Supplementary Tables

Supplementary Table T1

Figure	Name	Sequence	Company	Purification
4	DUX4_TCT	5'Atto647N-GCTAATCTAATCAACCGCAGGTTGATTAGATTAGC	1	1
4	SELEX#1	GCATAAACTTAATCAAGACCAGGTCATTGATTAACGCTCGTTTGG	1	2
4	SELEX#2	TGGTGAAGCGCAAGGAAACTTAATCACAAGGTGATTAATTGTTTG	1	2
4	SELEX#3	TCAATTTAACTTCAGCCTTTGAGAGAACCCTGATTAATCATTGAC	1	2
4	SELEX#4	TGATTAACCAACTTAGAGAACTGGTGATTAAGTTCATCATCGCGA	1	2
4	SELEX#5	CTAGATTAACCCGTTTACCAAGCGATGGAAACTTAATCTCCATGG	1	2
4	SELEX#6	GGTGGAAATCTAACTCAACTCGATGATTAAATTTCGGTGCGCTTG	1	2
4	SELEX#7	CCAGTAATTTAATCAACACAAATATGATTAACCCATCACTTCTGC	1	2
4	SELEX#8	CAACGATTAGCGTTCCGAGTGATAATTAACCTAATCGGACGTTGG	1	2
4	SELEX#9	TCAACTCAATCAGCACATGTACCCTGATTGACATGGTTGTCAAGG	1	2
5A	DUX4_TCT	5'Atto647N-GCTAATCTAATCAACCGCAGGTTGATTAGATTAGC	1	1
5A	DUX4_TCT	GCTAATCTAATCAACCGCAGGTTGATTAGATTAGC	1	2
5A	DUX4_TCT_F1CC	GCT**CC**AATCTAATCAACCGCAGGTTGATTAGATTAGC	1	2
5A	DUX4_TCT_F2CC	GCTA**CC**ATCTAATCAACCGCAGGTTGATTAGATTAGC	1	2
5A	DUX4_TCT_F3CC	GCTAA**CC**TCTAATCAACCGCAGGTTGATTAGATTAGC	1	2
5A	DUX4_TCT_F4CC	GCTAAT**CC**TAATCAACCGCAGGTTGATTAGATTAGC	1	2
5A	DUX4_TCT_F5CC	GCTAATC**CC**TAATCAACCGCAGGTTGATTAGATTAGC	1	2
5A	DUX4_TCT_F6CC	GCTAATCT**CC**AATCAACCGCAGGTTGATTAGATTAGC	1	2
5A	DUX4_TCT_F7CC	GCTAATCTA**CC**ATCAACCGCAGGTTGATTAGATTAGC	1	2
5A	DUX4_TCT_F8CC	GCTAATCTAA**CC**TCAACCGCAGGTTGATTAGATTAGC	1	2
5A	DUX4_TCT_F9CC	GCTAATCTAAT**CC**AACCGCAGGTTGATTAGATTAGC	1	2
5A	DUX4_TCT_F10CC	GCTAATCTAATC**CC**AACCGCAGGTTGATTAGATTAGC	1	2
5B	DUX4_TCT	5'Atto647N-GCTAATCTAATCAACCGCAGGTTGATTAGATTAGC	1	1
5B	DUX4_TCT	GCTAATCTAATCAACCGCAGGTTGATTAGATTAGC	1	2
5B	DUX4_TCT_R1CC	GCTAATCTAATCAACCGCAGGTT**CC**GATTAGATTAGC	1	2
5B	DUX4_TCT_R2CC	GCTAATCTAATCAACCGCAGGTTG**CC**ATTAGATTAGC	1	2
5B	DUX4_TCT_R3CC	GCTAATCTAATCAACCGCAGGTTGA**CC**TTAGATTAGC	1	2
5B	DUX4_TCT_R4CC	GCTAATCTAATCAACCGCAGGTTGAT**CC**TAGATTAGC	1	2
5B	DUX4_TCT_R5CC	GCTAATCTAATCAACCGCAGGTTGATT**CC**AGATTAGC	1	2
5B	DUX4_TCT_R6CC	GCTAATCTAATCAACCGCAGGTTGATTA**CC**GATTAGC	1	2
5B	DUX4_TCT_R7CC	GCTAATCTAATCAACCGCAGGTTGATTAG**CC**ATTAGC	1	2
5B	DUX4_TCT_R8CC	GCTAATCTAATCAACCGCAGGTTGATTAGA**CC**TTAGC	1	2
5B	DUX4_TCT_R9CC	GCTAATCTAATCAACCGCAGGTTGATTAGAT**CC**TAGC	1	2
5B	DUX4_TCT_R10CC	GCTAATCTAATCAACCGCAGGTTGATTAGATT**CC**AGC	1	2
5C	DUX4_TCT	5'Atto647N-GCTAATCTAATCAACCGCAGGTTGATTAGATTAGC	1	1
5C	DUX4_TCT	GCTAATCTAATCAACCGCAGGTTGATTAGATTAGC	1	2
5C	DUX4_TCT_F8C	GCTAATCTAA**C**TCAACCGCAGGTTGATTAGATTAGC	1	2
5C	DUX4_TCT_F8CC	GCTAATCTAA**CC**TCAACCGCAGGTTGATTAGATTAGC	1	2
5C	DUX4_TCT_F8CCC	GCTAATCTAA**CCC**TCAACCGCAGGTTGATTAGATTAGC	1	2
5C	DUX4_TCT_F8CCCC	GCTAATCTAA**CCCC**TCAACCGCAGGTTGATTAGATTAGC	1	2
5C	DUX4_TCT_F8CCCCC	GCTAATCTAA**CCCCC**TCAACCGCAGGTTGATTAGATTAGC	1	2
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5D	DUX4_TCT_R7C	GCTAATCTAATCAACCGCAGGTTGATTAG**C**ATTAGC	1	2
5D	DUX4_TCT_R7CC	GCTAATCTAATCAACCGCAGGTTGATTAG**CC**ATTAGC	1	2
5D	DUX4_TCT_R7CCC	GCTAATCTAATCAACCGCAGGTTGATTAG**CCC**ATTAGC	1	2
5D	DUX4_TCT_R7CCCC	GCTAATCTAATCAACCGCAGGTTGATTAG**CCCC**ATTAGC	1	2
5D	DUX4_TCT_R7CCCCC	GCTAATCTAATCAACCGCAGGTTGATTAG**CCCCC**ATTAGC	1	2
5E	DUX4_TCT	5'Atto647N-GCTAATCTAATCAACCGCAGGTTGATTAGATTAGC	1	1
5E	DUX4_TCT	GCTAATCTAATCAACCGCAGGTTGATTAGATTAGC	1	2
5E	DUX4_TCT_F8C	GCTAATCTAA**C**TCAACCGCAGGTTGATTAGATTAGC	1	2
5E	DUX4_TCT_F8T	GCTAATCTAA**T**TCAACCGCAGGTTGATTAGATTAGC	1	2
5E	DUX4_TCT_F8G	GCTAATCTAA**G**TCAACCGCAGGTTGATTAGATTAGC	1	2
5E	DUX4_TCT_F8A	GCTAATCTAA**A**TCAACCGCAGGTTGATTAGATTAGC	1	2
5E	DUX4_TCT_F8CT	GCTAATCTAA**CT**TCAACCGCAGGTTGATTAGATTAGC	1	2
5E	DUX4_TCT_F8CC	GCTAATCTAA**CC**TCAACCGCAGGTTGATTAGATTAGC	1	2
5E	DUX4_TCT_F8TT	GCTAATCTAA**TT**TCAACCGCAGGTTGATTAGATTAGC	1	2

