

A CRISPR/Cas9 screen to identify RNA Binding Proteins with oncogenic functions in colorectal cancer

Meghan Forsythe

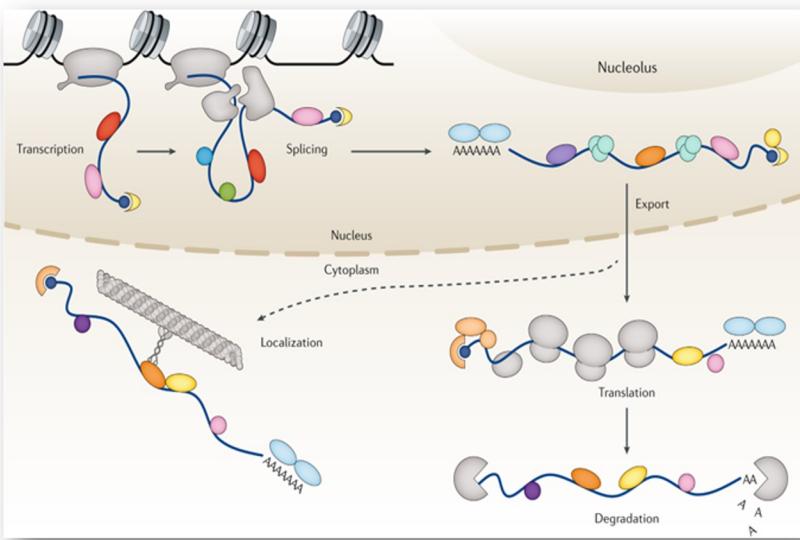
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Why study RNA Binding Proteins (RBPs)?

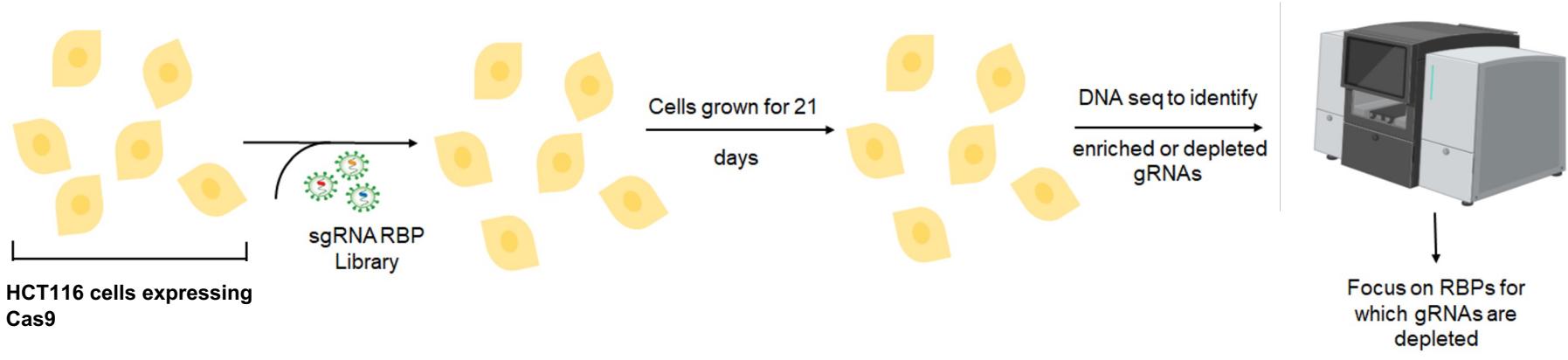
- ❖ RBPs needed for cell development and function
- ❖ They exert post-transcriptional control of RNA function
- ❖ RBP malfunction underlies the basis of numerous diseases, including cancer
- ❖ Not much is known about RBPs role in colorectal cancer (CRC)



(Gebauer et al, 2020)

The aim of this study is to examine the effect of RBPs on CRC.

A CRISPR/Cas9 screen to identify RBPs important in CRC



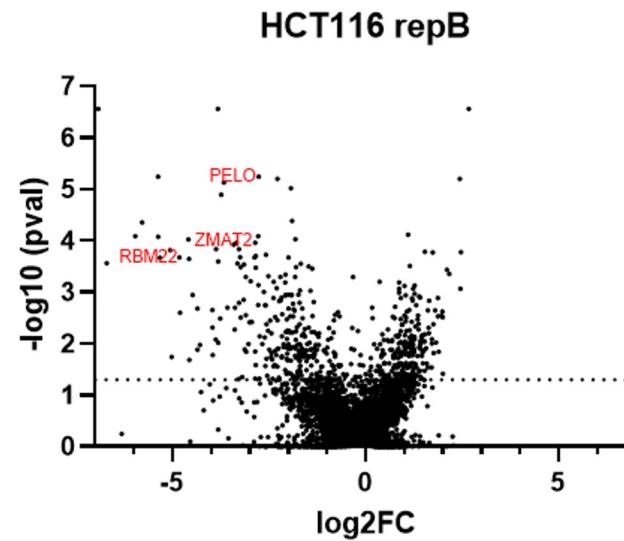
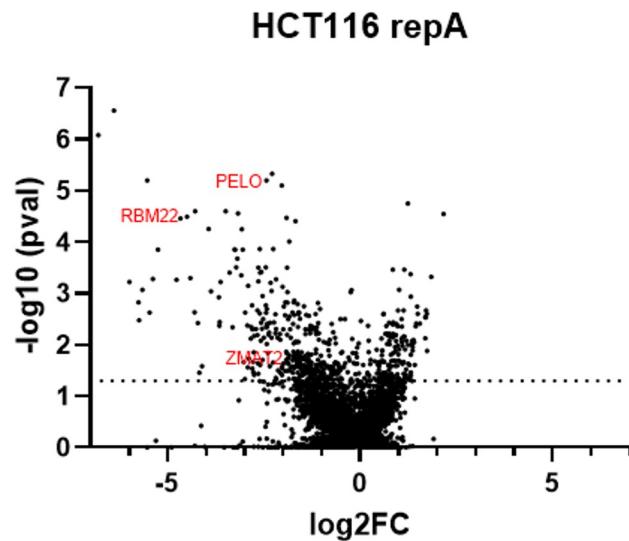
- ❖ HCT116 cells with stable expression of Cas9 transduced with a lentiviral gRNA library targeting RBPs
- ❖ Cells were grown for 21 days and DNA sequencing was performed to identify enriched or depleted guide RNAs
- ❖ We focused on RBPs for which gRNAs are depleted in the cell population

