Towards the development of a therapeutic RNA editing platform

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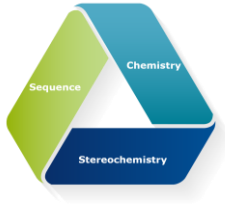
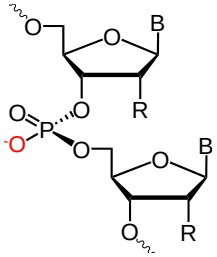
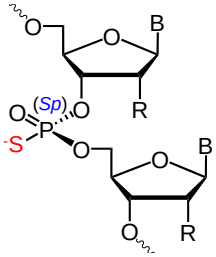
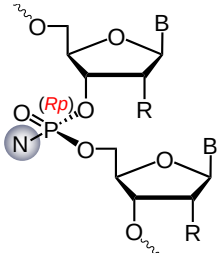
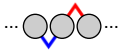
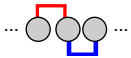
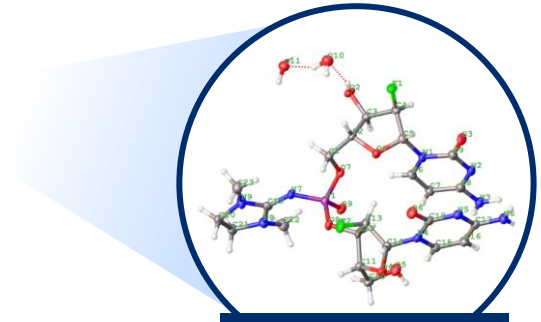
Innovating stereopure backbone chemistry modifications



PRISM backbone linkages

PO	PS	PN
		
		
Chirality None	**Chirality** PS backbone *Rp* PS backbone *Sp*	**Chirality** PN backbone *Rp* PN backbone *Sp*
Negative charge	**Negative charge**	**Neutral charge**



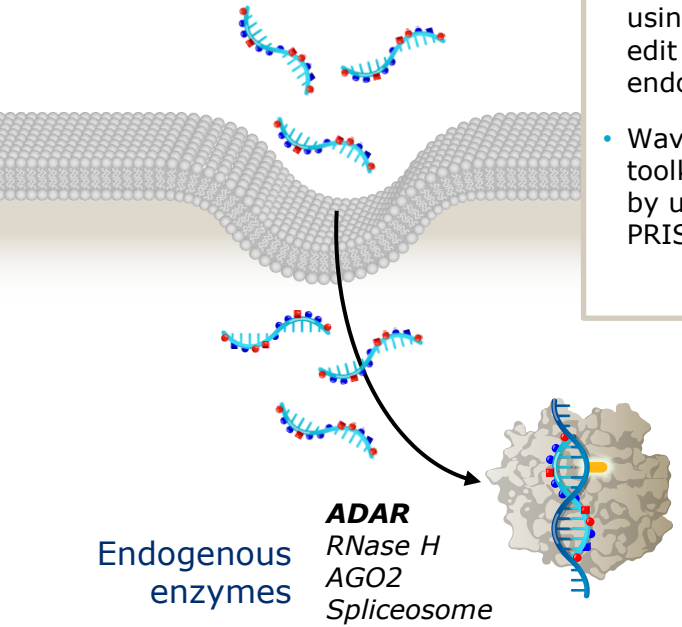
Phosphoryl guanidine x-ray structure

example

PO: phosphodiester PS: phosphorothioate PN: phosphoryl-guanidine

RNA editing with AIMers: A-to-I editing oligonucleotides

Free-uptake of chemically modified oligonucleotides



Endogenous enzymes

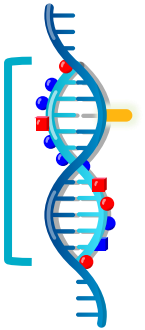
ADAR
RNase H
AGO2
Spliceosome

- First publication (1995) using oligonucleotide to edit RNA with endogenous ADAR[1]
- Wave goal: Expand toolkit to include editing by unlocking ADAR with PRISM oligonucleotides

- ✓ Learnings from biological concepts
- ✓ Applied to ASO structural concepts
- ✓ Applied Wave's proprietary PRISM chemistry

AIMer:

