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Supplemental information

Efficient *in vivo* prime editing corrects the most frequent phenylketonuria variant, associated with high unmet medical need

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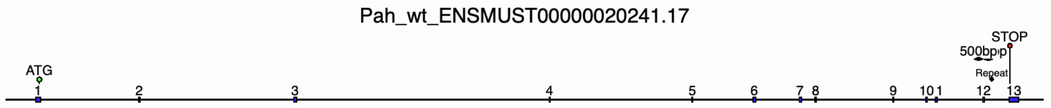
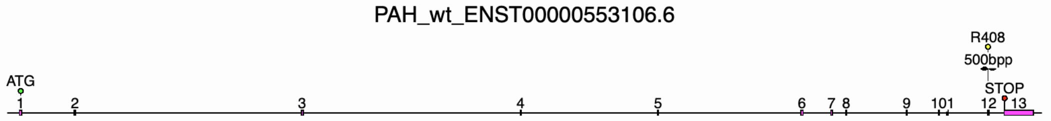
Figure S1. Scheme for Generation of Humanized R408W Mice.

Wildtype Locus of the Murine Pah Gene (standard project)



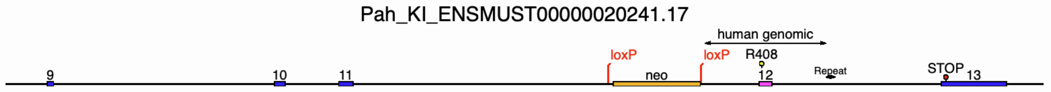
blue boxes: murine exons

Wildtype Locus of the Human Pah Gene (standard project)



pink boxes: human exons

Gene Targeted Locus of the Pah Gene (standard project)



blue boxes: mouse exons; pink boxes: human exons; neo: neomycin cassette for selection in ES cells; loxP: recognition sequence for cre recombinase mediated neo removal

Gene Targeted Locus after cre mediated neo removal (included in project)

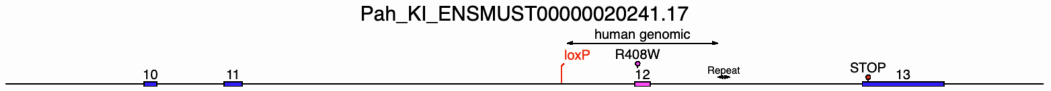


Figure S2. Phenylketonuria Phenotypes in Humanized R408W Mice.

(A) Homozygous R408W mouse with PKU as evidenced by hypopigmentation of the fur, on left, compared to heterozygous R408W non-PKU littermate, on right.

(B) Blood phenylalanine levels and weights of homozygous R408W PKU (*n* = 8 animals) and heterozygous R408W non-PKU (*n* = 6 animals) age-matched (6 weeks of age) colonymates. Lines = mean values. *P* values calculated with Mann-Whitney *U* test.

