

In Utero Gene Editing for Treatment of Duchenne Muscular Dystrophy (DMD): Systemic Delivery of Lipid Nanoparticles and Therapeutic Base Editing

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Background

Duchenne muscular dystrophy (DMD) is a debilitating congenital myopathy that affects 1 in 10,000 boys. It is caused by frameshifting or nonsense mutations that cluster in "hotspots" between exons 2 and 9 and exons 43 and 55 in the DMD gene, producing non-functional dystrophin. Linkage between F-actin in myocytes to the extracellular matrix is thus ablated, causing muscle atrophy and respiratory and cardiac dysfunction. Existing antisense oligonucleotide treatments must be dosed repeatedly. We demonstrate an adenine base editing strategy to mediate skipping of exon 45 at the DMD locus (applicable to 8% of DMD patients) via mutation of A to G at the splice acceptor site, thereby restoring a functional open reading frame. We hypothesize that systemic injection of lipid nanoparticles (LNPs) containing adenine base editors (ABEs) will mediate durable editing at greater efficiency in fetal satellite cells and myocytes compared to those of the adult, forming the basis for a "one-and-done" *in utero* gene therapy to treat DMD.

Experimental Workflow

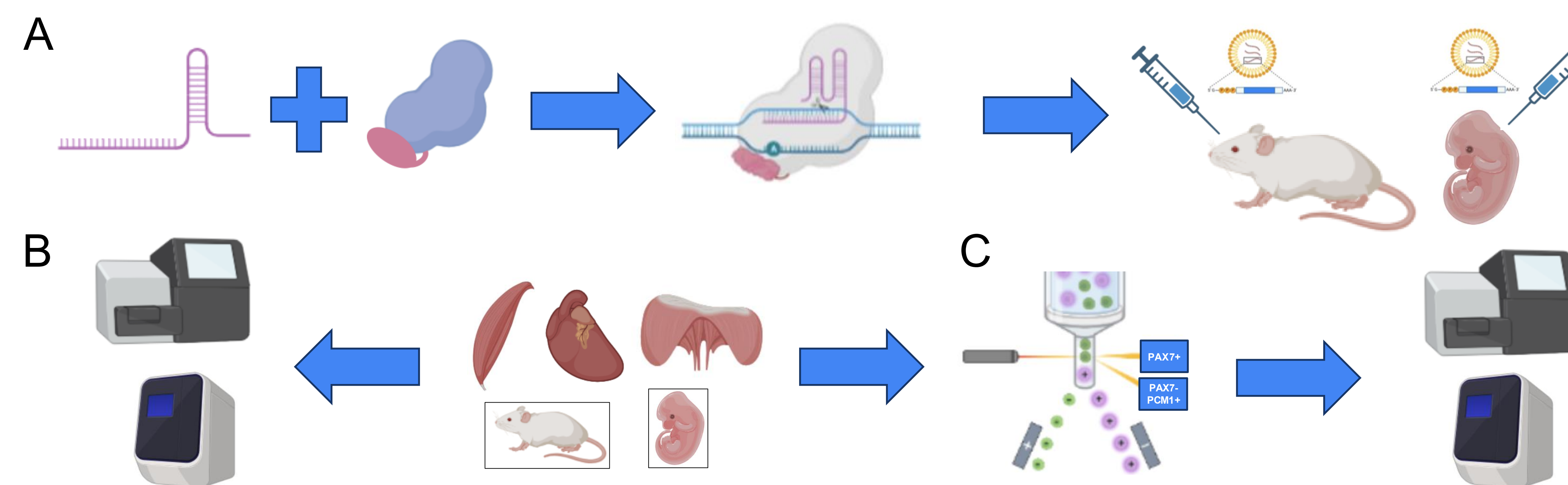


Figure 1. (A) A single guide RNA¹ (sgRNA) targeting the splice acceptor site immediately upstream of exon 45 in mouse DMD and ABE were optimized *in vitro*, encapsulated within LNPs, and then intravenously injected into adult and fetal mice. The Peranteau Lab aims to demonstrate that the fetus is the ideal candidate for gene therapy. (B) Hearts, quadriceps, and diaphragms (tissues implicated in DMD) are harvested from injected mice either one or twelve months post-injection. Total DNA is isolated from bulk tissues and sequenced via Illumina MiSeq to assess ABE editing efficiency at the DMD exon 45 splice acceptor site. In future experiments, total RNA will be harvested from these organs and used to perform RT-qPCR with a primer spanning the DMD exon 44-46 junction to quantify exon skipping efficiency. (C) Nuclei of satellite cells (responsible for regenerating myocytes) and myocytes are sorted from bulk tissues using antibodies staining for the nuclear markers PAX7 and PCM1, respectively. Nuclei of the same cell type from mouse replicates will be pooled to increase input DNA for sequencing, and exon skipping efficiency will be evaluated using total RNA and RT-qPCR.

