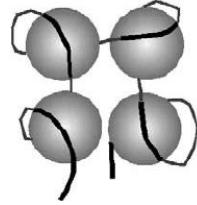


The Supraspliceosome

Joint CCA-Z Project meeting

Amsterdam, September 23, 2019



Targeting SF3B1 as a novel therapeutic strategy against diffuse malignant peritoneal mesothelioma

Elisa Giovannetti



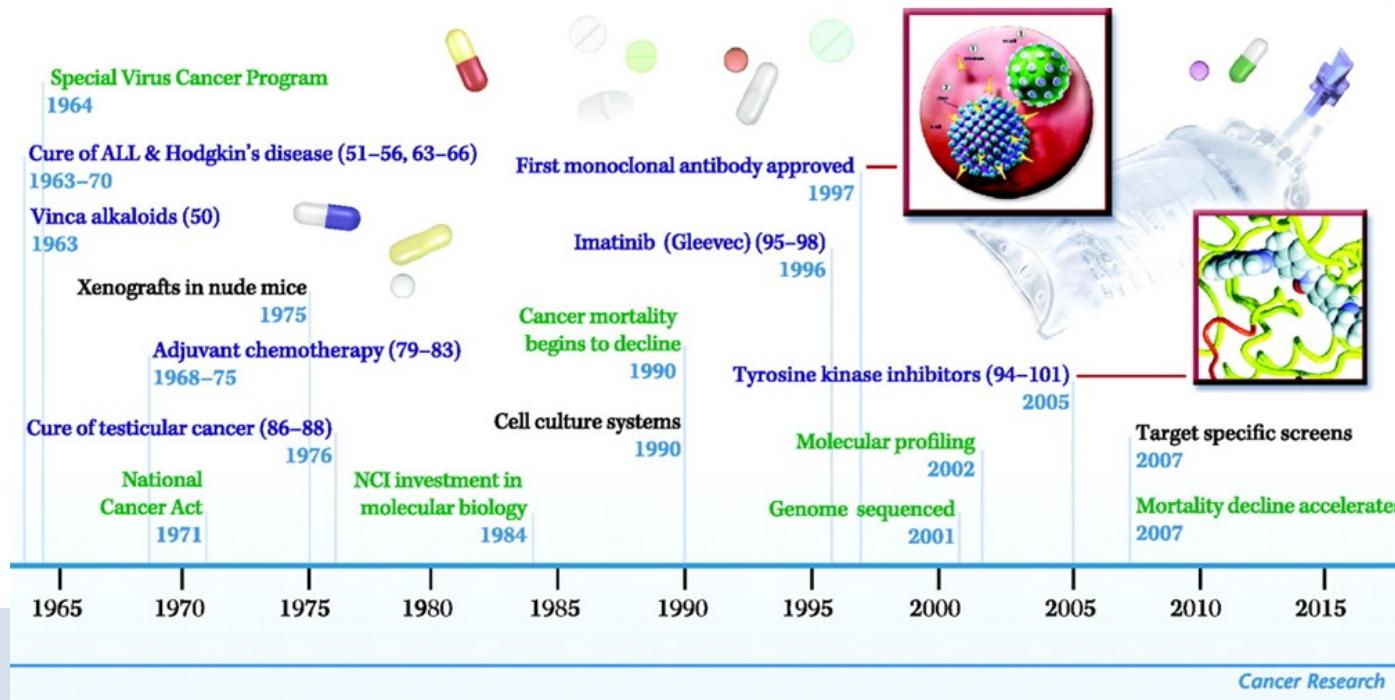
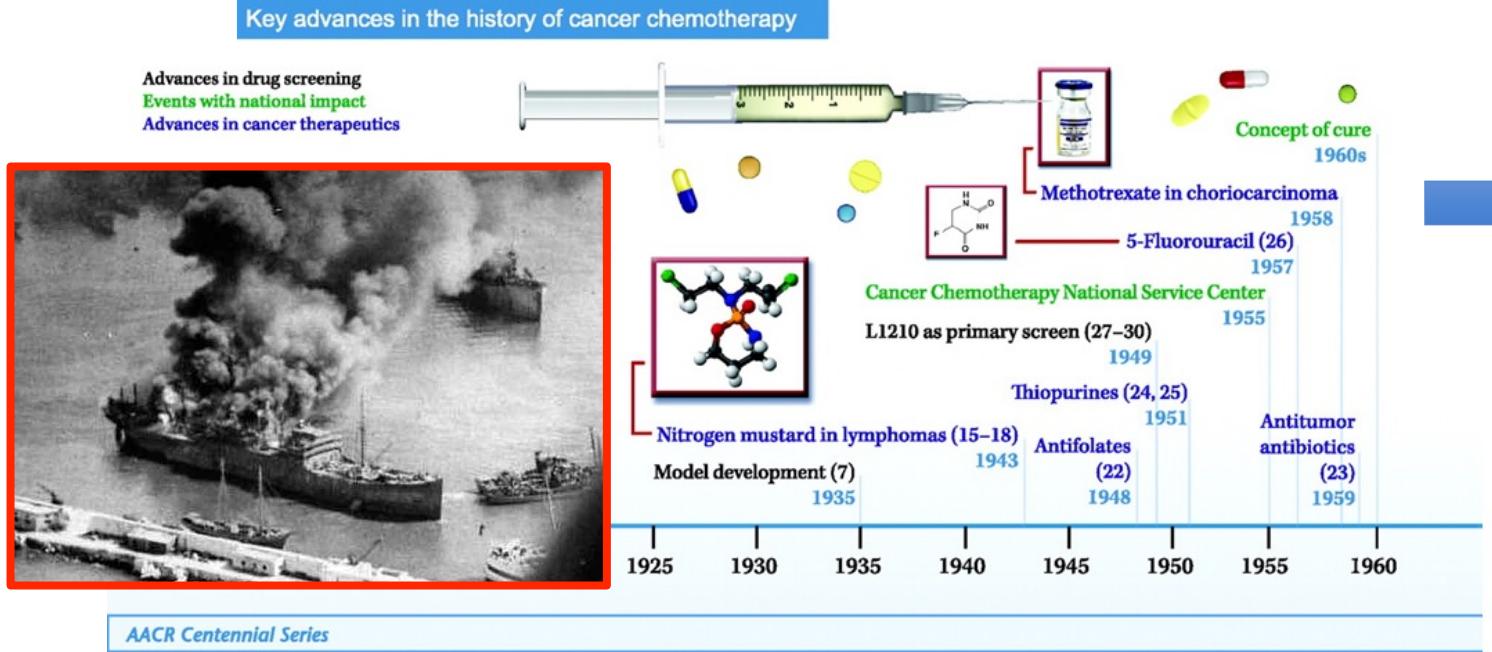
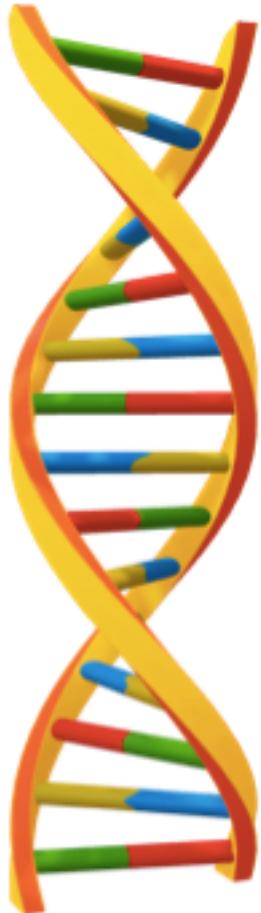
LabMO, Amsterdam UMC, Cancer Center Amsterdam
Cancer Pharmacology Lab, AIRC Start-Up Unit, FPS, Pisa



23rd September 2019

A T

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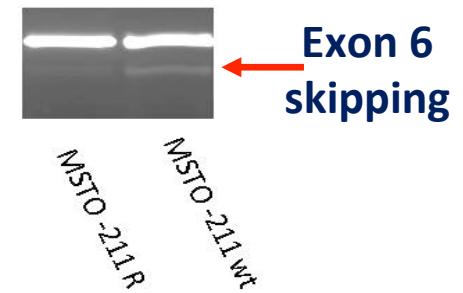
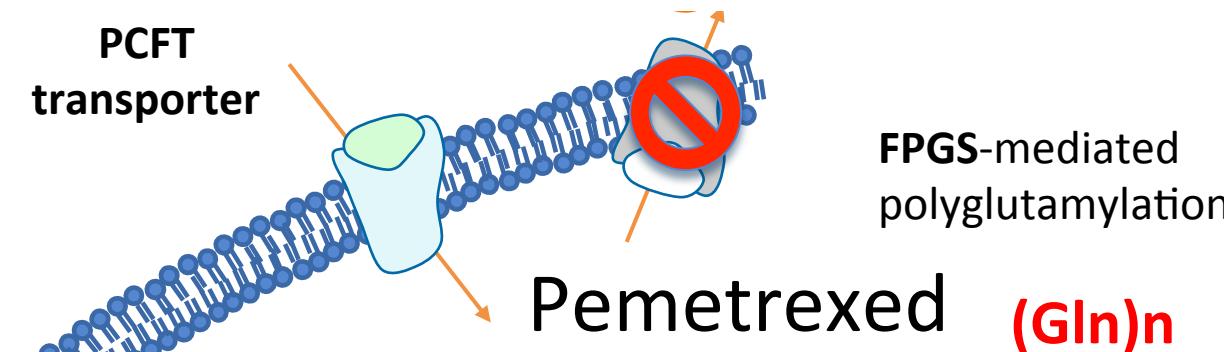
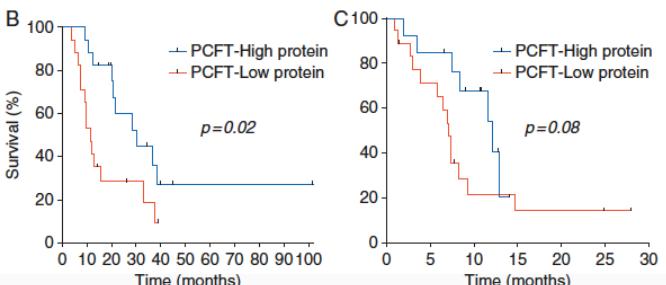
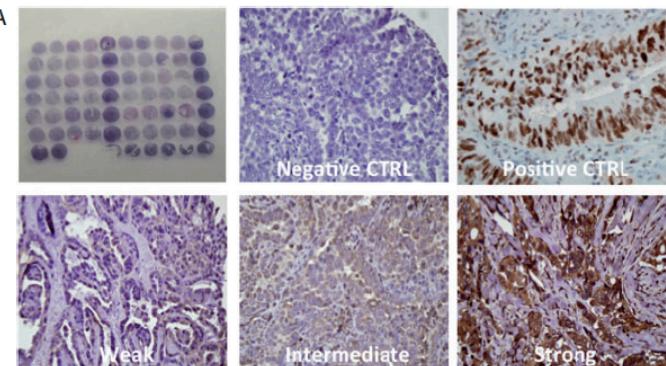
Antimetabolites & antifolates

