Scientific Poster Session

n-lorem FOUNDATION

Nano-rare Patient Colloquium 2025

Monday, October 20 | 5:15 – 6:15 pm EST

Tackling RNA-caused Diseases: A Focus on RNU4-2

Mutations in U-rich RNAs are increasingly recognized as drivers of neurodevelopmental disorders. Although their short length and structured nature presents challenges, ASO technology offers a unique therapeutic opportunity. This work focuses on a recently identified mutation in RNU4-2, highlighting its functional characterization and n-Lorem's therapeutic strategy to address its toxic effects.

Wei Zhang, Ph.D.

Associate Director, Core Research, n-Lorem

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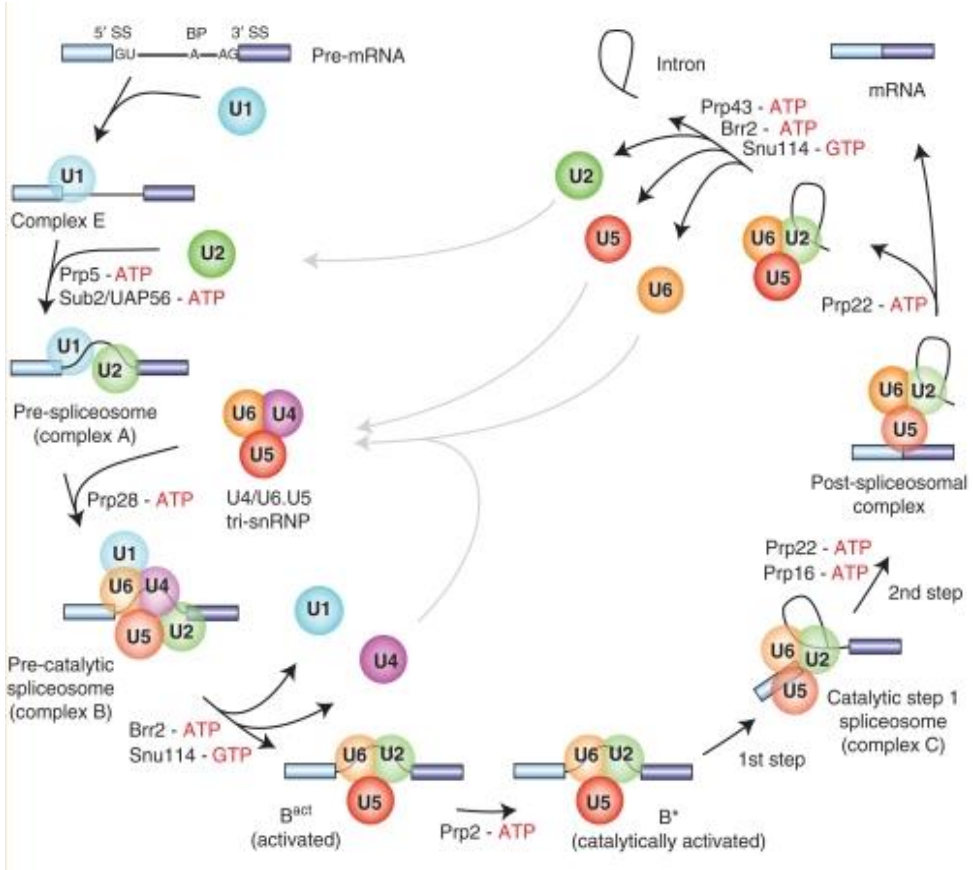
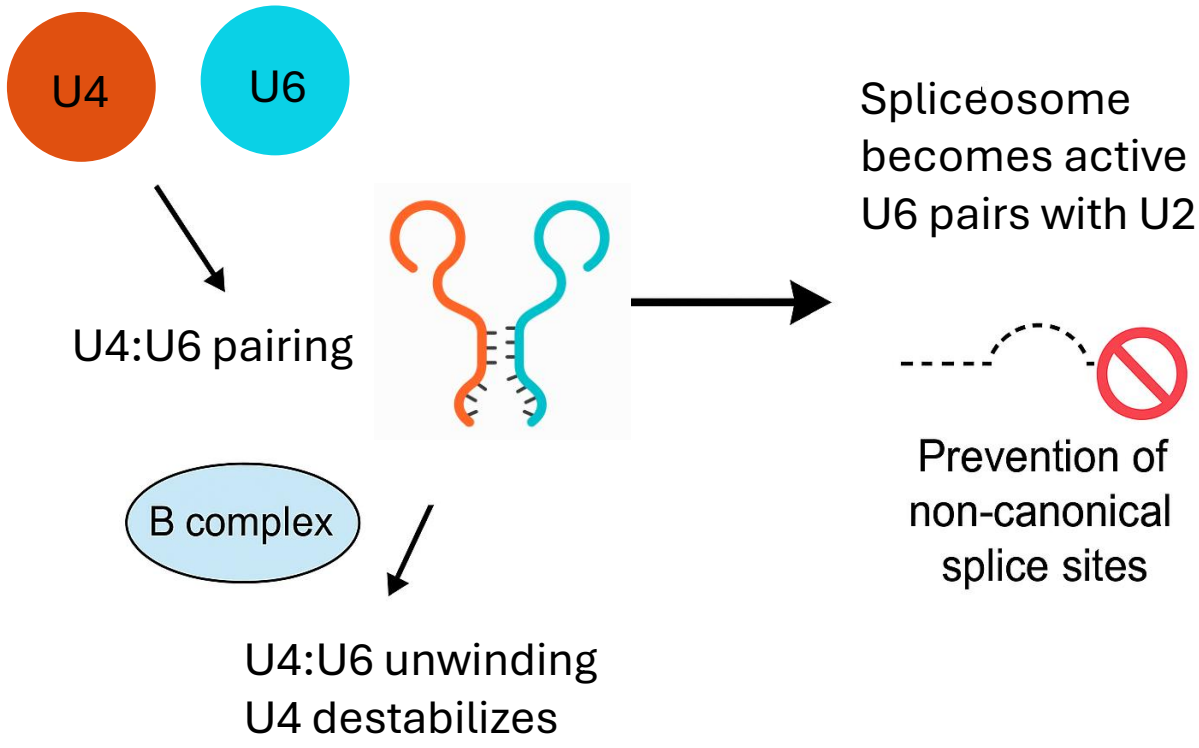
Biogen

