in vivo Base-Editing Corrects Metabolic Defects in Glycogen Storage Disease Type-Ia

Yvonne Aratyn-Schaus

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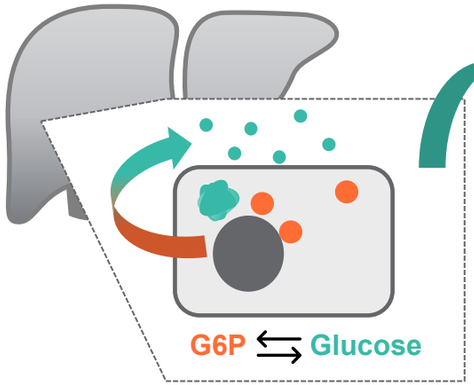
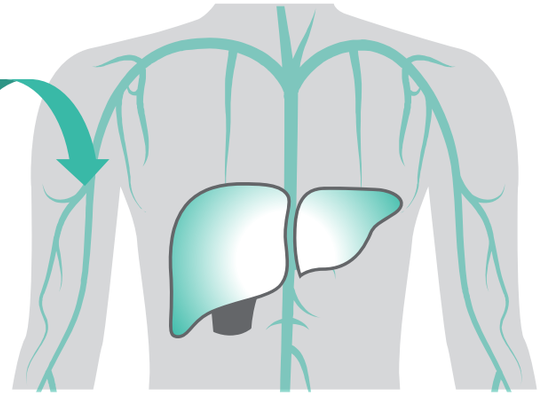
DISCLOSURE

- I am a Beam employee and shareholder

GSD-Ia overview

	Molecular	Liver	Circulation	Outcome
Normal G6PC Function	**Protein** ▲ **RNA** ▲ **DNA** **Gene** WT *G6PC* gene			• Glucose homeostasis is primarily maintained by the liver • When blood sugar drops below normal, G6P, produced in the terminal step of glycogenolysis and gluconeogenesis is converted to glucose by glucose-6-phosphatase-α (G6Pase) in the liver

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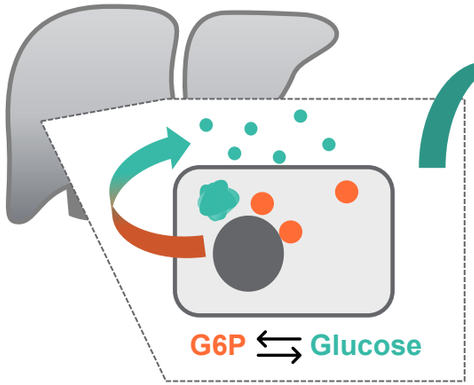
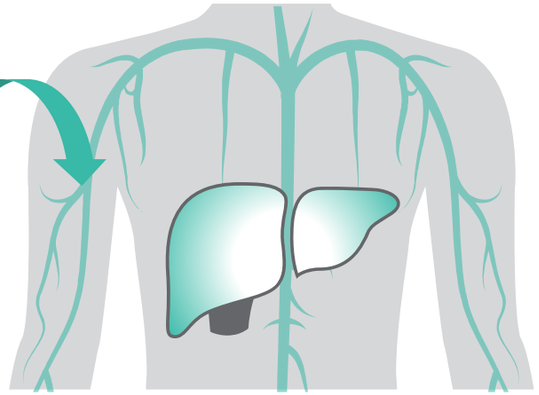
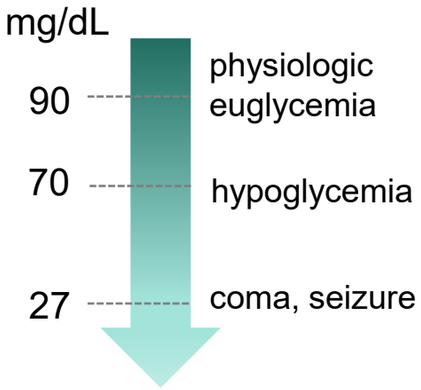
Normal G6PC Function	Protein RNA DNA Gene: WT *G6PC* gene			• Glucose homeostasis is primarily maintained by the liver • When blood sugar drops below normal, G6P, produced in the terminal step of glycogenolysis and gluconeogenesis is converted to glucose by glucose-6-phosphatase-α (G6Pase) in the liver
G6PC Loss-of-function	Protein RNA DNA Gene: Mutated *G6PC* gene (R83C)			• GSD-Ia patients lacking G6pase cannot produce endogenous glucose and suffer from fasting hypoglycemia which may result in seizures or death • Current treatment is dietary therapy • Hepatomegaly is clinical manifestation of GSD-Ia caused by glycogen accumulation

