Supporting Information

Rapid Identification of Specific DNA Aptamers Precisely Targeting CD33 Positive Leukemia Cells through Paired Cell Based Approach

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Supplementary Figure Legends

Fig.S1. Classical cell-based and protein-based systematic evolution of ligands by exponetial enrichment (SELEX) approach

A) Single stranded DNA pool containing different random sequences of 30-100n.t. (>10^{15} variant sequences) is incubated with a targeted positive live cells. Then, variants with the desired binding activity are recovered, followed by a negative selection step and then amplification of the enriched library by PCR. Employing this enriched PCR product, the single-stranded DNA pool is regenerated by template-strand removal. Typically, this process is repeated for several to more than 20 rounds for the identification of aptamers. **B)** Schematic representation of CD33 targeted aptamers selection by protein-based SELEX (for details please see experimental section).

Fig. S2. Binding affinity of ssDNA pool and selected aptamers to CD33 negative cells

Representative ssDNA pool from each selection round (6~8) by CD33 recombinant Protein-Based SELEX (**A**) or ssDNA pool from each selection round (1~3) by paired cell based approach (**B**) were incubated with CD33 negative cells (Jurkat cells) and resultant cell binding was examined by flow cytometry.

Fig. S3. The binding affinities of aptamer S30 and S28 to CD33 positive cells

Cell binding of FITC-labeled S30 **(A)** and S28 **(B)** aptamer was determined by flow cytometry after incubation with CD33 positive HL-60 cells at 37℃ for 30min. **C**) The free energies of all selected aptamers (including S15, 20, 23 ,28 and 30) were calculated by IDT-DNA. Data are representative of at least three independent experiments.

Fig.S4. Two-dimensional structure of S30 and its truncated aptamers

A) Two-dimensional secondary structure of S30 and its truncated aptamers S30-T1 and S30-T2 by S30 were predicated by IDT analysis. **B**) The free energies of all aptamers were calculated from IDT-DNA.

Fig.S5. Determination of binding affinity of S30 with CD33 antigen by computational method and confocal microscopy

A) Crystal structure of CD33 was simulated by *ab initio* method, as described in Method and Materials. CD33 antigen contains two major domains, namely, endo- and ectodomains, especially ectodomain further separated to C2 and V domains. **B**) Three-dimensional structures of selected DNA aptamers S15, 20, 23 and 30 were also further simulated by *ab initio* method and found that they contains 2 or 3 stem loops. **C**) In particular, aptamer S30 was found to specifically bind to C2 domain of CD33 by protein-DNA complex molecular dynamics simulation. Moreover, RFP-CD33 (red) transfected-293T cells (**D**) and CD33 positive HL-60 cells (**E**) were incubated with FITC-labeled S30 (green) at 4℃ for 30 min, and then determined by laser scanning confocal microscopy. Nuclei were stained with DAPI (blue).

Fig.S6. Isolation of CD33 positive cells from mixture of CD33 positive and negative cell population by S30

A) Separation procedure for isolating CD33 positive cells from mixture of CD33 positive (HL-60) and negative cell (Jurkat) population by S30. **B**) Each CD33 negative ($5x10^5$) and CD33 positive cells ($5x10^5$), or mixture of these two cells ($5x10^5$) were incubated with FITC-labeled S30 at 37℃ for 30min. After incubation, mixed cell populations were determined by flow cytometry. Mixed positive and negative cells without S30 were used as blank. Blue color indicates the binding of S30 with CD33 positive cells.

