

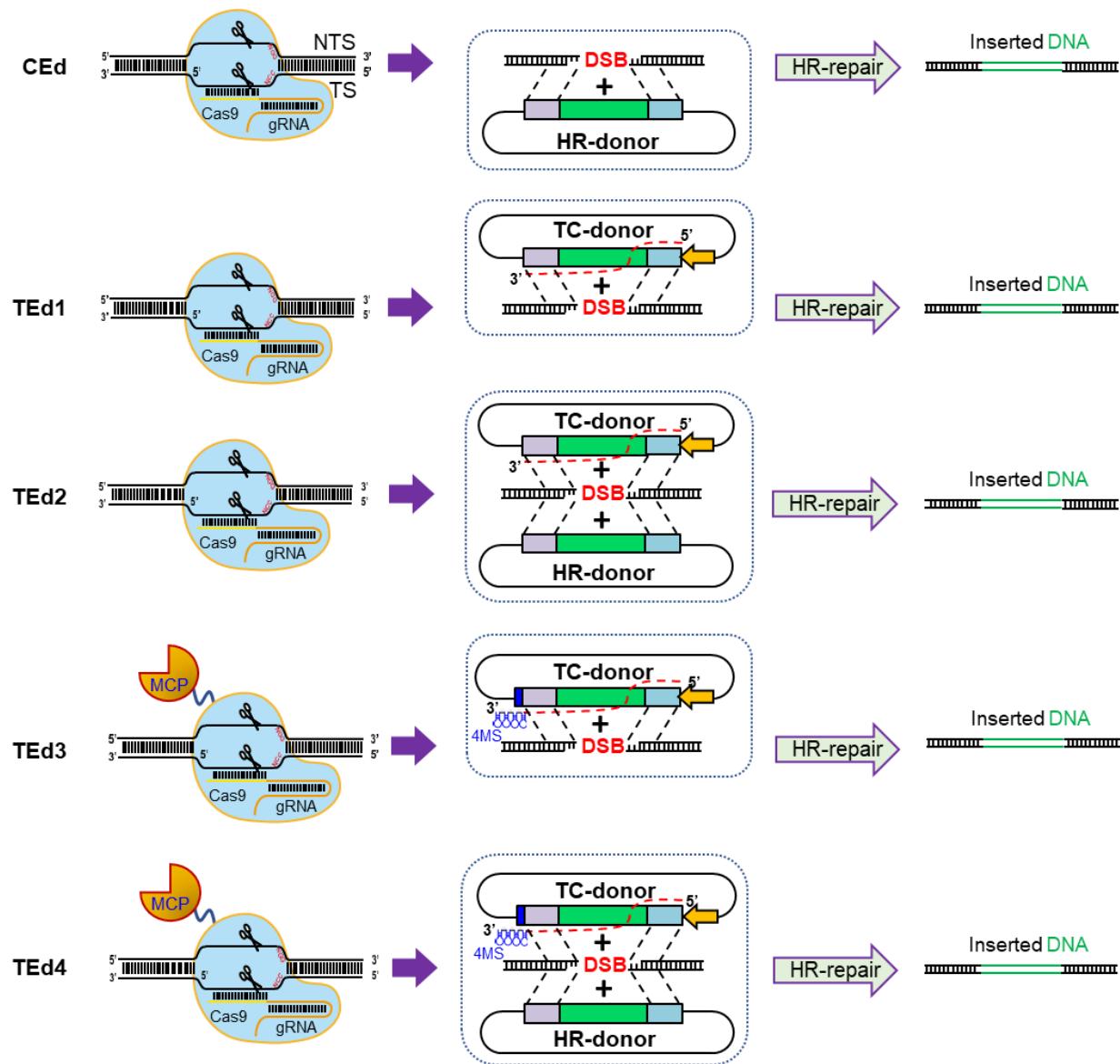
SUPPLEMENTARY DATA

Transcription-coupled Donor DNA Expression Increases Homologous Recombination for Efficient Genome Editing

Supplementary Figure S1 to S12

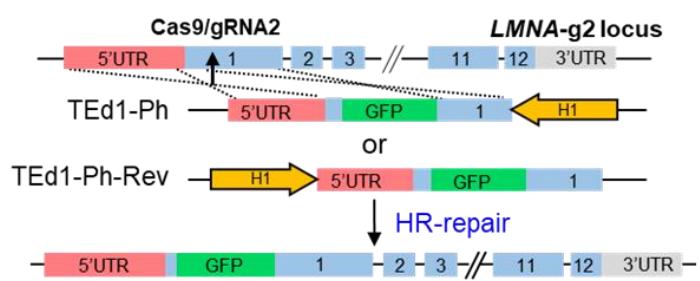
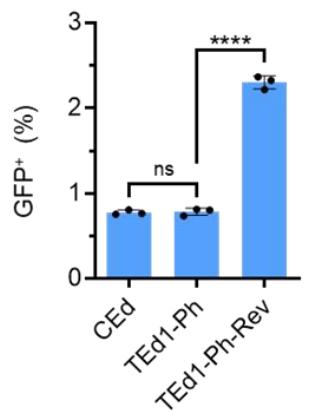
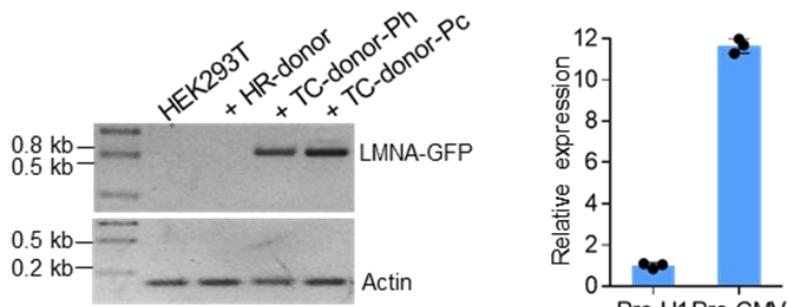
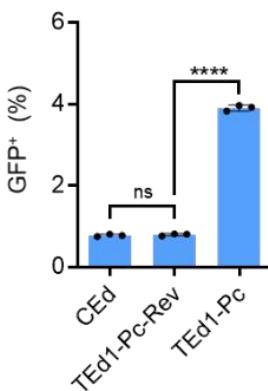
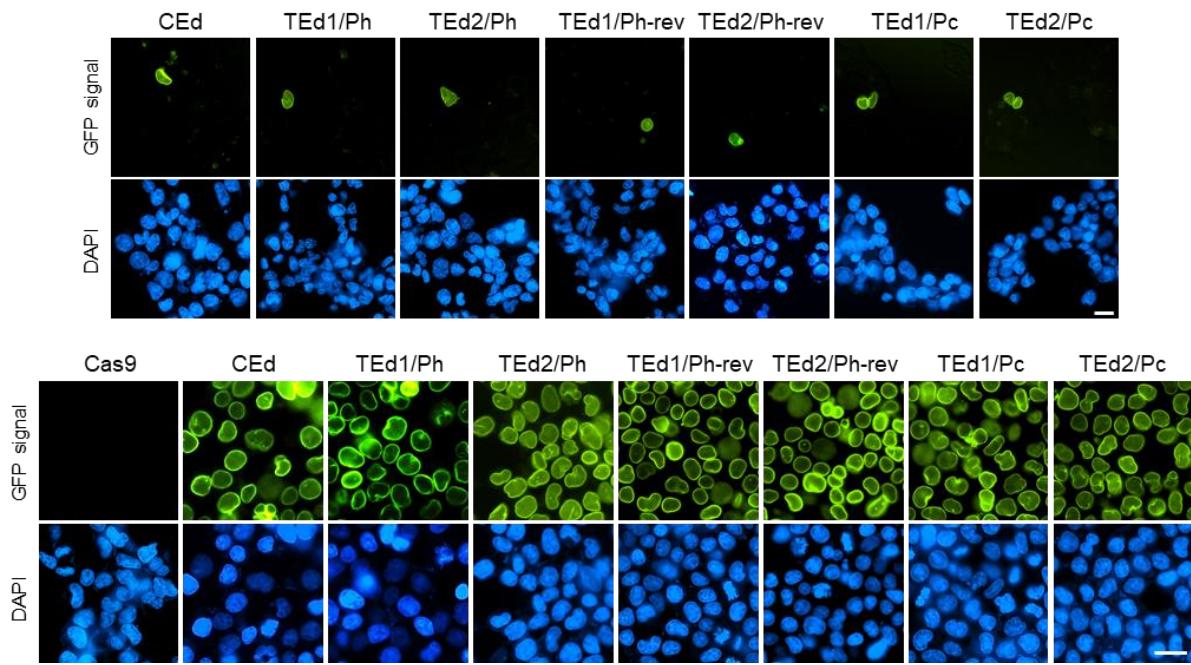
Supplementary Tables S1 to S4

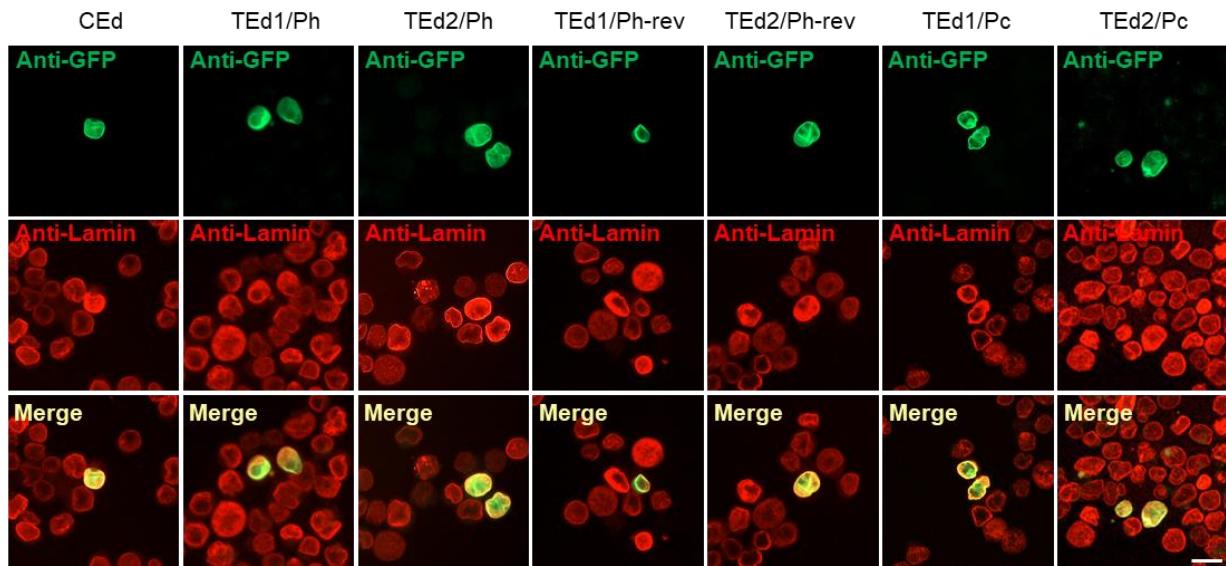
Supplementary Figures



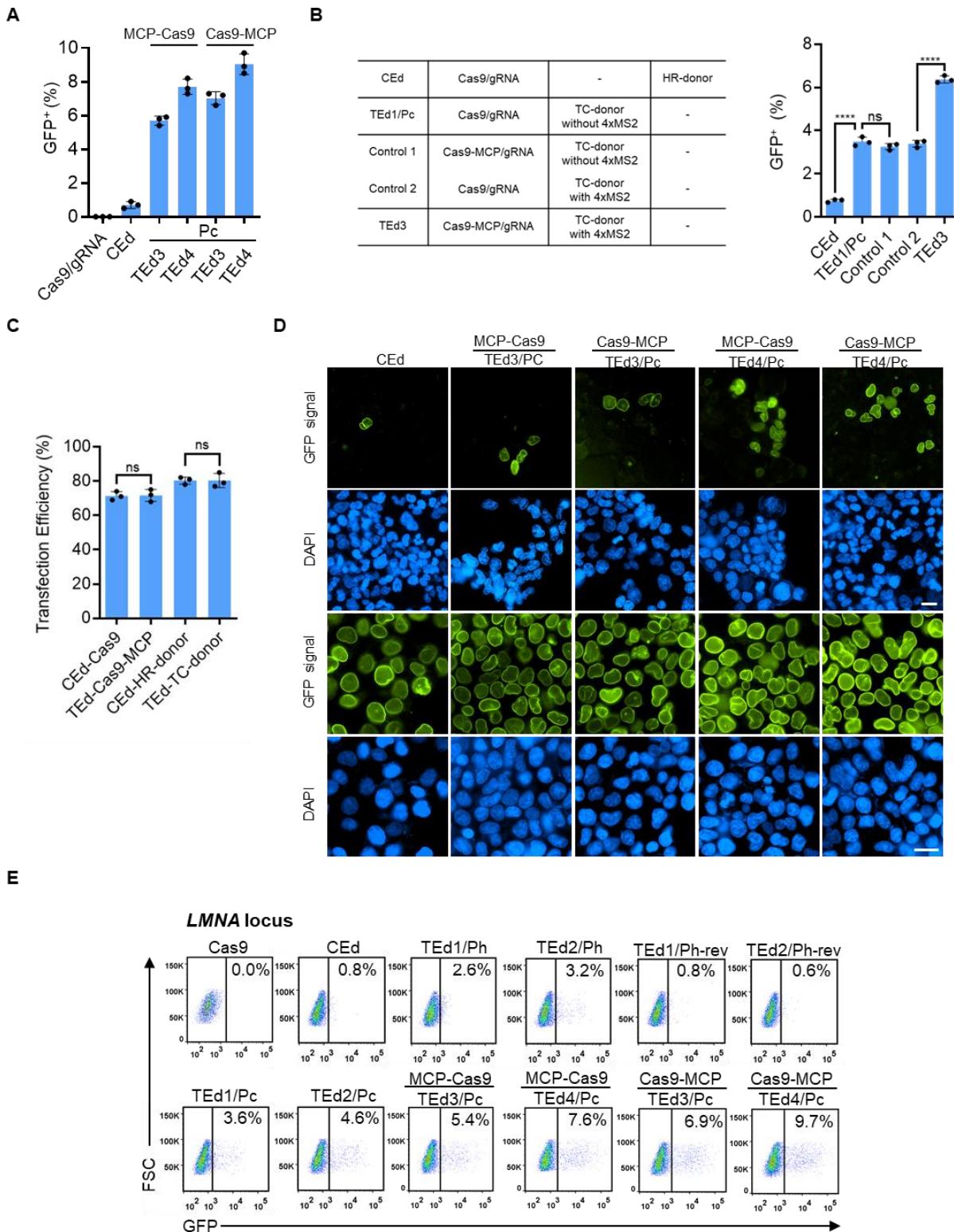
Supplementary Figure S1. Development of TEd methods.

Gene KI by CEd is mediated by a plasmid donor template (HR-donor) while TEd is mediated by a TC-donor (TEd1). In TEd2, TC-donor and HR-donor are used together. TEd3/4 additionally employ the MCP-MS2 system, in which the MCP protein is fused to Cas9, and the 4xMS2 sequence is fused to the 3' tail of TC-RNA.

A**B****C****D****E**

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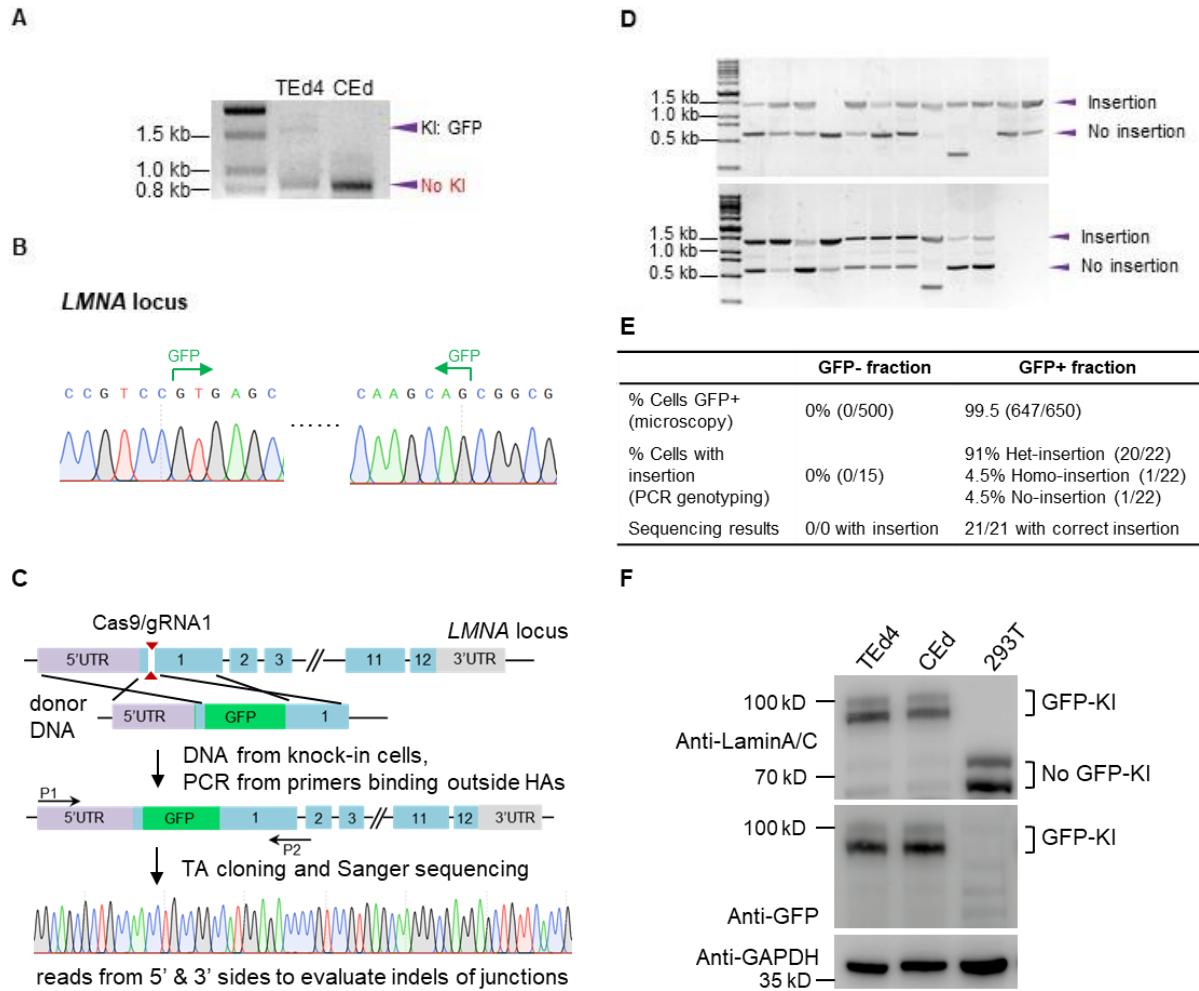
Supplementary Figure S2. Optimization of the promoter and transcriptional direction in the TEd-mediated knock-in strategy. **(A)**, Components of the TEd1 systems using original TC-donors at the *LMNA*-gRNA2 locus. **(B)**, GFP-KI efficiencies at the *LMNA*-gRNA2 locus in HEK293T cells using CEd, TEd1-Ph, and TEd1-Ph-Rev. **(C)**, Left, gel image showing TC-donor transcripts. Right, a histogram showing the relative expression of homologous TC-RNAs driven by H1 and CMV promoters (calculated by qRT-PCR). *GAPDH* served as the internal reference. **(D)**, GFP-KI efficiencies evaluated by FACS in HEK293T cells using CEd, TEd1-Pc, and TEd1-Pc-Rev at the *LMNA*-gRNA1 locus. **(E)**, Living imaging verification of GFP inserted into the *LMNA* locus by CEd and TEd in HEK293T cells before (upper) and after sorting (below). Scale bar, 50 μ m. **(F)**, Immunofluorescence imaging verification of GFP colocalization with *LMNA* for CEd and TEd. Scale bar, 50 μ m. Data are shown as individual data points and mean \pm s.d. for n=3 independent biological replicates. *P* values were obtained using the two-tailed Student's t-test. *****P* < 0.0001.



Supplementary Figure S3. Tethering TC-RNAs to DSBs by the MCP-MS system improves TEd-mediated GFP-KI.