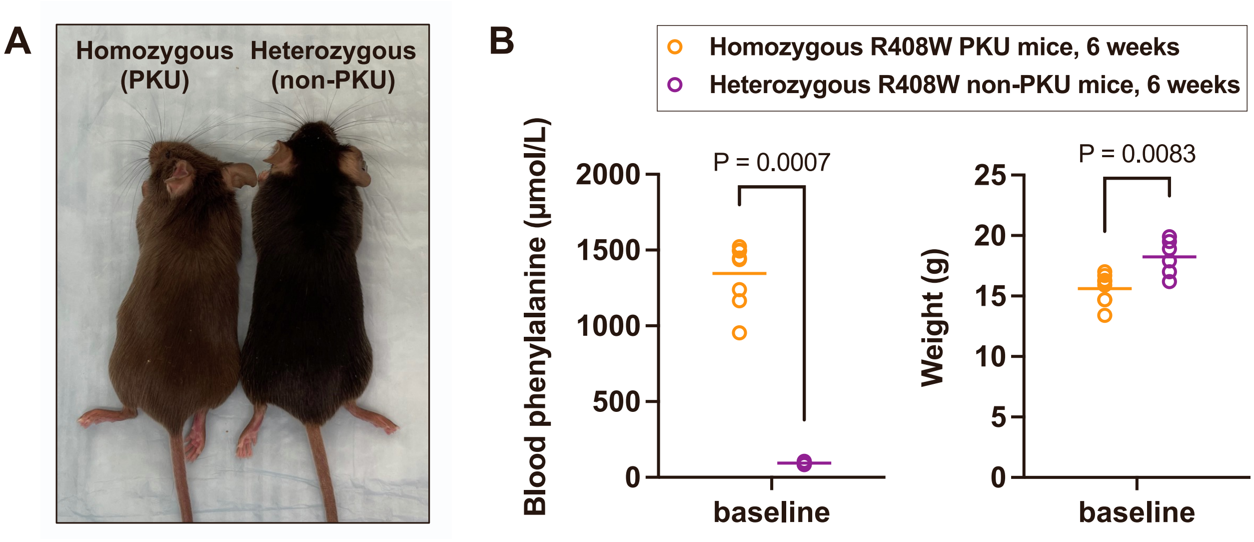
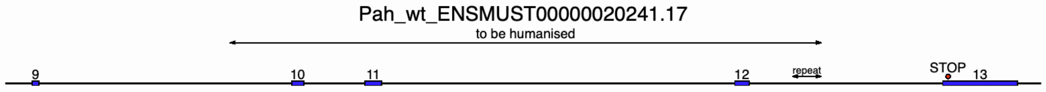


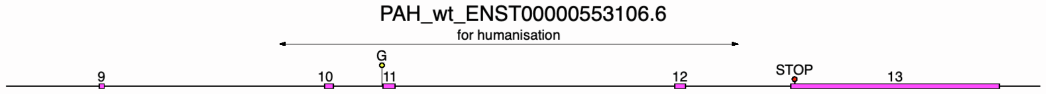
Figure S3. Scheme for Generation of Humanized 1066 Mice.

Wildtype Locus of the Murine Pah Gene (standard project)



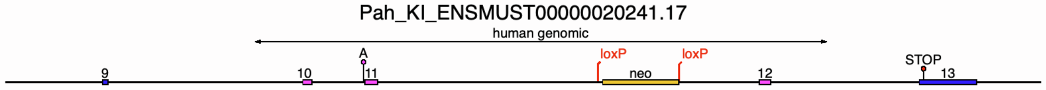
blue boxes: exons

Wildtype Locus of the Human Pah Gene (standard project)



pink boxes: human exons

Gene Targeted Locus of the Pah Gene (standard project)



blue boxes: murine exons; pink boxes: human exons; neo: neomycin cassette for selection in ES cells; loxP: recognition sequence for cre recombinase mediated neo removal

Gene Targeted Locus after cre mediated neo removal (included in project)

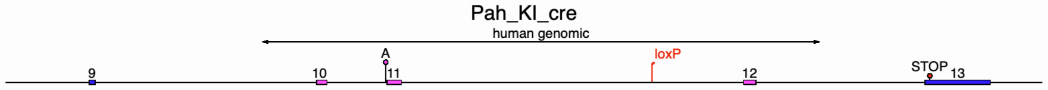


Figure S4. Schematic of Dual-AAV Prime Editing System.

PE3 = prime editor 3; AAV = adeno-associated virus; SpPE2 max = *Streptococcus pyogenes* prime editor 2 max; ITR = inverted terminal repeat; Cbh promoter = chicken β-actin promoter with cytomegalovirus early enhancer element; SpCas9max = *Streptococcus pyogenes* Cas9 max; Npu-N = *Nostoc punctiforme* N-terminal split intein; Npu-C = *Nostoc punctiforme* C-terminal split intein; MMLV = Moloney murine leukemia virus; RT = reverse transcriptase; RNaseH = ribonuclease H; SV40 = simian virus 40; pA = polyadenylation signal; mU6 = mouse U6 promoter; sgRNA = single guide RNA; hU6 = human U6 promoter; epegRNA = engineered prime editing guide RNA. This figure was reproduced without changes from reference 16 [Davis, J.R., Banskota, S., Levy, J.M., Newby, G.A., Wang, X., Anzalone, A.V., Nelson, A.T., Chen, P.J., Hennes, A.D., An, M., et al. (2023). Efficient prime editing in mouse brain, liver and heart with dual AAVs. Nat. Biotechnol. 10.1038/s41587-023-01758-z] in accordance with the terms of the Creative Commons Attribution 4.0 International License: https://creativecommons.org/licenses/by/4.0/.

