

Materials and Methods

Construction of ABE7.10, SaKKH-ABE , VQR-ABE and sgRNA expression vectors.

Human Codon-optimized TadA-linker-TadA* sequences were codon optimized and synthesized by Idobio (Nanjing, China). PCR was performed using KOD –Plus- Neo DNA Polymerase (TOYOBO, Code:KOD-401). Fragments TadA-linker-TadA* , Cas9n(D10A) (template: Addgene Plasmid #42335), SaKKH (template: Addgene Plasmid #85170) , VQR-Cas9 (template: Addgene plasmid #101715, the clone primer was the same as Cas9n(D10A)) were amplified with primers listed in table S3. Fragments TadA-linker-TadA* and corresponding fragment Cas9n(D10A) , SaKKH , VQR-Cas9 were assembled into the pCMV-BE3 (Addgene plasmid # 73021) (digested with NotI and PmeI) using ClonExpress MultiS One Step Cloning Kit (Vazyme) to generate plasmid ABE7.10, SaKKH-ABE, VQR-ABE, respectively (Supplementary sequence 1-3). U6-sgRNA scaffold for SpCas9 or SaCas9 were amplified from PX458 or BPK2660 (Addgene Plasmid #70709) respectively, and cloned into pcDNA 3.1(+) eGFP (Addgene Plasmid #78583) to generate U6-sgRNA(sp)-EF1 α -GFP or U6-sgRNA(sa)-EF1 α -GFP. T7-sgRNA scaffold for SpCas9 or SaCas9 were amplified from PX458 (Addgene Plasmid #48138) or BPK2660 (Addgene Plasmid #70709), and cloned into pcDNA 3.1(+) eGFP (Addgene Plasmid #78583) to generate T7-sgRNA sp-scaffold or T7-sgRNA sa-scaffold, respectively.

Preparation of sgRNA and mRNA

The annealed sgRNAs were cloned into the T7-sgRNA sp-scaffold or T7-sgRNA sa-scaffold. The T7 promoter and different sgRNA templates were amplified using primers IVT-PCF-F/R (sp) and IVT-PCF-F/R (sa) (table S5). The sgRNAs were then transcribed using the in vitro Transcription T7 Kit (MEGAscript™ Kit, Ambion). The T7 promoter was introduced to ABE7.10 or SaKKH-ABE or VQR-ABE mRNA template by PCR using primers IVT-T7-ABE7.10-F/R or IVT-T7-saKKH-ABE7.10-F/R (table S5). ABE7.10, SaKKH-ABE and VQR-ABE mRNAs were transcribed using

the in vitro RNA transcription kit (mMESSAGE mMACHINE® T7 Ultra Kit, Ambion). Both sgRNA and mRNA were purified with MEGAclean™ Kit (Ambion). Chemically modified cr-RNA and trac-RNA were synthesized by Genscript (Nan jing, China) (Supplementary sequence 4).

Cell culture and transfection.

HEK293T (ATCC CRL-3216) cells lines were maintained in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% FBS (Gibico), 100 U ml⁻¹ penicillin, and 100 mg ml⁻¹ streptomycin at 37 °C with 5% CO₂ incubation. Cells were seeded into 24-well plates (Corning) one day before transfection at a density of 200,000 cells per well, and transfected at 70–80% confluency using polyethylenimine (Polysciences) following the manufacturer's recommended protocol. For each well of a 24-well plate, a total of 500 ng DNA was used. 250 ng of SaKKH-ABE and 250 ng of sgRNA expression plasmids were transfected using 1.5 µl of polyethylenimine per well according to the manufacturer's protocol. The cells in a 24-well plate were seeded into a 12-well plate 3 days after transfection. Fluorescence-activated cell sorting (FACS) was performed 5 days after transfection following BD's recommended protocol.

Microinjection of zygotes.

For microinjection, solutions containing complexes of ABE7.10 (50 ng/µl), SaKKH-ABE (50 ng/µl) or VQR-ABE mRNA (50 ng/µl) and sgRNA (50 or 100 ng/µl) were diluted in Nuclease-free Water and injected into cytoplasm using an Eppendorf transferMan NK2 micromanipulator. Injected zygotes were transferred into pseudopregnant female mice or rats immediately after injection or after overnight culture in KSOM medium at 37 °C in a humidified atmosphere consisting of 5% CO₂ in air.

Animals.

C57/BL6 strain mice and Sprague-Dawley strain rats purchased from Shanghai Laboratory Animal Center were housed in standard cages in a specific pathogen-free facility on a 12 h light/dark cycle with ad libitum access to food and water. All animal

experiments conformed to the regulations drafted by the Association for Assessment and Accreditation of Laboratory Animal Care in Shanghai and were approved by the East China Normal University Center for Animal Research. To obtain homozygous mice carrying mutation on the TGA stop codon of *Fah*, the crossed founders were kept on 10 mg/L NTBC water.

Targeted deep sequencing of genomic DNA samples

HEK293T or mouse tail tip genomic DNA was isolated using the blood/cell/tissue DNA Isolation Kit (TianGen) according to the manufacturer's instructions. PCR products for targeted deep sequencing were prepared as described in HI-TOM Kits (Novogene). Mixed samples were sequenced on the Illumina HiSeq platform as previously described (www.hi-tom.net/hi-tom/documentation.html). All primers used for Hi-Tom deep sequencing are listed in table S6.

qPCR

Mice were sacrificed by carbon dioxide asphyxiation. Total RNA was extracted from mouse liver using RNAiso Plus (Takara) and reverse transcribed using HiScript II Q RT SuperMix (Vazyme). qPCRs were run on QuantStudio 3 (Applied Biosystems) using Hieff™ qPCR SYBR® Green Master Mix (YEASEN). Data were normalized to β -Actin. Primers used for qPCR are listed in table S4.

Western blot, histology and immunohistochemistry.

For western blot, mouse liver protein lysates were prepared in RIPA buffer with proteinase and phosphatase inhibitors. Proteins were separated on SDS/PAGE and transferred to nitrocellulose membranes for immunostaining with 1:500 anti-Fah antibody (AbboMax). For immunohistochemistry, mouse livers were fixed with 4% paraformaldehyde (PFA), embedded in paraffin, sectioned at 5 μ m and detected with 1:2000 anti-Fah antibody (AbboMax). For measuring glycogen content, rat muscles tissues were fixed with 4% PFA, frozen in Tissue Freezing Medium (Leica), sectioned at 5 μ m and stained using Glycogen Periodic Acid Schiff (PAS/Hematoxylin) Stain Kit (Solarbio).

Serum biochemical analysis.

Blood was collected using retro-orbital puncture and centrifuged at 12,000 r.p.m. at 4 for 5 min. The serum was frozen at -80°C before being sent to ADICON Clinical Laboratory to determine AST, ALT, TBIL and ALB levels.

