# EXPLORING THE THERAPEUTIC POTENTIAL OF RNA NEW EDITING TECHNOLOGIES LEVERAGING ADAR ENDOGENOUS MACHINERY

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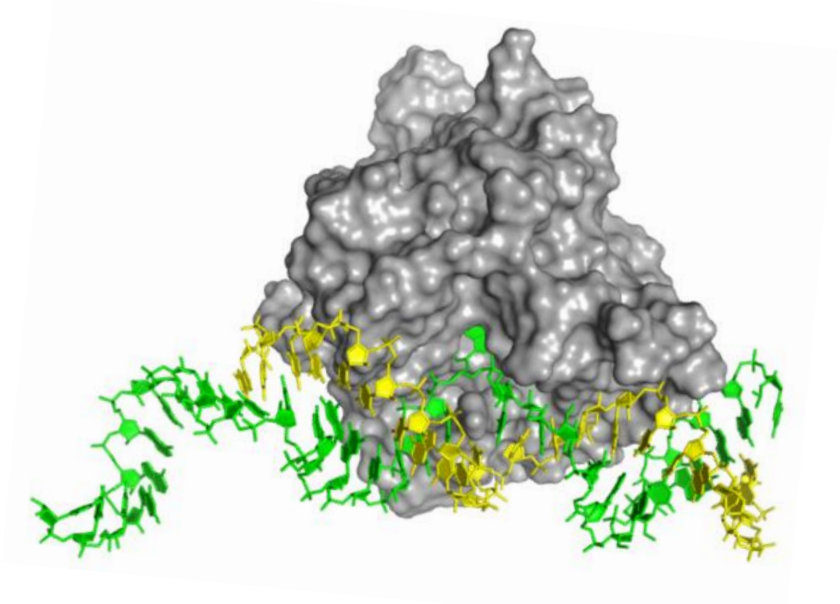
DATS - June 1, 2023

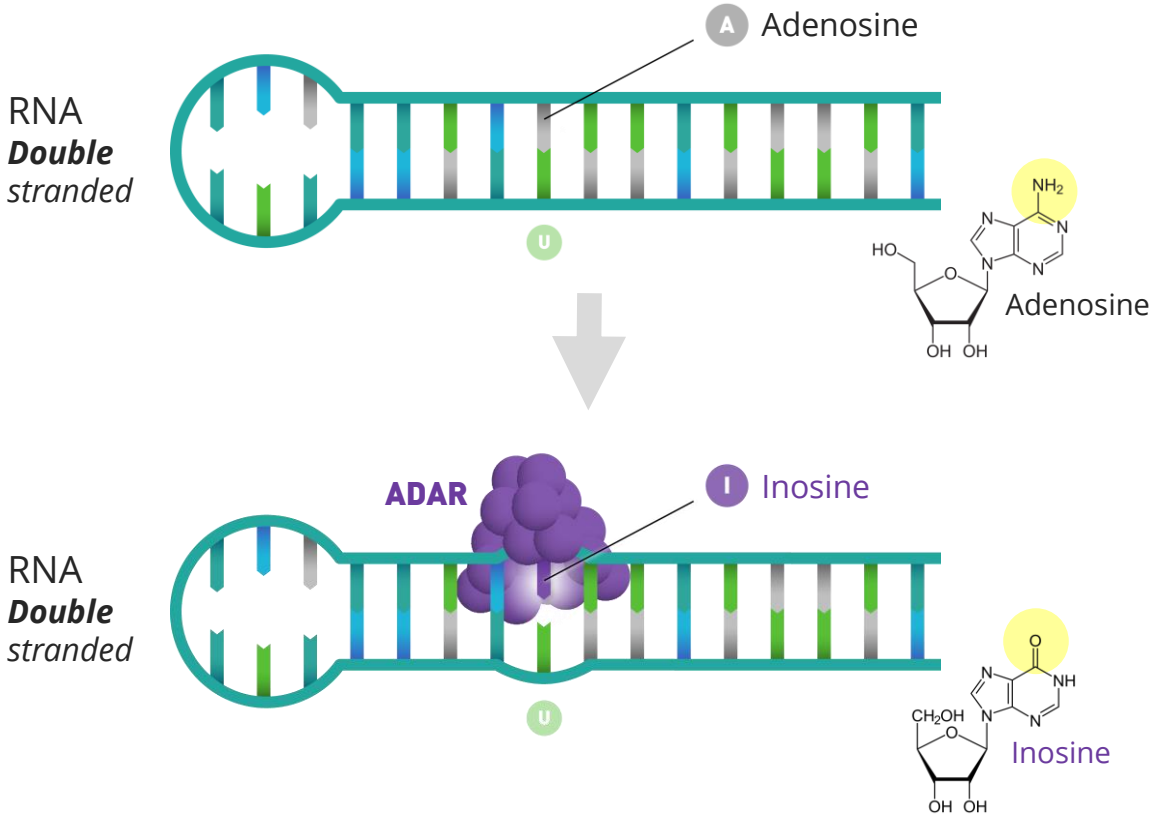
# What is ADAR editing?

**ADAR** *(Adenosine Deaminase Acting on RNA)*



Enzyme that performs specific form of natural RNA editing, called **A-to-I editing.** During A-to-I editing an **A nucleotide (adenosine)** is changed into an **I nucleotide (inosine)**

