

# CRISPR/Cas9-mediated Gene Knockout to Address Primary Hyperoxaluria

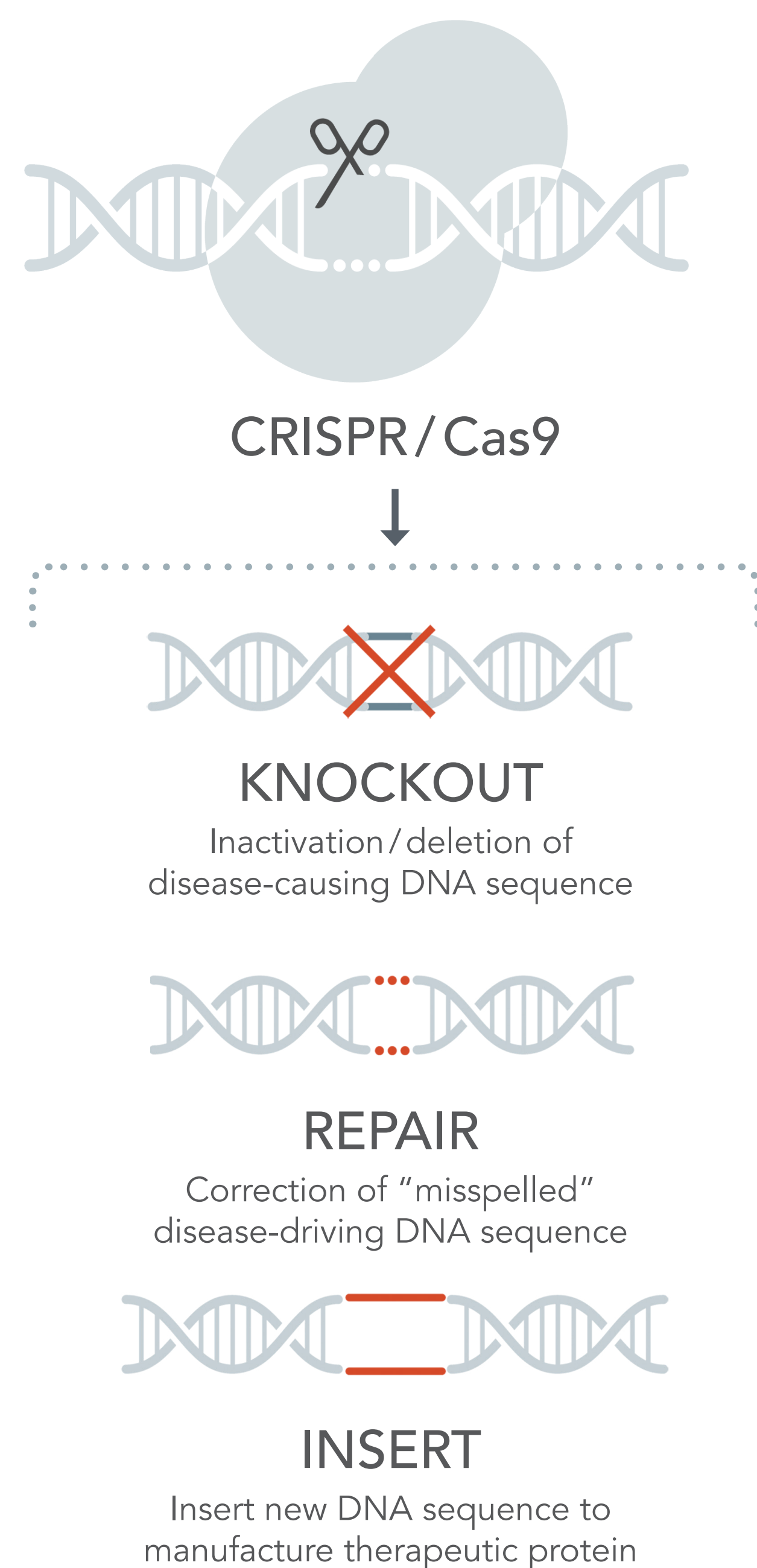
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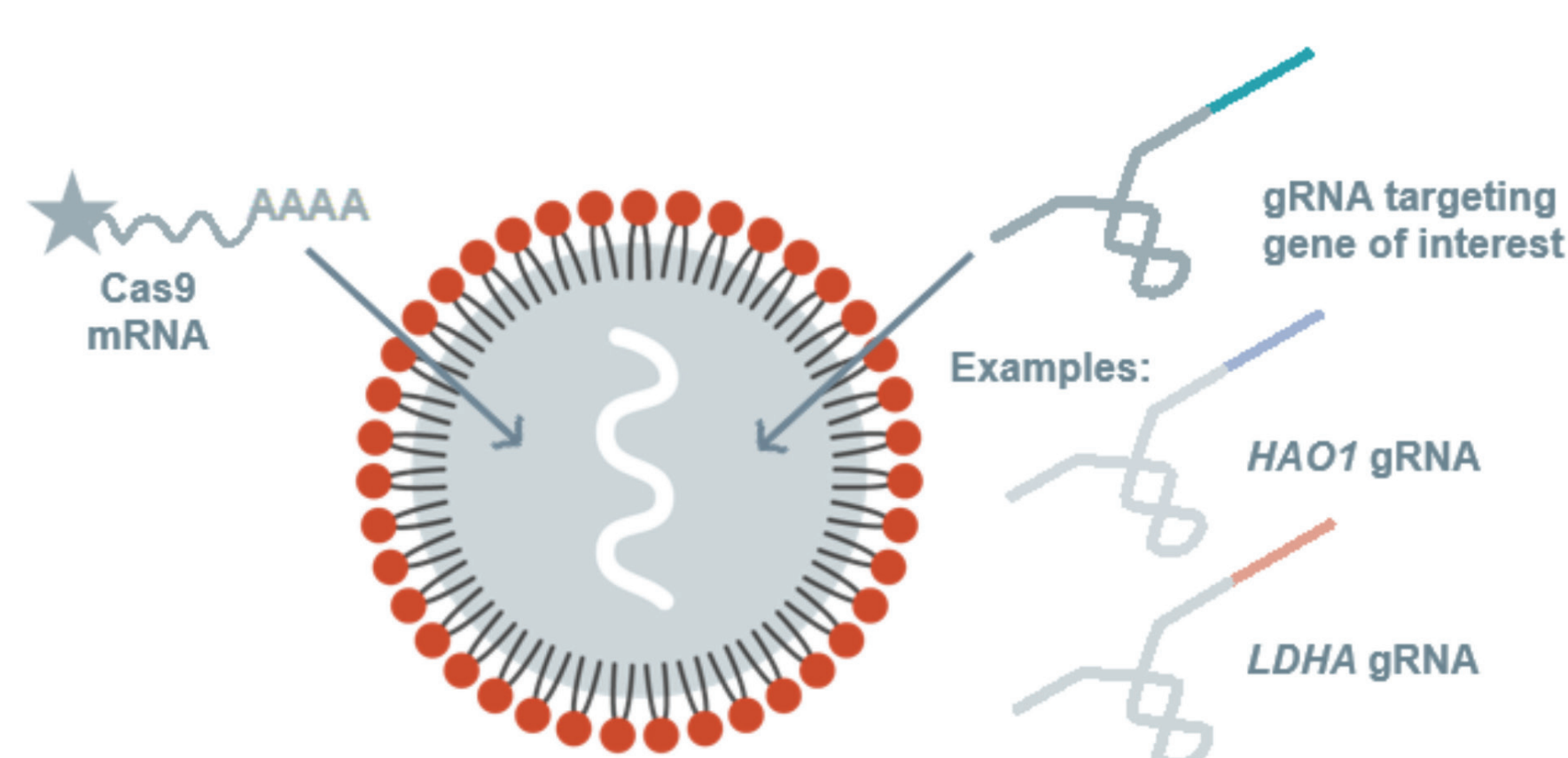
## INTRODUCTION

