



22nd International Conference on Human Retrovirology:
HTLV and Related Retroviruses
HTLV2026

June 3-6, 2026 | Philadelphia, Pennsylvania, USA

Symposium 1 - Host pathogen interaction

Chairs: Arnold Rabson and Lishomwa Ndhlovu

Investigating the role of PP2A-B56 in establishing HTLV-I infection

Goedele N. Maertens, Imperial College London

Jordan J. Minnell*, Emma Punch*, Roghaiyeh Safari, Nora Cronin,
Peter Cherepanov and Goedele Maertens

Investigating the role of PP2A-B56 in establishing HTLV-I infection

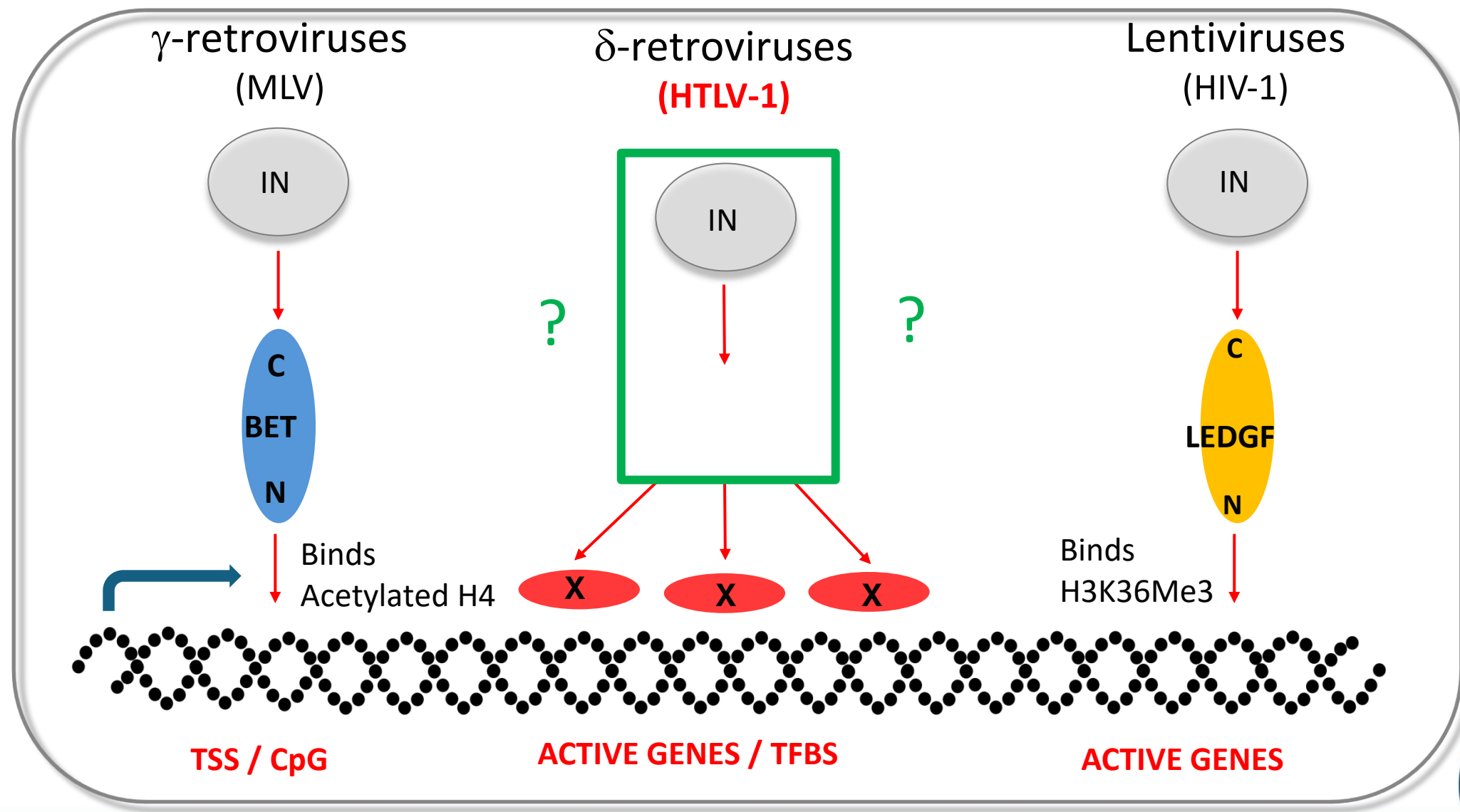
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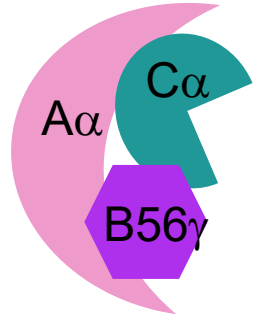
Conflicts of interest

I have no conflicts of interest

HOST DETERMINANTS OF RETROVIRAL INTEGRATION



PP2A-B56 holoenzyme



- A α : scaffold
 - C α : catalytic
 - B56: regulatory subunit
- } core
- B56: α , β , γ , δ and ϵ
 - PP2A-B56 critical for cell survival

> [Nucleic Acids Res.](#) 2016 Jan 8;44(1):364-76. doi: 10.1093/nar/gkv1347. Epub 2015 Dec 10.

B'-protein phosphatase 2A is a functional binding partner of delta-retroviral integrase

Goedele N Maertens ¹

Displays key features of an IN targeting/tethering factor

- ✓ Interaction is genus specific
- ✓ HTLV-1 IN and PP2A-B56 co-localize in the nucleus
- ✓ B56 stimulates concerted integration activity of HTLV-1/2 IN



δ -RETROVIRAL INTEGRASES MIMIC A SLIM



LxxIxEx

HTLV-1 IN 202 KTRWQLHHSPR**L**Q**P****I****P****E**THSLS 223
HTLV-2 IN KTRWQIIHHSP**P****L****P****P****I****P****E**ASTPP
BLV IN KTRWELHHSP**P****L****A****V****I****S****E**GGETP

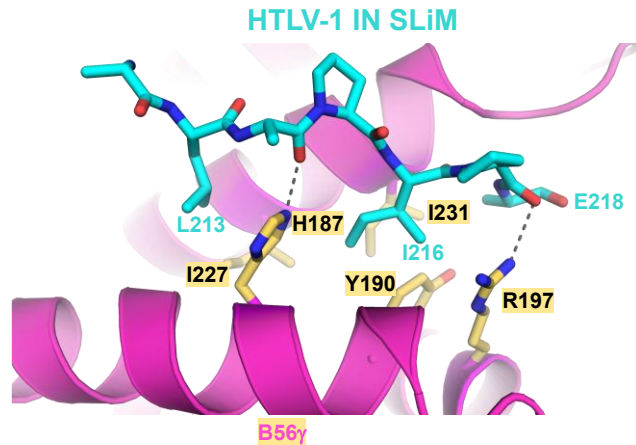
RepoMan GERDIASKK**P****L****S****P****I****P****E**LPEVP
BubR1 RDIASIK**K****L****S****P****I****I****E**DSREA

(Hertz 2016, Wang 2016)

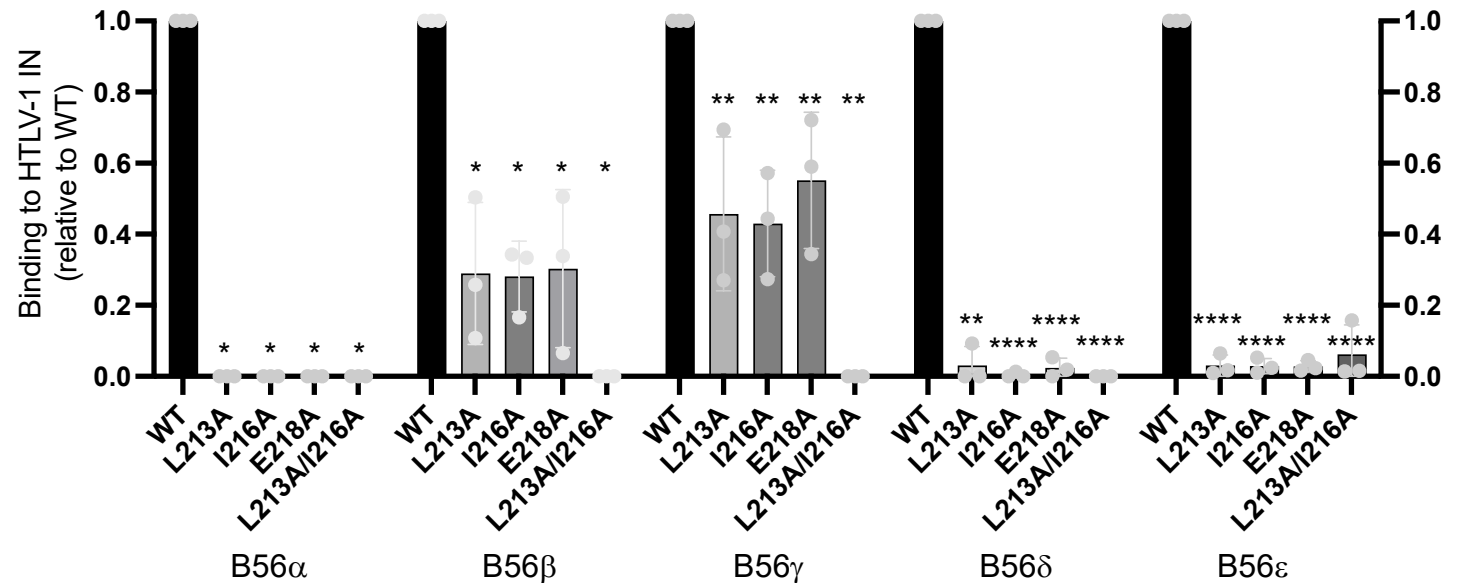
HOW DOES MUTATION OF IN SLiM AFFECT BINDING TO OTHER B56 (FULL-LENGTH) ENDOGENOUS ISOFORMS?



Flag-IP of HTLV-I IN WT and SLiM mutants: detection of endogenous B56 isoforms* by WB (densitometry, n=3).



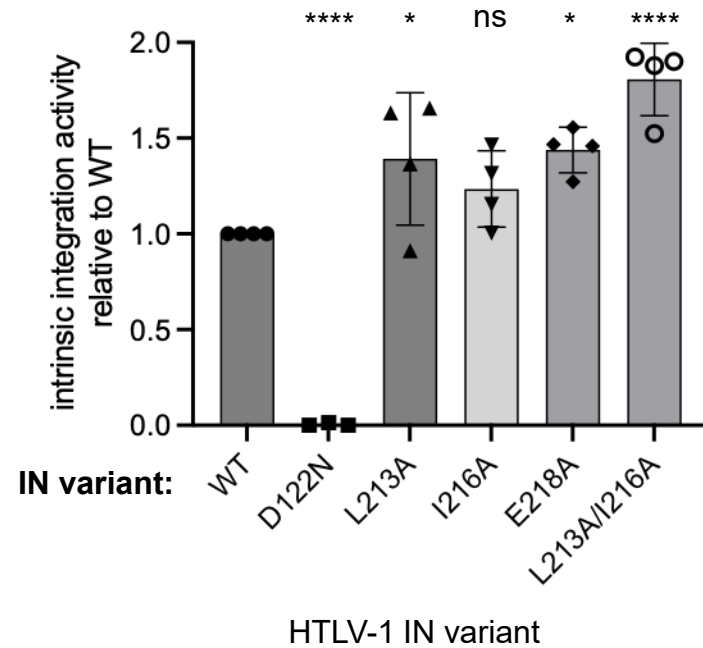
PDB ID: 6TOQ, Barski et al Nat Comms 2020



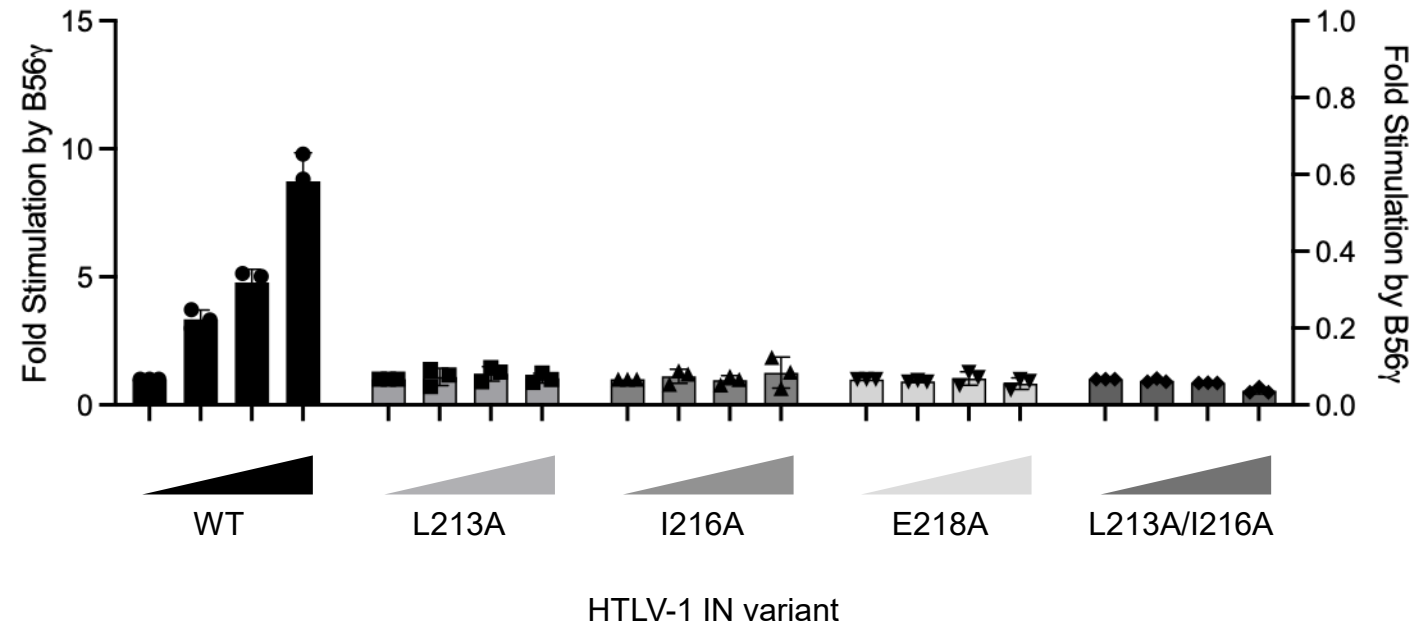
- ✓ IN SLiM mutants:
 - ✓ Complete loss of interaction with B56 α , δ and ϵ
 - ✓ Partial loss of interaction with B56 β (~70% loss) and B56 γ (~50%)



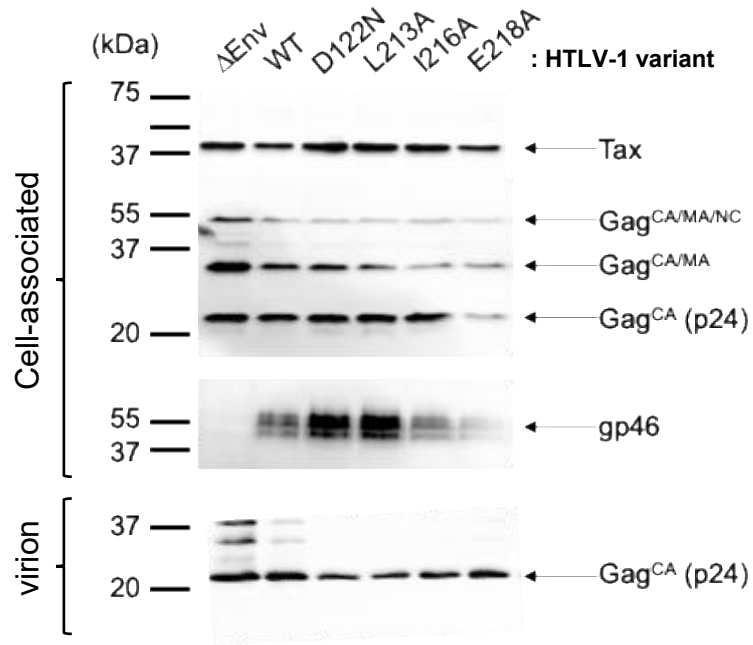
Intrinsic activity of IN mutants



IN mutants are not stimulated by B56

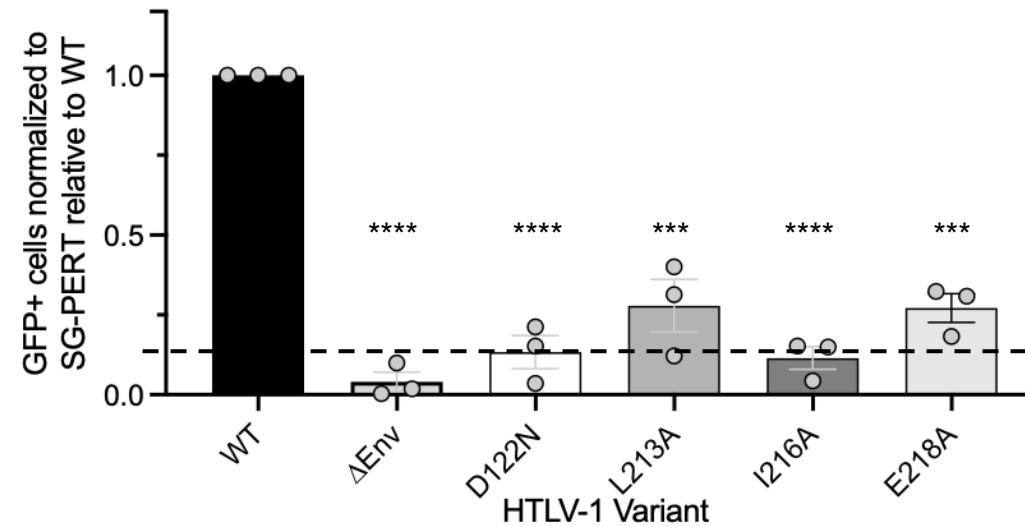


MUTATION OF THE IN SLiM RESULTS IN A LOSS OF INFECTION



Relative infection efficiency

HOS Tax- dependent GFP reporter cell line
(Zahoor et al, J Virol 2014)

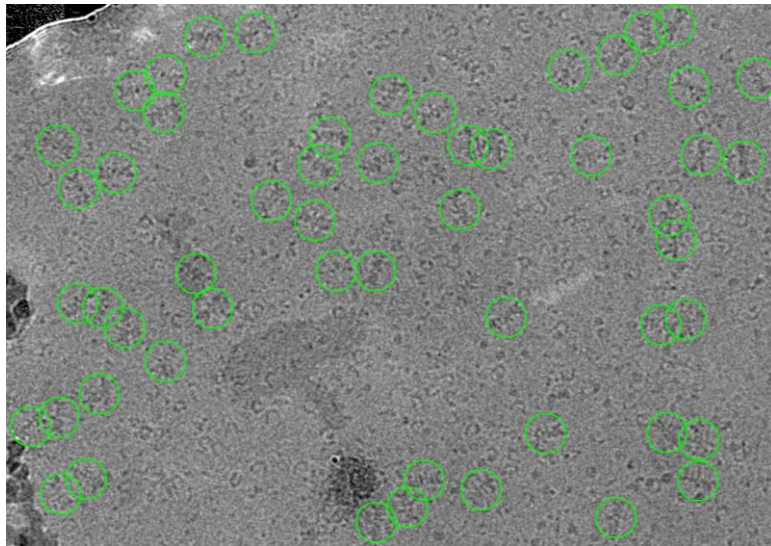


- ✓ IN SLiM mutants display reduced infection efficiency
- ✓ Also confirmed in single round infection assay

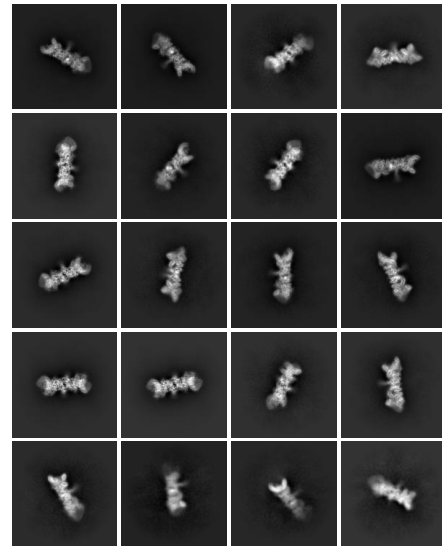
CRYO-EM OF STLV-1 INTASOME: PP2A-B56 HOLOENZYME COMPLEX



2D averages

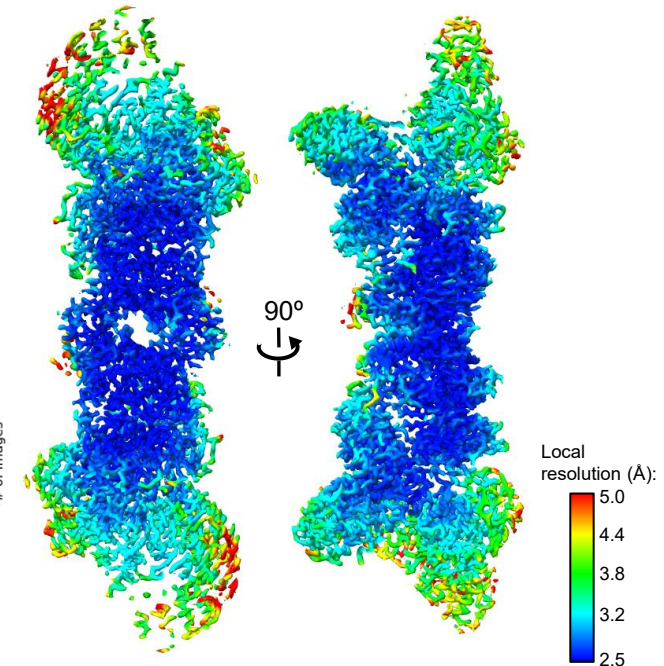
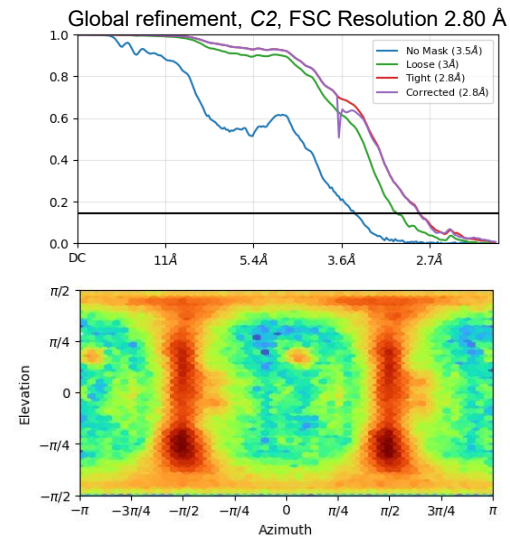


20 nm



20 nm

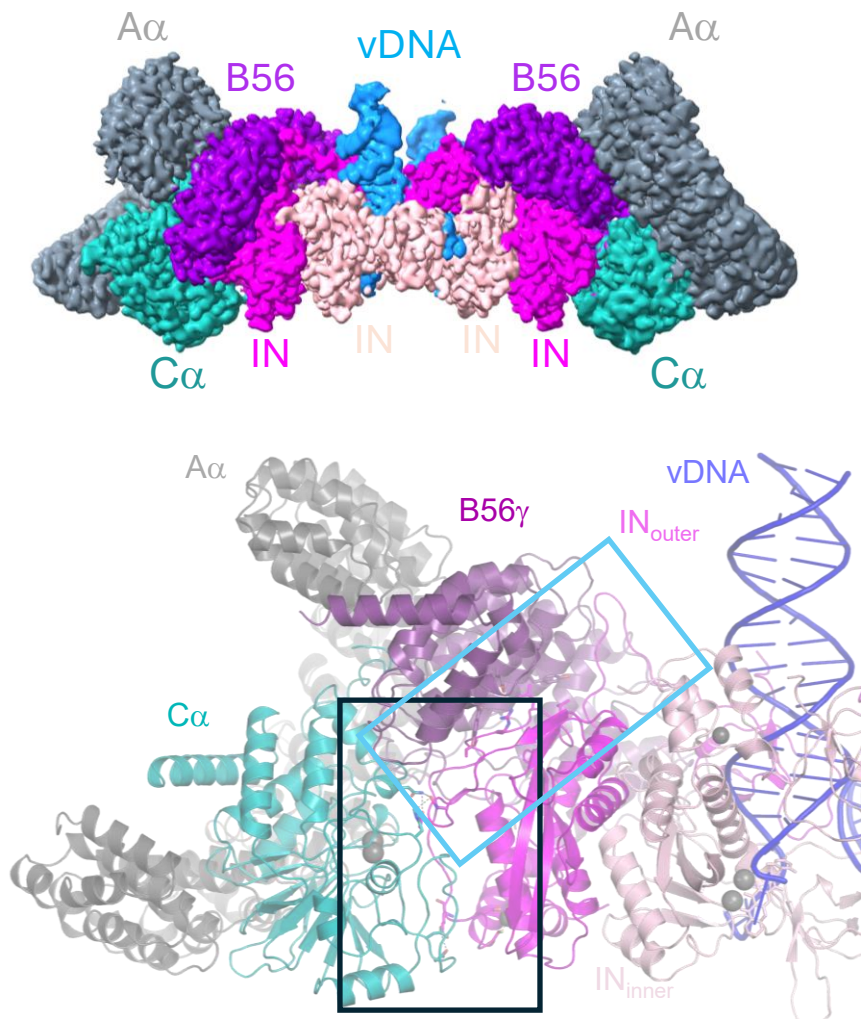
2.8 Å resolution



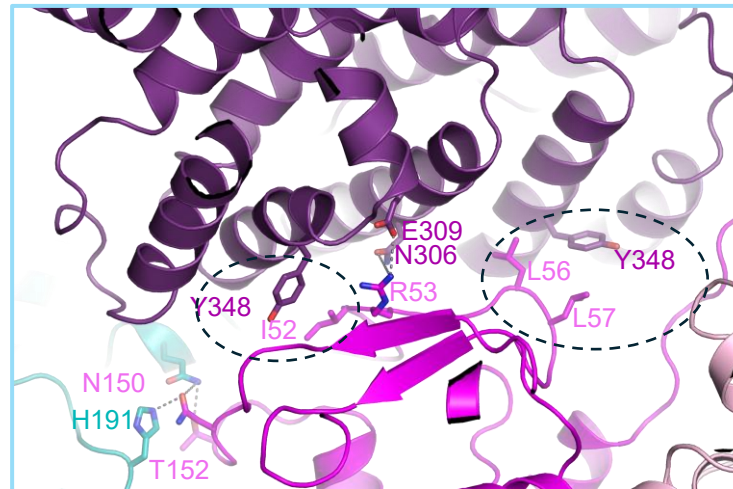
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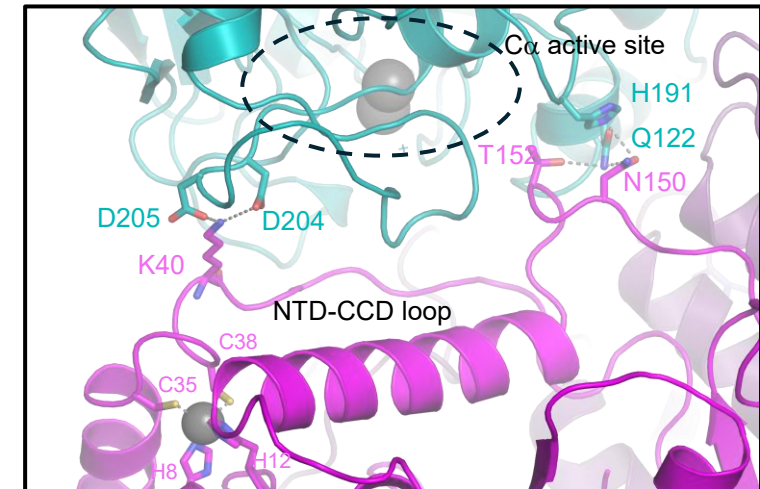
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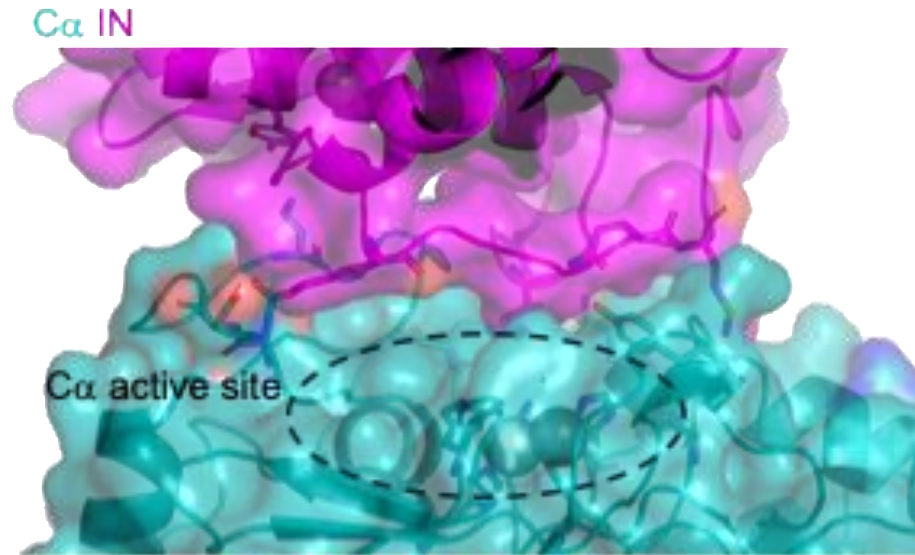
IN B56 γ C α



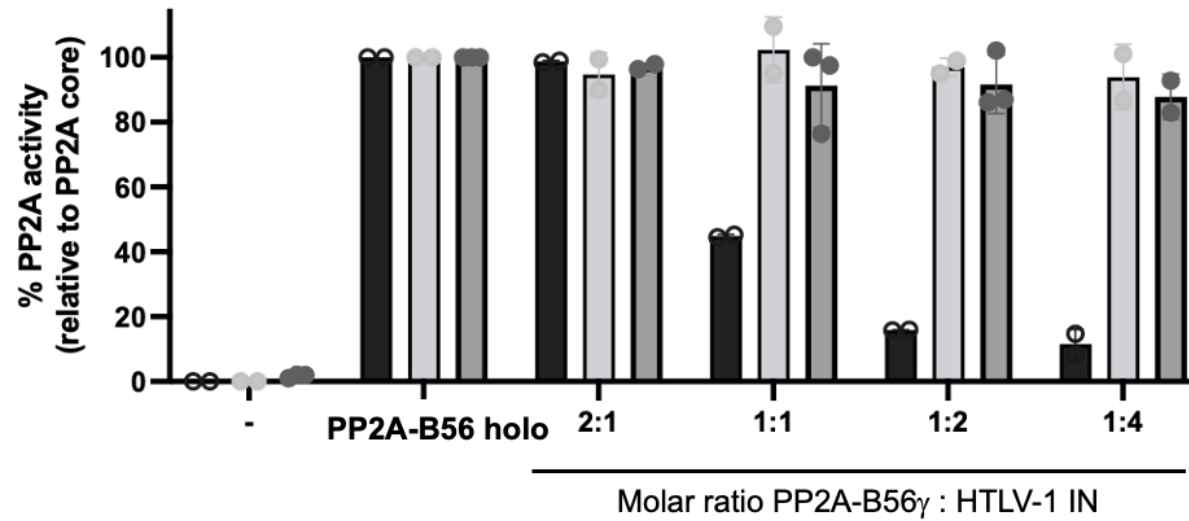
C α IN



STLV-1 IN BLOCKS PP2A-C α ACTIVE SITE?

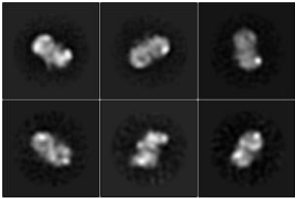


³⁹RKNNPQM⁵¹PRGH : IN residues

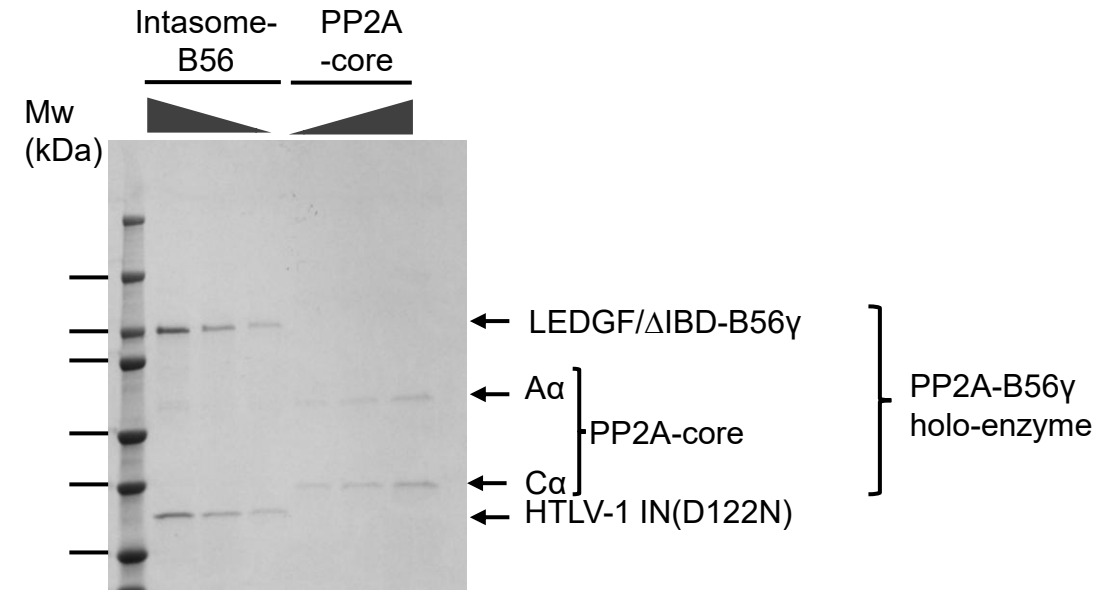
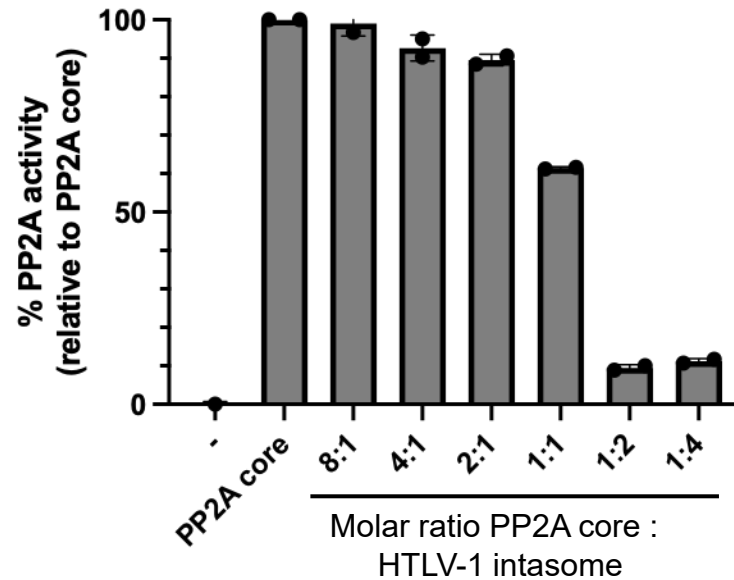
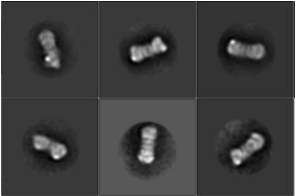


HTLV-1 INTASOME BLOCKS PP2A-B56 CATALYTIC ACTIVITY

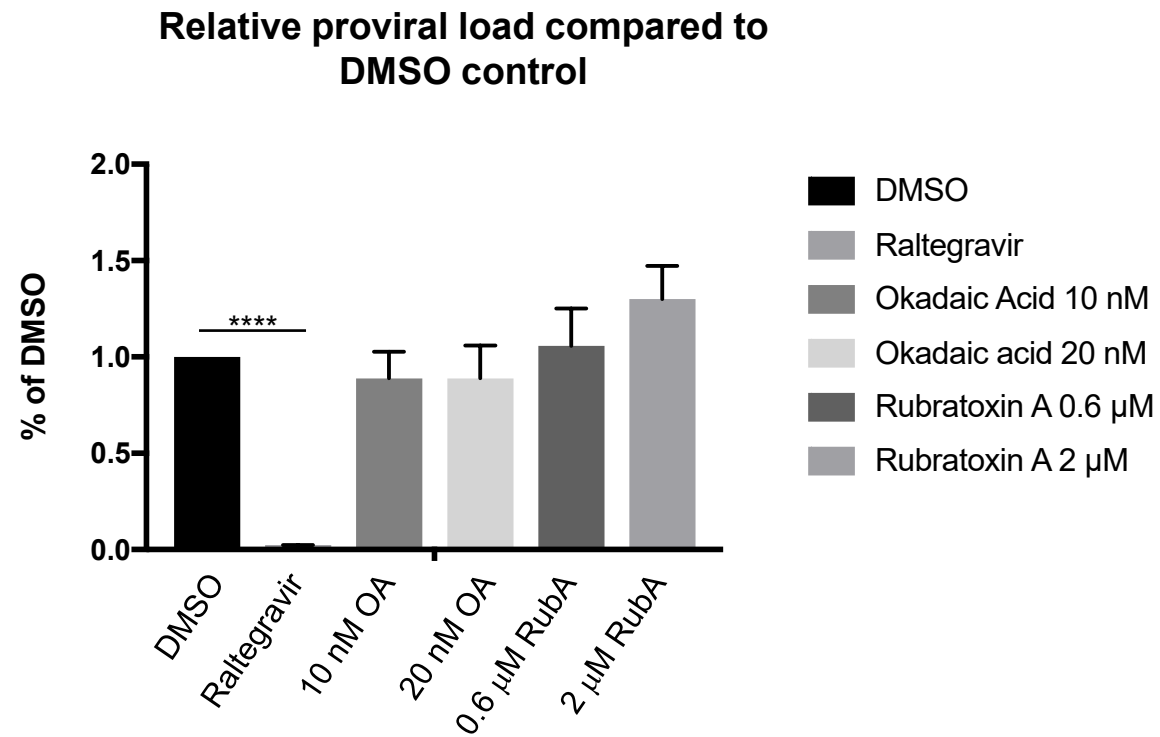
HTLV-1 intasome : B56γ



HTLV-1 intasome : PP2A-B56γ



IS PP2A CATALYTIC ACTIVITY CRITICAL TO ESTABLISH INFECTION?



CONCLUSIONS

- ✓ Interaction between HTLV-1 IN and PP2A-B56 is critical to establish infection
- ✓ Deltaretroviruses use molecular mimicry to gain access to PP2A-B56 (SLiM)
- ✓ The intasome blocks PP2A-B56 activity (similar to endogenous PP2A inhibitor ARPP19) – first example of viral protein to block PP2A-B56 active site

Why does the intasome block PP2A-B56 activity?

- ~ 398,000 copies of B56 δ e.g. in the cell (HeLa, Bekker-Jensen et al Cell Systems, 2017)
- Any retroviral particle contains 50-100 monomers of IN...
 - Unlikely role of IN is to block PP2A-B56 activity *per se*
 - PP2A activity is not essential in the context of HTLV-1 infection
 - **Interaction is for structural reasons**



ACKNOWLEDGMENTS



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Emily Ford

Christine Hilcenko

Owen Ambridge

NHLI FACS FACILITY

Radhika Patel

Yanping Guo

Francis Crick Institute

Cherepanov group

Emma Punch

LonCEM

Nora Cronin



Aaron
Talk
11:30am



National Institute of Health (NIH)

Chou-Zen Giam



Medical
Research
Council

THE ROYAL
SOCIETY



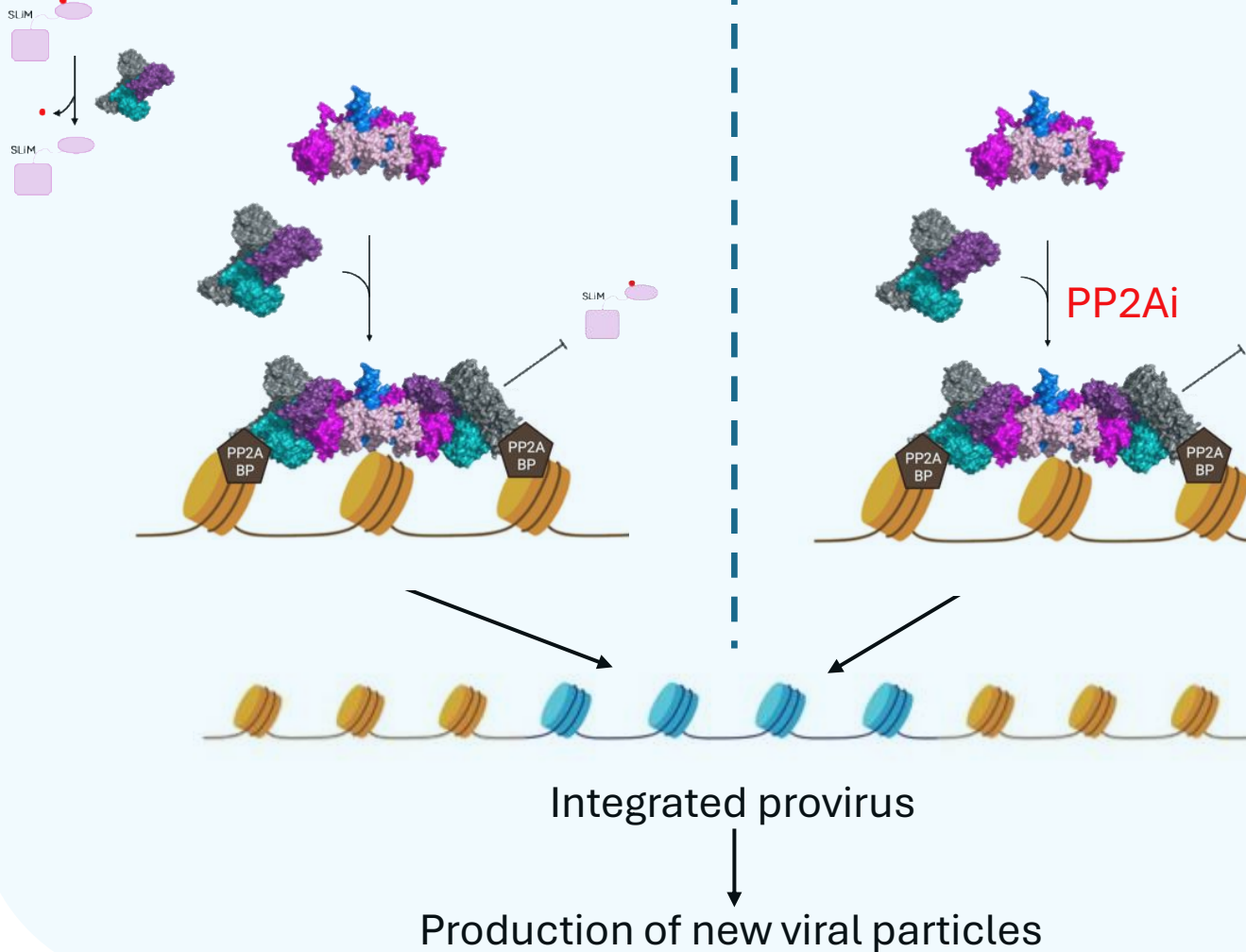
RECIPROCAL SPACE
RECIPROCAL SPACE



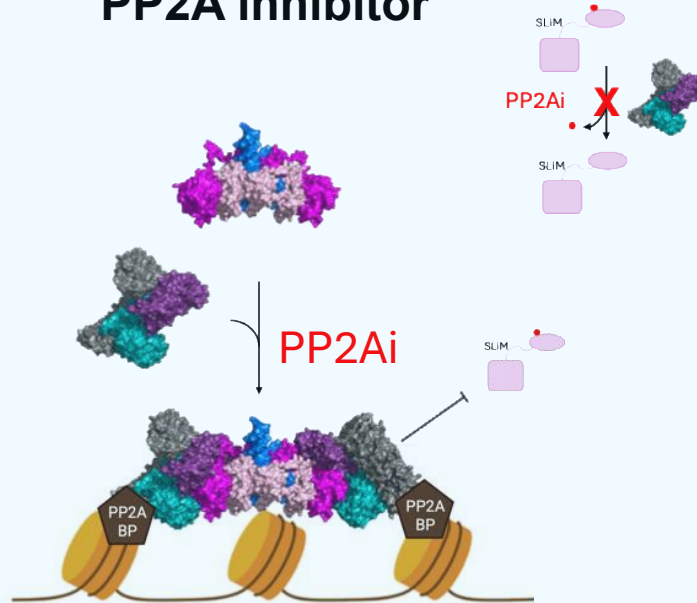
IMPERIAL

SUMMARY

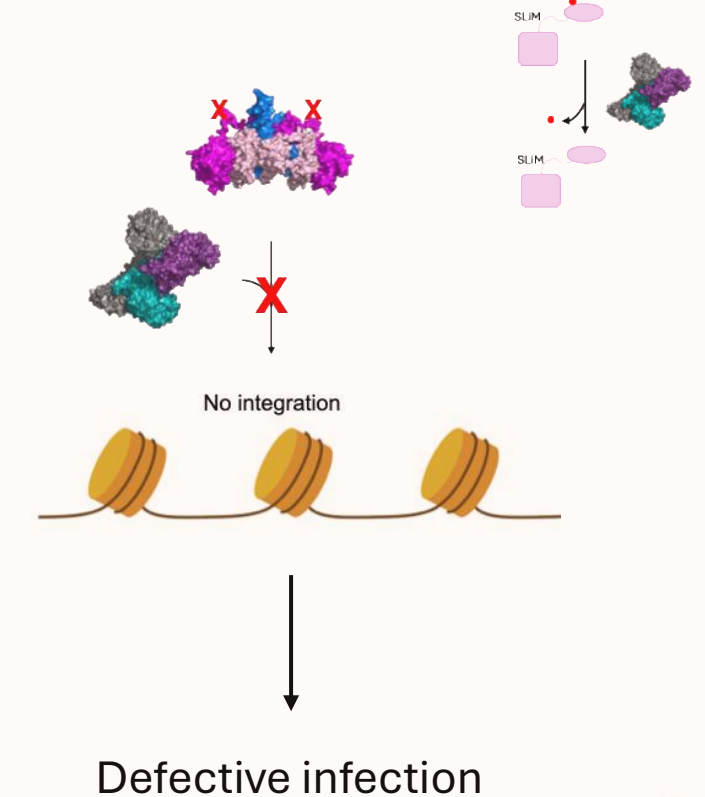
WT HTLV-1 integrase No PP2A inhibitor



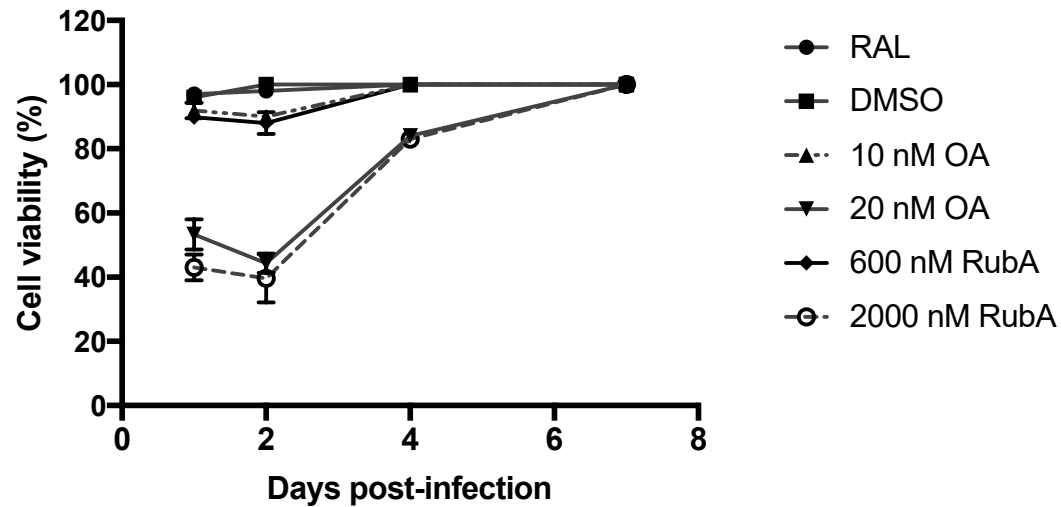
WT HTLV-1 integrase PP2A inhibitor



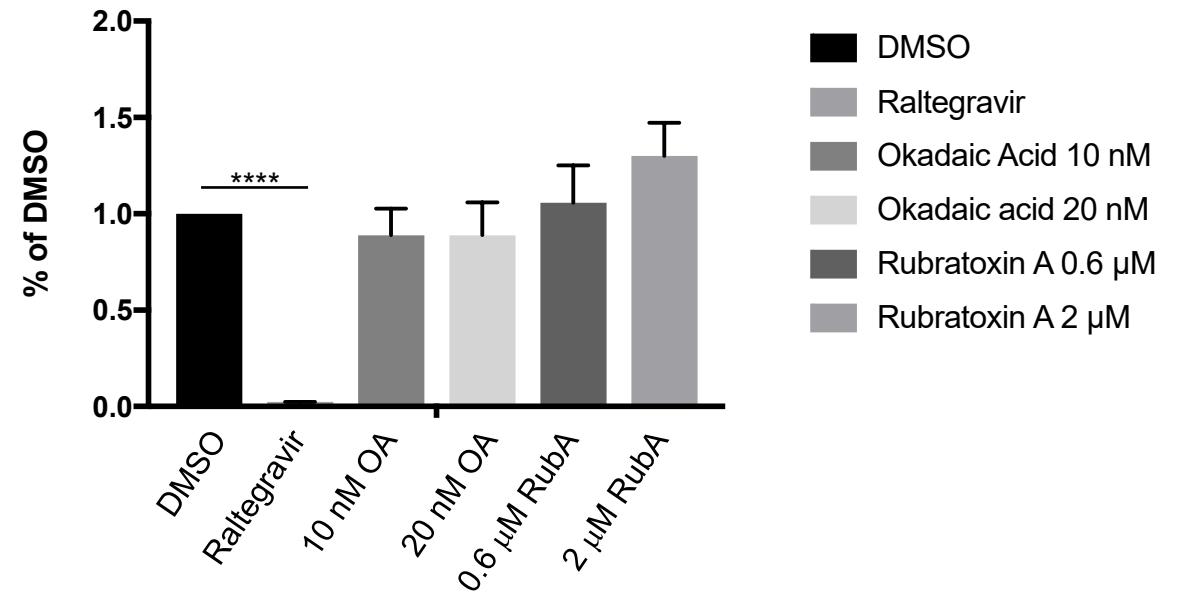
HTLV-1 IN SLiM mutant No PP2A inhibitor



IS PP2A CATALYTIC ACTIVITY CRITICAL TO ESTABLISH INFECTION?

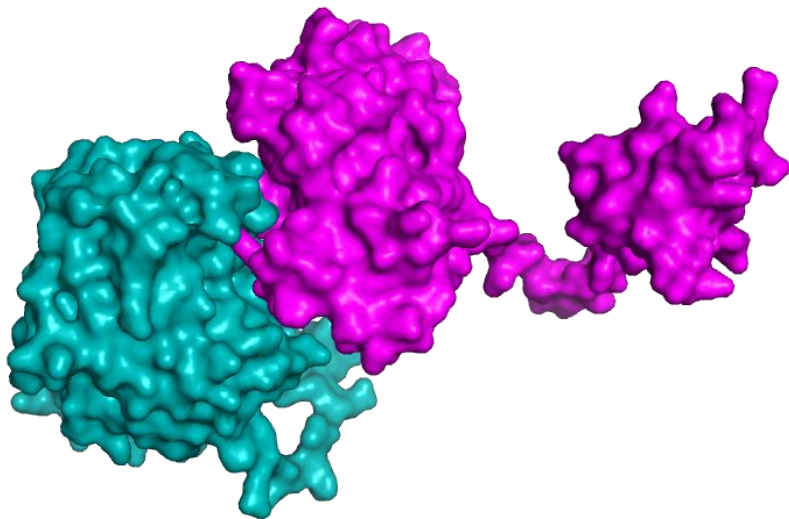
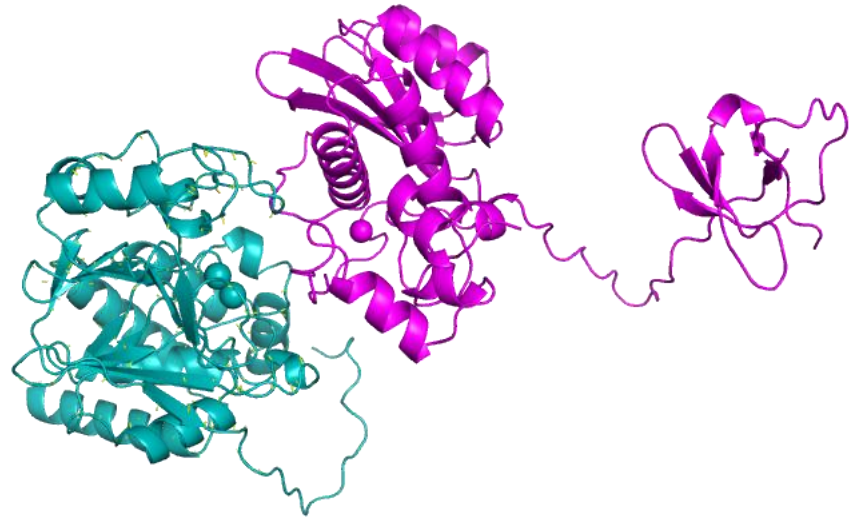


Relative proviral load compared to DMSO control

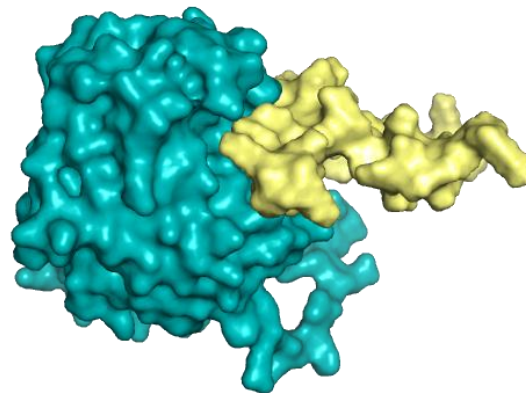
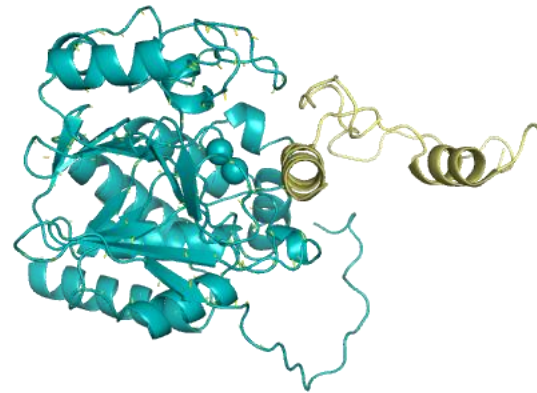


PP2A IS INHIBITED BY CELLULAR PROTEINS

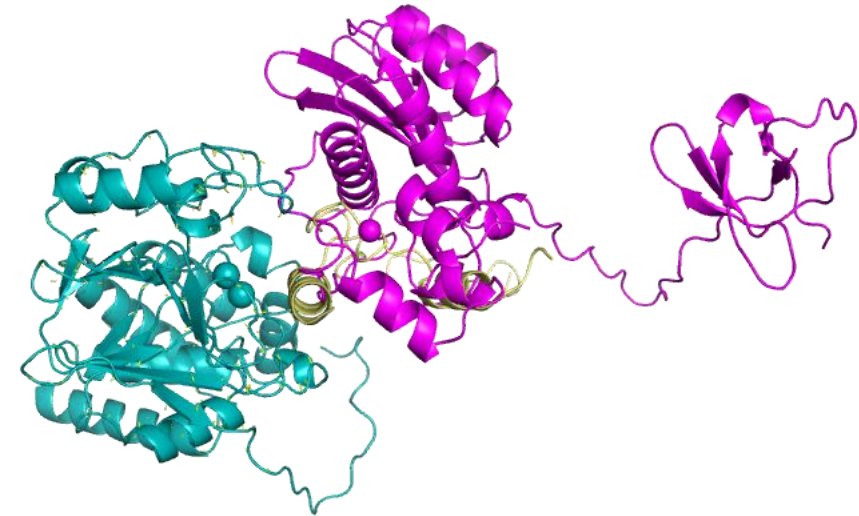
C α : STLV-1 IN



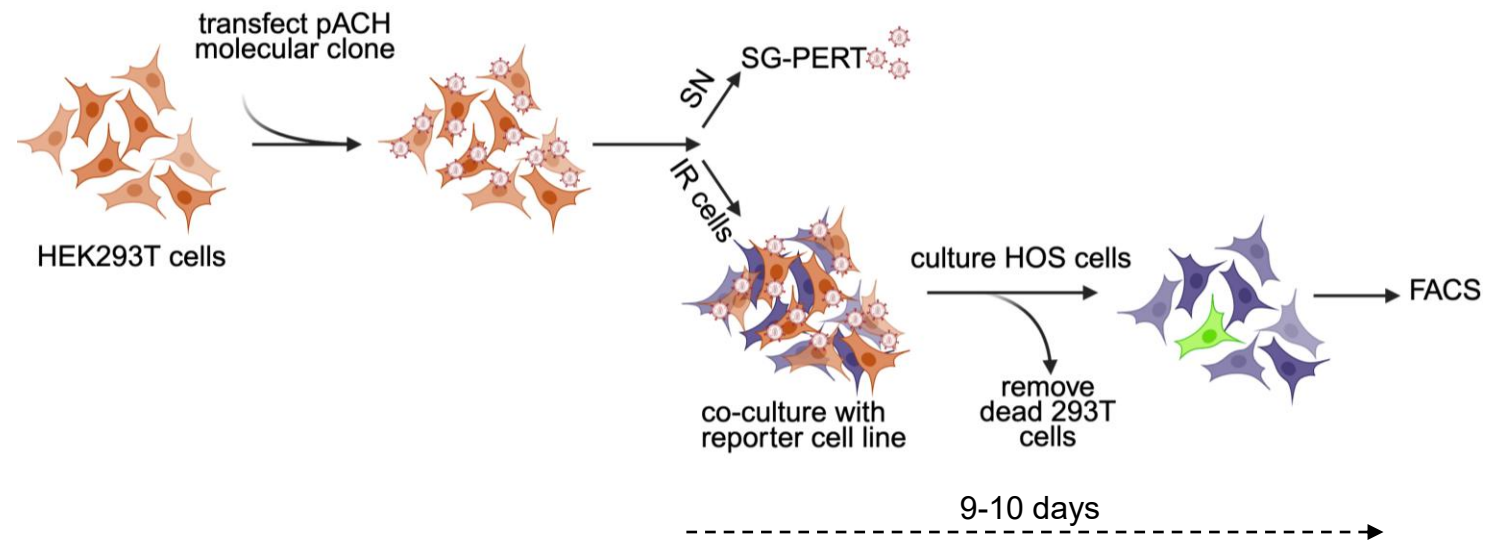
C α : ARPP19 (8TTB)



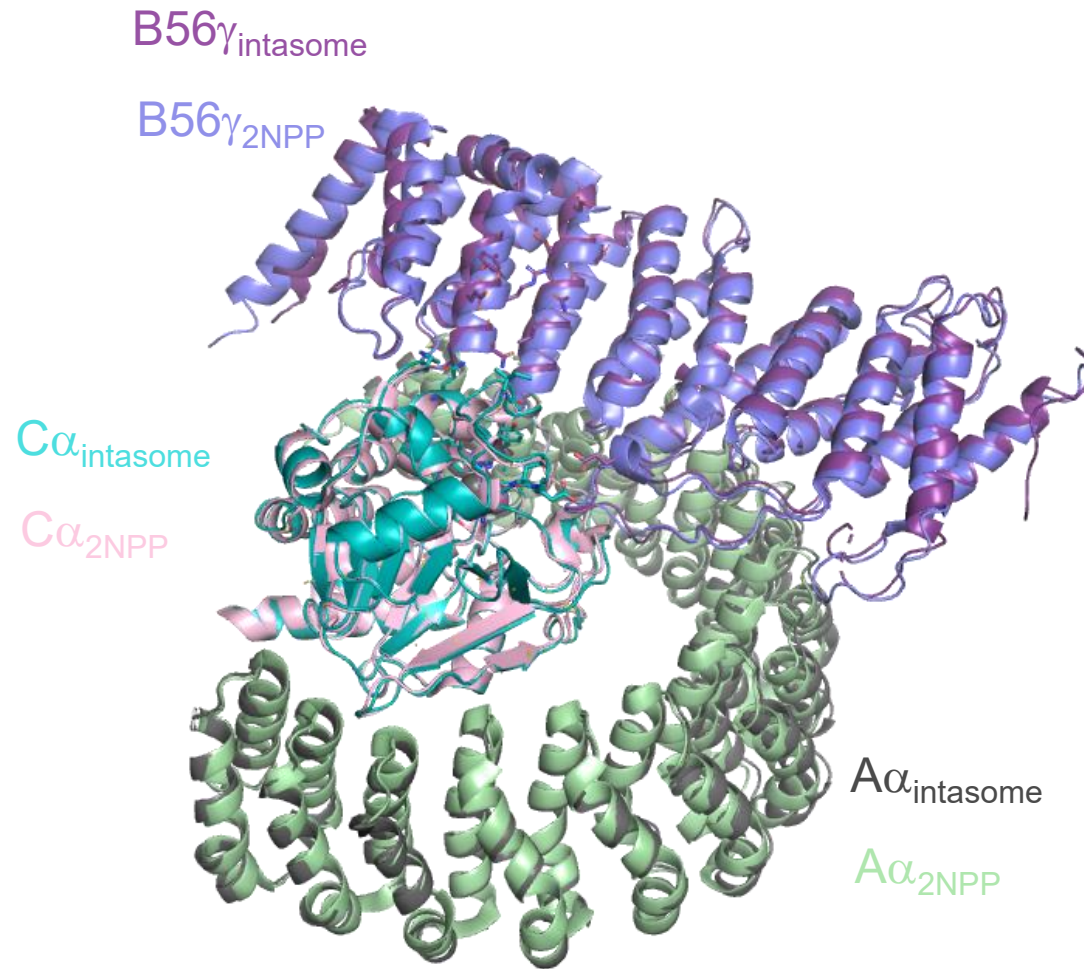
overlay



INFECTION ASSAY – CO-CULTURE WITH REPORTER HOS CELL LINE



SUPERIMPOSITION FREE PP2A-B56 HOLOENZYME ONTO PP2A-B56 STRUCTURE OF INTASOME COMPLEX



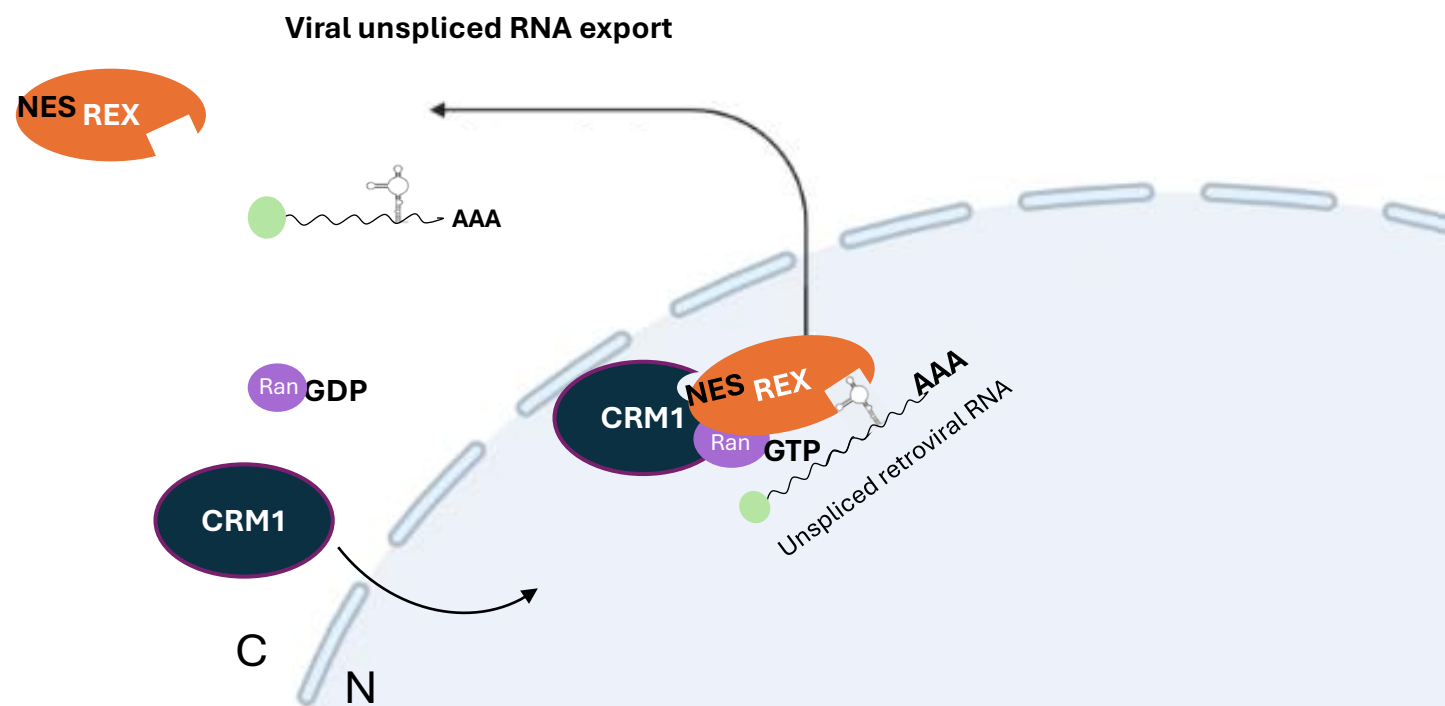
CRM1/XPO1 hijacking by HTLV-1 Rex reprograms the UPF1 helicase from antiviral to proviral functions

