# Translating Base Editing Technology into a Potential Treatment for Alpha-1 Antitrypsin Deficiency

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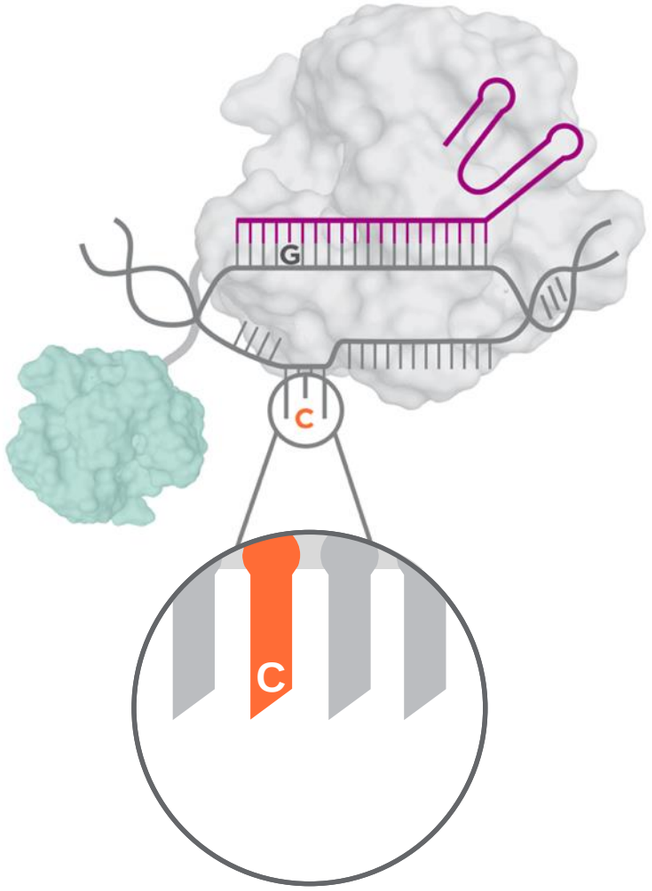
ASGCT Poster #1475

# DISCLOSURE

- I am a Beam employee and shareholder

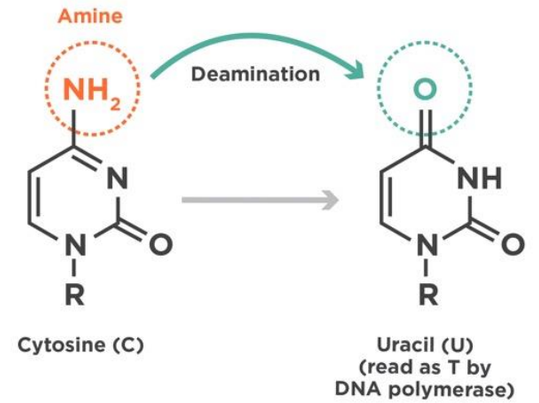
# Base Editors Chemically Modify Target Bases, Permanently and Predictably

## Base editor binds the target DNA and exposes a narrow editing window

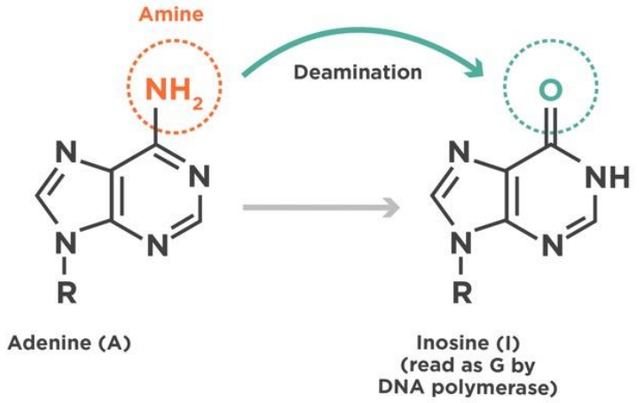



## Deaminase chemically modifies target base, permanently and predictably

**C-to-T base editor (“CBE”)**


**A-to-G base editor (“ABE”)**

# A Precise, Versatile Editing Technology

**Gene Correction**

Directly repair point mutations to restore gene function


**Gene Modification**

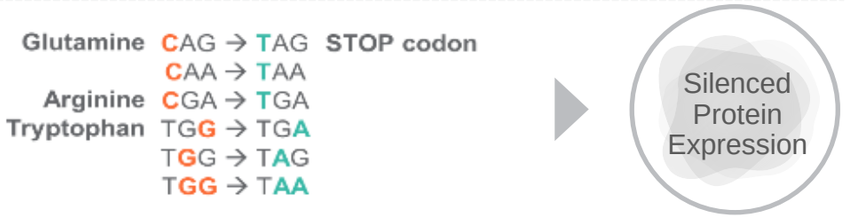
Insert protective clinical variants to prevent or modify risk of disease


**Gene Activation**

Edit regulatory elements to reactivate gene expression

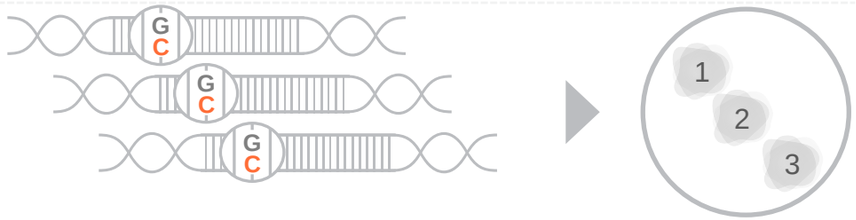

**Gene Silencing**

Edit stop codons or splice sites to silence expression



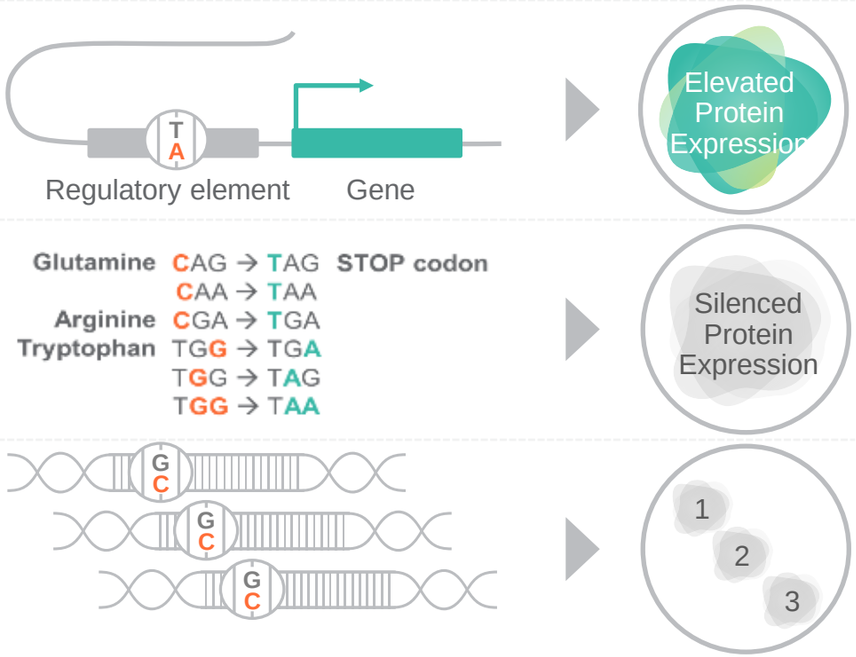

**Multiplex Editing**

Editing multiple sites simultaneously, with no detectable translocations



# A Precise, Versatile Editing Technology

| | |
|---|---|
| **Gene Correction** | **Today's presentation and poster #1475** |
| **Gene Modification** | **Oral presentation #1438: Vivek Chowdhary will present an allosteric compensatory mutation approach to A1AT deficiency** |
| **Gene Activation** | Edit regulatory elements to reactivate gene expression |
| **Gene Silencing** | Edit stop codons or splice sites to silence expression |
| **Multiplex Editing** | Editing multiple sites simultaneously, with no detectable translocations |



