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Regulation of Gene Expression via Unproductive Splicing

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Integrative transcriptomic analysis suggests new autoregulatory splicing events coupled with nonsense-mediated mRNA decay

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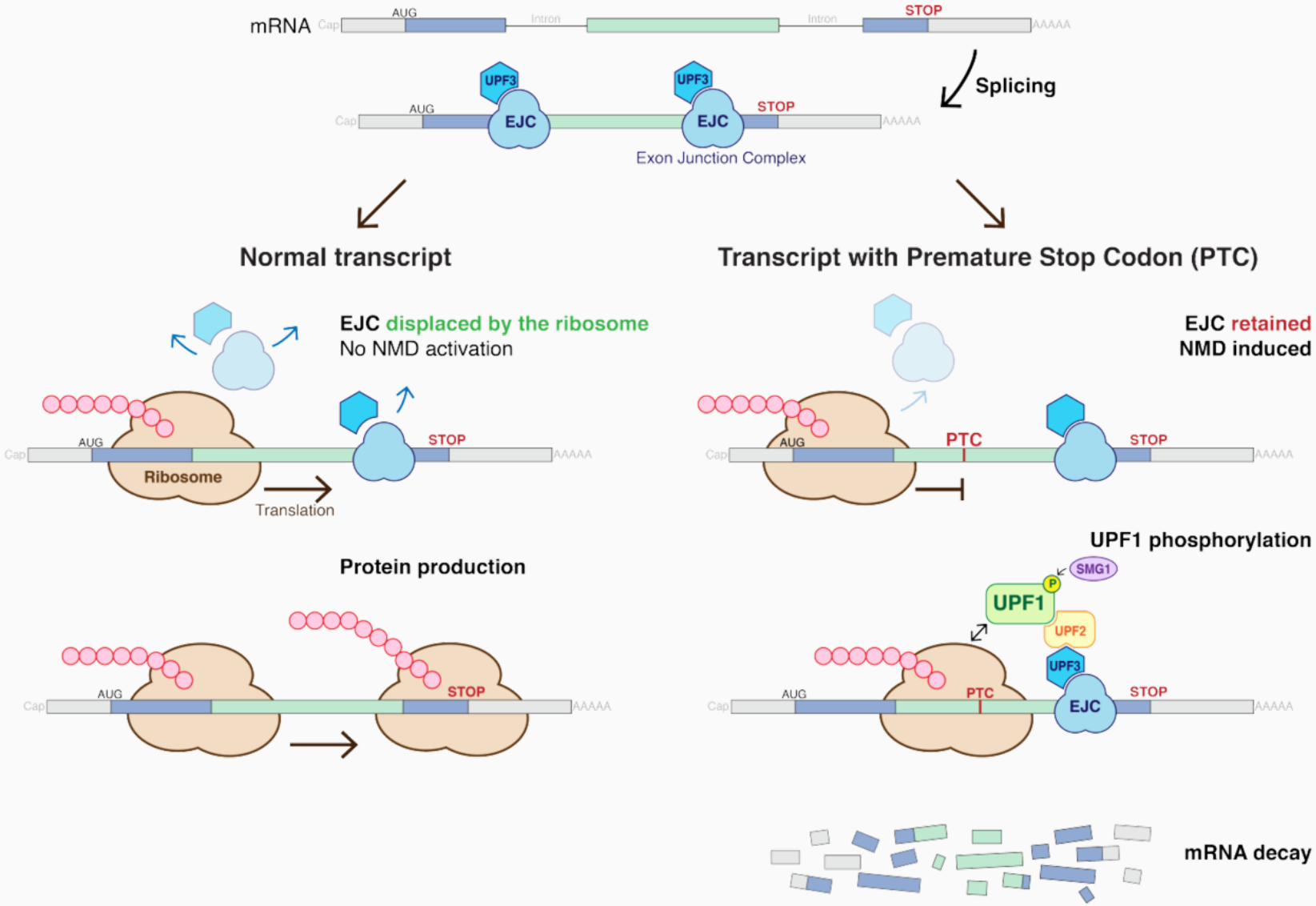
ABSTRACT

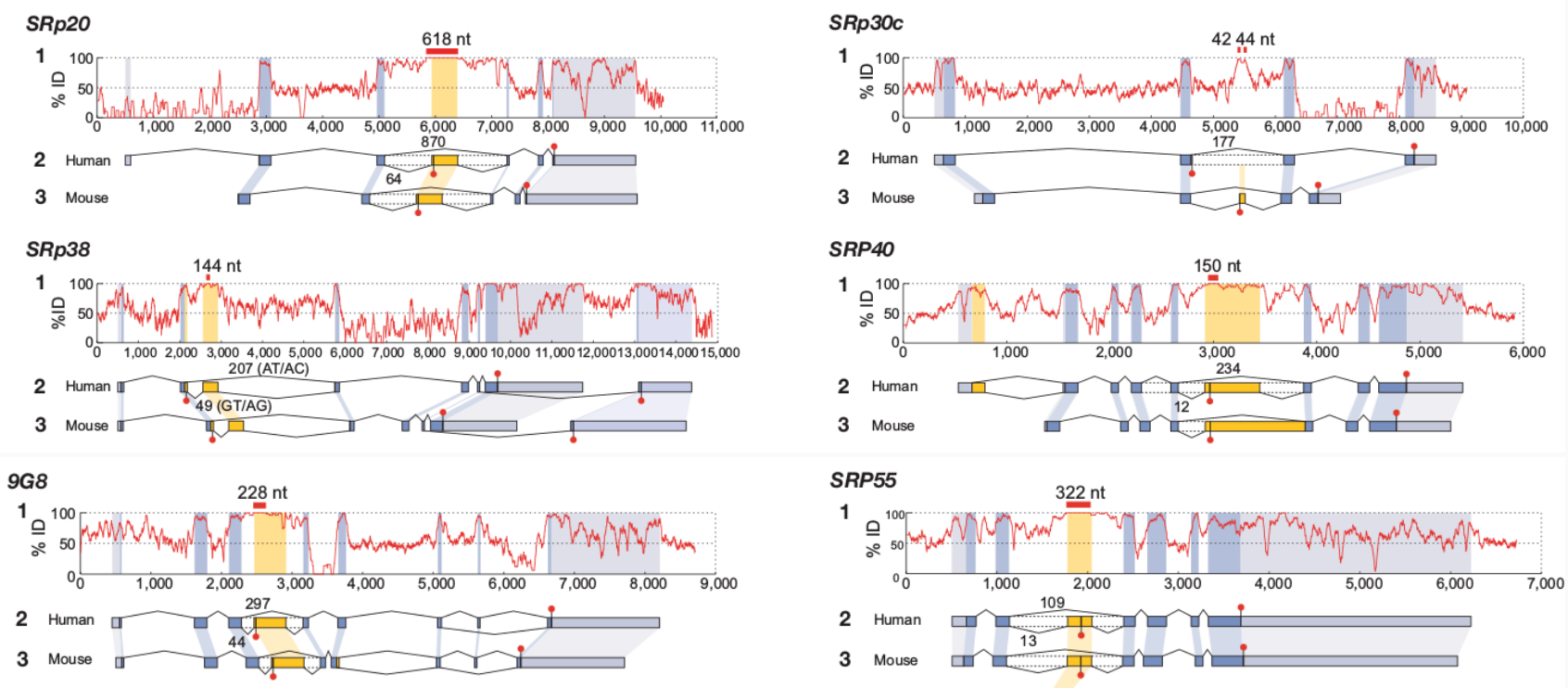
Nonsense-mediated decay (NMD) is a eukaryotic mRNA surveillance system that selectively is maintained by a large number of protein factors and *cis*-regulatory elements, which control the balance between mRNA production and degradation (1,2). Nonsense mutations and frame-shifting splicing errors induce premature

Nonsense-mediated mRNA decay[1]



[1]Kurosaki T, Maquat LE., Nonsense-mediated mRNA decay in humans at a glance., J Cell Sci. 2016 129(3):461-7

AS-NMD Events Associated With Ultraconserved DNA Elements[2]