Vincent Mocquet, PhD
INSERM, ENS de Lyon, France

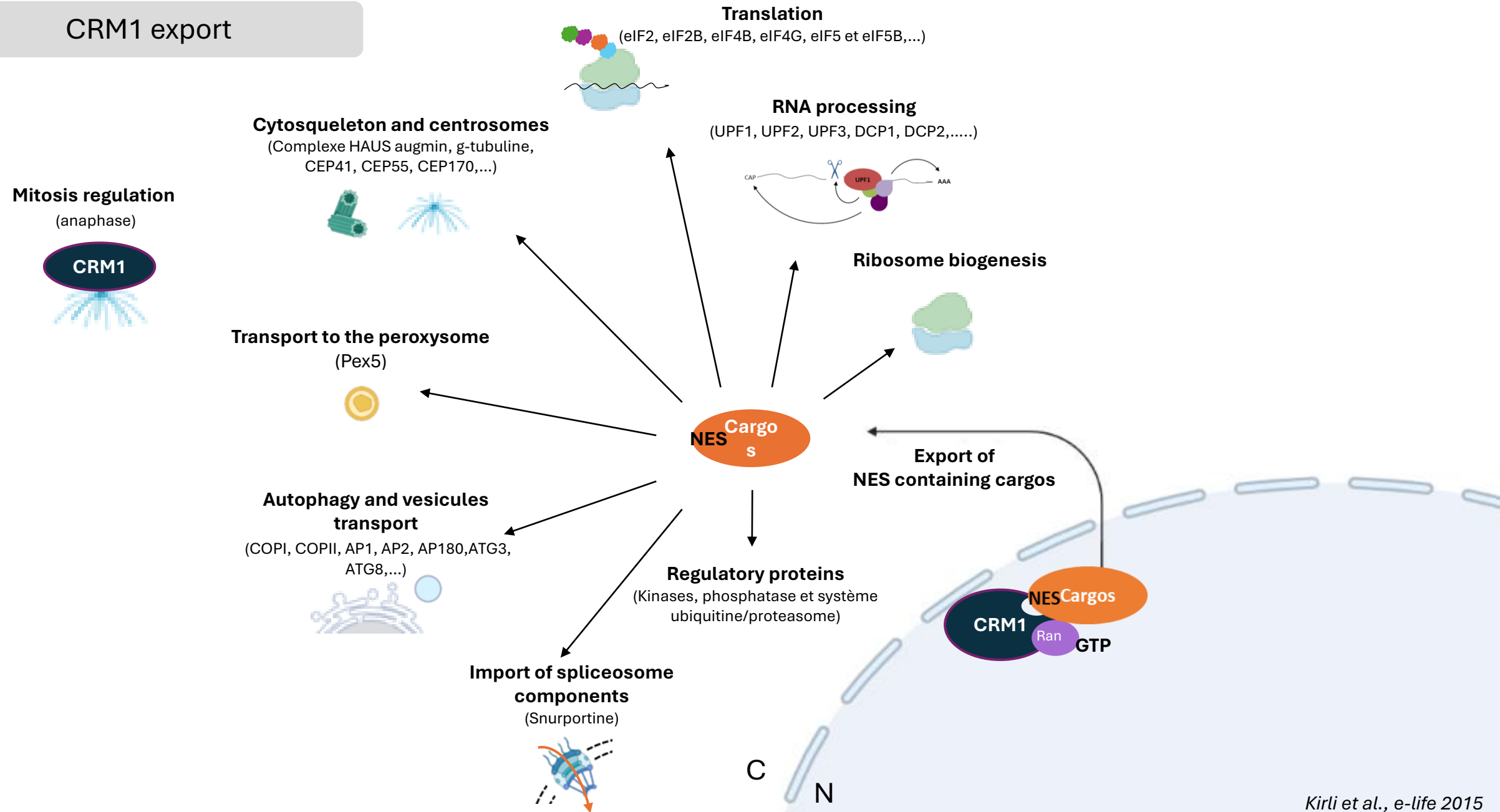
Conflicts of interest

I have no conflicts of interest

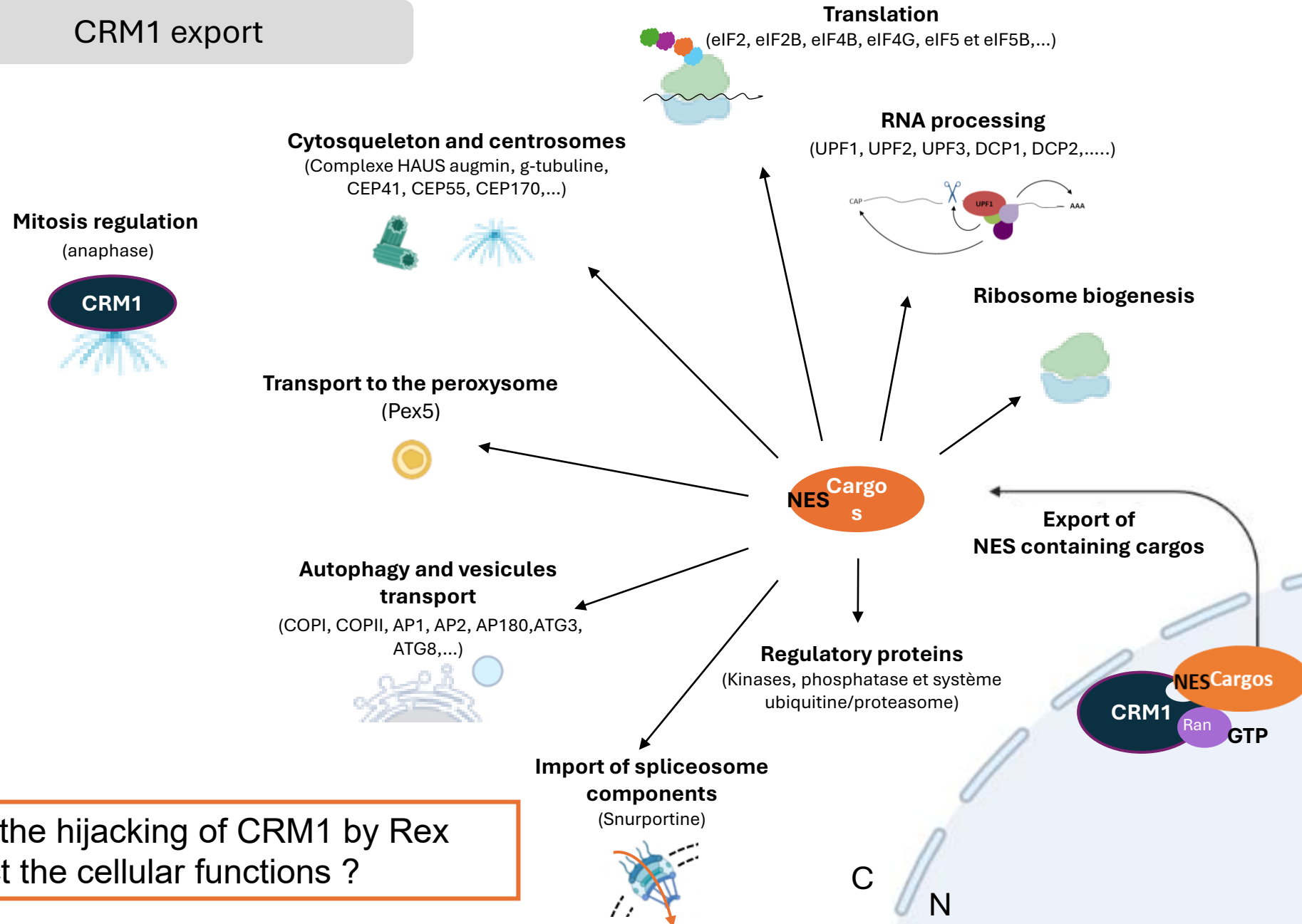
Unspliced vRNA export



CRM1 export

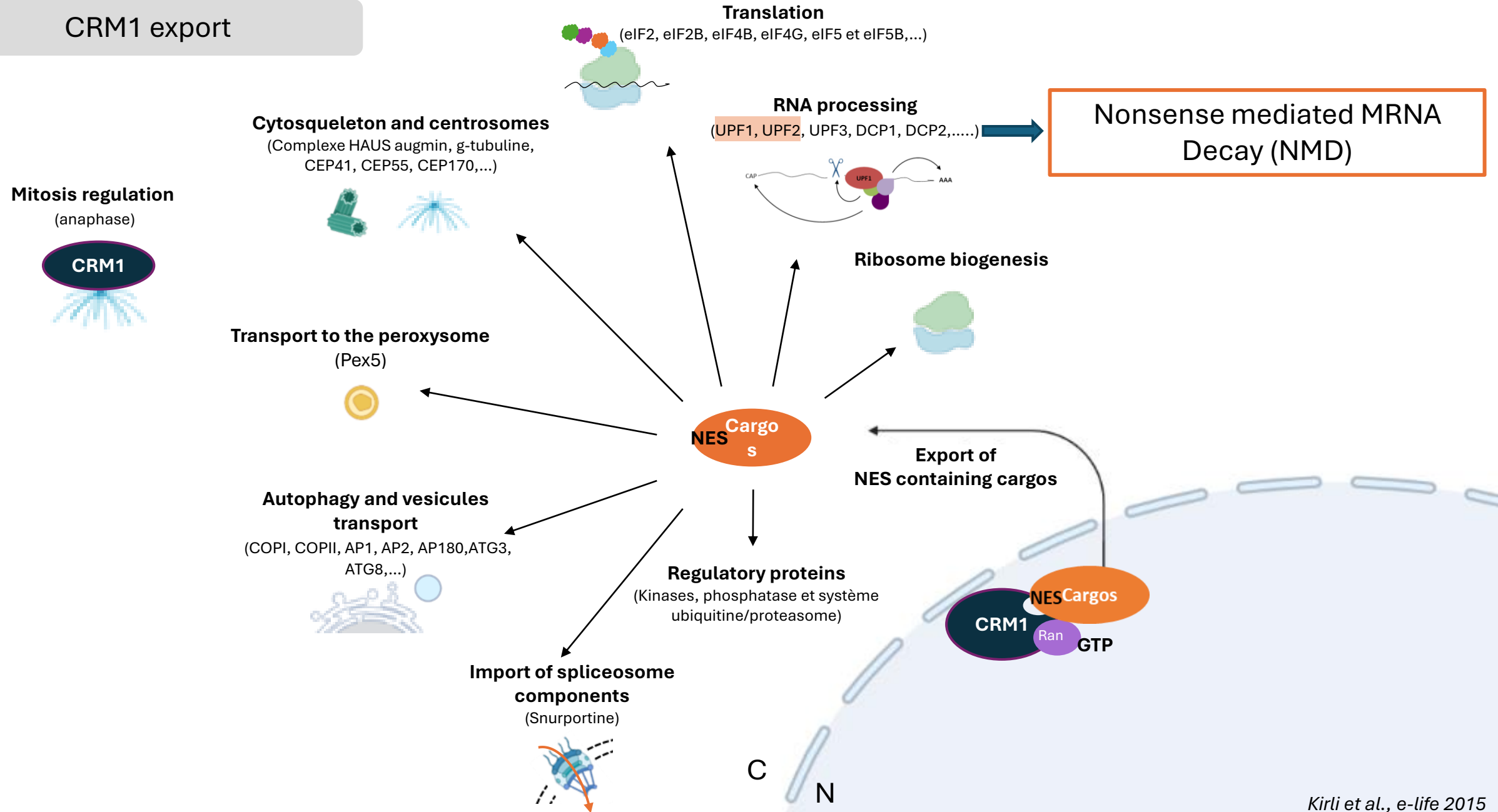


CRM1 export

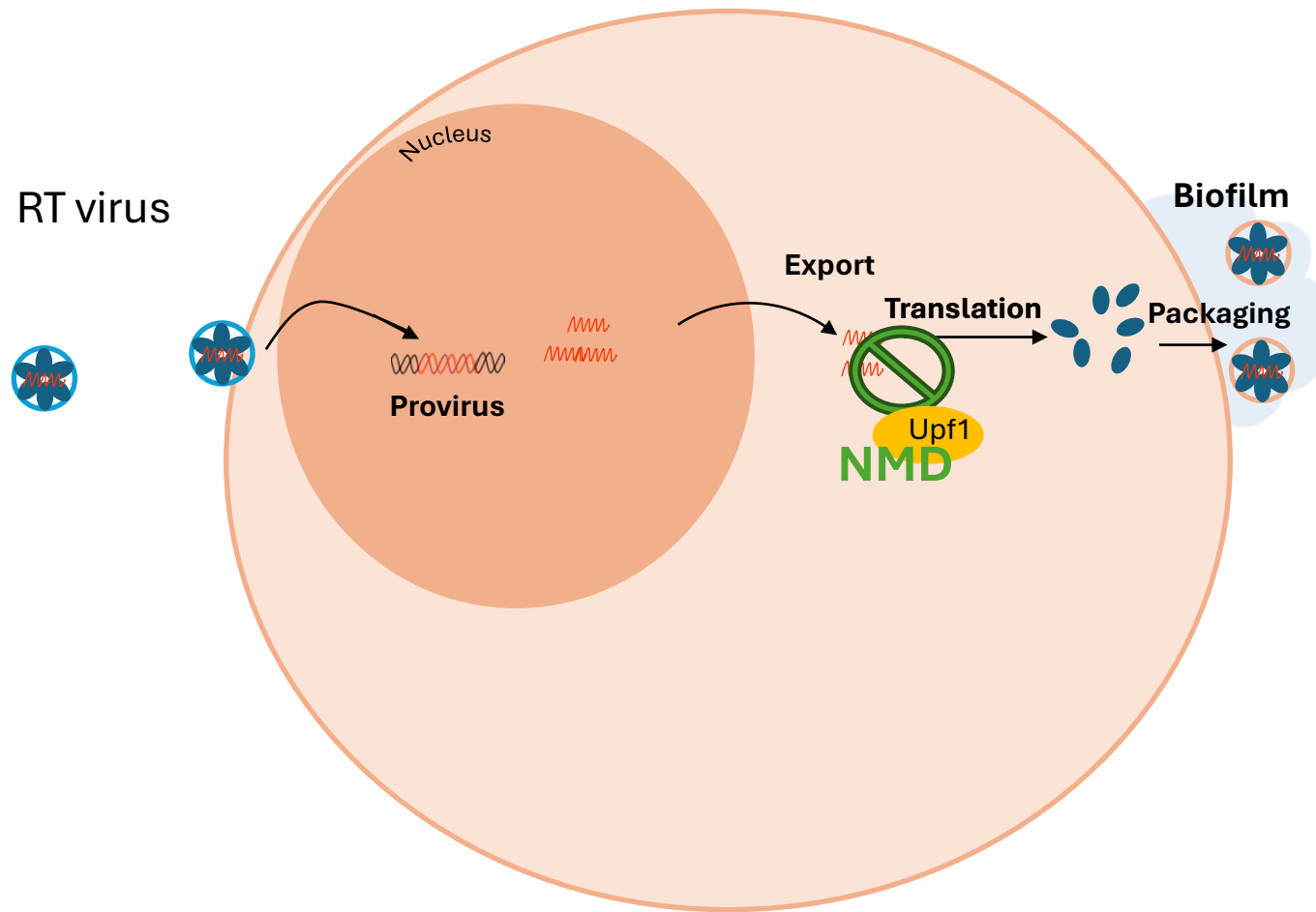


Does the hijacking of CRM1 by Rex impact the cellular functions ?

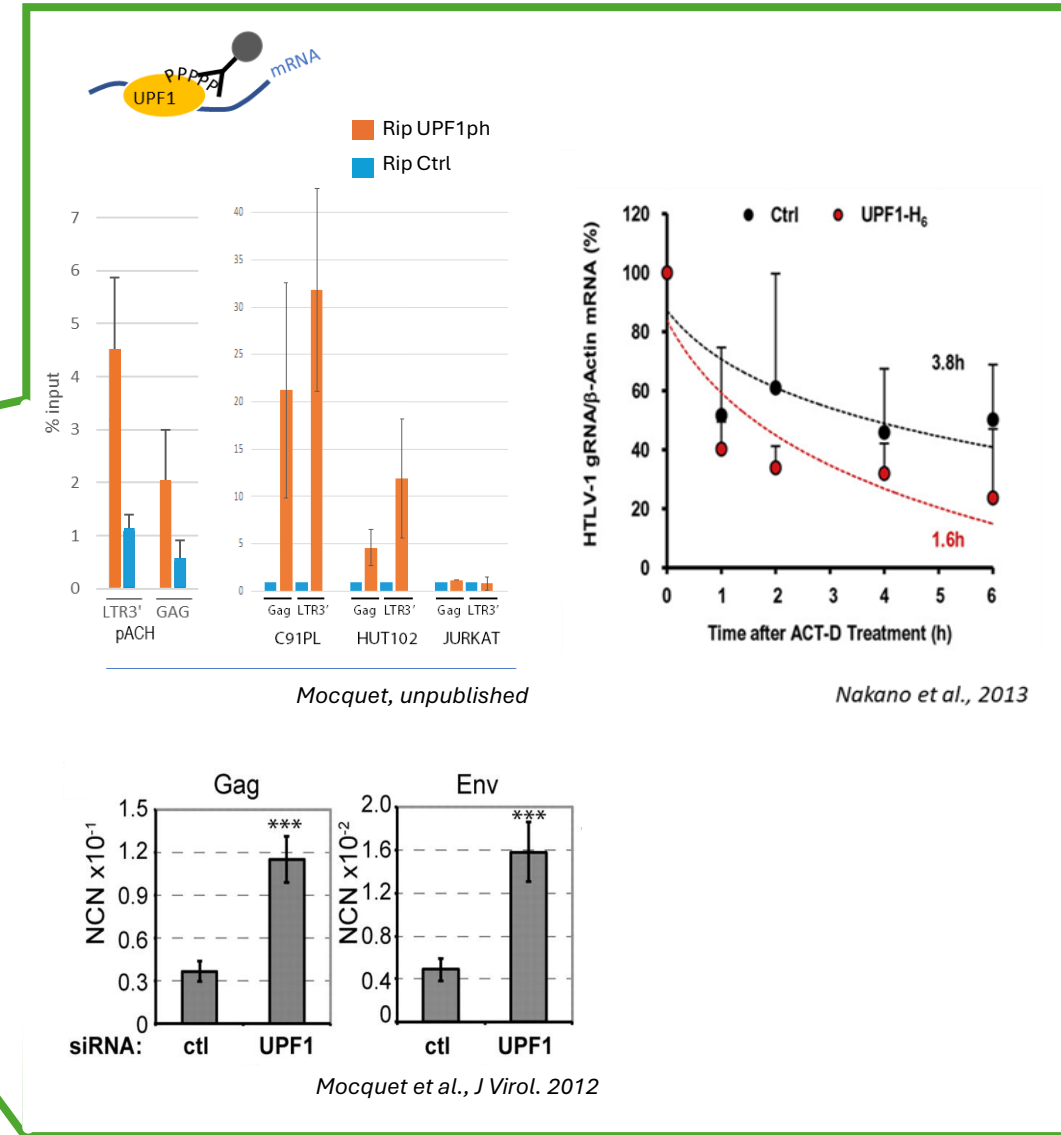
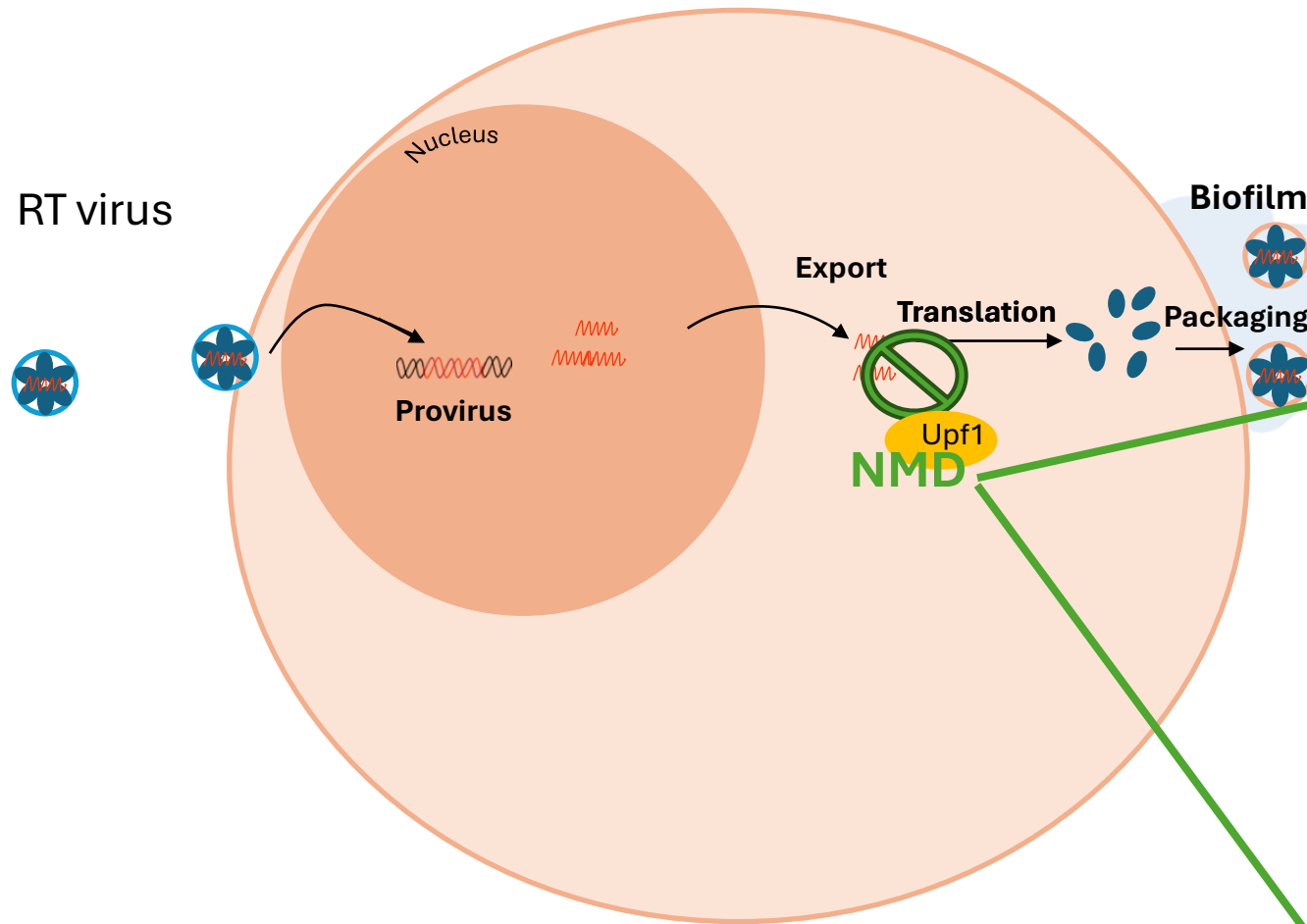
CRM1 export



NMD during infection



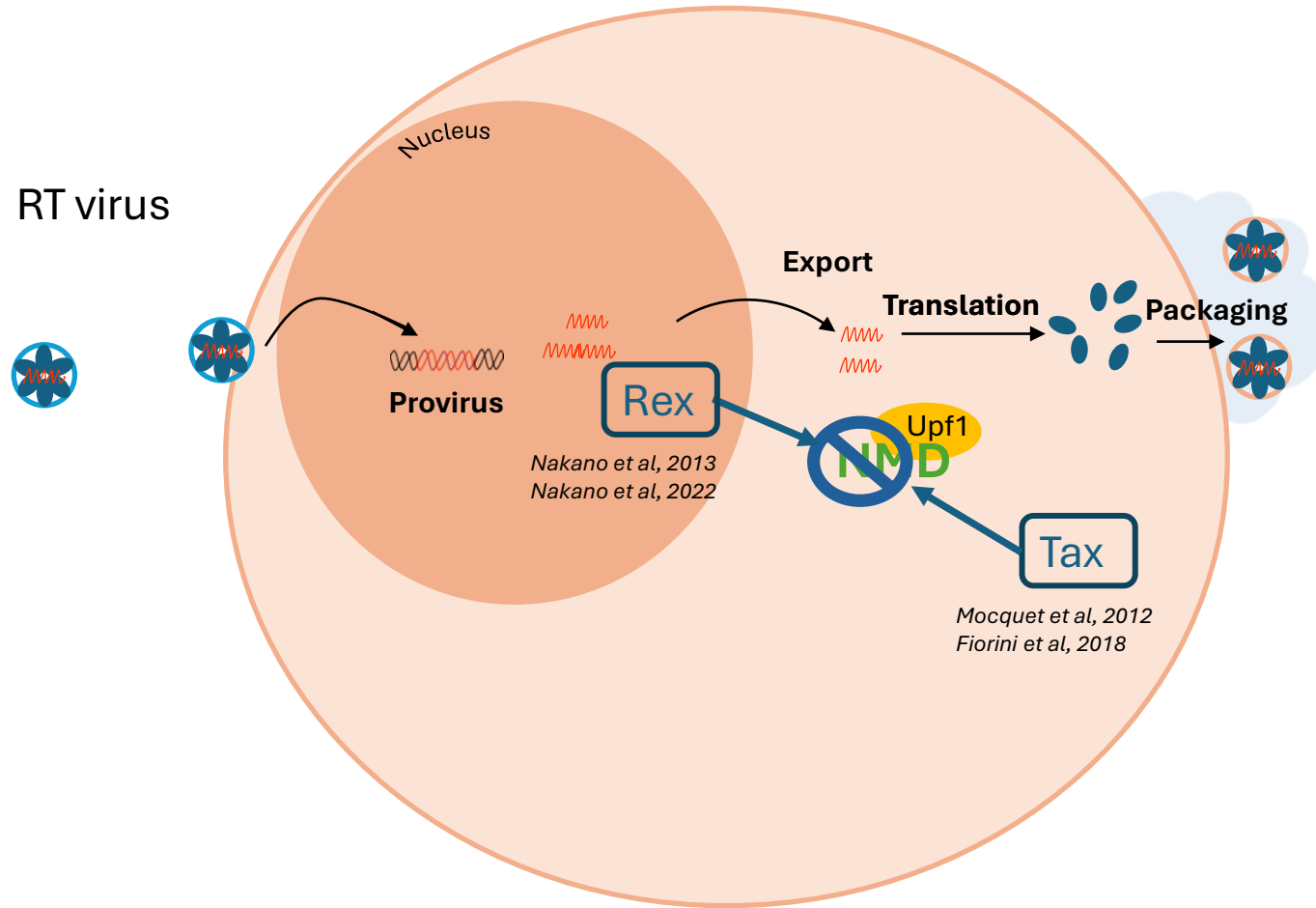
NMD during infection

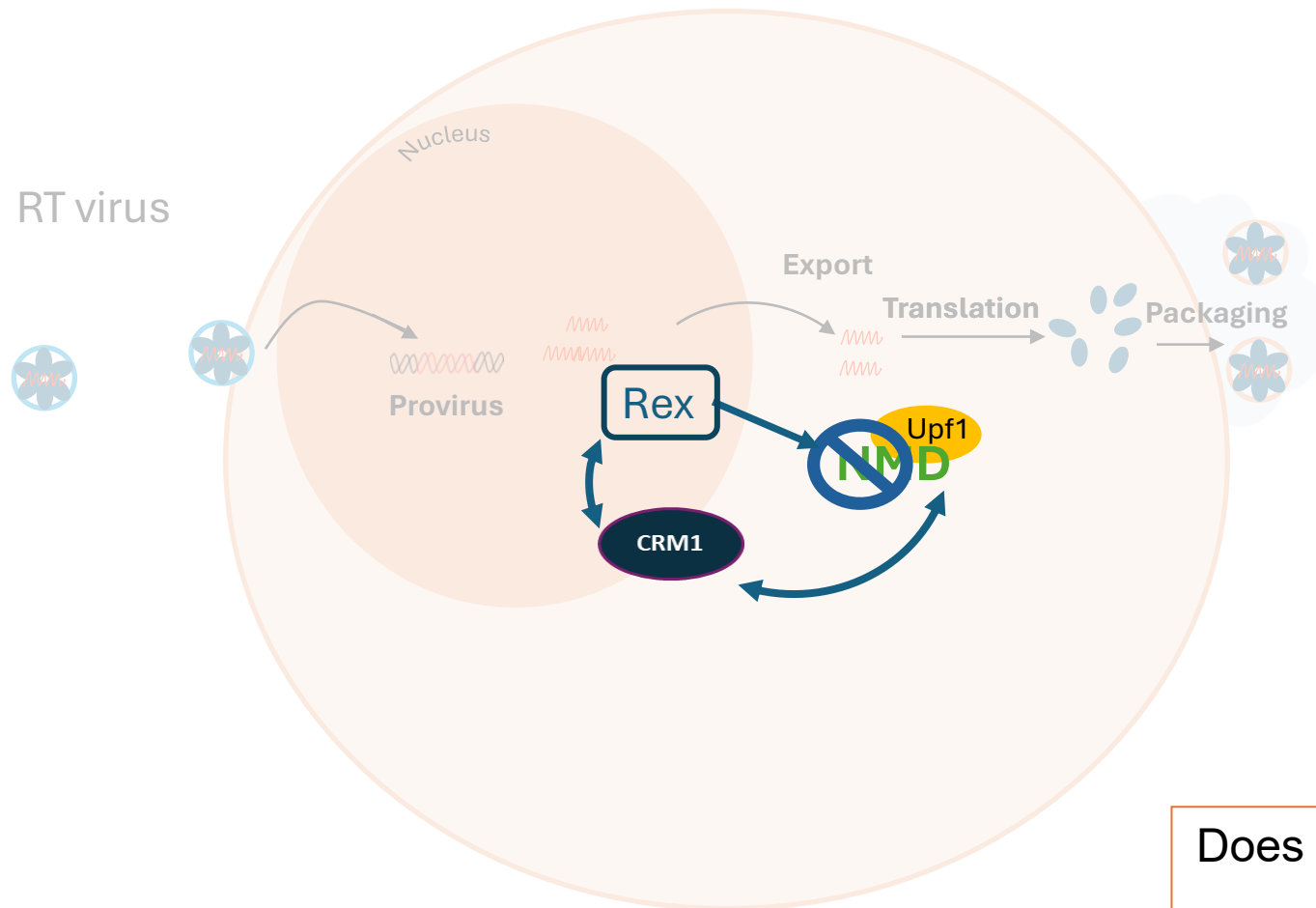


We and others showed that the retroviral mRNA is targeted by a contranlational mRNA decay pathway called NMD.

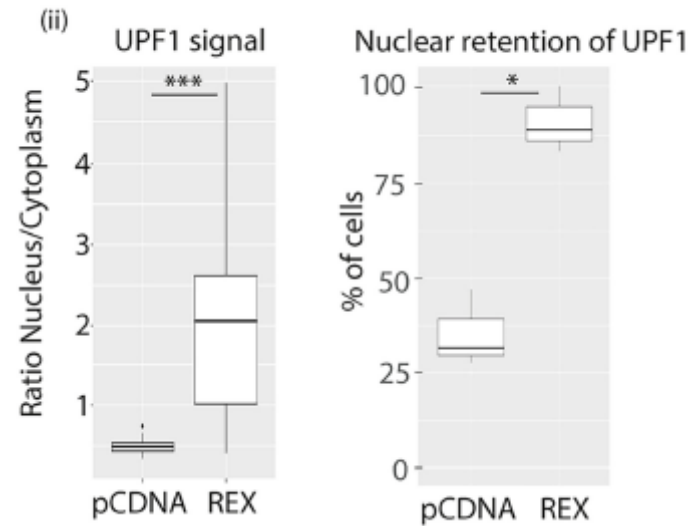
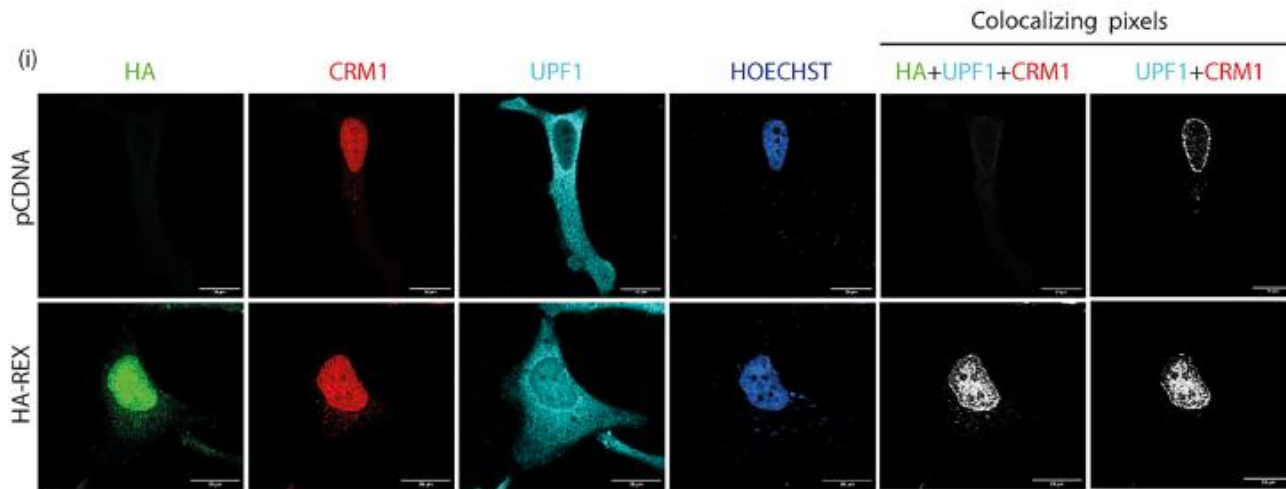
NMD during infection

RT virus

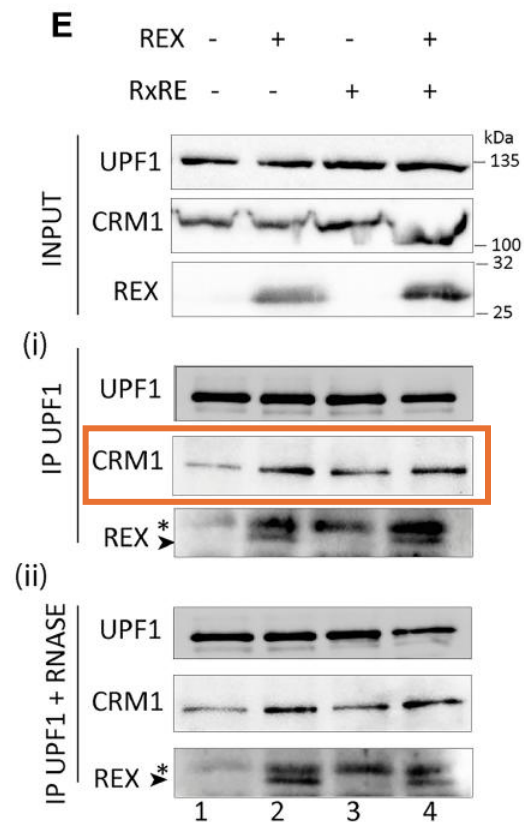
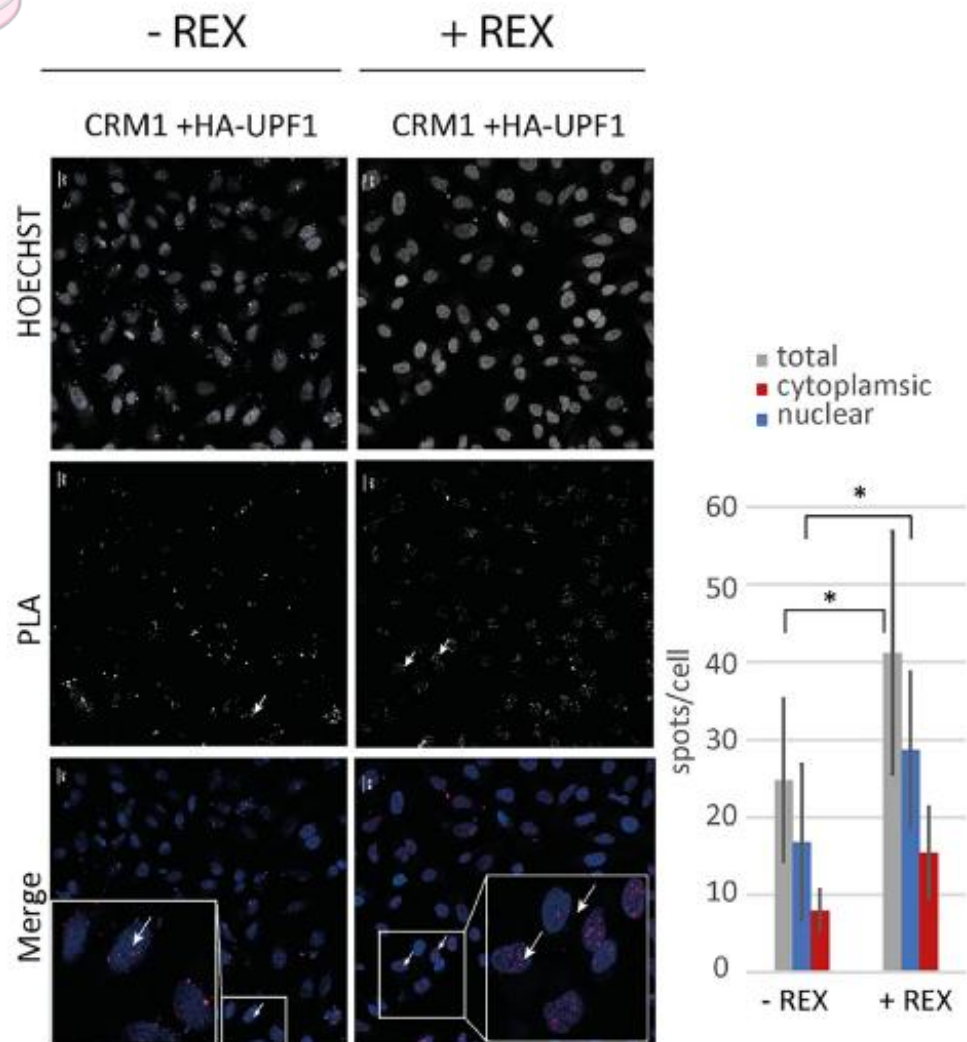




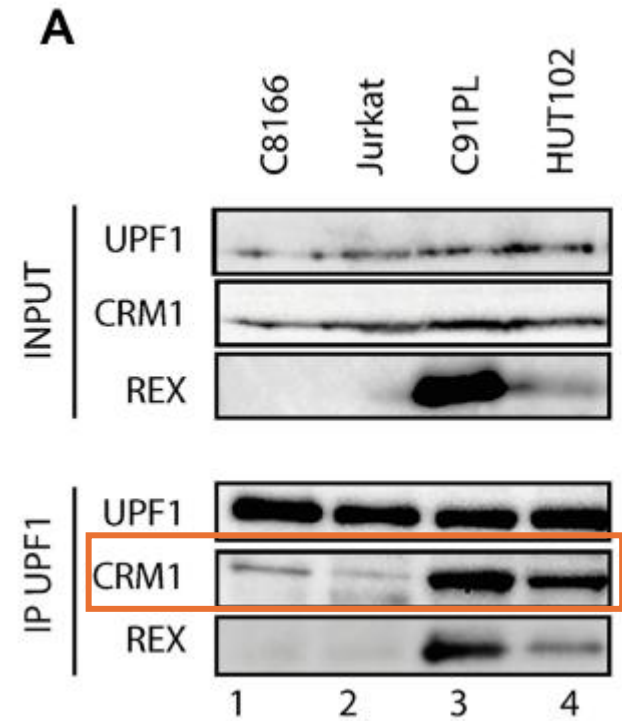
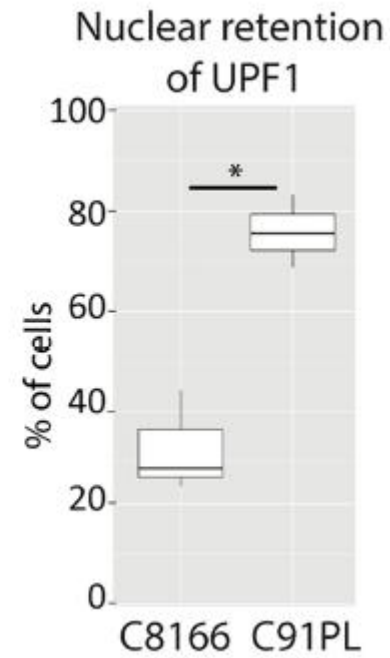
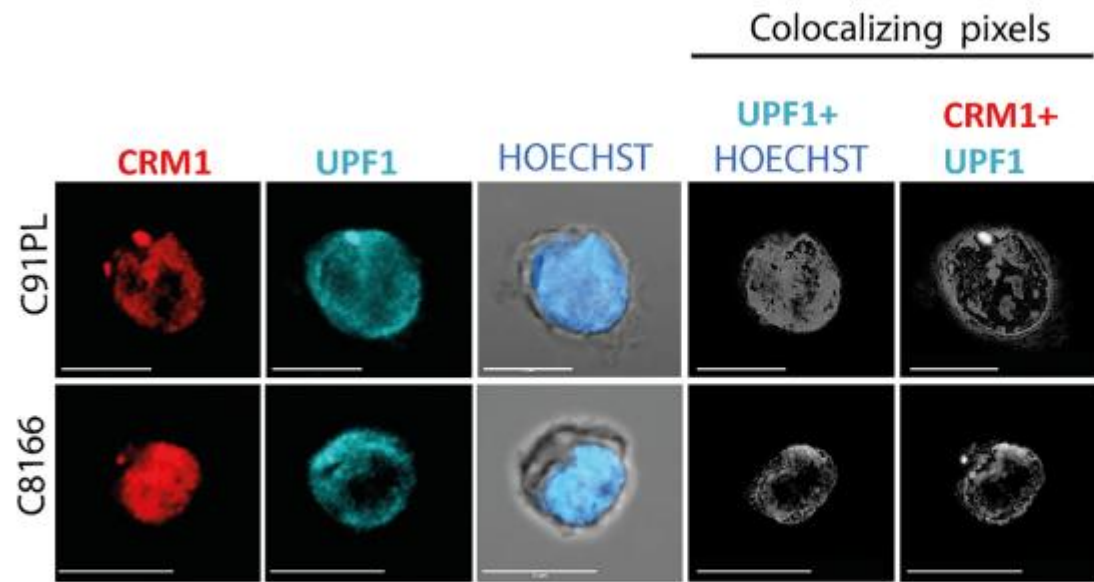
Does the hijacking of CRM1 by Rex impact NMD/UPF1 antiviral activity ?



Rex expression UPF1 nuclear retention

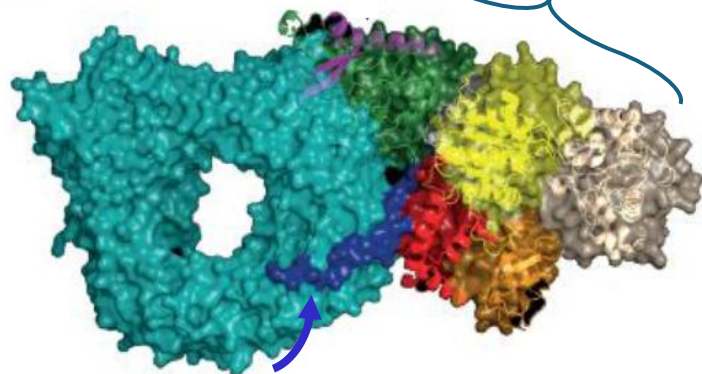


Rex expression Locking off the CRM1 UPF1 complex

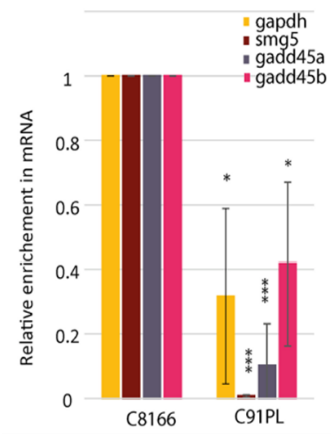
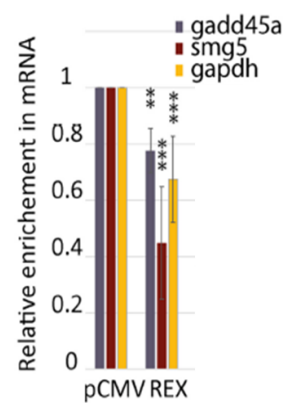
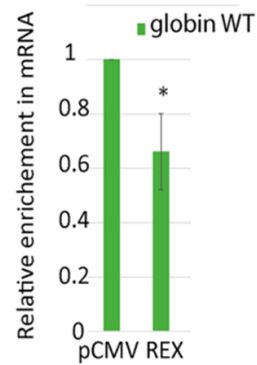
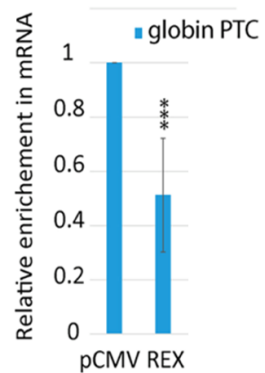


CRM1

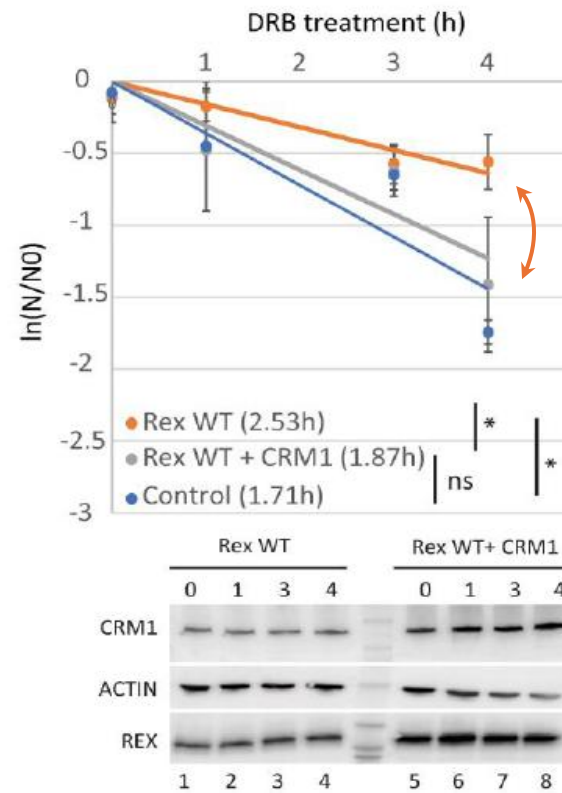
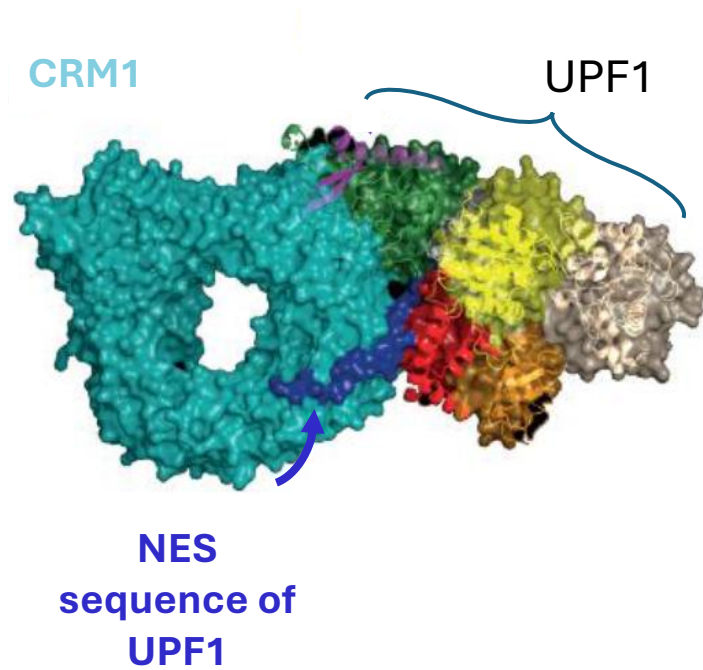
UPF1



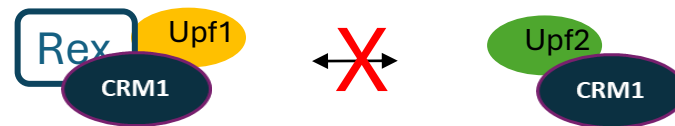
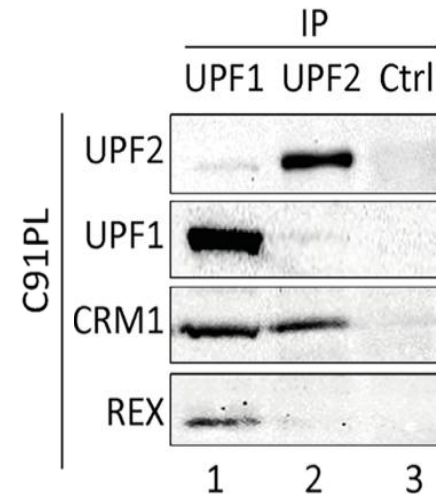
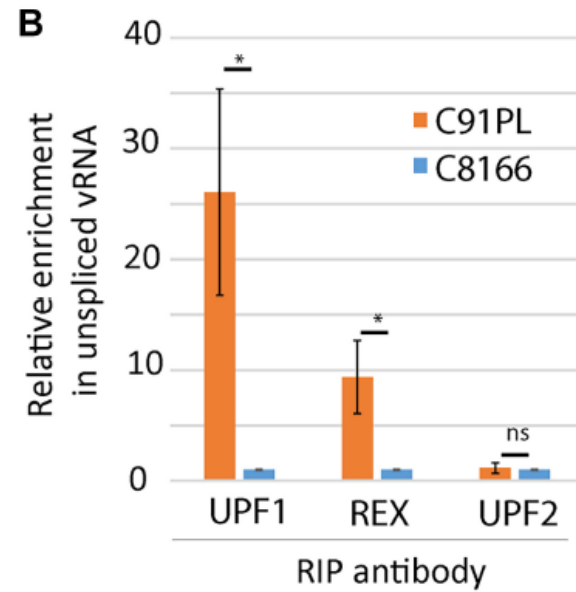
NES
sequence of
UPF1



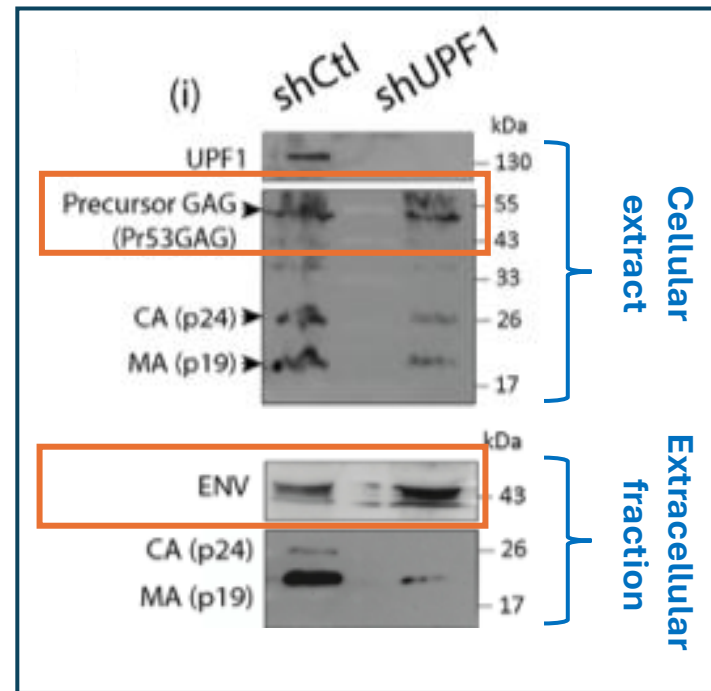
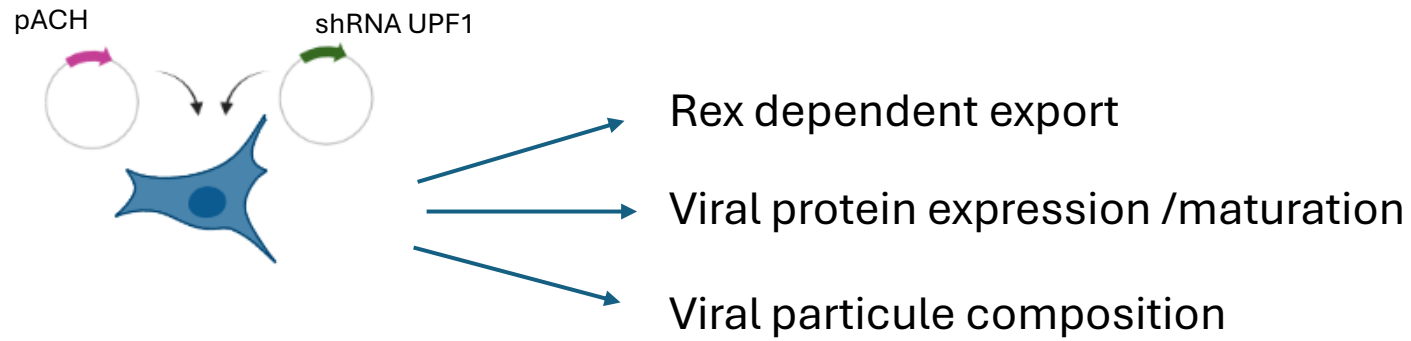
Rex expression — UPF1 binding to cellular RNA



Rex expression UPF1 binding to cellular RNA NMD inhibition

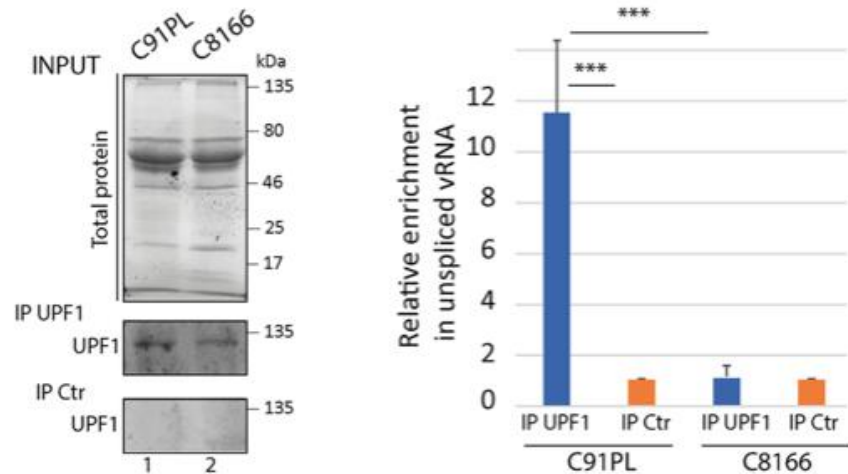
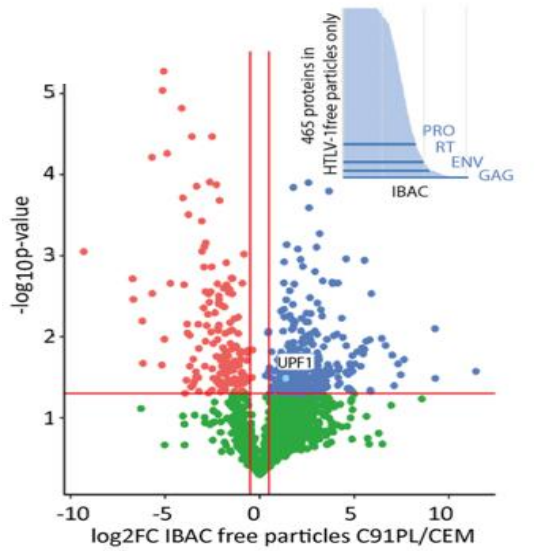


UPF1 is specifically redirected towards vRNA without its cofactor UPF2

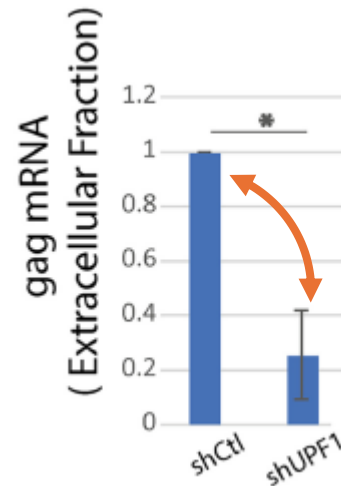
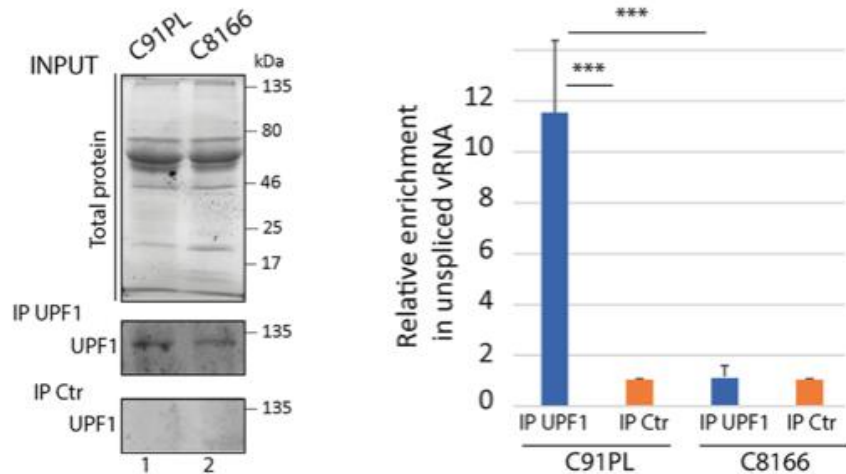
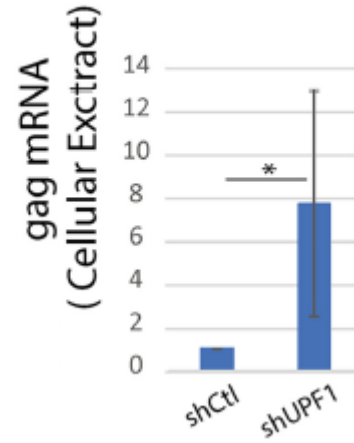
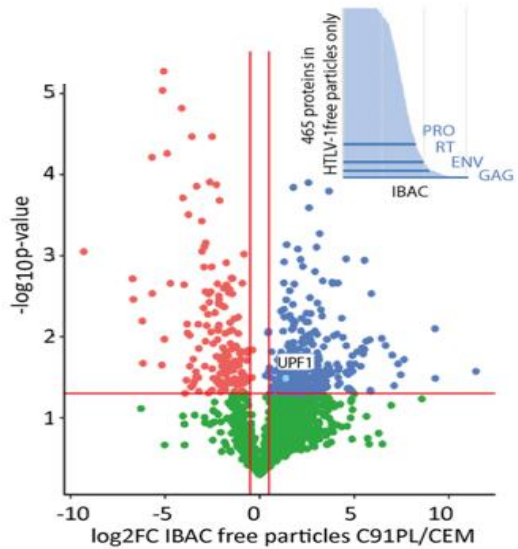


Translation / maturation

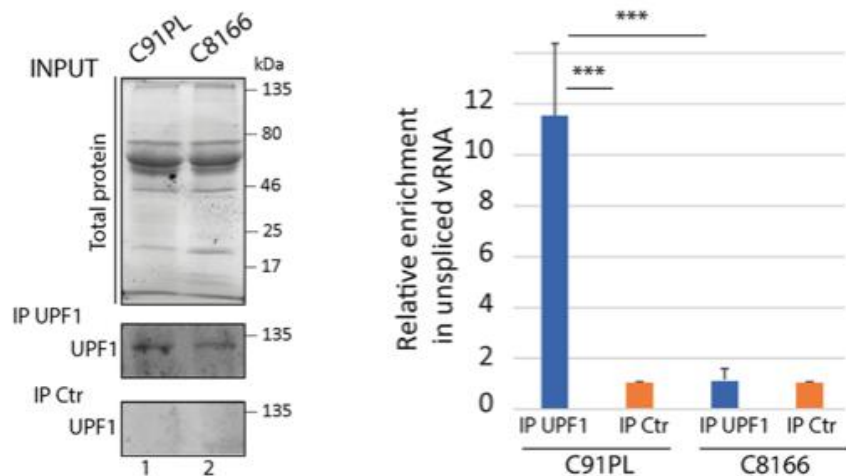
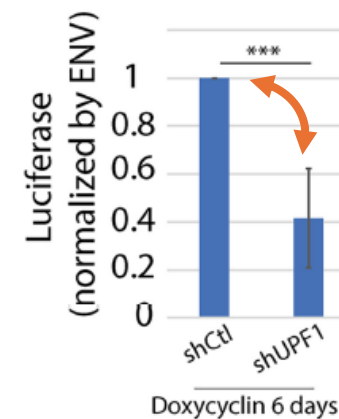
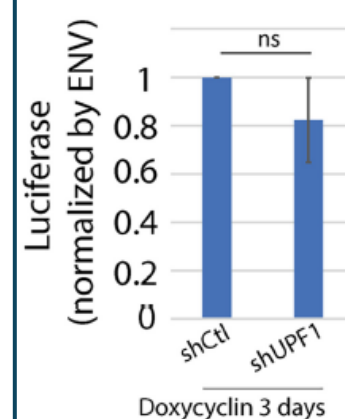
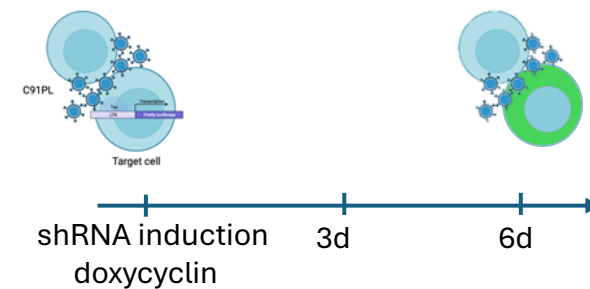
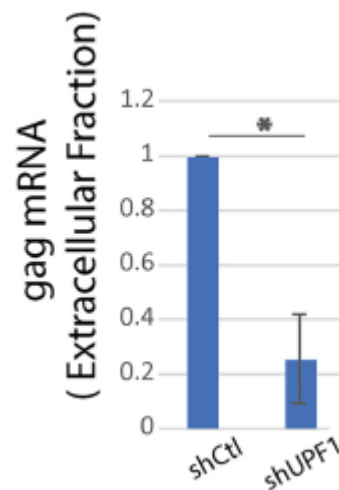
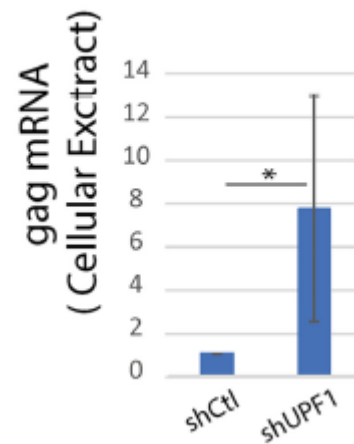
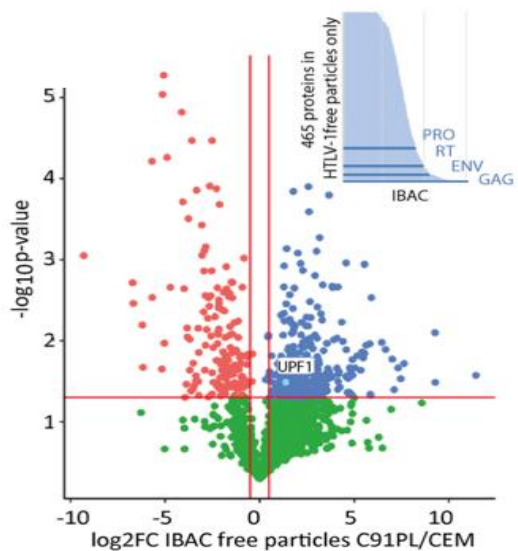
Viral particle composition and infectivity



Viral particle composition and infectivity



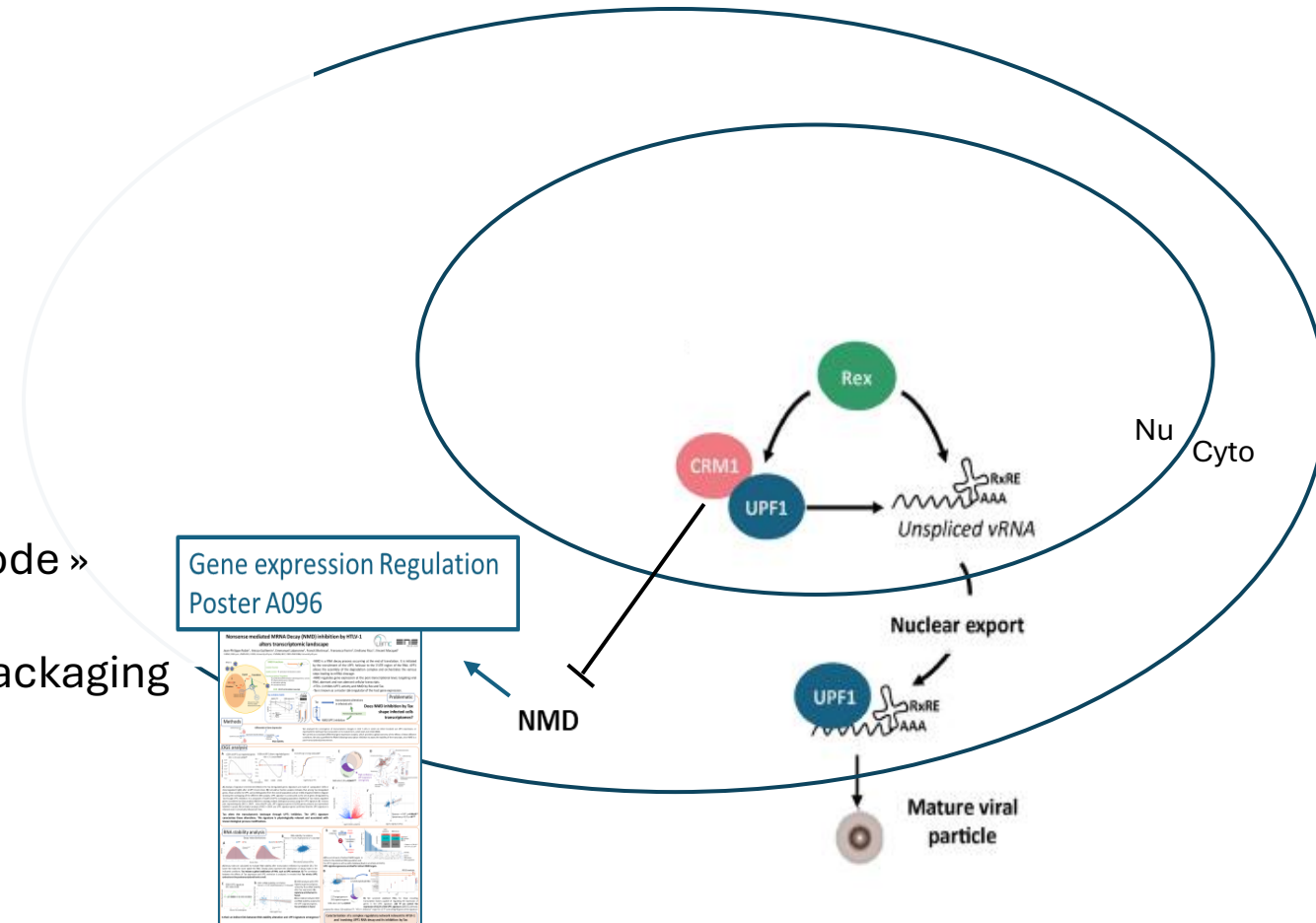
Viral particle composition and infectivity



CONCLUSION

CRM1 hijacking by Rex alters UPF1 functions :

- inhibition of NMD
- redirection of UPF1 on vRNA in a viral « safe mode »
- revealed an impact of UPF1 on vRNA export, packaging and viral particle maturation



➡ **Hijacked UPF1 shifts from antiviral to proviral activity**

➡ **Additional roles of Rex other than RNA export adapter and linked with CRM1 hijacking**



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Armelle Roisin
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Hub Biocomputing

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PSF
Platim
AniRA - Vectorologie
AniRA - Cytométrie



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A. Cimorelli H. Dutartre



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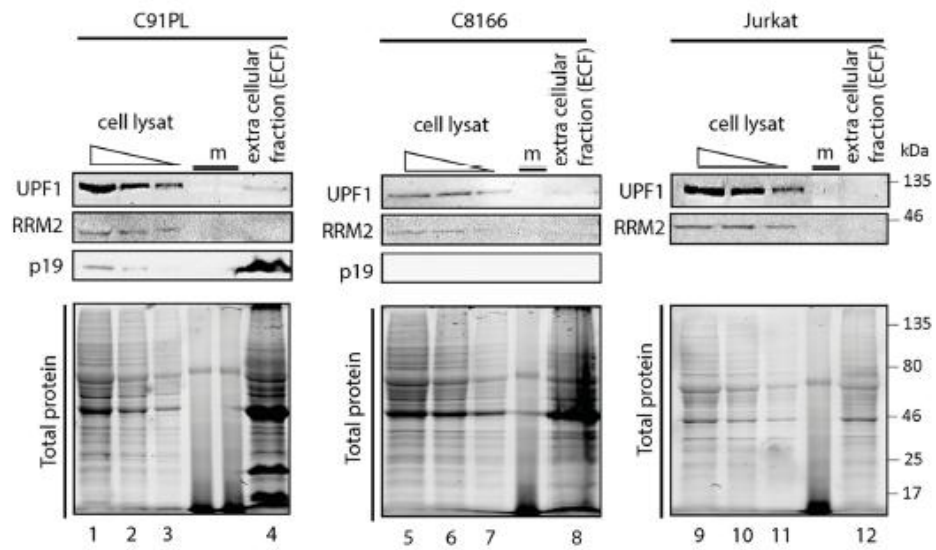
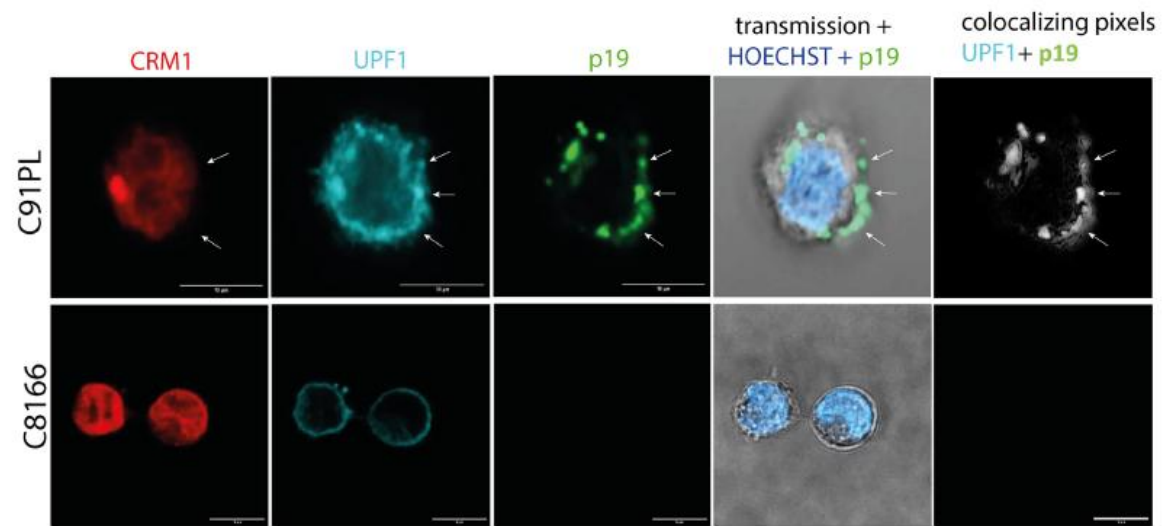