Conclusions

ABE-mediated editing of the exon 45 splice acceptor site at the DMD locus following systemic delivery via LNPs is more efficient in the fetus than in the adult. This result is supported by our lab's LNP biodistribution studies, which found that LNPs transfect fetal tissues with greater efficiency than adult tissues (confidential data).

To our knowledge, no published work to date has addressed the systemic delivery of gene therapy via LNPs to treat DMD *in utero*. Our study thus fills a gap in the literature and provides an additional treatment modality.

Future Directions

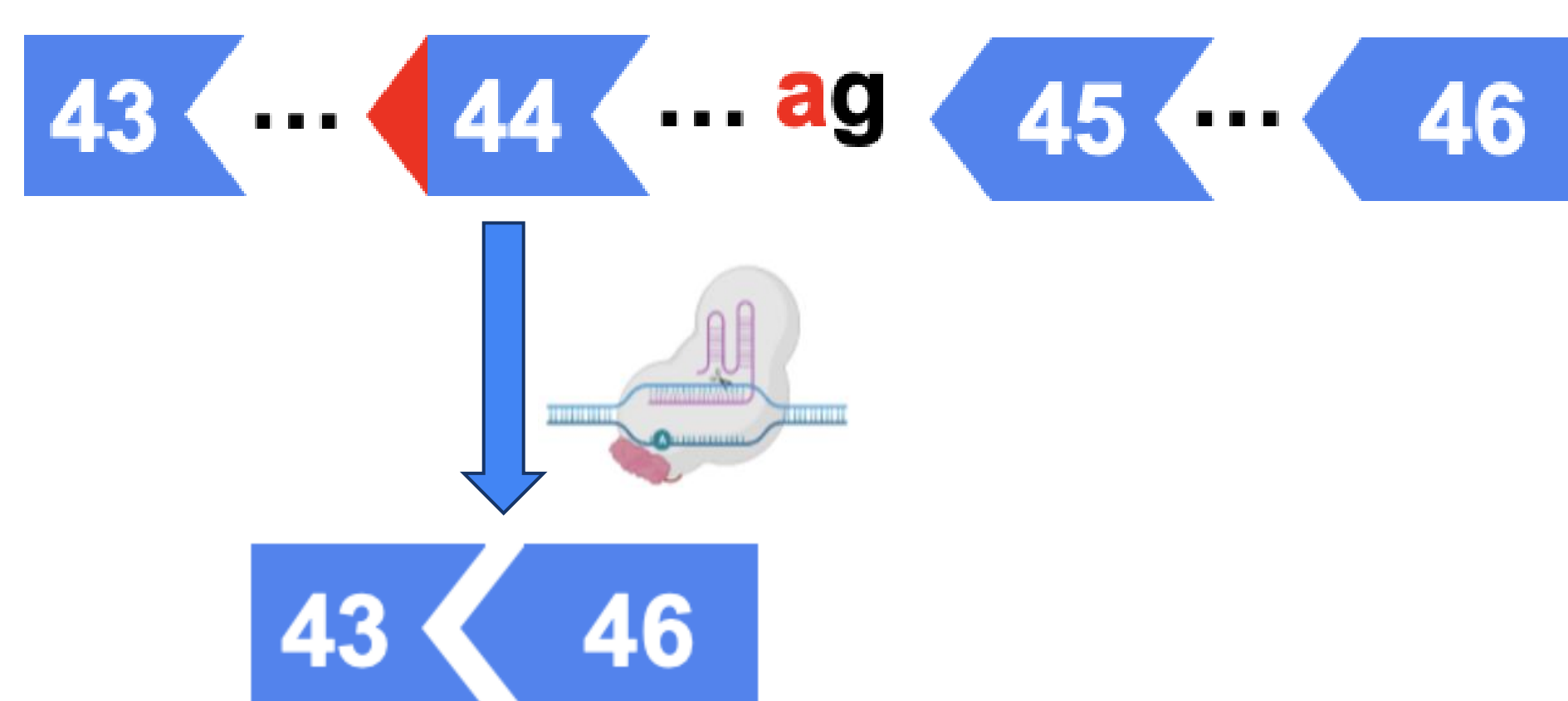
To obtain functional readouts of dystrophin restoration, we will quantify production of the DMD transcript whose exon 45 has been skipped, as outlined in Figure 1E, as well as dystrophin production in a DMD mouse model. Furthermore, we are interested in evaluating ABE editing efficiency on a cell-type-specific level in myocytes and satellite cells. We will also assess DMD editing in large animal models, including sheep and monkeys, as a continuation of our pre-clinical study.

