Development of a luciferase reporter mouse model for adenine base editing

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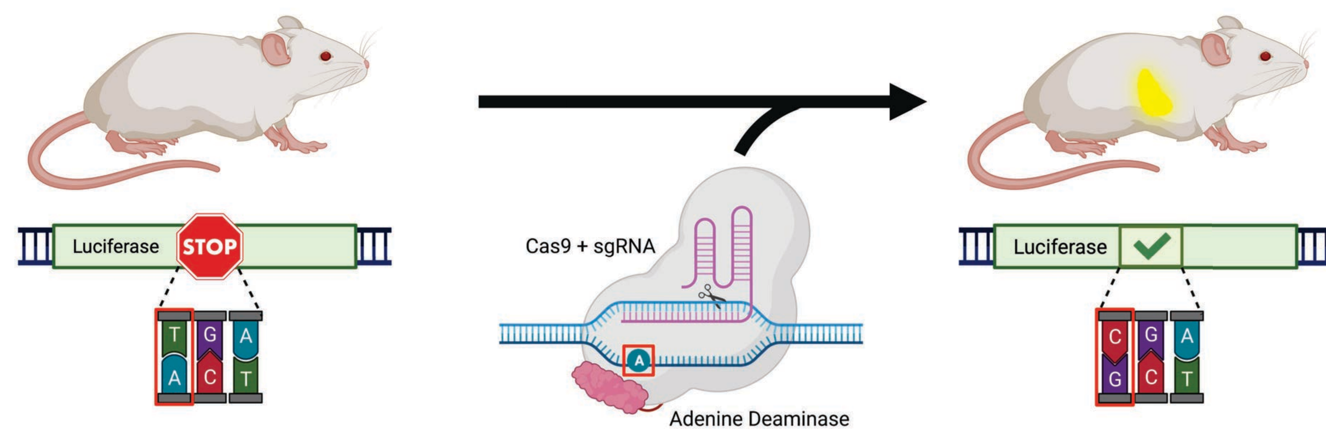
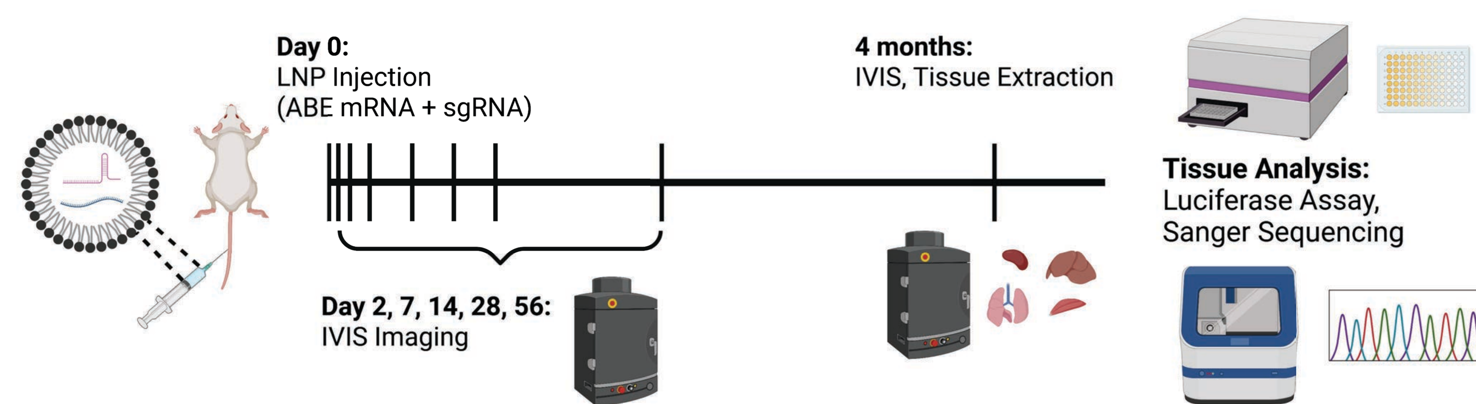
INTRODUCTION

