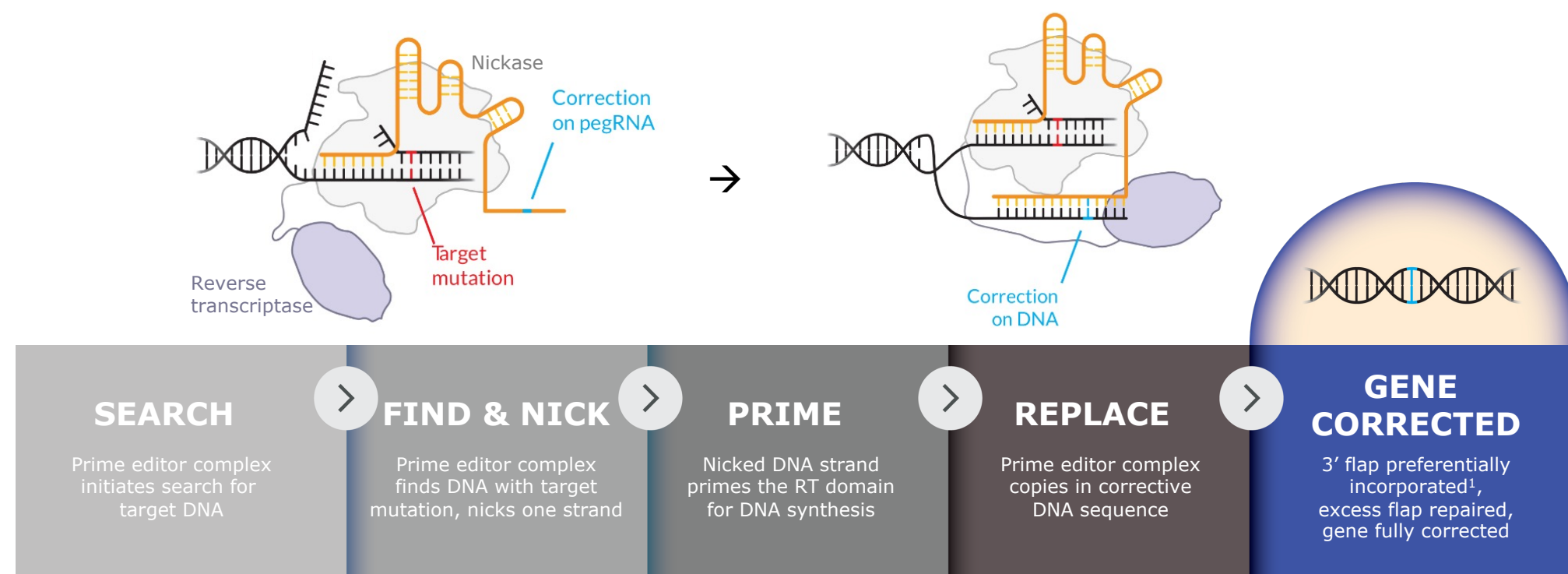


Prime Editing advancements enable *in vivo* therapeutic correction of *ATP7B* p.H1069Q and p.R778L mutations in Wilson's disease

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Prime Medicine, Inc. 60 First Street, Cambridge, MA, USA

