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Identify causal variants and estimate their effects on splicing in a Massively Parallel Splicing Assay (MaPSy)

Will Fairbrother
Hassenfeld Professor
MCB Department
Brown University

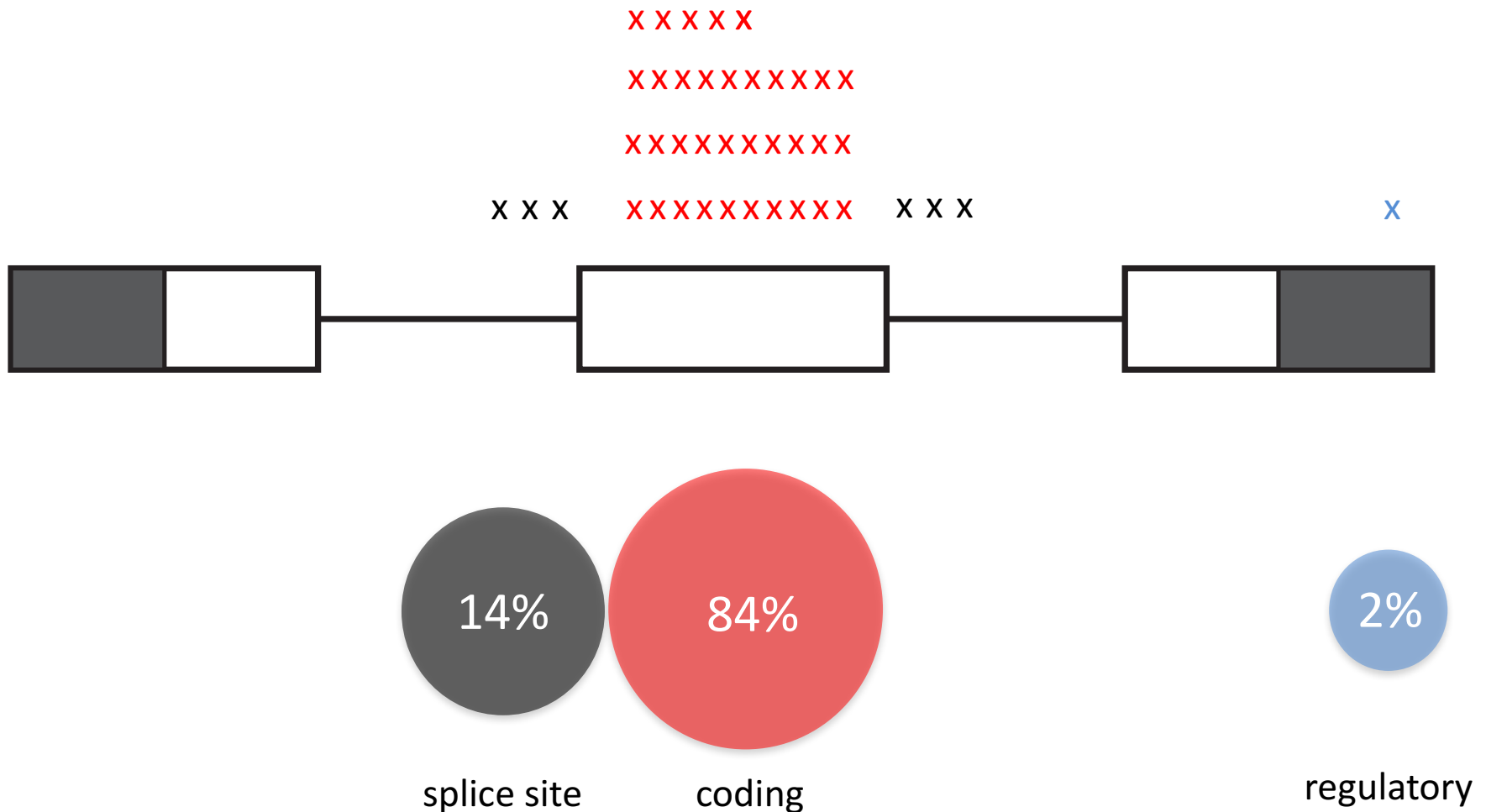


BROWN



Summary of mutation types in Human Gene Mutation Database

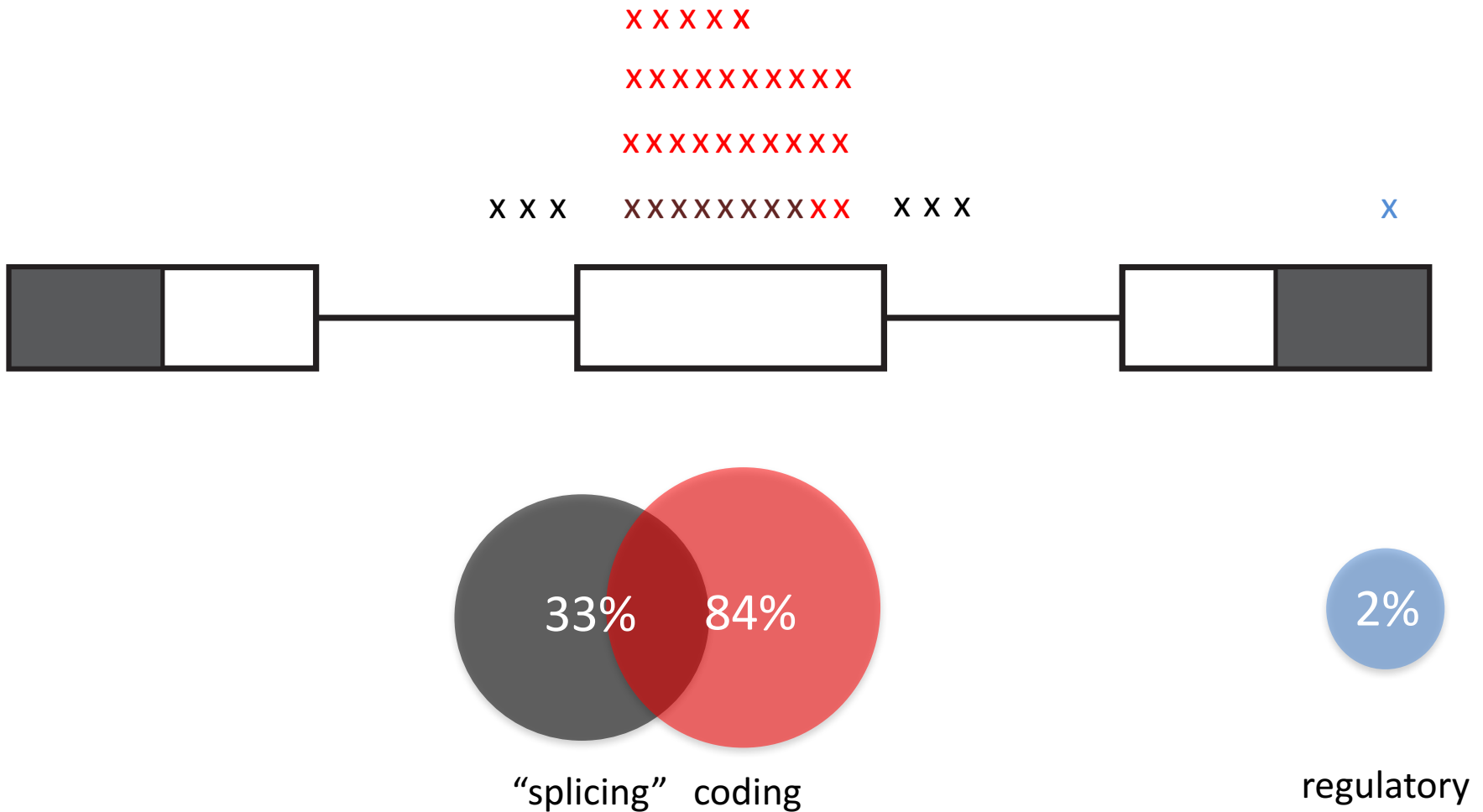
HGMD,
public 2008



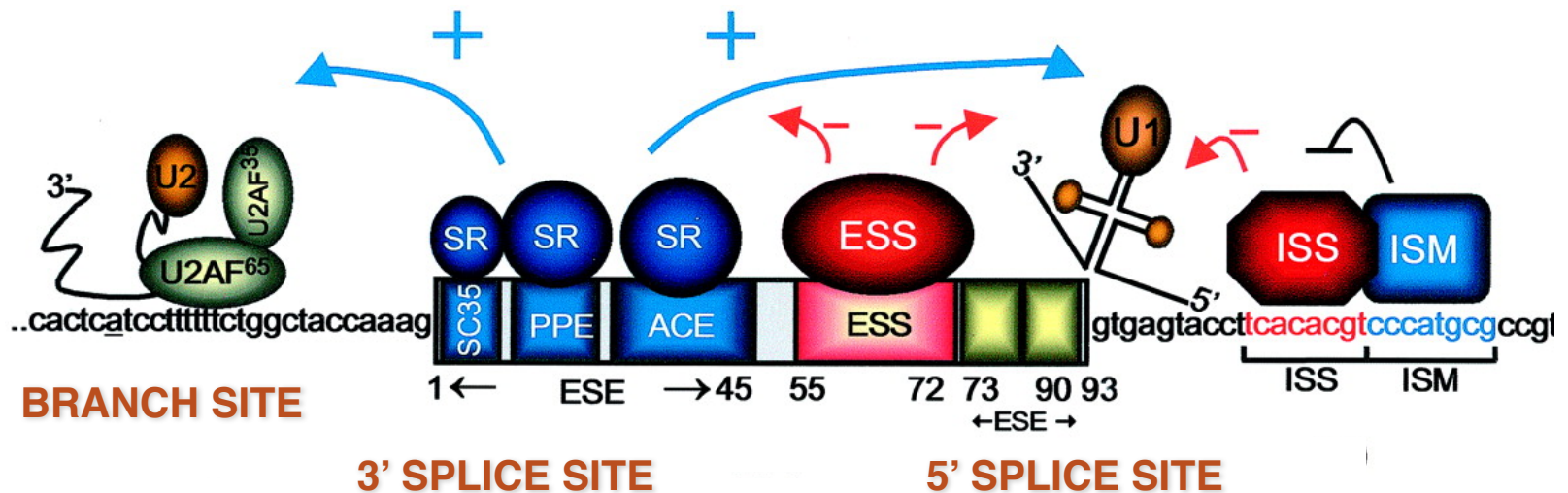