Aptamer sequences used in this work. Company indicators: 1) Microsynth, Balgach, Switzerland, 2) IDT Integrated DNA Technologies, Coralvill, US-IA; Purification indicators: 1) HPLC purification, 2) standard desalting.

5E	DUX4_TCT_F8TC	GCTAATCTAA**TC**TCAACCGCAGGTTGATTAGATTAGC	1	2
5E	DUX4_TCT_F8GG	GCTAATCTAA**GG**TCAACCGCAGGTTGATTAGATTAGC	1	2
5E	DUX4_TCT_F8AA	GCTAATCTAA**AA**TCAACCGCAGGTTGATTAGATTAGC	1	2
5F	DUX4_TCT	5'Atto647N-GCTAATCTAATCAACCGCAGGTTGATTAGATTAGC	1	1
5F	DUX4_TCT	GCTAATCTAATCAACCGCAGGTTGATTAGATTAGC	1	2
5F	DUX4_TCT_R7CCC	GCTAATCTAATCAACCGCAGGTTGATTAG**CCC**ATTAGC	1	2
5F	DUX4_TCT_R7TTT	GCTAATCTAATCAACCGCAGGTTGATTAG**TTT**ATTAGC	1	2
5F	DUX4_TCT_R7AAA	GCTAATCTAATCAACCGCAGGTTGATTAG**AAA**ATTAGC	1	2
5F	DUX4_TCT_R7GGG	GCTAATCTAATCAACCGCAGGTTGATTAG**GGG**ATTAGC	1	2
5G	DUX4_TCT	5'Atto647N-GCTAATCTAATCAACCGCAGGTTGATTAGATTAGC	1	1
5G	DUX4_CCT	GCTAA**CCT**AATCAACCGCAGGTTGATT**AGG**TTAGC	1	2
5G	DUX4_TCT	GCTAA**TCT**AATCAACCGCAGGTTGATT**AGA**TTAGC	1	2
5G	DUX4_CTT	GCTAA**CTT**AATCAACCGCAGGTTGATT**AAG**TTAGC	1	2
5G	DUX4_TTT	GCTAA**TTT**AATCAACCGCAGGTTGATT**AAA**TTAGC	1	2
5G	DUX4_CTC	GCTAA**CTC**AATCAACCGCAGGTTGATT**GAG**TTAGC	1	2
5G	DUX4_TCC	GCTAA**TCC**AATCAACCGCAGGTTGATT**GGA**TTAGC	1	2
5G	DUX4_CCC	GCTAA**CCC**AATCAACCGCAGGTTGATT**GGG**TTAGC	1	2
5G	DUX4_TTC	GCTAA**TTC**AATCAACCGCAGGTTGATT**GAA**TTAGC	1	2
5H	DUX4_TCT	5'Atto647N-GCTAATCTAATCAACCGCAGGTTGATTAGATTAGC	1	1
5H	DUX4_CTT	GCTAA**CTT**AATCAACCGCAGGTTGATT**AAG**TTAGC	1	2
5H	DUX4_CCT_R7CCC	GCTAA**CCT**AATCAACCGCAGGTTGATT**AGCCCG**TTAGC	1	2
5H	DUX4_TCT_R7CCC	GCTAA**TCT**AATCAACCGCAGGTTGATT**AGCCCA**TTAGC	1	2
5H	DUX4_CTT_R7CCC	GCTAA**CTT**AATCAACCGCAGGTTGATT**AACCCG**TTAGC	1	2
5H	DUX4_CTT_F8C_R7CCC	GCTAA**CTT**AA**C**TCAACCGCAGGTTGATT**AACCCG**TTAGC	1	2
5H	DUX4_CTT_F8CC_R7CCC	GCTAA**CTT**AA**CC**TCAACCGCAGGTTGATT**AACCCG**TTAGC	1	2
6	DUX4_CTT_R7CCC	5'FAM-AGCTAA**CCT**AATCAACCGCAGGTTGATT**AGCCCG**TTAGC	2	1
7A	PROP1 aptamer	5'Atto647N-GCTAATTTAATTAACCGCAGGTTAATTAAATTAGC	1	1
7A	PROP1 aptamer	GCTAATTTAATTAACCGCAGGTTAATTAAATTAGC	1	2
7A	DUX4_CTT	GCTAA**CTT**AATCAACCGCAGGTTGATT**AAG**TTAGC	1	2
7A	DUX4_CTT_R7CCC	GCTAA**CTT**AATCAACCGCAGGTTGATT**AACCCG**TTAGC	1	2
7A	DUX4_CTT_F8C_R7CCC	GCTAA**CTT**AA**C**TCAACCGCAGGTTGATT**AACCCG**TTAGC	1	2
7A	DUX4_TCT	GCTAA**TCT**AATCAACCGCAGGTTGATT**AGA**TTAGC	1	2
7A	DUX4_TCT_R8CCC	GCTAA**TCT**AATCAACCGCAGGTTGATT**AGA**TTAGC	1	2
7A	PAX7 aptamer	GCTAATCGATTAACCGCAGGTTAATCGATTAGC	1	2
7B	PAX7 aptamer	5'Atto647N-GCTAATCGATTAACCGCAGGTTAATCGATTAGC	1	1
7B	PAX7_T4C	GCTAA**C**CGATTAACCGCAGGTTAATCG**G**TTAGC	1	2
7B	PAX7_T9C	GCTAATCGAT**C**AACCGCAGGTT**G**ATCGATTAGC	1	2
7B	DUX4_CTT	GCTAA**CTT**AATCAACCGCAGGTTGATT**AAG**TTAGC	1	2
7B	DUX4_CTT_R7CCC	GCTAA**CTT**AATCAACCGCAGGTTGATT**AACCCG**TTAGC	1	2
7B	DUX4_CTT_F8C_R7CCC	GCTAA**CTT**AA**C**TCAACCGCAGGTTGATT**AACCCG**TTAGC	1	2
7B	DUX4_TCT	GCTAA**TCT**AATCAACCGCAGGTTGATT**AGA**TTAGC	1	2
7B	DUX4_TCT_R8CCC	GCTAA**TCT**AATCAACCGCAGGTTGATT**AGA**TTAGC	1	2
7B	PROP1 aptamer	GCTAATTTAATTAACCGCAGGTTAATTAAATTAGC	1	2
7C	DUX4_TCT_R8CCC	5'Atto647N-GCTAACTTAATCAACCGCAGGTTGATTAACCCGTTAGC	1	1
7C	DUX4_CTT	GCTAA**CTT**AATCAACCGCAGGTTGATT**AAG**TTAGC	1	2
7C	DUX4_CTT_R7CCC	GCTAA**CTT**AATCAACCGCAGGTTGATT**AACCCG**TTAGC	1	2
7C	DUX4_CTT_F8C_R7CCC	GCTAA**CTT**AA**C**TCAACCGCAGGTTGATT**AACCCG**TTAGC	1	2
7C	DUX4_TCT	GCTAA**TCT**AATCAACCGCAGGTTGATT**AGA**TTAGC	1	2
7C	DUX4_TCT_R8CCC	GCTAA**TCT**AATCAACCGCAGGTTGATT**AGA**TTAGC	1	2
7C	PROP1 aptamer	GCTAATTTAATTAACCGCAGGTTAATTAAATTAGC	1	2
7C	PAX7 aptamer	GCTAATCGATTAACCGCAGGTTAATCGATTAGC	1	2
7C	PAX7_T4C	GCTAA**C**CGATTAACCGCAGGTTAATCG**G**TTAGC	1	2
7C	PAX7_T9C	GCTAATCGAT**C**AACCGCAGGTT**G**ATCGATTAGC	1	2

