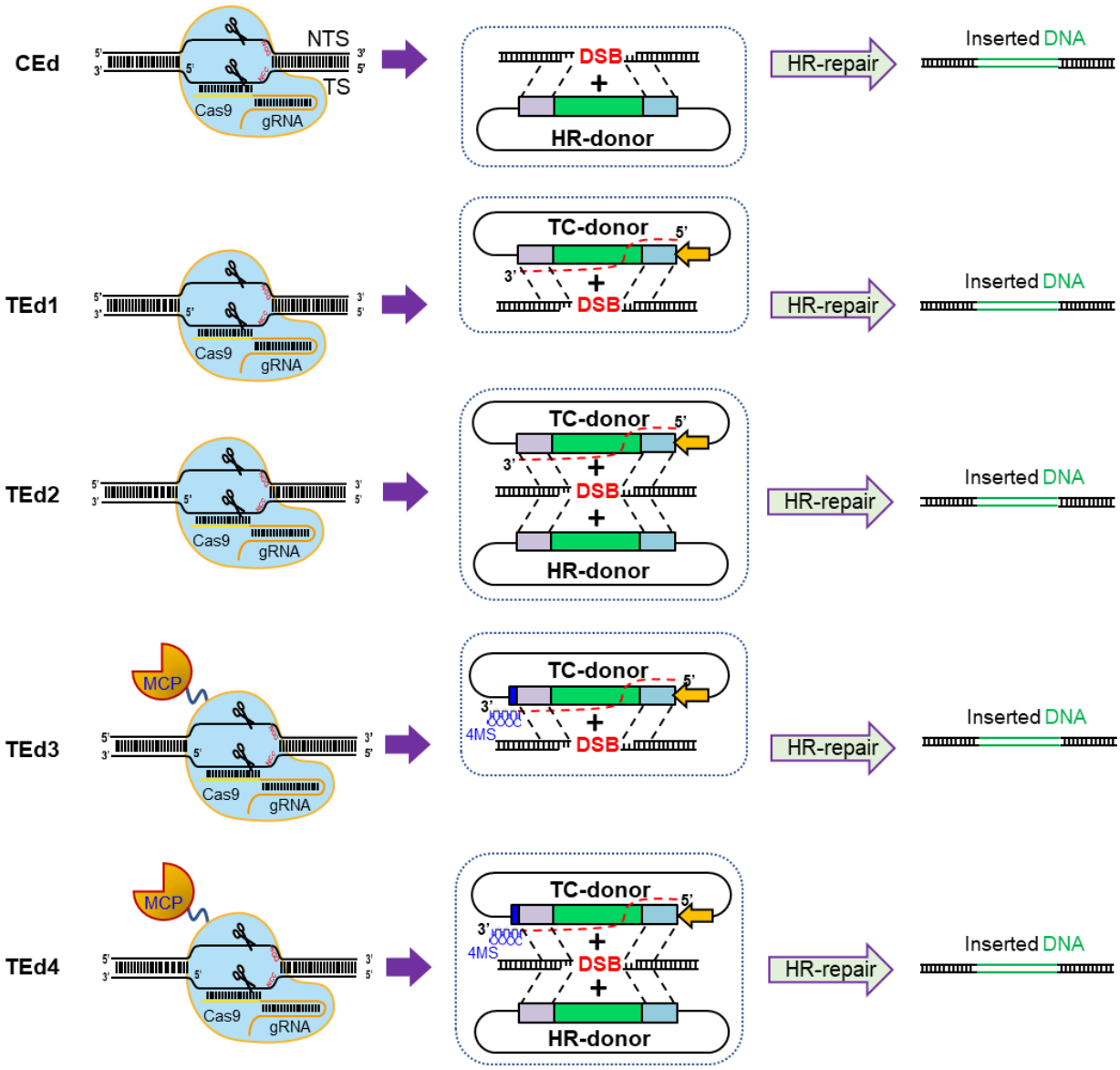
SUPPLEMENTARY DATA

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

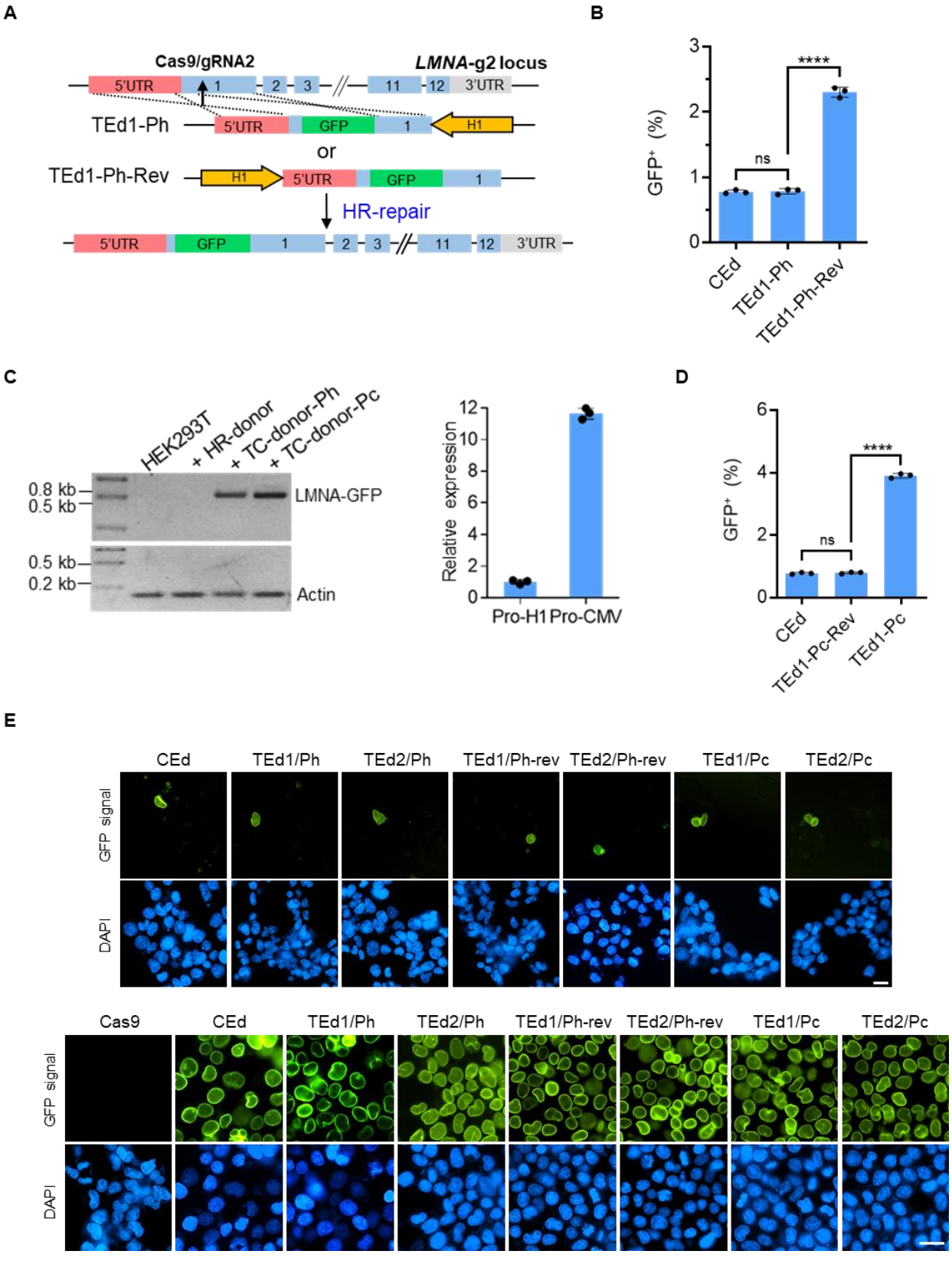
Supplementary Figure S1 to S12

Supplementary Tables S1 to S4

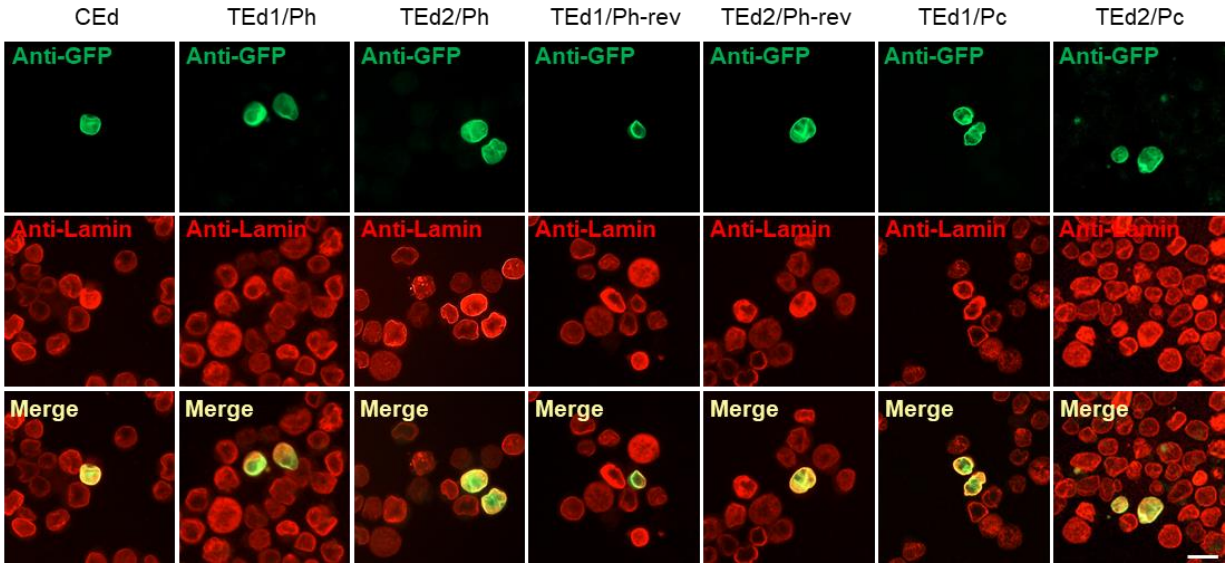
Supplementary Figures



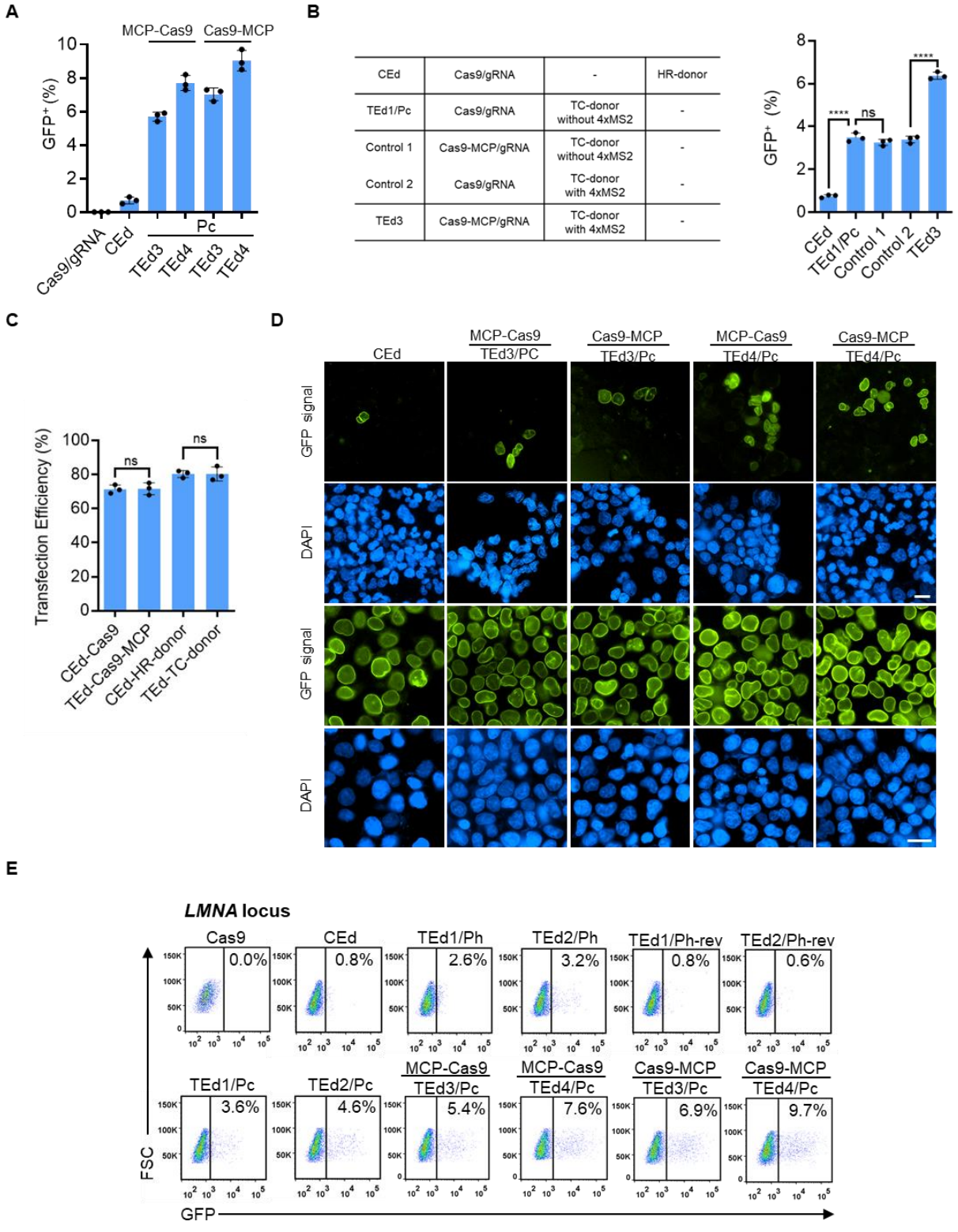
Supplementary Figure S1. Development of TEd methods.
Gene KI by CEd is mediated by a plasmid donor template (HR-donor) while TEd is mediated by a TC-donor (TEd1). In TEd2, TC-donor and HR-donor are used together. TEd3/4 additionally employ the MCP-MS2 system, in which the MCP protein is fused to Cas9, and the 4xMS2 sequence is fused to the 3' tail of TC-RNA.


