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## Supplemental information

# Haplotyping SNPs for allele-specific gene editing of the expanded huntingtin allele using long-read sequencing

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# Supplemental Figures

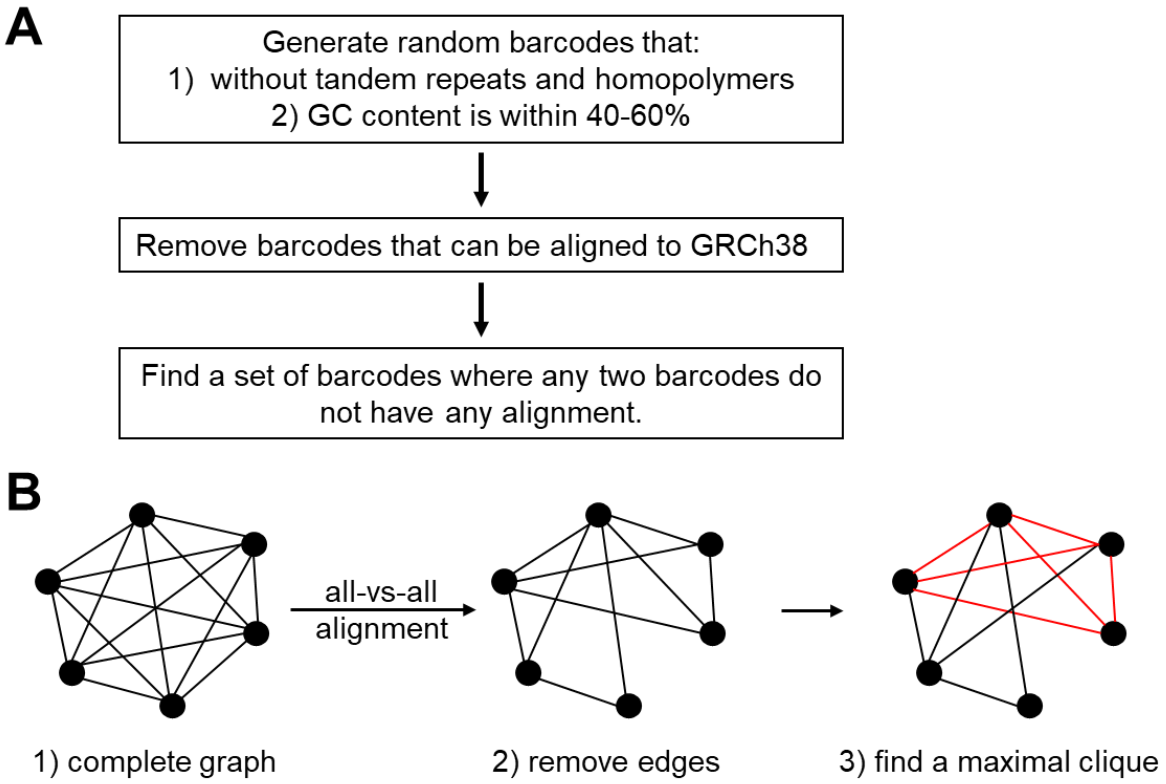
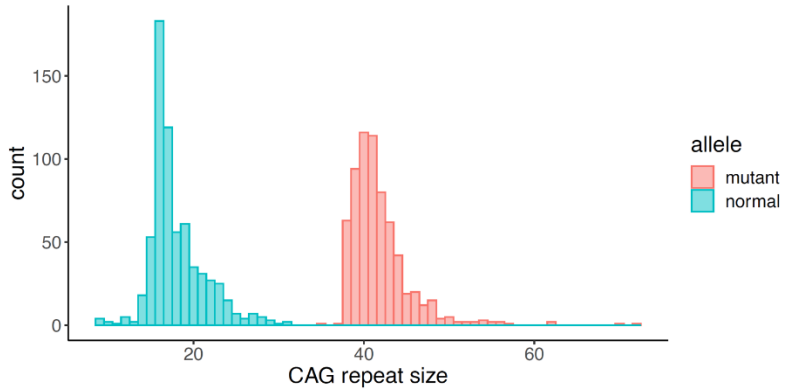


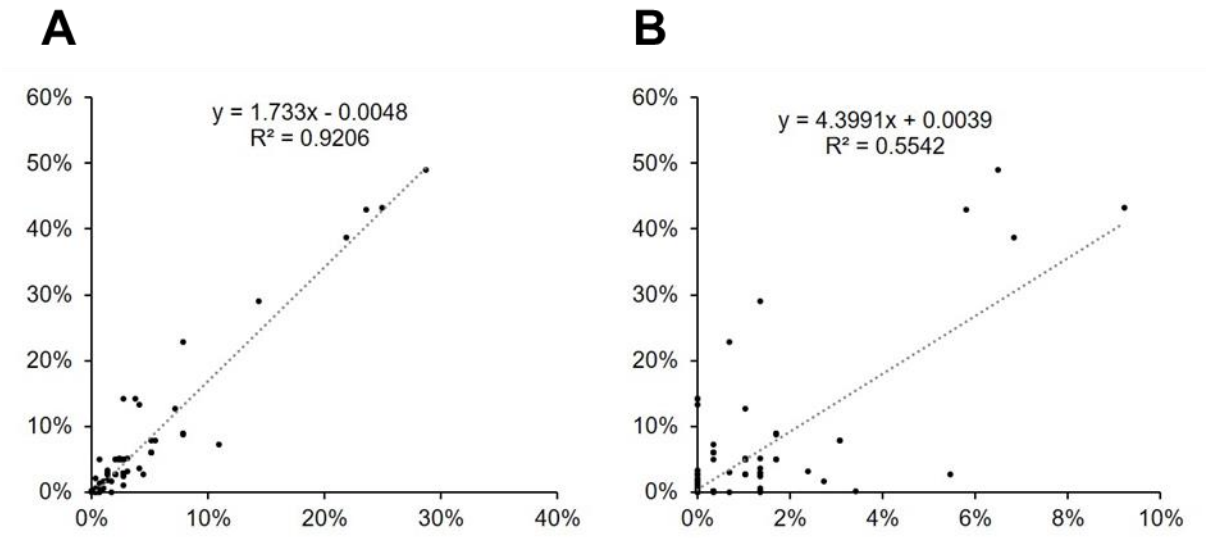

**Figure S1**

**Barcode design strategy used in this study**. **A**) The barcode design workflow. **B**) The algorithm to find a set of barcodes where any two barcodes do not have any alignment. 1) Each barcode was a node in the graph. Initially, all nodes are connected in the undirected graph. 2) An all-vs-all alignment of the barcode sequences was performed, and the edge between two barcodes (nodes) was removed if the two barcodes were aligned. 3) The remaining edges only connect barcode pairs that have no alignment. Therefore, a complete subgraph (clique) is a set of barcodes in which any two barcodes have no alignment.