Fig.S7. Internalization and release of S30-T1-Dox in CD33 negative cells

A) CD33 negative Jurkat cells were incubated with 1μM of S30-T1-Dox conjugates at 37℃ for 3h, and washed twice with PBS and then cultured in drug free fresh medium for up to 24h. Internalization and release of Dox were confirmed by confocal microscope

Fig S1

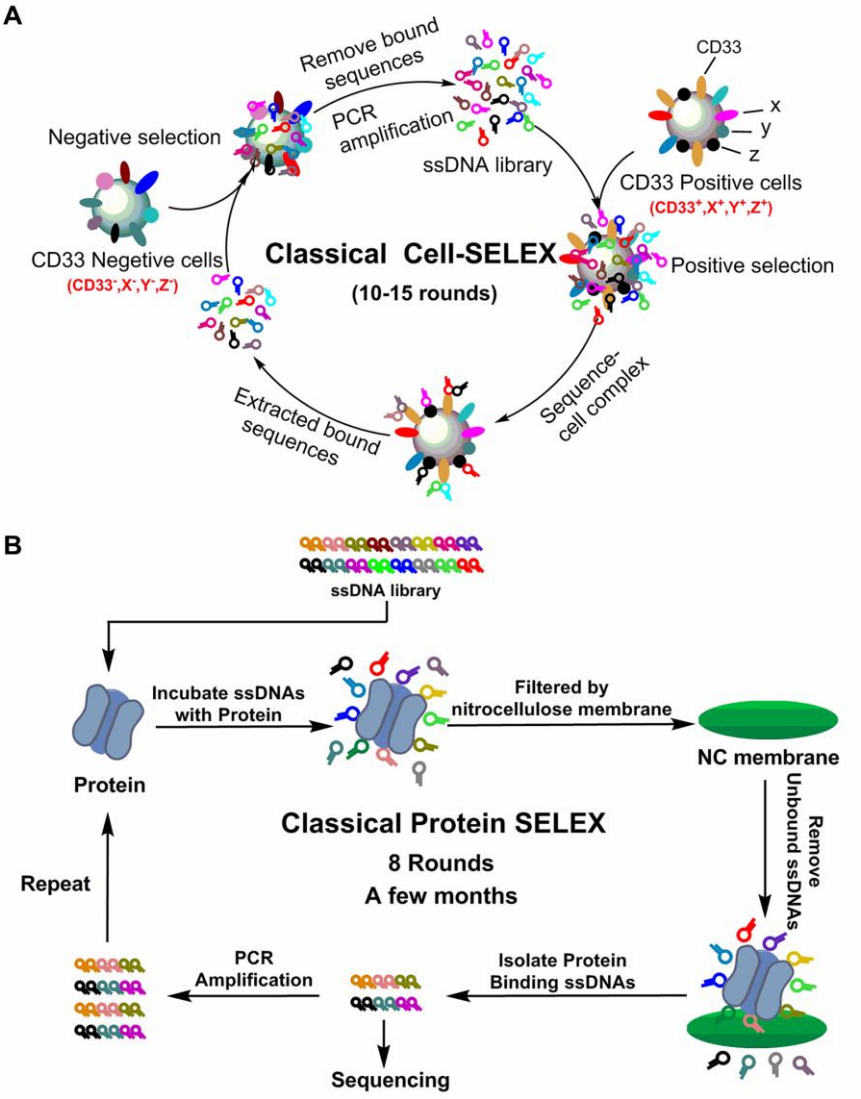

A
Remove bound sequences
PCR amplification
ssDNA library
CD33
x
y
z
CD33 Positive cells
(CD33+,X+,Y+,Z+)
Negative selection
CD33 Negetive cells
(CD33-,X-,Y-,Z-)
Classical Cell-SELEX
(10-15 rounds)
Positive selection
Sequence-cell complex
Extracted bound sequences
B
ssDNA library
Incubate ssDNAs with Protein
Filtered by nitrocellulose membrane
Protein
NC membrane
Classical Protein SELEX
8 Rounds
A few months
Remove Unbound ssDNAs
Repeat
PCR Amplification
Isolate Protein Binding ssDNAs
Sequencing

