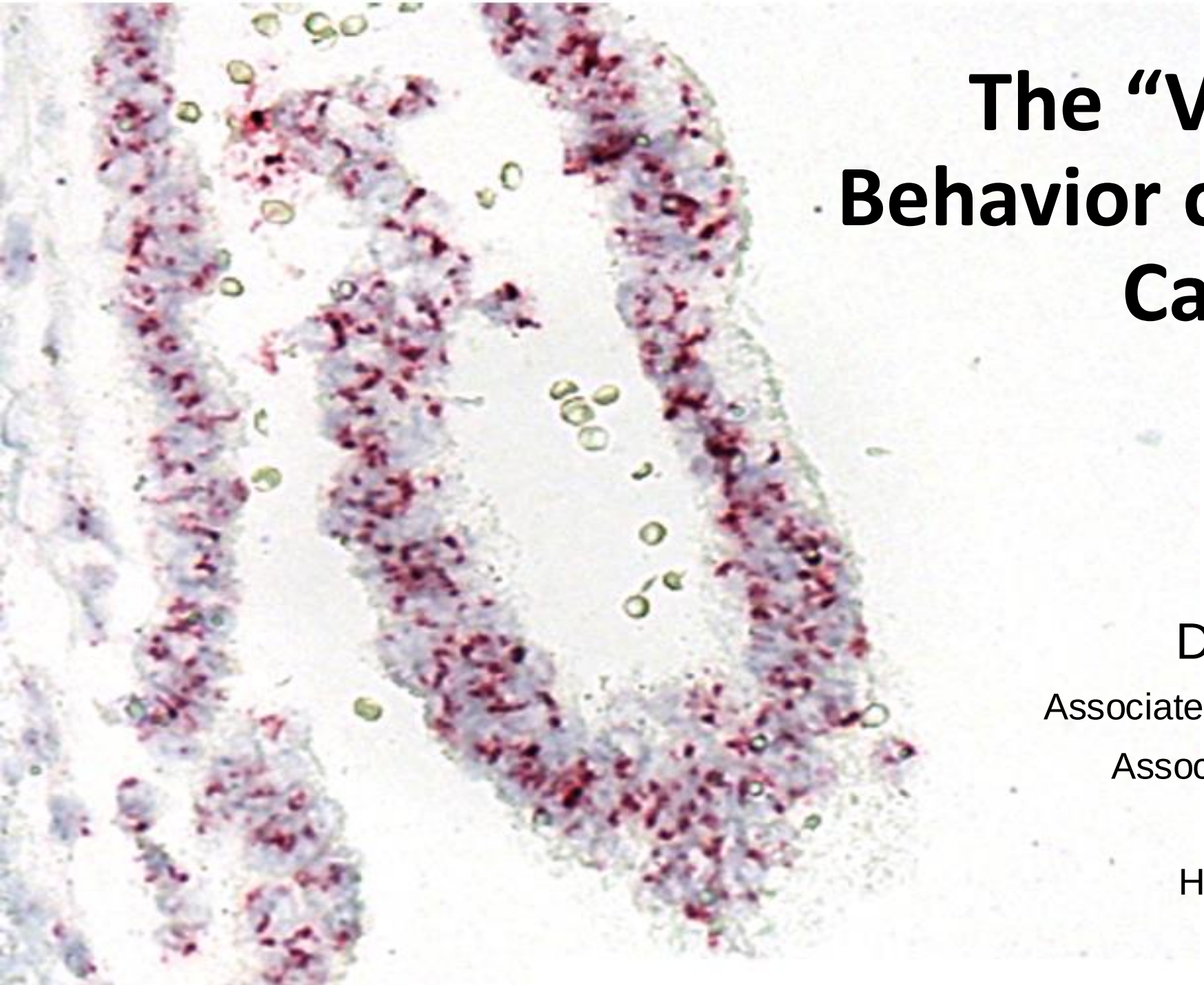


The “Viral-like” Behavior of Pancreatic Cancer

David T Ting, MD

Associate Clinical Director for Innovation

Associate Professor of Medicine

MGH Cancer Center

Harvard Medical School

Disclosure Information

David T. Ting, MD

I have the following financial relationships to disclose:

Consultant for: Astellas, Leica, PanTher Therapeutics, ROME Therapeutics, Sonata Therapeutics, abrdn

Honoraria from: AstraZeneca, Moderna, Ikena Oncology, Nanostring Technologies, Pfizer, Ventana-Roche, EMD Millipore Sigma, Foundation Medicine, Inc., Merrimack Pharmaceuticals

Funding from: Astellas, Sanofi, ACD-Biotechne, Incyte

Advisory Board for: PanTher Therapeutics, ROME Therapeutics, TellBio Inc., ImproveBio, Inc.

Founder for: PanTher Therapeutics, ROME Therapeutics, TellBio Inc.

ROME Therapeutics is a company focused on developing therapeutics on repeatome biology, but no funding from the company contributed to this work. Dr. Ting's interests were reviewed and are managed by Massachusetts General Brigham in accordance with their conflict of interest policies.

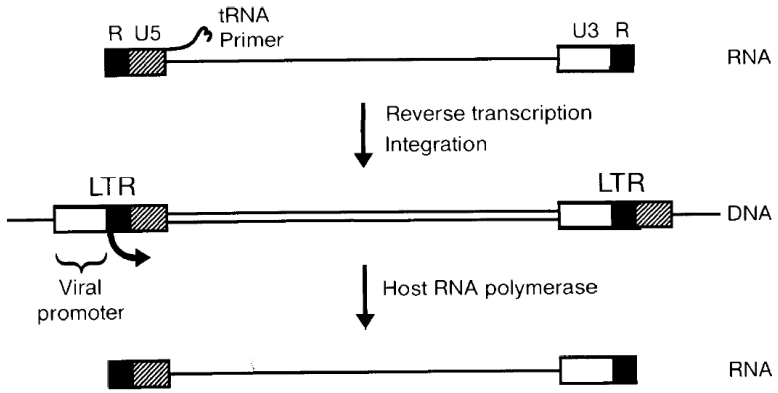
- and -

I will discuss off label use and/or investigational use in my presentation of a generic drug.

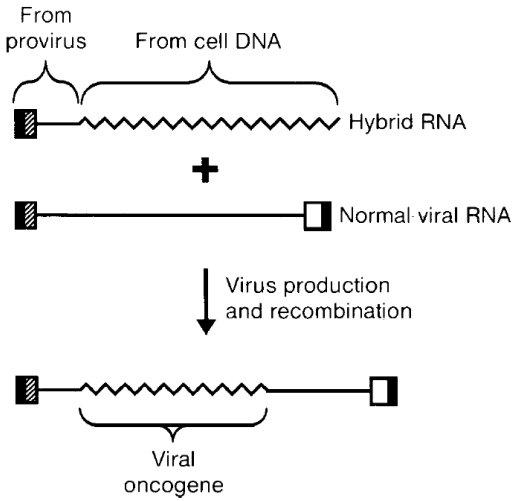
The Repeatome:
The Latent Virus Within

Bishop and Varmus Nobel Prize
“Discovery of the cellular origin of retroviral oncogenes."

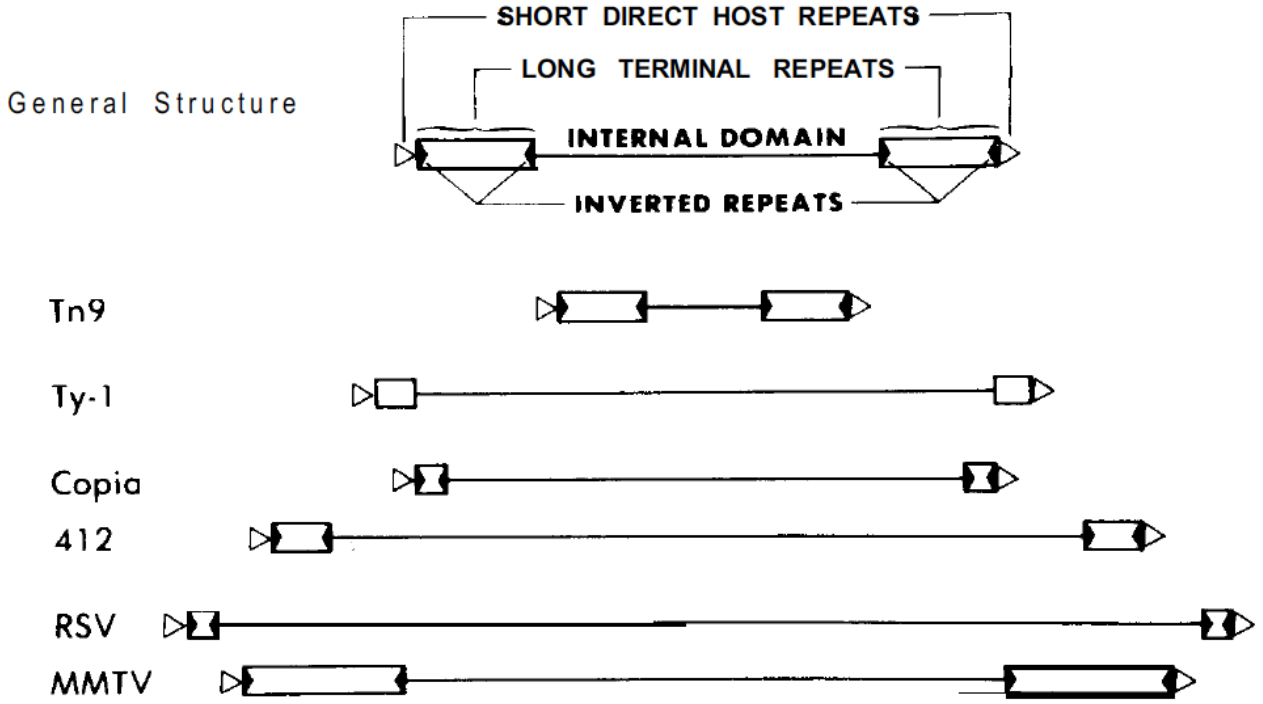
Retroviruses Replicate through Reverse Transcription



Retroviruses can pick up “oncogenes”

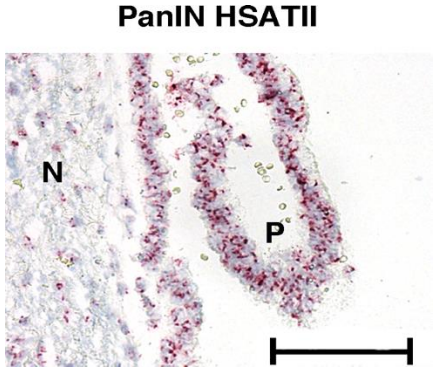


Genome “viral elements” (Repeatome) have the same structure as retroviruses



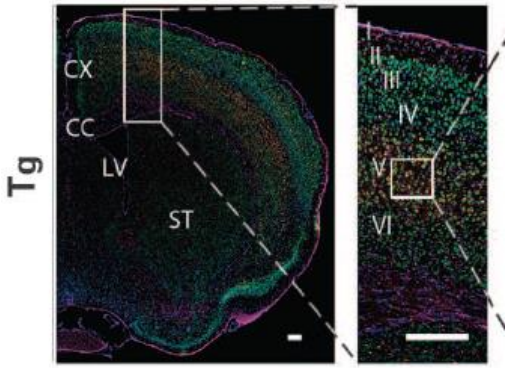
The ”Repeatome”: A Global Genomic Program in Human Disease

Repeat RNA Reactivated in Early Cancers



Ting et al ***Science*** (2011)

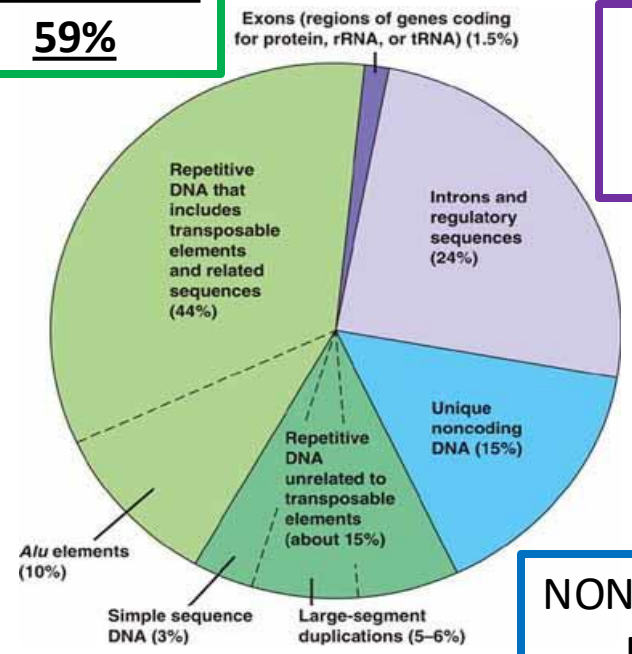
Repeat Dysregulation in Neurodegenerative diseases



Li et al. ***Science Trans. Medicine*** (2015)

Repeats Comprise Majority of Genome

REPEAT DNA 59%

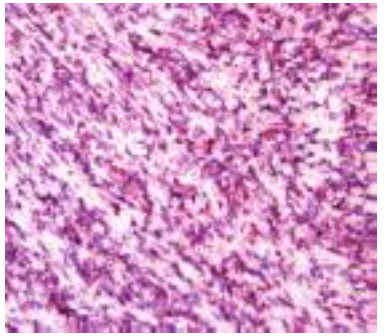


Pearson Ed, 2011

CODING DNA 26%

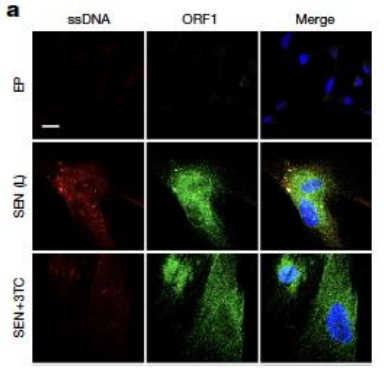
NONCODING DNA 15%

Repeat RNA Reactivated in Chronic Viral Infection



Nogalski et al. ***Nature Communications*** (2018)

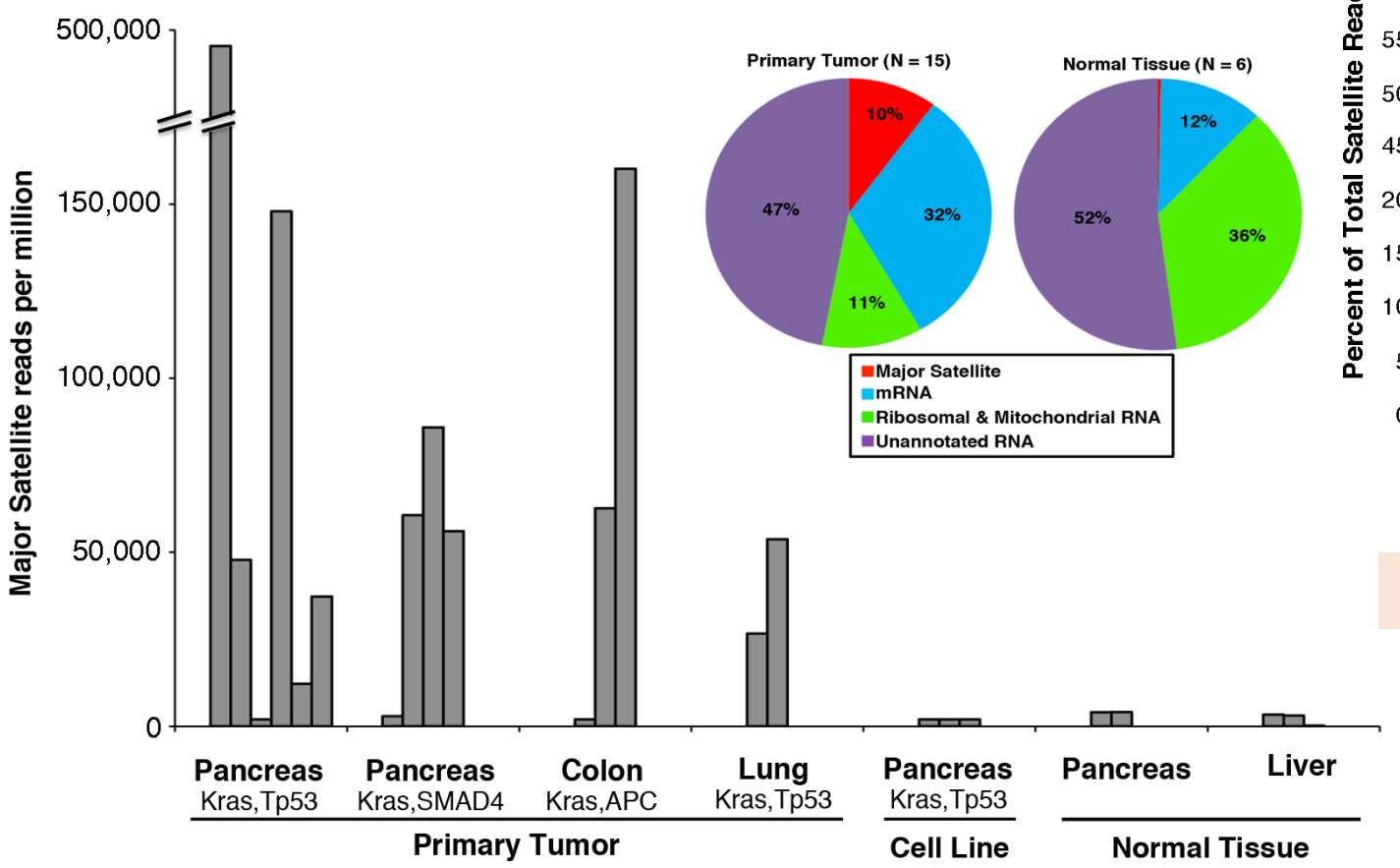
Repeat RNA Reactivated in Aging/Senescence



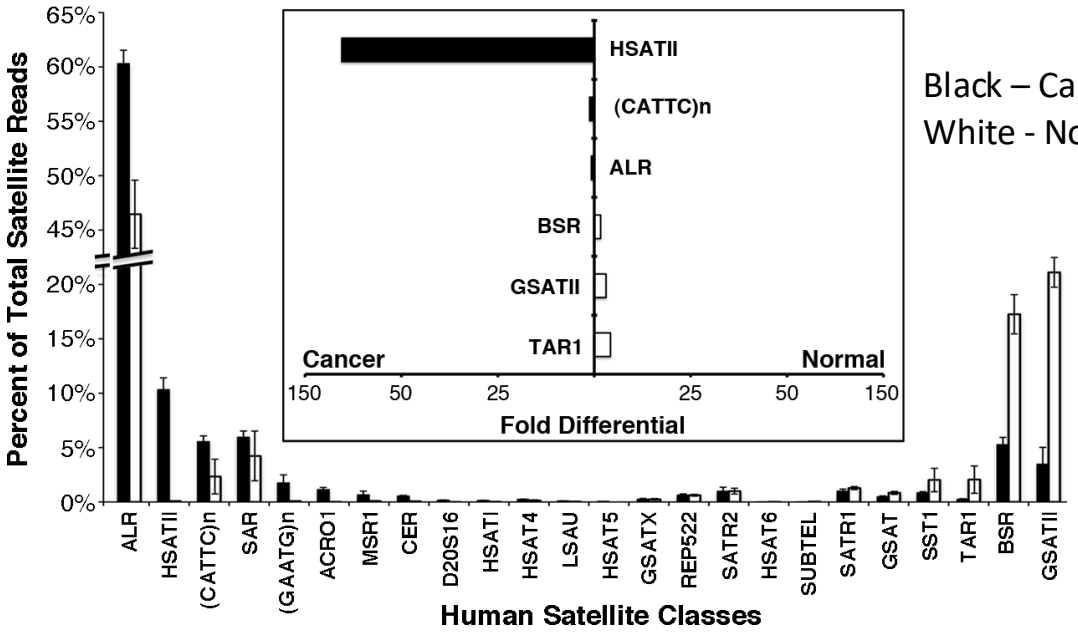
De Cecco et al. ***Nature*** (2019)

