8° WORKSHOP
IN EMATOLOGIA TRASLAZIONALE

DELLA SOCIETÀ ITALIANA DI EMATOLOGIA SPERIMENTALE

Firenze - Auditorium CTO - A.O.U. Careggi, 22-23 giugno 2023

Alterazioni dello Splicing nelle MDS a basso rischio

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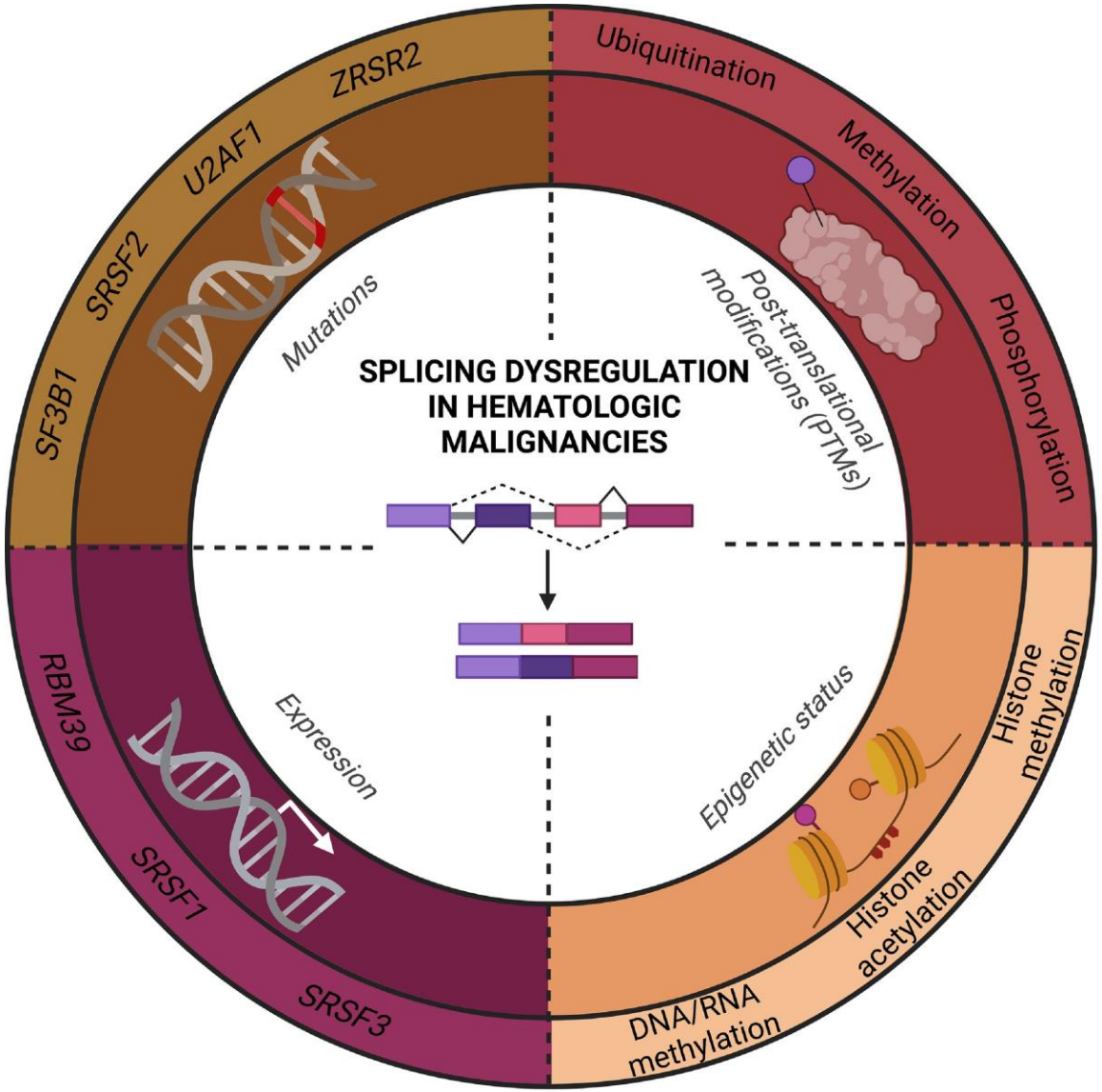
Disclosures of Emiliano Fabiani

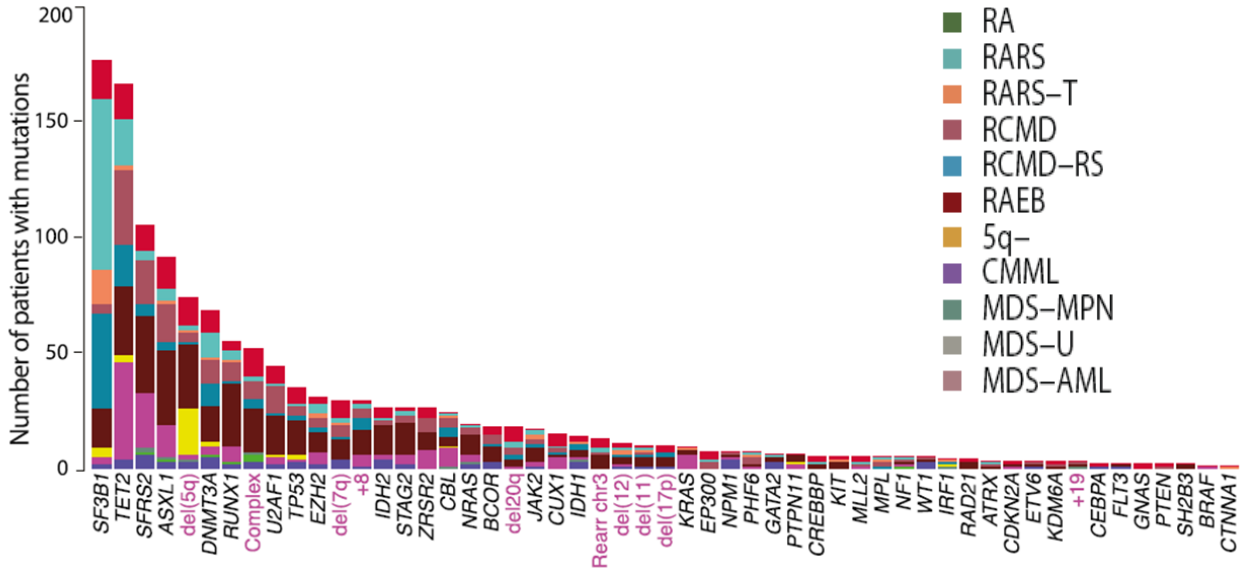
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

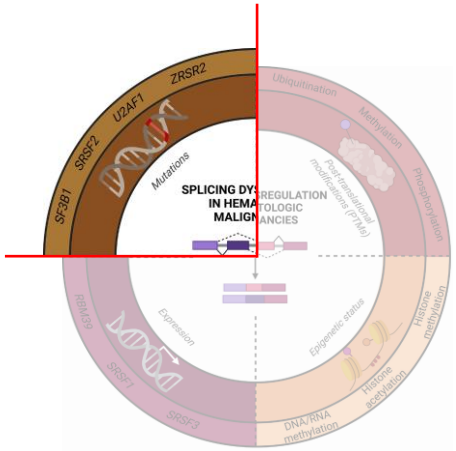
Molecular mechanisms that can drive aberrant splicing in hematologic malignancies



De Kesel et al., Trends Immunol 2022

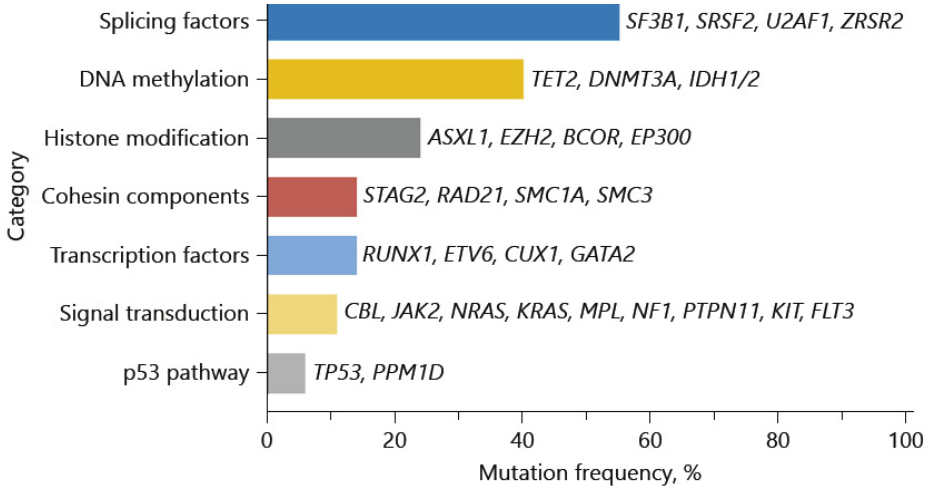
Mutational landscape of MDS





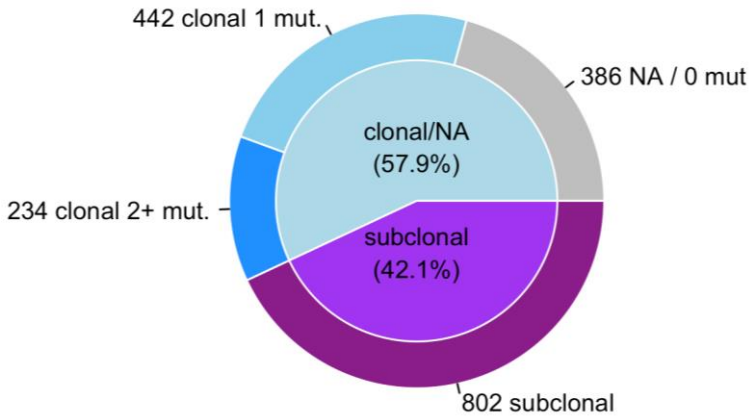
Mutant Gene	%
SF3B1	25-30%
TET2	20-25%
RUNX1	15-20%
ASXL1	10-15%
SRSF2	10%
TP53	5-10%
U2AF35	5-10%
NRAS/KRAS	5-10%
DNMT3A	5-10%
ZRSR2	5%

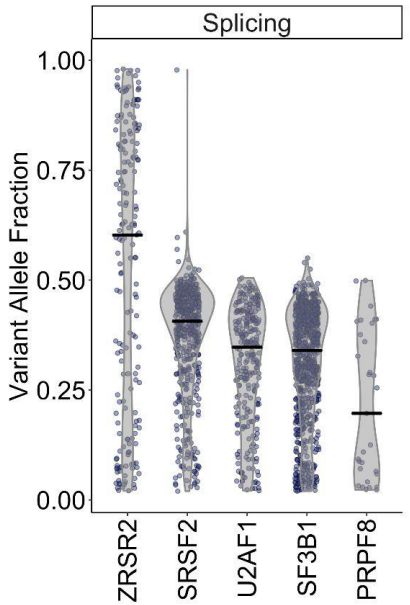
- About 95% of patients with MDS have at least one mutation (t-NGS 30-80 genes)
- Genes belonging to the splicing machinery (SF3B1, SRSF2, U2AF1 and ZRSR2) are the most frequently mutated genes in MDS (50-60%)
- SF3B1 is the most frequently mutated gene in MDS (25-35%)

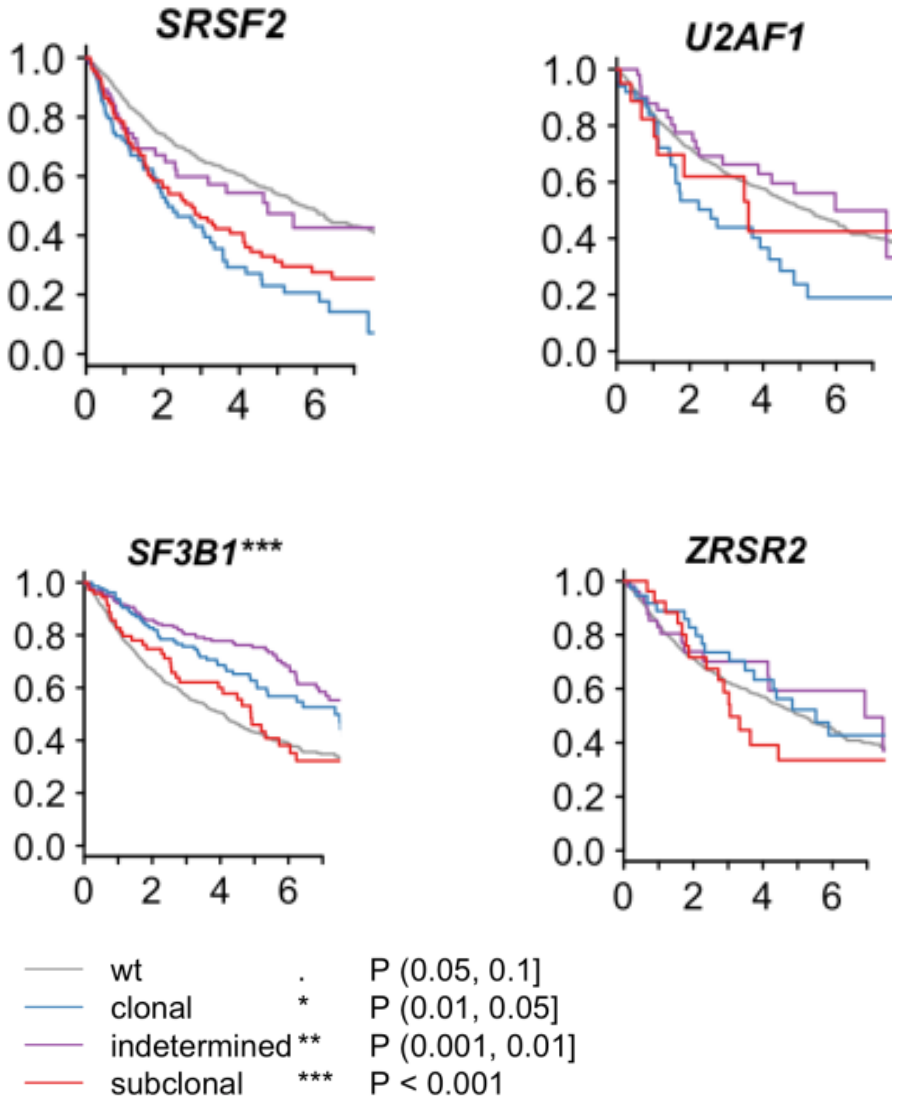


Data are from Papaemmanuil et al., Blood 2013, Haferlach et al., Leukemia 2014 and Kennedy et al., JCO 2017.

