Optimization of pre-mRNA exon editing for efficient rescue of protein expression

American Society for Gene and Cell Therapy, 2022 Annual Meeting abstract 829

Dr. Kirk Burkhart, Ph.D
May 18th, 2022

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Disclosure: Employee and shareholder of Ascidian Therapeutics

Gene therapy promises cures for many diseases, but has limitations

Limited cargo capacity of AAV

Over 300 disease genes with coding sequences > 4.7 kb that cannot be treated by traditional gene replacement

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Exon editing has the potential to overcome these limitations

Exon editing offers solutions to gene therapy limitations

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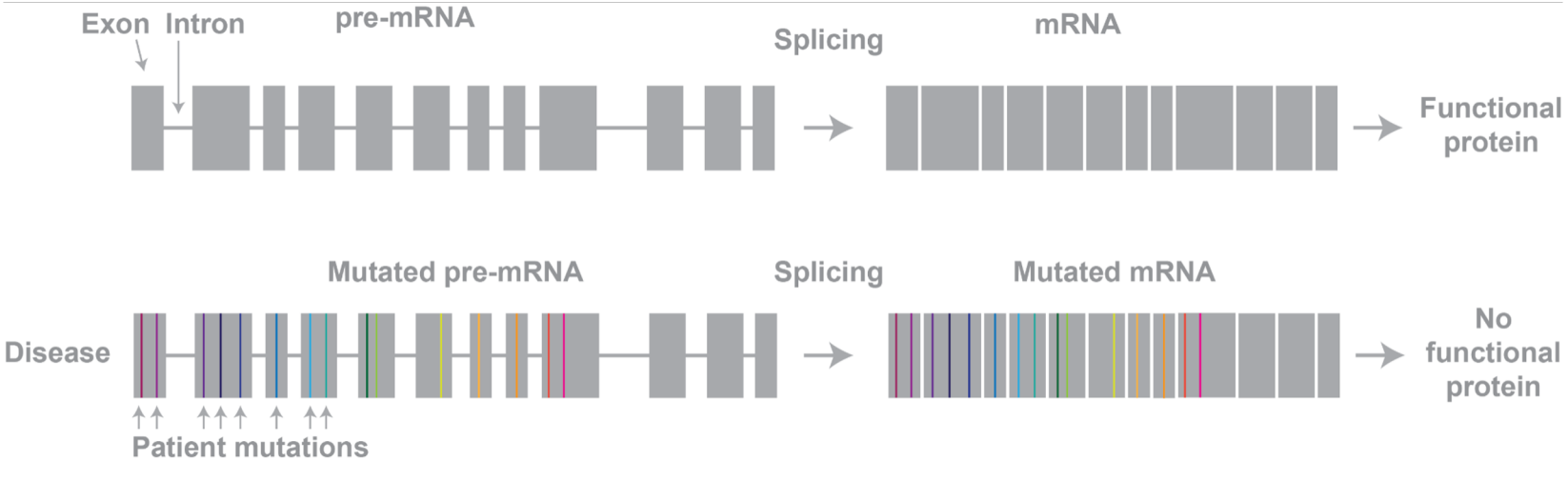
Exon editing offers solutions to gene therapy limitations



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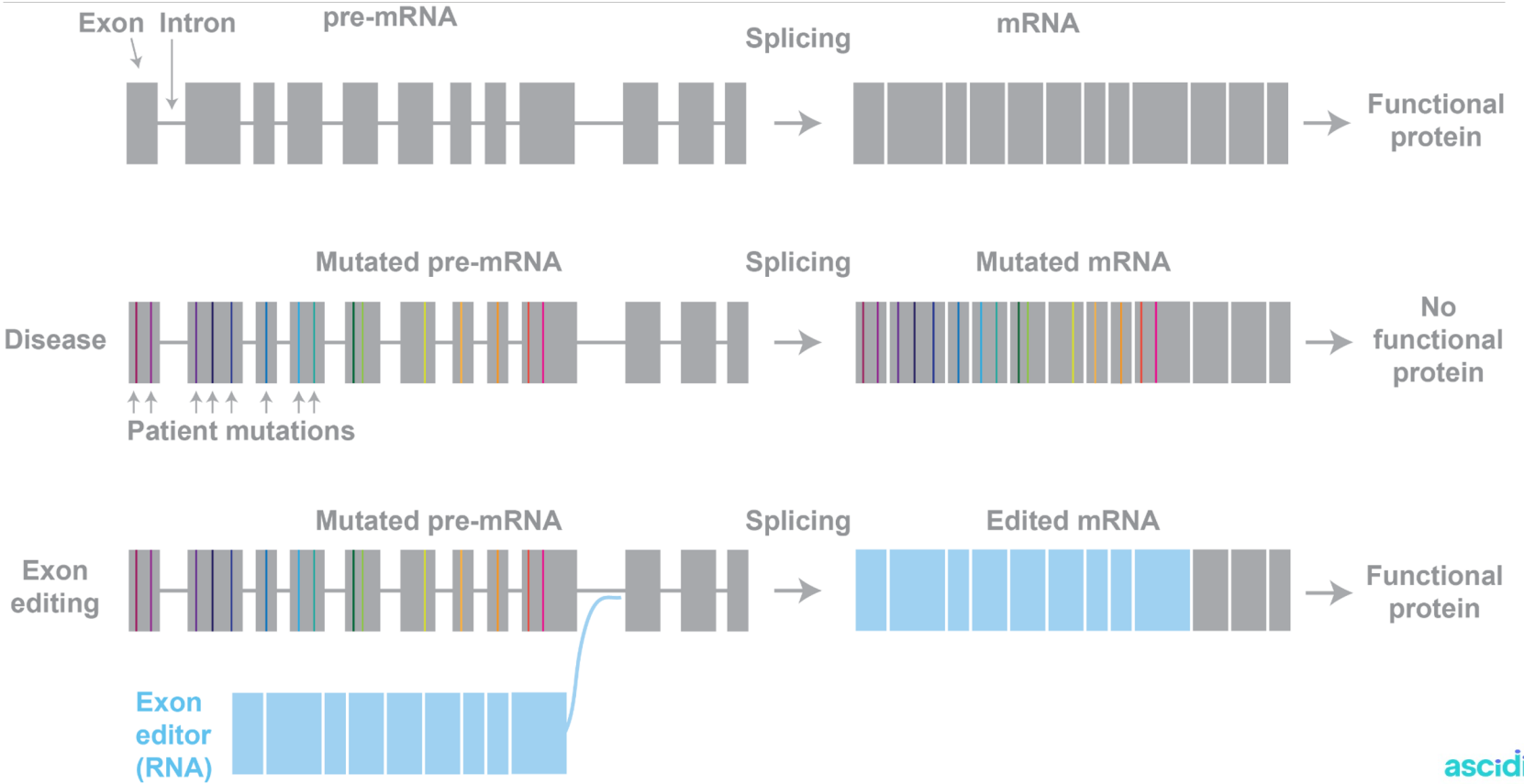
Exon editing offers solutions to gene therapy limitations



