SUPPLEMENTARY DATA

Highly stable hexitol based XNA aptamers targeting the vascular endothelial growth factor

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SUPPLEMENTARY METHODS

Hexitol nucleoside triphosphate (hNTP) synthesis (1). The synthesis of hNTPs was performed according to literature procedures starting from the corresponding nucleosides (2, 3) using the Ludwig method (4). Specifically, the regioselective phosphorylation of the 6'-hydroxy group of the sugar moiety was carried out in one-pot using a solution of phosphoryl oxychloride in trimethylphosphate followed by the addition of tetrabutylammonium pyrophosphate. The resulting triphosphates were isolated by ion-exchange chromatography, purified by RP HPLC, and finally precipitated as sodium salts.

Oligonucleotide preparation. All oligonucleotides, commercial [Integrated DNA Technologies (IDT), BVBA, Belgium] or laboratory synthesized, used in this study (Table S1) were purified by 15% denaturing polyacrylamide-7 M urea gel electrophoresis [PAGE, 19:1 = acrylamide:bisacrylamide (w:w) in a 1× Tris-boric acid-EDTA buffer (TBE)] according to a standard procedure (5). The sequences were eluted from the gel using a diffusion buffer (Table S2) by shaking overnight at 37 °C. The gel was washed twice with water for 1 h under shaking at 37 °C; the resulting eluted solutions were combined, desalted on a NAP-25 column, and precipitated using 3% $LiClO_4$ in acetone. The oligonucleotides were quantified using a ClarioStar microplate reader (VIS plate, BMG Labtech, Isogen Life Science).

Biotinylation of the target protein. For the biotinylation step, the biotinylation reagent EZ-Link Sulfo-NHS-LC-Biotin was used according to the manufacturer's instructions. Samples of the reagent containing 3-fold molar excess to the target were prepared in 100 µL of 1× PBS buffer (Table S2). The reactions were incubated for 2 h on ice, and then for 30 min at RT. The resulting protein was desalted using Micro-Spin chromatography P-6 columns. The protein samples (taken before or after the biotinylation reaction and desalting) were analysed by 12.5% SDS-PAGE and were either Coomassie stained or subjected to Western Blot analysis. The samples were quantified by bicinchoninic acid (BCA) assay and NanoDrop. The level of biotin incorporation was estimated using the Pierce Biotin Quantitation Kit (HABA assay, ThermoFisher Scientific) according to the manufacturer's instructions (in PBS-K buffer, Table S2) and measured using a ClarioStar microplate reader. According to the HABA (4'-hydroxyazobenzene-2-carboxylic acid) assay, samples of bio-rVEGF$_{164}$ contained at least 2.7±0.2 biotin molecules per molecule of protein.

Western Blot analysis of the biotinylated samples. The Trans-Blot Turbo Blotting System (Bio-Rad) was used as western blotting transfer system. The low fluorescent PVDF membrane, filter papers, and transfer buffer (TransBlot Turbo Mini-size, Bio-Rad) were used according to the manufacturer's instruction. The transfer proceeded at 25 V-1 A for 30 min. For the first membrane, a HRP-conjugated streptavidin substrate (1:10000 dilution) was used, while for the second membrane, the rabbit anti-VEGFA antibody (1:5000) was employed together with the HRP-conjugated anti-rabbit-IgG antibody (1:5000). Each antibody was used for 1 h upon rotation at RT and washed 4 times with a washing buffer (WB, Table S2) for 10 min. A chemiluminescent HRP substrate was applied to each membrane for 30 s. Visualisation of blots was performed using the chemiluminescence imaging system Fusion SOLO S (Viber Lourmat).

SUPPLEMENTARY TABLES

Table S1. Oligonucleotide sequences used in this study.

Name	Sequence 5'→3'
Pr1	CTCGCTATGACACTCGTCTCTT
Cy5-Pr1	/5Cy5/CTCGCTATGACACTCGTCTCTT
2'-OMe-Pr2	/56-FAM/mCmUmUmGmUmUmGmUmGmCmUmCmCmAmCmGmGmUmUmA
Bio-2'-OMe-Pr2	/5BiotinTEG/mCmUmUmGmUmUmGmUmGmCmUmCmCmAmCmGmGmUmUmA
Pr2	CTTGTTGTGCTCCACGGTTA
Cy3-Pr2	/5Cy3/CTTGTTGTGCTCCACGGTTA
P-Pr2	/5Phos/CTTGTTGTGCTCCACGGTTA
Lib25	CTCGCTATGACACTCGTCTCTT(N1:26262424)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)TAACCGTGGAGCACAACAAG
1-18 template	CTCGCTATGACACTCGTCTCTTGCACACACACTAGCCACCCCCGCATTTTAACCGTGGAGCACAACAAG
1-32 template	CTCGCTATGACACTCGTCTCTTACCATAATTCACACCCACATTCATCATAACCGTGGAGCACAACAAG
2-8 template	CTCGCTATGACACTCGTCTCTTCATGCTGATTAGTTTGTGTGTGGATTAACCGTGGAGCACAACAAG
2-15 template	CTCGCTATGACACTCGTCTCTTCTAACCATATCCGTATCACGTGTGTAACCGTGGAGCACAACAAG
2-18 template	CTCGCTATGACACTCGTCTCTTCATCAACTCAATCCGTGTCGGTTACTAACCGTGGAGCACAACAAG
2-21 template	CTCGCTATGACACTCGTCTCTTCAATGCACATGTACGCTCGTTAGTTAACCGTGGAGCACAACAAG
3-5 template	CTCGCTATGACACTCGTCTCTTACACTTACACATAAACACGCATATTAACCGTGGAGCACAACAAG
3-7 template	CTCGCTATGACACTCGTCTCTTATTCTCTCTAGATTGCGTTTCATAACCGTGGAGCACAACAAG
4-6 template	CTCGCTATGACACTCGTCTCTTCATGGTACCAGTAATGCCGCAATATAACCGTGGAGCACAACAAG
4-19 template	CTCGCTATGACACTCGTCTCTTAGGTCATTGCGGCTCGTATACATTAACCGTGGAGCACAACAAG
DNA Lib	/56-FAM/CTTGTTGTGCTCCACGGTTANNNNNNNNNNNNNNNNNNNNNNNNAAGAGACGAGTGTCATAGCGAG
DNA 2-15	/56-FAM/CTTGTTGTGCTCCACGGTTACACACGTGATACGGATATGGTTAGAAGAGACGAGTGTCATAGCGAG
DNA 2-21	/56-FAM/CTTGTTGTGCTCCACGGTTAACTAACGAGCGTACATGTGCATTGAAGAGACGAGTGTCATAGCGAG
DNA 4-6	/56-FAM/CTTGTTGTGCTCCACGGTTATATTGCGGCATTACTGGTACCATGAAGAGACGAGTGTCATAGCGAG
V7t1	/56-FAM/TGTGGGGGTGGACGGGCCGGGTAGA
Bio-V7t1	/5BiotinTEG/TGTGGGGGTGGACGGGCCGGGTAGA
HNA V7t1	hThGhThGhGhGhGhGhThGhGhAhChGhGhGhChChGhGhGhThAhGhA/36-FAM/
Macugen	/56-FAM//i2FC/mGmGrArA/i2FU//i2FC/mAmG/i2FU/mGmAmA/i2FU/mG/i2FC//i2FU//i2FU/mA/i2FU/mA/i2FC/mA/i2FU//i2FC//i2FC/mG

OligoT	TTTTTTTTTTTTTTT
TF-FW	GCCAGGAGAAAGGGGAAT
TF-RV	CAGTGCAATATAGCATTTGCAGTAGC
Actin-FW	TCACCCACACTGTGCCCATCTACGA
Actin-RV	CAGCGGAACCGCTCATTGCCAATGG

DNA sequences are written using IDT notations; mN, hN, and rA stand for 2'-OMe-ribonucleotide, hexitol nucleotide, and adenosine ribonucleotide, respectively; i2FC and i2FU denote internal 2'-fluoro-C and U, respectively; /5Cy5/, /5Cy3/, and /56-FAM/ indicate 5-cyanine, 3-cyanine, and 6-carboxyfluorescein 5'-fluorescent dye tags, respectively; /5BiotinTEG/ is a biotin-TEG tag, while /5Phos/ is a 5'-phosphoryl tag. V7t1 and Macugen are known anti-rVEGF$_{165}$ aptamers with K_d = 1400 and 50 pM, respectively (6–8). Underlined sequences indicate primer complementary regions on DNA templates.

Table S2. Buffers and solutions used in this study.

