Supplementary Information

Rational Design of Antisense Oligonucleotides Targeting Single Nucleotide Polymorphisms for Potent and Allele Selective Suppression of Mutant Huntingtin in the CNS

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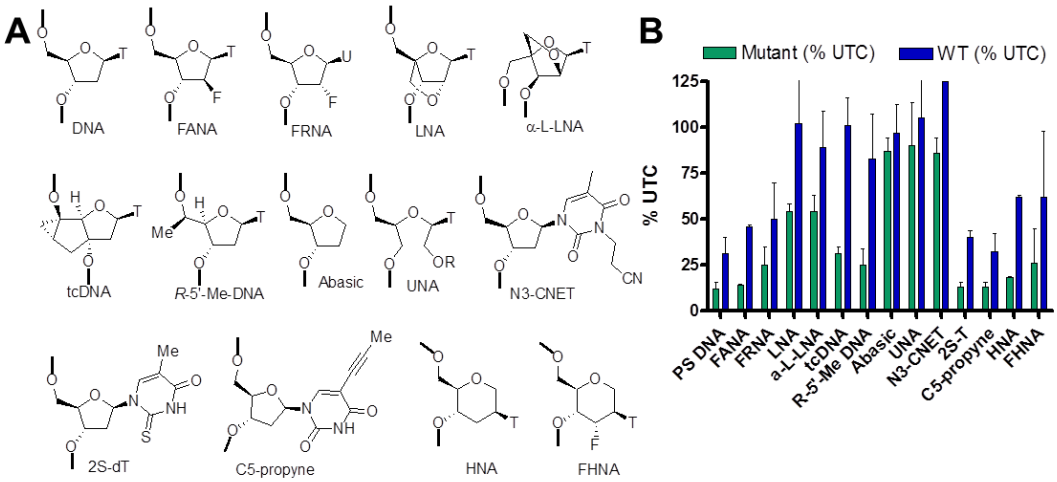
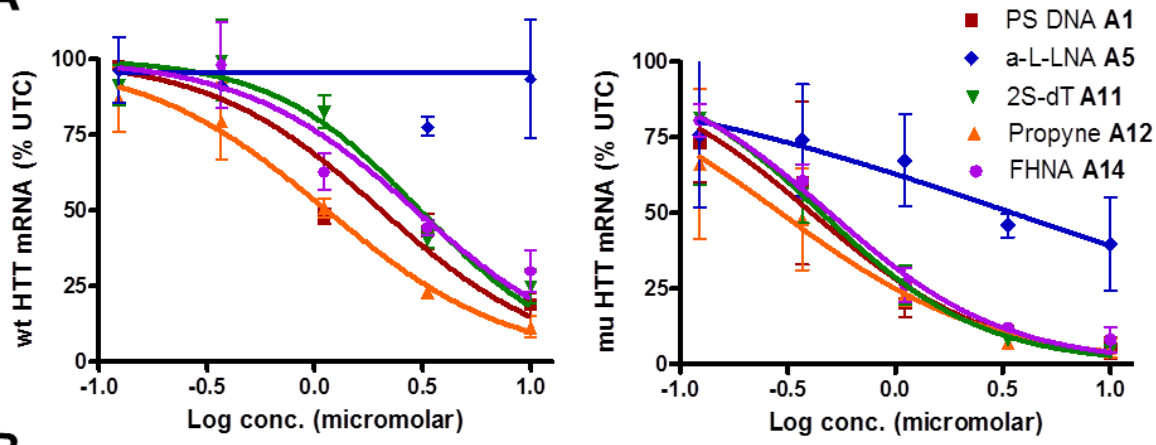