Pemetrexed

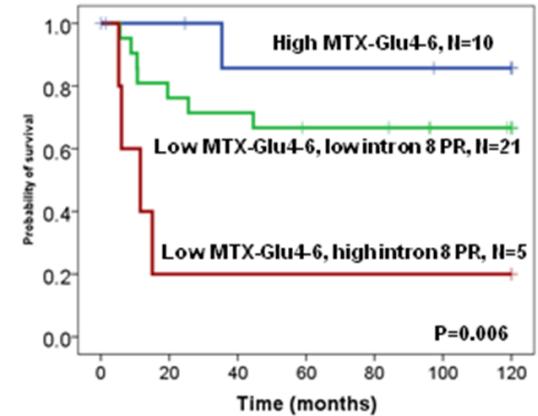


for NSCLC / MPM

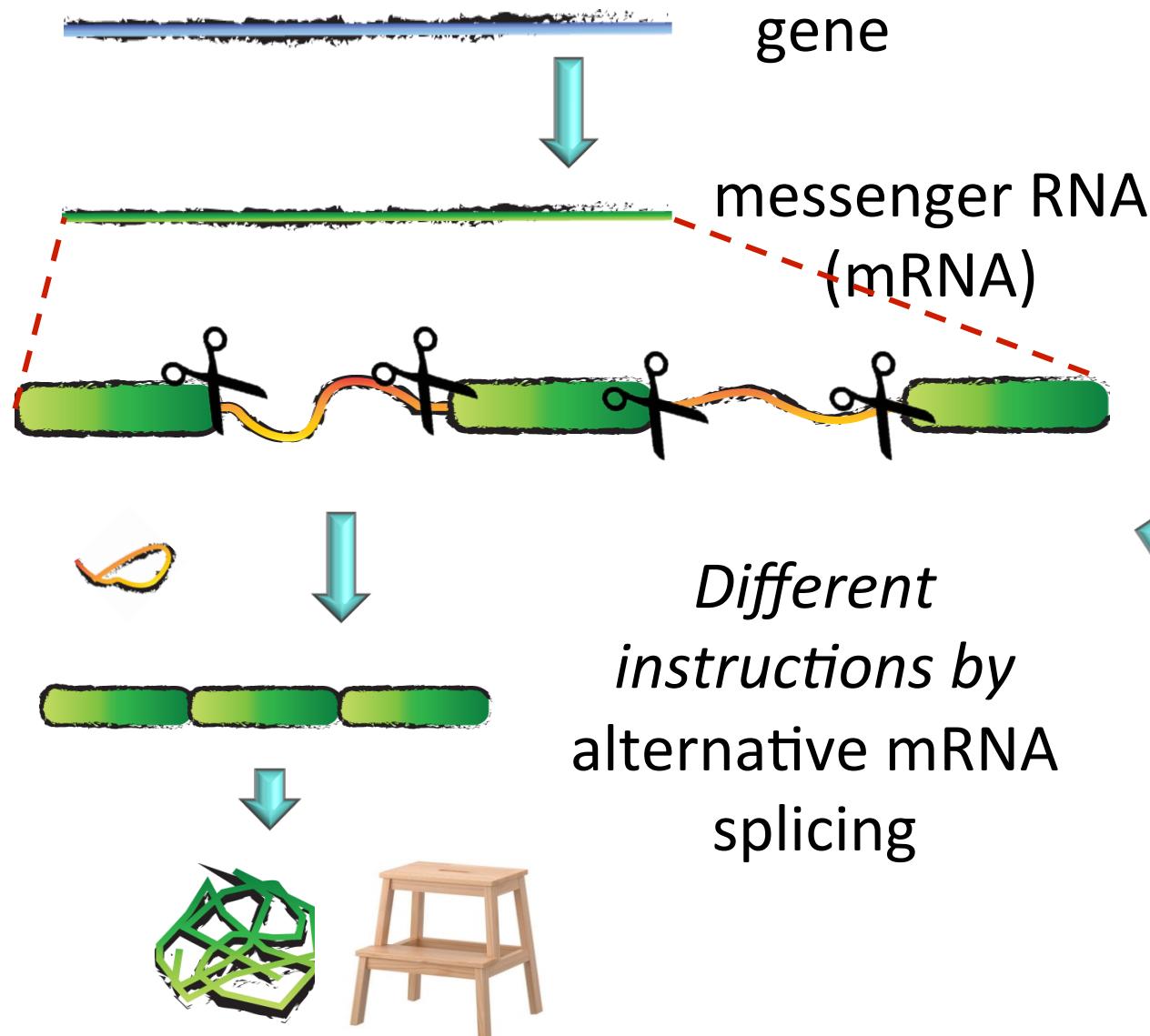
Giovannetti et al.
Ann Oncol 2017



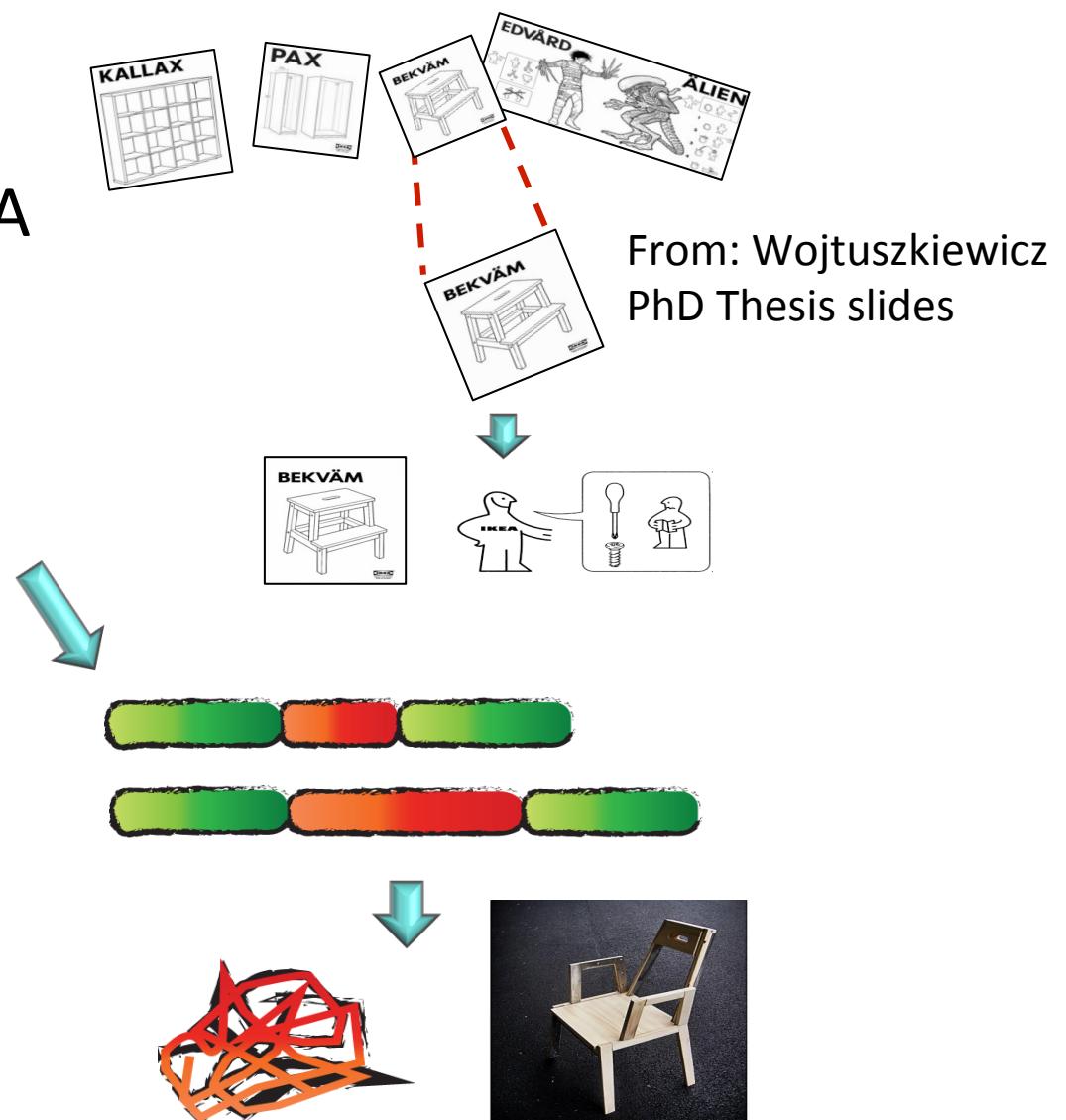
Wojtuszkiewicz et al.
Hematologica 2016



Galvani, Peters,
Giovannetti. *Exp Opin Invest Drugs* 2011

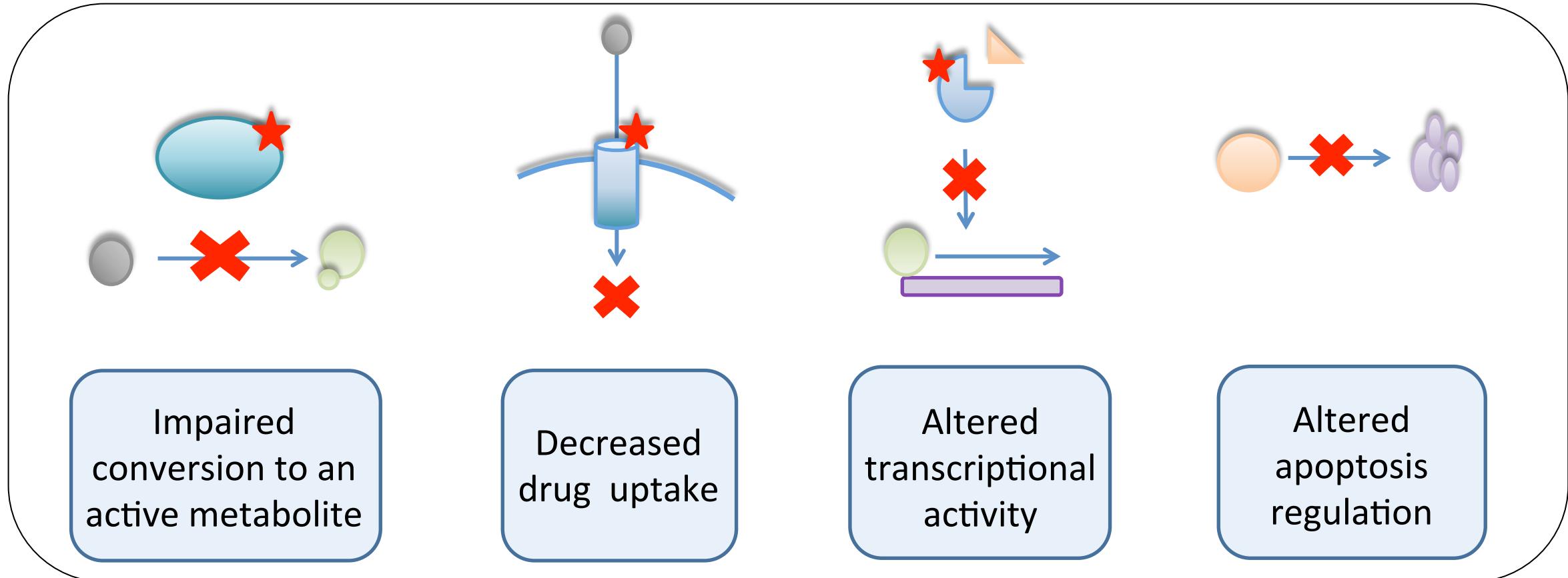


normal splicing



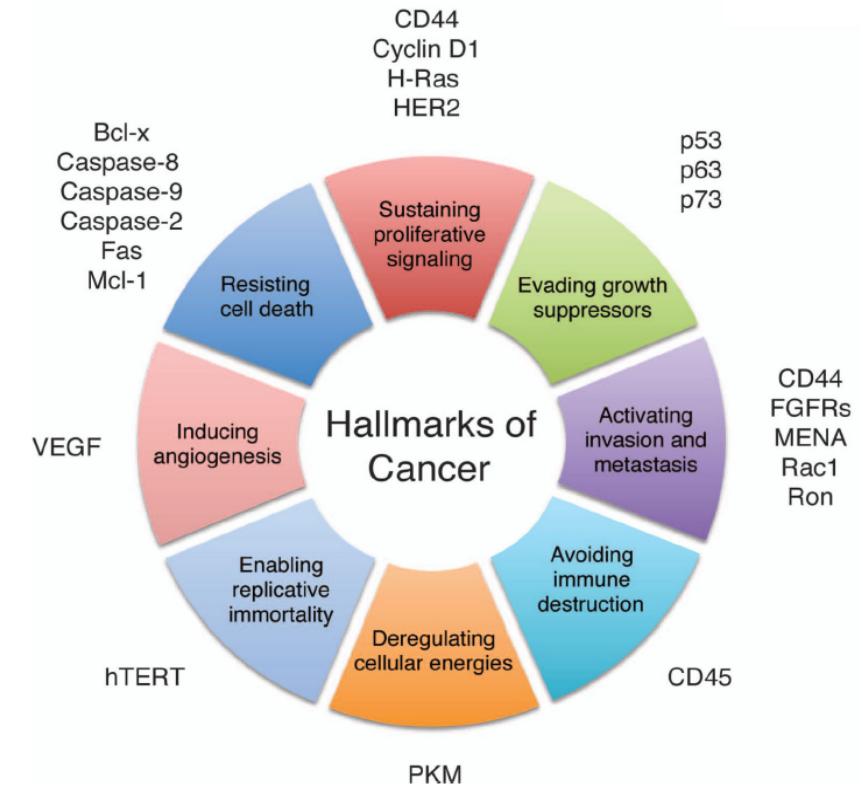
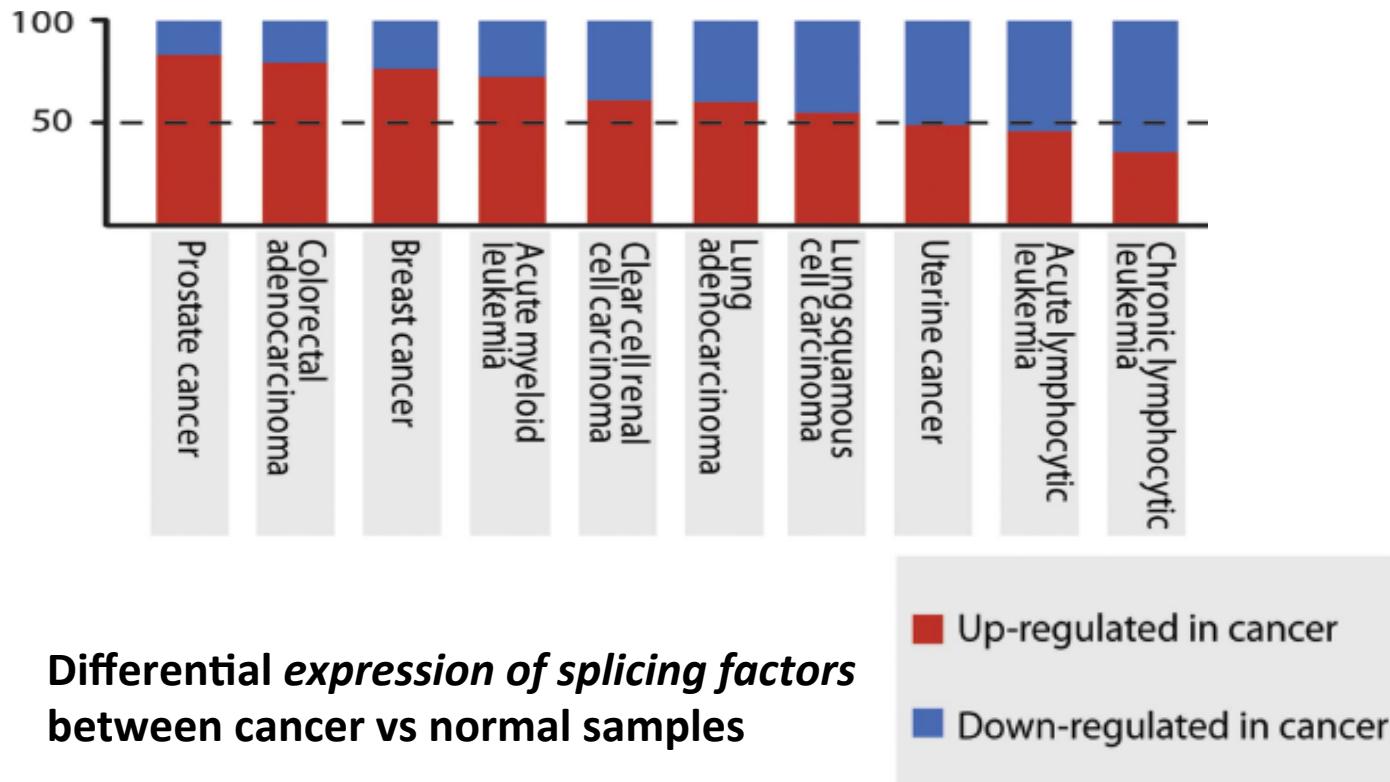
alternative splicing