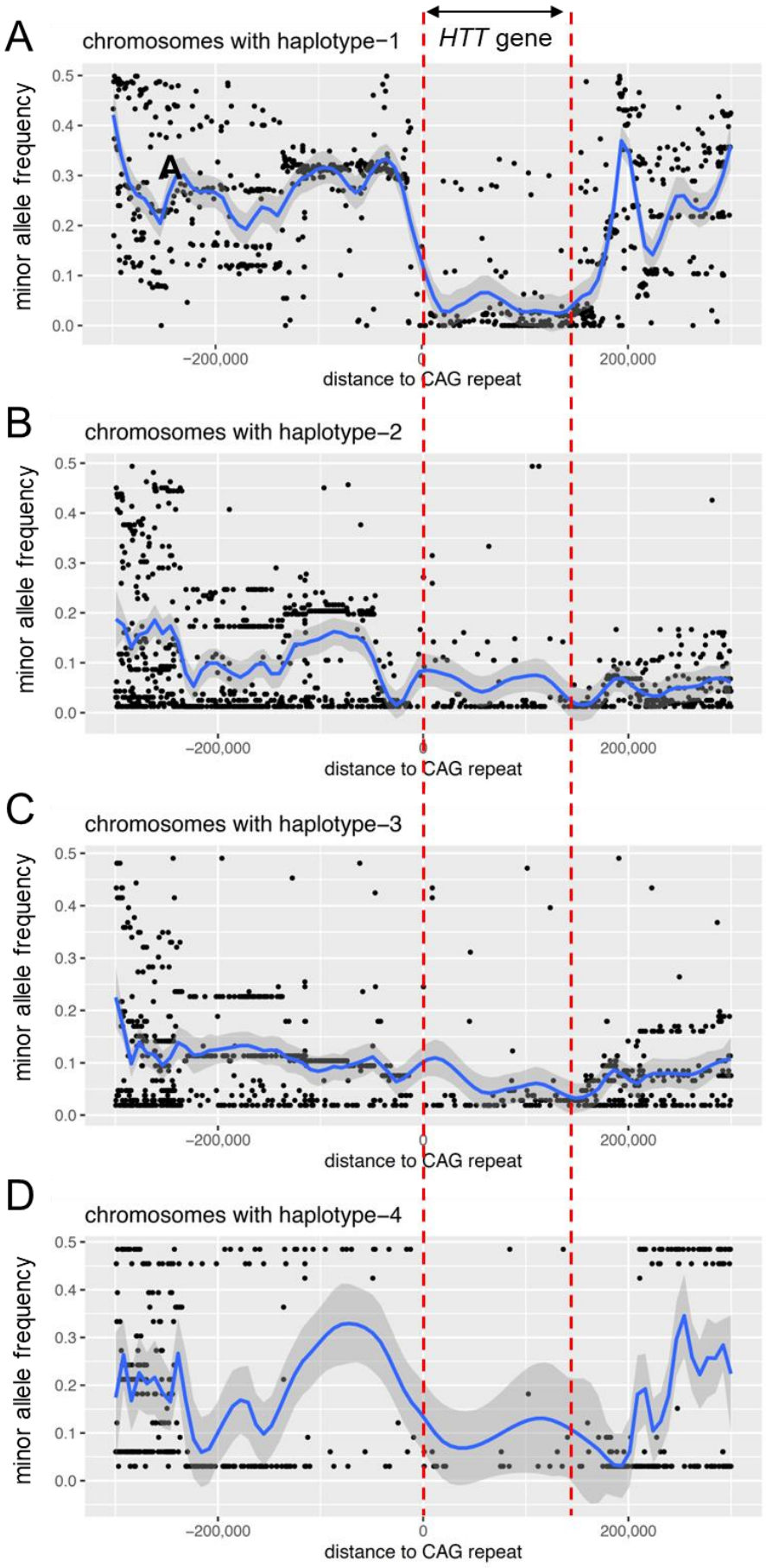

## Figure S2

**Histogram of the CAG repeat size of the CHDI cohort.** The repeat was quantified by AmpRepeat using the Oxford Nanopore long-read sequencing data.



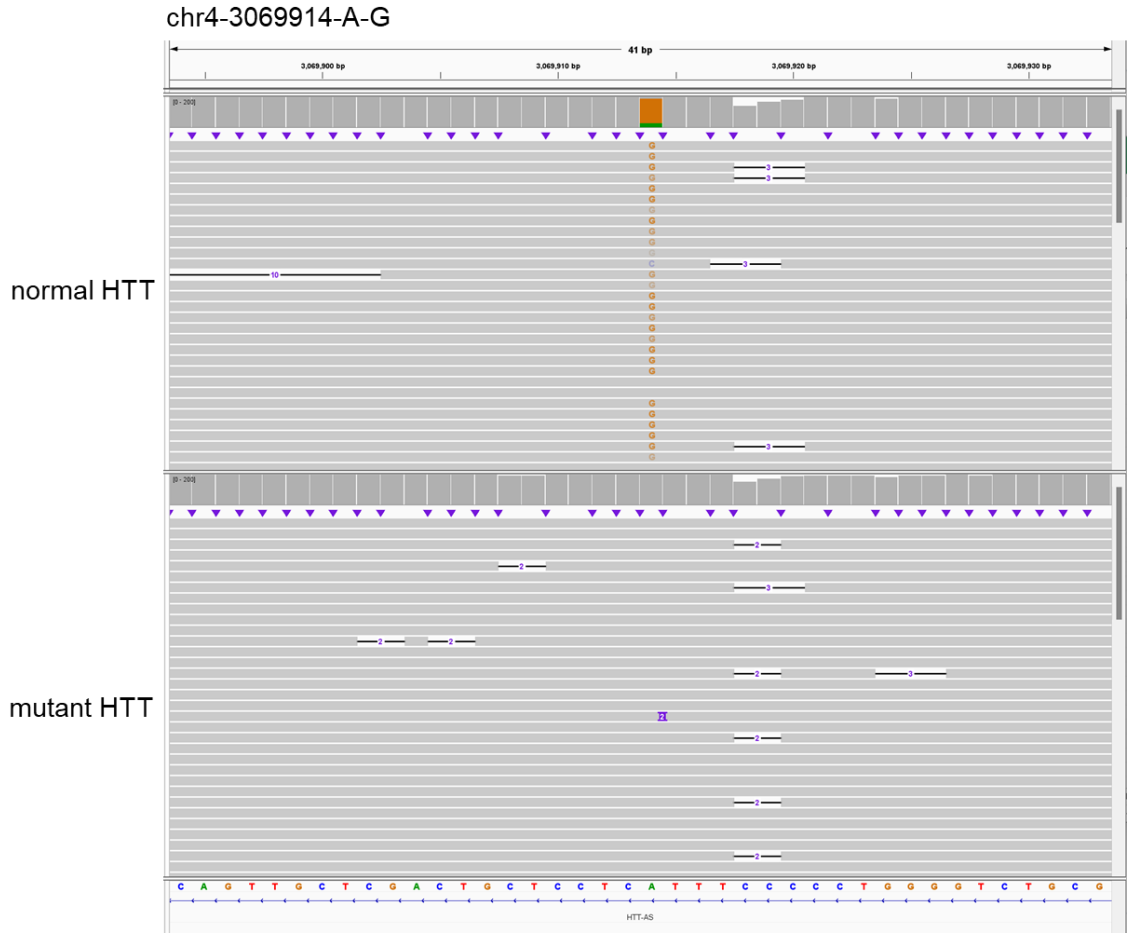

## Figure S3

**Scatter plots showing the of AFs of SNPs in the CHDI HD cohort (Caucasians) and the gnomAD database (non-Finnish European population).** a) normal alleles; b) *mHTT* alleles.


