Supplementary Information

Cytosine base editors induce off-target mutations and adverse phenotypic effects in transgenic mice

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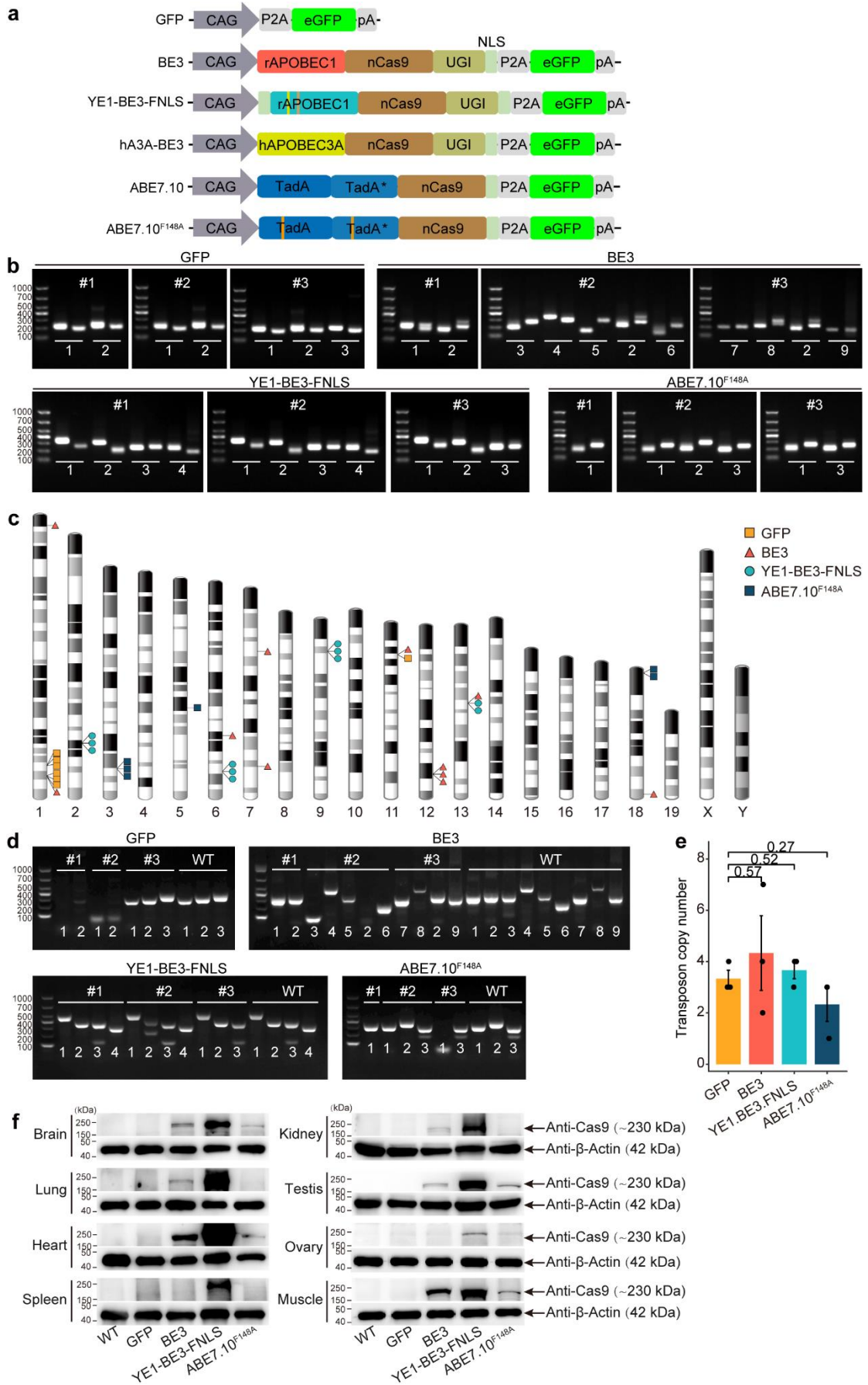[7]These authors contributed equally: Nana Yan, Hu Feng, Yongsen Sun, Ying Xin, Haihang Zhang

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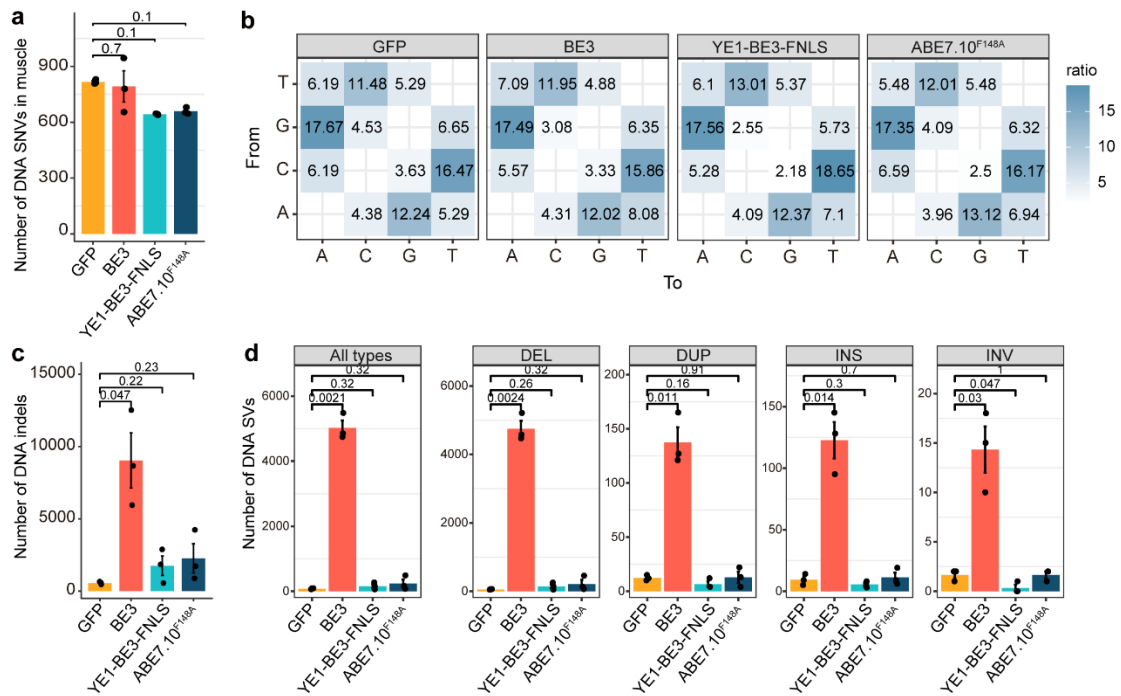
Supplementary Figures 1-11

Supplementary Tables 1-7

a

GFP — CAG ▶ P2A eGFP pA-

BE3 — CAG ▶ rAPOBEC1 nCas9 UGI NLS P2A eGFP pA-

YE1-BE3-FNLS — CAG ▶ rAPOBEC1 nCas9 UGI P2A eGFP pA-

hA3A-BE3 — CAG ▶ hAPOBEC3A nCas9 UGI P2A eGFP pA-

ABE7.10 — CAG ▶ TadA TadA* nCas9 P2A eGFP pA-

ABE7.10^F148A — CAG ▶ TadA TadA* nCas9 P2A eGFP pA-

b

GFP   BE3

YE1-BE3-FNLS   ABE7.10^F148A

c

GFP ■
BE3 ▲
YE1-BE3-FNLS ●
ABE7.10^F148A ■

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X Y

d

GFP   BE3

YE1-BE3-FNLS   ABE7.10^F148A

e

Transposon copy number

0.57
0.52
0.27

GFP   BE3   YE1.BE3.FNLS   ABE7.10^F148A

f

Brain   Kidney   Anti-Cas9 (~230 kDa)   Anti-β-Actin (42 kDa)