# Alpha-1 Antitrypsin (A1AT) Deficiency

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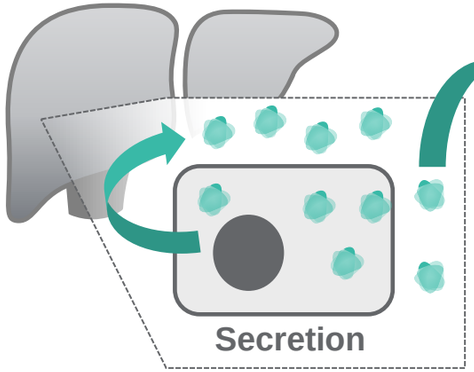
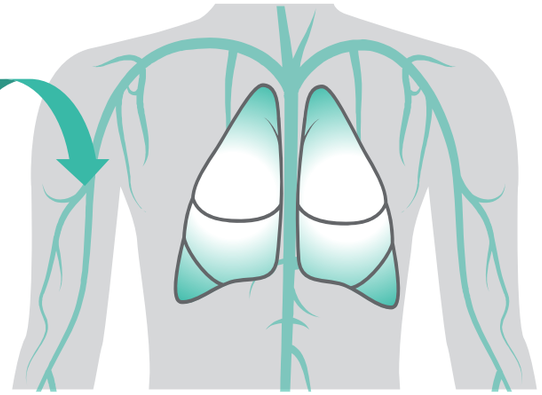
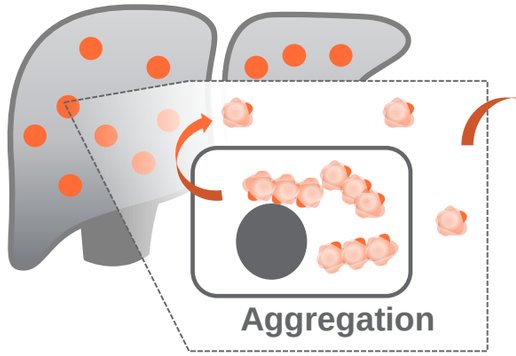
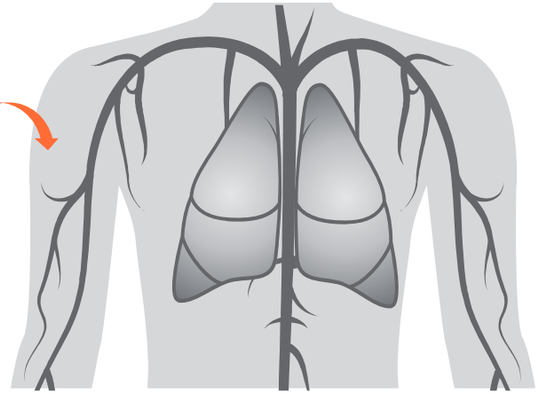
| | Molecular | Liver | Circulation | Outcome |
|---|---|---|---|---|
| **Normal A1AT Function** | Protein<br>RNA<br>DNA<br>Gene: WT “M” allele of SERPINA1 gene |  |  | • A1AT is produced and secreted from liver<br>• Circulating A1AT inhibits neutrophil elastase and protects lungs from proteases |
| **A1AT Deficiency** | Protein<br>RNA<br>DNA<br>Gene: E342K (PiZ) severe<br>E264V (PiS) mild |  |  | • A1AT ZZ proteins aggregate in the liver cause liver tissue damage<br>• Deficiency of A1AT in circulation leads to lung tissue damage<br>• Enzyme replacement slows but does not halt progression of lung disease |

## Today’s agenda

1. **Base editing**
   **Optimization of base editors for precise correction of PiZ**
2. **Liver phenotype**
   **Characterization of in vivo editing and liver A1AT aggregation**
3. **Circulating A1AT**
   **Measurement of circulating A1AT levels following precise correction**
4. **Next steps**

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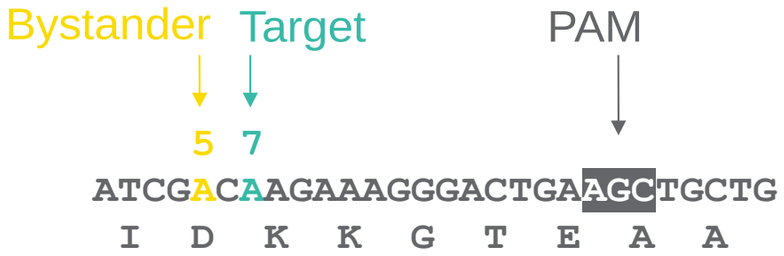
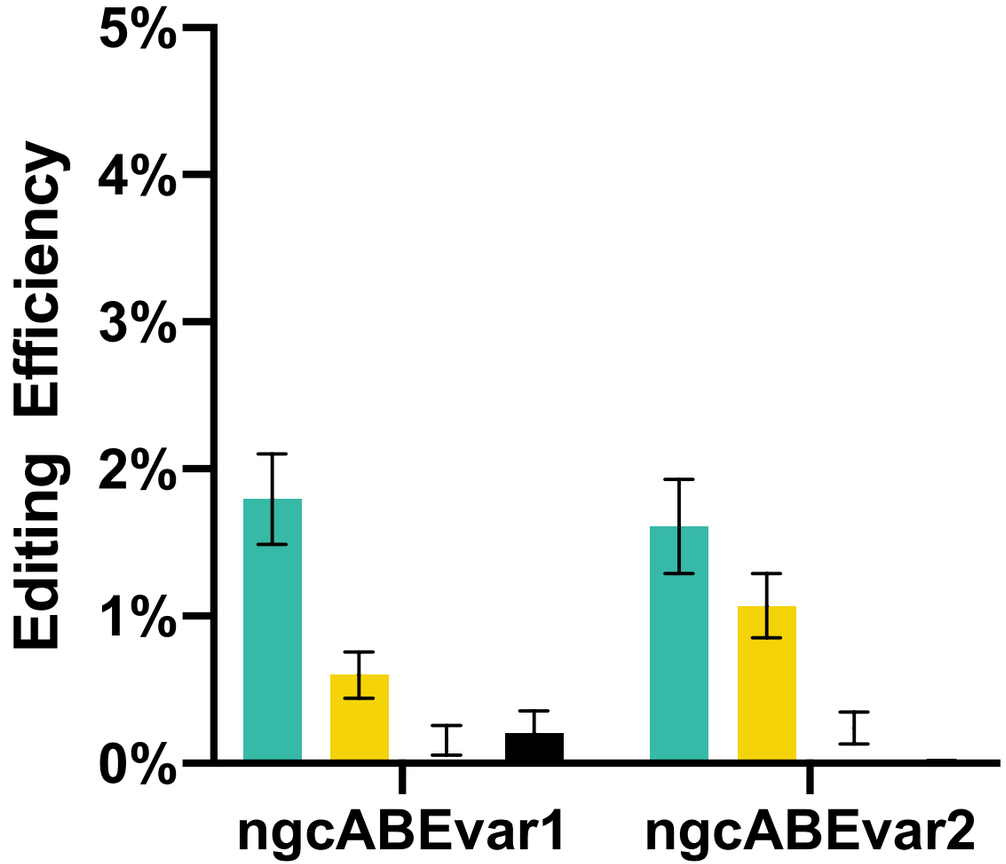
# Initial Screen in PiZZ Fibroblasts Reveals Low Rates of Precise Correction with Bystander Editing

