Supplementary Materials for

Haplotyping by CRISPR-mediated DNA circularization (CRISPR-hapC) broadens allele-specific gene editing

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List of supplementary materials:

1. Extended chapter for the CRISPR-hapC method.
2. Supplementary Figure Legends
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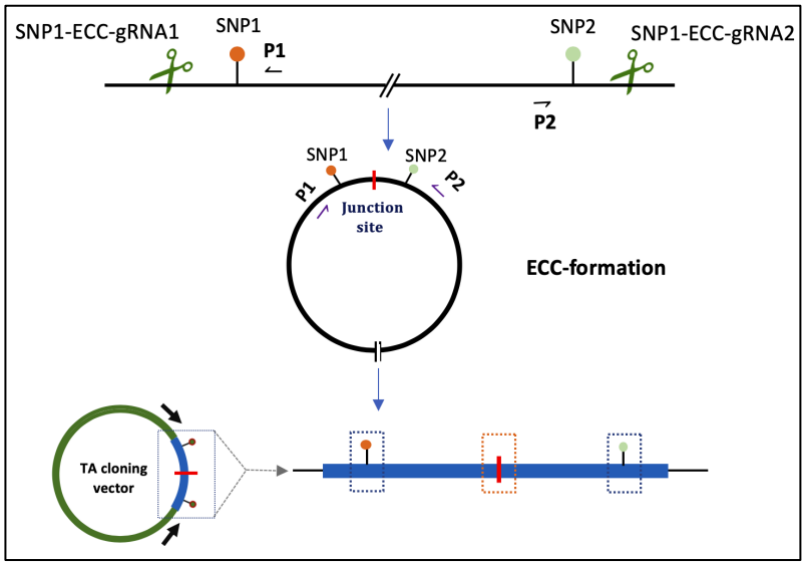
3. Supplementary Tables

1. Extended chapter for the CRISPR-hapC method

In this supplementary material, we provide a detail protocol for conducting the CRISPR-hapC method, which is a technique for haplotyping based on pair-gRNAs cleavage to form extrachromosomal circular DNA (eccDNA, or short for ECC in this protocol) in cells. The main application of CRISPR-hapC is to uncover the linkage between two heterozygous alleles in one chromosome, or known as haplotype (see extended **Fig. S1**). In this protocol, we exemplify the CRISPR-hapC with the 6 SNPs in chromosome 1 of HEK293 cells, of which the results were shown in Figure 1 and Supplementary Fig S1-S2.



Extended Fig.S1 Principle of CRISPR-hapC

The Workflow of CRISPR-hapC generally comprise the following four steps: 1) Define the SNPs of interest in genome of target cells; For example, the asPAM SNPs can be retrieved from our database. www.crispratlas.com; 2) Design CRISPR gRNAs in proximity to the SNPs which will be used to generate eccDNA in target cells by pair-gRNAs; 3) ECC detection by PCR; 4) TA-clone plus Sanger sequencing to uncover the linkage relationships of interested SNPs.

Materials:

1. Target cells;
2. Cell transfection reagents (in this study, we used lipofectamine 2000 or X-tremegene 9 to perform transduction);
3. CRISPR gRNA expression vectors (e.g. lentiCRISPRv2)
4. Protocols for generation of CRISPR vector can follow our simply LION method [1]. and vector construction related enzymes, reagents (for gRNA construction, please **refer to protocol in the appendix 1**)
5. PCR polymerase
6. TA-clone kit.
7. Competent cells
8. Plasmid safe DNA nuclease
9. Other reagents are listed in the protocol

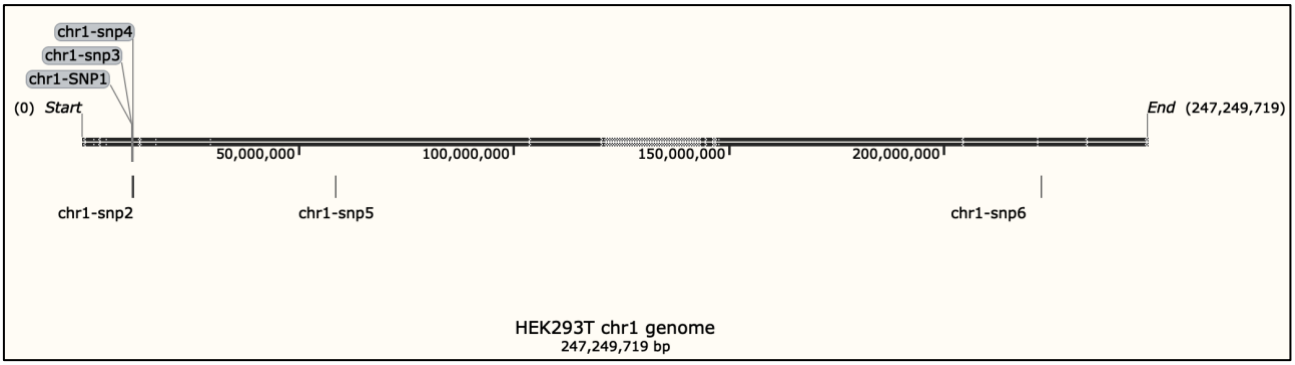
Step 1: prediction and validation of the SNPs in genome of target cells

The first step is to select the variants of interest, like the potential pathogenic SNPs, asPAM SNPs, etc. Generally, you can download the interested pathogenic variants from Clin Var database (https://www.ncbi.nlm.nih.gov/clinvar/). For asPAM SNPs, just use the database described in this study (http://www.crispratlas.com/knockout). Then confirm the existence of interested variants in your target cells by PCR and Sanger sequencing.

1) In this protocol, we selected 6 SNPs in chromosome 1 of HEK293T genome (Extended **Table S1 and Extended Fig S2**), which were downloaded from the HEK293genome database (http://hek293genome.org/v2/). We aimed to uncover the linkage relationships of SNP1 and the remaining SNPs.

Extended Table S1. HEK293T SNPs selected in this protocol

Number	CHROM	POS	REF	ALT	Distance from SNP1
SNP1	chr1	11427039	G	A	0 bp
SNP2	chr1	11427699	T	A	660 bp
SNP3	chr1	11443564	T	G	16.5 kb
SNP4	chr1	11733278	C	T	306.2 kb
SNP5	chr1	58707134	T	C	47.2 Mb
SNP6	chr1	222783385	G	A	211.3 Mb



Extended Fig. S2 selected SNPs in HEK293T chromosome 1

2) To make sure the SNPs you are interested are present in the genome of target cells, conduct PCR and Sanger sequencing to confirm it. The primers for the 6 HEK293T SNPs detection are showed in Extended **Table S2**.

Extended Table S2. Primers used for HEK293T Chr1-SNPs detection

Primer name	Oligo sequence (5'-3')	Product length
CHR1-SNP1-SCR-F	AAGGAAGGTCGGTGACTGGAGA	297 bp
CHR1-SNP1-SCR-R	AGGGAAGGAAGACAGCAGAGAG	
CHR1-SNP2-SCR-F	TCTAGCTCTGAGTGCCGCATCC	353 bp

CHR1-SNP2-SCR-R	CCTGACTGCCCACCTCTGTAAG	
CHR1-SNP3-SCR-F	GTGATTTGAATCTGGCAGCGTG	379 bp
CHR1-SNP3-SCR-R	AGTGACTCCTAGATCACACAGC	
CHR1-SNP4-SCR-F	AGTCCTGCCTGAGTGTTGCAAG	295 bp
CHR1-SNP4-SCR-R	CACAGCCCATCTACAGAATTCC	
CHR1-SNP5-SCR-F	AGGTCAACAGGGAGTCCTCATG	248 bp
CHR1-SNP5-SCR-R	AAGGTCAGCCAGGCGGAATGTT	
CHR1-SNP6-SCR-F	GCGCGTGGTCATTTAGGAAGCT	378 bp
CHR1-SNP6-SCR-R	TCTCTGTGCCAATGGATCACTG	

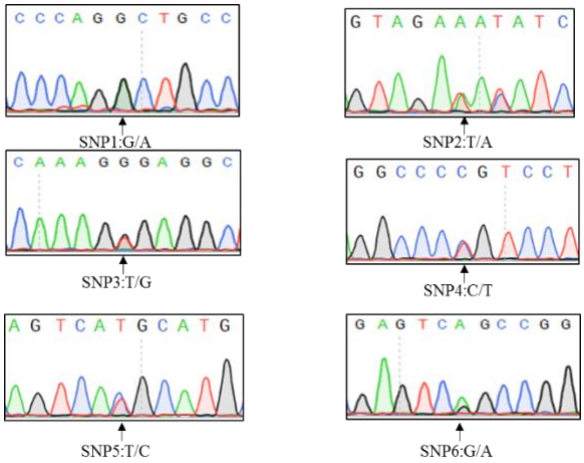
We recommend using high-fidelity DNA polymerase to avoid false-positive SNPs which are introduced by PCR. The PCR reaction program depends on the polymerase you choose. we used platinum pfx polymerase (ThermoFisher Scientific) to conduct PCR reaction in this protocol (Extended **Table S3**).

Extended Table S3. PCR reaction system for HEK293T Chr1 SNPs detection

Reagents	**Volume**
Genomic DNA	100 ng
Forward primer (5 μM)	0.6 μL
Reverse primer (5 μM)	0.6 μL
dNTP (10 mM)	0.6 μL
Mg_2SO_4	0.3 μL
platinum Pfx	0.24 μL
10×Enhancer	3 μL
10×Pfx buffer	3 μL
H2O	To 15 μL

Thermocycles: 94°C 2min, (94 °C/20 s, 58 °C/30 s, 68 °C/45 s) × 35cycles, 68 °C 7 min, 4 °C 5 min. For most PCR, 58°C annealing works well.

3) Column- or gel-purify the PCR products and send for Sanger sequencing to confirm the existence of target SNPs (Extended **Fig. S3**).



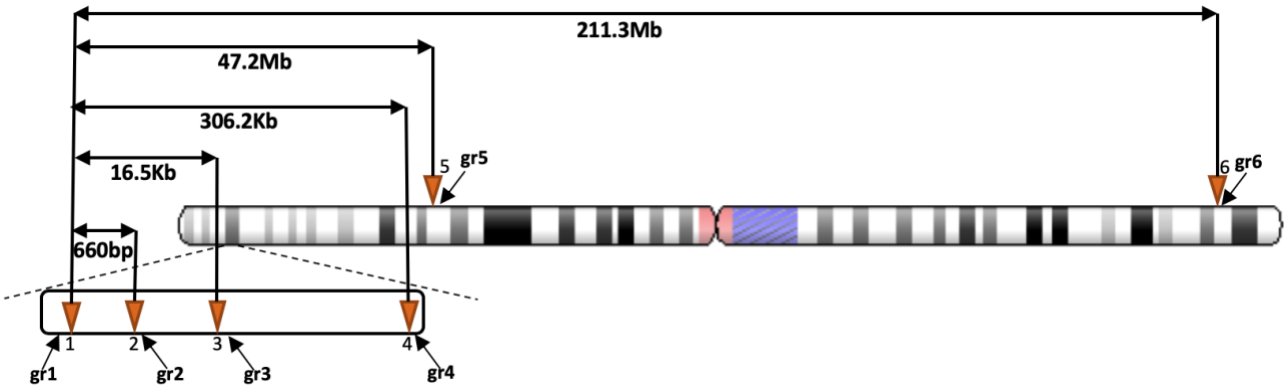
Extended Fig. S3 Validation of 6 selected Chr1-SNPs of HEK293 cells by Sanger sequencing.

Step 2: Generation of eccDNA by pair-gRNAs

1) CRISPR-C design. After the validation of the genotype of target SNPs, design and construct a pair of gRNAs for generating eccDNA in cultured cells. Here we use the SpCas9 system. The SaCas9 system also works for CRISPR-C. Taking haplotyping of SNP1 and SNP2 as an example, **a gRNA upstream of SNP1 and a gRNA downstream of SNP2** were designed (extended **Fig. S1**), or known as flanking gRNAs. **OBS!** The distance between the SNP site and gRNA cleavage site is better to be 20 ~ 150 bp. This makes it easier to perform the subsequent eccDNA amplification PCRs. The gRNAs we used in this protocol is showed in Extended **Table S4** and the physical positions of these gRNAs and SNPs are showed in Extended **Fig. S4**.

