Selective export of HIV mRNAs is regulated by compartmentalized interactions with Sam68, PTB and m6A RNA methylation in reactivated latently infected T-cells

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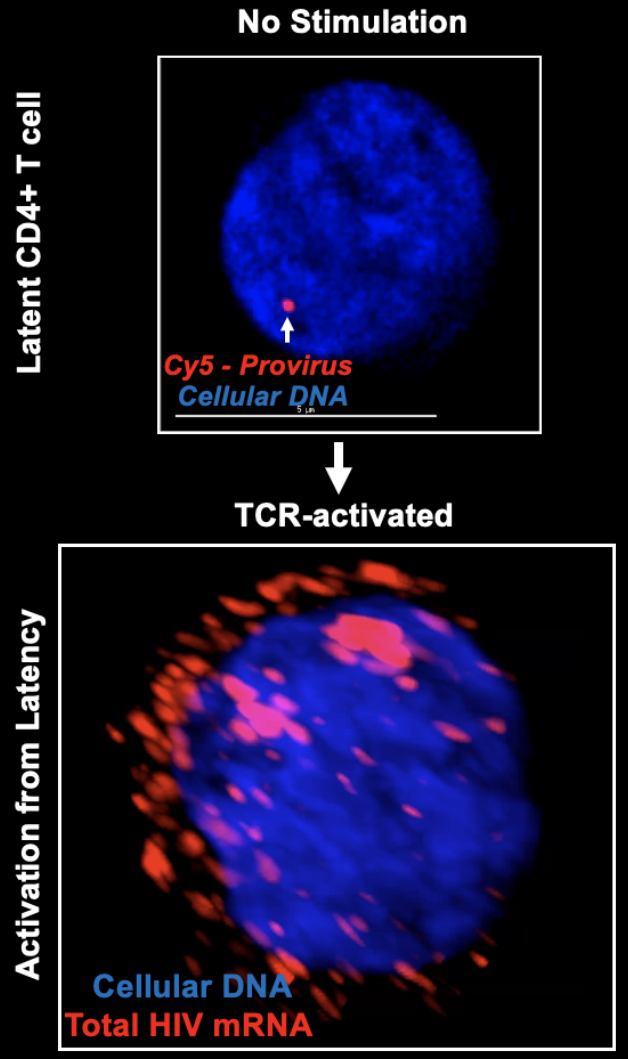
12.11.2024

DECLARATION

The Authors Declare No Competing Interests

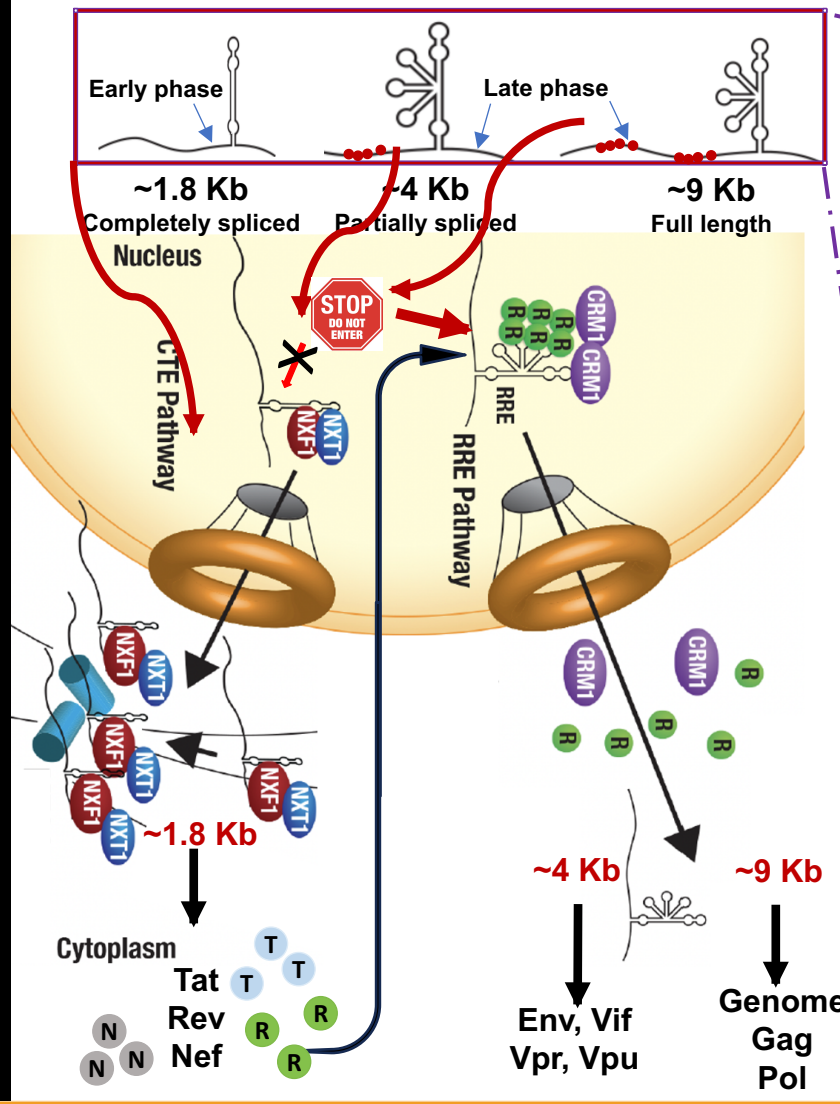
INTRODUCTION

HIV-1 Latency and subsequent reactivation

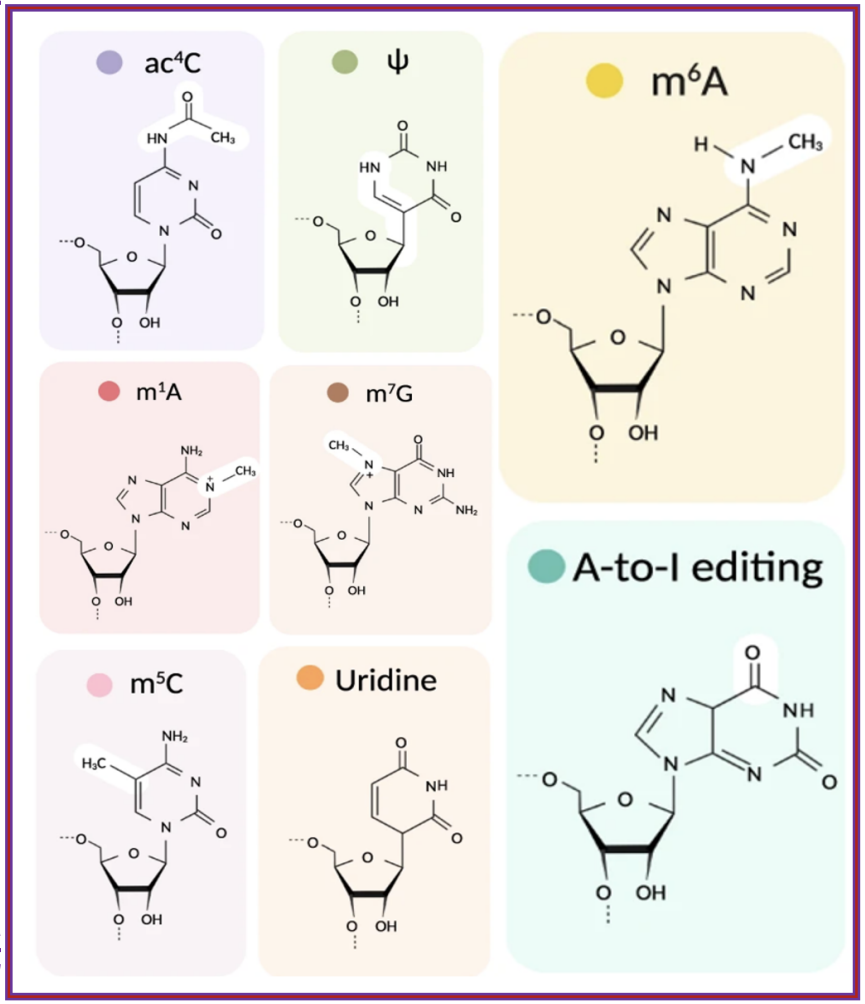


Fredrick Kizito, et al,. (To be published soon)

Alternative splicing products and export of HIV-RNA



RNA modifications



Liu, WW. et al. 2024

THE PROBLEM

CD4+ T cells sequester HIV-1 RNA. This is a supplementary mechanism of HIV-1 Persistence!

Sequestration of HIV-1 RNA in the Nucleus

Nuclear retention of multiply spliced HIV-1 RNA in resting CD4+ T cells.

Lassen KG[1], Ramyar KX, Bailey JR, Zhou Y, Siliciano RF.

Author information

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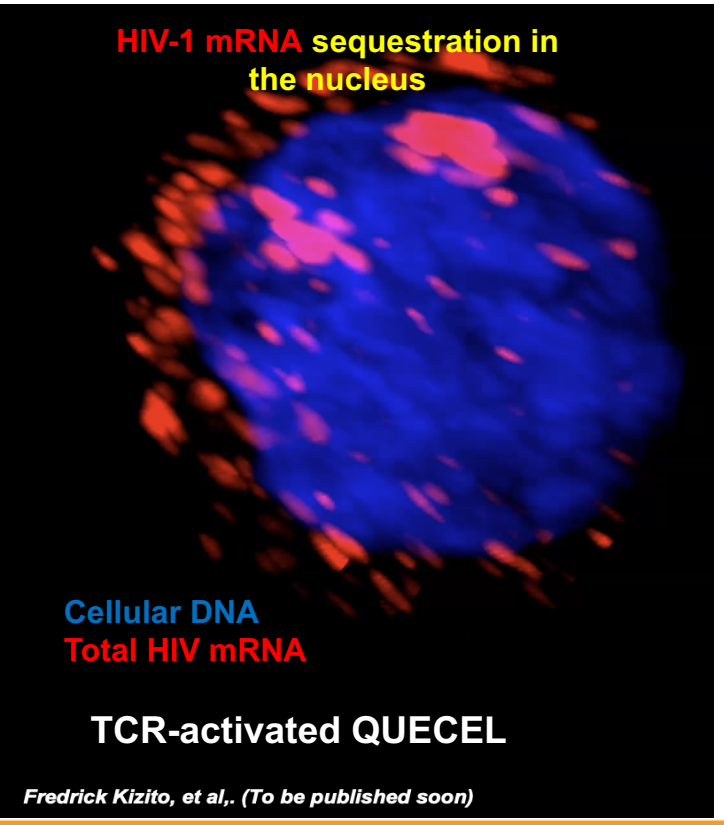
Why do we care?

