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

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

Symposium 5 - Animal models

Chairs: Patrick Green and Zafar Khan

Federal University of Bahia

Salvador - Brazil

DTG use is associated with sustained decrease in HTLV-1 proviral load that persists after 6 months of therapy interruption.

Carlos Brites
School of Medicine
Federal University of Bahia
Salvador, Brazil

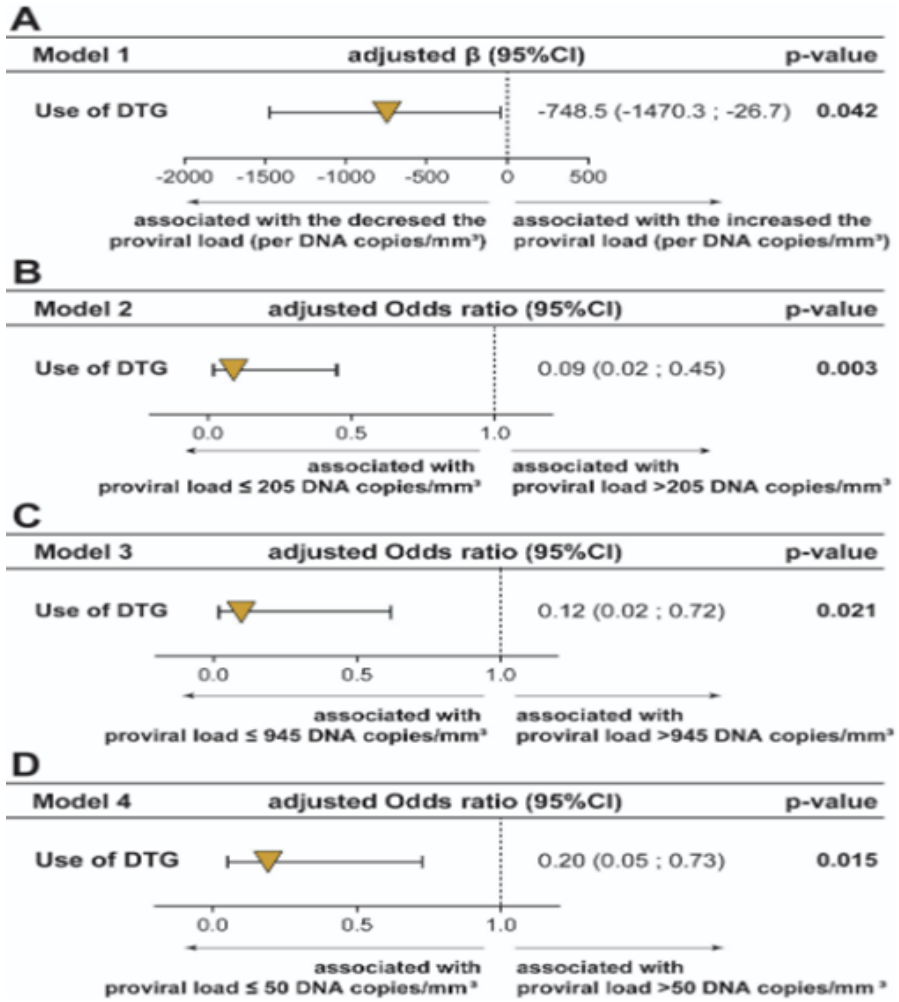
Background & Rationale

- High HTLV-1 proviral load is linked to persistence and disease progression
- No established antiviral therapy consistently reduces PVL
- Integrase inhibitors are active against HTLV infection
- Dolutegravir emerged as a promising candidate

Barski et al Front Microbiol. 2019
Enose-Akahata Y, Ann Clin Transl Neurol. 2021

Background & Rationale

A proof of concept study:



Fernandez T, et al. Comm Med 2025

Use of DTG > 6 months

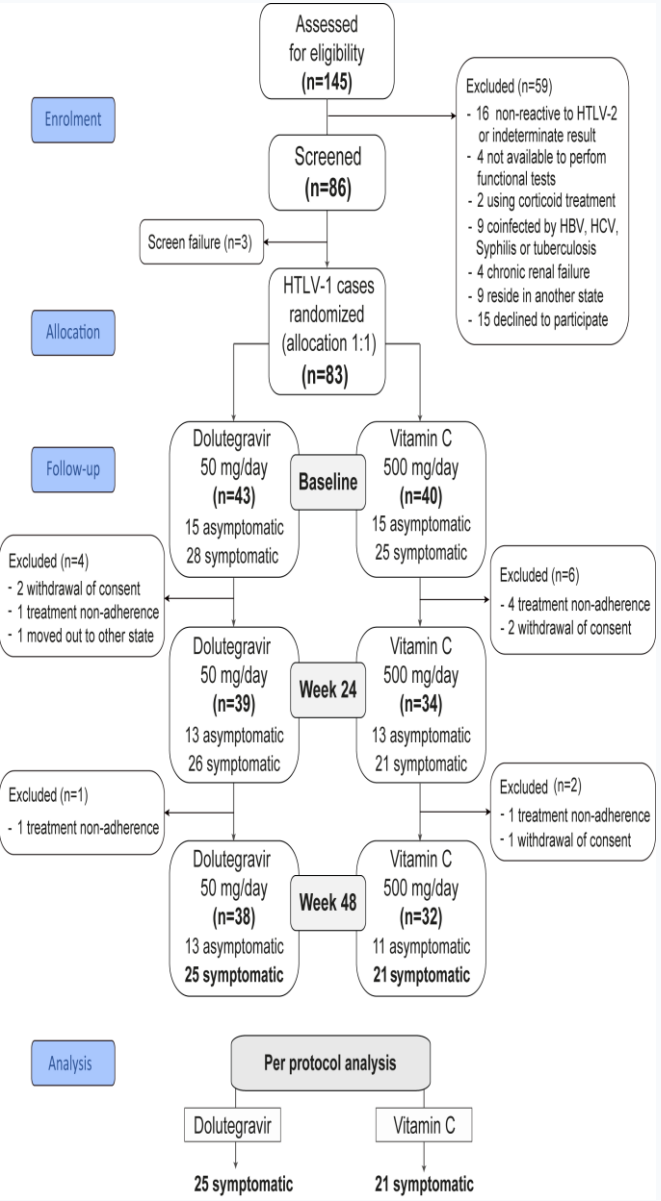
Proportion of patients with HTLV-1 PVL below the median Value