References

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Disruption of the DMD Exon 45 Splice Acceptor Site and Biodistribution of Lipid Nanoparticles

A Disease Model:



B Wild-Type:

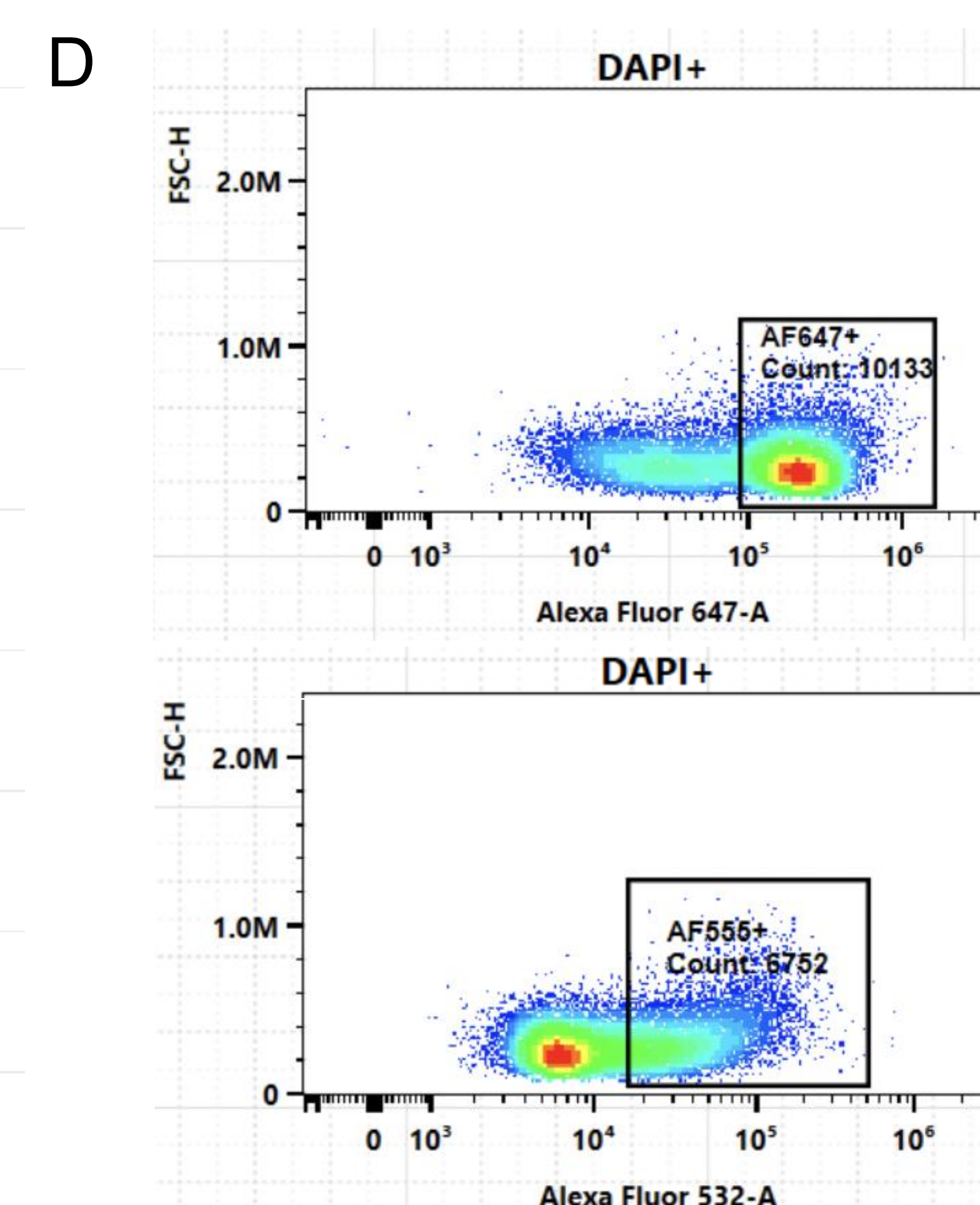
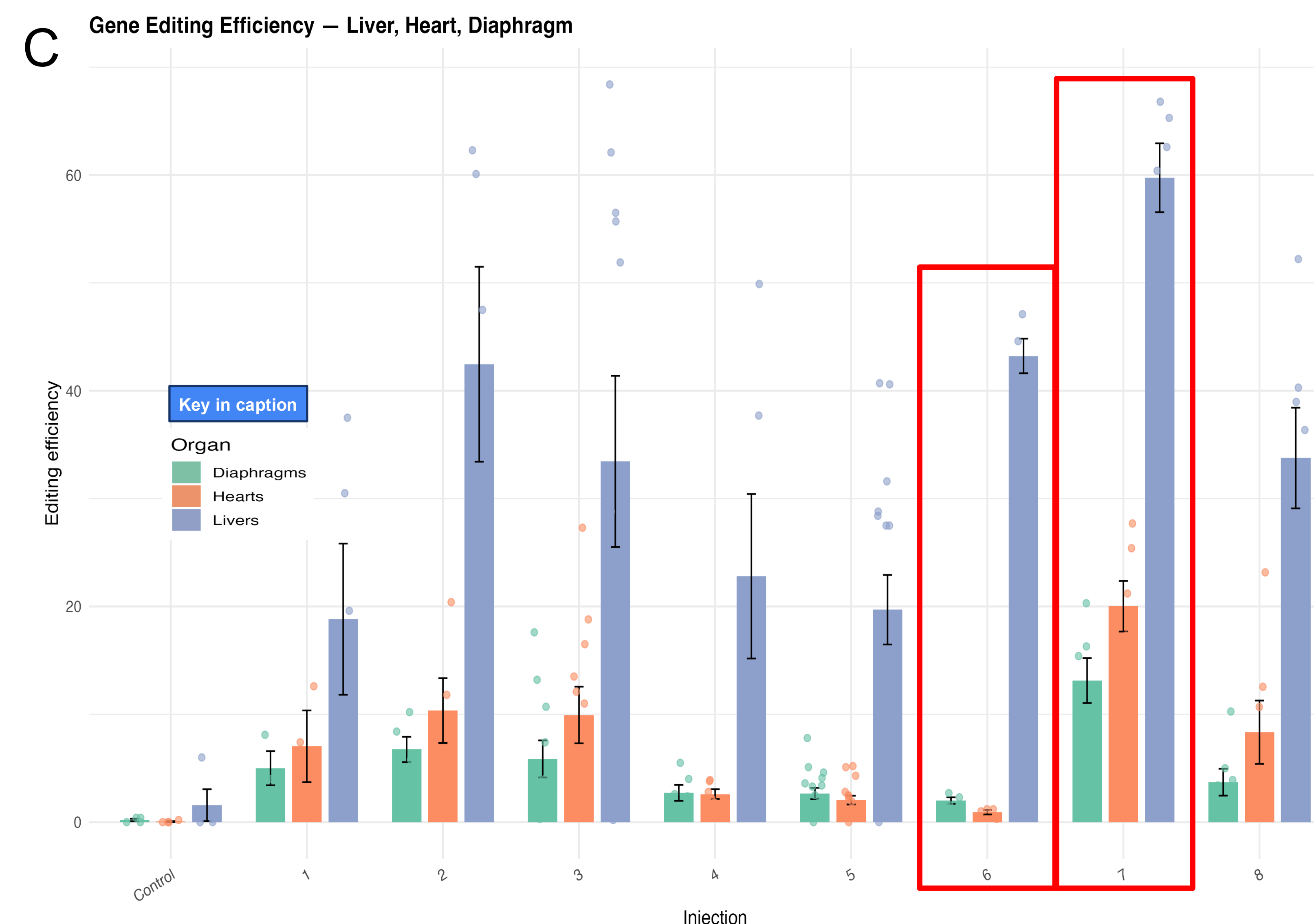
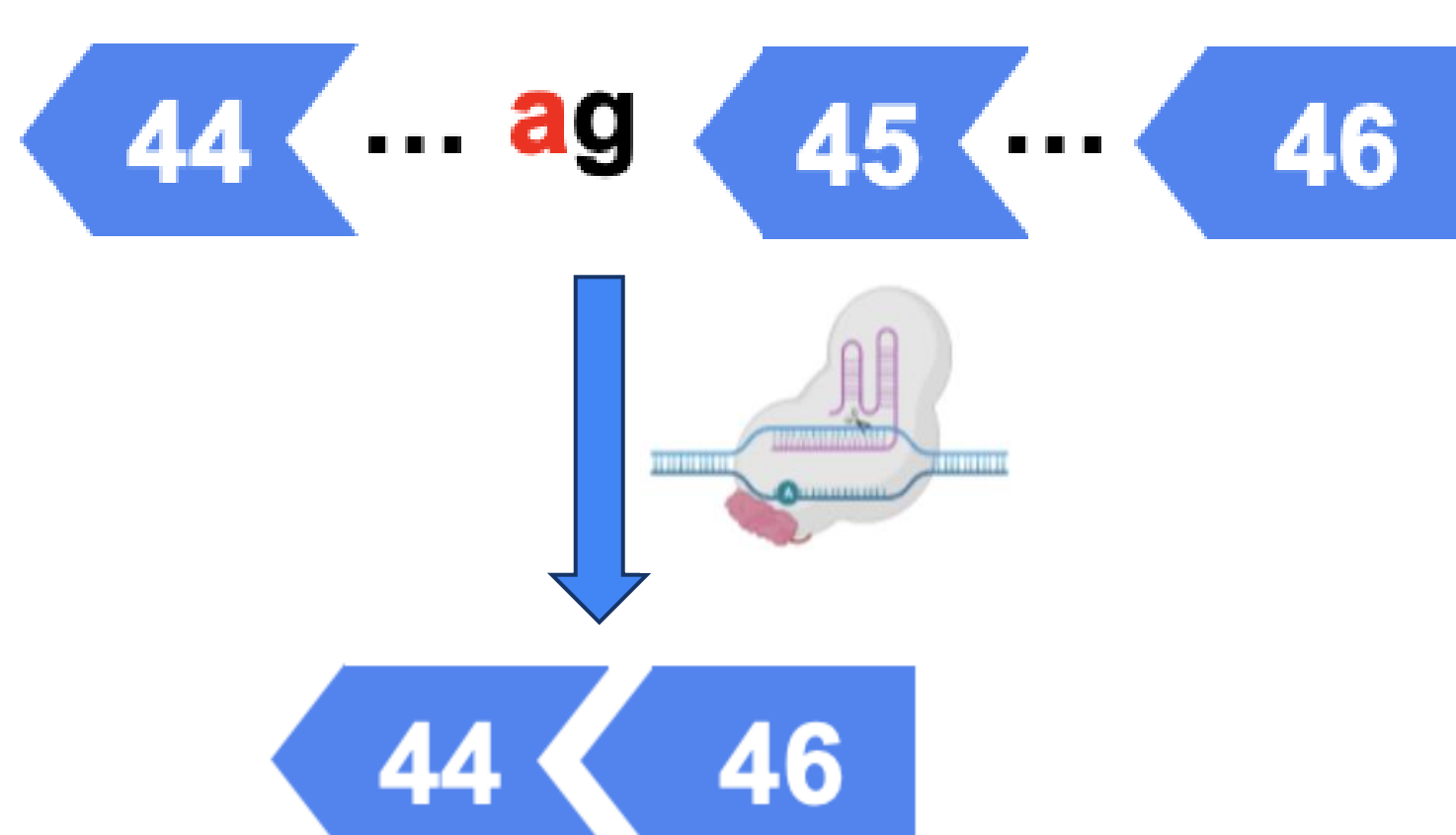


