

## Supplementary Information

### **Allele-Selective Inhibition of Mutant Huntingtin Expression with Antisense Oligonucleotides Targeting the Expanded CAG Repeat**

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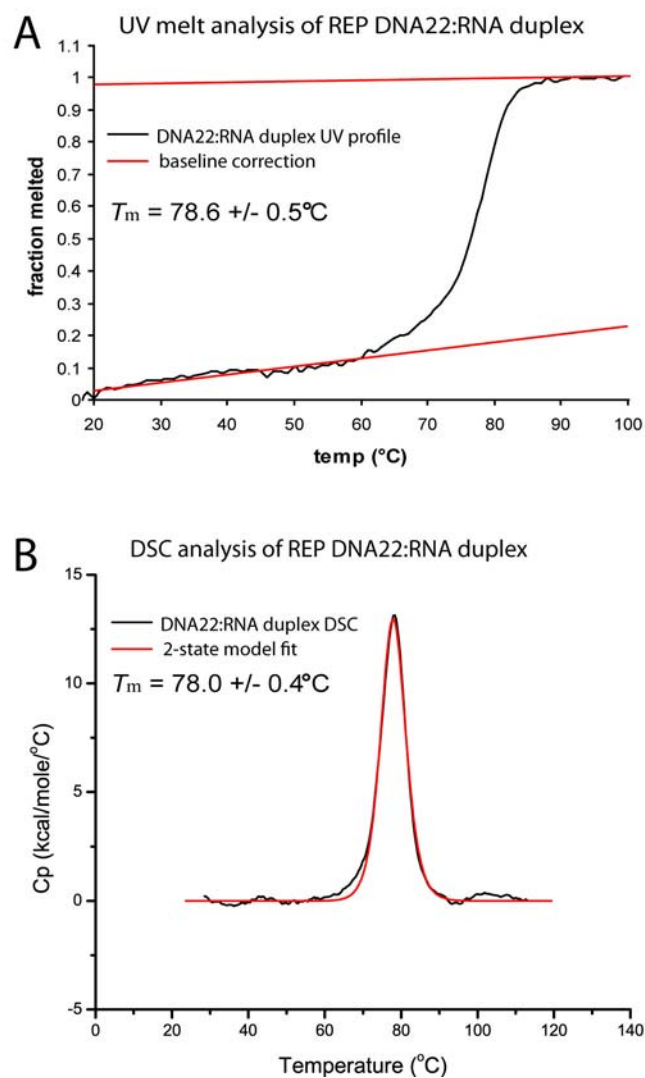
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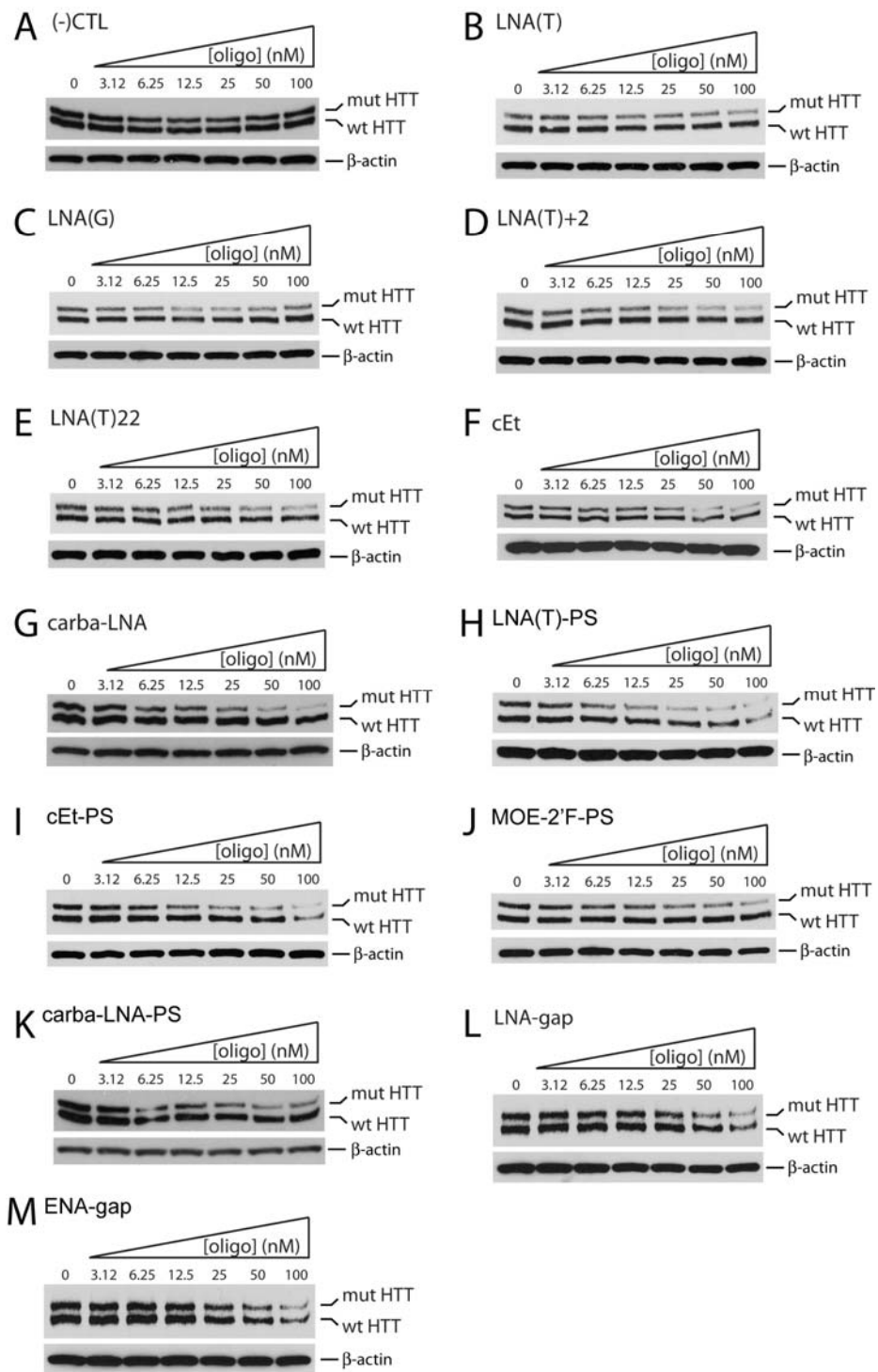