CRISPR screen suggests several RBPs promote proliferation in CRC

- ❖ From the screen, the three most promising proteins were *PELO*, *RBM22*, and *ZMAT2*
- ❖ Four gRNAs were used for each RBP & at least three of them showed an effect on cell proliferation to be considered a good candidate

Gene	1 st replicate			2 nd replicate		
	P-value	GoodsgRNA	log Fold Change	P-value	GoodsgRNA	log Fold Change
PELO	6.32E-06	4	-2.4291	5.77E-06	4	-2.7633
RBM22	3.49E-05	3	-4.6639	0.000212	4	-4.8164
RBMX	5.52E-05	4	-3.9332	0.099426	3	-1.6223
RBM42	3.94E-04	3	-2.5928	0.008126	4	-2.0365
HNRNPC	8.74E-03	4	-1.6808	0.000277	4	-3.3168
ZMAT2	1.89E-02	4	-1.8826	0.00011	4	-2.8574

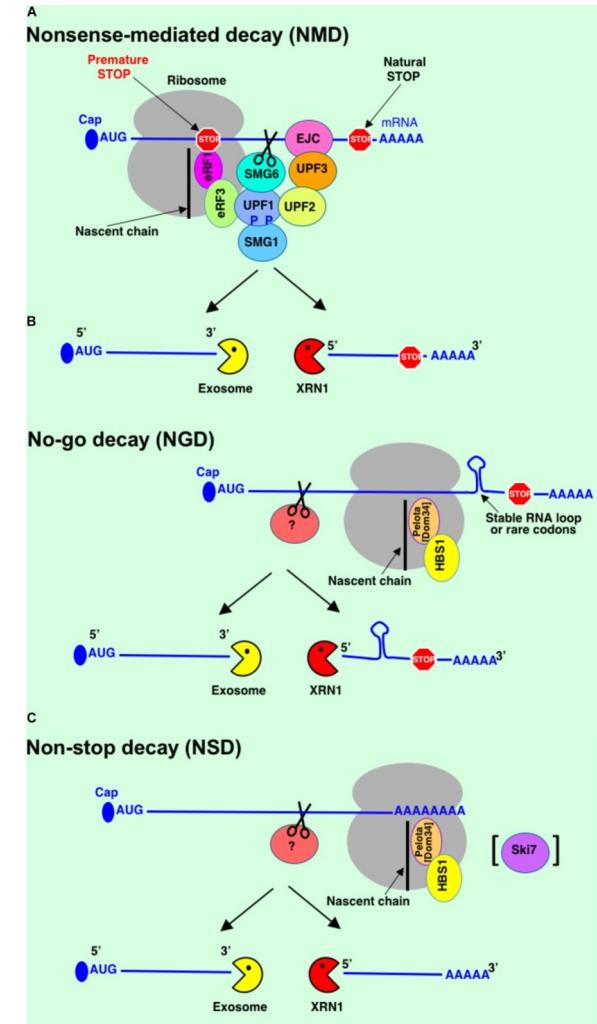
Volcano plots show how RBP gRNAs are enriched or depleted in the cell population



What is known about PELO?

Pelota mRNA Surveillance And Ribosome Rescue Factor (Protein Dom34 Homolog)

- ❖ Part of the No-Go Decay mechanism (NGD)
- ❖ Stalled ribosomes due to 2° structure or mRNAs without stop codons trigger NGD
- ❖ PELO recognizes ribosomes stalled at the 3' end of mRNA and promotes recruitment of ABCE1, which drives the disassembly of stalled ribosomes, followed by degradation of damaged mRNAs



PELO in cancer?

