Supporting Information

Takahashi et al. 10.1073/pnas.1012153107

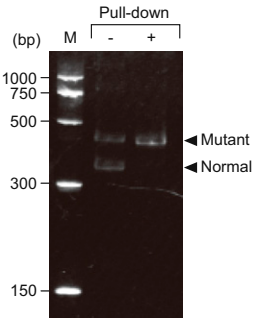

Fig. S1. Biased recovery of disease-causing *HTT* allele using a Huntington disease (HD) patient's peripheral blood cells. The cDNA prepared from peripheral blood sample directly drawn from HD patient (HD995) was subjected to the pull-down method to recover the disease-causing *HTT* cDNA. The recovered cDNA was examined by PCR analysis and SNP typing as in Fig. 2. As a result, biased recovery of the disease-causing *HTT* allele was successfully performed by the method, and the SNP typing determined that HD995 was homozygous at the examined cSNP sites (rs363099, rs362331, rs362273, and rs362272). M indicates the PCR marker (Promega).

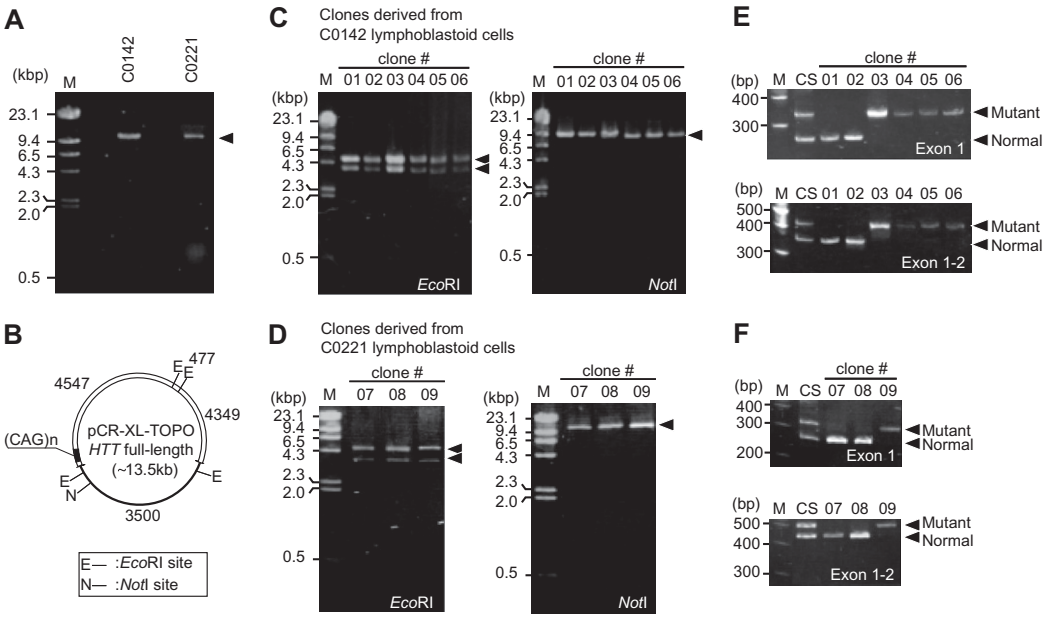

Fig. S2. Isolation of the *HTT* alleles by conventional methods. (*A*–*F*) Cloning of the *HTT* alleles. RT-PCR amplification of the whole ORF region of the *HTT* transcripts was carried out. (*A*) The resultant PCR products derived from the C0142 and C0221 total RNAs were examined by electrophoresis in 0.6% agarose gels, followed by ethidium bromide staining. M, HindIII-digested λDNA marker. (*B*) Schematic drawing of a plasmid containing the PCR product. The cloned PCR product and CAG trinucleotide repeat region [(CAG)n] are represented by open and solid curves, respectively. Restriction enzyme (EcoRI and NotI) sites are indicated, and figures (bp) indicate the expected lengths of the EcoRI restriction fragments. (*C* and *D*) Restriction enzyme digestion of isolated plasmid DNAs. Plasmid DNAs containing the PCR products derived from the C0142 (*C*) and C0221 (*D*) samples were examined by digestion with indicated restriction enzymes, followed by agarose gel electrophoresis analysis as in *A*. (*E* and *F*) PCR analysis of the CAG trinucleotide repeat in the *HTT* exon 1. To see the CAG trinucleotide repeats, the isolated plasmids were examined by PCR analysis using two sorts of PCR primer sets designed for amplification of the CAG trinucleotide repeat region. The expanded CAG repeat (mutant) alleles and normal alleles are indicated. The cDNA sources used in cloning were also examined as control samples (CS).

