

Supplemental Materials for

A HIT-trapping strategy for rapid generation of reversible and conditional alleles using a universal donor.

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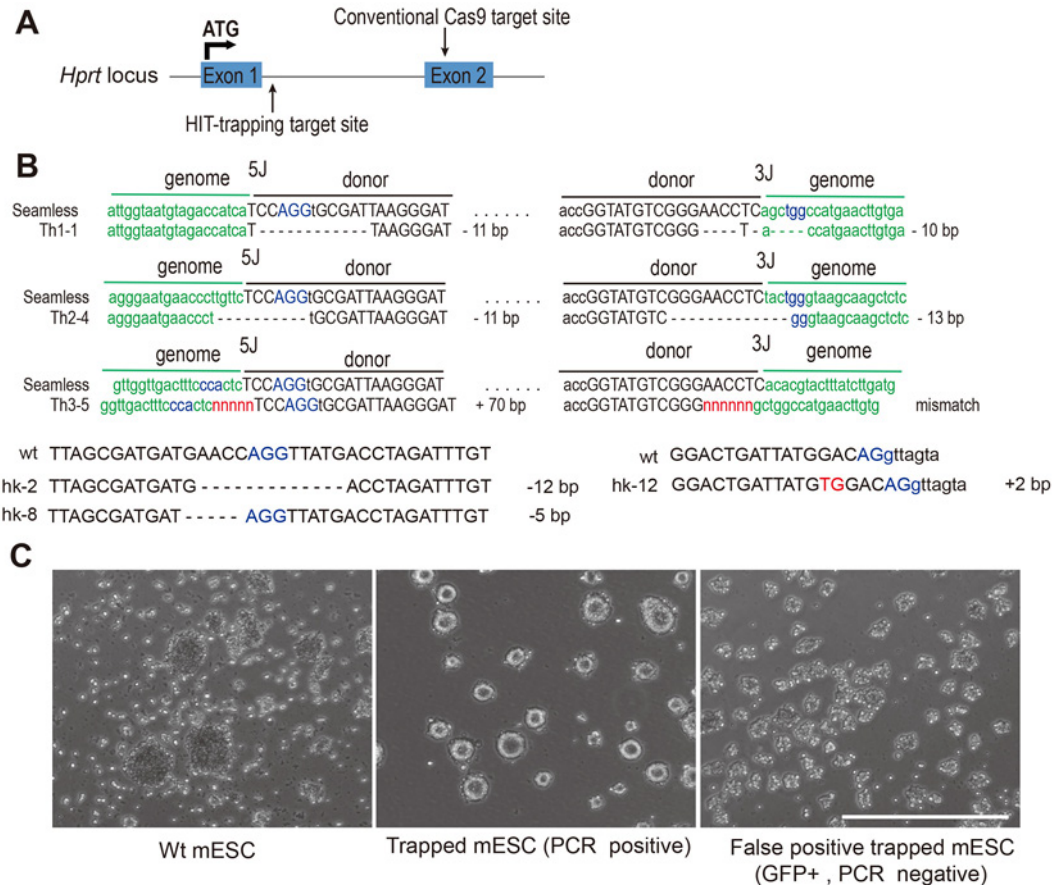
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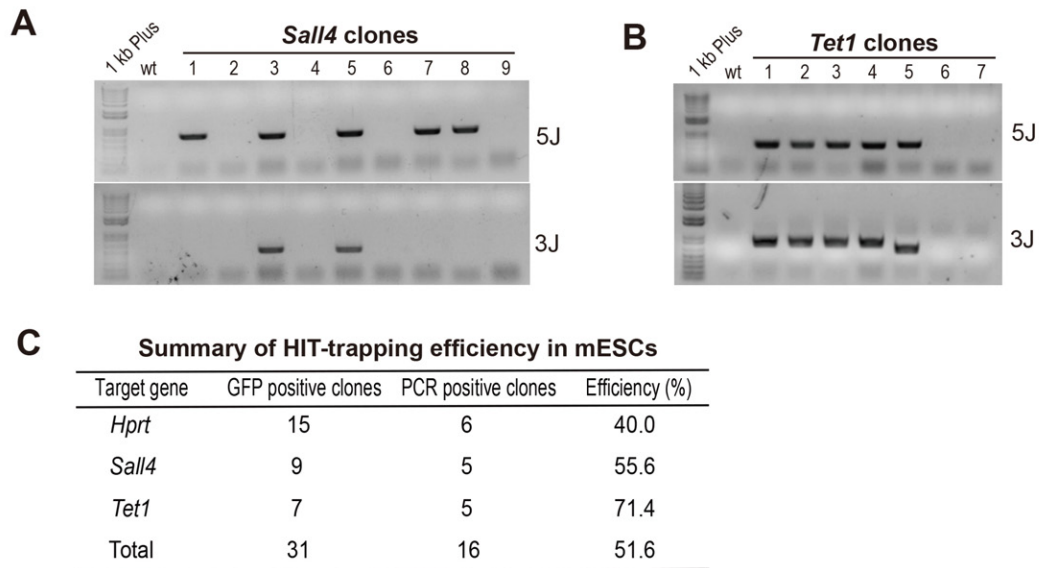
Supplemental Figures S1-S16

Supplemental Tables S1-S8

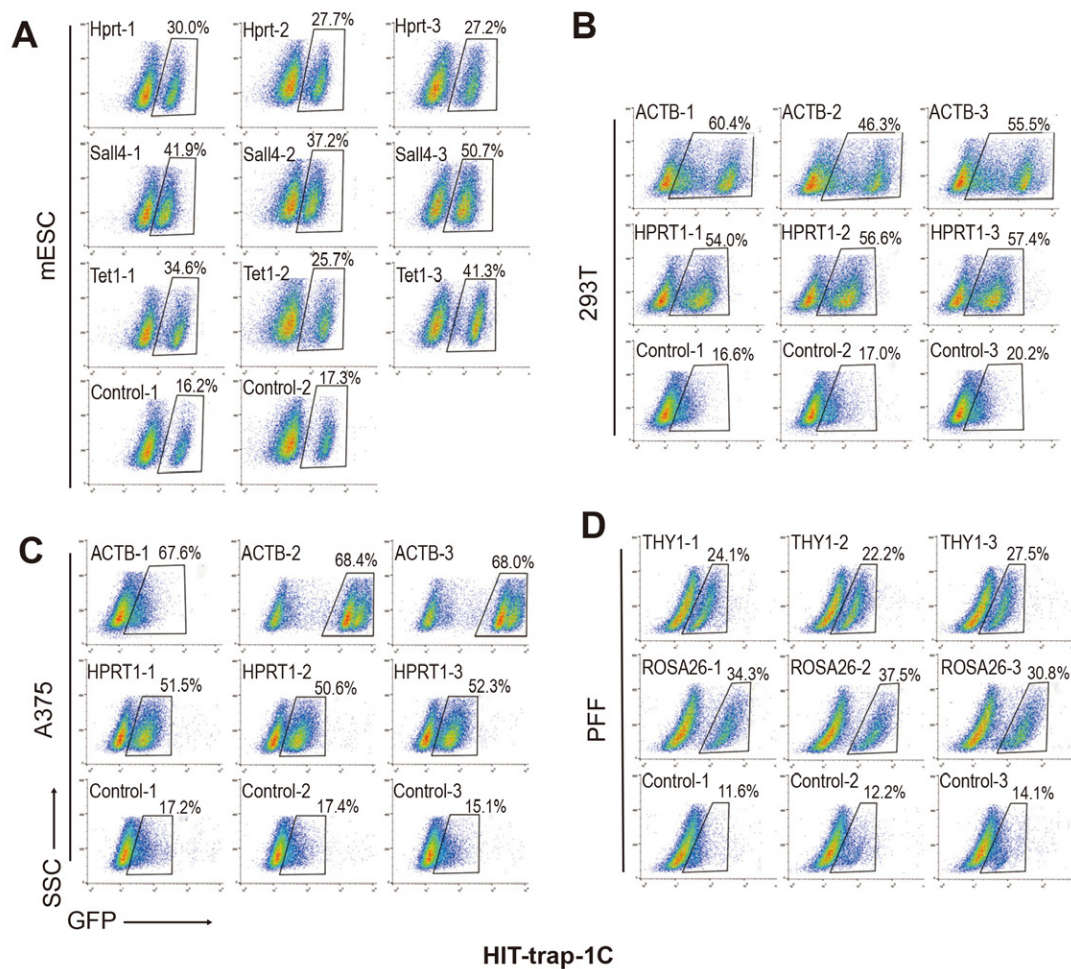


Supplemental Fig S1. Knock-out the *Hprt* gene in mESCs by HIT-trapping.

(A) Schematic showing the HIT-trapping target sites (within intron) and conventional Cas9 target site (within exon) selected to knock-out the *Hprt* gene. (B) Sequences of the HIT-trap clones and indel clones selected. Genome sequences are shown in green. PAM sequences are shown in blue. Insertions or mismatches are shown in red. Deletions are shown as dashed lines. 5/3J, 5'/3' junction. (C) Confirming phenotype of the HIT-trap clones generated by HIT-trapping. Only the PCR positive clones (middle) display resistance to 6-TG, indicating loss-of-function of the *Hprt* gene. Scale bar, 50 μ m.

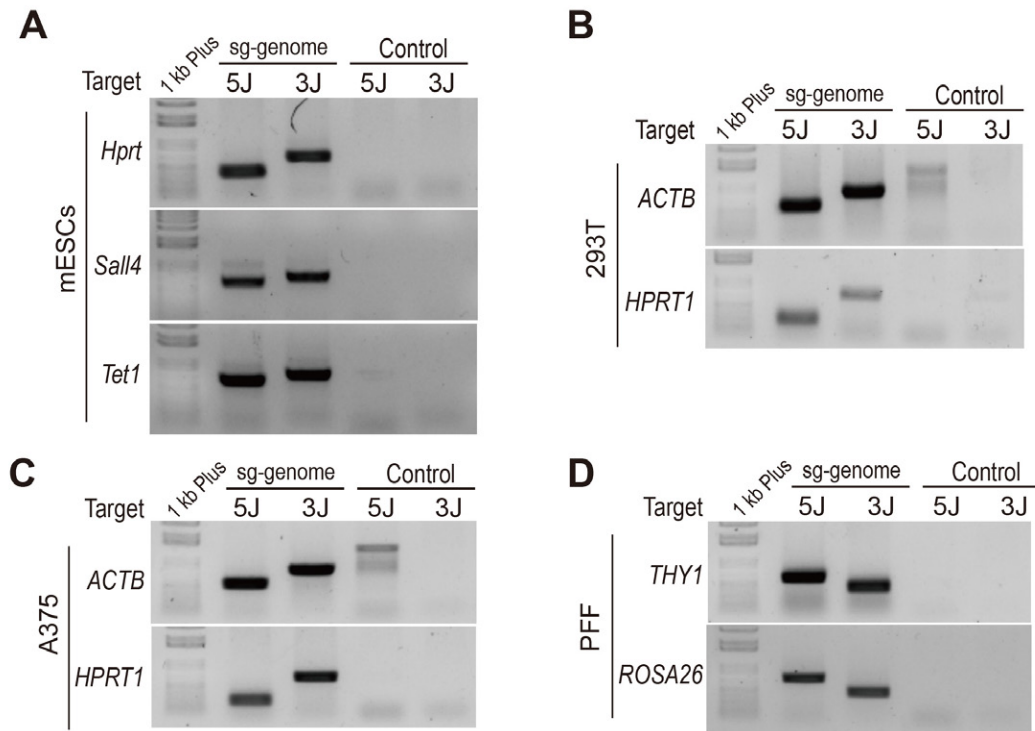


Supplemental Fig S2. Efficient HIT-trapping in mESCs. (A, B) PCR genotyping of GFP-positive clones confirmed the correct integration of HIT-trap donors at the *Sall4* and the *Tet1* loci. 5/3J, 5'/3' junction. (C) Summary of HIT-trapping efficiency in mESCs.