Buffer	Composition	Purpose
1× PBS	137 mM NaCl, 2.7 mM KCl, 1.14 mM $Na_2HPO_4 \cdot 7H_2O$, 1.5 mM $KH_2PO_4 \cdot 2H_2O$, pH 7.4	Biotinylation and magnetic beads coupling assay buffer
PBS-T	1× PBS with 0.05% Tween-20 and 0.1% BSA	Washing buffer for magnetic beads, coupling assay, and immunodetection analysis
PBS-T-5%BSA	PBS-T with 5% BSA	Blocking buffer for magnetic beads, coupling assay, and immunodetection analysis
PBS-K	150 mM NaCl, 70 mM $Na_2HPO4 \cdot 2H_2O$, 30 mM $NaH_2PO_4 \cdot 2H_2O$, pH 7.2	HABA assay
SB	40 mM Tris-HCl, 2 mM $MgCl_2$, 2 mM KCl, 100 mM NaCl, 1 mM $CaCl_2$, 0.05% Tween-20, pH 7.4	Selection buffer
WB	SB with 0.1% BSA	Washing buffer
EB	10mM EDTA in 95% formamide	Elution buffer
AB	SB with 1% BSA	Antibody dilution buffer
BB	SB with 5% BSA	Blocking buffer
2× gel loading buffer	95% formamide, 18 mM EDTA, 0.25% SDS, 0.05% bromophenol blue	Denaturing PAGE loading buffer
Diffusion buffer	500 mM ammonium acetate, 0.1% SDS, 1 mM EDTA, 10 mM magnesium acetate, pH 8.0	Elution of oligonucleotides from PAGE
1× ThermoPol Buffer purchased from NEB	20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% TritonX-100, pH 8.8 @ 25 °C	PCR buffer, HNA synthesis and RT reaction buffer
10% Native PAGE	Acrilamide:bisacrilamis = 29:1. For 100 mL, 25 mL 40% PAGE, 5 mL 10× TBE, 70 mL H_2O	Lambda exonuclease digestion detection
6% Native PAGE	Acrilamide:bisacrilamis = 29:1. For 100 mL, 15 mL 40% PAGE, 5 mL 10× TBE, 80 mL H_2O	Electrophoretic mobility shift assay (EMSA)

Table S3. Sequences of random regions of clones recovered after 7 SELEX rounds and grouped according to sequence identity.[a]

Group 1	
1-1	TACACAGAGACGACAATTTAGCCTAC
3-10	CCATAGAGGAGTTAACTTACCAG
4-12	ACATACATACGAGACACAGAGTGCC
Group 2	
1-2	AGAGCTACCAACTTGGATGAATCTG
1-4	TACACGGTGCTATTAACTGTTGGC
1-6	ATCCCGTTTGAGTTCATTGATTCCC
1-18	AAATGCGGGGGTGGCTAGTGTGTGTGC
1-27	CAACGACCTGGGTATATCTAACTG
1-32	TGATGAATGTGGGTGTGAATTATGGT
2-13	TGAGCATCCTAAATTGGAACCG
2-21	ACTAACGAGCGTACATGTGCATTG
3-6	CGAACGATGAATGCATGCCTG
3-12	TCAGCATGTCATAATCTGCACCTT
3-16	GCTAGCGTGCACGACTACTGCCTG
3-17	TCTGTAAGCGGCACTACTAACCTG
3-23	TCTGTAAGCGGCACTACTAACCTG
4-6	TATTGCGGCATTACTGGTACCATG
4-8	ACGTGCGAAATTAACTGAACCTG
4-18	ACAGGCGCATTAAGAGTTGTAG
4-19	ATGTATACGAGCCGCAATGACCT
4-22	ATACTAACGGGGGCTACAGTGCTGG
4-24	TCGCGGAGTTGATATCTACCTG
Group 3 G-rich	
1-5	GGGGGAGAATGAGTCGGGTAGT
1-8	GGAGGATGATGCTAGGGTTTGGGAT
1-18	AAATGCGGGGGTGGCTAGTGTGTGTGC
3-20	GGGGAGTGGTGGGGACTTTGTGGG
3-22	GGGAAGGACAGGCTTATGGGGGACC
4-14	TGGGGGCAGGGGGTGTG
4-15	GGGCTAGTAGAGTCGAGAGTGGAAT
Group 4	
1-10	GCGTGAACACGACCTGTTTG
1-21	AAATCGTGTTGGATTAGGTATTTGG
1-25	ATATCAGTGAAGTAGCAGTTGGGT
1-31	GATCAGAGTAAGCAATACCAGGTTG
2-15	CACACGTGATACGGATATGGTTAG
2-18	GTAACCGACACGGATTGAGTTGATG
Group 5	
1-14	GTGTGCAATAGCGTGTTGATGAT
2-16	TGAATGTGTGTTAGCGTGGCTTTGG
2-25	ATGCGCGGTAGCTTGGATTGG
3-5	ATATGCGTGTTTATGTGTAAGTGT
Group 6	
2-8	ATCCACACACAAACTAATCAGCATG
3-7	TGAAACGCAATCTAGAGAGAAT
3-27	TGCACAATCAAACTGCCTACGATCC
Group 7	
2-11	CGCGCACGACACACACATAGTTCC
4-12	ACATACATACGAGACACAGAGTGCC
4-15	GGGCTAGTAGAGTCGAGAGTGGAAT
4-16	GCGTGTTAAGCATAGATGAGAGTGG
Group 8	
3-3	TCGTTGTTGAGTGGAATATGATGGT
3-5	ATATGCGTGTTTATGTGTAAGTGT
3-11	AGTTGCAGCGTAATGTGATGGC
4-4	TGTGTATGGGATATGTGTGT
Group 9	
3-3	TCGTTGTTGAGTGGAATATGATGGT
3-8	GTACCAGCATGCGATGATG
3-11	AGTTGCAGCGTAATGTGATGGC
3-12	TCAGCATGTCATAATCTGCACCTT
Group 10	
1-21	AAATCGTGTTGGATTAGGTATTTGG
1-32	TGATGAATGTGGGTGTGAATTATGGT
2-16	TGAATGTGTGTTAGCGTGGCTTTGG
2-25	ATGCGCGGTAGCTTGGATTGG
3-11	AGTTGCAGCGTAATGTGATGGC
4-16	GCGTGTTAAGCATAGATGAGAGTGG
Group 11	
2-18	GTAACCGACACGGATTGAGTTGATG
3-4	TGCCGTCGACGAACTTTAATGTGCG
4-1	CCGTCGTGGTTATGTGATGTGTT
Group 12 Unsorted	
1-11	ACATACAGGCACAACTGTCCGATCC
1-16	CCTAGCCGATTGAAGTACGATTG
1-24	GCAGTCCACCCAATGCCCCATCCCT
2-10	AACTGTTGAAGCATTGGTATGTT
3-19	ATCATGAGGAGCTGTTGTTGCAGG

[a]Grouping was made according to the Clustal Omega and Clustal W2 algorithms available on the EMBL-EBI website. Sequences that appeared several times in different groups are highlighted in grey.

Table S4. Enzyme-linked oligonucleotide assay (ELONA) conditions.

	Microtiter plate	Reagent/ washing solution volumes	Immobilized reagent	Titrant	Capture/detecting antibodies (AB)	
					1st	2nd
ELONA1	96-well half-area microplate (Corning, #3690)	50 μL/125 μL	$rVEGF_{164}$ or competitors, 0.5 μg/mL in PBS	FAM-aptamers (0-200 or 1000 nM)	Anti-FAM AB HRP conjugate (1:3333)	-
ELONA2	Pirse NeutrAvidin coated plates (ThermoFisher Scientific, #15127)	100 μL/200 μL	Bio-aptamers, 50 nM in SB	$rVEGF_{164}$ (0-1000 nM)	Rabbit anti-VEGFA (1:500)	Anti-rabbit IgG HRP conjugate (1:2500)

Table S5. Examples of aptamers selected against the human $VEGF_{165}$ target.