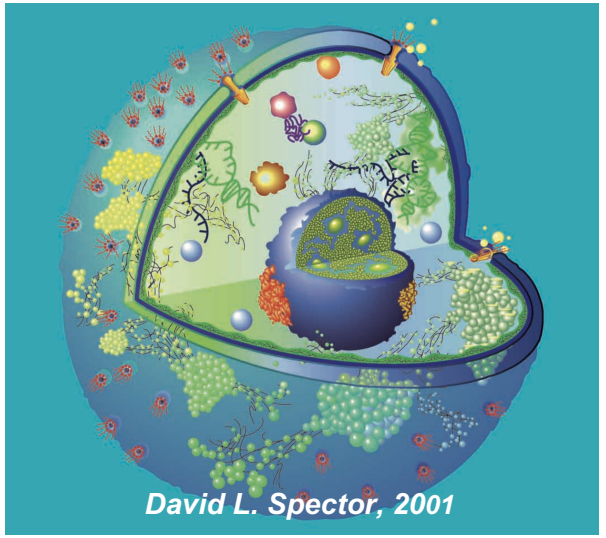
Gene expression and viral prodution in latently infected, resting CD4$^+$ T cells in viremic versus aviremic HIV-infected individuals

Tae-Wook Chun*†, J. Shawn Justement*, Richard A. Lempicki‡, Jun Yang‡, Glynn Dennis, Jr.‡, Claire W. Hallahan*, Christina Sanford*, Punita Pandya*, Shuying Liu*, Mary McLaughlin*, Linda A. Ehler*, Susan Moir*, and Anthony S. Fauci*



To-date, little is known about the critical nuclear compartmentation and chemical modifications which determine segregation, compartmentation and trafficking vis a vis subnuclear sequestration and retention

A Complex Nuclear Environment



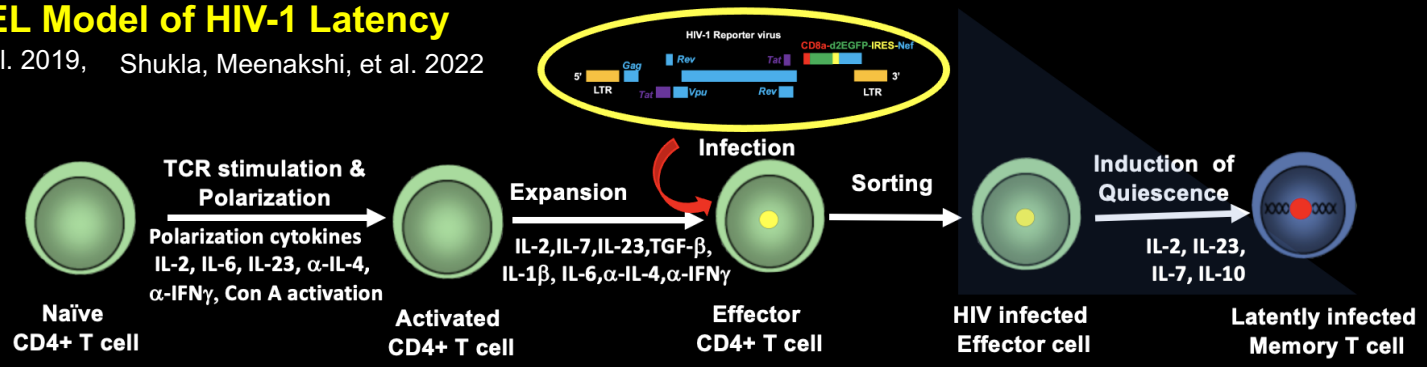
Aims

- Identify the subnuclear compartments, cellular factors for HIV-1 RNA segregation, sequestration and assembly and trafficking
- Identify the HIV-1 RNA modifications for sequestration and retention verses trafficking and export

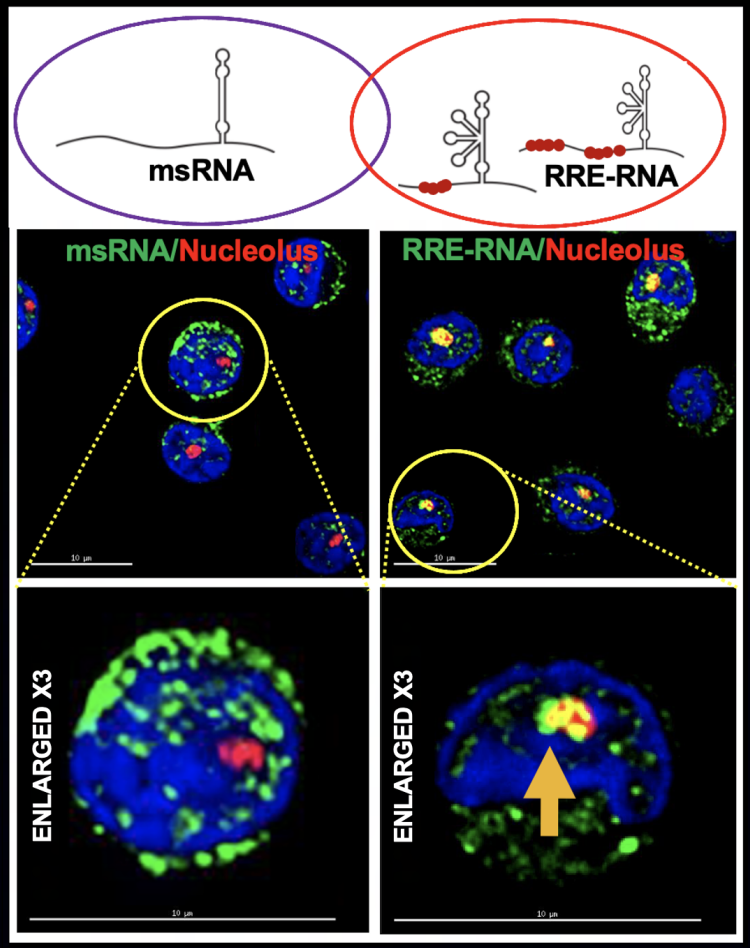
METHODS

The QUECEL Model of HIV-1 Latency

Dobrowolski et al. 2019, Shukla, Meenakshi, et al. 2022



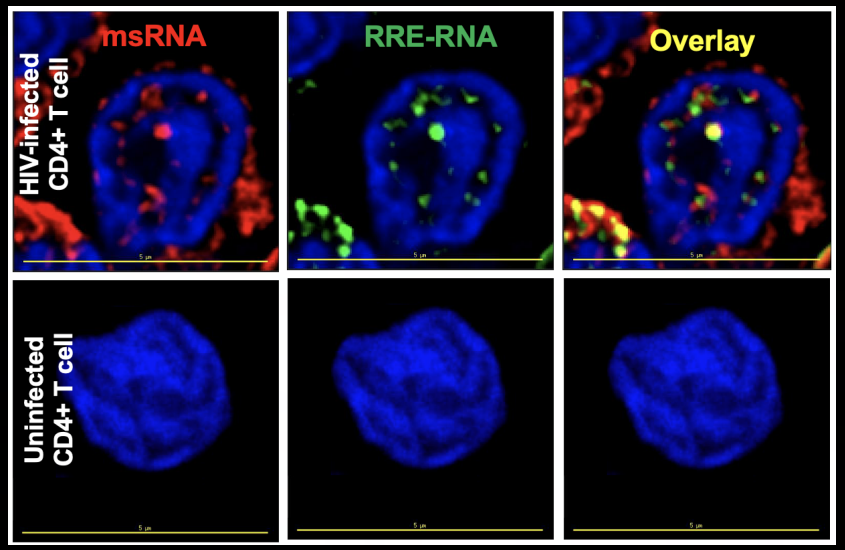
HIV mRNA interactions with the nucleolus



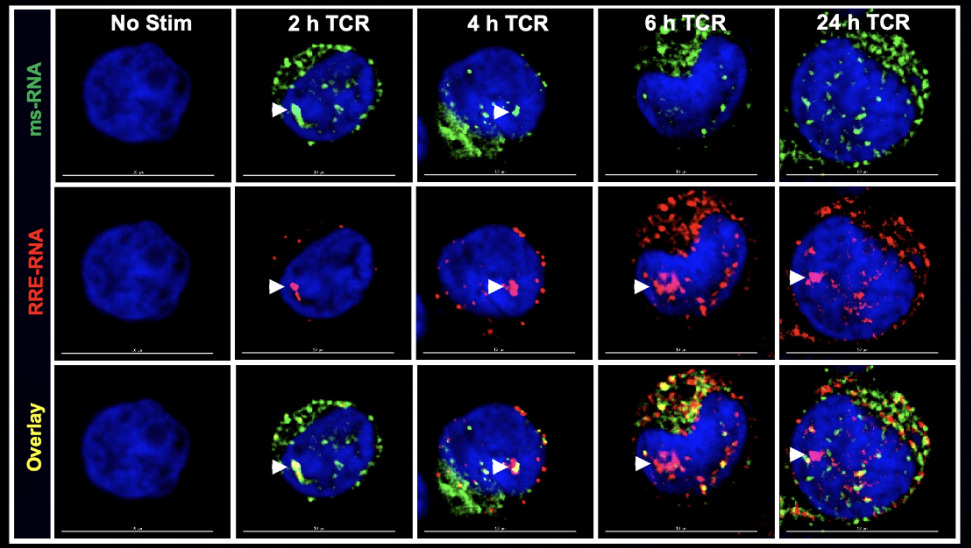
A Critical Validation

Imaging analysis to track spatiotemporal induction, segregation and subnuclear compartmentation of HIV-1 RNA, early RNPs assembly and export

Differential expression of msRNA and RRE-RNA



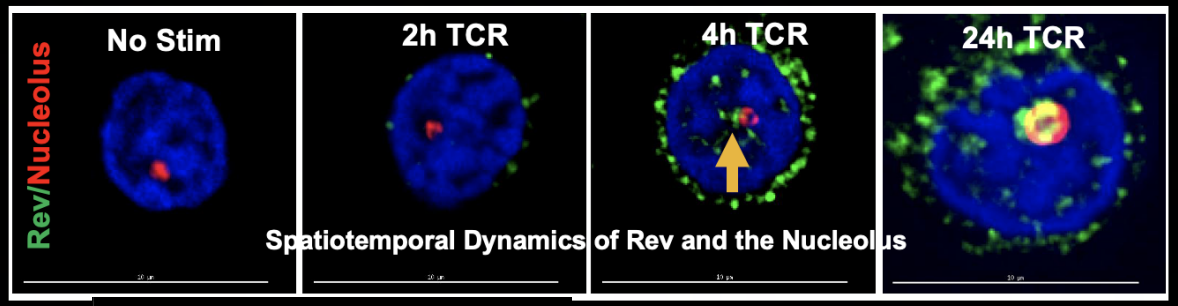
Nuclear dynamics of msRNA and RRE-RNA



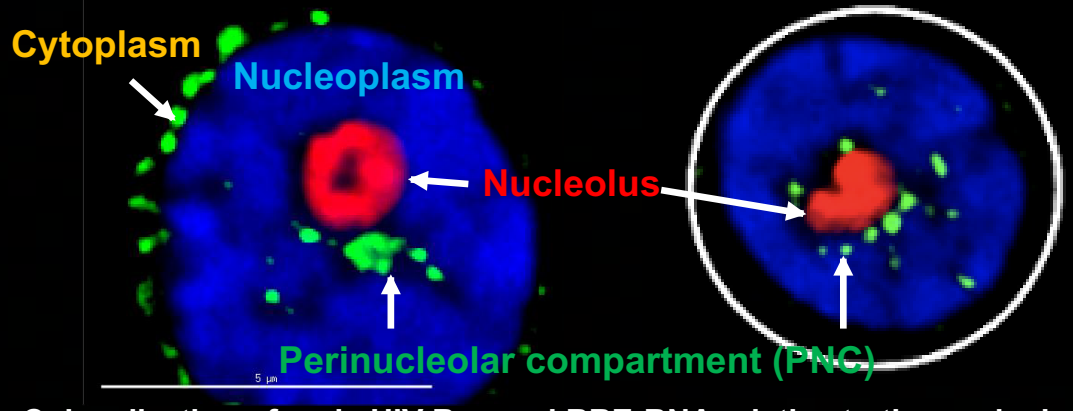
RESULTS

HIV-1 RNA accumulated in the Perinucleolar compartment (PNC)