A
chromosomes with haplotype−1
HTT gene
A
minor allele frequency
0.5
0.4
0.3
0.2
0.1
0.0
−200,000
0
200,000
distance to CAG repeat
B
chromosomes with haplotype−2
minor allele frequency
distance to CAG repeat
C
chromosomes with haplotype−3
minor allele frequency
distance to CAG repeat
D
chromosomes with haplotype−4
minor allele frequency
distance to CAG repeat

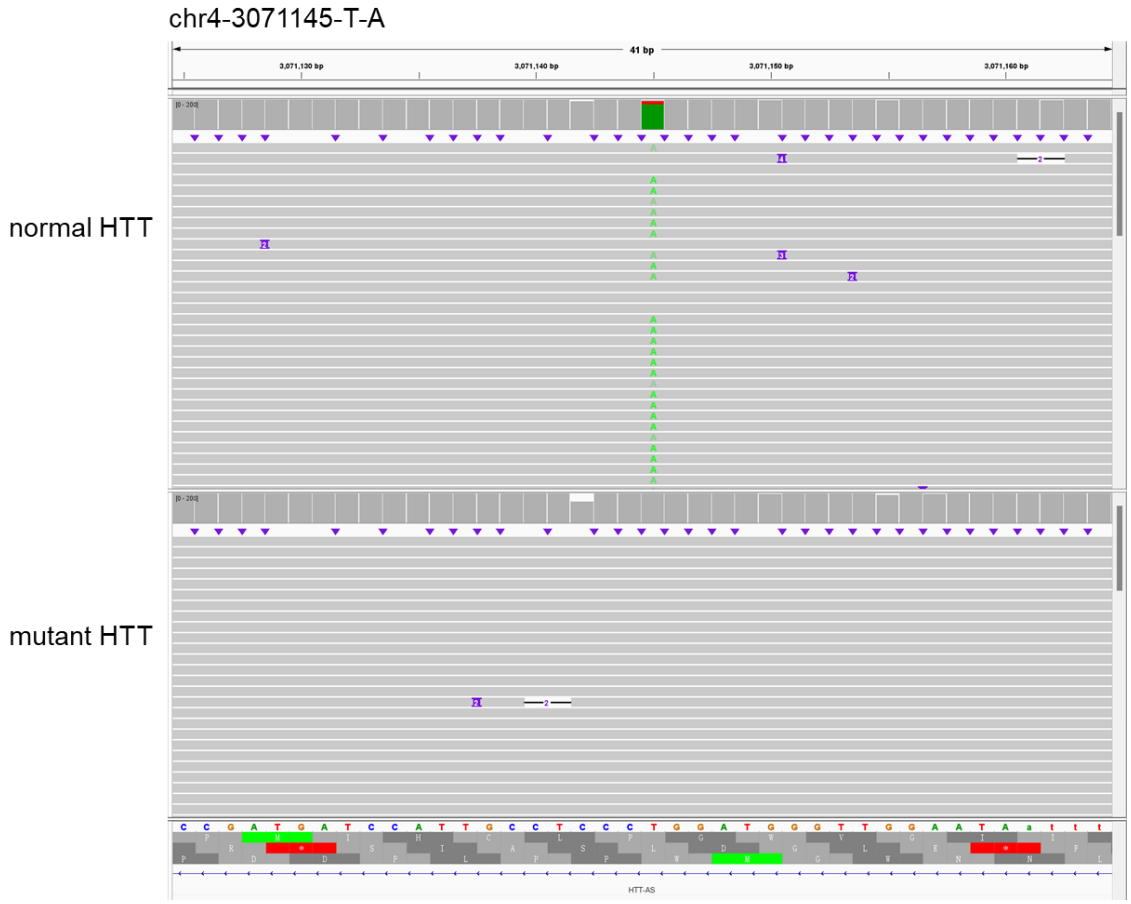
## Figure S4

**Minor allele frequencies of SNPs in chromosomes with haplotypes 1,2,3, and 4.** The data is based on 1000 Genomes individuals (phase 3 data set, non-Finnish European population). The region between dashed red lines is the HTT gene. Minor allele is the allele with frequency ≤ 0.5.



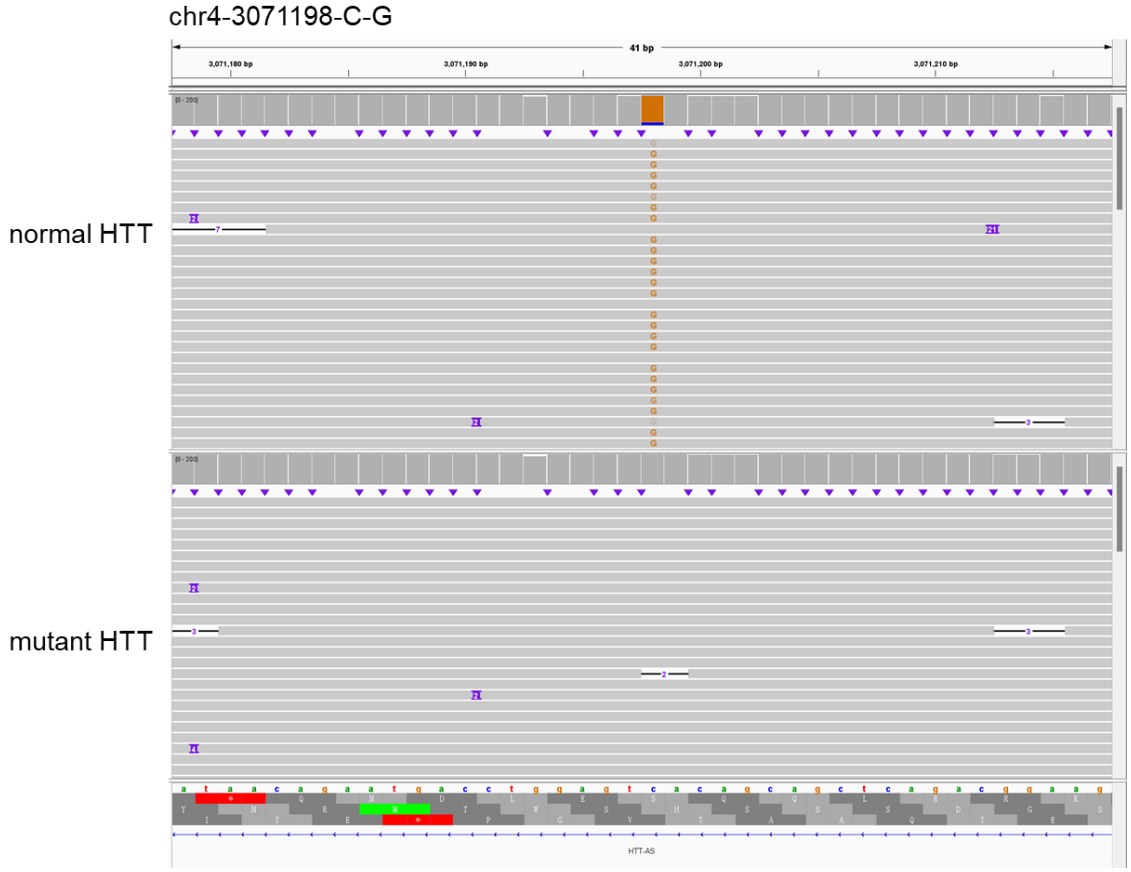

## Figure S5

**Integrative Genomics Viewer (IGV) showing the aligned sequences around chr4:3069914 (GRCh38).** Matched bases are in grey and mismatched bases are colored. The mismatched bases indicate an SNP in the normal HTT but not in the mutant HTT.