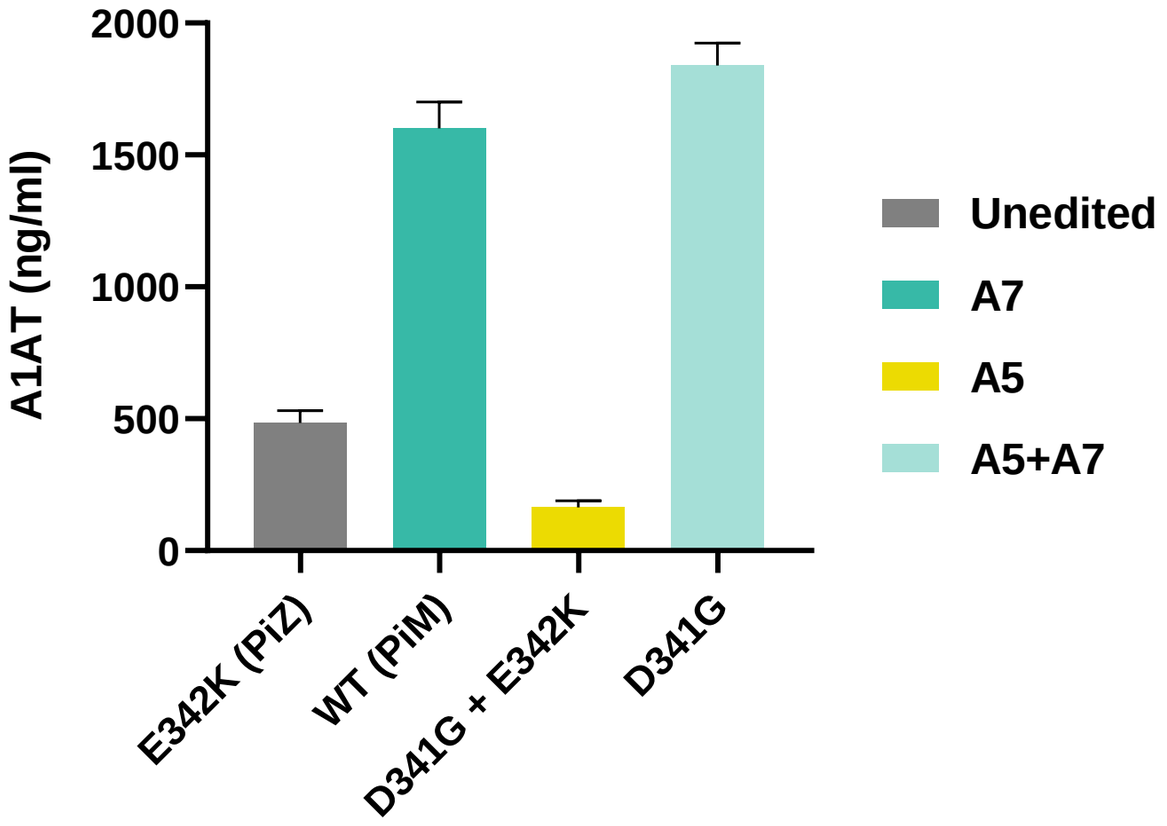


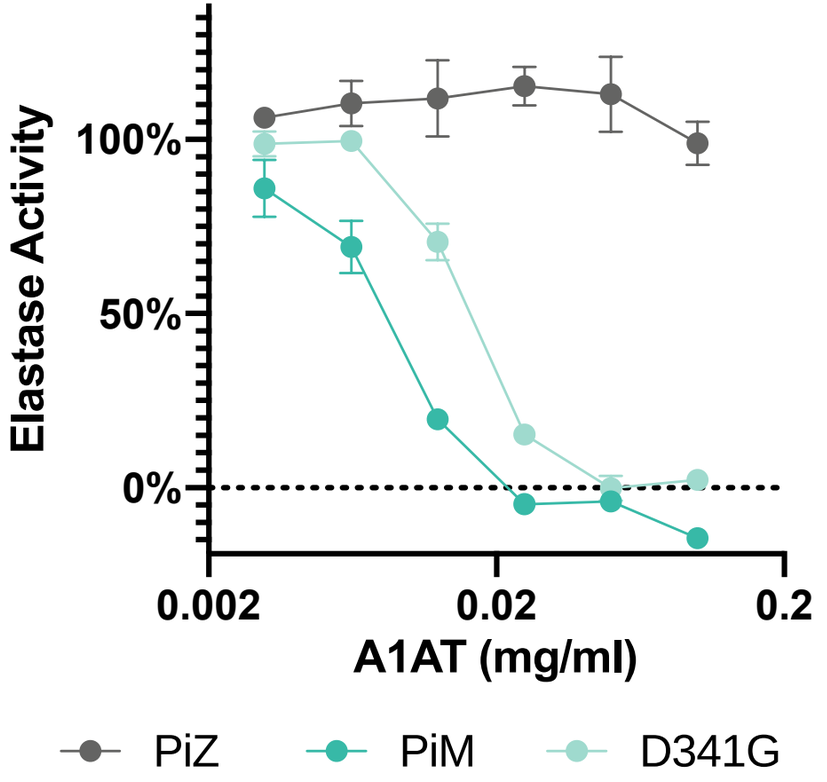

| Nucleotide Edits | | A1AT Mutant |
|---|---|---|
| Unedited | → | E342K (PiZ) |
| A7 | → | WT (PiM) |
| A5 | → | D341G+E342K |
| A5+A7 | → | D341G |
| Indels | | |




► What would be the biological consequence of these bystander edits?

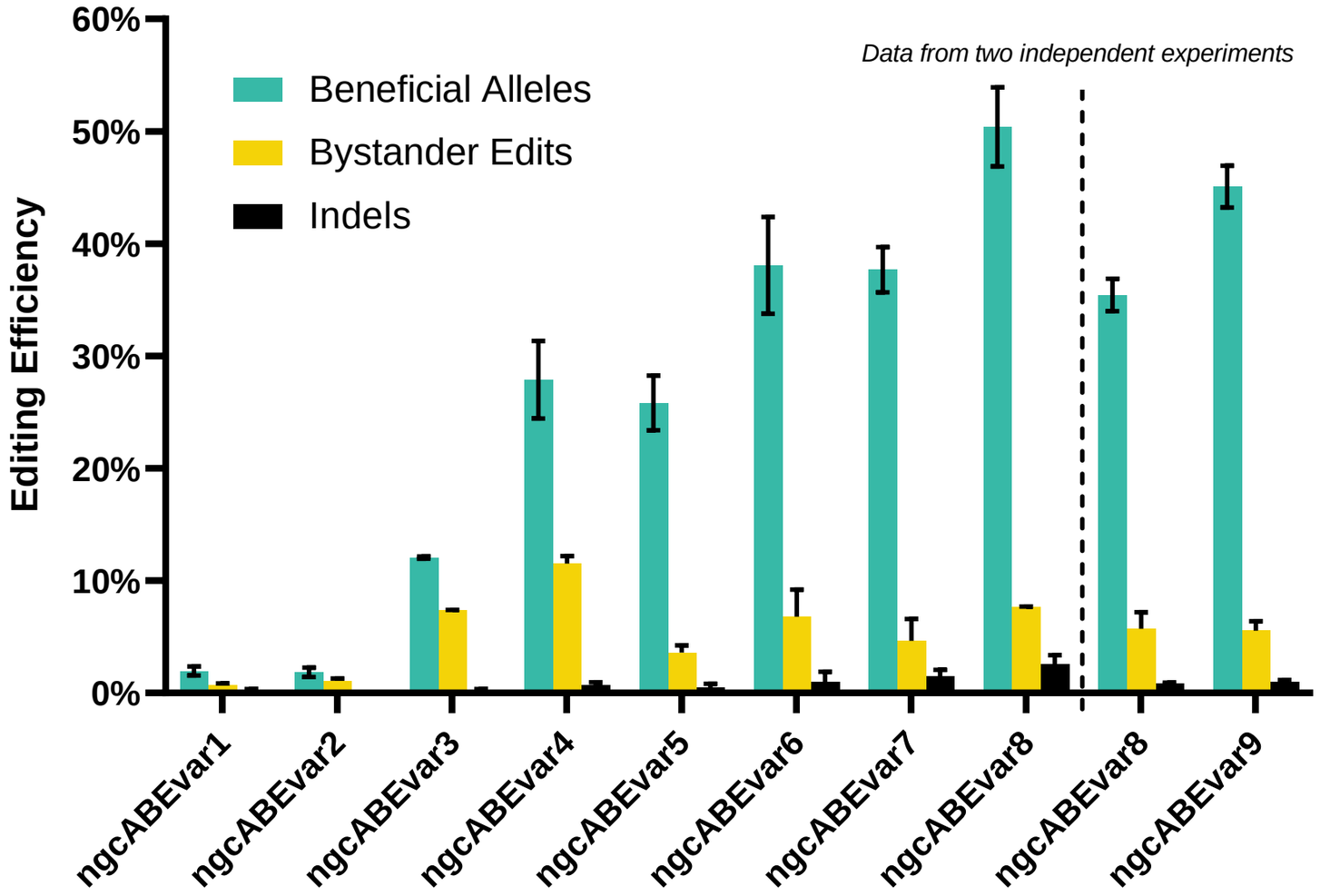
# The 5G+7G Allele Yields D341G A1AT Protein that Is Secreted and Functions Comparably to PiM




► Hereafter ‘beneficial alleles’ refers to the sum of A7 (WT) and A5+A7 (D341G)

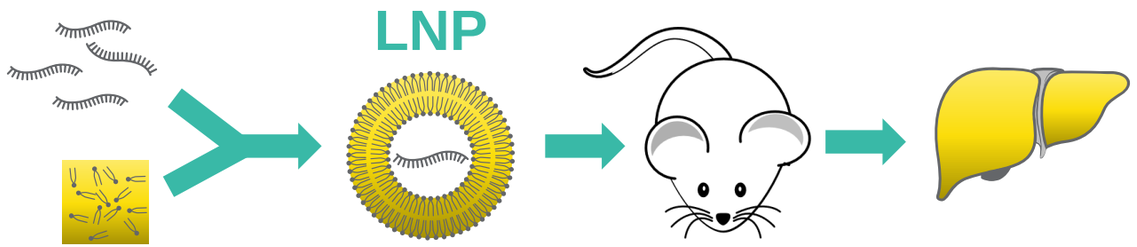
# Editor Engineering Significantly Improves Rates of Correction in Primary PiZZ Fibroblasts