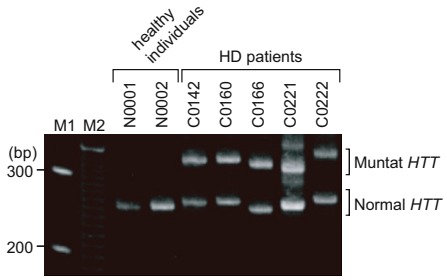

Fig. S3. PCR analysis of the CAG trinucleotide repeats in the *HTT* exon 1. Genomic DNAs isolated from HD patients (C0142, C0160, C0166, C0221, and C0222) and healthy individuals (N0001 and N0002) were examined by PCR analysis using the primer set designed for amplification of the CAG trinucleotide repeat region in the *HTT* exon 1. The PCR products were electrophoretically separated in 5% polyacrylamide gels and visualized by ethidium bromide staining. M1 and M2 represent the 100 and 10 base markers, respectively.

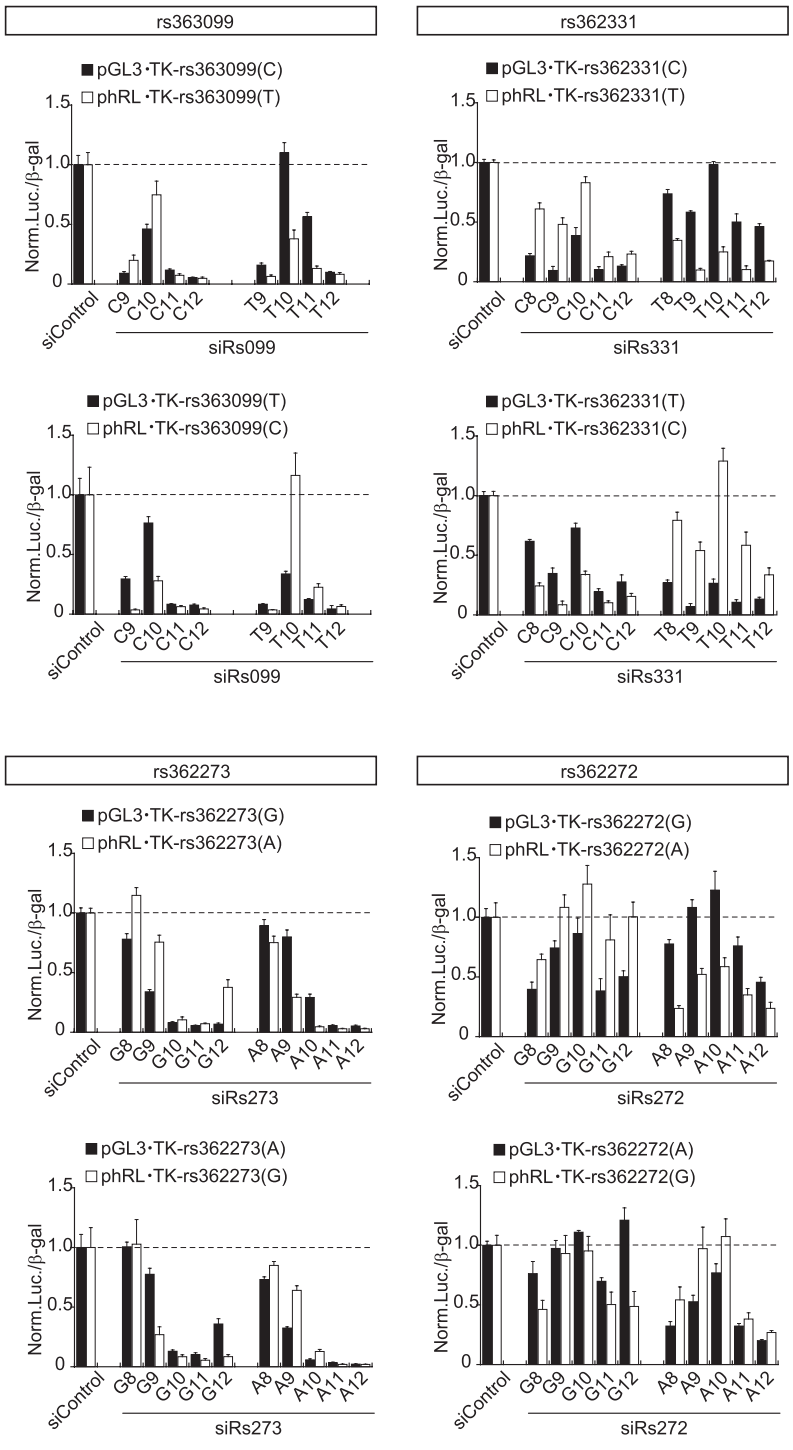

Fig. S4. Assessment of siRNAs conferring allele-specific gene silencing. siRNAs targeting the indicated cSNP sites (rs363099, rs362331, rs362273, and rs362272) in *HTT* were designed and synthesized. The sequences of the siRNAs are shown in Table S4. The synthetic siRNAs were assessed by our assay system with the luciferase reporter alleles [detailed in *Materials and Methods*]. The constructed reporter alleles (plasmids), whose allelic nucleotides at the SNP sites are shown in parentheses, and examined siRNAs are indicated. The ratios of the