Oncogene

www.nature.com/onc

ARTICLE

 Check for updates

PELO facilitates PLK1-induced the ubiquitination and degradation of Smad4 and promotes the progression of prostate cancer

Ping Gao  ^{1,5} , Jing-Lan Hao ^{1,5}, Qian-Wen Xie ^{1,5}, Gui-Qin Han¹, Bin-Bing Xu¹, Hang Hu¹, Na-Er Sa¹, Xiao-Wen Du¹, Hai-Long Tang², Jian Yan  ^{3,4} and Xiao-Ming Dong  ¹ 

- ❖ PELO facilitated the degradation of Smad4 by cooperating with PLK1, resulting in proliferation and metastasis of prostate cancer (PCa) cells

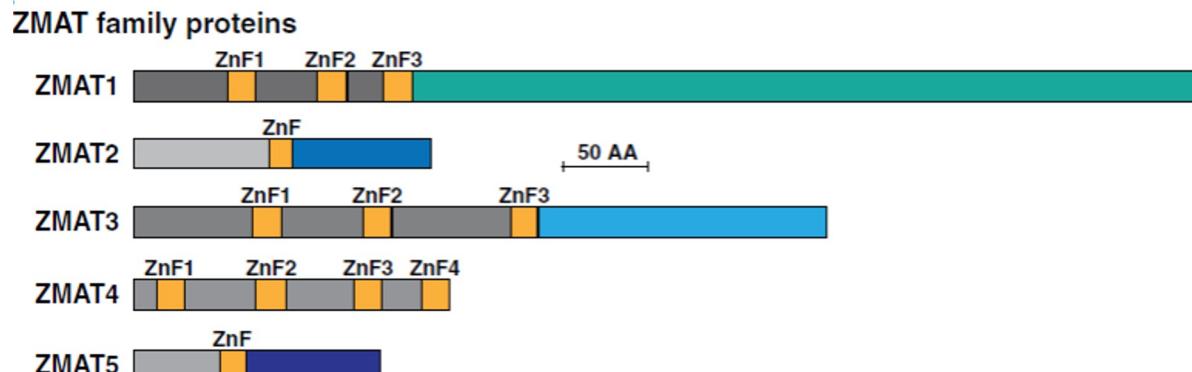
- ❖ PELO upregulation was positively associated with high grade PCa and knockdown of PELO expression significantly decreased PCa cell proliferation and metastasis

What is known about RBM22?

- ❖ *RBM22* is a gene that encodes an essential RNA-Binding Protein involved in pre-mRNA splicing and transcription, with a DNA-binding function
- ❖ Plays an essential role in maintaining the conformation of the catalytic core of the spliceosome & acts as a bridge between the catalytic core and other essential protein components of the spliceosome
- ❖ Due to RBM22's wide scope in the regulation of gene expression, RBM22 has been associated with several pathologies and, notably, with the aggressiveness of cancer cells.

What is known about ZMAT2?

- ❖ ZMAT2 (Zinc finger matrin-type protein 2) belongs to the family of ZMAT proteins
- ❖ Human ZMAT2 has been associated with the spliceosome



Baral & Rotwein, Sage., 2020

ZMAT2 in cancer

- ❖ A few different ZMAT2 coding changes appeared to be present in a range of human cancers, with 32 of 36 encoding single predicted AA substitutions

Table 5. Cancer-associated predicted mutations in ZMAT2.^a

MUTATION	CANCER TYPE	POPULATION VARIANT	GNOMAD PREVALENCE
G4V	Esophageal	None	–
G4R	Ovarian	G4R	1 allele
X6splice	Renal clear cell	None	–
N9K frameshift	Ewing sarcoma	N9S	1 allele
R13H	Colorectal	None	–
E22D	Uterine	None	–

Hypothesis

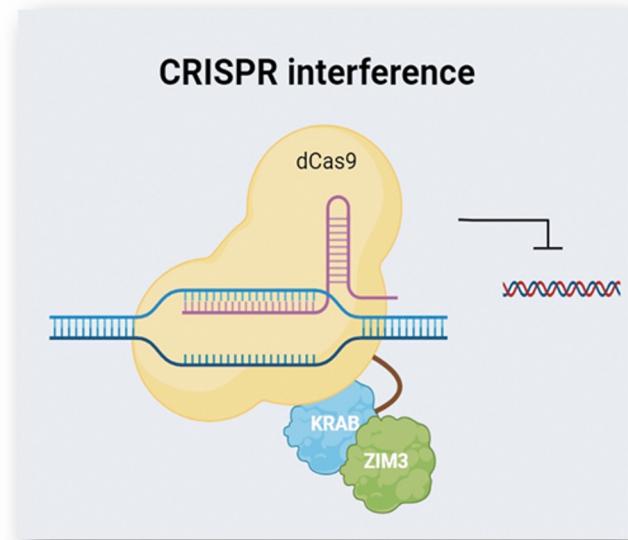
PELO, ZMAT2 & RBM22 promote cell proliferation in HCT116 CRC cells

Aims:

1. Validate the CRISPR screen result in HCT116 cells using CRISPRi
1. Assay for cell proliferation and other features of cancer cells (apoptosis, tumor growth, migration, & invasion)

