Improved specificity and efficiency of *in vivo* adenine base editing therapies with hybrid guide RNAs

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Abstract

Phenylketonuria (PKU), pseudoxanthoma elasticum (PXE), and hereditary tyrosinemia type 1 (HT1) are autosomal recessive disorders linked to the *PAH*, *ABCC6*, and *FAH* and *HPD* genes, respectively. Here we evaluated the off-target editing profiles of clinical lead guide RNAs (gRNAs) that, when combined with adenine base editors (ABEs), correct the recurrent *PAH* P281L variant, *PAH* R408W variant, or *ABCC6* R1164X variant or disrupt either of two sites in the *HPD* gene (a modifier gene of HT1) in human hepatocytes. To mitigate off-target mutagenesis, we systematically screened hybrid gRNAs with DNA nucleotide substitutions. Comprehensive and variant-aware specificity profiling of these hybrid gRNAs revealed dramatically reduced off-target editing and reduced bystander editing in cells. In humanized *PAH* P281L and *ABCC6* R1164X mouse models of PKU and PXE, we showed that when formulated in lipid nanoparticles (LNPs) with ABE mRNA, selected hybrid gRNAs reverted disease phenotypes, reduced off-target editing, increased on-target editing, and reduced bystander editing *in vivo*. These studies highlight the utility of hybrid gRNAs to improve the safety and efficiency of adenine base editing therapies.

Introduction

In vivo CRISPR base editing is an emerging therapeutic approach to efficiently and precisely make genomic alterations, including the direct correction of pathogenic variants, in a patient's body[1,2]. Early results from a clinical trial of base editing to inactivate the *PCSK9* gene in patients with familial hypercholesterolemia have provided a strong proof of concept of the efficacy of adenine base editing in the human liver *in vivo*[3]. These results motivate the development of liver-centered base-editing therapies to address a wide variety of genetic disorders. In previous

studies, we have demonstrated the ability of *in vivo* base editing to definitively address two such disorders in postnatal or prenatal mouse models: correction of the recurrent *PAH* P281L variant or *PAH* R408W variant in humanized mouse models of PKU, a metabolic disorder caused by increased blood phenylalanine (Phe) levels resulting from a defective phenylalanine hydroxylase (PAH) enzyme[4,5]; and inactivation of the mouse ortholog of the *HPD* gene in a mouse of model of HT1, a metabolic disorder caused by toxic metabolites resulting from a defective fumarylacetoacetate hydroxylase (FAH) enzyme, addressable by blocking the upstream 4-hydroxyphenylpyruvate dioxygenase (HPD) enzyme[6]. PXE is a connective tissue disorder marked by mineralization in various body tissues, associated with decreased blood pyrophosphate levels resulting from a defective ATP binding cassette subfamily C member 6 (ABCC6) transporter most highly expressed in the liver; the *ABCC6* R1164X variant is one of the most recurrent variants identified in PXE patients[7].

The eighth-generation ABE, ABE8.8-m (ABE8.8), has now been validated in preclinical non-human primate studies and in the aforementioned clinical trial.[3,8,9] As with other ABEs, it can use its adenosine deaminase domain to efficiently catalyze A→G edits on the sense or antisense strands of genes and correct a substantial proportion of human pathogenic variants. ABE8.8 also has among the narrowest editing windows of the eighth-generation ABEs[10]. If the position of the target adenine base lies within the window of ABE8.8, a narrow window limits the potential for two undesirable consequences of base editing: bystander editing and off-target editing. Bystander editing of one or more adenine bases near the target adenine base can be counterproductive if it introduces new pathogenic variants alongside the desired corrective editing of the original pathogenic variant. Off-target editing, a general concern with any type of CRISPR editing, entails mutagenesis at genomic sites other than the desired on-target site. Besides the use of a

narrow-window ABE, strategies that can simultaneously reduce off-target editing and bystander editing would be of substantial value in improving the safety and efficiency of ABE therapies.

In this work, we evaluated clinical lead gRNAs that, in combination with an ABE, achieve therapeutically desirable edits in *PAH*, *ABCC6*, or *HPD* in hepatocytes. To reduce the off-target editing by these gRNAs, we explored a previously reported strategy, the *in vitro* or *in cellulo* use of hybrid gRNAs in which certain positions of the spacer sequence are substituted with DNA nucleotides[11–17]—a strategy compatible with the use of mRNA-LNPs for *in vivo* delivery. In systematically assessing the effects of a series of hybrid gRNAs on the off-target profiles of clinical lead gRNAs, we unexpectedly observed that hybrid gRNAs have the potential to reduce bystander editing as well. Importantly, we found that hybrid gRNAs that reduced off-target editing by the lead *PAH* P281L-correcting and *ABCC6* R1164X-correcting ABE/gRNA combinations *in vivo*, in PKU and PXE humanized mouse models, did so without compromising *in vivo* editing efficiency. On the contrary, the hybrid gRNAs significantly increased the desired corrective editing in the liver while simultaneously reducing unwanted bystander editing.

Results

Optimizing correction of the *PAH* P281L variant *in cellulo*

In a previous study, we characterized a clinical lead gRNA (herein termed "PAH1") targeting the human-specific protospacer sequence TCAC**A**GTTCGGGGGTATACA, with the corresponding protospacer-adjacent motif (PAM) TGG, to correct the *PAH* P281L variant[4] (**Fig. 1a**). The variant adenine base lies in position 5 of the protospacer sequence. A potential bystander adenine base lies nearby in position 3; bystander A→G editing would result in a splice site disruption that has been reported to be pathogenic for PKU[18] and thus would be undesirable. In combination

with ABE8.8, the PAH1 gRNA results in efficient corrective editing of the P281L variant *in cellulo* (in HuH-7 hepatocytes homozygous for the P281L variant, derived from the HuH-7 human hepatoma cell line) and *in vivo* (in mouse liver), albeit with sizeable levels of bystander editing[4].

Most CRISPR-based off-target assay techniques are designed to detect double-strand breaks in DNA, whether *in vitro* or *in cellulo*. However, base editors like ABE8.8 do not directly make double-strand breaks, and so conventional off-target assays such as GUIDE-seq do not accurately reflect off-target editing by base editors. We therefore utilized an ABE-tailored version of OligoNucleotide Enrichment and sequencing (ONE-seq) to nominate off-target sites[8,19], followed by genomic sequencing to verify these candidate sites (**Fig. 1b**). ONE-seq is a biochemical assay that uses a synthetic genomic library, selected by similarity between the protospacer DNA sequence targeted by the gRNA and sequences anywhere in a reference human or mouse genome, using tools like Cas-OFFinder[20]. For any given gRNA, the library can comprise tens of thousands of oligonucleotides. The library is mixed *in vitro* with a ribonucleoprotein (RNP) comprising recombinant ABE protein complexed with the synthetic gRNA. The RNP nicks certain oligonucleotide sequences on one strand and deaminates an adenine base on the other strand. EndoV is used to cleave the other strand adjacent to the deaminated base, resulting in the equivalent of a double-strand break. Next-generation sequencing (NGS) then quantifies the frequency with which each unique oligonucleotide sequence was edited *in vitro*, generating a rank-ordered list of candidate off-target sites, normalized to the on-target site, which receives a ONE-seq score of 1.0. Typically, the on-target site is at or near the top of the list. A site with a ONE-seq score of 0.01 had one-hundredth the *in vitro* editing activity of the on-target site.

For the ABE8.8/PAH1 combination, we had previously performed a ONE-seq analysis followed by targeted amplicon sequencing of ≈50 top-ranked sites that verified one genomic site of low-level off-target mutagenesis in HuH-7 hepatocytes.[4] We now undertook a deeper analysis of the ONE-seq rank-ordered list, using hybrid capture sequencing to assess the entire set of 280 genomic sites with ONE-seq scores greater than 0.01 (**Supplementary Table 1**). We verified an additional six sites of off-target mutagenesis in ABE8.8/PAH1 mRNA/gRNA-transfected P281L HuH-7 hepatocytes (**Fig. 2a**). None of the seven sites raises a concern of oncogenic risk (see **Supplementary Note 1**). The third verified off-target site (PAH1_OT3) displayed the most off-target editing, with 1.3% editing.

To assess whether hybrid gRNAs could reduce the off-target editing at the verified sites, we designed a series of 21 synthetic hybrid gRNAs ("PAH1_hyb1" through "PAH1_hyb21") with single, double, or triple DNA nucleotide substitutions ranging from positions 3 through 10 in the spacer sequence (**Fig. 2b**). We transfected ABE8.8 mRNA in combination with the PAH1 standard gRNA and each of the hybrid gRNAs into P281L HuH-7 hepatocytes and assessed for (1) on-target P281L corrective editing, (2) bystander editing at the on-target site, and (3) PAH1_OT3 off-target editing in each of the samples (**Fig. 2c**). While most of the PAH1 hybrid gRNAs had comparable on-target editing to the PAH1 standard gRNA (≈90%), several had modestly decreased editing (as low as ≈80%). Notably and unexpectedly, most of the hybrid gRNAs reduced bystander editing (from 4.4% with the standard gRNA to as low as ≈1%). The PAH1 hybrid gRNAs had highly varied effects on PAH1_OT3 off-target editing. Although triple DNA nucleotide substitutions generally reduced PAH1_OT3 off-target editing more than single or double substitutions, a couple of gRNAs with triple substitutions had greater PAH1_OT3 off-target editing than the standard gRNA. None of the 21 PAH1 hybrid gRNA fully eliminated

PAH1_OT3 off-target editing. As an orthogonal method of off-target profiling, we performed ONE-seq with ABE8.8 and each of the 21 PAH1 hybrid gRNAs (**Fig. 2d**). We used the number of genomic sites with ONE-seq scores greater than 0.01 as a metric of gRNA specificity—the fewer the sites, the less potential for off-target editing. There was good agreement between the results of the site-specific PAH1_OT3 analysis and the more general ONE-seq off-target profiling for PAH1 hybrid gRNAs.

To further reduce off-target editing, we combined triple and double substitutions within a single hybrid gRNA. We chose the triple substitutions of PAH_hyb17 (positions 4, 5, and 6) or PAH_hyb16 (positions 3, 4, and 5), which maximally reduced PAH1_OT3 off-target editing and maximally reduced bystander editing while preserving on-target editing, and added the double substitutions of PAH_hyb15 (positions 9 and 10), which substantially reduced PAH1_OT3 off-target editing and bystander editing, yielding PAH1_hyb22 and PAH1_hyb23. We added an additional substitution in position 11 to PAH1_hyb23, yielding PAH1_hyb24. We compared these three hybrid gRNAs directly against the PAH1 standard gRNA and negative controls via mRNA/gRNA transfection in P281L HuH-7 hepatocytes; all three significantly reduced PAH1_OT3 off-target editing and bystander editing (**Fig. 2e**). ONE-seq confirmed the improved off-target profiles of the three new hybrid gRNAs relative to the 21 original hybrid gRNAs; indeed, PAH1_hyb24 had zero off-target sites with ONE-seq scores greater than 0.01 (**Fig. 2f**). At all seven verified PAH1 off-target sites, OT1 through OT7, PAH1_hyb24 reduced the detectable off-target editing to the background levels of the negative controls (**Fig. 2g**).

All standard off-target assessment techniques share a critical limitation: each is tied to the specific individual genome represented by the cells or by the *in vitro* genomic DNA sample used for analysis. For this reason, most off-target analyses have simply ignored the potential for

naturally occurring human genetic variation to create novel off-target editing sites in some patients. A cautionary tale is provided by the finding that the gRNA used in the recently approved CRISPR-based therapy for sickle cell disease, exa-cel, has substantial off-target editing in hematopoietic stem cells at a genomic site created by a genetic variant present in ≈10% of individuals of African ancestry, resulting in both indel mutations and chromosomal rearrangements[21]. The ONE-seq technique is uniquely capable of performing a variant-aware off-target analysis, accommodating naturally occurring human genetic variation by using oligonucleotide libraries designed not just using the reference human genome but also incorporating data from the 1000 Genomes Project, the Human Genome Diversity Project, etc. We used the bioinformatic tool CRISPRme[21] to design a variant-aware ONE-seq library for the PAH1 protospacer with ≈7,000 non-reference-genome oligonucleotides. In performing a variant-aware ONE-seq experiment with the ABE8.8/PAH1 combination, we identified 40 variant sites with ONE-seq scores greater than 0.01 (**Fig. 2h**). One of the top variant sites, with a ONE-seq score of 0.19, is a rare singleton 1-bp deletion that alters a reference genomic site that has 7 mismatches with the on-target protospacer, with an NTG PAM, into a site that has 3 mismatches, with an NGG PAM (**Extended Fig. 1**). Another top variant site, with a ONE-seq score of 0.11, is common enough to have been previously cataloged as rs76813758, a single-nucleotide variant that alters a reference genomic site that has 4 mismatches with the on-target protospacer, with an NGG PAM, into a site that has 3 mismatches, with an NGG PAM; the variant is present in ≈8% of individuals of African ancestry and ≈3% of individuals of European ancestry (**Extended Fig. 1**).

One limitation of variant-aware off-target analysis is that upon identifying candidate variant sites, it can be challenging to verify whether editing actually occurs at any of those sites in the

therapeutically relevant cells (e.g., hepatocytes) if there is no way to obtain such cells from individuals with those variants. We reasoned that the use of hybrid gRNAs should reduce the potential for off-target editing not only at reference genomic sites but also at variant sites, mitigating the need to evaluate variant sites *in cellulo*. Accordingly, we performed variant-aware ONE-seq experiments for the ABE8.8/PAH1_hyb22 and ABE8.8/PAH1_hyb24 combinations (**Fig. 2h**, **Extended Fig. 1**). With PAH1_hyb22, there were just 3 variant sites with ONE-seq scores greater than 0.01, and with PAH1_hyb24, there were no variant sites with ONE-seq scores greater than 0.01. For rs76813758, the ONE-seq score dropped from 0.11 to 0.004 with PAH1_hyb22 and to 0.0001 with PAH1_hyb24 (one-ten thousandth of the *in vitro* editing activity of the on-target site, within the background of the assay).

Optimizing correction of the *PAH* P281L variant *in vivo*

Although our rational exploration of hybrid gRNAs, culminating in PAH1_hyb24, was successful in rendering editing at the seven verified PAH1 off-target sites undetectable *in cellulo* while preserving on-target editing efficiency *in cellulo*, it remained to be answered whether hybrid gRNAs could function as effectively *in vivo*. We formulated LNPs with ABE8.8 mRNA and PAH1 standard gRNA, PAH1_hyb22 gRNA, PAH1_hyb23, or PAH1_hyb24, exactly paralleling LNPs we used in a recently published study[4]. We administered the LNPs to homozygous humanized P281L PKU mice at a dose of 2.5 mg/kg. We observed that only the PAH1_hyb23 and PAH1_hyb24 gRNAs resulted in complete normalization of blood Phe levels (mean <125 μmol/L) by 48 hours after treatment, significantly outperforming the PAH1 standard gRNA (**Extended Fig. 2**). With ABE8.8/PAH1 LNPs, there was mean 40% whole-liver P281L corrective editing; with each of the hybrid gRNAs, there was mean 50%–60% editing, establishing that the use of the hybrid gRNAs did not compromise and in fact significantly

improved on-target editing (**Fig. 2i**). The increased on-target editing was accompanied by significantly reduced bystander editing (mean 0.8% editing with standard gRNA compared to mean 0.2%–0.3% editing with hybrid gRNAs).

We performed ONE-seq for the ABE8.8/PAH1 combination against the reference mouse genome (**Supplementary Table 2**). In interrogating the candidate sites with the top ONE-seq scores in liver samples from the LNP-treated mice, we verified a site (PAH1_mOT3) with mean 2.1% off-target editing with the PAH1 standard gRNA (**Fig. 2i**). PAH1_hyb22 gRNA, PAH1_hyb23, and PAH1_hyb24 all significantly reduced the off-target editing at this site (<0.2%).

Optimizing correction of the *ABCC6* R1164X variant *in cellulo*

Out of the recurrent *ABCC6* variants causative of PXE, we focused on the R1164X variant because of features suggesting it would be amenable to adenine base editing (**Fig. 1a**, **Fig. 3a**), namely the positioning vis-à-vis a TGG PAM compatible with a gRNA (designated PXE1) with the protospacer sequence GTC**A**CGGGAAACTGATCCTC, the variant adenine base lying in position 4, and potential bystander adenine bases lying in positions 9, 10, and 11, outside the reported editing window of ABE8.8. We used prime editing to generate a homozygous R1164X HuH-7 cell line (**Supplementary Note 2**). Transfection of the cell line with ABE8.8 mRNA and the PXE1 gRNA achieved substantial corrective editing of the R1164X variant (32%), albeit with a moderate amount of unwanted bystander editing (6.3%) (**Fig. 3b**). For the ABE8.8/PXE1 combination, we performed a ONE-seq analysis followed by targeted amplicon sequencing of top-ranked sites that verified six genomic sites of off-target mutagenesis in the HuH-7 cells (**Supplementary Table 3**). None of the six sites raises a concern of oncogenic risk (see

Supplementary Note 1). The first and second verified off-target sites (PXE1_OT1, PXE1_OT2) displayed the most off-target editing.

As we had done with the PAH1 gRNA, we designed a series of 21 hybrid gRNAs for PXE1 (PXE1_hyb1 through PXE1_hyb21) with single, double, or triple DNA nucleotide substitutions ranging from positions 3 through 10 in the spacer sequence (**Fig. 3a**), matching the designs of the PAH1 hybrid gRNAs. Upon transfection of ABE8.8 mRNA and each of the gRNAs into R1164X HuH-7 hepatocytes, we found that all of the hybrid gRNAs reduced off-target editing at the OT1 and OT2 sites, some of the hybrid gRNAs increased on-target editing (to as high as ≈50%), and some of the hybrid gRNAs reduced bystander editing (to as low as ≈3%) (**Fig. 3b**). We performed ONE-seq for each of the hybrid gRNAs and, judging by the number of genomic sites with ONE-seq scores greater than 0.01, improvement of off-target editing was concordant with the effects on the individual OT1 and OT2 sites (**Fig. 3c**). We further evaluated PXE1_hyb18 and found that it significantly increased on-target editing and significantly, though not entirely, reduced off-target editing at the OT1 and OT2 sites (**Fig. 3d**). It is possible that additional DNA nucleotide substitutions would yield hybrid gRNAs that further reduce off-target editing.

Optimizing correction of the *ABCC6* R1164X variant *in vivo*

We used CRISPR-Cas9 targeting in mouse embryos to generate a humanized PXE model, in the C57BL/6J background, in which we replaced a small portion of the endogenous mouse *Abcc6* exon 24 with the orthologous human sequence spanning the PXE1 protospacer/PAM sequences and containing the R1164X variant (**Supplementary Note 3**). Consistent with prior studies of

Abcc6 knockout mice[7], homozygous R1164X mice had reduced blood pyrophosphate levels compared to wild-type littermates (**Extended Fig. 3**).

We formulated LNPs with ABE8.8 mRNA and either PXE1 standard gRNA or PXE1_hyb18 gRNA. We administered the LNPs to homozygous R1164X mice at a dose of 2.5 mg/kg. With ABE8.8/PXE1 LNPs, there was mean 24% whole-liver P281L corrective editing in the absence of bystander editing, and mean 3.5% corrective editing with bystander editing; with the hybrid gRNA, 29% and 2.1% editing, respectively (**Fig. 3e**). The PXE1 hybrid gRNA significantly increased the on-target editing and reduced bystander editing *in vivo*, as was observed with PAH1 hybrid gRNAs *in vivo*. LNP treatment also largely normalized the blood pyrophosphate levels in the homozygous R1164X mice (**Extended Fig. 3a**).

We performed ONE-seq for the ABE8.8/PXE1 combination against the reference mouse genome (**Supplementary Table 4**). In interrogating the candidate sites with the top ONE-seq scores in liver samples from the LNP-treated mice, we verified a site (PXE1_mOT24) with mean 8.4% off-target editing with the PXE1 standard gRNA (**Fig. 3e**). The PXE1_hyb18 gRNA significantly reduced the off-target editing at this site, with mean 0.6% editing. We also assessed blood alanine aminotransferase (ALT) and cytokine/chemokine levels in LNP-treated mice, and we observed similar safety profiles with the standard and hybrid gRNAs (**Extended Fig. 3b**, **Supplementary Table 5**).

Optimizing targeting of two distinct sites in the *HPD* gene with hybrid gRNAs

Lacking any previous data regarding adenine base editing to inactivate the *HPD* gene—our previous study assessed only cytosine base editing—we screened for precise disruption of a splice donor or acceptor site in the human *HPD* gene by adenine base editing (**Supplementary**

Note 4). We observed the highest levels of editing with the gRNAs designated "HPD20", "HPD3", "HPD4", and "HPD5", targeting either the splice acceptor of exon 7 or the splice donor of exon 8 (**Fig. 1a**, **Extended Fig. 4a**). To evaluate for off-target editing, we used two ONE-seq libraries against the reference human genome: a combined library for the cluster of closely spaced HPD3, HPD4, and HPD5 protospacers (consecutive protospacers shifted by one nucleotide), and a library for the HPD20 protospacer. Targeted amplicon sequencing of top-ranked sites in ABE8.8/gRNA-treated wild-type HuH-7 hepatocytes verified one genomic site of off-target mutagenesis each for HPD3 and HPD4, and none for HPD5 and HPD20 (**Extended Fig. 4b**, **Supplementary Tables 6–9**).

Because of its more favorable on-target and off-target editing profiles, we prioritized HPD20 for further study as a clinical lead gRNA. We assessed a full series of HPD20 hybrid gRNAs (HPD20_hyb1 through HPD20_hyb21) (**Fig. 4a**), matching the designs of the PAH1 hybrid gRNAs, via ABE8.8 mRNA/gRNA transfection in wild-type HuH-7 hepatocytes, for both editing of the target splice site adenine—in position 6 of the protospacer sequence, TCTGC**A**GAAAGCACGGGAAC, with a GGG PAM—and bystander editing of positions 8, 9, and/or 10 (**Fig. 4b**). Because we had not verified any individual genomic sites of off-target editing with the HPD20 standard gRNA, we used ONE-seq to assess the off-target editing profiles of the HPD20 hybrid gRNAs, with many showing decreased off-target potential (i.e., number of sites with ONE-seq scores greater than 0.01) but a few showing increased off-target potential (**Fig. 4c**). Two hybrid gRNAs, HPD20_hyb22 and HPD20_hyb23, with 5 DNA nucleotide substitutions each, maintained on-target editing, substantially reduced bystander editing, and had the fewest sites with ONE-seq scores greater than 0.01 (**Fig. 4a–c**).

We also assessed the HPD4 gRNA, having the protospacer sequence CACTC**A**CAGTTTAGGAAGTA and a GGG PAM, with the target adenine base in position 6 and a bystander adenine base in position 8. Having verified a site of off-target editing with the HPD4 standard gRNA (HPD4_OT13) with mean 4.9% editing, we assessed a full series of HPD4 hybrid gRNAs (HPD4_hyb1 through HPD4_hyb21) (**Fig. 4d**), matching the designs of the PAH1 hybrid gRNAs, via ABE8.8 mRNA/gRNA transfection in wild-type HuH-7 hepatocytes (**Fig. 4e**). All the hybrid gRNAs reduced OT13 off-target editing, some close to the background level, and many hybrid gRNAs reduced bystander editing while maintaining on-target editing.

Optimizing correction of the *PAH* R408W variant with a PAM-relaxed ABE

In a previous study, we characterized a gRNA (herein termed "PAH4") that corrects the *PAH* R408W variant *in cellulo* and *in vivo*[5]. Because there are no NGG PAMs situated appropriately for adenine base editing of the *PAH* R408W variant, we screened PAM-altered and PAM-relaxed versions[22] of ABEs and identified an optimal combination of SpRY-ABE8.8 and the protospacer sequence GGCC**A**AGGTATTGTGGCAGC with an AAA PAM (**Fig. 1a**). The variant adenine base lies in position 5 of the protospacer sequence. Potential bystander adenine bases lie in positions 6 and 10; bystander editing at position 6 would result in a benign synonymous variant, whereas editing at position 10 would result in a nonsynonymous variant that has been reported to be a pathogenic variant for PKU[23,24].