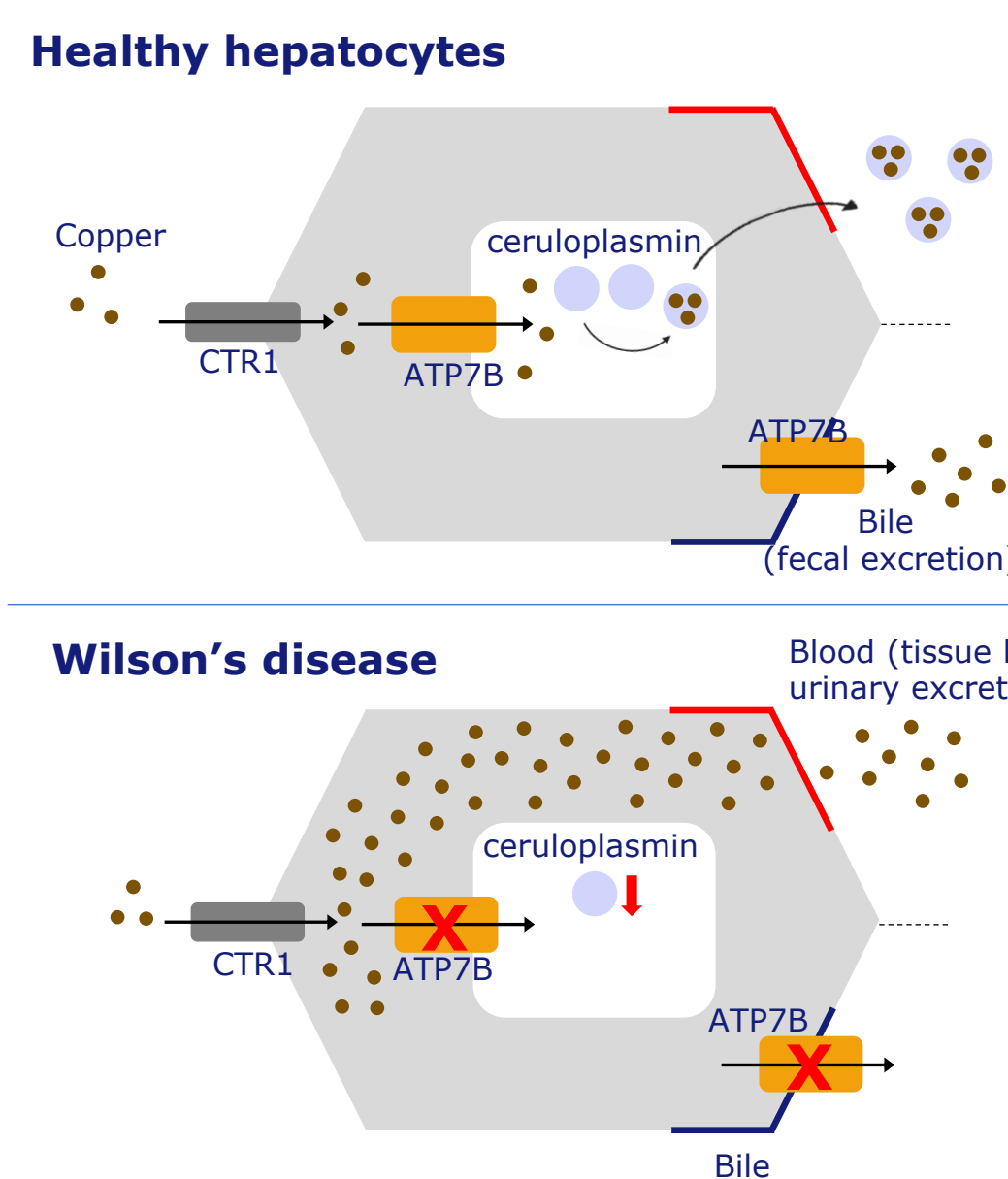
Background

How Prime Editing works



Prime Editing for safe and effective treatment of >90% of genetic diseases

- Targeted and precise search and replace genome editing enables highly effective disease-causing mutation correction while minimizing unintended edits



Wilson's Disease Overview

Disease severity

- Common liver and systemic disease presenting in teens to 20's (prevalence approx. 1:30,000)
- Leads to liver failure, neurocognitive decline and premature death

Human Biology

- Autosomal recessive due to loss of function mutations in *ATP7B*
- Affects copper homeostasis, leading to toxic accumulation of copper in liver and brain
- H1069Q and R778L are two prevalent mutations found in up to 50% of patients
- Correction of 20-30% of hepatocytes may be curative

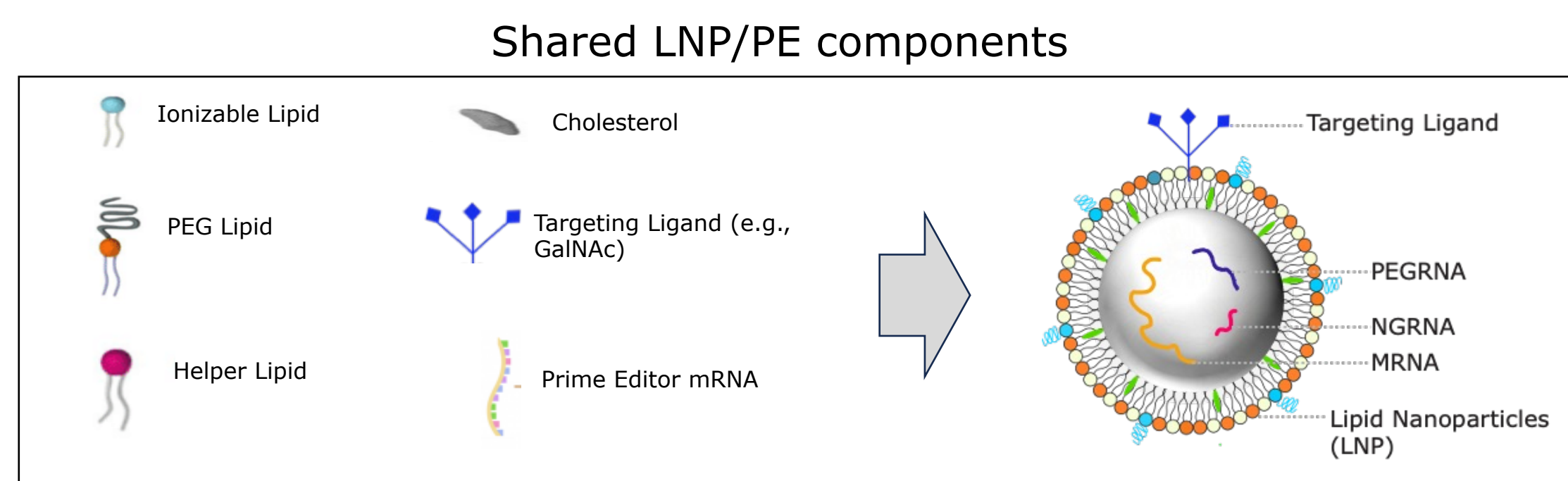
Prime Medicine therapeutic approach

- Prime's universal liver targeted LNP, to deliver RNA Prime Editors to patient liver to correct mutations in *ATP7B*