Fig S2

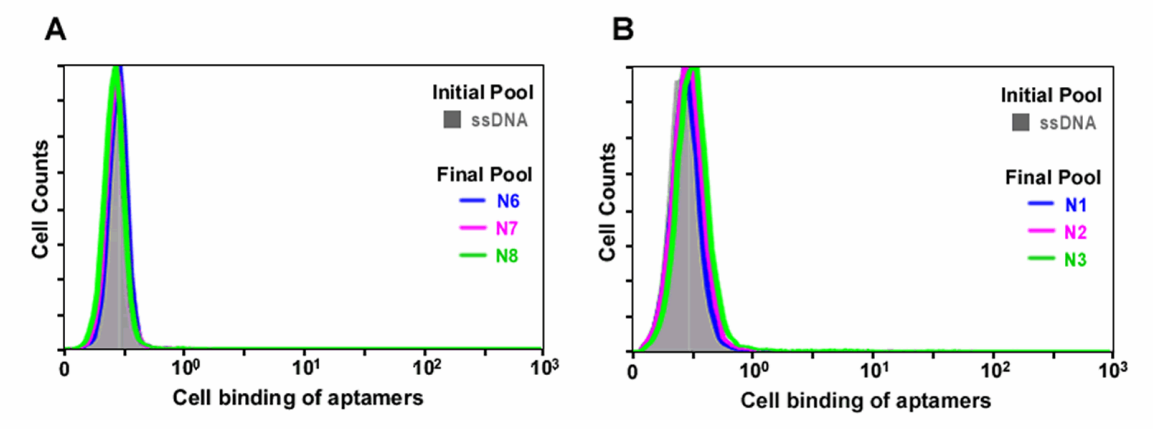
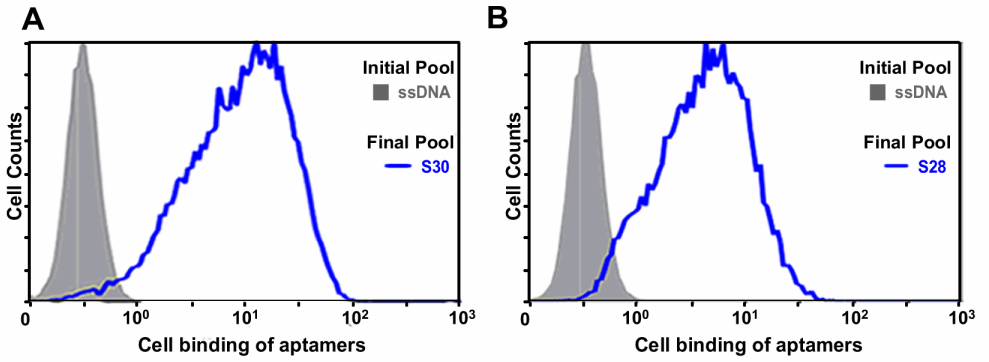

A
Cell Counts
Initial Pool
ssDNA
Final Pool
N6
N7
N8
0
10^0
10^1
10^2
10^3
Cell binding of aptamers
B
Cell Counts
Initial Pool
ssDNA
Final Pool
N1
N2
N3
0
10^0
10^1
10^2
10^3
Cell binding of aptamers