Editors based on the SpRY variant of *Streptococcus pyogenes* Cas9 are near-PAMless in their ability to engage genomic sites[22], greatly expanding their targeting range but also incurring an increased potential for off-target editing. To evaluate the SpRY-ABE8.8/PAH4 combination, which corrects the *PAH* R408W variant, we performed ONE-seq against the reference human

genome. Rather than interrogate candidate off-target sites nominated by ONE-seq with the PAH4 standard gRNA, we immediately tested a limited series of hybrid gRNAs (double/triple substitutions, PAH4_hyb9 through PAH4_hyb21, matching the designs of the analogous PAH1 hybrid gRNAs) with the goal of reducing the potential of SpRY-ABE8.8 for off-target editing and prospectively winnowing the list of candidate off-target sites for verification (**Fig. 5a**). Several of the PAH4 hybrid gRNAs displayed increased on-target editing and/or decreased bystander editing at protospacer position 10 (nonsynonymous variant) (**Fig. 5b**); ONE-seq demonstrated reduced off-target potential with some of these favorable hybrid gRNAs despite the use of a PAM-relaxed ABE (**Fig. 5c**).

Discussion

In this study, we investigated the off-target editing profiles of clinical lead gRNAs in combination with ABEs for the correction of pathogenic variants in PKU and PXE and for inactivation of the *HPD* gene, a modifier for HT1. We assessed the ability of hybrid gRNAs to mitigate the potential for off-target editing by the standard versions of the gRNAs and found that some hybrid gRNAs can dramatically reduce off-target editing while also reducing bystander editing while maintaining or even enhancing on-target editing efficiency *in cellulo* and *in vivo*. Our findings suggest that the use of hybrid gRNAs to reduce off-target and bystander editing by ABEs is generalizable across different target loci and underscores its potential as a broadly applicable strategy to improve the safety and efficiency of base-editing therapies for various genetic disorders.

We note the limitations of this study. All the work in this study focused on adenine base editing; it is possible that the advantages we observed with the use of hybrid gRNAs *in cellulo* and *in*

vivo are relevant to other editing modalities that rely on the use of gRNAs, such as nuclease editing, prime editing, and epigenome editing, but this would require validation for each modality. We present data for five distinct loci *in cellulo* and two of the loci *in vivo*, and in each case we were able to identify hybrid gRNAs that had favorable effects on editing specificity and efficiency, but it is possible that hybrid gRNAs would not be beneficial at all loci. In comparing our results across the five loci, our findings suggest that there will not be a single set of hybrid gRNA modifications that will fully optimize off-target and on-target editing across all genomic loci, although suggestive patterns are evident—e.g., the "hyb16" design (DNA nucleotide substitutions in protospacer positions 3, 4, and 5) substantially reduced off-target editing potential while maintaining on-target editing at all five tested loci, and so this design might be a reasonable starting point for future hybrid gRNA screening campaigns. Nonetheless, empirical testing will be needed for each new lead gRNA being considered for therapeutic use, at least for the foreseeable future. Although we have demonstrated that the use of a hybrid gRNA can substantially reduce gRNA-dependent off-target editing by an ABE both *in cellulo* and *in vivo*, it is not clear that use of a hybrid gRNA would affect gRNA-independent off-target editing, i.e., spurious RNA or DNA deamination by the ABE's adenosine deaminase domain[25,26]; further experimentation would be needed to evaluate this possibility. These issues notwithstanding, it is clear that hybrid gRNAs can substantially improve some mRNA-LNP adenine base editing therapies—such as the ABE8.8/PAH1_hyb24 combination for the treatment of PKU, which we intend to take forward into an early-phase clinical trial in the near future.

Methods

The research described here complied with all relevant regulations. All recombinant DNA research was approved by the Institutional Biosafety Committee at the University of Pennsylvania, where the studies were performed, and were consistent with local, state, and federal regulations as applicable, including the National Institutes of Health Guidelines for Research Involving Recombinant of Synthetic Nucleic Acid Molecules. All procedures used in animal studies were approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania (protocol #805887), where the studies were performed, and were consistent with local, state, and federal regulations as applicable, including the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

RNA production. 100-mer gRNAs were chemically synthesized under solid phase synthesis conditions by a commercial supplier (Agilent or Integrated DNA Technologies) with end-modifications as well as heavy 2'-O-methylribosugar modification as previously described[27]: for cellular studies, 5'-mX*mX*mX*XXXXXXXXXXXXXXXXXXGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*U*U*U-3'; for mouse studies, 5'-mX*mX*mX*XXXXXXXXXXXXXXXXXXGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU-3'; where the Xs indicate the spacer sequence positions (specified in Fig. 2b, Fig. 3a, Fig. 4a, Fig. 4d, and Fig. 5a), and "m" and * respectively indicate 2'-O-methylation and phosphorothioate linkage.

ABE8.8 mRNA and SpRY-ABE8.8 mRNA were produced via in vitro transcription (IVT) and purification as previously described[4,5]. In brief, a plasmid DNA template containing a codon-optimized ABE8.8 or SpRY-ABE8.8 coding sequence and a 3' polyadenylate sequence was linearized. An IVT reaction containing linearized DNA template, T7 RNA polymerase, NTPs, and cap analog was performed to produce mRNA containing N1-methylpseudouridine. After digestion of the DNA template with DNase I, the mRNA product underwent purification and buffer exchange, and the purity of the final mRNA product was assessed with spectrophotometry and capillary gel electrophoresis. Elimination of double-stranded RNA contaminants was assessed using dot blots and transfection into human dendritic cells. Endotoxin content was measured using a chromogenic Limulus amebocyte lysate (LAL) assay; all assays were negative.

LNP formulation. LNPs were formulated as previously described[4,5,28], with the lipid components (SM-102, 1,2-distearoyl-sn-glycero-3-phosphocholine, cholesterol, and PEG-2000 at molar ratios of 50:10:38.5:1.5) being rapidly mixed with an aqueous buffer solution containing ABE8.8 mRNA and a gRNA in a 1:1 ratio by weight in 25 mM sodium acetate (pH 4.0), with an N:P ratio of 5.6. The resulting LNP formulations were subsequently dialyzed against sucrose-containing buffer, concentrated using Amicon Ultra-15 mL Centrifugal Filter Units (Millipore Sigma), sterile-filtered using 0.2-µm filters, and frozen until use. The LNPs underwent quality control for particle size (Z-Ave, hydrodynamic diameter), polydispersity index as determined by dynamic light scattering (Malvern NanoZS Zetasizer) and total RNA encapsulation as measured by the Quant-iT Ribogreen Assay (Thermo Fisher Scientific).

Culture and transfection of HuH-7 cells. Wild-type HuH-7 cells, *PAH* P281L homozygous HuH-7 cells[4], *PAH* R408W homozygous HuH-7 cells[5], and *ABCC6* R1164X homozygous HuH-7 cells (**Supplementary Note 2**) were used for this study. HuH-7 cells were maintained in Dulbecco's modified Eagle's medium (containing 4 mM L-glutamine and 1 g/L glucose) with 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C with 5% CO_2. HuH-7 cells were seeded on 24-well plates (Corning) at 1.2×10^5 cells per well. For plasmid transfections, at 3–4 hours after seeding, cells were transfected at approximately 80–90% confluency with 1.5 µL TransIT®-LT1 Transfection Reagent (MIR2300, Mirus), 0.25 µg base editor plasmid, and 0.25 µg gRNA plasmid per well according to the manufacturer's instructions. An ABE8.8 (ABE8.8-m) expression plasmid[10] was used as previously described[4], and HPD3, HPD4, HPD5, HPD20, and PXE1 gRNA expression plasmids were newly generated for this study. For mRNA/gRNA transfections, in one tube, 3 µL Lipofectamine MessengerMax (LMRNA008, Thermo Fisher Scientific) was added to 50 µL of Opti-MEM and incubated for 10 minutes; in another tube, 0.5 µg mRNA and 0.5 µg gRNA was suspended in a total of 50 µL of Opti-MEM. The diluted RNA was added to the tube of diluted MessengerMAX, and the subsequent RNA/MessengerMax/Opti-MEM mixture was incubated for 5 minutes at room temperature and then added dropwise onto the cells. For each type of transfection, cells were cultured for 72 hours after transfection, and then media were removed, cells were washed with 1× DPBS (Corning), and genomic DNA was isolated using the DNeasy Blood and Tissue Kit (QIAGEN) according to the manufacturer's instructions.

ONE-seq. ONE-seq was performed as previously described[4,8,19]. The human ONE-seq libraries for the PAH1, PAH4, PXE1, HPD3/HPD4/HPD5 (combined library), and HPD20 gRNAs were

designed using the GRCh38 Ensembl v98 reference genome (ftp://ftp.ensembl.org/pub/release-98/fasta/homo_sapiens/dna/Homo_sapiens.GRCh38.dna.chromosome.{1-22,X,Y,MT}.fa, ftp://ftp.ensembl.org/pub/release-98/fasta/homo_sapiens/dna/Homo_sapiens.GRCh38.dna.nonchromosomal.fa), and the mouse ONE-seq libraries for the PAH1 and PXE1 gRNAs were designed using the GRCm39 Ensembl reference genome (ftp://ftp.ensembl.org/pub/release-113/fasta/mus_musculus/dna/Mus_musculus.GRCm39.dna.chromosome.{1-19,X,Y,MT}.fa, ftp://ftp.ensembl.org/pub/release-113/fasta/mus_musculus/dna/Mus_musculus.GRCm39.dna.nonchromosomal.fa). Sites with up to 6 mismatches and sites with up to 4 mismatches plus up to 2 DNA or RNA bulges, compared to the on-target site, were identified with Cas-Designer v1.2[20]. The variant-aware ONE-seq library for the PAH1 gRNA was designed using CRISPRme[21], with similar mismatch criteria. The final oligonucleotide sequences were generated with a script[19], and the oligonucleotide libraries were synthesized by Twist Biosciences. Recombinant ABE8.8 protein and SpRY-ABE8.8 protein were produced by GenScript. Duplicate ONE-seq experiments were previously performed for ABE8.8/PAH1[4]; mean ONE-seq scores were used for this study. All other ONE-seq experiments were performed specifically for this study as follows. Each library was PCR-amplified and subjected to 1.25× AMPure XP bead purification. After incubation at 25°C for 10 minutes in CutSmart buffer, RNP comprising 769 nM recombinant ABE8.8-m protein and 1.54 μM gRNA was mixed with 100 ng of the purified library and incubated at 37°C for 8 hours. Proteinase K was added to quench the reaction at 37°C for 45 minutes, followed by 2× AMPure XP bead purification. The reaction was then serially incubated with EndoV at 37°C for 30 minutes, Klenow Fragment (New England Biolabs) at 37°C for 30 minutes, and NEBNext Ultra

II End Prep Enzyme Mix (New England Biolabs) at 20°C for 30 minutes followed by 65°C for 30 minutes, with 2× AMPure XP bead purification after each incubation. The reaction was ligated with an annealed adaptor oligonucleotide duplex at 20°C for 1 hour to facilitate PCR amplification of the cleaved library products, followed by 2× AMPure XP bead purification. Size selection of the ligated reaction was performed on a BluePippin system (Sage Science) to isolate DNA of 150-200 bp on a 3% agarose gel cassette, followed by two rounds of PCR amplification to generate a barcoded library, which underwent paired-end sequencing on an Illumina MiSeq System as described below. The analysis pipeline[19] used for processing the data assigned a score quantifying the editing efficiency with respect to the on-target site to each potential off-target site. Sites were ranked based on this ONE-seq score, which was used for site prioritization.

Next-generation sequencing (NGS). NGS was performed as previously described[4]. For targeted amplicon sequencing, PCR reactions were performed using NEBNext Polymerase (NEB) using the primer sets listed in Supplementary Table 10, designed with Primer3 v4.1.0 (https://primer3.ut.ee/). The following program was used for all genomic DNA PCRs: 98°C for 20 seconds, 35× (98°C for 20 seconds, 57°C for 30 seconds, 72°C for 10 seconds), 72°C for 2 minutes. PCR products were visualized via capillary electrophoresis (QIAxcel, QIAGEN) and then purified and normalized via an NGS Normalization 96-Well Kit (Norgen Biotek Corporation). A secondary barcoding PCR was conducted to add Illumina barcodes (Nextera XT Index Kit V2 Set A and/or Nextera XT Index Kit V2 Set D) using ≈15 ng of first-round PCR product as template, followed by purification and normalization. Final pooled libraries were quantified using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific) and then after denaturation, dilution to 10 pM, and supplementation with 15% PhiX, underwent single-end or paired-end

sequencing on an Illumina MiSeq System. The amplicon sequencing data were analyzed with CRISPResso2 v2[29] and scripts to quantify editing. For on-target editing, A-to-G editing was quantified at the site of the target adenine and at the site(s) of each potential bystander adenine in the editing window; for candidate off-target sites, A-to-G editing was quantified throughout the editing window (positions 1 to 10 of the protospacer sequence). In some cases, PCR amplicons were subjected to confirmatory Sanger sequencing, performed by GENEWIZ.

Hybrid capture sequencing. The PAH1 probe library was generated to cover the 280 ONE-seq-nominated genomic sites with ONE-seq scores greater than 0.01, as well as additional sites. The probe library was generated serially using Agilent SureDesign, where probes were created using three rounds of generation settings. In the initial round, probes were generated with 2× tiling density, moderately stringent masking, and optimized performance with 90 minutes hybridization boosting. Regions that were not covered with the initial settings had probes generated with 2× tiling density, least stringent masking, and optimized performance with 90 minutes hybridization boosting. The remaining uncovered regions had probes generated with 1× tiling density, no masking, and no boosting. The hybrid capture procedure was conducted using the SureSelect XT HS2 DNA Reagent Kit (Agilent) as per the manufacturer's instructions. Briefly, 200 ng of genomic DNA from ABE8.8/PAH1 mRNA/gRNA-treated or control untreated HuH-7 cells, quantified on the TapeStation Genomic DNA ScreenTape platform (Agilent), were prepared and enzymatically fragmented with modifications for 2×150 reads. The DNA fragments were then processed for library construction using molecular barcodes. Adaptors were ligated to the DNA fragments, followed by repair, dA-tailing, and purification using AMPure XP beads. The libraries were amplified (8 cycles), indexed, and purified with AMPure XP beads, and their

quality was assessed via the TapeStation D1000 ScreenTape platform (Agilent); 1000 ng of each library was prepared and hybridized to the PAH1 probe library, captured using streptavidin beads, amplified (16 cycles), and purified using AMPure XP beads. The final libraries were purified and quantified via the TapeStation High Sensitivity D1000 ScreenTape platform (Agilent). The libraries were pooled and sequenced on an Illumina NovaSeq, performed by Novogene. The resulting reads were processed and analyzed using the Agilent Alissa Reporter software with the following settings: normal/tumor pair analysis mode for treated samples, and tumor only for control samples, molecular barcode deduplication mode, duplex MBC deduplication consensus mode, 2 read pairs per MBC minimum, 10 reads minimum coverage depth, 3 reads minimum supporting variant allele, and 0.001 minimum variant allele frequency. For each of the 280 sites, A-to-G editing was quantified throughout the editing window (Supplementary Table 1). For sites with any position displaying a mean net A-to-G editing level ≥ 0.1% in treated versus control samples, the sites were confirmed with targeted amplicon sequencing, as described above. Only 2 out of the 280 sites failed to be captured and sequenced, with both sites being on chromosome Y, which is absent from HuH-7 cells.

Mouse studies. Mouse studies were performed as previously described[4]. Mice were maintained on a 12-hour light/12-hour dark cycle, with a temperature range of 65°F to 75°F and a humidity range of 40% to 60%, and were fed ad libitum with a chow diet (LabDiet, Laboratory Autoclavable Rodent Diet 5010). Humanized PKU mice homozygous for the *PAH* P281L allele[4] or for the *ABCC6* R1164X allele (Supplementary Note 3) on the C57BL/6J background were generated as littermates/colonymates with wild-type C57BL/6J mice. Genotyping was performed using PCR amplification from genomic DNA samples (prepared from clipped tails/ears)

followed by Sanger sequencing or NGS. Roughly equal numbers of female and male littermates/colonymates were used for experiments at ages ranging from six to eight weeks, with random assignment of animals to various experimental groups when applicable, and with collection and analysis of data performed in a blinded fashion when possible. LNPs were administered to mice at a dose of 2.5 mg/kg via retro-orbital injection under anesthesia with 1%-2% inhaled isoflurane. Blood samples were collected via the tail tip at various timepoints: pre-treatment or 4 hours, 24 hours, 48 hours, or 7 days after treatment. Mice were euthanized 1 to 2 weeks after treatment, and 8 liver samples (2 from each lobe) were obtained on necropsy and processed with the DNeasy Blood and Tissue Kit (QIAGEN) as per the manufacturer's instructions to isolate genomic DNA. Euthanasia in all instances was achieved via terminal inhalation of carbon dioxide followed by secondary euthanasia through cervical dislocation or decapitation, consistent with the 2020 American Veterinary Medical Association Guidelines on Euthanasia. NGS results from the liver samples were averaged to provide quantification of whole-liver editing.

Measurement of blood phenylalanine. Blood phenylalanine levels were measured by an enzymatic method using the Phenylalanine Assay Kit (MAK005, Millipore Sigma) according to the manufacturers' instructions. Briefly, plasma samples were collected at timepoints in the early afternoon, to account for diurnal variation in blood phenylalanine levels, and deproteinized with a 10 kDa MWCO spin filter (CLS431478-25EA, Millipore Sigma) and pre-treated with 5 µL of tyrosinase for 10 minutes at room temperature prior to start of the assay. Reaction mixes were made according to the manufacturers' instructions, and the fluorescence intensity of each sample was measured (λ_{ex} = 535/λ_{em} = 587 nm).

Measurement of blood pyrophosphate. Plasma samples collected pre-treatment and 7 days post-treatment were diluted 1:1 in Tris-acetate pH 8.0 and centrifuged with a 30K filter (OD030C34, Cytiva) at 14,000 × g at 4°C for 20 minutes. The pyrophosphate concentrations were measured in platelet-free plasma using ATP sulfurylase to convert pyrophosphate into ATP in the presence of excess adenosine 5' phosphosulfate followed by a luminescent assay of ATP, as described previously[30,31].

Measurement of ALT, cytokines, and chemokines. Assays were performed as previously described[5]. Alanine aminotransferase (ALT) (MAK052-1KT, Millipore Sigma) activity was measured according to the manufacturer's instructions. The expression profile of cytokines and chemokines in mouse serum samples was determined using the BioLegend anti-virus response panel [13-plex bead-based assay for the quantification of interferons (α, β, γ), interleukins (1β, 6, 10, 12p70), and chemokines (MCP-1, RANTES, CXCL-1, IP-10, TNF-α, and GM-CSF)] according to the manufacturer's instructions. Briefly, kit components were thawed and reconstituted as needed. Standards were prepared by 1:4 serial dilution in assay buffer. Serum samples were diluted 1:3 in Matrix A. Filter bottom plates were prewetted with wash buffer before addition of standards and samples. 25 µL of each standard and sample was added to the plates in duplicates and mixed with 25 µL of premixed beads solution. Plates were incubated with shaking at 500 rpm for 2 hours and washed twice, followed by addition of 25 µL detection antibodies and incubation at 500 rpm for 1 hour. Without washing, 25 µL SA-PE was added to each well and incubated with shaking at 500 rpm for 30 minutes. Plates were washed twice, and beads were resuspended in 150 µL wash buffer, transferred to FACS tubes, and acquired on an

BD LSR flow cytometer. The standard curve was used to determine the concentrations of each analyte in the serum samples using a 5PL fitting.

Data analysis. Sequencing data were analyzed as described above. Other data were collected and analyzed using GraphPad Prism v10.4.1.

Data availability

DNA sequencing data that support the findings of this study will be deposited in the NCBI Sequence Read Archive upon publication of this manuscript. Other data supporting the findings of this study are available within the manuscript and its Supplementary Information. The GRCh38 Ensembl v98 reference genome (ftp://ftp.ensembl.org/pub/release-98/fasta/homo_sapiens/dna/Homo_sapiens.GRCh38.dna.chromosome.{1-22,X,Y,MT}.fa, ftp://ftp.ensembl.org/pub/release-98/fasta/homo_sapiens/dna/Homo_sapiens.GRCh38.dna.nonchromosomal.fa) annotation and the GRCm39 Ensembl reference genome (ftp://ftp.ensembl.org/pub/release-113/fasta/mus_musculus/dna/Mus_musculus.GRCm39.dna.chromosome.{1-19,X,Y,MT}.fa, ftp://ftp.ensembl.org/pub/release-113/fasta/mus_musculus/dna/Mus_musculus.GRCm39.dna.nonchromosomal.fa) annotation were used.

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Author contributions

K.M., M.-G.A., W.H.P., and X.W. supervised the work with intellectual input on experimental design and data analysis from J.Z.W., R.C.A.-N., and Q.L. M.N.W., L.C.T., D.L.B., A.Q., I.J., D.V., P.Q., Q.L., and X.W. contributed to web laboratory experiments. M.N.W. and K.M. contributed to bioinformatic analyses. H.S, G.D., and M.-G.A. contributed to mRNA production, LNP formulation, and cytokine/chemokine analysis. M.N.W., L.C.T., K.M., and X.W. drafted the manuscript, and all authors contributed to the editing of the manuscript.

Competing interests

M.N.W. is a fellow with Artis Ventures. J.Z.W. is an employee of Agilent Technologies. R.C.A.-N. is an advisor to Latus Bio. K.M. is an advisor to and holds equity in Verve Therapeutics and Variant Bio, is an advisor to LEXEO Therapeutics and Capstan Therapeutics, and receives research funding from Nava Therapeutics and Beam Therapeutics. M.-G.A. is an advisor to Afrigen Biologics. X.W. is an advisor to and receives research funding from Entact Bio. The University of Pennsylvania and Children's Hospital of Philadelphia have filed patent applications related to the use of base editing for the treatment of phenylketonuria and tyrosinemia (inventors include M.N.W., D.L.B., R.C.A.-N., K.M., W.H.P., and X.W.). The remaining authors declare no competing interests.

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Figure legends

Fig. 1 | Assessment of on-target and off-target editing by candidate clinical lead guide RNAs. a, Schematics of the genomic sites of the five loci that are evaluated in this study, adapted from the UCSC Genome Browser (GRCh38/hg38). Each vertical yellow bar indicates either the G altered to A (in red) by a pathogenic variant, or the target A (in red) at a splice site intended for disruption. Each horizontal grey bar indicates the protospacer (thick) and protospacer-adjacent motif (PAM) (thin) sequences targeted by the respective guide RNA (gRNA). **b**, Schematic of the ONE-seq methodology.

Fig. 2 | Optimizing correction of the *PAH* P281L variant *in cellulo* and *in vivo*. a, Hybrid capture sequencing of ONE-seq-nominated sites in treated vs. control *PAH* P281L HuH-7 cells with PAH1 gRNA in combination with ABE8.8 mRNA (*n* = 3 treated and 3 untreated biological replicates), with seven human sites verified to have off-target editing. **b**, Spacer sequences of PAH1 standard and hybrid gRNAs. "rN" = ribonucleotide; "dN" = deoxyribonucleotide; "m" = 2'-O-methylation; * = phosphorothioate linkage. DNA substitutions are in red bold and underlined. **c**, A-to-G editing of *PAH* on-target site (correction of P281L variant only, or correction plus unwanted nonsynonymous bystander editing) or verified PAH1 human off-target site (PAH1_OT3) in *PAH* P281L HuH-7 cells treated with PAH1 standard or hybrid gRNA in combination with ABE8.8 mRNA (*n* = 2–3 biological replicates per condition). **d**, Number of sites with ONE-seq score > 0.01 for each indicated PAH1 standard or hybrid gRNA with ABE8.8 protein. **e**, A-to-G editing of *PAH* on-target site or verified PAH1 human off-target site in *PAH* P281L HuH-7 cells treated with PAH1 standard or hybrid gRNA in combination with ABE8.8 mRNA (*n* = 11–12 biological replicates per condition). **f**, Number of sites with ONE-seq score > 0.01 for each indicated PAH1 standard or hybrid gRNA with ABE8.8 protein. **g**, Total A-to-G editing of *PAH* on-target site or any of seven verified PAH1 human off-target sites in untreated vs. treated *PAH* P281L HuH-7 cells with PAH1 standard or PAH1_hyb24 gRNA in combination with ABE8.8 mRNA (*n* = 11–12 biological replicates per condition). **h**, Number of variant (non-reference-genome) sites with ONE-seq score > 0.01 for each indicated PAH1 standard or hybrid gRNA with ABE8.8 protein. **i**, A-to-G editing of *PAH* on-target site or verified PAH1 mouse off-target site (PAH1_mOT3) in homozygous *PAH* P281L mice treated with 2.5 mg/kg dose of LNPs with PAH1 standard or hybrid gRNA in combination with ABE8.8 mRNA (*n* = 4–5 animals per group). Means are shown; *P* values were calculated with the Mann–Whitney *U* test.