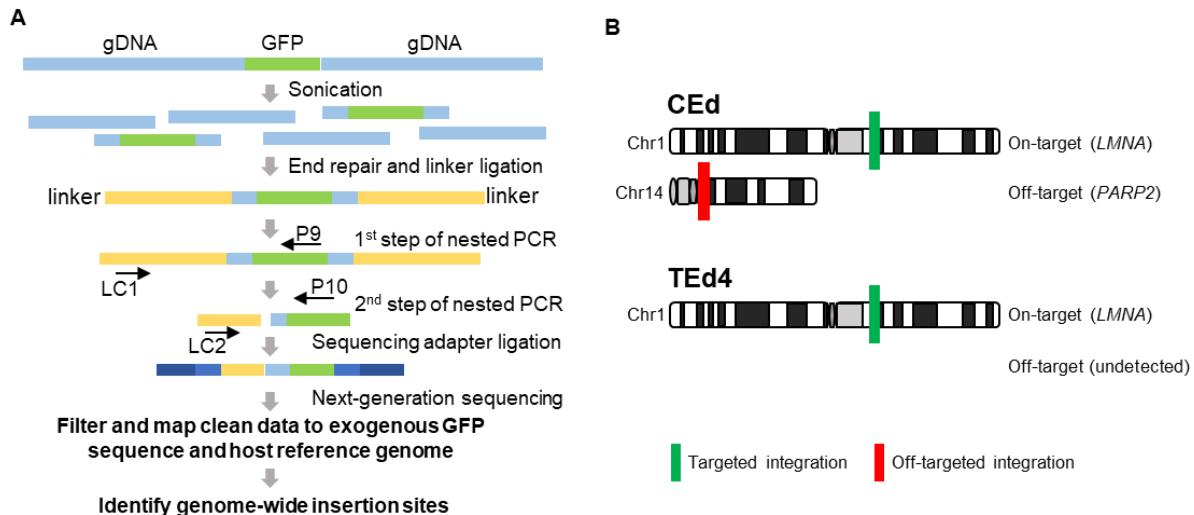
(A), A graph showing the percentage of GFP+ cells obtained after TEd3 and TEd4, in which MCP is fused to the N- or C-terminal of Cas9. (B), Left, Components of CEd, TEd1/3, and the two controls systems; Right,

GFP-KI efficiencies at the LMNA locus in HEK293T-cells for CEd and different TEd designs. (C), Transfection efficiencies of different plasmids used in CEd and TEd at the LMNA locus in HEK293T cells. (D), Imaging verification of GFP knock-in events at the LMNA locus for CEd and TEd (a) before sorting (left) and after sorting (right) in (A). Scale bar, 50 μ m. (E), Representative FACS analysis of GFP expression in cells edited by CEd and TEd. Data are shown as individual data points and mean \pm s.d. for n=3 independent biological replicates. P values were obtained using the two-tailed Student's t-test. ****P < 0.0001.



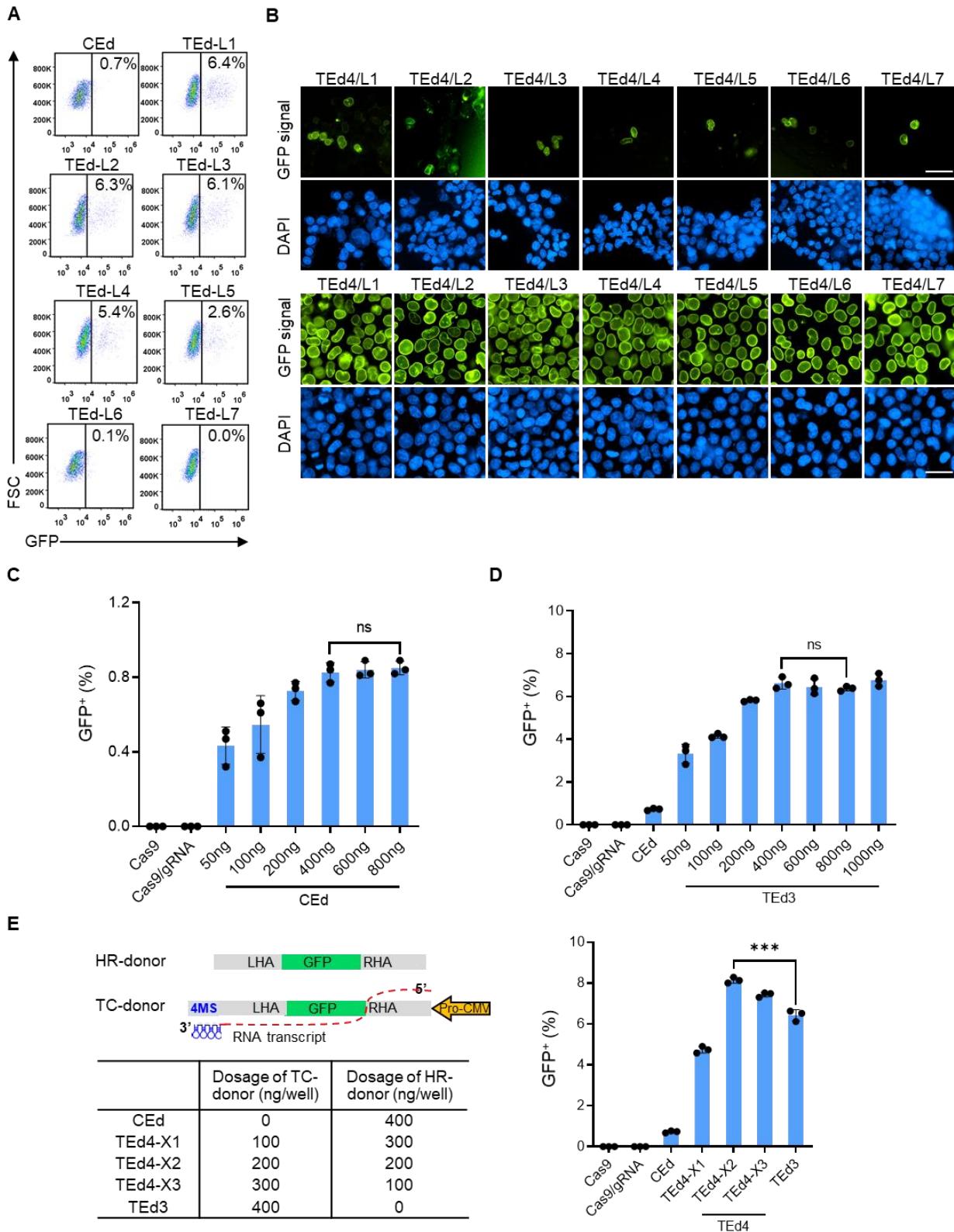
Supplementary Figure S4. Molecular identification of TEd-mediated GFP KI at the LMNA locus.

(A), Agarose gel showing representative PCR amplicons generated by specific primers outside the homology arms at the LMNA locus (P1 and P2 in Figure 1C). The KI products bands for CEd and TEd4 are marked with an arrow and the lower bands are marked as "No KI" with an arrow. (B), Sanger sequencing results of junction PCR of GFP positive single clones sorted from TEd4-edited cells. (C), Design of the junction profiling assay employing genomic PCR with specific primers (P1 and P2) that bind outside the homology arms, followed by isolation of knock-in TA-clones. Paired Sanger sequencing of the TA-clones reveal indel edits at the 5'- and/or 3'- junctions. (D), Genotyping PCR of 22 GFP positive single clones after the sorting of TEd4-edited cells. Genomic DNA was amplified using primers P1 and P2. (E), Statistics of GFP+ and GFP- monoclonal heterozygote genotyping results for TEd4 cells. (F), Western blot analysis showing expression of Lamin-GFP fusion proteins in CEd and TEd4 sorted cells. Upper bands of CEd and TEd4 (upper panel) showing the expected GFP-lamin fusion sizes. GAPDH was used as a loading control.



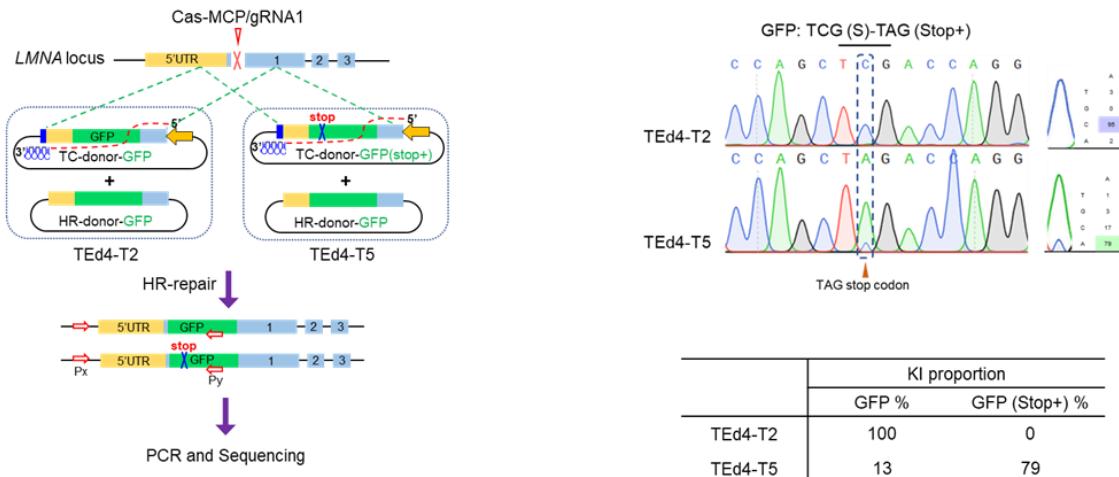
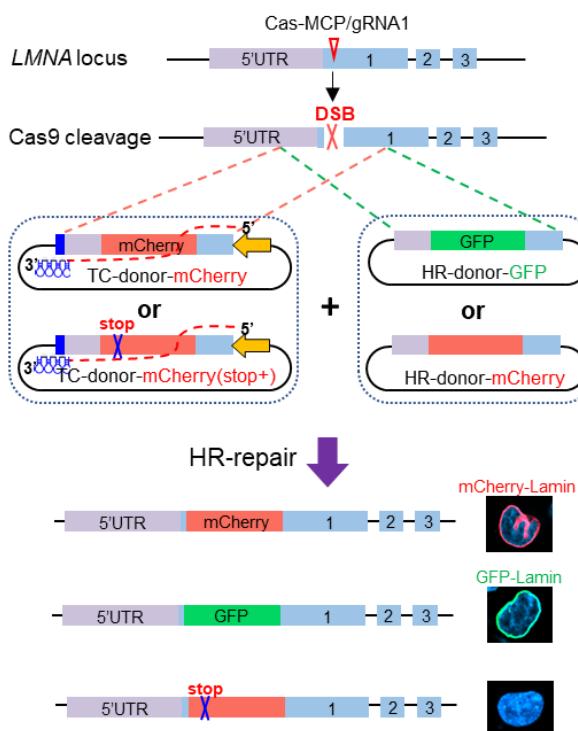
Supplementary Figure S5. Summary of off-target KI sites in CEd and TEd.

(A), Schematic presentation showing the design, procedures, and analysis steps for LM-PCR used to measure genome-wide KI sites of the GFP sequence. (B), Summary of the KI sites identified by LM-PCR, showing the expected on-target KI site and other identified off-target KI sites in CEd and TEd4. On-target KI at the LMNA locus is shown in green and off-target insertion is shown in red (Only insertion sites with more than three supporting reads are shown. Detailed sequence information is provided in Supplementary table S4).

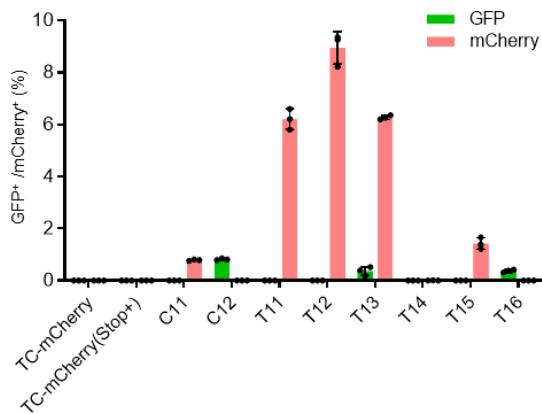


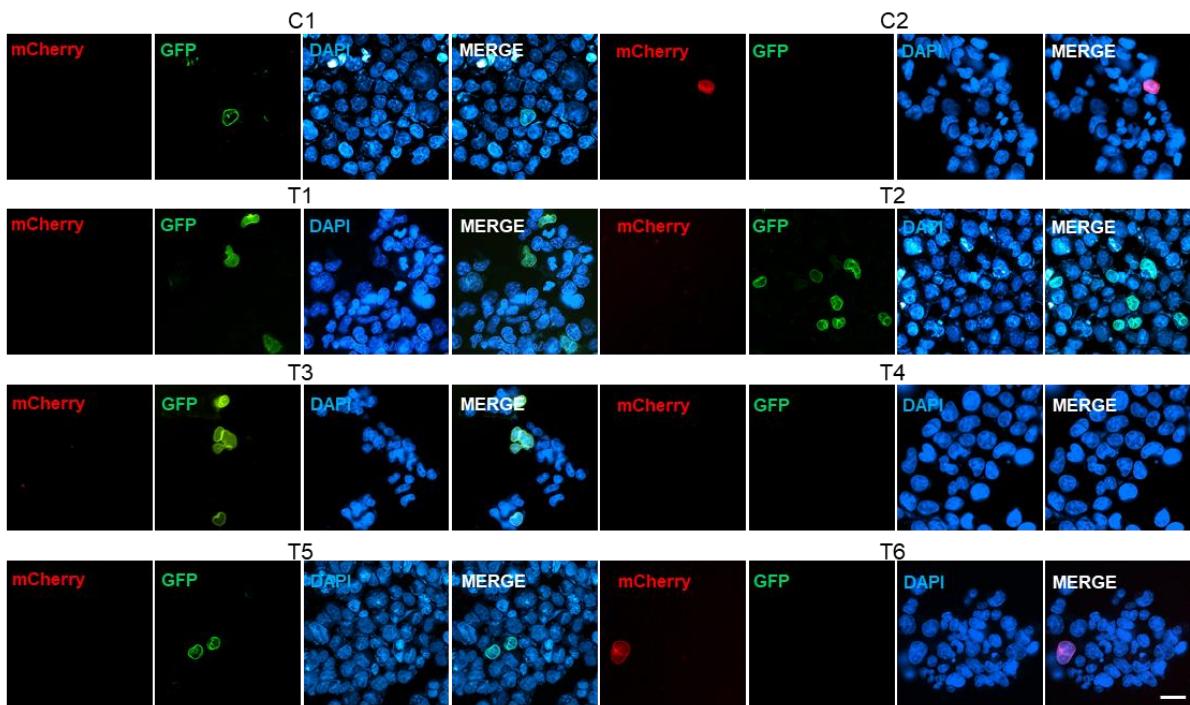
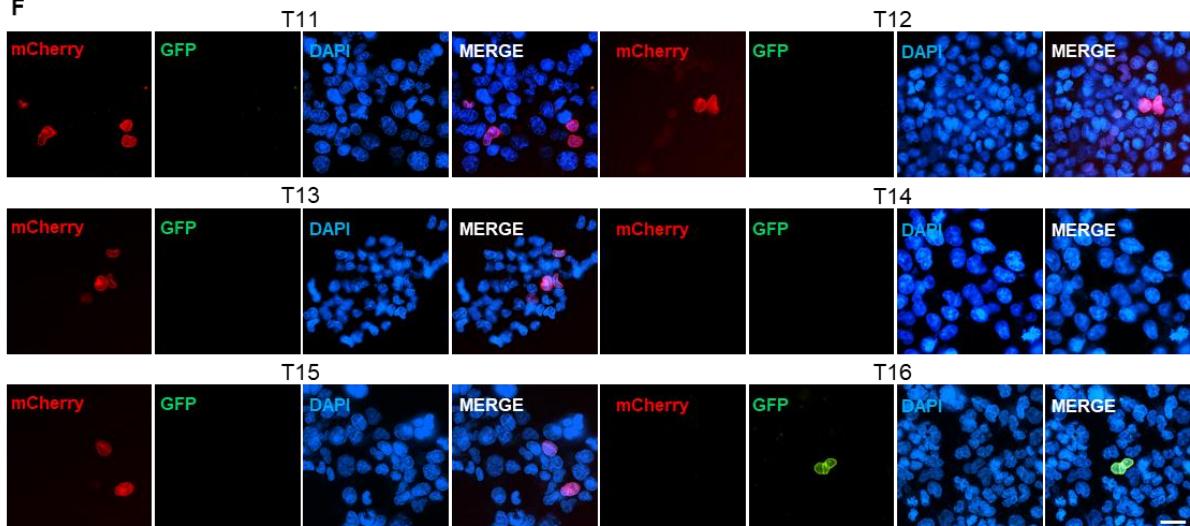
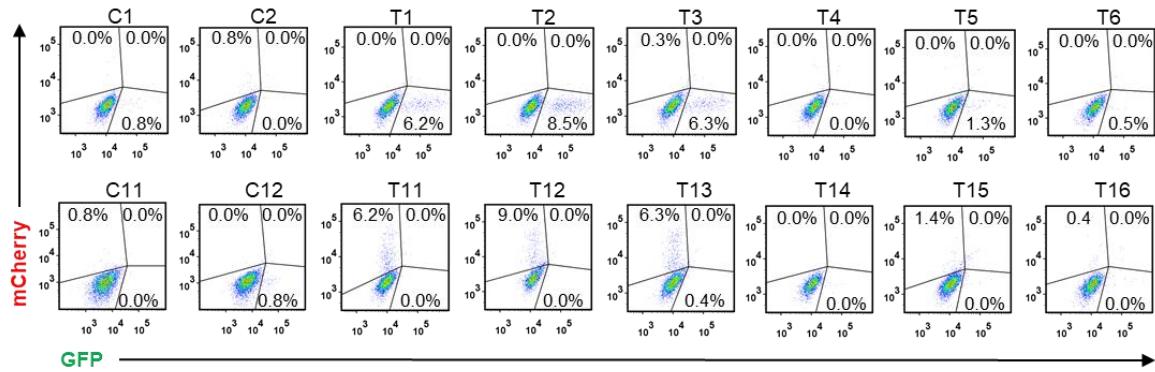
Supplementary Figure S6. Optimization of homologous arm lengths and plasmid concentrations for the TEd-mediated knock-in strategy.

(A), Representative FACS analysis of GFP expression in cells edited by TEd with different HA lengths or CEd 72h after transfection. (B), Living imaging verification of GFP KI at the LMNA locus in HEK293T cells edited by TEd with different HA lengths. Upper two rows show cells before sorting, and lower two rows show cells after sorting. Nuclei are stained with DAPI (blue). Scale bar, 50 μ m. (C) and (D), Plasmid concentration optimization of HR-donor in CEd (C) and TC-donor in TEd (D) at the LMNA locus in HEK293T cells. Graphs show the percentages of GFP+ cells obtained with different TC-donor plasmid concentrations 72 h after transfection. (E), Left, Donor plasmid concentration optimization for the TEd4 design. Right, Graphs showing percentage of GFP+ cells obtained at different donor plasmids concentrations 72 h after transfection. All data are shown as individual data points and mean \pm s.d for n=3 independent biological replicates. P values were obtained using the two-tailed Student's t-test. ***P < 0.001.