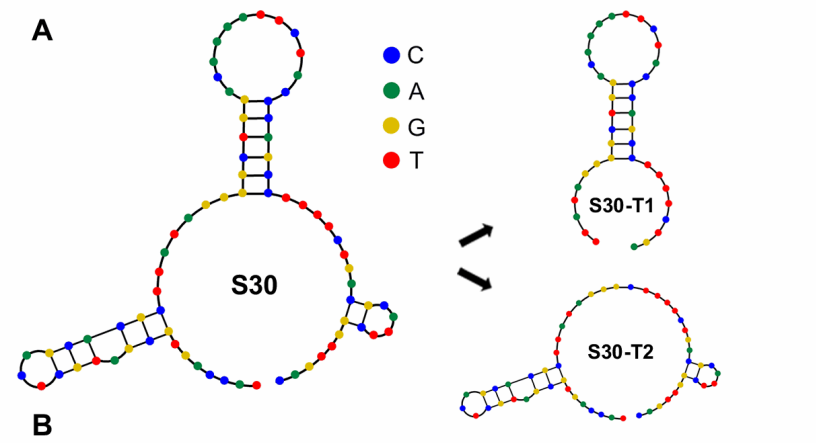
Fig S3



C

Name	Sequence	ΔG(kcal/mol)
S15	TACCAGTGCGATGCTCAGGATGCTCCGCAGGAGTCCGACCTGCCCGC AGCCTTCGAACCTGACGCATTCGGTTGAC	-9.46
S20	TACCAGTGCGATGCTCAGCCACACTGGCATGGAACGGGACTCAAGA GAAAGGCTCTGCCTGACGCATTCGGTTGAC	-9.05
S23	TACCAGTGCGATGCTCAGGCTCCATCAACCGGTCGAAAGTTCGAGCG CCTTTAAAGTACTGACGCATTCGGTTGAC	-9.52
S28	TACCAGTGCGATGCTCAGGTCAATGGAGGGATTGCGAACCTCTGTG GCGTTTTCTCTGCTGACGCATTCGGTTGAC	-6.29
S30	TACCAGTGCGATGCTCAGCACGCTTATAGGGGCTGGACAAAATTCTA CCCAGCCTTTTCTGACGCATTCGGTTGAC	-9.84

Fig S4



Name	Sequence	ΔG(kcal/mol)
S30	TACCAGTGCGATGCTCAGCACGCTTATAGGGGCTGGACAAA ATTCTACCCAGCCTTTTCTGACGCATTCGGTTGAC	-9.84
S30-T1	TTATAGGGGCTGGACAAAATTCTACCCAGCCTTTTCTGA	-5.87
S30-T2	TACCAGTGCGATGCTCAGCACGCTTATAGGGCTTTTCTGACG CATTCGGTTGAC	-3.98

