Supplementary information

Development of oligonucleotide-based antagonists of Ebola virus protein 24 inhibiting its interaction with karyopherin alpha 1

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Material & Methods

- HPLC and MS analysis of modified aptamers

HPLC analysis was performed using ACQUITY UPLC H-Class system (Waters) with ACQUITY UPLC Oligonucleotide BEH C18 Column (1.7 μm, 2.1 mm × 100 mm, 50 °C) determined by 260 nm absorbance. The sample was eluted (0.5 mL/min) with buffer A (15 mM trietylamine, 400 mM hexafluoro-2-propanol prepared in water) and buffer B (15 mM trietylamine, 400 mM hexafluoro-2-propanol prepared in MeOH) with the following: 0 min 5% B, 4 min 40% B, 4.5 min 80% B, 9.5 min 80% B, 10 min 5% B, 13 min 5% B.

MS analysis was performed using Xevo G2-S QTof (Waters) (negative mode).

Table S1 Sequences of library, primers, and aptamers.

* : Underlined T are substituted for dU^{ad} by one primer PCR.

Name	Sequence
T1F	FAM-TCGCCTTGCCGGATCGCAGA(N30)TGGTCCGTGAGCCTGACACC
T2H	HEX-GGTGTCAGGCTCACGGACCA(N30)TCTGCGATCCGGCAAGGCGA
P1F	FAM-TCGCCTTGCCGGATCGCAGA
P1P	phosphate-TCGCCTTGCCGGATCGCAGA
P2P	phosphate-GGTGTCAGGCTCACGGACCA
P2H	HEX-GGTGTCAGGCTCACGGACCA
P2H_C3	HEX-AAAAAAAAAAAAAAA-C3-GGTGTCAGGCTCACGGACCA
VPNA-1	TCGCCTTGCCGGATCGCAGAGACCCTCTGGGCATCGCCGCTTAGGACGCGTGGTCCGTGAGCCTGACACC
VPNA-2	TCGCCTTGCCGGATCGCAGAGACCCTTGTTCTTTCGGCCATCGCTAACGGTGGTCCGTGAGCCTGACACC
VPNA-3	TCGCCTTGCCGGATCGCAGAGACCCTCTTTAACCATTGTCCGTGGAGGACTGGTCCGTGAGCCTGACACC
VPNA-4	TCGCCTTGCCGGATCGCAGAGACCCTTTTTGCCACAGGGTGGCCCCCGGCTGGTCCGTGAGCCTGACACC
VPNA-5	TCGCCTTGCCGGATCGCAGAGACCCTTCACTCACGACCTGAAGCACACTTTGGTCCGTGAGCCTGACACC
VPKS-1*	TCGCCTTGCCGGATCGCAGACACGCGAGACTTCCCACTAGTGTAGTTGTTTGGTCCGTGAGCCTGACACC
VPKS-2*	TCGCCTTGCCGGATCGCAGAGACGCCCTACGTCAAATCGACCACGTCGCGTGGTCCGTGAGCCTGACACC
VPKS-4*	TCGCCTTGCCGGATCGCAGAGTAGCCTACGTAGGTGAGATCGAACACTGATGGTCCGTGAGCCTGACACC
VPKS-5*	TCGCCTTGCCGGATCGCAGAAGCGTGTAAACTACGTCGCAGCGAACACCCTGGTCCGTGAGCCTGACACC
VPKS-6*	TCGCCTTGCCGGATCGCAGACCGTCTCCGAGCTCCCTAATTGATGGACCATGGTCCGTGAGCCTGACACC

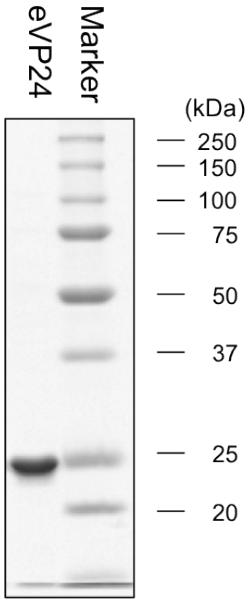


Figure S1 Purification confirmation of recombinant eVP24. Purified recombinant eVP24 was subjected to SDS-polyacrylamide gel electrophoresis (12% acrylamide). After electrophoresis, gel was stained with coomassie brilliant blue (CBB).

