Rewriting ABCA4 RNA for the Treatment of Stargardt Disease

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Disclosures
Robert Bell is an employee and shareholder of Ascidian Therapeutics
All animals treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research

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Ascidian Rewrites RNA To Treat Stargardt & Other Genetically Defined Diseases

Mechanism

- Exon editing occurs at the pre-mRNA level. Harnessing the cell's **endogenous splicing machinery**, two distinct RNA molecules are precisely linked to form a single mature mRNA

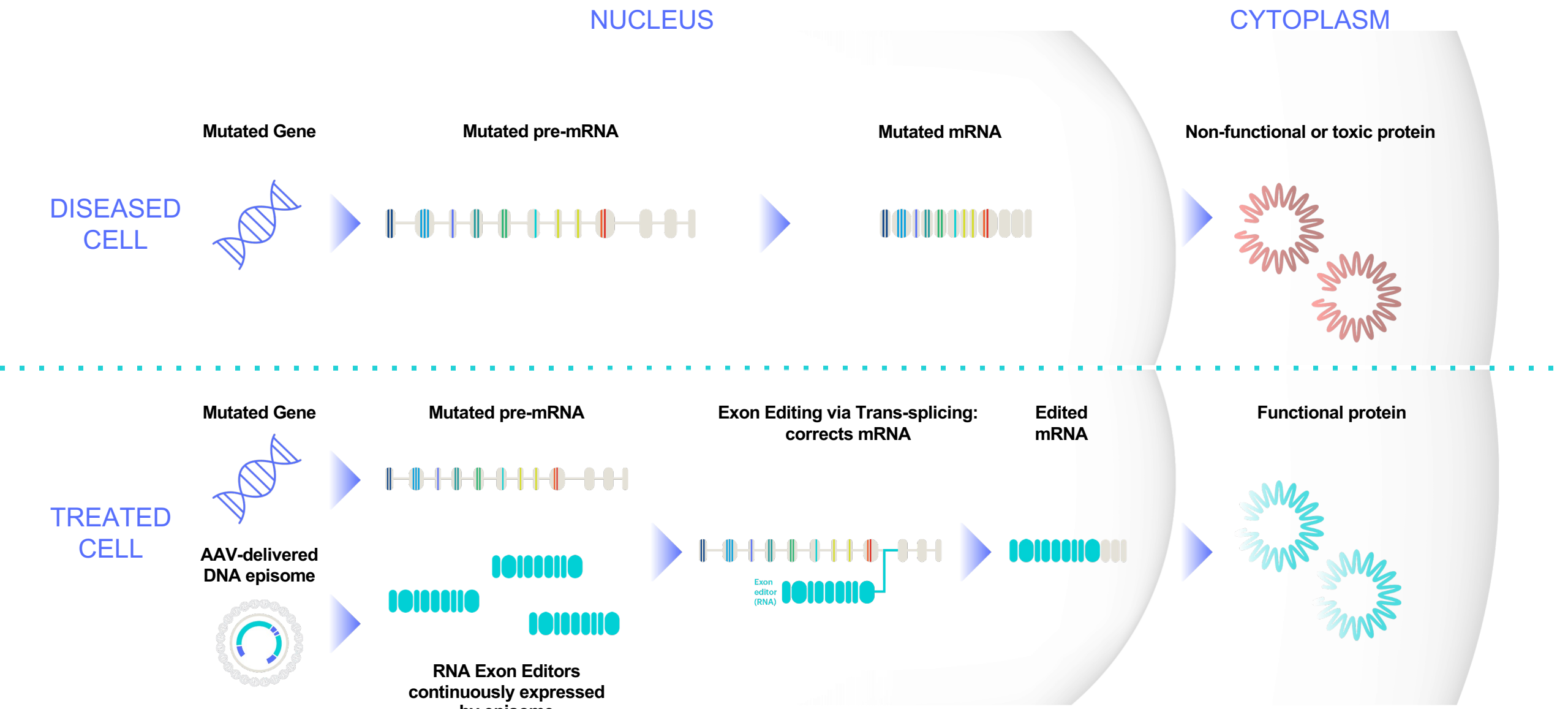
Advantages

- Exon editing provides **durability** of gene therapy **without DNA edits** or exogenous enzymes
  - Addresses genes **outside AAV package limits**
  - Addresses genes with **high mutational variance**
  - **Maintains native gene expression** patterns and levels; applicable to genes with a **narrow therapeutic index** where overexpression poses safety risks

Status

- **Lead program targeting ABCA4** retinopathies & Stargardt disease demonstrated *in vivo* exon editing in **NHP**, with clear path to clinic; CMC are on-line; IND enabling in progress
- Demonstrated exon editing in multiple genes and multiple cell types, including neurological disease targets