- From Variant 1 to Variant 9 we achieved over 20-fold improvement in correction of E342K.
- We also significantly decrease the ratio between the beneficial alleles and bystander edits.
- Next Step: In vivo Assessment

# In Vivo Evaluation of E342K Precise Correction in NSG-PiZ Mice Using Lipid Nanoparticles (LNP)

- **Beneficial Features of LNPs**
  - Efficient targeting to liver
  - Clinically validated
  - Potential for repeat dosing
  - Transient expression
- **Single IV administration to NSG-PiZ mice**
  - This model carries >10 PiZ transgene copies and retains functional mouse SERPINA1. It does not develop lung disease but does exhibit liver pathology.
- **Serum collection for A1AT assays**
- **Tissue collection for histology and NGS of total liver extracts**




| Group | Editor | Guide RNA | Endpoint (Days) |
|---|---|---|---|
| 1 | BE4 | PCSK9 Control (W159X)* | 7 |
| 2 | | | 84 |
| 3 | ngcABEvar9 | PiZ Correction | 7 |
| 4 | | | 84 |

* [Chadwick et L., ATVB vol 37: 1741-1747, 2017 ]

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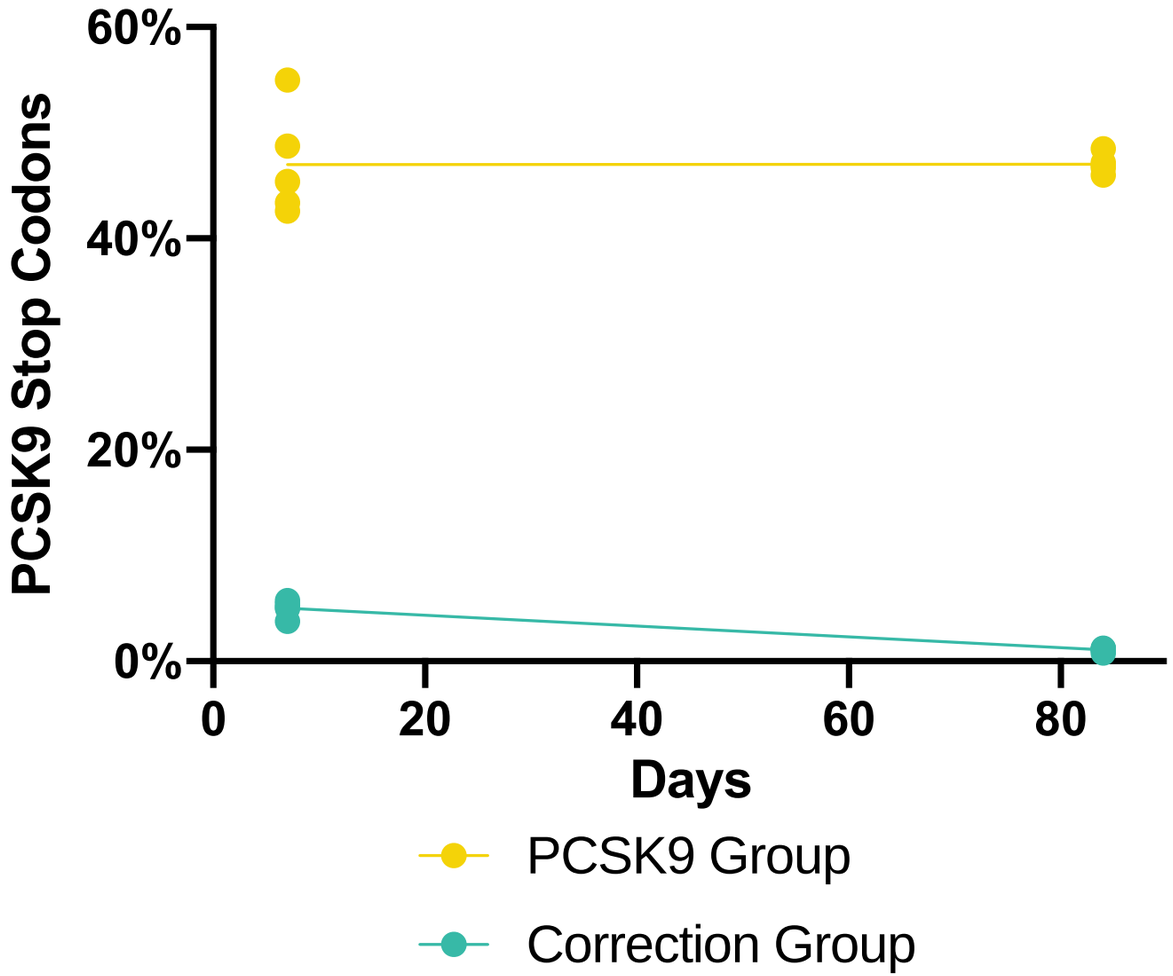
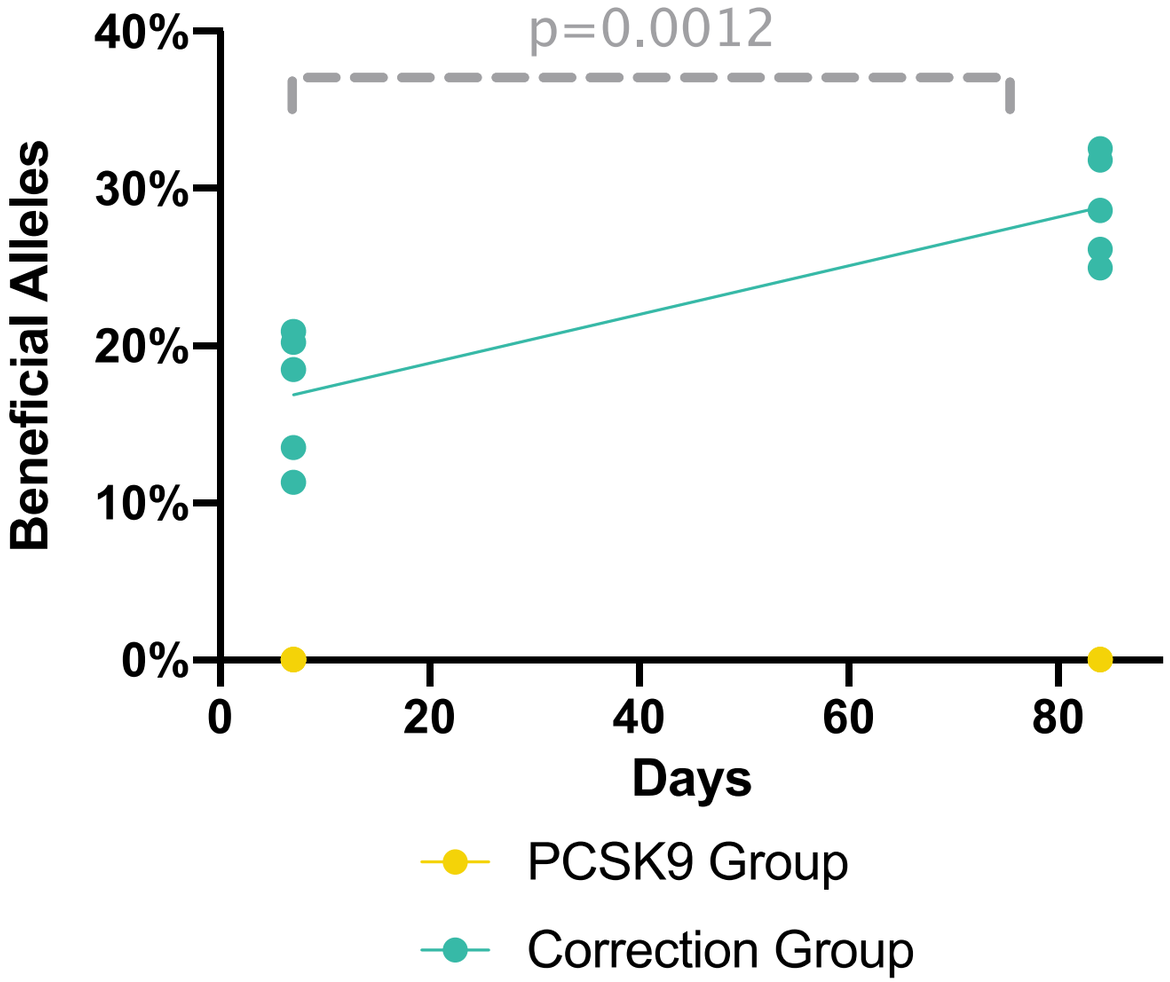
# Efficient LNP-mediated In Vivo Base Editing of PCSK9 Target Site