target and nontarget luciferase activities in the presence of the siRNAs were normalized against the control ratio obtained in the presence of the siControl duplex. Data are the average of at least four independent determinations. Error bars represent SDs. The siRs099_T10 and siRs331_T10 siRNAs targeting the rs363099 T-allele and rs362331 T-allele, respectively, seemed to confer marked allele-specific gene silencing.

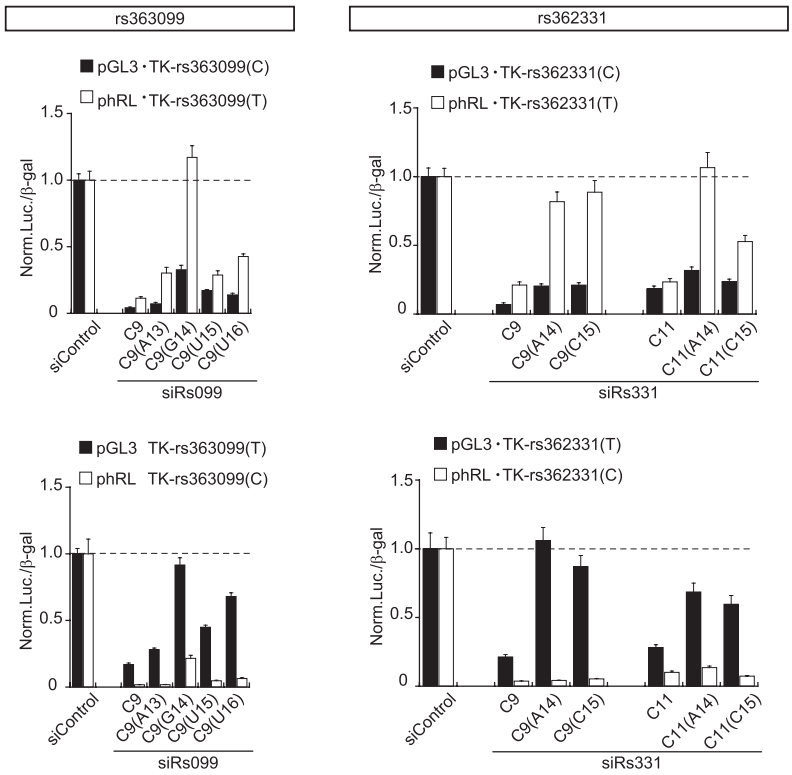

Fig. S5. Assessment of modified siRNAs. To improve allele-specific gene silencing by RNAi, the siRNAs examined in Fig. S4 were modified by introduction of base substitution into the siRNAs (Table S5), according to our previous study (1). The modified siRNAs, siRs099_C9(A13), C9(G14), C9(U15), and C9(U16) and siRs331_C9(A14), C9(C15), C11(A14), and C11(C15)—the modified bases and their positions are indicated in parentheses—were examined as in Fig. S4. The modified siRs099_C9(G14) and siRs331_C9(A14), C9(C15), and C11(A14) targeting the rs363099 C-allele and rs362331 C-allele, respectively, seem to gain marked allele discrimination, thereby conferring allele-specific gene silencing.