	Aptamer	Nature	Size (nt)	Structure	Modifications	K_D (pM)	Phase/ Status[a]	Ref.
1	Macugen/ Pegaptanib	RNA	27	Stem-loop	2-'F-Py and 2'-OMe-Pu[c]	50	III/ Approved	(7–9)
2	VGd1-2Ds-47[b]	DNA	47	Stem-loop	2 additional Ds bases	0.65	-	(10)
3	V7t1	DNA	25	G-quadruplex	-	1400	-	(6)
4	3R02 Bivalent	DNA	60	G-quadruplex dimer	-	30	-	(11)
5	SL2-B	DNA	26	Stem-loop	-	500	-	(12)
6	Anti-$VEGF_{165}$	DNA	26	Stem-loop	-	920	-	(13)
7	ARC245	RNA	23	Stem-loop	2'-OMe-RNA	2100	-	(14)
8	NX-213	RNA	24	-	2'-NH_2-Py and 2'-OMe-Pu	140	-	(15)

[a]Status or phase of clinical trial, as approved by FDA.
[b]Ds is 7-(2-thienyl)-imidazo[4,5-b] pyridine.
[c]Pu and Py stand for purine and pyrimidine nucleotides, respectively, within the aptamer sequence.

SUPPLEMENTARY FIGURES

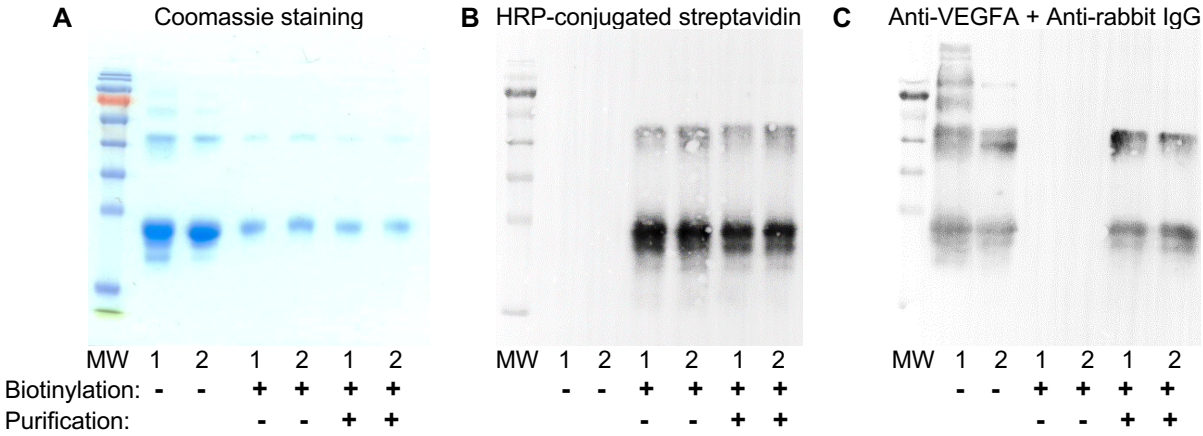


Figure S1. Biotinylation of the target protein rVEGF$_{164}$. (**A**) Image of 12.5% SDS-PAGE after Coomassie staining with different samples of non-biotinylated and biotinylated rVEGF$_{164}$, before and after desalting (all protein samples are visualised). (**B-C**) Western blot analysis of bio-rVEGF$_{164}$ with detection by streptavidin HRP conjugate (**B**, only biotinylated samples are visualised) or rabbit polyclonal anti-VEGFA and anti-rabbit IgG HRP conjugated antibodies (**C**, all VEGF-related samples with free epitopes are visualised) following by incubation with the HRP chemiluminescent substrate. Two different rVEGF$_{164}$ samples were used for biotinylation, and are indicated as 1 and 2. The protein samples were detected as expected, except for the unpurified bio-rVEGF$_{164}$ images from (**C**). The absence of samples might be due to the over-biotinylation of the protein (samples were stored overnight with an excess of biotin without desalting) resulting in the masking of the epitopes responsible for recognition by the anti-VEGFA antibody.

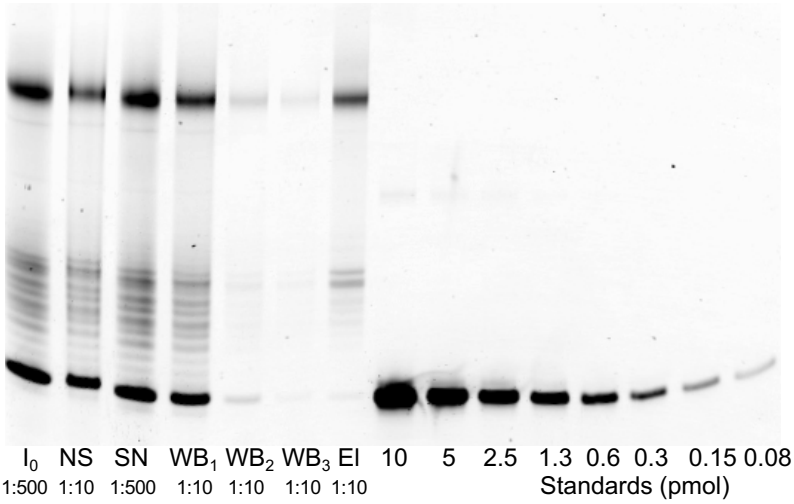


Figure S2. Example of PAGE analysis after selection round 7. I_0 – initial library before the selection round (1:500 dilution); NS – library washed away from the negative selection matrix by the elution buffer (magnetic beads without target protein, 1:10); SN – sample of the supernatant after incubation with the negative selection matrix (1:500); WB_1–WB_3 – samples from the washing steps after incubation of the library with the selection matrix (1:10); El – eluted sample (1:10). Dilutions of the samples are indicated for accurate comparison. Standards are the exact amount (0.078–10 pmol dilutions) of FAM-labelled 2'-OMe-Pr2 used for quantification of the eluate.

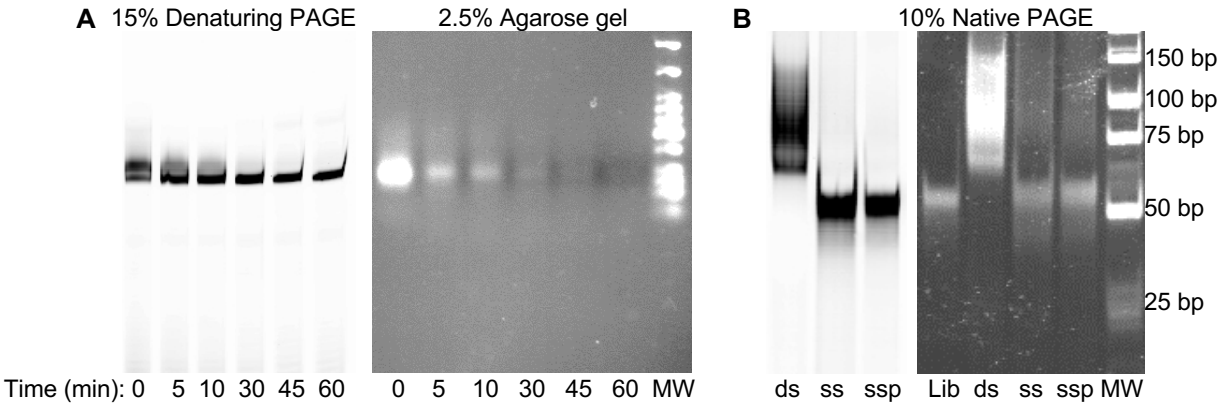


Figure S3. Example of Lambda exonuclease digestion of a double-stranded PCR product. (**A**) Images of λexo digests after different reaction times (0, 5, 10, 30, 45, and 60 min) with 0.2 U/µL λexo at 37 °C. The 5'-phosphorylated DNA strand is not labelled, while the non-phosphorylated strand is Cy5-tagged. On the left side, image of 15% denaturing gel scanned with the Cy5 channel showing ds and ss DNA. On the right side, image of 2.5% agarose gel stained with ethidium bromide showing only ds DNA. (**B**) Images of 10% native PAGE of the samples before (ds) and after (ss, ssp) the λexo digestion reaction. The reaction was performed in the presence of 0.2 U/µL λexo for 1 h. Ds stands for double-stranded product, while ss and ssp are single-stranded products before and after precipitation, respectively. Lib is the initial ss DNA Lib25. Visualisation was performed using scanning with the Cy5 channel (left) and SYBR Gold staining (right).