Results

1

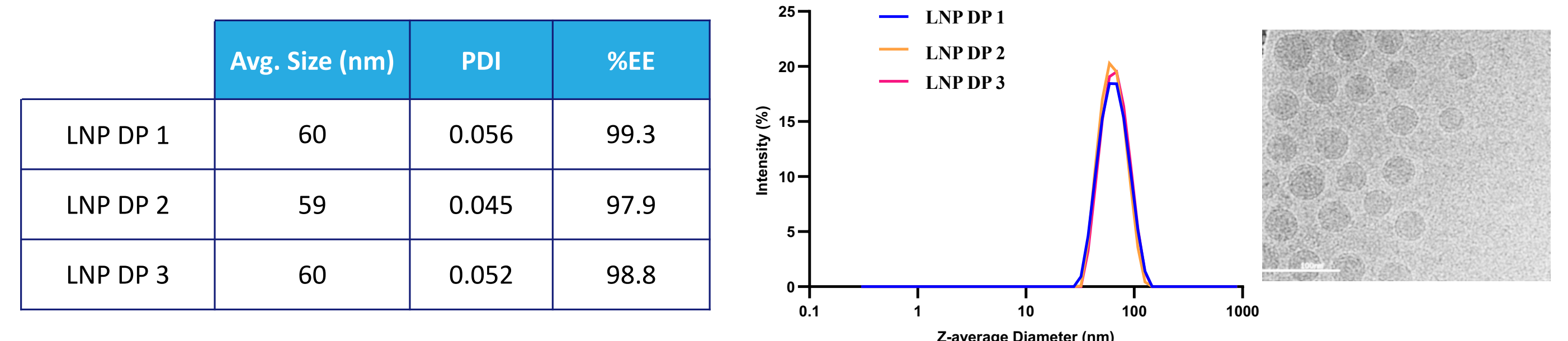
Prime Medicine's Universal LNP contains a novel GalNAc liver targeting ligand



- Validated as a delivery mechanism
- Increases potency
- Improves safety profile
- Improves biodistribution profile

2

Prime Medicine's Universal LNP can be used to generate multiple drug products

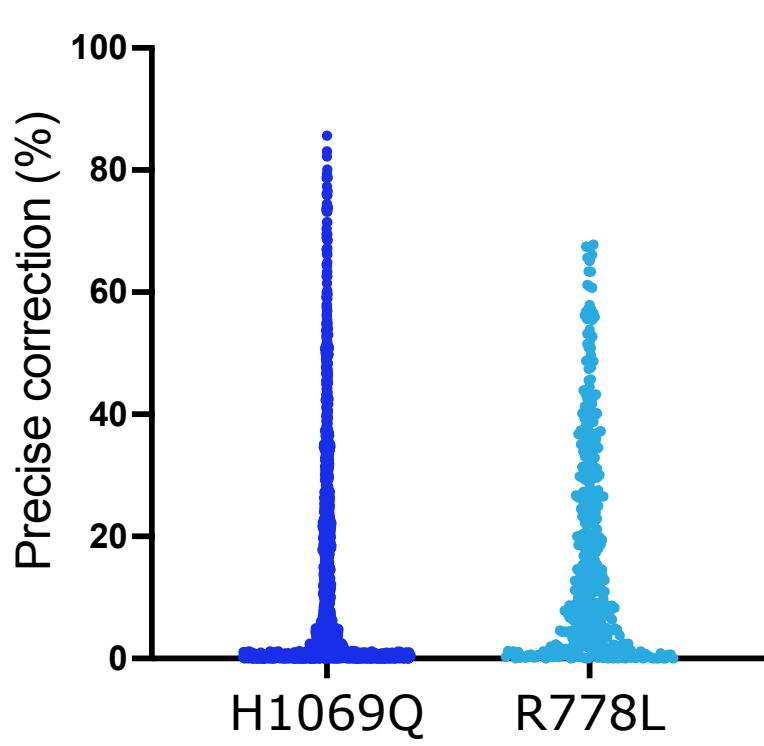


- Prime Medicine's Universal LNP keeps constant 6 out of the 8 components of the LNP and PE cargo for all liver programs
- By swapping out only the guide RNAs new drug products are made