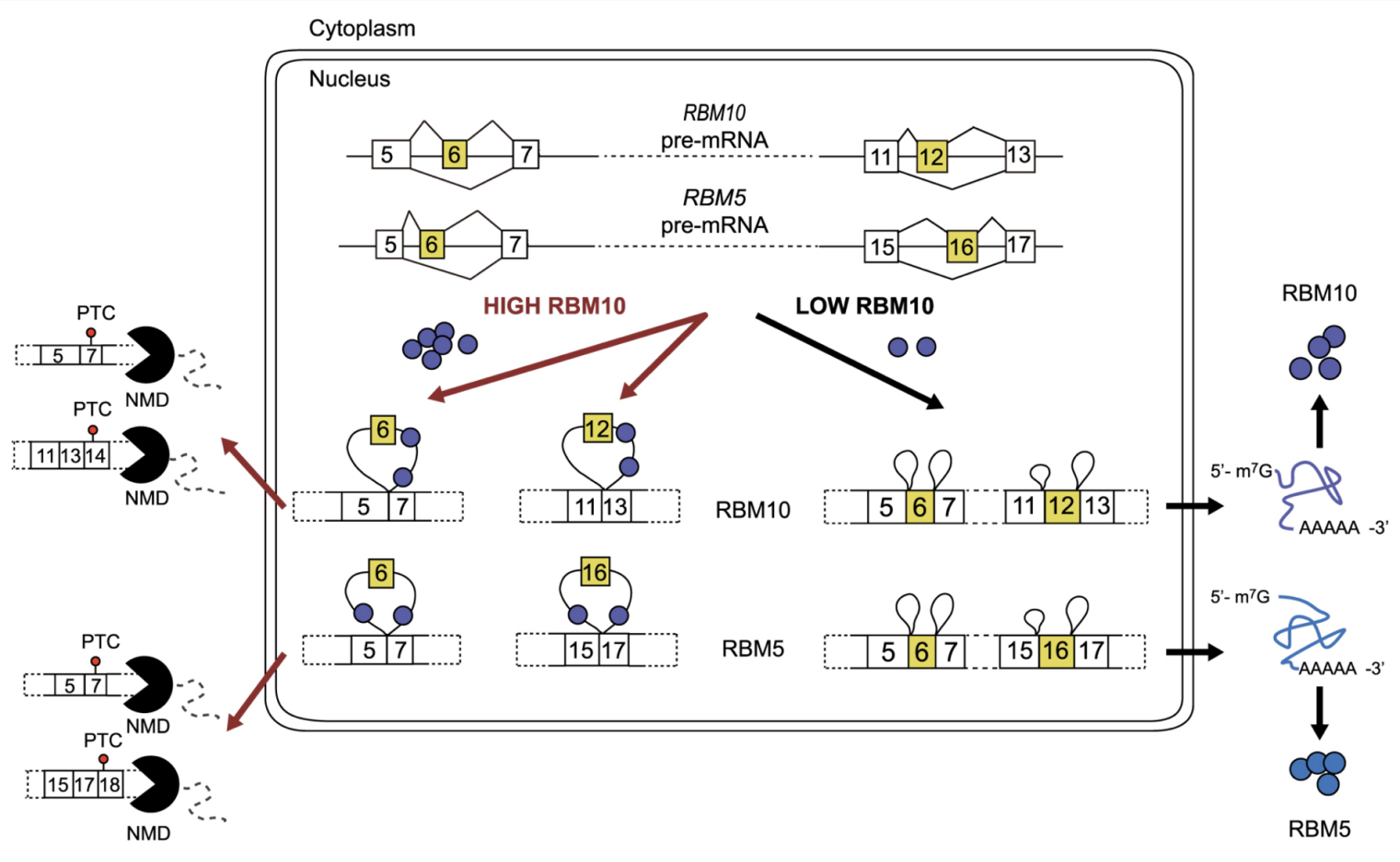


- AS-NMD is in every member of the human SR family
- Poison exons have evolved independently in most SR genes

[2]Lareau et al, Unproductive splicing of SR genes associated with highly conserved and ultraconserved DNA elements. Nature, 446(7138), 926-9.

Autoregulation of RBP by Nonsense-Mediated Decay (NMD)

- **Poison** exons cause NMD when included
- **Essential** exons cause NMD when skipped
- Exons 6 and 12 of RBM10 gene are essential[3]



[3] Yue Sun *et al.* NAR 45(14): 8524–8540, 2017

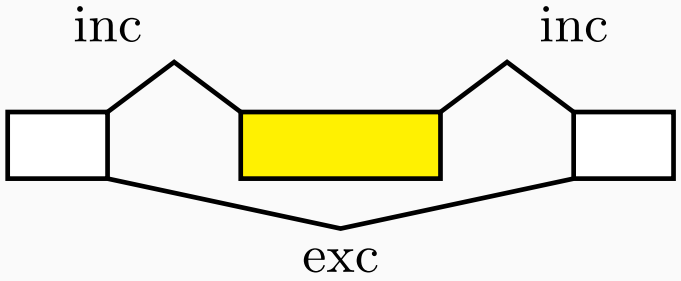
Can we identify autoregulatory feedback loops?

- Inactivation of NMD $\rightarrow$ poison and essential exons[1]
- RBP perturbation followed by RNA-seq $\rightarrow$ regulated exons[2]
- CLIP $\rightarrow$ RBP binding to RNA[2]

[1] Lykke-Andersen et al. Human NMD initiates widely by endonucleolysis and targets snoRNA host genes. Genes Dev. 2014

[2] Van Nostrand et al. A large-scale binding and functional map of human RNA-binding proteins. Nature 2020)

Percent-Spliced-In (PSI, Ψ)

