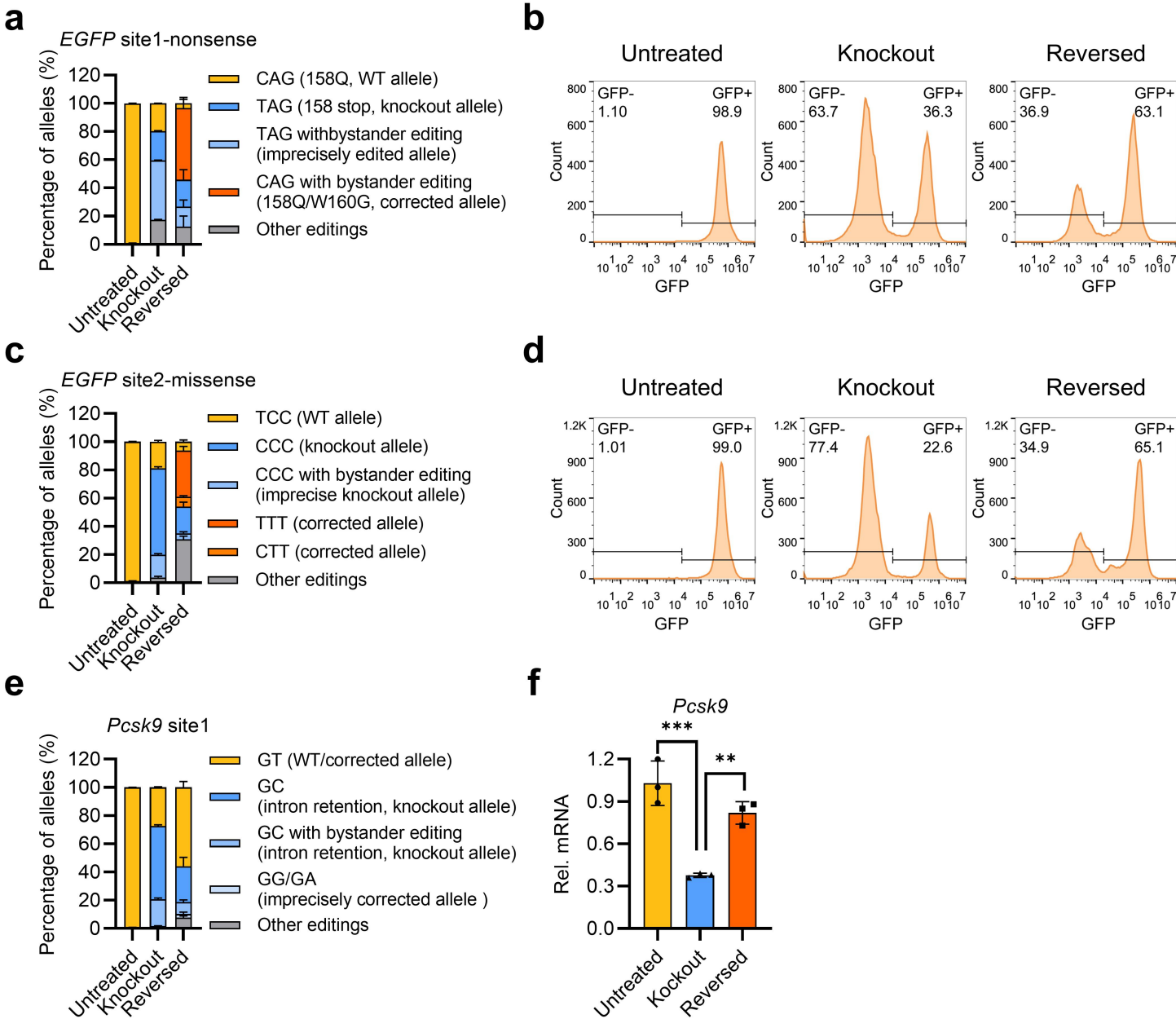


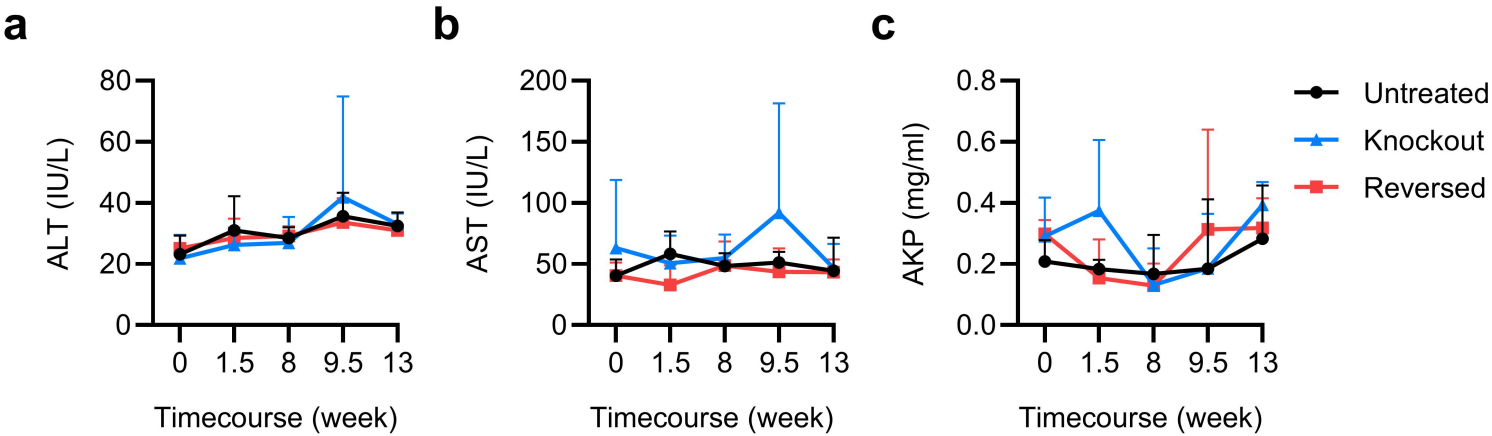
Supplementary Fig. S1



Supplementary Fig. S1 Reversible genome editing in bulk cells at *EGFP* site 1, *EGFP* site 2, and *Pcsk9* site 1, related to Fig. 1.

a, b Deep sequencing analysis of genomic knockout and reverse editing efficiencies (**a**) and flow cytometric quantification of EGFP functional impairment and recovery (**b**) in HEK293T-*EGFP* cells at *EGFP* site 1. CAG, wild-type allele encoding EGFP protein with normal fluorescence activity. TAG, knockout allele carrying a nonsense mutation that produces a truncated, non-fluorescent EGFP protein. TAG with bystander editing, imprecisely edited allele producing a truncated, mutant EGFP protein with no fluorescence activity. CAG with bystander editing, corrected allele encoding EGFP with the W160G mutation and retaining normal fluorescence activity. **c, d** Deep sequencing analysis of reverse editing efficiencies (**c**) and flow cytometric quantification of EGFP functional impairment and recovery (**d**) in HEK293T-*EGFP* cells at *EGFP* site 2. TCC, wild-type/corrected allele encoding serine at codon 31, producing EGFP with normal fluorescence activity. CCC, knockout allele encoding EGFP carrying the S31P mutation with abolished fluorescence activity. CCC with bystander editing, imprecise knockout allele encoding EGFP with abolished fluorescence activity. TTT/CTT, corrected alleles encoding fluorescent EGFP with the S31F or S31L mutations, respectively. **e** Deep sequencing analysis of genomic knockout and reverse editing efficiencies in Hepa1-6 cells at *Pcsk9* site 1. GT, wild-type/corrected allele encoding a fully functional PCSK9 protein. GC, knockout allele that causes intron retention and abolishes protein function. GC with bystander editing, knockout allele carrying bystander edits that abolishes protein function. GG/GA, imprecisely corrected alleles that cause intron retention and abolishes protein function. **f** qRT-PCR quantification of *Pcsk9* mRNA levels in above cells. Data are represented as mean $\pm$ SD of $n = 3$ independent biological replicates for **a, c, e, f**, and $n = 3$ independent experiments with similar results for **b** and **d**. $**P < 0.01$; $***P < 0.001$.