Role of splicing in anticancer drugs resistance

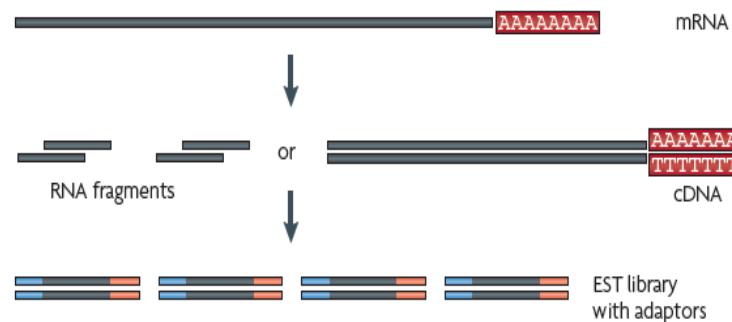


A novel hallmark of cancer

- Mutations in splicing factors in several types of cancer (sometimes prognostic)
- *Splice variants* related to specific tumor features

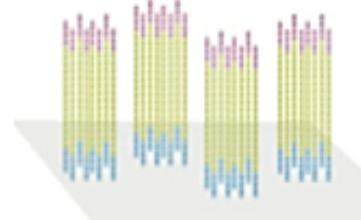


Expanding to a whole genome analysis with RNA sequencing



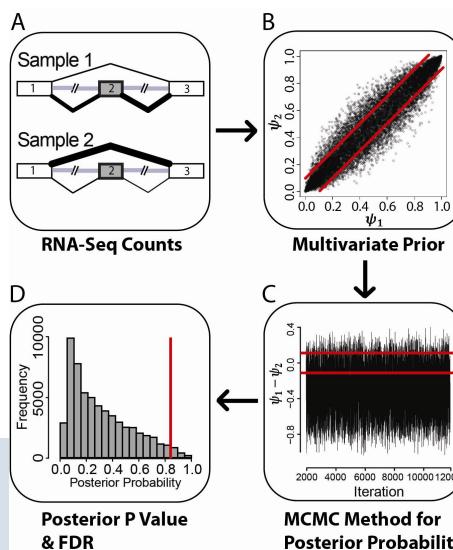
mRNA fragmentation

cDNA library (260-300 bp)



Massive parallel sequencing

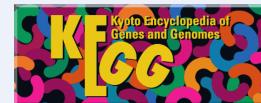
Multivariate Analysis of Transcript Splicing (MATS)



Top candidate genes

Annotated databases

- Gene Ontology
- KEGG
- REACTOME
- STRING



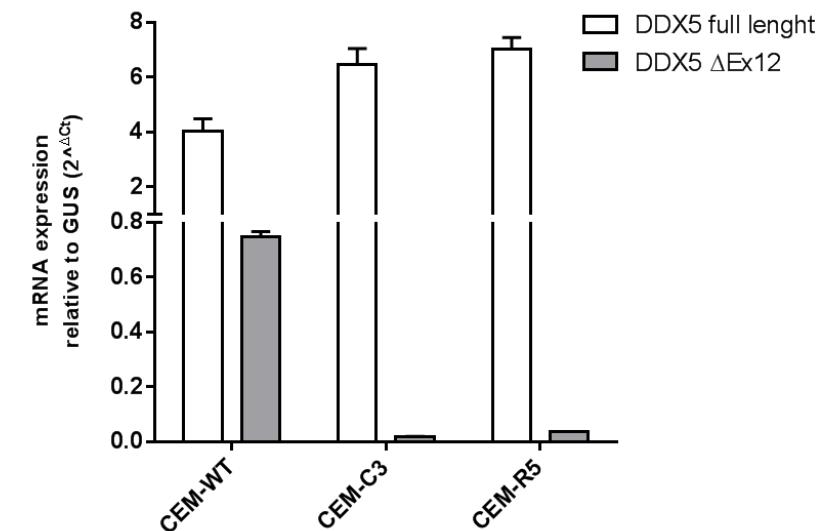
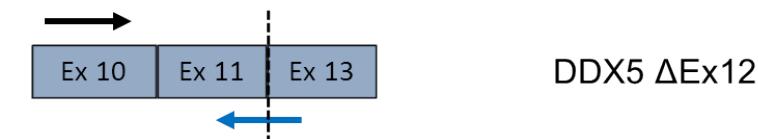
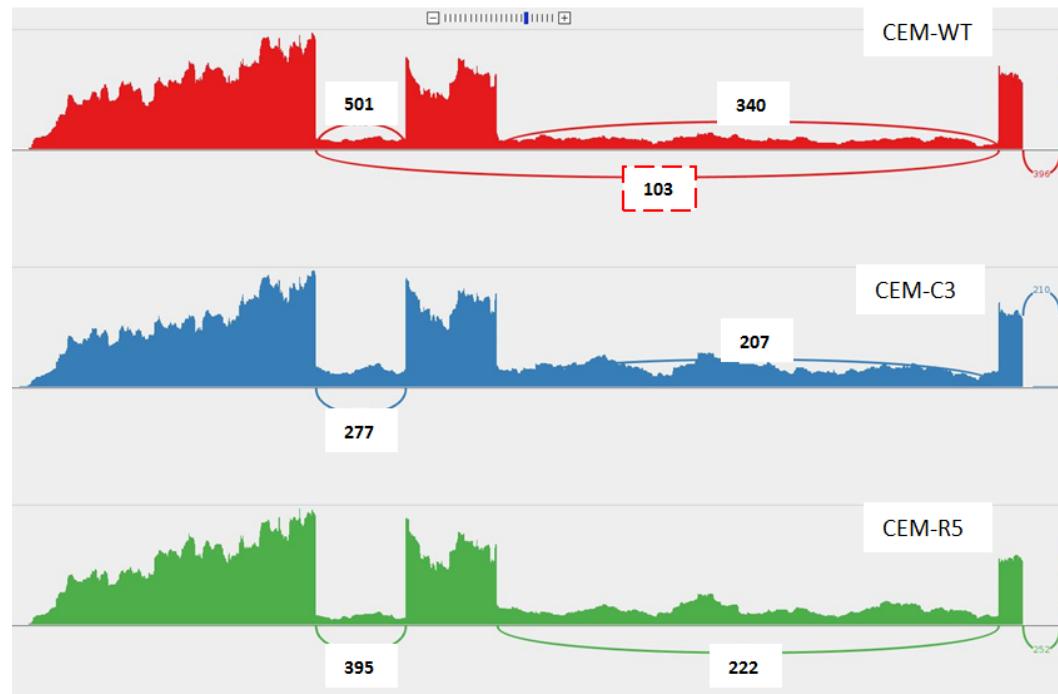
Biological interpretation

Functional validation

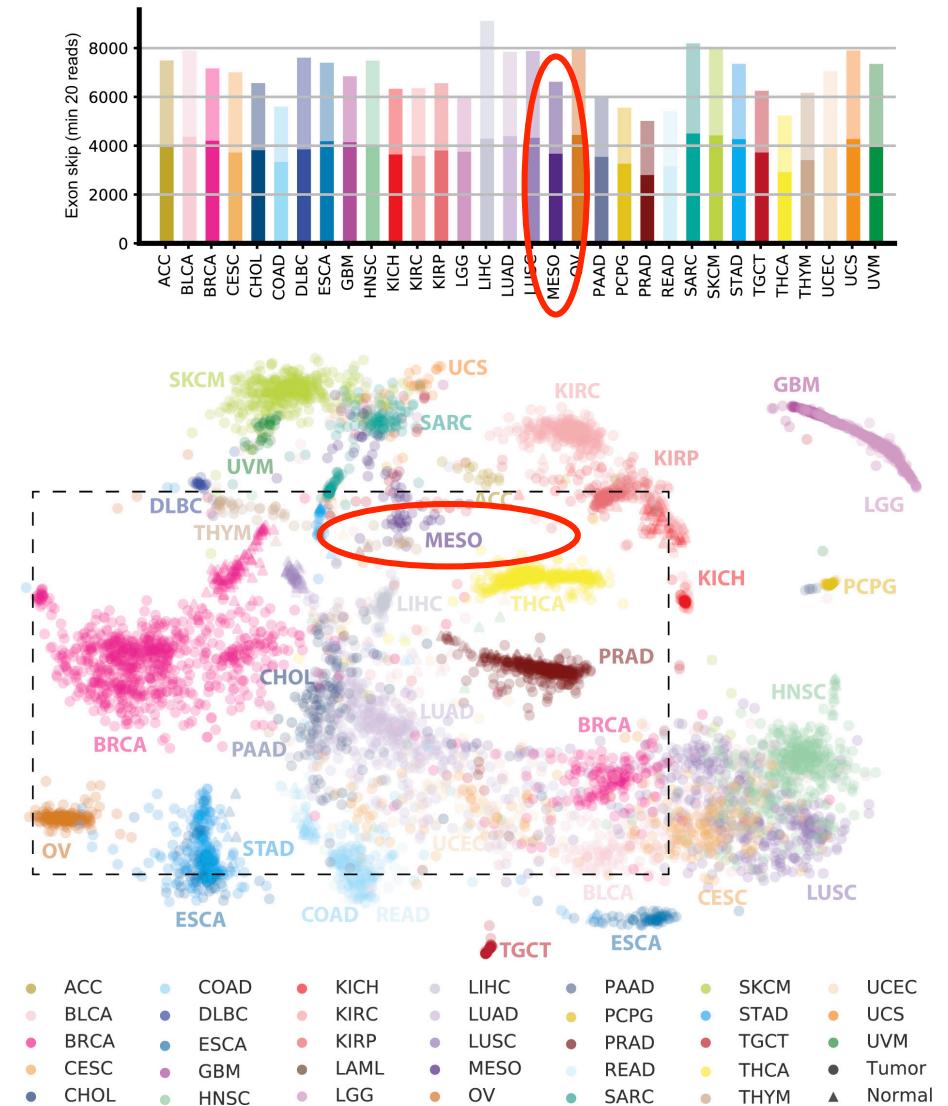
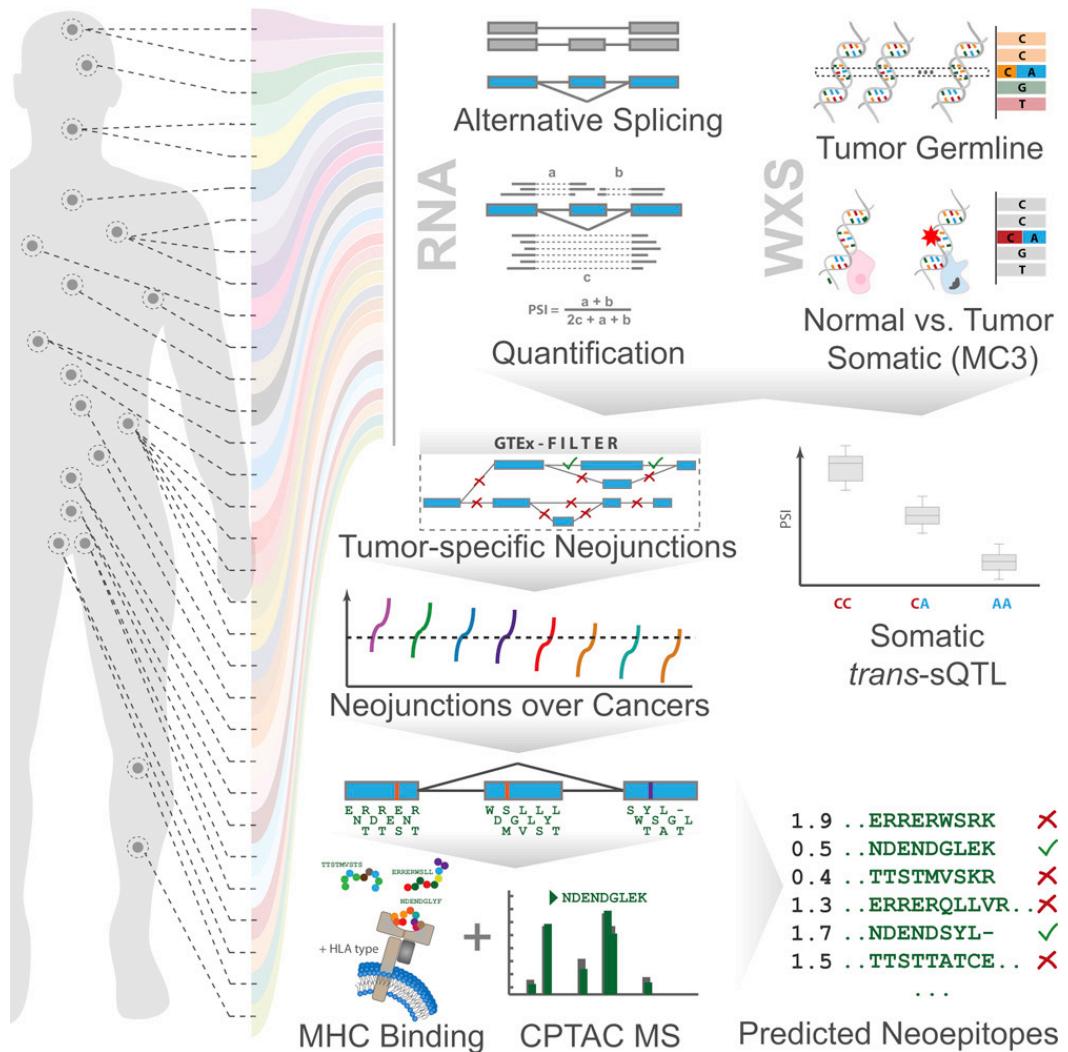
Establishment and validation of our method

