A versatile platform for ADAR-mediated RNA editing in vivo in preclinical models

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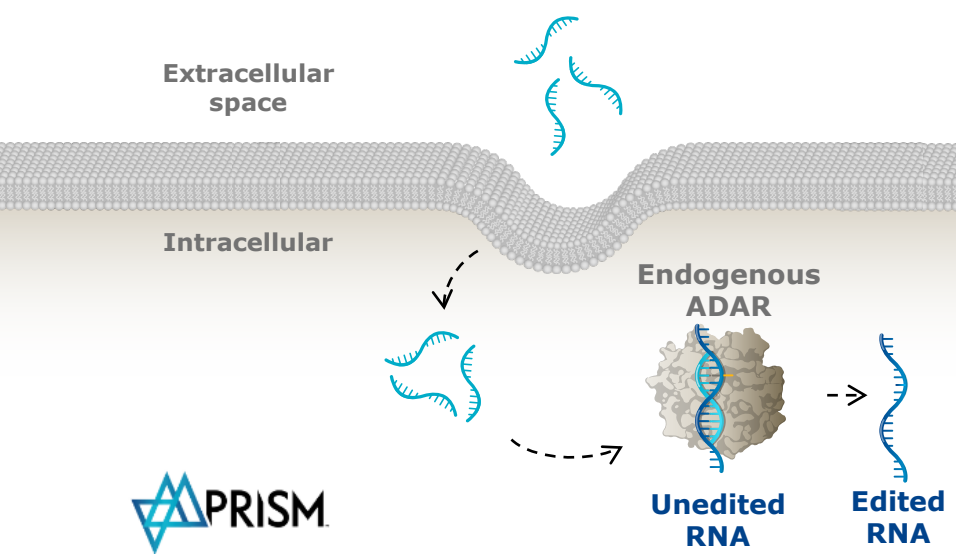
PRISM enables practical approach to RNA editing without need for viruses or exogenous protein

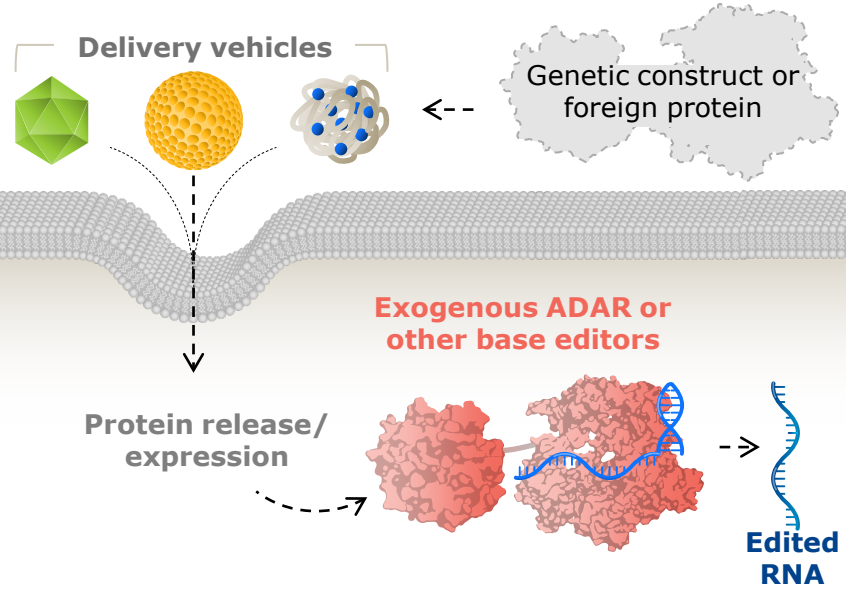
Wave ADAR-editing oligonucleotides



PRISM

- ✓No delivery vehicle required
- ✓No exogenous proteins necessary
- ✓Potential for reduced off-target effects

Alternative Base-Editing Systems



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PRISM platform enables rational drug design