1. Ohnishi Y, Tamura Y, Yoshida M, Tokunaga K, Hohjoh H (2008) Enhancement of allele discrimination by introduction of nucleotide mismatches into siRNA in allele-specific gene silencing by RNAi. *PLoS ONE* 3:e2248.

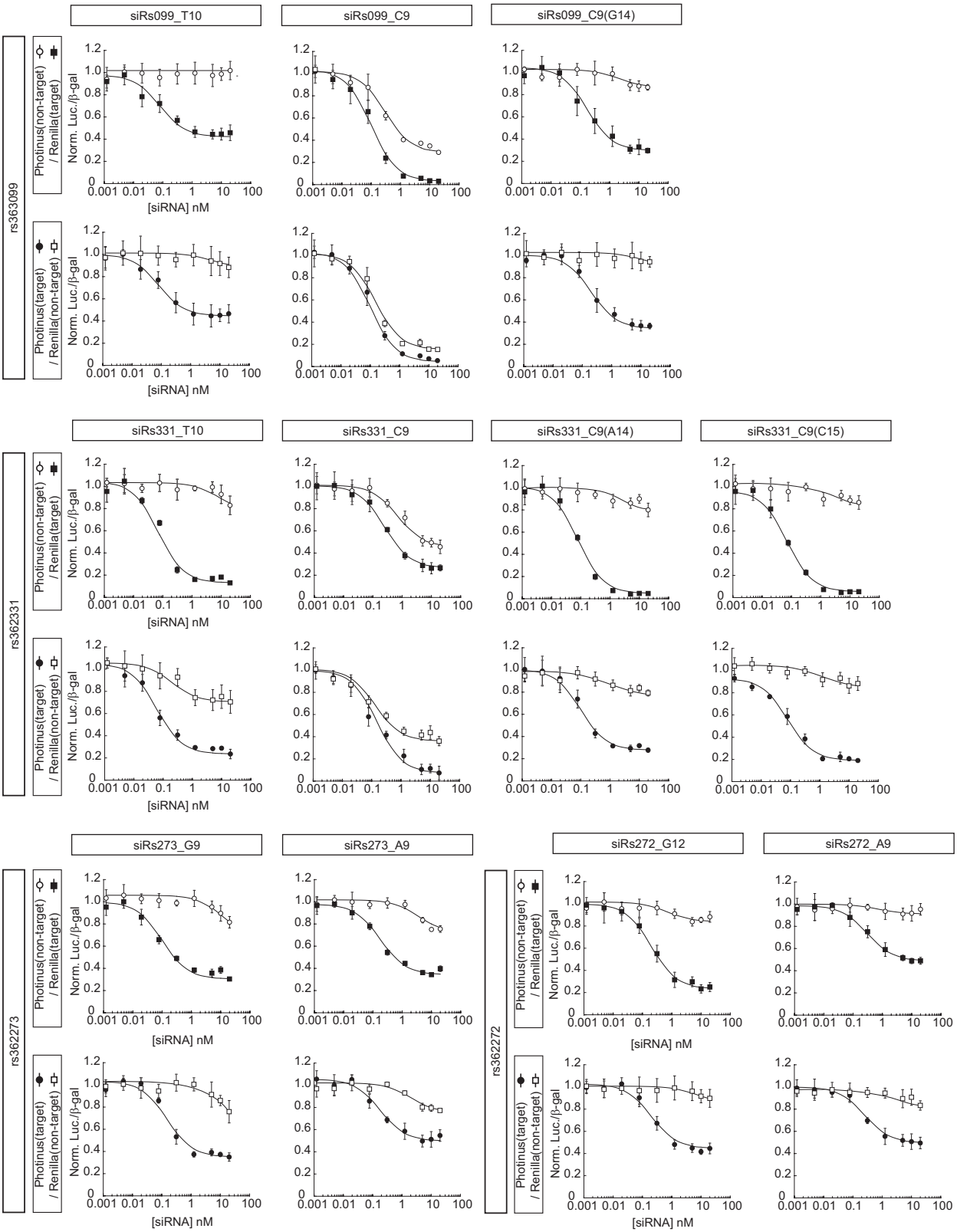

Fig. S6. IC_{50} analysis of the designed siRNAs. The effects of the designed siRNAs on suppression of target alleles and allele discrimination were investigated in detail by IC_{50} analysis. The assessment was carried out as in Figs. S4 and S5, with an increasing amount of each of the indicated siRNAs, from 0.001 nM to 20 nM. The data of the target and nontarget reporter alleles are represented by solid and open markers (circles, *Photinus* luciferase; squares, *Renilla* luciferase), respectively. Data are average of at least four independent determinations. Error bars represent SDs.