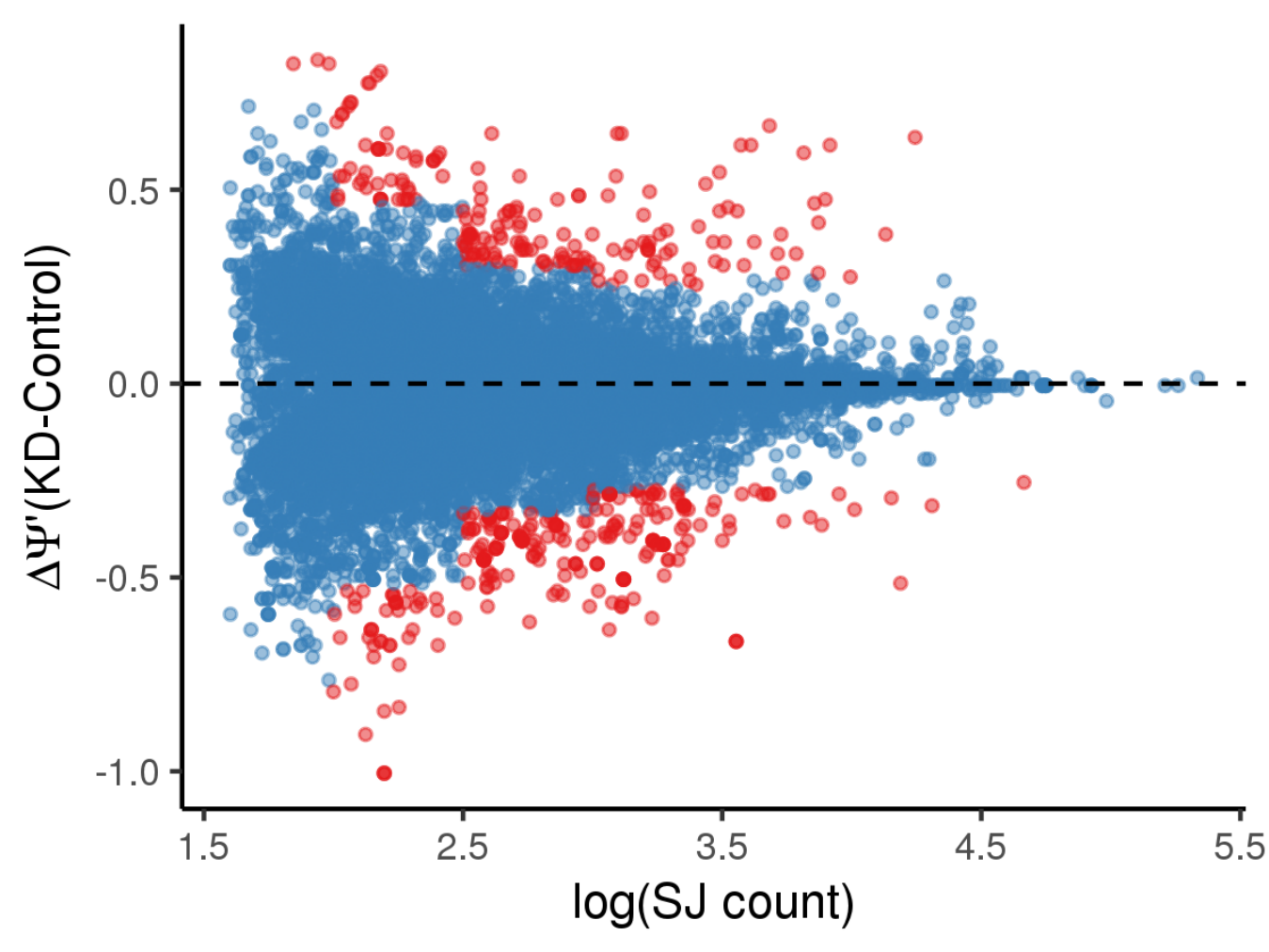


$$PSI = \Psi \simeq \text{proportion of transcripts}$$

$$\Psi = \frac{inc}{inc + exc}$$

$$SJ = inc + exc \simeq \text{local expression level}$$

Statistical Significance of Ψ



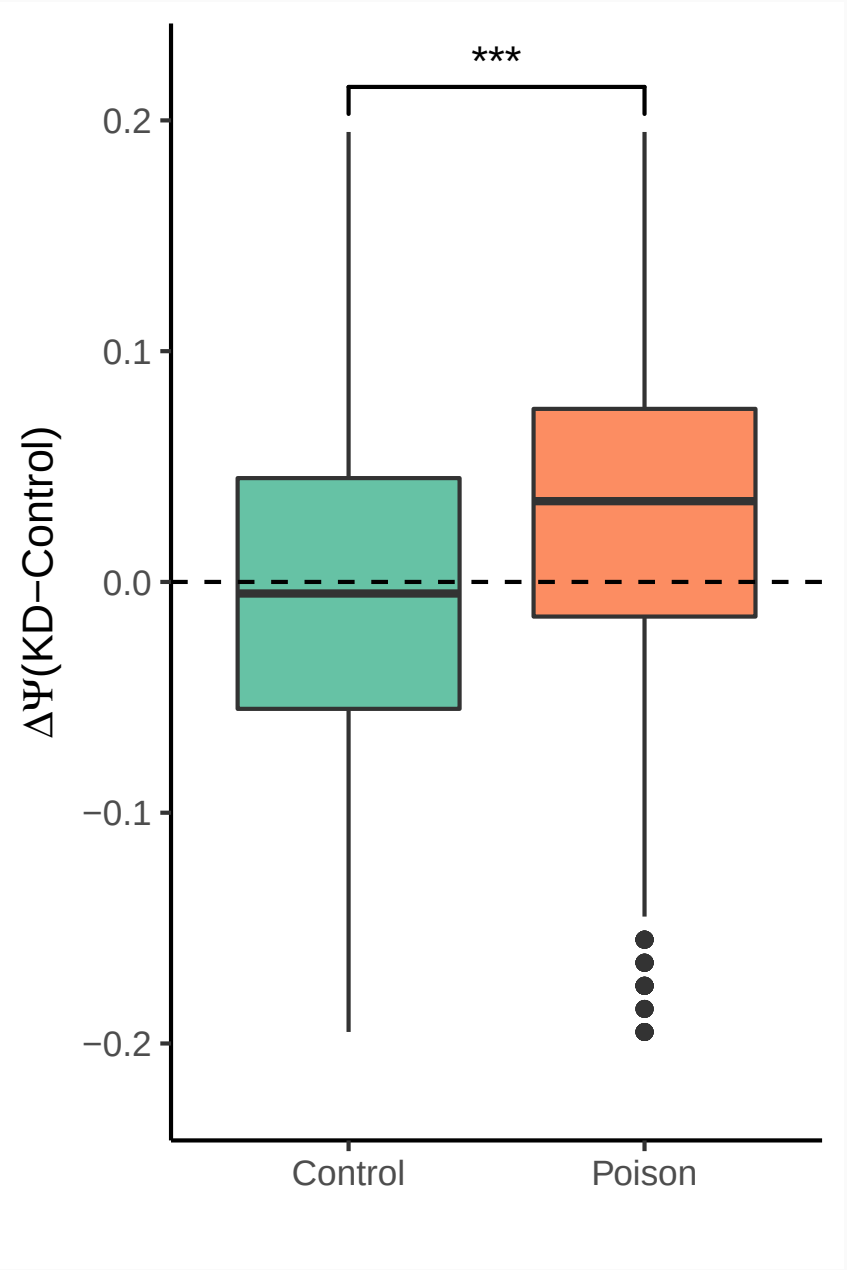
$$\Delta\Psi = \beta_0 + \beta_1 log_{10}(SJ) + e_i \rightarrow \text{residuals}$$

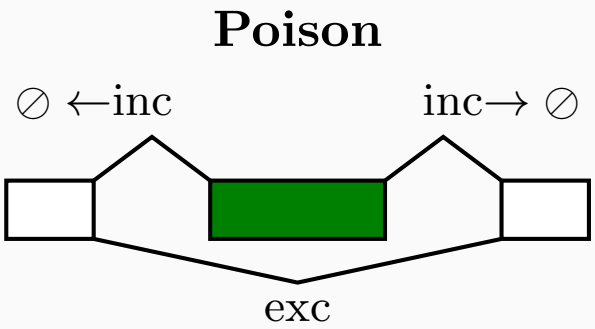
$$z = \frac{\Delta\Psi - \mu(\text{SJ})}{\sigma(\text{SJ})} \rightarrow \text{p-value} \rightarrow \text{q-value}$$

Splicing Factors Respond to UPF1/XRN1 Co-depletion

$$\Delta\Psi = \Psi(KD) - \Psi(Control)$$

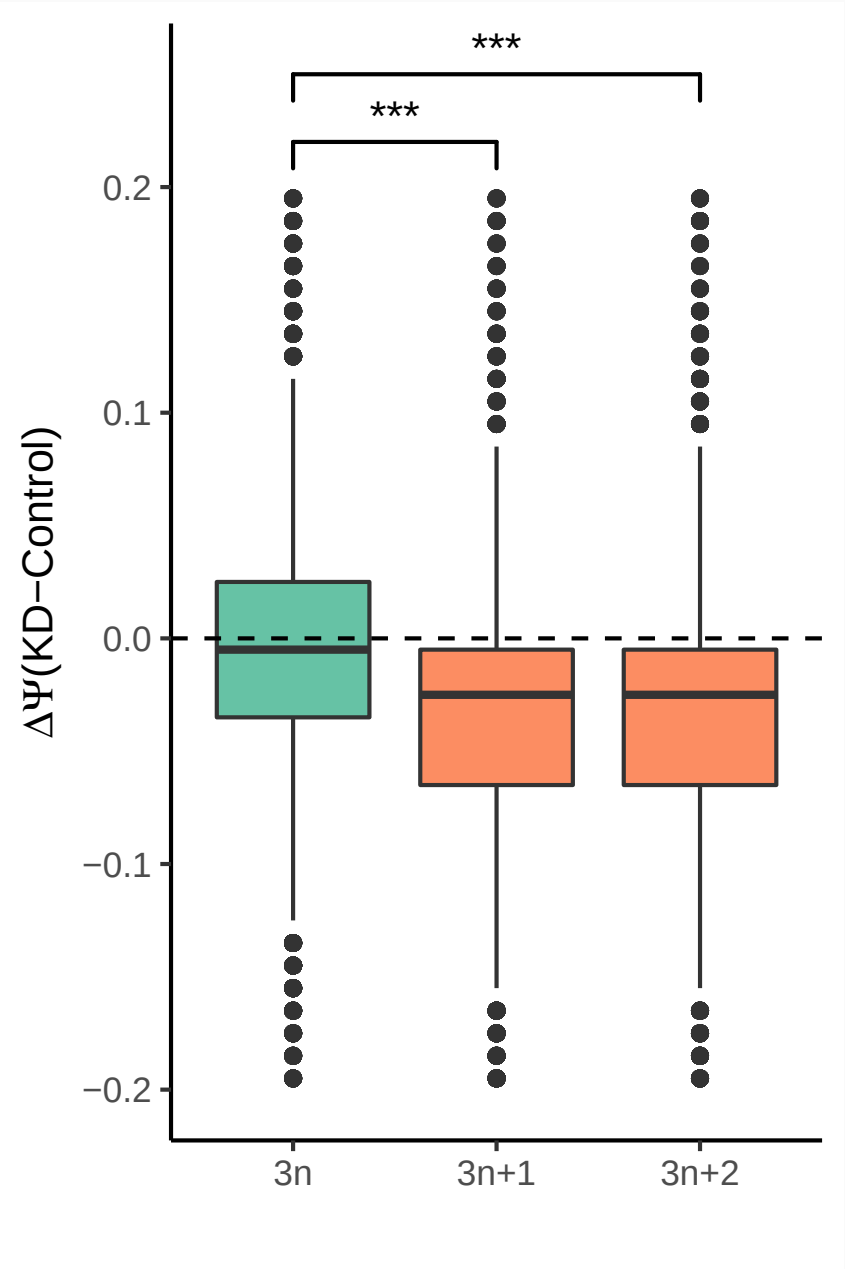
Poison exons are more included upon NMD inactivation