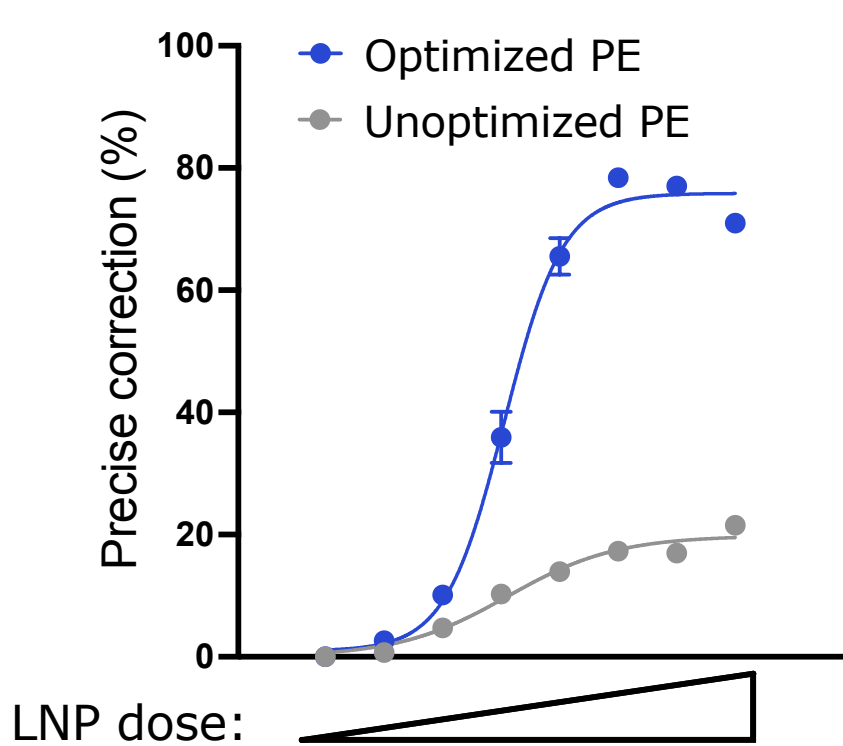
3

Screening and validation identifies highly active Prime Editors for H1069Q and R778L correction