Introduction: Splicing is a crucial process



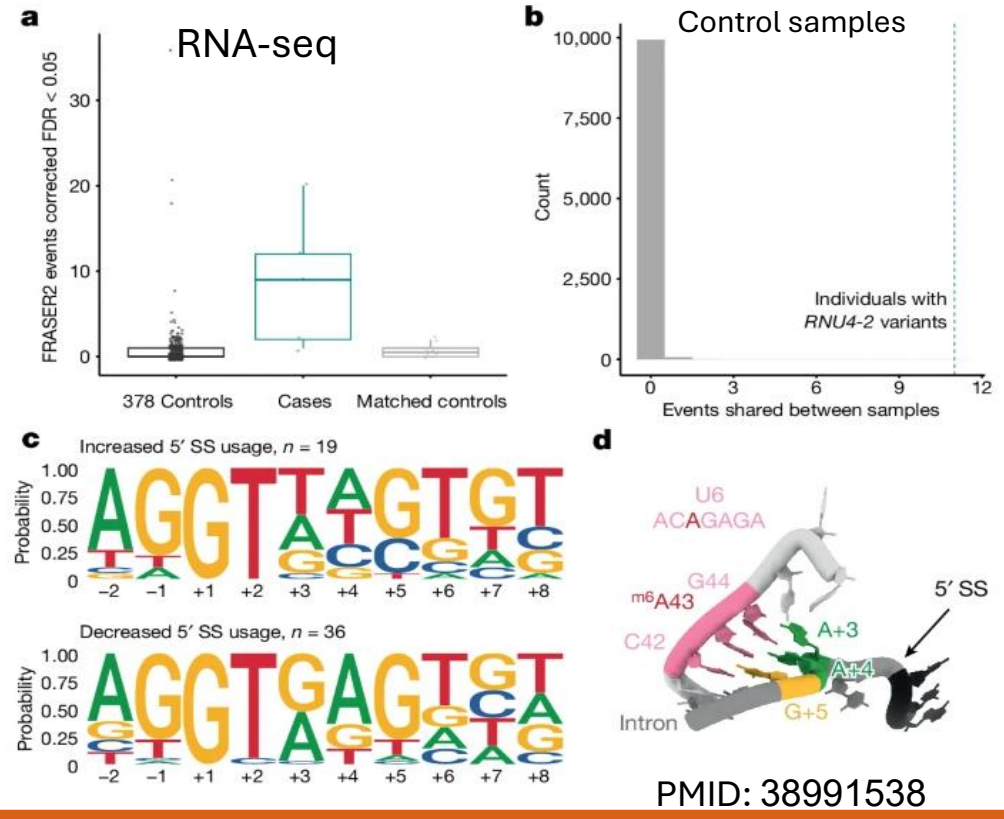
- Splicing is **very important and very well-understood**
- snRNAs are a subcategory of non-coding RNAs that are key components of the spliceosome.
- They are means of **Quality Control** as they ensure proper splicing
- The spliceosome assembly: Five snRNPs (U1, U2, U4, U5 and U6) mainly involved each containing a unique snRNA, and a total of approximately 100 additional proteins