Fig S5

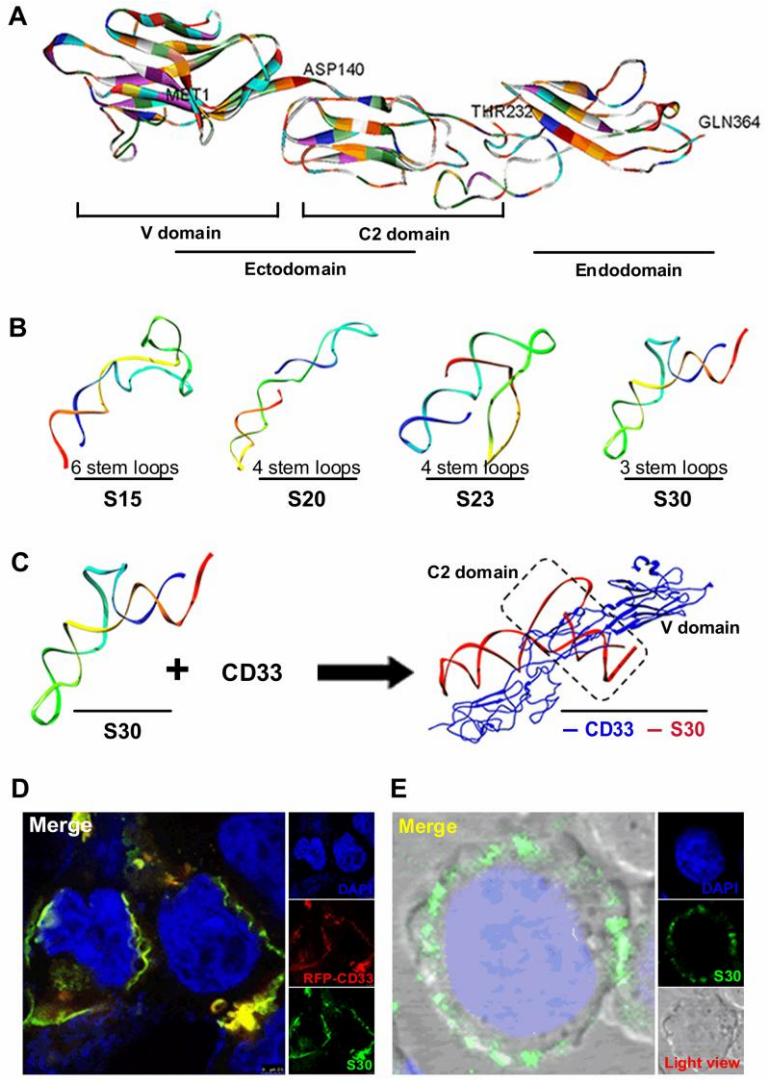

A
MET1
ASP140
THR232
GLN364
V domain
C2 domain
Ectodomain
Endodomain
B
6 stem loops
S15
4 stem loops
S20
4 stem loops
S23
3 stem loops
S30
C
S30
+
CD33
C2 domain
V domain
– CD33 – S30
D
Merge
DAPI
RFP-CD33
S30
E
Merge
DAPI
S30
Light view

Fig S6

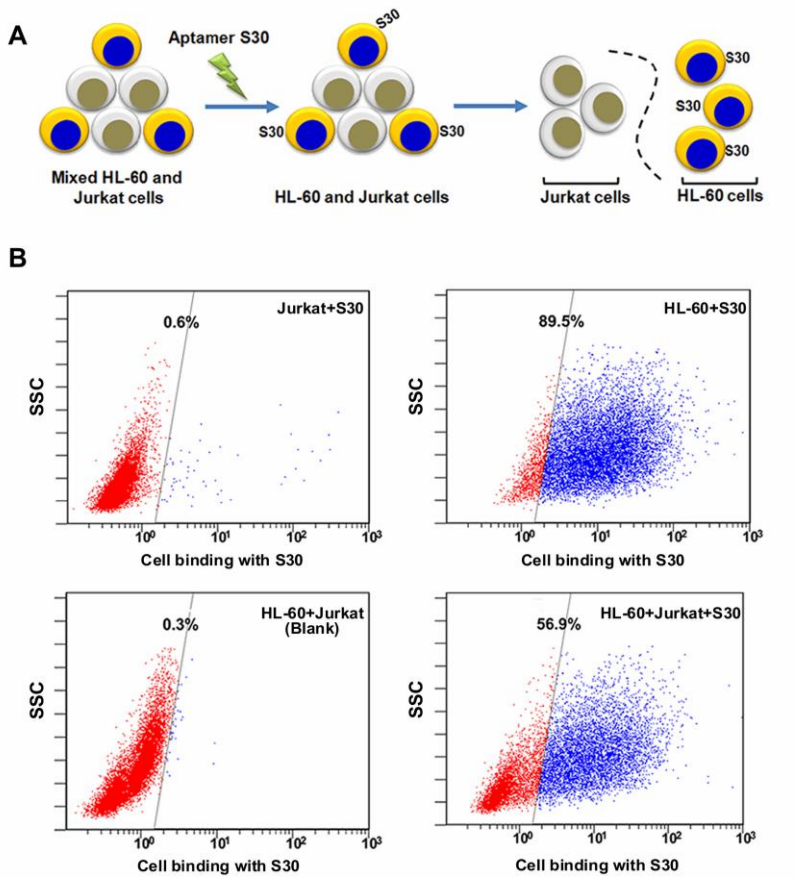

A
Aptamer S30
S30
S30
S30
S30
S30
S30
S30
Mixed HL-60 and Jurkat cells
HL-60 and Jurkat cells
Jurkat cells
HL-60 cells
B
Jurkat+S30
0.6%
HL-60+S30
89.5%
HL-60+Jurkat (Blank)
0.3%
HL-60+Jurkat+S30
56.9%
SSC
Cell binding with S30

Fig.S7