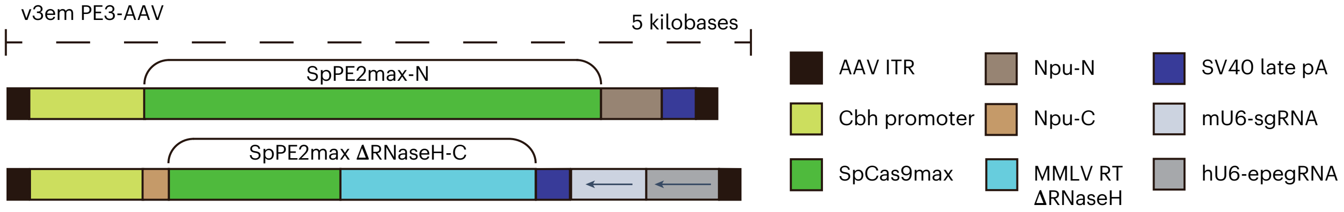


Figure S5. Liver Function Tests in Treated PKU and Control Non-PKU Groups of Mice, Related to Figure 5.

(A) Blood aspartate aminotransferase (AST) levels and alanine aminotransferase (ALT) levels in homozygous PKU mice following treatment with 8 × 10^{11} vg AAV dose (*n* = 4 animals) or with 4 × 10^{11} vg AAV dose (*n* = 3 animals), comparing levels at various timepoints up to 5 weeks following treatment to levels in untreated heterozygous non-PKU control (*n* = 3 animals) age-matched (6 weeks of age) colonymates (1 blood sample per timepoint).
(B) AST levels and ALT levels in homozygous PKU mice following treatment with 8 × 10^{11} vg AAV dose (*n* = 3 animals), comparing levels at various timepoints up to 5 weeks following treatment to levels in untreated heterozygous non-PKU control (*n* = 2 animals) age-matched (10 weeks of age) colonymates (1 blood sample per timepoint).
(C) AST levels and ALT levels in compound heterozygous PKU mice following treatment with 1 × 10^{12} vg AAV dose (*n* = 3 animals), comparing levels at various timepoints up to 5 weeks following treatment to levels in untreated heterozygous non-PKU control (*n* = 2 animals) age-matched (6 weeks of age) colonymates (1 blood sample per timepoint).
Lines in graphs = mean values.