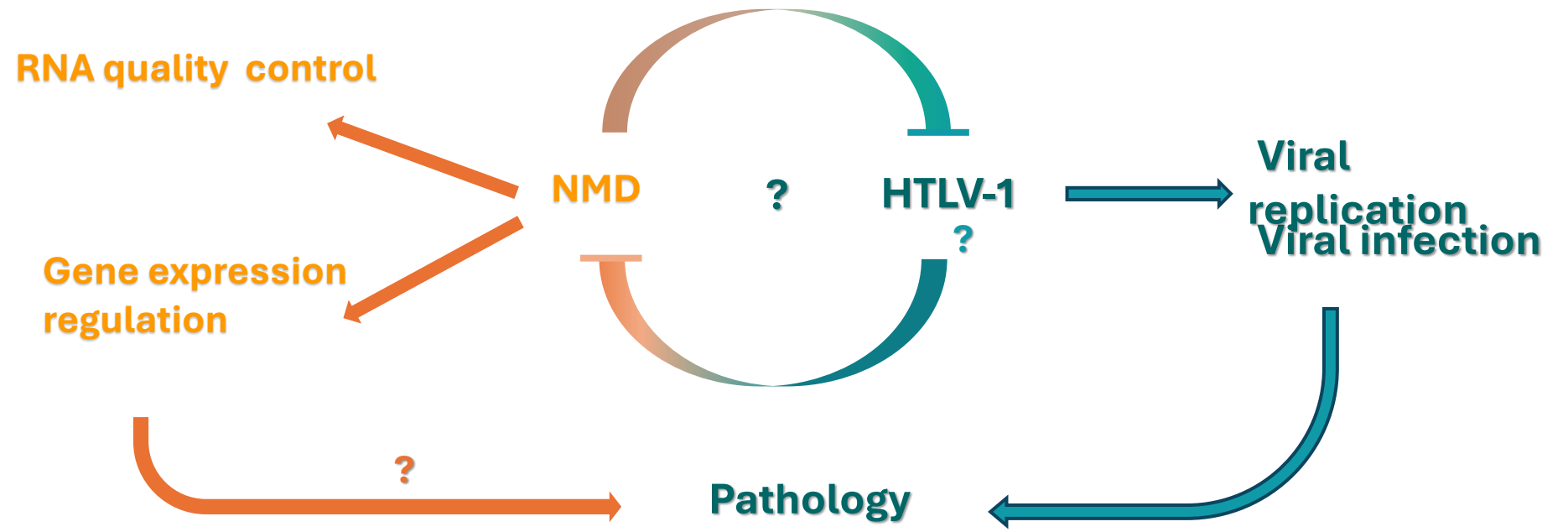
H. LeHir
V. Croquette

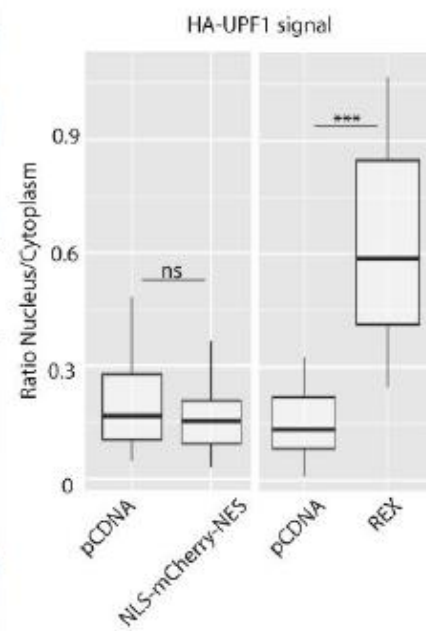
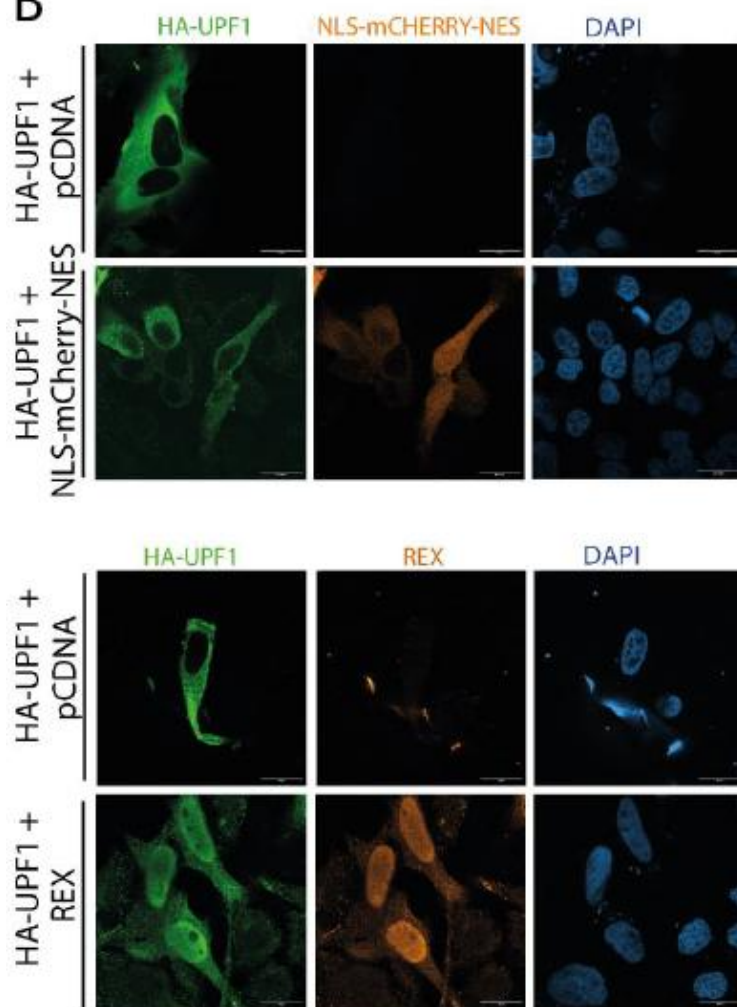


V. Ciminale
F. Rende



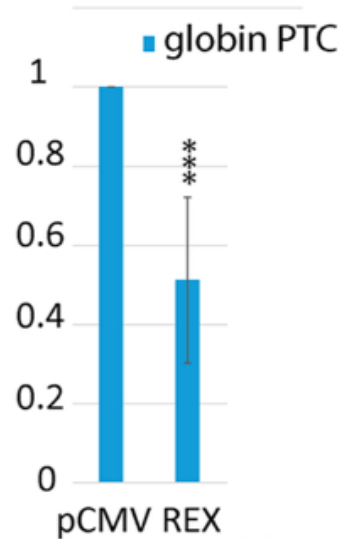




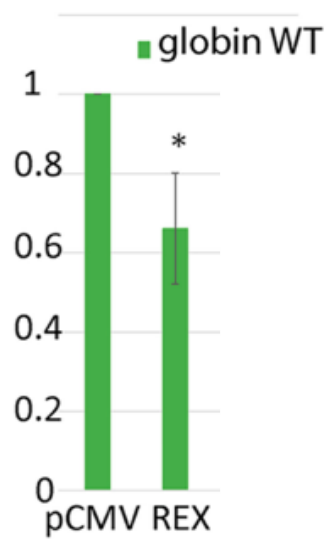
B

F

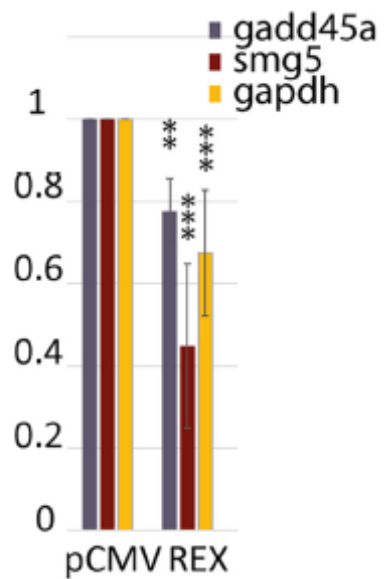
Relative enrichment in mRNA



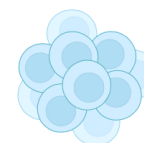
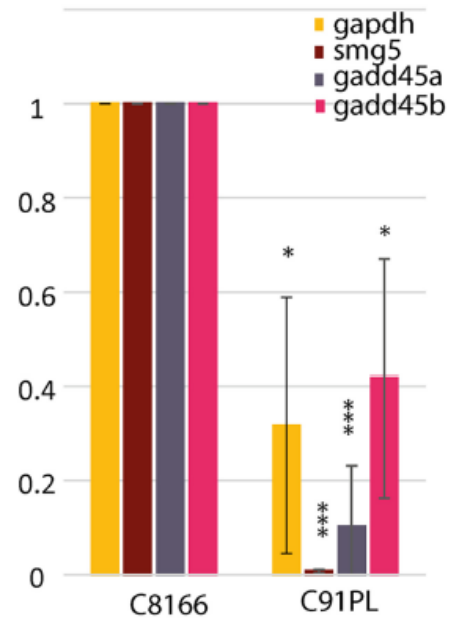
Relative enrichment in mRNA

**G**

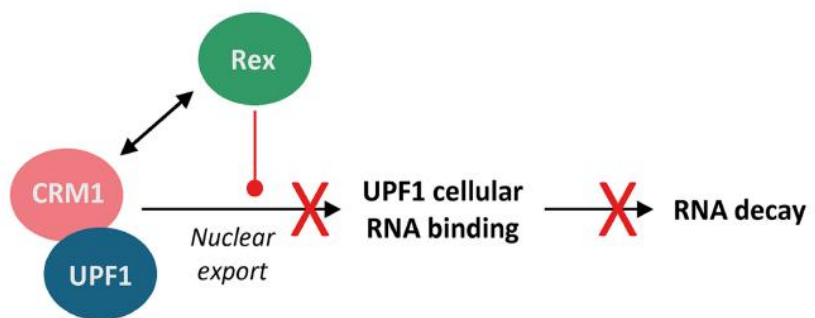
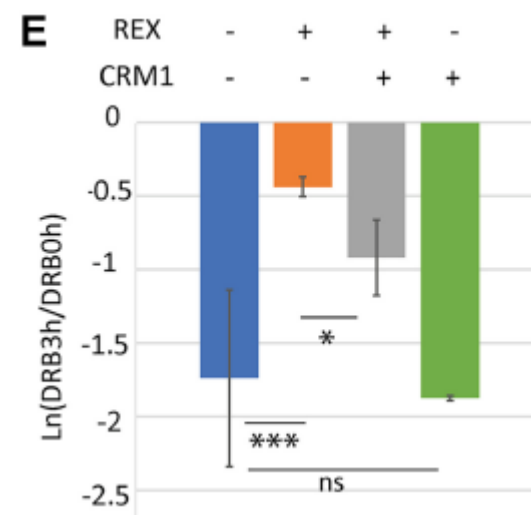
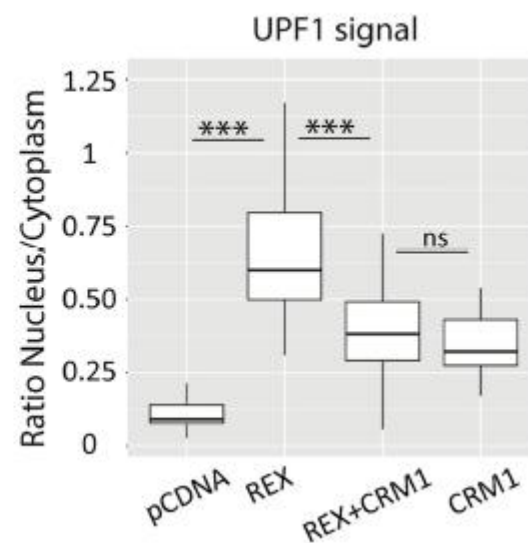
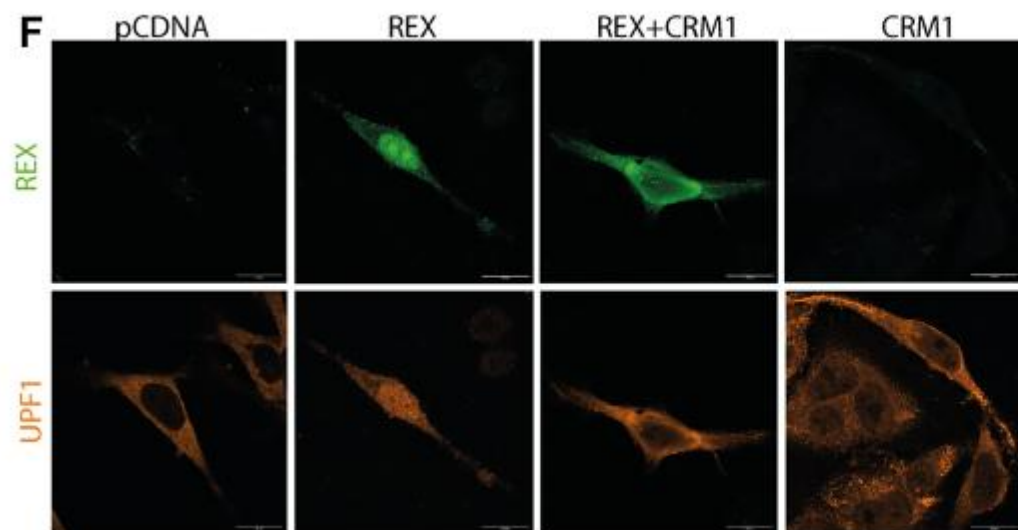
Relative enrichment in mRNA

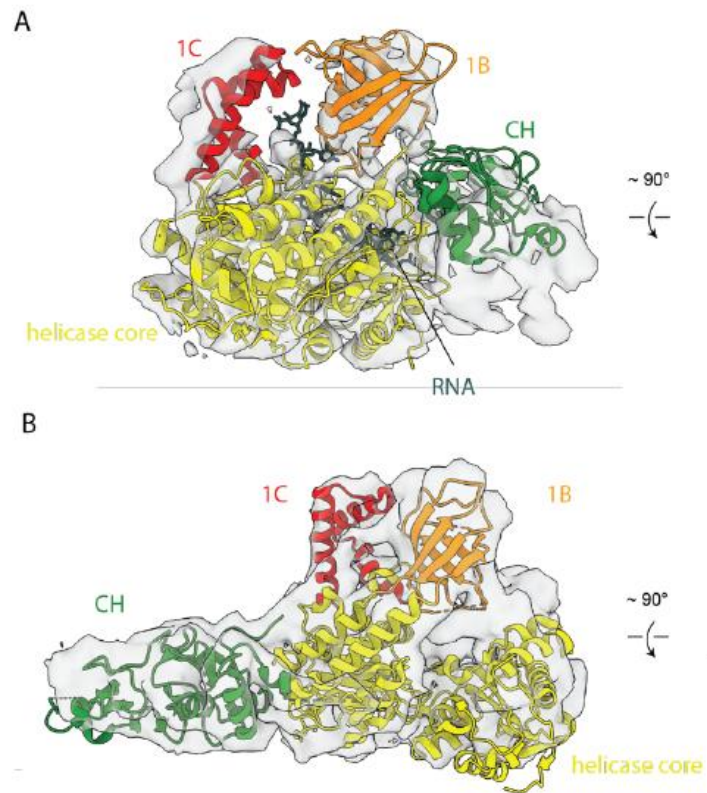
**D**

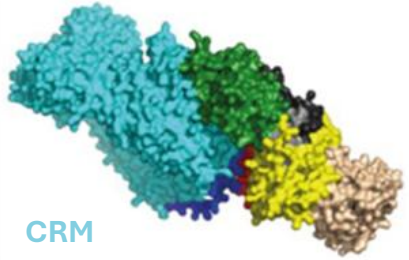
Relative enrichment in mRNA



- 10 min + 2 min de questions.
- - idées à poser :
- Rex altère l'export nucleocytoplasmique de façon globale. Bien que le mécanisme soit connu depuis longtemps une question a été éludée : quel est l'impact de ce détournement sur le fonctionnement cellulaire. Quelles fonctions cellulaires sont altérées ? Comprendre cela est d'autant plus important que ce mécanisme est partagé par plusieurs virus dont l'autre rétrovirus pathogène humain HIV;
- Introduction
- → CRM1 function in cells -> participe à l'homeostasie cellulaire en contrôlant la répartition nucléocytoplasmique des protéines.
- Impliqué dans de nombreuses fonctions dont RNA processing incluant les facteurs du NMD (description du mécanisme en 2 phrases)
- → fonction antivirale du NMD lors des infections virales et notamment HTLV (RNA decay nakano + RIP UPF1 phospho PRIO + siRNA)
- (NMD is of particular interest in the » infection » field because of its antiviral activity.
- Now that the context is described we analyzed the impact of Rex expression on UPF1 biology in lymphocytes chronically infected :
- Rex modifies the localisation of UPF1, favors its nuclear retention and its interaction with CRM1 in lymphocytes.
- Molecular dynamic analysis suggests that UPF1 CRM1 stabilized complex should have a reduced RNA binding affinity:
- This is validated experimentally and leads to NMD inhibition. (NMD inhibition depends on Rex binding to CRM1)
- Unexpectedly, When looking at UPF1 recruitment to vRNA the behaviour is opposite : UPF1 is recruited to vRNA on a Rex and CRM1 interacting manner. Without UPF2 the partner of UPF1 during NMD.
- Now considering the presence of UPF1 on vRNA likely in the nucleus, we checked its potential functions :
- -export,
- -translation
- -Encapsidation
- -Viral particle maturation.
- → to conclude that UPF1 expression stimulates cell to cell infection.

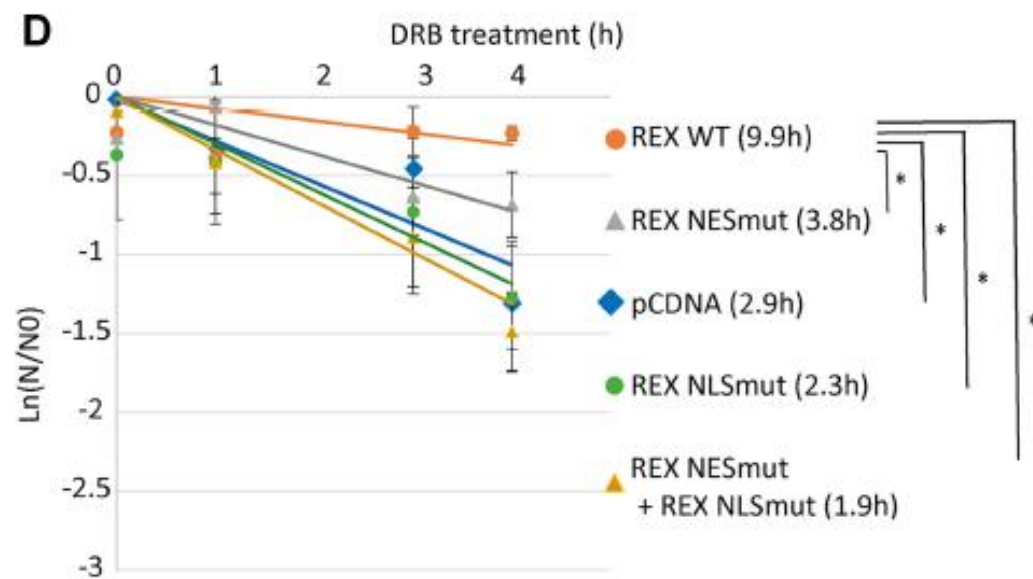
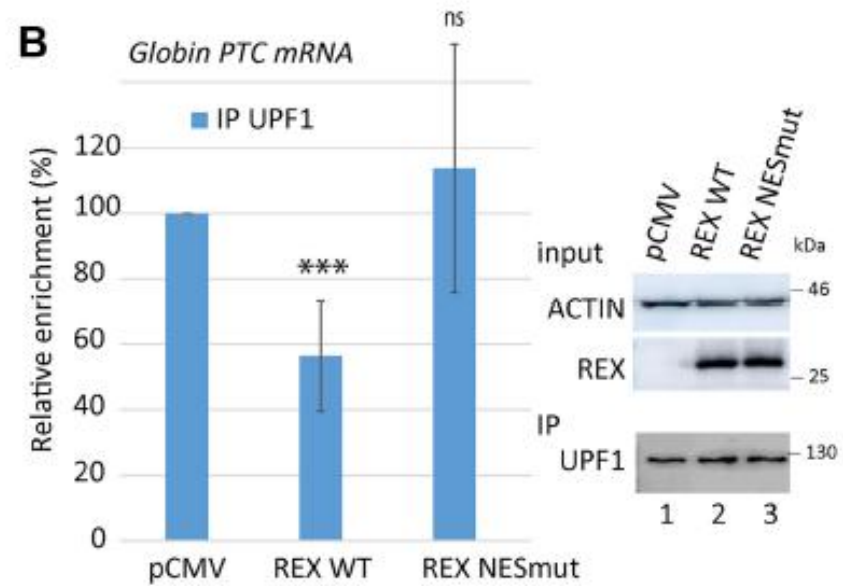


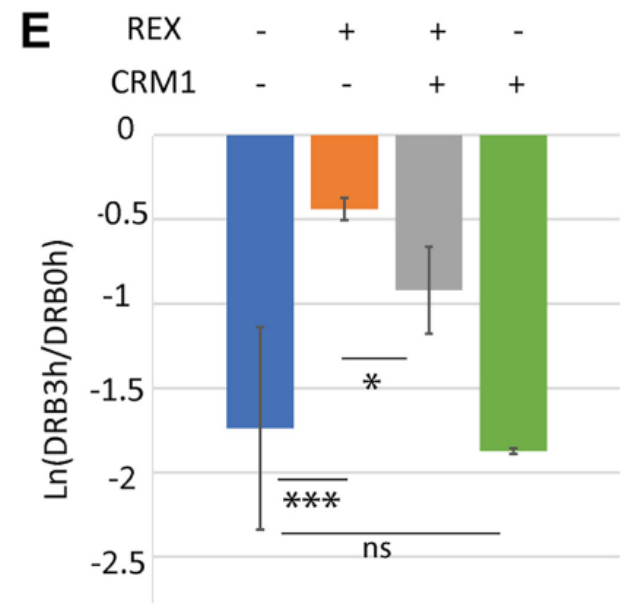
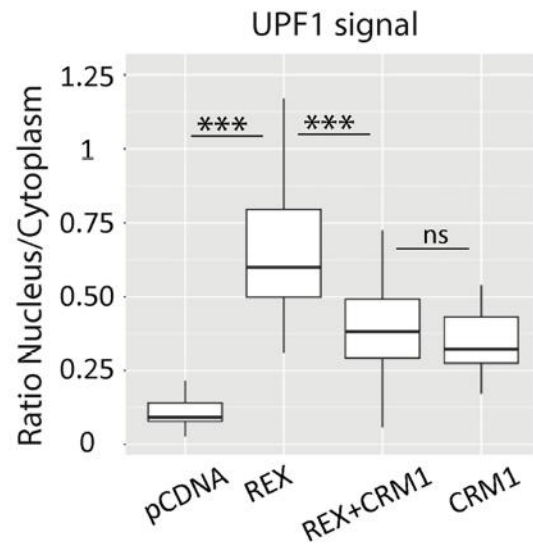
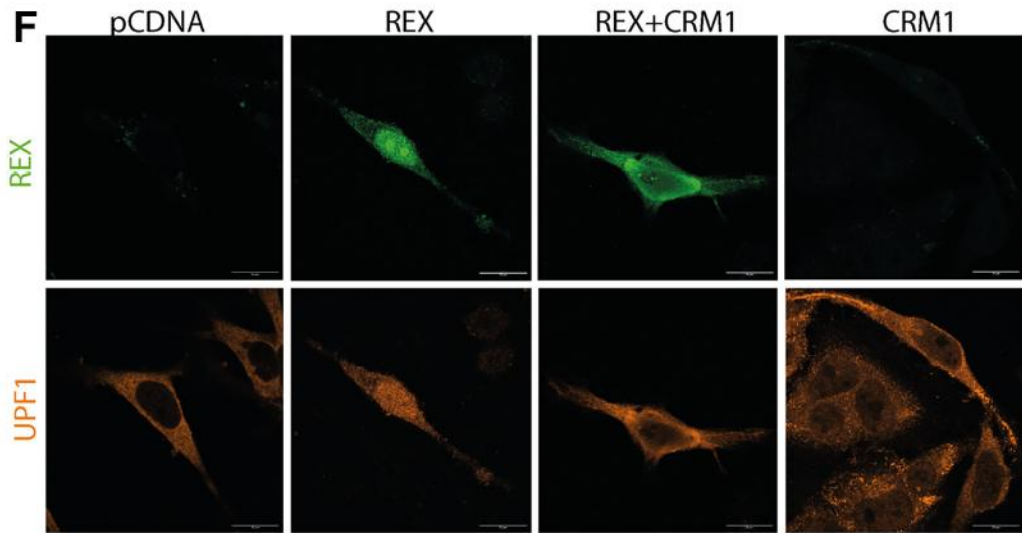


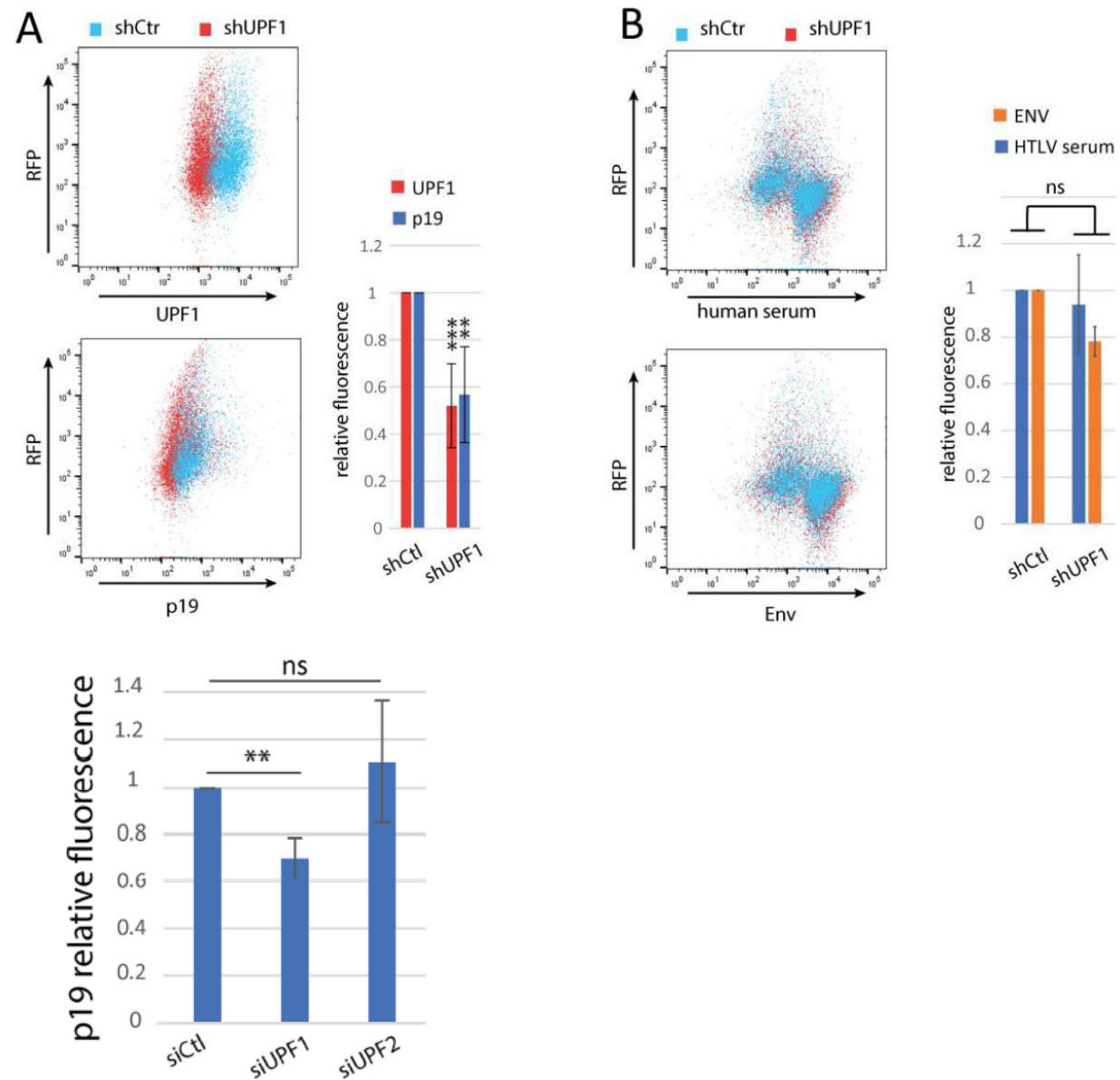
UPF1 conformation	open	closed
CRM1/UPF1 complex		
Rg (Å) pvalue : 0.001	47.05 ± 0.74	49.4 ± 0.65
BSA (Å²) pvalue : 0.049	8901 ± 1071	6351 ± 779

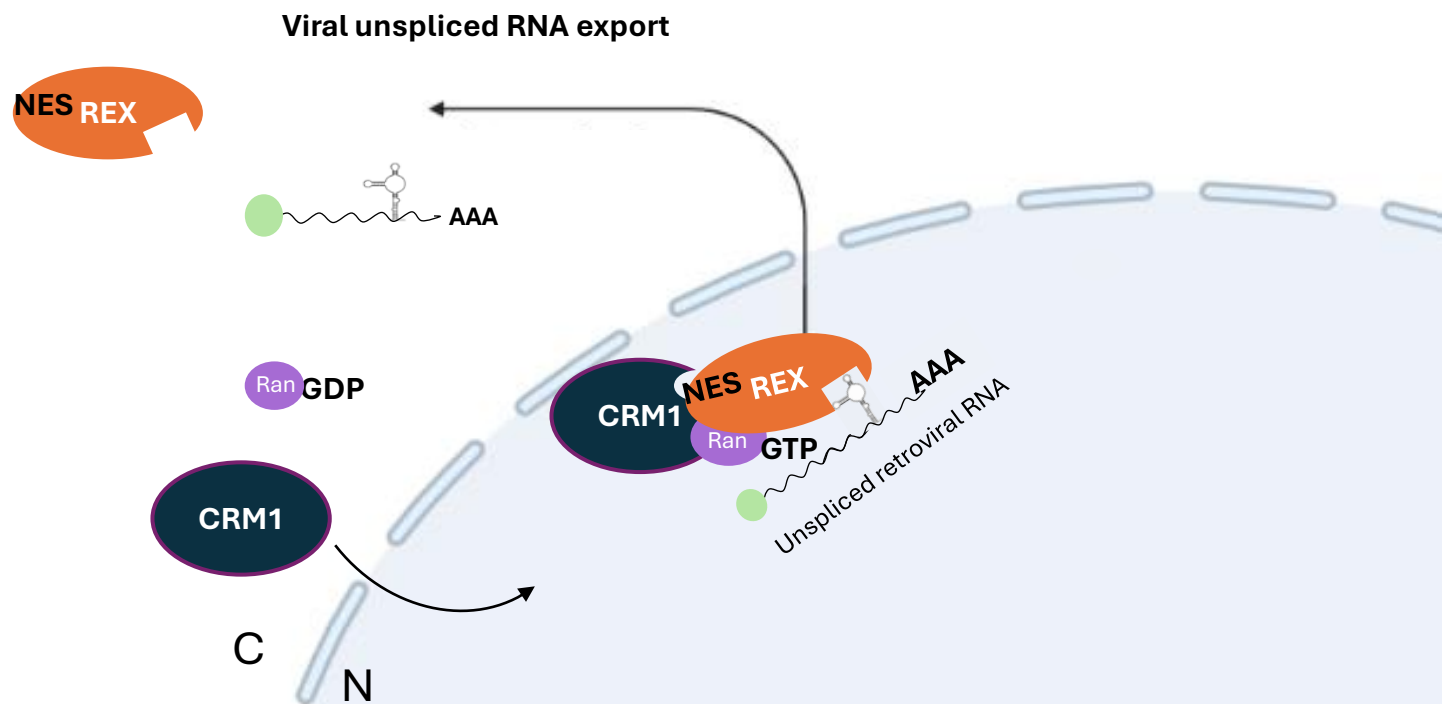
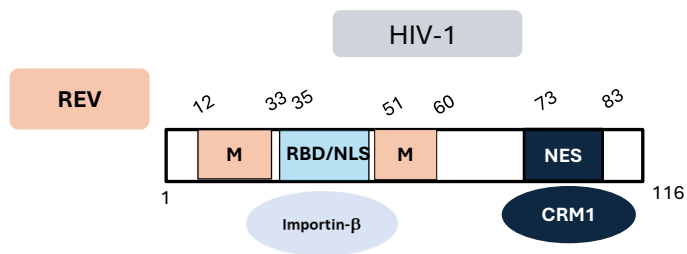
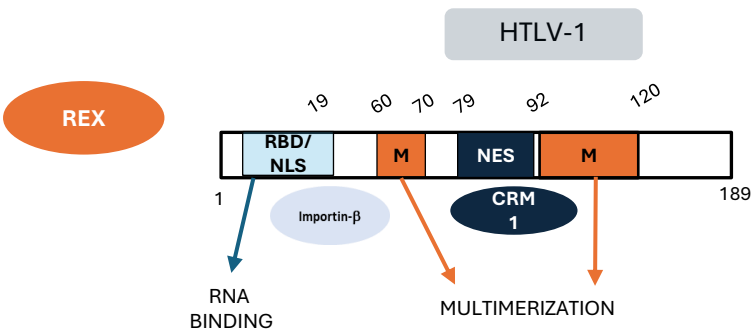
- Stabilization of CRM1/UPF1 interaction : involvement of “open” conformation of UPF1. “Open” conformation of UPF1 : less affinity to RNA.

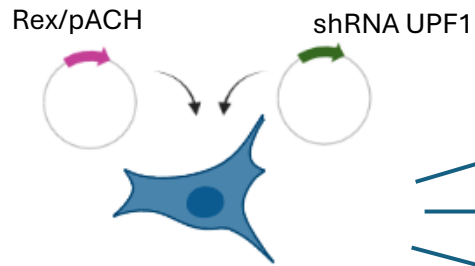
Langer, et al. NAR, 2024.







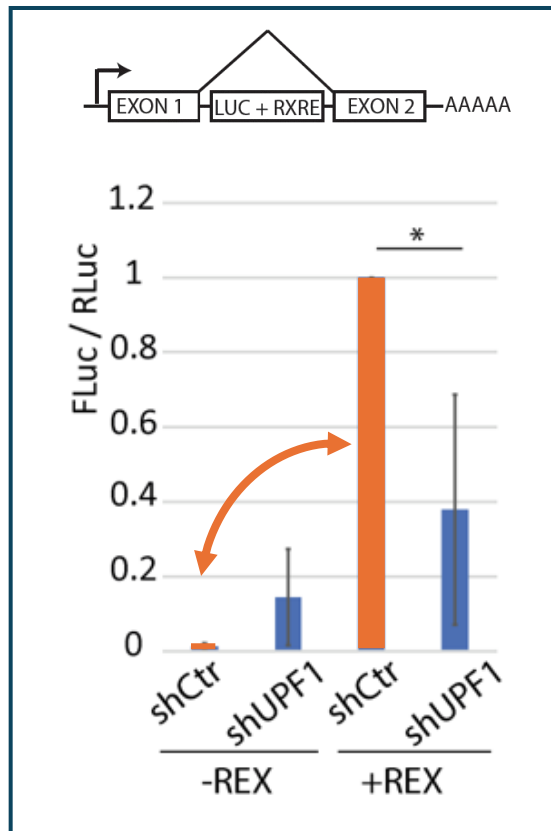




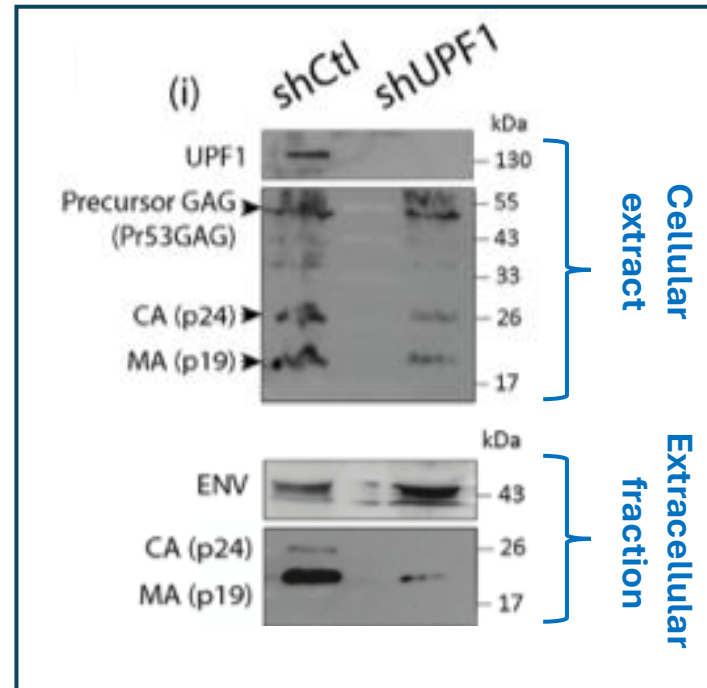
Rex dependent export

Viral protein expression /maturation

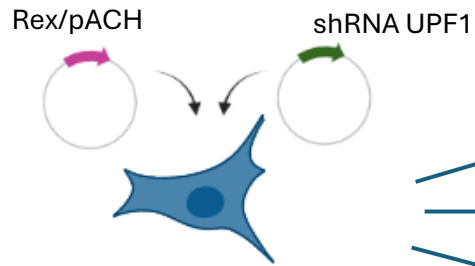
Viral particle composition



Export function



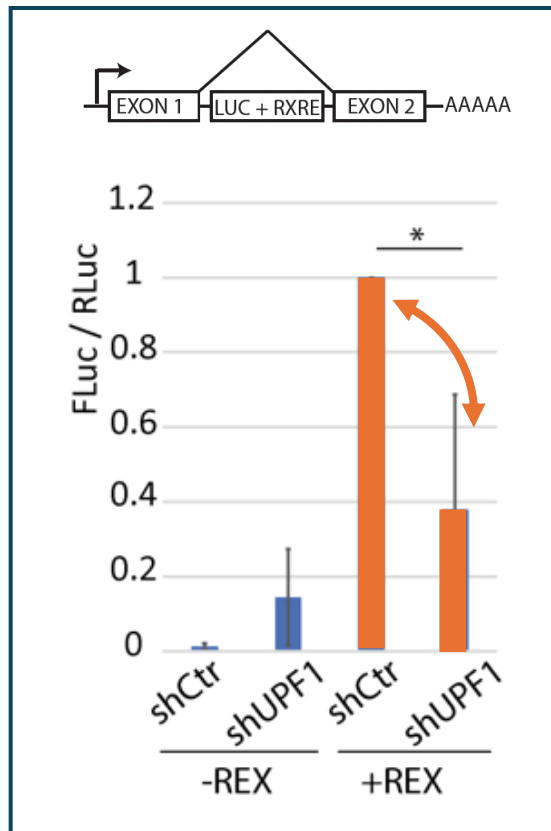
Translation / maturation



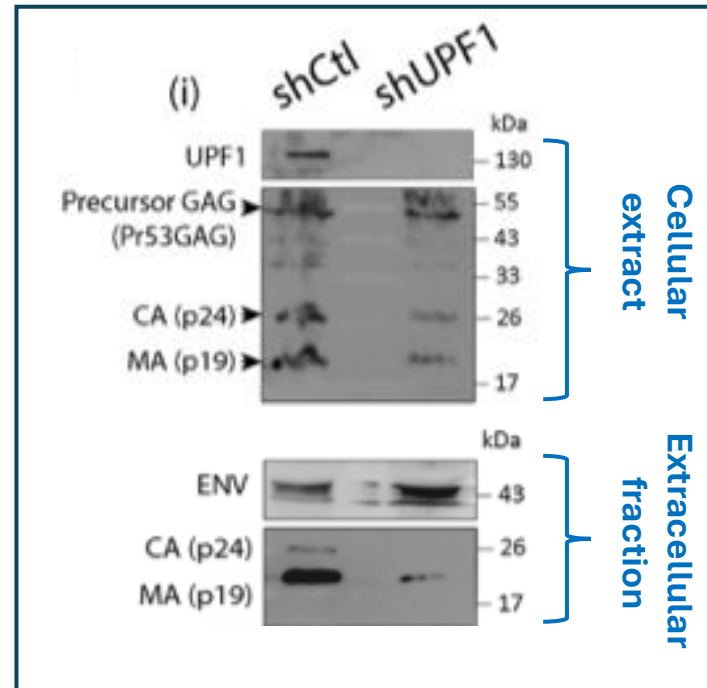
Rex dependent export

Viral protein expression /maturation

Viral particle composition



Export function



Translation / maturation

Hijacking antiviral defense: HLTF, a restriction factor that promotes clonal expansion in HTLV-1

Alexis Fontaine, Liège University - Belgium

Conflicts of interest

I have no conflict of interest

INTRODUCTION

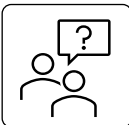
HLTF (Helicase Like Transcription factor) :

Functions

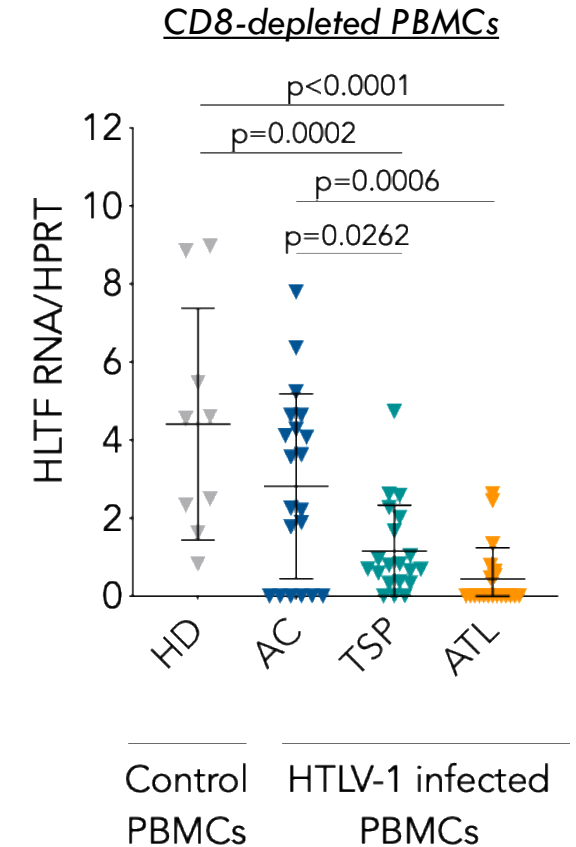
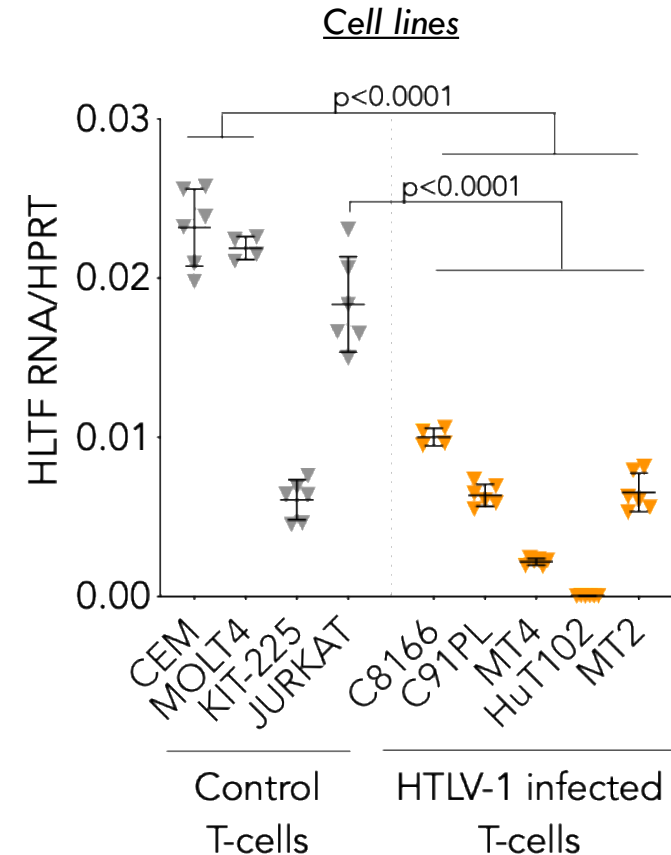
- Transcription factor.
- ATP-dependant chromatin remodelling.
- DNA damage tolerance pathways.

In HTLV-1

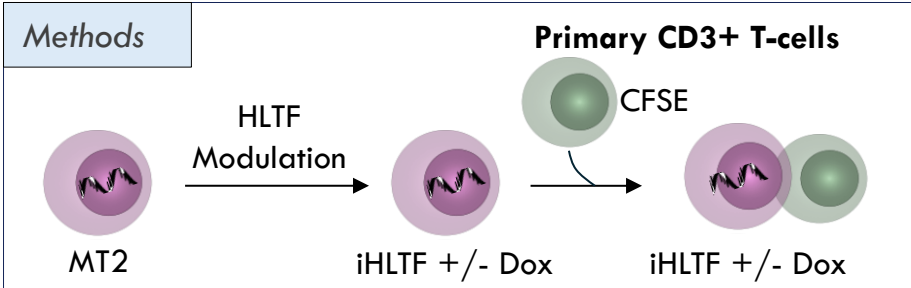
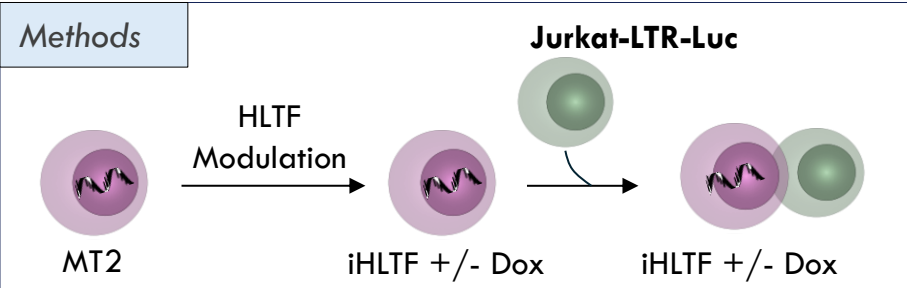
1 HLTF is **down-regulated** in HTLV-1 cell lines and in patients with HAM/TSP and ATL.



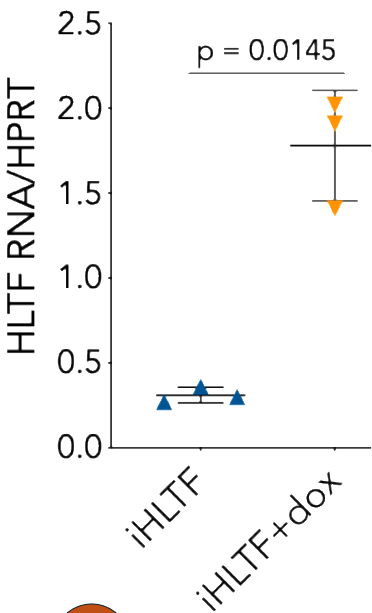
What is the role of **HLTF** in infected cells ?



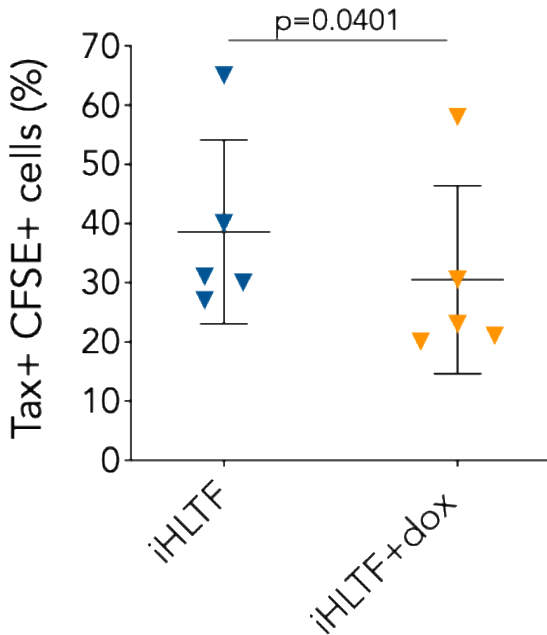
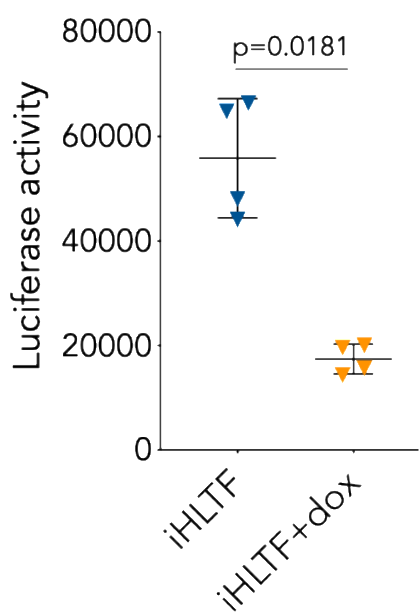
INTRODUCTION



HLTF Expression in MT2



Luciferase activity in Jurkat

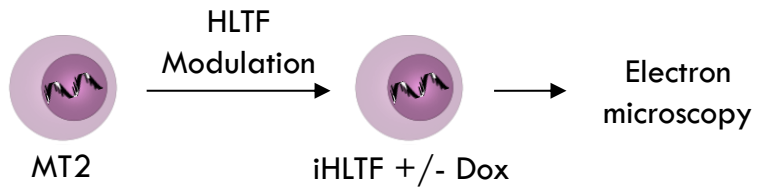


2

Restoring HLTF levels in HTLV-1-infected cells restricts infection of non-infected cells.

INTRODUCTION

Methods



Enveloped particles containing electron-dense cores

$\rightarrow 19.0 \pm 10.2 \%$

Mature

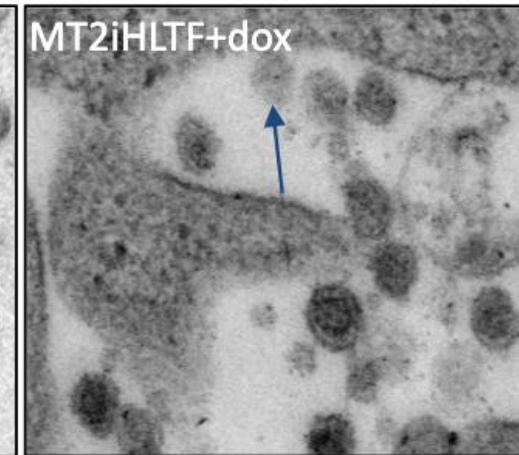
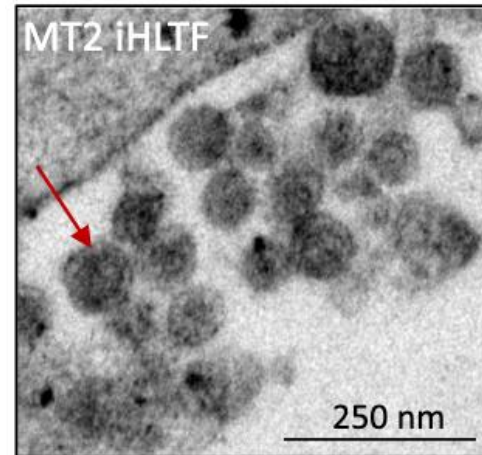


Viral particle size

$\rightarrow 69.8 \pm 20.33 \text{ nm}$

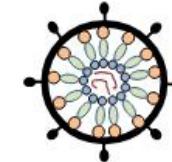
$p < 0.0001$

Extracellular viral particles



$11.3 \pm 5.7 \%$

Immature



$76.6 \pm 21.62 \text{ nm}$

2

Restoring HLTF decreases the number of mature viral particles.

CONCLUSIONS - QUESTIONS

1

HLTF is **down-regulated** in patients with HAM/TSP and ATL.

Beauvois, A. et al. PNAS (2023)

2

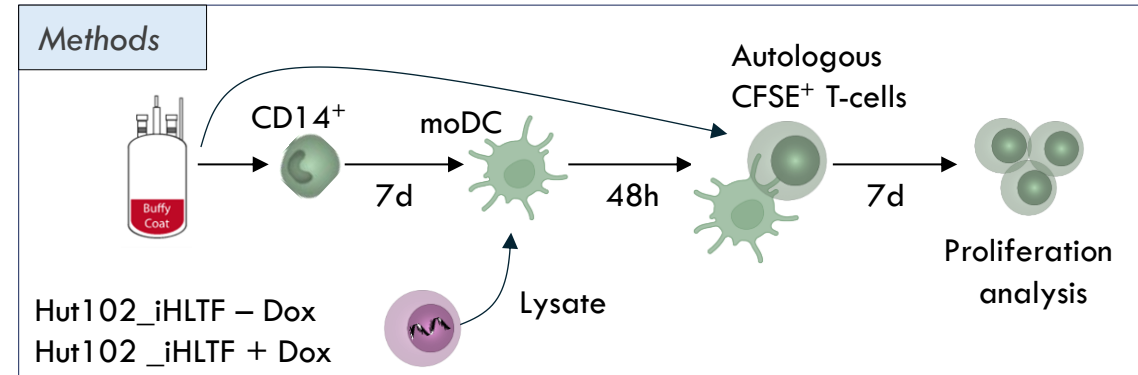
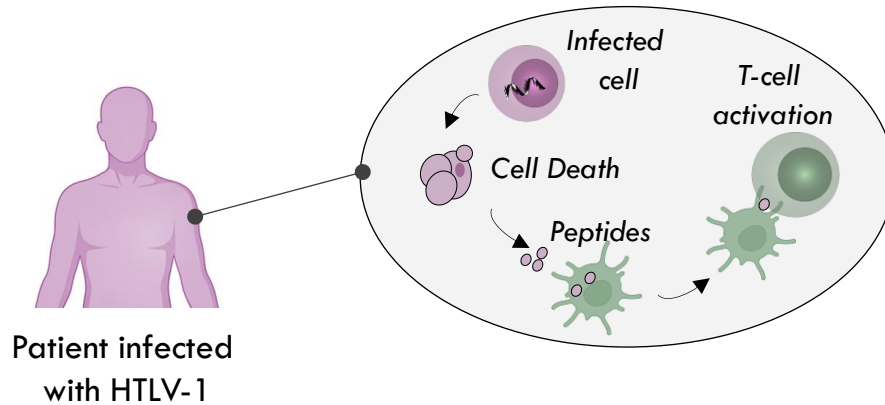
HLTF is **a restriction factor** of HTLV-1 infection.

Beauvois, A. et al. PNAS (2023)

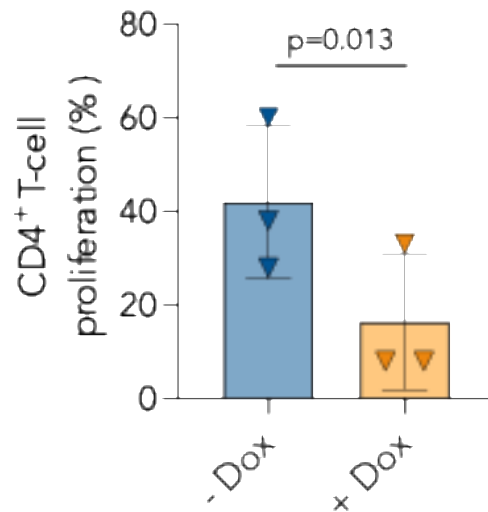


Does HLTF modulate immunogenicity of infected cells and the immune response ?

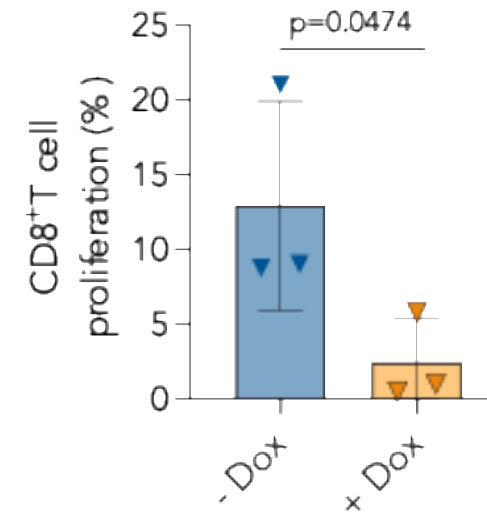
RESULTS



CD4 T-cell proliferation



CD8 T-cell proliferation



3

Restoring HLTF expression decreases DC-mediated CD4 and CD8 T-cell proliferation.

CONCLUSIONS - QUESTIONS

1

HLTF is **down-regulated** in patients with HAM/TSP and ATLL.

Beauvois, A. et al. PNAS (2023)

2

HLTF acts **as a restriction factor** of HTLV-1 infection.

Beauvois, A. et al. PNAS (2023)

3

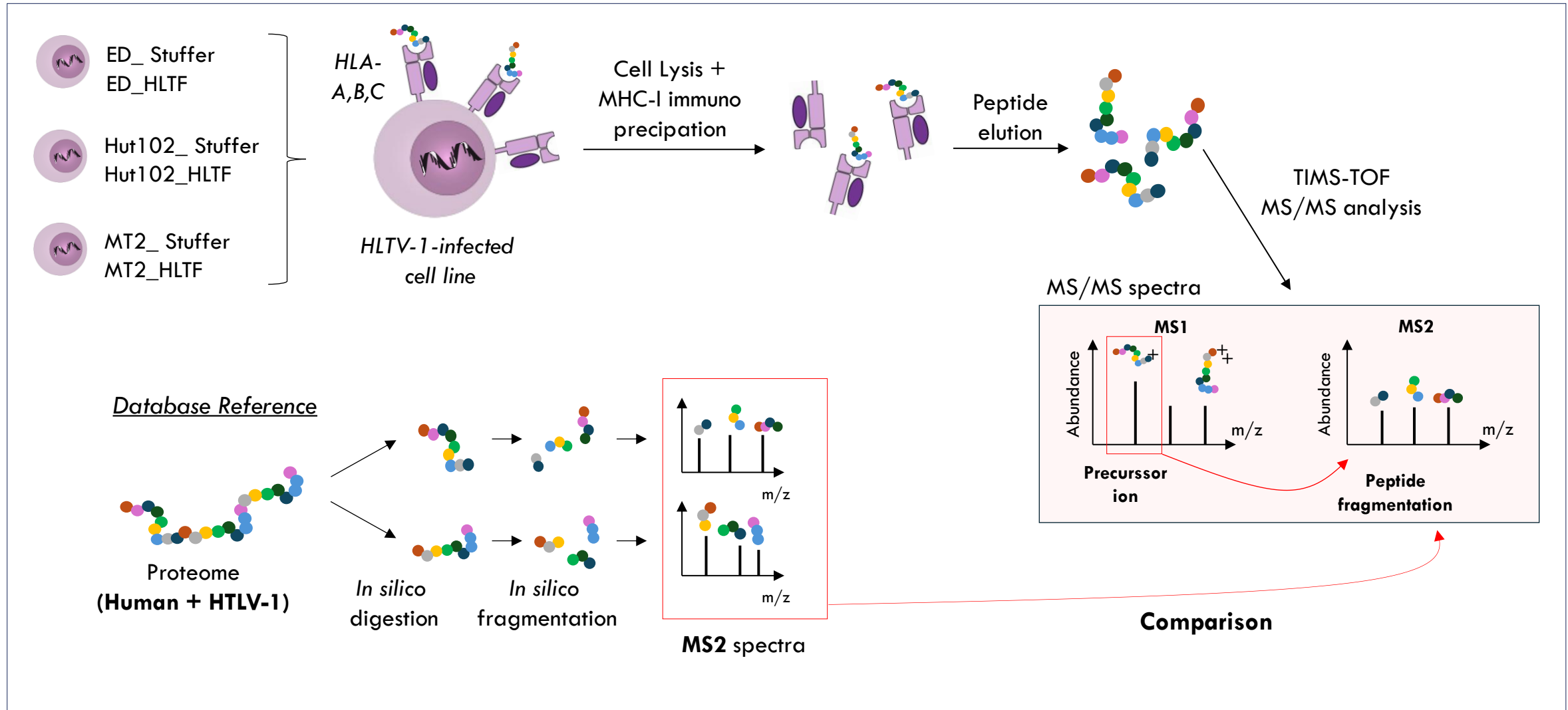
Restoring HLTF expression **decreases** DC-mediated **CD4⁺ and CD8⁺ T-cell proliferation**.



Does HLTF modulate peptides presented by MHC-I molecules of infected cells ?

Role of HLTF in the modulation of the MHC-I peptides

METHODS



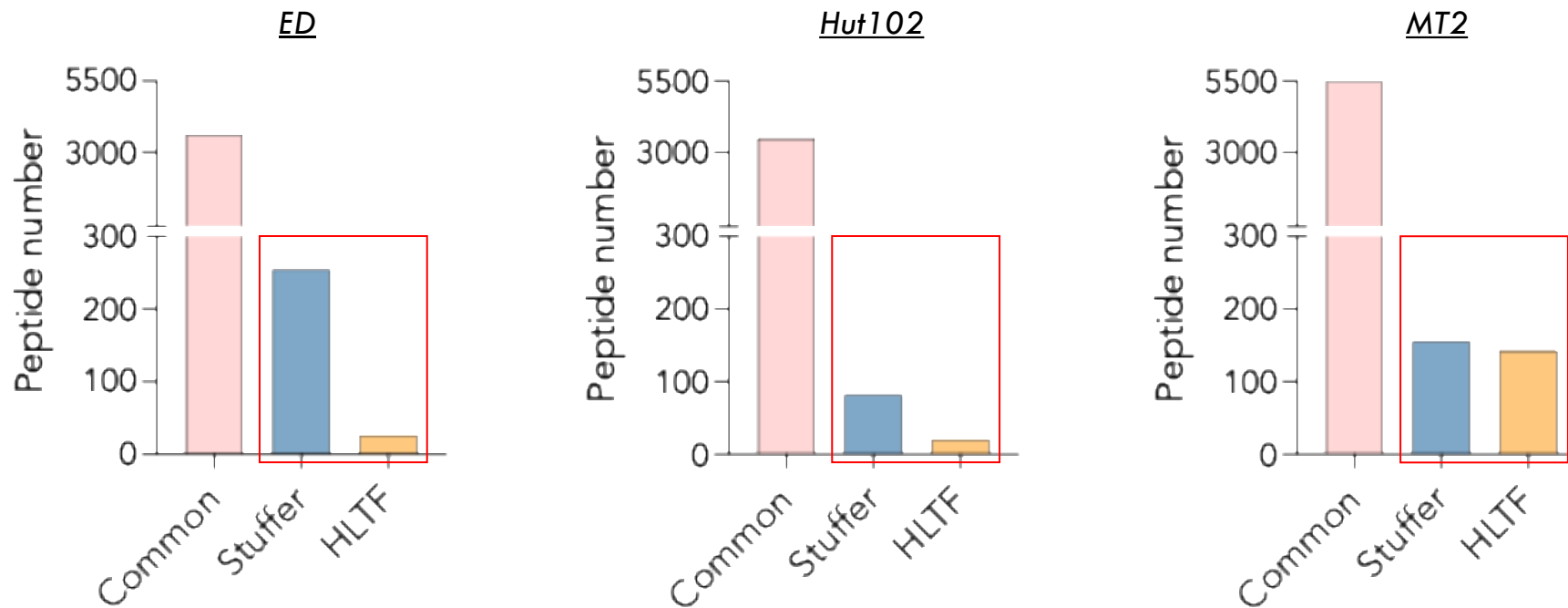
RESULTS

Abundance per peptide

Peptide	Stuffer_1	Stuffer_2	HLTF_1	HLTF_2
EELLYKISL	10	13	0	0
SYSSYHATY	21	22	18	23

→ “Stuffer-specific” (< LOQ)

→ Common peptide



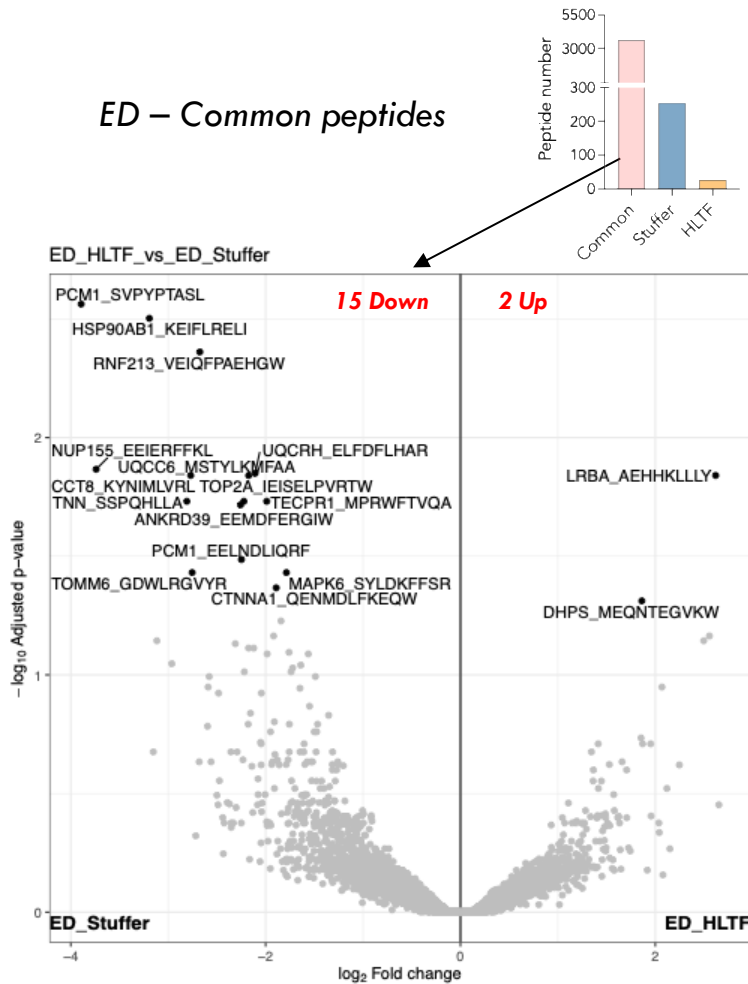
4

Restoring HLTF expression decreases the diversity of presented MHC-I peptides in ED and HUT102 cell lines.

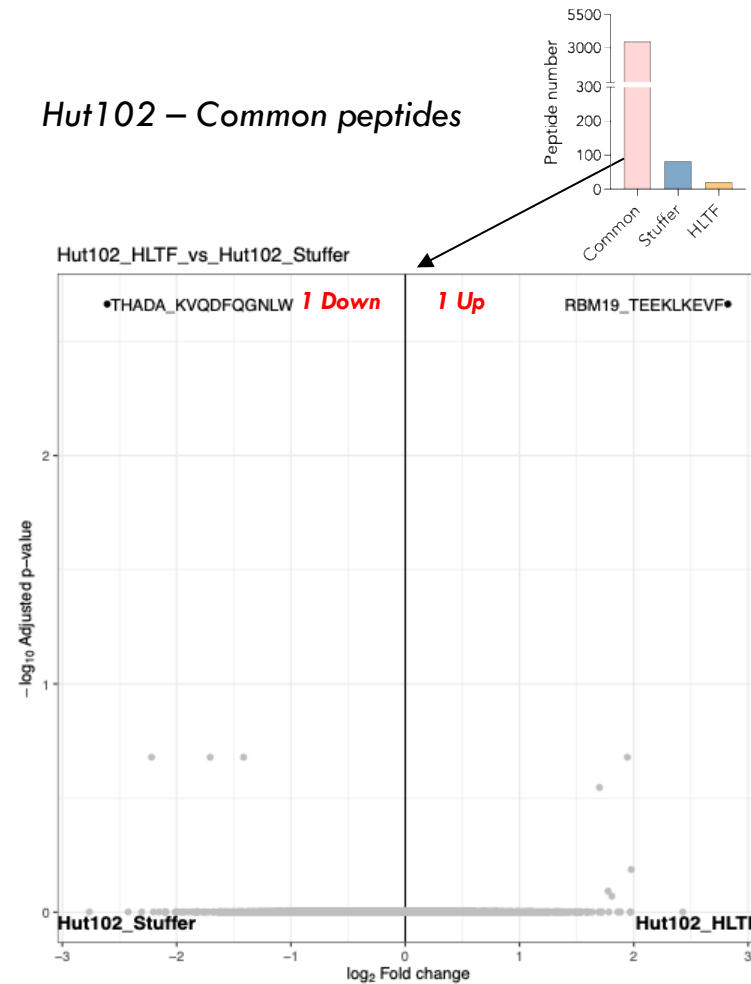
Role of HLTF in the modulation of the MHC-I peptides

RESULTS

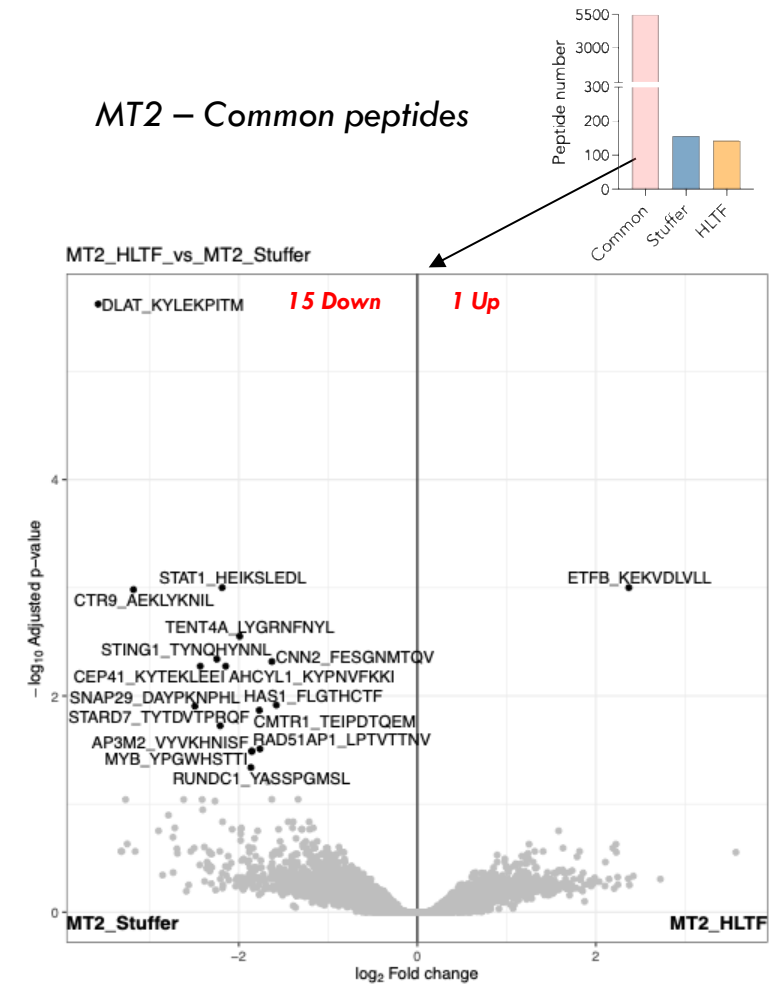
ED – Common peptides



Hut102 – Common peptides



MT2 – Common peptides



5

Restoring HLTF expression decreases the abundance of cellular peptides in ED and MT2 cell lines.

CONCLUSIONS - QUESTIONS

1

HLTF is **down-regulated** in patients with HAM/TSP and ATLL.

Beauvois, A. et al. PNAS (2023)

2

HLTF is **a restriction factor** of HTLV-1 infection.

Beauvois, A. et al. PNAS (2023)

3

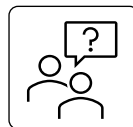
Restoring HLTF expression **decreases** DC-mediated **CD4⁺ and CD8⁺ T-cell proliferation**.

4

HLTF **decreases the diversity of presented MHC-I peptides** in ED and HUT102 cell lines.

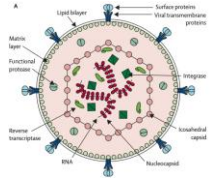
5

HLTF **decreases the abundance of cellular peptides** in ED and MT2 cell lines.