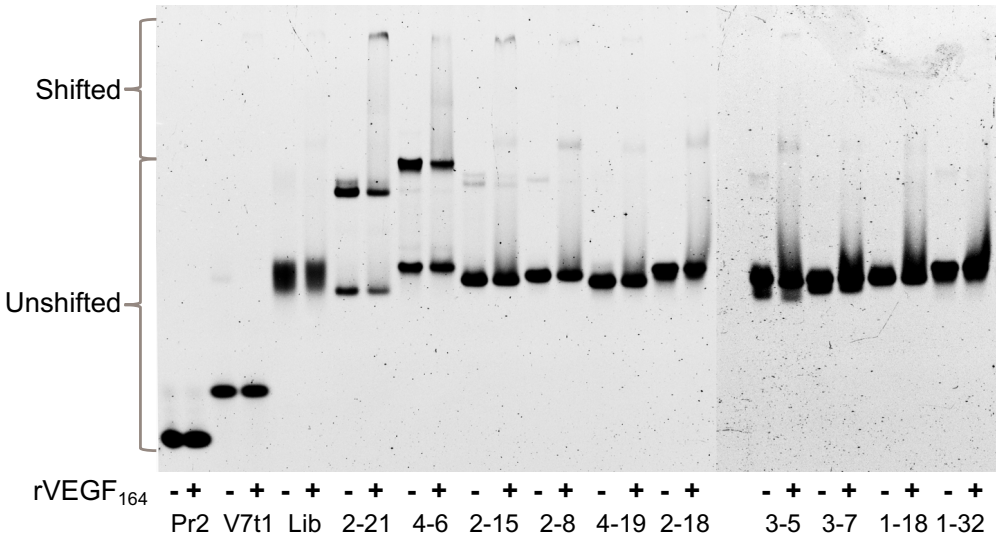


Figure S4. Electrophoretic gel mobility analysis of the selected aptamers. The 10 FAM-labelled MeORNA–HNA aptamers (2-21, 4-6, 2-15, 2-8, 4-19, 2-18, 3-5, 3-7, 1-18, and 1-32) and control sequences, i.e., 2'-OMe-Pr2, DNA V7t1, and MeORNA–HNA Lib25 were incubated at a 25 nM concentration for 2 h without (-) or with (+) 100 nM $rVEGF_{164}$ followed by separation using 6% native PAGE. The gel was scanned with Cy2-channel. Positions of shifted and upshifted material are indicated.

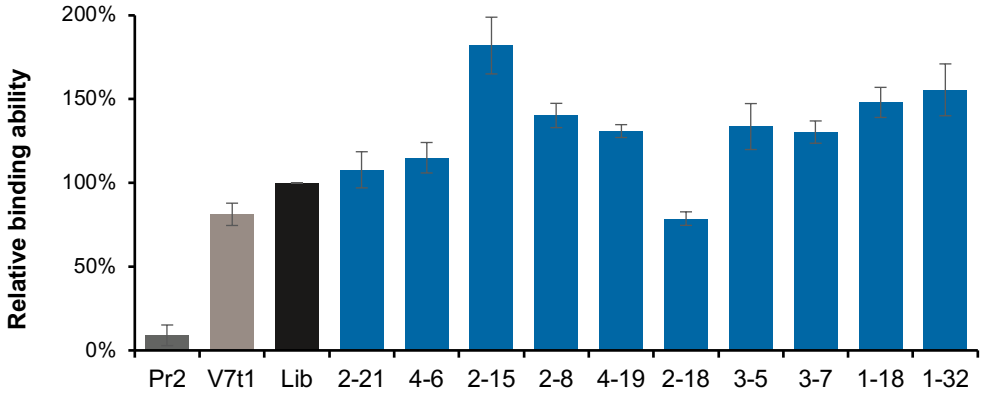


Figure S5. Fluorescence polarisation assay of selected MeORNA–HNA aptamers. Relative fluorescence polarisation changes of FAM-labelled aptamers (2-21, 4-6, 2-15, 2-8, 4-19, 2-18, 3-5, 3-7, 1-18, and 1-32) and control sequences, i.e., 2'-OMe-Pr2, DNA V7t1, and MeORNA–HNA Lib25 (as 100%), are shown. Oligonucleotides (10 nM) were incubated for 2 h with $rVEGF_{164}$ (400 nM) in 50 μL of SB buffer. The analysis was repeated 6 times.

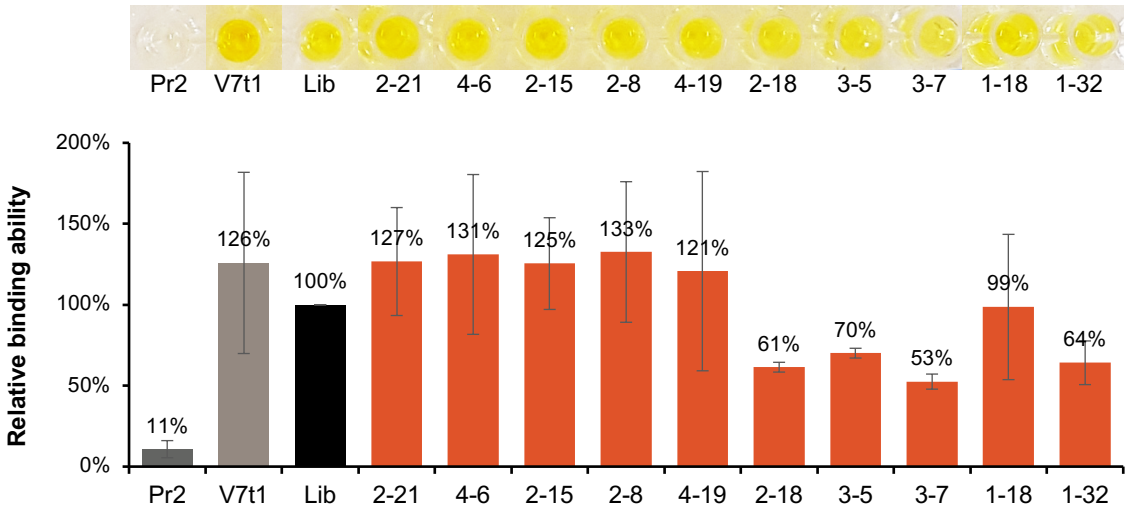


Figure S6. Binding analysis of MeORNA–HNA aptamer variants (25 nM) to rVEGF$_{164}$ (0.5 μg/mL) by ELONA1. An example of the ELONA specificity experiment (above) and the relative binding abilities of the aptamers (below) are shown. Binding of the initial library was considered as 100%. Mean values with standard deviations of 3-5 independent experiments are shown. The oligonucleotide-protein complexes were detected using the polyclonal anti-FAM HPR conjugated antibody with a calorimetric HPR substrate. Absorbance was measured at 450 nm.