Extended Table S4. gRNAs used to form ECCs for detection of SNP linkage

ECC-gRNA name	gRNA sequence	Distance from the target SNP site
CHR1-ECC-SNP1	GACTGGAGAGAAGCTTCCCTGGG	101 bp
CHR1-ECC-SNP2	TGACGGCACAGTTCGCAGAGAGG	36 bp
CHR1-ECC-SNP3	GGTCTTGCATGTCACCATGCTGG	87 bp
CHR1-ECC-SNP4	GCCTGGTCACATGGTGCCTAGG	28 bp
CHR1-ECC-SNP5	TATACAGTGTAGCGGCCATGGAGG	50 bp
CHR1-ECC-SNP6	TCCCTGCTCCCACCGGAAATGGG	44 bp



Extended **Fig. S4 Physical positions of the selected gRNAs in chromosome 1 of HEK293T.**

2) Transfection. Transfect pair-gRNAs in to the target cells. In our study, transfection was conducted with Lipofectamine 2000 transfection reagent (Invitrogen) or X-tremegene 9 (Roche) in 24-well plates according to the manufacturer's protocol.
Briefly, 60,000 cells/per well were seeded in 24 well plates and the media were changed when cell confluence reaches 50~70% before transfection (typically 24 hours after seeding). For pair-gRNA co-transfection, 500 ng plasmid DNA contains 250 ng upstream gRNA and 250 ng downstream gRNA (Cas9 expression cassette is in the same plasmid as the gRNA) and 1.5μL Lipofectamine 2000 were diluted separately in Opti-MEM (Gibco) to a total volume of 25μL. The diluted DNA was added to the diluted Lipofectamine and mixed gently. After 15 min incubation at room temperature, the transfection mixture was homogeneously added to the adherent cells in a dropwise manner. We change medium 24 hours after transfection and harvest cells 24-48 hours later for eccDNA detection. Transfection with a single gRNA was used as controls.

3) The eccDNA detection by PCRs. The ECC will be formed 48 hours after pair-gRNAs transfection [2]. We had previously compared three different methods for eccDNA purification. The convenient cell lysate approach was used here. Briefly, transfected cells were suspended in 0.2 mL lysis buffer (KCl 50 mM, MgCl2 1.5 mM, 0.5% NP40 and 0.5% Tween 20, 10 mM Tris pH 8.5) and incubated with 10 μl proteinase K (19.1 mg/mL, Thermo) for 2.5 hours at 55°C. Proteinase K was heat-inactivated at 95°C for 10 min, cooled down at room temperature for 15 min, and 32 μl DNA solution was transferred for linear DNA removal with plasmid safe DNA nuclease, storing the rest of the cell lysate at -20°C until PCR analysis.

The primers for ECC detection should be downstream of SNP1 and upstream of SNP2 (showed in extended **Fig. S1, or known as inverse PCR primers**). And we suggest the distance between primer and SNP site is better to be 100 ~ 400 bp, then the final ECC PCR product would be less than 1000 bp (the optimal size is 400~600 bp). Primers used in this protocol are showed in extended **Table S5**. PCR reaction system for ECC detection is showed in extended **Table S6**. And PCR results of Chr1-SNPs ECC detection is showed in extended **Fig. S5**.

Extended Table S5. Primers for Chr1 SNP-linkage detection (ECC)

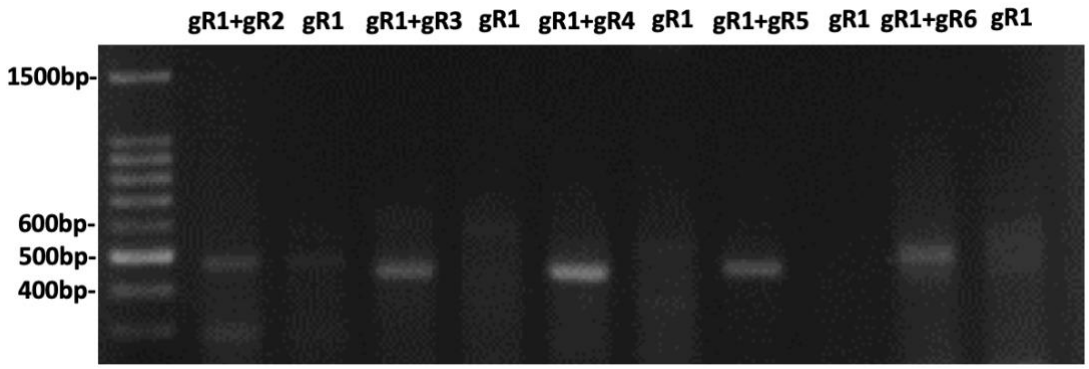
Primer combination	ECC product length
CHR1-SNP1-SCR-R + CHR1-SNP2-SCR-F	482 bp

CHR1-SNP1-SCR-R + CHR1-SNP3-SCR-F	457 bp
CHR1-SNP1-SCR-R + CHR1-SNP4-SCR-F	444 bp
CHR1-SNP1-SCR-R + CHR1-SNP5-SCR-F	456 bp
CHR1-SNP1-SCR-R + CHR1-SNP6-SCR-F	496 bp

Extended Table S6. PCR reaction system for ECC detection

Reagents	Volume
Genomic DNA	100 ng
Forward primer (5 μM)	0.6 μL
Reverse primer (5 μM)	0.6 μL
dNTP (10 mM)	0.6 μL
MgSO4	0.3 μL
platinum Pfx	0.24 μL
10×Enhancer	3 μL
10×Pfx buffer	3 μL
H2O	To 15 μL

Thermocycles: 94°C 2min, (94 °C/20 s, 58 °C/30 s, 68 °C/45 s) × 35cycles, 68 °C 7 min, 4 °C 5 min. For most PCR, 58°C annealing works well.



Extended Fig. S5 PCR results of Chr1-SNPs ECC detection

Step 3: TA clone and Sanger sequencing

1) Gel-purify the ECC-PCR products and clone to a TA-cloning vector. The PCR products amplified by pfx polymerase are blunt-ends. We firstly add an Adenine at 5'end of PCR fragments by rTaq polymerase (extended **Table S7**).

Extended Table S7. Adenine extension of blunt-end PCR products

Reagents	**Volume**
PCR products	17 μL
TAKARA rTaq (5 U/ μL)	0.1 μL
10 × PCR buffer	2 μL
dNTP (10 mM)	1 μL

Incubate at 72°C for 1 hour.

2) Next, we used a T-vector pMD19 (TAKARA) to conduct TA cloning (extended **Table S8**).

Extended Table S8. TA cloning

Reagents	**Volume**
T vector pMD19 (simple)	1 μL
PCR products with A	4 μL
Solution I	5 μL

Incubate at room temperature for 1 hour then transform competent cells (**Attachment 1**).

3) After transformation, pick up bacterial clones for Sanger sequencing to analyze the linkage between tested SNPs.

Attachment 1: protocol for gRNA assembly in our lab.

1. Oligos annealing

CrRNA oligo SS (100 μM)	1 μL
CrRNA oligo AS (100 μM)	1 μL
10X NEB Buffer2	2 μL
H2O	16 μL
Total	20 μL

95°C 5min, 95°C 10 sec (-0.5°C/cycle) X150 cycles, 20°C 5min, 4°C hold

2. Ligation

Annealed CrRNA	1 μL
LentiCRISPRv2	100ng
T4 ligase (FastDigest)	0.5 μL
T4 ligase buffer	2 μL
BsmBI (FastDigest)	0.5 μL
H2O	To 20 μL

10 cycles of 37°C for 5 min, 22C for 10 min
1 cycle of 37°C for 30 min,
1 cycle of 75°C for 15 min,
save at 4°C

3. Transformation
1）Prepare the following mixture and incubate on ice for 2-3min

Ligation product	1 μL
KCM buffer	5 μL
H2O	19 μL

2）add 25 μL competent cells into 1), pipette up and down gently, keep on ice for 20 min, then let them sit at room temperature for 10min;

3) add 150 μL LB medium, incubate at 37 °C in a shaking incubator for 1 hour.

4) plate 50 μL 3) on AmpR LB plate, incubate at 37°C up-side-down for 16 hours (±2 hours).

5) pick up 2 colonies for each crRNA and screen for positive colonies by PCR (pick up 1 colony using a 10 μL tip and re-suspend the colony in 6 μL ampR LB medium by pipetting up and down gently; 1 μL for PCR screening and the remaining 5 μL for expansion).

6) Store the plates at 4°C.

7) Expand 1 positive colony for each crRNA overnight and extract the plasmids the next day.

Reference

1. Xiang, X., et al., *LION: a simple and rapid method to achieve CRISPR gene editing.* Cell Mol Life Sci, 2019. **76**(13): p. 2633-2645.
2. Henrik Devitt Møller, L.L., Xi Xiang, Trine Skov Petersen, Jinrong Huang, Luhan Yang, Eigil Kjeldsen, Uffe Birk Jensen, Xiuqing Zhang, Xin Liu, Xun Xu, Jian Wang, Huanming Yang, George M Church, Lars Bolund, Birgitte Regenberg, Yonglun Luo;, *CRISPR-C: circularization of genes and chromosome by CRISPR in human cells.* Nucleic Acids Research, 2018: p. gky767.

2. Supplementary Figure Legends

Fig. S1. Genotyping of six SNPs in chromosome 1 of HEK293 cells

(A) A list of the six heterozygous SNPs in HEK293 cells from the HEK293 genome database.

(B) Genotyping of the six SNPs in our HEK293 cells by Sanger sequencing.

Fig. S2. Haplotyping of the six SNPs in Chromosome 1 of HEK293 cells with CRISPR-hapC

(A) PCR amplification of the eccDNA generated with the indicated CRISPR pair. Controls were transfection with single CRISPR vector (Cr1). White arrow heads indicate the corresponding eccDNA product.

(B) Illustration, Sanger sequencing and haplotyping of between SNP1 and the remaining SNPs.

(C) Summary of colonies with Sanger sequencing results supporting the haplotyping.

Fig. S3. Circos plot of the number of asPAM SNPs and CRISPRs across the human genome.

Resolution: number of SNPs per 1M bin for the hg19 genome. The color codes are corresponding to the type of Cas systems, SaCas9 (red), SpCas9(blue), xCas9(orange) and Cas12a (green).

Fig. S4. Summary of HTT SaCas9 asPAM SNPs.

Genome position, sequence for the reference and alternative SNP, heterozygous frequency based on human 1k genome, corresponding SNP if present in the human dbSNP, and consequence for PAM activity by the alternative SNP site are summarized.

Fig. S5. Illustration of the C-Check system for evaluating asPAM CRISPR specificity.

CC-aPAM: C-Check vector with the asPAM CRISPR target site (protospacer) and an active PAM; CC-dPAM: C-Check vector with the asPAM CRISPR target site (protospacer) and a dead PAM; asPAM-Cr: asPAM CRISPR.

Fig. S6. Summary of TTR asPAM SNPs.

Genome position, sequence for the reference and alternative SNP, heterozygous frequency based on human 1k genome, corresponding SNP if present in the human dbSNP, and consequence for PAM activity by the alternative SNP site are summarized.

Fig. S7. C-Check based evaluation of asPAM specificity

Representative FACS analysis for EGFP positive (reflecting cleavage activity) and AsRED positive (indicating transfection efficiency) in HEK293T cells. TTR asPAM SNP 5, 8 and 9 are presented here. Minimum 10,000 cells were analyzed for each group, n=3.

Fig. S8. HTT asPAM SNP genotyping by Sanger sequencing.

Sanger sequencing chromatograms for each HTT asPAM SNP are presented for three different cell lines. Black triangles indicate the SNP position. Sequences for heterozygous SNPs were indicated.

Fig. S9. Generation and evaluation of the activity of three HTT universal gRNAs.

(A) Illustration of the HTT universal gRNA (UnigRNA) target sites

(B) Table of HTT unigRNA spacer sequences
(C) Sanger sequencing of the unigRNA target sites (T1, T2, T3) in control cells and cells transient transfected with the HTT unigRNA CRISPR. Black triangle indicates the expected cleavage site.

Fig. S10. Chromatograms of Sanger sequencing-based genotyping of 14 asPAM SNPs in five cell lines.