## PCSK9 Editing is:

- Efficient (~45%)
- Specific to the appropriate treatment group
- Durable to 3 months

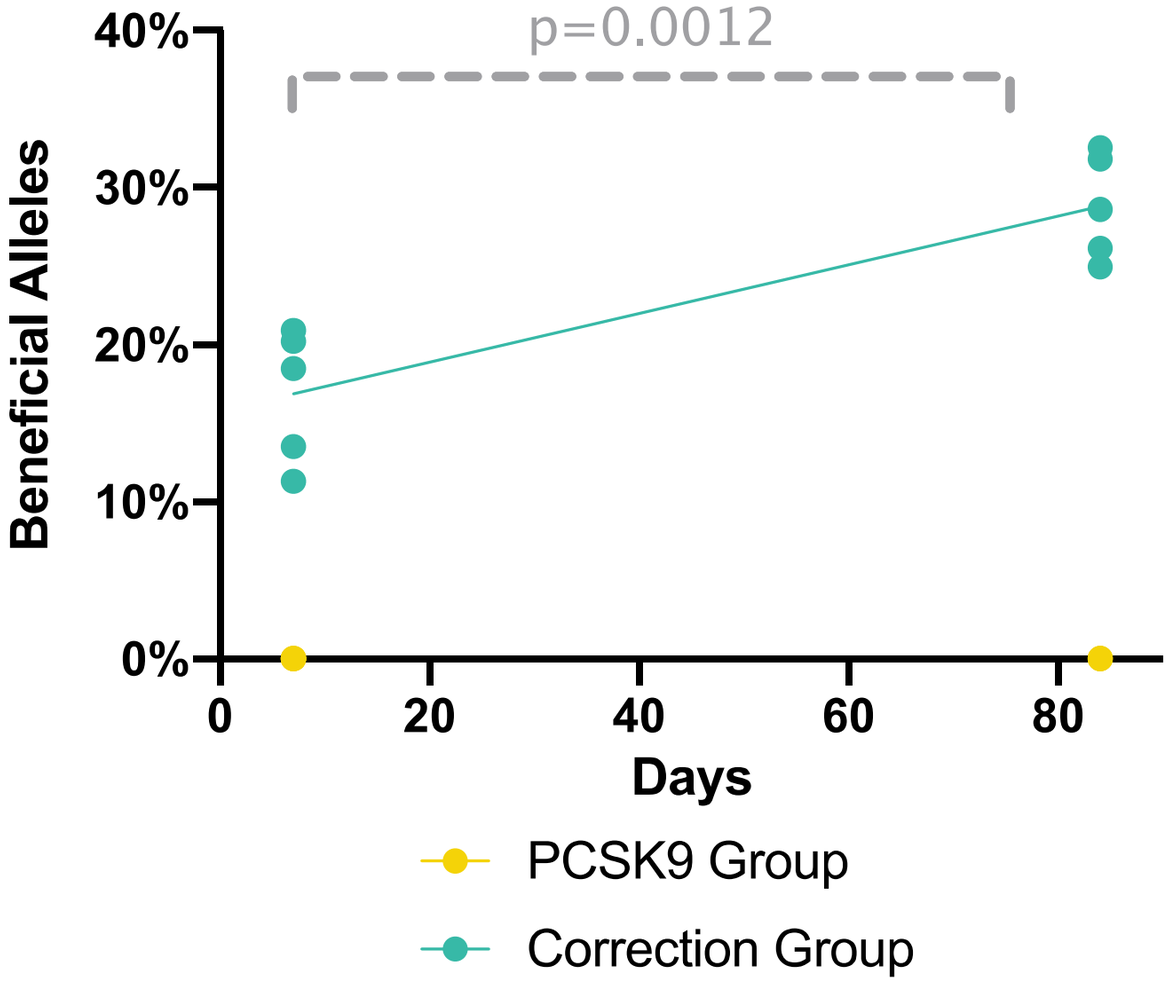
# LNP-mediated In Vivo Correction of the PiZ Mutation Increases Over Time




## PiZ Correction is:

- Specific to the appropriate treatment group
- May confer a proliferative advantage to edited hepatocytes

# LNP-mediated In Vivo Correction of the PiZ Mutation Increases Over Time



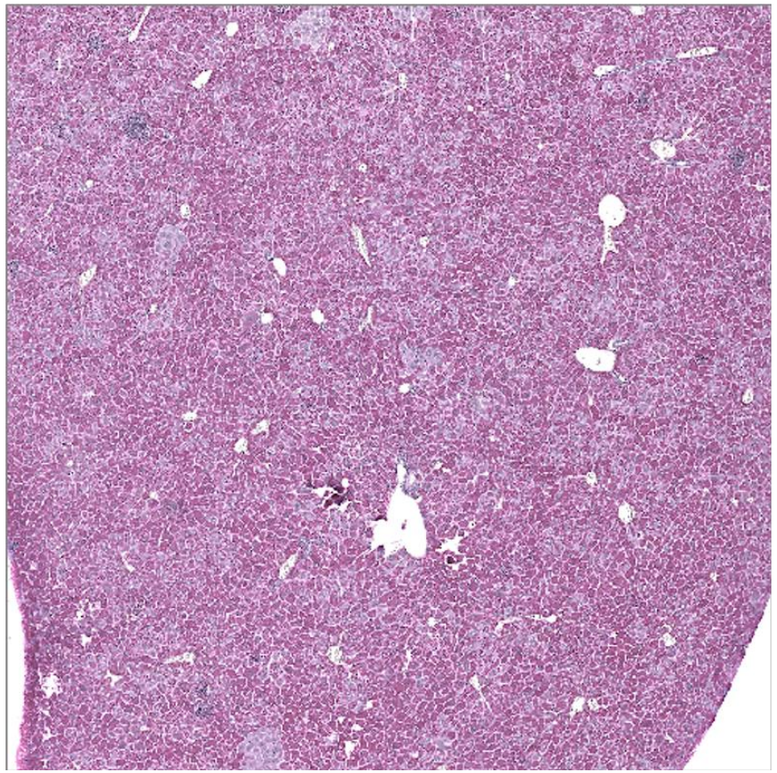

## PiZ Correction is:

- Specific to the appropriate treatment group
- May confer a proliferative advantage to edited hepatocytes

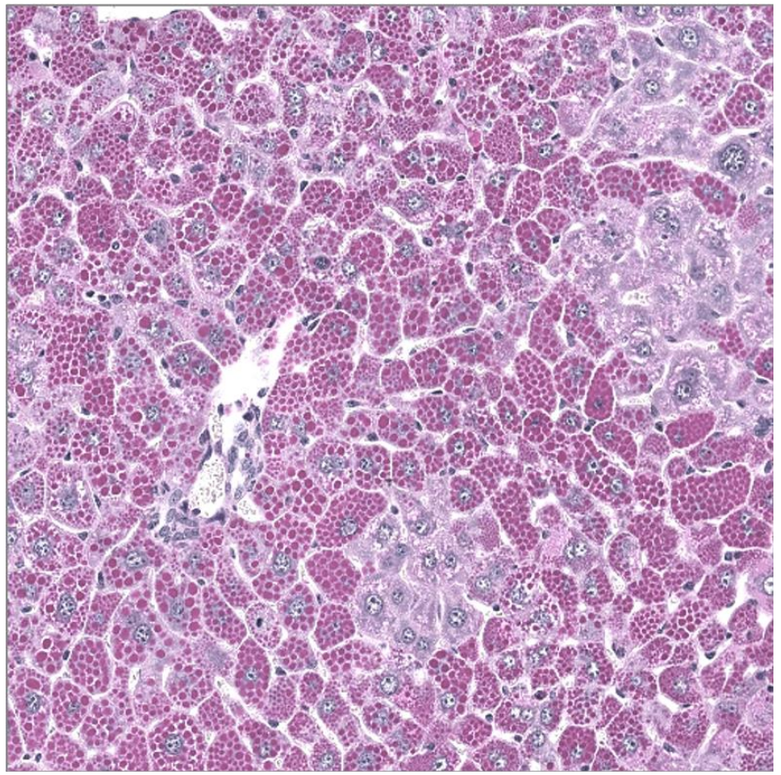
**Liver phenotype**
**A1AT secretion and function**