Primary hyperoxaluria (PH) is a rare genetic disease caused by mutations in one of three genes (*AGXT*, *GRHPR* and *HOGA1*) involved in the glyoxylate detoxification pathway, giving rise to PH types 1, 2, and 3, respectively. PH is characterized by excessive accumulation of the toxic waste product oxalate, which leads to formation of insoluble deposits in the kidney and other organs, resulting in renal failure and systemic oxalosis. Currently, the treatment of late-stage disease is limited to combined liver-kidney transplantation. Here, we tested the hypothesis that non-viral CRISPR/Cas9-mediated editing of two genes involved in oxalate formation, *Ldha* and *Hao1*, could significantly lower urinary oxalate in a mouse model of PH1, providing proof-of-concept for a one-time treatment approach for the disease.



## MODULAR NONVIRAL CRISPR DELIVERY SYSTEM

### Lipid Nanoparticles (LNPs)

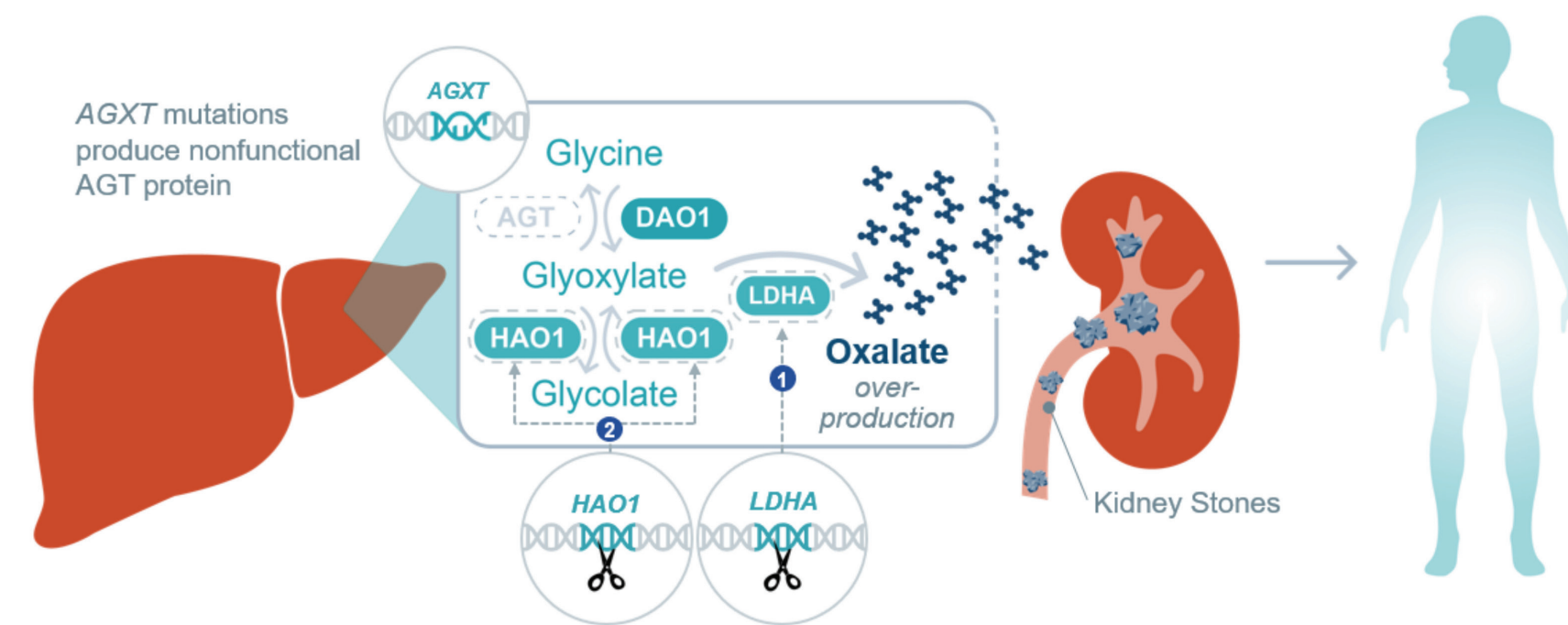


Variable portion of Intellia's modular LNP-based liver knockout approach is limited to 20-mer of gRNA

### Key Advantages of LNP Delivery

- Large cargo capacity for CRISPR/Cas9
- Transient expression
- Low immunogenicity with redosing capability
- Biodegradable and well-tolerated
- Scalable synthetic manufacturing

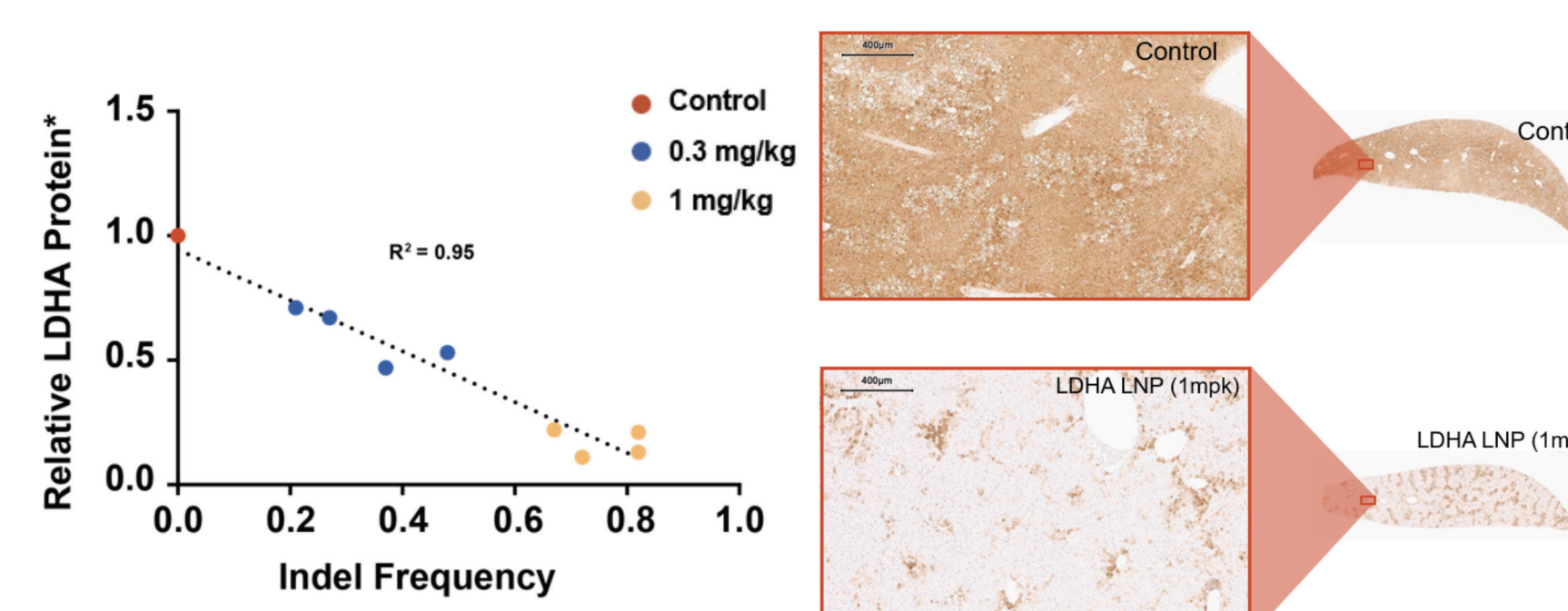
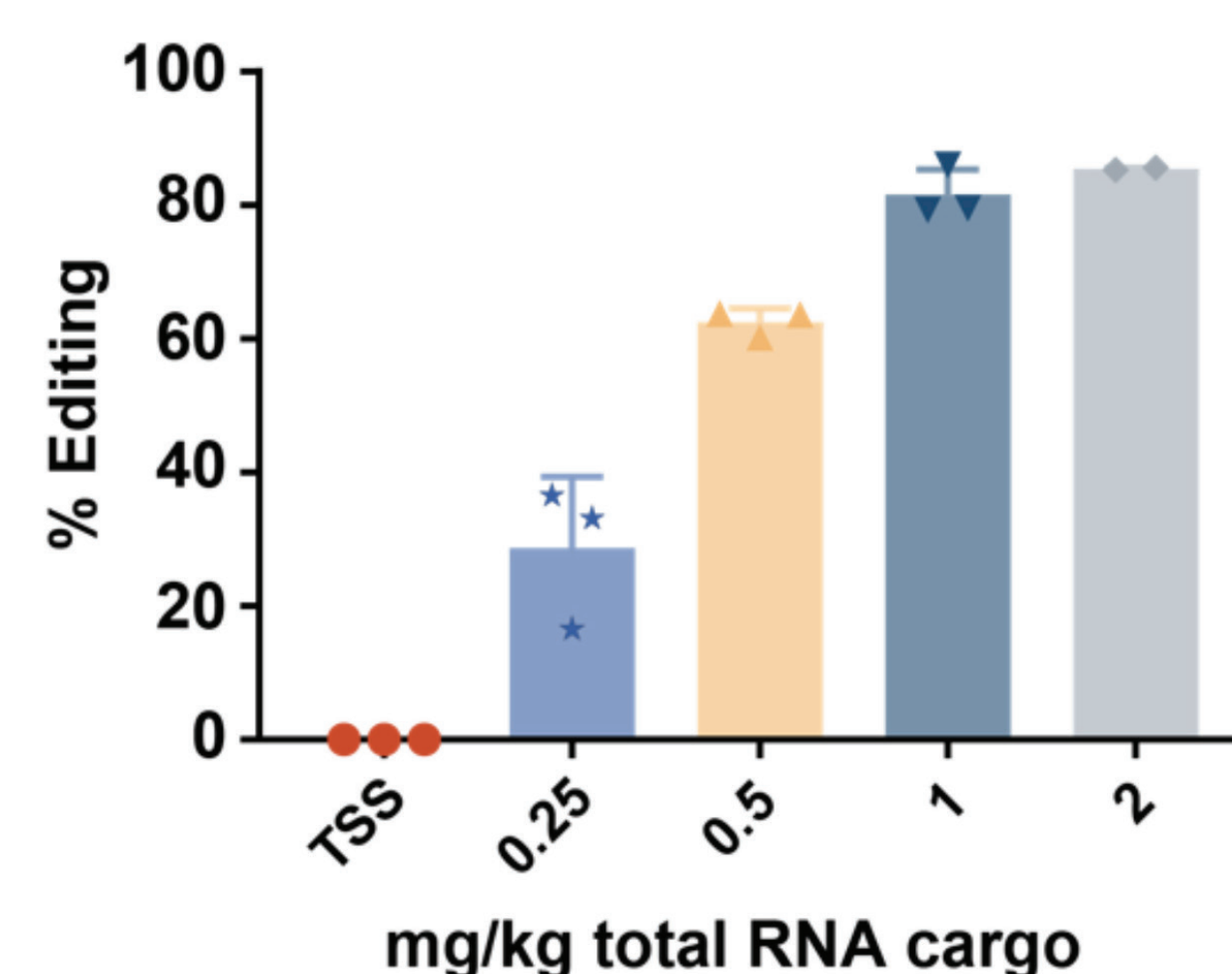
## RESULTS



