**Supplementary Table 1.** PNA, duplex RNAs, and LNA oligomers used in these studies.

| Name       | Sequence (length) |
|------------|------------------|
| **PNA-peptide conjugates** | |
| REP19      | K-GCTGCTGCTGCTGCTGCTG-K (19) |
| REP19N     | K-GCTGCTGCTGCTGCTGCTG-K (19) |
| REP19Arg   | K-GCTGCTGCTGCTGCTGCTG-R (19) |
| REP16      | K-GCTGCTGCTGCTGCTGCTG-K (16) |
| REP13      | K-GCTGCTGCTGCTG-K (13) |
| 5J/HTT     | K-GCTGCTGCTGAAAGGACTT-K (19) |
| 3J/HTT     | K-GCCGCCTGTGCTGCTGCTK (19) |
| +CTL       | K-GCTTTTCCAGGGTCGCCAT-K (19) |
| -CTL1      | K-GCTATACCCAGGTCGTCAT-K (19) |
| -CTL2      | K-ACCTACTGTCCTCGGCACCA-K (20) |
| 5J/ATX     | K-GCTGCTGCTGCTGCTGCTG-K (19) |
| 3J/ATX     | K-ATAGGTCCTCCTGCTGCTG-K (19) |
| **siRNAs** | |
| siRNA/REP  | GCUGCUGCUCCUGUCUCUGTT (21) |
| siRNA/5J   | GCUGCUGCUUGAAAGCCUUTT (21) |
| siRNA/3J   | GCGGCUGUUCUGUCUGCUTT (21) |
| siRNA/+CTL | GCUUUUCCAGGGUCCGCAUUTT (21) |
| siRNA/-CTL1| GCUAUACCAGGCUGUCAUUTT (21) |
| siRNA/-CTL2| GACGCGGUUGCUACUGUGTT (21) |
| siRNA/S4   | GAGGAAGAGGAGGAGCAGCATT (23) |
| **LNAs**   | |
| LNA/REP    | gCTgcTgcTgcTgcTgcTgcTgcTgcTgcTgcTgc (19) |
| LNA/5J     | gcTgcTgcTgcAagGacTcTc (19) |
| LNA/3J     | ggCggCttTgcTttGctgGctg (19) |
| LNA/+CTL   | gcTttTccAagGtcGgcAt (19) |
| LNA/-CTL   | gCTatAccAqcGtcGtcAt (19) |

PNAs are listed N to C terminal. siRNAs (antiense strands only) and LNAs are listed 5' to 3'. D-amino acids are used in all peptide conjugates. K=lysine, R=arginine. Mismatched bases are underlined. For LNAs, modified bases are represented as capital letters and DNA bases are lower case.
**Supplementary Table 2.** IC$_{50}$ values (µM) and statistical significance for inhibition of mutant and wild-type alleles of HTT and ataxin-3 in varied cell lines.

|                   | Rep19/wt | Rep19/mut | REP19N/wt | REP19N/mut | 3J/HTT/wt | 3J/HTT/mut | Rep16/wt | Rep16/mut | Rep13/wt | Rep13/mut | Rep19Arg/wt | Rep19Arg/mut | LNA/REP/wt | LNA/REP/mut | LNA/3J/wt | LNA/3J/mut | SiRNA/REP/wt | SiRNA/REP/mut | SiRNA/5J/wt | SiRNA/5J/mut |
|-------------------|----------|-----------|-----------|------------|-----------|------------|----------|-----------|----------|-----------|-------------|---------------|------------|-------------|----------|-----------|--------------|--------------|------------|-------------|
| **Inhibition of HTT** |          |           |           |            |           |            |          |           |          |           |              |                |            |              |          |           |              |              |            |              |
| GM04281           | 1.2 ± 0.3 | 0.34 ± 0.03 | > 16      | 2.1 ± 0.5  | 4.8 ± 1.8 | 0.96 ± 0.13 | 1.4 ± 0.3 | 0.39 ± 0.11 | 2.2 ± 0.4 | 0.47 ± 0.18 | 0.64 ± 0.08 | 0.33 ± 0.04  | > 0.1       | 0.017 ± 0.007 | > 0.1    | 0.086 ± 0.038 | 0.013 ± 0.005 | 0.005 ± 0.003 | 0.055 ± 0.023 | 0.018 ± 0.010 |
| GM04869           | 1.2 ± 0.4 | 0.58 ± 0.07 | > 8       | 2.3 ± 0.3  | n.d.      | n.d.       | n.d.     | n.d.      | n.d.     | n.d.      | n.d.         | n.d.          | n.d.       | n.d.        | n.d.     | n.d.       | n.d.         | n.d.          | n.d.       | n.d.        |
| GM04719           | 1.2 ± 0.4 | 0.66 ± 0.22 | > 8       | 4.5 ± 0.4  | n.d.      | n.d.       | n.d.     | n.d.      | n.d.     | n.d.      | n.d.         | n.d.          | n.d.       | n.d.        | n.d.     | n.d.       | n.d.         | n.d.          | n.d.       | n.d.        |
| GM04717           | 0.90 ± 0.07 | 0.76 ± 0.04 | > 8       | 5.4 ± 1.5  | n.d.      | n.d.       | n.d.     | n.d.      | n.d.     | n.d.      | n.d.         | n.d.          | n.d.       | n.d.        | n.d.     | n.d.       | n.d.         | n.d.          | n.d.       | n.d.        |