Figure 2. (A) Schematic of the DMD exon 45 skipping strategy via adenine base editing at the splice acceptor site to restore an open reading frame, resulting in a truncated but functional dystrophin product. In a disease model, a mutation at exon 44 in DMD may cause splicing errors that exclude exon 44 from the mature transcript. Splicing of exons 43 and 45 fails to produce a functional open reading frame. Skipping exon 45 by mutating its splice acceptor site causes exon 43 to splice with exon 46, which does create a functional open reading frame. (C) ABE editing efficiency in livers, hearts, and diaphragms of mice injected with various dosages and compositions of LNPs at various time points. Key: 1 = 2 mg/kg ABE 8e at E14; 2 = 2 mg/kg ABE 8e at E14 + chemically modified sgRNA; 4 = 2 mg/kg ABE 8e at E16; 5 = 2 mg/kg ABE 8e at E16 + chemically modified sgRNA; 6 = 3 mg/kg ABE 8e at 6 weeks + chemically modified sgRNA; 7 = 3 mg/kg ABE 8e at E14 + chemically modified sgRNA; 8 = 2 mg/kg ABE 8e at E14 + chemically modified sgRNA harvested at 12 months. Boxed graphs compare editing in mice injected as adults or as fetuses at equal doses of ABE8e. (D) Sorting of satellite cell and myocyte nuclei in skeletal muscle tissue.