Survival between clonal and subclonal mutations





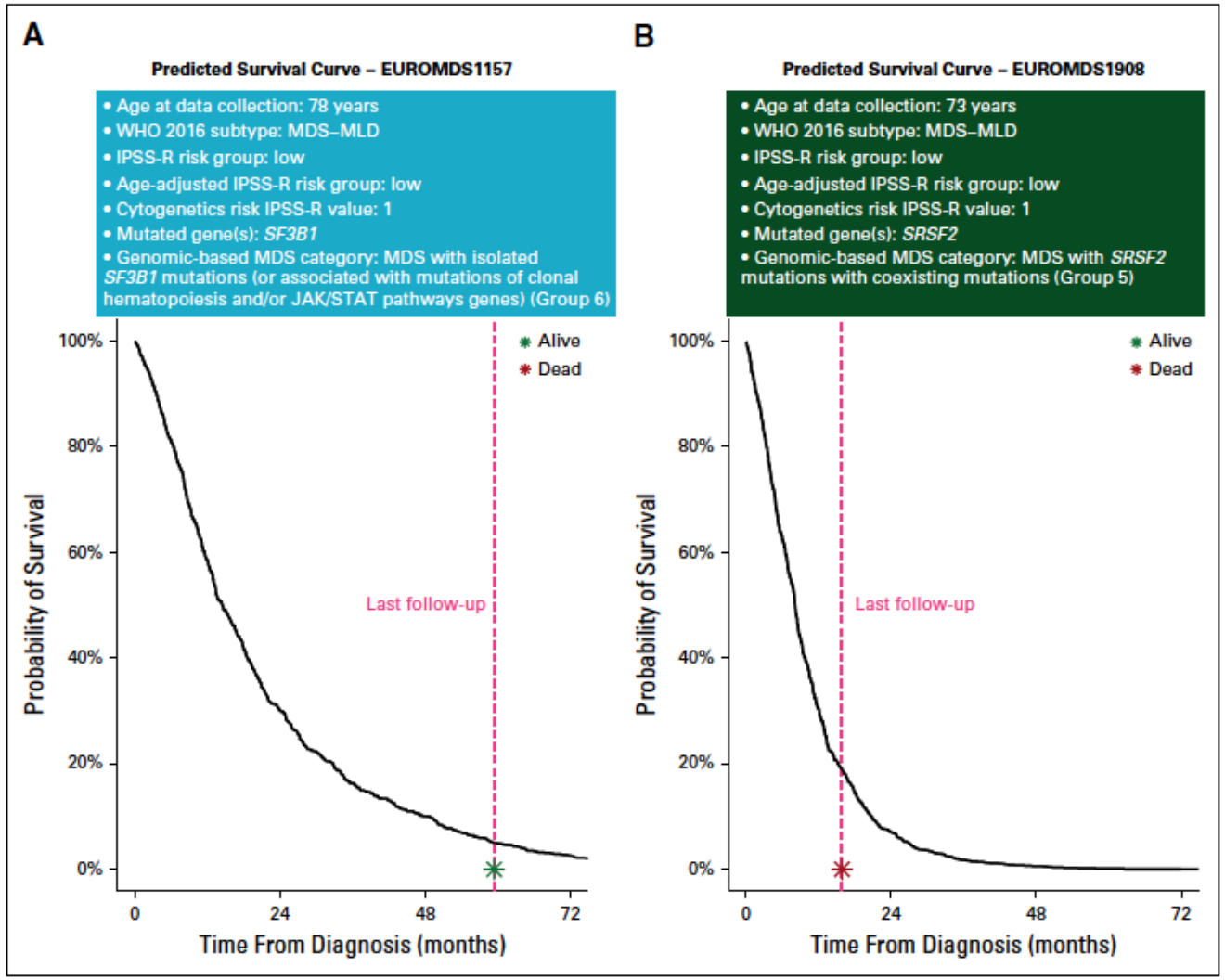


- No significant difference in survival between clonal and subclonal mutations for SRSF2, U2AF1 and ZRSR2
- The worse survival associated with subclonal SF3B1 suggests it belongs to a separate bystander clone, with a main clone driven by other mutations

Bersanelli et al., JCO 2021 and Bernard et al., NEJM Evidence 2022

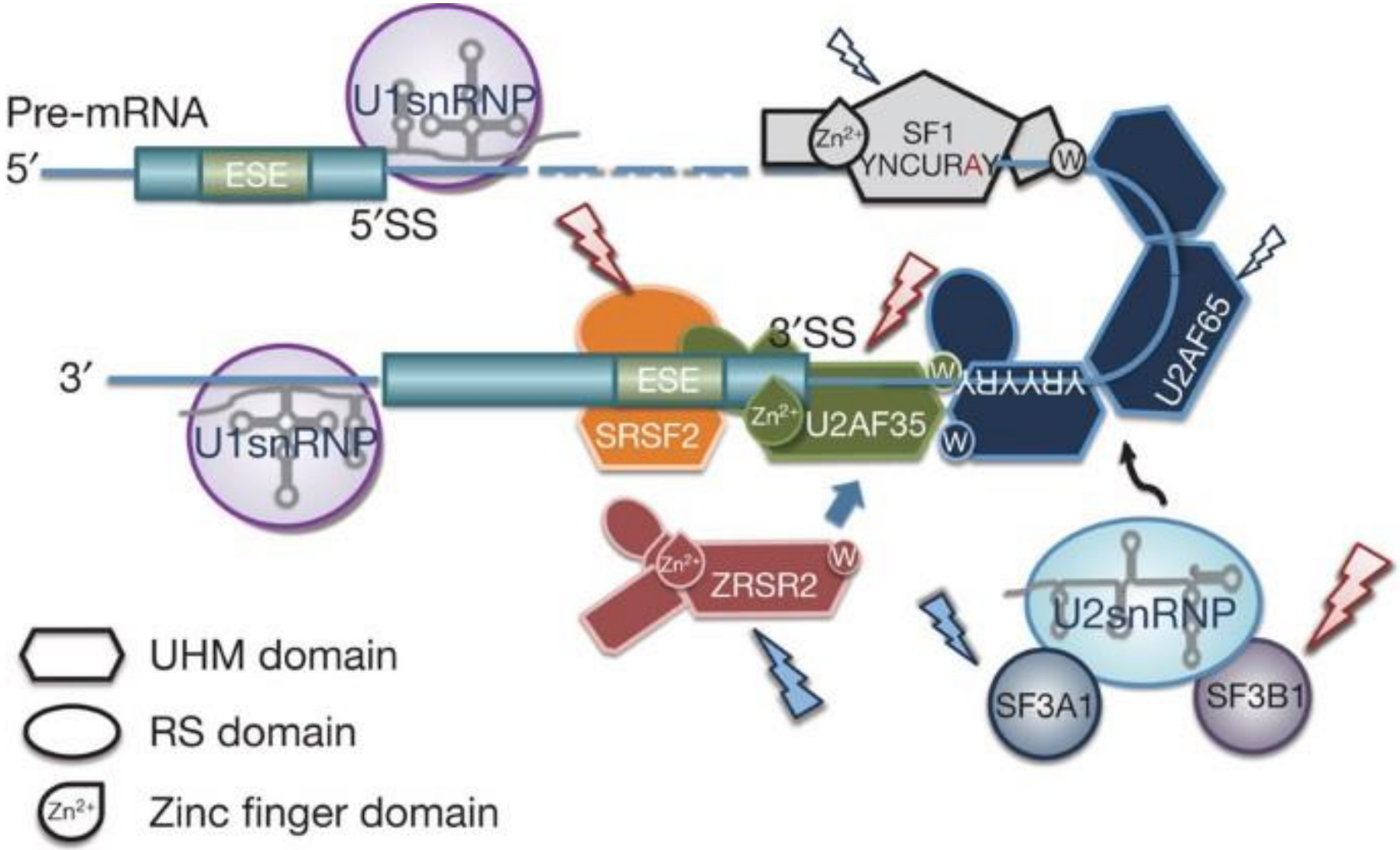
Impact of specific splicing mutations in LR-MDS

