Adenine Base Editing *in vivo* with a Single Adeno-Associated Virus Vector

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Abstract

Base editors (BEs) have opened new avenues for the treatment of genetic diseases. However, advances in delivery approaches are needed to enable disease targeting of a broad range of tissues and cell types. Adeno-associated virus (AAV) vectors remain one of the most promising delivery vehicles for gene therapies. Currently, most BE/guide combinations and their promoters exceed the packaging limit (~5 kb) of AAVs. Dual-AAV delivery strategies often require high viral doses that impose safety concerns. In this study, we engineered an adenine base editor using a compact Cas9 from *Neisseria meningitidis* (Nme2Cas9). Compared to the well-characterized *Streptococcus pyogenes* Cas9-containing ABEs, Nme2-ABE possesses a distinct PAM (N_4CC) and editing window, exhibits fewer off-target effects, and can efficiently install therapeutically relevant mutations in both human and mouse genomes. Importantly, we show that *in vivo* delivery of Nme2-ABE and its guide RNA by a single-AAV vector can efficiently edit mouse genomic loci and revert the disease mutation and phenotype in an adult mouse model of tyrosinemia. We anticipate that Nme2-ABE, by virtue of its compact size and broad targeting range, will enable a range of therapeutic applications with improved safety and efficacy due in part to packaging in a single-vector system.

Key words: AAV, base editing, genome editing, CRISPR, sgRNA, deaminase

Introduction

Point mutations represent the largest class of known human pathogenic genetic variants [1,2]. Base editors (BEs), which comprise a single-guide RNA (sgRNA) loaded onto a Cas9 (nuclease-inactivated or nickase form) fused to a deaminase enzyme, enable precise installation of A•T to G•C (in the case of adenine base editors (ABEs) [3]) or C•G to T•A (in the case of cytidine base editors (CBEs) [4]) substitutions. In contrast to traditional nuclease-dependent genome editing approaches, base editors do not generate double-stranded DNA breaks (DSBs), do not require a DNA donor template, and are more efficient in editing non-dividing cells, making them attractive agents for *in vivo* therapeutic genome editing.

While robust editing has been achieved in many cultured mammalian cell systems, safe and effective *in vivo* delivery of base editors remains a major challenge. To date, both non-viral and viral delivery methods have shown great promise for delivering base editors for *in vivo* therapeutic purposes in rodents and primates [5,6]. For example, *in vivo* delivery via AAVs has achieved efficient editing in a wide range of tissue and cell types including liver [5–10], heart [11], muscle [12,13], retina [14,15], inner ear [16], and central nervous system (CNS) [17,18]. However, the large coding size (5.2 kb) of the best-characterized *Streptococcus pyogenes* Cas9 (SpyCas9)-containing BEs exceed the packaging limit of AAV (5 kb) [19,20]. Currently, *in vivo* delivery of base editors by AAV has been approached by splitting the SpyCas9 base editor between two AAVs and relying on the use of intein trans-splicing for the assembly of the full-length effector [13,16–18,21,22]. Although effective, this approach requires transduction of the target cell by both AAVs and successful *in trans* splicing of the two intein halves. The requirement to deliver two AAV vectors also increases the viral dosage needed for a treatment, which raises safety concerns and adds burdens to AAV manufacturing [23–25].

Compact Cas9 orthologs are ideal candidates for engineering base editors suitable for single-AAV delivery [26–28]. For example, single-AAV delivery of a domain-inlaid *Staphylococcus aureus* Cas9 (SauCas9) ABE has been reported in cultured HEK293 cells [29]. Previously, we developed a *Neisseria meningitidis* Cas9 (Nme2Cas9) as an *in vivo* genome editing platform [27,30]. Nme2Cas9 is a compact, naturally accurate genome editor with a distinct N_4CC PAM specificity. Recently, two other groups have successfully implemented Nme2-CBEs in cultured mammalian cells and in rabbits [31], as well as Nme2-ABEs in rice [32]. Here, we develop ABEs using Nme2Cas9 (Nme2-ABEs) and define their editing efficiencies, editing windows, and off-target activities in comparison to those of the widely applied Spy-ABEs in cultured mammalian cells. Next, we show that Nme2-ABE can edit multiple therapeutically significant loci, including one of the most common mutations occurring in Rett syndrome patients that cannot be targeted by other compact ABEs (e.g. ABEs derived from SauCas9 and SauCas9-KKH [33–36]) due to PAM restrictions. Lastly, by optimizing the promoter and the nuclear localization signals, we show that Nme2-ABE and its guide can be packaged into a single-AAV vector genome for *in vivo* delivery. One systematic administration of the single-AAV vector encoding both Nme2-ABE and sgRNA readily corrects the disease-causing mutation and phenotype in an adult mouse model of hereditary tyrosinemia type 1 (HT1).

Results

Development of Nme2-ABE and comparison of editing windows and off-target effects to those of Spy-ABE

First, to evaluate base editing efficiency in a streamlined manner, we developed an ABE reporter cell line in which a G-to-A mutation in the mCherry coding sequence generates a nonsense mutation. Adenine base editing can reverse the mutation and recover red fluorescence, and the editing efficiency can be readily measured by fluorescence-activated cell sorting (FACS) (**Figure 1a, Supplementary Figure 1**). Initially, we constructed Nme2-ABE7.10 by linking a TadA-TadA7.10 dimer from the Spy-ABE7.10 to the N-terminus of the Nme2Cas9 HNH nickase [3]. However, by plasmid transient transfection, Nme2-ABE7.10 showed poor activity in the ABE reporter cell line. Because the evolved TadA8e is highly active and compatible with a wide range of Cas9s [35], we next engineered Nme2-ABE8e by linking TadA8e monomer to the N-terminus of the Nme2Cas9 HNH nickase (**Figure 1b**). We found that Nme2-ABE8e supports robust editing activity in the ABE reporter cell line (**Figure 1c**). Next, to define the editing window and editing efficiency of Nme2-ABE8e, and to compare them to those parameters of Spy-ABE7.10 and Spy-ABE8e, we transfected HEK293T cells with plasmids expressing each ABE along with sgRNAs targeting 12 human genomic loci for Nme2-ABE8e [including eight dual-target sites (target sites followed by NGGNCC PAMs for both SpyCas9 and Nme2Cas9) [30] and four Nme2Cas9-specific target sites]. We found that this first-generation Nme2-ABE8e has a broad but shallow editing window that, due to modest activity, was difficult to define clearly (**Figure 1d**).

We next sought to understand the potential extent of off-target editing by Nme2-ABE8e. It has been shown that the major source of DNA off-target base editing is Cas9-dependent [37,38], caused by Cas9 binding and unwinding at near-cognate sequences. Because Nme2Cas9 is highly accurate during nuclease-driven editing in cells and *in vivo* [27,30,39], we hypothesized that Nme2-ABE8e would exhibit similar accuracy advantages relative to Spy-ABE8e. As an initial test, we systematically investigated the tolerance for nucleotide mismatches between the guide and the target sequence for the two effectors. We designed a panel of guides targeting the ABE reporter with single- and di-nucleotide mismatches with the target sequence for both Nme2-ABE8e and Spy-ABE8e and measured their activities by plasmid transfection and FACS (**Figure 1e**). Considering the differences in on-target efficiencies between the two effectors, we further normalized the activities of the mismatched guides to those of the perfectly complementary guides for each effector. We found that Nme2-ABE8e exhibited significantly lower off-target editing propensity than Spy-ABE8e: while single-nucleotide mismatches in the seed region (guide nucleotide positions 17-24 for Nme2Cas9, and 10-20 for SpCas9) and the majority of dinucleotide mismatches significantly compromised the editing efficiency of Nme2-ABE8e, these near-cognate sequences were mostly efficiently edited by Spy-ABE8e (**Figure 1e**).

Inhibition of base editing by anti-CRISPR proteins that limit DNA binding activity

The development of Nme2Cas9 base editing platforms raises the possibility that regulation strategies developed for nuclease-based editing could be similarly implemented in the case of base editing. One such strategy is the use of anti-CRISPR (Acr) proteins that limit Cas9 DNA-binding activity [40–43], which have been deployed to reduce both off-target [44] and off-tissue editing [45]. AcrIIC3 and AcrIIC4 have been reported to reduce Nme2Cas9 DNA binding activity [41,43,46] but have no effect on SpyCas9 [41,43]. To test whether such Acr proteins can

146 function as off-switches for Nme2-ABE8e base editing, we co-transfected the ABE reporter cell
147 line described above with plasmids expressing Nme2-ABE8e, sgRNA, and Acr proteins. Spy-
148 ABE7.10 and AcrIIA4 (an anti-CRISPR that prevents SpyCas9 DNA binding [40], nuclease
149 editing [40,44], and base editing [47]) was used as a positive control. Conversely, AcrE2, which is
150 a Type I-E Acr [43] that has no effect on SpyCas9 or Nme2Cas9 activity, was used as a negative
151 control. As expected, Spy-ABE7.10 base editing was reduced to background levels by AcrIIA4,
152 but AcrE2, AcrIIC3, and AcrIIC4 had no effect (**Supplementary Figure 3a**). By contrast,
153 Nme2-ABE8e editing was strongly inhibited by AcrIIC3 and AcrIIC4, but AcrE2 and AcrIIA4
154 had no effect. These results confirm that these anti-CRISPRs can be effective off-switches for
155 Nme2-ABE8e editing.
156
157 Tissue-specific miRNAs, in combination with miRNA response elements [MREs] in the 3'UTR
158 of an Acr construct, have been used to restrict editing to cell types that express such miRNAs,
159 both in cultured cells [41,48] and *in vivo* [45]. To determine whether such a strategy could be
160 used to control Nme2-ABE8e, we inserted miR-122 MREs into the 3'UTRs of our AcrIIC3 and
161 AcrIIC4 constructs. Mir-122 is a hepatocyte-specific miRNA that is expressed in Huh7 cells but
162 not HEK293 cells, and has been used to validate this strategy for nuclease-based editing. Again,
163 AcrIIC3 and AcrIIC4 inhibited Nme2-ABE8e activity at an endogenous site in HEK293T cells,
164 and inhibition largely persisted even with the MREs (**Supplementary Figure 3b**), as expected
165 since miR-122 is not present to silence Acr expression. In Huh7 cells, which are transfected with
166 lower efficiencies, AcrIIC3 and AcrIIC4 again inhibited Nme2-ABE8e, but this inhibition of
167 editing activity was largely relieved by the insertion of the MRE122 sites. These results indicate
168 that miRNA-repressible anti-CRISPRs can be used to enforce the cell-type specificity of base
169 editing, as it can for nuclease editing.
170
171 **Installation of therapeutically relevant edits with Nme2-ABE8e**
172 We next tested the potential of Nme2-ABE8e to correct pathogenic mutations or to introduce
173 disease-suppressing mutations. One of the most common mutations that cause Rett syndrome is
174 a C•G to T•A base transition that produces a nonsense mutation in the human *MeCP2* gene
175 (c.502 C>T; p.R168X) [49–51]. Because the target adenine is within a cytidine-rich region, this
176 mutation is not readily accessible for ABEs based on other compact Cas9s such as SauCas9 and
177 SauCas9-KKH [33–36]. To test whether Nme2-ABE8e can correct this pathogenic mutation, we
178 electroporated Nme2-ABE8e mRNA with a synthetic sgRNA into a Rett syndrome patient-
179 derived fibroblast cell line that possesses this mutation. By amplicon deep sequencing, we found
180 that Nme2-ABE8e successfully edited the target adenine (A10) (**Figure 2a, 2b**). A bystander edit
181 at an upstream adenine (A16) causes a missense mutation (c.496 T>C; p.S166P), although this
182 occurs with only one-fourth the frequency of intended edit at A10. Because S166 has been shown
183 to be subject to phosphorylation in mice and is conserved from *X. laevis* to humans [52], further
184 investigation will be needed to determine whether bystander editing at A16 impairs functional
185 rescue of edited cells. However, as with other base editors [3,29,53–58], future protein
186 engineering efforts adjusting the editing window promise to provide greater control over Nme2-
187 ABE editing outcomes.
188
189 Next, we sought to generate a disease-suppressing mutation that has been shown to reverse
190 phenotypes of a validated Duchenne muscular dystrophy (DMD) mouse model (ΔEx51) [59].
191 The ΔEx51 mouse model was generated by deletion of the exon 51 in the *Dmd* gene, resulting in
192 a downstream premature stop codon in exon 52, causing the production of a nonfunctional

193 truncated dystrophin protein. Previously, it has been shown that the *Dmd* reading frame can be
194 restored by skipping exon 50 by adenine base editing (**Figure 2c**) [13]. However, *in vivo* base
195 editing using ABE_{max}-SpCas9-NG delivered by dual-AAV vectors was limited to local muscle
196 injection due to the high viral dosage required to achieve therapeutic benefit. We identified a
197 guide design for Nme2-ABE8e to target the adenine (A7) within the splice donor site downstream
198 of exon 50 (**Figure 2c**). By plasmid transfection in the mouse N2a cell line and amplicon deep
199 sequencing, we found that Nme2-ABE8e can generate 17.67 ± 4.57% editing at A7 (**Figure 2d**).
200 The efficient editing at multiple bystander adenines is not a concern in this case as those adenines
201 are within the skipped exon 50 or the intron.
202
203 **Optimization of an Nme2-ABE8e construct for single AAV delivery**
204 Previously, we showed that Nme2Cas9 with one or two sgRNAs can be packaged into a single
205 AAV vector and support efficient editing *in vivo* [27,30]. We reasoned, based upon the compact
206 sizes of Nme2Cas9 and TadA8e, that Nme2-ABE8e with a sgRNA could be packaged into a
207 single AAV for *in vivo* delivery. To achieve this, we first replaced NmeCas9 with Nme2-ABE8e in
208 the minimized all-in-one AAV vector reported previously [27]. We attached one cMyc NLS
209 sequence on each terminus of Nme2-ABE8e while retaining the original promoters for effector
210 and sgRNA expression (**Figure 3a,** 2x cMyc). By transient transfection of vector backbone
211 plasmids, the single-AAV construct successfully edited the ABE reporter cell line. To further
212 improve Nme2-ABE editing efficiency, we then tested three different NLS configurations: 1) one
213 cMyc NLS on the N-terminus and two cMyc NLSs on the C-terminus (3x cMyc); 2) one Ty1
214 NLS, which derived from the yeast Ty1 retrotransposon that supports robust nuclear localization
215 in dPspCas13b fusion proteins, on the N-terminus (Ty1) [60]; and 3) one bipartite SV40 NLS
216 (BP_SV40) on each terminus (2x BP_SV40) (**Figure 3a**) [61]. When transfecting the vector
217 plasmid into the ABE reporter cell line, the 2x BP_SV40 construct showed the highest editing
218 efficiency (**Figure 3b**).
219
220 The total length of the vector constructed with the 2xBP_SV40 NLS, hereafter Nme2-ABE8e-
221 U6, is 4998 bp, very close to the packaging limit of AAV. To test whether we could further
222 reduce the vector size without significantly compromising editing efficiency, we turned to a
223 recently reported "miniU6" promoter that has been shown to support sgRNA expression and
224 achieve comparable editing efficiencies as with the complete U6 promoter [62]. Upon
225 replacement of the U6 promoter with miniU6 promoter, the vector, hereafter Nme2-ABE8e-
226 miniU6, was shortened to 4860 bp, within the packaging limit of AAV (**Figure 3c**). Both
227 constructs induced robust editing in the ABE reporter cell line via transient transfection of the
228 vector plasmids (**Figure 3c**).
229
230 To avoid potential ABE reporter-specific effects, we further tested both single-AAV vector
231 backbone plasmids at two endogenous target sites: 1) one of the human dual-target sites, DS12,
232 and 2) a previously reported Nme2Cas9 target site in the mouse *Rosa26* gene [45]. By plasmid
233 transfection in human HEK293T or mouse N2a cells, we observed significant editing at these
234 loci by both vectors, although the Nme2-ABE8e-miniU6 vector was somewhat less efficient
235 (**Figure 3c**). We thus chose both vector designs for the subsequent *in vivo* study.
236
237 **Hydrodynamic injection of single-AAV vector plasmids corrects the disease**
238 **mutation and phenotype in an adult mouse model of HT1**