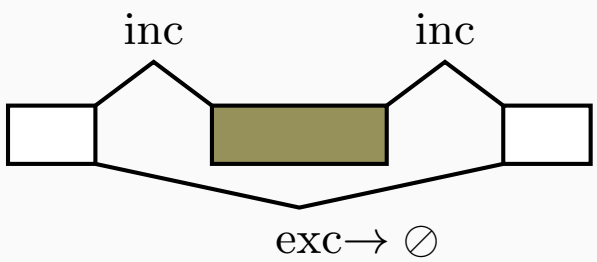


$$\Delta\Psi > 0$$

Essential exons are more skipped upon NMD inactivation

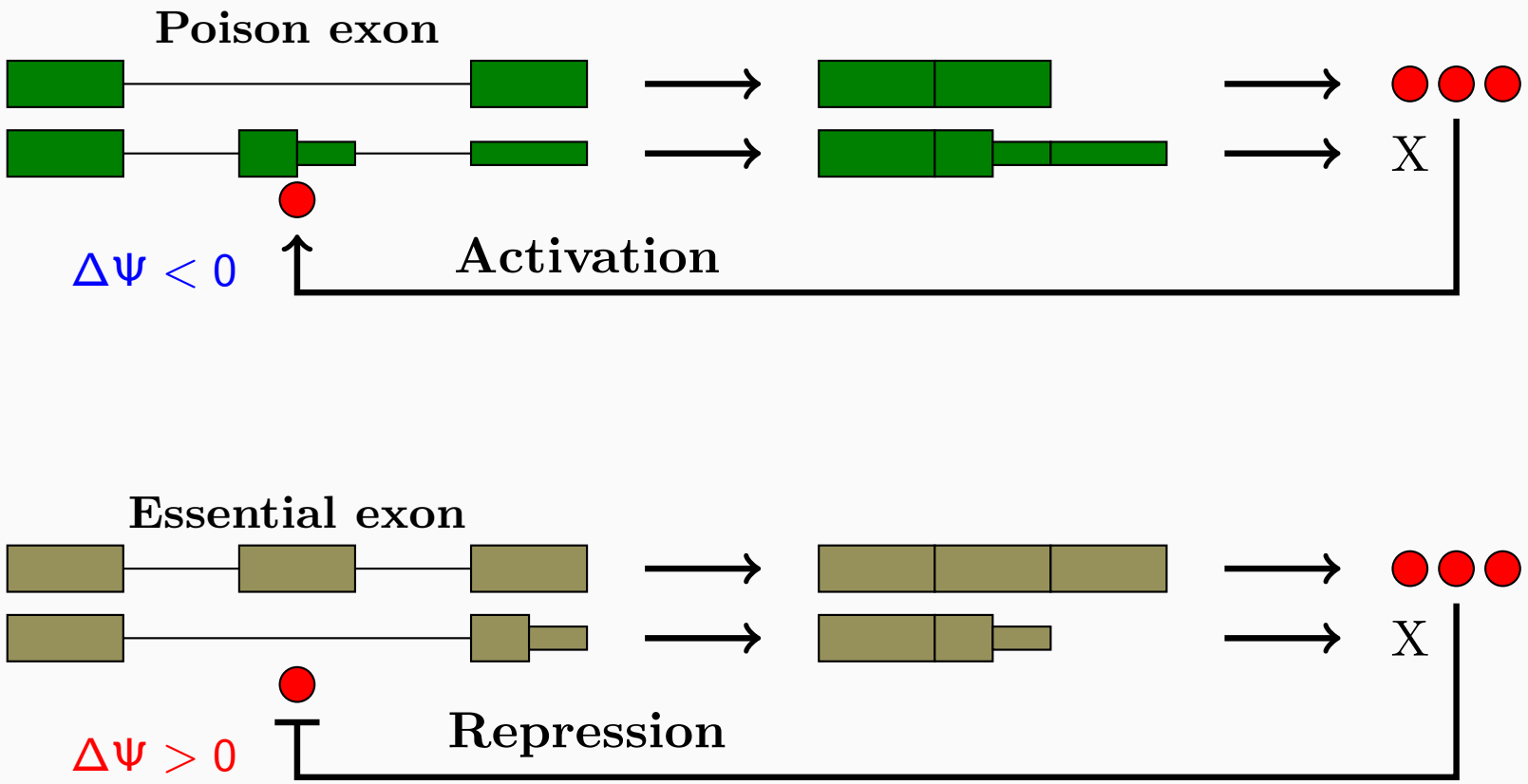


Essential

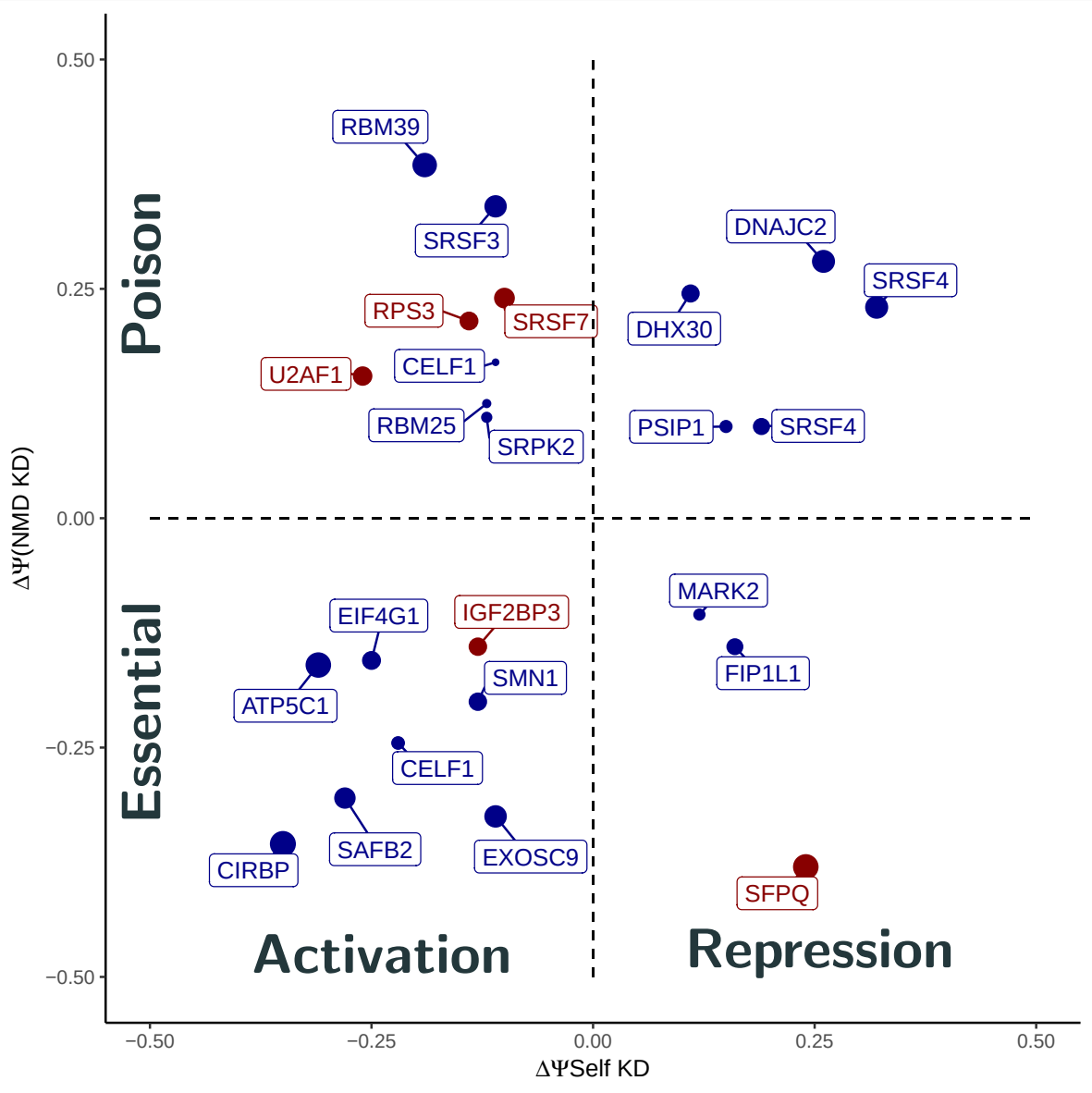


$$\Delta\Psi < 0$$

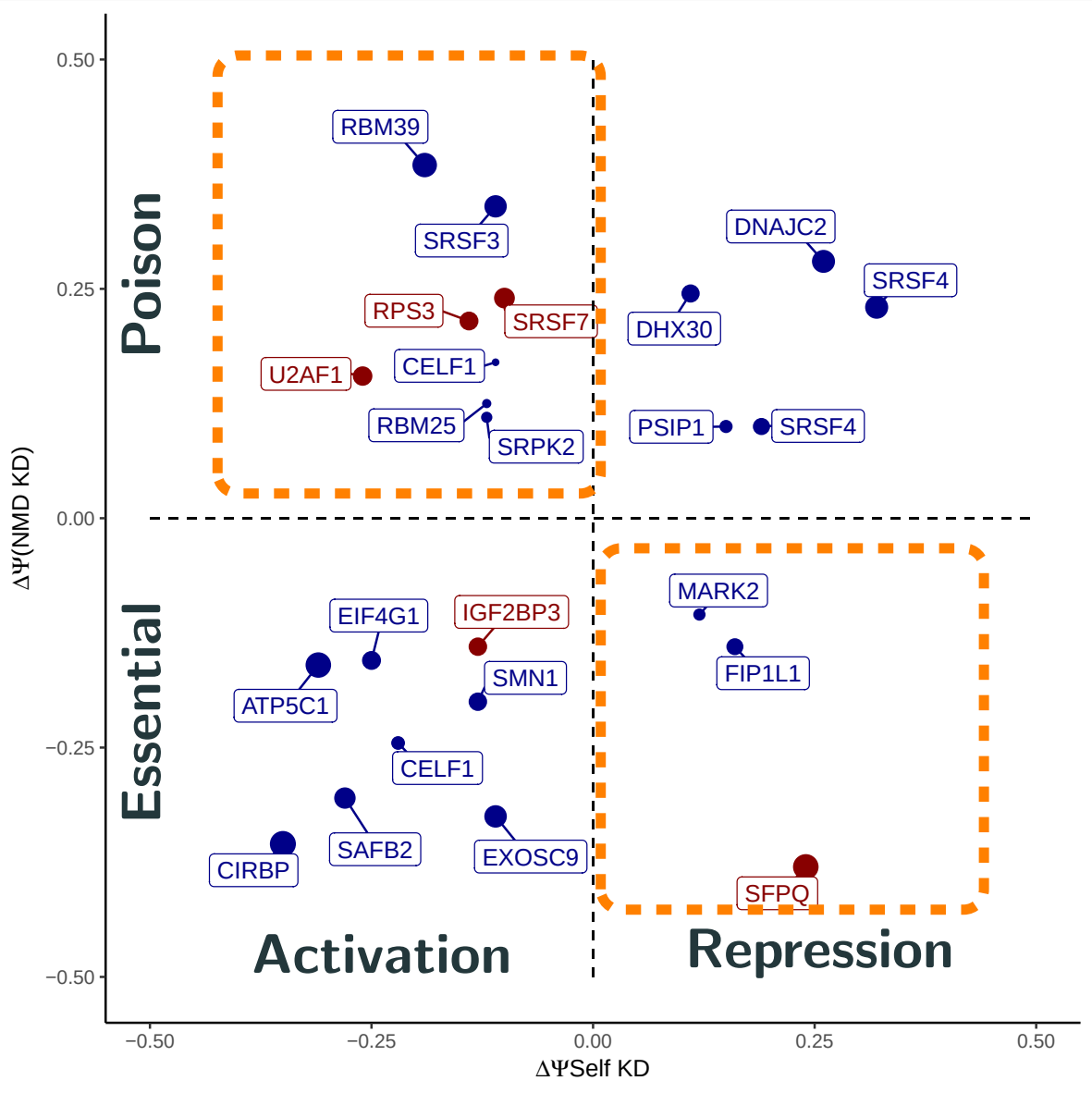
Response to the depletion of the host gene in a negative feedback loop