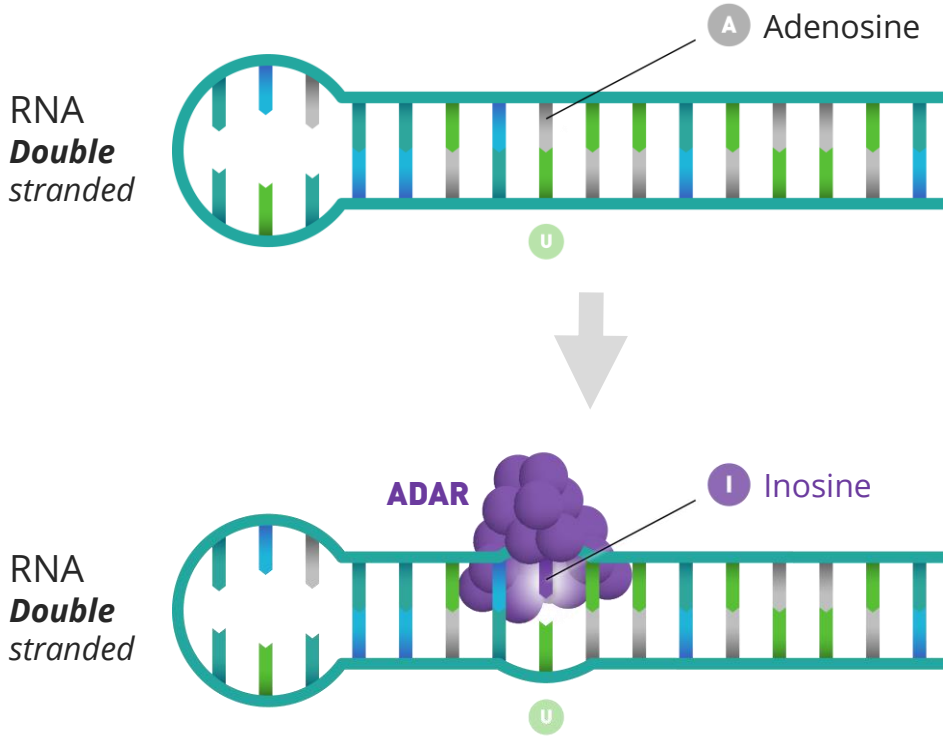
**Natural ADAR editing** *(A-to-I)*



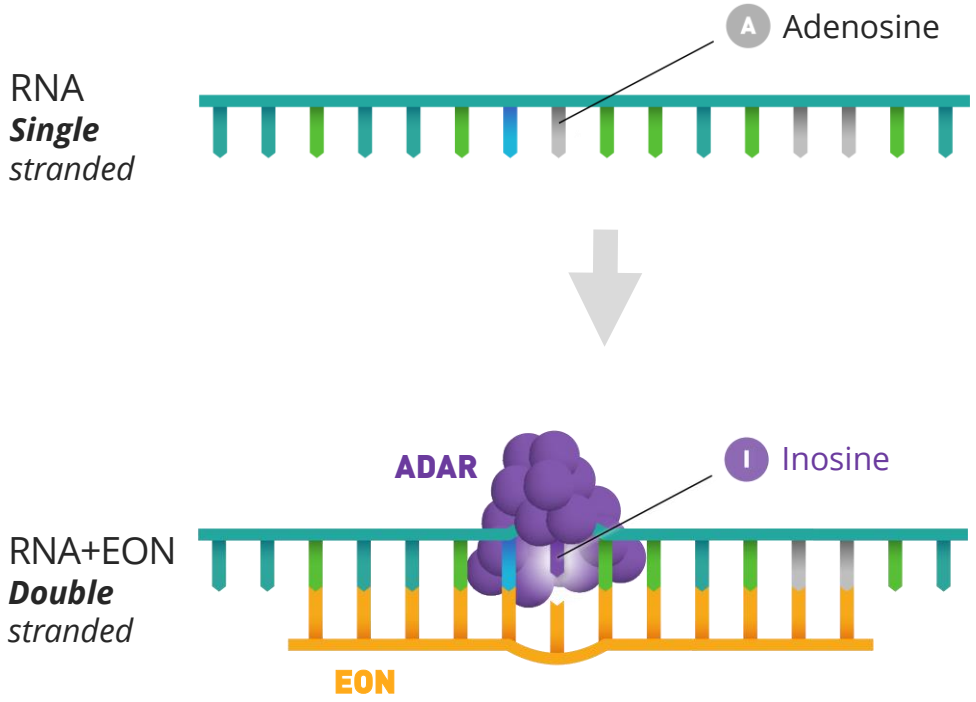
# Axiomer® EONs unlock cellular machinery potential to treat diseases

*By attracting ADARs and allowing highly specific editing*

**Natural ADAR editing** *(A-to-I)*




**Editing Oligonucleotide (EON)-directed therapeutic editing** *(A-to-I)*

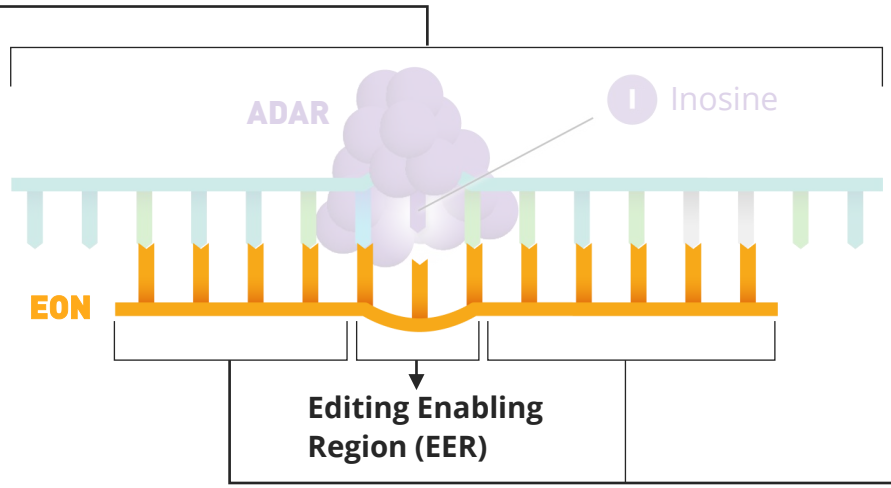



ADARs: Adenosine deaminases acting on RNA.

# Driving the development of optimized EONs for therapeutic use

**Sequence defines** target
RNA binding and provides stability

**EON total length**
Between ~25 - 30 nt




**ADAR-binding region (ABR)**
Backbone modifications enable ADAR binding, and disable off-target editing

## Optimized sequence and chemistry define functionality

- Increase editing efficacy
- Bring metabolic stability
- Prevent off-target ('bystander') editing
- Ensure bioavailability (cell and tissue uptake)
- Offer safety and tolerability at therapeutic doses

ADAR: Adenosine deaminase acting on RNA, EON: Editing oligonucleotide, Nt: nucleotides

# ProQR leading research to optimize EONs for therapeutic use

## Modification of the orphan base

in the EER confirm superiority of dZ base

## Positive impact of neutral linkage modifications in the ABR

Improvement in editing potential with PN linkages on EONs

## Structure–activity relationship (SAR) assessment

interrogating the impact of single change to define guiding principles

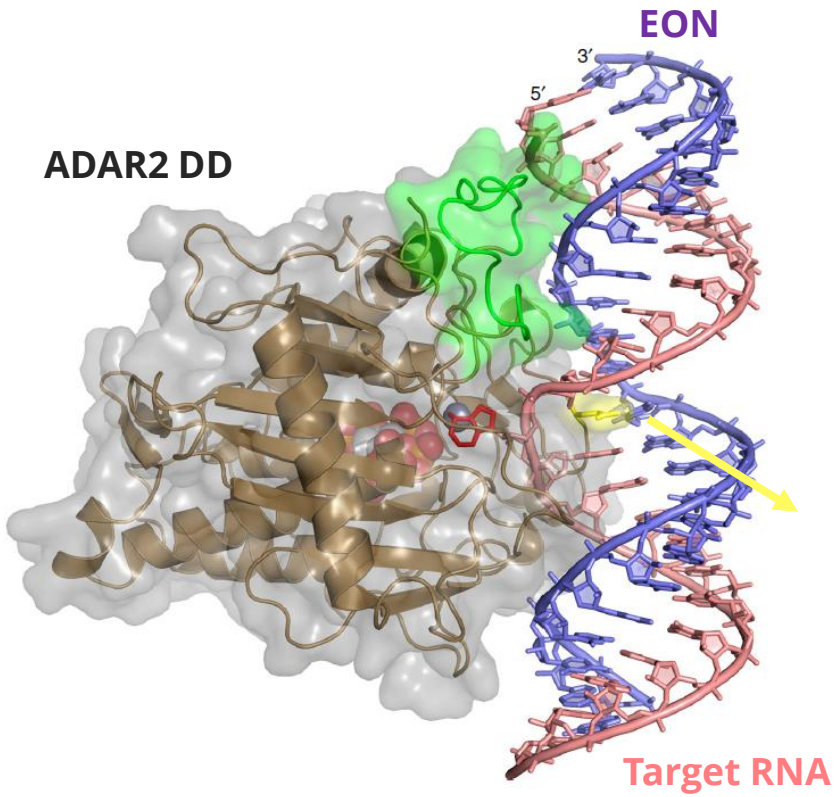
# Modification in the Editing Enabling Region (EER)