Supplementary Fig. S2

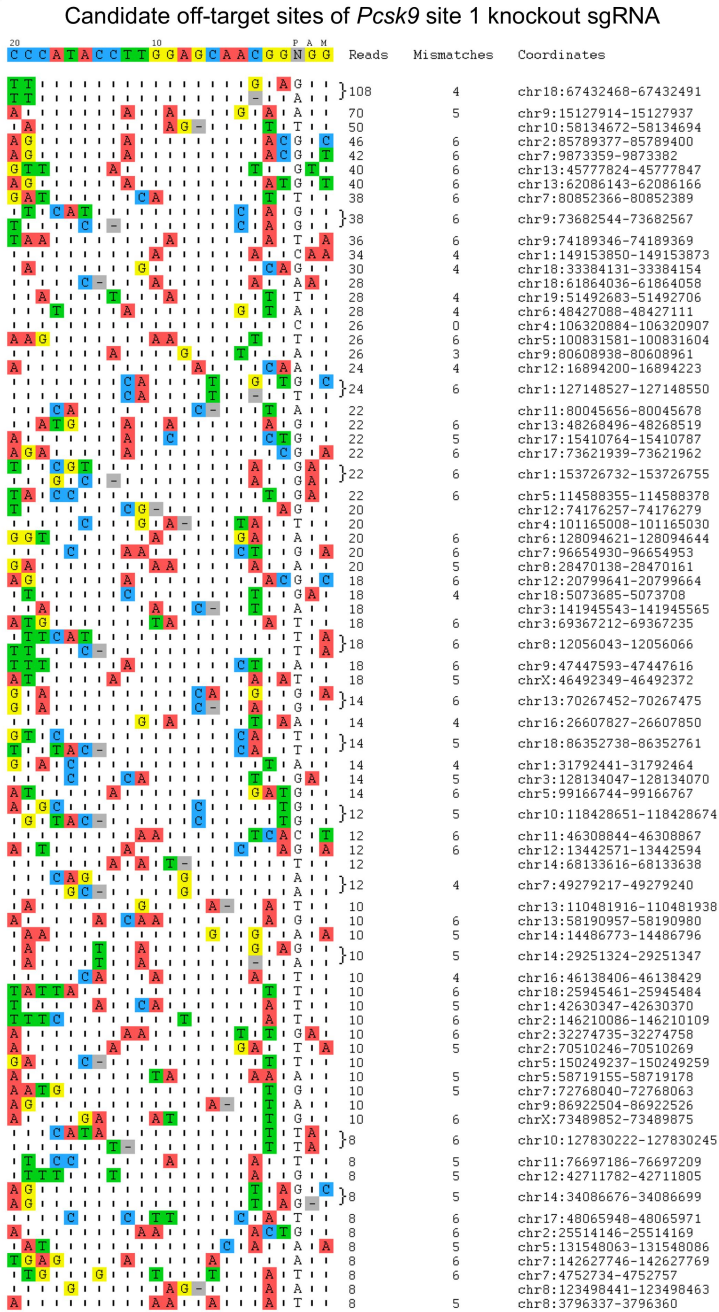


Supplementary Fig. S2 Liver function tests in mouse studies, related to Fig. 2.

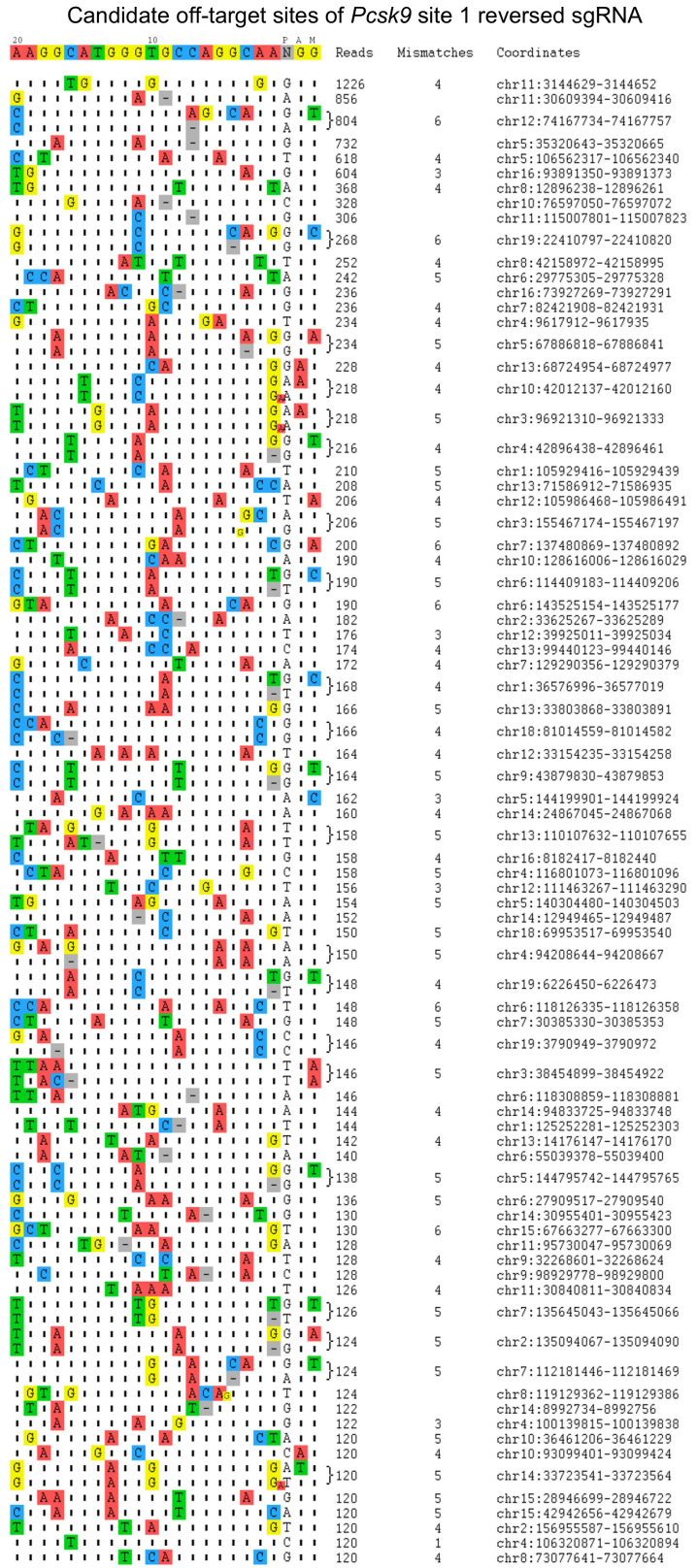
a–c Blood levels of alanine aminotransferase (ALT) (**a**), aspartate aminotransferase (AST) (**b**) and alkaline phosphatase (AKP) (**c**) from the start point to week 13 in mice in **Fig. 2**. Data are represented as mean $\pm$ SD of n = 6 mice for the untreated and knockout groups, and n = 4 mice for the reversed group.