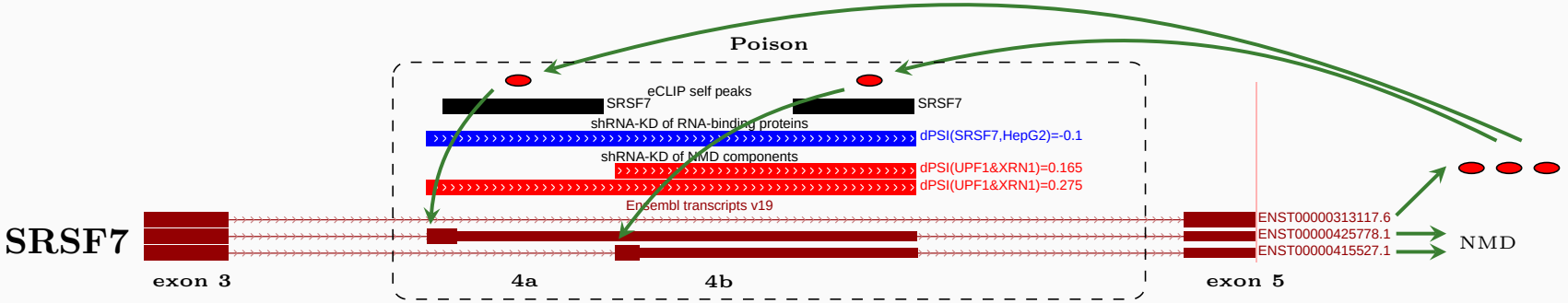
NMD inactivation, depletion of host gene, and eCLIP



NMD inactivation, depletion of host gene, and eCLIP



Serine And Arginine Rich Splicing Factor 7 (SRSF7)



- Splicing factor important for nuclear export and translation
- Overexpressed in colon and lung cancer tissues
- SRSF7 knockdown promotes apoptosis of colon and lung cancer cells
- SRSF7 regulates the splicing of the apoptosis regulator Fas
- SRSF7 maintains its homeostasis through the expression of Split-ORFs and nuclear body assembly (Königs et al, Nat Struct Mol Biol. 2020 Mar;27(3):260-273)

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Tissue-specific regulation of gene expression via unproductive splicing

Alexey Mironov, Maria Vlasenok, Sergei Margasyuk, Andrei A. Mironov, Dmitri D. Pervouchine

doi: https://doi.org/10.1101/2022.07.03.498634

This article is a preprint and has not been certified by peer review [what does this mean?].

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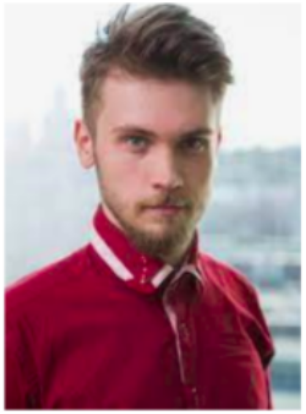
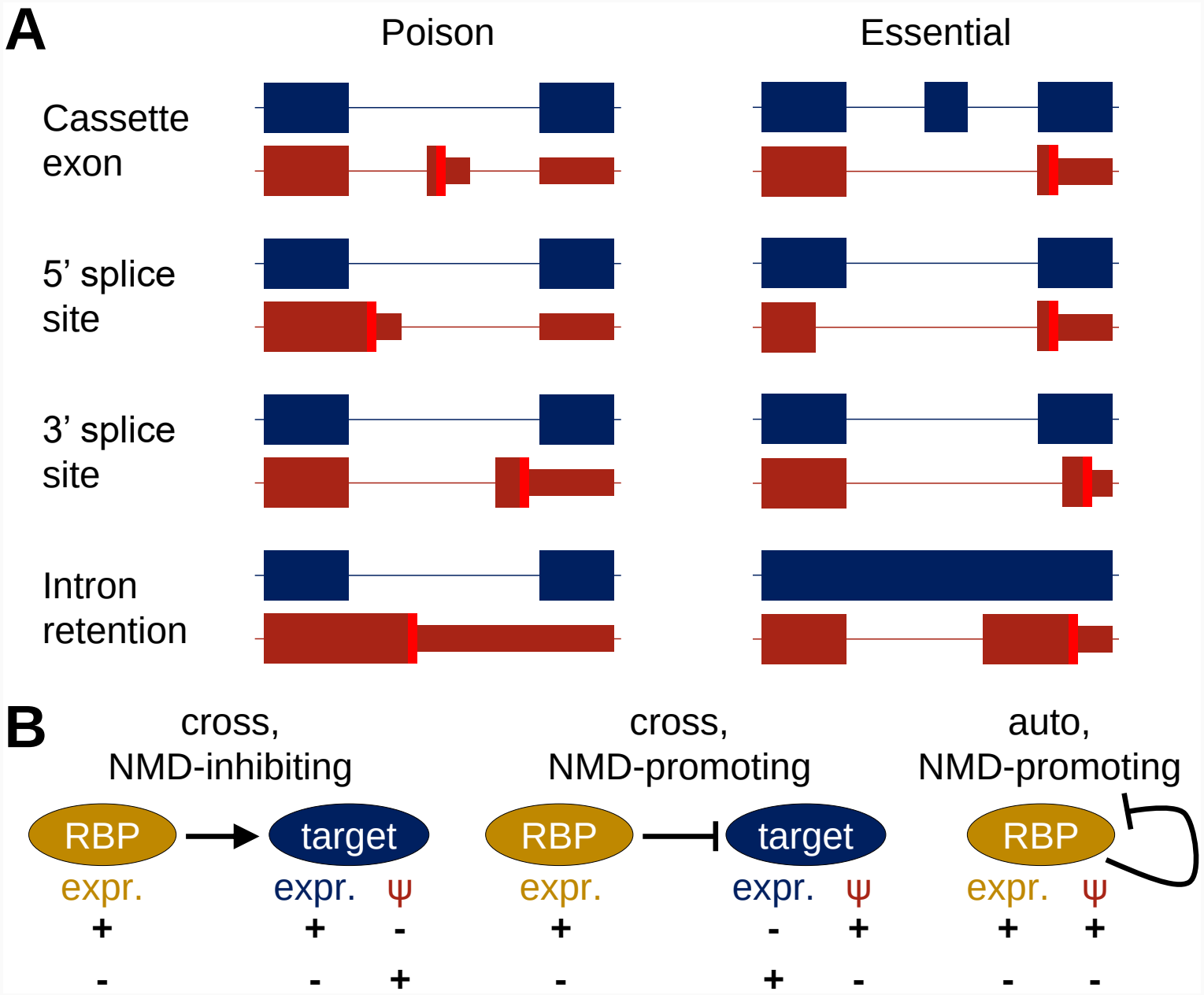
Abstract

Eukaryotic gene expression is regulated post-transcriptionally by a universal mechanism called unproductive splicing, in which mRNA is triggered to degradation by the nonsense-mediated decay (NMD) pathway as a result of alternative splicing (AS). Only a few dozen unproductive