C

Mod.	ASO	Sequence (5′ to 3′)	T_m mu	T_m wt	ΔT_m (mu-wt)	Ratio
PS DNA	**A1**	TAAATTGTCATCACC	53.7	52.2	1.5	2.6
FANA	**A2**	TAAATTGTCATCACC	53.8	52.5	1.3	3.4
FRNA	**A3**	TAAATTGTCATCACC	54.4	51.8	2.6	2.0
LNA	**A4**	TAAATTGTCATCACC	57.7	55.3	2.4	1.9
α-L-LNA	**A5**	TAAATTGTCATCACC	56.5	54.5	2.0	1.6
tcDNA	**A6**	TAAATTGTCATCACC	53.8	51.7	2.1	3.3
R-5'MeDNA	**A7**	TAAATTGTCATCACC	51.2	49.7	1.5	3.3
Abasic	**A8**	TAAATTGTCATCACC	43.1	43.8	-0.7	1.1
UNA	**A9**	TAAATTGTCATCACC	44.7	42.1	2.6	1.2
N^3-CNET	**A10**	TAAATTGTCATCACC	42.8	43.1	-0.3	1.6
2S-dT	**A11**	TAAATTGTCATCACC	53.8	49.1	***4.7***	3.1
C5-Propyne	**A12**	TAAATTGTCATCACC	54.1	50.8	***3.3***	2.5
HNA	**A13**	TAAATTGTCATCACC	55.4	50.5	***4.9***	3.5
FHNA	**A14**	TAAATTGTCATCACC	56.1	50.4	***5.7***	2.8

Supplementary Figure S1. Introducing chemical modifications into the ASO across from the SNP site does not improve allele selectivity substantially (A) Structures of modifications investigated. (B) Evaluation of ASOs **A1**-**A14** in GM4022 fibroblasts at a single dose of 2 μM delivered by electroporation. (C) Summary of sequence, modification patterns, T_m of matched versus mismatched RNA and ratio of wt*HTT*/mu*HTT* mRNA reduction in human GM4022 fibroblasts. Black letters indicate DNA, orange letters indicate MOE and blue nucleotides indicate *S*-cEt nucleotides, underlined red letter is the modified nucleotide across from the SNP, all internucleosidic linkages are phosphorothioate. All error bars are in ±std. dev.