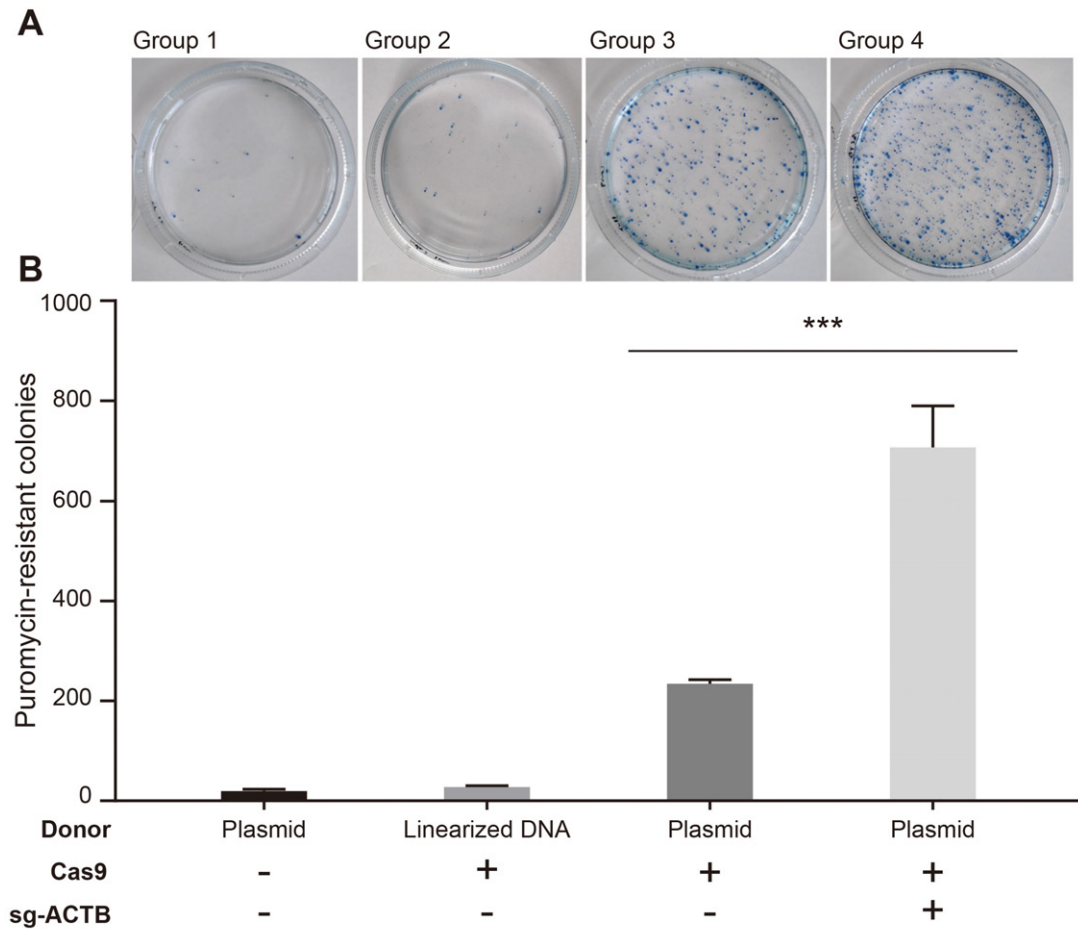


Supplemental Fig S3. Assessment of HIT-trapping efficiency via FACS in various mammalian cells. (A-D) FACS results showing the percentages of GFP-positive cells

produced in Fig. 2B.



Supplemental Fig S4. PCR genotyping confirming the targeted insertions in HIT-trapping. Genome PCR analysis of puromycin-resistant cells produced in Fig. 2B. For each target site, PCR amplification was carried out to confirm the specific genome-donor junction. 5/3J, 5'/3' junction.



Supplemental Fig S5. CRISPR-Cas9 mediated efficient integration of the HIT-trap donor. (A) Puromycin-resistant cells produced in Fig. 2B targeting *ACTB* (group 3 and 4) were stained with methylene blue to identify viable cell colonies. Group 1 and 2 are negative controls (without cutting the donor via CRISPR-Cas9 *in vivo*). For each transfection, approximately 5×10^5 A375 cells were transfected with the corresponding DNA mixture and seeded onto a 100 mm plate. Plasmid, HIT-trap-1C; Linearized DNA, *in vitro* linearized plasmid via *NedI* that disrupted the U6 promoter to abolish sgA expression. (B) Quantification of the number of puromycin-resistant colonies. Data are displayed as the mean $\pm$ s.d of three independent experiments. Significance was calculated using the Student's unpaired *t*-test: *** $P < 0.001$.

A

genome 5J donor

Seamless	attggtaatgtagaccatcaTCCAGG	IGCGATTAAGGGAT	
x 21	attggtaatgtagaccatcaTCCAGG	IGCGATTAAGGGAT	seamless
x 5	attggtaatgtagaccatc- - -	CAGGIGCGATTAAGGGAT	MMEJ, - 3,
x 3	attggtaatgtaga- - - - -	CCAGGIGCGATTAAGGGAT	MMEJ, - 7,
x 2	attggtaatgtagac- - - ca	TCCAGGIGCGATTAAGGGAT	MMEJ, - 3,
x 2	attggtga- - - - - - - a	TCCAGGIGCGATTAAGGGAT	MMEJ, - 12,
x 2	attggtaatgtagacca- - - - -	GGIGCGATTAAGGGAT	MMEJ, - 7,
	attggtaatgtagaccat- - - -	CAGGIGCGATTAAGGGAT	MMEJ, - 4,
		IGCGATTAAGGGAT	MMEJ, - 37,
	attggtGatgtaga- - - - -	CCAGGIGCGATTAAGGGAT	MMEJ, - 7,
	attggtaatgtagaccat- - - -	TCCAGGIGCGATTAAGGGAT	- 2
x 3	attggtaatgtagaccatca- - -	AGGIGCGATTAAGGGAT	- 3
	attggtaatgtag- - - - -	TCCAGGIGCGATTAAGGGAT	- 7
	attggtaatgtagaccat- - - - -	GIGCGATTACCGAAT	- 7
	attggtaatgtga- - - - -	CCAGGIGCGATTAAGGGAT	- 10
	attggtgaatg- - - - -	TCCAGGIGCGATTAAGGGAT	- 10
	attggtaatgt- - - - -	CCAGGIGCGATTAAGGGAT	- 10
	attggtaatgtagaccatca- - - -	GGGAT	- 15
	attggtaatgtagaccatca- - - - -		- 23
		TCCAGGIGCGATTAAGGGAT	- 28
	attg- - - - -	TAAGGGAT	- 28
x 2	attggtaatgtaga- - - - -		- 54
	attggtaatgtagaccatcaATCCAGG	IGCGATTAAGGGAT	+ 1
	attggtaatgtagaccatcaTCTCCAGG	IGCGATTAAGGGAT	+ 2
	attggtaatgtagaccaAATCcaTCCAGG	IGCGATTAAGGGAT	+ 3
x 3	attggtaatgtagaccatcaATCGCATCCAGG	IGCGATTAAG	+ 6
	attggtaatgtagaccatcaTTACAGG	IGCGATTAAGGGAT	+ 1, m 1
	attggtaatgtgaCCgGTaTGTGGGcaAcaTCCAGG	IGCG	+ 9, m 3
x 2	attgTACCCGtgatgCgGGaAcTACcaA	CCAGGIGCGATTA	indels
	attggtaatgtagaccaATTAcaTACCGAA	C - ATTAAGGGAT	indels
	attggtaatgtagaccat- - - - -	G-IGCGATTAACCGAA	indels

Seamless		accGGGTATGTCGGGAACCTCagctggccatgaactgtgga	seamless
ATC	x 22	accGGGTATGTCGGGAACCTCagctggccatgaactgtgga	MMEJ, - 12, GG
CC	x 3	accGGGTATGTCGGG ----- ccatgaactgtgga	MMEJ, - 27, TGT
CAT	x 3	accGGGTAT ----- tigtga	MMEJ, - 4, CT
AT		accGGGTATGTCGGGAACCT - - - tggccatgaactgtgga	MMEJ, - 5, CT
CA		accGGGTATGTCGGGAAC - - - ctggccatgaactgtgga	MMEJ, - 23, CAG
TG		accGGGTATGTCGGGAACCTCag ----- cccatgaactgtgga	MMEJ, - 42, CTC
CC, m 1		accGGGTATGTCGGGAACCTCag ----- cccatgaactgtgga	- 3
		accGGGTATGTCGGGAACCTCag ----- cccatgaactgtgga	- 4
		accGGGTATGTCGGGAACCTC - - - tggccatgaactgtgga	- 6
		accGGGTATGTCGGGAACCTC - - - ggccatgaactgtgga	- 10
		accGGGTATGTCGGGAACCTC - - - ctggccatgaactgtgga	- 12
		accGGGTATGTCGGGAACCTC - - - gaactgtgga	- 17
	x 2	accGGGTAT ----- gccatgaactgtgga	- 103
	x 3	----- agctggccatgaactgtgga	- 121
		----- agctggccatgaactgtgga	- 265
		accGGGTATGTCGGGAACCTC -----	- 279
		accGGGTATGTCGGGAACCTCagctggccatgaactgtgga	+ 1
		accGGGTATGTCGGGAACCTCTAGagctggccatgaact	+ 3
		accGGGTATGTCGGGAACCTC nnnn agctggccatgaact	+ 80
		accGG ----- nnnnn ----- tigtga	- 26, m 5
		accGGGTATGTCGGT ----- catgaactgtgga	- 13, m 1
		accGGGTATGT - - - A ----- catgaactgtgga	- 16, m 1
		accGGGTATGTCGG nnnnnnnnnnnnnn ccatgaactgtgga	+ 2, m 13
	x 2	accGGGTATGTC nnnnnnnnnnnn gctggccatgaactgtgga	+ 8, m 10
		accGGGTATGTCG ----- gctggccatgaactgtgga	+ 34, m 9
		a nnnnnnnnnnnnnnnnnnnnnnn agctggccatgaactgtgga	+ 35, m 19
		accGGGTATGTCGG nnnnnnnnnn gctggccatgaactgtgga	+ 36, m 8
		accGGGTATGTCGG nnnnnnnnnn gctggccatgaactgtgga	+ 36, m 7
		ATGTCGGGAACCTC nnnnc - - tggccatgaactgtgga	+ 41, m 2
		nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn cttgtga	indels
		accGGGTATGTCGG - TACCTCTAGagctggccatgaact	indels

B

genome ^{5J} donor

	Seamless	Seamless	Seamless
	cggccttgagtggtgatt	TCCAGG	ICGATTAAAGGGAT
x 53	cggccttgagtggtgatt	TCCAGG	ICGATTAAAGGGAT
	cggccttgagtggtg -		TAAGGGAT
	cggccttgagtggtgatt -		AGGGAT
	cggccttgagtggtgatt	CCAGG	ICGATTAAAGGGAT
	cggccttgagtggtgatt -	CAGG	ICGATTAAAGGGAT
	cggccttgagtggtg -		CGATTAAAGGGAT
	cggccttgagtggtg -		AT
	cggccttgagtggtgatt -		
	cggccttgagtggtgatt	TCCAGG	ICGATTAAAGGGAT
x 2	cggccttgagtggtgatt	TCCAGG	ICGATTAAAGGGAT
	cagccttgaagtgtatG -	TCCAGG	ICGATTAAAGGGAT

		3J		
		donor	genome	
Seamless		accGGTATGTCGGGAACCTC	<u>agtaggtgcacagtaggtct</u>	
	x 61	accGGTATGTCGGGAACCTC	<u>agtaggtgcacagtaggtct</u>	seamless
TA		accGGTATGTCGGGAAC	-----	MMEJ, - 27, AAC
TA		acc-----	-----ct	- 35
		accGGTATGTCGGGA	-----	- 73
		accGGTATGTCGGGAACCTC	<u>Aagtaggtgcacagtaggtct</u>	+ 1
		accGGTATGTCGGGAACCTC	<u>TAagtaggtgcacagtaggtct</u>	+ 2

Supplemental Fig S6 (continued)

C

PFF, *THY1*

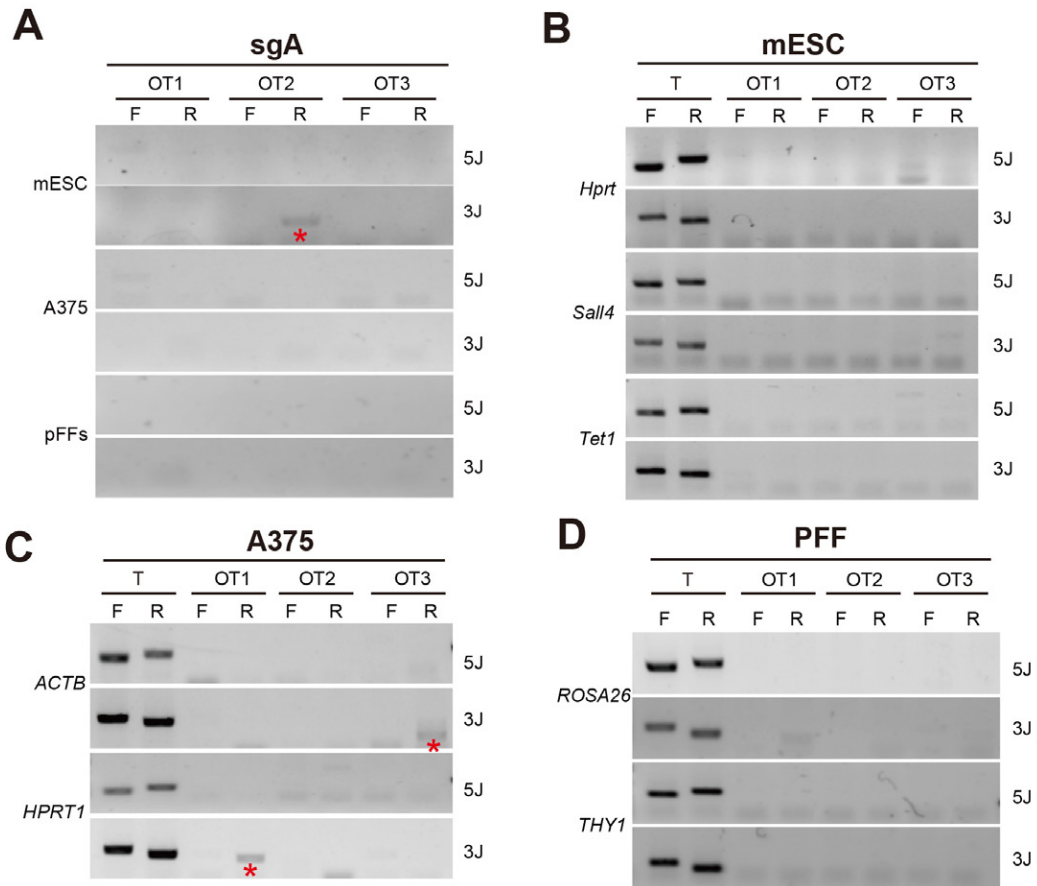
genome		5J	donor	donor		3J	genome
Seamless	tgatgatactgttcaaggg	TCCAGGtGCGATTAAGGGAT		Seamless	accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg	
x 52	tgatgatactgttcaaggg	TCCAGGtGCGATTAAGGGAT		seamless	x 58	accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	TCCAGGtGCGATTAAGGGAT				accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	TCCAGGtGCGATTAAGGGAT	MMEJ, - 7, TC			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	TCCAGGtGCGATTAAGGGAT	MMEJ, - 18, AA			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	TCCAGGtGCGATTAAGGGAT	MMEJ, - 113, TTC			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
x 2	tgatgatactgttcaaggg	- CAGGtGCGATTAAGGGAT	- 2			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	- gTCCAGGtGCGATTAAGGGAT	- 2			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	- gTCCAGGtGCGATTAAGGGAT	- 3			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	- - AGGtGCGATTAAGGGAT	- 3			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	- C - - - - - TTAAGGGAT	- 10			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	- gTCCAGGtGCGATTAAGGGAT	- 21			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	- - A - - - - - - - - - -	- 24			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	nnnn TCCAGGtGCGATTAAGGGAT	+ 175			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	- ATCCAGGtGCGATTAAGGGAT	- 2, m 1			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	TCCAGGtGCGATTAAGGGAT	m 1			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnn	+ 15, m 20			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnn	+ 171, m 33			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg
	tgatgatactgttcaaggg	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnn	+ 450, m 14			accGGTATGTCGGGAACCTC	ctcgggaactggcaaatggg