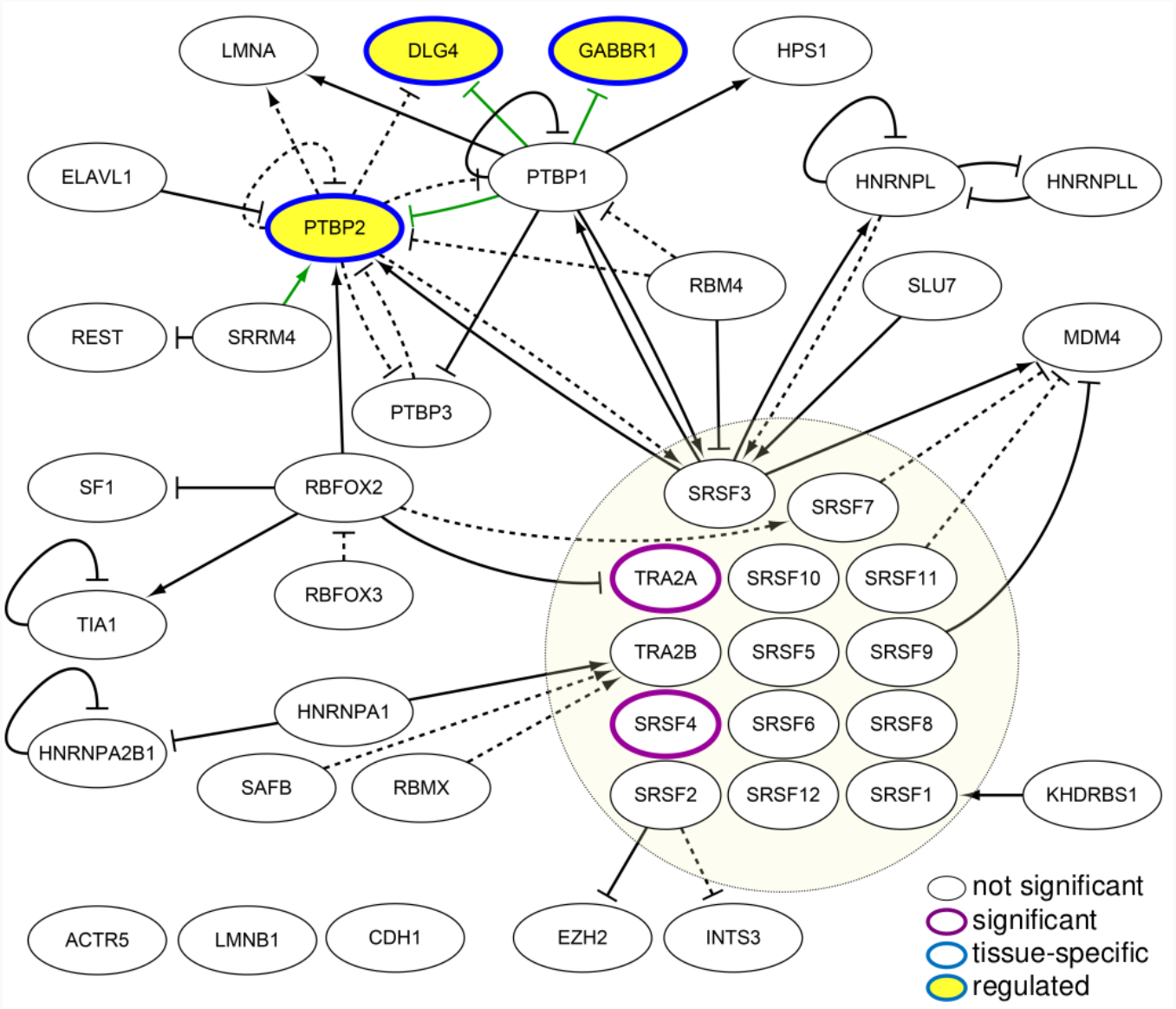


GTEx

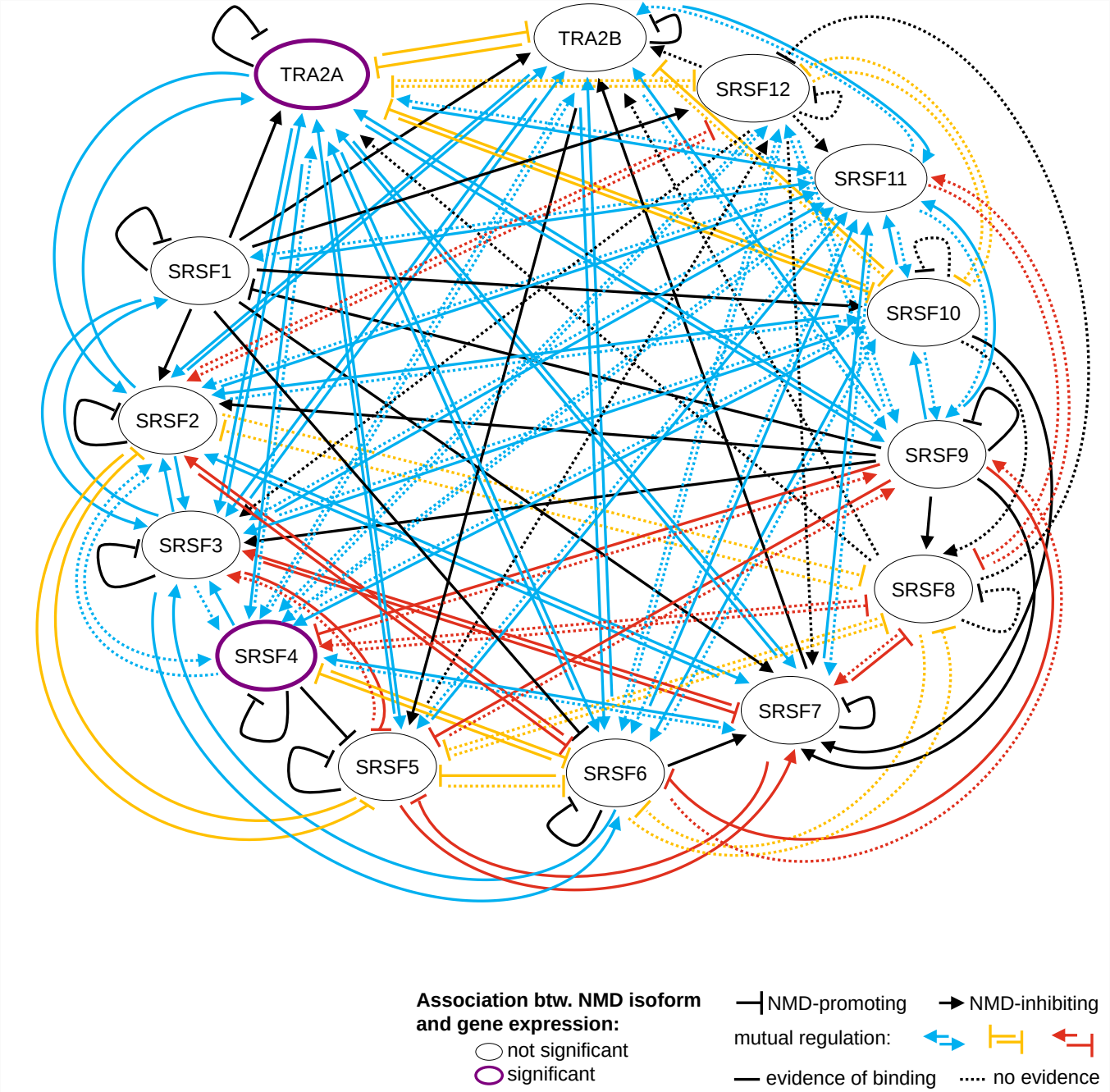
Unproductive Splicing Events (USE)