A
Cas9/gRNA2
LMNA-g2 locus
5'UTR
1
2
3
11
12
3'UTR
TEd1-Ph
GFP
H1
or
TEd1-Ph-Rev
HR-repair
B
GFP+ (%)
****
ns
CEd
TEd1-Ph
TEd1-Ph-Rev
C
HEK293T
+ HR-donor
+ TC-donor-Ph
+ TC-donor-Pc
0.8 kb
0.5 kb
LMNA-GFP
Actin
0.2 kb
Relative expression
Pro-H1
Pro-CMV
D
TEd1-Pc-Rev
TEd1-Pc
E
CEd
TEd1/Ph
TEd2/Ph
TEd1/Ph-rev
TEd2/Ph-rev
TEd1/Pc
TEd2/Pc
GFP signal
DAPI
Cas9

F



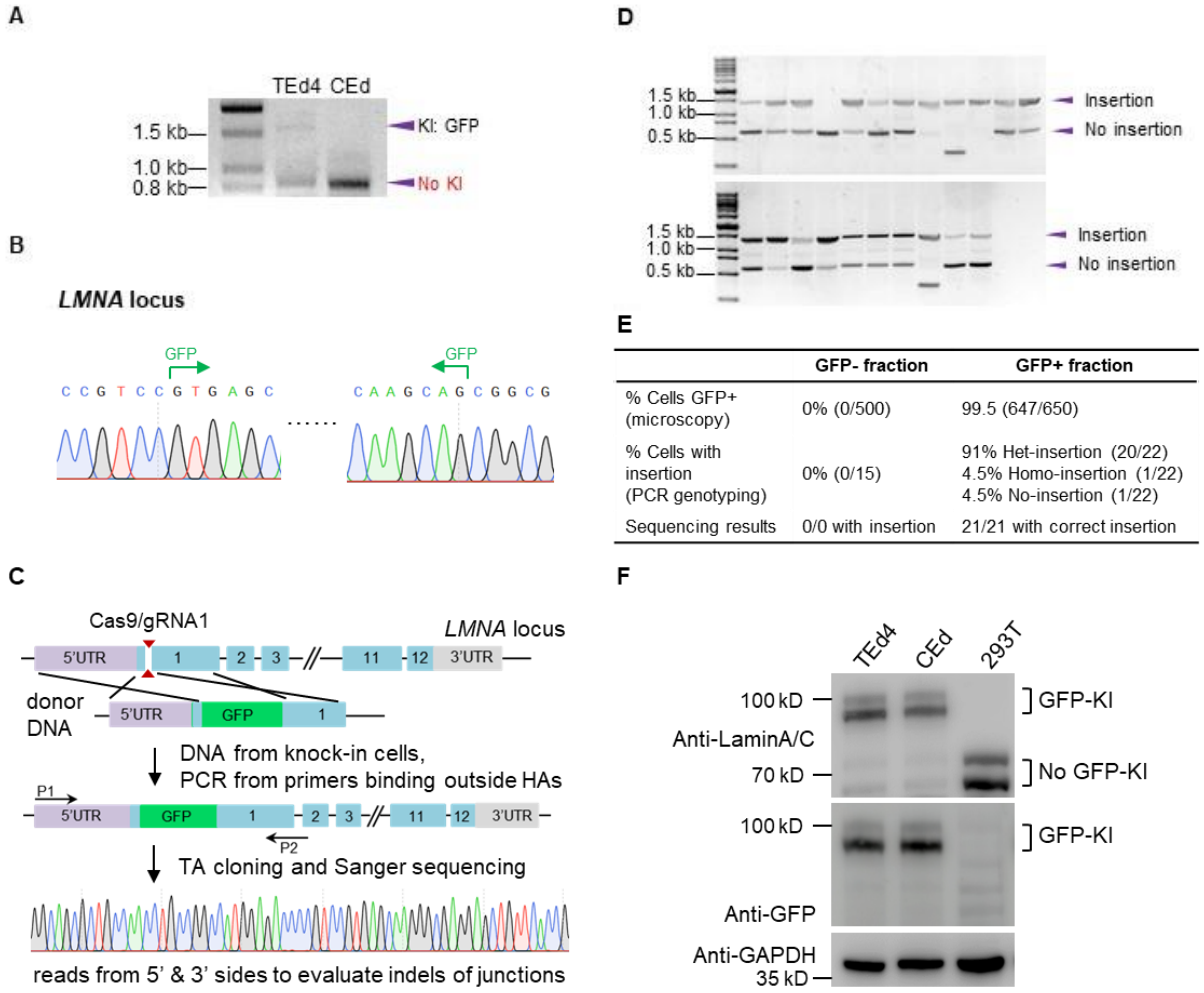
Supplementary Figure S2. Optimization of the promoter and transcriptional direction in the TEd-mediated knock-in strategy. (A), Components of the TEd1 systems using original TC-donors at the *LMNA*-gRNA2 locus. **(B),** GFP-KI efficiencies at the *LMNA*-gRNA2 locus in HEK293T cells using CEd, TEd1-Ph, 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 μm. **(F),** Immunofluorescence imaging verification of GFP colocalization with *LMNA* for CEd and TEd. Scale bar, 50 μm. Data are shown as individual data points and mean ± 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.

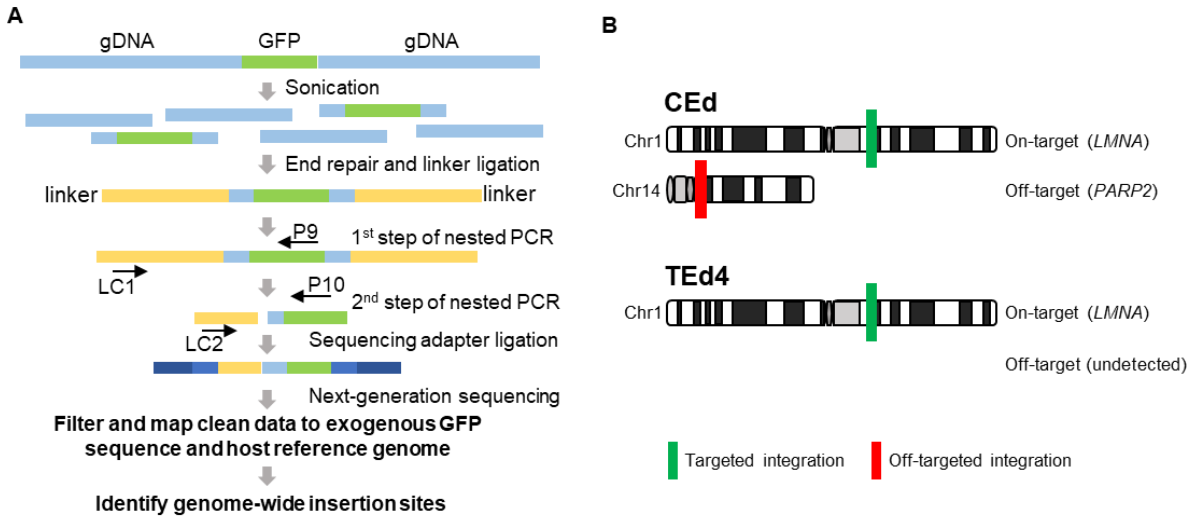
(A), A graph showing the percentage of GFP+ cells obtained after TEd3 and TEd4, in which MCP is fused to the N- or C-terminal of Cas9. (B), Left, Components of CEd, TEd1/3, and the two controls systems; Right,

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 µm. (E), Representative FACS analysis of GFP expression in cells edited by CEd and TEd. Data are shown as individual data points and mean ± s.d. for n=3 independent biological replicates. P values were obtained using the two-tailed Student's t-test. ****P < 0.0001.



	GFP- fraction	GFP+ fraction
% Cells GFP+ (microscopy)	0% (0/500)	99.5 (647/650)
% Cells with insertion (PCR genotyping)	0% (0/15)	91% Het-insertion (20/22) 4.5% Homo-insertion (1/22) 4.5% No-insertion (1/22)
Sequencing results	0/0 with insertion	21/21 with correct insertion

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).

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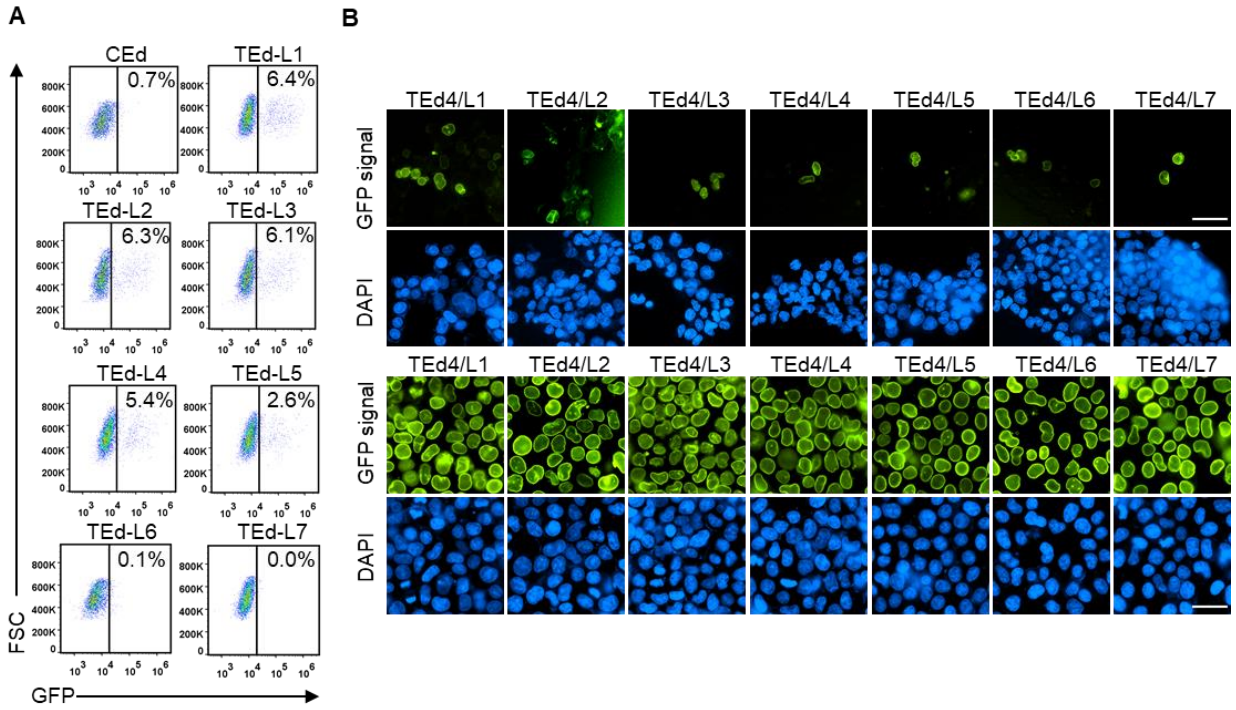
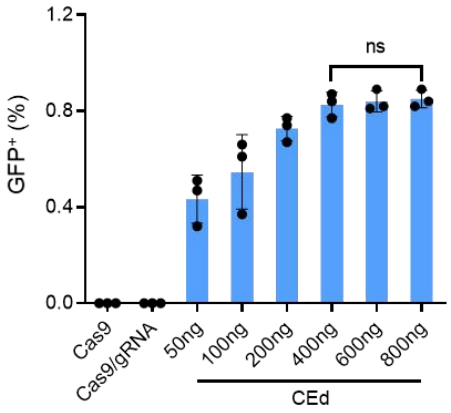
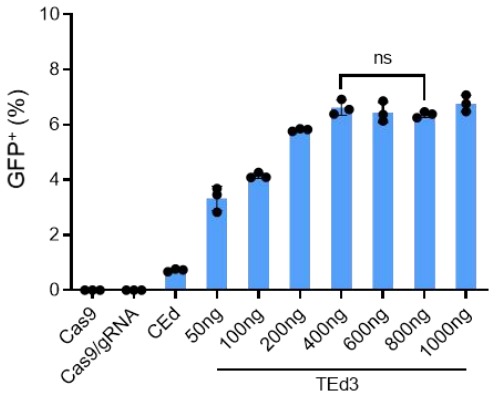
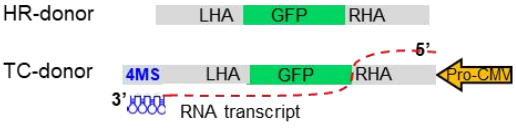
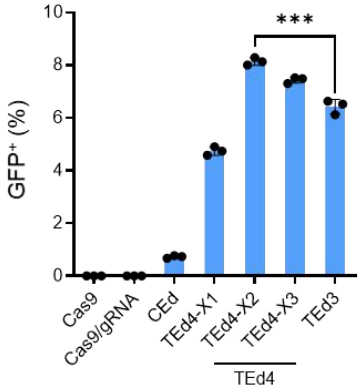








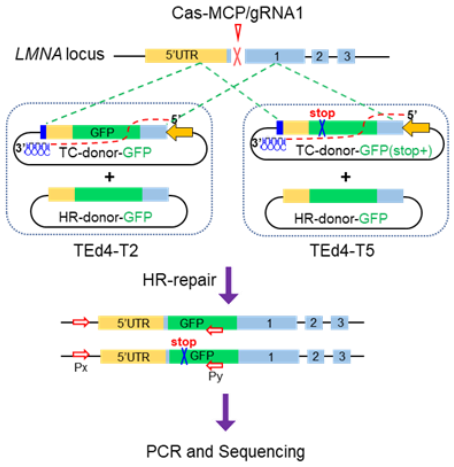
	Dosage of TC-donor (ng/well)	Dosage of HR-donor (ng/well)
CEd	0	400
TEd4-X1	100	300
TEd4-X2	200	200
TEd4-X3	300	100
TEd3	400	0

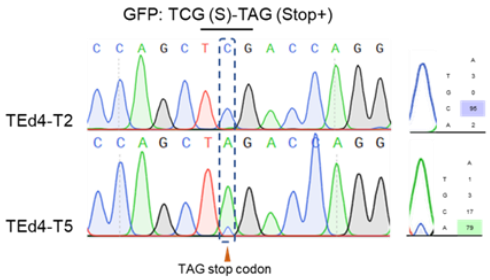


Supplementary Figure S6. Optimization of homologous arm lengths and plasmid concentrations for the TEd-mediated knock-in strategy.

(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 μm. (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 ± s.d for n=3 independent biological replicates. P values were obtained using the two-tailed Student's t-test. ***P < 0.001.