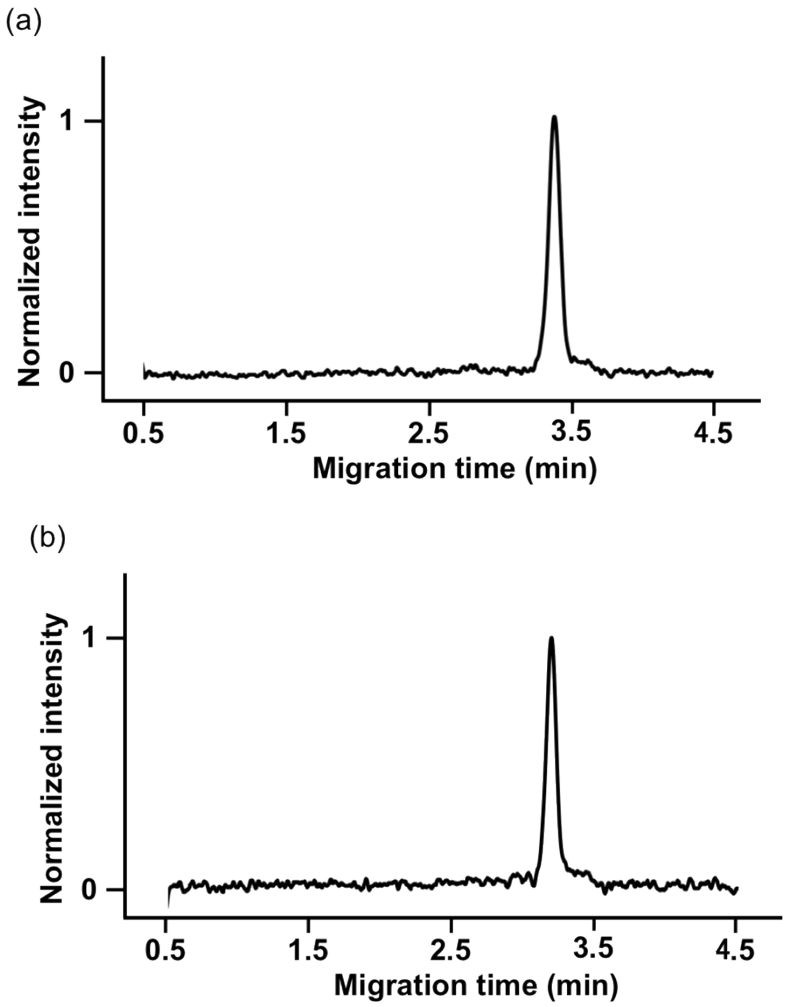
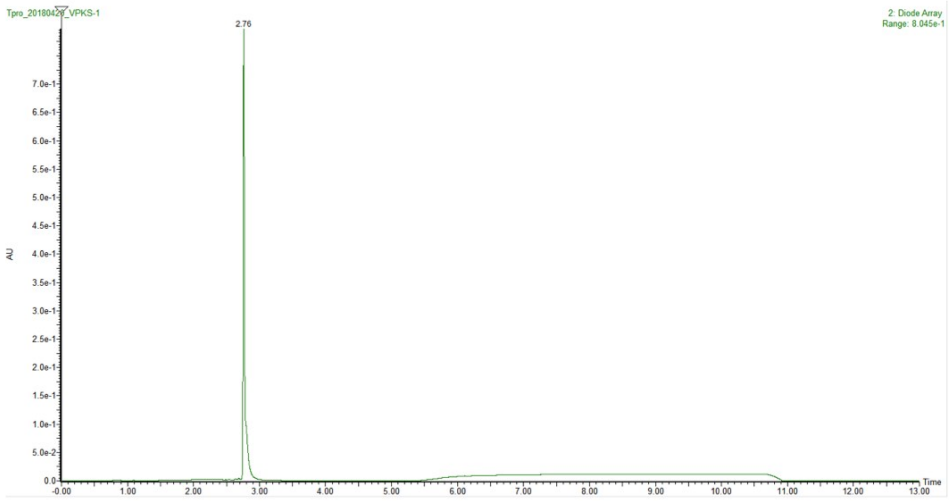
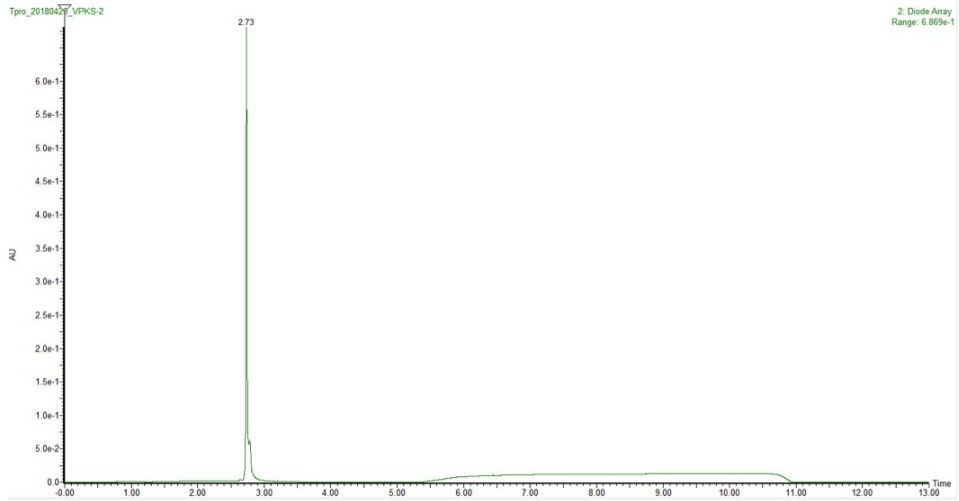