The rapid development of CRISPR-Cas9 genome editing technology has advanced our knowledge of biological systems and provided the potential to treat genetic diseases precisely and effectively more than ever before. However, efficient delivery of genome editors to affected tissues remains a key challenge. While *in vitro* cell culture systems play a key role in the initial development of genome editing therapeutics, they are not suitable to evaluate *in vivo* efficacy, as they cannot yet effectively predict the delivery and biodistribution of gene editing components. To address this problem, our lab has developed a novel reporter mouse model that couples the production of a luminescent signal with the physical locations of successful gene editing. This model is designed for use with an adenine base editor (ABE), a CRISPR/Cas9-based technology that converts A-T base pairs to G-C, providing the potential to treat ~48% of known human pathological single nucleotide variants.

METHODS



Mice carry a copy of the firefly (*Photinus pyralis*) luciferase gene containing a nonsense mutation inactivating its luminescence-producing enzymatic activity. Upon correction and restoration of enzymatic activity, luminescence produced by the functional luciferase protein can be used to visualize the biodistribution and efficacy of gene editing.



As an initial validation study, we use clinically approved lipid nanoparticle (LNP) formulations to deliver ABE mRNA/sgRNA via intravenous (IV) administration. The efficacy and biodistribution of gene editing was examined by whole-body IVIS imaging of live animals, luciferase enzyme assays of terminally collected tissues, and Sanger sequencing.

RESULTS

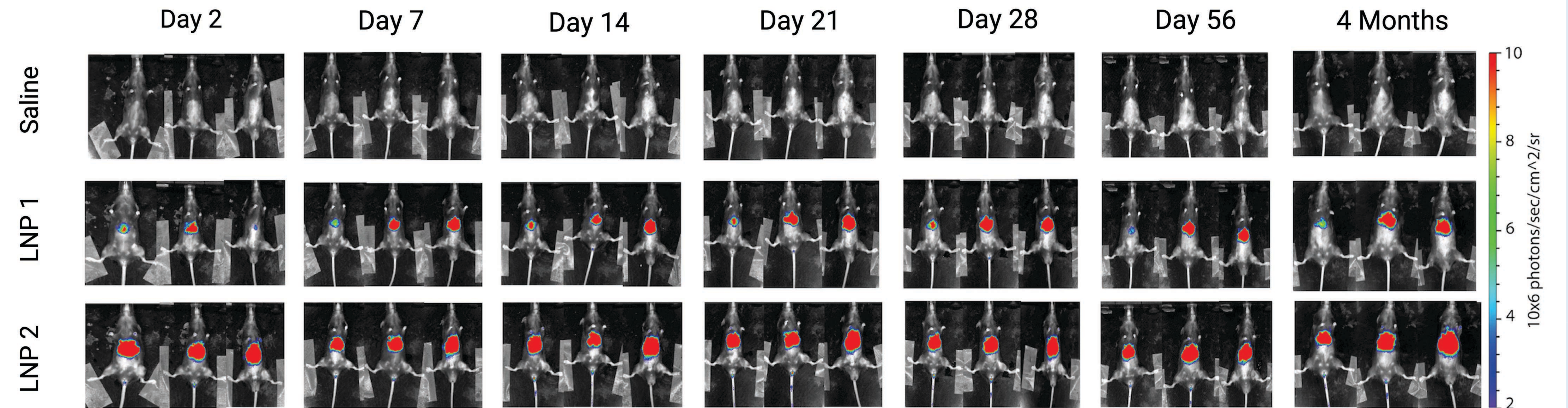


Figure 1. IVIS live imaging of luciferase reporter mice following a tail vein injection of either saline or LNPs encapsulating ABE mRNA + sgRNA (2 mg/kg total RNA). Images were taken 15 minutes following an intraperitoneal injection of D-luciferin (150 mg/kg) to induce luminescence (exposure time = 1s). Mice were aneasthetized throughout the duration of imaging via isofluorane. Signal indicates restoration of luminescence and is therefore representative of successful gene editing.

