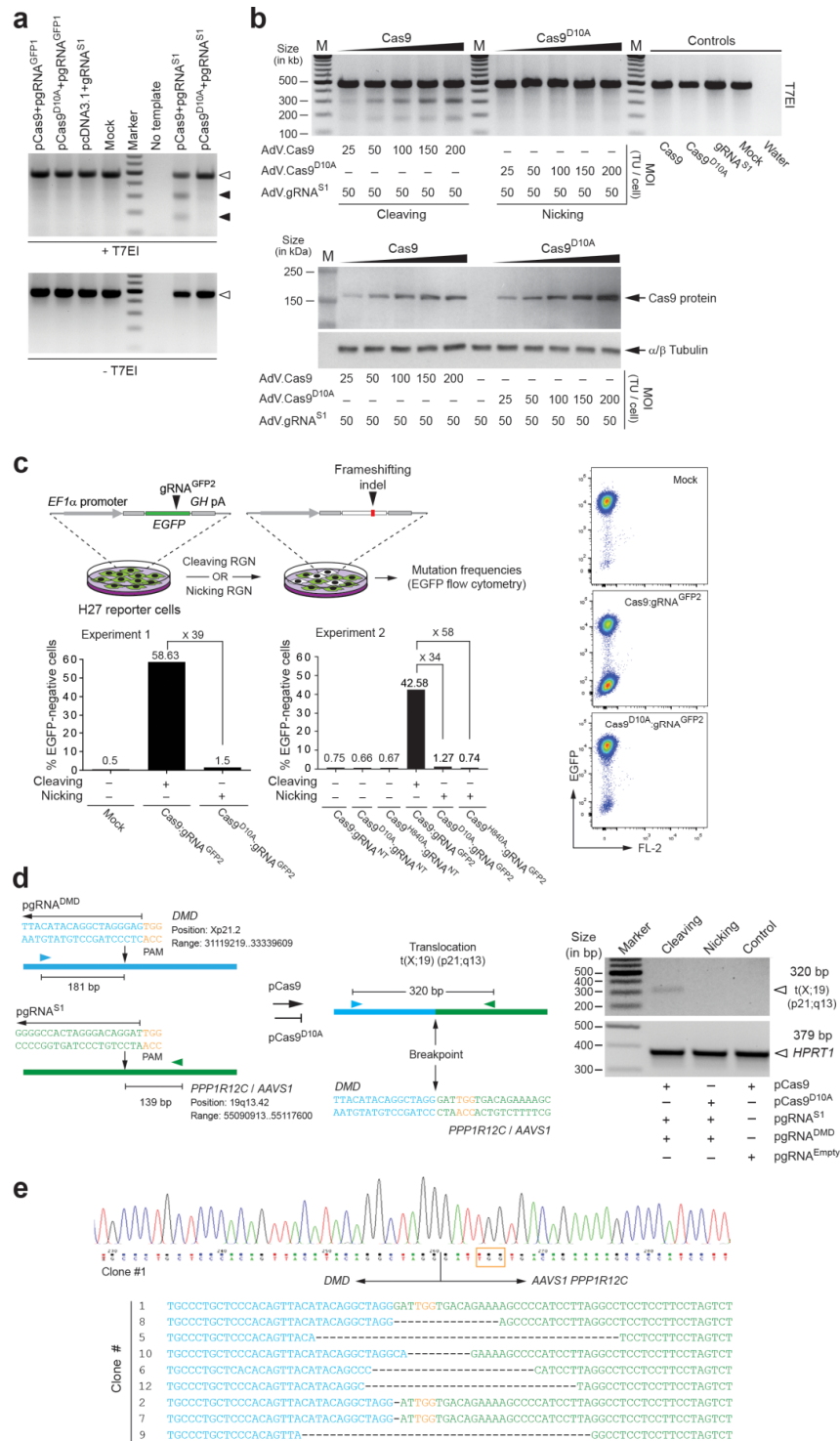


Description of Supplementary Files

Title: Supplementary Information

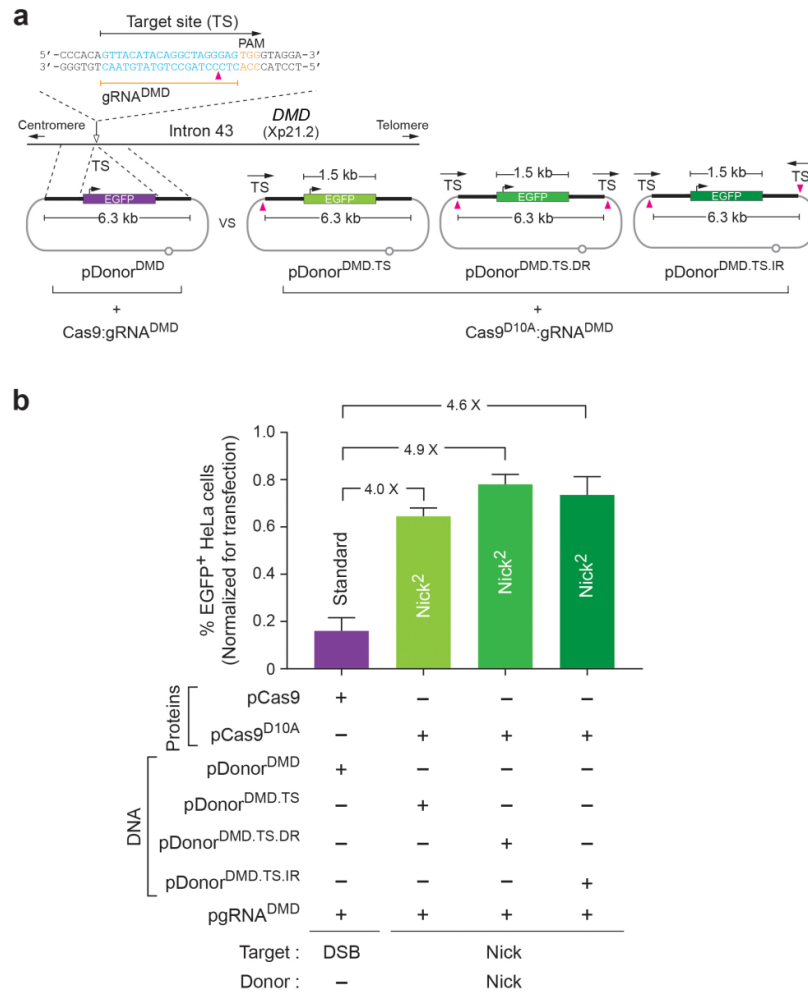
Description: Supplementary Figures and Supplementary Tables

Title: Peer Review File

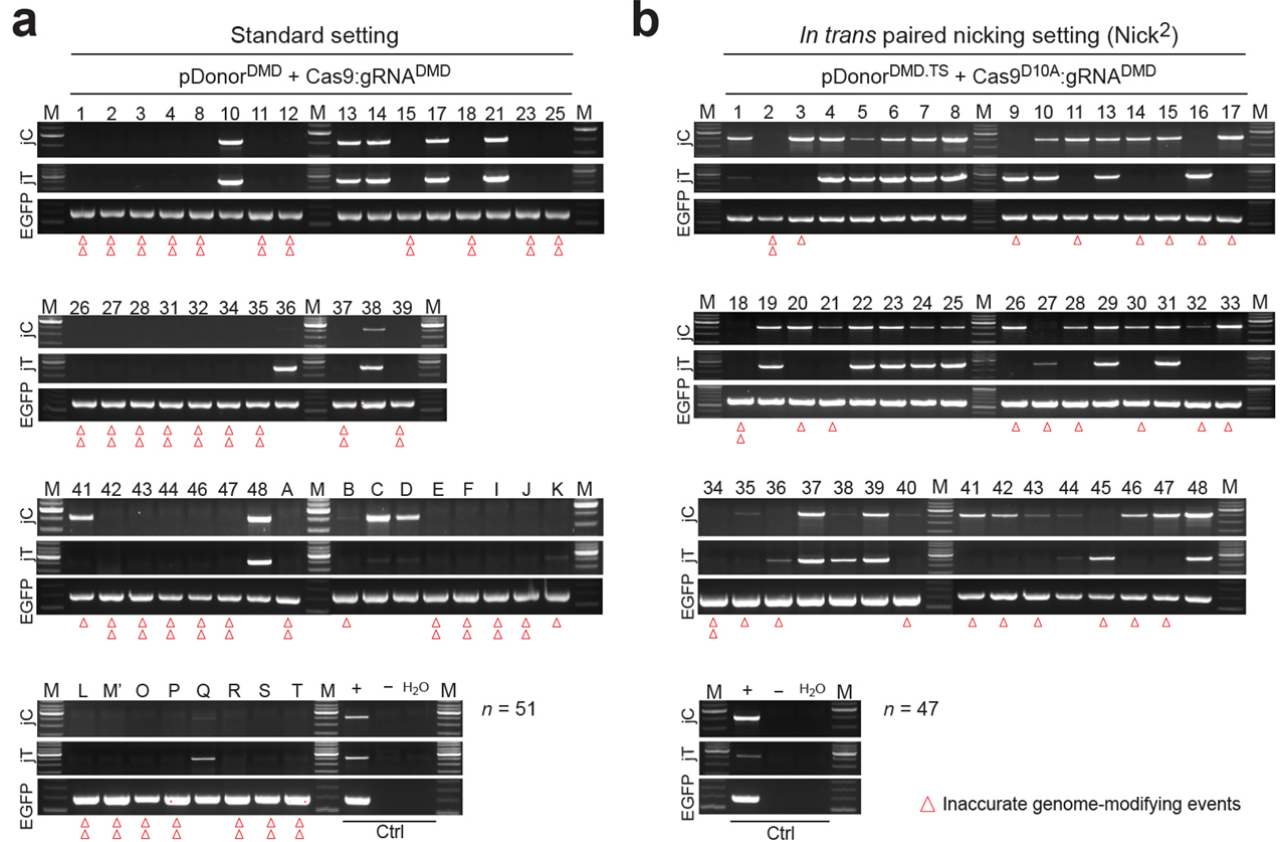


Supplementary Figure 1. Comparing the generation of allelic mutations and translocations in human cells provided with cleaving and nicking RGNs. (a) Detection of

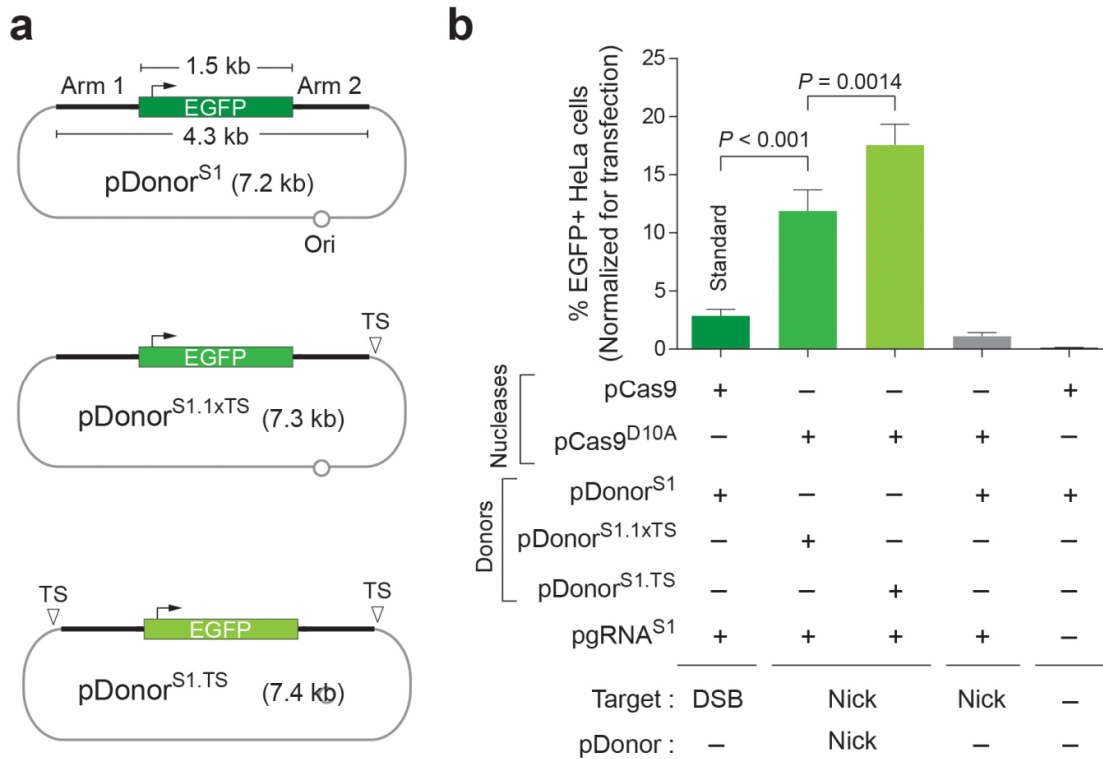
target gene mutagenesis induced by RGNs with Cas9 and Cas9^{D10A}. PCR products spanning an *AAVS1* target sequence retrieved from 293T cells transfected with the indicated plasmids were exposed to the mismatch-sensing T7EI. Indels formed by NHEJ-mediated DSB repair were detected by the appearance of T7EI-digested products (solid arrowheads). Experimental controls consisted of introducing only the *AAVS1*-targeting gRNA^{S1} or each nuclease together with the *EGFP*-specific gRNA^{GFP1}. Assay controls were provided by PCR samples containing DNA from mock-transfected cells or lacking either template DNA or T7EI. **(b)** Indel detection in cells exposed to increasing concentrations of cleaving Cas9 or nicking Cas9^{D10A}. HeLa cells were transduced in duplicate with the indicated doses and combinations of adenoviral vectors AdV.Cas9, AdV.Cas9^{D10A} and AdV.gRNA^{S1}. At 3 days post-transduction, indels and nucleases were detected by using T7EI-based genotyping assays (top panel) and western blot analysis (bottom panel), respectively. MOI, multiplicity-of-infection; TU/cell, transducing units per cell; Tubulin, protein loading control. **(c)** Gene knockout levels induced by RGNs containing Cas9, Cas9^{D10A}, or Cas9^{H840A}. The schematics of the experimental system (top) and the quantification of gene disruption frequencies (bottom) are presented. EGFP⁺ H27 cells were either mock-transfected or were co-transfected with the indicated plasmids. The gRNA^{GFP2} and gRNA^{NT} moieties address Cas9 proteins to, respectively, *EGFP* and an irrelevant I-SceI recognition sequence. The mutation rates caused by DSBs and SSBs were determined through flow cytometric quantification of EGFP-negative cells. The bars correspond to the mean values of two experiments done in duplicate. Representative dot plots are shown. **(d)** Probing for RGN-induced translocations. Genomic DNA from HeLa cells exposed to cleaving and nicking RGN pairs targeting *DMD* and *AAVS1* was subjected to the depicted PCR assay. Translocation-specific amplicons were detected using the indicated primer pair (arrowheads). *HPRT1* served as an internal control template for genomic DNA integrity. **(e)** Molecular characterization of translocation events. Sanger sequencing of cloned amplicons corresponding to individual chromosomal translocation events confirmed the presence of various t(X;19)(p21;q13) species in cell populations subjected to DSBs at *DMD* and *AAVS1*.



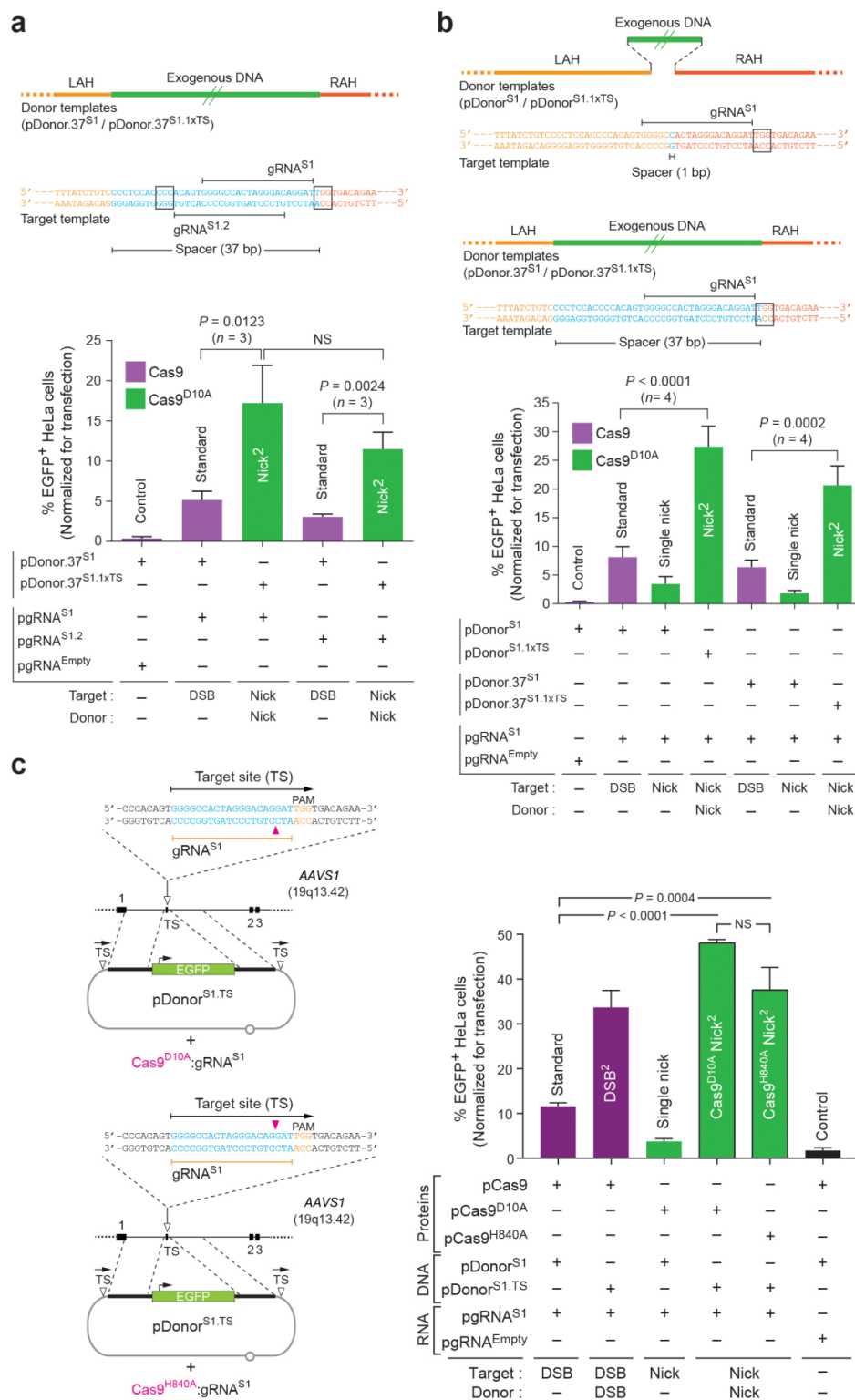
Supplementary Figure 2. Testing *in trans* paired nicking at *DMD* with donors containing different RGN target site arrangements. (a) Diagram of the standard and *in trans* paired nicking donor plasmids tested. Orange arrows define the orientations of the gRNA^{DMD} target site (TS). The plasmids pDonor^{DMD.TS.DR} and pDonor^{DMD.TS.IR} harbour the TS in a direct and inverted repeat configuration, respectively. Magenta arrowheads, position of sequence- and strand-specific DNA cleavage by Cas9^{D10A}. The transgene consists of the human *PGK1* promoter, the *EGFP* ORF and the bovine *GHI* polyadenylation signal. Cas9:gRNA^{DMD} and Cas9^{D10A}:gRNA^{DMD} are cleaving and nicking RGN complexes, respectively. PAM, protospacer adjacent motif; magenta arrowheads, position of sequence- and strand-specific DNA cleavage by Cas9^{D10A}. (b) Quantification of stable transfection levels by flow cytometry. Flow cytometric analysis of long-term HeLa cell cultures initially co-transfected with the indicated plasmids. The bars correspond to the mean $\pm$ s.d. of two independent experiments.



Supplementary Figure 3. Molecular characterization of human cells genetically modified by cleaving versus *in trans* paired nicking at *DMD*. (a) Screening of cells exposed to RGN-induced standard gene targeting. Junction PCR analyses of individual EGFP⁺ HeLa cell clones isolated from cultures co-transfected with pCas9, pgRNA^{DMD} and pDonor^{DMD}. (b) Screening of cells subjected to *in trans* paired nicking gene targeting (Nick²). Junction PCR analyses of individual EGFP⁺ HeLa cell clones derived from cultures co-transfected with pCas9^{D10A}, pgRNA^{DMD} and pDonor^{DMD.TS}. The different PCR screenings were carried out with the primer pairs shown in **Fig. 1a**. The resulting amplicons are specific for HR-derived telomeric and centromeric junctions formed between native DMD and exogenous DNA (jT and jC, respectively). PCR mixtures with DNA from parental cells or with nuclease-free water served as negative controls. PCR amplifications targeting *EGFP* served as internal controls (EGFP). Lanes M, GeneRuler DNA Ladder Mix molecular weight marker.

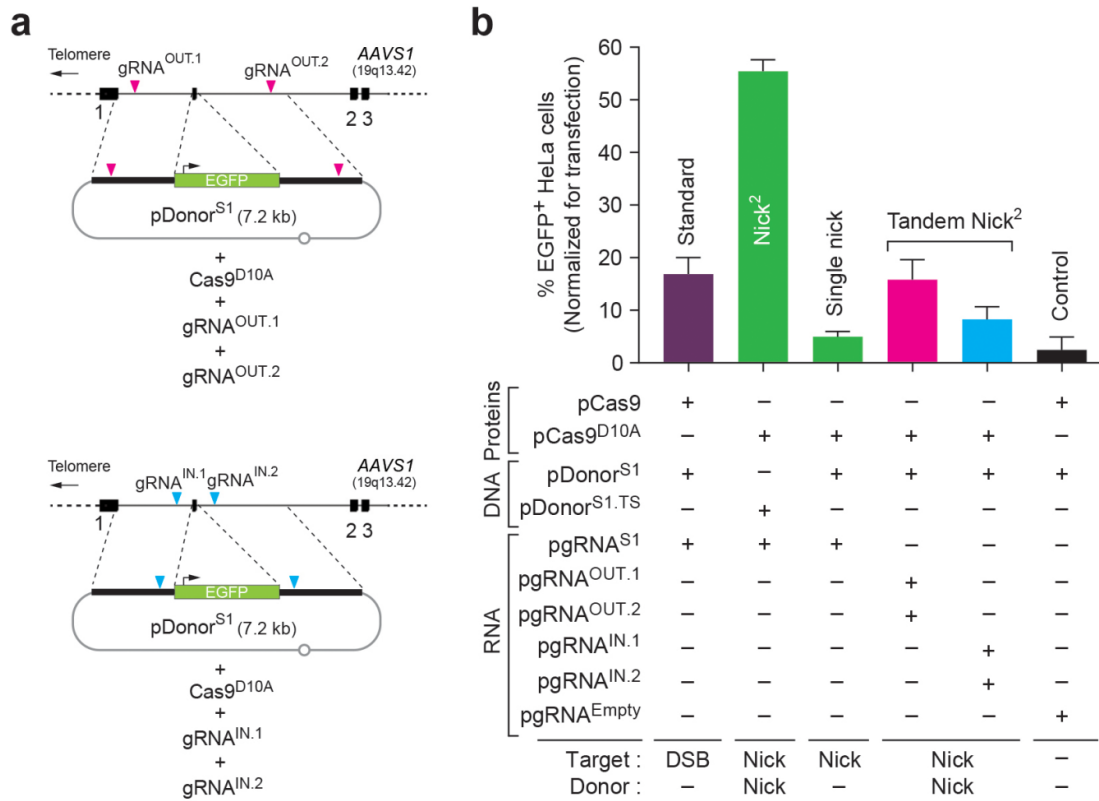


Supplementary Figure 4. Testing the performance of RGN-resistant versus RGN-susceptible donor plasmids. (a) Schematics of unmodified and modified *AAVS1*-targeting constructs. Arm 1 and Arm 2, DNA sequences sharing identity to those located “upstream” and “downstream”, respectively, the target site of RGNs containing the gRNA^{S1} moiety. EGFP, transgene consisting human *PGK1* promoter, *EGFP* ORF and bovine *GHI* polyadenylation signal sequences. The constructs pDonor^{S1}, pDonor^{S1.1xTS} and pDonor^{S1.TS} have zero, one and two target sites for *AAVS1*-specific RGN complexes (TS) located at the indicated positions. Ori, prokaryotic origin of replication. **(b)** Quantification of stable transfection levels by flow cytometry. EGFP-directed flow cytometry of HeLa cell populations transfected with the indicated plasmid combinations was done at 3 days and more than 2 weeks post-transfection, to determine transient and stable transfection levels, respectively. Ten thousand events, each corresponding to a single viable cell, were measured per sample. The bars represent mean $\pm$ s.d. of three independent experiments. The experimental groups were first compared by one-way ANOVA analysis. A subsequent statistical comparison between the indicated groups was carried out by Bonferroni analysis. The resulting *P* values are shown (*P* < 0.05 was considered significant).

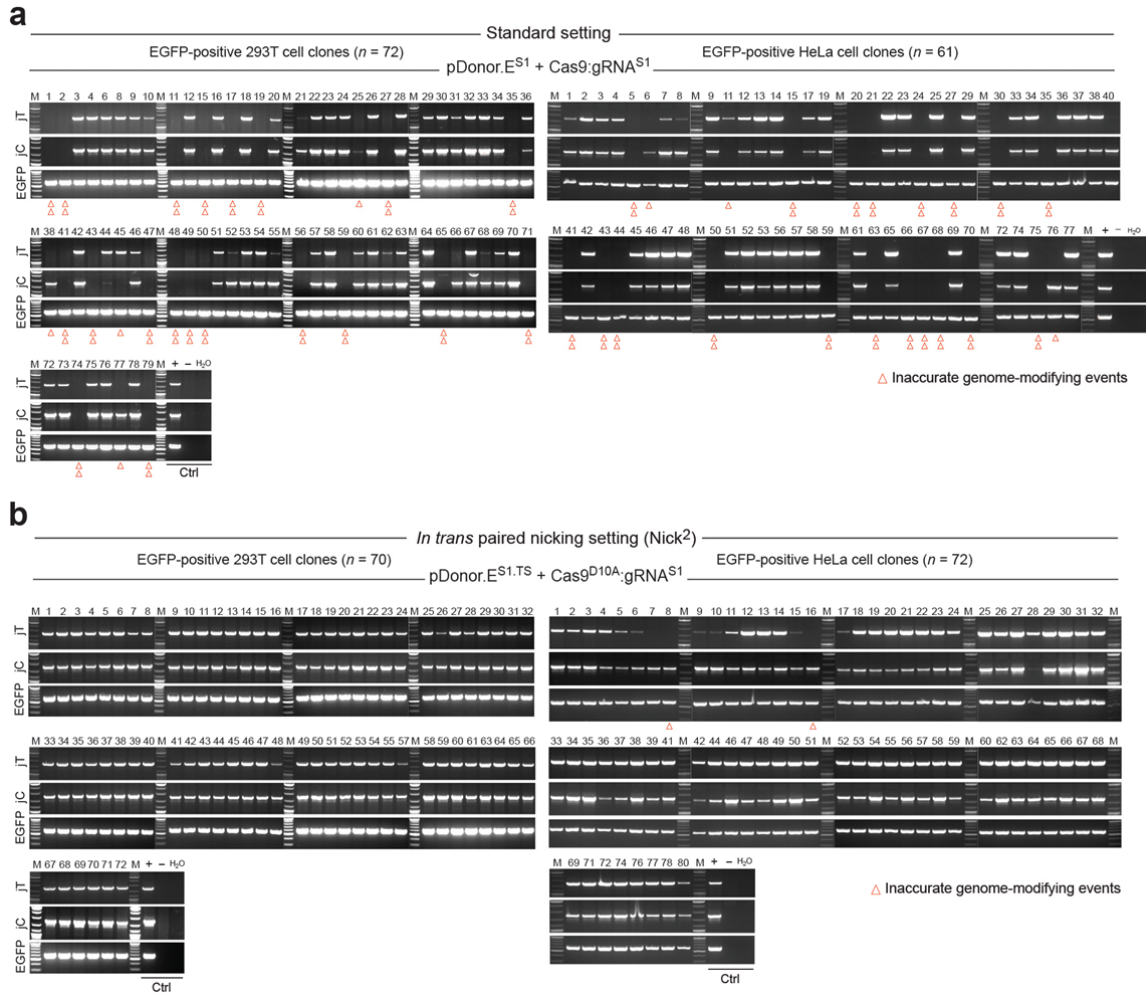


Supplementary Figure 5. Comparing the impact of different genome editing reagents target sequences and donor DNA compositions on *in trans* paired nicking. (a) Comparing standard versus *in trans* paired nicking using two different *AAVS1*-targeting RGN complexes.

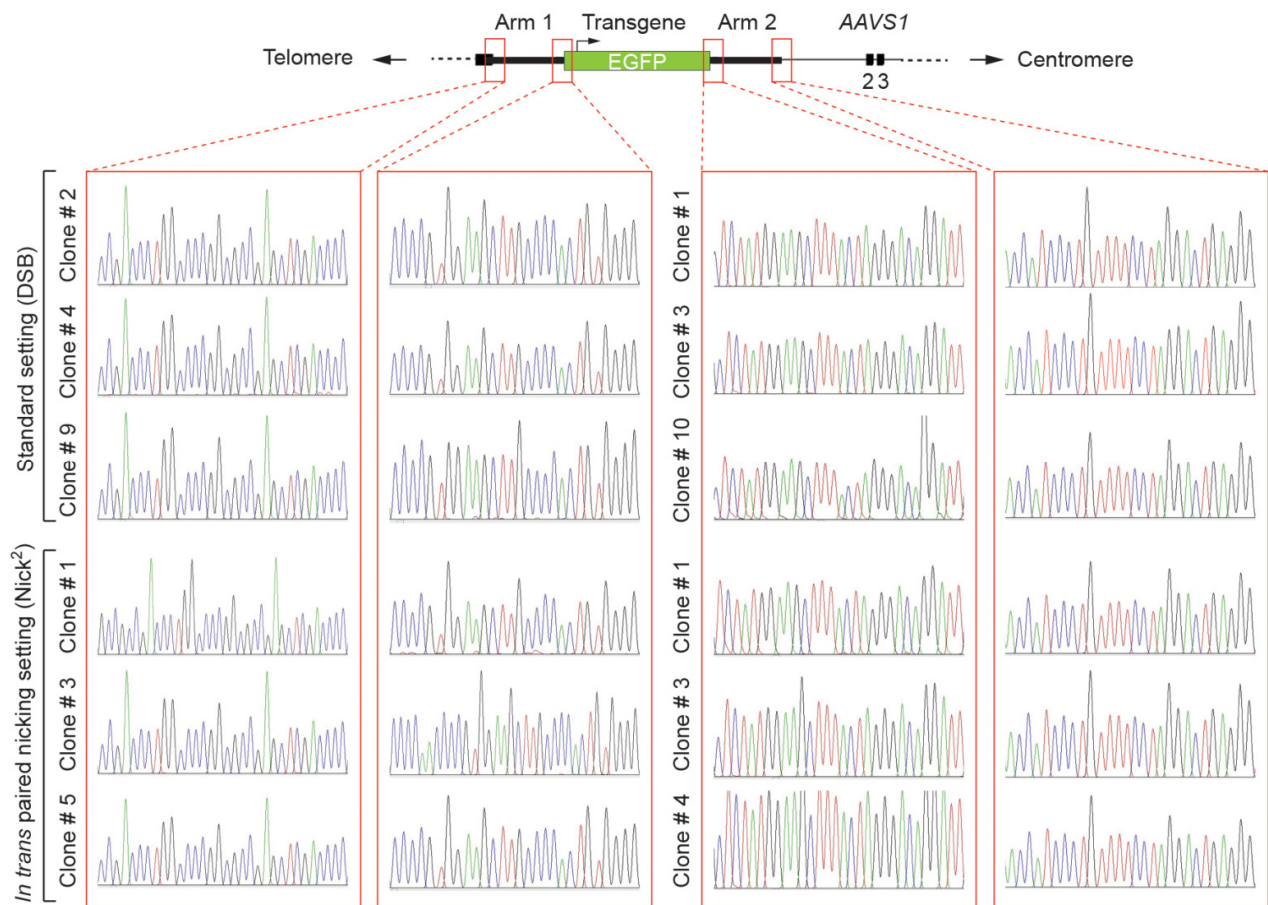
Top panel, Schematics of RGN-resistant pDonor.37^{S1} and RGN-susceptible pDonor.37^{S1.1xTS}, drawn in relation to their target DNA. The gRNAs gRNA^{S1} and gRNA^{S1.2} are also depicted in relation to their respective target sequences. Bottom panel, flow cytometric analysis of long-term HeLa cell cultures initially exposed to the indicated plasmid combinations. The bars correspond to mean $\pm$ s.d. of three independent experiments. *P* values were derived from two-tailed *t*-tests. **(b)** Comparing standard versus *in trans* paired nicking using *AAVS1*-targeting donors with different spacing between homology regions. Top panel, Schematics of donor DNA pairs pDonor^{S1}/pDonor^{S1.1xTS} and pDonor.37^{S1}/pDonor.37^{S1.1xTS} with 1-bp and 37-bp spacing between their arms of homology, respectively. LAH and RAH, “left” and “right” arms of homology, respectively. Bottom panel, flow cytometry of long-term HeLa cell cultures initially exposed to the indicated plasmid mixtures. The bars correspond to mean $\pm$ s.d. of four independent experiments. *P* values were obtained by applying two-tailed *t*-tests. **(c)** Comparing standard versus *in trans* paired nicking using different nicking Cas9 variants. The Cas9^{D10A} and Cas9^{H840A} proteins are sequence- and strand-specific endonucleases (“nickases”) owing to having their RuvC and HNH catalytic domains disabled, respectively. Magenta arrowheads, position of sequence- and strand-specific DNA cleavage by Cas9^{D10A} or Cas9^{H840A}. RGNs harbouring the former and latter proteins nick the DNA chain complementary and non-complementary to the gRNA spacer sequence, respectively (left panel). Flow cytometric analysis of long-term HeLa cell populations initially co-transfected with the indicated constructs (right panel). Ten thousand events, each corresponding to a single viable cells, were acquired per sample. The bars represent mean $\pm$ s.e.m. of three biological replicates from two independent experiments. *P* values were determined by two-tailed *t*-tests (*P* < 0.05 was considered significant; NS, non-significant).



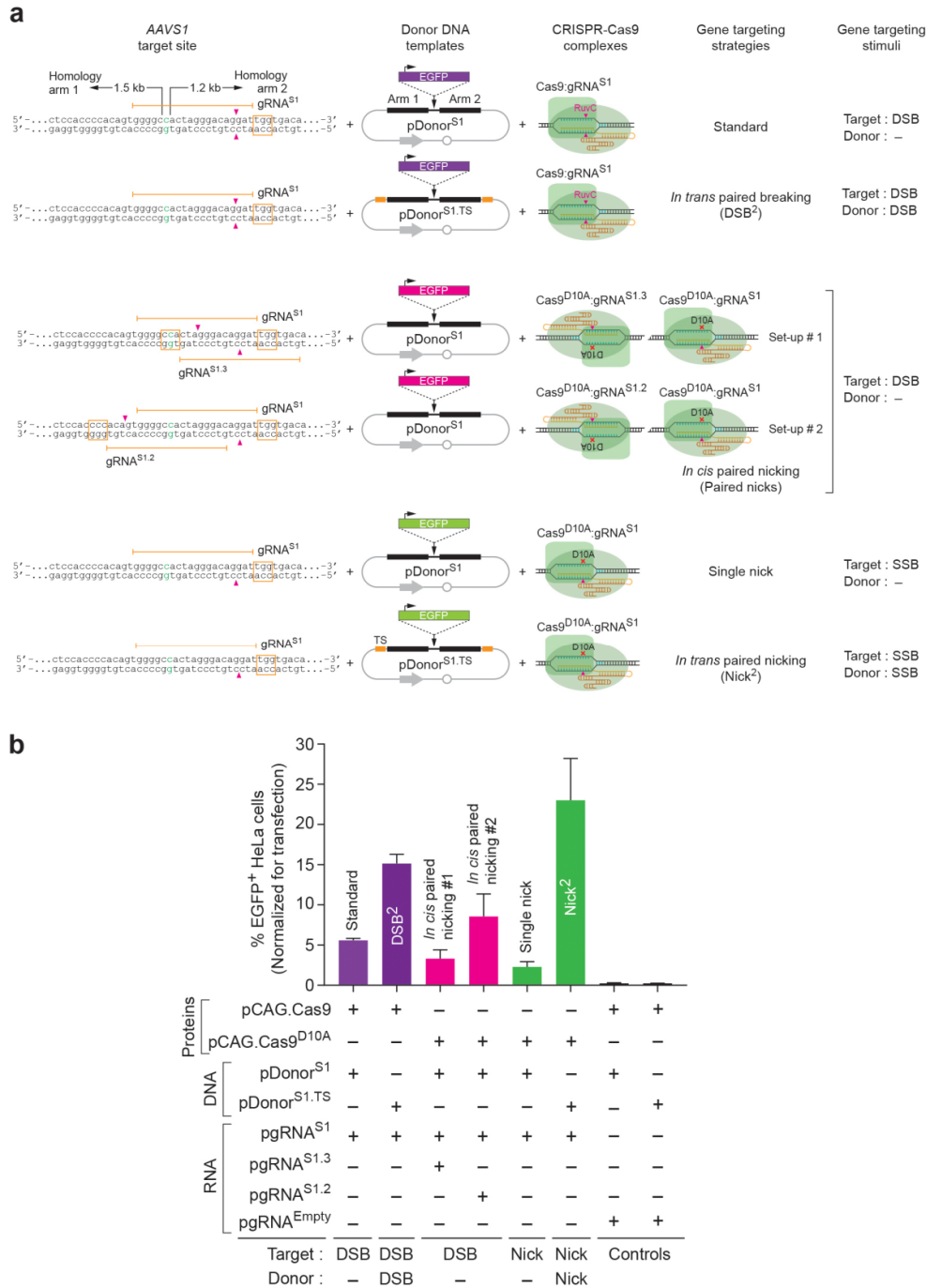
Supplementary Figure 6. Probing the tandem paired nicking concept. (a) Diagram of tandem paired nicking settings. The gRNAs used are drawn in relation to their target sequences located in *AAVS1* and in the “arms” of homology of donor plasmid pDonor^{S1}. Magenta and cyan arrowheads indicate the target sites of outward and inward gRNA pairs gRNA^{OUT.1}/gRNA^{OUT.2} and gRNA^{IN.1}/gRNA^{IN.2}, respectively. **(b)** Comparing standard with SSB-dependent gene targeting strategies. Quantification of stable transfection levels by EGFP-directed flow cytometry of HeLa cell populations initially transfected with the indicated plasmid mixtures. The bars correspond to mean $\pm$ s.d. of three independent experiments.