Figure S2 Confirmation of specificity of VPKS-2 and -5. The aptamers (1 nM)/FLAG-KPNA1 (4 nM) mixture was subjected to CE. (a) Electropherogram obtained for VPKS-2. (b) Electropherograme obtained for VPKS-5.

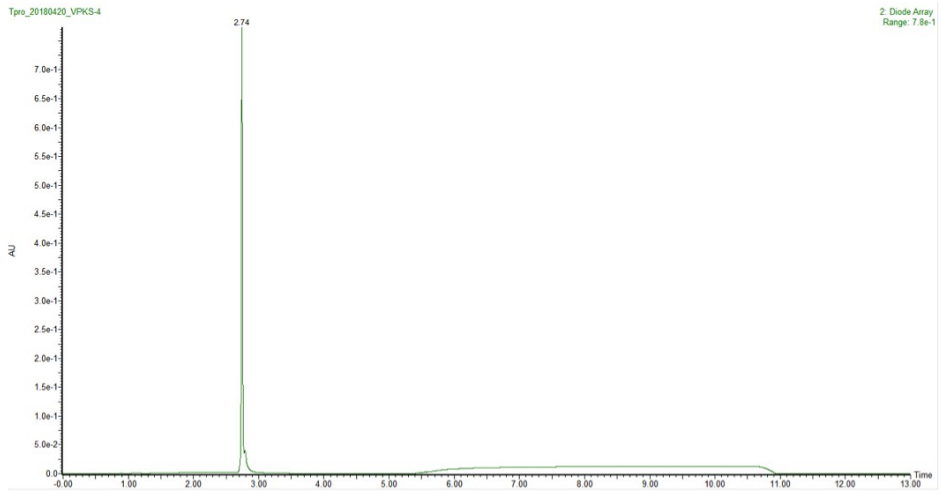
(a)


