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A luciferase reporter mouse model to optimize *in vivo* gene editing validated by lipid nanoparticle delivery of adenine base editors

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The rapid development of CRISPR genome editing technology has provided the potential to treat genetic diseases effectively and precisely. However, efficient and safe delivery of genome editors to affected tissues remains a challenge. Here, we developed luminescent ABE (LumA), a luciferase reporter mouse model containing the R387X mutation (c.A1159T) in the luciferase gene located in the *Rosa26* locus of the mouse genome. This mutation eliminates luciferase activity but can be restored upon A-to-G correction by SpCas9 adenine base editors (ABEs). The LumA mouse model was validated through intravenous injection of two FDA-approved lipid nanoparticle (LNP) formulations consisting of either MC3 or ALC-0315 ionizable cationic lipids, encapsulated with ABE mRNA and LucR387X-specific guide RNA (gRNA). Whole-body bioluminescence live imaging showed consistent restoration of luminescence lasting up to 4 months in treated mice. Compared with mice carrying the wild-type luciferase gene, the ALC-0315 and MC3 LNP groups showed 83.5% ± 17.5% and 8.4% ± 4.3% restoration of luciferase activity in the liver, respectively, as measured by tissue luciferase assays. These results demonstrated successful development of a luciferase reporter mouse model that can be used to evaluate the efficacy and safety of different genome editors, LNP formulations, and tissue-specific delivery systems for optimizing genome editing therapeutics.

INTRODUCTION

Base editors can precisely and permanently change a single base in the genome, providing the potential to address the root cause of genetic diseases.[1–3] The adenine base editor (ABE) is the fusion of an adenine deaminase with a catalytically inactive Cas protein. Most pathogenic human single-nucleotide polymorphisms (48%) are C-to-G to T-to-A conversions, making ABEs a desirable genome editing approach to treat diseases caused by such mutations.[1] Unlike conventional CRISPR-Cas9 systems, ABEs carry out precise A-to-G conversions at a specific location in the genome without introducing double-stranded DNA breaks (DSBs), which can create undesired insertions or deletions (indels). They can also act on both dividing and non-dividing cell types. While ABEs demonstrate great potential, the translation from cultured cells to therapeutics depends on their ability to edit the target gene safely and efficiently *in vivo*. Furthermore, many genetic diseases only affect specific tissues, making precise and targeted delivery a crucial factor in ensuring the safety and efficacy of gene editing.[4] Reporter animals, which facilitate the rapid visualization and quantification of editing at a tissue or organ level, are critical to advance the therapeutic applications of base editors. Fluorescence-based Cre-Lox reporter animals have been used for screening various delivery systems by delivering Cre mRNA to remove the STOP cassette and activate tdTomato expression.[5–7] To our knowledge, currently, only two fluorescence reporter animal models exist to visualize traditional DSB-mediated CRISPR-Cas9 editing *in vivo,* which are not suited for use with base editors.[8,9]

Here we report the development of luminescent ABE (LumA) reporter mice. These mice carry a firefly (*Photinus pyralis*) luciferase gene containing an ABE-correctable nonsense mutation that eliminates its activity. Following correction, restoration of enzymatic activity can be visualized by whole-body live luminescence imaging. We demonstrated that lipid nanoparticle (LNP)-mediated delivery of ABE (ABE8e) mRNA and mutation-specific guide RNA (gRNA) enables efficient repair of the mutation restoring luciferase activity in this reporter model.

RESULTS

Selection of the luciferase (Luc) R387X nonsense mutation

To develop this model, we took advantage of ABE's ability to convert stop codons back to an amino acid codon. The codons