GAA activity assay

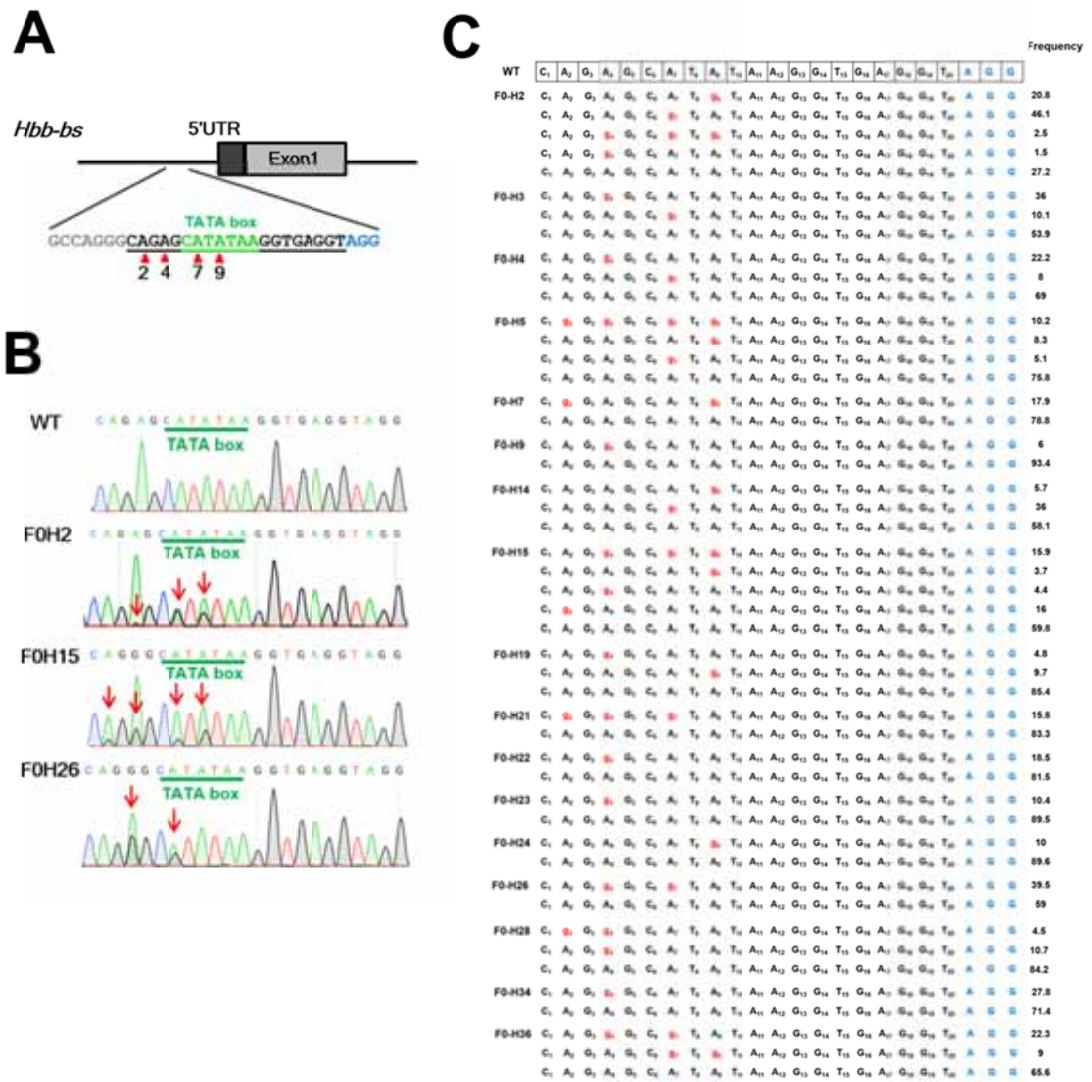
Substrate was 6 mM 4-methylumbelliferone- α -glucopyranoside (Sigma), prepared in 10% 2-methoxyethanol/0.2M phosphate citrate (PC) buffer (0.1M citric acid, 0.2 M Na_2HPO_4 in DI water, pH 4.3). Protein concentrations were determined by BCA Protein Assay Kit (Pierce). The acid α -glucosidase assay reaction mixture in each tube consisted of 50 μL muscle tissue homogenates, 10 μL 100 μM acarbose, and 50 μL 6mM 4-MUG. Two blank tubes with 50 μL water instead of homogenates were incubated along with the samples. All tubes were incubated for 1 hour at 37°C . The reaction was stopped by addition of 1.25 mL of Glycine Carbonate buffer (glycine, 0.17 M; Na_2CO_3 , 0.51M; pH 10.3). Muscle tissue homogenate (50 μL) was added to each blank tube after the reaction was stopped. Fluorescence intensity was measured with a VICTOR2 spectrofluorometer (Finland, Wallac). Acid β -galactosidase at pH4.0 was used as a control. Enzyme activity is expressed as nmol/hour/mg protein.

Statistics

Data are presented as means $\pm$ DS. Means of two groups were compared using Student's t-test (unpaired, 2-tailed), with $P < 0.05$ considered to be statistically significant.

Data availability.

Plasmids encoding ABE7.10 and SaKKH-ABE are available upon request from Addgene. See Supplementary Sequences 3 for full amino acid sequences. High-throughput sequencing data have been deposited in the NCBI Sequence Read Archive database under accession code PRJNA471163.



Supplementary Figure 1. Efficient A>G conversion by ABE on mouse *Hbb-bs* gene

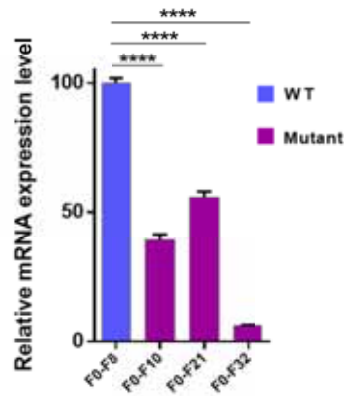
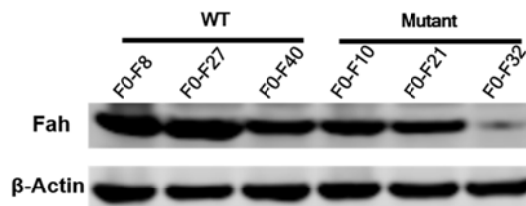
(A) schematic view of the target site in the *Hbb-bs* locus. Target sequence is underlined. PAM sequence is labeled in blue. TATA box is indicated by the green characters. Numbers represent the positions of the adenines within the editing window. (B) Sanger sequencing chromatograms of genomic DNA from WT and mutant F0 founders. Arrows indicate the double-peak signals caused by the A>G conversions (C) Deep sequencing results of the founders carrying mutations in the *Hbb-bs* locus. PAM sequences are labeled in blue. Base substitutions are labeled in red. Allele frequencies are listed to the right.