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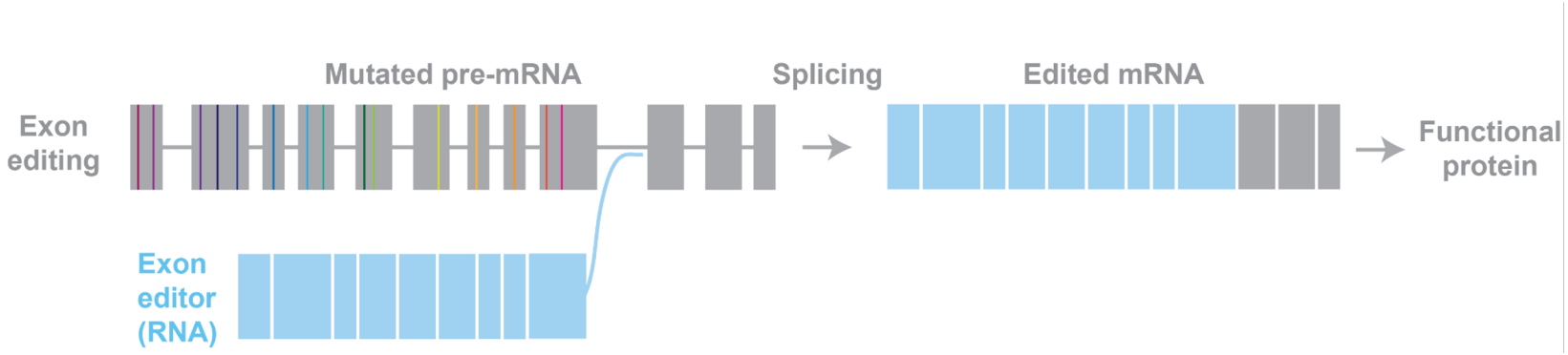

Limited cargo capacity of AAV

Only need to replace mutated exons

Diverse spectrum of patient mutations

Single exon editor can replace multiple exons

Difficulty controlling gene expression in different cell types

Expression level and cell-type specificity are determined by the target

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Early pre-mRNA trans-splicing work had important limitations

- Editors tested in artificial context
- Efficiency challenges
- Limited *in vivo* studies

Disease	Gene	Date(s) of report
Duchenne muscular dystrophy	*DMD*	2007, 2010
Dystrophic epidermolysis bullosa	*COL7A1, K14*	2007, 2013
Huntington's disease	*HTT*	2012, 2017
Cystic fibrosis	*CFTR*	2001, 2002, 2007
Spinal muscular atrophy	*SMN2*	2003, 2013, 2014
Dysferlinopathies / Titinopathies	*DYSF / TTN*	2005
Retinitis pigmentosa	*RHO*	2008
X-linked immunodeficiency with hyper-IgM	*CD40L*	2004

Berger A, Maire S, Gaillard MC, Sahel JA, Hantraye P, Bemelmans AP. mRNA trans-splicing in gene therapy for genetic diseases. *Wiley Interdiscip Rev RNA*. 2016

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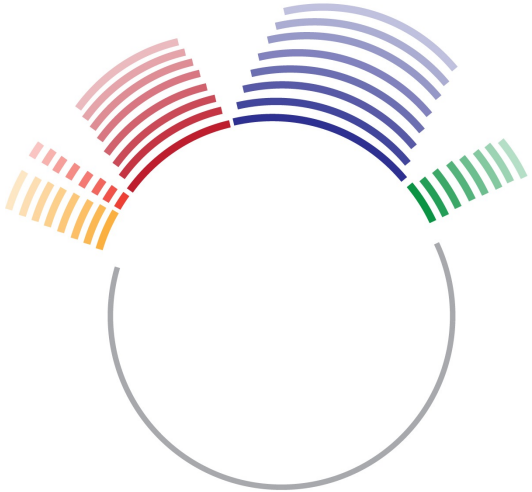
How can we make improved exon editors?

Berger A, Maire S, Gaillard MC, Sahel JA, Hantraye P, Bemelmans AP. mRNA trans-splicing in gene therapy for genetic diseases. *Wiley Interdiscip Rev RNA*. 2016

Advances in synthetic biology and next generation sequencing enable a high-throughput exon editing assay

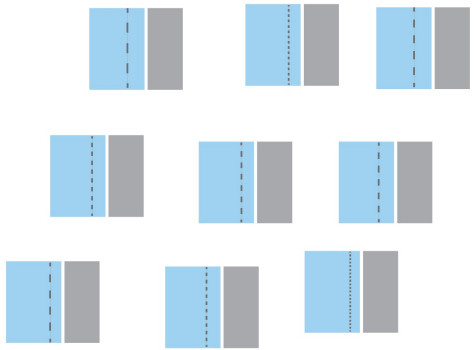
Building exon editors



Exon editor plasmid library

Testing exon editors

Edited mRNAs
(each with different barcode)



Barcoded RNA-seq

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Retinal disease gene ABCA4 excellent candidate for exon editing

- Mutations in *ABCA4* cause retinal degeneration and progressive vision loss
- The CDS of *ABCA4* (6.8kb) exceeds the AAV capacity
- Mutations are distributed throughout the gene

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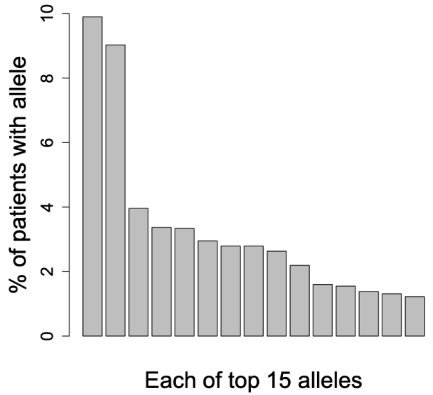