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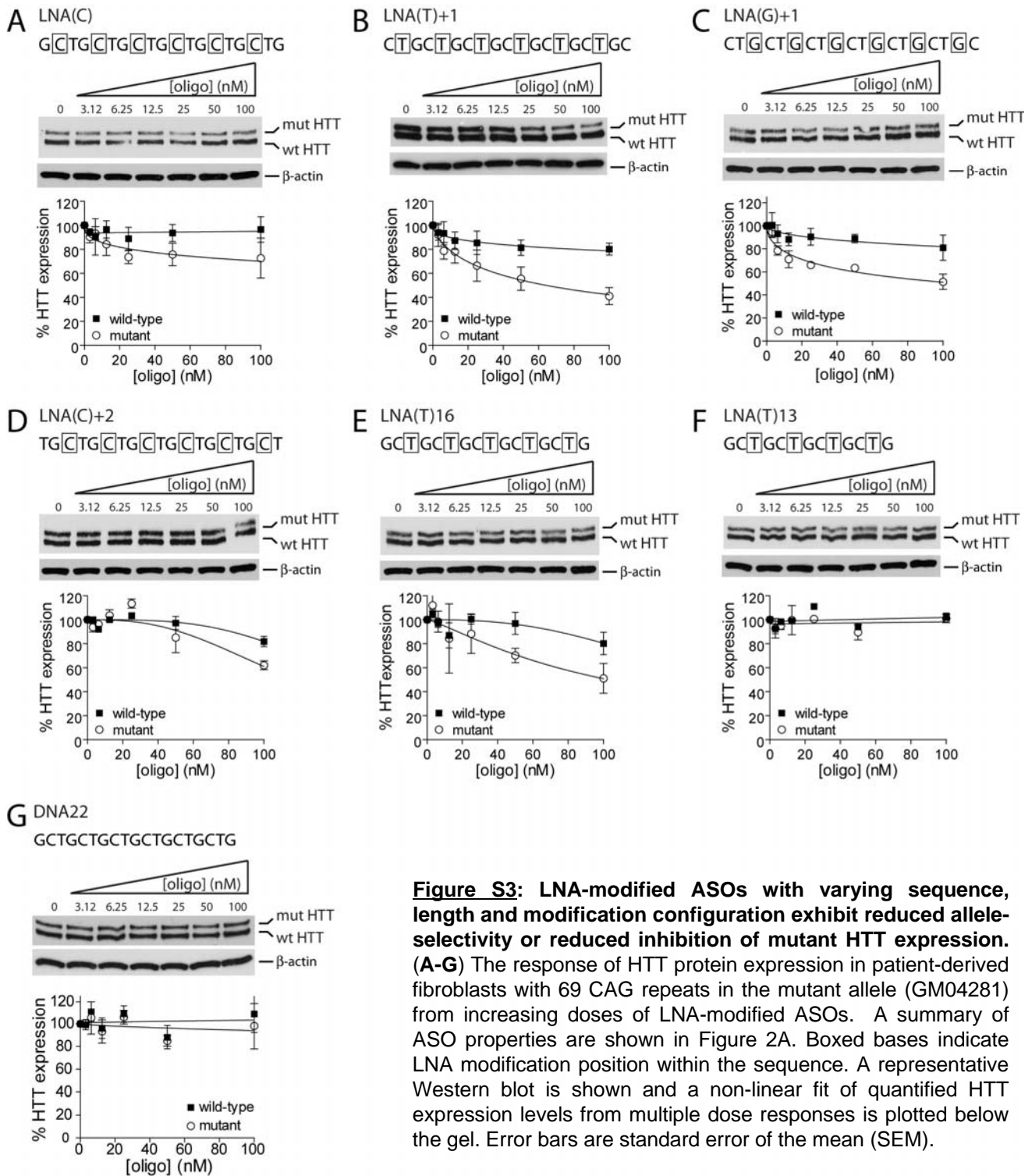
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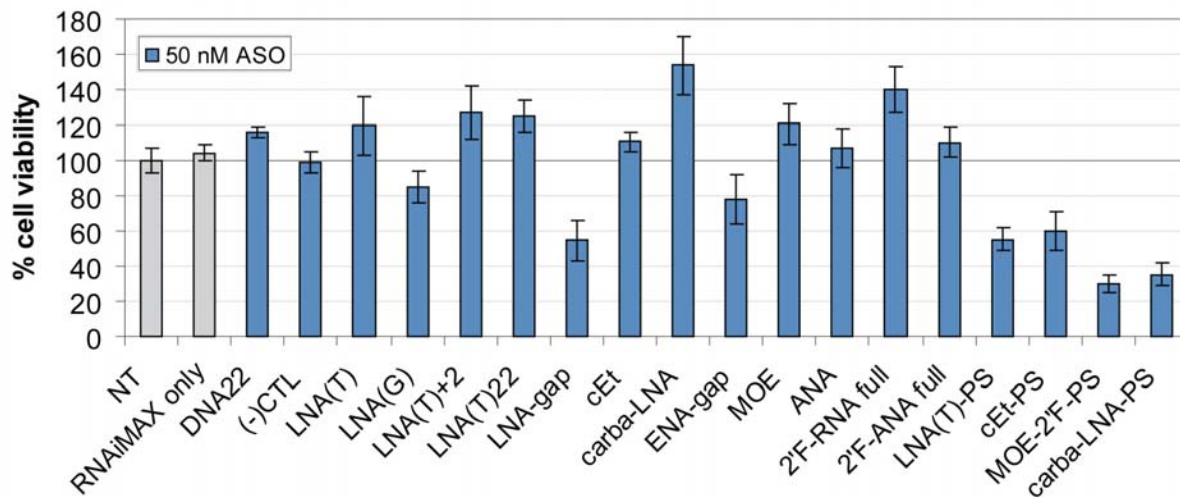