Lung   Testis   Anti-Cas9 (~230 kDa)   Anti-β-Actin (42 kDa)

Heart   Ovary   Anti-Cas9 (~230 kDa)   Anti-β-Actin (42 kDa)

Spleen   Muscle   Anti-Cas9 (~230 kDa)   Anti-β-Actin (42 kDa)

WT GFP BE3 YE1-BE3-FNLS ABE7.10^F148A

Supplementary Fig. 1 | Generation and identification of GFP, BE3, YE1-BE3-FNLS, ABE7.10^{F148A} transgenic mice. a, Schematics show the plasmids used for generating transgenic mice. **b,** Electrophoresis of agarose gel to confirm integration sites. The number below electrophoretic bands (left: 5 arm terminal; right: 3 arm terminal) represent corresponding integration sites in three individuals of GFP, BE3, YE1-BE3-FNLS or ABE7.10^{F148A} transgenic mice. **c,** Chromosome location of *PB* insertions in GFP, BE3, YE1-BE3-FNLS and ABE7.10^{F148A} transgenic mice. **d,** Electrophoresis of agarose gel to identify single or biallelic integration in each integration site. **e,** Transposon copy number of GFP, BE3, YE1-BE3-FNLS and ABE7.10^{F148A} transgenic mice. Data are presented as means ± SEM (n = 3). *P* values were calculated by two-sided, unpaired *t*-test. **f,** Western blot analysis showing the protein expression levels of BE3, YE1-BE3-FNLS and ABE7.10^{F148A} in the corresponding transgenic mice compared with WT and GFP mice. Source data are provided as a Source Data file.
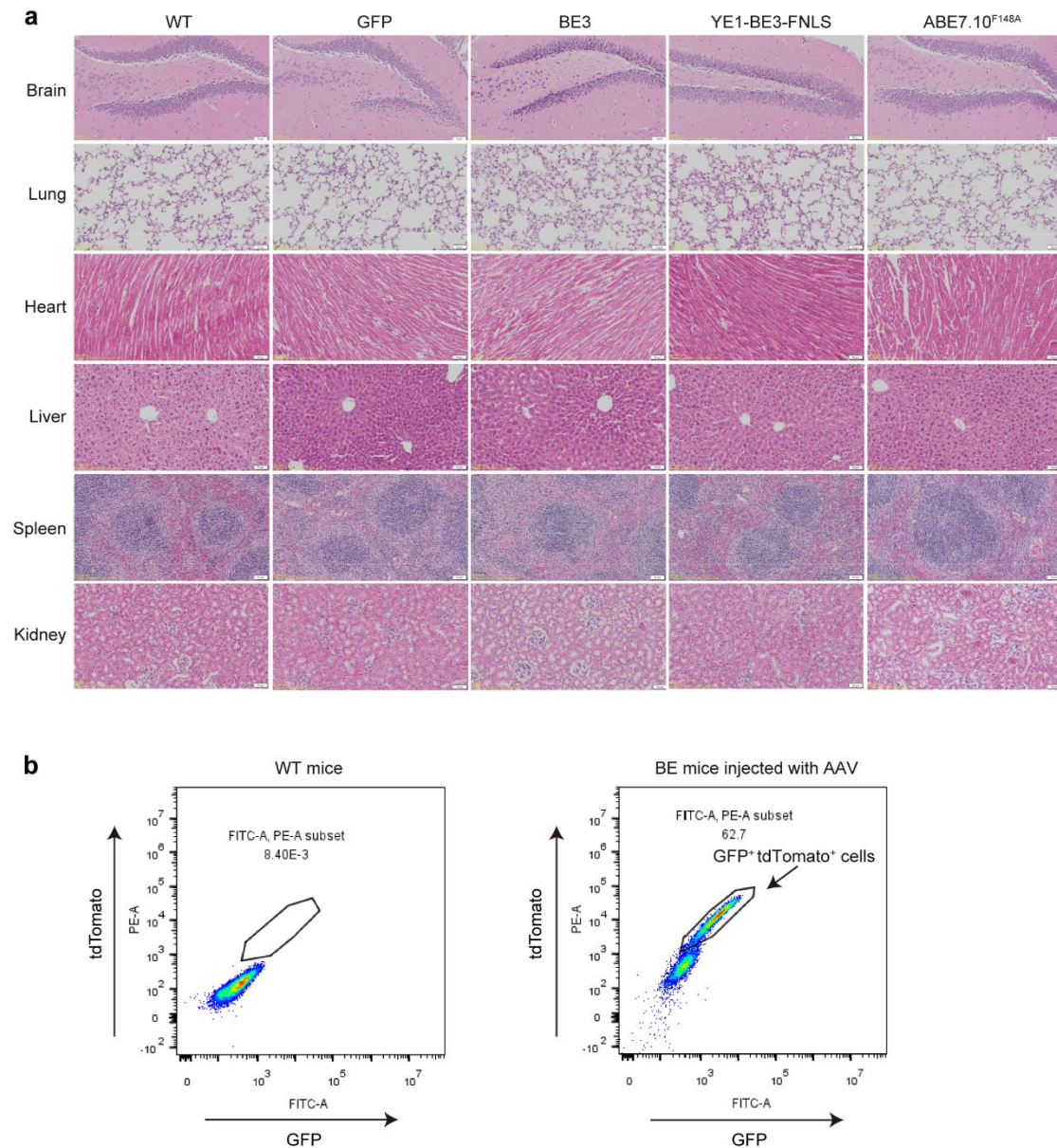
Supplementary Fig. 2 | H&E staining of the indicated tissues in WT, GFP, BE3, YE1-BE3-FNLS and ABE7.10^F148A mice and flow cytometry plots used for sorting GFP+tdTomato+ liver cells. a, H&E images showed no morphological difference between WT and the transgenic mice. **b,** Representative of the FACS gating strategy in the identification of GFP+ and tdTomato+ cells for on-target editing efficiency evaluation.