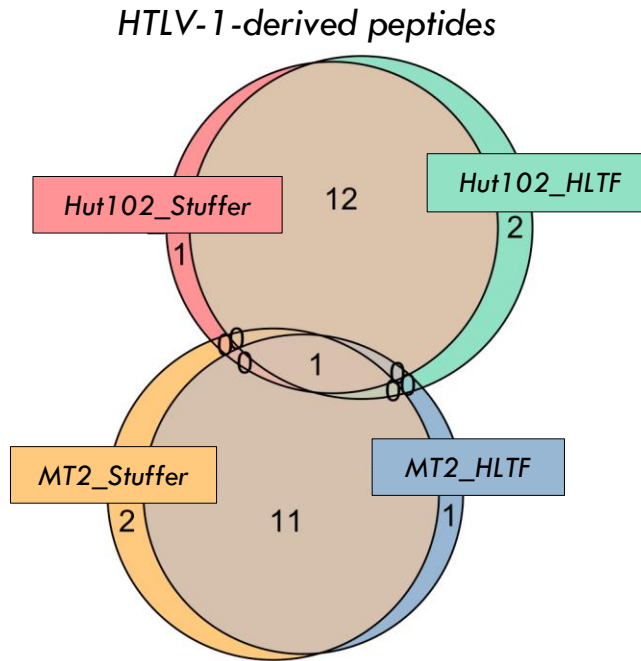
What about viral peptides ?

RESULTS



HTLV-1 proteins (9)
ATK-1, subtype A

- Gag – P03345
- Pro – P10274
- Pol – P03362
- Env – P03381
- p12i – P0C215
- p30II – P0C214
- Rex - P0C205
- Tax – P03409
- Hbz – P0C746



Hut102

Protein	Peptide seq.	Position	Specificity
Pol (2)	FAALPGSAK	251-259	Common
	HTNSPLGDM	318-326	Common
Env (5)	SSYHATYSL	74-82	Common
	RAVPVAVW	294-301	Stuffer
	SSSSTPLLY	249-257	Common
	VSPYWKWF	128-135	Common
	STVPYPSTPL	226-235	Common
Tax (8)	QSSSFIFHKF	272-281	Common
	KAYHPSFLL	284-292	Common
	ATCPEHQITW	47-56	Common
	AYHPSFLL	285-292	Common
	EELLYKISL	192-200	Common
	CPEHQITW	49-56	Common
	SGGLCSAR	32-39	Common
	KYSPFRNGY	111-119	HLTF
P30II (1)	SSFRIPSLR	168-176	HLTF

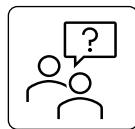
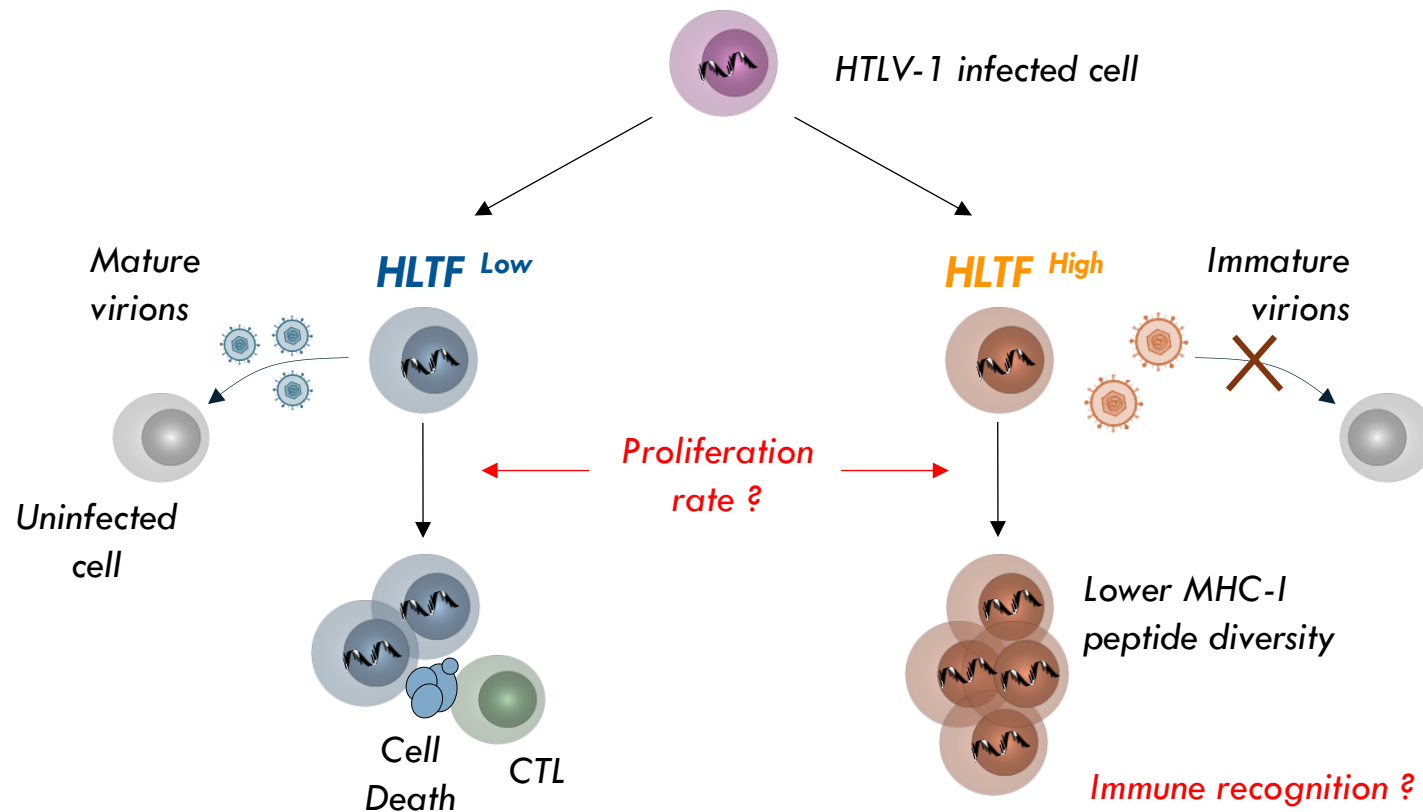
MT2

Protein	Peptide seq.	Position	Specificity
Pol (5)	DPASILPVI	440-448	Common
	DPPDSDPQI	109-117	Common
	RQWKGPQEAL	1405-414	Common
	KGYPGRVNEIL	74-84	Common
	LPMDNALS	1343-1351	Stuffer
Env (4)	HEVDKDISQL	348-357	Common
	SYSSYHATY	72-80	Common
	YSSYHATY	73-80	Common
	VPVAVWL	296-303	Common
Tax (4)	EELLYKISL	192-200	Common
	EYTNIPISL	311-319	Common
	PYKRIEELL	187-195	Common
	LYQLSPITW	156-165	HLTF
P30II (1)	VPYPSTPLL	227-235	Common
	VPYPSTPL	227-234	Stuffer

6

Restoring HLTF induces specific MHC-I viral peptides in Hut102 and MT2.

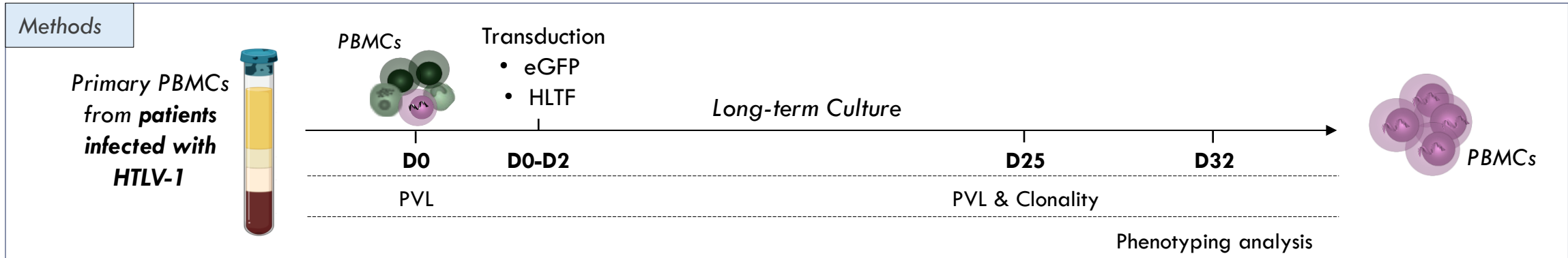
CONCLUSIONS - QUESTIONS



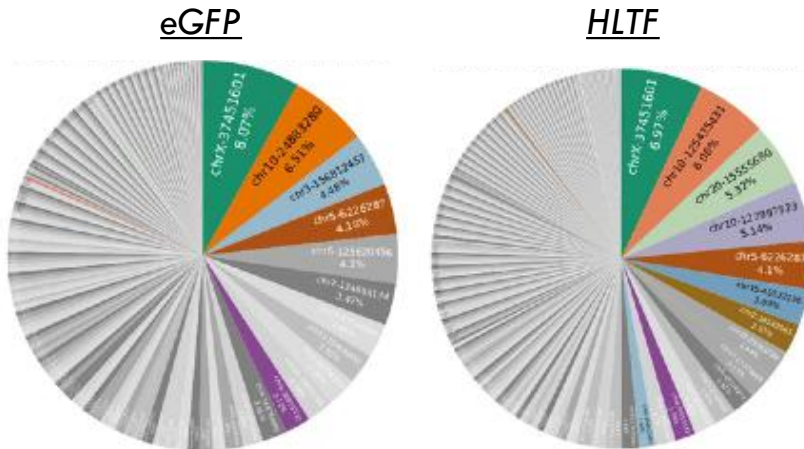
Does HLTF modulate the clonal expansion in an autologous system ?

Effect of HLTF on clonal expansion in primary cells

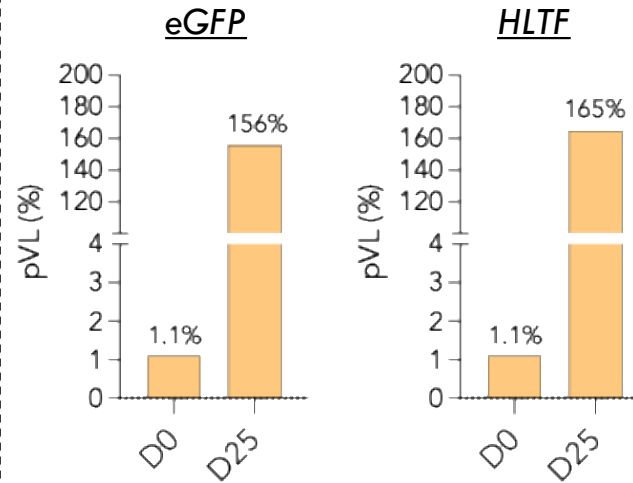
RESULTS (ONGOING)



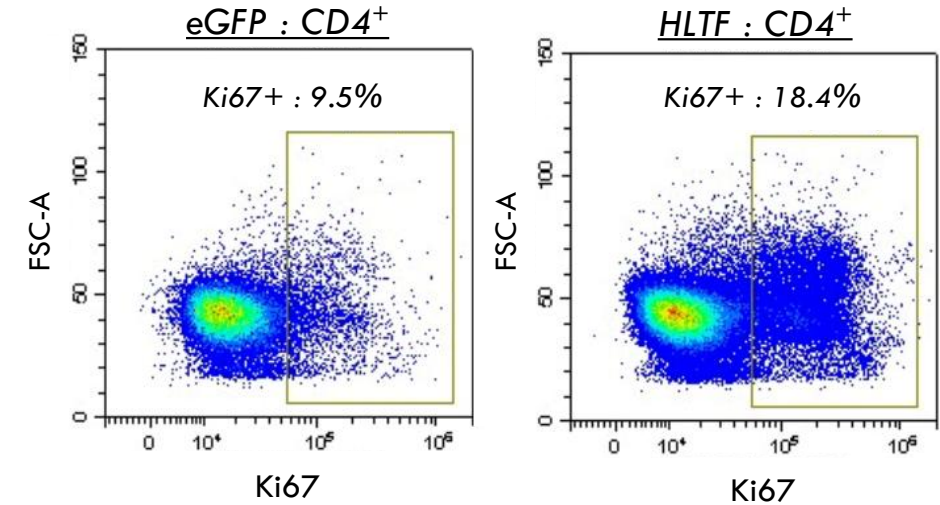
Clonal distribution (D25)



PVL (D25)



CD4⁺ Proliferation (D32)



Acknowledgments

ECM Lab (Liege University, Belgium)

Luc Willems
Malik Hamaidia
Alexis Fontaine
Thomas Jouant
Xavier Saintmard
Roxane Terres
Florine Doucet
Manon Zwaenepoel
Songkang Qin
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Proteomics platform (Liege University, Belgium)

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22nd International Conference on Human Retrovirology:
HTLV and Related Retroviruses
HTLV2026

June 3-6, 2026 | Philadelphia, Pennsylvania, USA

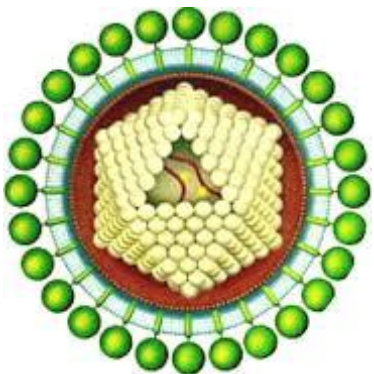
HTLV-1 Tax induces PINK1-Parkin-dependent mitophagy to mitigate activation of the cGAS-STING pathway

Edward Harhaj
Penn State College of Medicine, Hershey, USA

HTLV-1 Tax induces PINK1-Parkin-dependent mitophagy to mitigate activation of the cGAS-STING pathway

Ed Harhaj, Ph.D.

Penn State College of Medicine



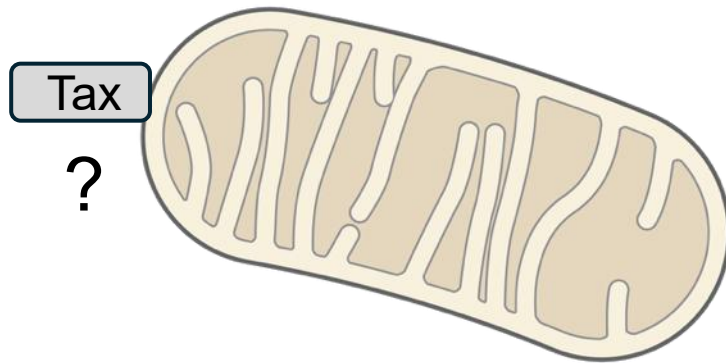
PennState

Conflict of Interest

In relation to this presentation, I declare that I have no conflict of interests

Background-Tax and mitochondria

- HTLV-1 Tax induces reactive oxygen species (ROS).
 - Kinjo et al. *J. Virol.* 2010 84: (5431-7)
 - Takahashi et al. *Blood* 2013 122: 715-25
- The HTLV-1 Tax interactome includes mitochondrial proteins.
 - Gao and Harhaj *J. Virol.* 2013 87: 13640-54.



1) Does Tax go to the mitochondria and if so how?

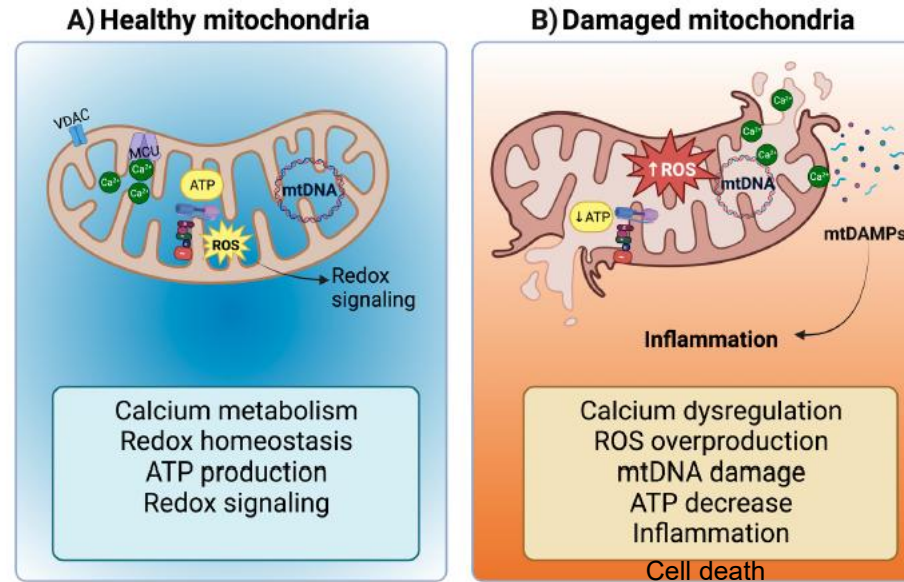
2) Does Tax target the mitochondria to suppress innate immune activation and cell death?

HTLV-1 Tax interacting proteins identified by LC-MS/MS (Gao and Harhaj *J. Virol.* 2013)

Protein	No. of unique peptides
Propionyl-coenzyme A carboxylase alpha chain	35
Methylcrotonoyl-coenzyme A carboxylase subunit alpha	25
Trifunctional enzyme subunit alpha	4
60-KDa heat shock protein, mitochondrial	29
Propionyl-coenzyme A carboxylase beta chain	27
ATP synthase subunit alpha	25
Mitochondrial import inner membrane translocase subunit TIM50	5
Phosphate carrier protein	8
ATP synthase subunit gamma	6
Serine/threonine-protein phosphatase PGAM5	4
Pyrroline-5-carboxylase reductase 1	3

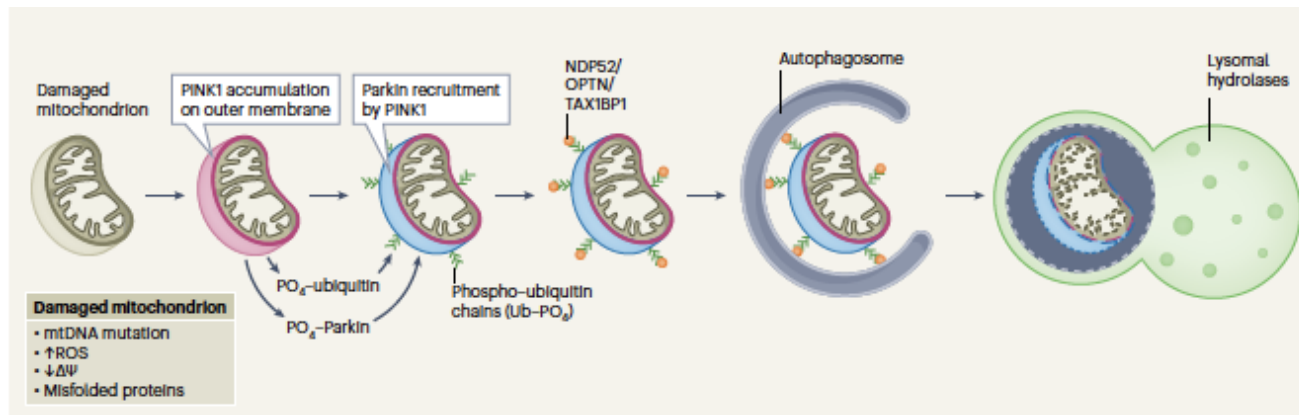
The PINK1-Parkin pathway clears damaged mitochondria

Mitochondria are the gatekeepers of metabolism, inflammation and cell death



Amador Martinez et al.
Int. J. Mol. Sci. 2023
24: 15875.

PINK1-Parkin mitophagy

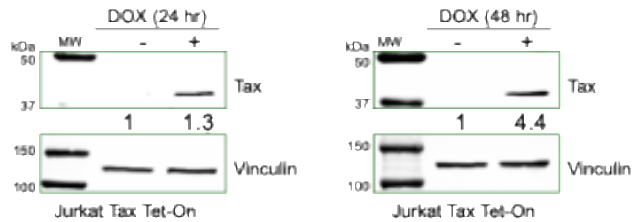


Many viruses including HBV, HCV, IAV, KSHV and others induce mitophagy to suppress innate immunity and prevent cell death.

Narendra and Youle
Nat. Cell Biol. 2024
26: 1639.

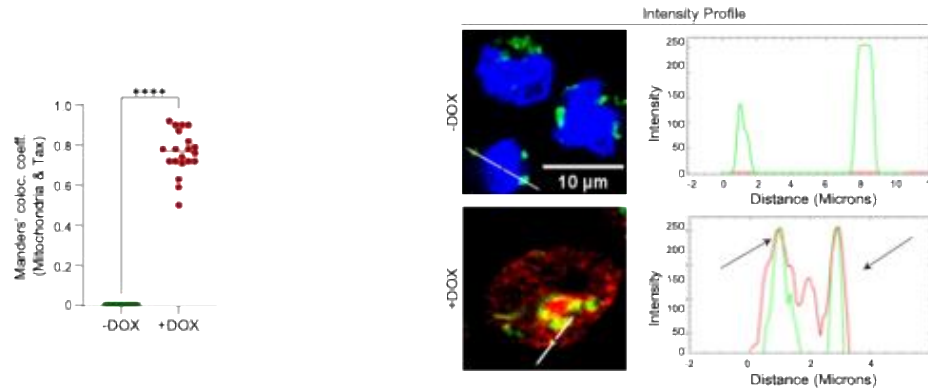
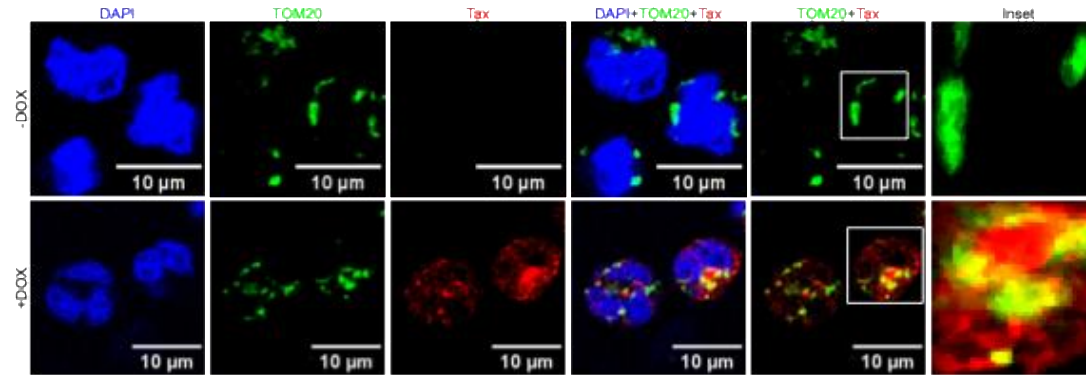
A subset of Tax localizes to the mitochondria in T cells

A



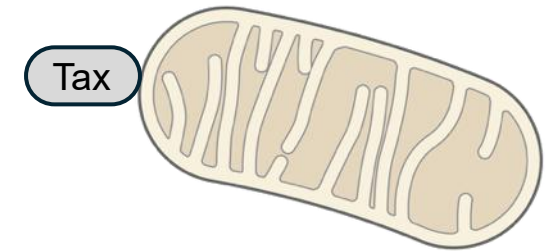
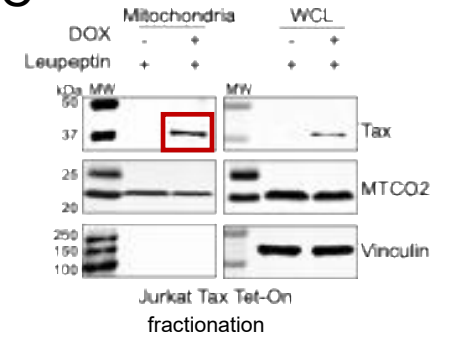
Jurkat Tax Tet-On cells
(Tax is induced by DOX treatment)

B

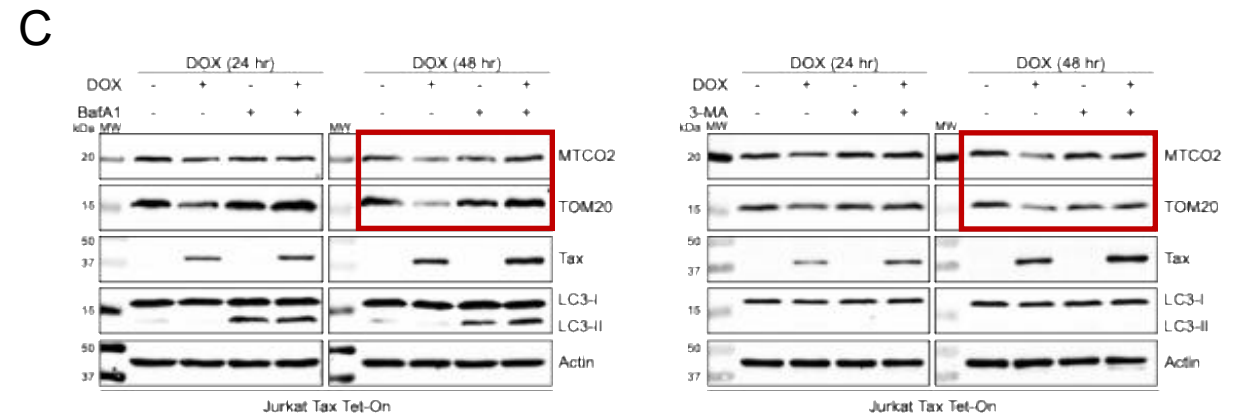
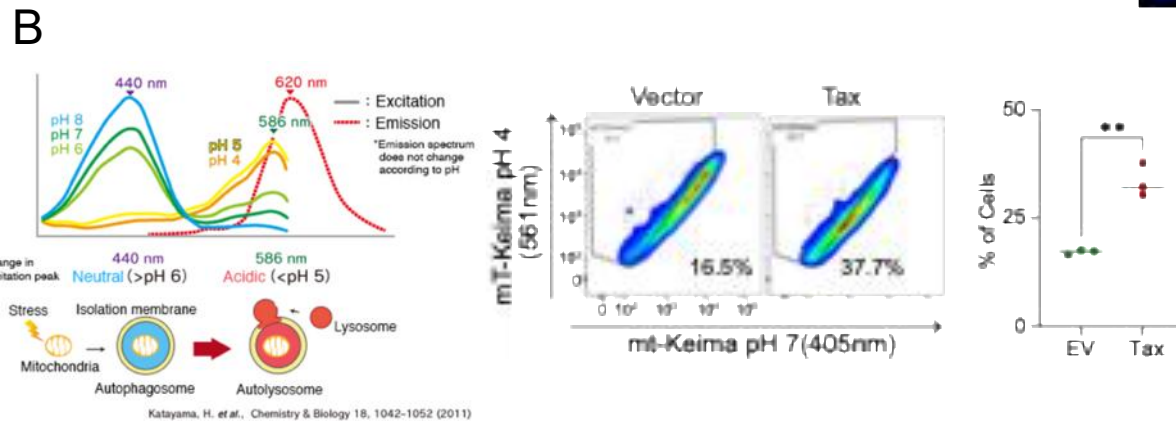
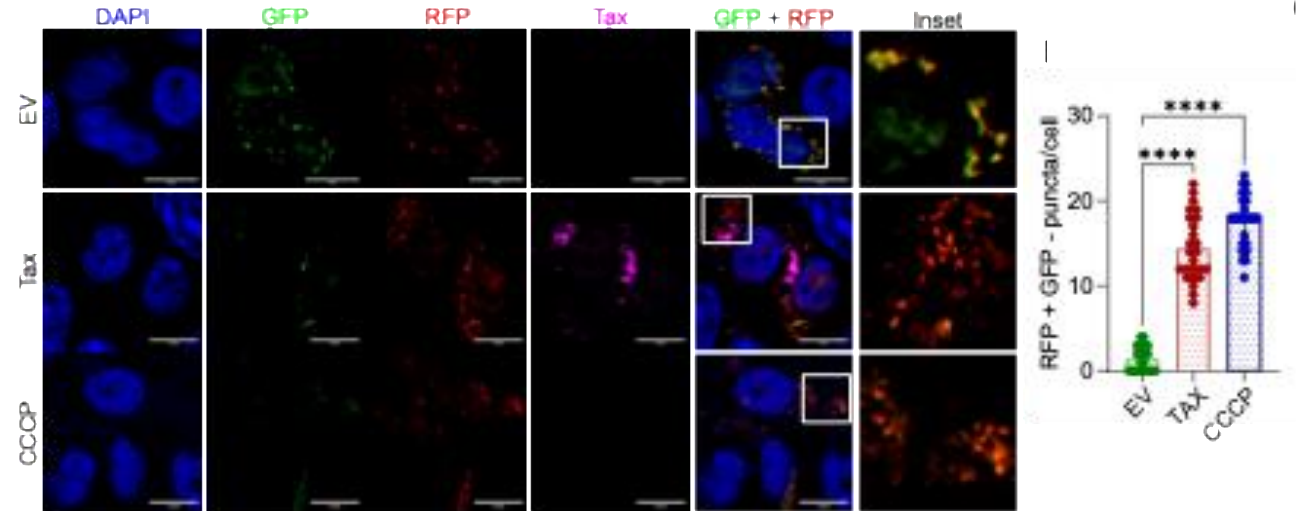
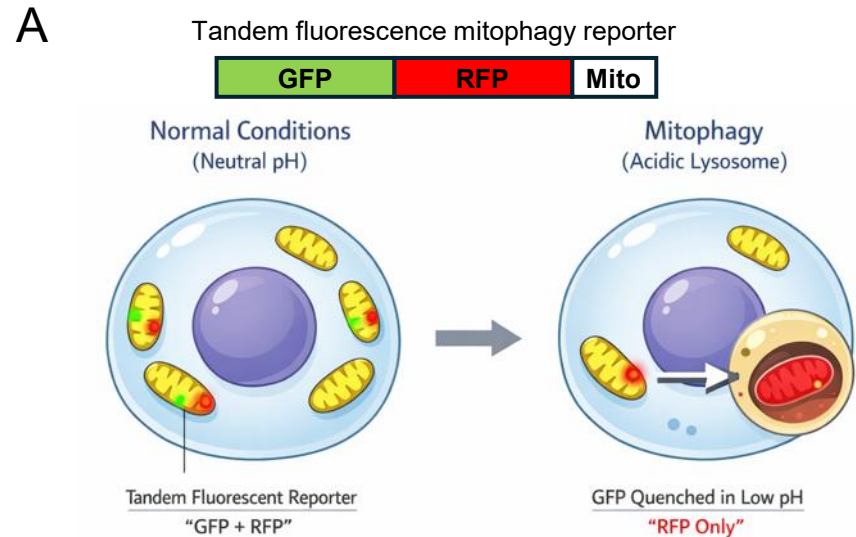


Tax colocalizes with mitochondria

C

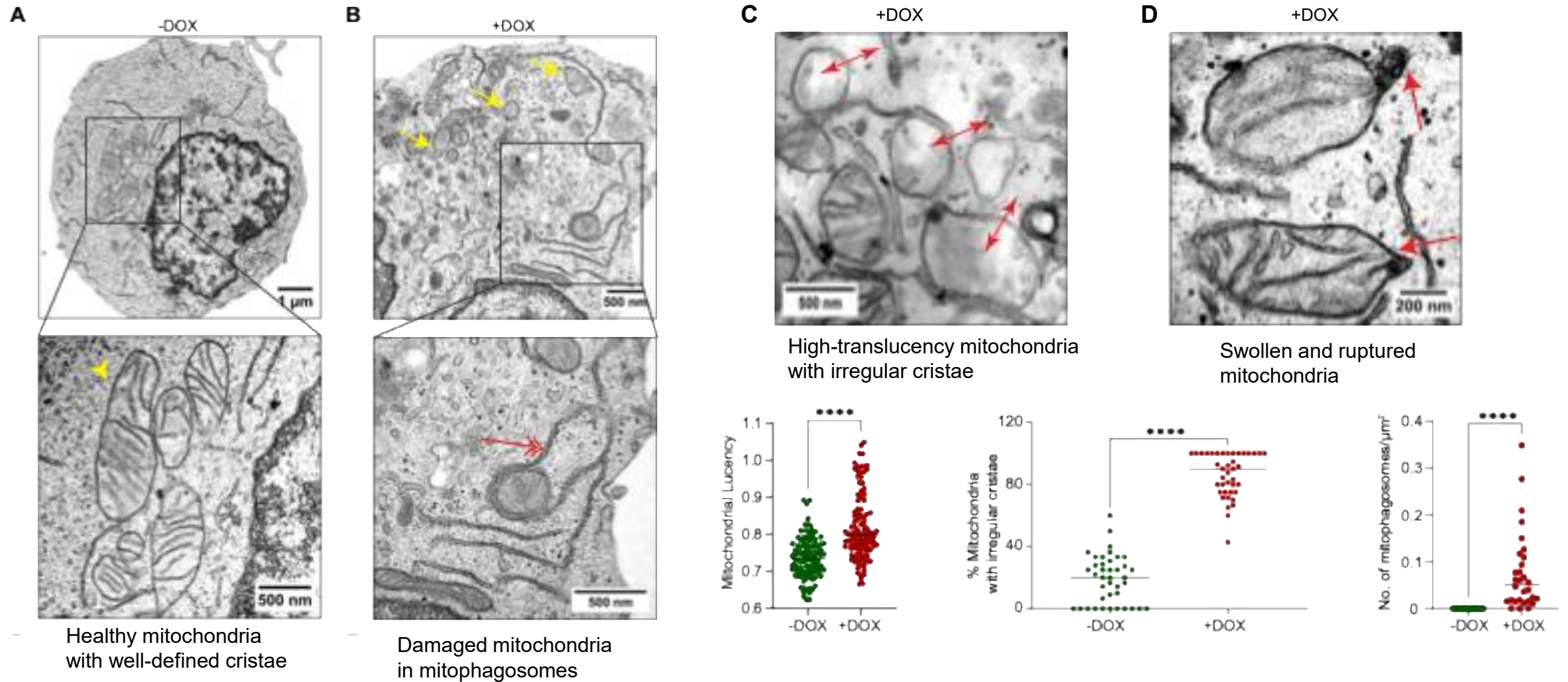


HTLV-1 Tax induces mitophagy



Mt-Keima assay

HTLV-1 Tax induces mitochondrial damage and mitophagy



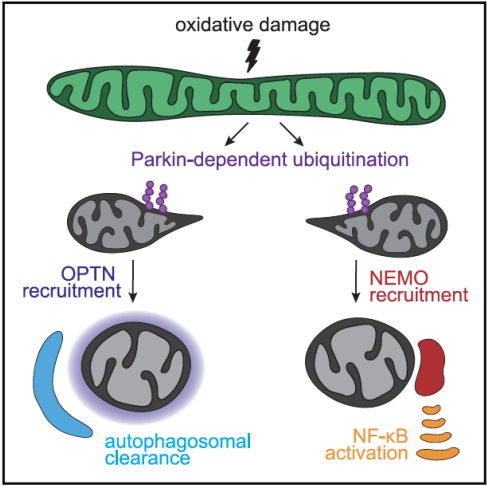
Tax requires NEMO for recruitment to damaged mitochondria

Molecular Cell

Article

Damaged mitochondria recruit the effector NEMO to activate NF- κ B signaling

Graphical abstract



Authors

Olivia Harding, Elisabeth Holzer, Julia F. Riley, Sascha Martens, Erika L.F. Holzbaur

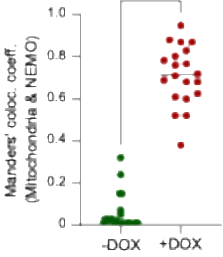
Correspondence

holzbaur@pennmedicine.upenn.edu

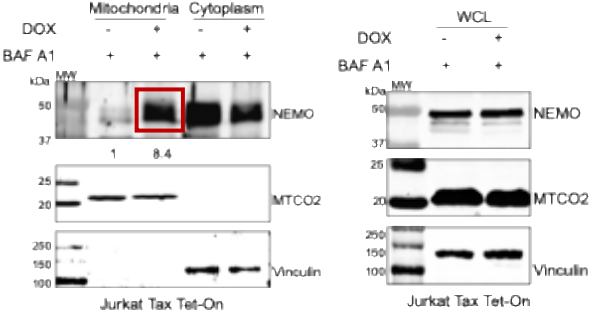
In brief

Mitochondrial dysfunction and neuroinflammation are implicated in neurodegeneration, prompting investigations into how these pathways intersect. Harding et al. identify an innate immune signaling mechanism initiating from damaged mitochondria via the NF- κ B effector NEMO. In HeLa cells, mitochondrial NEMO recruitment is Parkin dependent, and NF- κ B activation occurs in parallel to mitochondrial clearance.

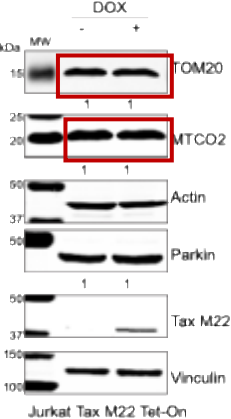
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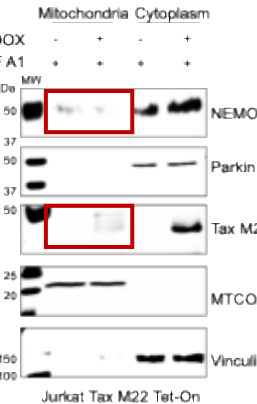
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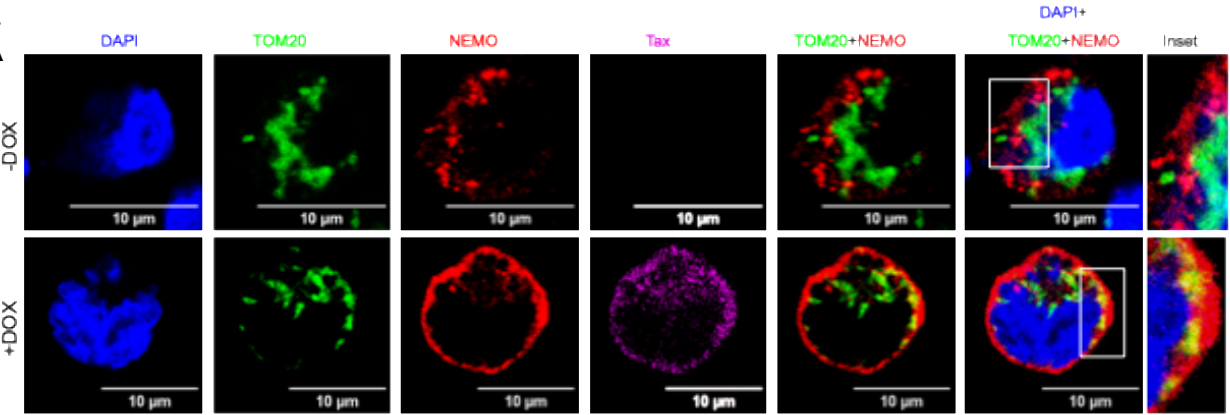
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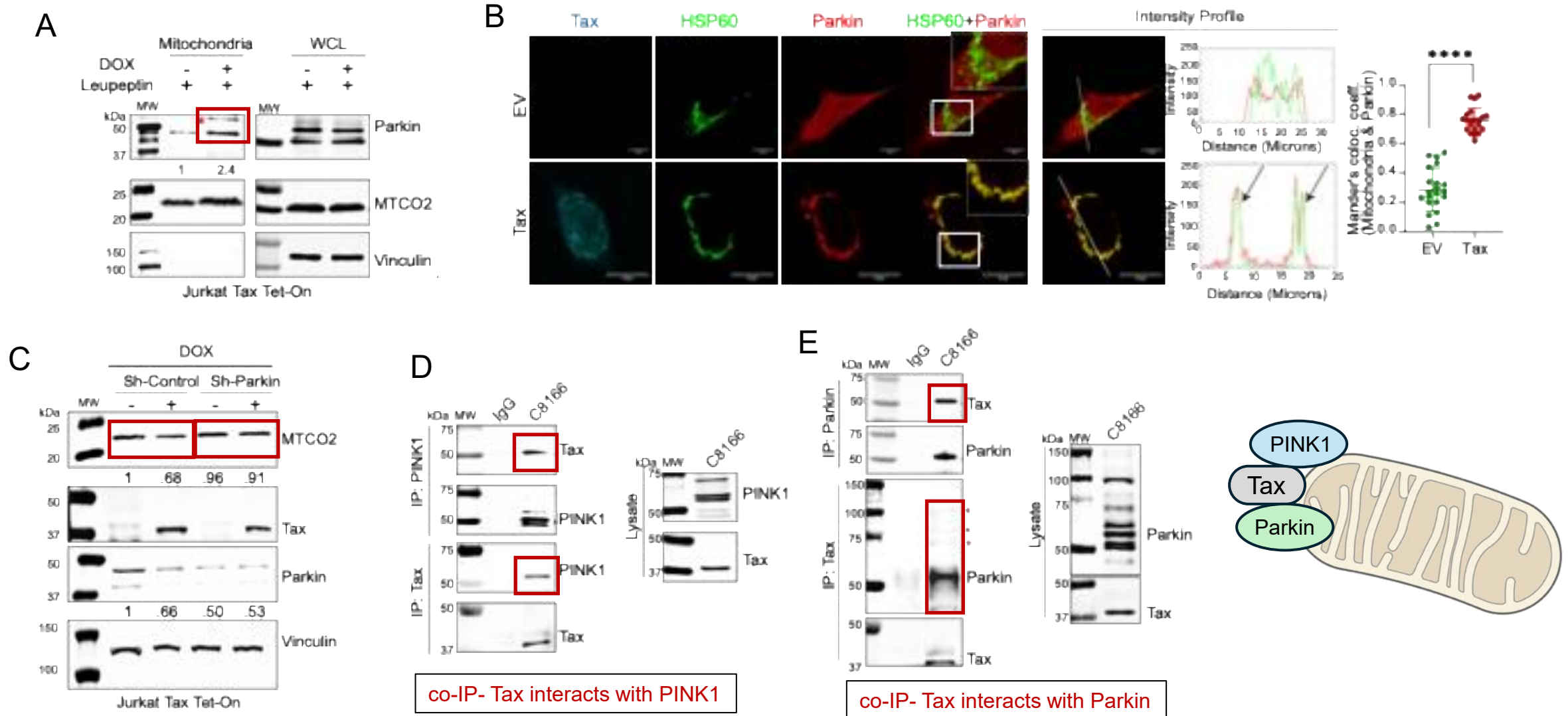


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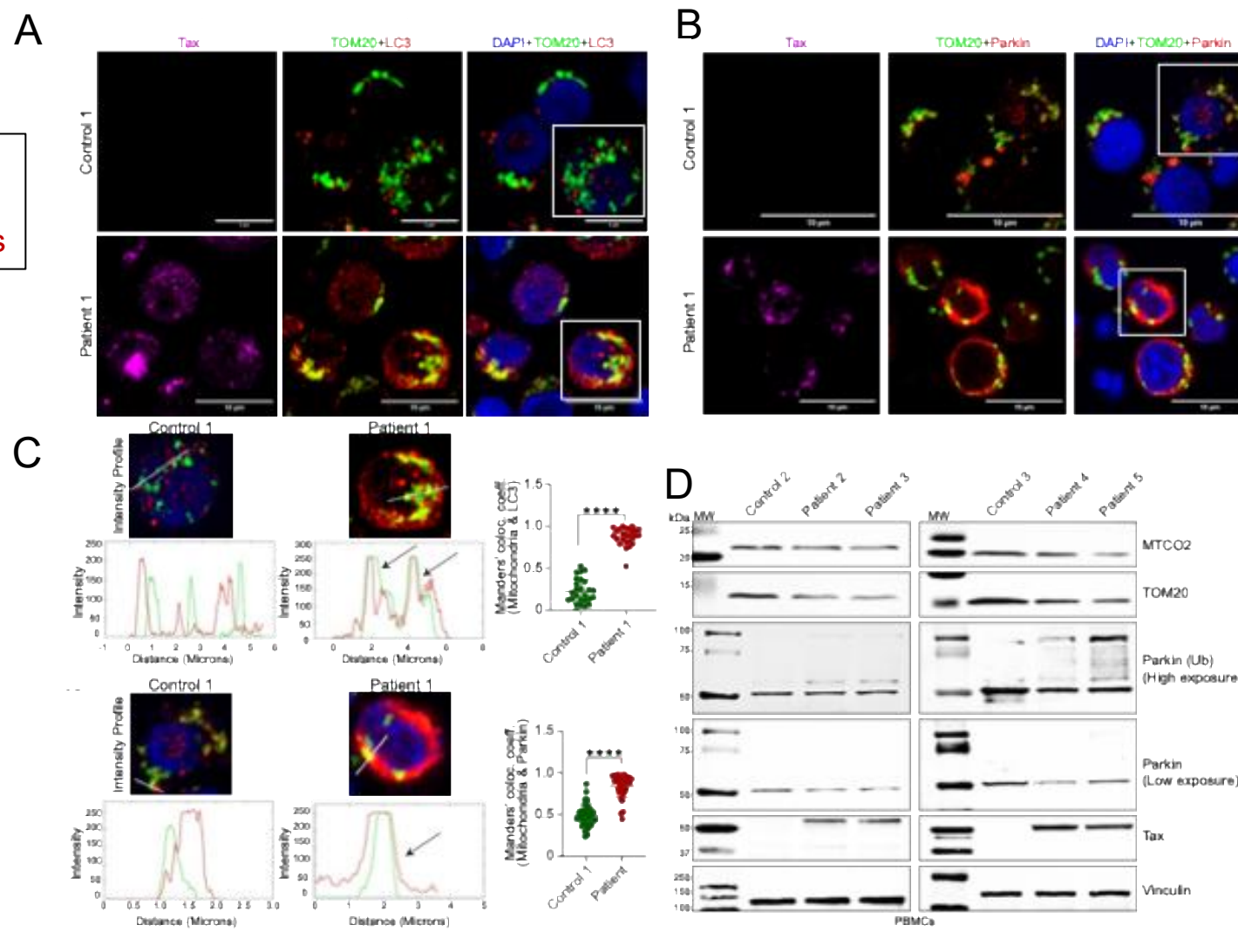
Tax M22 is impaired in NEMO binding/NF- κ B activation.
Tax M22 is impaired in mitochondrial localization and mitophagy.

HTLV-1 Tax induces Parkin-dependent mitophagy



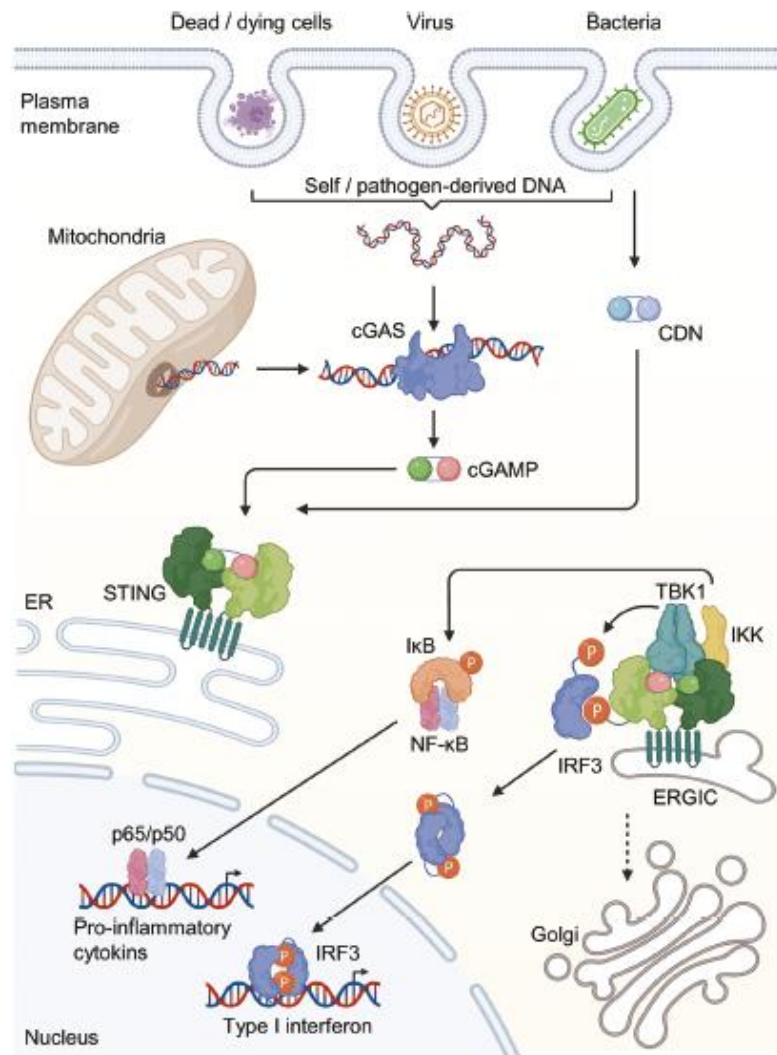
Chronic mitophagy in PBMCs from HAM/TSP patients

Primary PBMCs
from healthy controls
and HAM/TSP patients



- Similar results were obtained in HTLV-1-transformed cell lines

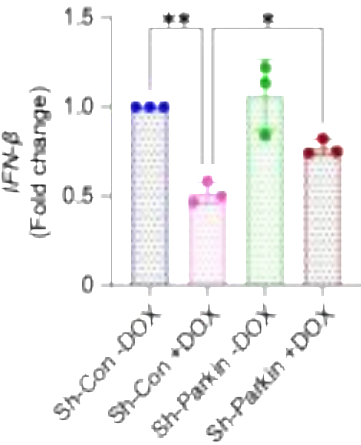
The cGAS-STING pathway



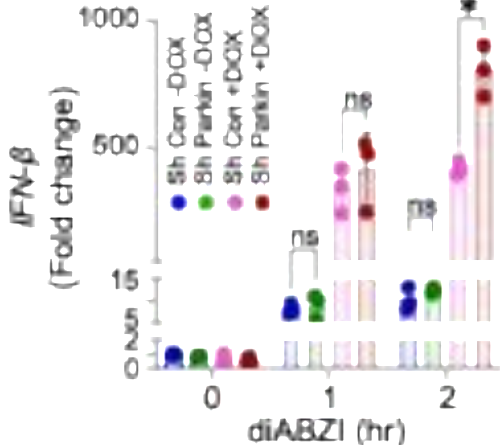
Kim et al. 2023
Exp. Mol. Med. 55: 510-519.

Tax-induced mitophagy limits cGAS-STING activation

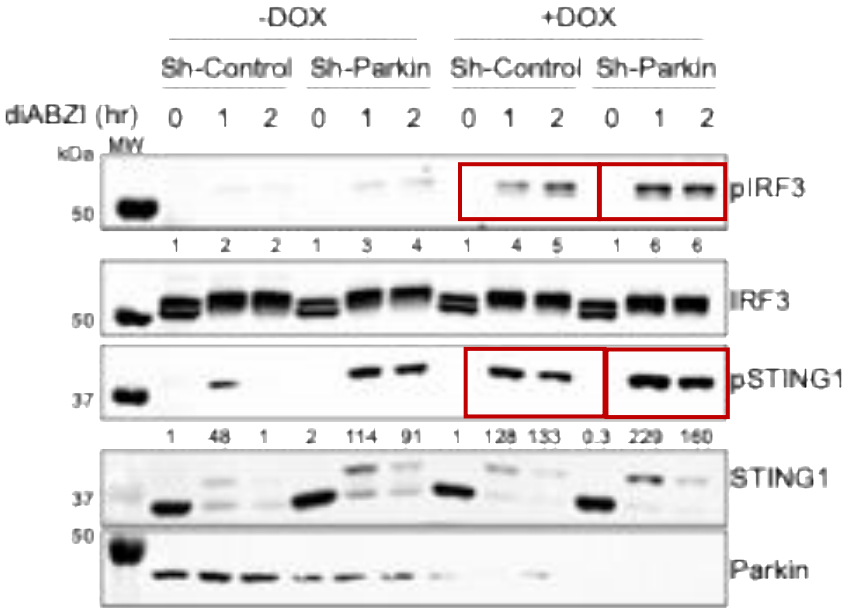
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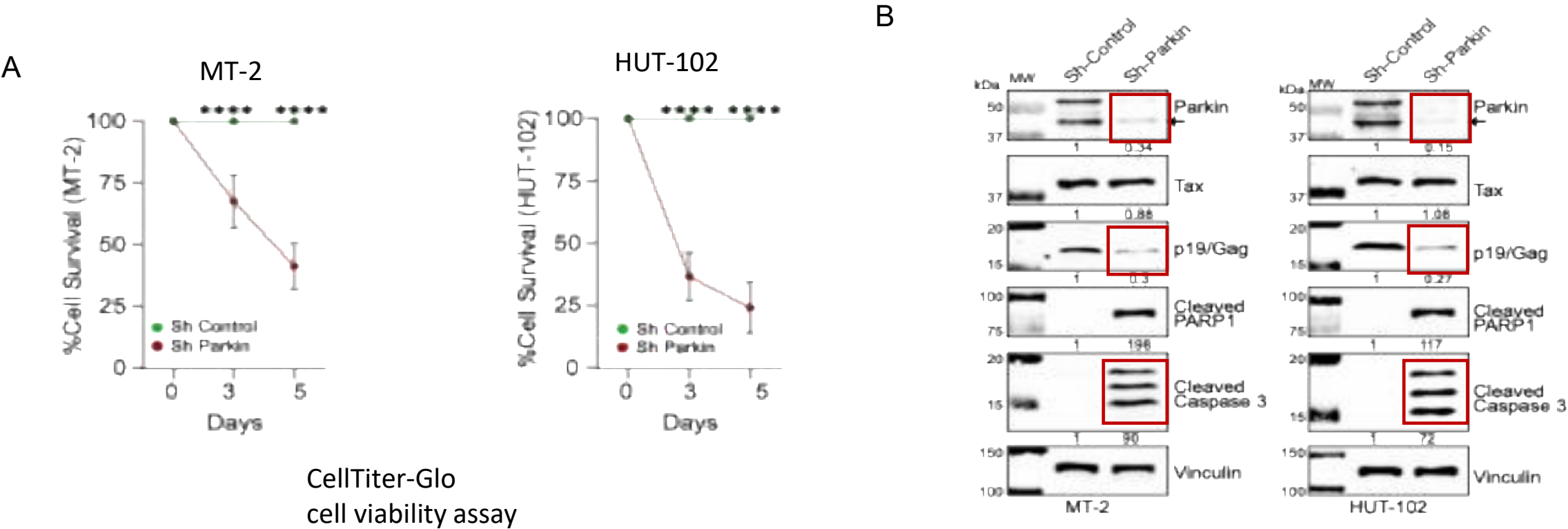
C



Jurkat Tax Tet-On

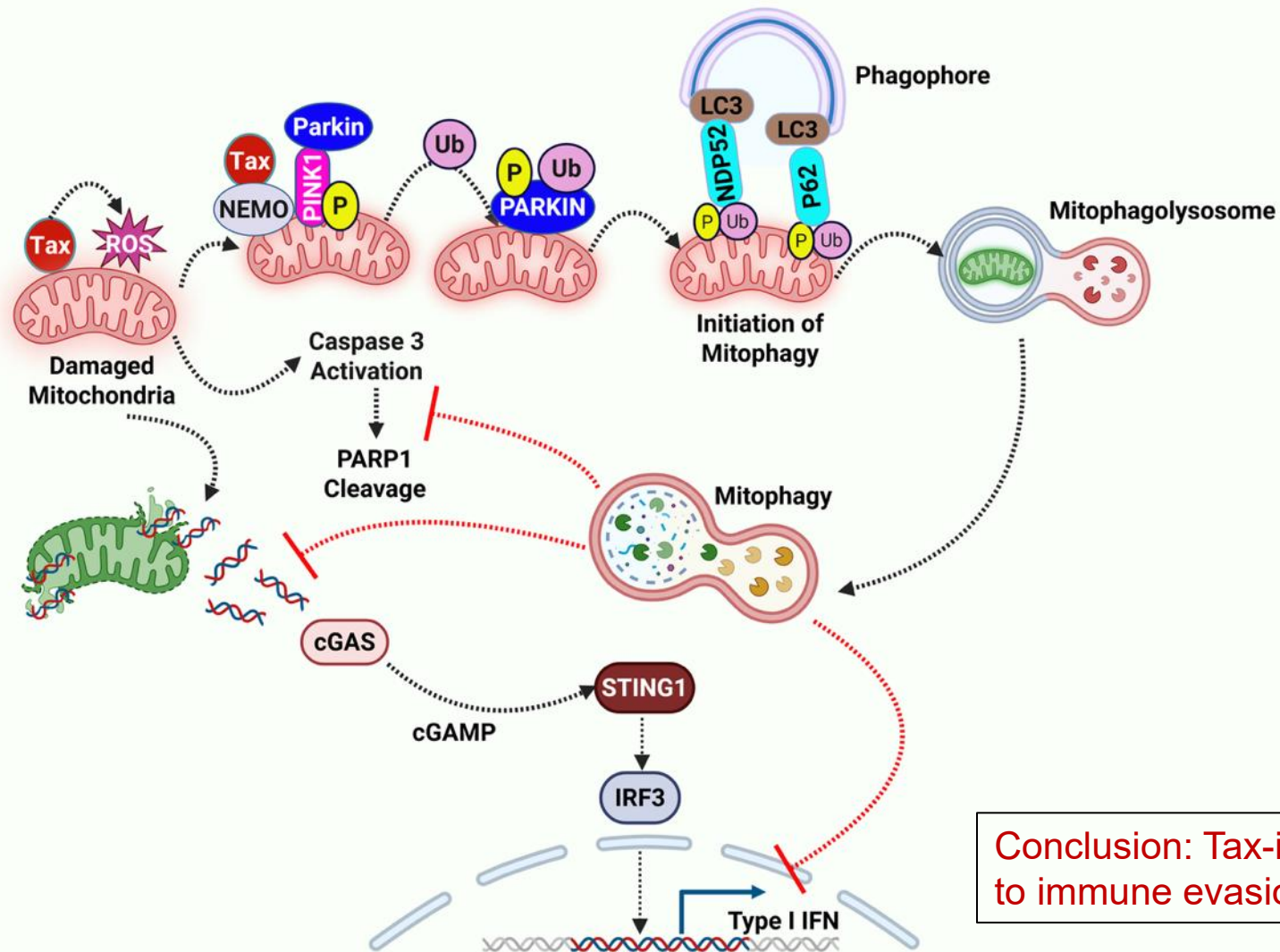
Tax likely primes cGAS-STING activation due to mitochondrial stress and DNA damage but utilizes Parkin to limit the extent of cGAS-STING activation.

Parkin is required for viral gene expression and survival of HTLV-1 transformed cells



Loss of Parkin in HTLV-1 transformed cell lines leads to decreased p19 Gag expression and the induction of apoptosis.

Model of Tax-induced mitophagy for inhibition of cGAS-STING and cell death



Conclusion: Tax-induced mitophagy contributes to immune evasion and cell survival.

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Current lab members

- **Sujit Suklabaidya, Ph.D.**
- Morgan Steele
- John Tawil
- Dominic Colter
- Cainan Riccio-Baum
- Callie Fry

Former lab members

- **Suchitra Mohanty, Ph.D. (Penn)**

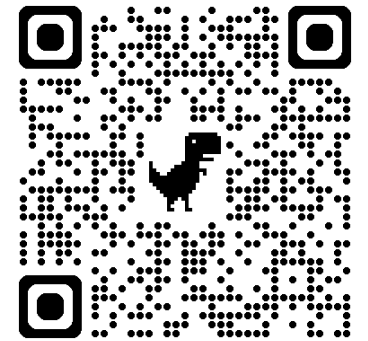
Collaborators/reagents

- Dr. Steve Jacobson (NIH/NINDS)
- Dr. Aleem Siddiqui (UCSD)
- Dr. Richard Youle (NIH/NINDS)
- Dr. Warner Greene (UCSF)
- Dr. Nelli Mnatsakanyan (PSU)

• A special thank you to the HAM/TSP patients who contributed to this study!