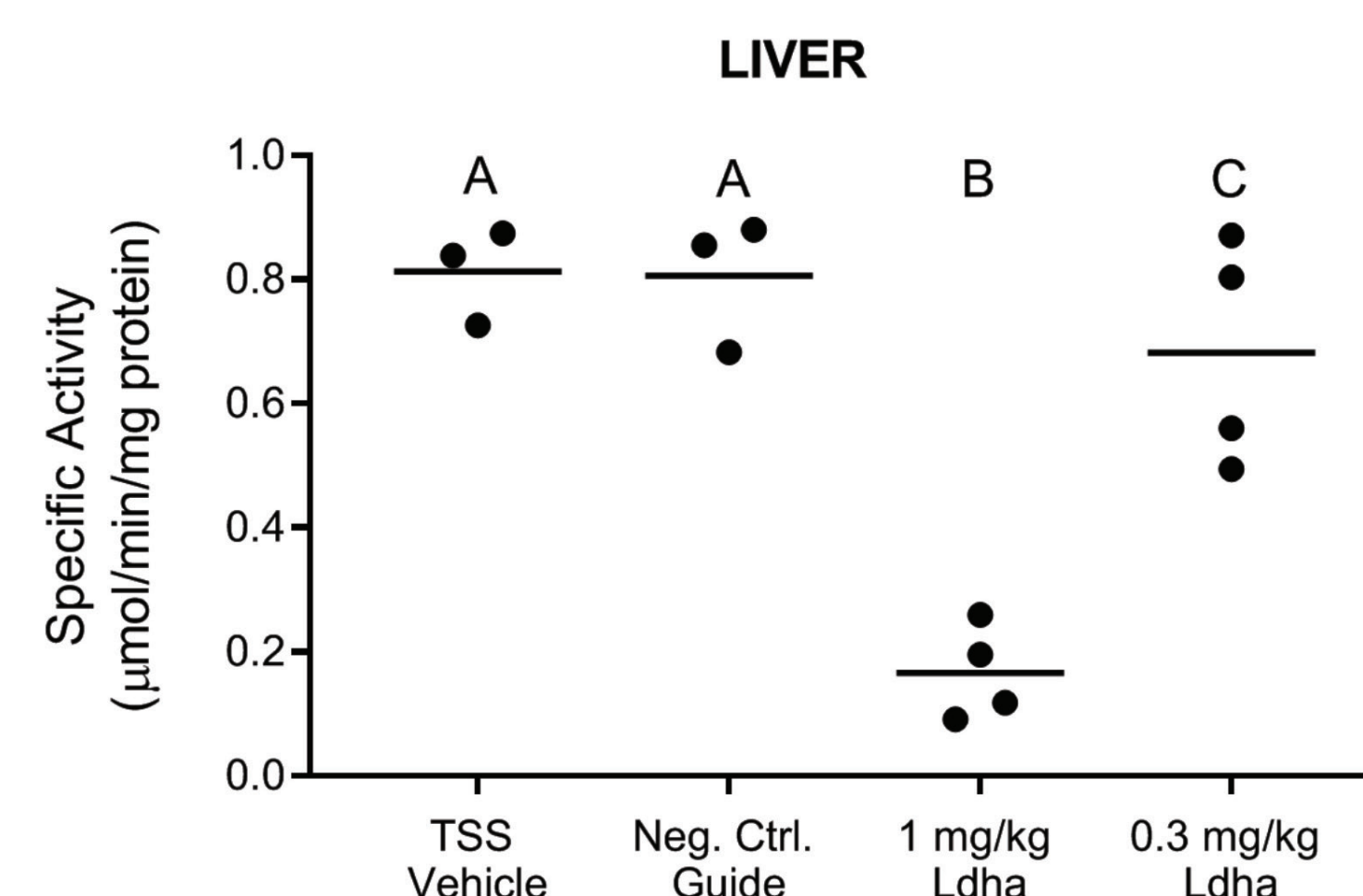
- Potential to treat PH1 with either:
1. CRISPR/Cas9-mediated knockout of *LDHA* or
  2. CRISPR/Cas9-mediated knockout of *HAO1*

