RNA Structures Regulating Alternative Splicing in Paralogs

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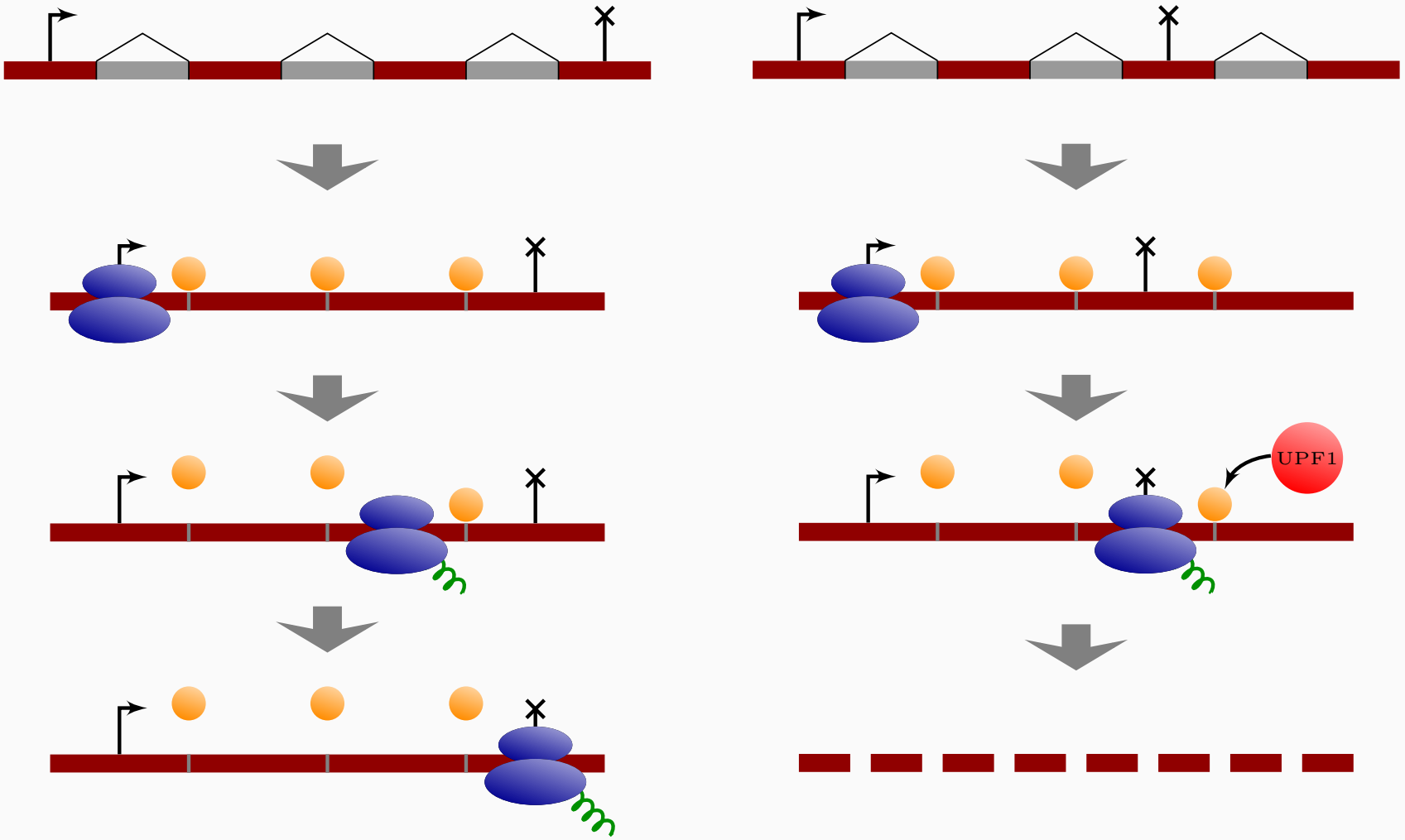
RNA structures regulating alternative splicing

Org.	Gene	AS event	IRBIS	PREPH	RIC-seq	Publ.
D. mel	CG33298	Alt. 5'ss	+	+		2009
	Gug	Alt. 3'ss	+	+		2009
	Nmnat	Alt. term. exon	+	+		2009
H. sap.	SF1	Intron retention	+	+		2012
	PHF20L1	Cassette exon	+	+	+	2023
	CASK	Cassette exon	+	+	+	2023
	ATE1	Mut. excl. exon	+	+	+/−	2021
	MARK2	Cassette exon	+	+	+	2023
	BRD2	Poison exon	+	+	+	2024
	BRD3	Poison exon	+	−	−	2024
	SCN9A	Mut. excl. exon	+	+	+	2026

Nonsense-mediated mRNA decay (NMD) pathway



NMD degrades mRNAs with premature termination codons (PTCs)

Poison and essential exons, unproductive splicing

- Poison exons generate PTCs when included

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- Essential exons generates PTCs when skipped

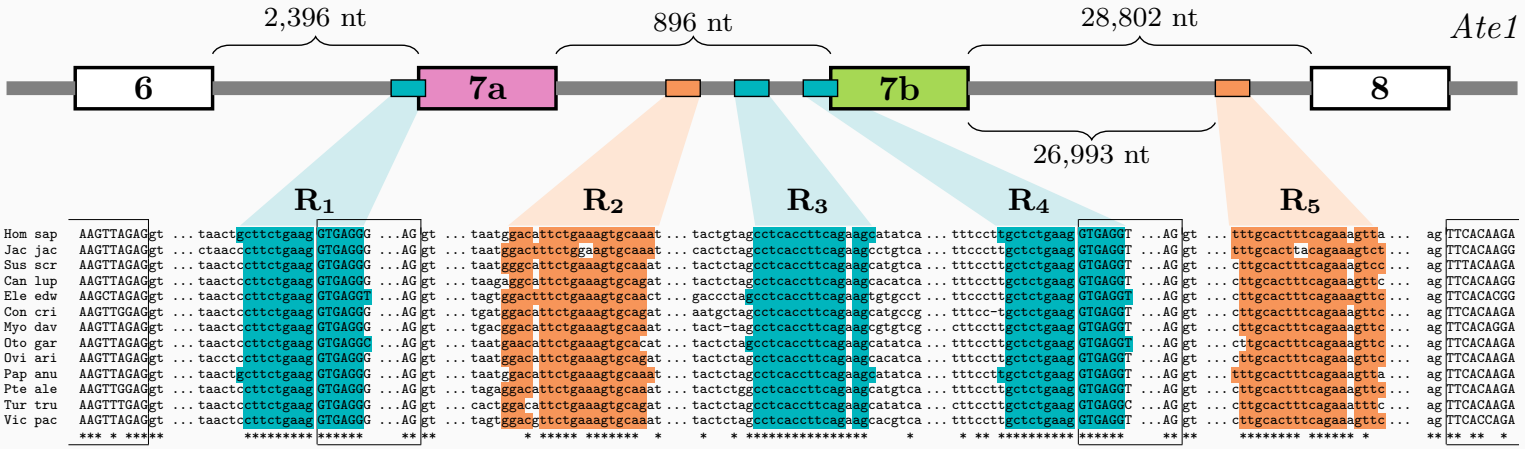
Poison and essential exons, unproductive splicing

- Poison exons generate PTCs when included

- Essential exons generates PTCs when skipped

- Pairs of mutually exclusive exons can generate PTCs

Competing RNA structures regulate mutually exclusive exons (MXE)



*Kalinina et al, Nucleic Acids Res. 49(1):479-490 (2021)