D

Summary of sequencing data

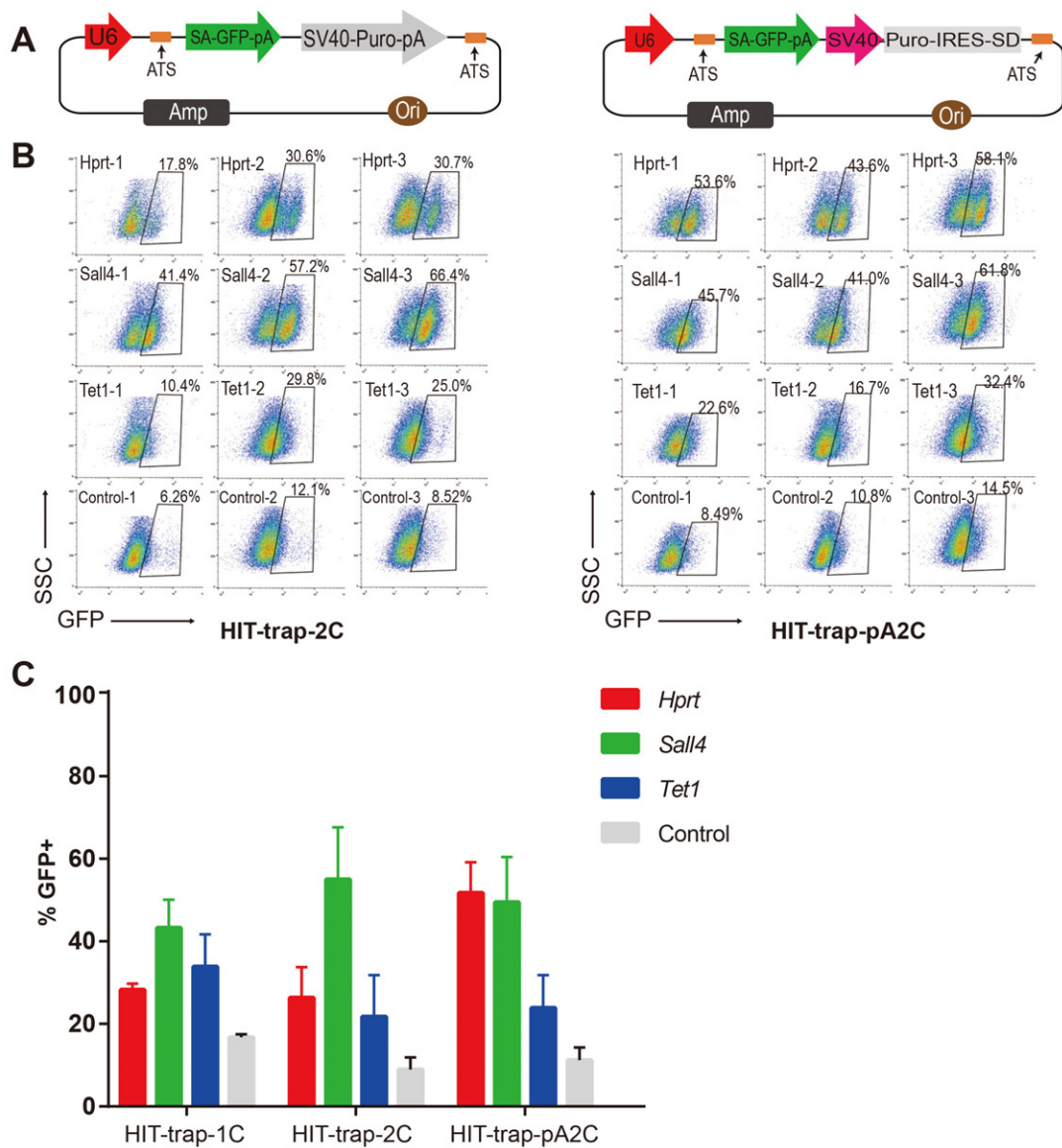
Cell type	Target	Junction	Seamless (%)	MMEJ (%)	Random deletion (%)	Insertion (%)	Complex (%)	n
mESC	<i>Hprt</i>	5J	21 (32.3)	17 (26.2)	15 (23.1)	6 (9.2)	6 (9.2)	65
		3J	22 (36.1)	10 (16.4)	13 (21.3)	3 (4.9)	13 (21.3)	61
A375	<i>ACTB</i>	5J	53 (82.8)	2 (3.1)	5 (7.8)	3 (4.7)	1 (1.6)	64
		3J	61 (92.4)	1 (1.5)	2 (3.0)	2 (3.0)	0 (-)	66
PFF	<i>THY1</i>	5J	52 (75.4)	3 (4.3)	8 (11.6)	1 (1.4)	5 (7.2)	69
		3J	58 (89.2)	2 (3.1)	2 (3.1)	1 (1.5)	2 (3.1)	65

Supplemental Fig S6. Junction assay of HIT-trapping. (A-C) Sequencing data of the corresponding samples in Fig. 2C. Genome sequences are shown in green. PAMs are shown in blue. Insertions or mismatches are shown in red. Deletions are shown as dashed lines. Microhomologies are shown in purple. MMEJ, microhomology-mediated end joining; m, mismatches; 5/3J, 5'/3' junction. (D) Summary of sequencing data.

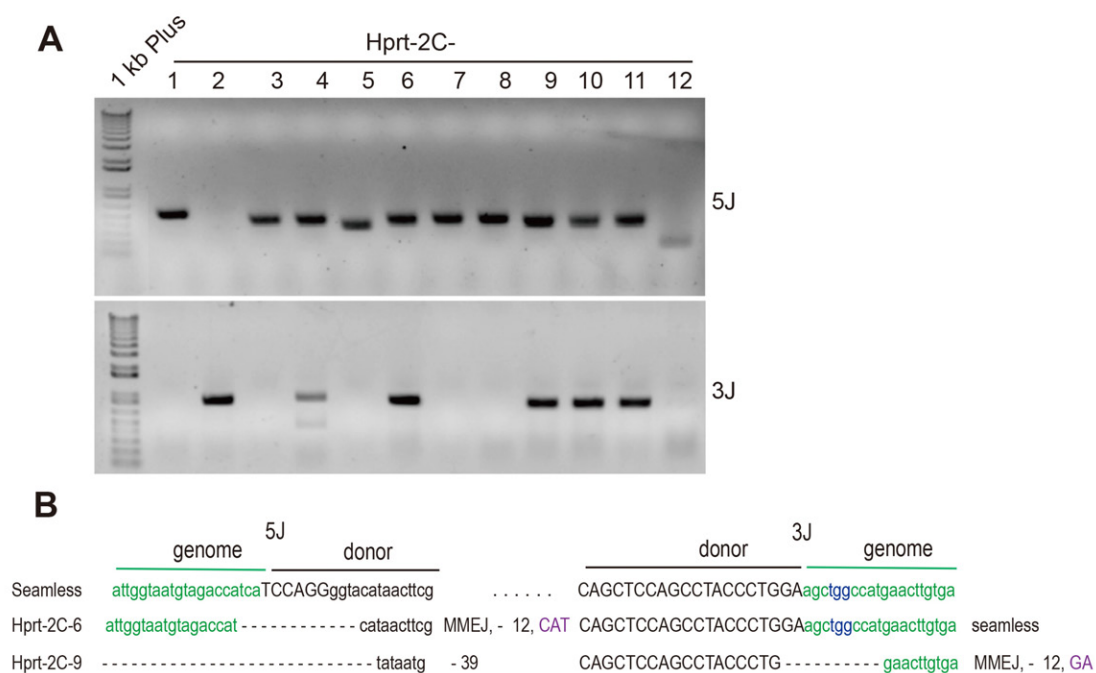


Supplemental Fig S7. Detection of off-target insertions in HIT-trapping.

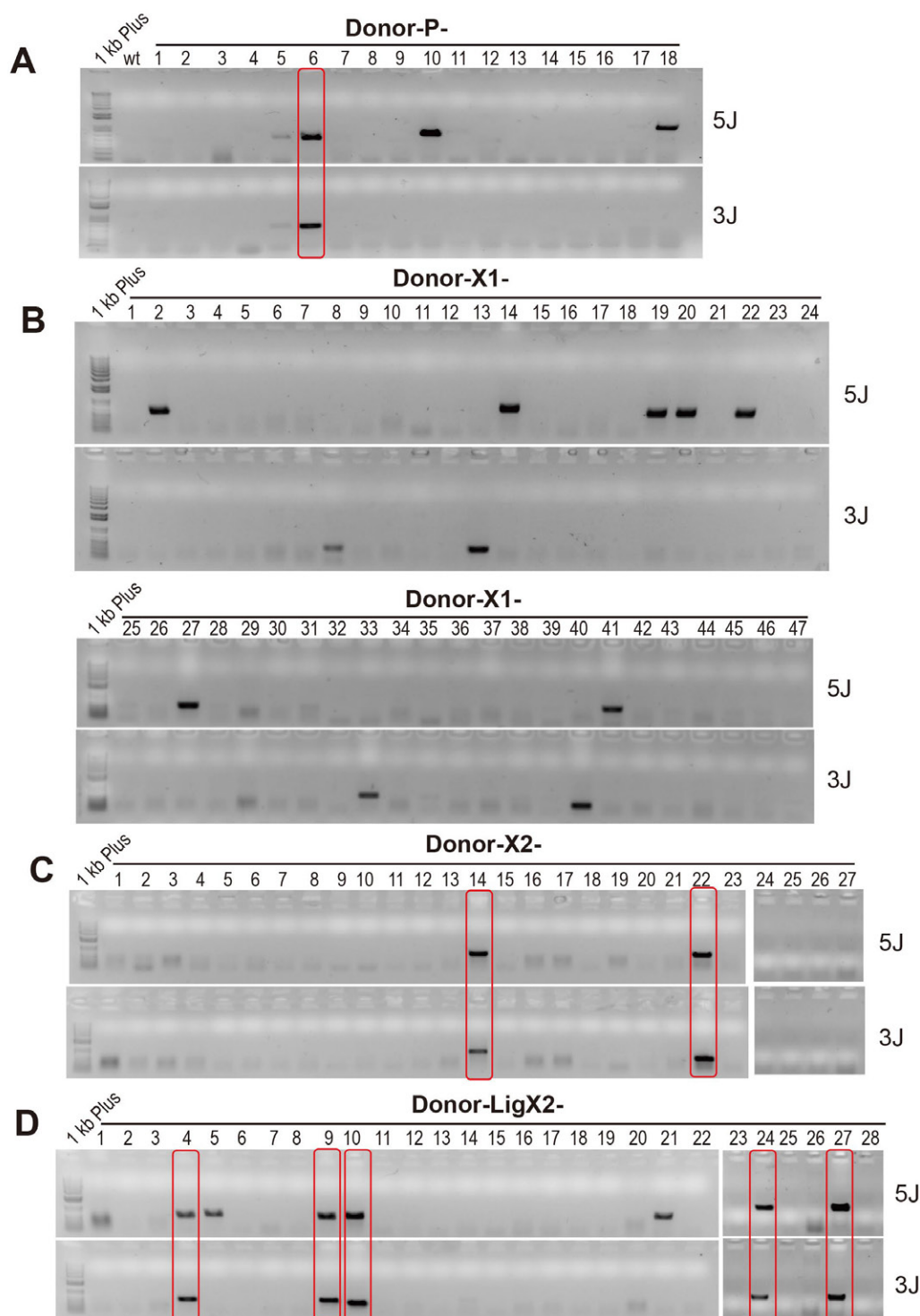
(A-D) PCR amplifications were carried out to detect the donor insertions at the top three potential off-target sites. Red asterisk indicates weak signals of off-target insertions (faint positive PCR bands). Potential off-target sites for sgRNAs targeting HIT-trap donor (sgA) and genome are listed in Supplemental Table S2. Genome-specific primers used are listed in Supplemental Table S5. F/R, F/R, forward/reverse integration of the donor; 5/3J, 5'/3' junction.