The screenshot shows the JoVE website interface. At the top, there is a search bar with placeholder text 'Search by keywords, for example: 'stem cells''. Below the search bar are buttons for 'Advanced', a magnifying glass icon, 'START A TRIAL', and 'LOG IN'. Underneath these are navigation links: 'ABOUT JoVE', 'FOR LIBRARIANS', 'VIDEO JOURNAL' (which is highlighted in blue), 'SCIENCE EDUCATION', and 'PUBLISH'. A 'CANCER RESEARCH' category is indicated by a blue dot. The main content area displays a research article with the title 'Using RNA-sequencing to Detect Novel Splice Variants Related to Drug Resistance in *In Vitro* Cancer Models'.

“Trusting” the NGS data? Ex: DDX5 Exon 12 skipping



Tumors with splicing aberrations

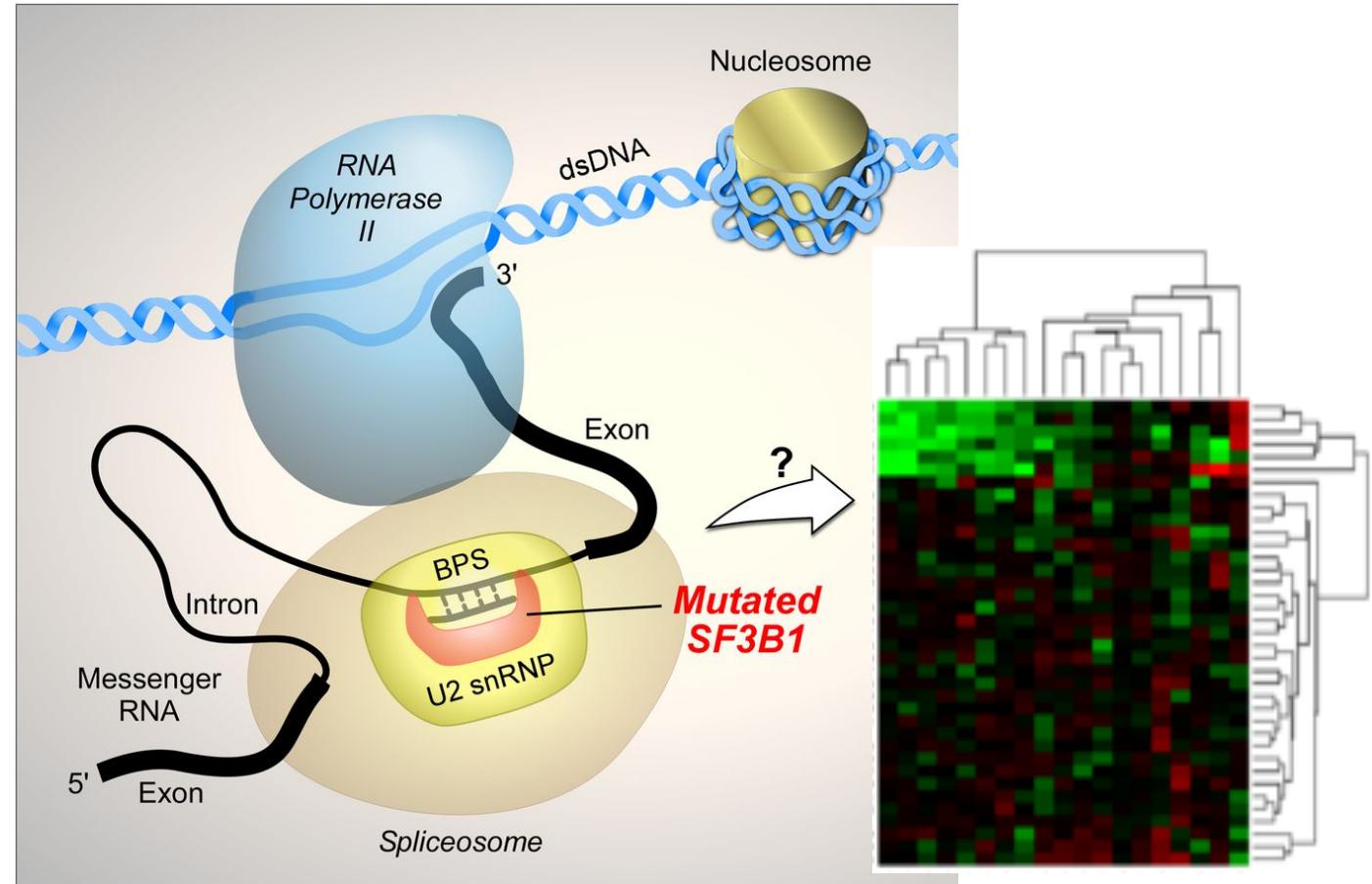


Splicing aberrations in Pleural Mesothelioma

Analyses of transcriptomes ($n = 211$), whole exomes ($n = 99$) and targeted exomes ($n = 103$) from 216 malignant pleural mesothelioma (MPM) tumors

Recurrent mutations in several genes involved in splicing, including SF3B1, SETD2, DDX51 and DDX3X

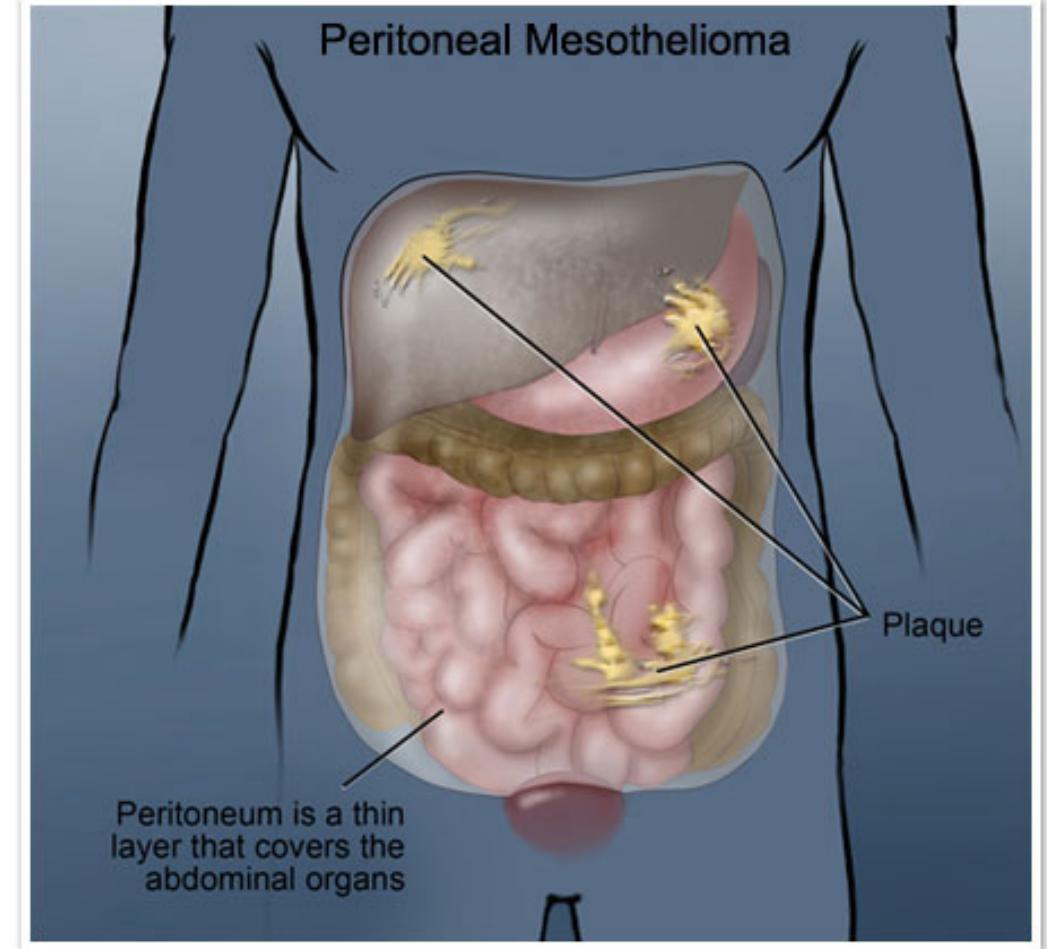
SF3B1-mutant samples showed a splicing profile distinct from that of wild-type tumors



Diffuse malignant peritoneal mesothelioma (DMPM)