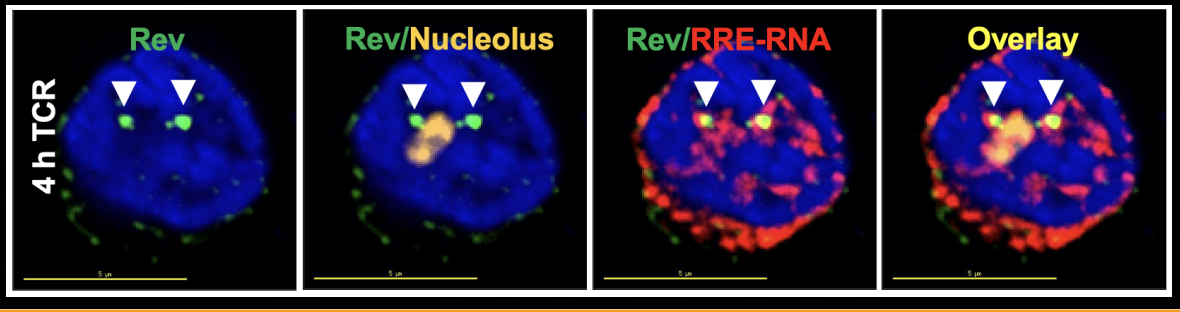
Cellular dynamics of HIV-Rev relative to the nucleolus



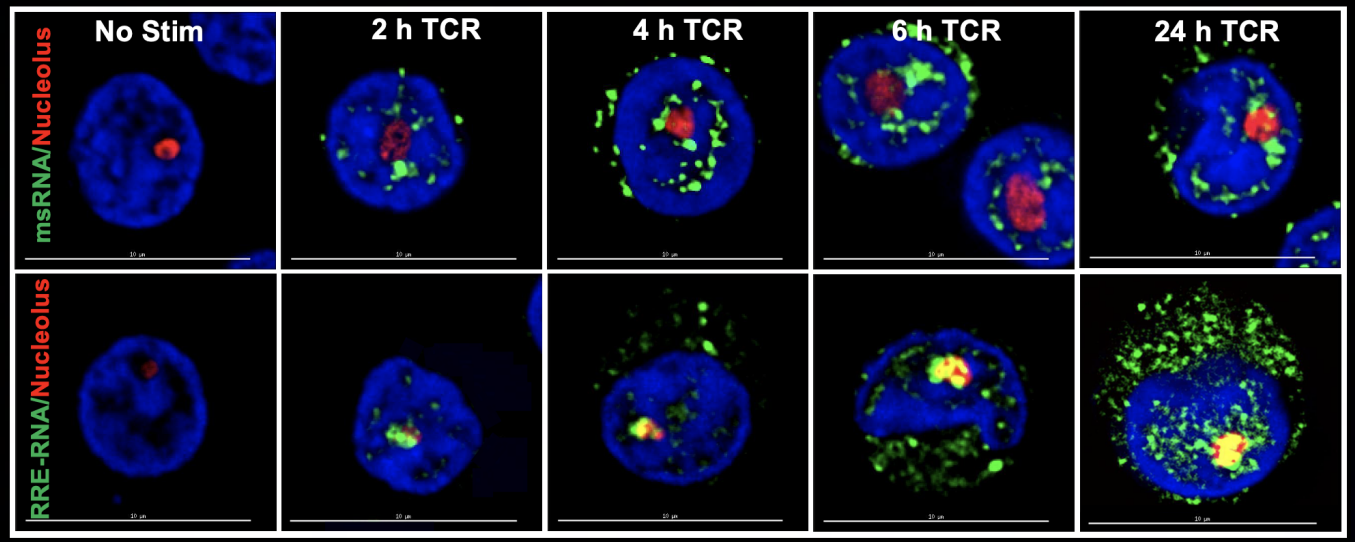
THE 4 MAIN SUBCELLULAR COMPARMENTS



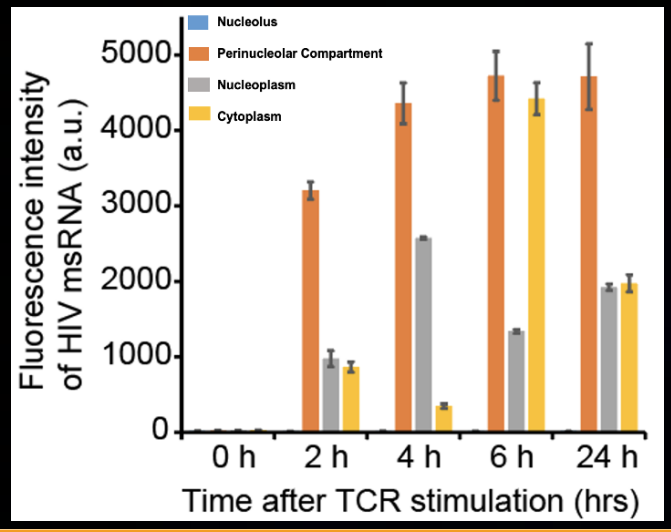
Colocalization of early HIV-Rev and RRE-RNA relative to the nucleolus

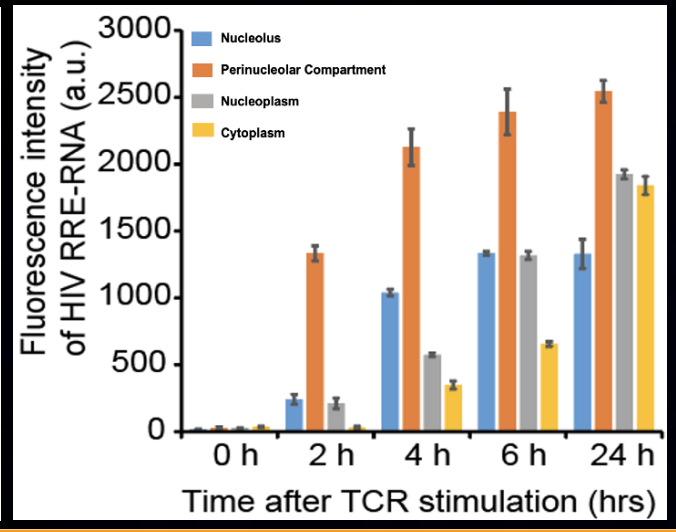


Sequestration of msRNA and RRE-RNA in the Perinucleolar compartment (PNC)



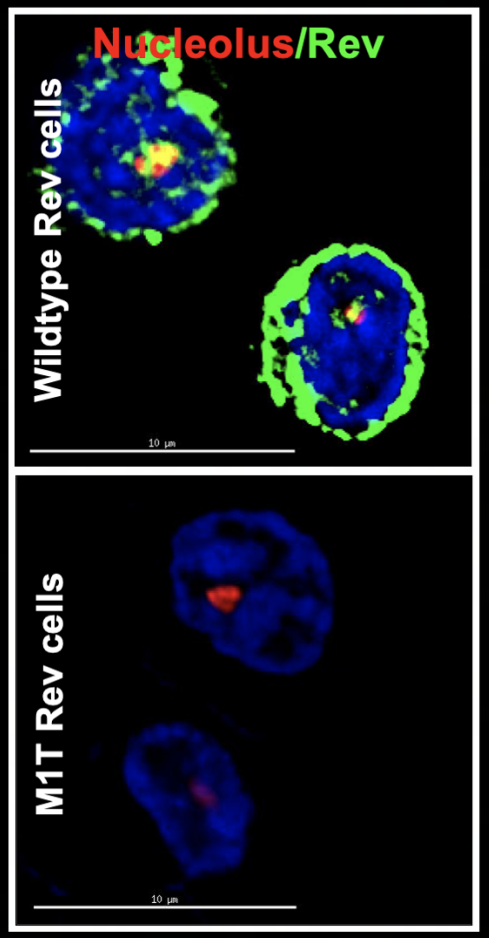
Subcellular distribution of msRNA and RRE-RNA