Sequence

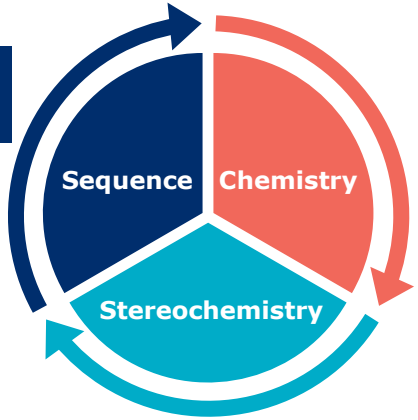
B: bases

A, T, C, mC, G, U,
other modified bases

Stereochemistry

Chiral control of
any stereocenter

Backbone modifications



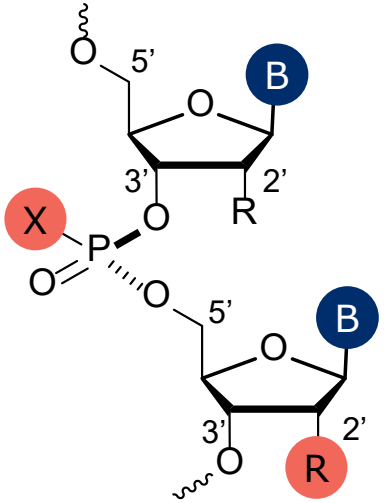
Chemistry

R: 2′ modifications

OMe, MOE, F,
other modifications

X: backbone chemistry

Phosphodiester (PO),
phosphorothioate (PS),
nitrogen-containing
backbone modifications
(PN)



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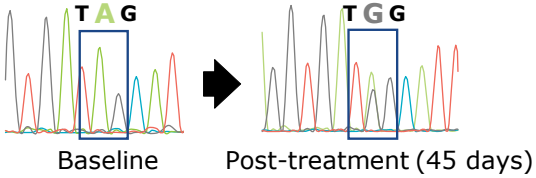
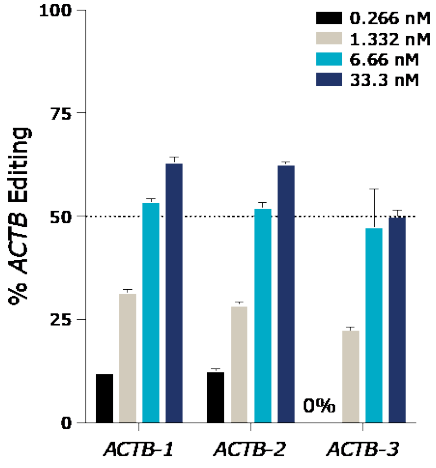
PRISM

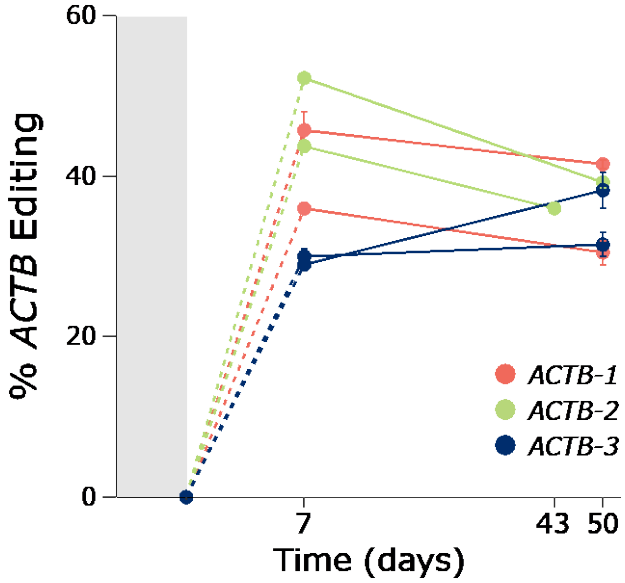
GalNAc-conjugated and unconjugated oligonucleotides support efficient RNA editing