Aberrant Expression of Repeat RNAs in Cancer

Aberrant Expression in Multiple Mouse Models

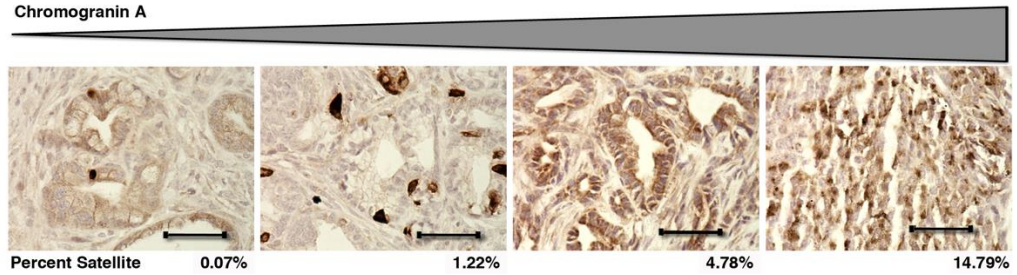


HSATII Specific in Human Cancers



Black – Cancer
White - Normal

Repeats Linked with Neuroendocrine Differentiation

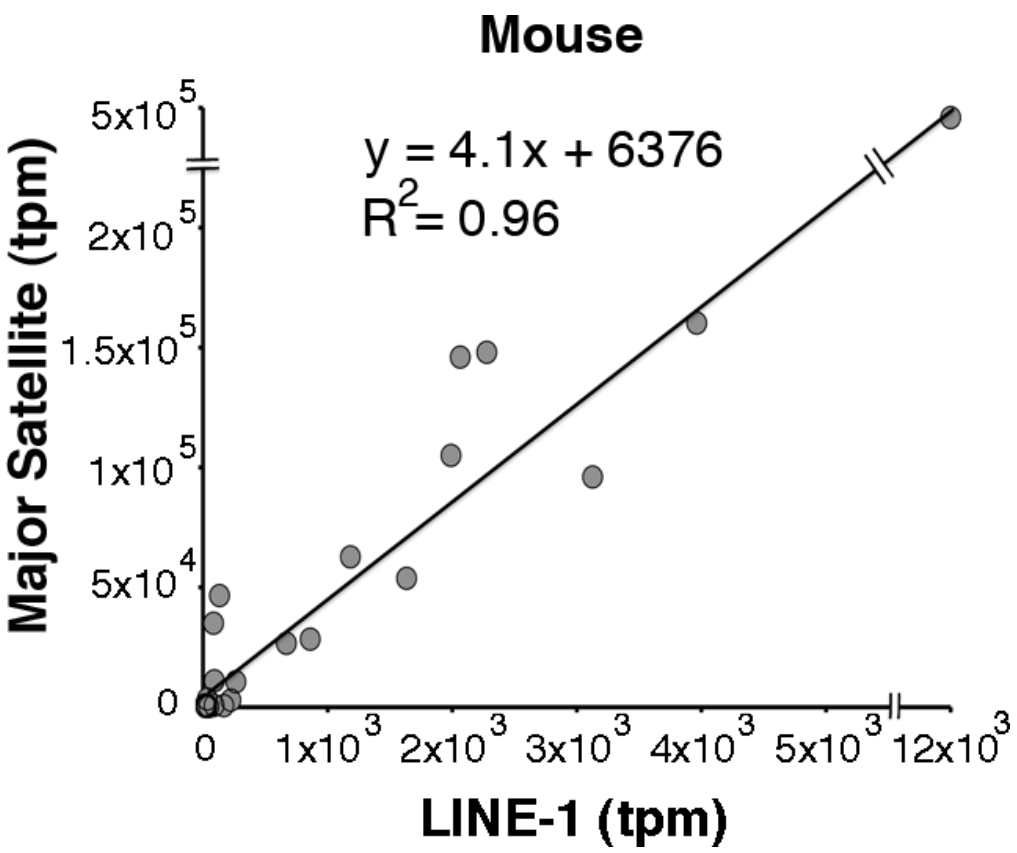


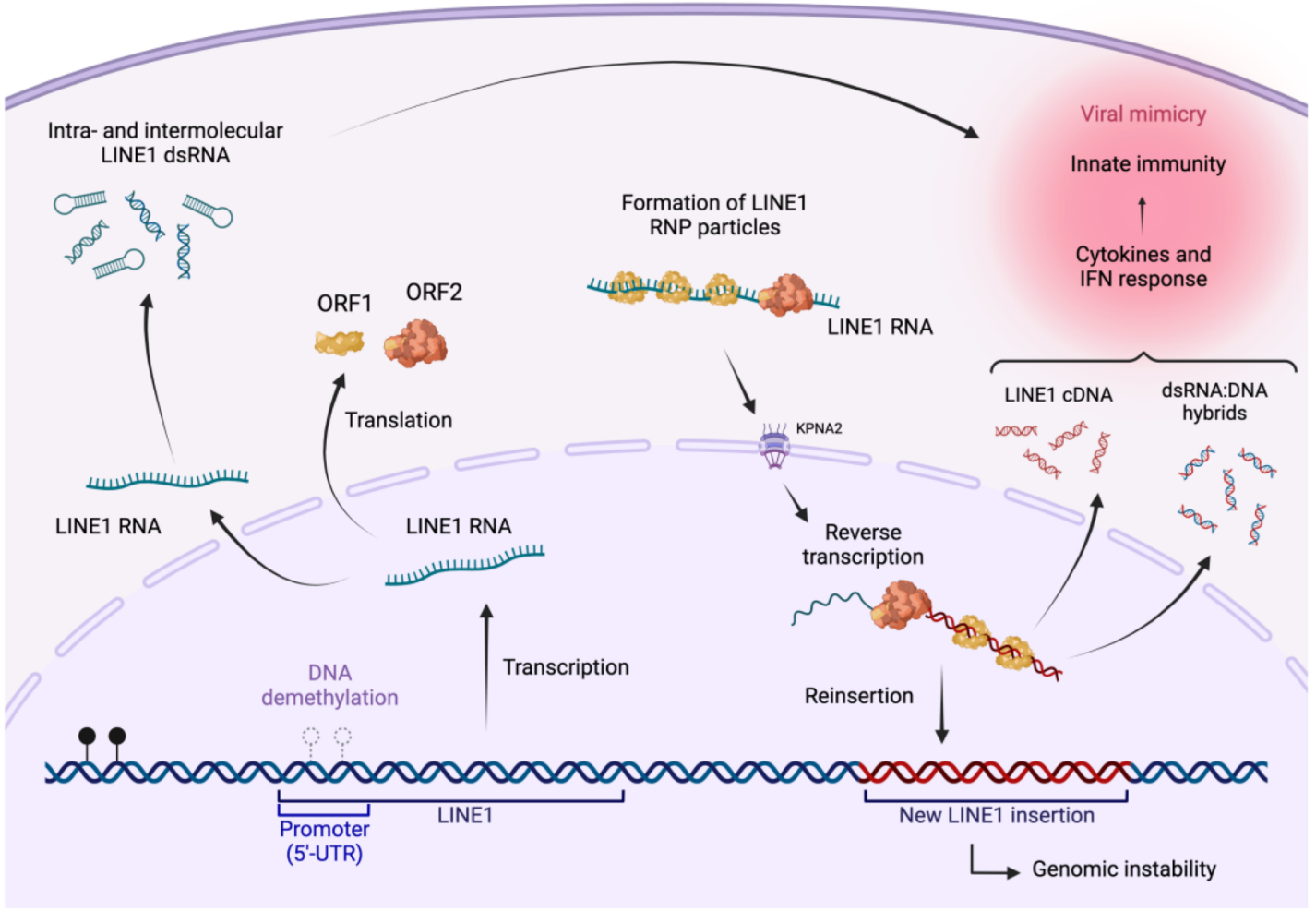
Satellite Correlated Genes (SCG)

Neural	Germ/Stem Cell	Zinc Finger Domain	HOX Related
120 (63%)	50 (26%)	16 (8%)	9 (5%)

Ting DT*, Lipson D*, et al. ***Science*** (2011)

LINE-1 Retrotransposon Highly Correlated with Satellite Expression

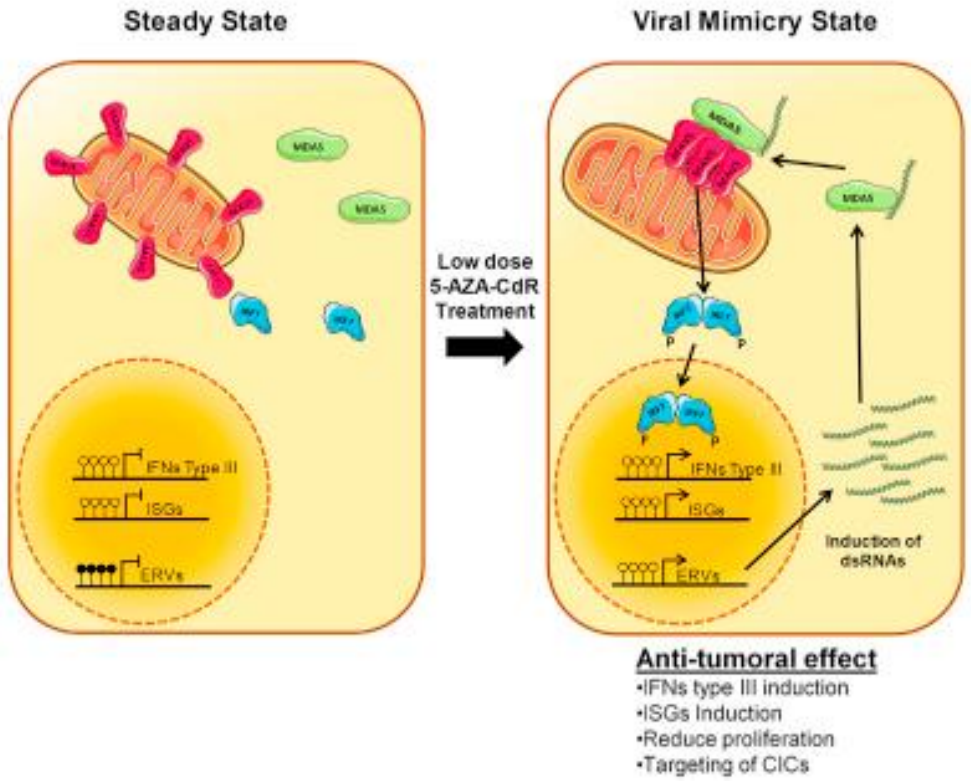


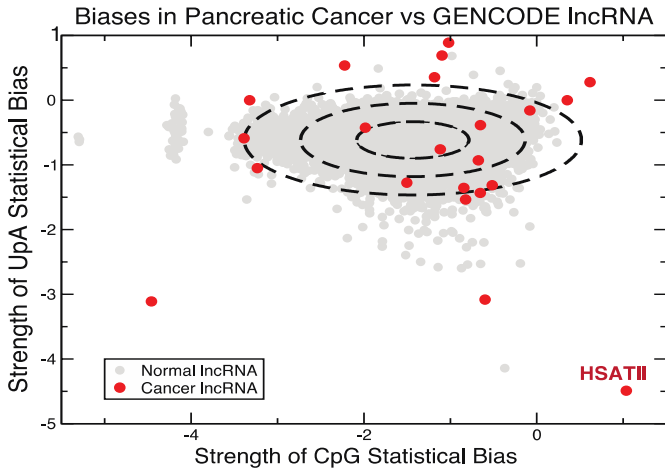


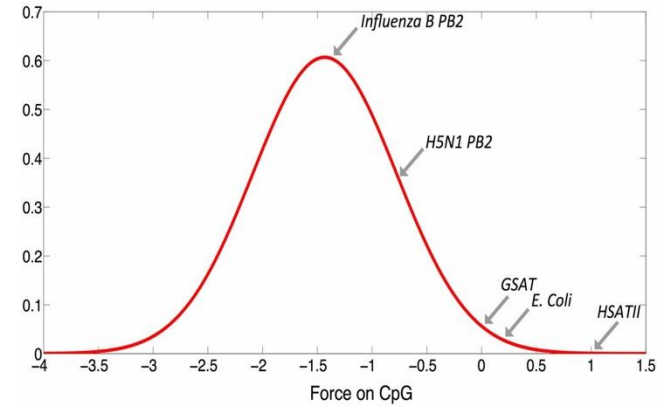
- LINE-1 Endogenous retrotransposon
- Insertions throughout the genome
- Comprises 16-20% of the entire genome

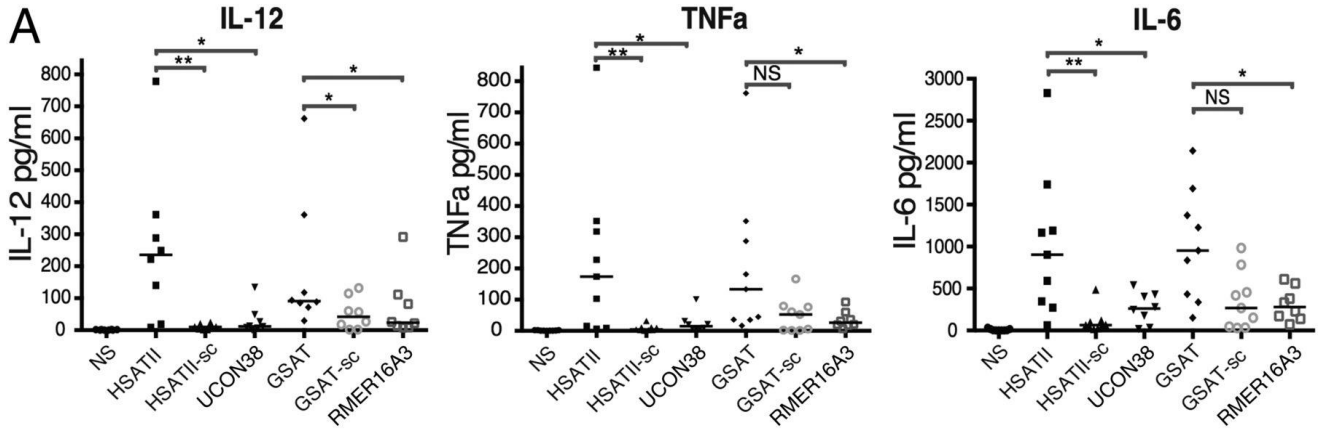
Do Repeats Look like Viruses?

Repeat RNAs Look Like Viruses
”Viral Mimicry”









Innate Immune Response to Repeat RNA Driven by CpG Motif Usage Similar to Pathogens

Tanne et al, ***PNAS*** (2015), Roulois et al. ***Cell*** (2015),
Chappinelli et al. ***Cell*** (2015), Sheng et al. ***Cell*** (2018)

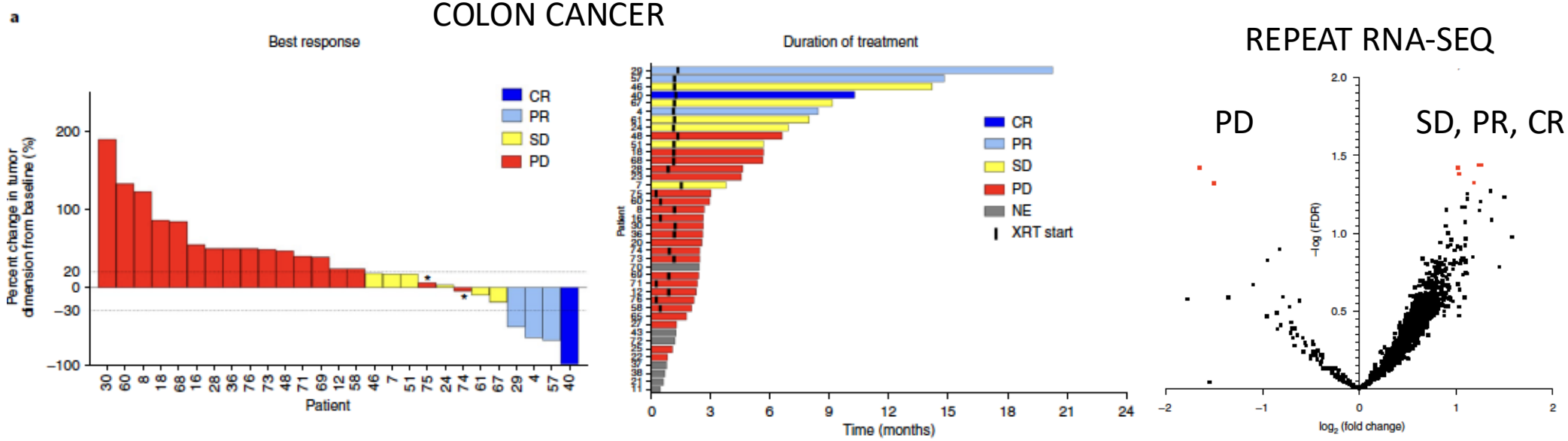
Certain Repeats Correlate with Immunotherapy Response in Colon and Pancreatic Cancer

Phase 2

Ipi/Nivo XRT

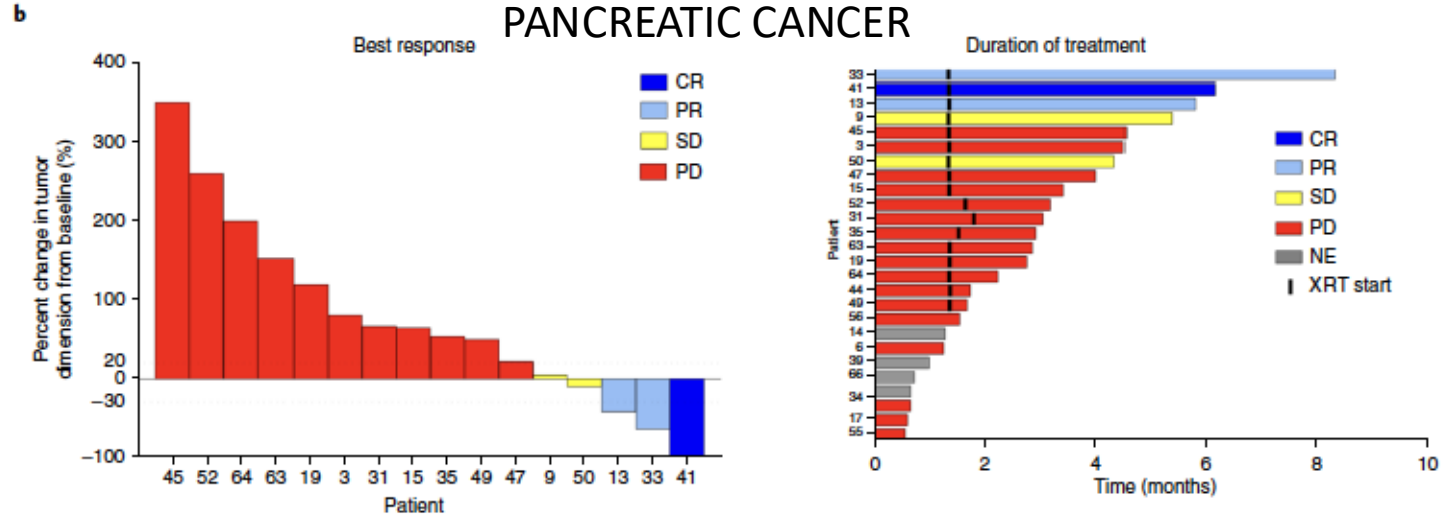
Metastatic CRC and PDAC

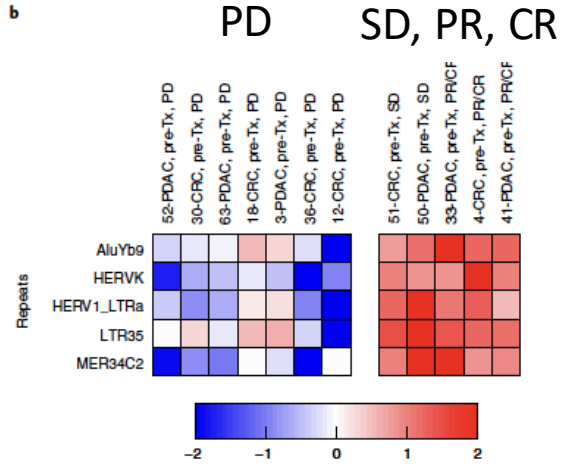
COLON CANCER



REPEAT RNA-SEQ

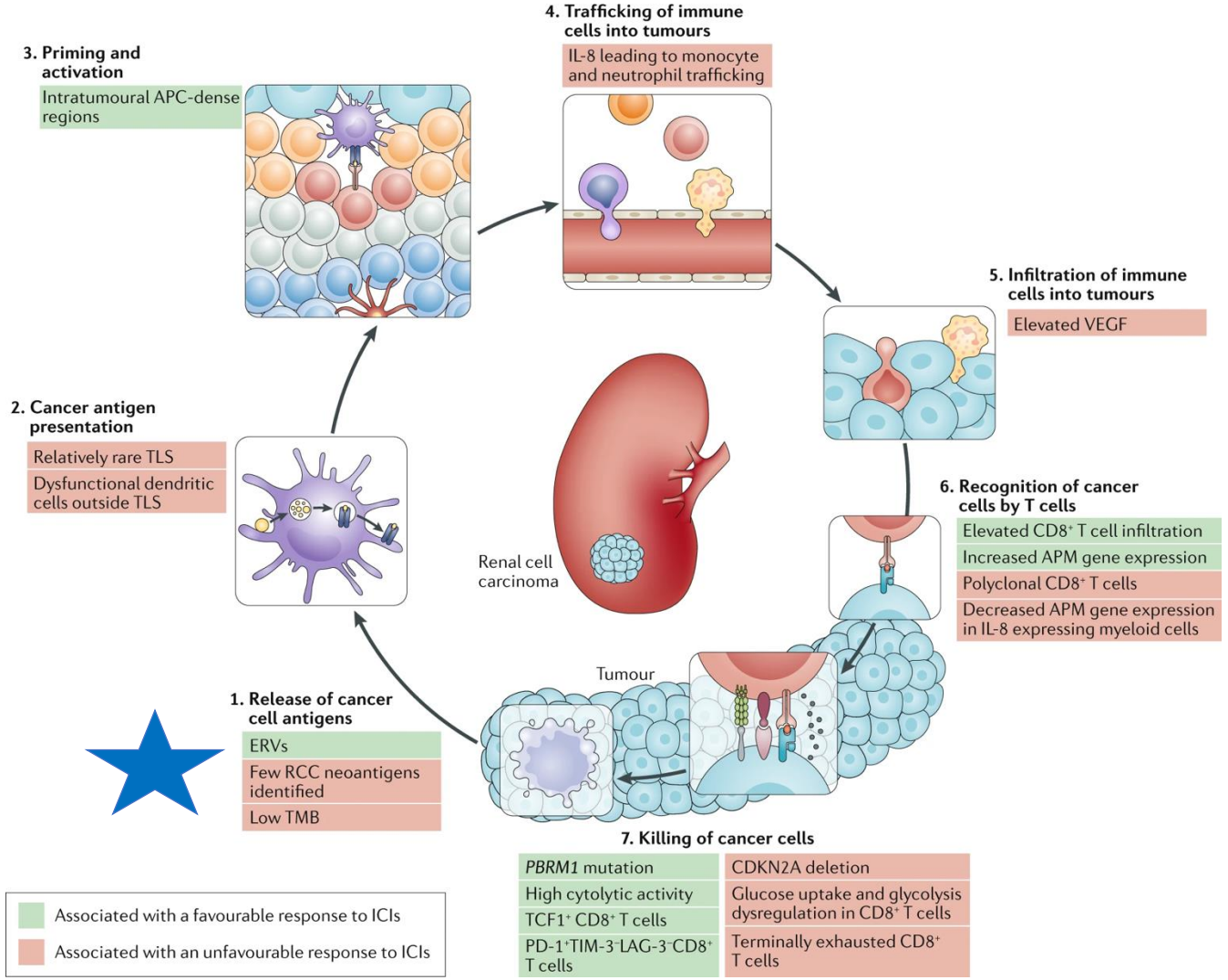
PANCREATIC CANCER





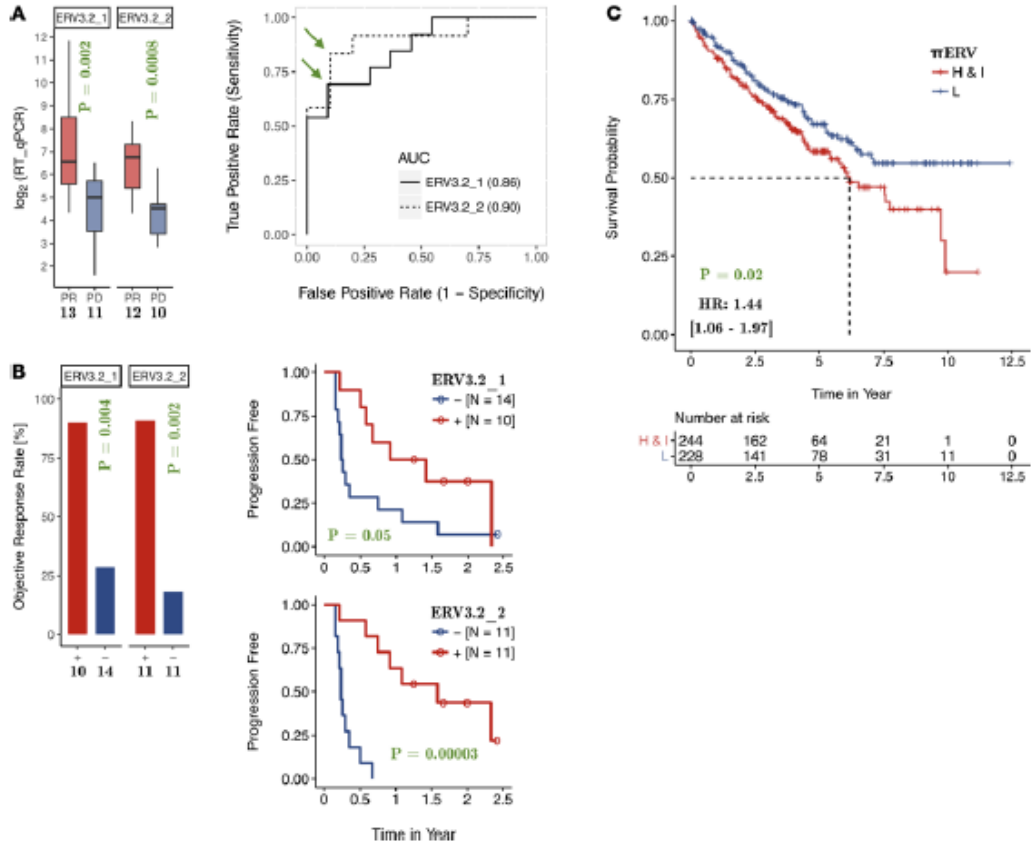
Parikh A, Szabolcs A, et al, ***Nat Cancer***, 2021

HERV Expression and Associated Immunotherapy Response in other Cancers



Braun D et al. Nat Rev Clin Onc 2021

Renal Cell Carcinoma Immunotherapy



Panda et al. JCI Insight 2018

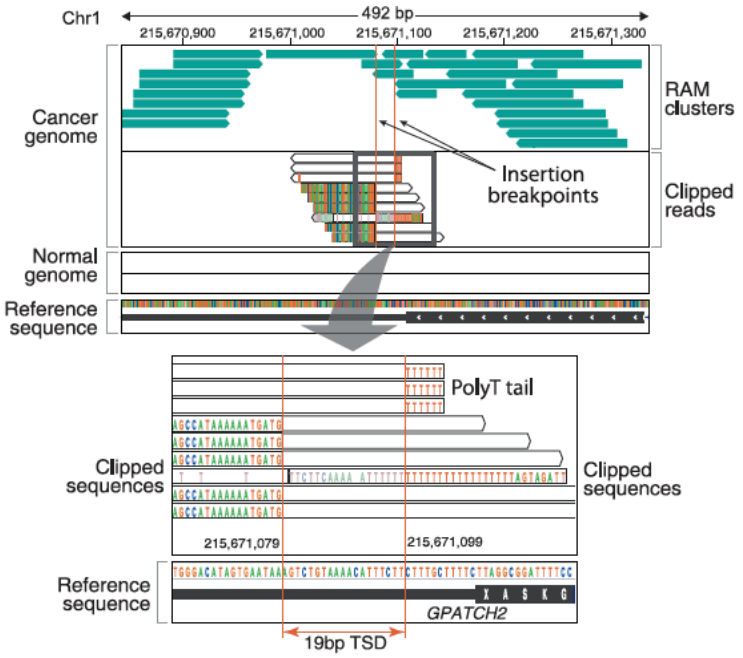
Can Repeats Replicate like a Virus?