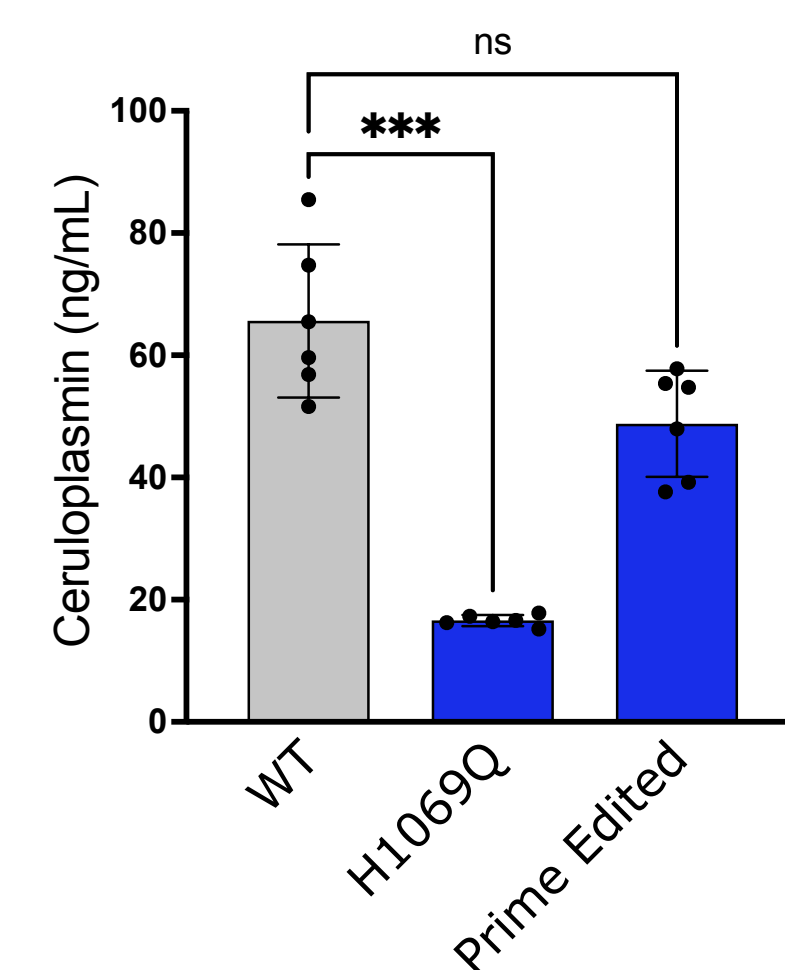
Prime Editor screening in cell lines



Hit validation in primary humanized mouse hepatocytes



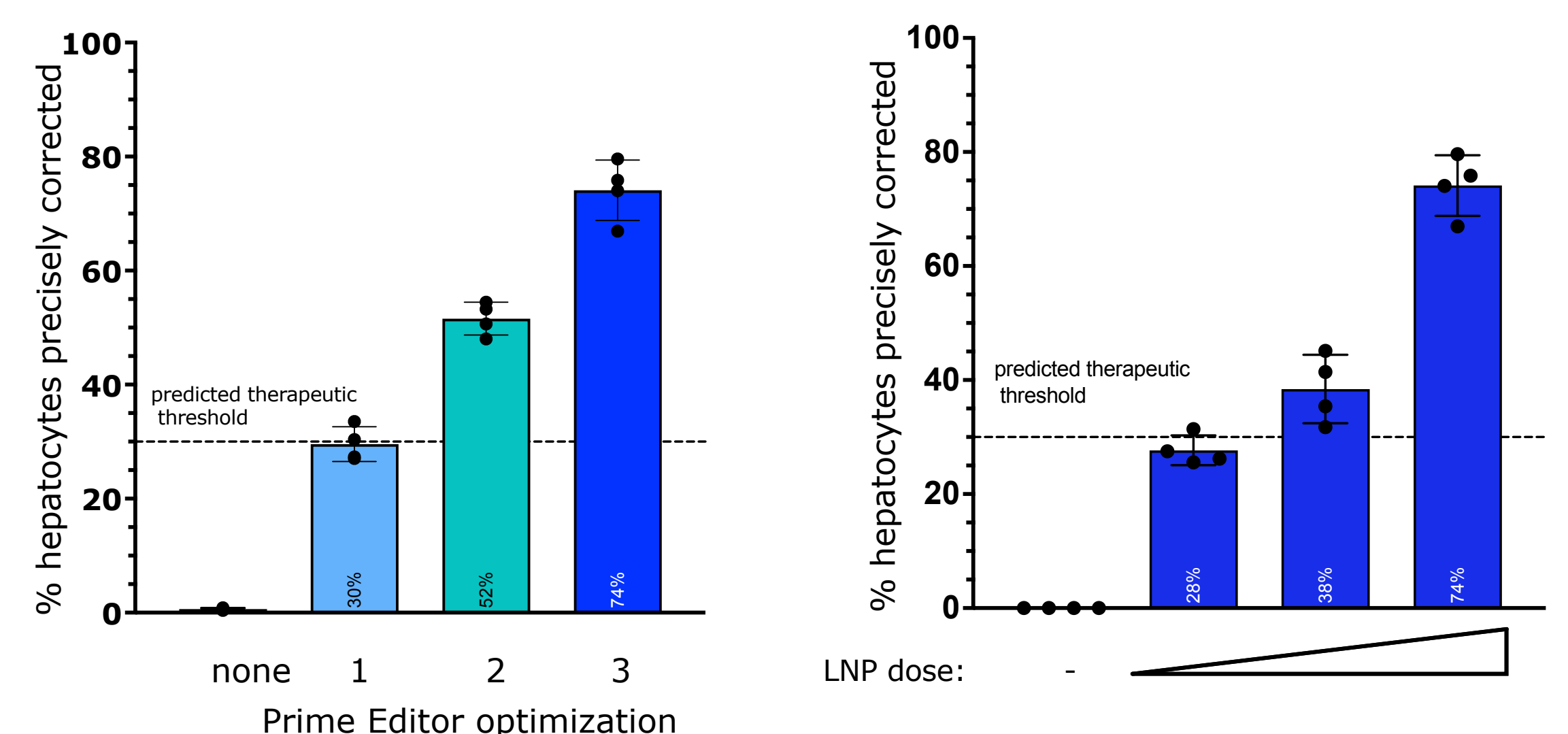
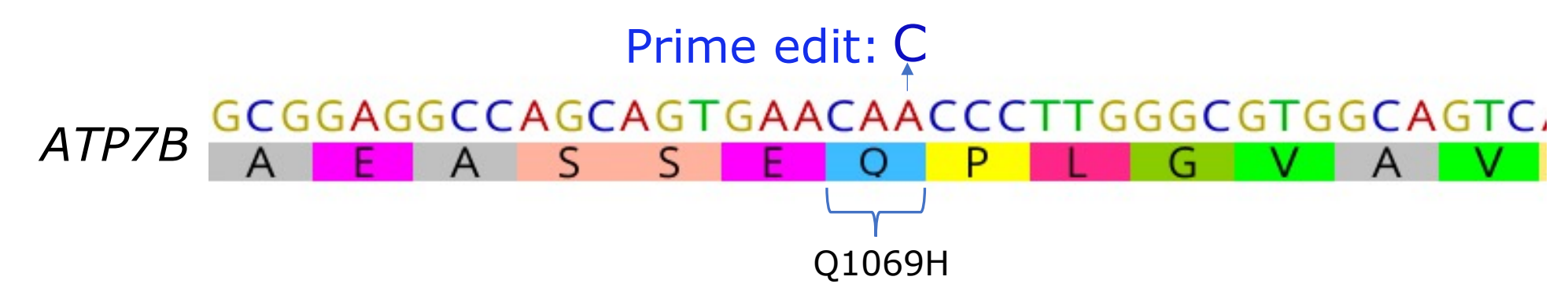
Ceruloplasmin in H1069Q patient iHeps



- High throughput screening in hepatocyte cell lines identifies several candidate Prime Editor components capable of >60% editing for H1069Q and R778L
- Further optimization of PE in *ATP7B* H1069Q humanized mouse hepatocytes greatly improves Prime Editor potency and efficacy
- Prime Editor normalizes ceruloplasmin abundance in H1069Q patient induced human hepatocytes (iHeps)