*Cytidine analogs as orphan base*

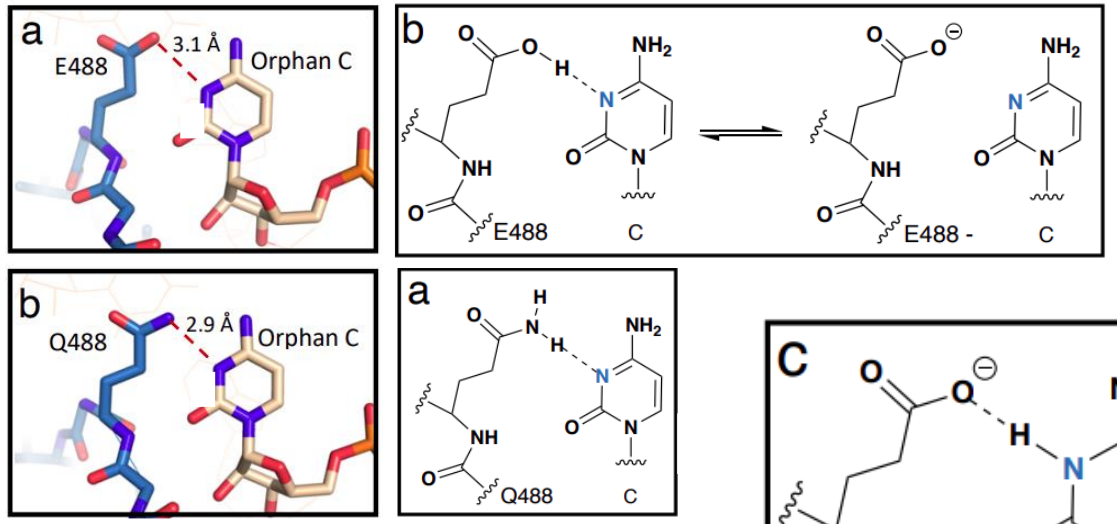
# A single base modification of the EER increases ADAR activity

*dZ base mimics E488Q mutation in ADAR2 causing hyperactivity*

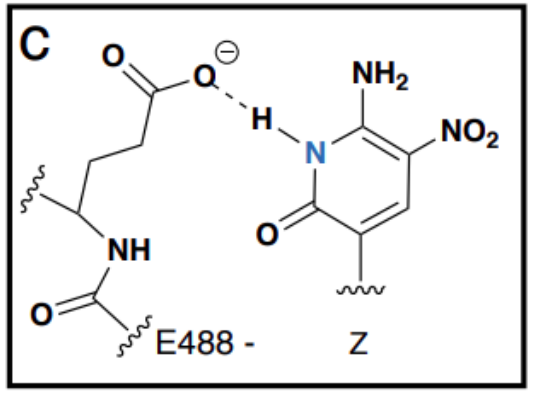
PROQR – UC DAVIS COLLABORATION




**Protonation dependent hydrogen bond – pH dependency**




**Protonation independent hydrogen bond**




**dZ base (dZ)**

*Metthews 2016, Nature Structural & Molecular Biology*

*Doherty et al., 2021, JACS, ProQR – UC Davis collaboration*

# dZ in the EER improves editing of *SERPINA1* E366K in A1AD patient hepatocytes

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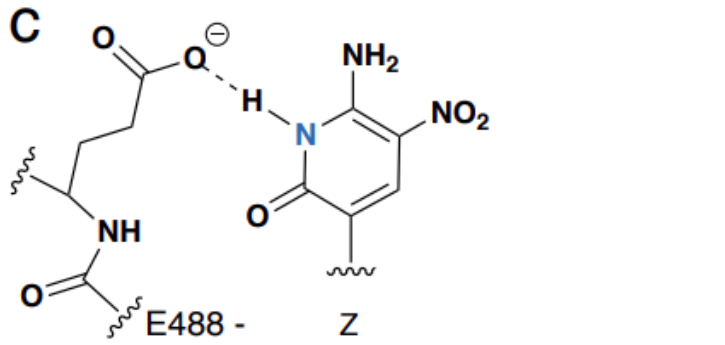
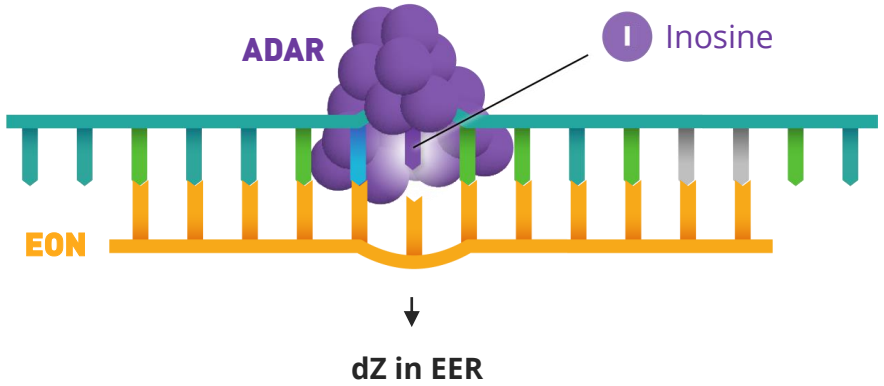
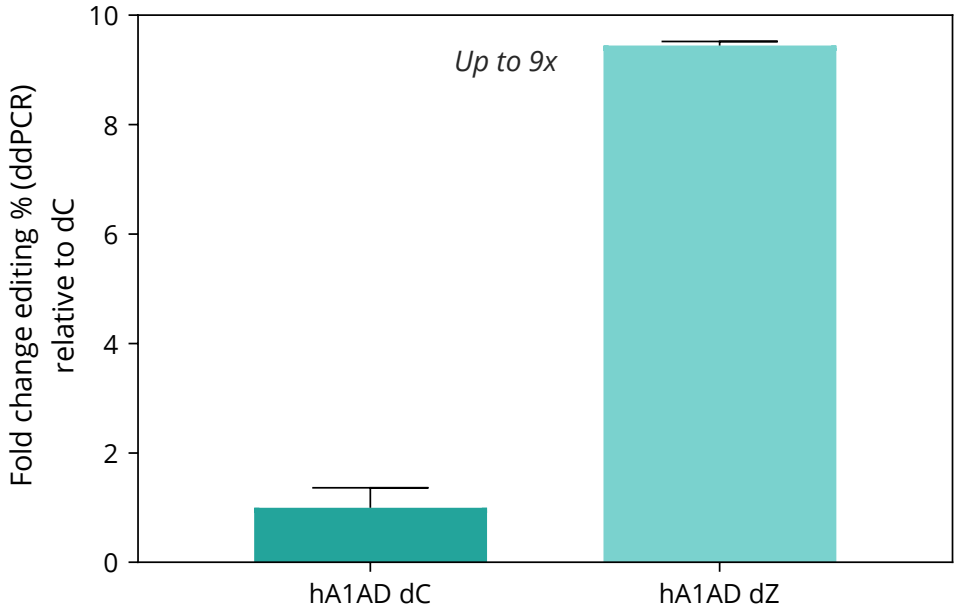



**RNA editing of *SERPINA1* E366K in A1AD patient hepatocytes**

*Transfection of 100nM EON, N=2, 48 hours*



# Improved editing obtained for several systems

*dZ improves editing in different cell types*

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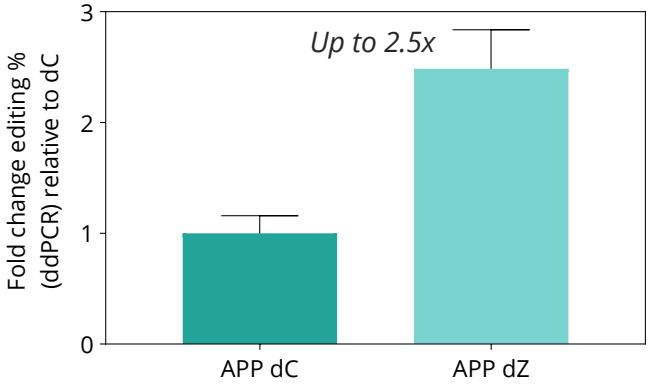
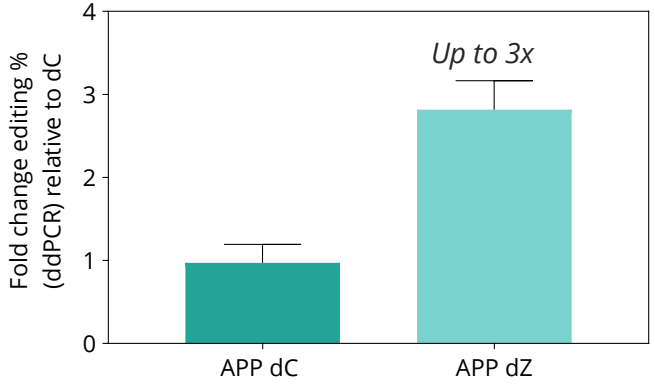
**Editing of WT *APP* RNA in human ARPE-19**