Wave's **A**-to-**I** editing oligonucleotides



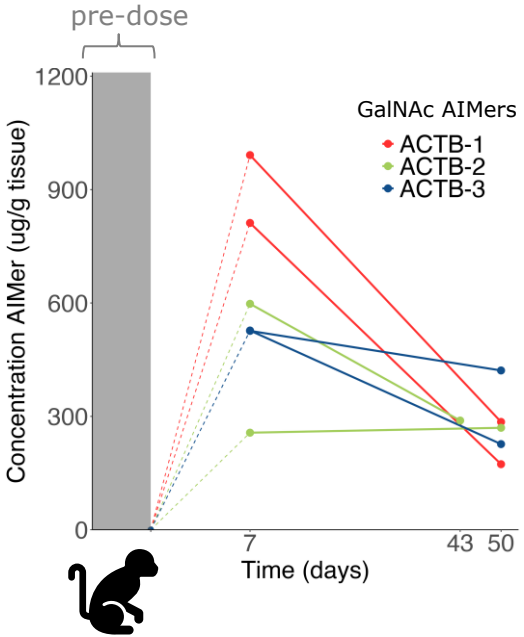
ADAR enzymes

- Catalyze conversion of A-to-I (G) in double-stranded RNA substrates
- A-to-I (G) edits are one of the most common post-transcriptional modifications
- ADAR1 is ubiquitously expressed across tissues, including liver and CNS

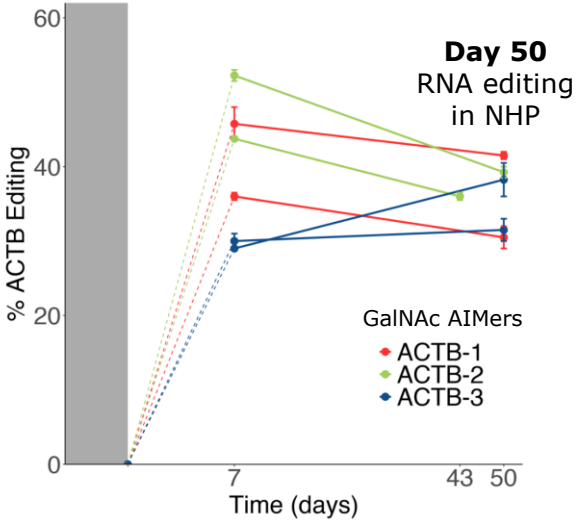
[1]Woolf et al., PNAS Vol. 92, pp. 8298-8302, 1995

GalNAc-AIMers enable durable and specific editing out to day 50 in liver of NHPs

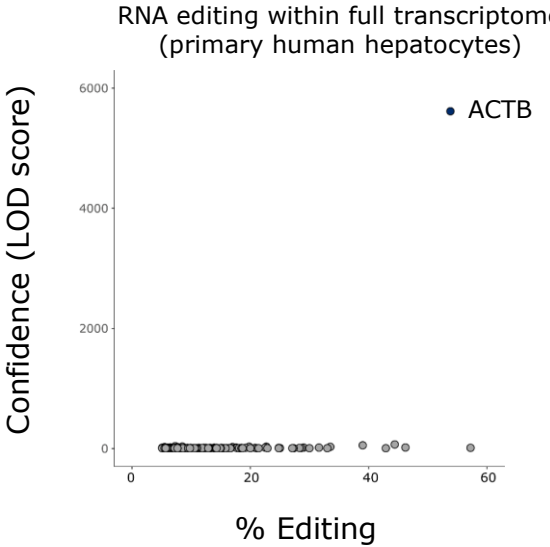
AIMers detected in liver of NHP at day 50