# PAS-D Specifically Stains Insoluble PiZ Globules in NSG-PiZ Mouse Liver Sections

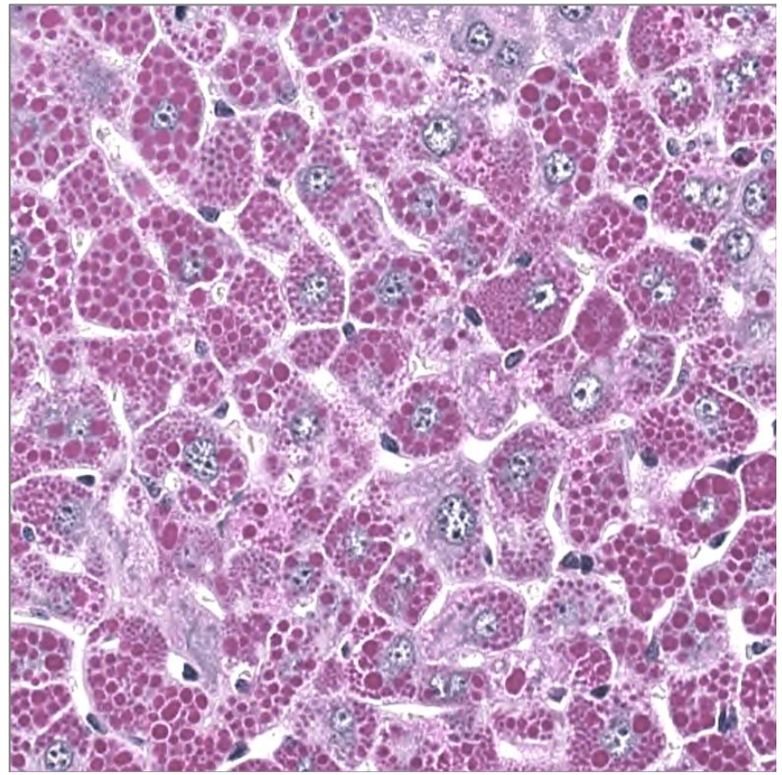
40x



100x

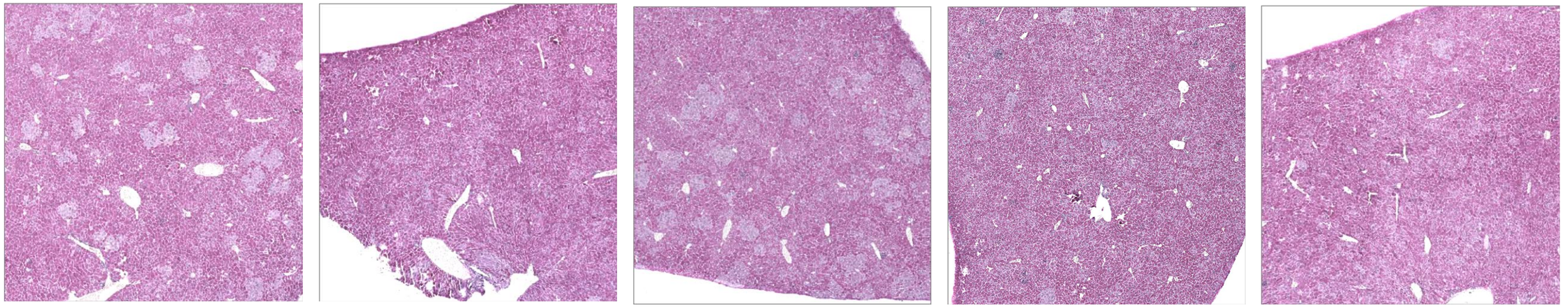


200x

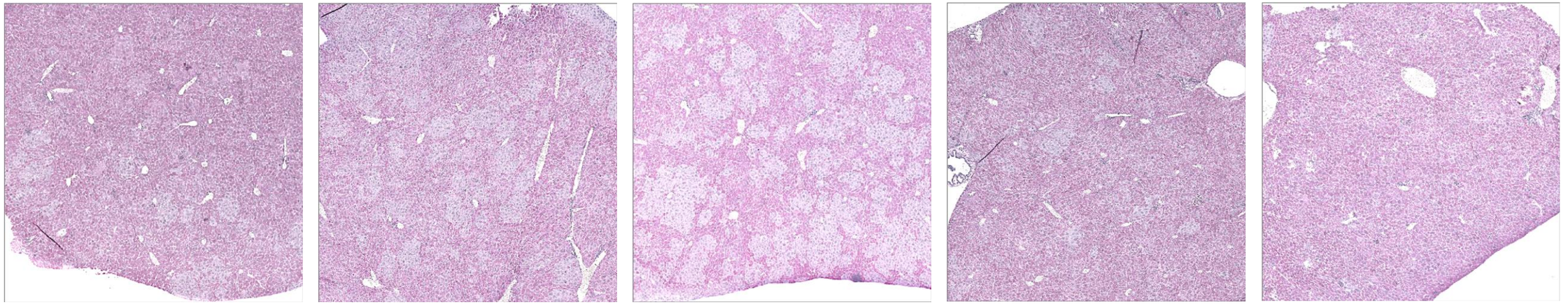


# In Vivo Correction of the PiZ Mutation Reduces PAS-D Globule Burden in Mouse Liver

## PCSK9

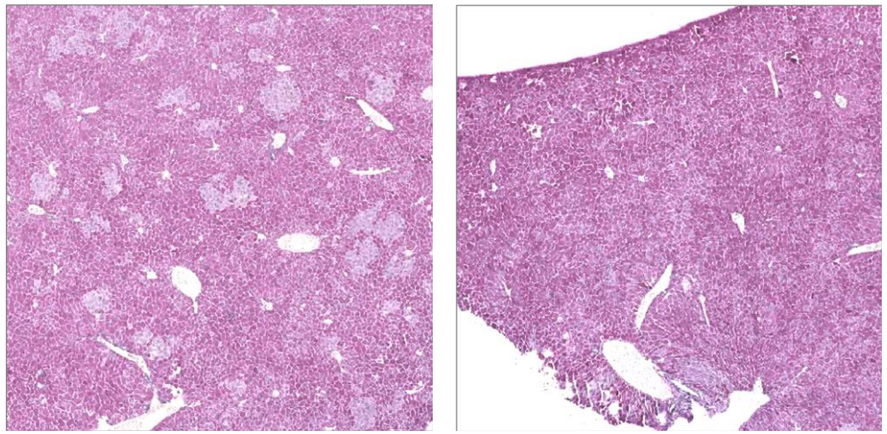


## Correction

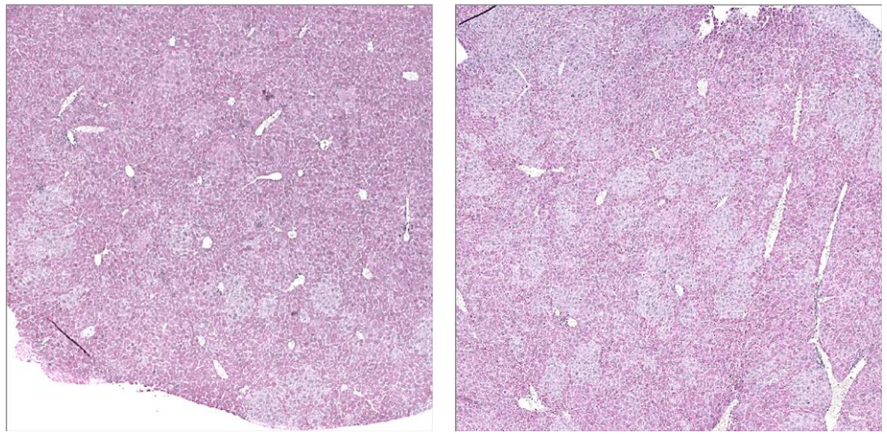


# In Vivo Correction of the PiZ Mutation Reduces PAS-D Globule Burden in Mouse Liver

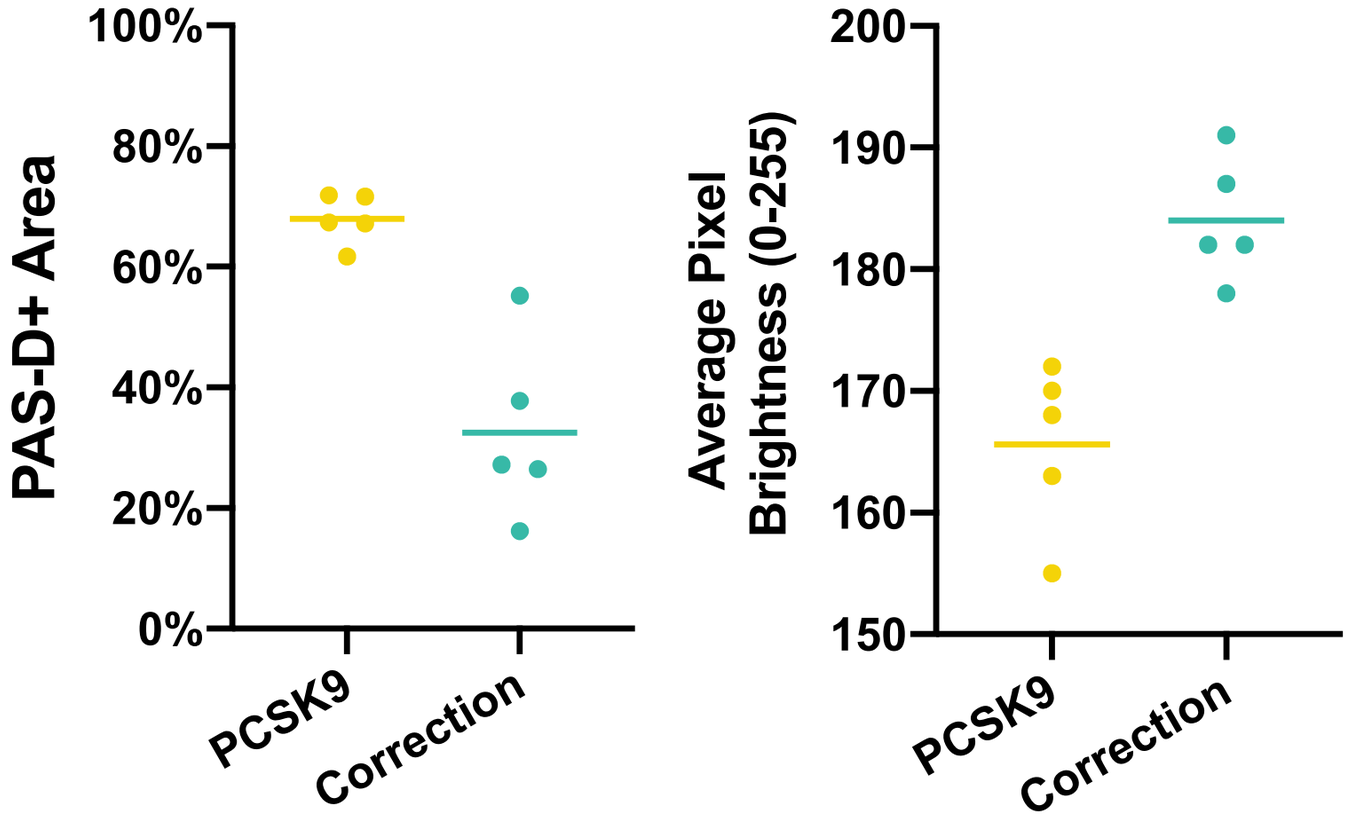
## PCSK9



## Correction

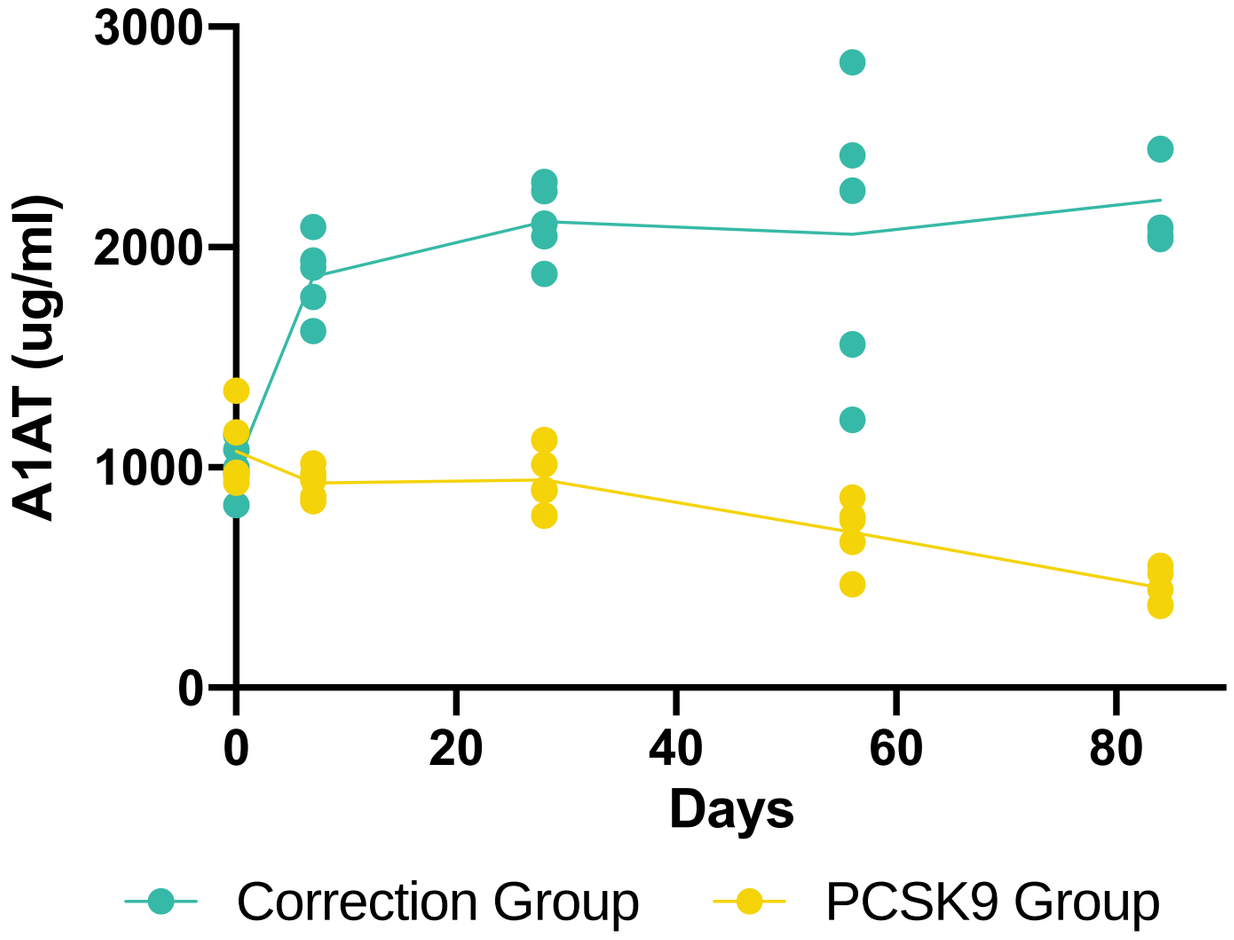


## Color Threshold Analysis



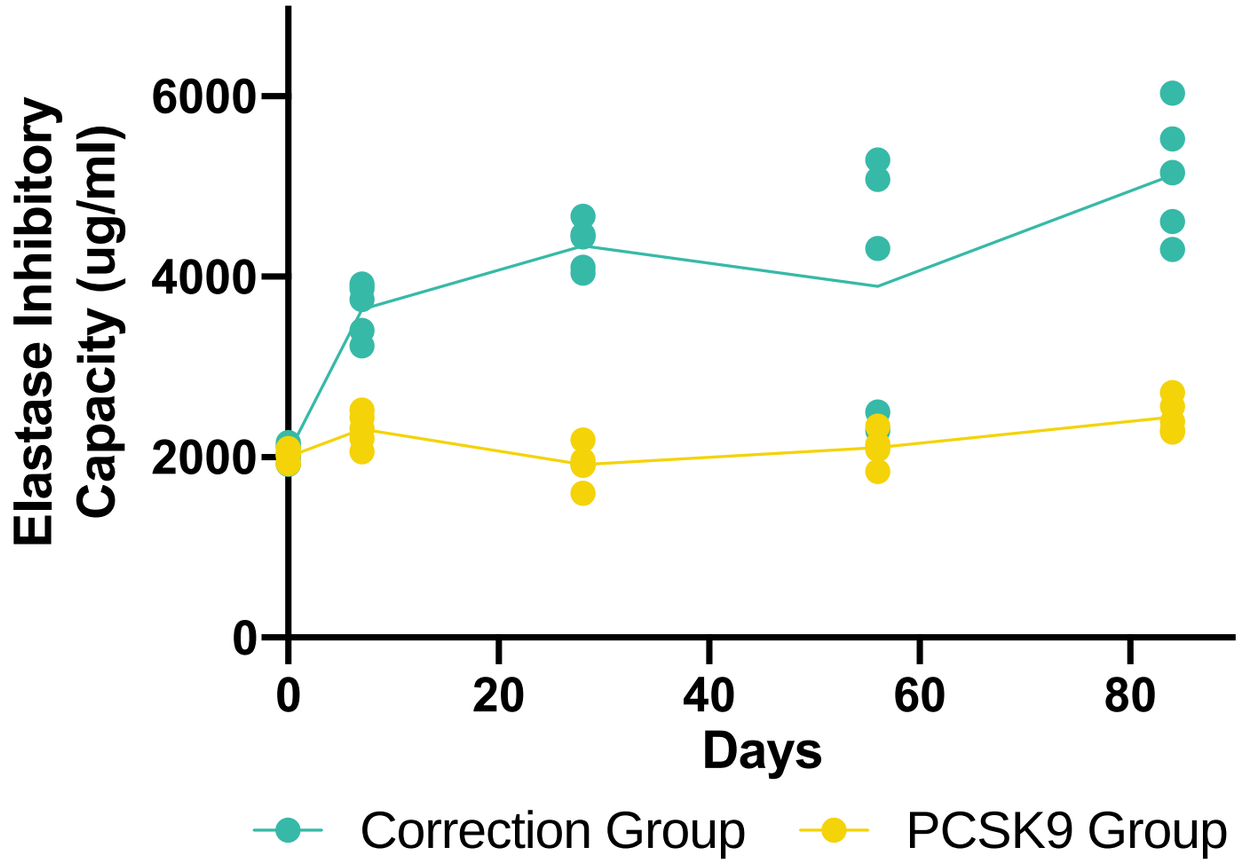

▶ Genetic correction of E342K decreases PASD staining density and intensity

# In Vivo Correction of the PiZ Mutation Increases Total Human A1AT in Serum



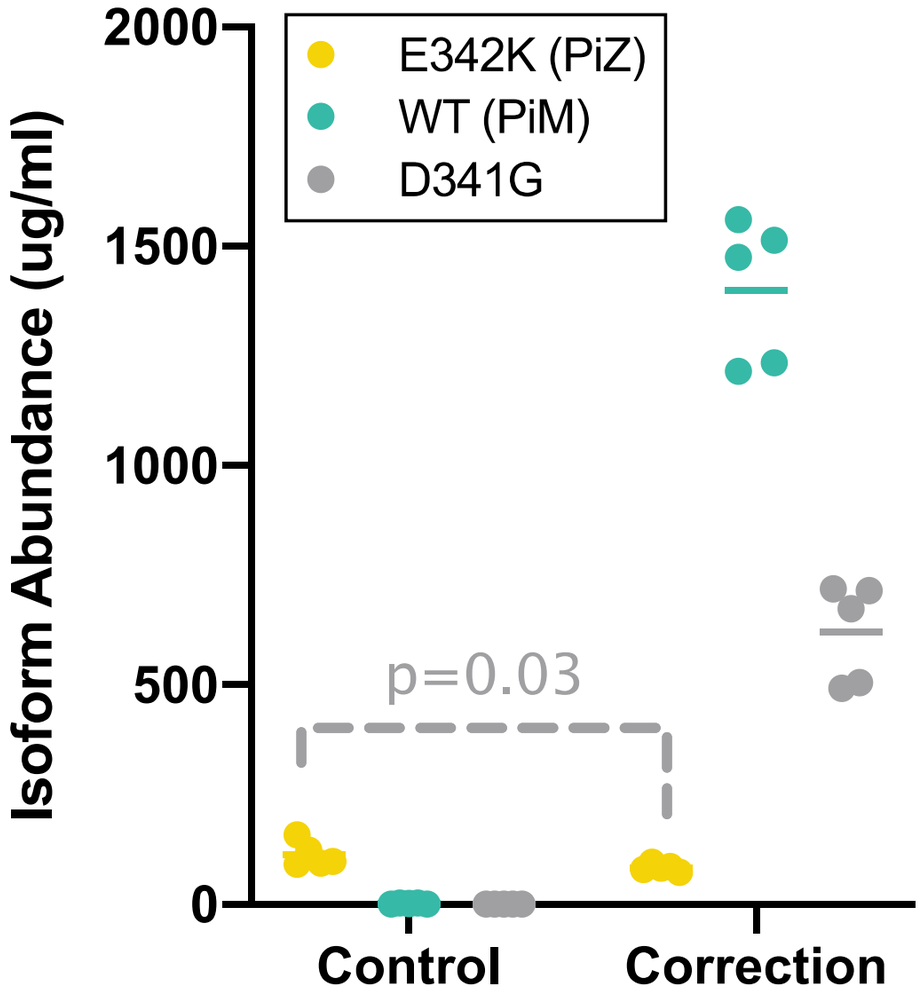

- Serum A1AT declines in the control group
- Upon genetic correction, a durable increase in serum A1AT is observed (4.9-fold at 3 months)
- This increase in serum A1AT, *if translated to humans*, could confer some degree of pulmonary protection

# In Vivo Correction of the PiZ Mutation Increases *Functional* A1AT in Serum




- **Genetic correction increases serum capacity to inhibit neutrophil elastase**