A

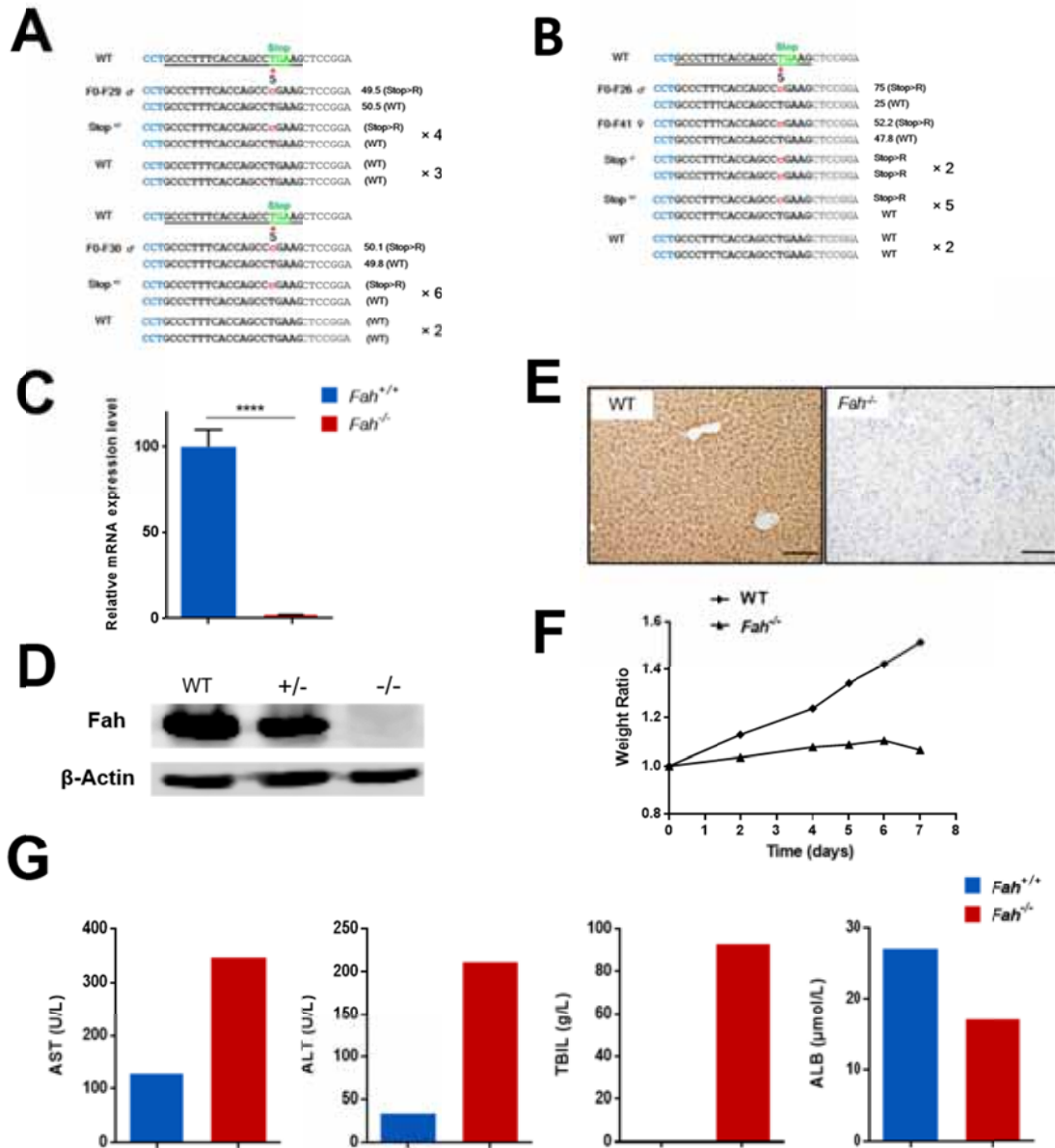
No.	Frequency (%)		No.	Frequency (%)	
	T>C	WT		T>C	WT
F0-F2	54.1	45.8	F0-F24	7.6	92.4
F0-F3	19.2	80.8	F0-F26	75	25
F0-F4	57.2	42.6	F0-F28	49.7	50.3
F0-F5	55.4	44.3	F0-F29	49.5	50.5
F0-F6	10.3	89.6	F0-F30	50.1	49.8
F0-F7	48.9	51	F0-F31	79.9	20.1
F0-F9	54.2	45.7	F0-F32	99	0
F0-F10	77.6	22.3	F0-F33	13.5	86.4
F0-F11	59.4	40.6	F0-F34	91.2	8.8
F0-F12	40.8	59.1	F0-F36	9.6	90.4
F0-F14	33.2	66.1	F0-F37	73	26.9
F0-F15	48.3	51.7	F0-F38	39	61
F0-F16	33.2	66.4	F0-F39	22	77.9
F0-F17	24.4	75.6	F0-F41	52.2	47.8
F0-F18	43.8	56.1	F0-F42	22.4	77.5
F0-F19	49.6	50.3	F0-F44	17.2	82.5
F0-F20	28.3	71.5	F0-F45	40.8	59.2
F0-F21	77	23	F0-F46	48.3	51.4
F0-F22	38.7	61	F0-F47	23.1	76.8
F0-F23	46	54			

B

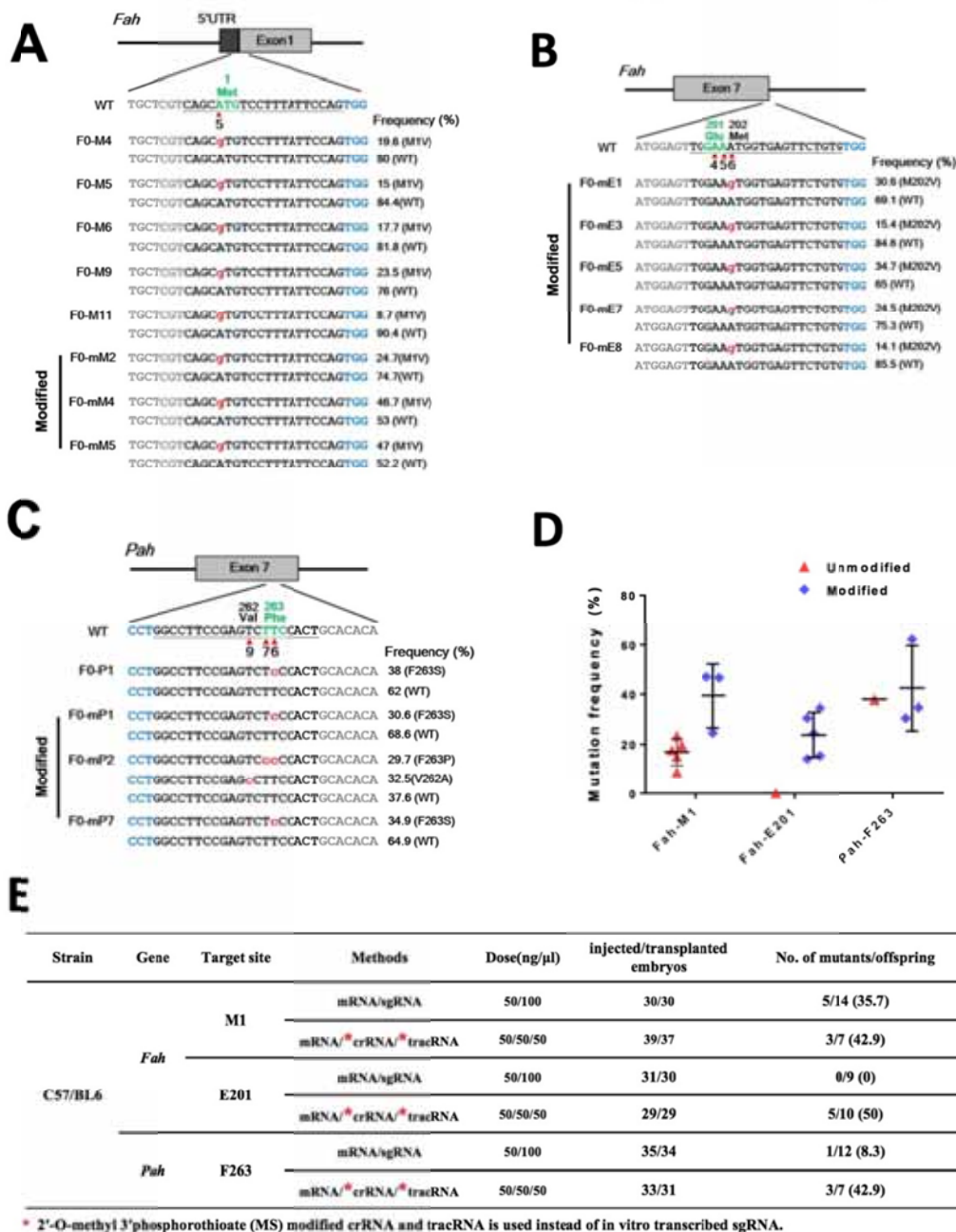
No.	Frequency (%)		No.	Frequency (%)	
	T>C	WT		T>C	WT
F0-hF1	65.5	34.5	F0-hF8	86.2	13.8
F0-hF2	65.7	34.3	F0-hF9	62.4	37.6
F0-hF3	86.1	13.6	F0-hF10	83.4	16.6
F0-hF4	82.9	16.6	F0-hF11	62.5	37.5
F0-hF5	73.5	26.4	F0-hF12	50.2	49.8
F0-hF6	71.6	28.4	F0-hF13	72	28
F0-hF7	56.8	43.2			