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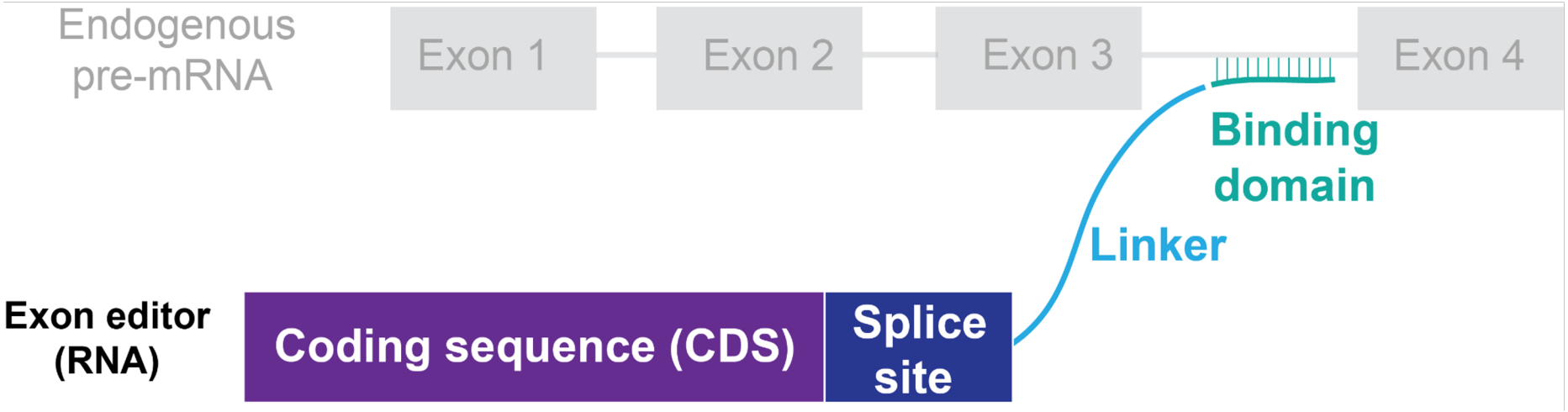
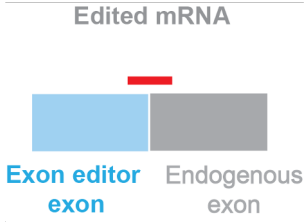
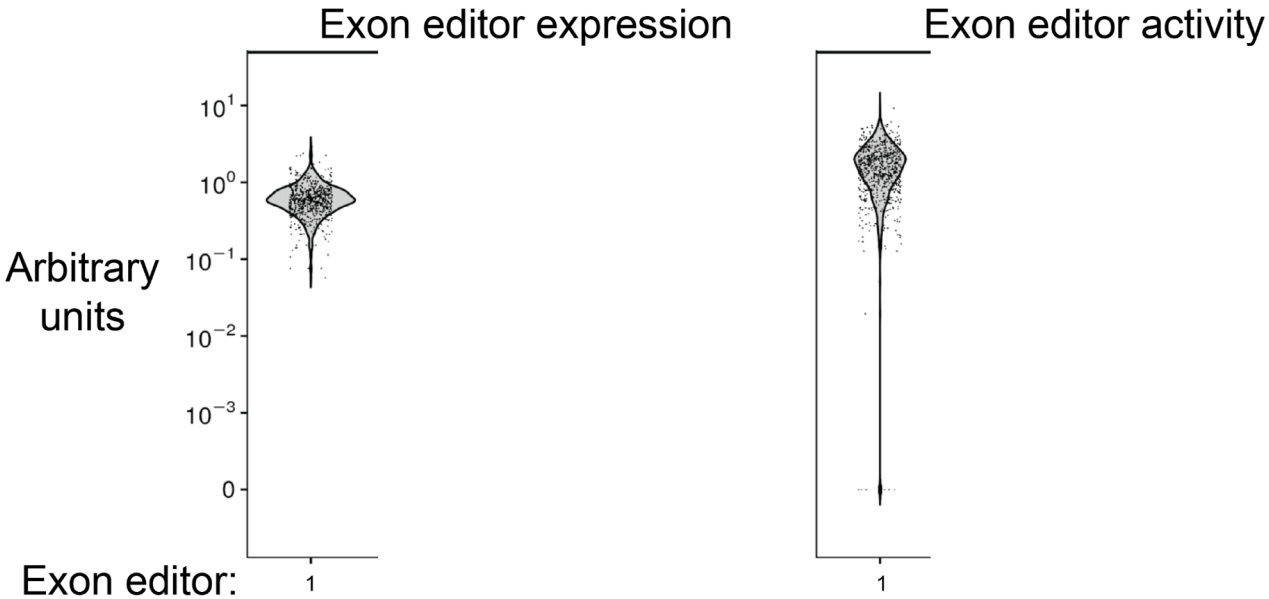
Essential components of an exon editor: Coding domain sequence, splice site, linker and binding domain



Establishing the screening engine: a high-throughput exon editing assay





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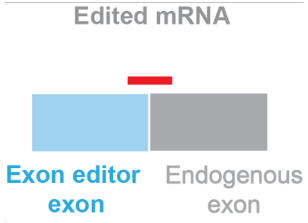

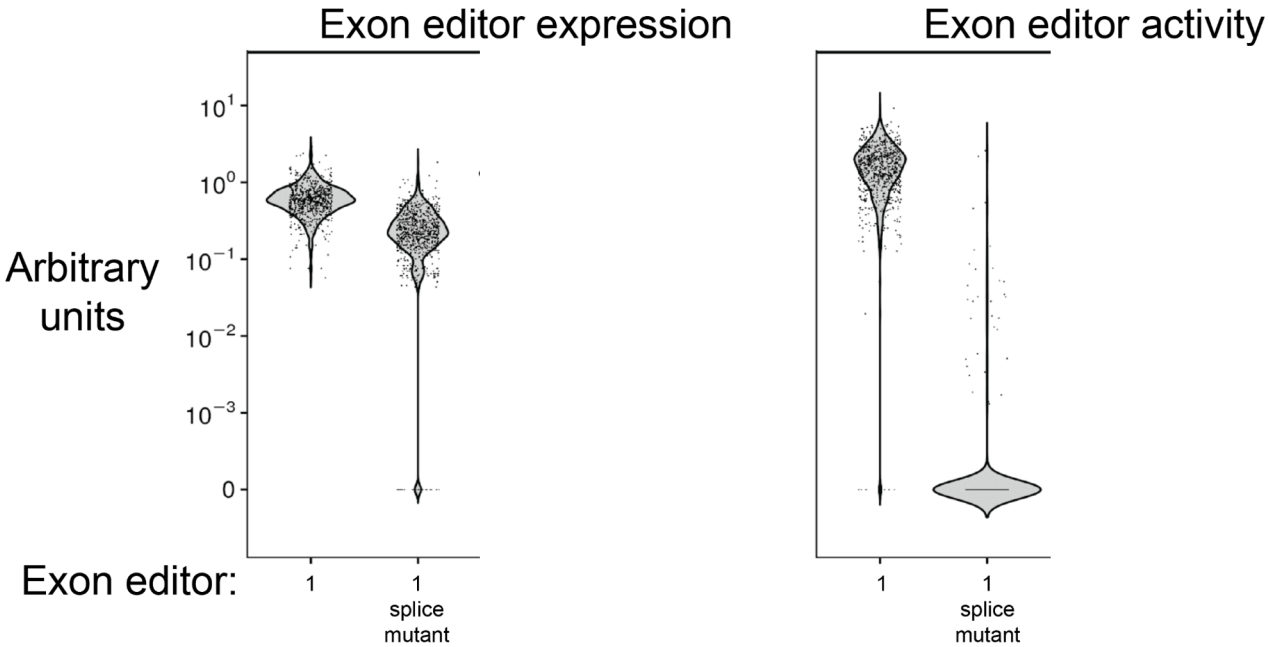

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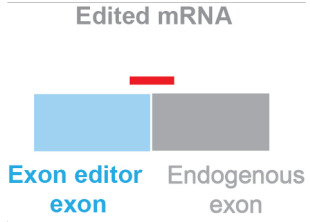