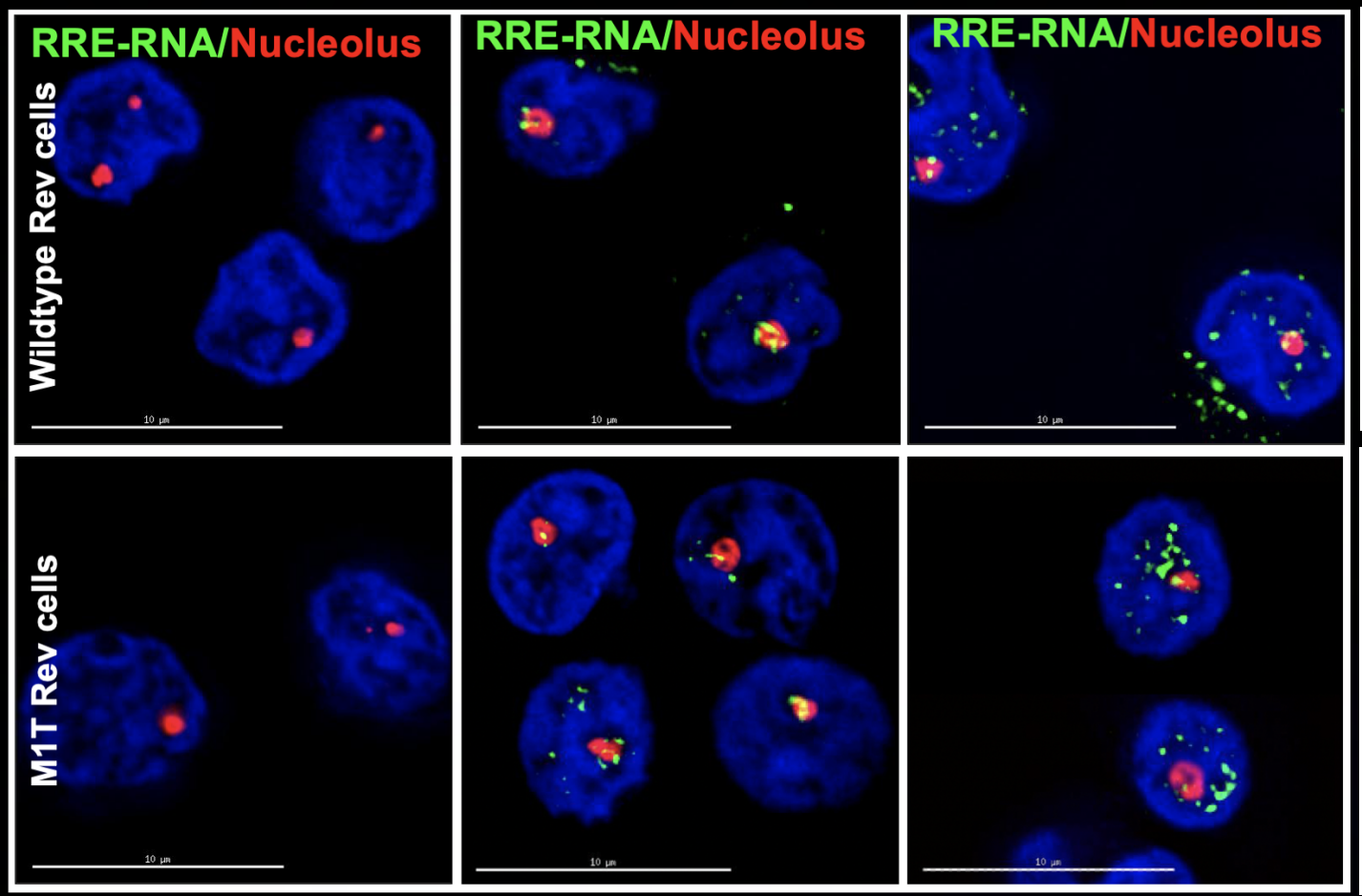


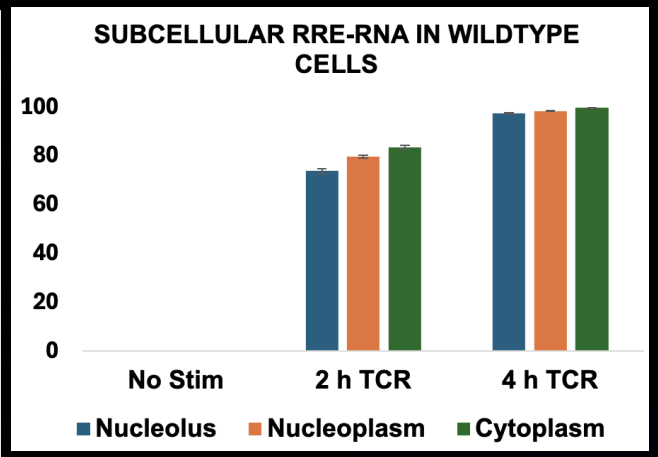


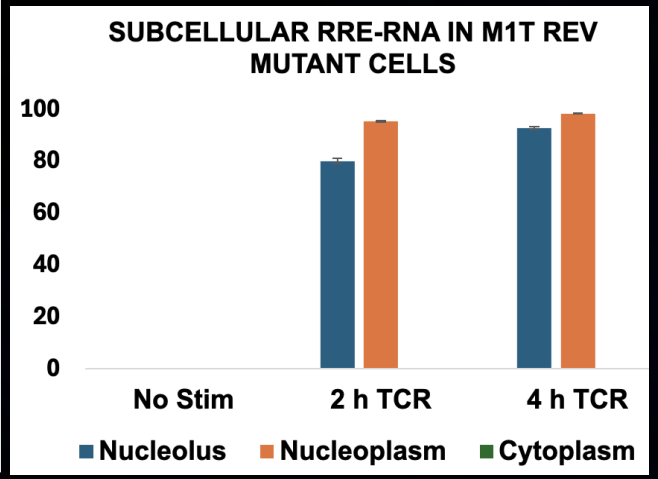
Mutagenesis studies of HIV-1 Rev

A portion of RRE-RNA appeared in the nucleolus in a Rev-independent manner









Kien Nguyen, PhD

✓RRE-RNA was dispersed in the nucleoplasm in a Rev-independent manner

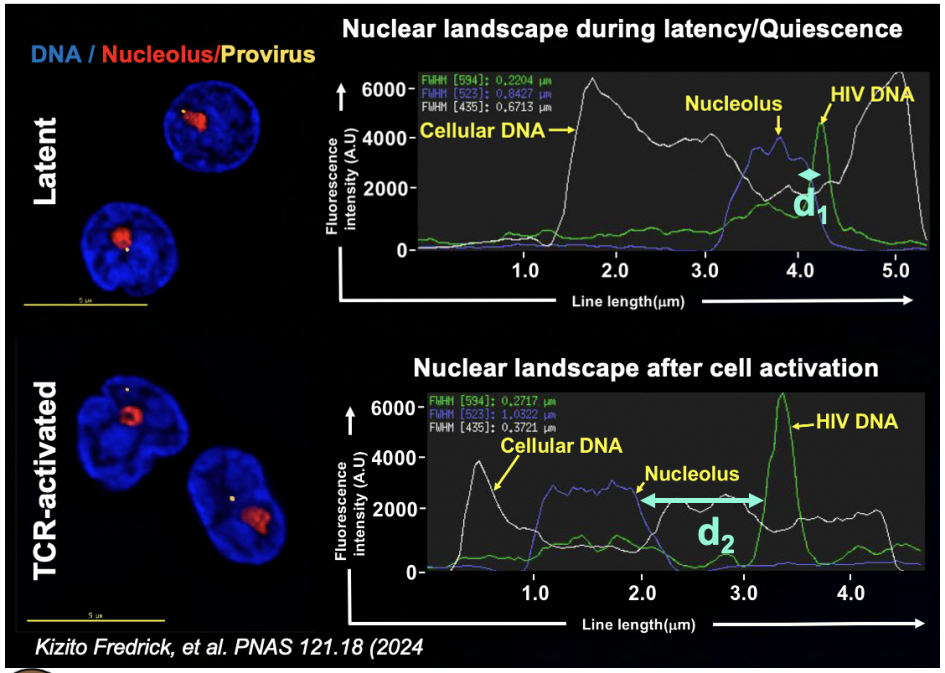
The PNC is critical in the establishment of HIV Latency and Reactivation

PNAS RESEARCH ARTICLE | MICROBIOLOGY OPEN ACCESS

Structural rearrangements in the nucleus localize latent HIV proviruses to a perinucleolar compartment supportive of reactivation

Fredrick Kizito[a], Kien Nguyen[a], Uri Mbonye[a], Meenakshi Shukla[a], Benjamin Luttge[a], Mary Ann Checkley[a], Anna Agaponova[a], Konstantin Leskov[a], and Jonathan Karn[a,1]

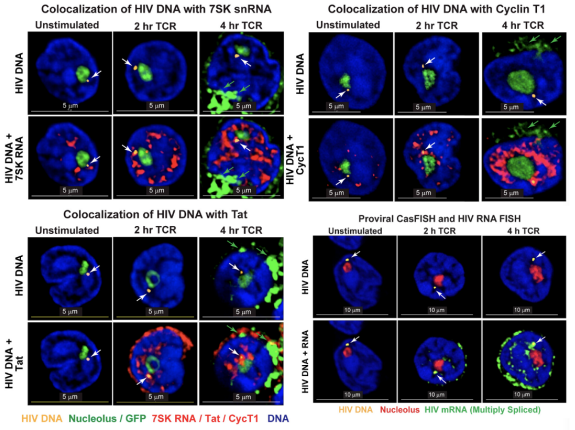
Locations of the Provirus in latent and reactivated CD4+ T cells

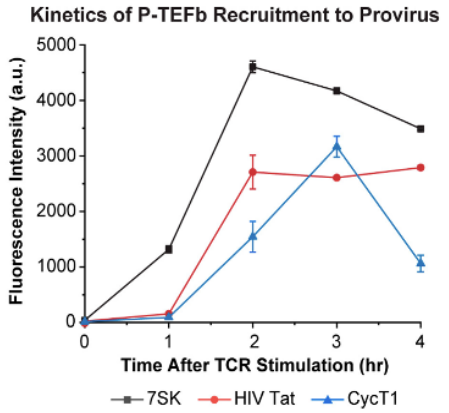


Kizito Fredrick, et al. PNAS 121.18 (2024

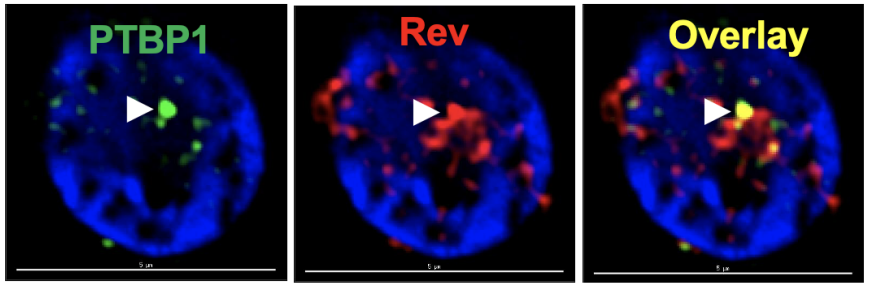
Is the PNC not only a high proviral transcription site but also High HIV-RNA regulatory site?