RNA Exon Editing Replaces Mutated Exons With Functional Exons



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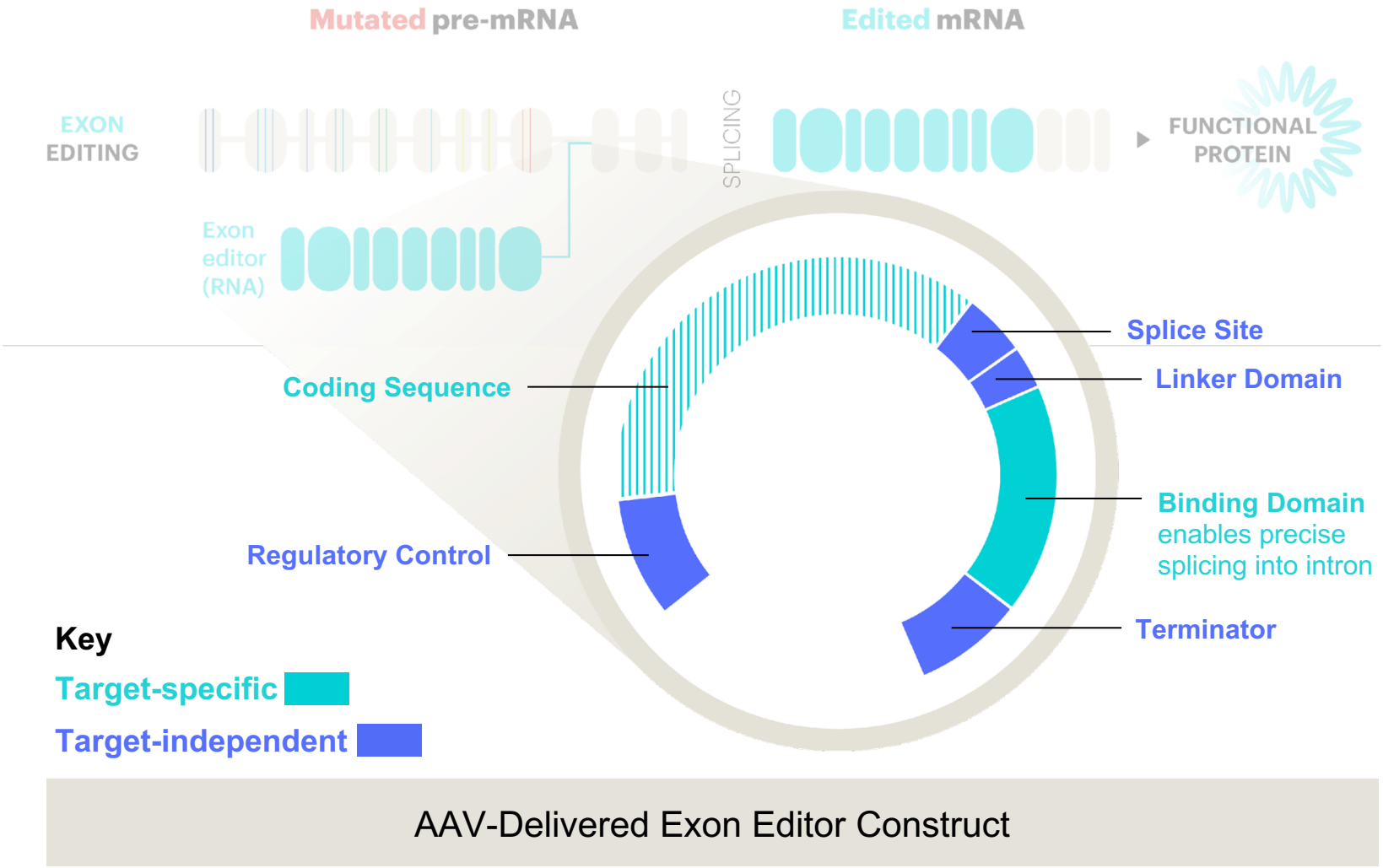
RNA Exon Editors are Modularly Designed to Provide Exquisite Control of Exon Replacement

Advances in **synthetic biology, computational sciences and RNA design** enable Ascidian to identify & design therapeutic RNA exon editing molecules

Ascidian has identified proprietary **RNA structural elements that significantly boost editing efficiency** and can be applied across targets



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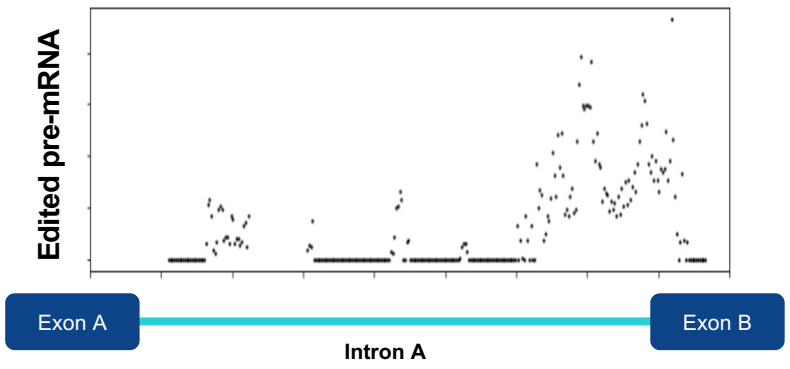
RNA Exon Editor Optimization Combines Modular, Target-Independent Components With Optimal Binding Domains

Iteration & Refinement

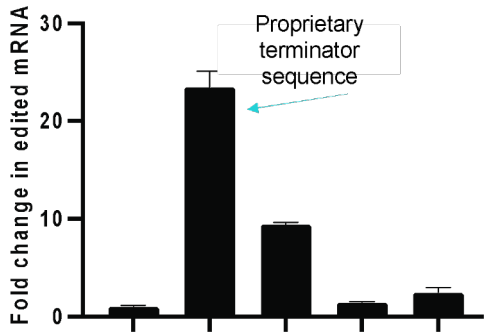
Intron Scan:
Identification of Binding Domain *Hot Spots*