Fig. S11. Generation of TTR mutated cells with CRISPR/SpCas9 and haplotyping with CRISPR-hapC.
(A) Genotyping of HepG2 clone 21. Blue: protospacer sequences; Red: SpCas9 PAM.
(B) Haplotyping of the TTR exon2 mutation allele with TTR asPAM SNP3 by CRISPR-hapC. Letters (P1 and P4) represent ECC genotyping primers. Top: Schematic illustration of the TTR locus and the procedure of CRISPR-hapC: ECC-DNA generated by CRISPR-C, ECC-DNA amplification by reverse PCR, subcloning and Sanger sequencing.

Fig. S12. Genotyping of haplotype-specific deletion of TTR by asPAM CRISPR
(A) Schematic illustration of the TTR locus, haplotypes, CRISPR target sites and genotyping primers (P6 – P9).
(B) Genotyping of asPAM specific TTR deletion by targeted PCR. Top, gel electrophoresis results of deletion specific PCR primers franking the TTR-Uni-Cr and either TTR-asPAM-Cr11 or TTR-asPAM-Cr14. Bottom, the expected PCR amplicon after targeted deletion were indicated with rectangles and sequenced with Sanger sequencing. The CRISPR gRNA spacers and PAM sequences were indicated.

Table S1. PCR primers for asPAM SNP genotyping
Table S2. CRISPR gRNA spacer sequences
Table S3. C-Check oligos
Table S4. PCR primers for CRISPR-hapC linkage analysis and PCRs
Table S5. Genotyping of TTR exon2 mutation clones and haplotype results by CRISPR-HapC.

Figure S1

A

Number	CHROM	POS	REF	ALT
SNP1	chr1	11427039	G	A
SNP2	chr1	11427699	T	A
SNP3	chr1	11443564	T	G
SNP4	chr1	11733278	C	T
SNP5	chr1	58707134	T	C
SNP6	chr1	222783385	G	A

B

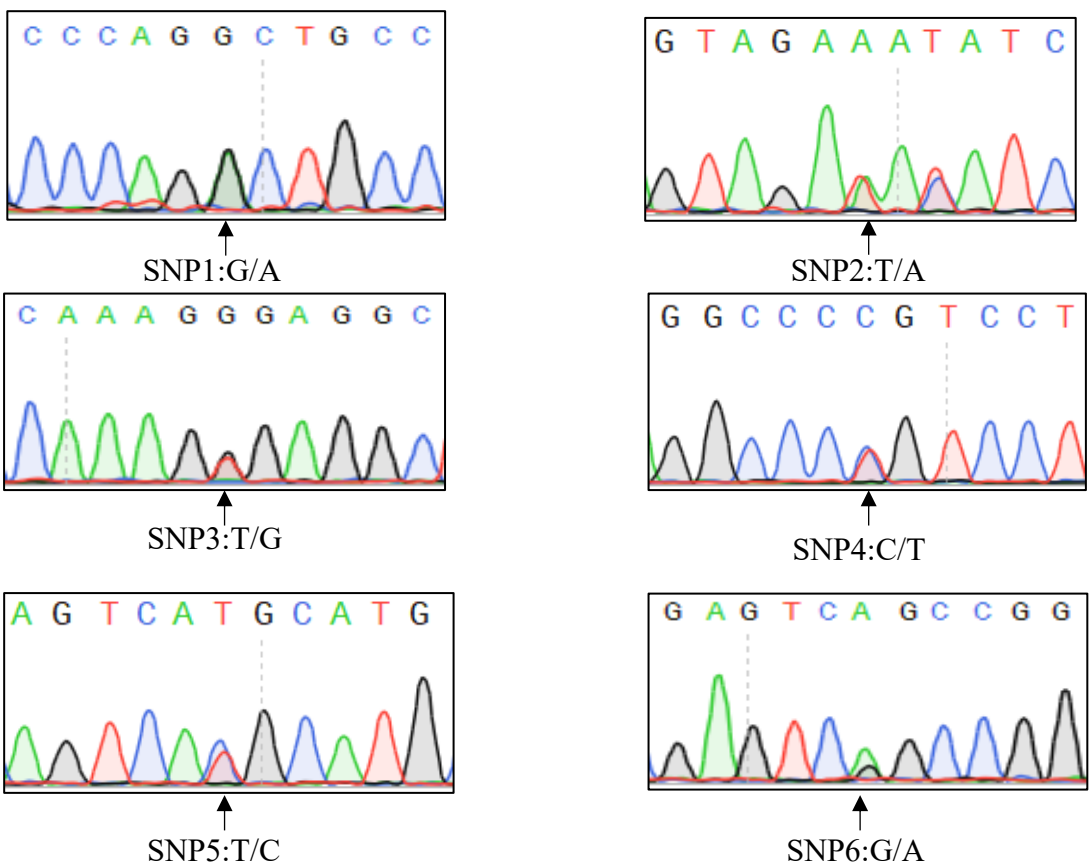


Figure S2

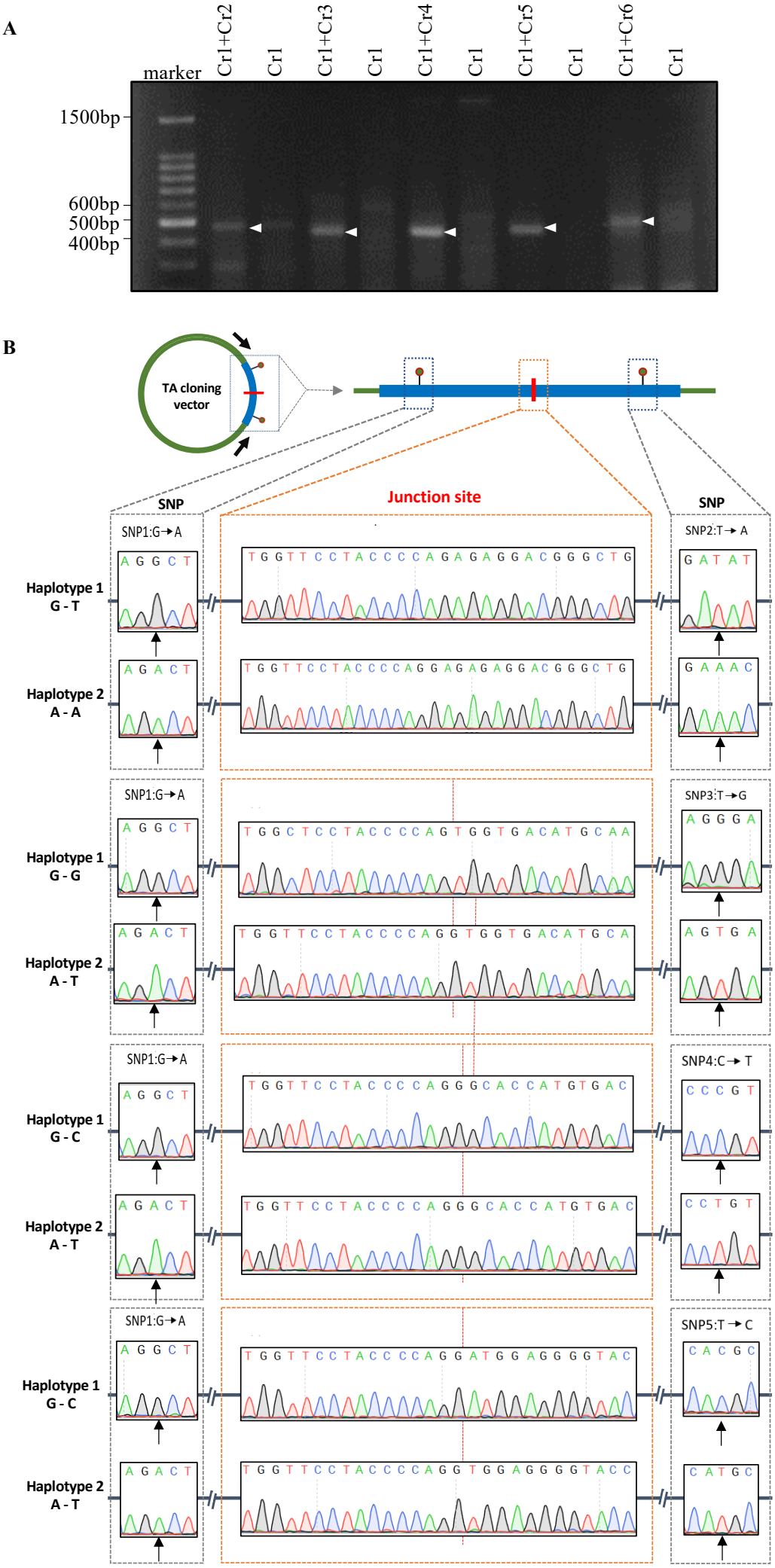
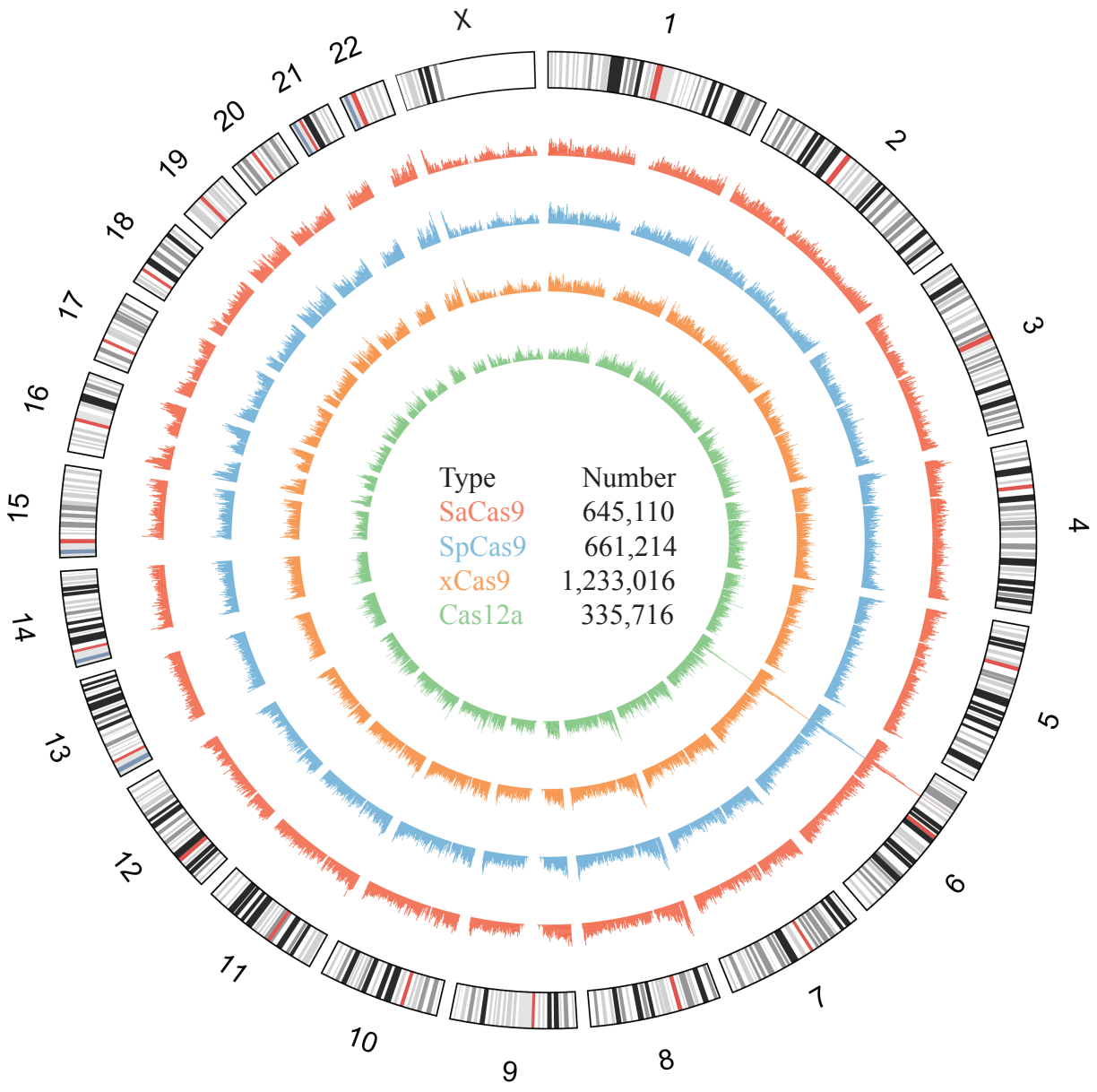