*Transfection of 100nM EON, N=3, 48 hours*



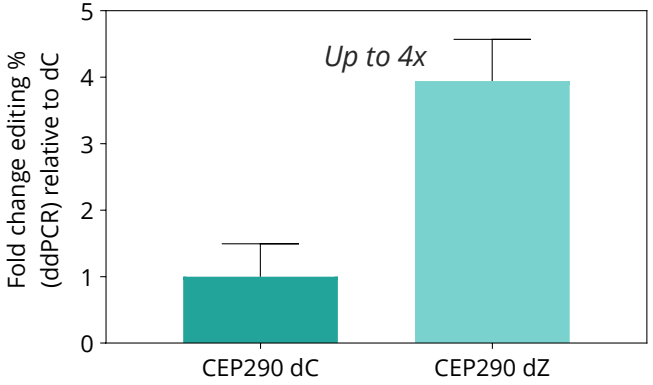

**Editing of *APP* WT RNA in human retinal organoids**

*Gymnosis, 10μM single dose + 40μM CQ, N=6, 4 weeks*



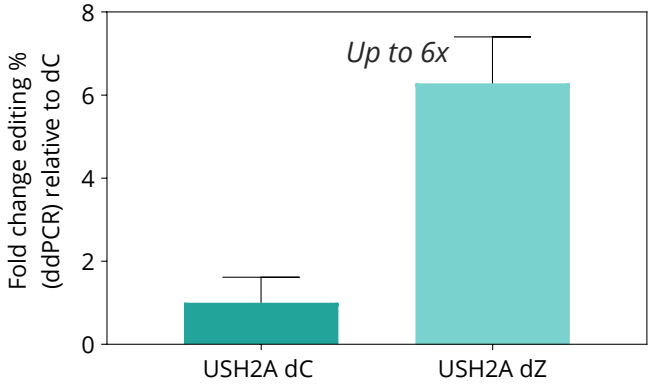

**Editing of *hCEP290* K1575X in human LCA retinal organoids**

*Gymnosis, 10μM single dose, N=8, 4 weeks*



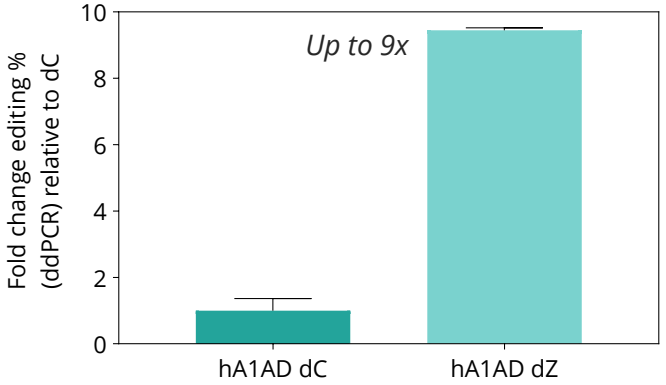

**Editing of *USH2A* WT RNA in human retinal organoids**

*Gymnosis, 15μM single dose + 40μM CQ, N=4, 4 weeks*




**Editing of *SERPINA1* E366K in A1AD patient hepatocytes**

*Transfection of 100nM EON, N=2, 48 hours*

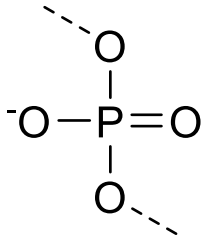


# Modification in the ADAR-binding region (ABR)

*Examples of structure–activity relationship (SAR) assessment interrogating the impact of neutral linkage modifications*

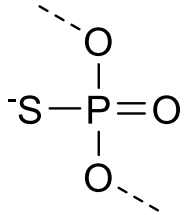
# Different linkage modifications commonly encountered in oligo therapeutics

**PO**



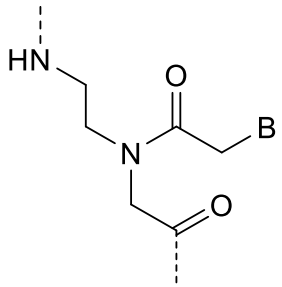
Low stability

**PS**

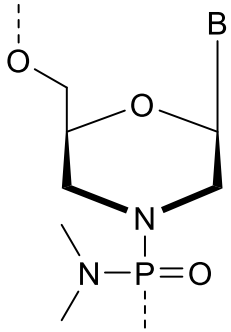


+ Increases cellular uptake and improves t1/2 *in vivo* by virtue of increased protein binding. Tolerability observations

**PNA**

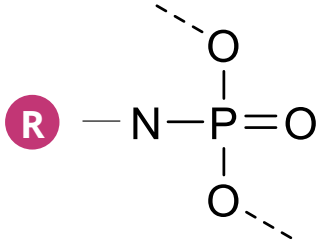


**PMO**

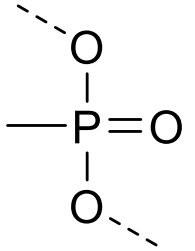


several RNA-modulating applications, challenge for A→I editing due to certain enzyme contacts being required for ADAR recognition

**PN**



**PMe**

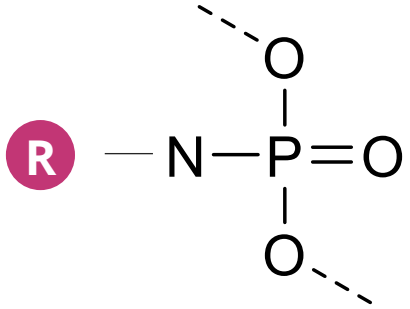


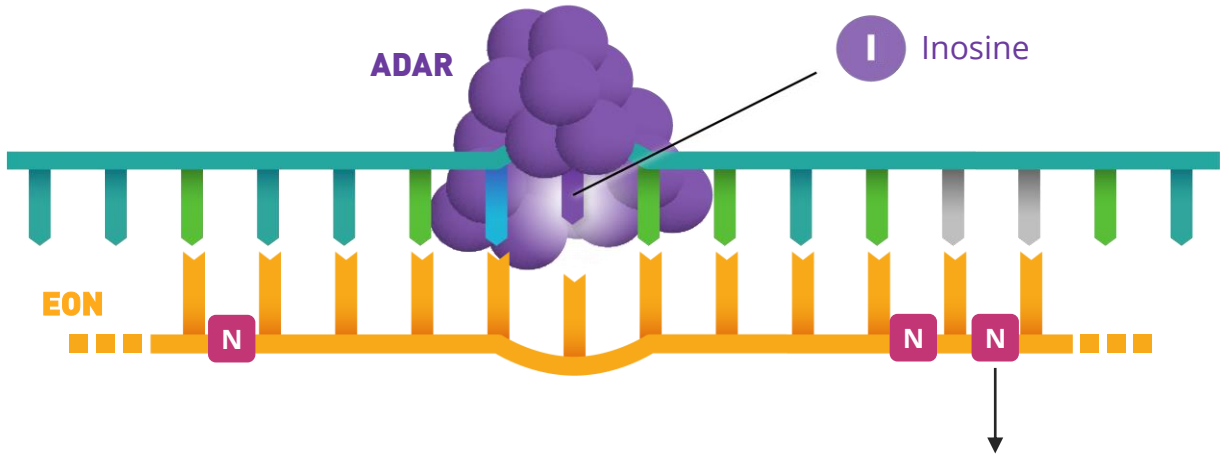
Neutral linkages
Decreased nuclease degradation,
Remove all PS