Repeats Replicate in the Genome like Viruses

Science AAAS

Landscape of Somatic Retrotransposition in Human Cancers

Eunjung Lee,[1,2] Rebecca Iskow,[3] Lixing Yang,[1] Omer Gokcumen,[3] Psalm Haseley,[1,2] Lovelace J. Luquette III,[1] Jens G. Lohr,[4,5] Christopher C. Harris,[6] Li Ding,[6] Richard K. Wilson,[6] David A. Wheeler,[7] Richard A. Gibbs,[7] Raju Kucherlapati,[2,8] Charles Lee,[3] Peter V. Kharchenko,[1,9]* Peter J. Park,[1,2,9]* and The Cancer Genome Atlas Research Network

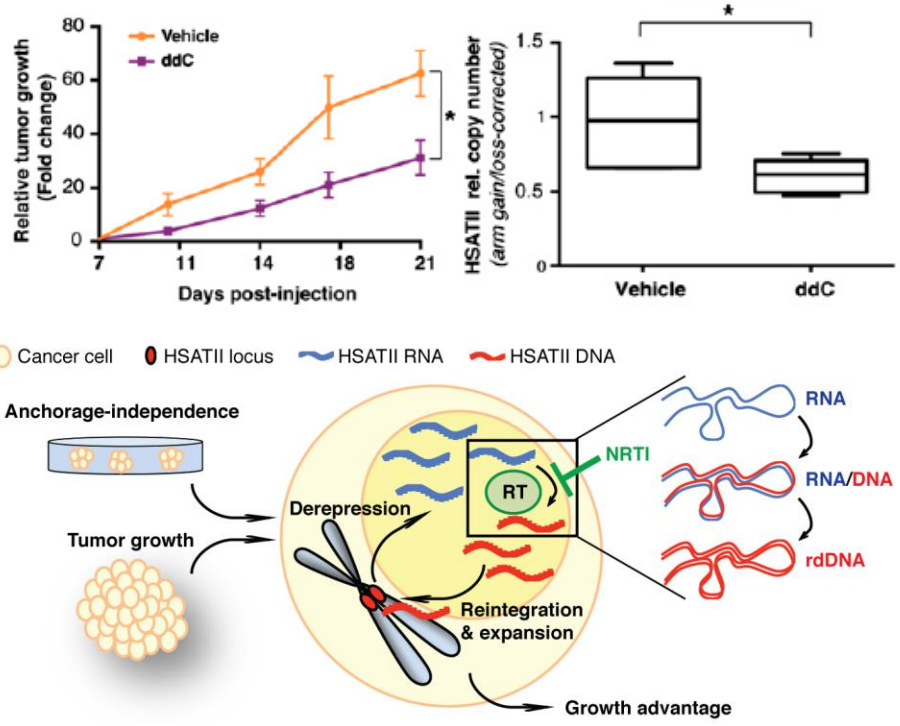


Lee et al, ***Science***. 2012

PNAS

Pericentromeric satellite repeat expansions through RNA-derived DNA intermediates in cancer

Francesca Bersani[a], Eunjung Lee[b,c], Peter V. Kharchenko[b,d], Andrew W. Xu[b], Mingzhu Liu[a,e], Kristina Xega[a], Olivia C. MacKenzie[a], Brian W. Brannigan[a], Ben S. Wittner[a], Hyunchul Jung[f], Sridhar Ramaswamy[a,g], Peter J. Park[b,c,h], Shyamala Maheswaran[a,i], David T. Ting[a,g,1,2], and Daniel A. Haber[a,e,g,1,2]



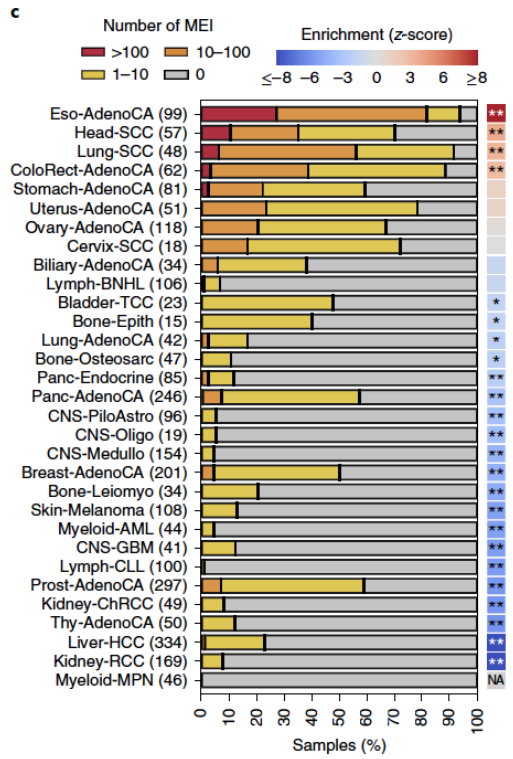
Bersani et al, ***PNAS***. 2015

nature genetics ARTICLES

https://doi.org/10.1038/s41588-019-0562-0

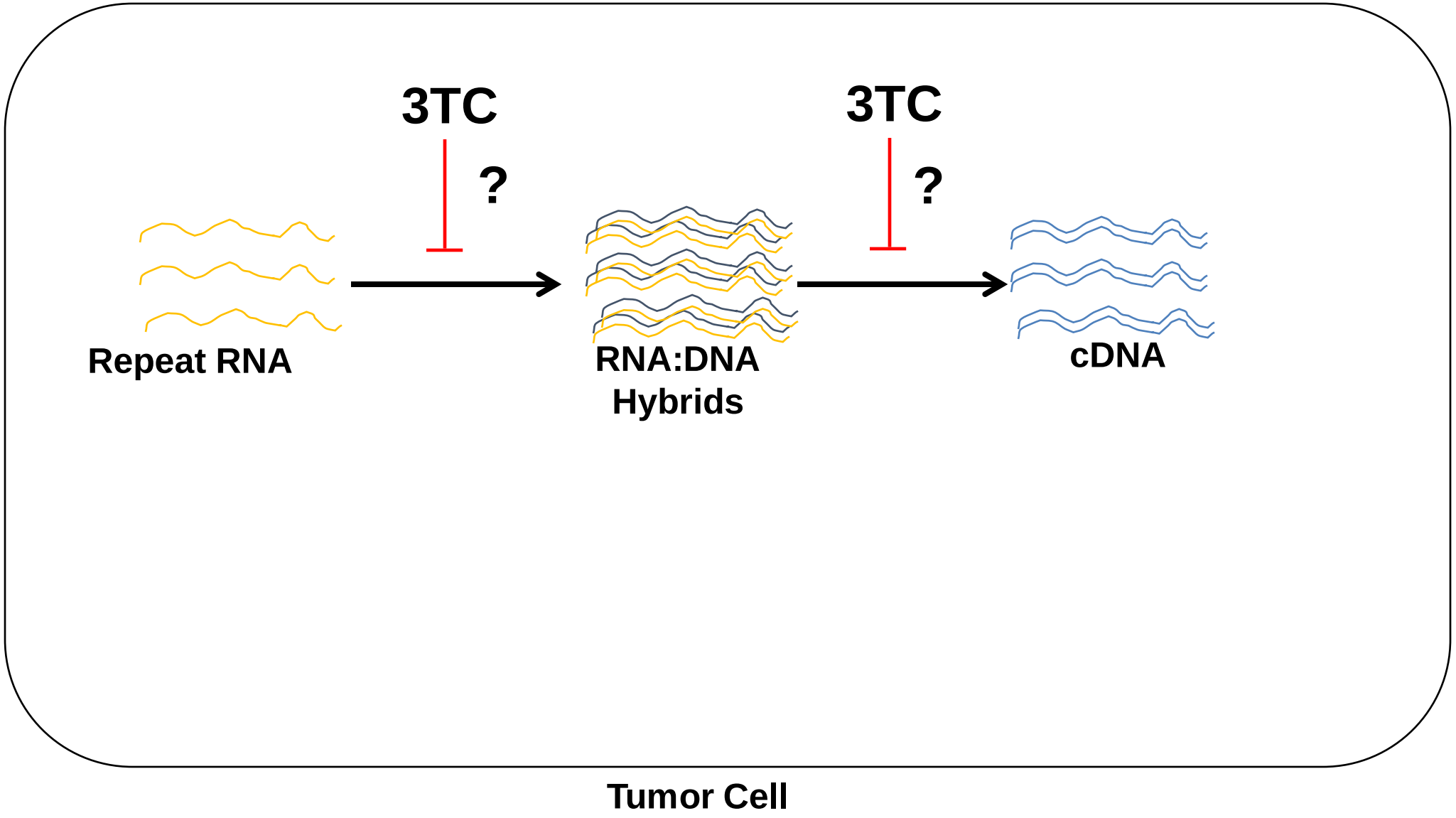
OPEN

Pan-cancer analysis of whole genomes identifies driver rearrangements promoted by LINE-1 retrotransposition

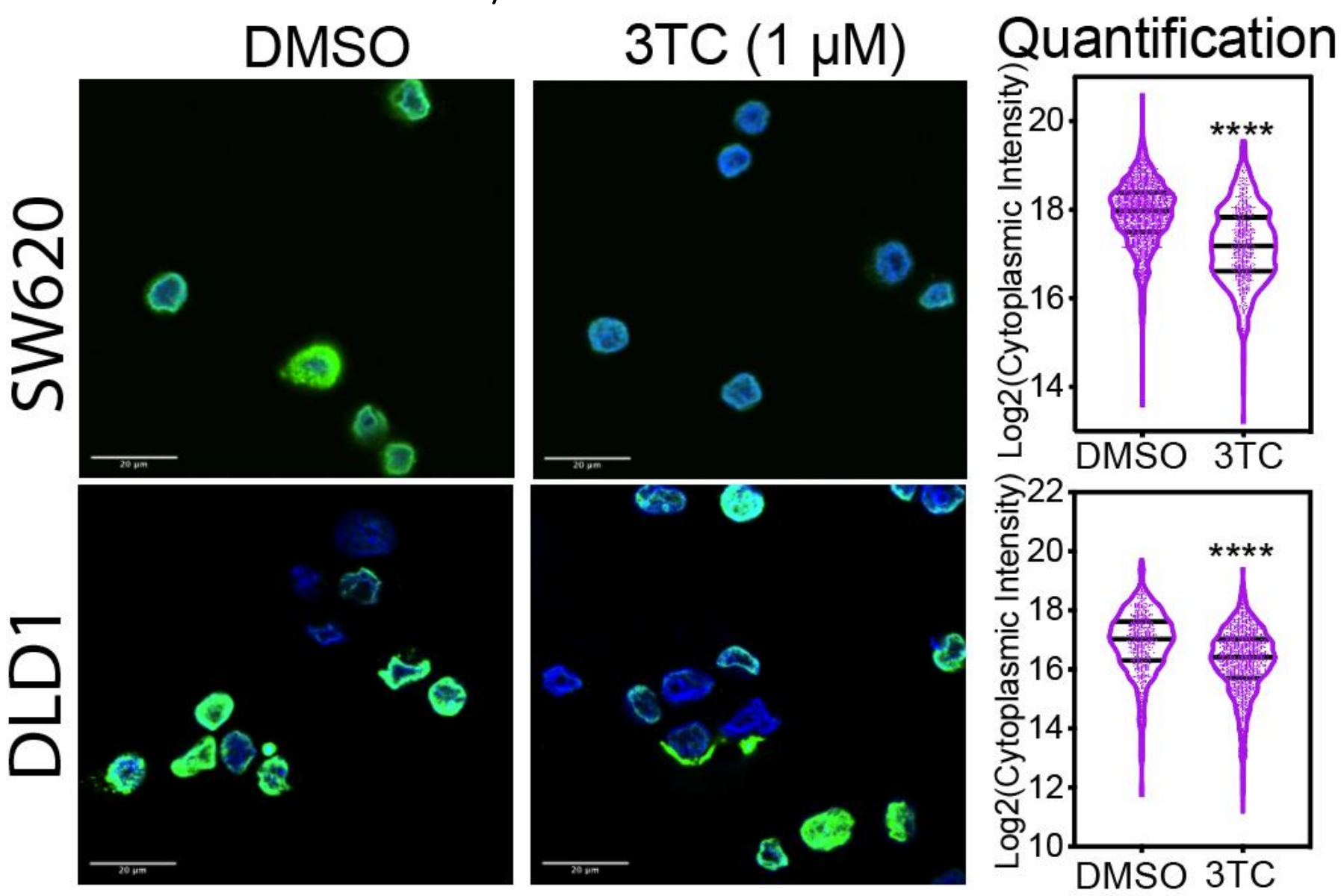


Rodriguez-Martin et al, ***Nat Genetics***. 2020

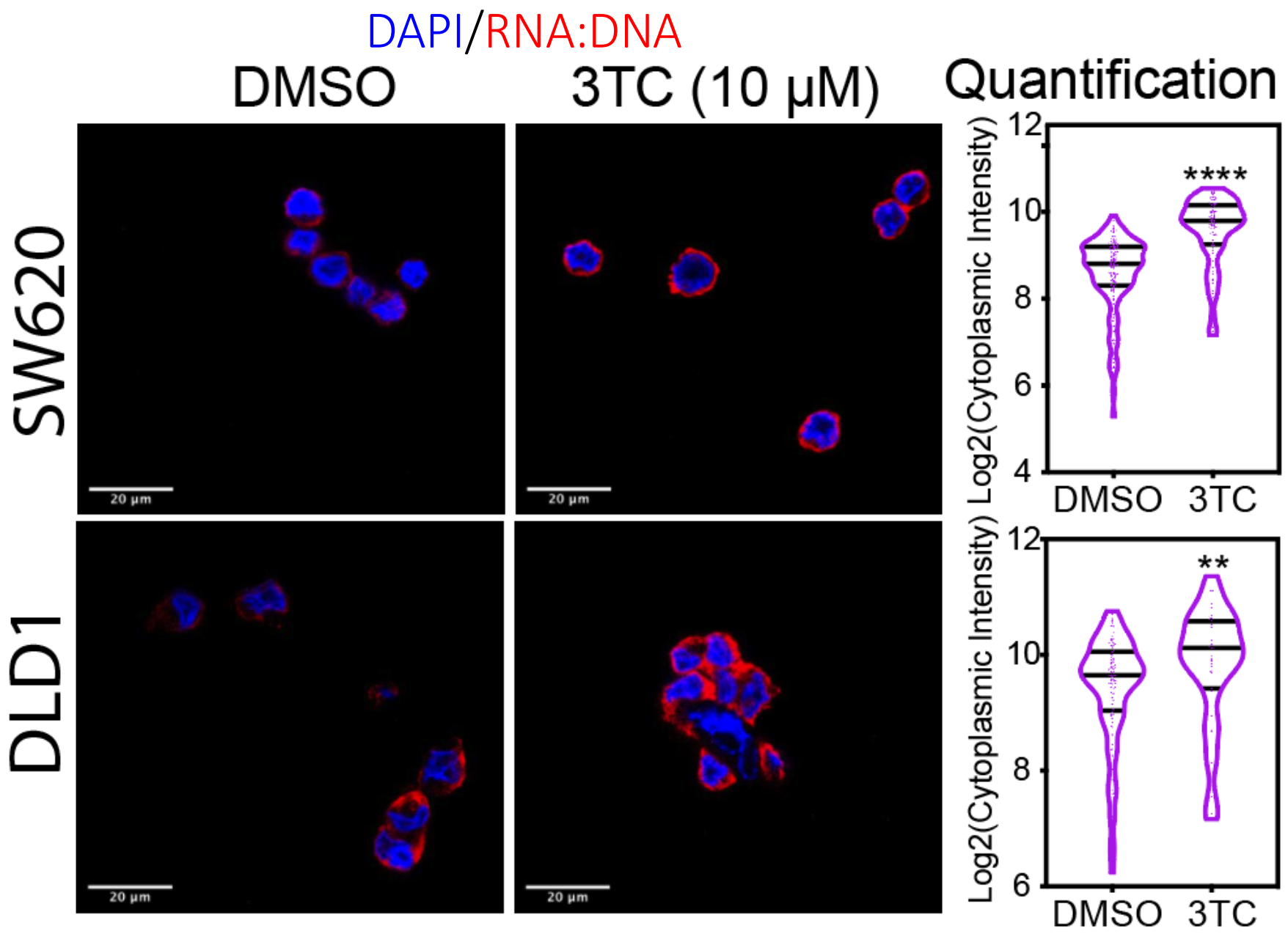
3TC Effects on Repeat RNA Reverse Transcriptase



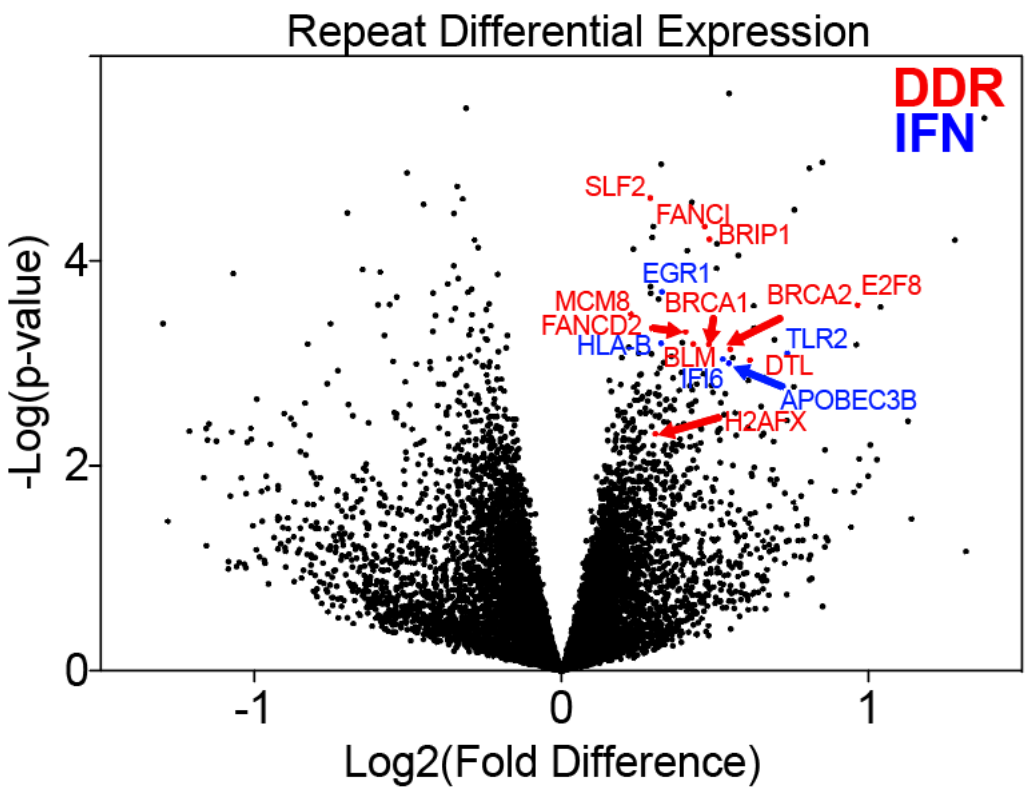
3TC treatment leads to decreased cytoplasmic DNA in CRC

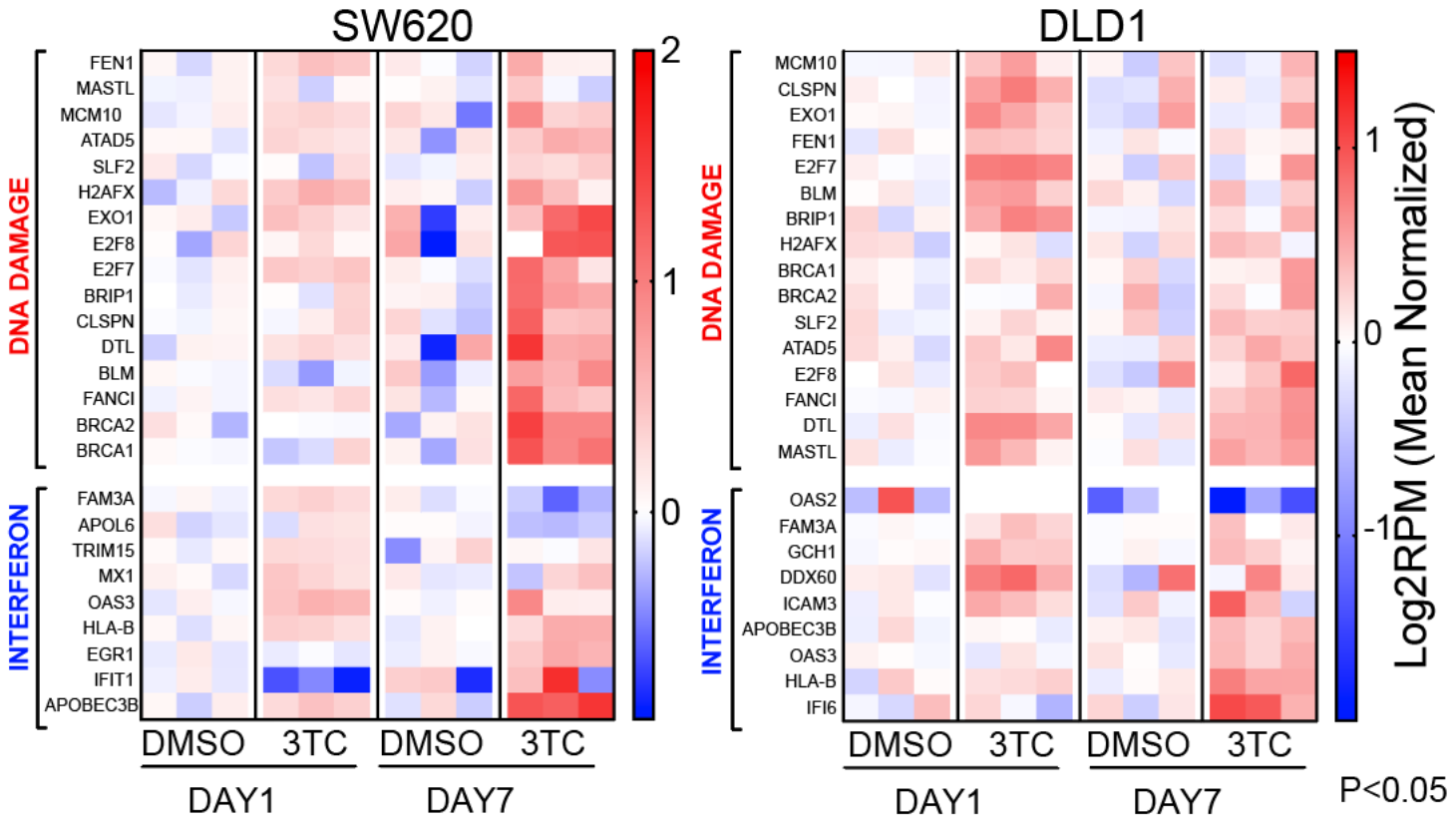


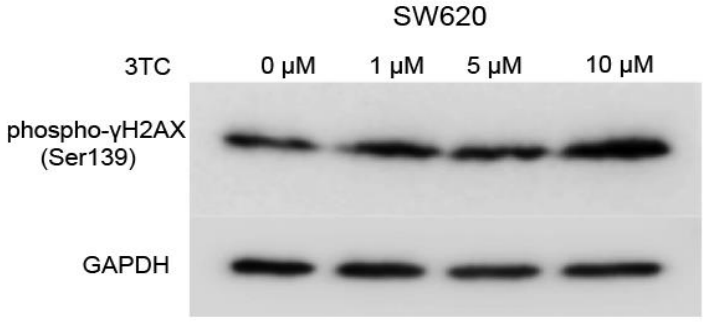
3TC treatment leads to increased cytoplasmic RNA:DNA hybrids in CRC