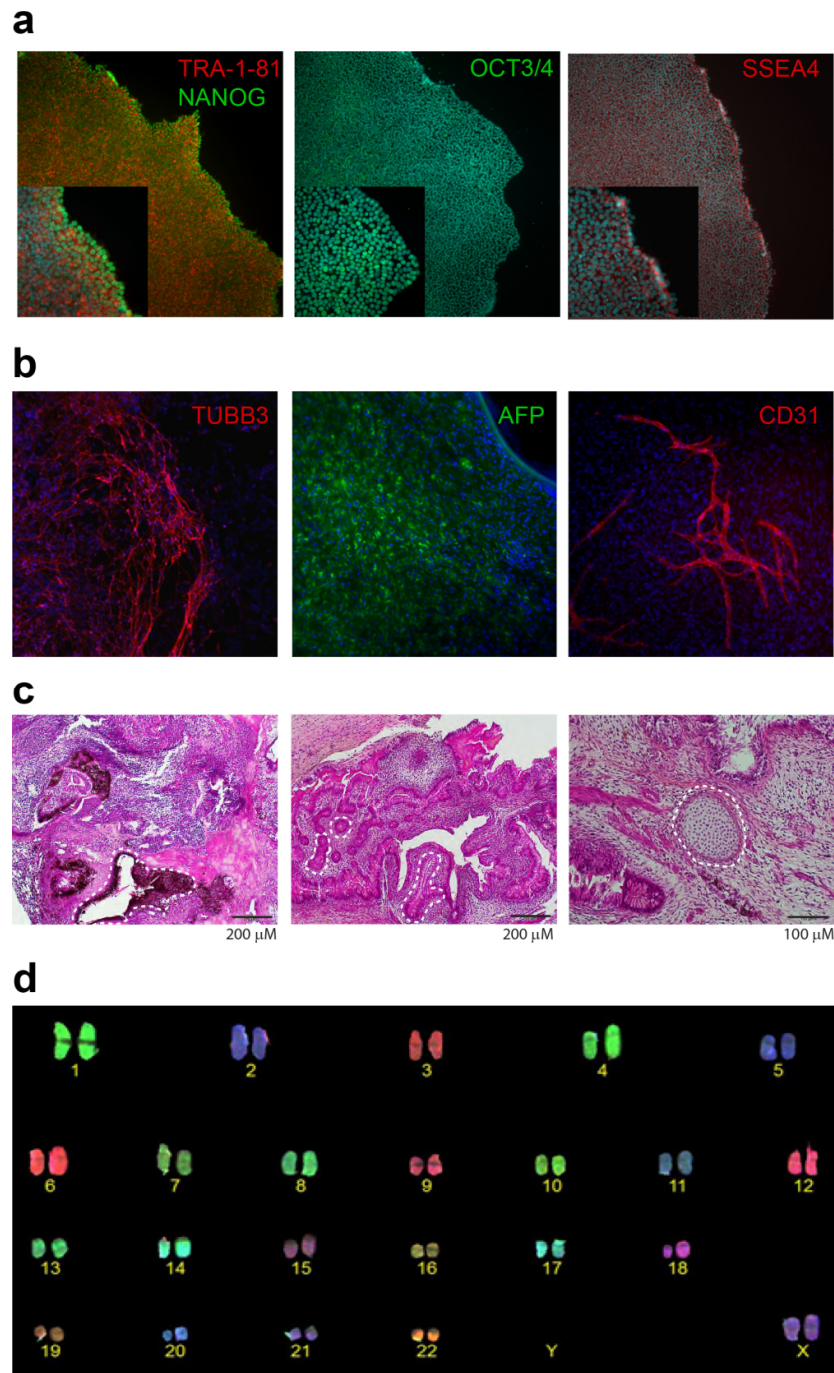
Supplementary Figure 7. Molecular characterization of human cells genetically modified by cleaving versus *in trans* paired nicking at *AAVS1*. (a) Screening of cells subjected to RGN-induced standard gene targeting. Junction PCR analyses of individual EGFP⁺ 293T and HeLa cell clones derived from cultures co-transfected with pCas9, pgRNA^{S1} and pDonor.E^{S1}. (b) Screening of cells exposed to *in trans* paired nicking gene targeting (Nick²). PCR analyses of individual EGFP⁺ 293T and HeLa cell clones isolated from cultures co-transfected with pCas9^{D10A}, pgRNA^{S1} and pDonor.E^{S1.TS}. The various PCR screenings were performed with the primer pairs depicted in **Fig. 2a**. The resulting amplicons are specific for HR-derived telomeric and centromeric junctions involving native *AAVS1* and exogenous DNA (jT and jC, respectively). PCR mixtures containing DNA from parental cells or with nuclease-free water served as negative controls. PCR amplifications targeting *EGFP* provided for internal controls (EGFP). Lanes M, GeneRuler DNA Ladder Mix molecular weight marker.



Supplementary Figure 8. DNA sequencing of transgenic-AAVS1 junctions. Amplicons corresponding to telomeric and centromeric exogenous-endogenous DNA junctions formed in 293T cell populations initially co-transfected with pCas9, pgRNA^{S1} and pDonor (DSB) or with pCas9^{D10A}, pgRNA^{S1} and pDonor^{TS} (Nick²), were cloned and sequenced. The chromatograms presented correspond to the border regions between the homology “arms” of donor templates and outward chromosomal DNA and between these “arms” and inward transgenic sequences. Six independent, randomly selected, molecular clones covering both integrant extremities were analysed per experimental condition. The sequence alignments detected neither large mutations (e.g. deletions, insertions or rearrangements) nor point mutations.

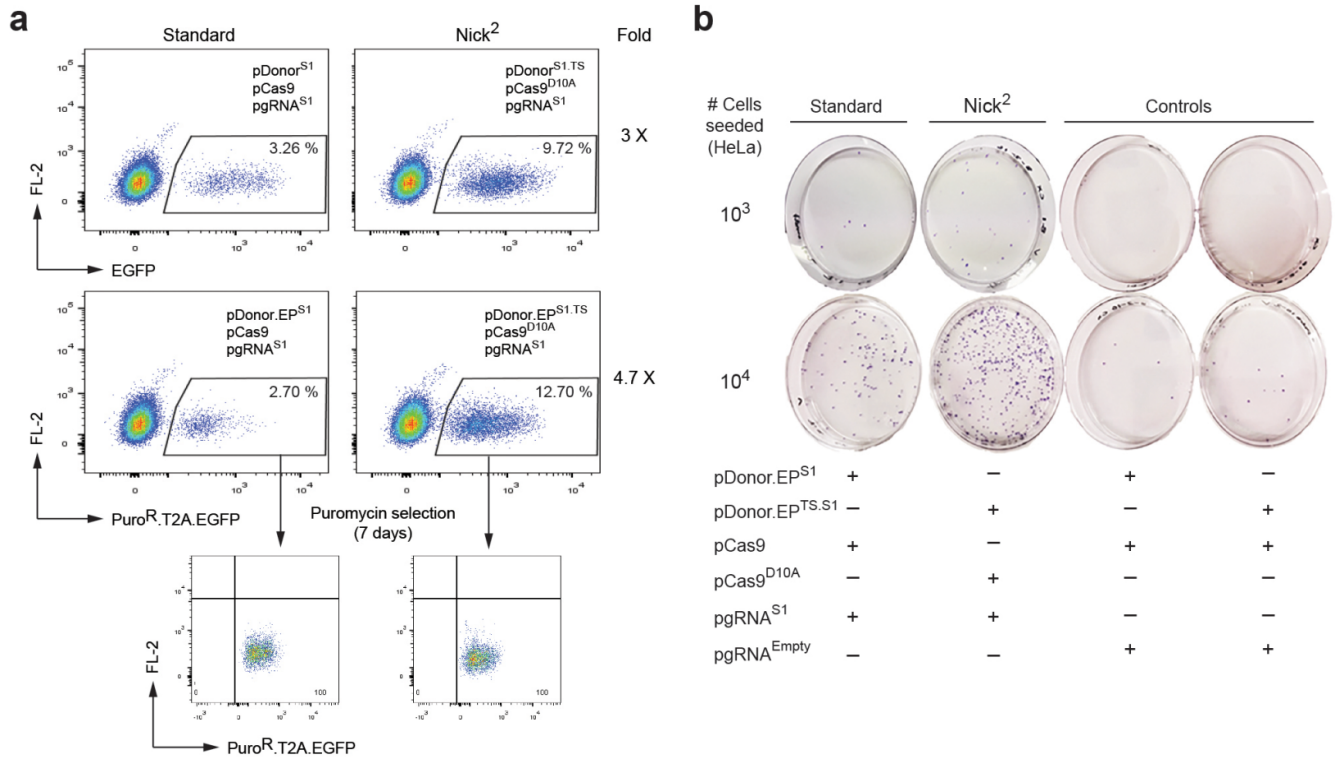


Supplementary Fig. 9. Comparing stable transfection levels achieved by using DSB- versus SSB-dependent gene targeting strategies. (a) Illustration of the different genome editing principles. Magenta arrowheads, position of sequence-specific DNA cleavage by Cas9 or Cas9^{D10A} **(b)** Quantification of stable transfection levels by flow cytometry. Flow cytometric analysis of long-term HeLa cell cultures initially co-transfected with the indicated plasmids. The bars correspond to the mean $\pm$ s.d. of two independent experiments.

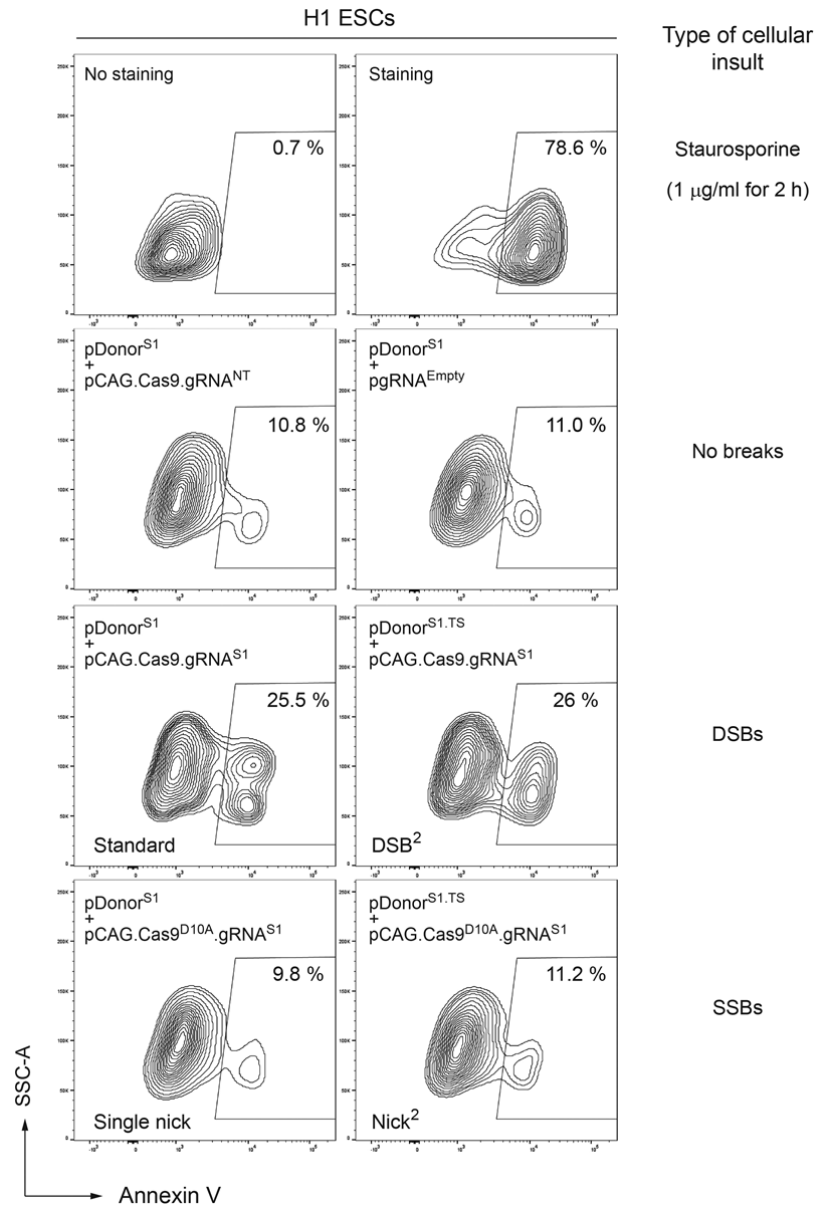


Supplementary Figure 10. Characterization of the iPSC line LUMC0044iCtrl44.9. (a) Immunofluorescence microscopy analysis of pluripotency markers in iPSCs. Undifferentiated iPSCs were stained for the pluripotent stem cell markers TRA-1-81, NANOG, OCT3/4 and SSEA4. (b) Analysis of *in vitro* differentiation of iPSCs by immunofluorescence microscopy. iPSCs were triggered to differentiate into cells belonging to the three different embryonic germ layers. Cells acquiring ectodermal, endodermal and mesodermal markers were

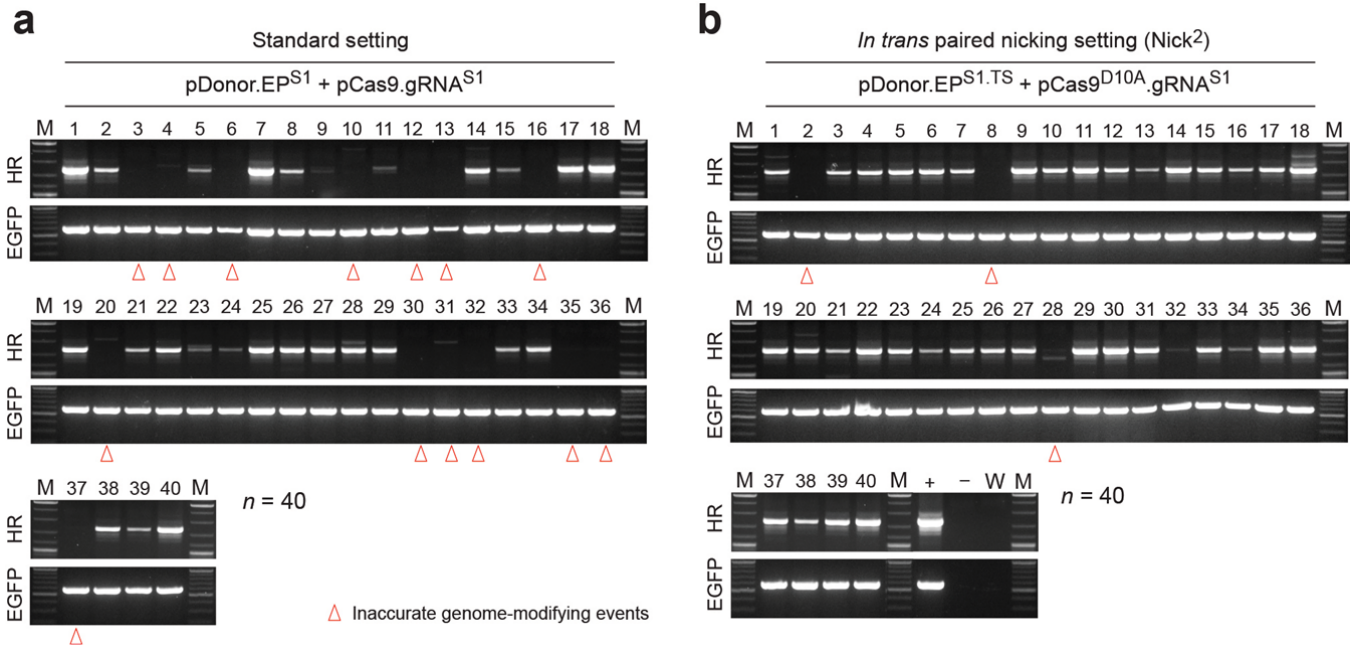
identified by staining with antibodies directed against tubulin beta 3 class III (TUBB3), alpha-fetoprotein (AFP) and platelet and endothelial cell adhesion molecule 1 (CD31). All immunofluorescence microscopy specimens were counterstained with the DNA dye DAPI (blue). **(c)** Analysis of *in vivo* differentiation of iPSCs by teratoma assays. iPSCs were injected subcutaneously into immunodeficient NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG) mice. Teratomas derived from iPSCs were analyzed by hematoxylin-phloxine-saffron (HPS) staining and visible light microscopy. Pigmented epithelium (left panel), trachea-like epithelium (middle panel) and cartilage tissue (right panel) belonging to ectodermal, endodermal and mesodermal germ layers, respectively, were identified. Representative morphological structures are outlined. **(d)** Karyotyping of iPSCs. COBRA-FISH analysis of undifferentiated iPSCs revealed a normal diploid karyotype (46, XX).



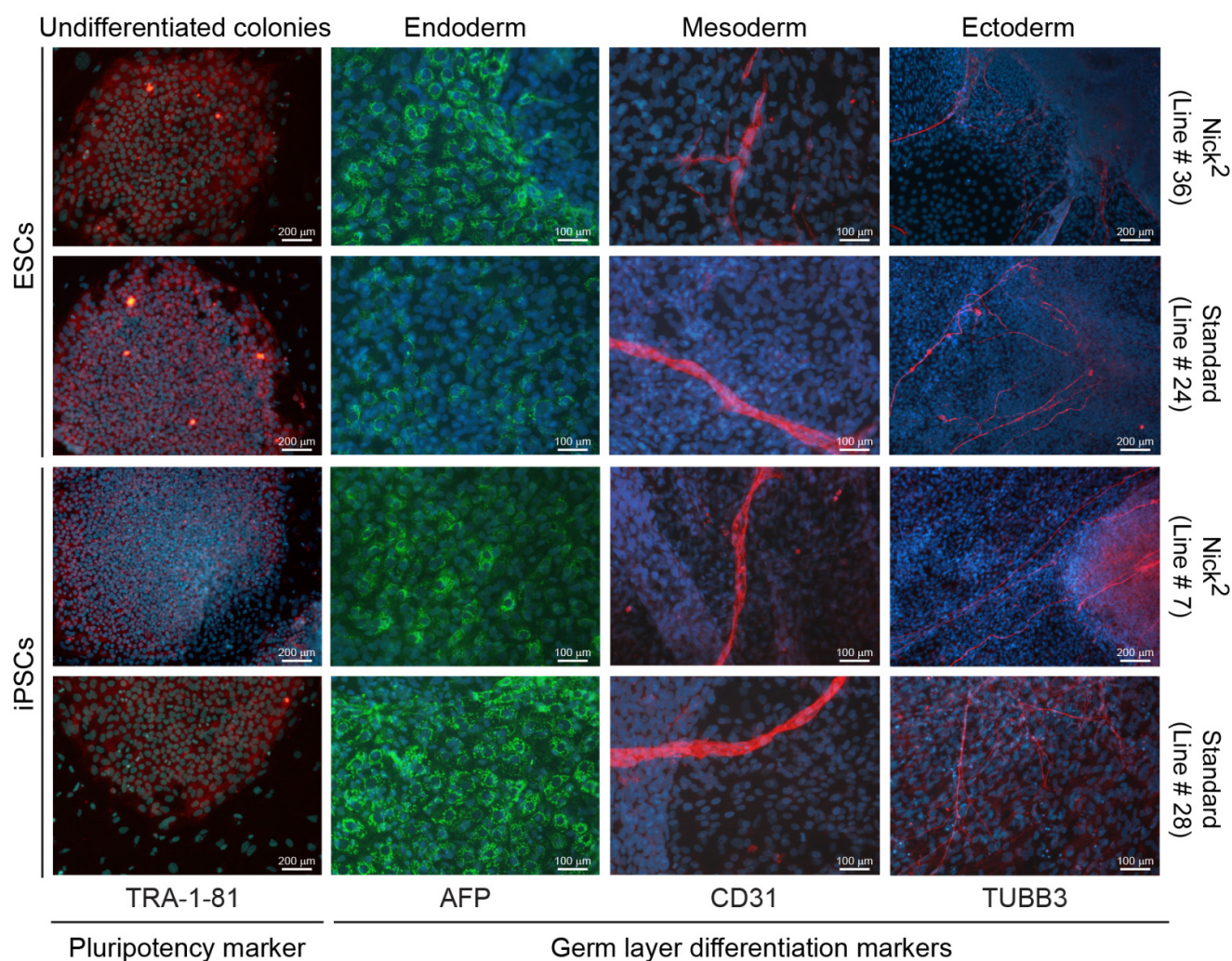
Supplementary Figure 11. Validation of *in trans* paired nicking with pDonor.EP^{S1} and pDonor.EP^{S1.TS}. (a) Quantification of genetically modified HeLa cells by flow cytometry. EGFP-directed flow cytometry dot plots of long-term HeLa cell cultures initially transfected with the indicated plasmids. The enhancement factors on the frequencies of stably transfected cells resulting from *in trans* paired nicking versus standard gene targeting approaches are indicated. Ten thousand events, each representing a single viable cell, were measured per sample. Stably transfected cells present in long-term HeLa cell cultures were selected by an incubation with puromycin for 7 days. (b) Identification of genetically modified HeLa cells by colony-formation assays. Thousand and ten-thousand cells from long-term HeLa cell cultures initially transfected with the indicated plasmids were treated for 7 days with puromycin. Puromycin-resistant colonies present in cells subjected to RGN-induced standard and *in trans* paired nicking (Nick²) gene targeting were identified by Giemsa staining.



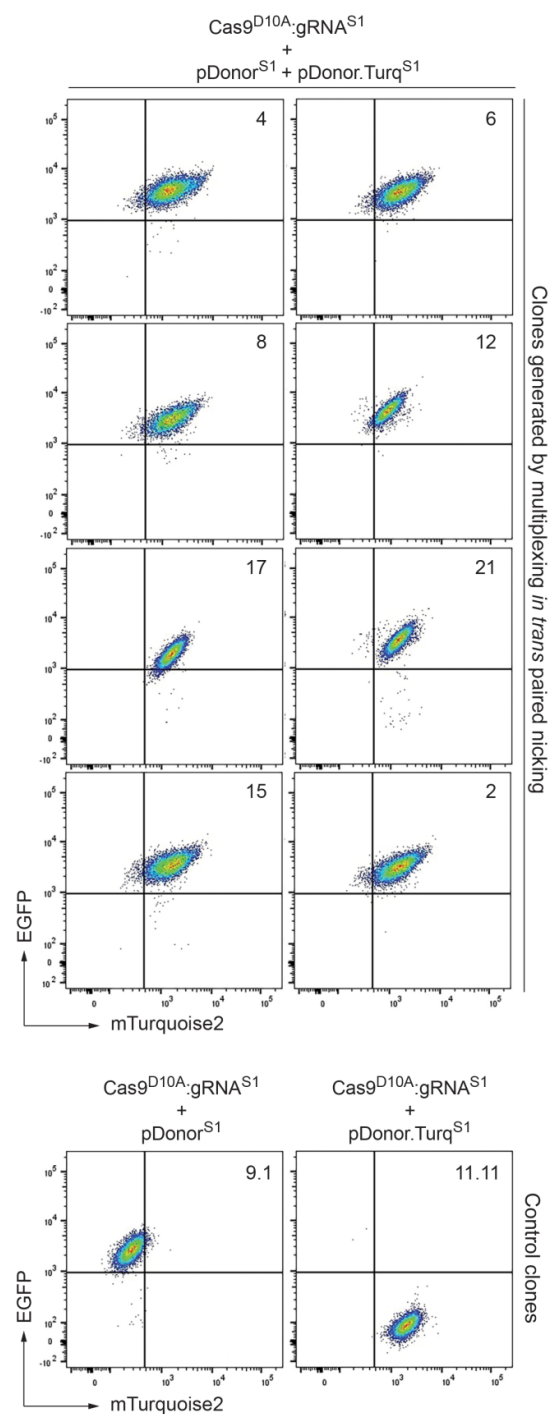
Supplementary Figure 12. Detection of apoptosis in ESCs subjected to DSB- versus SSB-dependent gene targeting strategies. Quantification of apoptosis by flow cytometric analysis of Annexin V binding to phosphatidylserine on ESCs treated with the indicated agents. The frequencies of EGFP⁺ and Annexin V-binding cells in transfected ESC cultures were determined at 2 days post-transfection. Staurosporine-incubated ESCs stained or not stained with fluorochrome-conjugated Annexin V served as positive and negative controls, respectively.



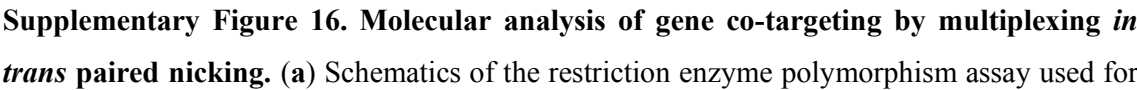
Supplementary Figure 13. Molecular characterization of iPSCs genetically modified by cleaving versus *in trans* paired nicking at *AAVS1*. (a) Screening of iPSCs subjected to RGN-induced standard gene targeting. Junction PCR analyses of individual puromycin-resistant iPSC colonies retrieved from cultures co-transfected with pDonor.EP^{S1} and pCAG.Cas9.gRNA^{S1}. (b) Screening of iPSCs treated subjected to *in trans* paired nicking gene targeting (Nick²). Junction PCR analyses of individual puromycin-resistant iPSC colonies isolated from cultures co-transfected with pDonor.EP^{S1.TS} and pCAG.Cas9^{D10A}.gRNA^{S1}. The PCR screening was carried out with the primers specific for the centromere-sided *AAVS1*-exogenous DNA junction (HR). PCR amplifications targeting EGFP provided for internal controls (EGFP). PCR mixtures containing unmodified genomic DNA (-) or nuclease-free water (W) served as negative controls. Genomic DNA from an *AAVS1*-targeted 293T clone was used as a positive control. Lanes M, GeneRuler DNA Ladder Mix molecular weight marker.



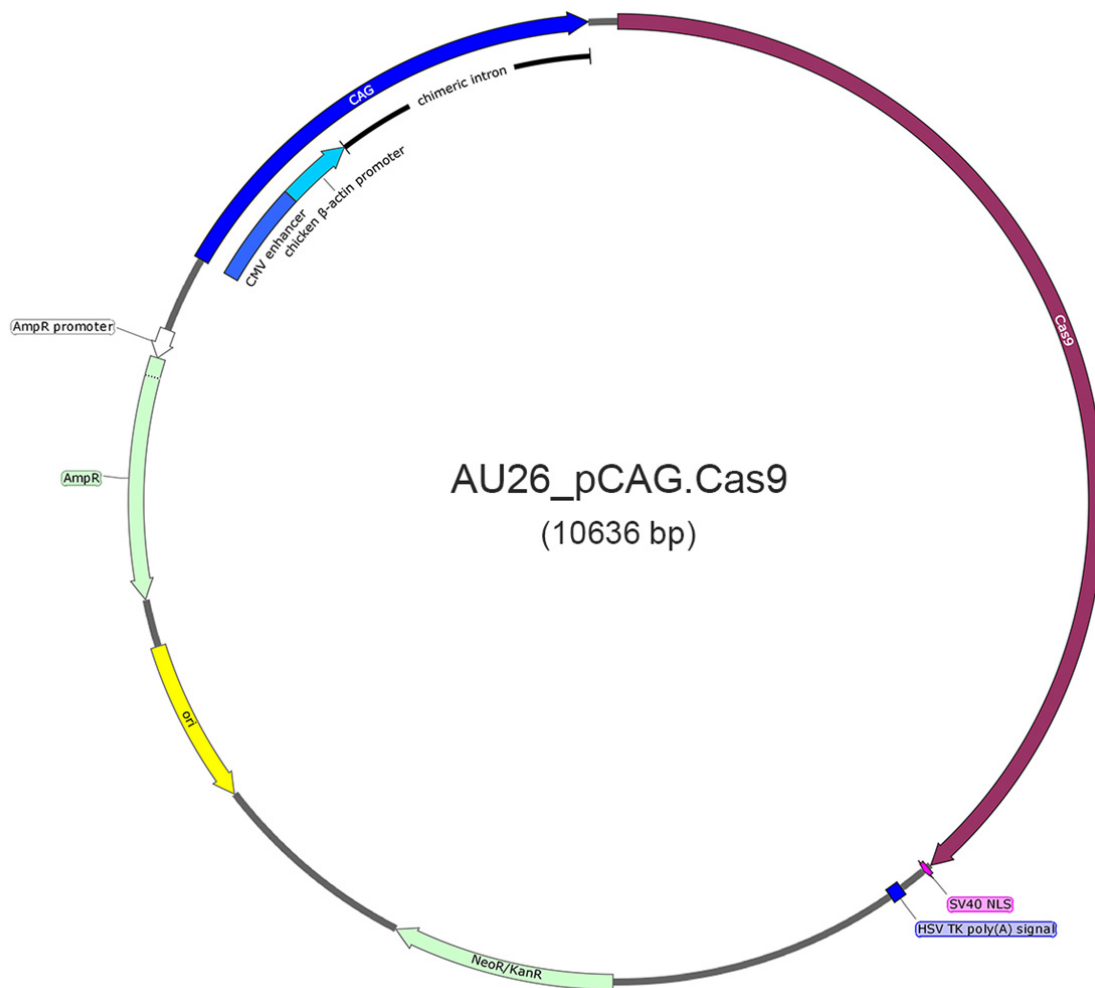
Supplementary Figure 14. Differentiation potential of PSC lines genetically modified by standard and *in trans* paired nicking. Immunofluorescence microscopy analysis of ESC and iPSC clones targeted at *AAVS1* by DSB-dependent (Standard) and *in trans* paired nicking (Nick²) protocols before and after differentiation. The marker for pluripotency and for each of the three embryo germ layers are indicated.



Supplementary Figure 15. Dual-colour flow cytometric analysis of gene co-targeting by multiplexing *in trans* paired nicking. Representative EGFP⁺/mTurquoise2⁺ HeLa clones generated by *in trans* paired nicking using two different donor plasmids. Inset numerals correspond to clone codes. Clones derived from HeLa cell cultures subjected to *in trans* paired nicking with a single donor plasmid aided in setting the fluorescence signal thresholds for the dual-colour flow cytometric analysis.



identifying co-targeting events at *AAVS1*. The presence and absence of a BlnI recognition site marks integrants resulting from HR events between *AAVS1* and, respectively, pDonor.Turq^{S1} and pDonor^{S1} sequences. The 2-kb amplicons susceptible and resistant to BlnI digestion are diagnostic for *mTurquoise2*- and *EGFP*-targeted *AAVS1* alleles, respectively. Solid boxes, *AAVS1* nucleotide sequences shared by target and donor DNA (“arms of homology”); grey boxes with and without broken arrow, human *PGK1* promoter and bovine *GHI* polyadenylation signal, respectively; cyan and green horizontal arrows, *mTurquoise2* and *EGFP* ORFs, respectively; half arrows, primers for amplifying 0.6 kb of recombinant DNA or 2.0 kb segments specific for recombinant-endogenous DNA junctions formed by HR events; horizontal magenta bars, ³²P-labelled Southern blotting probe **(b)** Assessing co-targeting of *AAVS1* alleles after multiplexing *in trans* paired nicking. Genomic DNA samples from EGFP⁺/mTurquoise2⁺ HeLa clones generated by multiplexing *in trans* paired nicking with donor plasmids pDonor^{S1} and pDonor.Turq^{S1}, were screened with the aid of the restriction enzyme polymorphism assay depicted in panel **a**. Genomic DNA samples from EGFP⁺ clone 9.2 and mTurquoise2⁺ clone 11.5 derived from HeLa cell cultures subjected to *in trans* paired nicking with pDonor^{S1} and pDonor.Turq^{S1}, respectively, served as controls. The 0.6 kb *mTurquoise2*- and *EGFP*-derived PCR products ascertained the integrity of the chromosomal DNA present in each sample. **(c)** Assessing co-targeting of *AAVS1* alleles after multiplexing *in trans* paired nicking by Southern blot analysis. BlnI-digested genomic DNA from randomly selected EGFP⁺/mTurquoise2⁺ HeLa clones were screened by Southern blotting using the ³²P-labelled probes shown in panel **a**. Nonspecific binding of the probes (background) to a DNA fragment from BlnI-digested pDonor.Turq^{S1} and BlnI-digested genomic DNA is indicated by the grey arrow. Green and cyan arrows point to the *EGFP*- and *mTurquoise2*-specific DNA fragments, respectively. Lane E, genomic DNA from an EGFP⁺ HeLa cell clone.



>AU26_pCAG.Cas9 (10,636 bp)

```

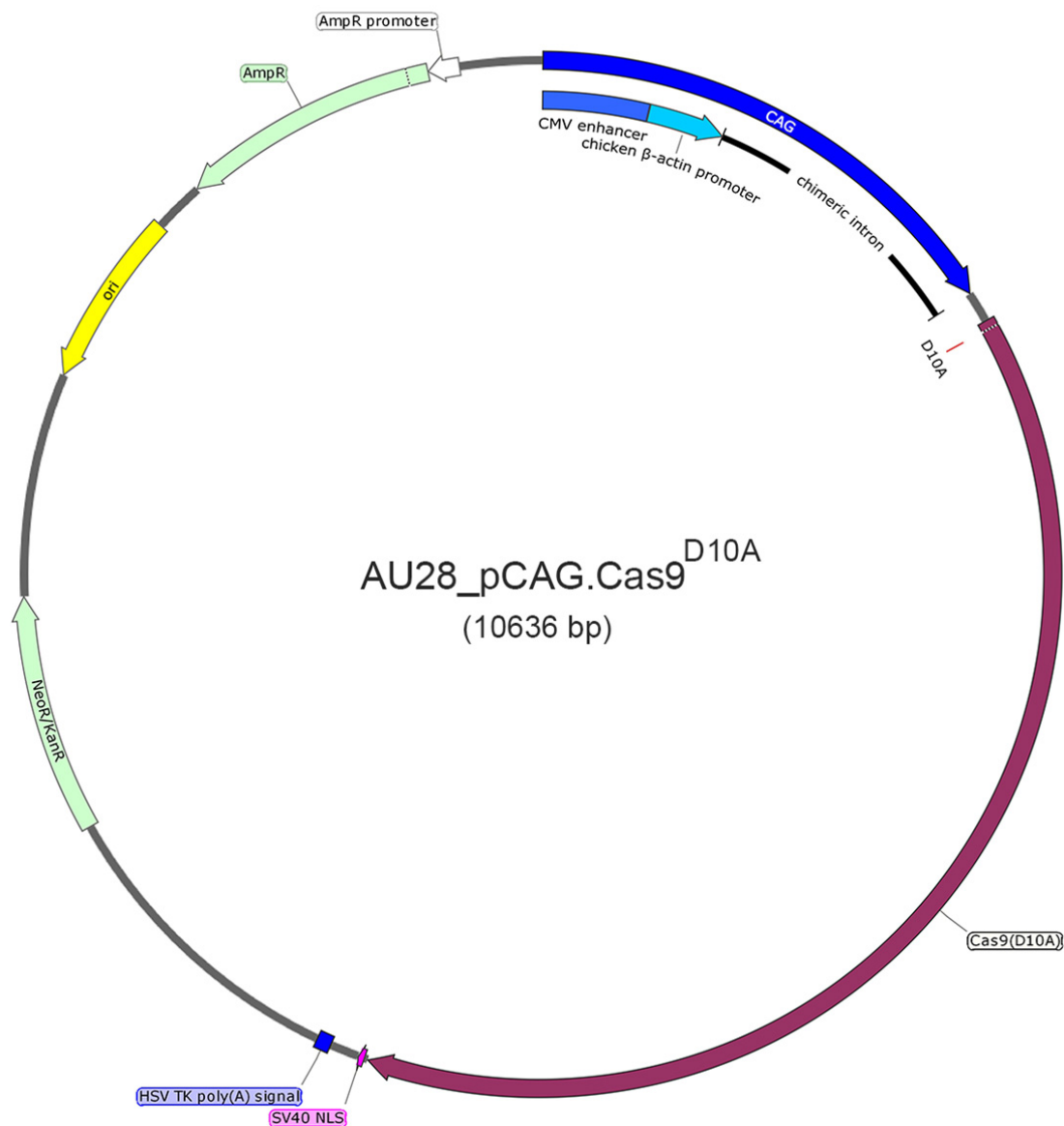
GCCACCATGGACAAGAAGTACTCCATTGGGCTCGATATCGGCACAAACAGCGTCGGCTGGGCCGTCATTACGGACGAGTA
CAAGGTGCCGAGCAAAAAATCAAAGTTCTGGGCAATACCGATCGCCACAGCATAAAGAAGAACCTCATTGGCGCCCTCC
TGTTCCGACTCCGGGAGACGGCCGAAGCCACGCGGCTCAAAGAACAGCAGCGCGCAGATATACCCGCAGAAAGAATCGG
ATCTGCTACCTGCAGGAGATCTTTAGTAATGAGATGGCTAAGGTGGATGACTCTTCTTCCATAGGCTGGAGGAGTCCTT
TTTGGTGGAGGAGATAAAAAGCACGAGCGCCACCCCAATCTTTGGCAATATCGTGACGAGGTGGCGTACCATGAAAAAGT
ACCCAACCATATATCATCTGAGGAAGAAGCTTGTAGACAGTACTGATAAGGCTGACTTGCGGTTGATCTATCTCGCGCTG
GCGCATATGATCAAAATTTCCGGGACACTTCCTCATCGAGGGGACCTGAACCCAGACAACAGCGATGTCGACAAACTCTT
TATCCAATCGGTTGAGACTTACAATCAGCTTTTGAAGAGAACCCGATCAACGCATCCGGAGTTGACGCCAAGCAATCC
TGAGCGCTAGGCTGTCCAAATCCCGGCGGCTCGAAAACCTCATCGCACAGCTCCCTGGGGAGAAGAAGAACGGCCTGTTT
GGTAATCTTATCGCCCTGTCACTCGGGCTGACCCCAACTTTAAATCTAACTTCGACCTGGCCGAAGATGCCAAGCTTCA
ACTGAGCAAAGACACCTACGATGATGATCTCGACAATCTGCTGGCCCAGATCGGCGACCAAGTACGCAGACCTTTTTTTGG
CGGCAAAGAACCTGTCAGACGCCATTCTGCTGAGTGATATCTGCGAGTGAACACGGAGATCACCAGCTCCGCTGAGC
GCTAGTATGATCAAGCGCTATGATGAGCACCACCAAGACTTGACTTTGCTGAAGGCCCTTGTCAGACAGCAACTGCCTGA
GAAGTACAAGGAAATTTCTTCGATCAGTCTAAAAATGGCTACGCCGGATACATTGACGGCGGAGCAAGCCAGGAGGAAT
TTTACAAATTTATTAAGCCCATCTTGGAATAAATGGACGGCACCCGAGGAGCTGCTGGTAAAGCTTAACAGAGAAGATCTG
TTGCGCAAACAGCGCACTTTGACAATGGAAGCATCCCCACCAGATTACCTGGGCGAACTGCACGCTATCTCAGGCG
GCAAGAGATTTTACCCCTTTTGAAGATAACAGGGAAAAGATTGAGAAAATCCTCACATTTTCGATACCTTACTATG
TAGGCCCCCTCGCCCGGGGAAATTCAGATTTCGCGTGGATGACTCGCAATCAGAAGAGACCATCACTCCCTGGAACCTC
GAGGAAGTCGTGGATAAGGGGGCCTCTGCCAGTCTTCATCGAAAGGATGACTAACTTTGATAAAAAATCTGCCTAACGA
AAAGGTGCTTCCTAAACACTCTCTGCTGTACGAGTACTTACAGTTTATAACGAGCTCACCAGGTCAAATACGTCACAG
AAGGGATGAGAAAGCCAGCATTCCTGTCTGGAGAGCAGAAGAAAGCTATCGTGGACCTCCTCTTCAAGACGAACCGGAAA
GTTACCGTGAAACAGCTCAAAGAAGACTATTTCAAAAAGATTGAATGTTTCGACTCTGTTGAAATCAGCGGAGTGGAGGA
TCGCTTCAACGCATCCCTGGGAACGTATCACGATCTCCTGAAAATCATTAAGACAAGGACTTCCTGGACAATGAGGAGA
ACGAGGACATTCTTGAGGACATTGTCTCACCTTACGTTGTTGAAGATAGGGAGATGATTGAAGAACGCTTGAAGAACT
TACGCTCATCTCTTCGACGACAAAAGTCATGAAACAGCTCAAGAGGCCGATATACAGGATGGGGGCGGCTGTCAAGAAA
ACTGATCAATGGGATCCGAGACAAGCAGAGTGGAAGACAATCCTGGATTTTCTTAAGTCCGATGGATTGCCAACCGGA