A**B****C**

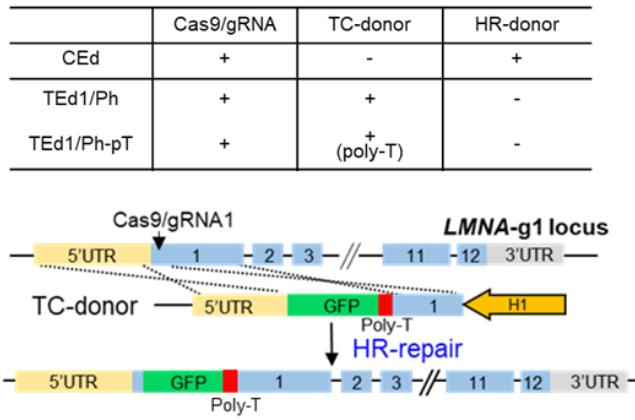
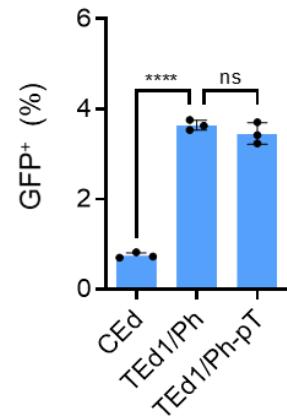
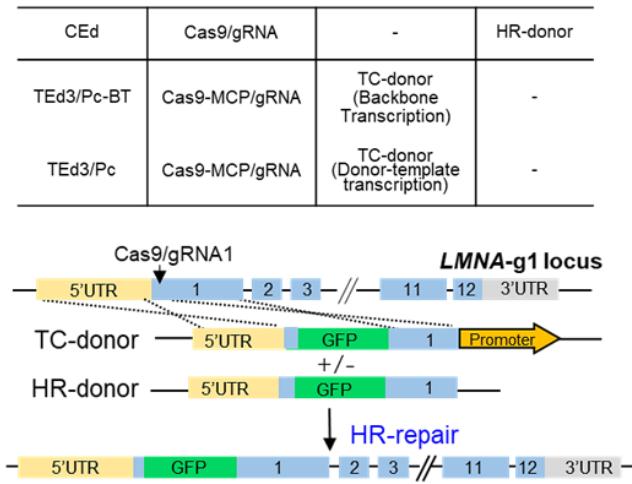
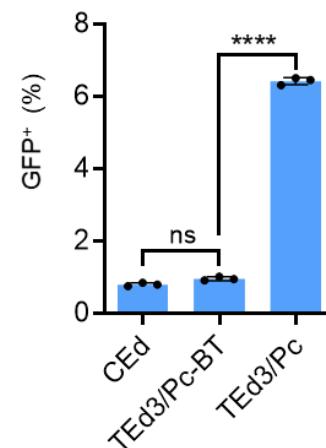
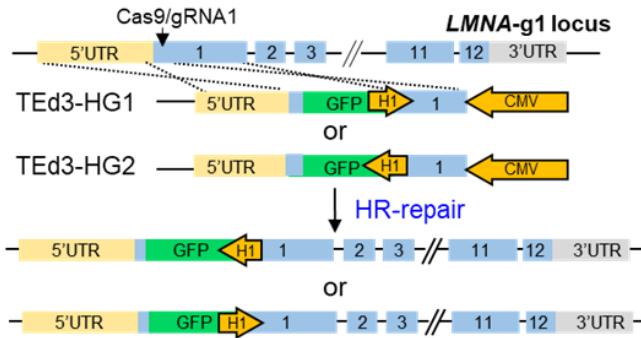
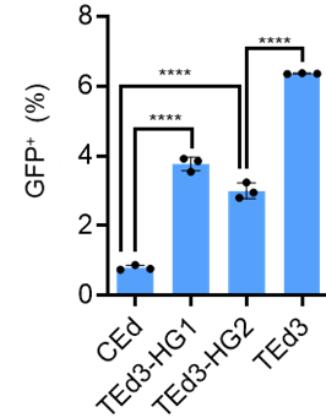
	TC-donor	HR-donor	Average KI efficiency	
			GFP (%)	mCherry(%)
C11	-		0.0	0.8
C12	-		0.8	0.0
T11			0.0	6.2
T12			0.0	9.0
T13	-		0.4	6.3
T14			0.0	0.0
T15			0.0	1.4
T16	-		0.4	0.0

D

E**F****G**

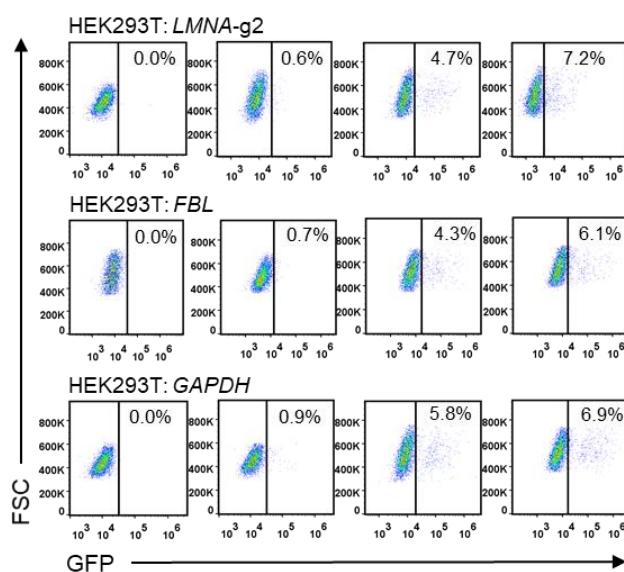
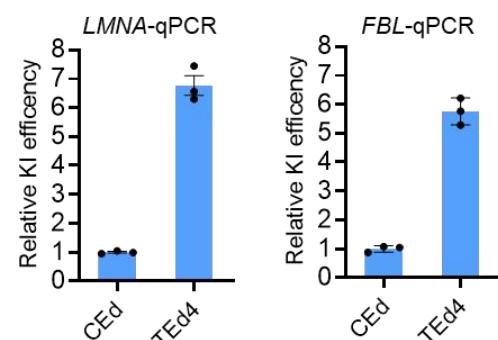
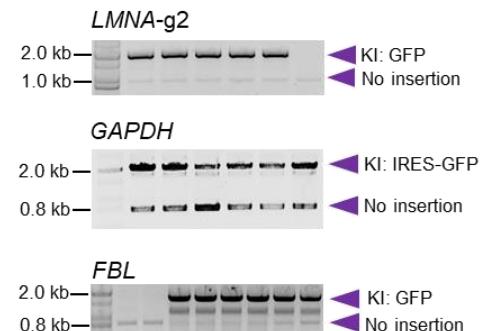
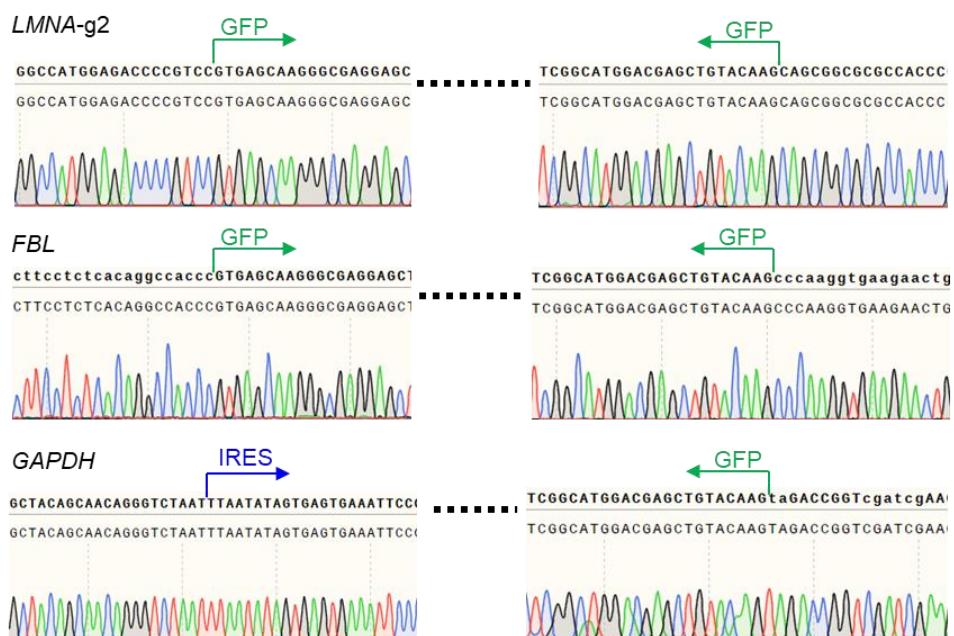
Supplementary Figure S7. Transcription of the TC-donor facilitates gene editing.

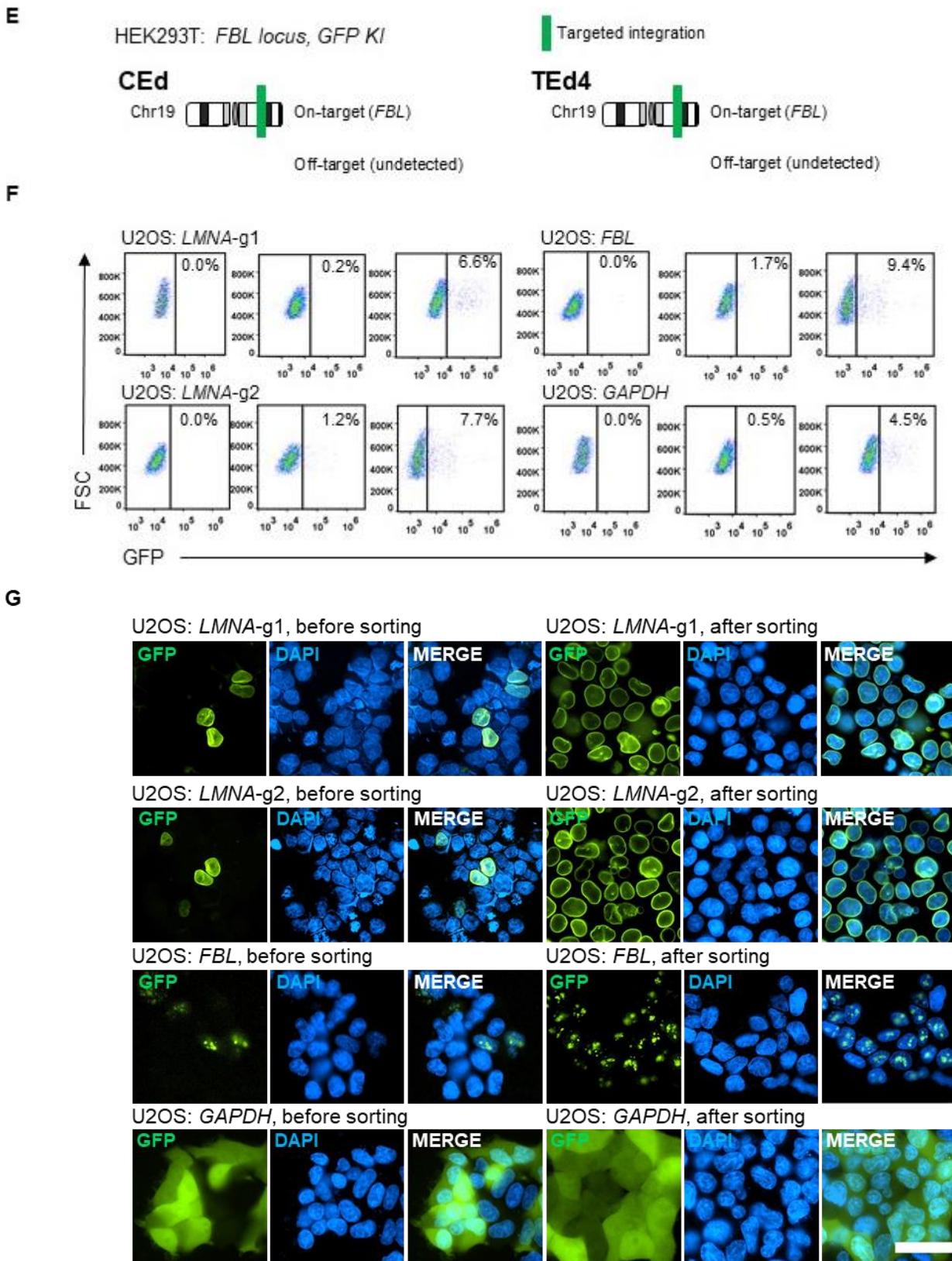
(A), Left, Schematics of TEd4-T2 and TEd4-T5 in Figure 2B showing HR-repair of a DSB at the LMNA locus with different TC-donors (GFP/GFP (Stop+)). Right upper, Sanger sequencing of genomic PCR products from HEK293T cells edited by the TEd4-T2 and TEd4-T5 designs in Figure 2B at the LMNA locus. The orange triangle indicates the mutated C-G to A-T base pairs of GFP. Right lower, Evaluation of GFP and GFP (Stop+) KI ratios in TEd4-T2 and TEd4-T5 by Sanger sequencing and TIDE online analysis. (B), Schematics showing HR-repair of a DSB at the LMNA locus with different GFP or mCherry donors. The HR-donor-GFP contains GFP edit (green) and HR-donor-mCherry contains mCherry edit (red). TC-donor-mCherry contains mCherry edit with no stop codon and TC-donor-mCherry-stop (+) contains mCherry edit with a stop codon (blue cross). (C), Summary of the GFP/mCherry KI efficiencies of CEd and TEd with different donors. (D), GFP/mCherry KI efficiencies of C11, C12, and T11~T16. Graphs showing the percentage of GFP+/mCherry+ cells (y-axis, as determined by FACS) for each donor combination (x-axis). The HR-donor alone and the corresponding TC-donor alone were used as controls. All data are shown as individual data points and mean \pm s.d for n=3 independent biological replicates. (E, F), Imaging verification of GFP or mCherry inserted at the LMNA site in HEK293T living cells edited by CEd or TEd designs with different HA lengths before (upper) and after sorting (lower) in Fig.2B and Supplementary Fig.7B. Scale bar, 50 μ m. (G), Representative FACS analysis of GFP or mCherry expression in HEK293T cells transfected with combinations of C1, C2, T1~T6, C11, C12, and T11~T16 listed in Figure 2B and Supplementary Figure S7B targeting the LMNA locus 72h after transfection.

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Supplementary Figure S8. Other factors that may affect TEd-mediated GFP-KI efficiency.

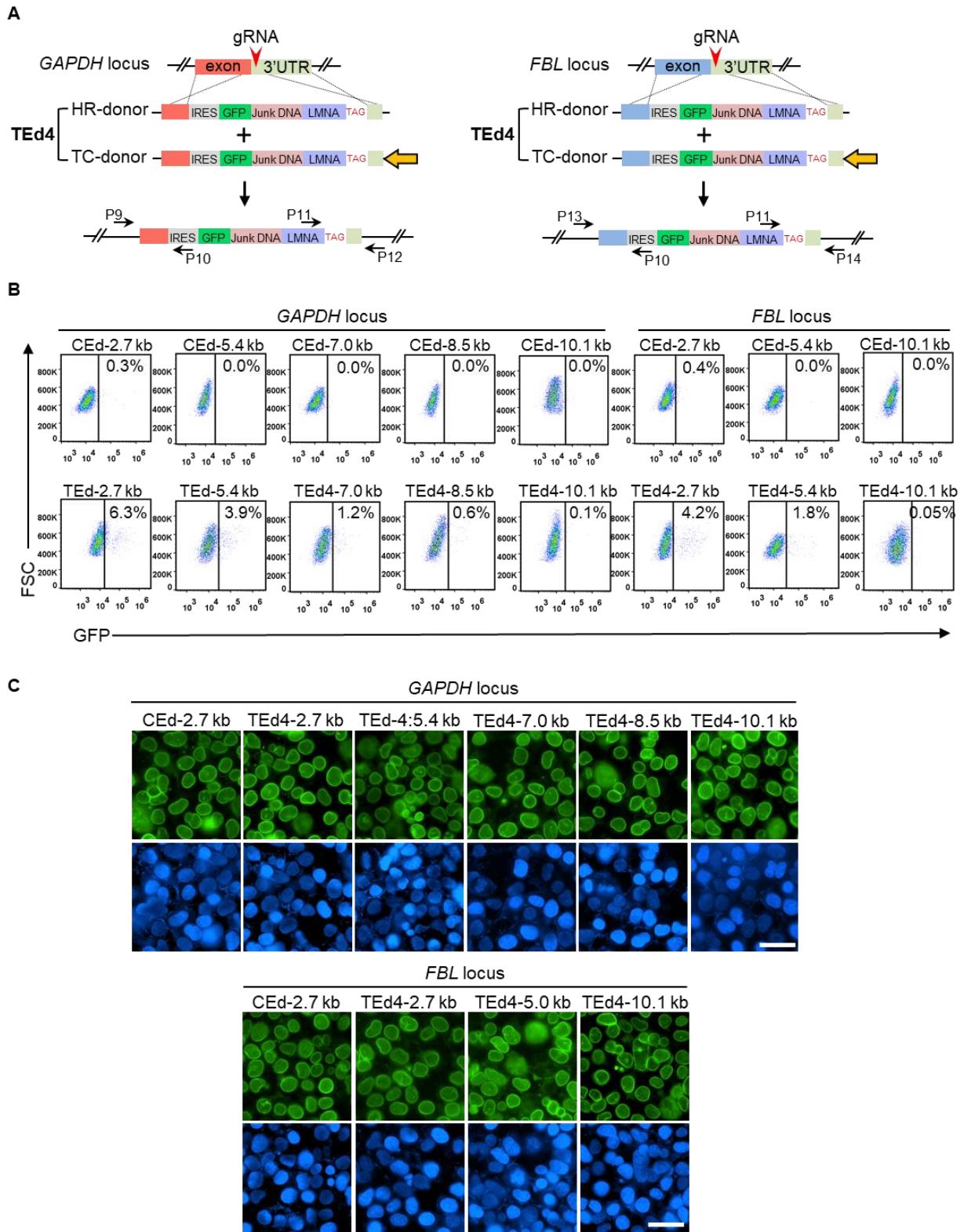
(A), Upper, Components of CEd and different TEd designs; lower, Schematic overview of the TEd1/Ph-pT with poly-T sequence mediated GFP-KI at the LMNA locus. A 9 bp sequence of thymine was inserted into the N-terminus of the GFP sequence, which would terminate transcription initiated from the H1 promoter. (B), GFP-KI efficiencies at the LMNA locus in HEK293T cells with CEd and the different TEd designs. (C), Upper, Components in the CEd and the two TEd3 systems; Lower, Schematic overview of TEd3/Pc-BT with a TC-donor, in which the CMV promoter initiates backbone-transcription (BT) to mediate GFP-KI at the LMNA locus. (D), GFP-KI efficiencies at the LMNA locus in HEK293T cells with CEd and different TEd designs. (E), Schematic overview of TEd3-mediated gene editing (TEd3-HG1/2, TC-donors with a functional H1 promoter at the 3' end of the GFP coding sequence). (F), GFP-KI efficiencies at the LMNA locus in HEK293T cells using CEd and different TEd3 designs. Data are shown as individual data points and mean \pm s.d. for $n = 3$ independent biological replicates. P values were obtained using the two-tailed Student's t-test. ****P < 0.0001.

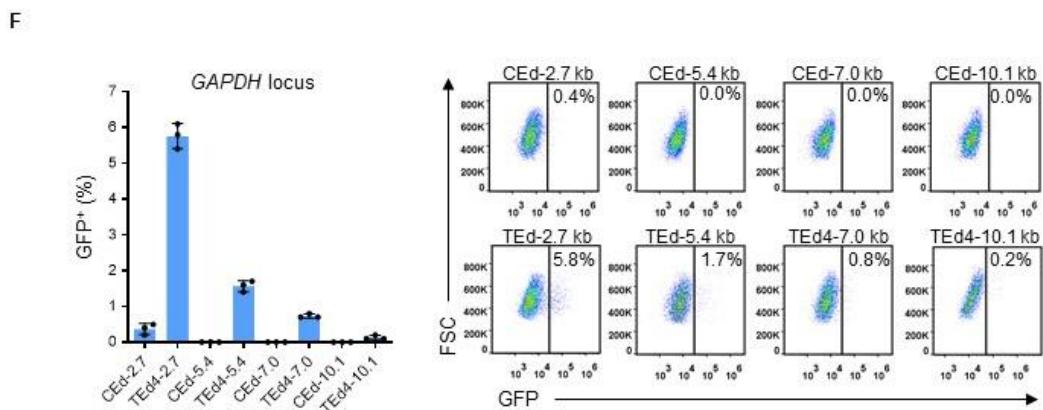
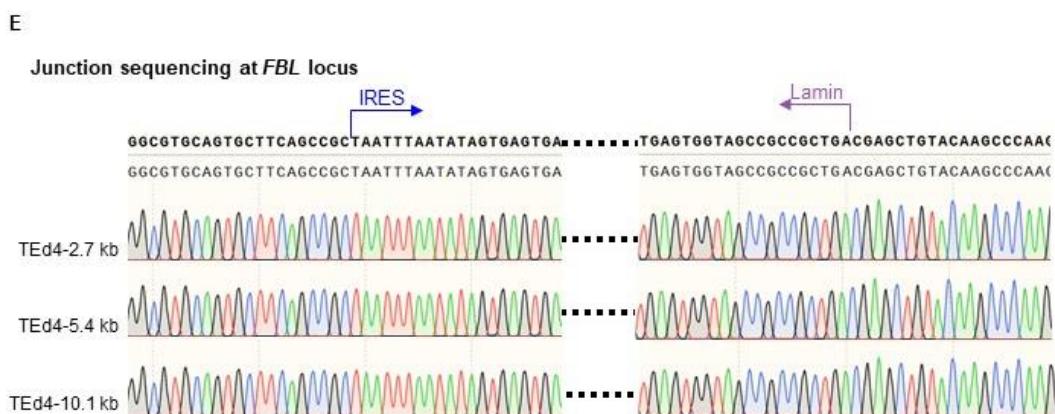
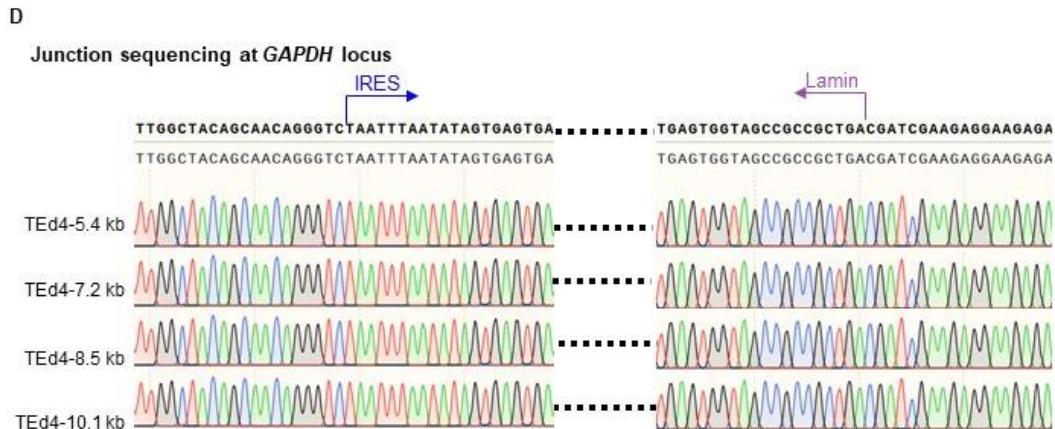
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Supplementary Figure S9. TED editing at other gene loci in different cell types.