Early synthesized 7SK, Tat and P-TEFb and HIV-RNA

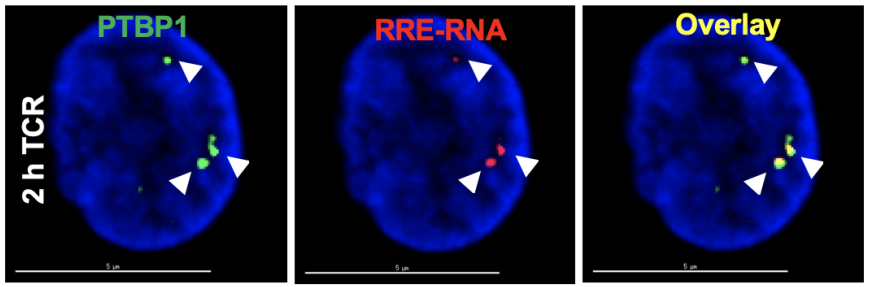




Basal PTBP1 colocalization with Rev

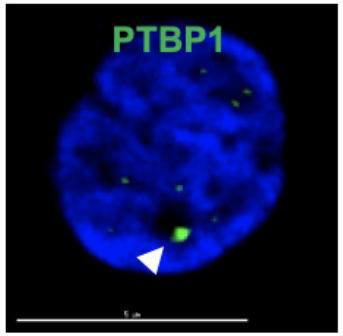


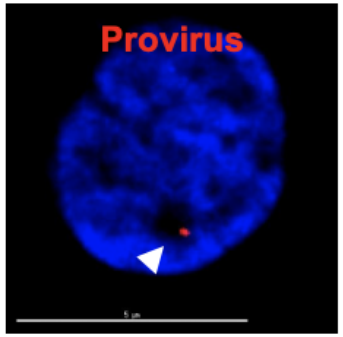
Basal PTBP1 colocalization with RRE-RNA

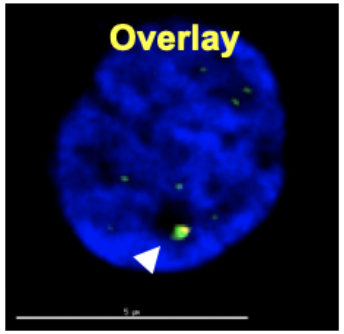


PTBP1 colocalization with the provirus

No Stimulation

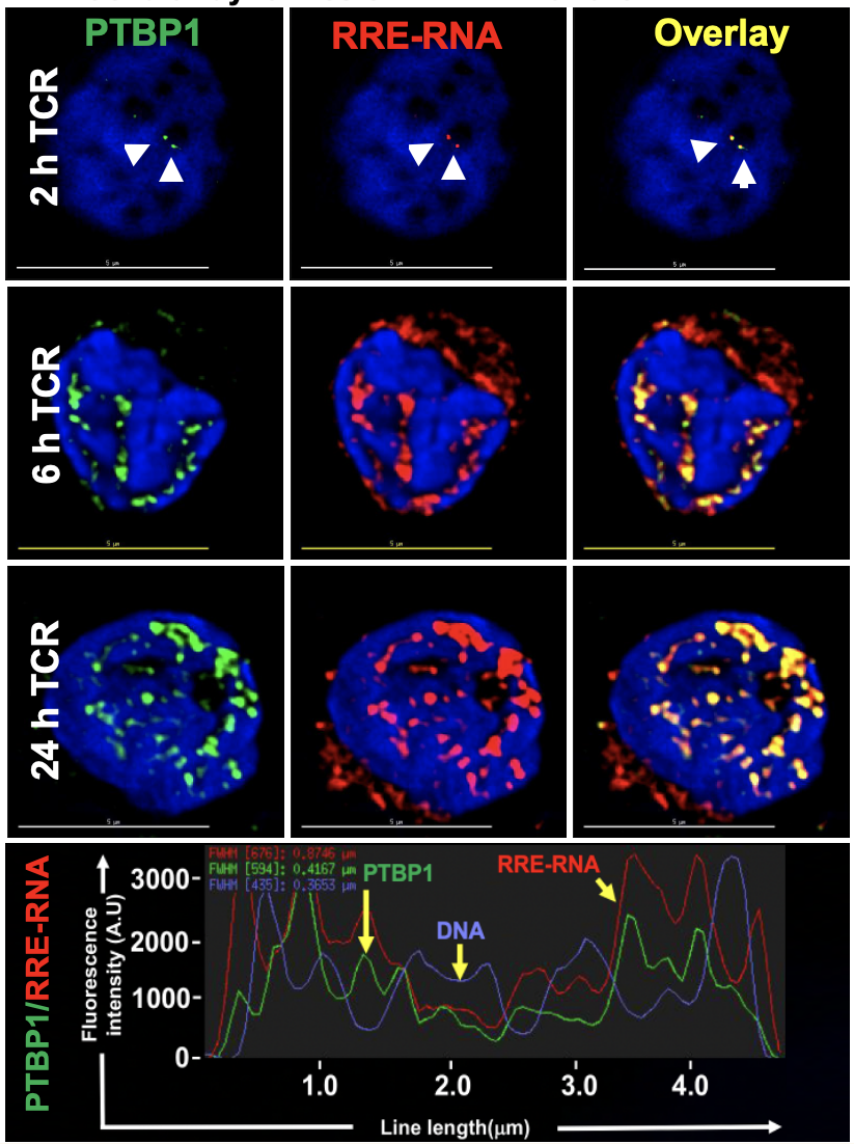




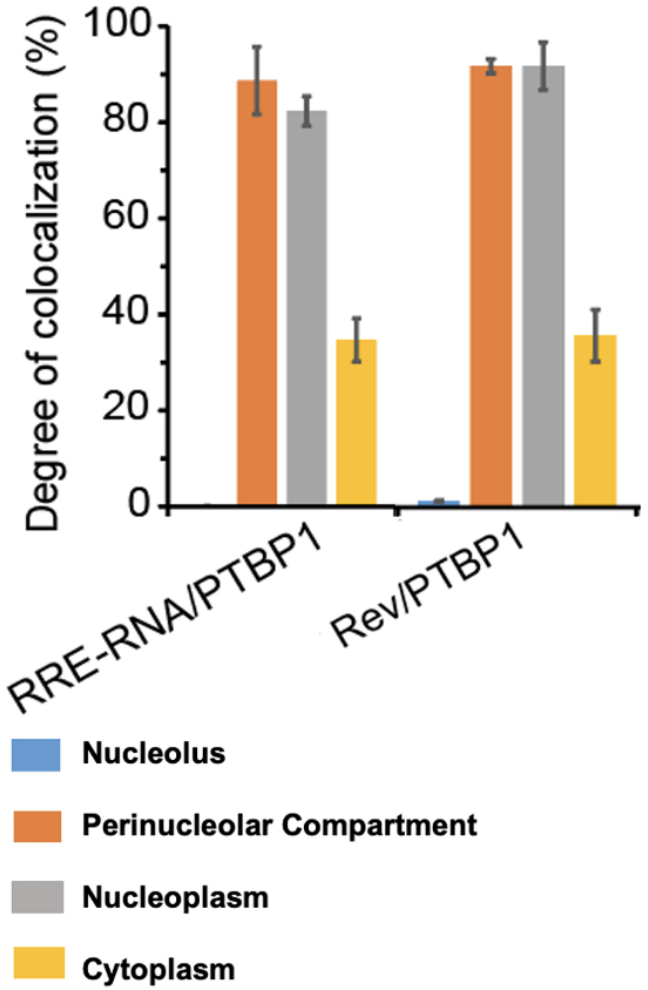


PTBP1 colocalized with RRE-RNA and Rev in the nucleoplasm, but not in the cytoplasm

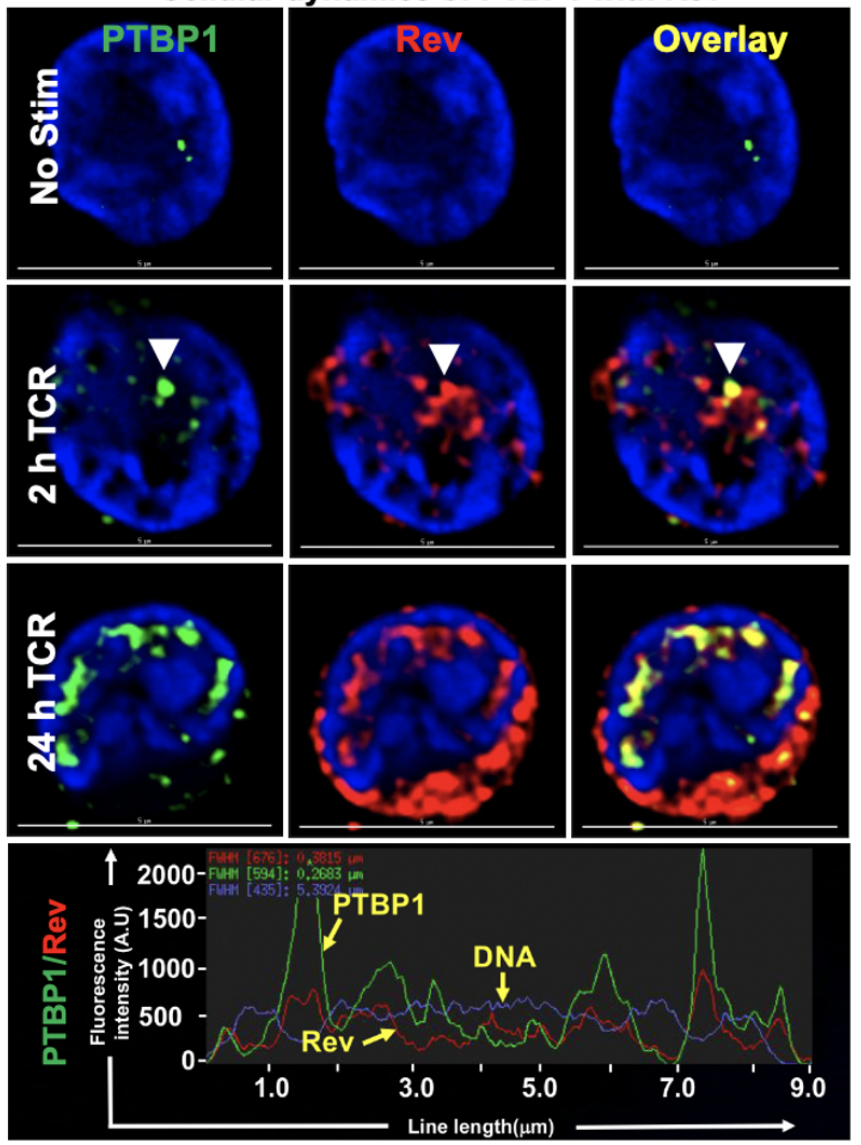
Cellular dynamics of PTBP1 with the RRE-RNA