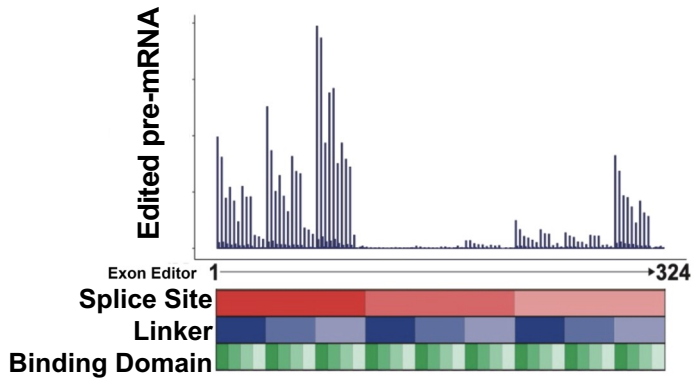
Target-Independent Components,
e.g., terminators (shown), splice sites

+

Target-Specific Components,
e.g., binding domains

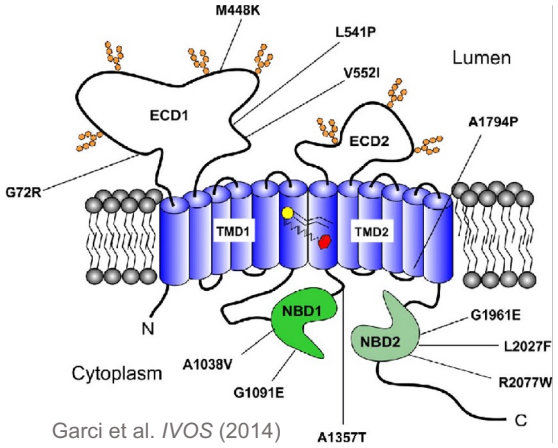


Combinatorial Assessment

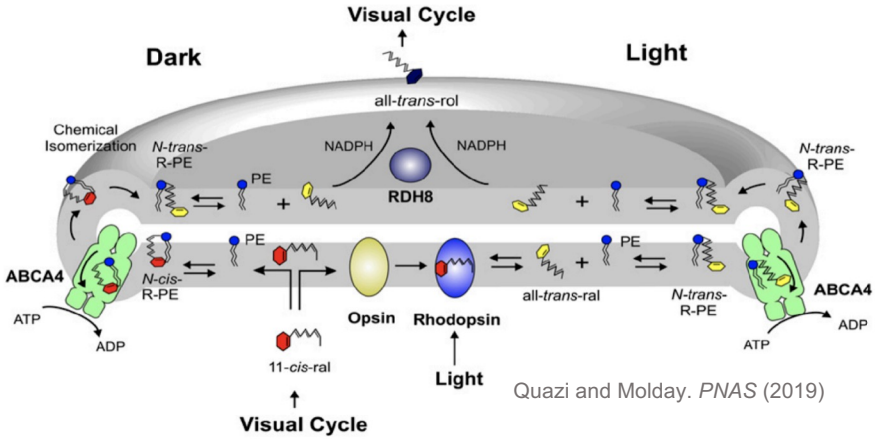


Ascidian's RNA design engine improves with each molecule developed as target-independent elements are repeatedly applied

ABCA4-Related Retinopathies have No Treatment, High Mutational Variance, and Cannot Be Addressed with Conventional Gene Therapy

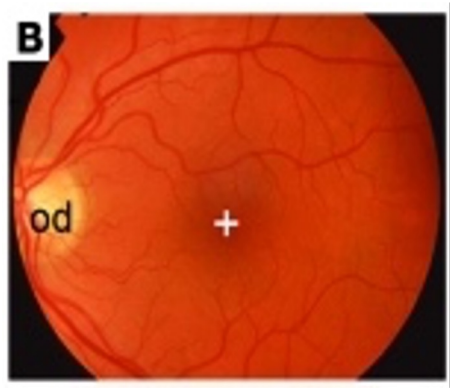


Garci et al. *IVOS* (2014)

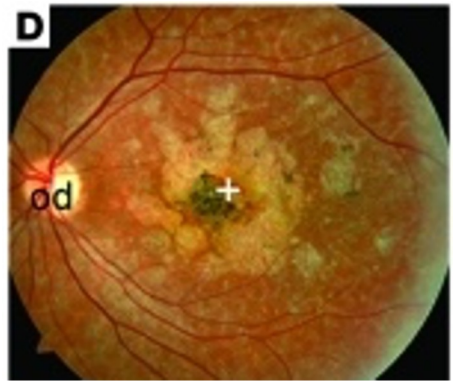


Quazi and Molday. *PNAS* (2019)