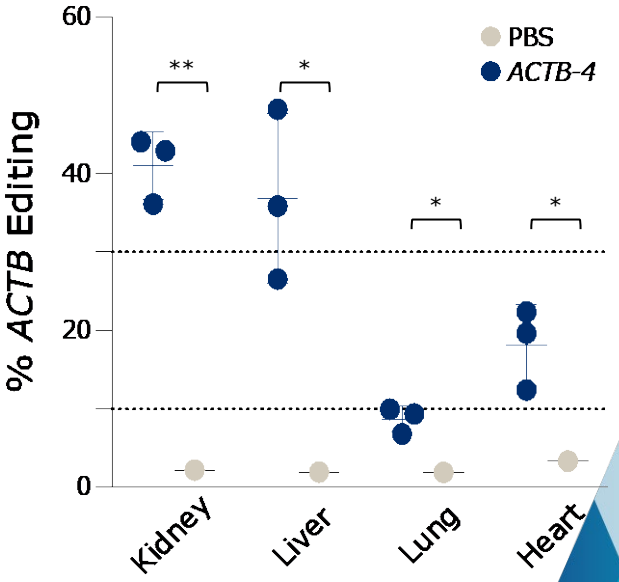
***In vitro* dose-response GalNAc conjugate (NHP hepatocytes)**



***In vivo* editing NHP liver GalNAc conjugate (Multiple 5 mpk, SC doses)**



Editing in NHP 1-week post-dose unconjugated (Single 50 mpk, SC dose)



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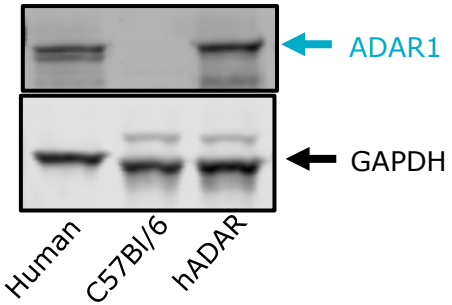
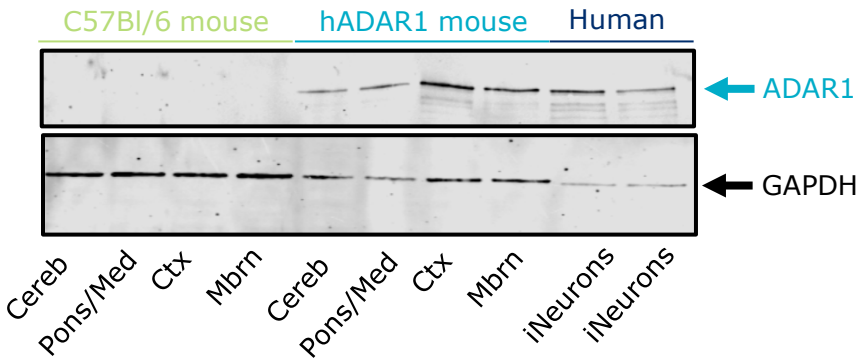
Left: Primary hepatocytes (NHP) were treated with GalNAc-conjugated oligo. Middle: GalNAc-conjugated oligo was administered once daily on days 1-5. Liver biopsies were collected on days 7 and 50. Right: Unconjugated oligo was administered once on day 1. Tissue biopsies were collected on day 8.
NHP nonhuman primate; ACTB β-actin; mpk mg/kg; SC subcutaneous; oligo A-to-I editing oligonucleotide
Stats: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; all comparisons to PBS-treated group by t test

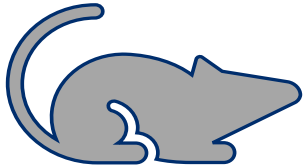
Humanized ADAR1 mouse

Expression in hepatocytes



Expression in neurons





- Transgenic mouse expressing **human ADAR1**
- Expression of ADAR in liver and neurons in mouse approximates expression in corresponding human tissues

