

Liver and Lung Manifestations of Alpha-1 Antitrypsin Deficiency

Alpha-1 Antitrypsin Deficiency (A1AD) is a rare genetic disease most commonly caused by a G>A transition mutation within the SERPINA1 gene referred to as the PiZ allele. The resulting PiZ protein containing a single amino acid substitute E342K is prone to polymerization within hepatocytes leading to cirrhosis in a subset of patients. The impaired secretion of alpha-1 antitrypsin (A1AT), an inhibitor of neutrophil elastase, leads to loss of lung elastin and progressive pulmonary disease. Although underdiagnosed, there are an estimated 60,000 PiZZ individuals in the US¹. Current therapy is plasma-derived protein augmentation to slow lung disease progression. Base editing has the potential to simultaneously address both liver and lung manifestations of A1AD.

Genome Editing with Adenine Base Editors

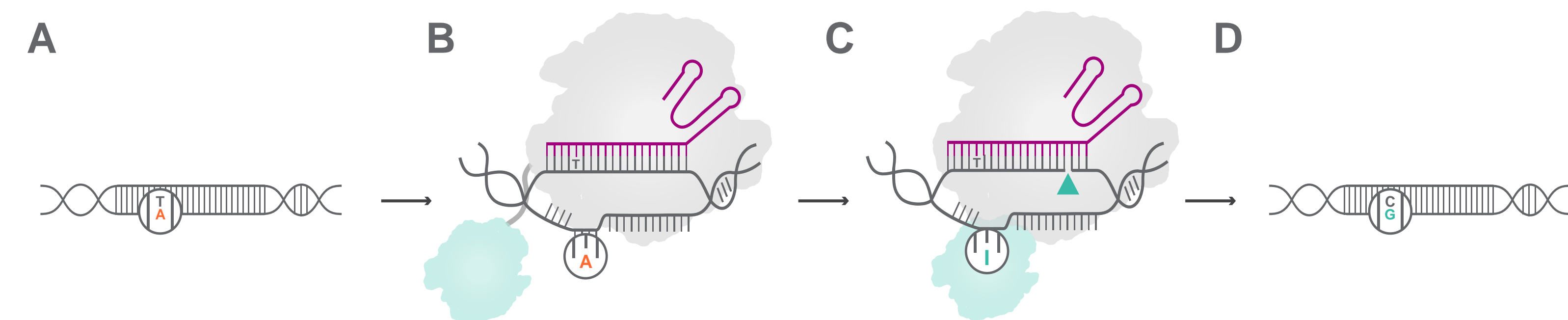


Figure 1. (A) Starting DNA sequence with the target base pair (A:T). (B) The adenine base editor (ABE) is a fusion protein consisting of an evolved TadA* deaminase (teal) connected to CRISPR-Cas enzyme (grey)². The base editor binds to a target sequence that is complementary to the guide-RNA (magenta) and exposes a stretch of single-stranded DNA. (C) The deaminase converts the target adenine into inosine (which is read as guanine by DNA polymerases) and the Cas enzyme nicks (▲) the opposite strand. (D) The nicked strand is repaired completing the conversion of an A:T to G:C base pair.