**Figure S1: UV melt and differential scanning calorimetry (DSC) for deriving ASO and ASO:RNA duplex melting temperatures.** Thermal denaturation of a DNA22:RNA duplex monitored by UV absorbance (**A**) or DSC (**B**).  $T_m$  is reported as mean +/- standard error of the mean (SEM) in panel A or as the temperature at maximum Cp +/- deviation from a 2-state unfolding model fit in panel B.

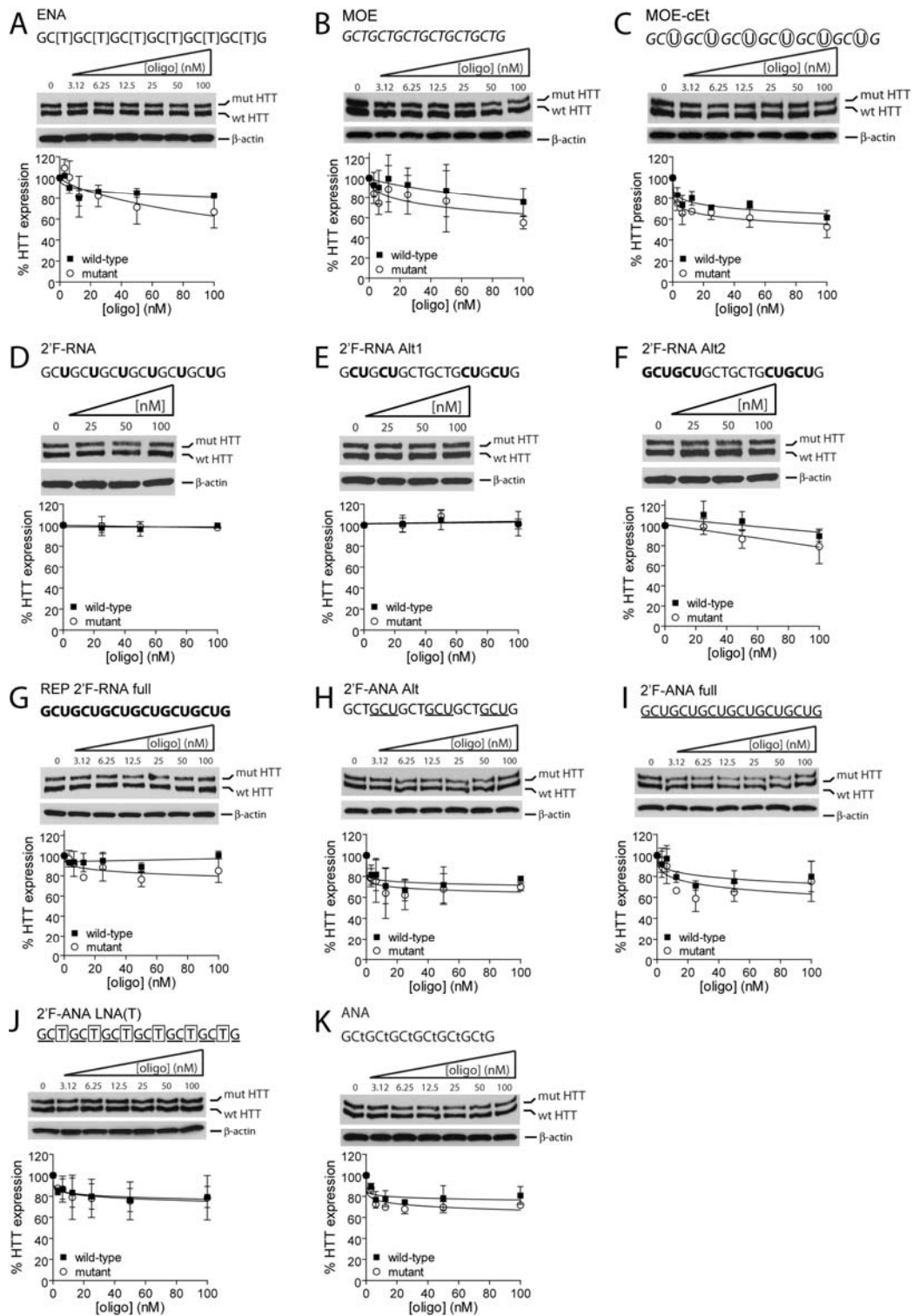


**Figure S2: Representative Western blots for quantification of HTT inhibition in patient-derived cells with 69 CAG repeats.** (A-M) The response of HTT protein expression in patient-derived fibroblasts with 69 CAG repeats in the mutant allele (GM04281) from increasing doses of allele-selective ASOs. Western blots are representative of 3 or more replicates and were used for quantification and IC<sub>50</sub> calculations presented in Figures 2, 3 and 4.