```

ACTTCATGCAGTTGATCCATGATGACTCTCTCACCTTTAAGGAGGACATCCAGAAAGCACAAGTTTCTGGCCAGGGGGAC
AGTCTTACGAGCAGCATCGTAATCTTGCAGGTAGCCCAGCTATCAAAAAGGGAATACTGCAGACCGTTAAGGTCGTGGA
TGAACCTCGTCAAAGTAATGGGAAGGCATAAGCCCCGAGAATATCGTTTATCGAGATGGCCCCGAGAGAACCAAACCTACCCAGA
AGGGACAGAAGAACAGTAGGGAAGGATGAAGAGGATTGAAGAGGGTATAAAGAAGCTGGGGTCCCAAATCCTTAAGGAA
CAGCCAGTTGAAAACACCCAGCTTCAAGATGAGAAGCTCTACCTGTACTACCTGCAGAACGGCAGGGACATGTACGTGGA
TCAGGAACTGGACATCAATCGGCTCTCCGACTACGACGTGGATCATATCGTGCCCCAGTCTTTTCTCAAAGATGATTCTA
TTGATAATAAAGTGTGACAAAGATCCGATAAAAAATAGAGGGAAGAGTGATAACGTCCTCCCTCAGAAGAAGTTGTCAGAAA
ATGAAAAATTATTTGGCGGCAGCTGCTGAACGCCAAACTGATCACACAACGGAAGTTTCGATAATCTGACTAAGGCTGAACG
AGGTGGCTGTCTGAGTTGGATAAAGCCGGCTTCATCAAAAGGCAGCTTGTGAGACACGCCAGATCACCAGCACGTGG
CCCAAATCTCGATTACGCATGAACACCAAGTACGATGAAAATGACAACTGATTTCGAGAGGTGAAAGTTATTTACTCTG
AAGTCTAAGCTGGTCTCAGATTTTCAGAAAGGACTTTTCAGTTTATAAGGTGAGAGAGATCAACAATTACCACCATGCGCA
TGATGCCCTACCTGAATGCAGTGGTAGGCATGCACCTTATCAAAAAATATCCCAAGCTTGAATCTGAATTTGTTTACGGAG
ACTATAAAGTGTACGATGTTAGGAAAATGATCGCAAAGTCTGAGCAGGAAATAGGCAAGGCCACCGCTAAGTACTTCTTT
TACAGCAATATTATGAATTTTTTCAAGACCGAGATTACACTGGCCAATGGAGAGATTTCGAAGCGACCACTTATCGAAAC
AAACGGAGAAACAGGAGAAATCGTGTGGGACAAGGGTAGGGATTTCCGCACAGTCCGGAAGGTCCGTGTCATGCCGAGG
TGAACATCGTTAAAAAGACCGAAGTACAGACCGGAGGCTTCTCCAAGGAAAGTATCTCCGAAAAGGAACAGCGACAAG
CTGATCGCACGCAAAAAAGATTGGGACCCCAAGAAATACGGCGGATTCGATTCTCCTACAGTCGCTTACAGTGTACTGGT
TGTGGCCAAAGTGAGAAAGGGAAGTCTAAAAAACTCAAAGCGTCAAGGAACTGCTGGGCATCACAAATCATGGAGCGAT
CAAGCTTCGAAAAAAACCCCATCGACTTTCTCGAGGCGAAAGGATATAAAGAGGTCAAAGACCTCATCATTAAGCTT
CCCAAGTACTCTCTCTTTGAGCTTGAAAACGGCCGGAACGAATGCTCGCTAGTGCAGGCGAGCTGCAGAAAGGTAACGA
GCTGGCACTGCCCTCTAATAACGTTAATTTCTGTATCTGGCCAGCCACTATGAAAAGCTCAAAGGGTCTCCCGAAGATA
ATGAGCAGACGCAAAAAAGATTGGGACCCCAAGAAATACGGCGGATTCGATTCTCCTACAGTCGCTTACAGTGTACTGGT
AGAGTGATCCTCGCCGACGCTAACCTCGATAAGGTGCTTTCTGCTTACAATAAGCACAGGGATAAGCCCATCAGGGAGCA
GGCAGAAAACATTATCCACTTGTCTTACTCTGACCAACTTGGGCGCGCTGCAGCCTTCAAGTACTTCGACACCACCATAG
ACAGAAAGCGGTACACCTCTACAAAGGAGGTCTGGACGCCACACTGATTATCAGTCAATTACGGGGCTCTATGAAACA
AGAATCGACCTCTCTCAGCTCGGTGGAGACAGCAGGGCTGACCCCAAGAAGAAGAGGAAGGTGTGAAAGGGTTCGATCCC
TACCGGTTAGTAATGTTTAAACGGGGGAGGCTAACCTGAAACAGGAAGGAGACAATACCGGAAGGAACCCGCGTATG
ACGGCAATAAAAAAGACAGAATAAAACGCACGGGTGTTGGGTGCTTTGTTTATAAACCGGGGTTCCGTCCCAGGGCTGGC
ACTCTGTGATACCCACCGAGACCCATTGGGGCCAATACGCCCGCTTTCTTCTTTTCCCCACCCACCCCAAGT
TCGGGTGAAGGCCAGGGCTCGCAGCCAACGTGGGGCGGCAGGCCCTGCCATAGCAGATCTGCGCAGCTGGGGCTCTAG
GGGGTATCCCCACGCGCCTGTAGCGGCGCATTAAGCGCGGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTG
CCAGCGCCTTAGCGCCGCTCTTTCTGCTTTCTTCCCTTCTTCTCGCCACGTTTCGCGGCTTTCCCGCTCAAGCTTFA
AATCGGGGCTCCCTTTAGGGTTCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTC
ACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGT
TCCAACTGGAACACACTCAACCTATCTCGGTCTATTCTTTGATTTATAAGGGATTTTGCCGATTTTCGGCTATTGG
TTAAAAAATGAGCTGATTTAAACAAAAATTTAACGCGAATTAATCTGTGGAATGTGTGTCAGTTAGGGTGTTGAAAGCTC
CCAGGTCCTCCGACAGGCAGAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCTAACTCCGCCATCCCCG
CCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCTAACTCCGCCATCCCCG
CCCTAACTCCGCCAGTTCCGCCCATTTCTCCGCCCATGGCTGACTAATTTTTTTTATTTATGACAGAGCCGAGGCCGCC
TCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCTAGGCTTTTGCAAAAAGCTCCCGGGAGCTTG
TATATCCATTTTCGGATCTGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAAGTT
CTCCGGCGCTTGGGTGGAGAGGCTATTTCGGCTATGATGAGGCAACAGACAAATCGGCTGCTCTGATGACCGCCGCTTC
CGGCTGTGACGCGAGGGGCGCCGCTTCTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAAGTGCAGGACGAGGC
AGCGCGGCTATCGTGGCTGGCCACGACGGGCTTCTTGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGACT
GGCTGCTATTGGGCGAAGTGCCGGGCGAGGATCTCCTGTCTCTACCTTGTCTGCGGAGAAAGTATCCATCATGGCT
GATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCATTTCGACCAACCAAGCGAAACATCGCATCGAGCGAGC
ACGTACTCGGATGGAAGCCGCTTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGT
TCGCCAGGCTCAAGGCGCGCATGCCGACGGCGAGGATCTCGTGTGACCCATGGCGATGCTGCTTGGCGAATATCATG
GTGAAAAATGGCCGCTTTTTCTGGATTATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGC
TACCCGTGATATTGCTGAAGAGCTTGCGGCGCAATGGGCTGACCGCTTCTCGTGCTTTACGGTATCGCCGCTCCCGATT
CGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGCGAAATGACCGACCAAGC
GACGCCCAACCTGCCATCACGAGATTTTCGATTCCACCGCTGCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCGGG
ACGCGGCTGGATGATCCTCCAGCGCGGGATCTCATGTGGAGTTCTTCGCCACCCCAACTTGTTTATTGCAGCTTAT
AATGGTTACAAATAAAGCAATAGCATCAAAATTTACAAATAAAGCAATTTTTTCACTGCATTTCTAGTTGTGGTTTGTG
CAAACCTCATCAATGTATCTTATCATGTCTGTATACCGTCGACCTTAGCTAGAGCTTGGCGTAATCATGGTCAATAGCTGT
TTCTGTGTGAAATTTGTTATCCGCTCACAATTCACACACATACGAGCCGGAAGCATAAAGTGTAAAGCTGGGGTGCC
TAATGAGTGTAGCTTAACATCAATTAATTTGCGTTGCGCTACTGCCCCGCTTTCCAGTCGGGAAACCTGTCTGCGCCAGTGC
TTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCATATTGGGCGCTCTTCCGCTTCTCGCTCACTGACTCGCTGC
GCTCGGTGTTGCGTGGCGGAGCGGTATCAGCTCACTCAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAAC
GCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTGTGCTGGCGTTTTTCCATAG
GCTCCGCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATAACC
AGGCTTTTCCCGCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCTTCCGCTTACCGGATACCTGTCCGCTTTCTC
CCTTCGGGAAGCGTGGCGCTTTCTCATAGCTACGCTGTAGGTATCTCAGTTCCGGTGTAGGTGCTGCTCCAGCTGGG
CTGTGTGCACGAACCCCGCTTTCAGCCGACCGCTGCGCTTATCCGGTAACATCGTCTTGAGTCCAACCCGTAAGAC
ACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGTACAGAGTTCTTG
AAGTGGTGGCCTAATACGGCTACACTAGAAGAACAGATTTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAA
AAGAGTTGGTAGCTCTTGATCCGGCAACAAACCCGCTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGCAGGATACGC
CGAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGAACGAAAACCTACGTTAA

GGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTAAATCAATCTA
AAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTC
GTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCA
ATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAG
TGGTCCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCGCGGAAGCTAGAGTAAGTAGTTCCGCAGTTAATA
GTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACAGCTCGTCGTTTGGTATGGCTTCATTACGCTCCGGT
TCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGT
CAGAAGTAAGTTGCCGCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAA
GATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCG
GCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAGTGCTCATCATTTGGAAAACGTTCTTCGGGGCGAAA
ACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTA
CTTTCACCGAGCGTTTCTGGGTGAGCAAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGT
TGAATACTCATACTCTTCTCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGA
ATGTATTTAGAAAAATAACAAATAGGGGTTCCGCGCACATTTCCCGAAAAGTGCCACCTGACGTGACGAGATCGGGAG
ATCTCCCGATCCCTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATCTGCTCCCTGCTT
GTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGACAATTGCATGAAG
AATCTGCTTAGGGTTAGGCGTTTTCGCTGCTTCGCGATGTACGGGCCAGATATACTcgagccatggattcGACATTGAT
TATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTTCATAGCCCATATATGGAGTTCGCGGTTACATAACTT
ACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAAC
GCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAGTGTATC
ATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGG
GACTTTTCCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTCA
CTCTCCCCATCTCCCCCCCCCCCCACCCCAATTTTGTATTTATTTATTTTAAATTATTTTGTGCAGCGATGGGGGCG
GGGGGGGGGGGGCGCGCGCAGGCGGGGCGGGGCGGGGCGAGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAG
CCAATCAGAGCGGCGCGCTCCGAAAGTTTCTTTTATGGCGAGGCGCGCGCGCGCGCCCTATAAAAAGCGAAGCGCG
CGGCGGGGCGGAGTCGCTGCGTTGCCCTTCGCCCGCTGCCCGCTCCGCGCGCGCTCGCGCGCGCGCGCGCGCGCTCTGACT
GACCGCGTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCGGGCTGTAATTAGCGCTTGGTTTAAAGACGGC
TCGTTTCTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGG
TGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCTGCGGCCCGCGCTGCCCGCGGCTGTGAGCGCTGCGGGCGCGGCGC
GGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGCGGCTGCCCGCGGTCGCGGGGGGCTGCGAGGGG
AACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGCGGTCGGGCTGTAACCCCCC
TGCACCCCCCTCCCGAGTTGCTGAGCACGGCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCTGGCGCGGGGCTCGCC
GTGCCGGGCGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCGGCTCGGGCGGGGAGGGCTCGGGGGAGGG
GCGCGGCGGCCCCGAGCGCGCGGCTGTGAGGCGCGCGAGCCGACGCTTATGCTTTTATGGTAATCGTGCGAGAG
GGCGCAGGGACTTCTTTGTCCCAATCTGGCGGAGCGCAATCTGGGAGGCGCGCGCACCCCTCTAGCGGGCGCGG
CGCAAGCGGTGCGGCGCGGCGAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCCGCGCGCGCGCTCCCTTCTCCA
TCTCAGGCTCGGGGCTGCCGACGGGGGACGGCTGCGCTTCGGGGGGGACGGGGCAGGGCGGGGTTGCGCTTCTGCGTGT
GACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCTACAGCTCCTGGGCAACGTGCTGGTTG
TTGTGCTGTCTCATATTTTGGCAAGAATTatcgcatgcctgcagagctctagagTCCTAGAGGATCGAACCTT

Supplementary Figure 17. Map and nucleotide sequence of expression plasmid AU26_pCAG.Cas9. CAG, hybrid promoter (CMV enhancer, human cytomegalovirus *immediate early* enhancer; chicken β -actin promoter; chimeric intron, fusion between introns from the chicken β -actin and rabbit β -globin genes); Cas9, human codon-optimized Cas9 (Csn1) ORF coding for the endonuclease from the *Streptococcus pyogenes* type II CRISPR/Cas system; SV40 NLS, nuclear localization signal SV40 large T antigen; HSV TK poly(A) signal, herpes simplex virus *thymidine kinase* polyadenylation signal; NeoR/KanR, transposon Tn5 *aminoglycoside phosphotransferase* conferring resistance to the antibiotics G418 (geneticin) and kanamycin; AmpR, β -lactamase ampicillin resistance gene; ori, high-copy number ColE1 prokaryotic origin of replication.



```

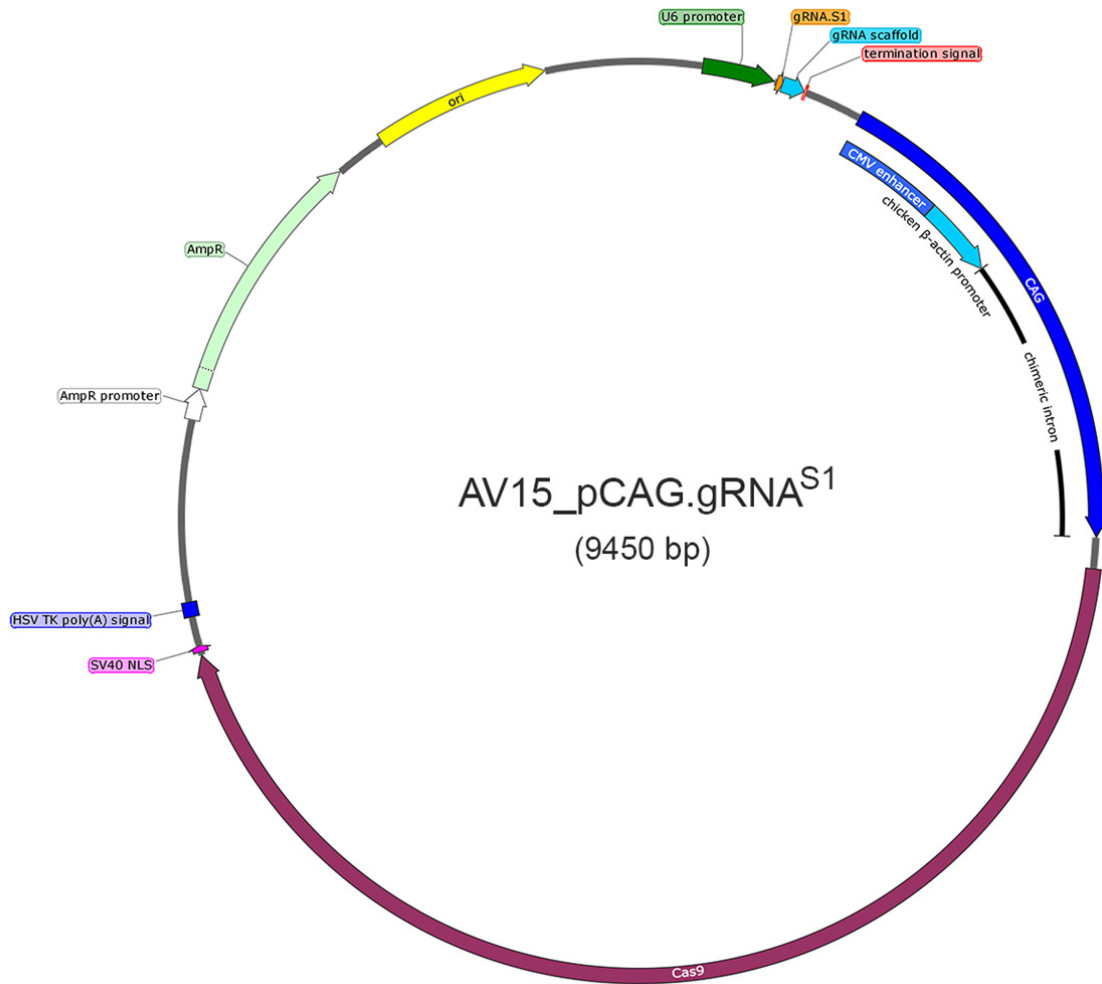
>AU28_pCAG.Cas9D10A (10,636 bp)
TCGAGCCATGGATTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
ATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAAT
AATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCC
ACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCAT
TATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCCG
AGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTGTATTTATTTATTTTAA
TTATTTTGTGTCAGCGATGGGGGCGGGGGGGGGGGGCGCGGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGG
CGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGG
CGGCCCTATAAAAAGCGAAGCGCGCGCGGGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCGCTCCGCGCCGCCCTC
GCGCGCCCGCCCGGCTCTGACTGACCGGTTACTCCACAGGTGAGCGGGCGGGACGCGCCCTTCTCCTCCGGGCTGTA
ATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTG
TGCGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGG
CTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGGCGGTGCCCC
GCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGC
GGCGGTGCGGGCTGTAACCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGT
GCGGGGCGTGGCGGGGGCTGCGCGTGCCGGGCGGGGGGTGCGGCGAGGTGGGGGTGCCGGGCGGGGCGGGGCGCGCTCG
GGCCGGGAGGGCTCGGGGAGGGGCGCGCGGCCCCGAGCGCGCGGCTGTGAGGCGCGGCGAGCCGACGCCATTG

```

CCTTTTATGGTAATCGTGCAGAGGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGGCGCCG
CCGCACCCCTCTAGCGGGCGCGGGCGAAGCGGTGCGGGCGCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCTC
GCCGCGCCGCGTCCCCTTCTCCATCTCCAGCCTCGGGGCTGCGCGAGGGGACGGCTGCCTTCGGGGGGGACGGGGCAG
GGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCTTCTAC
AGCTCCTGGGCAACGTGCTGGTTGTTGTGCTGCTCATCATTTTGGCAAAGAATTATCGCATGCCTGCAGAGCTCTAGAG
TCCTAGAGGATCGAACCTTGGCCACATGGACAAGAAGTACTCCATTGGGCTCGCTATCGGCACAAACAGCGTCGGCTGG
GCCGTCATTACGGACGAGTACAAGGTGCCGAGCAAAAAATTCAAAGTTCTGGGCAATACCGATCGCCACAGCATAAAGAA
GAACCTCATTGGCGCCCTCTGTTCGACTCCGGGAGACGGCCGAAGCCACGCGGCTCAAAAGAACAGCACGGCGCAGAT
ATACCCGAGAAAGAATCGGATCTGTACCTGCAGGAGATCTTTAGTAATGAGATGGCTAAGGTGGATGACTCTTTCTTCTC
CATAGGCTGGAGGAGTCTTTTGGTGAGGAGGATAAAAGCACGAGCGCCACCAATCTTTGGCAATATCGTGACGGA
GGTGGCGTACCATGAAAAGTACCAACCATATATCATCTGAGGAAGAAGCTTGTAGACAGTACTGATAAGGCTGACTTGC
GGTTGATCTATCTCGCGCTGGCGCATATGATCAAAATTCGGGGACACTTCTCATCGAGGGGACCTGAACCCAGACAAC
AGCGATGTCGACAACTCTTTATCCAACCTGGTTCAGACTTACAATCAGCTTTTTCGAAGAGAACCCGATCAACGCATCCGG
AGTTGACGCCAAAGCAATCCTGAGCGTAGGCTGTCCAAATCCCGGCGGCTCGAAAACCTCATCGCACAGCTCCCTGGGG
AGAAGAAGAACGGCTGTTTGGTAATCTTATCGCCCTGTCACTCGGGCTGACCCCAACTTTAAATCTAATCTCGACCTG
GCCGAAGATGCCAAGCTTCAACTGAGCAAAGACACCTACGATGATGATCTCGACAATCTGCTGGCCAGATCGGCGACCA
GTACGCAGACCTTTTGGCGGCAAGAACCTGTGAGACGCCATTCTGCTGAGTGATATCTGCGAGTGAACACGGAGA
TCACCAAGCTCCGCTGAGCGCTAGTATGATCAAGCGCTATGATGAGCACCACCAAGACTTGACTTTGCTGAAGGCCCTT
GTCAGACAGCAACTGCCTGAGAAGTACAAGGAAATTTTCTTCGATCAGTCTAAAAATGGTACGCGCGATACATTGACGG
CGGAGCAAGCCAGGAGGAATTTTACAATTTTATAAGCCCATCTTGGAAAAATGAGCGGCACCGAGGAGCTGCTGGTAA
AGCTTAACAGAGAAGATCTGTTGCGCAAAACAGCGCACTTTCGACAATGGAAGCATCCCCACCAGATTACCTGGGCGAA
CTGCACGACTATCTCAGGCGCAAGAGGATTTCTACCCCTTTTGAAGATAACAGGGAAGAAAGATTGAGAAATCCTCAC
ATTTCCGATACCTACTATGTAGGCCCTCGCCCGGGGAAATTCAGATTTCGCTGGATGACTCGCAAATCAGAAGAGA
CCATCACTCCCTGGAACCTTCGAGGAAGTCGTGGATAAGGGGGCCTCTGCCAGTCTTTCATCGAAAGGATGACTAATTT
GATAAAATCTGCCTAACGAAAAGGTCTTCTTAAACACTCTCTGCTGTACGAGTACTTACAGTTTATAACGAGCTCAC
CAAGGTCAAAATCGTACAGAAAGGATGAGAAAGCCAGCATCTCTGCTGAGAGCAGAAAGCTATCGTGGACCTCC
TCTTCAAGACGACGACCGGAAAGTTACCGTGAAACAGCTCAAGAAGACTATTTCAAAAAGATTGAATGTTTCGACTCTGTT
GAAATCAGCGAGTGGAGGATCGCTTCAACGCATCCCTGGGAACGTATCACGATCTCCTGAAAATCATTAAGACAAAGGA
CTTCTGAGCAATGAGGAGAACGAGGACATTTCTGAGGACATTGTCTCACCCTTACGTTGTTTGAAGATAGGGAGATGA
TTGAAGACGCTTGAACCTTACGCTCATCTCTTCGACGACAAAGTATGAAACAGCTCAAGAGGCGCCGATATACAGGA
TGGGGCGCGCTGTCAAGAAAATGATCAATGGGATCCGAGACAGAGTGGAAAGACAATCCTGGATTTCCTTAAGTC
CGATGGATTTCGCAACAGGAACCTTCAAGCTGATCCATGATGACTCTCTCACCCTTAAAGGAGGACATCCGACGACAC
AAGTTTCTGGCCAGGGGACAGTCTTACGAGCACATCGCTAATCTTGAGGTAGCCAGCTATCAAAAAGGGAATACTG
CAGACCGTTAAGGTCTGGATGAACCTCGTCAAAGTAATGGGAAGGCATAAGCCCGAGAATATCGTTATCGAGATGGCCG
AGAGAACCAAACTACCCAGAGGGACAGAAGAACAGTAGGGAAAGGATGAAGAGGATTGAAGAGGTATAAAGAAGCTGG
GGTCCCAATCCTTAAGGAACACCCAGTTGAAAACACCCAGCTTCAGAATGAGAAGCTCTACCTGTACTACCTCGAGAAC
GGCAGGGACATCTAGCTGGATCAGGAACCTGGACATCAATCGGCTCTCCGACTACGACGTGGATCATATCGTCCCGAGTC
TTTTCTCAAAGATGATTCTATTGATAATAAAGTGTGACAAGATCCGATAAAAAATAGAGGGAAGAGTGATAACGTCCCT
CAGAAGAAGTTGTCAAGAAAATGAAAATTTATTTGGCGGAGCTGCTGAACGCCAACTGATCACACAACGGAAGTTTCGAT
AATCTGACTAAGGTGAACGAGGTGGCTGTCTGAGTTGGATAAAGCCGGCTTCATCAAAGGCAGCTTGTGTAGACACG
CCAGATCACCAAGCAGTGGCCCAATCTCGATTACGCGATGAACACCAAGTACGATGAAAATGACAACTGATTTCGAG
AGGTGAAAGTTATTACTCTGAAGTCTAAGCTGGTCTCAGATTTCAGAAAGGACTTTTCAGTTTATAGGTGAGAGATC
AACAATTACCACCATGCGCATGATGCCTACCTGAATGCAGTGGTAGGCACTGCACTTATCAAAAAATATCCCAAGCTTGA
ATCTGAATTTGTTTACGGAGACTATAAAGTGTACGATGTTAGGAAAATGATCGCAAAGTCTGAGCAGGAAATAGGCAAGG
CCACCGCTAAGTACTTCTTTTACAGCAATATTATGAATTTTTTCAAGACCGAGATTACACTGGCCAAATGGAGAGATTCCG
AAGCGACCACTTATCGCAACAAACGGAGAAACAGGAGAAATCGTGGGACAAGGGTAGGGATTTCGCGCAGCTCCGAA
GGTCTGTCTTCCATGCGAGGTGAACATCGTTTAAAAGACCGAAGTACAGACCGGAGGCTTCTCAAAGGAAAGTCTCC
CGAAAAGGAACAGCGACAAGCTGATCGCACGCAAAAAGATTGGGACCCCAAGAAATACGGCGGATTTCGATTCTCTACA
GTCGCTTACAGTGTACTGGTTGTGGCCAAAGTGGAGAAAGGGAAGTCTAAAAAATCAAAAGCTCAAGGAAGTCTGGG
CATCACAATCATGGAGCGATCAAGCTTCGAAAAAAACCCCATCGACTTCTCGAGGCGAAAGGATATAAAGAGGTCAAAA
AAGACCTCATCATTAAAGCTTCCCAAGTACTCTCTCTTTGAGCTTGAACCGGCGGAAACGAATGCTCGCTAGTGGGGG
GAGCTGCAGAAAGGTAACGAGCTGGCACTGCCCTCTAAATACGTTAATTTCTTGTATCTGGCCAGCCACTATGAAAAGCT
CAAAGGTCTCCCGAAGATAATGAGCAGAAGCAGCTGTTCTGTGGAACAACACAAACACTACCTTGATGAGATCATCGAGC
AAATAAGCGAATTTCTCAAAAAGAGTGATCCTCGCCGACGCTAACCTCGATAAGGTGCTTTCTGCTTACAATAAGCACAGG
GATAAGCCCATCAGGGAGCAGGCAGAAAACATTATCCACTTGTTTTACTCTGACCAACTTGGGCGCGCTGCAGCCTTCAA
GTACTTCGACACCACCATAGACAGAAAGCGGTACACCTCTACAAGGAGGTCTTGGACGCCACACTGATTTCATCAGTCAA
TTACGGGGCTCTATGAAACAGAATCGACCTCTCTCAGCTCGGTGGAGACAGCAGGGCTGACCCCAAGAAAGAGGAAG
GTGTGAAAGGTTTCGATCCCTACCGGTTAGTAATGAGTTTAAACGGGGGAGGCTAACTGAAACACGGAAGGAGACAATAC
CGGAAGGAACCCGCGCTATGACGGCAATAAAAAGACAGAATAAAACGCACGGGTGTTGGGTGCTTTGTTTCATAAACCGG
GGTTTCGTTCCAGGGCTGGCACTCTGTGATACCCACCGAGACCCCATTTGGGGCCAATACGCCCCGCTTTCTCTCTTTT
CCCCCCCCACCCCAAGTTCCGGTGAAGGCCAGGGCTCGCAGCCAACGTGGGGCGGCGAGGCCCTGCCATAGCAGAT
CTGCGCATGGGCTCTAGGGGGTATCCCCACGCGCCCTGTAGCGGCGCATTAAGCGCGCGGGGTGTGGTGTACGCG
CAGCGTGACCGCTACACTTGCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTTCTCCCTTCTCTTCGCGCAGTTTCGCGG
GCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCAAAAAA
CTTGATTAGGGTGATGGTTACGTAAGTGGGCCATCGCCCTGATAGACGGTTTTTTCGCCCTTTGACGTTGGAGTCCACGTT
CTTTAATAGTGACTCTTGTTCAAACTGGAACAACACTCAACCCATCTCGGTCTATTCTTTGATTTATAAGGATTTT
TGCCGATTTTCGGCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTAATCTGTGGAATGTGTGTC
AGTTAGGGTGTTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGG

TGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCC
CCTAACTCCGCCCATCCCGCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCATGGCTGACTAATTTTTTTTATTT
ATGCAGAGGCCGAGGCCGCTCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCCTAGGCTTTTGC
AAAAAGCTCCCGGAGCTTGTATATCCATTTTCGGATCTGATCAAGAGACAGGATGAGGATCGTTTTCGCATGATTGAACA
AGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACACAGACAATCGGCT
GCTCTGATGCCCGCTGTTCGGCTGTGAGCGCAGGGGCGCCCGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTG
AATGAATTCAGGACGAGGCAGCGCGCTATCTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTCTGACGTTGT
CACTGAAGCGGGAAGGACTGGCTGCTATTGGCGAAGTGCCGGGCGAGGATCTCCTGTCATCTCACCTTGTCTCTGCCG
AGAAAGTATCCATCATGGCTGATGCAATGCGGCGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCG
AAACATCGCATCGAGCGAGCAGTACTCGGATGGAAGCCGCTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGG
GCTCGCGCCAGCCGAAGTGTTCGCCAGGCTCAAGGCGCGCATGCCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATG
CCTGCTTGCCGAATATCATGGTGGAATGGCCGCTTTTCTGGATTTCGACTGTGGCCGGCTGGGTGTGGCGGACCGC
TATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGCGAATGGGCTGACCGCTTCTCGTGCTTTA
CGGTATCGCCGCTCCCGATTTCGAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTT
CGCGAAATGACCGACCAAGCGACGCCAACCTGCCATCAGGAGATTTCGATTCCACCGCCGCTTCTATGAAGGTTGGG
CTTCGGAATCGTTTTCCGGGACGCCGCTGGATGATCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCCACCCCA
ACTTGTTTATTGCGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTTCACTG
CATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGTATACCGTCGACCTCTAGCTAGAGCTTGGC
GTAATCATGGTCATAGCTGTTTCTGTGTGAATTTGTTATCCGCTCACAAATCCACACAACATACGAGCCGGAAGCATAA
AGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATGCGTTGCGCTCACTGCCCCGCTTTCAGTCGGGA
AACCTGTCTGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCATTTGGGCGCTCTTCGCTTC
CTCGTCACTGACTGCTGCGCTCGGCTCGGTCGCTGCGCGGAGCGGTATCAGCTCACTCAAAGCGGTAATACGGTTAT
CCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCG
TTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC
GACAGGACTATAAAGATACAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCGACCCCTGCCGCTTACCG
GATACCTGTCCGCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCAGGCTGTAGGTATCTCAGTTCCGGTGTAG
GTCGTTTCGCTCAAGCTGGCTGTGTGCACGAACCCCGCTTACGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTT
TGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAG
GCGGTGTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTG
AAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAACAAACACCGCTGGTAGCGGTGGTTTTTTTGT
TTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGT
GGAACGAAAACTCAGTTAAGGATTTTGGTCAAGATTATGAGATTACAAAAAGGATCTTACCTAGATCTTTTAAATTA
TGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTAT
CTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTAC
CATCTGGCCCCAGTGCTGCATGATACCGCGAGACCCACGCTCACCAGGCTCCAGATTTATCAGCAATAAACCAGCCAGCC
GGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGT
AAGTAGTTTCGCCAGTTAATAGTTTGGCACAAGTTGTGCGCATGTTGCTGACAGGCATCGTGGTGTACAGCTCGTTTGGTA
TGGCTTCATTAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGTTAGCTCC
TTCGGTCTCCGATCGTTGTCAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCT
TACTGTGATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATCTGTAGAATAGTGTATGCGGC
GACCGAGTTGCTCTTGGCCGGCGTCAATACGGGATAATACCGGCCACATAGCAGAACTTTAAAGTGCTCATCATTTGGA
AAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGTGTGTGAGATCCAGTTTCGATGTAACCCACTCGTGCACCCAA
CTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAA
TAAGGGCGACACGGAATGTTGAATACTCATACTCTTCTTTTCAATATTATGAAGCATTATCAGGGTTATTGTCTC
ATGAGCGGATACATATTTGAATGTATTAGAAAAATAACAAATAGGGGTTCCGCGCACATTTCCCGGAAAAGTGCCACC
TGACGTCGACGGATCGGGAGATCTCCCGATCCCTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAG
CCAGTATCTGCTCCCTGCTTGTGTGGAGGTGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCT
TGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATGTACGGCCAGATATAC

Supplementary Figure 18. Map and nucleotide sequence of expression plasmid AU28_pCAG.Cas9^{D10A}. CAG, hybrid promoter (CMV enhancer, human cytomegalovirus *immediate early* enhancer; chicken β -actin promoter; chimeric intron, fusion between introns from the chicken β -actin and rabbit β -globin genes); Cas9, human codon-optimized Cas9 (Csn1) ORF coding for the endonuclease nicking variant D10A from the *Streptococcus pyogenes* type II CRISPR/Cas system; SV40 NLS, nuclear localization signal SV40 large T antigen; HSV TK poly(A) signal, herpes simplex virus *thymidine kinase* polyadenylation signal; NeoR/KanR, transposon Tn5 *aminoglycoside phosphotransferase* conferring resistance to the antibiotics G418 (geneticin) and kanamycin; AmpR, β -lactamase ampicillin resistance gene; ori, high-copy number ColE1 prokaryotic origin of replication.



>AV15_pCAG.Cas9.gRNA^{S1} (9450 bp)

```

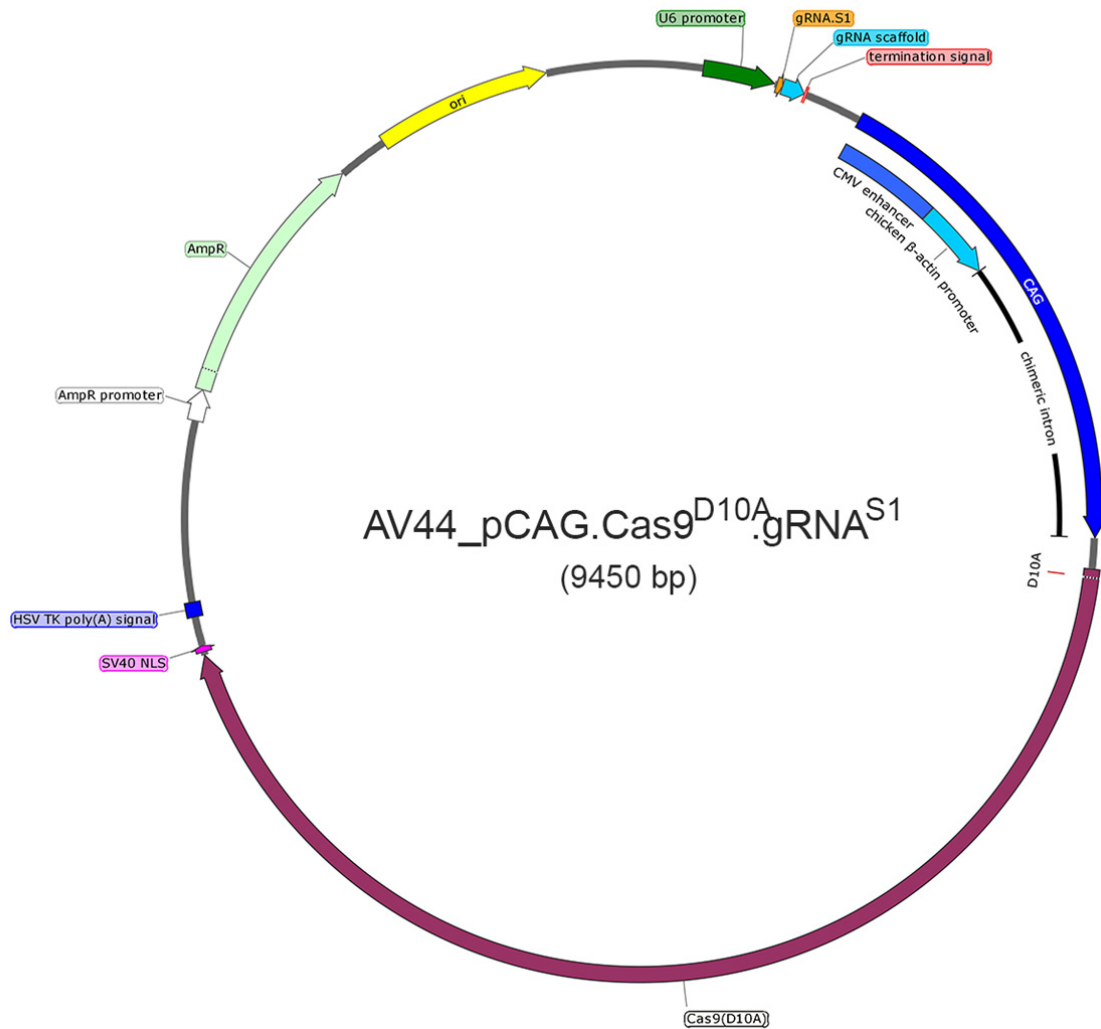
ATGTTATTCTCTTATTTTAAAGCTTAAGGTGCACGGCCACGTGGCCACTAGTACTTCTCGAGCTCTGTACATGTCCG
CGGTCCGACGTACGCGTATCGATGGCGCCAGCTGCAGGCGGCCCATATGCATCCTAGGCCTATTAATATTCGGAGT
ATACGTAGCCGGCTAACGTTGACTGGATCCGGTACCAAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCTTCATA
TTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATA
CGTGACGTAGAAAATAAATTTCTTGGGTAGTTGCAGTTTAAATTTATGTTTTAAATGGACTATCATATGCTTACC
GTAAGTTGAAAGTATTTTCGATTTCTTGGCTTTATATATCTTGTGAAAGGACGAAACACCGGGGCCACTAGGGACAGGAT
GTTTTAGAGCTAGAAATAGCAAGTTAAATAAGGCTAGTCCGTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT
TTTCTAGACCCAGCTTTCTTGTACAACAACCGGTACCTCTAGAACTATAGCTAGCATGCGCAAATTTAAAGCGCTGATAT
CGATCGCGCGCAGATCTGTCTATGATGATCATTGCAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTC
GCGATGTACGGGCCAGATATATCtcgagccatgattcGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGG
GTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAAATTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACG
ACCCCGCCCATTTGACGTCAATAATGACGTATGTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGGTG
GAGTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGA
CGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAG
TCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCATCTCCCCCCCCCTCCCCACCCCAAT
TTTGTATTATTATTTTAAATTATTTTGTGACAGCATGGGGGCGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGG
CGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCTTT
TATGGCGAGGCGGGCGGGCGGGCGGCCCTATAAAAGCGAAGCGCGCGGGCGGGGAGTCGCTGCGTTGCTTCGCCCC
GTGCCCCGCTCCGCGCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCACAGGTGAGCGGGCGGGG
GGCCCTTCTCTCCGGGCTGTAATTAGCGCTTGGTTTAAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAA
AGGGCTCCGGGAGGGCCCTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCCTGTGTGTGCGTGGGGAGCGCGCGG
TGCGGCGCGCGCTGCCCGCGGCTGTGAGCGCTGCGGGCGCGCGCGGGGCTTGTGCGCTCCGCGTGTGCGGAGGGGA
GCGCGGGCGGGGGCGGTGCCCGCGGCTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGG
GGTGAGCAGGGGGTGTGGGCGCGGGGTCGGGCTGTAACCCCCCTGCACCCCCCTCCCGAGTTGCTGAGCACGGGCC
GGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGGGGCTGCCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGTGC
CGGGCGGGGCGGGGCCCTCGGGCGGGGAGGGCTCGGGGAGGGGCGCGCGGCCCGGAGCGCGGGCGGCTGTCTGAG