Optimizing an ABE and gRNA to Correct the PiZ Mutation

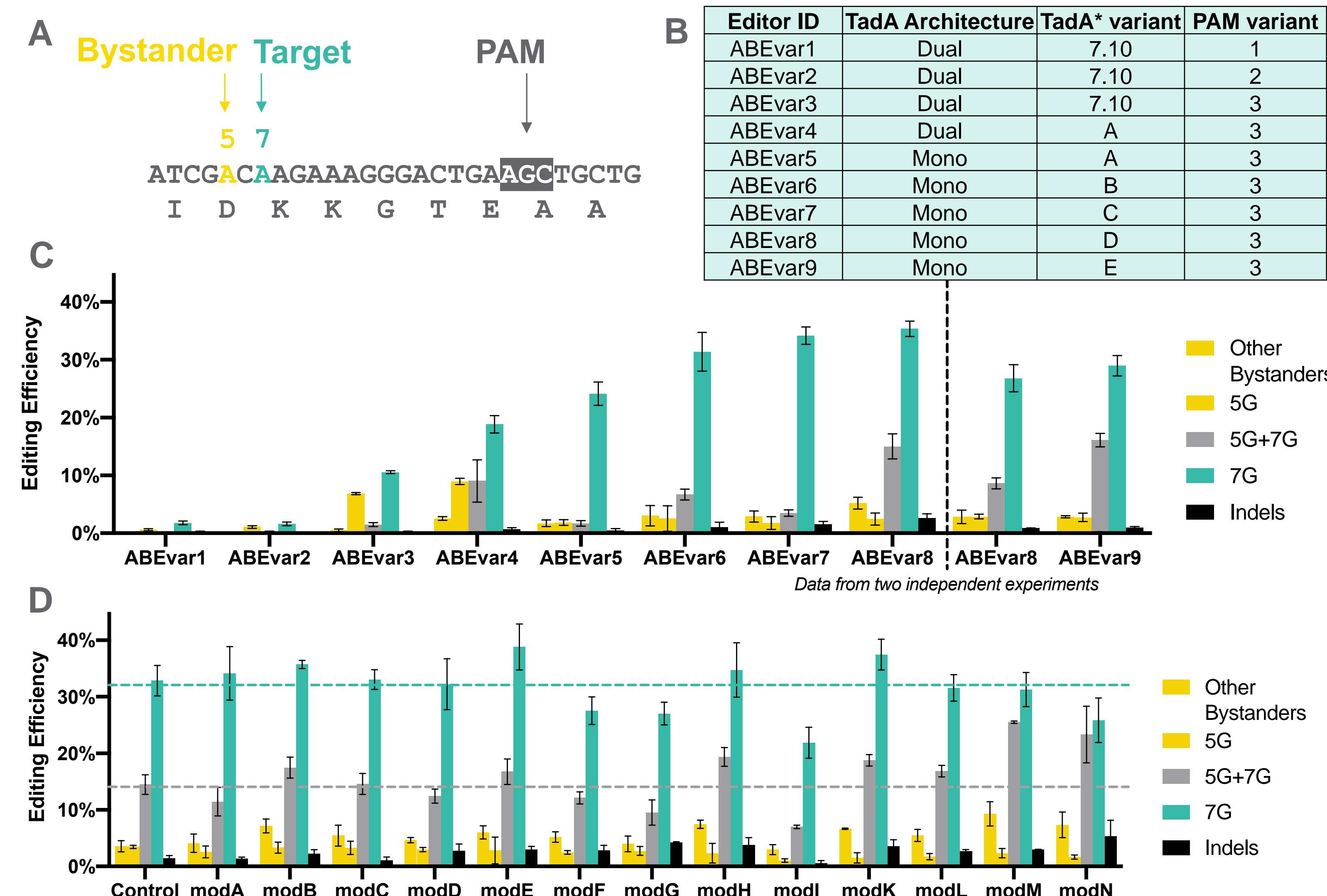


Figure 2. (A) Target DNA sequence and underlying amino acid translation for the E342K (PiZ) mutation. (B) A table outlining the iterative design process interrogating NGC PAM variants and then TadA* mutants. (C,D) Allele frequencies assessed by high-throughput targeted amplicon sequencing in primary PiZZ fibroblasts (GM11423, Coriell) transfected with base editor mRNA and gRNA. (C) Base editors were optimized for precise correction of E342K (7G) editing, although a significant amount of linked bystander editing (5G+7G) was also observed. (D) Chemical modifications within the variable region of the gRNA increase 7G and 5G+7G allele frequencies (see mod E, H, K, M, N).