C**D**

Supplementary Figure 2. Disruption of the stop codon on the mouse *Fah* gene via ABE (A-B) Allelic frequencies of each founder mutated by ABE via low sgRNA concentration (A) or high sgRNA concentration (B). (C) Relative mRNA expression of *Fah* gene from WT and three mutant F0 founders. Data presented as Mean $\pm$ SD (replicate = 3). ***, $p < 0.01$. (D) Western blot analysis of *Fah* expression in the liver tissue from the WT and mutant F0 founders.



Supplementary Figure 3. Pathological defects of F1 homozygotes generated by the ABE system (A-B) Germline transmission of mutations at the *Fah* stop codon through crossing founders with WT mice (A) or crossing between founders (B). (C) *Fah* mRNA expression in *Fah*^{+/+} and *Fah*^{-/-} mouse liver tissue. Data presented as Mean ± SD. ***, $p < 0.01$. (D) Western blot analysis of *Fah* expression in the liver tissue from WT, heterozygous and homozygous offspring. (E) IHC staining of *Fah* protein on liver sections from WT and *Fah*^{-/-} mouse. Scale bar, 100 μm. (F) Body weight ration of WT and *Fah*^{-/-} mouse after NTBC withdrawal. (G) Serum AST, ALT, TBIL and ALB levels of WT and *Fah*^{-/-} mice, 7 days after NTBC withdraw.



Supplementary Figure 4. Increasing ABE efficiency through increasing sgRNA stability (A-C) Mutation frequencies in individual mice induced by ABE with chemically modified cr-tracrRNAs in *Fah*-E201 site (A) , *Fah*-M1 site (B) and *Pah*-F263 site (C). PAM sequences are labeled in blue. Base substitutions are labeled in red. Allele frequencies are listed to the right. (D) The average mutation frequencies in each founder obtained via injection of modified crRNA/tracrRNAs or unmodified sgRNAs.(E) Micro-injection parameters used to inject ABE mRNA along with modified cr-tracrRNAs or unmodified sgRNAs

	644 655 666 Ala Asn Ser	Frequency (%)
WT	GCTGTGGCGACATCTCGGGTTCCAAGG 3 5 7	
FO-G4	GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG	42.7 (646V) 18.6 (D646G, 646V) 5.5 (D646I) 34.5 (WT)
FO-G5	GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGAGCGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG	23.8 (646V) 9.4 (D646G, 646V) 2.5 (D646I, 646V) 63.8 (WT)
FO-G6	GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG	52.9 (646V) 27.8 (D646G, 646V) 19.2 (WT)
FO-G7	GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG	48.5 (646G, 646V) 8.8 (646V) 44.2 (WT)
FO-G8	GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG	22.2 (D646G, 646V) 53.8 (WT) 23.9 (646V)
FO-G9	GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG	46.8 (D646G, 646V) 27.8 (646V) 25.3 (WT)
FO-G10	GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG	13.4 (646V) 90.1 (646V) 54.5 (WT)
FO-G11	GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG	29.5 (646V) 8.7 (D646I, 646V) 28 (D646G) 33.7 (WT)
FO-G13	GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG	14.4 (646V) 41.8 (646V) 44 (WT)
FO-G14	GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG	64.8 (646V) 35.4 (WT)
FO-G15	GCTGTGGCGACAGCTCTCGGGTTCCAAGG GCTGTGGCGACAGCTCTCGGGTTCCAAGG	58.4 (646V) 43.4 (WT)

F0-G18	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	14.5 (844bV)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	14.8 (D64SG, 844bV)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	7.7 (D64SG)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	62.3 (NT)
F0-G20	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	28.1 (844bV)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	72.8 (NT)
F0-G21	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	33.2 (844bV)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	88.4 (NT)
F0-G23	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	47.7 (D64SG, 844bV)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	31.3 (NT)
F0-G24	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	41.8 (844bV)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	7.2 (D64SG)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	50.3 (NT)
F0-G25	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	8.4 (D64SG, 844bV)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	30.2 (844bV)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	80.7 (NT)
F0-G27	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	32.2 (844bV)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	86.1 (NT)
F0-G28	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	55.1 (844bV)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	44.9 (NT)
F0-G29	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	41.7 (D64SG, 844bV)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	57 (844bV)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	1.3 (NT)
F0-G30	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	43.8 (844bV)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	5.8 (D64SG)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	50.3 (NT)
F0-G31	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	55.8 (844bV)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	43.8 (NT)
F0-G32	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	47.8 (844bV)
	GOTGGGGCGAGACATCTGCGGGCTTCCAGG	81.8 (NT)

Enzyme activity (nmol/h/mg)

Intercostal muscle

Erector spinae

Legend: F0-G16 (WT) (blue), F0-G22 (red), F0-G26 (green)

Tissue	F0-G16 (WT)	F0-G22	F0-G26
Intercostal muscle	~33	~9	~18
Erector spinae	~24	~9	~16

	544	545	546	
	Ala	Arg	Thr	
WT	GGTCGGG	<u>GCAGC</u>	ATCTCGGG	TTCAAGG
		4 5		
		3 5 7		
F0-G7 ♂	GGTCGGG	<u>GCAGC</u>	ATCTCGGG	TTCAAGG
		4 5		48.5 (D645G, I64V)
		3 5 7		8.8 (I64V)
				44.2 (WT)
F0-G12 ♀	GGTCGGG	<u>GCAGC</u>	ATCTCGGG	TTCAAGG
		4 5		56 (I64V)
		3 5 7		42.7 (D645G, I64V)
D645 ⁺ I646 ⁻	GGTCGGG	<u>GCAGC</u>	ATCTCGGG	TTCAAGG
		4 5		D645G, I64V
		3 5 7		WT
D645 ⁻ I646 ⁺	GGTCGGG	<u>GCAGC</u>	ATCTCGGG	TTCAAGG
		4 5		D645G, I64V
		3 5 7		D645G, I64V
F0-G14 ♀	GGTCGGG	<u>GCAGC</u>	ATCTCGGG	TTCAAGG
		4 5		84.8 (I64V)
		3 5 7		35.4 (WT)
F0-G4 ♂	GGTCGGG	<u>GCAGC</u>	ATCTCGGG	TTCAAGG
		4 5		42.7 (I64V)
		3 5 7		16.6 (D645G, I64V)
				5.5 (D645G)
				34.5 (WT)
I646 ⁻	GGTCGGG	<u>GCAGC</u>	ATCTCGGG	TTCAAGG
		4 5		I64V
		3 5 7		WT
I646 ⁺	GGTCGGG	<u>GCAGC</u>	ATCTCGGG	TTCAAGG
		4 5		I64V
		3 5 7		I64V
WT	GGTCGGG	<u>GCAGC</u>	ATCTCGGG	TTCAAGG
		4 5		WT
		3 5 7		WT