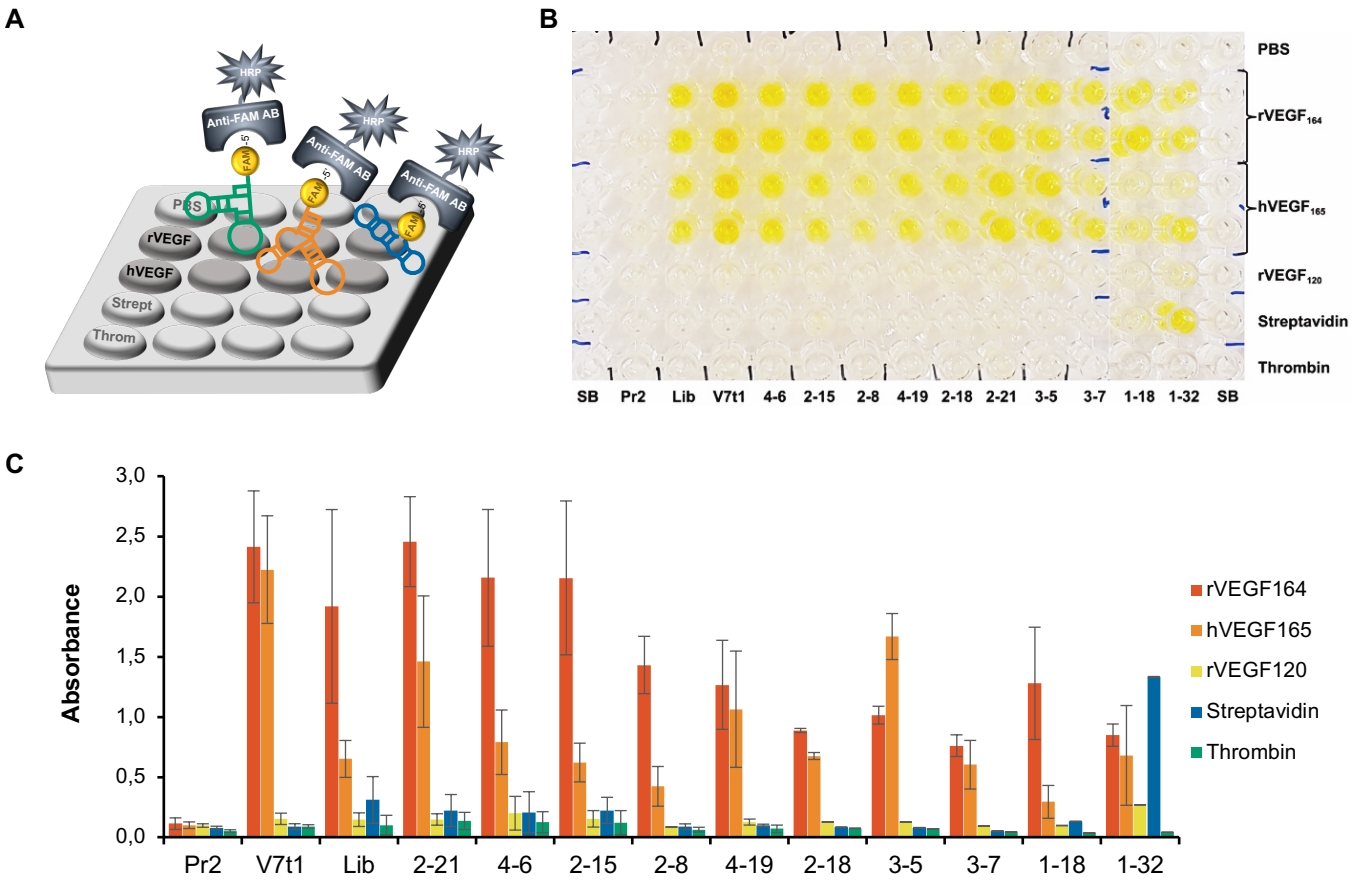


Figure S7. Determination of the specificity of anti-rVEGF$_{164}$ aptamers using ELONA. (**A**) Schematic illustration of the ELONA experiment. Immobilized rVEGF$_{164}$ target and competitors (all at a concentration of 0.5 µg/mL) were incubated with 25 nM FAM-labelled aptamers and control oligonucleotide sequences (SB – selection buffer, Pr2 – 2'-OMe-Pr2, V7t1 – DNA aptamer, Lib – initial MeORNA–HNA library). The oligonucleotide-protein complexes were detected using the polyclonal anti-FAM HPR conjugated antibody (Anti-FAM AB) with a calorimetric HPR substrate. (**B**) Example of the ELONA specificity experiment. (**C**) Binding abilities of aptamers. Absorbance was measured at 450 nm. Each experiment was performed 3-5 times.

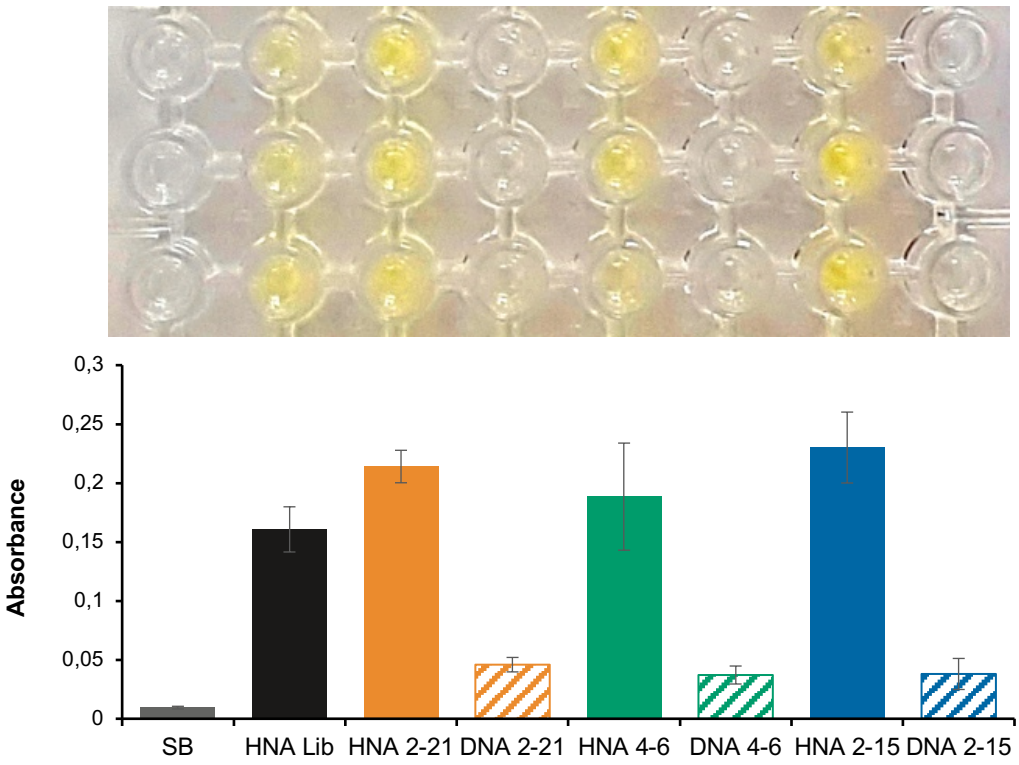


Figure S8. Comparison of the binding abilities of MeORNA–HNA (shortened as HNA) and DNA aptamer variants (10 nM) to $rVEGF_{164}$ (0.5 μg/mL) using ELONA1. SB is the selection buffer.