A
marker
Cr1+Cr2
Cr1
Cr1+Cr3
Cr1
Cr1+Cr4
Cr1
Cr1+Cr5
Cr1
Cr1+Cr6
Cr1
1500bp
600bp
500bp
400bp
B
TA cloning vector
SNP
Junction site
SNP
SNP1:G→A
SNP2:T→A
SNP3:T→G
SNP4:C→T
SNP5:T→C
Haplotype 1
G - T
Haplotype 2
A - A
Haplotype 1
G - G
Haplotype 2
A - T
Haplotype 1
G - C
Haplotype 2
A - T
Haplotype 1
G - C
Haplotype 2
A - T

Figure S2

C Summary of Sanger sequencing results

		SNP2		SNP3		SNP4		SNP5		SNP6	
		T	A	G	T	C	T	C	T	G	A
SNP1	G	3		1		1		4		1	
	A		5		3		4		3		2

Figure S3


X
1
2
3
4
5
6
7
8
9
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11
12
13
14
15
16
17
18
19
20
21
22
Type Number
SaCas9 645,110
SpCas9 661,214
xCas9 1,233,016
Cas12a 335,716

Figure S4

HTT asPAM SNP	Chr	Pos	REF	ALT	HetAF1000genomes	dbSNP	PAM alternation	PAM Strand
SNP1	chr4	3080173	G	A	G/A:0.47	rs3856973	dPAM	+strand
SNP2	chr4	3097495	G	A	G/A:0.38	rs28820097	dPAM	+strand
SNP3	chr4	3098321	C	G	C/G:0.40	N.A.	dPAM	-strand
SNP4	chr4	3113337	G	A	G/A:0.30	rs16843836	dPAM	+strand
SNP5	chr4	3151813	C	T	C/T:0.37	rs11731237	dPAM	-strand
SNP6	chr4	3166250	G	A	G/A:0.38	rs9884693	dPAM	+strand
SNP7	chr4	3216815	C	G	C/G:0.46	rs916171	aPAM	-strand
SNP8	chr4	3234828	G	T	G/T:0.47	rs2269478	dPAM	+strand
SNP9	chr4	3236135	G	T	G/T:0.38	rs362270	aPAM	-strand

Figure S5

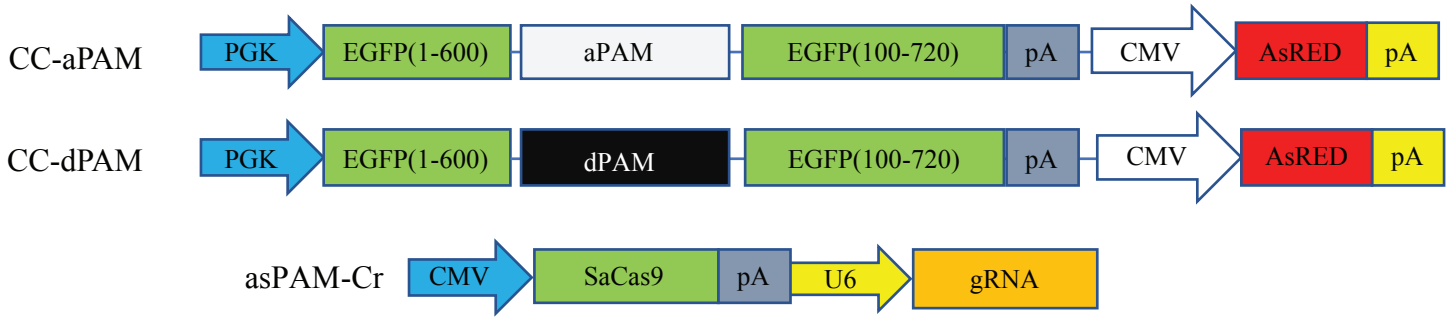

CC-aPAM
PGK
EGFP(1-600)
aPAM
EGFP(100-720)
pA
CMV
AsRED
pA
CC-dPAM
PGK
EGFP(1-600)
dPAM
EGFP(100-720)
pA
CMV
AsRED
pA
asPAM-Cr
CMV
SaCas9
pA
U6
gRNA

Figure S6

TTR asPAM SNP	CHROM	POS	REF	ALT	HetAF1000genomes	dbSNP	PAM alternation	PAM Strand
SNP1	chr4	29138749	G	A	G/A:0.36	rs1791202	Loss of PAM	+
SNP2	chr4	29139002	C	A,T	C/T:0.40	rs1667225	Loss of PAM	-
SNP3	chr4	29142751	C	G	C/G:0.45	-	Loss of PAM	-
SNP4	chr4	29146950	G	A,T	G/A:0.44	rs9963959	Loss of PAM	+
SNP5	chr4	29150290	G	A	G/A:0.46	rs9951041	Loss of PAM	+
SNP6	chr4	29151351	A	T	A/T:0.44	rs735329	Loss of PAM	+
SNP7	chr4	29155702	C	G	C/G:0.40	rs17660933	Loss of PAM	-
SNP8	chr4	29155949	G	C	G/C:0.40	rs11081702	Loss of PAM	+
SNP9	chr4	29156999	C	T	C/T:0.41	rs1375445	Loss of PAM	-
SNP10	chr4	29173680	C	G	C/G:0.42	-	Loss of PAM	-
SNP11	chr4	29176460	C	A	C/A:0.41	-	Loss of PAM	-
SNP12	chr4	29179040	C	T	C/T:0.35	rs1791228	Loss of PAM	-
SNP13	chr4	29189457	C	T	C/T:0.44	rs1791197	Loss of PAM	-
SNP14	chr4	29200724	G	A	G/A:0.43	rs974676	Loss of PAM	+

Figure S7

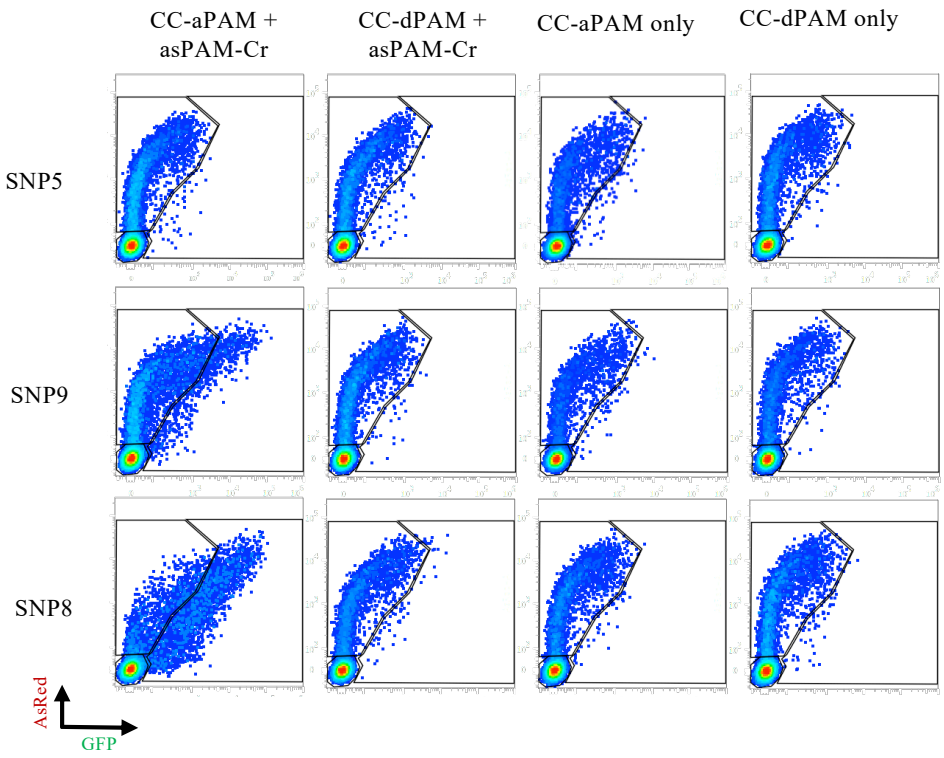

CC-aPAM + asPAM-Cr
CC-dPAM + asPAM-Cr
CC-aPAM only
CC-dPAM only
SNP5
SNP9
SNP8
AsRed
GFP

Figure S8

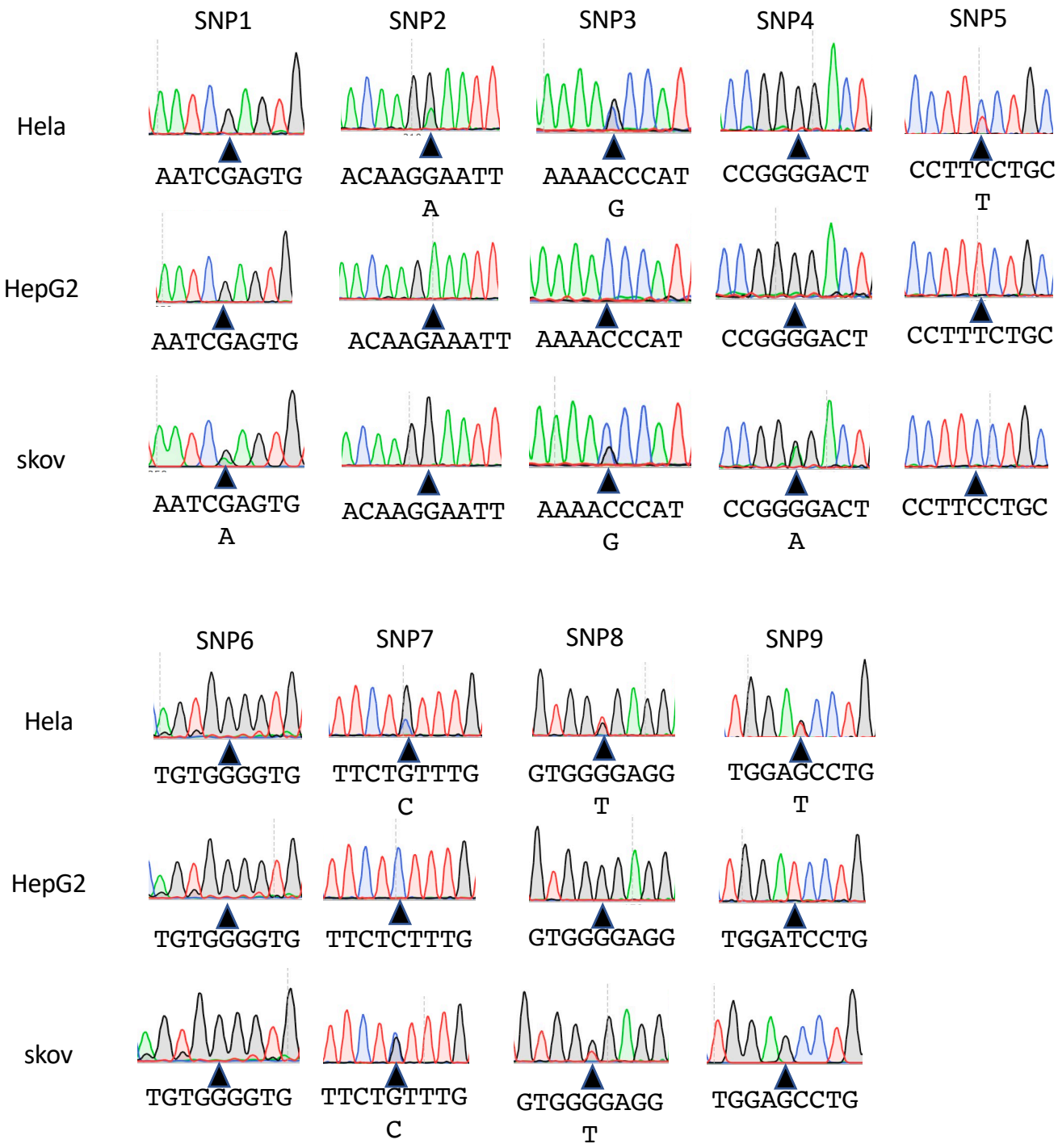
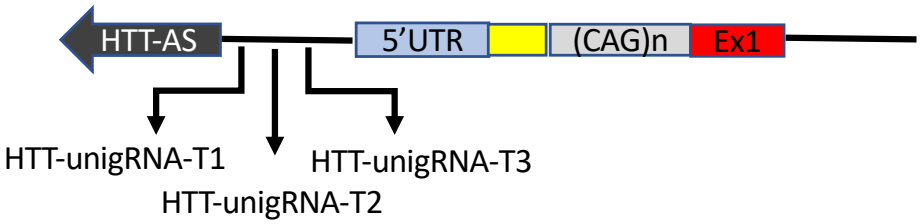