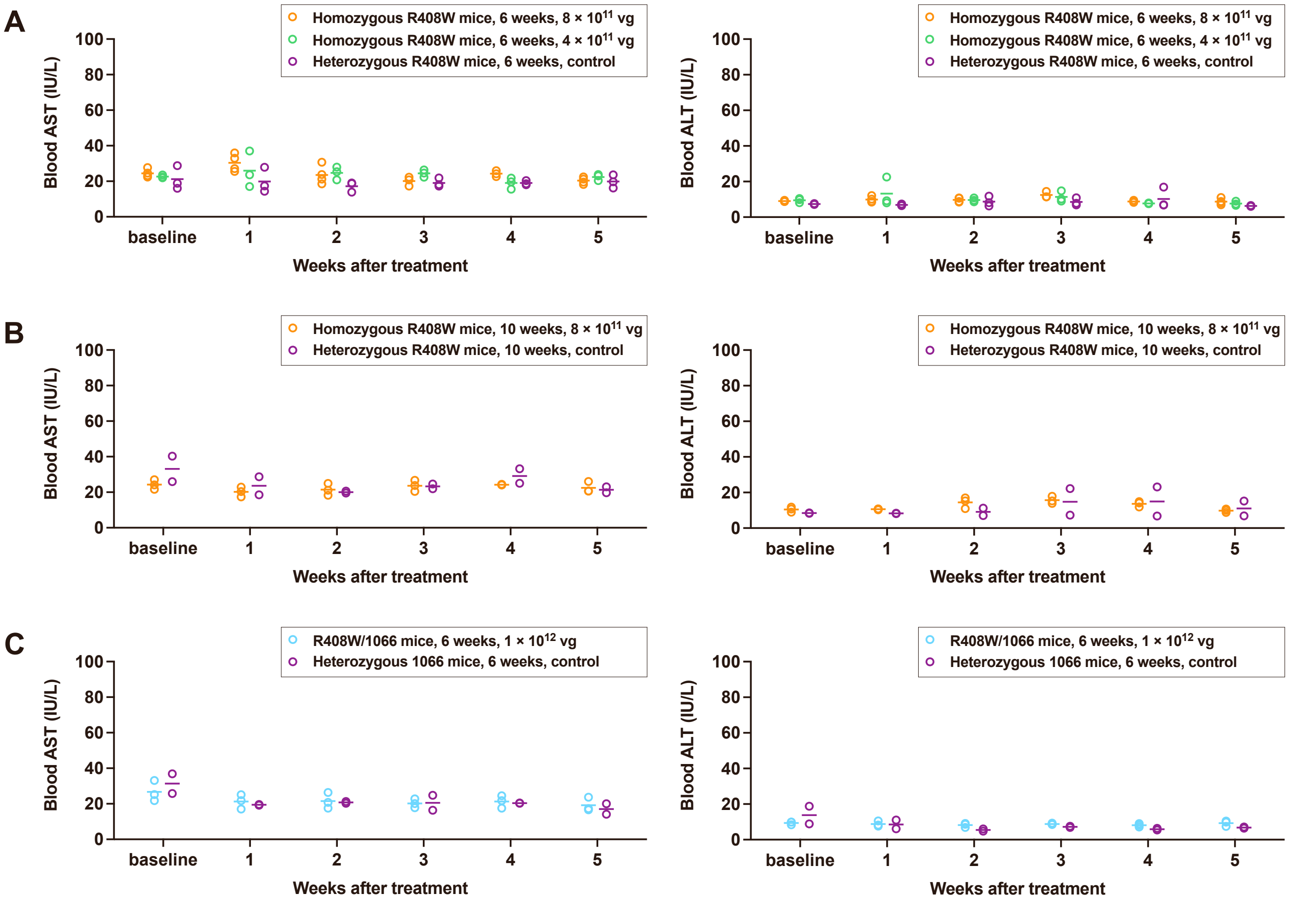


Table S1. pegRNAs.