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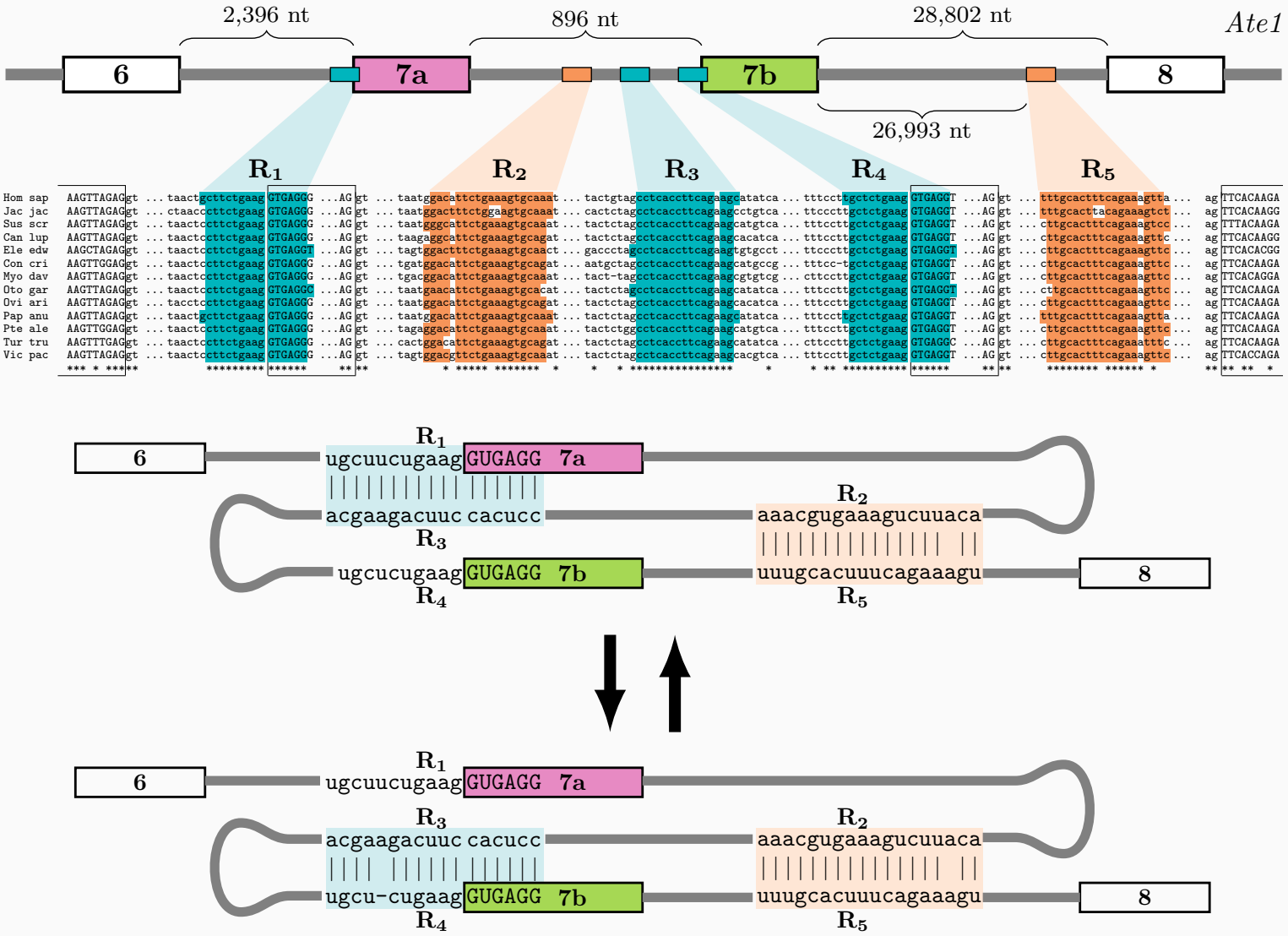
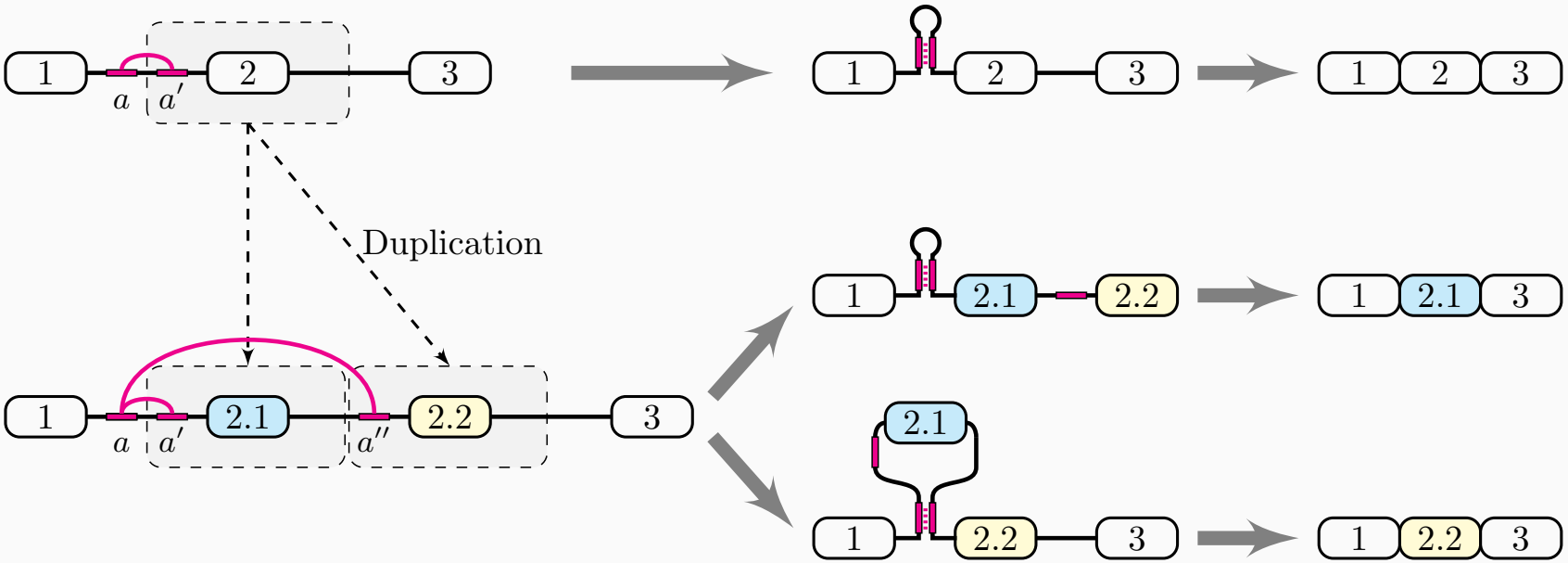


*Kalinina et al, Nucleic Acids Res. 49(1):479-490 (2021)

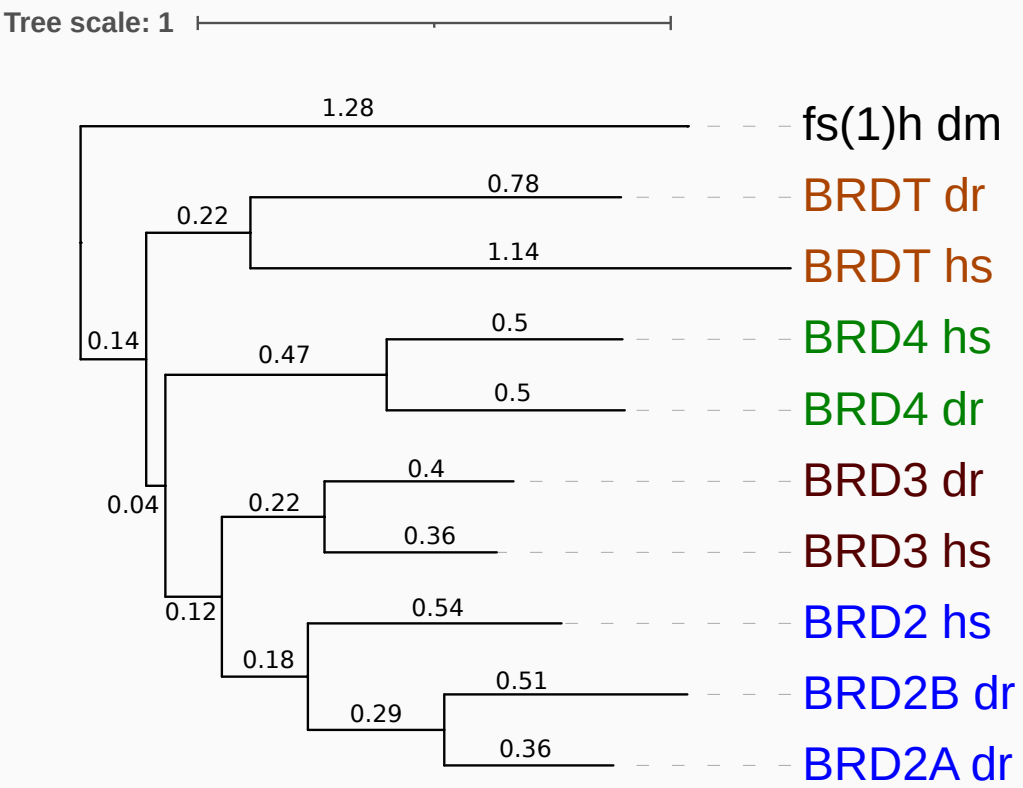
Tandem duplications generate competing RNA structures



*Ivanov & Pervouchine, Genes (Basel) 9(7):356 (2018).

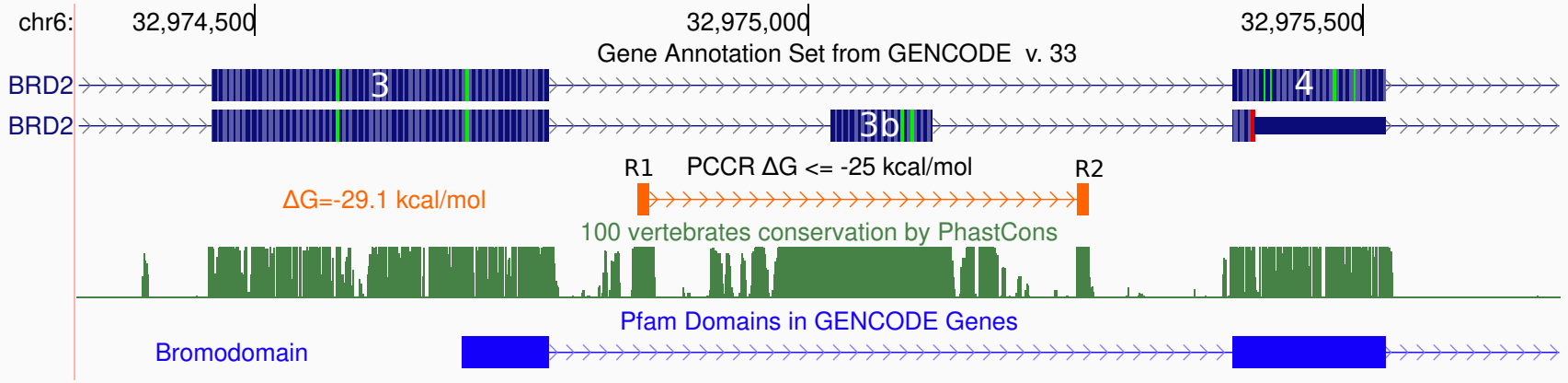
The Bromodomain and Extraterminal (BET) gene family

The BET gene family consists of four epigenetic readers regulating transcription widely implicated in cancer and inflammatory diseases