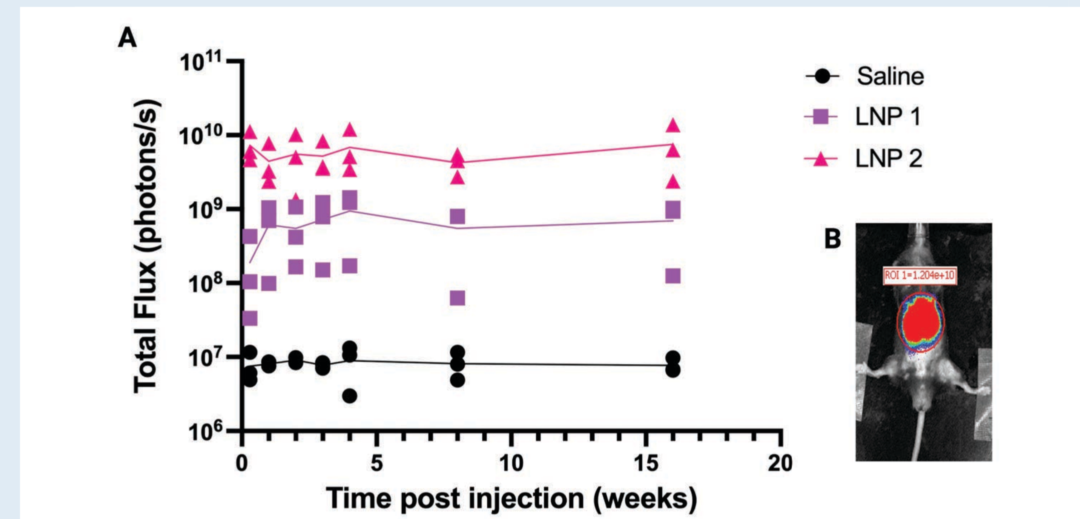


Figure 2. A. Quantification of luminescent signal shown in Figure 1. Signal was quantified using the IVIS Living Image software (PerkinElmer) by defining a region of interest (ROI) centered on and encompassing the signal, which was kept consistent between mice. The total flux in photons/second was then measured within the ROI. Consistent luminescent signal was observed over the 4-month experimental timeline, with peak signal being observed as early as 7 days. **B.** Representative image of ROI placement for quantification.

