

Generalizable anchor aptamer strategy for loading nucleic acid therapeutics on exosomes

Gang Han¹, Yao Zhang¹, Li Zhong¹, Biaobiao Wang¹, Shuai Qiu¹, Jun Song¹, Caorui
Lin¹, Fangdi Zou², Jingqiao Wu¹, Huanan Yu¹, Chao Liang³, Ke Wen⁴, Yiqi Seow⁵,
HaiFang Yin^{1,6*}

APPENDIX

Contents: Appendix Figures S1 and S2, Appendix Table S1

Appendix Figure S1- Page 2

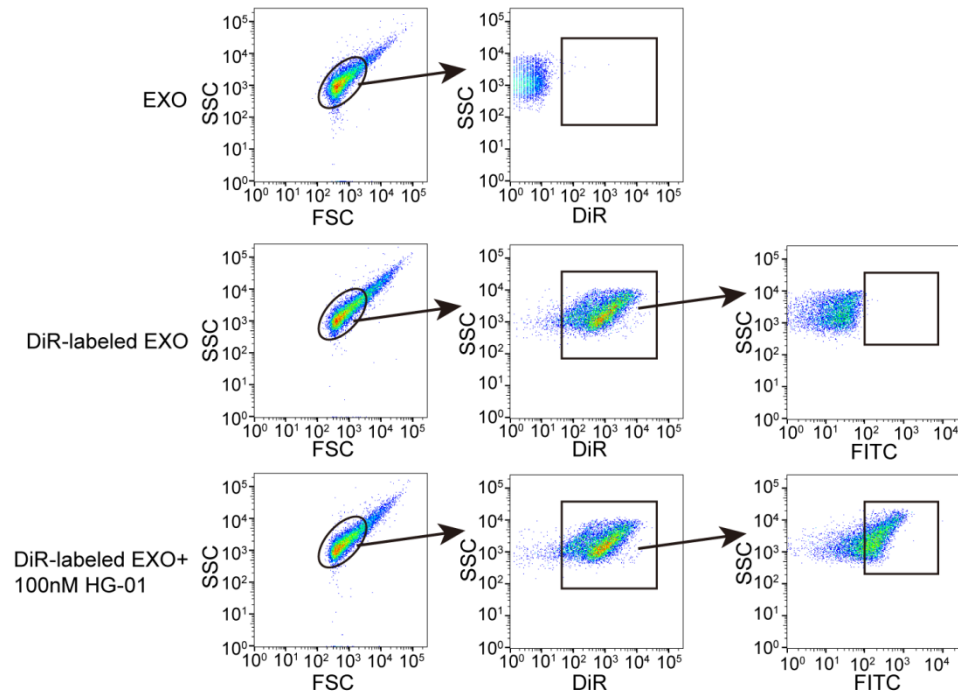
**Representative gating strategy for flow cytometric analysis of DiR- labeled
exosomes and DiR- and FITC-positive exosomes.**

Appendix Figure S2 - Page 2

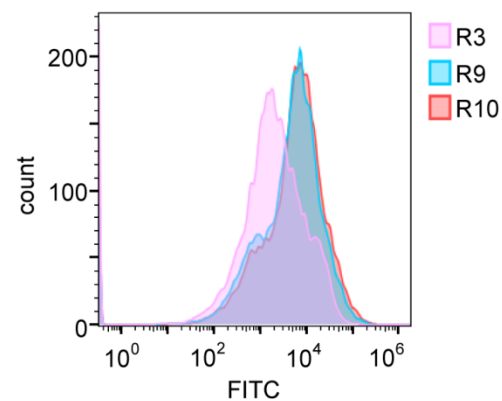
**Flow cytometric analysis of DNA aptamer enrichment for round 3, 9 and 10
during SELEX.**

Appendix Table S1 - Page 3-4

**Nomenclature and sequences for the DNA aptamer library, primers and tested
aptamers.**



Appendix Figure S1. Representative gating strategy for flow cytometric analysis of DiR-labeled exosomes and DiR- and FITC-positive exosomes. Exosomes were derived from differentiated C2C12 cells and labelled with DiR. HG-01 DNA aptamer was labelled with FITC.



Appendix Figure S2. Flow cytometric analysis of DNA aptamer enrichment for round 3, 9 and 10 during SELEX.