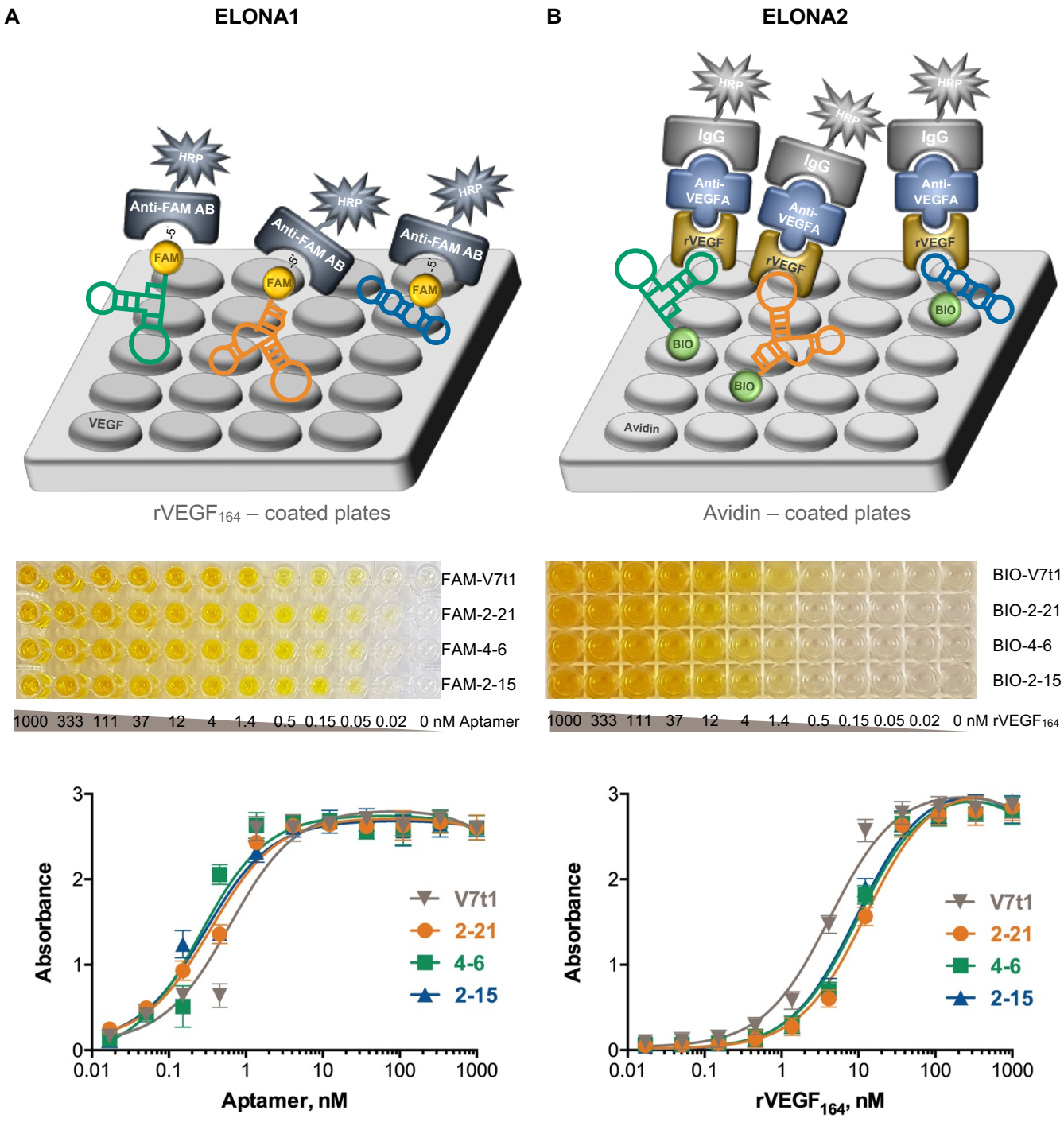


Figure S9. Enzyme-linked oligonucleotide assay (ELONA) used to study the binding affinity of the selected aptamers. (**A**) ELONA1 assay was performed with $rVEGF_{164}$ (1 μg/mL) coated plates. FAM-labelled aptamer solutions were applied to the plates, followed by serial dilutions of the anti-FAM HRP-conjugated antibody and TMB HRP substrate. (**B**) In the ELONA2 assay, pre-blocked NeutrAvidin coated plates were used and biotinylated aptamers were added to the wells, followed by serial dilutions of $rVEGF_{164}$, rabbit anti-VEGFA, and HRP-conjugated anti-rabbit-IgG antibodies. The binding was detected by applying the TMB HRP substrate to each well. The experiments were repeated three times, and the average data are plotted on the graphs.

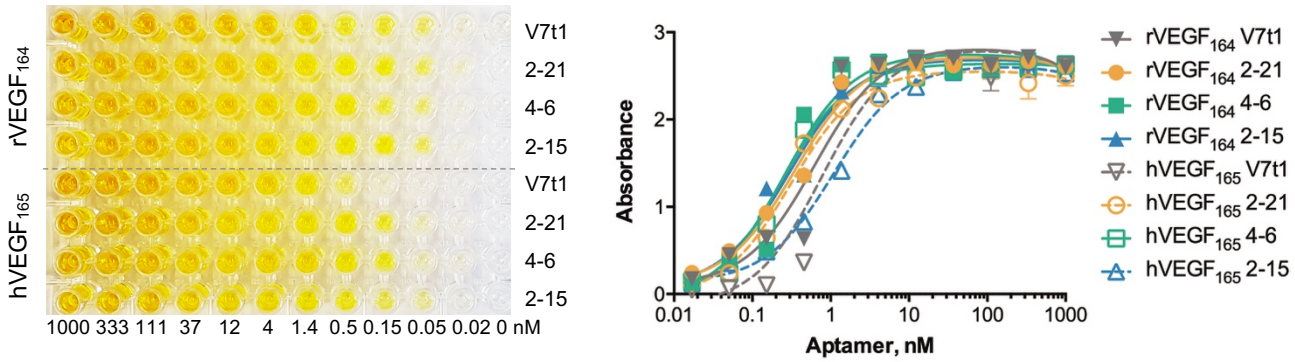


Figure S10. Comparison of aptamer binding affinities to the $rVEGF_{164}$ and $hVEGF_{165}$ targets (1 μg/mL) by using ELONA1. The experiment was repeated three times.

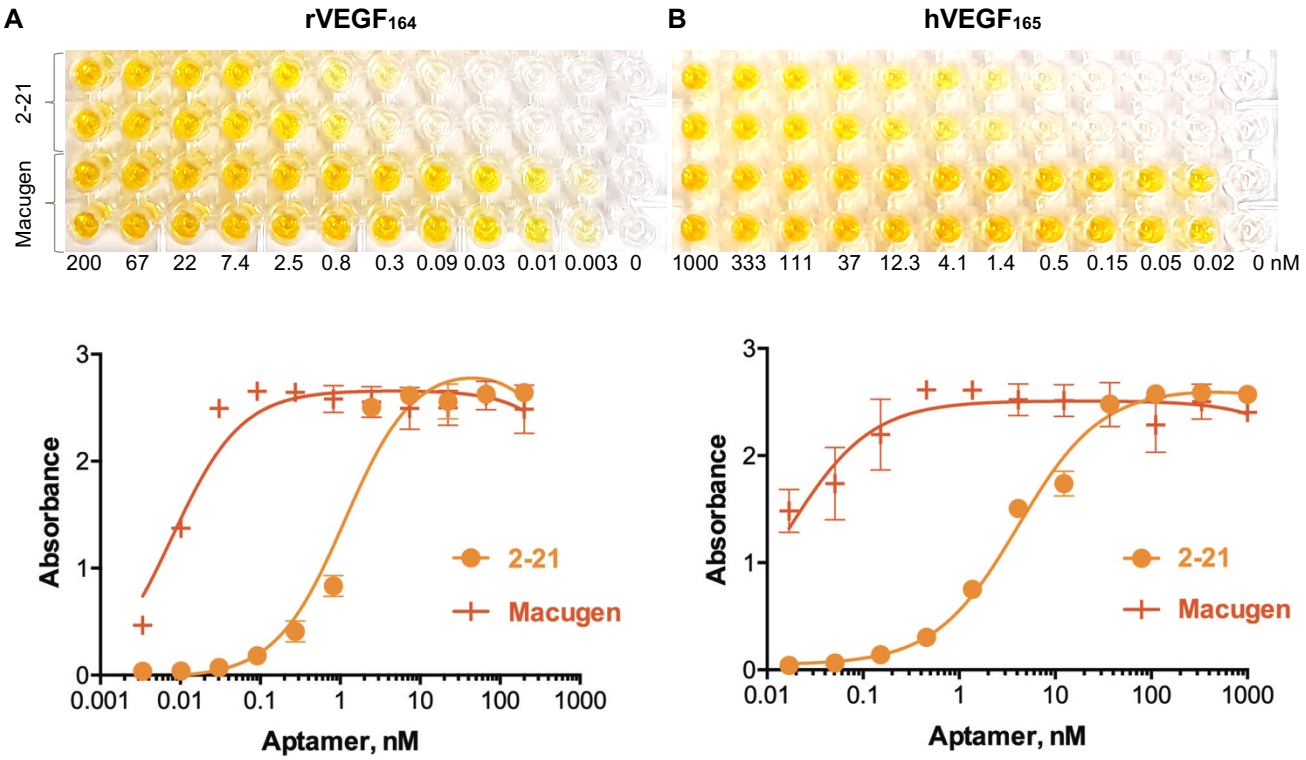
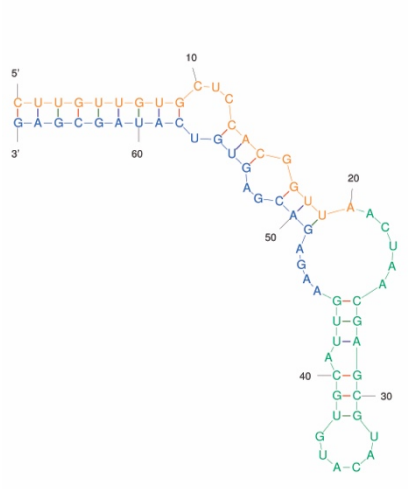


Figure S11. Comparison of MeORNA–HNA 2-21 (shortened as 2-21) and Macugen aptamers binding by using ELONA1. (**A**) Binding analysis of aptamers with $rVEGF_{164}$ (1 μg/mL). (**B**) Binding analysis of aptamers with $hVEGF_{165}$ (1 μg/mL).