A

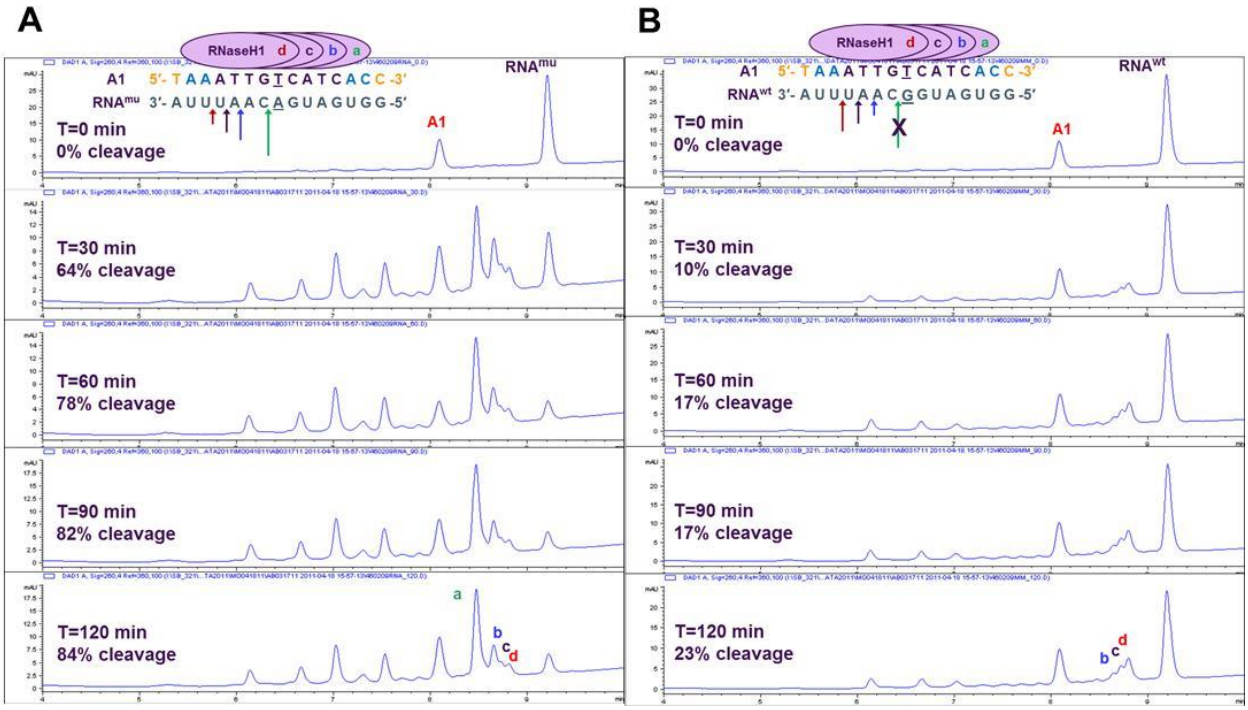


B

ASO	Sequence (5' to 3')	Chemistry	IC_{50} (μM)	Fold Select.	*T*m (Mu)	*T*m (WT)	Δ*T*m (Mu-WT)
A1	TAAATTGTCATCACC	PS DNA	0.41	5.0	53.7	52.2	1.5
A5	TAAATTGTCATCACC	α-L-LNA	3.5	>3	56.5	54.5	2.0
A11	TAAATTGTCATCACC	2S-dT	0.45	6.9	53.8	49.1	4.7
A12	TAAATTGUCATCACC	C5-propyne	0.29	3.8	54.1	50.8	3.3
A14	TAAATTGTCATCACC	FHNA	0.5	6.0	56.1	50.4	5.7

Supplementary Figure S2. Improved discrimination of GT wobble base-pair does not improve allele selectivity. (A) Dose dependent reduction of mu*HTT* mRNA and wt*HTT* mRNA using ASOs **A1**, **A5**, **A11**, **A12** and **A14** in human GM4022 fibroblasts. (B) Sequence, chemical design, potency and selectivity in cell culture, and T_m versus matched and mismatched RNA, of ASOs **A1**, **A5**, **A11**, **A12** and **A14.**

Supplementary Figure S3. (**A and B**)Time course for cleavage of **A1/RNA^{mu}** and **A1/RNA^{wt}** heteroduplexes by recombinant human RNase H1, using LCMS to monitor reaction progress. (**C**) Precise identity of cleavage fragments identified from LCMS assay (-4 charge state). Bold underlined nucleotide represents the SNP site while the nucleotides in blue represent the two nucleotide shift between cleavage sites “a” and “b”.



C.

Cleavage of fully complementary **RNA^{mu}** 5'-AGACUUUUUCUGGUGAUG**A**CAAUUUAUUAA

Cleavage	Fragment sequence	m/z found	m/z calculated	MW (Da)
a	5’-AGACUUUUUCUGGUGAUG**A**-3’	1509.1	1508.9	6039.8
	5’-AGACUUUUUCUGGUGAUG**A**C-3’	Not found	1585.2	6344.9
b	5’-AGACUUUUUCUGGUGAUG**A**CA-3’	1667.8	1667.5	6674.1
c	5’-AGACUUUUUCUGGUGAUG**A**CAA-3’	1750.0	1749.8	7003.2
d	5’-AGACUUUUUCUGGUGAUG**A**CAAU-3’	1825.9	1826.3	7309.4