(Continued Table T1) Aptamer sequences used in this work. Company indicators: 1) Microsynth, Balgach (Switzerland), 2) IDT Integrated DNA Technologies, Coralville (US-IA); Purification indicators: 1) HPLC purification, 2) standard desalting.

8	DUX4_TCT	5'Atto647N-GCTAATCTAATCAACCGCAGGTTGATTAGATTAGC	1	1
8	DUX4_TCT	GCTAATCTAATCAACCGCAGGTTGATTAGATTAGC	1	2
8	DUX4_TCT_R7CCC	GCTAATCTAATCAACCGCAGGTTGATTAGCCCATTAGC	1	2
8	DUX4_TCT_R7[ab][ab][ab]	GCTAATCTAATCAACCGCAGGTTGATTAG[dSpacer(abasic site)][dSpacer(abasic site)][dSpacer(abasic site)]ATTAGC	1	2
8	DUX4_TCT_R7[C3][C3][C3]	GCTAATCTAATCAACCGCAGGTTGATTAG[SpacerC3][SpacerC3][SpacerC3]ATTAGC	1	2
10	DUX4_TCT_R7CCC	GCTAA**TCT**AATCAACCGCAGGTTGATT**AGCCCA**TTAGC	2	1
10	DUX4_TCT_R7CCC (forward)	GCGTAATCTAATCAACA	2	1
10	DUX4_TCT_R7CCC (reverse)	TGTTGATTAGCCCATTACGC	2	1
S3A	DUX4_CTT_R7CCC	5'FAM-AGCTAACCTAATCAACCGCAGGTTGATTAGCCCGTTAGC	2	1
S3A	scr. DUX4_CTT_R7CCC	5'FAM-ACACTCGCTCCAAAGGACAGTCATTTTCAAAGATTTCGG	2	1
S3A	DUX4_CTT_R7CCC	AGCTAACCTAATCAACCGCAGGTTGATTAGCCCGTTAGC	2	2
S3B-C	DUX4_CTT_R7CCC	5'IRDye800-AGCTAACCTAATCAACCGCAGGTTGATTAGCCCGTTAGC	2	1
S3B-C	scr. DUX4_CTT_R7CCC	5'IRDye800-ACACTCGCTCCAAAGGACAGTCATTTTCAAAGATTTCGG	2	1
S3B-C	DUX4_CTT_R7CCC	AGCTAACCTAATCAACCGCAGGTTGATTAGCCCGTTAGC	2	2

(Continued Table T1) Aptamer sequences used in this work. Company indicators: 1) Microsynth, Balgach (Switzerland), 2) IDT Integrated DNA Technologies, Coralville (US-IA); Purification indicators: 1) HPLC purification, 2) standard desalting.