Fig. 3 | Optimizing correction of the *ABCC6* R1164X variant *in cellulo* and *in vivo*. a, Spacer sequences of PXE1 standard and hybrid gRNAs. "rN" = ribonucleotide; "dN" = deoxyribonucleotide; "m" = 2'-O-methylation; * = phosphorothioate linkage. DNA substitutions are in red bold and underlined. **b**, A-to-G editing of *ABCC6* on-target site (correction of R1164X variant only, or correction plus unwanted nonsynonymous bystander editing) or either of two verified PXE1 human off-target sites (PXE1_OT1, PXE_OT2) in *ABCC6* R1164X HuH-7 cells treated with PXE1 standard or hybrid gRNA in combination with ABE8.8 mRNA (*n* = 2–3 biological replicates per condition). **c**, Number of sites with ONE-seq score > 0.01 for each indicated PXE1 standard or hybrid gRNA with ABE8.8 protein. **d**, A-to-G editing of *ABCC6* on-target site or either of two verified PXE1 human off-target sites in *ABCC6* R1164X HuH-7 cells treated with PXE1 standard or PXE1_hyb18 gRNA in combination with ABE8.8 mRNA (*n* = 5–

6 biological replicates per condition). **e**, A-to-G editing of *ABCC6* on-target site or verified PXE1 mouse off-target site (PXE1_mOT24) in homozygous *ABCC6* R1164X mice treated with 2.5 mg/kg dose of LNPs with PXE1 standard or PXE1_hyb18 gRNA in combination with ABE8.8 mRNA (*n* = 6 animals per group). Means are shown; *P* values were calculated with the Mann–Whitney *U* test.

Fig. 4 | Optimizing targeting of two distinct sites in the *HPD* gene with hybrid gRNAs. a, Spacer sequences of HPD20 standard and hybrid gRNAs. "rN" = ribonucleotide; "dN" = deoxyribonucleotide; "m" = 2'-O-methylation; * = phosphorothioate linkage. DNA substitutions are in red bold and underlined. **b**, A-to-G editing of *HPD* on-target site (target adenine only, or target adenine plus bystander editing) in wild-type HuH-7 cells treated with HPD20 standard or hybrid gRNA in combination with ABE8.8 mRNA (*n* = 3 biological replicates per condition). **c**, Number of sites with ONE-seq score > 0.01 for each indicated HPD20 standard or hybrid gRNA with ABE8.8 protein. **d**, Spacer sequences of HPD4 standard and hybrid gRNAs. **e**, A-to-G editing of *HPD* on-target site (target adenine only, or target adenine plus bystander editing) or verified HPD4 off-target site (HPD4_OT13) in wild-type HuH-7 cells treated with HPD4 standard or hybrid gRNA in combination with ABE8.8 mRNA (*n* = 3 biological replicates per condition). Means are shown.

Fig. 5 | Optimizing correction of the *PAH* R408W variant with a PAM-relaxed ABE. a, Spacer sequences of PAH4 standard and hybrid gRNAs. "rN" = ribonucleotide; "dN" = deoxyribonucleotide; "m" = 2'-O-methylation; * = phosphorothioate linkage. DNA substitutions are in red bold and underlined. **b**, A-to-G editing of *PAH* on-target site (correction of R408W variant with or without synonymous bystander editing, or correction plus unwanted nonsynonymous bystander editing) in *PAH* R408W HuH-7 cells treated with PAH4 standard or hybrid gRNA in combination with SpRY-ABE8.8 mRNA (*n* = 3 biological replicates per condition). **c**, Number of sites with ONE-seq score > 0.01 for each indicated PAH4 standard or hybrid gRNA with SpRY-ABE8.8 protein.

Extended Fig. 1 | Reduction of variant-aware off-target editing by PAH1 hybrid guide RNAs. Examples of variant sites nominated by ONE-seq, with reductions in ONE-seq scores observed with PAH1 hybrid gRNAs. Differences between reference and variant versions of nominated sites are in bold and underlined.

Extended Fig. 2 | Reduction of blood phenylalanine levels by PAH1 hybrid guide RNAs. Blood phenylalanine levels in homozygous *PAH* P281L mice (*n* = 4–5 animals per group) before and 48 hours after treatment with 2.5 mg/kg dose of LNPs with PAH1 standard or hybrid gRNA in combination with ABE8.8 mRNA (1 blood sample per timepoint). The dotted line marks the upper end of the normal range for blood phenylalanine, 125 μmol/L. Means are shown; *P* values were calculated with the Mann–Whitney *U* test.

Extended Fig. 3 | Effects of PXE1 gRNA LNPs on blood pyrophosphate levels and ALT levels. a, Blood pyrophosphate levels in wild-type mice, before and 7 days after treatment with PBS vehicle (*n* = 10 animals), or in homozygous *ABCC6* R1164X mice, before and 7 days after treatment with 2.5 mg/kg dose of LNPs with PAH1 standard gRNA (*n* = 6 animals) or PAH1_hyb18 gRNA (*n* = 5 animals) in combination with ABE8.8 mRNA (1 blood sample per

timepoint). Means are shown; *P* values comparing genotype groups were calculated with the Mann–Whitney *U* test, and the *P* value comparison for the aggregated R1164X groups (before versus after treatment) was calculated with the Wilcoxon signed-rank test. *P* values were not calculated within each individual R1164X gRNA group (before versus after treatment) because of inadequate sample sizes for the Wilcoxon signed-rank test. **b**, Blood alanine aminotransferase (ALT) levels in wild-type mice (n = 4 animals per group) before and 24 hours after treatment with PBS vehicle or with 2.5 mg/kg dose of LNPs with PAH1 standard or hybrid gRNA in combination with ABE8.8 mRNA (1 blood sample per timepoint). No effects on ALT levels by LNP treatment were evident.

Extended Figure 4. Assessment of on-target and off-target editing by guide RNAs targeting the *HPD* gene. a, Lentiviral screening in primary human hepatocytes for adenine base editing of heterologous *HPD* splice site target sequences by various gRNAs in combination with ABE8.20; target adenines are in bold and underlined. MIT score = off-target/specificity score indicated by UCSC Genome Browser. Four candidate gRNAs (HPD3, HPD4, HPD5, and HPD20) were taken forward for off-target analyses. **b**, Targeted amplicon sequencing of ONE-seq-nominated sites in plasmid-treated vs. control wild-type HuH-7 cells with HPD3, HPD4, HPD5, or HPD20 gRNA in combination with ABE8.8 (n = 2 treated and 2 untreated biological replicates). Sites with unsuccessful sequencing are omitted. Arrows indicate verified sites of off-target editing. Means ± standard deviations are shown.

Figure 1

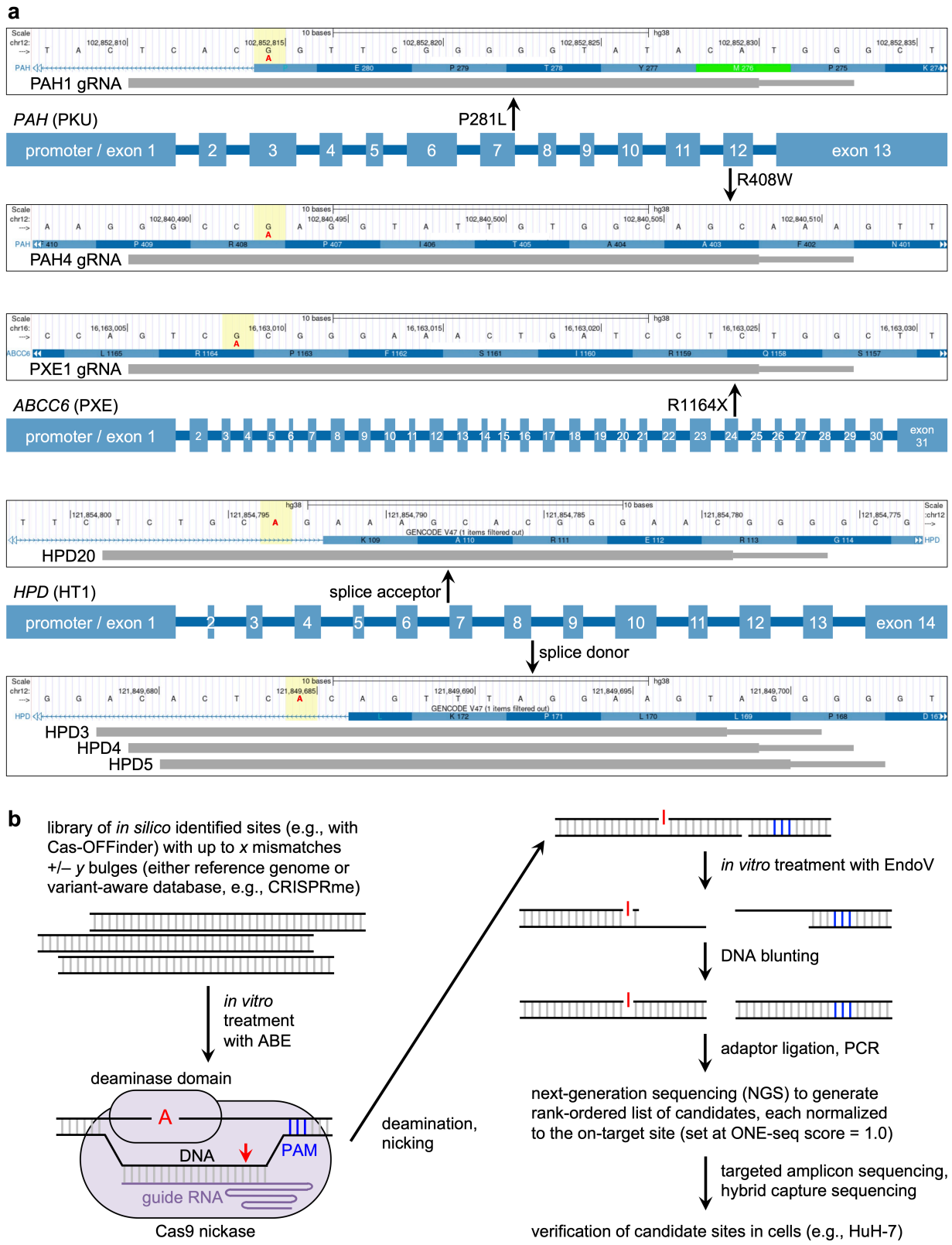
a
PAH1 gRNA
PAH (PKU)
P281L
promoter / exon 1
2
3
4
5
6
7
8
9
10
11
12
exon 13
R408W
PAH4 gRNA
PXE1 gRNA
ABCC6 (PXE)
R1164X
promoter / exon 1
exon 31
HPD20
HPD (HT1)
splice acceptor
promoter / exon 1
exon 14
splice donor
HPD3
HPD4
HPD5
b
library of in silico identified sites (e.g., with Cas-OFFinder) with up to x mismatches +/– y bulges (either reference genome or variant-aware database, e.g., CRISPRme)
in vitro treatment with ABE
deaminase domain
A
DNA
PAM
guide RNA
Cas9 nickase
deamination, nicking
in vitro treatment with EndoV
DNA blunting
adaptor ligation, PCR
next-generation sequencing (NGS) to generate rank-ordered list of candidates, each normalized to the on-target site (set at ONE-seq score = 1.0)
targeted amplicon sequencing, hybrid capture sequencing
verification of candidate sites in cells (e.g., HuH-7)

Figure 2

a

Verified PAH1 off-target sites in *PAH* P281L HuH-7 cells

Site	ONE-seq score	Protospacer/PAM	% net A>G editing
PAH P281L	1.00	TCACAGTTCGGGGGTATACATGG	94.6%
PAH1_OT1	0.74	TCACAGcTCGGtGGaATACAGGG	0.40%
PAH1_OT2	0.10	gCcttaTTCGGGGGTATACAAGA	0.41%
PAH1_OT3	0.08	TCACAG--aGaGGGTATACAAGG	1.26%
PAH1_OT4	0.05	gtAgtaTTCaGGGGTATACAAGG	0.95%
PAH1_OT5	0.03	TaACAGTaCaGaGGTATACAGGG	0.62%
PAH1_OT6	0.02	TttaAGTTCaGGGGTATACAAGG	0.22%
PAH1_OT7	0.01	gCACAG--aaaGGGTATACATGG	1.22%

b

PAH1 hybrid guide RNA designs

gRNA	Spacer sequence
PAH1	mU*mC*mA*rCrArGrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb1	mU*mC*dArCrArGrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb2	mU*mC*mA*dCrArGrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb3	mU*mC*mA*rCdArGrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb4	mU*mC*mA*rCrAdGrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb5	mU*mC*mA*rCrArGdTrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb6	mU*mC*mA*rCrArGrUdTrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb7	mU*mC*mA*rCrArGrUrUdCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb8	mU*mC*mA*rCrArGrUrUrCdGrGrGrGrGrUrArUrArCrA
PAH1_hyb9	mU*mC*dArCrArGrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb10	mU*mC*mA*dCdArGrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb11	mU*mC*mA*rCdAdGrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb12	mU*mC*mA*rCrAdGdTrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb13	mU*mC*mA*rCrArGdTdTrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb14	mU*mC*mA*rCrArGrUdTdCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb15	mU*mC*mA*rCrArGrUrUdCdGrGrGrGrGrUrArUrArCrA
PAH1_hyb16	mU*mC*dArCrArGrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb17	mU*mC*mA*dCdAdGrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb18	mU*mC*mA*rCdAdGdTrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb19	mU*mC*mA*rCrAdGdTdTrUrCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb20	mU*mC*mA*rCrArGdTdTdCrGrGrGrGrGrUrArUrArCrA
PAH1_hyb21	mU*mC*mA*rCrArGrUdTdCdGrGrGrGrGrUrArUrArCrA
PAH1_hyb22	mU*mC*mA*dCdAdGrUrUdCdGrGrGrGrGrUrArUrArCrA
PAH1_hyb23	mU*mC*dArCdArGrUrUdCdGrGrGrGrGrUrArUrArCrA
PAH1_hyb24	mU*mC*dArCdArGrUrUdCdGdGrGrGrGrGrUrArUrArCrA

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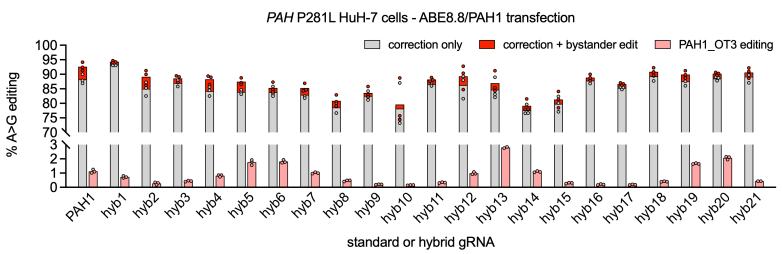

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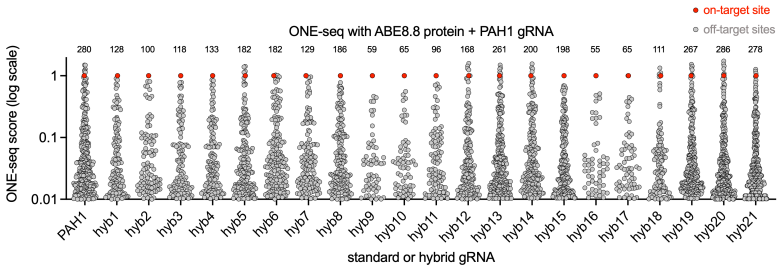

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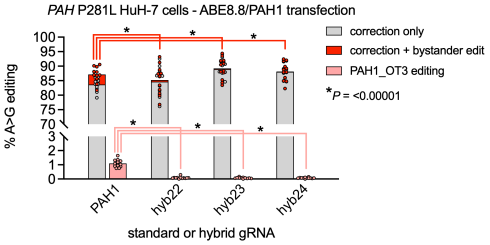

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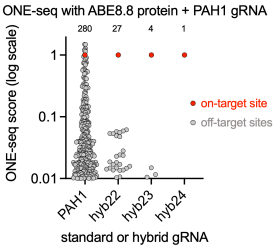

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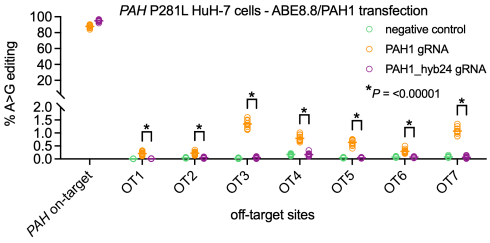

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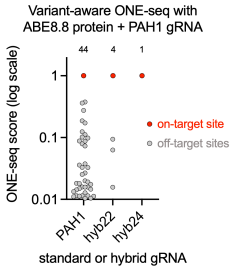

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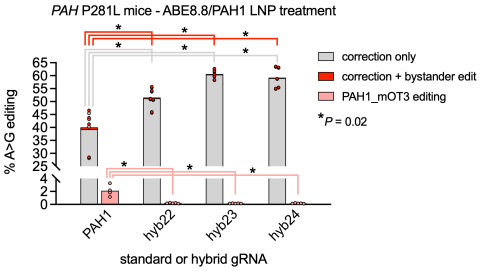

Figure 3

a

PXE1 hybrid guide RNA designs

gRNA	Spacer sequence
PXE1	mG*mU*mC*rArCrGrGrGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb1	mG*mU*dC*rArCrGrGrGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb2	mG*mU*mC*dArCrGrGrGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb3	mG*mU*mC*rAdCrGrGrGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb4	mG*mU*mC*rArCdGrGrGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb5	mG*mU*mC*rArCrGdGrGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb6	mG*mU*mC*rArCrGrGdGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb7	mG*mU*mC*rArCrGrGrGdArArArCrUrGrArUrCrCrUrC
PXE1_hyb8	mG*mU*mC*rArCrGrGrGrAdArArCrUrGrArUrCrCrUrC
PXE1_hyb9	mG*mU*dC*dArCrGrGrGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb10	mG*mU*mC*dAdCrGrGrGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb11	mG*mU*mC*rAdCdGrGrGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb12	mG*mU*mC*rArCdGdGrGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb13	mG*mU*mC*rArCrGdGdGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb14	mG*mU*mC*rArCrGrGdGdArArArCrUrGrArUrCrCrUrC
PXE1_hyb15	mG*mU*mC*rArCrGrGrGdAdArArCrUrGrArUrCrCrUrC
PXE1_hyb16	mG*mU*dC*dAdCrGrGrGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb17	mG*mU*mC*dAdCdGrGrGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb18	mG*mU*mC*rAdCdGdGrGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb19	mG*mU*mC*rArCdGdGdGrArArArCrUrGrArUrCrCrUrC
PXE1_hyb20	mG*mU*mC*rArCrGdGdGdArArArCrUrGrArUrCrCrUrC
PXE1_hyb21	mG*mU*mC*rArCrGrGdGdAdArArCrUrGrArUrCrCrUrC

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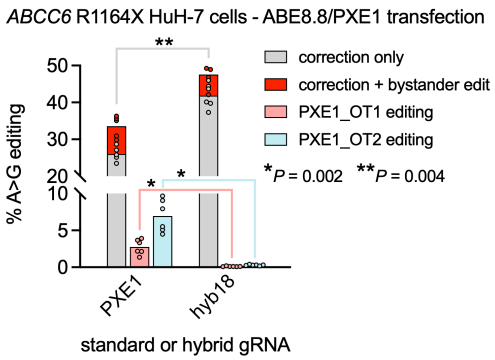

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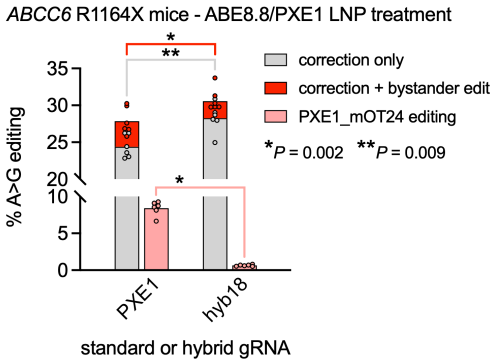

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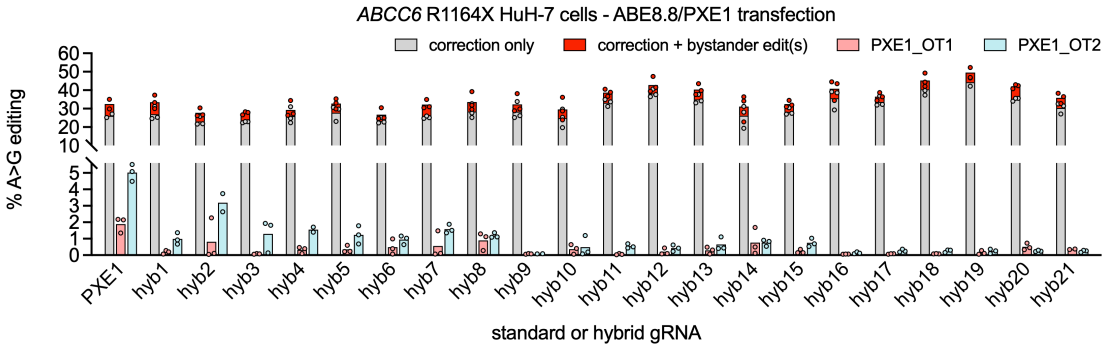

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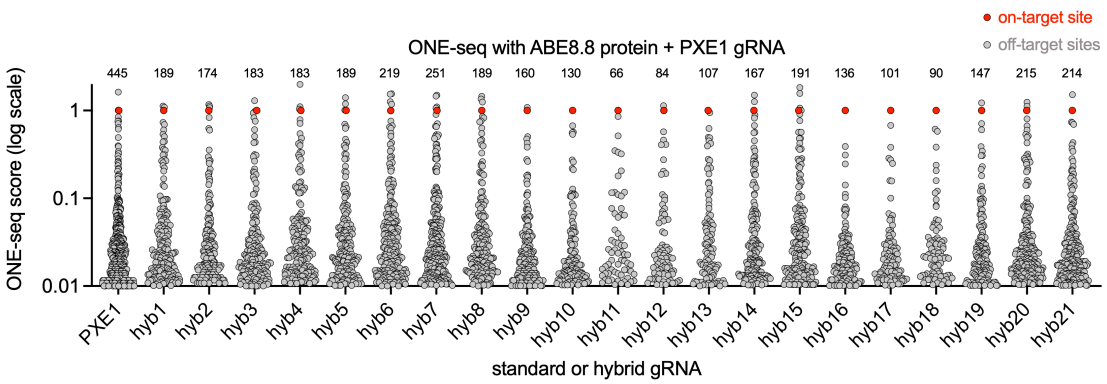