P1	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **GAAGGGCCaAGGUAUUGUGGCAGC**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P2	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **GAAGGGCCgAGGUAUUGUGGCAGC**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P3	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **GAAGGGuCgAGGUAUUGUGGCAGC**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P4	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **GAAGGGuCggGGUAUUGUGGCAGC**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P5	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **GAAaGGuCgAGGUAUUGUGGCAGC**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P6	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **GAAaGGuCggGGUAUUGUGGCAGC**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P7	**UAGCGAACUGAGAAGGGCCA**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **ACAAUACCUcGGCCCUUCUCAGUU**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P8	**UAGCGAACUGAGAAGGGCCA**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **ACAAUACCccGGCCCUUCUCAGUU**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P9	**UAGCGAACUGAGAAGGGCCA**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **ACAAUgCCccGGCCCUUCUCAGUU**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
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P12	**UGGGUCGUAGCGAACUGAGA**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **ACCUcGGCCCUUCUCAGUUCGCUACG**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
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P14	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **AGGGuCggGGUAUUGUGGCAG**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P15	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **AGGGuCggGGUAUUGUGGCAGC**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
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P26	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **AGAAGGGuCggGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P27	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **AGAAGGGuCggGGUAUUGUGGCAG**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P28	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **AGAAGGGuCggGGUAUUGUGGCAGC**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P29	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **AGAAGGGuCggGGUAUUGUGGCAGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P30	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC **AGAAGGGuCggGGUAUUGUGGCAGCAA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
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P40	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC**CUGAGAAGGGuCggGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
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P43	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC**GAACUGAGAAGGGuCggGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P44	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC**CGAACUGAGAAGGGuCggGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P45	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC**ACUGAGAAaGGuCggGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P46	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC**AACUGAGAAaGGuCggGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P47	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC**GAACUGAGAAaGGuCggGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P48	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC**CGAACUGAGAAaGGuCggGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P49	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC**GAGAAGGGuCgcGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P50	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC**GAGAAGGGuCguGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P51	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC**GAGAAGGGgCggGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P52	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC**GAGAAGGGgCgcGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P53	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC**GAGAAGGGgCguGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P54	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC**GAGAAGGGaCggGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P55	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC**GAGAAGGGaCgcGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA
P56	**ACUUUGCUGCCACAAUACCU**GUUUGAGAGCUAUGCUGGAAACAGCAUAGCAAGUUCAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC**GAGAAGGGaCguGGUAUUGUGGCA**CGCGGUUCUAUCUAGUUACGCGUUAAACCAACUAGAA

Black bold = spacer sequence; red bold = RTT; blue bold = PBS; lower case indicates nucleotide substitution to be introduced by RTT.

Table S2. ngRNAs.

N1	**UGAGAAGGGCCaAGGUAUUG**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N2	**UAGCGAACUGAGAAGGGCCa**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N3	**UGAGAAGGGCCgAGGUAUUG**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N4	**UGAGAAGGGuCgAGGUAUUG**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N5	**UGAGAAGGGuCggGGUAUUG**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N6	**UGAGAAaGGuCgAGGUAUUG**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N7	**UGAGAAaGGuCggGGUAUUG**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N8	**ACUUUGCUGCCACAAUACCU**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N9	**ACUUUGCUGCCACAAUACCc**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N10	**ACUUUGCUGCCACAAUgCCc**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N11	**ACUUUGCUGCCACgAUgCCc**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N12	**UGAGAAGGGuCgcGGUAUUG**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N13	**UGAGAAGGGuCguGGUAUUG**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N14	**UGAGAAGGGgCggGGUAUUG**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N15	**UGAGAAGGGgCgcGGUAUUG**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N16	**UGAGAAGGGgCguGGUAUUG**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N17	**UGAGAAGGGaCggGGUAUUG**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N18	**UGAGAAGGGaCgcGGUAUUG**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
N19	**UGAGAAGGGaCguGGUAUUG**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU

Black bold = spacer sequence; lower case indicates position complementary to nucleotide substitution introduced by RTT.

Table S3. Sequence of C-terminal AAV Vector With pegRNA/ngRNA P56/N19.

CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCCTTA
ATTAACCTAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATA
GCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCA
GCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCC
CTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGA
CGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCT
ATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTAT
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TGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTA
CATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCG
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AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGA
TCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATT
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GCCGGCAAGCTGACCATGGGACAGCCACTGGTCATCCTGGCACCACACGCAGTGGAGGCCCTGGTGAAGCAGCCTCCAGATCGCTGGCTGTCTAACGCCCGGATGACACAC
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GAGTTGG

Black bold = spacer sequence; red bold = RTT; blue bold = PBS; lower case indicates nucleotide substitution to be introduced by RTT.

Table S4. Assessment of Off-target Editing in R408W Homozygous HuH-7 Cells.