Supplemental Table T2

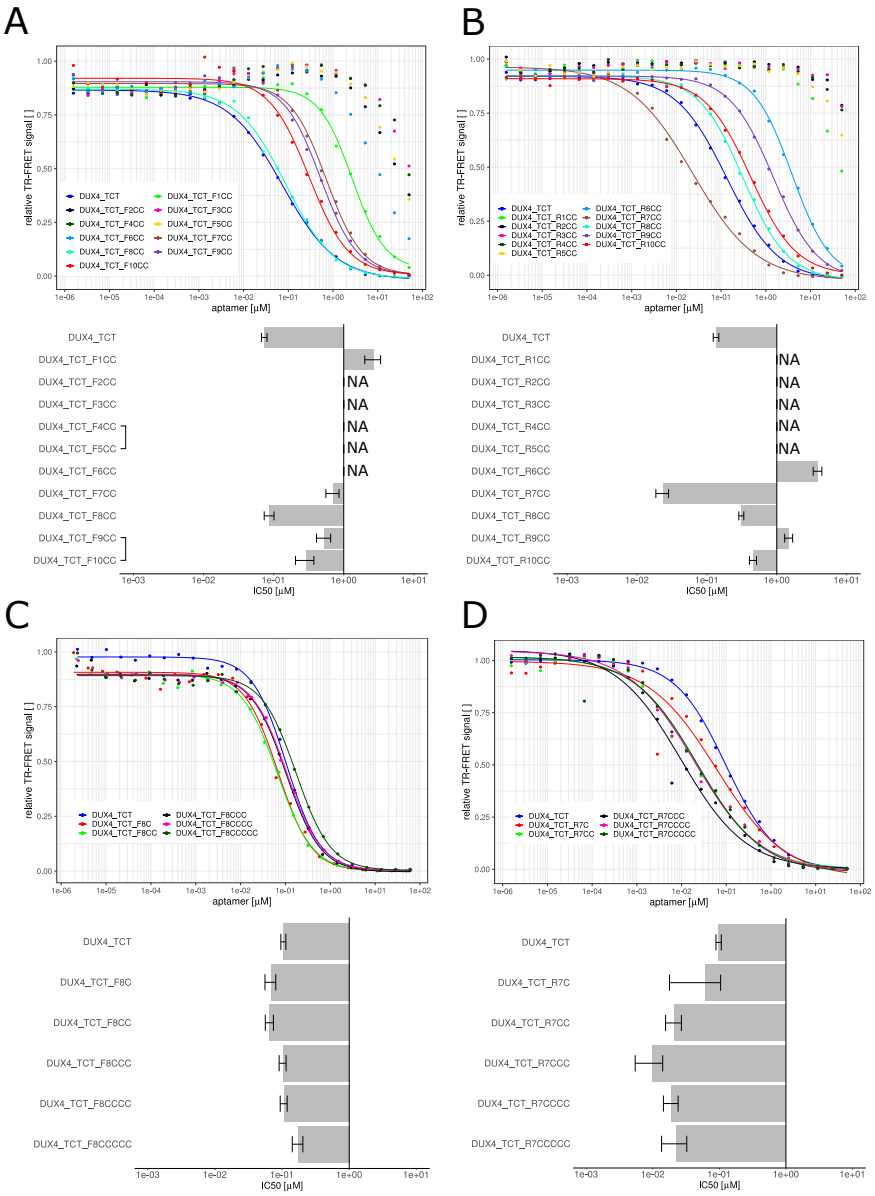
	strand-swapped	bulge/hairpin
Data collection		
Resolution range	46.3 - 2.34 (2.42 - 2.34)	58.5 - 3.21 (3.33 - 3.21)
Space group	$C222_1$	C2
Unit cell		
a,b,c (Å)	71.64, 73.19, 108.07	120.32, 75.67, 82.69
α,β,γ (˚)	90, 90, 90	90, 121.60, 90
Total reflections	86199 (5524)	32962 (3337)
Unique reflections	12294 (1174)	10380 (1005)
Multiplicity	7.0 (4.7)	3.2 (3.3)
Completeness (%)	97.08 (96.24)	97.54 (97.64)
I/σI	9.84 (0.65)	8.90 (1.05)
R-merge	0.104 (1.81)	0.085 (1.29)
R-meas	0.113 (2.04)	0.103 (1.53)
R-pim	0.042 (0.90)	0.057 (0.82)
$CC_{1/2}$	0.999 (0.628)	0.993 (0.604)
Refinement		
R-work/R-free	0.211/0.225	0.253/0.275
#non-H atoms	1906	3743
Macromolecules	1845	3743
Ligands	8	
Solvent	53	
RMS deviation		
Bond lengths (Å)	0.004	0.003
Bond angles (˚)	0.84	0.50
Ramachandran plot		
Favored (%)	96.15	98.85
Allowed (%)	3.85	1.15
Outliers (%)	0.00	0.00
Average B-factor	38.30	108.90
Macromolecules	38.52	108.90
Ligands	58.73	
Solvent	27.56	

Statistics for the highest-resolution shell are shown in parentheses.

Data collection and refinement statistics.