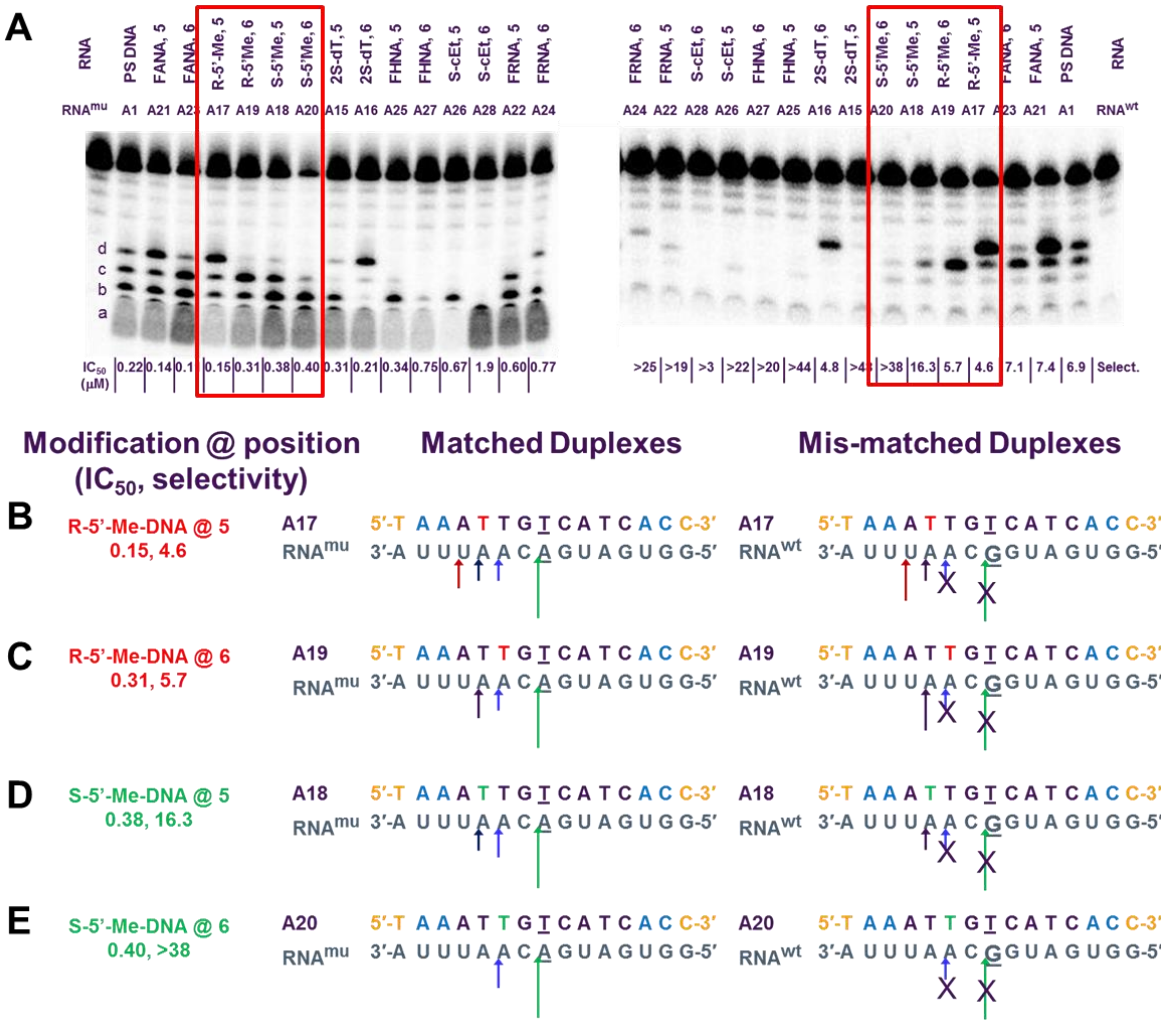
Cleavage of mismatched **RNA^{wt}** 5'-AGACUUUUUCUGGUGAUG**G**CAAUUUAUUAA