Normal Eye



ABCA4 Retinopathy: Stargardt's

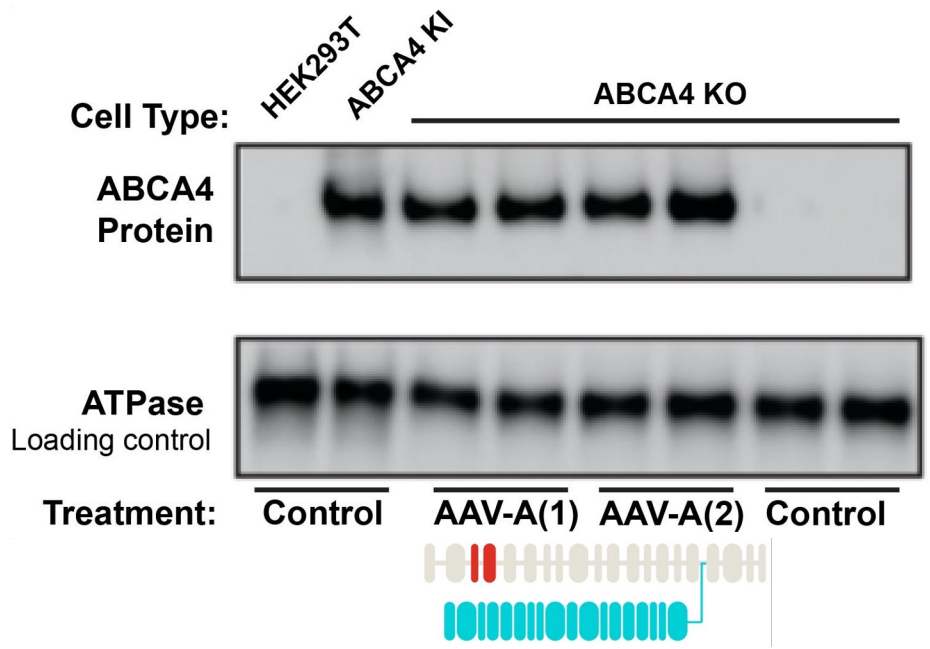


den Hollander, Black, Bennett, & Cremers 2010

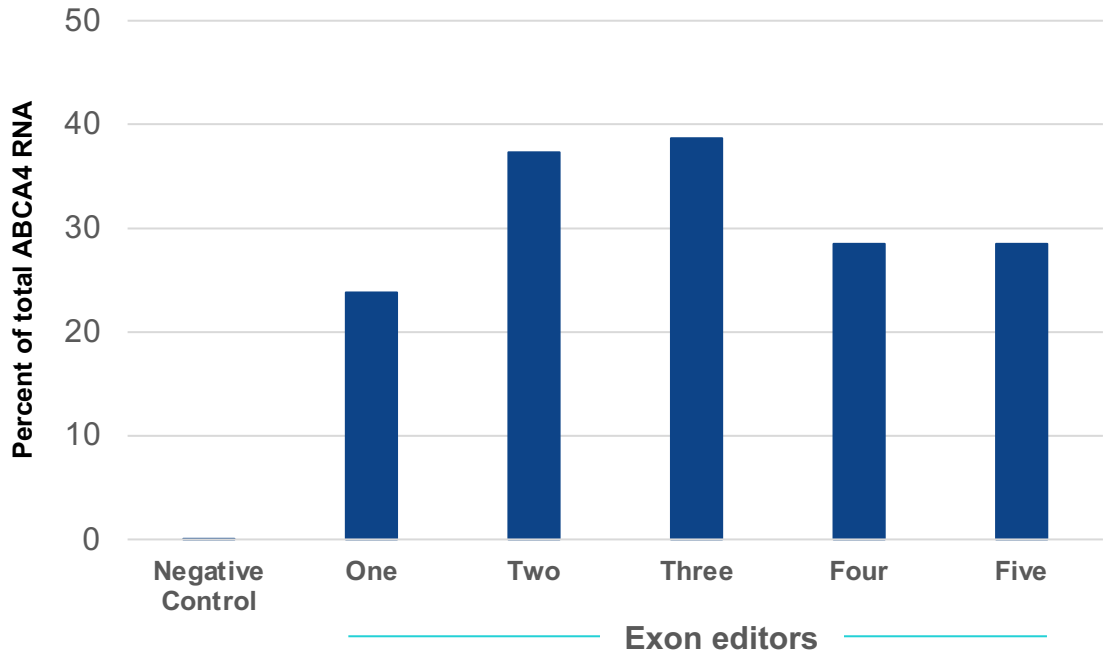
- ABCA4 retinopathies, such as Stargardt Disease, are caused by autosomal recessive mutations in the ATP-Binding Cassette sub-family A type 4 gene
- Loss of ABCA4 results in build-up of fatty byproducts (lipofuscin) in the macula leading to cellular toxicity and a progressive loss of vision
- ABCA4 is too large (6.8 kb) for AAV-mediated gene replacement and upwards of 900 unique mutations have been identified
- Ascidian's 5' ABCA4 RNA exon editor replaces exons 1-22 of ABCA4, covering ~60% of known pathogenic mutations, and offering potential treatment for up to 80% of patients

RNA Exon Editors Achieve Unprecedented Efficiency and Restore Wild-Type ABCA4 Protein Expression *in vitro*