```

GCGCGGCGAGCCGCGACCCATTGCCTTTTATGGTAATCGTGCAGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGG
AGCCGAAATCTGGGAGGCGCGCCGCGACCCCTTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGCGAGGAAGGAAATGG
GCGGGGAGGGCCTTCGTGCGTCGCCGCGCGCGCTCCCTTCTCCATCTCCAGCCTCGGGGCTGCCGCGAGGGGACGGCT
GCCTTCGGGGGGGACGGGCGAGGGCGGGTTCGGCTTCTGGCGTGTACCGGGCGGCTCTAGAGCCTCTGCTAACCATGTT
CATGCCTTCTTCTTTTCTACAGCTCCTGGGCAACGTGCTGGTTGTGTGCTGTCTCATCATTTTGGCAAAGAATtAtc
gcatgcctgcagagctctagagTCCTAGAGGATCGAACCCTTgccaccATGGACAAGAAGTACTCCATTGGGCTCGATAT
CGGCACAAACAGCGTCGGCTGGGCCGTCAATTACGGACGAGTACAAGGTGCCGAGCAAAAAATTCAAAGTTCTGGGCAATA
CCGATCGCCACAGCATAAAGAAGAACCTCATTGGCGCCCTCCTGTTGCGACTCCGGGGAGACGGCCGAAGCCACGCGGCTC
AAAAGAACAGCACGGCGCAGATATACCCGCGAGAAAGATCGGATCTGCTACCTGCAGGAGATCTTTAGTAATGAGATGGC
TAAGGTGGATGACTCTTTCTCCATAGGCTGGAGGAGTCTTTTGGTGGAGGAGGATAAAAAGCAGAGCGCCACCCAA
TCTTTGGCAATATCGTGGACGAGGTGGCGTACCATGAAAAGTACCCAACCATATATCATCTGAGGAAGAAGCTTGTAGAC
AGTACTGATAAAGGTGACTTGGGTTGATCTATCTCGCGTGGCGCATATGATCAAATTCGGGGACACTTCCTCATCGA
GGGGGACCTGAACCCAGACAACAGCGATGTGCAAACTCTTTATCCAAGTTCAGACTTACAATCAGCTTTTCGAAG
AGAACCCGATCAACGCTCCGGAGTTGACGCCAAAGCAATCCTGAGCGCTAGGCTGTCCAAATCCCGGGCGGCTCGAAAAC
CTCATCGCACAGCTCCCTGGGGAGAAGAAGAACGGCCTGTTTGGTAATCTTATCGCCCTGTCACTCGGGCTGACCCCAA
CTTTAAATCTAACTTCGACCTGGCCGAAGATGCCAAGCTTCAACTGAGCAAAGACACCTACGATGATGATCTCGACAATC
TGCTGGCCAGATCGGCGACAGTACGCAGACCTTTTTTGGCGGCAAGAACCTGTACAGCGCCATTCTGCTGAGTGAT
ATTCTGCGAGTGAACACGGAGATCACCAAGCTCCGCTGAGCGCTAGTATGATCAAGCGCTATGATGAGCACCACCAAGA
CTTGACTTTGCTGAAGGCCCTTGTGACACAGCAACTGCCTGAGAAGTACAAGGAAATTTTCTTCGATCAGTCTAAAAATG
GCTACGCCGATACATTGACGGCGGAGCAAGCCAGGAGGAAATTTTACAAATTTATTAAGCCCATCTTGGAAAAAATGGAC
GGCACCAGGAGGCTGCTGGTAAAGCTTAACAGAGAAGATCTGTTGCGCAACAGCGCACTTTTCGACAATGGAAGCATCC
CCACCAAGCTATCGTGGCGAAGTGCACGCTATCTCAGCGCGCAAGAGGATTCTACCCCTTTTTTGAAGAAATACAGGG
AAAAGATTGAGAAAACTCTCACATTTTCGGATACCTACTATGTAGGCCCCCTCGCCCGGGGAAATTCAGATTTCGCGTGG
ATGACTCGCAATCAGAAGAGACCATCACTCCCTGGAACCTTCGAGGAAGTCGTGGATAAGGGGGCTCTGCCAGTCCCT
CATCGAAAGGATGACTAACTTTGATAAAAATCTGCCTAACGAAAAGGTGCTTCTTAAACACTCTCTGCTGTACGAGTACT
TCACAGTTTATAACGAGCTCACCAAGGTCAAATACGTACAGAAAGGATGAGAAAGCCAGCATTCCTGTCTGGAGAGCAG
AAGAAAGCTATCGTGGAGCTCTCTTCAAGACGAACCGGAAGTTACCGTGAACAGCTCAAAGAAGAGCTTTTCAAAAA
GATTGAATGTTTCGACTCTGTTGAAATCAGCGGAGTGGAGGATCGCTTCAACGCATCCCTGGGAACGTATCACGATCTCC
TGAAAATCATTAAAGACAAGGACTTCCTGGACAATGAGGAGAACGAGGACATTCTTGAGGACATTGTCTCACCTTACG
TTGTTTGAAGATAGGAGATGATTGAAGAACGCTTGAAAATCTACGCTCATCTCTTCGACGACAAAGTCATGAACAGCT
CAAGAGGCGCGCATATACAGGATGGGGGCGGCTGTCAAGAAAACTGATCAATGGGATCCGAGACAAGCAGAGTGGAAAGA
CAATCTCGATTTTCTTAAAGTCCGATGGATTGTCACCCGAACTTTCATGCAGTTGATCCATGATGACTCTCTCACTTT
AAGGAGGACATCCAGAAAGCACAAAGTTTCTGGCCAGGGGACAGTCTTCACGAGCACATCGCTAATCTTGCAGGTAGCCC
AGCTATCAAAAAGGAATACTGCAGACCGTTAAGGTCTGGATGAACCTCGTCAAAGTAAATGGGAAGGCATAAGCCCGAGA
ATATCGTTATCGAGATGGCCCGAGAGAACCAACTACCCAGAAGGGACAGAAAGACAGTAGGGAAAGGATGAAGAGGATT
GAAGAGGGTATAAAAAGAACTGGGGTCCCAATCCTTAAGGAACACCCAGTTGAAAACACCCAGCTTCAGAATGAGAAGCT
CTACCTGTACTACCTGCAGAACGGCAGGGACATGTACGTGGATCAGGAAGTGCATCAATCGGCTCTCCGACTACGAGC
TGGATCATATCGTGCCCGAGTCTTTTCTCAAAGATGATTCTATTGATAATAAAGTGTGACAAGATCCGATAAAAAATAGA
GGGAAGAGTGATAACGTCCCTCAGAAGAAGTTGTCAAGAAAATGAAAAATTATTGGCGGCGAGTGTGAACGCCAACT
GATCACACAACGGAAGTTTCGATAATCTGACTAAGGCTGAACGAGGTGGCCTGTCTGAGTTGGATAAAGCCGGCTTCATCA
AAAGGCAGCTTGTGAGACACGCCAGATCACCAAGCACGTGGCCCAATTCCTGATTACGCATGAACACCAAGTACGAT
GAAAATGACAAACTGTTCGAGAGGTTGAAAGTTATTACTCTGAAGTCTAAGCTGGTCTCAGATTTTCAGAAAGGACTTTCA
GTTTTATAAGGTGAGAGAGATCAACAATTACCACCATGCGCATGATGCCTACCTGAATGCAGTGGTAGGCACTGCACCTTA
TCAAAAAATATCCCAAGCTTGAATCTGAATTTGTTTACGGAGACTATAAAGTGTACGATGTTAGGAAAATGATCGCAAAG
TCTGAGCAGGAAATAGGCAAGGCCACCGCTAAGTACTTCTTTTACAGCAATATATGAATTTTTTCAAGACCGAGATTAC
ACTGGCCAATGGAGAGATTTCGAAGCGACCTTATCGAAGAAACCGGAGAAACAGGAGAAATCGTGTGGGACAAGGGTA
GGGACTTTCGCGACAGTCCGGAAGGTCTGTCCATGCCCGAGGTGAACATCGTTAAAAAGACCGCAAGTACAGCAAGGAGGC
TTCTCCAAGGAAAGTATCTCCCGAAAAGGAACAGCGACAAGCTGATCGCACGCAAAAAAGATTGGGACCCCAAGAAATA
CGGCGGATTGATCTCTCTACAGTCTGCTTACAGTGTACTGGTTGTGGCCAAAGTGGAGAAAGGGAAGTCTAAAAACTCA
AAAGCGTCAAGGAAGTGTGGGCATCACAATCATGGAGCGATCAAGCTTCGAAAAAACCCTATCGACTTTCTCGAGGCG
AAAGGATATAAAGAGGTCAAAAAAGACCTCATCATTAAGCTTCCCAAGTACTCTCTCTTTGAGCTTGAACCGGCCGGAA
ACGAATGCTCGCTAGTGCGGGCGAGCTGCAGAAAGGTAACGAGCTGGCACTGGCCTCTAAATACGTTAATTTCTTGTATC
TGGCCAGCCACTATGAAAAGCTCAAAGGGTCTCCCGAAGATAATGAGCAGAAGCAGCTGTTCTGTTGAACAACACAAACAC
TACCTTGATGAGATCATCGAGCAAATAAGCGAATCTCCAAAAGAGTGATCCTCGCCGACGCTAACCTCGATAAGGTGCT
TTCTGCTTACAATAAGCACAGGGATAAGCCCATCAGGGAGCAGGCAGAAACATATCCACTTGTCTTACTCTGACCAACT
TGGGCGCGCCTGCAGCCTTCAAGTACTTCGACACCACATAGACGAGAAAGCGGTACACCTCTACAAAGGAGGTCTGGAC
GGCAGCTGATTCTCAGTCAATTACGGGGCTCTATGAAACAAGAAATCGACCTCTCTCAGCTCGGTGGAGACAGCGGC
TGACCCCAAGAAGAAGAGGAAGGTGTGAAAGGGTTCGATCCCTACCGGTTAGTAATGAGTTTAAACGGGGGAGGCTAACT
GAAACACGGAAGGAGACAATACCGGAAGGAACCCGCGCTATGACGGCAATAAAAAGACAGAATAAAACGCACGGGTGTTG
GGTCGTTTGTTCATAAACCGGGGGTTCGGTCCCAGGGCTGGCACTGTGCGATACCCACCGAGACCCCATTTGGGGCCAA
TACGGCCGCGTTTCTCTTTTCCCCACCCACCCCAAGTTTCGGGTGAAGGCCAGGGCTCGCAGCCAACGTTCGGGCG
GGCAGGCCCTGCCATGCGAGATCTGCCAGCTGGGGCTTATAGGGGTATCCCCACGCGCCCTGTAGCGCGGATTAAGCG
CGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCAGCGCCCTAGCGCCCGGGGTATAAATTACCTCAGGT
CGACGTCCCATGTGCAGGTGCTGAATTCGAAGGCATTCCAGCAGCTGGTCACCAGCCATGGCCATGTCCAATCCATCAA
ATCAGCTTGAGTAGCCATGCCATGGCTCCAGCTGTCTCGTACCAGCTTTGTTCCTTTAGTGAGGGGTAAATTCGAG
CTTGCGGTAAATCATGGTCATAGCTGTTTCCaattCTTGAAGACGAAAGGGCCTCGTGATACGCCATATTTTATAGGTTAA
TGTATGATAAATAATGGTTTCTTAGACGTCAAGTGGCACTTTTCGGGGAATGTGCGCGGAACCCCTATTTGTTTATTTT
TCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGT

ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCTTTTTTGCGGCATTTCCTTCCTGTTTTTGCTCACCAGAAAC
 GCTGGTGAAAGTAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAAG
 TCCTTGAGAGTTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCC
 CGTGTTCAGCGCCGGGCAAGAGCAACTCGGTGCGCGCATACACTATTCTCAGAACTGACTTGGTTGAGTACTCACCAGTCAC
 AGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTCTGCCATAACCATGAGTGATAACACTGCGGCCA
 ACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGACAACATGGGGGATCATGTAACCTCGCCTT
 GATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAAC
 GTTGCGCAAACTATTAACCTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAG
 TTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCT
 CGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAAC
 TATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACCTGTGACACCAAGTTTACT
 CATATATACTTTAGATTGATTAAAACTTCATTTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTTGATAATCTCATG
 ACCAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCC
 TTTTTTTCTGCGCGTAATCTGCTGCTGCAAAACAAAAAACACCGCTACCAGCGGTGGTTTGTTCGCCGATCAAGAGC
 TACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAACTACTGTCTTCTAGTGATAGCCGTAGTTA
 GGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGG
 CGATAAGTCGTGCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTTCGGGCTGAACGGGGGGTT
 CGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACG
 CTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGG
 GGGAAACGCTGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAG
 GGGGGCGGAGCCTATGGAACACGCCAGCAACGCGGCCTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATG
 TTCTTTCTGCGTTATCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCGAGCCG
 AACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCG
 GTATTTACACCGCATAGTAAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTGGAGCTTGGCGTAA
 TCATGGTCAT

Supplementary Figure 19. Map and nucleotide sequence of expression plasmid AV15_pCAG.Cas9gRNA^{S1}. CAG, hybrid promoter (CMV enhancer, human cytomegalovirus *immediate early* enhancer; chicken β -actin promoter; chimeric intron, fusion between introns from the chicken β -actin and rabbit β -globin genes); Cas9, human codon-optimized Cas9 (Csn1) ORF coding for the endonuclease from the *Streptococcus pyogenes* type II CRISPR/Cas system; SV40 NLS, nuclear localization signal SV40 large T antigen; HSV TK poly(A) signal, herpes simplex virus *thymidine kinase* polyadenylation signal; NeoR/KanR, transposon Tn5 *aminoglycoside phosphotransferase* conferring resistance to the antibiotics G418 (geneticin) and kanamycin; AmpR, β -lactamase ampicillin resistance gene; ori, high-copy number ColE1 prokaryotic origin of replication; U6 promoter, RNA polymerase III promoter for human snRNA; gRNA.S1, *AAVS1*-targeting gRNA^{S1} spacer; termination signal, RNA polymerase III termination signal.



```

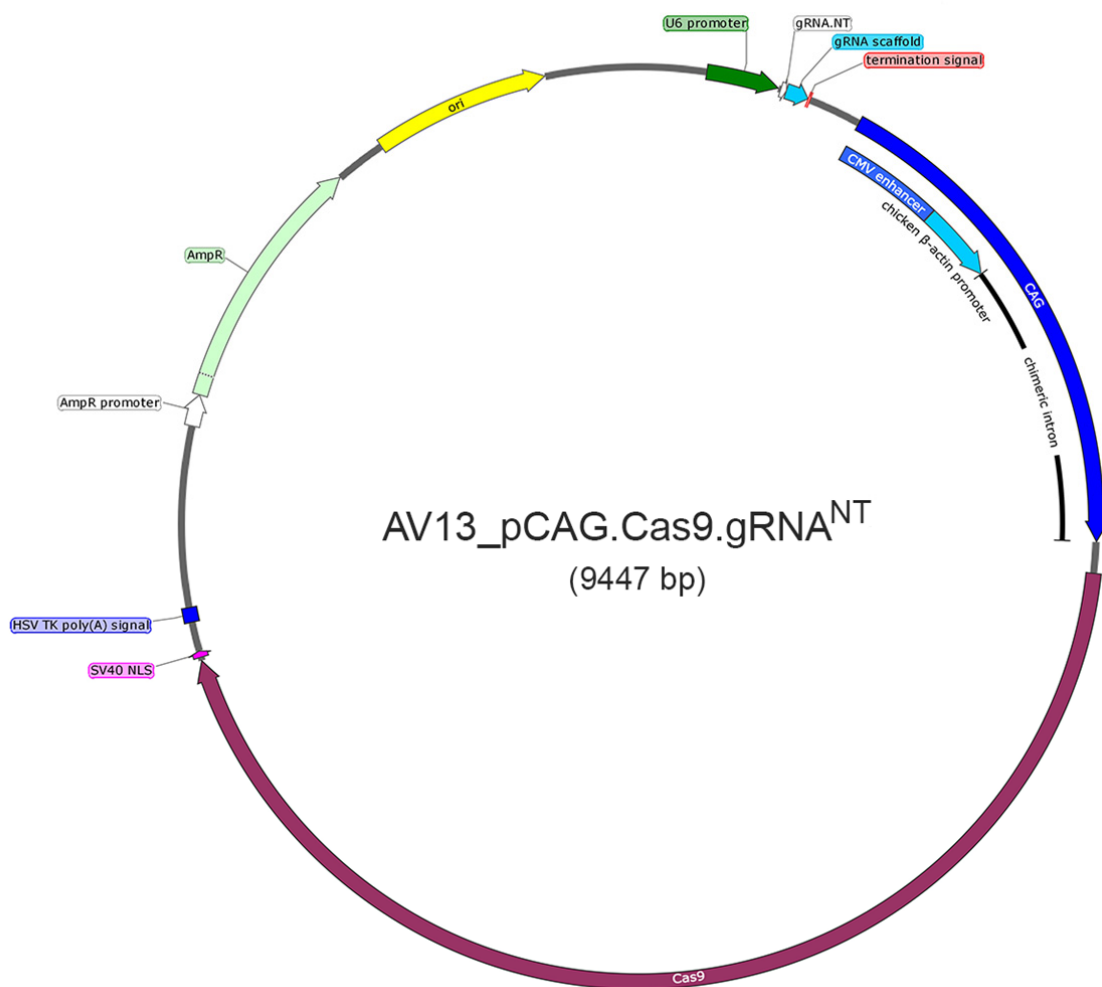
>AV44_p.CAG.Cas9D10A.gRNAS1 (9450 bp)
ATGTTATTCCTCCTTATTTAAAGCTTAAGGTGCACGGCCACGTGGCCACTAGTACTTCTCGAGCTCTGTACATGTCCG
CGGTCGCGACGTACGCGTATCGATGGCGCCAGCTGCAGGCGGCCGCCATATGCATCCTAGGCCTATTAATATTCGGAGT
ATACGTAGCCGGCTAACGTTGACTGGATCCGGTACCAAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCTTTCATA
TTTGATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATA
CGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTGCAGTTTAAATATATGTTTTAAATGGACTATCATATGCTTACC
GTAAC TTGAAAGTATTTTCGATTTCTTGGCTTTATATATCTTGTGGAAGGACGAAACACCGGGGCCACTAGGGACAGGAT
GTTTTAGAGCTAGAAATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT
TTTCTAGACCCAGCTTTCTTGTACAACAACCGGTACCTCTAGAATATAGCTAGCATGCGCAAATTTAAAGCGCTGATAT
CGATCGCGCGCAGATCTGTCTATGATGATCATTGCAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTC
GCGATGTACGGGCCAGATATACTCGAGCCATGGATTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGG
GTCATTAGTTTCATAGCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACG
ACCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTG
GAGTATTTACGGTAACTGCCCCTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGA
CGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCTTACTTGGCAGTACATCTACGTATTAG
TCATCGCTATTACCATGGGTGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCAAT
TTTGTATTATTTATTTTAAATTATTTTGTGTCAGCGATGGGGGCGGGGGGGGGGGGGCGCGCGCAGGCGGGGCGGGG
CGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTT
TATGGCGAGGCGGGCGGGCGGGCGGCCCTATAAAAGCGAAGCGCGCGGGCGGGGAGTCGCTGCGTGTGCTTTCGCCCC
GTGCCCCGCTCCGCGCGCCCTCGCGCCGCGCCCGCGGCTCTGACTGACCGCGTTACTCCACAGGTGAGCGGGCGGGGAC
GGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTAATGACGGCTCGTTTCTTTCTGTGGCTGCGTGAAAGCCTTAA
AGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGTGCTGTGTGCTGTGCTGTGCTGTGCTGTG
TGCGGCGCGCGCTGCCGCGGCGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGGAGGGGA
GCGCGGCGGGGGCGGTGCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGTGCGGGGTGTGTGCTGTGCGG
GGTGAGCAGGGGTGTGGGCGCGCGGTGCGGCTGTAACCCCCCTGCACCCCCCTCCCGAGTTGCTGAGCACGGCCC

```

GGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGC
CGGGCGGGGCGGGGCGCCCTCGGGCGGGGAGGGCTCGGGGAGGGGCGCGGGCGCCCCGGAGCGCGGGCTGTTCGAG
GCGCGGCGAGCCGCGAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGCGCG
AGCCGAAATCTGGGAGGCGCGCGCCGACCCCCCTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGCGAGGAAGGAAATGG
GCGGGGAGGGCCCTTCGTGCGTCGCGCGCGCGCGCTCCCTTCTCCTATCTCCAGCCTCGGGGCTGCCGCGAGGGGGACGGCT
GCCTTCGGGGGGGACGGGGCAGGGCGGGGTTTCGGCTTCTGGCGTGTGACCGGCGGGCTCTAGAGCCTCTGCTAACCATTGTT
CATGCCCTCTTCTTTTCCATAGCTCCTGGGCAACGTGCTGGTTGTGTGCTGTCTCATCATTTTTGGCAAAGAAATATC
GCATGCCTGCAGAGCTCTAGAGTCTAGAGGATCGAACCCTTGCCACCATTGGACAAGAAGTACTCCATTGGGCTCGCTAT
CGGCACAAACAGCGTCGGCTGGGCCGTATTACGGACGAGTACAAGGTGCCGAGCAAAAATTCAAAGTTCTGGGCAATA
CCGATCGCCACAGCATAAAGAAGAACCTCATTGGCGCCCTCCTGTTGACTCCGGGAGACGGCCGAAGCCACGCGGCTC
AAAAGAACAGCACGGCGCAGATATACCCGAGAAAGAATCGGATCTGCTACCTGCAGGAGATCTTTAGTAATGAGATGGC
TAAGGTGGATGACTCTTTCTTCCATAGGCTGGAGGAGTCTTTTGGTGAGGAGGATAAAAAGCAGAGCGCCACCCAA
TCTTTGGCAATATCGTGGACGAGGTGGCGTACCATGAAAAGTACCCAACCATATATCATCTGAGGAAGAAGCTTGTAGAC
AGTACTGATAAGGCTGACTTGCCTGTATCTCTCGCTGGCGCATATGATCAAATTTGGGGACACTTCTCATCGA
GGGGGACTGAACCCAGACACAGCGATGTGACAAACTCTTATCCAAGTGGTTCAGACTTACAATCAGCTTTTCGAAG
AGAACCCGATCAACGCATCCGGAGTTGACGCCAAAGCAATCCTGAGCGCTAGGCTGTCCAAATCCCGGGCGGCTCGAAAC
CTCATCGCACAGCTCCCTGGGGAGAAGAAGACGGCCTGTTGGTAATCTTATCGCCCTGTCACCTGGGCTGACCCCAA
CTTTAAATCTAACTTCGACCTGGCCGAAGATGCCAAGCTTCAACTGAGCAAAGACACCTACGATGATGATCTCGACAATC
TGCTGGCCGAGATCGGCGACAGTACGACAGCTTTTTTGGCGGCAAGAACCTGTGACAGCCATTCTGCTGAGTGAT
ATTCTGCGAGTGAACACGGAGATCACCAAAGCTCCGCTGAGCGCTAGTATGATCAAGCGCTATGATGAGCACCACCAAGA
CTTGACTTTGCTGAAGGCCCTTGTGACAGCAACTGCCTGAGAAAGTACAAGGAAATTTCTTCGATCAGTCTAAAAATG
GCTATCGCAGATGACTTACGGCGGAGCAAGCCAGGAGTAATTTACAAATTTATTAAGCCCATCTTGGAAAGAAATGGAC
GGCACCAGGAGCTGCTGGTAAAGCTTAACAGAGAAGATCTGTTGCGCAAACAGCGCACTTTTCGACAATGGAAGCATCCC
CCACCAGATTACCTGGGCGAACTGCACGCTATCCTCAGCGGCAAGAGGATTCTACCCCTTTTGAAGATAACAGGG
AAAAGATTGAGAAATCCTCACATTTTCGGATACCTACTATGTAGGCCCCCTCGCCCGGGGAAATTCAGATTTCGCGTGG
ATGACTCGCAAATCAGAAGAGACCATCACTCCCTGGAATTCGAGGAAGTCTGGATAAGGGGGCCTCTGCCAGTCCCT
CATCGAAGGATGACTAACTTTGATAAAAATCTGCCTAACGAAAGGTGCTTCTTAACACTCTCTCTGATGAGTACT
TCACAGTTTATAACGAGCTCACCAAGGTCAAATACGTACAGAAAGGATGAGAAAGCCAGCATTCCTGTCTGGAGAGCAG
AAGAAAGCTATCGTGGACCTCCTCTTCAAGACGAACCGGAAAGTTACCGTGAAACAGCTCAAAGAAGACTATTTCAAAA
GATTGAATGTTTTGACTCTGTTGAAATCAGCGGAGTGGAGGATCGCTTCAACGCATCCCTGGGAACGTATCAGGATCTCC
TGAAATCATTAAGACAAGGACTTCTTGACAATGAGGAGAACGAGGACATTCTTGAGGACATTGTCTCACCTTTACG
TTGTTTGAAGATAGGAGATGATTGAAGAAGCTTGAAACTTACGCTCATCTCTTCGACGACAAGGATGAAACAGCT
CAAGAGGCGCGGATATACAGGATGGGGGCGGCTGTCAAGAAAATGATCAATGGGATCCGAGACAAGCAGAGTGGAAGA
CAATCCTGGATTTTCTTAAGTCCGATGGATTTGCCAACAGGAATTCATGCAGTTGATCCATGATGACTCTCTCACCTTT
AAGGAGGACATCCAGAAAGCACAAGTTTCTGGCCAGGGGACAGCTTTCACGAGCACATCGCTAAATCTTGCAGGTAGCCC
AGCTATCAAAAAGGAATCTGCAGACCGTTAAGGTCTGGATGAACTCGTCAAAGTAATGGGAAGGCATAAGCCCCGAGA
ATATCGTTATCGAGATGGCCCGAGAGAACCAAACTACCCAGAAAGGACAGAAAGACAGTAGGGAAGGATGAGAGGATT
GAAGAGGGTATAAAAAGAACTGGGGTCCCAATCCTTAAGGAACACCCAGTTGAAAACACCCAGCTTCAGAATGAGAAGCT
CTACCTGTACTACCTGCAGAACGGCAGGGACATGTACGTGGATCAGGAAGTGGACATCAATCGGCTCTCCGACTACGACG
TGGATCATATCGTGCCCCAGTCTTTTCTCAAAGATGATTCTATTGATAATAAAGTGTTGACAAGATCCGATAAAAATAGA
GGGAAGAGTGATAACGTCCCTCAGAAGAAGTTGTCAAGAAAATGAAAATTTATTTGGCGGCGAGCTGCTGAACGCCAACT
GATCACACAACCGGAAGTTTCGATAATCTGACTAAGGCTGAACGAGGTGGCCTGTCTGAGTTGGATAAAGCCGGCTCATCA
AAAGGCAGCTTGTGAGACACGCCAGATCACCAAGCACGTGGGCCAAATTTCTCGATTACGCATGAACACCAAGTACGAT
GAAAATGACAACTGATTTCGAGAGGTGAAAGTTATTACTCTGAAGTCTAAGCTGGTCTCAGATTTTCAGAAAGGACTTTCA
GTTTTATAAGGTGAGAGAGATCAACAATTACCACCATGCGCATGATGCCTACCTGAATGCAGTGGTAGGCACTGCACCTTA
TCAAAAATATCCCAAGCTTGAATCTGAATTTGTTTACGGAGACTATAAAGTGTACGATGTTAGGAAAATGATCGCAAG
TCTGAGCAGGAAATAGGCAAGGCCACCGCTAAGTACTCTTTTACAGCAATATTATGAATTTTTTCAAGACCGAGATTAC
ACTGGCCAATGGAGAGATTTCGAAGCGACCACTTATCGAAACAAACGGAGAAACAGGAGAAATCGTGTGGGACAAGGGTA
GGGATTTTCGCGACAGTCCGGAAGGTCTGTCCATGCCGAGGTGAACATCGTTAAAAGACCGAAGTACAGACCGGAGGC
TTCTCCAAGGAAAGTATCTCCCGAAAAGGAACGCGACAGCTGATCGCACGCAAAAAGATTGGGACCCCAAGAAATA
CGGCGGATTTCGATTCTCTACAGTCGCTTACAGTGTACTGGTTGTGGCCAAAGTGGAGAAAGGGAAGTCTAAAAAACTCA
AAAGCGTCAAGGAAGCTGCTGGGCATCACAATCATGGAGGATCAAGCTTCGAAAAAAACCCCATCGACTTTCTCGAGGCG
AAAGGATATAAAGAGGTCAAAAAGACCTCATCATTAAGCTTCCCAAGTACTCTCTCTTTGAGCTTGAAAACGGCCGGAA
ACGAATGCTCGTAGTGCGGGCGAGCTGCAGAAAGGTAACGAGCTGGCACTGCCCTCTAAATACGTTAATTTCTGTATC
TGGCCAGCCACTATGAAAAGCTCAAAGGGTCTCCCGAAGATAATAGAGCAGAAGCAGCTGTTCGTGGAACAACACAAACAC
TACCTTGTAGATCATCGAGCAATAAAGCGAATTTCTCCAAAAGTGATCTCTCGCCGACGCTAACCTCGATAAGGTGCT
TTCTGCTTACAATAAGCACAGGGATAAGCCCATCAGGGAGCAGGCAGAAAACATTATCCACTTGTCTTACTCTGACCAACT
TGGGCGCGCCTGCAGCCTTCAAGTACTTCGACACCACCATAGACAGAAAGCGGTACACCTCTACAAAAGGAGGTCTGGAC
GCCACACTGATTATCAGTCAATTACGGGGCTCTATGAAACAAGAATCGACCTCTCTCAGCTCGGTGGAGACAGCAGGGC
TGACCCCAAGAAGAGAGGAAGGTGTGAAAGGGTTCGATCCCTACCGGTTAGTAATGAGTTTAAACGGGGGAGGCTAACT
GAAACACGGAAGGAGACAATACCGGAAGGAACCCGCGCTATGACGGCAATAAAAAGACAGAATAAAACGCACGGGTGTTG
GGTCGTTTGTTCATAAAGCAGGGGTTTCGGTCCAGGGCTGGCACTCTGTCTGATACCCACCGAGACCCCATTTGGGCCAA
TACGCCCGCGTTTCTTCTTTTCCCCACCCACCCCAAGTTTCGGGTGAAGGCCAGGGCTCGCAGCCAACGTCGGGGC
GGCAGGCCCTGCCATAGCAGATCTGCGCAGCTGGGGCTCTAGGGGGTATCCCCACGCGCCCTGTAGCGGCGCATTAAAGCG
CGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGGGGTTATAATTACCTCAGT
CGACGTCCTCATGTGACAGGTGCTGAATTCGAAGGCATTCACGACGCTGGTCACCAGCCATGGCCATGTCCAACCTCCATCA
ATCAGCTTGAGTAGCCATGCCCATGGCTCCAGCCTGTCTCTGATACAGCTTTTGTCCCTTTAGTGAGGGGTAATTTCCGAG
CTTGGCGTAATCATGGTCATAGCTGTTTCCAATCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAA

TGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTATTTT
TCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGT
ATGAGTATTCAACATTTCCGTGTCGCCCTTATCCCTTTTTTTCGGGCATTTTGCCTTCCTGTTTTTGCTCAGCCAGAAAC
GCTGGTAAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGA
TCCTTGAGAGTTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCC
CGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCAC
AGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGTGCCATAACCATGAGTGATAACACTGCGGCCA
ACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACCTCGCCTT
GATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAAC
GTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAG
TTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCT
CGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAAC
TATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTGAGACCAAGTTTACT
CATATATACTTTAGATTGATTTAAACTTCATTTTTTAATTTAAAGGATCTAGGTGAAGATCCTTTTTTGATAATCTCATG
ACCAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCC
TTTTTTTCTGCGCGTAATCTGCTGCTTGCAAAACAAAAAACCCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGC
TACCAACTCTTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAACTACTGTCCTTCTAGTGTAGCCGTAGTTA
GGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGG
CGATAAGTCGTGCTTACCAGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTT
CGTGACACAGCCAGCTTGGAGCGAAGCAGCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACG
CTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGG
GGGAAACGCCTGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAG
GGGGGCGGAGCCTATGGAACAAACGCCAGCAACCGCGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATG
TTCTTTCTGCGTTATCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCGAGCCG
AACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTCTCCTTACGCATCTGTGCG
GTATTTTACACCCGATAGTAAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTGGAGCTTGGCGTAA
TCATGGTCAT

Supplementary Figure 20. Map and nucleotide sequence of expression plasmid AV44_pCAG.Cas9^{D10A}.gRNA^{S1}. CAG, hybrid promoter (CMV enhancer, human cytomegalovirus *immediate early* enhancer; chicken β -actin promoter; chimeric intron, fusion between introns from the chicken β -actin and rabbit β -globin genes); Cas9, human codon-optimized Cas9 (Csn1) ORF coding for the endonuclease nicking variant D10A from the *Streptococcus pyogenes* type II CRISPR/Cas system; SV40 NLS, nuclear localization signal SV40 large T antigen; HSV TK poly(A) signal, herpes simplex virus *thymidine kinase* polyadenylation signal; NeoR/KanR, transposon Tn5 *aminoglycoside phosphotransferase* conferring resistance to the antibiotics G418 (geneticin) and kanamycin; AmpR, β -lactamase ampicillin resistance gene; ori, high-copy number ColE1 prokaryotic origin of replication; U6 promoter, RNA polymerase III promoter for human snRNA; gRNA.S1, *AAVS1*-targeting gRNA^{S1} spacer; termination signal, RNA polymerase III termination signal.



>AV13_pCAG.Cas9.gRNA^{NT} (9447 bp)

```

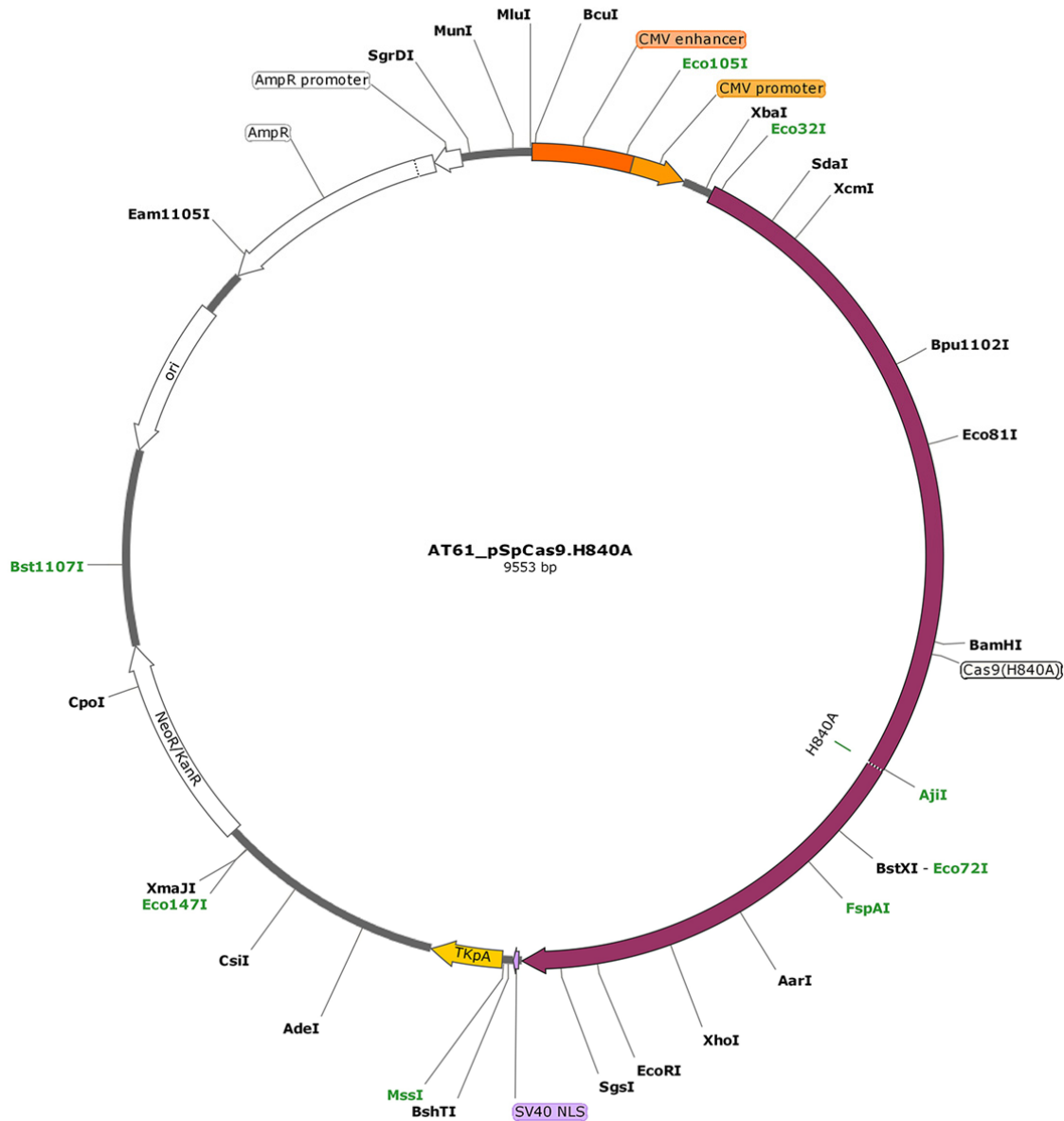
ATGTTATTCTCCTTATTTAAAGCTTAAGGTGCACGGCCACGTGGCCACTAGTACTTCTCGAGCTCTGTACATGTCCG
CGGTCGCGACGTACGCGTATCGATGGCGCCAGCTGCAGGCGGCCGCATATGCATCCTAGGCCTATTAATATCCGGAGT
ATACGTAGCCGGCTAACGTTatcgcatgcctgcagagctctagagtcCGGCCGCCCTTCACCGAGGGCCTATTTCCCA
TGATTCCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATA
TTAGTACAAAATACGTGACGTAGAAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAATATATGTTTTAAATGGACTA
TCATATGCTTACCGTAACTTGAAAGTATTTTCGATTTCTTGGCTTTATATATCTTGTGAAAGGACGAAACACCGGTGAGC
TCTTATTTGCGTAGTTTTAGAGCTAGAAATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCG
AGTCGGTGCTTTTTTTGaattAACAAACCGGTACCTCTAGAACTATAGCTAGCATGCGCAAATTTAAAGCGCTGATATCGA
TCGCGCGCAGATCTGTATGATGATCATTGCAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCG
ATGTACGGGCCAGATATACtgcagccatggattcGACATTGATTATGACTAGTTATTAATAGTAATCAATTACGGGGTC
ATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACC
CCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAG
TATTTACGGTAACTGCCCCTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGG
TAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTTACTTGGCAGTACATCTACGTATTAGTCA
TCGCTATTACCATGGgTCGAGGTGAGCCCCACGTTCTGCTTCACTTCCCCATCTCCCCCCCCCTCCCCCAATTTT
GTATTTATTTATTTTAAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGG
GGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGCGAGCCAAATCAGAGCGGCGCGCTCCGAAAGTTTCTTTTAT
GGCGAGGCGGGGCGGGGCGGGGCGGCCCCATAAAAAAGCGAAGCGCGGGGCGGGGAGTTCGCTGCGTTGCGCTTCGCCCCGTG
CCCCGCTCCGCGCGCCCTCGCGCGGCCCGCCCGGCTCTGACTGACCGCGTTACTCCACAGGTGAGCGGGCGGGACGGC
CCTTCTCCTCCGGGCTGTAATTAGCGCTTGTTTAAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGG
GCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGCGTGGGGAGCGCCGCGTGC
GGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGGAGGGGAGCG

```

CGGCCGGGGGCGGTGCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGT
GAGCAGGGGGTGTGGGCGCGCGGTGCGGGCTGTAAACCCCCCTGCAACCCCCCTCCCCGAGTTGCTGAGCACGGCCCCGGC
TTCGGGTGCGGGGCTCCGTGCGGGGCTGCGCGGGGCTCGCCGTGCGGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGG
GCGGGGCGGGGCCCTCGGGCCGGGAGGGCTCGGGGAGGGGCGCGGCGGGCCCCGAGCGCCGCGGCTGTGAGGGCG
CGGCGAGCCGAGCCATTGCTTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGC
CGAAATCTGGGAGGCGCGCGCACCCCTCTAGCGGGCGCGGGCGAAGCGGTGCGGCGCGGGCAGGAAGGAAATGGGCG
GGGAGGGCTTCGTGCGTGCCTGCGCGCGCGGCTCCCTTCTCCATCTCCAGCCTCGGGGTGCGCGCAGGGGGCAGGCTGCC
TTCGGGGGGGACGGGCGAGGCGGGGTTCGGCTTCTGCGGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTTCAT
GCCTTCTTCTTTTTCTACAGCTCCTGGGCAACGTGCTGTTGTTGTGCTGTCTCATCATTTTGGCAAAGAATTatcgca
tgctctgcagagctctagagTCTTAGAGGATCGAACCTTgcccaccATGGACAAGAAGTACTCCATTGGGCTCGATATCGG
CACAAACAGCGTCGGCTGGGCCGTCTTACGGACGAGTACAAGTGCCGAGCAAAAAATTCAAAGTCTGGGCAATACCG
ATCGCCACAGCATAAAGAAGAACCTCATTGGCGCCCTCCTGTTGACTCCGGGAGACGGCCGAAGCCACGCGCTCAA
AGAACAGCACGGCGCAGATATACCCGAGAAAAGTTCGGATCTGCTACCTGCAGGAGATCTTTAGTAATGAGATGGCTAA
GGTGGATGACTCTTCTTCCATAGGCTGGAGGAGTCTTTTTTGGTGGAGGAGGATAAAAAGCACGAGCGCCACCCAATCT
TTGGCAATATCGTGGACGAGGTGGCGTACCATGAAAAGTACCAACCATATATCATCTGAGGAAGAGCTTGTAGACAGT
ACTGATAAGGCTGACTTGCGGTTGATCTATCTCGCGTGGCGCATATGATCAAATTCGGGGACACTTCTCATCGAGGG
GGACCTGAACCCAGACAACAGCGATGTCGACAAACTCTTTATCCAACCTGGTTGAGACTTACAATCAGCTTTTCGAAGAGA
ACCCGATCAACGCATCCGGAGTTGACGCCAAAGCAATCTGAGCGCTAGGCTGTCCAAATCCCGCGGCTCGAAAACCTC
ATCGCACAGCTCCCTGGGGAGAAGAAGAACGGCTGTTTGGTAATCTTATCGCCCTGTCACTCGGGCTGACCCCCAATCT
TAAATCTAACTTCGACCTGGCCGAAGATGCCAAGCTTCAACTGAGCAAAGACACCTACGATGATGATCTCGACAATCTGC
TGGCCAGATCGGCGACAGTACGCAGACCTTTTTTGGCGGCAAGAAGCTGTCAGACGCCATTTCTGCTGAGTGAATAT
CTGCGAGTGAACCCAGACATCACCAGCTCCGCTGAGGCTAGTATGATCAAGCGCTATGATGAGCACCCACGAAGACTT
GACTTTGCTGAAGCCCTTGTGACAGACGAACTGCCTGAGAAGTACAAGGAAATTTTCTTCGATCAGTCTAAAAATGGCT
ACGCCGATACATTGACGGCGAGCAAGCCAGGAGGAATTTTACAAATTTATTAAGCCCATCTTGAAAAAATGGACGGC
ACCGAGGAGCTGCTGGTAAAGCTTAACAGAGAAGATCTGTTGCGCAACAGCGCACTTTCGACAATGGAAGCATCCCCA
CCAGATTACCTGGGCGAACTGCACGCTATCCTCAGGCGGCAAGAGGATTTCTACCCCTTTTGAAGATAACAGGGGAA
AGATTGAGAAATCTCACATTTTCGGATACCTTACTATGTAGGCCCCCTCGCCCGGGGAAATTCGAGATCCGATGAGTG
ACTCGCAAATCAGAAGAGACCATCACTCCCTGGAACCTCGAGGAAGTCGTGGATAAGGGGGCTCTGCCCAGTCCCTCAT
CGAAAGGATGACTAACTTTGATAAAAACTGCTTAACGAAAAGGTGCTTCTTAAACACTCTCTGCTGTACGAGTACTTCA
CAGTTTATAACGAGCTCACCAAGGTCAAATACGTCACAGAGGGATGAGAAAGCCAGCATTCCTGTCTGGAGAGCAGAAG
AAAGCTATCGTGACCTCCTCTTCAAGACGAACCGGAAGTACCCTGAAACAGCTCAAAGAAGACTATTTCAAAAGAT
TGAATGTTTCGACTCTGTTGAAATCAGCGGAGTGGAGGATGCTTCAACGCATCCCTGGGAACGTATCAGTATCTCTGA
AAATCATTAAAGACAAGGACTTCTTGGAATGAGGAGAACGAGGACATTTCTGAGGACATTGTCTCACCCCTACGTTG
TTTGAAGATAGGGAGATGATTGAAGAACGCTTGAAGAACTTACGCTCATCTCTTCGACGACAAAGTCATGAAACAGCTCAA
GAGGCGCGATATACAGGATGGGGGCGGCTGTCAAGAAAAGTATCAATGGGATCCGAGACAAGCAGAGTGGAAAGACAA
TCCTGGATTTCTTAAGTCCGATGGATTGCCAACCGGAACCTTCATGCAAGTATGATCCATGATGACTCTCTCACCTTTAAG
GAGGACATCGCAAGAGCACAAGTTTCTGCGAGGGGACAGTCTTCAAGGACACATCGCTAATCTTCAGGTAGCCCAAGC
TATCAAAAAGGGAATACTGCAGACCGTTAAGGTGCTGGATGAACTCGTCAAAGTAATGGGAAGGCATAAGCCCGAGAATA
TCGTTATCGAGATGGCCGAGAGAACCAAACCTACCCAGAAGGGACAGAAGAAGTAGGGAAAGGATGAAGAGGATTGAA
GAGGGTATAAAGAACTGGGGTCCCAATCCTTAAGGAACACCCAGTTGAAAACACCCAGCTTCAGAATGAGAAGCTCTA
CCTGTACTACCTGCGAAGCGCAGGACATGTACGTGGATCAGGAAGTGGACATCAATCGGCTCTCCGACTACGACGTGG
ATCATATCGTGCCCGAGTCTTTCTCAAAGATGATTCTGATGATAATAAAGTGTGACAAGATCCGATAAAAAATAGAGG
AAGAGTGATAACGTCCCTCAGAAGAAGTGTCAAGAAAATGAAAATTTATGGCGGCGAGCTGCTGAACGCCAACTGAT
CACACAACGGAAGTTCGATAATCTGACTAAGGCTGAACGAGGTGGCCTGTCTGAGTTGGATAAAGCCGGCTTCATCAAAA
GGCAGCTTGTGAGACACGCCAGATCACCAGCAGTGGCCCAATTCGATTCACGCATGAACACCAAGTACGATGAA
AATGACAACTGATTGAGAGGTGAAAGTTATTACTCTGAAGTCTAAGCTGGTCTCAGATTTAGAAAGGACTTTTCAGTT
TTATAAGGTGAGAGATCAACAATTAACACCATGCGCATGATGCTTACCTGATGATGAGTGGTAGGCATGACTTATCA
AAAAATATCCCAAGCTTGAATCTGAATTTGTTTACGGAGACTATAAAGTGTACGATGTTAGGAAAATGATCGCAAAGTCT
GAGCAGGAAATAGGCAAGGCCACCGCTAAGTACTTCTTTTACAGCAATATTATGAATTTTTCAGAACCGAGATTACACT
GGCCAATGGAGAGATTTCGAAGCGACCACTTATCGAAACAACCGGAGAAACAGGAGAAATCGTGTGGGACAAGGGTAGGG
ATTTCCGCGACAGTCCGGAAGGTCTGTCCATGCCGCGAGTGAACATCGTTAAAAAGACCGAAGTACAGACCGGAGGCTTC
TCCAAGGAAAGTATCCTCCCGAAAAGGAACAGCGACAAGCTGATCGCACGCAAAAAAGATTGGGACCCCAAGAAATACGG
CGGATTCGATTCTCTACAGTCGCTTACAGTGTACTGGTTGTGGCCAAAGTGGAGAAAGGGAAGTCTAAAAAATCAAAA
GCGTCAAGGAAGTCTGGGCATCACAATCATGGAGCGATCAAGCTTCGAAAAAACCCTATCGACTTTCTCGAGGCGAAA
GGATATAAAGAGGTCAAAAAAGACCTCATCATTAAGCTTCCCAAGTACTCTCTCTTTGAGCTTGAAAACGGCCGGAAACG
AATGCTCGCTAGTGCGGGCGAGCTGCAGAAAGGTAACGAGCTGGCACTGCCCTCTAAATACGTTAATTTCTTGATCTCG
CCAGCCATATGAAAAAGCTCAAAGGGTCTCCCGAAGATAATGAGCAGAAGCAGCTGTTCTGTGGAACCAACACAACTAC
CTTGATGAGATCATCGAGCAAAATAAGCGAATTTCTCCAAAAGAGTGATCCTCGCCGACGCTAACCTCGATAAGGTGCTTTC
TGCTTACAATAAGCACAGGGATAAGCCCATCAGGGAGCAGGCAGAAAACATTATCCACTTGTTTACTCTGACCAACTTGG
GCGCGCTGACAGCTTCAAGTACTTTCGACACCACCATAGACAGAAAGCGGTACACCTCTACAAAGGAGGTCTGACGCC
ACCTGATTTCATCAGTCAATTACGGGGCTCTATGAAACAAGAATCGACCTCTCTCAGCTCGGTGGAGACAGCAGGGCTGA
CCCAAGGAAGAAAGGAGGTGTGAAAGGGTTCGATCCCTACCGTTAGTAATGAGTTTAAACGGGGGAGGCTGATGAA
ACACGGAAGGAGACAATACCGGAAGGAACCCGCGCTATGACGGCAATAAAAAGACAGAATAAAACGCACGGGTGTTGGGT
CGTTTGTTCATAAAGCGGGGTTTCGGTCCCAGGGCTGGCACTCTGTGATACCCACCGAGACCCCATTTGGGGCCAATAC
GCCCCGCTTTCTTCTTTTCCCCACCCACCCCAAGTTTCGGGTGAAGGCCAGGGCTCGCAGCCAACGTCCGGGCGGC
AGGCCCTGCCATAGCATCTGCGCAGCTGGGGCTCTAGGGGATATCCCCACGCGCCCTGTAGCGGCGCATTAAGCGCGG
CGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGGCAGCGCCCTAGCGCCCGGGTTATAAATTACCTCAGTTCGA
CGTCCCATGTGACAGGTGCTGAATTCGAAGGCATTCCACGACGTGGTACCAGCCATGGCCATGTCCAACCTCATCAATC

AGCTTGAGTAGCCATGCCCATGGCTCCAGCCTGTCTCGTACCAGCTTTGTTCCCTTTAGTGAGGGGTAATTTTCGAGCTT
GGCGTAATCATGGTCATAGCTGTTTTCCaattcTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGT
CATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCT
AAATACATTCAAATATGTATCCGCTCATGAGACAATAACCTGATAAATGCTTCAATAATATTGAAAAGGAAGAGTATG
AGTATTCAACATTTCCGTGTCGCCCTTATTCCTTTTTTTCGGGCATTTTGCTTCCTGTTTTGCTCACCCAGAAACGCT
GGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAAGATCC
TTGAGAGTTTTTCGCCCCGAAGAAGCTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATATCCCGT
GTTGACGCCGGGCAAGAGCAACTCGGTGCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGA
AAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCACTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACT
TACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACCTCGCCTTGAT
CGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTT
GCGCAAACTATTAACCTGGCGAACTACTTACTCTAGCTTCCCGCAACAATTAATAGACTGGATGGAGCGGATAAAGTTG
CAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGC
GGTATCATTCGAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGAGTCAGGCAACTAT
GGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACGTGTCAGACCAAGTTTACTCAT
ATATACTTTAGATTGATTTAAACTTCATTTTTAATTTAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACC
AAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTT
TTTTCTGCGCGTAATCTGCTGCTTGCAAAACAAAAAACCCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTAC
CAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCTTCTAGTGATAGCCGTAGTTAGGC
CACCATTCAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGA
TAAGTCGTGTCTTACCGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGCTGAACGGGGGGTTCGT
GCACACAGCCAGCTTGAGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTT
CCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGG
AAACGCCCTGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGG
GGCGGAGCCTATGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCTGCGCTTTTGCTGGCCTTTTGCTCACATGTTTC
TTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGAGCCGAAC
GACCGAGCGCAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTCTCCTTACGCATCTGTGCGGTA
TTTACACCGCATAGTAAAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTGGAGCTTGGCGTAATCA
TGGTCAT

Supplementary Figure 21. Map and nucleotide sequence of expression plasmid AV13_pCAG.Cas9gRNA^{NT}. CAG, hybrid promoter (CMV enhancer, human cytomegalovirus *immediate early* enhancer; chicken β -actin promoter; chimeric intron, fusion between introns from the chicken β -actin and rabbit β -globin genes); Cas9, human codon-optimized Cas9 (Csn1) ORF coding for the endonuclease from the *Streptococcus pyogenes* type II CRISPR/Cas system; SV40 NLS, nuclear localization signal SV40 large T antigen; HSV TK poly(A) signal, herpes simplex virus *thymidine kinase* polyadenylation signal; NeoR/KanR, transposon Tn5 *aminoglycoside phosphotransferase* conferring resistance to the antibiotics G418 (geneticin) and kanamycin; AmpR, β -lactamase ampicillin resistance gene; ori, high-copy number ColE1 prokaryotic origin of replication; U6 promoter, RNA polymerase III promoter for human snRNA; gRNA.NT, gRNA^{NT} spacer targeting Cas9 to the recognition site of the *S. cerevisiae* I-SceI homing endonuclease; termination signal, RNA polymerase III termination signal.



>AT61_pSpCas9^{H840A}

```

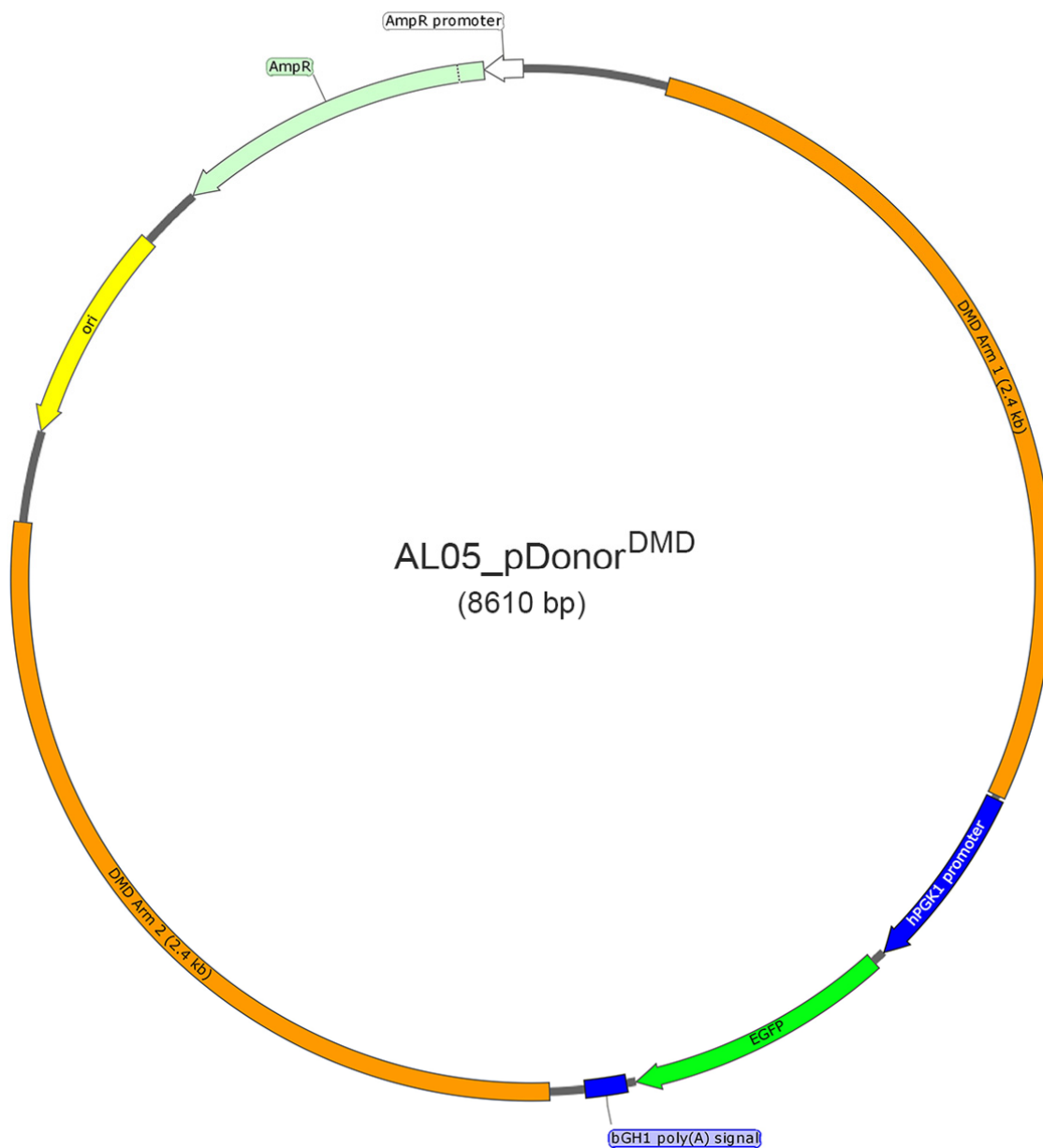
CGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGGC
GTTACATAACTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCC
CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAG
TGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTA
TGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGG
GCGTGGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATC
AACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATA
AGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACGGGGACCG
ATCCAGCCTCCGGACTCTAGAGGATCGAACCCTTgcccacATGGACAAGAAGTACTCCATGGGCTCGATATCGGCACAAACA
GCGTCGGCTGGGCCGTATTACGGACGAGTACAAGGTGCCGAGCAAAAAATTCAAAGTTCTGGGCAATACCGATCGCCACAGC
ATAAAGAAGAACCTCATTGGCGCCCTCCTGTTTCGACTCCGGGGAGACGGCCGAAGCCACGCGGCTCAAAGAAGACAGCAGCGC
CAGATATACCCGAGAAAGAATCGGATCTGCTACCTGCAGGAGATCTTTAGTAATGAGATGGCTAAGGTGGATGACTCTTTCT
TCCATAGGCTGGAGGAGTCTTTTTTGGTGGAGGAGGATAAAAAGCACGAGCGCCACCCCAATCTTTGGCAATATCGTGGACGAG
GTGGCGTACCATGAAAAGTACCAACCATATATCATCTGAGGAAGAAGCTTGTAGACAGTACTGATAAGGCTGACTTGCGGTT
GATCTATCTCGCGCTGGCGCATATGATCAAATTCGGGGACACTTCCTCATCGAGGGGGACCTGAACCCAGACAACAGCGATG
TCGACAAACTCTTTATCCAAGTGGTTCAGACTTACAATCAGCTTTTCGAAGAGAACCCGATCAACGCATCCGGAGTTGACGCC
AAAGCAATCCTGAGCGCTAGGCTGTCAAATCCGGCGGCTCGAAAACCTCATCGCACAGTCCCTGGGGAGAAGAAGAACGG

```

CCTGTTTGGTAACTCTTATCGCCCTGTCACTCGGGCTGACCCCCAACTTTAAATCTAACTTCGACCTGGCCGAAGATGCCAAGC
TTCAACTGAGCAAAGACACCTACGATGATGATCTCGACAATCTGCTGGCCAGATCGGCCAGCAGTACGACAGCCTTTTTTTTG
GCGGCAAAGAACCTGTGACAGCCATTCTGCTGAGTGATATTCTGCGAGTGAACACGGAGATCACCAAAGCTCCGCTGAGCGC
TAGTATGATCAAGCGCTATGATGAGCACCACCAAGACTTGACTTTGCTGAAGGCCCTTGTGACACAGCAACTGCCTGAGAAAGT
ACAAGGAAATTTTCTTCGATCAGTCTAAAAATGGCTACGCCGGATACATTGACGGCGGAGCAAGCCAGGAGGATTTTACAAA
TTTATTAAGCCCATCTTGGAAAAAATGGACGGCACCGGAGAGCTGCTGGTAAAGCTTAAACAGAGAAGATCTGTTGCGCAAAACA
GCGCACTTTCGACATAGGAAGCATCCCCACAGATTACCTGGGCGAACTGCACGCTATCCTCAGGCGGCAAGAGGATTTCT
ACCCCTTTTTGAAAGATAACAGGGAAGATTGAGAAAACTCTCACATTTTCGGATACCCCTACTATGTAGGCCCTCGCCCGG
GGAAATTCAGATTTCGCTGGATGACTCGCAAATCAGAAGAGACCATCACTCCCTGGAATTCGAGGAAGTCGTGGATAAGGG
GGCCTCTGCCAGTCCCTTCATCGAAAGGATGACTAACTTTGATAAAAAATCTGCCTAACGAAAAGGTGCTTCTTAAACACTCTC
TGCTGTACGAGTACTTCACAGTTTATAACGAGCTCACCAAGGTCAAATACGTCACAGAAGGGATGAGAAAGCCAGCATTCCTG
TCTGGAGAGCAGAAGAAAGCTATCGTGGACCTCCTCTTCAAGACGAACCGGAAAGTTACCGTGAACAGCTCAAAGAAGACTA
TTTCAAAAAGATTGAATGTTTCGACTCTGTTGAAATCAGCGGAGTGGAGGATCGCTTCAACGCATCCCTGGGAACGTATCACG
ATCTCCTGAAATCATTAAAGACAAGGACTTCTGGACAATGAGGAGAACGAGGACATTTCTGAGGACATTTGCTCTACCCCTT
ACGTTGTTGAAGATAGGGAGATGATTGAAGAACGCTTGAAAACTTACGCTCATCTCTTCGACGACAAAAGTCATGAAACAGCT
CAAGAGGCGCCGATATACAGGATGGGGGCGGCTGTCAAGAAAACTGATCAATGGGATCCGAGACAAGCAGAGTGGAAGACAA
TCCTGGATTTTCTTAAGTCCGATGGATTGCCCACCGGAACCTTCATGCAGTTGATCCATGATGACTCTCTCAGCTTTAAGGAG
GACATCCAGAAAGCACAAAGTTTCTGGCCAGGGGACAGTCTTACGAGCACATCGCTAATCTTGCAAGGTAGCCAGCTATCAA
AAAGGGAATACTGCAGACCGTTAAGGTCGTGGATGAACCTGTCAAAGTAATGGGAAGGCATAAGCCCAGAAATATCGTTATCG
AGATGGCCCGAGAGAACCAACTACCCAGAAGGGACAGAAGAAGTAGGGAAAGGATGAAGAGGATTGAAGAGGGTATAAAA
GAACCTGGGTCCTCAAACTCCTTAAGGAACCCAGTTGAAAAACCCAGCTTCAGAATGAGAAGCTTACCTGTACTACTCCTGCA
GAACGCGAGGACATGTACGTGGATCAGGAACCTGGACACTTTCAGTTTCAAGGCTTCCGACTACGACGTGGATGCTCTCCCGA
CTTTTCTCAAAGATGATTCTATTGATAATAAAGTGTGACAAGATCCGATAAAAAATAGAGGGAAGAGTGATAACGTCCCTCA
GAAGAAGTTGTCAAGAAAATGAAAAATTATTGGCGGCAGCTGCTGAACGCCAACTGATCACACAACGGAAGTTCGATAATCT
GACTAAGGCTGAACGAGGTGGCCTGTCTGAGTTGGATAAAGCCGGCTTCATCAAAAGGAGCTTGTGTGAGACACGCCAGATCA
CCAAGCAGTGGCCCAATTTCTCGATTACGCATGAACCAAGTACGATGAAAAATGACAACTGATTTCGAGAGGTGAAAGTT
ATTACTCTGAAGTCTAAGTCTGCTCAGATTTTCAAGAGACTTTTCAAGTTTATAAGGTGAGAGAGTCAACAAATTACACCA
TGCGCATGATGCCTACCTGAATGCAGTGGTAGGCACTGCACTTATCAAAAAATATCCCAAGCTTGAATCTGAATTTGTTTACG
GAGACTATAAAGTGTACGATGTTAGGAAAATGATCGCAAAGTCTGAGCAGGAAATAGGCAAGGCCACCGCTAAGTACTTCTTT
TACAGCAATATTATGAATTTTTTCAAGACCGAGATTACACTGGCCATGGAGAGATTTCGGAAGCGACCACTTATCGAAACAAA
CGGAGAAAACAGGAGAAATCGTGTGGGACAAGGGTAGGATTTCGCGACAGTCCGGAAGGTCTGTCCATGCCGCGAGGTGAACA
TCGTTAAAAAGTCCGAAGTACAGACCGGAGGCTTCTCCAAGGAAGTATCCTCCGAAAAGGAACGACGACAGCTGATCGCA
CGCAAAAAAGATTGGGACCCCAAGAAATACGCGGATTTCGATTCTCTACAGTCGCTTACAGTGTACTGGTTGTGGCCAAAGT
GGAGAAAGGAAGTCTAAAAAACTCAAAGCGTCAAGGAAGTCTGGGCATCACAAATCATGGAGCGATCAAGCTTCGAAAAAA
ACCCCATCGACTTCTCGAGGCGAAAGGATATAAAGAGGTCAAAAAAGACCTCATCATTAAAGCTTCCCAAGTACTCTCTCTT
GAGCTTGAACACGGCCGGAACGAATGCTCGCTAGTGGCGGCGAGCTGCAGAAAGGTAAACGAGCTGGCACTGCCCTTAAATA
CGTTAAATTTCTGTATCGGCCAGCCACTATGAAAAGTCAAAAGGTCTCCCGAAGATAATGAGCAGAAGCAGCTGTTCTGTG
AACAACACAAACTACCTTGATGAGATCATCGAGCAAATAAGCGAATTCTCCAAAAGAGTGATCCTCGCCGACGCTAACCTC
GATAAGGTGCTTTCTGCTTACAATAAGCACAGGGATAAGCCCATCAGGGAGCAGGCAGAAAACATTATCCACTTGTTTACTCT
GACCAACTTGGGCGCGCCTGACGCTTCAAGTACTTCGACACCACCATAGACAGAAAGCGGTACACCTCTACAAAGGAGGTCC
TGGACGCCACACTGATTATCAGTCAATTACGGGGCTCTATGAACAAAGATCGACCTCTCTCAGCTCGGTGGAGACAGCAGG
GCTGACCCCAAGAAAGAGGAAGGTGTGAAAGGGTTCGATCCCTACCGGTTAGTAATGAGTTTAAACGGGGGAGGCTGATG
AAACACGGAAGGAGACAATACCGGAAGGAACCCGCGCTATGACGGCAATAAAAAAGACAGAATAAAACGCACGGGTGTTGGGTC
GTTTGTTCATAAACGCGGGGTTTCGGTCCCAGGGCTGGCACTCTGTGATACCCACCGAGACCCATTGGGGCCAATACGCCC
GCGTTTCTTCTTTTCCCAACCCCAAGTTTCGGGTGAAGGCCAGGGCTCGCAGCCAACGTCGGGGCGGCAGGCCCT
GCCATAGCAGATCTGCGCAGCTGGGGCTCTAGGGGGTATCCCCACGCGCCCTGTAGCGGCGCATTAAGCGCGCGGGTGTGGT
GGTTACGCGCAGCGTACCGCTACACTTGCCAGCGCCCTAGCGCCGCTCCTTTTCGCTTCTTCCCTCTCTCTCGCCACGT
TCGCGGCTTTCCTTCCTCAAGCTCTAAATCGGGGCTCCTTTAGGGTTCCGATTAGTGCTTTACGGCACCTCGACCCAAA
AACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTT
CTTTAATAGTGGACTCTTGTTCAAACTGGAACAACACTCAACCTTATCTCGGTCTATTCTTTGATTTATAAGGGATTTTGC
CGATTTCCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTAATTCTGTGGAATGTGTGTCAGTTAG
GGTGTGGAAGATCCCAAGGCTCCCAAGCAGGCAGGAAGTATGCAAAAGCATGCATCTCAATTAGTCAGCAACCAAGGTGTGGAAG
TCCCAAGGCTCCCAAGCAGGCAGGAAGTATGCAAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCTAACTCCGCC
CATCCCGCCCTAACTCCGCCAGTTCGCCCATCTCCGCCCATGGCTGACTAATTTTTTTTATTATGACAGAGGCCGAGG
CCGCTCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCTAGGCTTTTGCAAAAAGCTCCCGGGAGCT
TGTATATCATTTTTCGGATCTGATCAAGAGACAGGATGAGGATCGTTTCGATGATGAACAAGATGGATTGCACGAGGTTC
TCGGCGGCTTGGGTGGAGAGGCTATTTCGGCTATGACTGGGCAACAACAGACAATCGGCTGCTGTGATGCGCGGCTGTTTCGG
TGTCAGCGCAGGGGCGCCGCTTCTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAAGTGCAGGACGAGGCAGCGCG
CTATCGTGGCTGGCCACGACGGCGTTCCTTGCGCAGCTGTGCTGACGTTGTCACTGAAGCGGGAAGGAGTGGCTGCTATT
GGGCGAAGTGCCGGGCGAGGATCTCCTGTCTATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGC
GGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACACCAAGCGAAACATCGCATCGAGCGACGATCTCGGATGGAA
GCGGCTCTGTCGATCAGGATGATCTGGACGAAGAGCATAGGGGCTCGCGCCAGCCGAACTGTTCCGCAAGGCTCAAGCGCG
CATGCCCGACGGCGAGGATCTCGTCTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTG
GATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTT
GGCGGCGAATGGGCTGACCGCTTCTCTGTGCTTTACGGTATCGCCGCTCCCGATTTCGACGCGCATCGCCTTCTATCGCCTTCT
TGACGAGTTCTTCTGAGCGGACTCTGGGGTTCGCGAAATGACCCACCAAGCGACGCCCAACCTGCCATCAGGATTTTCGAT
TCCACCGCCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCGGCTGGATGATCTCCGACGCGGGGATCT
CATGCTGGAGTTCTTCGCCCACCCAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCA

CAAATAAAGCATTTTTTCTACTGCATTCTAGTTGTGGTTTGTCCAACTCATCAATGTATCTTATCATGTCTGTATACCGTCG
 ACCTCTAGCTAGAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTGTTATCCGCTCACAAATCCACACACAT
 ACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCCG
 CTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGGGGAGAGGCGGTTTGGCTATTGGGCGC
 TCTTCCGCTTCTCGCTCACTGACTCGCTGCGCTCGGTCTTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAAT
 ACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGG
 CCGCGTTGCTGGCGTTTTTTCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAAC
 CCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCTGTTCGACCCCTGCCGCTTACCGG
 ATACCTGTCCGCTTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCG
 TTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTACGCCCCGACCGCTGCGCCTTATCCGGTAACATATCGTCTTGAGTCC
 AACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTAC
 AGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAGAAGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCT
 TCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATT
 ACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGAACGAAACTCACGTTA
 AGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAATAATGAAGTTTTAAATCAATCTAAA
 GTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCTGTTCA
 TCCATAGTTGCCCTGACTCCCCGTCTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTCAATGATACC
 GCGAGACCCACGCTCACCGCTCCAGATTTATCAGCAATAAACACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTTGCAA
 CTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCAGCAACGTT
 GTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTACGCTCCGGTTCCCAACGATCAAGGCG
 AGTTACATGATCCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTGAGAAGTAAGTTGGCCGCGAG
 TGTATCACTCATGGTTATGGCAGCACTGCATAATTCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAG
 TACTCAACCAAGTCATTCTGAGAATAGTGATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCC
 ACATAGCAGAACTTTAAAGTGCTCATCATTTGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGAT
 CCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTACGATCTTTTACTTTACACAGCGTTTCTGGGTGAGCAAAAAACA
 GGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCTCTTTTCAATATTATTG
 AAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCA
 CATTTCCCGAAAAGTGCCACCTGACGTGACGGATCGGGAGATCTCCCGATCCCCTATGGTGCATCTCAGTACAATCTGCT
 CTGATGCCGCATAGTTAAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTGCTGAGTAGTGCAGGAGCAAAATTTAAGCT
 ACAACAAGGCAAGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTTGCCTGCTTCGCGATGTACGGGC
 CAGATATA

Supplementary Figure 22. Map and nucleotide sequence of expression plasmid AT61_pSpCas9^{H840A}. CMV, regulatory sequences from the human cytomegalovirus *immediate-early* gene; nicking mutant of the Cas9 nuclease from the *Streptococcus pyogenes* Type II CRISPR/Cas system (together with an appropriate gRNA generates sequence- and strand-specific DNA breaks owing to the H840A mutation in its HNH catalytic domain); SV40 NLS, nuclear localization signal from the simian virus 40 large T antigen; TKpA, polyadenylation signal from the herpes simplex virus type 1 *thymidine kinase* gene; NeoR/KanR, gene coding for aminoglycoside phosphotransferase from transposon Tn5 (confers resistance to neomycin and kanamycin); AmpR, β -lactamase gene (confers resistance to ampicillin); Ori, prokaryotic ColE1 origin of replication.



```

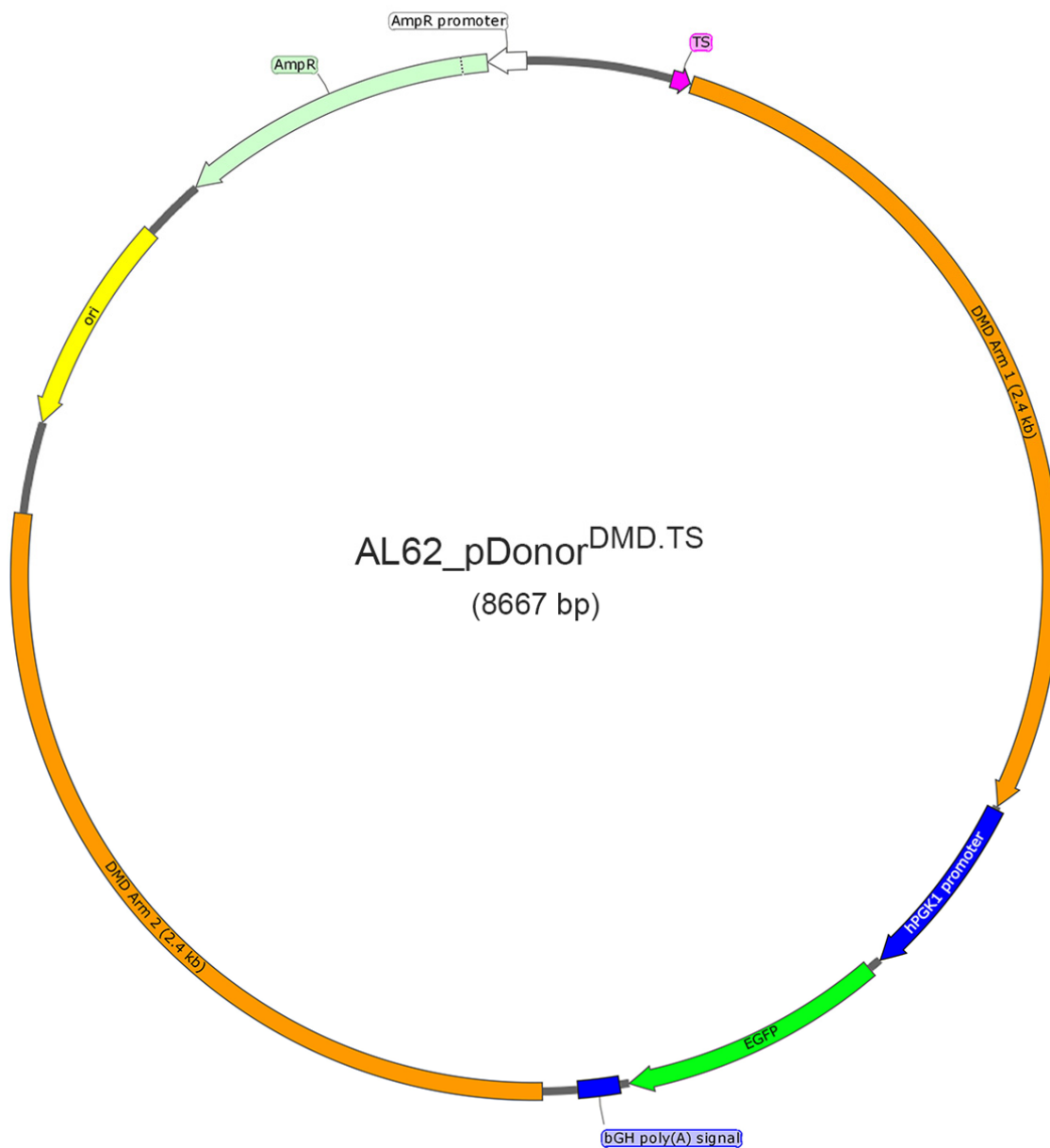
>AL05_pDonor.DMD (8610 bp)
CTAAATTGTAAGCGTTAATATTTTGTAAAAATTCGCGTTAAATTTTGTAAATCAGCTCATTCTTTTAACCAATAGGCCG
AAATCGGCAAAATCCCTTATAAATCAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATTTCGCC
ATTGAGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGCGGGCCTCTCGCTATTACGCCAGCTGGCGAAAAGGGGGATGT
GCTGCAAGGCGATTAAAGTTGGGTAACGCCAGGGTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGAGCGCGACGT
AATACGACTCACATATAGGGCGAATTGGCGGAAGGCCGTCAGGCCATAGGCCATAAGGTAAAGAGGACACCAATACAGTA
AATAATAACATAATAATAAGAAACCTTATGAATTCATTCAGTGATATCCATATTGAGGTATTTAAGGTGAATGATACT
GATGTCAGCAGTTTACCTGGATATGCATCAAAAAATGATTGATTGATGGATGGATCGGAGGAAGGAAAAGATATGTGAT
CAAAACGAGTATAGTAAAAATGTTTCATGGTGGAATCTAGATATATAGATATCACTTGTAATTTTAAAAATGTTATTGCAT
TTTTGAAATTTGTTATGTTAAAAATATTGGGTGAAAATTCGATGCCCTGGCCATGTCCCAATCAGTTGAATCAGATCTC
TGGTGTATTTTTTTTTTAAGATCCTTGATGACTCCATTGTACAGCAAGTTTGCAAACCACTATTCTAAGTCTCACCAC
CCCCTTCTTTATAGCTTACAAAATTATATTTTTAAATGTCAAATGTCACAATTCACCTTCTCTTCAAGAACTGATTA
GACTTCTAGCTTCTCCTATGCTCTCATCTTGCATAACGTTTCATTGTCAGTGGTTAAACTGATAGGCAATAAATAATAA
CCCATCTGTCTTTTCCCTGACCAACATAACTAAACCTTCTTCAAGGCGAAAATGAAGTATAATCTCACAGACAAGAG
TAGCTATAGACAATAATCTTCTGCTCTCGTGGTTCATGGGACTATTCACCACTGCCGTCCCTGCTGTCTATTTGGT
GGACCAGCCAGTTTACTGTGACCCATACCATGGCTCTTGAGAAGCATGAAGCTGCCGAAATCGCAGTTGTAGGGTTAAT
GGAAAACATGGTGAAGTATAGCTTACATATATATACAATTTGGCACAAAATGAAGCCTAAGAAATTGTACCGGGCTGGG
TGTGGTGACTCATGCCTGTAATCCCAGCACTTTGAGAGACCAAGGTGGGCGGATCACCTGAGGTCTGGGAGTTTCGAGACCA
GACTGAGCAACATGGAGAAACCTCTCTACTTAAAAATACAAAATTAGCCAGGCATGGTGGCGCATGCCTGTAACCCACAG