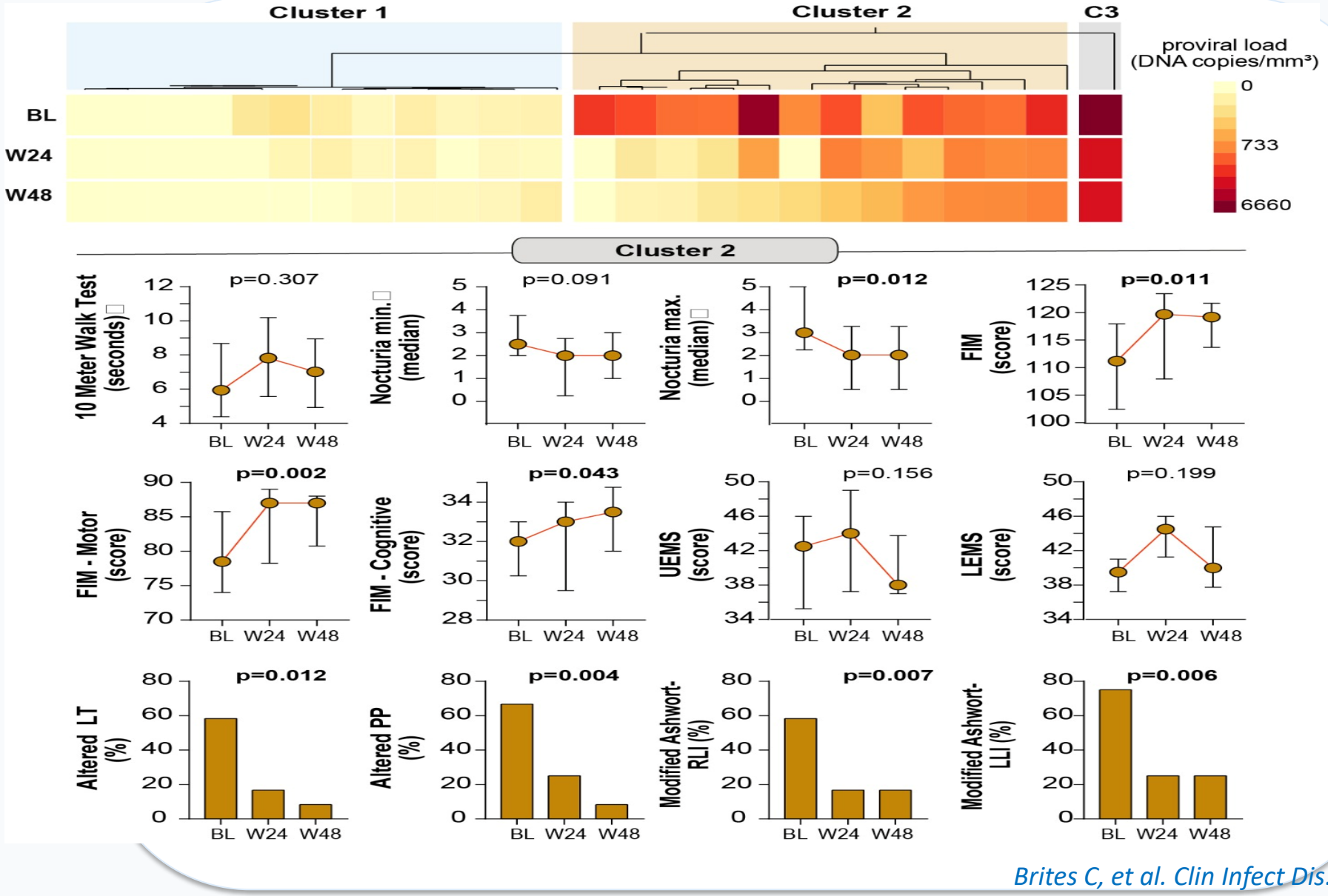
Proportion of patients with PVL< 75 IQR

Detectable HTLV-1 PVL vs. non-detected PVL

Brites C, et al. Clin Infect Dis. 2026

An exploratory trial:





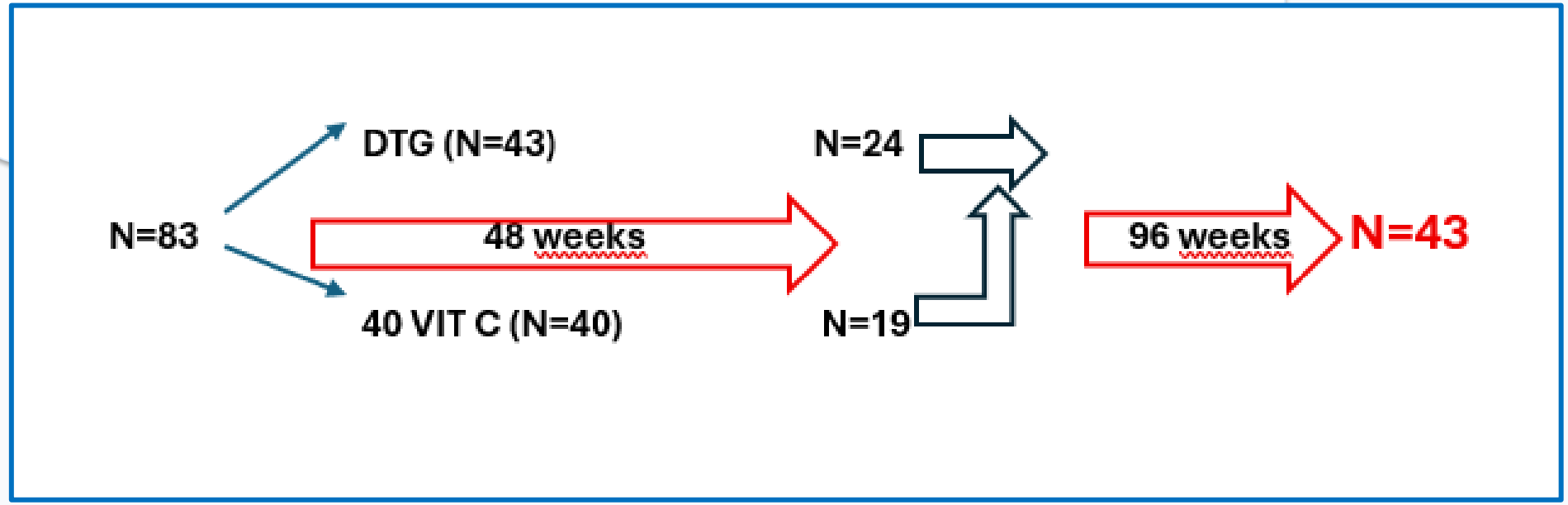
Brites C, et al. Clin Infect Dis. 2026

Study Objectives

- Evaluate the effect of DTG on HTLV-1 proviral load at 96 weeks, and 6 months post-therapy
- Assess viral dynamics after therapy interruption
- Explore response patterns using cluster analysis

Study Design

- Open-label randomized controlled trial
- DTG arm: baseline to week 96
- Vitamin C arm could switch to DTG at week 48



Analytical Approach

- Longitudinal PVL quantification (BL, 24, 48 and 96 weeks. *Post hoc* analysis at 24 weeks post-therapy)
- Delta PVL between visits
- Hierarchical cluster analysis
- Euclidean distance and dendogram analysis