One in three mutations predicted to have some effect on splicing

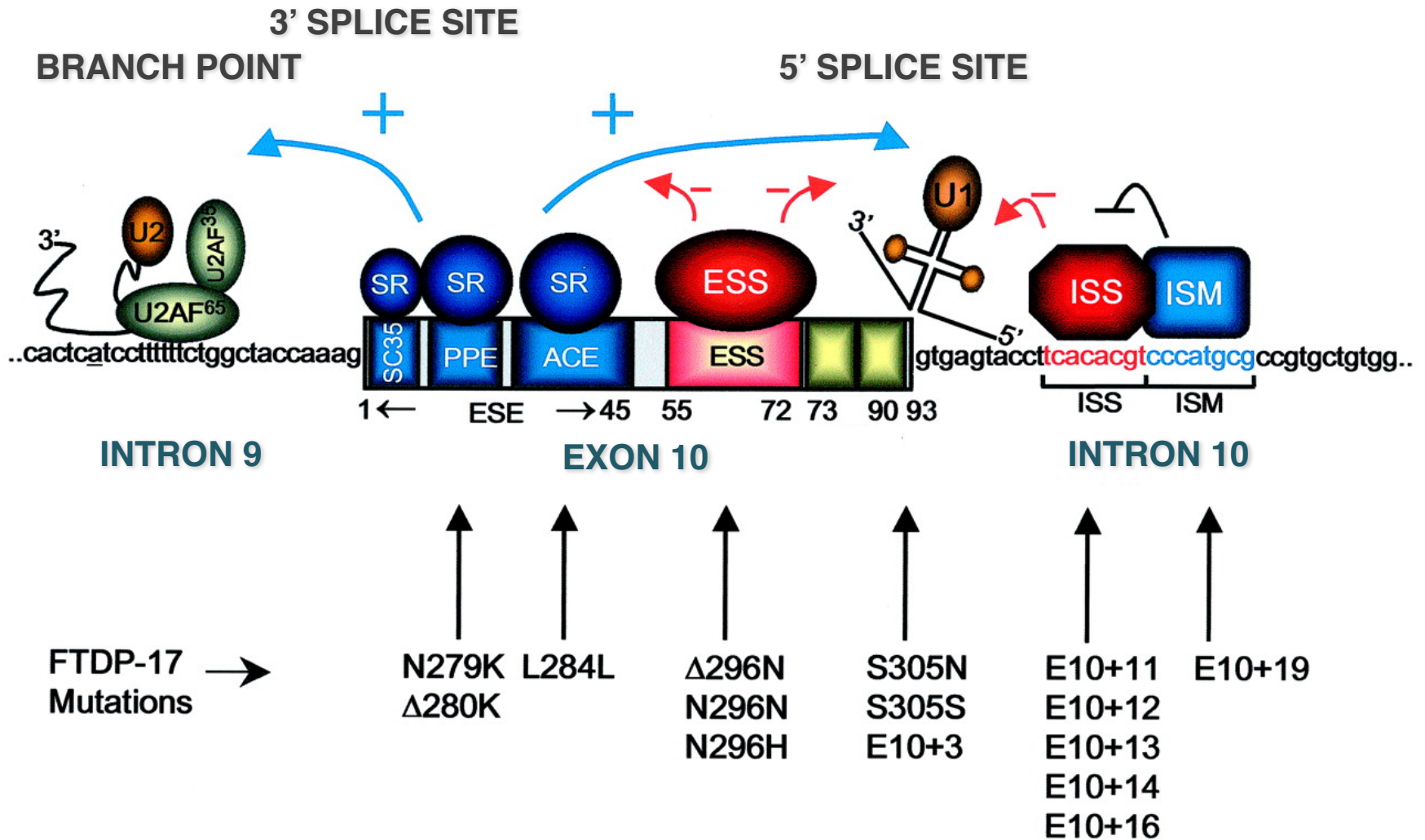
HGMD,
public 2008



MAPT EXON 10 SPLICING REGULATION

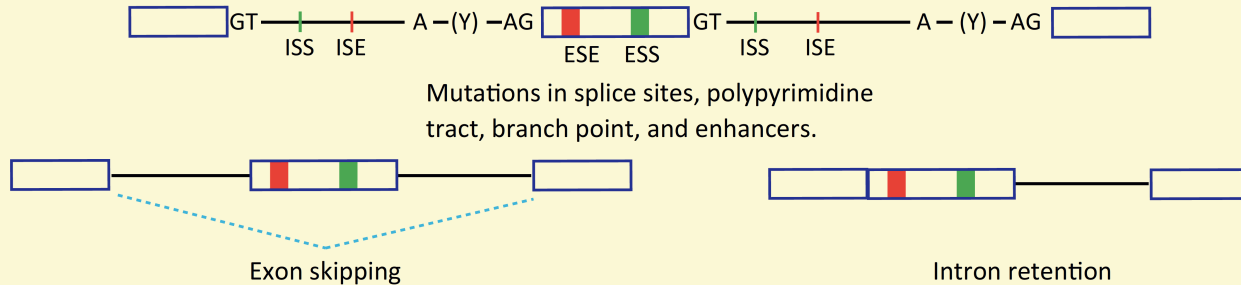


TAU EXON 10 SPLICING REGULATION

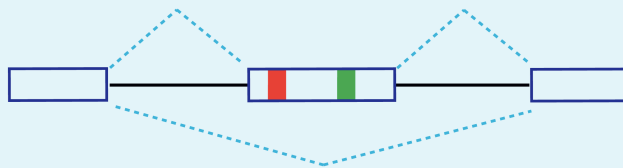


SEQUENCE VARIANTS OFTEN ALTER SPLICING

(a) Mutations affecting constitutive exon usage



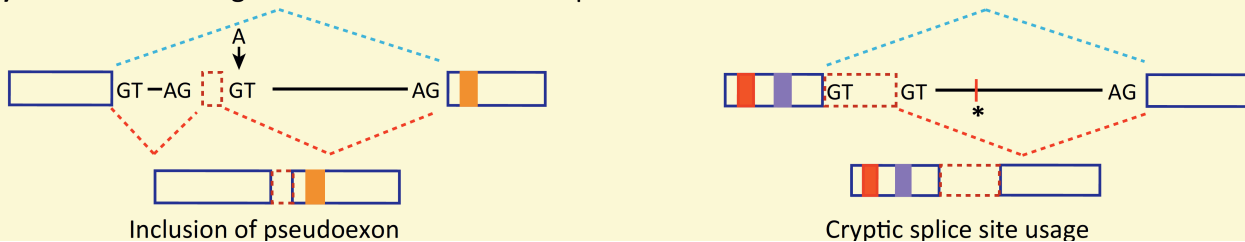
(b) Mutations affecting the ratio of alternative exons