## TARGETING *LDHA* WITH CRISPR/Cas9

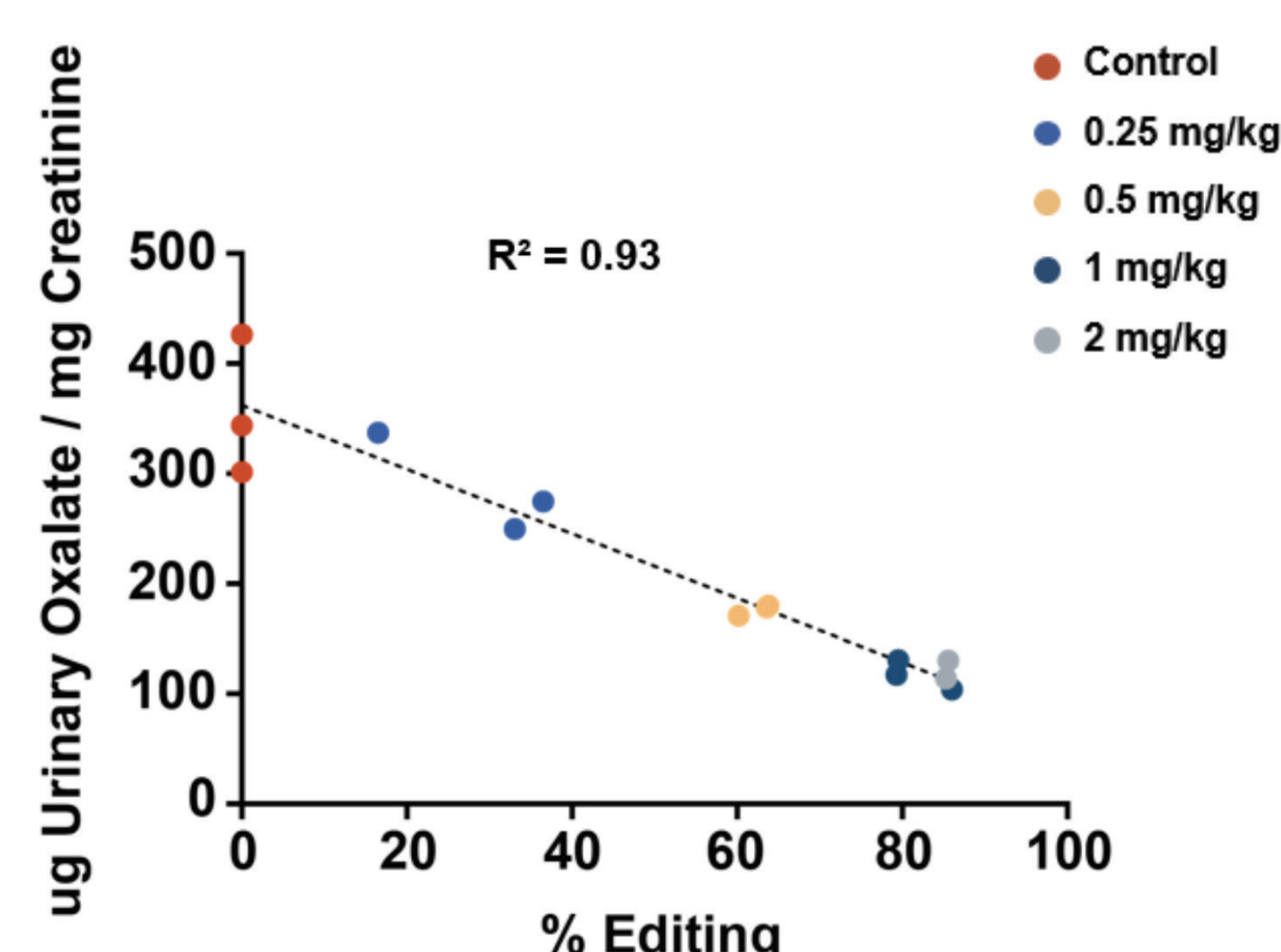
### Lead Guide Achieved Robust Editing and Protein Reduction\*