Supplementary Fig. 3 | WGS analysis in muscles of the GFP, BE3, YE1-BE3-FNLS and ABE7.10^{F148A} transgenic mice. a, The numbers of DNA SNVs detected in the WT mice with the transgenic mice as control from WGS data. **b,** Distribution of mutation types in muscles of GFP, BE3, YE1-BE3-FNLS and ABE7.10^{F148A} mice. The number indicates the percentage of a certain type of SNVs among all SNVs. **c,** The numbers of DNA indels detected in muscles of GFP, BE3 YE1-BE3-FNLS and ABE7.10^{F148A} mice. **d,** The numbers and distribution of DNA SVs in muscles of GFP, BE3 YE1-BE3-FNLS and ABE7.10^{F148A} mice. Data are presented as mean ± SEM (n = 3). *P* values were calculated by two-sided unpaired *t*-test. Source data are provided as a Source Data file.
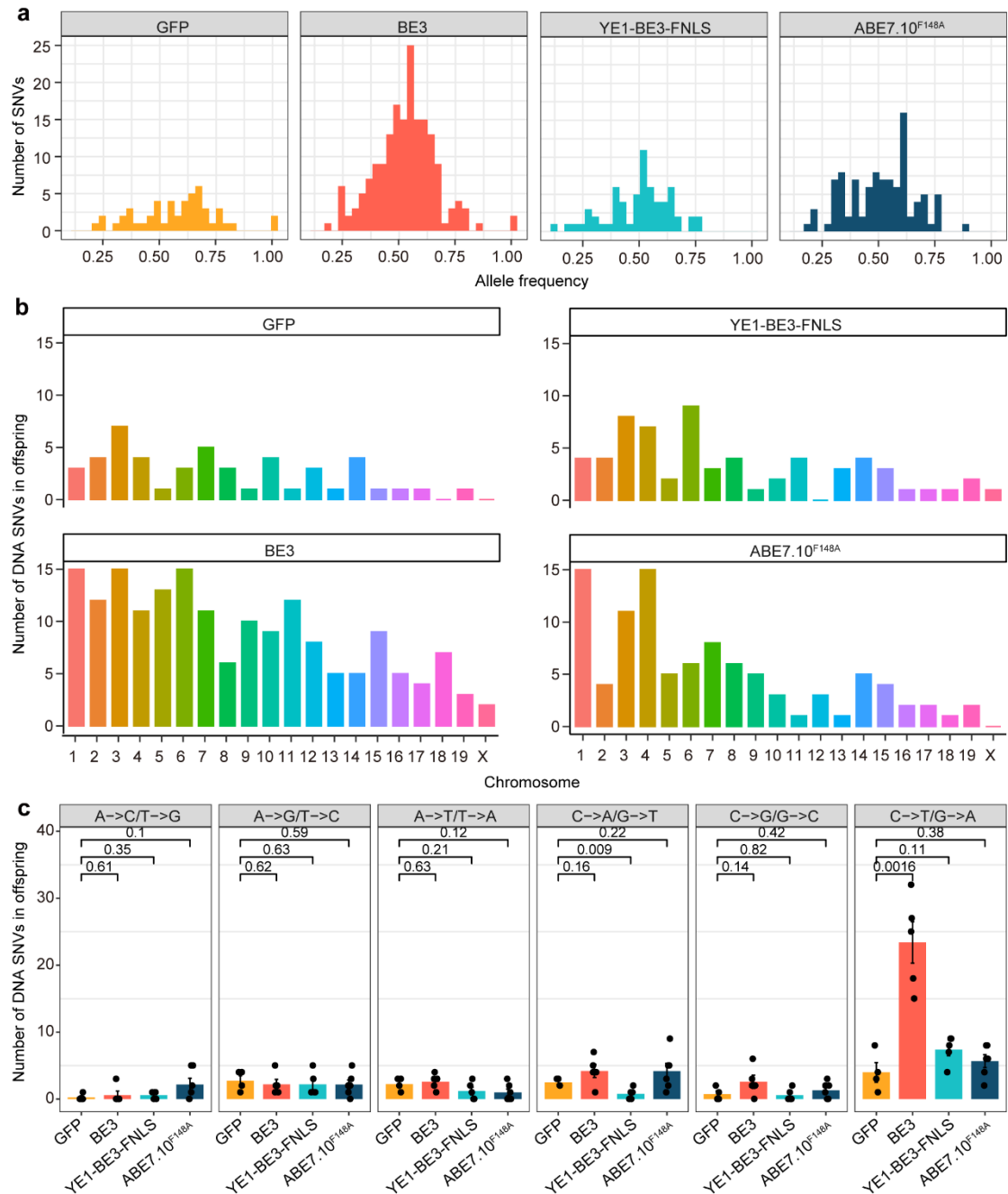
Supplementary Fig. 4 | WGS analysis for *de novo* off-target SNVs in the offspring of BE transgenic mice. a, The allele frequency distribution of *de novo* SNVs in the GFP and BE transgenic mice. **b,** Distributions of off-target RNA SNVs on chromosomes of the transgenic mice. **c,** Comparison of *de novo* SNV numbers in the offspring of different transgenic mice in the six indicated mutation types. Data are

presented as mean ± SEM, n = 4, 5, 5 and 6 for GFP, BE3, YE1-BE3-FNLS or

ABE7.10^{F148A} group, respectively. *P* values were calculated by two-sided unpaired *t*-

test. Source data are provided as a Source Data file.
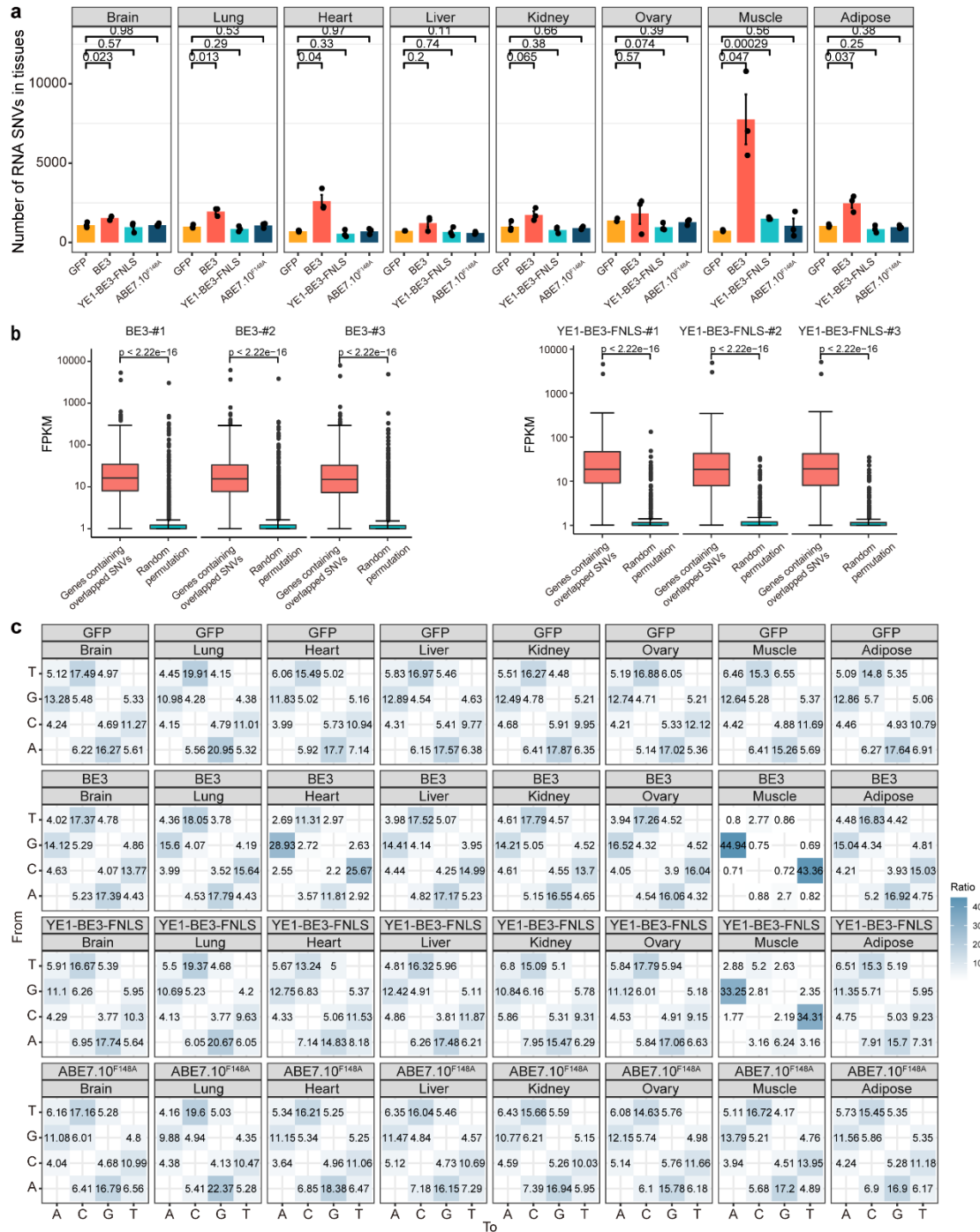
Supplementary Fig. 5 | RNA SNVs in various tissues of GFP, BE3, YE1-BE3-FNLS and ABE7.10^{F148A} mice. a, Comparison of RNA SNV numbers in brain, lung, heart, liver, kidney, ovary, muscle, and adipose tissues of the GFP and BE transgenic mice. Data are presented as mean ± SEM (n = 3). *P* values were calculated by two-sided unpaired *t*-test. **b,** Comparison of expression levels for genes containing the

overlapped off-target RNA SNVs and randomly simulated genes in muscles of the BE3

and YE1-BE3-FNLS mice. *P* values were calculated by two-sided unpaired *t*-test. The

center line indicates the median, and the bottom and top lines of the box represent the

first quartile and third quartile of the values, respectively. Tails extend to the minimum

and maximum values. **c,** Distribution of mutation types in the indicated tissues of the