Validated Unproductive Splicing Events

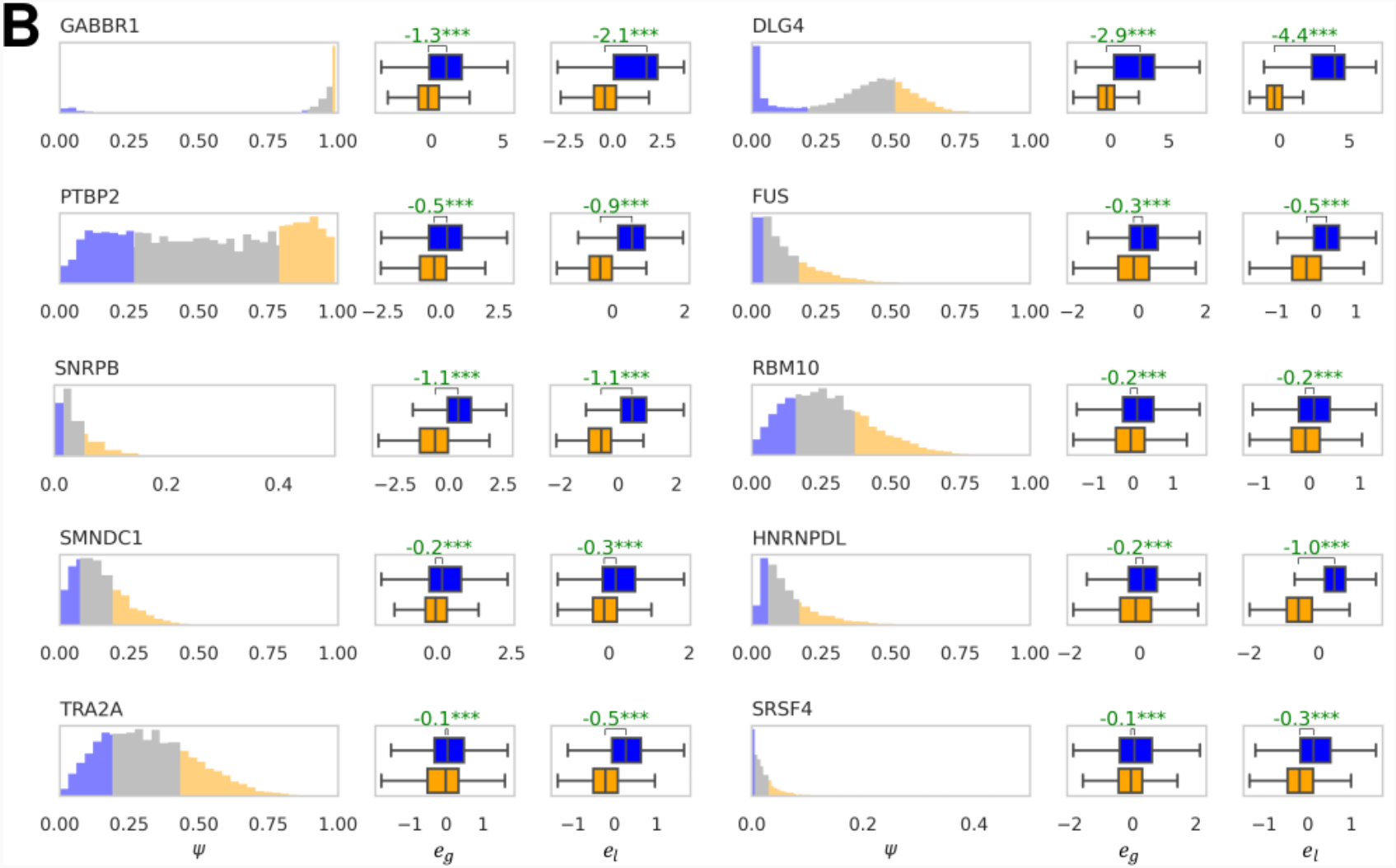


Validated Unproductive Splicing Events in SR proteins

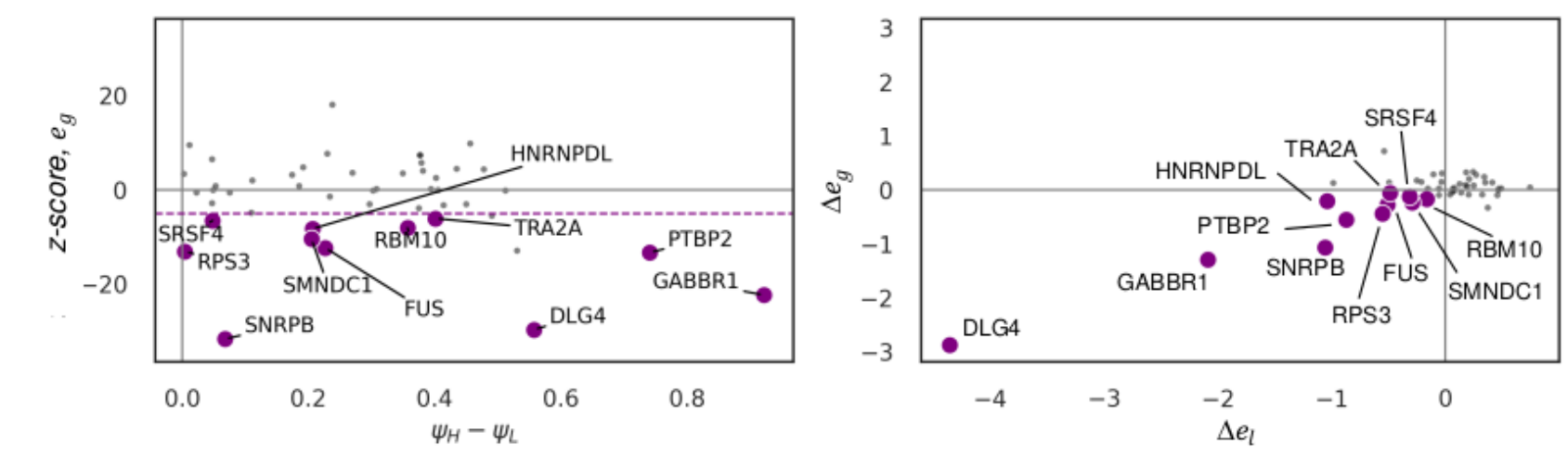


Validated Unproductive Splicing Events in GTEx

Estimating changes in gene expression between the upper and the lower quartile of Ψ distribution using Mann-Whitney U-test



Negative association between Ψ and host gene expression level



Ψ_H = median of the upper quartile

Ψ_L = median of the lower quartile

Δe_l = gene expression change (local)

Δe_g = gene expression change (global)

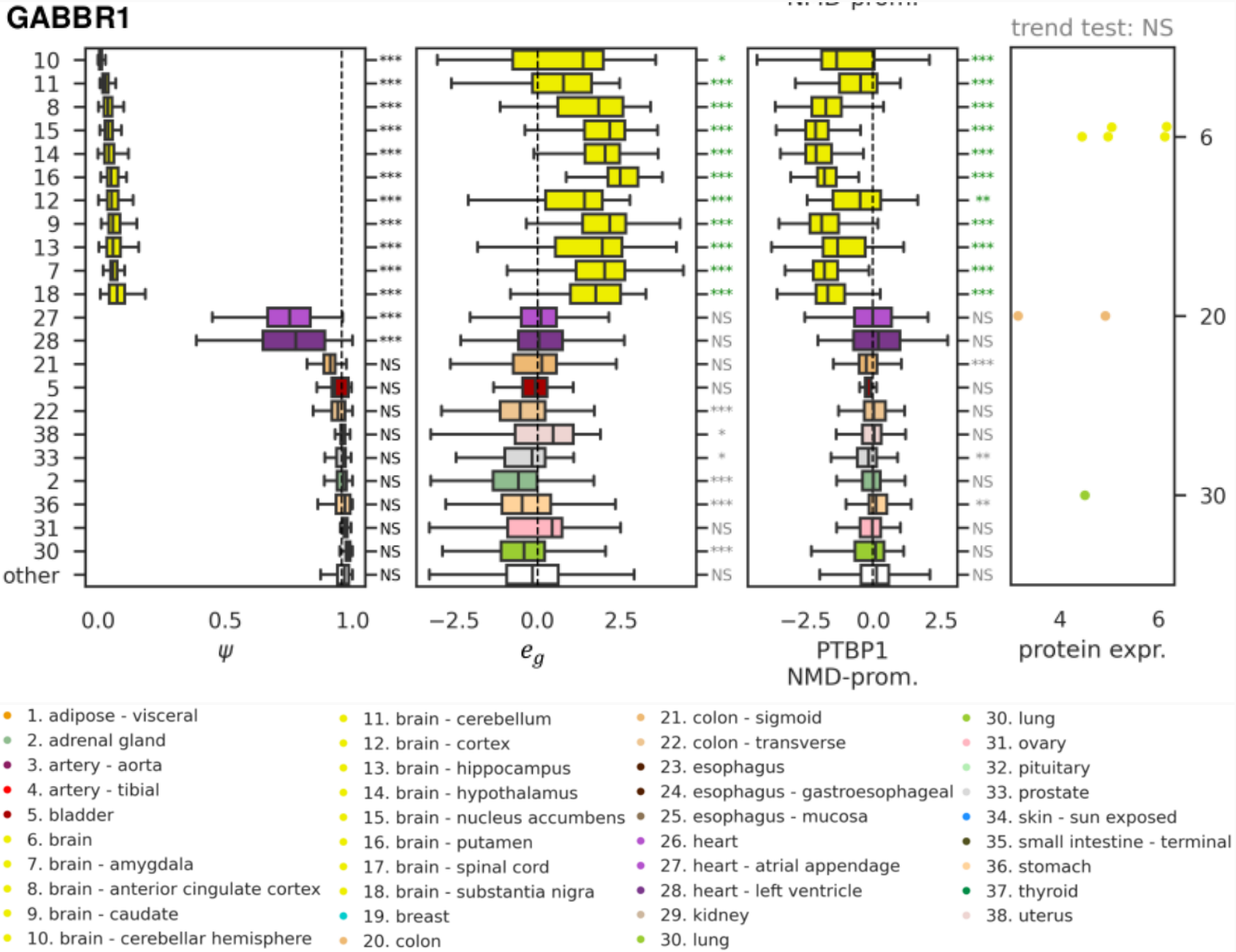
z = z-score of Mann-Whitney test for e_g

Prediction of regulation by RBP

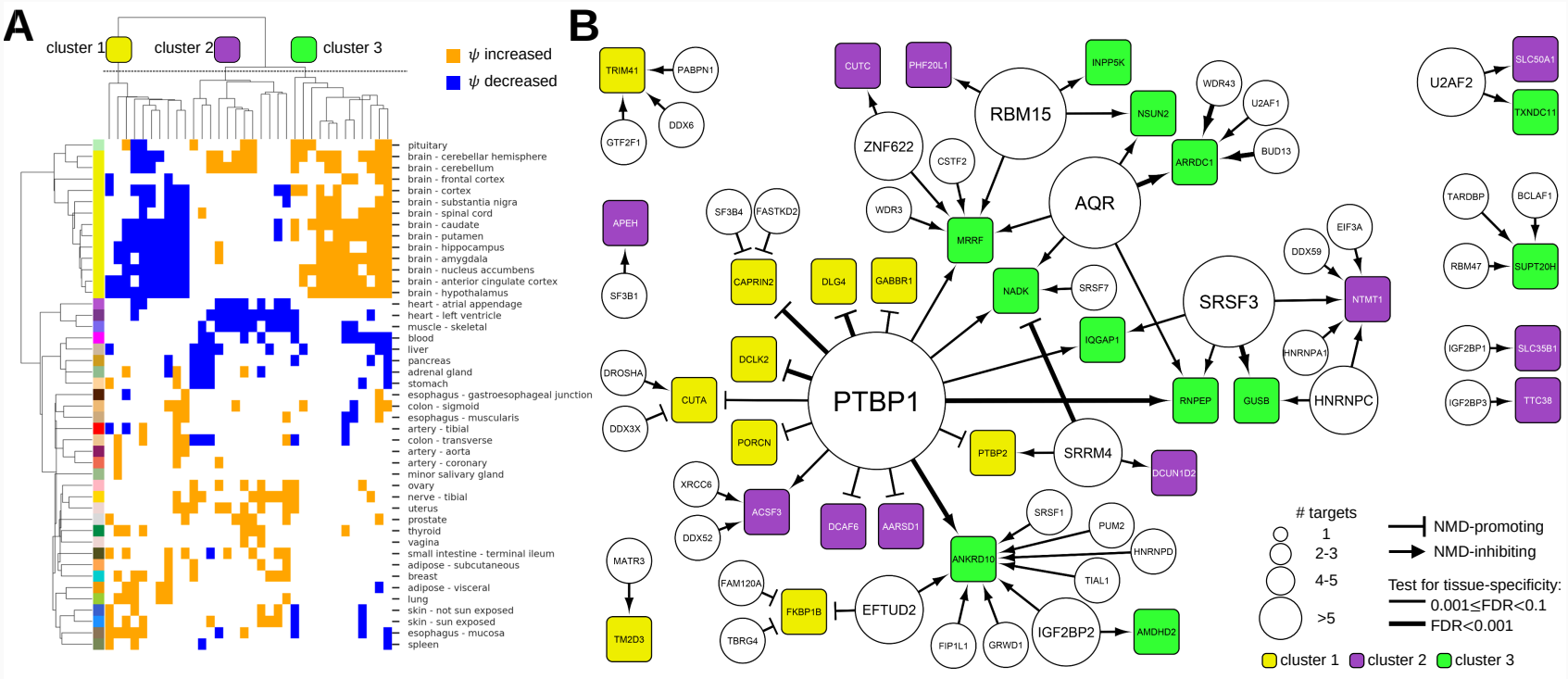
- Association of Ψ and e_g in GTEx: unproductive splicing
- Response of Ψ to RBP perturbations: potential regulators
- Association of RBP expression and e_g in GTEx: candidate regulators
- Additional evidence from CLIP, proteomics data etc