(A), Representative FACS analysis of GFP expression 72h after transfection at three targeting loci in HEK293T cells. (B), qPCR analyses of the GFP KI level at the LMNA-g2 and FBL loci. ACTB was used as internal genomic reference. Data are shown as individual data points and mean \pm s.d for n=3 independent biological replicates. (C), PCR genotyping of several GFP positive single clones of each target locus in HEK293T cells after sorting of TED4-edited cells, showing the heterozygosity of KI. Genomic DNA was amplified using primers outside HAs. HEK293T cells without transfection were used for controls. The GFP (or IRES-GFP) KI in each panel was of the expected size (upper bands). The lower bands of each panel show the expected sizes of endogenous genes. 293T cells were used as controls. (D), Sanger sequencing results of 5' and 3' junction PCR products of GFP positive single clones sorted after TED4 at three target loci in HEK293T cells. (E), Summary of the KI sites identified by LM-PCR, showing the on-target and off-target sites in CEd and TED4. On-target KI at the LMNA locus is shown in green (Only insertion sites with more than three supporting reads are shown. Detailed sequence information is provided in Supplementary table S4). (F), Representative FACS analysis of GFP expression at four target loci by CEd and TED4 in U2OS cells 72h after transfection. (G), (Left) Fluorescence imaging verification of GFP inserted into different target sites in U2OS before sorting. (Right) Imaging verification of GFP inserted into different sites in U2OS cells after sorting. Scale bar, 50 μ m.

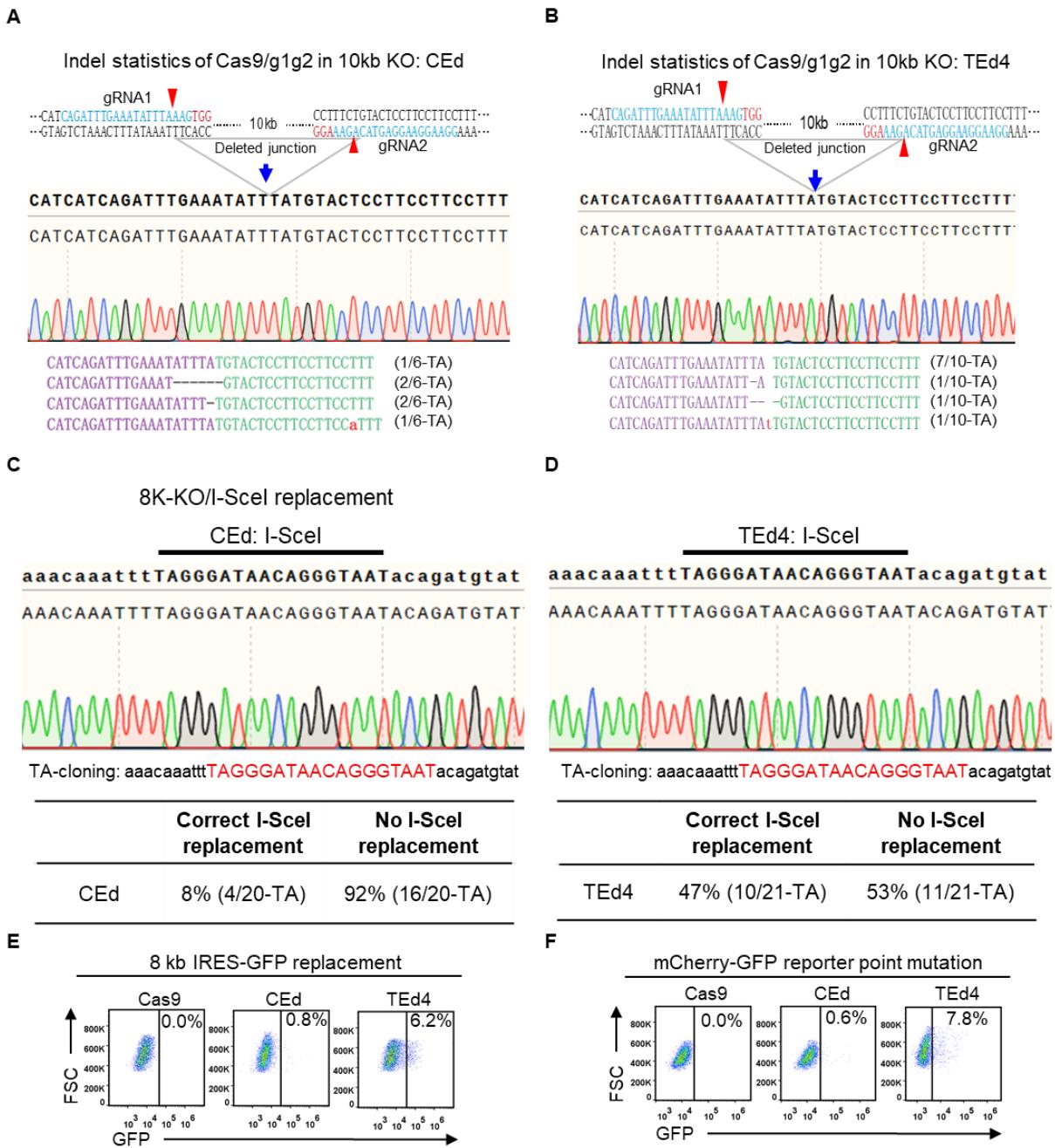




Supplementary Figure S10. TEd mediates long fragment KI.

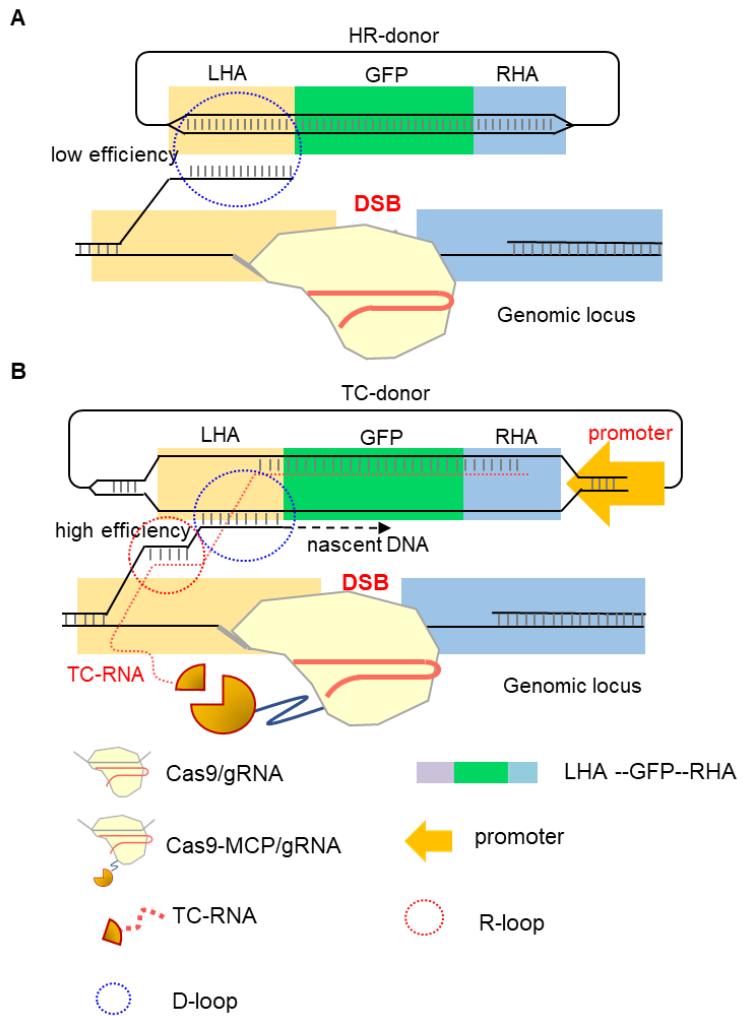
(A), Schematic of large fragment KI at the *GAPDH* locus (left) and *FBL* locus (right) by TEd4. HR-donor and TC-donor were designed to carry 2.7~10 kb edits. Both gRNAs were targeted to the 3'-UTR of genes. Primers used to amplify the junctions are marked as P9~P14. (B), Representative FACS analysis of GFP expression in HEK293T cells transfected with KI fragments of different sizes targeting the *GAPDH* and *FBL* sites listed in Fig. 4A. (C), Living fluorescence imaging of KI with different segment lengths at the *GAPDH* locus (upper) and *FBL* locus (lower) in HEK293T cells after sorting. GFP: green, DAPI: blue. Scale bar, 50 μ m. (D), Sanger sequencing results of the junction PCR products at the *GAPDH* locus using the specific primers shown in (A) (P9 and P10 for the 5'-junction, P11 and P12 for the 3'-junction). The two pairs of primers were designed on bind the IRES-GFP sequence near the junction site and to the outside of the

homology arms. (E), Sanger sequencing results of the 5' and 3' junction PCR products at the FBL locus using the specific primers listed in (A) (P10 and P13 for 5'-junction, P11 and P14 for 3'-junction). The two pairs of primers were designed to bind to the IRES-GFP sequence near the junction site and to the outside of the homology arms. (F), Representative FACS analysis of GFP expression after KI of segments of different lengths at the GAPHD locus using TEd4 and CEd in U2OS cells. All data are shown as individual data points and mean \pm s.d for n=3 independent biological replicates.



Supplementary Figure S11. Molecular validation of genome deletion/replacement and point mutations.

(A, B), Representative Sanger sequencing of TA-clones after 10 kb knockdown at the CDC42 locus in cells edited by CEd (A) and TEd4 (B). (C, D), Representative Sanger sequencing and statistics of I-SceI replacement TA-clones of genomic PCR at the CDC42 locus in cells edited by CEd (C) and TEd4 (D). (E), Representative FACS analysis of GFP expression in cells with replacement of IRES-GFP edited by CEd or TEd shown in Figure 5I. (F), Representative FACS analysis of GFP expression in mCherry-GFP reporter cells edited by CEd or TEd shown in Figure 6B.



Supplementary Figure S12. Model of the role of transcription-coupled DNA donor in the TED method. (A), The role of the classic HR-donor in canonical genome editing. (B), The possible functional mechanisms of the TC-donor in TED. Transcription of the TC-donor may play a major role in improving the efficiency of TED by facilitating the invasion of the 3' ssDNA of the DSB into the displaced single-stranded homology arm. Additionally, the TC-donor transcript (TC-RNA) may form transient RNA-DNA hybrids near the DSB, thus making it accessible to the repair machinery. Cas9-MCP may also help recruit TC-RNA/donor complexes to the DSB site by binding specifically to the MS2 aptamer, thereby increasing the local concentration of TC-donor templates around DSBs and facilitating HR.

Supplementary Table S1. The sequences of CRISPR/Cas9 target sites tested in this study. PAM sequences are underlined.

Target site	gRNA target site (5'-3')
<i>LMNA</i> gRNA1	CCATGGAGAC <u>CCC</u> G <u>CC</u> CAG <u>CGG</u>
<i>LMNA</i> gRNA2	GGGCGACAG <u>CGG</u> A <u>GTGG</u> A <u>GCTGG</u>
<i>FBL</i>	CTCTCACAGGCCAC <u>CCCCCA</u> AGG
<i>GAPDH</i>	AG <u>CCCC</u> CAGCAAGAGCACA <u>AGAGG</u>
<i>CDC42</i> gRNA1	CAGATTGAA <u>ATATTTAAAGTGG</u>
<i>CDC42</i> gRNA2	GGAAGGA <u>AGGGAGTACAGAAAGG</u>
<i>CDC42</i> gRNA3	CACAACAA <u>ACAAATTTCATCGG</u>
<i>CDC42</i> gRNA4	GA <u>CTAGAA</u> ATACATCT <u>GTGGTGTGG</u>
<i>mCherry-GFP Reporter</i>	GCGCCTGCTCG <u>GATGCTAGAGG</u>
<i>PRNP</i>	GCAGTGGTGGGGGG <u>CTTGGCGG</u>

Supplementary Table S2. Inserted sequences of donor plasmids used in this study. Restriction enzyme sites were underlined.

EGFP HR-donor plasmid, lamin A/C, 240bp HAs

GGTACCCCGGCGTGGTACTCAGTGGGAGCGCCGACCCTACACCAGCCACCCAGTCCCGAGGTCCGACAG
CGCCCGGCCCAGATCCCACGCCCTGGCCAGGAGCAAGCCGAGGCCAGCCGGCGCACTCCGACTCCGAGCAGTCT
GTCCTCGACCCGAGCCCCGCGCCCTTCGGGACCCCTGGCCAGCGGCGCAGCGCTGCCAACCTGCCGCCATGGAGAC
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GCTGCCGTGCCCTGGCCACCCCTCGTGTACCCGAGGCTACGGCGTCTAGCCGACCCATCTTCCAAGGACGACGGACATGAA
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AAGACCCGCGCCGAGGTGAAGTTCGGAGGGCGACACCCCTGGTGACCCGATCAGCTGAGGAGGACTCAGTCAAGGGAGA
CGGCAACATCTGGGGCAAGCTGGAGTACAACTACACAGCCACACGTCTATATCGACCCGACAGCAGAAGAACGGC
ATCAAGGTGAACTTCAGATCCGCCACACATCGAGGACGGCAGCGTGCAGCTCGCCACCCACTACCGCAGAACCCCC
ATCGGCAGGCCCCGTGCTGGAGCTCGTACCCGCGCCGGGATCACTCTCGGATGGACGGCTGTACAGCAGGGCG
GCGGATCACATGGTCTGGAGCTCGTACCCGCGCCGGGATCACTCTCGGATGGACGGCTGTACAGCAGGGCG
CGCCACCCGCAGCGGGGCGCAGGCCAGCTCCACTCGCGTCGCCACCCGATACCCGGCTGCAGGAGAAGGAGGAC
CTGCAGGAGCTAATGATCGCTGGCGGTCAATCGACCGTGTGCGCTCGTGAAAACGGAGAACGCAGGGGCTGCGCCT
CGCATCACCGAGTCTGAAGAGGTGGTCAGGGGCGAGGTGTCCGGATCAAGGCCCTACGAGGCCAGGCTCGGATCC

mCherry HR-donor plasmid, lamin A/C, 240bp HAs

GGTACCCCGGCGTGGTACTCAGTGGGAGCGCCGACCCTACACCAGCCACCCAGTCCCGAGGTCCGACAG
CGCCCGGCCCAGATCCCACGCCCTGGCCAGGAGCAAGCCGAGGCCAGCCGGCGCACTCCGACTCCGAGCAGTCT
GTCCTCGACCCGAGCCCCGCGCCCTTCGGGACCCCTGGCCAGCGGCGCAGCGCTGCCAACCTGCCGCCATGGAGAC
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CGTGAAGCCCCCCGCCACCCCGACTACTGAAGCTGCTCTCCCGAGGGCTCAAGTGGAGCGCGTGTGAACTT
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AGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGGACGGCGCCACTACGACGCTGAGGTCAAGACC
ACCTACAAGGCCAAAGACCCGTGCAGCTGCCGGCCTACACGTCAACATCAAGTGGACATCACCTCCACACGAG
GACTACACCATCGTGAAGACAGTCGAAACGGCAGGGGCCACTCCACCGGGGCCATGGACGAGCTGTACAGCAGCG
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GACCTGCAGGAGCTAATGATCGCTGGCGGTCAATCGACCGTGTGCGCTCGTGAAAACGGAGAACGCAGGGGCTGCGC
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EGFP TC-donor plasmid, lamin A/C, 240bp HAs

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CAAGTGTATATGCCAGTACGCCCTATTGACGTCAATGACGGAAATGGCCCTGGCATATGCCAGTACATGACCT
TATGGGACTTTCCACTTGGCAGTACATCTACGTATTAGTCATCGCTATTCGATACCGTGGTTGGCAGTACATCAATGG
CGTGGATAGCGGTTGACTCACGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGGCAGCAAAATCAA
CGGGACTTTCCAAAATGCGTAACAACTCCGCCCCATTGACGAAATGGCGGTAGGCGGTACGGTGGAGGTCTTATAAG
CAGAGCTGGTTAGTGAACCGTCAGATCCGCTCGCGTCTGGGCTGTATGCCGGACACCTCCGGGCTGACCCCTTCCAGACTCGGT
GTCGGCAGACGCGTGAGGCTCGGCTCGTAGGGCGCTGGTATGCCGGACACCTCCGGGCTGACCCCTTCCAGACTCGGT
ATGCCAAGGGCCAGCCCTGGCTCCGGCTTCCAGCGAGGGCCACACGGCTGATGACCGCCAGGCGATTTGACGCT
GCAGGTCCCTCTCTCGCAGCCGGGGTTATGCCGGAGCTGGAGCTGGAGCTGGCTGGCCCTGGCCCTGGGG
GCGCGCCCTGTGCTGTAAGCTGCCAGAGTGATCCGGCGGGCTACGAACTCCAGCGGACCATGTGATCG
CGCTTCCTGTTGGGCTTTGCTCAGGGGCGGACGGGTGCTCAGGTAGTGGTTGCTGGGGACGCAGCACGGGCGTCCG
ATGGGGGTTTCTGCTGGTAGGGTGCGGCGAGCTGACCGCTGCCCTCGATTTGTGGCGATCTTGAAGTTCACCTGA