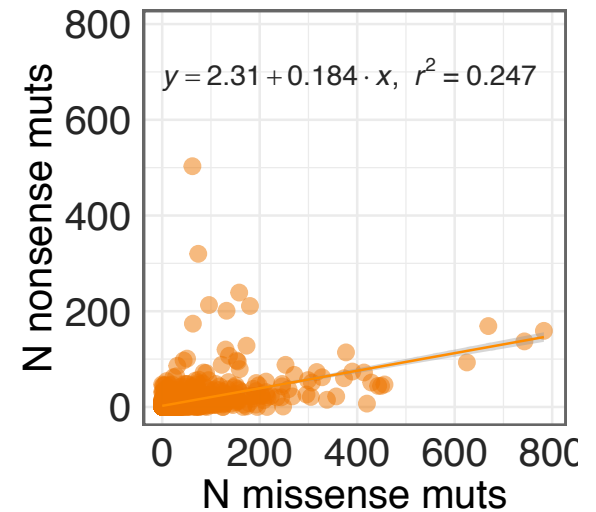
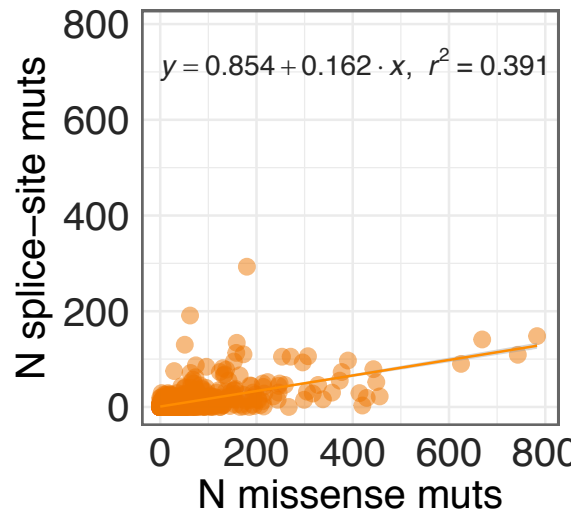
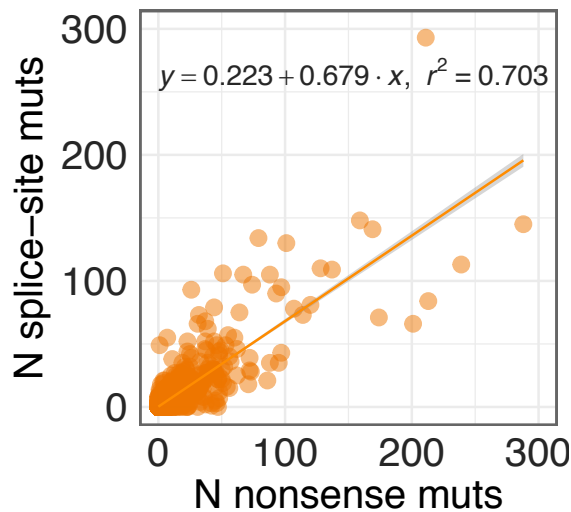
Normal	Mutations in silencers	Mutations in enhancers
70%	90%	30%
30%	10%	70%

scn2a

(c) Mutations leading to inclusion of intronic sequences



NONSENSE AND SPLICING MUTATIONS LIKELY SHARE LOSS-OF-FUNCTION DISEASE MECHANISM



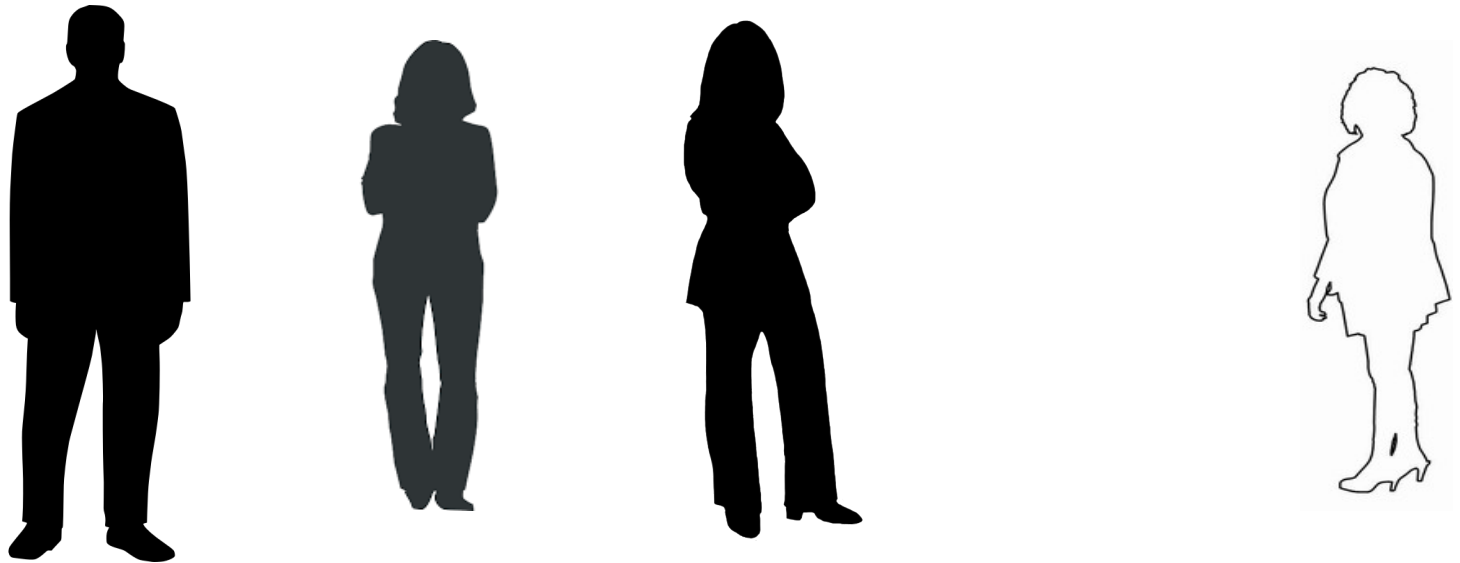
SPLICING THERAPIES UNDER DEVELOPMENT

Disease	Target gene	Therapeutic	Stage
Familial dysautonomia	IKBKAP	SM	Patients
Fanconi anemia	FANCC	U1 snRNA	Patient cells
Ataxia telangiectasia	ATM	ASO	Patient cells
Hutchinson-Gilford progeria	LMNA	ASO	Mouse model
Methylmalonic aciduria	MUT	ASO	Patient cells
Neurofibromatosis	NF1	ASO	Patient cells
Niemann-Pick type C	NPC1	ASO	Patient cells
Alzheimer's disease/FTDP-17	MAPT	ASO, SM	Cell lines
Spinal muscular atrophy	SMN2	ASO, SM	Clinical trials
Atherosclerosis (FHBL)	APOB	ASO	Cell lines
Immune-response	CD40	ASO	Cell lines
Influenza virus	TMPRSS2	ASO	Cell lines

SM, small molecule; ASO, antisense oligonucleotide.

No pathogenic variant detected -What next?

Which Variants Disrupt Splicing?



Computational Prediction of Splicing Mutation

Functional Assay (splicing assay) to screen SNV

Spliceman 2

Select your human assembly build (GRCh37/hg19 is the default)

Then, please either:

→ upload your .vcf file

OR

→ input one variant per line using the format below

☐ GRCh38/hg38 ☒ GRCh37/hg19

[chr variant_position(1-based) reference_allele alternative_allele] space
separated; for example: chr20 2301308 T G