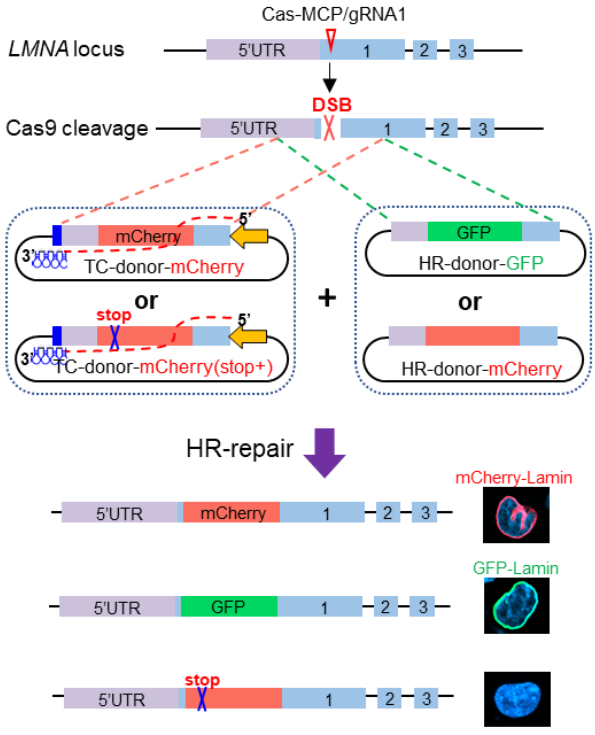
A





	KI proportion	
	GFP %	GFP (Stop+) %
TEd4-T2	100	0
TEd4-T5	13	79

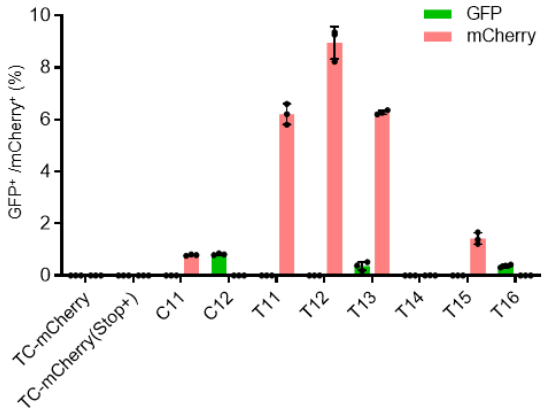
B

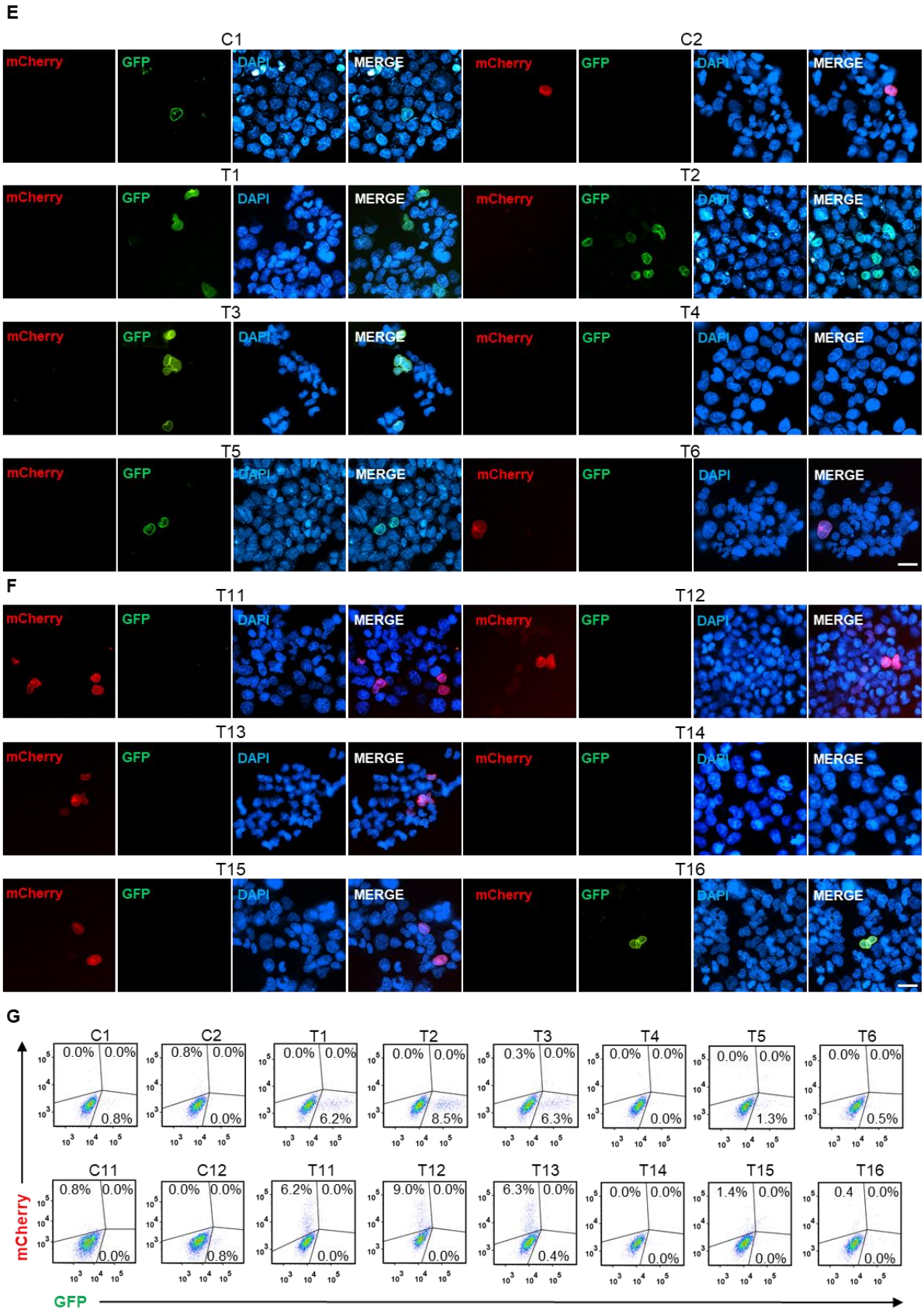


C

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

D

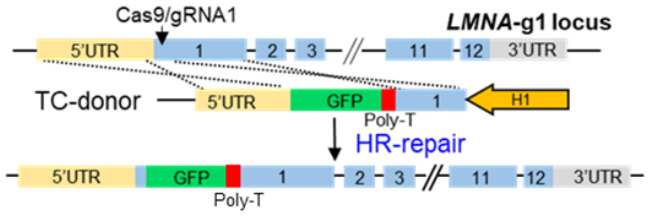



E
C1
C2
T1
T2
T3
T4
T5
T6
mCherry
GFP
DAPI
MERGE
F
T11
T12
T13
T14
T15
T16
G
C1 0.0% 0.0% 0.8%
C2 0.8% 0.0% 0.0%
T1 0.0% 0.0% 6.2%
T2 0.0% 0.0% 8.5%
T3 0.3% 0.0% 6.3%
T4 0.0% 0.0% 0.0%
T5 0.0% 0.0% 1.3%
T6 0.0% 0.0% 0.5%
C11 0.8% 0.0% 0.0%
C12 0.0% 0.0% 0.8%
T11 6.2% 0.0% 0.0%
T12 9.0% 0.0% 0.0%
T13 6.3% 0.0% 0.4%
T14 0.0% 0.0% 0.0%
T15 1.4% 0.0% 0.0%
T16 0.4 0.0% 0.0%
mCherry
GFP

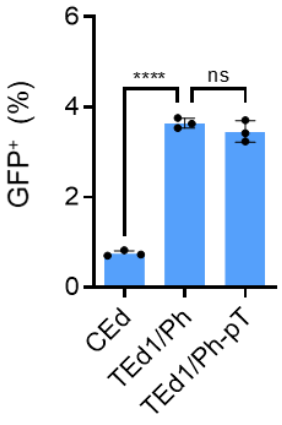
Supplementary Figure S7. Transcription of the TC-donor facilitates gene editing.
(A), Left, Schematics of TEd4-T2 and TEd4-T5 in Figure 2B showing HR-repair of a DSB at the LMNA locus with different TC-donors (GFP/GFP (Stop+)). Right upper, Sanger sequencing of genomic PCR products from HEK293T cells 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 ± 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 μm. (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.

A

	Cas9/gRNA	TC-donor	HR-donor
CEd	+	-	+
TEd1/Ph	+	+	-
TEd1/Ph-pT	+	+ (poly-T)	-

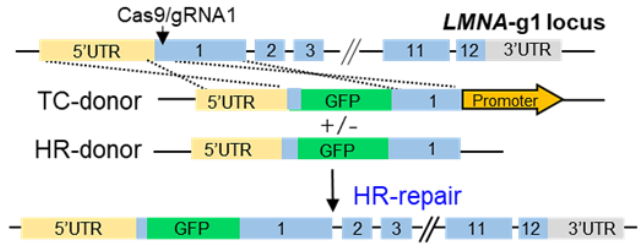


B

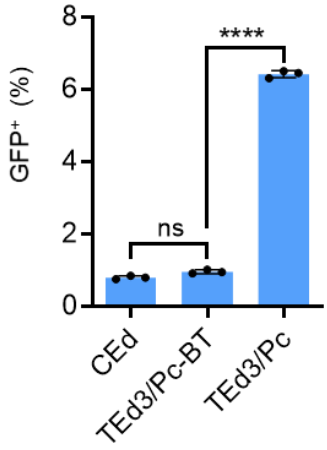


C

CEd	Cas9/gRNA	-	HR-donor
TEd3/Pc-BT	Cas9-MCP/gRNA	TC-donor (Backbone Transcription)	-
TEd3/Pc	Cas9-MCP/gRNA	TC-donor (Donor-template transcription)	-



D



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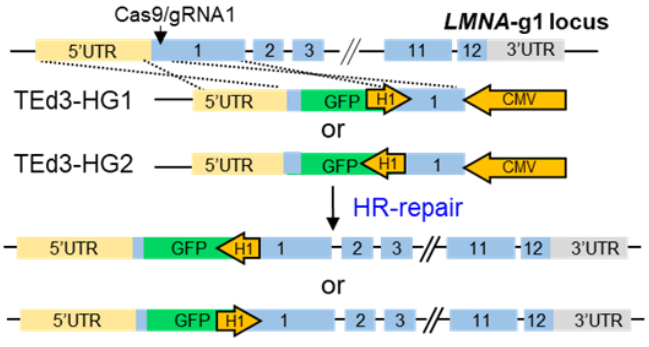

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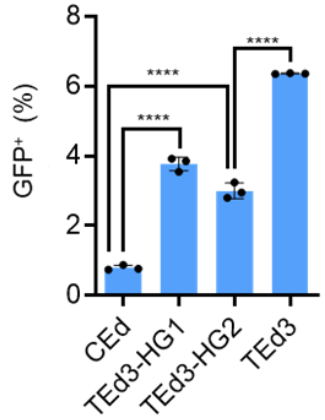
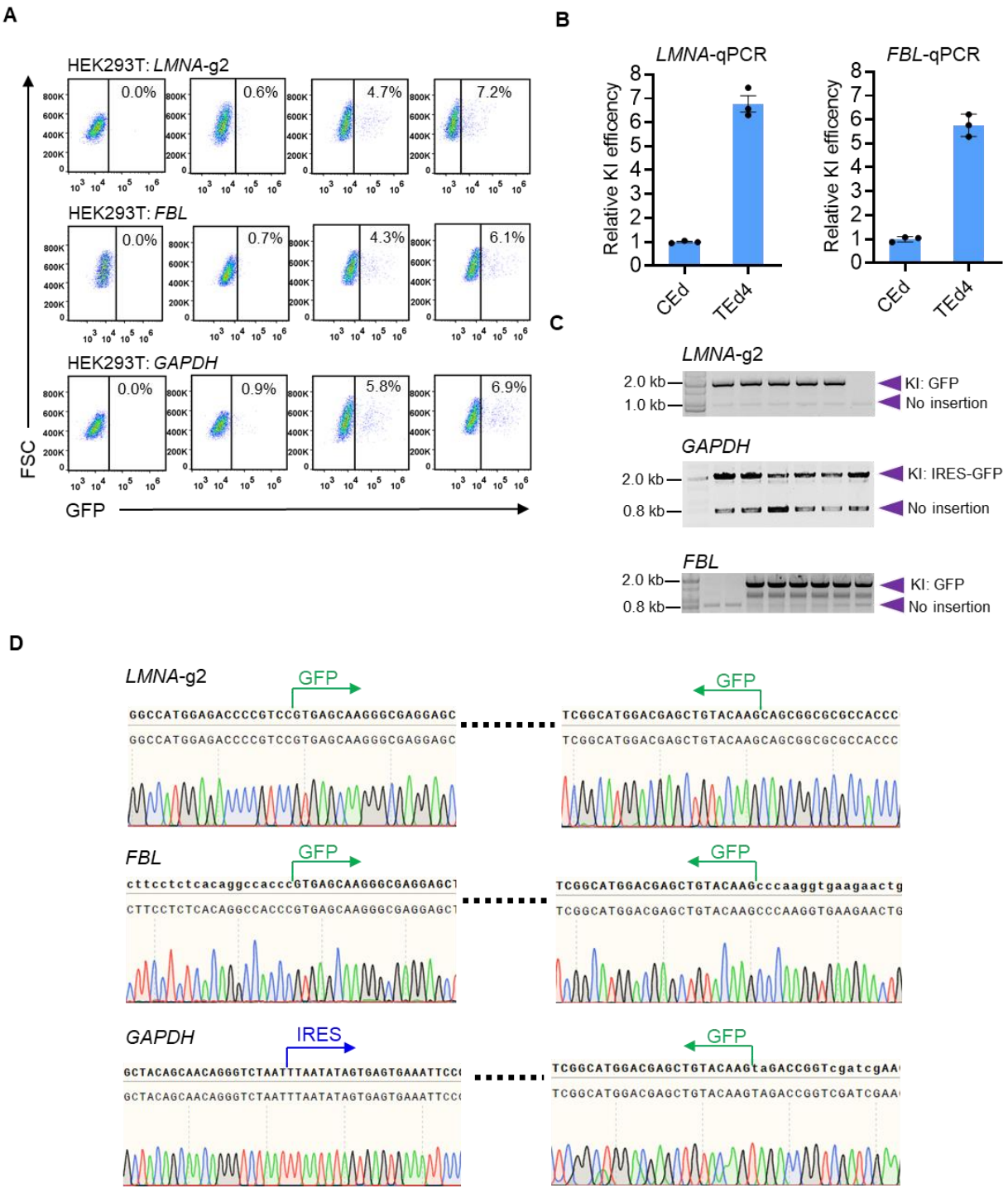