Figure 4

a

HPD20 hybrid guide RNA designs

gRNA	Spacer sequence
HPD20	mU*mC*mU*rGrCrArGrArArArGrCrArCrGrGrGrArArC
HPD20_hyb1	mU*mC*dT*rGrCrArGrArArArGrCrArCrGrGrGrArArC
HPD20_hyb2	mU*mC*mU*dGrCrArGrArArArGrCrArCrGrGrGrArArC
HPD20_hyb3	mU*mC*mU*rGdCrArGrArArArGrCrArCrGrGrGrArArC
HPD20_hyb4	mU*mC*mU*rGrCdArGrArArArGrCrArCrGrGrGrArArC
HPD20_hyb5	mU*mC*mU*rGrCrAdGrArArArGrCrArCrGrGrGrArArC
HPD20_hyb6	mU*mC*mU*rGrCrArGdArArArGrCrArCrGrGrGrArArC
HPD20_hyb7	mU*mC*mU*rGrCrArGrAdArArGrCrArCrGrGrGrArArC
HPD20_hyb8	mU*mC*mU*rGrCrArGrArAdArrGrCrArCrGrGrGrArArC
HPD20_hyb9	mU*mC*dT*dGrCrArGrArArArGrCrArCrGrGrGrArArC
HPD20_hyb10	mU*mC*mU*dGdCrArGrArArArGrCrArCrGrGrGrArArC
HPD20_hyb11	mU*mC*mU*rGdCdArGrArArArGrCrArCrGrGrGrArArC
HPD20_hyb12	mU*mC*mU*rGrCdAdGrArArArGrCrArCrGrGrGrArArC
HPD20_hyb13	mU*mC*mU*rGrCrAdGdArArArGrCrArCrGrGrGrArArC
HPD20_hyb14	mU*mC*mU*rGrCrArGdAdArArGrCrArCrGrGrGrArArC
HPD20_hyb15	mU*mC*mU*rGrCrArGrAdAdArGrCrArCrGrGrGrArArC
HPD20_hyb16	mU*mC*dT*dGdCrArGrArArArGrCrArCrGrGrGrArArC
HPD20_hyb17	mU*mC*mU*dGdCdArGrArArArGrCrArCrGrGrGrArArC
HPD20_hyb18	mU*mC*mU*rGdCdAdGrArArArGrCrArCrGrGrGrArArC
HPD20_hyb19	mU*mC*mU*rGrCdAdGdArArArGrCrArCrGrGrGrArArC
HPD20_hyb20	mU*mC*mU*rGrCrAdGdAdArArArGrCrArCrGrGrGrArArC
HPD20_hyb21	mU*mC*mU*rGrCrArGdAdAdArGrCrArCrGrGrGrArArC
HPD20_hyb22	mU*mC*mU*dGdCdArGdAdArArGrCrArCrGrGrGrArArC
HPD20_hyb23	mU*mC*dT*dGdCrArGdAdArArGrCrArCrGrGrGrArArC

d

HPD4 hybrid guide RNA designs

gRNA	Spacer sequence
HPD4	mC*mA*mC*rUrCrArCrArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb1	mC*mA*dC*rUrCrArCrArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb2	mC*mA*mC*dTrCrArCrArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb3	mC*mA*mC*rUdCrArCrArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb4	mC*mA*mC*rUrCdArCrArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb5	mC*mA*mC*rUrCrAdCrArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb6	mC*mA*mC*rUrCrArCdArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb7	mC*mA*mC*rUrCrArCrAdGrUrUrUrArGrGrArArGrUrA
HPD4_hyb8	mC*mA*mC*rUrCrArCrArGdTrUrUrArGrGrArArGrUrA
HPD4_hyb9	mC*mA*dC*dTrCrArCrArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb10	mC*mA*mC*dTdCrArCrArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb11	mC*mA*mC*rUdCdArCrArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb12	mC*mA*mC*rUrCdAdCrArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb13	mC*mA*mC*rUrCrAdCdArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb14	mC*mA*mC*rUrCrArCdAdGrUrUrUrArGrGrArArGrUrA
HPD4_hyb15	mC*mA*mC*rUrCrArCrAdGdTrUrUrArGrGrArArGrUrA
HPD4_hyb16	mC*mA*dC*dTdCrArCrArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb17	mC*mA*mC*dTdCdArCrArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb18	mC*mA*mC*rUdCdAdCrArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb19	mC*mA*mC*rUrCdAdCdArGrUrUrUrArGrGrArArGrUrA
HPD4_hyb20	mC*mA*mC*rUrCrAdCdAdGrUrUrUrArGrGrArArGrUrA
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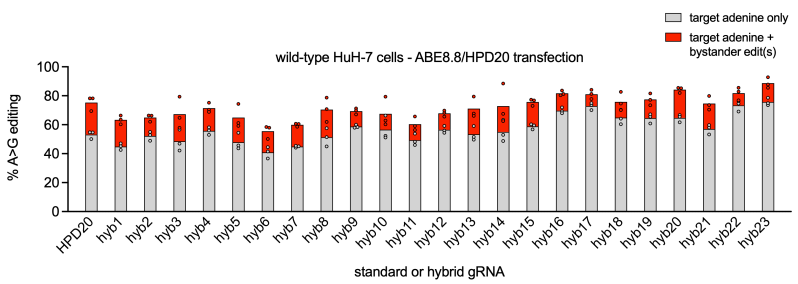

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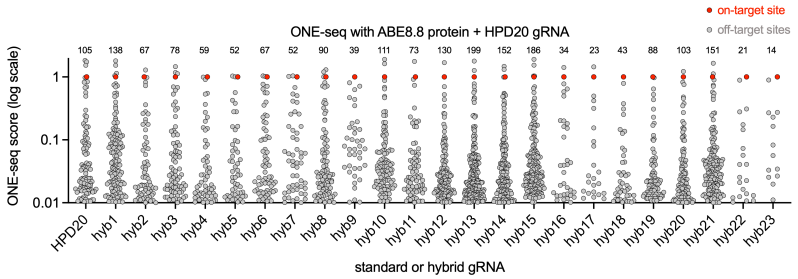

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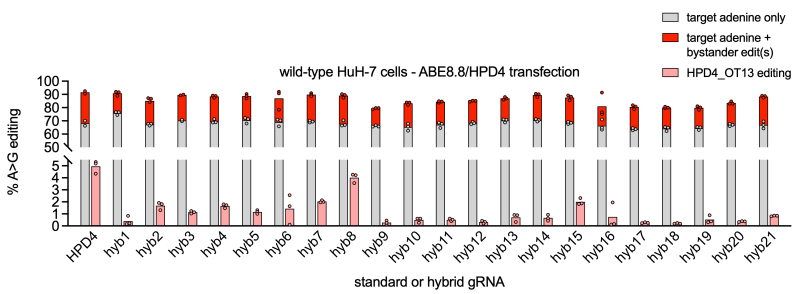

Figure 5

a

PAH4 hybrid guide RNA designs

gRNA	Spacer sequence
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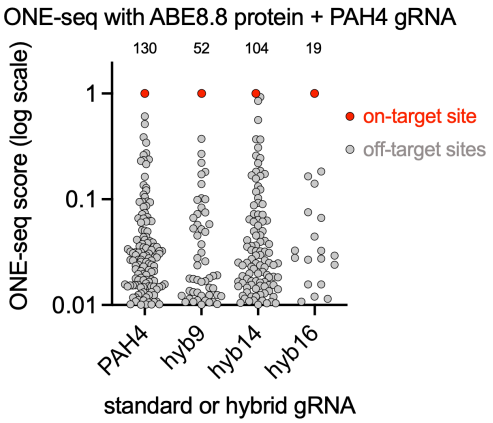
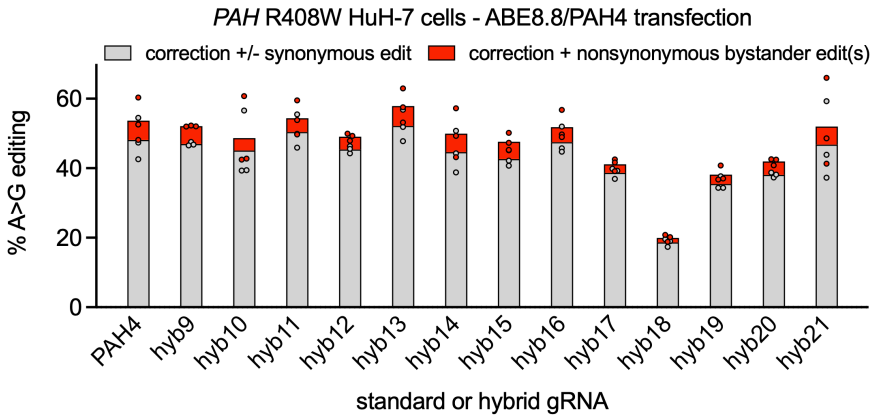
PAH4_hyb9	mU*mC*dA*dCrArGrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH4_hyb10	mU*mC*mA*dCdArGrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH4_hyb11	mU*mC*mA*rCdAdGrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH4_hyb12	mU*mC*mA*rCrAdGdTrUrCrGrGrGrGrGrUrArUrArCrA
PAH4_hyb13	mU*mC*mA*rCrArGdTdTrCrGrGrGrGrGrUrArUrArCrA
PAH4_hyb14	mU*mC*mA*rCrArGrUdTdCrGrGrGrGrGrUrArUrArCrA
PAH4_hyb15	mU*mC*mA*rCrArGrUrUdCdGrGrGrGrGrUrArUrArCrA
PAH4_hyb16	mU*mC*dA*dCdArGrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH4_hyb17	mU*mC*mA*dCdAdGrUrUrCrGrGrGrGrGrUrArUrArCrA
PAH4_hyb18	mU*mC*mA*rCdAdGdTrUrCrGrGrGrGrGrUrArUrArCrA
PAH4_hyb19	mU*mC*mA*rCrAdGdTdTrCrGrGrGrGrGrUrArUrArCrA
PAH4_hyb20	mU*mC*mA*rCrArGdTdTdCrGrGrGrGrGrUrArUrArCrA
PAH4_hyb21	mU*mC*mA*rCrArGrUdTdCdGrGrGrGrGrUrArUrArCrA

c



b

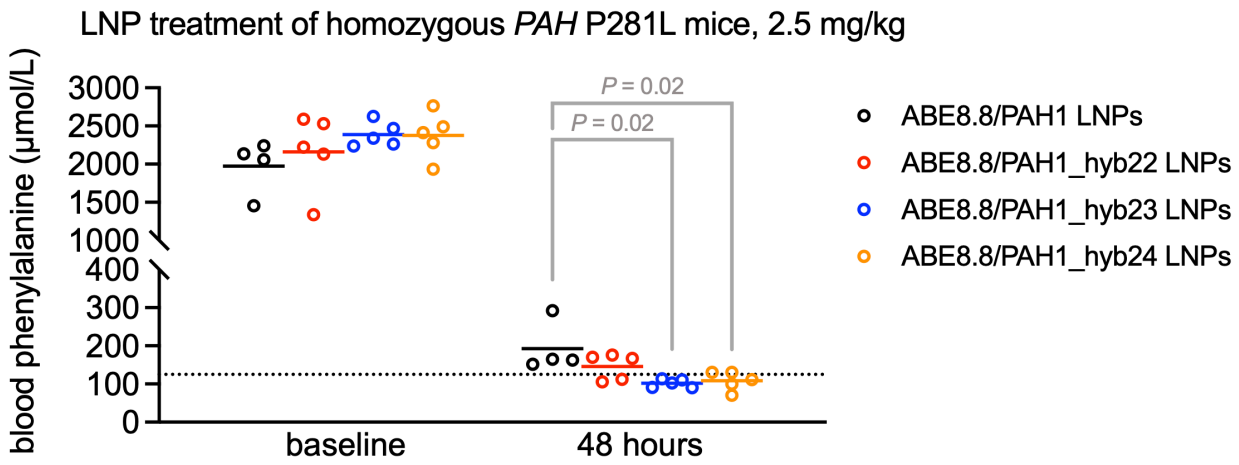


Extended Figure 1

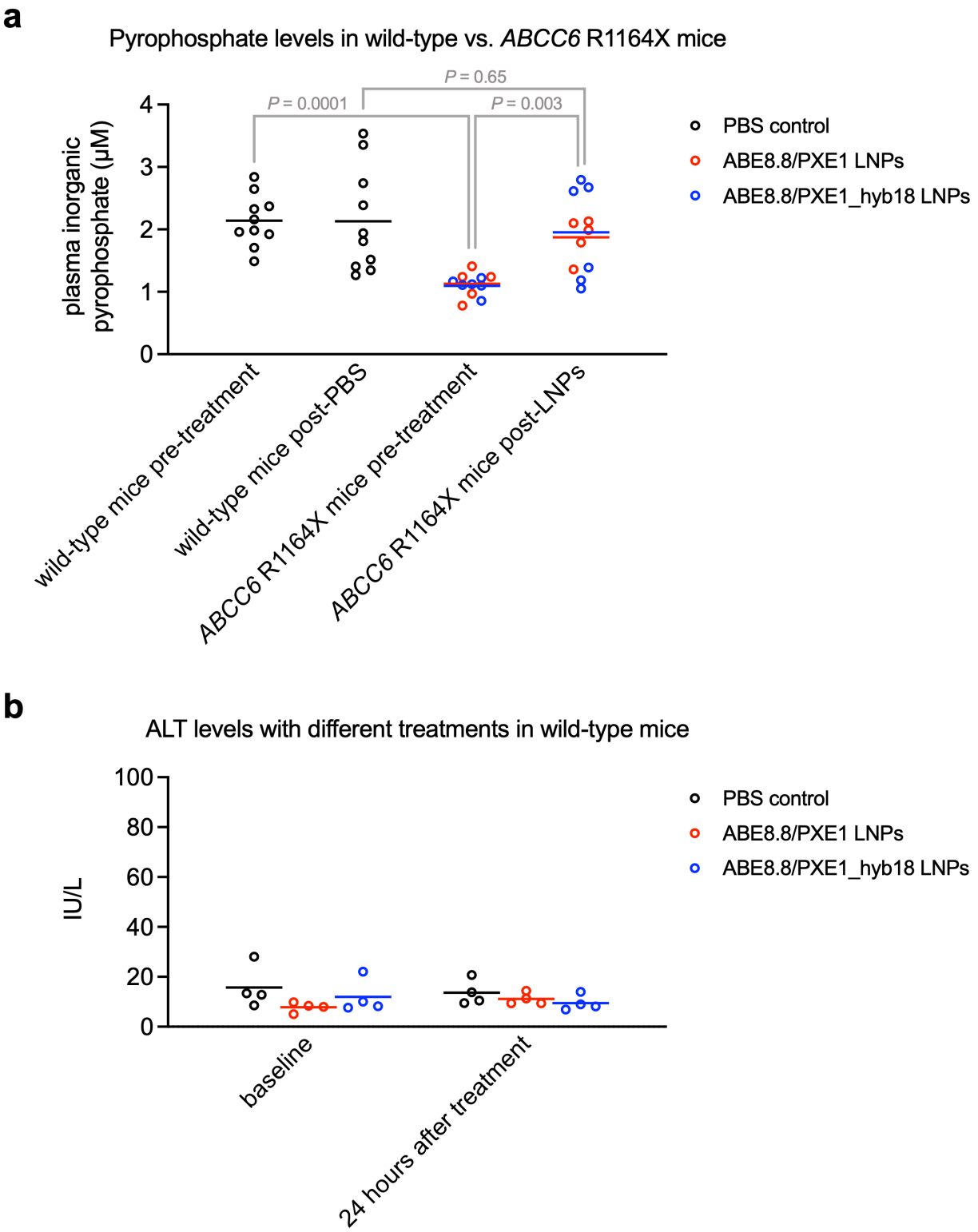
On-target site	TCACAGTTCGGGGGTATACA|TGG
Candidate site, reference version	TCACAGTcCGGGGGg**g**agac|AtGG
Candidate site, variant version (1-bp deletion)	TCACAGTcCGGGGGgAgACA|TGG
Minor allele frequency = 0.00001	
ONE-seq score: PAH1 gRNA = 0.19 / PAH1_hyb22 = 0.06 / PAH1_hyb24 = 0.00996	

On-target site	TCACAGTTCGGGGGTATACA|TGG
Candidate site, reference version	aCACAGTTCtaGGGTA**c**AgA|TGG
Candidate site, variant version (substitution)	aCACAGTTCtaGGGTA**T**AgA|TGG
Minor allele frequency = 0.02	
ONE-seq score: PAH1 gRNA = 0.11 / PAH1_hyb22 = 0.004 / PAH1_hyb24 = 0.0001	

Extended Figure 2



Extended Figure 3



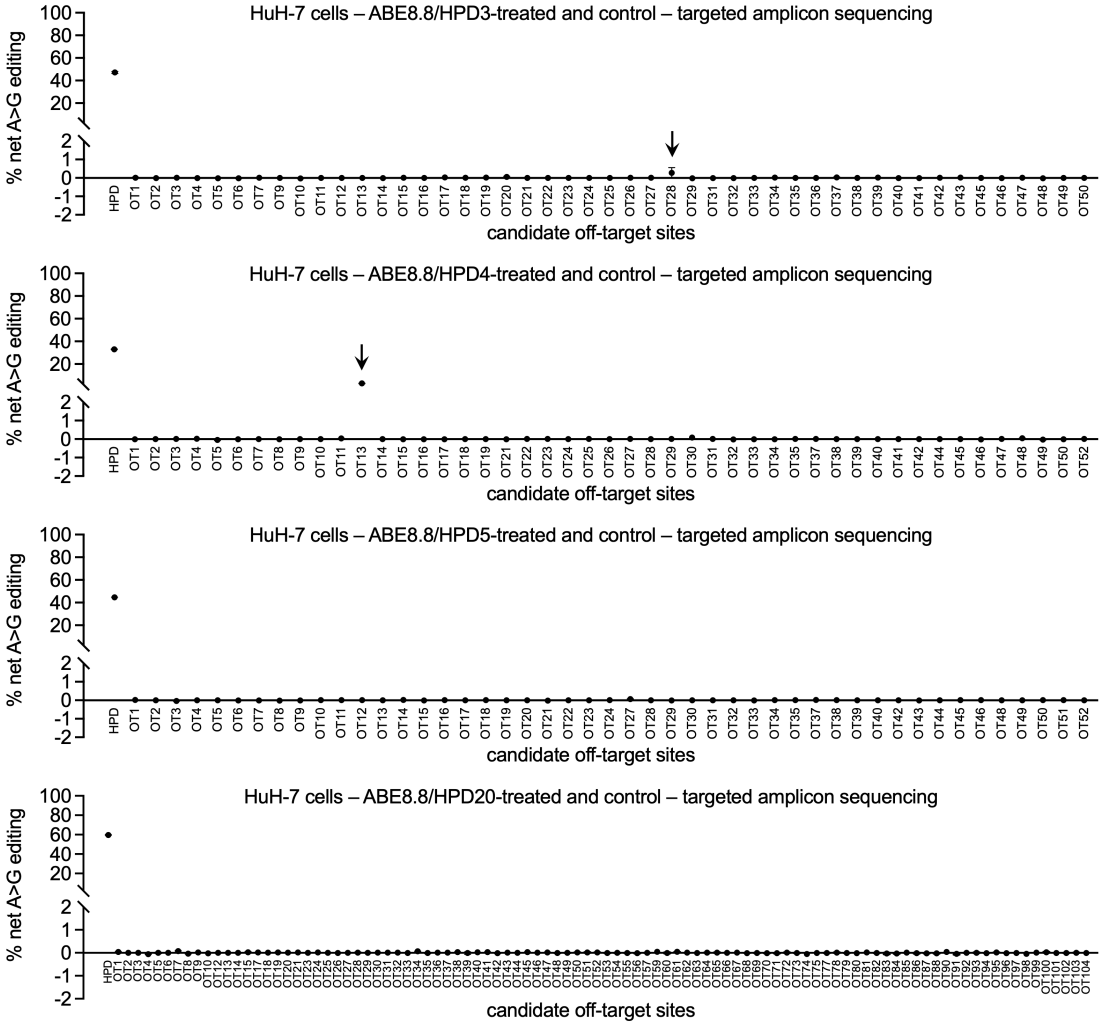
Extended Figure 4

a

ABE8.20 editing of human *HPD* sequences following lentiviral insertion into primary human hepatocytes

Label	Protospacer	PAM	Target Site	MIT Score	Desired A>G edit%	Any A>G edit%
A	GCACCTCACCTTTGTTCCAG	GGG	exon 5 splice donor	75	12%	12%
B	CACAGAGATGGGCGATCACC	TGG	exon 6 splice acceptor	76	13%	18%
C	CTCTGCAGAAAGCACGGGAA	CGG	exon 7 splice acceptor	60	21%	23%
D (HPD20)	TCTGCAGAAAGCACGGGAAC	GGG	exon 7 splice acceptor	83	32%	34%
E	CTGCAGAAAGCACGGGAACG	GGG	exon 7 splice acceptor	85	24%	25%
F (HPD3)	ACACTCACAGTTTAGGAAGT	AGG	exon 8 splice donor	65	45%	48%
G (HPD4)	CACTCACAGTTTAGGAAGTA	GGG	exon 8 splice donor	72	33%	34%
H (HPD5)	ACTCACAGTTTAGGAAGTAG	GGG	exon 8 splice donor	65	32%	33%
I	CTCACAGTTTAGGAAGTAGG	GGG	exon 8 splice donor	66	40%	42%
J	TCCCAGGCCCAAATGCAGTC	TGG	exon 9 splice acceptor	66	19%	19%
K	AAGGTCTCACCATTCGGAGG	CGG	exon 9 splice donor	90	11%	11%
L	CGCCTAGGAGCTGAAAATCC	TGG	exon 13 splice acceptor	79	18%	24%
M	CTAGGAGCTGAAAATCCTGG	TGG	exon 13 splice acceptor	66	11%	16%
N	GTACCTGGTGGTTGTGGCGC	TGG	exon 13 splice donor	82	21%	21%
O	CAAGCAGTACCTGGTGGTTG	TGG	exon 13 splice donor	67	28%	31%
P	CCGCCACAGGGTTTTGGAGC	CGG	exon 14 splice acceptor	79	7%	9%

b



Supplementary Note 1.

For the PAH1 gRNA, none of the seven verified sites of off-target editing raises a concern of oncogenic risk. The first site (PAH1_OT1) has 3 mismatches with the on-target protospacer, with an NGG PAM, and is in an intron of the *SPAG17* gene, which encodes a microtubule protein. PAH1_OT2 has 5 mismatches with the on-target protospacer, with an NGA PAM, and is in an intron of the *STYXL2* gene, which encodes a myosin-interacting protein. PAH1_OT3 has 2 mismatches and 2 consecutive bulges compared with the on-target protospacer, with an NGG PAM, and is in an intron of *LINC00882*, a long noncoding RNA. PAH1_OT4 has 6 mismatches with the on-target protospacer, with an NGG PAM, and is in an intron of DCLK1, which encodes a microtubule-associated protein. PAH1_OT5 and PAH1_OT6 each have 4 mismatches with the on-target protospacer, with an NGG PAM, and is intergenic and distant from any gene. PAH1_OT7 has 4 mismatches and 2 consecutive bulges compared with the on-target protospacer, with an NGG PAM, and is in an intron of *KCNJ10*, which encodes a potassium channel protein. None of the five implicated genes appear to be significantly expressed in the liver.

For the PXE1 gRNA, none of the six verified sites of off-target editing raises a concern of oncogenic risk. The first site (PXE1_OT1) has 2 mismatches and 1 bulge compared with the on-target protospacer, with an NGA PAM, and is intergenic and distant from any gene. PXE1_OT2 has 4 mismatches with the on-target protospacer, with an NGG PAM, and is in an intron of the *FGGY* gene, which encodes a carbohydrate kinase domain-containing protein expressed in fetal brain, with low expression in the liver. PXE1_OT8 has 4 mismatches with the on-target protospacer, with an NGG PAM, and is intergenic and distant from any gene. PXE1_OT10 has 3 mismatches with the on-target protospacer, with an NGG PAM, and is intergenic and distant from any gene. PXE1_OT14 has 6 mismatches with the on-target protospacer, with an NGG PAM, and is in the 5' untranslated region of the *KCNK2* gene, which encodes a neuronal potassium channel protein. PXE1_OT15 has 6 mismatches with the on-target protospacer, with an NGG PAM, and is intergenic and distant from any gene.

For the HPD4 gRNA, the verified site of off-target editing raises no concern of oncogenic risk. HPD4_OT13 has 2 mismatches and 1 bulge compared with the on-target protospacer, with an NGG PAM, and is intergenic and distant from any gene.

Supplementary Note 2.

We used prime editing, specifically PE5max with an engineered pegRNA (epegRNA), to introduce the *ABCC6* R1164X variant into HuH-7 human hepatoma cells. HuH-7 cells in a well of a 6-well plate were transfected with 9 µL TransIT®-LT1 Transfection Reagent, 1.5 µg PEmax plasmid (pCMV-PEmax-P2A-hMLH1dn; Addgene plasmid # 174828; http://n2t.net/addgene:174828; RRID:Addgene_174828), 0.75 µg epegRNA-expressing plasmid (pU6-tevopreq1-GG-acceptor; Addgene plasmid # 174038; http://n2t.net/addgene:174038; RRID:Addgene_174038; spacer sequence 5'-ACCUGUCAGCCACCAG UCGC-3'; RTT/PBS sequence 5'-AGUUUCCCG**U**GACUGGUGGCUGA-3'; the bold underlined position introduces the *ABCC6* R1164X variant), and 0.75 µg nicking gRNA plasmid (pGuide; Addgene plasmid # 64711; http://n2t.net/addgene:64711; RRID:Addgene_64711; spacer sequence 5'-GAUCAGU UUCCCGUGACUGG-3') as previously described[4]. Observing ≈30% editing in bulk HuH-7 cells, we used single-cell cloning to identify and expand a R1164X homozygous HuH-7 cell line. Cells were dissociated with trypsin 48 hours post-transfection and replated onto 10-cm plates (5,000 cells/plate) with conditioned medium to facilitate recovery, and genomic DNA was isolated from the remainder of the cells as a pool to perform PCR and Sanger sequencing of the *ABCC6* R1164X site. Single cells were permitted to expand for 7-14 days to establish clonal populations. Colonies were manually picked and replated into individual wells of a 96-well plate. Genomic DNA was isolated from individual clones, and PCR and Sanger sequencing was performed to identify R1164X homozygous HuH-7 clones. One representative clone was expanded for use in subsequent studies. This R1164X homozygous HuH-7 cell line is readily available from the authors via a Transfer of Research Material agreement with the University of Pennsylvania.