Choose File No file chosen

Load Sample Data

Reset Values

Recommend Variants for Submission

Process Sequences



CLARITY Challenge Winner



Boston Children's Hospital
Until every child is well™

MaPSy: A novel technology to find splicing mutants



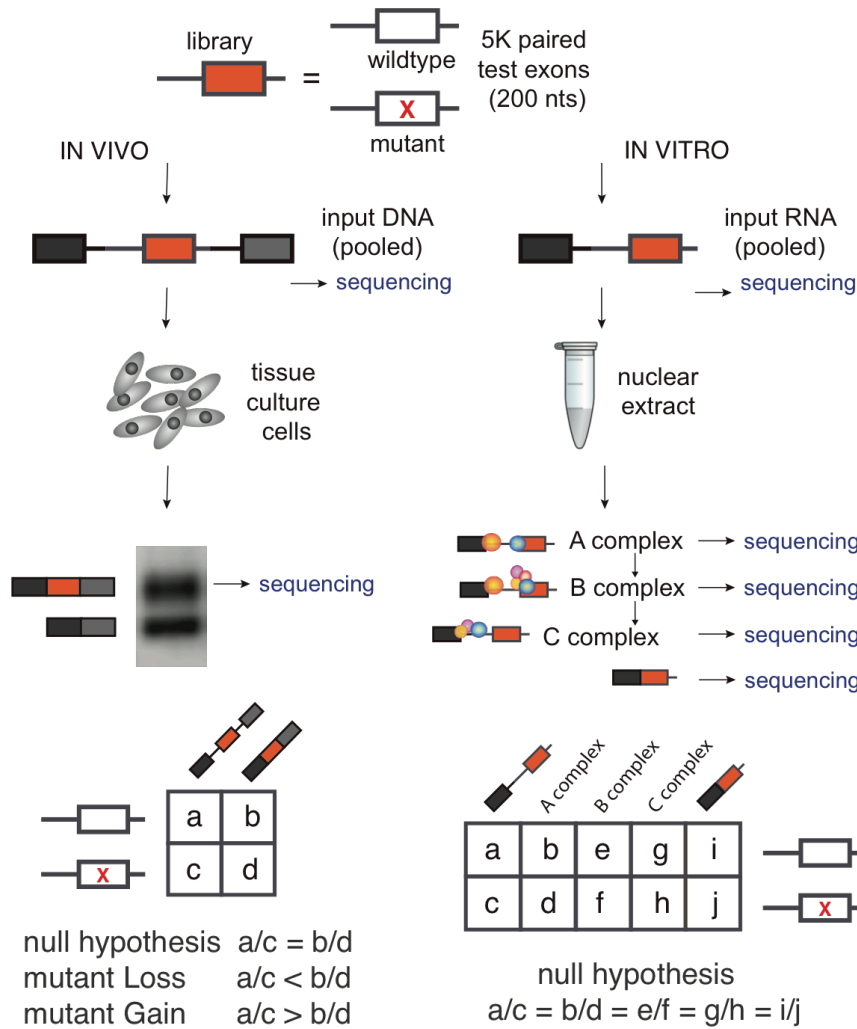
Dr. Rachel Soemedi



Kamil Cygan



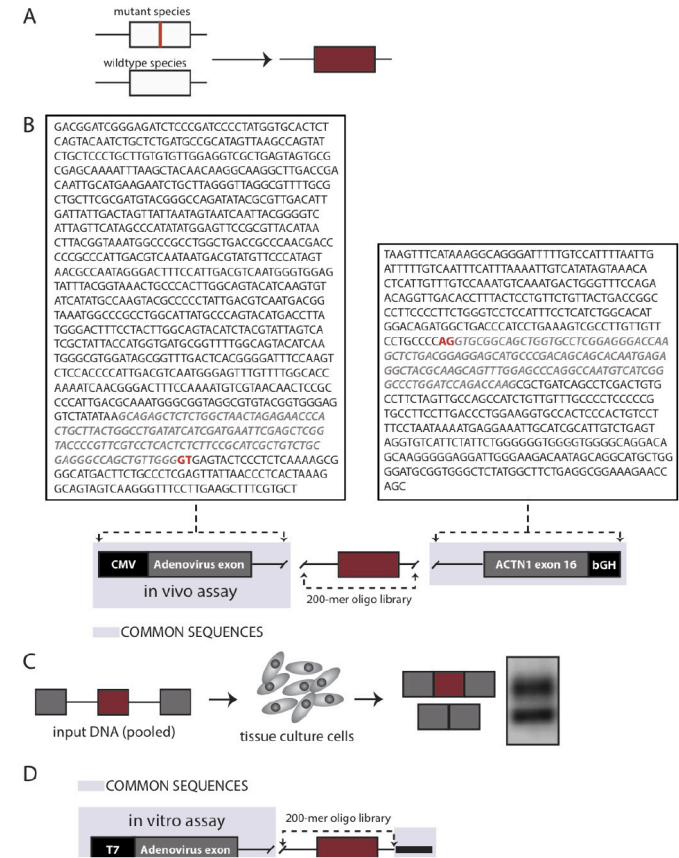
Massively Parallel Splicing Assay (MaPSy) for Exonic Splicing Mutations (ESM) detection



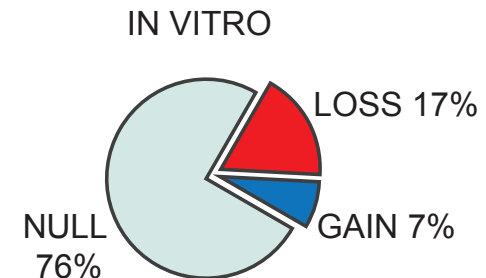
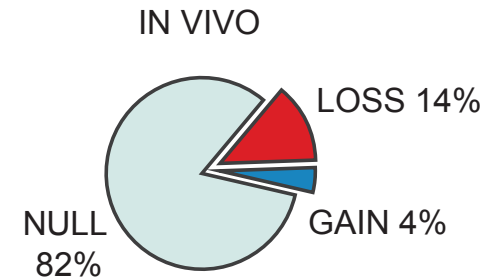
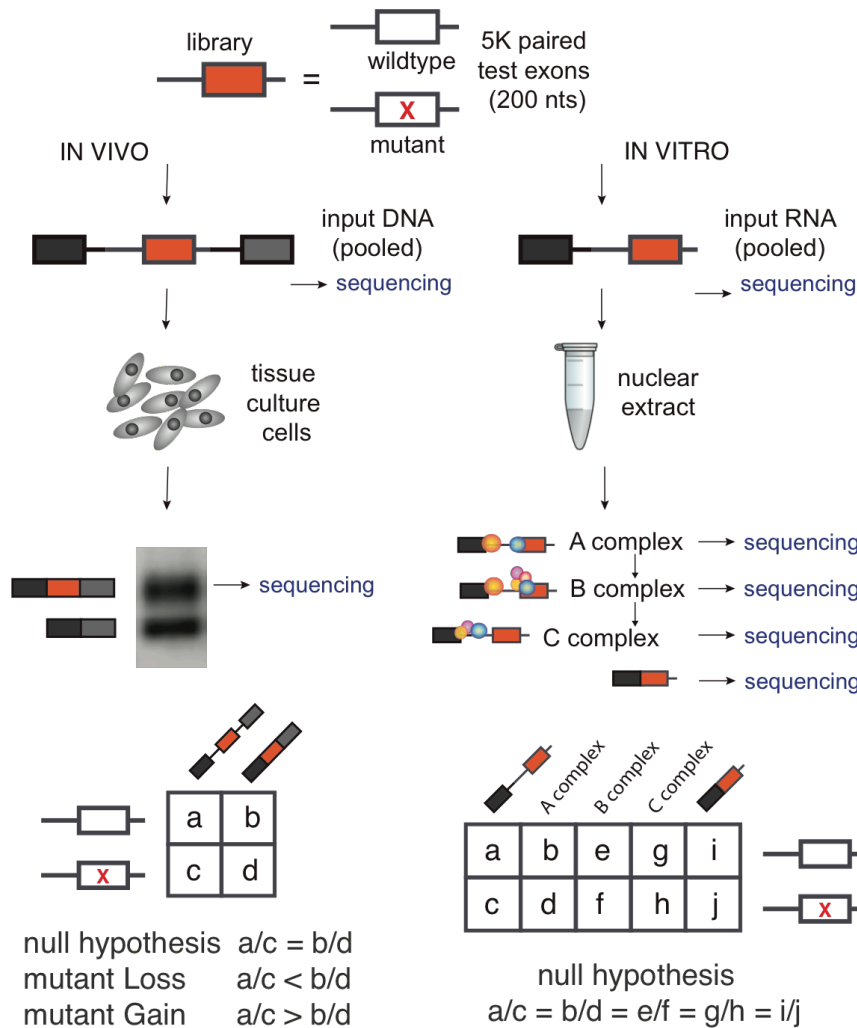
$$\log_2 \left(\frac{d/c}{b/a} \right)$$

significant at 5% FDR ; 1.5 fold

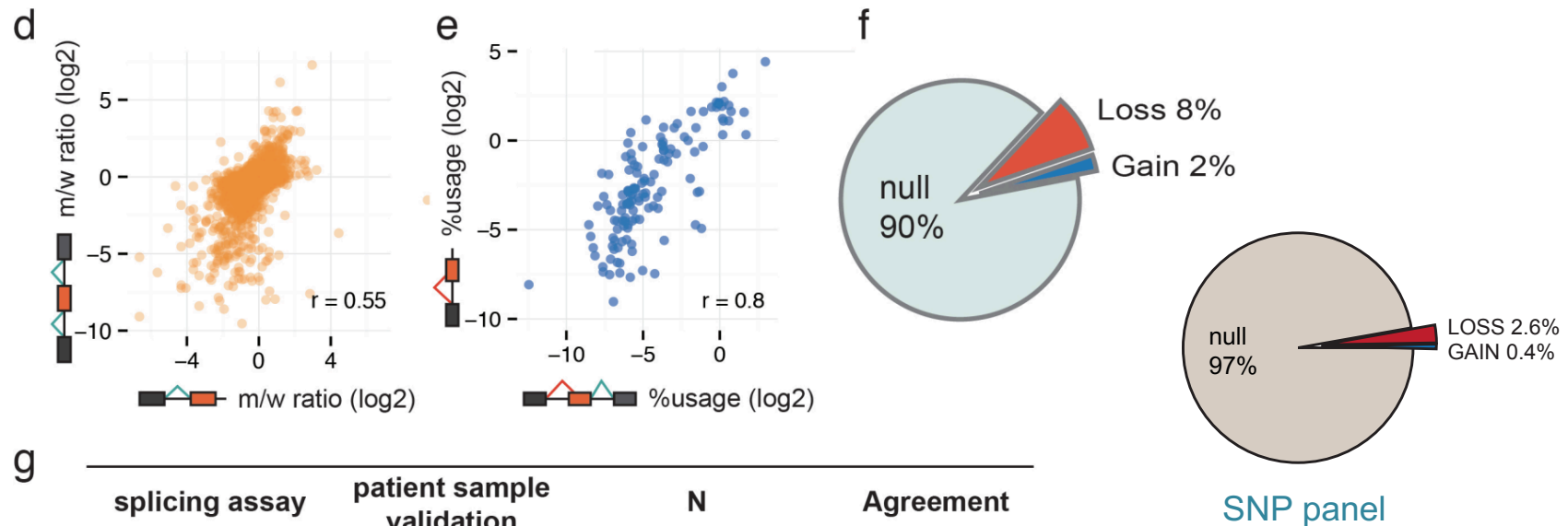
CHALLENGE



Massively Parallel Splicing Assay (MaPSy) for Exonic Splicing Mutations (ESM) detection