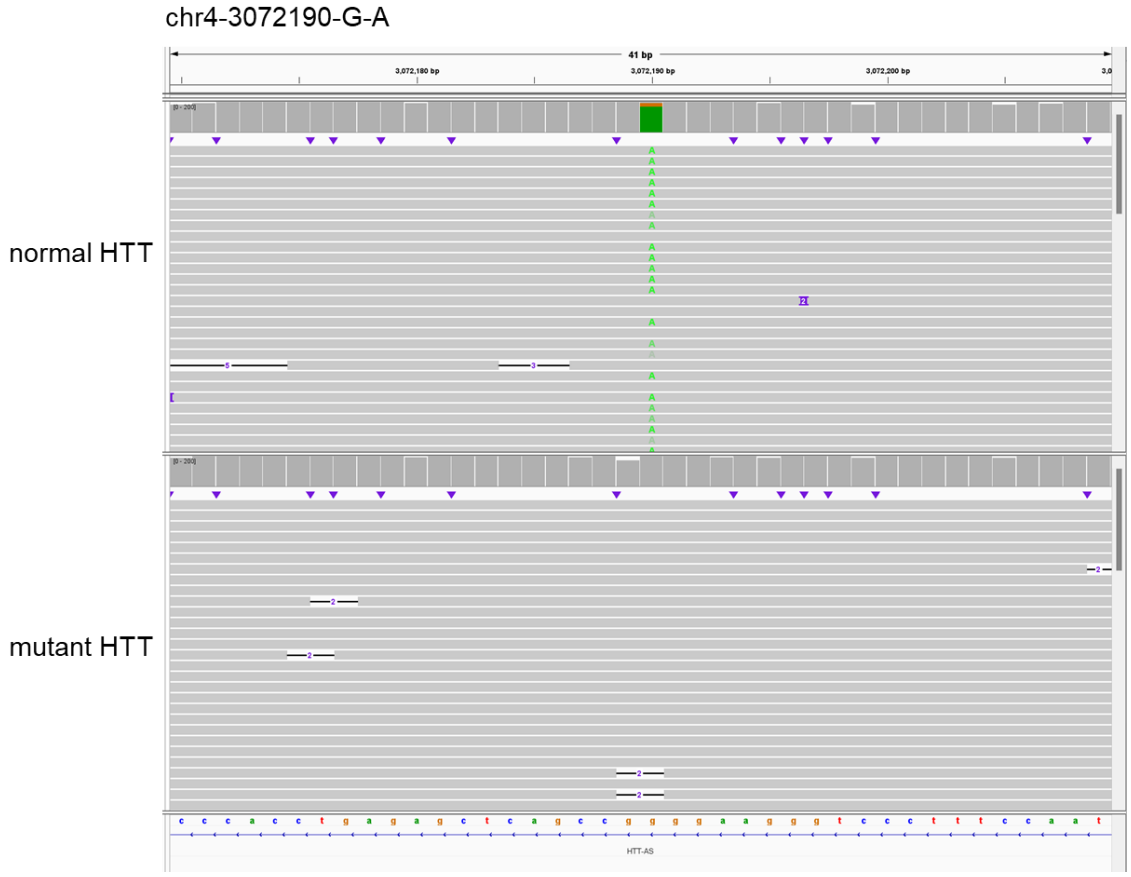

## Figure S6

**Integrative Genomics Viewer (IGV) showing the aligned sequences around chr4:3071145 (GRCh38).** Matched bases are in grey and mismatched bases are colored. The mismatched bases indicate an SNP in the normal HTT but not in the mutant HTT.



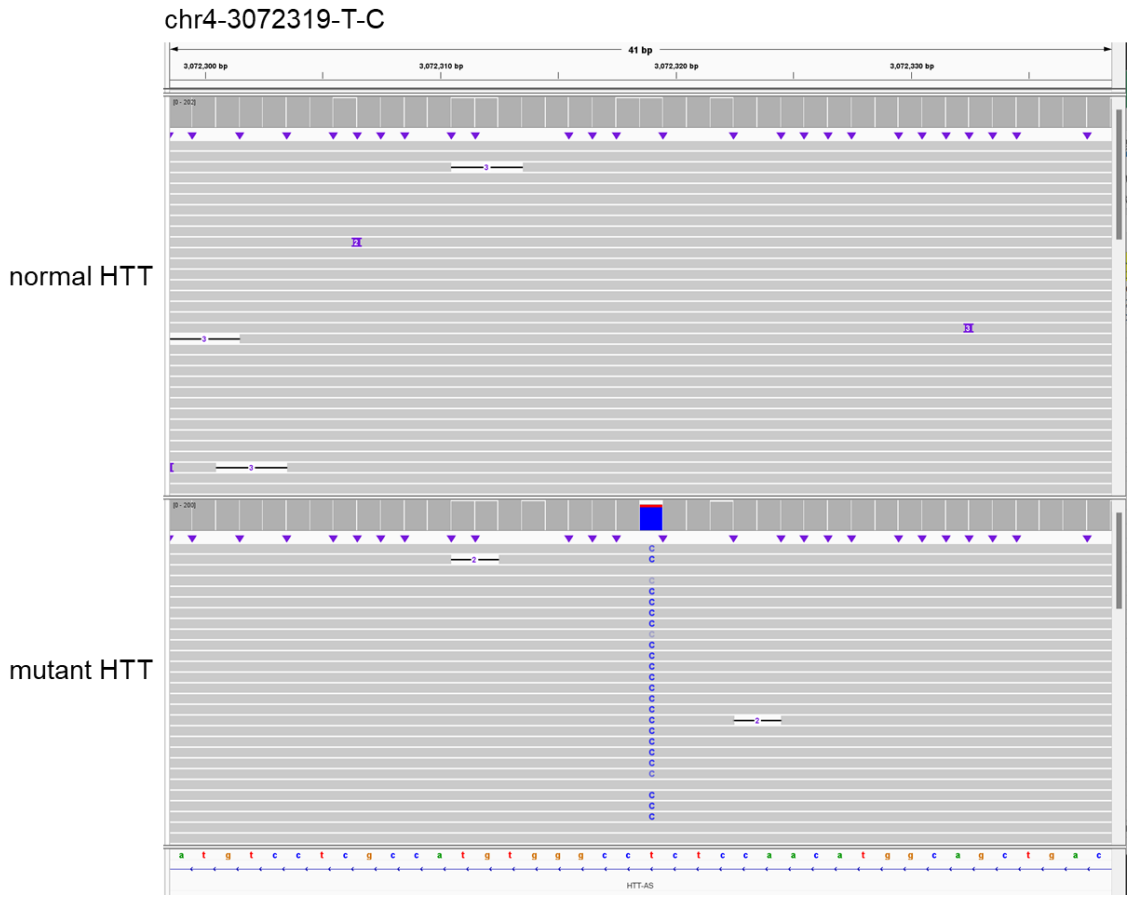

## Figure S7

**Integrative Genomics Viewer (IGV) showing the aligned sequences around chr4:3071198 (GRCh38).** Matched bases are in grey and mismatched bases are colored. The mismatched bases indicate an SNP in the normal HTT but not in the mutant HTT.