RNU4-2 is a Tiny RNA with a Big Role



- Assembly of the major spliceosome is initiated by binding of U1 and U2 snRNPs to the pre-mRNA strand.
- The interaction between **U4 and U6 snRNAs is crucial for stability of the complex and formation of active spliceosome**
- **Disassembly of the U4–U6 duplex** + pairing with **U2 snRNP at the 3' splice site = complete removal of the intron**
- **Ensures spliceosome recognition fidelity**

RNU4-2 n.64_65insT Variant Disrupts the 5’ Splice Site Usage



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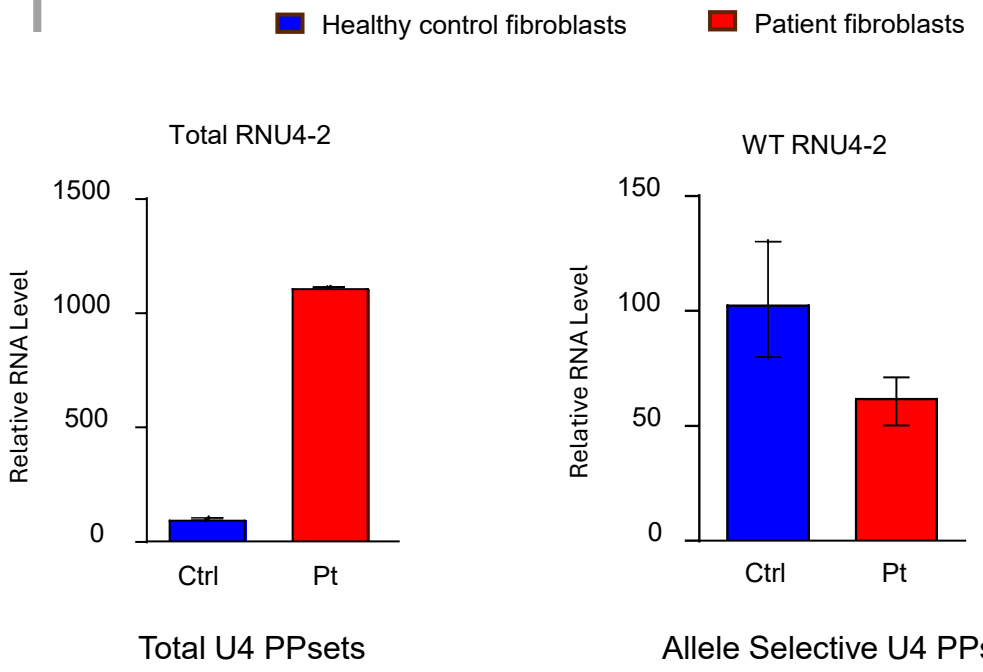
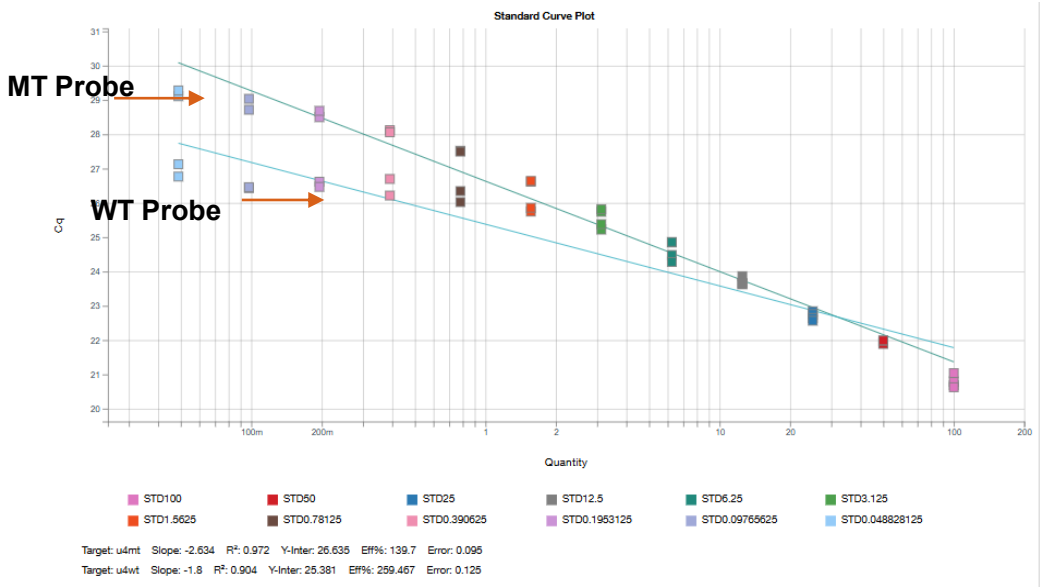
- Consistent with the importance of the critical region in **5′ splice-site recognition**, the most pronounced difference was observed for **abnormal splicing** events corresponding to **increased use of unannotated 5′ splice sites**
- **5** of the genes implicated in the 12 shared events are in the Developmental Disorders Genotype-to-Phenotype (DDG2P) database21 and/or were **associated with NDD** in a previous large-scale analysis (NDUFV1, H2AC6, JMJD1C, MAP4K4 and SF1)
- We have a clear read-out