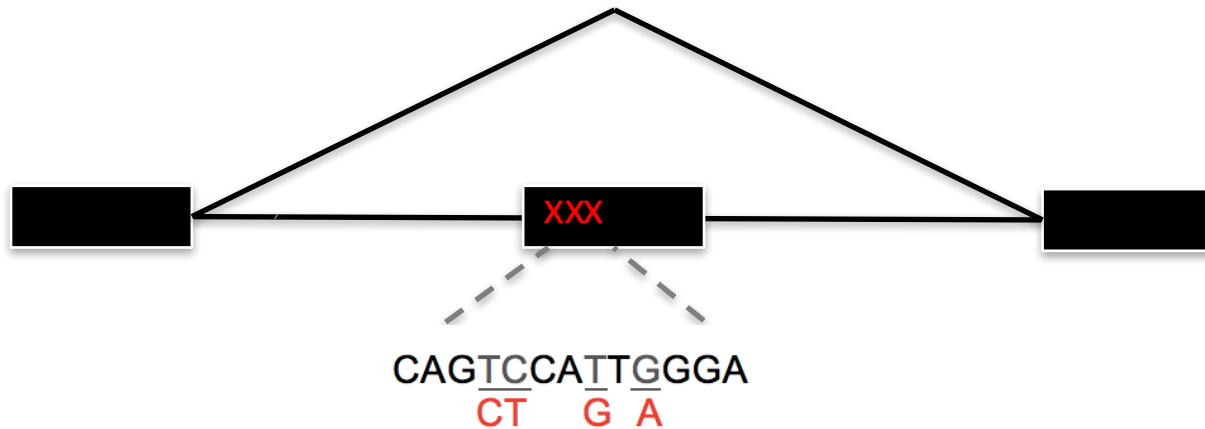


10% of coding mutations disrupt splicing in vivo AND in vitro



significant at 5% FDR ; 1.5 fold

Mutation re-occur in small clusters that appear to define ESEs



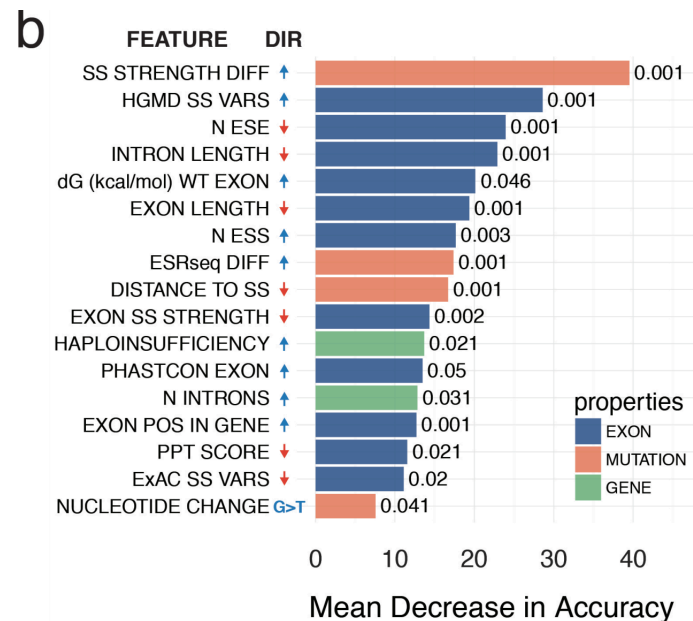
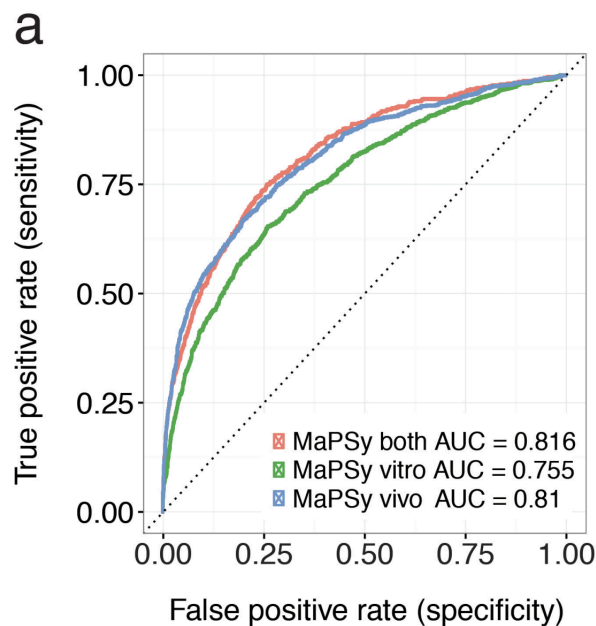
Gene	sequence	genomic position
<i>RXR2</i>	AATGGATCIGCA TC A	chr1:237944900-237944905
<i>KCNQ1</i>	CAGTCCATTGGGA CT G A	chr11:2798226-2798232
<i>BRCA1</i>	GTGGTTGTGCAGCCAG T G T G	chr17:41199693-41199702
<i>LMX1B</i>	TGGTCCAGGTCT C T C	chr9:129455849-129455854
<i>PLP1</i>	AGTICCAA C T	chrX:103044263-103044265
<i>HPRT1</i>	TCAGGGATT G T	chrX:133632704-133632707
<i>F8</i>	TTTCTGTAT A C	chrX:154215524-154215527

Red = loss of splicing

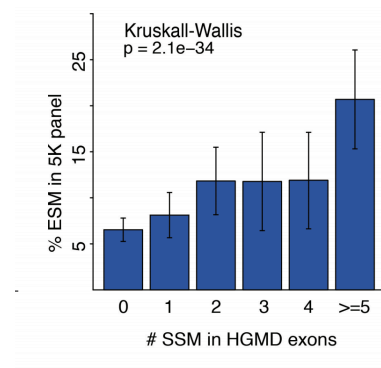
Blue = gain of splicing

significant at 5% FDR ; 1.5 fold

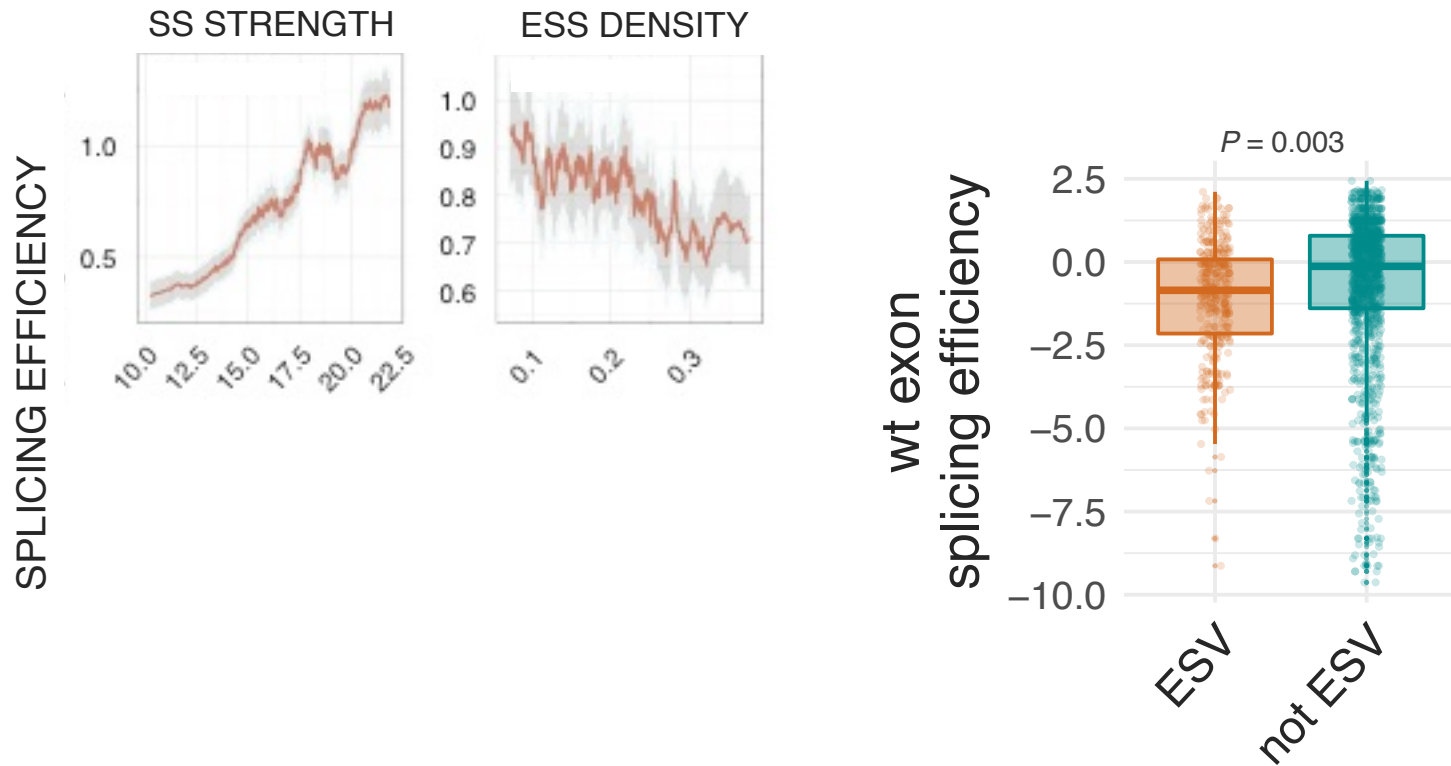
Dataset of 5K mutations was used to train a classifier and identify features