# Effect of phosphoroamidate linkage on EONs editing activity in different models

**Phosphoroamidate linkage**

**PN**

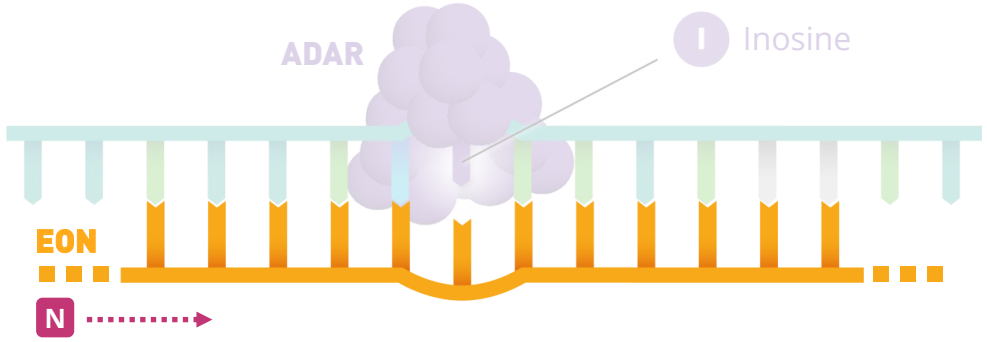



**Phosphoroamidate linkage modifications in the ABR**

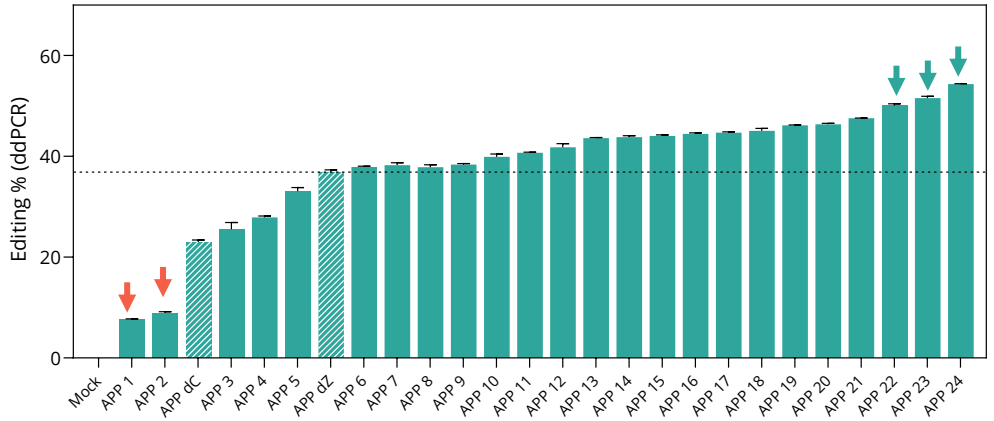
To enhance metabolic stability and activity

# Introduction of PN to EON showing the critical impact on editing efficiency




**RNA editing of WT *APP* in human ARPE-19**

*Transfection, N=2, 2 days, 100nM, ddPCR, Mean, SD*



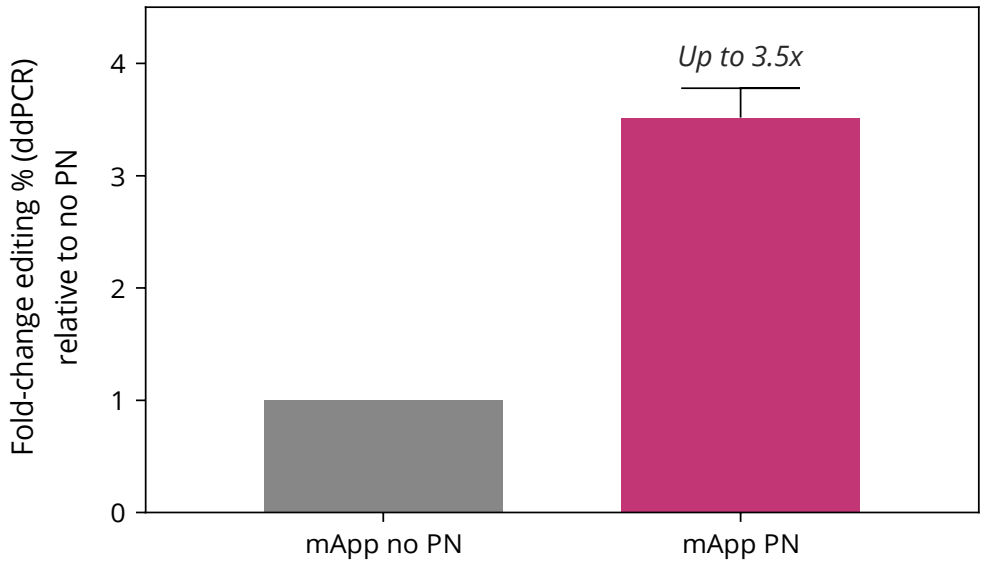

| EON | Structure |
|---|---|
| APP dC – No PN, dC base | $A_XU_XC_WA_XC_XU_XG_XU_XC_XG_ZC_XdCA_XU_YG_XA_XC_ZA_XA_XC_WA_XC_XC_XG_XC$ |
| APP dZ – No PN, dZ base | $A_XU_XC_WA_XC_XU_XG_XU_XC_XG_ZC_X\mathbf{dZ}A_XU_YG_XA_XC_ZA_XA_XC_WA_XC_XC_XG_XC$ |
| **APP 1-24 – dZ and PN at different positions** | $A_{N...}U_XC_WA_XC_XU_XG_XU_XC_XG_ZC_X\mathbf{dZ}A_XU_YG_XA_XC_ZA_XA_XC_WA_XC_XC_XG_XC$ |

- The sequences contain a mix of 2′-*O*-Me, DNA, PMe, PS, 2′-F, 2′-MOE
- The changing factor is +/- dZ in EER and +/- PN (N) with systematic change in location
- Each letter coding shows a combination of linkage and sugar modifications
- PN increases EON editing up to 1.5x and, in some positions, have negative effect on editing