239 To test the *in vivo* editing efficiency and therapeutic potential of the single-AAV constructs, we
240 chose to target a pathogenic mutation associated with the liver disease HT1. HT1 is caused by
241 mutations in fumarylacetoacetate hydrolase (FAH), which catalyzes one step of the tyrosine
242 catabolic pathway. FAH deficiency leads to accumulations of toxic fumarylacetoacetate and
243 succinyl acetoacetate, causing liver, kidney, and CNS damage [63]. The *Fah*$^{PM/PM}$ mouse model
244 possesses a G•C to A•T point mutation in the last nucleotide of exon 8, which causes skipping of
245 exon 8 and FAH deficiency (**Figure 4a**). Without treatment, FAH deficient mice will rapidly
246 lose weight and eventually die. The *Fah*$^{PM/PM}$ mouse can be treated with 2-(2-nitro-4-
247 trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC), an inhibitor of an enzyme upstream
248 within the tyrosine degradation pathway, which prevents toxin accumulation [64]. Previously, we
249 and others have tested various *in vivo* gene-editing tools to treat the *Fah*$^{PM/PM}$ mouse model,
250 including Cas9-directed HDR [27,65,66], base editing [67,68], microhomology-directed end
251 joining [69], and prime editing [70]. Among these, multiple approaches including AAV, lipid
252 nanoparticle (LNP), and plasmid hydrodynamic tail-vein injection have been used to deliver the
253 gene-editing agents into this mouse model. However, caveats should be considered when
254 comparing the efficiencies of different gene-editing strategies: only the initial editing efficiency
255 (measured before NTBC withdrawal) reflects the activity of the gene-editing agents, because after
256 NTBC withdraw, the hepatocytes in which *Fah* has been repaired will show clonal expansion
257 over time due to their survival advantage.
258
259 We first validated a guide targeting the point mutation by electroporation of the single-AAV
260 vector plasmids, either Nme2-ABE8e-U6 or Nme2-ABE8e-miniU6, into mouse embryonic
261 fibroblasts (MEFs) isolated from a *Fah*$^{PM/PM}$ mouse (**Figure 4b**). We detected modest but
262 significant editing by both vectors at the target adenine at position 13 (A13), despite low (~12%)
263 plasmid electroporation efficiencies. We also observed higher levels of bystander editing at A16
264 with both vectors, and a lower level of bystander editing at A10 for Nme2-ABE8e-U6. Bystander
265 editing at A10 (which changes an active-site-proximal serine into an alanine) has been observed
266 previously with Spy-ABE as well [67]. The effect (if any) of the intronic A16 edit on intron 8
267 splice donor activity has not been defined (**Figure 4c**).
268
269 To test our single-AAV vectors of Nme2-ABE8e *in vivo*, we first performed hydrodynamic tail-
270 vein injections of the AAV-vector plasmids into 10-week-old HT1 mice, or PBS injection as a
271 negative control group [71]. We also injected plasmids expressing Spy-RA6.3, which is a codon-
272 optimized Spy-ABE, as a positive control [67]. Seven days post-injection, we sacrificed 2 mice
273 from each experimental group, and 1 mouse from each control group, to measure the editing
274 efficiency before hepatocyte expansion. We then withdrew NTBC for the rest of the mice for
275 long-term phenotypic study**.** Before NTBC withdrawal, anti-FAH immunohistochemistry (IHC)
276 staining showed 4.58 ± 1.1% FAH^{+} hepatocytes from the group that was injected with the
277 Nme2-ABE8e-U6 plasmid, and 1.71 ± 0.49% from the group injected with the Nme2-ABE8e-
278 miniU6 plasmid (**Figure 4d**). The mouse injected with Spy-RA6.3 plasmid showed 4.5% FAH^{+}
279 hepatocytes, consistent with previously reported data [67] (**Figure 4d**). After NTBC withdrawal,
280 we monitored body weight changes. The PBS injected mice rapidly lost body weight after NTBC
281 withdrawal and were euthanized. By contrast, mice injected with either the Nme2-ABE8e-U6 or
282 Nme2-ABE8e-miniU6 plasmid gradually gained body weight, suggesting rescue of the
283 pathological phenotype (**Figure 4e)**. Forty days after NTBC withdrawal, we sacrificed mice
284 from all surviving groups. To determine whether Nme2-ABE8e successfully corrects the *Fah*
285 splicing defect, we extracted total RNA from the livers and performed reverse transcription PCR

286 (RT-PCR) using primers that spanned exons 5 and 9. By contrast to the PBS-injected mice,
287 which only showed a 305 bp PCR product corresponding to the truncated mRNA lacking exon
288 8, we observed that the 405 bp PCR product (containing exon 8) predominated in the Nme2-
289 ABE treated mice (**Figure 4f**). Sanger sequencing of the 405 bp bands further confirmed the
290 presence of the corrected G residue at the end of exon 8 (**Figure 4g**). When performing anti-
291 FAH IHC staining, we observed expansion of FAH^+ hepatocytes in the groups that were injected
292 with either of the single-AAV vector plasmids (**Figure 4h**). Amplicon deep sequencing of
293 genomic DNA from the livers of treated mice again provided evidence for Nme2-ABE activity
294 (**Figure 4i**). By contrast to the efficiencies achieved in the MEF cells, we observed a lower
295 editing at A16 and no significant editing at A10, likely due to partial (A16) or complete (A10)
296 selection against mice harboring bystander edits at those positions. These data indicate that our
297 Nme2-ABE8e single-AAV vector plasmids can correct the disease genotype and phenotype of the
298 *Fah*$^{PM/PM}$ mice *in vivo*.
299
300 ***In vivo* base editing by single AAV delivered Nme2-ABE8e in the *Fah*$^{PM/PM}$ mice**
301 Encouraged by the initial results, we packaged AAV9 with the Nme2-ABE8e-U6 construct, as
302 well as the Nme2-ABE8e-miniU6 construct, considering that the relatively smaller size of the
303 latter may potentially benefit packaging efficiency (**Figure 5a**). However, both constructs
304 yielded similar vector titers. Next, to confirm the AAV genome integrity, we performed AAV
305 genomic DNA extraction and alkaline gel electrophoresis. We did not observe any sign of
306 genome truncation (**Supplemental Figure 4**). We then tail-vein injected 8-week-old *Fah*$^{PM/PM}$
307 mice at a dosage of 4×10^{11} vg per mouse. We kept the mice on NTBC for one month before
308 analyzing the editing efficiency. One month after AAV injection and before NTBC withdrawal,
309 we sacrificed the mice and performed IHC staining using an anti-FAH antibody. The negative
310 control groups injected with AAV9 expressing Nme2-ABE and a sgRNA targeting the *Rosa26*
311 gene did not show any FAH^+ hepatocytes. In contrast, we observed $6.49 \pm 2.08\%$ FAH^+
312 hepatocytes in the AAV9-Nme2-ABE8e-U6-*Fah* treated group, and $1.62 \pm 0.49\%$ FAH^+
313 hepatocytes in AAV9-Nme2-ABE8e-miniU6-*Fah* treated group (**Figure 5b**). Because repair of
314 1/100,000 hepatocytes was reported to rescue the phenotype, both AAV constructs achieved
315 efficiencies that were well above the therapeutic threshold [72]. Moreover, the percentage of
316 edited hepatocytes by AAV9-Nme2-ABE8e-U6-*Fah* was higher than what has been reported
317 previously by other genome editing strategies [65–67,70]. By targeted deep sequencing, the
318 editing efficiency at the target adenine (A13) in the AAV9-Nme2-ABE8e-U6 treated group is
319 $0.34 \pm 0.14\%$, while no significant editing was observed in the AAV9-Nme2-ABE8e-miniU6
320 treated group, possibly due to low efficiency that was below the detection limit of amplicon deep
321 sequencing (**Figure 5c**). The reason for the higher frequency of FAH^+ hepatocytes than the
322 frequency of editing at the DNA level is likely due to hepatocyte polyploidy [73], as well as the
323 presence of genomic DNA from nonparenchymal cells. Similar distinctions in FAH^+ frequencies
324 and genomic readouts were also observed in previous studies using this mouse model [67,70]. We
325 also measured the editing efficiency at the *Rosa26* locus and observed ~5% editing efficiency at
326 the target site, indicating that the efficiency of AAV9-delivered Nme2-ABE8e is target site
327 dependent, and higher-efficiency sites can be identified (**Figure 5d**). We did not detect any
328 above-background level of indel, indicating that single-AAV delivered Nme2-ABE8e can install
329 precise editing *in vivo* without generating unwanted indels (**Figure 5c, d**).
330
331 Nme2Cas9 is known to be highly accurate in nuclease based editing in cells [27,30,39], and our
332 mismatch scanning experiments (**Figure 1e**) strongly suggest that the same will be true *in vivo*.

333 To evaluate potential Cas9-dependent off-target effects in the AAV9-injected mice, we searched
334 for genome-wide off-target sites for Nme2Cas9 using Cas-OFFinder [74], allowing for up to 6
335 mismatches. We then performed amplicon deep sequencing in the AAV9 treated livers at the two
336 top-ranking potential off-target sites, each including 5 mismatches. We did not detect any above-
337 background A•T-to-G•C editing at these sites **(Supplementary Figure 5)**.
338

339 **Optimizing effector and sgRNA arrangement improves editing efficiency by AAV**
340 **delivery**

341 Previous studies have shown that effector and sgRNA placement and orientation within the AAV
342 genome can affect transgene expression levels and editing efficiencies [18, 26, 85]. To test if
343 different arrangements of sgRNA and effector cassettes in the Nme2-ABE8e all-in-one AAV
344 construct can further increase *in vivo* editing efficiency, we moved the U6-sgRNA cassette to the
345 3' end of the AAV genome and reversed its orientation, similar to the optimal arrangement
346 reported by Fry *et al.* [85] (**Figure 6a**). Using the same *Rosa26* guide described above (**Figure**
347 **5d**), we packaged the rearranged construct in AAV9 capsids and performed tail-vein injections in
348 8-week-old mice. We observed significantly improved editing efficiency (34 ± 11.6%) with the
349 optimized construct (**Figure 6b**), which is significantly greater than the 4.7 ± 0.94% efficiency
350 with the original configuration, and comparable to the editing efficiency achieved previously by
351 dual-AAV delivered split-intein SpyCas9-ABEmax targeting the *Dnmt1* gene in adult mouse liver
352 (38 ± 2.9%) [18]. We conclude that efficient adenine base editing can be achieved *in vivo* via
353 single-AAV delivery of the Nme2-ABE8e system.
354

355 **Discussion**
356
357 Rapidly evolving and precise genome editing tools such as base editors and prime editors possess
358 great potential to address the root causes of human genetic diseases [2,75,76]. However, the safe
359 and effective delivery of genome editors remains a major challenge. Viral delivery using AAV,
360 which is the only FDA-approved *in vivo* gene therapy vector to date, has a limited packaging
361 capacity. Previous studies using AAV to deliver base editors or prime editors have been reported
362 to be effective in rodents [7,8,10–13,15–18,21], but all of them required multiple vectors and
363 most of them required high viral dosage [7,10–13,17,18]. To date, AAV administered at high
364 dose has been reported to relate to severe toxicity or even death in nonhuman primates, piglets,
365 and humans [23,24,77–79]. Engineering single-AAV deliverable genome editing tools has the
366 potential to achieve therapeutic benefits at lower dosage, which would not only ease
367 manufacturing burdens but also reduce likelihoods of serious adverse events [80,81].
368
369 Engineering new genome editors based on compact Cas9s is an alternative approach to address
370 this issue. Previously, single-AAV delivery of Cas9 nucleases has been reported to generate
371 NHEJ-based editing *in vivo* [26,30,82]. Precision editing via HDR has also been achieved with
372 single-AAV systems, albeit with low efficiencies [27,83]. The only single-AAV BE system
373 reported previously (which was not tested *in vivo*) was based on SauCas9, which has limited
374 targeting range due to its PAM constraints. In this study, we constructed and characterized a
375 compact, accurate adenine base editor, Nme2-ABE8e, which can target many sites that are
376 inaccessible to SauCas9 base editors due to their distinct PAMs. We showed that Nme2-ABE8e
377 with a sgRNA can be packaged into a single AAV vector, and a single intravenous injection in an
378 adult disease mouse model of tyrosinemia reversed both the disease mutation and phenotypes.
379 Furthermore, the dosage used in this study (4×10^{11} per mouse or 2×10^{13} vg/kg) is well below
380 the 1×10^{14} vg/kg systemic doses that have been tolerated in clinical trials [77,78].
381
382 Although the editing efficiency was modest at the *Fah* disease locus, we reached therapeutic
383 thresholds for HT1, and the initial editing efficiency of the AAV9-delivered Nme2-ABE8e-U6
384 construct exceeded that reported previously at this locus using other precision genome editing
385 tools and delivery approaches [65–67,70]. Moreover, by optimizing transgene orientation in the
386 AAV construct, we achieved significantly improved editing efficiency (~5-fold increase) by
387 AAV9-delivered Nme2-ABE8e targeting the *Rosa26* gene, comparable to that achieved
388 previously by dual-AAV delivered split-intein SpyCas9-ABEmax in adult mouse liver targeting
389 another genomic site [18]. Future optimization of this system promises to further improve
390 efficiency. There are multiple potential explanations for the inconsistent editing efficiencies we
391 achieved with our first-generation Nme2Cas9 ABE that suggest directions for future
392 improvement. First, structural analyses of Nme2Cas9 [84] indicate that the position of the N-
393 terminally fused TadA8e domain relative to the predicted path of the displaced strand is not
394 optimal. With other effectors, domain-inlaid deaminase fusions have proven to be advantageous
395 in some contexts [29,54], and the same is likely to be true with Nme2-ABEs. Second, the current
396 Nme2-ABE8e has a wide editing window that could result in increased bystander editing. For
397 example, we observed higher editing at a bystander adenine (A16) at the *Fah* locus compared to
398 the on-target adenine at A13. Optimizations of linker composition and length [3,53], as well as
399 domain-inlaid systems [29,54–58], may confer greater control over the editing window and
400 improved efficiency in editing the intended position.
401

402 Our studies also suggest factors that must be considered for optimal guide expression. In contrast
403 to a previous study that showed no significant difference between the U6 and miniU6 promoters
404 in supporting Cas9 editing efficiency [62], we found that the construct with the miniU6 promoter
405 was consistently less efficient than that with the U6 promoter, including via AAV delivery.
406 Because the previous study compared these promoters in T cells by lentivirus transduction, our
407 observations may only apply to certain delivery strategies and cellular contexts.
408
409 In summary, we have engineered and characterized Nme2-ABE8e editing in mammalian cell
410 culture and achieved efficient *in vivo* editing by delivery of a single AAV vector. To our
411 knowledge, this is the first single-AAV-delivered *in vivo* base editing reported to date [29]. We
412 anticipate that Nme2-ABE8e, with its distinct PAM specificity, editing window, and high
413 accuracy, will provide additional targetability, safety, and therapeutic potential for genome
414 engineering applications.
415

Methods

Cell culture. HEK293T cells (ATCC CRL-3216), ABE reporter cells, MEF cells, and mouse N2a cells (ATCC CCL-131) were cultured in in Dulbecco's Modified Eagle Media (DMEM, Genesee Scientific Cat. #: 25-500) supplemented with 10% Fetal Bovine Serum (Gibco Cat. #: 26140079). Rett syndrome human patient-derived fibroblasts (hPDFs) were obtained from the Rett Syndrome Research Trust and cultured with DMEM supplemented with 15% FBS and 1x non-essential amino acids (Gibco Cat. #: 11140050). All cells were incubated in a 37°C incubator with 5% CO_2.