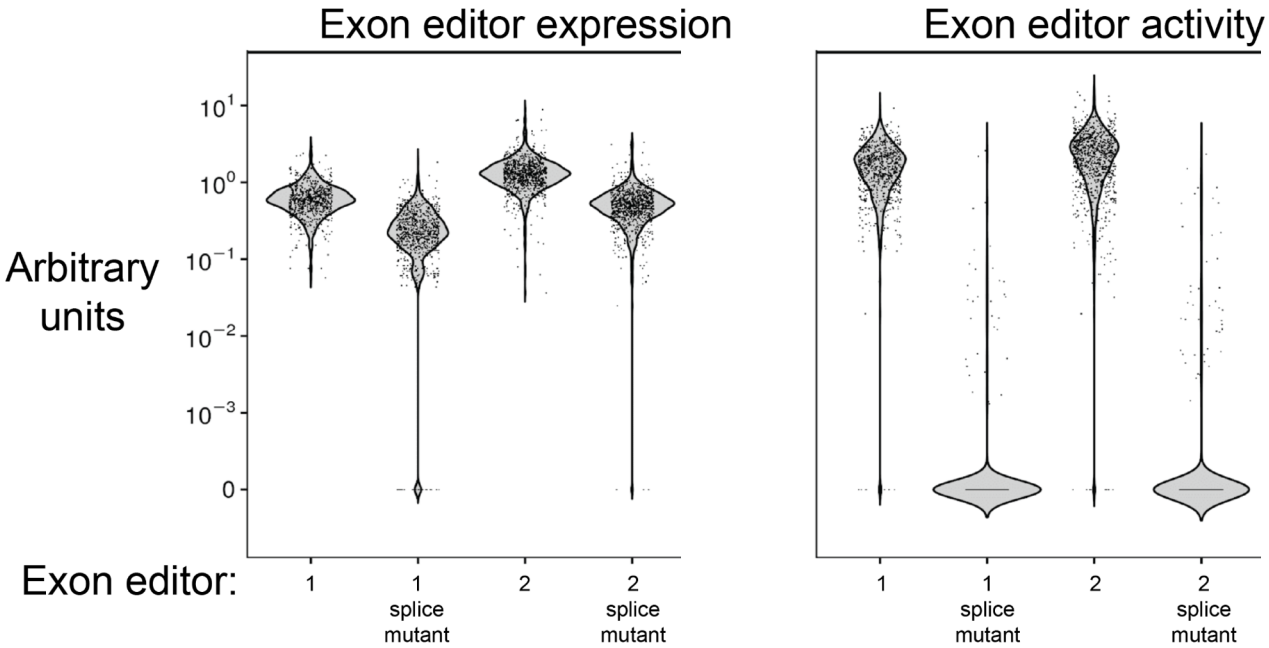

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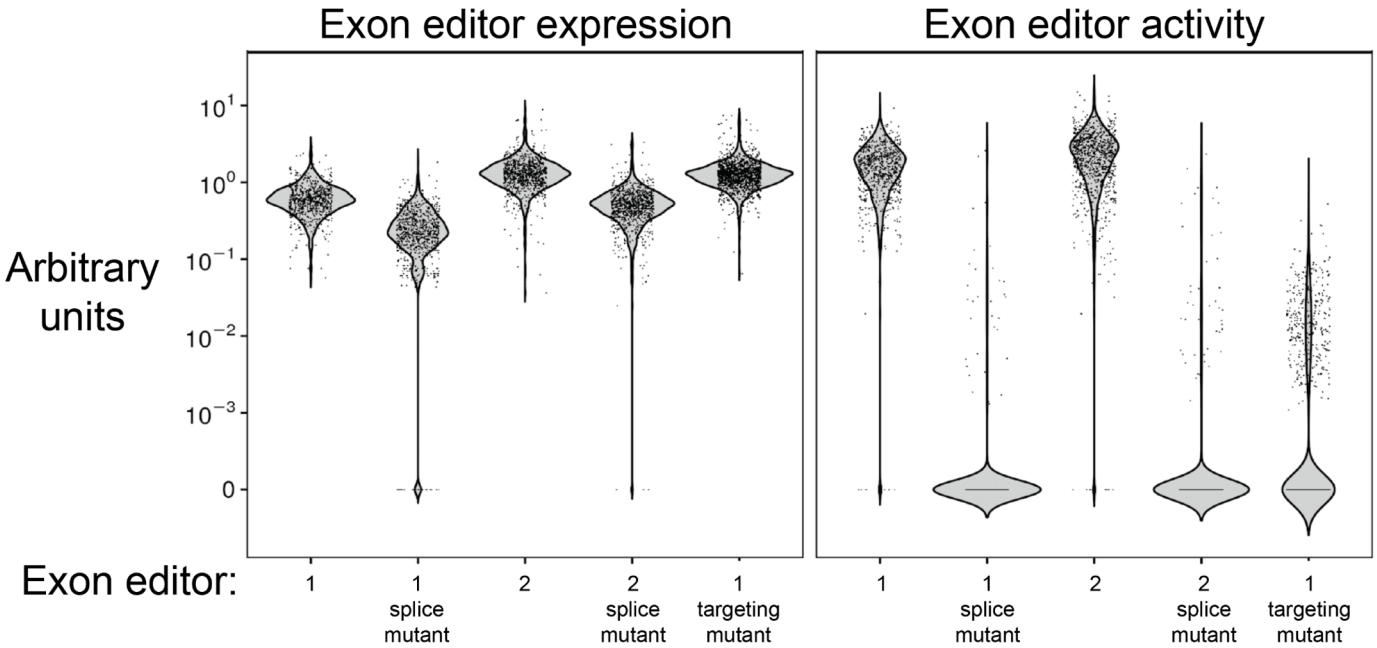

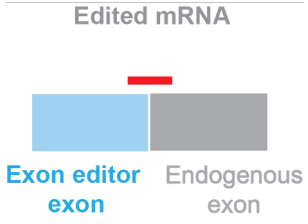

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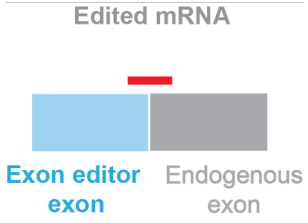