Indication that RNU4-2 mutation is a TGOF

Mutant RNU4-2 is 20 Times More Abundant Than Wild-type



RT-qPCR using allele-specific probes showed significantly earlier Cq values for the mutant probe -> higher abundance



Mutant RNU4-2 (MT-U4) is approximately 20 times more abundant than WT, despite being transcribed from only one allele

Indication that RNU4-2 mutation is a TGOF

Overexpression of RNU4-2 n.64_65insT variant increased cell proliferation but didn't affect cell viability

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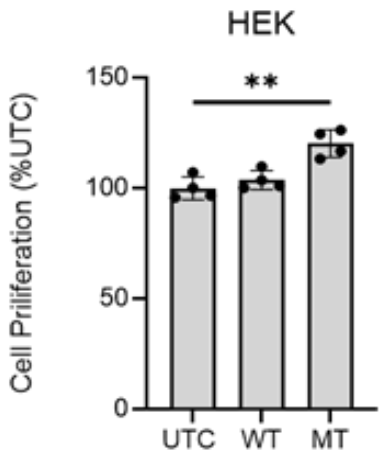

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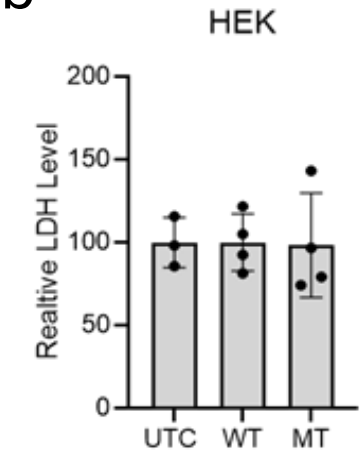

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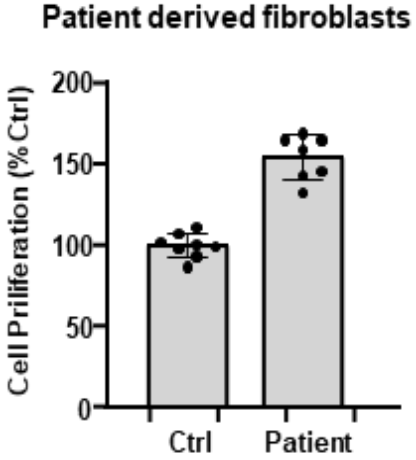