Substantial and durable editing in NHP liver *in vivo*



ADAR editing with ACTB AIMer is highly specific

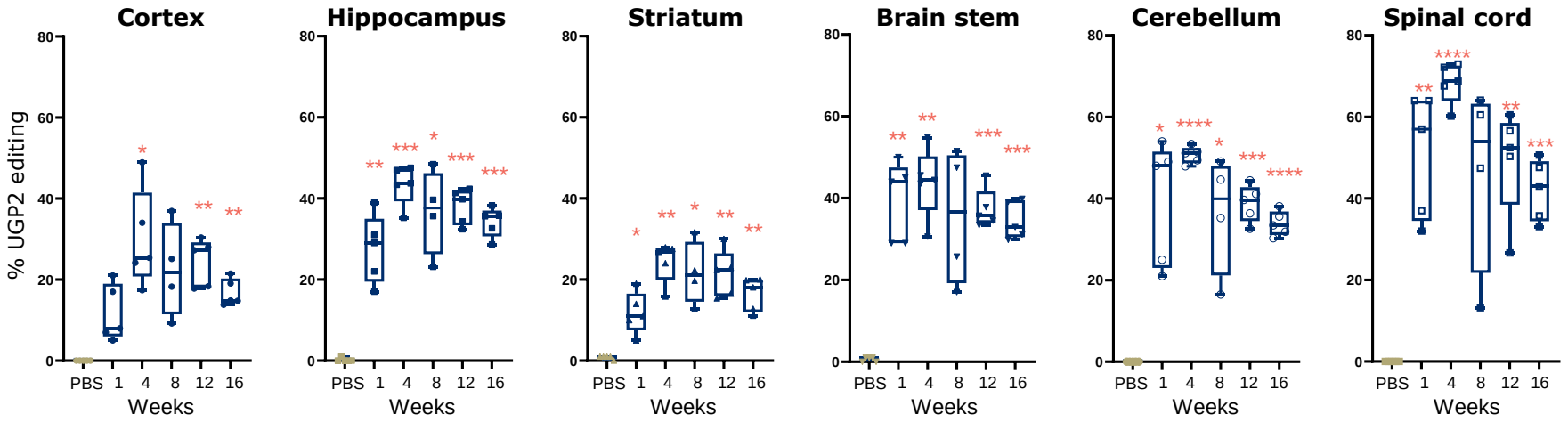


No bystander editing in ACTB

AIMer tissue concentration (left) and editing activity (middle) in liver after 5 mg/kg SC injections on days 1-5; Right: Hepatocytes treated with 1 uM AIMer, 48 hrs later RNA collected, RNAseq conducted using strand-specific libraries to quantify editing; plotted circles represent sites with LOD>3. NHP: non-human primate; ACTB: Beta-actin; Monian *et al.*, manuscript in press

Efficient and durable editing in mouse CNS with unconjugated AIMer

Peak editing observed 4-weeks post-single ICV dose across tissues



	Cortex	Hippocampus	Striatum	Brain stem	Cerebellum	Spinal cord
Peak editing	30%	>40%	25%	>40%	50%	>65%

Transgenic huADAR mice were administered 100 μg AIMer or PBS on day 0 and evaluated for UGP2 editing across CNS tissues at 1, 4, 8, 12 and 16-weeks post dose. Percentage UGP2 editing determined by Sanger sequencing. Stats: 2-way ANOVA with post-hoc comparison to PBS (n=5 per time point per treatment) *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. ICV intracerebroventricular; PBS phosphate buffered saline

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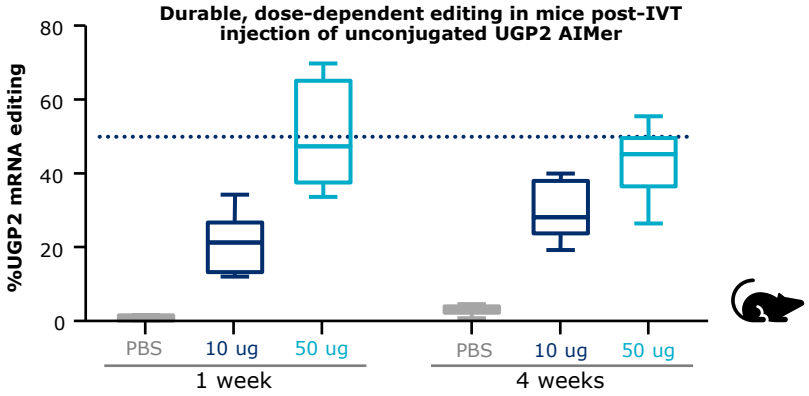
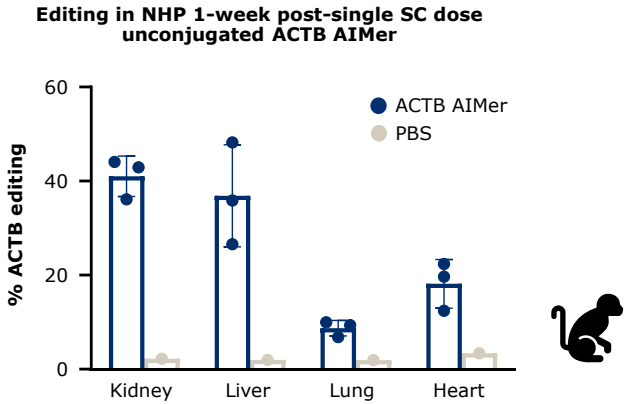
Productive editing beyond liver and CNS with unconjugated AIMers