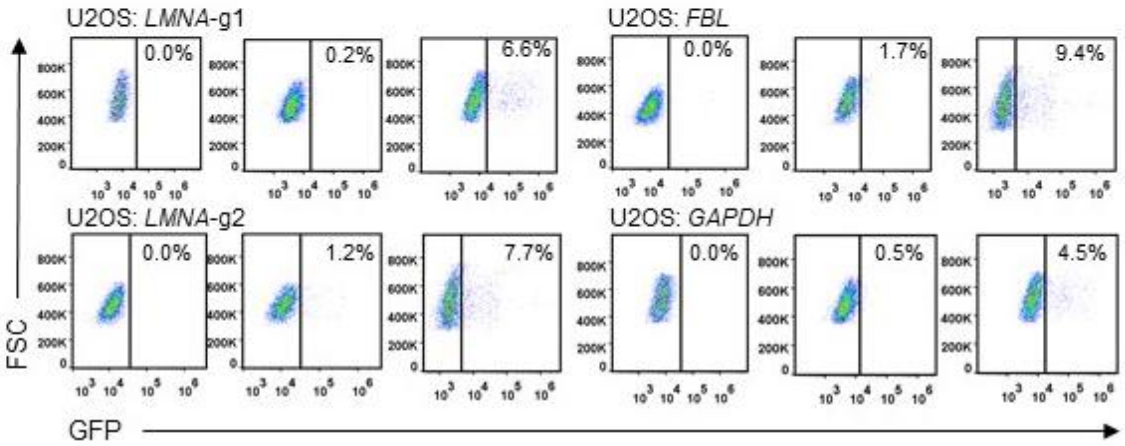
Supplementary Figure S8. Other factors that may affect TEd-mediated GFP-KI efficiency.
(A), Upper, Components of CEd and different TEd designs; lower, Schematic overview of the TEd1/Ph-pT with poly-T sequence mediated GFP-KI at the LMNA locus. A 9 bp sequence of thymine was inserted into the N-terminus 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 ± s.d. for n = 3 independent biological replicates. P values were obtained using the two-tailed Student's t-test. ****P < 0.0001.


A
HEK293T: LMNA-g2
0.0%
0.6%
4.7%
7.2%
HEK293T: FBL
0.0%
0.7%
4.3%
6.1%
HEK293T: GAPDH
0.0%
0.9%
5.8%
6.9%
FSC
GFP
B
LMNA-qPCR
FBL-qPCR
Relative KI efficency
CEd
TEd4
C
LMNA-g2
2.0 kb
1.0 kb
KI: GFP
No insertion
GAPDH
2.0 kb
0.8 kb
KI: IRES-GFP
No insertion
FBL
2.0 kb
0.8 kb
KI: GFP
No insertion
D
LMNA-g2
GFP
GGCCATGGAGACCCCGTCCGTGAGCAAGGGCGAGGAGC
TCGGCATGGACGAGCTGTACAAGCAGCGGCGCGCCACCC
FBL
GFP
cttcctctcacaggccacccGTGAGCAAGGGCGAGGAGCT
TCGGCATGGACGAGCTGTACAAGcccaaggtgaagaactg
GAPDH
IRES
GFP
GCTACAGCAACAGGGTCTAATTTAATATAGTGAGTGAAATTCCC
TCGGCATGGACGAGCTGTACAAGtaGACCGGTcgatcgAA

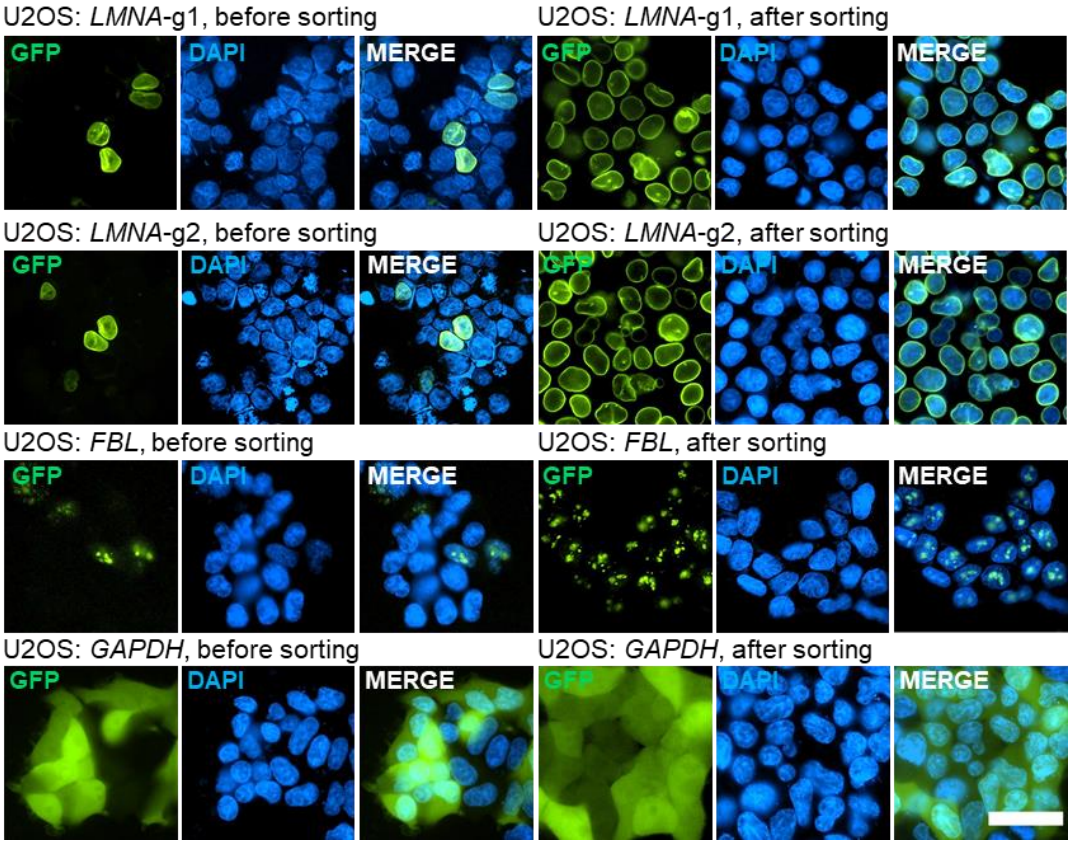
E


HEK293T: *FBL locus, GFP KI*
Targeted integration
CEd
Chr19
On-target (*FBL*)
Off-target (undetected)
TEd4
Chr19
On-target (*FBL*)
Off-target (undetected)

F

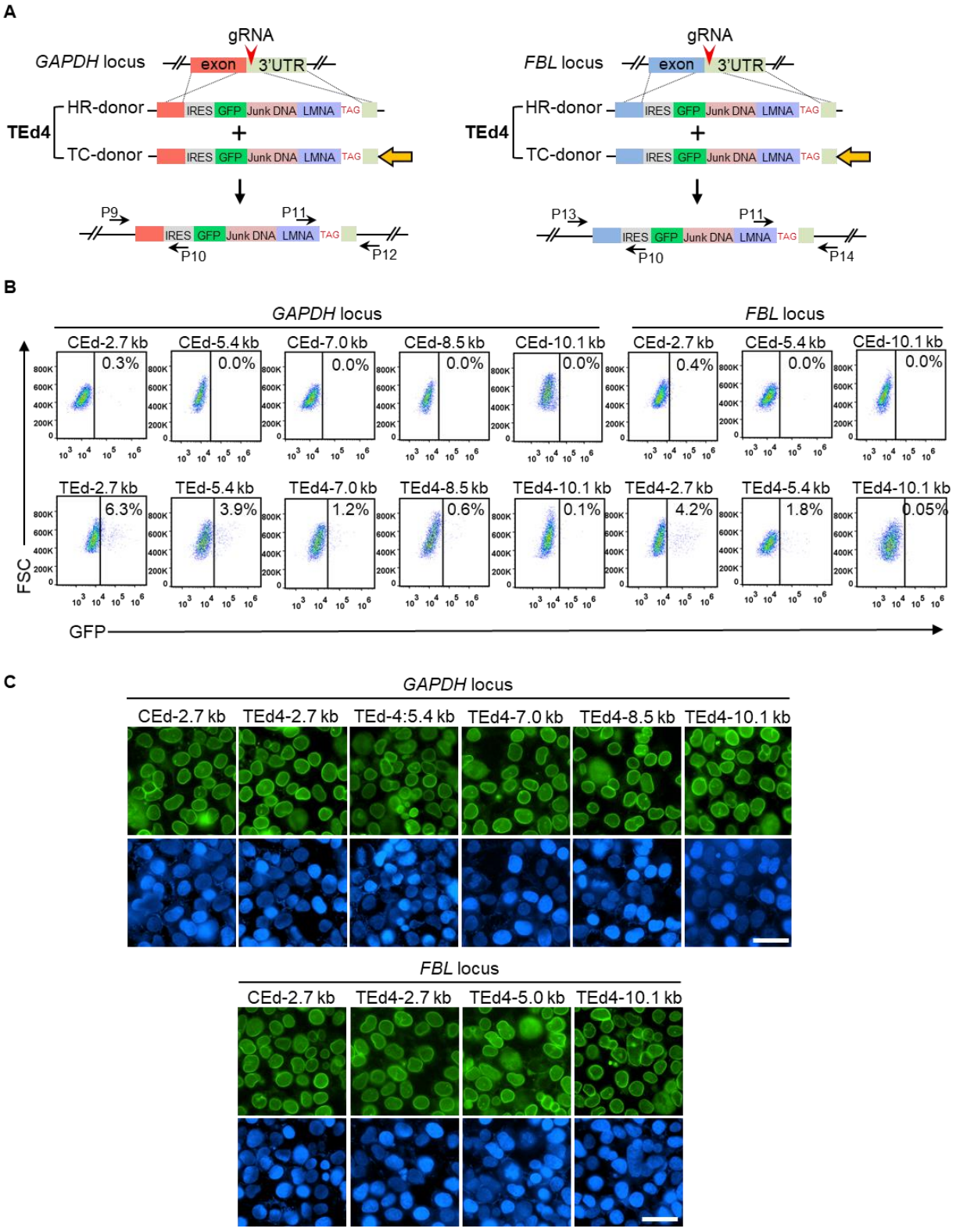

U2OS: *LMNA*-g1
0.0%
0.2%
6.6%
U2OS: *FBL*
0.0%
1.7%
9.4%
U2OS: *LMNA*-g2
0.0%
1.2%
7.7%
U2OS: *GAPDH*
0.0%
0.5%
4.5%
FSC
GFP

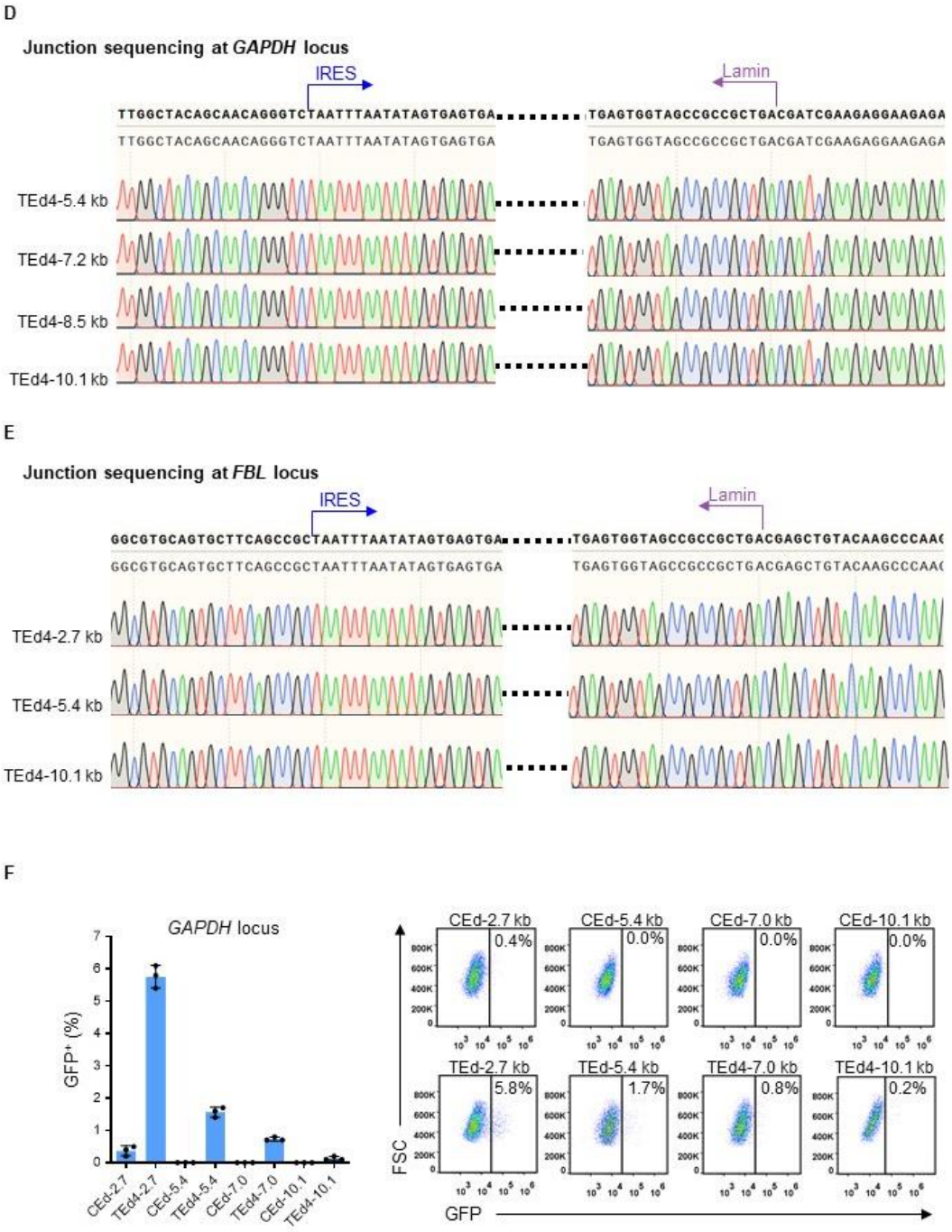
G


U2OS: *LMNA*-g1, before sorting
U2OS: *LMNA*-g1, after sorting
U2OS: *LMNA*-g2, before sorting
U2OS: *LMNA*-g2, after sorting
U2OS: *FBL*, before sorting
U2OS: *FBL*, after sorting
U2OS: *GAPDH*, before sorting
U2OS: *GAPDH*, after sorting
GFP
DAPI
MERGE