SNP1
SNP2
SNP3
SNP4
SNP5
Hela
AATCGAGTG
ACAAGGAATT
A
AAAACCCAT
G
CCGGGGACT
CCTTCCTGC
T
HepG2
AATCGAGTG
ACAAGAAATT
AAAACCCAT
CCGGGGACT
CCTTTCTGC
skov
AATCGAGTG
A
ACAAGGAATT
AAAACCCAT
G
CCGGGGACT
A
CCTTCCTGC
SNP6
SNP7
SNP8
SNP9
Hela
TGTGGGGTG
TTCTGTTTG
C
GTGGGGAGG
T
TGGAGCCTG
T
HepG2
TGTGGGGTG
TTCTCTTTG
GTGGGGAGG
TGGATCCTG
skov
TGTGGGGTG
TTCTGTTTG
C
GTGGGGAGG
T
TGGAGCCTG

Figure S9

a



b

Sa gRNA	target sequence
HTT-unigRNA-T1	GGTTCCCTGGCCAGCCATTGG
HTT-unigRNA-T2	GGCGCCGCTCAGCACCGGGGCA
HTT-unigRNA-T3	GCCCCGGTGCTGAGCGGCGCC

c

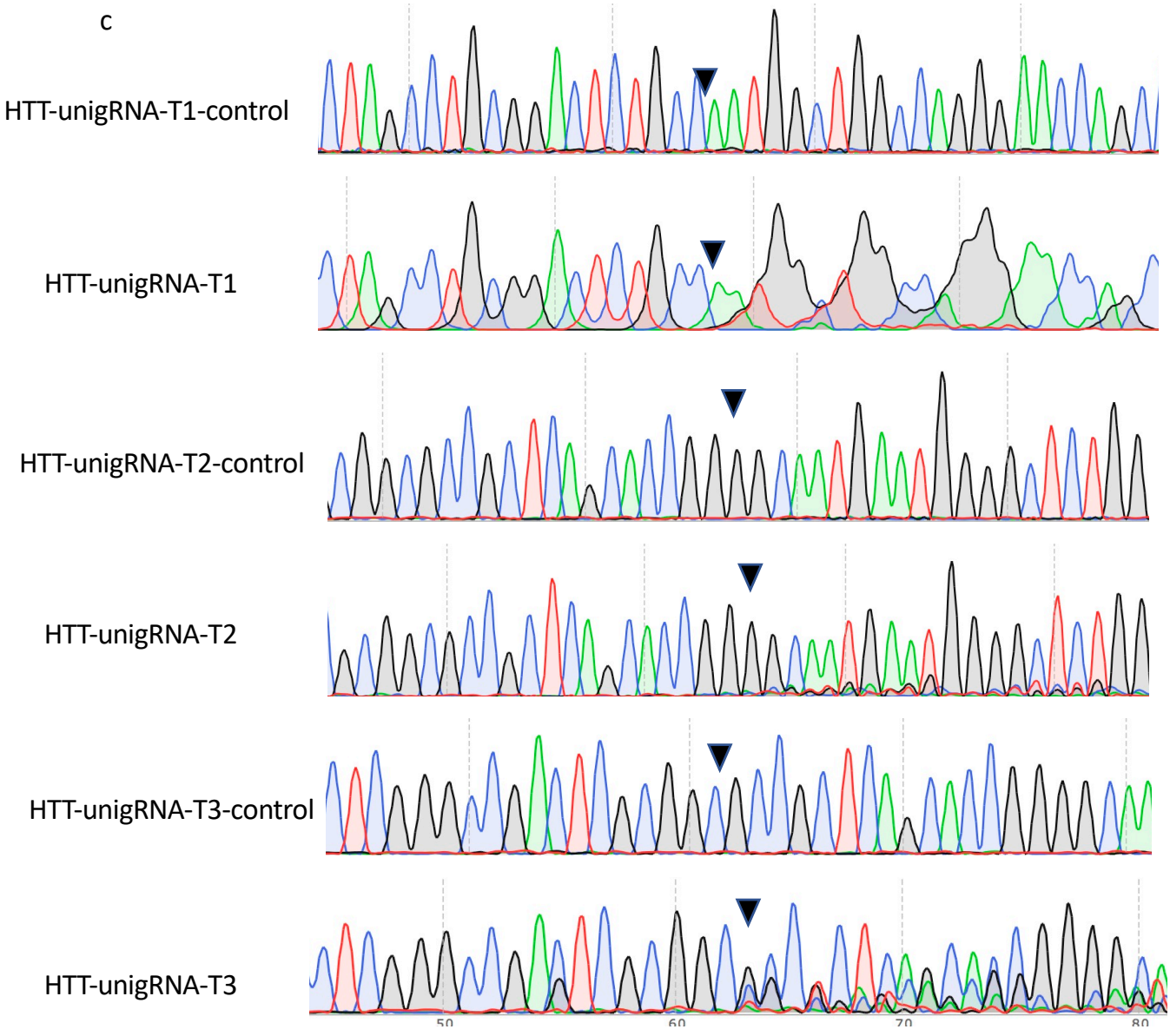


Figure S10

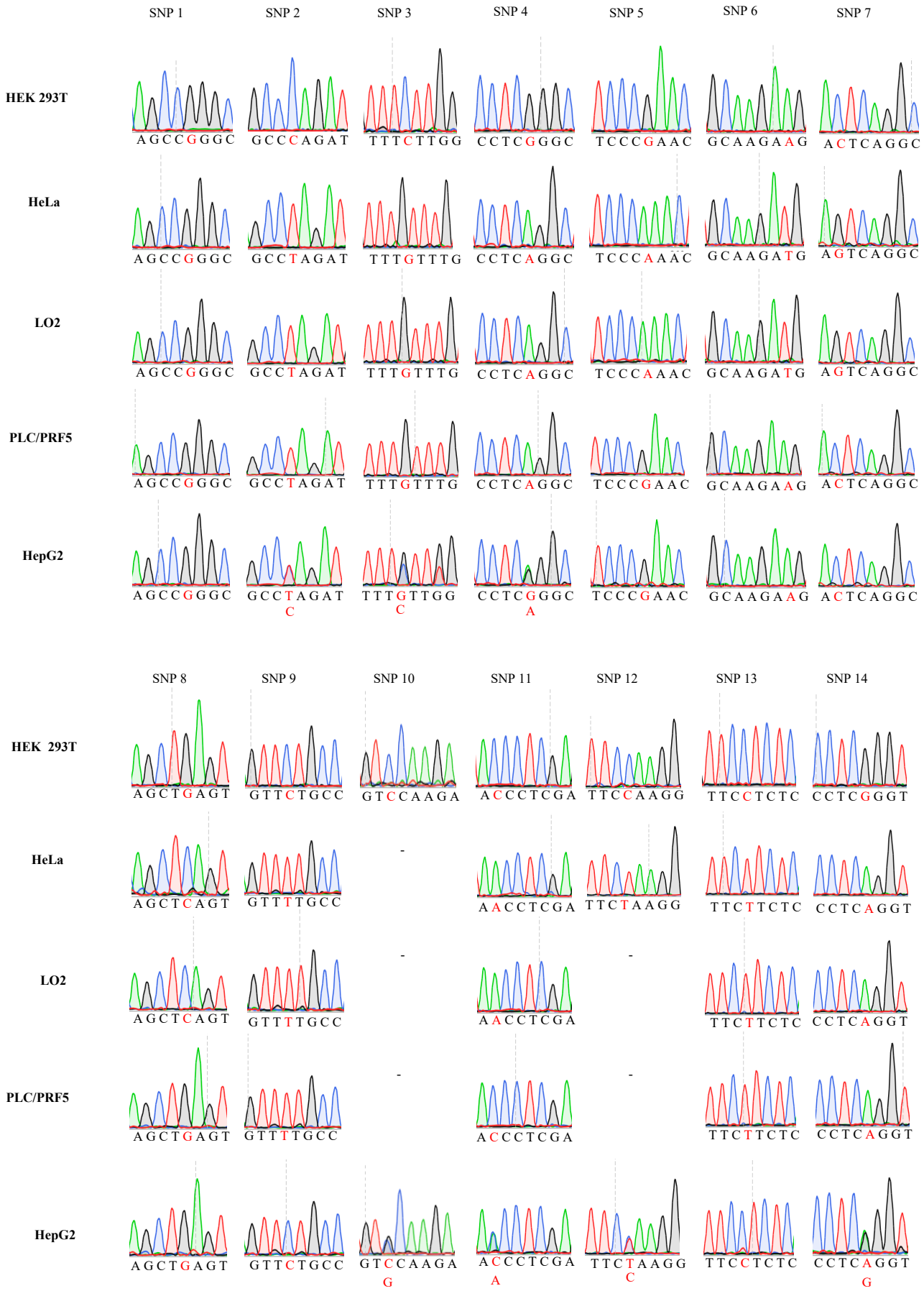
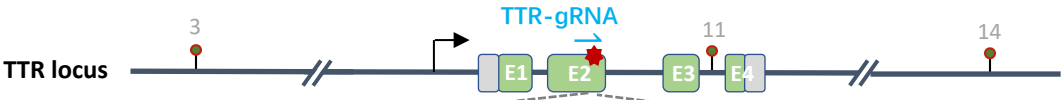

SNP 1
SNP 2
SNP 3
SNP 4
SNP 5
SNP 6
SNP 7
HEK 293T
AGCCGGGC
GCCCAGAT
TTTCTTGG
CCTCGGGC
TCCCGAAC
GCAAGAAG
ACTCAGGC
HeLa
AGCCGGGC
GCCTAGAT
TTTGTTTG
CCTCAGGC
TCCCAAAC
GCAAGATG
AGTCAGGC
LO2
AGCCGGGC
GCCTAGAT
TTTGTTTG
CCTCAGGC
TCCCAAAC
GCAAGATG
AGTCAGGC
PLC/PRF5
AGCCGGGC
GCCTAGAT
TTTGTTTG
CCTCAGGC
TCCCGAAC
GCAAGAAG
ACTCAGGC
HepG2
AGCCGGGC
GCCTAGAT
C
TTTGTTGG
C
CCTCGGGC
A
TCCCGAAC
GCAAGAAG
ACTCAGGC
SNP 8
SNP 9
SNP 10
SNP 11
SNP 12
SNP 13
SNP 14
HEK 293T
AGCTGAGT
GTTCTGCC
GTCCAAGA
ACCCTCGA
TTCCAAGG
TTCCTCTC
CCTCGGGT
HeLa
AGCTCAGT
GTTTTGCC
AACCTCGA
TTCTAAGG
TTCTTCTC
CCTCAGGT
LO2
AGCTCAGT
GTTTTGCC
AACCTCGA
TTCTTCTC
CCTCAGGT
PLC/PRF5
AGCTGAGT
GTTTTGCC
ACCCTCGA
TTCTTCTC
CCTCAGGT
HepG2
AGCTGAGT
GTTCTGCC
GTCCAAGA
G
ACCCTCGA
A
TTCTAAGG
C
TTCCTCTC
CCTCAGGT
G