GSD-Ia overview

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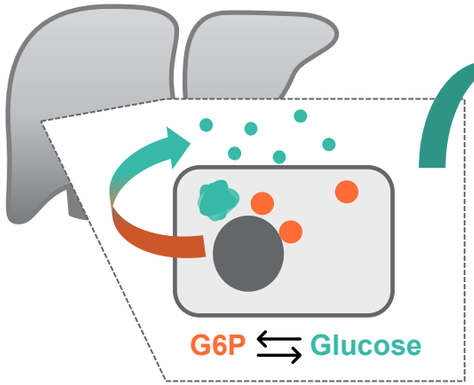
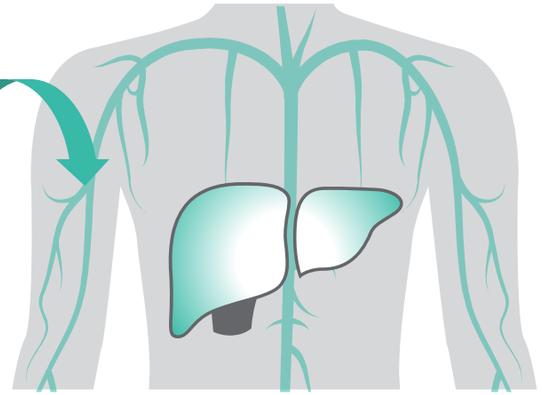
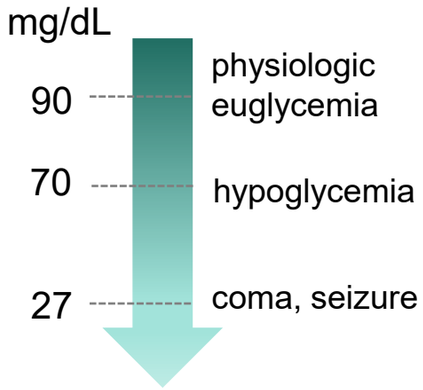
Normal G6PC Function	Protein RNA DNA Gene: WT *G6PC* gene			• Glucose homeostasis is primarily maintained by the liver • When blood sugar drops below normal, G6P, produced in the terminal step of glycogenolysis and gluconeogenesis is converted to glucose by glucose-6-phosphatase-α (G6Pase) in the liver
G6PC Loss-of-function	Protein RNA DNA Gene: Mutated *G6PC* gene (R83C)			• GSD-Ia patients lacking G6pase cannot produce endogenous glucose and suffer from fasting hypoglycemia which may result in seizures or death • Current treatment is dietary therapy • Hepatomegaly is clinical manifestation of GSD-Ia caused by glycogen accumulation

Today's agenda

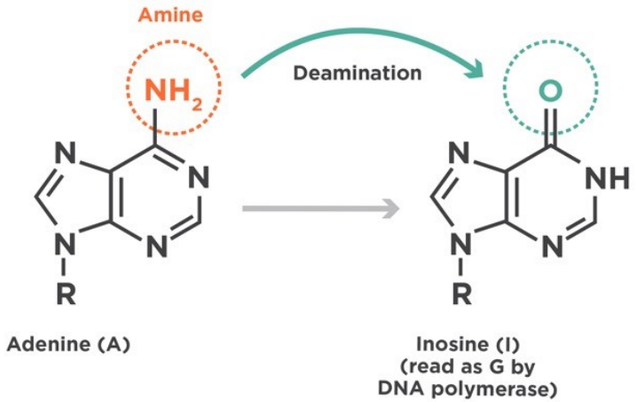
1. **Base editing**
   Optimization of base editors for precise correction of G6PC-R83C
2. **Disease model**
   Characterization of an R83C transgenic mouse model of GSD-Ia
3. **In vivo correction**
   In vivo base-editing and correction of metabolic defects associated with GSD-Ia
4. **Next steps**