Supplementary Fig. S3

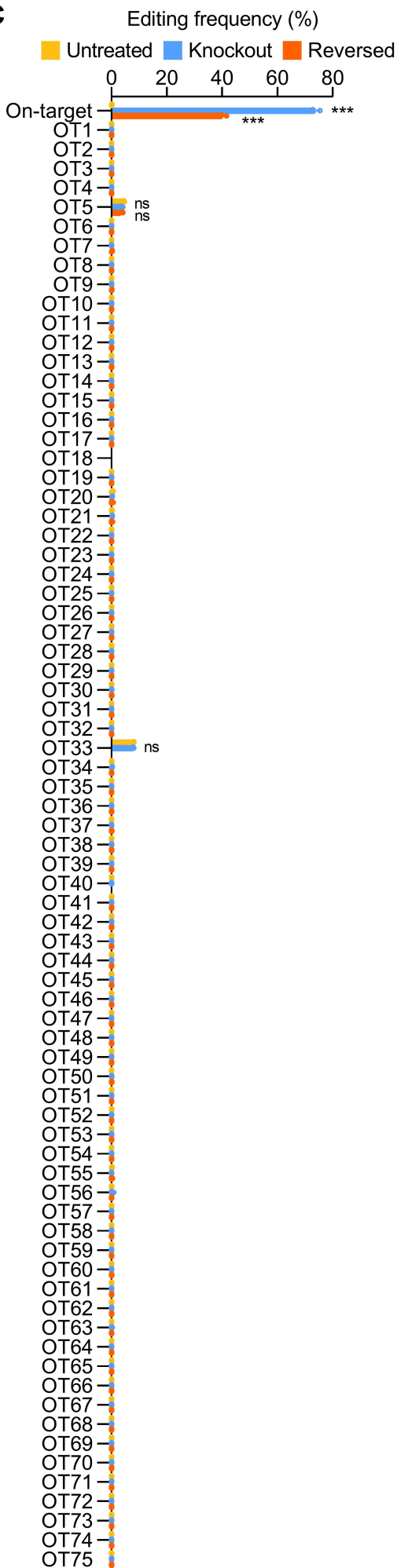
a



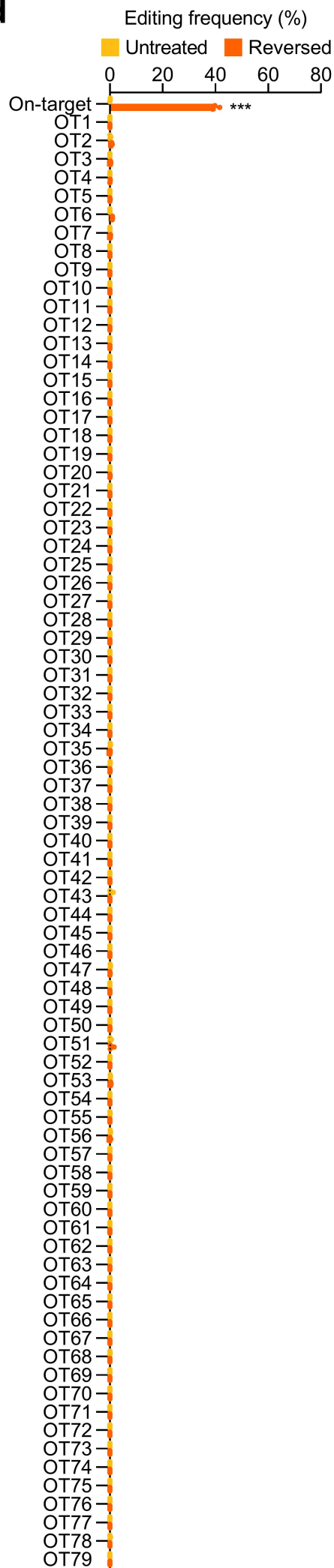
b



C



d



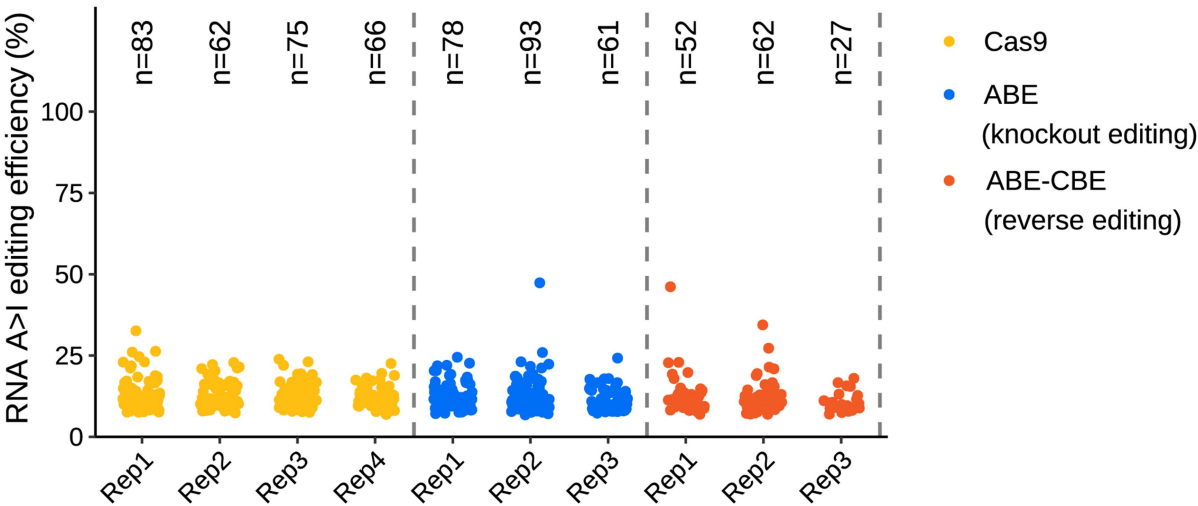
Supplementary Fig. S3 Assessment of sgRNA-dependent off-target effects for *Pcsk9* site 1-targeting sgRNAs using Change-seq, related to Fig. 2.

a, b Candidate off-target sites of *Pcsk9* site 1 knockout sgRNA (**a**) and reverse sgRNA (**b**) using