```

TACTCAGGAGGCTAAGGCAAGAGAATCTCTTCCACCCTGGAGGCATAGGTTGTGGTGAGAGAAGATCACACCATTGCACT
CCAGCTGGGGCAACAAGAGCGAAGCTCCGTCTCAATAAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAA
AAAAGAAAAAAGAAATGTACCAGGAAGTGATTTGGGAATGCTCTATATATTCTCCCCCTCCAAGAAGTTTTTTTAATTTA
TTGTATTGTGAAAGATCTGCAGCTATTTATTTTGTGGTACTCTTTTGCAATAAACTACTTCTGCATAAATGAAGTCAA
AACTTTTGATATTTCATGAACCTTCATAATGTTGATCTAGTACATCTTGCCCACTAGCCCTTGGGCCATTCTCTCCATGC
CACTGGTTGAAAAATATGCTCCCCACCCAGTAACATCAGCATCAGACCTCTGCAACCAGAAAGATTCCATAGGTAATGA
AATCCAAATGCTGGCATTGTGGAAGTCAAAACAGTGTATTTTGAATGGAAAGGCAAGGGGAAGTGCATGGAGCAAGTACA
GCATGTGCTACAACCTCAGTTCATACCTCCTTAAAATACTGTGCAACTAACTGTCAATTGCCCATTGATAGAGATAGT
ATCTTTTTTCACTTTACACCTTCCGAATTCAACACACTGCTAGCCTCAGGACTAAGCGCTCAATAAATGTTTATCAAAAG
ATTGAGTAAATGAATCAGTGGTGAATAAATGTACAAACACATACGCTTCATACACGTTTAGGAAGCATTCTAGTGAG
CTTTGTGGATATTTATTTCTTAAAGGTTCTTTTTCTTTCCATGTTTCATGTTATTCTTTATTTTTTAAAAATGTTGCTTCT
GCTTATTTCTCTTTCTACGGCAGCTATATTTACTTGGCTGAGAGCACAGCATATACTGATGTTAATCAAACCTTAGATGA
AACACCTAAAATGATAGAAAAGAAATCTGCTCTAATTAATAATAAAGCAACCCCTACAGGTTTAGACATGTGCCTTCCGG
TGTGGGAAAGAAAAATTTAATGAATGTAGTAGTTTATGCCAAGAACATTTCCCTCGTGCACCTGTGTTTTAATAAGAT
AGAATATAAAATAGCAAAAGGGGCCCCGACTTTTGTGATGATATTTACTCATAAGGTAGTAAGTCAGATGTGATGACATTT
TCTTTGACTTAGAGCTGCTATACTTGGGTGAGATTTTCAAGTTCAGTAAATTTGCAGTGAAGTTGTCTTTCTAACATGGTGT
CATCCTGGAACTGCCCTGCTCCACAGTAAGCTTCCACGGGGTGGGGTGGCGCTTTTCCAAGGCAGCCCTGGGTTTGC
GCAGGGACGCGGCTGCTCTGGGCGTGGTCCGGGAAACGACGCGGCGCCGACCTGGGTCTCGCACATTCTTACAGTCCG
TTCGACGCTCACCCGATCTTCCGCGCTACCTTGTGGGCCCCCGGCGACGCTTCCGTCTCGCCCCCTAAGTCGGGAA
GGTTCCCTTGGCGTTTCGCGGCGTCCCGACGTGACAAACGGAAGCCGCACGCTCTCACTAGTACCCTCGCAGACGGACAGCG
CCAGGGAGCAATGGCAGCGCGCCGACCGCGATGGGCTGTGGCCAAATAGCGGCTGCTCAGCAGGGCGCGCGGAGAGACGCG
CCGCGGTAAGGGCGGCGTCCGGGAGCGGGGTGTGGGCGGTAGTGTGGGCGCTTCTCTGCCCCGCGCGCTTTCCGCAATC
TGCAAGCCTCCGAGCGCACGTCGGCAGTCCGCTCCCTCGTTGACCGAATCACCGACCTCTCTCCCCACACGACTCTAGA
GGATCCCCGGGTACCGGTGCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGGCCATCTCGTGCAGC
TGGACGGCGACGTAACCGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCGT
AAGTTTCATCTGCACACCGGCAAGCTGCCCGTGCCCTGGCCACCCCTCGTGACCACCCCTGACCTACGGCGTGCAGTGCTT
CAGCCGTACCCCGACCAACATGAAGCAGCAGCACTTCTTCAAGTCCGCCATGCCGAAGGCTACGTCAGGACGCGACCA
TCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAG
CTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACACAGCCACAACGTCTA
TATCATGGCCGACAGCAGAGAAGCGCATCAAGGTGAACCTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGC
TCGCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCGTGTCTGCTGCCGACAACCCTACCTGAGCACCAG
TCCGCCCTGAGCAAGACCCCAACGAGAAGCGCGATCACATGGTCTGCTGGAGTTCTGTGACCGCGCGGATCACTCT
CGGCATGGACGAGCTGTACAAGTAAAGCGGCCGCGACTCTAGAATTTAAGCTGTGCCTTCTAGTTGCCAGCCATCTGTT
GTTTGGCCCTCCCCCGTGCCTTCTTGACCCTGGAAGGTGCCACTCCCACTGTCTTTCTTAATAAAATGAGGAAATTGC
ATCGCATTTGTCTGAGTAGGTGTCTTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACA
ATAGCAGGCATGCACGTGTACAGGCTAGGGAGTGGGTAGGAGTGGGGGTGAATCCTCTTAATGTTTATGGTGTGATGAG
ATTCAAACATAAAATAGCCTTACAGCCATCTCCTAATAAGGGGCCCTGGCATATTTAATTGATTAAACAAATTTATCA
AAAATAGATAAACTGAAATCTGCCTTGAAATTAATTACTGTATCTCTATTTTTATAAGAAAAATTTTTGGACCCGTTCCC
TCTGCCTTATGGGTGCAACTCCCGGCAGAATAGGTGATGTCTCCTGAAAATAGTTCTTATTTTCTTACTACTTATGACC
TCTTATAGCCTAGAGTTTTTCTTGTATTTCTAGTTGAAATGCTAATCTGGCATAATTTCTAGTTGAAATGCTAATCT
GGCATAATTTCAAGTAATTTTCTGTTAATGCCACTTGAACATCTAAATTCCTCTTTTTCAATAAATACTATATTTGT
GTGTTGCAAGCACACACAAATCATACTATATTTGTGTATATAGTAATAATCCAACCTATAAAAGTAAATTTGGAATTT
TTTTCTTTCTTTTGGAAAATATTTTATAATTGAGAATTATAAATGTCACTTTTTTAAATGCTGCAACCTTTGAGATTGG
TTTCAATAAAGTAAACTTAGTAAACATTAAGAAAAATGATAGCTTGATATGTTCACTAATATGGTAAATGAAAATTTT
ATGTGTGTATAGGCTCTTGTTAATTATAACTACCTTCACAGGAAAAACAGTCTTGTGGAAGGTAATGGTGCCAGTGAGAG
AAAATAGAGAAAGTAGAATAGTAGTAAGAGAGAAAAATAGAATTTCCCGTTTGAATAAAGCTAAGTAAAAAGTGAATTTG
TTCAGTTTTATTTATGATTTGTAAAGTGTGGTCTCATGCCATATATATTCAAAGATAGACAGAGATCTTAATCTTTAATTT
TTTTATGGCCAAAGAAAATGAGTCCCATGTAAAGGGACATTTTCGTAAGCTGATCTCAAGTGGTGTATTTTGATTGGCC
GCAGCAGCTATGGAAGAAATATCATTTTGGTGCCTAAGAAGAAAAATGTCATACCTTTCTATTTTTTATTCATACTCC
GCATATTTGGAATTCGTATTCTCACCATACTTTTGGGAAGATAACAGAAATTGCCTTACTTGCCTCGAAATCAAAATCT
GCCATTCGTTTTAAAAATAAATGGCTTTTTTCTATCTCATTTTCTTTAGCAGAAAAAGTAAAGTAAATTTTTTGTAGCT
GTGTTAAGTGTAAGTTTCTCCCTCGTTGAGAACGAAACAAAGACATATGATTCCATTTACTGTAATTTGTTTTGCTTGC
CCAATTACAATAGCAGTAAATCATTTACACATAGTAAATGTTTGAGCCTTAAAGGGCAAGAAGACTAAATTTAGCTGA
AGATATACAACCTTTTCATGTGCCAATGAGGAAATGTATAAAATCAGTTATACATATTTTCTCATTATAGATATTGAAAA
TATTCATATATGTGTACATGATTTTGATAACTTAATATTTTCTAAATAACAGAAATTATATACTGTATATATATTTGGAT
ATTATGATTATTGTTATGGCCACCATTTAGCACTTACTATGGGCTCAGAAAACCTTCACTGTATTTTAAATCTTATA
ACAACCTGTGAAATATATATATGCTTATTTTCAAAATGAGAACTCTGAAATCAGGAAATTAATTAACCTGTCTGAAGTT
TACAAAGCACTGTTAGGCAATAAAACAGAATTTCAAACCTCACGTTTGTCTTCAAATTTTGTATGCTGTACAACCTG
ATTGTTTTTACTACTTGTCTTAATTTCAAAAAAATCTTCATACTAAAAGATGATACTTTGGGAGTTCTAAAGAACATGT
TTTTTGGCCGGGCGGCTGGCTCACGCTGTAATCCAGCACTTTGGGAGGCCGAGGCAGAGGATCACGAGGTCAGGAGA
TCGAGACCGCTCTGGCCAAACAGGTGAAACCCGCTCTCTACTAAAAAATACAAAAAATAGCCGGGCGTGGTGGCGGGCG
CCTGTAGTCCGAGTACTCGGGAAGCTGAGCGGGGAGAAAGCGTGAACCTGGGAGGCAGAGCTTGTAGTGAGCCGAGAT
CGCGCCGCTGCACTCCAGCCTGGTTGACAGAGCGAGACTCCGTCTCAAAAAATAACAAAACAAAACAAAACAAAACAAA
AAACCATGTTTCTTTTCAAGAGGTATACTCATTTGAAGTGGATACCAATTATTTGTATTAAAATTACTTATGGATAAAT
TGAATCTGCAAAAAATTAAGTGAACATATTTTTTTGGCACTGTTATAGGAGGGTAACCTCGAGGGTACCTCTTAATTAAC
TGGCCTCATGGCCCTTCCGCTCACTGCCCCGCTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAACATGGTGCATAGC
TGTTTTCTTGCATTTGGCGCTCTCCGCTTCCGCTCACTGACTCGCTGCGCTCGGTGCTTCCGCTTAAAGCTGGGT
GCCTAATGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTGTCTGGCGTTTTTCCATAGGCTCCGCC

CCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTC
 CCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCGTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGA
 AGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCA
 CGAACCCCCGTTTACGCCCCACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTAT
 CGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGG
 CCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGG
 TAGCTCTTGATCCGGCAAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAA
 AAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTG
 GTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAAATGAAGTTTTAAATCAATCTAAAGTATATA
 TGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCTGTTTCATCCA
 TAGTTGCGCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCG
 CGAGAACCACGCTCACCGGCTCCAGATTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTCTGC
 AACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCA
 ACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTCAGCTCCGTTTCCCAACGA
 TCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCCTCCGATCGTTGTGAGAAGTAA
 GTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCTATGCCATCCGTAAGATGCTTTT
 CTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGATGCGGCGACCGAGTTGCTCTTGCCCGGCTCAATA
 CGGGATAATACCGCGCCACATAGCAGAACTTTAAAGTGCTCATCATTTGGAACGTTCTTCGGGCGCAAACTCTCAAG
 GATCTTACCGCTGTTGAGATCCAGTTCGATGTAAACCACTCGTGCACCAACTGATCTTCAGCATCTTTTACTTTACCA
 GCGTTTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTC
 ATACTCTTCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTGAATGTATTTA
 GAAAAATAACAAATAGGGGTTCCGCGCACATTTCCCGAAAAAGTGCCAC

Supplementary Figure 23. Map and nucleotide sequence of the unmodified donor construct targeting the human *DMD* locus. DNA sequences sharing identity to the human *DMD* gene are indicated in orange; hPGK1 promoter, human phosphoglycerate kinase 1 gene (*PGK1*) regulatory sequences; EGFP, enhanced green fluorescence protein gene (*EGFP*) open reading frame; bGH1 poly(A) signal, bovine growth hormone gene (*GHI*) polyadenylation signal; AmpR, β -lactamase ampicillin resistance gene; ori, high-copy number ColE1 prokaryotic origin of replication.



```

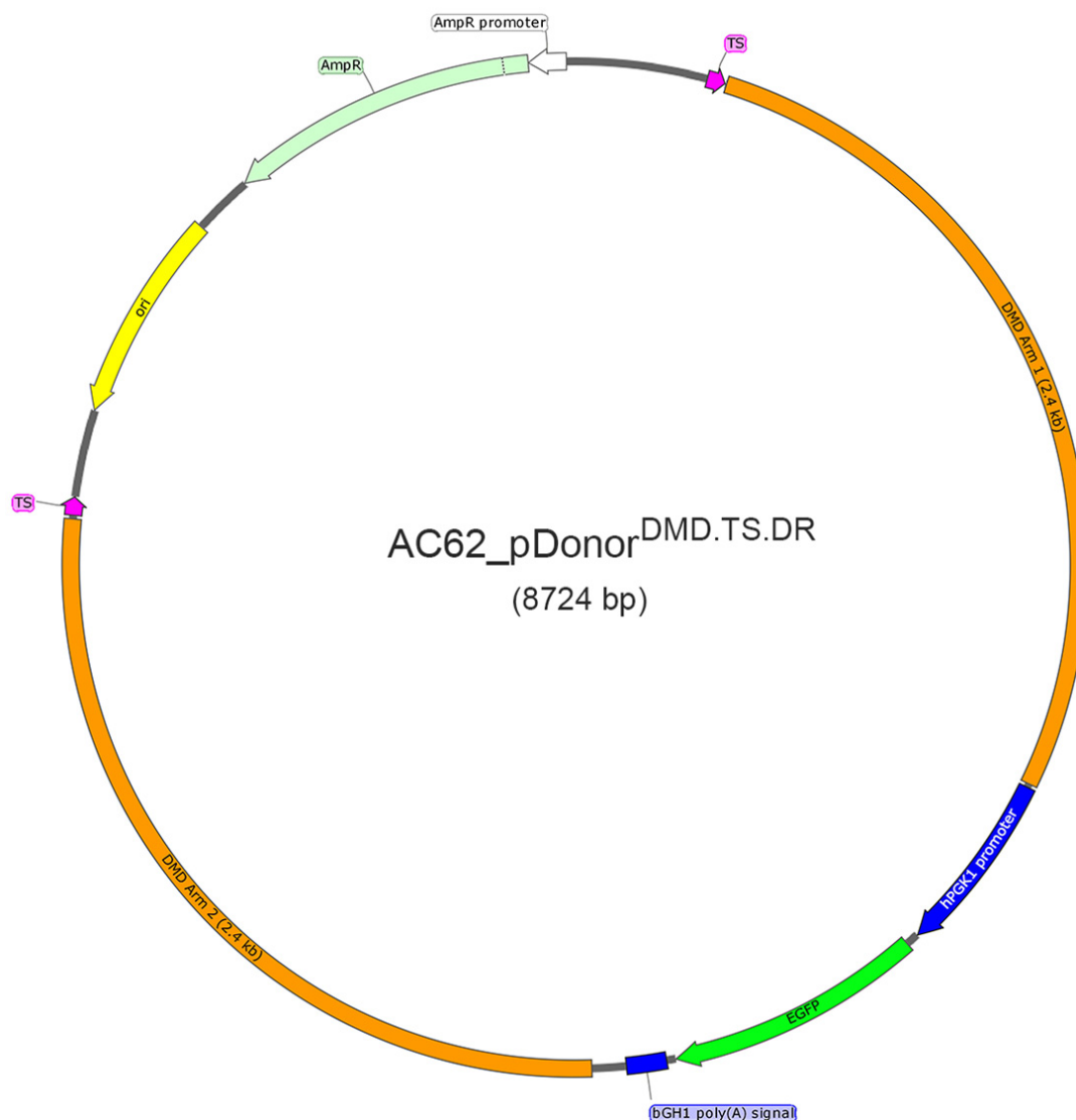
>AL62_pDonor.DMD.TS (8667 bp)
CTAAATTGTAAGCGTTAATATTTTGTAAAATTCGCGTTAAATTTTGTAAATCAGCTCATTTTTTAACCAATAGGCCG
AAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATTCGCC
ATTCAGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGT
GCTGCAAGGCGATTAAAGTTGGGTAACGCCAGGGTTTCCAGTCACGACGTTGTAACGACGCGCAGTGAGCGCGACGT
AATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAAGGCCTAGGTGGAAGTGCCTGCTCCACAGTTACATACA
GGCTAGGGAGTGGGTAGGAGCCTAGGCCATAAGGTAAAGAGGACCAAAATACAGTAAATAATAACATAATAATAAGAA
ACCTTATGAATTCATTTCAGTGATATCCATATTGAGGTATTTAAGGTGAATGATACTGATGTCAGCAGTTTACCTGGATA
TGCATCAAAAATATGATTGATTGATGGATGGATCGGAGGAAGGAAAAGATATGTGATCAAAACGAGTATAGTAAATGTT
CATGGTGAATCTAGATATATAGATATCACTTGTAAATTTTAAAAATGTTATTGCATTTTGAATTTGTTATGTTAAAA
TATTGGGTGAAAATCTGATGCCCTGGCCATGTCCCAATCAGTTGAATCAGATCTCTGGTGTATTTTTTTTTTAAGATC
CTTGGATGACTCCATTGTACAGCAAGTTTGCAAACTACTATTCTAAGTCTCACCACCCCTTCTTTATAGCTTACAAAA
TTATATTTTAAATGTCAAATGTCACAATTTCACTTTCCCTTCTTCAAGAACTGATTAGACTTCTAGCTTCTCCTATGCTC
TCATCTTGCAATACGTTTCACTTGCACGTGGTTAAACTGATAGGCAATAAATAATAACCCATCTGTCTTTTCTGACCCA
ACATAACTAAAACCTTCTTCAAGGCGAAAATGAAGTATAATCTCACAGACAGAGTAGCTATAGACAACATAATCTTT
CTGGTCTCTCGTGGTGCATGGGACTATTCACTGCGCTCCCTGCTGTCTATTGGTGGACAGCCAGTCTTACTGTGAC
CCATACCATGGCTCTTGAGAAGCATGAAGCTGCCGAAATCGCAGTTGTAGGGTTAATGGAAAACATGGTGAAGTATAGCT
TACATATATACAAATTTTGGCACAAAATGAAGCCTAAGAAATTTGACCGGGCTGGGTGTGGTGACTCATGCCCTGTAATC
CCAGCACTTTGAGAGACCAAGGTGGGCGGATCACCTGAGGTGGGAGTTTCGAGACCAGACTGAGCAACATGGAGAAACCC

```

TCTCTACTTAAAATACAAAATTAGCCAGGCATGGTGGCGCATGCCTGTAACCCCAGCTACTCAGGAGGCTAAGGCAAGAG
AATCTCTTCCACCTGGAGGCATAGGTTGTGGTGAGAGAAGATCACACCATTGCACTCCAGCTGGGGCAACAAGAGCGAA
GCTCCGCTCTCAATAAAATAAAATAAAATAAAATAAAATAAAATAAAATAAAATAAAAGAAAAAGAAATGTACCAG
GAAGTGGATTGGGAATGCTCTATATATCTCCCCCTCCAAGAAGTTTTTTAATTTATTGTATTGTGAAAGATCTGCAGC
TATTTATTTTGTGTGGTACTCTTTTGCATAAACTACTTCTGCATAAATGAATCAAAAACCTTTGATATTCAATGAACCTT
CATAATGTTGATCTAGTACATCTTGCCCAACTAGCCCTTGGGCCATTCTTCCATGCCACTGGTTGAAAAATATGCTCCC
CACCCCGTAACATCAGCATCAGACCTCTGCAACCAGAAAAGATTCCATAGGTAATGAAATCCAAATGCTGGCATTGGAA
GTCAAACCAAGTGATTTTGAATGGAAGGCAAGGGGAAGTGCATGGAGCAAGTACAGCATGTGTACAACCTCAGTTCA
TACCCTCCTTAAAATACTGTGCAACTAACTGTCAATTGCCCATTTGATAGAGATAGTATCTTTTTCAGCTTTACACCTTC
CGAATTCACACACTGCTAGCCTCAGGACTAAGCGCTCAATAAATGTTTATCAAAAGATGAGTAATGAATCAGTGGTG
AAATAAAATGTACAAACACATACGCTTCATACACGTTTAGGAAGCATTCTAGTGAGCTTTGTGGATATTTTATTCTTA
AAGTTCTTTTTCTTCCATGTTTATCTTTATTTTAAAAATGTTGCTTCTGCTTATTTCTTTCTACGGCAG
CTATATTTACTTGCTGAGAGCACAGCATATACTGATGTTAATCAAACCTTTAGATGAAACACCTAAAATGATAGAAAAGA
ATCTGCTCTAATTAATAATAAAGCAACCCCTACAGGTTTAGACATGTGCCTTCCGGTGTGGGAAAGAAAAATTTAATG
AATGTAGTAGTTTTATGCCAAGAACATTTCCCTCGTGCACTGTGTTTTAATAAGATAGAAATATAAAATAGCAAAAGGGG
CCGACCTTTGTGATGATATTTACTCATAAGGTAGTAAGTCAGATGTGATGACATTTCTTTGACTTAGAGCTGCTATAC
TTGGGTCAGATTTTCAAGTTTCAAGTTTGCAGTGAAGTTGTCTTTTAAACATGGTGTCTCCTGGAACTGCCCTGCTCCC
ACAGTAAGCTTCCACGGGGTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTGGCGAGGGACGCGGCTGCTCTGGGC
GTGGTTCCGGGAAACGCAGCGGCGCCGACCCTGGGTCTCGCACATTCTTACGTCCGTTTCGACGCTCACCCGGATCTTC
GCCGCTACCTTTGTGGGCCCCCGGCGACGCTTCTGCTCGCCCCTAAGTCGGGAAGGTTCTTTCGGGTTCCGCGCGTG
CCGGACGTGACAAACGGAAGCCGACGCTCTACTAGTACCCTCGCAGACGGACAGCGCCAGGGAGCAATGGCAGCGCGCC
GACCGCATGGGTGTGAGCAATAGCGGCTGCTCAGCAGGCGCGCCGAGAGCAGCGCGCGGAAGGGCGGCTGCGGGAG
GCGGGGTGTGGGCGGTAGTGTGGGCCTGTTCTGCCCCGCGCGGTGTTCCGCATTCTGCAAGCCTCCGGAGCGCACGTC
GGCAGTCGGCTCCCTCGTTGACCGAATCACCGACCTCTCTCCACACGACTCTAGAGGATCCCCGGGTACCGGTGCGCA
CCATGGTGAGCAAGGGCGAGGAGCTGTTACCCGGGGTGGTGCCCATCTGCTGAGCTGGACGGCGACGTAACCGGCCAC
AAGTTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTTCTGTCACCCGCGCA
GCTGCCCTGCCCTGTGAGCCACCTCGTGACACCTGACCTACCGCGTGCAAGTGTTCAGCCGCTACCCGACCACTAGA
AGCAGCAGCACTTCTTCAAGTCCGCCATGCCCCAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAAC
TACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCTTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGA
GGACGGCAACATCTGGGGCAAGCTGGAGTACAACACAGCCACAACGCTCTATATCATGGCCGACAAGCAGAAGA
ACCGCATCAAGGTGAATTCAGATCCGCCACAACATCGAGGACGGCAGCTGCAGCTCGCCGACCCTACAGCAGAAC
ACCCCCATCGCGCAGCGCCCGCTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAGACCCCA
CGAGAAGCGCATCACATGGTCTGCTGGAGTTCTGTGACCGCCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGT
AAAGCGGCGCGACTCTAGAATTTTAAGCTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTC
CTTGACCTGGAAGGTGCCACTCCACTGTCTTTCTTAATAAAATGAGGAAATGTCATCGCATTGTCTGAGTAGGTGTC
ATTTCTATTTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGAGGATTGGGAAGACAATAGCAGGCATGCAGTGTACAG
GCTAGCGAGTGGGTAGGAGTGGGGGTGAAATCCTCTTAATGTTTATGGTGTCAAGTATGATTAACAACTTACGCTTAC
AGCCATACTCCTAATAAGGGGCCCTGGCATATTTAATTGATTTAACAATTTATCAAAAATAGATAAACTGAAATCTGC
CTTGAAATTAATTACTGTATCTCTATTTTATAAGAAAATATTTTGGACCCGTTCCTCTGCCTTATGGGTGCAACTCCC
GGCAGAAATAGGTCTATGCTCTCTGAAATAGTTCTTATTTTCTTTACTACTTATGACCTCTTATAGCTTAGAAGTTTTCC
TTGCTATTTCTAGTTGAAATGCTAATCTGGCATAATTTCTAGTTGAAATGCTAATCTGGCATAATTTCAGAACTAATTTT
CTGTTAATGCCACTGTGGAACATCTAAATTCCTCTTTTCAATAAATACTATATTTGTGTGTTGCAAAACACACAAATCA
TACTATATTTGTGTGTATATATAGTAAATTCCAACCTTATAAAAGTAAAATTTGGAATTTTTTTCTTTTGGAAAATTTAT
TTTATAATTGAGAATTATAAATGTCACCTTTTTTAAATGCTGCAACCTTTGAGATTGGTTTCAATAAAGTAAAACCTTAGTA
AACATTAAAGAAAATGATAGCTTGATATGTTCTACTAATATGGTAAATGAAAACCTTTTATGTGTGTATAGGCTCTGTGTTAA
TTATAACTACCTTCACAGGAAAAACAGTCTTGTGGAAGGTAATGGTGCCAGTGAGAGAAAAATAGAGAAAGTAGAATAGTA
TAAAGAGAGAAAATAGAAATTTCCCGTTTGAATAAAGCTAAGTAAAAAGTGAATTTGTTTCAAGTTTATTTATTTGT
AAGTGTGGTCTCATGCCATATATATTCAAAGATAGACAGAGATCTTAATCTTTAATTTTTTATGGCCAAAGAAAATGAGT
CCCATGTAAAAGGACATTTTCGTAAGCTGATCTCAAGTGGTGTATTTGATTTGCCGAGCAGCCTATGGAAGAAATAT
CATTTTGGCTGCCTAAGAAGAAAAATGTCATACCTTTCTATTTTTTATTCATACTCCGCATATTTGGAATTCGTATTCTC
ACCATACTTTTGGGAAGATAACACGAAATTGCCCTTACTTGCCCTCGAAATCAAAATCTGCCATTGTTTTAAAAATAAAATG
GCTTTTTTCATCTCATTTTTCTTTAGCAGAAAAAAGTAAGTTAAATTTTTTTGTAGCTGTGTTAAGTGTACTGTTCTCCC
TCGTTGAGAACGAAACAAAGACATATGATTCCATTTACTGTAATTTGTTTTGGTTTGGCCAATTACAATAGCAGTAAAATC
ATTTACACATAGTAAAATGTTTGAGCCTTAAAGGGCAAGAAGACTAAATTTAGCTGAAGATATACAACCTTTTATGTGCCA
ATGAGGAATGTATAAAATCAGTTATACATATTTTCTCATTCATAGATATTTGAAAATATTCATATATGTGTACATGTAT
TTGATAACTTAATATTTTCTAAATAACAGAAATATATACTGTATATATTTGGATATTATGATTATTTGTTATTTGCCAC
CATTGAGCACTTACTATTTGGGCTCAGAAAACTTCACTGTATTTTAAATTTCTATAACAACCCCTGTGAAATATATATTA
TGCCTATTTTCAAAATGAGAACTCTGAAATCAGGAAATTAATCACTTGCTGAAGTTTACAAAGCACTGTTAGGCAATAA
AACAGAAATTTCAAACCTCACGTTTGCTTGTCTTCAAATTTTGTATGTGTACAACCTGATTGTTTTACTACTTGTCTTAA
TTTCAAAAAAATCTTCATACTAAAAGATGATACTTTGGGAGTTCTAAAGAACATGTTTTTGGCCGGGCGCGGTGGCTCA
CGCCTGTAATCCCAGCACTTTGGGAGGCGGAGGCAGAGGATCACGAGGTGAGAGATCGAGACCGTCTGGCCAACAAG
GTGAAACCCGCTCTACTAAAAAATACAAAAAATAGCCGGCGTGGTGGCGGGCGCCTGTAGTCCCGCTACTCGGA
AGCTGAGGCGGGAGAAAGCGGTGAACCTGGGAGGCGAGCTGTAGTGAGCCGAGATCGCGCGCTGCACCTCCAGCCTGG
TTGACAGAGCGAGACTCCGTCTCAAAAATAACAAAACAAAACAAAACAAAACAAAACAAAACAAAACAAAACAAAAC
TATACTCATTTGAAGTGGATACCAATTTTGTATTAAAAATTAATTTATGGATAAAATTTGAATCTGCAAAAAATTAAGTGCA
ACATTTATTTTGGCACCTGTTATAGGAGGGTAACTCGAGGTAACCTCTTAATTAAGTGGCCTCATGGGCCCTCCGCTCA
CTGCCGCTTTCCAGTCGGGAAACCTGTCTGCGCAGCTGCATTAACATGGTGTATAGCTGTTTCTTGGGTATTTGGGCGCT
CTCCGCTTCTCGCTCACTGACTCGCTGCGCTCGGTGCTTGGGTAAGCCTGGGGTGCCTAATGAGCAAAAGGCCAGCA

AAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATC
GACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGC
TCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTC
ACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTACGCCCCGACC
GCTGCGCCTTATCCGGTAACATATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGT
AACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAG
AACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAA
CCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTG
ATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGAT
CTTCACCTAGATCCTTTTAAATTAATAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTT
ACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTG
TAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGAACCACGCTCACC GGCTCC
AGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAAGTGGTCTGCAACTTTATCCGCTCCATCCAGT
CTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCAGCAACGTTGTTGCCATTGCTACAGGC
ATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTACGCTCCGTTCCCAACGATCAAGGCGAGTTACATGATCCCC
CATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCCTCCGATCGTTGTGCAAGTAAGTTGGCCGAGTGTATCACTCA
TGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACC
AAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGGCCACATAG
CAGAACTTTAAAAGTGCTCATCATTGGAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCA
GTTTCGATGTAAACCACTCGTGACCCCACTGATCTTCAGCATCTTTTACTTTACCAGCGTTTCTGGGTGAGCAAAAACA
GGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCTCTTTTCAATATTA
TTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGGTTT
CGCGCACATTTCCCCGAAAAGTGCCAC

Supplementary Figure 24. Map and nucleotide sequence of the unmodified donor construct targeting the human *DMD* locus. DNA sequences sharing identity to the human *DMD* gene are indicated in orange; Magenta arrow, gRNA^{DMD} target site (TS); hPGK1 promoter, human phosphoglycerate kinase 1 gene (*PGK1*) regulatory sequences; EGFP, enhanced green fluorescence protein gene (*EGFP*) open reading frame; bGH1 poly(A) signal, bovine growth hormone gene (*GHI*) polyadenylation signal; AmpR, β -lactamase ampicillin resistance gene; ori, high-copy number ColE1 prokaryotic origin of replication.



```

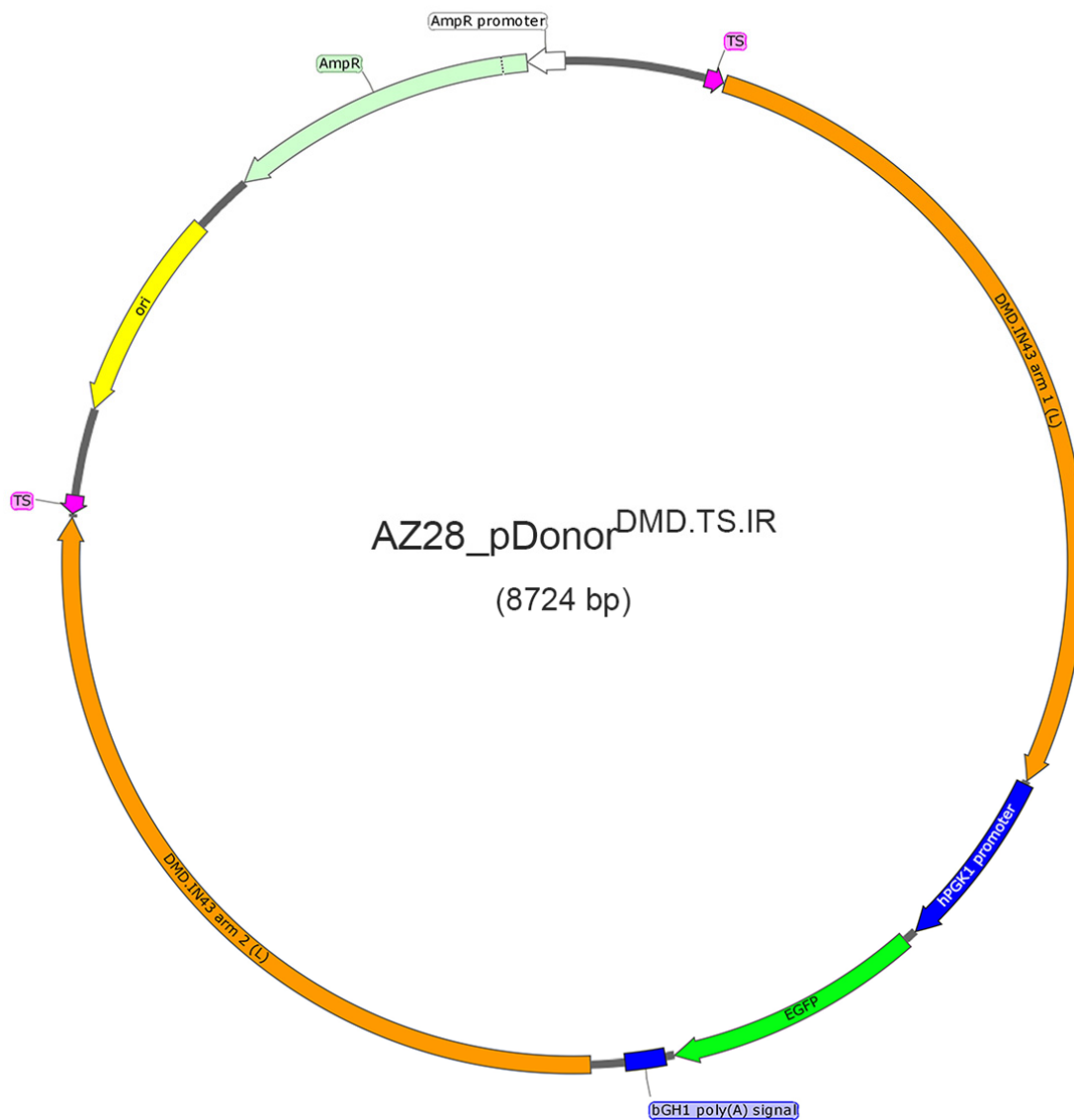
>AC62_pdonor.DMD.TS.DR (8724 bp)
CTAAATTGTAAGCGTTAATATTTTGTAAATTCGCGTTAAATTTTGTAAATCAGCTCATTTTTTAACCAATAGGCCG
AAATCGGC AAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATTCGCC
ATTGAGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGT
GCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTCCAGTCACGACGTTGTAAACGACGGCCAGTGAGCGCGACGT
AATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGCTCAAGGCC TAGGTGGAAGTGCCTGCCACAGTTACATACA
GGCTAGGGAGTGGTAGGAGCCTAGGCCATAAGGTAAAGAGGACACCAAATACAGTAAATAATAACATAATAATAAGAA
ACCTTATGAATTCATTTCAGTGATATCCATATTGAGGTATTTAAGGTGAATGATACTGATGTCAGCAGTTTACCTGGATA
TGCATCAAAAATATGATTGATTGATGGATGGATCGGAGGAAGGAAAAGATATGTGATCAAAACGAGTATAGTAAATGTT
CATGGTGAATCTAGATATATAGATATCACTTGTAAATTTTAAAAATGTTATTGCATTTTGAATTTGTTATGTTAAAA
TATTGGGTGAAAAATCTGATGCCCTGGCCATGTCCCAATCAGTTGAATCAGATCTCTGGTGTATTTTTTTTTTAAGATC
CTTGGATGACTCCATTGTACAGCAAGTTTGC AAACCACTATTCTAACTTCTCACCACCCCTTCTTTATAGCTTACAAA
TTATATTTTAAATGTCAAATGTCACAATTTCACTTTCCCTCTTCAAGAACTGATTAGACTTCTAGCTTCTCCTATGCTC
TCATCTTGCAATACGTTTCATTTCACGTGGTTAAAACTGATAGGCAATAAATAATAACCCATCTGTCTTTTCTGACCCA
ACATAACTAAAACCTTCTTCAAGGCGAAAATGAAGTATAATCTCACAGACAAGAGTAGCTATAGACAACATAATCTTT
CTGGTCTCTCGTGGTCATGGGACTATTCAACACTGCCGTCCTGCTGTCTATTGGTGGACCAGCCAGTTTACTGTGAC
CCATACCATGGCTCTTGAGAAGCATGAAGCTGCCGAAATCGCAGTTGTAGGGTTAATGAAAAACATGGTGAAGTATAGCT
TACATATATATACAATTTTGGCACAAAATGAAGCCTAAGAAATTGTACCGGGCTGGGTGTGGTGACTCATGCCTGTAATC

```

CCAGCACTTTTGAGAGACCAAGGTGGGCGGATCACCTGAGGTGCGGAGTTTCGAGACCAGACTGAGCAACATGGAGAAACCC
TCTCTACTTAAAAATACAAAAATAGCCAGGCATGGTGGCGCATGCCGTAAACCCAGCTACTCAGGAGGCTAAGGCAAGAG
AATCTCTTCCACCCTGGAGGCATAGGTTGTGGTGAGAGAAGATCACACCATTGCACTCCAGCTGGGGCAACAAGAGCGAA
GCTCCGTCTCAATAAAATAAAATAAAATAAAATAAAATAAAATAAAATAAAAGAAAAAGAAATTTGACCAG
GAAGTGGATTGGGAATGCTCTATATATCTCCCCCTCCAAGAAGTTTTTTTAAATTTATTGTATTGTGAAAGATCTGCAGC
TATTTATTTTGTGTGGTACTCTTTTGCATAAACTACTTCTGCATAAATGAACCAAACTTTGATATTTCATGAACCTT
CATAATGTTGATCTAGTACATCTTGCCCACTAGCCCTTGGGCCATTCCTTCCATGCCACTGGTTGAAAAATATGCTCCC
CACCCAGTAACATCAGCATCAGACCTCTGCAACCAGAAAGATTCCATAGGTAATGAAATCCAAATGCTGGCATTGGA
GTCAAACCAGTGTATTTTGAATGGAAAGGCAAGGGGAAGTGCATGGAGCAAGTACAGCATGTGTACAACCTCAGTTCA
TACCCTCCTTAAATACTGTGCAACTAACTGTCAATTGCCATTGATAGAGATAGTATCTTTTTCAGCTTTACACCTTC
CGAATTCAACACACTGCTAGCCTCAGGACTAAGCGCTCAATAAATGTTTATCAAAAGATTGAGTAAATGAATCAGTGGTG
AAATAAAATGTACAACACATACGCTTCATACACGTTTAGGAAGCATTTCTAGTGAGCTTTGTGGATATTTTATTTCTTA
AAGGTTCTTTTCTTTCCATGTTTCATGTTATTTCTTTATTTTAAATGTTGCTTCTGCTTATTTCTCTTTACGGCAG
CTATATTTACTTGCCTGAGAGCACAGCATATACTGATGTTAATCAAACTTTAGATGAAACACCTAAATGATAGAAAAGA
ATCTGCTCTAATTAATAATAAAGCAACCCCTACAGGTTTAGACATGTGCCTCCGGTGTGGGAAAGAAAAATTTAATG
AATGTAGTAGTTTTATGCCAAGAACATTTCCCTCGTGCACCTGTGTTTTAATAAGATAGAATATAAAATAGCAAAAGGGG
CCGCACTTTTGTGATGATATTACTCATAAGGTAGTAAGTCAGATGTGATGACATTTTCTTTGACTTAGAGCTGCTATAC
TTGGGTGAGATTTAGTTTCAGTAAATTTGCAGTGAAGTTGTCTTTCTAACATGGTGTCTCCTGGAACCTGCCTGCTCCC
ACAGTAAGCTTCCACGGGTTGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTGCAGCAGGACGCGGCTGCTCTGGGC
GTGGTTCCGGGAAACGCAGCGGCGCCGACCCTGGGTCTCGCACATTTCTACGTCCGTTTCGACGCTCACCCTGGATCTCT
CGCGTACCCTTGTGGGCCCCCGGCGACGCTTCCTGCTCCGCCCTAAGTCGGGAAGGTTTCTTGGGTTTCGCGGCTG
CCGCACTGTGACAAACGGAAGCGCACGCTCTCAGTAGTCTCGCAGACGGACAGCGCCAGGAGCAATGGCAGCGCGCC
GACCGCGATGGGCTGTGGCCAAATAGCGGCTGCTCAGCAGGCGCGCGGAGAGCAGCGCCGGGAAGGGGCGGTGCGGGAG
GCGGGGTGTGGGCGGTAGTGTGGGCCCTGTTCTGCCCCGCGGTTTCCGATTCTGCAAGCCTCCGAGCGCACGTC
GGCAGTCCGCTCCCTCGTTGACCGAATCACCGACCTCTCTCCCCACAGACTCTAGAGGATCCCCGGGTACCGGTGCGCA
CCATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCTGGTGCAGCTGGACGGCGACGTAACGGCCAC
AAGTTACGCGTGTCCGGCAGGGCGAGGCGATGCCACTACGCAAGCTGACCTGACCTGAAGTTTCATCTGCACACCGCCAA
GCTGCCCGTGCCCTGGCCCCACCCTCGTGACCAACCTGACCTACGGCGTGCACTGCTTCAGCCGCTACCCCGACCATGA
AGCAGCAGCACTTCTTCAAGTCCGCCATGCCCCAAGGCTACGTCCAGGAGCGACCATCTTCTTCAAGGACGACGGCAAC
TACAAGACCCGCGCGAGGTGAAGTTCGAGGGCGACACCCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGA
GACCGGCAACATCTCTGGGCAACAAGCTGGAGTACAACACAGCCACAACGCTCTATATCATGGCCGACAGCAGAAGA
ACGGCATCAAGGTGAAGTTCAAGATCCGCCACAACATCGAGGACGGCAGCTGCAGCTCGCCGACCATACAGCAGAAC
ACCCCATCGGCGACGGCCCCGTGCTGCTGCCGACAACCACTACCTGAGCACCAGTCCGCCCTGAGCAAAAGACCCCAA
CGAGAAGCGCATACATGGTCTGCTGGAGTTCTGTACCGCCGCGGGATCACTCTCGGCATGGACGAGCTGTACAAGT
AAAGCGGCGCGACTCTAGAAATTTAAGCTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCGTGCTTCT
CTTGACCTTGAAGGTGCCACTCCCCTGCTCTTCTTAATAAAATGAGGAATTTGCATCGCATTGTCTGAGTAGGTGTC
ATTCTATTCTAGTTGGGTTGGGTCAGGACAGCAAGGGGAGGATTGGGAAGACAATAGCAGGATGCTACAG
GCTAGGGAGTGGGTAGGAGTGGGGGTGAAATCCTCTTAATGTTTATGGTGTCACTAGATTCAAACATAAATAGCCTTAC
AGCCATACTCCTAATAAGGGGCCCTGGCATATTTAATTGATTTAACAATTTATCAAAATAGATAAACTGAAATCTGC
CTTGAAATTAATTAATCTGTATCTCTATTTTATAAGAAAATATTTTGGACCCGTCCCTCTGCCTTATGGGTGCAACTCCC
GGCAGAATAGGTCATGTCTCCTGAAATAGTTCTTATTTTCTTACTACTTATGACCTCTTATAGCCTAGAAGTTTTTCC
TTGCTATTCTAGTTGGGAGATAACACGAAATGGCTTACTTGCCCGAAATCAAAATCTGCCATTGCTTTTAAATAAAATG
CTGTTAATGCCACTTGAACATCTAAATTCCTCCTTTTTCAATAATACTATATTTGTGTGTTGCAAAACACACAAATCA
TACTATATTTGTGTATATATAGTAAATTTCAACTTATAAAAGTAAATTTGGAATTTTTTCTTTTGGAAATTTAT
TTTATAATTGAGAATTATAAATGTCATTTTTTAAATGCTGCAACCTTTGAGATTGGTTTCAATAAAGTAAACTTAGTA
AACATTAAGAAAATGATAGCTTGATATGTTCAATAATGGTAAATGAAAACCTTTATGTGTGTATAGGCTCTTGTAA
TTATAACTTACCTTACAGGAAAAACAGTCTTGTGGAAGGTAATGGTGCCAGTGAGAGAAAATAGAGAAAGTACATGA
GTAAGAGAGAAAAATAGAATTTCCCGTTTGAATAAAGCTAAGTAAAAAGTGAATTGTTTCAGTTTTATTTATGTATTTGT
AAGTGTGGTCTCATGCCATATATATTCAAAGATAGACAGAGATCTTAATCTTTAATTTTTTATGGCCAAAGAAAATGAGT
CCCATGTAAAAGGGACATTTTCGTAAGCTGATCTCAAGTGGTGTATTTTGATTGCGCGAGCAGCTATGGAAGAAATAT
CATTTTGGCTGCCTAAGAAGAAAAATGTCATACCTTTCTATTTTTTATTCTACTCCGCATATTTGGAATTCGTATTTCT
ACCATACTTTTGGGAAGATAACACGAAATGGCTTACTTGCCCTCGAAATCAAAATCTGCCATTGCTTTTAAATAAAATG
GCTTTTTCTATCTCATTTTTCTTTAGCAGAAAAAGTAAAGTTAAATTTTTTGTAGCTGTGTTAAGTGTACTGTTCTCCC
TCGTTGAGAACGAAACAAAGACATATGATTCCATTTACTGTAATTGTTTTGGTTTGGCCAATTACAATAGCAGTAAATC
ATTTACACATAGTAAATGTTTGAAGCTTAAAGGCAAGAAGACTAAATTTAGCTGAAGATATACAACTTTTCATGTGCCA
ATTGAGGAAATGTATAAAATCAGTTATACATATTTCTCATTCTAGATATTGAAAATATTTCATATATGTGTACATGTAT
TTGATAACTTAATATTTTCTAAATAACAGAAATTTATATCTGATATATATTTGGATATTTATGATTATTTGTTATGCCAC
CATTGAGCACTTACTATGGGCTCAGAAAACCTTCACTGTATTATTTTAAATTTCTATAACAACCTGTGAAATATATATTA
TGCCATTTTACAAATGAGAACTCTGAAATCAGGAAATTAATCATTGCTGAAGTTTACAAAGCACTGTTAGGCAATAA
AACAGAAATTTCAAACCTACGTTGTCTTCAAATTTGTATGCTGTACAACCTGATGTTTTTACTACTTGTCTTTAA
TTTCAAAAAAAATCTTCACTATAAAGATGATACTTTGGGAGTTCTAAAGAACATGTTTTTGGCCGGGCGCGGTGGCTCA
CGCCTGTAATCCCGCACTTTGGGAGCGCGAGGCAAGGATCACAGGTCAGGAGATCGAGACCTCCTGGCCCAACAG
GTGAAACCCCGTCTCTACTAAAAAATACAAAAAATAGCCGGGCGTGGTGGCGGGCGCCTGTAGTCCCAGCTACTCGGGA
AGCTGAGGCGGGAGAAAGGCGTGAACCTGGGAGGCGAGGCTGTAGTGAGCCGAGATCGCGCCGCTGCACTCCAGCCTGG
TTGACAGAGCGAGACTCCGTCTCAAAAATAACAAAACAAAACAAAACAAAACAAAACAAAACAAAACAAAACAAAACAAAAC
TATACTCATTTGAAGTGGATACCAATTATTTGTATTAATAATTAATTTATGGATAAAATTTGAATCTGCAAAAATTAAGTGCA
ACATATTTTTTTGGCACCCTGTATAGGAGGTAAGTCAAGTGAAGTGCCTGCTCCACAGTTACATACAGGCTAGGGA
GTGGGTAGGAGCTCGAGGGTACCTCTTAATTAAGTGGCCTCATGGGCTTCCGCTCACTGCCCGCTTTCCAGTCGGGAAA