4

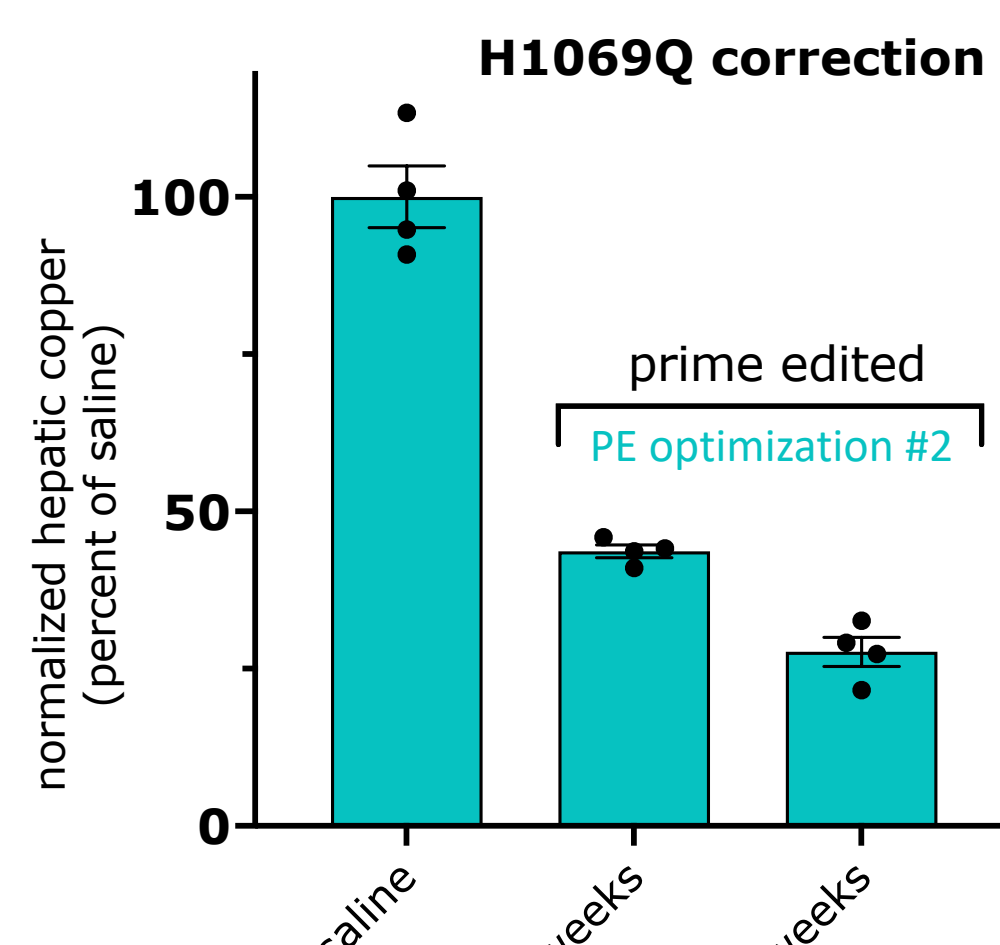
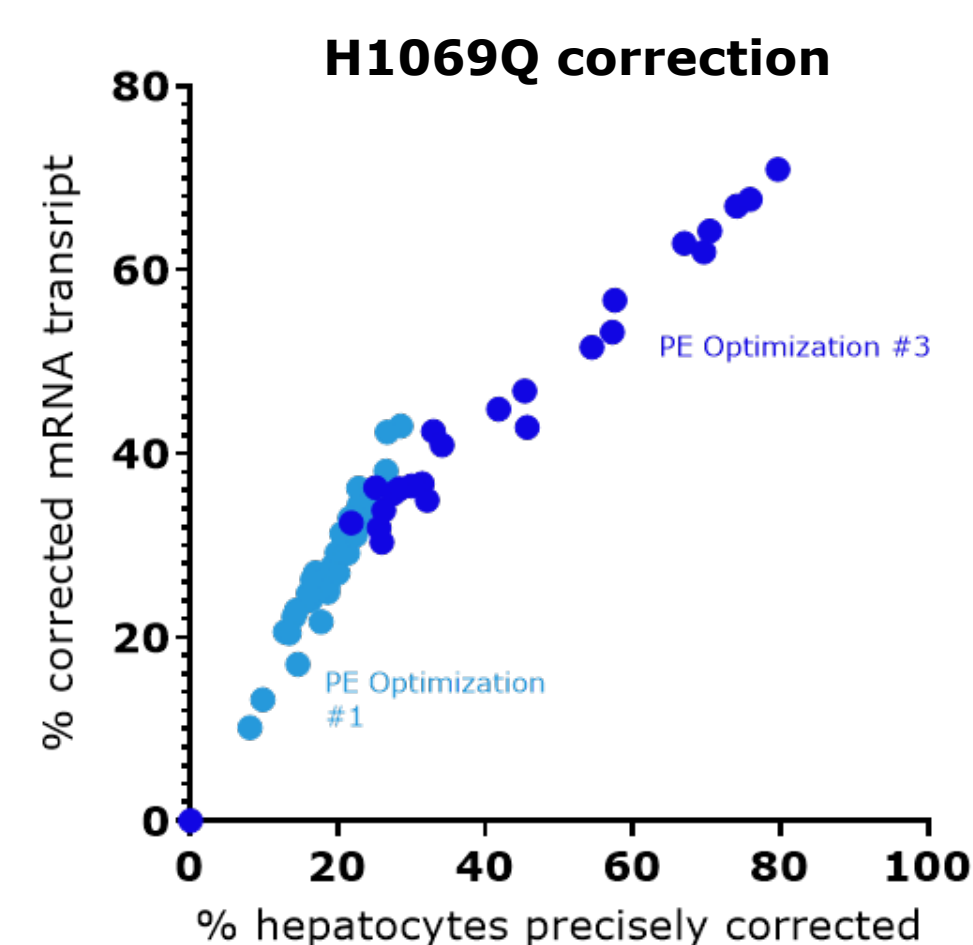
Improvements to Prime Editor cargo in Universal LNP enables therapeutic correction in fully humanized *ATP7B* H1069Q WD mouse models