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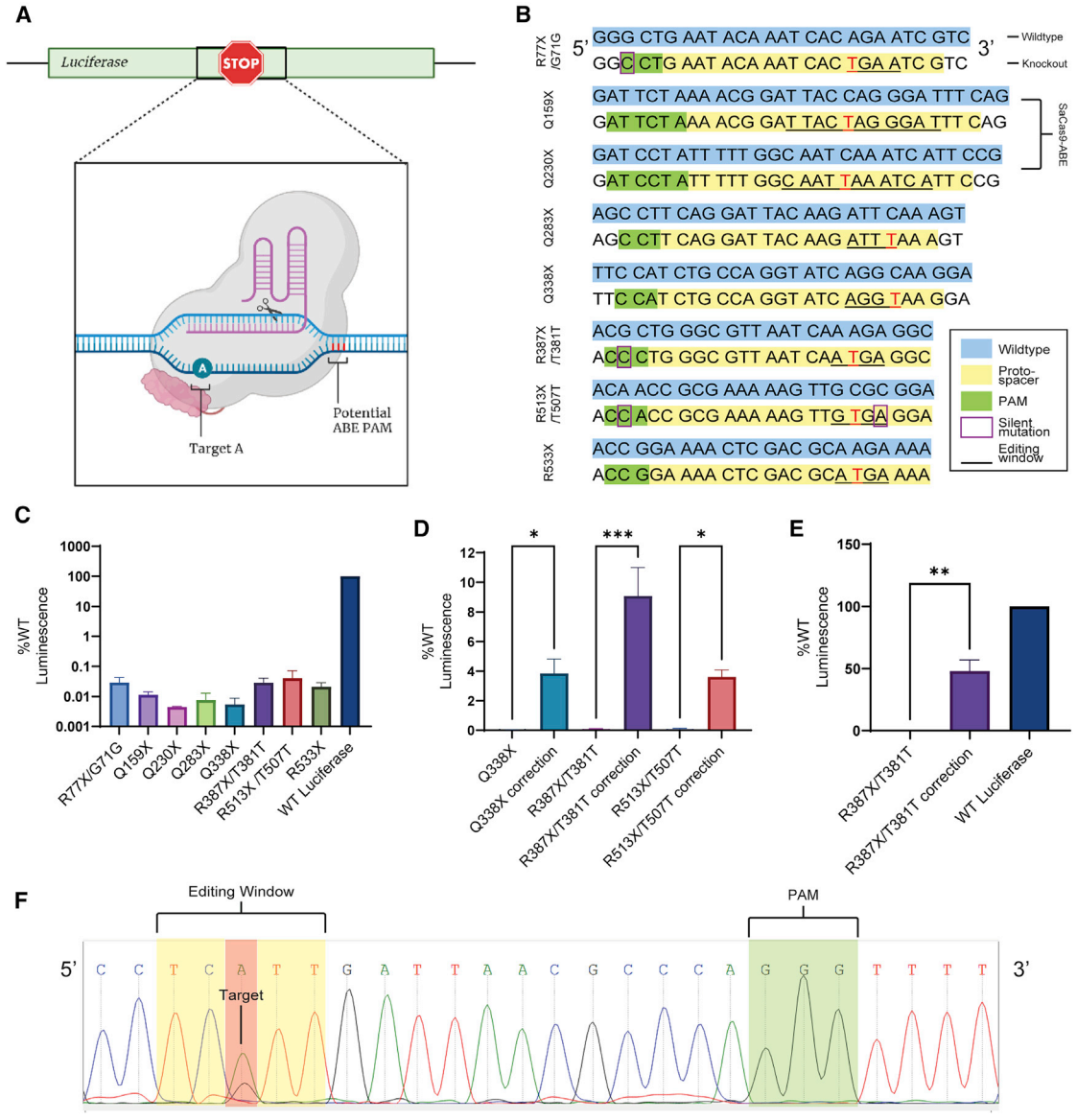

Figure 1. Mutation selection for generation of the luciferase reporter mouse model

(A) Schematic outline for designing ABE-correctable luciferase mutations. (B) Sequence of eight selected candidate mutations. Editing target nucleotide is in red. (C) Luciferase activity assay of the selected mutations R77X/G71G, Q159X, Q230X, Q283X, Q338X, R387X/T381T, R513X/T507T, and R533X by transient transfection of HEK293T cells. (D) Base editing correction analysis of the mutations R387X/T381T, R513X/T507T, and Q339X after co-transfection with ABE7.10 into HEK293T cells. (E) Base editing correction analysis of luciferase R387X/T381T in Flp-In Trex stable cell lines transfected with adenine base editor ABE8e. (F) Sanger sequencing of PCR-amplified genomic DNA extracted from base editing-corrected luciferase R387X/T381T stable cells. The editing efficiency was quantified with EditR.[10] Values and error bars indicate means ± SEM of n = 3 independent biological replicates. Means were compared using one-way ANOVA with Sidak correction. *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$.