Base Editors Generate Permanent and Predictable Single Nucleotide Substitutions

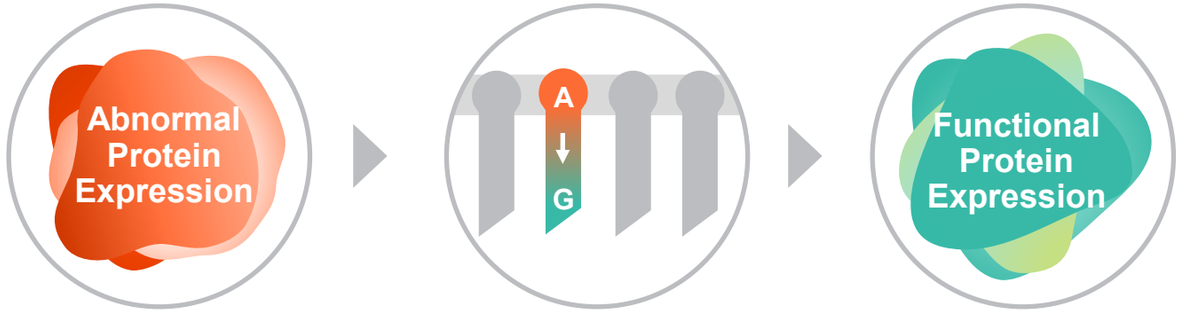
Base editor binds the target DNA and exposes a narrow editing window

A-to-G base editor ("ABE")

Deaminase chemically modifies target base, A>G edit made permanent by DNA repair/replication



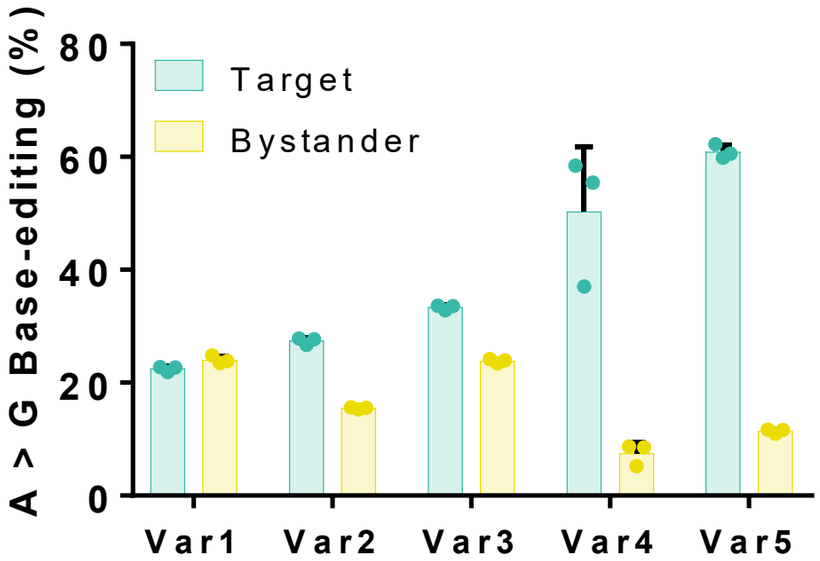
Gene Correction – Direct repair of point mutations to restore gene function



1

Base Editing: Lead optimization in immortalized HEK293 cells yields significant rate of precise correction of R83C