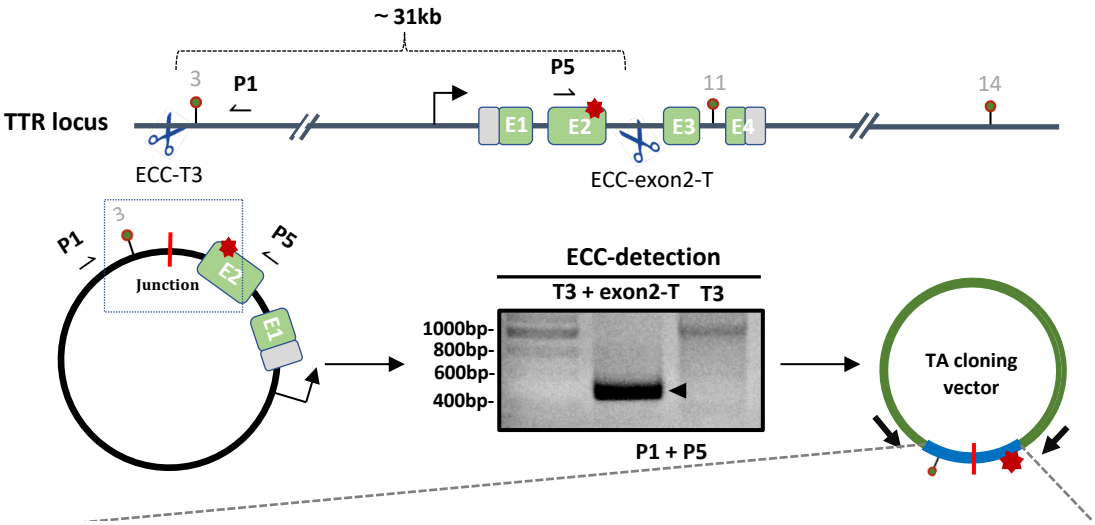
Figure S11

A. TTR-mutation cell model——Genotype of clone #21



WT	TGCTGTCCGAGGCAGTCCTGCCATCAATGTGGCCGTGCATGTGTTCAGAAAGGCTGCTGATGA	
21-1	TGCTGTCCGAGGCAGTCCTGCCATCAATGTGGCCGTGCTGCTGATG---- AGGCTGCTGATGA	Δ 4 bp
21-2	TGCTGTCCGAGGCAGTCCTGCCATCAATGTGGCCGTGCTGCTGATG---- AGGCTGCTGATGA	Δ 4 bp
21-3	TGCTGTCCGAGGCAGTCCTGCCATCAATGTGGCCGTGCATGTGTTC ---- AGGCTGCTGATGA	Δ 4 bp
21-4	TGCTGTCCGAGGCAGTCCTGCCATCAATGTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGATGA	+ 1 bp
21-5	TGCTGTCCGAGGCAGTCCTGCCATCAATGTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGATGA	+ 1 bp
21-6	TGCTGTCCGAGGCAGTCCTGCCATCAATGTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGATGA	+ 1 bp
21-7	TGCTGTCCGAGGCAGTCCTGCCATCAATGTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGATGA	+ 1 bp

B. TTR-mutation-SNP haplotyping



WT	TTTCTTGGTTTTTCTTTAAATGCCCTA —\\— CCATCAATGTGGCCGTGCATGTGTTCAGAAAGGCTGCTGATGACACCTGGGAG	
21-1	TTTCTTGGTTTTTCTTTAAATGCCCTA —\\— CCATCAATGTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGATGACACCTGGGAG	+ 1 bp
21-2	TTTCTTGGTTTTTCTTTAAATGCCCTA —\\— CCATCAATGTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGATGACACCTGGGAG	+ 1 bp
21-3	TTTGTTTGTTTTTCTTTAAATGCCCTA —\\— CCATCAATGTGGCCGTGCTGCTGATG ····· AGGCTGCTGATGACACCTGGGAG	△4 bp
21-4	TTTCTTGGTTTTTCTTTAAATGCCCTA —\\— CCATCAATGTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGATGACACCTGGGAG	+ 1 bp
21-5	TTTCTTGGTTTTTCTTTAAATGCCCTA —\\— CCATCAATGTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGATGACACCTGGGAG	+ 1 bp
21-6	TTTCTTGGTTTTTCTTTAAATGCCCTA —\\— CCATCAATGTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGATGACACCTGGGAG	+ 1 bp
21-7	TTTCTTGGTTTTTCTTTAAATGCCCTA —\\— CCATCAATGTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGATGACACCTGGGAG	+ 1 bp
21-8	TTTGTTTGTTTTTCTTTAAATGCCCTA —\\— CCATCAATGTGGCCGTGCTGCTGATG ····· AGGCTGCTGATGACACCTGGGAG	△4 bp
21-9	TTTGTTTGTTTTTCTTTAAATGCCCTA —\\— CCATCAATGTGGCCGTGCTGCTGATG ····· AGGCTGCTGATGACACCTGGGAG	△4 bp
21-10	TTTCTTGGTTTTTCTTTAAATGCCCTA —\\— CCATCAATGTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGATGACACCTGGGAG	+ 1 bp
21-11	TTTCTTGGTTTTTCTTTAAATGCCCTA —\\— CCATCAATGTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGATGACACCTGGGAG	+ 1 bp

Figure S12

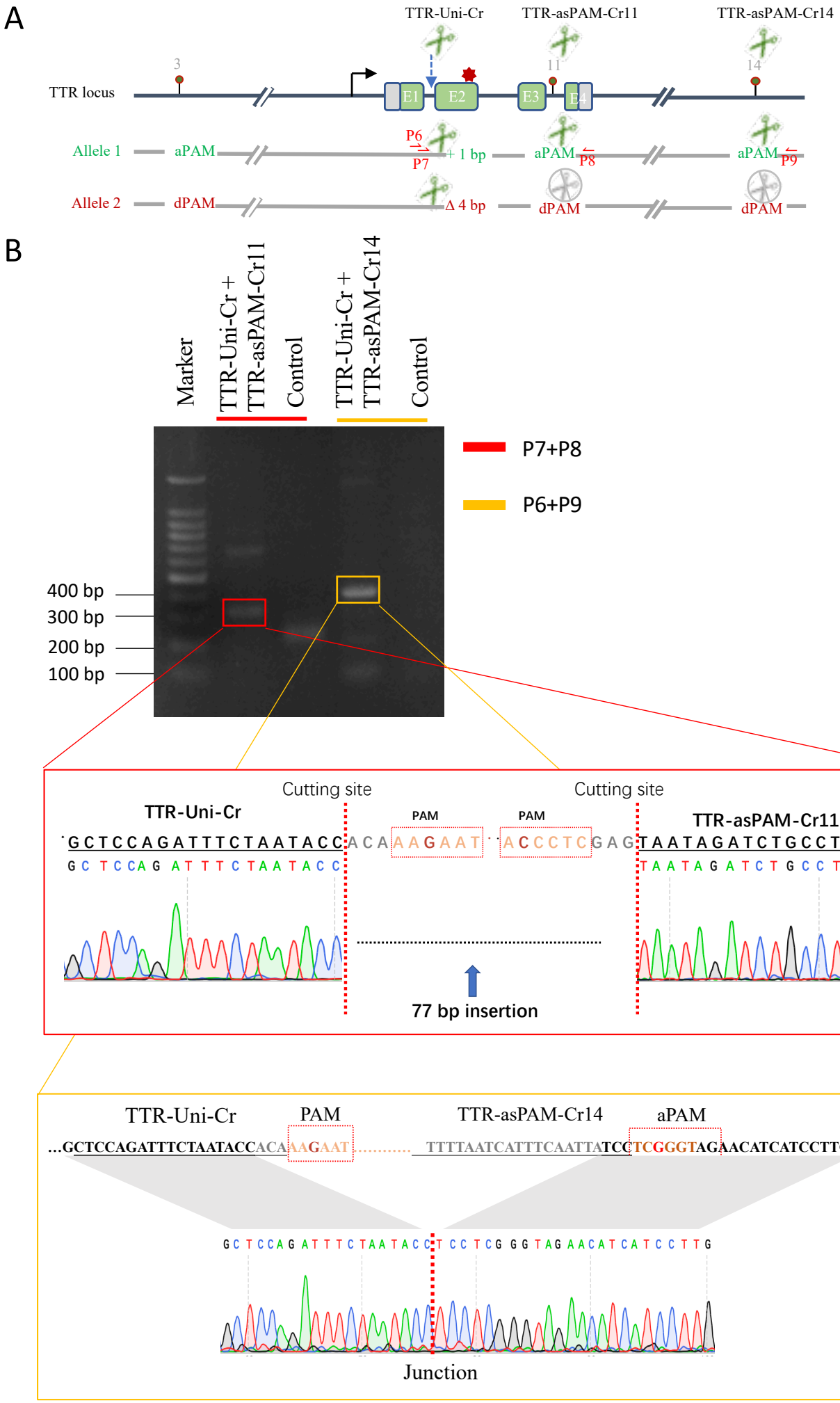

A
TTR-Uni-Cr
TTR-asPAM-Cr11
TTR-asPAM-Cr14
TTR locus
3
11
14
E1
E2
E3
E4
Allele 1
aPAM
P6
P7
+ 1 bp
aPAM
P8
aPAM
P9
Allele 2
dPAM
Δ 4 bp
dPAM
dPAM
B
Marker
TTR-Uni-Cr + TTR-asPAM-Cr11
Control
TTR-Uni-Cr + TTR-asPAM-Cr14
Control
P7+P8
P6+P9
400 bp
300 bp
200 bp
100 bp
Cutting site
Cutting site
TTR-Uni-Cr
PAM
PAM
TTR-asPAM-Cr11
77 bp insertion
TTR-Uni-Cr
PAM
TTR-asPAM-Cr14
aPAM
...GCTCCAGATTTCTAATACCACAAAGAAT.....TTTTAATCATTTCAATTATCCTCGGGTAGAACATCATCCTTG.....
Junction

Table S1. Primers for SNP genotyping PCRs

Primer name	Oligo sequence (5'-3')
CHR1-SNP1-SCR-F	AAGGAAGGTCGGTGACTGGAGA
CHR1-SNP1-SCR-R	AGGGAAGGAAGACAGCAGAGAG
CHR1-SNP2-SCR-F	TCTAGCTCTGAGTGCCGCATCC
CHR1-SNP2-SCR-R	CCTGACTGCCCACCTCTGTAAG
CHR1-SNP3-SCR-F	GTGATTTGAATCTGGCAGCGTG
CHR1-SNP3-SCR-R	AGTGACTCCTAGATCACACAGC
CHR1-SNP4-SCR-F	AGTCCTGCCTGAGTGTTGCAAG
CHR1-SNP4-SCR-R	CACAGCCCATCTACAGAATTCC
CHR1-SNP5-SCR-F	AGGTCAACAGGGAGTCCTCATG
CHR1-SNP5-SCR-R	AAGGTCAGCCAGGCGGAATGTT
CHR1-SNP6-SCR-F	GCGCGTGGTCATTTAGGAAGCT
CHR1-SNP6-SCR-R	TCTCTGTGCCAATGGATCACTG
TTR-SNP1/2-F	GTTCTCACTTATAAGTGGAAGC
TTR-SNP1/2-R	ATGAAACTGTGAAAGCTCCGAG
TTR-SNP3-F	CATCATCAGACAGTTCACCCCA
TTR-SNP3-R	AGCTCTGAGGCTTACAGGCAAT
TTR-SNP4-F	CATTGTGAGACAACCTGCAACC
TTR-SNP4-R	AAACCTGTCCCAACCCACTCCT
TTR-SNP5-F	CACCTAAACCTGAGCAGTGCTC
TTR-SNP5-R	GGGACTATGGGATATGCCACTA
TTR-SNP6-F	GGAGACAGTCAAAAGACCAGTG
TTR-SNP6-R	TATAAGGATAGACTGAGAGCCG
TTR-SNP7/8-F	ATTTCCACCTCTACCTGATGAG
TTR-SNP7/8-R	CAGCTTACCCATCTCAGGAACT
TTR-SNP9-F	TTCCTAAGCTAGTGCTGTCTGC
TTR-SNP9-R	CAGAATCATCTGGAGGGCTTGT
TTR-SNP10-F	GCTAAGTGTCCTTGTCTTAG
TTR-SNP10-R	CCACATGATTCTTCAACCAT
TTR-SNP11-F	CTGGCTTAGTCATGGATGAGAC
TTR-SNP11-R	GACAGATTAAGCAGGACCGTCA
TTR-SNP12-F	CTAGAAAGTATCTGGGCAGAAC
TTR-SNP12-R	TAGGCTGGTCTACGAACTCCT
TTR-SNP13-F	GGTCACACACCGAGTTACTA
TTR-SNP13-R	TGGTGTCAATACGCTACACAGT
TTR-SNP14-F	TGGCCGACGAATTTCAATTGT
TTR-SNP14-R	CATCTCCTTCTGAAGTACATG
HTT-SNP1-SCR-F	CAGCATACAGGATGCAGGAGTTC
HTT-SNP1-SCR-R	GCAGTGCATGTCTATAATCCCAGC
HTT-SNP2-SCR-F	GGGCGAGGGAATTCCTTAATTTC
HTT-SNP2-SCR-R	TGTTTCTTGCAGGCAGCAGCATA
HTT-SNP3-SCR-F	ATGCTGGGCCATAAAACAAG