TGCCGTTCTCTGCTTGTGGCCATGATAGACGTTGTGGCTGTTGAGTTGACTCCAGCTTGCCCCAGGATGTTGCCGT
 CCTCCTTGAAGTCGATGCCCTCAGCTCGATGCCGTTCACCAAGGGTGTGCGCCCTCGAACTTACCTCGGCCGGGTCTTGT
 GTTGCCGTCGTCCTGAAGAAGATGGTGCCTGGCAGCTAGCCTCGGGCATGGCAGCTGAAGAAGTCGTCGCT
 ATGTTGGTCGGGTTAGCGCTGAAGCACTGCACGCCGTAGGTGCAAGGGTGGTCAGCAGGGTGGGATCTGGCCGGACAGCT
 GCCGGTGGTGCAGATGAACCTCAGGGTCAAGCTCGCTAGGTGGCATGCCCTCGCCCTCGCCGGACACGCTGAAC
 GCCGTTTACGTCGCCGCCAGCTGCCAGCAGGATGGGCACCAACCCGGTAACAGCTCTCGCCCTGCTCACGGACGGGG
 CTCCATGCCGGCAGGTGGCAGCGCTGCCCGCGGGCAGGGTCCCGAAAGGGCGCGGGCTCGGGTGAAGGACAG
 AGACTGCTCGGAGTGGAGTGCGCCGGCGCTGGCTCTGGCTCTGGCTCCCGCAACACTGAGTCACCAGCAGGGCTCGAGCT
 GTCGGACCTCGGATCTGGGTTGGCTGGTAGGTGCGCGCTCCCGCAACACTGAGTCACCAGCAGGGCTCGAGCT
 AAGCTTCCACCGCAAATGCTTCTGAGATCTATTCGGCAAGAAGACGAGCATCAGCGTGCCTCAGGTGAATCTCAA
 ATTGACGAGCATCAGCCGTGGAGTCTATTCCGGCAAGAAGACGAGCATCAGCCGTGGCTCCAGGGTGAATCTCAA
 TGACGAGCATCAGCCGTGGAGTCAATATGTAAGTAAGTAAAGCTGGCTTTTTGTCAGGGTACCCGGCCATGGTCCCAGCC
 TCTCGCTGGCGCCGGTGGCAACATTCGGAGGGGACCGTCCCTCGGAATGGCAATGGGACGCGGCCGACTCTA
 GATCATAATCAGCCATACCACATTGTAGAGGTTTACTTGCTTAAAAAACCTCCACACCTCCCCCTGAACCTGAAACATAAAA
 TGAATGCAATTGTTGTTAACTGTTATTGCAAGTTATAATGGTACAAATAAGCAATGCACTACAAATTCAAAATAAG
 ATTGTTTCACTGCATTCTAGTTGTGGTTGTCCAAACTCATCAATGTATCTTAAG

mCherry TC-donor plasmid, lamin A/C, 240bp HAs

ACTAGTCGTTACATAACTACGGTAATGGCCCGCCTGGCTGACCGCCAACGACCCCCGCCATTGACGTCATAATGACGTA
 TGTCCCAGATAACGCCAATAGGGACTTCCATTGACGTCATGGGTGGAGTATTACGGTAACACTGCCCACTTGGCAGTACAT
 CAAGTGTATCATGCAAGTACGCCCTATTGACGTCATGCGTAATGCGGTAATGGCCCGCCTGGCATTATGCCCAGTACATGACCT
 TATGGGACTTCTACTTGGCAGTACATCTACGTTAGTACGCTATTACGCTCGTATTACATGGTATGCGGTTTGGCAGTACATCAATGG
 CGTGGATAGCGTTTACTCACGGGATTCCAAGTCTCCACCCATTGACGCAATGGCGGTAGGGCGTAGGGCGTAGCGTGGAGGTCTATAAG
 CAGAGCTGGTTAGTGAACCGTCAGATCCGCTAGCGCTGCCCTGGATGAGTCGCTGAGGACGAAACGGTACTCGTAC
 GTCGGCAGACCGTGAGCTCGGCCCTGAGGGCGCCTTATGCCGACACCTCGCGCTGACCCACTTCAAGACTCGGT
 ATGCGAAGGGCGAGCCCTCGCCTCCGGTACCGAGCGCACACGGTCACTGAGACCGGCAAGCGATCATTGAGCTCT
 GCAGGTCCCTCTCTCGCAGCCGGGTATGCCGAGCGAGCTGGAGCTGCCCTGCCGCGCTGCCGCGTGG
 GCGCCGCGCTGCTGTACAGCTCGCCATGCCGCGGTAGGGCGTAGGGCGCTGGCGCTGACTGTTCCACGATGGT
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 GGTGGTCTTGACCTCAGCGTCGAGTGGCCGGCTCTCAGCTTCACTGCTGATCTGCCCTCAGGGCTTCTGGCTTGA
 TCGGGGTACATCCGCTGGAGGAGGCCTCCAGCCATGGTCTTCTGCACTACGGGGCGTGGGAGGGAAAGTGGT
 CCGCGCAGCTTACCTTAGATGAACCTGCCCTCTGCAAGGGAGGAGTCTGGTACGGTACCCCGCCGCTCTCG
 AAGTTCATCACCGCTCCACTTGAAGCCCTGGGAAGGACAGCTCAAGTAGTCGGGATGTCGGCGGGTGTCTCACGT
 AGGCTTGGAGCGTACATGAACCTGAGGGGACAGGATGTCCTCAGGGCAAGGGGAGGGGCCACCTTGGTACCTTCAGCT
 TGGCGGCTGGGTGCCCTCGTAGGGGCGGCCCTGCCCTCGCATCTCGAACTCTGTCGGCTTCAAGGGGCCCTCA
 TGTGCACCTTGAAGCGCATGAACCTCTGTAGTGGCATGTTATCCTCTCGCCCTGCTCAGCATGGACGGGTCTCATG
 GCCGGCAGGGTGGCAGCGCTGCCCGGGCAGGGGCTCCCGAAAGGGCGCGGGCTGGGTCGAAGGACAGAGACTG
 TCGGAGTCGAGTGCGCCGGCGCTGGCTCTGGCTCAGGCGTGGGATCTGGCGGGCGTGTGGAC
 CTCGGGATCTGGTTGGCTGGTAGGTGCGCGCTCCCGCAACACTGAGTCACCGACGCCGGAGATCTCGAGCTCAAG
 CTCACCAGCAAATGTTCTGAGATCTATTCGGCAAGAAAGACGAGCATCAGCGTGCCTCAGGTGAATCTCAAATT
 GCACGAGCATCAGCGTGCAGTCAATGTAAGTAAGTCAGGTTTGTGACGGTACCCGGCCATGGTCCCAGCCTC
 CTCGCTGGCGCCGGTGGCAACATCCGGAGGGGACCGTCCCTCGTAATGGCAATGGGACGCGGCCGACTCTAGA
 TCATAATCAGCCATACCACTTGTAGAGGTTTACTTGCTTAAAAAACCTCCACACCTCCCCCTGAACCTGAAACATAAAATG
 AATGCAATTGTTGTTAACTGTTATTGCAAGTTATAATGGTACAAATAAGCAATGCACTACAAATTCAAAATAAG
 ATTGTTTCACTGCATTCTAGTTGTGGTTGTCCAAACTCATCAATGTATCTTAAG

EGFP HR-donor plasmid, FBL, 240bp HAs

GGTACCAAGATGCAACAGGAGAACATGAAGCCGAGGAGCAGTTGACCCCTTGAGGCCATATGAAAGAGACCATGCCGTGGTC
 TGGGAGTGTACAGGTGAGCAGGGGCCAGCAATAACCCAAGACAGACATCTGTCCTTGACCCCGAGTGCCTGATGACCT
 GGGGTAGTAGACCCCTCTCATCACCTATCTCTCAGGCCACCCGTGAGCAAGGGCGAGGAGCTGTTCACCGGGTG
 GTGCCCATCTGGTCAGCTGGACGCCAGTAAACGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCAC
 CTACGCCAGCTGACCTGAAAGTCTCATGCCACCCGGCAAGTGCCTGCCCTGCCACCTCGTGACCCACCTGAC
 TACGCCGTGAGTCTCATGCCACCCGGCAAGTGCCTGCCCTGCCACCTCGTGACCCACCTGAC
 TCCAGGAGCGCACCATTCTCTCAAGGACGACGCCAACTACAAGGCCCGCCAGGGTAAGTTCAGGGCGACACCTGG
 TGAACCGCAGTCAAGGAGGATCGACTCAAGGAGGAGGCAACATCTGGGCAAGAGCTGGAGTACAACACTAACAG
 CCACAACGTCTATCATGGCCACAAGCAGAAGAACGGCATCAAGGTGAACCTCAAGATGCCACACATCGAGGACGGCA
 GCGTGCAGCTGCCGACCAACTACCAGCAGAACACCCCCATGCCGACGGCCCCGTGCTGCTGCCGACAACCAACTACCTGA
 GCACCCAGTCCGCCCTGAGCAAAGACCCCAAGGAGAACGGCGATCACATGGTCTGCTGGAGTTGTCAGGCCGCCGG
 TCACTCTCGCATGGACGAGCTGACAAGGCCAAGGTGAAGAAGTCAAGTTCAGCGCTGTCAGGATTGCGAGAGATGTTG
 GATACTGTTGACGAGCTGTTTCTATTAAAGACTCATCCGCTCCCATGTCGCTGTCATTCTCCCCCTGACCTGCTGACA
 CAGGGAGCAGCCACCCCTGGTCAATTGGGGGTTGGTAAATTCTCACTCGGTACAGAGGGCATGCTCGTTAGCTG
 CCTTGCGCAGCGCAGCCTGGGATCC

EGFP TC-donor plasmid, FBL, 240bp HAs

ACTAGTATTTGCACTGCGCTATGTTCTGGGAAATCACCATAAACGTGAAATGTTGGATTGGGAAATCTTATAAGTTG
 ATGAGACCAACTCGACCGCTCCAGGCTGCCGCTGCGAAAGGCAGCTAGAAACGGAGCATGCCCTGTGACCGAGT
 GAGAATTTACCCAGCAAATTGACCAAGGGTGCCTCCCTGTGTCAGCAGGTCAAGGGGAGGAATGAGCAGCAGACATGG
 AGACGGATGAGTCTTTAAAGAAAACACACGTGCAACAGTCAACACACATCTCGCAATCTGACAGCGCTGAACCTCA
 GTTCTCACCTGGGTTGTACAGCTCGTCCATGCCGAGAGTGTACCCGGCGGGTCAAGAATCCAGCAGGACCATGTGA
 TCGCGCTTCTCGTGGGGTCTTGCTCAGGGGGACTGGGTGTCAGGTAGTGGTGTGGCGAGCAGCACGGGGCGTCG

CCGATGGGGTGTCTGCTGGTAGGGTCGGAGCTGCACGCTGCCCTCGATGTTGGCGGATCTGAAGTCACCT
 TGATGCCGTTCTGCTTGCGCATGATAGACGTTGGTAGTTGACTCCAGCTGTGCCCAAGGATGTTGC
 CGTCCCTCTGAAGTCGATGCCCTCAGCTCGATGCCGTTACCGAGGGTGTGCCCTCGAACTCACCTCGGCCGGTCTT
 GTAGTTGCCGCTGCTTAAGAAGATGGTCGGCTCAGCTCGATGCCGTTACCGAGGGTGTGCCCTCGAAGAAGTCGCTGC
 TTCATGTTGGCTGGGTAGCGGCTGAAGCACTGCACGCCGTAGGTCAAGGGTGGTCAGGAGGGGGCAGGGCACGGCAG
 CTTGCCGGTGGTGCAGATGAACCTCAGGGTCAGCTGCCGTAGGTGGCATGCCCTGCCCTGCCGGACACGCTAACATTG
 TGGCCGTTACGTCGCCGTCCAGCTGCCAGGGATGGGACCACCCCCGGTGAACAGCTCTGCCCTGTCACGGTGGC
 CTGTGAGAGGAAGATAGGTGATGAAGGAGGGTCACTACCCCAGGATATGGCACTCGGGTCAAGGGACAGAGATGTC
 TCTTGGTGATTGCTGGGCCCTGTCACCTGTAACCTCCACGCCAGGGCATGGCACTCGGGTCTTTCATATGGCTCAAGGGTCAACT
 GCTCCTGCCGCTCATGTTCTCTGGTCATCTCTCAGGCTAAGCTCCACGCCAAATGCTCTGAGATCTATTCGGCAA
 GAAAGCACGAGCATGCCGTGCCCTCAGGCTGAATCTCAAATTGCAAGGAGCATGCCGTGCCGATCAATATGTAAGAAG
 AAAGCACGAGCATGCCGTGCCCTCAGGCTGAATCTCAAATTGCAAGGAGCATGCCGTGCCGATCAATATGTAAGAAG
 AGCGTTTTTTGTCACGGTACGCCGGCATGGTCCCAGGCCCTCGTGGCCGGTGGCAACATTCCGAGGGGAA
 CCGTCCCCCTCGGAATGGCGAATGGGACGCCGGCGACTCTAGATCATAATCAGCCATACCACATTGAGGGTTACTTG
 CTTAAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTAACCTGTTATTGCAAGCTATA
 ATGGTTACAATAAAGCAATAGCATCACAAATTCAAAATAAGCATTTCAGCTGATTCTAGTTGTTAACCTGCAATTGTT
 TCAATGTATCTAAG

IRES-EGFP HR-donor plasmid, GAPDH, 500bp HAs

GGTACCGGGCTGTGGGCAAGGTCAACCTGAGCTGAACGGGAAGCTCACTGGCATGGCCTCCGTGCCCCACTGCCAACGT
 GTCACTGGGGACTGACCTGCCCTAGAAAACCTGCCAAATATGATGACATCAAGAAGGGTGTGAAAGCAGGGCTGGAG
 GGCCCCCTCAAGGGCATCTGGCTACACTGAGCACAGGTGGCTCTCTGACTTCAACAGCGACACCCACTCCTCCACCT
 TTGACGCTGGGGCTGGCATTCGCCCTAACGACCAACTTGTCAAGCTCATTCCTGGTATGGCTGGGGCAGAGACTGGCTC
 TAAAAAAGTGCAGGGCTGGGCCCTCTGGCTGGCTCAGAAAAAGGGCCCTGACAACACTCTTTCATCTTAGGTATGAC
 AACGAATTGGCTACAGCAACAGGGGGTGGACCTCATGGCCCACATGGCTCCAAGGAGTAAGACCCCTGGACCACAGCC
 CCAGCAAGAGCACTAATTAAATAGTGAGTGAATTCCCCTCCCTCCCCCCCCCTAACGTTACTGGCCAAGCCGCTGG
 AATAAGGCCGTGTGCGTTGCTATATGTTTTCCACCATATTGCCGTCTTGCAATGTGAGGGCCCGAAACCTGGGCC
 TGCTCTTGACGAGCATCCCTAGGGCTTCCCTGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAG
 TTCTCTGGAAAGCTCTGAAGACAAACAGCTGTAGGCCCTTGCAAGGGCAGGCGAACCCCCCCTGGCAGAGGTG
 CCTCTGCCAAAAGGCCACGTATAAGATACACCTGCAAGGGGCAACACCCCCAGTGCACAGTTGTGAGTTGGATAGTT
 TGAAAGAGTCAAATGGCTCTCTCAAGCGTATTCAACAAGGGCTGAAGGATGCCAGAAGGTACCCATTGATGGATCT
 GATCTGGGGCTCGGTGCACATGCTTACATGTTAGTCAGGTTAAACACGCTAGGCCCTCATCGTGAGCAAGGGCAGG
 GGTTTCTTGAAAAACACGATGATAATATGGCACAATGGTCGACCTCATCGTGAGCAAGGGCAGGAGCTGTCACCGGG
 GTGGTGCCCCATCTGGTGAGCTGGACGGCACGTAACGGCCACAAGTTCAGCGTGTCCGGCAGGGCGAGGGCGATGC
 CACCTACGGCAAGCTGACCCCTGAAGTTCATGCAACACCGCAAGCTGCCCTGGCCCACCCCTCGTGACCAACCC
 ACCTACGGCGTGCAGTGTCTCAGCGCTACCCGACCATGAGCACGACGACGACTTCTCAAGTCCGCCATGCCGAAGG
 ACCTCGAGGAGCGCACCATCTCTCAAGGACGACGGCAACTACAAGACCCGCCGGAGGTGAAGTTCGAGGGCGACAC
 TGGTGAACCCGATCGAGCTGAAGGGCATGACTCAAGGAGGACGCCAACATCTGGGGCACAAGCTGGAGTACAAC
 CAGCCACAACGCTATATCATGGCCACAAGCAGAACAGGGCATCAAGGTGAACCTCAAGATCCGCCACAACATCGAG
 GCAGCGTGCAGCTGCCGACCACTACCGAGAACACCCCCATGGCGACGGCCCCGTGCTGCTGCCGACAACCAACTACC
 TGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAACAGCGCATCACATGGCTCTGCTGGAGTTCGAC
 GGATCACTCTGGCATGGACGAGCTGTACAAGTAGACCGGGCGATGAGAGAGAGAGACCCACTGCTGGGGAGTC
 CCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCTCTCAGCTAACAGTGTGACCTTGAAGAGGGAGGGCCT
 AGGGAGGCCACCTGTGATGACCATCAATAAGTACCCCTGTGCAACCGAGTTACTTGTCTGTCTATTCTAGGGCTGG
 GCAGAGGGAGGGAAAGCTGGCTTGTGCAAGGTGAGACATTCTGTGGGGAGGGACCTGGTATGTTCTCAGACTGAG
 GGTAGGGCTTCAACACGCTTGTGCTCGAGAACATTGCTTCCGCTCAGCGTCTGAGTGTACAGGAAGCTGGCA
 CCAACTTCAGAGAACAGGCCCTTCTCTCGCTCAGTCTAGGCTATGCTGTTGGCAAACATGGAAGAACGTT
 TCTGTGGGAGCCCCAGGGAGGCTGACAGGGTGGAGGAAGTCAGGGCTCGGGATCC

mCherry-GFP reporter point mutation HR-donor plasmid, GAPDH, 240bp HAs

CTCGAGCGACGCTGAGGTCAAGACCACTACAAGGCCAAGAACGCCGTCAGCTGCCGCCCTACAACGTCAACATCAA
 GTGGACATCACCTCCCACAACGAGGACTACACCATGTCGAAACAGTACGAACGCCGAGGGCCCACTCCACCGCG
 CATGGACGAGCTGTACAAGCTGAGGGCGAGGAGGCAGCGCGAGGGAGGCAGCGCGAGGGAGGCAGCGCTGCTCG
 CGATGCTAGAGGGCTGCTCCAGTGAAGGCCAGGGAGGAGCTGTTACCGGGCTGGTCCACCTCTGGTCAGCTGGACGG
 GACGTAACGGCCACAAGTTCAAGCTGCTGAGGGAGGAGCTGCTGGGACCTACGGCAAGCTGACCCCTGAAGTTCATCT
 GCACCAACGGCAAGCTGCCGTGCCCTGGCCACCCCTCGTGACCAACCCCTGACCTACGGCGTGCAGTGCCTCAGCCGCTACC
 CCGACGCGT

mCherry-GFP reporter point mutation TC-donor plasmid, GAPDH, 240bp HAs

CGTTACATAACTACGGTAATGCCGCCCTGGCTGACCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCC
 CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGGAGTATTAACGTTAACTGCCACTTGGCAGTACATCAAGT
 TATCATATGCCAAGTACGCCCTATTGACGTCAATGACGTTAAATGCCGCCCTGGCATTATGCCACTACATGACCTTATGG
 ACTTCCCTACTTGGCAGTACATCTACGTTAGTCATCGCTATTACCATGGTATGCCGTTGGCAGTACATCAATGGCGTGG
 ATAGCGGTTGACTCACGGGATTTCAAGCTCCACCCATTGACGTCATGGGAGTTGGTGGCAGGGACAAAATCAACGGGA
 CTTCCAAAATGCTGTAACAAACTCCGCCATTGACGCAATGGGGCGTAGGGCTGTACGGTGGAGGTCTATAAGCAGAG
 CTGTTTAGTGAACCGCTGAGCTGCCAGGGCTGGATGAGTCCGTGAGGAGCAACGGTACTCGGTACCGTGG
 CAGACGCGTGGGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG
 TTGCCGGTGGTGCAGATGAACCTCAGGGTCAGCTGCCGTAGGTGGCATGCCCTGCCCTGCCGGACACGCTGAACCT
 GGCGTTACGTGCCGCCAGCTGACCAAGGGATGGGACCAACCCCCGGTGAACAGCTCTGCCCTGCTCACTGGCAGAG
 CTCCTAGCATCGCGAGCAGGCCGCTGCCCTCCGCCGCTGCCCTCCGCCGCTGCCCTGCCCTGAGCTTGAC
 CTCGTCATGCCGCCGGTGGAGTGGCAGGCCCTGCCGCTGGTCACTGTTCCAGATGGTGTAGTCTCTGTTGAGGAGG
 GATGTCCAACCTGATGTTGACGTTGAGGCGCCGGCAGCTGACGGCTTCTGGCCTGAGGTGGCTTGTGACCTCAGCG

TCGCTCGAGCTCAAGCTTCCACCGCAAATGCTCTGAGATCTATATTGGCCAAGAAAGCACGAGCATCAGCCGTGCCTCCAG
 GTCGAATCTTCAAATTGACGAGCATCAGCCGTGCGGATCTATATTGGCCAAGAAAGCACGAGCATCAGCCGTGCCTCCAGG
 TCGAATCTTCAAATTGACGAGCATCAGCCGTGCGGATCAATATGTAAGTAAGCTGGTTTGTGACGGTACCGCCGG
 CCATGGTCCCAGCCTCCCGTGGGGGGGGGGCAACATTCCGAGGGGGGACCGTCCCCTCGGTAATGGCAATGGGACG
 CGGCCGCGACTCTAGATCATACGCCATACCACATTGTAGAGGTTACTGCTTAAAAAACCTCCACACCTCCCCCTGA
 ACTCTGAAACATAAAATGAATGCAATTGTTGTTAACTTGCTTATTGAGCTTAAATGGTTACAATAAGCAATAGCATACAA
 ATTTCACAAATAAAGCATTTTTCACTGCATTCTAGTTGTTGCTAAACTCATCAATGTATCTA