SF3B1^{mut} Group 6 **SRSF2^{mut} Group 5**



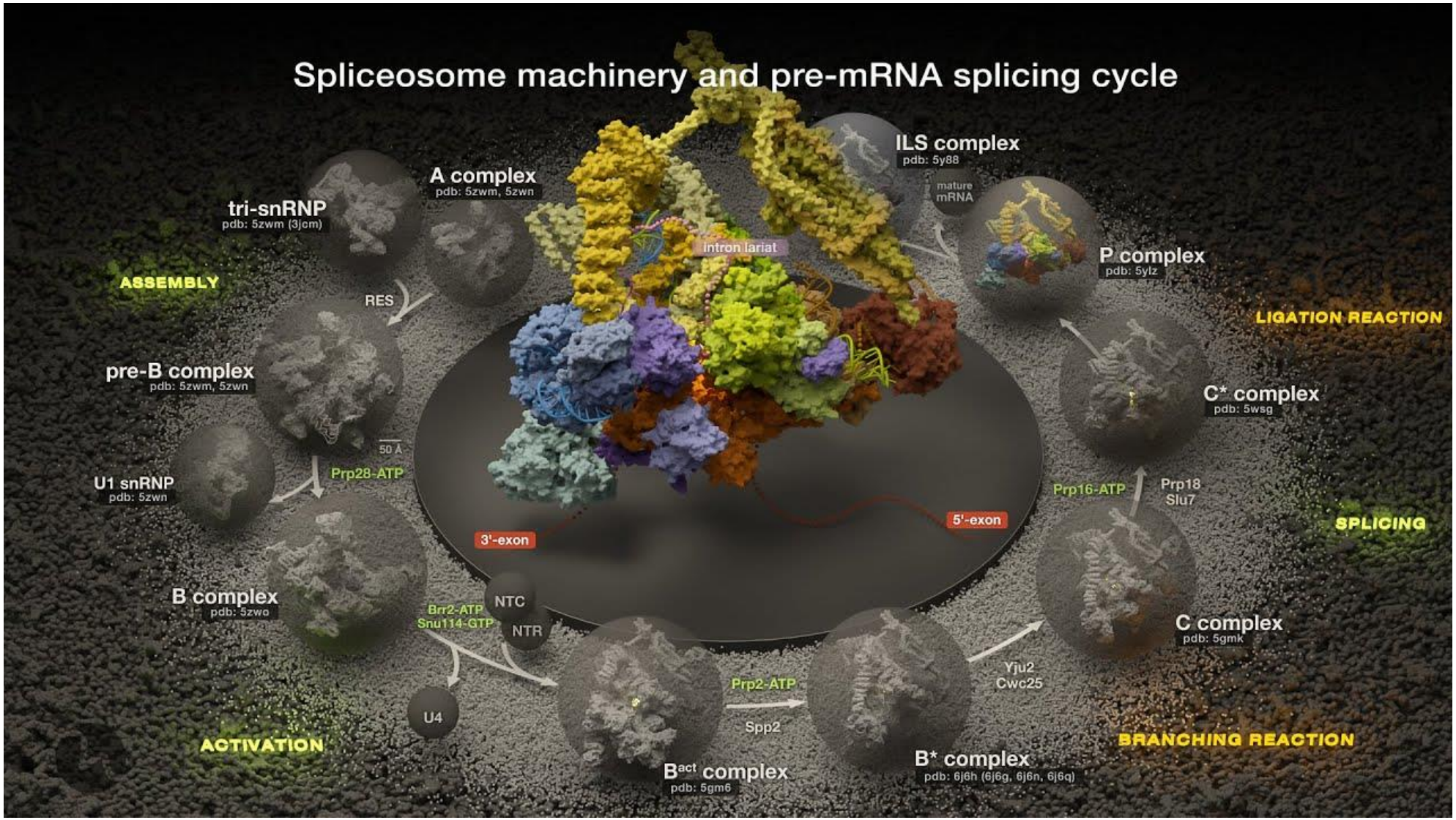
Bersanelli et al., JCO 2021

Splicing machinery



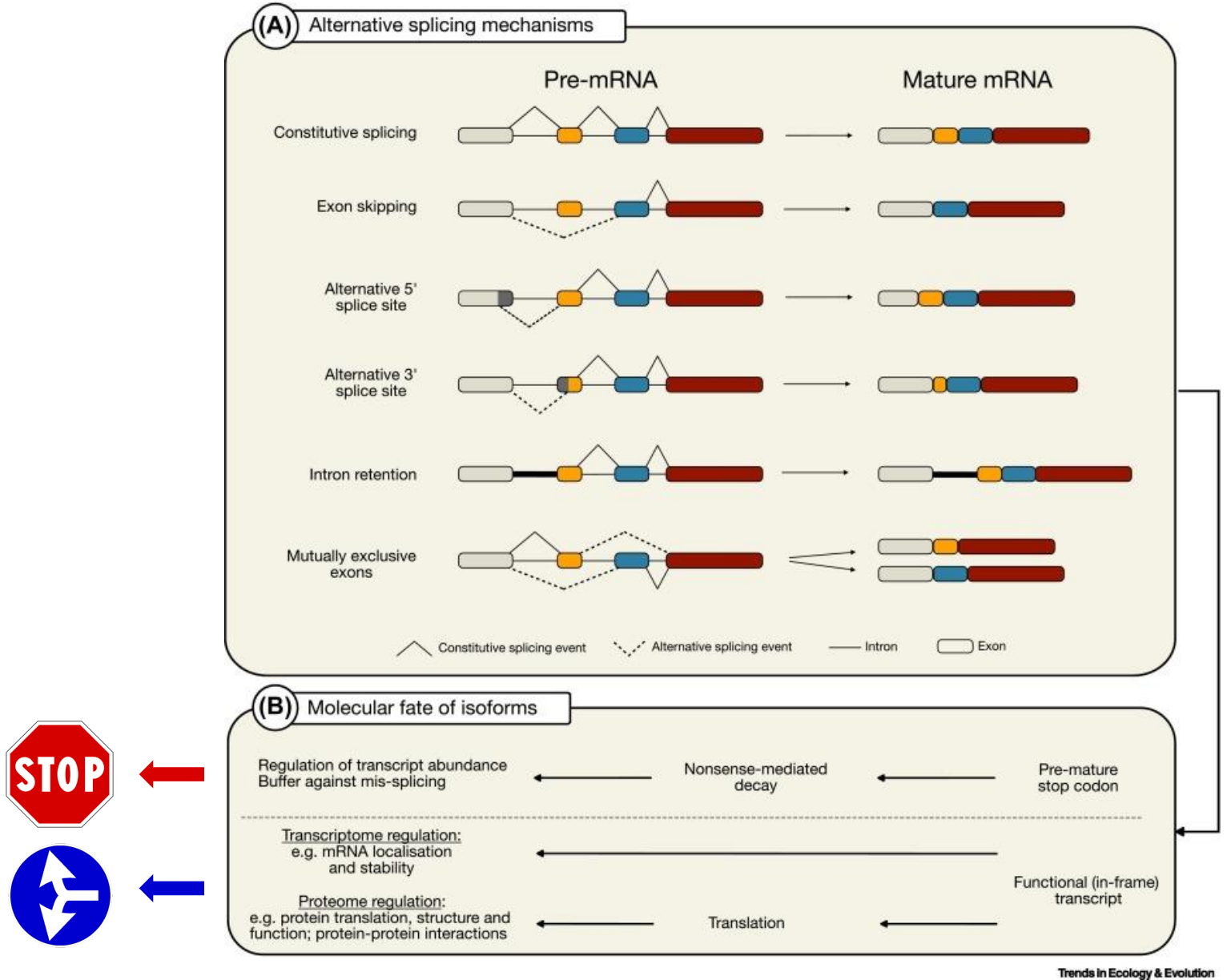
Yoshida et al., Nature 2011

Splicing machinery



Beauty of Science: https://youtu.be/OuAGeQYjfus

Alternative splicing events



Study cohort

Starting MDS cohort (n=300)

Selected cohort (n=50)

RNA-Seq (gene expression)

WGS (molecular *screening*)

Validation (qRT-PCR)

Validation (Sanger/ddPCR)

MLL: *Munich Leukemia Laboratory*

Patients' selection according to IPSS-R and splicing factors mutation profile

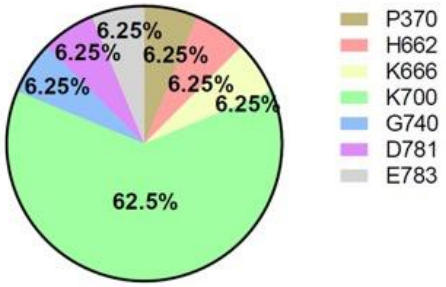
Gene	Target region (exon)	Gene	Target region (exon)	Gene	Target region (exon)
ABL	4-9	*FLT3*	13-15 and 20	*PTPN11*	3,7-13
ASXL1	9,11,12	*HRAS*	2,3	*RUNX1*	all
BRAF	15	*IDH1*	4	*SETBP1*	4
CALR	9	*IDH2*	4	*SF3B1*	10-16
CBL	8,9	*JAK2*	all	*SRSF2*	1
CEBPA	all	*KIT*	2,8-11, 13,17 and18	*TET2*	all
CSF3R	all	*KRAS*	2,3	*TP53*	all
DNMT3A	all	*MPL*	10	*U2AF1*	2,6
ETV6	all	*NPM1*	10,11	*WT1*	6-10
EZH2	all	*NRAS*	2,3	*ZRSR2*	all

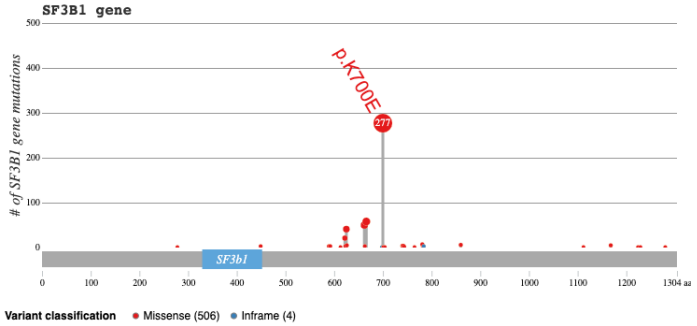
Coverage	1000X
***VAF**	> 1%

Fabiani *et al.,* Unpublished data

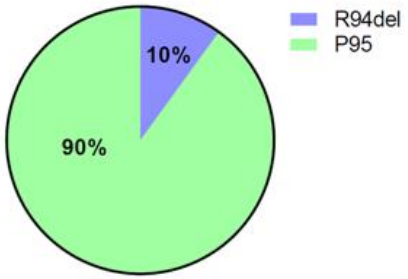
WGS analysis: mutation types and co-mutations pattern

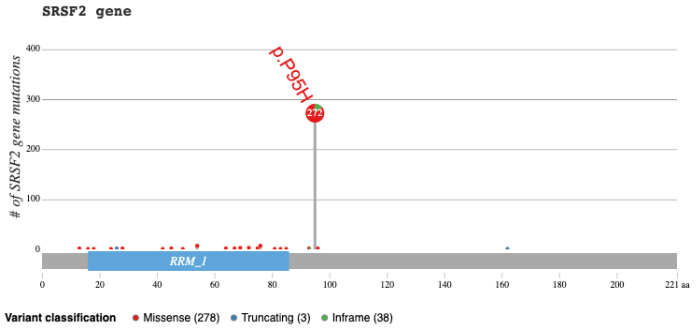
Type of mutations in SF3B1 gene



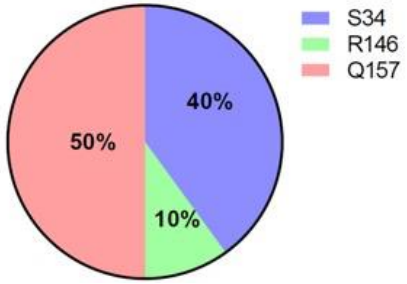


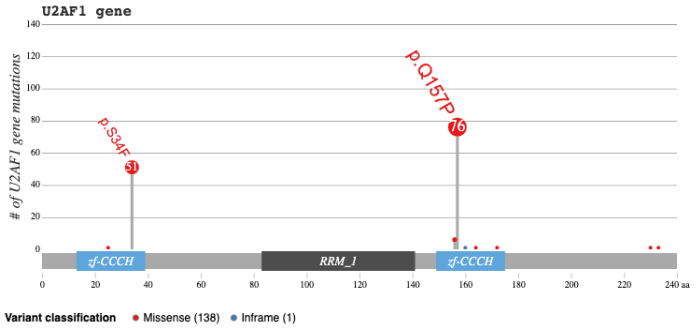
Type of mutations in SRSF2 gene





Type of mutations in U2AF1 gene



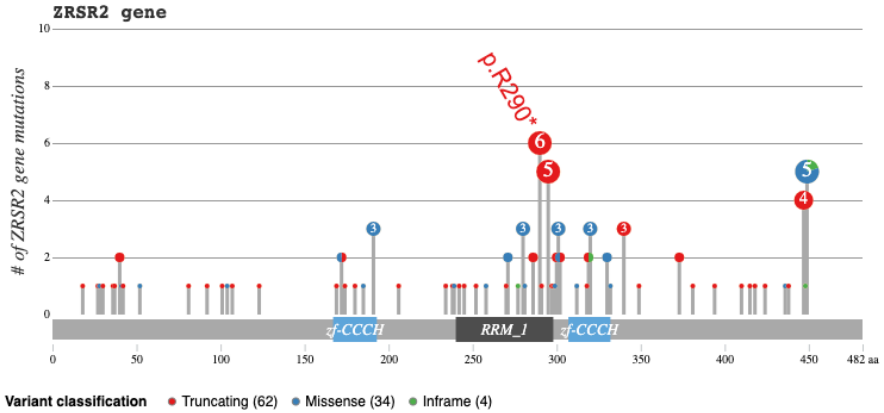


Fabiani *et al.,* Unpublished data

Bersanelli et al., JCO 2021

Exclusion criteria

ZRSR2 mutated patients

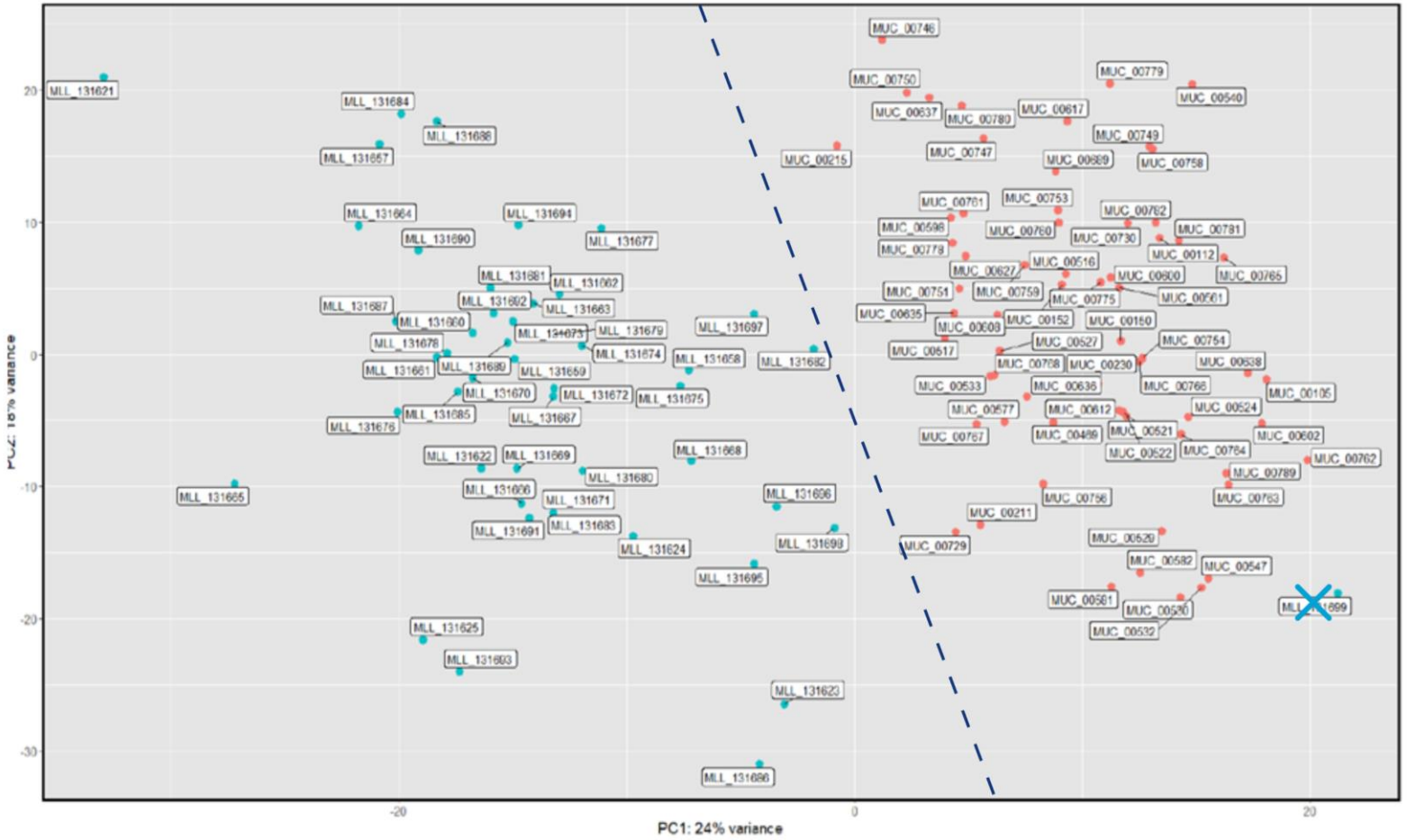


SF co-mutated patients

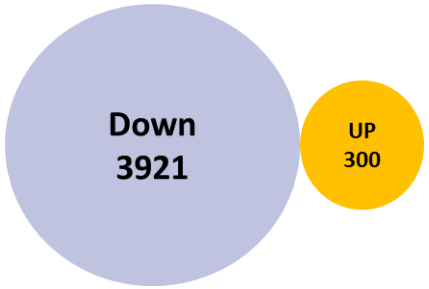
Patient ID	Mutations	VAF (%)	CO-Mutations	VAF (%)
UPN2	SF3B1 E738K	33,6	SRSF2 P95H	21
UPN13	SF3B1 P370T	4,5	SRSF2 P95H	31,4
UP16	U2AF1 Q157P	39,1	ZRSR2 K405Rfs*	74,2
UPN17	U2AF1 Q157P	11,7	ZRSR2 W340*	30
UPN27	SF3B1 K700E	42,1	SRSF2 R94H100del	29,9