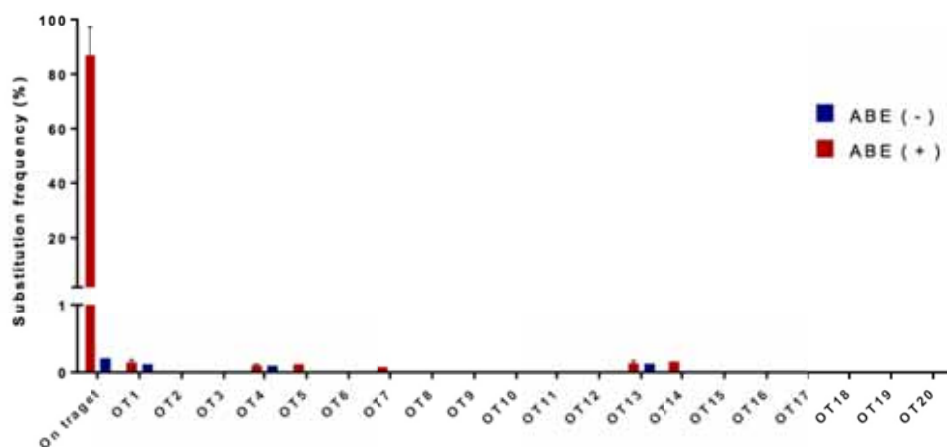
Enzyme activity (nmol/h/mg)

Muscle	WT	I646V	D645G/I646V
Heart	~17	~14	~2
Diaphragm	~22	~10	~2
Tibialis	~20	~19	~2

Legend: WT (blue), I646V (purple), D645G/I646V (red)

Significance levels: * p < 0.05, ** p < 0.01, *** p < 0.001.

Supplementary Figure 5. Generation of acid alpha-glucosidase mutant rats through the ABE system (A) Mutation frequencies of individual rat induced by ABE in the *Gaa* locus. PAM sequences are labeled in blue. Base substitutions are labeled in red. Allele frequencies are listed to the right. (B) *Gaa* enzyme activities in intercostal muscle and erector spinae from 4 week old WT and mutant founders. Data presented as Mean $\pm$ SD. (replicate = 3). ***, $p < 0.01$. (C) Germline transmission of mutations at the *Gaa* locus through crossing founders with other founders. PAM sequences are labeled in blue. Base substitutions are labeled in red. F1rat quantities are listed to the right. (D) *Gaa* enzyme activities in heart, diaphragm, and tibialis anterior from 3 week old WT, I656V and D645G/I646V homozygous rats. Data presented as Mean $\pm$ SD. (replicate = 3). *, $P < 0.05$. ***, $P < 0.01$. (E) PAS staining of rectus femoris cryosections from WT, I646V and D645G/I646V homozygous rats. Scale bar, 20 μ m.



Supplementary Figure 6. Editing frequencies of ABE in predicted off target sites.

Base editing frequencies of ABE at potential off target sites of Fah-stop predicted by <http://crispr.mit.edu/> were measured by Hi-Tom deep sequencing. Genomic DNA from 4 week old mouse tail tips was extracted and pcr amplified for Hi-Tom deep sequencing.

Supplementary Table 1. Target sites in mouse embryos, and HEK293T cells.

		No.	Sequence (5'-3')
ABE	Mouse embryos	Fah-stop	CTTCAGGCTGGTGAAAGGGCAGG
		Fah-E201	TGGAAATGGTGAGTTCTGTGTGG
		Fah-M1	CAGCATGTCCTTTATTCCAGTGG
		Pah-F263	AGTGGAAGACTCGGAAGGCCAGG
		GAA-D645	GCAGACATCTGCGGCTTCCAGGG
		Hbb-bs	CAGAGCATATAAGGTGAGGTAGG
SaKKH-ABE	HEK293T cells	EMX1 site 1	CGGATGCACGGTCAGCGCGGGGTGGT
		EMX1 site 2	CAACCACAAACCCACGAGGGCAGAGT
		RUNX1 site 1	AAAGAGAGATGTAGGGCTAGAGGGGT
		Hek293 site 1	AGAGACACAGAGATGTCATGGAGAGT
	Mouse embryos	Otc	CACAAGACATTCACTTGGGTGTGAAT
VQR-ABE	HEK293T cells	VEGFA Site 1	GCGAGCAGCGTCTTCGAGAGTGAG
		VEGFA Site 2	AAACAGAGAAACTAAGGGAAAGAC
		RUNX1 site 3	AGAAAGAGAGATGTAGGGCTAGAG
		RUNX1 site 4	CAGAAGAGGGTGCAATTTTCAGGAG
	Mouse embryos	Hbb-bs	AGAGCATATAAGGTGAGGTAGGAT

Supplementary Table 2. Potential off-target sites of Fah-stop sgRNA in the mouse genome.

No.	Sequence (5'-3')	Chromosome	Position	Direction	Mismatches
on target	CTTCAGGCTGGTGAAAGGGCAGG	chr7	91733777	-	0
OT1	CTTgAGGCTGGTaAAAGGGCTGG	chr2	110803644	+	2
OT2	CaTgAGtCTGGTGAAAGGGCTGG	chr7	124292990	+	3
OT3	CTTagGGCTGGgGAAAGGGCAGG	chr9	45541246	+	3
OT4	tTTCAGGCTGccGAAAGGGCAGG	chr15	67559328	+	3
OT5	CcTCAGGCaGGTGAAAtGGCAGG	chr19	58733308	+	3
OT6	CTTCAGGCaaGTGAAAGGaCAGG	chr18	52821719	-	3
OT7	CTTCAGtCTGaTaAAAGGGCAGG	chr7	118609893	-	3
OT8	CaTtAaGCTtGTGAAAGGGCAGG	chr9	15919356	-	4
OT9	gTcCAGGCTttTGAAAGGGCGGG	chrX	3518390	-	4
OT10	CTgggGGCTGGTGAAAtGGCAGG	chr4	62063879	+	4
OT11	ggTCAtGCTGGTGAAAtGGCTGG	chr8	9242931	-	4
OT12	CTTggGGCTaGTGAcAGGGCTGG	chr6	56312390	+	4
OT13	CTaCcGaCTGGTcAAAGGGCTGG	chr4	138147304	-	4
OT14	CTTAgGtCTGGTcAAAGGGCAGG	chr4	34446483	-	4
OT15	CaTCtGGaTGGTGAAAGGGtGGG	chr15	38502392	+	4
OT16	gTcCAGGCTGcTGAAAGGGaTGG	chr16	70689741	+	4
OT17	CTaCAGcaTGGaGAAAGGGCTGG	chr2	80450019	-	4
OT18	CTTggGgCTGGgGAAAGGGCTGG	chr7	87740211	-	4
OT19	CTTtcaGCTGGaGAAAGGGCTGG	chr3	149023365	-	4
OT20	gccCAGGCTGGTGAgAGGGCTGG	chr14	67495093	-	4

Supplementary Table 3. List of primers used for plasmid construction.