The 5G+7G Allele Yields a Functional D341G A1AT Protein

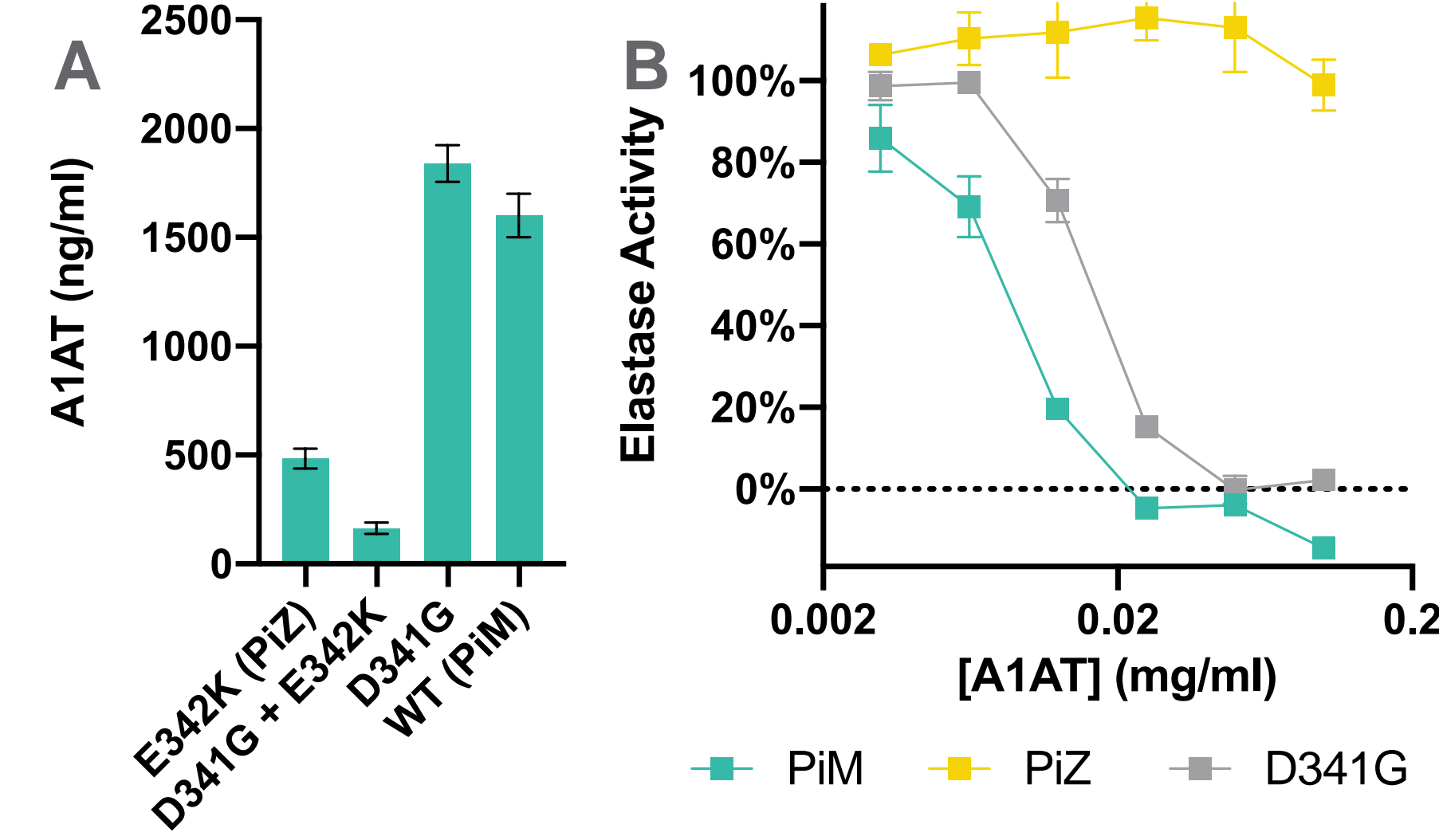


Figure 3. (A) A1AT secretion assay in HEK293T. Both E342K (PiZ) and D341G+E342K A1AT exhibit a secretion defect, whereas D341G A1AT is secreted at wild-type (PiM) levels. (B) Elastase inhibition assay with purified A1AT proteins. D341G A1AT exhibits wild-type levels of anti-elastase activity. We conclude that the 5G+7G and 7G edited alleles, yielding D341G and WT respectively, are both beneficial alleles.