CAA/CAG for glutamine (Q) and AGA/CGA for arginine (R) are potential sites to generate an in-frame, ABE-correctable stop codon, provided there is an appropriate PAM 12–17 bases downstream of the target nucleotide (Figure 1A). We screened the firefly luciferase sequence for CAA/CAG/CGA/AGA codons and the surrounding sequences for potential NGG PAM sites (SpCas9-ABE) or, less preferably, NNNRRT (SaCas9-ABE), which is associated with a broadened editing window (Figure S1). Eight candidate sites were identified (Figure 1B) and subsequently tested for luciferase activity by transfection of plasmids carrying each of the mutations into HEK293T cells (Figure 1C). All 8 mutations nearly eliminated luciferase function, with each demonstrating less than 0.012% activity of the wild-type control (Figure 1C). Potential bystander adenines within the editing window were then evaluated and resulted in

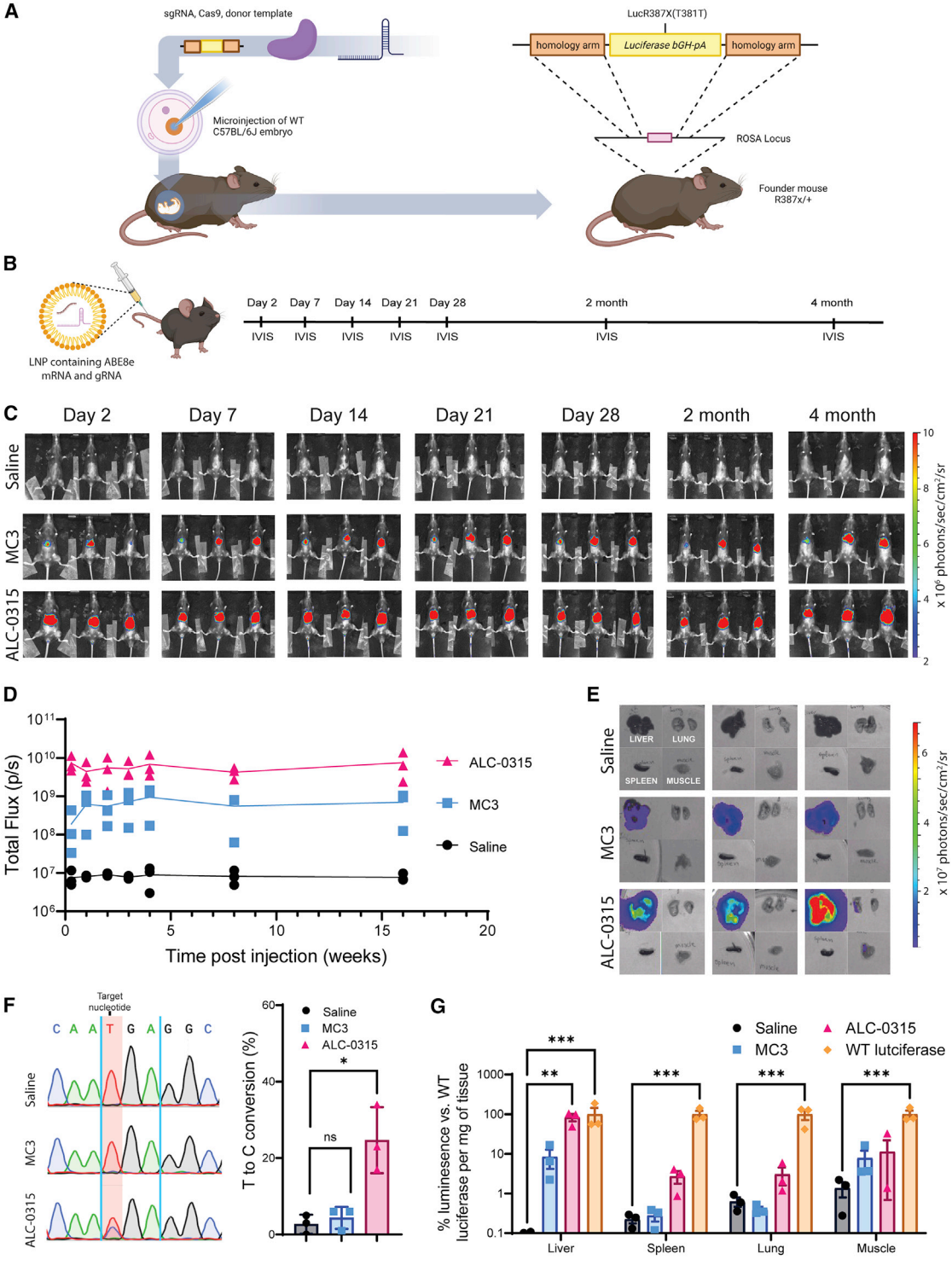

Figure 2. Lipid nanoparticle delivery of adenine base editor mRNA restored luciferase activity in the luciferase R387X mice