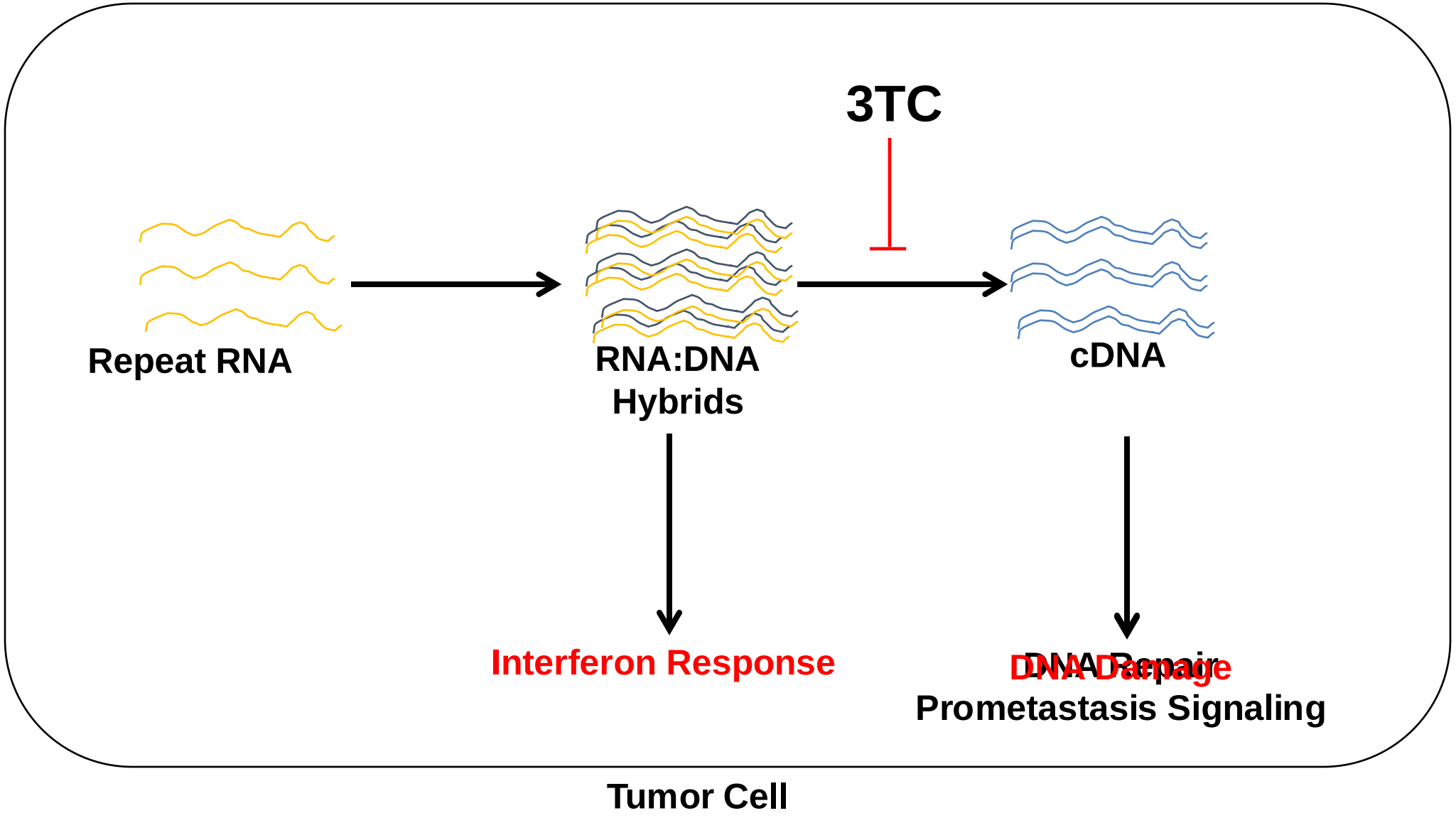
3TC activates Interferon and DNA damage response in CRC



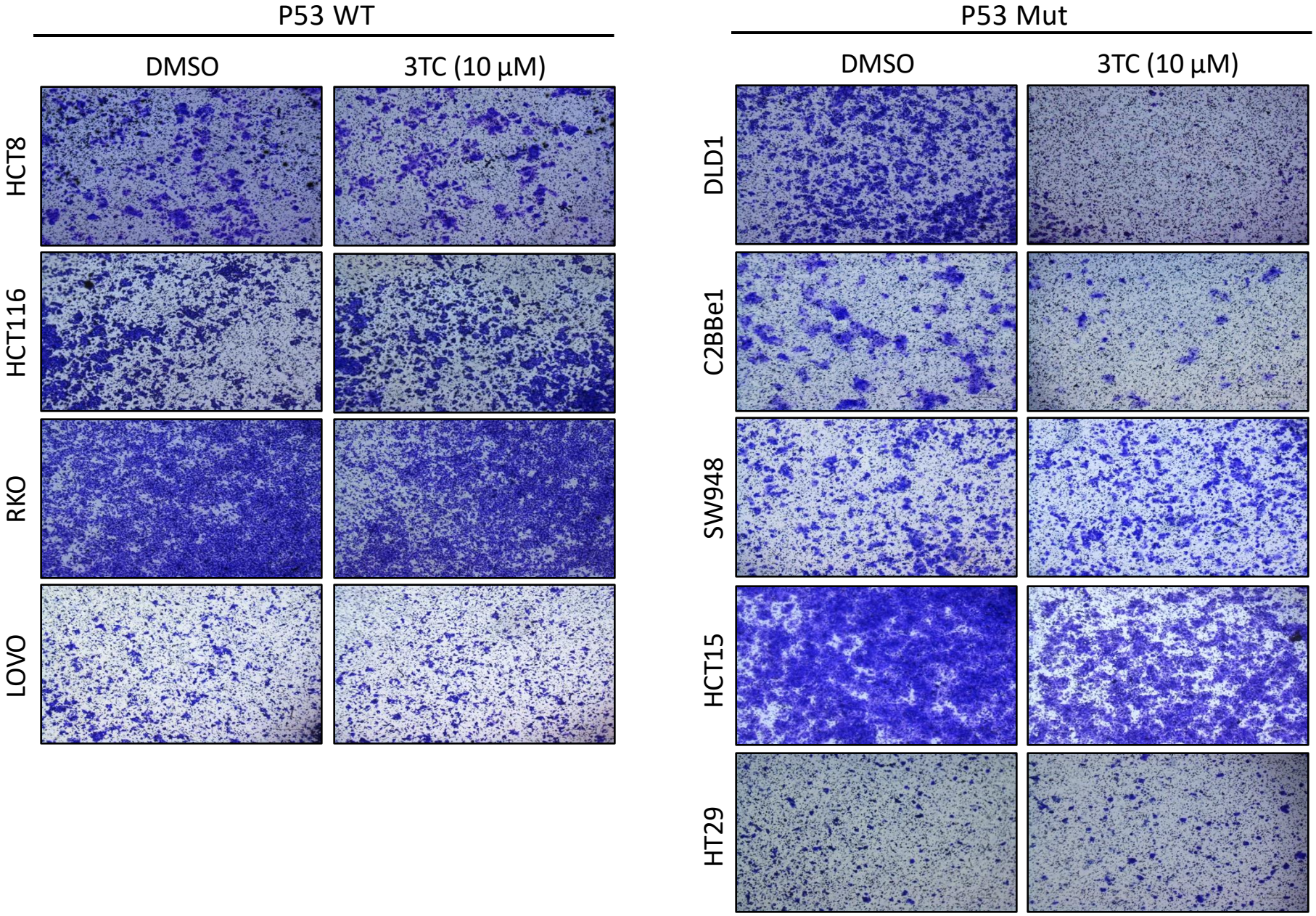




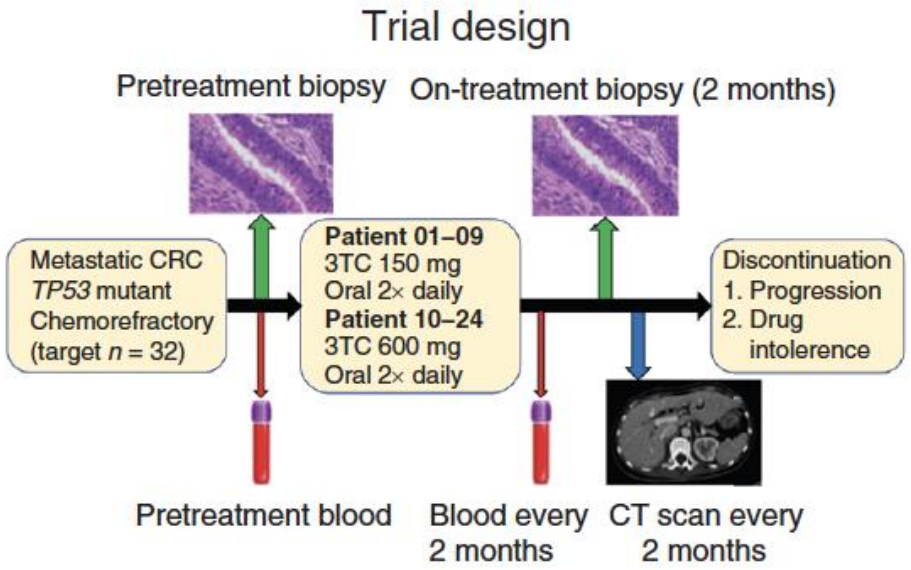
Protumorigenic Repeatome Circuitry of Cancer “Shorted” by NRTI



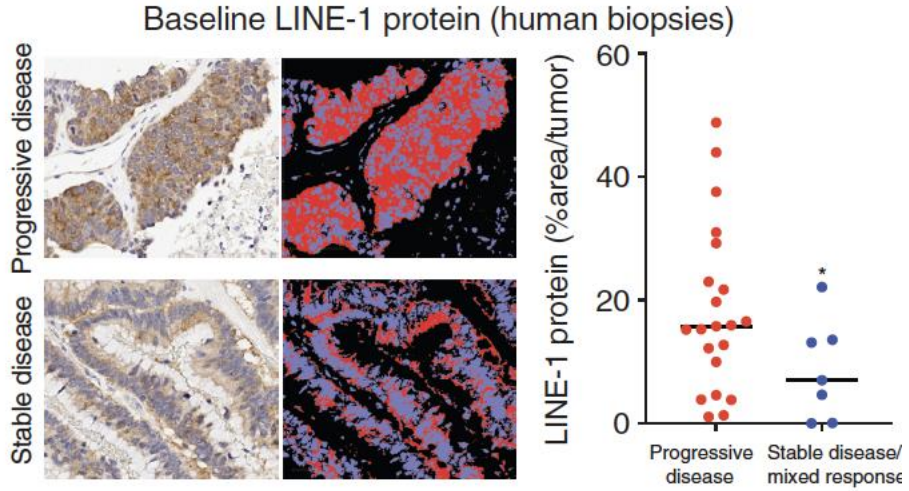
3TC with Significant Effects on Migration in P53 Mutant Cell Lines

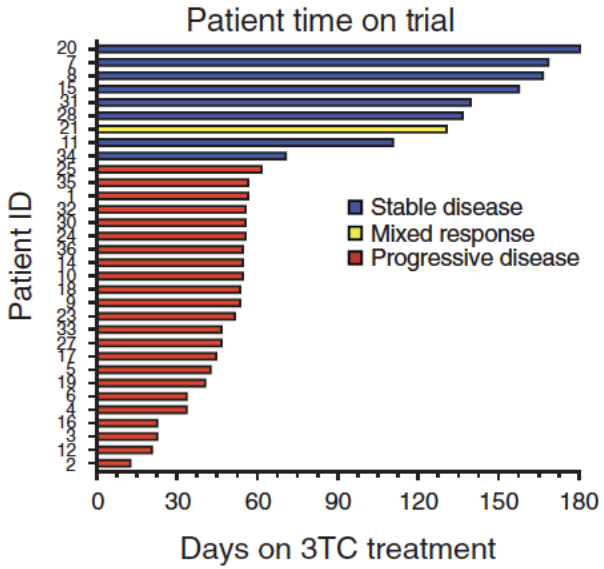


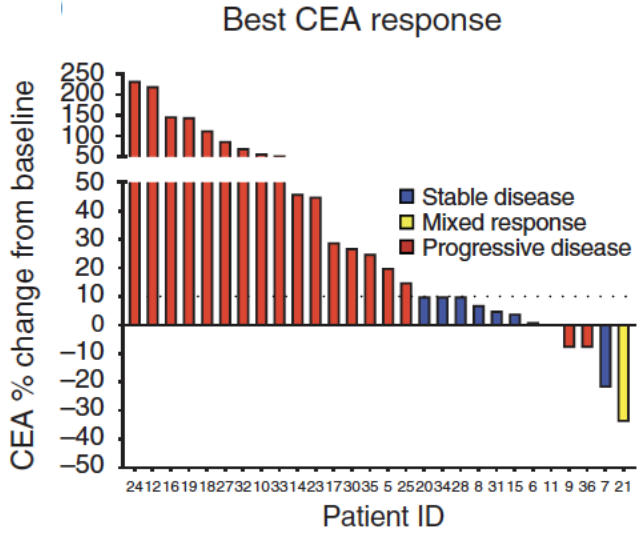
Phase II 3TC NRTI in P53 Mutant Metastatic Colorectal Cancer Disease Stability Correlated with LINE1 Protein Level

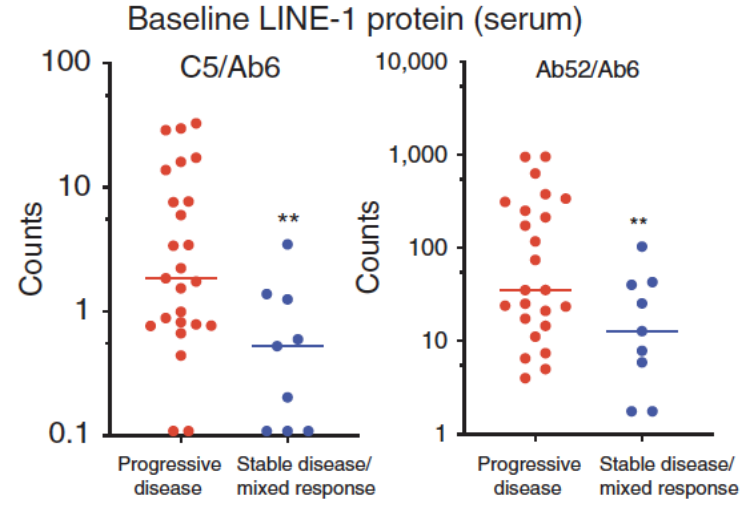


Low LINE1 Protein Correlates with Disease Stability









Rajurkar M, Parikh A, Solovyov A et al, **Cancer Discovery**. 2022

Epithelial Cancer Incidence in HIV Treated Patients are Lower than in General Population

ARTICLE

Risk of Breast, Prostate, and Colorectal Cancer Diagnoses Among HIV-Infected Individuals in the United States

Anna E. Coghill, Eric A. Engels, Maria J. Schymura, Parag Mahale, Meredith S. Shiels

	Overall	
Cancer diagnosis	No.	SIR (95% CI)
Invasive breast cancer	688	0.63 (0.58 to 0.68)
Estrogen receptor positive	305	0.55 (0.49 to 0.61)
Estrogen receptor negative	164	0.68 (0.58 to 0.79)
Prostate cancer	1522	0.48 (0.46 to 0.51)
Proximal colon cancer	269	0.67 (0.59 to 0.75)
Distal colon cancer	173	0.51 (0.43 to 0.59)
Rectal cancer	271	0.69 (0.61 to 0.77)

~50% Lower Incidence of Breast, Prostate, and Colon Cancer

"This set of inverse HIV-cancer associations is therefore unlikely to be due primarily to differential screening and may instead represent biological relationships requiring future investigation."

Coghill AE et al. ***JNCI*** (2018)

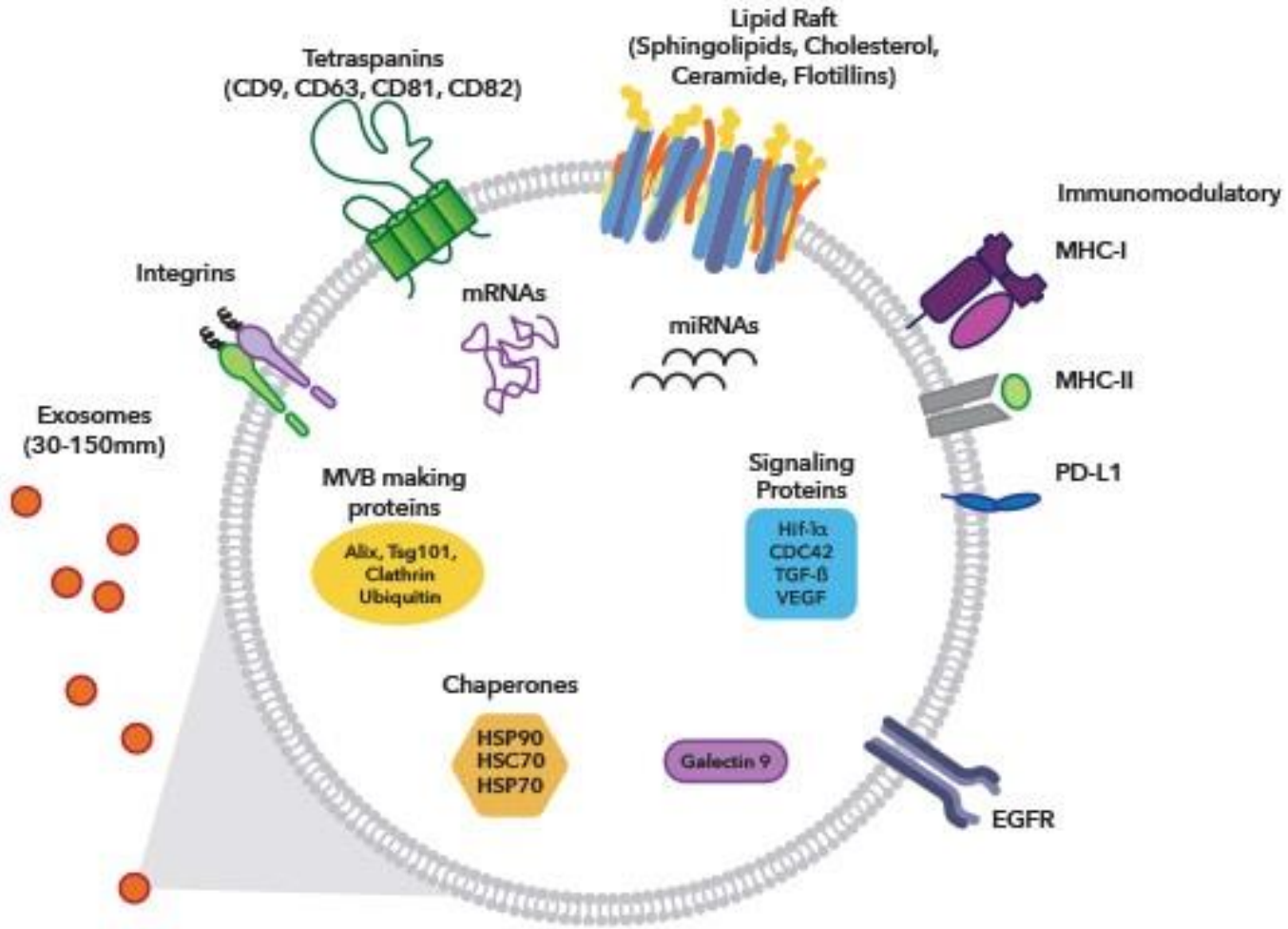
Can Repeats Infect like a Virus?

Eunae You PhD

You E et al. ***Cell*** 2024

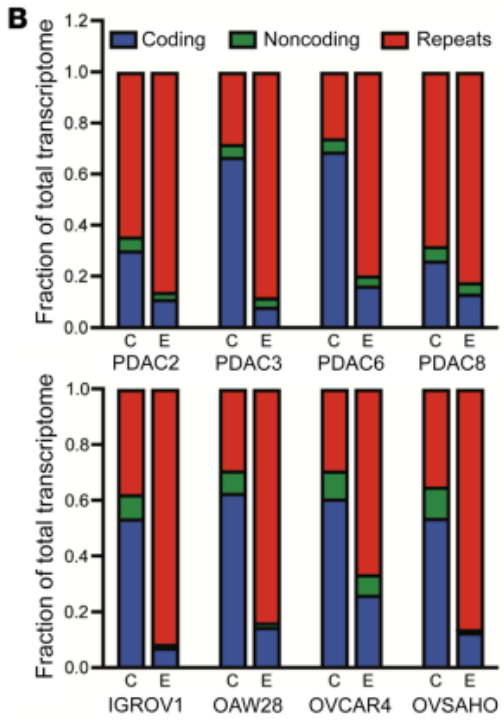
Extracellular Vesicles: The Viral Particle to Transmit Repeat RNAs?