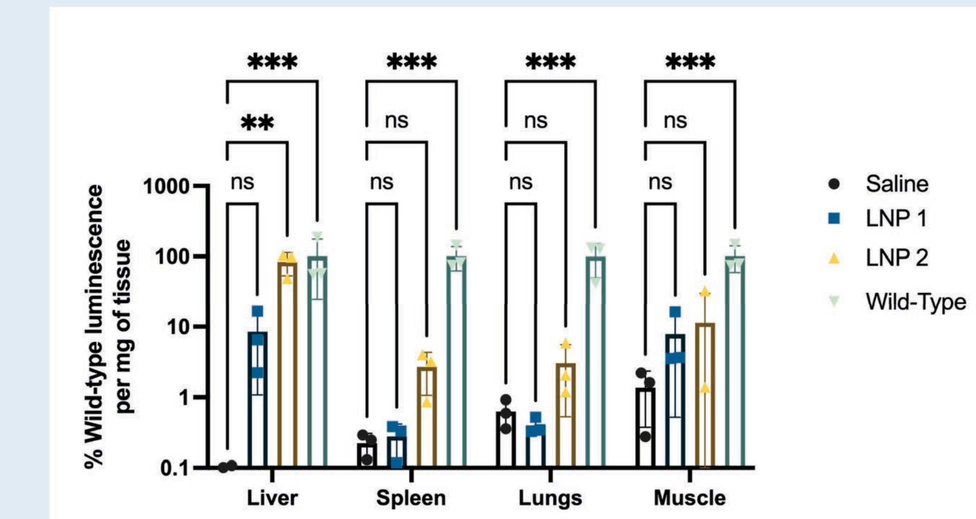


Figure 3. Luciferase assay of tissues extracted from reporter mice at experimental endpoint (t = 4 months). Extracted tissues were flash frozen and homogenized prior to performing the assay (Promega Steady-Glo Luciferase Assay). Data is shown as mean ± SD. A significant increase in luminescence for treated samples was only seen in the liver of LNP 2-treated mice (83.48 ± 30.30). Sample means were compared using a two-way ANOVA with post-hoc Dunnett's test. ns: no significance, **: p≤0.01, ***:p≤0.001.

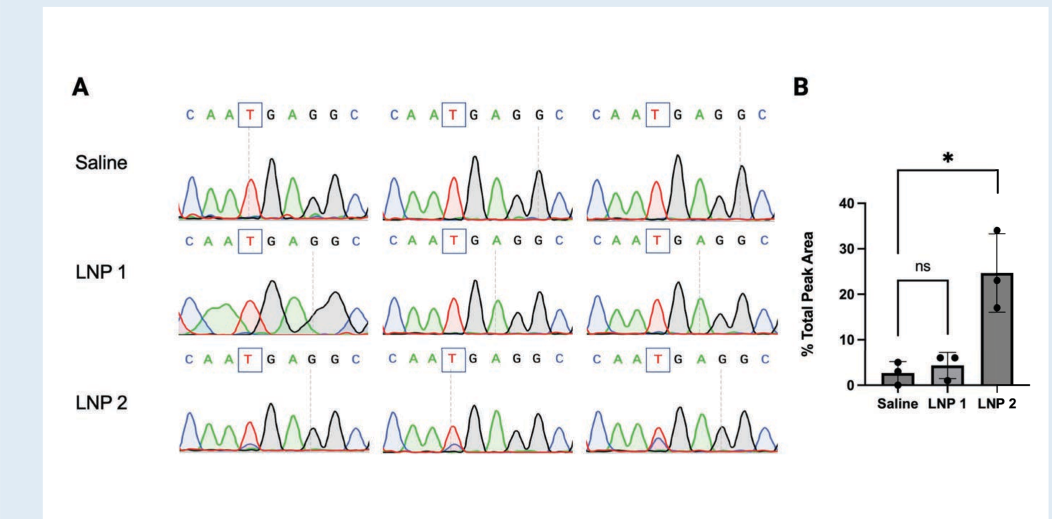


Figure 4. A. Sanger sequencing chromatograms of extracted and sequenced liver tissue at the editing target site. The target nucleotide is identified in the box, with a change from T to C being the desired outcome. **B.** Quantification of Sanger sequencing reads in terms of total peak area. Quantification was performed using the EditR software. Data is shown as mean ± SD, Saline: 2.67 ± 2.52, LNP 1: 4.33 ± 2.89, LNP 2: 24.67 ± 8.62. Samples were compared with a two-tailed unpaired t-test. ns: no significance, *: p≤0.05.

CONCLUSIONS/FUTURE DIRECTIONS

These initial experiments demonstrate the utility of our mouse model as a screening platform for *in vivo* genome editing via an adenine base editor. We observed significantly higher gene editing and luminescence restoration in the liver when using LNP 2, showing that our model can be used to evaluate the efficacy of different gene editing delivery methods. In future studies, we plan to use this system to evaluate gene editing mediated by novel LNP formulations with different tissue specificities. Additionally, this model may be extendable to other modern gene editing technologies, such as prime editing.

ACKNOWLEDGEMENTS

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