mouse liver genomic library. **c, d** Off-target editing frequency induced by knockout sgRNA (**c**) or reverse sgRNA (**d**). The same on-target editing efficiencies of the reverse-edited group are shown in both panels c and d for comparison purposes. Data are represented as mean $\pm$ SD of n = 3 biological replicates.

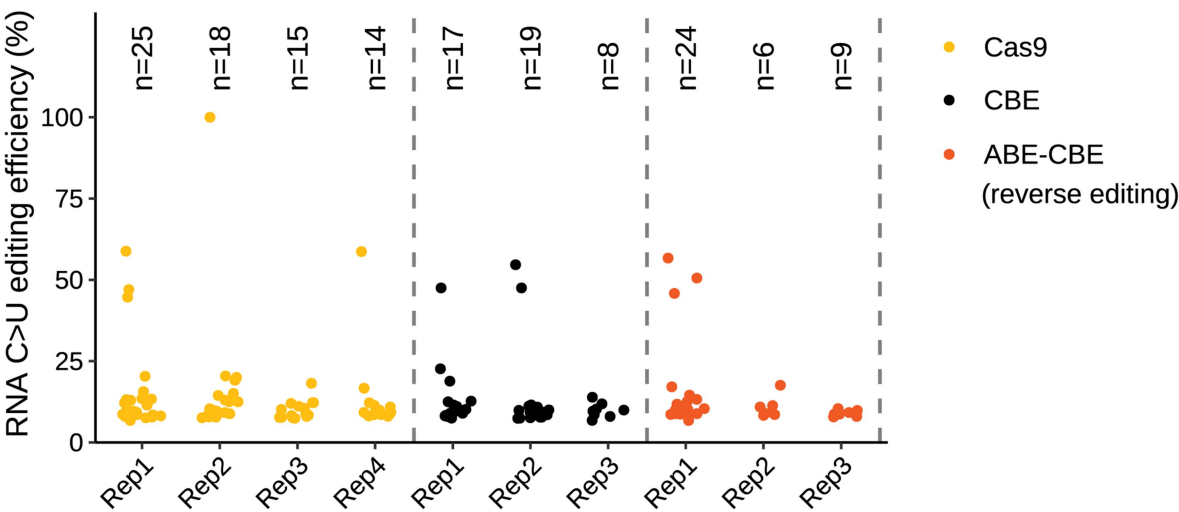
*** $P < 0.001$; ns, not significant ($P > 0.05$).