Supplementary Note 3.

The *ABCC6* R1164X mouse model of PXE was generated using in vitro transcribed Cas9 mRNA, a synthetic gRNA (spacer sequence 5'-GAGGGUCAGUUUCCCGAAAC-3') (Integrated DNA Technologies), and a synthetic single-strand DNA oligonucleotide (Integrated DNA Technologies) with homology arms matching the target site and harboring the R1164X variant and synonymous variants (bold with underline): 5'-TCCAGGGAAGTCTGGTGGTCAGGGCCTTCCGGGCCCAGGCATCCTT CACGGCTCAGCACGATGCTCTCATGGATGAGAACCAGAGG**A**TCAGTTTCCCG**TG**ACTGGTG GCTGACAGGTAGGATGAGCCTGGGATCAGAGTAGATCTGGAACCCACTCCCCACAAGAGC TGCACTCTGTAGTTAGAGGGGTCACATGGCTTG-3'. The mixture of the 3 components was injected into cytoplasm of fertilized oocytes from C57BL/6J mice at the Penn Vet Transgenic Mouse Core (https://www.vet.upenn.edu/research/core-resources-facilities/transgenic-mouse-core). Genomic DNA samples from founders were screened for knock-in of the desired sequence in the *Abcc6* locus via homology-directed repair. Founders with the humanized R1164X allele were bred through two generations to obtain homozygous mice. This humanized PXE mouse model is readily available from the authors via a Transfer of Research Material agreement with the University of Pennsylvania.

Supplementary Note 4.

We implemented a high-throughput screening platform in which to introduce a base editor in combination with a variety of gRNAs and rapidly evaluate the relative activities of the gRNAs in the target cell type of interest, primary human hepatocytes. We used lentiviral vectors harboring an expression cassette for an adenine base editor and a combination of an expression cassette for an individual gRNA and a genomic fragment that (1) spanned the potential target sequence of that gRNA, (2) included a unique barcode, and (3) was flanked by fixed sequences that allowed for PCR amplification with a common set of primers. For the screen, we chose ABE8.20, being a high-activity eighth-generation adenine base editor with a wide editing window (protospacer positions 3 to 9)[10] that permitted the greatest flexibility in gRNA selection for the disruption of splice donors (editing of the antisense A in the second position of the GT motif) or splice acceptors (editing of the sense A in the first position of the AG motif) in the *HPD* gene. We selected 16 gRNAs matched to protospacer sequences in the human *HPD* gene and with NGG PAMs, targeting splice sites ranged across the entire HPD gene (Extended Fig. 4a).

A pool of oligonucleotides spanning guide RNA sequences and target sequences, along with unique barcodes, was synthesized by Twist Bioscience and was amplified using KAPA HiFi HotStart ReadyMix (Roche), using various conditions to minimize template switching. After determining the optimal PCR strategy (48 parallel PCR reactions with 0.5 ng of the oligonucleotide pool per reaction, with 15 cycles), the amplified pool was cloned into a lentiviral vector using In-Fusion (Takara Bio) per the manufacturer's instructions. Lentiviruses with a gene encoding ABE8.20 (ABE8.20-m[10]) and with the cloned gRNA/barcode/target sequences were generated using the Broad Institute's RNAi Consortium protocol (https://portals.broadinstitute.org/gpp/public/dir/download?dirpath=protocols/production&filename=TRC%20shRNA%20sgRNA%20ORF%20Low%20Throughput%20Viral%20Production%20201506.pdf) for low-throughput lentiviral production and then concentrated using PEG-it virus precipitation solution (System Biosciences) per the manufacturer's instructions. Primary human hepatocytes (Gibco/Thermo Fisher Scientific) were thawed into a 24-well Collagen I coated plate (Gibco/Thermo Fisher Scientific) and transduced with the lentiviruses 6 hours later. Each well of cells was treated via addition of 1 µL of concentrated virus, with 8 biological replicates. Four days after transduction, each group was pooled and then harvested using the DNeasy Blood and Tissue Kit (QIAGEN). NGS library preparation was performed with KAPA HiFi HotStart ReadyMix (Roche), with 35 PCR cycles using a primer pair spanning the barcode and the target sequence. Following a secondary indexing PCR round and sequencing on an Illumina MiSeq system, target sequences were binned via the unique barcodes, and A-to-G editing was quantified in the editing window of each target sequence.

We observed the highest levels of editing with gRNAs targeting either the splice acceptor of exon 7 or the splice donor of exon 8 (Extended Fig. 4a). We prioritized the gRNAs designated "HPD3", "HPD4", "HPD5", and "HPD20" due to their efficient editing. Noting that these gRNAs in combination with ABE8.20 achieved editing of adenine bases towards the middle of the editing window (positions 4 through 7), for our subsequent studies we switched to ABE8.8 due to its narrow editing window. We retested the HPD3, HPD4, HPD5, and HPD20 gRNAs in combination with ABE8.8, now using plasmid transfection of ABE8.8 and gRNA in wild-type HuH-7 hepatocytes (Extended Fig. 4b). All demonstrated efficient editing of their target splice site adenine, with HPD20 performing the best. Of note, all gRNAs demonstrated bystander editing, but such editing is expected to be of little consequence with respect to the inactivation of the *HPD* gene.

Supplementary Table 1. Assessment of off-target editing with ABE8.8/PAH1 with mRNA delivery in HuH-7 cells via hybrid capture sequencing of all sites with ONE-seq score > 0.01.

See accompanying Excel spreadsheet.

Supplementary Table 2. Assessment of off-target editing with ABE8.8/PAH1 with LNP delivery in the liver in humanized *PAH* P281L mice via targeted amplicon sequencing.

Genomic site	Protospacer/PAM sequence*	Amplicon (chromosome: position range)†	ONE-seq score‡	Treated sample 1§	Treated sample 2	Control sample 1	Control sample 2	Mean net A→G editing %
PAH	TCACAGTTCGGGGGTATACATGG	—	1.00	48.06	44.23			
mOT1	TCACAGTTaaGGGGaATACAAAG	chr14:81039247-81039269	0.60	0.06	0.06	0.04	0.06	+0.01
mOT2	TCAtAGTTaGaGGcTATACAGGG	chr8:88571065-88571087	0.29	0.00	0.18	0.02	0.03	+0.06
mOT3	aCACAGTataGGGGTATACATGG	chr4:41324566+41324588	0.27	1.63	3.56	0.07	0.05	**+2.53**
mOT4	gtACAaTTCGaGaGTATACATGG	chr16:52540266-52540288	0.26	n.d.	0.00	0.09	0.00	–0.04
mOT5	TCACAGTTtGaGGGTAcACACGG	chr9:61015063-61015085	0.26	0.00	0.08	0.03	0.02	+0.05
mOT6	TCACAGTgtaGGGGaATACAGGG	chr12:57398816-57398838	0.23	0.00	0.09	0.09	0.05	–0.03
mOT7	TCACAGTgtaGGGGaATACAAGG	chr10:100928529-100928551	0.20	n.d.	n.d.	n.d.	n.d.	n.d.
mOT8	TCACAGTTaGGtGaTATACACAG	chr1:55570707+55570729	0.20	0.00	0.02	0.02	0.03	–0.01
mOT9	aCACAGTTCtGGGGgATACtGGG	chr1:23049779+23049801	0.17	n.d.	n.d.	n.d.	n.d.	n.d.
mOT10	TCACAGTggGGaGGaATACAAGG	chr1:39014077+39014099	0.16	0.03	0.03	0.00	0.03	+0.01
mOT11	aCACAGcTCGGGGGTATgCtGGG	chr11:16953736+16953758	0.16	0.05	0.04	0.03	0.04	+0.01
mOT12	TCACAGTTt--GGGTATACtGGG	chr18:78330714-78330734	0.16	0.03	0.02	0.02	0.02	+0.00
mOT13	aCACA-TTCaaGaGTATACAGGG	chr14:101284934+101284956	0.15	n.d.	0.02	0.04	0.07	–0.03
mOT14	cCACAGTTCtGGGGTATACAGTG	chr11:75831380-75831402	0.15	0.01	0.02	0.02	0.03	–0.01
mOT15	TCACAG-TCaaaGGTATACATAG	chr12:119728853+119728874	0.14	n.d.	n.d.	n.d.	n.d.	n.d.
mOT16	aCACAcagtGGGGGTATACtGGG	chr9:74279255-74279277	0.14	0.00	n.d.	0.00	0.07	–0.04
mOT17	cCAtAGTTaGGGGaTATACAGGG	chr3:13831914-13831936	0.14	0.00	0.00	0.03	0.07	–0.05
mOT18	atACAGcTCGtGGGTATACtAGG	chr16:23638895-23638917	0.14	0.08	0.04	0.03	0.04	+0.02
mOT19	TCACAGTaaGaGGaaATACAGGG	chr11:91245477+91245499	0.14	0.00	0.05	0.06	0.05	–0.03
mOT20	aCACAGTTCcaGGGTAgACAGGG	chr12:51713403+51713425	0.13	0.04	n.d.	0.04	0.03	+0.01
mOT21	TCACAaTTa-GGaGTATACAGGG	chr10:47705951-47705972	0.13	0.06	n.d.	0.05	0.00	+0.04
mOT22	agACAGTTCGGGGtTATACAAGA	chr3:9617766+9617788	0.13	0.14	n.d.	0.13	0.09	+0.03
mOT23	TCACAG-TataGGaTATACATGG	chr2:91587310+91587331	0.13	n.d.	n.d.	n.d.	n.d.	n.d.
mOT24	TCACAGcTCaGGGGTATttAGGG	chr8:50739969-50739991	0.11	0.04	0.03	0.03	0.04	+0.00

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

†Coordinates in the GRCm39/mm39 assembly of the mouse genome; "+" and "-" indicate forward or reverse orientation of protospacer/PAM sequence.

‡This table includes the top 25 ONE-seq-nominated sites, all of which have ONE-seq score > 0 01.

§n.d. = PCR was unsuccessful for the genomic site, or PCR was not attempted.

Supplementary Table 3. Assessment of off-target editing with ABE8.8/PXE1 with plasmid delivery in HuH-7 cells via targeted amplicon sequencing.

Genomic site	Protospacer/PAM sequence*	Amplicon (chromosome: position range)†	ONE-seq score‡	Treated sample 1§	Treated sample 2	Control sample 1	Control sample 2	Mean net A→G editing %
ABCC6	GTCACGGGAAACTGATCCTCTGG	chr16:16163006+16163028	1.00	57.82	69.34			
OT1	GTCACaaG-AACTGATCCTCTGA	chr1:214579555-214579576	0.55	5.97	6.11	0.10	0.04	**+5.97**
OT2	agCACaGGAAACTGATCCTaAGG	chr1:59349109+59349131	0.51	13.55	13.61	0.07	0.06	**+13.51**
OT3	GTCACtGGAAACTGATCtTCTGG	chrY:8758708+8758730	0.29	n.d.	n.d.	n.d.	n.d.	n.d.
OT4	GTCACGGG-AtCTGATCCcCTGG	chr19:41033388-41033409	0.26	n.d.	n.d.	n.d.	n.d.	n.d.
OT5	agCAaGGGAAACcGATCCcCGGG	chr4:8712135+8712157	0.23	0.04	0.06	0.07	0.10	–0.04
OT6	GTCACaaTGAAAtTGATCCTCTGG	chr12:29437669-29437692	0.21	0.03	0.03	0.01	0.07	–0.01
OT7	GTCAtaGGAAACaGATCCTCTAG	chr1:190789174-190789196	0.19	0.05	0.07	0.06	0.05	+0.00
OT8	tcCtCaGGAAACTGATCCTCAGG	chr7:45508698-45508720	0.18	2.65	2.50	0.00	0.06	**+2.55**
OT9	GTtAtatGAAACTGATCCTCAAG	chr6:143180833+143180855	0.15	n.d.	n.d.	n.d.	n.d.	n.d.
OT10	tTCtCaGGAAACTGATCCTCAGG	chr2:68105838+68105860	0.15	2.13	1.93	0.00	0.00	**+2.03**
OT11	tTCtCaGGAAACcGATCCTCAGG	chrX:16556252+16556274	0.14	n.d.	n.d.	n.d.	n.d.	n.d.
OT12	ccCtCaGGAAACTGATCCTCAGG	chr6:145016909-145016931	0.14	n.d.	n.d.	n.d.	n.d.	n.d.
OT13	GTCACcaGgAACTGATCCTgCTG	chrX:32534193-32534215	0.13	0.06	0.12	0.05	0.02	+0.06
OT14	tgtACaGGAAACTGATCtaCAGG	chr1:215005750+215005772	0.10	0.47	0.44	0.07	0.05	**+0.39**
OT15	agactaGGAAACTGATCCTCAGG	chr11:130576422+130576444	0.10	0.86	0.75	0.11	0.07	**+0.71**
OT16	cTCAtaGGAAACTGATCacCAGG	chr5:104709882-104709904	0.10	0.02	0.03	0.04	0.06	–0.03
OT17	agaACaGGAAACTGATCCTCAGA	chr10:6083650-6083672	0.10	0.14	0.21	0.12	0.13	+0.05
OT18	ccCttaGGAAACTGATCtTCAGG	chr12:21555583+21555605	0.09	0.03	0.13	0.06	0.04	+0.03
OT19	cTCACaGG-AACTGATCCctTGG	chr22:17173026-17173047	0.08	n.d.	n.d.	n.d.	n.d.	n.d.
OT20	tTCAtGGG-AACTGtTCCTCTGG	chr6:158115399+158115420	0.08	0.05	0.04	0.03	0.04	+0.01
OT21	GatAa-GGAAAtTGATCCTCAGG	chr2:114115050+114115071	0.08	0.08	0.09	0.11	0.12	–0.03
OT22	GggAaGGGAAACTGATCaTCTGG	chr9:94096996-94097018	0.08	0.08	0.07	0.09	0.08	–0.01
OT23	aTCACaAGGAAACTGATCCcaTGG	chr1:107618987+107619010	0.08	0.07	0.07	0.03	0.08	+0.01
OT24	tTCACaGaAAACTGATCCTgAGG	chrY:3172149+3172171	0.08	n.d.	n.d.	n.d.	n.d.	n.d.

*The *ABCC6* on-target sequence and top ONE-seq-nominated candidate off-target sequences with mismatched positions and bulges indicated by lower case letters and dashes.

†Coordinates in the GRCh38/hg38 assembly of the human genome; "+" and "-" indicate forward or reverse orientation of protospacer/PAM sequence.

‡This table includes the top 25 ONE-seq-nominated sites, all of which have ONE-seq score > 0 01.

§n.d. = PCR was unsuccessful for the genomic site, or PCR was not attempted.

Supplementary Table 4. Assessment of off-target editing with ABE8.8/PXE1 with LNP delivery in the liver in humanized *ABCC6* R1164X mice via targeted amplicon sequencing.

Genomic site	Protospacer/PAM sequence*	Amplicon (chromosome: position range)†	ONE-seq score‡	Treated sample 1§	Control sample 1	Mean net A→G editing %
ABCC6	GTCACGGGAAACTGATCCTCTGG	—	1.00	25.81		
mOT1	catACaGGAAACTGATCtTaGGG	chr18:41197176+41197198	0.93	0.04	0.00	+0.04
mOT2	catACaGGAAACTGATCtTaGGG	chr18:41196868+41196890	0.61	n.d.	n.d.	n.d.
mOT3	cTtACGGGAAACTcATCtcaGGG	chr17:65514023-65514045	0.60	n.d.	n.d.	n.d.
mOT4	GTCAtGGGAgACTGATCCctGGC	chr7:43275219+43275241	0.55	0.07	0.00	+0.07
mOT5	agCACGGGAAtCTGATCCatTGG	chr10:44556261+44556283	0.53	0.13	0.10	+0.03
mOT6	GgCACGGaAAACaGATCtcgGGG	chr17:27626778-27626800	0.53	0.14	0.09	+0.05
mOT7	cTtACaGGAAACTGATCtcaGGG	chr4:81719354+81719376	0.51	n.d.	n.d.	n.d.
mOT8	GTtAtGGGAAACTGATCCaaGAG	chr2:111739994+111740016	0.50	0.04	0.01	+0.03
mOT9	agCAgGGGAAACTGATCaTCTGG	chr17:56516740-56516762	0.48	0.05	0.07	–0.02
mOT10	cTtACaGGAAACTGATCtcaGGG	chr2:80870466+80870488	0.44	0.07	0.50	–0.42
mOT11	taatatGGAAACTGATCCTCTGG	chr4:104841320-104841342	0.42	0.35	0.04	+0.32
mOT12	agCACaGGAAACTGATtCTCAGG	chr1:183737754-183737776	0.41	0.12	0.08	+0.05
mOT13	tTCACaGGAAACTGATCtgCTGG	chrX:60012930-60012952	0.37	0.00	0.00	+0.00
mOT14	GTCtaGGGAAA-TGATCaTCTGG	chrX:168572179+168572200	0.37	0.03	0.11	–0.08
mOT15	GTCACaGGcAACTGATCacaGGC	chr9:70876453+70876475	0.35	0.09	0.05	+0.05
mOT16	cgCACaGGAAACTGAcCCTCTGG	chr4:143076499-143076521	0.35	0.00	0.00	+0.00
mOT17	cTtACGGGAAACTcATCtcaGGG	chr10:56498728-56498750	0.34	0.04	0.00	+0.04
mOT18	caCACaGGAAACTGA-CCTCAGG	chr4:24186788-24186809	0.34	0.00	0.00	+0.00
mOT19	tgtACtGGAAACTGATCCTCTGC	chr14:60497835-60497857	0.32	0.07	0.05	+0.02
mOT20	GTCACGaGAAACTGATaCTCGGA	chr14:120415487+120415509	0.31	0.00	0.00	+0.00
mOT21	cTCACaGGAAACTcATCtcaGGG	chr14:39236775+39236797	0.30	1.55	1.43	+0.12
mOT22	cTtACaGGAAACTGATCtcaGGG	chrX:102211744+102211766	0.30	n.d.	n.d.	n.d.
mOT23	agCACaGGAAACaGATCCTgTGG	chr1:69059975-69059997	0.26	0.11	0.09	+0.01
mOT24	cTCAC-aGAAACTGATCCTCTGG	chr4:11040762+11040783	0.26	5.76	0.02	**+5.74**

*The *ABCC6* on-target sequence and top ONE-seq-nominated candidate off-target sequences with mismatched positions and bulges indicated by lower case letters and dashes.

†Coordinates in the GRCm39/mm39 assembly of the mouse genome; "+" and "-" indicate forward or reverse orientation of protospacer/PAM sequence.

‡This table includes the top 25 ONE-seq-nominated sites, all of which have ONE-seq score > 0 01.

§n.d. = PCR was unsuccessful for the genomic site, or PCR was not attempted.

Supplementary Table 5. Mean cytokine and chemokine levels in age-matched wild-type mice following LNP treatment or vehicle treatment.

Time	IFN-γ	CXCL-1	TNF-α	MCP-1	IL-12p70	RANTES	IL-1β	IP-10	GM-CSF	IL-10	IFN-β	IFN-α	IL-6
Treatment with phosphate-buffered saline (PBS vehicle), *N* = 4 animals per group													
baseline	0.28	62.08	3.56	21.56	0.00	72.47	0.61	376.28	1.00	22.82	0.00	1.42	16.28
4 hours	0.00	90.95	3.62	51.41	0.00	32.38	0.72	288.18	0.00	0.00	0.00	0.00	398.51
24 hours	0.34	28.95	3.28	25.77	0.00	93.29	2.00	498.90	2.50	3.72	0.00	0.61	126.77
Treatment with ABE8.8/PXE1 LNPs, 2.5 mg/kg, *N* = 4 animals per group													
baseline	0.09	38.55	3.37	0.00	0.00	30.05	1.31	346.69	0.00	5.06	0.00	0.00	3.92
4 hours	20.10	334.44	117.68	2189.41	0.72	243.91	57.32	13436.10	9.64	21.68	341.12	985.17	868.78
24 hours	0.21	27.44	5.84	94.07	0.00	126.71	5.50	1146.90	0.00	0.48	0.65	222.65	37.88
Treatment with ABE8.8/PXE1_hyb18 LNPs, 2.5 mg/kg, *N* = 4 animals per group													
baseline	1.49	50.38	5.21	4.76	0.00	31.27	6.61	378.33	0.00	1.18	0.00	38.02	10.95
4 hours	4.63	405.06	57.88	689.26	0.35	91.57	25.67	5704.49	1.07	9.46	15.32	204.14	600.73
24 hours	5.70	38.33	13.49	54.62	0.21	77.39	15.20	1029.71	0.00	2.39	0.00	147.83	48.65

All cytokine/chemokine measurements are in units of pg/mL.

Supplementary Table 6. Assessment of off-target editing with ABE8.8/HPD3 with plasmid delivery in HuH-7 cells via targeted amplicon sequencing.