GFP, BE3, YE1-BE3-FNLS and ABE7.10^{F148A} mice. The number indicates the

percentage of a certain type of SNVs among all SNVs. Source data are provided as a

Source Data file.

Supplementary Fig. 6 | Sanger sequencing chromatograms show that the C-to-U RNA SNVs were only observed in RNA but not DNA in the muscles of the GFP and BE3 mice. Single nucleotide conversions are indicated by red arrows. Primers were listed in Supplementary Table 5.
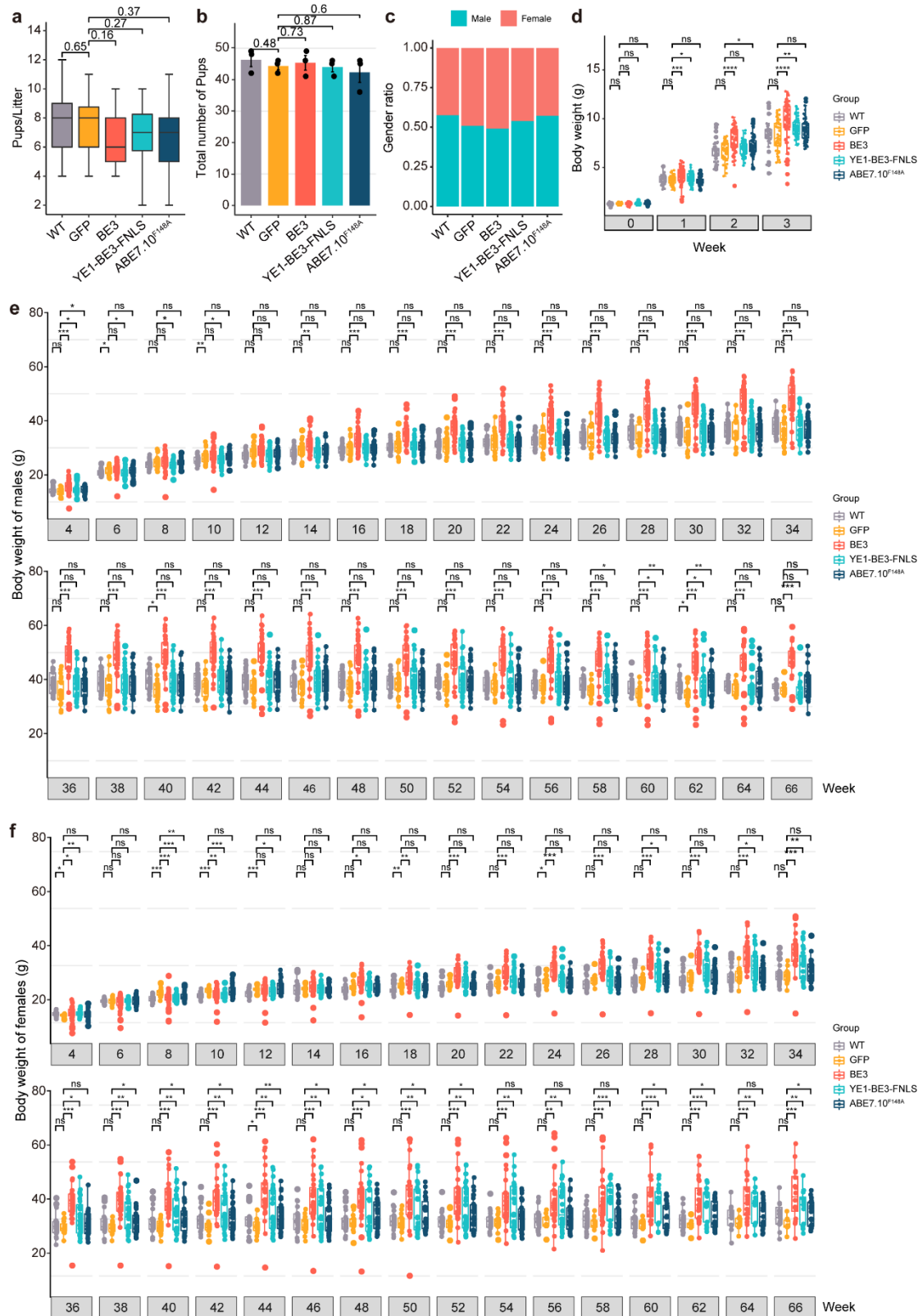
Supplementary Fig. 7 | The fertility and development phetotype monitoring of transgenic mice. a-c, Comparison of pups per litter **(a)**, total number of pups **(b)** and