Genomic site	Protospacer/PAM sequence[a]	Amplicon (chromosome: position range)[b]	Treated sample 1[c]	Treated sample 2	Treated sample 3	Control sample 1	Control sample 2	Control sample 3
PAH	ACTTTGCTGCCACAATACCTTGG	chr12:102840439-102840600	32.32	29.99	32.40			
OT1	ttTaTGCTGCCA-AATACCTAGG	chr8:78462251-78462495	0.62	0.47	0.48	0.55	0.60	0.57
OT2	AagcgtgTGCCACAATACCTGGG	chr2:13589808+13589996	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
OT3	tCTaTGCTGCCA-AATACCTGGG	chr4:169715128-169715285	0.95	0.61	0.66	0.59	0.75	0.89
OT4	AaTcTaCTGCCA-AATACCTGGG	chr8:76707015-76707213	0.43	0.39	0.45	0.41	0.48	0.47
OT5	AggTTG-TaCCACAATACCTTGG	chr10:113490901-113491091	0.51	0.51	0.48	0.49	0.50	0.45
OT6	AgcTgGCTG-CACAATACCTTGG	chr13:56216964-56217162	0.55	0.43	0.47	0.50	0.44	0.47
OT7	tCTgcGgTGCCACAATACCTTGG	chr12:98762684+98762848	0.59	0.61	0.56	0.75	0.52	0.71
OT8	AgagTGCaGCCACAATACCTAGG	chr9:28448755+28448930	0.71	0.73	0.67	0.64	0.72	0.68
OT9	ACaaTaCTGCCA-AATACCTAGG	chr6:45052043-45052196	0.71	0.64	0.78	1.53	0.78	1.39
OT10	AtTTATGaaGCCACAATACCTGGG	chr12:90309031+90309183	0.67	0.64	0.58	0.64	0.67	0.61
OT11	gtTTTGCTGCCACAAgACCTAGG	chr6:46216177-46216276	0.86	0.64	0.46	0.43	0.59	0.51
OT12	tgTTTGtTGCCA-AATACCTTGG	chr7:136729999-136730159	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
OT13	ACTTcaCTGCCAtAATACCTGGG	chr2:195824652-195824785	0.95	0.78	1.11	0.89	0.81	0.84
OT14	ACTTTatATGaCACAATACCTGGG	chr9:117378303+117378482	0.48	0.54	0.39	0.54	0.61	0.47
OT15	tCTcTGCT-CCACAATACCTAGG	chr3:25365542+25365698	0.50	0.44	0.47	0.52	0.45	0.55
OT16	tCTgTGCTGCC-CAATACCTTGG	chr13:35884818+35884996	0.72	0.56	0.60	0.64	0.66	0.52
OT17	cCTcTtCT-CCACAATACCTAGG	chr21:27362728-27362863	0.80	0.64	0.62	0.65	0.79	0.66
OT18	gCTTTcCTGCCAC-ATACCTGGG	chr1:84363379+84363574	0.55	0.56	0.57	0.47	0.57	0.47
OT19	Ag-aTGCTGCaACAATACCTAGG	chr18:3456273-3456460	0.80	0.54	0.61	0.62	0.54	0.55
OT20	AggTTcCTcCCACAATACCTGGG	chr5:2961395+2961503	0.70	0.61	0.62	0.78	0.76	0.72
OT21	ACTTTGCaGCCACAtTACCTGGG	chr17:61009795+61009954	0.67	0.72	0.83	0.84	n.d.	1.22
OT22	A-TgTGaTcCCACAATACCTGGG	chr12:5650929-5651072	0.54	0.63	0.64	0.66	0.60	0.56
OT23	AtTcT-CTGCCAtAATACCTGGG	chr8:108304580-108304753	0.52	0.56	0.65	1.10	0.48	0.61
OT24	cCTTTGCTcCCtCAATACCTAGG	chr12:53011957-53012129	0.70	0.62	0.66	0.61	0.71	0.58
OT25	ACTTTGCaGCCACAtgACCTGGG	chr17:75547288+75547430	0.56	0.53	0.78	0.69	0.58	0.63
OT26	ACTTTGCTtCCAaAATAgCTGGG	chr13:76387376-76387540	0.69	0.59	0.62	0.71	0.65	0.70
OT27	ACTTTGtTtCCACAATAgCTAGG	chr13:77055944-77056107	0.69	0.61	0.74	1.11	0.54	0.65
OT28	ACTTTGCTGCtACAATcCCTAGC	chr13:81974451+81974555	0.40	0.49	0.52	0.39	0.51	0.51
OT29	ACTTTGtTGCCACAcgACCTGGG	chr10:73226422+73226613	0.82	0.68	0.99	0.73	0.73	0.88