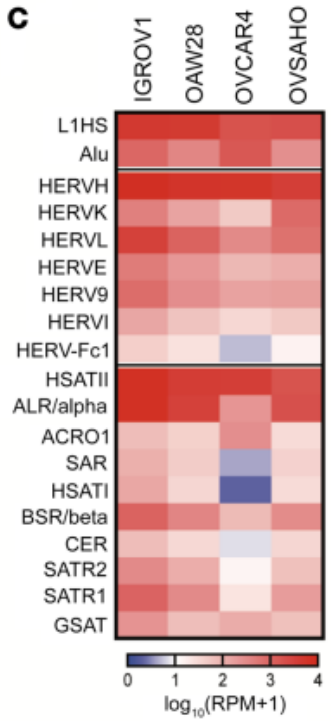
Extracellular vesicles (EVs) are lipid bound vesicles secreted by cells into the extracellular space.

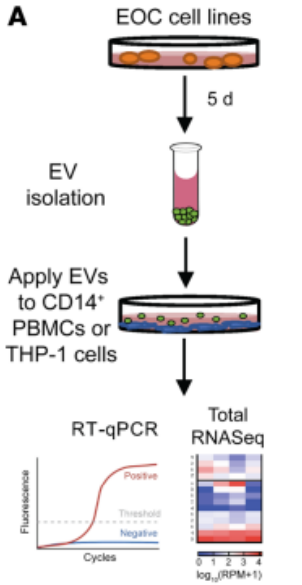


Karpman et al., (2017) **Nature Reviews Nephrology**

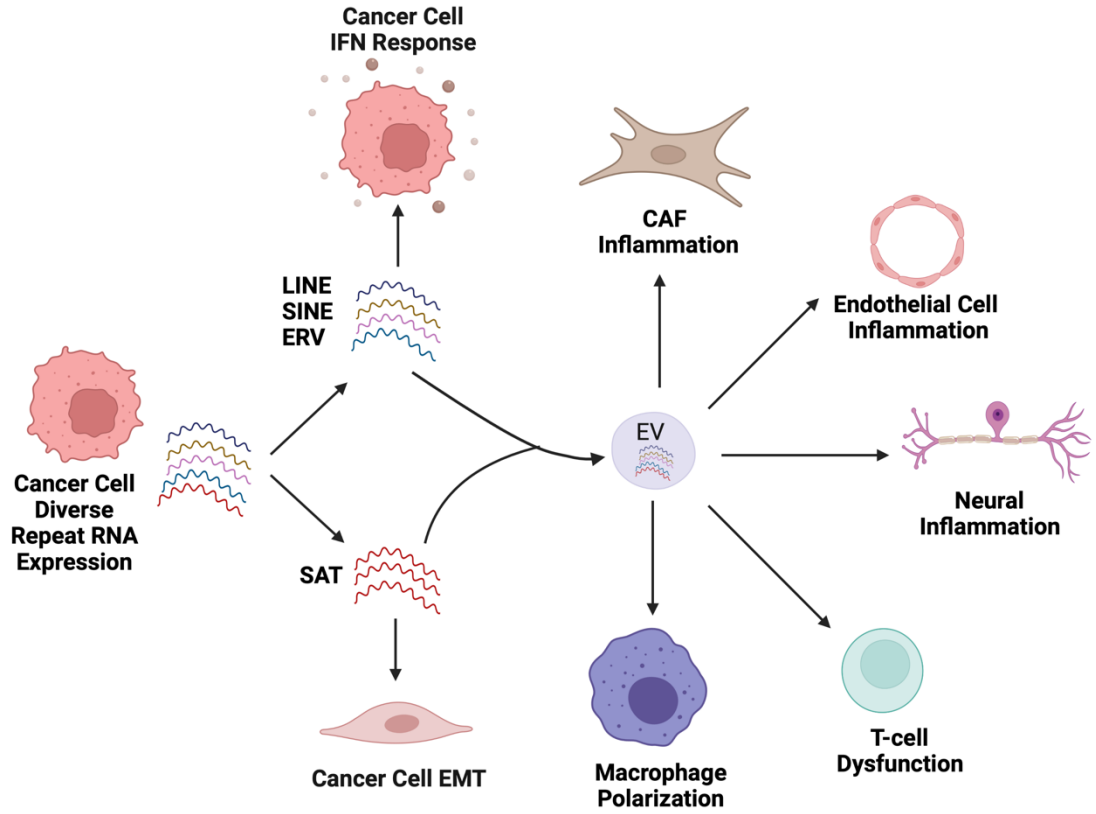
Repeat RNAs Enriched in Extracellular Vesicles as a Mechanism of Altering Immune Microenvironment







PBMC : peripheral blood mononuclear cell



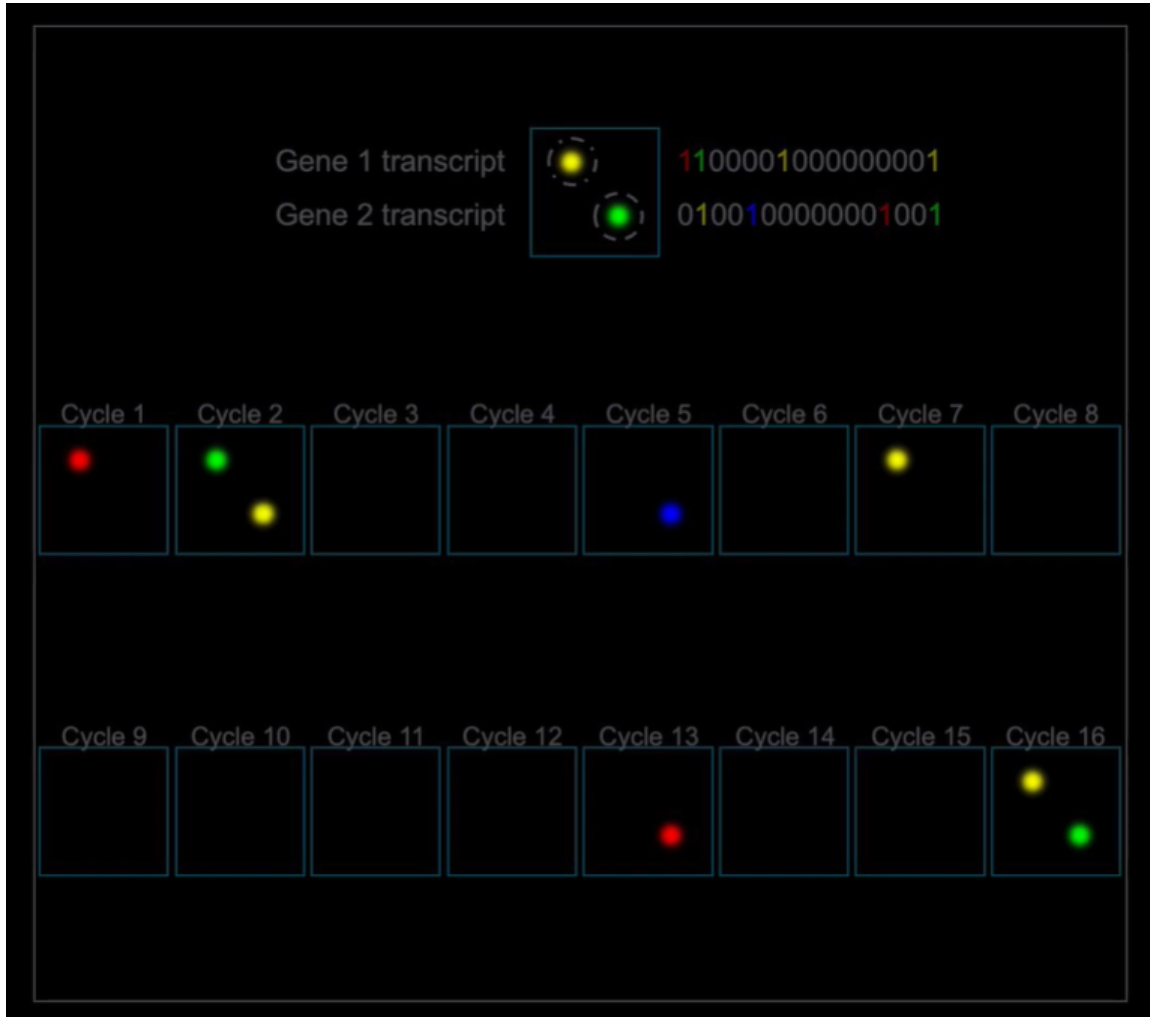
C : Cell

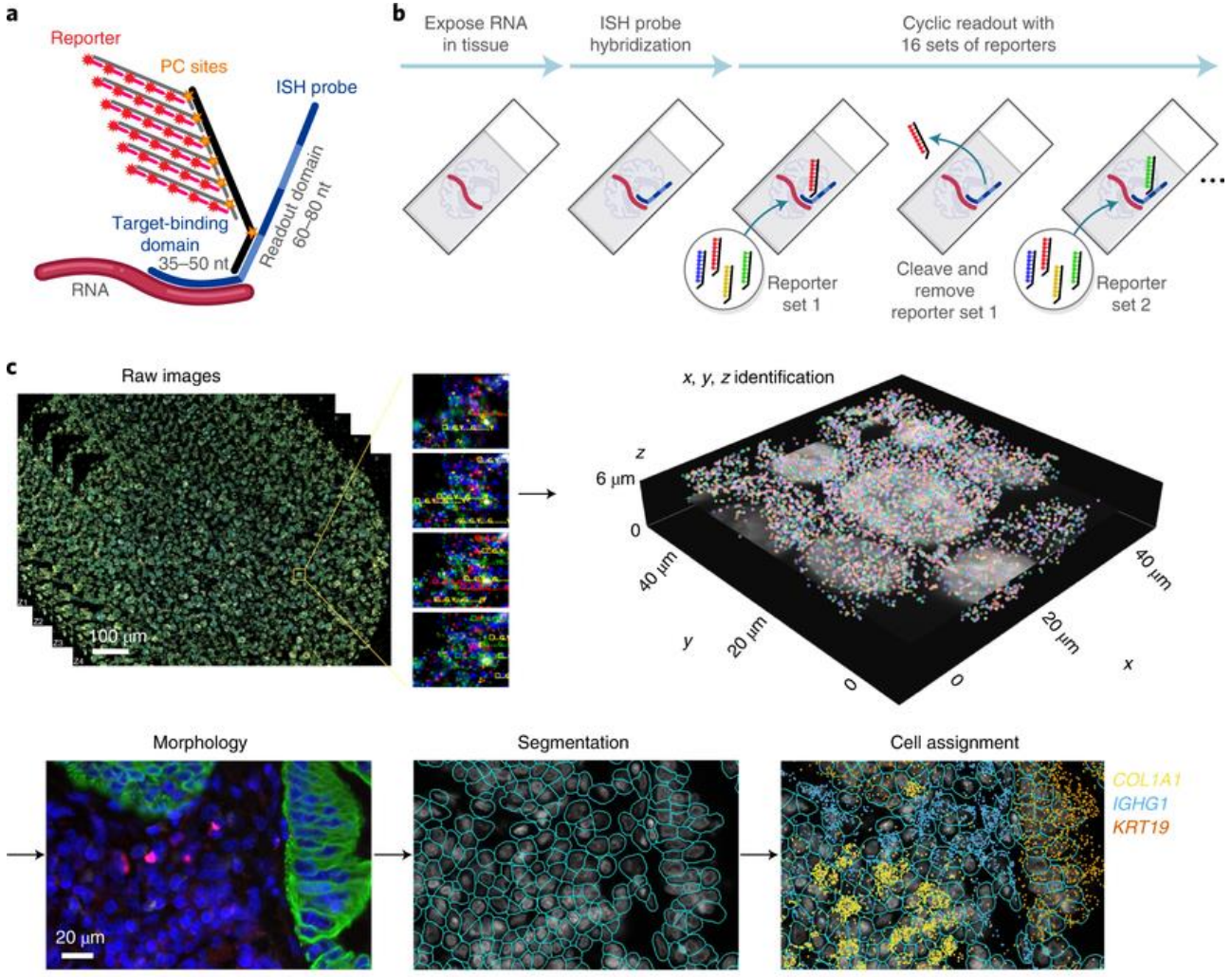
E : Extracellular vesicle

Extracellular Vesicle repeat RNA might have a potential function to modulate stromal microenvironment.

Porter et al., **J Clin Invest**, 2022

Single Cell Molecular Spatial Profiling: Nanostring CosMx

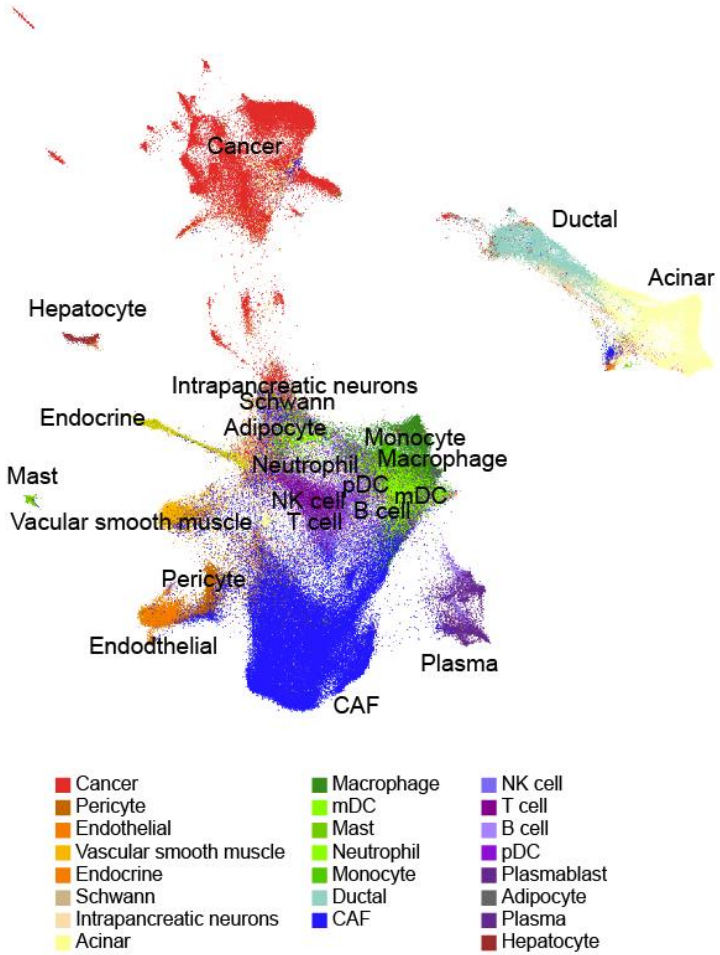


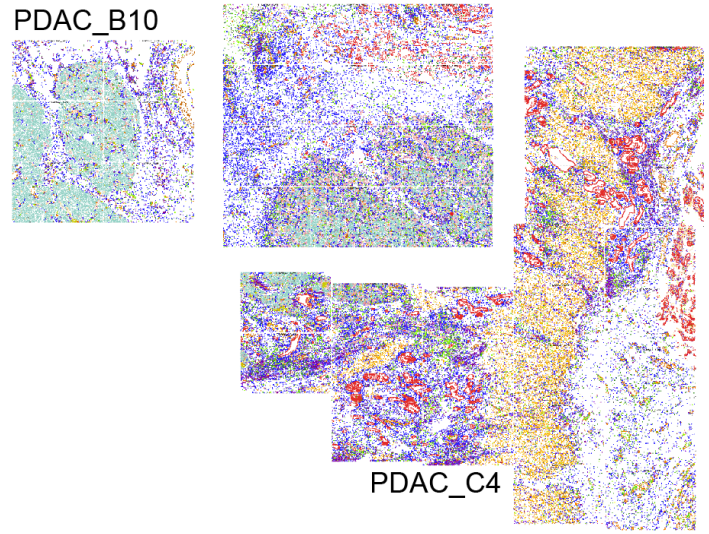


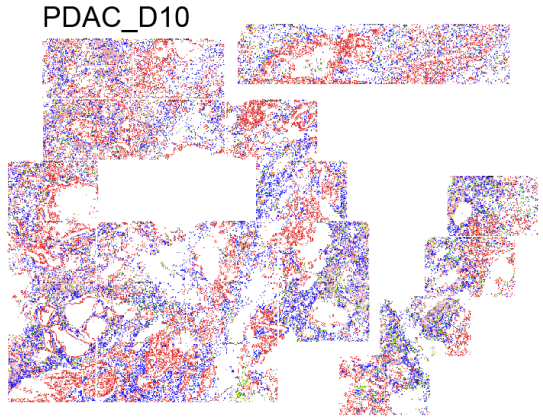
He et al., (2021) **Nature Biotechnology**

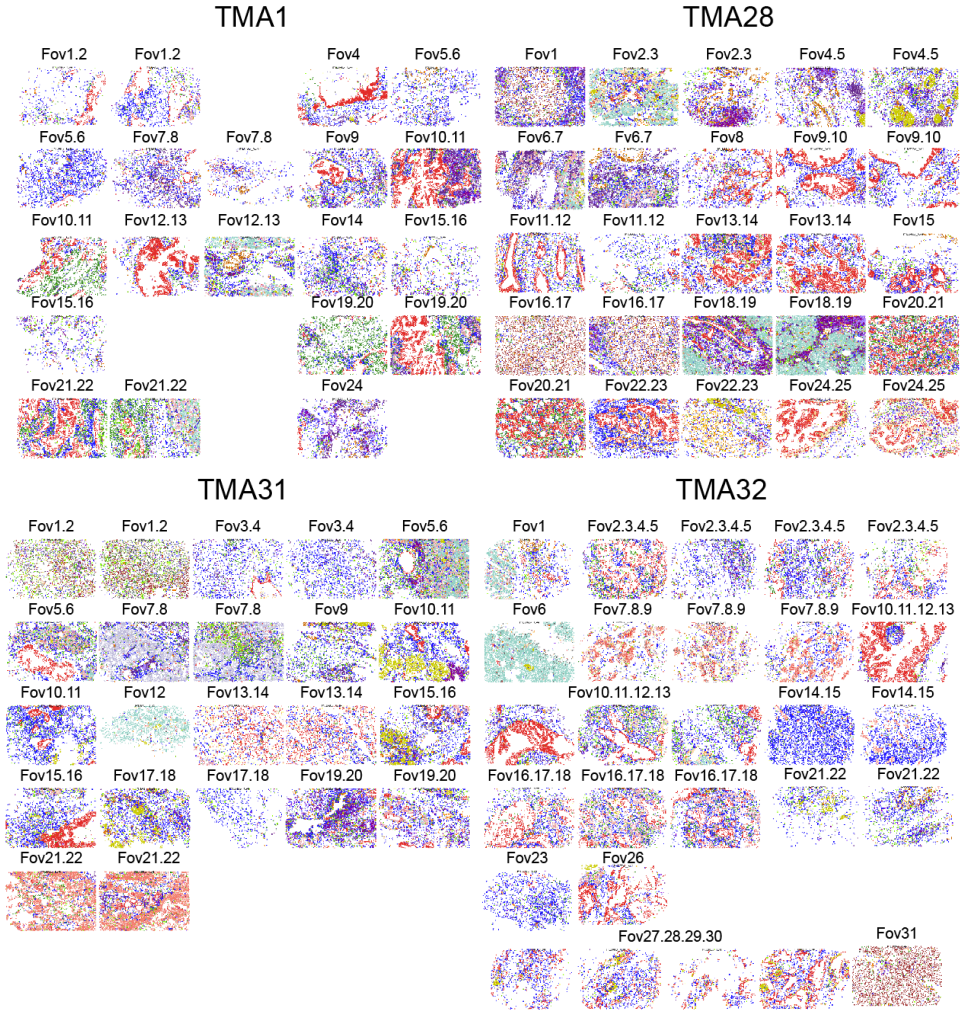
Building the Google Map of Pancreatic Cancer

Total Cell # : 418,224







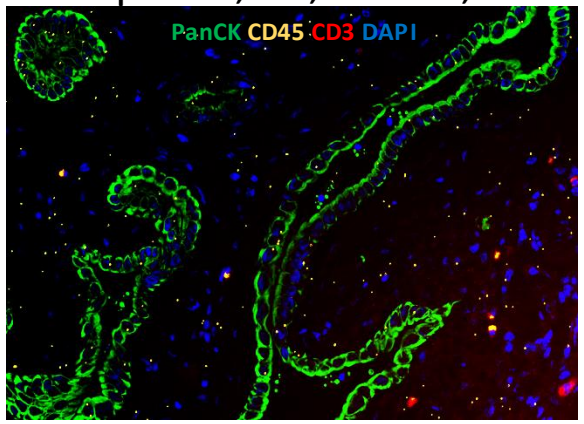


Custom Repeat RNA probes HSATII, LINE1, HERVK, HERVH

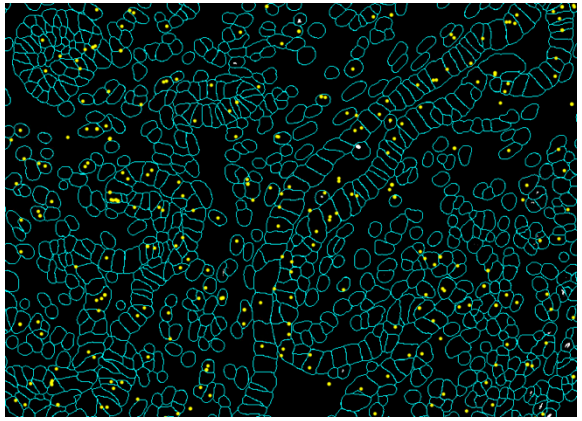
Patrick Danaher (Nanostring)