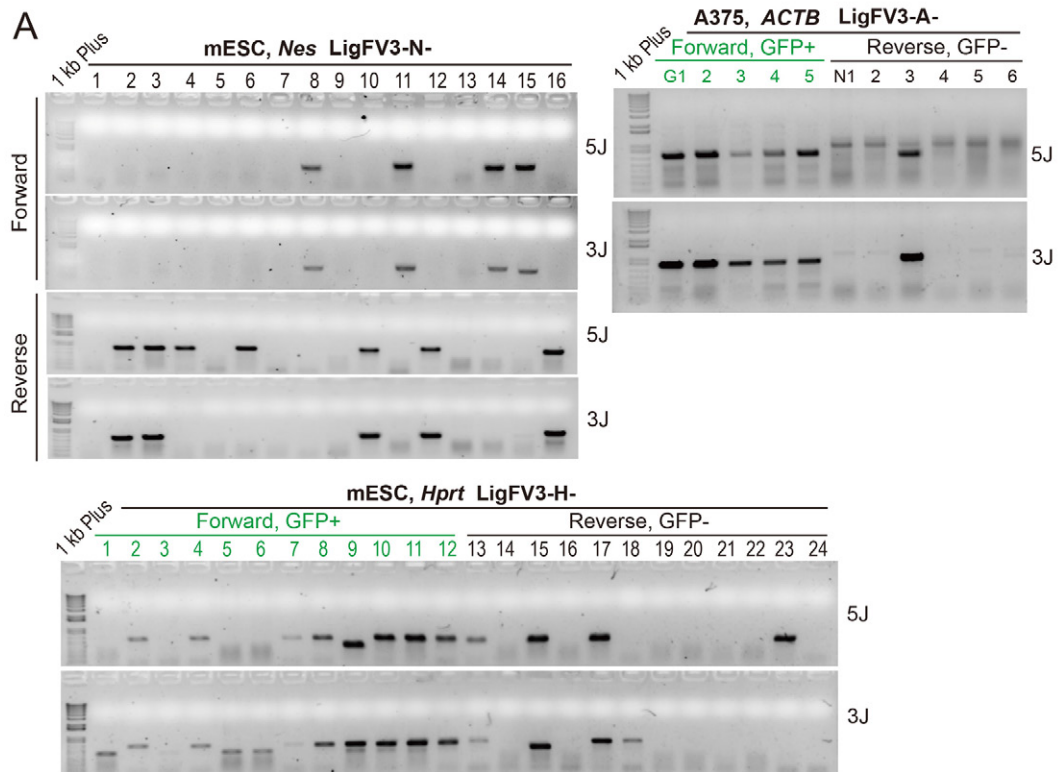
Supplemental Fig S8. Assessment of HIT-trapping efficiency by two-cut HIT-trap donors. (A) Schematic showing construct of HIT-trap-2C and HIT-trap-pA2C (upper panel). (B) FACS results showing the percentages of GFP-positive cells in the various groups (lower panel). (C) Summary of HIT-trapping efficiency by the various HIT-trap donors.



Supplemental Fig S9. Efficient knock-in without plasmid backbone at the *Hprt* locus by a two-cut HIT-trap donor. (A) PCR genotyping of screened clones by targeted trapping of the *Hprt* locus in mESC using donor HIT-trap-2C. (B) Sequencing confirmed removal of the vector backbone. Genome sequences are shown in green. PAMs are shown in blue. Deletions are shown as dashed lines. Microhomologies are shown in purple. 5/3J, 5'/3' junction; MMEJ, microhomology-mediated end joining.



Supplemental Fig S10. Detection of the desired knock-in pattern generated by different optimized HIT-trap donors. (A-D) PCR genotyping results of the puromycin-resistant clones by respective optimized HIT-trap donors. The lanes in red rectangles indicate desired knock-in events. 5/3J, 5'/3' junction.



B

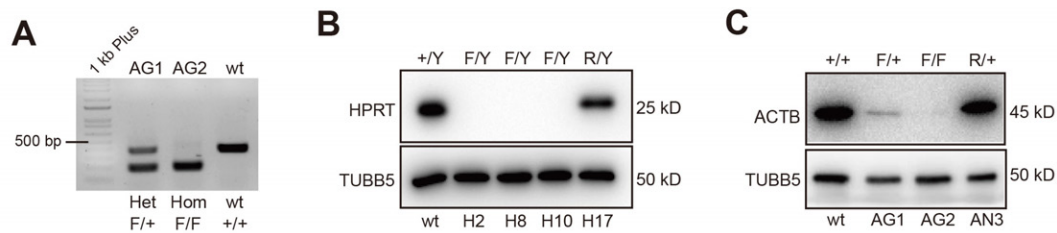
Nes. Forward:

Seamless	5'-gctttcctccaagcctctTCCAGGactcattttctag-3'	Seamless	5'-TCGGGTATGTCGGGAACCTCgaggcgactcttgataaat-3'
LigFV3-N8	5'-agcct-----nnnGGACCAGGactcattttctag-3'	LigFV3-N8	5'-CTATAGAG-----ttctgttcca-3'
N14	5'-gctttcctccaagcctctTCCAGGactcattttctag-3'	N14	5'-TCGGGTATGTCGGGAACCTCgaggcgactcttgataaat-3'
	- 40, m 15 seamless.		- 63 Seamless.

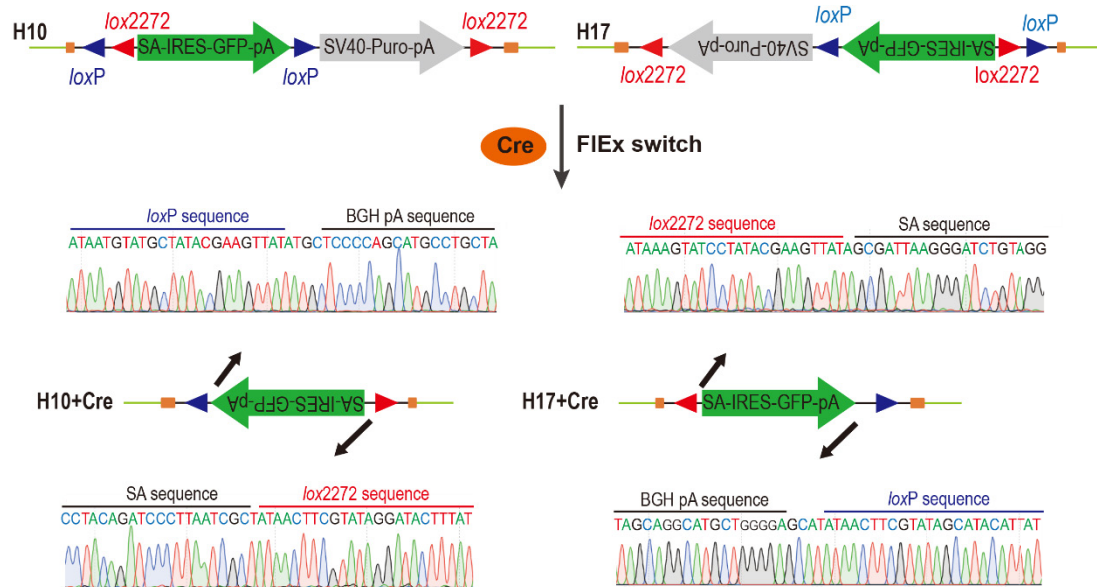
Nes. Reverse

Seamless	5'-gctttcctccaagcctctGAGGTTCCCGACATACCCGA-3'	Seamless	5'-ctagaaaatgaagtCCTGGAcaggcgactcttgataaat-3'
N3	5'-gctttcctccaagcctctGAGGTTCCCGACATACCCGA-3'	N3	5'-ctagaaaatgaagtCCTGGAcaggcgactcttgataaat-3'
N10	5'-gctttcctccaagcctctGAGGTTCCCGACATACCCGA-3'	N10	5'------Acaggcgactcttgataaat-3'
	seamless.		Seamless.
	seamless.		- 51

Supplemental Fig S11. Assessment of knock-in efficiency of the optimized donor LigFV3. (A) PCR genotyping results of respective samples in Fig. 5C. (B) Sequencing confirmation of the genome-donor junctions in the trapped clones. Genome sequences are shown in green. PAMs are shown in blue. Insertions or mismatches are shown in red. Deletions are shown as dashed lines. Microhomologies are shown in purple. 5/3J, 5'/3' junction; MMEJ, microhomology-mediated end joining; m, mismatches.

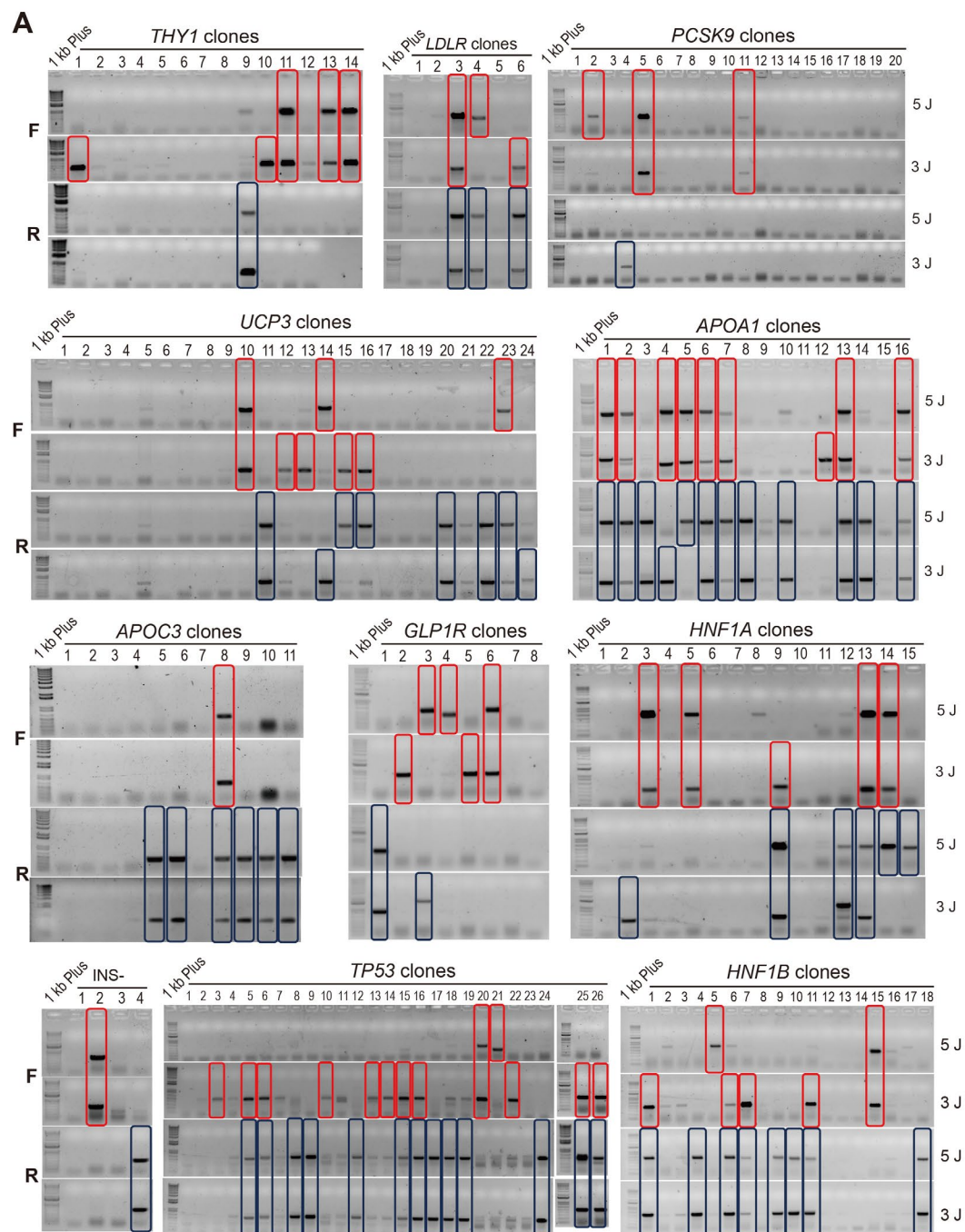


Supplemental Fig S12. Knockouts depend on the insertion orientation of the gene-trap. (A) Allele-specific PCR confirms the genotype of the representative *ACTB* clones in Fig. 5C. (B, C) Western blot analysis of the protein expression in clones bearing F/Ex-trap insertions at the *Hprt* locus in mESCs and *ACTB* locus in A375 cells, with TUBB5 as the loading control. Y, Y Chromosome (*Hprt* locus is located on the X Chromosome); F/R, forward/reverse integration of the donor.