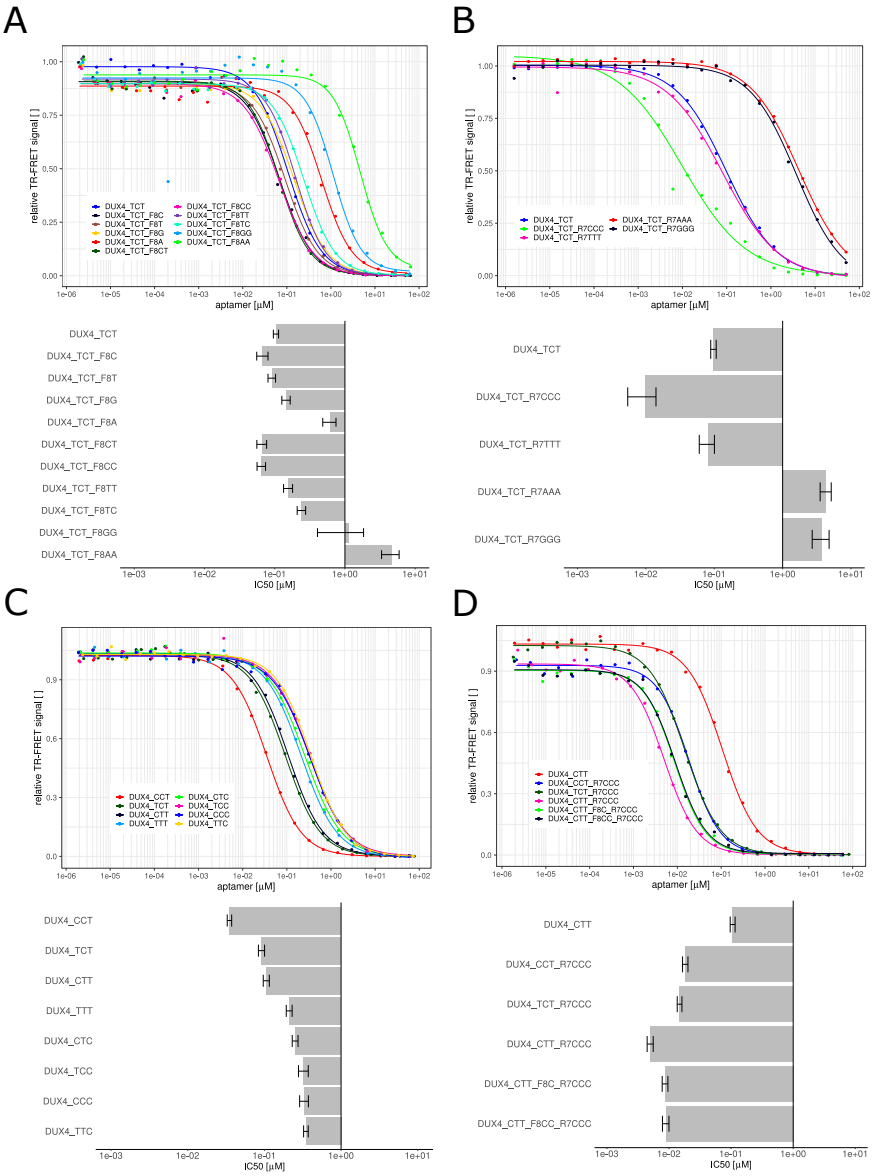
Supplementary Figures

Supplemental Figure S1



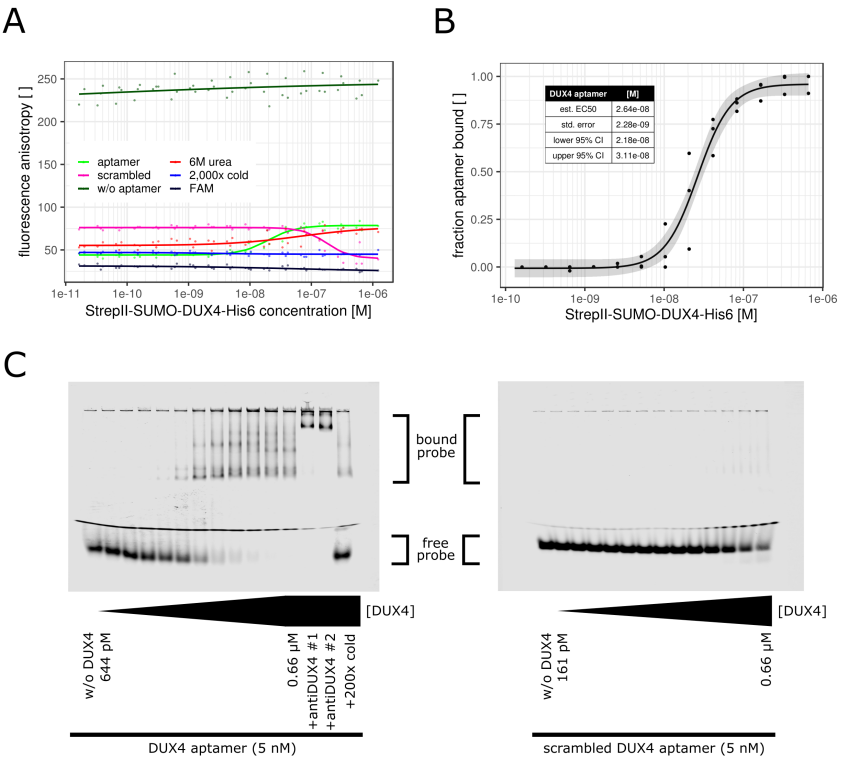
Comparing aptamer variants as part of an optimization process using TR-FRET assays. Different rationally designed aptamers were compared against a labelled template aptamer. IC50 values ±95% confidence interval (bar charts) gained by four-parameter logistic curve fitting of different data points (corresponding curves depicted above) are displayed. NA: IC50 not measurable because data points indicated an IC50>10 μM. (A) The position of a double-dC loop was varied on the forward DUX4 motif. Brackets indicate identical aptamers. (B) The position of a double-dC loop was screened on the reverse DUX4 target motif. (C) Loop size of a dC loop was varied from a single base to 5x dC on the forward motif. (D) Loop size of a dC loop was varied from a single base to 5x dC on the reverse motif.