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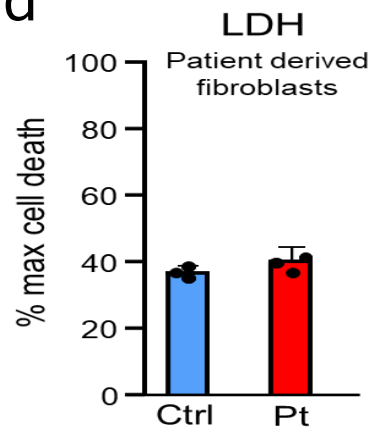
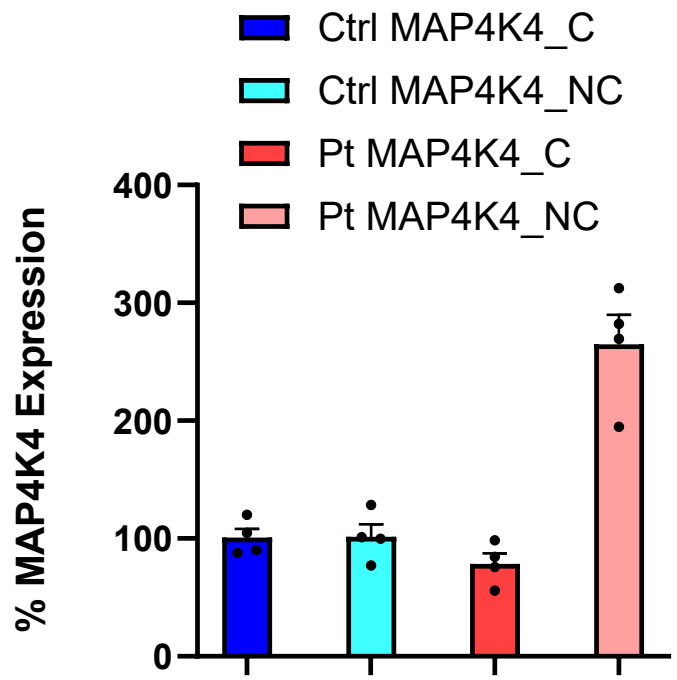
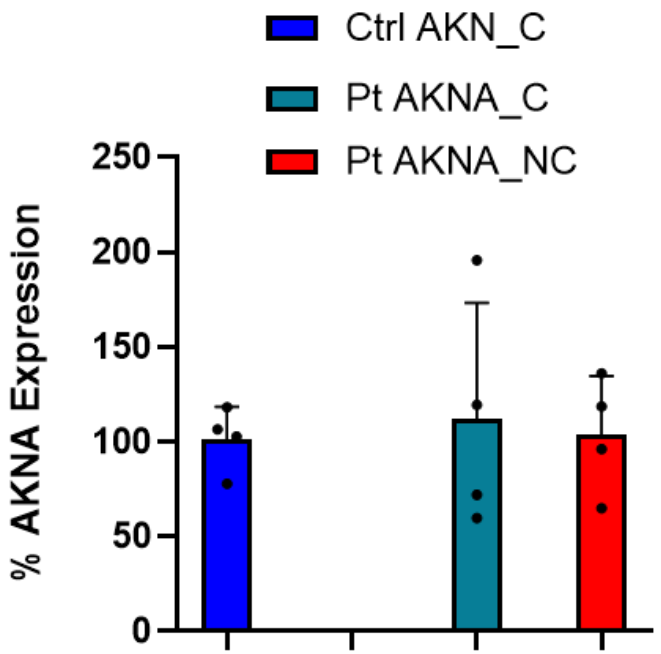
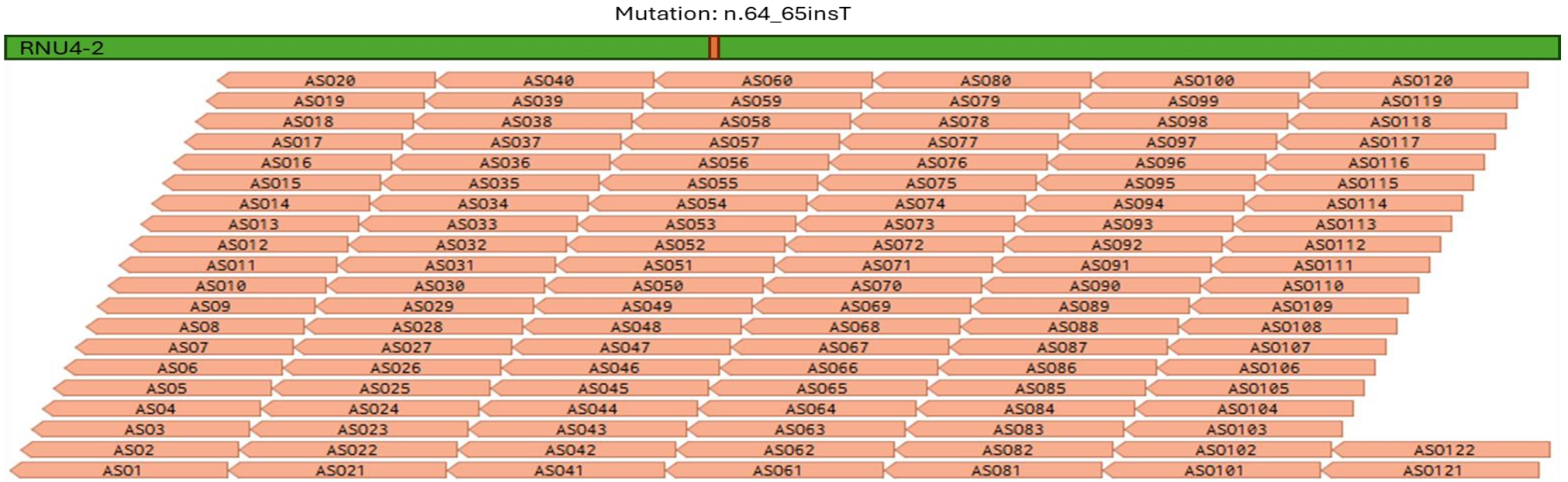