- most predictive features are features of the EXON not the mutation
- some features describe the strength of the splicing substrate
- some features measure the likelihood of disease

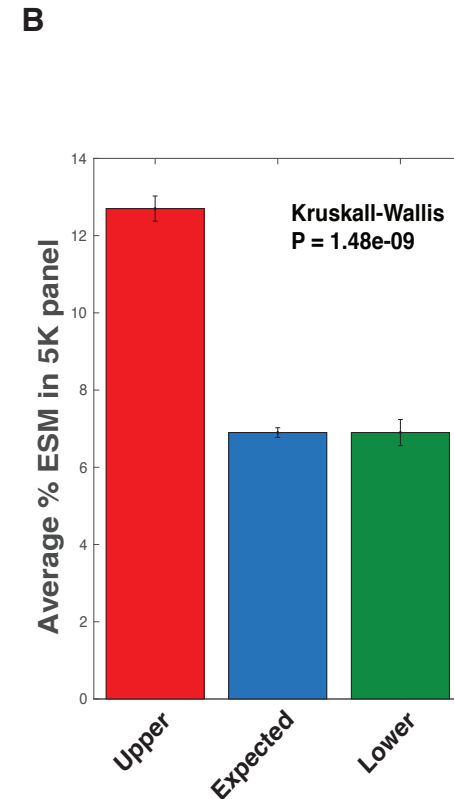
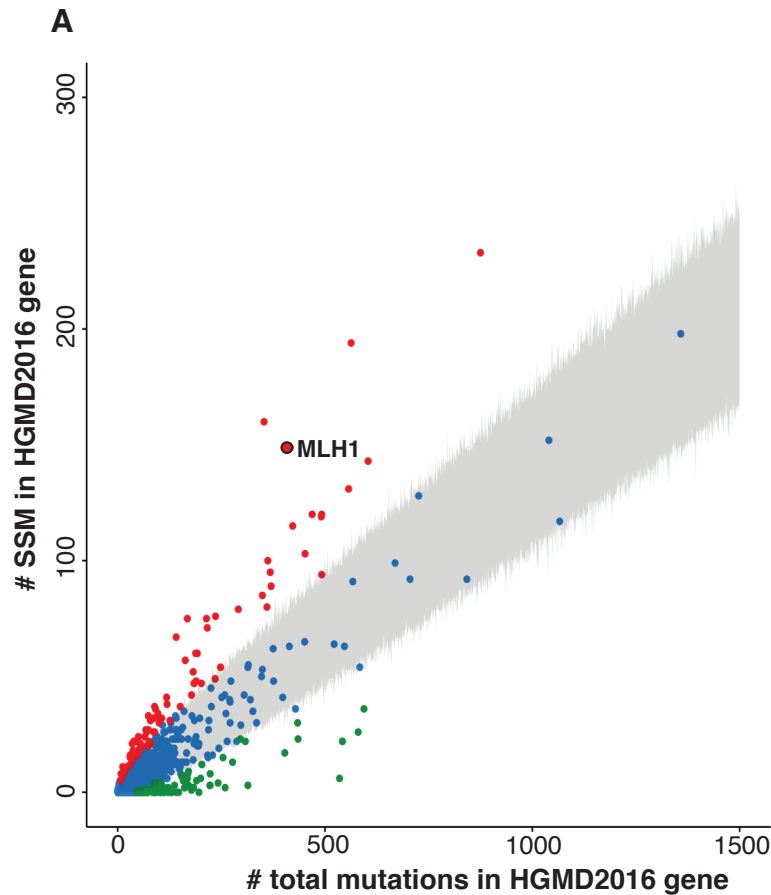


“WEAK EXONS” ARE SUSCEPTIBLE TO ESV



Splicing mutations are not distributed uniformly across diseases

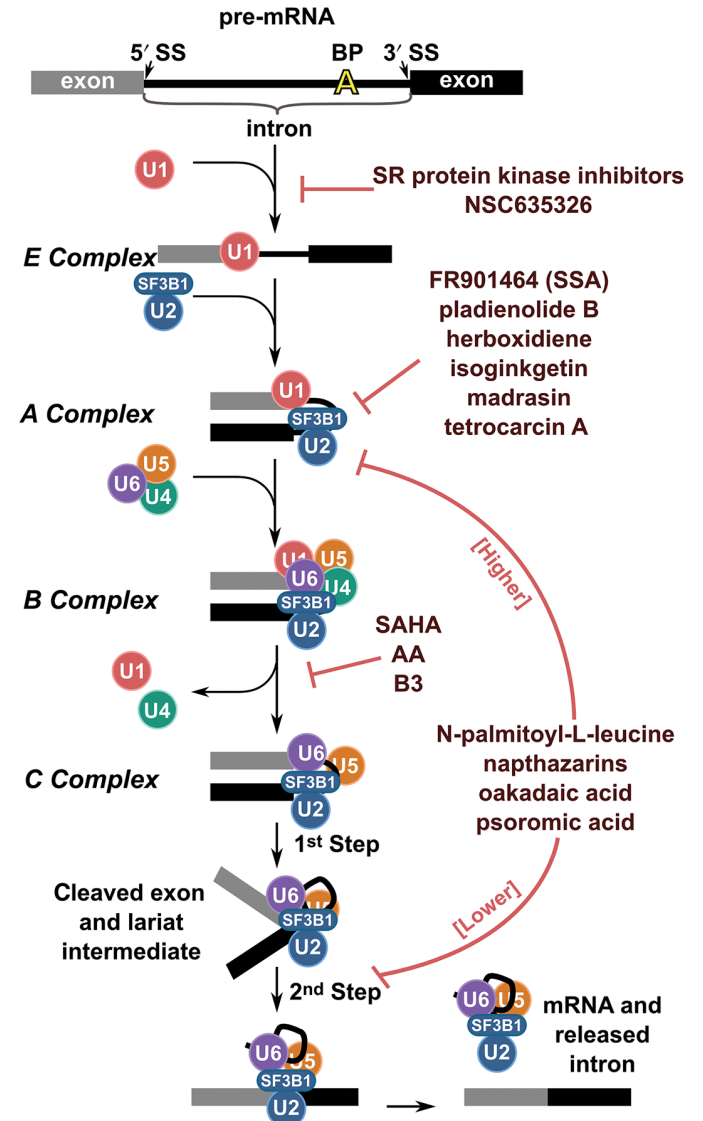
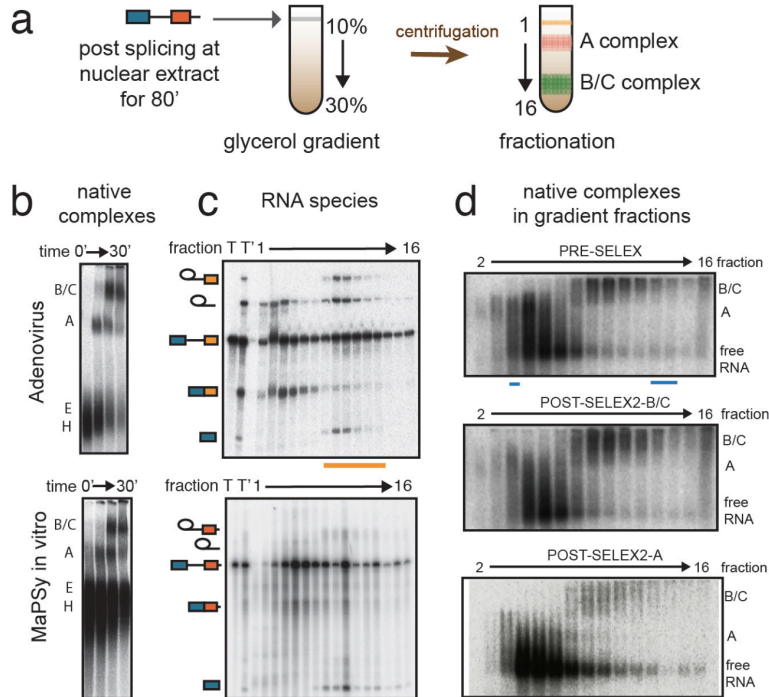
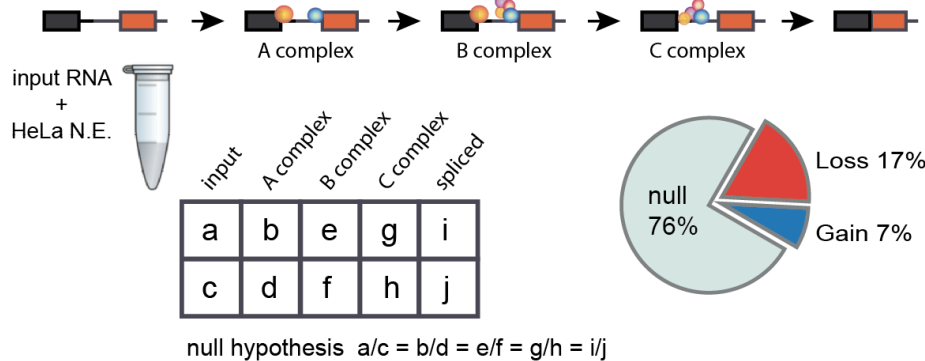
APC,
BRCA2,
CUL3,
EXT1,
SPAST,
STXBP1