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

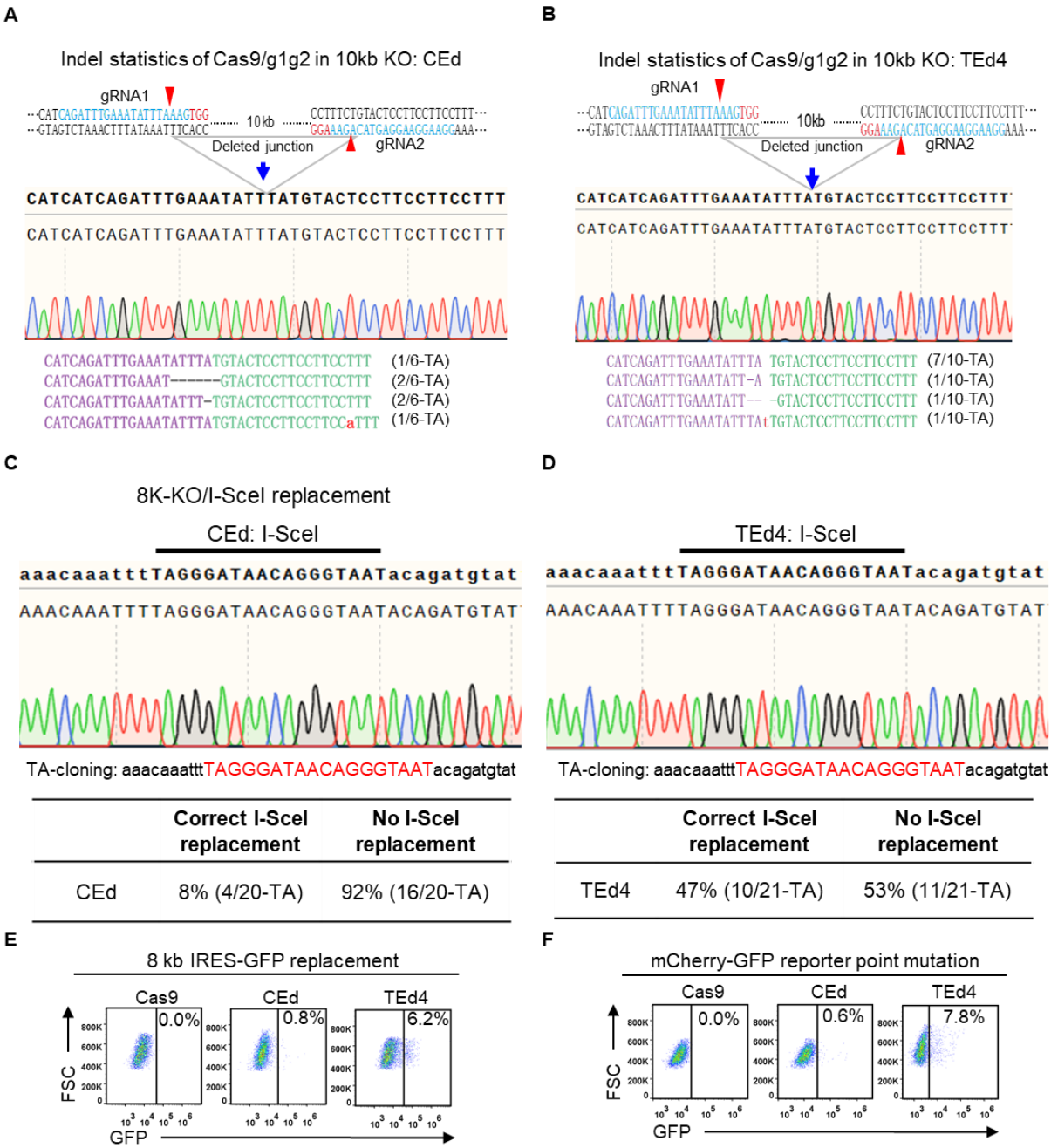
(A), Representative FACS analysis of GFP expression 72h after transfection at three targeting loci in HEK293T cells. (B), qPCR analyses of the GFP KI level at the LMNA-g2 and FBL loci. ACTB was used as internal genomic reference. Data are shown as individual data points and mean ± s.d for n=3 independent 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 μm.


A
gRNA
GAPDH locus
exon
3'UTR
HR-donor
TEd4
TC-donor
IRES GFP Junk DNA LMNA TAG
P9
P11
P10
P12
FBL locus
P13
P14
B
GAPDH locus
FBL locus
CEd-2.7 kb
CEd-5.4 kb
CEd-7.0 kb
CEd-8.5 kb
CEd-10.1 kb
0.3%
0.0%
0.0%
0.0%
0.0%
0.4%
0.0%
0.0%
TEd-2.7 kb
TEd-5.4 kb
TEd4-7.0 kb
TEd4-8.5 kb
TEd4-10.1 kb
TEd4-2.7 kb
TEd4-5.4 kb
TEd4-10.1 kb
6.3%
3.9%
1.2%
0.6%
0.1%
4.2%
1.8%
0.05%
FSC
GFP
C
GAPDH locus
CEd-2.7 kb
TEd4-2.7 kb
TEd-4:5.4 kb
TEd4-7.0 kb
TEd4-8.5 kb
TEd4-10.1 kb
FBL locus
CEd-2.7 kb
TEd4-2.7 kb
TEd4-5.0 kb
TEd4-10.1 kb



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 μm. (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 ± s.d for n=3 independent biological replicates.

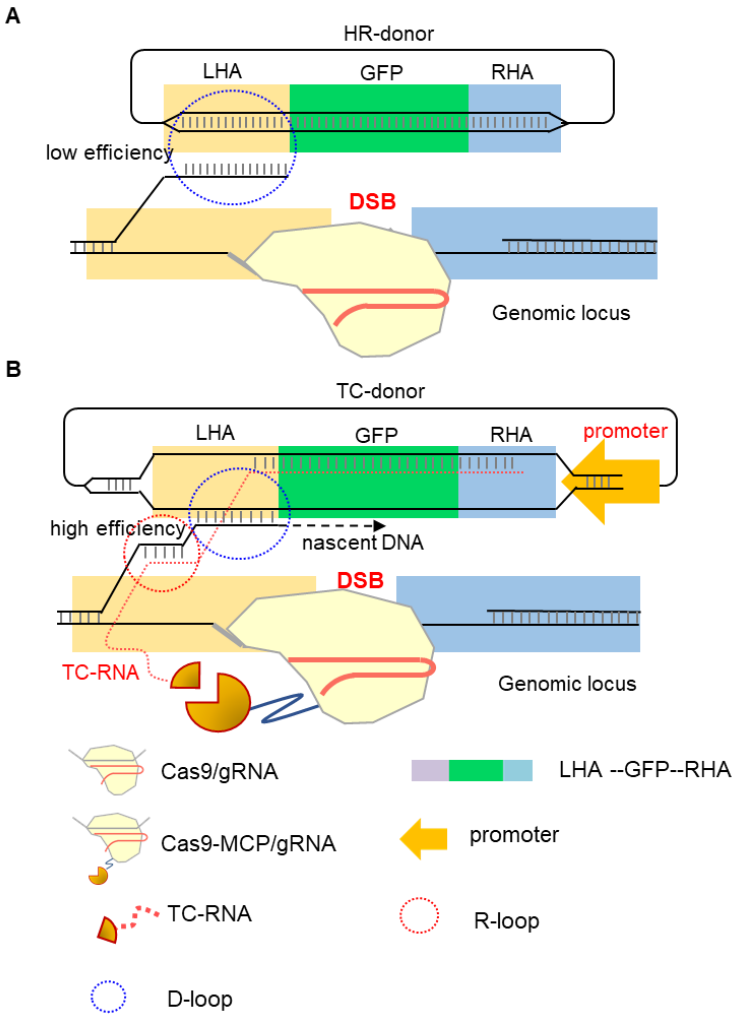


	Correct I-SceI replacement	No I-SceI replacement
CEd	8% (4/20-TA)	92% (16/20-TA)

	Correct I-SceI replacement	No I-SceI replacement
TEd4	47% (10/21-TA)	53% (11/21-TA)

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')
LMNA gRNA1	CCATGGAGACCCCGTCCCAGCGG
LMNA gRNA2	GGGCGACAGCGGAGTGGAGCTGG
FBL	CTCTCACAGGCCACCCCCCAAGG
GAPDH	AGCCCCAGCAAGAGCACAAGAGG
CDC42 gRNA1	CAGATTTGAAATATTTAAAGTGG
CDC42 gRNA2	GGAAGGAAGGAGTACAGAAAGG
CDC42 gRNA3	CACAACAAACAAATTTCCATCGG
CDC42 gRNA4	GACTAGAAATACATCTGTTTGTGG
mCherry-GFP Reporter	GCGCCTGCTCGCGATGCTAGAGG
PRNP	GCAGTGGTGGGGGGCCTTGGCGG

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

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

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

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

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EGFP HR-donor plasmid, FBL, 240bp HAs

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EGFP TC-donor plasmid, FBL, 240bp HAs

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

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

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CCGACGCGT

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

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TATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGG
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CTCGTCCATGCCGCCGGTGGAGTGGCGGCCCTCGGCGCGTTCGTACTGTTCCACGATGGTGTAGTCCTCGTTGTGGGAGGT
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CGGCCGCGACTCTAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGA
ACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAA
ATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTA

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

ACTAGTCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTA
TGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACAT
CAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCT
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CGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAA
CGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAG
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PRNP point mutation TC-donor plasmid, 240bp HAs

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CAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCT
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ATCTTCAAATTGCACGAGCATCAGCCGTGCGGATCAATATGTAAGTAAGTAAGCGTTTTTTTTGTCGACGGTACCGCCGGCCATG

GTCCCAGCCTCCTCGCTGGCGGCCGGTGGGCAACATTCCGAGGGGGACCGTCCCCTCGGTAATGGCGAATGGGACGCGGC
CGC

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

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CCTTCAGCTCGATGCGGTTCACCAGGGTGTCGCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTCGTCCTTGAA
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TGACCTTGCCCACAGCCCTCGAG

PRNP point mutation HR-donor plasmid, 240bp HAs

CTCCATCATCTTAACGTCGGTCTCGGTGAAGTTCTCCCCCTTGGTGGTTGTGGTGACCGTGTGCTGCTTGATTGTGATATTGAC
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ACGATAGTAACGGTCCTCATAGTCACTGCCGAAATGTATGATGGGCCTGCTCATGGCACTTCCCAGCATGTAGACGCCAAGGCC
CCCCACCACTGCCCCAGCTGCTGCAGCACCAGCCATGTGCTTCATGTTGGTTTTTGGCTTACTCGGCTTGTTCCACTGACTGT
GGGTGCCACCTCCTTGACCCCAGCCACCACCATGAGGCTGTCCCCAGCCACCACCATGGGGCTGCCCCCAGCCACCACCAT
GAGGCTGCCCCCAGCCACCACCATGAGGCTGCCCCCAGCCACCACCGCCCTGAGGTGGGTAGCGGT

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

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CCTCCCCTTGACCTGCTGACACAGGGAGCACGCACCCTTGGTCAATTTTGCGGGGTTGGGTAAATTCTCACTCGGTCACAGAG
CGCATGCTCCGTTTCTAGCTGCCTTTGCGCAGCGGCAGCCTGGACGCGT

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

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CCCCGGTGAACAGCTCCTCGCCCTTGCTCACGATGAGGTCGACCATTGTGGCCATATTATCATCGTGTTTTTCAAAGGAAAACC
ACGTCCCCGTGGTTCGGGGGGCCTAGACGTTTTTTTAACCTCGACTAAACACATGTAAAGCATGTGTACCGAGGCCCCAGATC
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AACTATCCAACTCACAACGTGGCACTGGGGTTGTGCCGCCTTTGCAGGTGTATCTTATACACGTGGCTTTTGGCCGCAGAGGC
ACCTGTCGCCAGGTGGGGGGTTCCGCTGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCAAGAAGCTTCCAGAGGAAC
TGCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTTGGCGAGAGGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAG
GGCCAGGTTTCCGGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATAACATATAGACAAACGCACACCGGCCTTATT

CCAAGCGGCTTCGGCCAGTAACGTTGGGTGGCCTGTGAGAGGAAGATAGGTGATGAAGGAGGGTCTACTACCCCAGGATCAT
GGCACTCGGGGGTGCAAGGGACAGAGATGTCTGTCTTGGTGTATTGCTGGGCCCCTGCTCACCTGTACACTCCCACGACCACG
GCATGGTCTCTTTCATATGGCTCAAGGGTCAACTGCTCCTGCGGCTTCATGTTCTCCTGTTGCATCTTCTCGAGCTCAAGCTTC
CACCGCAAATGCTTCTGAGATCTATATTCGGCCAAGAAAGCACGAGCATCAGCCGTGCCTCCAGGTCGAATCTTCAAATTGCAC
GAGCATCAGCCGTGCGGATCTATATTCGGCCAAGAAAGCACGAGCATCAGCCGTGCCTCCAGGTCGAATCTTCAAATTGCACG
AGCATCAGCCGTGCGGATCAATATGTAAGTAAGTAAGCGTTTTTTTTGTCGACGGTACCGCCGGCCATGGTCCCAGCCTCCTCG
CTGGCGGCCGGTGGGCAACATTCCGAGGGGGACCGTCCCCTCGGTAATGGCGAATGGGACACGCGT