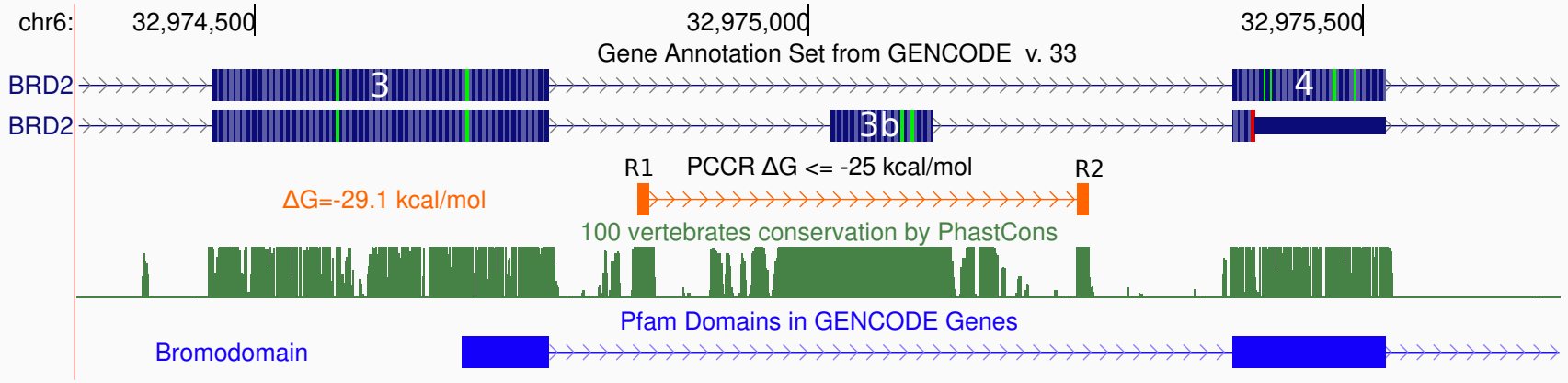


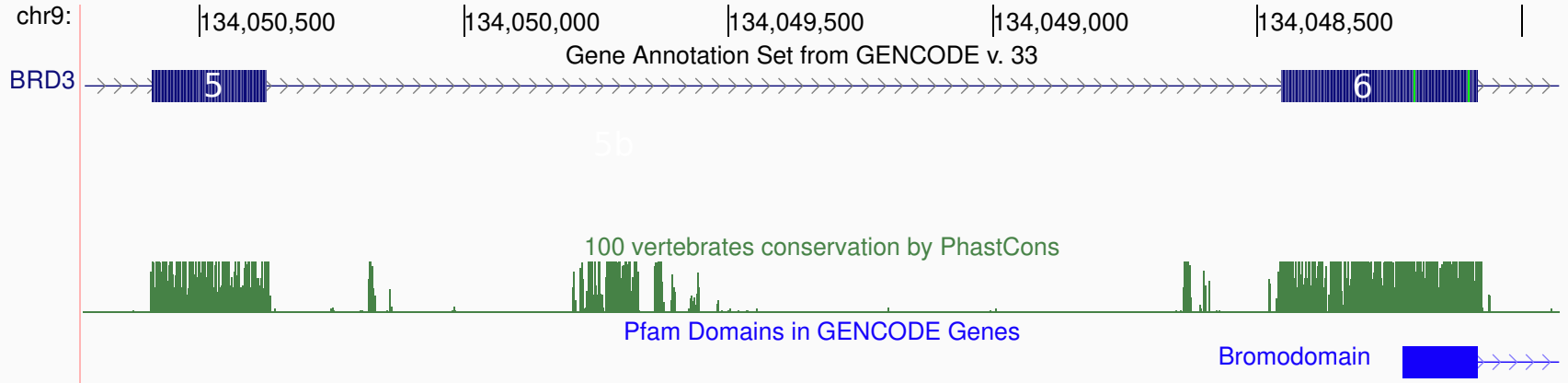
***Petrova et al NAR Genom Bioinform. 2024 Jan 15;6(1):lqad113**

Poison exons in the BET gene family



Poison exons in the BET gene family





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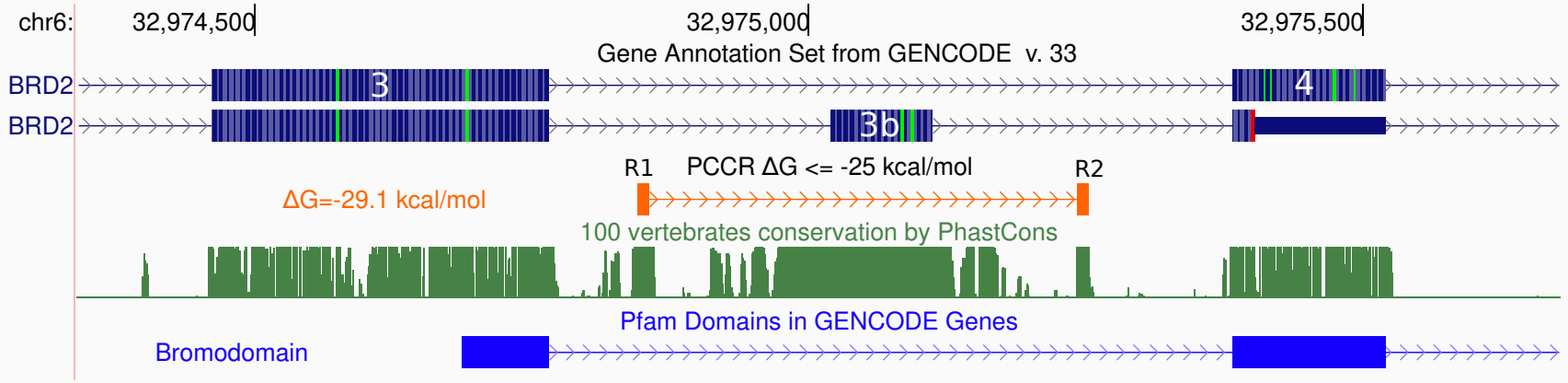
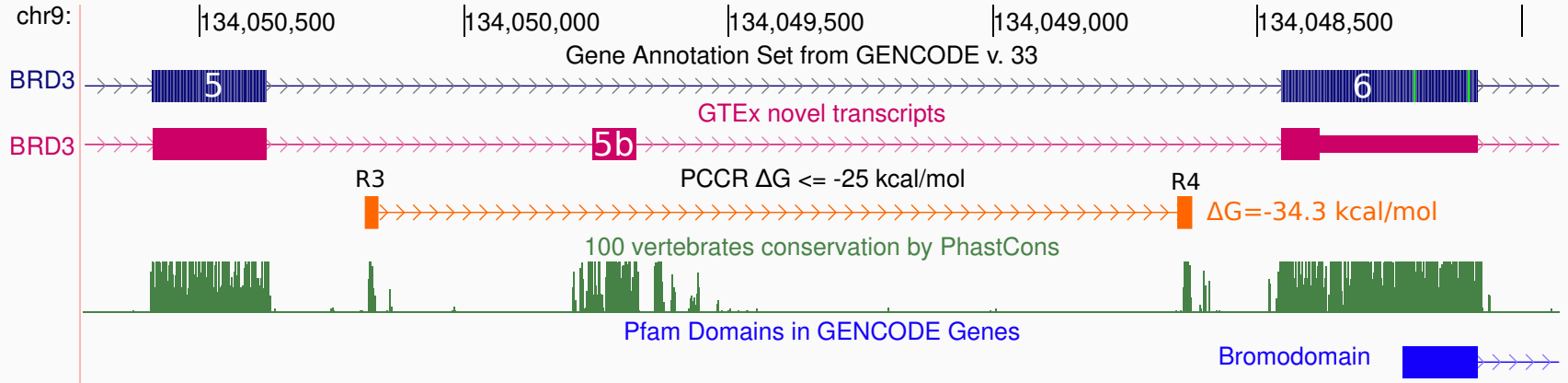
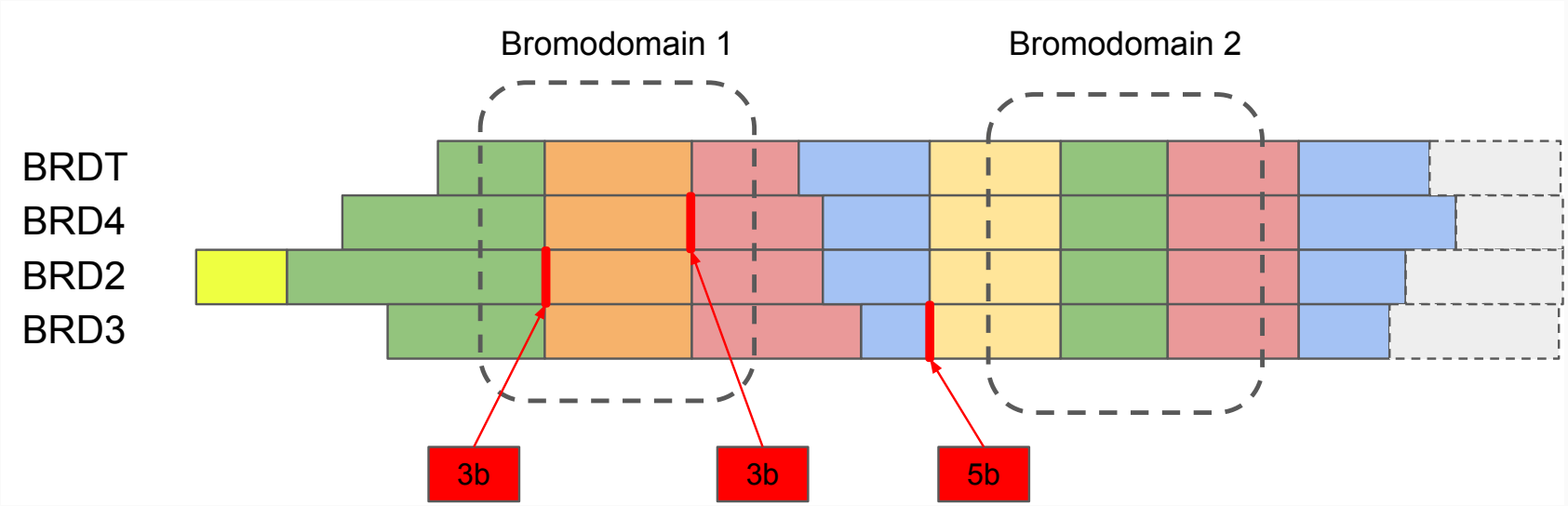




Poison exons located in non-homologous introns



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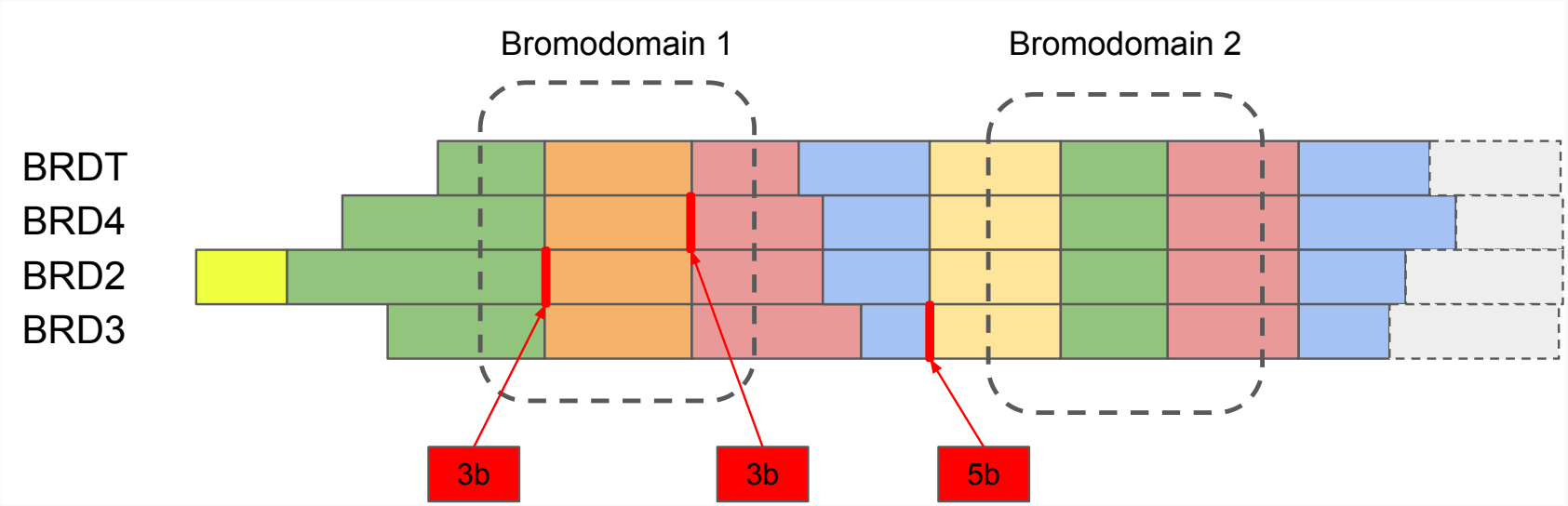

Poison exons and regulatory RNA structures around them were acquired independently in convergent evolution.