Molecular cloning. To generate the CMV-Nme2-ABE8e and the CMV-Nme2-ABE7.10 plasmids used in **Figure 1**, the Nme2-ABE8e, Nme2-ABE7.10, Spy-ABE8e, and Spy-ABE7.10 constructs were cloned into the pCMV-PE2 vector backbone (Addgene #132775) by Gibson assembly. Briefly, the pCMV-PE2 plasmid was digested with NotI and PmeI restriction enzymes, and the plasmid backbone was then Gibson-assembled with five fragments: N-terminal NLS, TadA8e (for Nme2-ABE8e or Spy-ABE8e), or TadA-TadA*7.10 (for Nme2-ABE7.10 or Spy-ABE7.10), the linker, Nme2Cas9-D16A or SpCas9-D10A nickase, and the C-terminal NLS. The ABE reporter construct was cloned by site-directed mutagenesis to change the 47th amino acid (Glutamine, CAG) of the mCherry coding sequence to a stop codon (TAG). The ABE reporter was further cloned into a lentiviral transfer vector backbone (Addgene #99373) by Gibson assembly. The sgRNA expression plasmids used in **Figure 1** were cloned from pBluescriptSKII (Addene #74705) in two steps. First, pBluescriptSKII was digested by NotI and XbaI, and a gene fragment that contains a U6 promoter, a type-IIS restriction cloning site (BfuAI), and a tracrRNA was assembled into the backbone by Gibson assembly. The plasmids were further digested with BfuAI and ligated to the annealed oligos to insert the guide sequences. The single-AAV vector plasmids in **Figure 3a** were cloned from the Addgene #119924 plasmid by replacing the Nme2Cas9 sequence with the Nme2-ABE8e sequence, and then subsequently re-cloned to encode different NLS configurations and the miniU6 promoter by restriction enzyme digestion and Gibson assembly. The NLS configuration of Nme2-ABE8e mRNA used in **Figure 2b** was 2x_BPSV40, and the plasmid used in **Figure 2d** was the single-AAV Nme2-ABE8e-U6 plasmid shown in **Figure 3a** (2xBP_SV40). To clone the AAV-Nme2-ABE8e_V2 plasmid in **Figure 6**, first, the 2xBP_SV40 plasmid from **Figure 3a** was digested by PmeI and NotI, the AAV backbone fragment was then Gibson-assembled with the fragment containing U1a-Nme2-ABE8e, and the fragment containing U6-sgRNA with homology sequences by overhang PCR. Most of the plasmids used in **Supplementary Figure 3a** were previously deposited in Addgene (AcrIIC3, Addgene #85713; AcrIIC4, Addgene #113434; Acr-E2, Addgene #85677). To clone the plasmid expressing AcrIIA4, Addgene #85713 plasmid was digested with XhoI and BamHI and Gibson-assembled with a gene fragment containing AcrIIA4 coding sequence [46]. In **Supplementary Figure 3b**, the AcrIIC3-MRE122 plasmid was cloned from Addgene plasmid #129531 by replacing the *Rosa26*-targeting guide with the DS12-targeting guide through restriction enzyme cloning. Subsequent replacement of the coding sequence of AcrIIC3 with AcrIIC4 generated the AcrIIC4-MRE122 plasmid. Sequences of plasmids first described in this paper can be found in the **Supplementary note** and will be made available from Addgene.

ABE reporter HEK293T cell line. Lentivirus was produced following instructions from Addgene (https://www.addgene.org/protocols/lentivirus-production/). Briefly, HEK293T cells were transfected with the transfer vector and the packaging plasmids psPAX2 (Addgene #12260)

463 and pMD2.G (Addgene #12259), using Lipofectamine 3000 (ThermoFisher Cat. #: L3000015).
464 Two days later, the medium was collected and filtered through a 0.45 µm filter (Cytiva Cat. #:
465 6780-25040) to remove cell debris. The viral titer was determined using Lenti-X™GoStix™
466 (Takara Bio Cat. #: 631280). HEK293T cells were transduced with lentivirus encoding the ABE
467 reporter at varying dilutions (1:10, 1:100, 1:500, and 1:1000) in the presence of 8 µg/ml
468 polybrene (Millipore Sigma Cat. #: TR-1003-G). Three days after transduction, the media was
469 removed, and fresh media was supplemented with 2 µg/ml Puromycin to select cells expressing
470 the full-length reporter construct. Seven days after selection, the puromycin-resistant cells were
471 collected. Single-cell clones were established by serial dilution in 96-well plates.
472
473 **Fluorescent reporter assay.** Forty-eight hours after transfection, cells were trypsinized and
474 harvested into microcentrifuge tubes. After centrifuging at 300 x g for 3 mins, cells were
475 resuspended into 150ul 1xPBS for flow cytometry analysis (MACSQuant VYB). For each
476 sample, 10,000 events were counted for FACS analysis. Data was analyzed using Flowjo v10.
477 Representative gating strategy can be found in **Supplementary Figure 2**.
478
479 ***In vitro* transcription of Nme2-ABE8e mRNA.** Nme2-ABE8e mRNA was *in vitro*
480 transcribed using the NEB HiScribe T7 RNA synthesis kit, from 500ng of a linearized template.
481 Uridine was fully substituted with 1-methylpseudouridine, and mRNA was capped co-
482 transcriptionally using CleanCap AG analog (TriLink Biotechnologies Cat. #N-1081 and N-
483 7113, respectively). All enzymes were purchased from New England Biolabs. Transcription was
484 conducted according to the manufacturer's protocol with the following amendments:
485 transcriptions were completed in 1× NEB HiScribe transcription buffer, and 4 mM CleanCap
486 AG was used during transcription. Transcription reactions were incubated at 37 °C for 2 hours
487 then treated with 0.4 U/µl DNase I (final concentration) for 15 min at 37 °C. mRNAs were
488 purified with NEB Monarch RNA purification columns and treated with pyrophosphatase for 1
489 hour with 0.25 U/µg Antarctic phosphatase (final concentration) in 1X Antarctic phosphatase
490 buffer. The final product was further purified with a NEB Monarch RNA column and eluted in
491 water.
492
493 **Transfection and electroporation**. For plasmid transfection, cells were seeded in 24-well
494 plates at 80,000 cells per well in culture media and incubated overnight. Briefly, plasmids were
495 transfected at 400ng per well when targeting the ABE reporter, and 1ug per well for the
496 endogenous target sites. Specifically, in **Figure 1c**, **1e**, **2d**, **3b**, **3c**, and **Supplementary**
497 **Figure 3**, plasmids were transfected using Lipofectamine3000 (ThermoFisher Cat #L3000001),
498 while in **Figure 1d** and **Supplementary Figure 2**, plasmids were transfected using
499 Lipofectamine2000 (ThermoFisher Cat #11668030), following the manufacturer's protocols.
500 Electroporation was performed using Neon TransfectionSystem 10 ul kit (ThermoFisher Cat #:
501 MPK 1096), with the following electroporation parameters: Pulse voltage (1650 v), Pulse width
502 (20 ms), Pulse number (1). Specifically, in **Figure 2a**, 264 ng Nme2-ABE8e mRNA and 100
503 pmole of sgRNA were electroporated into 50,000 Rett Syndrome hPDFs, while in **Figure 4c**, 1
504 ug plasmid DNA was electroporated into 100,000 MEF cells.
505
506 **AAV production**. AAV vector packaging was done at the Viral Vector Core of the Horae Gene
507 Therapy Center at the UMass Chan Medical School as previously described [86]. Constructs
508 were packaged in AAV9 capsids and viral titers were determined by digital droplet PCR and gel
509 electrophoresis followed by silver staining.

510
511 **AAV genomic DNA extraction and alkaline agarose gel electrophoresis.** Genomic
512 DNAs were extracted from 10^{11} vg AAV by incubating with 20 units of DNase I (ThermoFisher
513 Cat. #: EN0521) at 37 °C for 30 minutes and then with an equal volume of 2 x Pronase solution
514 (Sigma-Aldrich, Cat. # 10165921001) for 4 hours. The genomic DNA was subsequently purified
515 by phenol-chloroform (ThermoFisher Cat. #:15593-049) extraction and ethanol precipitation.
516 DNA pellets were resuspended in water and analyzed by alkaline agarose gel electrophoresis and
517 SYBR Gold staining.
518
519 **Mouse experiments.** All animal study protocols were approved by the Institutional Animal
520 Care and Use Committee (IACUC) at UMass Chan Medical School. The *Fah*$^{PM/PM}$ mice were
521 kept on water supplemented with 10 mg/L 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-
522 cyclohexanedione (NTBC; Sigma-Aldrich, Cat. #: PHR1731-1G). Mice with more than 20%
523 weight loss were humanely euthanized according to IACUC guidelines. For hydrodynamic tail
524 vein injections, plasmids were prepared by EndoFree Plasmid Maxi Kit (Qiagen Cat. #12362).
525 Briefly, 30 ug SpCas8-RA6.3 and 30 ug sgRNA plasmids, or 60 ug Nme2-ABE8e single AAV
526 plasmids were suspended in 2 ml saline and injected via tail vein within 5-7 seconds into 10-
527 week-old *Fah*$^{PM/PM}$ mice. Mice were euthanized 7 days after injection and livers were collected
528 for analysis. For AAV injection, a dosage of 4 x 10^{11} vg per mouse (in 200 ul saline) was tail-vein
529 injected into 8-week-old *Fah*$^{PM/PM}$ mice.
530
531 **Genomic DNA extraction from cultured cells and mouse liver.** For cultured cells,
532 genomic DNAs were extracted 72 hours after plasmid transfection, or 48 hours after mRNA and
533 sgRNA electroporation. Briefly, cell culture media were aspirated, and cells were washed with 1x
534 PBS. Genomic DNAs were prepared using QuickExtract DNA Extraction Solution (Lucigen)
535 following the manufacturer's protocols. To extract genomic DNA from mouse livers, all 5 lobes
536 of mouse liver were combined and pulverized in liquid nitrogen, and 15 mg of the tissue from
537 each mouse liver was used for genomic DNA extraction using GenElute Mammalian Genomic
538 DNA Miniprep Kit (Millipore Sigma Cat. #: G1N350).
539
540 **Amplicon deep sequencing and data analysis**. Genomic DNA was amplified by PCR
541 using Q5 High-Fidelity 2X Master Mix (NEB Cat. #: M0492) for 20 cycles. One microliter of
542 the unpurified PCR product was used as a template for 20 cycles of barcoding PCR. The
543 barcoding PCR reactions were further pooled and gel-extracted using Zymo gel extraction kit
544 and DNA clean & concentrator (Zymo research Cat. #: 11-301 and 11-303) and quantified by
545 Qubit 1x dsDNA HS assay kits (Thermo Fisher Scientific, Cat #: Q32851). Sequencing of the
546 pooled amplicons was performed using an Illumina MiniSeq system (300-cycles, FC-420-1004)
547 following the manufacturer's protocol. The raw MiniSeq output was de-multiplexed using
548 bcl2fastq2 (Illumina, version 2.20.0) with the flag --barcode-mismatches 0. To align the
549 generated fastq files and to quantify editing efficiency, CRISPResso2 [87] (version 2.0.40) was
550 used in batch mode with base editor output and the following flags: -w 15, -q 30. Indel frequency
551 = (insertions reads + deletions reads)/all aligned reads x 100%.
552
553 **Immunohistochemistry (IHC).** Mice were euthanized by CO_2 asphyxiation and livers were
554 fixed with 10% neutral buffered formalin (Epredia Cat. #: 5735), sectioned at 10um, and stained
555 with hematoxylin and eosin for pathology analysis. For IHC, liver sections were dewaxed,

556 rehydrated, and stained using an anti-FAH antibody (Abcam Cat. #: ab83770) at 1:400 dilution
557 as described previously [67].
558
559 **Reverse transcription PCR**. All 5 lobes of each mouse liver were combined and pulverized in
560 liquid nitrogen. Total RNA was extracted from 50 mg of liver tissue using TRIzol reagent
561 (ThermoFisher Cat. #: 15596026) and reverse-transcribed using SuperScript III First-Strand
562 Synthesis System (ThermoFisher Cat. #:18080051). PCR was performed using primers
563 previously described [67].
564
565 **Author contributions**
566
567 H.Z., X.D.G., and E.J.S conceived the study. H.Z. designed, performed, and analyzed the *in vivo*
568 experiments. H.Z., N.B., O.O., P.L., and X.D.G., designed, performed, and analyzed the *in vitro*
569 experiments. T.R. and H.C. analyzed the deep sequencing data. E.J.S., W.X., and S.A.W.
570 supervised research. H.Z. and E.J.S. wrote the manuscript with contributions from N.B., P.L.,
571 and H.C. All authors edited the manuscript.
572
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595
596 **Declaration of Competing Interests**
597
598 H.Z., N.B., P.L., X.D.G., S.A.W., W.X., and E.J.S. are co-inventors on patent filings related to
599 this work. G.G. is scientific co-founder, scientific advisor, and equity holder of Voyager
600 Therapeutics, Adrenas Therapeutics, and Aspa Therapeutics. E.J.S. is a co-founder, scientific
601 advisor, and equity holder of Intellia Therapeutics.
602

603 **References**

604 [1] M.J. Landrum, J.M. Lee, M. Benson, G. Brown, C. Chao, S. Chitipiralla, B. Gu, J. Hart,
605 D. Hoffman, J. Hoover, W. Jang, K. Katz, M. Ovetsky, G. Riley, A. Sethi, R. Tully, R.
606 Villamarin-Salomon, W. Rubinstein, D.R. Maglott, ClinVar: public archive of interpretations of
607 clinically relevant variants, Nucleic Acids Res. 44 (2016) D862–8.
608 [2] H.A. Rees, D.R. Liu, Base editing: precision chemistry on the genome and transcriptome
609 of living cells, Nat. Rev. Genet. 19 (2018) 770–788.
610 [3] N.M. Gaudelli, A.C. Komor, H.A. Rees, M.S. Packer, A.H. Badran, D.I. Bryson, D.R.
611 Liu, Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage,
612 Nature. 551 (2017) 464–471.
613 [4] A.C. Komor, Y.B. Kim, M.S. Packer, J.A. Zuris, D.R. Liu, Programmable editing of a
614 target base in genomic DNA without double-stranded DNA cleavage, Nature. 533 (2016) 420–
615 424.
616 [5] T. Rothgangl, M.K. Dennis, P.J.C. Lin, R. Oka, D. Witzigmann, L. Villiger, W. Qi, M.
617 Hruzova, L. Kissling, D. Lenggenhager, C. Borrelli, S. Egli, N. Frey, N. Bakker, J.A. Walker
618 2nd, A.P. Kadina, D.V. Victorov, M. Pacesa, S. Kreutzer, Z. Kontarakis, A. Moor, M. Jinek, D.
619 Weissman, M. Stoffel, R. van Boxtel, K. Holden, N. Pardi, B. Thöny, J. Häberle, Y.K. Tam,
620 S.C. Semple, G. Schwank, In vivo adenine base editing of PCSK9 in macaques reduces LDL
621 cholesterol levels, Nat. Biotechnol. 39 (2021) 949–957.
622 [6] K. Musunuru, A.C. Chadwick, T. Mizoguchi, S.P. Garcia, J.E. DeNizio, C.W. Reiss, K.
623 Wang, S. Iyer, C. Dutta, V. Clendaniel, M. Amaonye, A. Beach, K. Berth, S. Biswas, M.C.
624 Braun, H.-M. Chen, T.V. Colace, J.D. Ganey, S.A. Gangopadhyay, R. Garrity, L.N. Kasiewicz,
625 J. Lavoie, J.A. Madsen, Y. Matsumoto, A.M. Mazzola, Y.S. Nasrullah, J. Nneji, H. Ren, A.
626 Sanjeev, M. Shay, M.R. Stahley, S.H.Y. Fan, Y.K. Tam, N.M. Gaudelli, G. Ciaramella, L.E.
627 Stolz, P. Malyala, C.J. Cheng, K.G. Rajeev, E. Rohde, A.M. Bellinger, S. Kathiresan, In vivo
628 CRISPR base editing of PCSK9 durably lowers cholesterol in primates, Nature. 593 (2021) 429–
629 434.
630 [7] L. Wang, W. Xue, H. Zhang, R. Gao, H. Qiu, J. Wei, L. Zhou, Y.-N. Lei, X. Wu, X. Li,
631 C. Liu, J. Wu, Q. Chen, H. Ma, X. Huang, C. Cai, Y. Zhang, B. Yang, H. Yin, L. Yang, J.
632 Chen, Eliminating base-editor-induced genome-wide and transcriptome-wide off-target
633 mutations, Nat. Cell Biol. 23 (2021) 552–563.
634 [8] P. Liu, S.-Q. Liang, C. Zheng, E. Mintzer, Y.G. Zhao, K. Ponnienselvan, A. Mir, E.J.
635 Sontheimer, G. Gao, T.R. Flotte, S.A. Wolfe, W. Xue, Improved prime editors enable
636 pathogenic allele correction and cancer modelling in adult mice, Nat. Commun. 12 (2021).
637 https://doi.org/10.1038/s41467-021-22295-w.
638 [9] L. Villiger, T. Rothgangl, D. Witzigmann, R. Oka, P.J.C. Lin, W. Qi, S. Janjuha, C.
639 Berk, F. Ringnalda, M.B. Beattie, M. Stoffel, B. Thöny, J. Hall, H. Rehrauer, R. van Boxtel,
640 Y.K. Tam, G. Schwank, In vivo cytidine base editing of hepatocytes without detectable off-target
641 mutations in RNA and DNA, Nat Biomed Eng. 5 (2021) 179–189.
642 [10] D. Böck, T. Rothgangl, L. Villiger, L. Schmidheini, N. Mathis, E. Ioannidi, S. Kreutzer,
643 Z. Kontarakis, N. Rimann, H.M. Grisch-Chan, B. Thöny, G. Schwank, Treatment of a
644 metabolic liver disease by in vivo prime editing in mice, bioRxiv. (2021) 2021.08.17.456632.
645 https://doi.org/10.1101/2021.08.17.456632.
646 [11] L.W. Koblan, M.R. Erdos, C. Wilson, W.A. Cabral, J.M. Levy, Z.-M. Xiong, U.L.
647 Tavarez, L.M. Davison, Y.G. Gete, X. Mao, G.A. Newby, S.P. Doherty, N. Narisu, Q. Sheng,
648 C. Krilow, C.Y. Lin, L.B. Gordon, K. Cao, F.S. Collins, J.D. Brown, D.R. Liu, In vivo base

