGTCCTATGCGTGCTACCGTGAA	76	1.37E-08
COL1A1 03	TTCAGCACTCCACGCATAGCTTCCCTTAGGGGGGGCAGCTTATGGGAGGGAGGAAGCCTATGCGTGCTACCGTGAA	76	6.23E-07
COL1A1 17	TTCAGCACTCCACGCATAGCTTCCCTTAGGGGGGGCAGTTTATGGGAGGGAGGAAGCCTATGCGTGCTACCGTGAA	76	1.23E-06
IGFBP3 01	TCCAGCACTCCACGCATAACTGGTGGGTCAGGCGGTATCGGAGTTGTTCTAAGGGTGTTATGCGTGCTACCGTGAA	76	6.65E-08
IGFBP3 43	TCCAGCACTCCACGCATAACCTGGTGGTGGGTCATGGGAGGGTGGAGGGGGAATAGGTTATGCGTGCTACCGTGAA	76	5.77E-08
CXCL5 02	GCACGGACACAAGAACAAAGGCACGTTAGATGGGTGGTGGGGGCGGGTTGAGGATACTTGTGCTGCCTTTGTTCTG	76	2.09E-07
CXCL5 30	GCACGGACACAAGAACAAAGGCACGTTAGATGGGTGGTGGGGGTGGGTTGAGGATACTTGTGCTGCCTTTGTTCTG	76	1.08E-07
RENIN-01	GCACGGACACAAGAACAAAGTGGTTGGTTTGGTTGGATAAGCATAAAGGCGATTGACTTGTGCTGCCTTTGTTCTG	76	8.89E-8
RENIN-09	GCACGGACACAAGAACAAAGTGGTTGGTTTGGTTTGGTTTTAAGCACAAGAGCGGGCTTGTGCTGCCTTTGTTCTG	76	5.67E-8

Table S5. CQ values obtained from four library dilutions at four concentrations

lib18	Initial concentration (pM)	Final concentration（pM）	Cq Value	log Initial concentration
	10000	1000	4.24	3
	1000	100	6.76	2
	100	10	10.01	1
	10	1	13	0

lib13	Initial concentration (pM)	Final concentration（pM）	Cq Value	log Initial concentration
	10000	1000	4.34	3
	1000	100	6.6	2
	100	10	9.75	1
	10	1	13.25	0

lib10	Initial concentration (pM)	Final concentration（pM）	Cq Value	log Initial concentration
	10000	1000	3.92	3
	1000	100	6.21	2
	100	10	8.91	1
	10	1	11.13	0

lib24	Initial concentration (pM)	Final concentration（pM）	Cq Value	log Initial concentration
	10000	1000	4.09	3
	1000	100	6.41	2
	100	10	9	1
	10	1	12.88	0

The table is the Cq data obtained by qPCR of Fig S5 converted to log concentration.

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