Spatially-resolved Repeat RNAs in the PDAC Tumor Microenvironment

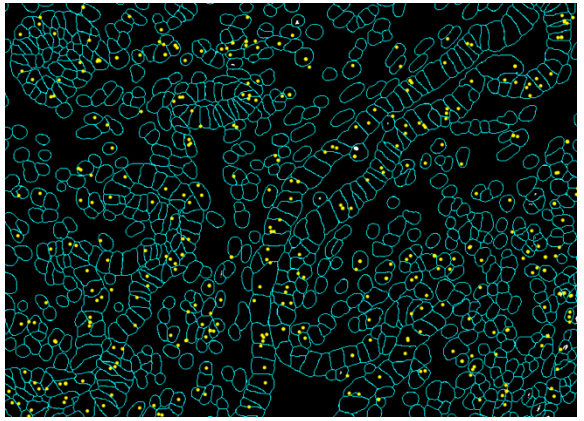
IF composite, C4, FOV18, zoom



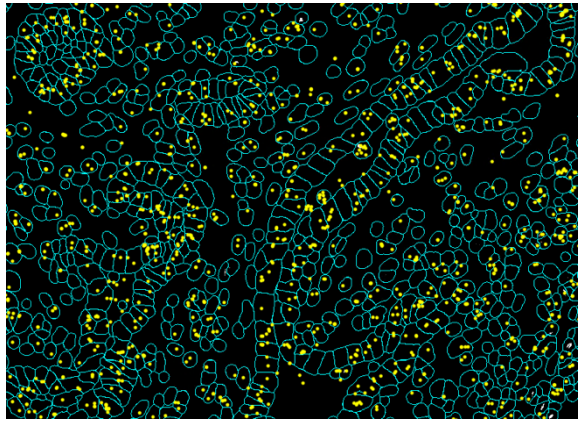
HERV-H



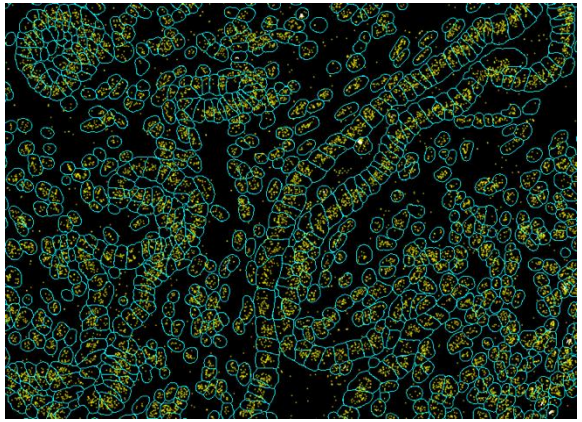
HERV-K



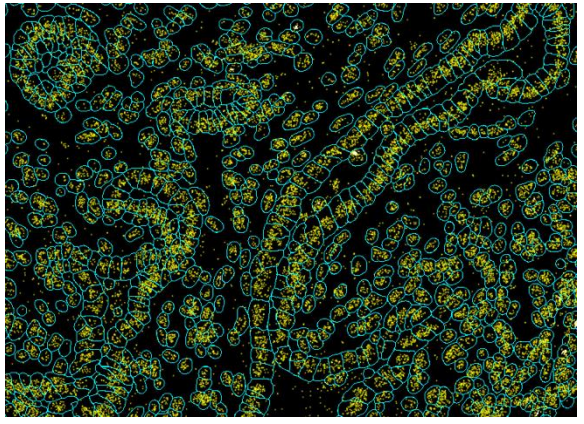
HSATII

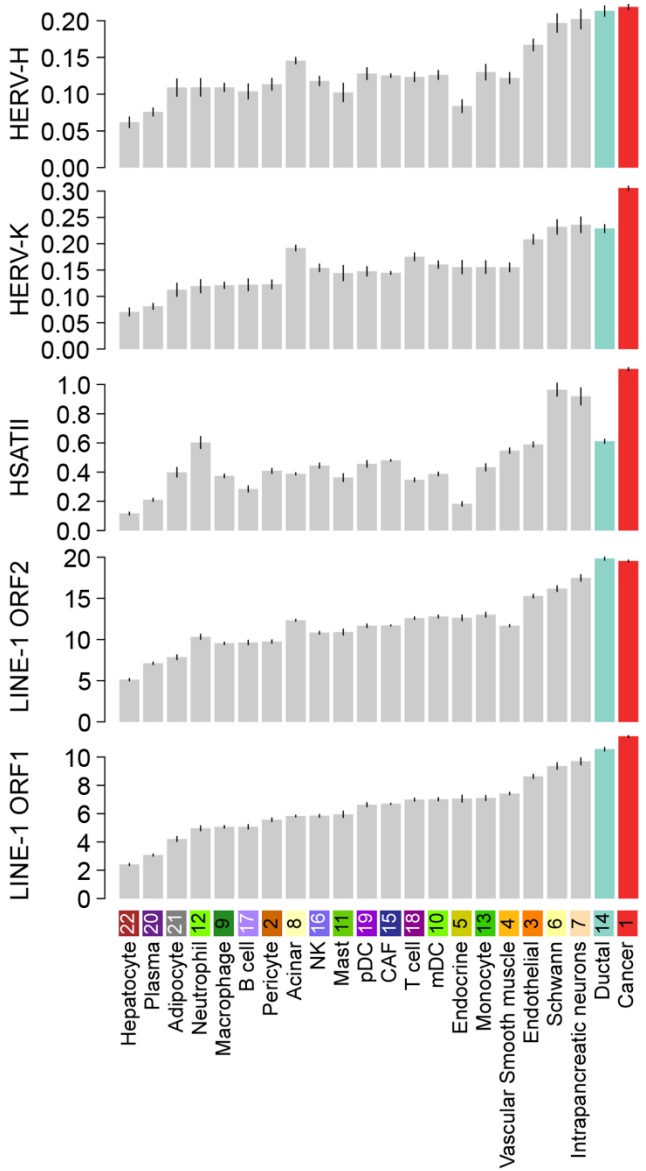


LINE-1 ORF1



LINE-1 ORF2

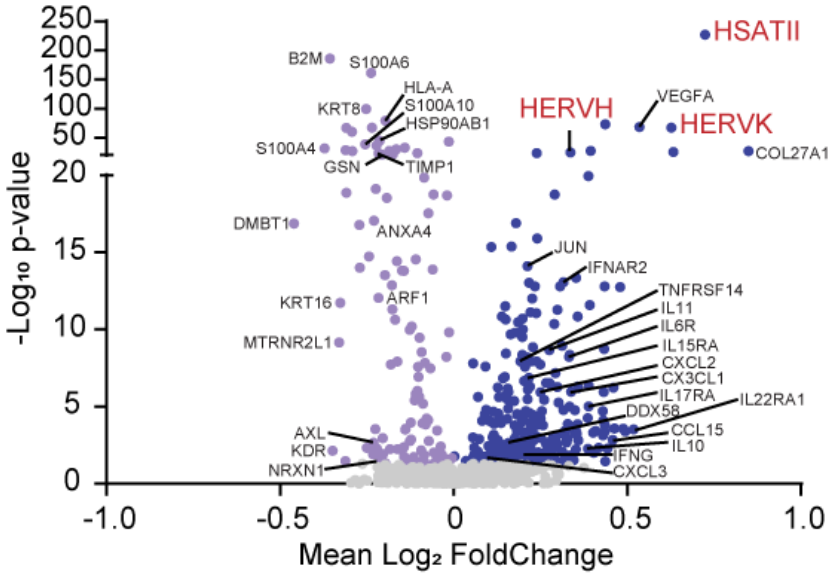




Repeats Highest in Cancer and Preneoplastic Cells

Repeat RNA Expression Anti-correlated with Epithelial Keratin Gene Expression

PDAC Cancer Cells

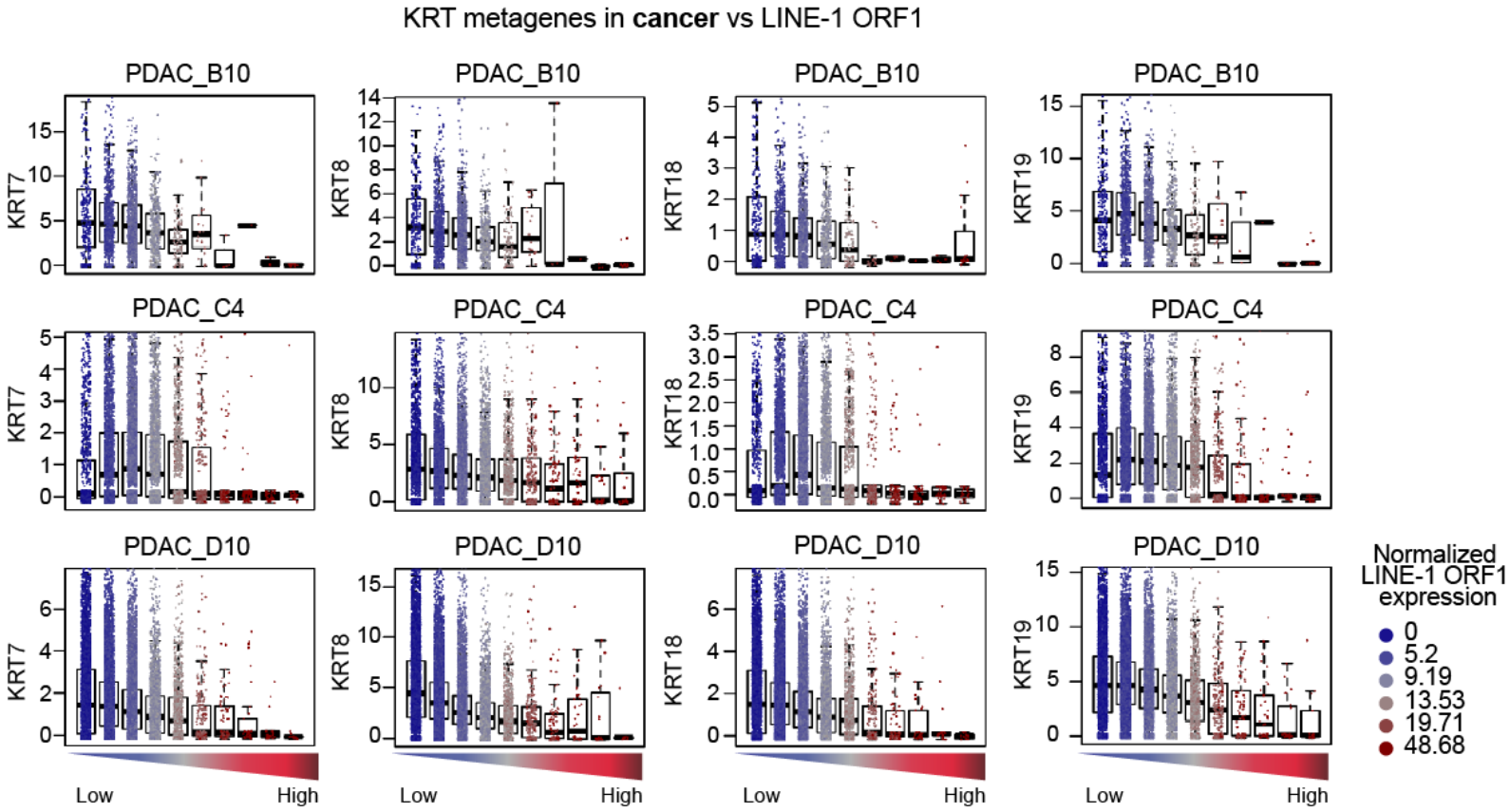


Low LINE1

KRT8
KRT18

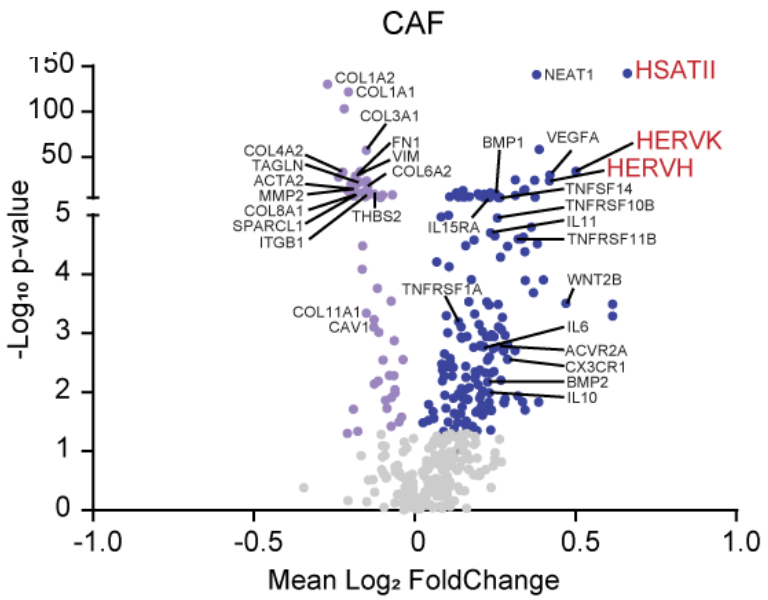
High LINE1

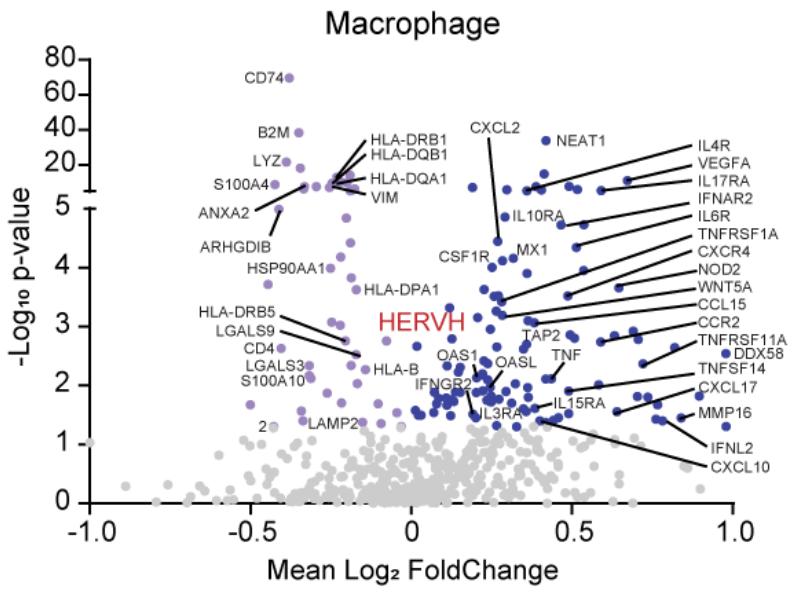
IFN Response genes

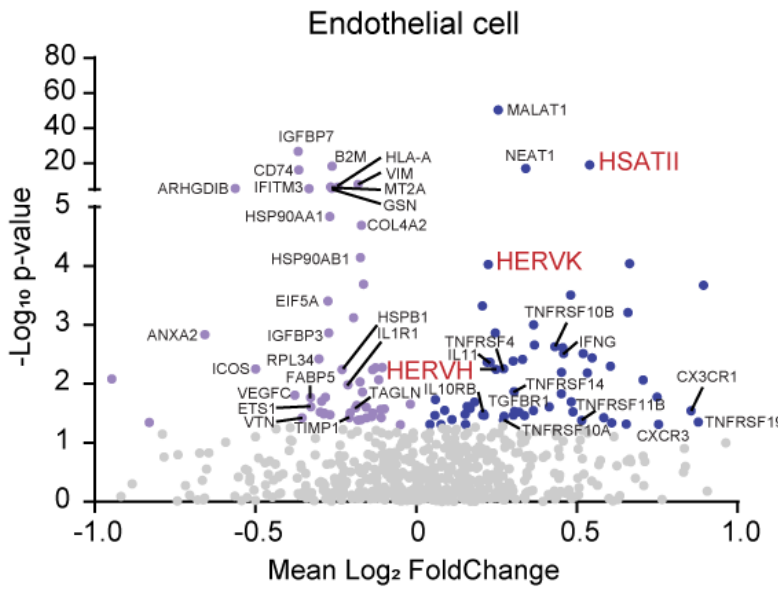


Patrick Danaher (Nanostring)

Multiple Cell Types in Tumor Microenvironment with Repeat RNA Expression Linked with IFN Response







Low LINE1 High LINE1

IFN Response genes

Low LINE1 High LINE1

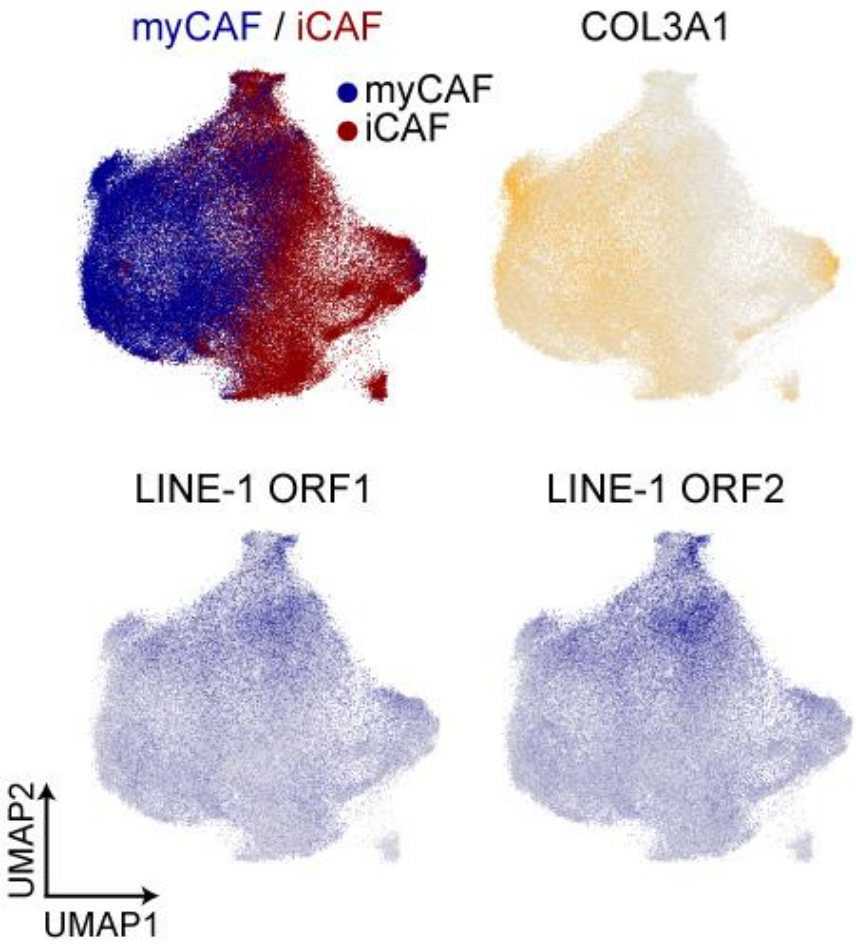
IFN Response genes

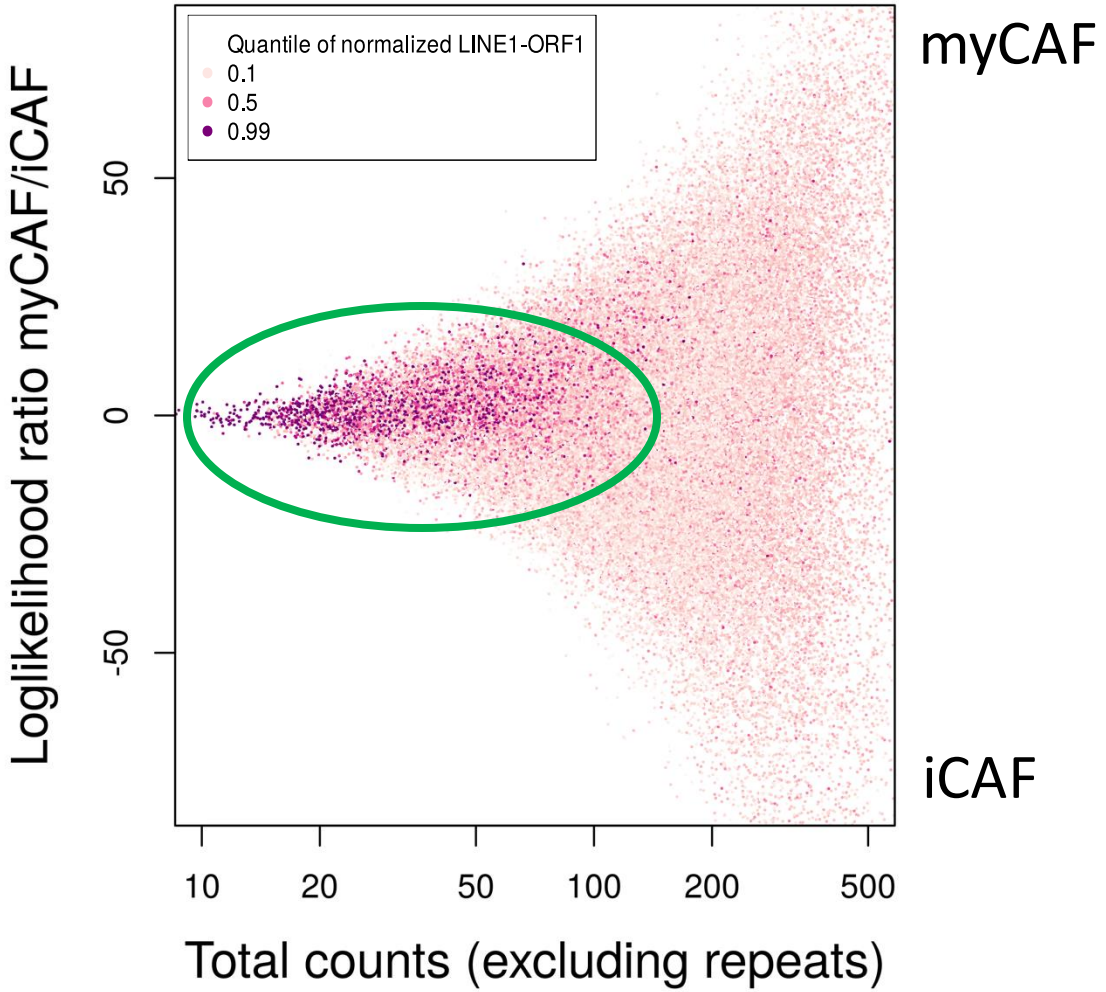
Low LINE1 High LINE1

IFN Response genes

High Repeat RNAs found in CAFs enriched in myCAF/iCAF intermediate state

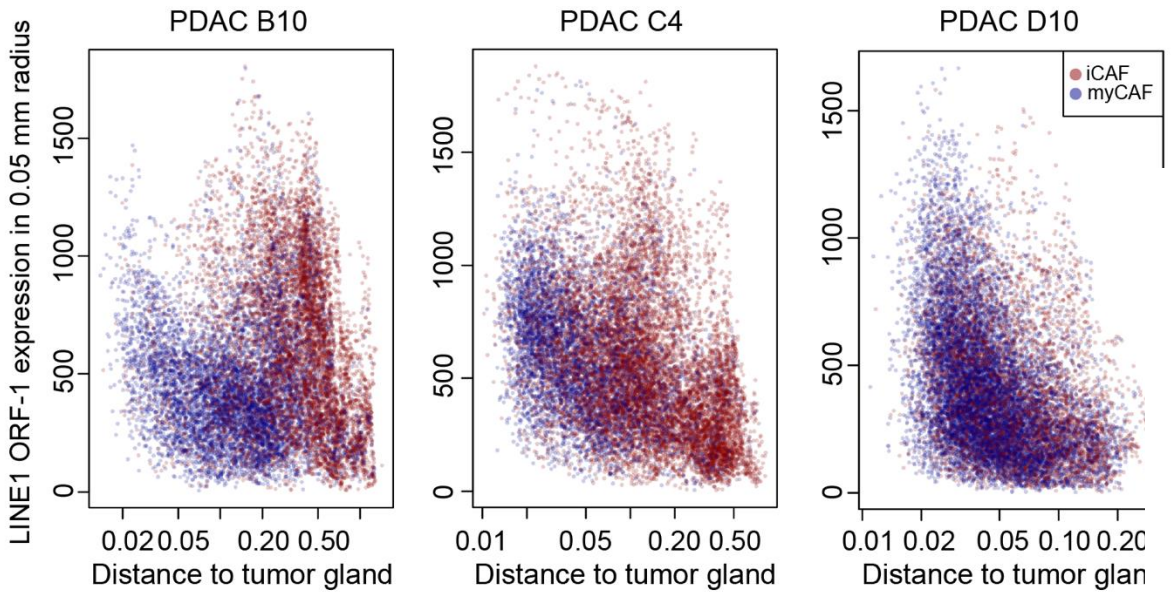
Myofibroblastic CAF = myCAF
Inflammatory CAF = iCAF