Sequence ID	Sequence (5'-3')
ABE7.10-F	AGATCCGCTAGAGATCCGCGGCCGCCACCATGAGCGAGGTGGA
ABE7.10-F(in)	GAGGCTCTTCTGGAGGATCAGATAAAAAGTATTCTATTGGTTTAGCC
ABE7.10-R(in)	TGATCCTCCAGAAGAGCCTCCG
ABE7.10-R	CGAGGCTGATCAGCGGGTTTAAACTTAGACTTTCCTCTTCTTCTTGGG
saKKH-ABE-F	AGATCCGCTAGAGATCCGCGGCCGCCACCATGAGCGAGGTGGA
SaKKH-ABE-F(in)	CAGCGGAGGCTCTTCTGGAGGATCAAAAAGGAACTACATTCTGGGGCTGGCC
SaKKH-ABE-R(in)	GTTCCCTTTTGATCCTCCAGAAGAGCCTCCGCTGCTCT
SaKKH-ABE-R	ACAGTCGAGGCTGATCAGCGGGTTTAAACTTAAGCGTAATCTGGTACGTCGTATGG

Supplementary Table 4. List of primers used for q-PCR and RT-PCR.

Sequence ID	Sequence (5'-3')
Fah-qPCR-F	CTTCGGCAGCGTGCATTC
Fah-qPCR-R	GCCATGGTATCCCACAGGTA
Mouse-Actin-F	GTGTGACGTTGACATCCGTAA
Mouse-Actin-R	CCACCGATCCACACAGAGTA

Supplementary Table 5. List of Oligonucleotides and Primers used for sgRNA and mRNA preparation.

sequence ID	Sequence (5'-3')
Fah-stop-up	TAGGCTTCAGGCTGGTGAAAGGGC
Fah-stop-dn	AAACGCCCTTTCACCAGCCTGAAG
Fah-E201-up	TAGGGTGGAAATGGTGAGTTCTGTG
Fah-E201-dn	AAACCACAGAACTCACCATTTCAC
Fah-M1-up	TAGGCAGCATGTCCTTTATCCAG
Fah-M1-dn	AAACCTGGAATAAAGGACATGCTG
Pah-F263-up	TAGGAGTGGAAGACTCGGAAGGCC
Pah-F263-dn	AAACGGCCTTCCGAGTCTTCCACT
GAA-D645-up	TAGGGCAGACATCTGCGGCTTCCA
GAA-D645-dn	AAACTGGAAGCCGCAGATGTCTGC
Hbb-bs-TATA-up	TAGGCAGAGCATATAAGGTGAGGT
Hbb-bs-TATA-dn	AAACACCTCACCTTATATGCTCTG
EMX1-site2-up	CACCGCAACCACAAACCCACGAGGG
EMX1-site2-dn	AAACCCCTCGTGGGTTTGTGGTTGC
EMX1-site1-up	CACCGCGGATGCACGGTCAGCGCGG
EMX1-site1-dn	AAACCCGCGCTGACCGTGCATCCGC
HEK293 site 2-up	CACCGAGAGACACAGAGATGTCATG
HEK293 site 2-dn	AAACCATGACATCTCTGTGTCTCTC
RUNX1-site1-up	CACCGAAAGAGAGATGTAGGGCTAG
RUNX1-site1-dn	AAACCTAGCCCTACATCTCTCTTTC
VEGFA-site1-up	CACCGCGAGCAGCGTCTTCGAGAG
VEGFA-site1-dn	AAACCTCTCGAAGACGCTGCTCGC
VEGFA-site2-up	CACCGAAACAGAGAACTAAGGGAA
VEGFA-site2-dn	AAACTTCCCTTAGTTTCTCTGTTTC
RUNX1-site3-up	CACCGAGAAAGAGAGATGTAGGGCT
RUNX1-site3-dn	AAACAGCCCTACATCTCTCTTTC
RUNX1-site4-up	CACCGCAGAAGAGGGTGCAATTTCA
RUNX1-site4-dn	AAACTGAAAATGCACCCTCTTCTGC
Hbb-bs-up	TAGGAGAGCATATAAGGTGAGGTA
Hbb-bs-dn	AAACTACCTCACCTTATATGCTCT
Mouse-Otc- T7-up	TAGGCACAAGACATTCACTTGGGT
Mouse-Otc- T7-dn	AAACACCCAAGTGAATGTCTGTG
IVT-PCF-F	GCGGCTTTGTTGAATAAATCGCATTCG
IVF-PCR-R(sp)	AGAGGATCCTTTAAAAGCACCGACTCGGTGCC
IVF-PCR-R(sa)	GAGGATCCAAAAAATCTCGCCAACAAGTTGACGA
IVT-T7-ABE7.10-F	TTAATACGACTCACTATAGGGAGAGCCGCCACCATGAGCGAGGTGGA
IVT-T7-ABE7.10-R	TCCGCCTCAGAAGCCATAGA
IVT-T7-saKKH-ABE-F	TTAATACGACTCACTATAGGGAGAATGAGCGAGGTGGAGTTCAGCC
IVT-T7-saKKH-ABE-R	TCGAGGCTGATCAGCGGGTTAAACTT

Supplementary Table 6. List of primers used for targeted deep sequencing.