(A) Schematic representation of the full-length luciferase gene containing the desired R387X/T381T mutations was introduced in the *Rosa26* locus of the WT C57BL/6J mice to generate founder mice. (B) Outline of animal experiment design. Homozygous luciferase R387X mice were injected with 2 mg/kg body weight total RNA (ABE8e mRNA and gRNA) encapsulated in different lipid nanoparticle (LNP) formulations or with saline as negative control and imaged in IVIS imager system at the time points as indicated. (C) IVIS imaging of the mice 15 min after injected intraperitoneally with 150 mg/kg luciferin (exposure = 1 s). (D) Quantification of the bioluminescent signal detected from the

(legend continued on next page)

the elimination of R77X (bystander site in position A5); Q283X (bystander sites in positions A7 and A8); Q159X (multiple As); and Q230X (multiple As). R533X was placed as backup due to its proximity to the C terminus. Three candidate mutations, Q338X, R387X(/T381T), and R513X(/T507T), were shortlisted and tested by ABE base editing correction analysis. The silent mutations, T381T and T507T, were added to create a new PAM site required for the binding of the base editor. Mouse codon usage was referenced to ensure expression in mice.

Co-transfection of the three luciferase mutants into HEK293T cells with ABE7.10 plasmid, and the corresponding gRNA showed that R387X(/T381T) had the highest correction rate (9.08% ± 1.92%) compared with the wild-type, followed by Q338X (3.85% ± 0.97%) and R513X/T507T (3.61% ± 0.47%) (Figure 1D). Furthermore, transfection of a Flp-In T-Rex 293 stable cell line expressing a single copy of R387X(/T381T) luciferase gene with ABE8e plasmid led to 29% A-to-G conversion as shown by Sanger sequencing and restored luciferase activity by 51.47% ± 6.28% compared with wild-type luciferase (Figures 1E and 1F). Thus, the R387X(/T381T) mutation was selected to generate our luciferase ABE mouse model.

Generate the LumA mouse model from C57BL/6J mice

Microinjection of CRISPR-Cas9 homology-directed repair (HDR) machinery was used to generate LumA mice. C57BL/6J mice were used as the starting point to introduce LucR387X/T381T at the *Rosa26* locus (Figure 2A). Founder mice were backcrossed for up to 5 generations to confirm that this mouse line ubiquitously expresses the designed mutations (Figure S2).

In vivo base editing restored luminescent signal in the LumA mouse model

To test if our luciferase knockin mice can serve as a suitable CRISPR base-editable model, we sought to repair the mutation by delivering the base editing cargo with LNPs. LNPs have been used to successfully deliver small interfering RNA (siRNA) molecules (Onpattro) for the treatment of the hereditary transthyretin-mediated amyloidosis and with the BioNTech and Moderna COVID-19 mRNA vaccines to deliver longer RNA molecules.[11–13] Two LNP formulations consisting of either DLin-MC3-DMA (MC3) or ALC-0315 ionizable cationic lipids, which are similar to either the Onpattro or BioNTech vaccine formulations, were chosen and co-encapsulated with ABE8e mRNA (~5,000 nucleotides) and mutation-specific gRNA (~100 nucleotides) (Figure 2B). Overall, no significant differences in LNP quality between formulations after encapsulation were observed (Table S1). The LNPs were administered intravenously, and successful base editing resulted in a luminescence signal that can be detectable by whole-body live imaging following intraperitoneal (i.p.) injection of luciferase substrate Luciferin.

Mice that received ABE mRNA/gRNA encapsulated in MC3 and ALC-0315 formulations produced significant luminescence signals in the liver 2 days after the administration, indicating successful base editing (Figure 2C). ALC-0315 mice showed higher levels of luminescence than the MC3 mice. Both formulations resulted in a stable, consistent luminescence signal after 7 days, lasting for at least 4 months after the initial single administration (Figures 2C and 2D).