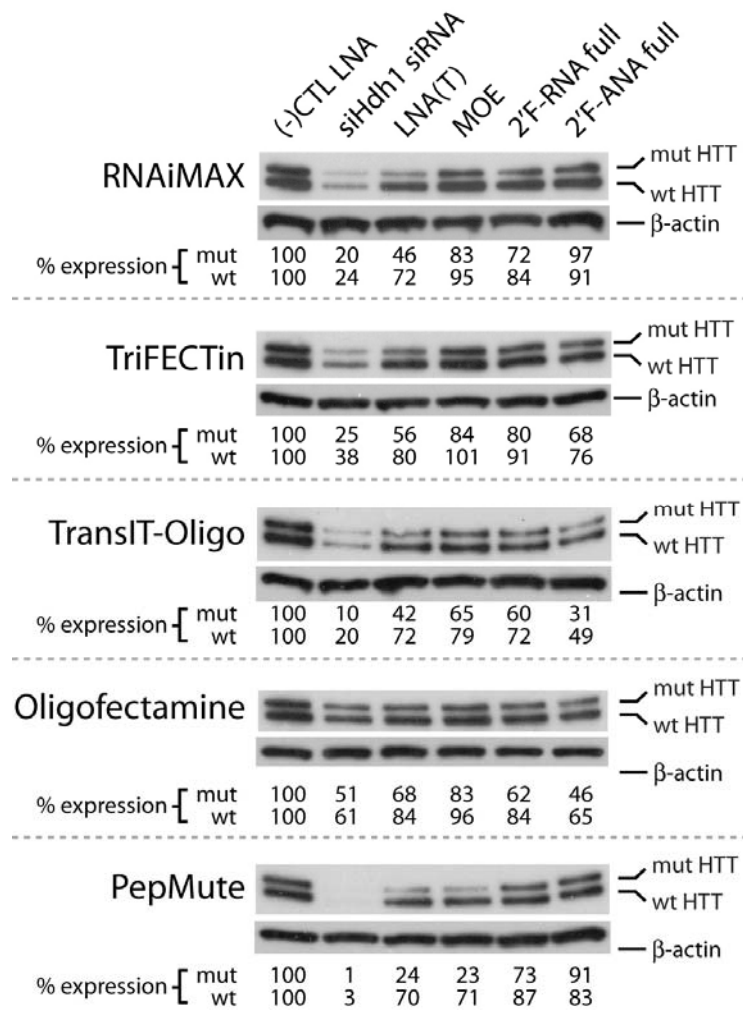




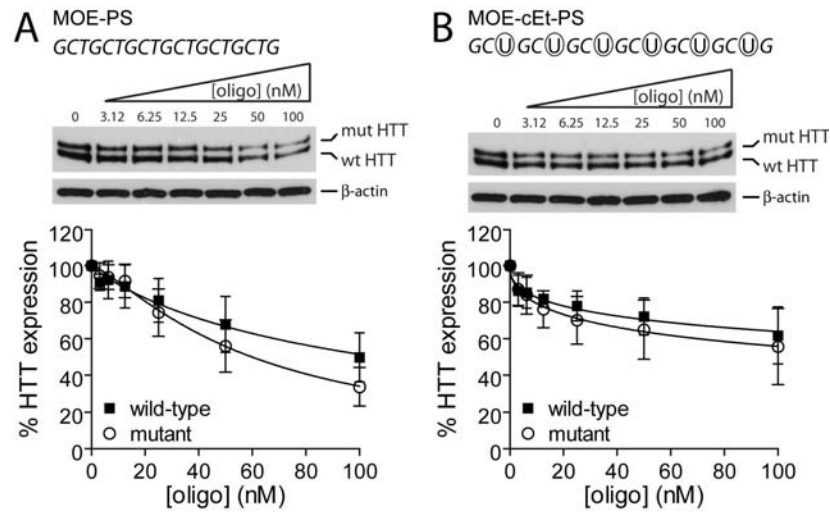
**Figure S4: Viability of patient-derived cells by MTS assay after treatment with various ASOs.** Patient-derived fibroblasts (GM04281) were transfected with 50 nM ASO and Lipofectamine RNAiMAX (see Methods) then assayed for cell viability 4 days after transfection with a CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (MTS) following the manufacturer's protocol. The assay was performed in triplicate. Error bars are standard deviation. NT, non-transfected; RNAiMAX only, cells treated with Lipofectamine RNAiMAX transfection reagent.