Tpro_20180420_VPKS-1
2.76
2: Diode Array
Range: 8.045e-1
AU
Time

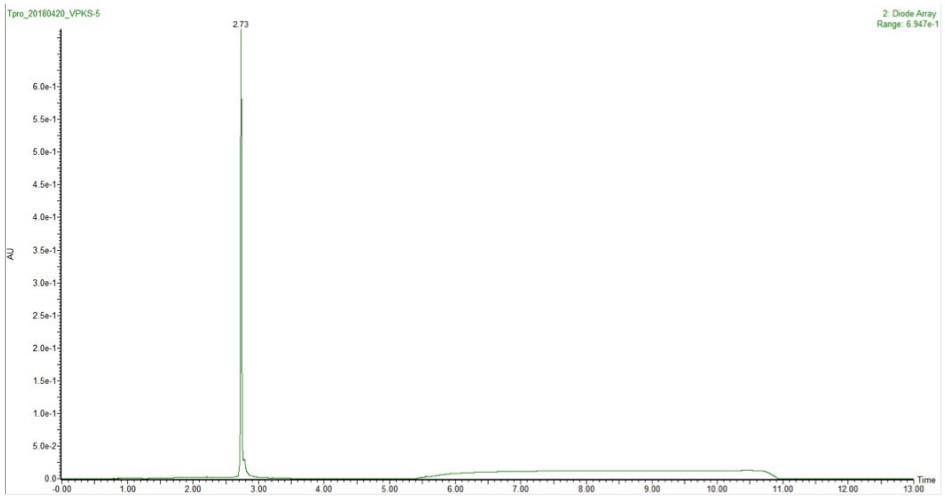
(b)


Tpro_20180420_VPKS-2
2.73
2: Diode Array
Range: 6.869e-1
AU
Time

(c)


Tpro_20180420_VPKS-4
2.74
2: Diode Array
Range: 7.8e-1
AU
Time

(d)



(e)