649 editing rescues Hutchinson-Gilford progeria syndrome in mice, Nature. 589 (2021) 608–614.
650 [12] S.-M. Ryu, T. Koo, K. Kim, K. Lim, G. Baek, S.-T. Kim, H.S. Kim, D.-E. Kim, H. Lee,
651 E. Chung, J.-S. Kim, Adenine base editing in mouse embryos and an adult mouse model of
652 Duchenne muscular dystrophy, (n.d.). https://doi.org/10.1038/nbt.xxxx.
653 [13] F. Chemello, A.C. Chai, H. Li, C. Rodriguez-Caycedo, E. Sanchez-Ortiz, A. Atmanli,
654 A.A. Mireault, N. Liu, R. Bassel-Duby, E.N. Olson, Precise correction of Duchenne muscular
655 dystrophy exon deletion mutations by base and prime editing, Sci Adv. 7 (2021).
656 https://doi.org/10.1126/sciadv.abg4910.
657 [14] S. Suh, E.H. Choi, H. Leinonen, A.T. Foik, G.A. Newby, W.-H. Yeh, Z. Dong, P.D.
658 Kiser, D.C. Lyon, D.R. Liu, K. Palczewski, Restoration of visual function in adult mice with an
659 inherited retinal disease via adenine base editing, Nat Biomed Eng. 5 (2021) 169–178.
660 [15] S. Zhi, Y. Chen, G. Wu, J. Wen, J. Wu, Q. Liu, Y. Li, R. Kang, S. Hu, J. Wang, P.
661 Liang, J. Huang, Dual-AAV delivering split prime editor system for in vivo genome editing, Mol.
662 Ther. (2021). https://doi.org/10.1016/j.ymthe.2021.07.011.
663 [16] W.-H. Yeh, O. Shubina-Oleinik, J.M. Levy, B. Pan, G.A. Newby, M. Wornow, R. Burt,
664 J.C. Chen, J.R. Holt, D.R. Liu, In vivo base editing restores sensory transduction and transiently
665 improves auditory function in a mouse model of recessive deafness, Sci. Transl. Med. 12 (2020).
666 https://doi.org/10.1126/scitranslmed.aay9101.
667 [17] C.K.W. Lim, M. Gapinske, A.K. Brooks, W.S. Woods, J.E. Powell, M.A. Zeballos C, J.
668 Winter, P. Perez-Pinera, T. Gaj, Treatment of a Mouse Model of ALS by In Vivo Base Editing,
669 Mol. Ther. 28 (2020) 1177–1189.
670 [18] J.M. Levy, W.-H. Yeh, N. Pendse, J.R. Davis, E. Hennessey, R. Butcher, L.W. Koblan, J.
671 Comander, Q. Liu, D.R. Liu, Cytosine and adenine base editing of the brain, liver, retina, heart
672 and skeletal muscle of mice via adeno-associated viruses, Nat Biomed Eng. 4 (2020) 97–110.
673 [19] Z. Wu, H. Yang, P. Colosi, Effect of genome size on AAV vector packaging, Mol. Ther.
674 18 (2010) 80–86.
675 [20] J.Y. Dong, P.D. Fan, R.A. Frizzell, Quantitative analysis of the packaging capacity of
676 recombinant adeno-associated virus, Hum. Gene Ther. 7 (1996) 2101–2112.
677 [21] L. Villiger, H.M. Grisch-Chan, H. Lindsay, F. Ringnalda, C.B. Pogliano, G. Allegri, R.
678 Fingerhut, J. Häberle, J. Matos, M.D. Robinson, B. Thöny, G. Schwank, Treatment of a
679 metabolic liver disease by in vivo genome base editing in adult mice, Nat. Med. 24 (2018) 1519–
680 1525.
681 [22] J. Winter, A. Luu, M. Gapinske, S. Manandhar, S. Shirguppe, W.S. Woods, J.S. Song, P.
682 Perez-Pinera, Targeted exon skipping with AAV-mediated split adenine base editors, Cell
683 Discov. 5 (2019) 41.
684 [23] C. Hinderer, N. Katz, E.L. Buza, C. Dyer, T. Goode, P. Bell, L.K. Richman, J.M.
685 Wilson, Severe Toxicity in Nonhuman Primates and Piglets Following High-Dose Intravenous
686 Administration of an Adeno-Associated Virus Vector Expressing Human SMN, Hum. Gene
687 Ther. 29 (2018) 285–298.
688 [24] J.M. Wilson, T.R. Flotte, Moving Forward After Two Deaths in a Gene Therapy Trial of
689 Myotubular Myopathy, Human Gene Therapy. 31 (2020) 695–696.
690 https://doi.org/10.1089/hum.2020.182.
691 [25] L. Wang, J. Smith, C. Breton, P. Clark, J. Zhang, L. Ying, Y. Che, J. Lape, P. Bell, R.
692 Calcedo, E.L. Buza, A. Saveliev, V.V. Bartsevich, Z. He, J. White, M. Li, D. Jantz, J.M. Wilson,
693 Meganuclease targeting of PCSK9 in macaque liver leads to stable reduction in serum
694 cholesterol, Nat. Biotechnol. 36 (2018) 717–725.
695 [26] F.A. Ran, L. Cong, W.X. Yan, D.A. Scott, J.S. Gootenberg, A.J. Kriz, B. Zetsche, O.

696 Shalem, X. Wu, K.S. Makarova, E.V. Koonin, P.A. Sharp, F. Zhang, In vivo genome editing
697 using Staphylococcus aureus Cas9, Nature. 520 (2015) 186–191.
698 [27] R. Ibraheim, P.W.L. Tai, A. Mir, N. Javeed, J. Wang, T.C. Rodríguez, S. Namkung, S.
699 Nelson, E.S. Khokhar, E. Mintzer, S. Maitland, Z. Chen, Y. Cao, E. Tsagkaraki, S.A. Wolfe, D.
700 Wang, A.A. Pai, W. Xue, G. Gao, E.J. Sontheimer, Self-inactivating, all-in-one AAV vectors for
701 precision Cas9 genome editing via homology-directed repair in vivo, Nat. Commun. 12 (2021)
702 6267.
703 [28] M.L. Maeder, M. Stefanidakis, C.J. Wilson, R. Baral, L.A. Barrera, G.S. Bounoutas, D.
704 Bumcrot, H. Chao, D.M. Ciulla, J.A. DaSilva, A. Dass, V. Dhanapal, T.J. Fennell, A.E.
705 Friedland, G. Giannoukos, S.W. Gloskowski, A. Glucksmann, G.M. Gotta, H. Jayaram, S.J.
706 Haskett, B. Hopkins, J.E. Horng, S. Joshi, E. Marco, R. Mepani, D. Reyon, T. Ta, D.G.
707 Tabbaa, S.J. Samuelsson, S. Shen, M.N. Skor, P. Stetkiewicz, T. Wang, C. Yudkoff, V.E. Myer,
708 C.F. Albright, H. Jiang, Development of a gene-editing approach to restore vision loss in Leber
709 congenital amaurosis type 10, Nat. Med. 25 (2019) 229–233.
710 [29] M.T. Nguyen Tran, M.K.N. Mohd Khalid, Q. Wang, J.K.R. Walker, G.E. Lidgerwood,
711 K.L. Dilworth, L. Lisowski, A. Pébay, A.W. Hewitt, Engineering domain-inlaid SaCas9 adenine
712 base editors with reduced RNA off-targets and increased on-target DNA editing, Nat. Commun.
713 11 (2020) 4871.
714 [30] A. Edraki, A. Mir, R. Ibraheim, I. Gainetdinov, Y. Yoon, C.-Q. Song, Y. Cao, J. Gallant,
715 W. Xue, J.A. Rivera-Pérez, E.J. Sontheimer, A Compact, High-Accuracy Cas9 with a
716 Dinucleotide PAM for In Vivo Genome Editing, Mol. Cell. 73 (2019) 714–726.e4.
717 [31] Z. Liu, S. Chen, Y. Jia, H. Shan, M. Chen, Y. Song, L. Lai, Z. Li, Efficient and high-
718 fidelity base editor with expanded PAM compatibility for cytidine dinucleotide, Sci. China Life
719 Sci. 64 (2021) 1355–1367.
720 [32] R. Xu, R. Qin, H. Xie, J. Li, X. Liu, M. Zhu, Y. Sun, Y. Yu, P. Lu, P. Wei, Genome
721 editing with type II-C CRISPR-Cas9 systems from Neisseria meningitidis in rice, Plant
722 Biotechnol. J. (2021). https://doi.org/10.1111/pbi.13716.
723 [33] K. Hua, X. Tao, J.-K. Zhu, Expanding the base editing scope in rice by using Cas9
724 variants, Plant Biotechnol. J. 17 (2019) 499–504.
725 [34] L. Yang, X. Zhang, L. Wang, S. Yin, B. Zhu, L. Xie, Q. Duan, H. Hu, R. Zheng, Y.
726 Wei, L. Peng, H. Han, J. Zhang, W. Qiu, H. Geng, S. Siwko, X. Zhang, M. Liu, D. Li,
727 Correction to: Increasing targeting scope of adenosine base editors in mouse and rat embryos
728 through fusion of TadA deaminase with Cas9 variants, Protein Cell. 10 (2019) 700.
729 [35] M.F. Richter, K.T. Zhao, E. Eton, A. Lapinaite, G.A. Newby, B.W. Thuronyi, C.
730 Wilson, L.W. Koblan, J. Zeng, D.E. Bauer, J.A. Doudna, D.R. Liu, Phage-assisted evolution of
731 an adenine base editor with improved Cas domain compatibility and activity, Nat. Biotechnol.
732 38 (2020) 883–891.
733 [36] N.M. Gaudelli, D.K. Lam, H.A. Rees, N.M. Solá-Esteves, L.A. Barrera, D.A. Born, A.
734 Edwards, J.M. Gehrke, S.-J. Lee, A.J. Liquori, R. Murray, M.S. Packer, C. Rinaldi, I.M.
735 Slaymaker, J. Yen, L.E. Young, G. Ciaramella, Directed evolution of adenine base editors with
736 increased activity and therapeutic application, Nat. Biotechnol. 38 (2020) 892–900.
737 [37] H.A. Rees, A.C. Komor, W.-H. Yeh, J. Caetano-Lopes, M. Warman, A.S.B. Edge, D.R.
738 Liu, Improving the DNA specificity and applicability of base editing through protein engineering
739 and protein delivery, Nat. Commun. 8 (2017) 15790.
740 [38] D. Kim, K. Lim, S.-T. Kim, S.-H. Yoon, K. Kim, S.-M. Ryu, J.-S. Kim, Genome-wide
741 target specificities of CRISPR RNA-guided programmable deaminases, Nat. Biotechnol. 35
742 (2017) 475–480.

743 [39] J. Wen, T. Cao, J. Wu, Y. Chen, S. Zhi, Y. Huang, P. Zhen, G. Wu, L. Aagaard, J.
744 Zhong, P. Liang, J. Huang, Single AAV-mediated CRISPR-Nme2Cas9 efficiently reduces
745 mutant hTTR expression in a transgenic mouse model of transthyretin amyloidosis, Mol. Ther.
746 (2021). https://doi.org/10.1016/j.ymthe.2021.05.010.
747 [40] B.J. Rauch, M.R. Silvis, J.F. Hultquist, C.S. Waters, M.J. McGregor, N.J. Krogan, J.
748 Bondy-Denomy, Inhibition of CRISPR-Cas9 with Bacteriophage Proteins, Cell. 168 (2017) 150–
749 158.e10.
750 [41] J. Lee, A. Mir, A. Edraki, B. Garcia, N. Amrani, H.E. Lou, I. Gainetdinov, A. Pawluk, R.
751 Ibraheim, X.D. Gao, P. Liu, A.R. Davidson, K.L. Maxwell, E.J. Sontheimer, Potent Cas9
752 Inhibition in Bacterial and Human Cells by AcrIIC4 and AcrIIC5 Anti-CRISPR Proteins,
753 MBio. 9 (2018). https://doi.org/10.1128/mBio.02321-18.
754 [42] S. Hwang, K.L. Maxwell, Meet the Anti-CRISPRs: Widespread Protein Inhibitors of
755 CRISPR-Cas Systems, CRISPR J. 2 (2019) 23–30.
756 [43] A. Pawluk, N. Amrani, Y. Zhang, B. Garcia, Y. Hidalgo-Reyes, J. Lee, A. Edraki, M.
757 Shah, E.J. Sontheimer, K.L. Maxwell, A.R. Davidson, Naturally Occurring Off-Switches for
758 CRISPR-Cas9, Cell. 167 (2016) 1829–1838.e9.
759 [44] J. Shin, F. Jiang, J.-J. Liu, N.L. Bray, B.J. Rauch, S.H. Baik, E. Nogales, J. Bondy-
760 Denomy, J.E. Corn, J.A. Doudna, Disabling Cas9 by an anti-CRISPR DNA mimic, Sci Adv. 3
761 (2017) e1701620.
762 [45] J. Lee, H. Mou, R. Ibraheim, S.-Q. Liang, P. Liu, W. Xue, E.J. Sontheimer, Tissue-
763 restricted genome editing in vivo specified by microRNA-repressible anti-CRISPR proteins,
764 RNA. 25 (2019) 1421–1431.
765 [46] L.B. Harrington, K.W. Doxzen, E. Ma, J.-J. Liu, G.J. Knott, A. Edraki, B. Garcia, N.
766 Amrani, J.S. Chen, J.C. Cofsky, P.J. Kranzusch, E.J. Sontheimer, A.R. Davidson, K.L. Maxwell,
767 J.A. Doudna, A Broad-Spectrum Inhibitor of CRISPR-Cas9, Cell. 170 (2017) 1224–1233.e15.
768 [47] M. Liang, T. Sui, Z. Liu, M. Chen, H. Liu, H. Shan, L. Lai, Z. Li, AcrIIA5 Suppresses
769 Base Editors and Reduces Their Off-Target Effects, Cells. 9 (2020).
770 https://doi.org/10.3390/cells9081786.
771 [48] M.D. Hoffmann, S. Aschenbrenner, S. Grosse, K. Rapti, C. Domenger, J. Fakhiri, M.
772 Mastel, K. Börner, R. Eils, D. Grimm, D. Niopek, Cell-specific CRISPR–Cas9 activation by
773 microRNA-dependent expression of anti-CRISPR proteins, Nucleic Acids Res. 47 (2019) e75–
774 e75.
775 [49] T. Bienvenu, A. Carrié, N. de Roux, M.C. Vinet, P. Jonveaux, P. Couvert, L. Villard, A.
776 Arzimanoglou, C. Beldjord, M. Fontes, M. Tardieu, J. Chelly, MECP2 mutations account for
777 most cases of typical forms of Rett syndrome, Hum. Mol. Genet. 9 (2000) 1377–1384.
778 [50] M.J. Lyst, A. Bird, Rett syndrome: a complex disorder with simple roots, Nat. Rev.
779 Genet. 16 (2015) 261–275.
780 [51] R. Krishnaraj, G. Ho, J. Christodoulou, RettBASE: Rett syndrome database update,
781 Hum. Mutat. 38 (2017) 922–931.
782 [52] G. Stefanelli, A. Gandaglia, M. Costa, M.S. Cheema, D. Di Marino, I. Barbiero, C.
783 Kilstrup-Nielsen, J. Ausió, N. Landsberger, Brain phosphorylation of MeCP2 at serine 164 is
784 developmentally regulated and globally alters its chromatin association, Sci. Rep. 6 (2016) 28295.
785 [53] J. Tan, F. Zhang, D. Karcher, R. Bock, Engineering of high-precision base editors for
786 site-specific single nucleotide replacement, Nature Communications. 10 (2019).
787 https://doi.org/10.1038/s41467-018-08034-8.
788 [54] S.H. Chu, M. Packer, H. Rees, D. Lam, Y. Yu, J. Marshall, L.-I. Cheng, D. Lam, J.
789 Olins, F.A. Ran, A. Liquori, B. Gantzer, J. Decker, D. Born, L. Barrera, A. Hartigan, N.