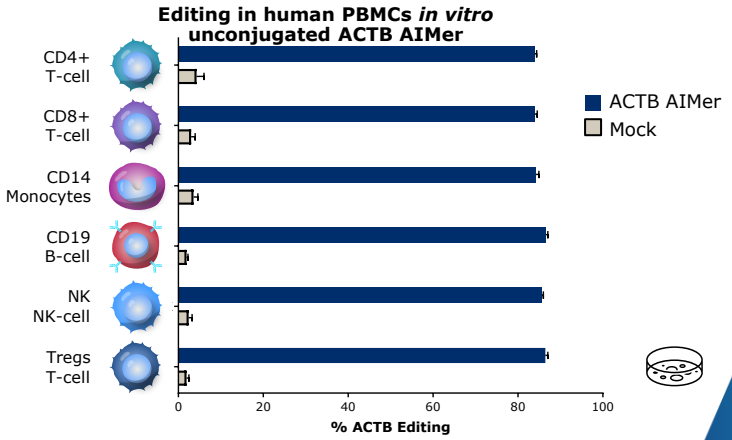
Ophthalmology



Kidney, liver, lung, heart



Immune cells



(left): non-human primate (NHP) 50 mg/kg beta-Actin (ACTB) AIMer, SC (subcutaneous) on day 1; Necropsy for editing day 8; (top right): Mice received 10 or 50 μg UGP2 AIMer intravitreal (IVT), eye collected for analysis 1 or 4 weeks later. (lower right): Human PBMCs dosed with 10 μM ACTB AIMers, under activating conditions (PHA). After 4 days, different cell types isolated, quantitated for editing.

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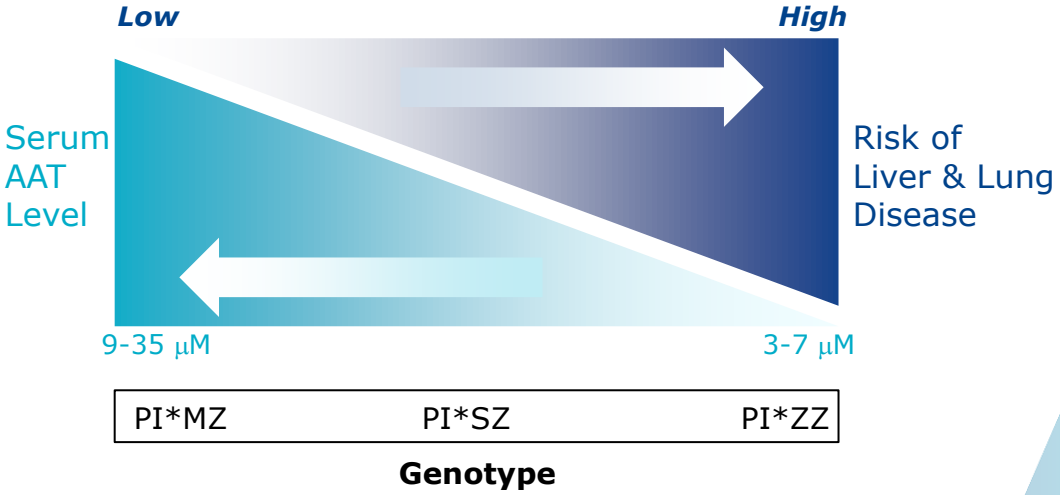
An ADAR editing approach to correct Alpha-1 antitrypsin deficiency (AATD)

Objectives

- Recruit endogenous ADAR enzyme to edit SERPINA1 Z mRNA
- Restore circulating M-AAT protein to expected therapeutic threshold (11 μM)
- Confirm functionality of M-AAT
- Confirm specificity of SERPINA1 editing

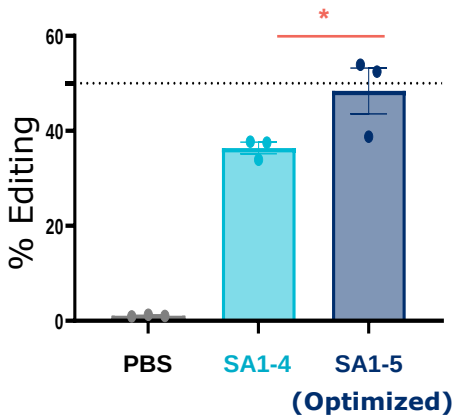
Inverse relationship between circulating AAT levels and disease risk