Cleavage	Fragment sequence	m/z found	m/z calculated	MW (Da)
a	5’-AGACUUUUUCUGGUGAUG**G**-3’	not found	1508.9	6039.8
	5’-AGACUUUUUCUGGUGAUG**G**C-3’	not found	1589.2	6360.9
b	5’-AGACUUUUUCUGGUGAUG**G**CA-3’	1671.2	1671.5	6690.1
c	5’-AGACUUUUUCUGGUGAUG**G**CAA-3’	1755.0	1753.8	7019.2
d	5’-AGACUUUUUCUGGUGAUG**G**CAAU-3’	1831.3	1830.3	7325.3

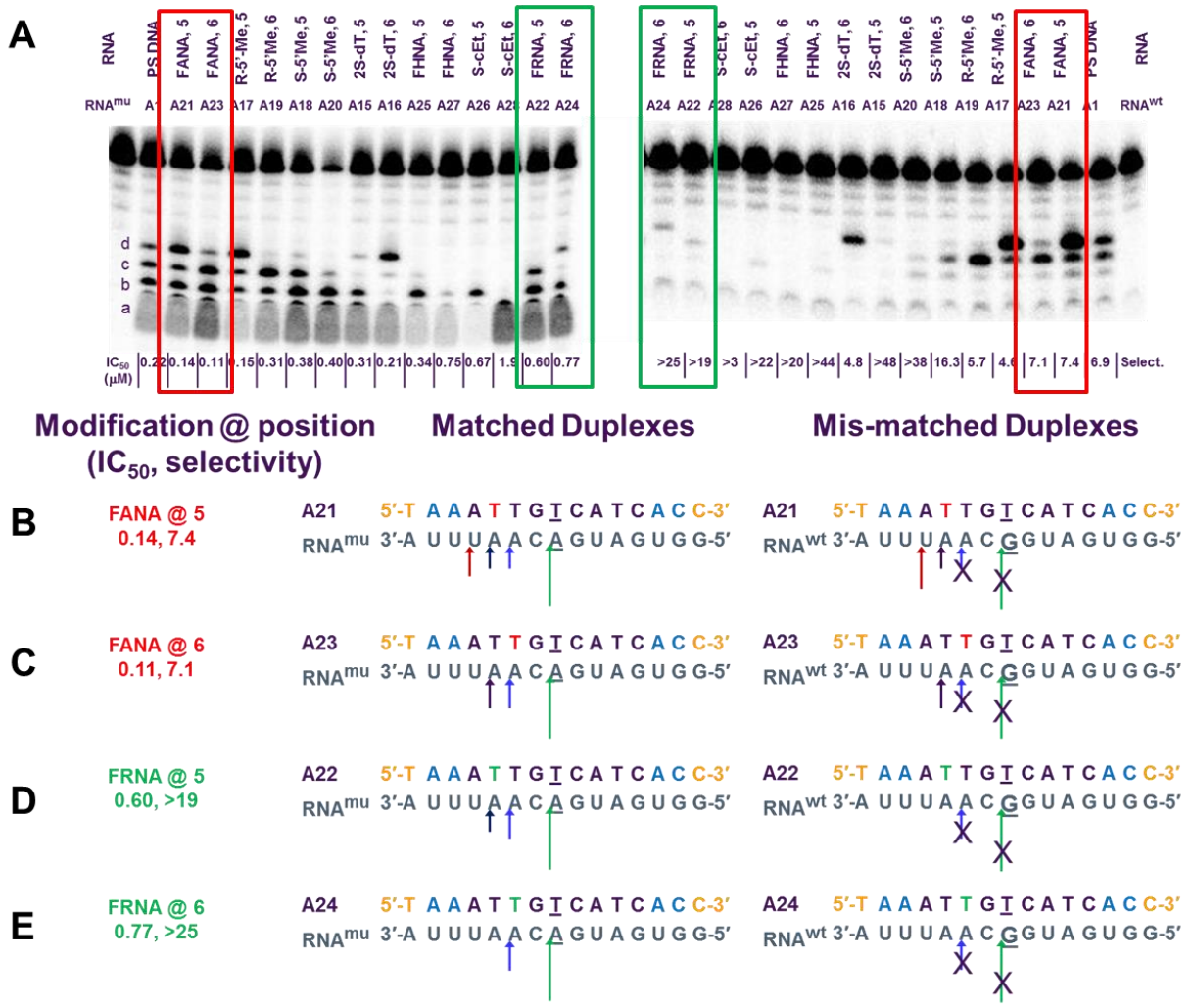
Supplementary Figure 4. T_m of selected ASOs versus matched and mismatched RNA showing that chemical modification at positions 5 and 6 do not alter binding affinity of the ASO for the matched and mismatched RNA.