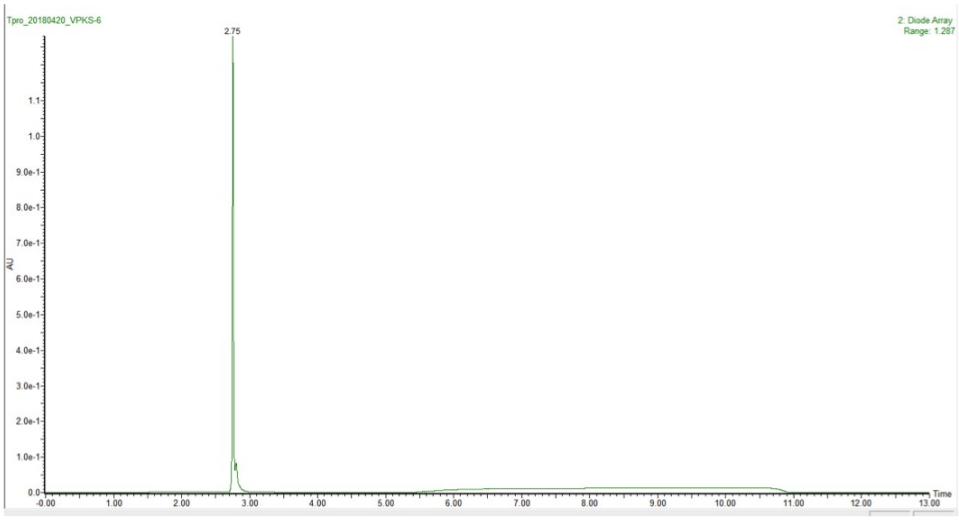
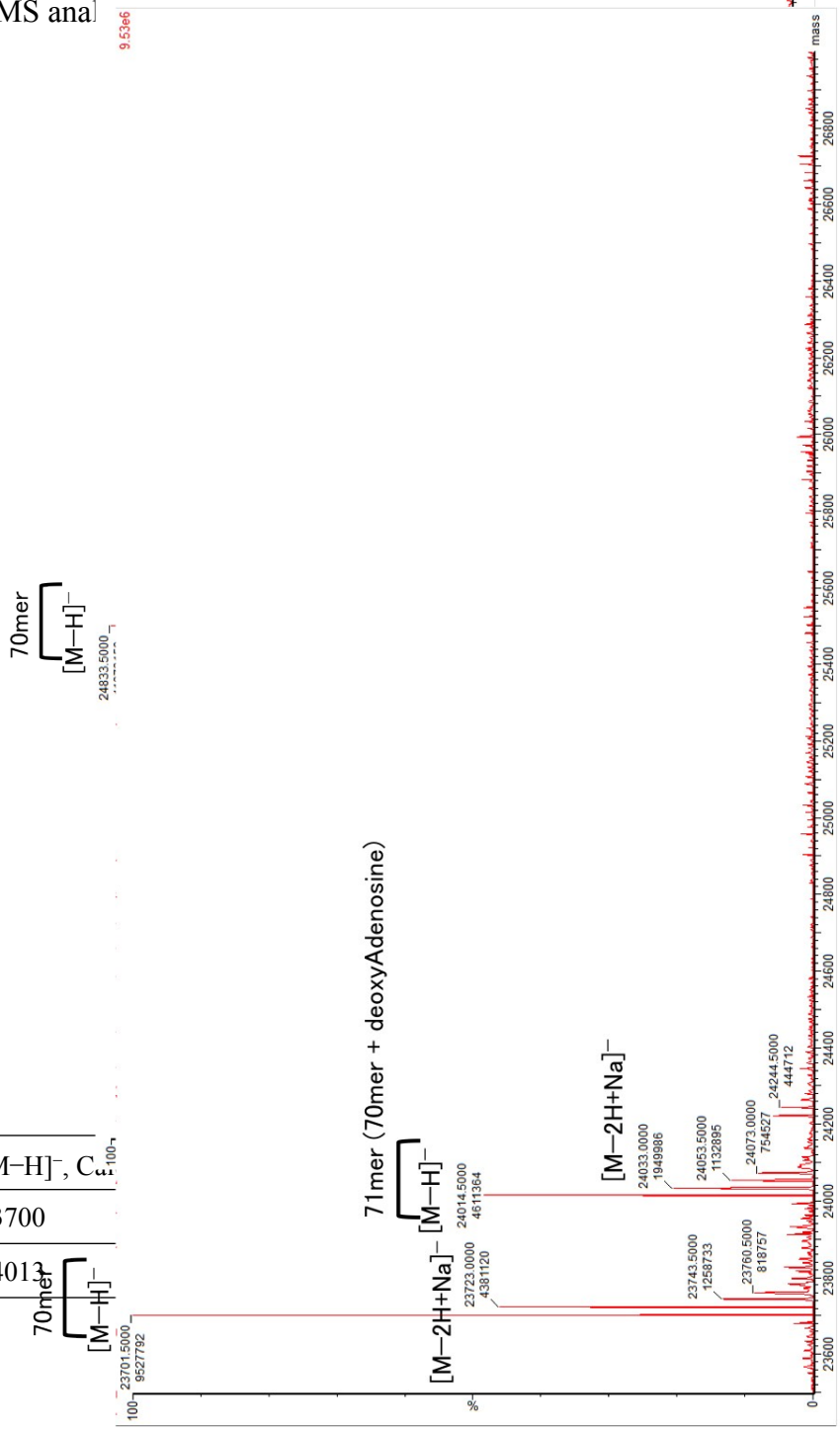


Figure S3 HPLC analysis of modified aptamers. (a) 5'-FAM-VPKS-1. Purity was estimated to be 81%. (b) 5'-FAM-VPKS-2. Purity was estimated to be 90%. (c) 5'-FAM-VPKS-4. Purity was estimated to be 94%. (d) 5'-FAM-VPKS-5. Purity was estimated to be 94%. (e) 5'-FAM-VPKS-6. Purity was estimated to be 92%.