CCTGTCGTGCCAGCTGCATTAACATGGTCATAGCTGTTTCCTTGCGTATTGGGCGCTCTCCGCTTCCTCGCTCACTGACT
 CGCTGCGCTCGGTGCTTCGGGTAAAGCCTGGGGTGCCCTAATGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGG
 CCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGA
 AACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCTGCCGCT
 TACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCCG
 TGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTAT
 CGTCTTGAGTCCAAACCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTA
 TGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTC
 TGCTGAAGCCAGTTACCTTCGGAAGAGATTGGTAGCTCTTGATCCGGCAAAACAAACCACCGCTGGTAGCGGTGGTTTT
 TTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGC
 TCAGTGAACGAAAACTCACGTTAAGGGATTTTGGTTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATT
 AAAAAATGAAGTTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCA
 CCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGG
 CTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGAACACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGC
 CAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCT
 AGAGTAAGTAGTTCGCCAGTTAATAGTTTGCAGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACAGCTCGTCGTT
 TGGTATGGCTTCATTCAGCTCCGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTA
 GCTCCTTCGGTCTCCGATCGTTGTGCAAGTAAGTTGGCCGAGTGTTATCACTCATGGTTATGGCAGCACTGCATAAT
 TCTCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGTAGTACTCAACCAAGTCATTCTGAGAATAGTGTAT
 GCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGTCTATCA
 TTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCA
 CCCAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAA
 GGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTATCAGGGTTATT
 GTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTG
 CCAC

Supplementary Figure 25. Map and nucleotide sequence of the unmodified donor construct targeting the human *DMD* locus. DNA sequences sharing identity to the human *DMD* gene are indicated in orange; Magenta arrows, gRNA^{DMD} target site (TS); hPGK1 promoter, human phosphoglycerate kinase 1 gene (*PGK1*) regulatory sequences; EGFP, enhanced green fluorescence protein gene (*EGFP*) open reading frame; bGH1 poly(A) signal, bovine growth hormone gene (*GHI*) polyadenylation signal; AmpR, β -lactamase ampicillin resistance gene; ori, high-copy number ColE1 prokaryotic origin of replication.

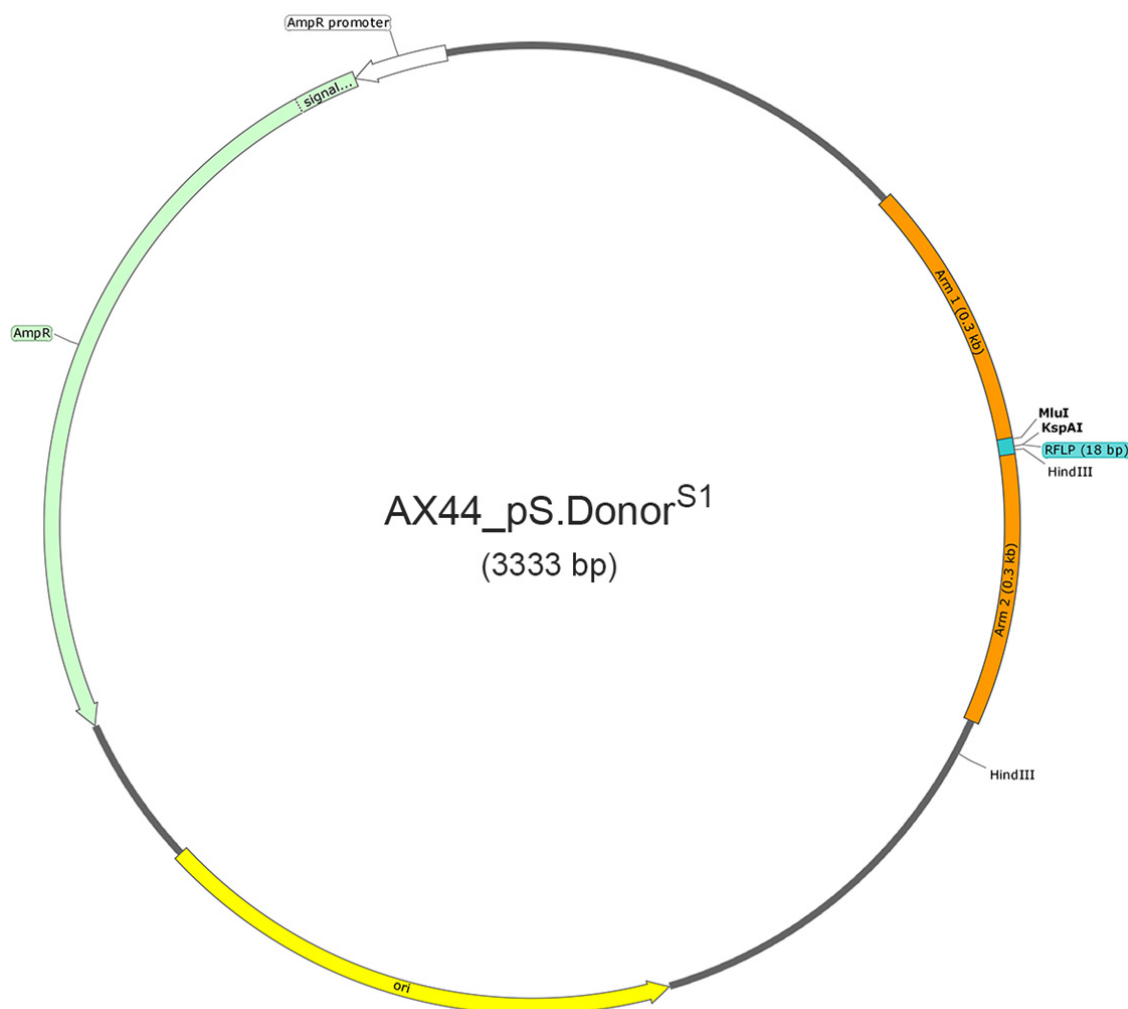


```
>AZ28_pdonor.DMD.TS.IR (8724 bp)
CTAAATGTGAAGCGTTAATATTTTGTAAAAATTCGCGTTAAATTTTGTAAATCAGCTCATTTTTTAACCAATAGGCCG
AAATCGGC AAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATTCGCC
ATTGAGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGT
GCTGCAAGGCGATTAAAGTTGGGTAACGCCAGGGTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGAGCGCGACGT
AATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAAGGCC TAGGTGGAAGTGCCTGCTCCACAGTTACATACA
GGCTAGGGAGTGGGTAGGAGCCTAGGCCATAAGGTAAGAGGACACCAAATACAGTAAATAATAACATAATAATAAGAA
ACCTTATGAATTCTATTTCAGTGATATCCATATTGAGGTATTTAAGGTGAATGATACTGATGTCAGCAGTTTACCTGGATA
TGCATCAAAAATATGATTGATTGATGGATGGATCGGAGGAAGGAAAAGATATGTGATCAAAACGAGTATAGTAAATGTT
CATGGTGGAAATCTAGATATATAGATATCACTTGTAAATTTTAAAAATGTTATTGCATTTTGAATTTGTTATGTTAAAA
TATTGGGTGAAAATTCTGATGCCCTGGCCATGTCCCAAATCAGTTGAATCAGATCTCTGGTGTATTTTTTTTTTAAGATC
CTTGGATGACTCCATTGTACAGCAAGTTTGCAAACCACTATTCTAACTTCTCACCACCCCTTCTTTATAGCTTACAAA
TTATATTTTAAATGTCAAATGTCACAATTTCACTTTCTTCTCAGAAGTATTAGACTTCTAGCTTCTCTCTATGCTC
TCATCTTGCAACGTTTCATTGTCAGCTGGTTAAACTGATAGGCAATAAATAATAACCCATCTGTCTTTTCTGACCCA
ACATAACTAAAACCTTCTTTCAAGGCGAAAATGAAGTATAATCTCACAGACAAGAGTAGCTATAGACAACATAATCTTT
CTGGTCTCTCGTGGTCATGGGACTATTCAACCACTGCCGTCCTGCTGTCTATTGGTGGACAGCCAGTTTACTGTGAC
CCATACCATGGCTCTTGAGAAGCATGAAGCTGCCGAAATCGCAGTTGTAGGGTTAATGGAAAACATGGTGAAGTATAGCT
TACATATATATACATTTTGGCACAAAATGAAGCCTAAGAAATGTACCGGGCTGGGTGTGGTGACTCATGCTGTAATC
CCAGCACTTTGAGAGACCAAGGTGGGCGGATCACCTGAGGTCGGGAGTTTCGAGACCAGACTGAGCAACATGGAGAAACCC
TCTCTACTTAAAAATACAAAATTAGCCAGGCATGGTGGCGCATGCCGTGAACCCAGCTACTCAGGAGGCTAAGGCAAGAG
AATCTCTTCCACCCTGGAGGCATAGGTTGTGGTGAGAGAAGATCACACCATTGCACTCCAGCTGGGGCAACAAGAGCGAA
```

GCTCCGCTCTCAATAAAATAAAATAAAATAAAATAAAATAAAATAAAATAAAATAAAAGAAAAAGAAATTGTACCAG
GAAGTGGATTGGGAATGCTCTATATATCTCCCCCTCCAAGAGTTTTTTTAATTTATTGTATTGTGAAAGATCTGCAGC
TATTTATTTTGTGTGGTACTCTTTTGAATAAACTACTTCTGCATAAATGAACCAAAAACCTTTGATATTTCATGAACCTT
CATAATGTTGATCTAGTACATCTTGCCCACTAGCCCTTGGGCCATTCTTCCATGCCACTGGTTGAAAAATATGCTCCC
CACCCAGTAACATCAGCATCAGACCTCTGCAACCAGAAAGATTCCATAGGTAATGAAATCCAAATGCTGGCATTGTGGAA
GTCAAACCAAGTGTATTTGAAATGGAAAGGCAAGGGGAAGTGCATGGAGCAAGTACAGCATGTGCTACAACCTCAGTTCA
TACCTCCTTAAATCTACTGTGCAACTAACTGTCAATTGCCCATTTGATAGAGATAGTATCTTTTTCAGCTTTACACCTTC
CGAATTCAACACACTGCTAGCCTCAGGACTAAGCGCTCAATAAATGTTTATCAAAAGATTGAGTAAATGAATCAGTGGTG
AAATAAAATGTACAAACACATACGCTTCATACACGTTTAGGAAGCATTTCTAGTGAGCTTTGTGGATATTTTATTTCTTA
AAGGTTCTTTTTCTTTCCATGTTTCATGTTATTTCTTTATTTTTTAAATGTTGCTTCTGCTTATTTCTCTTTACGGCAG
CTATATTTACTTGCTGAGAGCACAGCATATACTGATGTTAATCAAACCTTTAGATGAAACACCTAAAAATGATAGAAAAGA
ATCTGCTCTAATTTAAATAAATAAAGCAACCCCTACAGGTTTAGACATGTGCCCTCCGGTGTGGGAAAGAAAAATTTAATG
AATGTAGTAGTTTATGCCAAGAACATTTCCCTCGTGCACCTGTGTTTTAATAAGATAGAATATAAAATAGCAAAAGGGG
CCCGACTTTTGTGATGATATTTACTCATAAGGTAGTAAGTCAGATGTGATGACATTTTCTTTGACTTAGAGCTGCTATAC
TTGGGTGAGATTTTCAAGTTCAGTAAATTTGCAGTGAAGTTGCTTTTCAACATGGTGTCTCCTGGAACTGCCCTGCTCCC
ACAGTAAGCTTCCACGGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTGGCGAGGGACGCGGCTGCTCTGGG
GTGGTTCCGGGAAACGCAGCGCGCCGACCTGGGTCTCGACATTTCTCACGTCCGTTTCGACGCTCACCCGGATCTTC
GCCGCTACCTTTGTGGGCCCCCGGCGACGCTTCCTGCTCCGCCCTAAGTCGGGAAGGTTTCCTTGGGTTTCGCGGCTG
CCGGACGTGACAAACGGAAGCCGACGCTCTACTAGTACCTTCGACAGCGGACAGCGCCAGGGAGCAATGGCAGCGCGCC
GACCGCATGGGCTGTGGCCAAATAGCGGCTGCTCAGCAGGGCGCGCCGAGAGCAGCGGCCGGGAAGGGGCGGTGCGGGG
GCGGGGTGTGGGGCGGTAGTGTGGGCCCTGTTCTGCGCGCGGTGTTCCGCTTCTGCAAGCCTCCGGAGCGCACGCTC
GGCAGTCCGGTCCGTTTCGTTGACCGAATCACCGACCTCTCTCCACACGACTCTAGAGGATCCCGGGTACCCGGATCTTC
CCATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAACGGCCAC
AAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCTGAAGTTCATCTGCACCACCGGCAA
GCTGCCGTGCCCCGGCCCCACCTCGTGACACCTGACCTACGGCGTGACGTGCTTCAGCCGCTACCCCGACCATGA
AGCAGCAGCACTTCTTCAAGTCCGCCATGCCCCAAGGCTACGTCAGGAGCGCACCATCTTCTCAAGGACGACGGCAAC
TACAAAGACCCGCGCGAGGTGAAGTTCGAGGGCGACACCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCGGA
GGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACACAGCCACAACGCTCTATATCATGGCCGACAAGCAGAAGA
ACGGCATCAAGGTGAACCTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAAC
ACCCCCATCGGGCAGCGCCCCGTGCTGCTGCCGACAACCACTACCTGAGCAGCCAGTCCGCCCTGAGCAAAAGACCCCAA
CGAAGACCGCATCACATGGTCTGCTGGAGTTCGTGACCGCGCGGGATCACTCTCGGCATGGACGAGGTGTAACAGT
AAAGCGCGCGCATCTAGAAATTTAAGCTGTGCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCGTCTCTTC
CTTGACCTGGAAGGTGCCACTCCACTGTCTTTTCTAATAAAATGAGGAAATTCATCGCATTGTCTGAGTAGGTGTC
ATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGAGGATTTGGGAAGACAATAGCAGGCATGCACGTGTACAG
GCTAGGAGTGGGTAGGAGTGGGGGTGAAATCCTCTTAATGTTTATGGTGTGATGATTCAAACTAAATTTAGCCTTAC
AGCCATACTCCTAATAAGGGGCCCCCTGGCATATTTAATTGATTTAACAATTTATCAAAAATAGATAAACTGAAATCTGC
CTTGAAATTTAATCTCTCTATTTTATAAGAAAATTTTGGACCCGTCCCTCTGCCTTATGGGTGCAACTCCC
GGCAGAATAGGTGATGCTCTCTGAAAATAGTTCTTATTTTCTTACTACTTATGACCTCTTATAGCCTAGAAGTTTTTCC
TTGCTATTCTAGTTGAAATGCTAATCTGGCATAATTTCTAGTTGAAATGCTAATCTGGCATAATTTCAGAATAATTTT
CTGTTAATGCCACTTGAACATCTAAATTCCTCCTTTTTCAATAATACTATATTTGTGTGTTGCAACACACACAAATCA
TACTATATTTGTGTATATATAGTAAATCCAACTTATAAAAGTAAATTTGGAATTTTTTCTTTTGGAAAATTTAT
TTTATAATTGAGAATTATAAATGTCACTTTTTTAAATGCTGCAACCTTTGAGATTGGTTTCAATAAAGTAAACTTAGTA
AACATTAAGAAAAATGATAGCTTGATATGTTCACTAATATGGTAAATGAAAACCTTTTATGTGTGTATAGGCTCTGTAA
TTATACTACCTTCACAGGAAAAACAGTCTTGTGGAAGGTAATGGTGCCAGTGAGAGAAAATAGAGAAAGTAGAATAGTA
GTAAGAGAGAAAAATGAATTTCCCGTTTGAATAAAGCTAAGTAAAAAGTGAATGTTTCAGTTTTATTTATGATTTTGT
AAGTGTGGTCTCATGCCATATATTTCAAAGATAGACAGAGATCTTAATCTTTAATTTTTTATGGCCAAAGAAAATGAGT
CCCATGTAAAAAGGACATTTTCGTAAGCTGATCTCAAGTGGTGATTTTGGATTGTCGCGCAGCAGCTTGGAGAAATAT
CATTTTGGCTGCCTAAGAAGAAAAATGTCATACCTTTCTATTTTTTATTCATACTCCGCATATTTGGAATTCGTATTCTC
ACCATACTTTTGGGAAGATAACACGAAATTCCTTACTTGCTCGAAATCAAAATCTGCCATTGTTTTAAATAAAATG
GCTTTTTCATCTCATTTTCTTTAGCAGAAAAAGTAAGTTAAATTTTTTGTAGCTGTGTTAAGTGTACTGTCTCTCC
TCGTTGAGAACGAAACAAAGACATATGATTCCATTTACTGTAATGTTTTGGTTTGGCCAATTACAATAGCAGTAAATC
ATTTACACATAGTAAATGTTTGAGCCTTAAAGGGCAAGAGACTAAATTTAGCTGAAGATATACAACTTTTCATGTGCCA
ATGAGGAAATGTATAAAATCAGTTATACATATTTTCTCATTCATAGATATTGAAAATATTCATATATGTGTACATGTAT
TTGATACTTAATATTTTCTAAATAACAGAAATTATATACTGTATATATTTGGATATTATGATTATTGTTATTGCCAC
CATTTAGCACTTACTATGGGCTCAGAAAACCTCACTGTATATTTTTAATTTCTATAACAACCCGTGAAATATATATTA
TGCCATTTTCAAAATGAGAACTCTGAAATCAGGAAATTAATCACTTGTCTGAAGTTTACAAAGCACTGTTAGGCAATAA
AACAGAAATTTCAAACTCAGTTTGTCTTCAAAATTTGTATGCTGTACAACCTGATTGTTTTTCACTACTTGTCTTAA
TTTCAAAAAAATCTTCACTAAAAGATGATACTTTGGGAGTTCTAAAGAACATGTTTTTGGCCGGGCGCGGTGGCTCA
CGCCTGTAATCCAGCACTTTGGGAGGCCGAGGCAGAAGGATCACGAGGTGAGAGATCGAGACCGTCTGGCCAACAAG
GTGAAACCCCGTCTCTACTAAAAAATACAAAAAATAGCCGGGCGTGGTGGCGGGCGCTGTAGTCCAGCTACTCGGGA
AGCTGAGGCGGGAGAAAGCGGTGAACCTGGGAGGCAGAGCTGTAGTGAGCCGAGATCGCGCCGTGCACCTCCAGCTGG
TTGACAGAGCGAGACTCCGTCTCAAAAATAACAAAAACAAAAACAAAAAACCATTGTTTTTCTTTTCAAGAGG
TATACTCATTTGAAGTGATACCAATATTTGTATTTAAATTTACTTATGGATAAAATTTGAATCTGCAAAAAATTAAGTGCA
ACATTATTTTTTGGCACCTGTTATAGGAGGTAACCTGAGCTCCTACCCACTCCCTAGCTGTATGTAAGTGTGGGAGCA
GGGCAGTTCCACTCGAGGTACCTCTTAATTAACCTGGCCTCATGGGCTTCCGCTCACTGCCCGCTTTCCAGTCGGGAAA
CCTGTCGTGCCAGCTGCATTAACATGGTCATAGCTGTTTCTTTCGCTATTGGGCGCTCTCCGCTTCTCGCTCACTGACT
CGCTCGCTCGTTCGGGTAAAGCTGGGGTGCTTAATGAGCAAAAGGCCAGCAAAAGCCGAGGAAACCGTAAAGAGG
CCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGA

AACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCT
TACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCCG
TG TAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTTACGCCCCGACCGCTGCGCCTTATCCGGTAACATAT
CGTCTTGAAGTCCAAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTA
TG TAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTC
TGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTT
TTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGC
TCAGTGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATT
AAAAATGAAGTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCA
CCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGG
CTTACCATCTGGCCCCAGTGTGCAATGATACCGCGAGAACCGCTCACCGGCTCCAGATTTATCAGCAATAAACACAGC
CAGCCGGAAGGGCCGAGCGCAGAAAGTGGTCTTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGAAGCT
AGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACAGCTCGTCGTT
TGGTATGGCTTCATTCAGCTCCGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTA
GCTCCTTCGGTCCCTCCGATCGTTGTCAGAAGTAAGTTGGCGCAGTGTATCACTCATGGTTATGGCAGCACTGCATAAT
TCTCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTAT
GCGGCGACCGAGTTGCTCTTGCCCGCGTCAATACGGGATAATACCGGCCACATAGCAGAAGTTTAAAGTGCTCATCA
TTGGAAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCA
CCCAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAA
GGGAATAAGGGCGACACGGAATGTTGAATACTCATCTCTTCTTTTCAATATTATTGAAGCATTATCAGGGTTATT
GTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTG
CCAC

Supplementary Figure 26. Map and nucleotide sequence of the unmodified donor construct targeting the human *DMD* locus. DNA sequences sharing identity to the human *DMD* gene are indicated in orange; Magenta arrows, gRNA^{DMD} target site (TS); hPGK1 promoter, human phosphoglycerate kinase 1 gene (*PGK1*) regulatory sequences; EGFP, enhanced green fluorescence protein gene (*EGFP*) open reading frame; bGH1 poly(A) signal, bovine growth hormone gene (*GHI*) polyadenylation signal; AmpR, β -lactamase ampicillin resistance gene; ori, high-copy number ColE1 prokaryotic origin of replication.

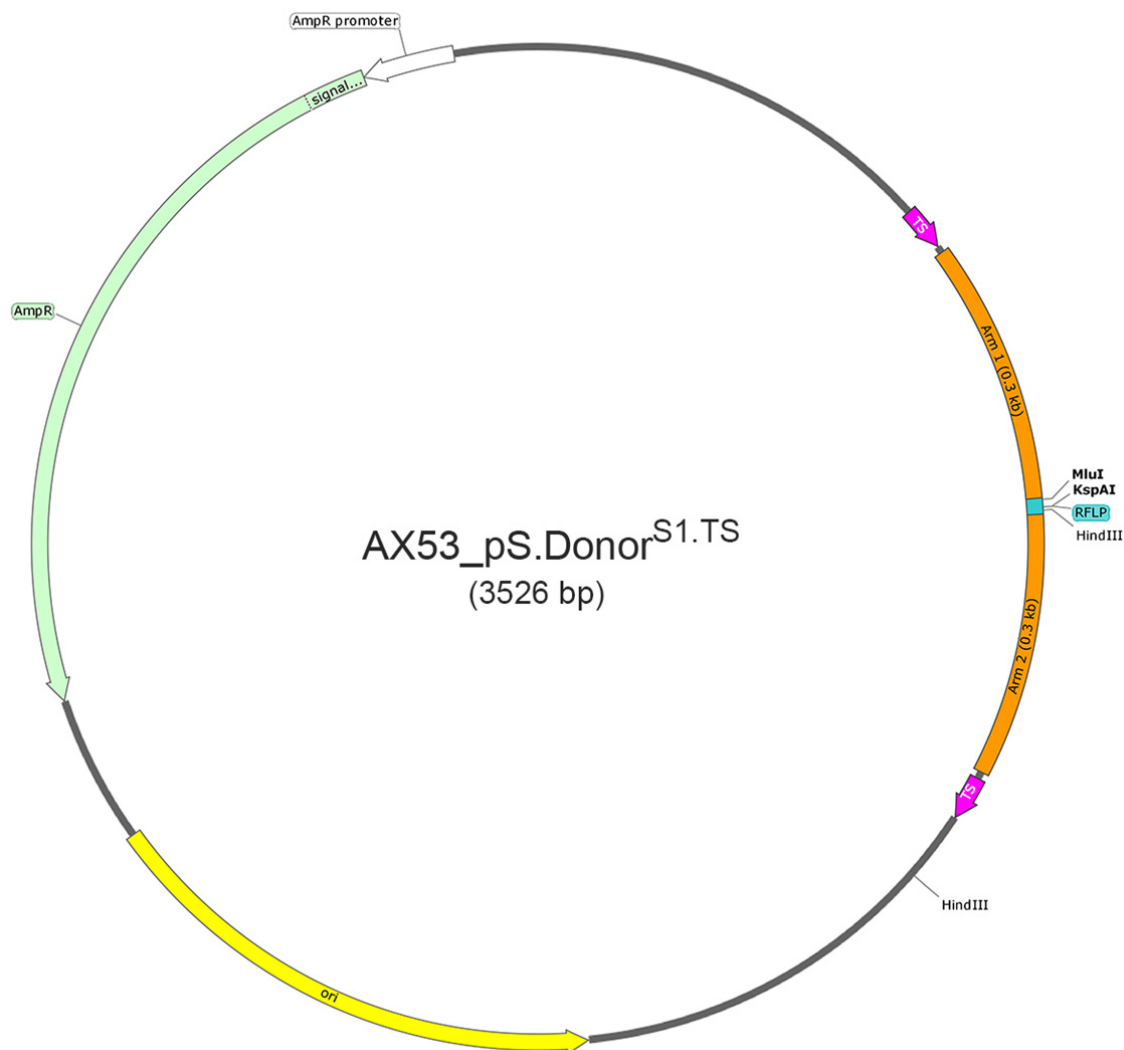


>AX44_pS.Donor.S1 (3333 bp)

```
TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGAT
GCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGCGGGCTGGCTTAACTATGCGGCATCAGA
GCAGATTGTAAGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGCGCC
ATTCGCCATTTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGG
GGATGTGCTGCAAGGCGATTAAAGTTGGGTAACGCCAGGGGTTTCCAGTCACGACGTTGTAAGAACGACGGCCAGTGAATT
CGAGCTCGGTACCTCGCGAATGCATCTAGATGGCCGCGGATTGGGTACCTCTCACTCCTTTCAATTGGGCAGCTCCCC
TACCCCTTACCTCTCTAGTCTGTGCTAGCTCTCCAGCCCCCTGTCATGGCATCTTCCAGGGGTCCGAGAGCTCAGCT
AGTCTTCTTCTTCCAAACCCGGGCCCCATGTCCACTTCAGGACAGCATGTTTGTGCTCCAGGGATCCTGTGTCCCGGA
GCTGGGACCACTTATATCCAGGGCCGGTTAATGTGGCTCTGGTCTGGGTACTTTTATCTGTCCCTCCACCCACA
GTGGGGCCACTAGGGACACGCGTGTAAACAAGCTTAGGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT
AGTCTCCTGATATTGGGTCTAACCCCACTCCTGTTAGGCAGATTCTTATCTGGTGACACACCCCACTTCTCCTGGAGC
CATCTCTCTCCTTCCAGAACCTCTAAGGTTTGCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGGG
GGTGGGAGGGAAGGGGGGATGCGTGACCTGCCCGGTTCTCAGTGGCCACCCTGCGCTACCTCTCCAGAACCTGAGCT
GCTCTGACGCGGCCATCGGATCCCGGGCCGTCGACTGCAGAGGCCATGTCATGCAAGCTTGGCGTAATCATGTCATAGCT
GTTTCTCTGTGTAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTG
CCTAATGAGTGAGCTAACTACATTAATTGCGTTGCGCTCACTGCCCGCTTCCAGTCGGGAAACCTGTCGTGCCAGCTG
CATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTTCCGCTTCTCCTCGCTCACTGACTCGCT
GCGCTCGGTGCTTCCGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATA
ACGAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCAT
AGGCTCCGCCCCCTGACGAGCATCAGAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAGATA
CCAGGCGTTTCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTTC
TCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTACGCTTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAGCTG
GGCTGTGTGCAGAACCCCGGTTTCCAGCCGACCGCTGCGCCTTATCCGGTAACCTATCGTCTTGAAGTCCAAACCCGTAAG
ACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCT
```

TGAAGTGGTGGCCTAACTACGGCTACACTAGAGAAGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGA
 AAAAGAGTTGGTAGCTCTTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGGTTTGCAAGCAGCAGATTAC
 GCGCAGAAAAAAGGATCTCAAGAAGATCCTTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTT
 AAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATC
 TAAAGTATATATAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATT
 TCGTTTCATCCATAGTTGCCGTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTG
 CAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGA
 AGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAA
 TAGTTTGGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCG
 GTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGTCCTCCGATCGTT
 GTCAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTTCTACTGTTCATGCCATCCGT
 AAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCC
 CGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAAGTTTAAAGTGCTCATCATTTGAAAAACGTTCTTCGGGGCGA
 AAATCTCAAGGATCTTACCGCTGTTGAGATCCAGTTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTT
 TACTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAAAT
 GTTGAATACTCATACTCTTCCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATT
 GAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCAT
 TATTATCATGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTTCGTC

Supplementary Figure 27. Map and nucleotide sequence of *AAVS1*-targeting plasmid AX44_pS.Donor^{S1}. Orange regions, sequences homologous to the human *AAVS1* “safe harbour” locus; RFLP, restriction fragment length polymorphism; AmpR, β -lactamase ampicillin resistance gene; high-copy number ColE1 origin of replication.



>AX53_pS.Donor.S1.TS (3526 bp)
 TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGAT
 GCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTTCGGGGCTGGCTTAACTATGCGGCATCAGA
 GCAGATTGTACTGAGAGTGCAACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGCGCC
 ATTCGCCATTTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGG
 GGATGTGCTGCAAGGCGATTAAAGTTGGGTAACGCCAGGGTTTCCAGTCACGACGTTGTAAACGACGGCCAGTGAATT
 CGAGCTCGGTACCTCGCGAATGCATCTAGATATCTAGGGATAACAGGGTAATGTCGAGGCCGGATTAAATCTGTCCCTT
 CCACCCACAGTGGGGCCACTAGGGACAGGATTGGTGACAGAGCGCCGCGGATTTCGGGTCACCTCTCACTCCTTTTCATT
 TGGGCAGCTCCCCCTACCCCTTACCTCTCTAGTCTGTGCTAGCTCTTCCAGCCCCCTGTCATGGCATCTTCCAGGGGTC
 CGAGAGCTCAGCTAGTCTTCTTCTCCTCAACCCGGGCCCTATGTCCACTTCAGGACAGCATGTTTGTGCTCCAGGGAT
 CCTGTGTCCTCCGAGCTGGGACACCTTATATTCCAGGGCGGGTAAATGTGGCTCTGGTCTGGGTACTTTTATCTGTCC
 CCTCCACCCACAGTGGGGCCACTAGGGACACGCGTGTAAACAGCTTAGGATTGGTGACAGAAAAGCCCCATCCTTAGG
 CCTCCTCCTTCTTAGTCTCCTGATATTGGGTCTAACCCCACTCCTGTTAGGCAGATTCTTATCTGGTGACACACCCCT
 CATTTCTGGAGCCATCTCTCTCCTTGCCAGAACCTCTAAGGTTTGCCTTACGATGGAGCCAGAGAGGATCCTGGGAGGGA
 GAGCTTGGCAGGGGTGGGAGGGAAGGGGGGATGCGTGACCTGCCCCGTTCTCAGTGGCCACCCTGCGCTACCTCTCC
 CAGAACCTGAGCTGCTCTGACGCGGCCGCGCCGCGCTGTCCCTCCACCCACAGTGGGGCCACTAGGGACAGGATTGG
 TGACAGAGTTTAACTAGGGATAACAGGGTAATGTCGAGGCCGGGATATCGGATCCCGGGCCCGTCTGACTGCAGAGGCC
 GCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCCTGTGTGAAATTGTTATCCGCTCACAATTCACACAACATAC
 GAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCC
 GCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGGGGAGAGGCGGTTTTCGTATTGG
 GCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGG
 CGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAAC
 CGTAAAAAGGCCGCTTGGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCA
 GAGGTGGCGAAACCCGACAGGACTATAAAGATACAGGCGTTTCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGA
 CCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCAGCTGTAGGTAT

CTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTAGCCCGACCGCTGCGCCTTATC
CGGTAACATATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCA
GAGCGAGGTATGTAGGCGGTGCTACAGAGTTCCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGT
ATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAG
CGGTGGTTTTTTTGTTTGCAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGG
GGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATC
CTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAAT
CAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCGTCGTGTAGATAAATACGA
TACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCAGGCTCCAGATTTATCAGCA
ATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTG
CCGGGAAGCTAGAGTAAGTAGTTTCGCCAGTTAATAGTTTGCACAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTAC
GCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAA
AAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTGTCAGAAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGC
ACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAG
AATAGTGTATGCGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAA
GTGCTCATCATTTGAAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTTCGATGTAACC
CACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATG
CCGCAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTTCCTTTTCAATATTATTGAAGCATTAT
CAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGGTTCCGCGCACATTTCC
CCGAAAAGTGCCACCTGACGTCTAAGAAACATTATTATCATGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCT
TTCGTC

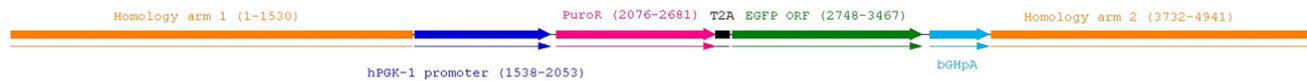
Supplementary Figure 28. Map and nucleotide sequence of *AAVSI*-targeting plasmid AX53_pS.Donor^{S1.TS}. Orange regions, sequences homologous to the human *AAVSI* “safe harbour” locus; RFLP, restriction fragment length polymorphism; Magenta arrows, gRNA^{S1} target site (TS); AmpR, *β-lactamase* ampicillin resistance gene; high-copy number ColE1 origin of replication.

ACCCCCCGTTTACGCCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGC
 CACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCT
 AACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAG
 CTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAG
 GATCTCAAGAAGATCCTTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTC
 ATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAATGAAGTTTAAATCAATCTAAAGTATATATGA
 GTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAG
 TTGCCTGACTCCCCGTGCTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGA
 GACCCACGCTCACCAGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAAC
 TTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCCGCCAGTTAATAGTTTGCACAACG
 TTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTAGCTCCGGTTCCCAACGATCA
 AGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGTTAGCTCCTTCGGTCCTCCGATCGTTGTGAGAAGTAAGTT
 GGCCGCGAGTGTTTACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTG
 TGAAGTGGTGAAGTCAACCAAGTCATCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGG
 GATAATACCGCGCCACATAGCAGAACTTTAAAGTGCTCATCATTTGGAACGTTCTTCGGGGCGAAAACTCTCAAGGAT
 CTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCAACTGATCTTCAGCATCTTTTACTTTTACCAGCG
 TTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGAATAAGGGCGACACGGAATGTTGAATACTCATA
 CTCTTCCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTGGAATGTATTTAGAA
 AAATAACAAATAGGGGTTCGCGCACATTTCCCGAAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACAT
 TAACCTATAAAAAATAGGCGTATCACGAGGCCCTTTCGTC

Supplementary Figure 29. Map and nucleotide sequence of *CCR5*-targeting plasmid AY42_pS.Donor^{R5}. Orange regions, sequences homologous to the human *CCR5* “safe harbour” locus; RFLP, restriction fragment length polymorphism; AmpR, *β*-lactamase ampicillin resistance gene; high-copy number ColE1 origin of replication.

GAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGG
AAGTCCCTCGTGCCTCTCCTGTTCCGACCCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGG
CGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCC
CCCGTTACAGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACT
GGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACT
ACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCT
TGATCCGGCAAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATC
TCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGA
GATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAA
ACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGC
CTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACC
CACGCTCACCGGCTCCAGATTTATCAGCAATAAACCCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTA
TCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCACAACGTTGT
TGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGC
GAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGTCTCCTTCGGTCCCTCCGATCGTTGTCAGAAGTAAGTTGGCC
GCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCTATGCCATCCGTAAGATGCTTTTCTGTGAC
TGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATA
ATACCGCGCCACATAGCAGAAGCTTTAAAAGTGCTCATCATTGGAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTA
CCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTC
TGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCT
TCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAAT
AAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAAC
CTATAAAAATAGGCGTATCACGAGGCCCTTTTCGTC

Supplementary Figure 30. Map and nucleotide sequence of *CCR5*-targeting plasmid AY10_pS.Donor^{R5.TS}. Orange regions, sequences homologous to the human *AAVS1* “safe harbour” locus; RFLP, restriction fragment length polymorphism; Magenta arrows, gRNA^{R5.1} target site (TS); AmpR, *β-lactamase* ampicillin resistance gene; high-copy number ColE1 origin of replication.