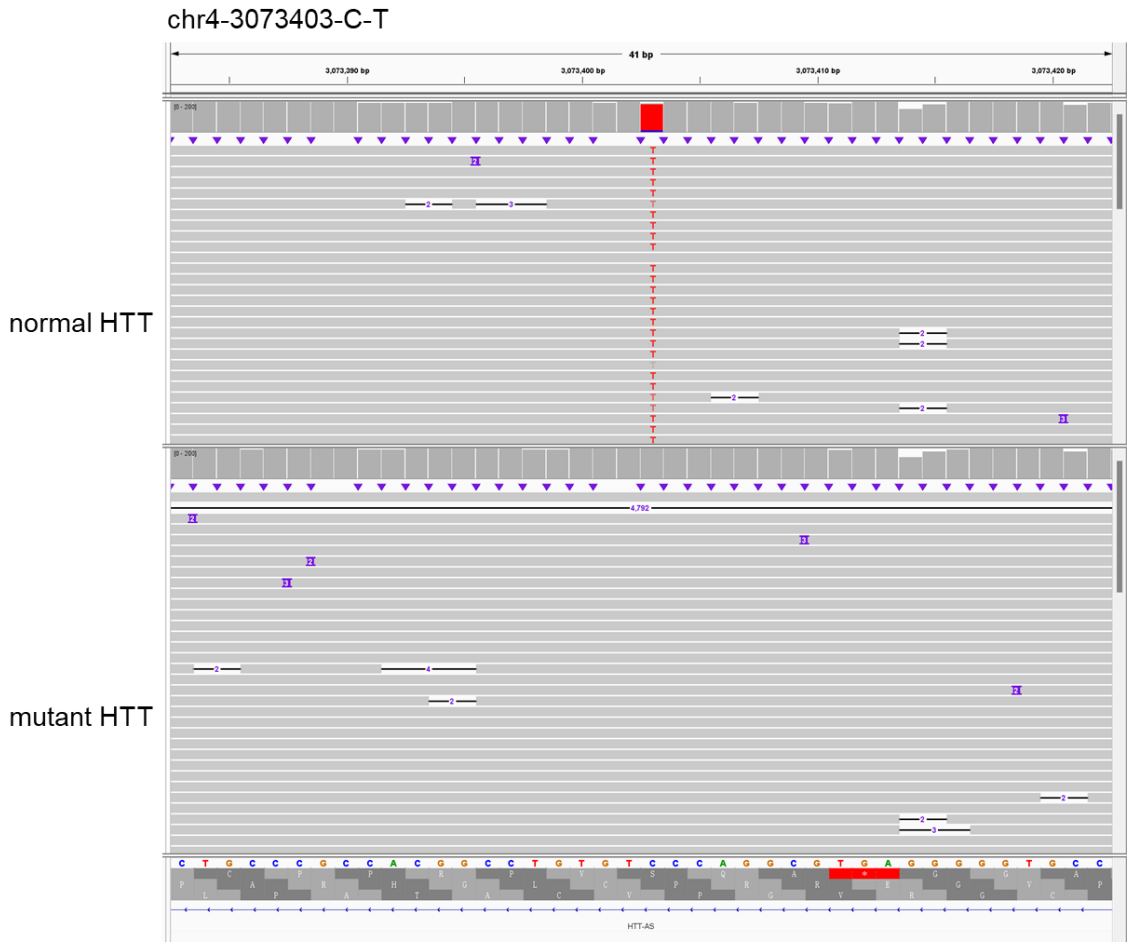

## Figure S8

**Integrative Genomics Viewer (IGV) showing the aligned sequences around chr4:3072190 (GRCh38).** Matched bases are in grey and mismatched bases are colored. The mismatched bases indicate an SNP in the normal HTT but not in the mutant HTT.




## Figure S9

**Integrative Genomics Viewer (IGV) showing the aligned sequences around chr4:3072319 (GRCh38).** Matched bases are in grey and mismatched bases are colored. The mismatched bases indicate an SNP in the mutant HTT but not in the normal HTT.

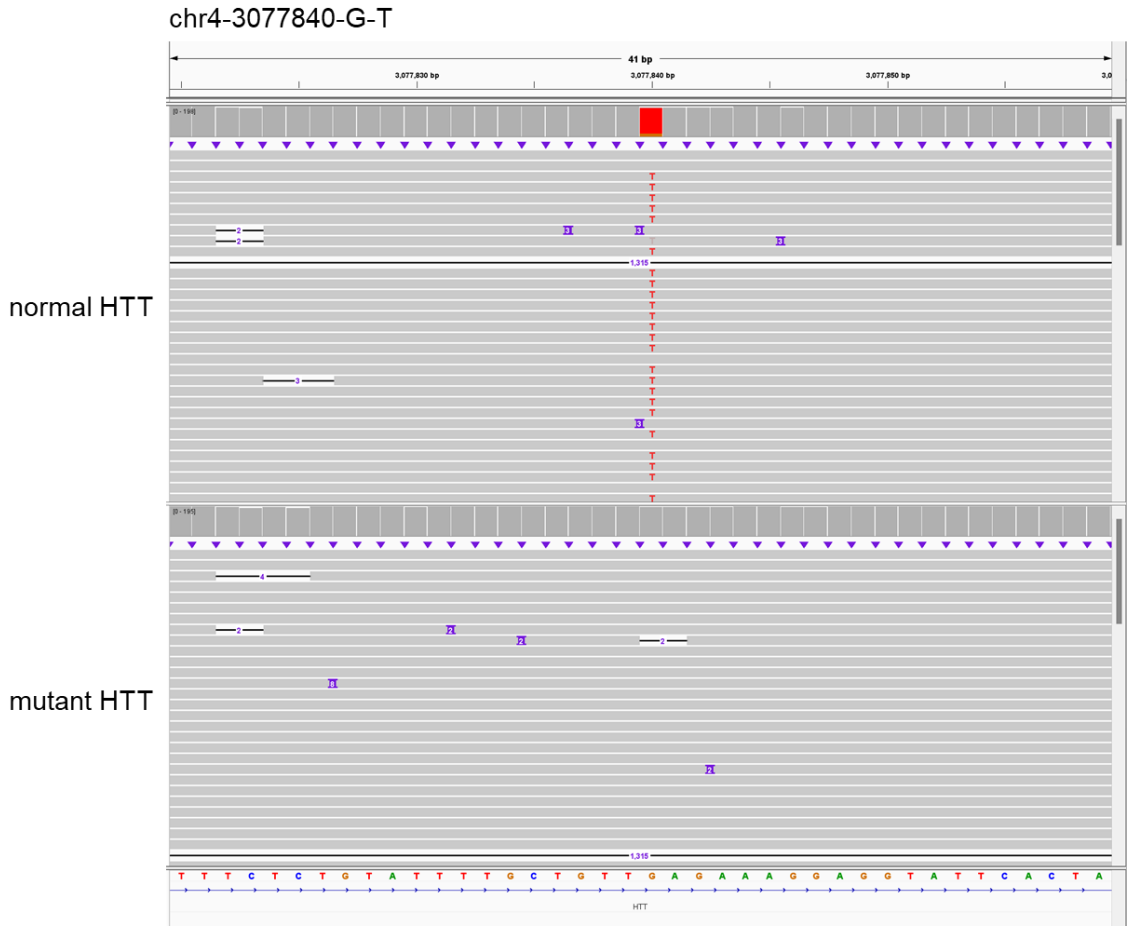



## Figure S10

**Integrative Genomics Viewer (IGV) showing the aligned sequences around chr4:3073403 (GRCh38).** Matched bases are in grey and mismatched bases are colored. The mismatched bases indicate an SNP in the normal HTT but not in the mutant HTT.


## Figure S11

**Integrative Genomics Viewer (IGV) showing the aligned sequences around chr4:3076208 (GRCh38).** Matched bases are in grey and mismatched bases are colored. The mismatched bases indicate an SNP in the mutant HTT but not in the normal HTT.




## Figure S12

**Integrative Genomics Viewer (IGV) showing the aligned sequences around chr4:3077840 (GRCh38).** Matched bases are in grey and mismatched bases are colored. The mismatched bases indicate an SNP in the normal HTT but not in the mutant HTT.