Western Blot of HEK293T Engineered ABCA4 Protein KO cell line



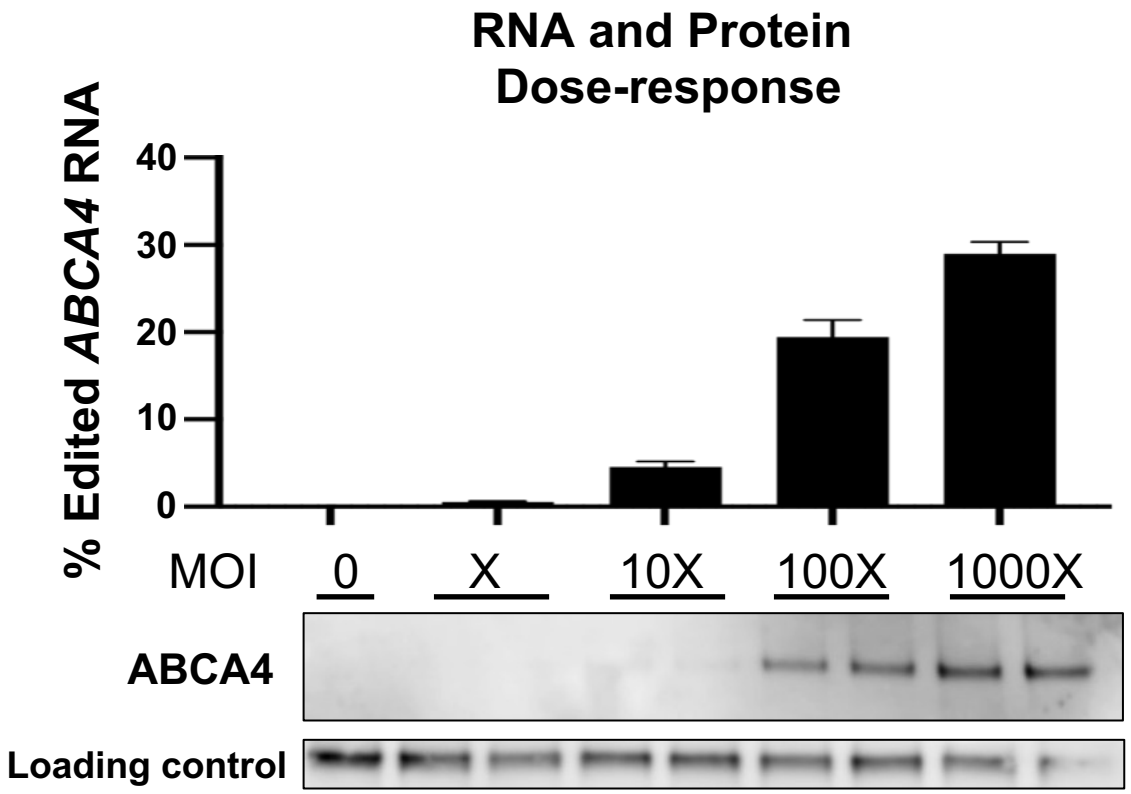
Exon Edited RNA (Percent of total ABCA4 RNA)
optimized transduction protocol (total cells)

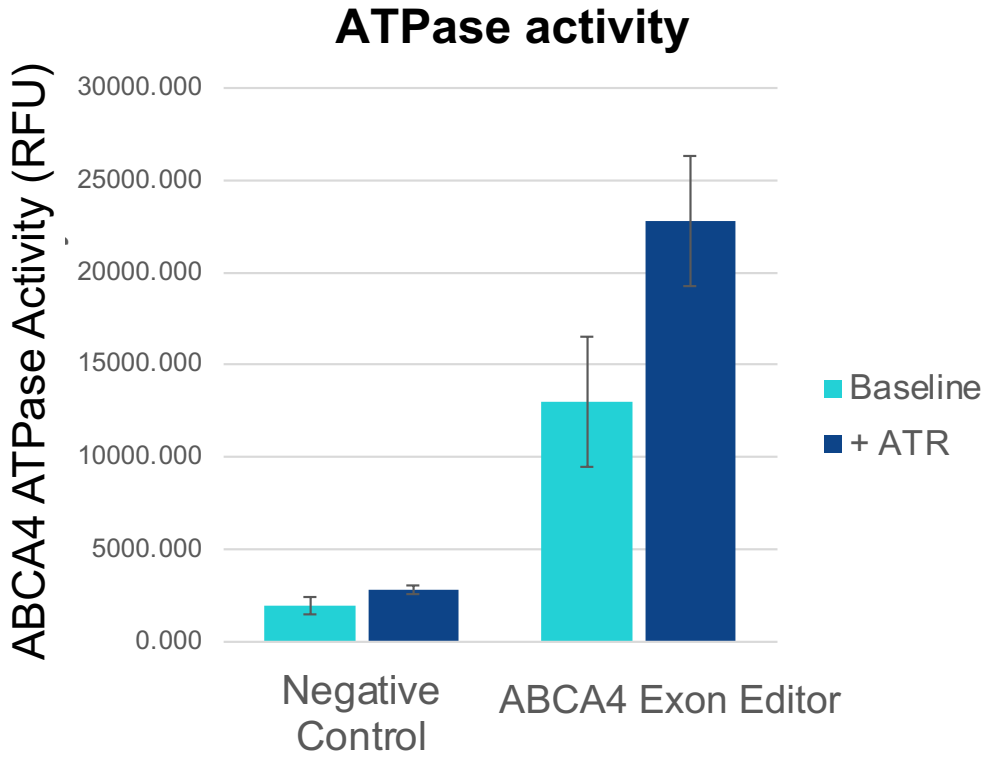


AAV delivered Exon Editors restore protein levels *in vitro* in ABCA4 protein knockout cells

AAV-A(1) and AAV-A(2) are identical exon editor constructs packaged using unique AAV-ITR plasmid backbones.
Data from biological duplicates shown.