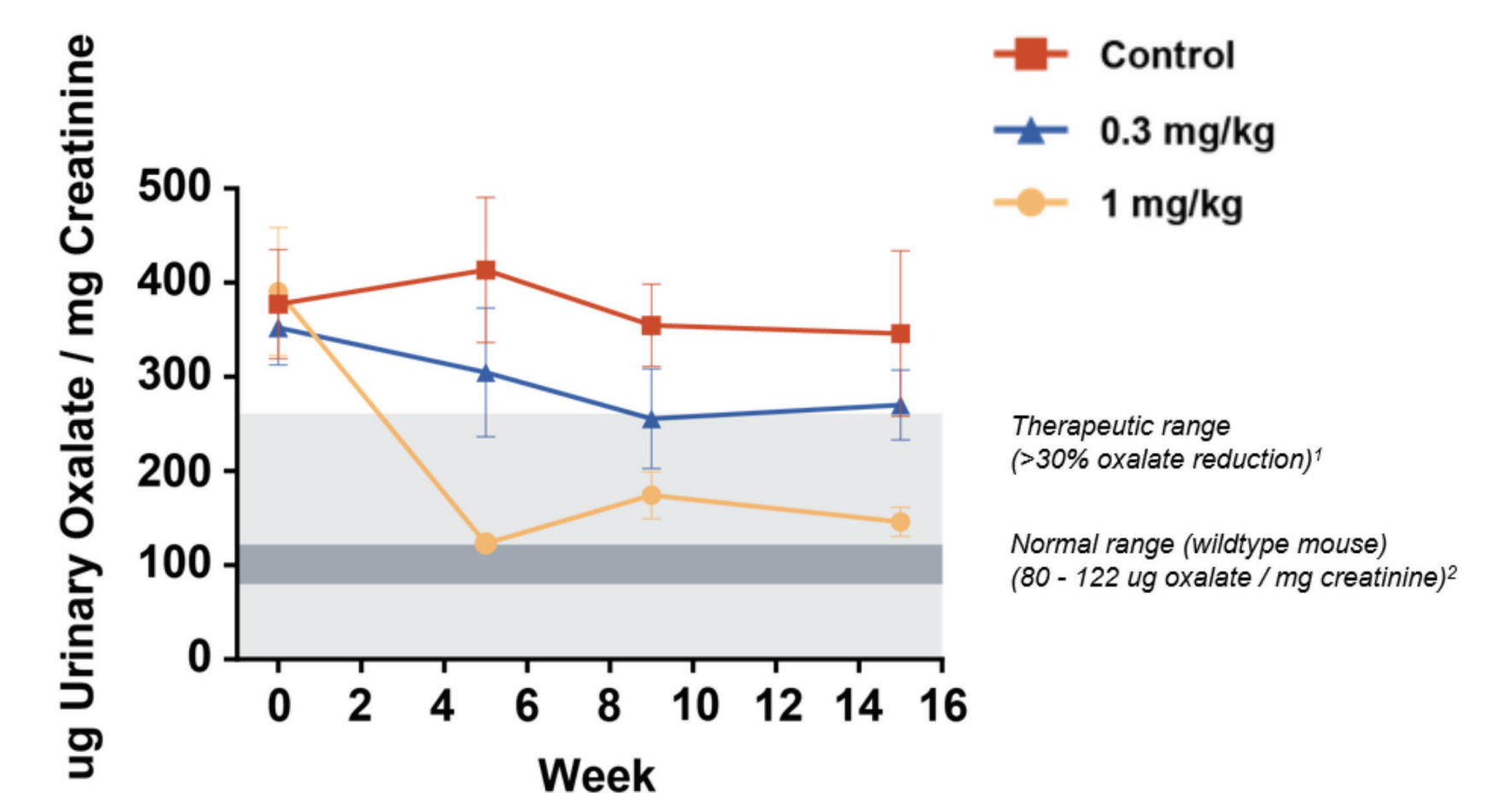
### Editing of *Ldha* Gene Reduces Liver LDH Enzyme Activity\*