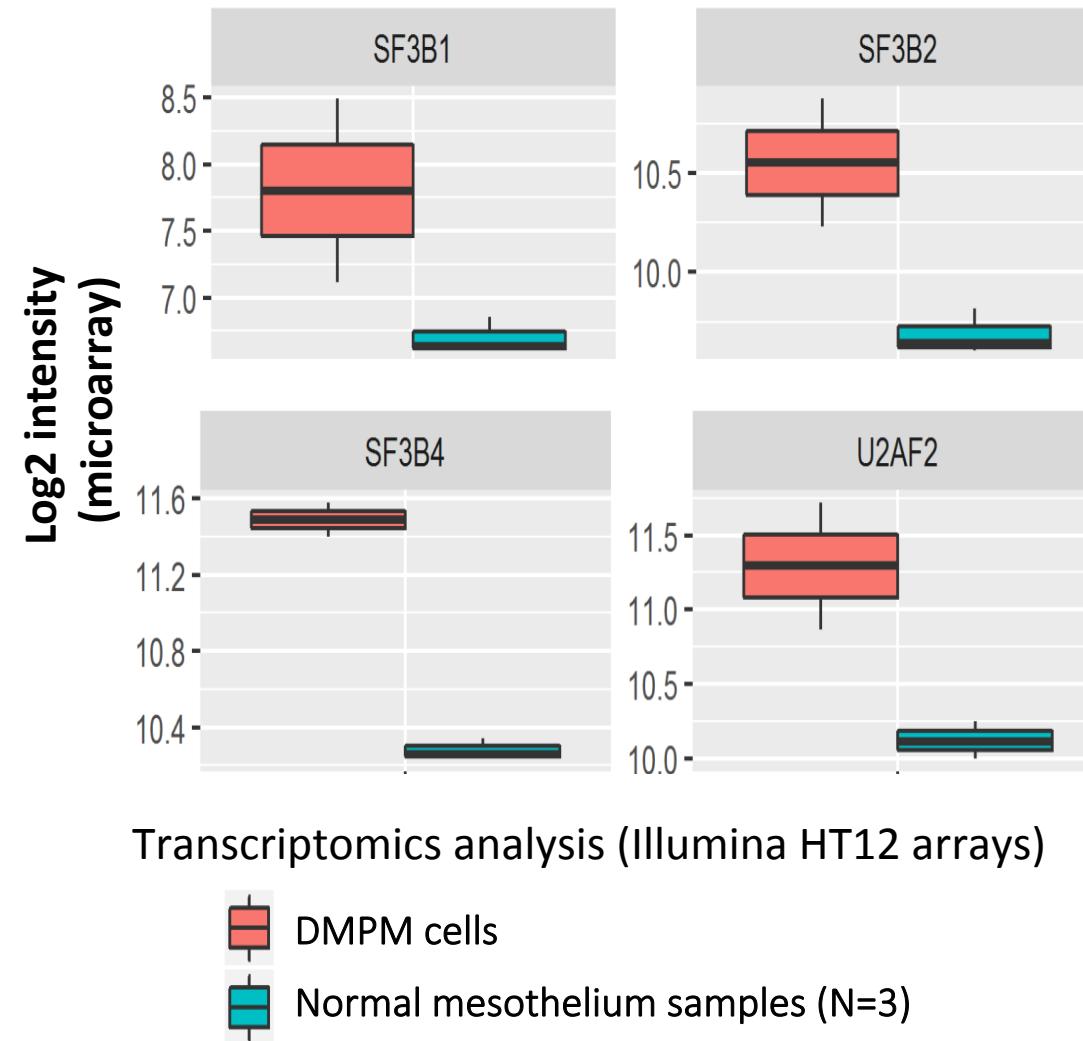
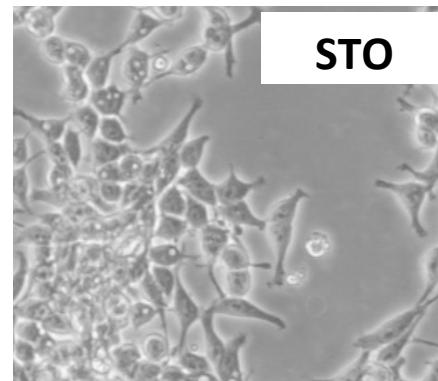
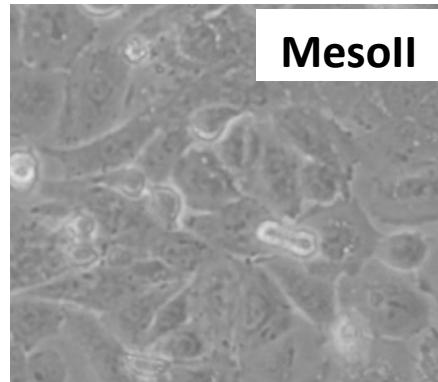
Neoplasm arising from mesothelial cells lining peritoneal cavity

- 20-30% of all mesotheliomas
- 250-500 new cases annually in US
- Asbestos exposure is main risk factor
- Surgery and heated chemotherapy (HIPEC) are the most effective treatments
- 5-year survival rate of 41% and median OS of 53 months

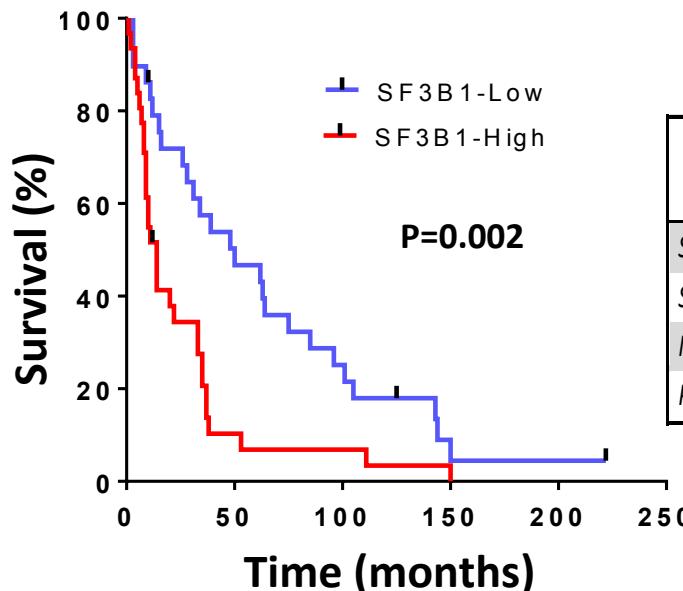
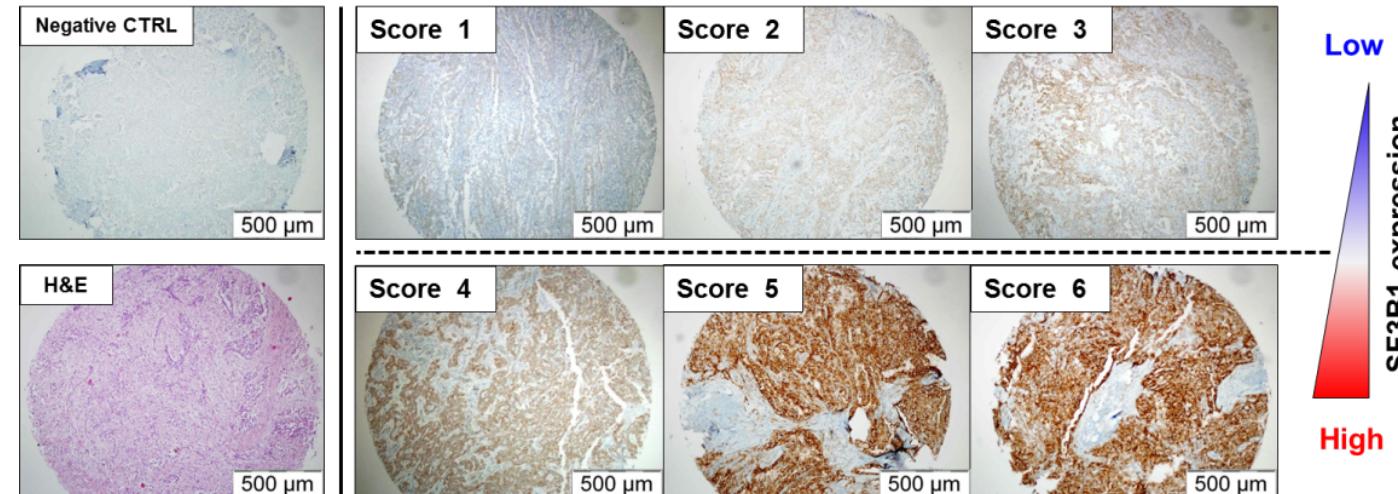


In collaboration with

SF3B1 in Diffuse Peritoneal Mesothelioma (DMPM)



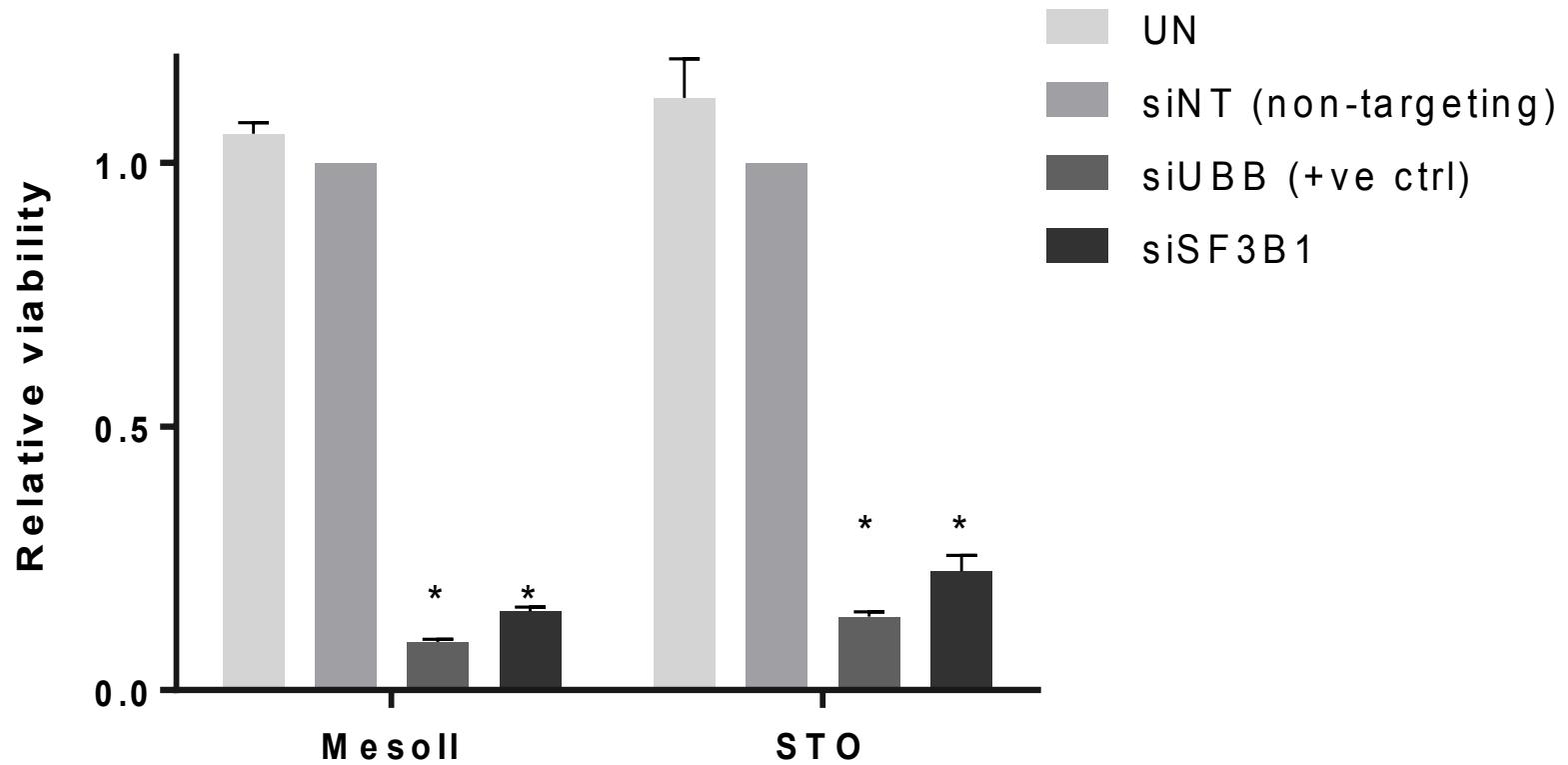
SF3B1 in Diffuse Peritoneal Mesothelioma (DMPM)



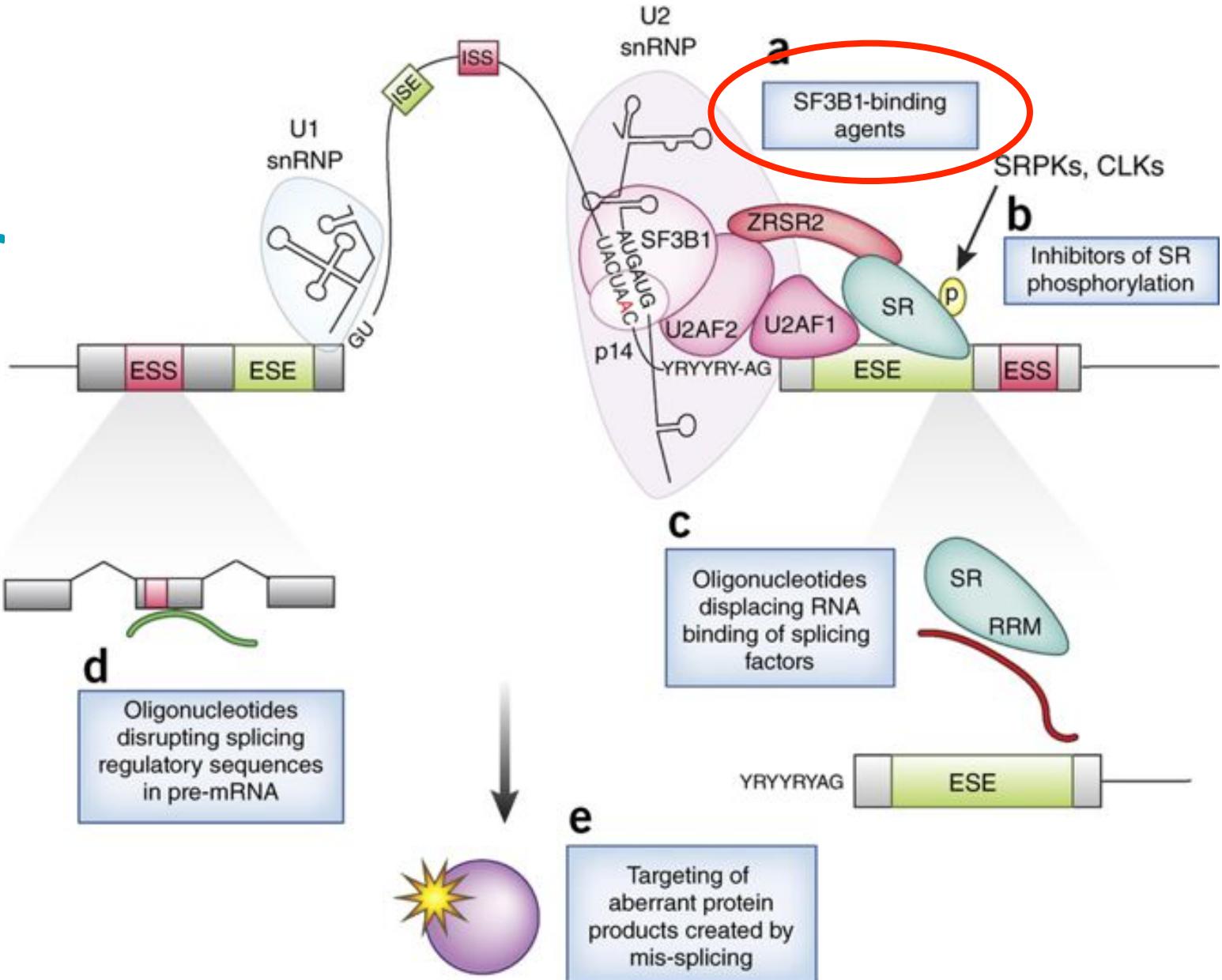
Multivariate	df	Risk of death HR (95% CI)	P
SF3B1 expression	1	2.0 (1.1-3.5)	0.018
Subtyping	1	2.4 (1.0-5.4)	0.033
N-stage	1	2.0 (0.9-4.5)	0.088
Performance Status	1	1.9 (0.9-4.2)	0.080