Main Results

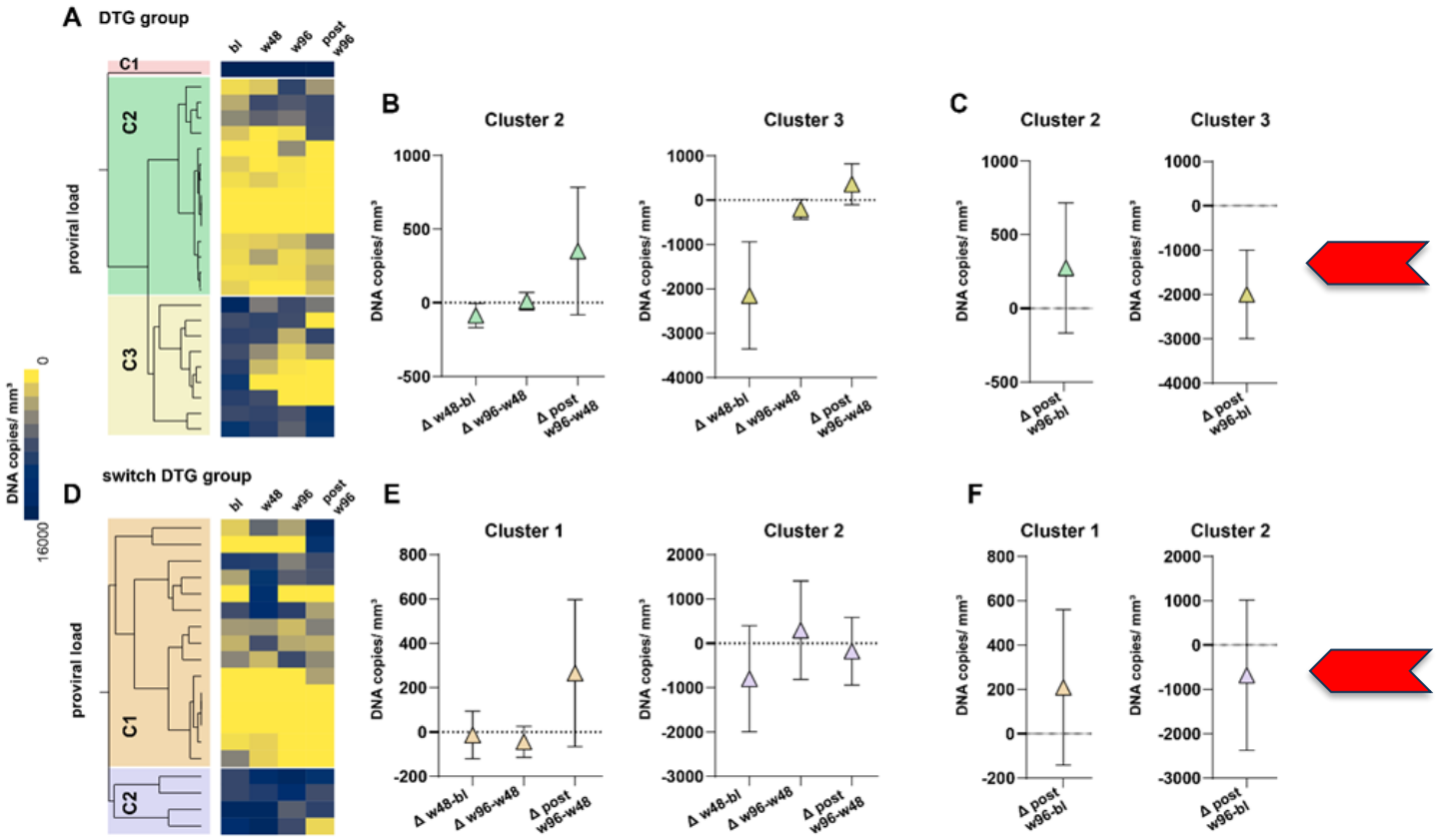
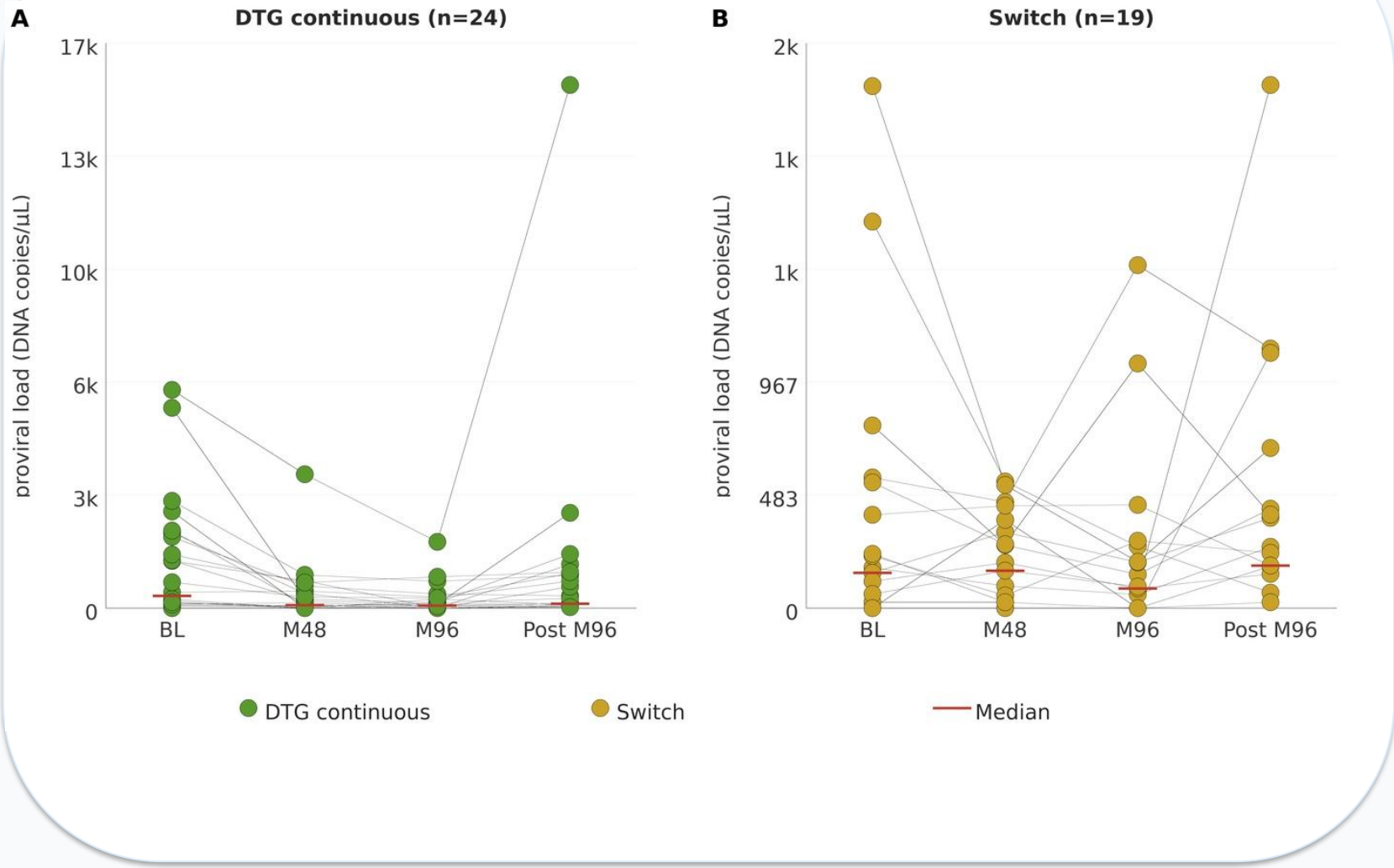


Figure 1. Variation in proviral load values by study visit. Hierarchical cluster analysis employing Ward's unsupervised method between **(A)** Dolutegravir (DTG) and **(D)** switch DTG groups. Panels show delta (Δ) and standard deviation of proviral load (PVL) values in each identified cluster in **(B)** DTG and **(E)** switch DTG groups. Additionally, we calculated the PVL delta of post week 96 and baseline for each study group (**C** and **F**).

Cluster Analysis

- Distinct responder patterns identified
- Largest PVL reductions clustered together
- Part of responders maintained lower PVL post-treatment
- Suggests biological heterogeneity

Viral kinetics



Intragroup comparisons

Comparison (Wilcoxon signed-rank)	T1 median [IQR]	T2 median [IQR]	Statistic	p-value	
DTG continuous (n=24)					
CPV 1 → M48	375 [102–2208]	92 [0–465]	W=9	<0.001	
M48 → M96	92 [0–465]	76 [0–284]	W=42	0.060	
M96 → Post M96	76 [0–284]	134 [49–1019]	W=34	<0.001	
CPV 1 → Post M96	375 [102–2208]	134 [49–1019]	W=109	0.241	
Switch (n=19)					
CPV 1 → M48	151 [0–468]	160 [12–351]	W=25	0.168	
M48 → M96	160 [12–351]	84 [0–231]	W=37	0.330	
M96 → Post M96	84 [0–231]	182 [25–412]	W=63	0.196	
CPV 1 → Post M96	151 [0–468]	182 [25–412]	W=75	0.647	

Main Results

- Progressive PVL reduction during DTG exposure
- Greater reductions with longer therapy
- Persistent effect after treatment interruption in some cases (1 case remains with non-detectable PVL after 3 Years off-therapy
- These findings suggest durable biological effect