Read our paper in BioRxiv:



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Dendritic cells pulsed with HAM/TSP exosomes sensitize CD4 T cells to enhance HTLV-1 infection, induce helper T-cell polarization, and decrease cytotoxic T-cell response

Thomas A. Premeaux PhD, BBA

Assistant Professor of Immunology and Infectious Diseases in Medicine

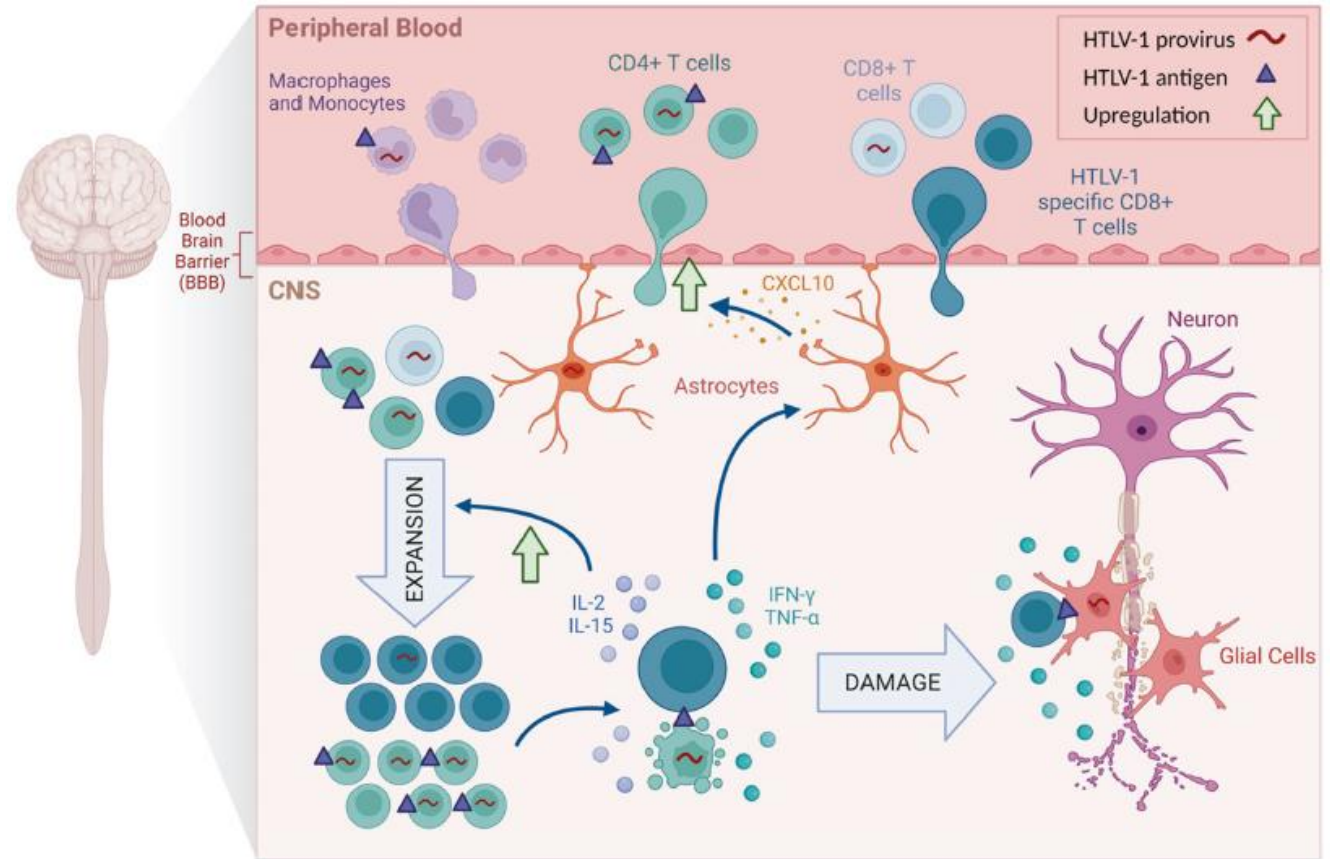
Weill Cornell Medicine, Department of Medicine, Division of Infectious Diseases



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HTLV2026

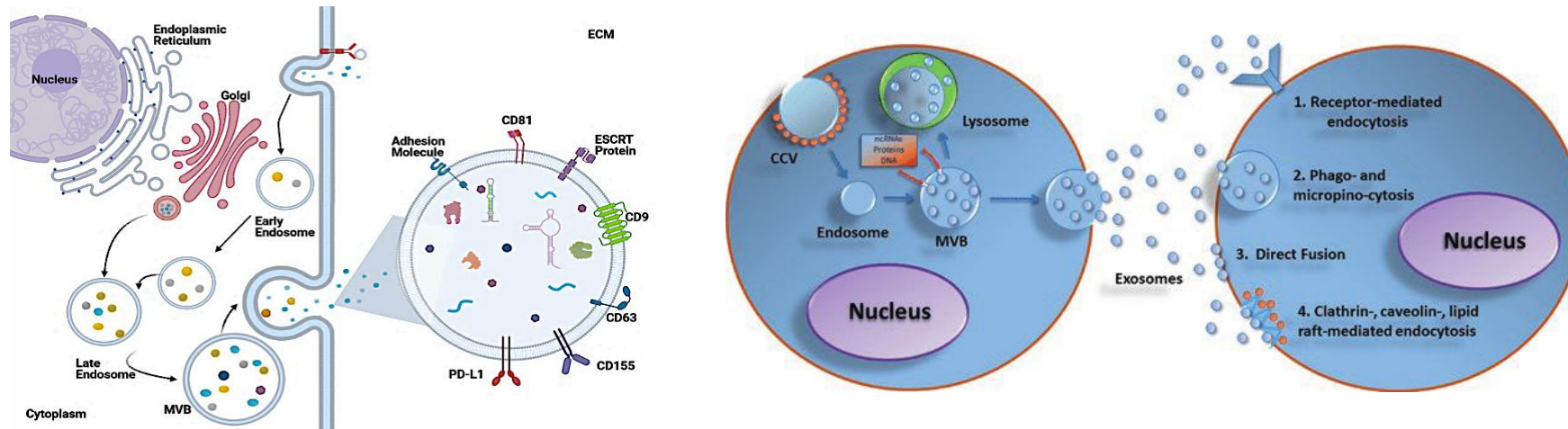
HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)

- Demyelination, neuronal death, and tissue atrophy due to bystander effects
 - Chronic inflammatory state
- Dysfunctional anti-viral responses
 - Hyperactive and exhausted T cells



Exosomal cargo and communication in HTLV-1

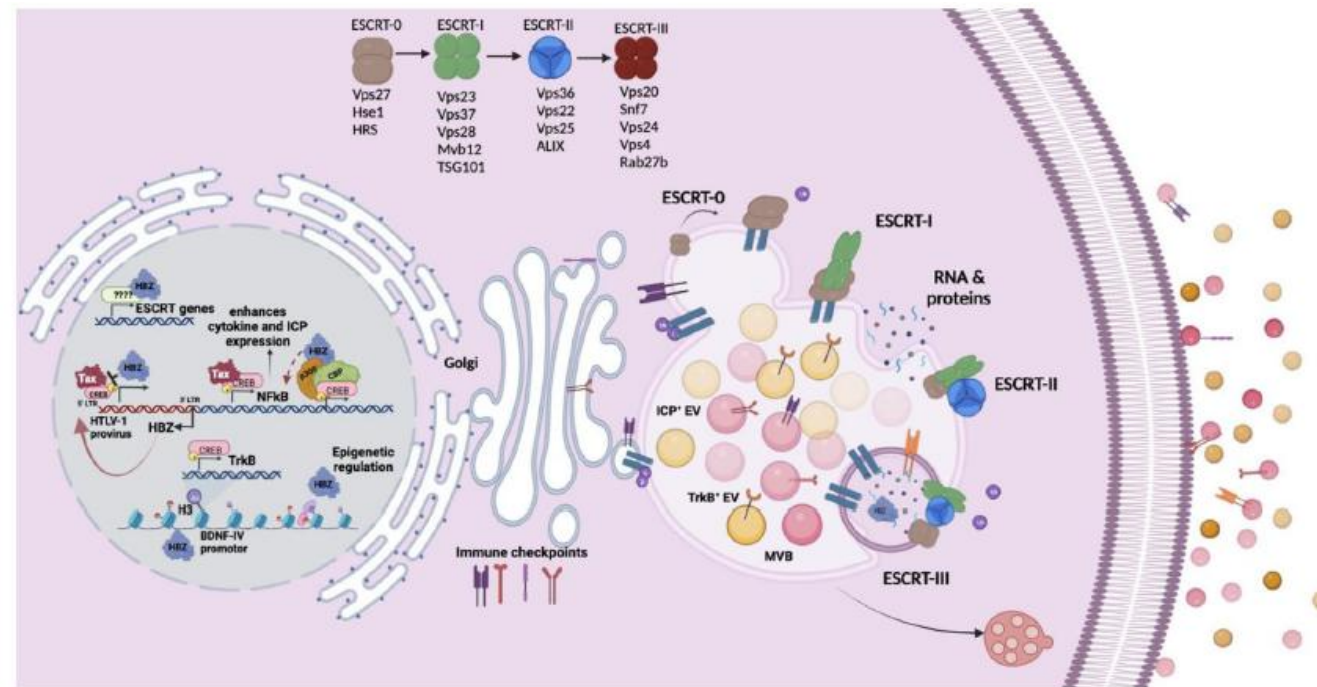
- Extracellular vesicles secreted by cells
 - Carry nucleic acids, proteins, lipids, and other bioactive substances
 - Promote cell-to-cell communication



- HTLV-1 infected cells can secrete exosomes that:
 - Contain Tax protein, TAX mRNA, cytokines, and other immunoregulatory proteins
 - Modulate targets cells and facilitate viral spread
- Exosomes containing viral antigens are detectable in the cerebrospinal fluid of people with HTLV-1

Retroviral b-Zip protein (HBZ) contributes to the release of soluble and exosomal immune checkpoint molecules in the context of neuroinflammation

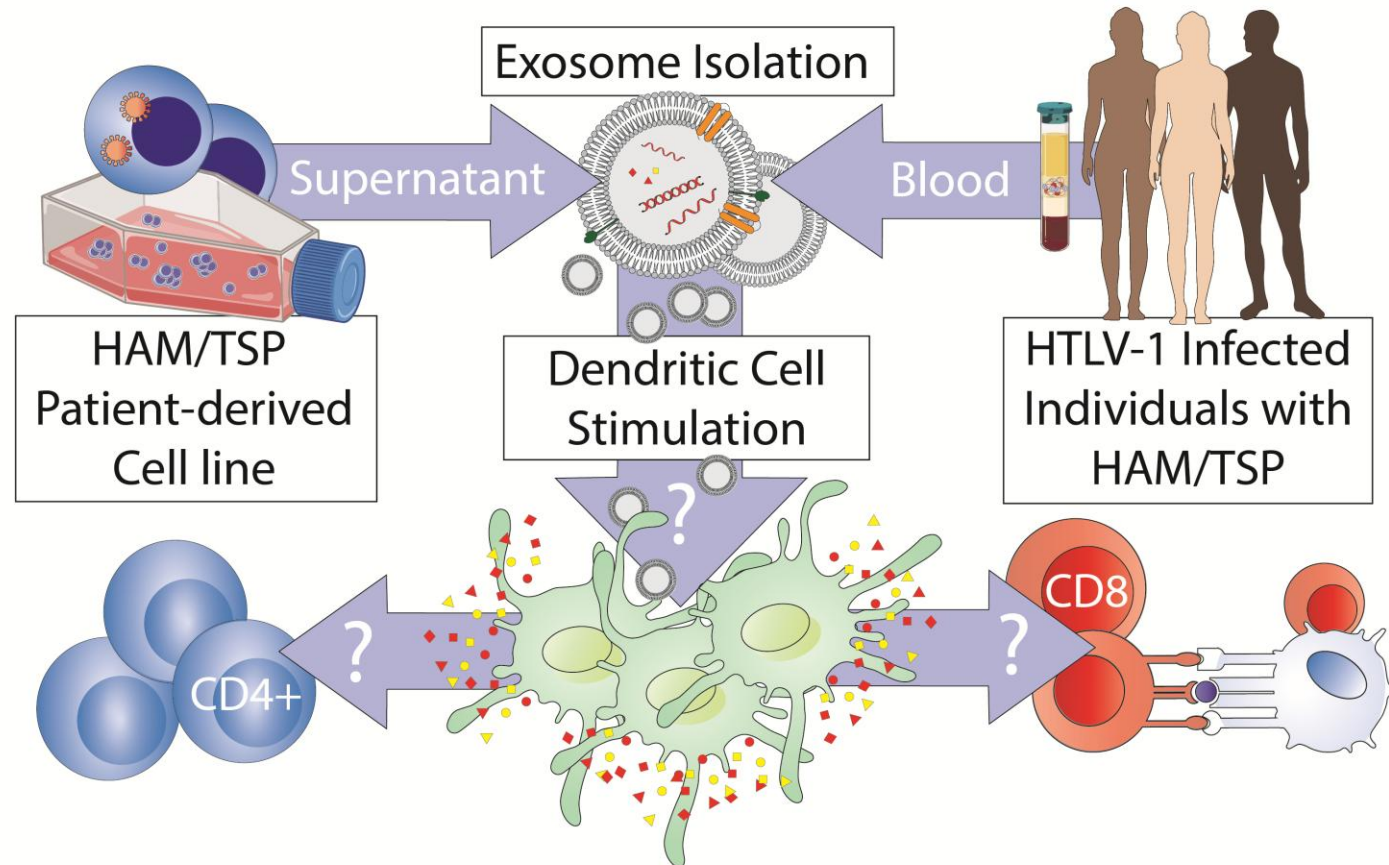
Julie Joseph¹ | Thomas A. Premeaux² | Daniel O. Pinto^{3,4} | Abhishek Rao¹ |
Shrobona Guha⁵ | Amanda R. Panfil⁶ | Alison J. Carey^{1,7} | Lishomwa C. Ndhlovu² |
Elke S. Bergmann-Leitner³ | Pooja Jain^{1,5} 



- Stimulatory and inhibitory immune checkpoint proteins carried on exosomes and in circulation in the context of HAM/TSP
- HBZ in enhancing the production and release of exosomes (ESCT pathway upregulation) and immune checkpoint proteins

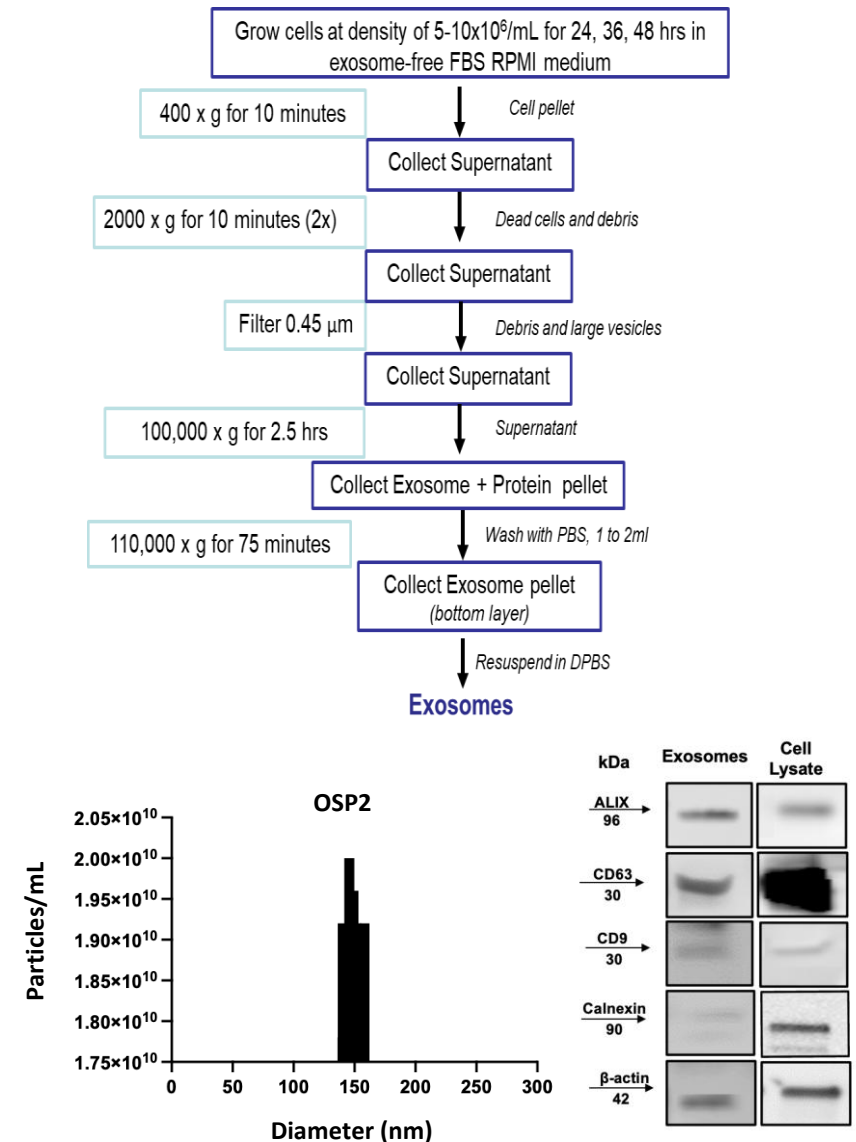
Objective

Determine whether exosomes in the context of HAM/TSP influence phenotypical and functional changes in dendritic cells, CD4+ T cells, and CD8+ T cells

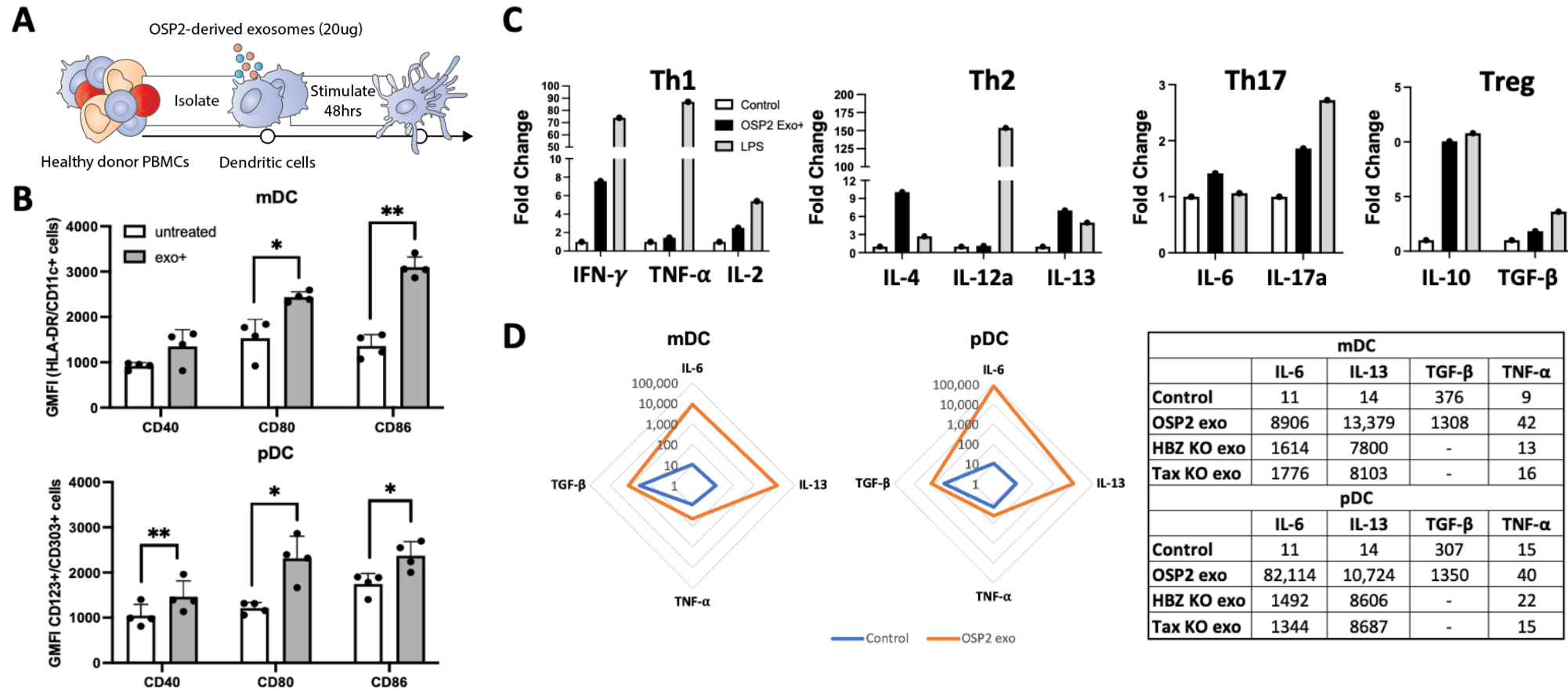


Methods

- Exosomes were isolated from:
 - OSP-2 cell line: immortalized HTLV-1 infected CD4+ T cell line derived from an individual with HAM/TSP
 - Plasma of individuals with HAM/TSP
- Isolated by ultracentrifugation
- Exosome validation, characterization, and purity
 - Western blot, Nanoparticle Tracking Analysis (NTA), anti-p19 ELISA
- Dendritic cells, CD4+ T cells, and CD8+ T cells were enriched from healthy donors or donors with HAM/TSP
- Flow cytometry, immunoassay, qPCR

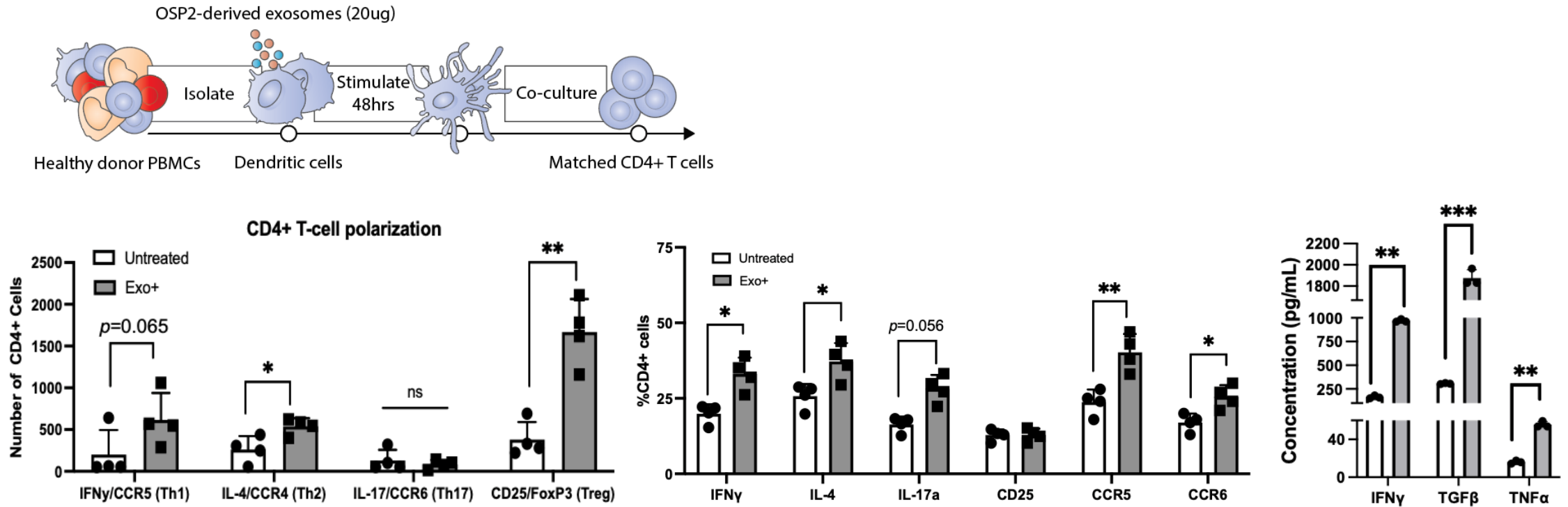


HAM/TSP cell line-derived exosomes on dendritic cell phenotype and function



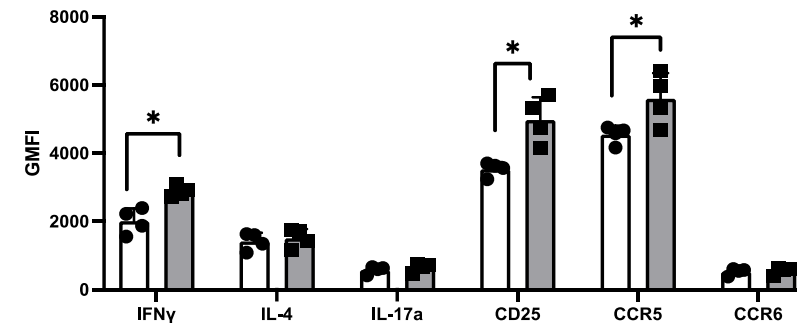
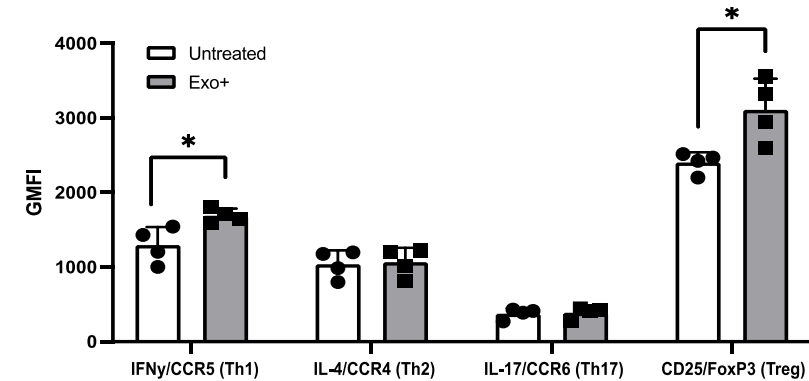
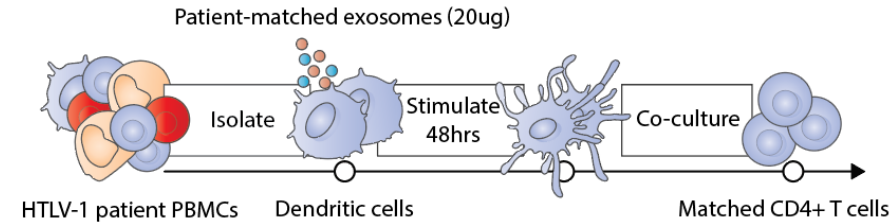
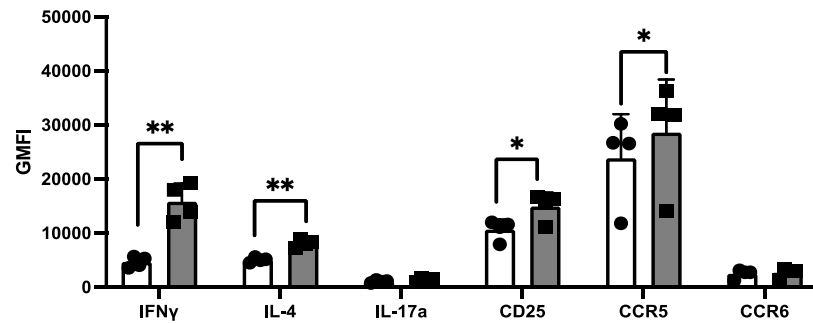
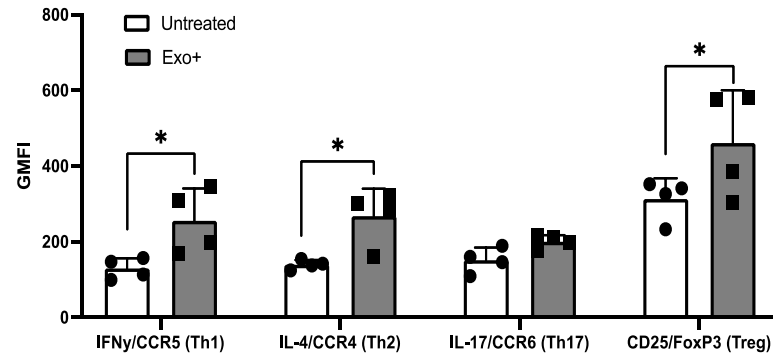
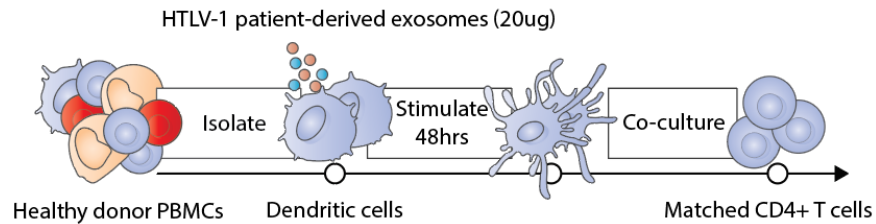
HAM/TSP exosomes increased DC activation and the production of cytokines that skew CD4⁺ T cell frequencies primarily to Th1 and Treg phenotypes

Effect of exosomes derived from HTLV-infected cell lines on CD4+ T-cell phenotypes



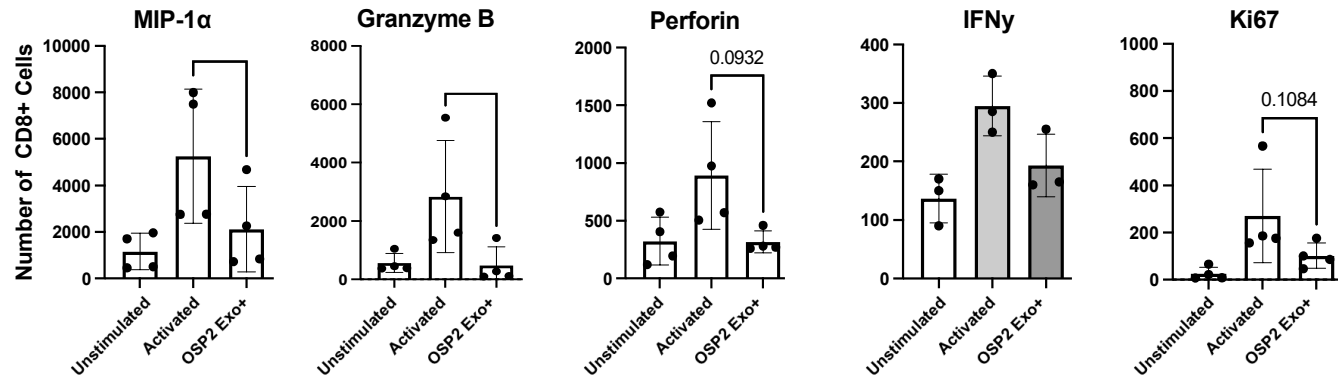
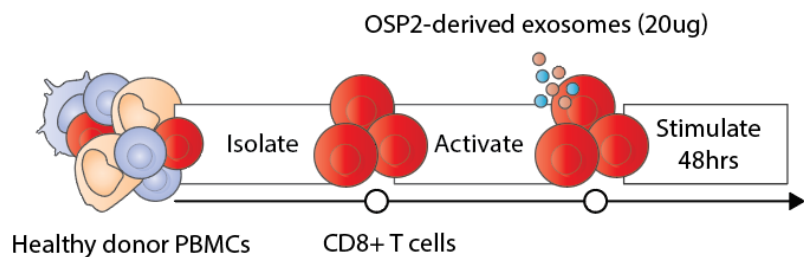
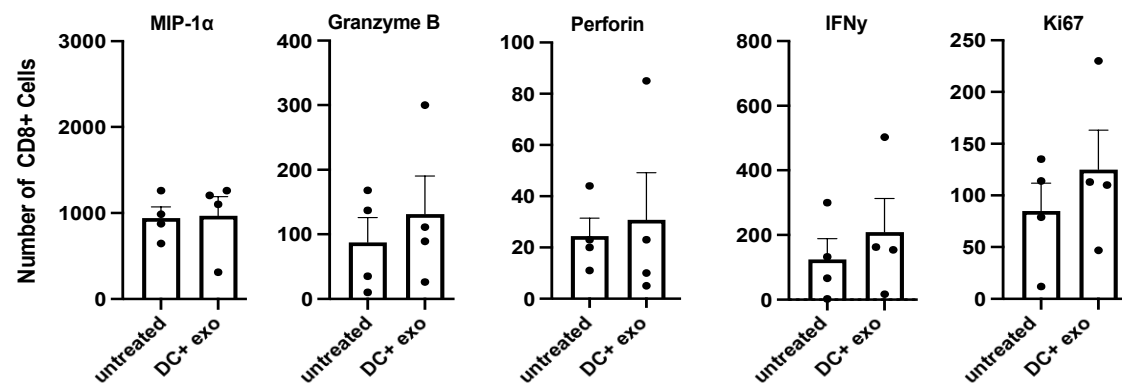
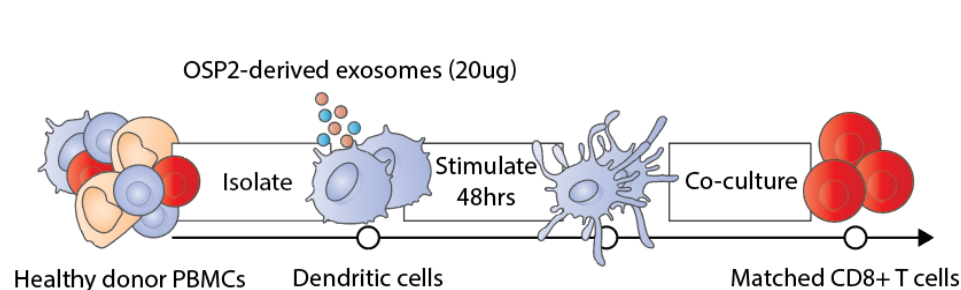
HAM/TSP cell line-derived exosomes promote CD4+ T cell subtype expansion/polarization

HTLV-1 patient-derived exosomes and CD4+ T-cells



Exosomes from individuals with HAM/TSP similarly drives CD4+ T cell polarization in healthy donors and matched HAM/TSP donors

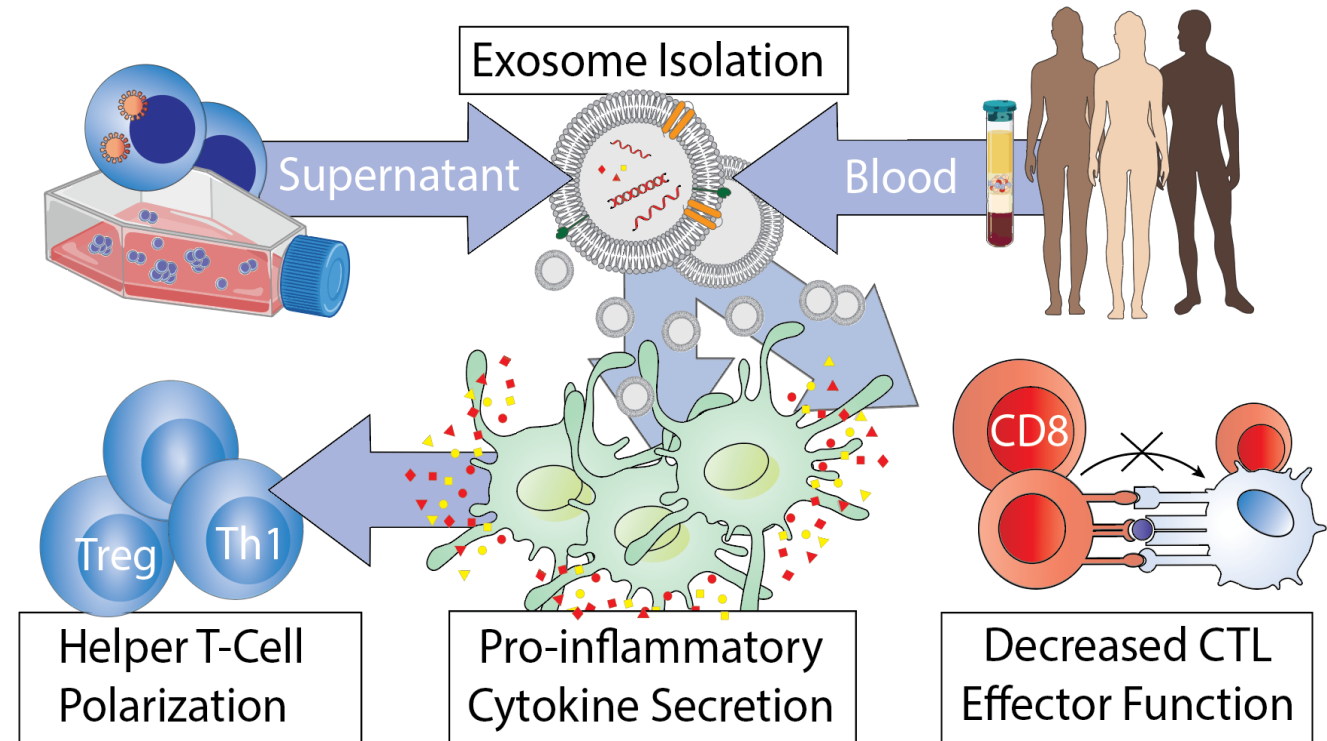
OSP2-derived exosomes suppress CD8+ T cell function



Effector function of activated CD8+ T cells is decreased when directly treated with OSP2-derived exosomes

Summary

- HAM/TSP-derived exosomes activate dendritic cells and stimulate the release of proinflammatory cytokines
- Exosome stimulated DCs skews CD4⁺ T-cell polarization/expansion towards Th1/Treg phenotypes
- OSP2-derived exosomes decreases the effector function of activated CD8⁺ T cells



- Study results suggests the potential of exosomes in HAM/TSP pathogenesis that may lead to targeted therapeutic strategies

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Weill Cornell Medicine

Lishomwa Ndhlovu

University of Pennsylvania

Wei Guo

Jingbo Yang

Luca Musante

WRAIR

Daniel Pinto

Elke Bergmann-Leitner

UCSF

Edward Murphy

Ohio State University

Amanda Panfil



**Weill Cornell
Medicine**

Funding Sources

R01NS097147 NIH/NINDS (PJ)

INSTITUTE FOR MOLECULAR MEDICINE & INFECTIOUS DISEASE

NIMH NEUROHIV TRAINING GRANT



Drexel Component
Interdisciplinary and
Translation Research
Training in NeuroHIV

National Institute
of Mental Health
(5T32MH079785)
A collaborative graduate
student training program between
Temple and Drexel involving
joint activities with Penn

Institute for Molecular Medicine & Infectious Disease

INSTITUTE FOR MOLECULAR MEDICINE & INFECTIOUS DISEASE

GRADUATE RESEARCH TRAINING



22nd International Conference on Human Retrovirology:
HTLV and Related Retroviruses
HTLV2026

June 3-6, 2026 | Philadelphia, Pennsylvania, USA

Symposium 2 - Viral Proteins and Molecular Biology

Chairs: Jianping Li and Louis Mansky

Identifying key HTLV-1 integrase residues for catalysis, host-factor binding, and intasome formation

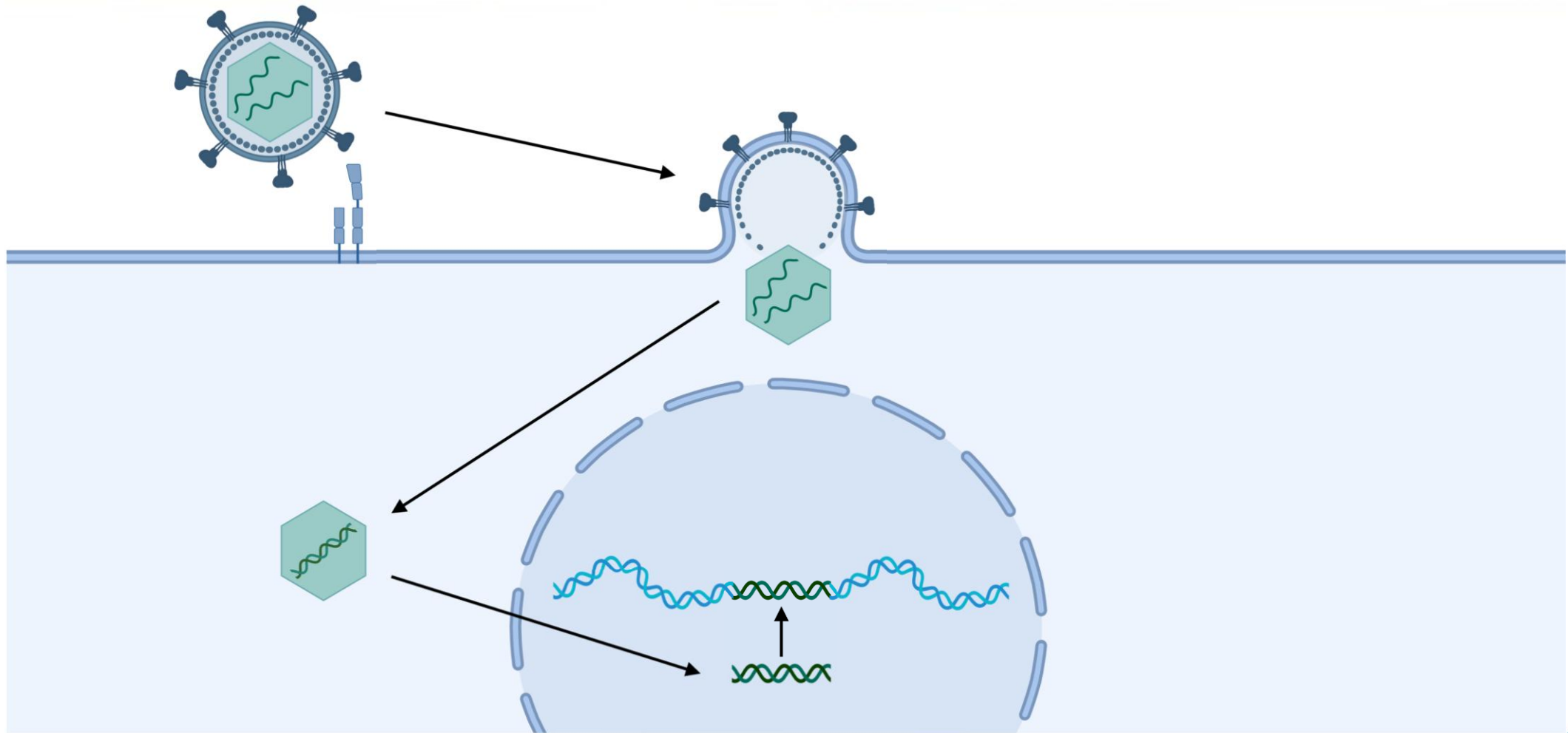
Aaron Mamane-Logsdon, Imperial College London

Conflicts of interest

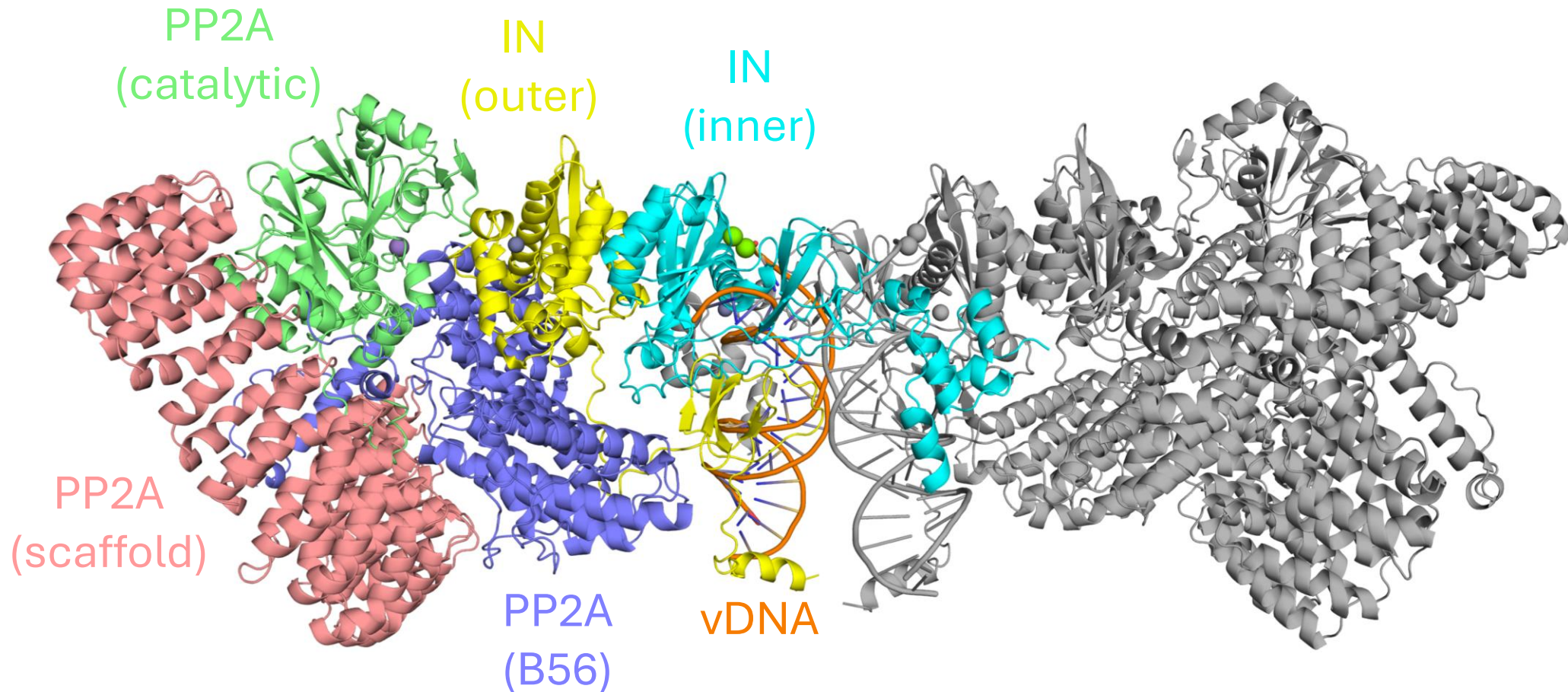
Please add your conflicts of interest or declare:

I have no conflicts of interest

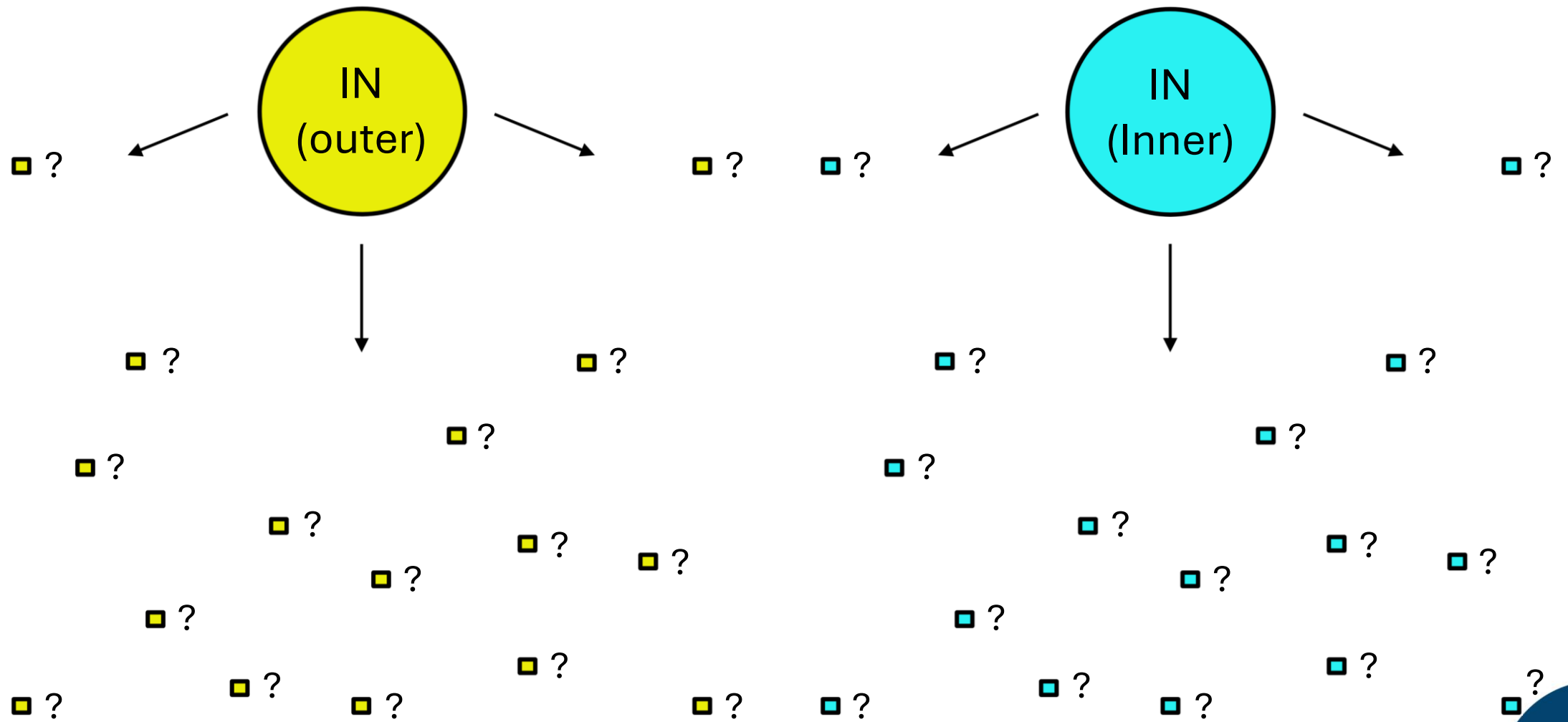
Integration is needed to establish HTLV-1 infection



Integration is mediated by the intasome



IN residue roles in integration are mostly unknown



Novel methods of variant prediction are required

What type of task is this? **Scanning mutagenesis.**

What problems does this raise? **Time and money.**

What is the solution? **Targeted mutagenesis by upstream prediction.**

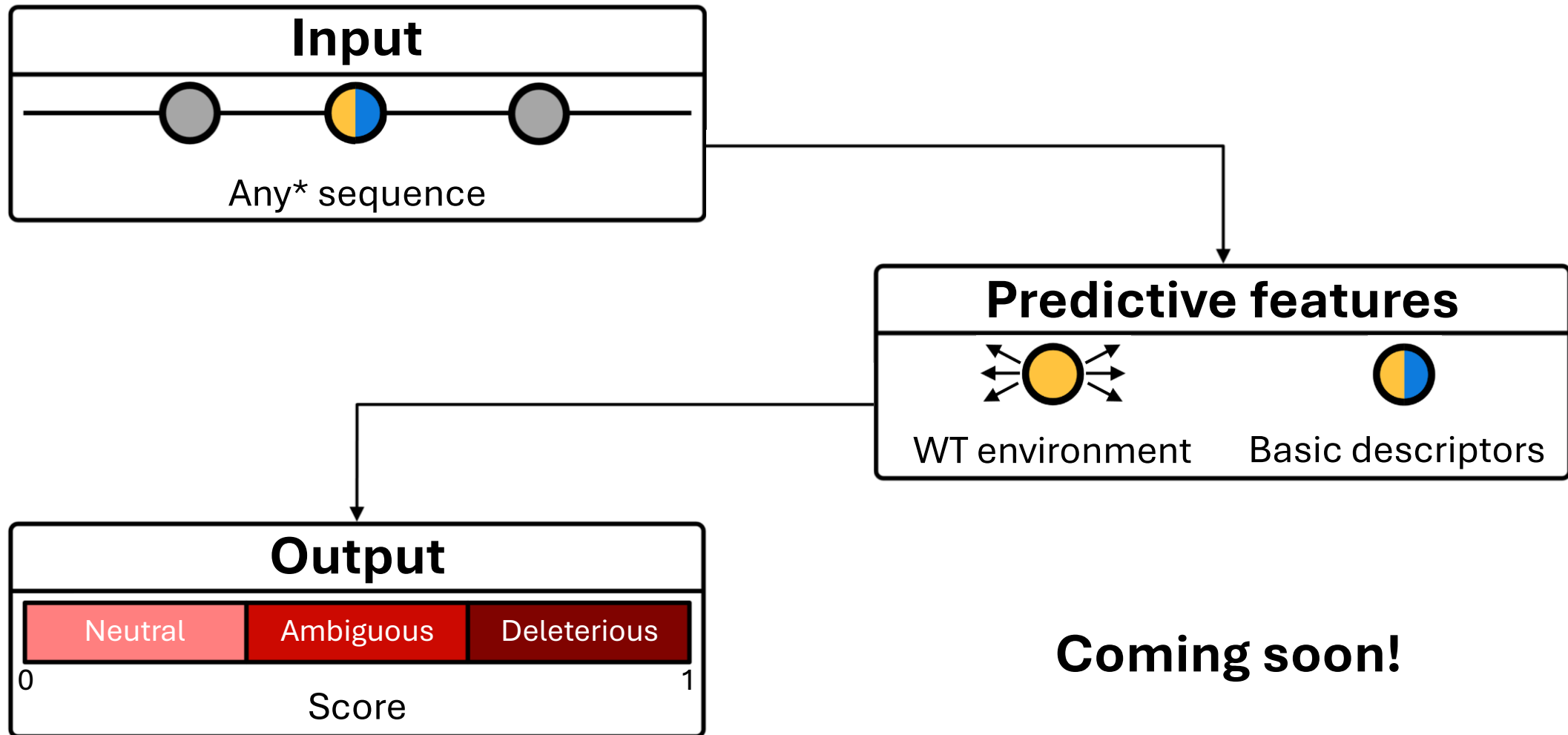
Is there software for this? **Yes.**

Can we reliably use it? **No.**

Why? **Static, human-centric, protein-centric, ...**

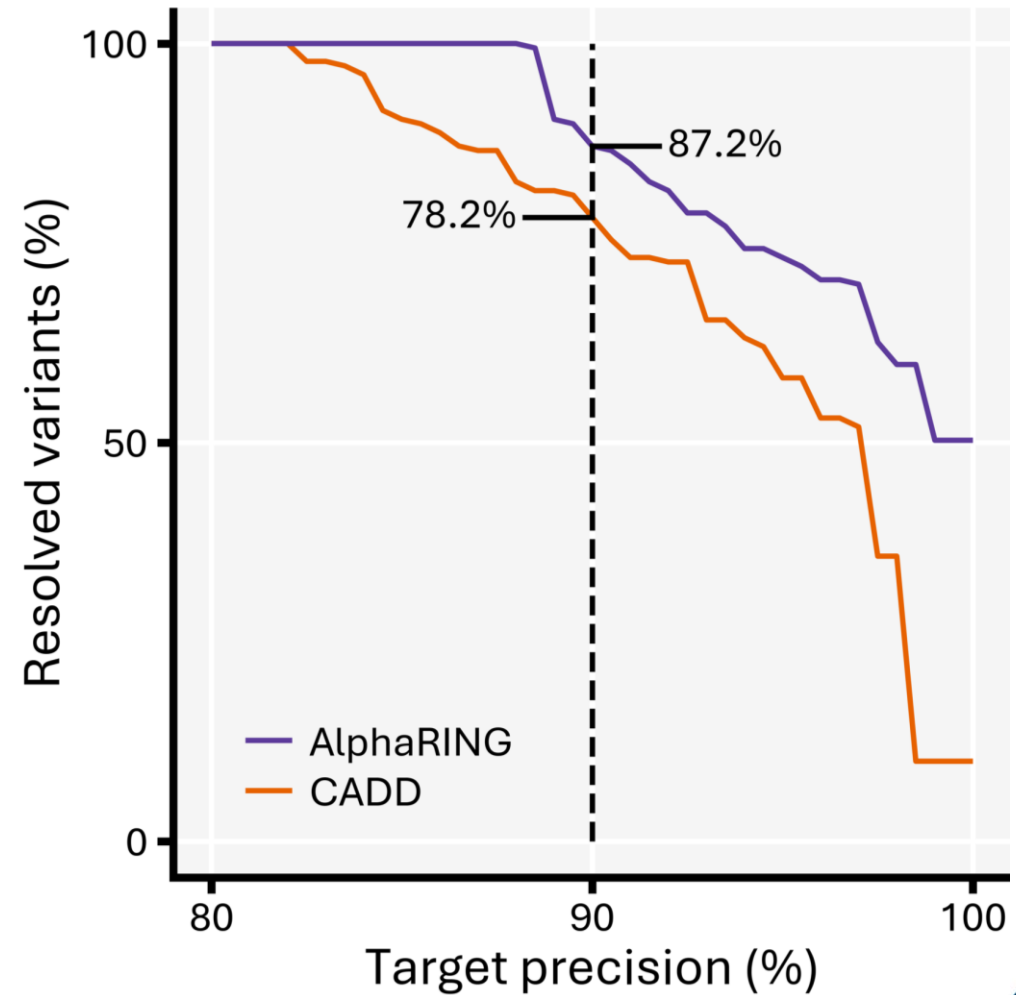
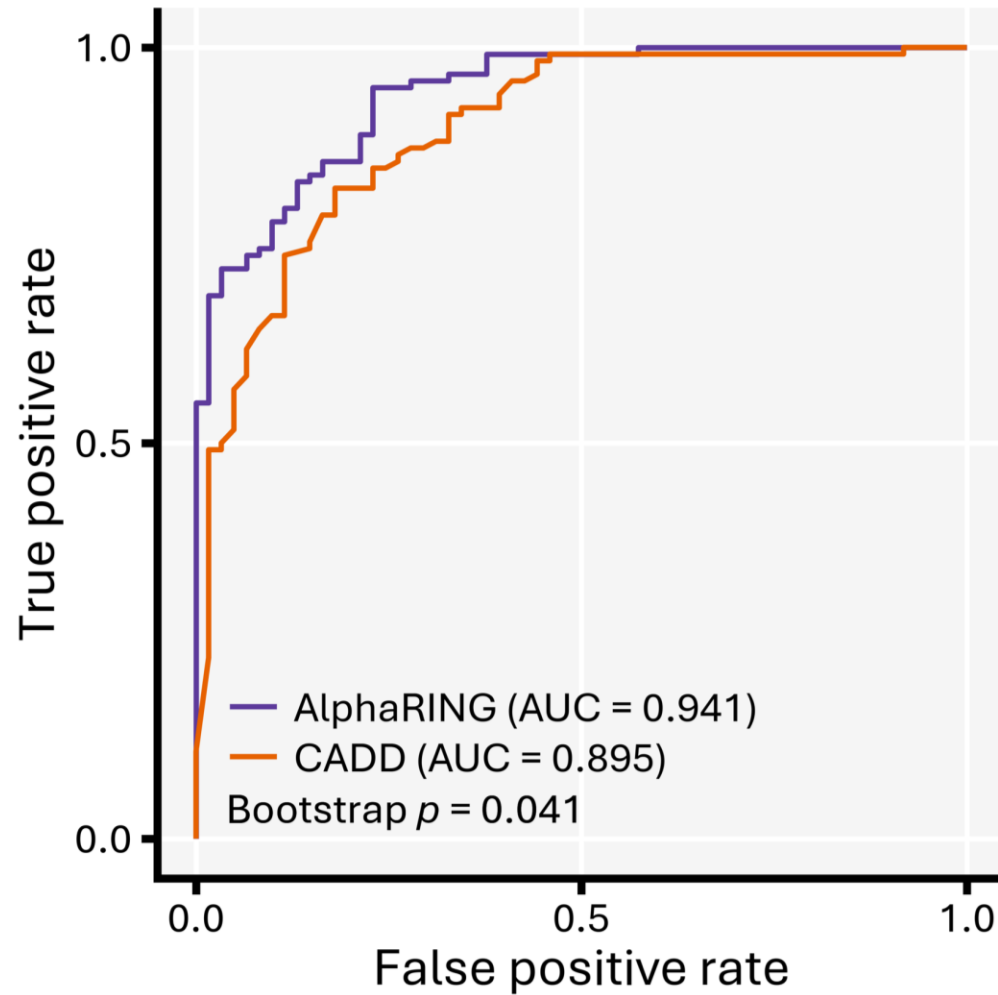


AlphaRING (v3): a solution to the problem

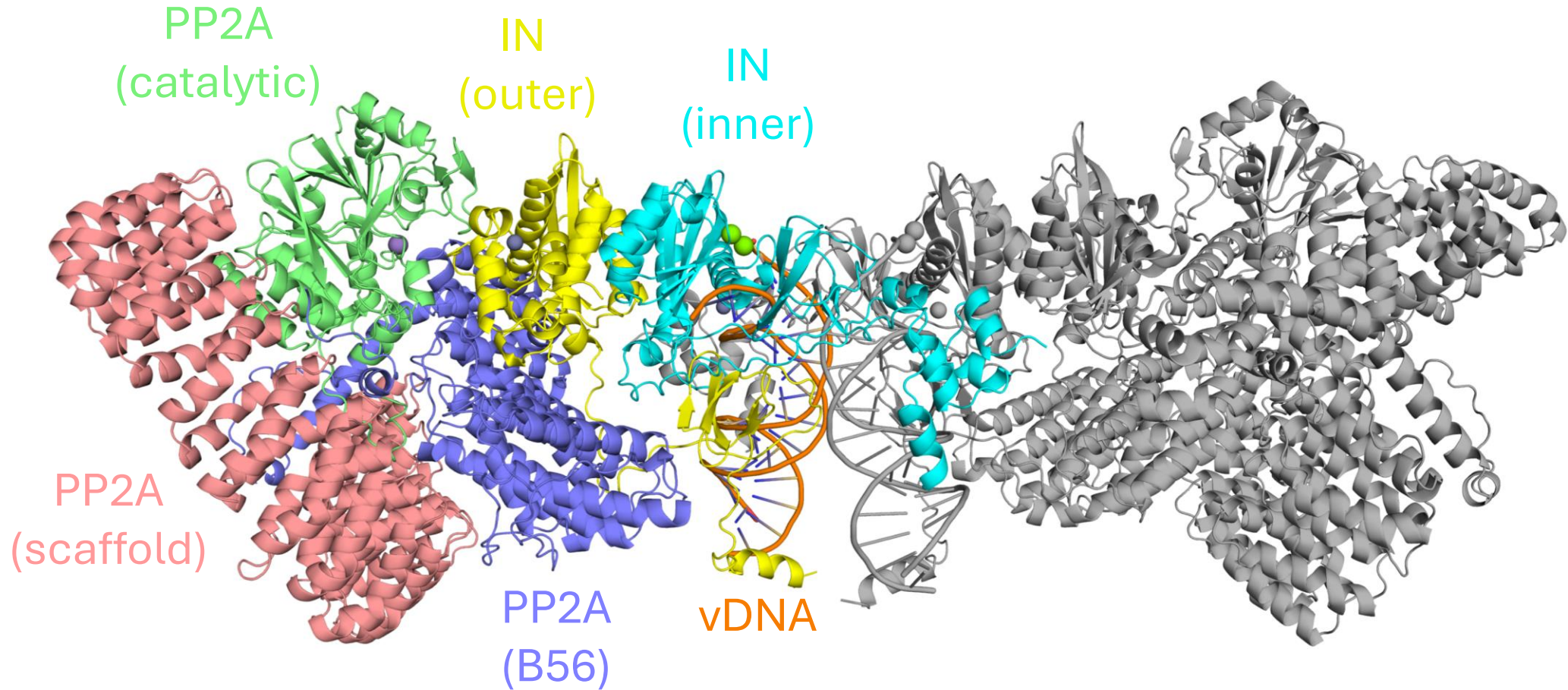


Coming soon!

AlphaRING is highly accurate and decisive



Selection of IN variants for AlphaRING validation



AlphaRING predicted deleterious IN variants

Variant	Reason	Score (in/out)	Validation
F10D	Intasome formation	0.896/0.900	Unstable IN
W189D	Intasome formation	0.918/0.918	Unstable IN
W205R	Intasome formation	0.908/0.913	TBD
R53D	Host-factor binding	0.707/0.911	Insoluble IN
H201D	Host-factor binding	0.643/0.899	TBD
R159A	Host-factor binding	0.605/0.864	B56:IN binding reduced
R159E	Multiple	0.645/0.869	Insoluble IN



Conclusions

- Prediction of HTLV-1 IN variant deleteriousness can expedite the characterisation of residue roles in integration.
- To do this, we developed AlphaRING, which outperformed gold-standard tools.
- AlphaRING identified novel IN variants that were confirmed to be deleterious.
- Non-alanine substitutions were too deleterious for functional characterisation.
- Going forward, we will focus on alanine substitutions.



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- Dr Christine Hilcenko
- Ben Rebairo

Barnard Lab

- Dr Anna Barnard



Bellos Lab

- Dr Evangelos Bellos



Thank you





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Small-molecule modulators of BLV and HTLV proteases: from enzymatic screening to antiviral activity

Camila Sagasti
Institut Pasteur de Montevideo, Montevideo, Uruguay
Facultad de Medicina, UdelaR, Montevideo, Uruguay

Small-molecule modulators of BLV and HTLV proteases: from enzymatic screening to antiviral activity

Camila Sagasti; Mariana Salinas; Florencia Rammauro; Yrupé Arhancet; Agustín Demarco;
Melissa De León; Jeniffer Chá; Federico Carrión; Natalia Olivero; Martín Fló.

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Unidad Académica de Inmunobiología, Facultad de Medicina, UdelaR, Montevideo,
Uruguay

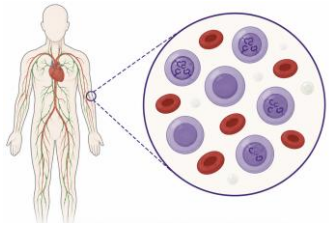
Conflicts of interest

I have no conflicts of interest

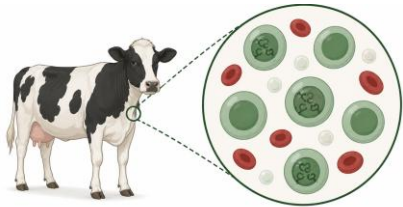
Introduction

BLV and HTLV-1: related deltaretroviruses with global impact

HTLV-1 ~ 20 million people worldwide infected. Shows a global distribution with endemic regions.

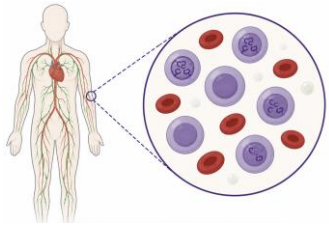


BLV (agent of enzootic bovine leukosis) globally distributed and highly relevant in **livestock-producing countries**.

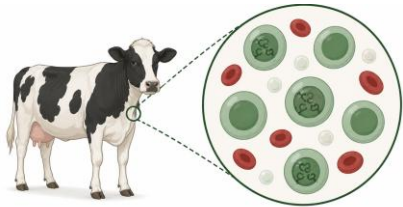


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Uruguay as an example



Modified from WAHIS (woah.org)
2020-2023

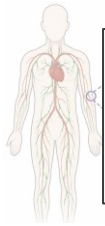
BLV prevalence >80%
Positive animals in ~100% of dairy farms studied



Estimated economic losses of 2.5–3.5% in the dairy industry.

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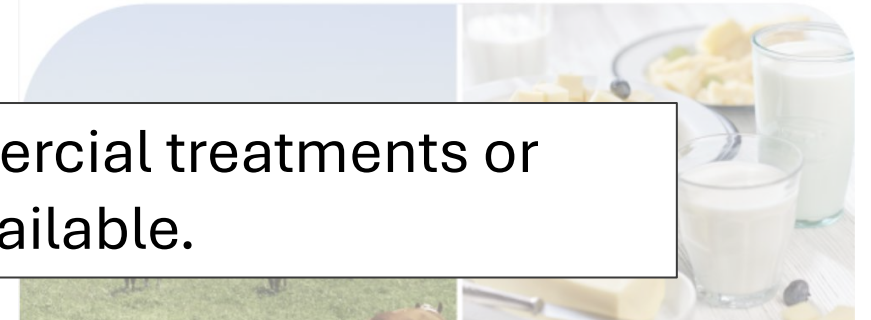
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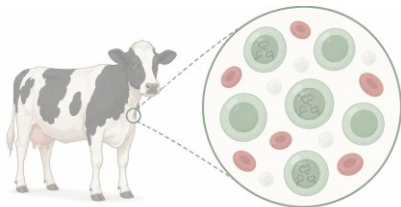
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BLV (agent of enzootic bovine leukosis) globally distributed and highly relevant in **livestock-producing countries**.



For HTLV, as well as for BLV, no commercial treatments or vaccines are currently available.



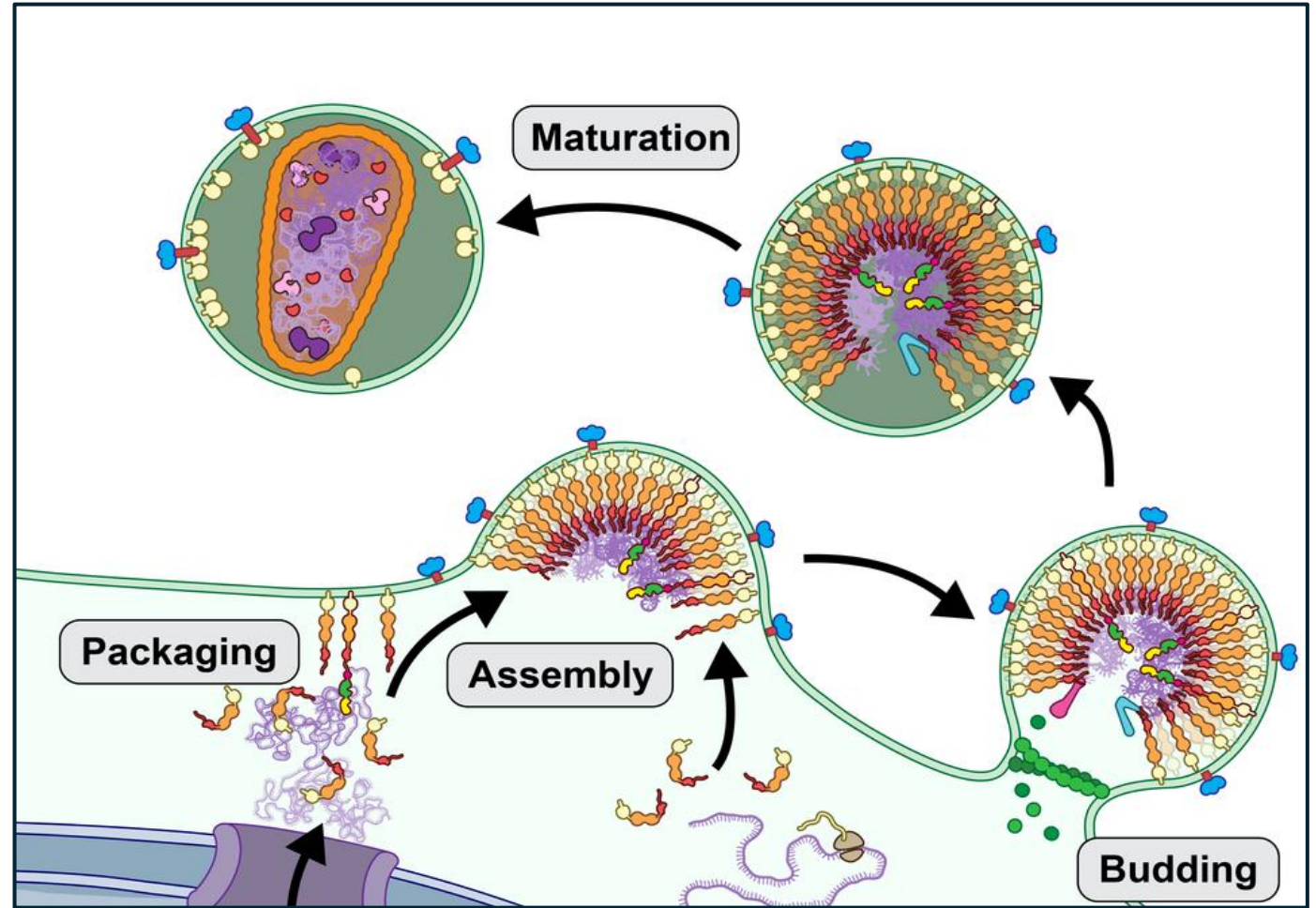
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The retroviral protease

The virus buds in an immature form

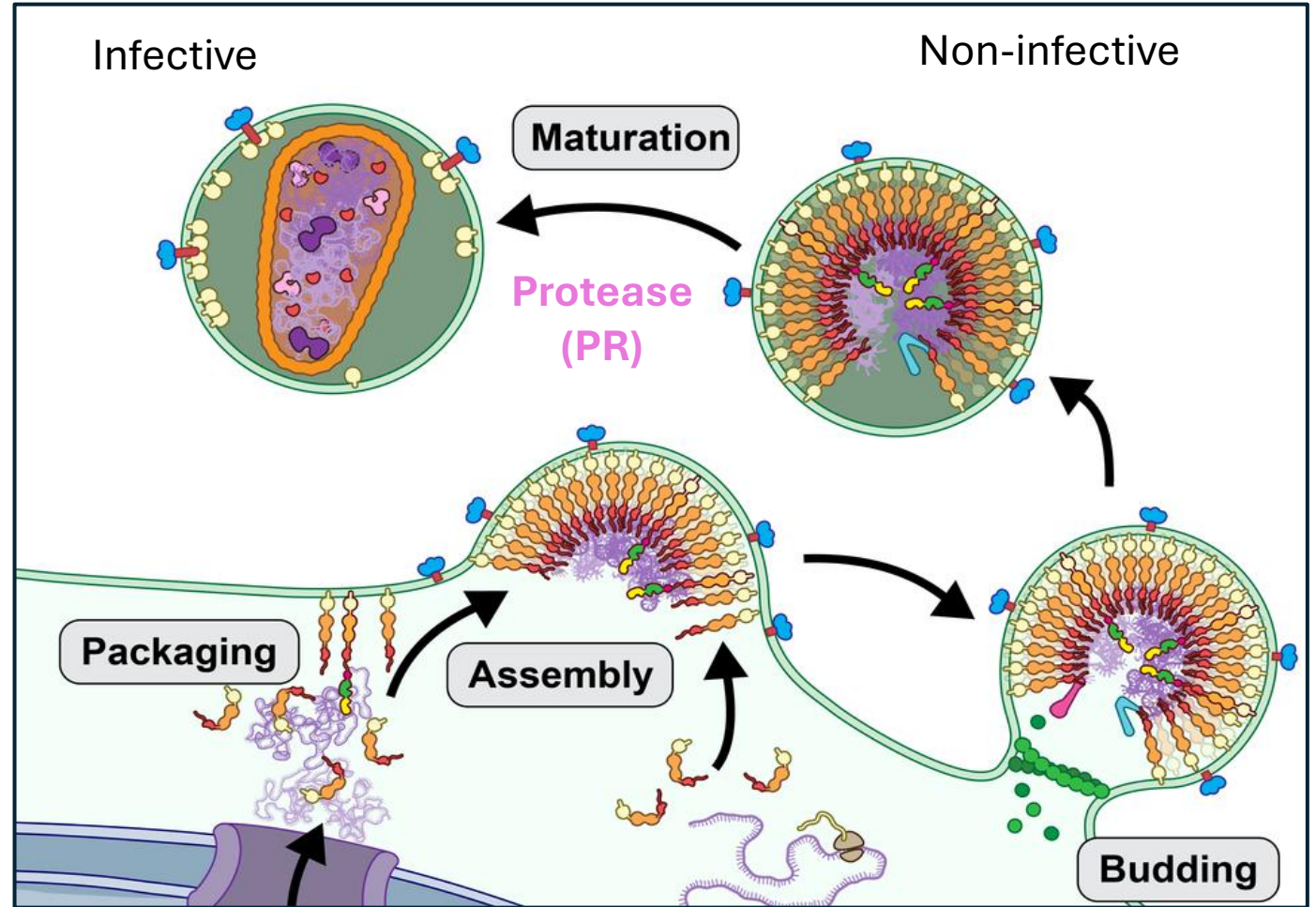


Modificado de Freed *et al*, 2019

The retroviral protease

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Protease-mediated cleavage of structural proteins drives viral maturation and infectivity

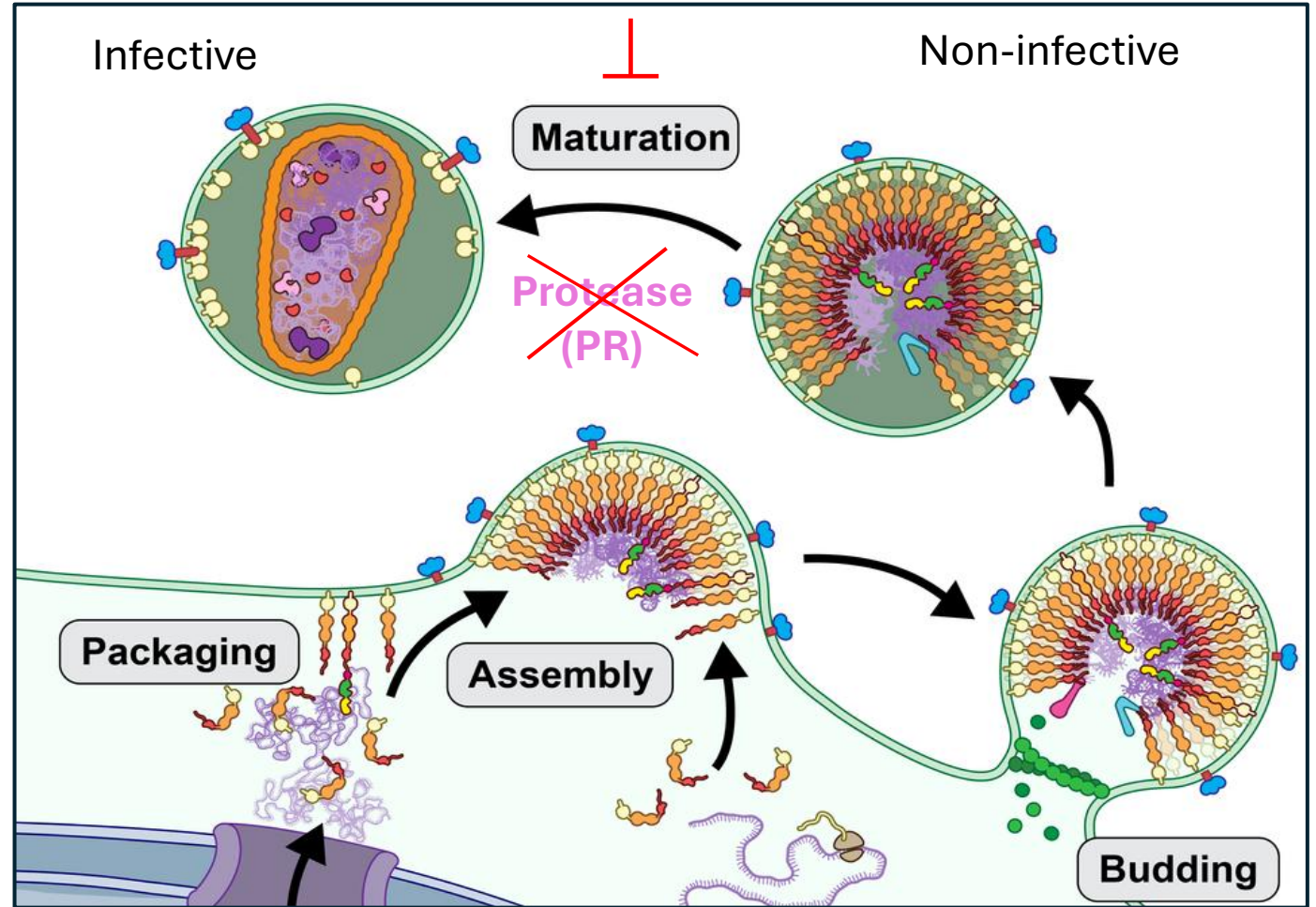
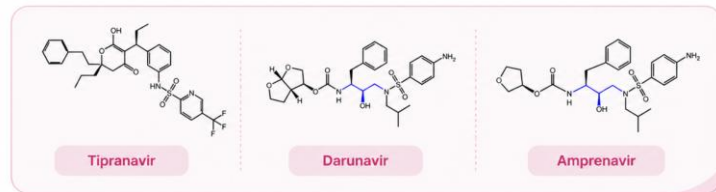


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The use of protease inhibitors has been crucial for **HIV** treatment.