# Effect of PN linkages on EONs editing activity in different models

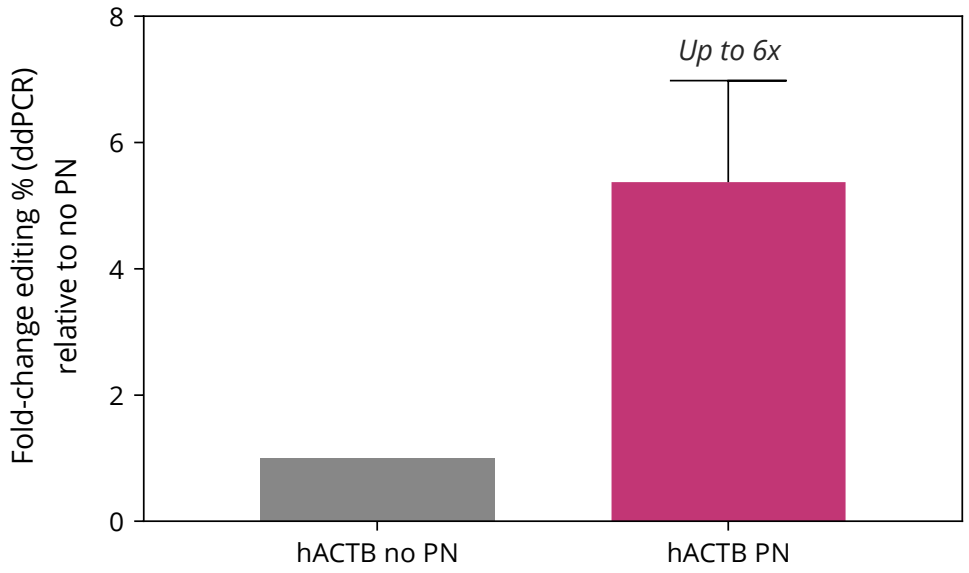
**RNA editing of m*App in mRPE cells***

*Gymnosis, 5µM, single dose, N=2, 5 days, ddPCR, mean, SEM*




**RNA editing of *hACTB* in Weri-rb1 cells**

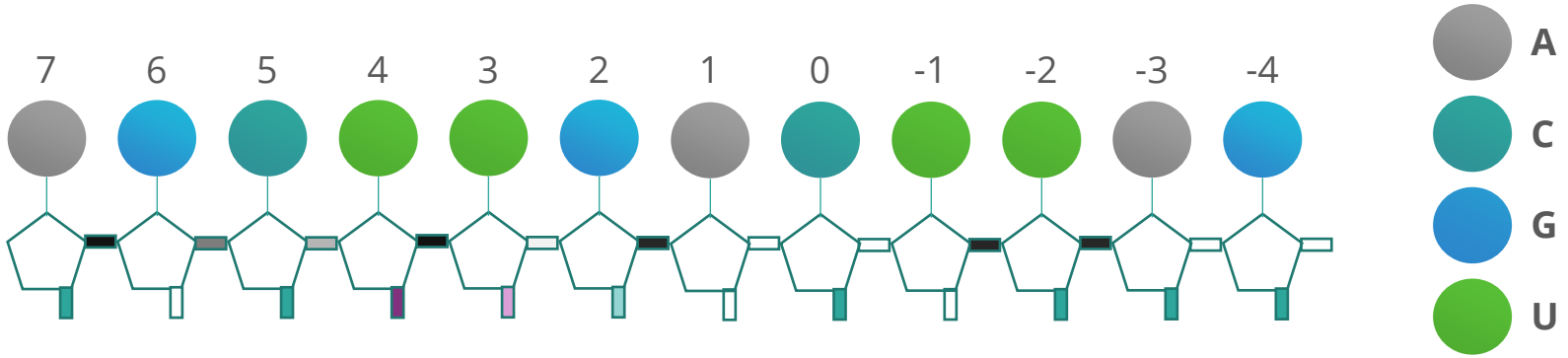
*Gymnosis, 5µM single dose, N=3, 5 days, ddPCR, mean, SEM*




- The sequences contain a mix of 2'-O-Me, DNA, PMe, PS, 2'-F, 2'-MOE
- The changing factor is +/- 2 PNs at the same locations

# Accelerating program advancement with focus on design principles

EON







| Aspect | Determined by | Modifications | Effects |
|---|---|---|---|
| Base | Target RNA | Mismatches and analogs (**dZ**) | Improved PD |
| Ribose modification | ADAR structure | 2′-H, 2′-*O*-Me, 2′-MOE, 2′-F, 2′-$NH_2$, LNA, TNA, UNA, 2′,2′-diF, FANA | Improved PK and PD |
| Linkage | ADAR structure | PO; PS; **PN**; PMe; PAc | Improved PK and PD |

ADAR: Adenosine deaminase acting on RNA, EON: Editing oligonucleotide, PD: pharmacodynamic, PK: pharmacokinetic

# Advancing Axiomer® development across different models and targets in the liver

**Liver**

Targeting liver originated diseases

**Cell models**

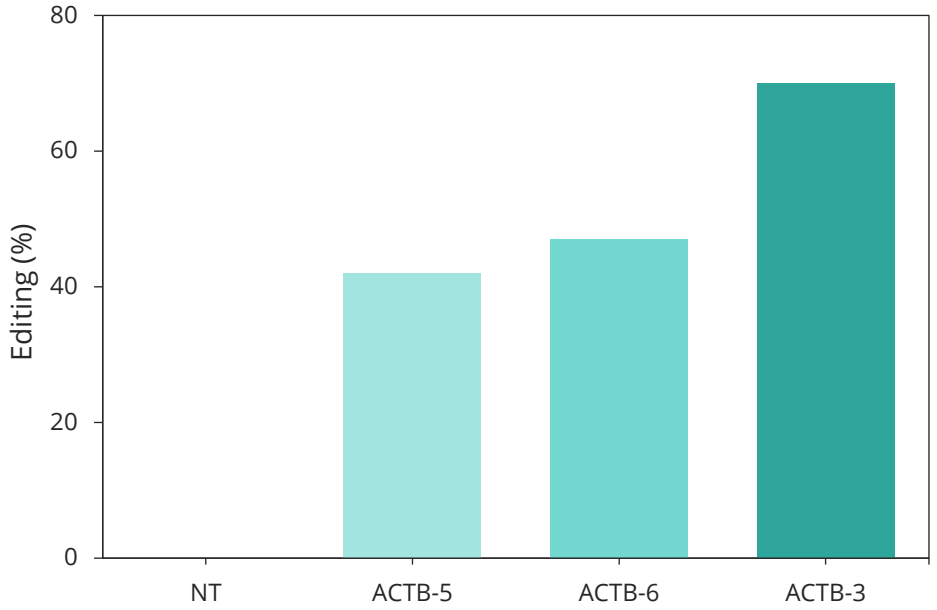
**Organoids**

**Mice *in vivo***

# Up to 70% RNA editing in human primary hepatocytes

**RNA editing of *ACTB* in human primary hepatocytes**

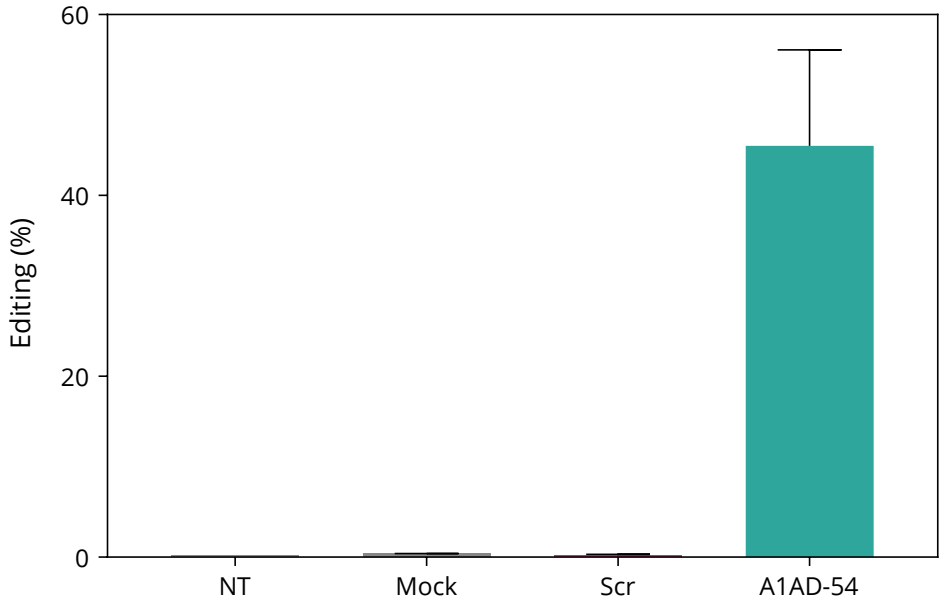
*Gymnosis, 10µM, single dose, n=1, 48 hours, dPCR*




Similar levels of RNA editing of *ACTB* achieved in several models of liver origin (not presented here)

**RNA editing of *SERPINA1* E366K in human A1AD patient hepatocytes**

*Transfection, 100 nM, single dose, n=2, 47 hours, dPCR, mean, SD*




>50% RNA editing of *SERPINA1* E366K in human A1AD patient hepatocytes

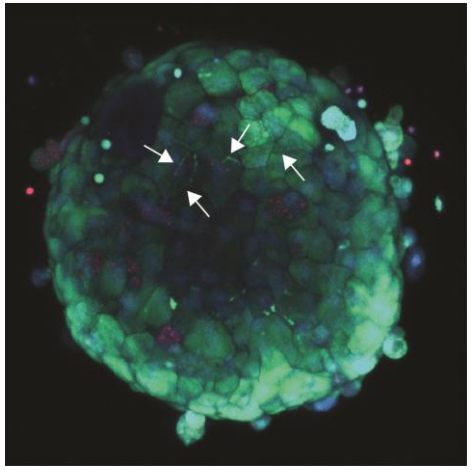
A1AD: Alpha-1 antitrypsin deficiency.