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

CTCGAGGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGCTCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGT
GTCAGTGGTGGACCTGACCTGCCGTCTAGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAG
GGCCCCCTCAAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACTCCTCCACCT
TTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTGGTATGTGGCTGGGGCCAGAGACTGGCTC
TTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTGGCTCAGAAAAAGGGCCCTGACAACTCTTTTCATCTTCTAGGTATGAC
AACGAATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGGAGTAAGACCCCTGGACCACCAGCC
CCAGCAAGAGCACTAATTTAATATAGTGAGTGAAATTCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGG
AATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCC
TGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAG
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TGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCT
GATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGT
GGTTTTCCTTTGAAAAACACGATGATAATATGGCCACAATGGTCGACCTCATCGTGAGCAAGGGCGAGGAGCTGTTCACCGGG
GTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGC
CACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTG
ACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCT
ACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCC
TGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAA
CAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACG
GCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACC
TGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCG
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TTCTGTTCCATATGGAGATTTAGCTGTGGTTTTGGTATTCACTGTATGTCATATCCCCTTCCACCTGCCCTGCACTGGTGGTGTT
GCTTGCCGGTTGCCTTAGGAATAGCAAGCGCTCTAGGTTAGGCAGAAAGTCTGATTAGAAACAGATGGTTGTGGGAACTAAGC
AACCATATTTTCTCTATAGCTCCTGAGACATACAGCAAGTGGTGATGGTCCCCTTCTCCATGTGAACTGTTTAAGCGTTTTATTCC
CAAACTCATAAAGGAGTTAATGTAGTTTTCACTCTGTCCCCATCCCTAAGGTAACTGCTTTTGGCTGAAAACCATTTACCCTAGAG
CTAGAAACTTTGGGGACAATAAGAAGGTTGTGACCTTTCTAGTATATGCCAAATACAAGATTTTTTTTTTCTTTTTAGTATGAAATAC
TTTTCACAACCGCATTGTTTGTTGTATGGATTCACAAAACTAGGACCATTTGGTATCTGTCTTCAGAAAGTTTTTACGTCTGATATC
CTTGTTGGTAACCGCTGTTTCTAGGGGTATCATATCATCCCATTTAAAAGAAATGCAAACTGCAGATTATAGAGTGCAGCTACACA
TATATATAATGGGGTGGATAGTTTATAGTGTGCTCATTGCTGCTTTGTTATTATTAGTGTTGAGAGTTCCTGTGCTGTGTGGAATTC
AACACTAATCTGCTGTAAGTATGGAGCTGGGTATGTGGAACATTTGCAGGGAAGTTTGTTTCTCCGCTTGTTTTTCCAGGGGTAT
TCGAGATGACATTGAAGAGGAAGATGACCAAGTGAGTTCCTATGCAGTATTTCTATCTTTTATTTTTATCCACAAAGTCTATACTGG
GGACAAGCTAGAGCTTGCCTTCACTCAAAATGGCCATTCCTGACATTCAGCAGGGAATGTCATTTGATTTGCTCCTTTTGTACCT
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GAGGAAGAGAGAGACCCTCACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTT
GCCATGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAGTACCCTGTGCTCAAC
CAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGGGAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTG
GGGAGGGACCTGGTATGTTCTCCTCAGACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCG
CTCAGACGTCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGCTCCAGTCCTAGG
CTATCTGCTGTTGGCCAAACATGGAAGAAGCTATTCTGTGGGCAGCCCCAGGGAGGCTGACAGGTGGAGGAAGTCAGGGCTC
GCACGCGT

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

CTCGAGCCGTGTGATCGTGTGGTGCTCTGCTGCTTGTTGGTCAGAGCCTGAGTAAGGCTGAATAAGGCTGGATCATGAACCAT
TCTTTAAAGCAGTGGCCTTTGTATTGCAGTCTAGTTGACATACATGCATACATACAGGTGTGTGTGCACGTGTAACTGAAGAACA
GCTTTCACAAAATAATATTTAGCACACTGTGGAATTTTCTCTGATACAGCCTCTTCTCTTTCATGATAAAAATAAATTCGCTGATTAT
CACACCACTTTGGACCTTTTAAAAATAACACTTAAGAAGTTGGAATAGCCAGGGATCAGAAACTTAGAAATGGACCTTATTTGTTA
TTGTAACTTATAGCAAGTCATTGTGTCTCTTATTGGGTTCTTTTAGCCATAATAATTGTGCTAGTTCTTTTCTTGCTCTGAGTGCCT
GAACCTGTTGCTAAGTGAGGAAGAAAAATCCATATGTAAGTATAAATAAACAAATGTCTTTAATCTTTTTGCAGGTCATCATCAGAT
TTGAAATATTTTGTACTCCTTCCTTCCTTTTATATTTTACTGCCAGAGTTGGTTCCTAGATGTGCAGAAAGAATTGCAGTGGAATTT
TAACTTTTATGTGGCATTTCTACTTTGTAAATGATGTAGAAAGAGGAAGGCTTTTTAACTTTTGCTGCTTTTAAAGAATTTACTGCT
GTTTCTGATTTATTTTGTGTCTAATGTGACATGTGGGTACCAGTCCTGTGGGATGTCATGGATGGGGGCAGGGTGGAGGTGGGA
ACACTTGGGGTGGTCTGGGGTTGGCACTTACAATTGGAGCTGTGTTCTGGGACTTTTAGGACATATTTACTTGGGATCAAACTG
ATCAAACTAAAGAACATGTGAAACAACCTTGGGTGGTAAACTTGTGGCTTCAGATTTAGGTGATTTTAGGTGAGTCTGTAGACCT
GGGTCCTAAGTACCACCAATGCTGAGTCACACTGTCATGTTGGAGTTTCTGGCTGAGGTGTAAGTGTATTTCTGTATCTTAATCC
CCGACGCGT

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

CTCGAGACTAGTCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAAT
GACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCA
GTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACA
TGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATC
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AAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCT
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CCAACCCCAGACCACCCCAAGTGTTCCCACCTCCACCCTGCCCCCATCCATGACATCCCACAGGACTGGTACCCACATGTCAC
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AAGTAGAAATGCCACATAAAAGTTAAAATTCCACTGCAATTCTTTCTGCACATCTAGGAACCAACTCTGGCAGTAAAATATAAAAG
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AGTGTGCTAAATATTATTTTGTGAAAGCTGTTCTTCAGTTACACGTGCACACACACCTGTATGTATGCATGTATGTCAACTAGACT
GCAATACAAAGGCCACTGCTTTAAAGAATGGTTCATGATCCAGCCTTATTCAGCCTTACTCAGGCTCTGACCAACAAGCAGCAG
AGCACCACACGATCACACGGCTCGAGCTCAAGCTTCCACCGCAAATGCTTCTGAGATCTATATTCGGCCAAGAAAGCACGAGC
ATCAGCCGTGCCTCCAGGTCGAATCTTCAAATTGCACGAGCATCAGCCGTGCGGATCTATATTCGGCCAAGAAAGCACGAGCAT
CAGCCGTGCCTCCAGGTCGAATCTTCAAATTGCACGAGCATCAGCCGTGCGGATCAATATGTAAGTAAGTAAGCGTTTTTTTTGT
CGACGGTACCGCCGGCCATGGTCCCAGCCTCCTCGCTGGCGGCCGGTGGGCAACATTCCGAGGGGGACCGTCCCCTCGGTA
ATGGCGAATGGGACGCGGCCGCGACGCGT

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

CTCGAGCAGGTGTGTGTGCACGTGTAACTGAAGAACAGCTTTCACAAAATAATATTTAGCACACTGTGGAATTTTCTCTGATACA
GCCTCTTCTCTTTCATGATAAAAATAAATTCGCTGATTATCACACCACTTTGGACCTTTTAAAAATAACACTTAAGAAGTTGGAATA
GCCAGGGATCAGAAACTTAGAAATGGACCTTATTTGTTATTGTAACTTATAGCAAGTCATTGTGTCTCTTATTGGGTTCTTTTAGCC
ATAATAATTGTGCTAGTTCTTTTCTTGCTCTGAGTGCCTGAACCTGTTGCTAAGTGAGGAAGAAAAATCCATATGTAAGTATAAATA
AACAAATGTCTTTAATCTTTTTGCAGGTCATCATCAGATTTGAAATATTTAAAGTGGATACAAAACTATTTCAGCAATGCAGACAATT
AAGTGTGTTGTTGTGGGCGATGGTGCTGTTGGTAAAACATGTCTCCTGATATCCTACACAACAAACAAATTTTAGGGATAACAGG
GTAATACAGATGTATTTCTAGTCTGTTTTTCAGTGGTCTCTCCATCTTCATTTGAAAACGTGAAAGAAAAGGTAAGCTGATCAGAT
ACTCTTGCCCTAAGAAGATCATCTCAGAATTTCTACTGACCTGTTATAAATAGCATTAGAGGCTTGTTGATTAACAAAGGTGTATTT
TAAAATACCTTTTTTTAGTGGGTGCCTGAGATAACTCACCACTGTCCAAAGACTCCTTTCTTGCTTGTTGGGACTCAAATTGATCT
CAGAGATGACCCCTCTACTATTGAGAAACTTGCCAAGAACAAACAGAAGCCTATCACTCCAGAGACTGCTGAAAAGCTGGCCC
GTGACCTGAAGGCTGTCAAGTATGTGGAGTGTTCTGCACTTACACAGGTAAGAATGGCATGAAACCCCATGTGTATTTATGGTC
GAGTCATTTATTAGAGCATTAGGATACAGGAGTTATTTCTAACAGTGATCAGCAGTGCTCAAAGATGCCCAGCAAGACGCGT

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

CTCGAGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGT
ATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACA
TCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACC
TTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGG
GCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCA
ACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAA
GCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGCGCGTCTGCCCTGGATGAGTCCGTGAGGACGAAACGGTACTCGGTAC
CGTCGGCAGACGCGTCTTGCTGGGCATCTTTGAGCACTGCTGATCACTGTTAGAAATAACTCCTGTATCCTAATGCTCTAATAAA
TGACTCGACCATAAATACACATGGGGTTTCATGCCATTCTTACCTGTGTAAGTGCAGAACACTCCACATACTTGACAGCCTTCAG
GTCACGGGCCAGCTTTTCAGCAGTCTCTGGAGTGATAGGCTTCTGTTTGTTCTTGGCAAGTTTCTCAATAGTAGAGGGGTCATC
TCTGAGATCAATTTGAGTCCCAACAAGCAAGAAAGGAGTCTTTGGACAGTGGTGAGTTATCTCAGGCACCCACTAAAAAAAGGT
ATTTTAAAATACACCTTTGTTAATCAACAAGCCTCTAATGCTATTTATAACAGGTCAGTAGAAATTCTGAGATGATCTTCTTAGGGC
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TATTACCCTGTTATCCCTAAAATTTGTTTGTTGTGTAGGATATCAGGAGACATGTTTTACCAACAGCACCATCGCCCACAACAACA
CACTTAATTGTCTGCATTGCTGAAATAGTTTTGTATCCACTTTAAATATTTCAAATCTGATGATGACCTGCAAAAAGATTAAAGACAT
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TCGAGCTCAAGCTTCCACCGCAAATGCTTCTGAGATCTATATTCGGCCAAGAAAGCACGAGCATCAGCCGTGCCTCCAGGTCG
AATCTTCAAATTGCACGAGCATCAGCCGTGCGGATCTATATTCGGCCAAGAAAGCACGAGCATCAGCCGTGCCTCCAGGTCGA
ATCTTCAAATTGCACGAGCATCAGCCGTGCGGATCAATATGTAAGTAAGTAAGCGTTTTTTTTGTCGACGGTACCGCCGGCCATG
GTCCCAGCCTCCTCGCTGGCGGCCGGTGGGCAACATTCCGAGGGGGACCGTCCCCTCGGTAATGGCGAATGGGACACGCGT

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

CTCGAGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGT
ATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACA
TCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACC
TTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGG
GCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCA
ACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAA

GCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGCGCGTCTGCCCTGGATGAGTCCGTGAGGACGAAACGGTACTCGGTAC
CGTCGGCAGACGCGTACTGTTAGAAATAACTCCTGTATCCTAATGCTCTAATAAATGACTCGACCATAAATACACATGGGGTTTCA
TGCCATTCTTACCTGTGTAAGTGCAGAACACTCCACATACTTGACAGCCTTCAGGTCACGGGCCAGCTTTTCAGCAGTCTCTGG
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CGTTTTCAAATGAAGATGGAGAGACCACTGAAAAACAGACTAGAAATACATCTGTATTACCCTGTTATCTACTTGTACAGCTCGTC
CATGCCGAGAGTGATCCCGGCGGCGGTCACGAACTCCAGCAGGACCATGTGATCGCGCTTCTCGTTGGGGTCTTTGCTCAGG
GCGGACTGGGTGCTCAGGTAGTGGTTGTCGGGCAGCAGCACGGGGCCGTCGCCGATGGGGGTGTTCTGCTGGTAGTGGTC
GGCGAGCTGCACGCTGCCGTCCTCGATGTTGTGGCGGATCTTGAAGTTCACCTTGATGCCGTTCTTCTGCTTGTCGGCCATGA
TATAGACGTTGTGGCTGTTGTAGTTGTACTCCAGCTTGTGCCCCAGGATGTTGCCGTCCTCCTTGAAGTCGATGCCCTTCAGCT
CGATGCGGTTCACCAGGGTGTCGCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTCGTCCTTGAAGAAGATGGT
GCGCTCCTGGACGTAGCCTTCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGGTCGGGGTAGCGGCTGAAGCA
CTGCACGCCGTAGGTCAGGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGG
TCAGCTTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGAC
CAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACGATGAGGTCGACCATTGTGGCCATATTATCATCGTGTT
TTTCAAAGGAAAACCACGTCCCCGTGGTTCGGGGGGCCTAGACGTTTTTTTAACCTCGACTAAACACATGTAAAGCATGTGCAC
CGAGGCCCCAGATCAGATCCCATACAATGGGGTACCTTCTGGGCATCCTTCAGCCCCTTGTTGAATACGCTTGAGGAGAGCCA
TTTGACTCTTTCCACAACTATCCAACTCACAACGTGGCACTGGGGTTGTGCCGCCTTTGCAGGTGTATCTTATACACGTGGCTTT
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GCTTCCAGAGGAACTGCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTTGGCGAGAGGGGAAAGACCCCTAGGAATGCT
CGTCAAGAAGACAGGGCCAGGTTTCCGGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATAACATATAGACAAACG
CACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGGGGGGGGGGAGGGAGAGGGGCCTAAAATTTGTTTGTTGTG
TAGGATATCAGGAGACATGTTTTACCAACAGCACCATCGCCCACAACAACACACTTAATTGTCTGCATTGCTGAAATAGTTTTGTA
TCCACTTTAAATATTTCAAATCTGATGATGACCTGCAAAAAGATTAAAGACATTTGTTTATTTATACTTACATATGGATTTTTCTTCCT
CACTTAGCAACAGGTTCAGGCACTCAGAGCAAGAAAAGAACTAGCACAATTATTATGGCTAAAAGAACCCAATAAGAGACACAAT
GACTTGCTATAAGTTACAATAACAAATAAGGTCCATTTCTAAGTTTCTGATCCCTGGCTATTCCAACTTCTTAAGTGTTATTTTTAAA
AGGTCCAAAGTGGTGTGATAATCAGCGAATTTATTTTTATCATGAAAGAGAAGAGGCTGTATCAGAGAAAATTCCACAGTGTGCT
AAATATTATTTTGTGAAAGCTGTTCTTCAGTTACACGTGCACACACACCTGCTCGAGCTCAAGCTTCCACCGCAAATGCTTCTGA
GATCTATATTCGGCCAAGAAAGCACGAGCATCAGCCGTGCCTCCAGGTCGAATCTTCAAATTGCACGAGCATCAGCCGTGCGG
ATCTATATTCGGCCAAGAAAGCACGAGCATCAGCCGTGCCTCCAGGTCGAATCTTCAAATTGCACGAGCATCAGCCGTGCGGAT
CAATATGTAAGTAAGTAAGCGTTTTTTTTGTCGACGGTACCGCCGGCCATGGTCCCAGCCTCCTCGCTGGCGGCCGGTGGGCA
ACATTCCGAGGGGGACCGTCCCCTCGGTAATGGCGAATGGGACACGCGT