In Vivo Correction of E342K May Confer a Proliferative Advantage

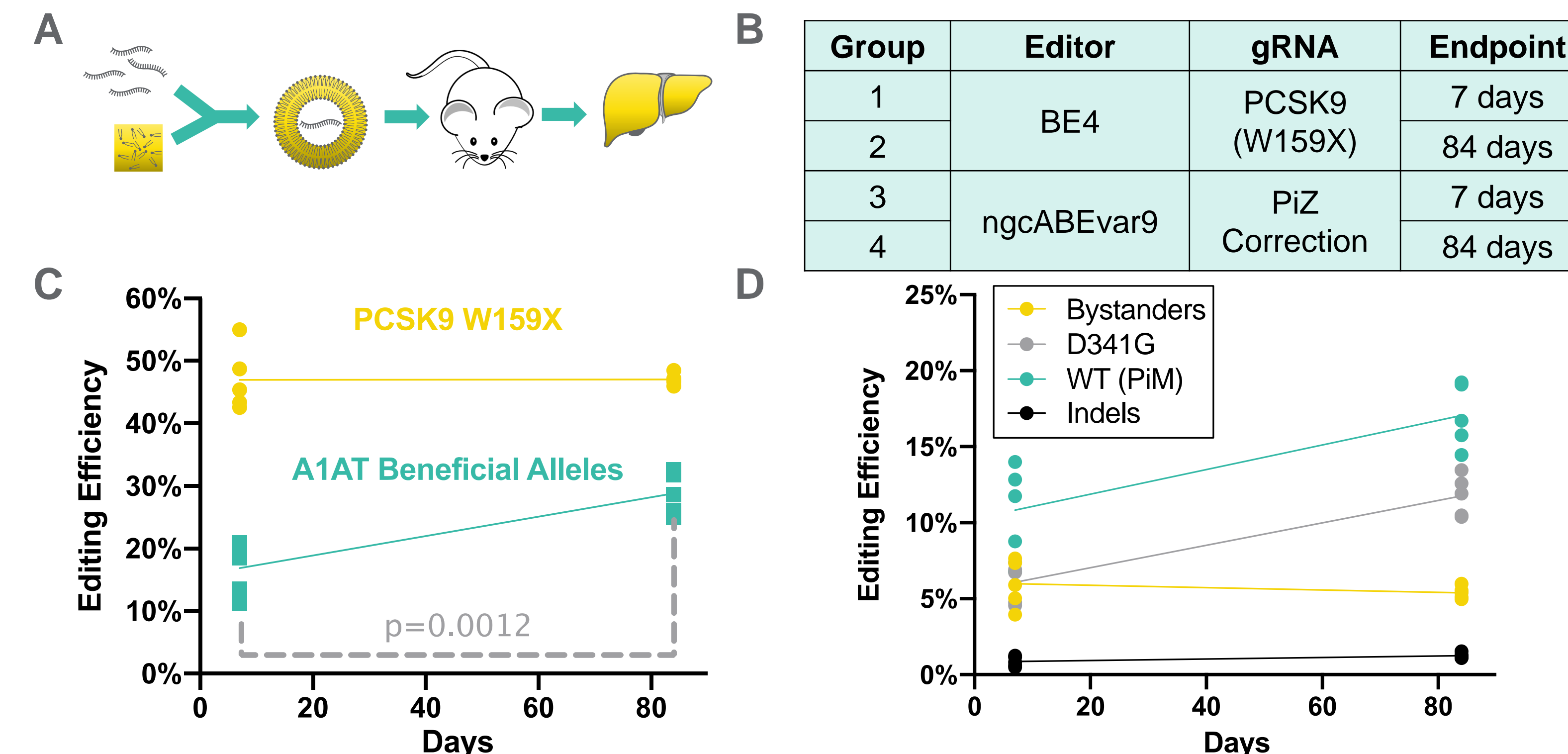


Figure 4. (A) Schematic depicting in vivo workflow. Lipid nanoparticles (LNP) carrying base editor mRNA and gRNA were dosed via IV injection in NSG-PiZ transgenic mice (Jackson Laboratory). (B) Table describing four treatment groups (N=5) including correction and a control formulation targeting the PCSK9 gene⁶. (C) Base editing efficiency in *total liver extracts*. PCSK9 editing is efficient and durable. A1AT beneficial alleles increase significantly at three months indicating that corrected hepatocytes may have a proliferative advantage. (D) Allele frequencies within the PiZ correction group. Both D341G and WT (PiM) alleles increase at 3 months, while Indel and bystander edits remain at low levels.