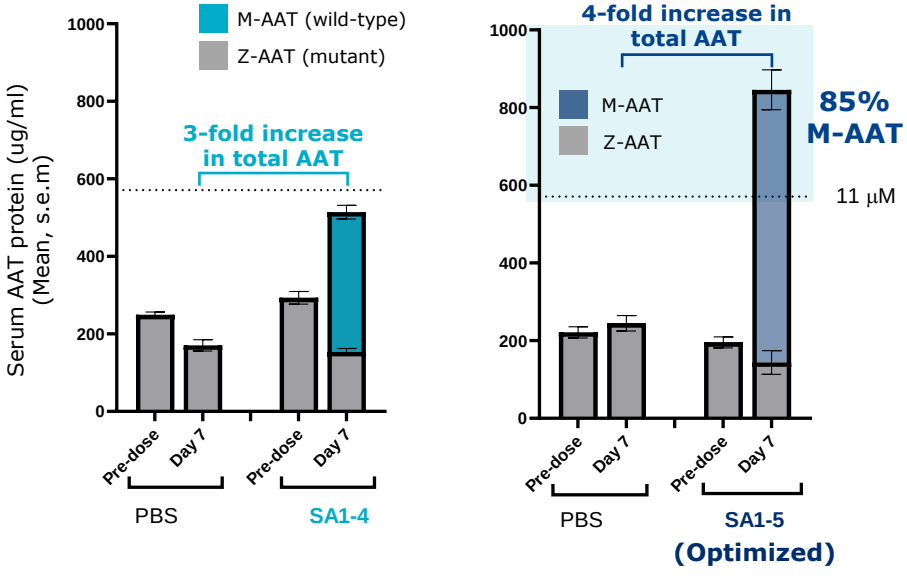
Sources: Strnad 2020; Blanco 2017; Remih 2021

Optimized AIMers achieve ~50% mRNA editing and restore AAT protein well above therapeutic threshold in mouse model

SERPINA1 RNA editing huADAR mouse
(3x5 mg/kg, SC)



AAT protein concentration in serum
(3x10 mg/kg, SC)



Serum levels

PI*MZ	~3-5-fold
PI*SZ	~2-fold
PI*ZZ	~3-7 μM

✓ **Z allele mRNA editing *in vivo***

✓ **Restoration of AAT protein expression *in vivo***

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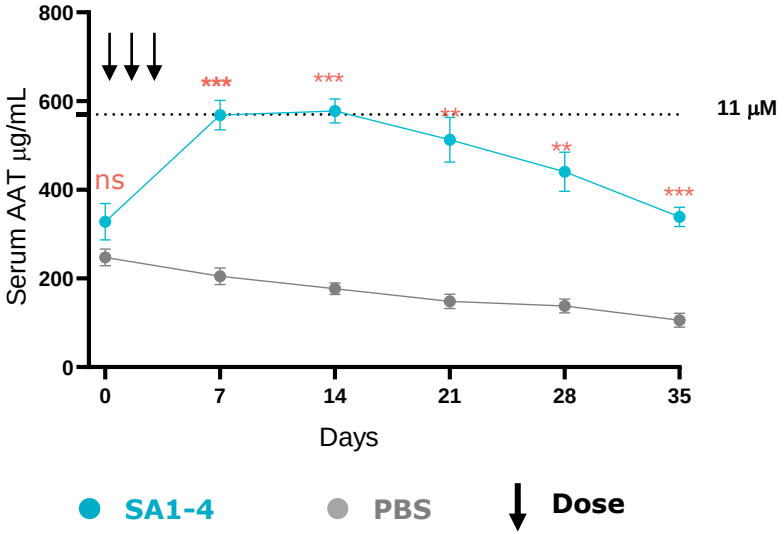
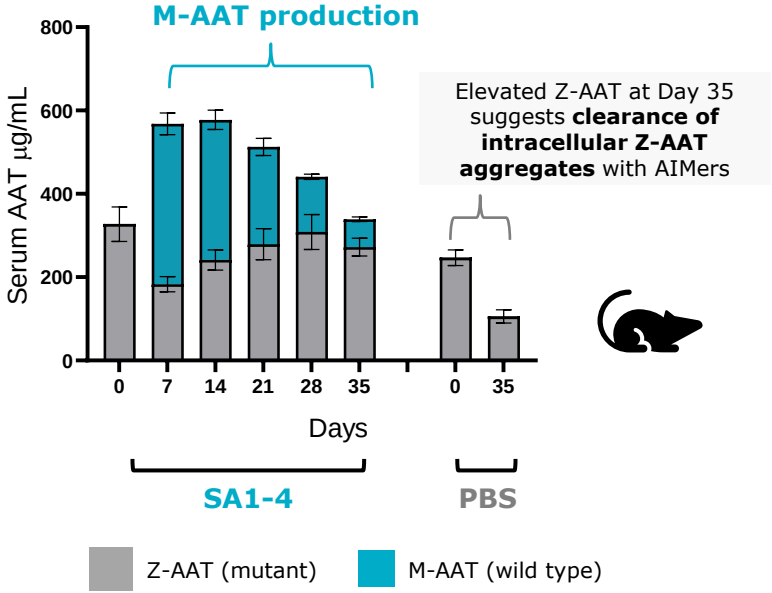
Left: AIMers administered hu*ADAR/SERPINA1* mice (3x5 mg/kg) on days 0, 2, and 4. Livers collected on day 7, and SERPINA1 editing was quantified by Sanger sequencing (shown as mean ±. sem) Stats: One-way ANOVA was used to test for differences in editing between SA1-4 and other oligos * P<0.05
Right: hu*ADAR/SERPINA1* mice administered PBS or 3 x 10 mg/kg AIMer (days 0, 2, and 4) SC. Proportion of AAT protein in serum, Z type or M type, measured by mass spectrometry, total AAT protein levels quantified by ELISA.