# Editing in InSphero Human Liver microtissues (LMTs)

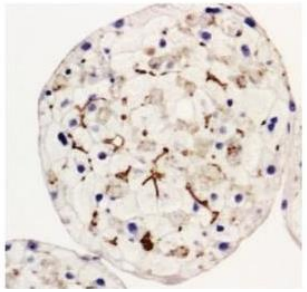
*Primary hepatocytes, Kupffer cells and liver endothelial cells in 3D spheroid*

## Live imaging of LMT

*Stained with 5-CFDA (green), PI (red) and Hoechst (blue)*



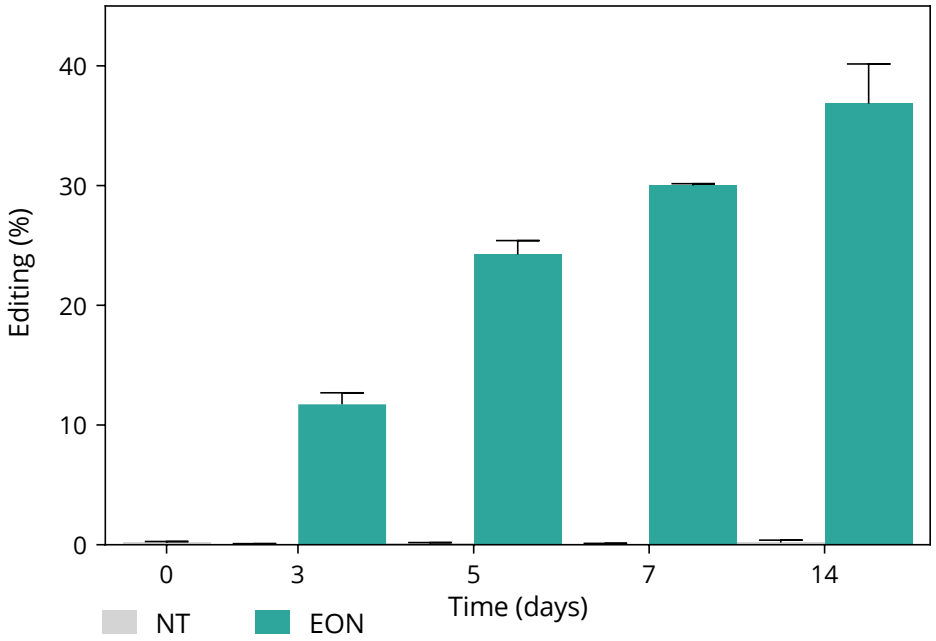
**BSEP** Bile Canaliculi (InSphero data)



Presence of bile channels in LMTs by day 7 fluorescent dye 5-CFDA secreted from healthy cells into bile channels (canaliculi)

## Editing of *ACTB* in human LMTs

*Gymnosis, 1µM, constant dose, 3 pools of 24 LMTs per condition, 14 days, dPCR, mean, SD*



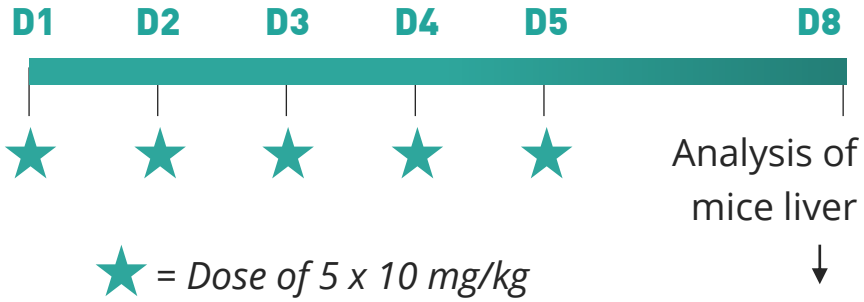

Treatment of LMTs with 1µM EON for 14 days results in up to 40% RNA editing of *ACTB*

BSEP: Bile salt export pump, LMTs: Liver Microtissues constituted of primary hepatocytes, Kupffer cells and liver endothelial cells in 3D spheroid.

# Up to 50% RNA editing of *ActB* in liver of mice

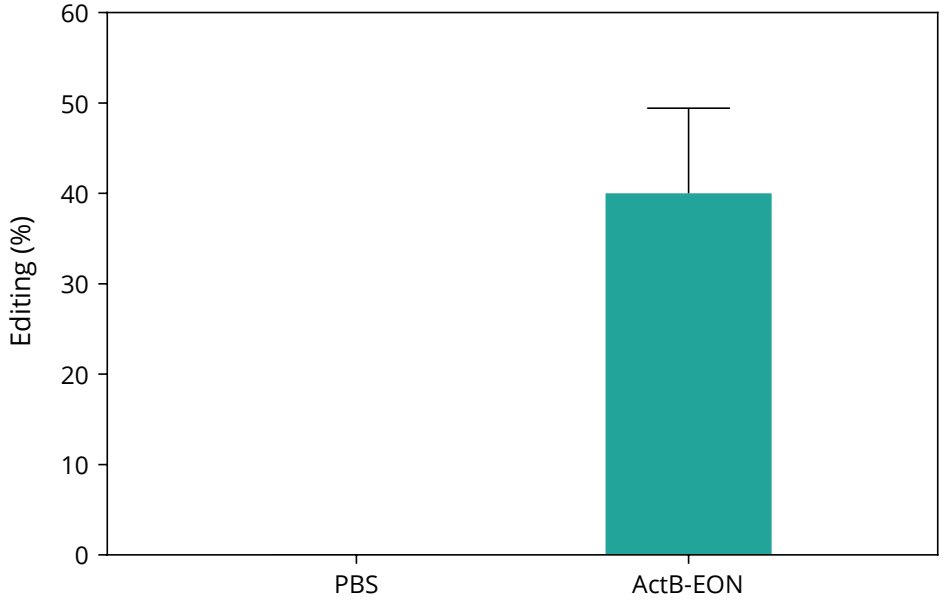
**Mice treatment**

*In vivo*




**RNA editing of *ActB* in liver of mice**

*SC, 5 daily doses of 10 mg/kg, N=4, 1 week, ddPCR, mean, SD*




High *in vivo RNA* editing of *ActB* in the liver of mice reaching up to 50%

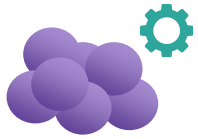
# Axiomer® creating a new class of medicines with broad therapeutic potential

## Correction

**Mutations correction**

Thousands of G-to-A mutations, many of them described in literature

## Protein modulation

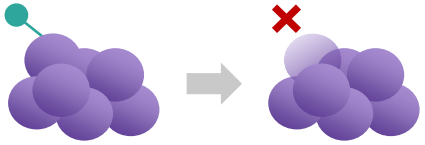


**Alter protein function or include protective variants**

Modified proteins achieving loss- or gain-of-functions that help addressing or preventing diseases

**Disrupt >400 different types of PTMs**

Regulate protein activity, change localization, folding, preventing immune escape or slowing down degradation



**Change protein interactions**

Changes localization, folding, protein function or prevents immune escape of glycosylated tumor antigens

### BROAD THERAPEUTIC POTENTIAL

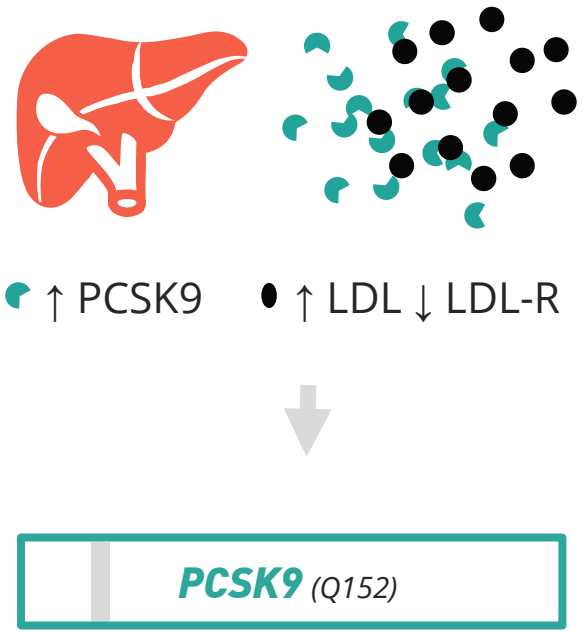
- Common diseases
- Rare diseases
- Target a wide variety of organs
- Treat so-far undruggable targets

PTMs: Post-translational modifications.