Appendix Table S1

Nomenclature	Sequence (from 5' to 3' end)
sDNA aptamer library	ATCCAGAGTGACGCAGCA-40N-TGGACACGGTGGCTTAGT
Forward Primer for ssDNA Pool	FITC-ATCCAGAGTGACGCAGCA
Reverse Primer for ssDNA Pool	biotin-ACTAAGCCACCGTGTCCA
HG-01	FITC- <u>ATCCAGAGTGACGCAGCAGGGGGTTTGGTTTGGC</u> GGGGTGGCTCCCCGGGGTTGGTTAT <u>TGGACACGGTGGCT</u> <u>TAGT</u>
HG-02	FITC- <u>ATCCAGAGTGACGCAGCAAATGGTTGGGCCGGAT</u> AGG TTGGTTG GATACGGTTTTGCT <u>TGGACACGGTG</u> <u>GCTTAGT</u>
HG-03	<u>ATCCAGAGTGACGCAGCAGGATTTGTGGGTGGGTGG</u> GCGGACCGCGAGTCGAACGTTCT <u>TGGACACGGTGGCT</u> <u>TAGT</u>
HG-04	<u>ATCCAGAGTGACGCAGCATAGGTTTGGGGTTGGAGGG</u> AGGTCGT TCGCGGATCTGGATT <u>TGGACACGGTGGC</u> <u>TTAGT</u>
HG-05	<u>ATCCAGAGTGACGCAGCAGAAAGTGCGGGGGACTGCTC</u> GGGATT GCGGATCGTTCTCGCT <u>TGGACACGGTGGCT</u> <u>TAGT</u>
HG-06:	<u>ATCCAGAGTGACGCAGCATGTGGGCGGGTGGGTGGTC</u> TTGCGGC ATCTCGGAGGCAGGT <u>TGGACACGGTGGC</u> <u>TTAGT</u>
HG-07:	<u>ATCCAGAGTGACGCAGCACTAGGTTGGAGTAGGTTTGG</u> TGTGCTGGTTATTTCACTCGT <u>TGGACACGGTGGCTTAGT</u>

HG-08:	<u>ATCCAGAGTGACGCAGCACCGGGGATGTAGGGGCAG</u> GGGTTCG AGTGAGGAGTATTAT <u>TGGACACGGTGGCTT</u> <u>AGT</u>
HG-09	FITC- <u>ATCCAGAGTGACGCAGCAGGGT</u> GGAACGAGAAG GCGGATCGCG CGGCCTGGACCAATGT <u>TGGACACGGTGG</u> <u>CTTAGT</u>
EAA	FITC/Cy5-GGGGGTTTGGTTTGGCGGGGTGGCTCCCCGG GGT TGGTTA
CD63 aptamer	FITC/Cy5-CACCCACCTCGCTCCCGTGACACTAATGCTA
EAA-NU172	FITC- <i>GGGGTTTGGTTTGGCGGGGTGGCTCCCCGGGGTTG</i> <i>GTTA</i> <u>TGGAC</u> CGCCTAGGTTGGGTAGGGTGGTGGCG
NU172	FITC-CGCCTAGGTTGGGTAGGGTGGTGGCG
EAA-linker	FITC- <i>GGGGTTTGGTTTGGCGGGGTGGCTCCCCGGGGTTG</i> <i>GTTA</i> <u>TGGACACGGTG</u>
linker-FIXa	FITC- <u>rCrArCrCrGrUrGrUrCrCrAr</u> ArUrGrGrGrGrArCrUrA rUrArCrCrGrCrGrUrArArUrGrCrUrGrCrCrUrCrCrCrArU
EAA-PMOC	FITC- <i>GGGGGTTTGGTTTGGCGGGGTGGCTCCCCGGGGTT</i> <i>GGTTAATTCAGGTAAGCCGAGGTTTGGCC</i>
PMO	FITC-GGCCAAACCTCGGCTTACCTGAAAT

Appendix Table S1. Nomenclature and sequences for the DNA aptamer library, primers and tested aptamers. Underlined letters refer to the 18-base linker. Italic letters refer to EAA sequences and underlined bold letters represent linker sequences for different aptamers. r means RNA. Bold letters represent the sequences complementary to PMO. FITC, Cy5 and Biotin refer to the dyes used.