Durable restoration of functional, M-AAT protein with ADAR editing

Human AAT serum concentration ≥3-fold higher over 30 days post-last dose



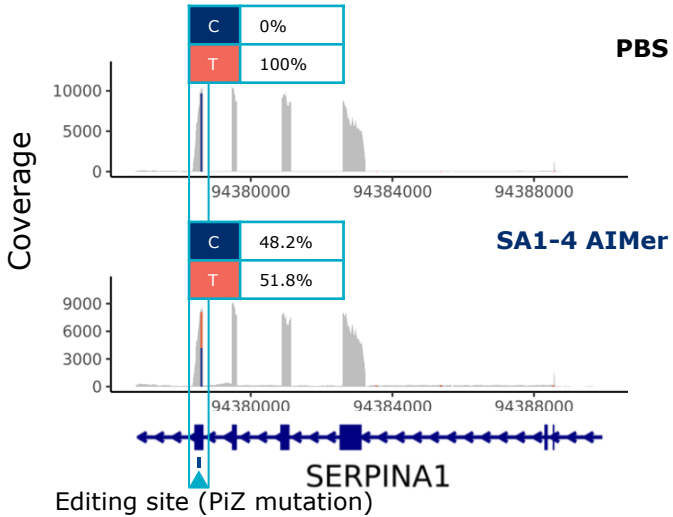
Restored wild-type M-AAT detected over 30 days post-last dose



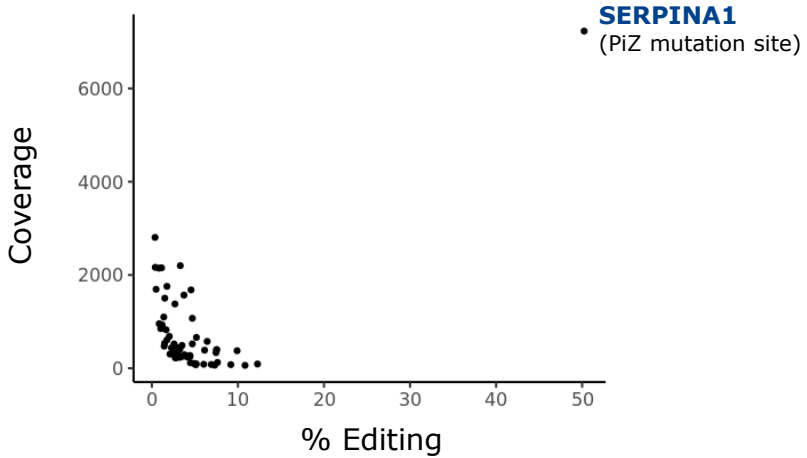
(left) SA1-4 (GalNAc AIMer) or PBS administered to hu*ADAR/SERPINA1* mice (3 x 10 mg/kg on days 0, 2, 4) SC. Serum AAT quantified by ELISA. (right) AAT quantified by ELISA; M-AAT and Z-AAT distinguished by mass spectrometry.