790 Gaudelli, G. Ciaramella, I.M. Slaymaker, Rationally Designed Base Editors for Precise Editing
791 of the Sickle Cell Disease Mutation, CRISPR J. 4 (2021) 169–177.
792 [55] T.P. Huang, K.T. Zhao, S.M. Miller, N.M. Gaudelli, B.L. Oakes, C. Fellmann, D.F.
793 Savage, D.R. Liu, Circularly permuted and PAM-modified Cas9 variants broaden the targeting
794 scope of base editors, Nat. Biotechnol. 37 (2019) 626–631.
795 [56] Y. Wang, L. Zhou, N. Liu, S. Yao, BE-PIGS: a base-editing tool with deaminases inlaid
796 into Cas9 PI domain significantly expanded the editing scope, Signal Transduct Target Ther. 4
797 (2019) 36.
798 [57] S. Li, B. Yuan, J. Cao, J. Chen, J. Chen, J. Qiu, X.-M. Zhao, X. Wang, Z. Qiu, T.-L.
799 Cheng, Docking sites inside Cas9 for adenine base editing diversification and RNA off-target
800 elimination, Nat. Commun. 11 (2020) 5827.
801 [58] Y. Liu, C. Zhou, S. Huang, L. Dang, Y. Wei, J. He, Y. Zhou, S. Mao, W. Tao, Y.
802 Zhang, H. Yang, X. Huang, T. Chi, A Cas-embedding strategy for minimizing off-target effects
803 of DNA base editors, Nat. Commun. 11 (2020) 6073.
804 [59] F. Chemello, Z. Wang, H. Li, J.R. McAnally, N. Liu, R. Bassel-Duby, E.N. Olson,
805 Degenerative and regenerative pathways underlying Duchenne muscular dystrophy revealed by
806 single-nucleus RNA sequencing, Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 29691–29701.
807 [60] K.M. Anderson, P. Poosala, S.R. Lindley, D.M. Anderson, Targeted Cleavage and
808 Polyadenylation of RNA by CRISPR-Cas13, bioRxiv. (2019) 531111.
809 https://doi.org/10.1101/531111.
810 [61] L.W. Koblan, J.L. Doman, C. Wilson, J.M. Levy, T. Tay, G.A. Newby, J.P. Maianti, A.
811 Raguram, D.R. Liu, Improving cytidine and adenine base editors by expression optimization
812 and ancestral reconstruction, Nat. Biotechnol. 36 (2018) 843–846.
813 [62] R. Preece, C. Georgiadis, S.A. Gkazi, A. Etuk, A. Christi, W. Qasim, "Mini" U6 Pol III
814 promoter exhibits nucleosome redundancy and supports multiplexed coupling of CRISPR/Cas9
815 effects, Gene Ther. 27 (2020) 451–458.
816 [63] J.L. Aponte, G.A. Sega, L.J. Hauser, M.S. Dhar, C.M. Withrow, D.A. Carpenter, E.M.
817 Rinchik, C.T. Culiat, D.K. Johnson, Point mutations in the murine fumarylacetoacetate
818 hydrolase gene: Animal models for the human genetic disorder hereditary tyrosinemia type 1,
819 Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 641–645.
820 [64] S. Lindstedt, Treatment of hereditary tyrosinaemia type I by inhibition of 4-
821 hydroxyphenylpyruvate dioxygenase, The Lancet. 340 (1992) 813–817.
822 https://doi.org/10.1016/0140-6736(92)92685-9.
823 [65] H. Yin, W. Xue, S. Chen, R.L. Bogorad, E. Benedetti, M. Grompe, V. Koteliansky, P.A.
824 Sharp, T. Jacks, D.G. Anderson, Genome editing with Cas9 in adult mice corrects a disease
825 mutation and phenotype, Nat. Biotechnol. 32 (2014) 551–553.
826 [66] H. Yin, C.-Q. Song, J.R. Dorkin, L.J. Zhu, Y. Li, Q. Wu, A. Park, J. Yang, S. Suresh, A.
827 Bizhanova, A. Gupta, M.F. Bolukbasi, S. Walsh, R.L. Bogorad, G. Gao, Z. Weng, Y. Dong, V.
828 Koteliansky, S.A. Wolfe, R. Langer, W. Xue, D.G. Anderson, Therapeutic genome editing by
829 combined viral and non-viral delivery of CRISPR system components in vivo, Nat. Biotechnol.
830 34 (2016) 328–333.
831 [67] C.-Q. Song, T. Jiang, M. Richter, L.H. Rhym, L.W. Koblan, M.P. Zafra, E.M. Schatoff,
832 J.L. Doman, Y. Cao, L.E. Dow, L.J. Zhu, D.G. Anderson, D.R. Liu, H. Yin, W. Xue, Adenine
833 base editing in an adult mouse model of tyrosinaemia, Nat Biomed Eng. 4 (2020) 125–130.
834 [68] T. Jiang, J.M. Henderson, K. Coote, Y. Cheng, H.C. Valley, X.-O. Zhang, Q. Wang,
835 L.H. Rhym, Y. Cao, G.A. Newby, H. Bihler, M. Mense, Z. Weng, D.G. Anderson, A.P.
836 McCaffrey, D.R. Liu, W. Xue, Chemical modifications of adenine base editor mRNA and guide

837 RNA expand its application scope, Nat. Commun. 11 (2020) 1979.
838 [69] J.H. Shin, S. Jung, S. Ramakrishna, H.H. Kim, J. Lee, In vivo gene correction with
839 targeted sequence substitution through microhomology-mediated end joining, Biochem. Biophys.
840 Res. Commun. 502 (2018) 116–122.
841 [70] H. Jang, D.H. Jo, C.S. Cho, J.H. Shin, J.H. Seo, G. Yu, R. Gopalappa, D. Kim, S.-R.
842 Cho, J.H. Kim, H.H. Kim, Application of prime editing to the correction of mutations and
843 phenotypes in adult mice with liver and eye diseases, Nat Biomed Eng. (2021).
844 https://doi.org/10.1038/s41551-021-00788-9.
845 [71] G. Zhang, V. Budker, J.A. Wolff, High levels of foreign gene expression in hepatocytes
846 after tail vein injections of naked plasmid DNA, Hum. Gene Ther. 10 (1999) 1735–1737.
847 [72] N.K. Paulk, K. Wursthorn, Z. Wang, M.J. Finegold, M.A. Kay, M. Grompe, Adeno-
848 associated virus gene repair corrects a mouse model of hereditary tyrosinemia in vivo,
849 Hepatology. 51 (2010) 1200–1208. https://doi.org/10.1002/hep.23481.
850 [73] M.-J. Wang, F. Chen, J.T.Y. Lau, Y.-P. Hu, Hepatocyte polyploidization and its
851 association with pathophysiological processes, Cell Death Dis. 8 (2017) e2805.
852 [74] S. Bae, J. Park, J.-S. Kim, Cas-OFFinder: a fast and versatile algorithm that searches for
853 potential off-target sites of Cas9 RNA-guided endonucleases, Bioinformatics. 30 (2014) 1473–
854 1475.
855 [75] A.V. Anzalone, L.W. Koblan, D.R. Liu, Genome editing with CRISPR-Cas nucleases,
856 base editors, transposases and prime editors, Nat. Biotechnol. 38 (2020) 824–844.
857 [76] G.A. Newby, D.R. Liu, In vivo somatic cell base editing and prime editing, Mol. Ther.
858 (2021). https://doi.org/10.1016/j.ymthe.2021.09.002.
859 [77] High-dose AAV gene therapy deaths, Nat. Biotechnol. 38 (2020) 910.
860 [78] L. Morales, Y. Gambhir, J. Bennett, H.H. Stedman, Broader Implications of Progressive
861 Liver Dysfunction and Lethal Sepsis in Two Boys following Systemic High-Dose AAV, Mol.
862 Ther. 28 (2020) 1753–1755.
863 [79] T.R. Flotte, Revisiting the "New" Inflammatory Toxicities of Adeno-Associated Virus
864 Vectors, Hum. Gene Ther. 31 (2020) 398–399.
865 [80] D. Wang, P.W.L. Tai, G. Gao, Adeno-associated virus vector as a platform for gene
866 therapy delivery, Nat. Rev. Drug Discov. 18 (2019) 358–378.
867 [81] D. Wang, F. Zhang, G. Gao, CRISPR-Based Therapeutic Genome Editing: Strategies
868 and In Vivo Delivery by AAV Vectors, Cell. 181 (2020) 136–150.
869 [82] R. Ibraheim, C.-Q. Song, A. Mir, N. Amrani, W. Xue, E.J. Sontheimer, All-in-one
870 adeno-associated virus delivery and genome editing by Neisseria meningitidis Cas9 in vivo,
871 Genome Biol. 19 (2018) 137.
872 [83] S.A. Krooss, Z. Dai, F. Schmidt, A. Rovai, J. Fakhiri, A. Dhingra, Q. Yuan, T. Yang, A.
873 Balakrishnan, L. Steinbrück, S. Srivaratharajan, M.P. Manns, A. Schambach, D. Grimm, J.
874 Bohne, A.D. Sharma, H. Büning, M. Ott, Ex Vivo/In vivo Gene Editing in Hepatocytes Using
875 "All-in-One" CRISPR-Adeno-Associated Virus Vectors with a Self-Linearizing Repair
876 Template, iScience. 23 (2020) 100764. https://doi.org/10.1016/j.isci.2019.100764.
877 [84] W. Sun, J. Yang, Z. Cheng, N. Amrani, C. Liu, K. Wang, R. Ibraheim, A. Edraki, X.
878 Huang, M. Wang, J. Wang, L. Liu, G. Sheng, Y. Yang, J. Lou, E.J. Sontheimer, Y. Wang,
879 Structures of Neisseria meningitidis Cas9 Complexes in Catalytically Poised and Anti-CRISPR-
880 Inhibited States, Mol. Cell. 76 (2019) 938–952.e5.
881 [85] L.E. Fry, C.F. Peddle, M. Stevanovic, A.R. Barnard, M.E. McClements, R.E. MacLaren,
882 Promoter Orientation within an AAV-CRISPR Vector Affects Cas9 Expression and Gene
883 Editing Efficiency, CRISPR J. 3 (2020) 276–283.

884 [86] M. Sena-Esteves, G. Gao, Introducing Genes into Mammalian Cells: Viral Vectors, Cold
885 Spring Harb. Protoc. 2020 (2020) 095513.
886 [87] K. Clement, H. Rees, M.C. Canver, J.M. Gehrke, R. Farouni, J.Y. Hsu, M.A. Cole, D.R.
887 Liu, J.K. Joung, D.E. Bauer, L. Pinello, CRISPResso2 provides accurate and rapid genome
888 editing sequence analysis, Nat. Biotechnol. 37 (2019) 224–226.

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Figures

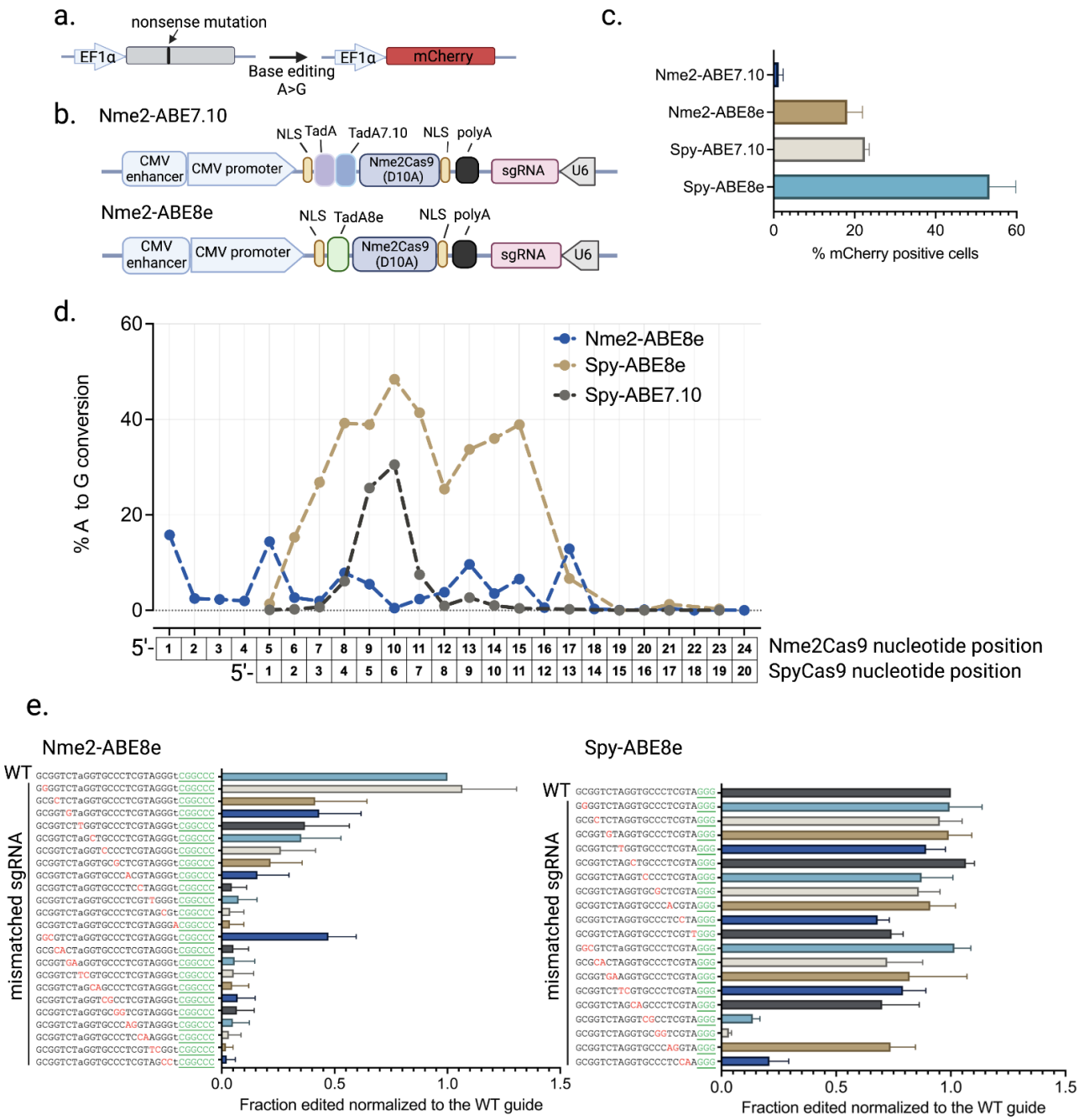


Figure 1

a). Schematic representation of the ABE reporter HEK293T cell line. **b).** Schematic representation of the Nme2-ABE constructs. **c).** Comparison of editing efficiencies of Nme2-ABEs to those of Spy-ABEs in the ABE reporter cell line by plasmid transient transfection (n = 3 biological replicates). **d).** Summary of editing windows and comparison of editing efficiencies for the ABEs at endogenous genomic loci. Each data point represents the mean A-to-G editing efficiency at the indicated position of the spacer across 12 Nme2Cas9 target sites and 8 SpyCas9 target sites, respectively (n = 3 biological replicates). Summary of the individual A-to-G conversion and indel efficiencies at each target site can be found in **Supplementary Figure 2**. **e).** Comparison of Nme2-ABE8e mismatch tolerance to that of Spy-ABE8e in the ABE reporter cell line. The activities of the effectors with the mismatched guides are normalized to that of the perfectly complementary (WT) guide. Red, mismatched nucleotides; green, PAM sequence (n = 3 biological replicates).