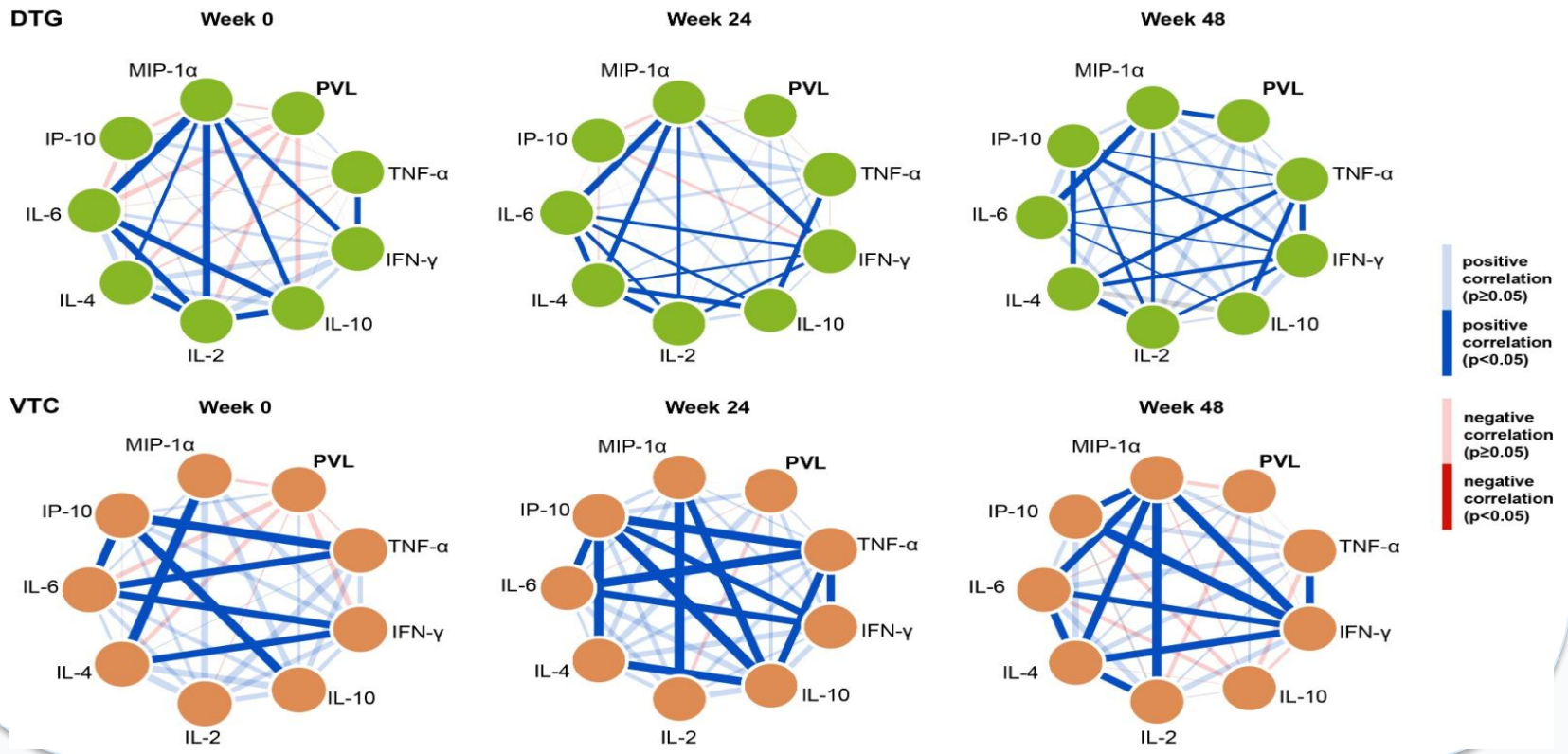
Biological Interpretation of DTG effects

- Possible modulation of infected cell expansion
- Potential effect on clonal proliferation
- May alter HTLV persistence dynamics
- Supports antiviral activity beyond transient suppression

Sustained decrease in PVL may modulate the immune response, favouring a better immune control of HTLV infection

Biological Interpretation

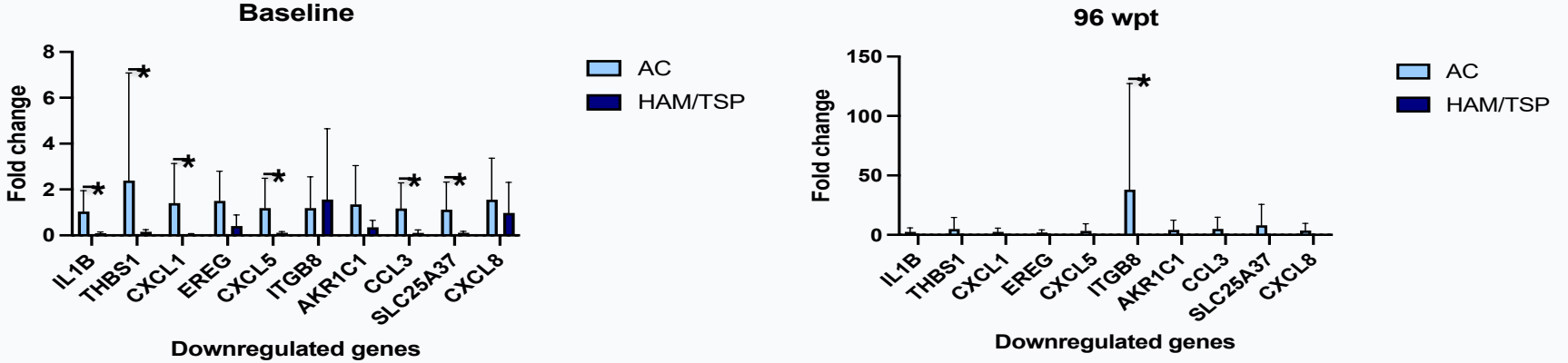
Correlations between cytokine levels during the study, by group



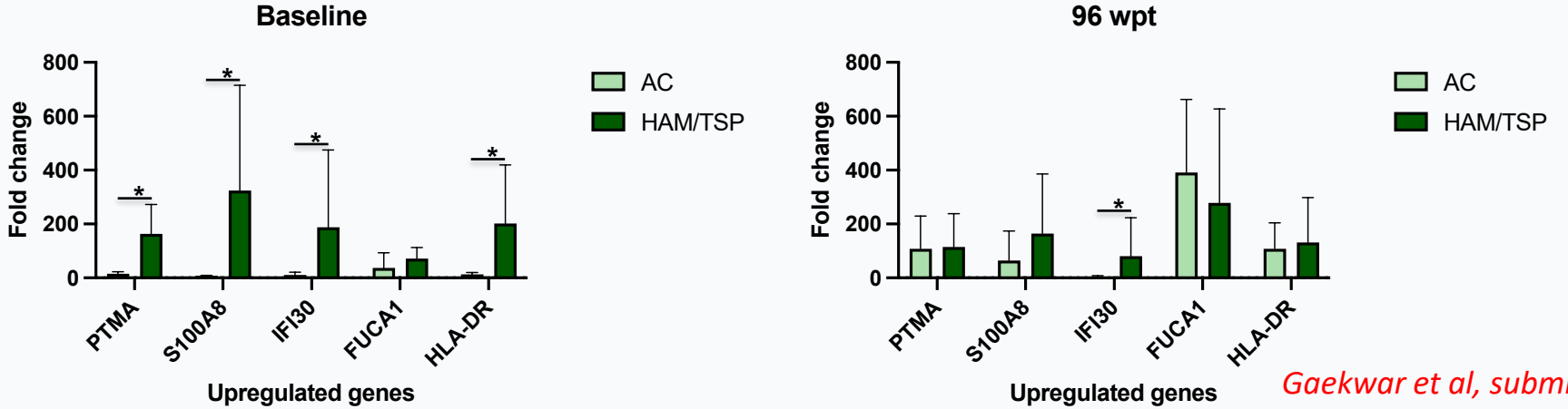
Brites et al, CID 2026

Transcriptional results of single cell RNA sequencing

- Most chemokines and inflammation-modulating genes are suppressed in HAM/TSP at baseline, but rebound following 96 wpt with DTG



- Immune activation signatures observed in HAM/TSP patients at baseline are attenuated after 96 wpt with DTG



Gaekwar et al, submitted

Biological Interpretation

- Our transcriptional data reinforce the immune modulation effects, wherein monocytes from HAM/TSP patients show upregulation of genes involved in antigen presentation and inflammatory activation, indicating they are not passive bystanders but immunologically activated in disease
- After DTG treatment, we observed broad transcriptional reprogramming in PBMCs, including downregulation of pro-inflammatory pathways, and restoration of a more regulated immune profile
- This suggests that DTG may not only block viral replication but also reprogram the host immune environment, relieving chronic immune activation and potentially improving clinical outcomes and viral replication control