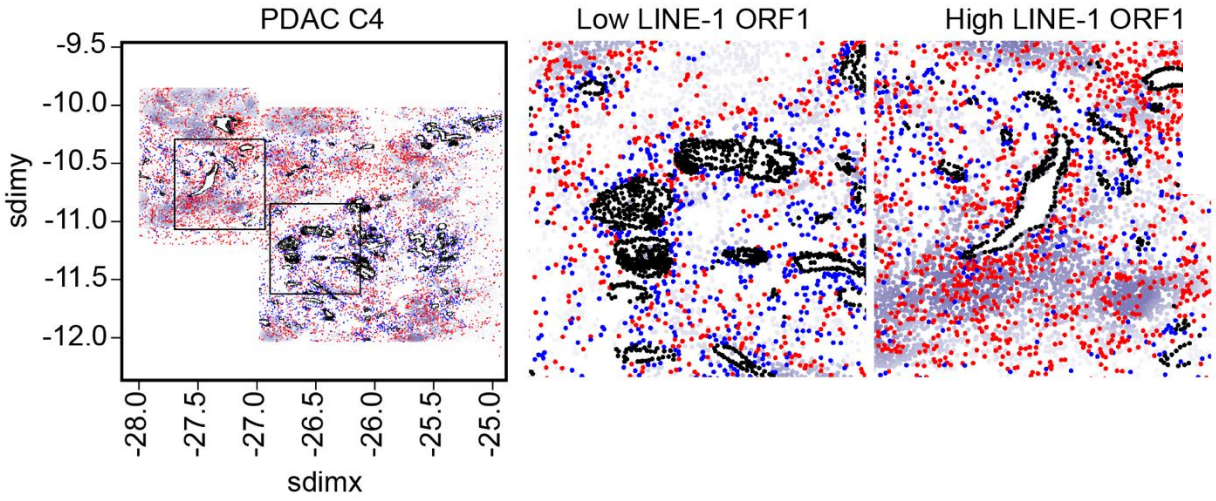




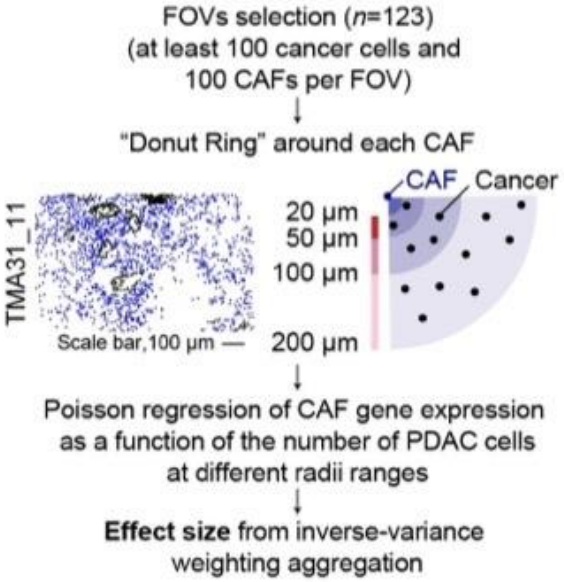
Relationship between LINE-1 ORF1 and CAF spatial distribution

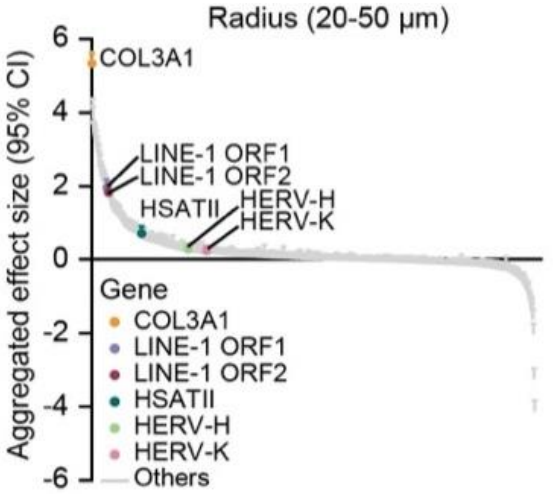
myCAFs with high LINE1 enriched close to Cancer Cells



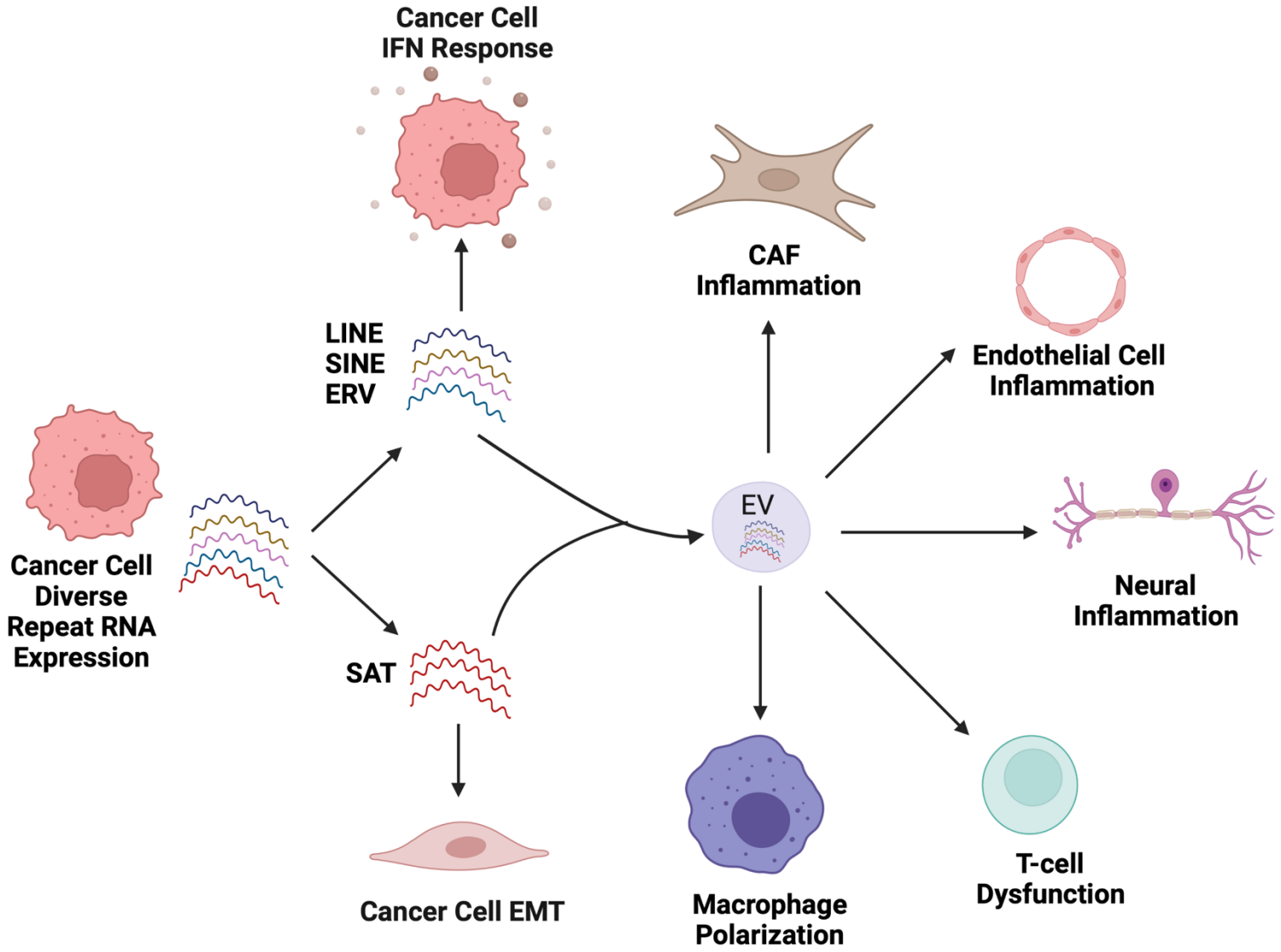


Relationship of CAF gene expression as a function of distance from Cancer Cells

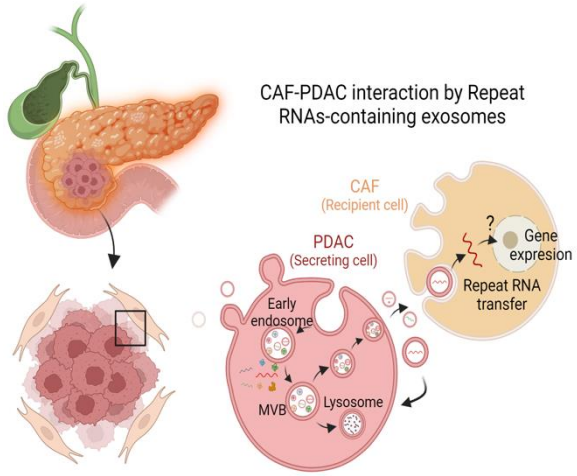


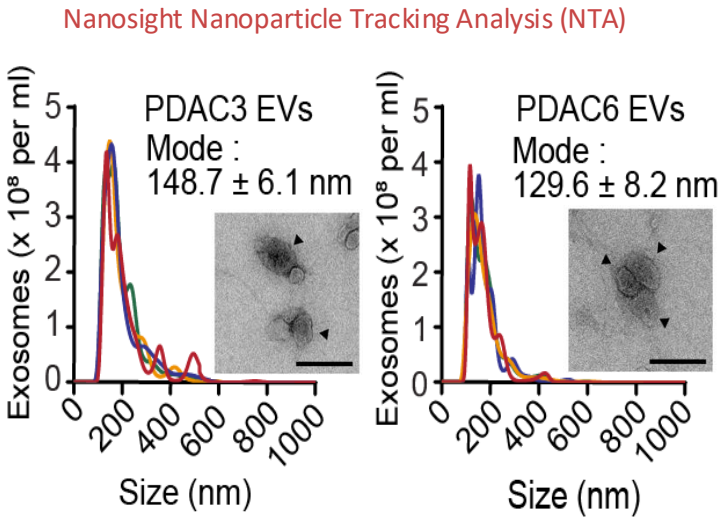


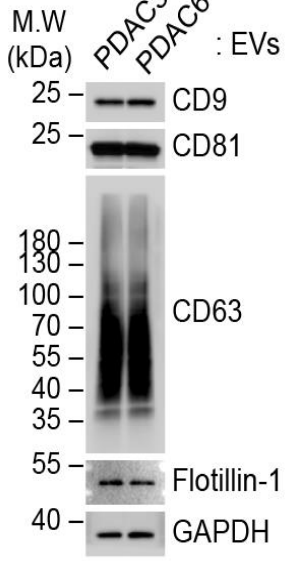
Could EV “Infection” Explain Spatial Distribution of Repeats

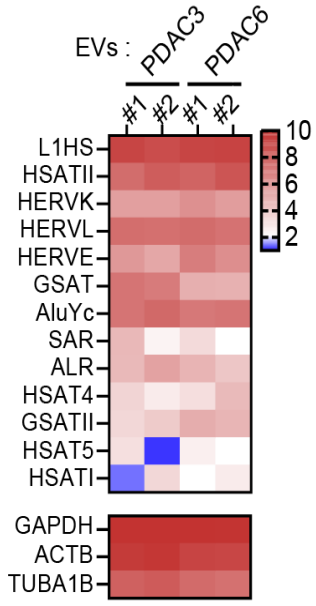


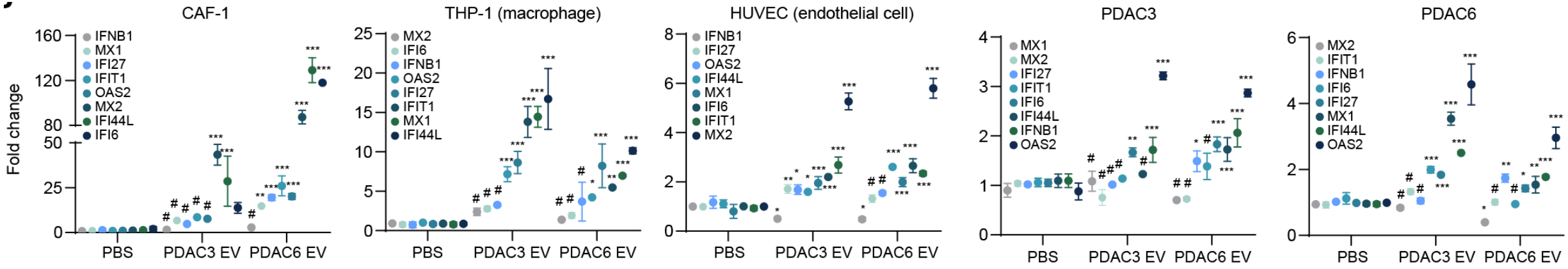
PDAC EVs with High Repeat RNA Content Induce IFN Response Across Cell Types





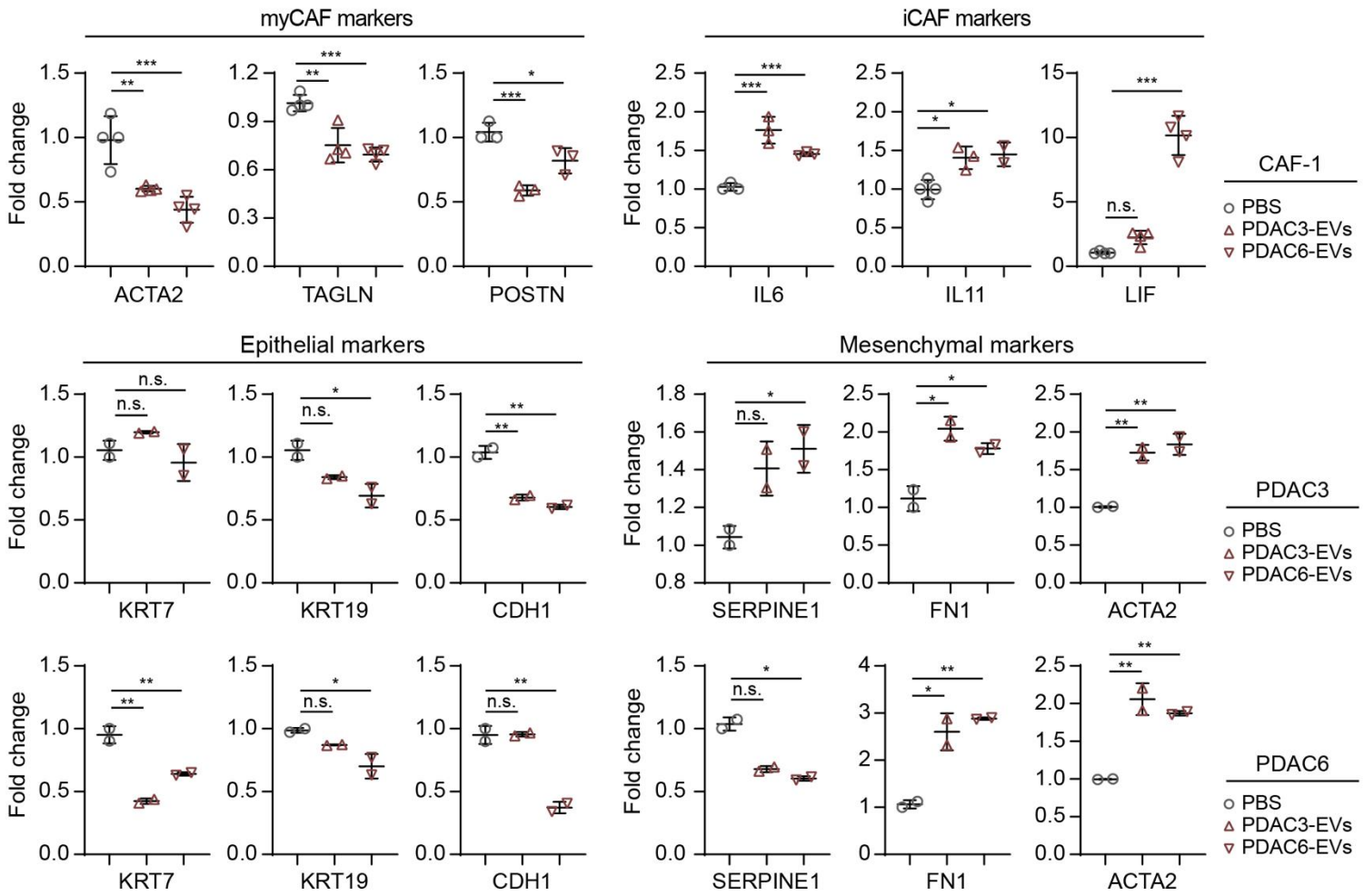




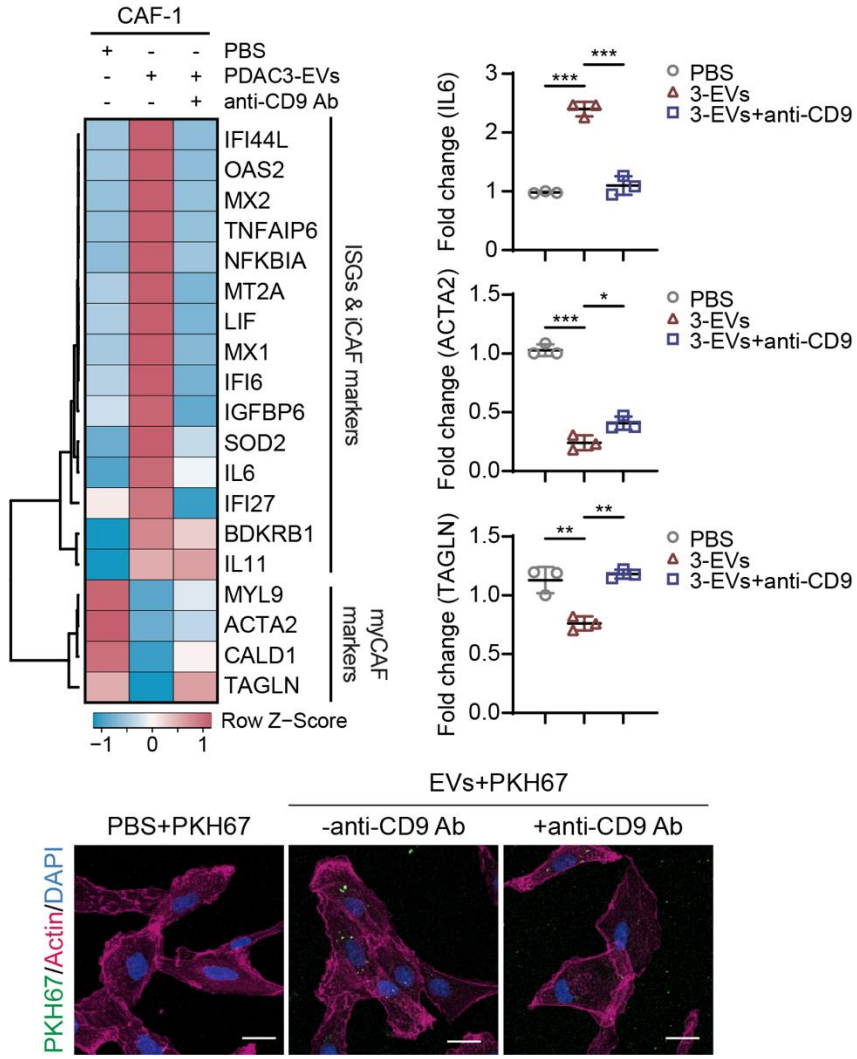


Divergence in Mesenchymal Gene Expression between PDAC and CAF cells in Response to EVs

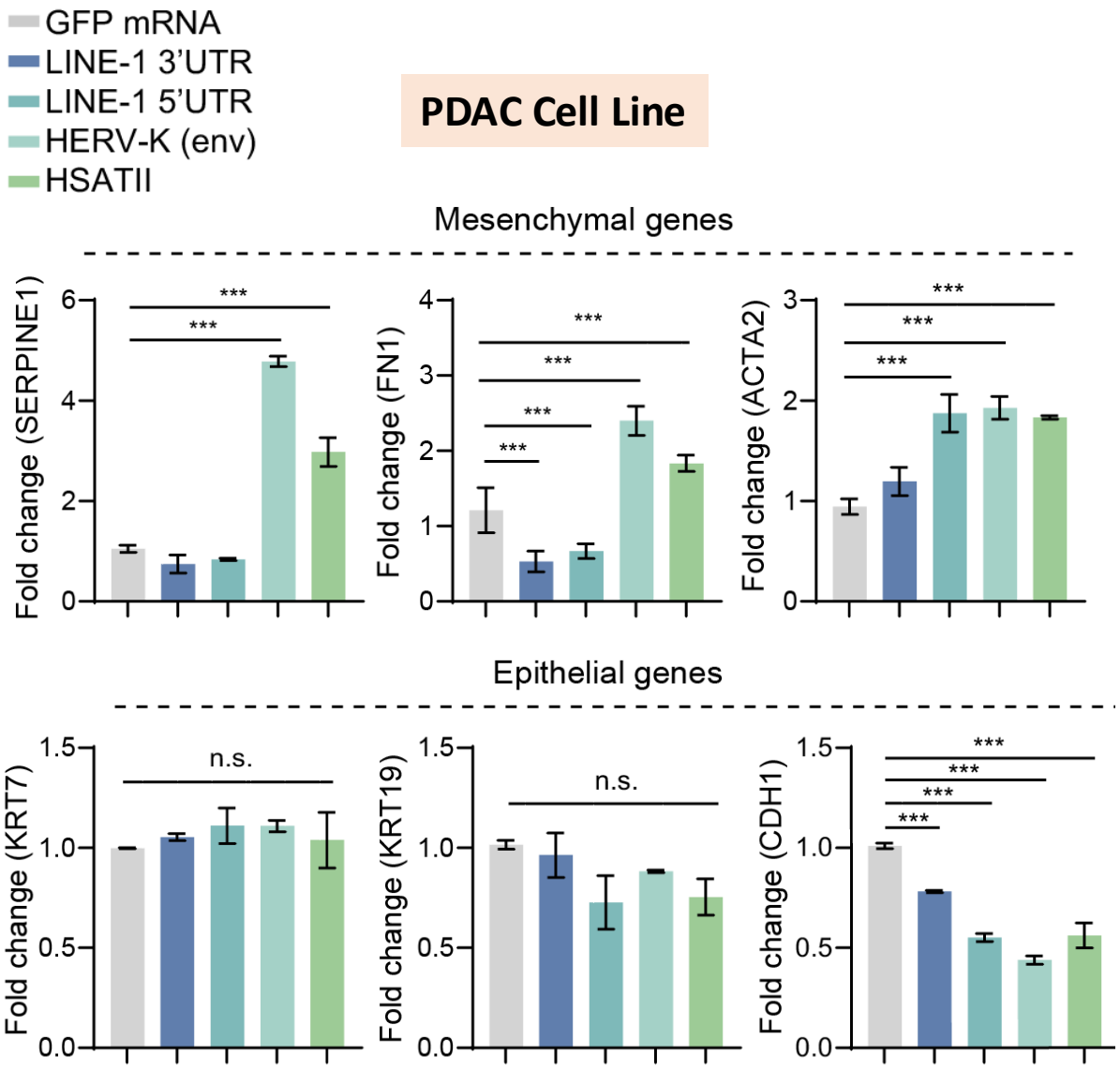
PDAC-derived EVs induce CAF loss (myCAF->iCAF) and PDAC gain (E->M) of mesenchymal genes