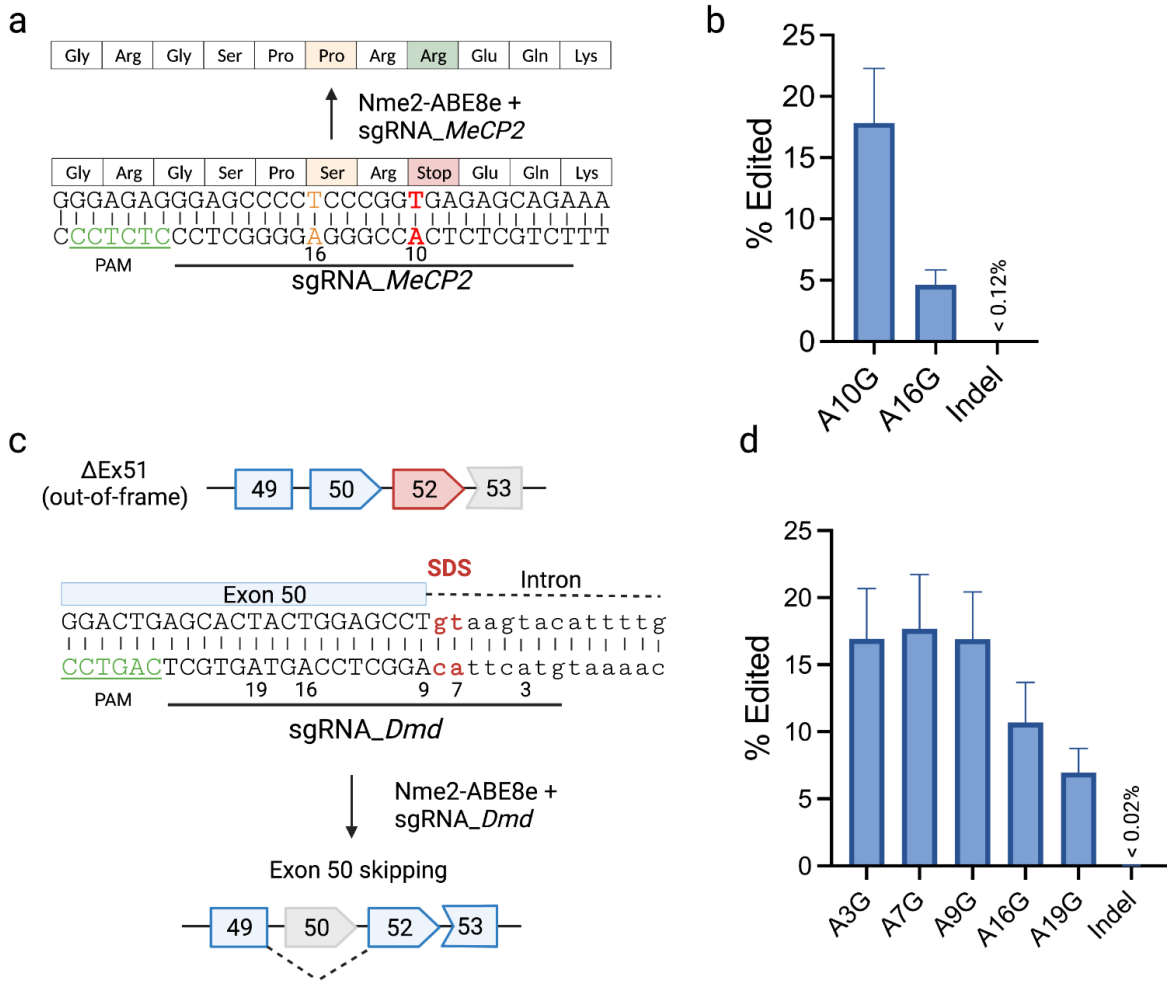


Figure 2

a). Schematic representation of a nonsense mutation in the human *MeCP2* gene (c.502 C>T; p.R168X) that causes Rett Syndrome. The black underline denotes the target sequence of an Nme2-ABE8e guide for reverting the mutant A to G (wildtype) at position 10 (red, bold). The PAM region is underlined in green. A bystander edit at position 16 (orange) can generate a missense mutation (c.496 T>C; p.S166P). **b).** Amplicon deep sequencing quantifying the editing efficiency in Rett patient-derived fibroblasts transfected with the Nme2-ABE8e mRNA and the synthetic sgRNA_*MeCP2* noted in **a** (n = 3 biological replicates). **c).** Schematic representation of the exon skipping strategy that restores the reading frame of the mouse *Dmd* transcript. Deletion of exon 51 (ΔEx51) can alter the reading frame and generate a premature stop codon in exon 52 (red). Adenine base editing at the splice site of exon 50 (red) by Nme2-ABE8e can cause exon 50 skipping (gray) and restore the *Dmd* reading frame. The PAM region is underlined in green. **d).** Amplicon deep sequencing quantifies the editing efficiency at the target site in mouse N2a cells transfected with the Nme2-ABE8e and sgRNA_*Dmd* expression plasmid (n = 3 biological replicates).

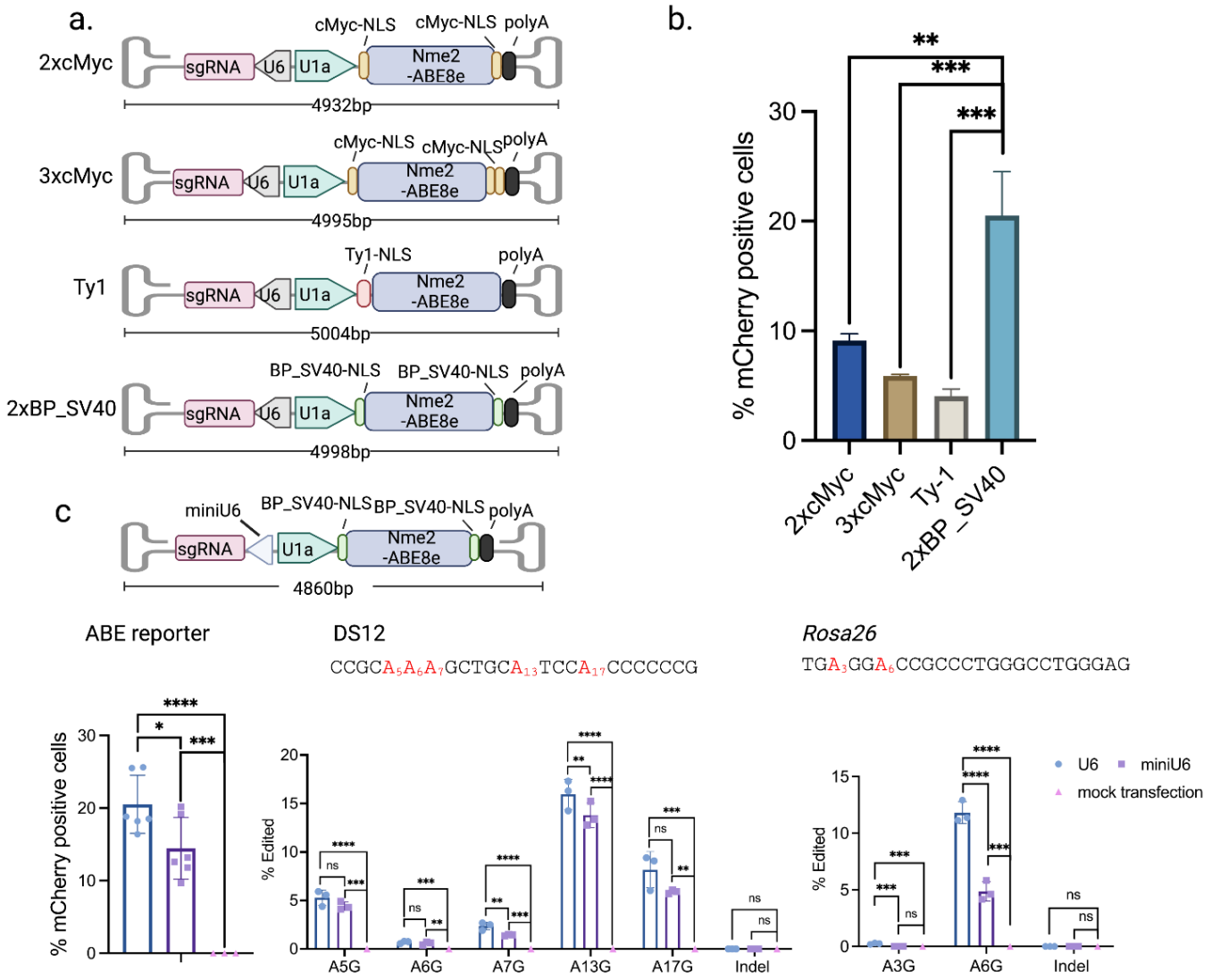
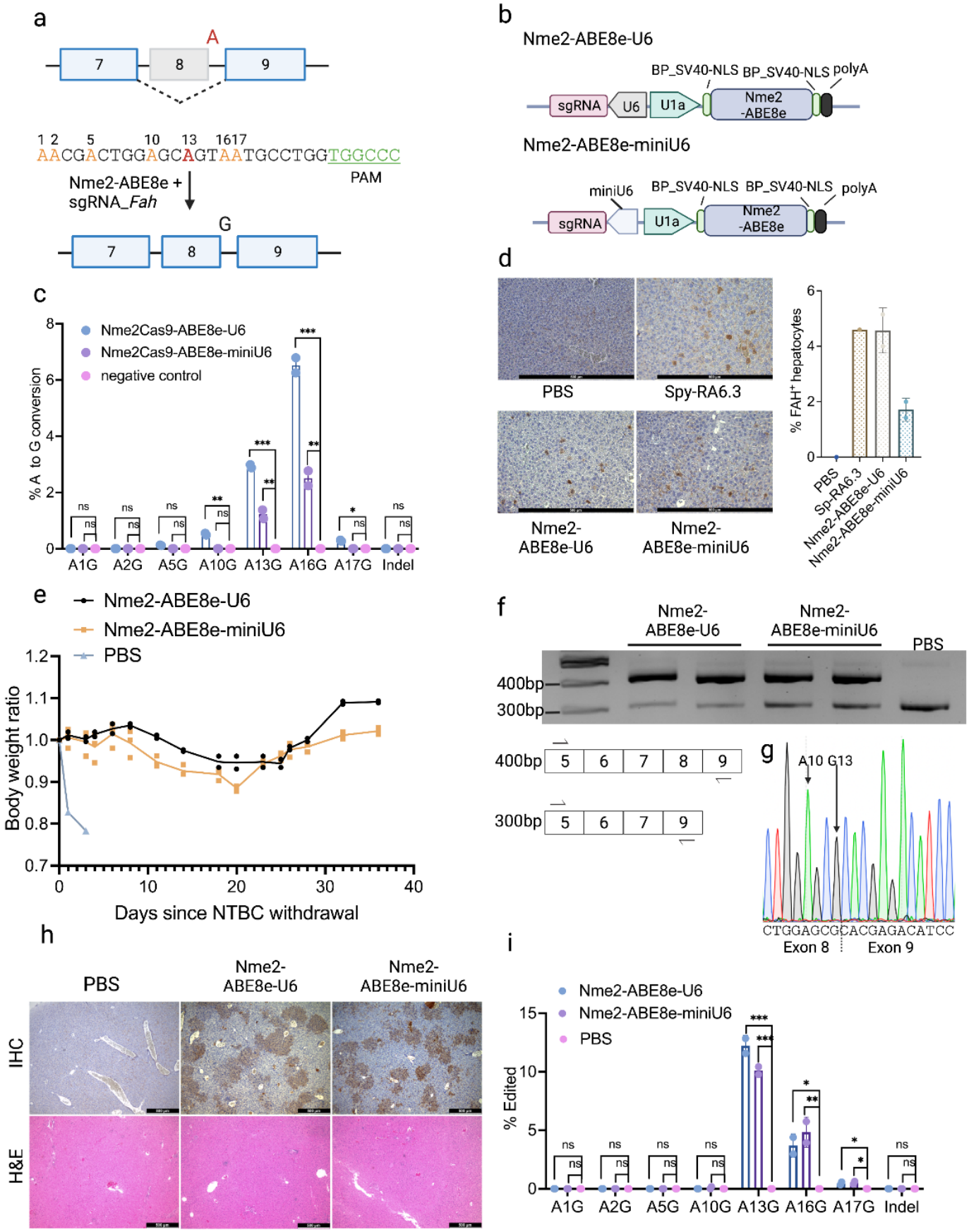


Figure 3

a). Schematic representation of single AAV constructs with different NLS configurations. **b).** Comparison of different NLS configurations by plasmid transfection in the ABE reporter cell line. **c).** Comparison of Nme2-ABE activity when sgRNA expression is driven by the U6 or miniU6 promoters in the 2xBP_SV40 NLS construct targeting the ABE reporter site (left) or endogenous human (middle) and mouse (right) genomic sites by plasmid transfection in cultured cells followed by amplicon deep sequencing (n = 3 biological replicates). Data represent mean ± SD; ns, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001 (one-way ANOVA).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1 2 5 10 13 1617
AACGACTGGAGCAGTAATGCCTGGTGGCCC
PAM
Nme2-ABE8e +
sgRNA_Fah
G
b
Nme2-ABE8e-U6
BP_SV40-NLS BP_SV40-NLS polyA
sgRNA U6 U1a Nme2 -ABE8e
Nme2-ABE8e-miniU6
miniU6 BP_SV40-NLS BP_SV40-NLS polyA
c
Nme2Cas9-ABE8e-U6
Nme2Cas9-ABE8e-miniU6
negative control
% A to G conversion
A1G A2G A5G A10G A13G A16G A17G Indel
d
PBS
Spy-RA6.3
Nme2-ABE8e-U6
Nme2-ABE8e-miniU6
% FAH+ hepatocytes
e
Nme2-ABE8e-U6
Nme2-ABE8e-miniU6
PBS
Body weight ratio
Days since NTBC withdrawal
f
Nme2-ABE8e-U6
Nme2-ABE8e-miniU6
PBS
400bp
300bp
g
A10 G13
CTGGAGCGCACGAGACATCC
Exon 8
Exon 9
h
PBS
Nme2-ABE8e-U6
Nme2-ABE8e-miniU6
IHC
H&E
i
Nme2-ABE8e-U6
Nme2-ABE8e-miniU6
PBS
% Edited
A1G A2G A5G A10G A13G A16G A17G Indel

Figure 4

a). Illustration of the pathogenic point mutation in the *Fah*$^{PM/PM}$ mouse model that causes exon 8 skipping of the *Fah* gene, and the guide design for Nme2-ABE8e to correct the point mutation. Red and bold, target adenine; orange, other bystander adenines; green and underlined, PAM. **b).** Illustration of constructs of the single-AAV vector plasmids used in *in vivo* studies. **c).** Editing efficiencies at the *Fah* mutant site by AAV plasmid electroporation in MEF cells derived from the *Fah*$^{PM/PM}$ mouse. Data are from amplicon deep sequencing (n = 2 biological replicates). **d).** Anti-FAH immunohistochemistry (IHC) staining showing FAH^{+} hepatocytes, before NTBC withdrawal, in the *Fah*$^{PM/PM}$ mouse hydrodynamically injected with the indicated plasmid. The bar graph quantifies the percentage of FAH^{+} hepatocytes detected by IHC (n = 2 mice per group). Scale bars, 500 μm. **e).** Body weight plot of mice injected with the single-AAV vector plasmid showing gradual weight gain over a month after NTBC withdrawal. **f).** RT–PCR analysis of the plasmid- or PBS-injected mouse livers using primers that span exons 5 and 9. The wild-type amplicon is 405 bp and exon 8 skipped amplicon is 305 bp. **g).** Representative Sanger sequencing trace of the 405 bp RT-PCR band. **h).** Anti-FAH IHC staining showing expansion of FAH^{+} hepatocytes 40 days post NTBC withdrawal. Scale bars, 500 μm. **i).** Quantification of the editing efficiency by amplicon deep sequencing of genomic DNA of the treated mouse livers harvested 40 days post NTBC withdrawal. **d-i**, n = 2 mice per group). Data represent mean ± SD; ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (one-way ANOVA).