5'- mCmUmUmGmUmUmGmUmGmCmUmCmCmAmCmGmGmUmUmA **-(hN)$_{25}$-** hAhAGhAhGhAhChGhAhGhThGhThChAhThAhGhChGhAhG -3'

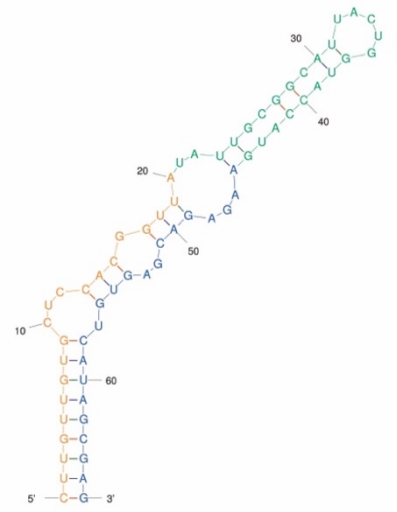
MeORNA-HNA Lib

2-21



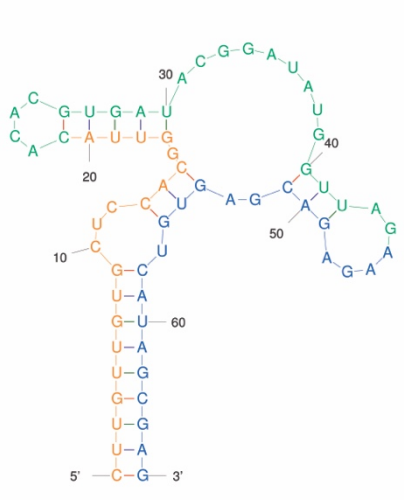
ΔG = -13.30

4-6

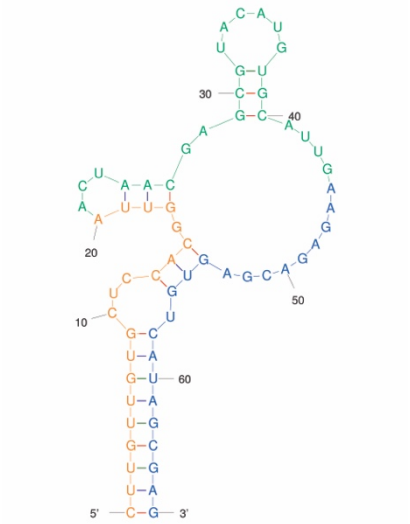


ΔG = -12.40

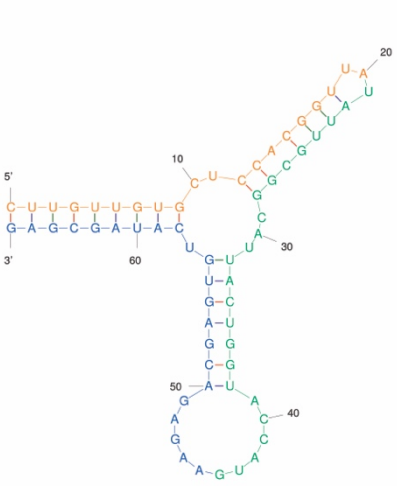
2-15



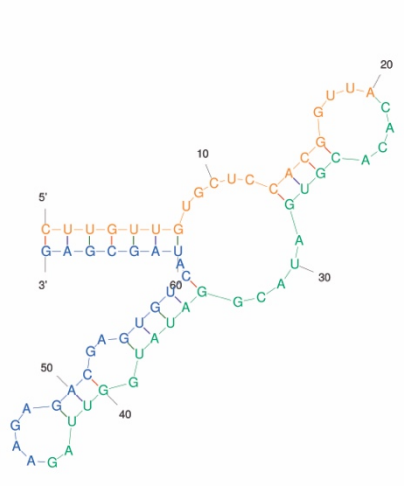
ΔG = - 12.74



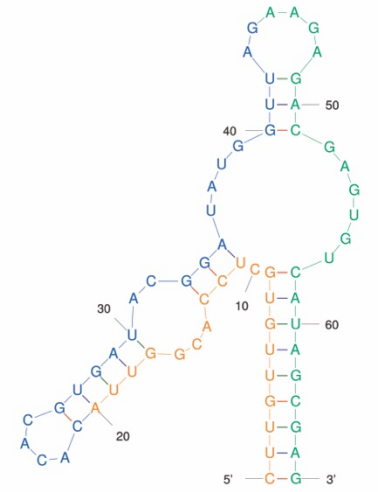
ΔG = -8.42



ΔG = -11.30



ΔG = - 12.13



ΔG = - 11.03

Figure S12. Predicted structures of selected full-length MeORNA–HNA aptamers recovered from the 7th round of SELEX. The RNA folding method was employed for predicting the secondary structures using the Mfold software. The first 20 nt starting from the 5'-end are 2'-OMe-RNA residues (orange) followed by 46 hexitol nucleotides (random HNA region is shown in green, HNA primer sequence is shown in blue). Gibbs free energies (ΔG) are expressed in kcal/mol.

5'- mCmUmUmGmUmUmGmUmGmCmUmCmCmAmCmGmGmUmUmA **-(hN)$_{25}$-** hAhAGhAhGhAhChGhAhGhThGhThChAhThAhGhChGhAhG -3'

MeORNA-HNA Lib

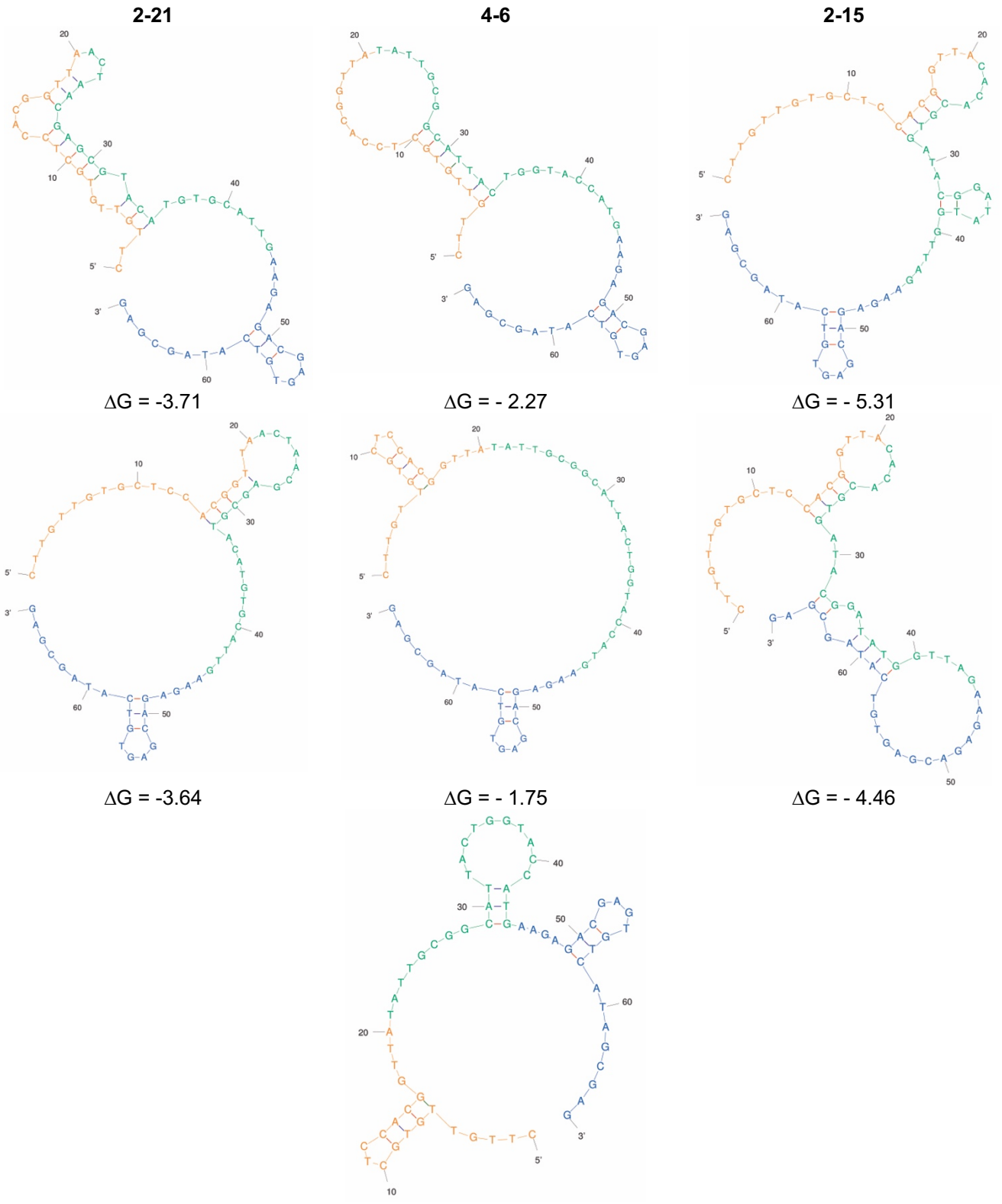


Figure S13. Predicted structures of the selected full-length MeORNA–HNA aptamers recovered from the 7th round of SELEX. The DNA folding method was employed for predicting the secondary structures using the Mfold software. The first 20 nt starting from the 5'-end are 2'-OMe-RNA residues (orange) followed by 46 hexitol nucleotides (random HNA region is shown in green, HNA primer sequence is shown in blue). Gibbs free energies (ΔG) are expressed in kcal/mol.