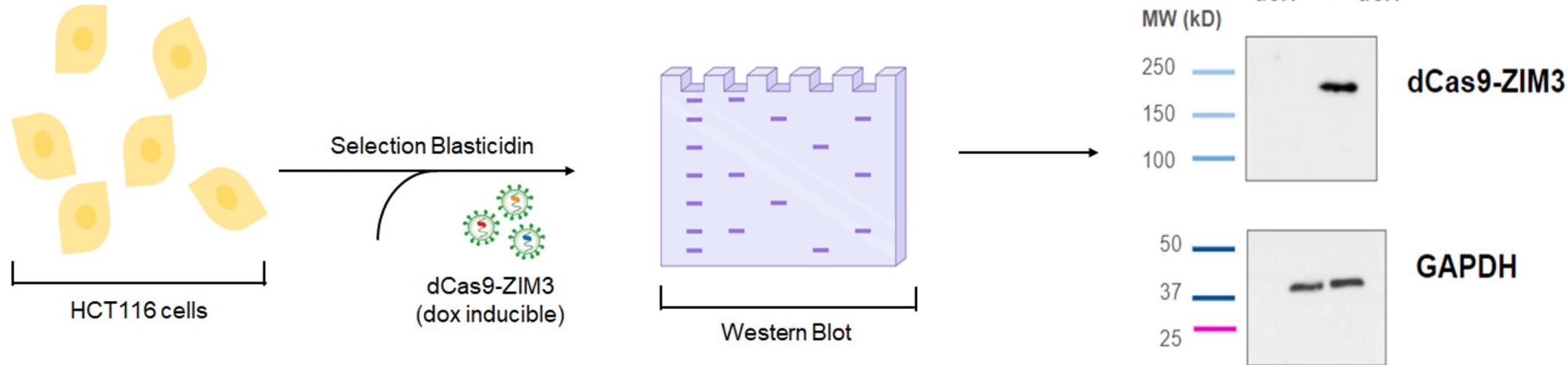
What is CRISPRi?

- ❖ Inactive form of Cas9, dCas9, is fused to KRAB
- ❖ dCas9-KRAB is recruited with the guide RNA (gRNA)
- ❖ KRAB mediates the transcriptional repression of the targeted gene



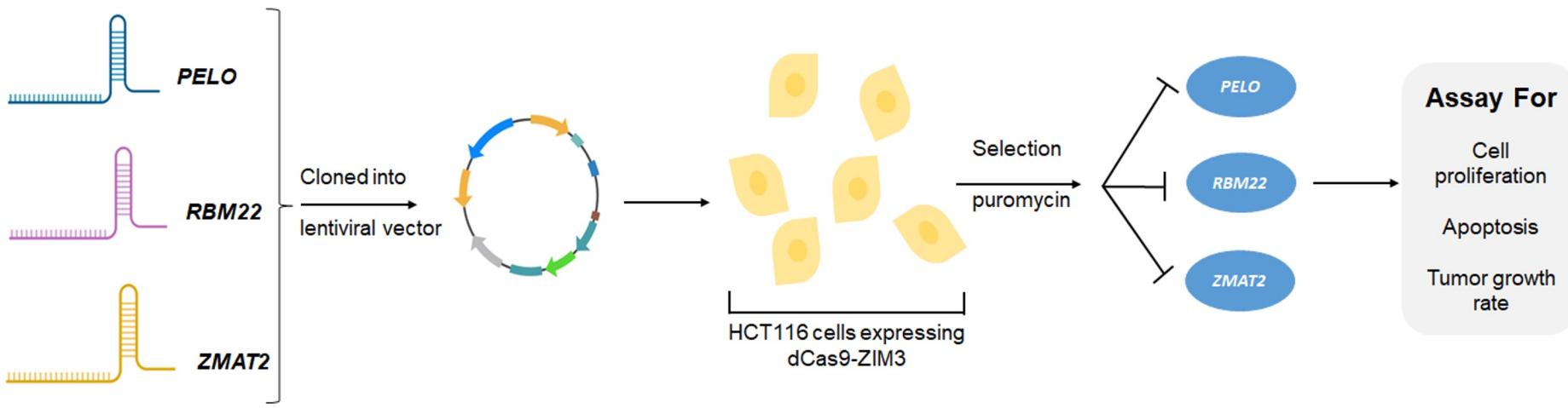
(Mohamad et al, 2024)