TMA with tissues and data
from 60 DMPM patients

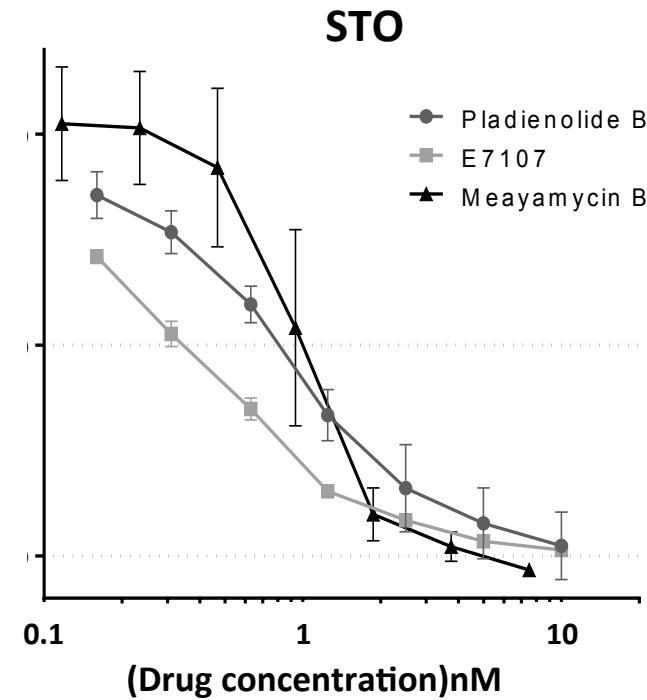
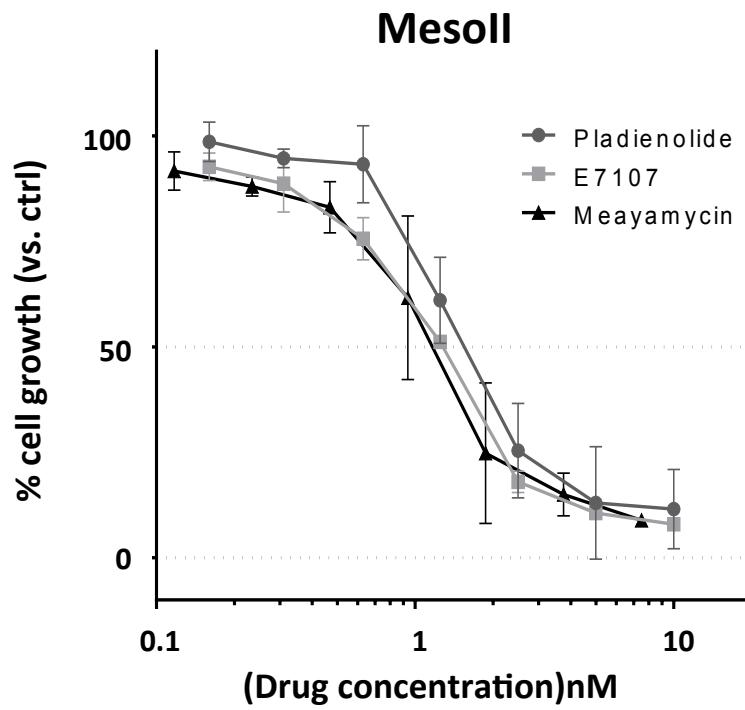
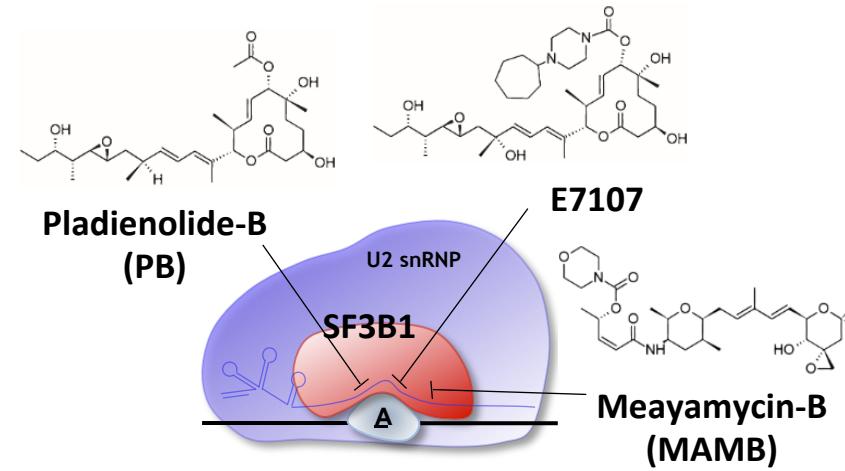
siRNA of SF3B1 in DMPM cells



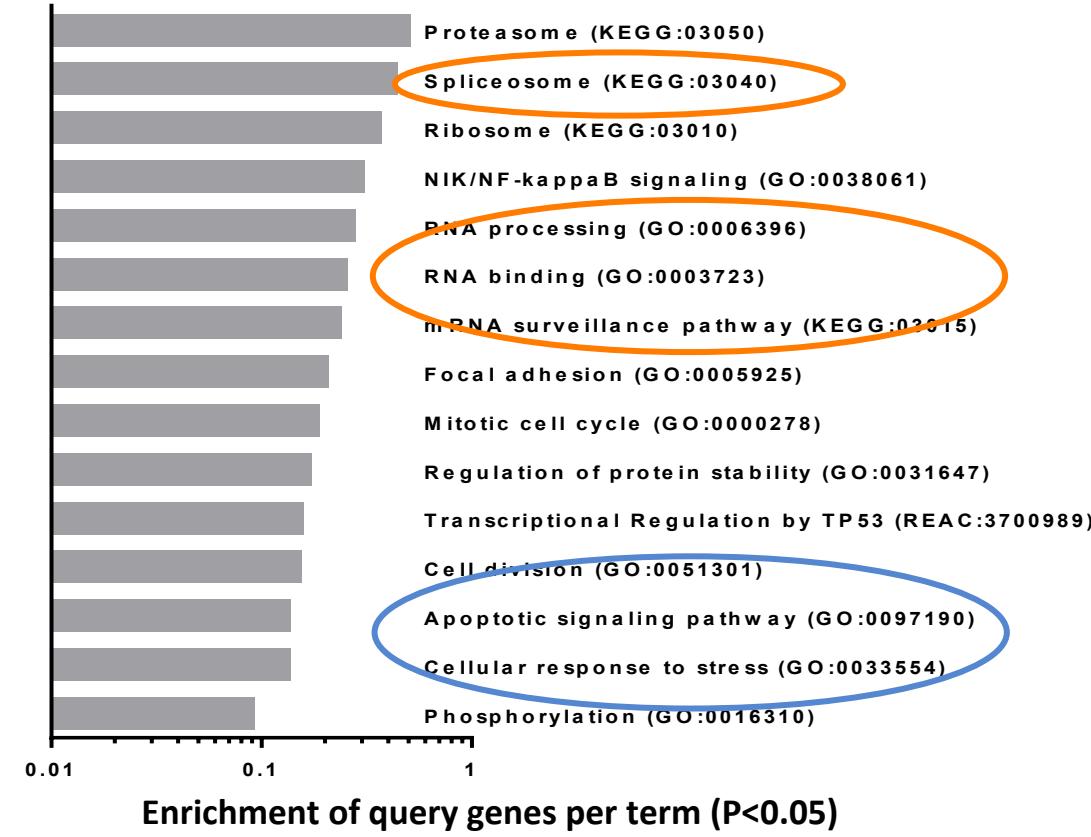
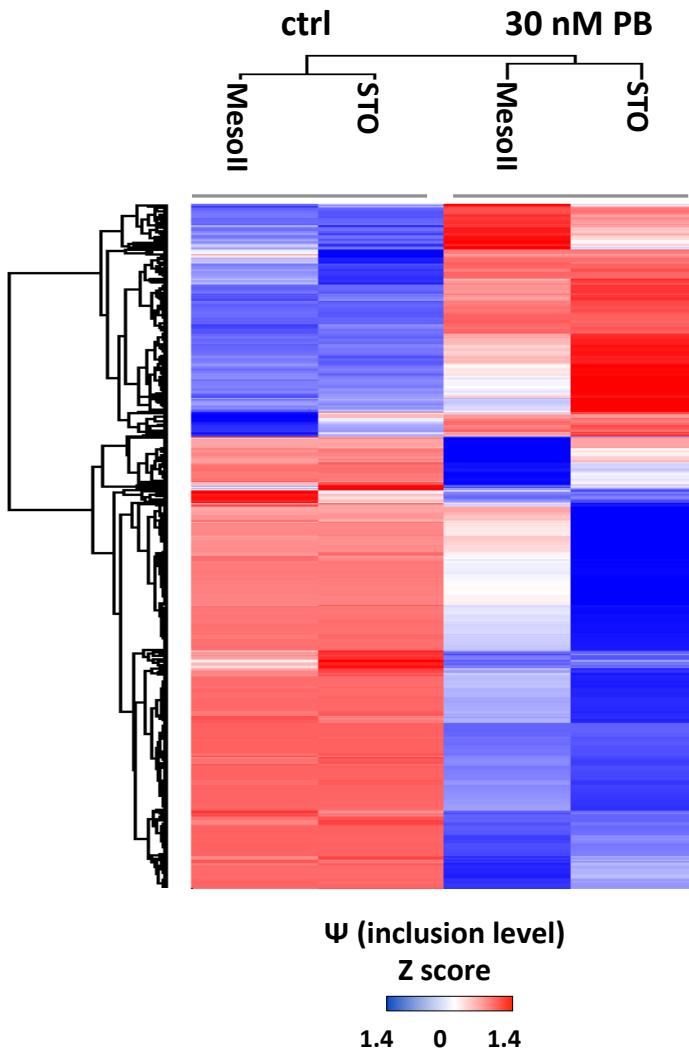
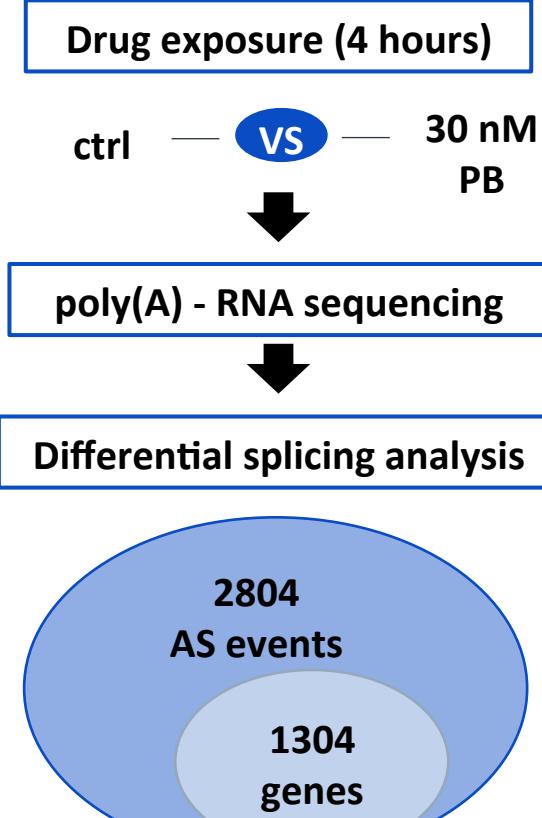
Methods by which splicing may be modulated for cancer therapy