Supplemental Figure S2



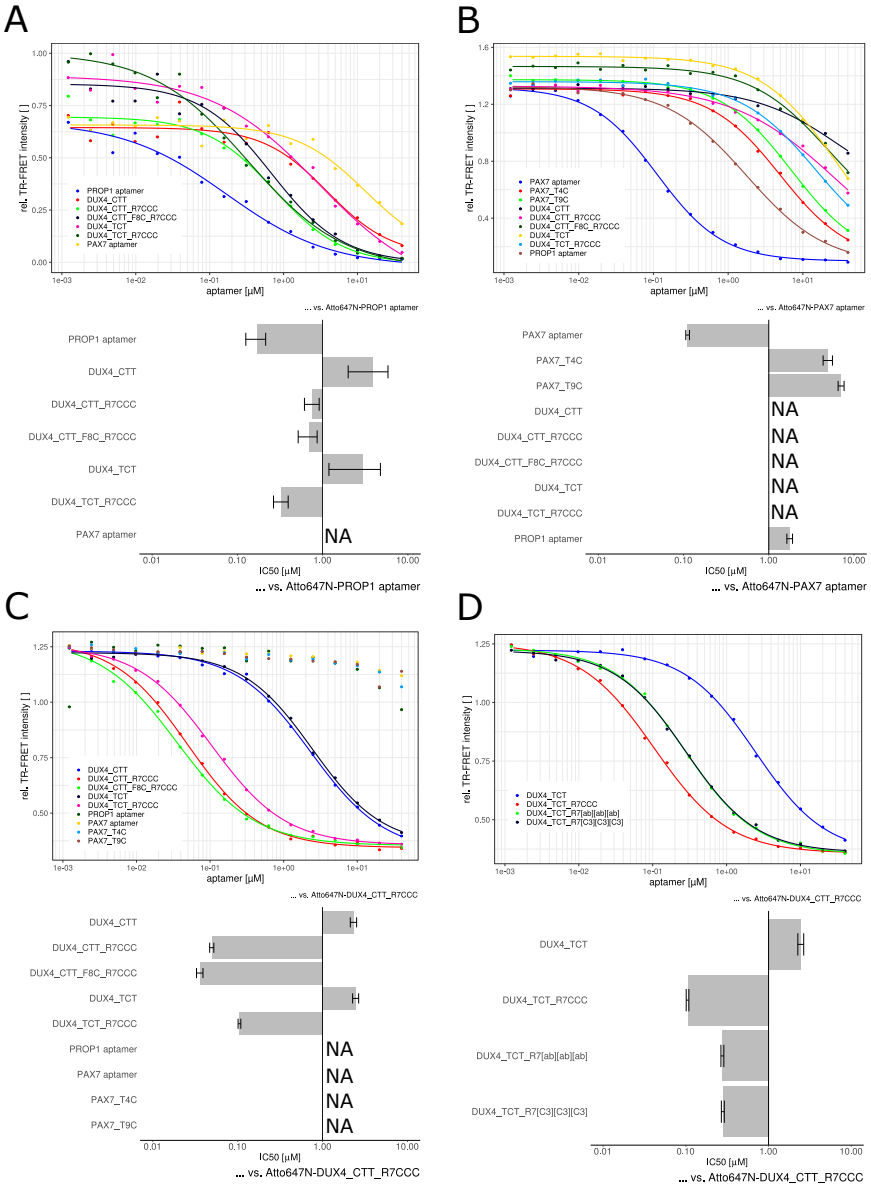
Comparing aptamer variants as part of an optimization process using TR-FRET assays. Different rationally designed aptamers were compared against a labelled template aptamer. IC50 values ±95% confidence interval (bar charts) gained by four-parameter logistic curve fitting of different data points (corresponding curves depicted above) are displayed. NA: IC50 not measurable because data points indicated an IC50>10 μM. (A) Selected sequences of single base and double base loops were tested on the forward motif between A8 and T9. (B) Base composition of a triple base loop between G7 and A8 of the reverse motif was examined. (C) The triplet sequence after A3 of the forward motif was varied by permuting dC and dT. The reverse motif was altered correspondingly to retain base pairing. (D) Bulge occurrence at two positions of the DUX4 motif in combination with three selected base triplets after A3 on the forward motif were tested.