**Figure S5: ASOs with a variety of modifications known to enhance nucleic acid hybridization and improve nuclease resistance show little or no selective inhibition of mutant HTT expression. (A-L)** The response of HTT protein expression in patient-derived fibroblasts with 69 CAG repeats in the mutant allele (GM04281) from increasing doses of modified ASOs. Position of nucleotide modifications within the sequence are indicated as follows: boxed, LNA; circled, cET; bracketed, ENA; italicized, MOE; lowercase, ANA; bold, 2'F-RNA; underlined, 2'F-ANA. A representative Western blot is shown and a non-linear fit of quantified HTT expression levels from multiple dose responses is plotted below the gel. Error bars are standard error of the mean (SEM).

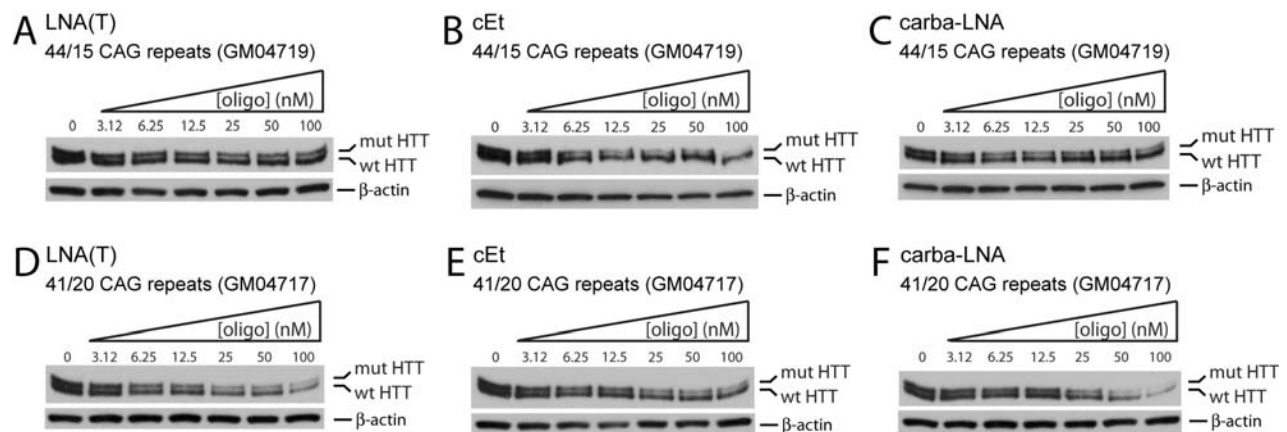


**Figure S6: Side-by-side testing of different transfection reagents with oligonucleotides substituted with various chemical modifications.** Antisense oligonucleotides representing distinct chemistries were transfected into patient-derived fibroblasts (GM04281) with different transfection reagents. HTT protein expression was assayed 4 days after transfection by Western blot analysis (see Methods). Expression of mutant (mut) and wild-type (wt) HTT was quantified and is shown beneath each blot as percent expression.

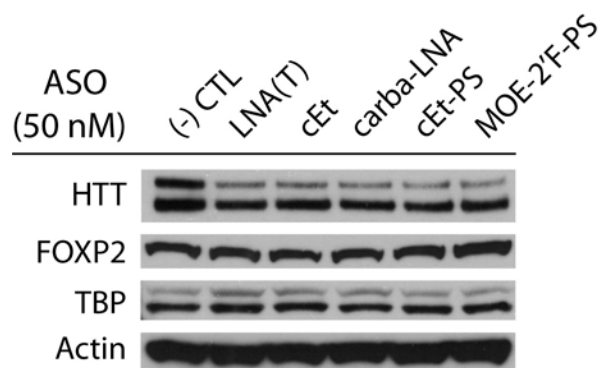


**Figure S7: Two non-selective ASOs modestly inhibit HTT expression when uniformly modified with a phosphorothioate (PS) backbone. (A-B)** The response of HTT protein expression in patient-derived fibroblasts with 69 CAG repeats in the mutant allele (GM04281) from increasing doses of modified ASO. MOE and cEt modifications are indicated as italicized or circled within the ASO sequence, respectively. A representative Western blot is shown and a non-linear fit of quantified HTT expression levels from multiple dose responses is plotted below the gel. Error bars are standard error of the mean (SEM).