- Optimization of LNP components improve Prime Editing to up to 80% of hepatocytes in humanized *ATP7B* H1069Q mouse models
- Dose-dependent editing was observed for H1069Q correction, exceeding 30% therapeutic threshold at clinically relevant dose

5

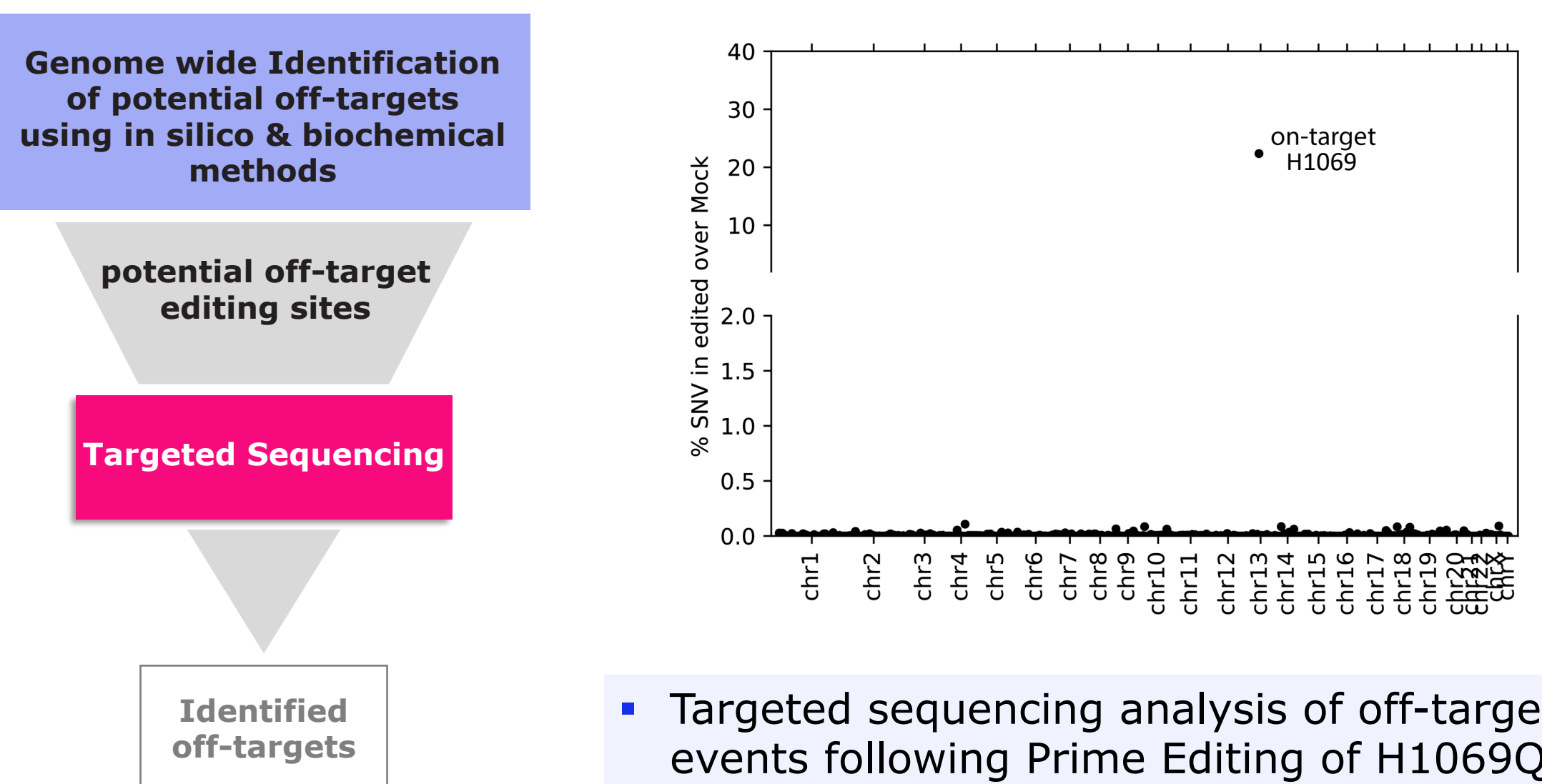
Correction of H1069Q in humanized mouse models produces corrected mRNA transcripts and reduces liver copper



- DNA correction of H1069Q by Prime Editing correlates with corrected mRNA
- Time-dependent 75% reduction in liver copper using optimization 2 Prime Editor
- Further copper studies ongoing with Prime Editor Optimization #3

6

Preliminary off-target analysis for H1069Q Prime Editor does not identify off-target (SNVs or indels) events genome-wide