IRES-EGFP TC-donor plasmid, GAPDH, 500bp HAs

ACTAGTCGTTACATAACTACGGTAAATGGCCGCCCTGGCTGACCGCCAACGACCCCGCCATTGACGTCAATAATGACGT
 TGGTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGGTTGAGTATTACGGTAAACTGCCACTGGCAGTACAT
 CAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAATGGCCCGCTGGCATTATGCCAGTACATGACCT
 TATGGGACTTCCACTTGGCAGTACATCACGGTATTACGTATTAGTCATCGCTATTACCGTGGATCGCGTTTGGCAGTACATGGG
 CGTGGGATAGCGGTTGACTCACGGGATTCCAAGTCCACCCATTGACGCAATGGCGGTAGGCCTGTACGGTGGGAGGTCTATAAG
 CGGGACTTCCAAAATGCGTAACAACCTCCGCCCCATTGACGCAATGGCGGTAGGCCTGTACGGTGGGAGGTCTATAAG
 CAGAGCTGGTTAGTGAACCGTCAGATCCGCTAGCGCTGCCCCCTGGGCTGCCACAGAATAGCTTCCATGTT
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 CAGCAGCACGGGCCGTCGCCATGGGGTGTCTGTTGTAGTGGTGGCGAGCTGACCGCTGCCCTCGATGTTG
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 CGCCGGACACGCTGAACCTGTGGCGTTACGTCGCGTCCAGCTGACCCAGGATGGCACCCAGGTTGAGTGGCAG
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 GGGCTAGACGTTTTAACCTGACTAAACACATGTAAGCATGTGACCGAGGCCAGATCAGATCCCATAACATGGG
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 TCAACATTGCCAAAAGACGGCAATATGGGAAAACACATAGACAAACGCAACACGGCCTTATTCAAGGGCCTTC
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 AGCCACATACCAGGAAATGAGCTGACAAGTGGTGTGAGGGCAATGCCAGGCCAGCGTCAAGGTGGAGGAGTGGG
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 CCACCTCTTGATGTCACTATTTGCGCAGGTTCTAGACGGCAGGTCAGGTGCCACACTGACACGTCAGTGGGACA
 CGGAAGGCCATGCGCAGTCCCGTCAAGCTCAGGGATGACCTTCCACAGCCCTGAGCTCAAGCTTCCACCGCAA
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 CGCTGCGGATCTATTGCGCAAGAAAGCAGAGCATCAGCCGTGCCCTCAGGGTCAATCTCAATTGACGAGCATCAG
 CGTGGGAGTCAATATGTAAGTAAGCTGTTGCTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
 CGGTGGGCAACATCCGAGGGGGACCGTCCCTCGGAATGGCAATGGACGCCGCGACTAGATCATAATCAGCA
 TACCACATTGAGGGTTACTGCTTAAAAAACCTCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTT
 TTGTTAACTTGTGTTATTGAGCTTAAATGGTTACAATAAGCAATAGCATACAAATTTCACAAATAAGCATT
 TTCTAGTTGTTGCTTAACTCATCAATGTATCTAAG

PRNP point mutation TC-donor plasmid, 240bp HAs

ACTAGTCGTTACATAACTACGGTAAATGGCCGCCCTGGCTGACCGCCAACGACCCCGCCATTGACGTCAATAATGACGT
 TGGTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGGTTGAGTATTACGGTAAACTGCCACTGGCAGTACAT
 CAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAATGGCCCGCTGGCATTATGCCAGTACATGACCT
 TATGGGACTTCCACTTGGCAGTACATCACGTATTAGTCATCGCTATTACCATGGTGTGCGGTTTGGCAGTACATCAATGGG
 CGTGGATAGCGGTTGACTCACGGGATTCCAAGTCTCACCCATTGACGTCAATGGGAGTTGTTGGCAGGAAACATCAA
 CGGGACTTCCAAAATGCGTAACAACCTCCGCCCCATTGACGCAATGGCGGTAGGCCTGTACGGTGGAGGTCTATAAG
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 CCAGCCACCAACATGAGGCTGCCACCCAGCCACCCATGGGCTGCCACCCAGCCACCCAGCCACCCAGCC
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 AAATCTCAAATTGACGAGCATCAGCGTGCAGGATCTATATTGGCCAAGAAAGCACGAGCATCAGCCGTGCCCTCAGGT
 ATCTCAAATTGACGAGCATCAGCCGTGCCAGGATCAATGTAAGTAAGTAAGCGTTTTTGTGACGGTACCGCCGCCATG

GTCCCAGCCTCGCTGGCGGCCGGTGGCAACATTCGAGGGGGACCGTCCCTCGTAATGGCGAATGGGACGCGGC
CGC

IRES-EGFP-Lamin TC-donor plasmid, GAPDH, 500bp HAs

ACTAGTCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACGCCAACGACCCCCGCCATTGACGTCAATAATGACGT
TGTTCCCAGTAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACAT
CAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGCCCGCCCTGGCATTATGCCCACTGACCT
TATGGGACTTCTACTTGGCAGTACATCTACGTATTAGTCATCGTCAATTACCATGGTATGCCAGTACATCAATGGG
CGTGGATAGCGGTTGACTCACGGGATTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTGGCAGTACATCAATGGG
CGGGACTTCCAAGATGCTGAACACTGCCCTATTGACGCAAATGGCGTAGCGCTGAGCTCCCTGGGCTGCCACAGAATAGCTT
CAGAGCTGGTTAGTGAACCGTCAAGTCCCTAGCGCTGAGCCCTGACTTCCACCTGTCAGCCTCCCTGGGCTGCCACAGAATAGCTT
GTCGGCAGACGCGTGCAGGCCCTGACTTCCACCTGTCAGCCTCCCTGGGCTGCCACAGAATAGCTT
GGCCAACAGCAGATAGCTAGGACTGGAGCAGGAGAGAAAAGGCCCTGTTCTGAAGTAGTGGTGCAGCTCCTGAG
CACTCAAGACGTCTAGCGGGAAAGCAAATGGTCTCGAAGCAAGCAAGGCTTTGGAGGCCCTACCCAGTCTGAGGAGA
ACATACCAGGCTCCCTCCCCAGCAAGAAATGTCACCTTGACACAAGCCCAGCTCCCTCCCTGCCCCAGACCCCTAGAATA
AGACAGGACAAGTACTGGTTAGCACAGGGTACTTTATTGATGGTACATGACAAGGTGGCTCCCTAGGCCCTCCCT
TCAAGGGTCTACATGGCAACTGTGAGGGGGAGATTAGTGTGGTGGGGACTGAGTGTGGCAGGGACTCCCGAGCAGT
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GATGTTACCAAGGGCATCAATAGATCCCACACTACTTACCAACTTGAATTATGAGTCAAAACATTAAATTGGATTAAAAAGAAG
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AAACTATCCACCCATTATATATATGTGAGTGTGACTCTATAATCTGAGTTGCAATTCTTAAATGGGATGATGATACCCCT
AGAAAACAGGGTACCAAAAGGATATCAGAGTAAAACATTCTGAAGAGCATACCAAAATGGTCTAGTTGTAATCCATA
CAACAAACAAATCGGGTTGAAAAGATTCTACTAAAAAGAAAAAAATCTGATTGGCATACTAGAAAGGTCAACAC
CTTCTATTGCCCCAAAGTTCTAGCTCTAGGGTAAATGGTTTCAGCCAAAGCAGTTACCTTAGGGATGGGACAGAGTGA
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CGGCCATGATAGACGTTGTGGCTGTTGACTCCAGCTGCCCCAGGATGTCCTGCTGCTGCTGCTGCTGCTG
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GACAAACGCACACCGCCATTCCAAGCGGCTCGGCCAGTAACGTTAGGGGGGGAGGGAGAGGGGAAATTCACTCA
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GGCAGGTCAAGGTCCACCAACTGACACGTTGCAGTGGGACACGGAAGGCCATGCCAGTGGCTGAGCTCCCGTTGAGCTCAGGG
TGACCTTGGCCACAGCCCTCGAG

PRNP point mutation HR-donor plasmid, 240bp HAs

CTCCATCATCTAACGTCGGTCTCGGTGAAGTTCCCCCTGGTGGTGGTACCGTGTGCTGCTTATTGTGATATTGAC
GCAGTCGTGCACAAAGTTCTGGTTCTGACTCATCCATGGGCCCTGTAAGTACACTGGTGGGGTAACGGTGATGTTTC
ACGATAGTAACGGTCTCATAGTCACTGGCAAATGTATGATGGGCCCTGCTCATGGCACTTCCCAGCATGAGGCCAAGGCC
CCCCACCACTGGCCCAAGCTGCAAGCAGCATGGTCTCATGGTGGTTTGGCTTACTGGCTTGTGCTTCACTGACTGT
GGGTGCCCCACCTCCTTGGACCCAGCCACCACCATGAGGCTGCCCCAGGCCACCATGGGCTGCCCCAGGCCACCATGG
GAGGCTGCCCCAGCCACCACATGAGGCTGCCCCAGCCACCACGCCCTGAGGTGGTAGCGGT

IRES-GFP-Lamin HR-donor plasmid, FBL, 240bp HAs

CTCGAGAAGATGCAACAGGGAAACATGAAGCCGCAGGAGCAGTTGACCCCTTGAGCCATATGAAAGAGACCATGCCGTGGTC
TGGGAGTGTACAGGTGAGCAGGGGCCAGCAATACACCAAGACAGACATCTGTCCCTTGCACCCCGAGTGCATGATCCT
GGGGTAGTAGACCCTCCTCATCACCTATCTCCTCTCACAGGCCACCCAACTGAGCTACTGGCGAAGCCGCTTGGAAATAAGGCC
GGTGTGCGTTGTATATGTTATTTCCACCATATTGCGCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCT
GACGAGCATTCCTAGGGGTTTCCCCCTCTGCCAAAAGGAATGCAAGGTCTGTTAGTGTGAGGAAGCAGTCTCTGG
AAGCTCTGAAGACAAACAGCTGTAGCGACCCCTTGAGGCCAGCGAACCCCCCCTGGCGACAGGTGCTCTGGCG

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 CCTCGGTACACATGCTTACATGTGTTAGTCGAGGTTAAAAAAACGCTAGGCCCCCGAACCACGGGGACGTGGTTCCCT
 TGAAAACACGATGATAATATGGCCACAATGGTCGACCTCATCGTAGACAAGGGCGAGGGAGCTTCACCGGGGTGGC
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 GGCATGGACGAGCTGACAAGACGGTATGCGACCACTCTGTGCTTCTGCTCTCGTGTGCGGGCTGCGGGTGTG
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 GGGTGGAGGAGCCGGCTGCTGCTGCTGCTCCGTGATGATTCTCTGCCGGAACCTCTTCTGATCTCCAG
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 GGACTTACGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
 GGATTGCGAGAGATGTGTTGATACTGTTGACGTGTTGTTCTATTAAAGACTCATCCGCTCCCATG
 CCTCCCTTGACCTGCTGACACAGGGAGCACGCCCTGGTCAATTGCGGGGTTGGTAAATTCT
 ACTCGGTGAGGAGCAGCTGGACGCTGGACG
 CGCATGCTCCGTTCTAGCTGCCCTGGCAGCGGAGCCTGG

IRES-GFP-Lamin TC-donor plasmid, FBL, 240bp HA

CTCGAGATATTGCACTGCGCTATGTGTTGGAAATCACCATAAACGTAATGCTTTGGATTTGGAAATCTTATAAGTTCTG
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 TGTCGACCGAGAAGTCTCCAGCCATGAGCGGGCGCCTCTGCAAGGCCATCTGGAGCAGCAGGAGCAGGATGAGGAG
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 CGCAAGGGAGGTGGACTCGACGCACTGACGGAGAAGCAGTGGCTCAAGGCCATCGAGGAGGAGCAGCTGGAGGAG
 CGAAGAGGGAGGTGGCAGAAGAAATCATCACCGAAGGCCAAGCGAGAGACAGGCCAGGGGGCTCTCCACCCCC
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 CGTGTGTTTCAAAGGAAAC
 CCCGGTGAACAGCTCCCGCCCTGCTCACGATGAGGTCGACCATGGCCATTATCAT
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 GGCGAGGTTCCGGGCCACATTGCAAAAGACGGCAATGGTGA
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CACCGCAAATGCTTCTGAGATCTATTCGGCCAAGAAAGCACGAGCATCAGCCGTCGCTCCAGGTCGAATCTCAAATTGAC
GAGCATCAGCCGTGCGGATCTATTCGGCCAAGAAAGCACGAGCATCAGCCGTGCTCCAGGTCGAATCTCAAATTGAC
AGCATCAGCCGTGCGGATCAATATGTAAGTAAGTAAGCGTTTTTGTCGACGGTACCGCCGCCATGGTCCCAGCCTCTCG
CTGGCGGCCGGTGGCAACATTCCAGGGGACGTCCCTCGTAATGGCAATGGCAATGGGACACCGCG

IRES-EGFP-Lamin HR-donor plasmid, GAPDH, 500bp HA

CTCGAGGGCTGTGGCAAGGTCACTCCCTGAGCTGAACGGGAAGCTCACTGGCATGGCCTCCGTGTCCCCACTGCCAACGT
GTCAGTGGTGGACCTGACCTGCCGCTAGAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAG
GGCCCCCTCAAGGGCATCCTGGGCTACACTGAGCACCGAGTGGCTCCTCTGACTTCACCGCAGACCCACTCCTCCACCT
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AACCATATTCTCTATAGCTCTGAGACATACAGCAAGTGGTGTGGTCCCTCCATGTCAGTTAAAGCGTTTATTCC
CAAACCTATAAAGGAGTTATGAGTTTCACTGTGCTCCCATCCTTAAGGTACTGCTTGGTGAACCTTACCCATTAG
CTAGAAACCTTGGGACAATAAGAAGGGTGTGACCTTCTAGTATGCAAAATACAAGTTTTTTCTTTAGTATGAAATAC
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CAGTTACTGTCTCTGTTCTAGGCTCTGGGAGGGAGGGAAAGCTGGCTGTGTCAGGTGAGACATTCTGCTG
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CTCAGACGCTTGTGACTGTCACAGGAAGCTGGCACCACACTTCAGAGAACAGGCCCTTCTCTCTGCTCCAGTCTAGG
CTATCTGCTGTTGGCAACATGGAGAACGCTATTCTGTTGGCAGCCCCAGGGAGGCTGACAGGTGGAGGAAGTCAGGGCTC
GCACCGCGT

10 kb KO HR-donor plasmid, CDC42, 500bp HA

CTCGAGCCGTGTGATCGTGTGGCTCTGCTGCTGGTGTGGTCAGAGCCTGAGTAAGGCTGAATAAGGCTGGATCATGAACCAT
TCTTAAAGCAGTGGCCTTGTATTGCACTGAGTCACTGACATACATGCAACATACAGGTGTGTCACGTGTAACGTGAAAGAAC
GCTTCACAAAATAATTTAGCACACTGTGAAATTCTCTGATACAGCCTTCTCTTCATGATAAAAATAATTGCTGATT
CACACCACTTGGACCTTTAAATACACTTAAGAAGTGGAAATGCGAGGGATCAGAAACTTAGAAATGGACCTTATTGTTA
TTGTAACTTATAGCAAGTCATTGTCCTTATTGGGTTCTGCCTGCAATAATTGTCAGTTCTTCTGCTGAGTGCCT
GAACCTGTTGCTAAGTGGAGGAAAGAAAATCCATGTAAGTAAATAAAACAAATGCTTAACTCTTCTGAGTGCCT
TTGAAATATTGTACTCCTCCTTATTAGTACTGCAAGTGGTCTAGGCTTCTGAGTGCAGAAAGAATTGCACTGG
TAACCTTCTGAGTGCATTCTACTTGTAAATGATGAGAAGAGGAGGGCTTAACTTTGCTGCTTAAAGAATTACTGCT
GTTCTGATTATTGTTGCTAATGTCAGATGTTGAGGCTTCTGAGTGCAGTTAGGTGATGGGACTTTAGGACATATT
ACTGGGATCAAACGCT
ACACTGGGGTGGCTGGGTTGGCACTTACAATTGGAGCTGTTCTGGGACTTTAGGACATATTACTGGGATCAAACG
ATCAAACAAAGAACATGTGAAACAACCTGGGTTAAACTTGTGGCTCAGATTAGGTGATTTAGGTGAGTCTGTA
GGGTCTAAGTACCAATGCTGAGTCACACTGTCATGTTGGAGTTCTGGCTGAGGTGAAGTGTATTCTGATCTTA
CCGACCGCGT

10 kb KO TC-donor plasmid, CDC42, 500bp HAs

CTCGAGACTAGTCGTTACATAACTTACGGTAATGGCCGCCCTGGCTGACCGCCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTCATAGTAAACGCCAATAGGGACTTCCATTGACGTCAATGGGGAGTATTACGGTAAACTGCCACTGGCA GTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAATGGCCGCCCTGGCATTATGCCAGTACA TGACCTTATGGACTTCCACTTGCAGTACATCTACGGTATTAGTCATCGCTTACCATGGTATGCCGGTTTGGCAGTACATC AATGGGGCTGGATAGCGGTTGACTCACGGGGATTCCAAGTCTCACCCCCATTGACGTCAATGGGAGTTTGGCACC AAAATCACCGGACTTCCAAAATGTCGAACACTCCGCCCTATTGACGAATGGCGGTAGGCCTGTCAGCTGGAGGTCT ATATAAGCAGAGCTGGTTAGTGAACCGTCAGATCCGTAGCGCTGCCCCGGATGAGTCCTGAGGGACGAAACGGTACTC GGTACCGCTGGCAGACCGCTGGGATTAAGATACAGAAATACACTTACACCTCAGCCAGAAACTCCAAACATGACAGTGTGAC TCAGCATTGGTGGTACTTAGGACCCAGGTCTACAGACTCACCTAAATCACCTAAATCTGAAGGCCACAAGTTACCACCAAGG TTGTTTACATGTTCTTAGTTGATCAGTTGATCCCAAGTAAATATGTCCTAAAGTCCAGAACACAGCTCCAATTGTAAGT GCCAACCCAGACCACCCCAAGTGTCCACCTCCACCCCTGCCCATCCATGACATCCACAGGACTGGTACCCACATGTAC ATTAGACACAAAATAATCAGAAACAGCAGTAAATCTTAAAGCAGCAAAGTAAAGCCTTCCTTCTACATTACATTTACA AAGTAGAAATGCCACATAAAAGTAAATTCCTACTGCAATTCTTCTGCACATCTAGGAACCAACTTGGCAGTAAATATAAAAG GAAGGAAAGGAGTACAAAATATTCAAATCTGATGATGACCTGCAAAAGAGTAAAGACATTGTTATTACTACATATGGATT TTCTTCCTCACTTAGCAACAGGTTAGGCACTCAGAGCAAGAAAAGAAACTAGCACAATTATTGGCTAAAGAACCAATAAGA GACACAATGACTTGTATAAGTACAATAACAAATAAGGTCATTCTAAGTTCTGATCCCTGGCTATCCAACTTCTTAAGTGT TTTTTAAAGGTCAAAGTGGTGTGATAATCAGCGAATTATTATTCATGAAAGAGAAGAGGCTGTATCAGAGAAAATTCCAC AGTGTGCTAAATATTATTTGTGAAAGCTGTTCTCAGTTACACGTCACACACACCTGTATGTATGCTAGTCAACTAGACT GCAATACAAAGGCCACTGTTAAAGATGGTCTAGTACGCCCTATTGACGCCCTACTCAGGCTCTGACCAACAAGCAGCAG AGCACCAACAGCATCACCGCTGAGCTCAAGCTCCACCGCAAATGCTCTGAGATCTATTCGGCCAAGAAAGCACGAGCAG ATCAGCGGTGCTCCAGGTGAAATCTTCAAATTGACCGAGCATCAGCGTGCGGATCTATTCGGCCAAGAAAGCACGAGCAT CAGCGGTGCTCCAGGTGAAATCTTCAAATTGACCGAGCATCAGCGTGCGGATCTATGTAAGTAAAGTAAAGCCTTTTGT CGACGGTACCGCCGCCATGGTCCCAGGCCCTCTCGCTGGCGGCCGTGGCAACATTGAGGGGGACCGTCCCCTCGTA ATGGCGAATGGGACGCCGCCGACGCGT