**Figure S8: Representative Western blots for quantification of HTT inhibition in patient-derived fibroblasts with 44 or 41 CAG repeats. (A-M)** The response of HTT protein expression in patient-derived fibroblasts with 44 (GM04719) or 41 (GM04717) CAG repeats in the mutant allele from increasing doses of allele-selective ASOs. Western blots are representative of 3 or more replicates and were used for quantification and  $IC_{50}$  calculations presented in Figure 5.



**Figure S9: ASOs targeted to CAG repeats are specific for mutant HTT at concentrations that elicit allele-selective inhibition.** Western blots showing expression of endogenous CAG-repeat containing genes after treatment with 50 nM ASO in patient-derived fibroblasts containing 69 CAG repeats (GM04281). ASO treatment is indicated above each lane and the protein probed by Western blot is indicated to the left.

**A**

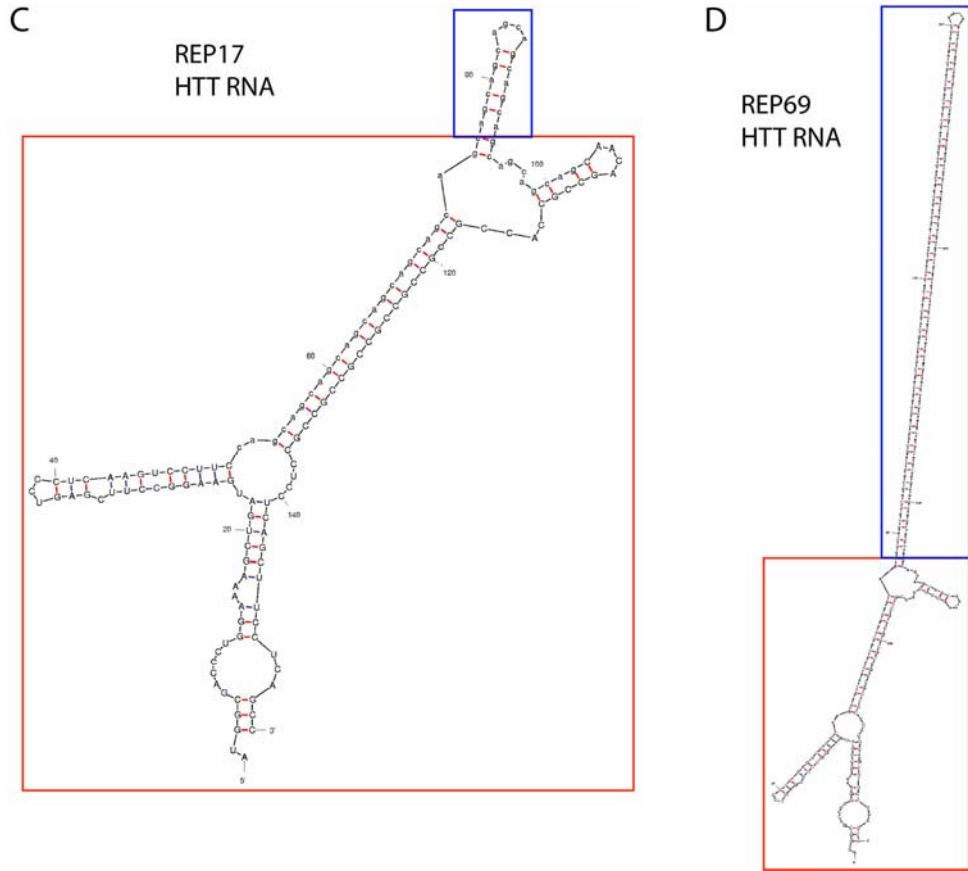
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      10      20      30      40      50      60      70      80
REP17 HTT RNA  ATGGCGACCC TGGAAAAGCTGATGAAGGCCTTCGAGTCCCTCAAGTCC TTCcagcagcagcagcagcagcagcagcagca
      90      100     110     120     130     140     150
REP17 HTT RNA  gcagcagcagcagcagcagcagcagcagCAACAGCCGCCACCGCCGCCGCCGCCGCCGCCGCCCTCCTCAGCTTCCTCAGCC
  