Four months after LNP-ABE8e injection, mice were sacrificed, and the liver, lung, spleen, and gastrocnemius muscle were collected and imaged with the IVIS imaging system (Figure 2E). Overall, high luminescence was detected in the liver of mice in the ALC-0315 group, and a lower signal was seen in the MC3 group. No detectable signal was observed by IVIS in other organs collected for either formulation. Sanger sequencing of genomic DNA from mouse liver samples showed an average A-to-G editing efficiency of 24.6% ± 5% for the ALC-0315 formulation, while only a trace correction was observed for the MC3 formulation (Figure 2F).

To further examine the effect of LNP-delivered ABE at the organ levels, a tissue luciferase assay was performed (Figure 2G). Liver luciferase assay results showed an average restoration of 83.5% ± 17.5% relative to wild-type (WT) luminescence in ALC-0315 mice, whereas an average restoration of 8.4% ± 4.3% of WT luminescence was observed in the MC3 mice (Figure 2G). No luciferase signal above the background level was detected in the lungs and muscle for both formulations (Figure 2G). Only a low luciferase signal was detected in the spleens of the mice injected with the ALC-0315 formulation (Figure 2G), which was not detectable when directly imaged in the IVIS system.

DISCUSSION

The field of genome editing has progressed rapidly. Newer generations of base editors have been developed that overcome the initial PAM site limitations by accepting alternative sequences beyond the original NGG.[14–18] New Cas9 and deaminases have also been engineered to increase on-target editing and reduce off-target editing.[19–23] These innovative gene editors have broadened the potential therapeutic applications of genome editing. However, the safe and effective delivery of gene editing components remains a major hurdle to overcome for future therapeutic applications.

Several *in vitro* base editing reporter systems have been developed.[24–31] One key advantage of these systems is that they will only be turned on after base editing and not by indels, making them very suitable models

experiment in areas of interest. (E) IVIS imaging of freshly isolated tissues. (F) Representative Sanger sequencing results from each group. The percentage of T-to-C conversion was quantified with EditR and presented as a bar graph.[10] n = 3 animals. Means were compared using two-way ANOVA with Tukey's test. (G) Luciferase activity of homogenates from isolated liver, spleen, lungs, and muscle. The luciferase activity was measured using Steady Glo luciferase system (Promega). The luminescence readings were standardized based on the weight of the tissue and expressed as percentage of signal from the wild-type tissue. Values represent mean ± SEM of n = 3 animals. Means were compared using two-way ANOVA with Dunnet correction, and treatment groups were compared with saline for each tissue. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

to compare the editing efficiency of different base editors or to enrich for base-edited cells. However, all current base editing reporter systems have been created in cell lines only. In addition, as these systems typically rely on green fluorescent proteins (GFP), which permit gene editing at the single-cell level through flow cytometry, their *in vivo* sensitivity may be affected by inherent cellular background fluorescence. In contrast, a bioluminescence-based reporter system, such as our LumA mice, is sensitive, and luciferase activity will only be measured from live cells. *In vivo* luminescence measurement is non-invasive and allows long-term assessment of gene editing since animals do not need to be euthanized.

In this article, we developed a luciferase mouse model, named LumA mice, containing the R387X/T381T that is correctable by SpCas9-ABE. The introduced R387X mutation can be corrected *in vivo* by intravenous injection of LNPs encapsulated with the ABE8e mRNA and the mutation-specific gRNA. These results demonstrated that just a single intravenous injection of LNPs encapsulating base editing components resulted in effective editing to permanently restore luciferase activity up to 83% in the liver of LumA mice. We have also shown that successful gene editing in the LumA mice was sustained up to 4 months without changes in luminescence signal. Thus, the LumA mouse model provides a valuable tool to study the long-term effects of gene editing and to evaluate the efficacy and safety of repeated doses/injections without sacrificing the mice.

The field of gene editing has progressed rapidly in the last few years. However, rigorous testing and optimization in animal models are required to examine the efficacy and safety of genome editing prior to clinical applications. To help achieve this, LumA, a reporter gene mouse model, was generated and is compatible with base editing. Combining this with the stable cell line containing the same mutation, it is now possible to systematically test different aspects of gene editing both *in vitro* and *in vivo*. This includes, but is not limited to, comparing different gene editors (base editing, prime editing, and the conventional HDR-based CRISPR-Cas9 system, etc.), optimizing ratios of gene editor to gRNA to improve on-target editing efficiency, exploring the effects of different modifications on stabilizing the gene editor mRNA and/or gRNA, and evaluating gRNA-dependent off-target effects using deep-sequencing approaches.