Differentially expressed genes by RNA-Seq analysis

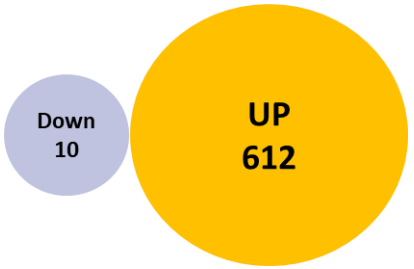
MDS patients | **Controls**



LR-MDS vs CTRL



***SF3B1* mut vs SF WT**



***SRSF2* mut vs SF WT**

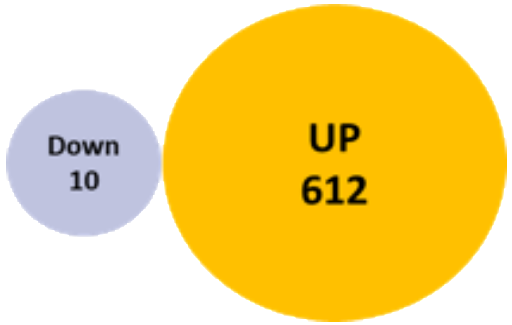
***U2AF1* mut vs SF WT**

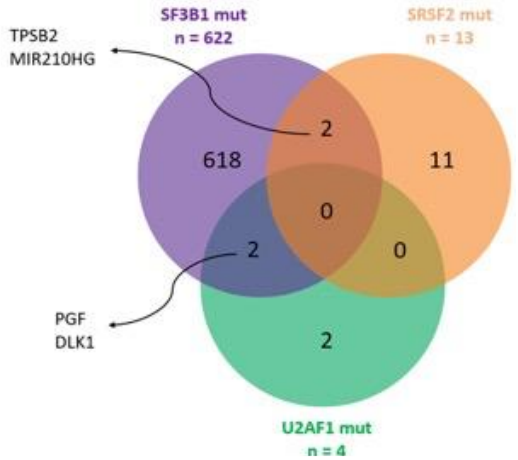
***p-adj* < 0,05; Log2 FC ≥2 e log2 FC≤- 2**

Fabiani *et al.*, Unpublished data

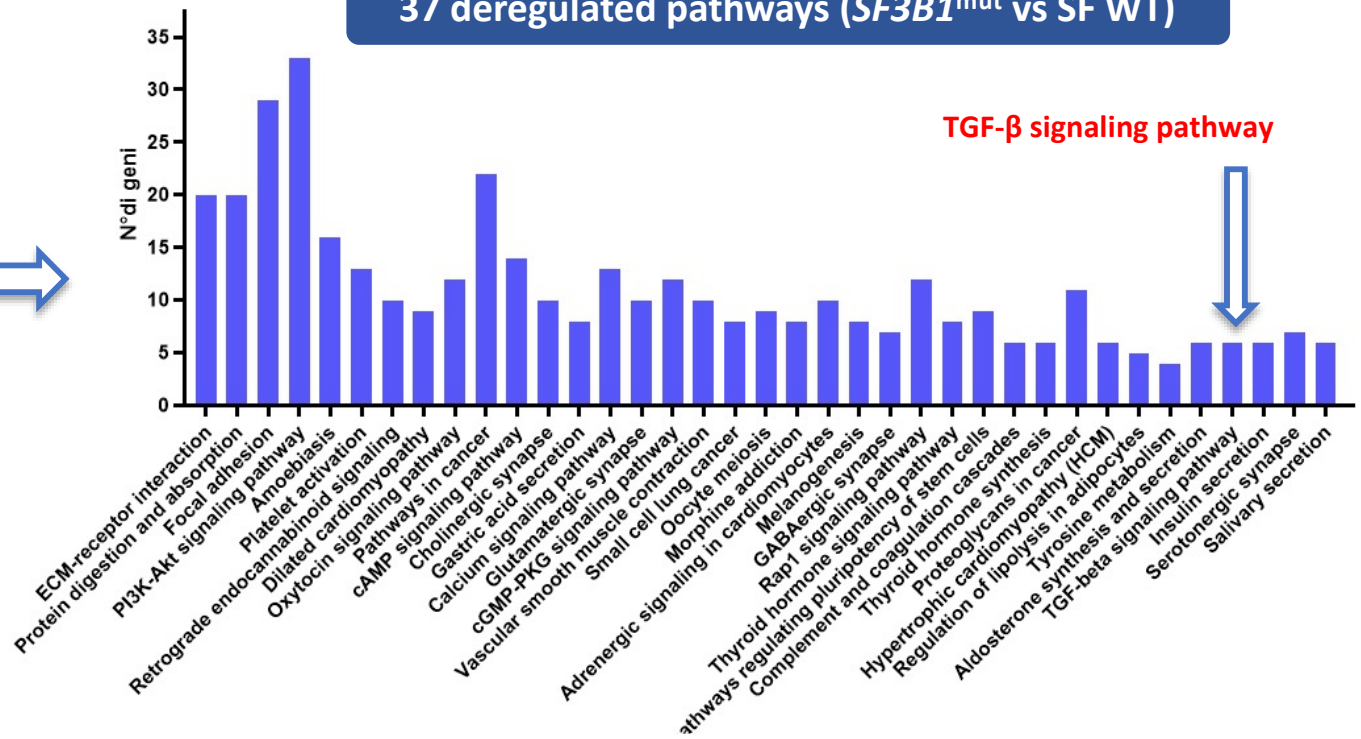
Differentially expressed genes in LR-MDS SF3B1^{mut}

***SF3B1*^{mut} (13) vs SF WT (8)**





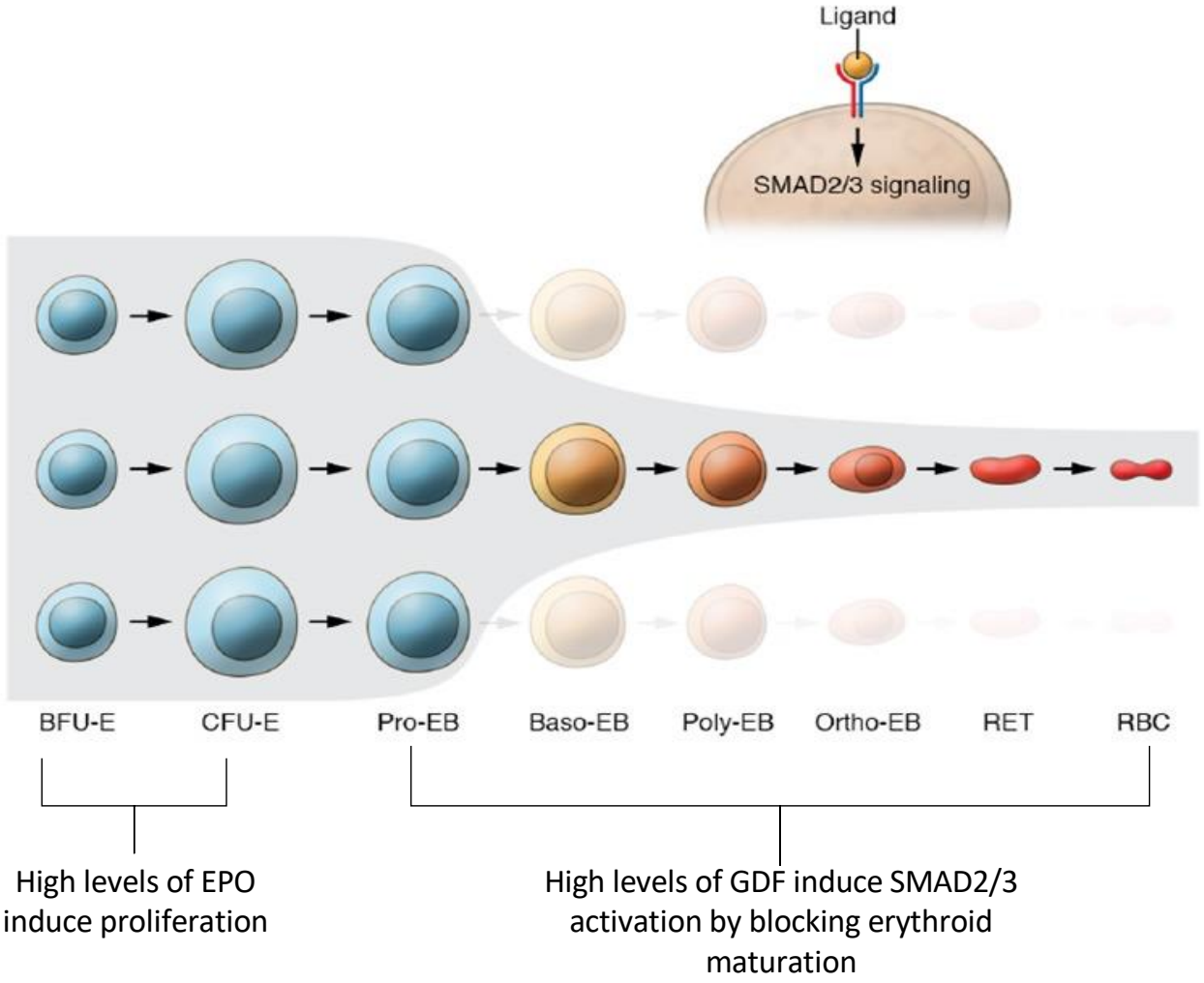
37 deregulated pathways (*SF3B1*^{mut} vs SF WT)



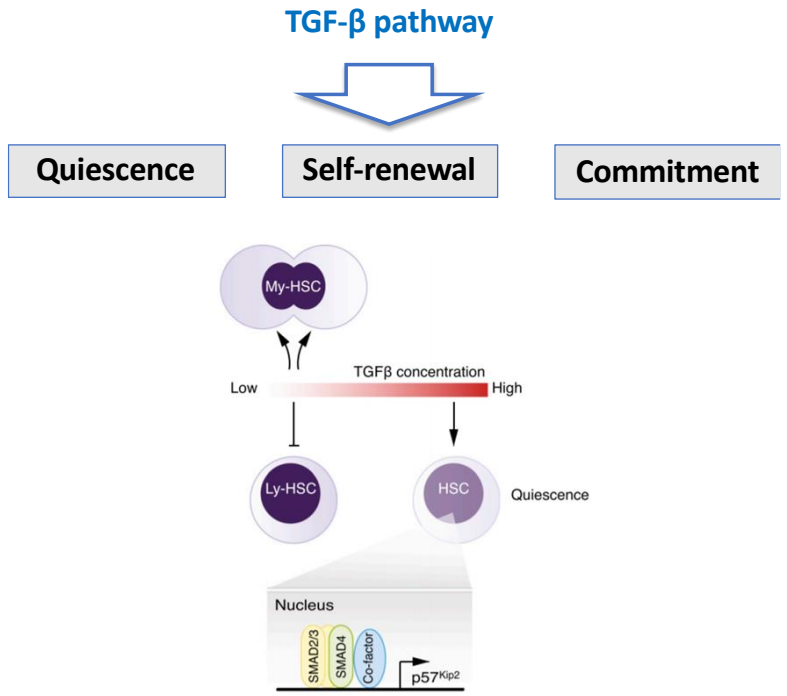
DAVID Bioinformatics Resources 6.8
Laboratory of Human Retrovirology and Immunoinformatics (LHRI)