Figure S4 MS analysis of 5’-FAM-VPKS-1

	[M−H]⁻, C	]Na]⁻, Found.
70mer	24832	
71mer	25145	

Figure S5 MS anal

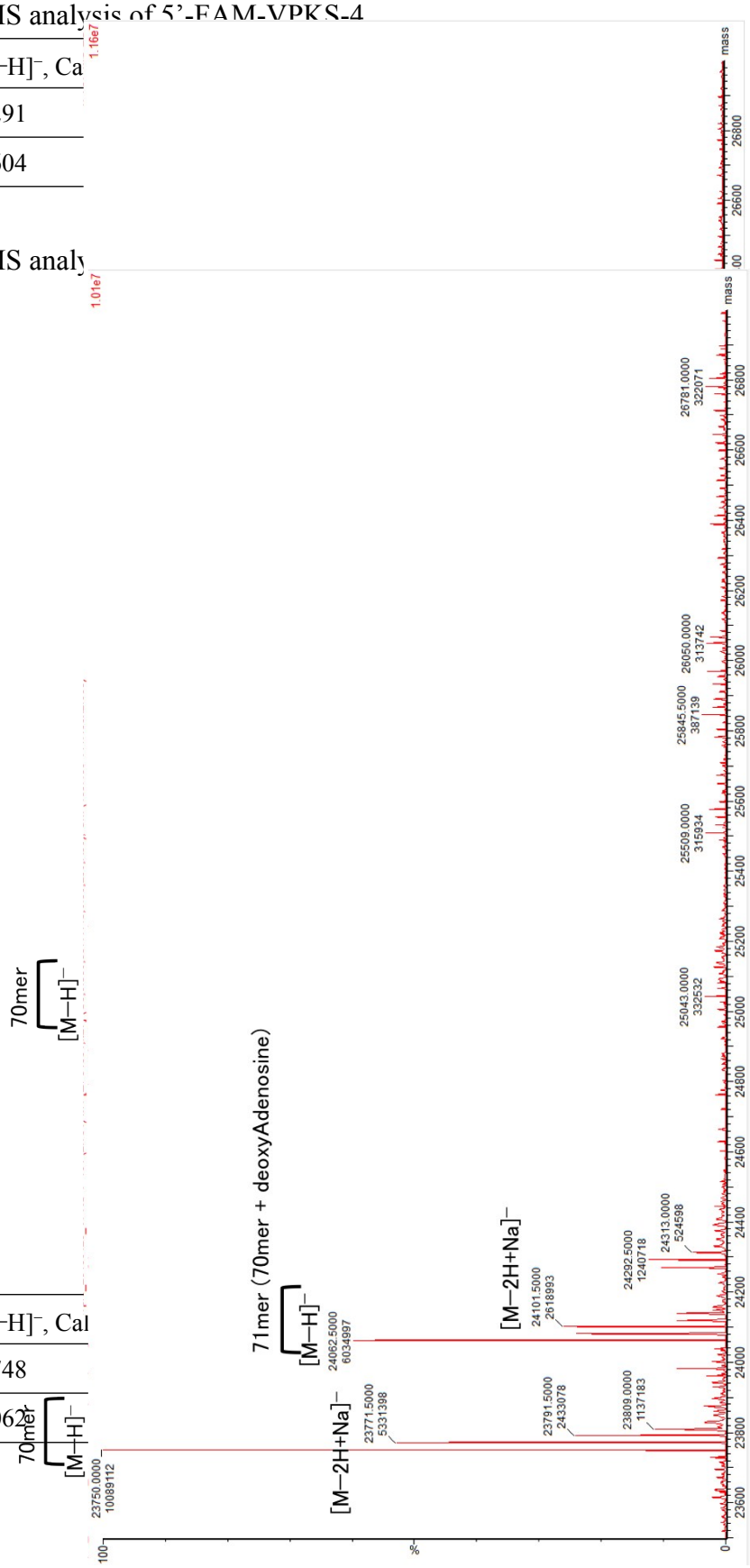


	[M−H]⁻, Ca	]⁻, Found.
70mer	23700	
71mer	24013	

Figure S6 MS analysis of 5’-FAM-VPKS-4

	[M−H]⁻, Ca	]⁻, Found.
70mer	24291	
71mer	24604	

Figure S7 MS analy



	[M−H]⁻, Cal	]⁻, Found.
70mer	23748	
71mer	24062	

Figure S8 MS analy

	[M−H]⁻, Ca	]⁻, Found.
70mer	24330	
71mer	24644