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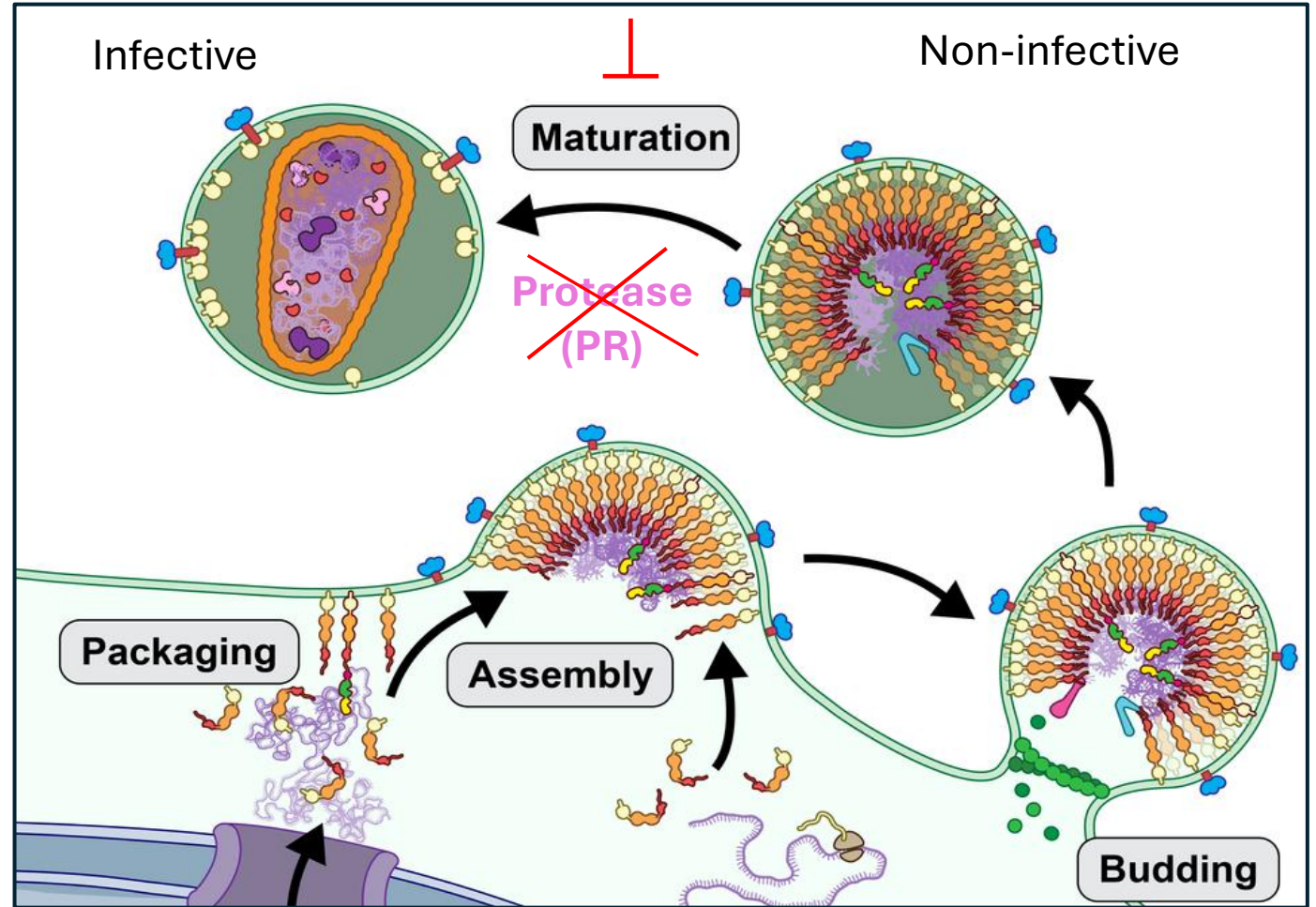
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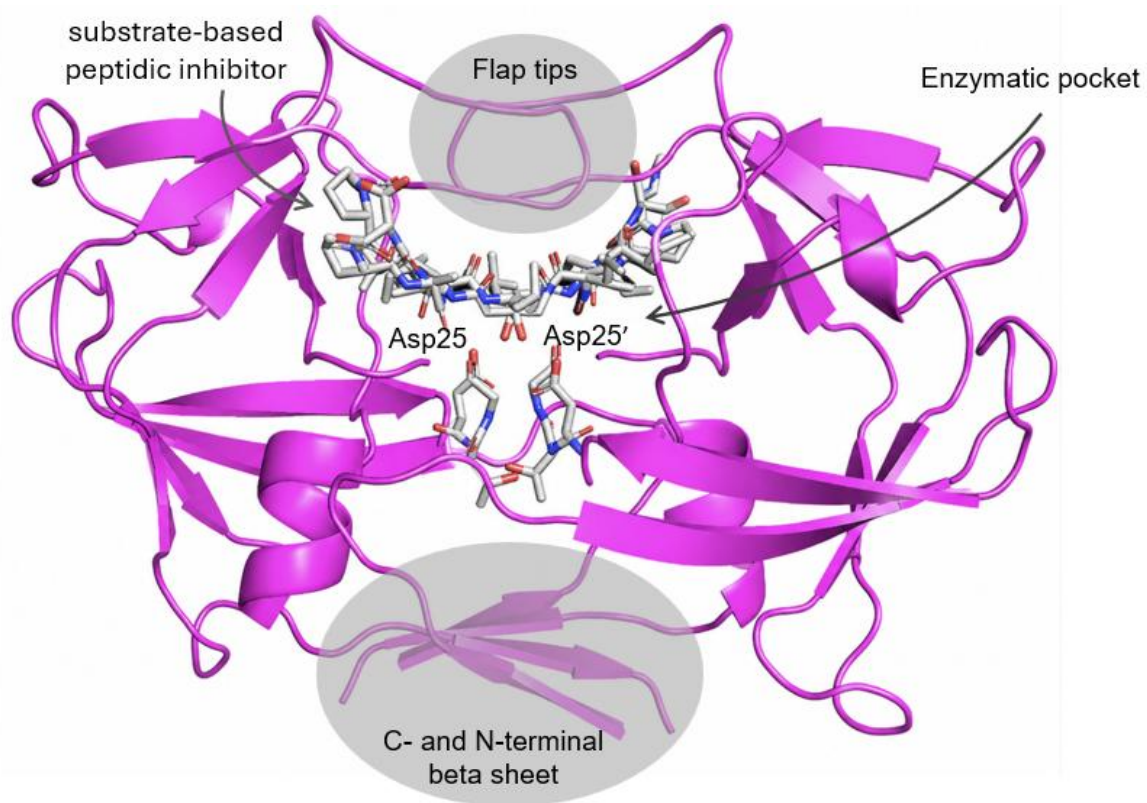
HTLV-PR inhibitors have been reported, including HIV-1 PR inhibitors-derived scaffolds. Only few mostly peptidic inhibitors described for **BLV-PR**



BLV and HTLV proteases

Retroviral protease (structure)

Homodimeric aspartic protease with ~14 kDa/monomer

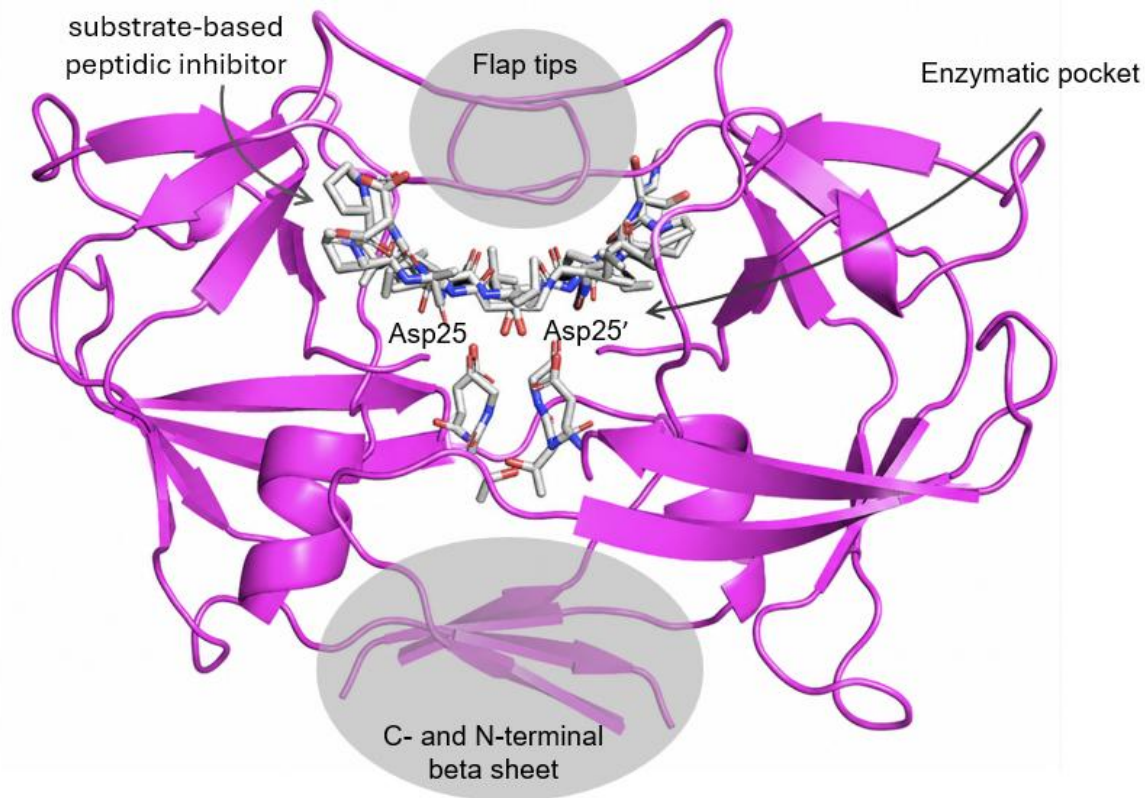


BLV crystallographic structure. Fló, et al. (in preparation)

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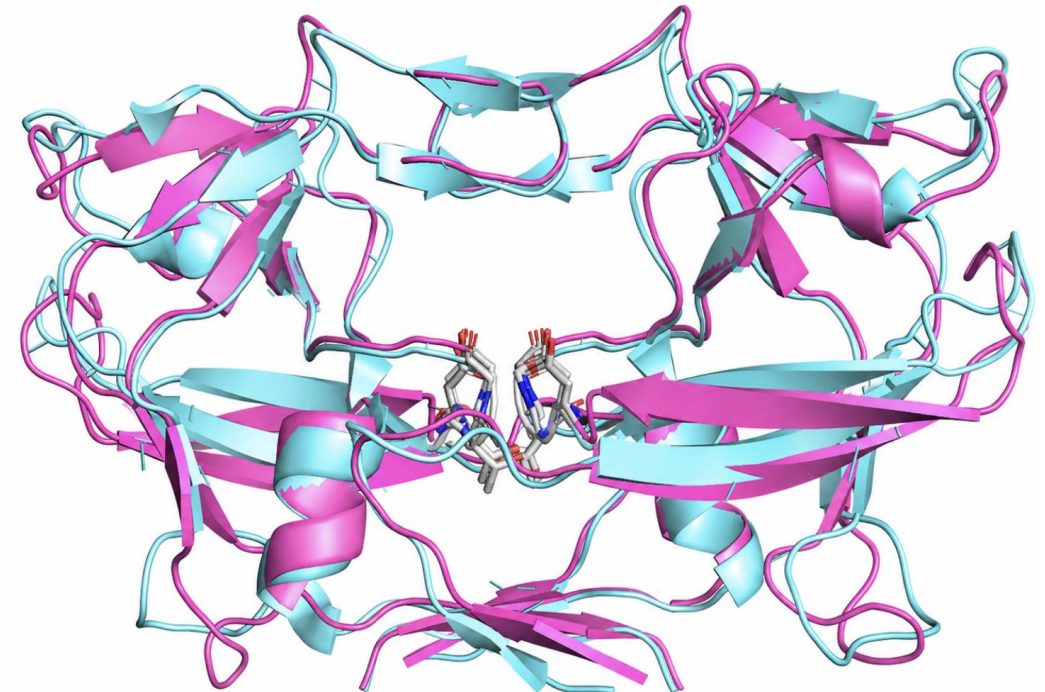
BLV crystallographic structure. Fló, et al. (in preparation)

3D structure alignment ■ BLV-PR ■ HTLV-PR

Highly conserved structural core and enzymatic pocket

Strong overlap of catalytic residues. RMSD $\approx 1.1 \text{ \AA}$

BLV and HTLV-1 PR share substrates and peptidic inhibitors



HTLV-1 protease structure (PDB: 2B7F)

Research questions

Key points

Several **HTLV-PR** inhibitors have been reported but **no antiviral** has been approved.

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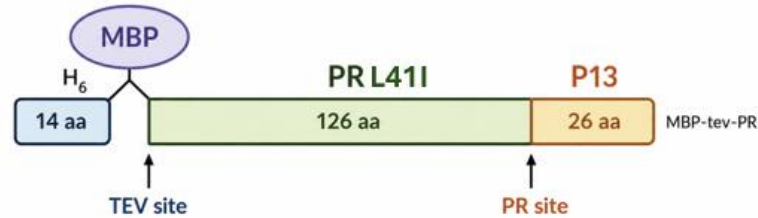
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Experimental strategy

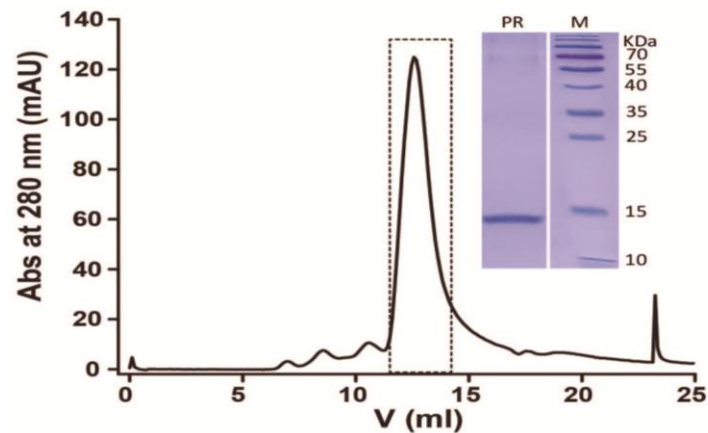
Recombinant expression and purification

BLV-PR

Expression construct for stable variant production



SEC and SDS-page of last peak

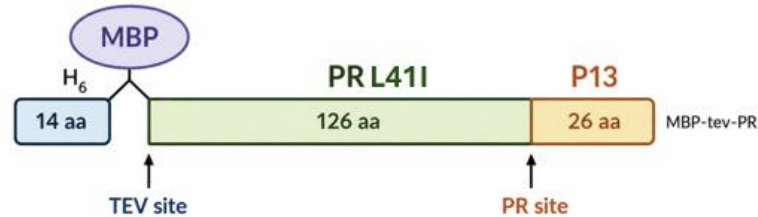


**Yield: 5 mg
per culture
liter**

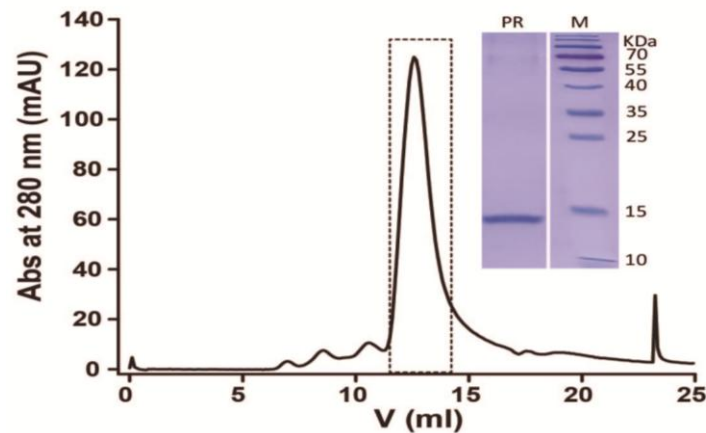
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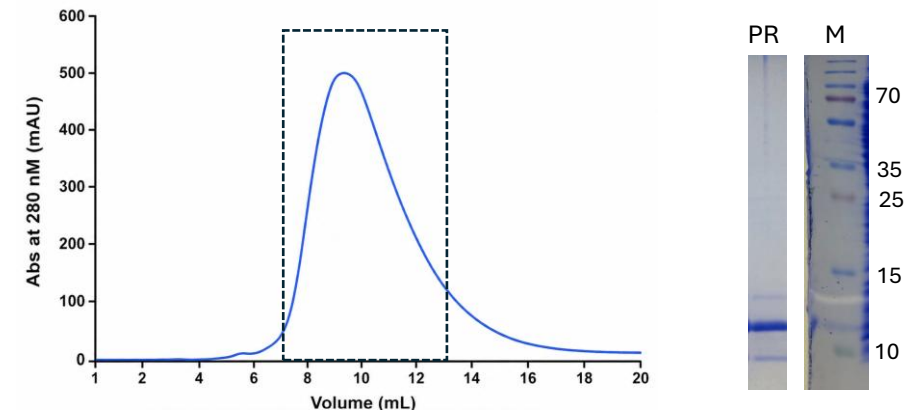
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Flo et al, 2022

HTLV-PR

Purified from inclusion bodies as in Li et al, 2005.

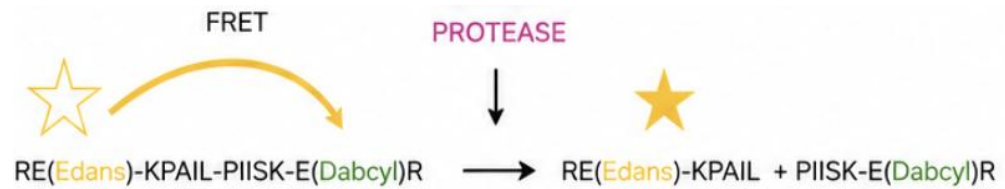
Cationic exchange and SDS-page of last peak



Yield: 2 mg per culture liter

Enzymatic assays with fluorogenic substrate

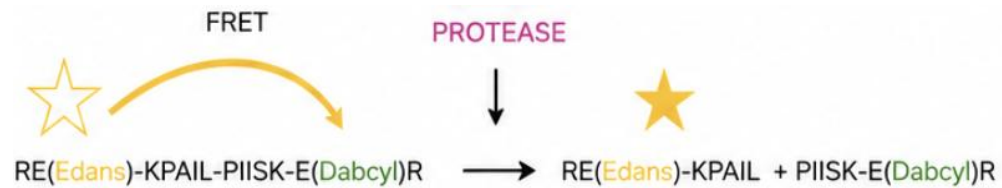
Fluorogenic substrate for BLV-PR



(Analog substrate was synthesized for HTLV-PR)

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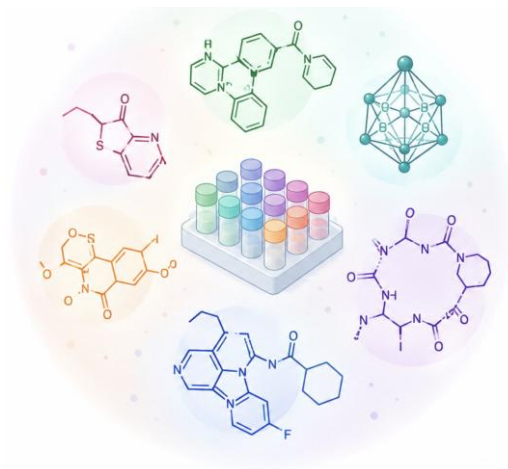
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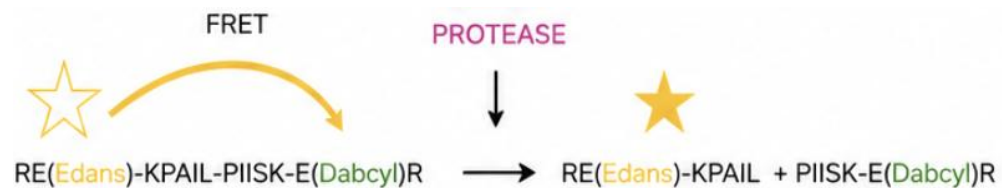
Local chemical library

Highly diverse chemical
library (>2000
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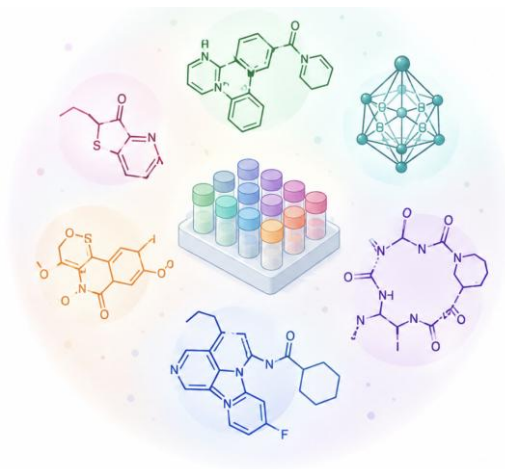
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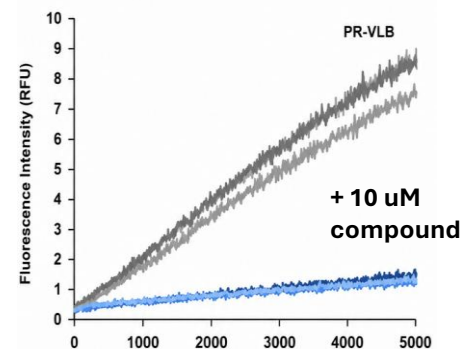
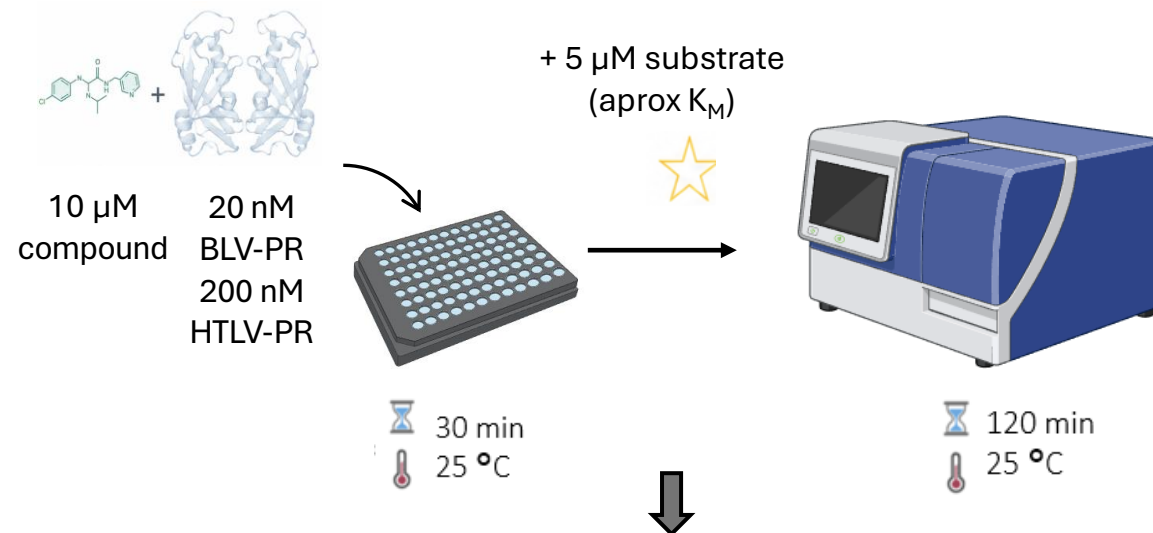
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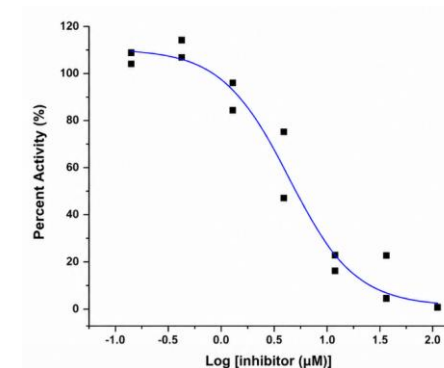
Highly diverse chemical library (>**2000 compounds**) from local research groups



Assay conditions for compound screening



HIT =
≥ 50%
activity
inhibition



Results

Screening for BLV-PR inhibitors



>2000
compounds
pre-selected for
HIV inhibitors-
related scaffolds

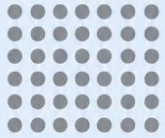


Mariana Salinas

Screening for BLV-PR inhibitors



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250
compounds
screened



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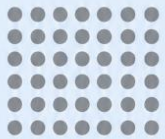
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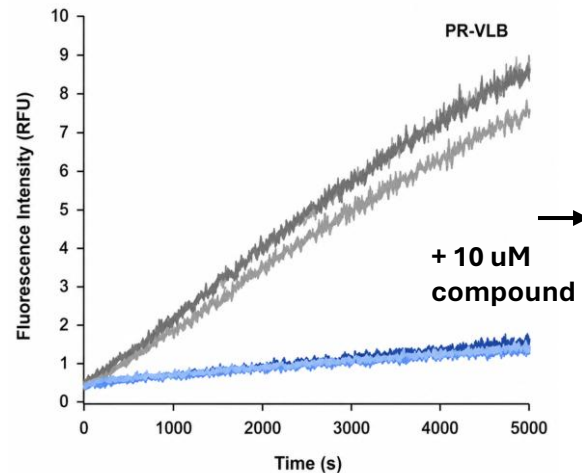


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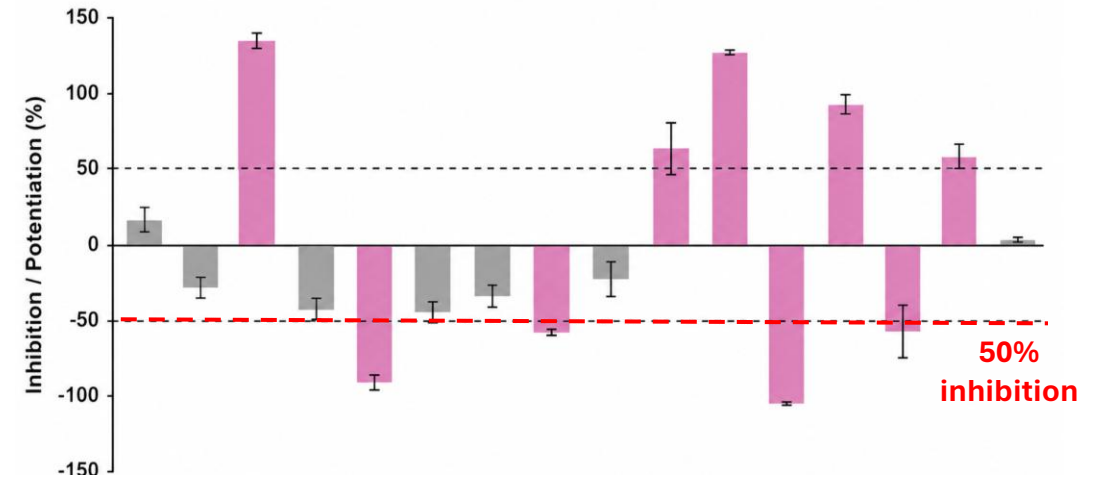


40 HITS
4 chemical
families
~ 24% HIT rate

Fluorescence time-course curves



Inhibition percentage graph - Family 2 compounds



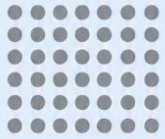
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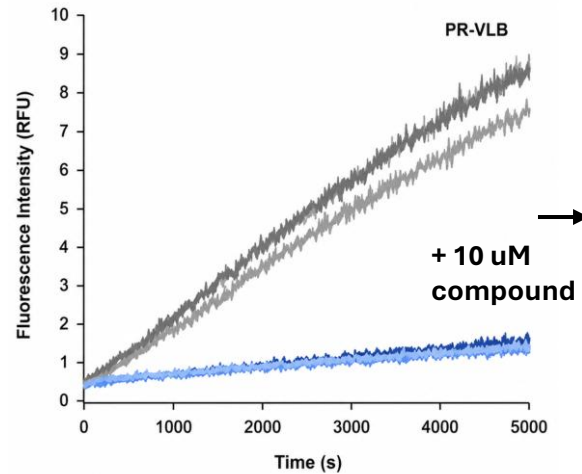


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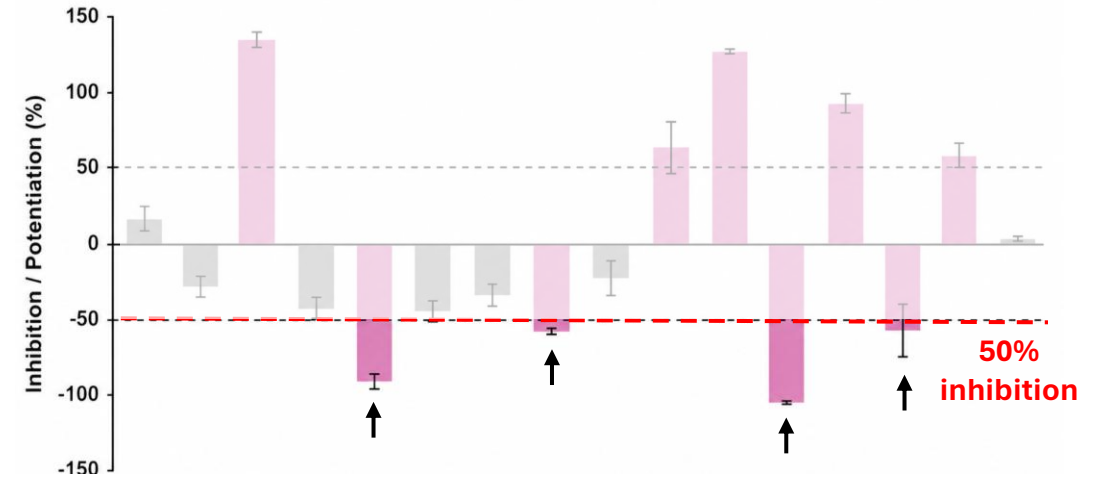


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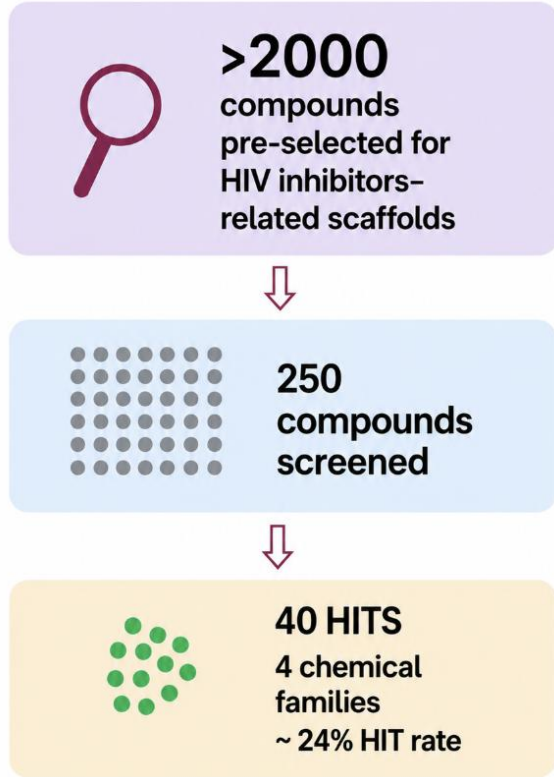
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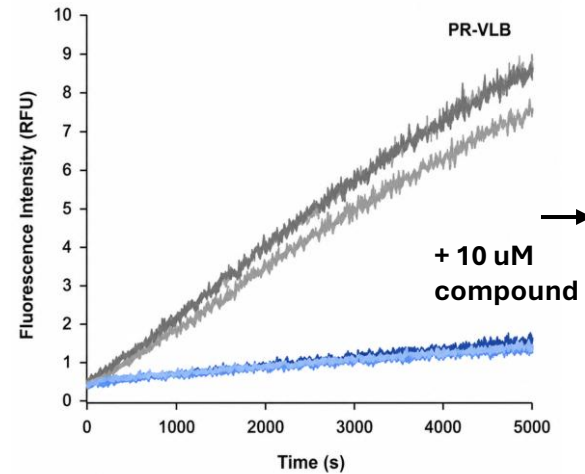
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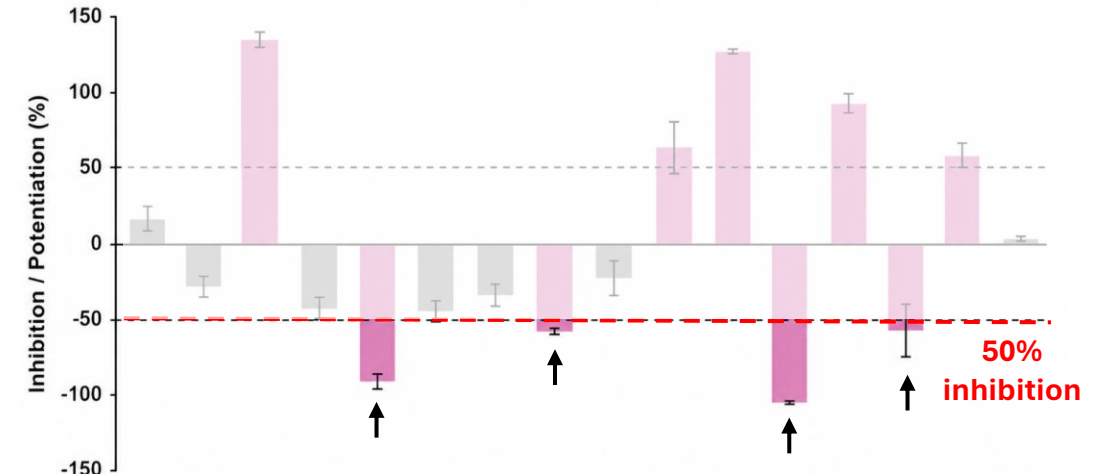
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Inhibition percentage graph -
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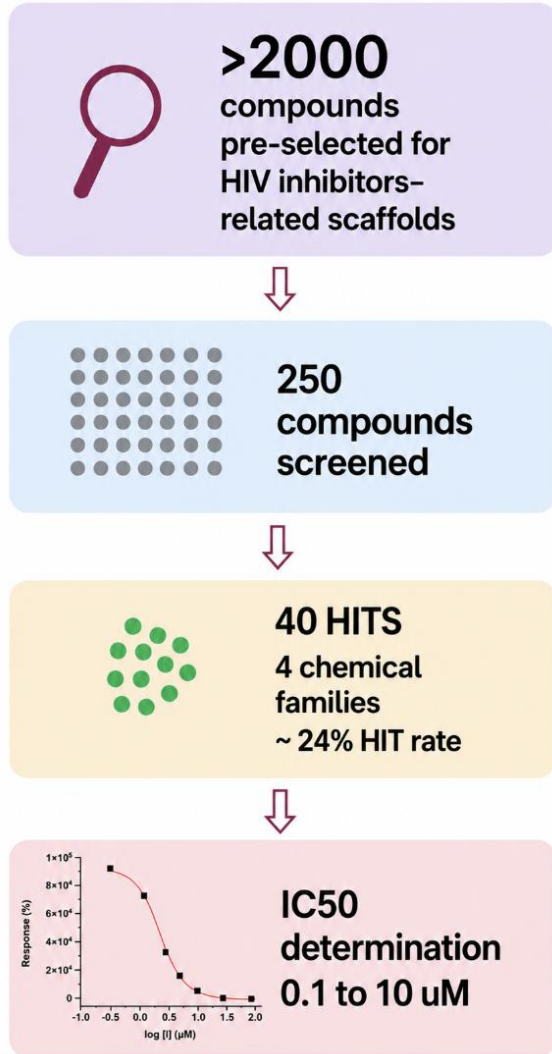


Family	Compounds tested	HITS	HIT %
Family 1	54	10	19%
Family 2	16	4	25%
Family 3	14	5	36%
Family 4	166	25	15%

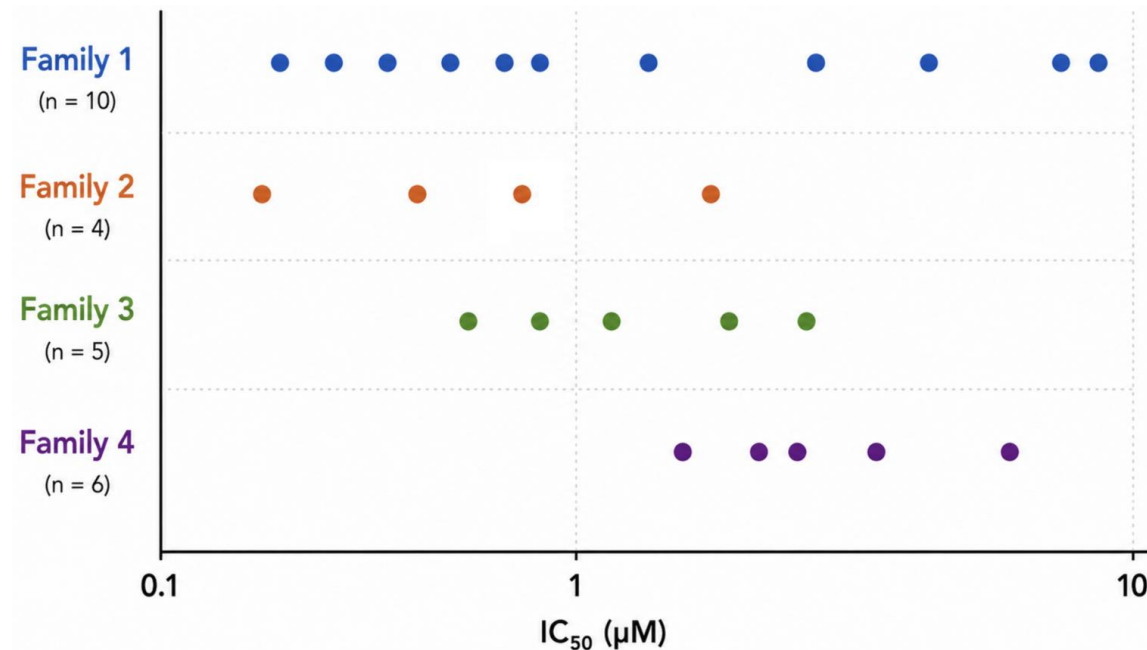
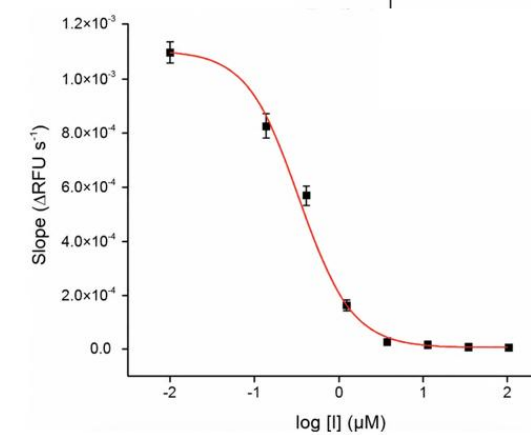
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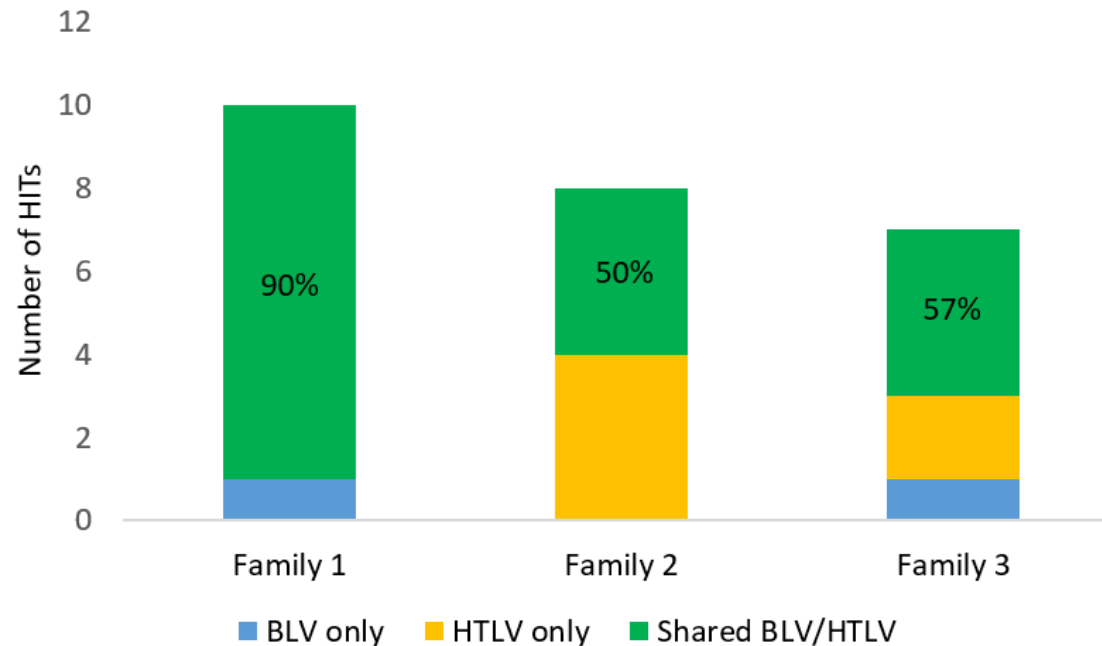


IC₅₀ determination →



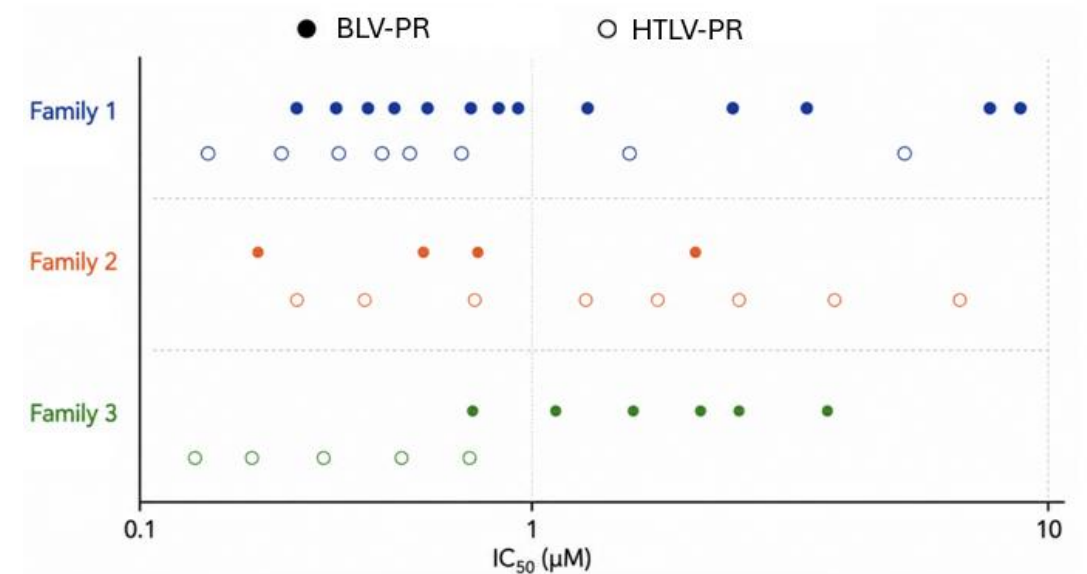
Screening for HTLV-PR inhibitors

Initial compound screening for BLV and HTLV summary



≤50% overlap in inhibitory activity between both targets

IC₅₀ values comparison



Family 3 shows lower IC₅₀ values for HTLV PR

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High structural and functional similarity between both proteases, **shared substrates** and **peptidic inhibitors**

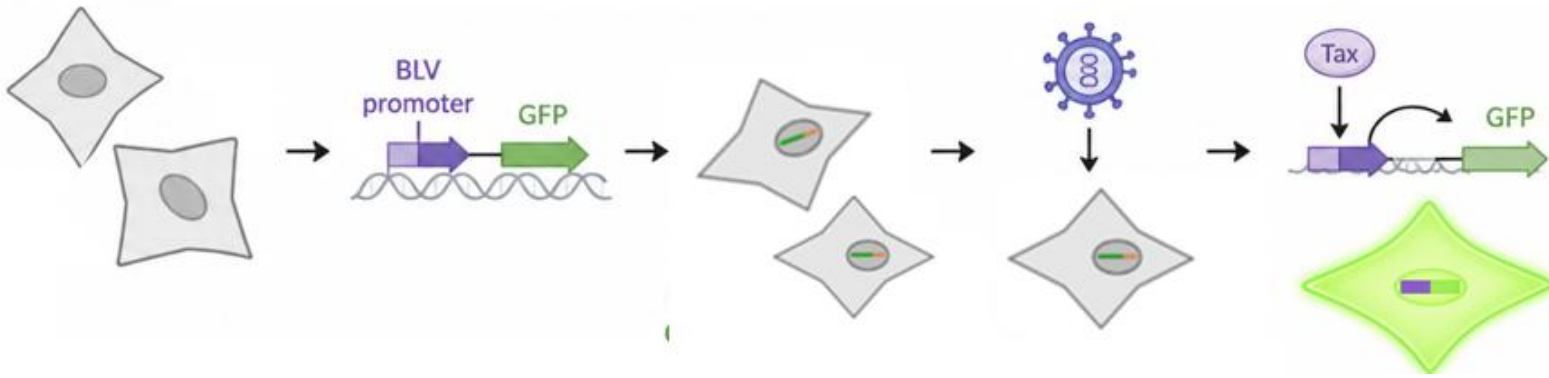
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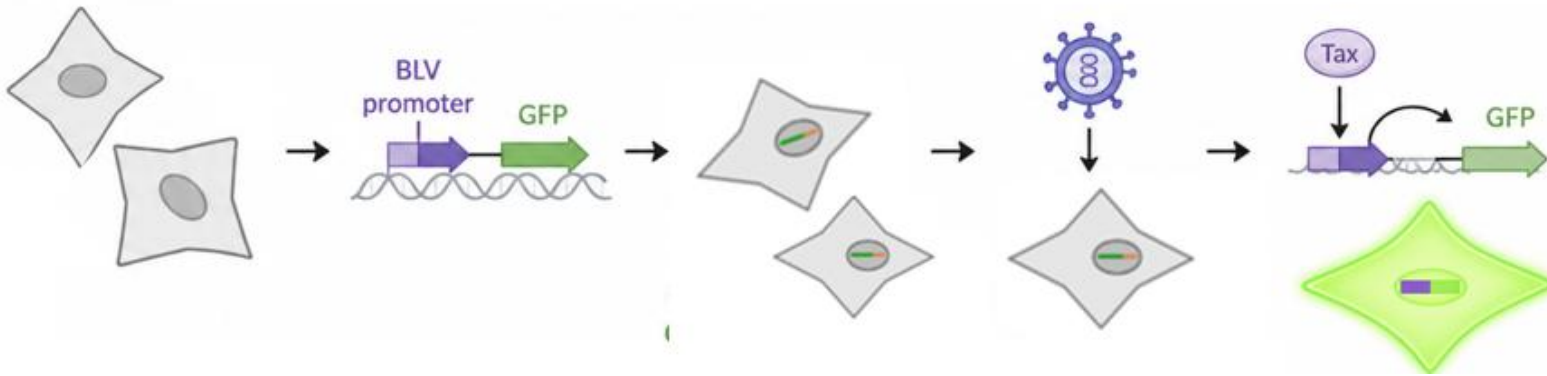
Viral infectivity assay

Development of GFP-based reporter cell line for BLV infection

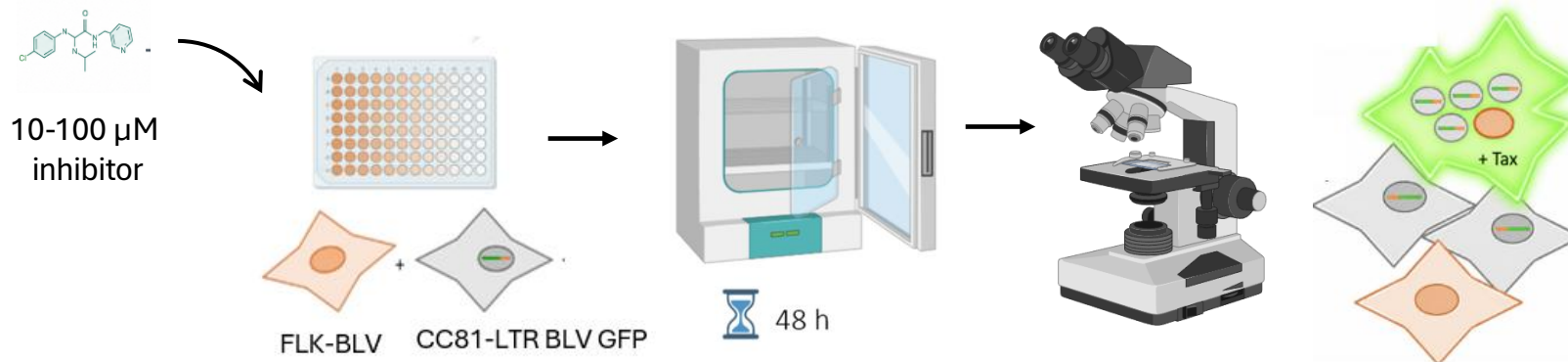


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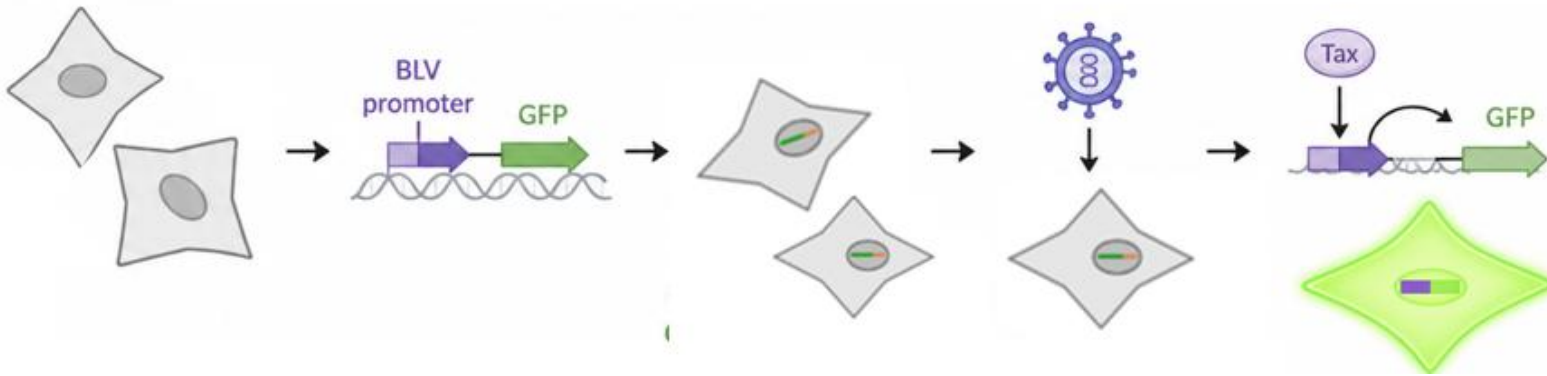


FLK/CC81 co-culture based infectivity assay

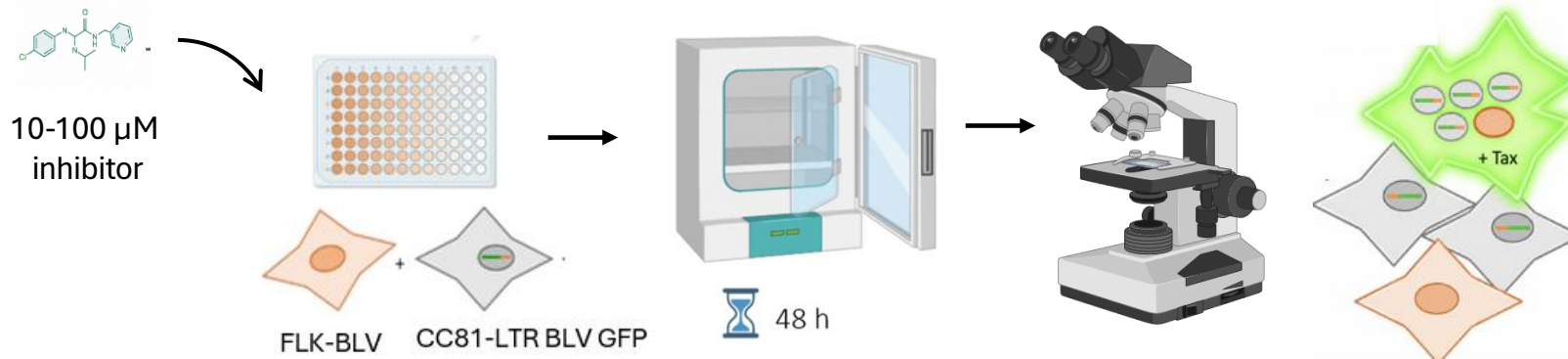


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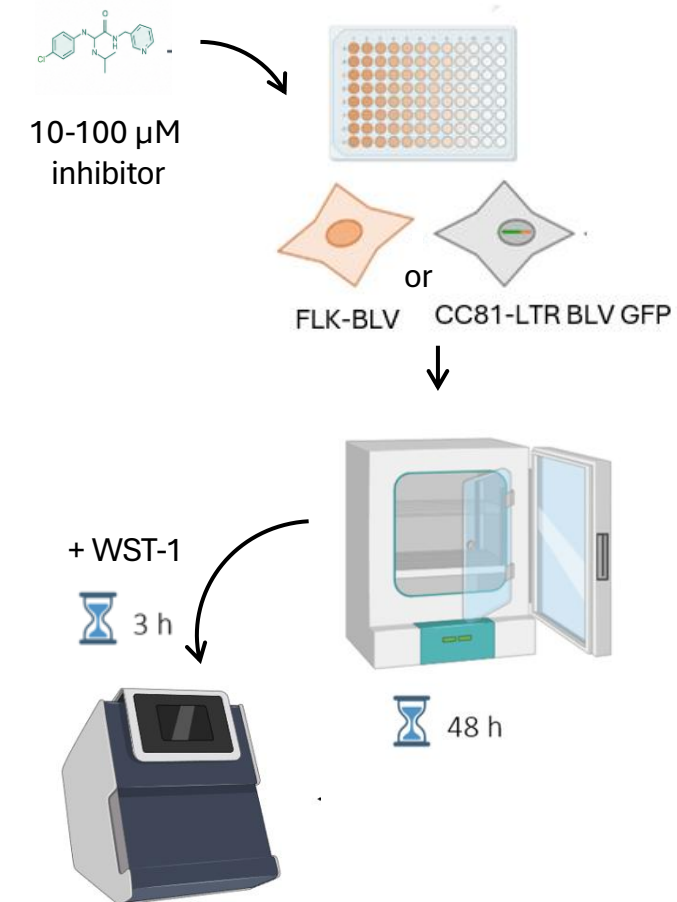
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WST-1 viability assay

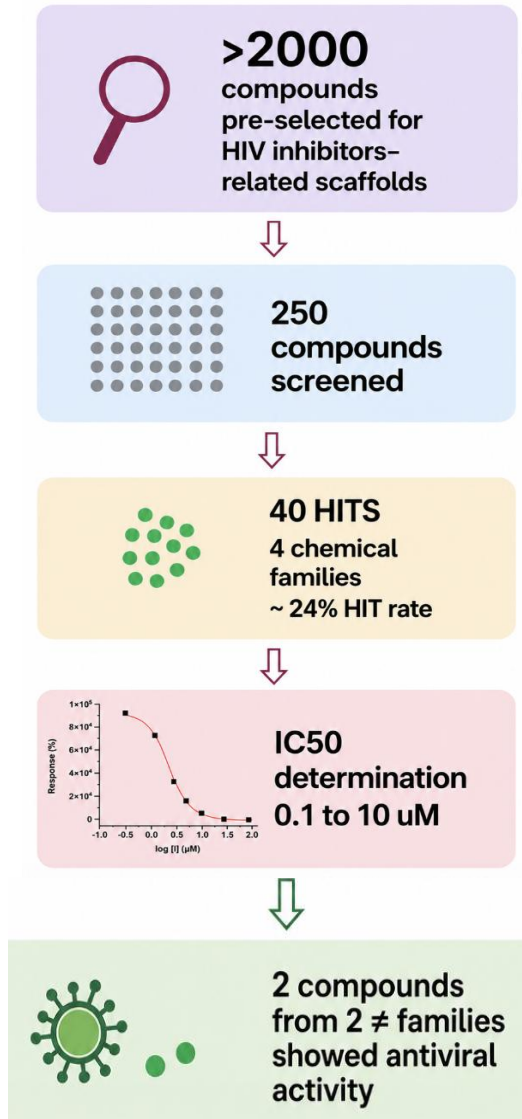


Results

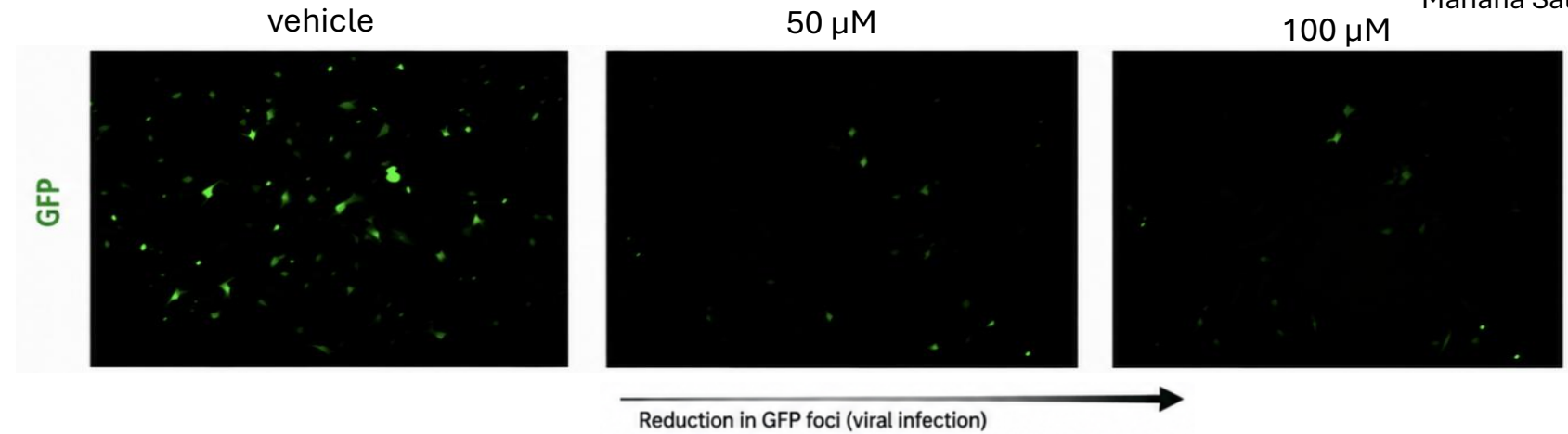
Effects on viability and viral infectivity



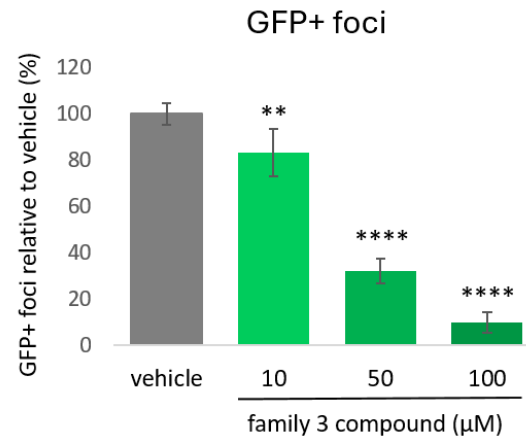
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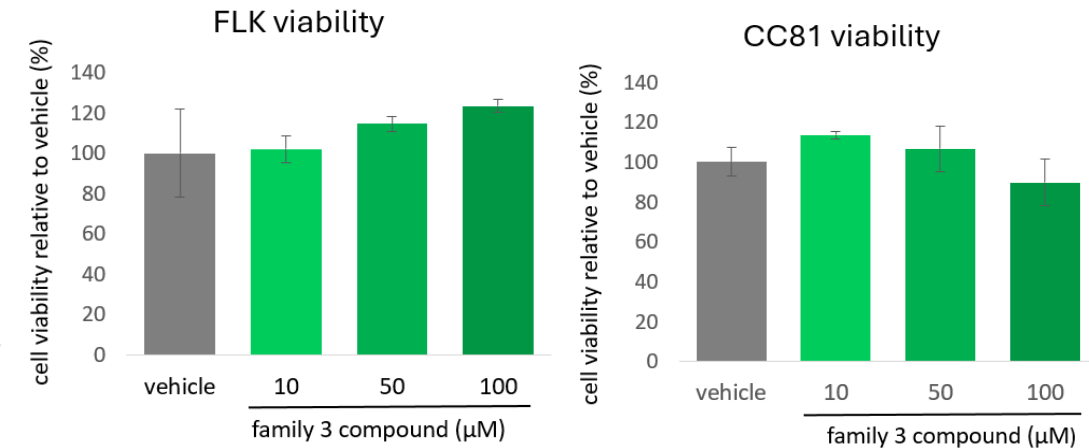
Antiviral activity - CC81/FLK co-culture



Antiviral activity



WST-1 citotoxicity assay



Conclusions and Perspectives

Final remarks

1. **Can we identify novel inhibitors of BLV and HTLV-1 proteases?**

We identified 40 new non-peptidic inhibitors of BLV-PR expanding the currently limited inhibitor set

We identified 24 new inhibitors of HTLV-PR

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We established a robust platform for the identification of BLV-PR inhibitors.

All families active against BLV-PR also inhibited HTLV-PR, with at least a 50% overlap in HITs.

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3. Among the identified inhibitors, can we find non-cytotoxic compounds with antiviral activity?

We found 2 compounds that showed antiviral activity in an BLV infection model

Next steps

- Expanding the screening to larger libraries
- Further physicochemical and kinetic characterization of the identified HITs.
- Structural characterization of PR-BLV:inhibitor complexes to guide rational inhibitor optimization.
- Evaluation of selected non-toxic compounds for antiviral activity against HTLV-1 within an ongoing collaborative international project.

Acknowledgments

- Supervisors: Dr. Martin Fló and Dr. Natalia Olivero
- Members of the Immunovirology Laboratory – Institut Pasteur de Montevideo

- Funding agencies





22nd International Conference on Human Retrovirology:
HTLV and Related Retroviruses
HTLV2026

June 3-6, 2026 | Philadelphia, Pennsylvania, USA

HTLV-1C p16 protein contributes to virus production and dissemination

Cynthia Pise-Masison
National Cancer Institute, Bethesda, USA

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Cynthia Pise-Masison, PhD, National Cancer Institute

Conflicts of interest

I have no conflicts of interest

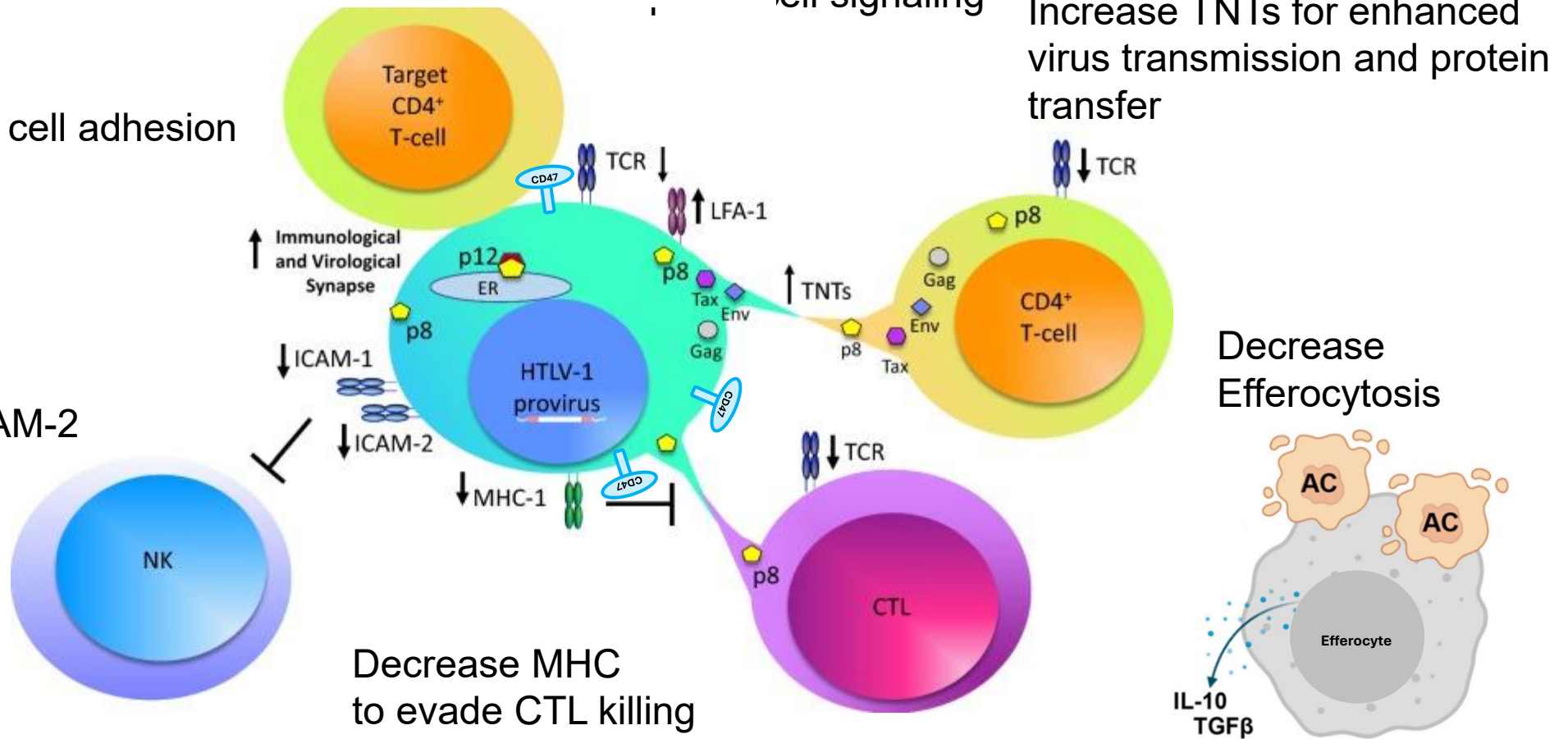
HTLV-1A Orf-1 protein (p12/p8) modulates the host immune response

Critical for infection of Rhesus macaques

Ratio of p12 to p8 expression correlates with proviral load in humans

Enhance cell adhesion

Decrease ICAM-1 and ICAM-2
to evade NK cell killing



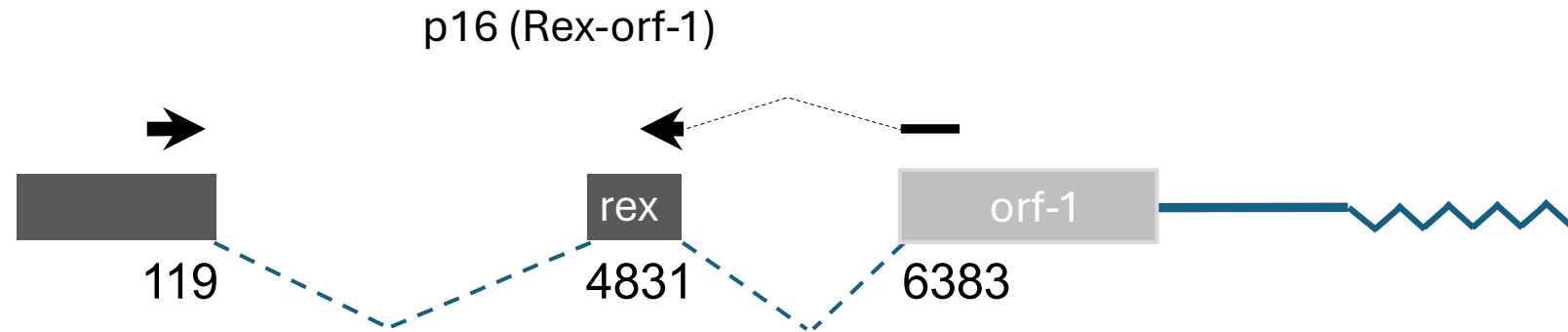
Decrease
Efferocytosis

Expression of Orf-1 in HTLV-1ACo-L virus infected cells

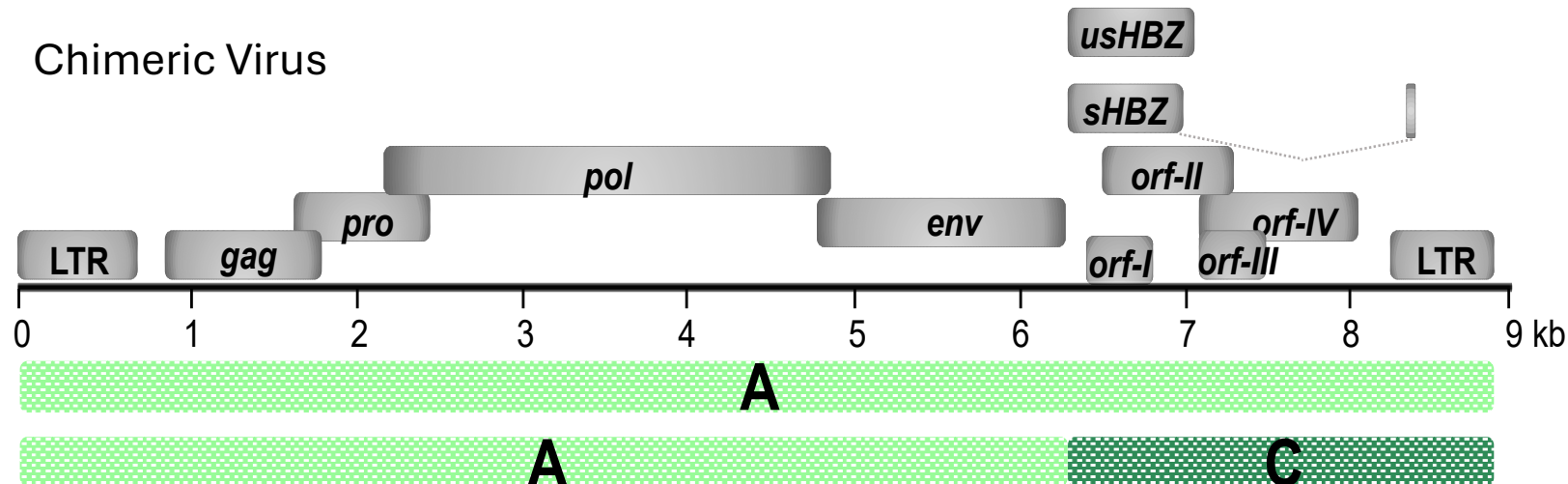
<i>orf-I</i>	
HTLV-1A	HTLV-1C
AUG	ACG

Met
p12/p8

Thr
p12~~x~~p8



Chimeric Virus

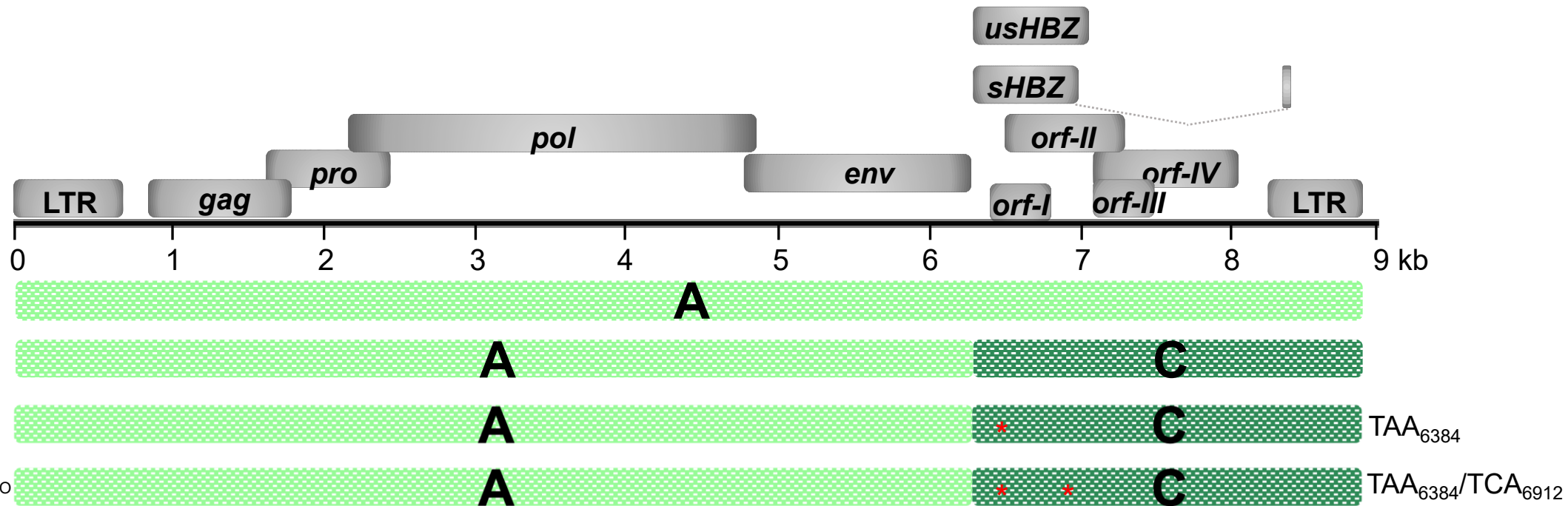


Summary

- HTLV-1C encodes an alternatively spliced mRNA producing p16-contains the RNA binding and NLS domains of Rex in frame with the p12 protein.
- p16 modulates the host immune response --decreased surface expression of ICAM-1 and decreased efferocytosis (a crucial process for maintaining tissue health, preventing autoimmunity and resolving inflammation).
- Equivalent infection of HTLV-1A and chimeric HTLV-1ACo-L virus in Rhesus macaques.
- High levels of p16 and HBZ mRNA detected in the lung of HTLV-1ACo-L infected macaques.