Gaekwar et al, submitted

Conclusions

- DTG use was associated with sustained PVL reduction
- In some cases, the effect persisted at 6 months after interruption
- Supports further HTLV therapeutic trials
- Potential new approach for HTLV antiviral therapy

Acknowledgments

LAPI - Federal University of Bahia

Tatiana Fernandez
Iris Montaño-Castellon
Cleyde Marconi
Maria B. Arriaga
Eduardo M. Netto
Rafaela Mayoral
Estela Luz
Felice Deminco
Carlos Brites

Herrera Lab
- Bobby Brooke Herrera, PhD
- **Prince Baffour Tonto, PhD**
- **Reem Alatrash**
- Sainetra Sridhar
- Sebastian Gallon
- Lily Lumkong
- Varun Vaidya
- Josh Ding
- Sanjana Iyer

CHINJ
- Arnold Rabson, MD
- Cindy Lin, PhD
- Stephanie Peeters
- Kathey Semler
- Guillermo Cardona

Rutgers RWJMS
- Keith Kaye, MD

Rutgers Global Health Institute
- Ric Marlink, MD

Drexel University, College of Medicine
- **Pooja Jain, PhD**
- **Salwa Ahmed**

Rutgers New Jersey Medical School
- **Alexander Lemenze, PhD**

MINISTÉRIO DA SAÚDE

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

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

Efficient induction of anti-HTLV-1 NAbs by immunization with LNP-mRNA expressing a membrane-anchored Env SU trimer

Hiroshi Ishii[1], Midori Nakamura-Hoshi[1], Rena Sakamoto[1], Akira Ainai[1], Yasuhiro Hayashi[1,2], Theodore Asigbee Worlanyo[1], Tetsuro Matano[1,2,3]

[1] National Institute of Infectious Diseases, Japan Institute for Health Security, Tokyo, Japan
[2] Institute of Medical Science, University of Tokyo, Tokyo, Japan
[3] Joint Research Center for Human Retrovirus, Kumamoto University, Kumamoto, Japan

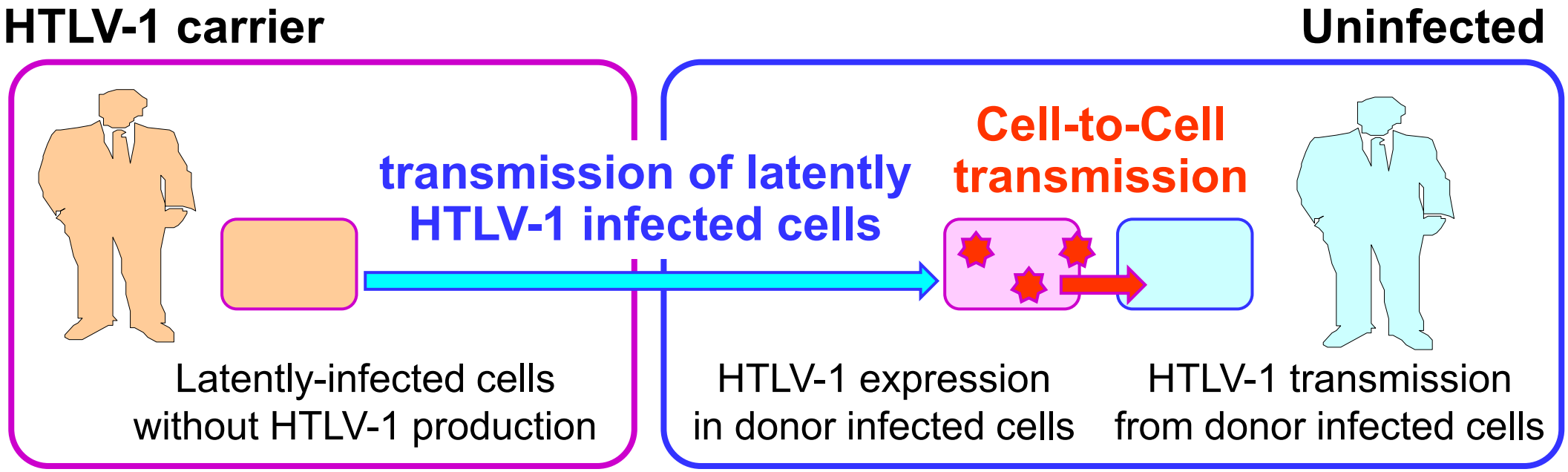
Conflicts of interest

I have no conflicts of interest

HTLV-1 transmission

- In asymptomatic infected individuals (HTLV-1 carriers), almost no HTLV-1 expression or production is detectable (**latent infection**).
- In HTLV-1 transmission, it is believed that latently infected cells transmitted from HTLV-1 carriers to uninfected begin to express/produce HTLV-1 after transmission, resulting in **cell-to-cell** HTLV-1 transmission to recipient cells.

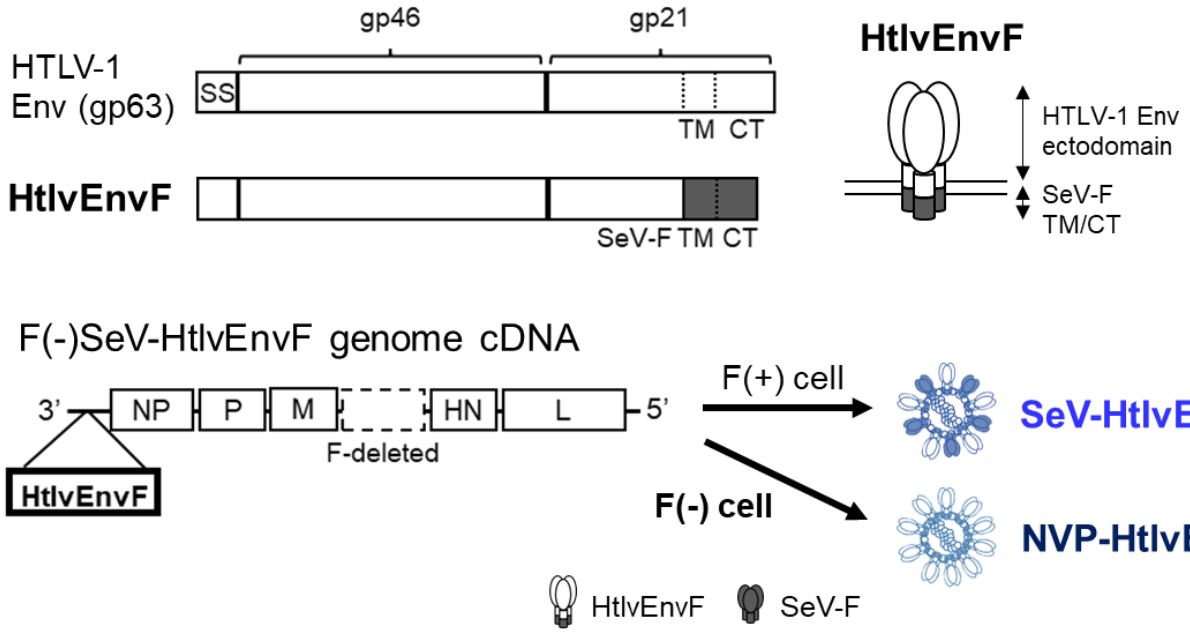
➢ **It is a great challenge to know whether vaccine-induced antibodies can have a protective efficacy against cell-to-cell HTLV-1 transmission.**



A vaccine inducing anti-HTLV-1 Env antibodies

Sendai virus expressing HtlvEnvF (provided by ID Pharma)

- We have developed a chimeric antigen **HtlvEnvF** consisting of the HTLV-1 gp63 ectodomain and the SeV F transmembrane-cytoplasmic domain.
- ➢ SeV-HtlvEnvF vaccination induced detectable anti-HTLV-1 NAb responses in 8 out of 10 cynomolgus macaques (except for VA2, VA5).