Supplementary Fig. S4

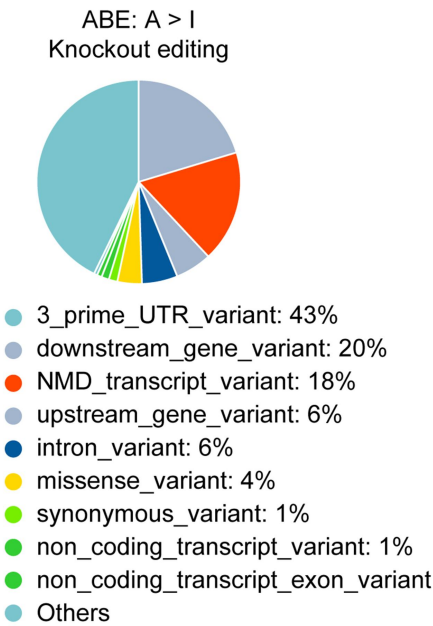
a



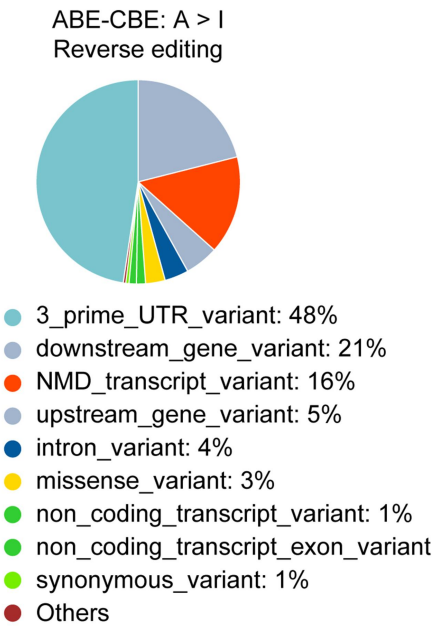
b



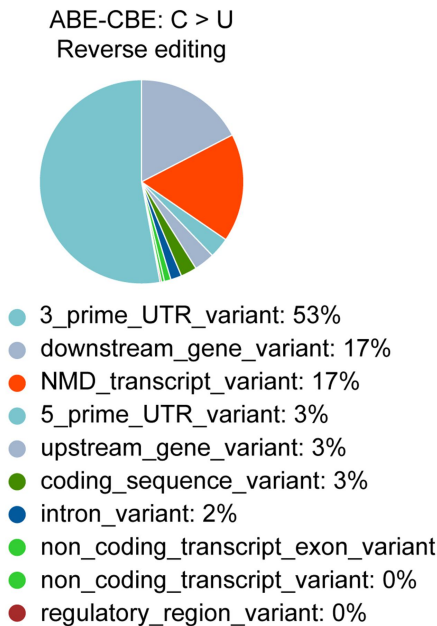
c



d



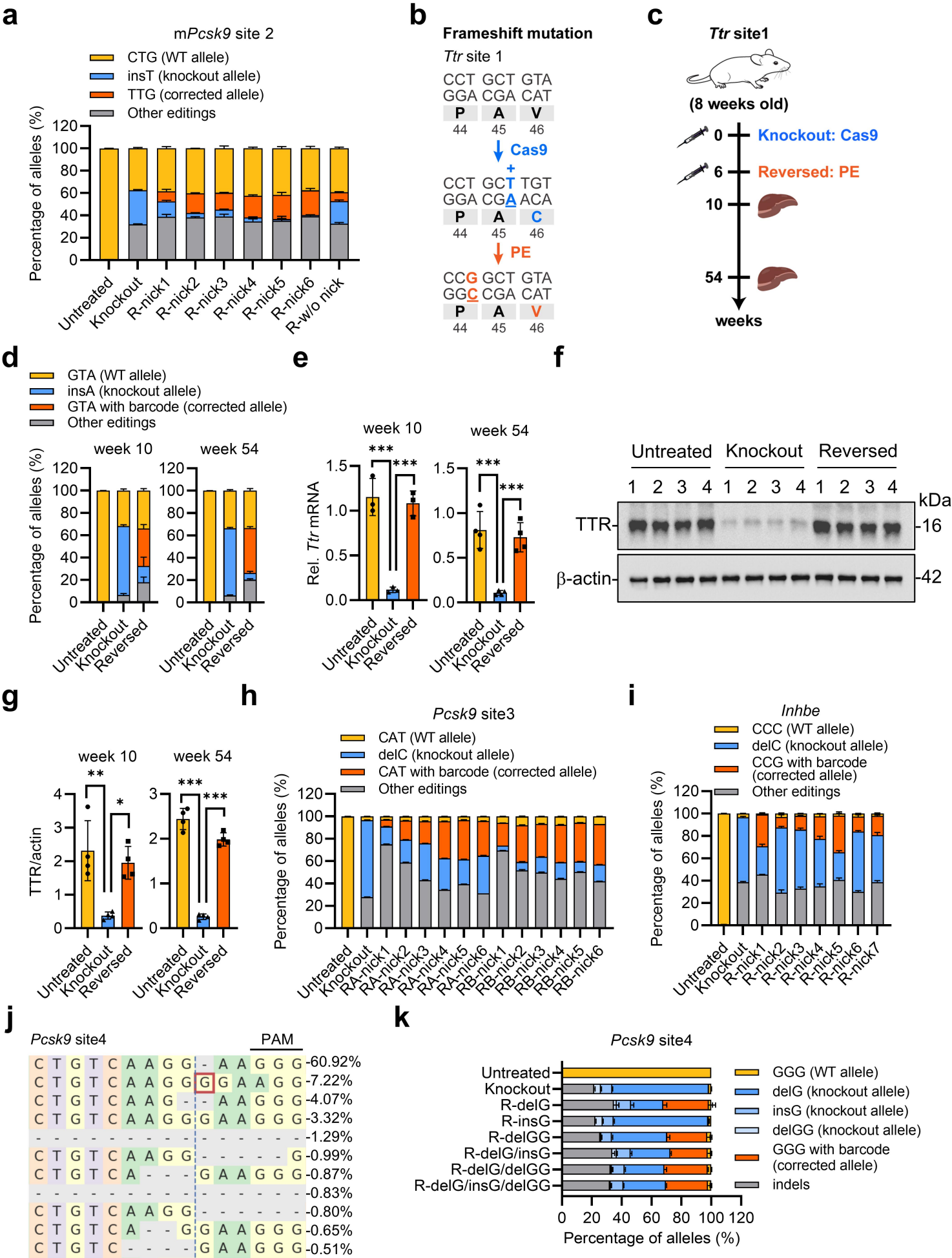
e



Supplementary Fig. S4 Assessment of sgRNA-independent RNA editing using RNA-seq, related to Fig. 2.

a Transcriptome-wide off-target RNA editing (A-to-I conversions) in mouse livers subjected to either knockout editing with ABE7.10 RA alone, or reverse editing with ABE7.10 RA followed by A3A (N57Q)-BE3. Cas9 treated mice were served as a control. **b** Transcriptome-wide off-target RNA editing (C-to-U conversions) in mouse livers subjected to reverse editing with ABE7.10 RA followed by A3A (N57Q)-BE3. Cas9 and A3A (N57Q)-BE3 alone served as controls. **c–e** Global distribution of off-target editing sites identified by RNA-seq. For panels (**a–e**): n = 4 mice for the Cas9 treated group, n = 3 mice for the ABE only, CBE only and ABE-CBE treated groups.