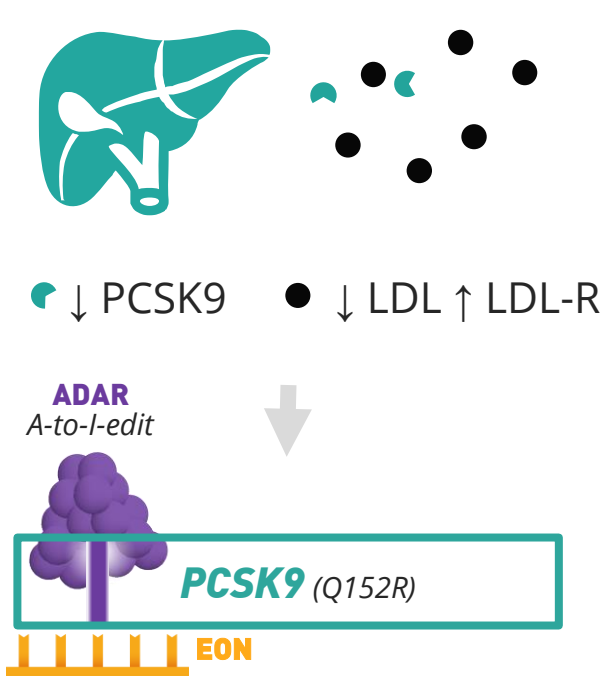
# Changing the autocleavage site with Axiomer® leads to a LOF in PCSK9

*Generation of a loss-of-function variant to lower PCSK9*

**Individuals with hypercholesterolemia**




**Axiomer® edit**




**Disruption of PCSK9 autocleavage site reduces protein in bloodstream**

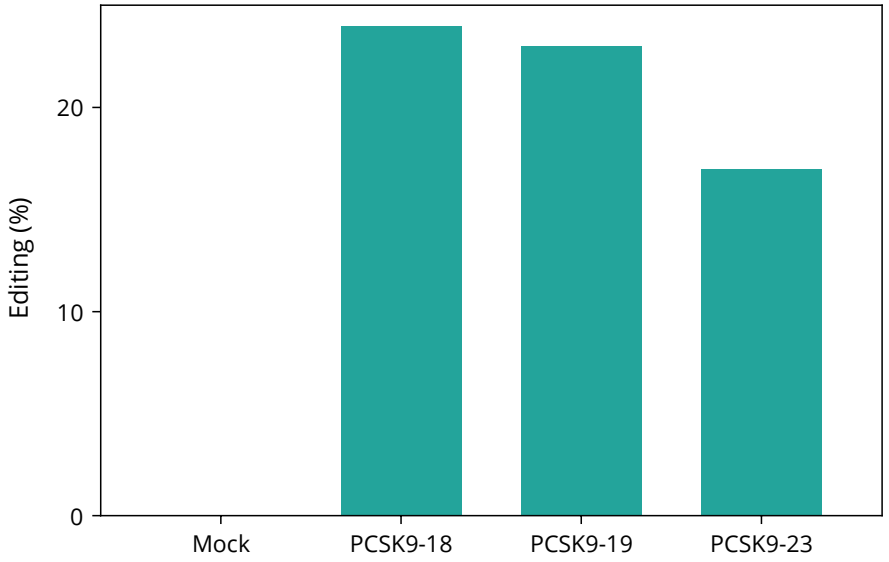
- Less PCSK9 leads to increase of LDL-R on cells, decrease of 'bad' LDL in bloodstream
- Loss-of-function *PCSK9* variant Q152H is associated with low plasma LDL cholesterol in a French-Canadian family and with impaired processing and secretion in cell culture

LDL: Low density lipoprotein, LDL-R: Low density lipoprotein receptor. LOF: Loss of function. Reference: Mayne J, et al. Clin Chem. 2011 Oct;57(10):1415-23.

# Editing of *PCSK9* RNA results in a proenzyme with dominant negative properties

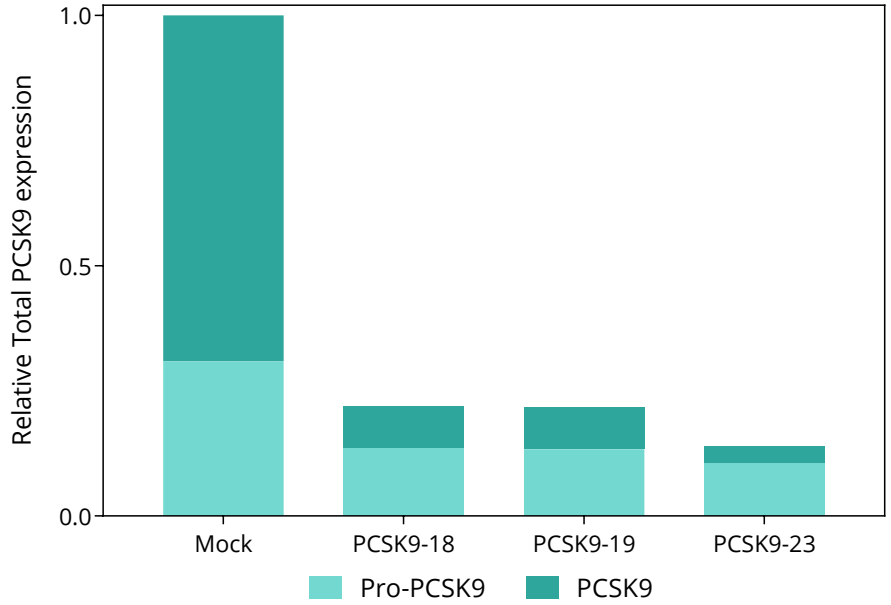
**RNA editing of *PCSK9* in HeLa cells**

*Transfection, 100nM, single dose, N=2, 48 hours, ddPCR*




**PCSK9 protein expression in HeLa cells**

*Transfection, 100nM, single dose, N=2, 48 hours, western blot*




- Up to 25% A-to-I editing of *PCSK9* RNA detected using ddPCR assays leading up to 80% reduction of total PCSK9 protein
- Shift in the ratio cleaved to uncleaved PCSK9 observed; 70%:30% in mock to 25%:75% in treated samples
- The inability to undergo autocleavage likely retains the proenzyme in the endoplasmic reticulum where it can act as a dominant negative protein, preventing the exit of the wild-type form of PCSK9.

# ProQR leading research to optimize EONs for therapeutic use

**Modification of the orphan base**
in the EER confirm superiority of dZ base

**Structure–activity relationship (SAR) assessment**
to define guiding principles

**Further improvements led to approx. 50% editing**
in liver mice

**Positive impact of neutral linkage modifications**
in the ABR (PN)

**New optimizations combined for pipeline development**
targeting liver originated disorders

ProQR®

# IT'S IN OUR RNA