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

CTCGAGCAGGTGTGTGTGCACGTGTAACTGAAGAACAGCTTTCACAAAATAATATTTAGCACACTGTGGAATTTTCTCTGATACA
GCCTCTTCTCTTTCATGATAAAAATAAATTCGCTGATTATCACACCACTTTGGACCTTTTAAAAATAACACTTAAGAAGTTGGAATA
GCCAGGGATCAGAAACTTAGAAATGGACCTTATTTGTTATTGTAACTTATAGCAAGTCATTGTGTCTCTTATTGGGTTCTTTTAGCC
ATAATAATTGTGCTAGTTCTTTTCTTGCTCTGAGTGCCTGAACCTGTTGCTAAGTGAGGAAGAAAAATCCATATGTAAGTATAAATA
AACAAATGTCTTTAATCTTTTTGCAGGTCATCATCAGATTTGAAATATTTAAAGTGGATACAAAACTATTTCAGCAATGCAGACAATT
AAGTGTGTTGTTGTGGGCGATGGTGCTGTTGGTAAAACATGTCTCCTGATATCCTACACAACAAACAAATTTTAGGCCCCTCTCC
CTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTG
CCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAA
AGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCC
TTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGC
GGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGC
TGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGT
TAAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATGATAATATGGCCACAATGGTCGAC
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AAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAG
CTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAG
CAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAA
GACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGG
CAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCA
AGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGG
CGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGA
TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAGATAACAGGGTAAT
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TGCCCTAAGAAGATCATCTCAGAATTTCTACTGACCTGTTATAAATAGCATTAGAGGCTTGTTGATTAACAAAGGTGTATTTTAAAA
TACCTTTTTTTAGTGGGTGCCTGAGATAACTCACCACTGTCCAAAGACTCCTTTCTTGCTTGTTGGGACTCAAATTGATCTCAGA
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CTGAAGGCTGTCAAGTATGTGGAGTGTTCTGCACTTACACAGGTAAGAATGGCATGAAACCCCATGTGTATTTATGGTCGAGTC
ATTTATTAGAGCATTAGGATACAGGAGTTATTTCTAACAGTACGCGT

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

Primers used for the cloning of HR and TC-donors	
H1-F	AATTCATATTTGCATGTCGCTATGTGTTC
H1-R	CGAGTGGTCTCATACAGAACTTATAAG
CMV-F	CGTTACATAACTTACGGTAAATGGCCC
CMV-R	AGCTCTGCTTATATAGACCTCCCACC
LMNA-HA-F1	CCCGGCGTCGGTGACTCAGT
LMNA-HA-R1	GGACGGGGTCTCCATGGCCG
LMNA-HA-F2	CAGCGGCGCGCCACCCGC
LMNA-HA-R2	GAGCTCGGCCTCGTAGGCG
FBL-HA-F1	AAGATGCAACAGGAGAACATGAAGC
FBL-HA-R1	GGGTGGCCTGTGAGAGGAAGATAG
FBL-HA-F2	CCCAAGGTGAAGAACTGAAGTTCAGCG
FBL-HA-R2	CCAGGCTGCCGCTGCGCAAA
GAPDH-HA-F1	CCATCACTGCCACCCAGAAGACT
GAPDH-HA-R1	ATGAGGTCGACGACCCTGTTG
GAPDH-HA-F2	ACCACCAGCCCCAGCAAGAG
GAPDH-HA-R2	GACTTCCTCCACCTGTCAGCCTC
GFP-F	GTGAGCAAGGGCGAGGAGC
GFP-R	CTTGTACAGCTCGTCCATGCCG
CDC-HA-F1	CCGTGTGATCGTGTGGTGCTCTGC
CDC-HA-R1	AAATATTTCAAATCTGATGATGACCTGCAAAAAGATT
CDC-HA-F2	TGTACTCCTTCCTTCCTTTTATATTTTACTGCC
CDC-HA-R2	CGGGGATTAAGATACAGAAATACACTTACACC
CDC-HA-F3	CAGGTGTGTGTGCACGTGTAACTG
CDC-HA-R3	AAATTTGTTTGTTGTGTAGGATATCAGGAGAC
CDC-HA-F4	ACAGATGTATTTCTAGTCTGTTTTTCAGTGGTC
CDC-HA-R4	ACTGTTAGAAATAACTCCTGTATCCTAATGCTC
IRES-F	TTTTAGGCCCCTCTCCCTCCC
IRES-R	CGACCATTGTGGCCATATTATCATCGTG
PM-HA-F	GTGCTTCAGCCGCTACCCCG
PM-HA-R	CGACGCTGAGGTCAAGACCACC
Primers used in gRNA synthesis	
LMNAg1-F	ACCGCCATGGAGACCCCGTCCCAG
LMNAg1-R	AAACCTGGGACGGGGTCTCCATGG
LMNAg2-F	ACCGGGGCGACAGCGGAGTGGAGC
LMNAg2-R	AAACGCTCCACTCCGCTGTCGCCC
gFBL-F	ACCGCTCTCACAGGCCACCCCCCA
gFBL-R	AAACTGGGGGGTGGCCTGTGAGAG
gGAPDH-F	ACCGAGCCCCAGCAAGAGCACAAG
gGAPDH-R	AAACCTTGTGCTCTTGCTGGGGCT
CDCg1-F	ACCGCAGATTTGAAATATTTAAAG
CDCg1-R	AAACCTTTAAATATTTCAAATCTG
CDCg2-F	ACCGGGAAGGAAGGAGTACAGAA
CDCg2-R	AAACTTCTGTACTCCTTCCTTCC
CDCg3-F	ACCGCACAACAAACAAATTTCCAT
CDCg3-R	AAACATGGAAATTTGTTTGTTGTG
CDCg4-F	ACCGGACTAGAAATACATCTGTTTG
CDCg4-R	AAACCAAACAGATGTATTTCTAGTC
Primers used in gRNA synthesis	
gReporter-F	ACCGGCGCCTGCTCGCGATGCTAG
gReporter-R	AAACCTAGCATCGCGAGCAGGCGC
gPRNP-F	ACCGGCAGTGGTGGGGGGCCTTGG
gPRNP-R	AAACCCAAGGCCCCCCACCACTGC

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

Primers used in genotyping PCR	
P1	GACCCTTTTGCCCACCCACTCT
P2	AAACTCCTCACGCACTTTGCTCAG
P3	CCGGCGTCGGTGACTCAGTGTTC
P4	GAGCTCGGCCTCGTAGGCGGCC
P5	GTGTGTGCACGTGTAACTGAAGAACAGC
P6	GCATACACTTCAGTTATTCAGCAATGTTC
P7	CGTGTGATCGTGTGGTGCTCTGC
P8	GGTTCATGATCCAGCCTTATTCAGC
P9	ATGACATCAAGAAGGTGGTGAAGCA
P10	GCCTTGTTCTCTGAAGTAGTGGTGC
P11	GGTTCGCCGTCGCCCATCGAGAAC
P12	GACTCCTTGGAGAATCCGCCTGTC
P13	CTCCACAGCCTCAGCCGAGGCC
P14	GCATGCGCAGCGAGCTACCAATCC
GFP-N137-R	AACTTCAGGGTCAGCTTGCCGTA
GFP-C175-F	ACTACCAGCAGAACACCCCCATC
mCherry-GFP-F	GGACATCACCTCCCACAACGA
mCherry-GFP-R	GTCGTCCTTGAAGAAGATGGTGC
PRNP-F	GAAGCCTGGAGGATGGAACACTG
PRNP-R	ACCGTGTGCTGCTTGATTGTGAT
Primers used in qPCR	
LMNA-F	GACTCAGTGTTCGCGGGAGCG
LMNA-R	CGGTGAACAGCTCCTCGCCC
FBL-F	GCATTGACTCCACAGCCTCAGCC
FBL-R	CCCGGTGAACAGCTCCTCGC
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	GTCGTCCTTGAAGAAGATGGTGC
PRNP-F12	GGCAACCGCTACCCACCTCA
PRNP-R12	GAAGTTCTCCCCCTTGGTGGTT
LMNA-ISCE-F13	GCGTCGGTGACTCAGTGTTCG
LMNA-ISCE-R13	CTCGTAGGCGGCCTTGATGC

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

Primers used in LM-PCR	
P15	TCCAGCTCGACCAGGATG
P16	CCGGTGAACAGCTCCTCG
NGS barcoding PCR primer	
I5	AATGATACGGCGACCACCGAGATCTACACNNNNNNNNACACTCTTTCCCTAC ACGACGCTCTTCCGATCT
I7	CAAGCAGAAGACGGCATACGAGATNNNNNNNNGTGACTGGAGTTCAGACG TGTGCTCTTCCGATC

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	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGCGGGGCAGGGGTCCCGGAAAG GGCGCGGGGCTCGGGTCGAAGGACAGAGACTGCTCGGAGTCGGAG TGCGCCGGCCGGCTGGCTCTCGGCTTGCTCCTGGCAGGCGTGGGG ATCTGGGCCGGGCGCTGTCGGACCTCGGGATCTGGGTTGGCTGGT GTAGGTGCGGCGCTCCCGCGAACA
chr14	20811578	RAPP2	5	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CGGATCCGAGTGGTCTCATACAGAACTTATAAGATTCCCAAATCCAA AGACATTTCACGTTTATGGTGATTTCCCAGAACACATAGCGACATGCA AATATTGCAGGGCGCCACTCCCCTGTCCCTCACAGCCATCTTCCTGC CAGGGCGCACGCGCGCTGGGTGTTCCCGCCTAGTGACACTGGGCC CGCGATTCCTTGGAGCG
chr20	30049696	DEFB124	3	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACACGAATAAGCCAG AAAAATCCCTCCTTGAGCCAGTGCCTGAATGAGTGGGAGATGGGAAT TGGATTATTTCCTGGGCAGGGAAAGCTTCTGGCAGTGAAGACATCAC ATTGCATTGTGTGATTCCACTGTGGAAAGATACTTATTTTTCACCAAA AAAAATGGTGCCTAAACATAACCAACCACTTCCTTTAATGCACGGCCA GAGAAATGTGATAT
chr2	1.27E+08	GYPC	3	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGCGGGGCAGGGGTCCCGGAAAG GGCGCGGGGCTCGGGTCGAAGGACAGAGACTGCTCGGAGTCGGAG TGCGCCGGCCGGCTGGCTATATGGCTAACGTATAGAGAATATCATGC ACTGAAACCCAACTTTCCTCTTTGTAGTCTGGTCTGATGGTTATTCCC CTGCTAATACCATAGCTTC
chr17	3700587	ITGAE	3	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGCGGGGCAGGGGTCCCGGAAAG GGCGCGGGGCTCGGGTCGAAGGACATGTAGCTGGGACTACAGGCA CCCGCCACCATGCCCAGCTAATTTTTTGTGTTTTTAGTAGAGACGGA GTTTCACTGTGTTAGCCAGGATGGTCTCGATCTCCTGACCTCATGAT CTGCCCACCTTGGCCTCCTA
chr1	1.11E+08	KCNA3	3	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGCGGGGCAGGGGCTGTTAGATT ATTGGTCCAATTTTAATTTACATAATTATTTAATAGTATGCAGTGGTAC AGTTCTTTGAAACAAAAAAGGGTTAAAAATAAGAGTAACATTTTATTTA AACATAATCATTCTGTTTCATATACATTATATAAATTATACATTATCTAC AGTTGTTTTTT
chr13	91832047	LINC00379	3	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACACACTGACTAGTC GGAGGGAACGTTGTCGGATCTCACCAATGGTCAGTCCAAGTCGACT CGTCAGTGTAAAAAAAAGCATTTAATCAAAAGCACTTGATCAAATTAT TTAGAGCAATAATTTTATATTGTATTTGTTTTGTGGAACTTTAATAAAAT TACGGTCTTTGCTATGTTTATTGGTGAACTTTTATACTTTAGCGGCATT TTAAACATGTT
chr5	1.61E+08	LINC02159	3	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGCGGGGCAGGGGTCCCGGATCA GGAAGCTGAGGCAGGAGAATTGCTTGAAGCCAGGAGGCAGAGATTG CAATGAGCGGAGATCACGCCACTGCACTGCAGCCTGGGTGACAACA GTGAAACTCGTTCTCAAACAAACAAACAAAAAAACACTGCCCAGATTT TTTTTTAATTGTTAGGCAG
chr12	65780874	MSRB3	3	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGCGGGGCAGGGGTCCCGGAAAG GGCGCGGGGCTCGGGTCGAAGGACAGAGACTGCTCGGAGTCGGAG TGCGCCGGCCGGCTGGCTCTCATGAGATACTGCTGTTGTACTGTAAT CCTGTCACCCATTAGCTCTACAATAGCCTTACATAAATAAACTCAGAA TTGAAATAATGAGATAAGA
chr11	64834438	NAALADL1	3	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGCGGGGCAGGGGTCCCGGAAAG GGCGCGGGGTCAGTCCAAGTCGACTCGTCAGTGTCACCTGGTCCTA TAGCCACCTGGGACCAGTGTTTCAGTAAGAGCCCAGTCTTCCTGTCC CGGCAAAACCACAGGTGGGATGGGAATTGTTCTCCCTGAGTGATTG 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	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGCGGGGCAGGGGTCCCGGAAAG GGCGCGGGGCTCGGGTCGAAGGACAGAGACTGCTCGGAGTCGGAG TGCGCCGGCCGGCTGGCTCTCGGCTTGCTCCTGGCAGGCGTGGGG