Mod.	ASO	Sequence (5′ to 3′)	T_m (mu)	T_m (wt)	IC_{50} (μM) mu*HTT*	Fold Select.
PS DNA	**A1**	**TAAATTGTCATCACC**	53.7	52.2	0.29	6.9
2S-dT	**A16**	**TAAATTGTCATCACC**	54.8	52.8	0.31	>48
2S-dT	**A17**	**TAAATTGTCATCACC**	55.6	53.9	0.21	4.8
R-5'Me-DNA	**A18**	**TAAATTGTCATCACC**	52.2	50.0	0.15	4.6
R-5'Me-DNA	**A19**	**TAAATTGTCATCACC**	52.0	49.7	0.31	5.7
FANA	**A22**	**TAAATTGTCATCACC**	53.4	51.3	0.14	7.4
FANA	**A23**	**TAAATTGTCATCACC**	55.0	53.3	0.11	7.1

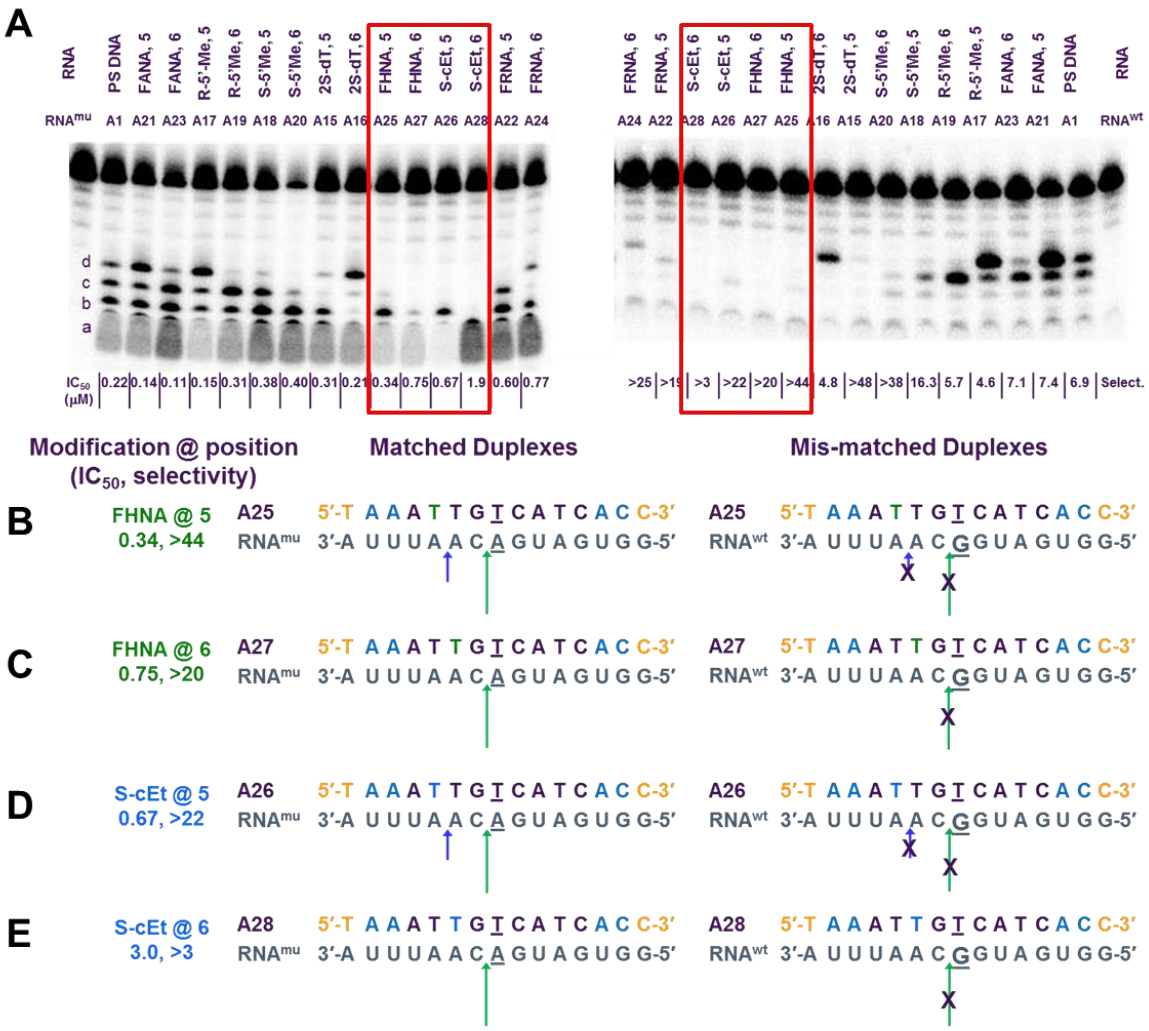
Black letters indicate DNA, orange letters indicate MOE and blue nucleotides indicate *S*-cEt nucleotides, underlined letter is the nucleotide across from the SNP site, red letter indicates site of chemical modification. All internucleosidic linkages are phosphorothioate.