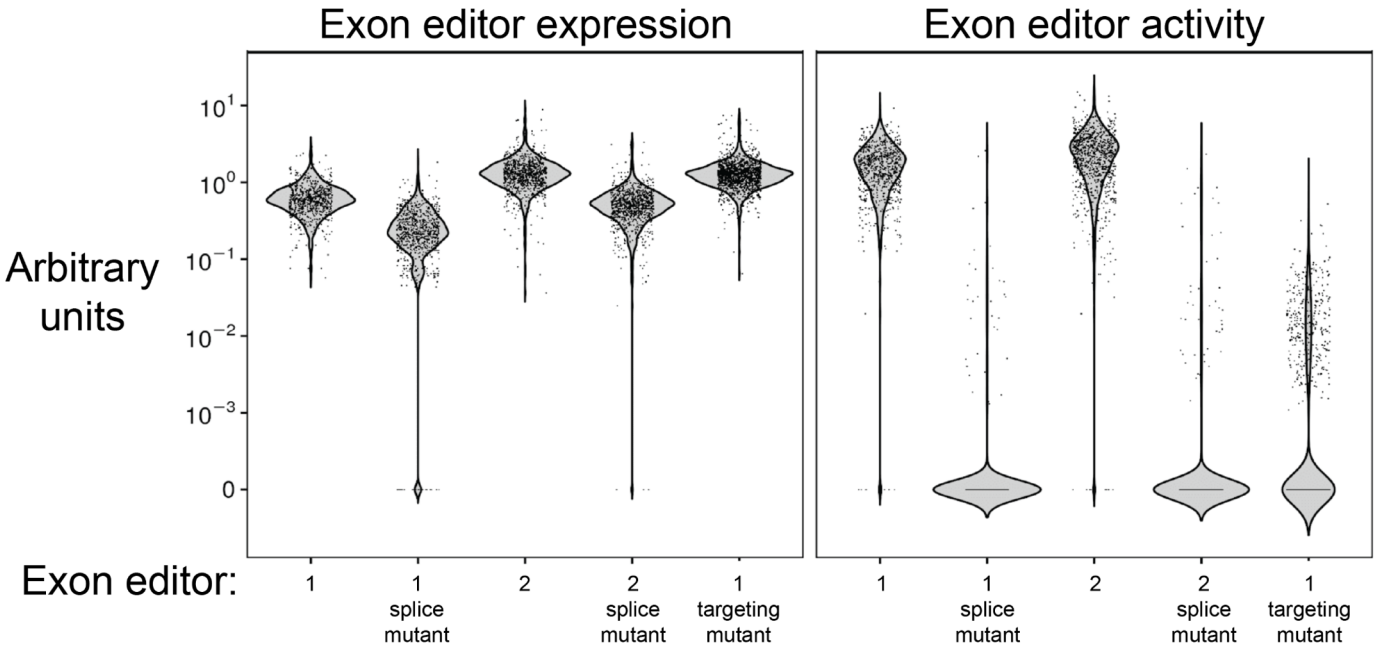

Libraries of thousands of exon editors assayed in a single experiment

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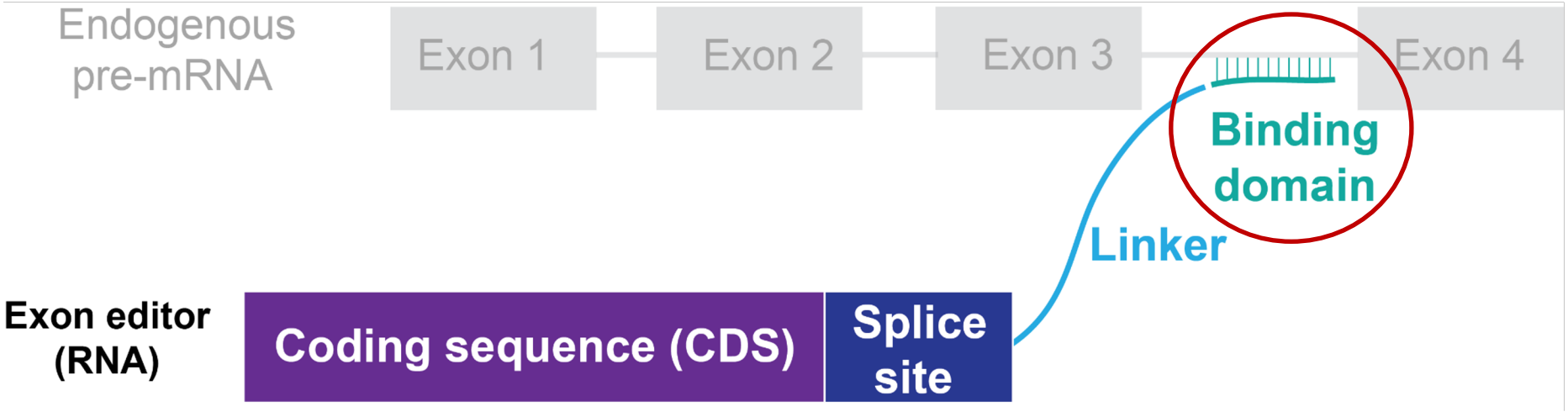
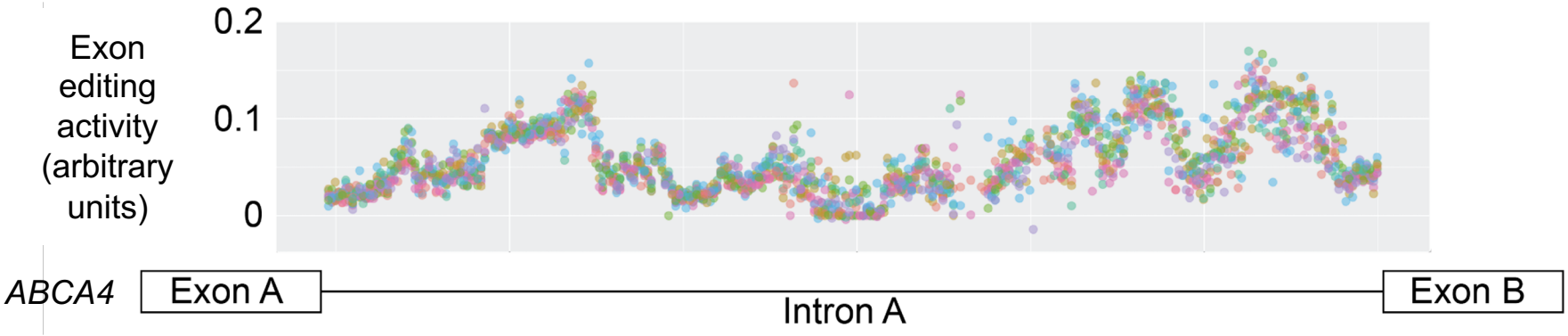
Base pairing between the binding domain and target intron recruits the exon editor to the target