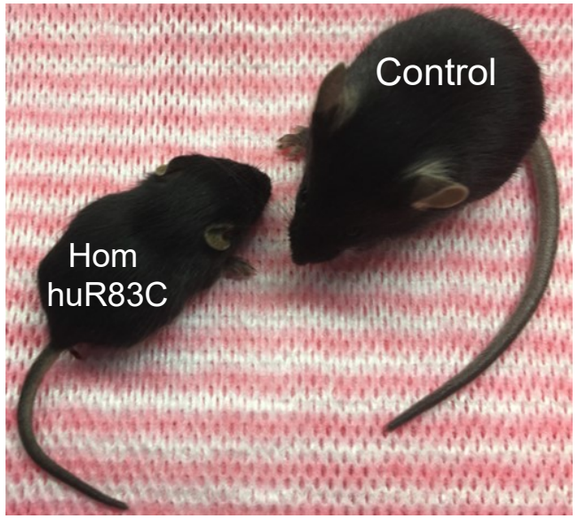
Nucleotide Edits		G6PC Mutation
Unedited	→	R83C
A12	→	WT (R83R)
A10	→	Synonymous
A6	→	Y85H



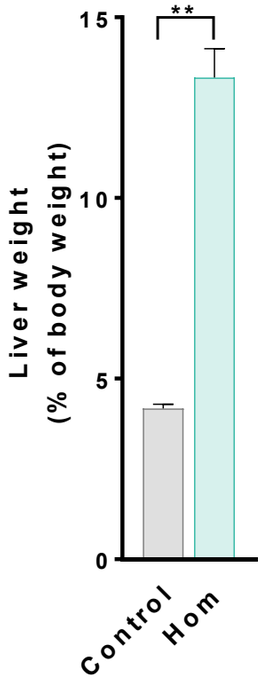
- Lead optimization yields **~60% targeted base-editing efficiency, reduced bystander editing**
- What is the functional benefit of R83C correction via base-editing in a GSD-Ia mouse model?