RNU4-2 n.64_65insT variant increased non-canonical NDD related transcripts





Allele-selective ASOs were designed and screened



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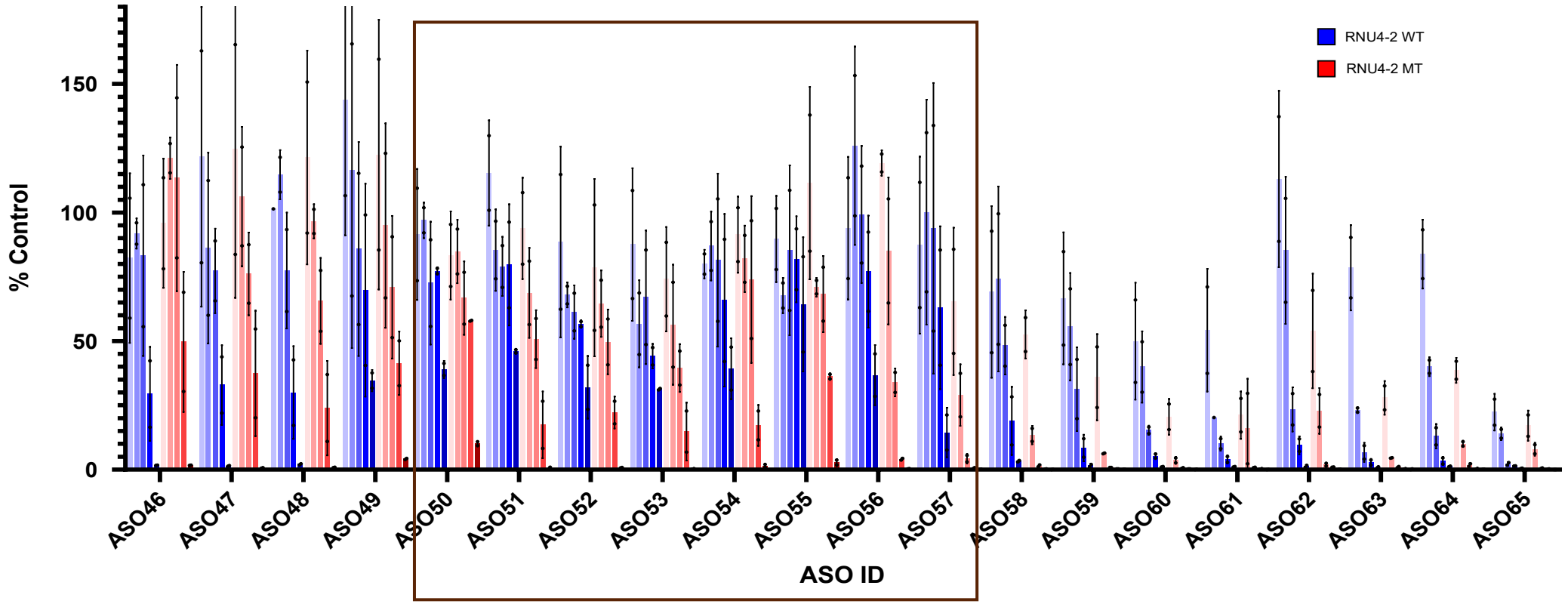
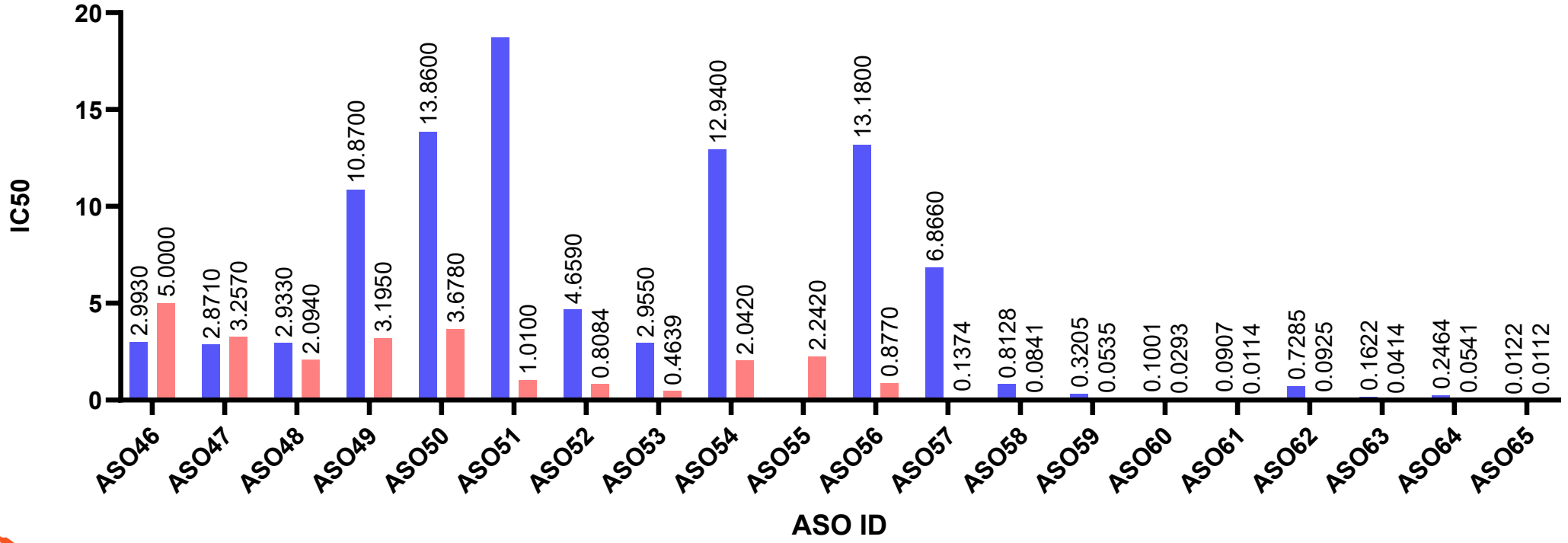
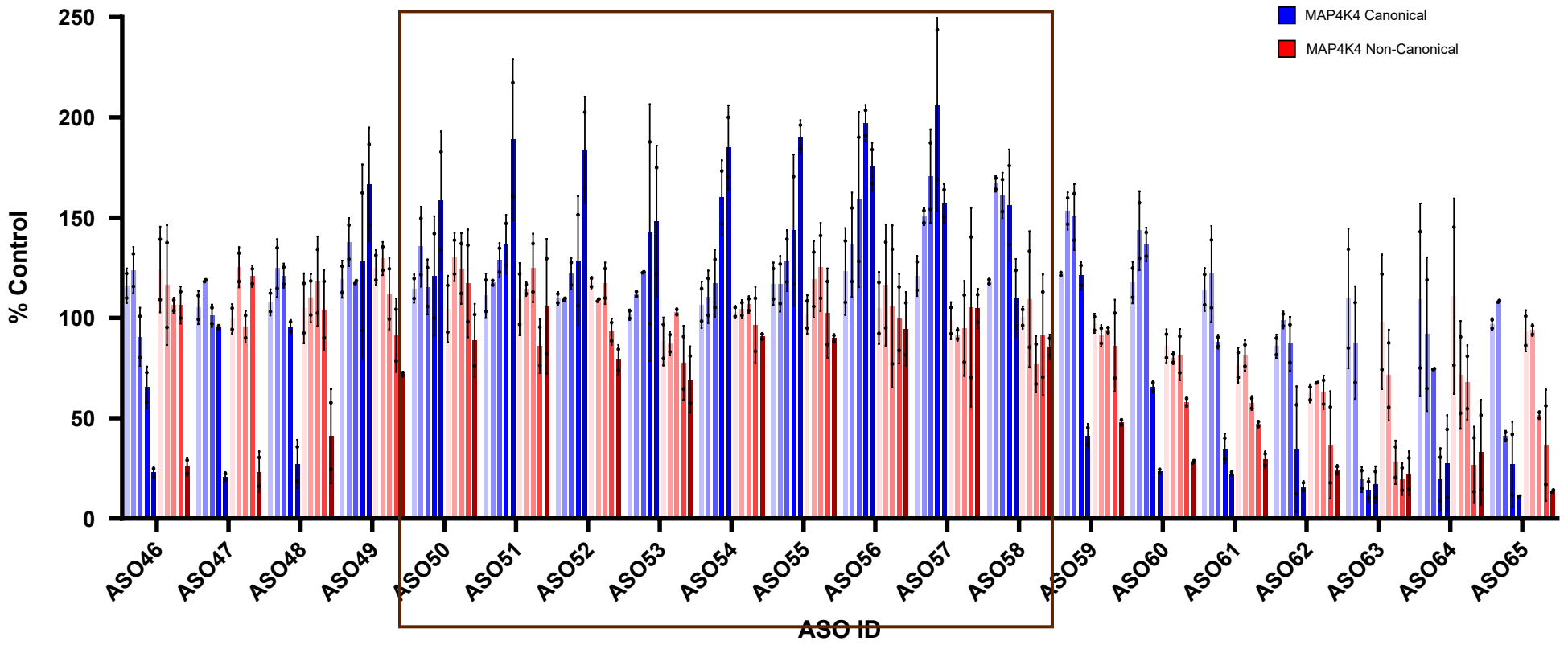