8 kb KO and I-SceI Replacement HR-donor plasmid, CDC42, 500bp HAs

CTCGAGCAGGTGTGTCACCGTAACTGAAGAACAGCTTCAAAAATAATTAGCACACTGTGGAATTCTCTGATACA
GCCTCTTCTTCTGATAAAAATAATTGCTGATTATCACACCCTTGGACCTTTAAAATAACACTTAAGAAGTGGAAATA
GCCAGGGATCAGAAACTTAGAAATGGACCTTATTGTTATTGTAACCTAGCAAGTCATTGTCCTTATTGGGTTCTTAGCC
ATAATAATTGCTAGTCTTCTGCTGAGTCCTGAACCTGCTAAGTGAGGAAGAAAAATCCATATGTAAGTATAAATA
AACAAATGCTTTAATCTTTGCAAGGTCACTCATCAGATTGAAATATTAAAGTGGATACAAAACATTTCAGCAATGCAGACAAATT
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GTAATACAGATGATTCTGTTCTGTTCTGTTCTCAGTGGCTCTCCATCTTCAAGAACAGTGAAGAAGGGATAAGCTGATCAGAT
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TAAAATACCTTTTTAGTGGGTCGCTGAGATAACTCACCACGTCAAAGACTCTTCTGTTGGACTCAATTGATCT
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GTGACCTGAAGGCTGCAAGTATGTTGAGTGTGCACTTACACAGTAAGAATGGCATGAAACCCATGTGATTGTC
GAGTCATTATTAGAGCATTAGGATACAGGAGTTTCAACAGTGTAGCAGTCTAAAGATGCCAGCAAGACCGT

8 kb KO and I-SceI Replacement TC-donor plasmid, CDC42, 500bp HAs

CTCGAGCGTTACATAACTTACGTTAATGGCCGCCCTGGCTGACCCCCCAACGACCCCCCCCATTGACGTCAATAATGACGT
ATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCATGGGTGGAGTATTACGTTAACTGCCCACTTGGCAGTACA
TCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCATGCCGAAATGGCCGCCCTGGCATTATGCCAGTACATGACC
TTATGGGACTTCCACTTGGCAGTACATCTAGCTTACGTTAGTCATCGCTTACCGTATTACCGTATGGTGATGCCAGTACATCAATGG
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ACGGGACTTCCAAAATGCTGAACAATCCTGCCCTATTGACGCCAATGGCCGGTAGCGCTGTACGGTGGGAGGTATATAA
GCAGAGCTGGTTAGTGAACCGTCAGATCCGCTAGCGCTGCCCTGGATAGTCCGTGAGGACGAAACGGTACTCGGTAC
CGTCGGCAGACGCCCTTGGCATCTTGAGCAGTGTGATCACTGTTAGAAATAACTCCTGTATCCTAATGCTCTAATAAA
TGACTCGACCATAAATACACATGGGGTTCATGCCATTCTACCTGTGTAAGTGCAGAACACTCCACATACTTGACAGCCTTCAG
GTCACGGCCAGCTTCACTGGAGTGTAGGCTCTGGCAAGTTCTCAATAGTAGAGGGGTCACTC
TCTGAGATCAATTGAGTCCCAAAGCAAGAAAGGAGTCTTGGACAGTGGTAGTTATCTCAGGCACCCACTAAAAAAAGGT
ATTTAAACACCTTGTAAATCAACAAGCCTTAATGCTATTAAACAGGTCTAGAGAAATTCTGAGATGATCTCTTAGGGC
AAGAGTATCTGATCAGCTTACCTTTTCACTGGTAAATGAAGATGGAGAGACCACTGAAAAAACAGACTAGAAATACTCTG
TATTACCTTGTATCCCTAAATTGTTGTTGTGAGGATATCAGGAGACATGTTACCAACAGCACCATGCCAACACAACA
CACTTAATTGTCGATTGCTGAAATAGTTGTATCCACTTAAATATTCAAATCTGATGATGACCTGCAAAAAGATAAAGACAT
TTGTTTATTATACTTACATATGGATTTCCTTCACTTAGCAACAGGTTAGGCACACTCAGAGCAAGAAAAGAAACTAGCACAAATT
ATTATGGCTAAAGAACCAATAAGAGACACAATGACTTGTCTATAAGTTACAATAACAAATAAGGTCTTCAAGTTCTGATCC
CTGGCTATTCAACTCTTAAAGTGTATTAAAGGCCAAGTGGTGTGATAATCAGCAATTATTTATCATGAAAGAGAA
GAGGCTGATCAGAGAAAATTCCACAGTGTCTAAATATTGTTGAAAGCTTCTCAGTTACACCGTCACACACACTGC
TCGAGCTCAAGCTTCCACCGCAATGCTTGTGAGATCTATTCGGCCAAGAACAGCAGGATCAGCCGTGCCCTGGCT
AAATCTTCAAATTGCACTGAGGATCAGCCGTGCCGTATCTTCCGGCAAGAACAGCAGGATCAGCCGTGCCCTCAGGTGCA
ATCTTCAAATTGCACTGAGGATCAGCCGTGCCGTATCTTCCGGCAAGAACAGCAGGATCAGCCGTGCCCTCAGGTGCA
GTCCCCAGCCTCTCGCTGGCGCCGGTGGCAACATTCCGAGGGGGACCGTCCCCCTCGGTAATGGCAATGGGACACGCGT

8 kb IRES-GFP Replacement TC-donor plasmid, CDC42, 500bp HAs

CTCGAGCGTTACATAACTTACGGTAATAGGCCGCTGGCTGACCCCCCAACGACCCCCCCCATTGACGTAAATGACGT
ATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTACGTAACGTCCACTTGGCAGTACA
TCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAATGGCCGCCCTGGCATTATGCCAGTACATGACC
TTATGGGACTTCTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTATGGCTTGGCAGTACATCAATGG
GGCTGGATAGCGGGTGTGACTCACGGGATTTCAAGTCATCCACCCATTGACGTCAATGGGAGTTGGCAGGACCAAAATCA
ACGGGACTTCCAAAATGTCGTAACACTCCGCCCTATTGACGCAATGGGCGGTAGGGCGTGTACGGTGGGAGGTCTATATAA

GCAGAGCTGGTTAGTGAACCGTCAGATCCGCTAGCGCTGCCCCGGATGAGTCGTGAGGACGAAACGGTACTCGGTAC
 CGTCGGCAGACCGTACTGTTAGAATAACTCCTGATCCCTAATGCTTAATAAATGACTCGACCAATAATACACATGGGTTCA
 TGCCATTCTTACCTGTGAACTGCAAGAACACTCCACATACTTGACAGCCTCAGGTACGGTCACGGGCCAGCTTCTGAGCAGTCTCTGG
 AGTGATAGGGCTTGTGTTCTGGCAAGTCTCAATAGTAGAGGGGTCTGAGATCAATTGAGTCCCACAAAGCAAG
 AAAGGAGTCTTGGACAGTGGTAGTTATCTCAGGCACCCACTAAAAAAAGGTATTAAAATACACCTTGTAAATCAACAAGC
 CTCTAATGCTATTATAACAGGTCACTAGAAATTCTGAGATGATCTCTAGGGCAAGAGTATCTGATCAGCTTACCTTCTTC
 CGTTTCAAATGAAGATGGAGAGACACTGAAAAACAGACTAGAAATACATCTGTATTACCTGTTACTTGTACAGCTCGT
 CATGCCAGAGTGATCCCGCGGCCAGCAGAACTCCAGCAGGACCATGTGATCGCCTCTCGTTGGGGTCTTGTCAAG
 GCGGACTGGGTGCTCAGGTAGTGGTGTGGCAGCAGCACGGGCCGTCGCCATGGGGGTCTGCTGGTAGTGGT
 GCGCAGCTGCACGCTGCCCTCGATGGTGTGGGATCTGAAGTTCACCTGTGCGCTTCTGCTGTGCGGCCATGA
 TATAGACGTTGTGCTGTGTTAGTGTACTCCAGCTTGTGCCCCAGGTGTTGCCCTCTGAAGTCCGATGCCCTCAGCT
 CGATGCGTTTACCAAGGGTGTGCCCTCGAACCTCAGCTGCCCTGGCAGGACAGCTGAACCTGTGGCCCTTACGTGCCGTCAGCTGAC
 GCGCTCTGGACGTAGCCTCGGGCATGGCGACTTGAAGAAGTGTGCTCTGTTGATGGTGTGGGAGCAGCTTGTGCGTGAAGCA
 CTGCACGCCGTAGGTCAAGGGTGGTACGAGGGTGGGCCAGGGCACGGGAGCAGCTTGTGCGTGTGAGTGAACCTCAGGG
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 TTTCAAAGGAAAACACGGTCCCCGTGGTCTGGGGGCTAGACGTTTTAAACCTGACTAAACACATGTAAGCATGTCAC
 CGAGGCCAGATCAGATCCCATAATGGGGTACCTCTGGGATCTGCCAGCAGCTTGTGAGGAGACGTTGAGGAGAGCA
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 TGGCCCGAGAGGACCTGTGCCAGGTGGGGGCTCCTGCTGCCAGCAGGTTGGGGGCTCACATTGCCAAAGGGTGTGCT
 GCTCCAGAGGAACCTGCTCTCACGACATTCAACAGACCTGCTTGGCAGAGGGGAAAGACCCCTAGGAATGCT
 CGTCAAGAAGACAGGGCAGGTTCCGGGCTCACATTGCCAAAGACGGCAATATGGGAAAATAACATATAGACAAACG
 CACACGGCCTATTCCAAGCGGCTCGGCCAGTAACGTTAGGGGGGGAGGGAGAGGGGCTAAATTGTTGTG
 TAGGATATCAGGAGACATGTTTACCAACAGCACCAGCCACAACACACTTAATTGTCATTGCTGAAATAGTTGT
 TCCACTTAAATTTCAATCTGATGACCTGCAAAAAGATTAAAGACATTGTTATTACATATGGATTCTCT
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 GACTGCTATAAGTACAATAACAAATAAGGTCATTCTAAGTTCTGATCCCTGGCTATTCAACTTAAAGTGTATT
 AGGTCAAAGTGGTGTGATAATCAGCGAATTATTATCATGAAAGAGAAGGGCTGATCAGGAAAATTCACAGTGT
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 GATCTATATTGGCCAAGAAAGCAGGACATCAGCGTGCCTCCAGGTGAATCTCAAATTGCAAGGACATCAGCCGTGCGG
 ATCTATATTGGCCAAGAAAGCAGGACATCAGCGTGCCTCCAGGTGAATCTCAAATTGCAAGGACATCAGCCGTGCGG
 CAATATGTAAGTAAGTAAAGCTTTTTGTGACGGTACGCCGGCATGGTCCAGGCTCTCGCTGGGGCCGGTGGCA
 ACATTCCGAGGGGACCGTCCCCTCGTAATGGCAATGGACACCGT

8 kb IRES-GFP Replacement HR-donor plasmid, CD42, 500bp HAs

CTCGAGCAGGTGTGTCACGTAACTGAAGAACAGCTTCAACAAAATAATATTAGCACACTGTGGAATTCTCTGATACA
 GCCCTTCTCTTCTGATGAAAAATAATTGCTGATTATCACCACCTTGGACCTTAAAGAACACTTAAGAAGTGGAAATA
 GCCAGGAGTCAGAAACTTAGAAATGGACCTTATTGTTAGTGTAACTTATAGCAAGTCATTGTCCTTATTGGTTCTTCT
 ATAATAATTGCTAGTTCTTCTGCTCTGAGTCCTGAACTGTGTTGCAAGTGGAGAAGAAAATCCATATGTAAGTATAATA
 AACAAATGCTTTAATCTTTCAGGTCTCATCAGATTGAAATATTAAAGTGGATACAAACACTTACAGCAATGCAAGACAATT
 AAGTGTGTTGTGGCGATGGTGTGGTAAAACATGTCCTGATATCCTACACAACAAACATTAGGCCCTCTCC
 CTCCCCCCCCCTAACGTTACTGGCGAAGCCCTGGAAATAAGGCCGTGTGCGTTGTCTATGTTATTCCACCATATTG
 CCGTCTTGGCAATGTGAGGGCCCGAACCTGGCCCTGTCTTGTGACGAGCATCCTCTAGGGGTCTTCCCTCGCCAA
 AGGAATGCAAGGTCTGTAATGTCGAAGGAAGCAGTCTCTGAAAGACAAACACGCTGTAGCAGGCC
 TTTGCAAGGCGCCAGGAAACCCCACCTGGCGACAGGTGCTCTGGCCAGGAAAGACCTCTGCTGAGGAGCTTCTGAAAGACAAACGCTGTAGCAGGCC
 GGCACAACCCAGTGCACAGTTGTGAGTTGGATAGTTGTTGAGCTGATCTGGGGCCTCGGTGACATGCTTACATGTTAGTCAGG
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 CAGCAGCAACTTCTCAAGTCGCCATGCCGAAGGCTACCTCCAGGGACGCCACATCTTCAAGGACGACGGCAACTCAA
 GACCCGCCGGAGGTGAAGTCCAGGGGACACCCCTGGTGAACGGCAGCTGACTTCAAGGAGGAC
 CAACATCTGGGGCAAGCTGGAGTACAACACAGCCACAACGTCATATCATGGCGACAAGCAGAAGAACGGCATCA
 AGGTGAACCTCAAGATCGCCACAAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCAACTACAGCAGAACACCCCCCATCG
 CGACGGCCCCGTGCTGTCGCCCCGACAACCAACTACCTGAGCACCCAGTCGCCCTGAGCAAAGACCCCAACGAGAACGGCG
 TCACATGGTCTGCTGGAGTCTGACGCCGGGATCACTCTCGGATGGACGAGCTGACAAGTAGATAACAGGGTAAT
 ACAGATGATTCTAGTGTGTTCTGAGTGTCTCCATCTCATTGAAAACGTTGAAAGAAAAGGTAGCTGATCAGATACTCT
 TGCCCTAAGAAGATCATCTCAGAATTCTACTGACCTGTTATAATAGCATTAGGGCTTGTGATTAACAAAGGTGATT
 TACCTTGTGAGGAAACTTGCCAAGAACAAACAGAACAGCTCCTACTCCAGAGACTGCTGAAAGCTGCCCCGTGAC
 CTGAAGGCTGTCAGTATGAGGAGTGTCTGCACTTACACAGTAAGAATGGCATGAAACCCATGTGATTGTCAGTC
 ATTATTAGAGCATTAGGATAACAGGAGTTTCTAACAGTACCGCGT

Supplementary Table S3. Sequences of primers (5'→3') used in this study.

Primers used for the cloning of HR and TC-donors	
<i>H1-F</i>	AATTCATATTCGATGTCGCTATGTGTT
<i>H1-R</i>	CGAGTGGTCTCATACAGAACTTATAAG
<i>CMV-F</i>	CGTTACATAACTTACGGTAAATGGCCC
<i>CMV-R</i>	AGCTCTGTTATAGACCTCCCACC
<i>LMNA-HA-F1</i>	CCCGGCGTCGGTGACTCAGT
<i>LMNA-HA-R1</i>	GGACGGGGTCTCCATGGCCG
<i>LMNA-HA-F2</i>	CAGCGGCCGCCACCCGC
<i>LMNA-HA-R2</i>	GAGCTCGGCCCTCGTAGGCG
<i>FBL-HA-F1</i>	AAGATGCAACAGGAGAACATGAAGC
<i>FBL-HA-R1</i>	GGGTGGCCTGTGAGAGGAAGATAG
<i>FBL-HA-F2</i>	CCCAAGGTGAAGAACTGAAGTTCAGCG
<i>FBL-HA-R2</i>	CCAGGCTGCCGCTGCGAAA
<i>GAPDH-HA-F1</i>	CCATCACTGCCACCCAGAAGACT
<i>GAPDH-HA-R1</i>	ATGAGGTCGACGACCCCTGTT
<i>GAPDH-HA-F2</i>	ACCACCAAGCCCCAGCAAGAG
<i>GAPDH-HA-R2</i>	GACTTCCTCCACCTGTCAGCCTC
<i>GFP-F</i>	GTGAGCAAGGGCGAGGAGC
<i>GFP-R</i>	CTTGACAGCTCGTCATGCCG
<i>CDC-HA-F1</i>	CCGTGTGATCGTGTGGTGCTCTGC
<i>CDC-HA-R1</i>	AAATATTCAAATCTGATGATGACCTGCAAAAAGATT
<i>CDC-HA-F2</i>	TGTACTCCCTCCCTCCCTTATTTACTGCC
<i>CDC-HA-R2</i>	CGGGGATTAAGATAACAGAAATACACTTACACC
<i>CDC-HA-F3</i>	CAGGTGTGTGTCACGTGTAAGT
<i>CDC-HA-R3</i>	AAATTGTTGTTGTGAGGATATCAGGAGAC
<i>CDC-HA-F4</i>	ACAGATGTTAGCTAGTGTGTTTCAGTGGTC
<i>CDC-HA-R4</i>	ACTGTTAGAAATAACTCCTGTATCCTAATGCTC
<i>IRES-F</i>	TTTTAGGCCCTCTCCCTCC
<i>IRES-R</i>	CGACCATTGTGCCATATTATCATCGTG
<i>PM-HA-F</i>	GTGCTTCAGCCGCTACCCCG
<i>PM-HA-R</i>	CGACGCTGAGGTCAAGACCACC
Primers used in gRNA synthesis	
<i>LMNAg1-F</i>	ACCGCCATGGAGACCCCGTCCCAG
<i>LMNAg1-R</i>	AAACCTGGGACGGGGTCTCCATGG
<i>LMNAg2-F</i>	ACCGGGGCGACAGCGGAGTGGAGC
<i>LMNAg2-R</i>	AAACGCTCCACTCCGCTGCGCCC
<i>gFBL-F</i>	ACCGCTCTCACAGGCCACCCCCCA
<i>gFBL-R</i>	AAACTGGGGGTGGCCTGTGAGAG
<i>gGAPDH-F</i>	ACCGAGCCCCAGCAAGAGCACAAG
<i>gGAPDH-R</i>	AAACCTTGCTCTTGCTGGGGCT
<i>CDCg1-F</i>	ACCGCAGATTGAAATATTTAAAG
<i>CDCg1-R</i>	AAACCTTAAATATTCAAATCTG
<i>CDCg2-F</i>	ACCGGGAAAGGAAGGAGTACAGAA
<i>CDCg2-R</i>	AAACTTCTGTACTCCTCCCTCC
<i>CDCg3-F</i>	ACCGCACACAAACAAATTCCAT
<i>CDCg3-R</i>	AAACATGGAAATTGTTGTGTTG
<i>CDCg4-F</i>	ACCGGACTAGAAATACATCTGTTG
<i>CDCg4-R</i>	AAACCAACAGATGTTCTAGTC
Primers used in gRNA synthesis	
<i>gReporter-F</i>	ACCGGCGCCTGCTCGCGATGCTAG
<i>gReporter-R</i>	AAACCTAGCATCGCGAGCAGGCGC
<i>gPRNP-F</i>	ACCGGCAGTGGTGGGGGGCCTGG
<i>gPRNP-R</i>	AAACCCAAGGCCCCCACCACGTG

Supplementary Table S3. Sequences of primers (5'→3') used in this study.