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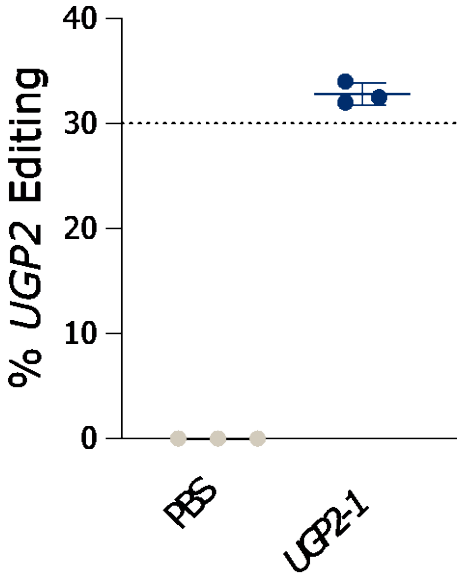
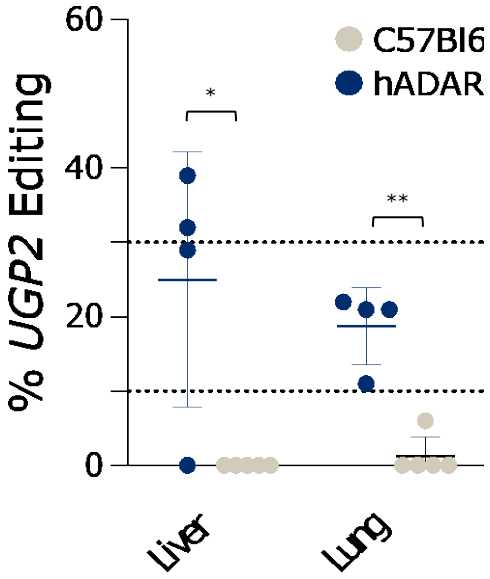
Western blots showing expression of ADAR1 and GAPDH proteins in the indicated tissue
Left: Protein extracts from human hepatocytes, C57Bl/6 or hADAR1 mouse liver; Right: Protein extracts from cerebellum (Cereb), pons/medulla (pons/med), cortex (Ctx), midbrain (Mbrn), or human iCell neurons (iNeurons)

Humanized ADAR mouse model editing similar to editing in NHPs

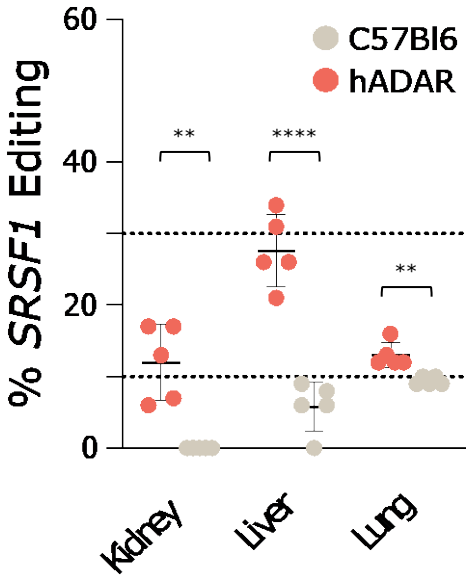
Editing *UGP2* in liver
3-days post-dose
(GalNAc conjugate)



Editing *UGP2*
1-week post-dose
(Unconjugated)