Genomic site	Protospacer/PAM sequence*	Amplicon (chromosome: position range)†	ONE-seq score‡	Treated sample 1§	Treated sample 2	Control sample 1	Control sample 2	Mean net A→G editing %
HPD	CACTCACAGTTTAGGAAGTAGGG	chr12:121849613+121849785	1.00	46.56	47.71			
OT1	CACaCACAGTTTAGatAGTAGGC	chr9:71133899-71134050	6.72	0.07	0.12	0.06	0.11	+0.01
OT2	CACaCACAGTTTAG--AGTtGGC	chr8:129535012-129535209	4.12	0.06	0.06	0.08	0.05	–0.01
OT3	CACTCTACAGTTTAGGAAtgtGGA	chr11:34141552-34141694	1.15	0.02	0.05	0.03	0.03	+0.01
OT4	CAtaCACAGTTTAGaAAtTtGGT	chr1:179641516+179641660	0.90	0.02	0.03	0.04	0.02	–0.01
OT5	tcCTCACAGTTcAGGAgGTAGGA	chr5:13257359+13257546	0.59	0.02	0.01	0.03	0.03	–0.02
OT6	CACTCtCAGTTTAGGgAGagGAG	chr15:52222324+52222506	0.57	0.00	0.02	0.04	0.01	–0.02
OT7	CAtTCACAGTTTAGaAAGTgAGG	chr14:20158742-20158893	0.56	0.04	0.04	0.02	0.04	+0.01
OT8	CACaCACAGTTTgGaAAtTgGGC	chr3:164629339+164629484	0.56	n.d.	n.d.	n.d.	n.d.	n.d.
OT9	aACcCACAGTTTAGG-AGTgGGA	chr2:240663681+240663791	0.43	0.05	0.04	n.d.	0.04	+0.00
OT10	CACTCACAcTcTAGGAAcTgGGA	chr1:153768158+153768304	0.43	0.03	0.02	0.06	0.04	–0.03
OT11	tcCTCACAGTccAGGAgGTgGGG	chr19:10914311+10914483	0.41	0.05	0.04	n.d.	0.04	+0.00
OT12	CACaCACAGTTTAGGtctggGGG	chr17:65841698+65841939	0.39	0.12	0.08	0.06	0.13	+0.00
OT13	CAtTCACAGTTTAGaAtGTgGGG	chr12:100653598+100653785	0.37	0.05	0.05	0.06	0.03	+0.00
OT14	CtaTCACAGTTcAGGAAGTgTGC	chr12:61254986-61255170	0.36	0.03	0.04	0.05	0.05	–0.01
OT15	CACTCACAGGTcTAGGAtGTtGGT	chr1:184773598-184773733	0.36	0.04	0.03	0.03	0.04	+0.01
OT16	ttCTCACAGTTcAGGAAGctGGG	chr8:125682758+125682876	0.35	0.03	0.02	0.03	0.02	+0.00
OT17	gACTCACAGTTcAGGAgGTtGGG	chr11:101382779-101382932	0.31	0.07	0.03	0.03	0.03	+0.02
OT18	CACatACAGTTTAGaAAGTAAGT	chr5:156260561+156260739	0.28	0.06	0.05	0.06	0.04	+0.01
OT19	tACTCACAGaaTAGGAAa-AGGG	chr9:104188183+104188347	0.28	0.05	0.07	0.05	0.04	+0.02
OT20	agCTCACAGTTTAGGAAGgAGGC	chr17:62097919-62098019	0.27	0.08	0.13	0.06	0.05	+0.05
OT21	CACTCACAGTTTA-GAAGagGGA	chr21:37868854+37869027	0.26	0.04	0.05	0.05	0.04	+0.00
OT22	ttCTCACAGTTcAGGAgGTtGGA	chr13:87903905+87904103	0.26	0.03	0.03	0.04	0.02	+0.00
OT23	CACTCACAGTaaAGGAgGTgGGA	chr1:208850269+208850445	0.26	0.05	0.02	0.02	0.04	+0.00
OT24	ttCTCACAGTTcAGGAgGTtGGA	chr2:196572793-196572973	0.26	0.02	0.04	0.04	0.03	+0.00
OT25	tACaCACAGTTTAGG-AGTtGGA	chr5:28033147+28033325	0.24	0.05	0.06	0.05	0.07	+0.00
OT26	CACcCACAGTTTAGGAAGcAGCT	chr5:6741426-6741552	0.23	0.03	0.05	0.04	0.03	+0.01
OT27	CACTCACAa-TTAGGAAGgtCGG	chr2:101723864+101723991	0.22	0.05	0.07	0.06	0.04	+0.01
OT28	tgCTCACAGTTTAGGAAGctGGG	chr12:32901606-32901769	0.22	0.10	0.50	0.03	0.03	**+0.27**
OT29	CAtTCACAGTTTAGGAAcTcAGA	chr13:108113244-108113356	0.19	0.05	0.03	0.06	0.06	–0.02
OT30	ttCTCACAGTTTAGGAtGTgGGG	chrX:79848611-79848728	0.19	n.d.	n.d.	n.d.	n.d.	n.d.
OT31	CACTCACAtTTTAGGgAaTtGGC	chr4:144387480-144387659	0.19	0.03	0.03	0.03	0.04	0.00
OT32	ACACcCACAG-TcAGGAAcTGGG	chr3:11222120+11222285	0.18	0.02	0.04	0.03	0.06	–0.01
OT33	CACTCACAGagTAGGAAtatGGC	chr2:213478565-213478798	0.18	0.08	0.05	0.07	0.06	+0.00
OT34	gtCTCACAGTTT-GGAAGTtGGG	chr9:90910874-90911035	0.17	0.05	0.06	0.04	0.04	+0.02
OT35	ttCTCACAGTTcAGGAAGTtAGG	chr15:76465133+76465249	0.16	0.03	0.00	0.02	0.01	+0.00
OT36	CACTCACAGTTgAGGAAtagGGT	chr5:75926694+75926854	0.16	0.04	0.04	0.04	0.04	+0.00
OT37	ACACT-ACAGaTTAGGAAGaGGA	chr19:49249583-49249695	0.16	0.05	0.09	0.05	0.03	+0.03
OT38	tgCTCACAGTTcAGGAgGTtGGG	chr3:107403236-107403382	0.15	0.03	0.02	0.03	0.03	+0.00
OT39	aACTCACAGTTcAGaAAcTgGGG	chr16:49598950-49599074	0.15	0.06	0.05	0.03	0.03	+0.02
OT40	CgCTCACAGTTTAGGAAaTtGGT	chr7:45486206+45486388	0.14	0.03	0.03	0.04	0.03	–0.01
OT41	CACTCAgAGTcTAaGAAGTcAGC	chr6:120673970+120674124	0.14	0.04	0.05	0.06	0.05	–0.01
OT42	aACaCACAGTTTAGGtAGTtGGC	chr5:73985188+73985369	0.14	0.09	0.10	0.10	0.06	+0.01
OT43	gtCTCACAGTTcAGGAAGTtAGA	chr2:226416848+226416985	0.13	0.05	0.03	0.03	0.02	+0.01
OT44	ttCTCACAGTTTAGGAtGcAGGT	chr14:41204728+41204884	0.13	n.d.	n.d.	n.d.	n.d.	n.d.
OT45	aACTCACAG-TTAGGAAcTtGGA	chr4:59970165+59970299	0.12	0.04	0.05	0.05	0.03	+0.00
OT46	CtCTCACAGTTcAGGAAGccGGA	chr11:71457536+71457667	0.12	0.02	0.02	0.02	0.03	+0.00
OT47	gtCTCACAGTTTAGGAgGgtAGG	chr13:21460941+21461098	0.12	0.05	0.04	0.04	0.03	+0.01
OT48	CACaCACAGTTT-GGtAGTtGGC	chr8:96405209-96405328	0.12	0.03	0.03	0.03	0.07	–0.02
OT49	tACTCACAcTTTAaGAAGTAGGA	chr10:74459927+74460112	0.12	0.04	0.03	0.03	0.04	+0.00
OT50	ttCTCACAGTTcAGGAAGctGGG	chr10:14951533+14951651	0.11	0.02	0.02	0.02	0.03	+0.00

*The *HPD* on-target sequence and top ONE-seq-nominated candidate off-target sequences with mismatched positions and bulges indicated by lower case letters and dashes.

†Coordinates in the GRCh38/hg38 assembly of the human genome; "+" and "-" indicate forward or reverse orientation of protospacer/PAM sequence.

‡This table includes the top 51 ONE-seq-nominated sites, all of which have ONE-seq score > 0 01.

§n.d. = PCR was unsuccessful for the genomic site, or PCR was not attempted.

Supplementary Table 7. Assessment of off-target editing with ABE8.8/HPD4 with plasmid delivery in HuH-7 cells via targeted amplicon sequencing.

Genomic site	Protospacer/PAM sequence*	Amplicon (chromosome: position range)†	ONE-seq score‡	Treated sample 1§	Treated sample 2	Control sample 1	Control sample 2	Mean net A→G editing %
HPD	CACTCACAGTTTAGGAAGTAGGG	chr12:121849585+121849753	1.00	32.80	33.23			
OT1	tACaCACAG-aTAGGAAGTAAGG	chr21:20182393-20182543	1.72	0.06	0.07	0.08	0.08	–0.01
OT2	CACTCACAGaTcAGGAAcTATGG	chr3:9092925+9093061	1.44	0.05	0.06	0.04	0.06	+0.00
OT3	CAtTCACAGTTTAGaAAGTgAGG	chr14:20158742-20158893	0.46	0.06	0.05	0.06	0.04	+0.01
OT4	CACTtACAGTTTAGGAAtacAGC	chr12:10379646+10379843	0.46	0.08	0.14	0.10	0.08	+0.02
OT5	CACaCACAGTTTAGatAGTAGGC	chr9:71133899-71134050	0.41	0.03	0.07	0.07	0.14	–0.05
OT6	CACTCTACAGTTTAGGAAtgtGGA	chr11:34141552-34141694	0.41	0.05	0.03	0.05	0.04	–0.01
OT7	CACTCACt-TTcAGGAAGTAAAG	chr16:86145755-86145933	0.34	0.03	0.02	0.03	0.03	+0.00
OT8	tgCTCACAG-TTAGGAAGTtTGG	chr12:119662566+119662714	0.34	0.03	0.03	0.04	0.05	–0.01
OT9	tACTCACAG-aTAGGAAGcAAGG	chr1:105956790-105956927	0.33	0.07	0.05	0.06	0.05	+0.00
OT10	CACcCACAGTTTAGGAgGTgAGG	chr8:123436530+123436719	0.32	0.03	0.04	0.04	0.04	+0.00
OT11	aACTCACAG-aaAGGAAGTAGGG	chr3:126880143+126880286	0.31	0.12	0.12	0.08	0.08	+0.04
OT12	CACTCACAG-aaAGGAAGccAGG	chr13:111545332+111545529	0.24	n.d.	n.d.	n.d.	n.d.	n.d.
OT13	CACTCACAG-aTAGGAAGTgGGG	chr15:96510560+96510735	0.23	3.15	2.82	0.07	0.07	+**2.92**
OT14	agCTCACAG-TTAGGAAGcAAGG	chr1:234082875-234083074	0.22	0.04	0.05	0.04	0.04	+0.00
OT15	aACaCACAGTTTAGGtAGTtGGC	chr5:73985188+73985369	0.20	0.07	0.06	0.07	0.07	–0.01
OT16	CACTCACAGagTAGGAAtatGGC	chr2:213478565-213478798	0.19	0.06	0.06	0.07	0.06	–0.01
OT17	tACTtACAG-aTAGGAAGcAAGG	chr8:53729339+53729495	0.18	0.06	0.03	0.06	0.05	–0.01
OT18	aAgTCACAGTTcAGGAAaTgAGG	chr6:28301059+28301330	0.17	0.21	0.14	0.20	0.15	+0.00
OT19	tcCTCACAGTTgAGGAAGTAGGG	chr10:34725514-34725668	0.16	0.03	0.03	0.03	0.03	+0.00
OT20	CACTCACAG-aaAGGAAGcgAGG	chr13:111545396+111545569	0.15	n.d.	n.d.	n.d.	n.d.	n.d.
OT21	CACTCA-tcTTTAGGAAGTATGA	chr6:102884454-102884607	0.14	0.02	0.02	0.03	0.03	–0.01
OT22	tACTCACAcTTTAaGAAGTAGGA	chr10:74459927+74460112	0.13	0.04	0.05	0.03	0.04	+0.01
OT23	aACcCACAGTTcAGGAAtTATGG	chr15:59358774-59358929	0.13	0.05	0.05	0.04	0.04	+0.01
OT24	agCTCACA-TTTAaGAAGTgGGG	chr8:98563913-98564062	0.12	0.05	0.03	0.04	0.03	+0.00
OT25	CACTCACAaTTT-GGAAGTATGG	chr5:62633698+62633838	0.11	0.08	0.06	0.05	0.06	+0.01
OT26	tACTtACAG-aTAGGAAGcAAGG	chr4:2363523-2363691	0.11	0.06	0.07	0.07	0.07	+0.00
OT27	aACTCACt-TTTAaGAAGTtGGG	chr11:58388631-58388793	0.11	0.05	0.04	0.03	0.03	+0.01
OT28	tACTCACA-TTcAGGAAGgACGG	chr20:23706230-23706385	0.11	0.05	0.02	0.04	0.04	+0.00
OT29	aACTCACAG-aaAGGAAGTAGTG	chr19:5249437+5249599	0.10	0.10	0.07	0.08	0.09	+0.01
OT30	gtaTCACAGTTTA-GAAGTAGAG	chr15:59025979+59026237	0.09	0.16	0.12	0.07	0.04	+0.08
OT31	CACT-ACAGTccAGGAAaTATGG	chr1:174531664+174531804	0.09	0.05	0.05	0.05	0.03	+0.01
OT32	tACaCACAGTTcAaGAAGTAAGG	chr9:97753663+97753822	0.09	0.05	0.04	0.06	0.07	–0.02
OT33	CgCTCACAGTTTAGGAAaTtGGT	chr7:45486206+45486388	0.09	0.03	0.03	0.03	0.04	–0.01
OT34	ttCTCACAGTTcAGGAAGTtAGG	chr15:76465133+76465249	0.09	0.03	0.02	0.03	0.04	–0.01
OT35	tACaCACAG-aTAGGAAGaAAGG	chr6:106865425+106865557	0.09	0.10	0.08	0.07	0.09	+0.01
OT36	aACTCAtAGTTTAGGAAGagGGC	chr3:171833685-171833914	0.08	n.d.	n.d.	n.d.	n.d.	n.d.
OT37	tACTCACA-TgaAGGAAGTAAGG	chr6:64294296-64294544	0.08	0.06	0.05	0.06	0.03	+0.01
OT38	gtCTCACAGTTT-GGAAGTtGGG	chr9:90910874-90911035	0.08	0.03	0.05	0.04	0.03	+0.00
OT39	agCTCACAGTcTA-GAAGTgGGG	chr1:58937014+58937174	0.08	0.05	0.04	0.05	0.05	+0.00
OT40	ataTCACAGTTTAaGAAaTATGG	chrX:129100077+129100226	0.08	0.06	0.06	0.07	0.05	+0.00
OT41	CACTCAC-GTaTtGGAAGTAGAG	chr2:218276927+218277070	0.07	0.06	0.05	0.05	0.05	+0.00
OT42	CACTCACAGTTgAGGAAtagGGT	chr5:75926694+75926854	0.07	0.05	0.04	0.04	0.04	+0.00
OT43	gACTCACAa-TTAGaAAGTAGGG	chrX:83541561+83541739	0.07	n.d.	n.d.	n.d.	n.d.	n.d.
OT44	ttCTCACAGTTTtGGAAGTgGGG	chrX:108926545-108926663	0.07	0.03	0.04	0.04	0.03	+0.00
OT45	attTCACAGTTTA-GAAGTAGAG	chr5:102250459+102250630	0.06	0.06	0.07	0.07	0.06	+0.00
OT46	CctTCACAGTTTAGGAAGcAAGG	chr13:30173299+30173455	0.06	0.03	0.04	0.06	0.05	–0.02
OT47	tACTCACAGaaTAGGAAa-AGGG	chr9:104188183+104188416	0.06	0.06	0.05	0.06	0.04	+0.01
OT48	tgCTCACAGTTTAGGAAGctGGG	chr12:32901606-32901769	0.06	0.06	0.08	0.03	0.03	+0.04
OT49	gACTCACAGTTaAGGgAagtAGG	chr9:82247483+82247626	0.06	0.04	0.07	0.11	0.07	–0.03
OT50	tACTCACAG-aTAGGgAGcAGGG	chr15:26019155-26019299	0.06	0.06	0.06	0.06	0.07	–0.01
OT51	tACTCACAGTTctGGAAGTAGGG	chr14:26888839+26889031	0.06	n.d.	n.d.	n.d.	n.d.	n.d.
OT52	CACTCACAa-TTAGGAAGgtCGG	chr2:101723864+101723991	0.06	0.06	0.06	0.04	0.05	+0.01

*The *HPD* on-target sequence and top ONE-seq-nominated candidate off-target sequences with mismatched positions and bulges indicated by lower case letters and dashes.

†Coordinates in the GRCh38/hg38 assembly of the human genome; "+" and "-" indicate forward or reverse orientation of protospacer/PAM sequence.

‡This table includes the top 53 ONE-seq-nominated sites, all of which have ONE-seq score > 0 01.

§n.d. = PCR was unsuccessful for the genomic site, or PCR was not attempted.

Supplementary Table 8. Assessment of off-target editing with ABE8.8/HPD5 with plasmid delivery in HuH-7 cells via targeted amplicon sequencing.

Genomic site	Protospacer/PAM sequence*	Amplicon (chromosome: position range)†	ONE-seq score‡	Treated sample 1§	Treated sample 2	Control sample 1	Control sample 2	Mean net A→G editing %
HPD	CACTCACAGTTTAGGAAGTAGGG	chr12:121849585+121849753	1.00	44.77	44.62			
OT1	aACTtACAGTTTAGGAAGaAGAA	chr2:149730234-149730433	0.75	0.05	0.06	0.02	0.06	+0.02
OT2	tACTtACAGTTTAGGAAaaAGAG	chr7:7216857-7217047	0.62	0.04	0.03	0.04	0.03	+0.00
OT3	tACTCACAcTTTAaGAAGTAGGA	chr10:74459927+74460112	0.60	0.02	0.04	0.03	0.09	–0.04
OT4	CACcCACAGTTgAGGAAGcAGAG	chr5:134574934+134575076	0.55	0.03	0.03	0.03	0.04	+0.00
OT5	aACTCACAGTTaAGGgAGTAGTG	chr14:42104487+42104676	0.53	0.07	0.04	0.04	0.06	+0.00
OT6	gACTCAgAGTTTAGGAAGaACAG	chr13:67489114+67489255	0.48	0.04	0.02	0.03	0.04	–0.01
OT7	tCTCACAG-TaAGGAAGcAGAGG	chr20:34952014+34952156	0.43	0.02	0.03	0.03	0.04	–0.01
OT8	gAaTCACAGTTTAaGAAGaAGAG	chr2:63499942-63500082	0.38	0.04	0.02	0.03	0.07	–0.02
OT9	CctTCACAGTTTAGGAAGcAAGG	chr13:30173296+30173430	0.37	0.01	0.02	0.02	0.02	–0.01
OT10	atCTCACAG--aAGGAAGTAGGG	chr3:105980524-105980663	0.36	0.05	0.06	0.04	0.05	+0.01
OT11	ACTCACAG-aaAGGAAaTAGGTG	chr5:114806740+114806991	0.34	0.13	0.09	0.12	0.08	+0.01
OT12	tACTCACAG-aTAGGtAGTAAGG	chr1:217473036-217473177	0.31	0.04	0.04	0.03	0.03	+0.01
OT13	tACTCACAGaTTAGGAtGTACAG	chr4:100586150+100586282	0.26	0.02	0.03	0.03	0.03	+0.00
OT14	CACTCACAGgTTAGGAgGaAGAG	chr1:222073075+222073242	0.25	0.05	0.07	0.05	0.04	+0.02
OT15	tACTCACAG--TAGGAAGTAGCA	chr5:150433268-150433412	0.23	0.01	0.03	0.02	0.04	-0.01
OT16	tACTCACAG-TcAGaAAGTAGAG	chr2:46394123+46394265	0.21	0.03	0.05	0.02	0.04	+0.01
OT17	agCTCACAGTTTAGGAAGgAGGC	chr17:62097919-62098019	0.20	0.03	0.03	0.03	0.03	+0.00
OT18	aACTCACAGacTAGGAAGcATAG	chr6:148367572-148367728	0.19	0.04	0.04	0.03	0.03	+0.01
OT19	tAagCACAGTTTAGGAAGgtGAG	chr6:71011412-71011565	0.18	0.06	0.05	0.05	0.05	+0.00
OT20	aACTtACAGTTaAGaAAGTAGAG	chr2:216658842+216659027	0.17	0.03	0.03	0.04	0.03	+0.00
OT21	aACTCACAG-aaAGGAAGTAGGG	chr3:126880143+126880286	0.17	0.03	0.04	0.06	0.04	–0.02
OT22	aACTCACAGTTTgGGAAGaAAGA	chr3:188702412-188702578	0.16	0.04	0.04	0.04	0.04	+0.00
OT23	CAtTCACAGTTcAGGgAGaAGAG	chr12:13031726-13031879	0.16	0.04	0.04	0.04	0.03	+0.00
OT24	aACaCACAGTcTAGGAAGaAAAG	chr2:179266080-179266250	0.15	0.06	0.06	0.05	0.05	+0.01
OT25	gAaaCACAGTTTAGGAAGTtTTG	chr9:39981045+39981248	0.15	n.d.	n.d.	n.d.	n.d.	n.d.
OT26	gACTCACAGagAGaAAGaAGGG	chr8:59224924+59225128	0.13	n.d.	n.d.	n.d.	n.d.	n.d.
OT27	tACTCACAGTTctGGAAGTAGGG	chr14:26888839+26889031	0.12	0.06	0.08	0.00	0.00	+0.07
OT28	tcCTCACAGTTgAGGAAGTAGGG	chr10:34725514-34725668	0.11	0.02	0.03	0.02	0.03	+0.00
OT29	ACTCACAG--TcaGgAaTAGGGG	chr1:98462417-98462579	0.11	0.03	0.03	0.03	0.04	–0.01
OT30	CACTCACAG-aTAGGAAGTgGGG	chr15:96510560+96510735	0.11	0.05	0.05	0.06	0.04	+0.00
OT31	tACTCACAG-aTAGGgAGcAGGG	chr15:26019155-26019299	0.10	0.04	0.04	0.03	0.05	+0.00
OT32	ttCTCACAGTTTAGGgAtTACAG	chr9:114758066-114758199	0.10	0.03	0.03	0.02	0.04	+0.00
OT33	gAaaCACAGTTTAGGAAGTtTTG	chr9:41482259+41482469	0.09	0.11	0.10	0.12	0.12	–0.01
OT34	ttTCACAGaTTAGGAAGctGAGG	chr1:204565742-204565921	0.09	0.04	0.05	0.03	0.03	+0.01
OT35	tACaCACAGTTcAaGAAGTAAGG	chr9:97753663+97753822	0.09	0.04	0.04	0.03	0.03	+0.01
OT36	gAaaCACAGTTTAGGAAGTtTTG	chr9:62269477+62269674	0.09	n.d.	n.d.	n.d.	n.d.	n.d.
OT37	tACTCACAG-aTAGGAAGcAAGG	chr1:105956790-105956927	0.09	n.d.	0.05	0.04	0.03	+0.01
OT38	CAtTCACAGTTTAGaAAGTgAGG	chr14:20158742-20158893	0.08	0.04	0.04	0.03	0.04	+0.01
OT39	gAaTCACAGTTTAGGgAGagTGG	chr5:143910524-143910667	0.08	0.04	0.05	0.05	0.04	+0.00
OT40	cCTCACAG-TTAGGAAaTAGTTG	chr14:51982054-51982213	0.08	0.03	0.03	0.02	0.02	+0.00
OT41	gtCaCACAGTTTAGGAAGTAGCT	chr20:42662345-42662525	0.08	n.d.	n.d.	n.d.	n.d.	n.d.
OT42	gACTCACA-TTcAGGAAGgtAGG	chr2:81325426+81325576	0.08	0.02	0.02	0.02	0.03	+0.00
OT43	gtCTCACAGTTcAGGAAGTtAGA	chr2:226416848+226416985	0.08	0.02	0.01	0.03	0.03	–0.01
OT44	aAtTCACAGTTTAGGAAGTAGCA	chr14:32471376-32471550	0.08	0.02	0.04	0.02	0.04	+0.00
OT45	tACTCACtG--TAGcAAGTAGAG	chr12:33782613-33782771	0.07	0.03	0.01	0.01	0.02	+0.01
OT46	CACTgACAGTTTAGGAAGaAGTG	chr19:4998447-4998567	0.07	0.03	0.04	0.03	0.02	+0.01
OT47	ACTCACAGTaagGGAAaTAGAAG	chr17:12794963-12795150	0.07	n.d.	n.d.	n.d.	n.d.	n.d.
OT48	tACcCACAGTTTAGGAgGctGAG	chr2:31854443-31854570	0.07	0.03	0.03	0.03	0.04	+0.00
OT49	aACTCACAGaTaAGGAAtTAGCA	chr2:147463226-147463390	0.06	0.05	0.03	0.04	0.03	+0.00
OT50	tCTCACAG--TAGGgAGaAGTGG	chr3:46953117+46953280	0.06	0.04	0.02	0.01	0.02	+0.01
OT51	tAtTCACAG--TAGGAAGTAGGA	chr2:4649666+4649776	0.06	0.02	0.04	0.02	0.03	+0.01
OT52	gACTCACA-TTTAGGgAGTAGCA	chr5:153457501+153457622	0.06	0.04	0.04	0.06	0.01	+0.00

*The *HPD* on-target sequence and top ONE-seq-nominated candidate off-target sequences with mismatched positions and bulges indicated by lower case letters and dashes.

†Coordinates in the GRCh38/hg38 assembly of the human genome; "+" and "-" indicate forward or reverse orientation of protospacer/PAM sequence.

‡This table includes the top 53 ONE-seq-nominated sites, all of which have ONE-seq score > 0 01.

§n.d. = PCR was unsuccessful for the genomic site, or PCR was not attempted.

Supplementary Table 9. Assessment of off-target editing with ABE8.8/HPD20 with plasmid delivery in HuH-7 cells via targeted amplicon sequencing.