Fabiani *et al.*, Unpublished data

TGF-β pathway and modulators in SF3B1^{mut}

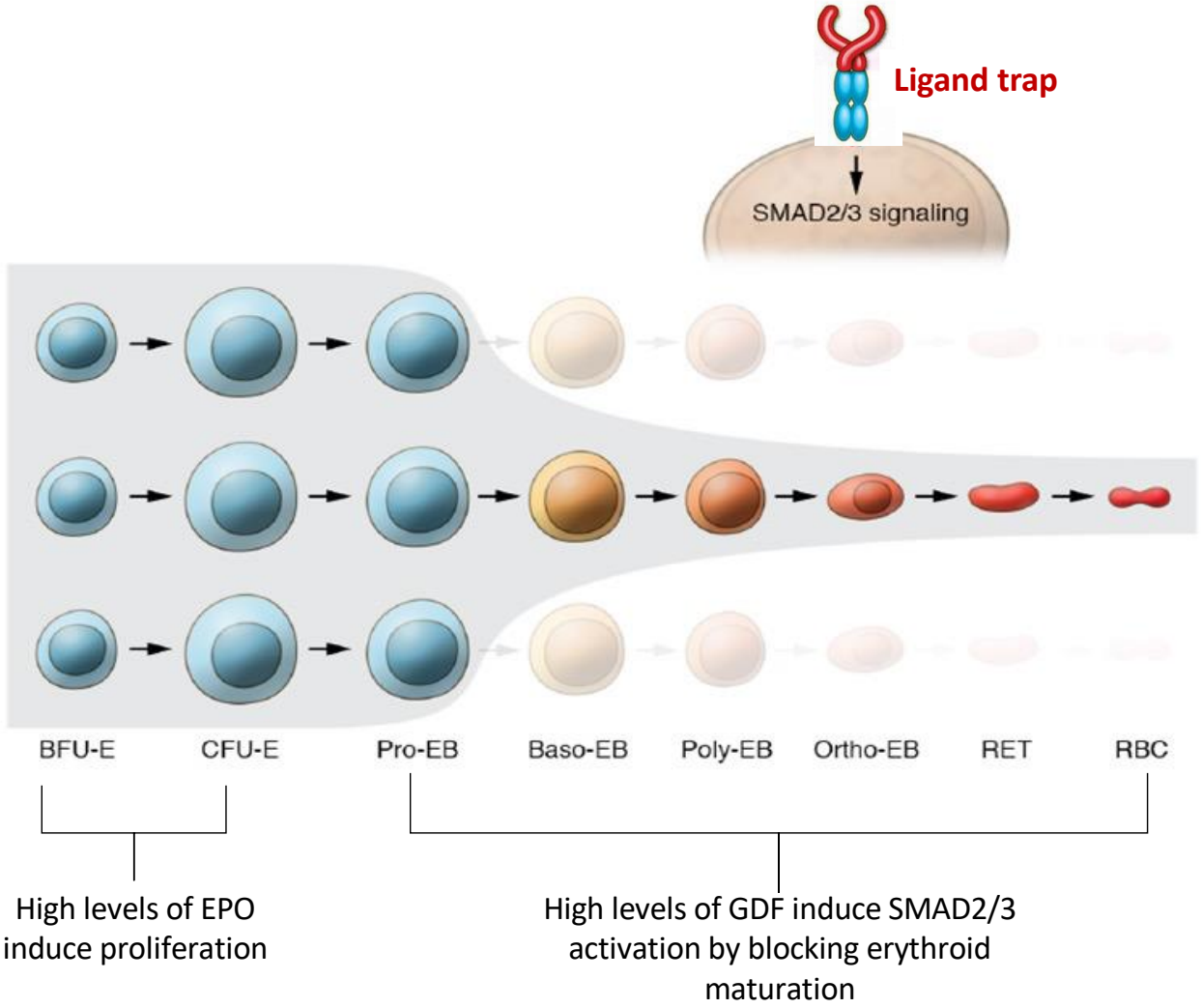


- Transforming growth factor beta (TGF-β) signaling pathway is key to hematopoiesis regulation
- Up-regulation of TGF-β signaling has been proposed as one of the causes of ineffective hematopoiesis



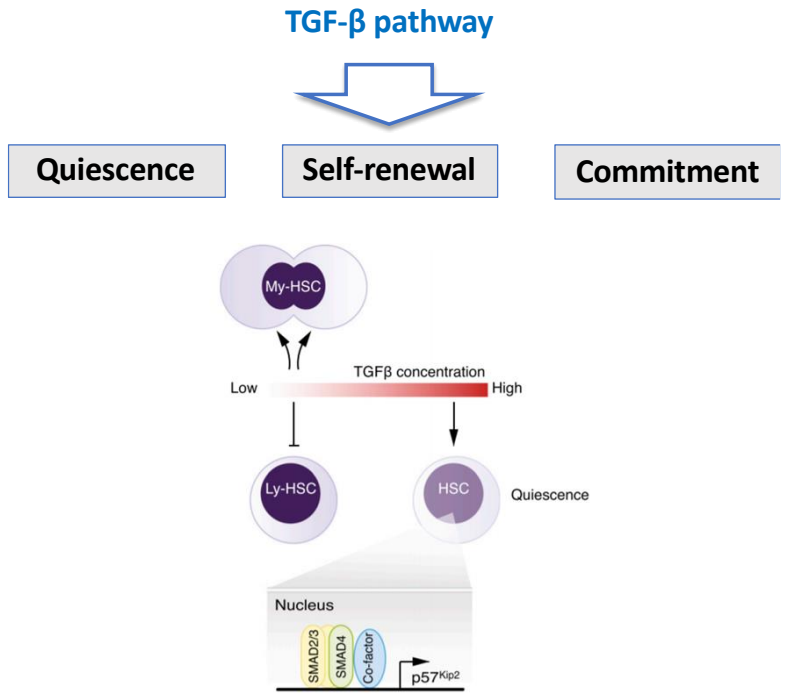
Verma A, et al. J Clin Invest. 2020; Zhou L, et al. Blood 2008

TGF-β pathway and modulators in SF3B1^{mut}



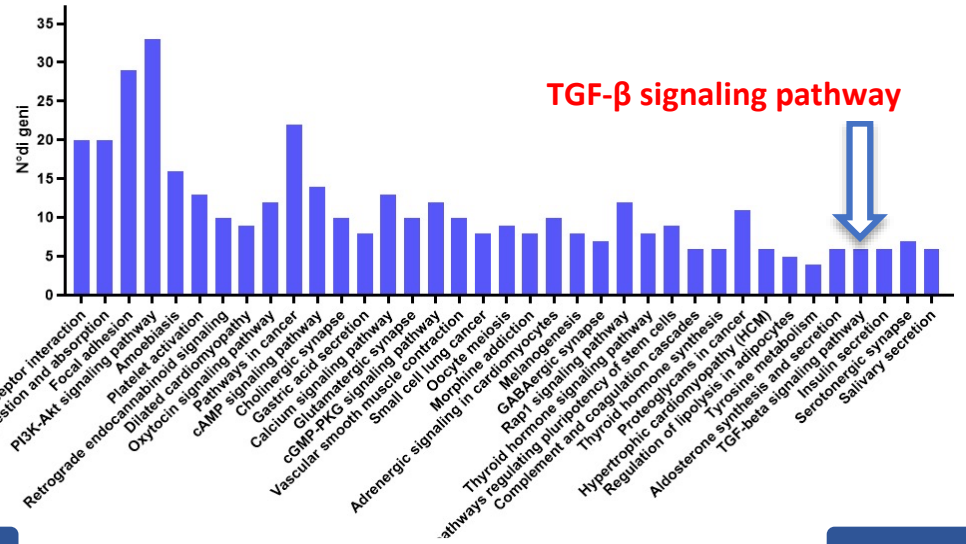
Ligand trap

- It binds to GDF11 and other members of the TGF-B superfamily, inhibiting their binding to the activin IIB receptor.
- Thus, it prevents the signal activation of SMAD2 and SMAD3.

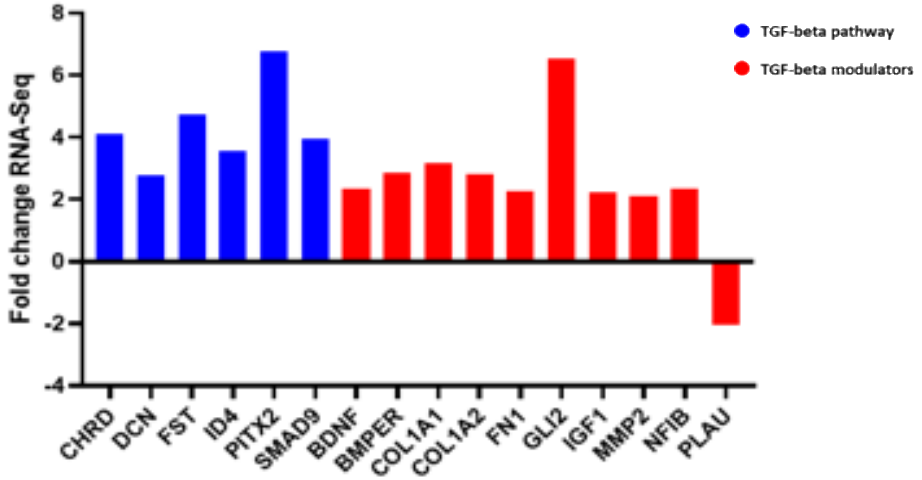


Verma A, et al. J Clin Invest. 2020; Zhou L, et al. Blood 2008

TGF-β pathway and modulators in SF3B1^{mut}



TGF-beta pathway and modulators



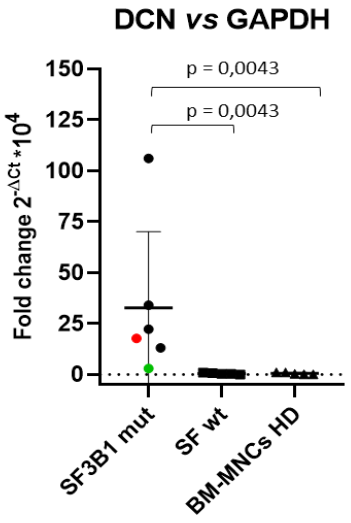
Validation

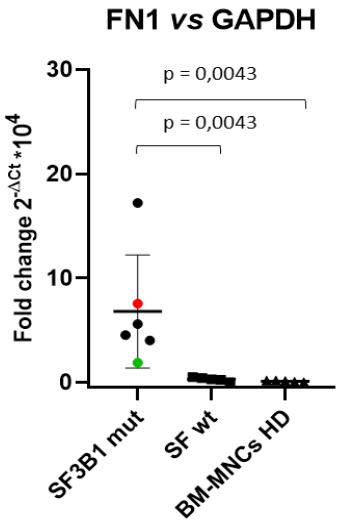
Q-RT-PCR validation

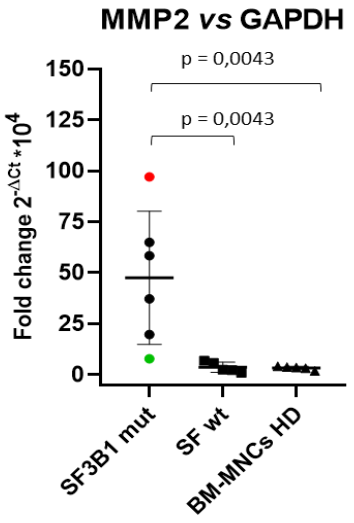
SMAD9 (SMAD family member 9)
DCN (Decorin)
FST (Follistatin)
FN1 (Fibronectin)
IGF1 (Insulin-like growth factor I)
MMP2 (Matrix metallopeptidase 2)
PLAU (Plasminogen activator, urokinase)

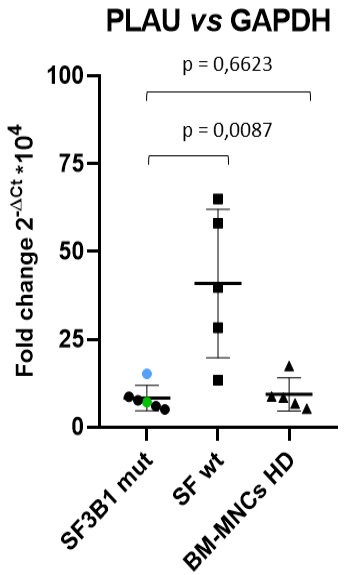
Fabiani *et al.,* Unpublished data

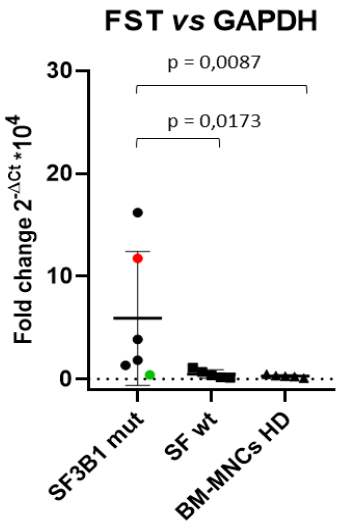
TGF-β pathway and modulators in SF3B1^{mut}