| **Inhibition of Ataxin-3** |          |           |           |            |           |            |          |           |          |           |              |                |            |              |          |           |              |              |            |              |
| GM06151           |          |           |           |            |           |            |          |           |          |           |              |                |            |              |          |           |              |              |            |              |
| Rep19/wt          | 0.99 ± 0.06 | 0.36 ± 0.09 |           |           |           |            |          |           |          |           |              |                |            |              |          |           |              |              |            |              |
| Rep19/mut         | 0.36 ± 0.09 |           |           |           |           |            |          |           |          |           |              |                |            |              |          |           |              |              |            |              |
| 3J/ATX/wt         | 5.4 ± 0.9  |           |           |           |           |            |          |           |          |           |              |                |            |              |          |           |              |              |            |              |
| 3J/ATX/mut        | 2.2 ± 1.1  |           |           |           |           |            |          |           |          |           |              |                |            |              |          |           |              |              |            |              |
| 5J/ATX/wt         | 1.7 ± 0.3  |           |           |           |           |            |          |           |          |           |              |                |            |              |          |           |              |              |            |              |
| 5J/ATX/mut        | 0.7 ± 0.2  |           |           |           |           |            |          |           |          |           |              |                |            |              |          |           |              |              |            |              |
| SiRNA/REP/wt      | 0.024 ± 0.009 |           |           |           |           |            |          |           |          |           |              |                |            |              |          |           |              |              |            |              |
| SiRNA/REP/mut     | 0.012 ± 0.004 |           |           |           |           |            |          |           |          |           |              |                |            |              |          |           |              |              |            |              |

**wt:** IC$_{50}$ value (µM) for inhibition of wild-type protein. **Mut:** IC$_{50}$ value (µM) for inhibition of mutant protein. Values represent the mean ± s.d. (n=3–6). * p < 0.05, ** p < 0.01, *** p < 0.001.
SUPPLEMENTARY METHODS

Analysis of HTT mRNA level by Quantitative PCR. Total RNA from treated and untreated fibroblast cells was extracted using TRIzol (Invitrogen) 3 days after transfection. Samples were then treated with DNase I at 25°C for 10 min. Reverse transcription reactions were done using High Capacity Reverse Transcription Kit (Applied Biosystems) according to the manufacturer’s protocol. Quantitative PCR was performed on a 7500 real-time PCR system (Applied Biosystems) using iTaq SYBR Green Supermix (Bio-rad). Data was normalized relative to levels of GAPDH mRNA. Primer sequences specific for HTT are as follows: forward primer, 5′–CGACAGCGAGTCAGTAATG–3′; reverse primer, 5′–ACCACTCTGGCTTCACAAGG–3′. Primers specific for GAPDH were obtained from Applied Biosystems.