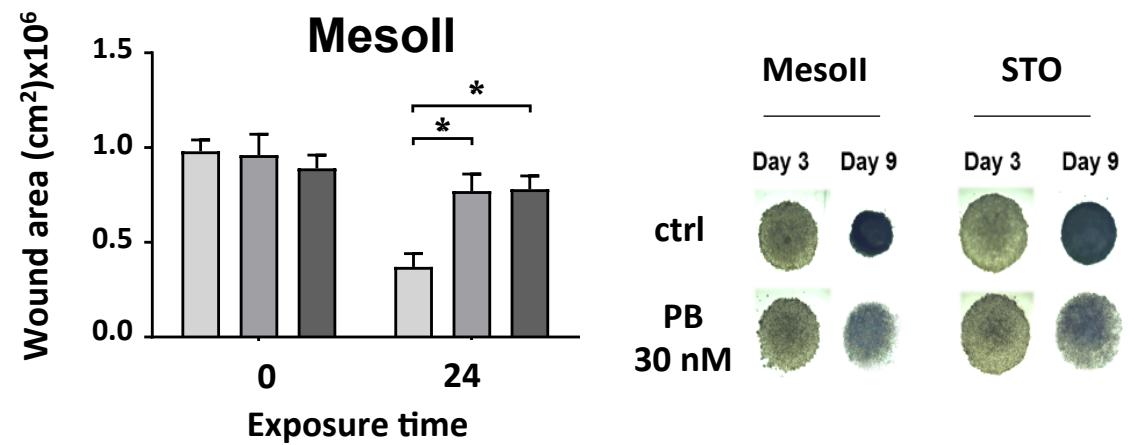
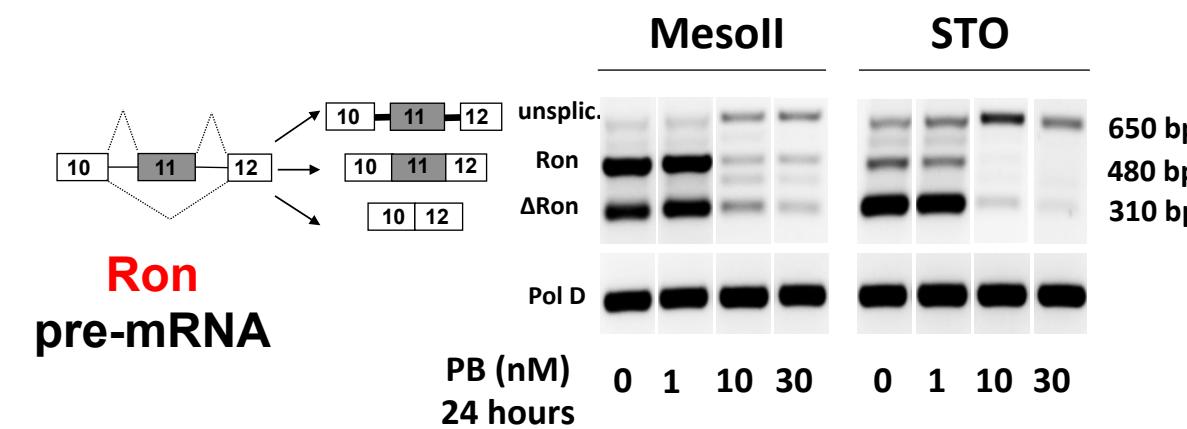
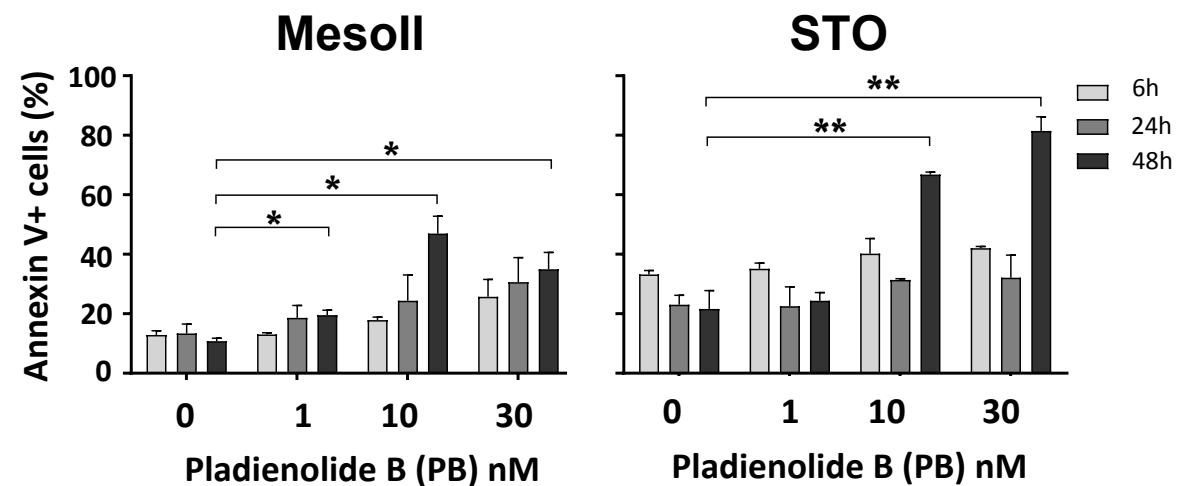
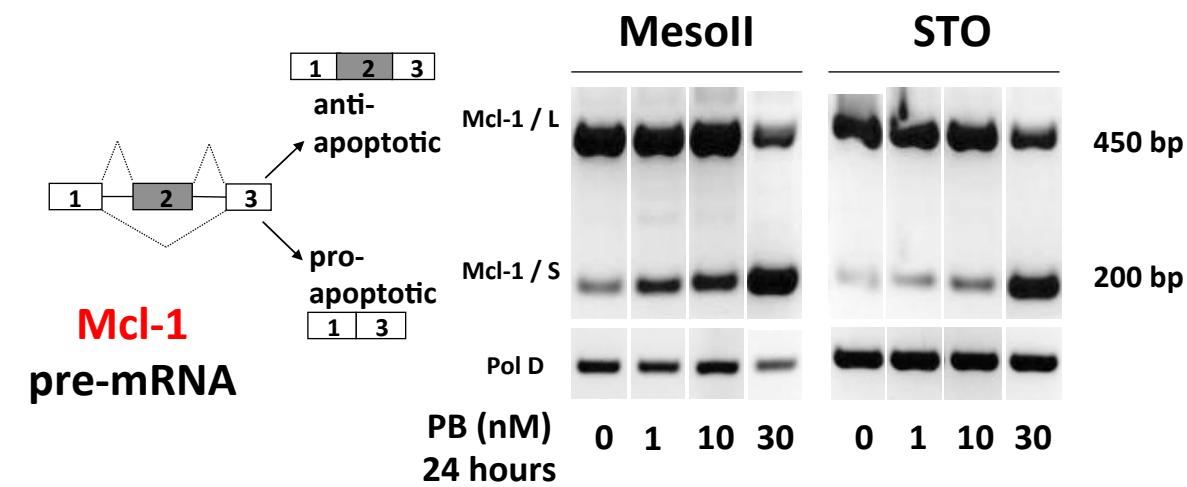
Targeting SF3B1 in DMPM cells



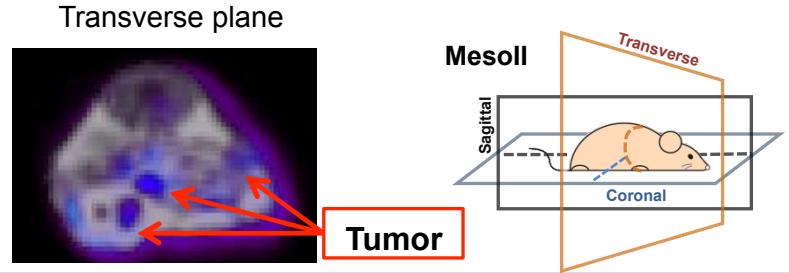
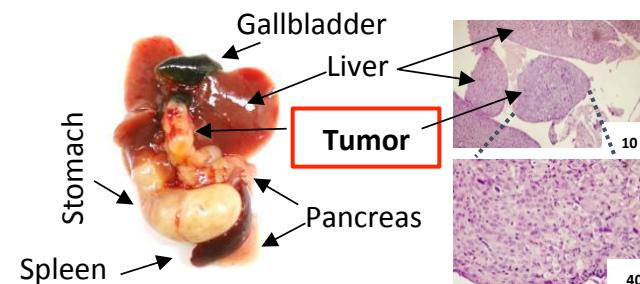
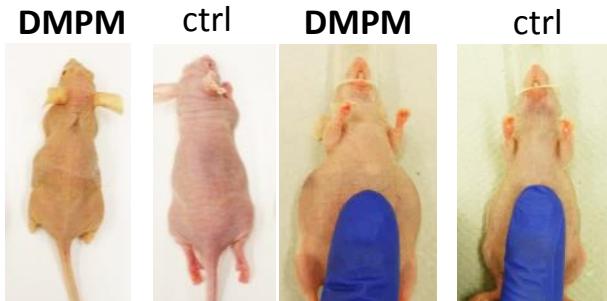
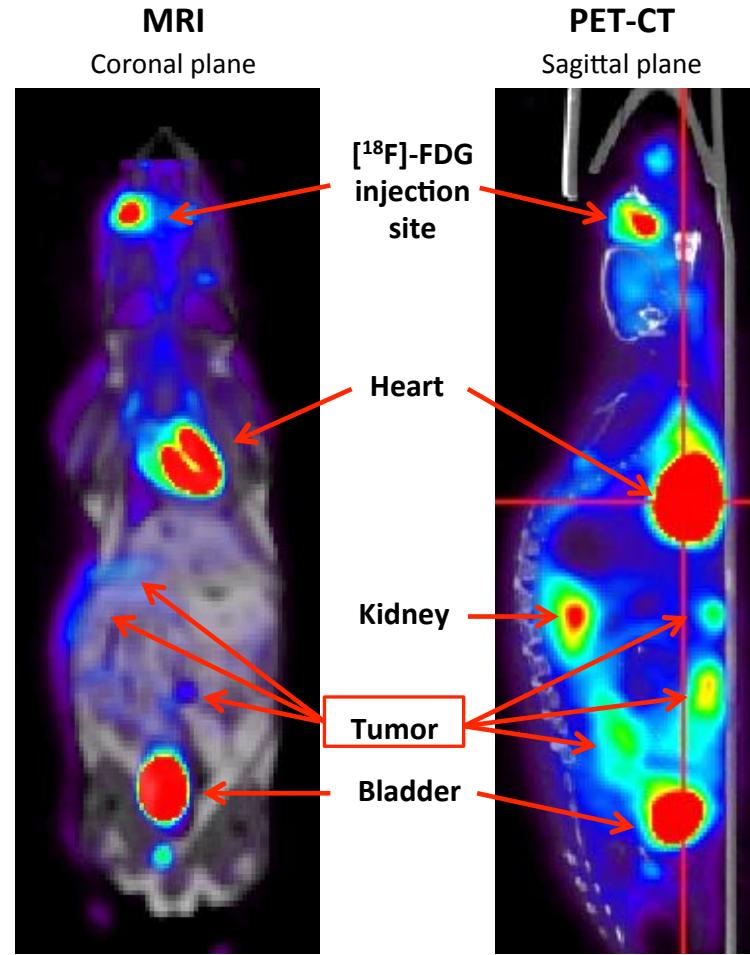
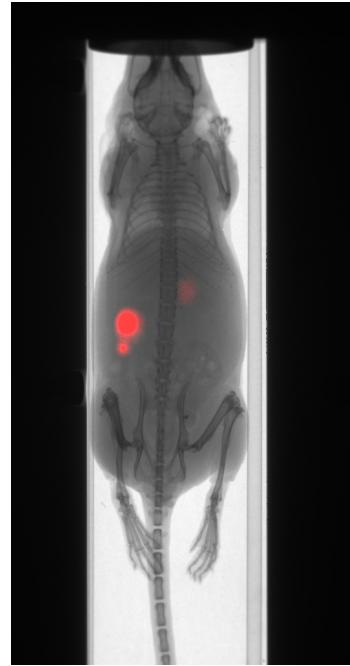
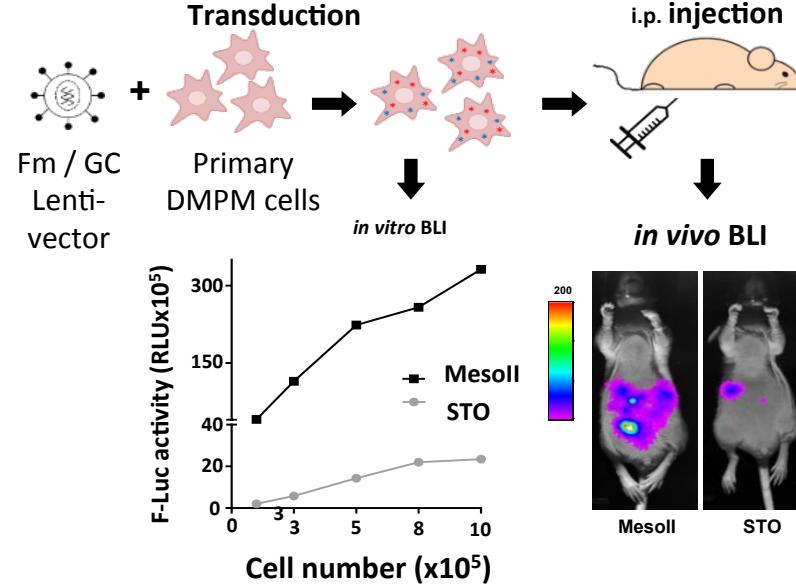
Next generation sequencing