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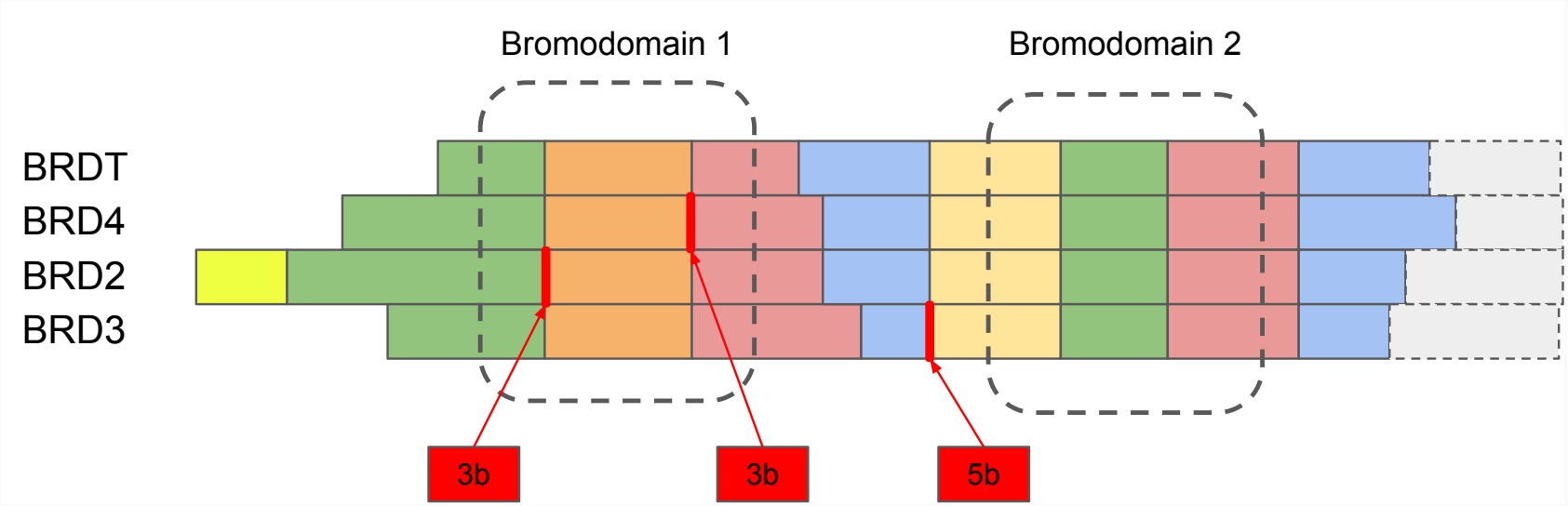
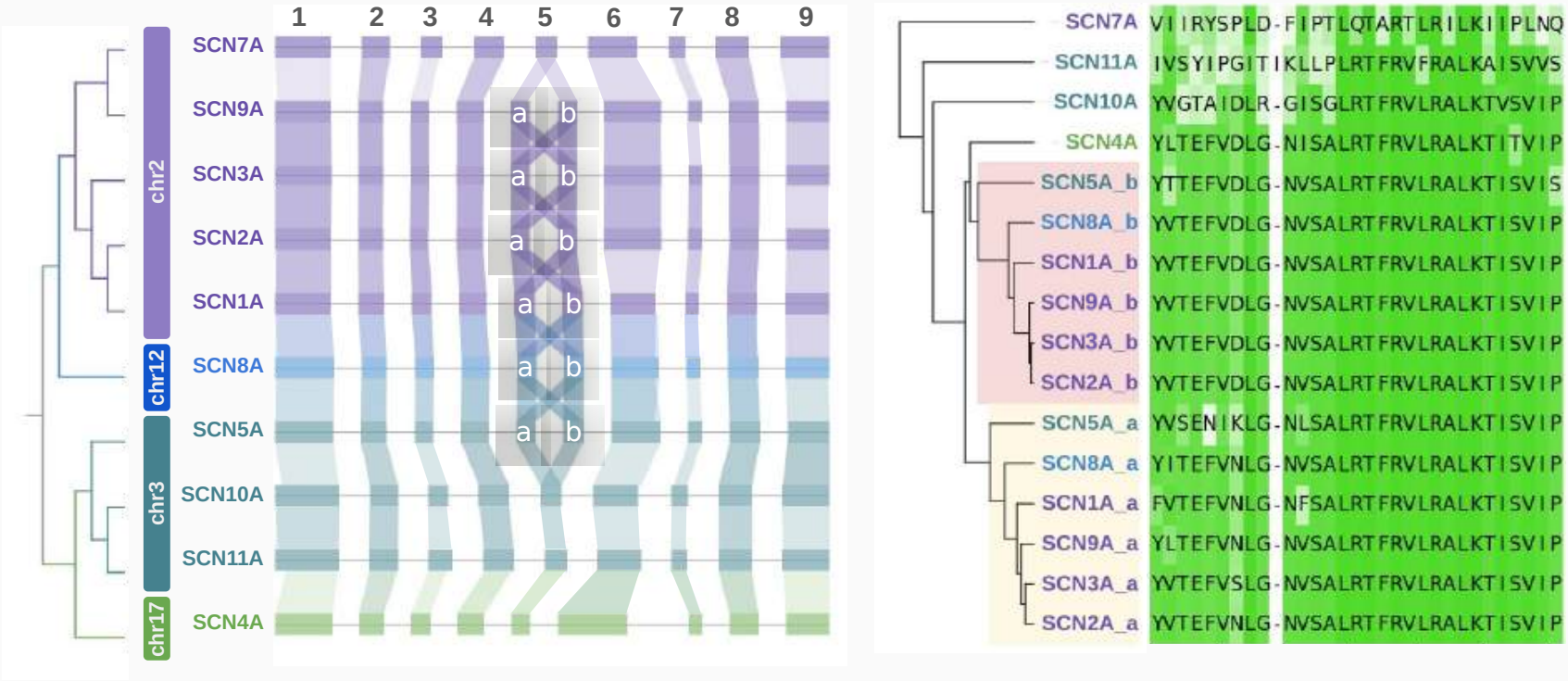


Poison exons and regulatory RNA structures around them were acquired independently in convergent evolution.

Possibly to enable tissue-specific regulation through RNAPII elongation rate

The SCNα (Na^+ voltage-gated channel α subunit) gene family

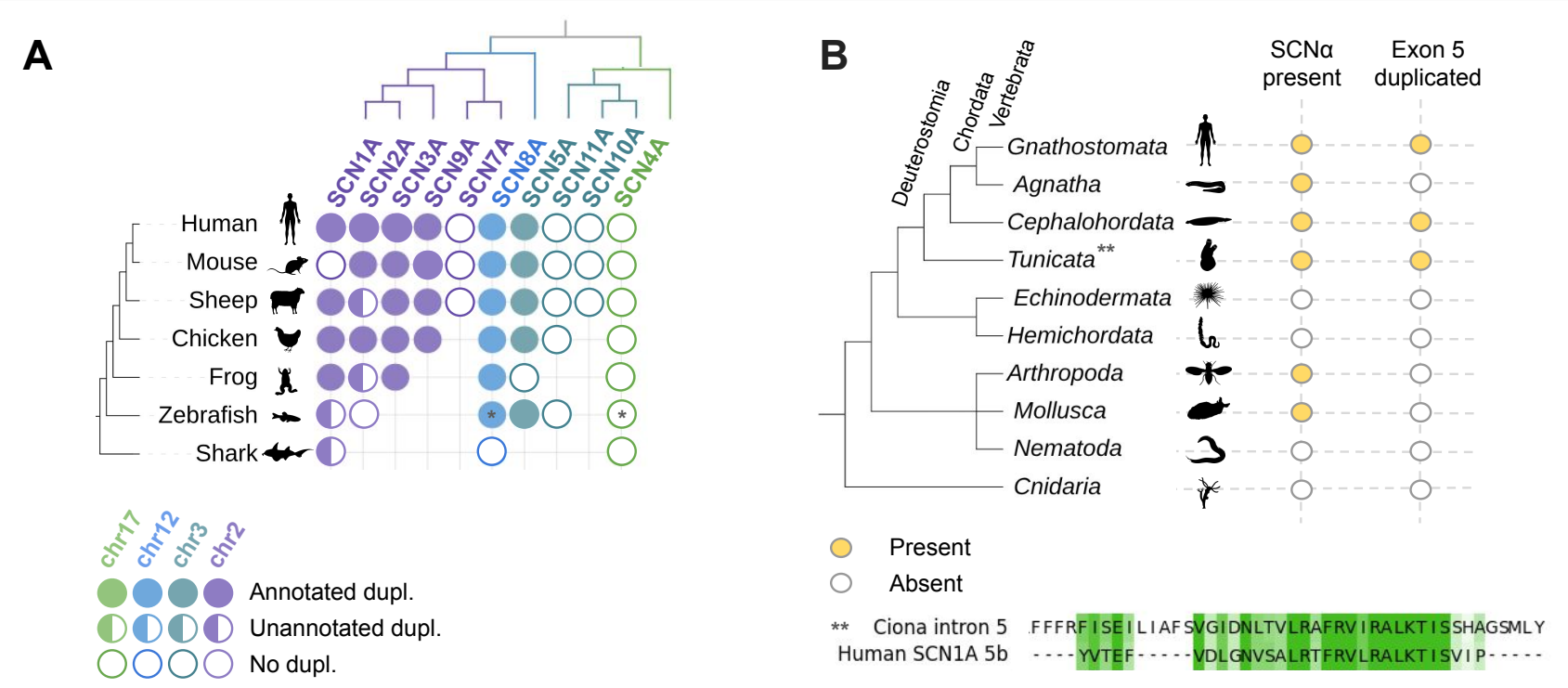
SCNα genes encodes the principal pore-forming proteins of voltage-gated sodium channels.



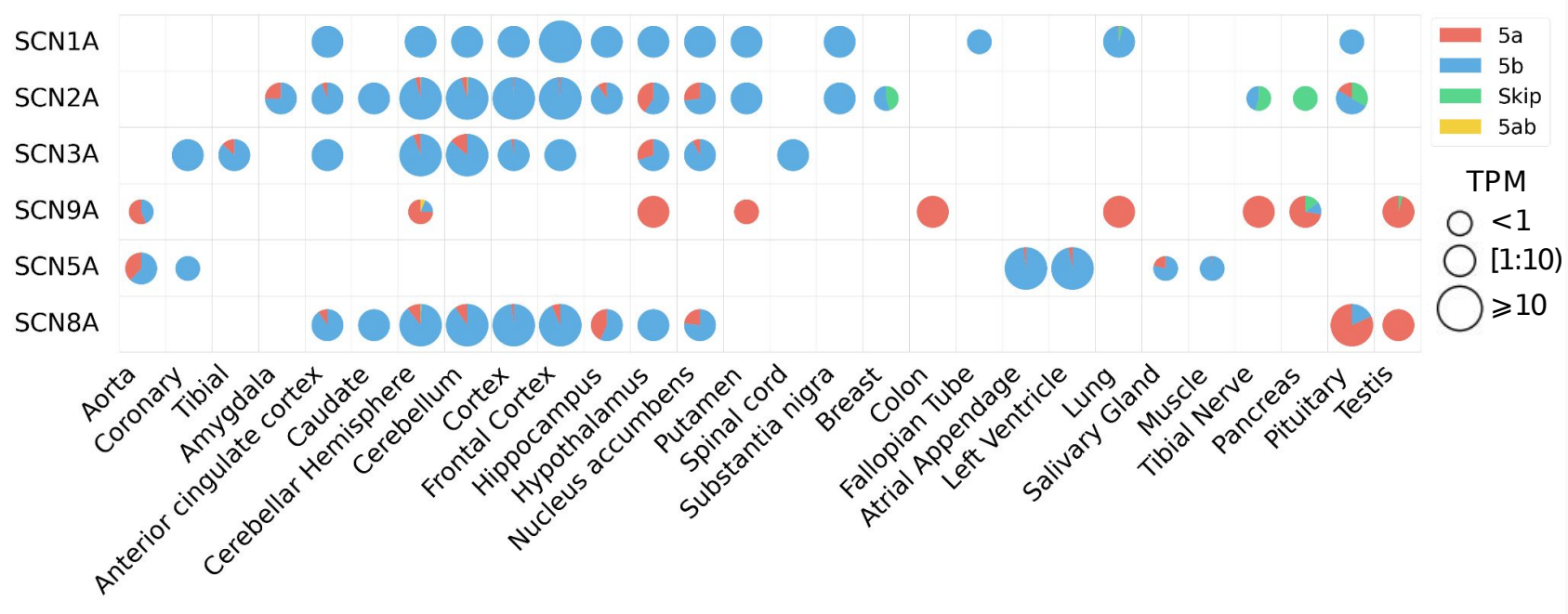
Exon 5 duplication happened in the common ancestor of (some) SCNα genes, followed by gene duplication

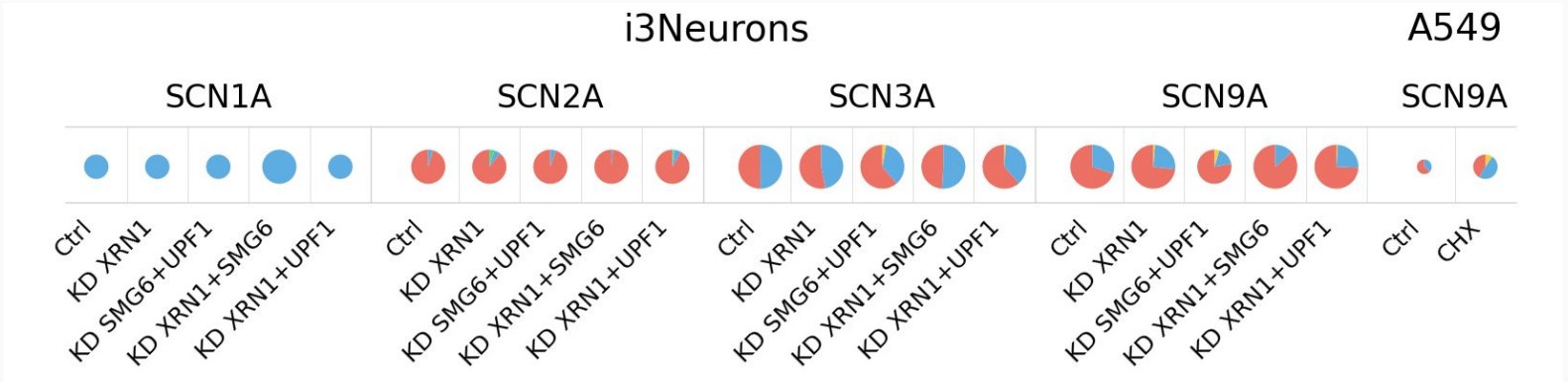
***Chernyavskaya et al, RNA 18;32(6):827-842 (2026).**

Exon 5 duplication was present in Deuterostomia

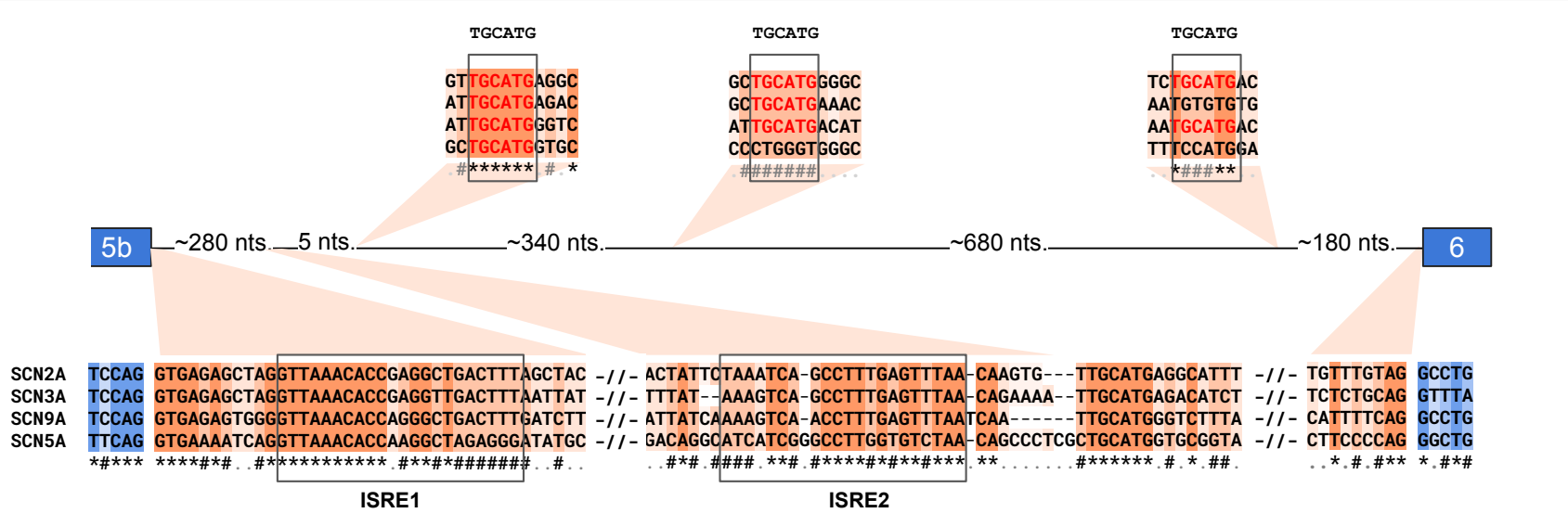


Exons 5a and 5b are mutually exclusive not due to NMD



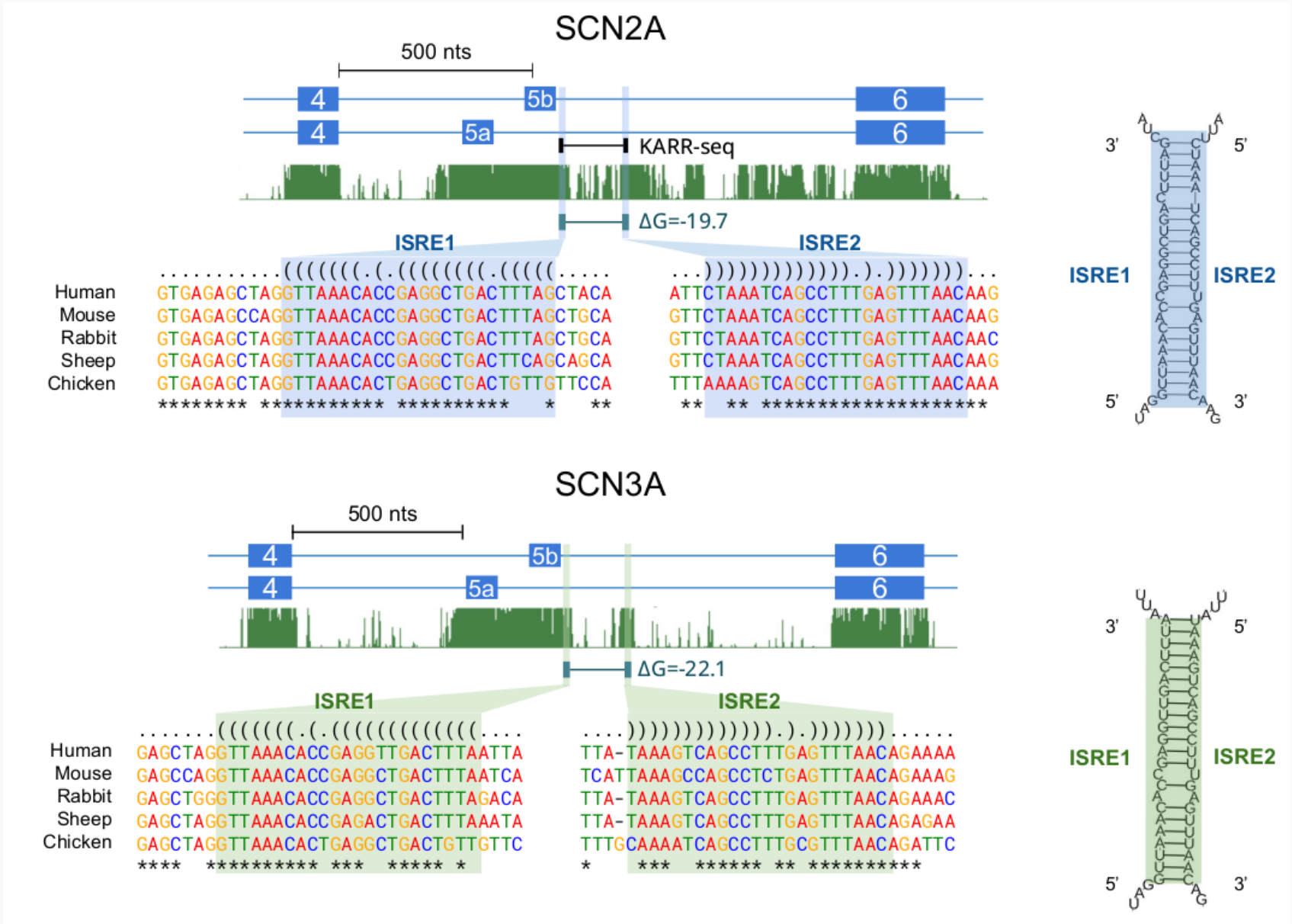


SCNα genes share intronic splicing regulatory elements (ISRE)



- ISRE1 and ISRE2 can form a long dsRNA helix
- Base pairing is supported by KARR-seq and RIC-seq
- TGCATG is a binding site of RBFOX2
- ISRE are very conserved in orthologs (no variation)
- ISRE are homologous between paralogs
- As ancient as the common ancestor!

ISRE1 and ISRE2 are complementary



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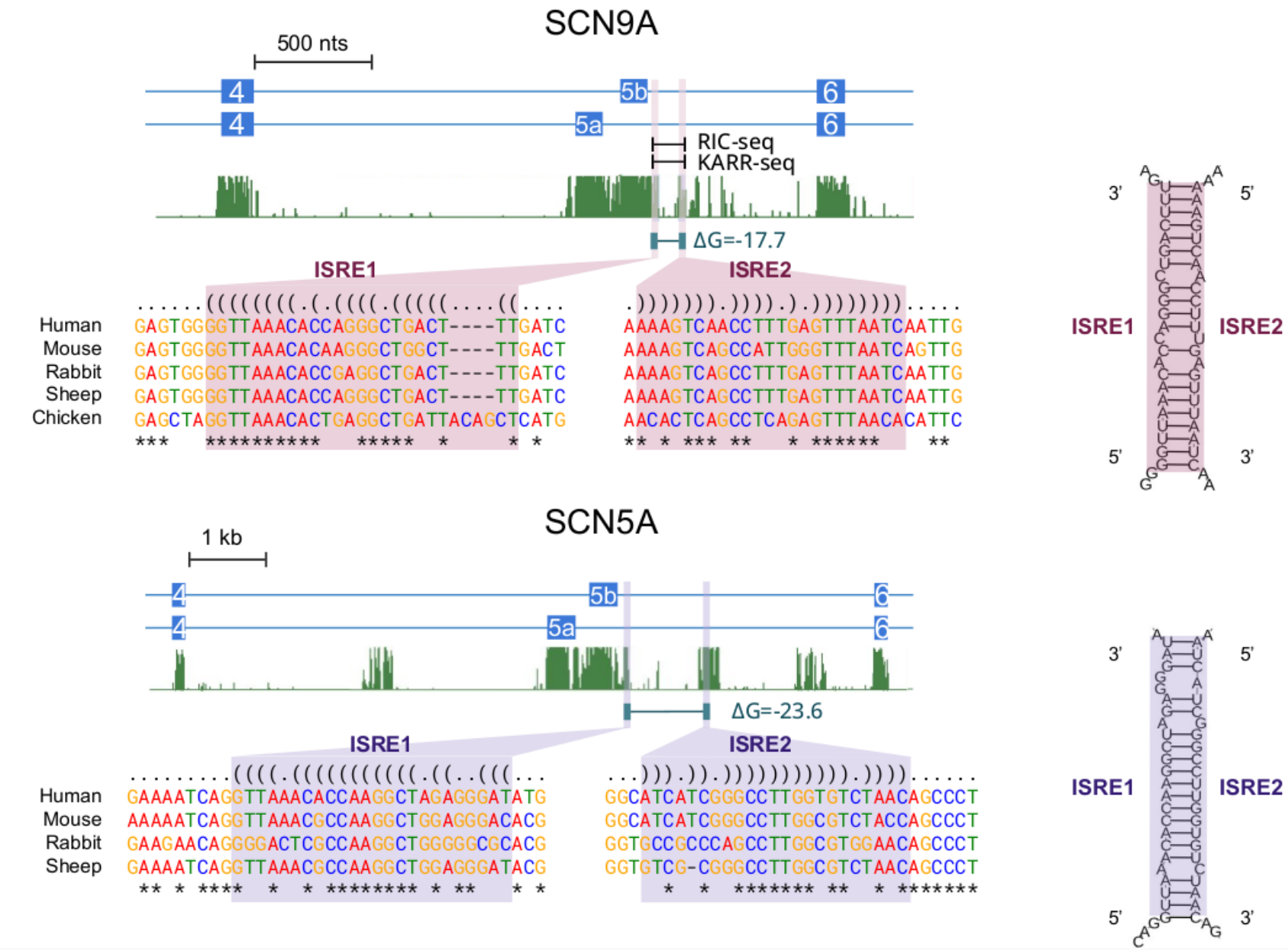
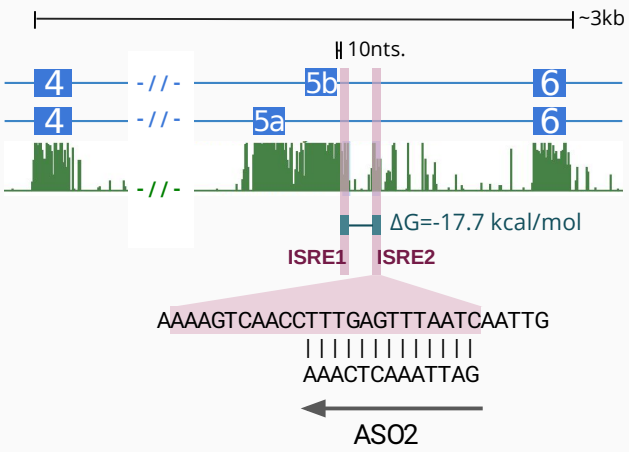
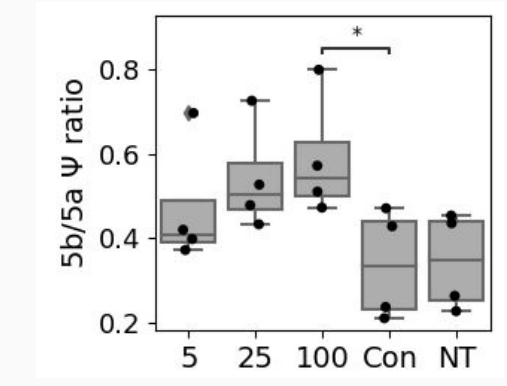
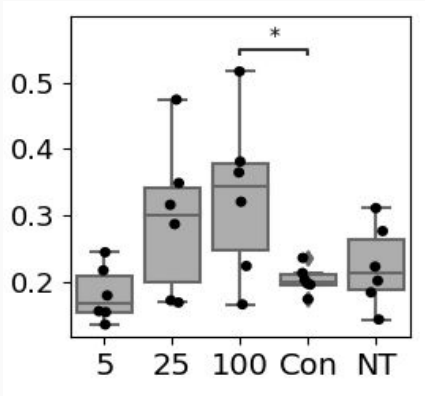
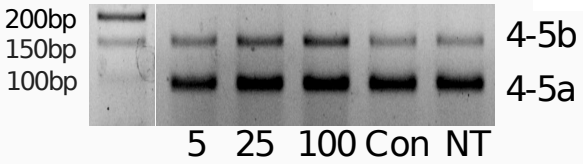


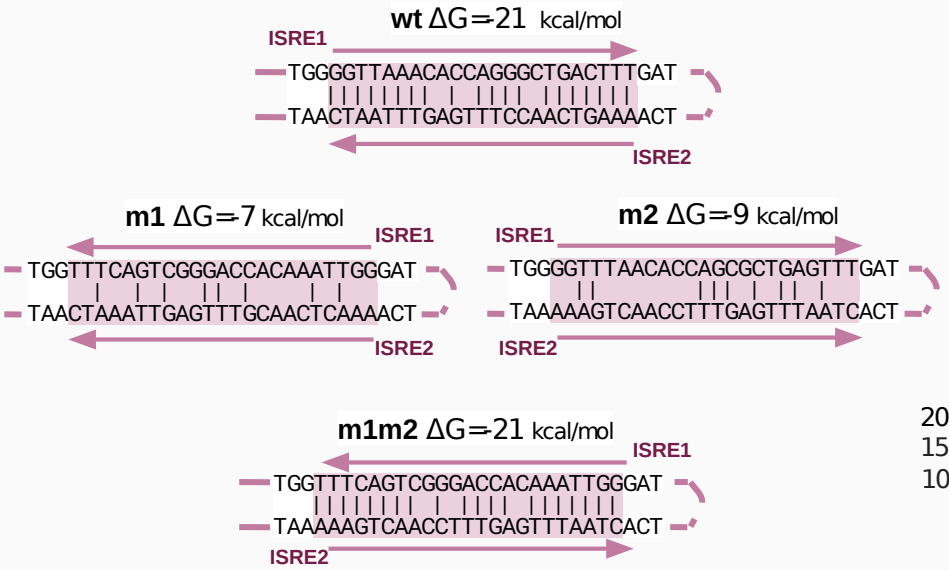
Disruption of RNA structure promotes exon 5b inclusion

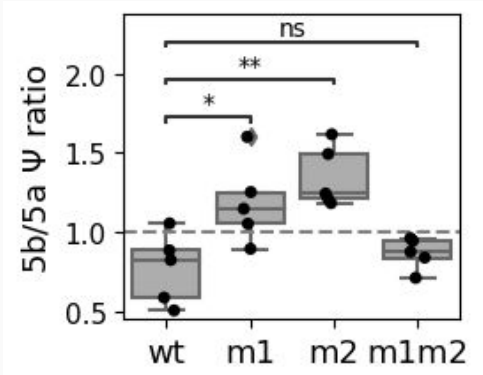


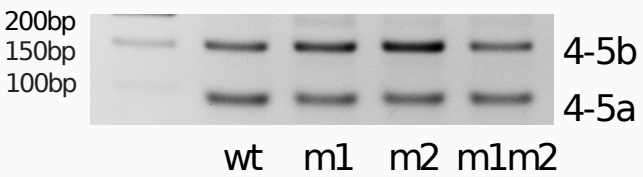




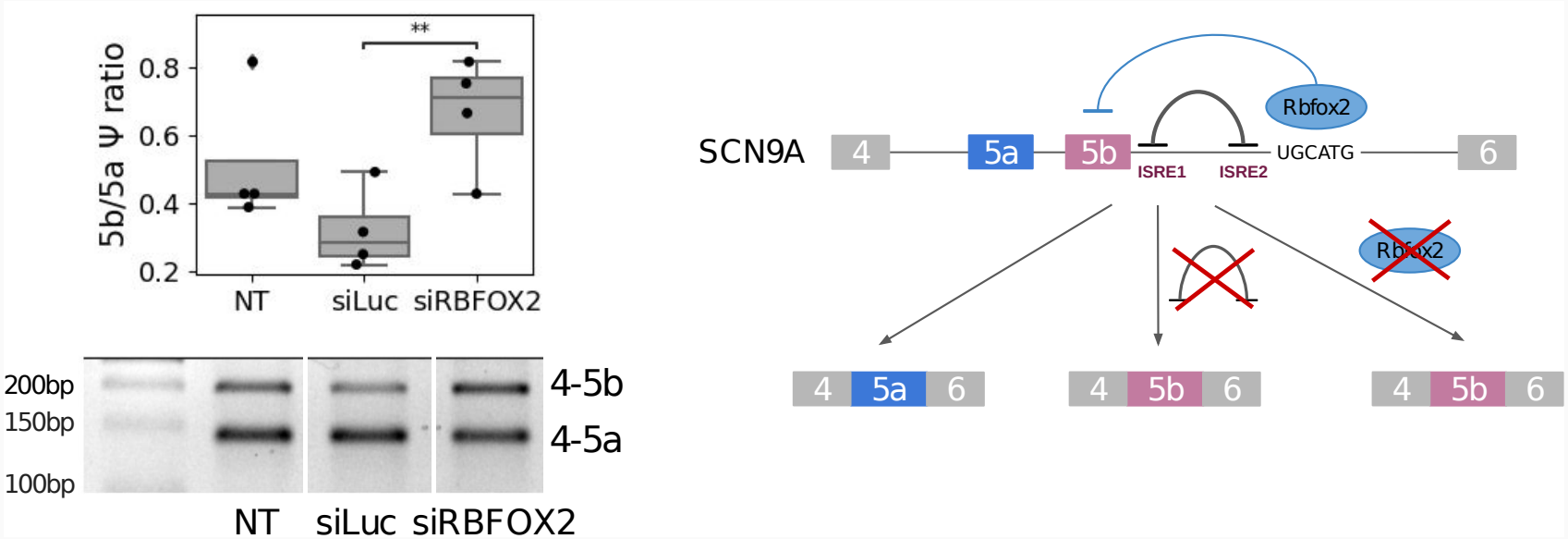








RBFOX2 is suppressing exon 5b through an RNA bridge



- RBFOX2 usually acts as splicing activator, not repressor

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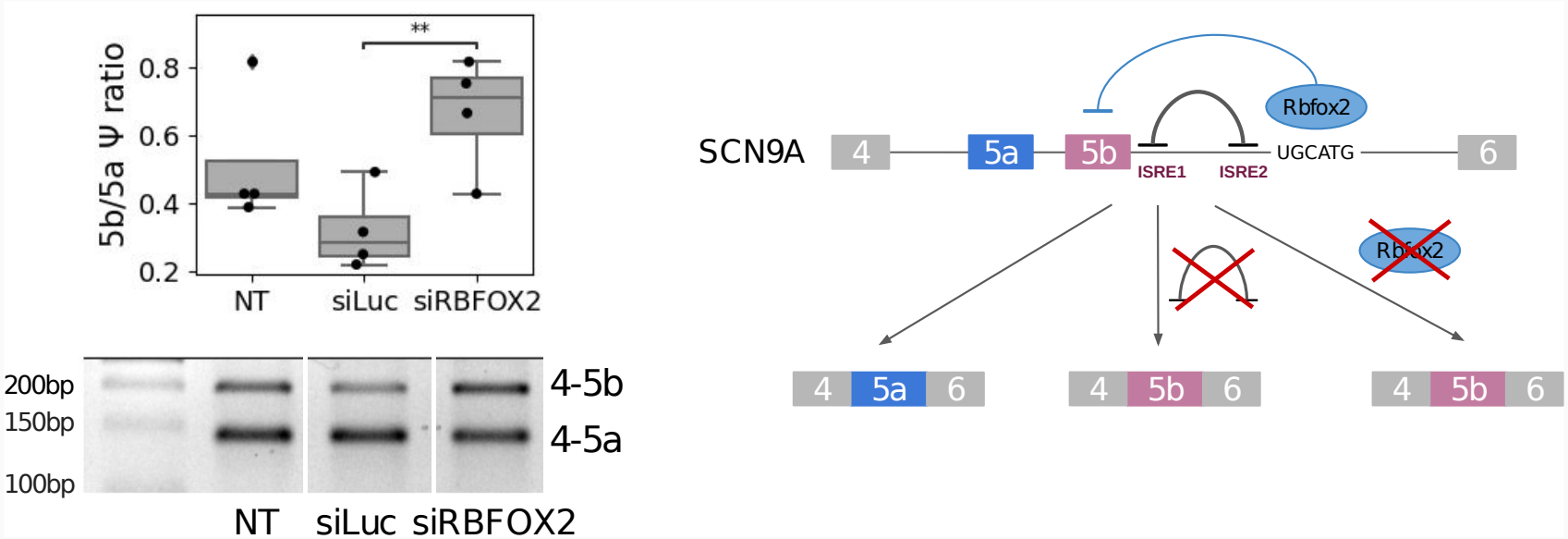
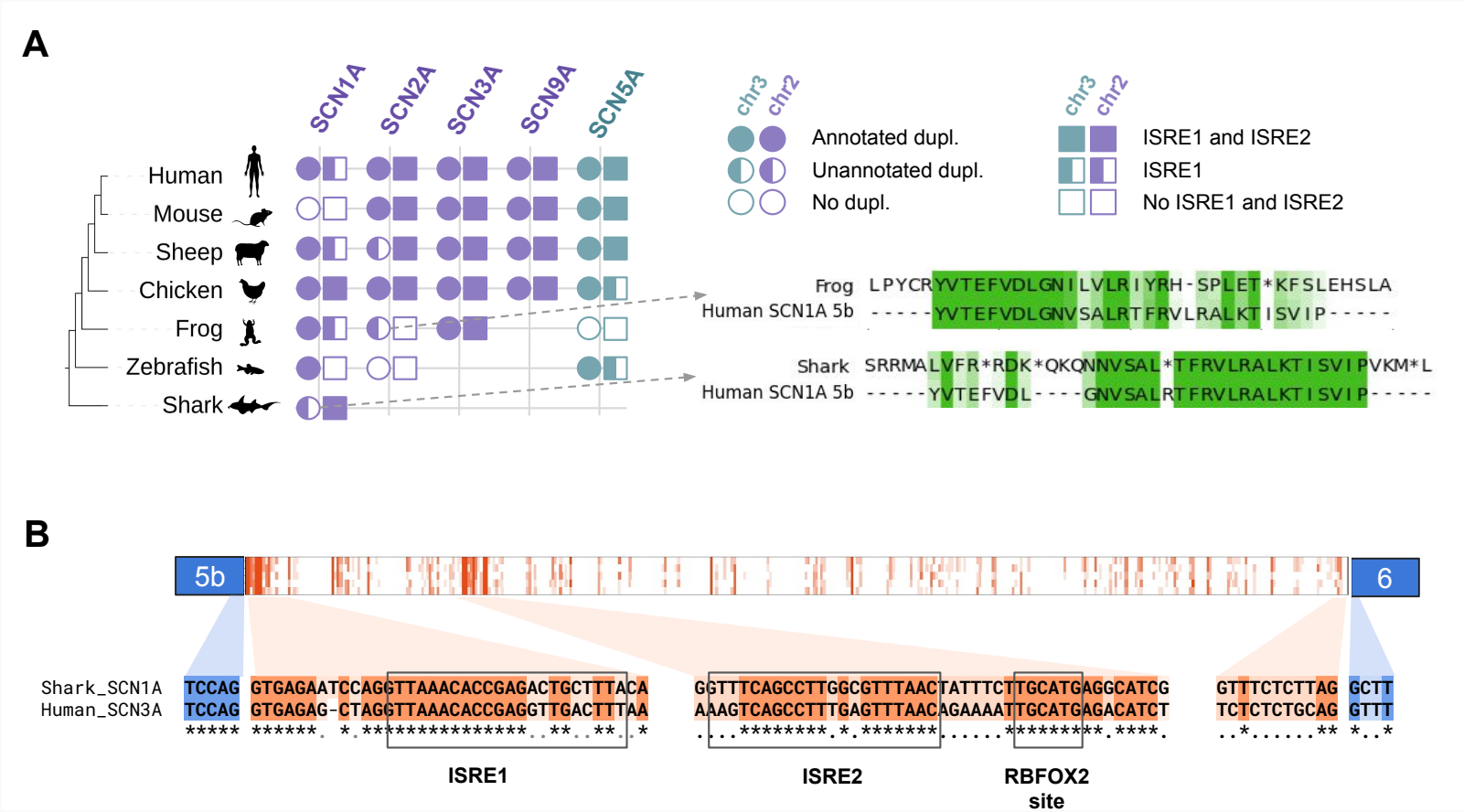


- RBFOX2 usually acts as splicing activator, not repressor
- The experiment was done in the *huh7* cell line, where the direction of regulation may be different

ISRE1 and ISRE2 date back to elephant shark



Conclusion

- In order to discover functional RNA structures through covariation, one needs variation
- Most known functional RNA structures in vertebrates lack sufficient level of sequence variation
- Comparing homologous non-coding sequences in paralogs (e.g., introns) helps discover functional regulatory elements including but not limited to RNA structures

Acknowledgments

Maria Vlasenok

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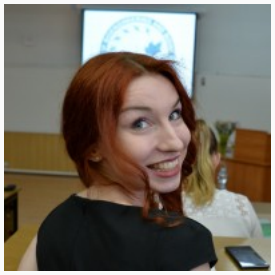
Rita Vorobeva



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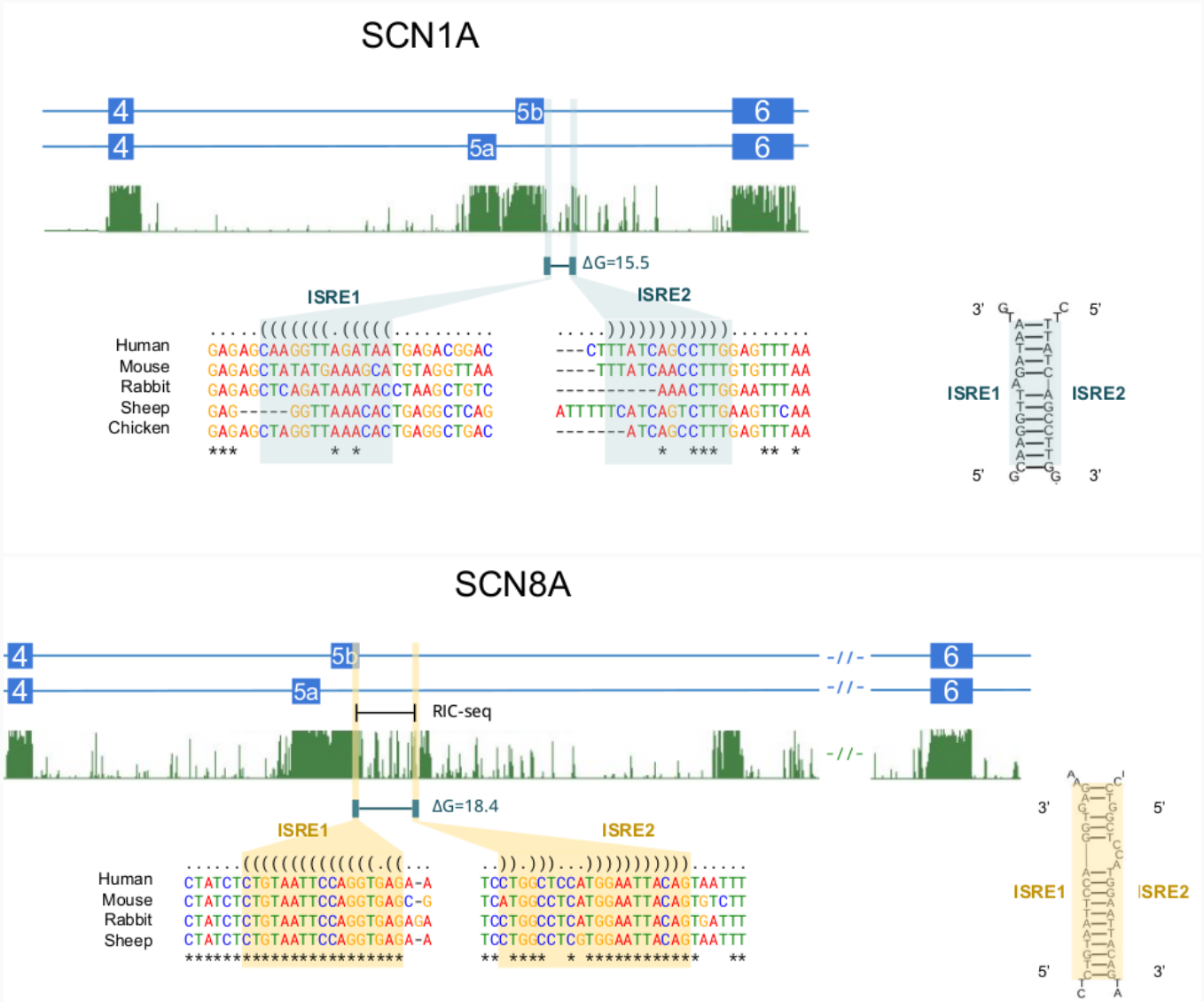
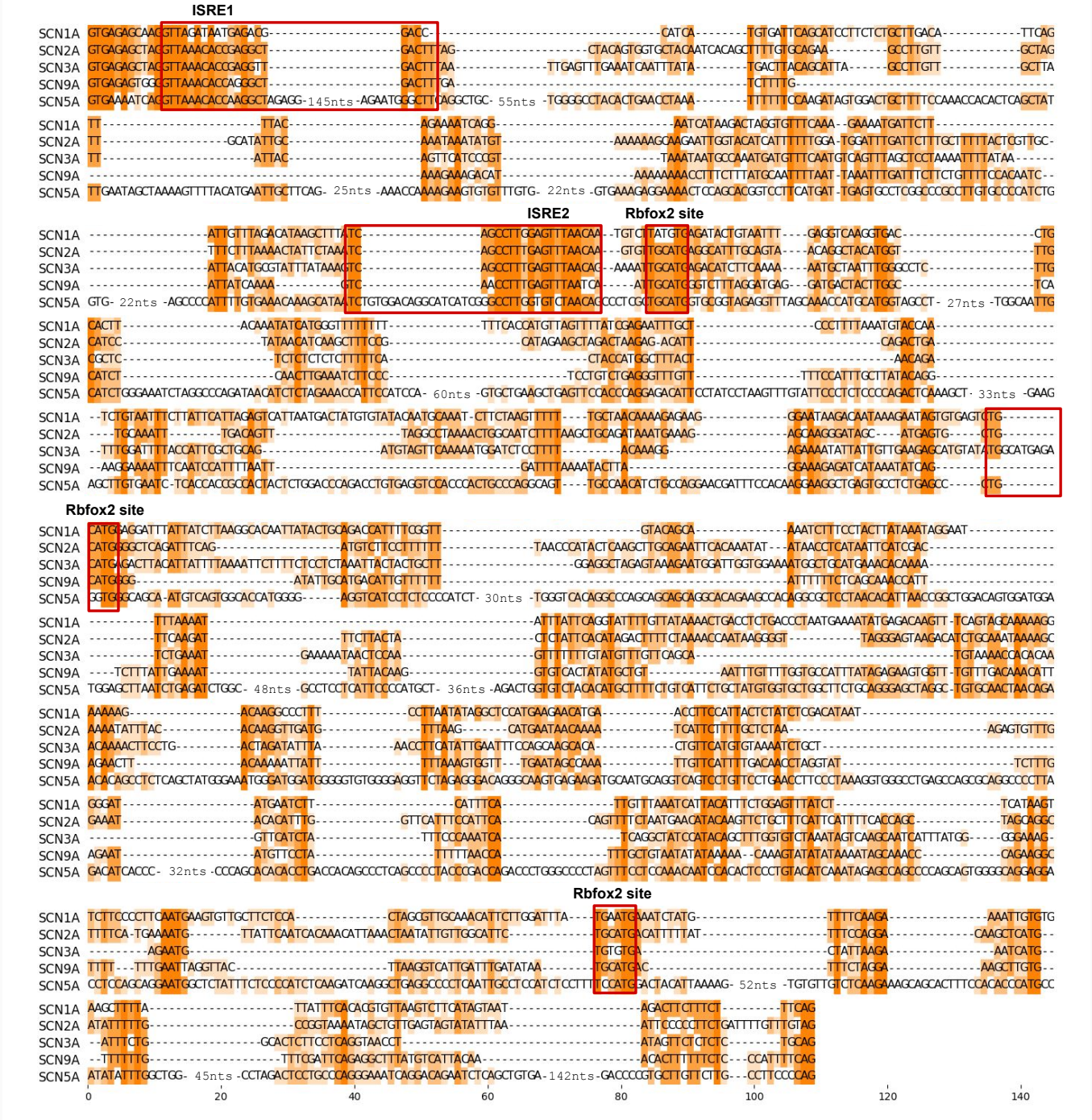
RNA Structures in SCN5α and SCN8α




ISRE1
ISRE2
Rbfox2 site
Rbfox2 site
Rbfox2 site
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SCN2A
SCN3A
SCN9A
SCN5A
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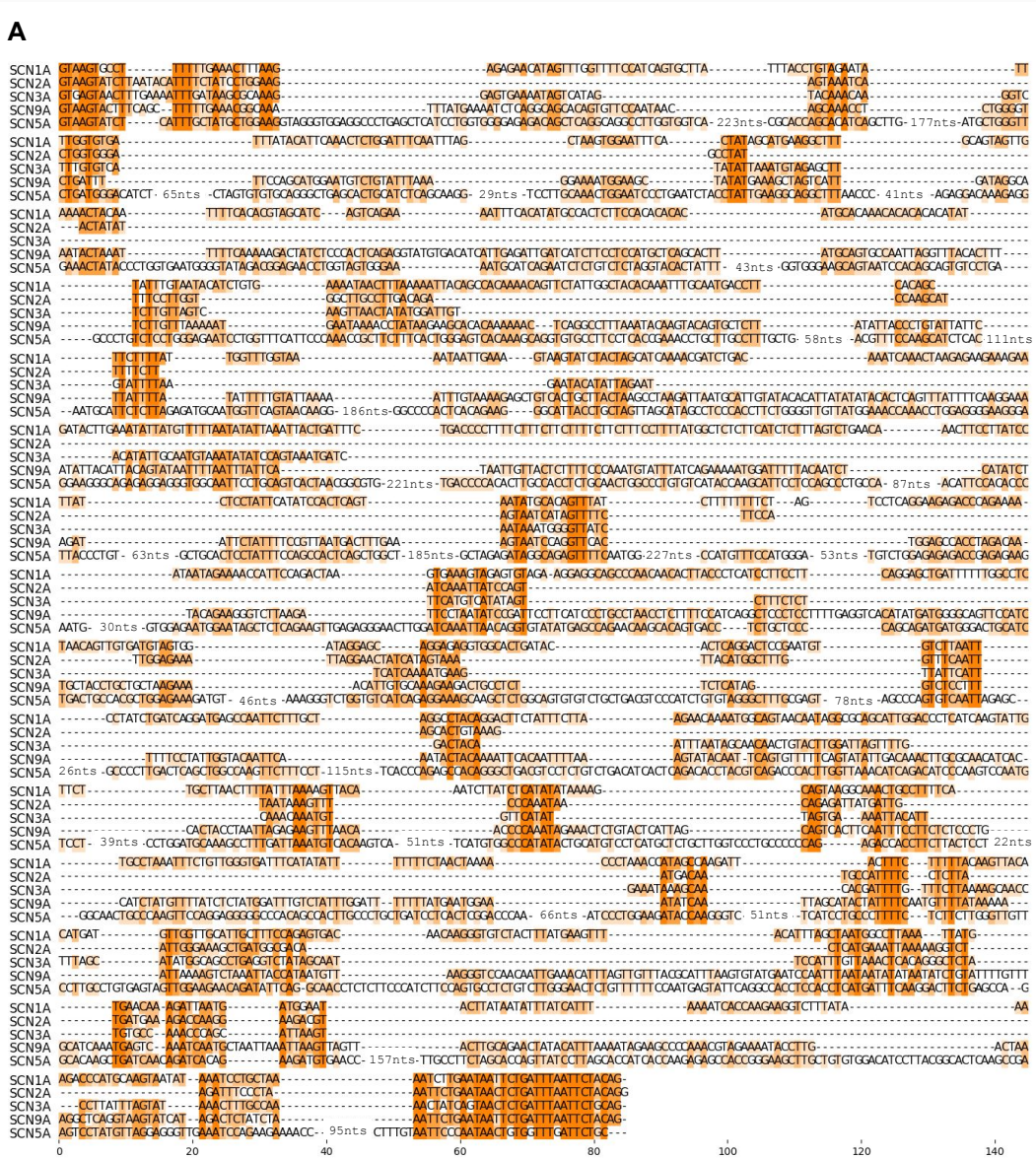
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