sex ratio **(c)** between GFP and BE transgenic mice. Three breeding pairs of the four transgenic mouse lines were mated for 6 months and the numbers of pups were recorded. Box-and-whisker plots: the center line indicates median, box represents first and third quantiles, and whiskers indicate maximum and minimum values. **d,** Comparison of body weights between WT (n = 46), GFP (n = 48) and BE3 (n = 74), YE1-BE3-FNLS (n = 64) and ABE7.10^{F148A} (n = 63) transgenic mice from birth to 3 weeks. **e, f,** Comparison of body weights between WT (n > 13 for males and > 11 for females), GFP (n > 12 for males and > 9 for females) and BE3 (n > 22 for males and > 16 for females), YE1-BE3-FNLS (n > 20 for males and > 12 for females) and ABE7.10^{F148A} (n > 22 for males and > 21 for females) transgenic male **(e)** and female **(f)** mice from 4 to 66 weeks. Data are presented as mean ± SEM, *P < 0.01, **P < 0.01, ***P < 0.001, ****P < 0.0001 by two-sided unpaired t-test. P values are listed in Supplementary Data 1. Source data are provided as a Source Data file.
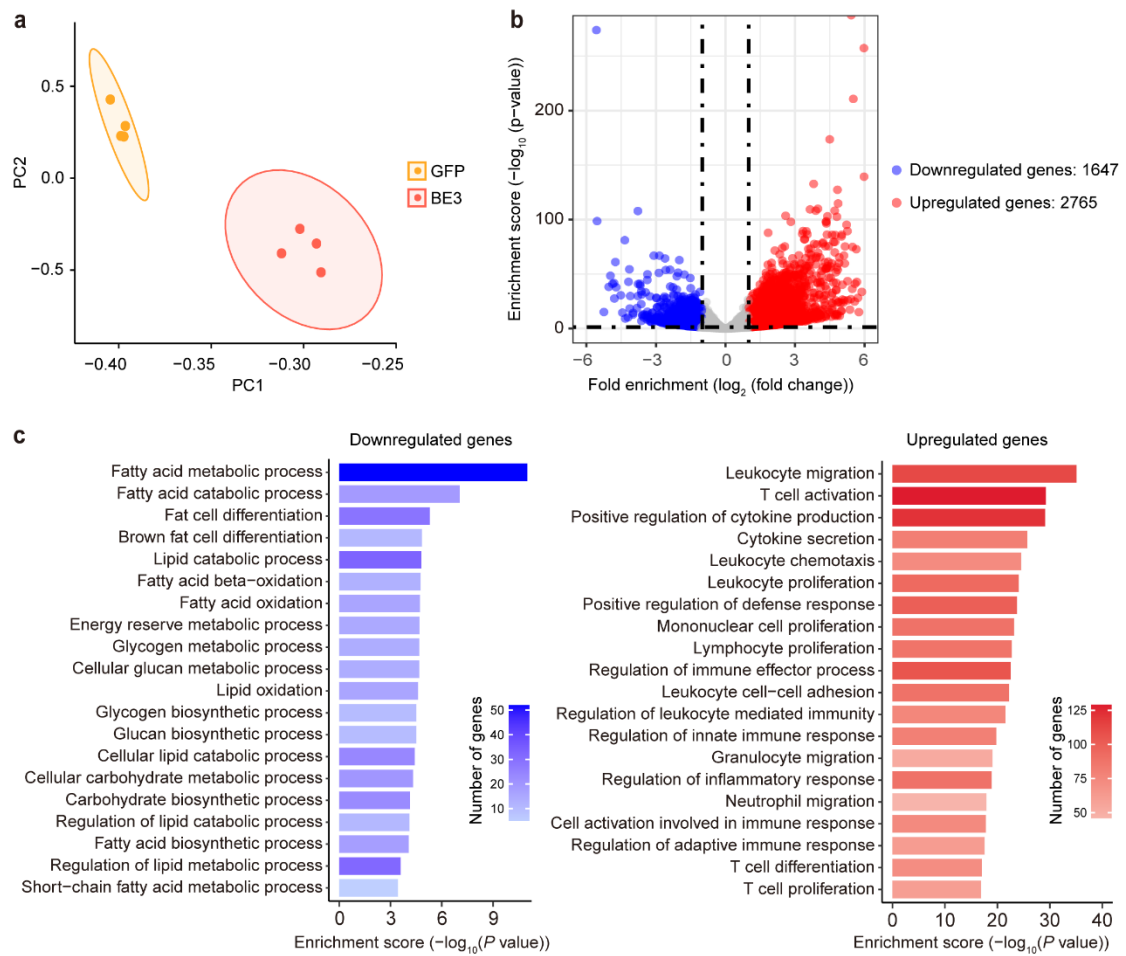
Supplementary Fig. 8 | RNA-seq analysis for adipose tissues between 30-week-old BE3 and GFP mice. a, Principal Component Analysis (PCA) analysis of transcriptome-wide gene expression levels between 30-week-old GFP and BE3 male mice. **b,** Volcano plot of the DEGs in adipose tissues between BE3 and GFP mice. **c,** GO analysis of downregulated genes and upregulated genes.
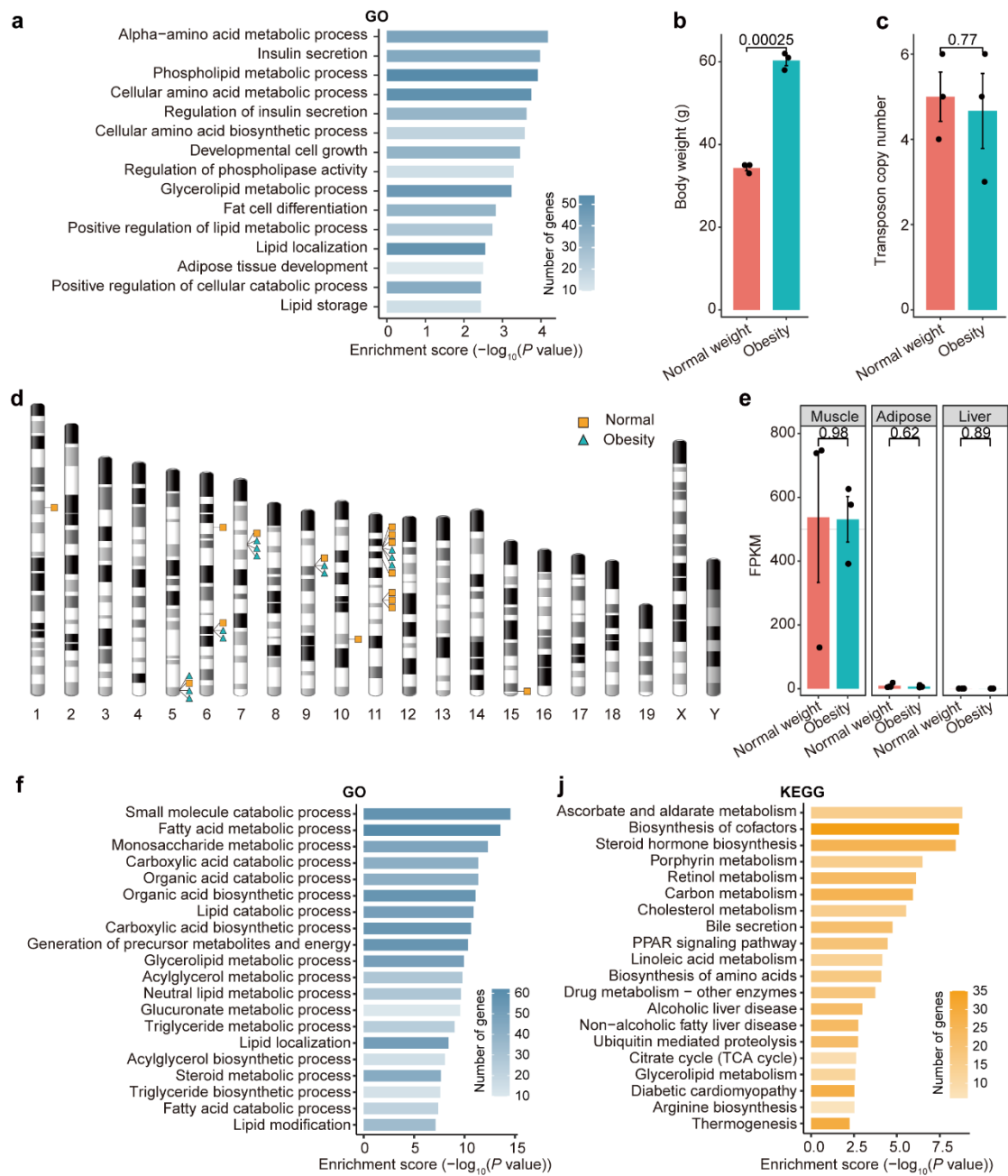
Supplementary Fig. 9 | Body weight, copy number, integration sites, WGS and RNAseq analysis of BE3 transgenic mice showing obesity and with normal weight phenotype. a, GO enrichment analysis for DNA SNVs in 30-week-old male mice from BE3 groups. **b,** Body weight of BE3 transgenic mice showing obesity and with normal weight phenotype. **c,** BE3 copy number in obesity and normal weight mice. **d,** Chromosome location of BE3 insertions in obesity and normal weight mice. **e,** The BE3