In Vivo Correction Reduces PiZ Globule Burden in Mouse Liver

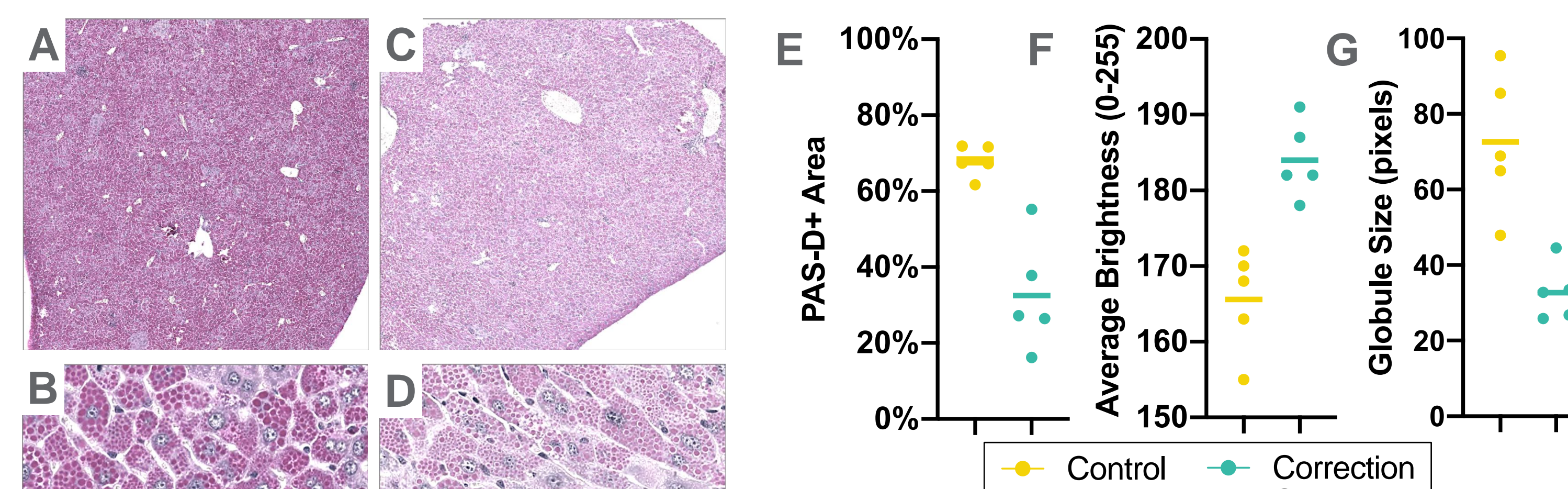


Figure 5. (A-D) Representative Periodic Acid-Schiff-Diastase (PAS-D) stained sections of mouse livers from PCSK9 control (A,B) and correction groups (C,D). PAS-D efficiently stains the insoluble PiZ globules, a hallmark of A1AT liver disease. Images at 40x magnification were subject to color thresholding (ImageJ) to calculate (E) percent PAS-D stained area and (F) average pixel brightness in PAS-D stained regions. (G) Globule sizes were calculated at 200x magnification. Genetic correction reduces PAS-D stain density, intensity, and globule size.