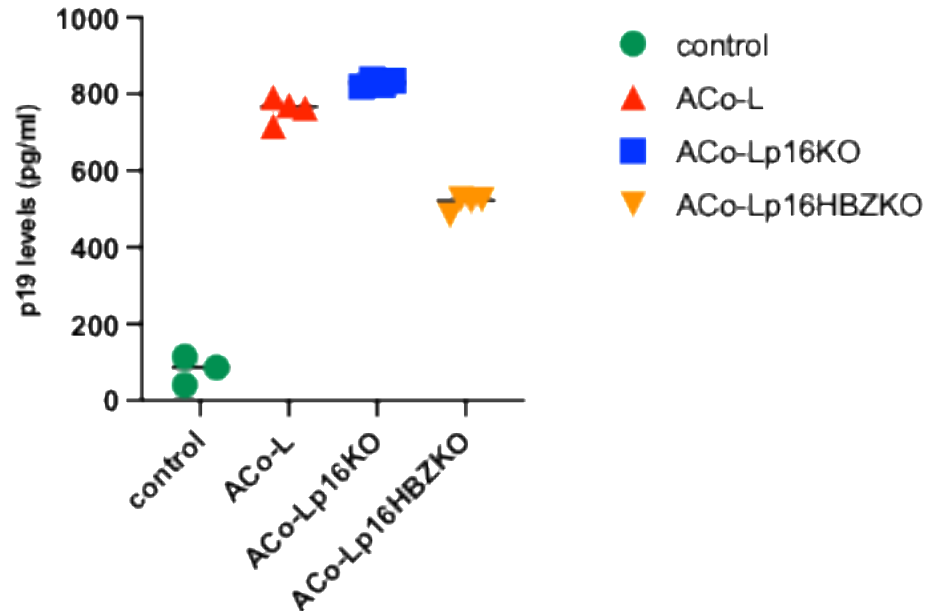
Is p16 critical for persistent infection in Rhesus macaques?

Construction of p16 knockout molecular clones

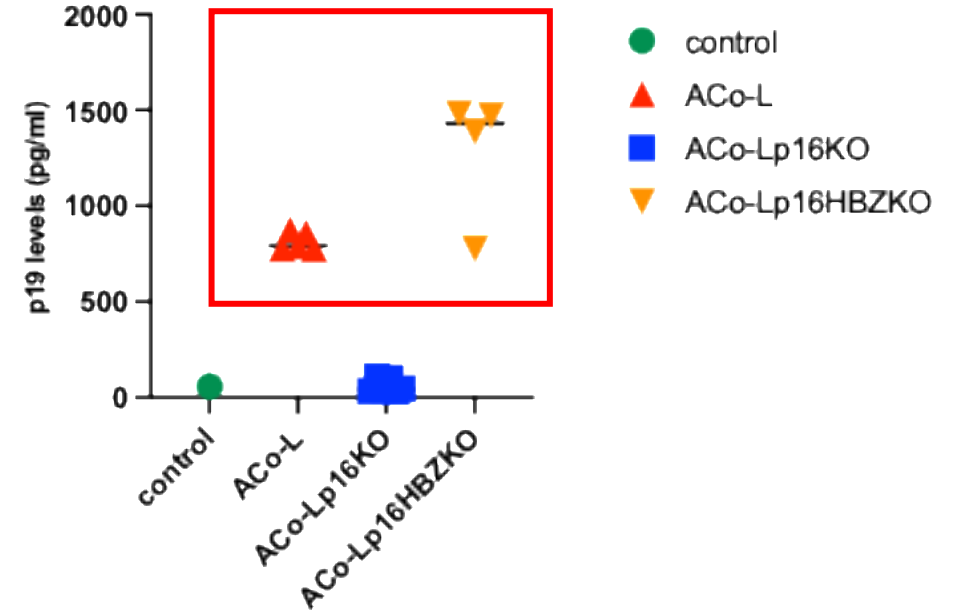


Viral expression in Transient and Stable Transfection

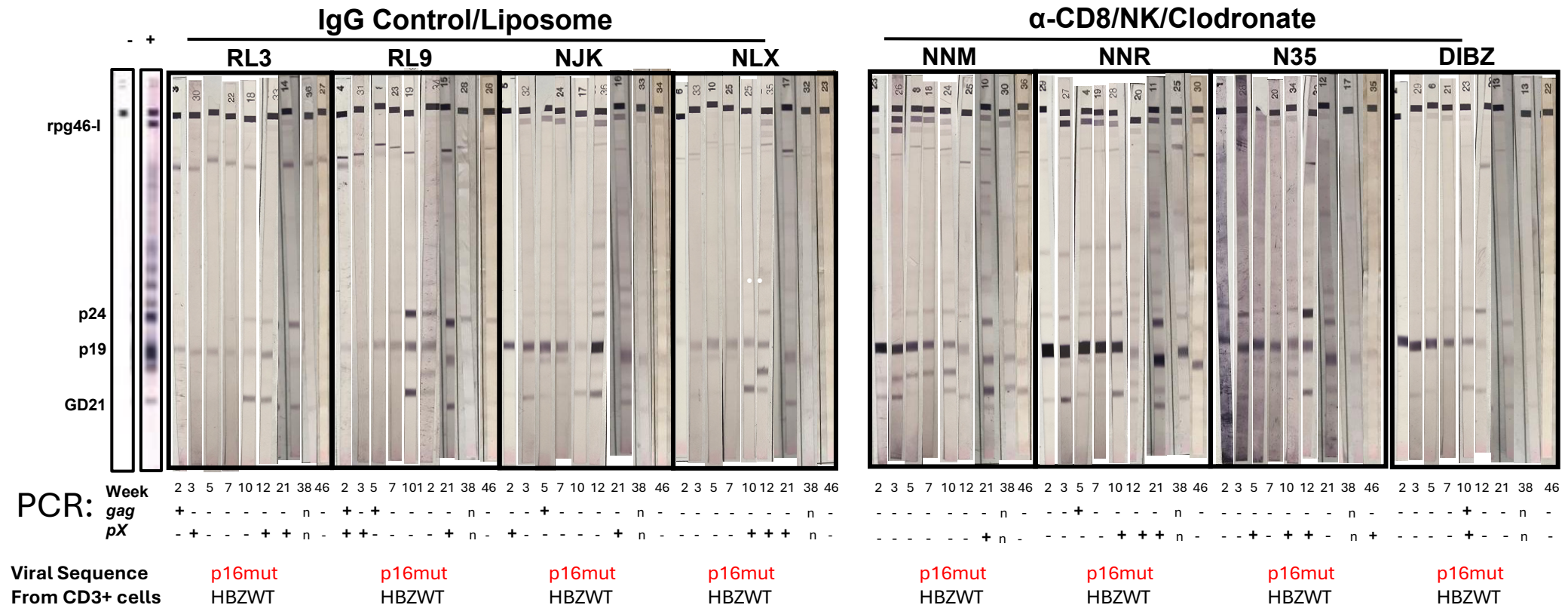
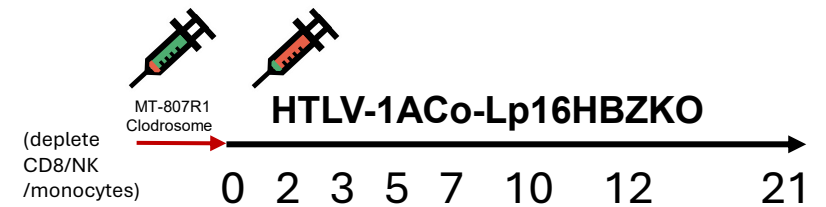
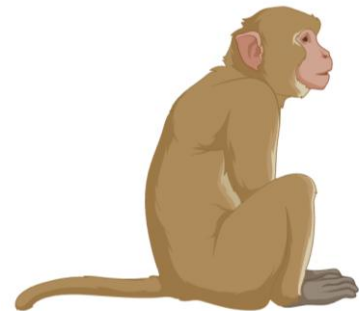
293T Transient Transfection



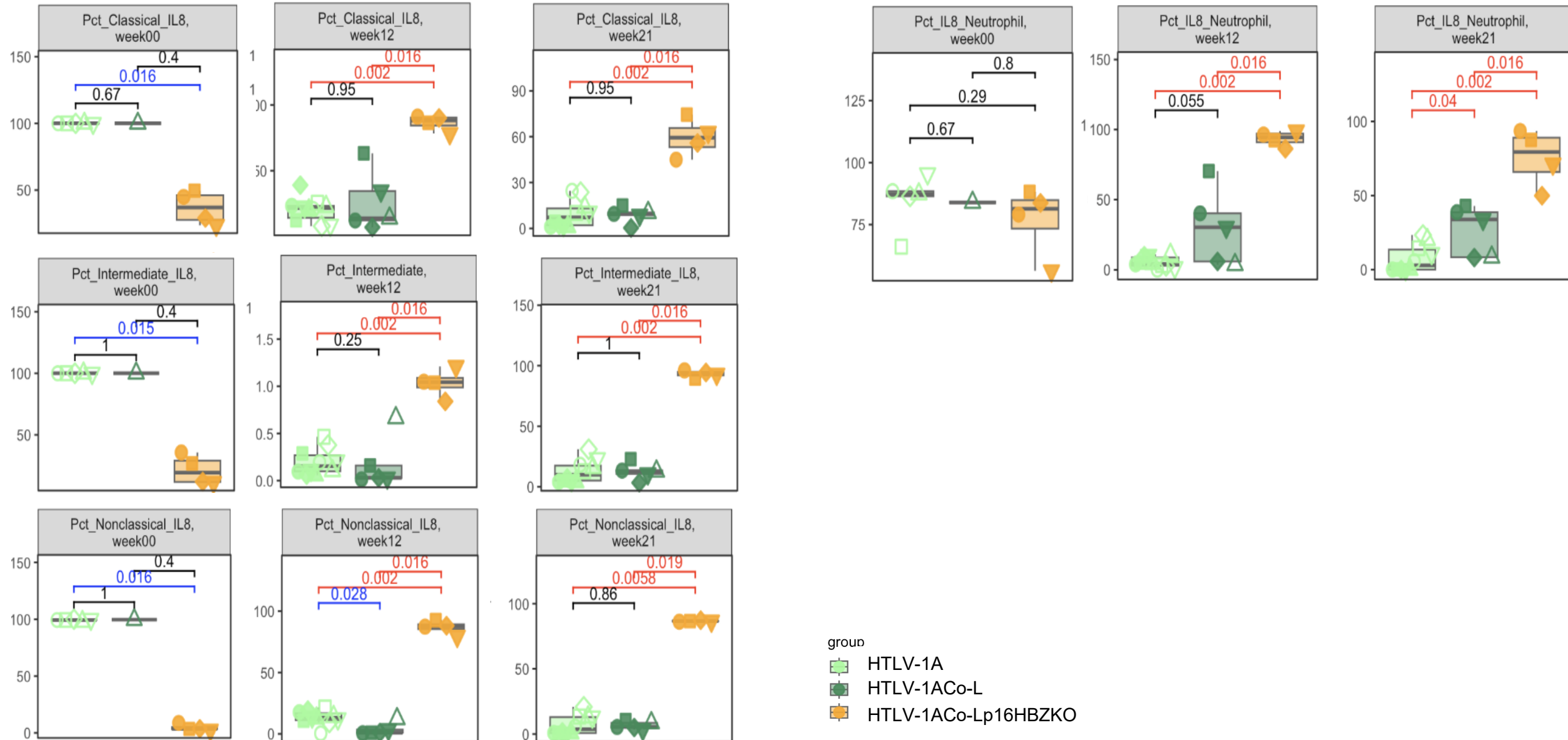
729 producing cells



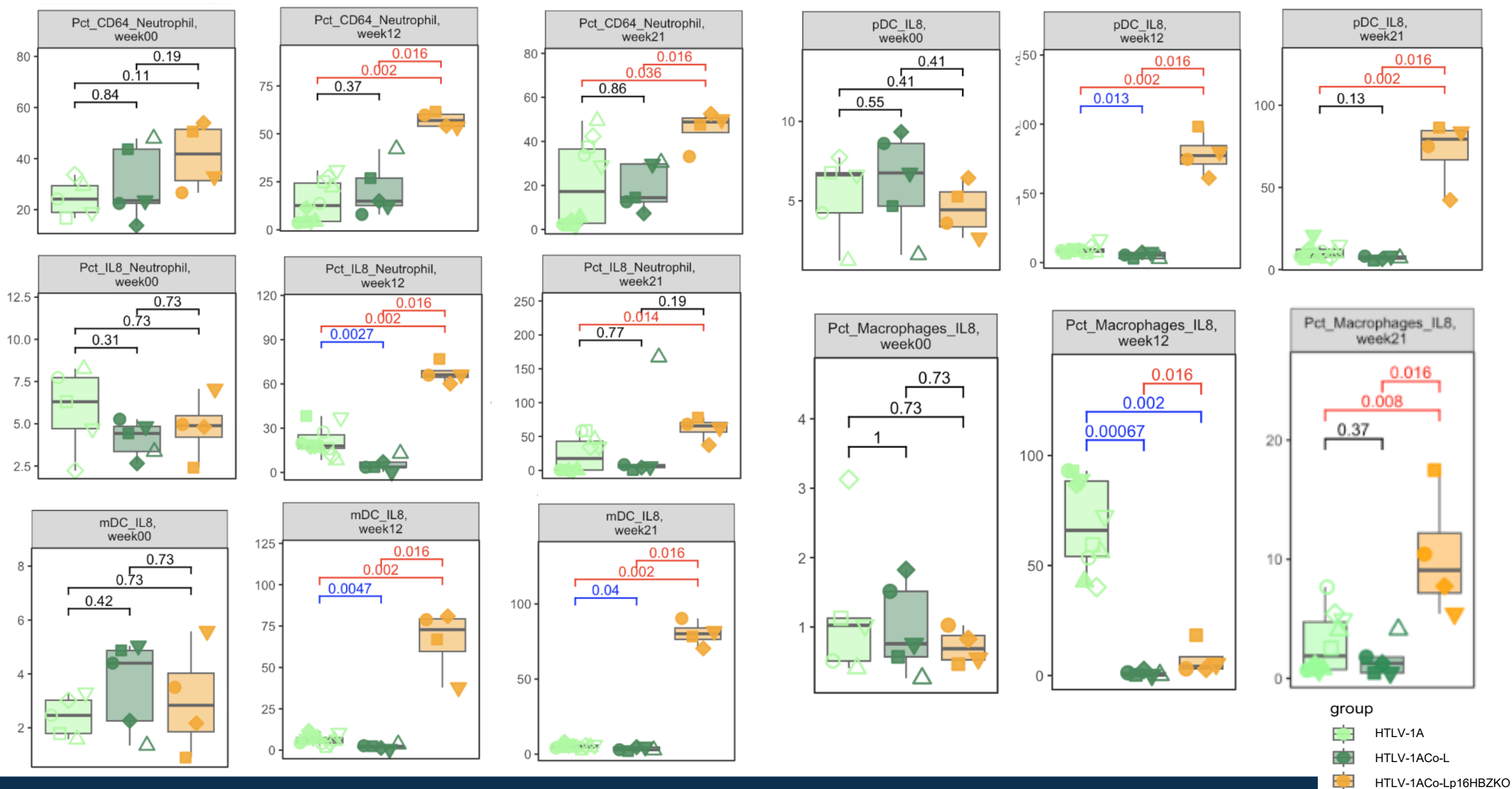
- No detectable viral RNAs for HBZ or p30 in 729 p16KO
- Adding p16 by lentivirus transduction to 729 p16KO cells does not relieve repression
- PVL in 729 p16KO cells decreases with time
- See accumulation of mutations in the pX region

A cartoon illustration of a brown monkey sitting on the ground, facing right. The monkey has a long tail curled on the ground and is looking towards the right side of the frame.

Increased IL-8 Positive Cells in Blood



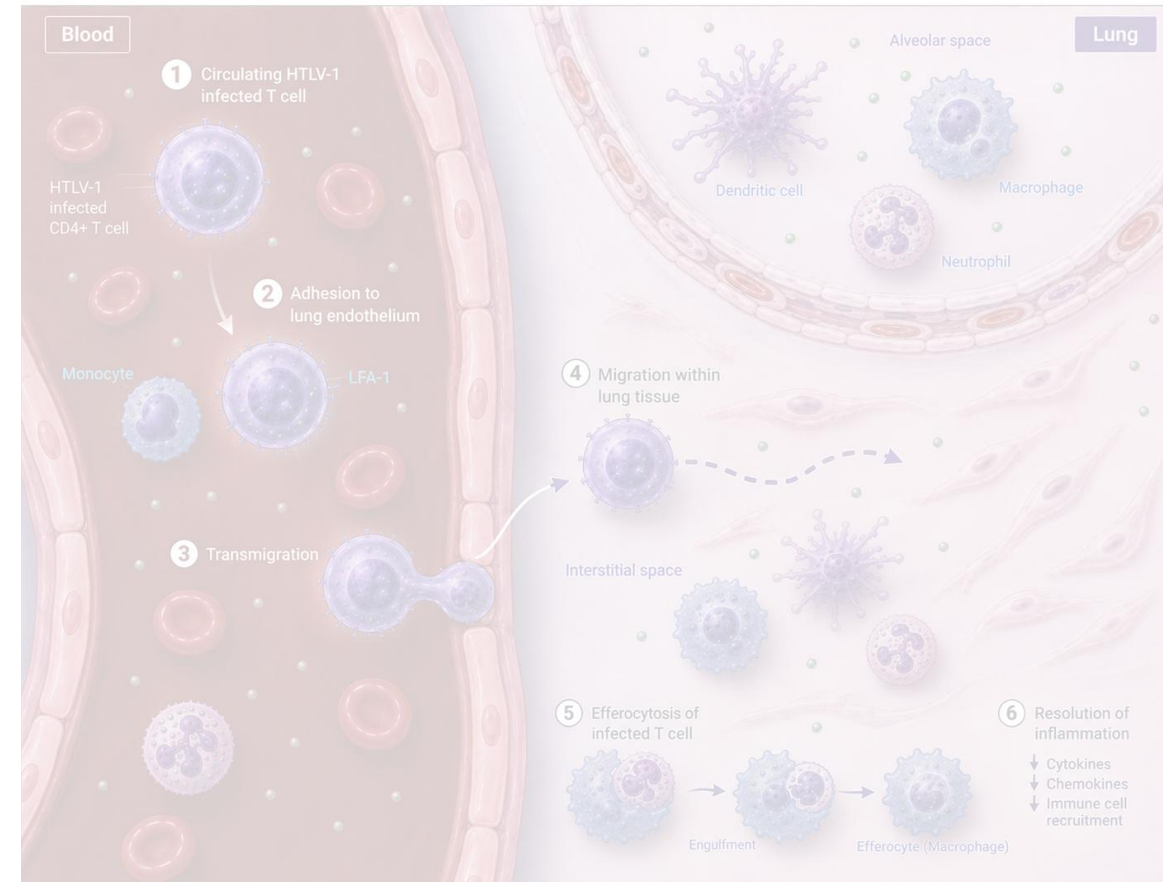
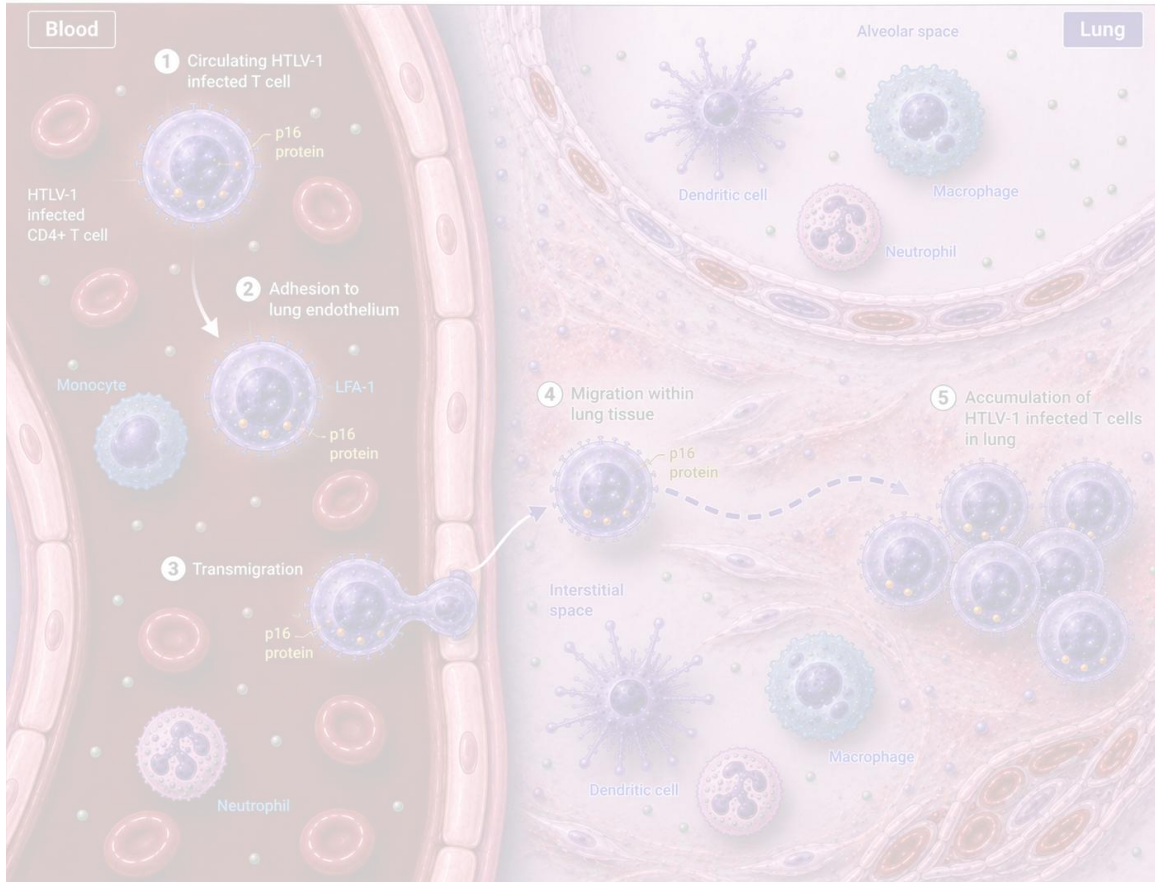
Increased IL-8 Positive Cells in the Lung



Summary

- p16KO molecular clone is productive in 293T cells, but not 729 cells.
- In contrast, p16HBZKO molecular clone is productive in both 293T and 729 cells, suggesting an interplay between p16 and HBZ.
- p16HBZKO virus can infect macaques but HBZ reverts to wild type while p16 knockout mutation is retained.
- A more robust seroconversion in animals transiently depleted for NK/CTL/monocytes prior to infection occurred. However, seroconversion in all infected animal decreased after week 21 and viral DNA detection in PBMCs low and sporadic.
- Higher percentages of IL-8 producing inflammatory cells in the blood and BAL of HTLV-1ACp16HBZKO infected animals compared to wild type HTLV-1AC or HTLV-1A animals at weeks 12 and 21 post infection.

Role of p16 in persistent infection



Acknowledgments

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Past members

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Arif Rahman

Luca Schifanella

Melvin Doster

Rubel Hoq

Collaborators

University of Melbourne

Damian Purcell

Samantha Grimley

NIAID

Tongqing Zhou

NCI

Kate Goldfarburmen-CCBR and FNL

FlowCytometry Core

Katherine McKinnon

Sophia Brown

NCI LASP

Michelle Metrisko

Kristin E. Killoran

Joshua Kramer

LASP facility staff

Whole genome HTLV-1 amplification using a simplified long-fragment polymerase chain reaction workflow optimized for next-generation sequencing

Felice Deminco

Federal University of Bahia (UFBA), Salvador, Brazil

Conflicts of interest

I have no conflicts of interest

Problem: Limited Whole Genome Data Restricts HTLV-1 Molecular Surveillance

HTLV-1 was the first human retrovirus discovered in 1980

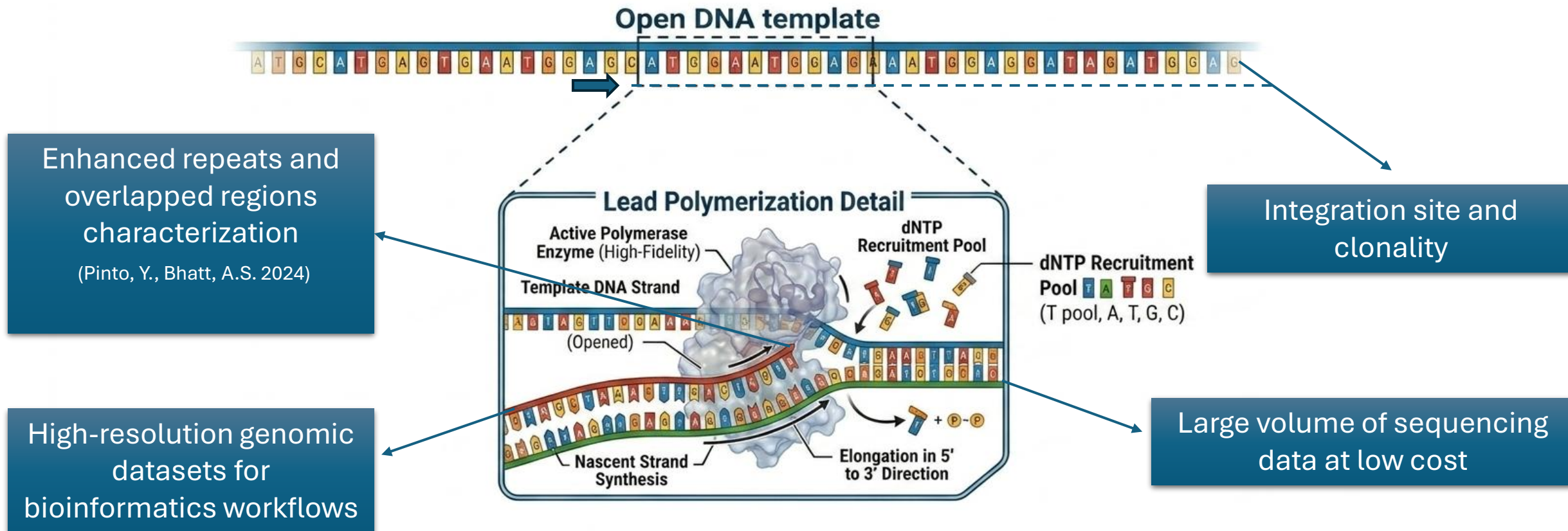
HTLV-1 causes ATLL and HAM/TSP, with 10-20 million people infected worldwide

Despite being identified earlier than HIV-1, there are limited HTLV-1 genomes available, impacting the development of therapeutics and antiviral

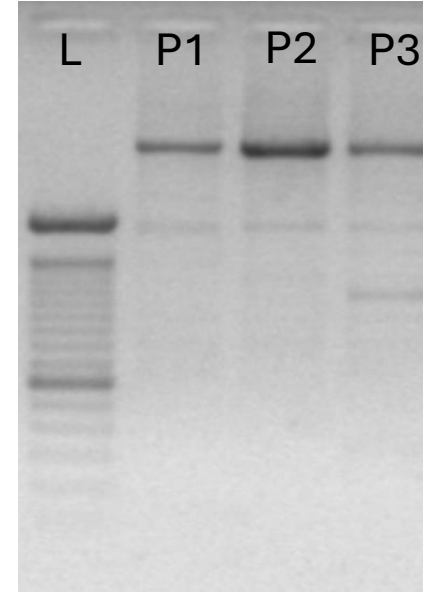
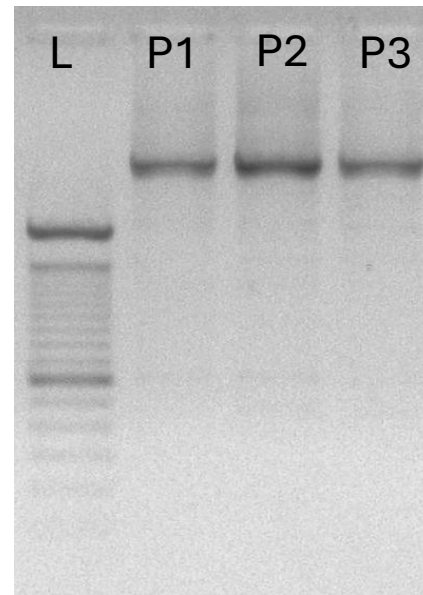
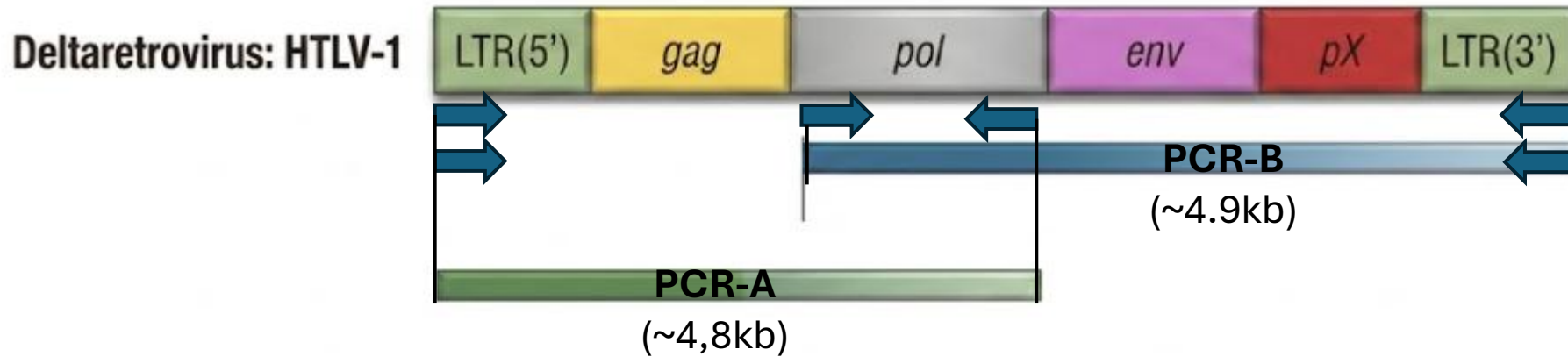
HIV-1 vs HTLV-1 Genome Data on NCBI (2026)

HIV-1	HTLV-1
1,456,3305	11,006
↓	↓
11,364 Full Genome	525 Full Genome

Solution: Long-Fragment PCR Offers Advantages for HTLV-1 Whole Genome Sequencing (WGS)



We Developed a Simplified One-Step LF-PCR Workflow for HTLV-1 WGS



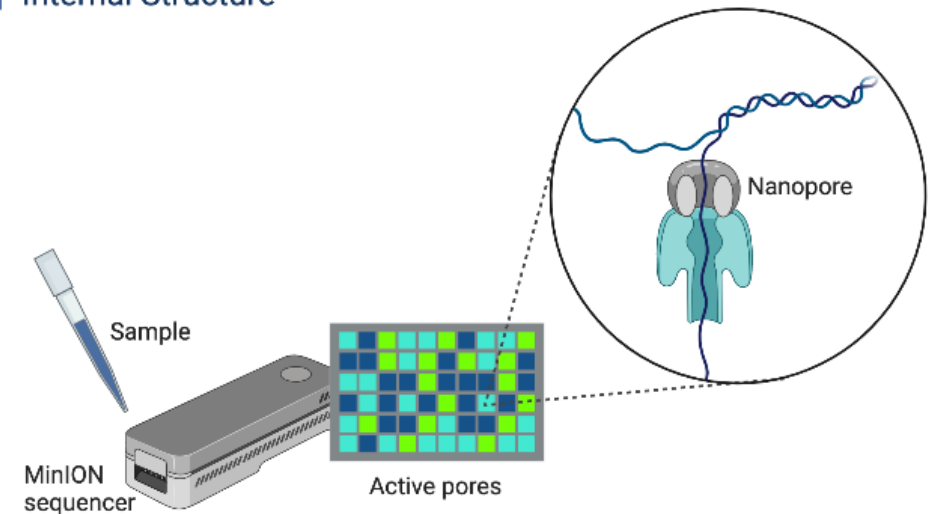
MinION Sequencing Enables Low-Cost HTLV-1 WGS

Portability and Field-Deployability: MinION is a pocket-sized device, making it ideal for in-field, point of care, or remote sequencing

Ultra-Long Reads: MinION can produce very long, single-molecule reads (often >50 kb and up to 2 Mb), which are crucial for resolving complex genomic regions

Cost-Effective and Flexible: It has low capital costs and requires minimal infrastructure compared to traditional, large-scale sequencers

MinION Sequencer
Internal Structure



Patients Samples from Clinical Study with Proviral Load Reduction were selected for LF-PCR workflow

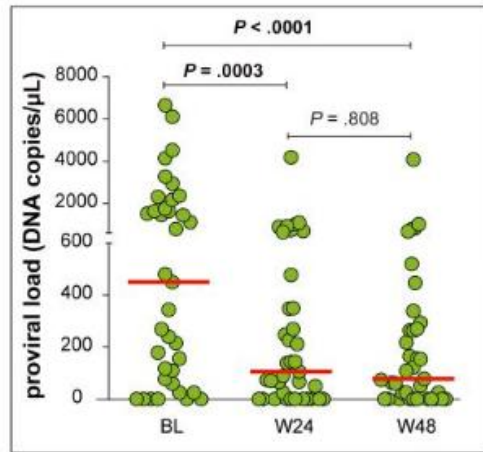
Clinical Infectious Diseases

MAJOR ARTICLE

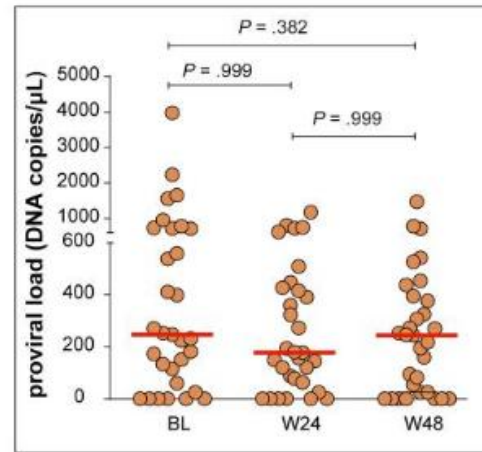


Dolutegravir Reduces Human T-Cell Lymphotropic Virus Type 1 Proviral Load and Improves Neurological Outcomes in a Phase 2 Controlled Trial

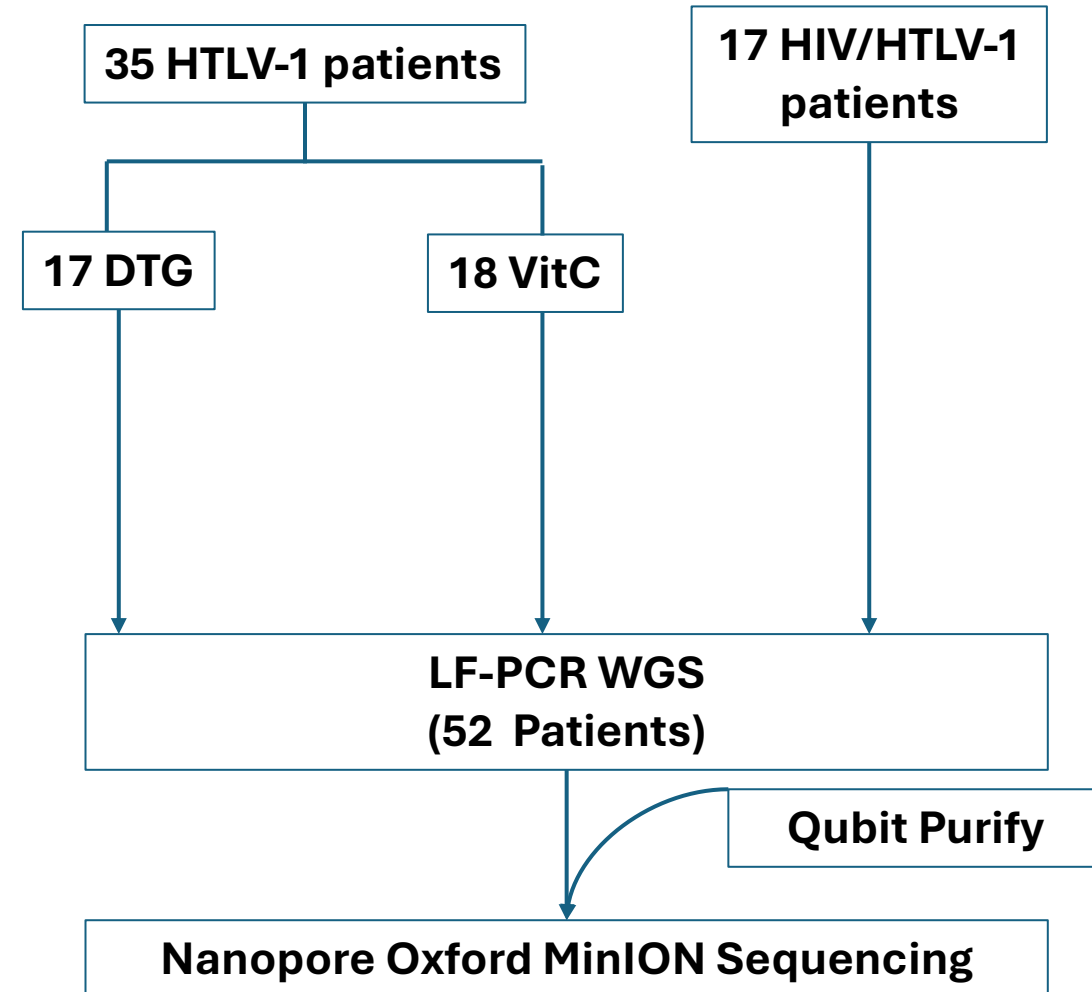
Carlos Brites,^{1,2} Iris Montañó-Castellón,² María B. Arriaga,^{2,3} Cleyde Marconi,¹ Rafaela Mayoral,² Estela Luz,² Camila A. Figueredo,³ Bianca S. D. Fiuza,³ and Eduardo M. Netto^{1,2}



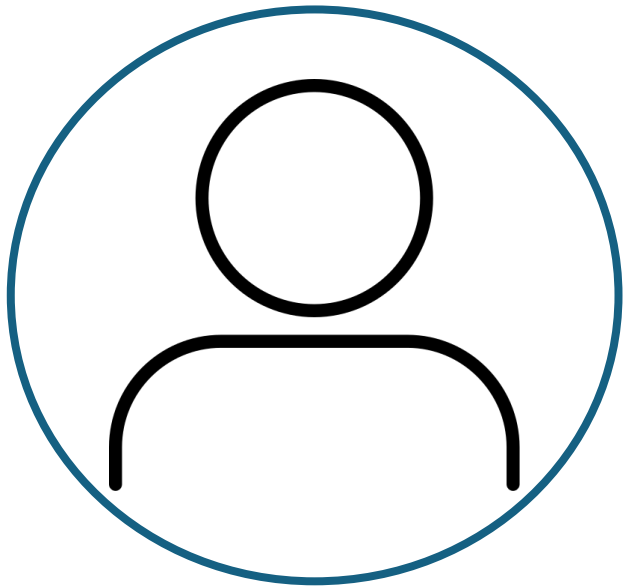
DTG



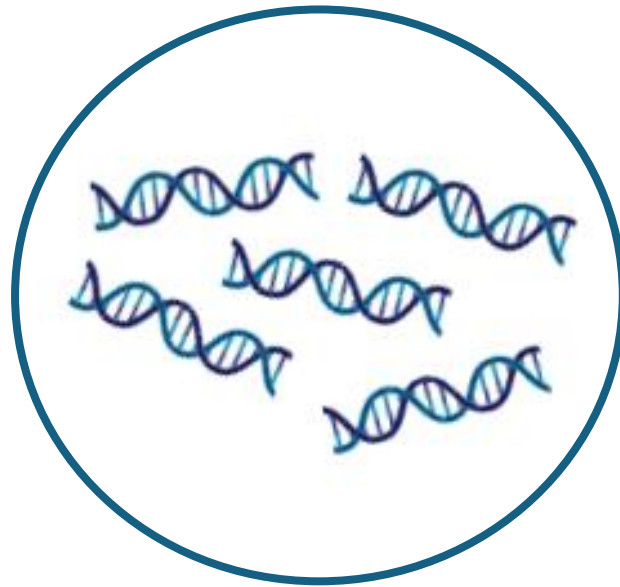
VitC



LF-PCR Successfully Amplified 95% of Samples and Produced High Sequencing Genome Coverage and Read Depth



95% success rate

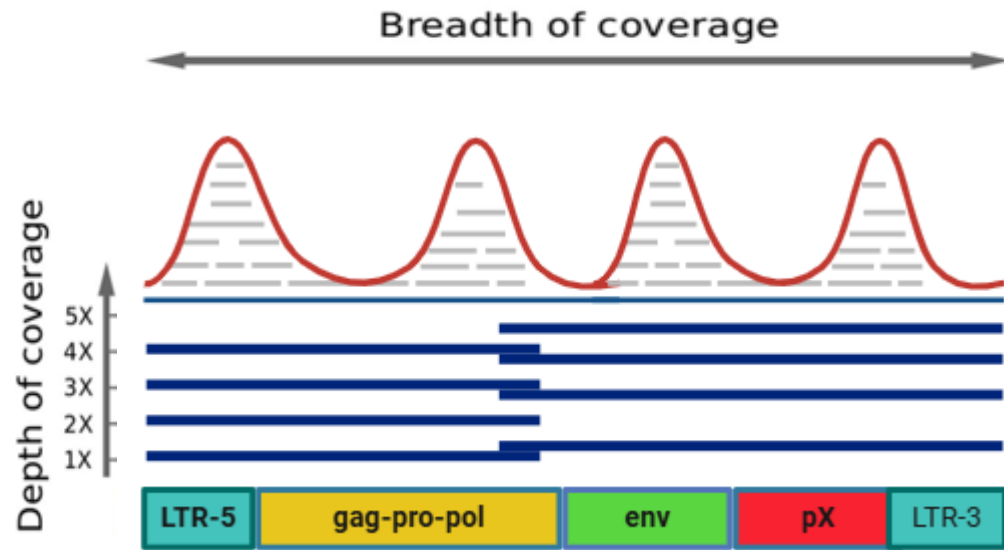


High amplification yield



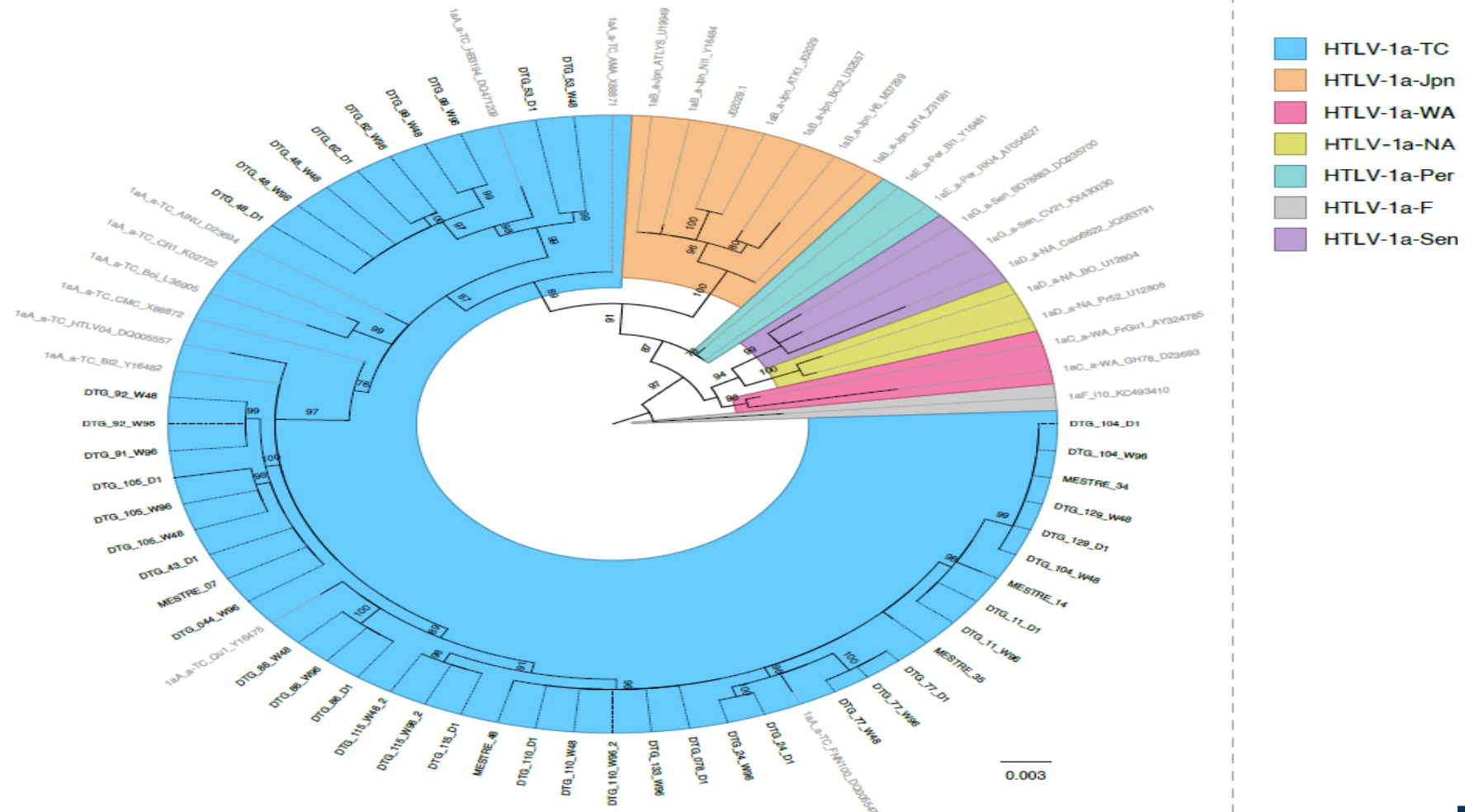
High genome coverage and depth

Viral DNA input Concentration Correlates With LF-PCR Sequencing Depth; however, Coverage was Great Overall



	High		Low	
	Average	% samples	Average	% samples
Mean_A+B	23.62	51.1%	12.96	48.9%
CTvalue	26.99	51.1%	27.54	48.9%
Coverage (%)	99.11	51.1%	99.11	48.9%
Depth	476.30	51.1%	261.52	48.9%

Phylogenetic analyses reveals that the HTLV-1 sequences clustered exclusively with the Cosmopolitan-Transcontinental genotype



Summary

- Developed a one-step LF-PCR workflow which relies on amplification of two PCR fragments
- LF-PCR protocol amplified both fragments with high efficiency
- Long-read MinION sequencing had high coverage and depth, even for low-input DNA samples
- Streamlined, high-throughput compatible workflow for HTLV-1 genomic characterization and variability for many studies

Future Directions

Clinical Infectious Diseases

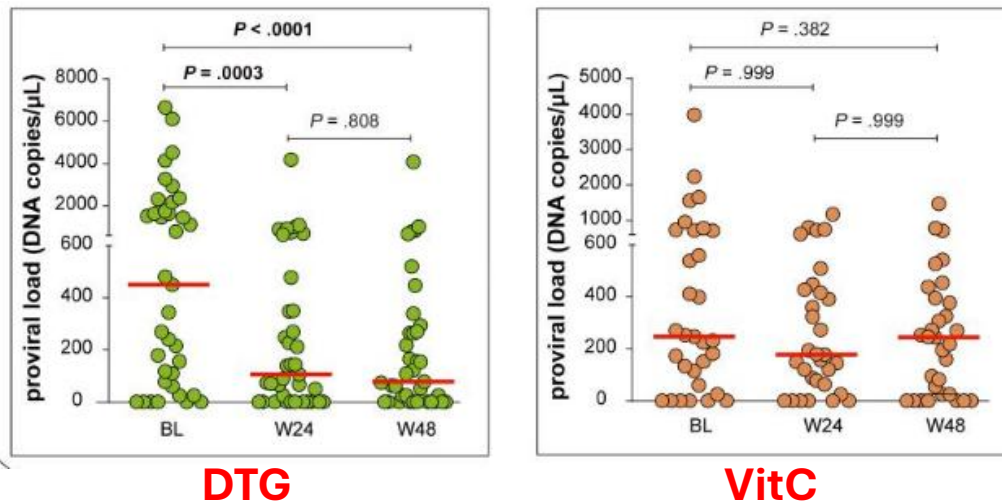
MAJOR ARTICLE



Dolutegravir Reduces Human T-Cell Lymphotropic Virus Type 1 Proviral Load and Improves Neurological Outcomes in a Phase 2 Controlled Trial

Carlos Brites,^{1,2} Iris Montaña-Castellón,² María B. Arriaga,^{2,3} Cleyde Marconi,¹ Rafaela Mayoral,² Estela Luz,² Camila A. Figueredo,³ Bianca S. D. Fiuza,³ and Eduardo M. Netto^{1,2}

- Hypothesis: Genome mutation & integration site characterization will provide insights into DTG treatment outcomes



- WGS HTLV-1 from patients that responded to treatment and those that did not (baseline vs 48 weeks) vs VitC controls

Acknowledgements



LAPI - UFBA



**Dr. Brites
UFBA**

**Dr. Herrera
Rutgers**



LAPI - UFBA



22nd International Conference on Human Retrovirology:
HTLV and Related Retroviruses
HTLV2026

June 3-6, 2026 | Philadelphia, Pennsylvania, USA

PRMT5 regulates HTLV-1 p30 stability and cell cycle progression

Bethany Pepple
The Ohio State University, Columbus, USA

PRMT5 regulates HTLV-1 p30 stability and cell cycle progression

Bethany Pepple

22nd International Conference on Human Retrovirology:
HTLV and Related Retroviruses

Laboratory of Amanda Panfil, PhD



THE OHIO STATE UNIVERSITY
CENTER FOR RETROVIRUS RESEARCH



Conflicts of interest:

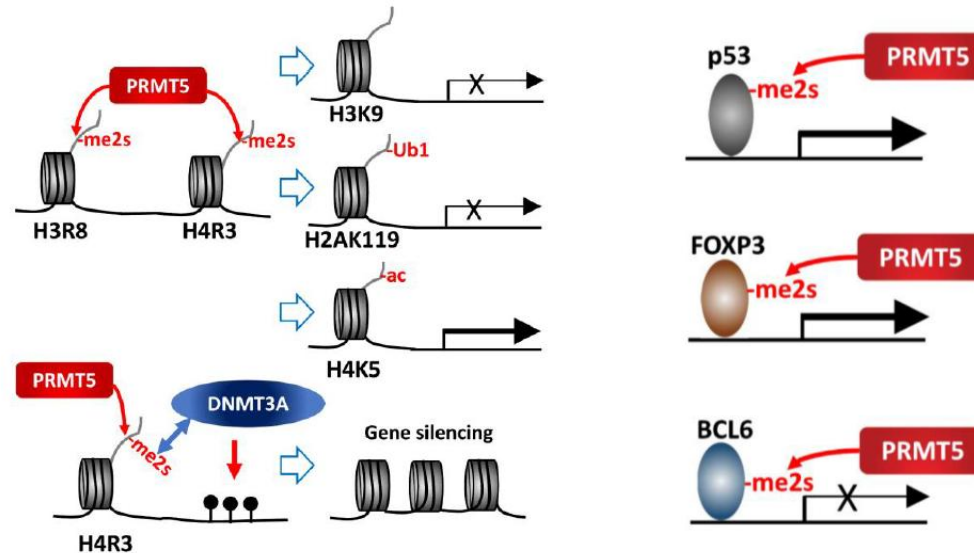
I have no conflicts of interest.

The viral accessory protein p30 interacts with the cellular epigenetic factor PRMT5

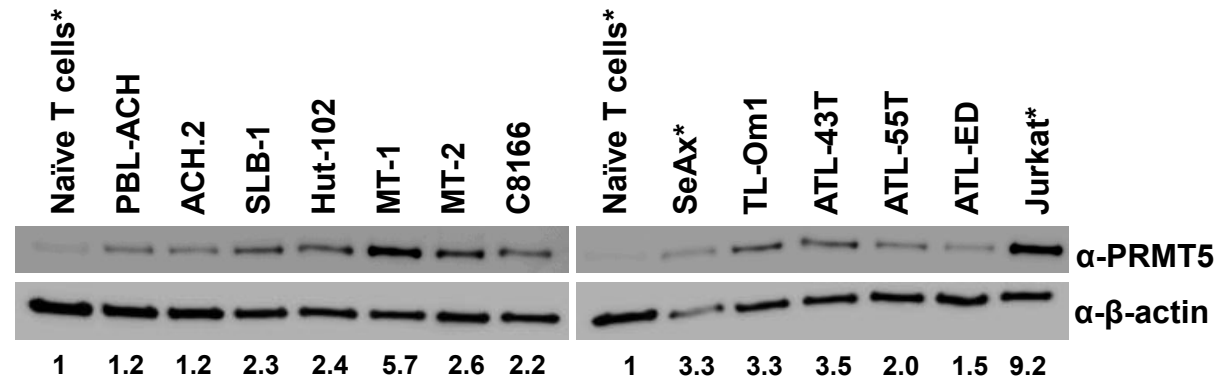
HTLV-1 p30-interacting host proteins			
Name of the protein	Molecular function	Unweighted spectrum count	% coverage
	Cell cycle and protein degradation		
Proteasome activator complex subunit		21	54
Heat shock protein 90 beta	Protein folding	9	14
Heat shock protein 90 alpha	Protein folding	7	8.2
Methylosome subunit pICln	Methylosome component	5	37
Reticulocalbin-2	Calcium binding	5	20
Succinate dehydrogenase [ubiquinone] flavoprotein subunit	Electron transport	5	9.2
Elongation factor Tu	Translation	5	17
Clathrin heavy chain 1	Vesicular transport	5	5.4
Protein arginine N-methyltransferase 5	Methyltransferase activity	4	16

Protein arginine methyltransferase 5 (PRMT5)

- Catalyzes symmetric dimethylation of arginine residues
- Controls gene expression by histone methylation
 - Transcriptional activator or repressor
 - H4R3, H2AR3, H3R8, H3R2
- Targets several non-histone proteins with tumor promoting or suppressing functions
- One of the most highly expressed PRMT family members in cancer



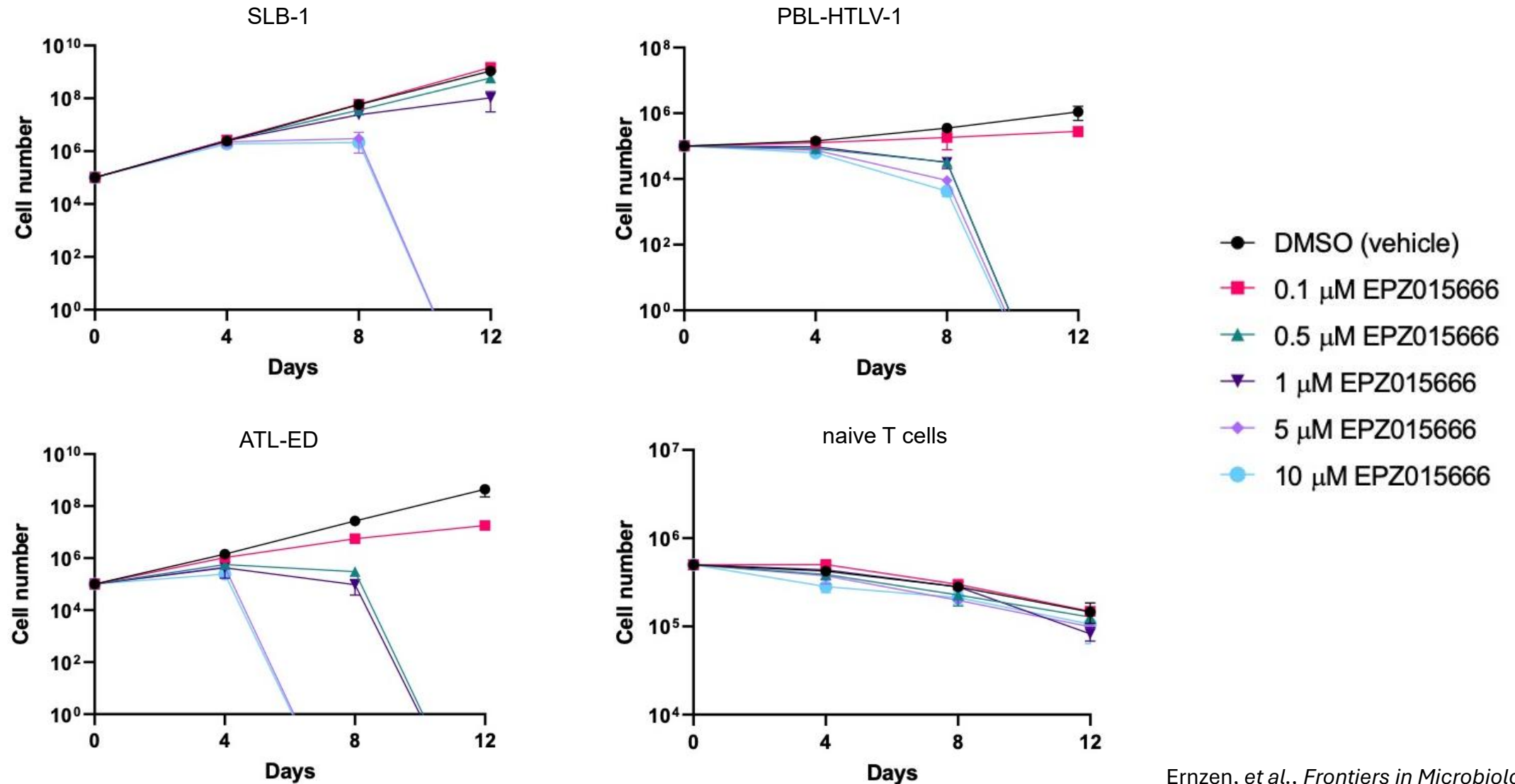
PRMT5 is over-expressed in T-cell leukemia/lymphoma cell lines



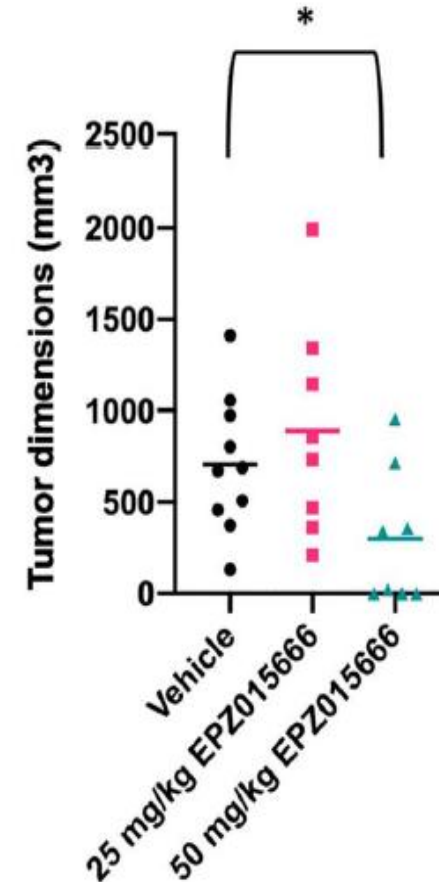
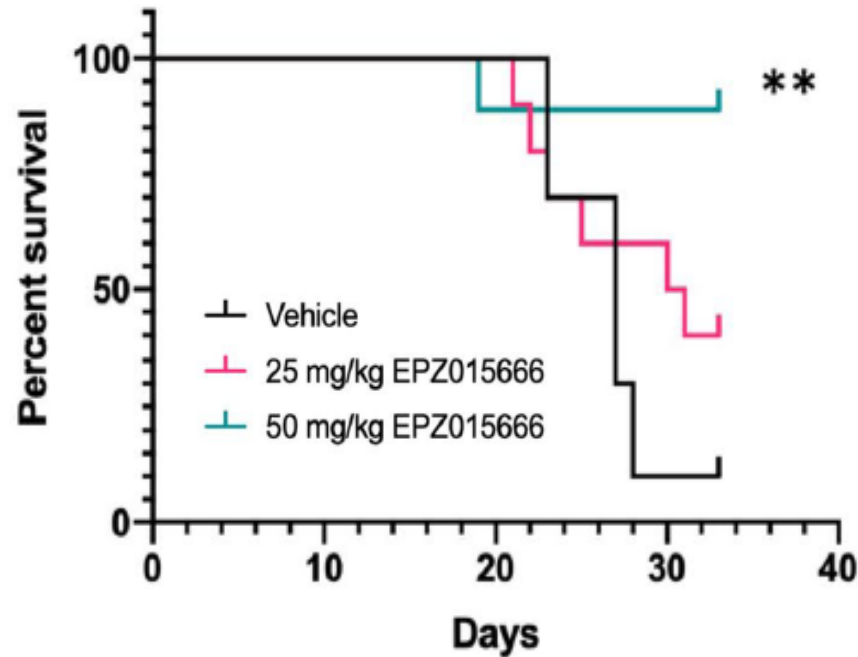
* Denotes HTLV-1 negative cell line

Panfil, *et al.*, *Viruses* (2015)

PRMT5 enzymatic inhibition is selectively toxic in transformed T-cell lines

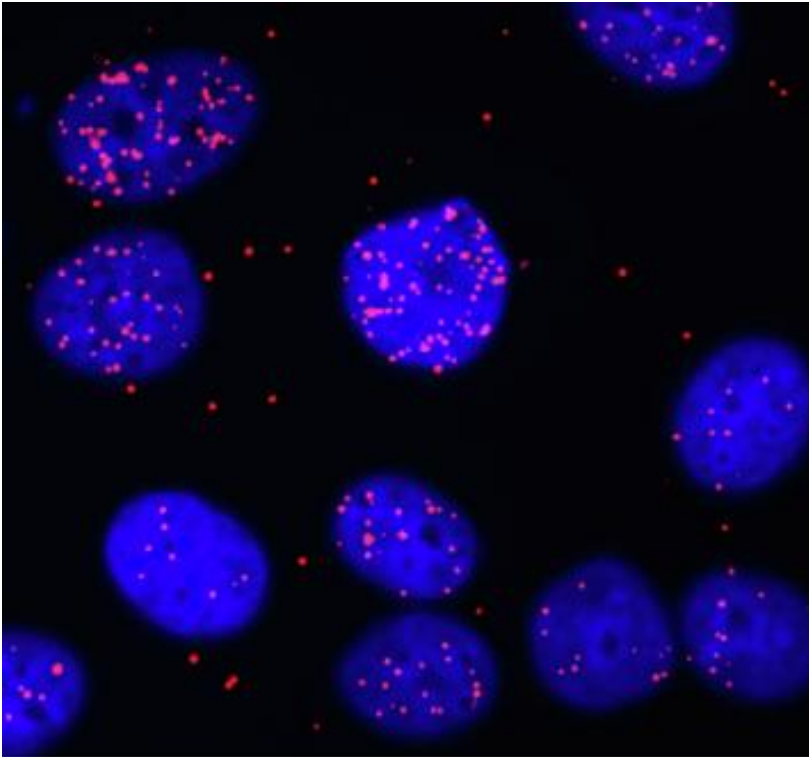


PRMT5 inhibition increases survival and decreases tumor cell proliferation *in vivo*

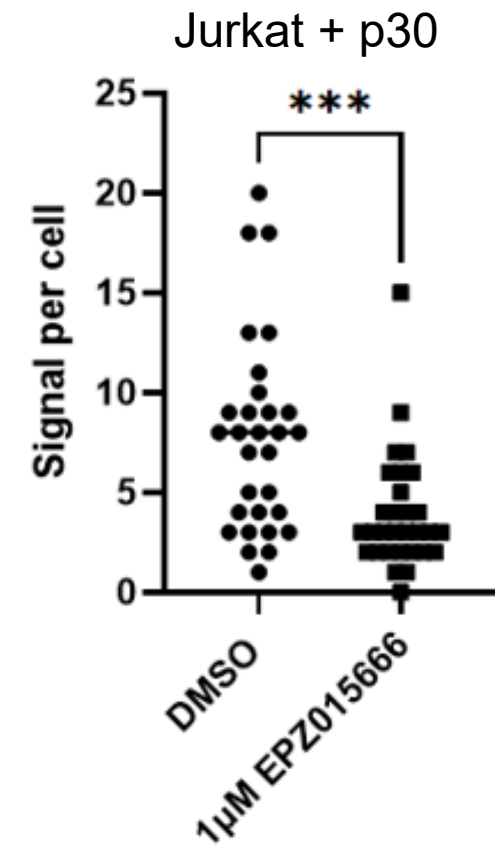
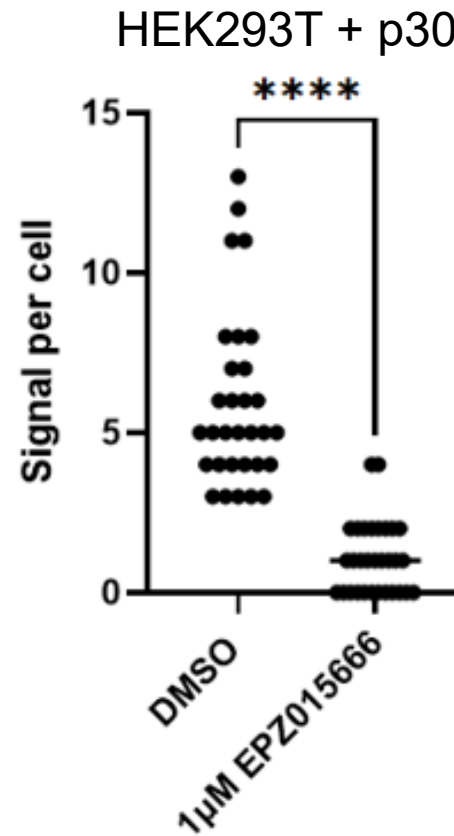