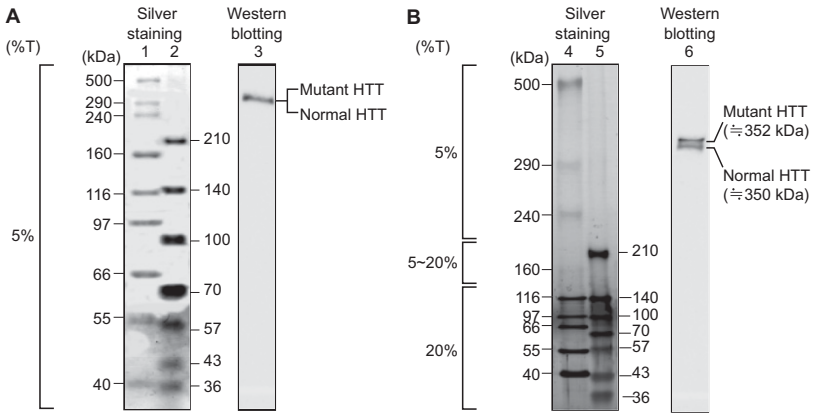

Fig. S7. Separation of the mutant and wild-type HTT isoforms by modified step-gradient gels. Protein extract from C0142 lymphoblastoid cells was separated by SDS/PAGE with (*A*) conventional 5% polyacrylamide gels (3.3%C; 29: 1) and (*B*) modified 5–20% (step-gradient) polyacrylamide gels (0.45%C; 30: 0.135), which were prepared in 380 mM Tris-acetate (pH 8.8) containing 0.1% SDS. The separated proteins were examined by Western blotting with anti-human HTT antibody as described in *Materials and Methods*. Size marker proteins indicated (lanes 1, 2, 4, and 5) were also electrophoretically separated in the same gels and visualized by silver staining. It is apparent that the modified gels have improved the separation of high-molecular-mass proteins from ≈200 to 500 kDa (lanes 4 and 5), thereby allowing for detection of both the normal and mutant HTT isoforms (lane 6). In addition, actin (≈42 kDa) and tubulin (≈55 kDa), which are often examined as controls, can be also detected on the same membranes (Fig. 4 and Fig. S8).

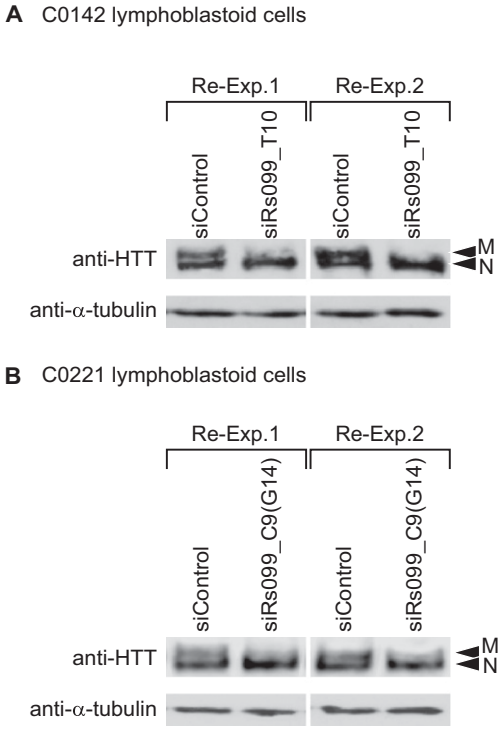

Fig. S8. Reproducibility of disease-causing allele-specific silencing against the endogenous mutant *HTT* alleles in C0142 and C0221 lymphoblastoid cells. To examine the reproducibility of the disease-causing *HTT* allele-specific knockdown, the same experiments as in Fig. 4*B* were repeated [re-experiments (Re-Exp) 1 and 2] in either of (*A*) C0142 and (*B*) C0221 lymphoblastoid cells. Used siRNAs and antibodies are indicated. The mutant and normal HTT isoforms are indicated by M and N, respectively.

Other Supporting Information Files

Table S1 (PDF)
Table S2 (PDF)
Table S3 (PDF)
Table S4 (PDF)
Table S5 (PDF)
Table S6 (PDF)
Table S7 (PDF)