sequence ID	Sequence (5'-3')
Fah-stop- hi-tom-F	GGAGTGAGTACGGTGTGCCAGTGATGTGGCTGATCCCA
Fah-stop- hi-tom-R	GAGTTGGATGCTGGATGGACTGATGCAGTGGTAGCATGA
Fah-E201- hi-tom-F	GGAGTGAGTACGGTGTGCAGCTCTGTAGCCTGGTATTGATG
Fah-E201- hi-tom-R	GAGTTGGATGCTGGATGGCCAAACAGGTGTGAAGTGCCG
Pah-F263- hi-tom-F	GGAGTGAGTACGGTGTGCCTTTCCAGCTTGACTGGTTTC
Pah-F263- hi-tom-F-R	GAGTTGGATGCTGGATGGTTTGAGCATCCATTGTGGTTGG
Fah-M1-hi-tom-F	GGAGTGAGTACGGTGTGCTAAAGGCCCTCGGCTAGTCT
Fah-M1-hitom-R	GAGTTGGATGCTGGATGGGCTCACGTTGCTTTGAGTGG
GAA-D645- hi-tom-F	GGAGTGAGTACGGTGTGCTTGGTAACCTGGCACCCTC
GAA-D645- hi-tom-R	GAGTTGGATGCTGGATGGAGGTCATTGTGGTTCCGCAT
Hbb-bs-F	TTGTCATCACCGAAGCCTGAT
Hbb-bs-R	AAGCACCAACTTCTTGTGAG
Hbb-bs-hi-tom-F	GGAGTGAGTACGGTGTGCGATTCCGTAGAGCCACACCC
Hbb-bs-hi-tom-R	GAGTTGGATGCTGGATGGCAGCAGCCTTCTCAGCATCA
EMX1-site2- hi-tom-F	GGAGTGAGTACGGTGTGCAAGAAGGGCTCCCATCACATCAACC
EMX1-site2- hi-tom-R	GAGTTGGATGCTGGATGGGAGTGGCCAGAGTCCAGCTGGG
EMX1-site1- hi-tom-F	GGAGTGAGTACGGTGTGCCTTCGTGAGTGGCTTCCCTGCC
EMX1-site1- hi-tom-F	GAGTTGGATGCTGGATGGGAAGAAGGAGTGCGGGGGCTG
RUNX1-site1- hi-tom-F	GGAGTGAGTACGGTGTGCTTTAATAGGGCTTGGGGAGTCCCAG
RUNX1-site1- hi-tom-R	GAGTTGGATGCTGGATGGCATCGCTTCCTCCTGAAAAATGCACC
HEK293 site2-hi-tom-F	GGAGTGAGTACGGTGTGCCTGGGTAAGGTCTACTGTGA
HEK293 site2-hi-tom-R	GAGTTGGATGCTGGATGGTCATTAAATAAATTACAAAG
VEGFA-site1-hi-tom-F	GGAGTGAGTACGGTGTGCGTGTGCAGACGGCAGTCACTAGG
VEGFA-site1-hi-tom-R	GAGTTGGATGCTGGATGGCTATTGGAATCCTGGAGTGACCCCT
VEGFA-site2-hi-tom-F	GGAGTGAGTACGGTGTGCTGAGCAATGAACCATTTGGAATTGA
VEGFA-site2-hi-tom-R	GAGTTGGATGCTGGATGGACCACTCCAGCAGAGACAACAACAT
RUNX1-site3-hi-tom-F	GGAGTGAGTACGGTGTGCTTTAATAGGGCTTGGGGAGTCCCAG
RUNX1-site3-hi-tom-R	GAGTTGGATGCTGGATGGCATCGCTTCCTCCTGAAAAATGCACC
RUNX1-site4-hi-tom-F	GGAGTGAGTACGGTGTGCTTTAATAGGGCTTGGGGAGTCCCAG
RUNX1-site4-hi-tom-R	GAGTTGGATGCTGGATGGCATCGCTTCCTCCTGAAAAATGCACC
Mouse-Otc- hi-tom-F	GGAGTGAGTACGGTGTGCATAGCTGGTGCAAGTACTGATGCCT
Mouse-Otc- hi-tom-R	GAGTTGGATGCTGGATGGTCTGTGAGACTTTCATTACACCCA

Supplementary Table 7. List of primers used for deep sequencing of potential off-target sites of Fah-stop site.

sequence ID	Sequence (5'-3')
Fah-stop-OT1-F	GGAGTGAGTACGGTGTGCTATCCTTCAACGCTCTCCTTGAT
Fah-stop-OT1-R	GAGTTGGATGCTGGATGGTTGACACTGTGACTTGTGGCT
Fah-stop-OT2-F	GGAGTGAGTACGGTGTGCTTGCAGTGACTTGACCAACC
Fah-stop-OT2-R	GAGTTGGATGCTGGATGGTGGACATTTCTATGCCTGTGTTTAG
Fah-stop-OT3-F	GGAGTGAGTACGGTGTGCTTCCTCAGACAGGAGCAGGT
Fah-stop-OT3-R	GAGTTGGATGCTGGATGGTGCAGATCTGTGGCTGATGG
Fah-stop-OT4-F	GGAGTGAGTACGGTGTGCTCTGGCAGAAATGTTCTCATCC
Fah-stop-OT4-R	GAGTTGGATGCTGGATGGAAAAGCATCCGTGGCTGTATC
Fah-stop-OT5-F	GGAGTGAGTACGGTGTGCCACACAGATGGAAAGGGGGA
Fah-stop-OT5-R	GAGTTGGATGCTGGATGGAGACCCCCATCCAAACCTCT
Fah-stop-OT6-F	GGAGTGAGTACGGTGTGCATGATGGTGGGCAATCCCTG
Fah-stop-OT6-R	GAGTTGGATGCTGGATGGGTATGGGTGAGGAGATGCCA
Fah-stop-OT7-F	GGAGTGAGTACGGTGTGCCATGCATCATGGTGCTAAAAGTG
Fah-stop-OT7-R	GAGTTGGATGCTGGATGGAAACTGTCCAAGATGTGACCC
Fah-stop-OT8-F	GGAGTGAGTACGGTGTGCATGCTCCCCAACTGGGTCAA
Fah-stop-OT8-R	GAGTTGGATGCTGGATGGTATTAGCACGTCCTATGGGCA
Fah-stop-OT9-F	GGAGTGAGTACGGTGTGCCAAAGGCAAATGACCTGGACC
Fah-stop-OT9-R	GAGTTGGATGCTGGATGGAAGCCTGAATTTGCGCGG
Fah-stop-OT10-F	GGAGTGAGTACGGTGTGCACTCCTCAATGTCGTCGAGC
Fah-stop-OT10-R	GAGTTGGATGCTGGATGGACTGTTCTGTGTACAGATTATC
Fah-stop-OT11-F	GGAGTGAGTACGGTGTGCAGGGTTAGTCTCAAAGAGGGGT
Fah-stop-OT11-R	GAGTTGGATGCTGGATGGTGATACTCAGATCTGCTACAAGAGA
Fah-stop-OT12-F	GGAGTGAGTACGGTGTGCCAAGCCCTGCTGACATGCAA
Fah-stop-OT12-R	GAGTTGGATGCTGGATGGCGGGCAGCCATCATTGTAGG
Fah-stop-OT13-F	GGAGTGAGTACGGTGTGCGGACACTGGAGGCCCTTCTAT
Fah-stop-OT13-R	GAGTTGGATGCTGGATGGATCCCACTGGGGACAATGATAC
Fah-stop-OT14-F	GGAGTGAGTACGGTGTGCCCAGGGATCTCAAAAATCTAGCC
Fah-stop-OT14-R	GAGTTGGATGCTGGATGGTAAGGCACTGCAGGTAACCTCC
Fah-stop-OT15-F	GGAGTGAGTACGGTGTGCATGAGGGTGTACCTTAGAGAGGC
Fah-stop-OT15-R	GAGTTGGATGCTGGATGGAGGATGGCGTCAGCAGCAAA
Fah-stop-OT16-F	GGAGTGAGTACGGTGTGCTCTGAGTGTTCTTTCAGCCATA
Fah-stop-OT16-R	GAGTTGGATGCTGGATGGCATGAAGGCATGCTAAGTGAAAC
Fah-stop-OT17-F	GGAGTGAGTACGGTGTGCACCCAGCTCCATTGCTTTCA
Fah-stop-OT17-R	GAGTTGGATGCTGGATGGTCTAACGGGGTAACCAAAGC
Fah-stop-OT18-F	GGAGTGAGTACGGTGTGCGGGGTTTTTCTCTGTGATTTCG

Fah-stop-OT18-R	GAGTTGGATGCTGGATGGTAGCAGGTTTGTGATGTTTATGC
Fah-Stop-OT19-F	GGAGTGAGTACGGTGTGCGAGGGTGTGTGGCTTCAACTG
Fah-stop-OT19-R	GAGTTGGATGCTGGATGGTCTTGCAAAAGTTTAAGAAGAGCCT
Fah-Stop-OT20-F	GGAGTGAGTACGGTGTGCCCTTCTAGATCGCAGCCTCTT
Fah-stop-OT20-R	GAGTTGGATGCTGGATGGTGCTAGAGTAGGGCCAAGAAC

Supplementary Sequences 1. Amino acid sequences of ABE.