OT30	ACaTTGCTaCCACAATACCTAGT	chr11:1493022+1493184	0.72	0.71	0.64	0.65	0.69	0.64
OT31	AaTTTGCTGaCACAAgACCTTGG	chr11:10677426+10677544	0.50	0.59	0.35	0.46	0.47	0.53
OT32	AgTTTGCTtCCACAATACaTAGG	chr11:36513516+36513638	0.59	0.52	0.64	0.56	0.51	0.51
OT33	taTTTGCTGCCAgAATACCTTGG	chr9:73068606-73068772	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
OT34	ACagTGCTGCCAaAATACCTGGG	chr15:78935858+78935995	0.62	0.64	0.77	0.61	0.52	0.63
OT35	ACTTgcCTGCCACcATACCTGGG	chrY:7231179+7231337	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
OT36	ACTTTGaTGCCACAATAtCaTGG	chr7:24649482-24649678	0.64	0.63	0.73	0.59	0.74	0.71
OT37	ACTTTtCTGCCtCcATACCTTGG	chr3:70299910+70300073	1.19	0.88	1.00	0.76	1.06	0.86
OT38	ACTTTGCT-CCACAATgCCTGGG	chr1:227324114-227324224	0.50	0.56	0.48	0.50	0.57	0.48
OT39	ACTTTGCTGCCACtgGTACCTGGG	chr4:20389722-20389855	0.62	0.52	0.53	0.51	1.02	0.48
OT40	ACTcTGCT-CCACAATAtCTGGG	chr4:101789193-101789357	0.58	0.64	0.56	0.69	0.64	0.66
OT41	tCTTTGCT-CCACAATAtCTGGG	chr4:129408329+129408552	0.74	0.91	0.87	0.81	0.96	1.04
OT42	ACTTgGCTGCCACcA-ACCTGGG	chr18:23806155+23806259	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
OT43	ACTTTGCTG-CACcATACCTGGC	chr1:70805762-70805954	0.57	0.55	0.53	0.61	0.53	0.50
OT44	ACTTTGCTGCCAC-ATACCaTGT	chr1:216169709+216169886	0.51	0.50	0.51	0.78	0.47	0.65
OT45	AC-TTGCTGCCAaAATgCCTTGG	chr5:52278218-52278389	0.66	0.96	0.67	0.59	0.68	0.60
OT46	ACTTTGCTTGCCAaAATACCTGGA	chr5:59665136-59665275	0.45	0.50	0.47	1.43	0.51	0.51
OT47	ACTTTGCTGCC-CAAgACtTTGG	chr19:49082325+49082466	1.33	1.35	1.33	1.48	1.41	1.42
OT48	ACaTTGCTG-CAgAATACCTTGG	chr12:50837080-50837226	0.79	0.77	0.67	0.72	0.76	0.75
OT49	AC-TgGCTGCCACcATACCTGGG	chr12:79983294-79983445	1.22	0.85	0.83	0.88	0.86	0.74
OT50	ACTTT-CTGCCACAtgACCTGGG	chr12:126286065-126286233	0.65	0.80	0.76	0.72	0.82	0.79
OT51	ACTTTGCTGatAC-ATACCTGGG	chr16:50829626+50829775	0.63	0.71	0.84	0.78	0.69	0.64
OT52	ACTTTGgTGCCAaACATACCTGGG	chr16:67891109-67891286	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
OT53	ACcTTGCTG-CAgAATACCTTGG	chr22:41811842+41811995	0.54	0.53	n.d.	0.48	0.69	0.46
OT54	ACTTCTGCTGgCACAAaACCTTGG	chr8:10512237+10512356	0.56	0.42	0.43	0.44	0.45	0.48
OT55	ACTTaGCTGCCAgAA-ACCTTGG	chr8:130760741+130760888	0.40	0.41	n.d.	0.45	0.43	0.56
OT56	AC-TTGCTGCCACAATAaCTTTG	chr14:93610395+93610551	0.65	0.48	0.70	0.55	0.49	0.58
OT57	AaTTTGCTGCCACAATt-CTAGG	chr10:20442761-20442922	0.51	0.50	0.52	0.49	0.46	0.53
OT58	ACTTTGCT-CCACAtTACCcAGG	chr11:58176050-58176199	0.80	0.76	0.67	0.78	0.81	0.92
OT59	tCTTTGCTGCCA-AtTACCTGGG	chr15:29093086+29093232	0.65	0.58	0.59	0.55	0.63	0.62
OT60	ACTTTGCTG-CAaAATACCTTGC	chr21:39088389+39088584	0.45	0.56	n.d.	0.49	0.46	0.61
OT61	ACcTTGCTGCCA-AAcACCTTGG	chr2:160662807-160662949	0.72	0.60	0.51	0.66	0.52	0.52