Colocalization profiles of PTBP1 with the RRE-RNA and Rev

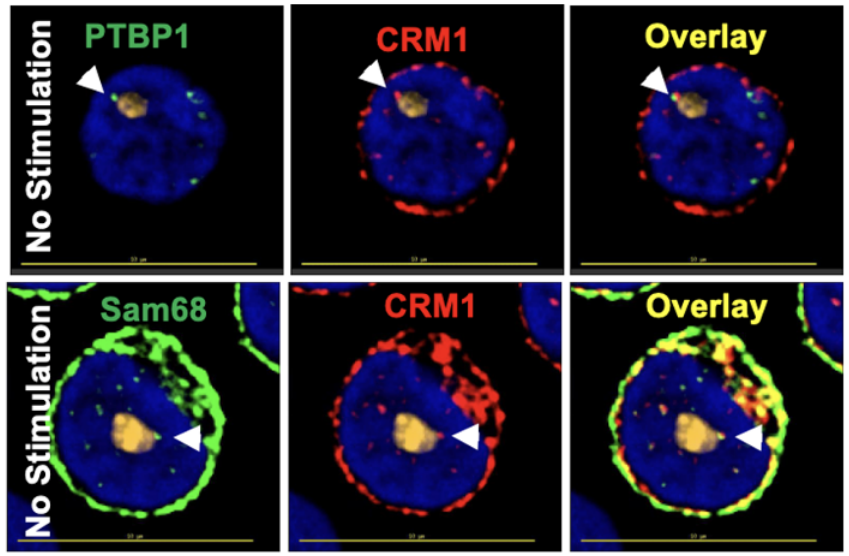


Cellular dynamics of PTBP1 with Rev

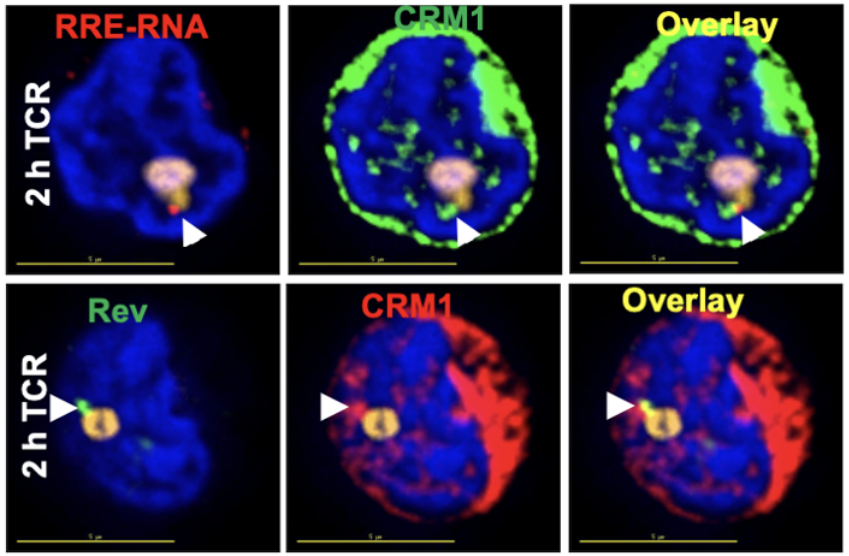


Imaging analysis of the RRE-RNP macromolecular export complex assembly in the PNC

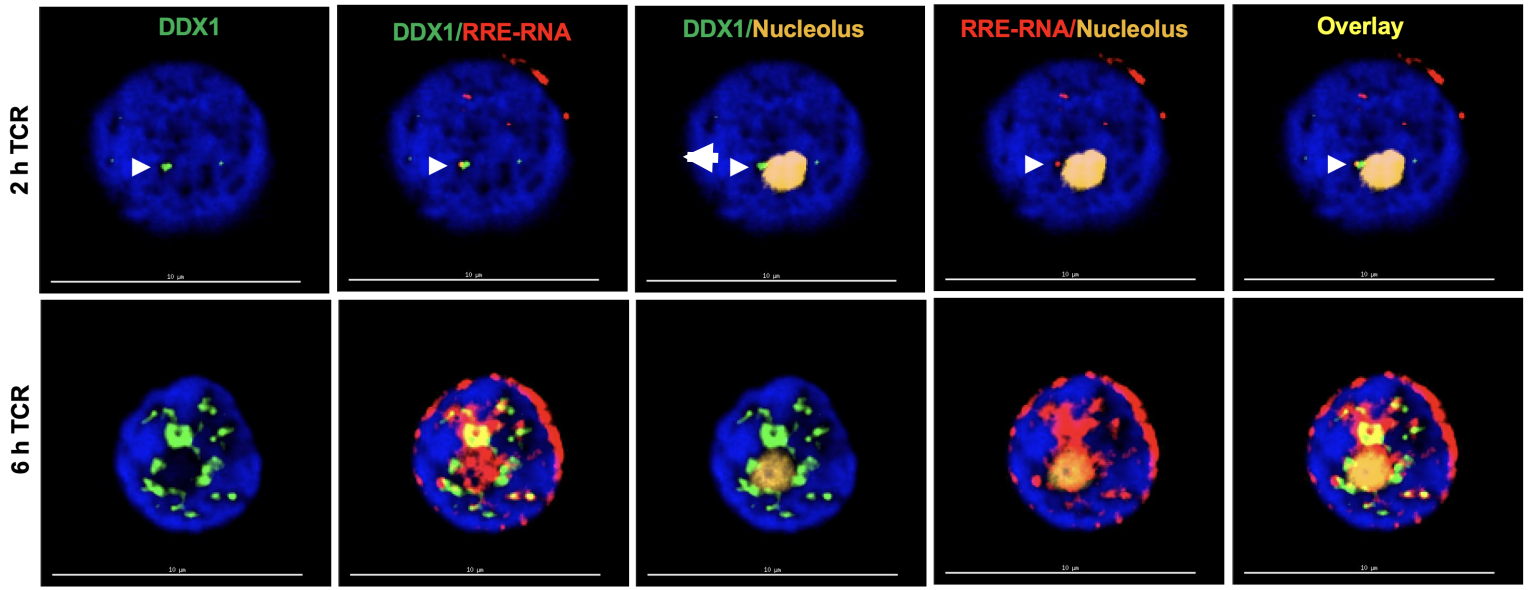
PNC-mediated interactions in latently infected resting CD4+ T cells



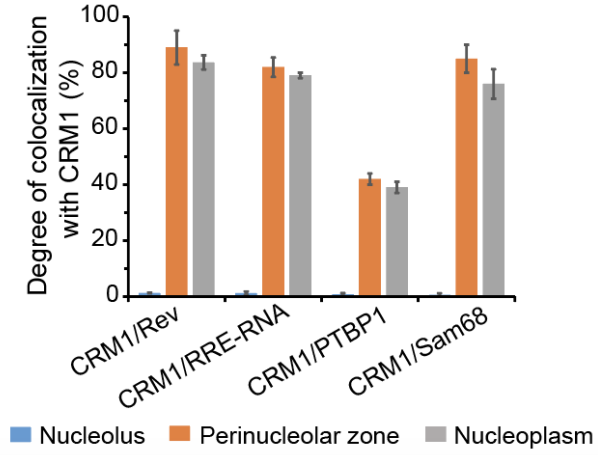
PNC-mediated interactions in activated resting CD4+ T cells



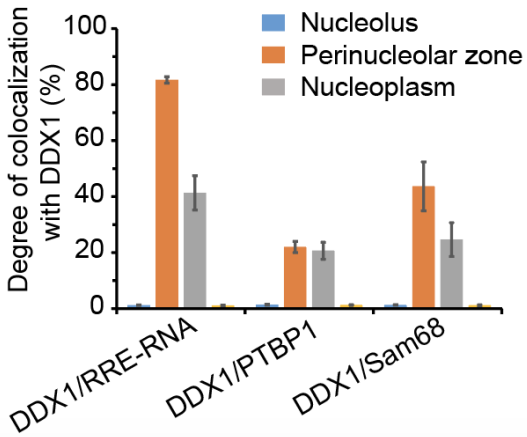
Initial DDX1 and RRE-RNA colocalization in the PNC



Colocalization profile of CRM1 with Rev, RRE-RNA, PTBP1 and Sam68

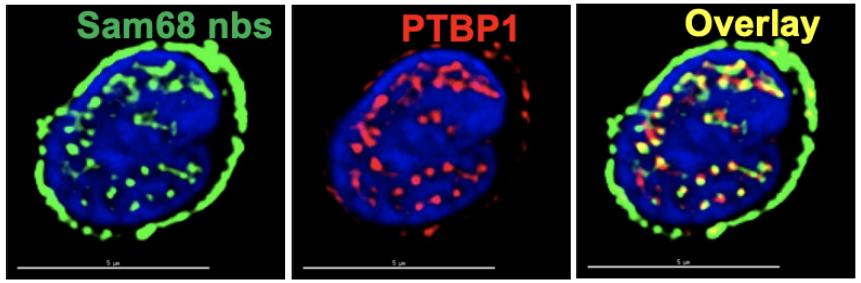


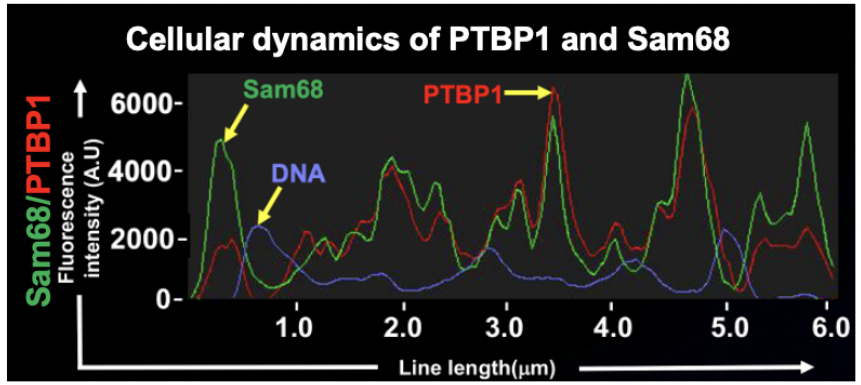
Colocalization profiles of DDX1 with the RRE-RNA, PTBP1 and Sam68



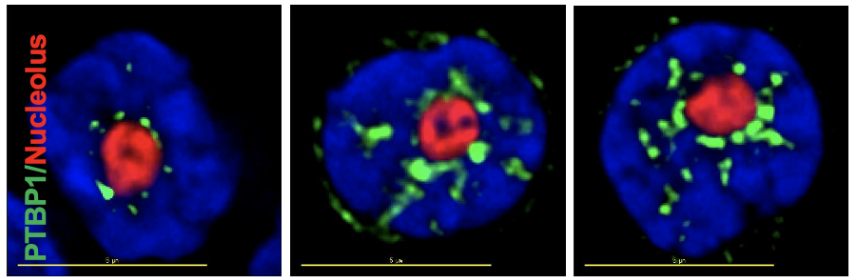
Sam68 colocalized with PTBP1 in the nucleoplasm