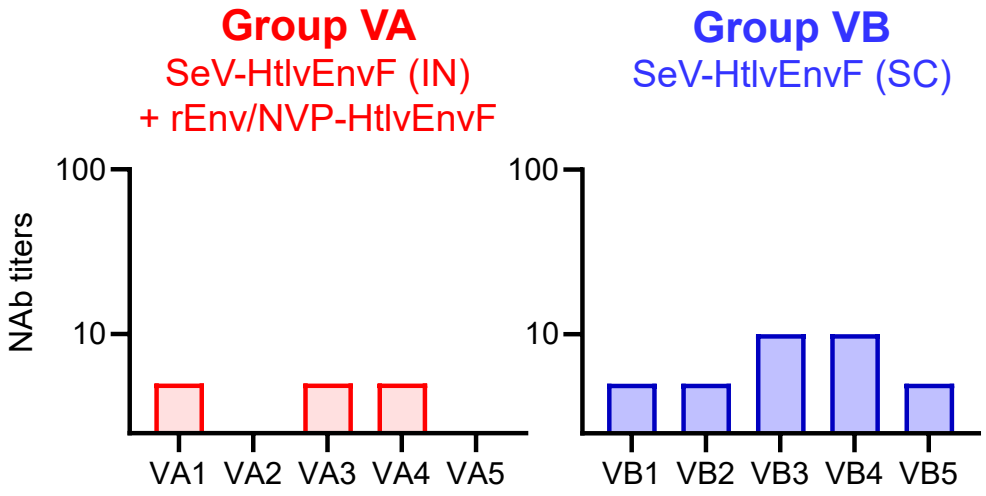


(Ishii H, Nakamura-Hoshi M, et al. Vaccine, 2022)

Anti-HTLV-1 NAb titers

(Syncytium Inhibition Assay)

4 wks after the last vaccination

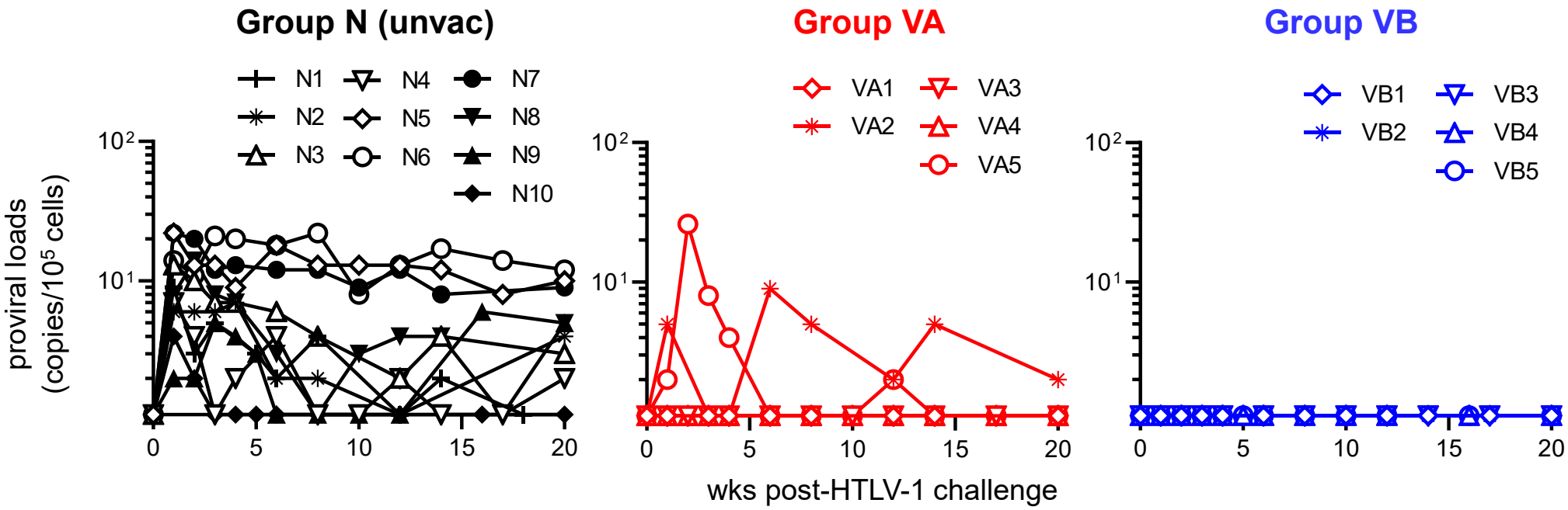


(Nakamura-Hoshi M, et al. Mol Ther, 2024)

Protection from HTLV-1 Challenge in NAb+ animals

- SeV-HtlvEnvF vaccinated (n = 10) and unvaccinated (n = 10) macaques were intravenously challenged with 10^8 of HTLV-1-producing cells (ATL040).
- The eight macaques with detectable NAbs following vaccination were protected (no detectable provirus), whereas the remaining two vaccinees without detectable NAbs (VA2, VA5) were infected with HTLV-1.

These results suggest involvement of vaccine-induced NAbs in protection from HTLV-1 challenge.



(Nakamura-Hoshi M, et al. Mol Ther, 2024)

- In our previous macaque study, SeV-HtlvEnvF vaccinated macaques with NAb induction showed no detectable HTLV-1 provirus after challenge with HTLV-1-producing cells.

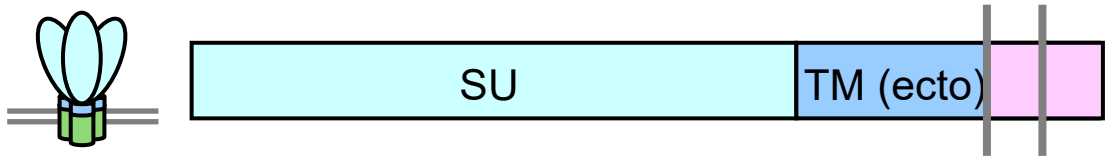
- ✓ These results indicate that a vaccine inducing NAbs can confer sterile protection against HTLV-1 transmission.

- ✓ **This study provides a rationale for strategies aimed at anti-Env antibody induction in prophylactic vaccine development.**

- **It is necessary to improve immunogenicity of vaccines (potency and duration of NAb responses) for clinical application.**
- **It is known that expression of full-length native HTLV-1 Env is inefficient.**
- **Now, we have decided to use LNP-mRNA modality and are trying to optimize HTLV-1 Env immunogen for efficient NAb induction.**

HTLV-1 Env immunogen design for LNP-mRNA

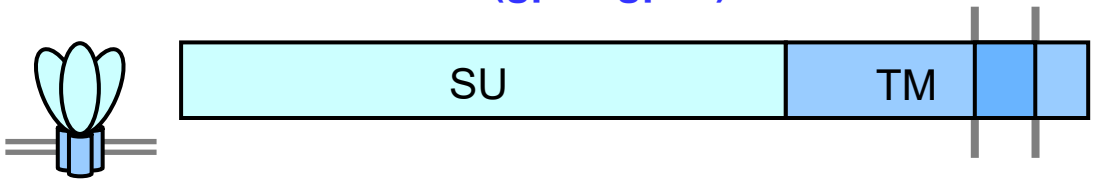
EnvF: Env ecto/SeV-F TM



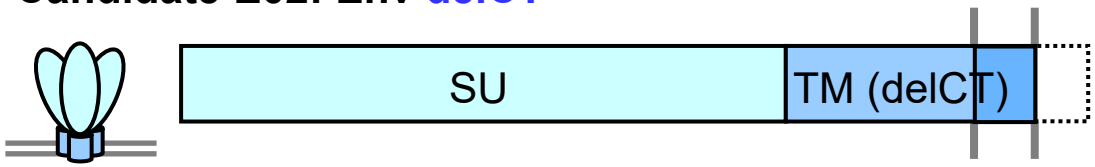
TM and CT
derived from SeV F

- **Optimization of Env antigen for efficient NAb induction by LNP-mRNA (collaborating with Moderna).**