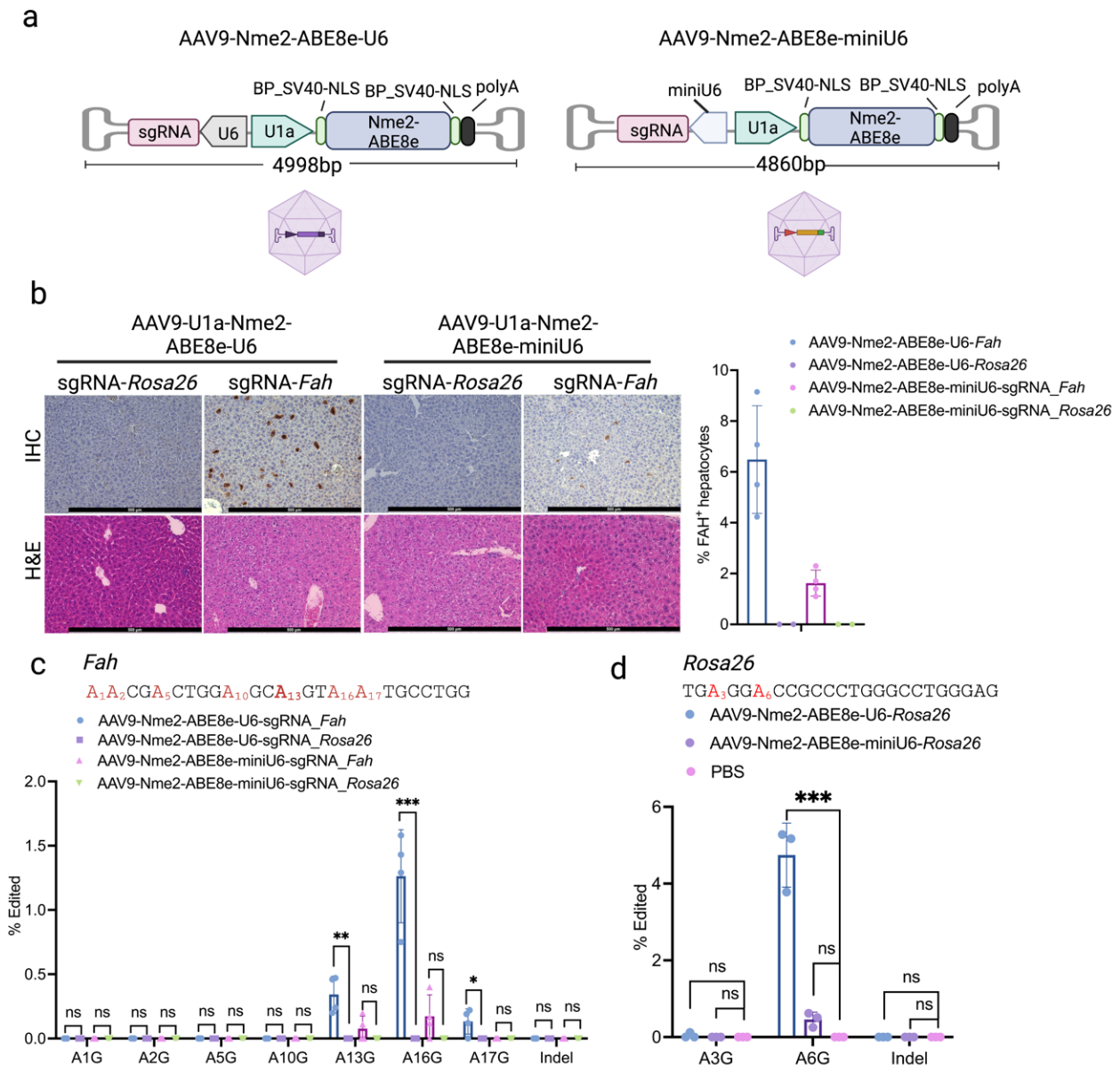


Figure 5

a). Schematic representation of the AAV constructs. **b).** Anti-FAH immunohistochemistry (IHC) staining showing FAH$^+$ hepatocytes, before NTBC withdrawal, in the *Fah*$^{PM/PM}$ mouse injected with AAV9 expressing Nme2-ABE8e with a sgRNA targeting either the *Fah* gene, or the *Rosa26* gene that serves as a negative control. Scale bar, 500 µm. The bar graph quantifies the percentage of FAH$^+$ hepatocytes detected by IHC (n = 4 mice in the *Fah* targeting group, n = 3 mice in the *Rosa26* targeting group). **c, d).** Quantification of the editing efficiency by amplicon deep sequencing of genomic DNA from the AAV9-injected mouse livers harvested before NTBC withdrawal (n = 4 mice in the *Fah* targeting group, n = 3 mice in the *Rosa26* targeting group and the PBS control group). Data represent mean ± SD; ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (one-way ANOVA).

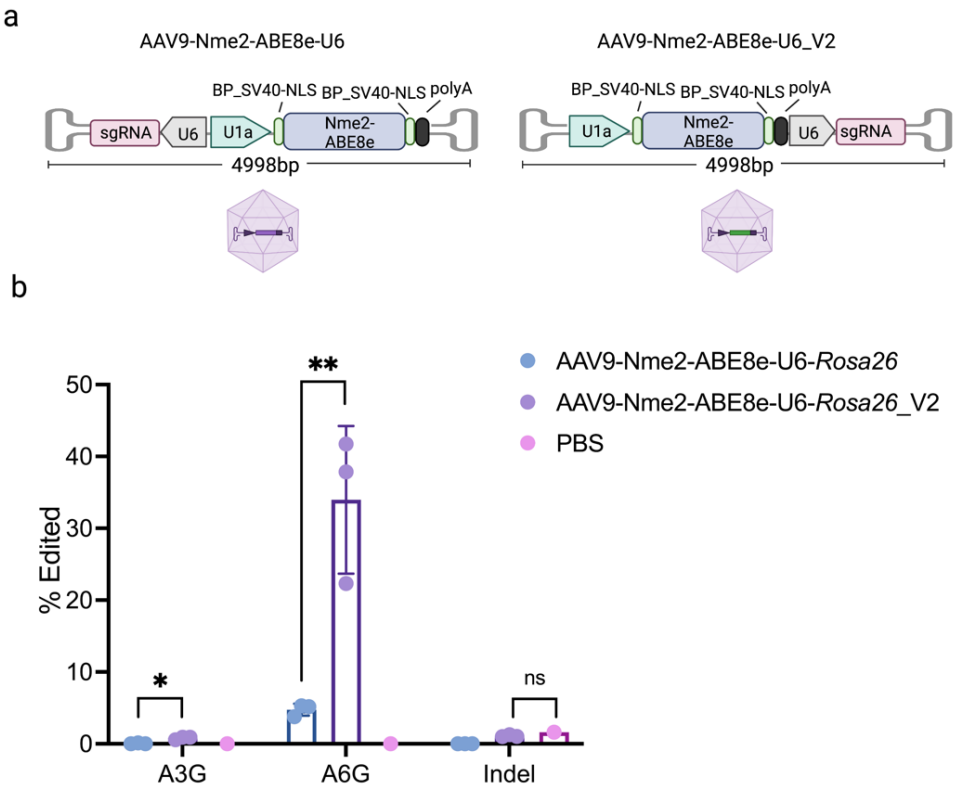
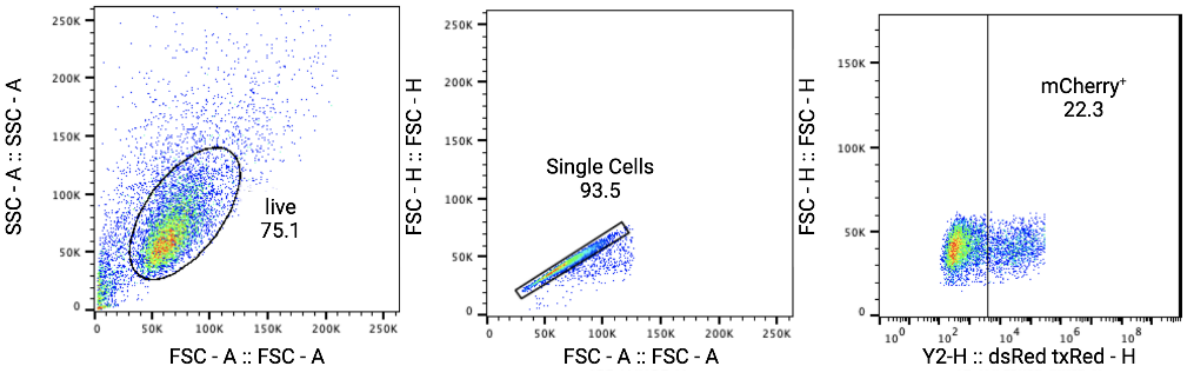
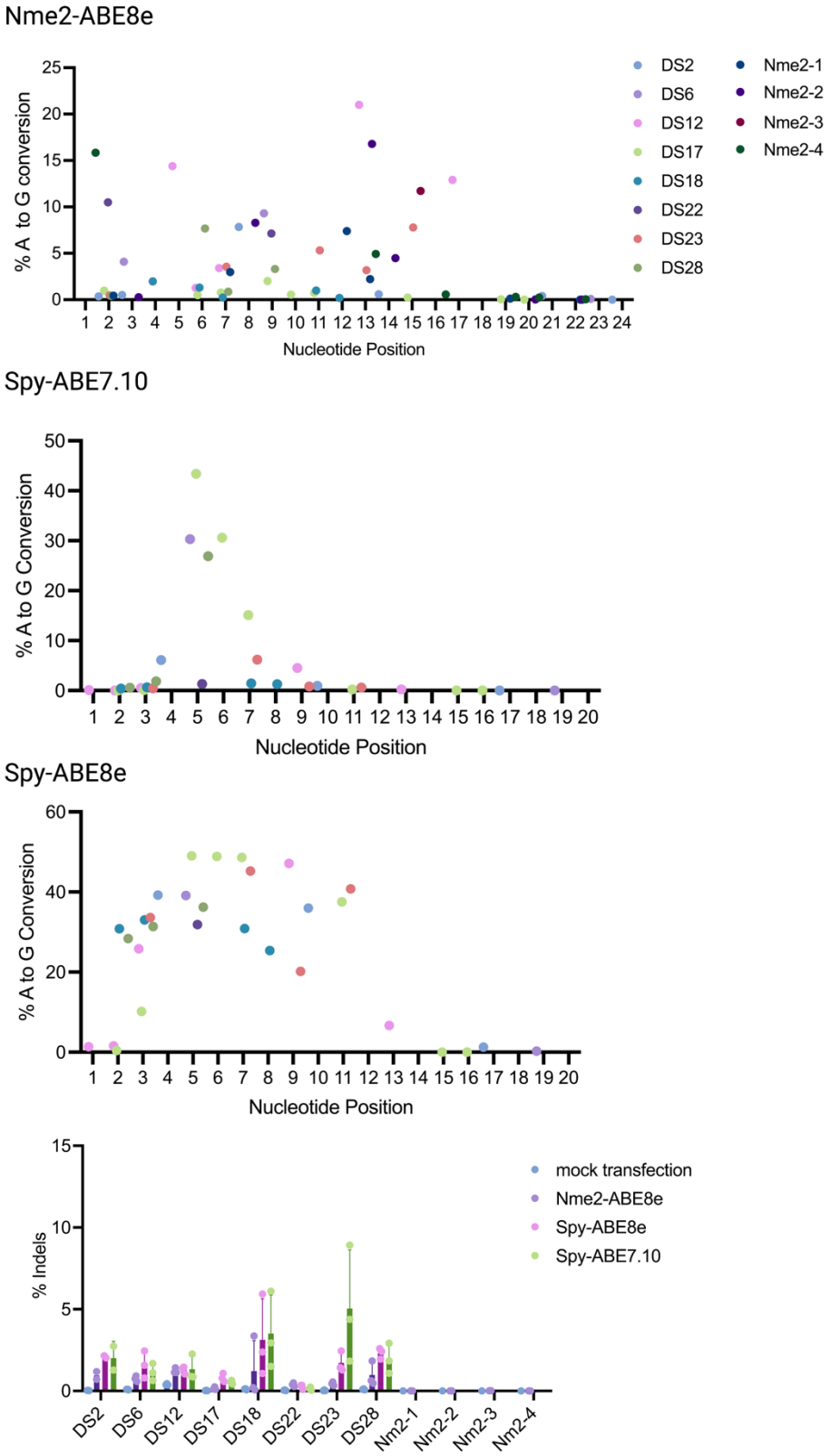


Figure 6

a). Schematic representation of the original (left) and the optimized AAV constructs (AAV9-Nm2-ABE8e-U6_V2, right). **b).** Quantification of the editing efficiency at the *Rosa26* locus by amplicon deep sequencing using mouse livers injected with indicated AAV (n = 3 mice in the AAV injected group, n = 1 mouse in the PBS injected group). Data from the original AAV configuration (**a**, left) is the same as that from the experiment shown in **Figure 5d**. Data represent mean ± SD, ns, P > 0.05; *, P<0.1; **, P < 0.01(one-way ANOVA).

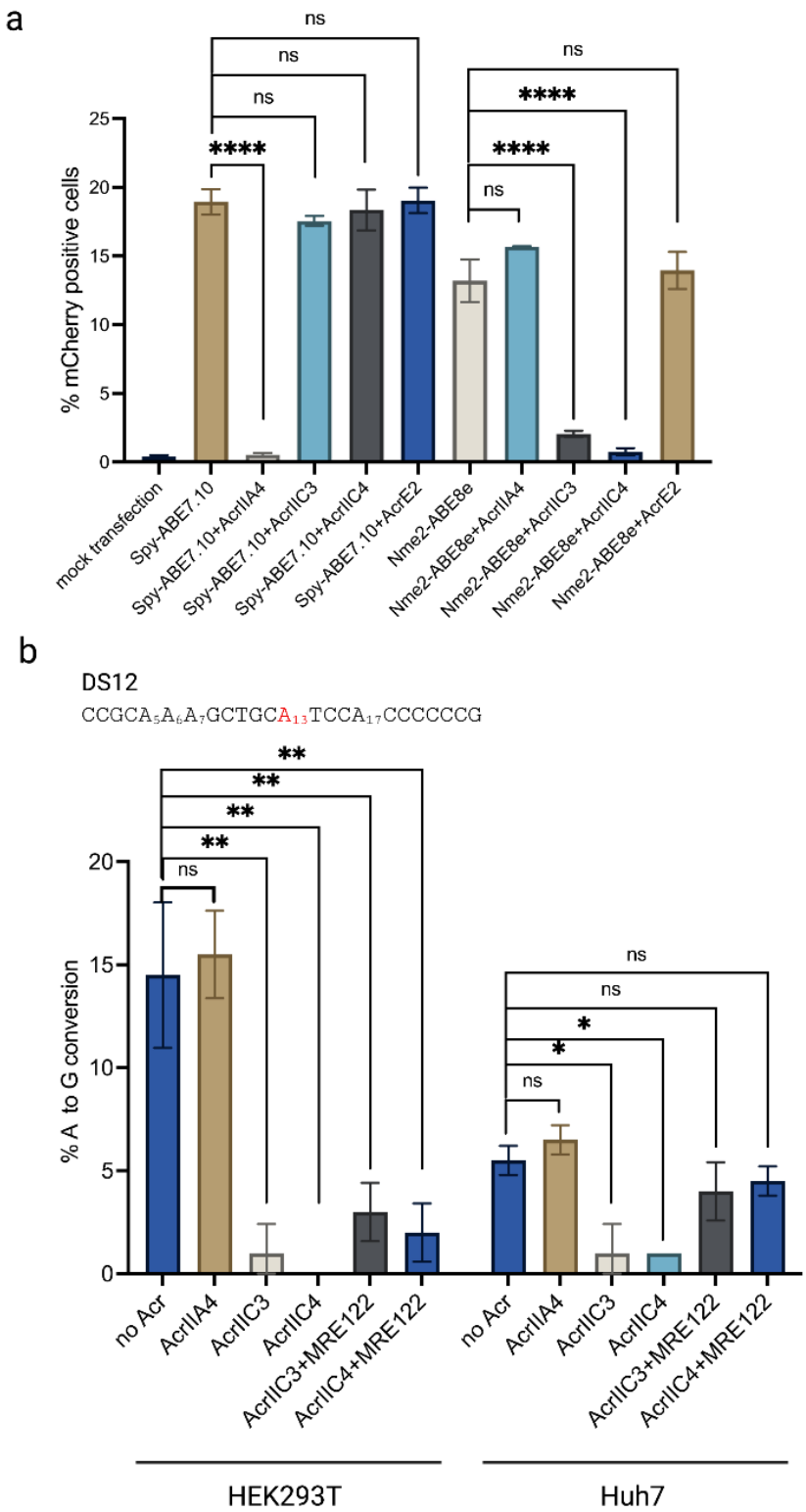


Supplementary Figure 1
Representative flow cytometry gating strategy for the ABE reporter cell line.