Supplemental Figure S3



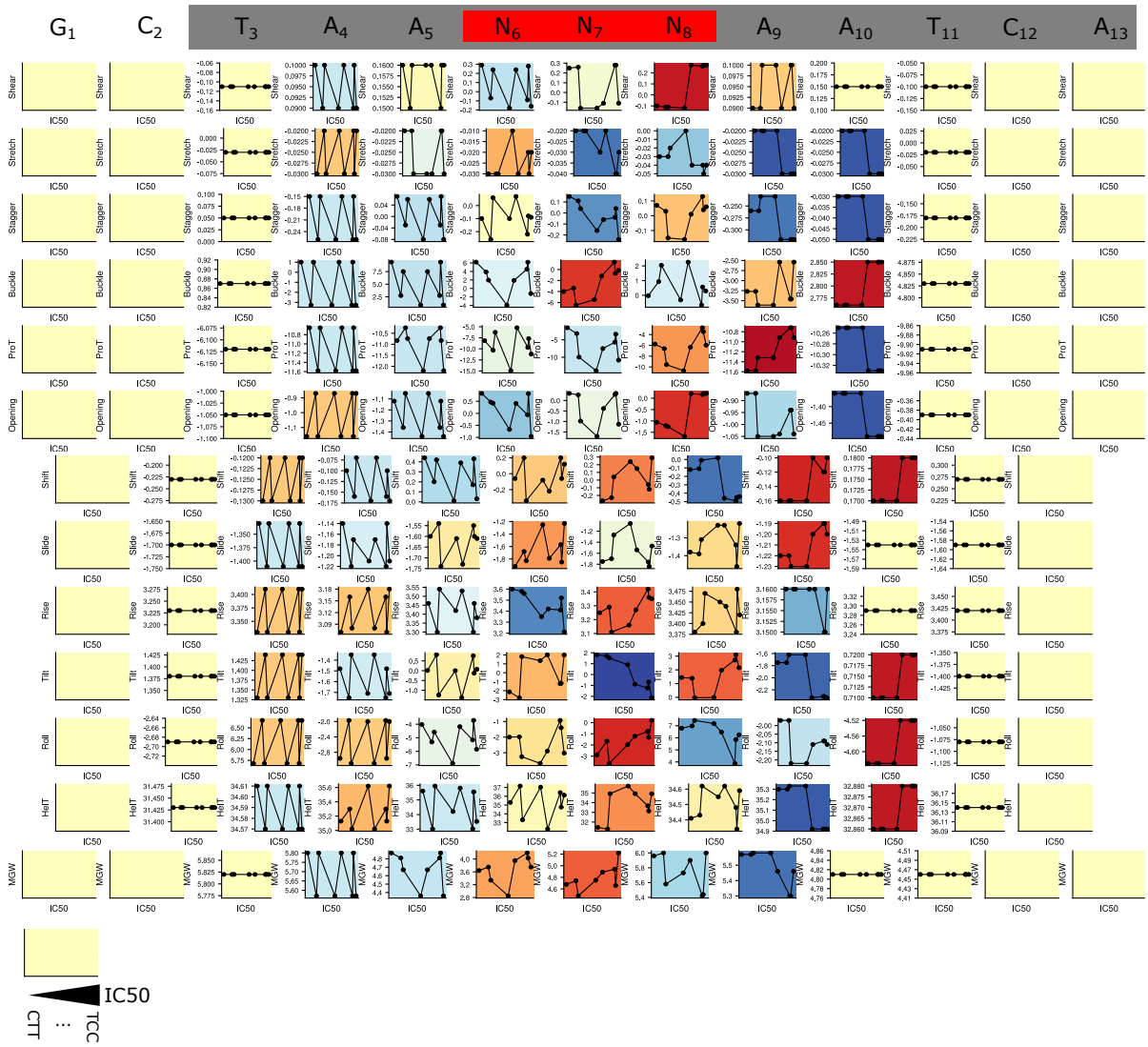
Calculating K_D value of strongest aptamer from the optimization procedure. (A) K_D values was assessed by means of fluorescence polarization assay using 5 nM 5'-end FAM-labelled 5'-GCTAA**CTT**AATCAACCGCAGGTTGATTAG*CCC*ATTAGC-3' in reaction buffer together with different concentrations of StrepII-SUMO-DUX4-His6 for 24 h at ambient temperature. For estimating unspecific binding of the protein, the experiments was also conducted with or without 2,000x excess of unlabelled aptamer, with a scrambled variant of the aptamer, fluorescein to exclude unspecific binding of the fluorophore, buffer without FAM-labelled aptamer to subtract background, and buffer containing 6 M urea to test unspecific binding of the aptamer under denaturating conditions. Data is based on four independent technical replicates using four different recombinant protein batches. (B-C) Gel shift assay of 5 nM of the 5'-IRDye800-labelled DUX4 aptamer and its scrambled variant incubated in EMSA buffer (modified from (28)) containing 100 mM HEPES pH 7.6, 5 mM EDTA, 50 mM $(NH_4)_2SO_4$, 1% Tween20 (W/V), 150 mM KCl, and 11.25% glycerol with different concentrations of StrepII-SUMO-DUX4-His4 and with or without 1 μM unlabelled DUX4 aptamer, #1 antiDUX4 (P4H2) antibody (Novus Biologicals, LLC, Centennial, US-CO), or #2 antiDUX4 [E5-5] (ab124699) antibody (abcam, Cambridge, UK) for 1 h at ambient temperature and run on a 6% non-denaturating acrylamide gel at 150 V for 4 h at ambient temperature in running buffer containing 25 mM Tris pH 8, 190 mM glycine, and 1 mM EDTA. Labelled aptamer was visualized by a Odyssey CLx Imager (LI-COR Bioscience, Lincoln, US-NE). Representative gels are depicted (C) together with the densitometric evaluation (B) using ImageJ software (National Institutes of Health, Bethesda, US-MD). Data was fitted to a four parameter logistic using the R package "drc". Data curve of the bound fraction of the DUX4 aptamer is displayed with the 95% confidence band in gray, n=3.