Generation of dCas9-ZIM3 expressing cells



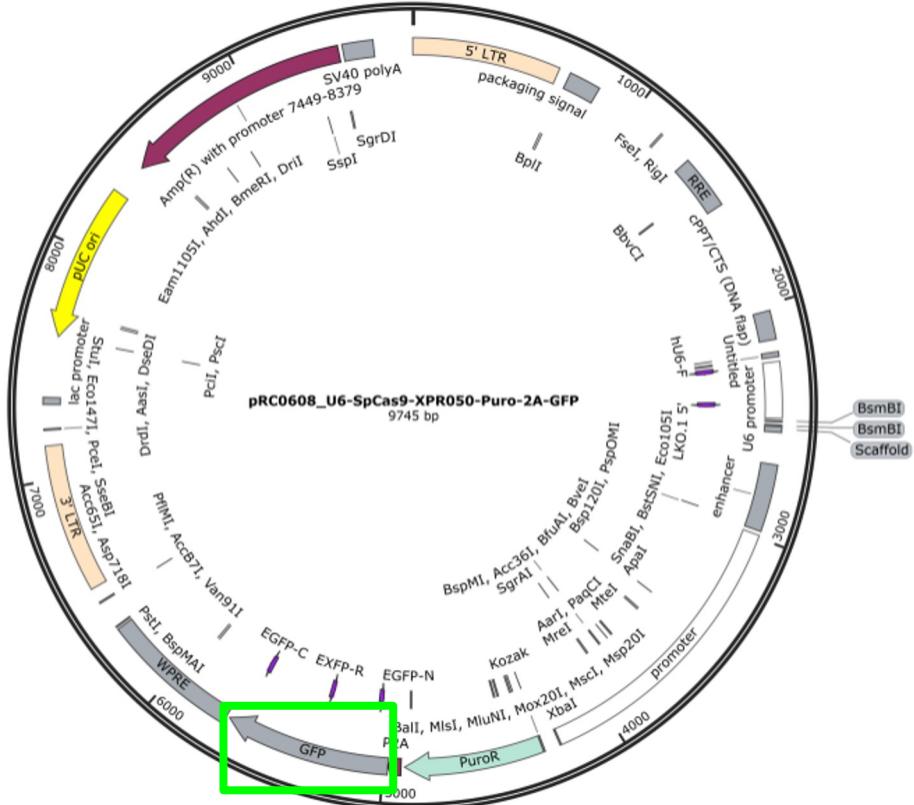
- ❖ HCT116 cells were transduced with the lentivirus expressing dCas9-ZIM3 under a doxycycline inducible promoter
- ❖ After blasticidin selection, cells were treated with doxycycline and Western Blot was performed to confirm dCas9-ZIM3 expression

Summary of method



- ❖ gRNAs targeting *PELO*, *RBM22*, and *ZMAT2* were cloned into a lentiviral vector
- ❖ I generated viral particles with the vector expressing gRNAs
- ❖ HCT116 cells expressing dCas9-ZIM3 were transduced with the virus and puromycin was added to the cells for selection
- ❖ Effect of gRNAs in the RBPs expression is currently being investigated

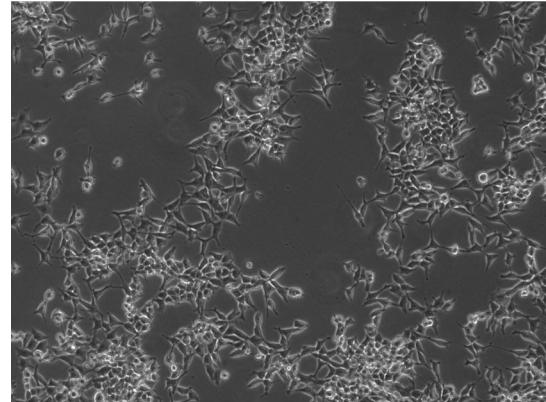
Confirmation of lentiviral vector expression with GFP



Lentiviral vector expressing gRNA

Green fluorescent protein (GFP)

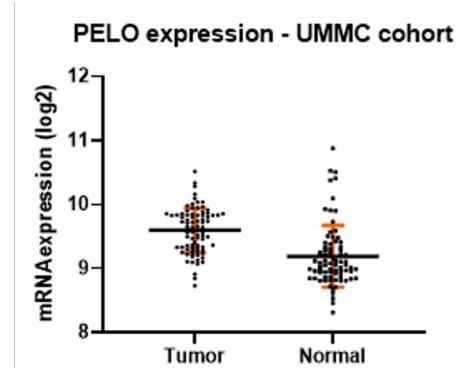
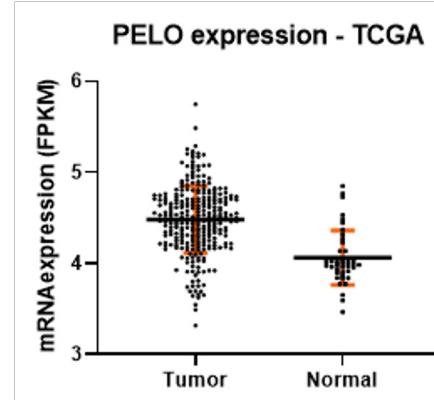
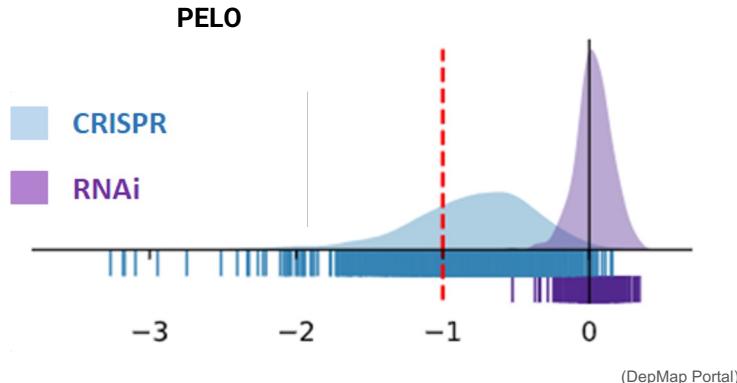
Transparent