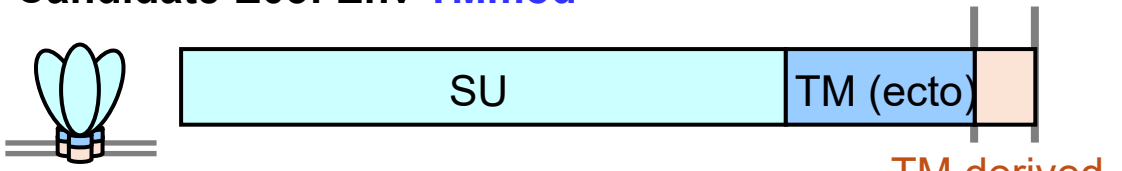
Candidate E01: Env full (gp46/gp21)



Candidate E02: Env delCT

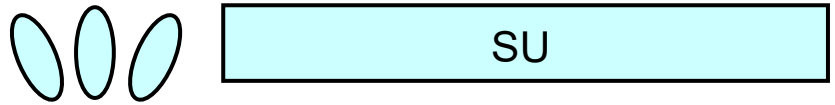


Candidate E03: Env TMmod

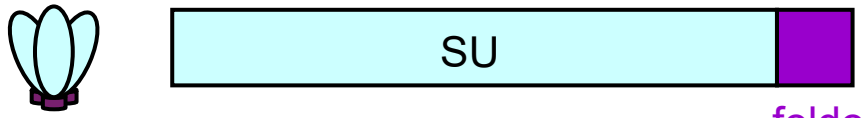


TM derived
from Flu HA

Candidate E04: Env SU (gp46)

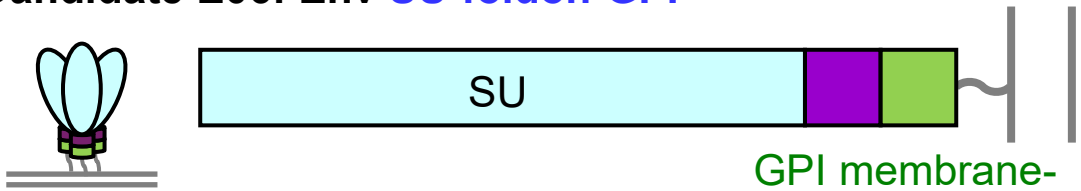


Candidate E05: Env SU-foldon



foldon trimerizing
sequence

Candidate E06: Env SU-foldon-GPI



GPI membrane-
anchoring sequence

HTLV-1 Env expression from mRNA *in vitro*

- **SU**, **SU-foldon**, and **SU-foldon-GPI** expression by mRNA was efficient in 293T cells, while full-length Env expression was poor..

mRNA-Env
(0.5 μg)

↓ transfection
(Lipo Messenger MAX)

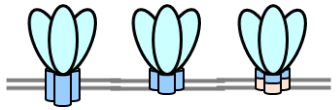
293T cell
(1 x 10^5 cells)

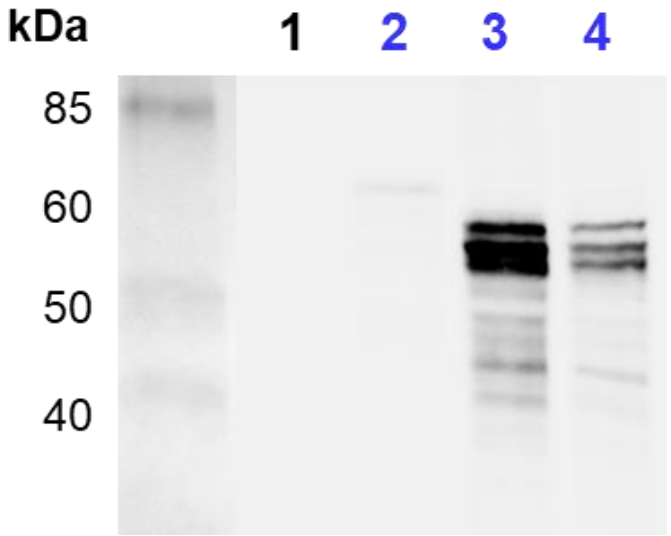
⇩ 48 hrs

WB (cell lysate)
1st: anti-gp46 mouse IgG
2nd: anti-mouse IgG-HRP

Loading: **15 μl**/well
Long exposure

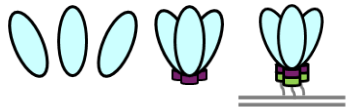
1. mock
2. Env full
3. Env delCT
4. Env TMmod

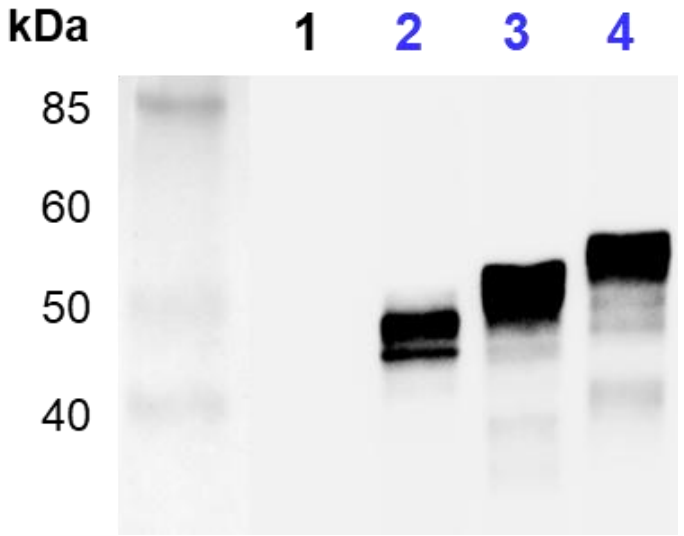




Loading: **5 μl**/well
Short exposure

1. mock
2. Env SU
3. Env SU-foldon
4. Env SU-foldon-GPI





HTLV-1 Env expression from mRNA *in vitro*

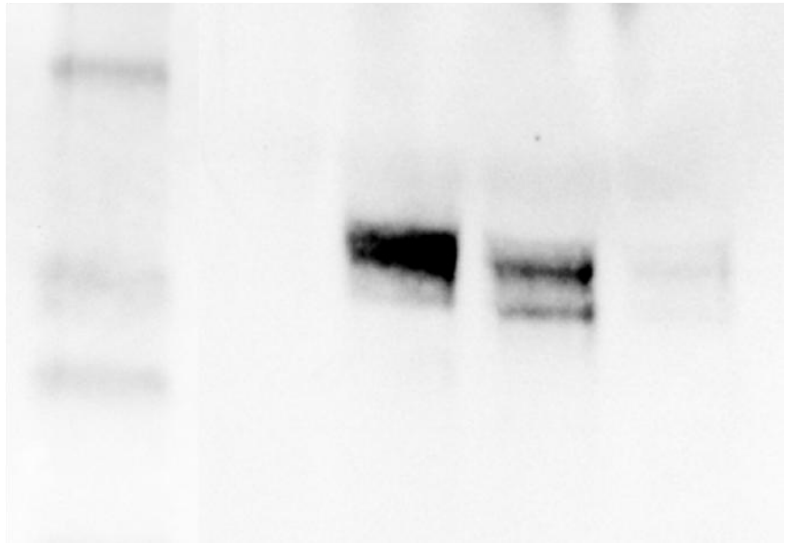
➢ **SU-foldon-GPI** expressed by mRNA was anchored on the cell surface.