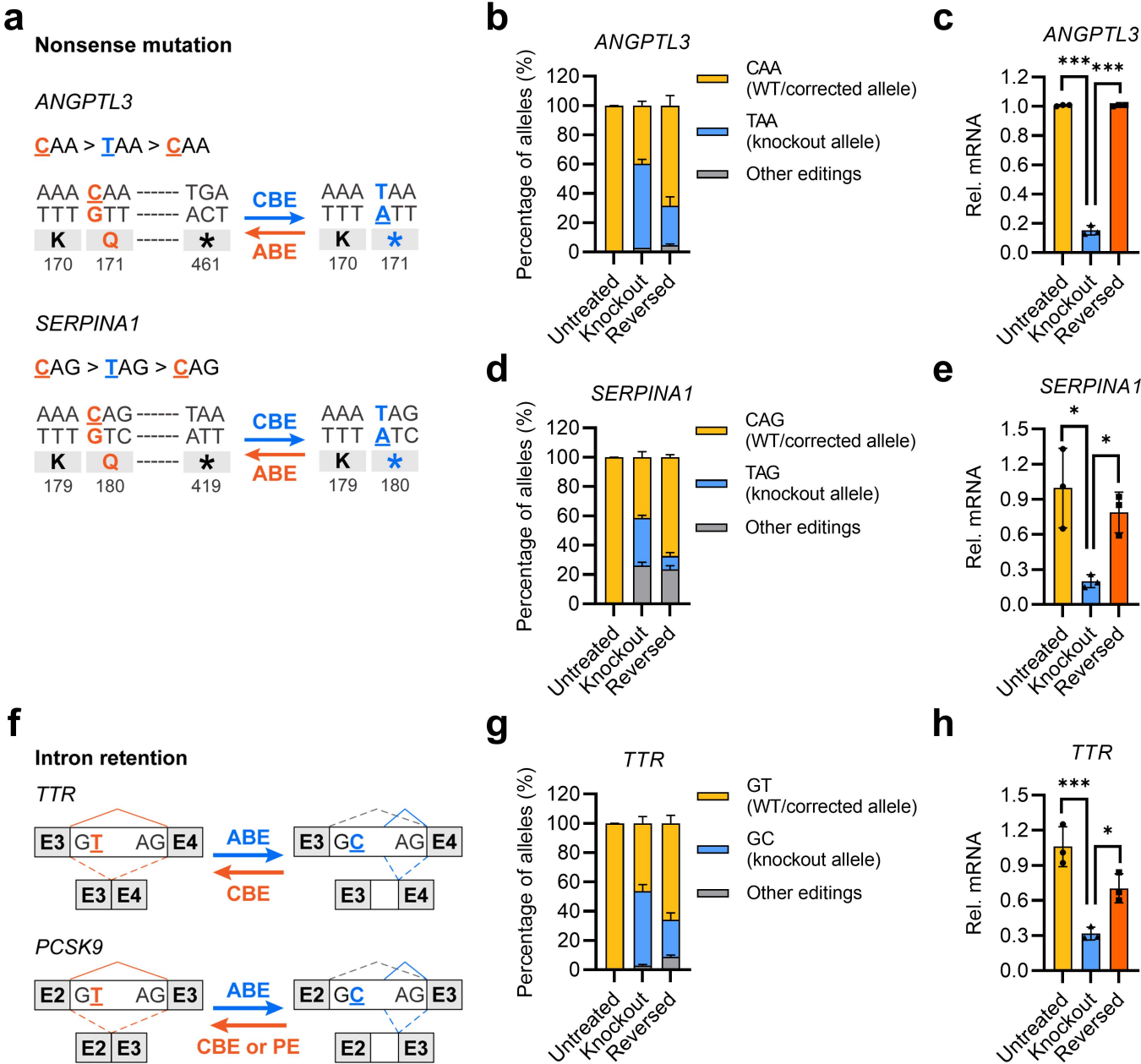
Supplementary Fig. S5



Supplementary Fig. S5 Reversible genome editing via a Cas9 knockout and PE restoration strategy at *Pcsk9* site2, *Ttr* site1, and other therapeutic targets, related to Figs. 3–4.

a Deep sequencing analysis of genomic knockout and reverse editing efficiencies in Hepa1-6 cells at *Pcsk9* site 2 using PE with different nick sgRNAs. CTG, wild-type allele encoding PCSK9 with Leu250. insT, knockout allele that causes frameshift mutation and abolishes PCSK9 expression. TTG, corrected allele with synonymous mutations, encoding functional PCSK9. **b** Schematic illustration of the editing strategy for *Ttr* locus. Edited nucleotides are indicated in colors. **c** Timeline and workflow of *in vivo* experiments. **d** Deep sequencing analysis of genomic knockout and reverse editing efficiencies at *Ttr* site1 by Cas9 and PE in mouse liver. GTA, wild-type allele encoding TTR with Val46. insA, knockout allele that causes frameshift mutation and abolishes TTR expression. GTA with barcode, corrected allele with synonymous mutations, encoding functional TTR. **e–g** qRT-PCR and western blot quantification of *Ttr* mRNA (**e**) and protein (**f, g**) expression levels in mouse liver. **h** Deep sequencing analysis of genomic knockout and reverse editing efficiencies using Cas9 and PE in Hepa1-6 cells at *Pcsk9* site 3. CAT, wild-type allele encoding functional PCSK9. delC, knockout allele that causes frameshift mutation and abolishes PCSK9 expression. CAT with barcode, corrected allele with synonymous mutations, encoding functional PCSK9. **i** Deep sequencing analysis of genomic knockout and reverse editing efficiencies using Cas9 and PE in Hepa1-6 cells at *Inhbe* site 1. CCC, wild-type allele encoding functional INHBE. delC, knockout allele that causes frameshift mutation and abolishes INHBE expression. CCG with barcode, corrected allele with synonymous mutations, encoding functional INHBE. **j** Allele frequencies of knockout editing products at mouse *Pcsk9* site 4. **k** Deep-sequencing