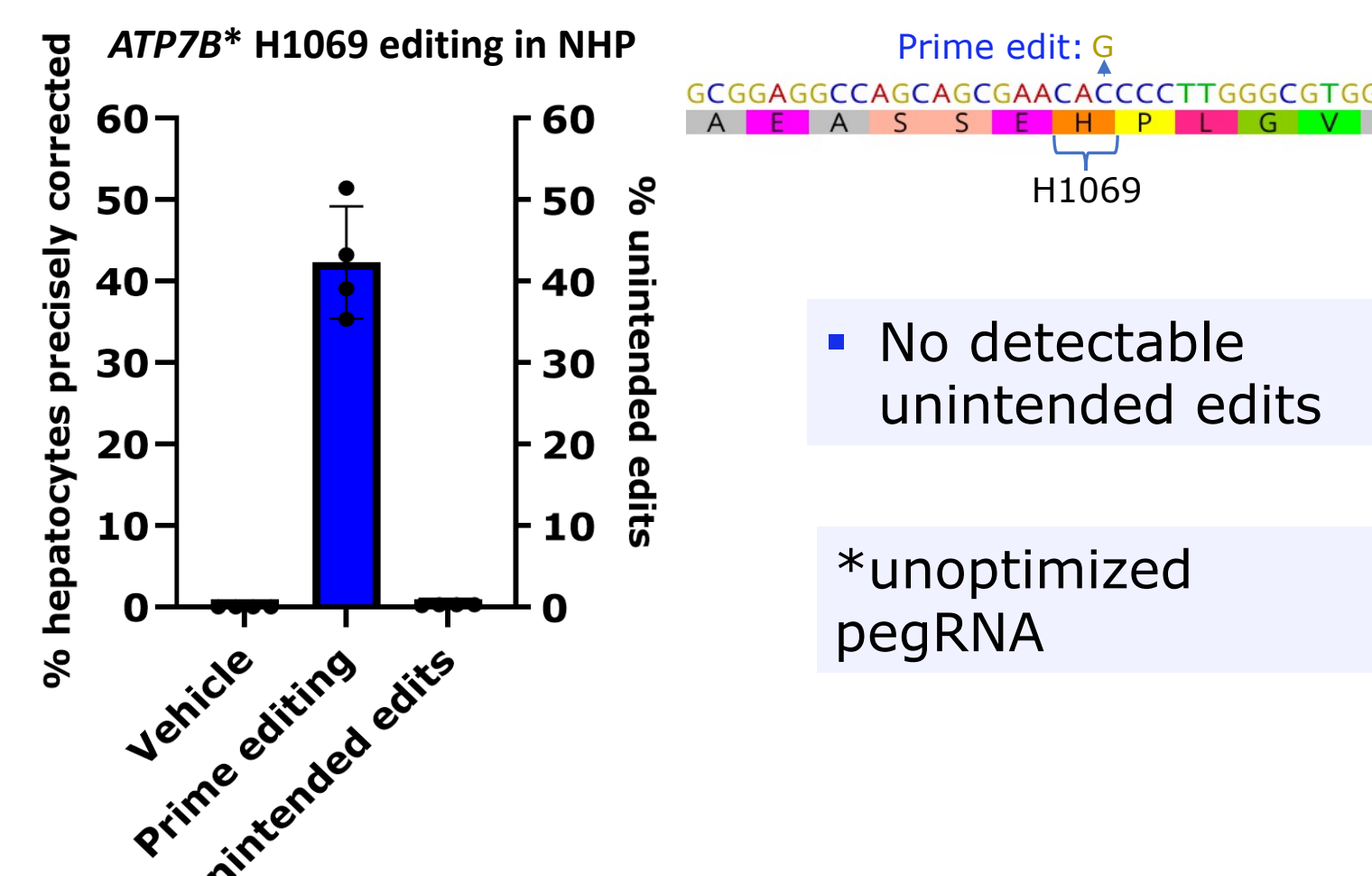
- Targeted sequencing analysis of off-target events following Prime Editing of H1069Q in patient-derived iPSC

7

Universal LNP-formulated surrogate H1069 Prime Editor *in vivo* in NHP achieves up to 51% precise editing in liver hepatocytes

Prime's Universal LNP has an excellent safety profile in NHP

- Well-tolerated with no acute reactions, clinical observations, or body weight changes
- Minimal LFT abnormalities
- No change in platelet, coagulation, or blood count
- No change in blood biochemistry
- Minimal change in IL6 production
- No other cytokine changes
- No change in liver histopathology (H&E)



- No detectable unintended edits

*unoptimized pegRNA

Conclusions

- Successfully identified several candidate Prime Editor components capable of >60% editing for *ATP7B* p.H1069Q and p.R778L
- LNP-formulated Prime Editors using Prime Medicine's Universal liver targeted LNP efficiently correct (up to 80%) the H1069Q mutation in a fully humanized *ATP7B* Wilson's disease mouse model without detectable unintended edits
- Preliminary off-target analysis demonstrated H1069Q Prime Editors do not result in detectable off-target events in patient-derived iPSC
- LNP-formulated Prime Editors resolve copper accumulation in humanized mouse livers
- Using Prime Medicine's Universal LNP with a surrogate Prime Editor RNA cargo, up to 51% editing at the H1069 locus was observed in NHP *in vivo*
- Prime Medicine's Universal LNP is well-tolerated in NHP with a favorable safety profile
- These data support the advancement of a potential one-time, curative approach for Wilson's disease patients H1069Q or R778L mutations