				ATCTGGGCCGGGCGCTGTCGGACCTCGGGATCTGGGTTGGCTGGT GTAGGTGCGGCGCTCCCGCGAACA
chr20	25483286	NINL	3	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTTCAAATGGTTTAGCACCATCCCTCTTAGTACTGACCT CATGATAGTGAGTGAATTCTCATGAGACCTGGTTGTTTAAAAGTGTGT CACACCTCCCCCCTCGCTTTCTTGCTCCTGCTCTGGCCATGTGACCT GCCTGCACCCCTTCACCTTCTGCCATGATTGTAAGCTTCCTGAGGCC TCCCCAGAAGCAGCC
chr9	92748202	MIR4290	3	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGGCAACAGGATTCAGGGCTGTCTCACTGCAATGTCA GTGCAACAAGAATGGAGTCTGGCAGAGAAGGGAGACACCACAATGG CATCTTACCTAAACTTGCACTGTCACAGGCTTACCATGAGGGTTACG GCTACTCGCCTCCAACCAATAAGCAAATAACTTGGGTTGACTCCCAG TGCCTGTTGGAAAATCAA
chr10	75584440	NDST2	3	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGGCAGCGCCCAGTCACCCCCCTGCTGACTCTCTAGT TAGCCACCACTAGCAGAGGGACCTCCAAGAGCACACAGAACCCCTG GAGGAGCCCAGAGAGAACTTAGCCAGCATGAGGCACTCCAGGAGCC TGGAGACCTCAGCACCATCCAGAGGTGGGGACCCCAGCCCTCTTTC CTGTTTCTGGCTTTGCAAAG
chr14	20811578	RAPP2	3	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGATACGGTGTCTCCAT GGCGGATCCGAGTGGTCTCATACAGAACTTATAAGATTCCCAAATCC AAAGACATTTCACGTTTATGGTGATTTCCCAGAACACATAGCGACATG CAAATATTGCAGGGCGCCACTCCCCTGTCCCTCACAGCCATCTTCCT GCCAGGGCGCACGCGCGCTGGGTGTTCCCGCCTAGTGACACTGGG CCCGCGATTCCTTGGAGCG
chr17	56991606	PPM1E	3	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGCGGGGCAGGGGTCCCGGAAAG GGCGCGGGGCTCGGGTCGAAGGACAGATAGCCAACCAGCATTTGTT CATTACTGTTAACTACATTCCAGACTTACCTTGTATTTCATCAGCTTTT CCACTAATGTCCTTTTTCTGTTCTAGGATCCAATCCAGGATACTACAT TGCATCACGTTTATTT
chr12	5891999	ANO2	2	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGCGGGGCAGGGGTCCCGGAAAG GGCGCGATGGAAAATATCCACATTTAATGTTTTTAAAACATCTTTTGA GCCAAATCTTAAAAATGTCTACTAGCAGAAATCTTCCTTGAGATCACA GAATGCCACATCTGTGTATCTTCCCTACTGCCAATCTACCACTTTAAA TGGGGTGCCCAGAAC
chr8	62045358	CLVS1	2	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGCGGGGCAGGGGTCCCGGAAAG GGCGCGGGGCTCGGGTCGAAGGACAGAGACTGCTCGGAGTCGGGT CAGGCATTGTGTCCCAGCAGCGACACTGGGGAACTTCCCCCACTTC ATCACTCTGCAGCTCACTGGGCCCTGCATCCCGCTTTTTAGAACATC TGAAGCTTCATGCGGAAGCCT
chr1	2.32E+08	DISC2	2	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGCGGGGCAGGGGTCCCGGAAAG GGCGCGGCAAAATGATTACAAATAATTTCAGGTGTGCCTTGATACAA ATCAGATCTGTATACTTGTGTTTTCCTGTTGACATCTGTCATTTGCAG TCAGTGGCTTAAATTAAAATTTTTTGGTCTTAGGGAAAATCATGTGAG GGTTTCCCTGTTGCCT
chr7	1.26E+08	MIR592	2	CCGGTGAACAGCTCCTCGCCCTTGCTCACGGACGGGGTCTCCATGG CCGGCAGGTTGGCAGCGCTGCCCGCGGGGCAGGGGTCCCGGAAAG GCTAAAATATTTATAGTAAAATAGCAAAATGAAATATTCAATAGTTCTA CCTTTTAAAAAGTTAATAAGAGGTGGGTTGTGTTGTGTTTTTGGTAAT GAAATAATGTATATGGTTTTGGAATTAACATTTCTGACATACTTTAGAA CTTTGTTTTACTT
LM-PCR results of GFP insertion at *FBL* loci by CEd system				
Chr	start	gene	frequency	Validation Seq
chr19	40325195	FBL	319743	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGTG TTTTTCTATTAAAAGACTCATCCGTCTCCCATGTCTGCTGCTCATTCC TCCCCTTGACCTGCTGACACAGGGAGCACGCACCCTTGGTCAATTTT GCGGGGTTGGGTAAATTCTCACTCGGTCACAGAGCGCATGCTCCGT TTCTAGCTGCCTTTGC
chr15	68132383	SKOR1	3	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGAGCGGTGTTTCGTCCTT TCCACAAGATATATAAAGCCAAGAAATCGAAATACTTTCAAGTTACGG TAAGCATATGATAGTCCATTTTAAAACATAATTTTAAAACTGCAAACTA

				CCCAAGAAATTATTACTTTCTACGTCACGTATTTTGTACTAATATCTTTGTGTTTACAGT
chr6	1.03E+08	GRIK2	2	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAGCGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGTGTTTTTTCTATTAAAAGACTCATCCGTCTCCCATCTACTAGAATCATACAAAGAAGTCAGTTCTGTTAGCACTGTTTGTCTCTATAGCTATCTCTCCAGATTTTGGTGCAGCTTTAGTGTAGGACTATGTGAAGTTTATTAATAATCACACTCAGAAAT
chr4	54519321	LNX1	2	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAGCGCATATAGGATTCTGTGGAGAATTAAATAAAATAATGCAGGTCAGGTAATTACTACAGAACCTGGTAGACGGTAAGCACTTAATAAATGTTAGCAAAGATGCTGCAGCCACTGACGATGATGATGATGGTGATGCTGAAGAATGAGCAGAAGAAATCCTTATTTAAACAATAATTCTATATCTTTAGAGTTAAGAGTCTTAAA
chr3	1.87E+08	ADIPOQ	2	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAGCGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGTGTTTTTTCTATTAAAAGACTCATGGAACCCTCTGCAAATCTTACATGGCAGGTGGGTGACCAGAGGAGCCATTCCAATACCAGTCATTGCTGGATGTTTCTGGGGTGCTGTCCCAGGTGCTGTGGAGCATATCTACTCTGTCTAGTCTGTCTTTCCATT
chr6	11700243	ADTRP	2	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAGCGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGTGTTTTTTCTATTAAAAGACTCATCCGTCTCCCATGTCTGCTGCTCAGTGTCTTACTTGTTATAGATGCCCCATAAATATTTGTTAGATGAGTGTCTGAAAATCAAATTTCTAAAACACTGTTTTATCTTTTCAGAAGGTGGATTAACTATTTGCCTAAA
chr1	36411242	AGO3	1	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAGCGCTGTCAGGATTGCGAGAGATGTGTGTTGATAAAGCCTTGTCAACCAGAGTATATTAGAATTATAAGTACAGCTGATAATGGCTAAAACCTAGTTCTGCAAGATGACATCATTTCTAACACTTCAACTTCTGTCACCCCAGATTTCCTCTCTTCATTAAAAATAAACACAGAAAACCGTATGCCTCTGGAAAACTGTTCTGGA
chrX	40267960	ATP6AP2	1	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAGCGCTGACACTGACGAGTCGACTTGGACTGACTATTTCTTTCTTTCCAATTTTTTGTTTGTTTCTTTTTCTTGTTTTATTGCACTGGCAAAGACCTCTAGTACAATGCTGAATAGAAGTGATGAGAATGGAGATCTTTGTCTTATTCCTAATCTTAGAGTGAAAGCAGTCTTTTATGATATAAGCTGTAGGTTTTTTGGTAGATAC
chr10	1.25E+08	BUB3	1	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAGCGCTGTCAGGATTGCGAGAGACACTGACGAGTCGACTTGGACTGACCCCAGGAGGTTAAAAGCAGCAGCTTTAAAACGTTCTGGAAGCTGAGGCCAAGAATTGGCATGGTATCACTTTCCCTGCATTTTATTGGTTAAAGCAGGTTGCAGTCTCAGTCCAGATTCAAGAGGAGGGAACTACACAAAGGCCTGAATATGAGGAGG
chr11	6264195	CNGA4	1	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAGCGCTGTCAGGATTGCGAGAGATGTGTGTTGATACAGCCTTATGCTGAGGGCAGTAGGAAACTATTGAAGTATTTTAAGCAGGGAGCTGTATGATATCCTTTGCAGTTTAGAAAGATGTTGGTGGAGAATGGGTGGGGAGATAGGTGAGGCTGAATGCTGCAAAGTCTTAGAGCTAGAAGGACCTTGGACAAGGGAAAGGGAGCT
LM-PCR results of GFP insertion at *FBL* loci by TEd system				
Chr	start	gene	frequency	Validation Seq
chr19	40325195	FBL	374604	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAGCGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGTGTTTTTTCTATTAAAAGACTCATCCGTCTCCCATGTCTGCTGCTCATTCCTCCCCTTGACCTGCTGACACAGGGAGCACGCACCCTTGGTCAATTTTGCGGGGTTGGGTAAATTCTCACTCGGTCACAGAGCGCATGCTCCGTTTCTAGCTGCCTTTGA
chr1	63842205	ALG6	2	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCATGGATGGTACTCTAAAGAAAATGTATAGCTTAAATGATAGTGTTACAGAGACTGAAACTTAAGAGCTAAAGCATCCAATTTAGTAAATTTAAAAAAGAACAACCCCAAGGATATCACTGAGAGAATGGGCAAAGCTCAGATTAGTTATTTGAAAAATACGGCTATGTACCACAATAATGACATCTCAGTCAACAACGAACTGTAT
chr7	6078400	ANKRD61	2	GGCATGGACGAGCTGTACAAGTCCAAGTCGACTCGTCAGTGTAGTAGGCCTCTAGGGAAGTCATTAAACCTGTGTGGACTTCTCTTTGCAAGACAGAAGTGTCCTCAGGACACAGGCCCACCCAAGAAGCCCAAAGTCTAATGAAAGTGACAGATAAGGAGACTGATAATTAAAACCGGGTGTTGT

				AATACCAGCAGAAGGGGGGCCAGGCTGAAGTCAGGCAGGGGTCCA ATGGCAGAAGCTCTCAACT
chr12	1.06E+08	APPL2	2	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGTG TTTTTCTATTAAAAGACTCATCCGTCTCCCATGTCTGCTGCTCATTCC TCCCCTTGACCTGCTGACACAGGGAGCACATGTAAAGGAGTCATAG GCTAAGGTCAACATTTCTGGAATGAGTGTCCTGACAGGGGACGATGC ACAGGACAGGAGTGTG
chr4	94646574	ATOH1	2	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGTG TTTTTCTATTAAAAGACTCATCCGTCTCCCATGTCTGGAACAGTGCCGCA ATAAACATACATGTGCATGTGTCTTTATAGCAGCATGATTTATAATCCT TTGGGTATATACCCAGTAATGGGATGGCTGGGTCAAATGGTATTTCT AGTTCTAGATCCT
chr15	51601395	CYP19A1	2	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGTG TTTTTCTATTAAAAGACTCATCCGTCTCCCATGTCTGACACTGACGAG TCGACTTGGACTGACCTCTTCTTACAGAGGCACCAATCCAATTCATGT GGGCTCCACCCTCATGACCTAATCACCTCAAAAGGTCCCACCACATA ATACCATCACACTG
chr15	68132383	SKOR1	2	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAG CGCTGTCAGGATTGCGAGAGATGTGTGTTGATACTGTTGCACGTGTG TTTTTCTATTAAAAGACTCATCCGTCTCCCATGTCTGCCTAAAACTGG GGGGTGGCCTGTGAGAGCGGTGTTTCGTCCTTTCCACAAGATATATA AAGCCAAGAAATCGAAATACTTTCAAGTTACGGTAAGCATATGATAGT CCATTTTAAAACAT
chr6	1.64E+08	CAHM	1	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTGAT AAAATATACTTAAAAATTACCATATGACCATTTTAAAGTGTACAATTCA TTGGCATTAACTACATATACAATGTTGTGTAACCATCACCAACGATTT CCAGAACTTTGTCATTATCCCCAGCAGAAATCCTGAATACTTTAAACA GGAACTCCCAGCCCTCCCCTCCCCACAACATGAACAAGATTGATTAG GACTCTGTTCTT
chr17	32745159	CCL1	1	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAG CGCTGTCACACTGACGAGTCGACTTGGACTGACCTTTTCTTTGTTTTG CCTGCTTGTAATTCTTTTTTCCCCCTTTTTCACATAGTTGAGTTCATAC TGACTGTACAAATTCTCCATATCCTGATTTCACTCACACTATCATAAGT GTTTTCCATGTTATTACACGTACTTCATAAATGCTGTTCTGAAGGCAA CAAGCTCACT
chr11	65074588	CDC42EP2	1	GGCATGGACGAGCTGTACAAGCCCAAGGTGAAGAACTGAAGTTCAG CGCTGTCAGGATTGCGAGAGATGACACTGACGAGTCGGATCACCTTT TGTCGGATCTCACCAATGCGGTTGACTGTCAAGCACGCCTCGCTAAT TTTTGTATTTTTAGTAGAGACGGGTTTTCACCATGTTCCCCAGGCTGG TCTCGAACTCCTAACCTCAGCTGATCCACCCGCCTTGGCCTCCCAAA 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.