PRMT5 symmetrically dimethylates HTLV-1 p30 protein

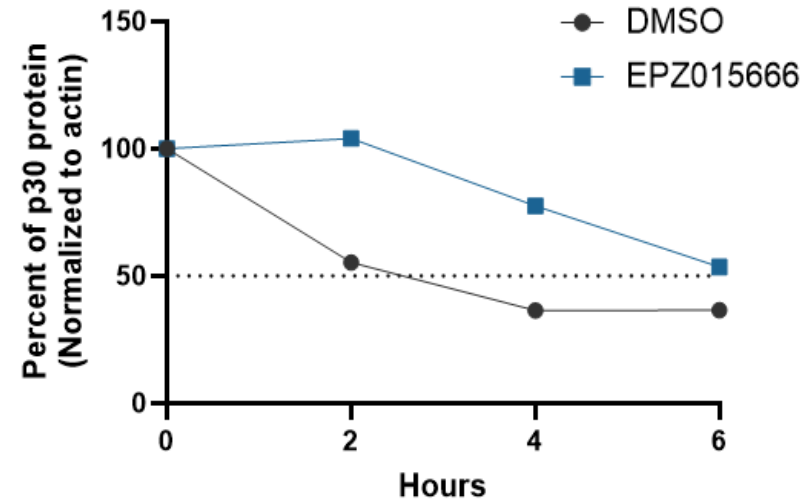
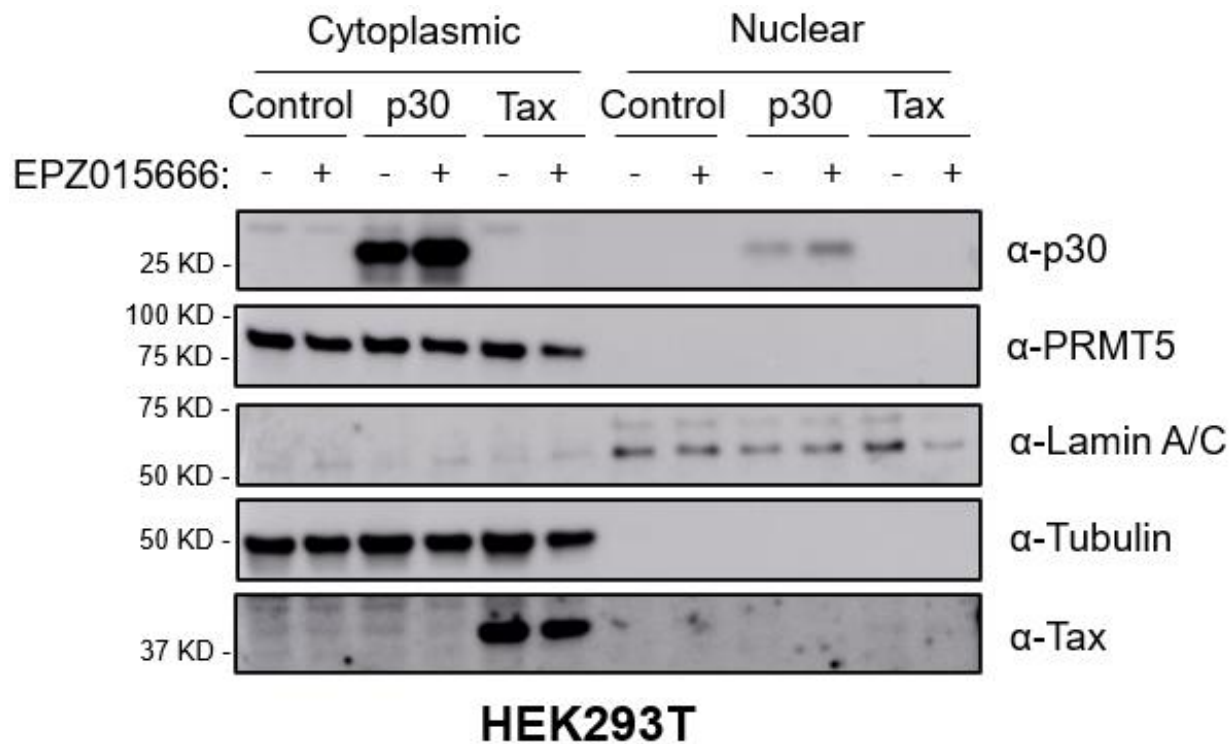
Proximity ligation assay (PLA)



Poulard, C. et al., *Methods* (2020)

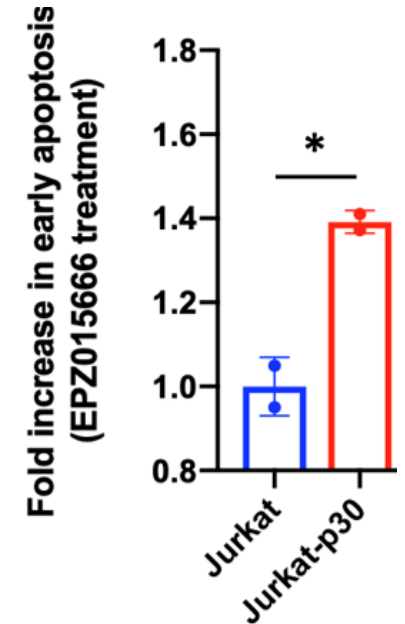
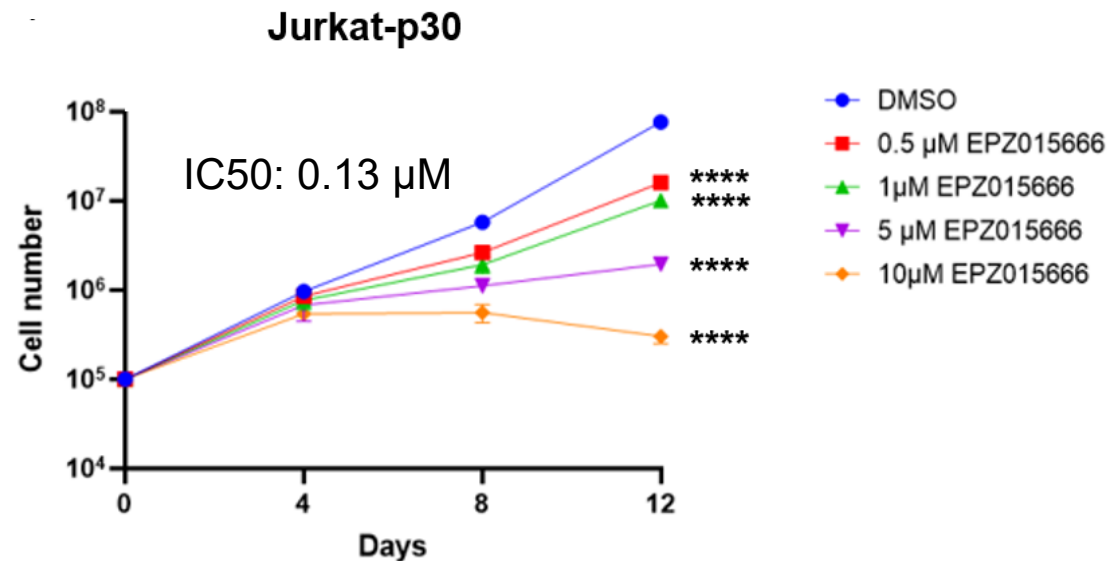
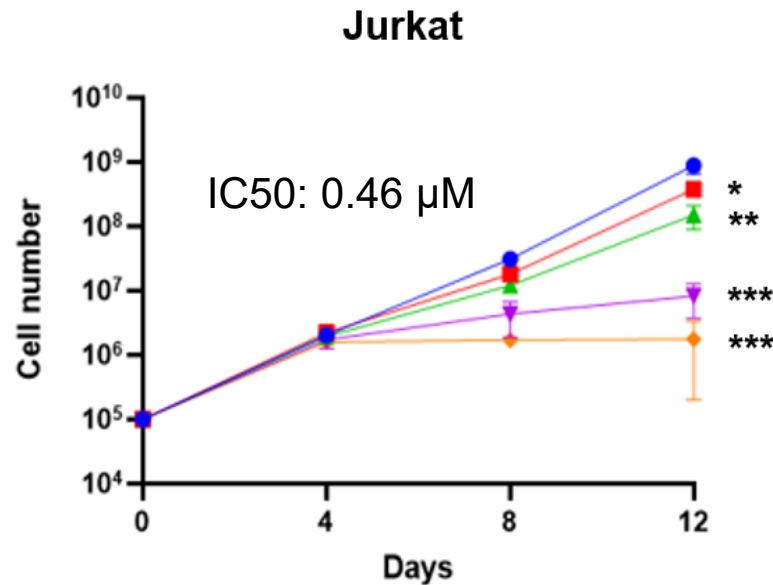
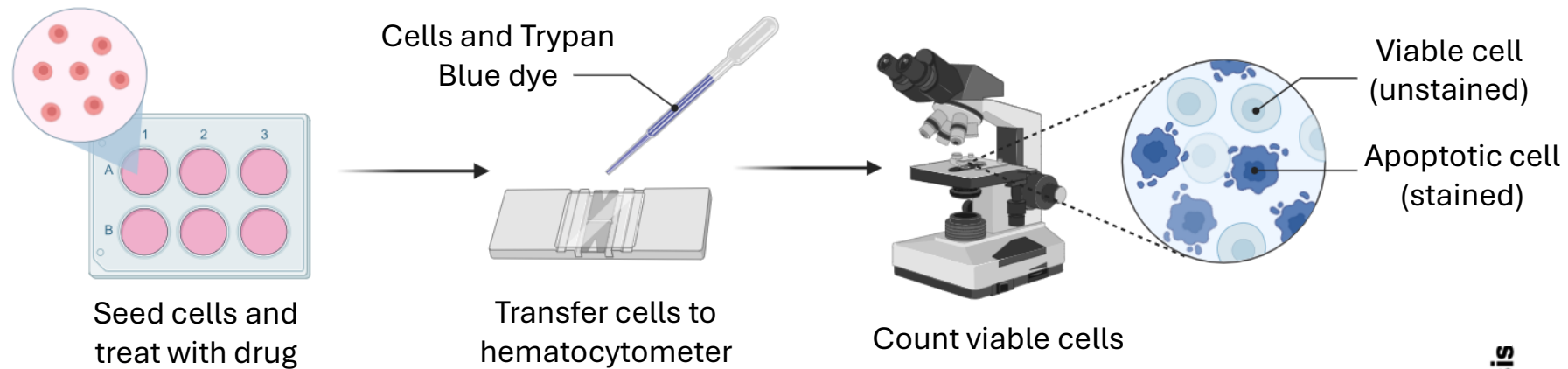


PRMT5 regulates HTLV-1 p30 protein stability

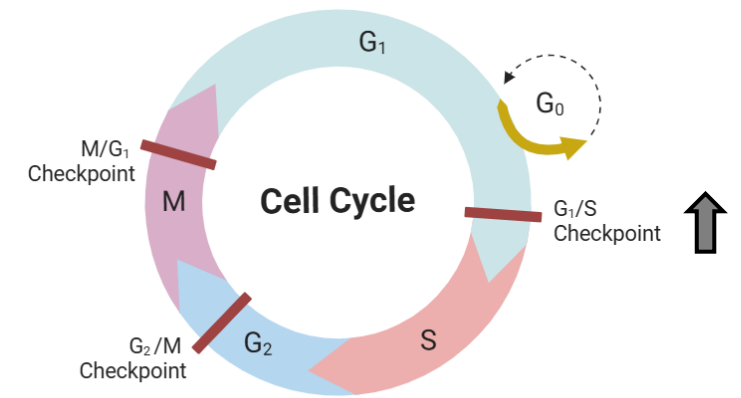
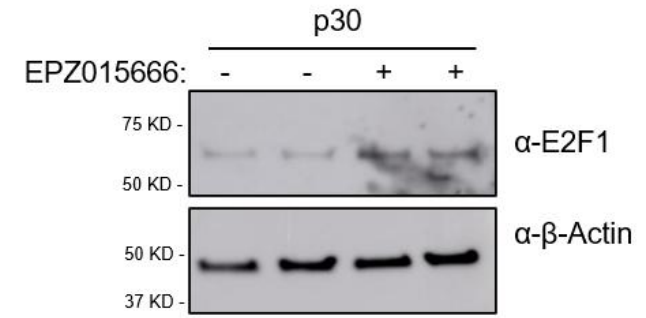
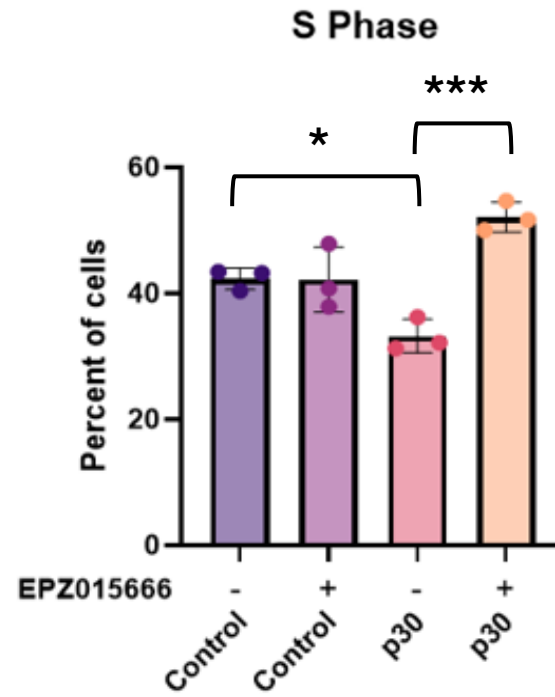
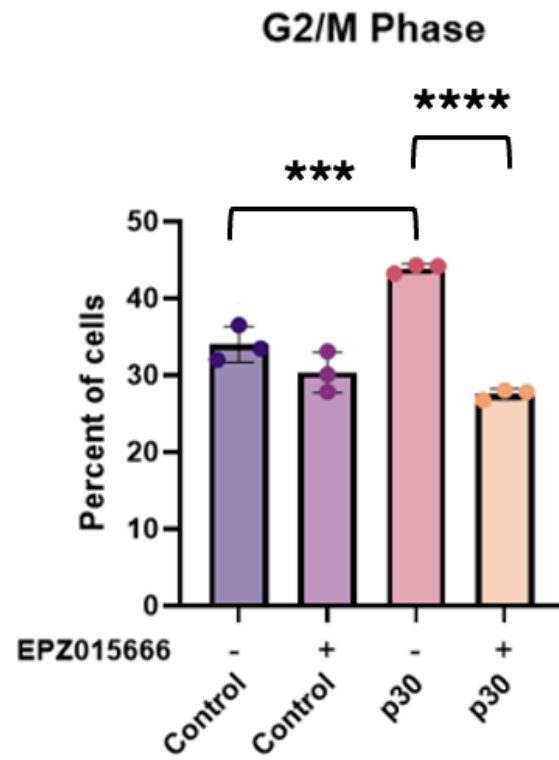


Condition	Half-Life
DMSO	2.6 hours
EPZ015666	6.3 hours

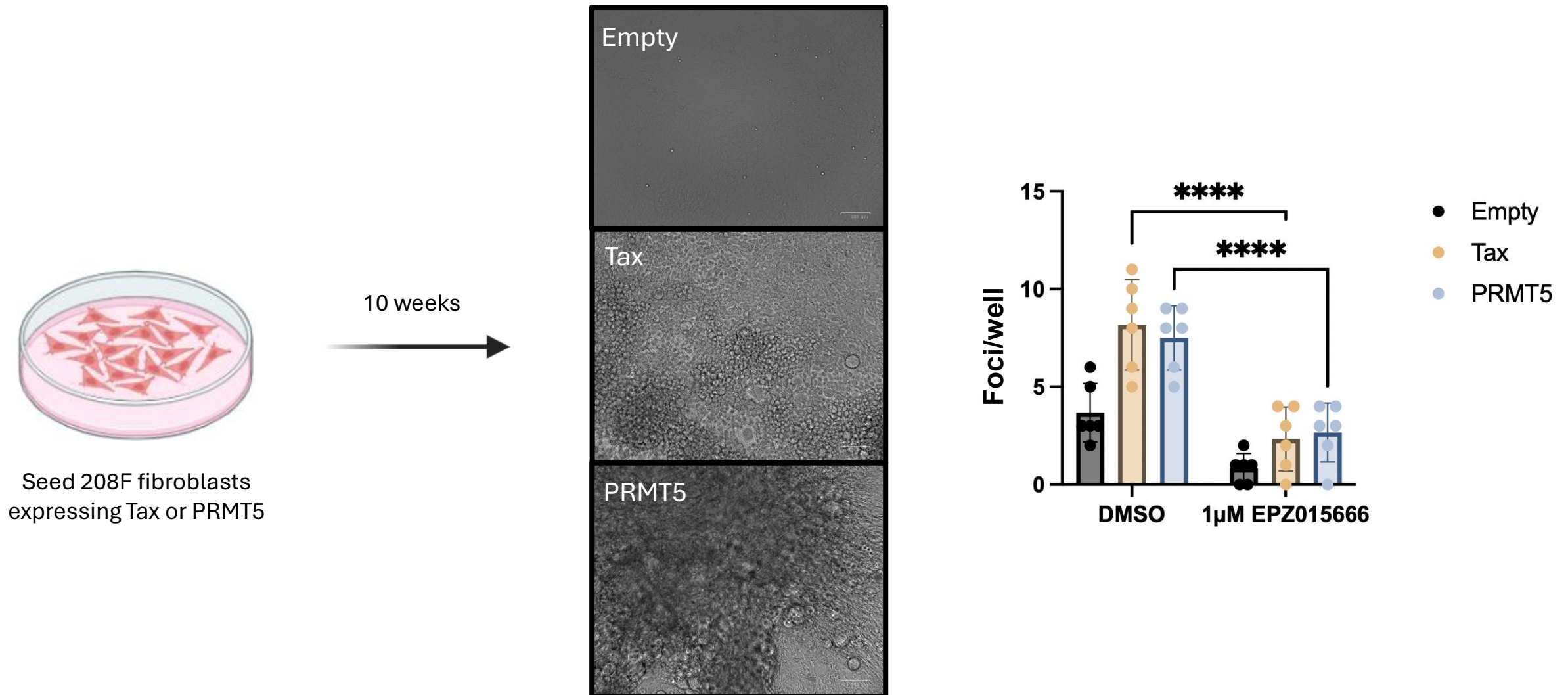
HTLV-1 p30 enhances cell sensitivity to EPZ015666-induced cell death and apoptosis



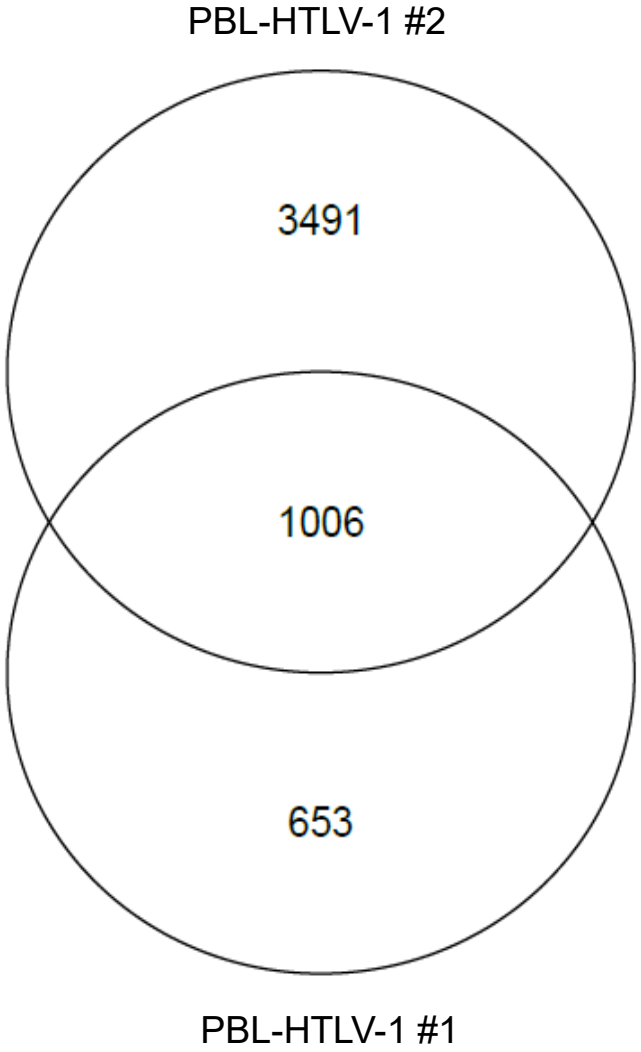
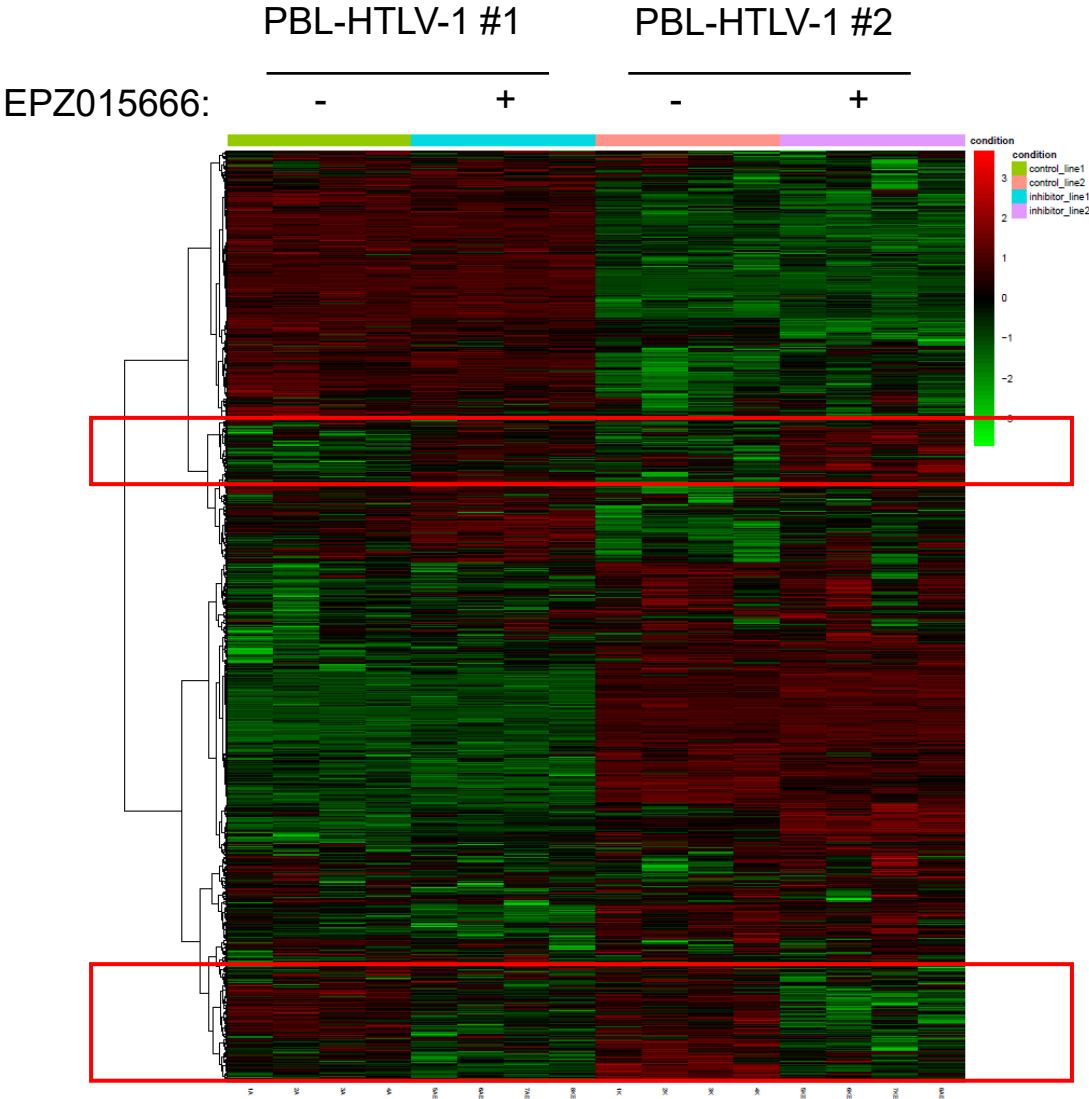
PRMT5 inhibition alters HTLV-1 p30-mediated cell cycle regulation



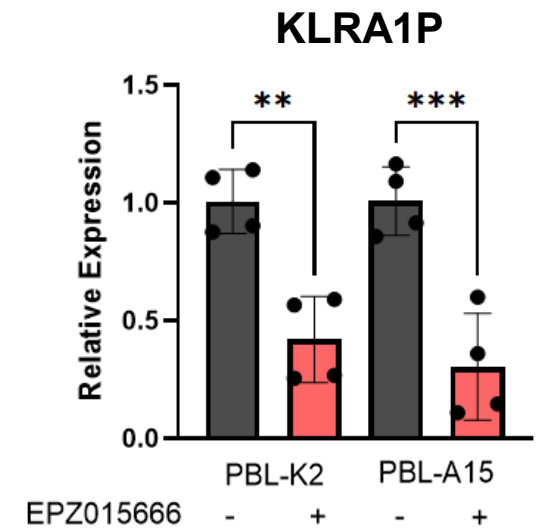
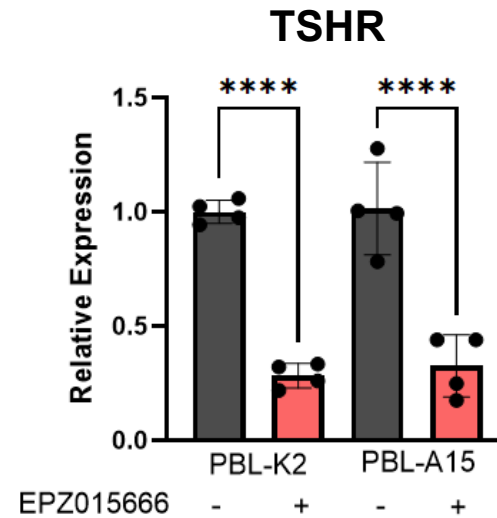
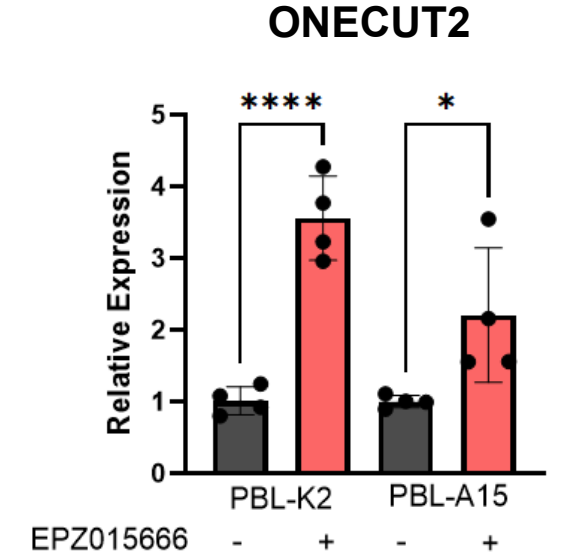
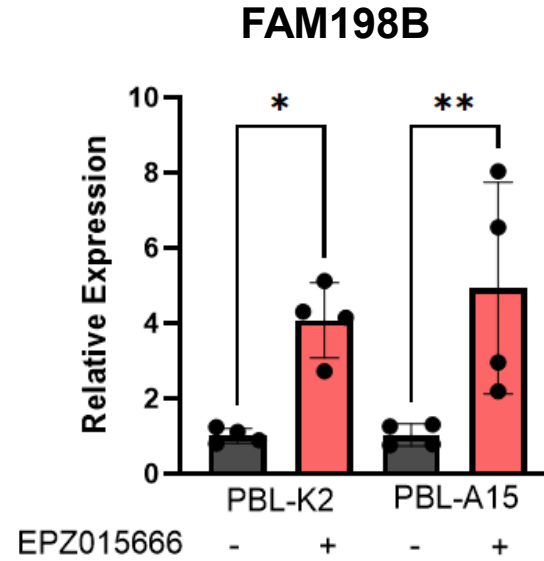
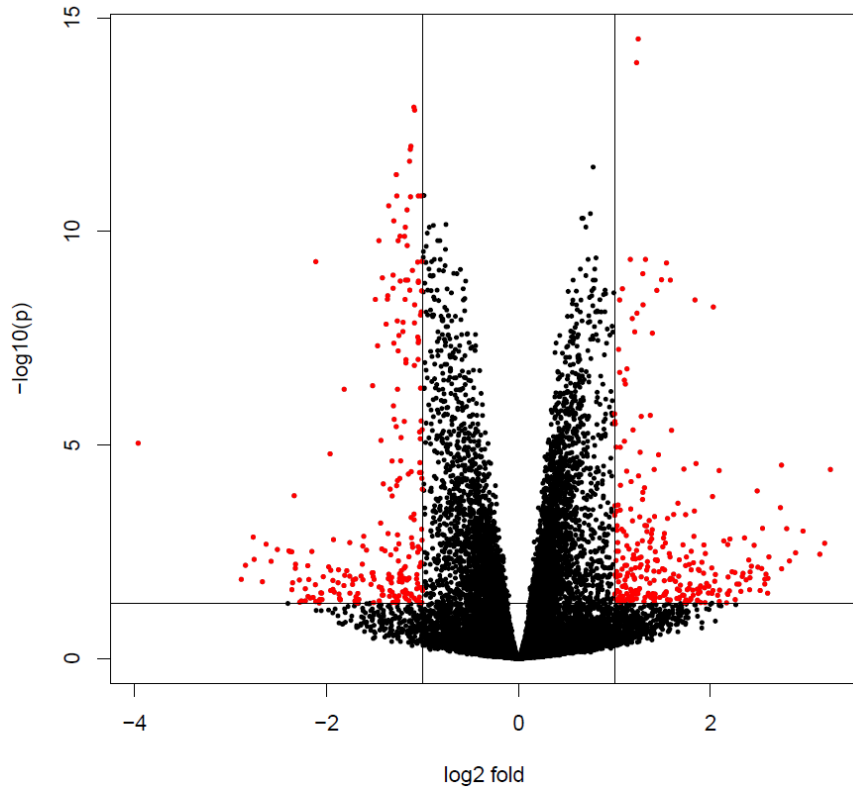
PRMT5 induces transformation in 208F fibroblasts



PRMT5 inhibition alters cellular gene expression



PRMT5 inhibition alters cellular gene expression



Summary

- HTLV-1 p30 protein is symmetrically dimethylated by PRMT5
- PRMT5-mediated methylation destabilizes p30
- p30 expression increases cellular sensitivity to PRMT5 inhibitor-induced apoptosis
- PRMT5 inhibition modulates p30-dependent cell-cycle regulation
- PRMT5 promotes fibroblast transformation
- Pharmacological inhibition of PRMT5 broadly alters cellular transcriptional programs

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- Rani Chunduri, MS PhD
- Amelie Mockbee
- Cameron Phelps
- Rocio Zaldivar, MS
- **Kyle Ernzen, PhD**

Collaborator

- Shan-Lu Liu, MD PhD

Resources

- OSU CCC
- NCI P01CA100730





22nd International Conference on Human Retrovirology:
HTLV and Related Retroviruses
HTLV2026

June 3-6, 2026 | Philadelphia, Pennsylvania, USA

Characterisation of proviral mutation diversity in HTLV-1-infected clones

Anat Melamed
Imperial College London, London, UK

Characterisation of proviral mutation diversity in HTLV-1- infected clones

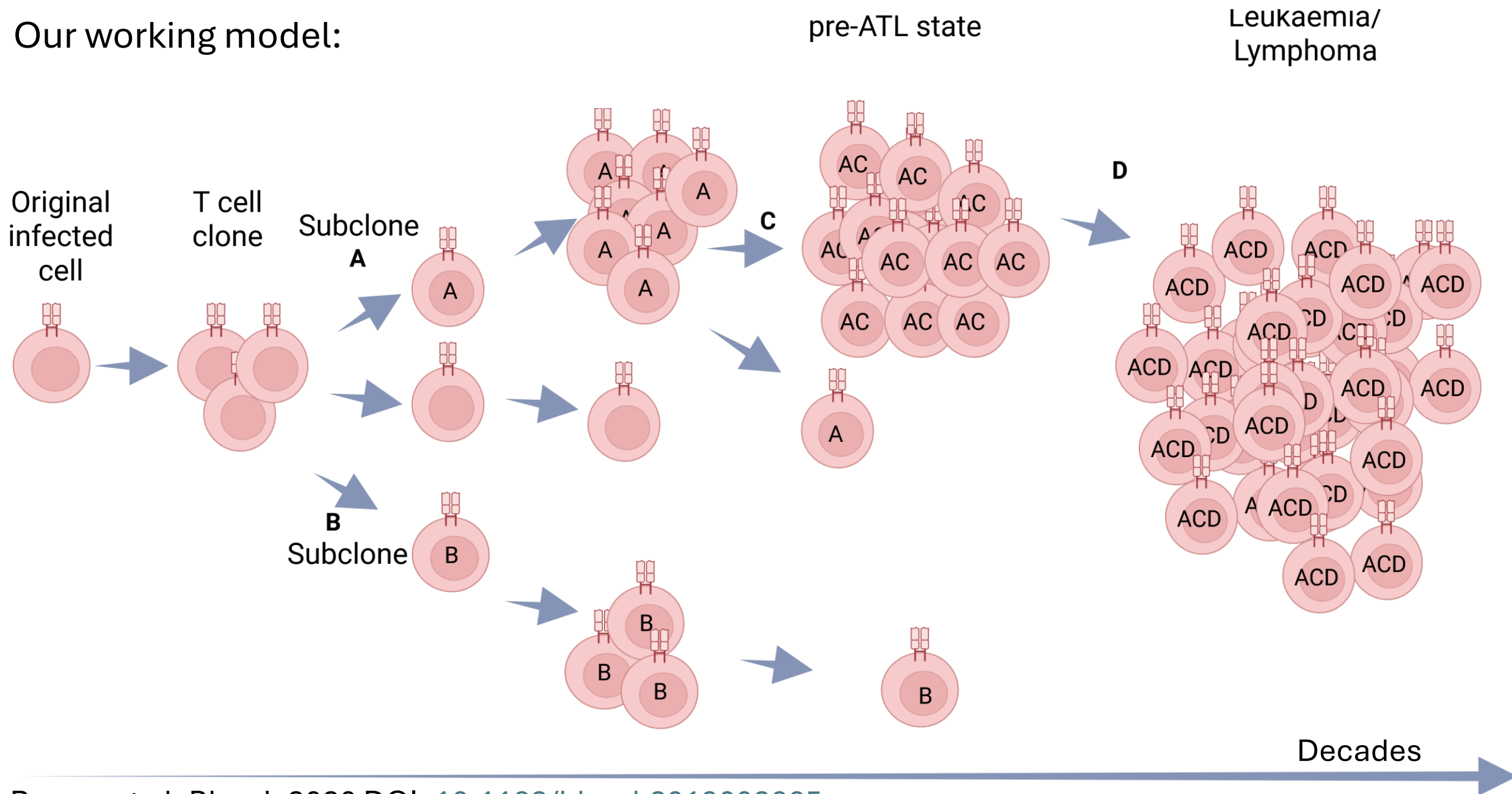
Anat Melamed
Rowan lab

Dept of infectious Disease
Imperial College London

HTLV-1 sequence diversity

- HTLV-1 causes a persistent infection, maintained by a set of long-lived clones in infected individuals, each of which carries an integrated provirus.
- While HTLV-1 is considered to be genetically stable, it is known that HTLV-1 can accumulate somatic changes leading to abrogation of Tax expression.
- It is not known what is the timing of acquisition / accumulation of these variants.

Our working model:



HTLV-1 sequence diversity

- Historically –
 - focus on consensus sequences or on a limited part of the genome.
 - PCR-dependent protocols limited to the presence of primer binding sites
 - narrow patient focus
- Here we aim to illuminate the sequence diversity underlying the consensus sequences
 - Genome wide
 - Healthy carriage and disease
 - PCR-independent

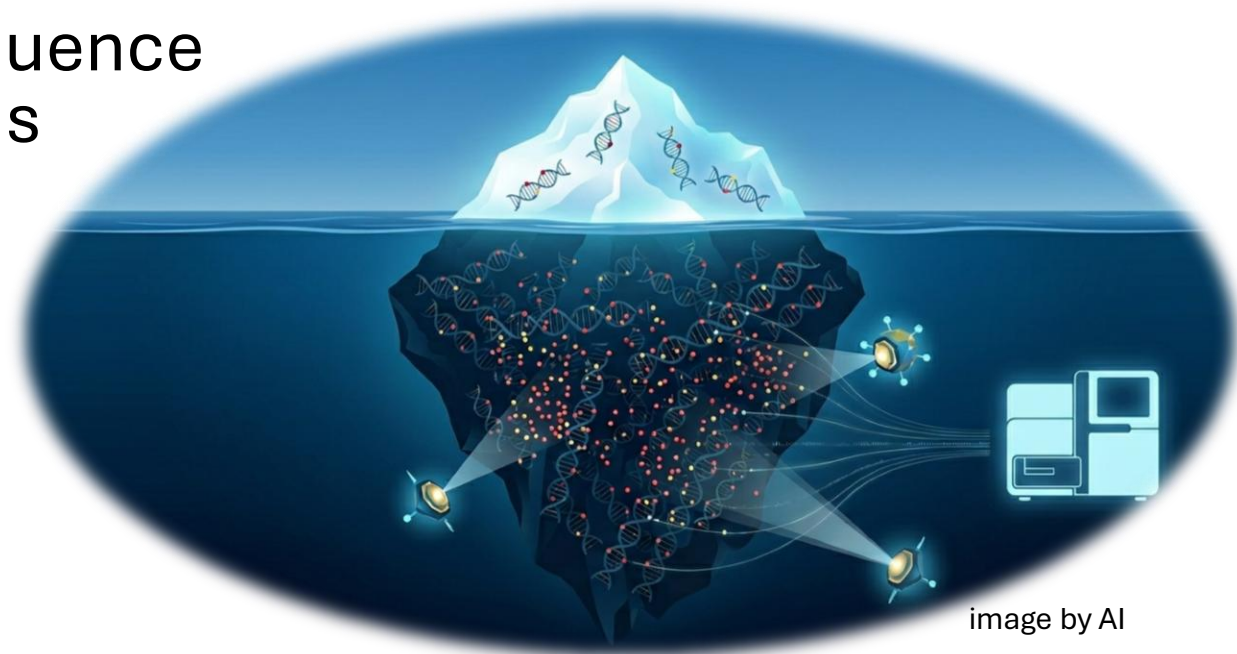


image by AI

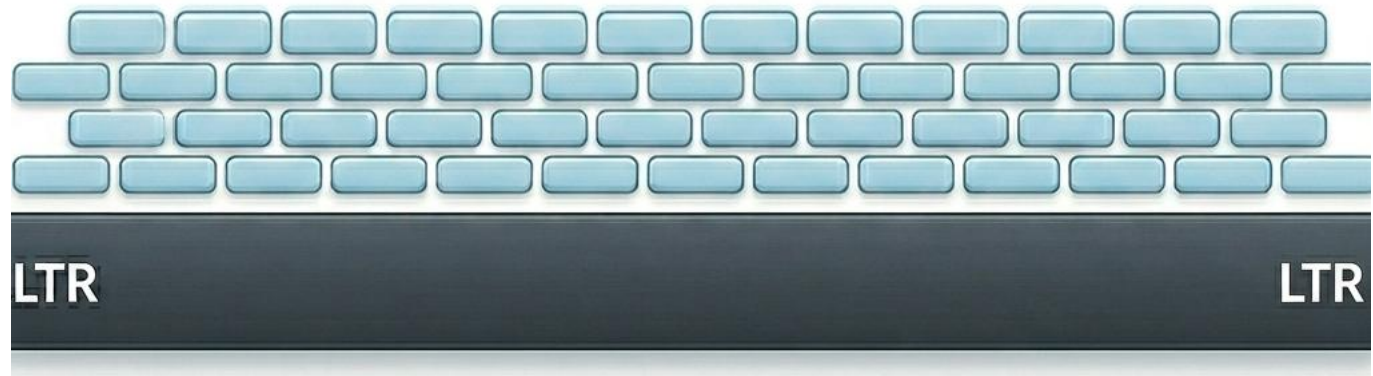
Method

- Genotypically-agnostic Probe-capture enrichment of proviral sequences from DNA sequencing libraries
- Ultra-deep sequencing of proviral genomes from naturally-infected individuals.
- Bespoke pipeline to identify somatic variants.



Probe-set design

- All HTLV-1 complete sequences available from NCBI, published as of by August 2023, using the following parameters (Total – **198 sequences**)
 - COMPLETE[All Fields] AND "Human T-cell leukemia virus type I"[Primary Organism] AND (("9000"[SLEN] : "9050"[SLEN]) AND ("1993/01/01"[PDAT] : "2024/12/31"[PDAT]))
- Probe design for Agilent Sure-select by proprietary algorithm across all genomes identifying most informative set.

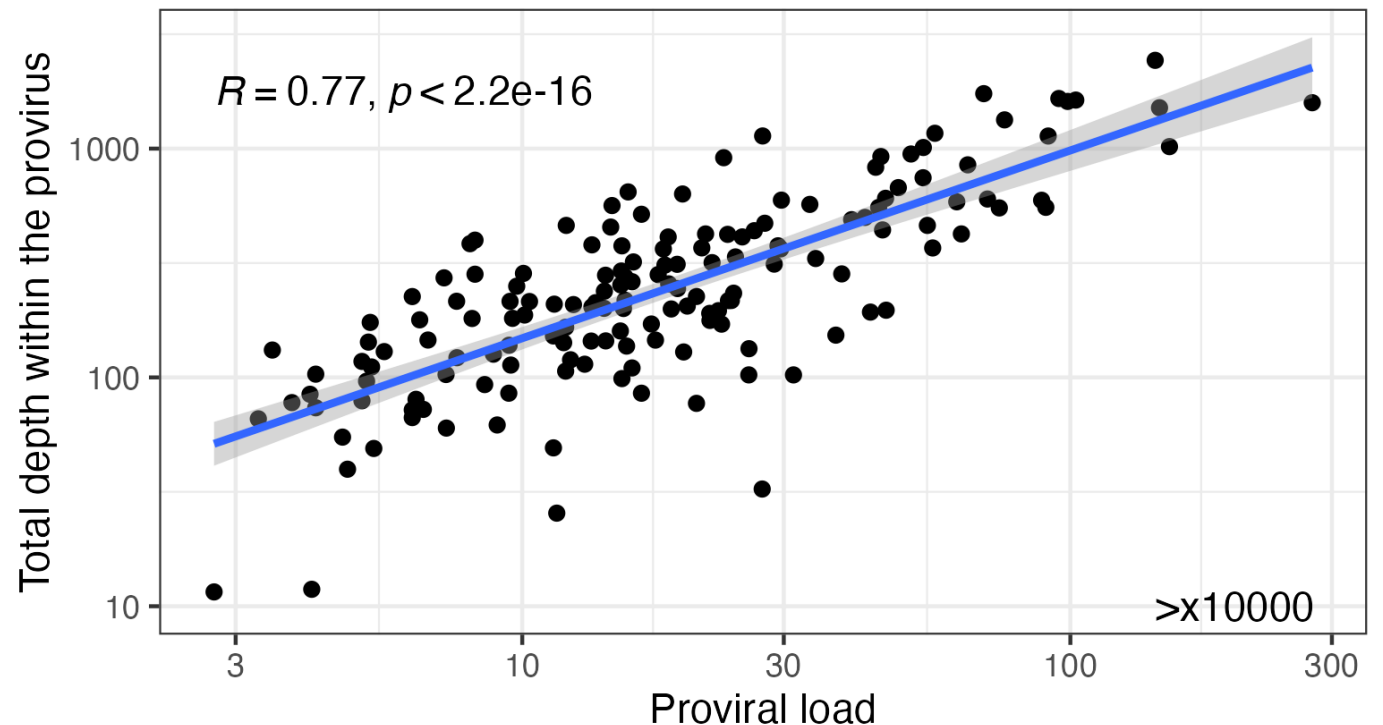
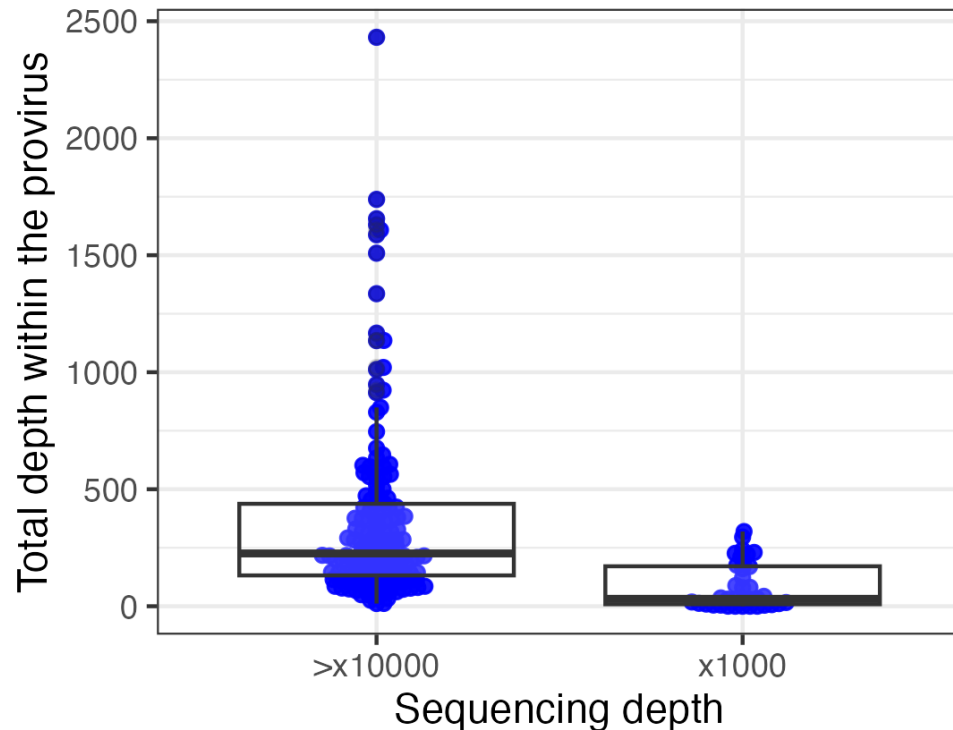


Samples

- 153 PBMC samples from 107 HTLV-1-infected individuals, including:
 - 66 Individuals with ATL
 - 23 individuals at high risk for ATL (OCI > 0.77 and PVL > 4%)
 - 9 samples sorted for expression of the most frequent Vb.

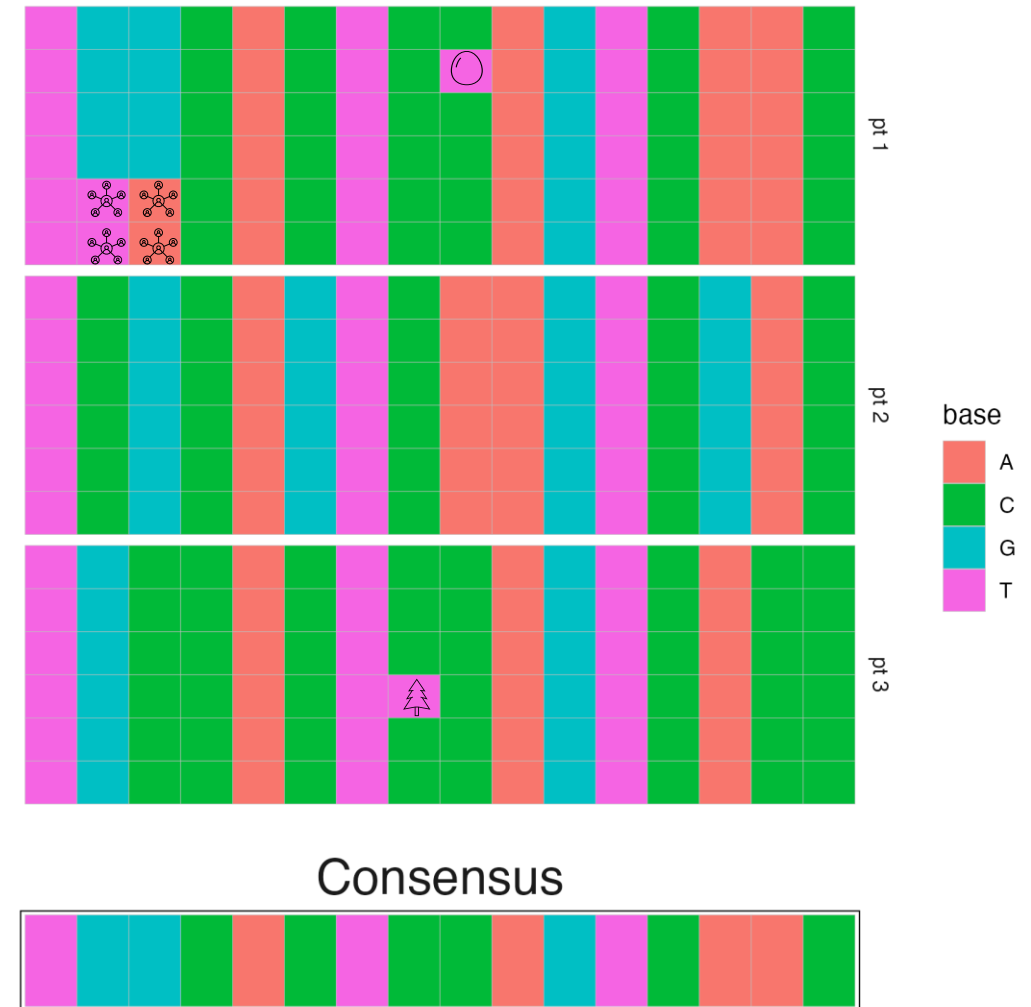
Sequencing depth

- Median Sequencing depth – 225 for ultra-deep sequencing
- Correlates with proviral load



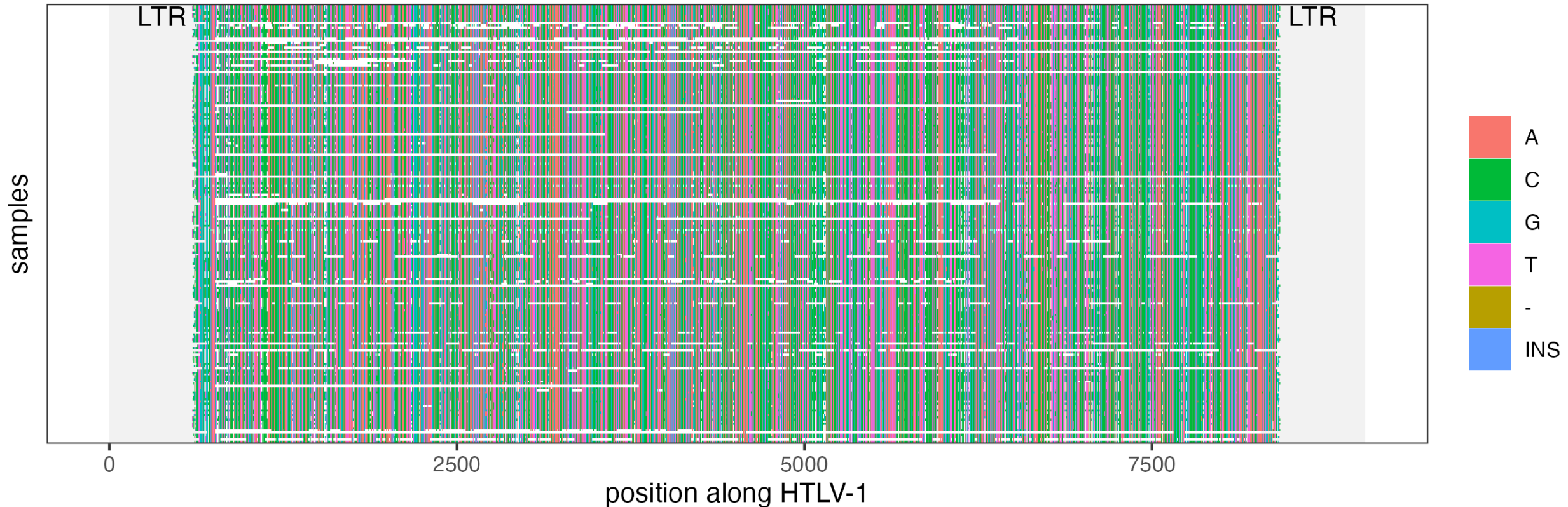
Approach – somatic variant calling

- Alignment to a single HTLV-1 reference
- Base calling at each position.
 - Stringent filters
- Define a ‘consensus HTLV’ for cohort, call all variants from this ‘consensus HTLV’.
- Identify putative ‘founder’, somatic variants
- Annotate HTLV-1 coding sequence and predict outcome.



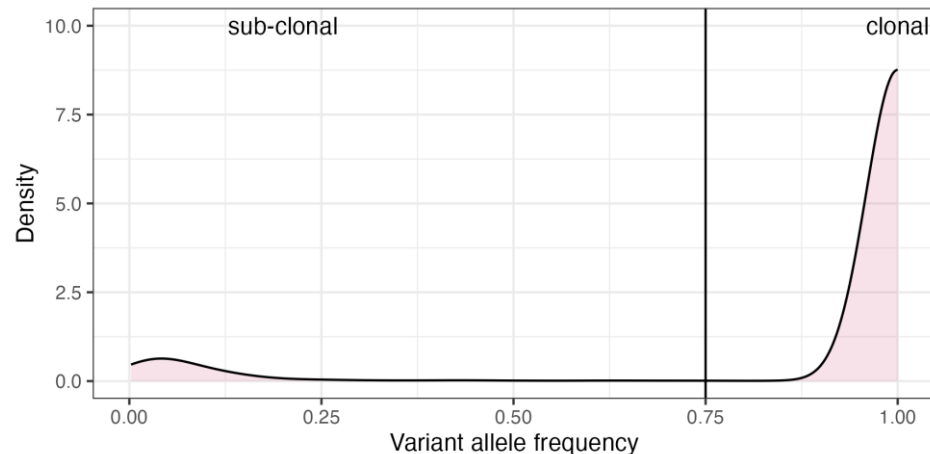
Variant coverage

- Variant analysis covers 87% of the HTLV-1 genome, LTR having reduced coverage due to low confidence mapping.



Variant filtering

- After exclusion of ‘consensus’ variants, 8230 variants remain across all samples.
- The majority of variants are clonal – many will be ‘founder’



Confidently called
bases

1339137

Excluding
‘consensus’ variants

8230

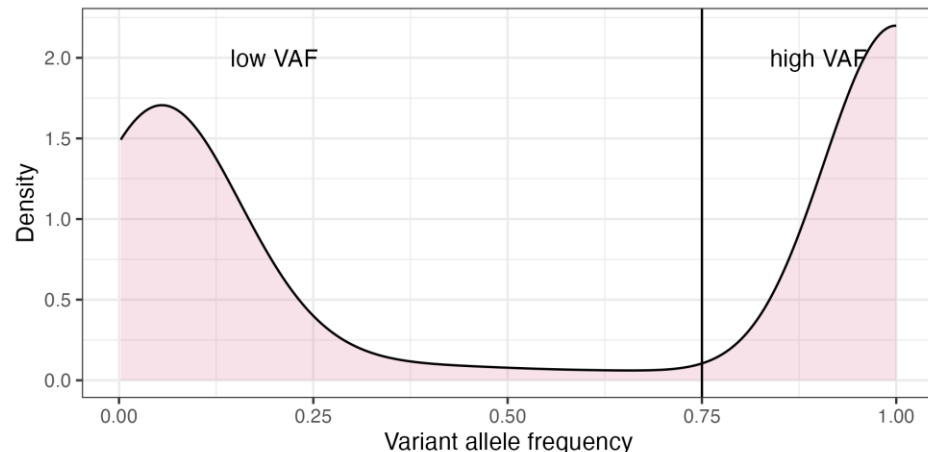
Excluding ‘founder’
variants

1754

Annotate

Variant filtering

- Suspected ‘founder’:
 - Found clonally in samples from > 1 individuals (excluding high OCI individuals)
 - Found clonally in samples from > 1 sorted samples of same individual
 - Total – 428 variants – excluded from further analysis.



Confidently called
bases

1339137

Excluding
‘consensus’ variants

8230

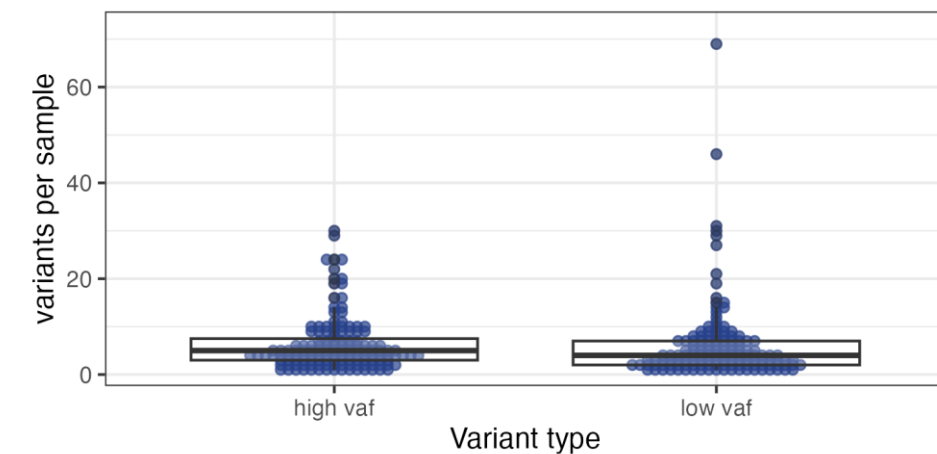
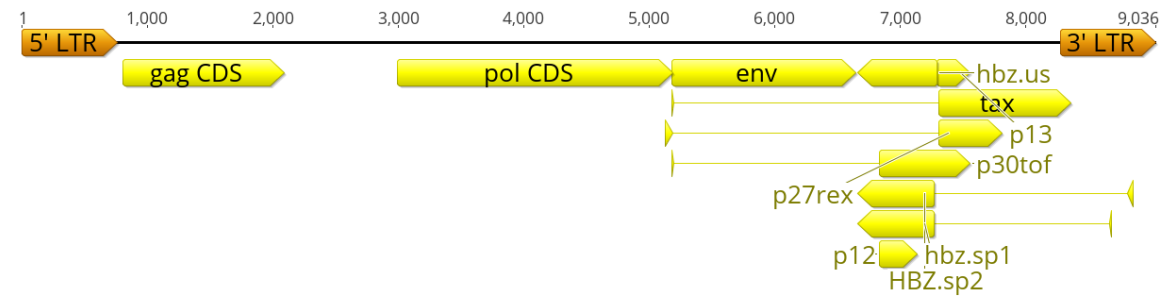
Excluding ‘founder’
variants

1754

Annotate

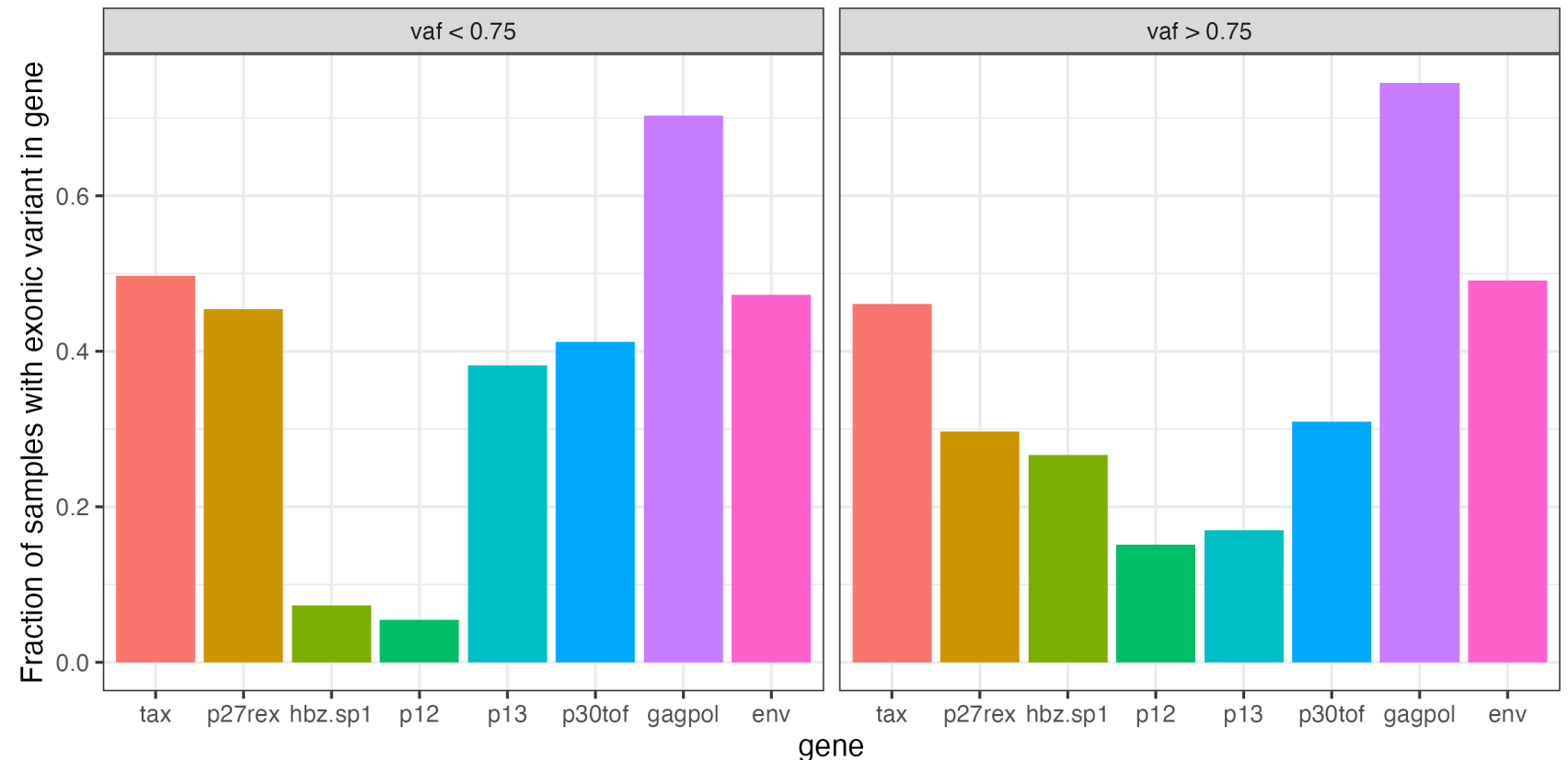
HTLV-1 variant distribution

- up to 78 variants found per sample
- Low-VAF variants not equally distributed along provirus.

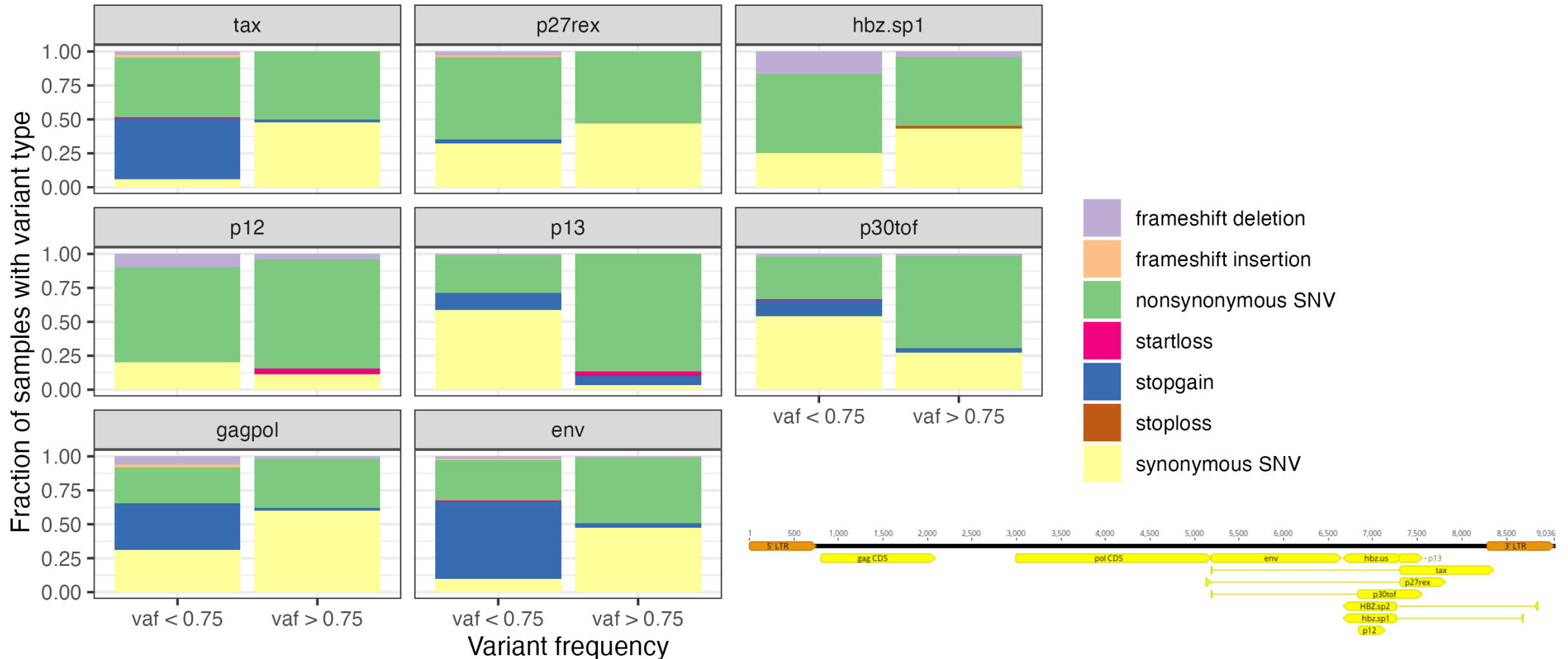


Somatic emergence of mutations in the blood

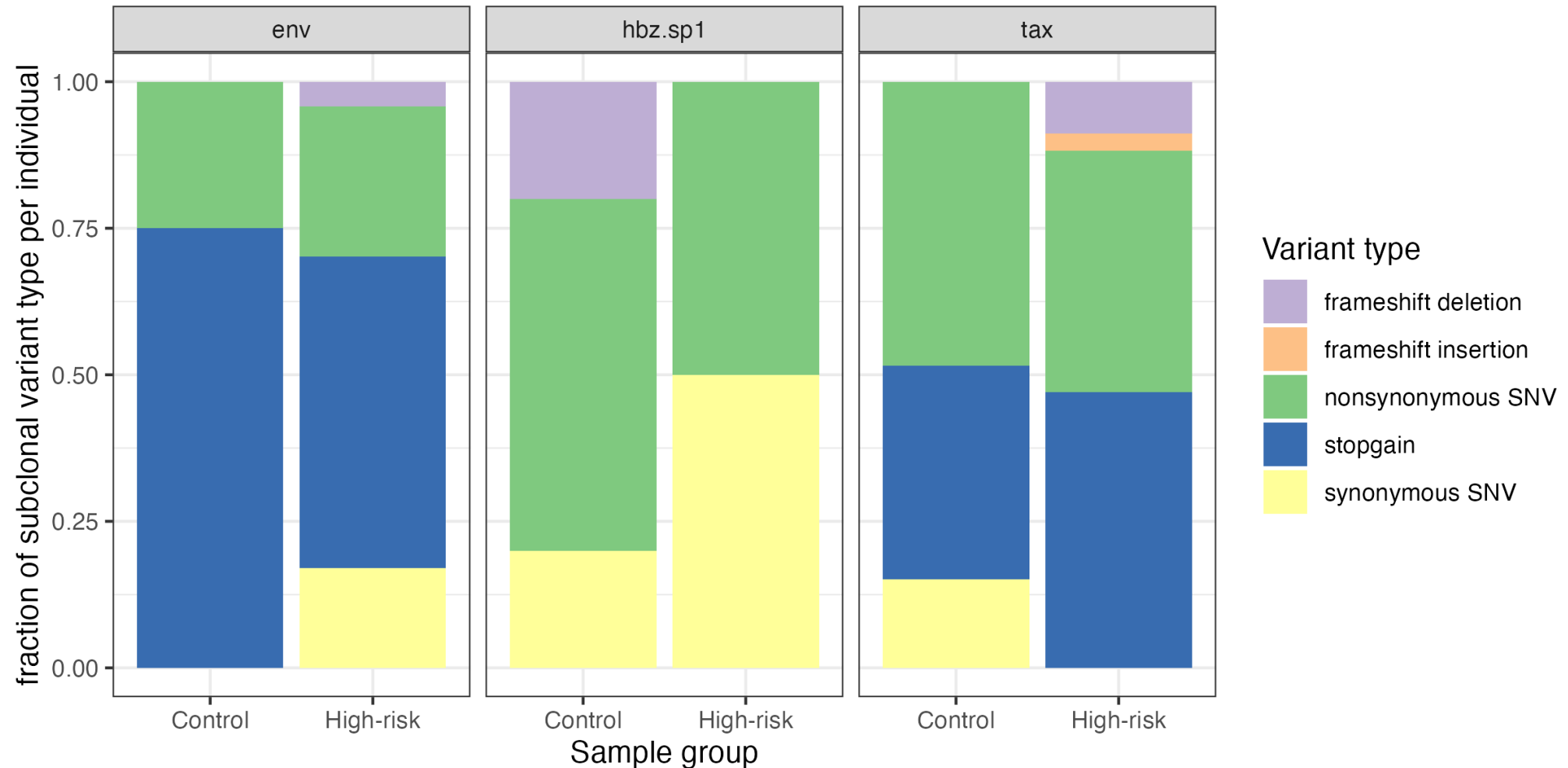
- Approx 50% of samples had a variant in Tax coding region.
- < 10% of emerging variants had a variant in HBZ coding region



Somatic emergence of mutations in the blood



Individuals with an expanded clone are more likely to have nonsense mutations in tax, env



Summary and outlook

- Ultra-deep sequencing of HTLV-1 proviral genomes allows investigation of somatic variants and proviral deletions, including subclonally.

Previous work	This work
Consensus sequence	Emerging variants
PCR (genotype)-dependent	PCR (genotype)-independent
Can be done with Sanger sequencing	Requires deep sequencing tech
Narrow patient focus	Wide patient cohort
Often not genome wide	Genome wide

- Next:
 - further characterise observed variants
 - quantify subclonal deletions
 - Identify variants common to ATL – can these be detected early?

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- Patient donors and staff at the HTLV-1 clinic





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Lunch