Table S1. Primers for SNP genotyping PCRs

HTT-SNP3-SCR-R	TGGATTCCATTTGCTTGTCA
HTT-SNP4-SCR-F	AGTAGAGCCAGAGTTTCACCATG
HTT-SNP4-SCR-R	AAGAAGAGCTCGGGCTTTGGAG
HTT-SNP5-SCR-F	ACATCTCTGGGCACTGCTCT
HTT-SNP5-SCR-R	CCCTGCCCAATACTGGAGCCA
HTT-SNP6-SCR-F	GCAATGTGGTGCTGAGAAGAATG
HTT-SNP6-SCR-R2	ACAAAGAGACTCCCACCCGG
HTT-SNP7-SCR-F	GAGATGGCCAACAGGAGACAGTC
HTT-SNP7-SCR-R	TGGACTACAGCCTCCCTGACAG
HTT-SNP8-SCR-F	GCAAGTTCTCAGCACCAACCCT
HTT-SNP8-SCR-R	CTGCTTCCTTCACAGCTCATTCTG
HTT-SNP9-SCR-F	TTCAGACAGGAGGGAGGTGTGAG
HTT-SNP9-SCR-R	CGTGCCCAGTGATGCGTATATCT

Table S2. List of gRNAs used in the study

Chromosome 1

gRNA name	gRNA target site (5'- 3) * PAM	Function of gRNA
CHR1-ECC-Cr1	GACTGGAGAGAAGCTTCCCTGGG	SNP-linkage by CRISPR-hapC
CHR1-ECC-Cr2	TGACGGCACAGTTCGCAGAGAGG	SNP-linkage by CRISPR-hapC
CHR1-ECC-Cr3	GGTCTTGCATGTCACCATGCTGG	SNP-linkage by CRISPR-hapC
CHR1-ECC-Cr4	GCCTGGTCACATGGTGCCTAGG	SNP-linkage by CRISPR-hapC
CHR1-ECC-Cr5	TATACAGTGTAGCGGCCATGGAGG	SNP-linkage by CRISPR-hapC
CHR1-ECC-Cr6	TCCCTGCTCCCACCGGAAATGGG	SNP-linkage by CRISPR-hapC

Table S2. List of gRNAs used in the study

TTR locus

gRNA name	gRNA target site (5'- 3) * PAM	Function of gRNA (strand +/-)
TTR-asPAM-Cr1	GAGCATGTAAACAATCTGCAGCCGGGC	Allele-specific target +
TTR-asPAM-Cr2	GCCCAGATGACAAAGACTATGCTTTAA	Allele-specific target -
TTR-asPAM-Cr3	TTTCTTGGTTTTTCTTTAAATGCCCTA	Allele-specific target -
TTR-asPAM-Cr4	TATGCAATCTCCATGCTTTCCTCGGGC	Allele-specific target +
TTR-asPAM-Cr5	GACTTGGGCCTTGAGAGGTTCCCGAAC	Allele-specific target +
TTR-asPAM-Cr6	ACAAATGCACCGCACCGATGCAAGAAG	Allele-specific target +
TTR-asPAM-Cr7	ACTCAGGCAAAGTCAGACTCAGACCAA	Allele-specific target -
TTR-asPAM-Cr8	CCCTGTCCACAGAAGTCCCAGCTGAGT	Allele-specific target +
TTR-asPAM-Cr9	GTTCTGCCCTGTTAACGAAATAGGAAT	Allele-specific target -
TTR-asPAM-Cr10	GTCCAAGAGTGGCTTCTCACCTTCATT	Allele-specific target -
TTR-asPAM-Cr11	ACCCTCGAGTAATAGATCTGCCTACCT	Allele-specific target -
TTR-asPAM-Cr12	TTCCAAGGGCCCACTTCTTTACTGTGG	Allele-specific target -
TTR-asPAM-Cr13	TTCCTCTCAGCGCGTTACATCTAGGAG	Allele-specific target -
TTR-asPAM-Cr14	TTTTAATCATTTCAATTATCCTCGGGT	Allele-specific target +
TTR-Uni-Cr	GCTCCAGATTTCTAATACCACAAAGAAT	Allele-specific KO assistant gRNA +
TTR-ECC-Cr3	CCAAGGTTAAGGGCACTTCAGAA	SNP-linkage detection gRNA -
TTR-ECC-Cr11	GAGCTGGGTCTCAGCCTGATGGG	SNP-linkage detection gRNA +
TTR-ECC-Cr14	TGTTTCACAGATAATGGCAGAGG	SNP-linkage detection gRNA +
TTR-exon2-T	TTGCCAAAGAACCCTCCCACAGG	TTR-mutation cell model construction +

Table S2. List of gRNAs used in the study

HTT locus

HTT-asPAM-Cr1	GATAGGGAAATGTCAGGGTTAATCGAGT
HTT-asPAM-Cr2	GCAACAACTAAAAGCACAACAAGGAAT
HTT-asPAM-Cr3	ACCCATTTCCACATGGCCCATGGGTCAG
HTT-asPAM-Cr4	GGCTATGTTTATCCTGCAACCGGGGA
HTT-asPAM-Cr5	TTCCTGCCCACCAGCACATGCTTTCTA
HTT-asPAM-Cr6	TTGATCTGCCTAATATTGACAGTGGGGT
HTT-asPAM-Cr7	CTCTTTGTTCTGTTGTAATTTTAGTTGC
HTT-asPAM-Cr8	GCAGCGCCCCCGCCTCGGCTGTGGGGA
HTT-asPAM-Cr9	AGCCTGGCATAGGGCCAAGTCACACGG
HTT-Uni-Cr1	GGTTCCCTGGCCAGCCATTGGCAGAGT
HTT-Uni-Cr2	ATTCATTGCCCCGGTGCTGAGCGGCGCC
HTT-Uni-Cr3	GCCCCGGTGCTGAGCGGCGCCGCGAGT
HTT-ECC-Cr1	ACCCAGAAAGCCTGCTAGACAAATTCC
HTT-ECC-Cr2	GGAGATTAGAATAATAAAAATGGTGAAT
HTT-ECC-Cr3	AAACAATAGATTTCTAAACTTGTGGGGT
HTT-ECC-Cr4	ATCCCTCTCAATCTTTGCCGGAGGTGGG
HTT-ECC-Cr5	GTCTCTGGGCCAGTGCTGTTCTAGAGAGT
HTT-ECC-Cr6	GTAGGTCTCTAAGAACTTGCTTCATGAAT
HTT-ECC-Cr7	ATCCAAACACACAGAGTAAGTCTCAGGAC
HTT-ECC-Cr8	CAGGAGCAGCCACCTGCCCAGCAGGGT
HTT-ECC-Cr9	GGGCATGGAGGACTCAGGGAAGGAGAGT

Table S3. C-Check oligos used in the research (HTT and TTR locus)

SagRNA-HTT-T1Ref-CC-SS	GTCGGAtgatagggaaatgtcagggttaatcgagtataGGT
SagRNA-HTT-T2Ref-CC-SS	GTCGGAtgcaacaactaaaagcacaacaaggaatataGGT
SagRNA-HTT-T3Ref-CC-SS	GTCGGAtgctgacccatgggccatgtggaaatgggtataGGT
SagRNA-HTT-T4Ref-CC-SS	GTCGGAtggctatgtttatcctgcaaccggggacataGGT
SagRNA-HTT-T5Ref-CC-SS	GTCGGAtgtagaaagcatgtgctggtgggcaggaagataGGT
SagRNA-HTT-T6Ref-CC-SS	GTCGGAtgttgatctgcctaatattgacagtggggtataGGT
SagRNA-HTT-T7Ref-CC-SS	GTCGGAtgcaactaaaattacaacagaacaaagagaataGGT
SagRNA-HTT-T8Ref-CC-SS	GTCGGAtgcagcgcccccgcctcggctgtggggagataGGT
SagRNA-HTT-T9Ref-CC-SS	GTCGGAtgccgtgtgacttggccctatgccaggctataGGT
SagRNA-HTT-T1Alt-CC-SS	GTCGGAtgatagggaaatgtcagggttaatcaagtataGGT
SagRNA-HTT-T2Alt-CC-SS	GTCGGAtgcaacaactaaaagcacaacaagaaatataGGT
SagRNA-HTT-T3Alt-CC-SS	GTCGGAtgctgacccatgggccatgtggaaatggctataGGT
SagRNA-HTT-T4Alt-CC-SS	GTCGGAtggctatgtttatcctgcaaccggagacataGGT
SagRNA-HTT-T5Alt-CC-SS	GTCGGAtgtagaaagcatgtgctggtgggcagaaagataGGT
SagRNA-HTT-T6Alt-CC-SS	GTCGGAtgttgatctgcctaatattgacagtgaggtataGGT
SagRNA-HTT-T7Alt-CC-SS	GTCGGAtgcaactaaaattacaacagaacaaacagaataGGT
SagRNA-HTT-T8Alt-CC-SS	GTCGGAtgcagcgcccccgcctcggctgtggtgagataGGT
SagRNA-HTT-T9Alt-CC-SS	GTCGGAtgccgtgtgacttggccctatgccaggatataGGT
SagRNA-HTT-T1Ref-CC-AS	CGGTACCtatACT CGA TTA ACC CTG ACA TTT CCC TAT CaTC
SagRNA-HTT-T2Ref-CC-AS	CGGTACCtatATT CCT TGT TGT GCT TTT AGT TGT TGCaTC
SagRNA-HTT-T3Ref-CC-AS	CGGTACCtatACC CAT TTC CAC ATG GCC CAT GGG TCA GCaTC
SagRNA-HTT-T4Ref-CC-AS	CGGTACCtatGTC CCC GGT TGC AGG ATA AAC ATA GCCaTC
SagRNA-HTT-T5Ref-CC-AS	CGGTACCtatCTT CCT GCC CAC CAG CAC ATG CTT TCT ACaTC
SagRNA-HTT-T6Ref-CC-AS	CGGTACCtatACC CCA CTG TCA ATA TTA GGC AGA TCA ACaTC
SagRNA-HTT-T7Ref-CC-AS	CGGTACCtatTCT CTT TGT TCT GTT GTA ATT TTA GTT GCaTC
SagRNA-HTT-T8Ref-CC-AS	CGGTACCtatCTC CCC ACA GCC GAG GCG GGG GCG CTG CaTC
SagRNA-HTT-T9Ref-CC-AS	CGGTACCtatAGC CTG GCA TAG GGC CAA GTC ACA CGG CaTC

Table S3. C-Check oligos used in the research (HTT and TTR locus)

SagRNA-HTT-T1Alt-CC-AS	CGGTACCtatACT TGA TTA ACC CTG ACA TTT CCC TAT CaTC
SagRNA-HTT-T2Alt-CC-AS	CGGTACCtatATT TCT TGT TGT GCT TTT AGT TGT TGCaTC
SagRNA-HTT-T3Alt-CC-AS	CGGTACCtatAGC CAT TTC CAC ATG GCC CAT GGG TCA GCaTC
SagRNA-HTT-T4Alt-CC-AS	CGGTACCtatGTC TCC GGT TGC AGG ATA AAC ATA GCCaTC
SagRNA-HTT-T5Alt-CC-AS	CGGTACCtatCTT TCT GCC CAC CAG CAC ATG CTT TCT ACaTC
SagRNA-HTT-T6Alt-CC-AS	CGGTACCtatACC TCA CTG TCA ATA TTA GGC AGA TCA ACaTC
SagRNA-HTT-T7Alt-CC-AS	CGGTACCtatTCT GTT TGT TCT GTT GTA ATT TTA GTT GCaTC
SagRNA-HTT-T8Alt-CC-AS	CGGTACCtatCTC ACC ACA GCC GAG GCG GGG GCG CTG CaTC
SagRNA-HTT-T9Alt-CC-AS	CGGTACCtatATC CTG GCA TAG GGC CAA GTC ACA CGG CaTC

Table S3. C-Check oligos used in the research (HTT and TTR locus)