ADAR editing is highly specific; no bystander editing observed on SERPINA1 transcript

RNA editing only detected at PiZ mutation site in SERPINA1 transcript
(mouse liver)



RNA editing within transcriptome
(mouse liver)

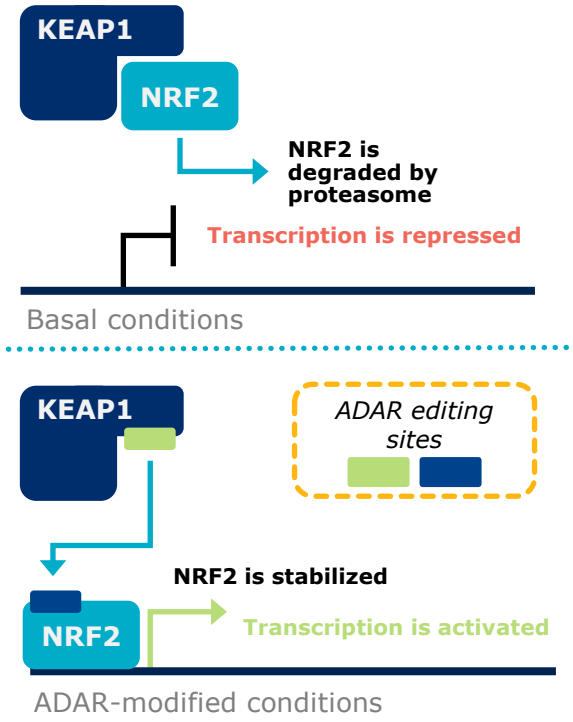


No bystander editing in SERPINA1

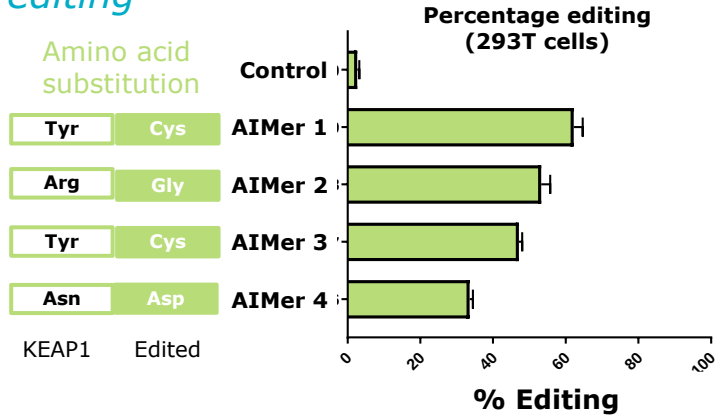
Editing is specific *in vivo*

Dose 3 x 10mg/kg days (0, 2, 4) SC. Liver biopsies day 7. RNAseq, To quantify on-target SERPINA1 editing reads mapped to human SERPINA1, to quantify off-target editing reads mapped to entire mouse genome; plotted circles represent sites with LOD>3 (N=4); Analyst and Investor Research Webcast September 28, 2021

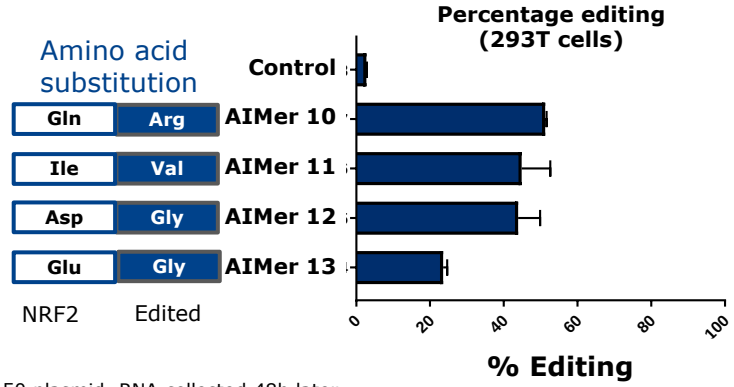
Apply AIMers to modify protein-protein interactions