# Supplemental Tables

## Table S1

**Barcoded primers for amplicon-1**. The left part in the sequence is the barcode.

| primer ID | primer sequence | direction |
|---|---|---|
| A1B01F | 5'-AATTCGCCAGTGATGC-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A1B02F | 5'-CAGCCATTGATGTCGA-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A1B03F | 5'-GGCCGCTAGTAATTCA-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A1B04F | 5'-TCAGGCGCCGATTAAT-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A1B05F | 5'-ACTAAGCGAGGTCTCT-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A1B06F | 5'-CGTTCCATCGAGTAAG-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A1B07F | 5'-ACTGTTCAGACGATCG-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A1B08F | 5'-CCTCGACGTGGATAAT-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A1B09F | 5'-GTACATCGGATGATCC-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A1B10F | 5'-TACGGCGCTATTGAAC-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A1B01R | 5'-AATTCGCCAGTGATGC-GAGGGAAGTGGCACTGAGCAAATCT-3' | reverse |
| A1B02R | 5'-CAGCCATTGATGTCGA-GAGGGAAGTGGCACTGAGCAAATCT-3' | reverse |
| A1B03R | 5'-GGCCGCTAGTAATTCA-GAGGGAAGTGGCACTGAGCAAATCT-3' | reverse |
| A1B04R | 5'-TCAGGCGCCGATTAAT-GAGGGAAGTGGCACTGAGCAAATCT-3' | reverse |
| A1B05R | 5'-ACTAAGCGAGGTCTCT-GAGGGAAGTGGCACTGAGCAAATCT-3' | reverse |
| A1B06R | 5'-CGTTCCATCGAGTAAG-GAGGGAAGTGGCACTGAGCAAATCT-3' | reverse |
| A1B07R | 5'-ACTGTTCAGACGATCG-GAGGGAAGTGGCACTGAGCAAATCT-3' | reverse |
| A1B08R | 5'-CCTCGACGTGGATAAT-GAGGGAAGTGGCACTGAGCAAATCT-3' | reverse |
| A1B09R | 5'-GTACATCGGATGATCC-GAGGGAAGTGGCACTGAGCAAATCT-3' | reverse |
| A1B10R | 5'-TACGGCGCTATTGAAC-GAGGGAAGTGGCACTGAGCAAATCT-3' | reverse |

## Table S2

**Barcoded primers for amplicon-2**. The left part in the sequence is the barcode.

| primer ID | primer sequence | direction |
|---|---|---|
| A2B01F | 5'-CGTCGTTAACAGCGTACAGCCATTGATGTCGA-AAAGTCCCGATGATCCATTGCCTCC-3' | forward |
| A2B02F | 5'-CGATAGTCTTACGAGCGGCCGCTAGTAATTCA-AAAGTCCCGATGATCCATTGCCTCC-3' | forward |
| A2B03F | 5'-GCGAACGATCAGTCTTTCAGGCGCCGATTAAT-AAAGTCCCGATGATCCATTGCCTCC-3' | forward |
| A2B04F | 5'-TGCCATGGCGTATACAACTAAGCGAGGTCTCT-AAAGTCCCGATGATCCATTGCCTCC-3' | forward |
| A2B05F | 5'-AGCGCATCATTGGCATCGTTCCATCGAGTAAG-AAAGTCCCGATGATCCATTGCCTCC-3' | forward |
| A2B06F | 5'-GACGACGTATGTACCTAATTCGCCAGTGATGC-AAAGTCCCGATGATCCATTGCCTCC-3' | forward |
| A2B07F | 5'-ATAAGTTGCGCACGCTACTGTTCAGACGATCG-AAAGTCCCGATGATCCATTGCCTCC-3' | forward |
| A2B08F | 5'-ATAACACGGTCCGGTTCCTCGACGTGGATAAT-AAAGTCCCGATGATCCATTGCCTCC-3' | forward |
| A2B09F | 5'-GGTTAGATTCACGACCGTACATCGGATGATCC-AAAGTCCCGATGATCCATTGCCTCC-3' | forward |
| A2B10F | 5'-AACGGTTCATGAGCCTTACGGCGCTATTGAAC-AAAGTCCCGATGATCCATTGCCTCC-3' | forward |
| A2B01R | 5'-CGTCGTTAACAGCGTACAGCCATTGATGTCGA-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A2B02R | 5'-CGATAGTCTTACGAGCGGCCGCTAGTAATTCA-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A2B03R | 5'-GCGAACGATCAGTCTTTCAGGCGCCGATTAAT-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A2B04R | 5'-TGCCATGGCGTATACAACTAAGCGAGGTCTCT-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A2B05R | 5'-AGCGCATCATTGGCATCGTTCCATCGAGTAAG-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A2B06R | 5'-GACGACGTATGTACCTAATTCGCCAGTGATGC-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A2B07R | 5'-ATAAGTTGCGCACGCTACTGTTCAGACGATCG-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A2B08R | 5'-ATAACACGGTCCGGTTCCTCGACGTGGATAAT-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A2B09R | 5'-AACGGTTCATGAGCCTTACGGCGCTATTGAAC-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A2B10R | 5'-GGTTAGATTCACGACCGTACATCGGATGATCC-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |

## Table S3

**Barcoded primers for amplicon-3.** The left part of the sequence is the barcode.

| primer ID | primer sequence | direction |
|---|---|---|
| A3B01F | 5'-TCGTATCGTGAGCGTCAACCGACTGAGCATAA-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B02F | 5'-TTAGTCACTGTACAGCGTGAGGCGTAGTTCAC-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B03F | 5'-ATCAGTACGTTGCTAGCTTGAGCGATAGCCAG-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B04F | 5'-AGTATGCACGACCGGATCTGTCAACGATACGT-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B05F | 5'-GAAGTCTAGATCAATCGTTAGCATCTGCTCGC-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B06F | 5'-AGACGTCTGACGATGCTCATAACCTGGACATC-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B07F | 5'-CTCATTCGATGTATGTCGCAGGTAGCAAGCAT-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B08F | 5'-TACTGTCGATTCGACCACGACTAGGCTATGCT-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B09F | 5'-CTGTACTCCGATGAACGGCGATCTAGTCTACG-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B10F | 5'-CGATGGTACTCAGATCGGCGACATCAGTTGAT-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B11F | 5'-AGTGCTAGTCGATGCCGCTGCATACCTATGAT-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B12F | 5'-ACATCTTACGGCTCGACTGGCACGATGTCTGA-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B13F | 5'-ACTTAAGTCGAGTCGCATGCCTGTCGCTAGAT-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B14F | 5'-ATCAAGATGTACCACGTCAGGCTAGTACTGCT-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B15F | 5'-TCGAGCTTCGAGTGATAACGTAACGCTGCGTA-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B16F | 5'-GCAGATGACCACTACGTCGAACTGACTTGACT-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B17F | 5'-TCAGCATAGCGTCGATCACCAATGCATGCTAG-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B18F | 5'-CTCGATGACAGATGCGATACTGGCGTTCAATG-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B19F | 5'-GCGTCAGCTACGATTGTATCCAAGTGCTCGAT-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B20F | 5'-GACATTGACTGCTATGACGCCTTGAGTAGCAG-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B21F | 5'-AGCAACGCTAGTGGCCGCTATGTACTAGCTCG-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B22F | 5'-TACATCTGGCGAGTATGATCCTACGGTGAGTC-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B23F | 5'-ATCAGCTGTTACGATAGGCGACTCGCCATCGA-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B24F | 5'-CGCACTAGTATCAGCCTAAGCACTCGTGATGG-AAAACGAGGGTTGTCAAAGACCCCA-3' | forward |
| A3B01R | 5'-TCGTATCGTGAGCGTCAACCGACTGAGCATAA-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B02R | 5'-TTAGTCACTGTACAGCGTGAGGCGTAGTTCAC-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B03R | 5'-ATCAGTACGTTGCTAGCTTGAGCGATAGCCAG-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B04R | 5'-AGTATGCACGACCGGATCTGTCAACGATACGT-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B05R | 5'-GAAGTCTAGATCAATCGTTAGCATCTGCTCGC-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B06R | 5'-AGACGTCTGACGATGCTCATAACCTGGACATC-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B07R | 5'-CTCATTCGATGTATGTCGCAGGTAGCAAGCAT-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B08R | 5'-TACTGTCGATTCGACCACGACTAGGCTATGCT-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B09R | 5'-CTGTACTCCGATGAACGGCGATCTAGTCTACG-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B10R | 5'-CGATGGTACTCAGATCGGCGACATCAGTTGAT-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B11R | 5'-AGTGCTAGTCGATGCCGCTGCATACCTATGAT-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B12R | 5'-ACATCTTACGGCTCGACTGGCACGATGTCTGA-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B13R | 5'-ACTTAAGTCGAGTCGCATGCCTGTCGCTAGAT-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B14R | 5'-ATCAAGATGTACCACGTCAGGCTAGTACTGCT-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B15R | 5'-TCGAGCTTCGAGTGATAACGTAACGCTGCGTA-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B16R | 5'-GCAGATGACCACTACGTCGAACTGACTTGACT-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B17R | 5'-TCAGCATAGCGTCGATCACCAATGCATGCTAG-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B18R | 5'-CTCGATGACAGATGCGATACTGGCGTTCAATG-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B19R | 5'-GCGTCAGCTACGATTGTATCCAAGTGCTCGAT-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B20R | 5'-GACATTGACTGCTATGACGCCTTGAGTAGCAG-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |

| | | |
|---|---|---|
| A3B21R | 5'-AGCAACGCTAGTGGCCGCTATGTACTAGCTCG-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B22R | 5'-TACATCTGGCGAGTATGATCCTACGGTGAGTC-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B23R | 5'-ATCAGCTGTTACGATAGGCGACTCGCCATCGA-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |
| A3B24R | 5'-CGCACTAGTATCAGCCTAAGCACTCGTGATGG-ACAAACCTGATAACGCAAGCTACTGC-3' | reverse |

## Table S4

**SNP frequencies in normal and mutant HTT.** The SNPs are annotated with frequencies in different databases including GnomAD v3.0, GnomAD v2.1.1, and 1000 Genomes Project Phase 3. (in a separate Excel file)

## Table S5

**CRISPR enzymes and PAMs analyzed in this study.**

| Enzyme | High efficiency PAMs (included) | Low efficiency PAMs (excluded) | Reference |
|---|---|---|---|
| SpCas9 | NGG, NAG | - | - |
| SpCas9_VQR | NGAG, NGAT, NGAC, NGAA, NGCG, NGTG, NGGG, NAAG | NGGA, NGGT, NGGC | (1) |
| SpCas9_EQR | NGAG, NGCG, NGAT, NGAA | NGGG, NGTG, NGAC | (1) |
| SpCas9_VRER | NGCG | - | (1) |
| SaCas9 | NGGGT, NGAAT, NGAGT, NGGAT | NGGAA, NGGAC, NGACT, NGGCA, NGGGA, NGATC, NGGAG, NGATT, NGGTA, NGGTT, NGACA, NGATA, NGGGC, NGGGG, NGAGC, NGACC, NGAAG, NGGCT, NGCAT, NGACG, NGCGT, NGCAC, NGATG, NAAGT, NAGGT, NGGCG, NGTTT, NGGCC, NGCTT, NCAGT, NGCGA, NGGTG, NGGTC, NCGAT, NGCAA, NAGAT, NGTGT | (1) |
| AsCpf1 | TTTN | - | (2) |

## Table S6

**SNPs carried by each haplotype.** The genome coordinates are based on GRCh38. ‘0’ indicates the reference allele and ‘1’ indicates the alternative allele. (in a separate Excel file)

## Table S7

**Upstream SNPs with AF > 20% in the chromosomes with haplotype-1**. The analysis was based on the 1000 Genomes Project Phase 3 dataset. AF NFE means the allele frequency of the non-Finnish European population.

| Position (GRCh38) | Ref allele | Alt allele | Distance to exon-1 | AF NFE | AF NFE with Hap1 | AF NFE without Hap1 | Ref motif | Alt motif | Enzyme | Strand | Effect on the PAM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3062277 | G | A | -12404 | 36.8% | 61.3% | 16.3% | GG[C]G | GG[T]G | SpCas9_VRER | negative | loss |
| 3060438 | A | G | -14243 | 48.1% | 70.6% | 29.5% | TT[T]G | TT[C]G | AsCpf1 | negative | loss |
| 3060438 | A | G | -14243 | 48.1% | 70.6% | 29.5% | C[A]AA | C[G]AA | SpCas9_VQR | positive | gain |
| 3060438 | A | G | -14243 | 48.1% | 70.6% | 29.5% | C[A]AA | C[G]AA | SpCas9_EQR | positive | gain |
| 3059924 | G | T | -14757 | 51.7% | 71.4% | 35.4% | TT[G]T | TT[T]T | AsCpf1 | positive | gain |
| 3059924 | G | T | -14757 | 51.7% | 71.4% | 35.4% | T[G]TT | T[T]TT | AsCpf1 | positive | gain |
| 3059924 | G | T | -14757 | 51.7% | 71.4% | 35.4% | [G]TTT | [T]TTT | AsCpf1 | positive | gain |
| 3058322 | G | C | -16359 | 50.2% | 69.8% | 34.0% | GA[C] | GA[G] | SpCas9 | negative | gain |
| 3058322 | G | C | -16359 | 50.2% | 69.8% | 34.0% | A[C]AG | A[G]AG | SpCas9_VQR | negative | gain |
| 3058322 | G | C | -16359 | 50.2% | 69.8% | 34.0% | A[C]AG | A[G]AG | SpCas9_EQR | negative | gain |
| 3056856 | A | G | -17825 | 47.8% | 69.8% | 29.5% | CA[A] | CA[G] | SpCas9 | positive | gain |
| 3056181 | T | C | -18500 | 52.0% | 70.0% | 37.0% | C[A]TG | C[G]TG | SpCas9_VQR | negative | gain |
| 3056082 | A | G | -18599 | 54.8% | 70.6% | 41.7% | T[T]TC | T[C]TC | AsCpf1 | negative | loss |
| 3056082 | A | G | -18599 | 54.8% | 70.6% | 41.7% | GA[A] | GA[G] | SpCas9 | positive | gain |
| 3056082 | A | G | -18599 | 54.8% | 70.6% | 41.7% | A[A]AT | A[G]AT | SpCas9_VQR | positive | gain |
| 3056082 | A | G | -18599 | 54.8% | 70.6% | 41.7% | A[A]AT | A[G]AT | SpCas9_EQR | positive | gain |
| 3055248 | T | G | -19433 | 54.8% | 70.6% | 41.7% | TG[T] | TG[G] | SpCas9 | positive | gain |
| 3055248 | T | G | -19433 | 54.8% | 70.6% | 41.7% | G[T]G | G[G]G | SpCas9 | positive | gain |
| 3055248 | T | G | -19433 | 54.8% | 70.6% | 41.7% | TG[T]GAT | TG[G]GAT | SaCas9 | positive | gain |