mRNA expression levels in obesity and normal weight mice. FPKM, Fragments Per Kilobase Million. **f-j,** GO enrichment analysis and KEGG pathway enrichment analysis for unique RNA SNVs detected in liver of obesity mice. Data are presented as mean ± SEM (n = 3). *P* values were calculated by two-sided unpaired *t*-test. Source data are provided as a Source Data file.
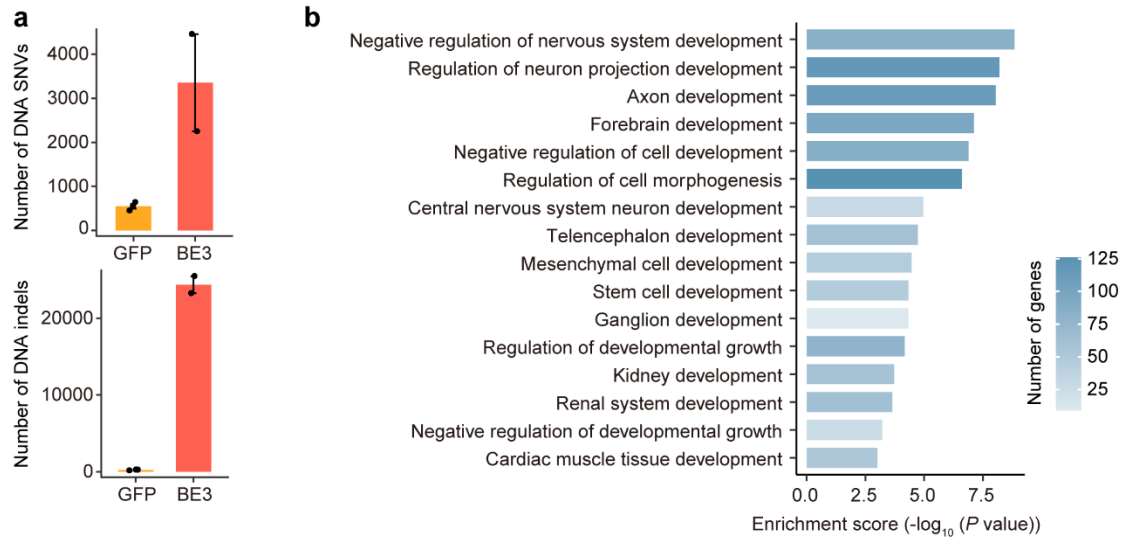
Supplementary Fig. 10 | WGS analysis of two dead BE3 mice with developmental delay. a, The numbers of DNA SNVs and indels in the dead BE3 mice (n = 2) compared with the GFP group (n = 3). **b,** GO analysis of genes with DNA mutations in the two dead BE3 mice. Source data are provided as a Source Data file.
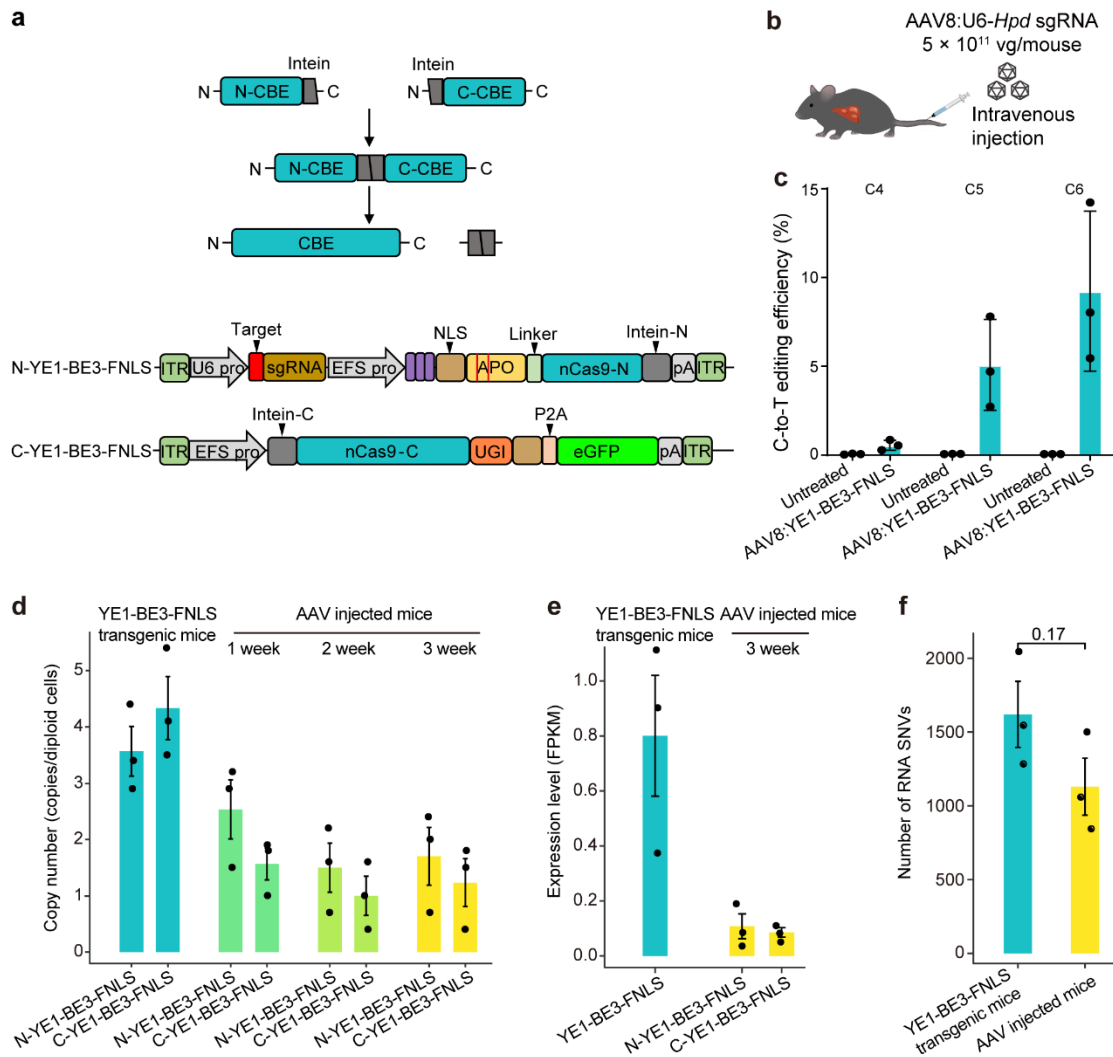
Supplementary Fig. 11 | Comparison of transgene copy numbers and off-target RNA SNVs in the YE1-BE3-FNLS transgenic mice and in mice with AAV-delivered YE1-BE3-FNLS. a, Schematic diagram of dual-split YE1-BE3-FNLS for AAV package. **b**, Schematic showing the experimental procedure for delivery of dual-split YE1-BE3-FNLS through AAV8 into mice by tail-vein injection. **c**, Deep sequencing analysis of the C-to-T base editing efficiency at the *Hpd* target site in mice after 3 weeks of AAV injection. **d**, DdPCR detection of YE1-BE3-FNLS copy numbers per diploid cells in the liver of transgenic mice (at 8 weeks old) and AAV-delivered mice (at 1-, 2-, and 3-week post-injection). **e**, Expression levels of transgenes in the

liver of transgenic mice and AAV-delivered mice (at 3-week post-injection). **f**, Numbers of RNA SNVs in the liver of transgenic mice and AAV-delivered mice (at 3-week post-injection). Data are presented as mean ± SEM (n = 3). *P* values were calculated by two-sided unpaired *t*-test. Source data are provided as a Source Data file.

Supplementary Table 1. Primes for genotyping.