Targeting different positions along an intron reveals the best binding domains



- >2,000 exon editors
- Tiled every 5 nt along the intron

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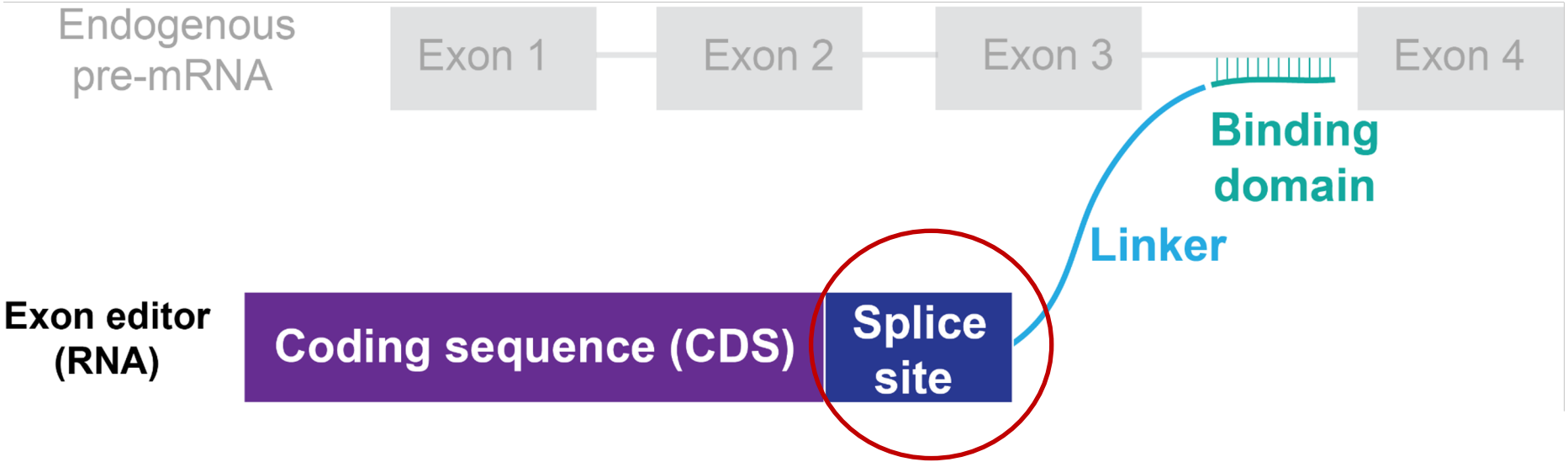
The splice site of the exon editor recruits the spliceosome and is essential for exon editing



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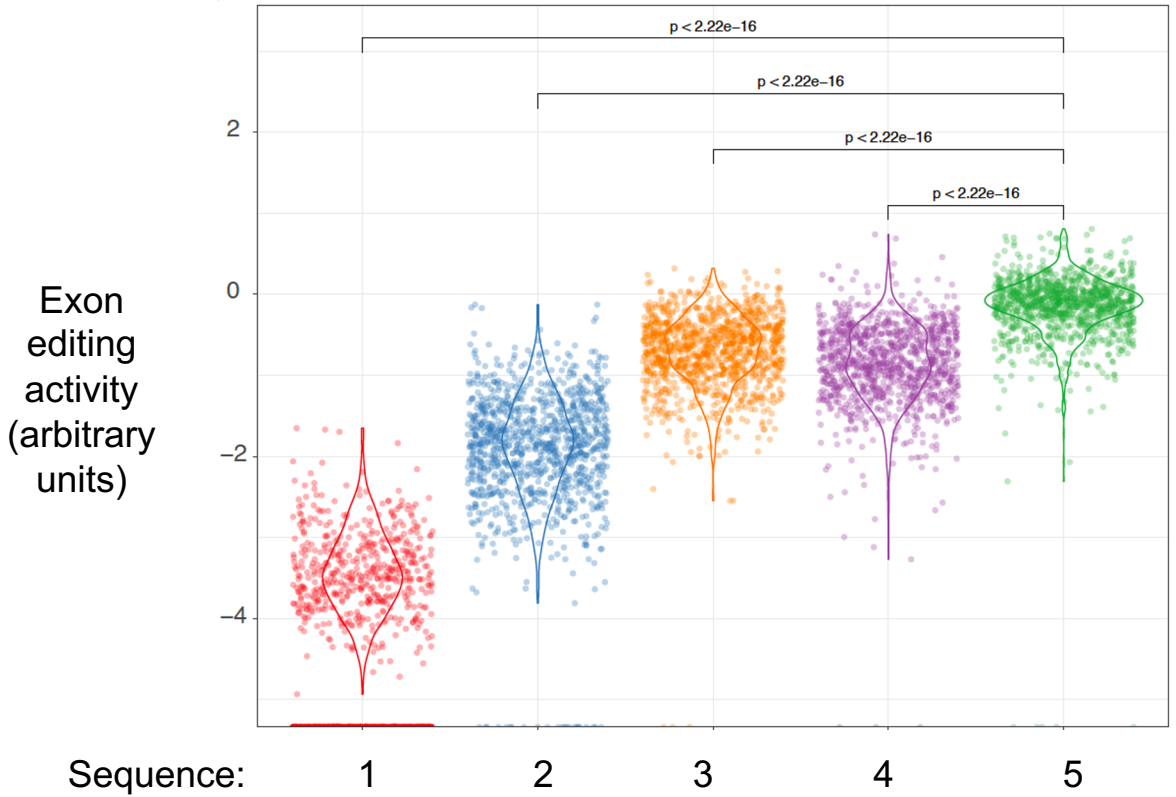
The splice site is critical for exon editor activity

- 5 different splice sites
- 550 linkers



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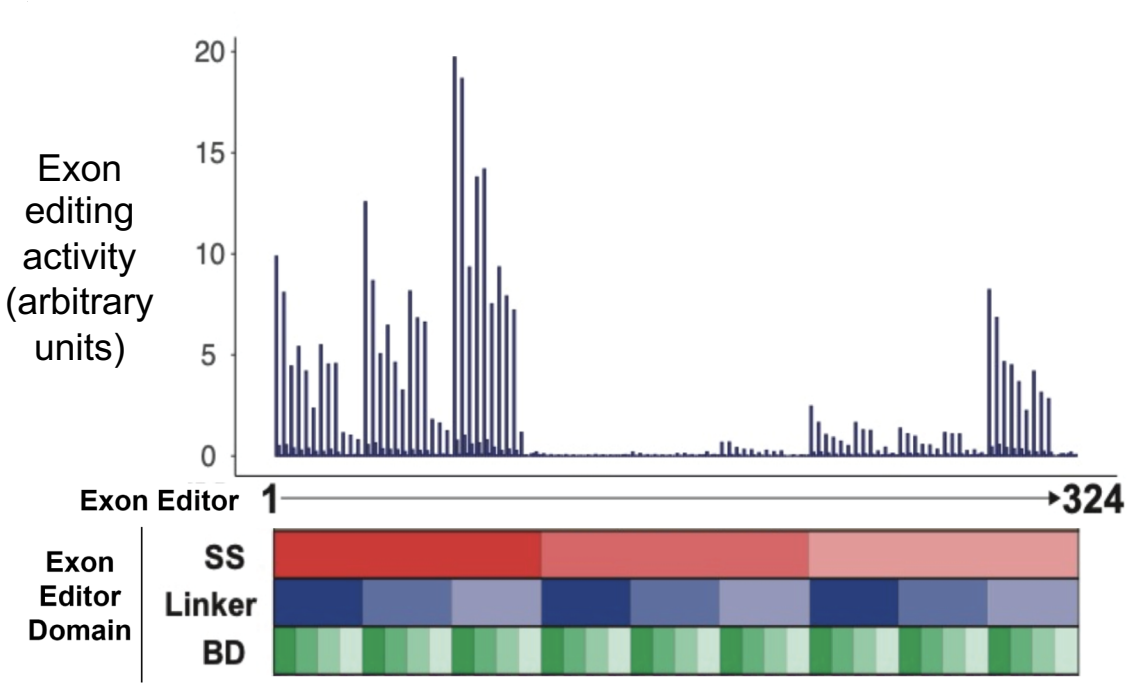
Testing optimized components in combination identifies highest performing editors



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What percent of *ABCA4* is replaced by our exon editors?

$$\frac{\text{Exon-edited } \textit{ABCA4} \text{ mRNA}}{\text{Total } \textit{ABCA4} \text{ mRNA}} \times 100$$

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What percent of *ABCA4* is replaced by our exon editors?