Confirmation of Exon Editor Dose-Response and Functional ATPase Activity

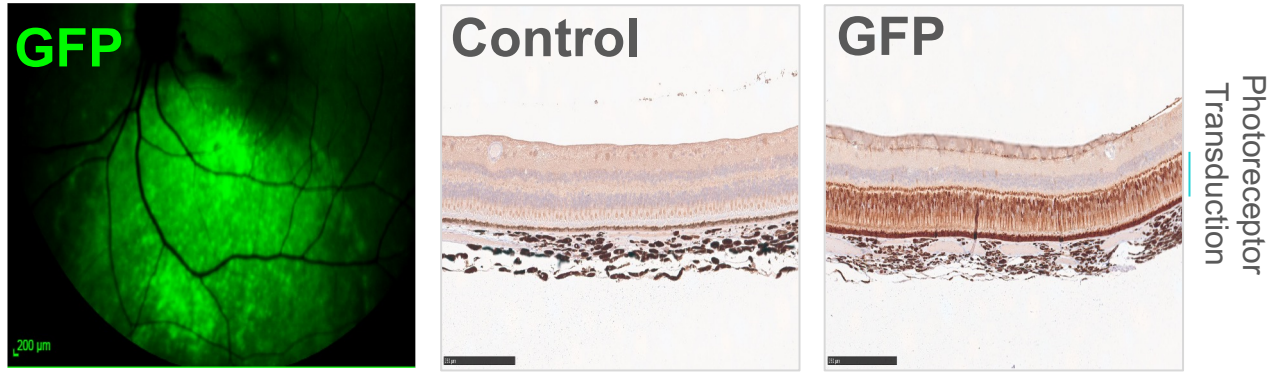




Clear dose response between RNA editing levels and protein expression, and confirmation that Exon Edited ABCA4 protein retains normal functional ATPase Activity

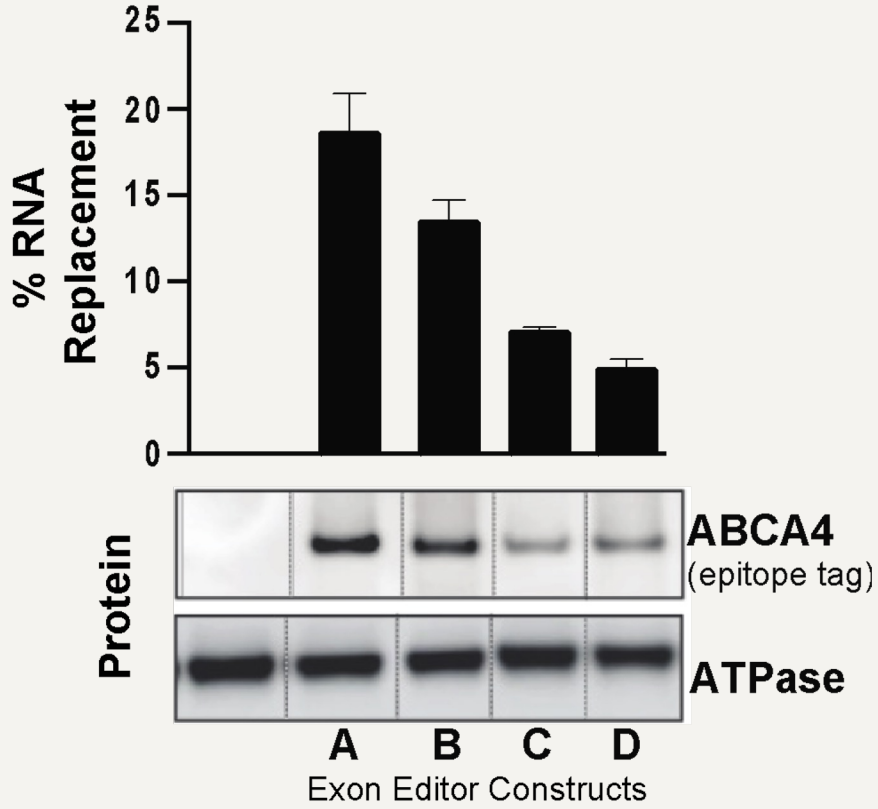
in vivo Proof of Principle: RNA Exon Editing and Full-Length Protein Production in NHP Retina

Transduction (GFP) in NHP retina validates AAV capsid and regulatory elements of lead candidate



- Subretinal route of delivery and clinical device precedent by Luxturna
- Robust Non-Human Primate Retinal Transduction Achieved
- First time full-length ABCA4 protein expressed in NHP retina after single AAV administration

RT-qPCR and Western Blot analysis of human-NHP edited ABCA4 *in vivo*



NHP: Non-Human Primates. GFP: Green fluorescent protein
All animals treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research