Fragment name	Primers	Sequence
GFP	Forward primer	cctacagatccttaattaagcgacg
	Reverse primer	gtagcggctgaagcactgca
BE3	Forward primer	tgctaaccatgttcatgccttc
	Reverse primer	taccttctccgagcggttcg
YE1-BE3-FNLS	Forward primer	accatgttcatgccttcttc
	Reverse primer	cataggttttcagccgttcc
hA3A-BE3	Forward primer	tgctaaccatgttcatgccttc
	Reverse primer	taccttctccgagcggttcg
ABE7.10	Forward primer	tgctaaccatgttcatgccttc
	Reverse primer	taccttctccgagcggttcg
ABE7.10^{F148A}	Forward primer	tgctaaccatgttcatgccttc
	Reverse primer	taccttctccgagcggttcg
Tyr sgRNA	Forward primer	tcaagaactctgtagcaccgcc
	Reverse primer	tgacgtcaatggaaagtccc

Supplementary Table 2. Insert locations of GFP, BE3, YE1-BE3-FNLS and ABE7.10^{F148A} mice in corresponding transgenic mouse lines.

Mouse lines		Insertion name	Integration site	Location
GFP	#1	GFP-insert 1	chr1:172468937-172468938	Gm7804 **Intronic**
		GFP-insert 2	chr1:179249731-179249732	Smyd3 **Intronic**
	#2	GFP-insert 1	chr1:172468937-172468938	Gm7804 **Intronic**
		GFP-insert 2	chr1:179249731-179249732	Smyd3 **Intronic**
	#3	GFP-insert 1	chr1:172468937-172468938	Gm7804 **Intronic**
		GFP-insert 2	chr1:179249731-179249732	Smyd3 **Intronic**
		GFP-insert 3	chr11:23139312-23139313	Gm12059|1700061J23Rik **Intergenic**
BE3	#1	BE3-insert 1	chr11:23139312-23139313	Gm12059|1700061J23Rik **Intergenic**
		BE3-insert 2	chr12:102836616-102836617	Btbd7 **Intronic**
	#2	BE3-insert 3	chr1:8155815-8155816	Gm38216|Gm38024
		BE3-insert 4	chr6:106085816-106085817	Cntn4 **Intronic**
		BE3-insert 5	chr7:122759154-122759155	Cacng3 **Intronic**
		BE3-insert 2	chr12:102836616-102836617	Btbd7 **Intronic**
		BE3-insert 6	chr18:87054755-87054756	Gm5971|Gm5234 **Intergenic**
	#3	BE3-insert 7	chr1:179249731-179249732	Smyd3 **Intronic**
		BE3-insert 8	chr7:44299731-44299732	Gm18307|Gm7238 **Intergenic**
		BE3-insert 2	chr12:102836616-102836617	Btbd7 **Intronic**
		BE3-insert 9	chr13:54600579-54600580	Cltb **Intronic**
YE1-BE3-FNLS	#1	YE1-BE3-FNLS-insert 1	chr2:143578196-143578197	Pcsk2 **Intronic**
		YE1-BE3-FNLS-insert 2	chr6:130690506-130690507	Gm27013 **Intronic**
		YE1-BE3-FNLS-insert 3	chr9:23270781-23270782	Bmper **Intronic**
		YE1-BE3-FNLS-insert 4	chr13:54600579-54600580	Cltb **Intronic**
	#2	YE1-BE3-FNLS-insert 1	chr2:143578196-143578197	Pcsk2 **Intronic**
		YE1-BE3-FNLS-insert 2	chr6:130690506-130690507	Gm27013 **Intronic**
		YE1-BE3-FNLS-insert 3	chr9:23270781-23270782	Bmper **Intronic**
		YE1-BE3-FNLS-insert 4	chr13:54600579-54600580	Cltb **Intronic**
	#3	YE1-BE3-FNLS-insert 1	chr2:143578196-143578197	Pcsk2 **Intronic**
		YE1-BE3-FNLS-insert 2	chr6:130690506-130690507	Gm27013 **Intronic**
		YE1-BE3-FNLS-insert 3	chr9:23270781-23270782	Bmper **Intronic**
ABE7.10^{F148A}	#1	ABE7.10^{F148A}-insert 1	chr3:139126962-139126963	Rap1gds1|Gm4862 **Intergenic**
	#2	ABE7.10^{F148A}-insert 1	chr3:139126962-139126963	Rap1gds1|Gm4862 **Intergenic**
		ABE7.10^{F148A}-insert 2	chr5:89060403-89060404	Slc4a4 **Intronic**
		ABE7.10^{F148A}-insert 3	chr18:4283951-4283952	Gm10557|Gm7400 **Intergenic**
	#3	ABE7.10F148A-insert 1	chr3:139126962-139126963	Rap1gds1|Gm4862 **Intergenic**
		ABE7.10F148A-insert 3	chr18:4283951-4283952	Gm10557|Gm7400 **Intergenic**

Supplementary Table 3. sgRNA sequences and primers for Sanger sequencing.

Locus	Target sequences	Primers	Sequence
Hpd	caacccagaaggtcaccgag	Outer forward primer	gaaatgtcttaggaggttagcc
		Outer reverse primer	ggttccaaagtgccagtcct
		Inner forward primer	agatgggagcagggtgagtc
		Inner reverse primer	ctcgcactagccaatcccag
Dmd	ataataggggacgaacaggg	Outer forward primer	ccattgcattctaggtcatgag
		Outer reverse primer	ctgacaggaagcagcacaac
		Inner forward primer	ggcctatagagttcctgtatcc
		Inner reverse primer	cttatgcaagtaacctgctgtc
Tyr	acctcagttccccttcaaag	Outer forward primer	gtctgtgacactcattaacc
		Outer reverse primer	aaatgtggctgctgaagtac
		Inner forward primer	gtattgccttctgtggagtt
		Inner reverse primer	ctgctgaagtaccagtcttt

Supplementary Table 4. Primers for targeted deep sequencing.

Locus	group	Primers	Sequence
Hpd	GFP-#1	Forward primer	tgctcaacgaatgctgcccaccatggat
		Reverse primer	gtggtaaaacttacagtttccgcagttg
	GFP-#2	Forward primer	ctacgaaagaatgctgcccaccatggat
		Reverse primer	gtggtaaaacttacagtttccgcagttg
	GFP-#3	Forward primer	gtcaacccgaatgctgcccaccatggat
		Reverse primer	gtggtaaaacttacagtttccgcagttg
	BE3-#1	Forward primer	tagttatcgaatgctgcccaccatggat
		Reverse primer	gtggtaaaacttacagtttccgcagttg
	BE3-#2	Forward primer	gaaaaactgaatgctgcccaccatggat
		Reverse primer	gtggtaaaacttacagtttccgcagttg
	BE3-#3	Forward primer	gagcgtttgaatgctgcccaccatggat
		Reverse primer	gtggtaaaacttacagtttccgcagttg
	YE1-BE3-FNLS-#1	Forward primer	tcgctgaagaatgctgcccaccatggat
		Reverse primer	gacacaagacttacagtttccgcagttg
	YE1-BE3-FNLS-#2	Forward primer	gaaaaactgaatgctgcccaccatggat
		Reverse primer	gacacaagacttacagtttccgcagttg
	YE1-BE3-FNLS-#3	Forward primer	gagcgtttgaatgctgcccaccatggat
		Reverse primer	gacacaagacttacagtttccgcagttg
Dmd	GFP-#1	Forward primer	atcacgagggccctcttgcccccttaac
		Reverse primer	acagtggaatgcagagttaatacacacg
	GFP-#2	Forward primer	cgatgtagggccctcttgcccccttaac
		Reverse primer	acagtggaatgcagagttaatacacacg
	GFP-#3	Forward primer	ttaggcagggccctcttgcccccttaac
		Reverse primer	acagtggaatgcagagttaatacacacg
	ABE7.10$^{\text{F148A}}$-#1	Forward primer	atcacgagggccctcttgcccccttaac
		Reverse primer	gccaatgaatgcagagttaatacacacg
	ABE7.10$^{\text{F148A}}$-#2	Forward primer	cgatgtagggccctcttgcccccttaac
		Reverse primer	gccaatgaatgcagagttaatacacacg
	ABE7.10$^{\text{F148A}}$-#4	Forward primer	tgaccaagggccctcttgcccccttaac
		Reverse primer	gccaatgaatgcagagttaatacacacg

Supplementary Table 5. Primers for verification of random selected RNA SNVs in BE3 muscle.