Oligo name	Ordered oligo sequence(5’ -3’)
TTR-T1 (R-CC)-SS	GTCGGAtgagcatgtaaacaatctgcagCCGGGCataGGT
TTR-T1 (R-CC)-AS	CGGTACCtatGCCCGGctgcagattgtttacatgctcaTC
TTR-T1 (A-CC)-SS	GTCGGAtgagcatgtaaacaatctgcagCCAGGCataGGT
TTR-T1 (A-CC)-AS	CGGTACCtatGCCTGGctgcagattgtttacatgctcaTC
TTR-T2 (R-CC)-SS	GTCGGAtttaaagcatagtctttgtcatCTGGGCataGGT
TTR-T2 (R-CC)-AS	CGGTACCtatGCCCAGatgacaaagactatgctttaaaTC
TTR-T2 (A-CC)-SS	GTCGGAtttaaagcatagtctttgtcatCTAGGCataGGT
TTR-T2 (A-CC)-AS	CGGTACCtatGCCTAGatgacaaagactatgctttaaaTC
TTR-T3 (R-CC)-SS	GTCGGAttagggcatttaaagaaaaaccAAGAAAataGGT
TTR-T3 (R-CC)-AS	CGGTACCtatTTTCTTggtttttctttaaatgccctaaTC
TTR-T3 (A-CC)-SS	GTCGGAttagggcatttaaagaaaaaccAACAAAataGGT
TTR-T3 (A-CC)-AS	CGGTACCtatTTTGTTggtttttctttaaatgccctaaTC
TTR-T4 (R-CC)-SS	GTCGGAtgtatgcaatctccatgctttccTCGGGCataGGT
TTR-T4 (R-CC)-AS	CGGTACCtatGCCCGAggaaagcatggagattgcatacaTC
TTR-T4 (A-CC)-SS	GTCGGAtgtatgcaatctccatgctttccTCAGGCataGGT
TTR-T4 (A-CC)-AS	CGGTACCtatGCCTGAggaaagcatggagattgcatacaTC
TTR-T5 (R-CC)-SS	GTCGGAtgacttgggccttgagaggttcCCGAACataGGT
TTR-T5 (R-CC)-AS	CGGTACCtatGTTCGGgaacctctcaaggcccaagtcaTC
TTR-T5 (A-CC)-SS	GTCGGAtgacttgggccttgagaggttcCCAAACataGGT
TTR-T5 (A-CC)-AS	CGGTACCtatGTTTGGgaacctctcaaggcccaagtcaTC
TTR-T6 (R-CC)-SS	GTCGGAtacaaatgcaccgcaccgatgcAAGAAGataGGT
TTR-T6 (R-CC)-AS	CGGTACCtatCTTCTTgcatcggtgcggtgcatttgtaTC
TTR-T6 (A-CC)-SS	GTCGGAtacaaatgcaccgcaccgatgcAAGATGataGGT
TTR-T6 (A-CC)-AS	CGGTACCtatCATCTTgcatcggtgcggtgcatttgtaTC
TTR-T7 (R-CC)-SS	GTCGGAtttggtctgagtctgactttgcCTGAGTataGGT
TTR-T7 (R-CC)-AS	CGGTACCtatACTCAGgcaaagtcagactcagaccaaaTC
TTR-T7 (A-CC)-SS	GTCGGAtttggtctgagtctgactttgcCTGACTataGGT
TTR-T7 (A-CC)-AS	CGGTACCtatAGTCAGgcaaagtcagactcagaccaaaTC
TTR-T8 (R-CC)-SS	GTCGGAtccctgtccacagaagtcccagCTGAGTataGGT
TTR-T8 (R-CC)-AS	CGGTACCtatACTCAGctgggacttctgtggacagggaTC
TTR-T8 (A-CC)-SS	GTCGGAtccctgtccacagaagtcccagCTCAGTataGGT
TTR-T8 (A-CC)-AS	CGGTACCtatACTGAGctgggacttctgtggacagggaTC
TTR-T9 (R-CC)-SS	GTCGGAtattcctatttcgttaacagggCAGAACataGGT
TTR-T9 (R-CC)-AS	CGGTACCtatGTTCTGccctgttaacgaaataggaataTC
TTR-T9 (A-CC)-SS	GTCGGAtattcctatttcgttaacagggCAAAACataGGT
TTR-T9 (A-CC)-AS	CGGTACCtatGTTTTGccctgttaacgaaataggaataTC
TTR-T10 (R-CC)-SS	GTCGGAtaatgaaggtgagaagccactcTTGGACataGGT
TTR-T10 (R-CC)-AS	CGGTACCtatGTCCAAgagtggcttctcaccttcattaTC
TTR-T10 (A-CC)-SS	GTCGGAtaatgaaggtgagaagccactcTTGCACataGGT
TTR-T10 (A-CC)-AS	CGGTACCtatGTGCAAgagtggcttctcaccttcattaTC
TTR-T11 (R-CC)-SS	GTCGGAtaggtaggcagatctattactcGAGGGTataGGT
TTR-T11 (R-CC)-AS	CGGTACCtatACCCTCgagtaatagatctgcctacctaTC
TTR-T11 (A-CC)-SS	GTCGGAtaggtaggcagatctattactcGAGGTTataGGT
TTR-T11 (A-CC)-AS	CGGTACCtatAACCTCgagtaatagatctgcctacctaTC

Table S3. C-Check oligos used in the research (HTT and TTR locus)

TTR-T12 (R-CC)-SS	GTCGGAtccacagtaaagaagtgggcccTTGGAAataGGT
TTR-T12 (R-CC)-AS	CGGTACCtatTTCCAAgggcccacttctttactgtggaTC
TTR-T12 (A-CC)-SS	GTCGGAtccacagtaaagaagtgggcccTTAGAAataGGT
TTR-T12 (A-CC)-AS	CGGTACCtatTTCTAAgggcccacttctttactgtggaTC
TTR-T13 (R-CC)-SS	GTCGGAtctcctagatgtaacgcgctgaGAGGAAataGGT
TTR-T13 (R-CC)-AS	CGGTACCtatTTCCTCtcagcgcgttacatctaggagaTC
TTR-T13 (A-CC)-SS	GTCGGAtctcctagatgtaacgcgctgaGAAGAAataGGT
TTR-T13 (A-CC)-AS	CGGTACCtatTTCTTCtcagcgcgttacatctaggagaTC
TTR-T14 (R-CC)-SS	GTCGGAtttttaatcatttcaattatccTCGGGTataGGT
TTR-T14 (R-CC)-AS	CGGTACCtatACCCGAggataattgaaatgattaaaaaTC
TTR-T14 (A-CC)-SS	GTCGGAtttttaatcatttcaattatccTCAGGTataGGT
TTR-T14 (A-CC)-AS	CGGTACCtatACCTGAggataattgaaatgattaaaaaTC

Table S4. Primers for CRISPR-hapC and eccDNA PCR-based genotyping

Primer name	Oligo sequence (5’ -3’)
TTR Gene	
P1	GCCGTGCCTTGTGATAACTCTG
P2	CTGGCTTAGTCATGGATGAGAC
P3	CACATACATGATCACATGTAGG
P4	CTCTACTGTCTGCCCCTAAATG
P5	TGATAGCAGTGTGTCTGGAGGC
P6	TACGTCTGTGTTATACTGAGTAGG
P7	CTGAGTAGGGAAGCTCATTAATTG
P8	GAGCAGACAAGGAAGCTAAGATCT
P9	TTCTCCAGCACGTATTTCTCAG
HTT Gene	
HTT-del-scr-R	ggttgctgggtcactctgtc
HTT-ECCuni-TIDE-F	tcgcctcaccccattacagtct
HD1	ccttcgagtccctcaagtccttc
HD3	cggcggtggcggctgttg

Primers combinations for PCRs

Primers combination	Primers combination	Purpose	PCR product length
TTR	P1+P2	TTR SNP3-SNP11 linkeage	624 bp
	P1+P3	TTR SNP3-SNP14 linkeage	636 bp
	P1+P5	TTR SNP3-Exon2 mutation linkeage	688 bp
	P2+P4	TTR Allele-specific KO detection(ECC)	672 bp
	P3+P4	TTR Allele-specific KO detection(ECC)	648 bp
	P7+P8	Allele-specific KO detection (del)	305 bp
	P6+P9	Allele-specific KO detection (del)	399 bp
HTT	HTT-SNP2-scr-F + HTT-del-scr-R	CAG repeat-SNP linkeage	939 bp (23 repeat)
	HTT-SNP3-scr-F + HTT-del-scr-R	CAG repeat-SNP linkeage	888 bp (23 repeat)
	HTT-SNP5-scr-F + HTT-del-scr-R	CAG repeat-SNP linkeage	893 bp (23 repeat)
	HTT-SNP7-scr-F + HTT-del-scr-R	CAG repeat-SNP linkeage	1015 bp (23 repeat)
	HTT-SNP8-scr-F + HTT-del-scr-R	CAG repeat-SNP linkeage	983 bp (23 repeat)
	HTT-SNP9-scr-F + HTT-del-scr-R	CAG repeat-SNP linkeage	1065 bp (23 repeat)

Table S4. Primers for CRISPR-hapC and eccDNA PCR-based genotyping

	HTT-ECCuni-TIDE-F + HTT-SNP3-scr-R	Junction sequence detection	373 bp for uni-T1 and SNP3
	HTT-ECCuni-TIDE-F + HTT-SNP9-scr-R	Junction sequence detection	496 bp for uni-T1 and SNP9
	HTT-SNP3-scr-F + HTT-del-scr-R	Nest PCR for Allele-specific KO detection-1st round	
	HTT-SNP9-scr-F + HTT-del-scr-R	Nest PCR for Allele-specific KO detection-1st round	
	HD1 +HD3	Nest PCR for Allele-specific KO detection-2nd round	

Table S5. genotype and linkeage pattern of TTR-mutation cell clones tested in this research

TTR Cell Clone ID	Alleles	SNP3	sequence at TTR mutation locus	Mutation genotype	SNP11	SNP14
#2	1	aPAM	GTGGCCGTG……CTGCTGAT	Δ 16 bp	aPAM	aPAM
	2	dPAM	GTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGAT	+ 1 bp	dPAM	dPAM
	3	dPAM	GTGGCCGTGCATGTGTTCAGAAAGGCTGCTGAT	WT	dPAM	dPAM
#7	1	aPAM	GTGGCCGTGCATGTGTT……GCTGAT	Δ 10 bp	aPAM	aPAM
	2	aPAM	GTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGAT	+ 1 bp	aPAM	aPAM
	3	dPAM			dPAM	dPAM
#12	1	aPAM	GTGGCCGTGCATGTGTT……GCTGAT	Δ 10 bp	aPAM	aPAM
	2	aPAM	GTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGAT	+ 1 bp	aPAM	aPAM
	3	dPAM	GTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGAT	+ 1 bp	dPAM	dPAM
#13	1	aPAM	GTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGAT	+ 1 bp	aPAM	aPAM
	2	aPAM	GTGGCCGTGCATGTGTTCAGAAAGGCTGCTGAT	WT	aPAM	aPAM
	3	dPAM	GTGGCCGTGCATGTGTTCAGAAAGGCTGCTGAT	WT	dPAM	dPAM
	4	dPAM	GTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGAT	+ 1 bp	dPAM	dPAM
#14	1	aPAM	GTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGAT	+ 1 bp	aPAM	aPAM
	2	aPAM	GTGGCCGTGCATGTGTTCAGAAAGGCTGCTGAT	WT	aPAM	aPAM
	3	dPAM			dPAM	dPAM
#16	1	aPAM	GTGGCCGTGCATGTGTTCAG……CTGCTGAT	Δ 5 bp	aPAM	aPAM
	2	aPAM	GTGGC……TGCTGAT	Δ 21 bp	aPAM	aPAM
	3	dPAM	GTGGC……TGCTGAT	Δ 21 bp	dPAM	dPAM
#21	1	aPAM	GTGGCCGTGCATGTGTTCAAGAAAGGCTGCTGAT	+ 1 bp	aPAM	aPAM
	2	dPAM	GTGGCCGTGCTGCTGATG……AGGCTGCTGAT	Δ 4 bp	dPAM	dPAM
	3	*ND	GTGGCCGTGCATGTGTTC……AGGCTGCTGAT	Δ 4 bp	ND	ND

***ND means not detected**