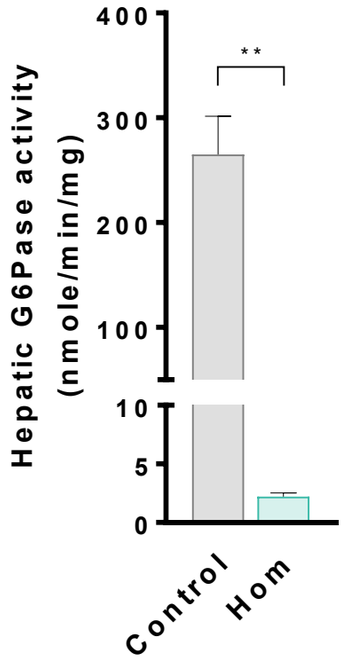
2

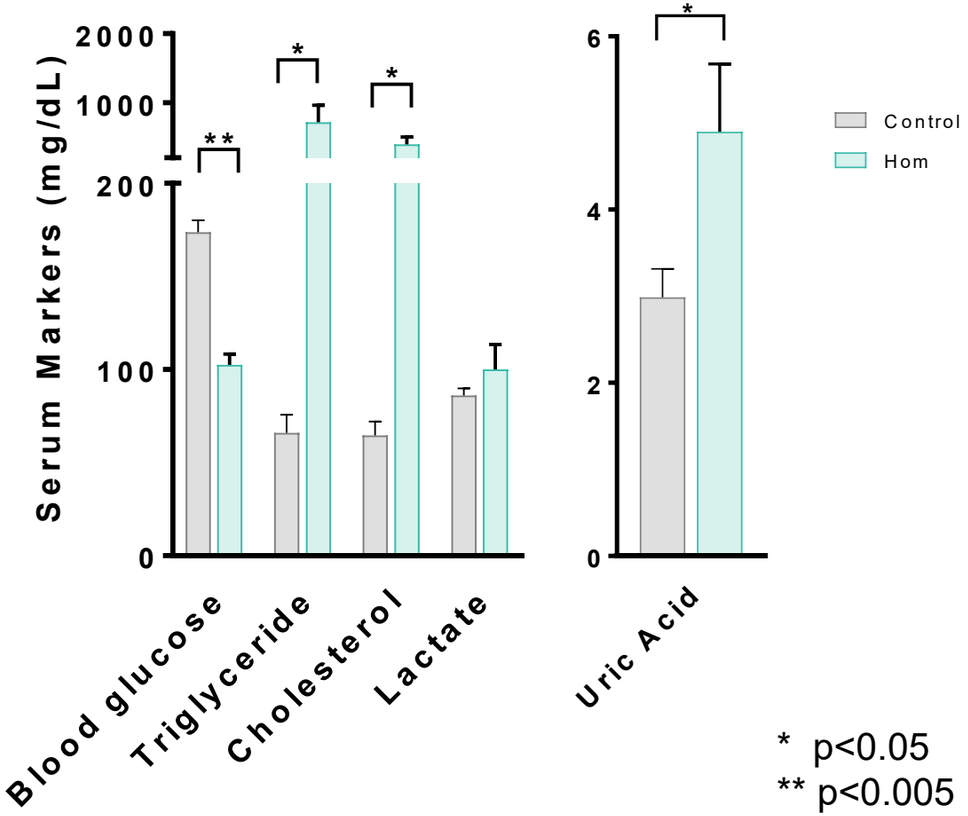
Disease Model: 3-week-old homozygous huR83C mice exhibit expected growth impairment and metabolic defects



The homozygous huR83C mouse is a novel GSD-Ia model in which a human *G6PC*-R83C transgene replaces mouse *G6pc*