Chromatin immunoprecipitation (ChIP) of RNA polymerase 2 (RNAP2). GM04281 fibroblast cells used for ChIP were seeded at 1.8 million cells in 15 cm dishes. Two dishes were treated with mismatch-containing PNA –CTL1 and two with REP19. Transfections were performed as described above and cells were crosslinked with formaldehyde (1%) three days after transfection. Before crosslinking, a small sample was collected for western analysis. Cells were recovered by scraping and nuclei isolated. Nuclei were lysed in 1 mL lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl pH 8.1, 1x Roche protease inhibitors cocktail) and sonicated (2 pulses, 40% power, 30 sec). 100 µL of lysate was incubated overnight with 4 µg of monoclonal anti-RNAP2 antibody (Millipore 05-623) or mouse IgG negative control antibody (Millipore 12-371) diluted in 900 µL of immunoprecipitation Buffer (0.01% SDS, 1.1% Triton-X, 1.2 mM EDTA, 16.7 mM Tris pH 8.1, 167 mM NaCl, and 1x Roche protease inhibitors cocktail). Antibody was recovered with 60 µL of Protein A/G beads (Calbiochem EMD IP05). Beads were washed with low salt (0.1% SDS, 1% Triton-X, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 150 mM NaCl), high salt (see low salt but with 500 mM NaCl), LiCl solution
(0.25 M LiCl, 1% NP40, 1% deoxycholate, 1 mM EDTA, and 10 mM Tris-HCl pH 8.1), and Tris-EDTA pH 8 (Ambion AM9863) washes. Protein was eluted with 500 µL of 1% SDS, 0.1M NaHCO₃ for 30 minutes at room temperature. Crosslinking was reversed by adding NaCl to 200 mM and heating at 65 °C for at least 2 hours. Protein was digested by Proteinase K followed by extraction using an equal volume of phenol:chloroform:isoamyl alcohol and centrifugation. DNA in the aqueous layer was precipitated using 1/10 volume sodium acetate/ 2 volumes ethanol. The pellet was resuspended in 50 µL of nuclease-free water. Real time PCR was performed using Biorad iTaq SYBR® Supermix.

*MTS assay.* GM04281 fibroblast cells (5,000 cells/well) in 96-well plate were plated 1 day before transfection. The cells were transfected with PNA-peptides. On day 4, cell viability for each sample was measured using CellTiter 96® AQueous One Solution Cell Proliferation Assay Kit (Promega) according to the manufacturer’s protocol.

*Evaluation of HTT mRNA stability.* M04281 fibroblast cells (60,000 cells/well) were plated and transfected. For no treatment samples (NT1 and NT2), the media were just changed with OptiMEM. One day after transfection, the media were removed and replaced by fresh supplemented MEM. On day 3, actinomycin D (5 µg/mL) was added to each sample except for NT1 to stop transcriptions. Each sample was harvested using TRIzol at the specific time points (0, 2, 4, and 6 h), and cDNAs were prepared for qPCR to measure HTT and GAPDH mRNA levels.

*Western Analysis of HTT and ataxin-3 expression: blot preparation and cropping.* For separation of HTT allelic variants, cell lysates from fibroblasts were loaded on 5% Tris-acetate gels. This type of gel is not commercially available and preparing by pouring acrylamide solution into empty Ready Gel cassettes (Bio-Rad). Gel formulation: separating gel, 5 % acrylamide-bisacrylamide/34.7:1,450 mM Tris-acetate pH 8.8; stacking gel: 4% acrylamide-bisacrylamide/34.7:1, 150 mM Tris-acetate pH 6.8. The separating gel is about 4.5 cm in length.
Gels were run at 70V for 15 min followed by 100V for 4 h. The electrophoresis apparatus was placed in ice-water bath to prevent excess heating that prevents good separation of the HTT variants. Under these conditions, HTT protein migrates to the bottom of the gel. Proteins were transferred to Hybond-C Extra membrane (100V for 2.5 h). After Ponceau S staining to reveal the location of protein bands, the membrane was cut from 1.5 to 3.5 cm from the bottom (the 250 kD protein maker we use to measure the progress of the electrophoresis is run off the gel in most cases).

For separation of HTT variants containing shorter CAG repeats, gels were run at 70 V for 15 min, then 110 V for 6 h. After transfer to the membrane, the blot was cut 2 cm above the bottom. The cropped blot was incubated in 5% milk, then probed with anti-HTT antibody (MAB2166. 1:10000; Chemicon).