A major hurdle for application of therapeutic gene editing is delivery to the target tissue. The field of nanomedicine exploded after the development of mRNA-based COVID-19 vaccines developed by Pfizer-BioNTech and Moderna. Efforts have already been shifted to focus on LNP-mediated delivery of CRISPR-Cas9 systems. For instance, ionizable cationic lipid formulations can deliver base editor mRNA and sgPCSK9 both *in vitro* (primary human and cynomolgus monkey hepatocytes) and *in vivo* (mouse and cynomolgus monkey livers).[32,33] Recently, the first base editing phase Ib clinical trial for heterozygous familial hypercholesterolemia started in July 2022 (ClinicalTrials.gov: NCT05398029).[34] This trial targets the liver—a common site for LNP delivery and gene editing. The fact that the LumA mouse model contains the luciferase nonsense mutation in all tissues in the body makes it an excellent model to test different LNP formulations targeting many tissues such as the spleen and lungs. It is possible to further streamline the process of vetting novel formulations, allowing for rapid testing of thousands of formulations with an iterative process. One innovative way for high-throughput *in vivo* screen of functional mRNA delivery is Fast Identification of Nanoparticle Delivery (FIND), which quantifies functional RNA delivery mediated by LNPs.[35–37] This approach can simultaneously perform multiple LNP delivery experiments in a single mouse by encapsulating RNA (siRNA/Cre mRNA) and a DNA barcode that allows for cell sorting and sequencing to identify tissue specific LNP delivery. We believe the LumA mouse model complements the FIND approach, where newly identified LNPs can then be analyzed in our model for its compatibility with *in vivo* CRISPR-Cas9 gene editing. Furthermore, different routes of administration can also lead to accumulation of LNPs to various tissues, which will be a fascinating area of research that lends particularly well to the LumA mouse model. An important characteristic of our luciferase models is that they are sensitive such that restoration of even 0.1% of WT signal can still be easily imaged using the IVIS instrument.

In summary, the LumA reporter mouse model provides an *in vivo* platform for developing new genome editing therapeutics, testing various genome editing cargo, rapidly evaluating and optimizing different delivery systems, and screening tissue-specific targeting.

MATERIALS AND METHODS

Luciferase plasmids subcloning

The WT and mutant firefly luciferase sequences (custom synthesized, GenScript) were excised with HindIII and ApaI (New England Biolabs) from pcDNA3.1 (Addgene plasmid #18964) and cloned into pcDNA5/FRT/TO vector (Thermo Fisher Scientific) and confirmed through Sanger sequencing.

ABE mRNA and gRNA

Base editor mRNA was purchased from TriLink. Single gRNAs were purchased from Synthego or Integrated DNA Technologies (IDT). When separate sequence-specific CRISPR RNAs (crRNAs) and SpCas9 universal tracrRNA were used, prehybridization was performed according to manufacturer's protocol (IDT). gRNA expression plasmids (under the human U6 promoter) were prepared according to protocol previously described.[38]

Cell culture and transfections

HEK293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (Gibco, qualified), Antibiotic-Antimycotic (Gibco), and L-glutamine (Gibco). The Flp-In-T-Rex cells (Thermo Fisher Scientific) were cultured in the same media with additional hygromycin at 200 μg/mL (Gibco). All cell types were incubated, maintained, and cultured at 37°C with 5% CO_2.

HEK293T cells were seeded in 96-well plates and transfected with 0.3 μL Lipofectamine 2000 (Thermo Fisher Scientific) according to

the manufacturer's protocols, and either 20 ng pcDNA3 plasmid containing candidate luciferase mutation sequences only or with 75 ng ABE7.10 plasmid (Addgene), 25 ng prehybridized gRNA, and 2 ng Renilla luciferase as transfection control. Cells were cultured for 2 days following transfection, the media were removed, and the cells were lysed using dual-luciferase assay (Promega) according to manufacturer's protocols for luciferase activity assay.