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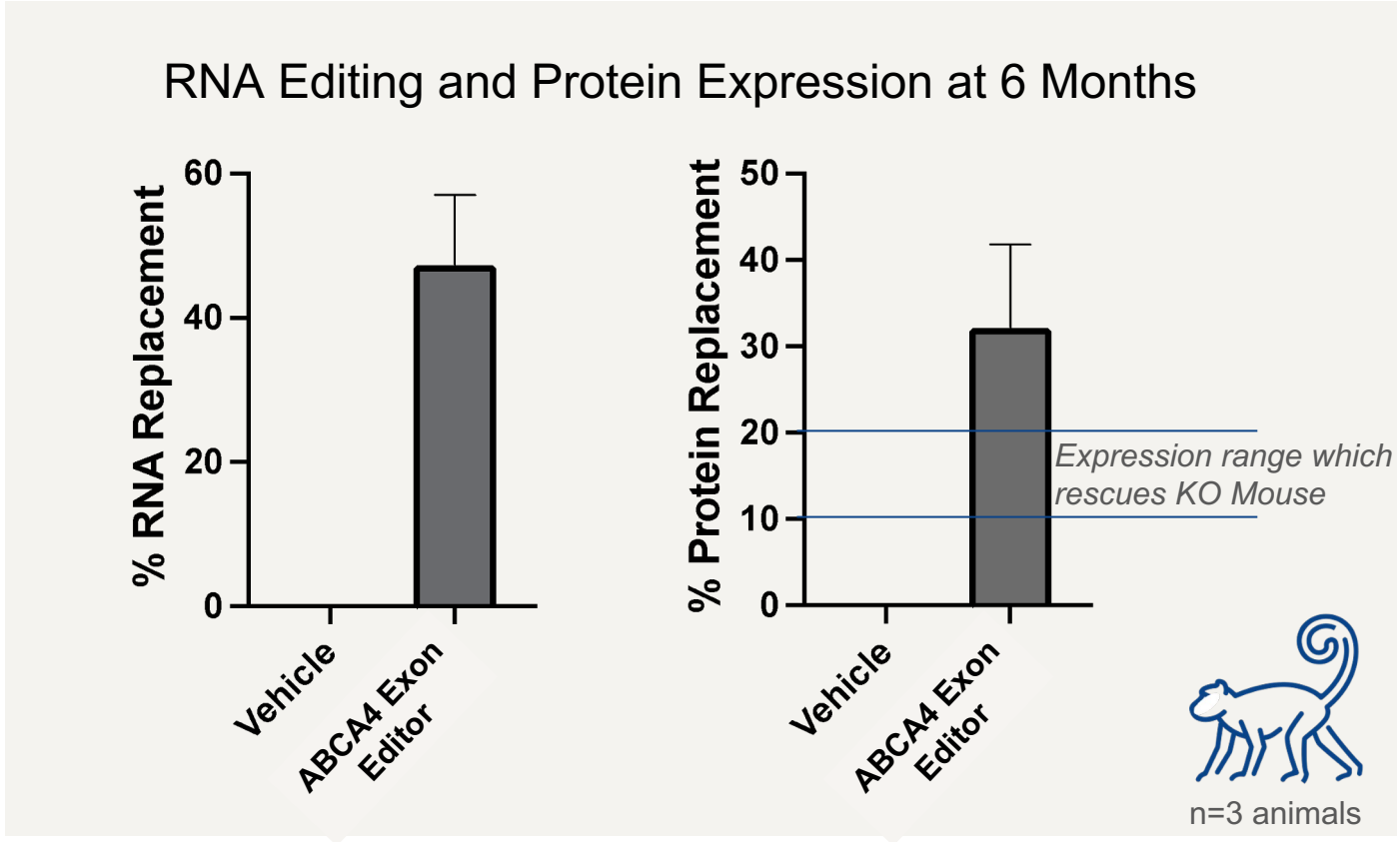
Durable RNA Exon Editing and Therapeutic Levels of ABCA4 Protein Confirmed with Development Candidate out to 6 Months

- Single sub-retinal administration of development candidate from CMC engineering run
- No prophylactic steroid regimen for investigative NHP studies
- Persistent expression and activity of ABCA4 exon editor at therapeutically relevant levels
- Full dose-response and GLP tox studies underway in preparation for IND filing



Treatment results in greater than 30% ABCA4 protein replacement in NHP at a well-tolerable dose

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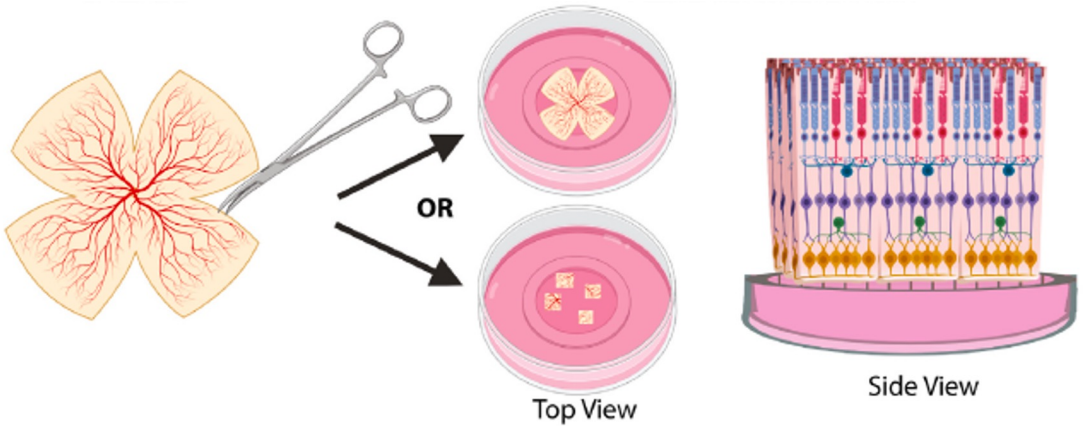
Robust ABCA4 RNA Exon Editing Achieved in Human Retinal Explants