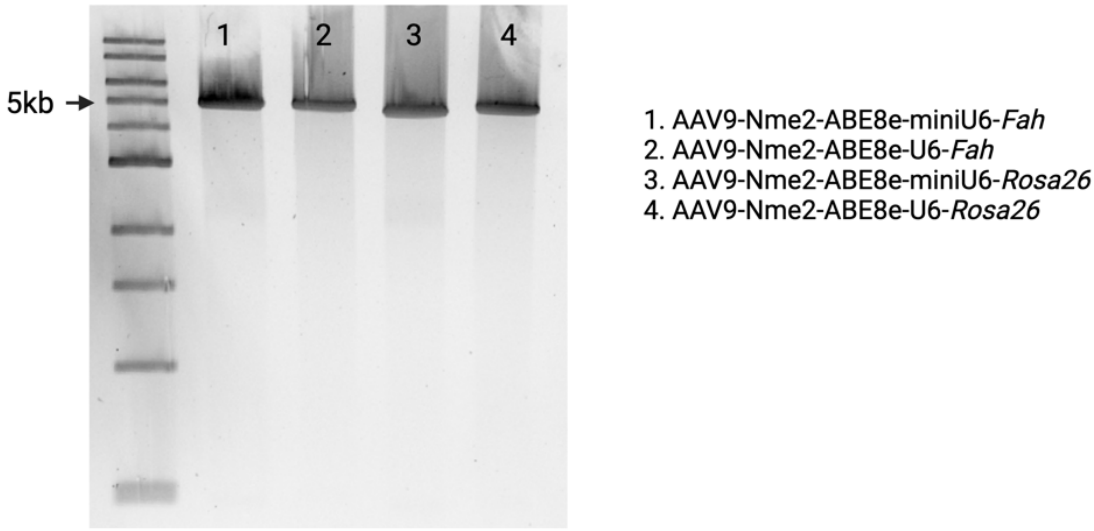
Supplementary Figure 2

Summary of the individual A-to-G conversion efficiency and the indel efficiency (bottom) at 12 target sites for Nme2-ABE8e, which includes 8 dual-target sites (DS 2 - 28) and 4 Nme2Cas9-specific target sites (Nme2 1 - 4), and 8 dual-target sites for Spy-ABE7.10 and Spy-ABE8e. Each data point represents the A-to-G conversion efficiency at the indicated nucleotide position measured by amplicon deep sequencing (n = 3 biological replicates).

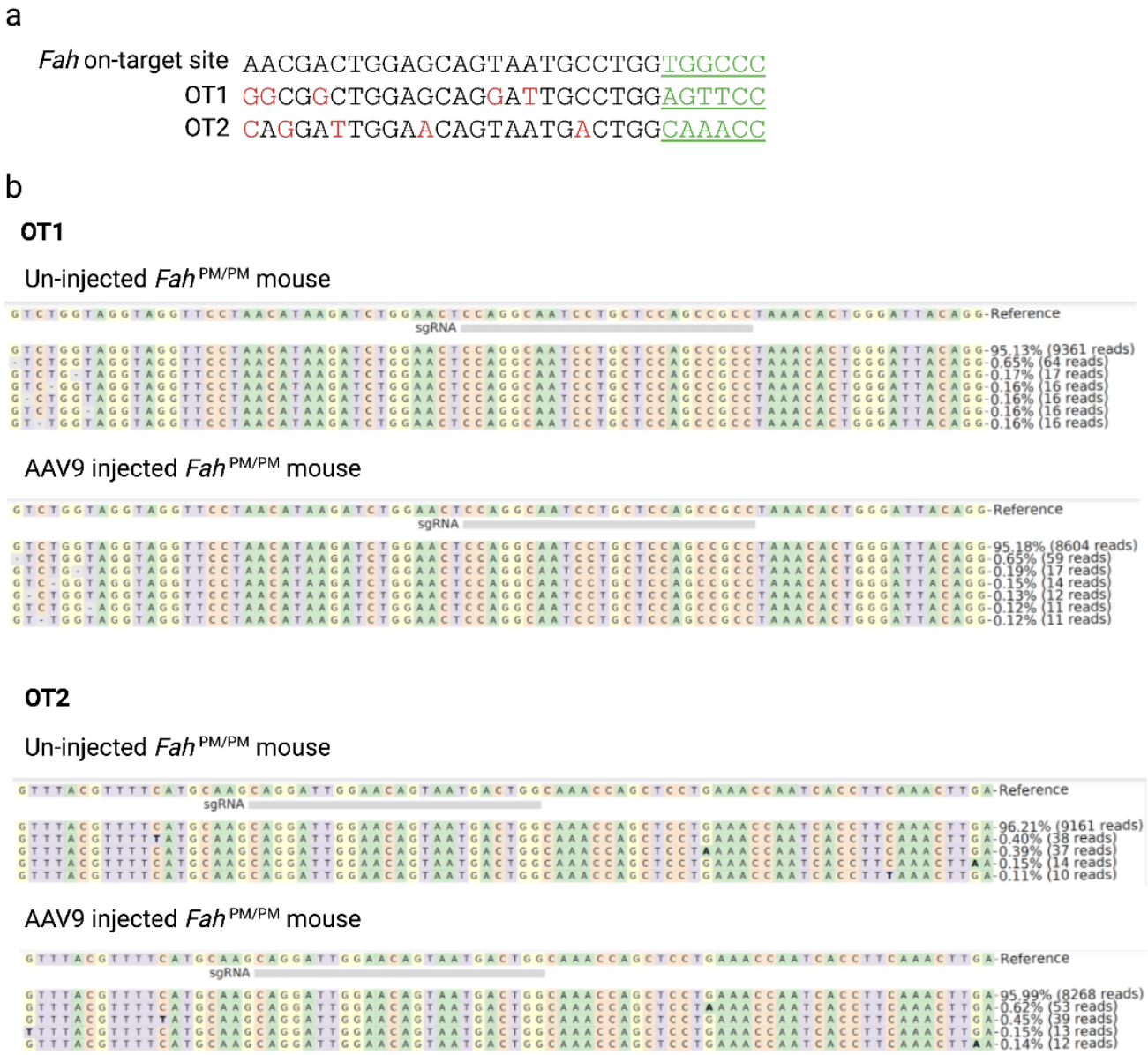


Supplementary Figure 3

a). Anti-CRISPR proteins inhibit the activity of either Spy-ABE7.10 or Nme2-ABE8e in the ABE reporter cell line as a function of their previously defined nuclease specificity (n = 2 biological replicates). **b).** Editing efficiency at the A12 (the highest edited adenine within the target) of the DS12 site by Nme2-ABE8e controlled by microRNA-repressible anti-CRIPSR proteins. Data generated by Sanger sequencing and EditR analysis (n = 2 biological replicates). Data represent mean ± SD; ns, P > 0.05; *, P < 0.05; **, P < 0.01; ****, P < 0.0001 (one-way ANOVA).



Supplementary Figure 4
Alkaline gel electrophoresis of AAV9 genomic DNA.



Supplementary Figure 5

a). Sequence of the *Fah* on-target site and two top-rated Cas-OFFinder predicted off-target sites for Nme2-ABE8e. Bases that are different from the on-target site are labeled in red. PAM, green, underlined. **b).** Representative amplicon deep sequencing reads at the predicted off-target sites in mice injected with AAV9 expressing Nme2-ABE8e and sgRNA-*Fah*.

Supplementary note

Nucleotide sequences of plasmids

Nme2-ABE7.10 construct in **Figure 1**

Legend: CMV enhancer, CMV promoter, NLS, **TadA**, linker, TadA7.10, Nme2Cas9-D16A, 3xHA, poly-A signal

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Nme2-ABE8e construct in **Figure 1 and Supplementary Figure 3a**

Legend: CMV enhancer, CMV promoter, NLS, TadA8e, linker, Nme2Cas9-D16A, 3xHA, poly-A signal

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CCGCCAAGCGCGTGAAGCTGGACGGCGGCAGCGGCGGCGGCAGCGGCGGCGGCAGCGGCggcggtagcggcggaggcagcg
gtggcggcagcggcAAGCGCACCGCCGACAGCCAGCACAGCACCCCCCCCAAGACCAAGCGCAAGGTGGGCGGCAGCGGCG
GCGGCAGCGGCGGCGGCAGCGGCTCCGGACCGGCAGCTAAGAGGGTGAAACTGGATtgaTAAgcggccgccaccgcggtggag
ctccagcttttgttccctttagtgagggttaatGAATTCGAGCCCAAGAAGAAGAGGAAAGTCTAACCGGTCATCATCACCATCACCAT
TGAGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCT
TGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGAAAATTGCATCGCATTGTCTGAGTAGGTGTCATT
CTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCG
GTGGGCTCTATGG

Spy-ABE7.10 construct in **Figure 1 and Supplementary Figure 3a**

Legend: CMV enhancer, CMV promoter, NLS, **TadA**, linker, TadA7.10, SpyCas9-D10A, 3xHA, poly-A signal

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTA
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TAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAA
GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACC
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CGGCAGCGGCCCGGCAGCTAAGAGGGTGAAACTGGATggcggtagcggcggaggcagcggtggcggcagcggccccctcgagccaaaga

agaagcggaaagtc**tctgaagtcgagtttagccacgagtattggatgaggcacgcactgaccctggcaaagcgagcatgggatgaaagagaagtccccgt**
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AATGAGGAGAACGAGGACATTCTTGAGGACATTGTCCTCACCCTTACGTTGTTTGAAGATAGGGAGATGATTGAAGAAC
GCTTGAAAACTTACGCTCATCTCTTCGACGACAAAGTCATGAAACAGCTCAAGAGACGCCGATATACAGGATGGGGGCG
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GGCCAGGGGGACAGTCTTCACGAGCACATCGCTAATCTTGCAGGTAGCCCAGCTATCAAAAAGGGAATACTGCAGACCG
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CCCAAATCCTTAAGGAACACCCAGTTGAAAACACCCAGCTTCAGAATGAGAAGCTCTACCTGTACTACCTGCAGAACGGC
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ACAGTCGCTTACAGTGTACTGGTTGTGGCCAAAGTGGAGAAAGGGAAGTCTAAAAAACTCAAAAGCGTCAAGGAACTGC
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GGGCGGCAGCGGCGGCGGCAGCGGCGGCGGCAGCGGCTCCGGACCGGCAGCTAAGAGGGTGAAACTGGATtgaTAAgc
ggccgccaccgcggtggagctccagcttttgttccctttagtgagggttaatGAATTCGAGCCCAAGAAGAAGAGGAAAGTCTAACCGGTCA
TCATCACCATCACCATTGAGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCC
TCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGAAAATTGCATCGCATTGTC
TGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGG
CATGCTGGGGATGCGGTGGGCTCTATGG

Spy-ABE8e construct in **Figure 1**

Legend: CMV enhancer, CMV promoter, NLS, TadA8e, linker, SpyCas9-D10A, 3xHA, poly-A signal

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTA
CATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCA
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GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACC
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ACAAGGTGCCGAGCAAAAAATTCAAAGTTCTGGGCAATACCGATCGCCACAGCATAAAGAAGAACCTCATTGGAGCCCT
CCTGTTCGACTCCGGGGAGACGGCCGAAGCCACGCGGCTCAAAAGAACAGCACGGCGCAGATATACCCGCAGAAAGAA
TCGGATCTGCTACCTGCAGGAGATCTTTAGTAATGAGATGGCTAAGGTGGATGACTCTTTCTTCCATAGGCTGGAGGAGT

CCTTTTTGGTGGAGGAGGATAAAAAGCACGAGCGCCACCCAATCTTTGGCAATATCGTGGACGAGGTGGCGTACCATGA
AAAGTACCCAACCATATATCATCTGAGGAAGAAGCTGGTAGACAGTACTGATAAGGCTGACTTGCGGTTGATCTATCTCG
CGCTGGCGCACATGATCAAATTTCGGGGACACTTCCTCATCGAGGGGGACCTGAACCCAGACAACAGCGATGTCGACAA
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GGCAGCGGCGGCGGCAGCGGCGGCGGCAGCGGCTCCGGACCGGCAGCTAAGAGGGTGAAACTGGATtgaTAAgcggccg
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CACCATCACCATTGAGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCC
CCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGAAAATTGCATCGCATTGTCTGA
GTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCAT
GCTGGGGATGCGGTGGGCTCTATGG

U6-sgRNA construct for Nme2-ABE in **Figure 1**
Legend: U6 promoter, **guide sequence**, guide RNA scaffold, U6 termination signal

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGACT
GTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGT
TTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGAC**NN**
NNNNNNNNNNNNNNNNNNNNNNGTTGTAGCTCCCTTTCTCATTTCGGAAACGAAATGAGAACCGTTGCTACAATAAG
GCCGTCTGAAAAGATGTGCCGCAACGCTCTGCCCCTTAAAGCTTCTGCTTTAAGGGGCATCGTTTATTTTTTT

U6-sgRNA construct for Spy-ABE in **Figure 1**
Legend: U6 promoter**, guide sequence**, guide RNA scaffold, U6 termination signal
GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGACT
GTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGT
TTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGAC**NN**
NNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAG
TGGCACCGAGTCGGTGCTTTTTTT

Single-AAV vector with 2xcMyc NLS in **Figure 3a**
Legend: ITR, U1a promoter, cMyc NLS, TadA8e, linker, Nme2Cas9-D16A, short poly-A signal, U6 promoter,
guide sequence, guide RNA scaffold, U6 termination signal
Cctgcaggcagctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgtcgggcgacctttggtcgcccggcctcagtgagcgagcgagcgc
gcagagagggagtggccaactccatcactaggggttcctgcggcctctagaGTTTAAACAAAAAAA
TAAACGATGCCCCTTAAAGCAGAAGCTTTAAGGGGCAGAGCGTTGCGGCACATCTTTTCAGACGGCCTTATTGTAGCAAC
GTTTCGGGAGCTACAAC**NNNNNNNNNNNNNNNNNNNNNNNN**GGTGTTTCGTCCTTTCCACAAGATATATAAAGCCA
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gg

Single-AAV vector with 3xcMyc NLS in **Figure 3a**

Legend: ITR, U1a promoter, cMyc NLS, TadA8e, linker, Nme2Cas9-D16A, short poly-A signal, U6 promoter, **guide sequence**, guide RNA scaffold, U6 termination signal

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Single-AAV vector with Ty1 NLS in **Figure 3a**

Legend: ITR, U1a promoter, Ty1 NLS, TadA8e, linker, Nme2Cas9-D16A, short poly-A signal, U6 promoter, **guide sequence**, guide RNA scaffold, U6 termination signal

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Single-AAV vector with 2xBP_SV40 NLS in **Figure 2d, 3a, and Supplementary Figure 3b**
Legend: ITR, U1a promoter, BP_SV40 NLS, TadA8e, linker, Nme2Cas9-D16A, short poly-A signal, U6 promoter, **guide sequence**, guide RNA scaffold, U6 termination signal

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Single-AAV vector with miniU6 promoter in **Figure 3c**
Legend: ITR, U1a promoter, BP_SV40 NLS, TadA8e, linker, Nme2Cas9-D16A, short poly-A signal, miniU6 promoter, **guide sequence**, guide RNA scaffold, U6 termination signal
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AAV-Nm2-ABE8e-U6_V2 in **Figure 6**

Legend: ITR, U1a promoter, BP_SV40 NLS, TadA8e, linker, Nme2Cas9-D16A, short poly-A signal, U6 promoter, **guide sequence**, guide RNA scaffold, U6 termination signal

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AcrIIA4 plasmid in **Supplementary Figure 3**
Legend: CMV IE94 promoter, AcrIIA4, SV40 polyA signal,
GACCATAGCCAATTCAATATGGCGTATATGGACTCATGCCAATTCAATATGGTGGATCTGGACCTGTGCCAATTCAATATG
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GAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTT

AcrIIC4-MRE122 plasmid in **Supplementary Figure 3b**
Legend: ITR, CMV enhancer, CB promoter, SV40 interon, AcrIIC4, FLAG, SV40 NLS, 3 x MRE122, BGh polyA, U6 promoter, **guide sequence**, guide RNA scaffold, U6 termination signal
ctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgtcgggcgacctttggtcgcccggcctcagtgagcgagcgagcgcgcagagaggga
gtgtagccatgctctaggaagatcaattcggtacaattcagctagcGTTTAAACAAAAAAATAAACGATGCCCCTTAAAGCAGAAGCTTTA
AGGGGCAGAGCGTTGCGGCACATCTTTTCAGACGGCCTTATTGTAGCAACGTTTCGGGAGCTACAAC**NNNNNNNNNNNN**
NNNNNNNNNNggtgtttcgtcctttccacaagatatataaagccaagaaatcgaaatactttcaagttacggtaagcatatgatagtccattttaaaaca
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ACCATTGTCACACTCCAACAAACACCATTGTCACACTCCACCATAgAGCTcgctgatcagcctcgactgtgccttctagttgccagccatct
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