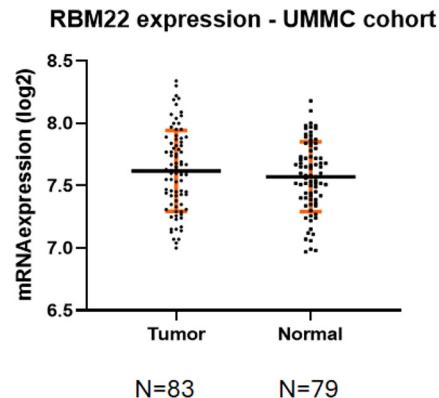
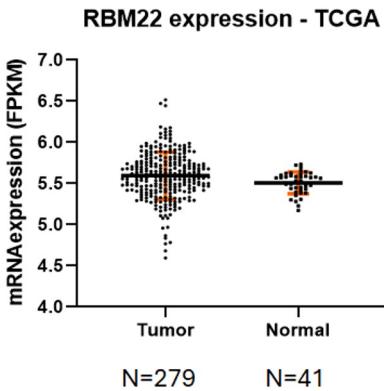
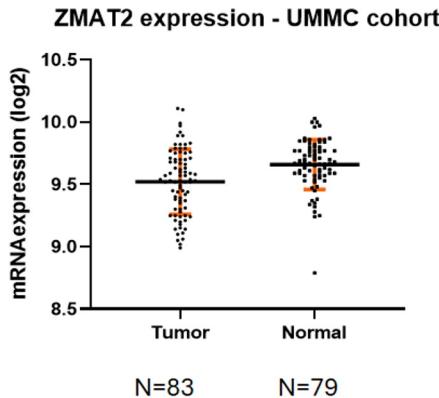
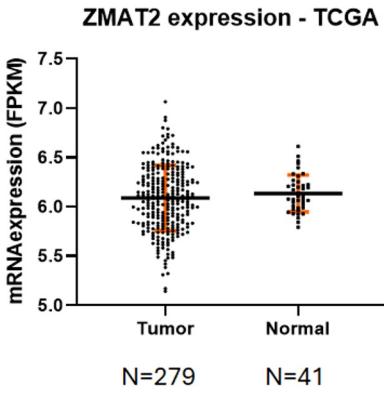
A fluorescence micrograph showing bright green punctate signals against a dark background. The signals are concentrated in several distinct clusters or regions across the field of view.

PELO is upregulated in CRC

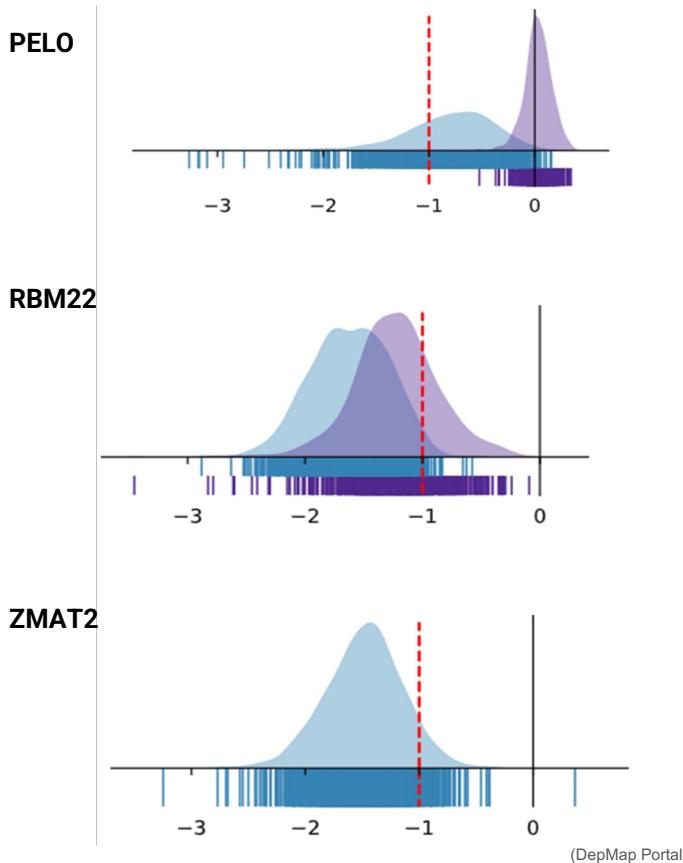
- ❖ Data from TCGA and from a cohort of patients from the University of Maryland show that *PELO* was expressed higher in CRC patients
- ❖ Evidence also supported by DepMap portal



No significant difference is observed for *RBM22* and *ZMAT2*



DepMap data shows *PELO*, *RBM22*, & *ZMAT2* are essential genes



- ❖ Score of zero means interfering with a gene has little to no effect on a target cell line. A negative score indicates the gene is more likely to be dependent on a given cell line
- ❖ **PELO** shows the gene is essential with CRISPR but nonessential with RNAi
- ❖ **RBM22** shows the gene is essential with both CRISPR and RNAi
- ❖ **ZMAT2** shows the gene is essential with CRISPR



Summary and future directions

- ❖ We identified RBPs playing a role in proliferation in the context of colorectal cancer
- ❖ Depletion of PELO, RBM22, and ZMAT2 resulted in a decrease in cell proliferation
- ❖ *PELO* is expressed higher in CRC patients
- ❖ These proteins were selected for further validation and their effect in the CRC cell line, HCT116 is being investigated
- ❖ Best RBP candidates will be further tested for global post-transcriptional gene regulation