Supplemental Figure S4



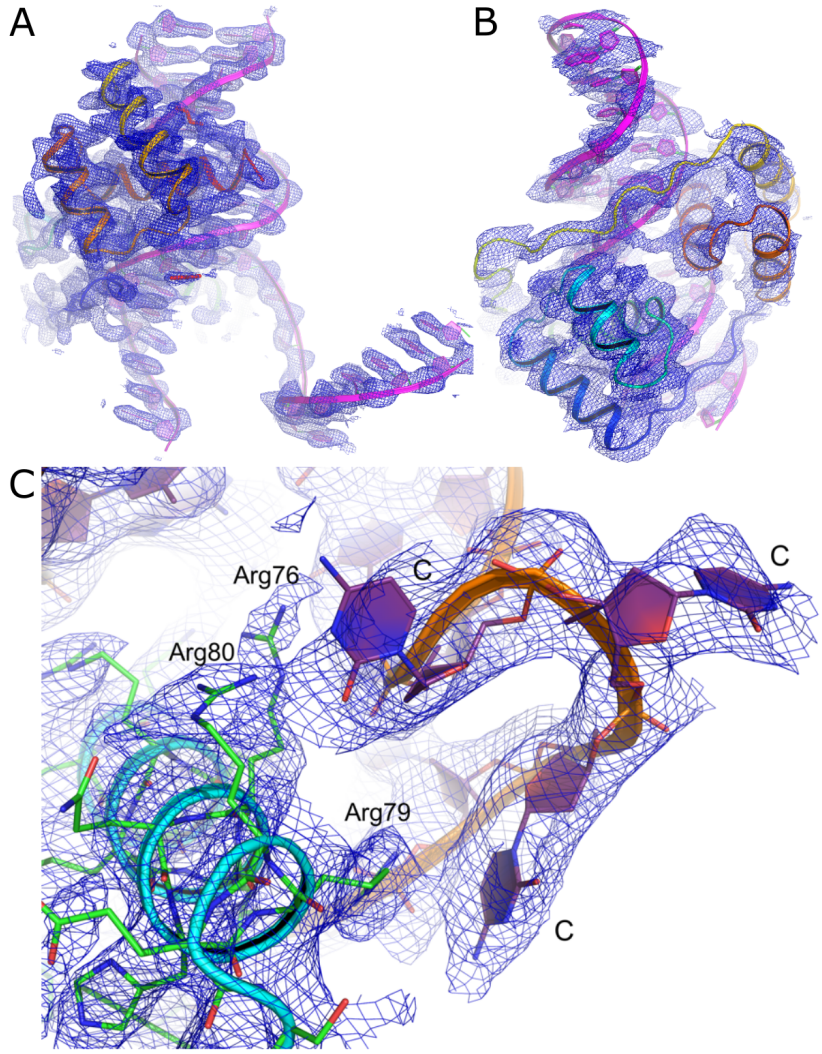
Specificity of DUX4 aptamers. (A) Different aptamers were tested against the consensus PROP1 binding sequence inserted into the aptamer backbone by means of a TR-FRET competition assays. Single experiment data is shown as IC50 values including fitted curves of various aptamers ± standard error of the data fitting to a four-parameter logistic using R package "drc". (B) TR-FRET competition assay was performed to compare the binding of different aptamers to PAX7. (C) TR-FRET competition assay was performed to compare the binding of different aptamers to DUX4. (D) TR-FRET competition assay was performed to compare the affinity of DUX4 aptamers with chemical loop modifications. An unmodified triple-dC loop and an aptamer without loops were compared to aptamers with loop lacking the nucleobases (abasic) and an aptamer with loops having aliphatic C3 spacer (no deoxyribose) between the phosphates. The chemical structures of the loops are depicted below. Single experiment data is shown as IC50 ± standard error of fitting to a four-parameter logistic using R package "drc".

Supplementary Figure S5



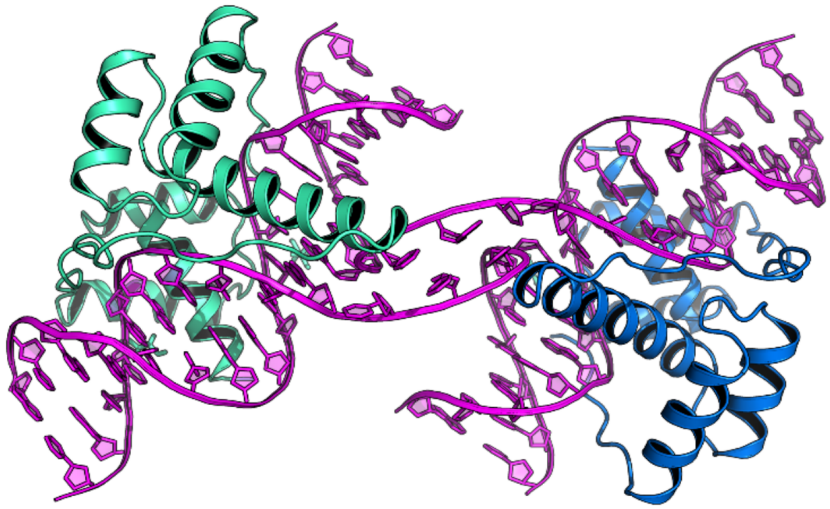
Correlation between DNA shape parameters and DUX4 motif alterations. The less conserved second base triplet of the DUX4 motif has effects on the affinity to DUX4. To explain the effect of the nine triplets (in order of increasing IC50 values: "CCT","TCT","CTT","TTT","CTC","TCC","CCC","TTC") on the affinity, pearson correlation coefficients between predicted DNA shape parameters and the IC50 values of the triplet permutations described in this work were calculated and depicted in this matrix as color code from blue (negative correlation) to red (positive correlation) with yellow as no correlation. The graphs show the correlation between the DNA shape parameters (ordinate in [°] or in [Å] for MGW) and the IC50 values (abscissa, triplets sorted by increasing IC50 values) at base position 1 to 13 of the DUX4 aptamer containing the DUX4 motif at 3-13 and the altered triplet from 6 to 8.

Supplemental Figure S6



Electron density maps for the two distinct DUX4-DNA complex structures. (A) Strand-swapped, Holliday junction-like structure obtained with the blunt-ended DNA with CCC insertion refined to 2.3 Å resolution. The σA-weighted 2mFo-DFc electron density map contoured at 1.0σ is shown in blue mesh, DNA molecule in magenta, and DUX4 protein in rainbow color. One protein molecule with the bound DNA contained the asymmetric unit of the crystal is shown. (B) The complex with the DNA aptamer with CCC bulge and GCA hairpin, refined to 3.2 Å. The σA-weighted 2mFo-DFc electron density map contoured at 1.0σ is overlaid on the structure. (C) A close-up view centered on the CCC bulge and the interacting α3 helix. The arginine side chains that make contacts with the DNA bulge are labelled.

Supplemental Figure S7



Holliday junction-like conformation of DUX4 together with the DNA aptamer. Crystal structure of the DUX4 double homeodomain bound to a blunt-ended DNA with the trinucleotide (-CCC-) insertion, observed in a strand-swapped, Holliday junction-like conformation. Instead of forming a bulge, the CCC segment served as a cross-over linker. DNA strands are colored in magenta and DUX4 in green and blue respectively.