Genomic site	Protospacer/PAM sequence*	Amplicon (chromosome: position range)†	ONE-seq score‡	Treated sample 1§	Treated sample 2	Control sample 1	Control sample 2	Mean net A→G editing %
HPD	TCTGCAGAAAGCACGGGAACGGG	chr12:121854709-121854850	1.00	59.38	59.98			
OT1	gCTGCAGAAAGCACaGGtcCAGG	chr1:24300946+24301142	1.82	0.11	0.08	0.06	0.06	+0.03
OT2	TCTGCAGAAAGCACGGcAcaCAG	chr3:46293912+46294063	1.73	0.05	0.05	0.06	0.04	+0.00
OT3	aCTGCAGAAAGCtCaGGAAtCAG	chr11:119970623-119970822	1.53	0.06	0.07	0.07	0.06	+0.00
OT4	gaaGCAGAAAGCACGGGAgtCGG	chr13:42673670+42673866	0.98	0.05	0.08	0.14	0.11	–0.06
OT5	TCTGCAGtAAGCACtGGgAaCAG	chr5:35954904+35955063	0.85	0.03	0.05	0.06	0.03	+0.00
OT6	cCTGCAGAAAaCAtGGGAcCAGG	chr8:25687286-25687419	0.84	0.06	0.04	0.05	0.04	+0.00
OT7	TCTcCAGAAAGCACGGGAcCTGG	chr15:41845393-41845492	0.83	0.08	0.12	0.02	0.03	+0.07
OT8	cCTGCAGAAAGCAtGGaAACAAG	chr3:78206254-78206486	0.69	0.02	0.04	0.08	0.05	–0.03
OT9	gtaGCAGAAAGCACaGGAAtTGG	chr1:165659845+165660004	0.68	0.07	0.09	0.05	0.08	+0.01
OT10	TaTGCAGAAAGCACaGGgcaCAG	chr12:57481823-57481952	0.56	n.d.	0.05	0.06	0.09	–0.02
OT11	caTGCAGAAAGCAaGGGAgCGGG	chr19:10984549-10984826	0.49	n.d.	n.d.	n.d.	n.d.	n.d.
OT12	aCTGCAGAAAGCAgGGagACGGG	chr11:69529236-69529341	0.30	0.08	0.05	0.07	0.05	+0.00
OT13	ggTGCAGAAAaCACaGGAtCTGG	chr22:45315767-45315879	0.24	0.05	0.03	0.04	0.05	+0.00
OT14	cCTGCAG-AAGCACGGGctCTGG	chr15:89333349-89333503	0.23	0.04	0.01	0.02	0.03	+0.00
OT15	ctgGCAGAAAGCAtGGGAACAGG	chr11:6002392+6002543	0.20	0.12	0.06	n.d.	0.07	+0.02
OT16	TCTGCAGAAAGCACGGtccaGGG	chr20:772807+772980	0.18	n.d.	n.d.	n.d.	n.d.	n.d.
OT17	TaTGCAGAAAaCAtGGaAACAGG	chr1:208900627-208900791	0.17	0.08	0.08	0.07	0.07	+0.01
OT18	TgTGCAGAAAGCACGGGAcCAGC	chr7:27496094+27496275	0.16	0.05	0.05	0.04	0.05	+0.00
OT19	aCTGCAGAgAGCACaGGActCTG	chr12:3473405-3473596	0.16	0.09	0.06	0.06	0.07	+0.02
OT20	TCTaCAGAAAaCA-GGaAACAGG	chr11:94882630-94882780	0.13	0.05	0.06	0.04	0.05	+0.01
OT21	TCTaCAGgAAGCACaGGctCAGG	chr5:80631206+80631387	0.13	0.05	0.03	0.02	0.04	+0.01
OT22	TCTGCAGAtAGCACaGGAAaCAG	chr14:93434139-93434369	0.12	n.d.	n.d.	n.d.	n.d.	n.d.
OT23	cCTGCAG-AAGCACaGGAtCTGG	chr2:85318510+85318630	0.11	0.05	0.03	0.02	0.05	+0.00
OT24	cCaGCAGAAAGCACtGGgAaCAG	chr20:44128949+44129094	0.11	0.06	0.06	0.05	0.04	+0.02
OT25	TCTGCAGgcAGCACaaGAACAGG	chr3:131568483+131568700	0.10	0.04	0.02	0.02	0.04	+0.00
OT26	aCTGCAtgAAGCAtGaGAACGGG	chr2:45775271-45775462	0.09	0.05	0.06	0.09	0.04	–0.01
OT27	aCTGCAGAAAGacCGGGgACGGG	chr19:52962559+52962740	0.09	0.08	0.07	0.08	0.07	+0.00
OT28	TCTGCAGAcAGCACaGGtAaCAG	chr10:2911412-2911569	0.09	0.03	0.04	0.02	0.03	+0.01
OT29	TCaGCAcAAAGCAaGGGAtCAGG	chr7:126635688-126635920	0.09	0.09	0.06	0.09	0.06	+0.00
OT30	aCTGCAG-AAGCACtGGgACTGG	chr20:45497078-45497239	0.08	0.05	0.05	0.03	0.06	+0.01
OT31	caTGCAGAAAGCA-GGGcACAGG	chr3:52371719+52371838	0.08	0.06	0.07	0.07	0.06	+0.00
OT32	TCTGCAGttAaCACaGGAACAAG	chr1:230774607-230774718	0.08	0.02	0.03	0.01	0.02	+0.01
OT33	TCTGCAGAcAGCACtGGAACAAG	chr14:104387236-104387343	0.07	0.03	0.03	0.03	0.06	–0.01
OT34	gaTaCAGAAAGCACaGGAACAGG	chr2:100149130+100149266	0.07	0.13	0.14	0.06	0.07	+0.07
OT35	TCTGCAGAAAGCAgGGaccCTGG	chr7:157785580-157785682	0.06	0.08	0.04	0.04	0.10	–0.01
OT36	TCTGCAGgAAGCACaGGcACATG	chr17:32929801+32929935	0.06	0.05	0.04	0.03	0.04	+0.01
OT37	TCTGCAGAAAGCAtGGGtgaTGG	chr11:126901459+126901631	0.06	0.05	0.06	0.06	0.05	+0.01
OT38	aCTaCAGAAAGtA-GGGAACAGG	chr10:46703078-46703275	0.06	0.12	0.13	0.06	0.13	+0.03
OT39	aCTGCAGgAAGCAtGGGAgtGGG	chrX:47225699-47225859	0.04	0.08	0.00	0.04	0.06	–0.01
OT40	TCTGCAGAAAGCACaGtAgaCAG	chr16:60622413+60622573	0.04	0.05	0.05	0.04	0.03	+0.02
OT41	aCTaCAGAAAGtA-GGGAACAGG	chr10:47637113+47637310	0.04	0.12	0.13	0.06	0.13	+0.03
OT42	TgTaCAGAAAGCAgGGaAAtGGC	chr5:137991546+137991760	0.04	0.03	0.06	0.07	0.05	–0.02
OT43	TCTGCAGAgAGaAaGGGAACTGG	chr16:5870906-5871083	0.04	0.04	0.05	0.03	0.04	+0.01
OT44	gtTGCAcAAAGCACaGGAACTGG	chr14:62033276+62033525	0.04	0.04	0.04	0.04	0.05	+0.00
OT45	aCTaCAGAAAGtA-GGGAACAGG	chr10:46856878-46857075	0.04	0.12	0.13	0.06	0.13	+0.03
OT46	TCTGtAGAAAGCAgGGaAAgGGG	chr17:50179782-50179903	0.04	0.06	0.04	0.04	0.05	+0.01
OT47	TCTGCAG-gAGCACaGGAcCCGG	chr20:57125614+57125735	0.04	0.08	0.05	0.03	0.06	+0.01
OT48	ctTGCAGAAAcCACaGGAtCGGG	chr12:69091620-69091827	0.03	0.02	0.03	0.03	0.04	–0.01
OT49	aCTGCAGAAAGCA-GaaAACAGG	chr5:170436647-170436760	0.03	0.07	0.07	0.09	0.05	+0.00
OT50	TCTGCAGtAAtCAtGGGAtCAGG	chr8:37073890+37074041	0.03	0.04	0.05	0.04	0.03	+0.01
OT51	TCTGCAGAAtGCACaGGAAaCAG	chr8:109854275-109854473	0.03	0.07	0.05	0.02	0.06	+0.01
OT52	TCTGCAGgAAGCACaGagAaCTG	chr18:66087503+66087640	0.03	0.06	0.04	0.04	0.03	+0.02
OT53	agaGCAGAAAGCACGGGtAgGGG	chr15:101961283+101961394	0.02	0.06	0.08	0.08	0.08	–0.01
OT54	cgTGCAGgAAGCACGGGAAaGGG	chr16:1525089+1525237	0.02	0.05	0.06	0.07	0.03	+0.00
OT55	agaGCAGAAAGCACGGGtAgGGG	chr9:29346-29457	0.02	0.06	0.08	0.08	0.08	–0.01
OT56	TCTGCAGAAAGCtaGGGActTGG	chr11:74019360+74019555	0.02	0.04	0.06	0.07	0.07	–0.02
OT57	TCTGCA-AAAGCACGGGAtaAGG	chr16:12159771-12159942	0.02	0.05	0.04	0.05	0.05	+0.00
OT58	TgTaCAGAAAGC-CaGGAACAGG	chr3:125754761-125754903	0.02	n.d.	n.d.	n.d.	n.d.	n.d.
OT59	TgTaCAGAAAGCA-GGGAAtGGG	chr2:122750544+122750732	0.02	0.08	0.08	0.04	n.d.	+0.05

OT60	ctgGCAGAAAaCACGGGAAaGGG	chr6:6918026+6918222	0.02	0.02	0.06	0.05	0.04	+0.00
OT61	TCaGCAGAgAGCACaGGAAtGGG	chr13:21004935+21005116	0.02	0.09	0.09	0.06	0.04	+0.04
OT62	TCTGCAGcAAGCACaGGgACACA	chr16:29941570-29941696	0.02	0.04	0.04	0.05	0.02	+0.00
OT63	aCTGCAGAAAGCcaGGGAAgAGG	chr4:140941644+140941750	0.02	0.05	0.05	0.06	0.05	–0.01
OT64	cCTaCAagAAGCACGGGAAaGGG	chr12:38832160+38832334	0.02	0.05	0.05	0.05	0.04	+0.01
OT65	aaaGCAGAAAGCACaGcAACCGG	chr5:148658160+148658297	0.02	0.06	0.09	0.07	0.07	+0.01
OT66	atgGCAGAAAGCA-GGGAgCCGG	chr6:77706756-77706905	0.02	0.08	0.08	0.08	0.07	+0.00
OT67	TCTGCAGAgAGCACGaaAAaTGG	chr18:11223190+11223381	0.02	0.04	0.04	0.02	0.04	+0.01
OT68	gCTGCAGAAAGCAtaGGActTGG	chr5:7514969-7515126	0.02	0.05	0.05	0.06	0.06	–0.01
OT69	agaGCAGAAAGCACGGGtAgGGG	chr1:200084-200195	0.02	0.06	0.08	0.08	0.08	–0.01
OT70	TgTGCAGAAAGCtCaGGAtCAGG	chr20:36414475+36414635	0.02	0.02	0.03	0.04	0.04	–0.01
OT71	cCTGCAG-AAGCACtGGAAtAGG	chr3:186709662+186709845	0.02	0.02	0.04	0.04	0.05	–0.02
OT72	aCTGCAGAAAGCAgGGaAAtGGG	chr3:111673780+111673941	0.02	0.08	0.06	0.06	0.06	+0.01
OT73	agaGCAGAAAGCACGGGtAgGGG	chr2:113583783+113583894	0.02	0.06	0.08	0.08	0.08	–0.01
OT74	TgTGCAGAAAGCA-GGGctCAGG	chr15:22404602-22404777	0.02	0.04	0.03	0.07	0.10	–0.05
OT75	TtaGCAGAAAGCACaaGAcCAGG	chr11:126623596+126623735	0.02	0.07	0.07	0.09	0.08	–0.01
OT76	TCTGCAGAAAaCACGaGAAtTAG	chr10:68737295+68737439	0.02	n.d.	n.d.	n.d.	n.d.	n.d.
OT77	agaGCAGAAAGCACGGGtAgGGG	chr12:31622-31733	0.02	0.06	0.08	0.08	0.08	–0.01
OT78	aggGCAGAAAGCA-GGaAACAGG	chr1:55818798-55818959	0.02	0.08	0.06	0.06	0.06	+0.01
OT79	gCTGCAGAAAGCA-GaGAAtGGG	chr5:55825784-55825928	0.02	0.05	0.06	0.06	0.07	–0.01
OT80	gtaGCAGAAAcCACaGGAACGGG	chr11:62464098+62464250	0.02	0.05	0.05	0.07	0.06	–0.01
OT81	cCTGCAGAAAGCAACaGGAACTGA	chr7:32749854-32749979	0.02	0.06	0.06	0.06	0.04	+0.01
OT82	agaGCAGAAAGCACGGGtAgGGG	chr19:71175-71286	0.02	0.06	0.08	0.08	0.08	–0.01
OT83	TCTGCAGAAAGCAgGGGAAaGGC	chr1:18711522+18711659	0.02	0.05	n.d.	0.05	0.06	+0.00
OT84	TgTGCAGAAAGCA-GGGctCAGG	chr15:20595609-20595784	0.02	0.07	0.05	0.12	0.07	–0.03
OT85	aCTGCAGAGAAaCACGGaAACTAG	chr11:108335836+108336029	0.02	0.05	0.06	0.05	0.04	+0.01
OT86	TtTGCAGAAAaCAaGGGAAaGGG	chr7:26649004+26649176	0.02	0.04	0.07	0.07	0.07	–0.01
OT87	gtTGCAGAAAtCACGGGAgCTGG	chr16:73307950-73308078	0.02	0.04	0.05	0.06	0.08	–0.02
OT88	TaTGCAGAAAGCA--GGAACTGG	chr6:107887698-107887870	0.01	0.07	0.05	0.05	0.11	–0.02
OT89	TgTaCAGAAAGC-CaGGAACAGG	chr11:71901084-71901226	0.01	n.d.	n.d.	n.d.	n.d.	n.d.
OT90	TCaGCAGAAAGCACaGagtCAGG	chr2:109710574+109710842	0.01	0.25	0.08	0.14	0.12	+0.04
OT91	ctTaCAGAAAGaACaGGAACAGG	chr6:13703726-13703885	0.01	0.00	0.05	0.09	0.05	–0.05
OT92	TtaGCAGAAtGCACGGGAAaGGG	chr4:78528872-78529015	0.01	0.04	0.05	0.03	0.07	+0.00
OT93	ctaGCAGAAAGCACaGacACAGG	chr2:96700005+96700195	0.01	0.07	0.05	0.06	0.06	+0.00
OT94	TCTGC--AAAGCACaGGAtCGGG	chr10:79195199-79195302	0.01	0.03	0.04	0.06	0.03	–0.01
OT95	aCTGCAAGgAAaCACGGGAAtGGG	chr3:9010345+9010449	0.01	0.06	0.05	0.04	0.03	+0.02
OT96	TCTGCAGAAg--caGGGAcCTGG	chr17:79426956+79427100	0.01	0.04	0.03	0.05	0.04	–0.01
OT97	aCTGCAtAAAGCACaaGAAaCAG	chr10:47347282-47347458	0.01	0.04	0.05	0.04	0.06	+0.00
OT98	TgaGCAGAAAGCA-GaGAACAGG	chr13:96513276-96513427	0.01	0.07	0.07	0.12	0.11	–0.04
OT99	aCTGtAGAAAGCACGGGccCCAG	chr17:59398252+59398419	0.01	0.06	0.06	0.05	0.05	+0.01
OT100	gCTGCAGAAAaCACGGaAAaGGA	chrX:136061616-136061778	0.01	0.07	0.04	0.03	0.04	+0.02
OT101	TCTGCAGAAAGCAC--aAACAGG	chr8:130448699-130448838	0.01	0.05	0.05	0.08	0.03	–0.01
OT102	agaGCAGAAAGCACGGGtAgGGG	chr1:29568-29679	0.01	0.06	0.08	0.08	0.08	–0.01
OT103	cagGCAGAAAGCAgGGaAcCTGG	chr1:160175195-160175392	0.01	0.07	0.08	0.08	0.07	+0.00
OT104	gCTGCAGAAAGCACaGGggaAGC	chr2:112721634-112721775	0.01	n.d.	0.04	0.05	0.05	–0.01

*The *HPD* on-target sequence and top ONE-seq-nominated candidate off-target sequences with mismatched positions and bulges indicated by lower case letters and dashes.

†Coordinates in the GRCh38/hg38 assembly of the human genome; "+" and "-" indicate forward or reverse orientation of protospacer/PAM sequence.

‡This table includes all sites with ONE-seq score > 0 01.

§n.d. = PCR was unsuccessful for the genomic site, or PCR was not attempted.

Supplementary Table 10. PCR primers for next-generation sequencing.

Site*	Forward primer	Reverse primer
PAH1 on-target site (human)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACCTCATTCTTGCAGCAGGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCTCGGGATTTCTTGGGTGG
PAH1 OT1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TTCTGTAGTTCTTTCTGTTCTCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACATCTACAATGGTGGCACT
PAH1 OT2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCACCTATCCCAACCACACA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GAAAATTCAGCATTATGGTCCCA
PAH1 OT3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACAGCCATACCCGTAAGTCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACTGAAGAAAGAGCTAAAGTGGT
PAH1 OT4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGCTCAAGTCACCAGGCTTA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CAAAGCTCACAGTTGACACCA
PAH1 OT5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGGCCTGAGGAGAGATGAACTGAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCCGCTCTCCGAGAAACACCGAAGAATGATC
PAH1 OT6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGTTCCATCCATGTTGTTGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACCTGGGTGATGAAATAATCTGT
PAH1 OT7	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGTGTTGTGCCATCGGAAAA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACTTTGTGGCATGGAGAAGG
PAH1 on-target site (humanized mice)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGCTGGCTTACTGTCGTCTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GAGCATCCATTGTGGTTGGC
PAH1 mOT1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGCCACTCTTTGTTGGACAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCCAAATTATCAAGCTAGCACGA
PAH1 mOT2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGCCCTGTCCCATCCATTTT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCACTGGGAGGGCAAGAAAG
PAH1 mOT3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTACTCCATGGCCGAGCATA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATCCTGCCACACTGACAGTT
PAH1 mOT4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGTTGTCCTCCTGGCTAAGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCCAGAGTTCCAGGAGACAT
PAH1 mOT5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGTCTGGGAGAAGCAGGAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GAAGGAGAAGGGAGACAGCA
PAH1 mOT6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGCAGAGTATGGCCTTGTCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGAAACACCCTTCCTCTCCC
PAH1 mOT7	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGGAGTACACATGGAGGGAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCCCATCCTCTCTCCTCCTG
PAH1 mOT8	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ATTGGGAAATGAGGGGCAGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCACCTTGTCTGAAACCCTG
PAH1 mOT9	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GACTAGGCCATGCTCTGCTA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AACACAGAGCCTCCAATGGA
PAH1 mOT10	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTCCTGCACTTTGGCCATAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCCCCTTTGATGTCTGGAA
PAH1 mOT11	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TTTGGAAAAGGAGGGCACTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGATAAAGCATGGCACGGTG
PAH1 mOT12	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGCAGCTATAAACGTCCCTCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCTTGTTGGTCAGGAATGC
PAH1 mOT13	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCGCCCTAGTAACCAACAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCTGGTGGTGGTAGAGTGTT
PAH1 mOT14	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGTGACTCTAGAGAACCCTGAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTACGATGCCCCTCACAAGA
PAH1 mOT15	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGAGTCCGAGCTGATGTTCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCACCTATAAGAACTTTGCCCA
PAH1 mOT16	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCTAGCCAGGACACACACAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCCCTGCCTCAATATCCCAA
PAH1 mOT17	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGCTTCAGTGGGAGATAACG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCCACACACTCCCTACTTGT

PAH1 mOT18	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCAAACATCAGCCTCTAATGACT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AACCCCTGCAACCCTTCTTA
PAH1 mOT19	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CAAGCTGCTGTGAGAGTTCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCCCATGTGCCCATATCTTCA
PAH1 mOT20	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGTGCATGCCTTTAATCCCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCTACTTCCCTTCCTACTTTGT
PAH1 mOT21	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TTGTCTAAGAATTGAGGCCTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGTTCTGTAATATCCACAACCTT
PAH1 mOT22	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCAATGACTGAAGTGGAGGGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCCATAGAGACTGCTGCCTA
PAH1 mOT23	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGATTGAGCTACCCACACCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGGAGAAGTACTGTTTGGGTTTG
PAH1 mOT24	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CAATGATGCCACAGACACCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGGTAAGGCAATCTCACTCC
PXE1 on-target site (human)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCAAGGTCTTCTCTGCCCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGTGGCTCAGAACAATGCTC
PXE1 OT1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TTTTGAGACAGAGGCCCGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGTTGCTGTCTGTTCATTTGGT
PXE1 OT2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CGGTACCCTGCTTTGAGTTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGGAGCCCAGCAGACATG
PXE1 OT3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ATCTCCATGGACAGGTACCG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACCCGATTCTTGCACACAG
PXE1 OT4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCCTCACTGCTGTCTTGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GATGGCACCTGGAAAATCGG
PXE1 OT5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGTGAGAGTCTGCCAGCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTCTGTCAACCTGCCCTCTC
PXE1 OT6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AACCATTAGCCTCTTTTGATAGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CAGGAAAACTCTGCCCTCTT
PXE1 OT7	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGTTCATCGTTGCTGGGGTA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCAGGCAAATTGTCACAGT
PXE1 OT8	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGGCTCAATTGTCCCAGAGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GATGAATGAGGGGCCTGGAT
PXE1 OT9	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TAAACCCCACCCCTCTTCAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGGGAAAGACAGATGCACAA
PXE1 OT10	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCCTCTTGGTCTTAAAGCTTGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GATGAGGGGTCTGGTGTCTT
PXE1 OT11	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GAAATTGACCCTCCCTAGTTACA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AAACAGGAATCAGGGAGGGG
PXE1 OT12	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AACATAGTGATTGACCCTCCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGGATGTGGTGATCTGGTGA
PXE1 OT13	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCATAAGATTTGGGAAGGGACG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CAGCCACTTATTTCTCGCAGT
PXE1 OT14	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AACAGAGGATTTGAGTAGCACA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACTAACAAGCCATCGTCCCA
PXE1 OT15	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGGACTACTGGCAAAAGTTGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCACCAGCTTTGAATTCACACA
PXE1 OT16	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CACATCTGCACAGTCCACAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTCTCTGCCTTCTCCCAGTT
PXE1 OT17	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GTGTGCCAGGGGTTTTAGTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTCCGACTTCAGCAAATCCC
PXE1 OT18	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGACACTTCTGATCTTAAAACT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGATAAAGAAGCAATCATGAGGA
PXE1 OT19	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CAAATGACGCCTTCCCATGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCTGACTTCATCTGAGCTTACA
PXE1 OT20	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GACCGCTGATGACCACACT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCCCTTCGTGTCCTCAAAGA
PXE1 OT21	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCAATTTCTTAGCAACTCTGAGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGCATACATGTGTGAGGAGGT

PXE1 OT22	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGTCATCTCAGTAGCCCAGAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGGATTTGCTTTCTTCTGATGGA
PXE1 OT23	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AAGACTTAAACTTGCACCAATGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCATGTGTTTCTCTCCCTCC
PXE1 OT24	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGGCCATCCTAGTCTTAAAGCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCAGGTGGTCAGTTAGGAA
PXE1 on-target site (humanized mice)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCCATGTGACCCCTCTAACT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CAGGGAAGTCTGGTGGTCA
PXE1 mOT1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGGGCAGCCACAATAAAACA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTCCAGCTTGTTTCTTCATACCA
PXE1 mOT2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACTAACAGACTGGCTACACAAAT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCTTGTTTCTTCATACCATTTGC
PXE1 mOT3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCAATGGACTCAACTCCCCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCCTTTCATTCTGGGGTAGT
PXE1 mOT4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACAGAACTTTCCCCTTGAGCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACCTCAGTCATGCACGGTAA
PXE1 mOT5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGTTGGATAAGTGTCAAAGGGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGTGAGGAGTGAACAGGCTG
PXE1 mOT6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACGGAGACTGGAGATGCTTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GAGCCTCAGTTTCTGCATCG
PXE1 mOT7	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTGGCTACACAAACAGGACC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCAGCTTGTTTCTTCAGACCA
PXE1 mOT8	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGATAGGACATTAGAGGGGCAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GAGCAAGGACTGTCTCTGAC
PXE1 mOT9	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GACCAAGAGCCTCATGAAACC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCCGAGCCAATCAGATTTCT
PXE1 mOT10	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGTCTACACAAACACAACCCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCAGCTTGTTTCTTCAGACCA
PXE1 mOT11	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGCAGCCAACAACTTCATAGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CAGAGCCACCTTCAGACCAT
PXE1 mOT12	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGAGGCCAGACACAGAGATG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AACACAAAGGCCCAATGGTT
PXE1 mOT13	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGCACAGAAATGGACAGCTT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACTGTGATAAGTGTTGCAATGT
PXE1 mOT14	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACAGACAGCTCTATTTCATTGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCAGAACACCAGGGATGTAA
PXE1 mOT15	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCAGATCCTAGGAGTGGGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGTCAACTTTCATTGGACACCA
PXE1 mOT16	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGAGGGAGGAGGAAGATGAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTCCCTCCACGATCTCCAC
PXE1 mOT17	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCAATGATCTCAACTCCCCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCCATCCTTTCATTCCTAGGT
PXE1 mOT18	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGGCTACATAAACAGAACCCAA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCAGCTTGTTTCTTCAGACCA
PXE1 mOT19	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCAGAAGAGGGTGTTGGGTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACATTTGTCAGGGCTGGAGA
PXE1 mOT20	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CAGAACATTGGACCCCGAAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTGAGTGAGCTTGGTTTTGG
PXE1 mOT21	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTGGCTACACAAACAGGACC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCAGCTTGTTTCTTCAGACCA
PXE1 mOT22	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTGGCTACACAAACAGGACAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACCCCAGCTTGTTTCTTCAG
PXE1 mOT23	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACTTGATACAGAACTTTAGTGCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCAGGTACCATTCGCATTCA
PXE1 mOT24	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGGCAGTCCTGTTAGTGAGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGCAGAGGAGGGACAGAAAG

HPD lentiviral screen	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ATCTTGTGGAAAGGACGAAA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTAGCTAGCGAATTCAAAAC
HPD3 on-target site	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACATGGAGGAAGGGGCATTA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGACACCACACACACCCT
HPD3 OT1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGATGCAAACACAGACACGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTGAAAACCACCTCTGCTGC
HPD3 OT2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCTTGGGAAACTCTGCTCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACAGAACCGAATCCAAACCA
HPD3 OT3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCAGAAGATGAAGGAGCAGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTGTTCTTCAGCTCTTCCCG
HPD3 OT4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGCGAAACTCCGTCTCAGAA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AAAACCCTCGCTCCCAACAT
HPD3 OT5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGGCTCTCTTCTGGTATCCG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACACAGCAAGAAGACAGCC
HPD3 OT6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTACCGTTCCCTGTGTCCAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCCTCTCTGCCTTGTTCTCT
HPD3 OT7	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGCCCTCTCAAGTTCTCCAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGTGTCTTGTAGCCGGTTGA
HPD3 OT8	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACTGTCTCTCACACACACACA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGGACAACTTAAGATTACCTGCT
HPD3 OT9	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCCCCTCCCTCCTTTCCATA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGATGCTACTCTTCCCTCAAG
HPD3 OT10	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TTGTCCTCTGATGGCTCACC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGAGGAGAAAGGGCCGATAC
HPD3 OT11	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GTCCCATGTCCTGCTCCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCTCCTCACACACGTACACT
HPD3 OT12	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTAATTTTTCTGATTTGATCACAACACTTTGCA AGAATG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTGCCCTAAGTCTCGTGTGTGTGTGTG
HPD3 OT13	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGGAGCTTCTGGACATTTGAA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGCCTCCCACTAAACCCAA
HPD3 OT14	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GTCAGAATAAGCCACTCATTGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCTGTCGTTATAGAGGGAAC
HPD3 OT15	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CAACCATGTGACTGCAACCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTCTACTGGATGGTGGGCAA
HPD3 OT16	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCTTAGTCGGATTGGGCTTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AAATCGGCCGGCACTTTGAT
HPD3 OT17	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACTTGAGACTGGGTAATTTGTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTTCCCTTTCGCTTCTCTGC
HPD3 OT18	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TTGCTAAATTTACTTATTAGCTCAAGTAGGTTGT GTATCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTCAGTGCTTCTATTCAAAATTTTACTGGAGCTCC C
HPD3 OT19	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGGCAGTGGGATTTCAGGAT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCCTGACCTTTTACCACCA
HPD3 OT20	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACAATAACACAGCTTTGCCAGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGAAATGTCCCATTCTACCTC
HPD3 OT21	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TTCAGAATCCACAGTACACAAGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCACTGTGAATTTCTTGCCCA
HPD3 OT22	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACTTTCATGTAAGGGAGTGAGAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTGGGCTCTCACCTGACACTGAATTTGCC
HPD3 OT23	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGAAGTGTAATCCCCAGACCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCTCCCTTCTGTCTGTCTGG
HPD3 OT24	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACACGTCTTAGTCAGCTCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTACACAGCCACAAGACAGC
HPD3 OT25	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCTAGTTTTCCTGGAGCCCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACAGTGGGTATTAGTGGACAGA
HPD3 OT26	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACTCATCAGAAACTGCTTTGGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGAGAGTGCCAGGAGTGATT

HPD3 OT27	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCATGTCAGTCCTTTGCCCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGAGCAGAAGTTACAGGGAAGA
HPD3 OT28	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AACAGCCAAACACCGCAAAA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGGCCCTGTCATGTTCTTCT
HPD3 OT29	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GTGCAGTCTCTCCTCTCACC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCCTACATATCCTTTTCAGTGCT
HPD3 OT30	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCTTAGTCTGTTCAGGCTGCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTCACCAAATCTGCCAGTGT
HPD3 OT31	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGCCTCTAAAACTGTGAGAAGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGTTTCCAGCTTGCTGACTG
HPD3 OT32	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCAGATTGTACAGGGGAGCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTTCCAGCTGTGCACACTTC
HPD3 OT33	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTCCTTGATTTTAGCTTCCCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACTCTCGAGGATGGTGATTCA
HPD3 OT34	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGGGCTTCCTGTCAATACCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGGCATACACACTGGGAGAC
HPD3 OT35	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGACTTATTCCATTTGAGCTGCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCACTTCTGCCAGCATCTTG
HPD3 OT36	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGGCCAGACTGTGCATATGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACAAGAGATTAAAAGGAACGGGA
HPD3 OT37	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCATGCAAACCAATGAGAAAGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCCTGCTGAATTGGCTCATT
HPD3 OT38	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGGGCTGATACAATGAACTGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACACTGTCTATGAGCCAGAGT
HPD3 OT39	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGGGAGGGATTTCTGCTGAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AAGTGGGGTGACTATTGGCT
HPD3 OT40	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CAGGGAACAGTCTGTCAAACT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGCATTTTCTAGAGCCAAGCA
HPD3 OT41	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCCATTGTCTTGACCCTGCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCCAAATGACTCTAGTACACTCT
HPD3 OT42	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTTCTTGTCAACGTGGAGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCCACACAGAAAGTACATTGT
HPD3 OT43	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGATCACACATTAGCGCATTGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGAACCAACCCTGACAATACC
HPD3 OT44	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TACACTACGGTCAGCCTTGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACACACGTATTAGAAGCTGAA
HPD3 OT45	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACCCATGCAGTTGTTTCCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGTGGCTTTTGTTCTGAGTGT
HPD3 OT46	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ATTAATTCCTGTGGCTGCCG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTTCGGAGGGAGCCAGAC
HPD3 OT47	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CACATTCGTTTCCTAGGGTGAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACATGGGATTTGGAGGGGAC
HPD3 OT48	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTACTCTCTCAGCACAGCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGAGTGGGTGTTATTGCTGC
HPD3 OT49	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CAACCCAAGCCTGTGATCAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTGGAGCCGAGAAACAGTTG
HPD3 OT50	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CACCAGCGCTGTAAGGAAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGCCCTCTCCAGAAACCAAA
HPD4 on-target site	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ATTCTGCCCCAACACACATG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACTACATCGGCCAATTCTTGC
HPD4 OT1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TATGGTTTGCATGCATCCCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTGCACAACTGTCCTGAACC
HPD4 OT2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGATTGTGTCCCTGATGTCGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCCCTGTCACTTCCCATG
HPD4 OT3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGCCCTCTCAAGTTCTCCAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGTGTCTTGTAGCCGGTTGA
HPD4 OT4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CATTAGCATTGTTAAATTGACAATGTTGCAATCT ACTTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCCAAACCTTTTATGCTTCTTTCTCTGATCTGTG

HPD4 OT5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGATGCAAACACAGACACGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTGAAAACCACCTCTGCTGC
HPD4 OT6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCAGAAGATGAAGGAGCAGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTGTTCTTCAGCTCTTCCCG
HPD4 OT7	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCACACAGACACTACACCTGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGTATTAATCTTGATCACCTGGC
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HPD4 OT11	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTGGGATAAGAGGAGGGCAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCATCACTCAGTCCCAACCT
HPD4 OT12	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCATCCTCAAGTCTCATCCCTCAAATTAACAAAC CAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CAGGCATACAGAAAGTGCTCAGTTAACACTCAC
HPD4 OT13	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTGCAGTAACCCAGGAGATG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AAAGAGCCAAGCGTGTGAAG
HPD4 OT14	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGCCTTCTCTCTCTTATTCTCCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACCTGGGAATTATGGGAGCC
HPD4 OT15	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTTCTTGTCAACGTGGAGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCCACACAGAAAGTACATTGT
HPD4 OT16	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTCCTTGATTTTAGCTTCCCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACTCTCGAGGATGGTGATTCA
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HPD4 OT18	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGTCAGCACTGGATTCTGGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGCATCTCTAACCAGGTTCTG
HPD4 OT19	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGAACCACCATGTATTCCCTCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CGTTGCCATTCTGATAAGCCA
HPD4 OT20	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCAAGTGATACCCGTGAATGTTAACTGATCAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CATACAGAAAGTGCTCAGTTAACAGTCACGG
HPD4 OT21	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GATTACTTAGAACTAGTGCCACA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGTTCTCCCTGCATTATTTTACT
HPD4 OT22	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CAACCCAAGCCTGTGATCAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTGGAGCCGAGAAACAGTTG
HPD4 OT23	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGGTCTCTCAGTTACTTGGCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACCACTTTGAACCTCTCTTCTAA
HPD4 OT24	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGATCACATGACTGAGGTAGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACTCCATCCTCAAGAAAGTGG
HPD4 OT25	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGGAATGCTCAACCAGGTGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCTTTCTAAAATAGCCAAGTCCC
HPD4 OT26	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GTTTAGTCCTCCATGCGCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TAGCCCTGGTGCAAAGAGAA
HPD4 OT27	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGACCTGATTTGGGAGATGGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCAGTGCAATTAGTGGCTAAC
HPD4 OT28	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGATTGGTGTAGGGGATGGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACCAACATCCAGAGGCAGAA
HPD4 OT29	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGCAGATGAGGGAACAGAGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACTTGGCCGTAATCCTCGTT
HPD4 OT30	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTTAGCCGCCCAAAGTGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGTTTATGTGAGCAAAAGGAGGA
HPD4 OT31	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TTGAGGCTGTAGTGTGCTTT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCCTAGGGTTCCACAATTACTCA
HPD4 OT32	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCCTTTCCCCATAGTCTACTCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGCTTACCTATCCTGCGTCA
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HPD4 OT34	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGACTTATTCCATTTGAGCTGCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCACTTCTGCCAGCATCTTG
HPD4 OT35	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGGACATACAGCACCTCAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTCTTCATATGCCCAGCCCC
HPD4 OT36	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AACCTTTCTCTCCCCATGGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GAGGACTCACATGTAATAGGGGA
HPD4 OT37	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGAAGACGACATGTGATGCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACTGCAAAGAAGAGGTGAGGA
HPD4 OT38	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGGGCTTCCTGTCAATACCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGGCATACACACTGGGAGAC
HPD4 OT39	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACTATGTGCGTGCCATTGTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTGTTCTGGACCCCTCAAGT
HPD4 OT40	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCTCTGCTCACCATCTCAATCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGGGCTTTCAGTGTCTCAGT
HPD4 OT41	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCCTGCGAGACCATGCTATA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTGTGTCCTGGGAATTGTGC
HPD4 OT42	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGGCCAGACTGTGCATATGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACAAGAGATTAAAAGGAACGGGA
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HPD4 OT52	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCATGTCAGTCCTTTGCCCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGAGCAGAAGTTACAGGGAAGA
HPD5 on-target site	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ATTCTGCCCCAACACACATG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACTACATCGGCCAATTCTTGC
HPD5 OT1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTGCCTCTGTTGATTGCTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CATGATGTGGTATGGCAGTGT
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HPD5 OT12	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCTCACTAAGGCAGAACACACT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTATCAGTCCCAGGCAGCTT
HPD5 OT13	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACACAAGCTCTTAGACTCTTTGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GATGAAGTCGGCCCTGAGAG
HPD5 OT14	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCCTCTCTTTACATCATTTTGCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGGGCTCCTCTGACTCTAAG
HPD5 OT15	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GTGAGCCAAGATGCCGTTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTACTTCCTTACACCTCTGATGG
HPD5 OT16	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGTAGACCCATCCCCTCTCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TACTCTGGTCAAGCTCAGCC
HPD5 OT17	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACAATAACACAGCTTTGCCAGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATGAAATGTCCCATTCTACCTC
HPD5 OT18	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ATCCCCACCAACTCCATCAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACCAGGGTGAAGCAGGAT
HPD5 OT19	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGGGACTGTGTAATTGTTGGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTTTGTTGTGCATTCCTTGACT
HPD5 OT20	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACACCCGGCCTAAGTCATC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGTAGCCGGGTTAAAGTCCA
HPD5 OT21	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTGGGATAAGAGGAGGGCAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCATCACTCAGTCCCAACCT
HPD5 OT22	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GATTACCAAGTGCCAAGAACT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CAGCCTTCTTCCGTCTCAGA
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HPD5 OT26	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGAGGGAAGTGGCCAAGATG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTTCCCTTGGCTCCATGTTG
HPD5 OT27	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCTTAGTTTATTCAGTTGCTGCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGGACCCAGTAAGAAGGCAC
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HPD5 OT31	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCCAAGACGAGTGAGACAGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATCTGCTCTGGGAAGGCTTC
HPD5 OT32	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGTGTCGAATTTCTGGCCTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTTCTCTGAGCCTAGCCCAC
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HPD5 OT35	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCCTTTCCCCATAGTCTACTCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGCTTACCTATCCTGCGTCA
HPD5 OT36	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTGGCCAATGACTGAGCATG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTTCAGTGATGCAAGCCTGG
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HPD5 OT38	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGCCCTCTCAAGTTCTCCAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGTGTCTTGTAGCCGGTTGA
HPD5 OT39	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CAAGGAAGCATCATGGCCTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTTTAACATGTAGCTACTTTGGG

HPD5 OT40	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCCAATGAGCCCAACTTTCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCAAAGACTGCCCATCTCCA
HPD5 OT41	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TTACCCCACTGACTTTGCCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTCTATGGTCAGCTGGGTCC
HPD5 OT42	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACTCTCCAAACACAGTCTTCCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCTCCTTTTACCTGCCCCAA
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HPD5 OT44	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AACTAAGTCTCAGCTCTCGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGACCAGTTCTGTAATGCGT
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HPD5 OT46	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGGGAAATACACACTGAAGGAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACATTCCCTGCTTTGCCTT
HPD5 OT47	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GACGAGTTTGGGTCCGCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTTCTAAGAGCCGTGACCCA
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HPD5 OT50	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCCAAGAACTCCAGTCCCAA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCTGGCCCATCATGTGAATG
HPD5 OT51	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTTTCCATCACCGTTGCAA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGCAAATCACCTCCAACCTC
HPD5 OT52	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGTGACCTGCCGAATTCTCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGTGGAAAGCAACAGGTCAC
HPD20 on-target site	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCAAGGGTTCTGGAAGGCTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CAGCACAGCAAACTTCACCT
HPD20 OT1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACTTGGAATGAGGGGCGAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGGAGAAGGCAGTGTTGTCA
HPD20 OT2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGTTTCTGAGCTGCGTCTTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCCCCATCCACATCTCACTA
HPD20 OT3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CAGAACTAACTGCTCCGCG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATAGGACAGTCAAGGGCTCG
HPD20 OT4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CAGTGACAGGGGTGGGATAT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTCCACTCCTCAGCTCTTGT
HPD20 OT5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CAAGAAGGTCACTATGCCGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCCCAACACAAAAGAGTAGAGAG
HPD20 OT6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AAGACTCTAGGGCACAGCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGGTATAGGTGGGTGGGAGT
HPD20 OT7	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCCTGCCTCCTTCCCAAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GATTGCAGATGTCACACCCA
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HPD20 OT10	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGTCCTTAGCAGCACATGGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCTTCCTCTGCCTGTTCCTT
HPD20 OT11	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CGCAGGTGAGGTAAAGACAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTCAGACCCCAGCCTGTG
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HPD20 OT13	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTGCAAAGGAGAGGCTTAGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGGGGAAAGGGTGGTGTTT
HPD20 OT14	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CGAGCAAATCTTCGGGCAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCAGGTTGTCCCCGTAGAG
HPD20 OT15	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGAGCTGATTGAAAGTGATGTCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGTTATAGCCCGGAATGCCT
HPD20 OT16	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTGAATGGGGAATGTGCCG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCCTCCTAGCTGTCCTTTGA

HPD20 OT17	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CGAAGATCATGCCACCGTAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCACTTGATTGTTGTTTTGGA
HPD20 OT18	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGCCATTCTTTCACATTGCTATA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCCTTGTGATGCCCCTATTC
HPD20 OT19	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CAGTGGGGAGTTGTCAGCAA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCTCCTCTGCCTCTCTAGCT
HPD20 OT20	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGCTGGGGATGTTACTAAGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGGAGGGGAAGATGAAAGCC
HPD20 OT21	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GAGGTGGAGGTTGCAATGAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGGCATACTCGTCCACATGA
HPD20 OT22	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACTCTTCTGCAATCACCTGAGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGAGGCGGAGATTGCTATGA
HPD20 OT23	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GTCACGCCAAATCCAACCTT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCTATGGGCGTGATGAGAAC
HPD20 OT24	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CGAGGTTATGAAGAGCCCCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCAACACCACCCTTACTCCT
HPD20 OT25	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CAGAGCCACCACGTTTTCAT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACTTGGTGTCATGGAGGGG
HPD20 OT26	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGGCCCCAGAAATTCAAAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TACTGTCTTCCTGCTCCGTG
HPD20 OT27	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGTGAGGACTTGAAACAGCG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATTGAAACGTCCGGGTGAGA
HPD20 OT28	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGCCATTGTTTCTGCTCAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGAGCCCTACACAGTCCTG
HPD20 OT29	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ATGATGTGTAAATGCTTGTGTGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGCAAAATTCCCTATCCAAATGC
HPD20 OT30	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GAACCATGCAGCAGTCACAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCCTCACCCTTCACCCTAAT
HPD20 OT31	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGCTCAGAGTCCCCATTCTT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTTGGCAAGCGGGTCCAG
HPD20 OT32	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AAGCGAGAGAGAAGCACAGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTGCTCTGGATGATGGGGAA
HPD20 OT33	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CGCTCCTAAAATCCCATCGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGAGGTGAAGGAGATACGCT
HPD20 OT34	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GAGACACTCAATCTGCTGGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCCTCTGACATGCTTTAATACCA
HPD20 OT35	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTTGGTGTGCTGTGCCCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTGGACAGACATGGAGGGAA
HPD20 OT36	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTCTAGCCTCACCACTCCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGAGGCAGACAGGTGACAAA
HPD20 OT37	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ATCCAGCTCCATTCTCTCCG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGATAATGGTTCAGGTGCTTTCA
HPD20 OT38	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCCGATATATACCACTCAAGGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCTGCTGCAAATGTTTTCTAGG
HPD20 OT39	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCAAAGACAAGGGTGAGGGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTCCTTGTCCTCACCTCTGT
HPD20 OT40	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AACAACACAATCCCACCAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TAGACCTGTGCTTGCCTCTC
HPD20 OT41	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCCGATATATACCACTCAAGGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCTGCTGCAAATGTTTTCTAGG
HPD20 OT42	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACCTCAAATACCATAAAGCCAA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCATGTGTGAGGTTGGGGC
HPD20 OT43	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCTTGCTGTCATGTTTTCAAAGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AAGCAGAACAAACCCCACCT
HPD20 OT44	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCTCCTGAAAGTCAAAGTGGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AACAACTCAACTGATTCATGA
HPD20 OT45	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCCGATATATACCACTCAAGGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCTGCTGCAAATGTTTTCTAGG
HPD20 OT46	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTTCGCTGCTGAGTTCACG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCTGGTTTCATGGCTCTTCC

HPD20 OT47	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GACCTTGTGCCCATTGACAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GATGGATGGGGATGTGGTGA
HPD20 OT48	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGGAGGGACATGAGGCTAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACCCGGAAAGCACCTACTA
HPD20 OT49	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TAGACTGGGCCTCTGGTCAA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTGTACTTGTGTCTGTGCCC
HPD20 OT50	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CGATGATGATGCTGTTCCCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGAGCTGGCTGTACTTGAGG
HPD20 OT51	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGCATGCCTTTTGTATTAGTCCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCCATGTAAGATGCCTGCTC
HPD20 OT52	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ATGGACTCCGCATTAACCCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACCCCTAGTATCCCTGGTGT
HPD20 OT53	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTGAGCTTGAGGGGAAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CATCCGTTGTCTTCCGAGC
HPD20 OT54	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTGGCATGGCTCTGAGGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGGATAAGTGAGTGTCCCCA
HPD20 OT55	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTGAGCTTGAGGGGAAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CATCCGTTGTCTTCCGAGC
HPD20 OT56	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CGTGGCCAAGTTGCTTATCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGGGTTTCTTCTCAGCCTGT
HPD20 OT57	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GAAGTGGCTGCCTAGATGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTCATGGGTAGTGGGCTCAG
HPD20 OT58	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGGAAGCCAAACTCCGAATTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGACAGCTGAGATCACATCG
HPD20 OT59	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGCATGCTTCTTCCATTTATCCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGGGCTGGGTACATTGTTACA
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HPD20 OT61	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTCCAAGGGACAGAGACCAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCATGACACCAAGCCCTCTT
HPD20 OT62	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCGGTTCCTGGTAGTGACTA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATCCCAGAATCCCCAACAGG
HPD20 OT63	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTTAAAGAGCACGAGTAGAAGCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCTGGTTTCTGTGACTATCGCT
HPD20 OT64	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGGCCAGAACTCCACTAGAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACTTAGTTTTCTCCTGGGGCT
HPD20 OT65	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TGAGATACCAGGAAGCAAGCT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCCATTCACCATGTCAGGA
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HPD20 OT68	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCAGCCGAACAAAGGTAGAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGTTACATCAGCCTCAAGAAGC
HPD20 OT69	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTGAGCTTGAGGGGAAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CATCCGTTGTCTTCCGAGC
HPD20 OT70	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TCTGTTGACTAGCACTTCTCCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCTCACGCTCCACTTTGCTA
HPD20 OT71	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCTGCTTGAATGGGGAGAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GAGCTTCAACTCCTCTGTATTCA
HPD20 OT72	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CGGTGTCCCTGTGATTTTCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTGGAAAGAGTCTCGCTGGT
HPD20 OT73	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTGAGCTTGAGGGGAAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CATCCGTTGTCTTCCGAGC
HPD20 OT74	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCCCAGAGTCAGGCAGAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTCCTGAGCCTTGATCCTGG
HPD20 OT75	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGGGATAACAGCTGATGGGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTCAATGGCTTTCTCCTCGC
HPD20 OT76	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGGAATTTGAGACCAGCCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCTCAACCTCCCAATGCTCA

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HPD20 OT78	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACCCCTCTCTATGTCCAGGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCTGCCTCCTGTCTTCTTGG
HPD20 OT79	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CAGCCAAGCATTCTGTACCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TCAAGGAATCTGTGTAAGCTGC
HPD20 OT80	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CATCATTGCAGCCTCAACCA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GTCTGTCTCTACACCGCCC
HPD20 OT81	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACACCTTTTCTTGAACCCTTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATCTCAGTCAAGCCGTTCCT
HPD20 OT82	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTGAGCTTGAGGGGAAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CATCCGTTGTCTTCCGAGC
HPD20 OT83	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGGGTTCTGCTGACCTAAGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AAGCACTCCTCCATCAGCTC
HPD20 OT84	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCCCAGAGTCAGGCAGAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTCCTGAGCCTTGATCCTGG
HPD20 OT85	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ATTCTGAAGGGCCGTGATGA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCACTTCACCCAACCAAATG
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HPD20 OT94	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCCTGCTTTGCTGAGTGAAT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTGGTATTTGACAGGCTGCC
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HPD20 OT100	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACAAGGAGGCTGTGGGAATT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCTCTGATCTGCACGTGATG
HPD20 OT101	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTAGCAAAGCCTCGCATTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGGAGGAGGAGGGTGGTATT
HPD20 OT102	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTGAGCTTGAGGGGAAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CATCCGTTGTCTTCCGAGC
HPD20 OT103	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ACCAAGCAAAGTGTCAGGAT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGAGCCCAATTACACACCCT
HPD20 OT104	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCCAGAATCCAAGGCCCAA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TTGCCTTCAGACACACATGG
PAH4 on-target site	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG ATCTTAAGCTGCTGGGTATTGT	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCCCTTCACTCAAGCCTGT