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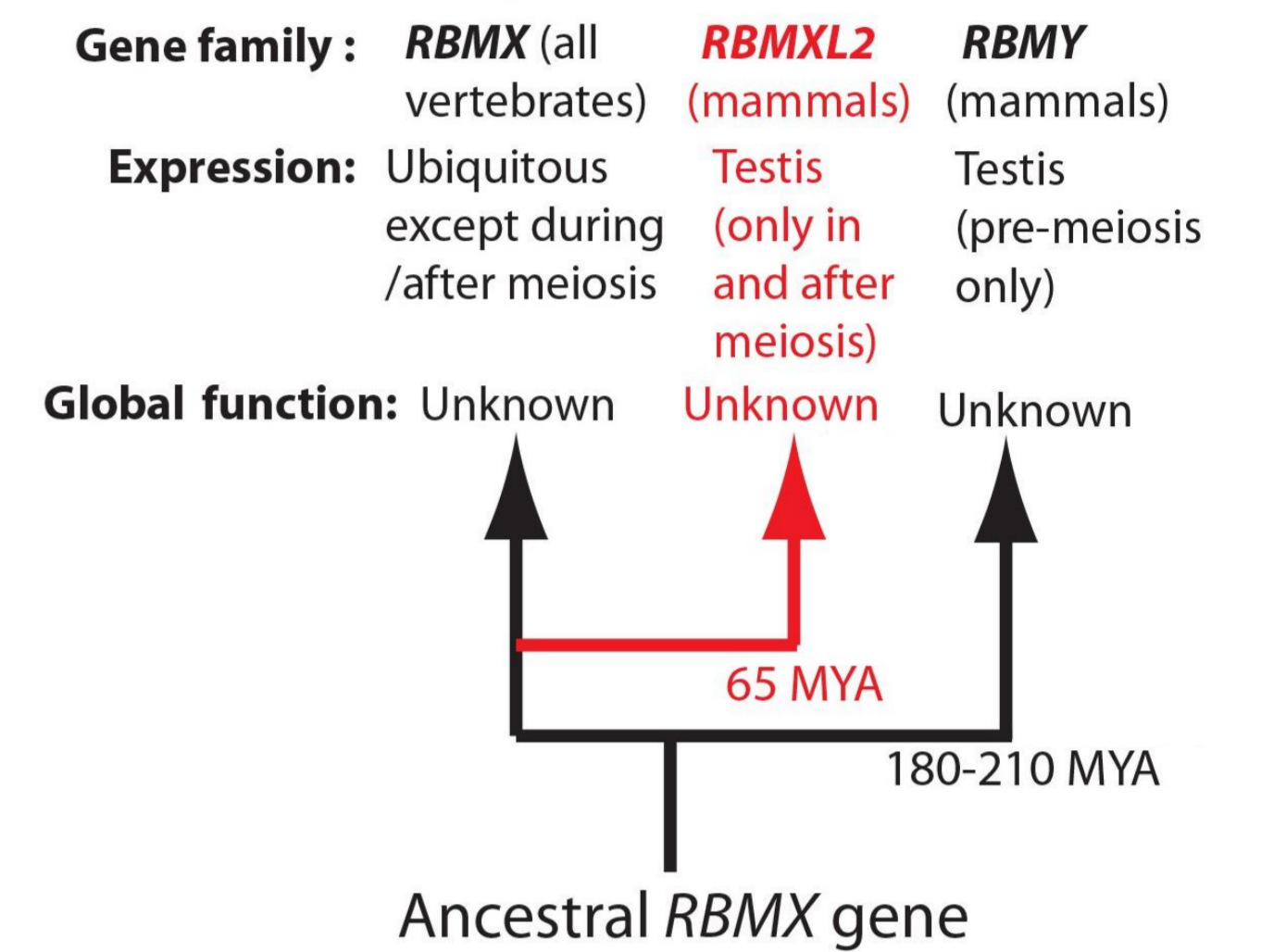
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THE RBMX RNA-BINDING PROTEIN: AN INTRODUCTION

- **RBMX** and **RBMXL2** are **hnRNP** proteins that belong to an ancient and conserved gene family of **RNA-binding proteins**¹.
- **RBMX** is **expressed** in somatic cells **except during meiosis**, while **RBMXL2** is only expressed **during meiosis**¹.
- **RBMX** and **RBMXL2** **both interact** with the SR protein and splicing regulator **Tra2β**, which often has a role in splicing opposite to RBMX and RBMXL2^{2,3}.
- **RBMX** is **mutated** in a type of X-Linked **Intellectual Disability Syndrome** called Shashi Syndrome¹.
- **RBMX** **activates** some **splicing sites** recognising m6A RNA methylation⁴ and has a potential function in **transcription**¹.
- **RBMX** **localises** to stalled replication forks and **sites of DNA damage**, promoting recruitment of DNA damage response proteins, and regulates **sister chromatids cohesion**^{5,6,7}.
- **Loss of RBMX** leads to **genome instability**^{5,6,7} and **reduced expression of BRCA2** tumour suppressor⁵. **HOW RBMX REGULATES BRCA2 IS UNKNOWN. DOES THE ROLE OF RBMX IN SPLICING CONTRIBUTE TO GENOME STABILITY?**
- **Loss of RBMXL2** in mice causes **meiotic arrest during spermatogenesis**² and **use of cryptic** (not normally used) **splice sites** within genes important for meiosis and **genome maintenance**².
- **RBMXL2 suppresses cryptic splicing of Brca2** gene in mouse testes².
- **RBMXL2** shares **73% protein identity to RBMX** and has a similar modular structure¹. **DOES RBMX ALSO REPRESS CRYPTIC SPLICING, SIMILARLY TO RBMXL2?**



RESULTS

RBMX represses cryptic RNA processing of genes important for genome maintenance

RNA-seq in MDA-MB-231 breast cancer cells treated with siRNA against RBMX

SUPPA2⁸ and MAJIQ⁹ bioinformatic analyses

~ 6700 differentially processed RNA isoforms upon RBMX depletion

Visual inspection on IGV genome browser¹⁰

155 strongly defective RNA processing events upon RBMX depletion

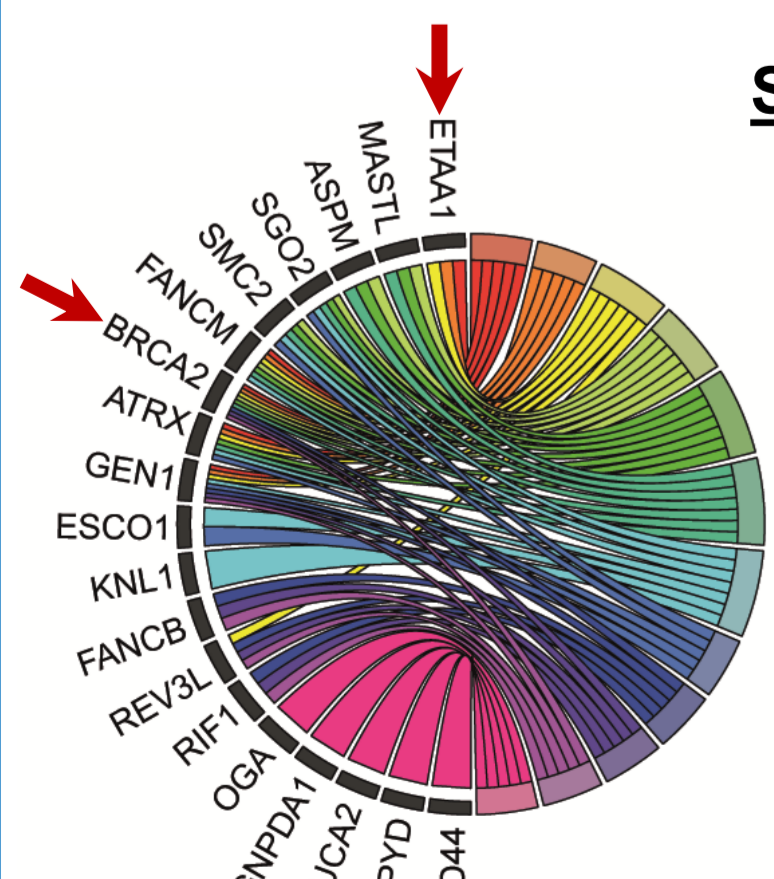
Gene ontology: Cell cycle, DNA replication, DNA repair, etc...

Strongly defective events

Activated by RBMX (n=26) → decreased RNA processing in knock-down cells

46% of repressed events observed also in RBMX-depleted HEK293 cells⁴

Repressed by RBMX (n=127) → RNA processing rare/absent in control cells

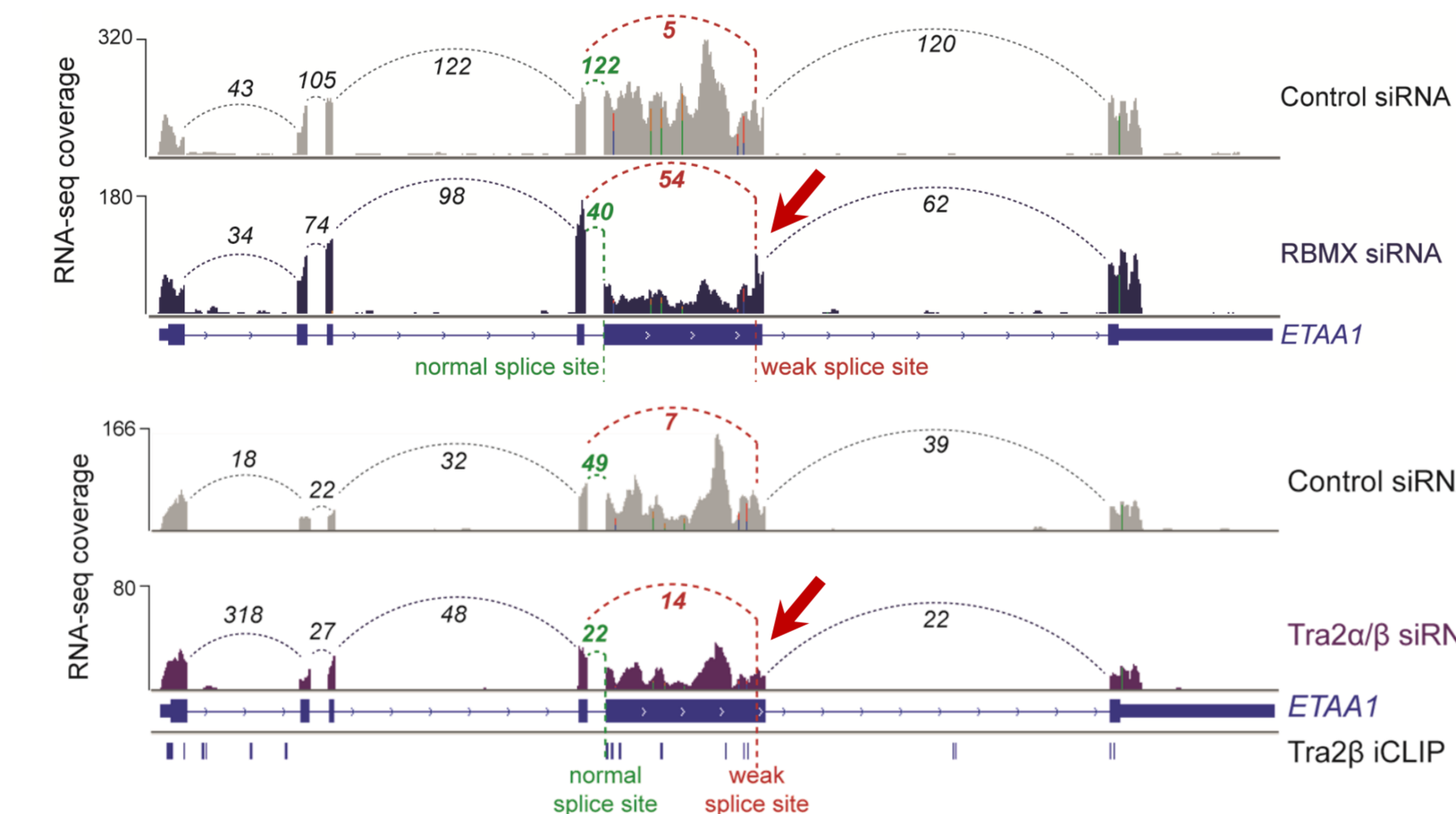


Strongly defective events – Gene Ontology

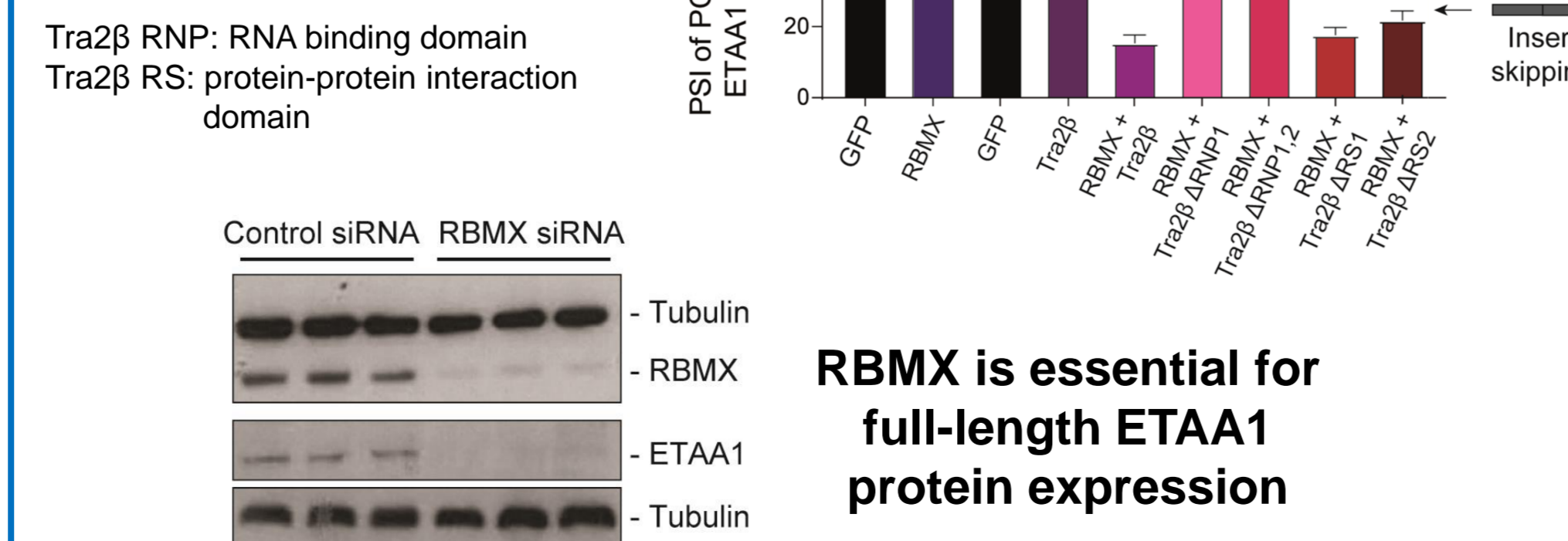
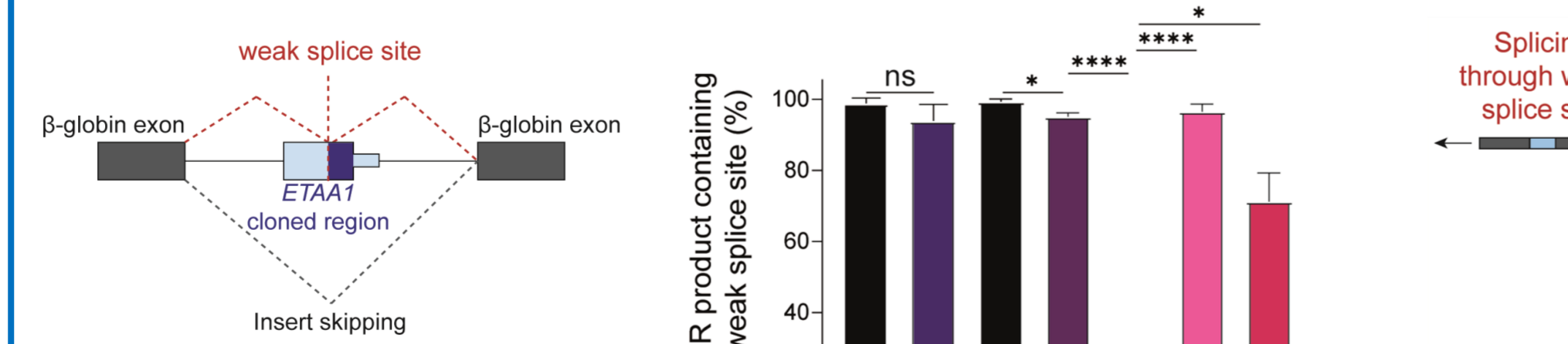
- replication fork processing
- DNA-dependent DNA replication maintenance of fidelity
- DNA-dependent DNA replication
- meiotic nuclear division
- meiotic cell cycle process
- meiotic cell cycle
- nuclear chromosome segregation
- sister chromatid segregation
- double-strand break repair via homologous recombination
- recombinational repair
- double-strand break repair
- carbohydrate derivative catabolic process

Splicing control by RBMX is required for expression of ETAA1

ETAA1 binds ssDNA at stalled replication forks and activates ATR. RNA-seq: depletion of either RBMX or the SR proteins Tra2α/β leads to use of a cryptic 3' splice site within the ETAA1 gene that produces a shorter RNA isoform



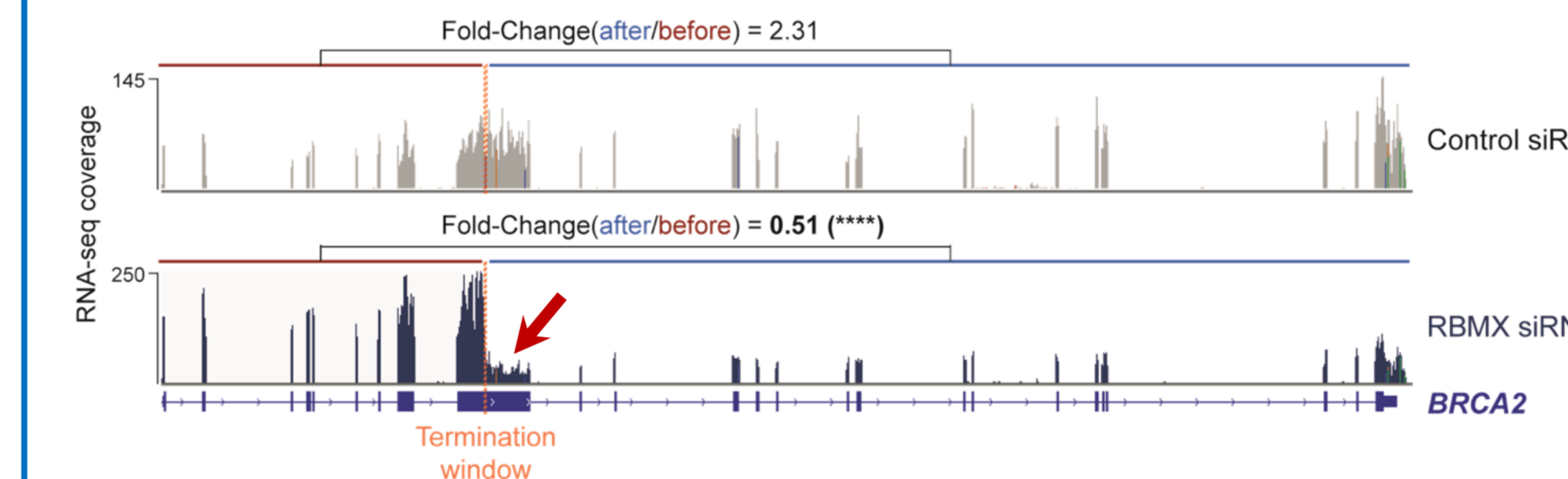
Joint overexpression of RBMX & RNA-binding Tra2β can repress use of ETAA1 cryptic splice site within a minigene reporter



RBMX is essential for full-length ETAA1 protein expression

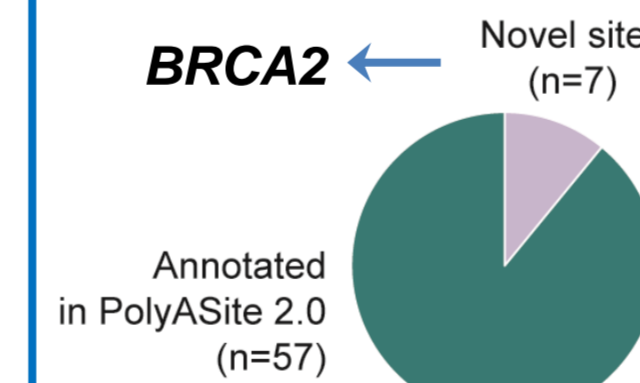
RBMX suppresses upstream polyA sites within BRCA2 and other genes important for genome stability

BRCA2 regulates homologous recombination and protects stalled replication forks. RBMX-depleted cells show drop of RNA-seq reads within the gene body of BRCA2 and other genome stability genes

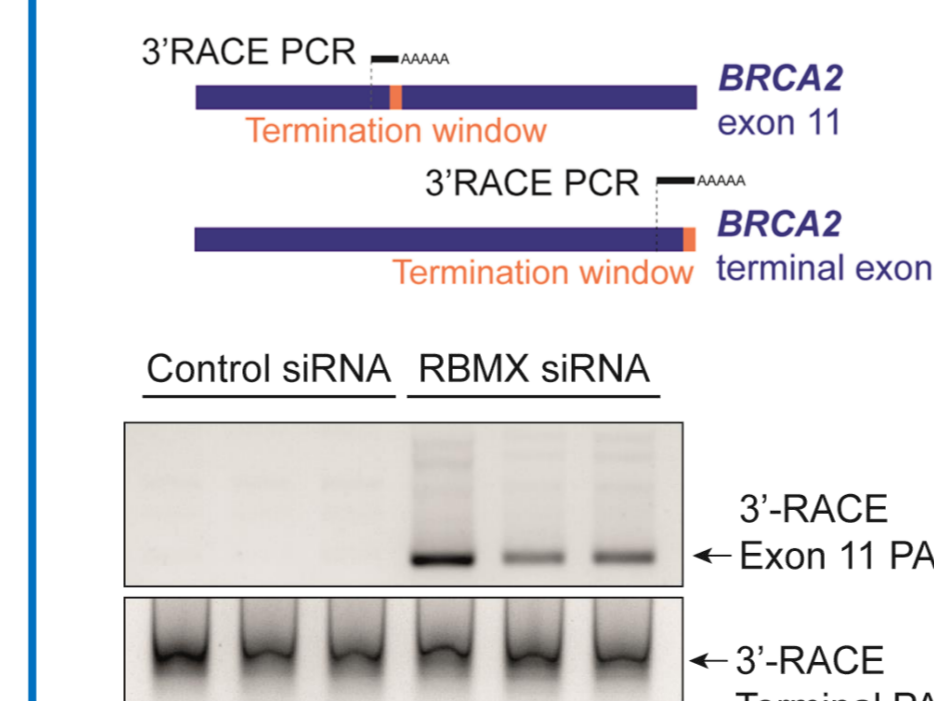


Most RNA-seq drops correspond to annotated polyA sites¹¹

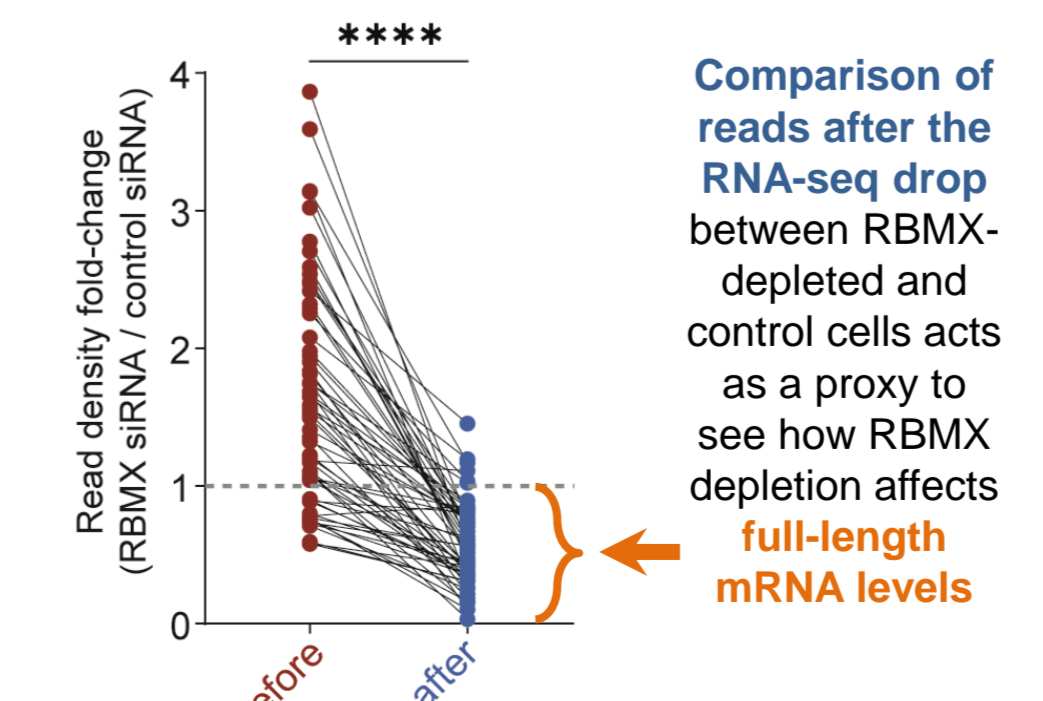
RNA-seq drop within BRCA2 exon 11 does not occur at an annotated polyA site, but still at a consensus polyA sequence



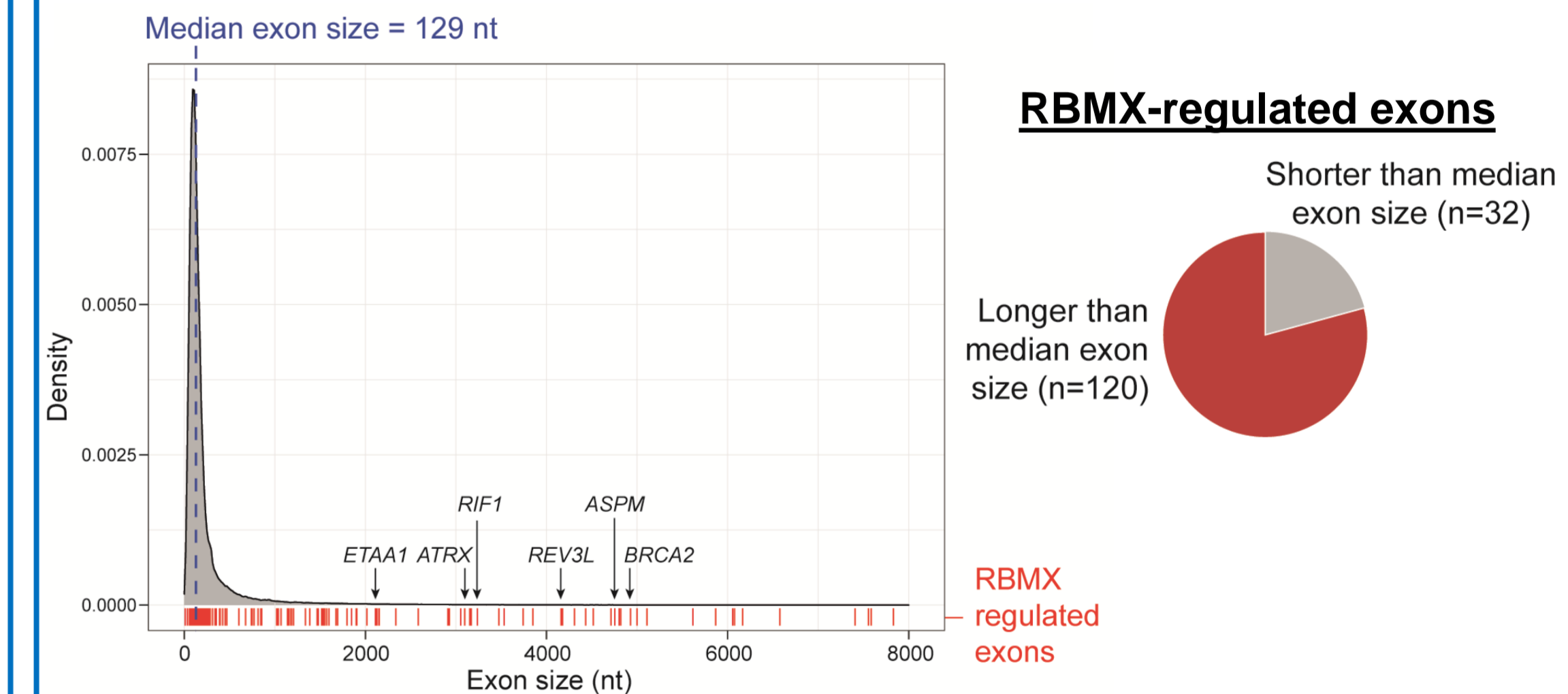
3-RACE reveals internal polyadenylation of BRCA2 exon 11 upon RBMX depletion



Downregulation of full-length mRNAs upon RBMX depletion suggests premature transcription termination



RBMX represses RNA processing sites within ultra-long exons



RBMX function in repressing cryptic RNA processing sites within long exons might help guide correct exon recognition when splice sites are very far apart

MODEL

RBMX ensures productive expression of full-length protein isoforms that maintain genome stability at replication forks