We monitor β-actin expression as a loading control to allow us to normalize HTT levels during quantitation. In parallel with analysis for HTT expression, an equivalent portion of each protein lysate was analyzed for β-actin expression by 7.5% Tris-HCl pre-cast gels (Bio-Rad). These gels were run at 70 V for 15 min followed by 100 V for 1 h. After transferring to the membrane, the blot was probed by anti-β-actin antibody (1:10,000; Sigma). It is necessary to run a separate gel because actin protein (~42 kDa) migrates much faster than HTT and runs off the gels used to separate HTT allelic variants. Under the conditions used to analyze β-actin, HTT appears as one band with no separation between the variants.

Ataxin-3 was analyzed by SDS-PAGE (7.5 % Tris-HCl pre-cast gels; Bio-Rad). After transferring to the membrane, the blot was probed with anti-ataxin-3 antibody (MAB5360; 1:10000; Chemicon). After treating with western blot stripping buffer (Fisher), the blot was then re-probed for β-actin.
Supplementary Figure 1. Potential hairpin structures at CAG repeats. (a) Potential hairpin structure formed by CAG repeats. (b) (c) CONTRAfold predictions of the structure of HTT mRNA with 17 (a typical number of wild-type repeats) or 69 (the number found in the mutant HTT allele of GM 04281 patient-derived fibroblast cells) CAG repeats respectively. The sequence is from base +121 ~ +360, NM_002111.
**Supplementary Figure 2.**

(a) Sequences of PNAs that are complementary to target sites that overlap the 3’ junction between the CAG repeat and downstream regions of HTT mRNA.

(b) Effect on HTT expression of adding 2 µM PNA conjugates 3J0, 3J4, 3J6, 3J/HTT, and 3J10 that target related sequences at the 3’ junction and have 0, 4, 6, 8, and 10 bases of overlap with the CAG repeat respectively.