KEAP1 editing

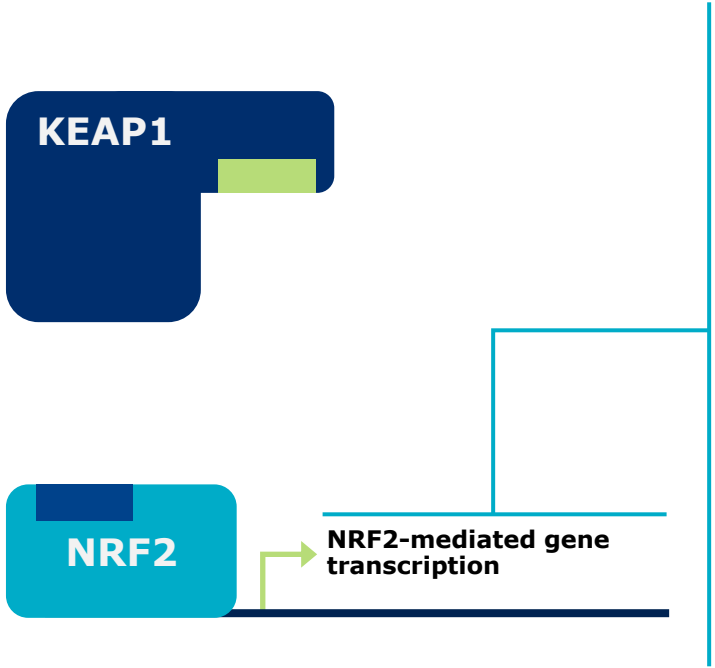


NRF2 editing

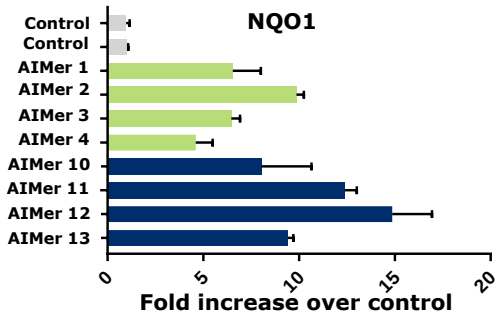


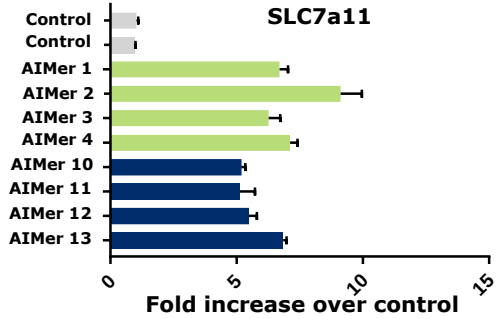
293T cells transfected with 20 nM of AIMer, ADAR-p110 or ADAR-p150 plasmid. RNA collected 48h later, editing quantified by PCR and Sanger (n=2).

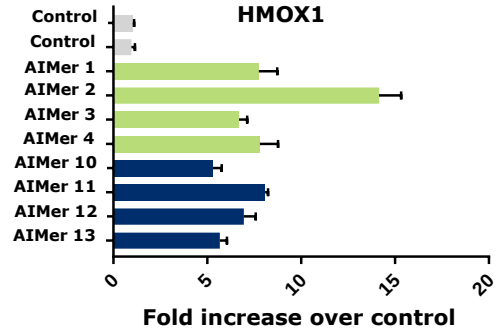
ADAR editing activates multiple genes, confirming disrupted protein-protein interaction *in vitro*

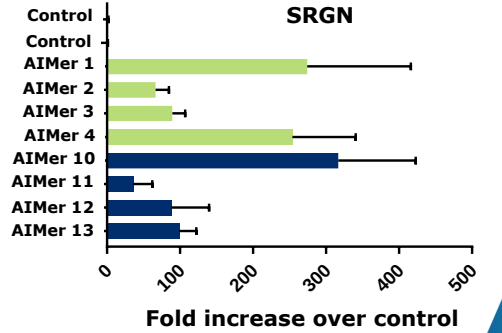


ADAR editing of either KEAP1 or NRF2 directs gene activation









Gene expression quantified by PCR (n=2) after transfection into 293T cells

Summary

- AIMers represent Wave's therapeutic RNA editing platform that leverages endogenous ADAR proteins
  - Achieve potent and specific editing
  - Support durable activity
  - Amenable to multiple routes of administration
  - Active in animals as GalNAc-conjugates or unconjugated
- AIMers restore expression of functional protein
  - Correct SERPINA1 Z mutation in mouse hepatocytes to durably express functional, secreted Z-AAT protein
- AIMers modulate protein-protein interactions
  - Disrupt KEAP1-NRF2 interface to activate downstream transcription in cells