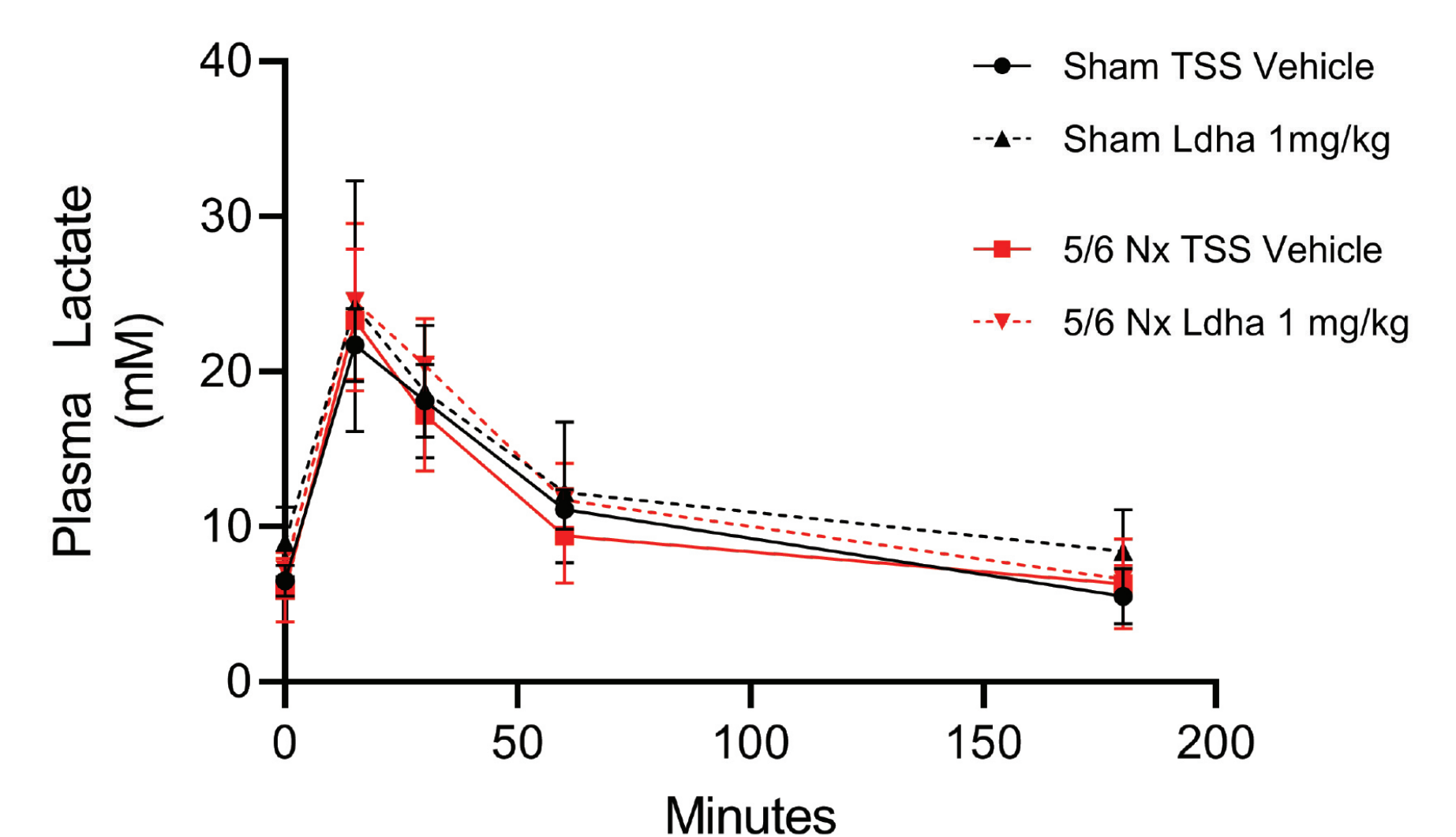
### Increased Editing and Reduction in Urinary Oxalate are Dose-Responsive\*