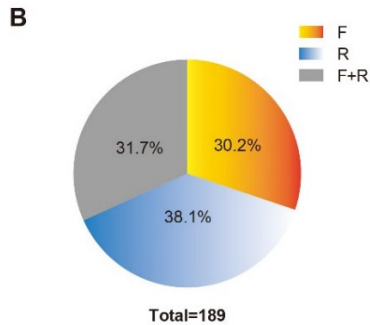
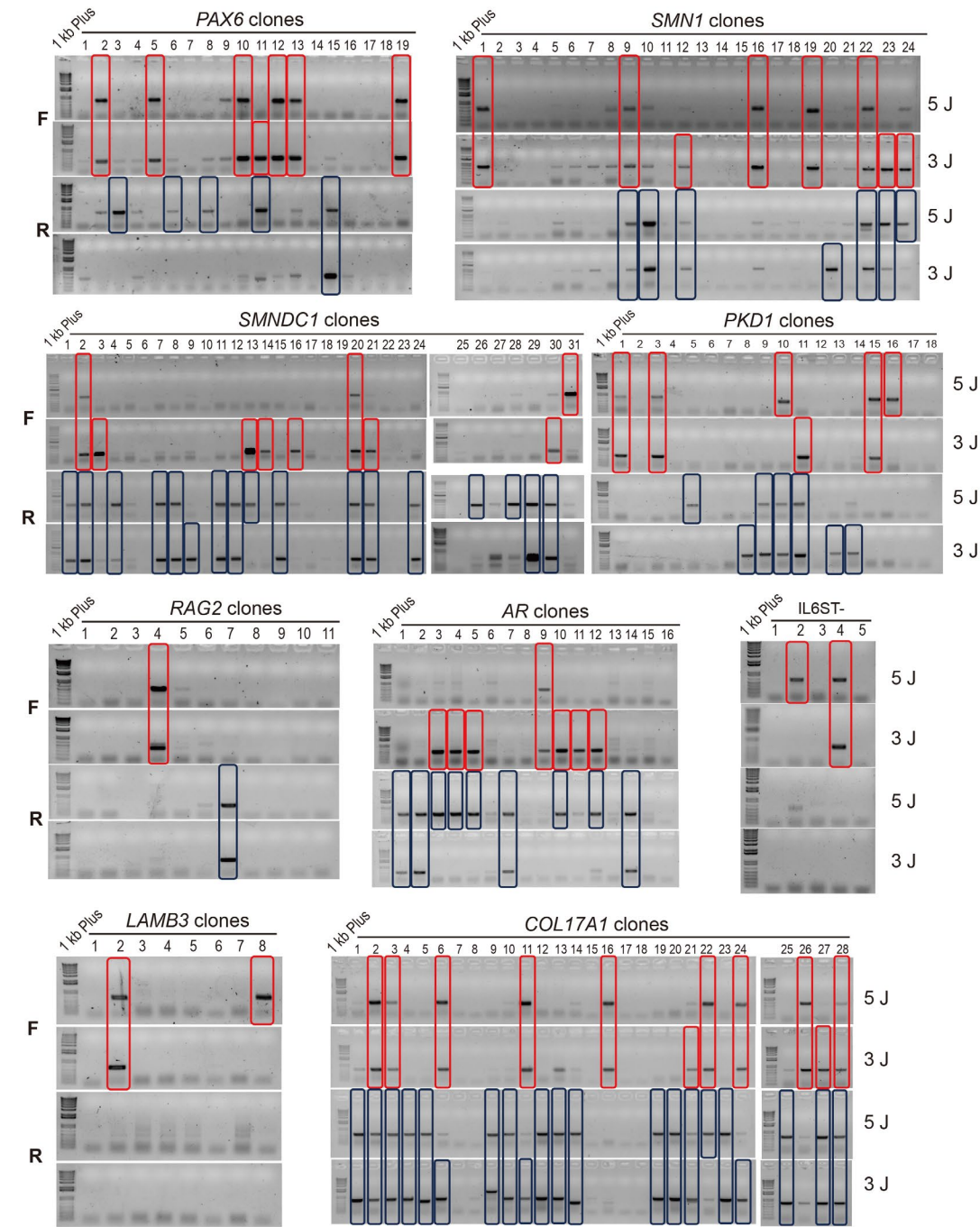


Supplemental Fig S13. Confirmation of the F/Ex switch at the *Hprt* locus by sequencing. Sequencing confirms the new genome-donor junctions created upon Cre-mediated F/Ex switch.

Supplemental Fig S14



Supplemental Fig S14 (continued)



Supplemental Fig S14. Supplementary PCR genotyping results of respective samples in Table 1. (A) Supplementary PCR genotyping results of PFF clones produced via

Supplemental Fig S15

THY1 Forward

THY1 Forward			THY1 Reverse		
genome	5J	donor	genome	5J	donor
Seamless	5'-tgatgatactgttcaagggtccaggaactcattcttagt-3'		Seamless	5'-tgatgatactgttcaagggtccaggaactcattcttagt-3'	
THY1-11	5'-tgatgatactgttcaagggnnnnnTCCAGGactcattcttagt-3'	+ 22	THY1-9	5'-tgatgatactgttcaagggtccaggaactcattcttagt-3'	seamless
THY1-13	5'-tgatgatactgttcaagggtccaggaactcattcttagt-3'	seamless	THY1-13	5'-tgatgatactgttcaagggtccaggaactcattcttagt-3'	seamless
THY1-14	5'-tgatgatactgttcaagggtccaggaactcattcttagt-3'	seamless	THY1-14	5'-tgatgatactgttcaagggtccaggaactcattcttagt-3'	seamless
LDLR Forward			LDLR Reverse		
genome	5J	donor	genome	5J	donor
Seamless	5'-tatactctttgcatctccatccaggaactcatttcttagt-3'		Seamless	5'-tatactctttgcatctccatccaggaactcatttcttagt-3'	
LDLR-3	5'-tatactctttgcatctccatccaggaactcatttcttagt-3'	seamless	LDLR-3	5'-tatactctttgcatctccatccaggaactcatttcttagt-3'	seamless
PCSK9 Forward			PCSK9 Reverse		
genome	5J	donor	genome	5J	donor
Seamless	5'-ttggacgagcggccaccccccTCCAGGactcatttcttagt-3'		Seamless	5'-ttggacgagcggccaccccccTCCAGGactcatttcttagt-3'	
PCSK9-5	5'-ttggacgagcggccaccccccTCCAGGactcatttcttagt-3'	seamless	PCSK9-5	5'-ttggacgagcggccaccccccTCCAGGactcatttcttagt-3'	seamless
UCP3 Forward			UCP3 Reverse		
genome	5J	donor	genome	5J	donor
Seamless	5'-cccccaactcagctccttactTCCAGGactcatttcttagt-3'		Seamless	5'-cccccaactcagctccttactTCCAGGactcatttcttagt-3'	
UCP3-10	5'-cccccaactcagctccttactTCCAGGactcatttcttagt-3'	seamless	UCP3-10	5'-cccccaactcagctccttactTCCAGGactcatttcttagt-3'	seamless
APOA1 Forward			APOA1 Reverse		
genome	5J	donor	genome	5J	donor
Seamless	5'-ctctcgtgtctatctgtgtgTCCAGGactcatttcttagt-3'		Seamless	5'-ctctcgtgtctatctgtgtgTCCAGGactcatttcttagt-3'	
APOA1-5	5'-ctctcgtgtctatctgtgtgTCCAGGactcatttcttagt-3'	seamless	APOA1-5	5'-ctctcgtgtctatctgtgtgTCCAGGactcatttcttagt-3'	seamless
APOC3 Forward			APOC3 Reverse		
genome	5J	donor	genome	5J	donor
Seamless	5'-accgcgcttgccggcgctTCCAGGactcatttcttagt-3'		Seamless	5'-accgcgcttgccggcgctTCCAGGactcatttcttagt-3'	
APOC3-8	5'-accgcgcttgccggcgctTCCAGGactcatttcttagt-3'	seamless	APOC3-8	5'-accgcgcttgccggcgctTCCAGGactcatttcttagt-3'	seamless

Supplemental Fig S15 (continued)

GLP1R Forward

	genome	5J	donor	
Seamless	5'-attccctgtgctcctcggg	TCCAGG	acttcattttctag-3'	
GLP1R-3	5'-ctcctcgtctatctggtcg	TCCAGG	acttcattttctag-3'	seamless
GLP1R-4	5'-attccctgtgctcctcggg	TCCAGG	acttcattttctag-3'	seamless
GLP1R-6	5'-attccctgtgctcctcggg	TCCAGG	acttcattttctag-3'	seamless

	donor	3J	genome	
Seamless	5'-TCGGGTATGTCGGGAACCTC	agcagg	gacctggcctttcc-3'	

GLP1R Reverse

	genome	5J	donor	
Seamless	5'-attccctgtgctcctcggg	GAGGTTCCCGACATACCCGA-3'		
GLP1R-1	5'-attccctgtgctcctcggg	GAGGTTCCCGACATACCCGA-3'		seamless

	donor	3J	genome	
Seamless	5'-ctagaaaatgaagtCCTGGA	agcagg	gacctggcctttcc-3'	

HNF1A Forward

	genome	5J	donor	
Seamless	5'-gcagggtcctgagtcctccat	TCCAGG	acttcattttctag-3'	
HNF1A-3	5'-gcagggtcctgagtcctccat	TCCAGG	acttcattttctag-3'	
HNF1A-5	5'-gcagggtcctgagtcctccat	TCCAGG	acttcattttctag-3'	seamless
HNF1A-13	5'-gcagggtcctgagtcctccat	TCCAGG	acttcattttctag-3'	seamless
HNF1A-14	5'-gcagggtcctgagtcctccat	TCCAGG	acttcattttctag-3'	seamless

	donor	3J	genome	
Seamless	5'-TCGGGTATGTCGGGAACCTC	gagctaaggcgttcg	CCCG-3'	
HNF1A-3	5'-TCGGGTATGTCGGGAACCTC	gagctaaggcgttcg	CCCG-3'	seamless
HNF1A-5	5'-TCGGGTATGTCGGGAACCTC	gagctaaggcgttcg	CCCG-3'	seamless

HNF1A Reverse

	genome	5J	donor	
Seamless	5'-gcagggtcctgagtcctccat	GAGGTTCCCGACATACCCGA-3'		
HNF1A-9	5'-gcagggtcctgagtcctccat	-----GA-3'	MMEJ, -18	
HNF1A-13	5'-gcagggtcctgagtcctccat	GAGGTTCCCGACATACCCGA-3'		seamless
HNF1A-14	5'-gcagggtcctgagtcctccat	GAGGTTCCCGACATACCCGA-3'		seamless

	donor	3J	genome	
Seamless	5'-ctagaaaatgaagtCCTGGA	agagctaaggcgttcg	CCCG-3'	
HNF1A-9	5'-ctagaaaatgaagtCCTGGA	nnnnnn	gagctaaggcgttc-3'	+ 35
HNF1A-13	5'-ctagaaaatgaagtCCTGGA	agagctaaggcgttcg	CCCG-3'	seamless

INS Forward

	genome	5J	donor	
Seamless	5'-cgtctgcggcccatgc	TCCAGG	acttcattttctag-3'	
INS-2	5'-cgtctgcggcccatgc	TCCAGG	acttcattttctag-3'	seamless

	donor	3J	genome	
Seamless	5'-TCGGGTATGTCGGGAACCTC	cgtggg	gatgcccacccc-3'	
INS-2	5'-TCGGGTATGTCGGGAACCTC	cgtggg	gatgcccacccc-3'	seamless

INS Reverse

	genome	5J	donor	
Seamless	5'-cgtctgcggcccatgc	GAGGTTCCCGACATACCCGA-3'		
INS-4	5'-cgtctgcggcccatgc	GAGGTTCCCGACATACCCGA-3'		Large size deletion impaired the lox site

	donor	3J	genome	
Seamless	5'-ctagaaaatgaagtCCTGGA	atggggtgcagtgtgtggc-3'		
INS-4	5'-ctagaaaatgaagtCCTGGA	atggggtgcagtgtgtggc-3'		seamless

TP53 Forward

	genome	5J	donor	
Seamless	5'-tggttaaggactggggcgcg	TCCAGG	acttcattttctag-3'	
TP53-20	5'-tggttaaggactggggcgcg	TCCAGG	acttcattttctag-3'	seamless
TP53-21	5'-tggttaaggactggggcgcg	TCCAGG	acttcattttctag-3'	Large size deletion impaired the lox site

	donor	3J	genome	
Seamless	5'-TCGGGTATGTCGGGAACCTC	caagggcaggggcctggggg-3'		
TP53-20	5'-TCGGGTATGTCGGGAACCTC	caagggcaggggcctggggg-3'		seamless

TP53 Reverse

	genome	5J	donor	
Seamless	5'-tggttaaggactggggcgcg	GAGGTTCCCGACATACCCGA-3'		
TP53-9	5'-tggttaaggactggggcgcg	GAGGTTCCCGACATACCCGA-3'		seamless
TP53-17	5'-tggttaaggactggggcgcg	GAGGTTCCCGACATACCCGA-3'		seamless
TP53-18	5'-tggttaaggactggggcgcg	GAGGTTCCCGACATACCCGA-3'		seamless
TP53-19	5'-tggttaaggactggggcgcg	GAGGTTCCCGACATACCCGA-3'		seamless
TP53-24	5'-tggttaaggactggggcgcg	GAGGTTCCCGACATACCCGA-3'		seamless

	donor	3J	genome	
Seamless	5'-ctagaaaatgaagtCCTGGA	caagggcaggggcctggggg-3'		
TP53-8	5'-ctagaaaatgaagtCCTGGA	caagggcaggggcctggggg-3'		seamless
TP53-9	5'-ctagaaaatgaagtCCTGGA	caagggcaggggcctggggg-3'		seamless
TP53-18	5'-ctagaaaatgaagtCCTGGA	caagggcaggggcctggggg-3'		seamless
TP53-19	5'-ctagaaaatgaagtCCT	-----gacctggggg-3'	MMEJ, -13	
TP53-24	5'-ctagaaaatgaagtCCTGGA	caagggcaggggcctggggg-3'		seamless