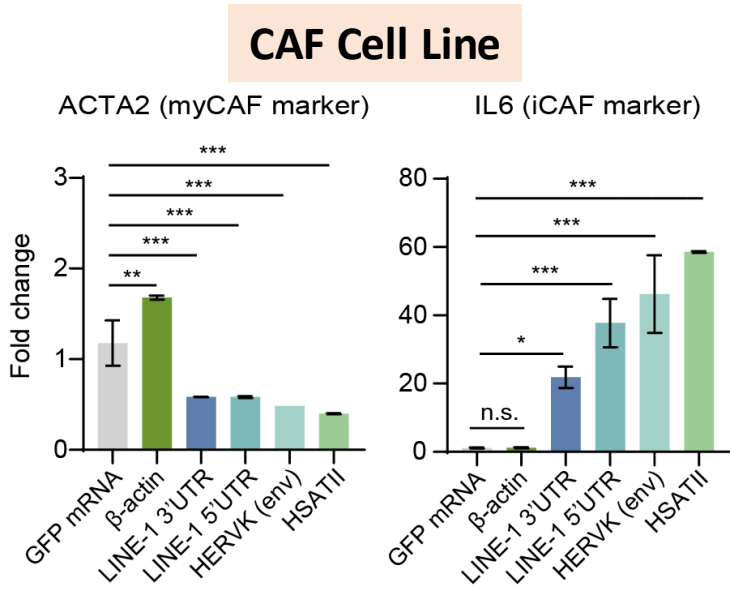


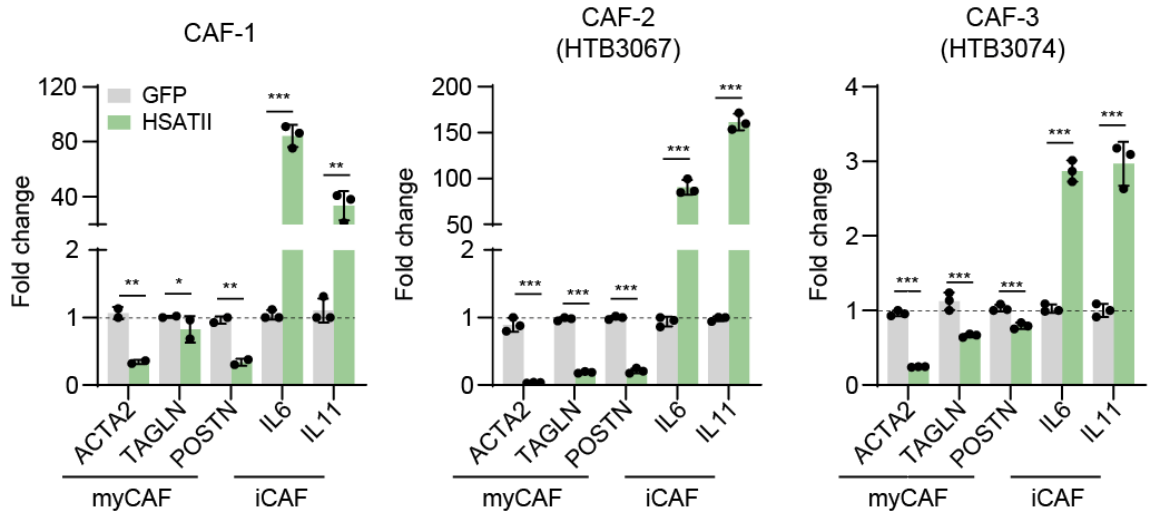
Anti-CD9 Ab (EV marker) Blocks EV induced changes in CAFs



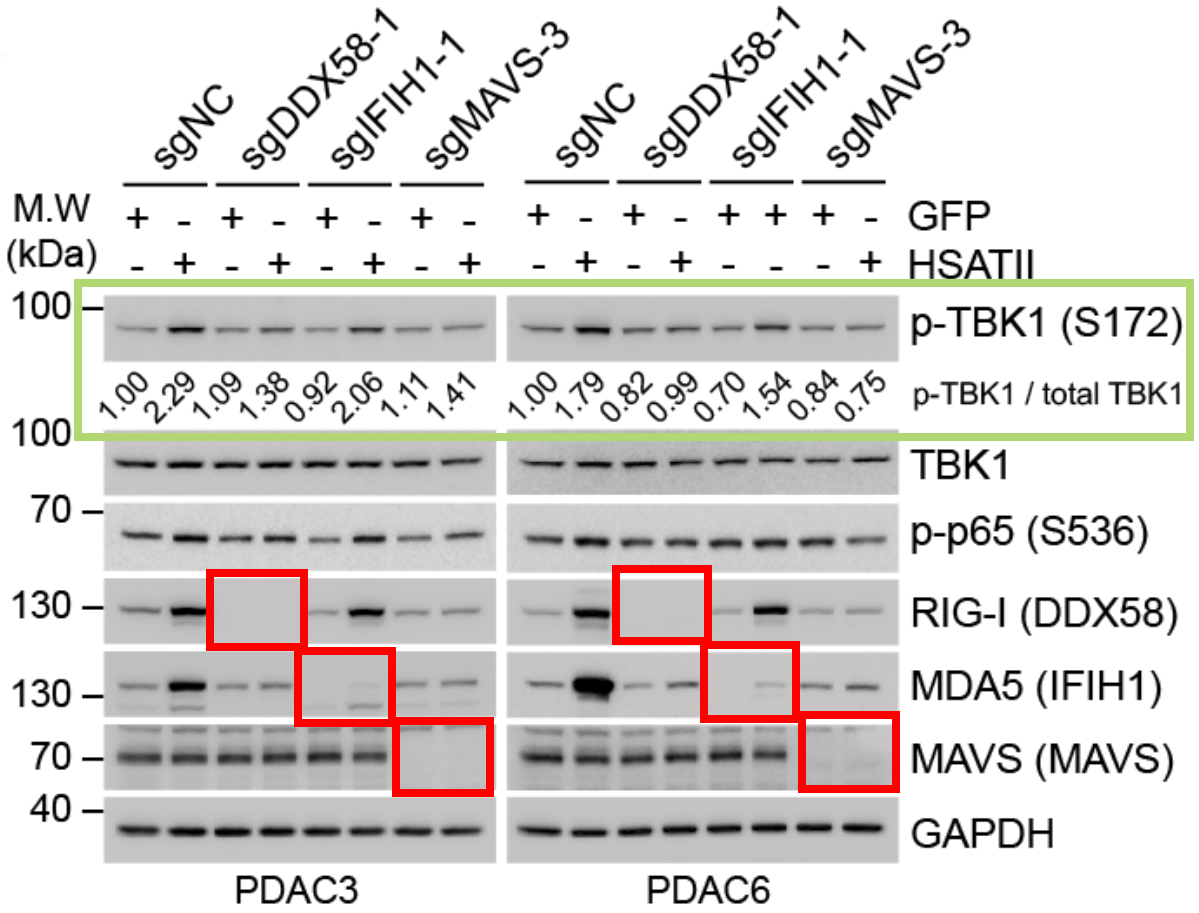
Repeat RNA Transfection Sufficient to Induce Mesenchymal Gene Changes

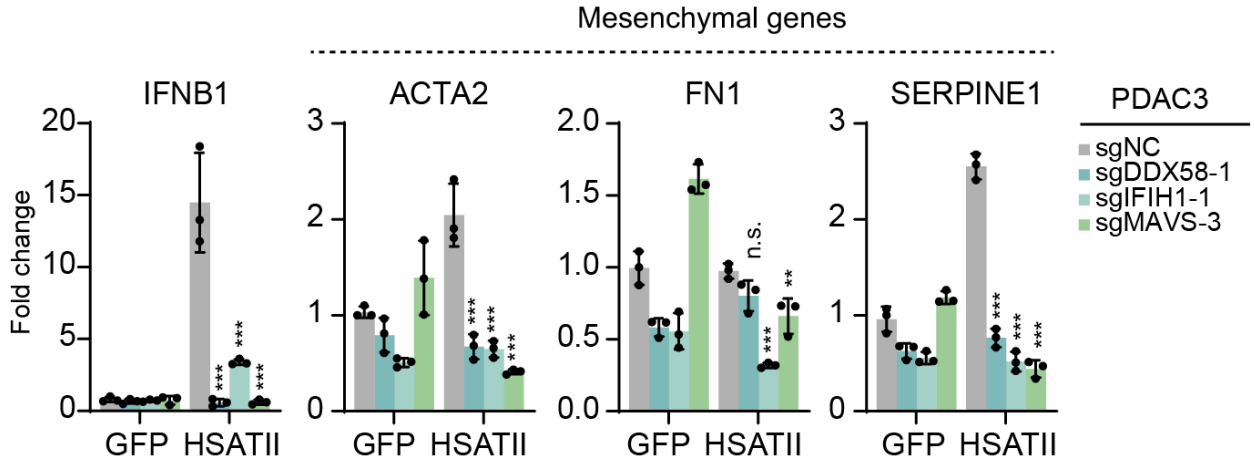


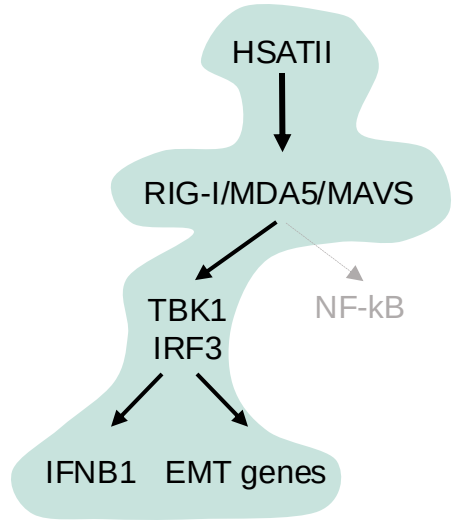




HSATII RNA sensing pathways in PDAC cells Driven by RIG-I/MDA5/MAVS

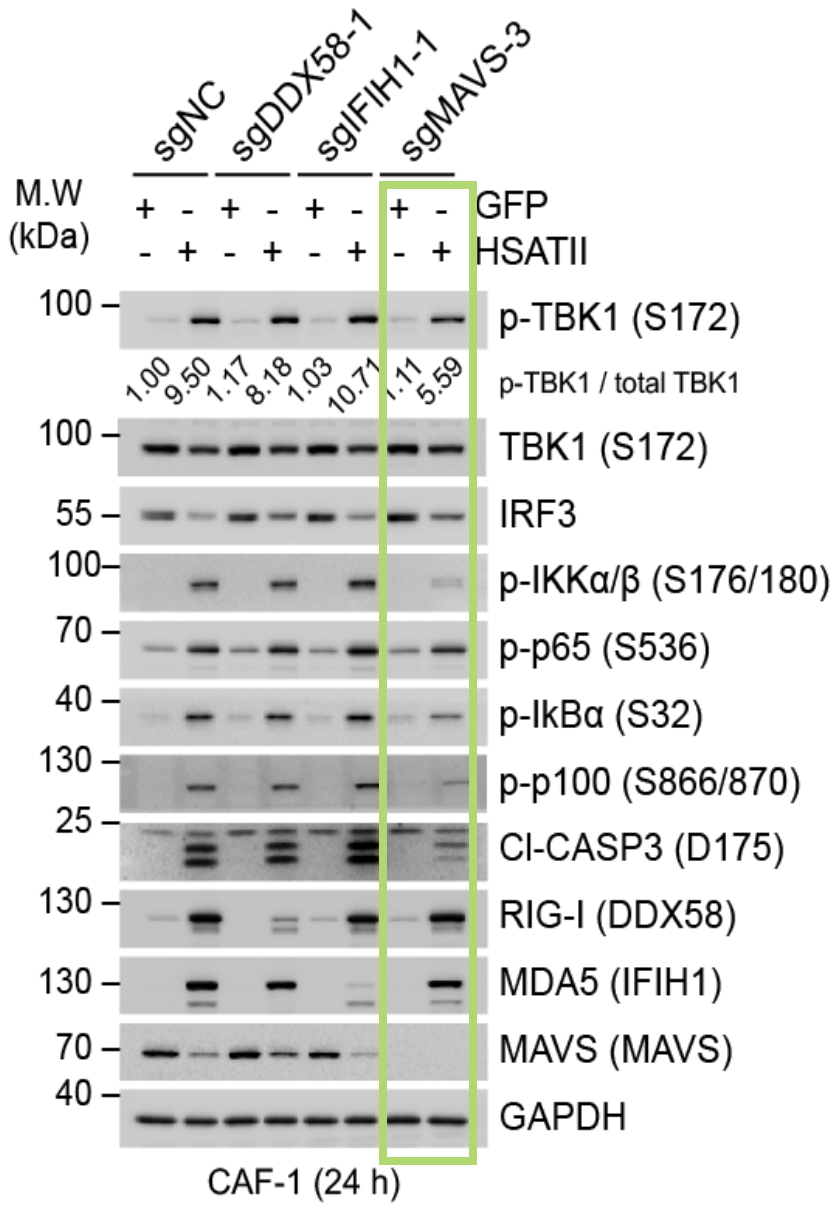


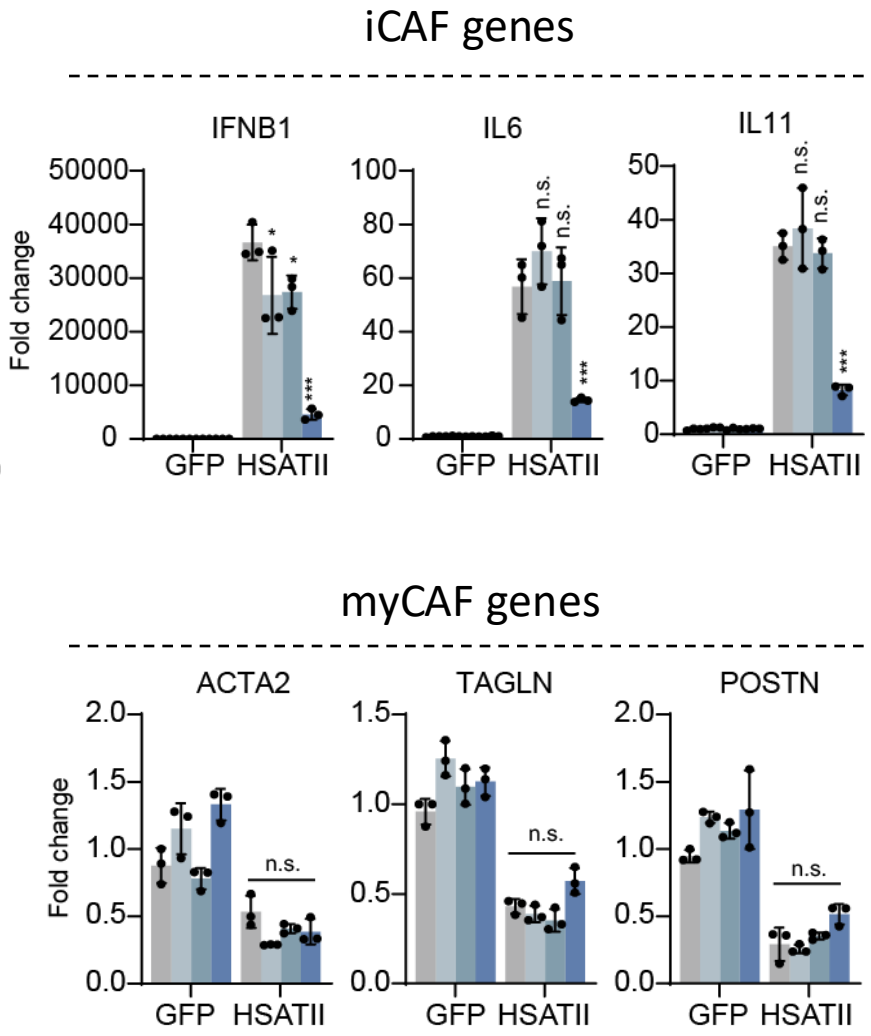


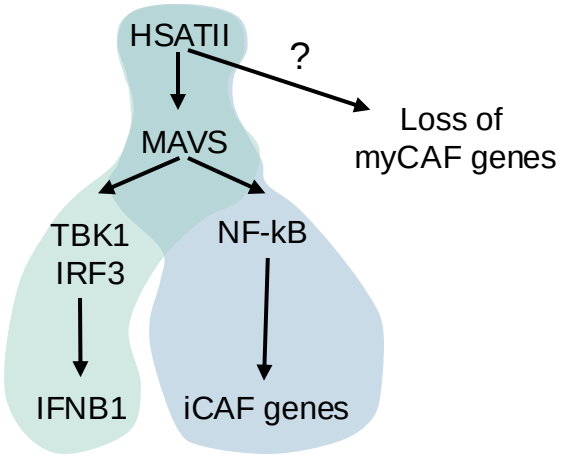


Induction of EMT genes are regulated by RLRs/TBK1/IRF3 pathway in PDAC cell lines

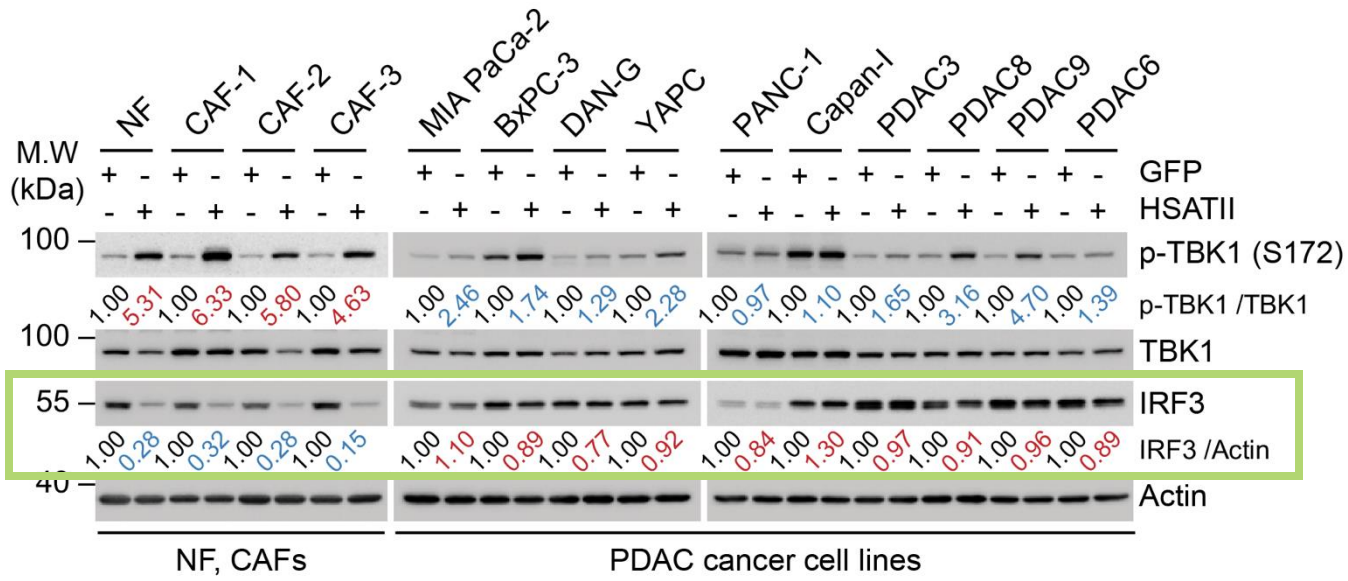
HSATII RNA sensing pathways in CAF cells Driven by MAVS

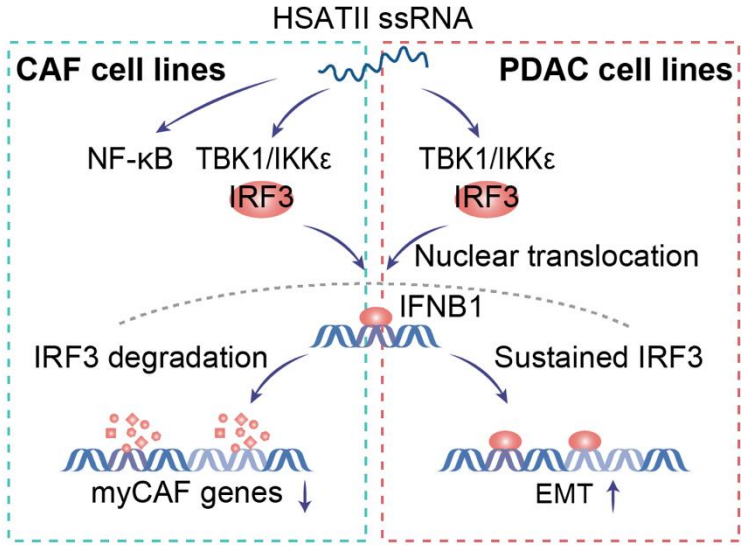


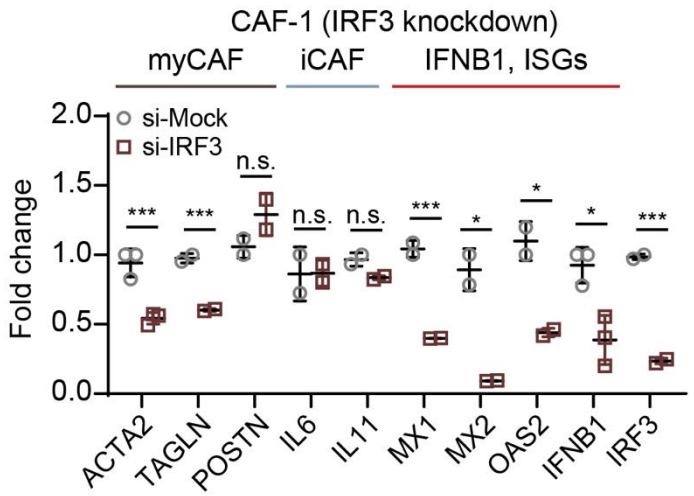


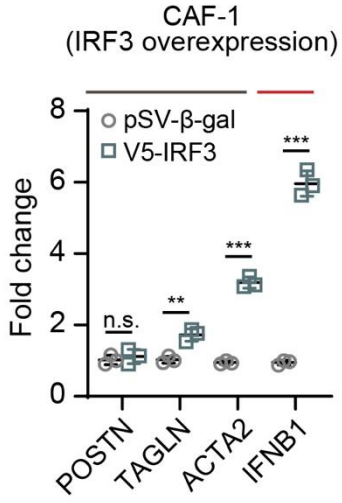


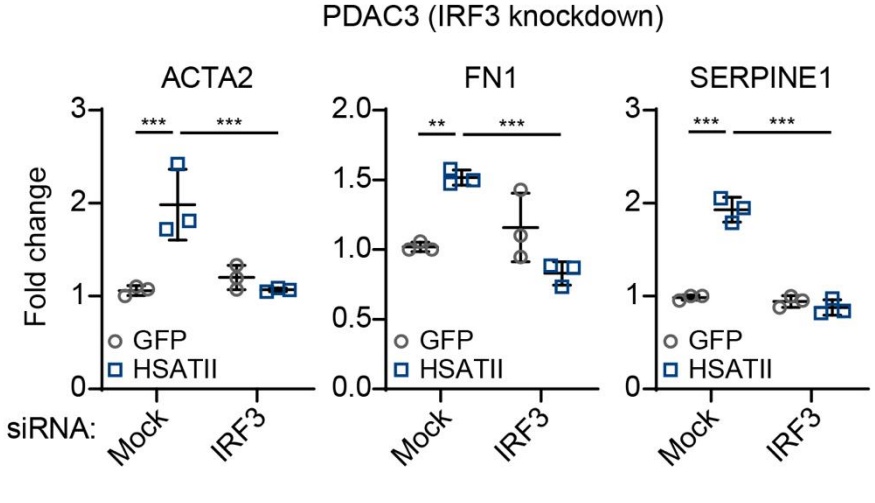
CAF and PDAC divergent response to repeat RNAs is IRF3 dependent











Summary and Future Directions

- Repeat RNA and protein biomarkers for early detection, immune response, and therapy resistance
- Impact of targeting repeat RNA biology to alter cellular plasticity
- Repeat RNA response affects cellular plasticity in PDAC and CAFs
- Spatial transcriptomics as a tool to study repeat RNA effects in cancer progression across many cell types

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