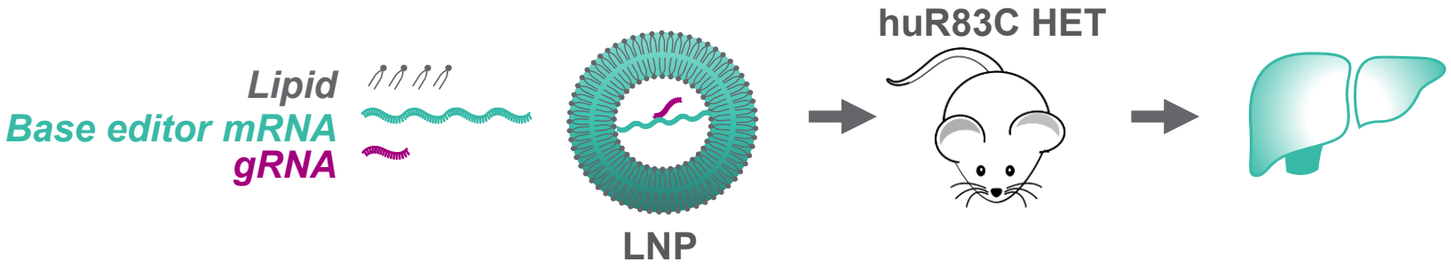


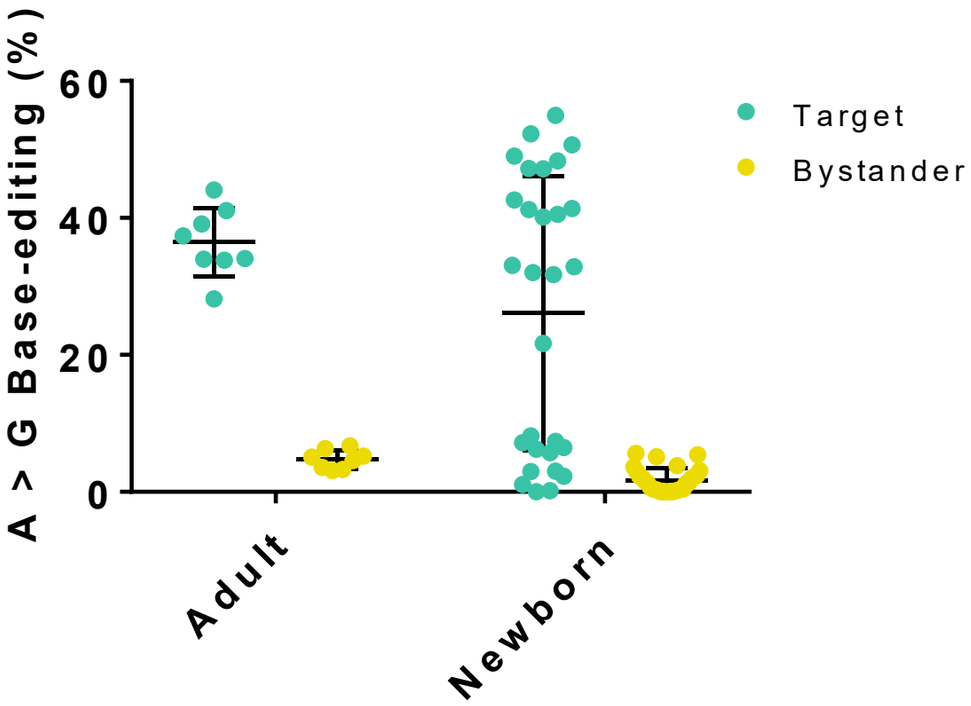
* p<0.05
** p<0.005

Relative to littermate controls, GSD-Ia mice homozygous for huG6PC-R83C exhibit

- **Postnatal lethality**
- **Lower body weight**
- **Enlarged livers**
- **Significant G6Pase inhibition**
- **Abnormal serum metabolites**

3 *In vivo* correction: Efficient LNP-mediated base editing in livers of adult and newborn heterozygous huR83C mice





- Given neonatal lethality of the GSD-Ia mouse model, we explored LNP-dosing shortly after birth via the temporal vein