  - **Normalized to a purified human A1AT standard**

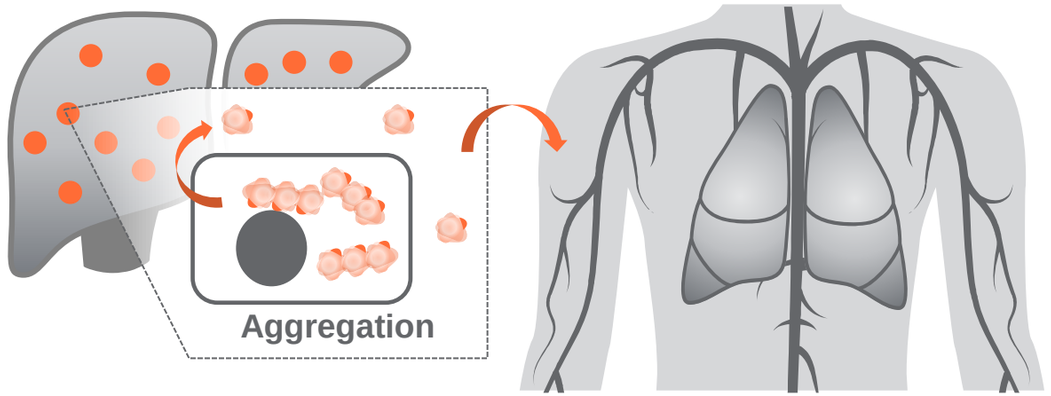
# Mass Spectrometry Confirms the Emergence of WT (PiM) and D341G A1AT




- Isoform abundance correlates with allele frequencies (PiM>D341G)
- Genetic correction decreases E342K (PiZ) abundance

# Progress Towards an A1AT Deficiency Base Editing Therapeutic

| | Molecular | Liver | Circulation | Outcome |
|---|---|---|---|---|
| **A1AT Deficiency** | Protein<br>RNA<br>DNA<br>Gene: E342K (PiZ) severe<br>E264V (PiS) mild | Aggregation | | • A1AT ZZ proteins aggregate in the liver cause liver tissue damage<br>• Deficiency of A1AT in circulation leads to lung tissue damage<br>• Enzyme replacement slows but does not halt progression of lung disease |






- **ABE was optimized to correct the PiZ mutation in cell cultures** ✓
- **LNP delivery mediates efficient genetic correction in livers of NSG-PiZ mice**
- **PAS-D stain reveals decreased PiZ globule burden** ✓
- **Genetic correction increases circulating levels of A1AT potentially providing pulmonary protection** ✓

# Next Steps & Conclusions

- **Conclusions:**
  - **Taken together, our results indicate that the precise correction of the PiZ mutation with an adenine base editor represents a feasible approach for the treatment of A1AD lung and liver disease.**

- **Next steps:**
  - **Further optimization of our proprietary LNP formulation is progressing**
  - **Additional improvements to editor and gRNA are ongoing**
  - **Off-target characterization has been initiated**

# Thank You

**Liver Therapeutics**
- Francine Gregoire
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- Aalok Shah
- Robert Dorkin

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- Krishna Ramanan
- Richard Dutko

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- Jeremy Decker

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- Valentina McEneany

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- Jeff Marshall

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- Seda Gyonjyan

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- John Evans
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- Sylvia Eash
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- Steve Cavnar

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- Vivek Chowdhary

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# Questions!

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