- AAV transduced exon editors in to cultured human cells

- qPCR to quantify copy number of mRNA

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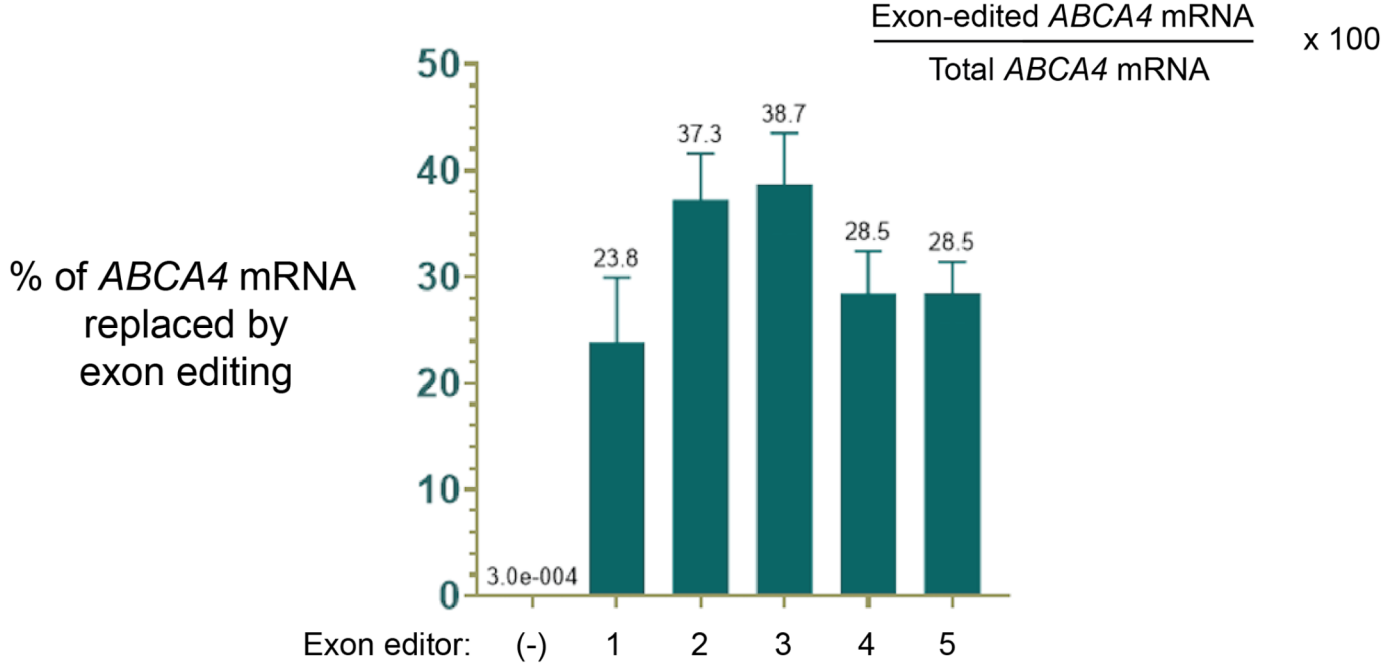

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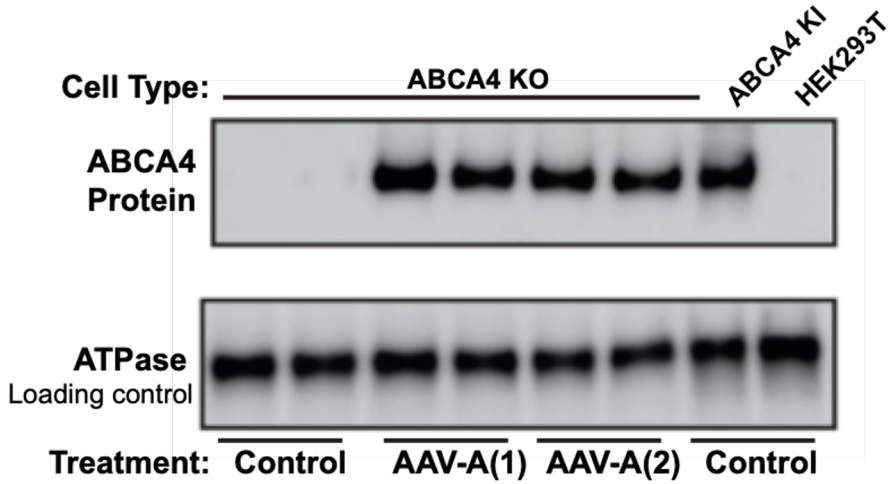
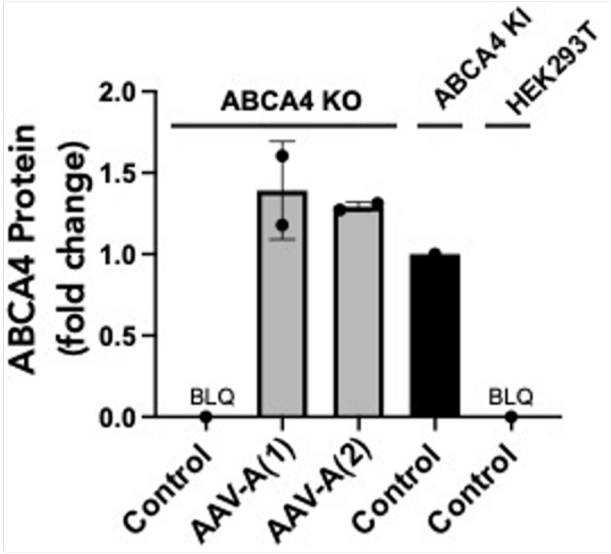
Edited mRNA restores wild-type ABCA4 protein expression in knock-out cells *in vitro*