Generation of Flp-In-T-Rex stable cell lines

Stable cell lines harboring WT or mutant luciferase sequences were generated using the Flp-In T-Rex System, which contains a hygromycin-resistant gene (Thermo Fisher Scientific) for selection, according to manufacturer's protocols. Briefly, Flp-In T-Rex host cells were seeded in a 6-well plate (Corning), and 4 h post-seeding, cells were transfected at 70%–90% confluency with 6 μL Lipofectamine 2000 (Thermo Fisher Scientific), and 1,500 ng total plasmid consists of 1,350 ng pOG44 recombinase and 150 ng either luciferase WT or mutant plasmids at 9:1 weight ratio. 48 h post-transfection, cells were split, diluted, and placed into new 6-well cell culture plates, and 50 μg/mL hygromycin was added to the culture media for selection of cells that were transfected and successfully incorporated the luciferase sequences into the genome. Culture media containing 50 μg/mL hygromycin were changed every 4–5 days for continuous selection of cells that have stably integrated the desired DNA sequences.

In vitro base editing analysis

Cells expressing the WT luciferase and luciferase mutations were seeded on 48-well poly-D-lysine-coated plates and transfected with 0.75 μL Lipofectamine 3000 (Thermo Fisher Scientific) according to the manufacturer's protocols and 750 ng ABE8e plasmid, 250 ng prehybridized gRNA plasmid, and 2 ng Renilla luciferase as transfection control. Cells were cultured 3 days following transfection, the media were removed, and the cells were collected for genomic DNA extraction or lysed for the dual-luciferase assay (Promega) according to the manufacturer's protocols. Genomic DNA was extracted by using the Dneasy Blood & Tissue Kits (Qiagen) for PCR amplification and Sanger sequencing.

Luciferase assay

Dual-luciferase assays were performed according to manufacturer's protocol (Promega). Briefly, 3 days post-transfection, cell culture media were removed, and cells were lysed with 75 μL passive lysis provided in the kit and incubated for 15 min with gentle shaking to dislodge the cells. Then, 70 μL lysed cells were transferred to 96-well white assay plates. To measure the luminescence from the firefly luciferase, 70 μL LARII was added per well, and the luminescence was measured using the FlexStation 3 (Molecular Devices). The luminescence from the Renilla luciferase was measured by adding 70 μL Stop & Glow reagent per well.

Mouse embryo microinjections to generate the LumA mice

CRISPR-Cas9 HDR was used to generate the LumA mice by microinjection of SpCas9 protein, gRNA (5′-GCAGATCACGAGGGAAGAGG-3′), and a DNA donor template sequence. The full-length luciferase gene containing the R387X/T381T mutation was introduced directly into the *Rosa26* locus of WT C57BL/6J mice. Five founders (N0 mice) were obtained from the microinjection, and Sanger sequencing confirmed the desired full-length luciferase knockin allele. All founders were backcrossed to generate thirteen N1 animals that were positive for the luciferase R387X/T381T insertion in the *Rosa26* locus. The microinjection, founder and N1 generation genotyping were done by the Jackson Laboratory. Subsequently, the luciferase R387X/T381T N1 mice were backcrossed with C57BL/6J mice for up to 5 generations. Consistent genotyping and sequencing results from each generation showed that this mouse line has been stably established and expresses the luciferase R387X/T381T mutation ubiquitously under the endogenous *Rosa26* promoter.

Mouse genomic DNA extraction and genotyping

Mice were ear notched for identification and genotyping. Genomic DNA was extracted by adding 50 mM NaOH and incubating at 95°C for 10 min. Then, 0.1 M Tris (pH 8) was added per sample before centrifugation for 6 min at 13,000 RPM. Luciferase genotyping was done with primers LucF664 (5′-GCCAGAGATCCTATTTTTGGC-3′)/LucR1626 (5′-CGATCTTTCCGCCCTTCTTG-3′). Sanger sequencing was used to examine the incorporation of desired nucleotides. For confirmation, the full-length luciferase gene containing the R387X/T381T mutations was inserted into the *Rosa26* locus, and primers ROSA-F9386 (5′- TTGCTCTCCCAAAGTCGCTC-3′)/ROSA-R9601 (5′-CTACTCAGACAATGCGATGC-3′) were used to confirm incorporation at the correct location.

Mouse breeding

Five founder (N0) mice were obtained and backcross bred with WT C57BL/6J to generate N1 mice. Genotyping and sequencing were performed to confirm the successful incorporation of luciferase, mutations, and transmissions at the *Rosa26* locus in mouse genome. Once N5 mice were obtained, the mice were inbred to obtain those that were homozygous for the LucF387X/T381T mutations.