Acknowledgments

Ashish Lal
Ioannis Grammatikakis
Ragini Singh
Lynn Li
Ravi Kumar
Bruna Muys

Collaborators

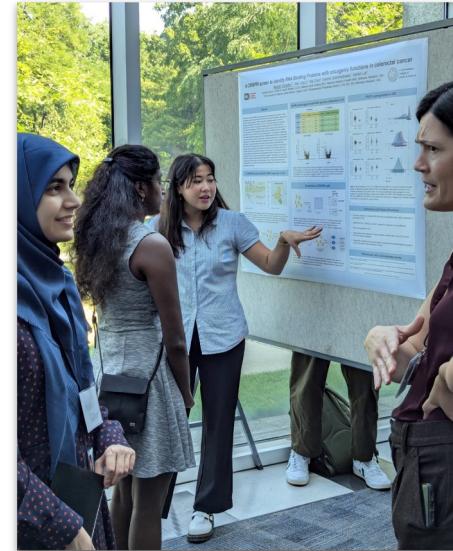
**Developmental Therapeutics
Branch (CCR, NCI, NIH)**
Raj Chari



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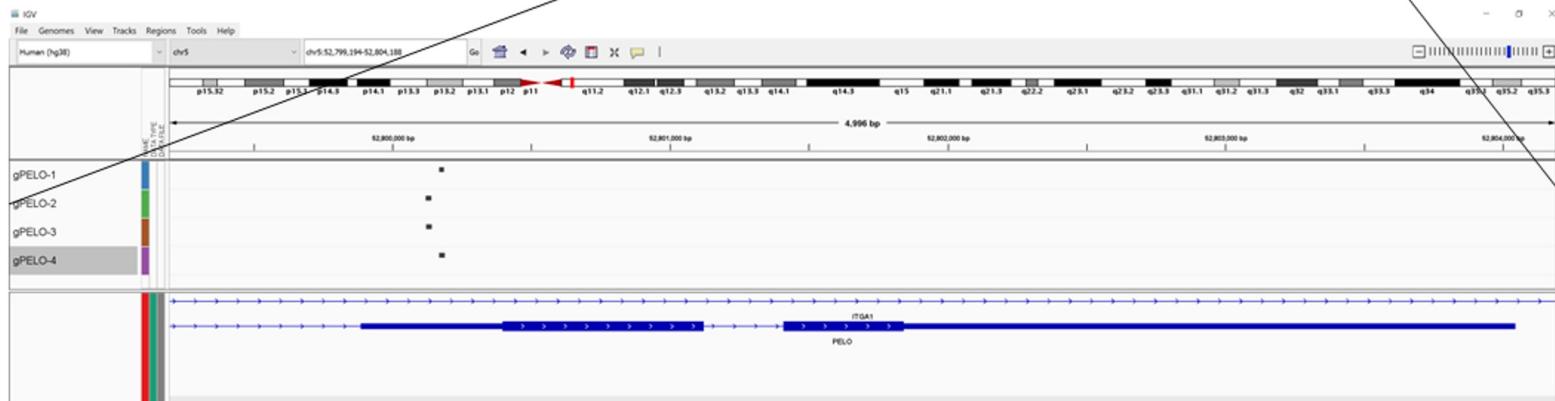
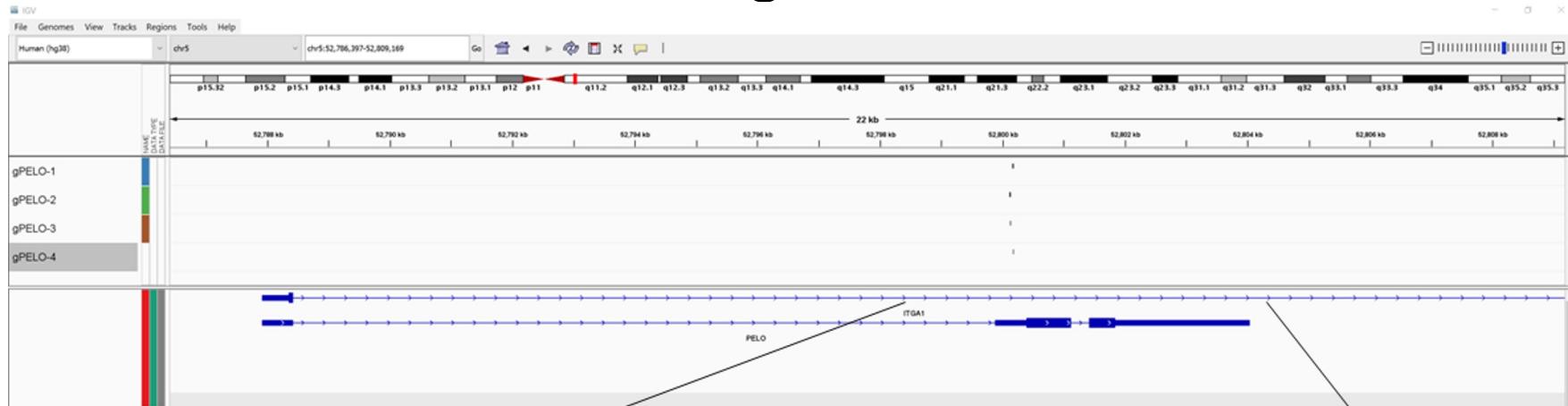
Thank You!