Supplementary Figure S5. Rationale for improved allele selectivity observed with *S*-5'-Me-DNA modified ASOs **A18** and **A20** versus *R*-5'-Me-DNA ASOs **A17** and **A19**. (A) Mapping human RNase H cleavage patterns for modified ASOs versus matched (**RNA^{mu}**) and mismatched (**RNA^{wt}**) RNA using gel electrophoresis. Pictorial representation of RNase H1 cleavage patterns versus matched (**RNA^{mu}**) and mismatched (**RNA^{wt}**) RNA for (B) **A17** with *R*-5'-Me-DNA at position 5 (C) **A19** with *R*-5'-Me-DNA at position 6 (D) **A18** with *S*-5'-Me-DNA at position 5 (E) **A20** with *S*-5'-Me-DNA at position 6.



Supplementary Figure S6. Rationale for improved allele selectivity observed with FRNA modified ASOs **A22** and **A24** versus FANA modified ASOs **A21** and **A23**. (A) Mapping RNase H1 cleavage patterns for modified ASOs versus matched (**RNA^{mu}**) and mismatched (**RNA^{wt}**) RNA using gel electrophoresis. Pictorial representation of RNase H1 cleavage patterns versus matched (**RNA^{mu}**) and mismatched (**RNA^{wt}**) RNA for (B) **A22** with FANA at position 5 (C) **A24** with FANA at position 6 (D) **A22** with FRNA at position 5 (E) **A24** with FRNA at position 6.



Supplementary Figure S7. Rationale for improved allele selectivity observed with FHNA (**A25** and **A27**) and *S*-cEt (**A26** and **A28**) modified ASOs. (a) Mapping RNase H1 cleavage patterns for modified ASOs versus matched (**RNA^{mu}**) and mismatched (**RNA^{wt}**) RNA using gel electrophoresis. Pictorial representation of RNase H1 cleavage patterns versus matched (**RNA^{mu}**) and mismatched (**RNA^{wt}**) RNA for (b) **A25** with FHNA at position 5 (c) **A27** with FHNA at position 6 (D) **A26** with *S*-cEt at position 5 (E) **A28** with *S*-cEt at position 6.