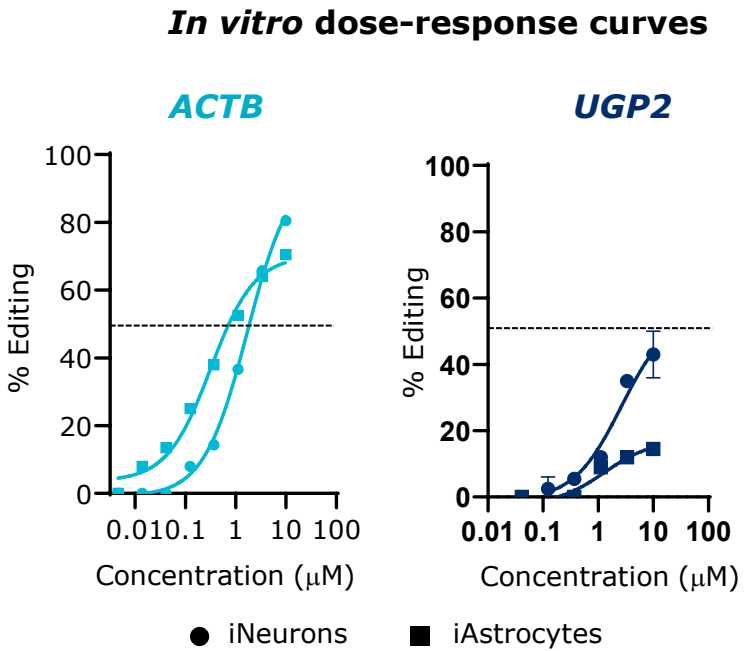


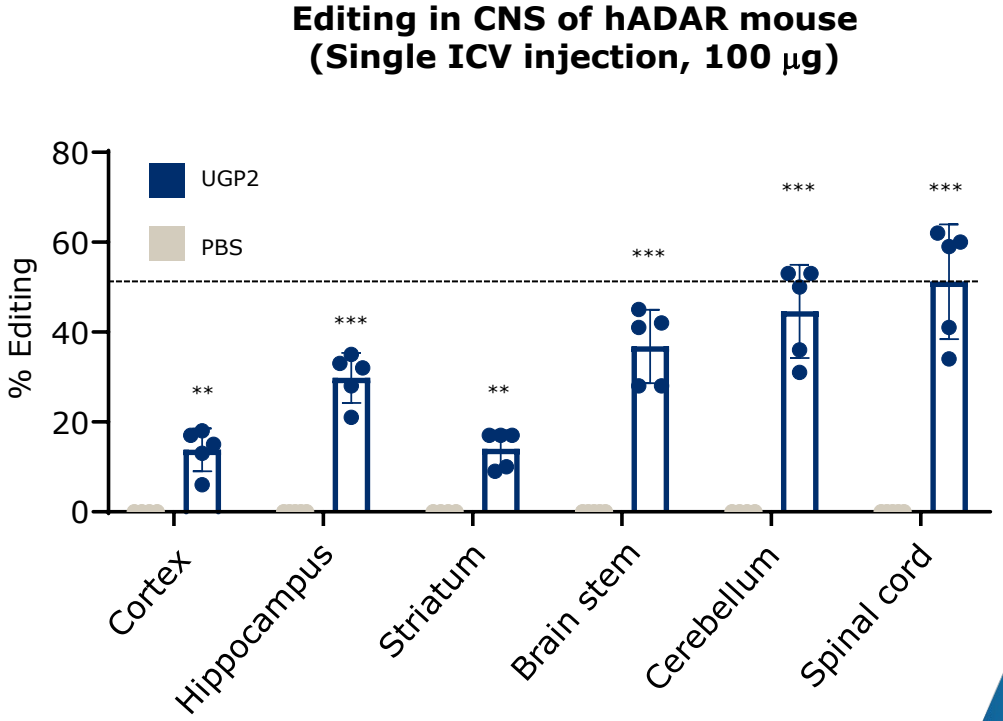
Editing *SRSF1*
1-week post-dose
(Unconjugated)



Left: GalNAc-conjugated oligo was administered by SC injection to hADAR mice once daily on days 1-3. Liver biopsies were collected on day 8.
Middle/Right: For the unconjugated AIMer, a single 100 mg/kg SC dose was administered on day 1. Tissue biopsies were collected on day 8. UGP2 UDP-glucose pyrophosphorylase 2; SRSF1 serine and arginine rich splicing factor 1
Stats: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 by t test