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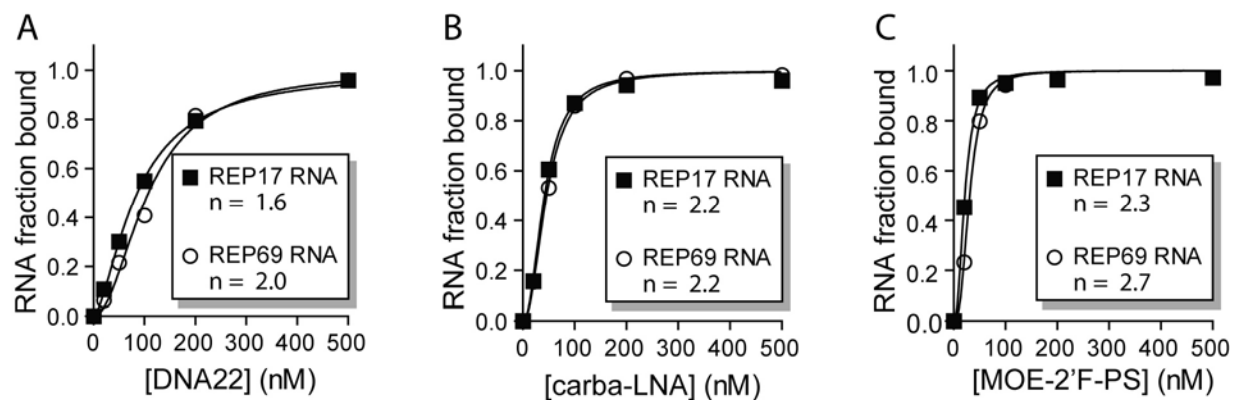
**B**

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      10      20      30      40      50      60      70      80
REP69 HTT RNA  ATGGCGACCC TGGAAAAGCTGATGAAGGCCTTCGAGTCCCTCAAGTCC TTCcagcagcagcagcagcagcagcagcagca
      90      100     110     120     130     140     150     160
REP69 HTT RNA  gcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagc
      170     180     190     200     210     220     230     240
REP69 HTT RNA  agcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcag
      250     260     270     280     290     300     310
REP69 HTT RNA  cagcagcagcagcagcagcagCAACAGCCGCCACCGCCGCCGCCGCCGCCGCCGCCCTCCTCAGCTTCCTCAGCC
  
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**Figure S10: Sequence and predicted secondary structure of 5'-end transcripts of HTT mRNA.** Truncated normal (A) and mutant (B) HTT mRNA sequences *in vitro* transcribed for use in RNase H assays and EMSA. REP17 contains 17 CAG repeats and was derived from the normal HTT mRNA while REP69 contains 69 CAG repeats and was derived from the mutant HTT mRNA. The CAG repeat sequence is shown in lowercase. Clones were sequenced to verify sequence accuracy. Secondary structure prediction for REP17 (C) and REP69 (D) RNA using mFold. Both are predicted to contain a conserved structure flanking the CAG repeat sequence (red box). The CAG repeat in REP17 is predicted to form a short hairpin whereas the CAG repeat expansion in REP69 is predicted to form an extended hairpin (blue box).



**Figure S11: Multiple ASOs appear to cooperatively bind the HTT 5'-end RNA transcripts REP17 and REP69.** (A-B) Quantification of DNA22, carba-LNA and MOE-2'F-PS binding to REP17 and REP69 HTT mRNA transcripts indicates cooperative ASO binding. ASO-bound RNA in gel shifts from panels A and B of figure 8 were quantified and plotted as a function of concentration. Fitting to the Hill equation revealed sigmoidal binding curves and Hill coefficients ( $n$ ) near 2, suggesting cooperative ASO binding, even for the unmodified, native DNA oligonucleotide DNA22.