Mice expressing the WT luciferase gene that acted as positive control were obtained after breeding FVB.129S6(B6)-Gt(ROSA)26Sortm1(Luc)Kael/J (the Jackson Laboratory strain #005125) with B6N.FVB-Tmem163Tg(ACTB-cre)2Mrt/CjDswJ (the Jackson Laboratory strain #019099). The offspring were further bred with C57BL/6J mice to obtain WT luciferase mice on the same C57BL/6J background as LumA mice. After five backcross generations, the offspring were then inbred to produce mice that were homozygous for the luciferase gene, which were used as positive control for the animal study.

LNP formulation

LNPs were prepared by injecting volumes of a lipid mixture (ionizable lipid, DSPC, cholesterol, and PEG-DMG with an amine-to-phosphate ratio [N/P] of 6) at appropriate ratios dissolved at 10 mM total lipid concentration in anhydrous ethanol with an aqueous phase containing mRNA dissolved in 25 mM sodium acetate (pH 4) through a Tee-junction with a 3:1 aqueous:ethanol (v/v). Flow rates were 5 mL/min

for the ethanolic lipid phase and 15 mL/min for the aqueous mRNA phase for a total output flow rate of 20 mL/min. The resulting formulations were then dialyzed in Spectra/Por 2 dialysis tubing with a 12–14 kD MWCO (Repligen, Waltham, MA, USA) against 1,000-fold volumes of phosphate-buffered saline (pH 7.4) over a period of 16 h at room temperature. Formulations were then concentrated by centrifugation at 1,500 × *g* in Amicon Ultra-15 Centrifugal Filter Units (MilliporeSigma, Burlington, MA, USA).

Animal experiments

Mouse experiments were performed in accordance with protocols approved by the University of British Columbia (UBC) animal care committee. Mice were housed in a pathogen-free animal facility at the Modified Barrier Facility at UBC and kept in a temperature- and humidity-controlled room on a 12 h light/dark cycle.

Mice were intravenously injected with 2 mg/kg total RNA encapsulated in LNPs. Mice from both sexes were used (2 females and 1 male per group).

D-luciferin (potassium salt) powder (Cayman Chemical) was dissolved in DPBS to make a fresh stock solution at 15 mg/mL and filter sterilized through a 0.2 μm filter. Mice were injected with luciferin at 150 mg/kg i.p. 15 min before *in vivo* imaging with IVIS Lumina II.

For mouse tissue imaging, mice were sacrificed by deep anesthesia under isoflurane followed by cervical dislocation. Livers, lungs, spleens, and muscles were harvested and placed on petri dishes. Tissues were imaged at 60 s exposure and then cut into three pieces and snap frozen individually with liquid nitrogen.

Tissue luciferase assay

Luciferase activity from the tissues was measured with the Steady Glo Luciferase Assay System (Promega). Mouse tissues were weighed before adding 500 μL GLO lysis buffer. Tissues were then homogenized with FastPrep homogenizer (MP Biomedicals) at speed "6" for 20 s for a total of 3 rounds. The homogenized samples were diluted with GLO lysis buffer (50 μL stock lysate +150 μL lysis buffer) on a clear plate. Then, 50 μL diluted sample was transferred to a new empty white plate, where 50 μL Steady Glo substrate was added per well and read with the FlexStation 3 microplate reader (Molecular Devices). The luminescence reading of the tissues was normalized to the weight of tissue in the homogenate.

Statistical analysis

Statistical analysis was performed in GraphPad Prism.

DATA AVAILABILITY

All data that support the conclusions of this article can be found in the main text or the supplemental material. LumA mice are available under materials transfer agreements (MTAs) with the University of British Columbia (UBC) under the stock number JAX Stock No. 038165 LumA.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.ymthe.2023.02.009.

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AUTHOR CONTRIBUTIONS

C.J.D.R., L.-H.Z., S.-Y.Y., and T.C. conceived the research. S.-Y.Y., T.T., A.B., G.B., D.K., and C.H. performed the experiments. This work was supervised by C.J.D.R., L.-H.Z., and J.K. S.-Y.Y. wrote the original draft, and S.-Y.Y., T.C., T.T., A.B., G.B., D.K., C.H., J.K., L.-H.Z., and C.J.D.R. reviewed and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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