SEQUENCE

TCGACCCCGCCGCGCCGCGCCCGCCGCGCCGTGCTGGACTCCACCAACGCCGACGGTATCAGCGCCCTGCACCAGGTCAGC
 GCCCCCGCCGCGCTCTCCCGGGGCAGGTCCACCCTCTGCTGCGCCACCTGGGGCATCCTCCTTCCCCGTGCGCAGTCTCG
 ATCCGCCCCGTCGTTCTGGCCCTGGGCTTTGCCACCCTATGCTGACACCCCGTCCCAGTCCCCCTTACCATTCCCCCTCGAC
 CACCCCACTTCCGAATTGGAGCCGCTTCAACTGGCCCTGGGCTTAGCCACTCTGTGCTGACCACTCTGCCCCAGGCCTCCTTA
 CCATTCCCCCTTCGACCTACTCTCTTCCGATTGGAGTCGCTTAACTGGCCCTGGCTTTGGCAGCCTGTGCTGACCCATGCAG
 TCCTCCTTACCATCCCTCCCTCGACTTCCCTCTTCCGATGTTGAGCCCCCTCAGCCGGTCTTGACTTTGTCTCCTTCCCTG
 CCCTGCCCTCTCCTGAACCTGAGCCAGCTCCCATAGCTCAGTCTGTGCTATCTGCCTGGCCCTGGCCATTGTCACTTTGGCT
 GCCCTCCTCTCGCCCCGAGTGCCCTTGTGTGCGCGCCGAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGAC
 CACTTTGAGCTCTACTGGCTTCTGCGCGCCCTCTGGCCCACTGTTTCCCCCTTCCCAGGCAGGTCTTGCTTTCTCTGACCTGCA
 TTCTCTCCCCCTGGGCTGTGCGGCTTCTGTCTGCAGCTTGTGGCTGGGTCACTCTACGGCTGGCCAGATCCTTCCCTGC
 CGCCTCCTTCAGGTTCCGCTTCTCTCCACTCCCTCTTCCCTTGTCTCTGTGTGTGTGTGCTGCCCCAAGGATGCTCTTCCGGA
 GCATTCTCTTCTCGGCGCTGCACCAGTGATGTCTCTGAGCGGATCCTCCCCGTGTCTGGGTCTCTCCGGGCATCTCTCT
 CCCTCACCAACCCATGCCGTCTTCACTCGCTGGGTTCCTTTTCTCTCTTCTGCGGCTGTGCCATCTCTCGTTTCTT
 AGGATGGCCTTCTCCGACGGATGTCTCCCTTGGCTCCCGCTCCCTTCTCTTGTAGGCCTGCATCATCACCGTTTTCTGGACA
 ACCCAAAGTACCCGCTCTCCCTGGCTTTAGCCACCTCTCCATCCTCTTGCTTTCTTTGCCTGGACACCCCGTTCTCTGTGG
 ATTCGGGTCACTCTCACTCCTTTTCAATTGGGCAGCTCCCTACCCCCCTTACCTCTCTAGTCTGTGCTAGCTCTTCCAGCCC
 CCTGTCTATGGCATCTTCCAGGGGTCCGAGAGCTCAGTAGTCTTCTTCTTCCCAACCCGGGCCCTATGTCCACTTCAGGACAG
 CATGTTTGTGCTGCCCTCCAGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATCCCAGGGCCGGTTAATGTGGCTCTGGTTC
 TGGGTACTTTTATCTGTCCCTCCACCCACAGTGGGGCAAGCTTCCACGGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCT
 GGGTTTGGCAGGACGCGGCTGTCTGGGCGTGGTTCCGGGAAACGACGCGCGCCGACCCTGGGTCTCGCACATTCTTCAC
 GTCCGTTTCGACGCTACCCGGATCTTCGCGGCTACCTTGTGGGCCCCCGCGACGCTTCTGTCTCGCCCTTAAGTCGGG
 AAGGTTCTTTCGGGTTTCGCGGCGTGGCGGACGTGACAAACGGAAGCCGACGCTCTCACTAGTACCCTCGCAGACGGACAGCGC
 CAGGGAGCAATGGCAGCGCGCCGACCGCATGGGCTGTGGCCAATAGCGGTGCTCAGCAGGGCGCGCCGAGAGCAGCGGCCG
 GGAAGGGGCGGTGCGGGAGGCGGGGTGTGGGGCGGTAGTGTGGGCCTGTTCCTGCCCCGCGGTTCTCCGATTCTGCAAGC
 CTCCGGAGCGCAGTTCGGCAGTCCGCTCCCTCGTTGACCGAATCACGACCTCTCTCCCCACGGGTACGTCGCTAGCCACC
 ATGACCGAGTACAAGCCACGGTGCGCTCGCCACCCGCGACGACGTCCCCCGGGCCGTACGCACCCTCGCCGCCGCTTCGC
 CGACTACCCGCCACGCGCCACACCGTCGACCCGGACCGCCACATCGAGCGGGTCACCGAGCTGCAAGAATCTTCTCTACGC
 GCGTGGGCTCGACATCGGCAAGGTGTGGGTGCGGACGACGGCGCGCGGTGGCGGTCTGGACCACGCCGAGAGCGTCGAA
 GCGGGGCGGTGTTCGCGAGATCGGCGCGCATGGCCGAGTTGAGCGGTTCCTCGGCTGGCCGCGCAGCAACAGATGGAAGG
 CCTCTGGCGCCGACCGGCCCAAGGAGCCCGGTGGTTCTGGCCACCGTCGGCGTCTCGCCGACCAACAGGGCAAGGGTC
 TGGGCAGCGCGTGTGCTCCCCGAGTGGAGGCGGCCGAGCGCGCGGGGTGCCGCTTCTGGAGACCTCCGCGCCCCGC
 AACCTCCCCCTTCTACGAGCGCTCGGCTTACCGTCAACGCCGACGTCGAGGTGCCCGAAGGACCGCGCACCTGGTGATGAC
 CCGCAAGCCCGGTGCCGATCGGGAGAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAGGAGAATCTGGCCCCACCGG
 TCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGC
 CACAAGTTCAGCGTGTCCGGCAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCTGAAGTTTCACTGCAACACCGGCAA
 GCTGCCCGTGCCCTGGCCACCCCTCGTGACCAACCTGACCTACGGCGTGCAGTGCTTACGCCGTACCCCGACCATGAAGC
 AGCAGACTTCTTCAAGTCCGCCATGCCGAAGGCTACGTCCAGGAGCGACCATCTTCTTCAAGGACGACGGCAACTACAAG
 ACCCGCGCCGAGGTGAAGTTTCGAGGGCGACACCTGGTGACCCGATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAA
 CATCTGGGGCACAGCTGGAGTACAACATAACAGCCACAACGCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGG
 TGAAGTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGCGAC
 GGCCCCGTGCTGTGCTGCCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCATCAT
 GGTCTGTGAGGTTCTGTACCGCGCGCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCGCGTCTGAGTCTA
 GGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCTTGACCTGGAAGGTGC
 CACTCCCCTGTCTTTCTTAATAAAATGAGGAAATGTCATCGCATGTCTGAGTAGGTGTATTCTATTCTGGGGGTGGGG
 TGGGGCAGGACAGCAAGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAAGCTTTACTA
 GGGACAGGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCTAGTCTCCTGATATTGGGTCTAACCCCCACCTCC
 TGTTAGGCAGATTCTTATCTGGTGACACACCCCAATTTCTTGAGCCATCTCTCTCCTTGCCAGAACCTCTAAGGTTTGCTT
 ACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGGGGTGGGAGGGAAGGGGGGATGCGTGACCTGCCGGGTTT
 TCAGTGGCCACCTGCGCTACCTCTCCAGAACCTGAGTGCTCTGACGCGGCTGTCTGGTGCGTTTCACTGATCTGGTGC
 TGCAGCTTCCTTACACTTCCCAAGAGGAGAAGCAGTTTGGAAAAACAAATCAGAATAAGTTGGTCCTGAGTTCTAAGTTTGG

CTCTTCACCTTTCTAGTCCCCAATTTATATTGTTCTCCGTGCGTCAGTTTTACCTGTGAGATAAGGCCAGTAGCCAGCCCCG
TCCTGGCAGGGCTGTGGTGAGGAGGGGGGTGTCCGTGTGGAAACTCCCTTTGTGAGAATGGTGCGTCCTAGGTGTTACCAG
GTCGTGGCCGCTCTACTCCCTTTCTCTTTCTCCATCCTTCTTCCCTTAAAGAGTCCCAGTGCTATCTGGGACATATTCTC
CGCCCAGAGCAGGGTCCCGCTTCCCTAAGGCCCTGCTCTGGGCTTCTGGGTTTGAGTCCTTGGCAAGCCCAGGAGAGGCGCTC
AGGCTTCCTGTCCCCCTTCTCGTCCACCATCTCATGCCCTGGCTCTCCTGCCCTTCCCTACAGGGGTTCTGGCTCTGC
TCTTCAGACTGAGCCCCGTCCCTGCATCCCCGTCCCTGCATCCCCCTTCCCTGCATCCCCCAGAGGCCCCAGGCCACC
TACTTGGCCTGGACCCACGAGAGGCCACCCAGCCCTGTCTACCAGGCTGCCTTTTGGGTGGATTCTCTCCAACGTGGGG
TGACTGCTTGGCAAACCTCACTCTTCGGGGTATCCAGGAGGCTGGAGCATTGGGGTGGGCTGGGGTTCAGAGAGGAGGGATT
CCCTTCTCAGGTTACGTGGCCAAGAAGCAGGGGAGCTGGGTTTGGGTCAGGTCTGGGTGTGGGTGACCAGCTTATGCTGTTT
GCCCAGGACAGCCTAGTTTTAGCACTGAAACCCTCAGTCCTAGG

Supplementary Figure 31. Map and nucleotide sequence of the targeting module in the Puro^R.T2A.EGFP-encoding plasmids pDonor.EP^{S1} and pDonor.EP^{S1.TS}. Construct pDonor.EP^{S1.TS} differs from pDonor.EP^{S1} in that it has its *AAVS1*-targeting module bracketed by gRNA^{S1} target sites. The regions of homology in pDonor.EP^{S1.TS} and pDonor.EP^{S1} are identical to those present in their EGFP-encoding counterparts. hPGK-1 promoter, human phosphoglycerate kinase 1 gene (*PGK1*) regulatory sequences; PuroR, *Streptomyces* puromycin N-acetyl-transferase (*pac*) gene; T2A, *Thoseaasigna* virus 2A “self-cleaving” peptide coding sequence; EGFP, enhanced green fluorescence protein gene (*EGFP*) open reading frame; bGHpA, bovine growth hormone gene (*GHI*) polyadenylation signal; DNA sequences sharing identity to the human *AAVS1* “safe harbour” locus are indicated in orange.

Supplementary Table 1. Oligonucleotide pairs to generate the gRNA expression constructs used in this study.

Plasmids	Oligonucleotide pairs
AL8_pgRNA ^{DMD}	5' -ACCGTTACATACAGGCTAGGGAG-3' 5' -AAACCTGCCTAGCCTGTATGTAA-3'
AD19_pgRNA ^{SI.2}	5' -ACCGTCCCTAGTGGCCCCACTGT-3' 5' -AAACACAGTGGGGCCACTAGGGA-3'
AD13_pgRNA ^{SI.3}	5' -ACCGTCACCAATCCTGTCCCTAG-3' 5' -AAACCTAGGGACAGGATTGGTGA-3'
AY22_pgRNA ^{R5.1}	5' -ACCGGAGAGCTTGGCTCTGTTGG-3' 5' -AAACCCAACAGAGCCAAGCTCTC-3'
AY23_pgRNA ^{R5.2}	5' -ACCGAGCCAAGCTCTCCATCTAG-3' 5' -AAACCTAGATGGAGAGCTTGGCT-3'
L06_pgRNA ^{OUT.1}	5' -ACCGCCCCGTCGTTCCCTGGCCCT-3' 5' -AAACAGGGCCAGGAACGACGGGG-3'
AA44_pgRNA ^{OUT.2}	5' -ACCGATTCCCTTCTCAGGTTACG-3' 5' -AAACCGTAACCTGAGAAGGGAAT-3'
X32_pgRNA ^{IN.1}	5' -ACCGTTAATGTGGCTCTGGTTCT-3' 5' -AAACAGAACCAGAGCCACATTAA-3'
AA48_pgRNA ^{IN.2}	5' -ACCGTTAGGCAGATTCCTTATC-3' 5' -AAACGATAAGGAATCTGCCTAA-3'

Supplementary Table 2. Transfection scheme for the experiments presented in **Supplementary Fig. 1a** assessing disruption of allelic *AAVS1* target sequences by Cas9 versus Cas9^{D10A}.

HEK 293T cells	24-well plate 1.0×10 ⁵ cells/well (1.65 µl PEI/well, medium replaced at 24 h p.t.)					DNA break type
	pCas9	pCas9 ^{D10A}	pgRNA ^{GFP1}	pgRNA ^{S1}	pcDNA3.1	
Size (bp)	9553	9553	3974	3973	5428	
DNA (ng/well)	250			250		DSB
		250		250		Nick
	250		250			Control
		250	250			Control
				250	250	Control

Post-transfection (p.t.)

Supplementary Table 3. Transfection scheme for the experiments shown in **Supplementary Fig. 1c** assessing target gene mutagenesis by Cas9 versus Cas9^{D10A}.

H27 cells	24-well plate 0.8×10 ⁵ cells/well (1.65 µl PEI/well, medium replaced at 24 h p.t.)				DNA break type
	pCas9	pCas9 ^{D10A}	pgRNA ^{GFP2}	pgRNA ^{NT}	
Size (bp)	9553	9553	3974	3045	
DNA (ng/well)	364		116		DSB
		364	116		Nick
	364			116	Control
		364		116	Control

Supplementary Table 4. Transfection scheme for the experiments presented in **Supplementary Figs. 1d** and **1e** probing for *DMD-AAVS1* translocations.

HeLa cells	24-well plate 0.6×10 ⁵ cells/well (1.65 µl PEI/well, medium replaced at 24 h p.t.)					DNA break type
	pCas9	pCas9 ^{D10A}	pgRNA ^{S1}	pgRNA ^{DMD}	pcDNA3.1	
Size (bp)	9553	9553	3973	3046	5428	
DNA (ng/well)	180		160	160		DSB
		180	160	160		Nick
	180					Control

Supplementary Table 5. Transfection scheme for the *DMD*-targeting experiments shown in **Figs. 1b** and **1c**.

HeLa cells	24-well plate 0.6×10 ⁵ cells/well (2.19 µl PEI/well, medium replaced at 6 h p.t.)						Gene targeting strategy
	pCas9	pCas9 ^{D10A}	pgRNA ^{Empty}	pgRNA ^{DMD}	pDonor ^{DMD}	pDonor ^{DMD.TS}	
Size (bp)	9553	9553	3915	3046	8610	8667	
DNA (ng/well)	150			150	200		Standard
	150			150		200	DSB ²
		150		150	200		Nick
		150		150		200	Nick ²
	150		150		200		Control

Supplementary Table 6. Transfection scheme for the *DMD*-targeting experiments shown in **Supplementary Fig. 2**.

HeLA cells	24-well plate 0.4×10 ⁵ cells/well (2.19 µl PEI/well, medium replaced at 6 h p.t.)								Gene targeting strategy
	pCAG.Cas9	pCAG.Cas9 ^{D10A}	pgRNA ^{DMD}	pgRNA ^{Empty}	pDonor ^{DMD}	pDonor ^{DMD1.TS}	pDonor ^{DMD2.TS}	pDMD ^{Donor1.V.TS}	
Size (bp)	10636	10636	3047	3915	8610	8667	8724	8724	
DNA (ng/well)	286,26		82,01		231,73				Standard
		286,26	82,01			231,73			Nick ²
		286,26	82,01				231,73		Nick ²
		286,26	82,01					231,73	Nick ²
		286,26	82,01		231,73				Nick
	286,26			82,01	231,73				Ctrl
	286,26			82,01		231,73			Ctrl

Supplementary Table 7. Transfection scheme for the *AAVS1*-targeting experiments in 293T cells presented in **Fig. 2**.

HEK293T cells	24-well plate 1.0×10 ⁵ cells/well (1.65 µl PEI/well, medium replaced at 24 h p.t.)						Gene targeting strategy
	pCas9	pCas9 ^{D10A}	pgRNA ^{Empty}	pgRNA ^{S1}	pDonorE ^{S1}	pDonorE ^{S1.TS}	
Size (bp)	9553	9553	3915	3973	11375	11465	
DNA (ng/well)	150			150	200		Standard
	150			150		200	DSB ²
		150		150	200		Nick
		150		150		200	Nick ²
	150		150		200		Control
	150		150			200	Control
		150	150		200		Control
		150	150			200	Control

Supplementary Table 8. Transfection scheme for the *AAVSI*-targeting experiments in HeLa cells shown in **Fig. 2**.

HeLa cells	24-well plate 0.6×10 ⁵ cells/well (1.32 µl PEI/well, medium replaced at 24 h p.t.)						Gene targeting strategy
	pCas9	pCas9 ^{D10A}	pgRNA ^{Empty}	pgRNA ^{S1}	pDonorE ^{S1}	pDonorE ^{S1.TS}	
Size (bp)	9553	9553	3915	3973	11375	11465	
DNA (ng/well)	120			120	200		Standard
	120			120		200	DSB ²
		120		120	200		Nick
		120		120		200	Nick ²
	120		120		160		Control
	120		120			160	Control
		120	120		160		Control
		120	120			160	Control

Supplementary Table 9. Transfection scheme for the experiments shown in **Supplementary Fig. 4** comparing donors with no, one or two gRNA^{S1} target sites.

HeLa Cells	24-well plate 0.6×10 ⁵ cells/well (2.19 µl PEI/well, medium replaced at 6 h p.t.)							Gene targeting strategy
	pCas9	pCas9 ^{D10A}	pgRNA ^{Empty}	pgRNA ^{S1}	pDonor ^{S1}	pDonor ^{S1.1×TS}	pDonor ^{S1.TS}	
Size (bp)	9553	9553	3915	3973	7199	7251	7359	
DNA (ng/well)	150			150	200			Standard
		150		150	200			Nick (0×TS)
		150		150		200		Nick ² (1×TS)
		150		150			200	Nick ² (2×TS)
	150		150		200			Control

Supplementary Table 10. Transfection scheme for the experiments presented in **Supplementary Fig. 5a** testing different *AAVSI*-targeting reagents.

HeLa Cells	24-well plate 0.6×10 ⁵ cells/well (2.19 µl PEI/well, medium replaced at 6 h p.t.)							Gene targeting strategy
	pCas9	pCas9 ^{D10A}	pgRNA ^{Empty}	pgRNA ^{S1}	pgRNA ^{S1.2}	pDonor ^{S1}	pDonor ^{S1.1×TS}	
Size(bp)	9553	9553	3915	3973	3046	5966	6078	
DNA (ng/well)	150			150		200	200	Standard
		150		150				Nick ²
	150				150		200	Standard
		150			150			Nick ²
	150		150			200		Control

Supplementary Table 11. Transfection scheme for the experiments shown in **Supplementary Fig. 5b** testing different *AAVS1*-targeting reagents.

HeLa Cells	24-well plate 0.6×10 ⁵ cells/well (2.19 µl PEI/well, medium replaced at 6 h p.t.)								
	pCas9	pCas9 ^{D10A}	pgRNA ^{Empty}	pgRNA ^{S1}	pDonor ^{S1}	pDonor ^{S1.1×TS}	pDonor.37 ^{S1}	pDonor.37 ^{S1.1×TS}	
Size(bp)	9553	9553	3915	3973	7199	7251	5966	6078	
DNA (ng/well)	150			150	200				Standard
		150		150	200				Nick
		150		150		200			Nick ²
	150			150			200		Standard
		150		150			200		Nick
		150		150				200	Nick ²
	150		150		200				Control

Supplementary Table 12. Transfection scheme for the experiments shown in **Fig. 5c** assessing *AAVS1*-targeting induced by Cas9^{D10A} versus Cas9^{H840A}.

HeLA cells	24-well plate 0.4×10 ⁵ cells/well (2.19 µl PEI/well, medium replaced at 6 h p.t.)							
	pCas9	pCas9 ^{D10A}	pCas9 ^{H840A}	pgRNA ^{Empty}	pgRNA ^{S1}	pDonorE ^{S1}	pDonorE ^{S1.TS}	Gene targeting strategy
Size (bp)	9553	9553	9553	3915	3973	11375	11465	
DNA (ng/well)	150				150	200		Standard
	150				150		200	DSB ²
		150			150	200		Nick
		150			150		200	Nick ²
			150		150	200		Nick
			150		150		200	Nick ²
	150			150		200		Control

Supplementary Table 13. Transfection scheme for the *AAVS1* editing experiments presented in **Fig. 2e**.

HeLA cells	24-well plate 0.4×10 ⁵ cells/well (2.19 µl PEI/well, medium replaced at 6 h p.t.)					
	pCAG.Cas9	pCAG.Cas9 ^{D10A}	pgRNA ^{S1}	pS.Donor ^{S1}	pS.Donor ^{S1.TS}	Gene targeting strategy
Size (bp)	10636	10636	3973	3333	3526	
DNA (ng/well)	371		106	123		Standard
		371	106		123	Nick ²
		371	106	123		Nick
	371		106		123	DSB ²

Supplementary Table 14. Transfection scheme for the experiments shown in **Supplementary Fig. 9** comparing DSB- versus SSB-dependent gene targeting at *AAVS1*.

HeLa cells	24-well plate 0.4×10 ⁵ cells/well (2.19 µl PEI/well, medium replaced at 6 h p.t.)								Gene targeting strategy
	pCAG.Cas9	pCAG.Cas9 ^{D10A}	pgRNA ^{S1}	pgRNA ^{S1.2}	pgRNA ^{S1.3}	pDonor ^{S1}	pDonor ^{S1.TS}	pgRNA ^{Empty}	
Size (bp)	10636	10636	3973	3046	3046	7199	7359	3915	
DNA (ng/well)	306		114			207			Standard
	306		114				207		DSB ²
	306		57	57		207			DSB
	306		57		57	207			DSB
		306	114			207			Nick
		306	114				207		Nick ²
	306					207		114	Ctrl
	306						207	114	Ctrl

Supplementary Table 15. Transfection scheme for the donor competition experiments shown in **Fig. 4**.

HeLa Cells	24-well plate 0.8×10 ⁵ cells/well (2.63 µl PEI/well, medium replaced at 6 h p.t.)					
	pCas9 ^{D10A}	pgRNA ^{S1}	pDonor ^{S1}	pDonor ^{S1.TS}	pDonor.Turq ^{S1}	pDonor.Turq ^{S1.TS}
Size(bp)	9553	3973	7199	7359	7203	7359
DNA (ng/well)	164	68	124			124
	164	68		124	124	

Supplementary Table 16. Transfection scheme for the multiplexing gene targeting experiments presented in **Fig. 5**.

HeLa Cells	24-well plate 0.6×10 ⁵ cells/well (2.19 μl PEI/well, medium replaced at 6 h p.t.)									Gene targeting strategy
	pCas9	pCas9 ^{D10A}	gRNA ^{S1}	pDonor ^{S1}	pDonor ^{S1.TS}	pDonor.Turq ^{S1}	pDonor.Turq ^{S1.TS}	gRNA ^{Empty}	pLacZ	
Size (bp)	9553	9553	3973	7199	7359	7203	7359	3915		
DNA (ng/we ll)	164		68	124		124				Standard
		164	68		124		124			Nick ²
	164		68	124					124	Standard
		164	68		124				124	Nick ²
	164		68			124			124	Standard
		164	68				124		124	Nick ²
	164			124				68	124	Ctrl
	164					124		68	124	Ctrl

Supplementary Table 17. Transfection scheme for the *CCR5* editing experiments presented in **Fig. 6c**.

HeLA cells	24-well plate 0.4×10 ⁵ cells/well (2.19 µl PEI/well, medium replaced at 6 h p.t.)					
	pCAG.Cas9	pCAG.Cas9 ^{D10A}	pgRNA ^{R5.1}	pDonor ^{R5}	pDonor ^{R5.TS}	Gene targeting strategy
Size (bp)	10636	10636	3046	3559	3715	
DNA (ng/well)	370		106	124		Standard
		370	106		124	Nick ²
		370	106	124		Nick
	370		106		124	DSB ²

Supplementary Table 18. Transfection scheme for the *CCR5* editing experiments presented in **Fig. 6d** (upper panel).

HeLA cells	24-well plate 0.4×10 ⁵ cells/well (2.19 µl PEI/well, medium replaced at 6 h p.t.)							
	pCAG.Cas9	pCAG.Cas9 ^{D10A}	pgRNA ^{R5.1}	pgRNA ^{R5.2}	pDonor ^{R5}	pDonor ^{R5.TS}	pgRNA ^{Empty}	Gene targeting strategy
Size (bp)	10636	10636	3046	3046	3559	3715	3915	
DNA (ng/well)	371		106		123			Standard
		371	106			123		Nick ²
		371	106		123			Nick
	371		106			123		DSB ²
	371		53	53	123			DSB
	371				123		106	Ctrl
	371					123	106	Ctrl

Supplementary Table 19. Transfection scheme for the *CCR5* mutagenesis experiments presented in **Fig. 6d** (lower panel).

HeLA cells	24-well plate 0.4×10 ⁵ cells/well (2.19 µl PEI/well, medium replaced at 6 h p.t.)						
	pCAG.Cas9	pCAG.Cas9 ^{D10A}	pgRNA ^{R5.1}	pgRNA ^{R5.2}	pgRNA ^{Empty}	pcDNA3.1	Gene targeting strategy
Size (bp)	10636		3046	3046	3915	5428	
DNA (ng/well)	371		106			123	DSB
	371			106		123	DSB
		371	106			123	Nick
		371	53	53		123	DSB
	371				106	123	Ctrl

Supplementary Table 20. Transfection scheme for the experiment investigating RGN-induced gene targeting by tandem paired nicking presented in **Supplementary Fig. 6**.

HeLA cells	24-well plate 0.6×10 ⁵ cells/well (2.19 µl PEI/well, medium replaced at 6 h p.t.)										Gene targeting strategy
	pCas9	pCas9 ^{D10A}	pgRNA ^{S1}	pgRNA ^{OUT.1}	pgRNA ^{OUT.2}	pgRNA ^{IN.1}	pgRNA ^{IN.2}	pDonorE ^{S1}	pDonorE ^{S1.TS}	pgRNA ^{empty}	
Size (bp)	9553	9553	3973	3046	3046	3046	3045	11375	11465	3915	
DNA (ng/well)	150		75					200		75	Standard
		150	75						200	75	Nick ²
		150	75					200		75	Nick ²
		150		75	75			200			Tandem Nick ²
		150				75	75	200			Tandem Nick ²
	150							200		150	Control

Supplementary Table 21. Transfection scheme for the gene targeting experiments in human PSCs presented in Fig. 3.

LUMC0044iCtrl44.9 iPSCs (Condition A)	1.1×10 ⁶ cells per well (6-well plate) 5 µl Lipofectamine TM 2000 per experimental condition						Gene targeting strategy
	pCAG.Cas9	pCAG.Cas9 ^{D10A}	gRNA ^{Empty}	gRNA ^{S1}	pDonor ^{S1}	pDonor ^{S1.TS}	
Size (bp)	10636	10636	3915	3973	7199	7359	
DNA (ng/well)	480			480	640		Standard
		480		480		640	Nick ²
		480	480		640		Nick
					640		Control
LUMC0044iCtrl44.9 iPSCs (Condition B)	0.5×10 ⁶ cells per well (6-well plate) 10 µl Lipofectamine TM 2000 per experimental condition						Gene targeting strategy
	pCAG.Cas9	pCAG.Cas9 ^{D10A}	gRNA ^{Empty}	gRNA ^{S1}	pDonor ^{S1}	pDonor ^{S1.TS}	
Size (bp)	10636	10636	3915	3973	7199	7359	
DNA (ng/well)	1200			1200	1600		Standard
		1200		1200		1600	Nick ²
		1200	1200		1600		Nick
					1600		Control
H1 ESCs (Condition C)	0.5×10 ⁶ cells per well (6-well plate) 5 µl Lipofectamine TM 2000 per experimental condition						Gene targeting strategy
	pCas9.gRNA ^{S1}	pCas9 ^{D10A} .gRNA ^{S1}	pCas9.gRNA ^{NT}	pDonor.EP ^{S1}	pDonor.EP ^{S1.TS}		
Size (bp)	9450	9450	9447	7881	8041		
DNA(ng/well)	1000			1000			Standard
		1000			1000		Nick ²
		1000		1000			Nick
			1000	1000			Control
H1 ESCs (Condition D)	0.3×10 ⁶ cells per well (6-well plate) 1 µl Lipofectamine TM 3000 + 2 µl P3000 per experimental condition						Gene targeting strategy
	pCas9.gRNA ^{S1}	pCas9 ^{D10A} .gRNA ^{S1}	pCas9.gRNA ^{NT}	pDonor.EP ^{S1}	pDonor.EP ^{S1.TS}		
Size (bp)	9450	9450	9447	7881	8041		
DNA (ng/well)	500			500			Standard
		500			500		Nick ²
		500		500			Nick
			500	500			Control
LUMC0044iCtrl44 iPSCs (Condition E)	0.3×10 ⁶ cells per well (6-well plate) 1 µl Lipofectamine TM 3000 + 2 µl P3000 per experimental condition						Gene targeting strategy
	pCas9.gRNA ^{S1}	pCas9 ^{D10A} .gRNA ^{S1}	pCas9.gRNA ^{NT}	pDonor.EP ^{S1}	pDonor.EP ^{S1.TS}		
Size (bp)	9450	9450	9447	7881	8041		
DNA (ng/well)	500			500			Standard
		500			500		Nick ²
		500		500			Nick
			500	500			Control

Supplementary Table 22. Transfection scheme for the experiment investigating apoptosis in ESCs triggered by Cas9 versus Cas9^{D10A} presented in **supplementary Fig. 12**.

H1 ESCs	5×10 ⁵ cells per well (6-well plate) 1 µl Lipofectamine TM 3000 + 2 µl P3000 per experimental condition						Gene targeting strategy
	pCas9.gRNA ^{S1}	pCas9 ^{D10A} .gRNA ^{S1}	pCas9.gRNA ^{NT}	pDonor ^{S1}	pDonor ^{S1.TS}	pgRNA ^{Empty}	
Size (bp)	9450	9450	9447	7199	7359	3915	
DNA(ng/well)	500			500			Standard
		500			500		Nick ²
		500		500			Nick
	500				500		DSB ²
			500	500			Ctrl
			500			500	Ctrl

Supplementary Table 23. Primer pairs and composition of PCR mixtures used for the analyses of genome-modifying events (**Supplementary Fig. 1a**, **1b** and **1d**, **Fig. 2c** and **2e**, **Fig. 4d**, **Fig 5d**, **Fig. 6c**, **6d** and **6e**).

Target	Primer code	Primers (5' → 3') / final concentrations (mM)	dNTPs (mM)	MgCl ₂ (mM)	GoTaq Flexi Buffer	GoTaq (Units)
<i>AAVS1</i> (T7EI assay)	#999	TTCGGGTCACCTCTCACTCC / 0.4	0.4	1	1×	2
	#1000	GGCTCCATCGTAAGCAAACC / 0.4				
<i>jC-AAVS1</i>	#1046	CGACAACCACTACCTGAGCA / 0.4	0.4	1	1×	2.5
	#1047	GACCTGCCTGGAGAAGGAT / 0.4				
<i>jH-T</i> concatemer	#1061	TGCTCTAAGGGCGAATTCG / 0.4	0.4	2	1×	2.5
	#1062	ATGGTCATAGCTGTTTCTCTG / 0.4				
<i>EGFP</i>	#978	GAGCTGGACGGCGACGTAAACG / 0.4	0.4	1	1×	2.5
	#979	CGCTTCTCGTTGGGGTCTTTGCT / 0.4				
<i>HPRT1</i>	#1005	TCACTGTATTGCCAGGTTGGTG / 0.2	0.4	3	1×	2
	#1006	GAAAGCAAGTATGGTTTGACAGAGAT / 0.2				
<i>DMD</i> (T7EI assay)	#994	AGACATGACCTATTCTGCCG / 0.2	0.4	1	1×	2
	#1052	GGTAGTAAGTCAGATGTGATG / 0.2				
t(X;19)(p21; q13)	#1000	GGCTCCATCGTAAGCAAACC / 0.2	0.4	1	1×	2.5
	#1052	GGTAGTAAGTCAGATGTGATG / 0.2				
<i>j-mTurquoise2</i>	#1251	ACTACCTGAGCACCCAGTCCAAG / 0.2	0.4	1	1×	2.5
	#1253	TCACCCAGAGACAGTGACCAACC / 0.2				
<i>j-EGFP</i>	#1252	ACCTGAGCACCCAGTCCGCC / 0.2	0.4	1	1×	2.5
	#1253	TCACCCAGAGACAGTGACCAACC / 0.2				
<i>AAVS1</i> (RFLP analysis)	#1257	AGCCACCTCTCCATCCTCTT / 0.2	0.4	1	1×	2.5
	#1258	AGGGAGTTTTCCACACGGAC / 0.2				
<i>CCR5</i> (RFLP analysis)	#1280	GTGCATGTTCTTTGTGGGCT / 0.2	0.4	1	1×	2.5
	#1281	GGTCAGAGATGGCCAGGTTG / 0.2				

Supplementary Table 24. PCR cycling parameters used for the analyses of genome-modifying events (**Supplementary Fig. 1a ,1b and 1d , Fig. 2c and 2e, Fig. 4d, Fig 5d, Fig. 6c, 6d and 6e**).

Target	Initial denaturation	Denaturation	Annealing	Elongation	Cycles	Final elongation
<i>AAVS1</i> (T7EI assay)	95 °C	95 °C	61 °C	72 °C	40	72°C
	3 min	30 s	30 s	30 s		5 min
<i>jC-AAVS1</i>	95 °C	95 °C	61 °C	72 °C	40	72 °C
	5 min	30 s	30 s	120 s		5 min
<i>jH-T</i> concatemer	95 °C	95 °C	57 °C	72 °C	35	72 °C
	3 min	25 s	25 s	20 s		2 min
<i>EGFP</i>	95 °C	95 °C	62 °C	72 °C	40	72 °C
	3 min	30 s	30 s	40 s		3 min
<i>HPRT1</i>	95 °C	95 °C	63 °C (decrease of 0.5 °C per cycle)	72 °C	10	
	3 min	25 s	30 s	25 s		
		95 °C	58 °C	72 °C	25	72 °C
		25 s	25 s	25 s		3 min
<i>DMD</i> (T7EI assay)	95 °C	95 °C	66 °C (decrease of 0.5 °C per cycle)	72 °C	10	
	5 min	30 s	30 s	30 s		
		95 °C	61 °C	72 °C	25	72 °C
		30 s	30 s	30 s		3 min
t(X;19)(p21;q13)	95 °C	95 °C	60 °C	72 °C	40	72 °C
	5 min	30 s	30 s	35 s		5 min
<i>j-mTurquoise2</i>	95 °C	95 °C	68 °C	72 °C	40	72 °C
	5 min	30 s	30 s	100 s		5 min
<i>j-EGFP</i>	95 °C	95 °C	67.4 °C	72 °C	40	72 °C
	5 min	30 s	30 s	100 s		5 min
<i>AAVS1</i> (RFLP analysis)	95 °C	95 °C	58.3 °C	72 °C	40	72 °C
	5 min	30 s	30 s	60 s		5 min
<i>CCR5</i> (RFLP analysis)	95 °C	95 °C	58.7 °C	72 °C	40	72 °C
	5 min	30 s	30 s	55 s		5 min

Supplementary Table 25. Thermocycler program for generating T7EI heteroduplex substrates spanning nuclease target sites.

Temperature	Time
95 °C	10 min
95 °C to 85 °C	-2.0 °C/s
85 °C	1 min
85 °C to 75 °C	-0.3 °C/s
75 °C	1 min
75 °C to 65 °C	-0.3 °C/s
65 °C	1 min
65 °C to 55 °C	-0.3 °C/s
55 °C	1 min
55 °C to 45 °C	-0.3 °C/s
45 °C	1 min
45 °C to 35 °C	-0.3 °C/s
35 °C	1 min
35 °C to 25 °C	-0.3 °C/s
25 °C	1 min
16 °C	Hold

Source: SURVEYOR Mutation Detection Kit (Transgenomic)

Supplementary Table 26. Primer pairs and composition of PCR mixtures used for the analyses of individual gene-edited cells (**Fig. 1c**, **Fig. 2d** and **Fig. 3d** gathering the data from **Supplementary Figs. 3, 7 and 13**, respectively, and **Supplementary Fig. 16**).

Target	Primer code	Primers (5' → 3') @ 0.5 mM final concentration	Master mix (mM)	Primer (μM)	DMSO
jC- <i>AAVSI</i>	#1046	CGACAACCACTACCTGAGCA	0.4	0.5	-
	#1047	GACCTGCCTGGAGAAGGAT			
jT- <i>AAVSI</i>	#986	AACCCCAACCCCGTGGAAG	0.4	0.5	2%
	#1004	GCACCGTCCGCTTCGAG			
<i>EGFP</i>	#978	CTGCATTCTAGTTGTGGTTTG	0.4	0.5	-
	#979	CTAACATGCGGTGACGTGG			
jC- <i>DMD</i>	#1026	TGCCTTGGAAAAGGCGC	0.4	0.5	-
	#1170	AGGGACCACTGTTTTATGGGA			
jT- <i>DMD</i>	#217	CGCATTTGTCTGAGTAGGTGTC	0.4	0.5	-
	#1172	ACACTCCTTTTGTCTGCTT			
<i>AAVSI</i>	#999	TTCGGGTCACCTCTCACTCC	0.4	0.5	-
	#1000	GGCTCCATCGTAAGCAAACC			
<i>mTurq.2/EGFP</i> (RFLP analysis)	#47	ACCCCGACCACATGAAGCAGC	0.4	0.5	-
	#1253	TCACCCAGAGACAGTGACCAACC			

Supplementary Table 27. Cycling parameters used for the junction PCR analyses of individual gene-edited cells (**Fig. 1c**, **Fig. 2d** and **Fig. 3d** gathering the data from **Supplementary Figs. 3, 7 and 13**, respectively, and **Supplementary Fig. 16**).

Target	Initial denaturation	Denaturation	Annealing	elongation	Cycles	Final elongation
jC- <i>AAVSI</i>	98 °C	98 °C	64.5 °C	72 °C	35	72 °C
	5 min	7 s	7 s	35 s		1 min
jT- <i>AAVSI</i>	98 °C	98 °C	72 °C		35	72 °C
	5 min	7 s	30 s			2 min
<i>EGFP</i>	98 °C	98 °C	72 °C		35	72 °C
	5 min	5 s	20 s			2 min
jC- <i>DMD</i>	98 °C	98 °C	70 °C	72 °C	40	72 °C
	5 min	7 s	7 s	50 s		2 min
jT- <i>DMD</i>	98 °C	98 °C	66 °C	72 °C	40	72 °C
	5 min	7 s	7 s	50 s		2 min
<i>AAVSI</i>	98 °C	98 °C	66 °C	72 °C	40	72 °C
	5 min	5 s	5 s	20 s		1 min
<i>mTurq.2/EGFP</i> (RFLP analysis)	98 °C	98 °C	72 °C		40	72 °C
	5 min	5 s	1 min			2 min

Supplementary Table 28. Restriction Fragment Length Polymorphism analysis presented in **Fig. 2e**, **Fig. 6c** and **6d** and **Supplementary Fig. 16**.

Target	PCR kit	Restriction Enzyme	Buffer	PCR product	Total volume	Incubation time
<i>AAVS1</i>	GoTaq G2 Flexi DNA polymerase (Promega)	3.3 U HindIII (ThermoFisher Scientific)	Buffer Red	20 µl	30 µl	4 h
<i>CCR5</i>	GoTaq G2 Flexi DNA polymerase (Promega)	3.3 U MluI (ThermoFisher Scientific)	Buffer Red	20 µl	30 µl	4 h
<i>EGFP/mTurquoise2-AAVS1</i>	Phire Tissue Direct PCR Master Mix (ThermoFisher Scientific)	3.3 U BspI (New England BioLab)	Cutsmart	10 µl	20 µl	Overnight