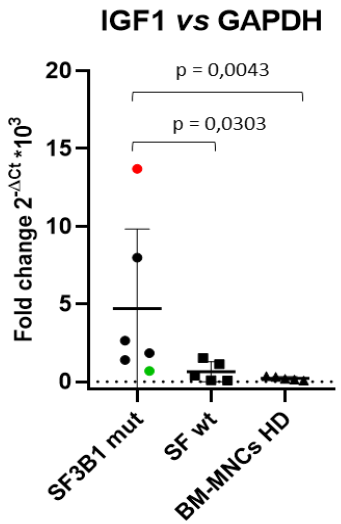


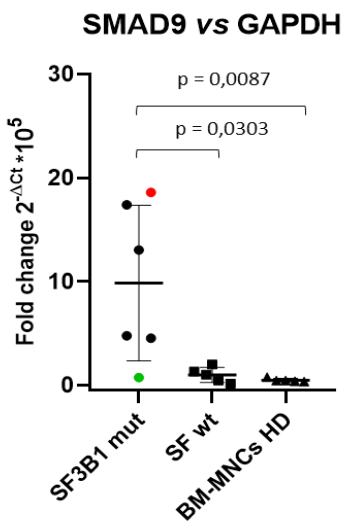






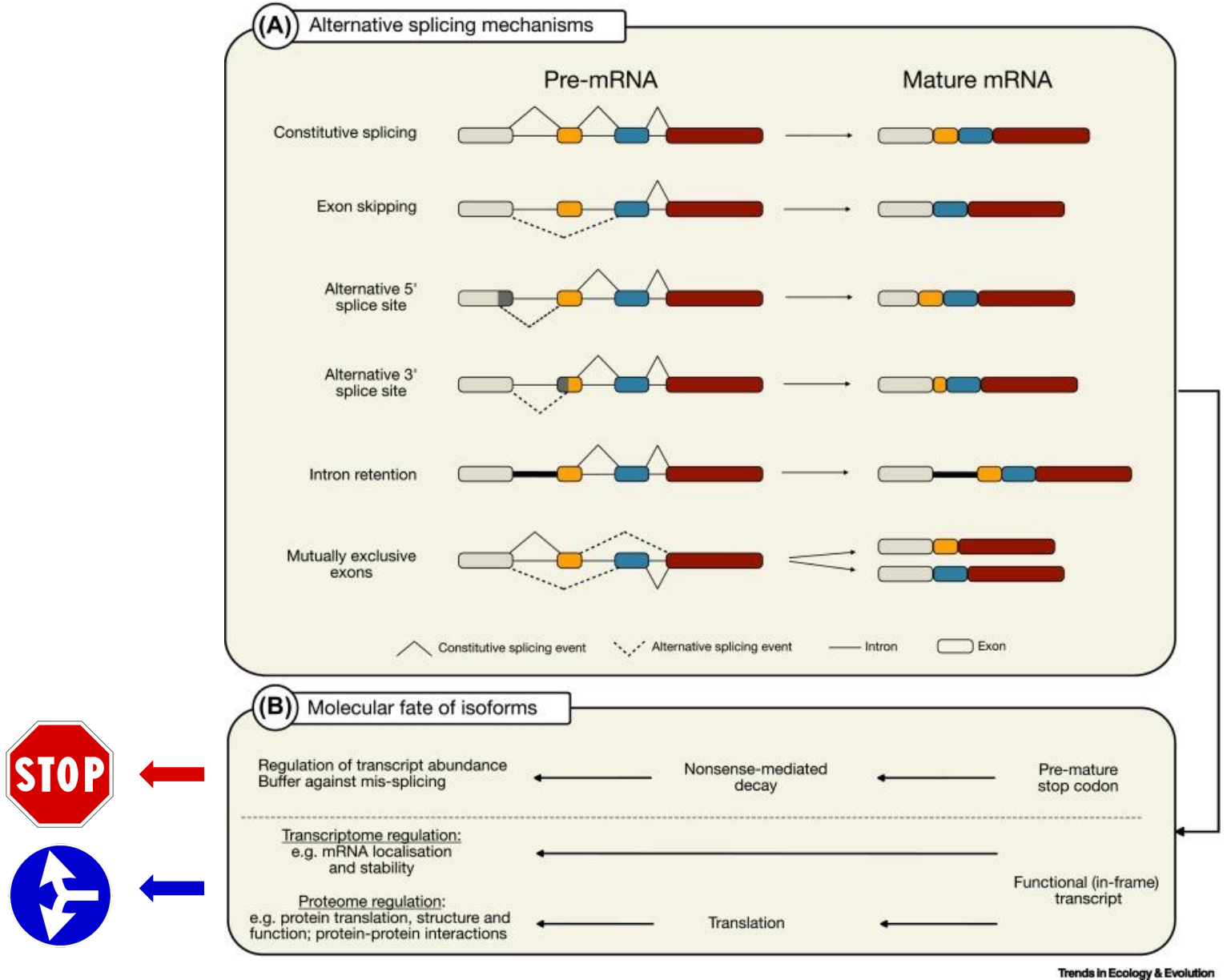






Fabiani *et al.,* Unpublished data

Alternative splicing events

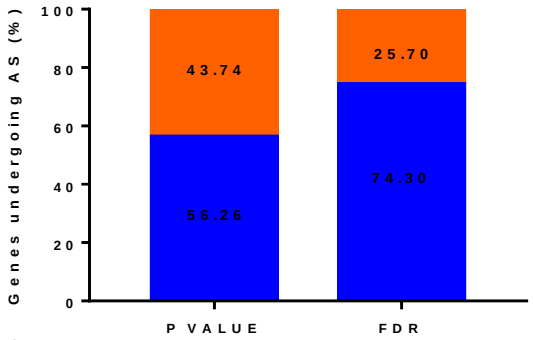


Percentage of genes subjected to AS regulation

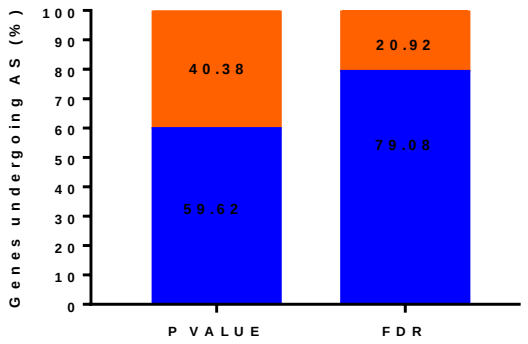
Regulated
Unregulated

A3SS: alternative 3'ss
A5SS: alternative 5'ss
RI: intron retention
SE: exon skipping
MXE: mutually exclusive exons
EV MIX: >1 AS events

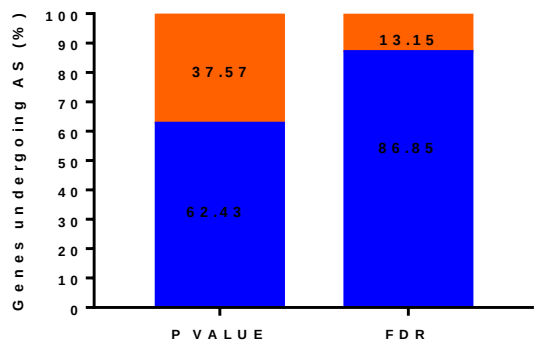
SF3B1-K700E



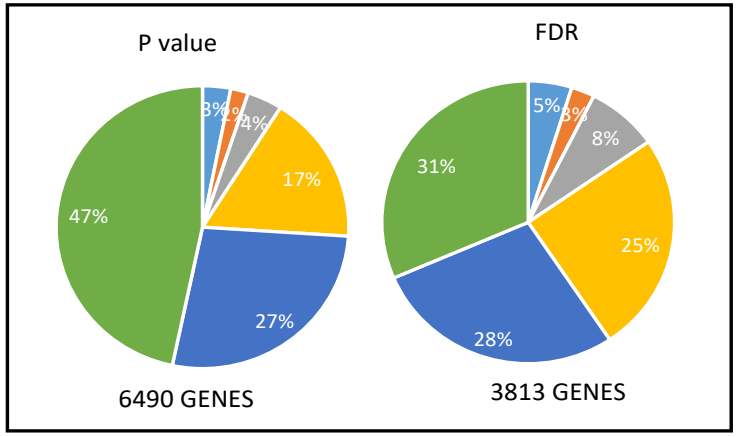
SRSF2-P95



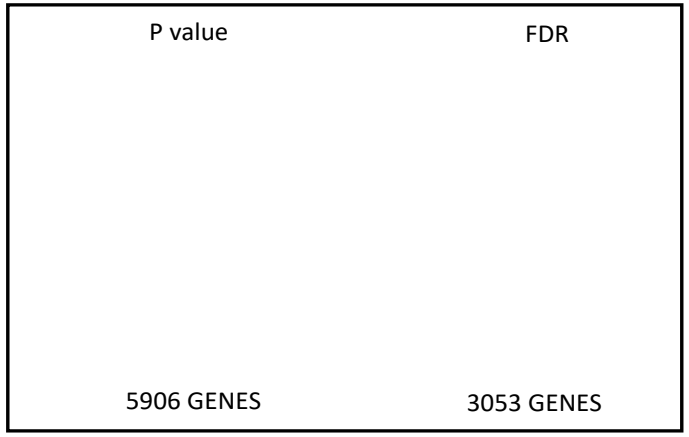
U2AF1



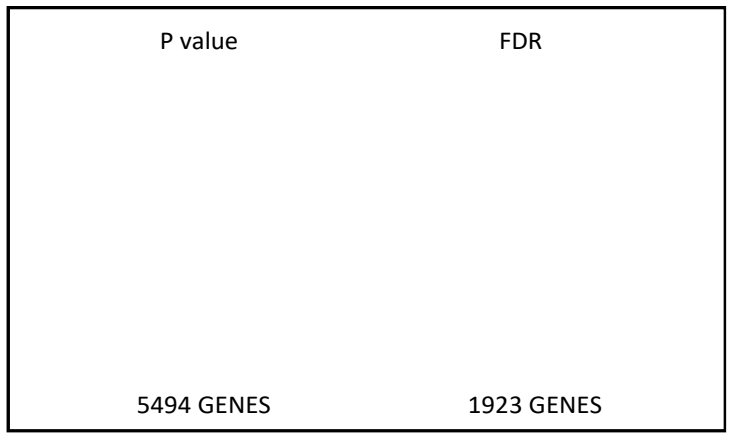
SF3B1-K700E MDS



SRSF2 MDS



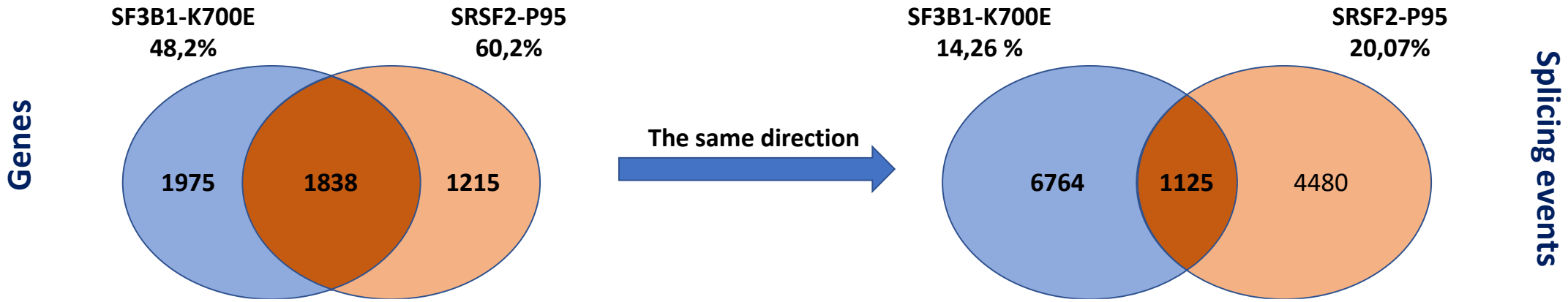
U2AF1 MDS



A3SS A5SS RI MXE SE EV MIX

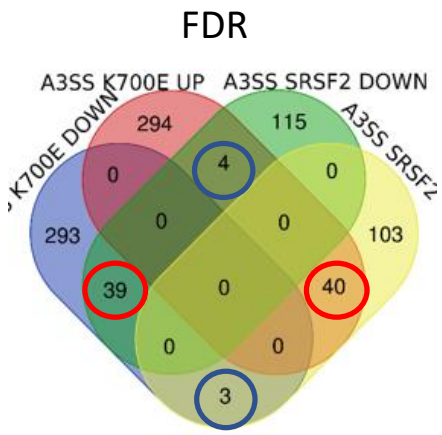
Fabiani *et al.*, Unpublished data

Overlap of genes/events undergoing AS regulation in SF3B1-K700E vs SRSF2-P95 mutated patients

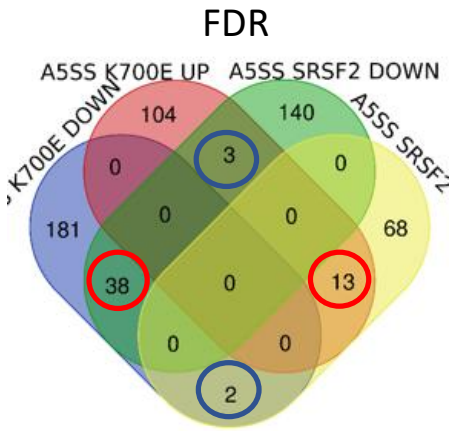


Red dashed circle = AS regulated in the same direction; **Blue dashed circle** = AS regulated in opposite direction

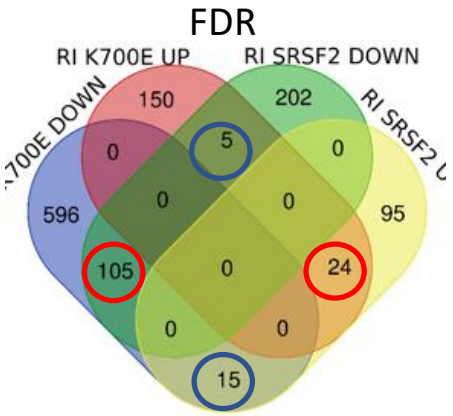
A3ss



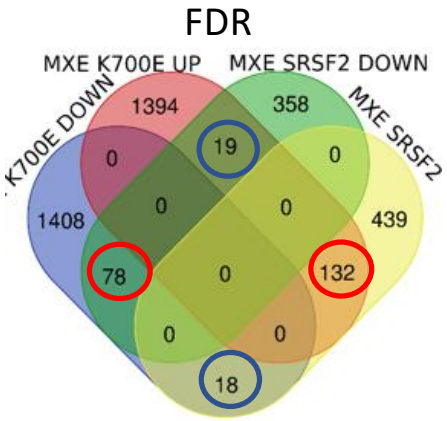
A5ss



RI



MXE



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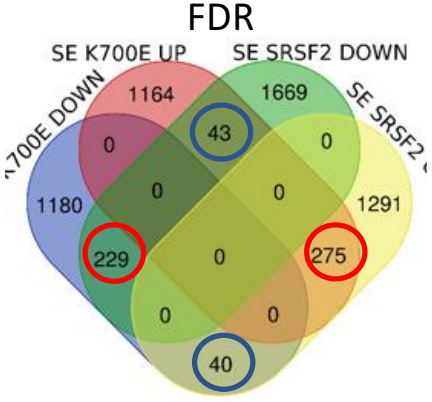
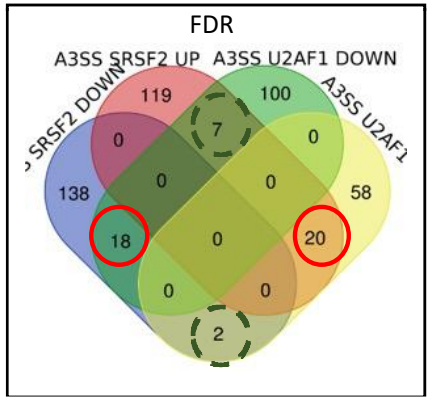