PTBP1 colocalization with Sam68

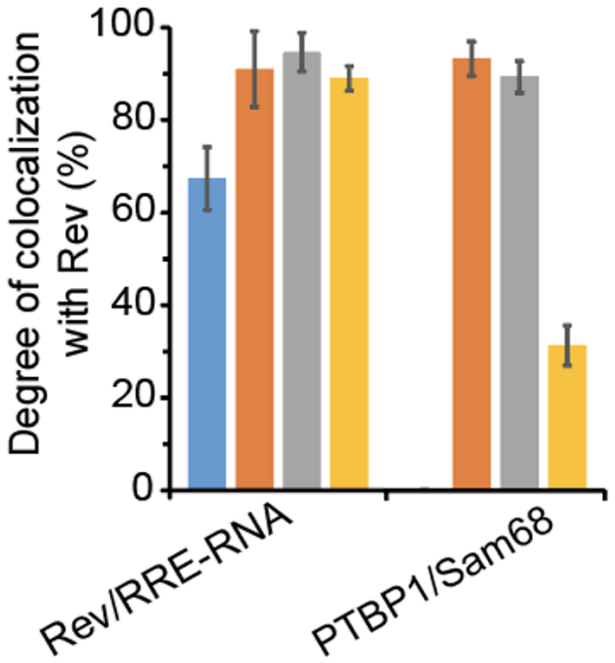




Cellular dynamics of PTBP1 relative to the nucleolus

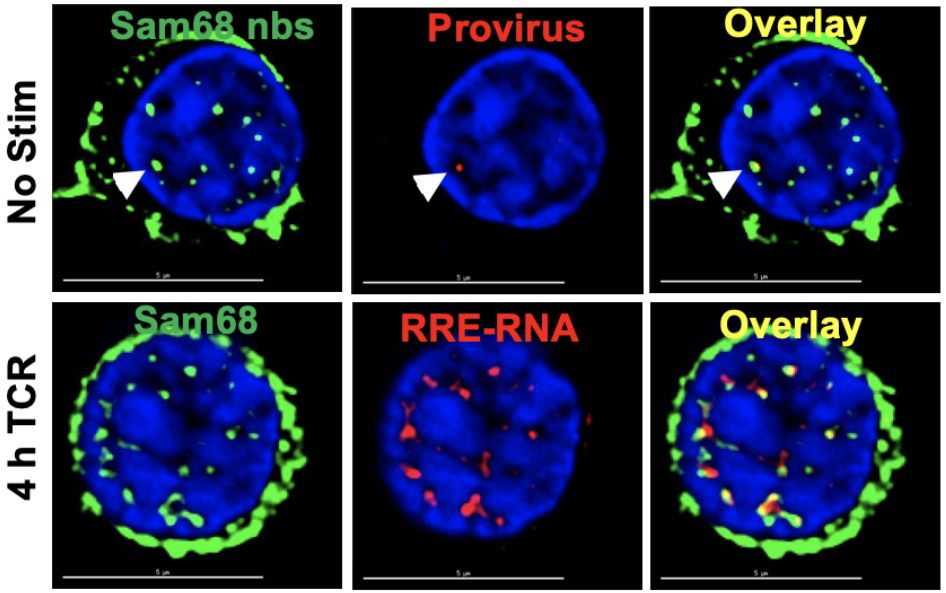


Colocalization profiles of PTBP1 with Sam68, and Rev with RRE-RNA

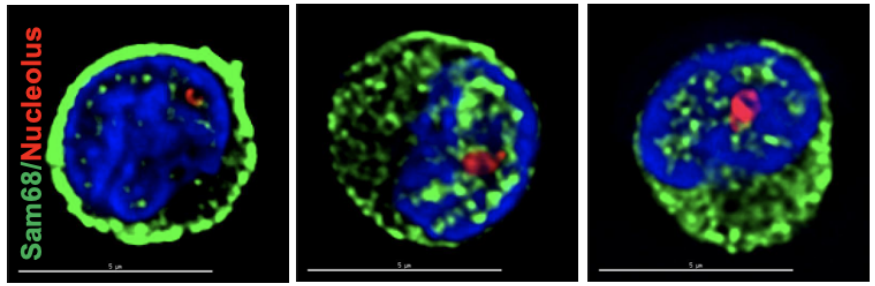


- Nucleolus
- Perinucleolar Compartment
- Nucleoplasm
- Cytoplasm

Sam68 colocalization with the provirus and RRE-RNA

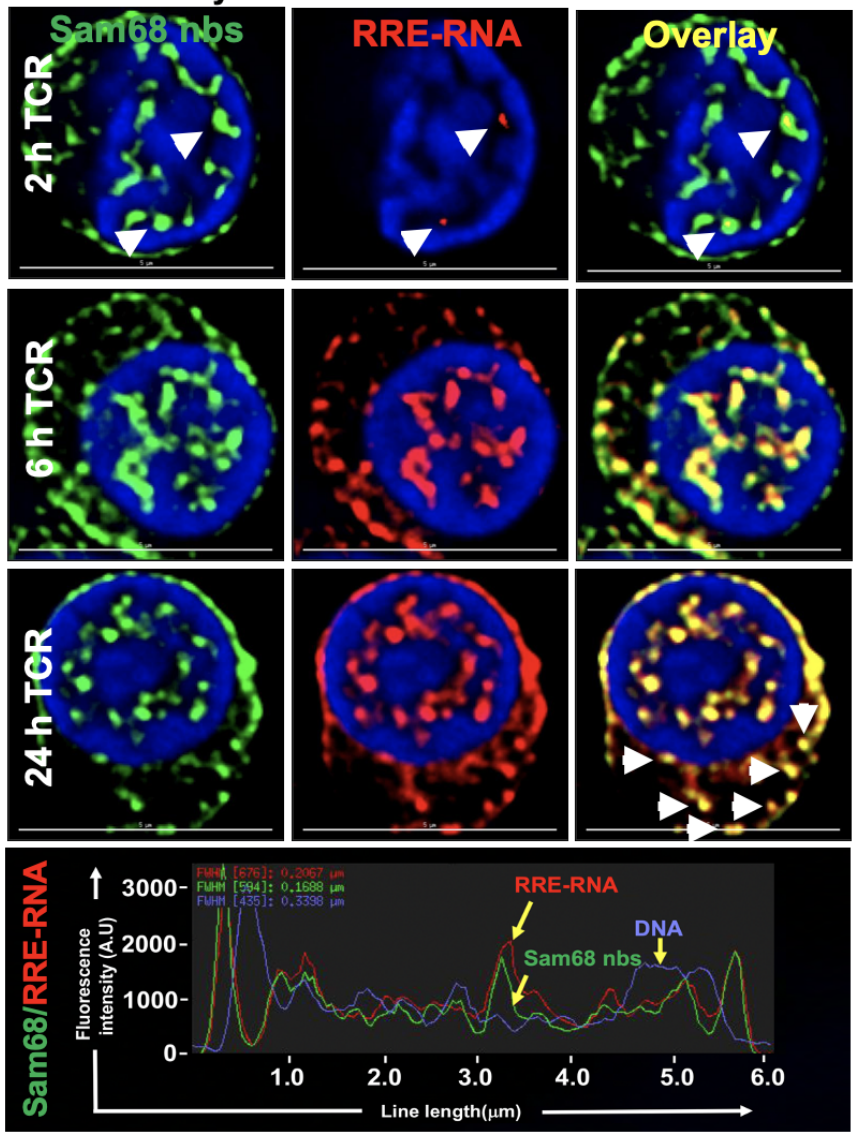


Cellular dynamics of Sam68 relative to the nucleolus

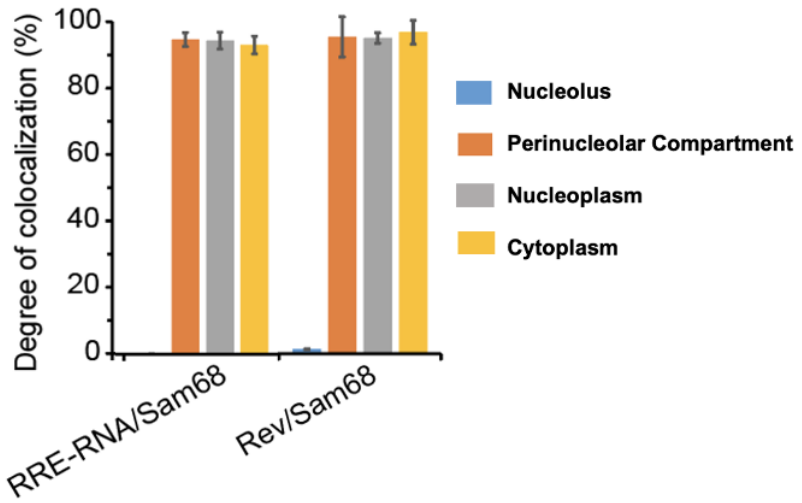


Sam68 colocalized with the RRE-RNA in the nucleoplasm and cytoplasm

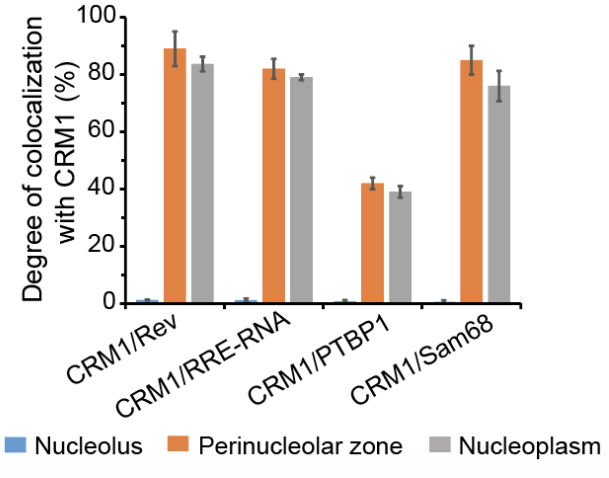
Cellular dynamics of Sam68 and the RRE-RNA



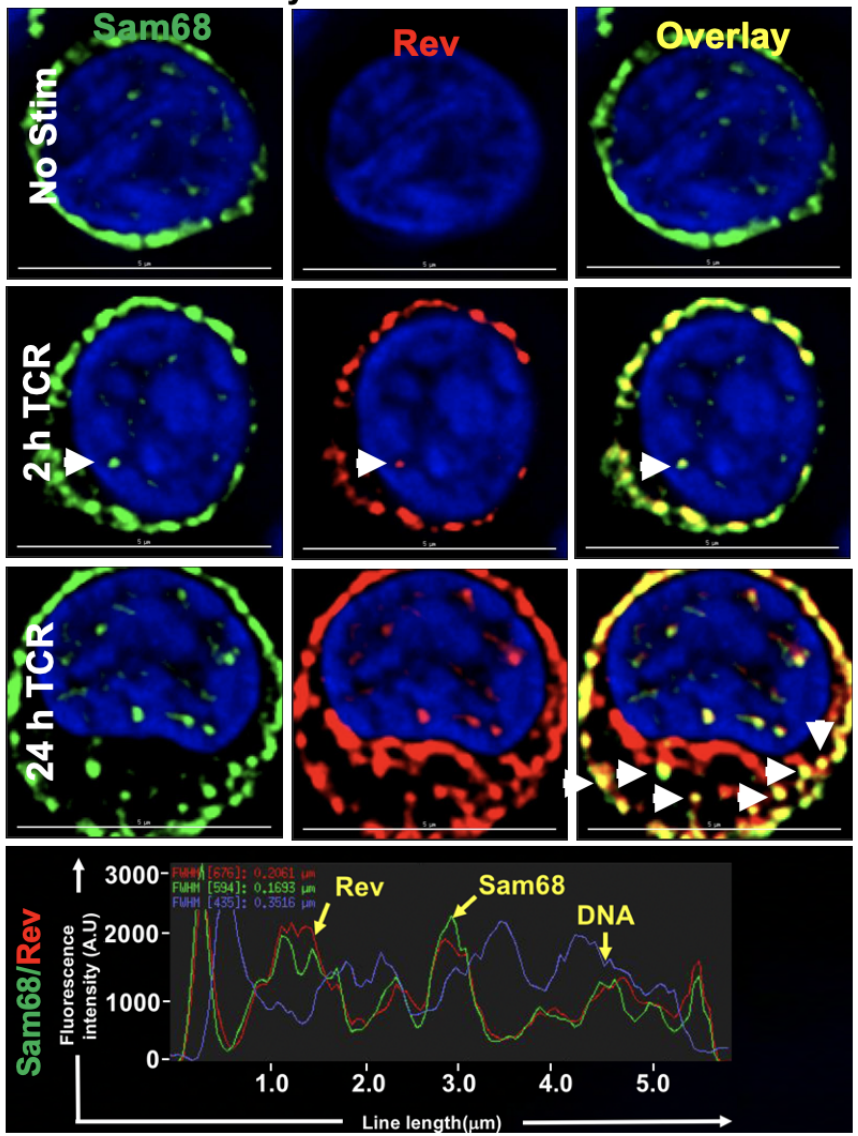
Colocalization of Sam68 with the RRE-RNA and Rev