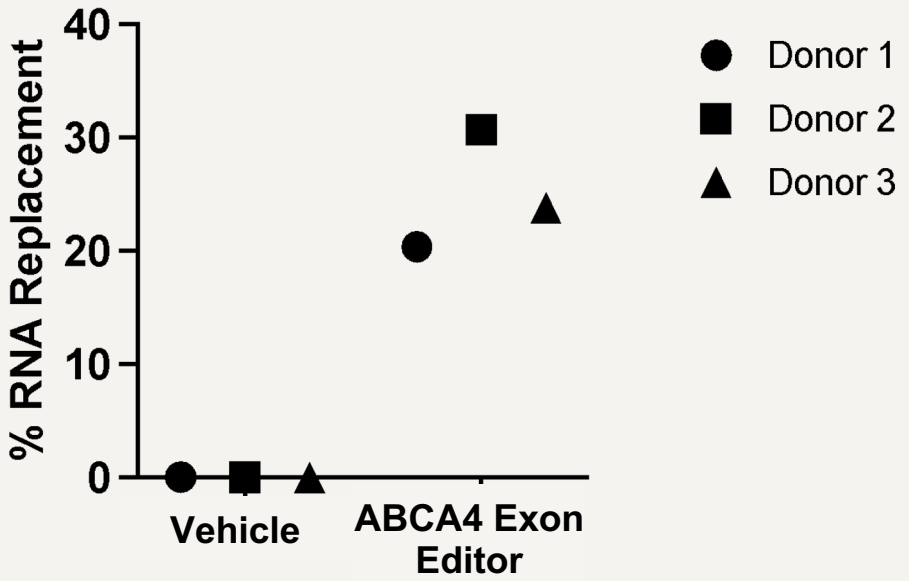
Lions Eye Institute
FOR TRANSPLANT & RESEARCH

Donor tissue (used for corneal transplant) enables testing in human photoreceptors



Modified from Eldred and Reh. *Dev Bio* (2021)

Three donor explants 21 days after ABCA4 Exon Editor treatment



First demonstration of therapeutically relevant levels of RNA exon editing reported in human photoreceptors

Lessons From ABCA4 Program Applied To Achieve Efficient Editing in Other Targets and Tissues

Intelligence from ABCA4

Knowledge Applied to Other Targets, includes:

- Efficiency-boosting elements (e.g., terminator sequence)
- NGS Intron Screening Capabilities
- Translatability to *in vivo* Studies
- Strategies to Minimize Off-Target Edits
- FDA Input on Off-Target Approach

Progress Across Targets

	Retinal *In Vitro*		CNS *In Vitro*				Other Tissue *In Vitro*	*Ex Vivo*	*In Vivo*	
	ABCA4 5' Editor in HEK Cells	Ocular Target 3' Editor in Mutant Cell Line	CNS "Target A" Editor in HEK Cells	CNS "Target A" Editor in Glioblastoma Cells	CNS "Target B" Editor in HEK Cells	CNS "Target C" Editor in HEK Cells	Other "Target D" Editor in HEK Cells	ABCA4 5' Editor in Human Explants	ABCA4 5' Editor in NHP	CNS "Target A" Editor in KO Mice
RNA Editing	✓	✓	✓	✓	✓	✓	✓	✓	✓	*In Progress*
Protein Rescue	✓	✓	✓	✓	✓	*In Progress*	*In Progress*	✓	✓	*In Progress*

Across multiple cell types, multiple genes, and multiple model systems, Ascidian can achieve therapeutically relevant levels of RNA exon editing and protein production

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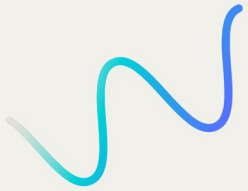
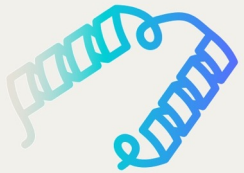
Summary of RNA Exon Editing Technology and Delivery



Edits RNA, not DNA

Edits whole exons, not only single bases



No foreign enzymes

Maintains native gene expression

Agnostic to delivery vehicle

- Clinically-validated subretinal AAV delivery enables the lead ABCA4 program
- Focal tissue delivery holds promise for many indications in the CNS and other organs
- Progress in AAV capsid and delivery device engineering is enabling broad biodistribution of innovative genetic medicines, including RNA Exon Editors and other novel payloads
- We are looking forward to the combination of novel payloads and next generation AAV capsids and other delivery technologies

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