Unproductive Splicing	Validated	Novel	Total
All	48	2,831	2,879
Significant	11	568	579
Tissue-specific	5	86	91
Regulated	3	47	50
CLIP in the gene	3	31	34
Local CLIP support	3	14	17

Brain-specific expression of GABBR1



Predicted network of unproductive splicing events

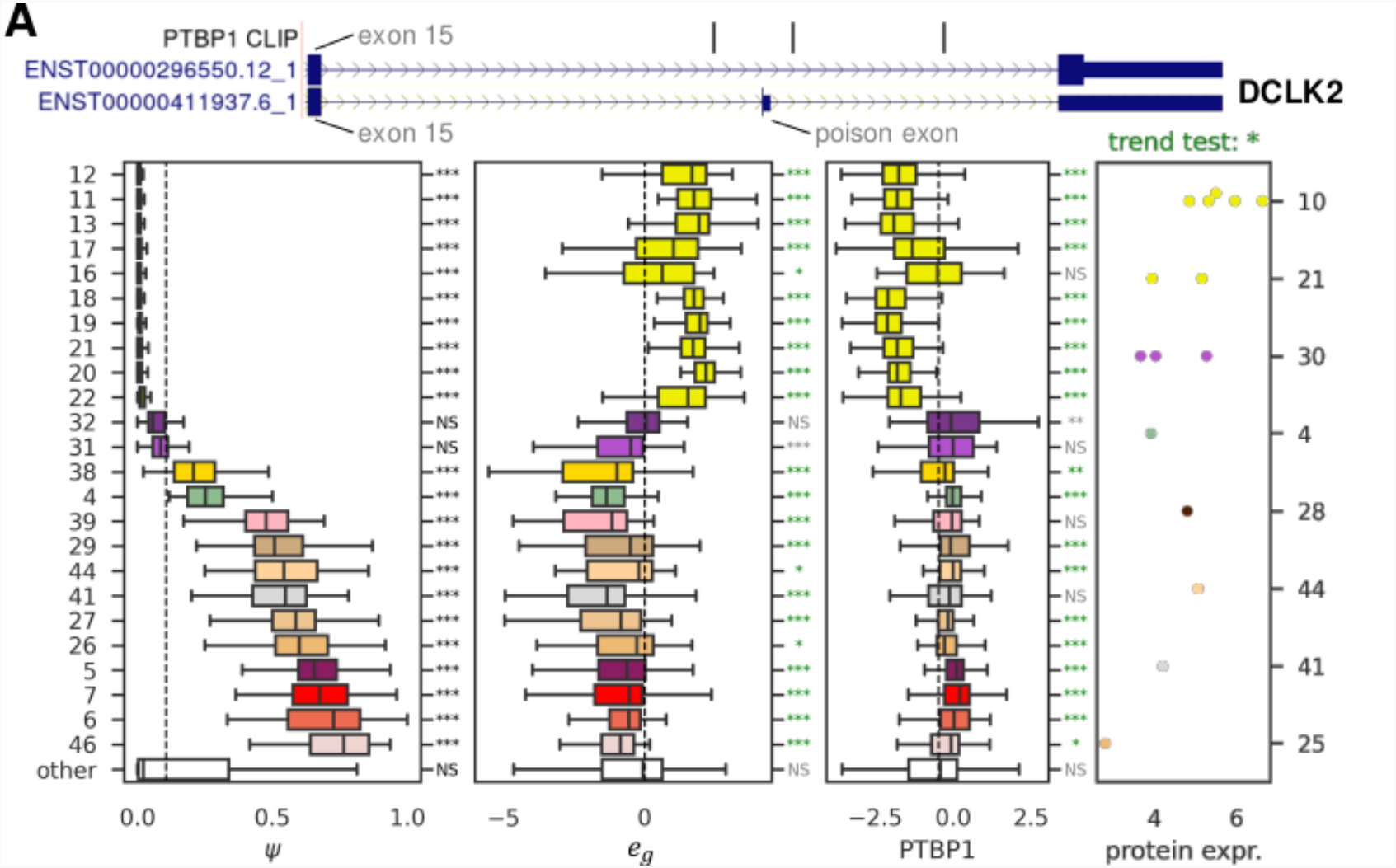


cluster 1: increased Ψ in the brain

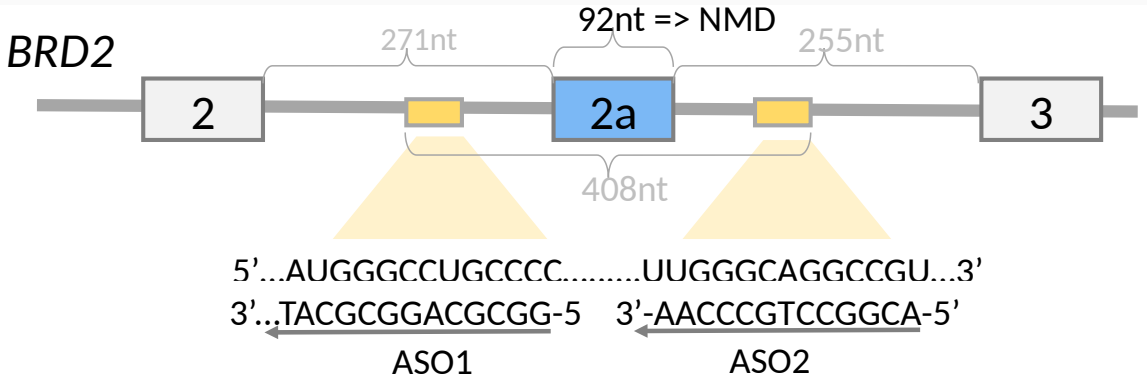
cluster 2: increased Ψ in the muscle

cluster 3: decreased Ψ in the brain

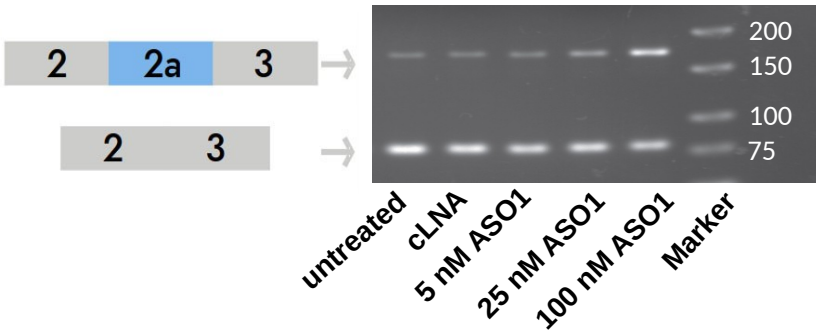
Brain-specific expression of DCLK4 is regulated by PTBP1 (unpublished)



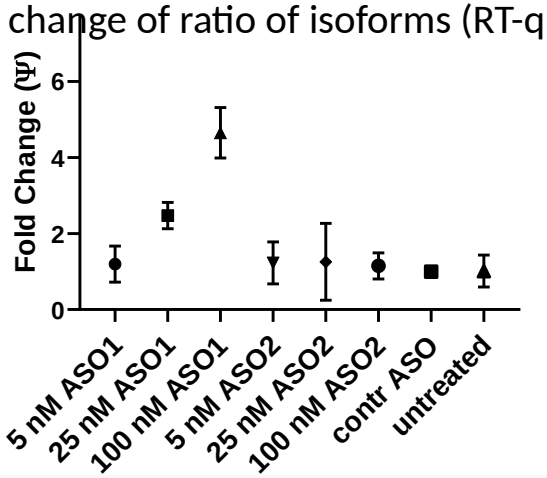
BRD2: long-range RNA structure around poison exon (unpublished)



BRD2 isoforms after ASO1 treatment (PCR)



Fold change of ratio of isoforms (RT-qPCR)



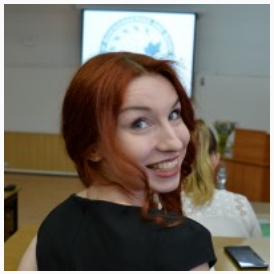
Credits to Marina Petrova and Dmitry Skvortsov

Summary

- Auto- and cross-regulatory networks of unproductive splicing can be identified using large panels of transcriptomic data
- Integrative analysis of transcriptomic data brings novel insights into the structure of regulatory unproductive splicing networks, i.e., identification of novel targets and regulators
- RNA structure is involved in unproductive splicing regulation, possibly mediating the connection between protein binding and alternative splicing
- Positive feedback loops?
- Many other questions...

Acknowledgments

Sveta Kalmykova



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Marina Petrova

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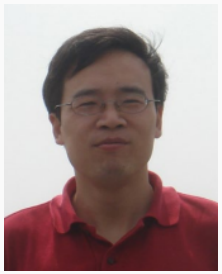

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