Fabiani *et al.*, Unpublished data

Overlap of genes/events undergoing AS regulation in SRSF2-P95 *vs* U2AF1 and SF3B1-K700E *vs* U2AF1 mutated patients

AS regulation in SRSF2-P95 *vs* U2AF1

A3'ss



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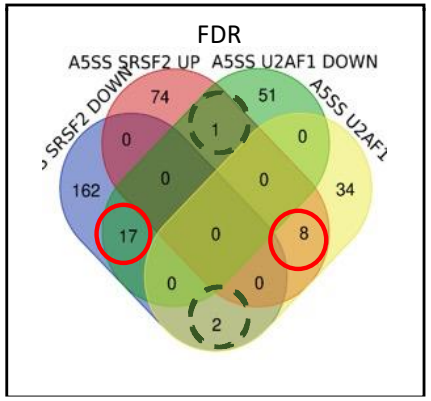

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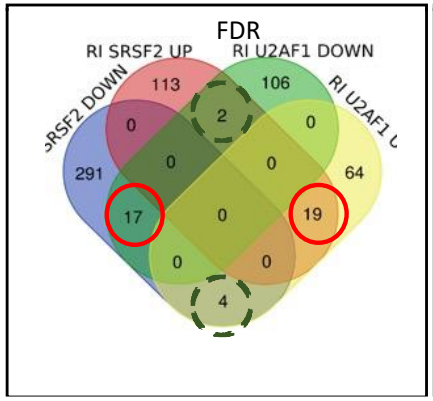

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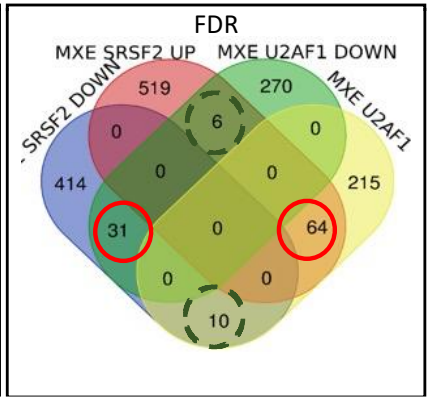
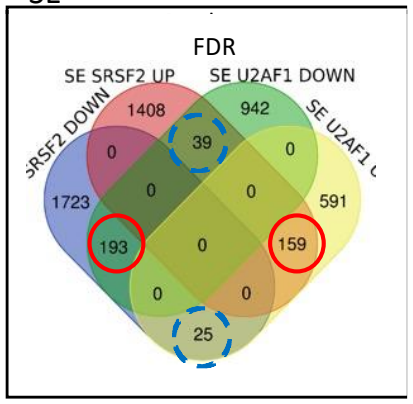
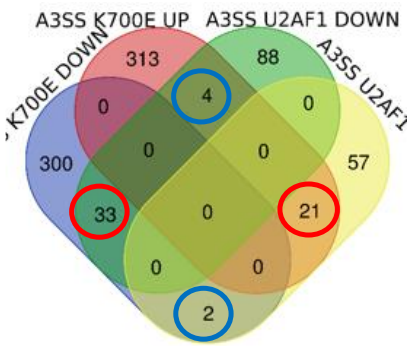
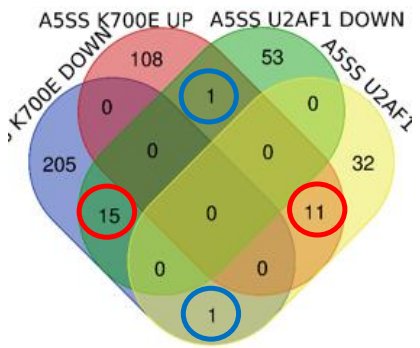


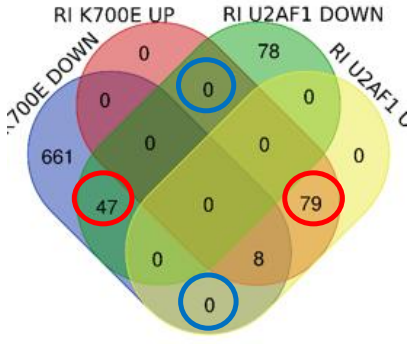
SE

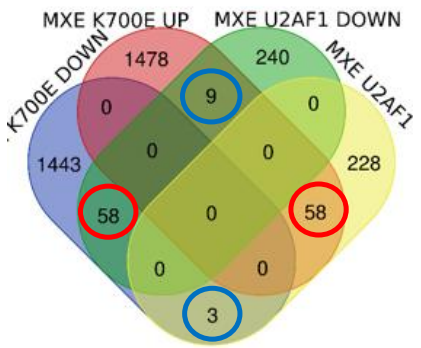


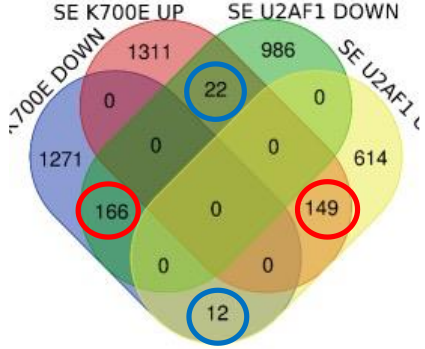
AS regulation in SF3B1-K700E *vs* U2AF1





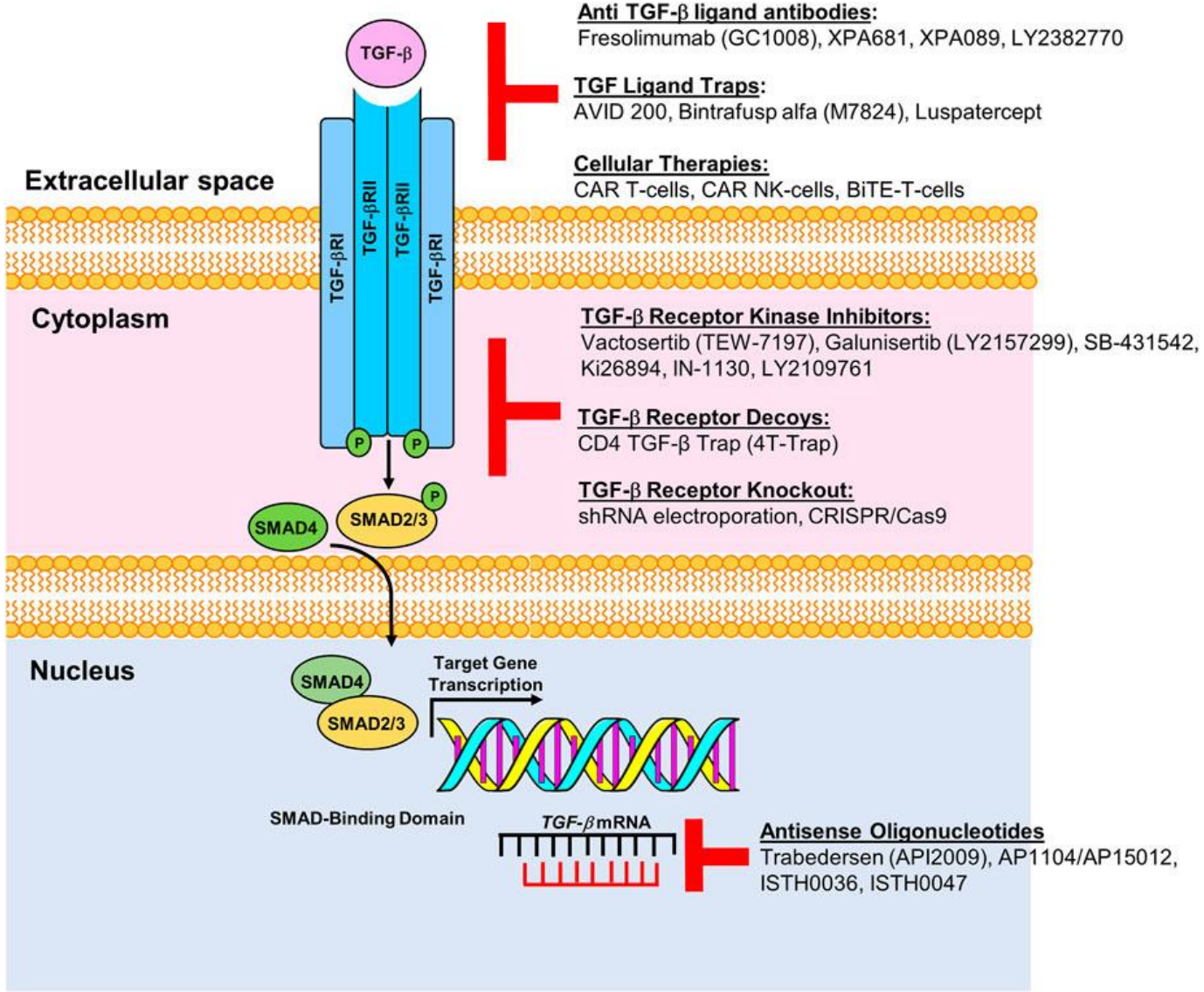






Fabiani *et al.,* Unpublished data

Alternative Splicing in TGF-β Signaling Pathway



TGFBR2, transforming growth factor, beta receptor II

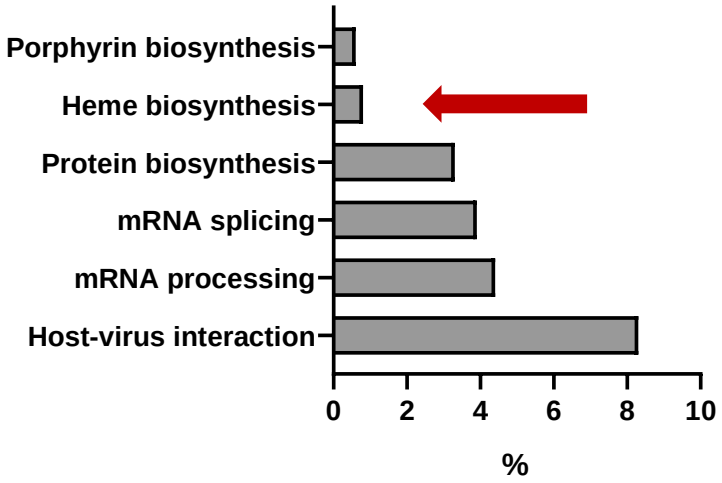
- The protein encoded by this gene is a transmembrane protein that has a protein kinase domain, forms a heterodimeric complex with TGF-beta receptor type-1, and binds TGF-beta
- This receptor/ligand complex phosphorylates proteins, which then enter the nucleus and regulate the transcription of genes related to cell proliferation, cell cycle arrest and tumorigenesis
- Deregulation of TGF-β pathway can be overcome by targeting the TGF-β receptors with ligand antibodies, ligand traps or by inhibiting TGF-β receptors using specific kinase inhibitors or by knocking out the TGF receptor genes with antisense oligonucleotides (e.g., AP11014 and AP15012)

Rana *et al.,* Front Cell Dev Biol 2022

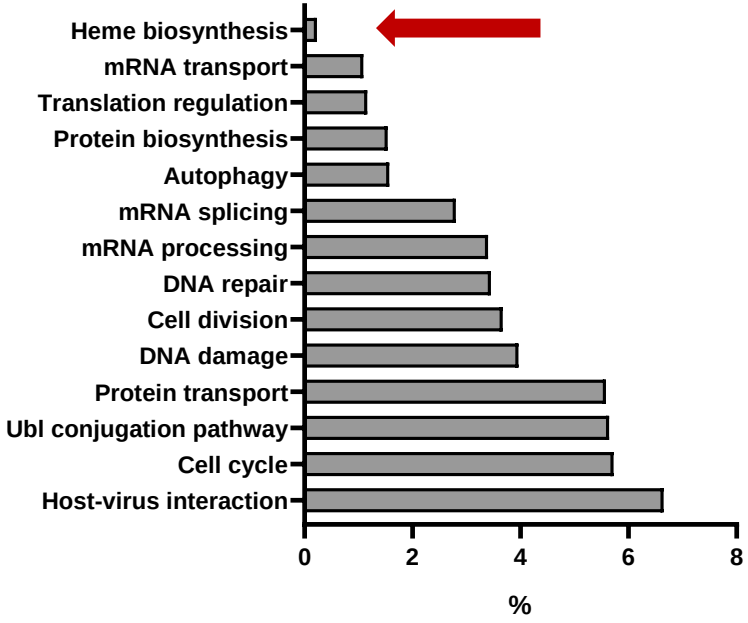
Functional annotation of AS events based on mutational status

The heme biosynthesis pathway is altered in LR-MDS SF3B1 and SRSF2 mutated patients