### *Ldha* Knockout Results in Sustained Oxalate Reduction\*

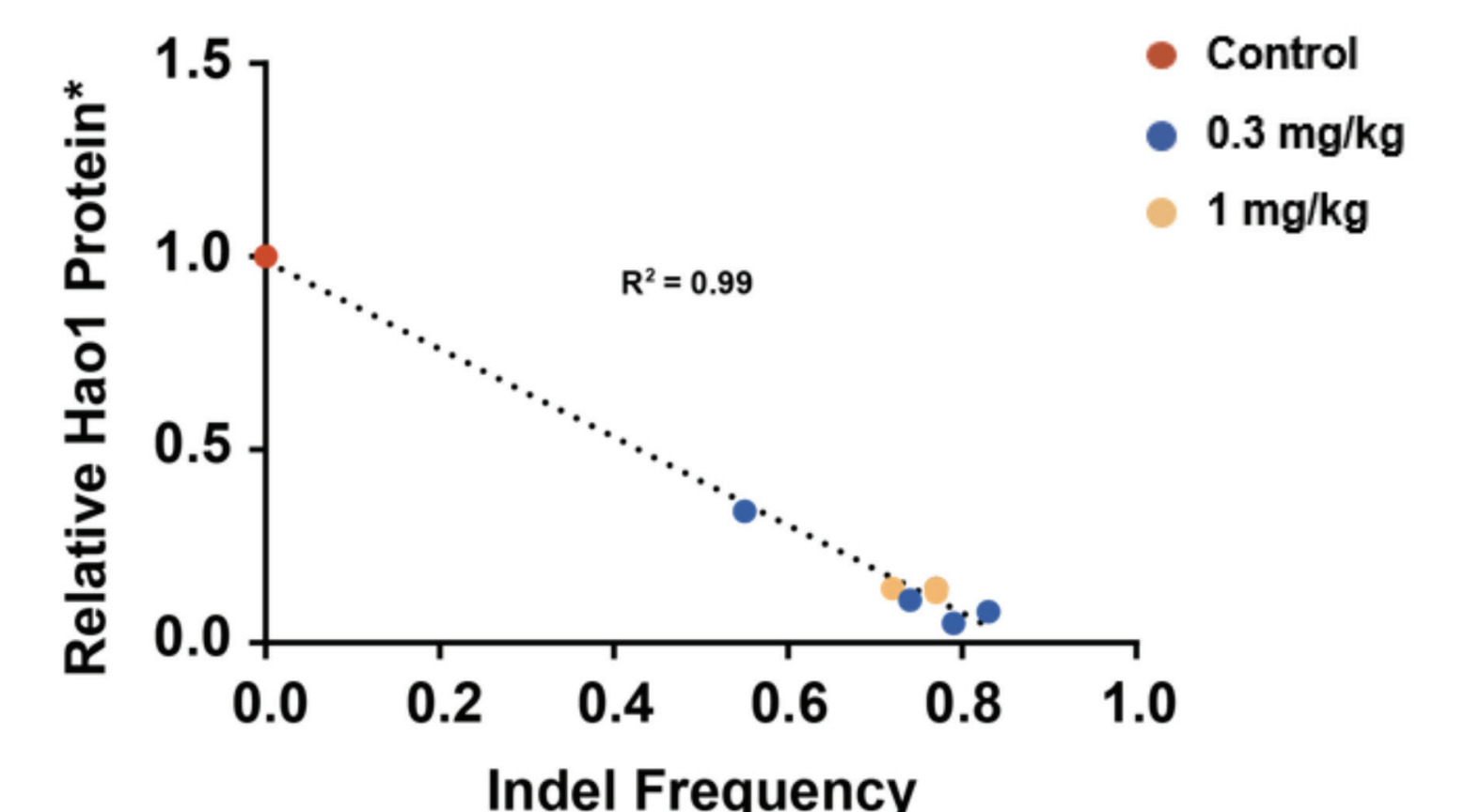


### Lactate Disposition is Preserved in Wild Type and 5/6th Nephrectomy Mice with Edited *Ldha*

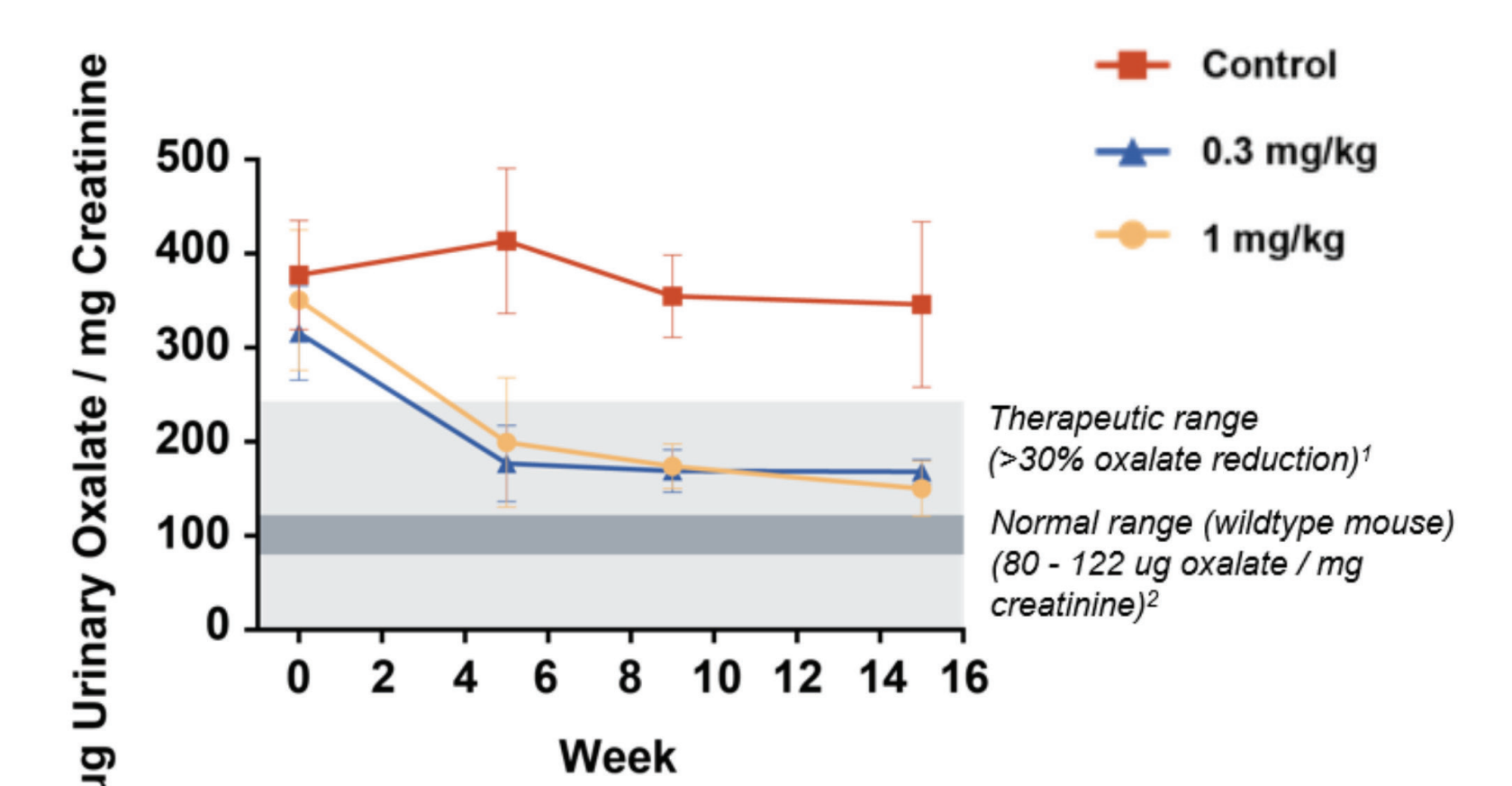


## TARGETING *HAO1* WITH CRISPR/Cas9

### *HAO1* Protein Reduction Correlates with Indel Frequency\*



### *Hao1* Knockout Results in Sustained Oxalate Reduction\*



\**Agxt*<sup>-/-</sup> mouse

## CONCLUSIONS

- Modular LNP-based CRISPR system enables efficient knockout of genes involved in oxalate production
- Single treatment targeting either *Hao1* or *Ldha* leads to a dose-dependent and persistent reduction of urinary oxalate levels in the *Agxt*<sup>-/-</sup> mouse model of PH1
- *Ldha* gene disruption decreased LDH enzyme activity in the liver, yet did not impair the disposition of lactate in either wild type or renally-impaired mice
- These results suggest the promise of LNP-delivered CRISPR for treating genetic forms of hyperoxaluria using a single-course treatment paradigm