Christy Rhine
MCB Jing Wang

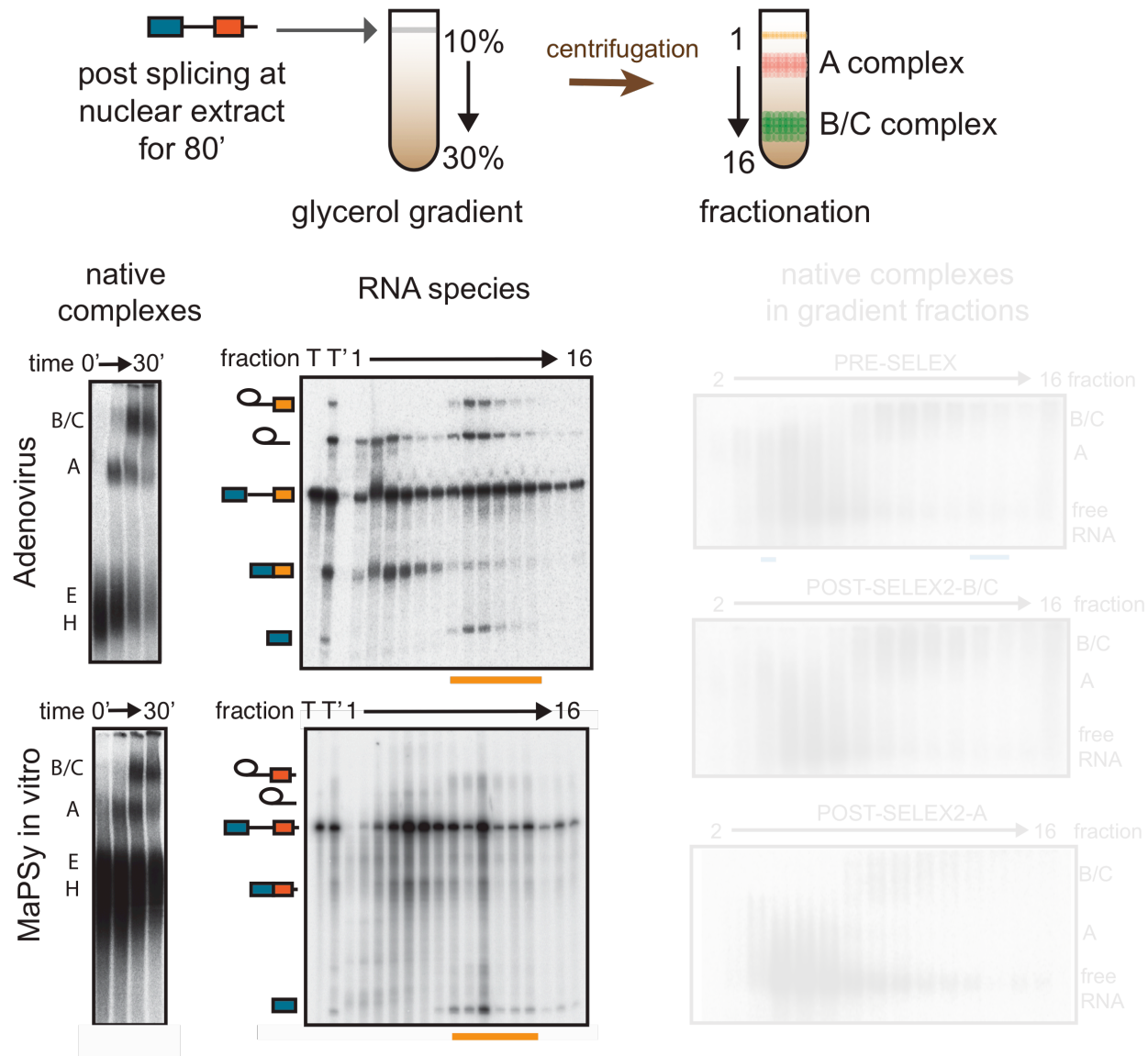
In vitro splicing assay as a platform for drug discovery

in vitro splicing / spliceosome assembly assay

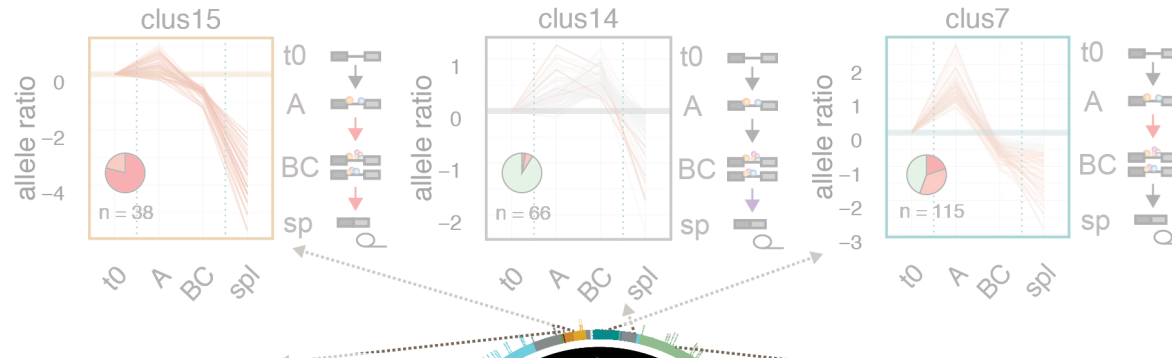


significant at 5% FDR ; 1.5 fold

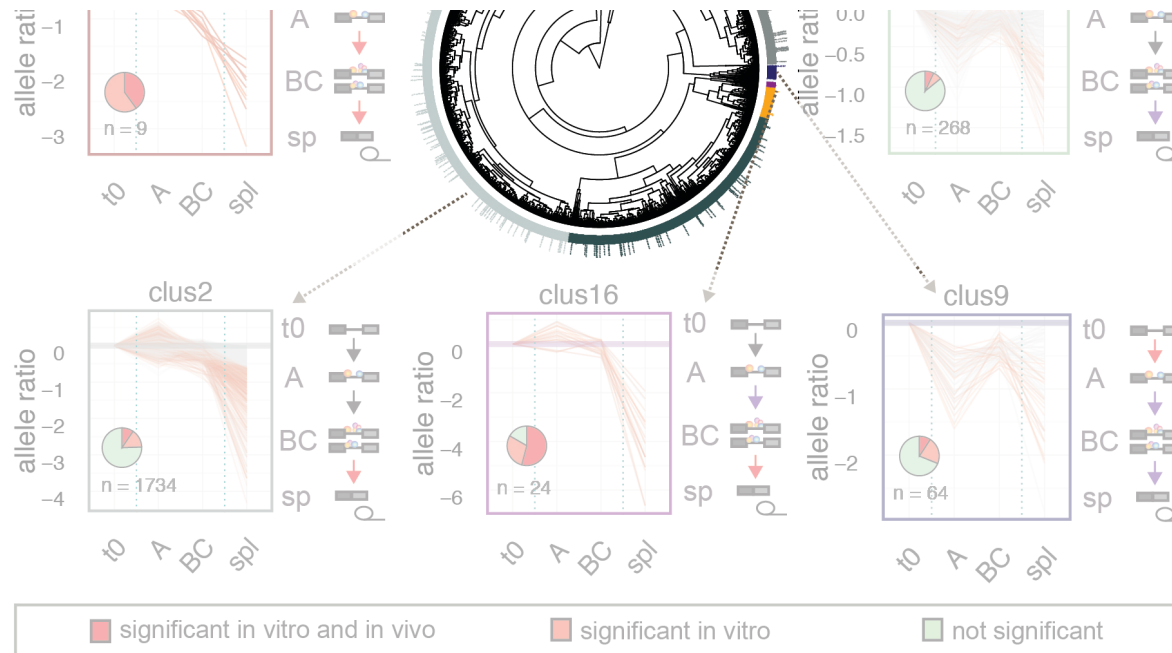
Isolation of spliceosomal intermediates of ESV