HNF1B Forward

	genome	5J	donor	
Seamless	5'-ggacccctccagtccttca	TCCAGG	acttcattttctag-3'	
HNF1B-6	5'-ggacccctccagtccttca	TCCAGG	acttcattttctag-3'	seamless
HNF1B-16	5'-ggacccctccagtccttca	TCCAGG	acttcattttctag-3'	Large size deletion impaired the lox site

	donor	3J	genome	
Seamless	5'-TCGGGTATGTCGGGAACCTC	caccgacgaccgagccccg-3'		

Supplemental Fig S15 (continued)

HNF1B Reverse

	genome	5J	donor			donor	3J	genome	
Seamless	5'-ggaccctccagtccttca	GAGGTTCCCGACATACCCGA-3'		Seamless	5'-ctagaaatgaagtCCTGGA	accggacgacccgagcccg-3'			
HNF1B-1	5'-ggaccctccagtccttca	GAGGTTCCCGACATACCCGA-3'	seamless	HNF1B-1	5'-ctagaaatgaagtCCTGGA	accggacgacccgagcccg-3'	seamless		
HNF1B-4	5'-ggaccctccagtccttca	GAGGTTCCCGACATACCCGA-3'	seamless	HNF1B-4	5'-ctagaaatgaagtCCTGGA	accggacgacccgagcccg-3'	seamless		
HNF1B-6	5'-ggaccctccagtccttca	GAGGTTCCCGACATACCCGA-3'	seamless	HNF1B-6	5'-ctagaaatgaagtCCTGGA	accggacgacccgagcccg-3'	seamless		
HNF1B-7	5'-ggaccctccagtccttca	GAGGTTCCCGACATACCCGA-3'	seamless	HNF1B-7	5'-ctagaaatgaagtCCTGGA	accggacgacccgagcccg-3'	seamless		
HNF1B-9	5'-ggaccctccagtccttca	nnnnnnGAGGTTCCCGACATAC-3'	+17	HNF1B-9	5'-ctagaaatgaagtCCTGGA	accggacgacccgagcccg-3'	seamless		
HNF1B-10	5'-ggaccctccagtccttca	GAGGTTCCCGACATACCCGA-3'	seamless	HNF1B-10	5'-ctagaaatgaagtCCTGGA	accggacgacccgagcccg-3'	seamless		
HNF1B-11	5'-ggaccctccagtccttca	GAGGTTCCCGACATACCCGA-3'	seamless	HNF1B-11	5'-ctagaaatgaagtCCTGGA	accggacgacccgagcccg-3'	seamless		
HNF1B-18	5'-ggaccctccagtcctt-	GAGGTTCCCGACATACCCGA-3'	-2	HNF1B-18	5'-ctagaaatgaagtCCTGGA	accggacgacccgagcccg-3'	seamless		

PAX6 Forward

	genome	5J	donor			donor	3J	genome	
Seamless	5'-tctgtaggaccccccccg	TCCAGGactcattttctag-3'		Seamless	5'-TCGGGTATGTCGGGAACCTC	ttagccttgacactogaaga-3'			
PAX6-2	5'-tctgtaggaccccccccg	- CCAGGactcattttctag-3'	-1	PAX6-2	5'-TCGGGTATGTCGGGAACCTC	ttagccttgacactogaaga-3'	seamless		
PAX6-5	5'-tctgtaggaccccccccg	TCCAGGactcattttctag-3'	seamless	PAX6-5	5'-TCGGGTATGTCGGGA	-----ttagccttgacactogaaga-3'	-5		
PAX6-10	5'-tctgtaggaccccccccg	TCCAGGactcattttctag-3'	seamless	PAX6-9	5'-TCGGGTATGTCGGGAACCTC	ttagccttgacactogaaga-3'	seamless		
PAX6-12	5'-tctgtaggaccccccccg	TTCCAGGactcattttctag-3'	+1	PAX6-10	5'-TCGGGTATGTCGGGAACCTC	ttagccttgacactogaaga-3'	seamless		
PAX6-13	5'-tctgtaggaccccccccg	TTTTCCAGGactcattttctag-3'	+3	PAX6-12	5'-TCGGGTATGTCGGGAACCTC	ttagccttgacactogaaga-3'	seamless		
				PAX6-13	5'-TCGGGTATGTCGGGAACCTC	ttagccttgacactogaaga-3'	seamless		
				PAX6-19	5'-TCGGGTATGTCGGGAACCTC	ttagccttgacactogaaga-3'	seamless		

PAX6 Reverse

	genome	5J	donor			donor	3J	genome	
Seamless	5'-tctgtaggaccccccccg	GAGGTTCCCGACATACCCGA-3'		Seamless	5'-ctagaaatgaagtCCTGGA	ttagccttgacactogaaga-3'			
PAX6-15	5'-tctgtaggaccccccccg	TTTTGAGGTTCCCGACATACCCGA-3'	+3	PAX6-11	Large size deletion impaired the lox site				
				PAX6-15	5'-ctagaaatgaagtCCTGGA	ttagccttgacactogaaga-3'	seamless		

SMN1 Forward

	genome	5J	donor			donor	3J	genome	
Seamless	5'-ttgcttaggattcggttaa	TCCAGGactcattttctag-3'		Seamless	5'-TCGGGTATGTCGGGAACCTC	taagggtttgagagaagaaa-3'			
SMN1-1	5'-ttgcttaggattcggttaa	TCCAGGactcattttctag-3'	seamless	SMN1-1	5'-TCGGGTATGTCGGGAACCTC	taagggtttgagagaagaaa-3'	seamless		
SMN1-9	5'-ttgcttaggattcggttaa	-----3'	-36	SMN1-9	5'-TCGGGTATGTCGGGAACCTC	taagggtttgagagaagaaa-3'	seamless		
SMN1-16	5'-ttgcttaggattcggttaa	TCCAGGactcattttctag-3'	seamless	SMN1-16	5'-TCGGGTATGTCGGGAACCTC	taagggtttgagagaagaaa-3'	seamless		
SMN1-19	Large size deletion impaired the lox site			SMN1-19	5'-TCGGGTATGTCGGGAACCTC	taagggtttgagagaagaaa-3'	seamless		
SMN1-22	5'-ttgcttaggattcggttaa	TCCAGGactcattttctag-3'	seamless	SMN1-22	5'-TCGGGTATGTCGGGAACCTC	taagggtttgagagaagaaa-3'	seamless		

SMN1 Reverse

	genome	5J	donor			donor	3J	genome	
Seamless	5'-ttgcttaggattcggttaa	GAGGTTCCCGACATACCCGA-3'		Seamless	5'-ctagaaatgaagtCCTGGA	taagggtttgagagaagaaa-3'			
SMN1-10	5'-ttgcttaggattcggttaa	GAGGTTCCCGACATACCCGA-3'	seamless	SMN1-9	5'-ctagaaatgaagtCCTGGA	taagggtttgagagaagaaa-3'	seamless		
SMN1-22	5'-ttgcttaggattcggttaa	GAGGTTCCCGACATACCCGA-3'	seamless	SMN1-10	5'-ctagaaatgaagtCCTGGA	taagggtttgagagaagaaa-3'	seamless		
				SMN1-22	5'-ctagaaatgaagtCCTGGA	taagggtttgagagaagaaa-3'	seamless		

SMNDC1 Reverse

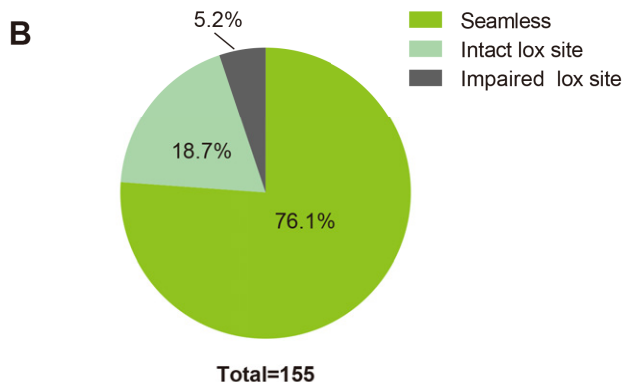
	genome	5J	donor			donor	3J	genome	
Seamless	5'-tagagacgcccgtgccccttc	GAGGTTCCCGACATACCCGA-3'		Seamless	5'-ctagaaatgaagtCCTGGA	ggtgcccgcgtgtttgcgg-3'			
SMNDC1-1	5'-tagagacgcccgtgccccttc	GAGGTTCCCGACATACCCGA-3'	seamless	SMNDC1-1	5'-ctagaaatgaagtCCTGGA	ggtgcccgcgtgtttgcgg-3'	seamless		
SMNDC1-20	5'-tagagacgcccgtgccccttc	GAGGTTCCCGACATACCCGA-3'	seamless	SMNDC1-20	5'-ctagaaatgaagtCCTGGA	ggtgcccgcgtgtttgcgg-3'	seamless		
SMNDC1-29	5'-tagagacgcccgtgccccttc	GAGGTTCCCGACATACCCGA-3'	seamless	SMNDC1-29	5'-ctagaaatgaagtCCTGGA	ggtgcccgcgtgtttgcgg-3'	seamless		

PKD1 Forward

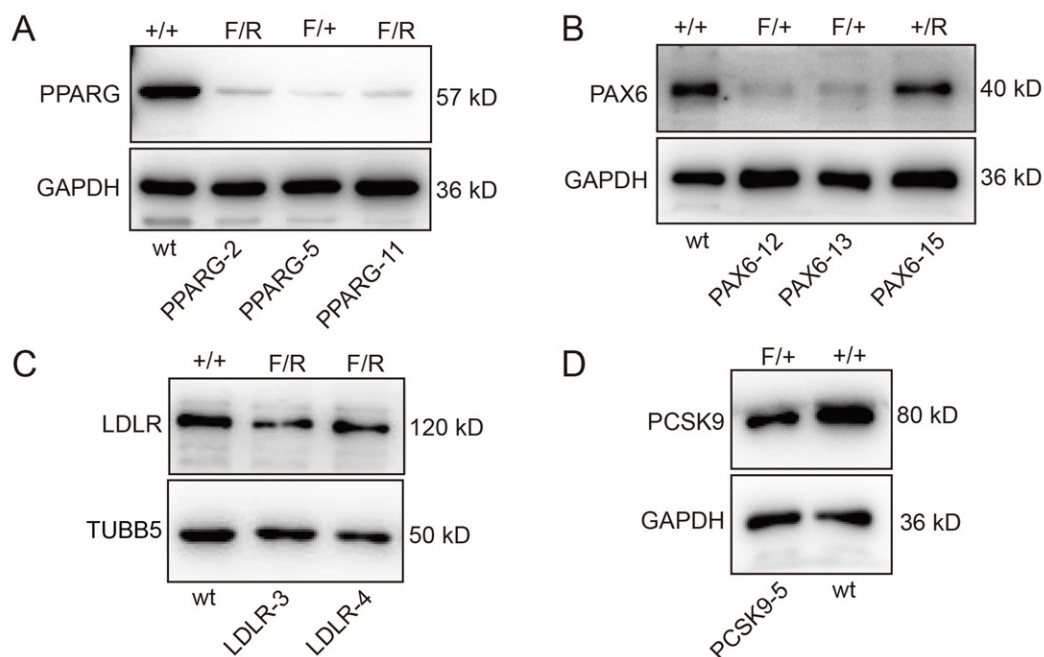
	genome	5J	donor			donor	3J	genome	
Seamless	5'-gggctgaggcctgccagcg	TCCAGGactcattttctag-3'		Seamless	5'-TCGGGTATGTCGGGAACCTC	ggggcgcttccaccttgc-3'			
PKD1-1	5'-gggctgaggcctgccagcg	TCTCCAGGactcattttctag-3'	+2	PKD1-1	5'-GG - - - CGGGTATGTCGGGA	-----Cggggcgcttcca-3'	-7		
PKD1-3	5'-gggctgaggcctgccagcg	TCCAGGactcattttctag-3'	seamless	PKD1-3	5'-TCGGGTATGTCGGGAACCTC	ggggcgcttccaccttgc-3'	seamless		
PKD1-15	5'-gggctgaggcctgccagcg	TCCAGGactcattttctag-3'	seamless	PKD1-15	5'-TCGGGTATGTCGGGAACCTC	ggggcgcttccaccttgc-3'	seamless		

Supplemental Fig S15 (continued)