Special thank you to Dr. Lal,
Ioannis, and the Lal group for
fostering a wonderful first research
experience and providing me with
extraordinary mentorship.

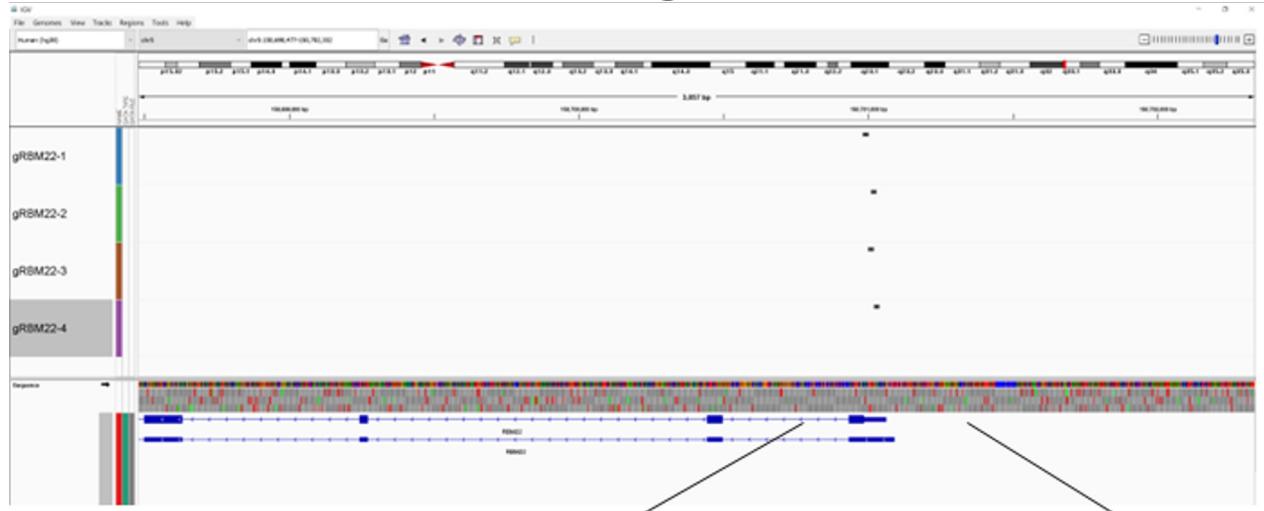


Poster Day 2024

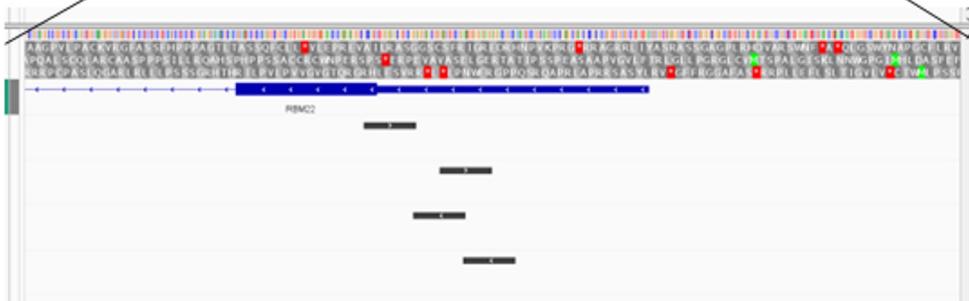
PELO gRNAs



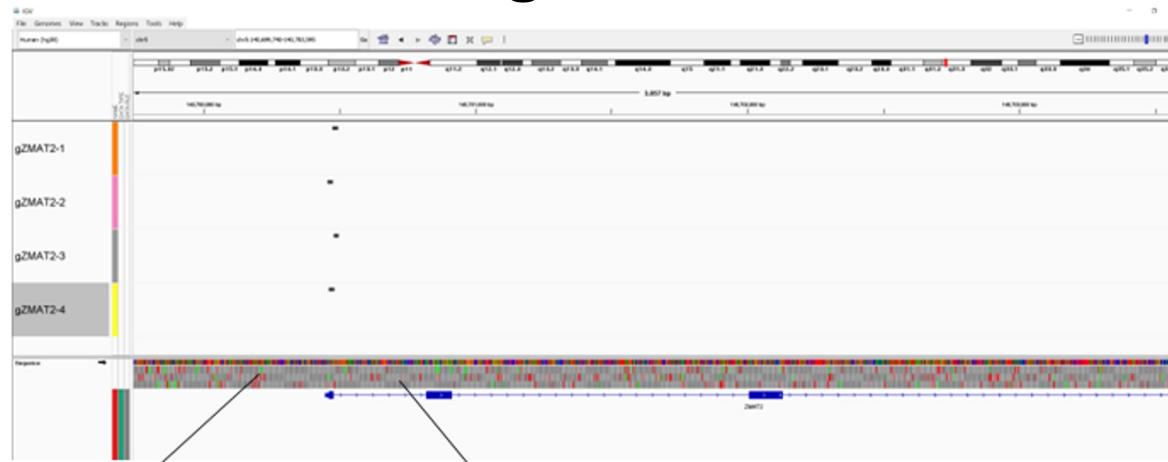
RBM22 gRNAs



RBM22



ZMAT2 gRNAs



ZMAT2