Colocalization profile of CRM1 with Rev, RRE-RNA, PTBP1 and Sam68

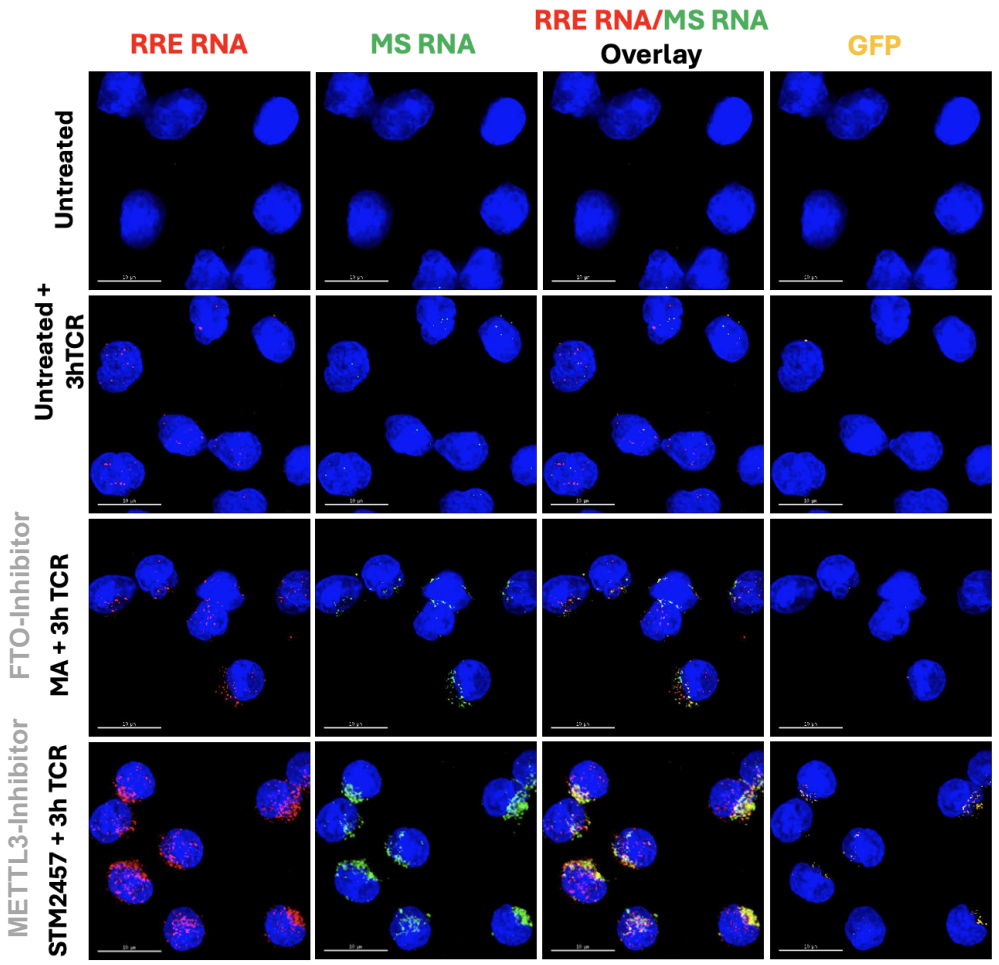


Cellular dynamics of Sam68 with Rev

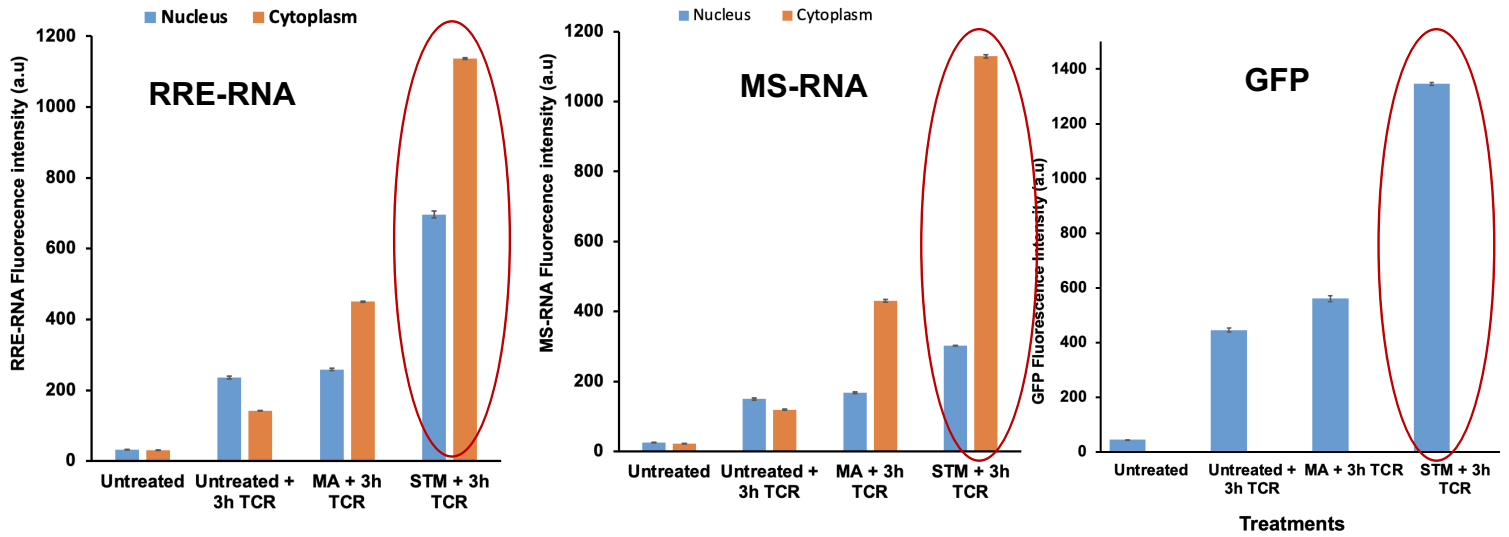


Reduced M6A modifications increased selective export and translation of HIV-1 RRE-RNA

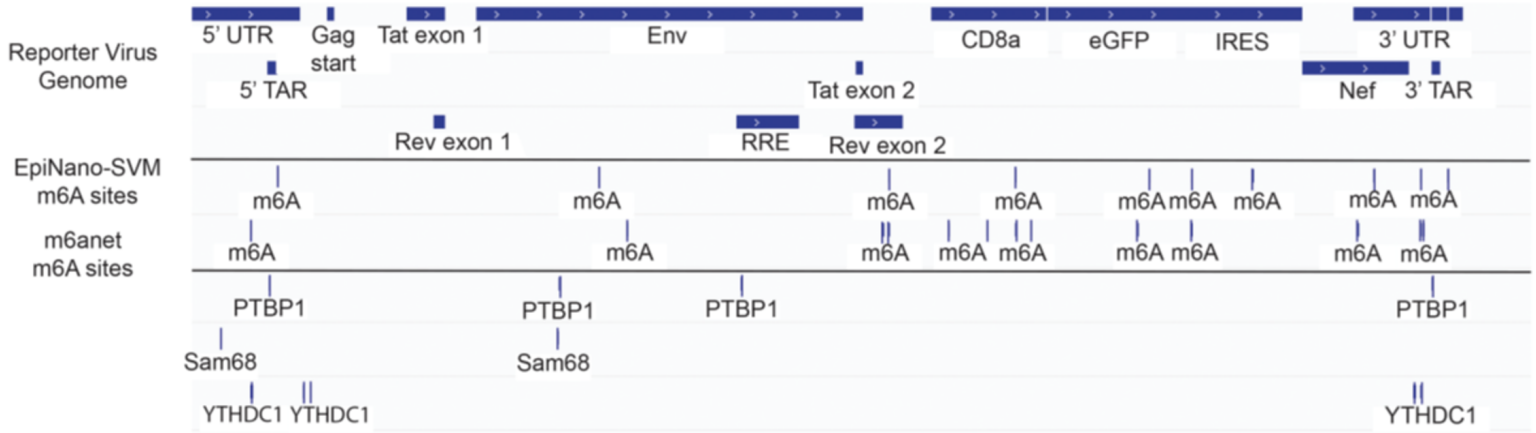
Differential effects of FTO and METTL3 inhibitors on the expression of HIV-1 RNA transcripts



Expression profiles of RRE-RNA, MS-RNA and GFP in untreated and inhibitor-treated cells



Potential overlap of M6A sites with PTBP1, Sam68 and canonical M6A reader, YTHDC1



TAKE HOME

- ✓ The Perinucleolar compartment was the 'Hotspot' subnuclear compartment for segregation and initial assembly of HIV-RNA-macromolecular export complexes in reactivation T-Cells
- ✓ RRE-RNA appearance in the nucleolus and distribution in the nucleoplasm were Rev-independent processes
- ✓ PTBP1 was the critical mediator of HIV-RNA segregation and assembly in the Perinucleolar compartment and mediated an independent RRE-RNA subnuclear trafficking pathway from that of the nucleolus
- ✓ Sam68 was the critical mediator of Rev-mediated nucleo-cytoplasmic export of RRE-RNA
- ✓ M6A modifications likely affect the export and translation kinetics of HIV-1 RNA

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Fengchun Ye, PhD

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Anna Agaponova, M.S

Principal Investigator

Prof. Jonathan Karn, PhD

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R61 AI169629

CRISPR for Cure Collaboratory
UM1 AI164568

Rustbelt Center for AIDS Research
P30 AI036219

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Sheetal Sreeram, M.S

Yoelvis Garcia, PhD

Ethan Honeycutt, B.S

Isabella Hendrickson

Muda Yang B.S

Mbonye Lab

Uri Mbonye, PhD

Amie Donner B.S