RAG2 Forward							
	genome	5J	donor		donor	3J	genome
Seamless	5'-aattcaatccactacaagc	TCCAGG	acttcattttctag-3'	Seamless	5'-TCGGGTATGTCGGGAACCTC	ttgtggaacaactctctttt-3'	
RAG2-4	5'-aattcaatccactacaagc	TCCAGG	acttcattttctag-3'	seamless	RAG2-4	Large size deletion	impaired the lox site
RAG2 Reverse							
	genome	5J	donor		donor	3J	genome
Seamless	5'-aattcaatccactacaagc	GAGGTTCCCGACATACCCGA-3'		Seamless	5'-ctagaaaaatgaagtCCTGGA	ttgtggaacaactctctttt-3'	
RAG-7	5'-aattcaatccactacaagc	GAGGTTCCCGACATACCCGA-3'	seamless	RAG-7	5'-ctagaaaaatgaagtCCTGGA	ttgtggaacaactctctttt-3'	seamless
AR Forward							
	genome	5J	donor		donor	3J	genome
Seamless	5'-agaagtagggccctttatat	TCCAGG	acttcattttctag-3'	Seamless	5'-TCGGGTATGTCGGGAACCTC	ccaggaaactgcataataa-3'	
AR-9	5'-agaagtagggccctttatat	TCCAGG	acttcattttctag-3'	seamless	AR-9	5'-TCGGGTATGTCGGGAACCTC	ccaggaaactgcataataa-3'
AR Reverse							
	genome	5J	donor		donor	3J	genome
Seamless	5'-agaagtagggccctttatat	GAGGTTCCCGACATACCCGA-3'		Seamless	5'-ctagaaaaatgaagtCCTGGA	ccaggaaactgcataataa-3'	
AR-2	5'-agaagtagggccctttatat	GAGGTTCCCGACATACCCG - - CG-3'	- 2	AR-2	5'-ctagaaaaatgaagtCCTGGA	ccaggaaactgcataataa-3'	seamless
AR-14	5'-agaagtagggccctttatat	GAGGTTCCCGACATACCCGA-3'	seamless	AR-14	5'-ctagaaaaatgaagtCCTGGA	ccaggaaactgcataataa-3'	seamless
IL6ST Forward							
	genome	5J	donor		donor	3J	genome
Seamless	5'-gcttttgttaaaaccagta	TCCAGG	acttcattttctag-3'	Seamless	5'-TCGGGTATGTCGGGAACCTC	latggggacactactgtataa-3'	
IL6ST-4	5'-gcttttgttaaaaccagta	TCCAGG	acttcattttctag-3'	seamless	IL6ST-4	5'-TCGGGTATGTCGGGAACCTC	latggggacactactgtataa-3'
LAMB3 Forward							
	genome	5J	donor		donor	3J	genome
Seamless	5'-ttatggcgtcctctataac	TCCAGG	acttcattttctag-3'	Seamless	5'-TCGGGTATGTCGGGAACCTC	ccgtggaagttccctggcta-3'	
LAMB3-2	5'-ttatggcgtcctctataac	TCCAGG	acttcattttctag-3'	seamless	LAMB3-2	5'-TCGGGTATGTCGGGAACCTC	ccgtggaagttccctggcta-3'
COL17A1 Forward							
	genome	5J	donor		donor	3J	genome
Seamless	5'-attgtacctatgtgcttata	TCCAGG	acttcattttctag-3'	Seamless	5'-TCGGGTATGTCGGGAACCTC	cggtgggtcagatcacactc-3'	
COL17A1-16	5'-attgtacctatgtgcttata	TCCAGG	acttcattttctag-3'	seamless	COL17A1-16	5'-TCGGGTATGTCGGGAACCTC	cggtgggtcagatcacactc-3'
COL17A1-26	5'-attgtacctatgtgcttata	TCCAGG	acttcattttctag-3'	seamless	COL17A1-26	5'-TC - - - - ATGTCGGGAACCTC	cggtgggtcagatcacactc-3'
COL17A1 Reverse							
	genome	5J	donor		donor	3J	genome
Seamless	5'-attgtacctatgtgcttata	GAGGTTCCCGACATACCCGA-3'		Seamless	5'-ctagaaaaatgaagtCCTGGA	cggtgggtcagatcacactc-3'	
COL17A1-1	5'-attgtacctatgtgcttata	GAGGTTCCCGACATACCCGA-3'	seamless	COL17A1-1	5'-ctagaaaaatgaagtCCTGGA	cggtgggtcagatcacactc-3'	seamless
COL17A1-4	5'-attgtacctatgtgcttata	GAGGTTCCCGACATACCCGA-3'	seamless	COL17A1-4	5'-ctagaaaaatgaagtCCTGGA	cggtgggtcagatcacactc-3'	seamless
COL17A1-23	5'-attgtacctatgtgcttata	GAGGTTCCCGACATACCCGA-3'	seamless	COL17A1-23	5'-ctagaaaaatgaagtCCTGGA	cggtgggtcagatcacactc-3'	seamless



Supplemental Fig S15. Supplementary sequencing data of PFF clonal cell lines produced via HIT-trapping. (A) Sequencing confirmation of gene-trap insertions at the target loci in PFFs. Genome sequences are shown in green. PAMs are shown in blue. Insertions or mismatches are shown in red. Deletions are shown as dashed lines. Microhomologies are shown in purple. 5/3J, 5'/3' junction; MMEJ, microhomology-mediated end joining. (B) Summary of sequencing data.



Supplemental Fig S16. HIT-trapping mediated heterozygous knockouts in PFFs. (A-D) Western blots for PFF clones bearing FLETrap insertions at *PPARG*, *PAX6*, *LDLR*, and *PCSK9*, with GAPDH or TUBB5 as the loading control. F/R, forward/reverse integration of the donor.

Supplemental Table S1. Genes and target sites selected for HIT-trapping in mammalian cells

Target gene	Species	Target sequence (PAM)
<i>Hprt</i>	mouse	ggtaatgtagaccatcaagc tgg (mTh1)
		ggaatgaaccctgtgtctac tgg (mTh2)
		caagataaagtacgtgtgag tgg (mTh3)
		gtggacagacttaactaggt agg
		ggactttaattattaggtga tgg
<i>Sall4</i>	human	tatcaagagtcgcctcgaga agg
<i>Tet1</i>		ggactttaattattaggtga tgg
<i>Nes</i>		ggactttaattattaggtga tgg
<i>Olig2</i>	human	ggatgggtggcatctacgccg cgg
<i>ACTB</i>		ccttggagtggtgattaagt agg
<i>HPRT1</i>		tcttaaccgtaatcagcctc tgg
<i>ROSA26</i>	pig	tgtgggaatatagagaaatt ggg
<i>THY1</i>		tgatactgttcaagggtc cgg

Supplemental Table S2. Potential off-target sites for sgRNAs targeting HIT-trap donor and genome. Red denotes mismatches.

Coordinates	Strand	MM	Target_seq	PAM	Distance		Gene name	Gene id	Note
chr3:94884551-94884573	-	4	GTTT GA [GGGAACCTCTCC]	AGG	98	I	<i>Psmb4</i>	ENSMUSG00000005779	sgA-OT1
chr9:56362583-56362605	-	4	GCT TA C[GTGAACCTCTCC]	TGG	11771	I	<i>Peak1</i>	ENSMUSG00000074305	sgA-OT2
chr17:37728440-37728462	-	3	GG AA TGTA[GGG G ACCTCTCC]	AGG	1449	-	<i>Olfr120</i>	ENSMUSG00000063994	sgA-OT3
chrX:52989368-52989390	+	0	GGTAATGT[AGACCATCAAGC]	TGG	1118	I	<i>Hprt</i>	ENSMUSG00000025630	<i>Hprt</i>
chr7:119274211-119274233	+	4	CATC AGGT[AGACCATCAAGC]	CGG	31890	-	<i>Gm23321</i>	ENSMUSG00000077653	OT1
chr9:57141691-57141713	+	4	AGTGCTG [AGACCATCAAGC]	AGG	0	E	<i>Man2c1</i>	ENSMUSG00000032295	OT2
chr10:11419222-11419244	-	4	ATTAATAC [AGACCATCAAGC]	CGG	28237	I	<i>Epm2a</i>	ENSMUSG00000055493	OT3
chr2:168761544-168761566	-	0	GTGGACAG[ACTTAAGTAGGT]	AGG	0	E	<i>Sall4</i>	ENSMUSG00000027547	<i>Sall4</i> mESC
chr9:71695872-71695894	-	3	GTG TGG AG[ACTTAAGTAGGT]	GGG	9254	I	<i>Cgnl1</i>	ENSMUSG00000032232	OT1
chr7:116904417-116904439	-	4	GT TC ACTG[ATTTAACTAGGT]	AGG	48150	-	<i>Gm26054</i>	ENSMUSG00000064971	OT2
chr18:55032118-55032140	-	4	AGGGACA [AC A TAACTAGGT]	AGG	13179	I	<i>Gm4221</i>	ENSMUSG00000096948	OT3
chr10:62874866-62874888	-	0	GGACTTGA[ATTATTAGGTGA]	TGG	3264	I	<i>Tet1</i>	ENSMUSG00000047146	<i>Tet1</i>
chr9:75326093-75326115	-	4	TGGCTTTG [AC T ATTAGGTGA]	AGG	1018	I	<i>Gnb5</i>	ENSMUSG00000032192	OT1
chr16:88457269-88457291	+	4	GT CC ATTA[ATT G TTAGGTGA]	TGG	48516	-	<i>Cldn17</i>	ENSMUSG00000055811	OT2
chr6:50651337-50651359	-	4	GGG CC TTA[G T CATTAGGTGA]	TGG	415	I	<i>Npvf</i>	ENSMUSG00000029831	OT3
chr1:149876764-149876786	-	4	TATAGGG C[GGGAACCTCTCC]	GGG	7673	I	<i>HIST2H2BE</i>	ENSG00000184678	sgA-OT1
chr3:124352067-124352089	+	4	G CACTG GC[GGGAACCTCTCC]	TGG	4802	I	<i>KALRN</i>	ENSG00000160145	sgA-OT2
chr17:27759823-27759845	+	4	GGT TA GT[C T GAACCTCTCC]	AGG	185	I	<i>NOS2</i>	ENSG00000007171	sgA-OT3
chr7:5528863-5528885	-	0	CCTTGGAG[TGTGTATTAAGT]	AGG	0	E	<i>ACTB</i>	ENSG00000075624	<i>ACTB</i> A375
chr20:16632206-16632228	-	3	TCTTGGT G[AGTGTATTAAGT]	TGG	38413	-	<i>Y_RNA</i>	ENSG00000200494	OT1
chr8:17144942-17144964	-	4	CTATG AA [TGTGTATTAAGT]	TGG	4541	I	<i>RP11-468H14.2</i>	ENSG00000249258	OT2
chr19:45040682-45040704	+	4	AATAG GAG[TG A GTATTAAGT]	TGG	0	E	<i>CLASRP</i>	ENSG00000104859	OT3

chrX:134461050-134461072	+	0	TCTTAACC[GTAATCAGCCTC]	TGG	515	I	HPRT1	ENSG00000165704	HPRT1	
chr8:132428436-132428458	+	4	T TAA AACC[G CA ATCAGCCTC]	TGG	18743	I	KCNQ3	ENSG00000184156	OT1	
chr17:7627563-7627585	+	3	TCTT C ACC[G CG AATCAGCCTC]	AGG	0	E	SAT2	ENSG00000141504	OT2	
chr16:87526018-87526040	+	4	T G TTA ATT [T T AATCAGCCTC]	TGG	10383	-	RP11-482M8.1	ENSG00000260750	OT3	
chr7:44977776-44977798	+	4	TCTG TG T [GGGAACCTCTCC]	AGG	181	I	TMEM63B	ENSSSCG00000001697	sgA-OT1	
chr14:44538663-44538685	+	4	AGTTG GT G [GGGAACCTCTCC]	AGG	1106	I	SSH1	ENSSSCG00000009943	sgA-OT2	
chr12:12723335-12723357	-	3	GGTA AGAC [A GGAACCTCTCC]	AGG	61958	-	AXIN2	ENSSSCG00000017265	sgA-OT3	
chr13:72804431-72804453	-	0	TGTGGGAA[TATAGAGAAATT]	GGG	7375	-		ENSSSCG00000030273	ROSA26	
chr11:63921198-63921220	-	2	TTTGGGAA[GATAGAGAAATT]	TGG	NA	-	NA	NA	OT1	
chr9:104586306-104586328	+	4	ATAGAG AA[TATAGAGAAATT]	TGG	13428	-	U6	ENSSSCG00000023059	OT2	pFF
chr9:104431560-104431582	-	4	ATAGAG AA[TATAGAGAAATT]	TGG	19435	-	U6	ENSSSCG00000022170	OT3	
chr9:51640854-51640876	-	0	TGATACTT[GTTCAAGGGCTC]	CGG	159	I	THY1	ENSSSCG00000015122	THY1	
GL894591-2:27644-27666	-	2	TGATAC CT [C TTCAAGGGCTC]	AGG	13175	I		ENSSSCG00000029982	OT1	
chrX:7253745-7253767	-	4	T CGC AC G T[GTTCAAGGGCTC]	GGG	5148	I	WWC3	ENSSSCG00000012108	OT2	
chr1:103509445-103509467	+	4	CTG TACTT[C TTCAAGGGCTC]	TGG	0	E	MTO1	ENSSSCG00000004487	OT3	

Supplemental Table S3. Disease-related genes and target sites selected for HIT-trapping in PFFs

Target Gene	Relate disease	Target sequence (PAM)
<i>THY1</i>	cancer	TGATACTTGTTCAAGGGCTC CGG
<i>LDLR</i>	Hypercholesterolemia	GAGATAGATCTACCTCATGG AGG
<i>PCSK9</i>	Hypercholesterolemia	ACTTTGACTAAAGCAGATGG GGG
<i>UCP3</i>	Obesity	CCACTCAGCTCCTTACTGCA GGG
<i>APOA1</i>	Familial HDL deficiency	CTCGGTCTATCTGGTCGATG GGG
<i>APOC3</i>	Non-alcoholic fatty liver disease	CGCCTTGGCCGGCGCTTCCA TGG
<i>GLP1R</i>	Diabetes mellitus	CCCTGTGCCTCCTCGGGAGC AGG
<i>PPARG</i>	Familial partial lipodystrophy	CTCGCGGTATTAATCACTAG TGG
<i>HNF1A</i>	Diabetes mellitus	GCGGAACGCCTTAGCTCATG GGG
<i>INS</i>	Diabetes mellitus	CTGCCGGCCACATGCCCGT GGG
<i>TP53</i>	Cancer	TAAGGACTGGGGCGCGGCAA GGG
<i>HNF1B</i>	Cancer	GGCTCGGGTCGTCCGGTTGA AGG
<i>PAX6</i>	Ophthalmology disease	TCGAGTGTCAAGGCAAACGG GGG
<i>SMN1</i>	Spinal muscular atrophy (SMA)	GCTTATGGATTCGGTAATAA AGG
<i>SMNDC1</i>	Spinal muscular atrophy (SMA)	TTCGGTGCGGAGCGTGTTTG CGG
<i>PKD1</i>	Polycystic kidney disease	AAGGTGGAAGCGCGCCCCGC TGG
<i>RAG2</i>	Omenn syndrome	TCAATCCCACTACAAGCTTG TGG
<i>AR</i>	Androgen insensitivity syndrome	AGTAGGGCCCTTTATATCCC AGG
<i>IL6ST</i>	Rheumatoid arthritis	TACAGTAGGTCCCCATATAC TGG
<i>LAMB3</i>	Junctional epidermolysis bullosa (JEB)	ATGGCTGCTCCTATAACCCG TGG
<i>COL17A1</i>	Junctional epidermolysis bullosa (JEB)	GTACCTATGTGCTTATACGG TGG

* Gene related diseases referenced from NIH (<https://ghr.nlm.nih.gov/>)

Supplemental Table S4. Primers used for plasmid construction. All oligos are in the 5' to 3' direction.