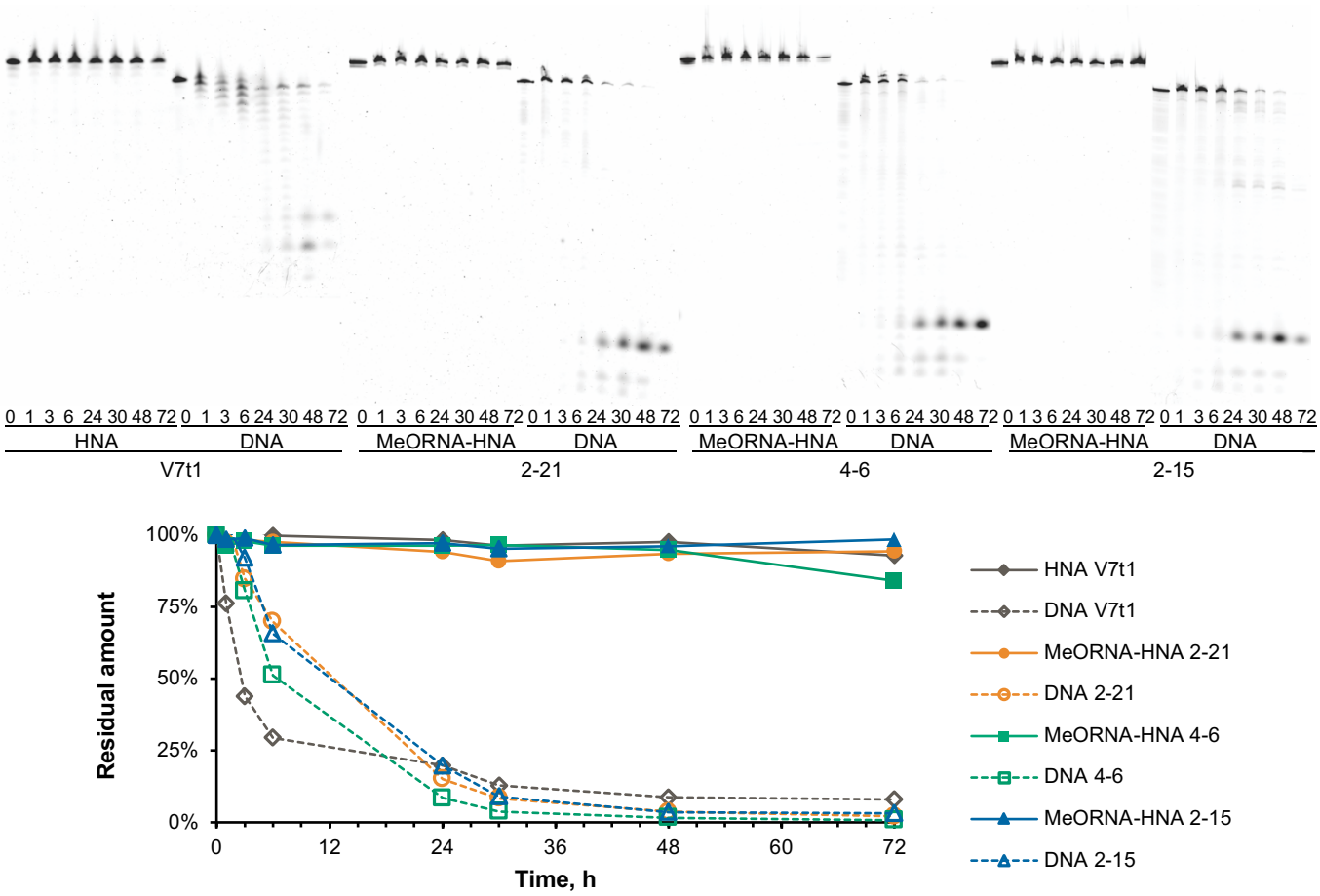


Figure S14. Nuclease resistance of MeORNA–HNA and DNA aptamer variants in 95% whole human serum at 37 °C for up to 72 h.

MeORNA-HNA 2-21 (66 nt)

5’-FAM-mCmUmUmGmUmUmGmUmGmCmUmCmCmAmCmGmGmUmUmAhAhChThAhAhChGhAhGhChGhThAhChAhThGhThGhChAhThThGhAhAhGhAhGhAhChGhAhGhThGhThChAhThAhGhChGhAhG

Macugen (27 nt)

5’-FAM-fCmGmGrArAfUfCmAmGfUmGmAmAfUmGfCfUfUmAfUmAfCmAfUfCfCmG

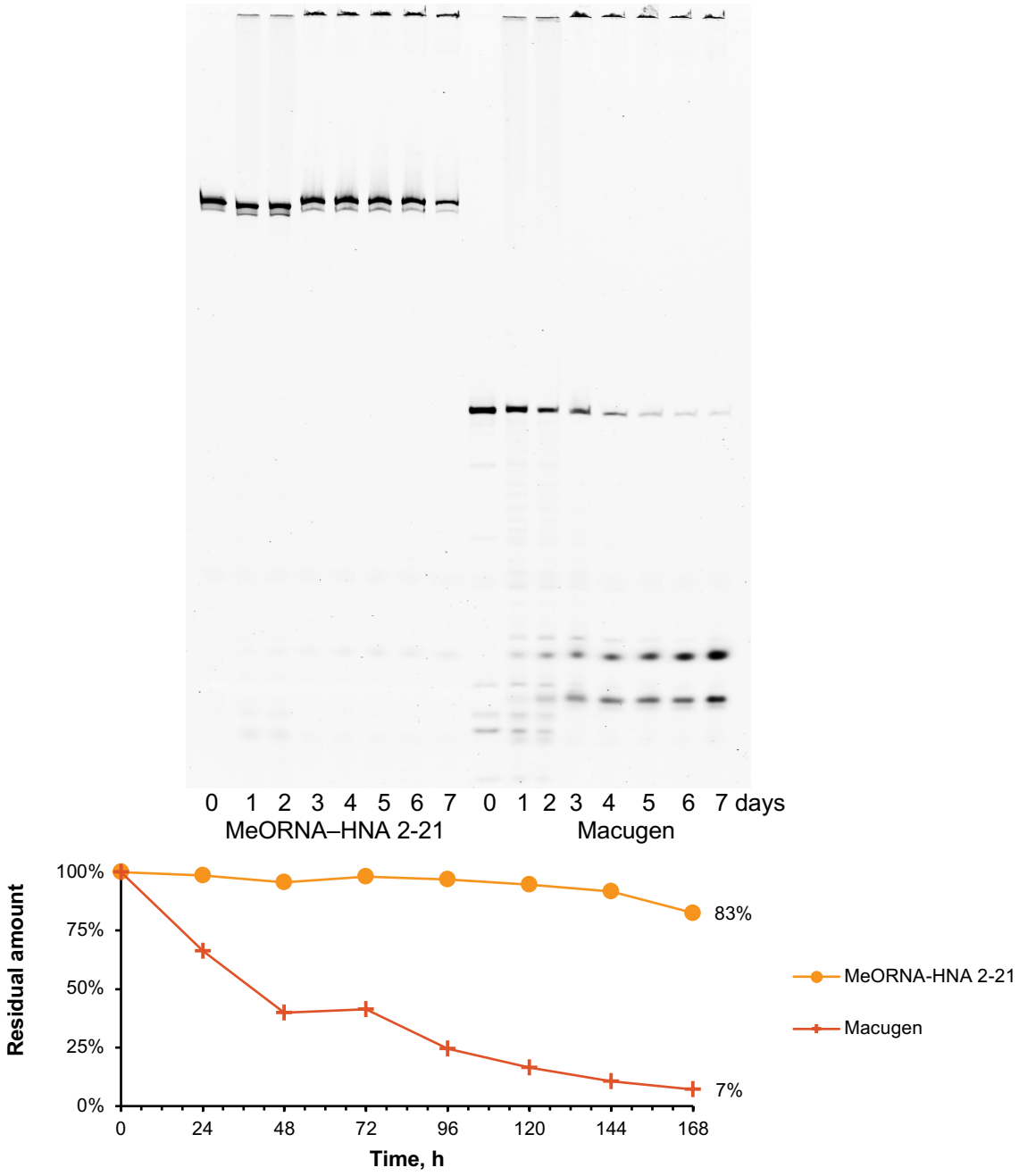


Figure S15. Nuclease resistance of MeORNA–HNA 2-21 and 2’F-/2’-OMe-RNA Macugen aptamers in 95% whole human serum at 37 °C for up to 7 days (168 h). Partial degradation of the MeORNA–HNA 2-21 aptamer after 6 days incubation might be due to the digestion of the FAM-2’-OMe-RNA part of the aptamer rather than that of the HNA sequence.

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