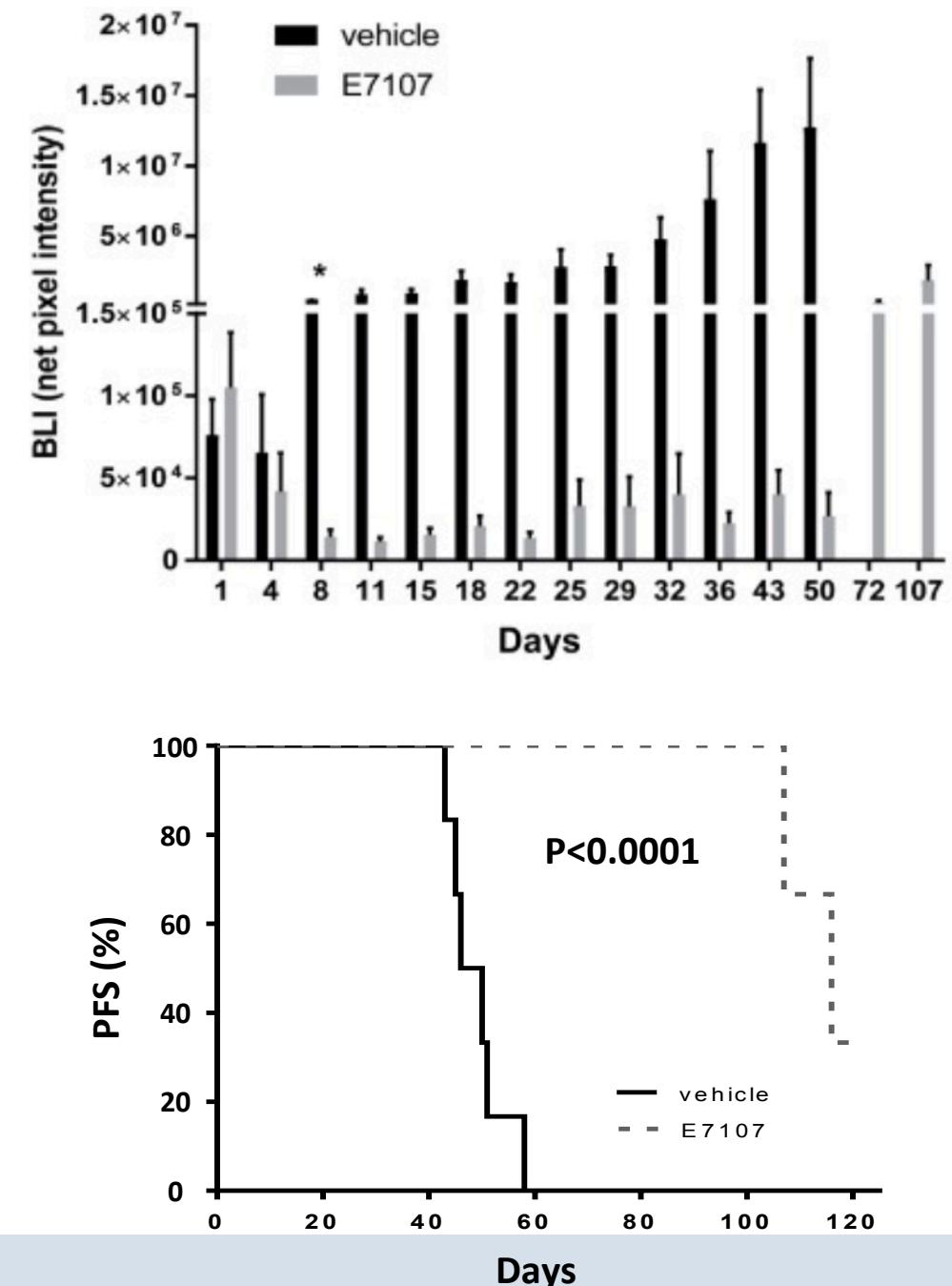
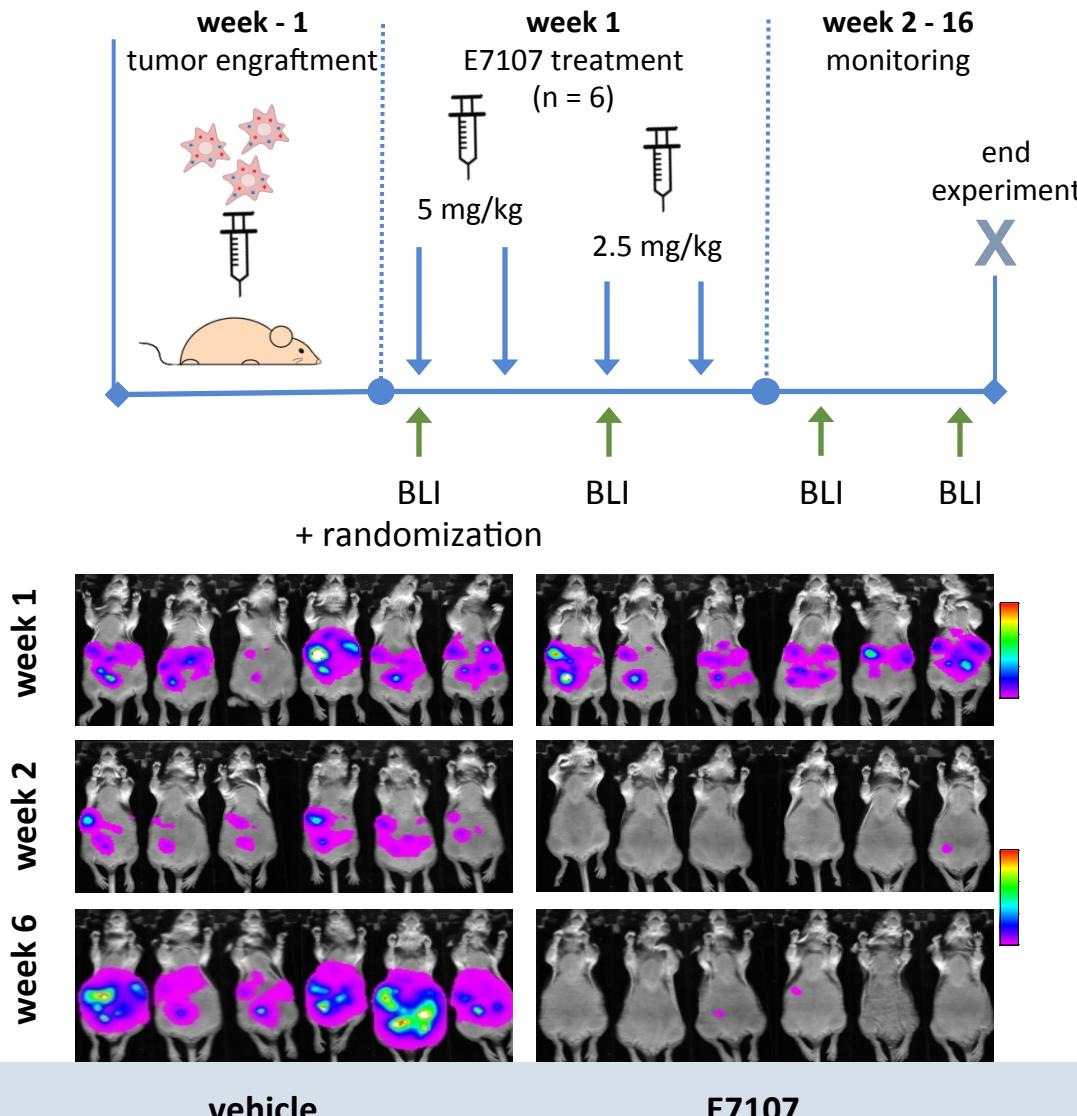
PCR & Functional validation



Characterization of *in vivo* models



Antitumor activity of E7107



Conclusions

- SF3B1 emerged as a novel potential prognostic factor in DMPM
- Splicing modulators markedly impair cancer cell viability
- Differential splicing analysis of Pladienolide-B-treated cells revealed abundant alterations of transcripts involved in apoptosis and other oncogenic pathways
- E7107 demonstrated remarkable *in vivo* antitumor efficacy
- Our data designate splicing as a promising therapeutic target in DMPM

Acknowledgements

Rocco Sciarrillo



Anna Wojtuszkiewicz

Niccola Funel

Btissame El Hassouni



Cancer Pharmacology Lab
AIRC Start-up Unit

Tonny Lagerweij

Maxime Blijlevens

Silvia Buonamici

Eveline A. Zeeuw van der Laan



Tom Würdinger

Carla F.M. Molthoff

Gerrit Jansen

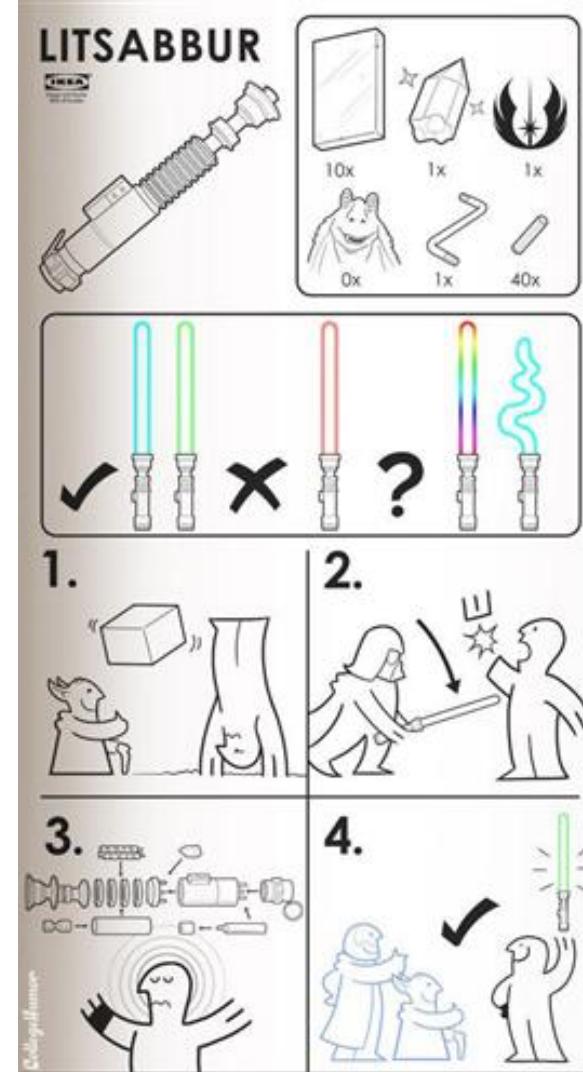
Gertjan J.L. Kaspers

Godefridus J. Peters

Jacqueline Cloos

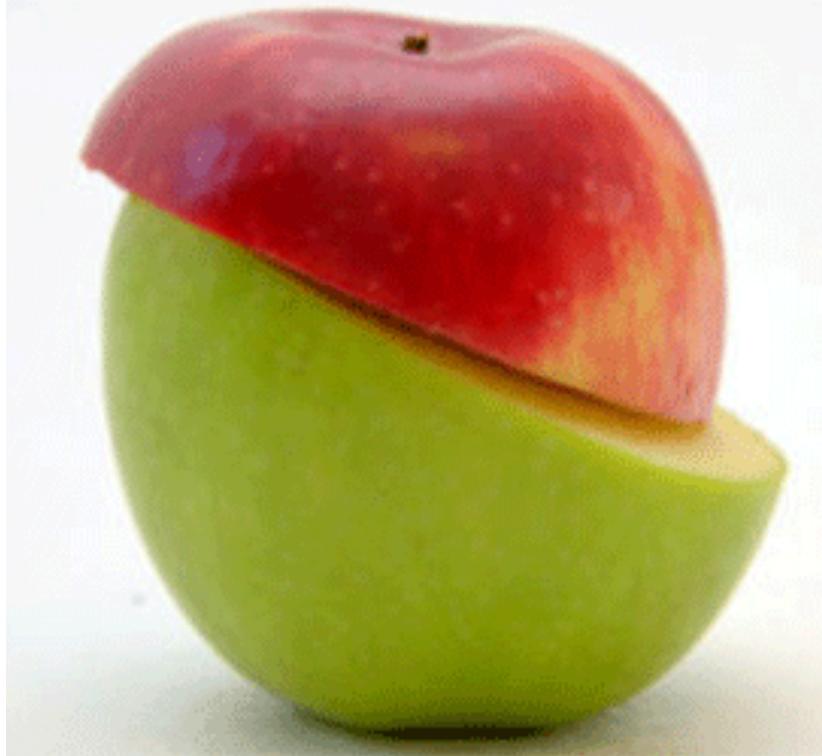


Fondazione IRCCS
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Cancer Center
Amsterdam
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Another Story Behind the Apple...