Several potent and selective ASOs



Allele selective ASOs selectively reduced non-canonical 5’ splicing induced by RNU4-2 mutation



Allele-selective ASOs showed no cytotoxicity on cells

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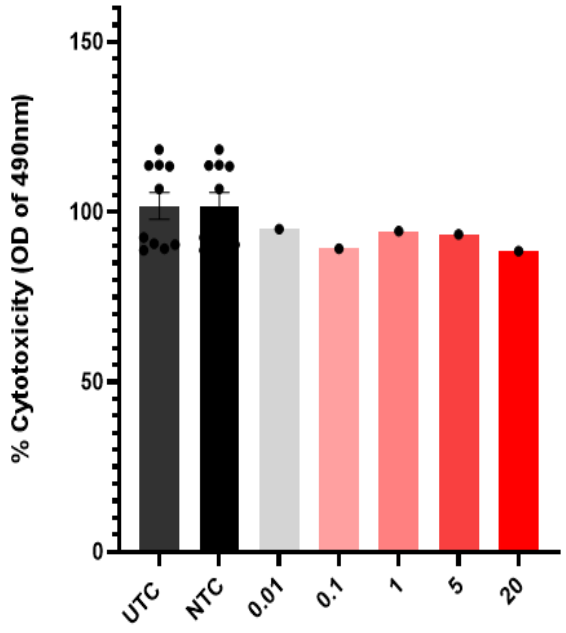

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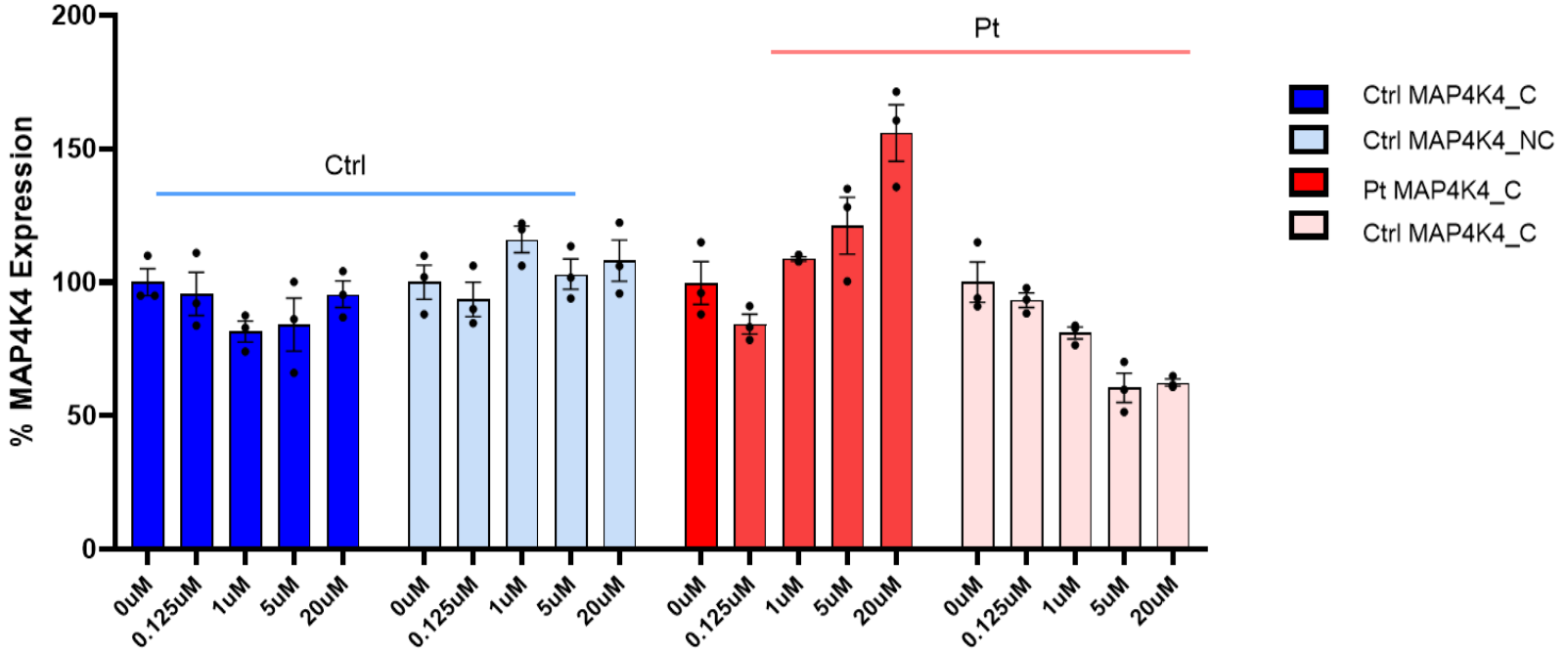

Conclusion

- RNU4-2 mutations disrupt 5’ splice site fidelity and drive TGOF pathology
- A subset of lead ASOs shows strong allele-selective knockdown of mutant RNA and restoration of downstream splicing
- Lead ASOs don’t show cytotoxicity on cell viability
- We developed highly promising allele-selective ASOs for the treatment of ReNU syndrome

Acknowledgement

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Thank you

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