- LNP administered via tail vein (adult) or temporal vein (newborn) in heterozygous huR83C mice
- **Next-gen. sequencing analysis in total liver extracts yield**
  - **~40% base-editing efficiency** in adults
  - A range, **up to ~60%** in newborns
- Next step: Correction in newborn homozygotes

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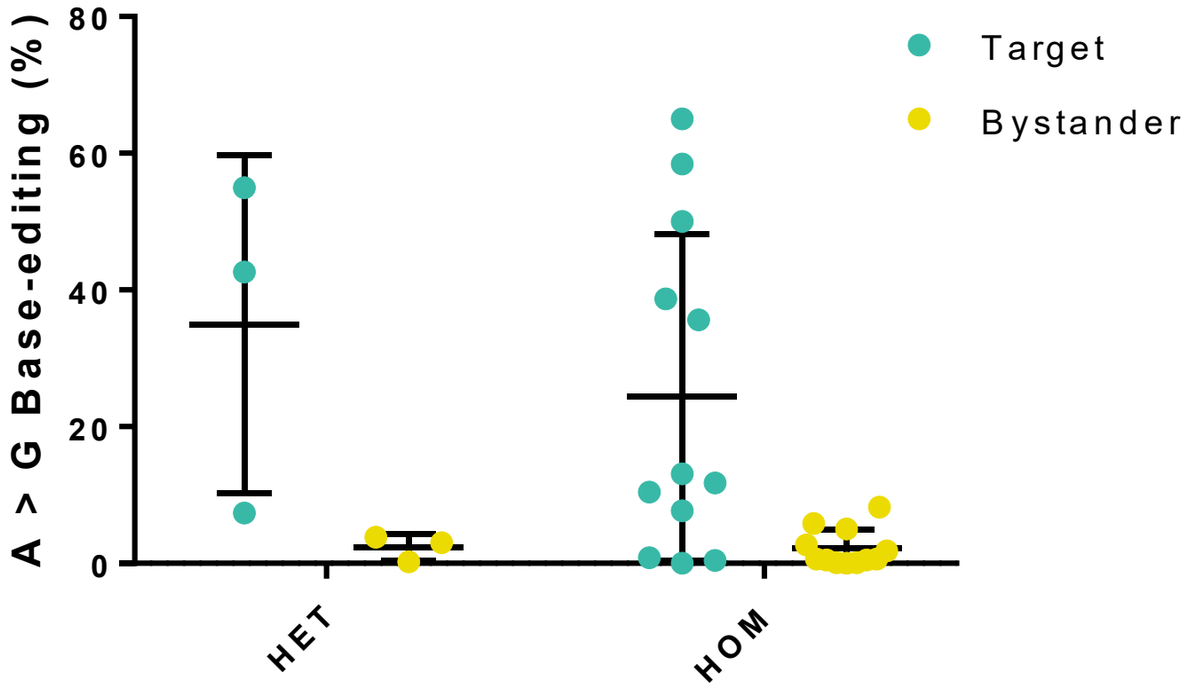
In vivo correction: LNP-mediated R83C correction is associated with survival of homozygous huR83C mice