Locus	Primers	Sequence
NC_000067.6-93508362	Outer forward primer	atagctggcctgactaatca
	Outer reverse primer	tcactgaaaaggggaagagt
	Inner forward primer	tgcttactagaattgaagtcaca
	Inner reverse primer	caaccctgaaatcctgcttt
NC_000070.6-15285798	Outer forward primer	gtattcccaaggtaccagga
	Outer reverse primer	ttaacaggtgtgatgctgtg
	Inner forward primer	ccacctagttttcaatctggt
	Inner reverse primer	tccaacaatgagacttgataaca
NC_000076.6-33367128	Outer forward primer	tgggtctcagtgataagctt
	Outer reverse primer	agccttttgtagttcccaaa
	Inner forward primer	tgtgaagctaaaggaatggtc
	Inner reverse primer	gttcttacttaacttatgtgcagt
NC_000082.6-64766215	Outer forward primer	gagcagcggattttgagata
	Outer reverse primer	aaaatgtgcgtcttctcctc
	Inner forward primer	ggaccgcaataagttctcag
	Inner reverse primer	aagaagctgcagaaacaaca
NC_000086.7-9654816	Outer forward primer	tgagccttcaacaacaacaa
	Outer reverse primer	aggtacctatcggatgtgtg
	Inner forward primer	gactgactttgcatttggtg
	Inner reverse primer	atgtagagttaggacaaatggg
NC_000068.7-119602997	Outer forward primer	catctgacttgggtgttgag
	Outer reverse primer	ctgggaccctgcatatctaa
	Inner forward primer	aagtagttgctttgttctgaaaa
	Inner reverse primer	cggcaggactctatttctaaa
NC_000073.6-131445809	Outer forward primer	gtgaagccattgatcagtgt
	Outer reverse primer	gatccaacagccatgaactt
	Inner forward primer	tttagaccttgaatccaaagaga
	Inner reverse primer	ttacaggagtggaaggtgtc
NC_000076.6-61429145	Outer forward primer	ccagagaaacaaaggggaag
	Outer reverse primer	gtgacaggtcctcagttttc
	Inner forward primer	attcatgggagtctgggtc
	Inner reverse primer	gcctgctcagctctttatag
NC_000086.7-159386968	Outer forward primer	cttgagaggaaatgaggggga
	Outer reverse primer	caaaccgaggagatgaagtc
	Inner forward primer	ggatcttgtcgtctggtttt
	Inner reverse primer	cagcatgtctagtgagttcc
NC_000068.7-120507290	Outer forward primer	cctttccttctccactctct
	Outer reverse primer	acagtctgaggctacacttt
	Inner forward primer	gcttcctcctttccttccta
	Inner reverse primer	agtggggttgaacagtaagt
NC_000071.6-20759239	Outer forward primer	tcctagaactctactgctgc
	Outer reverse primer	gccatgtgttaaatgttgcc
	Inner forward primer	gatacaacaatctcactttattcct
	Inner reverse primer	ggctttgtctgatatgttcct
NC_000074.6-124909354	Outer forward primer	caacaatgccaaacagttcc
	Outer reverse primer	cgtgaatgtggtatgtggtt
	Inner forward primer	cggctctacttggttgtttt
	Inner reverse primer	gccaaaatgtgtgtatgcag

Supplementary Table 6. Insert locations of BE3 in BE3 mice with normal weight and obesity.

Group		Integration name	Integration site	Location
BE3 mice with normal weight	# 1	BE3-insert 10	chr1:69683654-69683655	Ikzf2 **Intronic**
		BE3-insert 11	chr9:37485434-37485435	Gm47945|Gm47963 **Intergenic**
		BE3-insert 1	chr11:23139312-23139313	Gm12059|1700061J23Rik **Intergenic**
		BE3-insert 12	chr11:58169177-58169178	Gemin5|Mrpl22 **Intergenic**
	# 2	BE3-insert 13	chr5:148296121-148296122	Mtus2 **Intronic**
		BE3-insert 4	chr6:106085816-106085817	Cntn4 **Intronic**
		BE3-insert 8	chr7:44299731-44299732	Gm18307|Gm7238 **Intergenic**
		BE3-insert 14	chr10:92916728-92916729	Cfap54 **Intronic**
		BE3-insert 1	chr11:23139312-23139313	Gm12059|1700061J23Rik **Intergenic**
		BE3-insert 12	chr11:58169177-58169178	Gemin5|Mrpl22 **Intergenic**
	# 3	BE3-insert 15	chr6:37202544-37202545	Dgki **Intronic**
		BE3-insert 1	chr11:23139312-23139313	Gm12059|1700061J23Rik **Intergenic**
		BE3-insert 16	chr11:30464035-30464036	4930505A04Rik **Intronic**
		BE3-insert 12	chr11:58169177-58169178	Gemin5|Mrpl22 **Intergenic**
		BE3-insert 17	chr15:101114617-101114618	Fignl2|Ankrd33 **Intergenic**
BE3 mice with obesity	# 1	BE3-insert 13	chr5:148296121-148296122	Mtus2 **Intronic**
		BE3-insert 4	chr6:106085816-106085817	Cntn4 **Intronic**
		BE3-insert 8	chr7:44299731-44299732	Gm18307|Gm7238 **Intergenic**
		BE3-insert 11	chr9:37485434-37485435	Gm47945|Gm47963 **Intergenic**
		BE3-insert 1	chr11:23139312-23139313	Gm12059|1700061J23Rik **Intergenic**
	# 2	BE3-insert 13	chr5:148296121-148296122	Mtus2 **Intronic**
		BE3-insert 4	chr6:106085816-106085817	Cntn4 **Intronic**
		BE3-insert 8	chr7:44299731-44299732	Gm18307|Gm7238 **Intergenic**
		BE3-insert 11	chr9:37485434-37485435	Gm47945|Gm47963 **Intergenic**
		BE3-insert 1	chr11:23139312-23139313	Gm12059|1700061J23Rik **Intergenic**
	# 3	BE3-insert 13	chr5:148296121-148296122	Mtus2 **Intronic**
		BE3-insert 8	chr7:44299731-44299732	Gm18307|Gm7238 **Intergenic**
		BE3-insert 1	chr11:23139312-23139313	Gm12059|1700061J23Rik **Intergenic**

Supplementary Table 7. Primes and probes for droplet digital PCR reactions.

Gene	Primers	Sequence
N-YE1-BE3-FNLS	Primer F	gtatccacggagtcccagcag
	Primer R	cgtaaagcaggcaggtctcc
	Probe	5'VIC/ggcggatcgagccccatgag/BHQ-1
C-YE1-BE3-FNLS	Primer F	gtcgaagagaatcccgggcca
	Primer R	cgccggacacgctgaacttg
	Probe	5'VIC/ccatcctggtcgagctggacggcg/BHQ-1
Sod2 (Reference)	Primer F	aaggagcaaggtcgcttaca
	Primer R	ggcactgacatgggaagagt
	Probe	5'6-FAM/caggacccattgcaaggaaca/BHQ-1