Plasmid	Sequence	Cassette
HIT-trap-1C	CACCgGGTATGTCTGGGAACCTCTCC	sgA-linker
	aaacGGAGAGGTTCCCGACATACCC	
	gacaaatggctctagaggtaccGGTATGTCTGGGAACCTCTCCAGGtGC	SA-IRES-GFP
	GATTAAGGGATCTGTAGG	
	CCAAACTCATCAATGTATCTTAAGgcaggaaccctagtgat	Sv40-Puro-pA
	GCCTCAGGACTCTTCCTgcTCCCCAGCATGCCTGCTATTc	
HIT-trap-pA1C/2C	gAATAGCAGGCATGCTGGGGAgcAGGAAGAGTCCTGAGGC	SA-GFP
	attctgcagacaaatggctctAGAGGTACGGTATGTCTGGGAACCTCTC	
	CAGGggtaccTAGGGCGCAGTAgtCCAGGGTTTC	
	CTCCTCGCCCTTGCTCACCATACTGGAAAGACCGCAAGA	
	G	
	CTCTTCGCGGTCTTTCCAGTATGGTGAGCAAGGGCGAGGA	
	G	

[illegible]

Primer name	Sequence	Cell type	sgRNA
msgA-OT1	TAACCGGGTGAGAAATGGGC	mESCs	sgA
msgA-OT1	TCTGCCTTTGAGCCAGCTAC		
msgA-OT2-F	GACCCTCACCTGAAAGCCTC		
msgA-OT2-R	TAGAGCAGCGCAGTGTGTAC		
msgA-OT3-F	GGCTCGTGATTGGTTGGTCA		
msgA-OT3-R	AGGGTGGTGAAGTGTGTGTG		
mH-OT1-F	TGATGGCTTAGTCCCTGGGA	mESCs	sg-Hprt
mH-OT1-R	AACAAAACCCTCCCACAGCA		

mH-OT2-F	CCCAGGCTCCTTCCAAGAAG		
mH-OT2-R	GAGGGAAAGGTGACCAGTGG		
mH-OT3-F	TCTGCTAACCTCTGCCTCCT		
mH-OT3-R	AGGAAGGAATGGACACAGGC		
mS-OT1-F	CAAGGACAGCAGCAAAAGGG		
mS-OT1-R	TCCACCCTACCCATCACAGA		
mS-OT2-F	ACTTTTCCTCGTTGCCTGCT		sg-Sall4
mS-OT2-R	CATAGCCATACTCCGCGTGT		
mS-OT3-F	ATGCCTCTCTCTGTCTCCCC		
mS-OT3-R	ATGAGAAACCACCTGCAGCT		
mT-OT1-F	CTGCCTGAGTGAAGCGAGAA		
mT-OT1-R	ATGCCTCCTTTCCTGTGCTC		
mT-OT2-F	TGAGGTGCTTGTGGGTTGAA		sg-Tet1
mT-OT2-R	AAGAGAACAAGGTGGCCACA		
mT-OT3-F	TGGAGGGTGGGTAGACTCTG		
mT-OT3-R	TCTGTGAGTCTGAGCCAAGC		
hsgA-OT1-F	CCAGTCACAATGGCCAGCTA		
hsgA-OT1-R	CATATATAGGCGGGGCGTGG		
hsgA-OT2-F	CAGAGTGAAGGCCCAGGAAG		sgA
hsgA-OT2-R	TGATTTGGTGCTCTGAGGCT		
hsgA-OT3-F	GAGGAGATGCTGGAGATGGC		
hsgA-OT3-R	ATGTGTGAAGGCCGAGAGTC		
hAC-OT1-F	GTCAGGAGAGCACACGGAAT		
hAC-OT1-R	CTGCCCCACTTCCAGTCTTC		
hAC-OT2-F	GCATGCGCCACCACTTTAAA	A375	sg-ACTB
hAC-OT2-R	GCACTGCAGAGGGATAGTCA		
hAC-OT3-F	CCTTTTGTCTGGGGTGAGGG		
hAC-OT3-R	CATGCACTACAGCCTCCTCC		
hH-OT1-F	AGGTTTGTTCCTCCCTCAGT		
hH-OT1-R	GTGCCATTCTCCTACCACCC		
hH-OT2-F	CTGGCCTACTCTCTCCTCCC		sg-HPRT1
hH-OT2-R	AAGAGGACGTGGGTGTATGC		
hH-OT3-F	ACGGCATGAACTCACTCCAG		
hH-OT3-R	ACAGCATTGGTCGTGTGTGA		
psgA-OT1-F	GCAGCTATTTCGGTTTGAgta		
psgA-OT1-R	agaggtgttcccagaacctgt		
psgA-OT2-F	tctccccctgaaaataaccac	PFFs	SgA
psgA-OT2-R	cacaaaacacactgccttct		
psgA-OT3-F	atgttctgcttcgtgcaaact		
psgA-OT3-R	ttttgtgcttttgcacacac		

pR-OT1-F	tcttgggtatggcataagctg	
pR-OT1-R	ctgtctttgagggactgggtca	
pR-OT2-F	cttctgggtgactgaggcaaag	sg-ROSA26
pR-OT2-R	atacttgattgccccagaac	
pR-OT3-F	atttcttgcaattccattgtgg	
pR-OT3-R	atttcgctgagcataattcaggc	
pT-OT1-F	ctccgaagtgggtactgcttg	
pT-OT1-R	gactaatgtcgccggttgta	
pT-OT2-F	cagggccacttctaaagagc	sg-THY1
pT-OT2-R	gccctggattcggttttagt	
pT-OT3-F	aagttcccaagccaggtattg	
pT-OT3-R	ccacttcccaaacacgaacta	

Supplemental Table S6. Primers used for knock-in detection. All oligos are in the 5' to 3' direction.

Genome-specific primers			
Primer name	Sequence	Species	Target gene
lhx227-Trap-mhprr-F	cccctatcaatcccaacgcc	Mouse	<i>Hprt</i>
lhx228-Trap-mhprr-F	ggcaatgggtgcacaggaag		
lhx207-mSall4-F	ccaactttgtctgctctccacct		<i>Sall4</i>
lhx208-mSall4-R	tcaggctttgcctcaattct		
lhx209-mTet1-F	gagtatgggtggaactgccc		<i>Tet1</i>
lhx210-mTet1-R	agcccttgtctcaaaacccaaa		
lhx287-mNES-F	cactgtagctgctcctcactc		<i>Nes</i>
lhx288-mNES-R	gccatgactctctcactgct		
lhx289-mOlig2-F	ttacagaccgagccaacacc		<i>Olig2</i>
lhx289-mOlig2-R	ttacagaccgagccaacacc		
lhx269-hACTB-F	agaaaatctggcaccacacc	Human	<i>ACTB</i>
lhx270-hACTB-R	aggaaggaaggctggaagag		
lhx147-hHPRT-F	ctagggaagagcattaggtcat		<i>HPRT1</i>
lhx148-hHPRT-R	aagctggattcccagtttagactc		
lhx271-pRosa-F	ggtccaatcgagtggtagt	Pig	<i>ROSA26</i>
lhx272-pRosa-R	ggagtgccgcaataacctta		
lhx273-pThy1-F	cactcctaccgctctcgaac		<i>THY1</i>
lhx274-pThy1-R	cttatgccccacacttgac		
lhx387-pLDLR-F	ctgccagcctacatcacagc		<i>LDLR</i>
lhx388-pLDLR-R	ctgtggctcgggtttgatcc		
lhx447-pPCSK9-F	gtgttgacagacgcagaag		<i>PCSK9</i>
lhx448-pPCSK9-R	gatgacgccggagagtagag		
lhx375-pUCP3-F	aggatcaaatgccacaccatg		<i>UCP3</i>

lhx376-pUCP3-R	ggttccagagcctccttgat	
lhx471-pAPOA1-F	tgggccccatagaagacctg	<i>APOA1</i>
lhx472-pAPOA1-R	gaagtcgtccaggtagggct	
lhx473-pAPOC3-F	acccacaggacccagtttca	<i>APOC3</i>
lhx474-pAPOC3-R	gtgatgggaccaccagcaag	
lhx361-pGL1R-F	gtccctctcagaacagtgc	<i>GLP1R</i>
lhx362-pGL1R-R	tagtcgtcaaagggtccggttg	
lhx357-pPP-F	acactgtcctttgcagcgga	<i>PPARG</i>
lhx358-pPP-R	accattgctctcattcaggca	
lhx379-pHa-F	cagtctagagtcctgtgagt	<i>HNF1A</i>
lhx380-pHa-R	tagtaaccagggtcagctctgc	
lhx483-pINS-F	gagcgcggtcttcttacac	<i>INS</i>
lhx484-pINS-R	tattgggttttggggtgcgg	
lhx423-pTP53-F	caatggaggagtcgcagtc	<i>TP53</i>
lhx424-pTP53-R	ctggatcctggcctttctcc	
lhx365-pHb-F	tcttcacactctcaccaacg	<i>HNF1B</i>
lhx366-pHb-R	cacaagaggcaccaaggagaa	
lhx353-pPA-F	tctgtccttggctgtgggga	<i>PAX6</i>
lhx354-pPA-R	gagaacacacgcacctcgcta	
lhx369-pSMN-F	ctggcagttagggtctgtaa	<i>SMN1</i>
lhx370-pSMN-R	ttcacaatctcctgcccattc	
lhx371-pSDC-F	ctttccagtgcccagtttctg	<i>SMNDC1</i>
lhx372-pSDC-R	caaagaggtagagcgagcca	
lhx401-pPKD1-F	tccatcatctgcttgctagt	<i>PKD1</i>
lhx402-pPKD1-R	ctggaggggacacttacaact	
lhx469-pRAG2-F	cctggggcaataaagacaaa	<i>RAG2</i>
lhx470-pRAG2-R	agcggagaggaggaaggtag	
lhx477-pAR-F	gagaggagagaggcccaagg	<i>AR</i>
lhx478-pAR-R	tggaggggagaggagatgga	
lhx481-pIL6ST-F	ctggatgcctggatgggagt	<i>IL6ST</i>
lhx482-pIL6ST-R	atggtttgcctgcagcatga	
lhx381-pLamb3-F	atcaaacctgcctctctgg	<i>LAMB3</i>
lhx382-pLamb3-R	gcctacctcatcactgggt	
lhx439-pCOL17A1-F	gatgtccttgagacacctca	<i>COL17A1</i>
lhx440-pCOL17A1-R	gggggtgcacaactctgtaa	
Donor-specific primers		
Primer name	Sequence	Donor
lhx213	ATGCTGGAGTTCTTCGCCAC	HIT-trap-FlEx
lhx215	AAAGGACGAAACACCGGTA	HIT-trap-1C
lhx220	GACCGCGAAGAGTTTGTCTT	Universal

lhx217-R	CTGGGGTTCGAAATGACCGA	HIT-trap-2C
lhx266	CTACTGCACCCTTGGTCCTC	
1C-1-F	gaaaggacgaaacaccgGGTA	HIT-trap-
1C-2-R	agggaagaaagcgaaaggagc	pA1C/2C

Supplemental Table S7. Primers used for RT-PCR. All oligos are in the 5' to 3' direction.

Primer name	Sequence	gene
hx256-qmHprt-F	TGGTTAAGCAGTACAGCCCC	<i>Hprt</i>
lhx257-qmHprt-R	ACGTGATTCAAATCCCTGAAGT	
lhxq19-mGAPDH-F	AGGTCGGTGTGAACGGATTTG	
lhxq20-mGAPDH-R	TGTAGACCATGTAGTTGAGGTCA	<i>GAPDH</i>

Supplemental Table S8. Antibodies used for Western blotting.

Antibodies	Catalog
HPRT1	Proteintech, 15059-1-AP
TUBB5	Beyotime Biotechnology, AF1216
ACTB	ABclonal, AC038
GAPDH	Beyotime Biotechnology, AF5009
PPARG	ABclonal, A19676
PAX6	Abcam, ab195045
LDLR	ABclonal, A14996
PCSK9	ABclonal, A11532