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWN-
RPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVF-
GARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQK-
KAQSSTDSSGGSSGGSSGSETPGTSESATPESSGGSSGSSSEVEFSHEYWMRHALTLAKRAR-
DEREVPVGAVLVHNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVT-
FEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALL-
CYFFRMPRQVFNAQKKAQSSTDSSGGSSGGSSGSETPGTSESATPESSGGSSGSSDKKYSIG-
LAIGTNSVGWAVITDEYKVPSSKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRL-
KRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLVESFLVEEDKKHERHPIFGNI-
VDEVAYHEKYPTIYHLRKKLV DSTDKADLR LIYL-
ALAHMIKFRGHFLIEGDLNPDNSDVKLFQILVQTYNQLFEENPINASGVDAKAIL-
SARLSKSRRLLENLIAQLPGEKKNGLFGNLIALSLGLTPNFK-
SNFDLAEDAKLQLSKD TYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVN-
TEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDG-
GASQEEFYKFIKPILEKMDGTEELLVKNLREDLLRKQRTFDNGSIPHQIHLGELHAIL-
RRQEDFYPFLLKDNREKIEKILTFRIPIYVGPLARGNSRFAWMTRKSEETIT-
PWNFEVVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSLLYEYFTVYNELTKVKYVTEGMRK-
PAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRF-
NASLGTYYHDLKIIKDKDFLDNEENEDILEDIVLTTLTLFEDREMI-
EERLKTYYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRN-
FMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHK-
PENIVIEMARENQTTQKGQKNSRERMKRIE EGikelGSQILKE-
HPVENTQLQNEKLYLYYLQNGRDMYVDQELD-
INRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNY-
WRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRM-
NTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIK-
KYPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKR-
PLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF-
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVEKGK-
SKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLA-
SAGELQKGNELALPSKYVNFYLYLASHYEKLKG-
SPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVLSAYNKH RDKPIREQAE-
NIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ SITGLYETRIDLSQLGG-
DSGGSPKKKRKV*

Supplementary Sequences 2. Amino acid sequences of SaKKH-ABE.

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWN-
RPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVF-
GARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAK-
KAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGSSSEVEFSHEYWMRHALTLAKRAR-
DEREVPVGAVLVHNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVT-
FEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVE-
ITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGS-
ETPGTSESATPESSGGSSGSKRNYILGLAIGITSVGYGI-
IDYETRDVIDAGVRLFKENVENNEGRRSKRGARRL-
KRRRRHRIQRVKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEFSAALL-
HLAKRRGVHNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRG-
SINRFTSDYVKEAKQLLVQKAYHQLDQSFIDTYIDLLETRRTYYEGPGE-
SPFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYNALNDLNNL-
VITRDENEKLEYEYEFQIENVFQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFT-
NLKVYHDIKDITARKEIENAEELLDQIA-
KILTIYQSSEDIQEELTNLNSLTQEEIEQISNLKGYTGTHNLSLKAINLILDELWHTND-
NQIAIFNRLKLVPKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIK-
KYGLPNDIIIELAREKNSKDAQKMINEMQKRNRQTNERIEEIIRTTGKENAKYLIEKI-
KLHDMQEGKCLYSLEAIPLEDLLNPNFNYEVDHIIPRSVSFDNSFNN-
KVLVKQEENSKKGNRTPFQYLSSSDSKISYETFKKHILNLAKGKGRIS-
KTKKEYLLEERDINRFSVQKDFINRNLVDTRYATRGLMNLLRSYFRVNNLDVKVKS-
INGGFTSFLRRKWKFKKERNKGYKHHAEADALIINANAD-
FIFKEWKKLDKAKKVMENQMFEKQAESMPEIETEQEYKEIFITPHQIKHIK-
DFKDYKYSHRVDKKPNRKLINDTLYSTRKDDKGNTLIVNNLNGLYDKDNDKLKLINK-
SPEKLLMYHHPQTYQKLKLIMEQYG-
DEKNPLYKYYEETGNYLTKYSKKDNGPVIKKIKYYGNKLNALHDITDDYPNSRN-
KVVKLSLKPYRFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKCYEEAKKLKKISNQAE-
FIASFYKNDLIKINGELYRVIGVNNDLLNRIEVNMIDITYREYLENMNDKRPPHIK-
TIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKGGSPKKKRKVSSYPYDVPDYA*

Supplementary Sequences 3. Amino acid sequences of VQR-ABE.

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWN-
RPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVF-
GARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAK-
KAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGSSEVEFSHEYWMRHALTLAKRAR-
DEREVPVGAVLVHNNRVIGEGWNRAGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVT-
FEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVE-
ITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGS-
ETPGTSESATPESSGGSSGGSDKKYSIG-
LAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRL-
KRTARRRYTRRKNRICYLQEIFSNEMAKVDDSSFFHRLEESFLVEEDKKHERHPIFGNI-
VDEVAYHEKYPTIYHLRKKLVDSTDKADRLIYL-
ALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEENPINASGVDAKAIL-
SARLSKSRRLLENLIAQLPGEKKNGLFGNLIASLGLTPNFK-
SNFDLAEDAKLQLSKDQYDDDLNLLAQIGDQYADLFLAAKNLSDAILSDILRVN-
TEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDG-
GASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAIL-
RRQEDFYPLKDNREKIEKILTFRIPIYYVGPLARGNSRFAWMTRKSEETIT-
PWNFEVVDKGASAQSFIERMTNFDKNLPNEKVLPHSLLYEYFTVYNELT-
KVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEIS-
GVEDRFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTTLTFEDREMI-
EERLKTIAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRN-
FMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIAK-
KGILQTVKVVDDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRERMKRIEE-
GIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELD-
INRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKMKMKNY-
WRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRM-
NTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTA-
LIK-KYPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKR-
PLIETNGETGEIVWDKGRDFATVRKVLSPQVNVKKEVQTGGF-
SKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKEVGK-
SKKLKSVKELLGITIMERSSEFKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLA-
SAGELQKGNELALPSKYVNFLYLASHYEKLKG-
SPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVLSAYNKHHRDKPIREQAE-
NIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSTGLYETRIDLSQLGG-
DSGGSPKKKRKV*

Supplementary Sequences 4. Chemically modified crRNA and tracrRNA used in this study. The 2'-O-methyl 3'phosphorothioate (MS) modified bases are colored *red* and marked with *.

Fah-E201-crRNA

U*G*G*AAAUGGUGAGUUCUGUGGUUUUAGAGCUAUGCUGUUUUG

Pah-F263-crRNA

A*G*U*GGAAGACUCGGAAGGCCGUUUUAGAGCUAUGCUGUUUUG

Fah-M1-crRNA

C*A*G*CAUGUCCUUUAUCCAGGUUUUAGAGCUAUGCUGUUUUG

tracrRNA

ACAGCAUAGCAAGUUA AAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCAC-
CGAGUCGGUGCU*U*U*U