In Vivo Correction Increases Functional Human A1AT in Serum

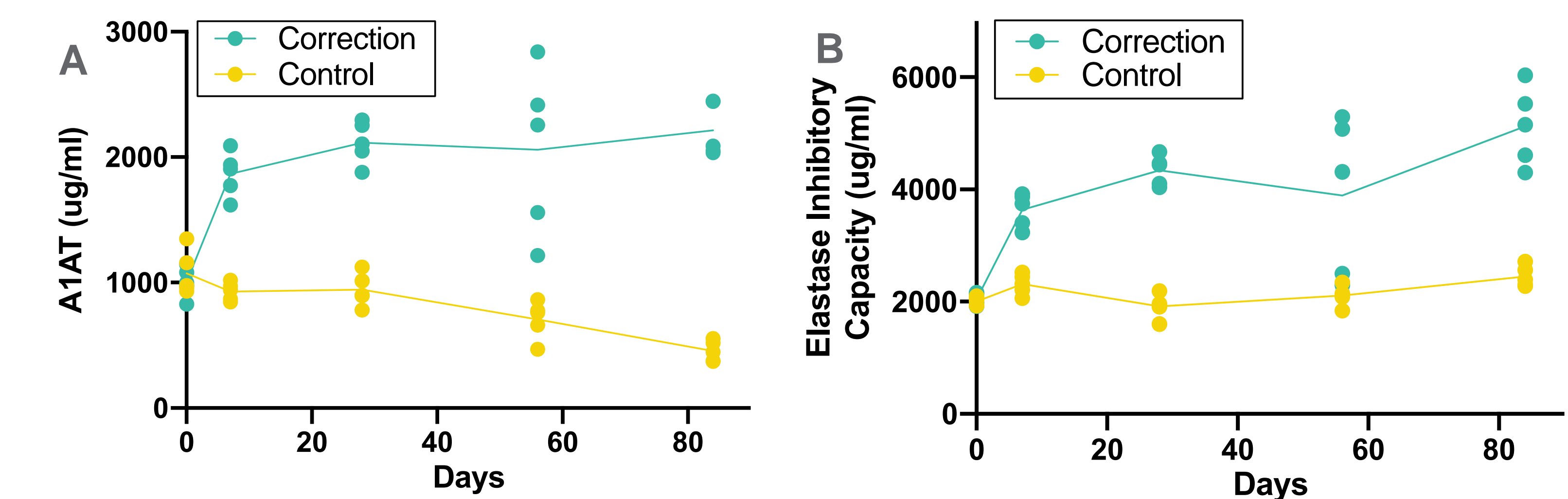


Figure 6. (A) Serum human A1AT measured by immunoassay (Meso Scale Discovery). Upon genetic correction, we observe an increase in serum A1AT that is durable and is roughly 4.9-fold higher than controls at 3 months. (B) Human neutrophil elastase inhibition capacity of serum samples. We observe that genetic correction produces a durable increase in the functional elastase inhibitory capacity. (C) A1AT isoform abundance measured by mass spectrometry of trypsin-digested sera. Upon genetic correction, we observe the appearance of the D341G and WT (PiM) isoforms and a significant decrease in the E342K (PiZ) isoform.

Conclusions

In the course of this work, adenine base editors were engineered to correct the disease-causing PiZ mutation in SERPINA1. As a consequence of this narrow focus, the optimized base editor does not conform to more general recommendations for TadA* deaminase variants or spCas9 PAM variants^{3,4,5}. Although precise correction of the PiZ mutation was the most common editing outcome, the second most abundant allele (D341G A1AT) exhibited secretion and elastase inhibitory properties comparable to wild-type.

Using LNP-delivery technology, we assessed our base editing approach in the NSG-PiZ transgenic mouse model. We observed an average of 16.9% beneficial alleles at 7 days and 28.8% at three months, at which point treated animals exhibited a 4.9-fold increase in serum A1AT relative to control animals. This result was further substantiated by an increase in elastase inhibitory capacity and mass-spectrometry quantification of individual A1AT isoforms. Liver pathology was also markedly improved as measured by a reduction in PAS-D stained PiZ globules. These results indicate that base editing has the potential to address both lung and liver disease in A1AT deficiency.

Future Directions

We have initiated studies to characterize the potential for off-target genome modification with these reagents. Further development of this therapeutic program will require refinements to the mRNA, gRNA, and LNP formulation to achieve safety and efficacy.

References and Disclosures

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