## Table S8

**Downstream SNPs with AF > 20% in the chromosomes with haplotype-1.** The analysis was based on the 1000 Genomes Project Phase 3 dataset. AF NFE means the allele frequency of the non-Finnish European population.

| Position (GRCh38) | Accession Number | Nearest exon | Distance to exon-1 | AF NFE | AF NFE with Hap1 | AF NFE without Hap1 | Ref motif | Alt motif | Enzyme | Strand | Effect on PAM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3095768 | rs28820097 | exon-3 | 20680 | 39.9% | 71.1% | 13.8% | TT[C]C | TT[T]C | AsCpf1 | negative | gain |
| 3095768 | rs28820097 | exon-3 | 20680 | 39.9% | 71.1% | 13.8% | AG[G] | AG[A] | SpCas9 | positive | loss |
| 3095768 | rs28820097 | exon-3 | 20680 | 39.9% | 71.1% | 13.8% | G[G]AA | G[A]AA | SpCas9_VQR | positive | loss |
| 3095768 | rs28820097 | exon-3 | 20680 | 39.9% | 71.1% | 13.8% | AG[G]A | AG[A]A | SpCas9_EQR | positive | gain |
| 3095768 | rs28820097 | exon-3 | 20680 | 39.9% | 71.1% | 13.8% | G[G]AA | G[A]AA | SpCas9_EQR | positive | loss |
| 3095768 | rs28820097 | exon-3 | 20680 | 39.9% | 71.1% | 13.8% | AG[G]AAT | AG[A]AAT | SaCas9 | positive | loss |
| 3107715 | rs10015979 | exon-6 | 32627 | 40.6% | 71.4% | 15.0% | TA[A] | TA[G] | SpCas9 | positive | gain |
| 3132184 | rs363080 | exon-17 | 57096 | 16.5% | 27.2% | 7.5% | C[G]AG | C[A]AG | SpCas9_EQR | negative | loss |
| 3142714 | rs363107 | exon-23 | 67626 | 16.8% | 28.1% | 7.5% | TT[T]A | TT[C]A | AsCpf1 | negative | loss |
| 3142714 | rs363107 | exon-23 | 67626 | 16.8% | 28.1% | 7.5% | T[A]AA | T[G]AA | SpCas9_VQR | positive | gain |
| 3142714 | rs363107 | exon-23 | 67626 | 16.8% | 28.1% | 7.5% | T[A]AA | T[G]AA | SpCas9_EQR | positive | gain |
| 3150086 | rs11731237 | exon-26 | 74998 | 35.5% | 66.8% | 9.5% | TT[C]C | TT[T]C | AsCpf1 | positive | gain |
| 3150086 | rs11731237 | exon-26 | 74998 | 35.5% | 66.8% | 9.5% | AG[G] | AG[A] | SpCas9 | negative | loss |
| 3150086 | rs11731237 | exon-26 | 74998 | 35.5% | 66.8% | 9.5% | G[G]AA | G[A]AA | SpCas9_VQR | negative | loss |
| 3150086 | rs11731237 | exon-26 | 74998 | 35.5% | 66.8% | 9.5% | AG[G]A | AG[A]A | SpCas9_EQR | negative | gain |
| 3150086 | rs11731237 | exon-26 | 74998 | 35.5% | 66.8% | 9.5% | G[G]AA | G[A]AA | SpCas9_EQR | negative | loss |
| 3158750 | rs363146 | exon-29 | 83662 | 100.0% | 100.0% | 100.0% | TG[A] | TG[G] | SpCas9 | positive | gain |
| 3158750 | rs363146 | exon-29 | 83662 | 100.0% | 100.0% | 100.0% | G[A]GG | G[G]GG | SpCas9_VQR | positive | gain |
| 3164523 | rs9884693 | exon-29 | 89435 | 38.5% | 67.6% | 14.3% | TG[G] | TG[A] | SpCas9 | positive | loss |
| 3164523 | rs9884693 | exon-29 | 89435 | 38.5% | 67.6% | 14.3% | G[G]GG | G[A]GG | SpCas9_VQR | positive | loss |

## Table S9

**Estimated miss-classification rate of demultiplexing.** Each sequencing run has 95 real samples and 5 blank samples. The mis-classification rate was calculated as the average number of reads in blank samples divided by the average number of reads in real samples.

| | Round 1 PCR (16 bp barcode) | | | | | Round 2 PCR (32 bp barcode) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | plate1 | plate2 | plate3 | plate4 | plate5 | plate 1 | plate 2 | plate 3 | plate 4 |
| number of reads assigned to one of the 100 bins | 29872 | 50653 | 48452 | 98692 | 117591 | 520487 | 426535 | 602341 | 378664 |
| number of reads assigned to 95 samples | 29870 | 50649 | 48451 | 98689 | 117582 | 520343 | 426465 | 602265 | 378569 |
| number of reads assigned to 5 blank samples | 2 | 4 | 1 | 3 | 9 | 144 | 70 | 76 | 95 |
| miss-classification rate | 0.13% | 0.15% | 0.04% | 0.06% | 0.15% | 0.53% | 0.31% | 0.24% | 0.48% |
| average number of reads per sample | 314 | 533 | 510 | 1039 | 1238 | 5477 | 4489 | 6340 | 3985 |

**Table S10**

**The list of filtered STR regions in CHM13 for evaluation of repeat quantification.** This list includes all STR regions that are > 100 bp and not within a 500 bp flanking region of another STR. We removed adjacent STRs because many of the adjacent STRs have similar sequences and it is hard to tell if they need to be merged or not without manual examination. Percent_match and percent_indel were calculated by Tandem Repeat Finder (TRF) v4.09. (in a separate Excel file)

**Table S11**

**Detailed information of the CHDI cohort.** Race, sex, region, and CAG repeat size (measured by PCR-based Fragment Analysis) of each subject are shown. Subjects are deidentified. This information was provided by the CHDI foundation. (in a separate Excel file)

**Table S12**

**Number of samples of each ethnic group included in the CHDI cohort.**

| | # of samples | # of QC-passed samples |
|---|---|---|
| American Black | 22 | 16 |
| American Indian | 6 | 5 |
| Asian | 6 | 5 |
| Caucasian | 825 | 610 |
| Hispanic or Latino Origin | 73 | 53 |
| Mixed | 18 | 12 |
| Other | 10 | 7 |

**Table S13**

**Phased SNPs of each individual in the French cohort (in a separate Excel file).** The genome coordinates are based on GRCh38. (in a separate Excel file)

**Table S14**

**Phased SNPs of each individual in the CHDI cohort (in a separate Excel file).** The genome coordinates are based on GRCh38. (in a separate Excel file)

**Table S15**

**CAG and CCG repeat sizes for the French cohort.** The repeat sizes were quantified by NanoRepeat from Oxford Nanopore long reads. (in a separate Excel file).

**Table S16**

**CAG and CCG repeat sizes for the CHDI cohort.** The repeat sizes were quantified by NanoRepeat from Oxford Nanopore long reads. (in a separate Excel file).

# Supplemental References


1. Kleinstiver BP, Prew MS, Tsai SQ, Topkar VV, Nguyen NT, Zheng Z, et al. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. Nature. 2015;523(7561):481-5.
2. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell. 2015;163(3):759-71.