**Supplementary Figure 2. Inhibition of HTT protein expression by PNA conjugates that overlap the CAG/3’ junction.** (a) Sequences of PNAs that are complementary to target sites that overlap the 3’ junction between the CAG repeat and downstream regions of HTT mRNA. (b) Effect on HTT expression of adding 2 µM PNA conjugates 3J0, 3J4, 3J6, 3J/HTT, and 3J10 that target related sequences at the 3’ junction and have 0, 4, 6, 8, and 10 bases of overlap with the CAG repeat respectively.
Supplementary Figure 3. Non-target effects of adding PNAs. (a) Effect of adding PNA REP19 on the expression of other proteins that contain CAG repeats within their mRNAs including AAK1, Pou, and TBP. Actin expression was monitored as an internal loading control. (b) Toxicity at PNA concentrations necessary for allele-specific inhibition of HTT expression (0.5 or 1 µM). No treatment (white bar). Fibroblast GM04281 cells were treated with REP19 (gray bar) or -CTL1 (black bar). (c) Toxicity at a wider range of concentrations, addition of PNAs REP19, REP19N, and -CTL1 at 0.5 to 16 µM. No treatment (white bar). Fibroblast GM04281 cells were treated with REP19 (dark gray bar), REP19N (light gray bar), and negative control -CTL1 (black bar). For (b) and (c) cell viability was evaluated using MTS assay (Promega). Error bars indicate s.d.; n=3 (b), n=4 (c).
Supplementary Figure 4. Inhibition of MSN/glial cell mixture by PNA REP19. Medium striatal spiny neurons (MSN) and supporting glial cells were obtained from YAC128 mice and cultured as described in Materials and Methods. HTT protein levels were analyzed by western analysis. The top HTT band is human HTT and the bottom is murine HTT. PNAs REP19, -CTL1, or -CTL2 were added at the indicated concentrations. The amount of MSN relative to glial cells was estimated using light microscopy. (a) Effect of PNAs on MSN/glial cell mixture. Because glial cells make up approximately half of the cells in culture, data should not be taken as a direct indication of the level of inhibition of HTT in MSN cells. (b) Effect of PNAs on cells harvested with cell dissociation solution (Sigma). This dissociation protocol enriches MSN relative to glial cells (MSN:glials = 2:1) and offers a better indication of inhibition in MSN. Note that the apparent potency of inhibition increases with the percentage of striatal cells.
Supplementary Figure 5. Anti-HTT PNAs do not reduce HTT mRNA levels. (a) Western analysis and qPCR showing effect on levels of HTT mRNA of adding PNA-peptide conjugates at 1 µM, a concentration that achieves allele-selective inhibition. Measurements used GM04281 fibroblast cells. *P<0.05 as compared to mismatch PNA peptide -CTL1. Error bars indicate s.d., n=4. (b) Western analysis and qPCR showing effect of adding REP19 at varied concentrations on expression of HTT mRNA. ** p < 0.01 as compared to mismatch PNA-peptide –CTL1; Error bars indicate s.d.; n=3. (c) Chromatin immunoprecipitation of RNAP2 on the HTT gene after addition of 1 µM of REP19 or mismatch-containing PNA –CTL1 shows no reduction of polymerase recruitment. Inset shows selective knockdown of mutant HTT by REP19 by western blot. Immunoprecipitation by a mouse IgG was included for comparison. (d) Effect of adding actinomycin D on levels of HTT mRNA after treatment with REP19. Four days after transfection ([REP19] or [-CTL1]=2 µM), each cell except for NT1 (no treatment sample 1) was treated with actinomycin D (5 µg/mL) for the indicated time (0, 2, 4, or 6 h). HTT mRNA level at each time point was determined by qPCR. GAPDH mRNA level was used for normalization. NT1: Not treated with PNA and not treated with actinomycin D. NT2: Treated with actinomycin D, but not treated with PNA.
Supplementary Figure 6. Effect of oligomer concentration, target sequence, length and chemical modification: Western analysis. Typical western gels underlying the graphical data in Figures 1 and 2. Effect on HTT expression of adding increasing concentrations of (a) REP19, (b) 3J/HTT, (c) REP16, (d) REP13, (e) REP19Arg, (f) REP19N, (g) LNA/REP, (h) LNA/3J, (i) siRNA/REP and, (j) siRNA/5J.
Supplementary Figure 7. Additional data on conjugates REP13 and REP19N. (a) Effect of adding REP 13 or REP19N on expression of other proteins with mRNAs that contain CAG repeats. (b) Effect of adding REP19N on cell toxicity measured by monitoring levels of caspase 3. Caspase-3 activity was measured by hydrolysis of Ac-DEVD-pNA. **p < 0.01, n = 3.
Supplementary Figure 8. Additional data on the effects of LNAs. (a) Effect on HTT expression of adding 100 nM LNAs. (b) Western analysis showing effect of adding LNA/REP on the expression of other proteins containing CAG repeats. (c) Western analysis and qPCR showing effect on HTT expression upon adding LNAs at 100 nM. ** p < 0.01, * p < 0.05 as compared to mismatch LNA (–CTL); Error bars indicate s.d.; n=6.
Supplementary Figure 9. Selectivity is affected by the number of repeats: Western analysis. Typical western gels underlying the graphical data in Figure 3. (a) Effect on HTT expression of adding REP19 to GM04869 cells. (b) Effect on HTT expression of adding REP19N to GM04869 cells. (c) Effect on HTT expression of adding REP19 to GM04719 cells. (d) Effect on HTT expression of adding REP19N to GM04719 cells. (e) Effect on HTT expression of adding REP19 to GM04717 cells. (f) Effect on HTT expression of adding REP19N to GM04717 cells.
**Supplementary Figure 10.** Potent and selective inhibition of mutant ataxin-3. Typical western gels underlying the graphical data in Figure 4. All data show analysis of ataxin-3 expression in GM06151 fibroblast cells. (a) Inhibition of ataxin-3 expression by PNA conjugate REP19. (b) Inhibition of ataxin-3 expression by PNA conjugate 3J/ATX. (c) Inhibition of ataxin-3 expression by PNA conjugate 5J/ATX. (d) Inhibition of ataxin-3 expression by siRNA/REP.
Supplementary Figure 11. Comparison of strategies targeting a deletion polymorphism to targeting CAG repeats. Effects on expression of HTT in GM09197 fibroblast cells (mutant allele with 151 CAG repeats/ wild-type allele with 21 CAG repeats). (a) Inhibition of HTT expression by siRNA S4 targeting a deletion polymorphism (Δ2642). (b) Inhibition of HTT expression by LNA/REP.