Primers used in genotyping PCR	
P1	GACCCTTTGCCACCCACTCT
P2	AAACTCCTCACGCACTTGCTAG
P3	CCGGCGTCGGTGAATCAGTGTC
P4	GAGCTCGGCCTCGTAGGCGGCC
P5	GTGTGTGACGTGTAATGAAGAACAGC
P6	GCATACACTTCAGTTATTCAAGCAATGTT
P7	CGTGTGATCGTGTGGTGCTCTGC
P8	GGTCATGATCCAGCCTTATTCAAGC
P9	ATGACATCAAGAAGGTGGTGAAGCA
P10	GCCTTGTCTCTGAAGTAGTGGTGC
P11	GGTCGCCGTCGCCATCGAGAAC
P12	GACTCCTGGAGAACCCGCTGTC
P13	CTCCACAGCCTCAGCCGAGGCC
P14	GCATGCGCAGCGAGCTACCAATCC
GFP-N137-R	AACCTCAGGGTCAGCTGCCGTA
GFP-C175-F	ACTACCAGCAGAACACCCCCATC
mCherry-GFP-F	GGACATCACCTCCCACAACGA
mCherry-GFP-R	GTCGTCTTGAAAGAACATGGTGC
PRNP-F	GAAGCCTGGAGGATGGAACACTG
PRNP-R	ACCGTGTGCTGCTTGATTGTGAT
Primers used in qPCR	
LMNA-F	GAATCAGTGTTCGCGGGAGCG
LMNA-R	CGGTGAACAGCTCCTCGCCC
FBL-F	GCATTGACTCCACAGCCTCAGCC
FBL-R	CCCGGTAAACAGCTCCTCGC
EGFP-RT-F	TCCAGCAGGACCATGTGATC
EGFP-RT-R	AGTCCGCCCTGAGCAAAGA
GAPDH-RT-F	TGCACCACCAACTGCTTAGC
GAPDH-RT-R	GGCATGGACTGTGGTCATGAG
36B4-F	CAGCAAGTGGGAAGGTGTAATCC
36B4-R	CCCATTCTATCATCAACGGGTACAA
Primers used in Next-generation sequencing	
mCherry-GFP-F11	GGACATCACCTCCCACAACGA
mCherry-GFP-R11	GTCGTCTTGAAAGAACATGGTGC
PRNP-F12	GGCAACCGCTACCCACCTCA
PRNP-R12	GAAGTTCTCCCCCTGGTGGTT
LMNA-ISCE-F13	GCGTCGGTGAATCAGTGTCG
LMNA-ISCE-R13	CTCGTAGGCAGGCCCTGATGC

Supplementary Table S3. Sequences of primers (5'→3') used in this study.

Primers used in LM-PCR	
<i>P15</i>	TCCAGCTCGACCAGGATG
<i>P16</i>	CCGGTGAACAGCTCCTCG
NGS barcoding PCR primer	
<i>I5</i>	AATGATAACGGCGACCACCGAGATCTACACNNNNNNNNACACTTTCCCTAC ACGACGCTTCCGATCT
<i>I7</i>	CAAGCAGAAGACGGCATACGAGATNNNNNNNGTACTGGAGTTAGACG TGTGCTTTCCGATC

Supplementary Tables S4. On-target and off-target insertion sites of LM-PCR

LM-PCR results of GFP insertion at LMNA loci by CEd system				
Chr	start	gene	frequency	Validation Seq
chr1	1.56E+08	LMNA	587142	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGCAGCGCTGCCGCCGGGGCAGGGGTCCCGAAAG GGCGCGGGGCTCGGGTCGAAGGACAGAGACTGCTCGGAGTCGGAG TGCGCCGGCCGGCTGGCTCGGCTCGGCTCGGACCTCGGGATCTGGGTTGGCTGGT GTAGGTGCGGCCCTCCCGCAACA
chr14	20811578	RAPP2	5	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGTCTCCATGG CGGATCCGAGTGGTCTCATACAGAACTATAAGATTCCCAAATCCAA AGACATTTCACGTTATGGTATTTCAGAACACATAGCGACATGCA AATATTGCAGGGCGCCACTCCCCTGTCCTCACAGCCATCTTCTGC CAGGGCGCACGCCGCGCTGGGTGTTCCCGCTAGTGACACTGGCC CGCATTCCCTGGAGCG
chr20	30049696	DEFB124	3	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACACGAATAAGCCAG AAAATCCCTCCTGGCTAGCCAGTCCTGAATGAGTGGGAGATGGAAAT TGGATTATTCCCTGGGAGGGAAAGCTCTGGCAGTGAAAGACATCAC ATTGCAATTGTGTGATTCCACTGTGGAAAGATACTTATTTTACCAAA AAAATGGTGCCTAACATAACCAACCACCTCCTTAATGCACGGCCA GAGAAATGTGATAT
chr2	1.27E+08	GYPC	3	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGCAGCGCTGCCGCCGGGGCAGGGGTCCCGAAAG GGCGCGGGGCTCGGGTCGAAGGACAGAGACTGCTCGGAGTCGGAG TGCGCCGGCCGGCTGGCTATGGCTAACGTATAAGAAATATCATGC ACTGAAACCCAACCTTCCTCTTGTAGTCTGGCTGATGGTTATCCC CTGCTAATACCATAGCTTC
chr17	3700587	ITGAE	3	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGCAGCGCTGCCGCCGGGGCAGGGGTCCCGAAAG GGCGCGGGGCTCGGGTCGAAGGACATGTAGCTGGACTACAGGCA CCCGCACCATGCCAGCTAATTTTTGTGTTTTAGTAGAGACGGA GTTTCACTGTGTAGCCAGGATGGTCTCGATCCTGACCTCATGAT CTGCCACCTGGCCTCTTA
chr1	1.11E+08	KCNA3	3	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGCAGCGCTGCCGCCGGGGCAGGGGTCTGGTAGATT ATTGGTCCAATTAAATTACATAATTAAATTAGTATGCAGTGGTAC AGTTCTTGAAACAAAAAGGGTAAAATAAGAGTAACATTATTTAA AACATAATCATTGTGTTCATACATTATAAAATTACATTATCTAC AGTTTTTTT
chr13	91832047	LINC00379	3	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGTCTCCATGG GGAGGAAACGTTGTCGGATCTACCAATGGTCAGTCAAGTCGACT CGTCAGTGTAAAAAAAGCATTAATCAAAGCACTTGATCAAATTAT TTAGAGCAATAATTATATTGTATTGTTGTGGAACCTTAATAAAAT TACGGTCTTGCTATGTTATTGGTGAACCTTATACTTACGGGCATT TTAACATGTT
chr5	1.61E+08	LINC02159	3	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGCAGCGCTGCCGCCGGGGCAGGGGTCCCGGAATCA GGAAGCTGAGGGCAGGAGAAATTGCTTGAAGCCAGGAGGAGGATTG CAATGAGCGGAGATCAGCCACTGCACTGCAGCCTGGGTGACAACA GTGAAACTCGTTCTCAAACAAACAAACAAAAAAACTGCCAGATT TTTTTAATTGTAGGCAG
chr12	65780874	MSRB3	3	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGCAGCGCTGCCGCCGGGGCAGGGGTCCCGAAAG GGCGCGGGGCTCGGGTCGAAGGACAGAGACTGCTCGGAGTCGGAG TGCGCCGGCCGGCTGGCTCATGAGATACTGCTGTTGACTGTAAT CCTGTCACCCATTAGCTACAATAGCCTACATAAATAAAACTCAGAA TTGAAATAATGAGATAAGA
chr11	64834438	NAALADL1	3	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGCAGCGCTGCCGCCGGGGCAGGGGTCCCGAAAG GGCGCGGGGCTCGGGTCGAAGGACAGAGACTGCTCGGAGTCGGAG TAGCACCTGGGACCACTGTTCAAGAGCCAGTCTCCCTGTCC CGGCAAAACCACAGGTGGGATGGGATTGTTCTCCCTGAGTGAATTG GTAGCAGGGGACCGAGAGAG
LM-PCR results of GFP insertion at LMNA loci by TEd system				
Chr	start	gene	frequency	Validation Seq
chr1	1.56E+08	LMNA	534989	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGCAGCGCTGCCGCCGGGGCAGGGGTCCCGAAAG GGCGCGGGGCTCGGGTCGAAGGACAGAGACTGCTCGGAGTCGGAG TGCGCCGGCCGGCTGGCTCGGCTTGCTGCTCCCTGGCAGGCGTGGGG

				ATCTGGGCCGGCGCTGTCGGACCTCGGATCTGGGGCTGGTGT GTAGGTGCGGCCGCTCCCGCGAACAA
chr20	25483286	NINL	3	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGCTCCATGG CCGGCAGGTTTCAAATGGTTAGCACCATCCCTTAGTACTGACCT CATGATAGTGAGTAATTCTCATGAGACCTGGTTAAAAGTGTGT CACACCTCCCCCTCGCTTCTGCTCTGCTCGGCCATGTGACCT GCCTGCACCCCTCACCTCTGCCATGATTGTAAGCTTCTGAGGCC TCCCAGAAGCAGCC
chr9	92748202	MIR4290	3	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGCTCCATGG CCGGCAGGTTGGCAACAGGATTCAAGGGCTGTCTACTGCAATGTCA GTGACAACAAGAAAGGACTGGCAGAGAAGGGAGACACCACAAATGG CATCTTACCTAAACTTGCACTGTACAGGCTTACCATGAGGGTACG GCTACTCGCCTCCAACCAATAAGCAAATAACTGGGTTACTCCAG TGCCTGTTGGAAAATCAA
chr10	75584440	NDST2	3	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGCTCCATGG CCGGCAGGTTGGCAGCGCAGCAGTCACCCCCCTGCTGACTCTAGT TAGCCACCAACTAGCAGAGGGACCTCCAAGAGCACACAGAACCCCTG GAGGAGCCCAGAGAGAACTTAGCCAGCATGAGGCAGTCCAGGAGCC TGGAGACCTCAGCACCCATCCAGAGGTGGGACCCAGCCCTTTTC CTGTTTCTGGCTTGTCAAAG
chr14	20811578	RAPP2	3	CCGGTGAACAGCTCCTGCCCTTGCTCACGGATACGGTGTCTCCAT GGCGGATCCGAGTGGTCTCATACAGAACTTAAAGATCCCAAATCC AAAGACATTCACGTTATGGTATTCCAGAACACATAGCGACATG CAAATATTGCAAGGGCGCCACTCCCTGTCCTCACAGCCATCTTCT GCCAGGGCGACCGCGCTGGGTGTTCCGCCTAGTGAACACTGGG CCCGCGATTCTGGAGCG
chr17	56991606	PPM1E	3	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGGGGGAGGGGCTCCGGAAAG GGCGCGGGGCTGGGTCAAGGACAGATGCCAACAGCATTGTT CATTACTGTTAACATCCAGACTTACCTTGTTATTCATCAGCTTT CCACTAATGTCCTTTCTGTTCTAGGATCCAATCCAGGATACTACAT TGCATCACGTTTATT
chr12	5891999	ANO2	2	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGGGGGAGGGGCTCCGGAAAG GGCGCGATGGAAAATATCCACATTAACTGTTTAAACATCTTGA GCCAAATCTAAAGATGTCACTAGCAGAAATCTCCTGAGATCACA GAATGCCACATCTGTGTTACTCCCTACTGCCAATCTACCACTTTAA TGGGGTGCCAGAAC
chr8	62045358	CLVS1	2	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGGGGGAGGGGCTCCGGAAAG GGCGCGGGGCTGGGTCAAGGACAGAGACTGCTCGGAGTCGGGT CAGGCATTGTGCTCCAGCAGCGACACTGGGAACCTCCCCCACTTC ATCAGCTCGCAGCTCACTGGGCCCTGCATCCGTTAGAACATC TGAAGCTTCATCGGGAAAGCCT
chr1	2.32E+08	DISC2	2	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGGGGGAGGGGCTCCGGAAAG GGCGCGGGAAATGATTACAATAATTCAAGGTGTCCTGATACAA ATCAGATCTGTATACTTGTGTTCTGTTGACATCTGTCAATTGAG TCAGTGGCTTAATTAAAATTGGTCTAGGGAAAATCATGTGAG GGTTTCCCTGTTGCCT
chr7	1.26E+08	MIR592	2	CCGGTGAACAGCTCCTGCCCTTGCTCACGGACGGGGCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGGGGGAGGGGCTCCGGAAAG GCTAAATATTAGTAAAGCAAAATGAAATATTCAATAGTTCTA CCTTTAAAAGTAAATAAGGGTGGGTTGTTGTTGGTAAT GAAATAATGTATAGGTTTGAATTACATTCTGACACTTTAGAA CTTTGTTTACTT
LM-PCR results of GFP insertion at <i>FBL</i> loci by CEd system				
Chr	start	gene	frequency	Validation Seq
chr19	40325195	FBL	319743	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGACACTGTTGCACTGTG TTTTCTTAAAGACTCATCCGCTCCATGTCCTGCTCATTC TCCCTTGACCTGACACAGGGAGCACGCACTGGTCAATT GGGGGTTGGTAAATTCTACTCGGTACAGAGCGCATGCTCGT TTCTAGCTGCCTTGC
chr15	68132383	SKOR1	3	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGAGCGTGTTCGCT TCCACAAGATATAAGCCAAGAAATCGAAATACTTCAAGTACGG TAAGCATATGATAGTCCATTAAAACATAATTAAAAGTACGAAACTA

				CCCAAGAAAATTATTACTTCTACGTACGTATTTGACTAATATCTT GTGTTTACAGT
chr6	1.03E+08	GRIK2	2	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGT TTTTCTATTAAAAGACTCATCCGTCCTCCA TCTACTAGAATCATACAA AGAACGTAGTTCTGTTAGCACTGTTGTCCTATAGCTATCTCCAG ATTTGGTGCAGCTTAGTGTAGGACTATGTGAAGTTATTAAATAAC ACACTCAGAAAT
chr4	54519321	LNX1	2	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAG CGCTATAGGATTCTGTTGAGAATTAAAATAATGCAGGTAGGT AATTACTACAGAACCTGGTAGACGGTAAGCACTTAATAAATGTTAGCA AAGATGCTGCAGCCACTGACGATGATGATGGTATGCTGAAGAA TGAGCAGAAGAACTCTTATTAAACAATAATTCTATCTTAGAGTT AAGAGTCTAA
chr3	1.87E+08	ADIPOQ	2	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGT TTTTCTATTAAAAGACTCATGGACCCTCTGCAAATCTTACATGGCA GGTGGGTGACCAAGAGGAGCCATTCCAATACCAGTCATTGCTGATG TTCTGGGGTGTGTCCTCAGGTGCTGTTGAGCATATCTACTCTGTCT AGTCCTGCTTCCATT
chr6	11700243	ADTRP	2	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGT TTTTCTATTAAAAGACTCATCCGTCCTCATGTCGCTGCTCAGTGT CTTACTTGTATAGATGCCCATAAATATTGTTAGATGAGTGTCTGA AAATCAAATTCTAAACACTGTTTATCTTTCAGAAGGTGGATTAAC TATTGCTCTAA
chr1	36411242	AGO3	1	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGATA AAAGCCTTGTCAACC AGATATATTAGAATTATAAGTACAGCTGATAATGGCTAAAACCTAGT TCGCAAGATGACATCATTCTAACACTCACTCTGTCACCCAGA TTCCCTCTCTCATTTAAACACAGAAAACCGTATGCCTCTGGA AAACTGTTCTGGA
chrX	40267960	ATP6AP2	1	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAG CGCTGACACTGACGAGTCGACTTGGACTGACT TTTTCTTCTTCTCAA TGTGTTGTTCTTCTGTTTATGCACTGGCAAAGACCTCTA GTACAATGCTGAATAGAAGTGTGAGAATGGAGATCTTGCTCTTAC CTAATCTTAGAGTGAAGCAGTCTTATGATATAAGCTGTAGGTTT TTGGTAGATAC
chr10	1.25E+08	BUB3	1	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAG CGCTGTCAGGATTGCGAGAGACACTGACGAGTCGACTTGGACTGAC CCCAGGAGGTTAAAGCAGCAGCTTAAACGTTCTGGAAGCTGAGG CCAAGAATTGGCATGGTATCACTTCCCTGCATTTTATGGTAAAGC AGGTGAGTCAGTCAGTCAGATTCAAGAGGGAAACTACACAAAG GCCTGAATATGAGGAGG
chr11	6264195	CNGA4	1	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGATA ACGCTTATGCTGA GGGCAGTAGGAAACTATTGAGTATTAAAGCAGGGAGCTGTATGAT ATCCTTGCAGTTAGAAAGTGTGGTGGAGAATGGTGGGGAGAT AGGTGAGGCTGAATGCTGCAAAGCTTAGAGCTAGAAGGACCTGG ACAAGGGAAAGGGAGCT
LM-PCR results of GFP insertion at <i>FBL</i> loci by TEd system				
Chr	start	gene	frequency	Validation Seq
chr19	40325195	FBL	374604	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGT TTTTCTATTAAAAGACTCATCCGTCCTCCA TCCCTTGACCTGCTGACACAGGGAGCACGCACCCCTGGTCAATT GCGGGGTTGGGAAATTCTACTCGGTACAGAGCGCATGCTCCGT TTCTAGCTGCCTT
chr1	63842205	ALG6	2	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAT GGATGGTACTCTAAAGAAAATGTATAGCTTAATGATAGTGTACAGA GACTGAAACTTAAGAGCTAACAGCATCCAATTAGTAAATTAAAAAG AACAAACCCAAGGAGATCAGTGGAGAGAATGGGCAAAGCTCAGATTAG TTATTGAAAATACGGCTATGTACCAATAATGACATCTCAGTC CAACGAACGTAT
chr7	6078400	ANKRD61	2	GGCATGGACGAGCTGTACAAGTCCAAGTCGACTCGTCAGTGTAGTA GGCCTCTAGGGAAGTCATTAACCTGTGTTGACTCTCTTGTCAAGA CAGAAGTGTCCCTCAGGACACAGGCCACCCAAGAACGCCAAAGTCT AATGAAAGTGAAGATAAGGAGACTGATAATTAAACCGGGTGTGT

				AATACCAGCAGAAGGGGGGCCAGGCTGAAGTCAGGCAGGGTCCA ATGGCAGAACGCTCTCAACT
chr12	1.06E+08	APPL2	2	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGTG TTTTCTATTAAAAGACTCATCCGTCTCCCATGTCTGCTGCTCATTCC TCCCCCTGACCTGCTGACACAGGGAGCACATGTAAGGAGTCATAG GCTAAGGTCAACATTCTGGAATGAGTGTCTGACAGGGACGATGC ACAGGACAGGAGTGTG
chr4	94646574	ATOH1	2	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGTG TTTTCTATTAAAAGACTCATCCGTCTCCCATGTCTGCTGCTCATTCC ATAAACATACATGTCATGTCTTATAGCAGCATGATTATACTCT TTGGGTATATACCCAGTAATGGGATGGCTGGTCAAATGGTATTCT AGTTCTAGATCT
chr15	51601395	CYP19A1	2	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGTG TTTTCTATTAAAAGACTCATCCGTCTCCCATGTCTGCTGACTGACGAG TCGACTTGGACTGACCTCTTCTACAGAGGCCAACATCAATTCTGATGT GGGCTCCACCCCTCATGACCTTAATCACCTCAAAGGTCCCACACATA ATACCATCACACT
chr15	68132383	SKOR1	2	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGTG TTTTCTATTAAAAGACTCATCCGTCTCCCATGTCTGCTAAACTGG GGGGTGGCCTGTGAGAGCGGTGTTCTCCTTCCACAAGATATATA AAGCCAAGAAATCGAAATACTTCAAGTTACGGTAAGCATATGATAGT CCATTAAAACAT
chr6	1.64E+08	CAHM	1	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTTGAT AAAATACTAAAAATTACCATGACCATTTAAAGTGTACAATCA TTGGCATTAACTACATACATACATGTTGTAACCATCACCAACGATT CCAGAACTTGTCTTACATCCCCAGCAGAAATCCTGAATACTTAAACA GGAACCTCCAGCCCTCCCCCACACATGAACAAAGATTGATTAG GAECTGTTCTT
chr17	32745159	CCL1	1	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAG CGCTGTCACACTGACGAGTCGACTTGGACTGACCTTTCTTGTGTTTG CCTGCTTGTAAATTCTTTTCCCCCTTTACATAGTTGAGTTCATAC TGACTGTACAAATTCTCCATACCTGATTCACTCACACTATCATAAAGT GTTTCCATGTTTACACGTACTTCATAAATGCTGTTGAAGGCAA CAAGCTCACT
chr11	65074588	CDC42EP2	1	GGCATGGACGAGCTGTACAAGCCCAGGTGAAGAACTGAAGTCAG CGCTGTCAGGATTGCGAGAGATGACACTGACGAGTCGGATCACCTT TGTGGATCTCACCAATGCGGTTGACTGTCAAGCACGCCCTCGTAAT TTTGTTAGTAGAGACGGGTTTACCATGTTCCCCAGGCTGG TCTCGAACTCCTAACCTCAGTGTCCACCCGCCCTGGCCTCCAAA GTGCTGGGATTACAG

The top 10 most frequent NGS read sequences showing the on-target and off-target sequences. Sequences in green are on-target; Sequences in red are off-target; Sequences in black are sequences derived from the donor template.