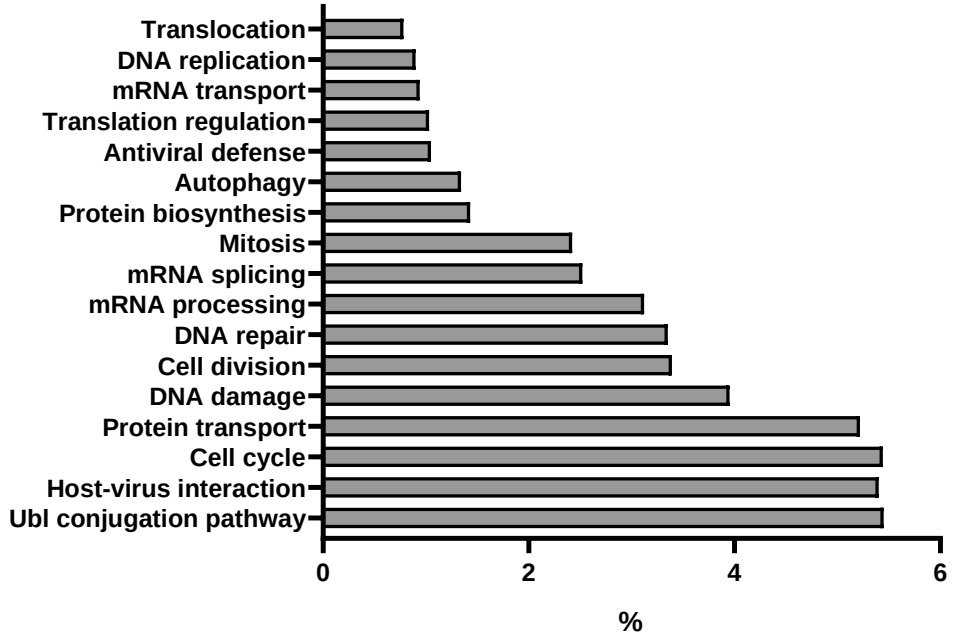
SF3B1-K700E



SRSF2- P95H/L/A

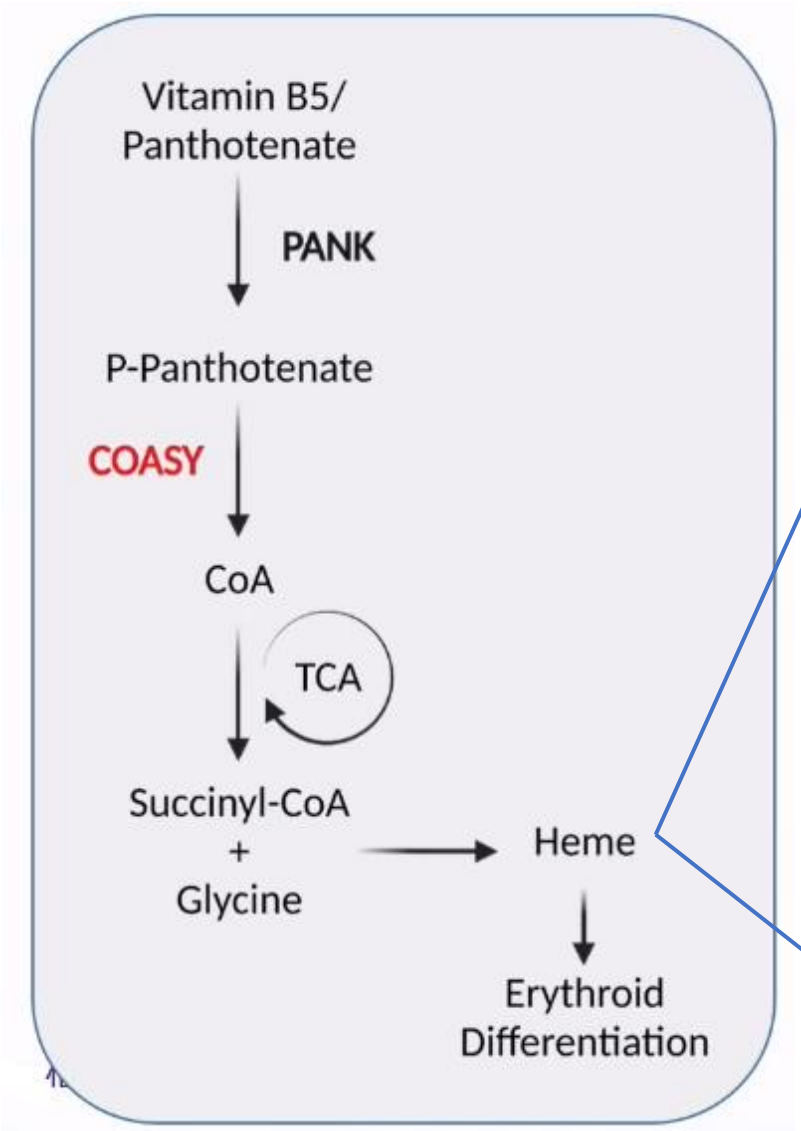


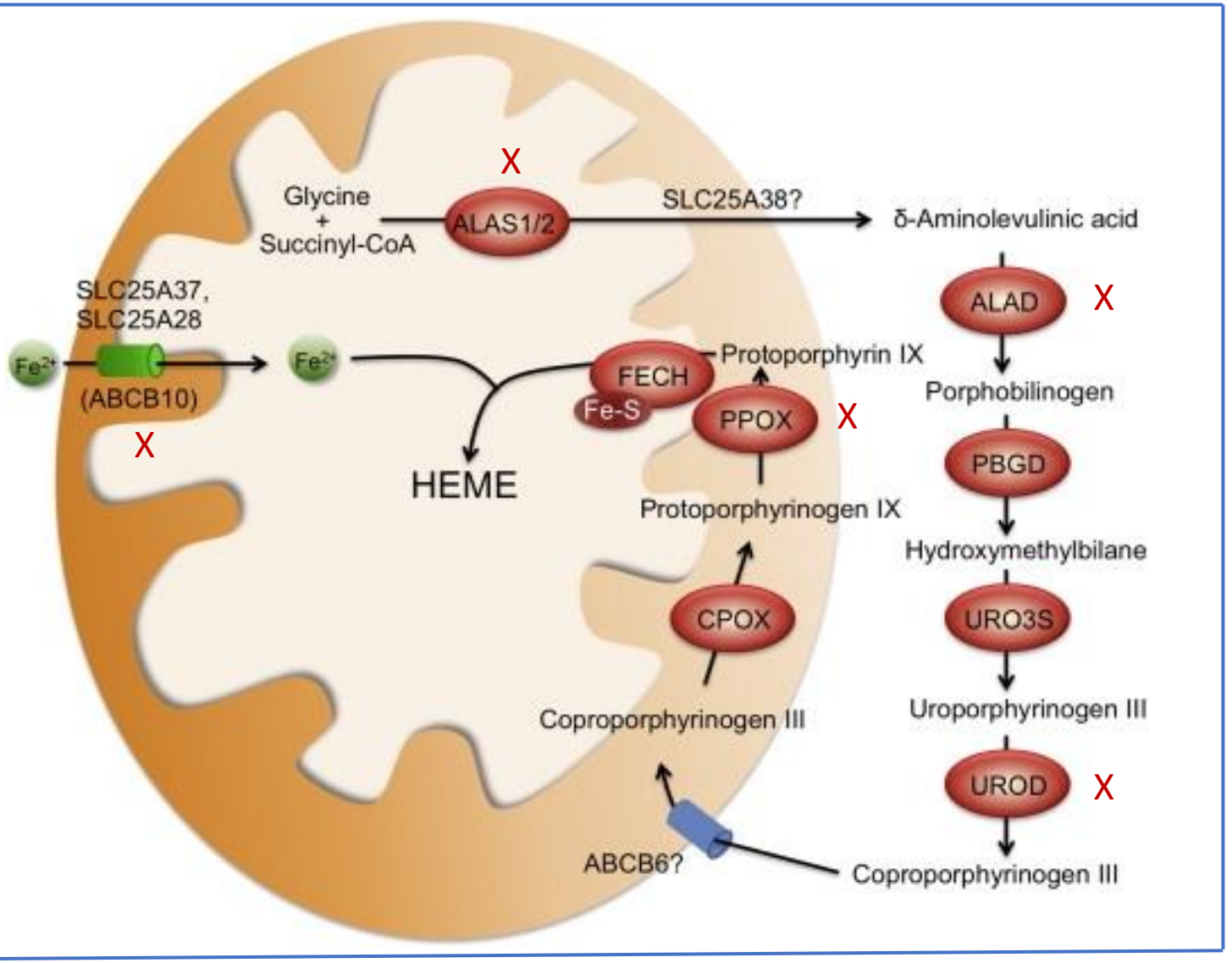
U2AF1-S34 and -Q157



Fabiani *et al.,* Unpublished data

COASY deregulation impacts CoA synthesis and erythroid differentiation



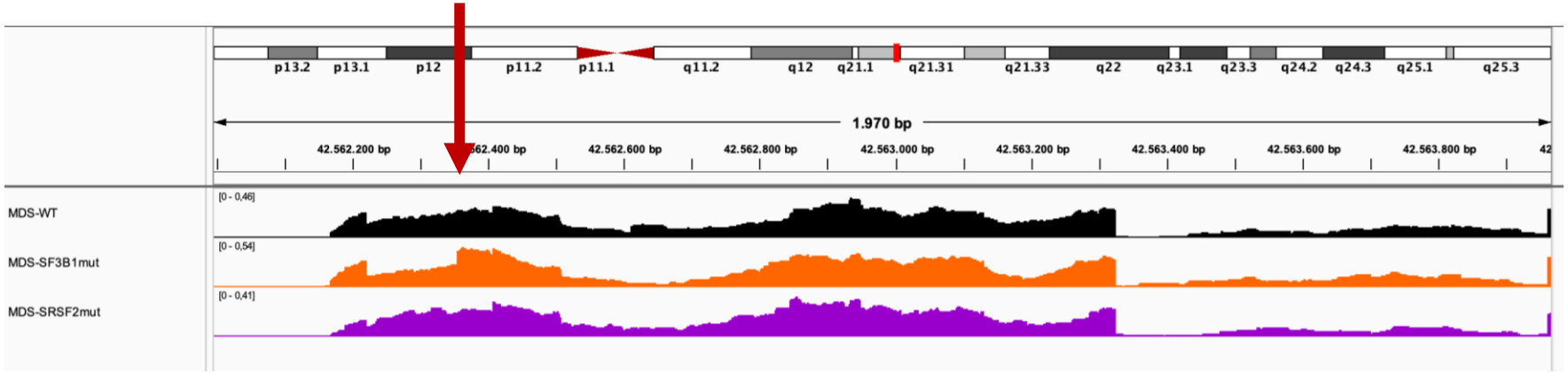


Philippe et al. Marseille, MDS 2023

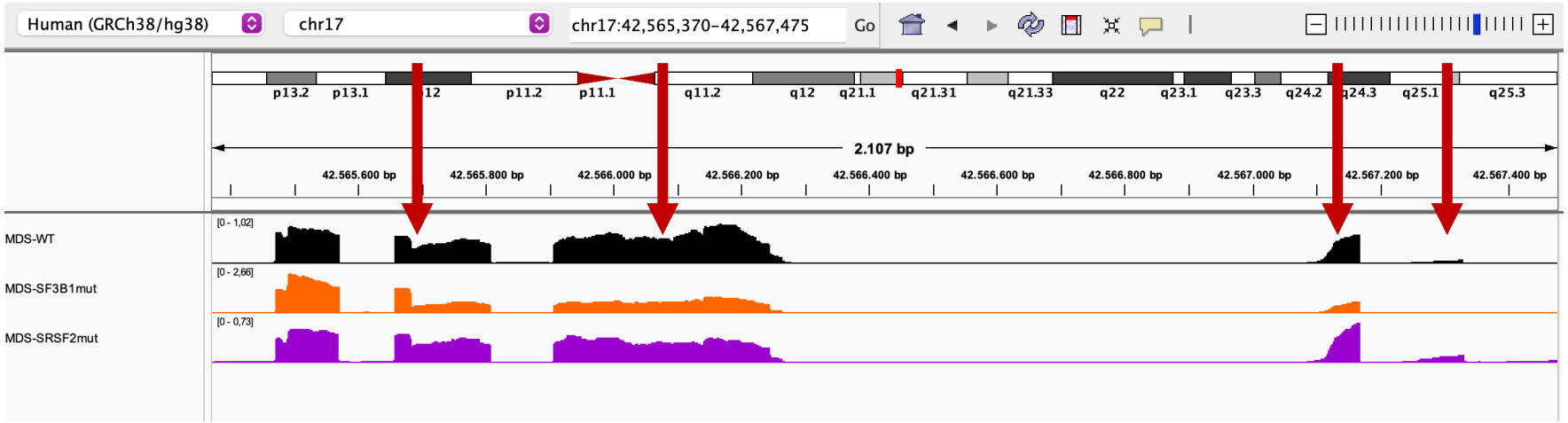
Fabiani *et al.,* Unpublished data

COASY: Alternative Splicing Events

Annotated event (PubMed)



Unannotated events



Fabiani *et al.,* Unpublished data

Conclusions

- RNA-Seq analysis showed a strong difference in gene expression profile between LR-MDS and non-hematological patients
- MDS with *SF3B1*^{K700E} showed a distinctive transcriptomic profile, while SRSF2 and U2AF1 mutated patients showed a more heterogeneous one
- In splicing factors mutated patients the alternative splicing events seem to be prominent compared to gene expression profile
- TGF-β pathway was identified as one of the biological pathways differently expressed in *SF3B1*^{K700E} *patients, suggesting its potential role* in the pathogenesis of MDS
- Recent findings suggest the involvement of COASY enzyme and genes belonging to HEME metabolism in erythroid differentiation
- Vitamin B5 and succinyl-CoA may improve ineffective erythropoiesis in SF3B1 mutated MDS (Philippe *et al.,* 2023)

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8° WORKSHOP
In Ematologia Traslazionale
Della Società Italiana di Ematologia Sperimentale