analysis of editing efficiency for single- and multi-pegRNA reverse editing. GGG, wild-type allele. DelG/insG/delGG, knockout alleles. GGG with barcode, corrected allele. Data are represented as mean $\pm$ SD. For panels (**a, h, i, j, k**): $n = 3$ independent biological replicates. For panel (**d**): $n = 6$ mice for the untreated group, $n = 7$ mice for the knockout and reversed groups at week 10; $n = 5$ mice per group at week 54. For panel (**e**): $n = 3$ mice per group at week 10; $n = 4$ mice per group at week 54. For panels (**f, g**): $n = 4$ mice per group. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Supplementary Fig. S6



Supplementary Fig. S6 Reversible genome editing in bulk cells at therapeutic relevant human genomic target sites, related to Fig. 5.

a Detailed schematic illustration of reversible genome editing for human genes *ANGPTL3*, and *SERPINA1*. **b, c** Deep sequencing analysis of knockout and reverse editing efficiencies (**b**) and qRT-PCR quantification of mRNA level (**c**) of *ANGPTL3* in bulk cells. CAA, wild-type/corrected allele encoding functional ANGPTL3 proteins. TAA, knockout allele carrying a nonsense mutation that produces non-functional ANGPTL3 proteins. **d, e** Deep sequencing analysis of knockout and reverse editing efficiencies (**d**) and qRT-PCR quantification of mRNA level (**e**) of *SERPINA1* in bulk cells. CAG, wild-type/corrected allele encoding functional SERPINA1 proteins. TAG, knockout allele carrying a nonsense mutation that produces non-functional SERPINA1 proteins. **f** Detailed schematic illustration of reversible genome editing for human genes *TTR* and *PCSK9*. **g, h** Deep sequencing analysis of knockout and reverse editing efficiencies (**g**) and qRT-PCR quantification of mRNA level (**h**) of *TTR* in bulk cells. GT, wild-type/corrected allele encoding fully functional TTR proteins. GC, knockout allele that causes intron retention and abolishes protein function. Data are represented as mean $\pm$ SD of n = 3 independent biological replicates. * $P < 0.05$; *** $P < 0.001$.