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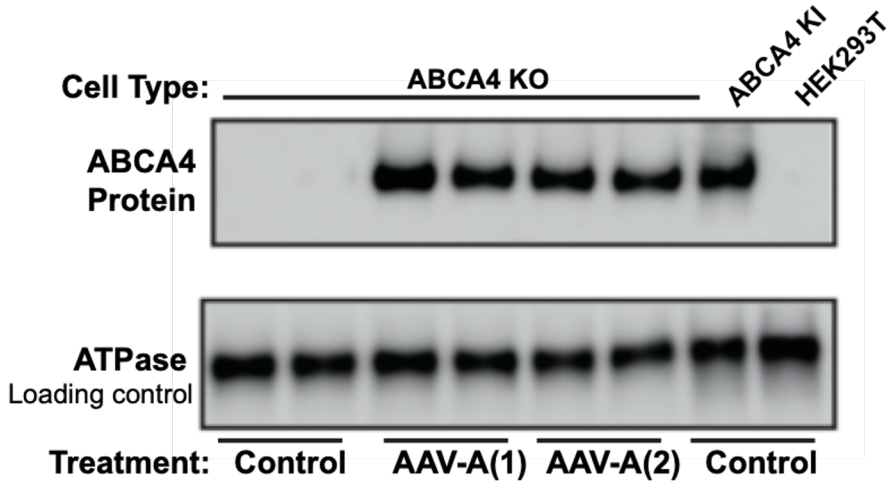
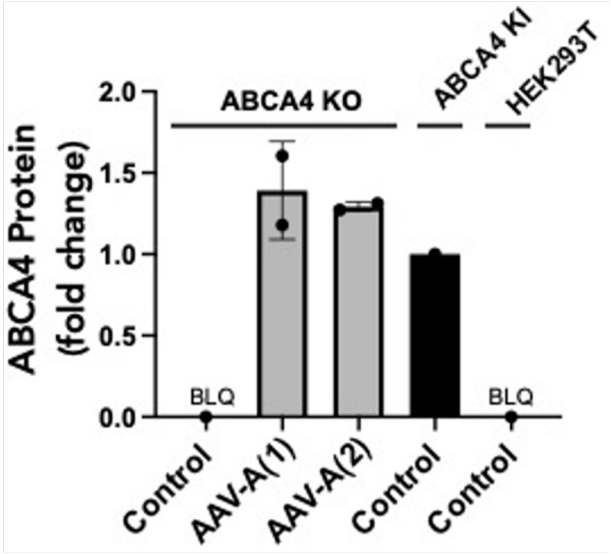
Edited mRNA fully restores wild-type ABCA4 protein expression in knock-out cells *in vitro*





Full rescue of protein expression in knock-out cells

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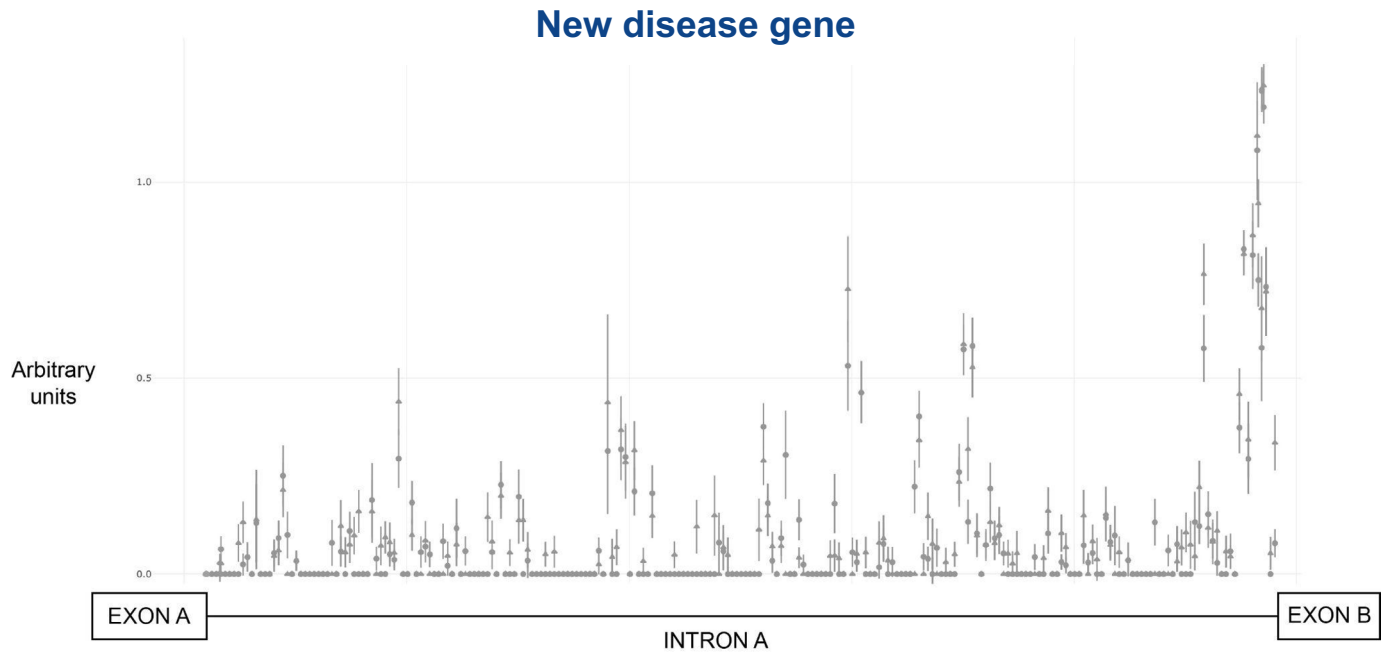
Summary

- Ascidian has established the first high throughput RNA exon editing Screening Engine
  - RNAseq-based assay capable of testing thousands of exon editors in a single experiment
- *ABCA4* is an excellent candidate for initial testing of this engine:
  - Too large to fit into AAV capsid envelope
  - High mutational variance
  - Significant area of unmet medical need
- *ABCA4* exon editors were optimized via iterative testing in the screening engine
- Optimized *ABCA4* exon editors have achieved potentially therapeutic levels of efficiency
- Further development of exon editing therapeutics is warranted by these results

Expanding exon editing to other genes …



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Acknowledgements

Kelly Becker
Rebekka Krumbach
John Bachmann
Bennett Bremer
Eric Carlin
Adam Clemens
Tayla Cormier
Conor Delaney

Akiko Doi
Lai Mung Fong
Kirk Gosik
Yevgenia Khodor
Xiaojin Liu
Savita Matapurkar
Julia Meredith
Akiko Noma
Anika Pavis
Lingtao Peng

Madison Stulir
Dennis Wu
Qin Yu
Laura Barrio Real
Scott Dooley
Gabrielle Hillman
Krishna Jawaharial
Linda Liu
Bruce C. Schnepp

Jasmine Shong
Philip R. Johnson
Robert D. Bell
Carrie Wager
Crystal Shih Byers
Shimyn Slomovic
Jesse Gray
Daniel Rosan
Michael D. Ehlers

Abstract 300

Dr. Rebekka Krumbach

Evaluation of ABCA4 RNA Exon Editing and Replacement in Non-Human Primate

Monday (5/16) 5:30 - 6:30PM

Abstract 1217

Dr. Robert Bell

A Large-Scale Exon Editing Solution for Treating Genetic and Complex Disorders

Thursday (5/19) 10:45AM

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