OT62	ACaTTGCTGgCACAAT-CCTGGG	chr2:213255950+213256111	0.65	0.57	0.52	0.87	0.54	0.55
OT63	ACTTTGgTtCC-CAATACCTTGG	chr2:196036434-196036580	0.68	0.62	0.73	0.56	0.60	0.72
OT64	ACTcT-CTGCCACAATACCTAGA	chrY:13436340-13436535	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
OT65	ACTTTtCT-CCACAtTACCTTGG	chr6:39041870+39042064	0.56	0.75	0.50	0.59	0.82	0.73
OT66	ACcTTGCTG-CAgAATACCTTGG	chr6:27785457+27785633	0.58	0.62	0.59	n.d.	0.67	0.65
OT67	AgTTTGCTGCCACAA-ACCaAGG	chr6:9683015-9683167	0.47	0.57	0.67	0.60	0.57	n.d.
OT68	ACTTTGg-GCCAgAATACCTAGG	chrX:13570761-13570950	0.51	0.49	1.25	0.64	0.56	0.55
OT69	ACcTTGtTGCCA-AATACCTTGG	chrX:48090226-48090407	0.62	0.64	0.71	0.81	0.65	0.75
OT70	AtTTTGCTGCCACAA-ACCaAGG	chrX:106057761-106057923	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
OT71	ACTgTGCTGCaACAA-ACCTTGG	chrX:143235479-143235619	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
OT72	ACTTTGCT-CCACAATgCCTGGT	chr7:155177942+155178085	0.60	0.56	0.62	0.58	0.57	0.55
OT73	AC-TTGaTGCCACAATACtTGGG	chr3:72392649-72392845	0.44	0.56	0.38	0.45	0.48	0.43
OT74	ACTcTGCTGCCACAgT-CCTAGG	chr3:133006042+133006198	0.48	0.40	0.44	0.51	0.31	n.d.
OT75	ACTcTGaTGCCACAGATACCTGGG	chr3:171524879+171525051	0.58	0.79	0.51	0.60	0.45	0.72
OT76	tCTTTGCT-CCACAATAtCTGGG	chr3:169677452-169677627	0.48	0.54	0.60	0.58	0.59	0.51
OT77	tCTTTGCTGCCAC--TACCTTGG	chr1:184534886-184535019	0.91	0.86	0.99	0.99	0.69	0.80
OT78	ACTTTGCTGC--CAATACtTTGG	chr15:40233992+40234119	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
OT79	ACcTTGCTGCCAC--TACCTGGG	chr2:98883002-98883164	0.72	0.59	0.62	0.74	0.65	0.63

[a] The *PAH* on-target sequence and top ONE-seq-nominated and in-silico-nominated candidate off-target sequences with mismatched positions and bulges indicated by lower case letters and dashes.

[b] Coordinates in the GRCh38/hg38 assembly of the human genome; “+” and “-” indicate forward or reverse orientation of protospacer/PAM sequence.

[c] n.d. = PCR was unsuccessful for the genomic site.