- LNP-dosed homozygous huR83C mice survived to 3 weeks of age without glucose therapy
- Up to **~60% R83C correction**

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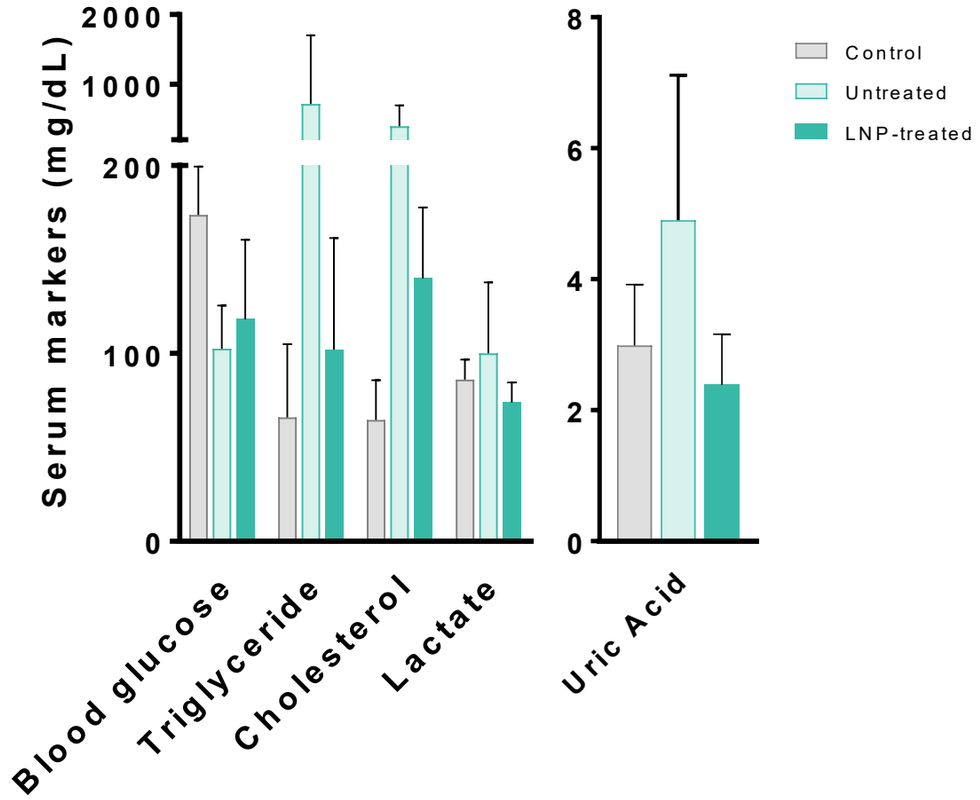
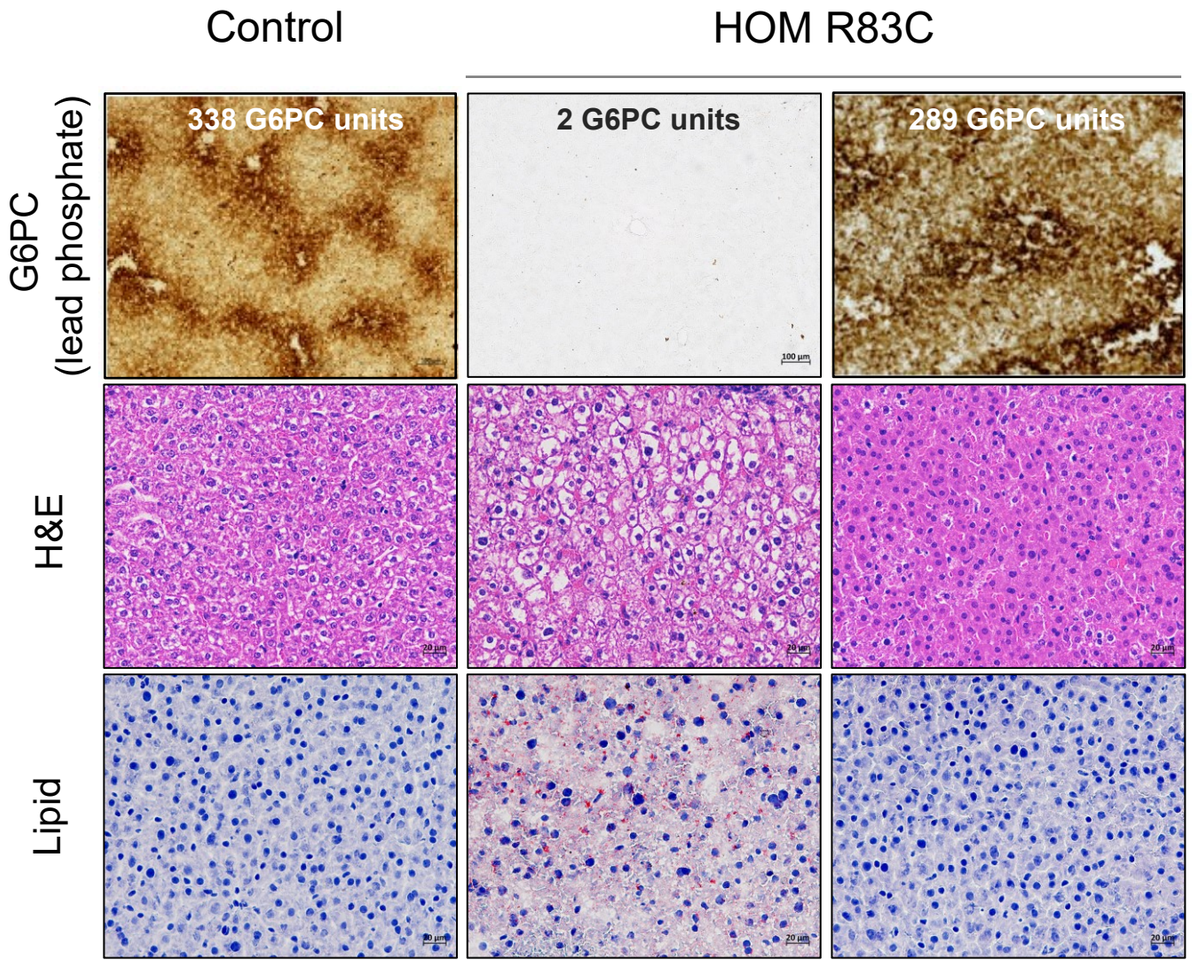
In vivo correction: Base editing reverses GSD-Ia pathology





R83C correction is associated with restoration of **near-normal serum metabolites, G6PC activity, hepatic morphology and lipid deposition**

4

Summary and Next Steps

Summary

- Base editor and guide RNA optimized for correction of R83C in vitro
- Transgenic huR83C mice exhibit expected GSD-Ia phenotypes
- LNP-mediated base editing yields up to ~60% R83C correction and restoration of function in treated homozygous huR83C mice

Next steps

- *in vivo* fasting challenge studies
- Correlation of base-editing efficiency and metabolic function

Thank You

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