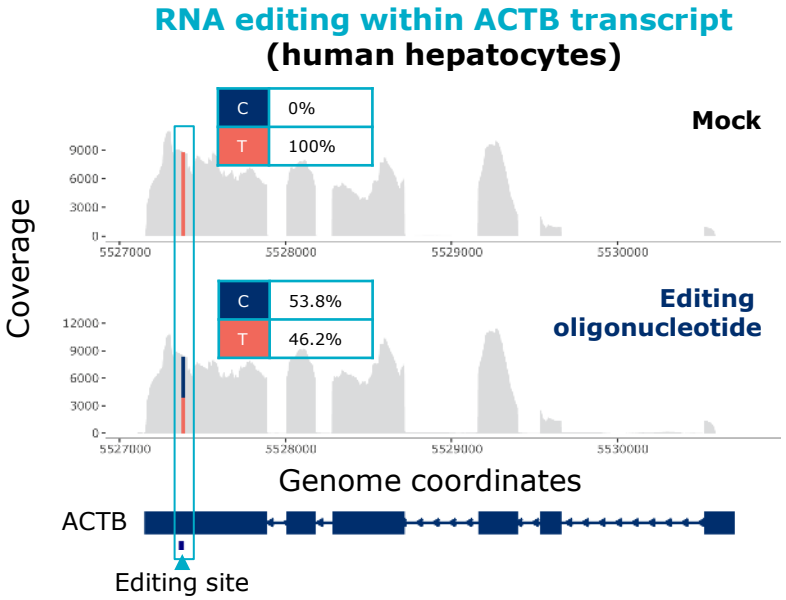
Oligonucleotides direct editing throughout CNS of hADAR mouse

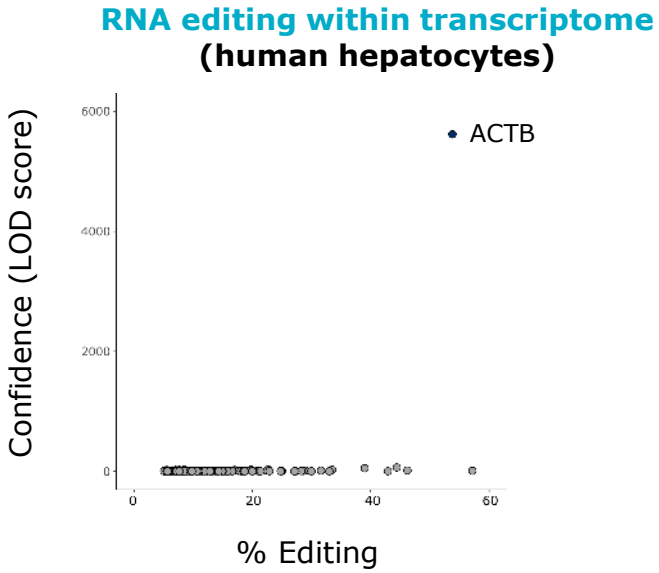




hADAR: human ADAR; UGP2: Glucose Pyrophosphorylase 2; CNS: central nervous system; Editing observed across all tested tissues of human-ADAR-transgenic mice by intracerebroventricular (ICV) injection. 5 mice in each group were injected with PBS or a single 100 μg dose on day 0. Animals were necropsied on day 7. RNA was harvested and editing measured by Sanger sequencing. UGP2 UDP-glucose pyrophosphorylase 2; ACTB β-actin
Stats: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; all comparisons to PBS-treated group by t test (P values Bonferroni corrected)

Wave ADAR editing oligonucleotides are highly specific





Human hepatocytes were dosed with 1um oligonucleotide, 48 hours later RNA was collected and sent for RNA sequencing. RNAseq conducted using strand-specific libraries to quantify on-target ACTB editing and off-target editing in primary human hepatocytes; plotted circles represent sites with LOD>3

Summary

- Stereopure oligonucleotides, generated with PRISM, promote RNA editing with endogenous ADAR enzymes in cellular and animal models
- GalNAc-conjugated and unconjugated oligonucleotides elicit robust editing in primary hepatocytes and liver of NHP and hADAR mice
- There are species-specific differences in ADAR enzymes, and editing in hADAR mouse is similar to editing observed in NHPs
- Oligonucleotides targeting distinct transcripts support editing throughout CNS of hADAR mouse after a single dose

This work was supported by Wave Life Sciences