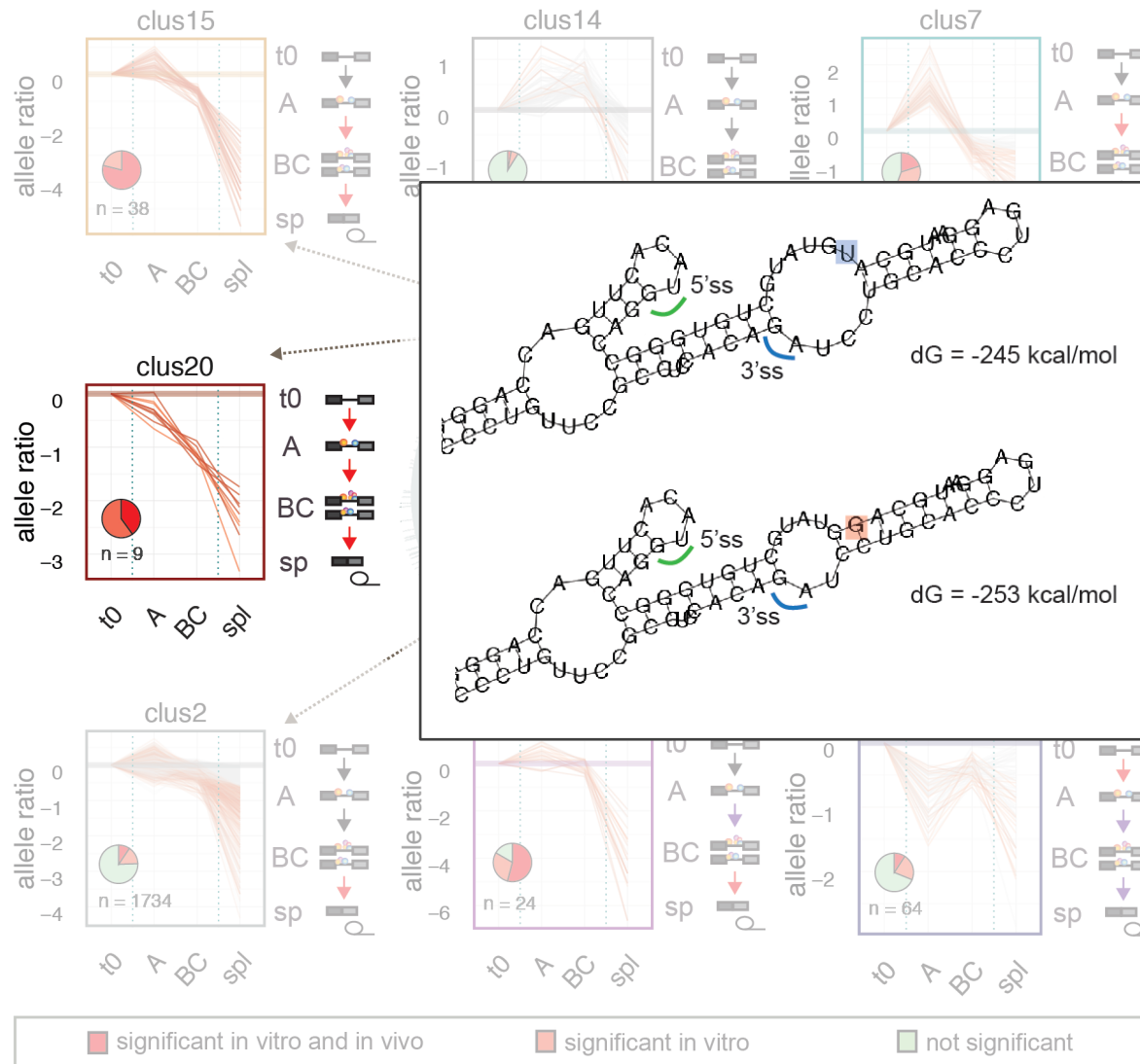
ESV MECHANISTIC DIVERSITY



Mutations in the same exon cluster together ($P=0.008$)



ESV MECHANISTIC DIVERSITY



http://fairbrother.biomed.brown.edu/ESM_browser/search

the fairbrother lab

home

upload

results

methods/design

hello, labmin

logout

manage

web management:

generate link

generate link (new)

search/edit contacts

send e-mail

followups

Visualization Tool

Data for: ENG
[Experimental Overview](#)

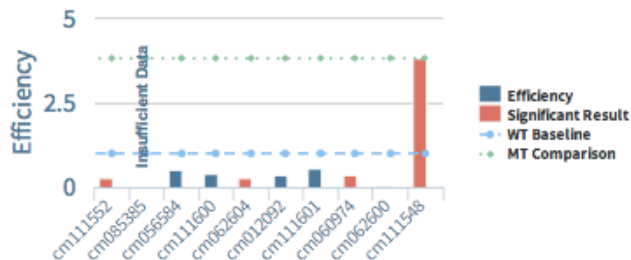
Results For:
eng

Experiment
[In Vivo](#)

Visualization



Efficiency of Mutant Types (Relative to Wild Type)

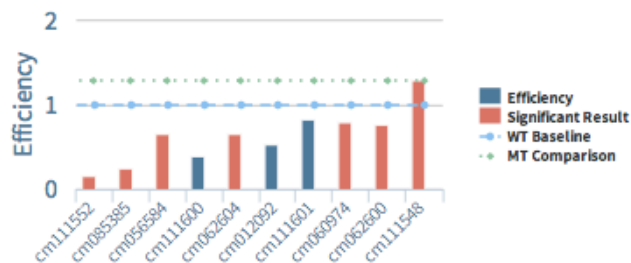


Highcharts.com

In Vitro



Efficiency of Mutant Types (Relative to Wild Type)

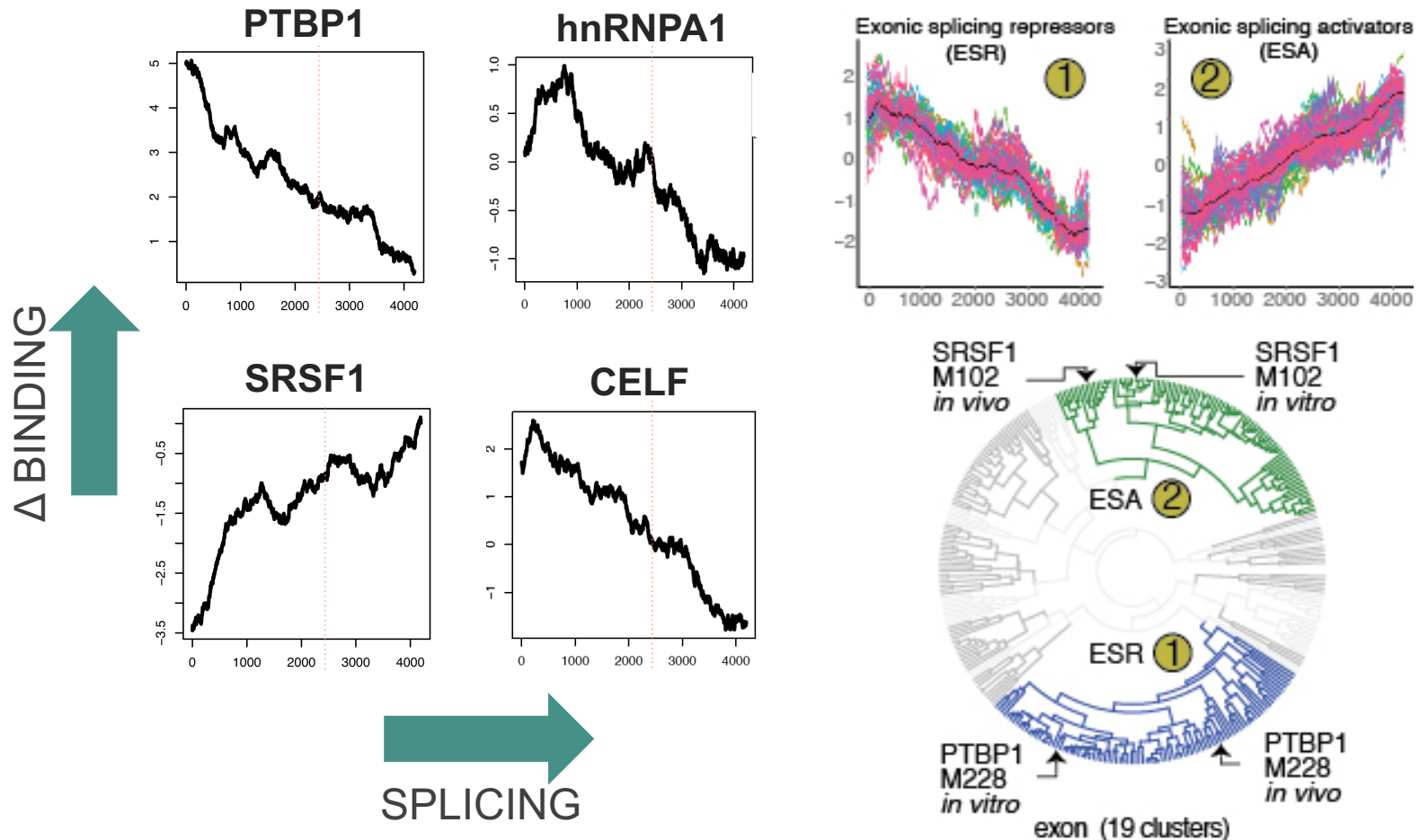


Highcharts.com

upload new variant(s) for
analysis

submit patient sample for
validation

RNA Binding Protein (RBP) motif loss/gain correlates with loss/gain of splicing in MaPSy



Acknowledgements

Will Fairbrother (PI)

Rachel Soemedi

Kamil Cygan

Christy Rhine

Chien-Ling Lin

Allison Taggart

Alger Fredericks

David Glidden

Seong Won (Mingo) Kim

Barsha Shrestha

Jing wang



Past members

Charlston Bulacan

Zheng (John) Yang

Arun Janssens

Karina Villanueva

Alex LeBlang



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the fairbrother lab 



Visualization tool of the 5K panel:

<http://fairbrother.biomed.brown.edu/dashboard/search>