Supernatant
(concentrated by Amicon Ultra-30K)

1. mock
2. Env SU
3. Env SU-foldon
4. Env SU-foldon-GPI

1 2 3 4

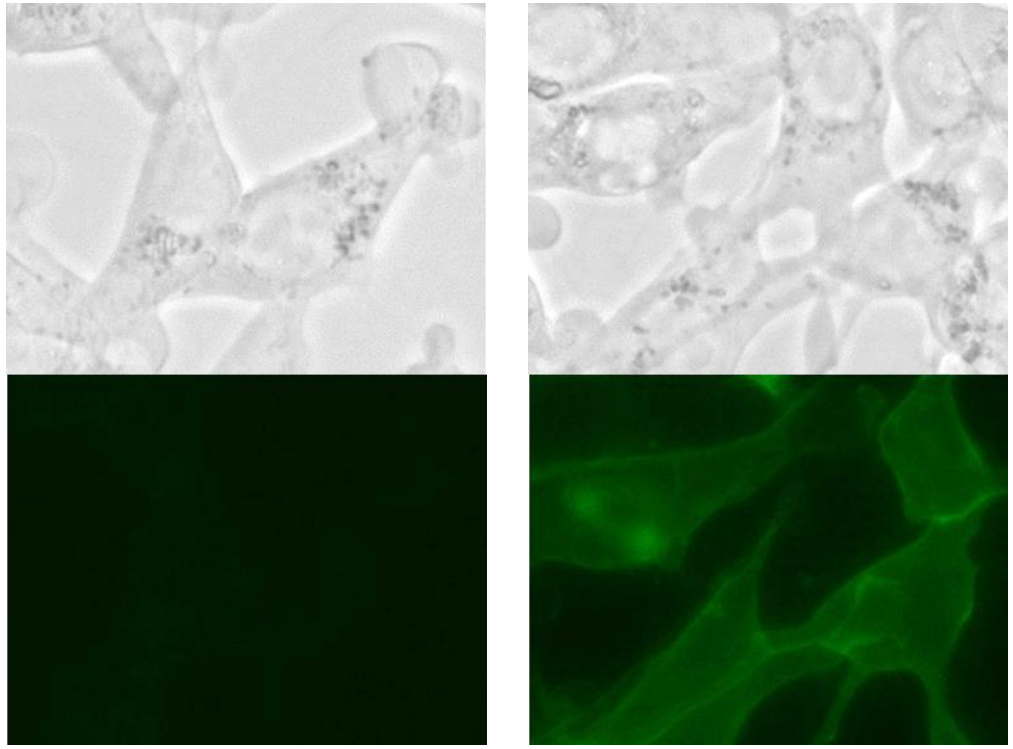
kDa
85
60
50
40



Immunostaining (without detergent)

SU-foldon

SU-foldon-GPI

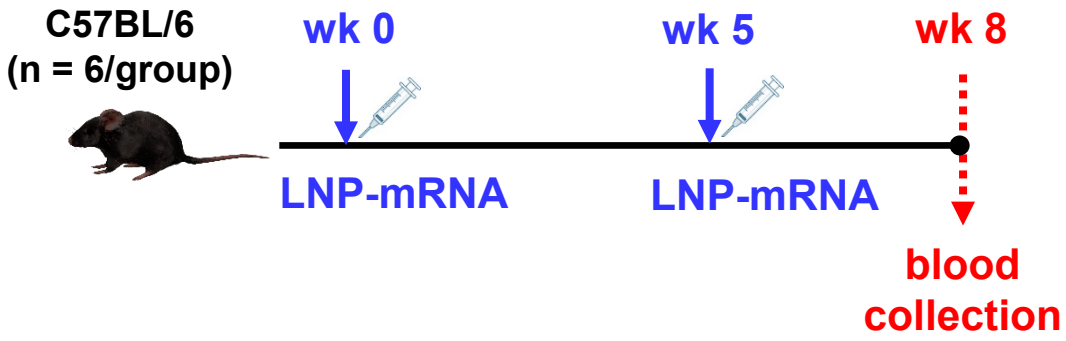


bright

anti-gp46
FITC

Mouse experiment to investigate LNP-mRNA-Env immunogenicity

➢ Antibody responses induced by LNP-mRNA-Env were analyzed in B6 mice.



Assay

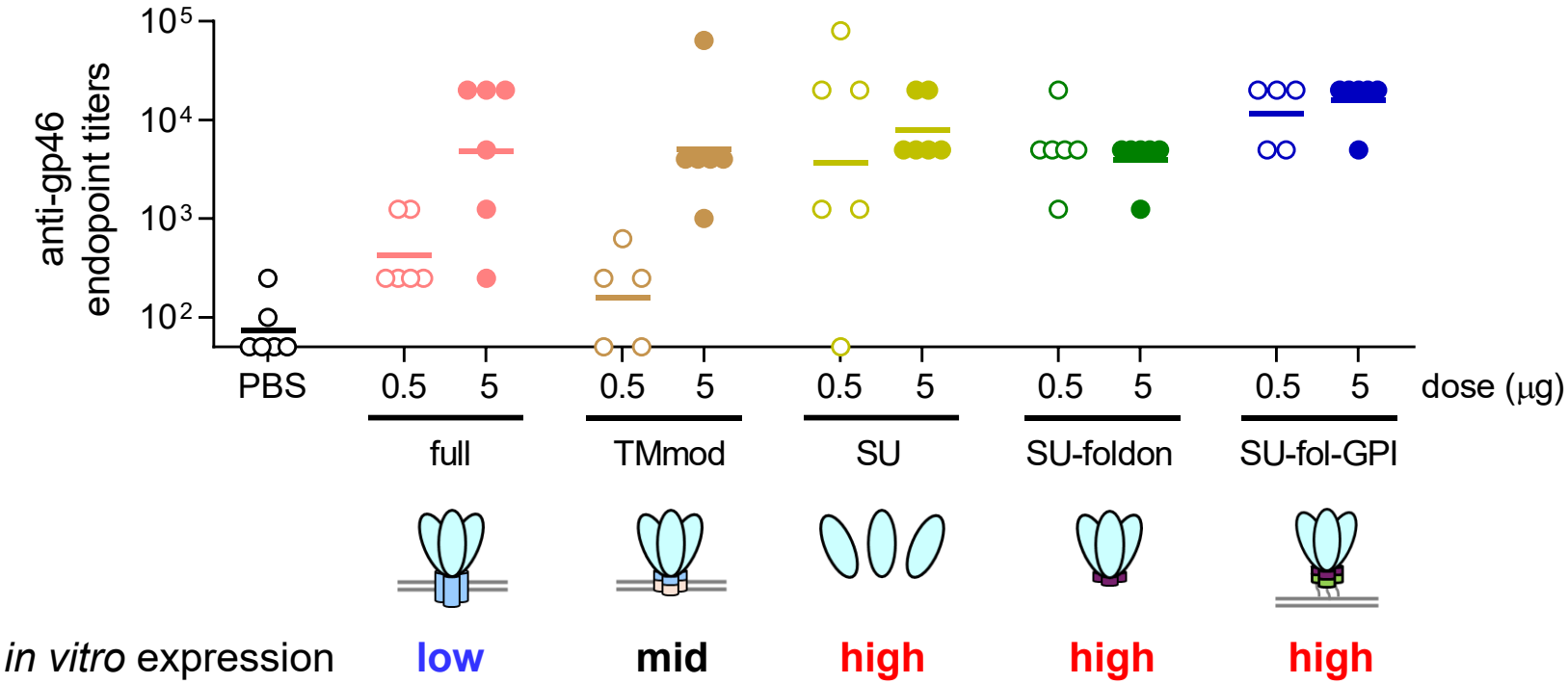
- **Binding antibody**
  IgG ELISA (recombinant gp46)
- **Neutralization activity**
  syncytium inhibition (ATL040/Jurkat)

group	antigen	dose
1	-	-
2	Env-full	IM 0.5 μg
3	Env-full	IM 5 μg
4	TMmod	IM 0.5 μg
5	TMmod	IM 5 μg
6	Env-SU	IM 0.5 μg
7	Env-SU	IM 5 μg
8	SU-foldon	IM 0.5 μg
9	SU-foldon	IM 5 μg
10	SU-foldon-GPI	IM 0.5 μg
11	SU-foldon-GPI	IM 5 μg

Mouse experiment to investigate LNP-mRNA-Env immunogenicity

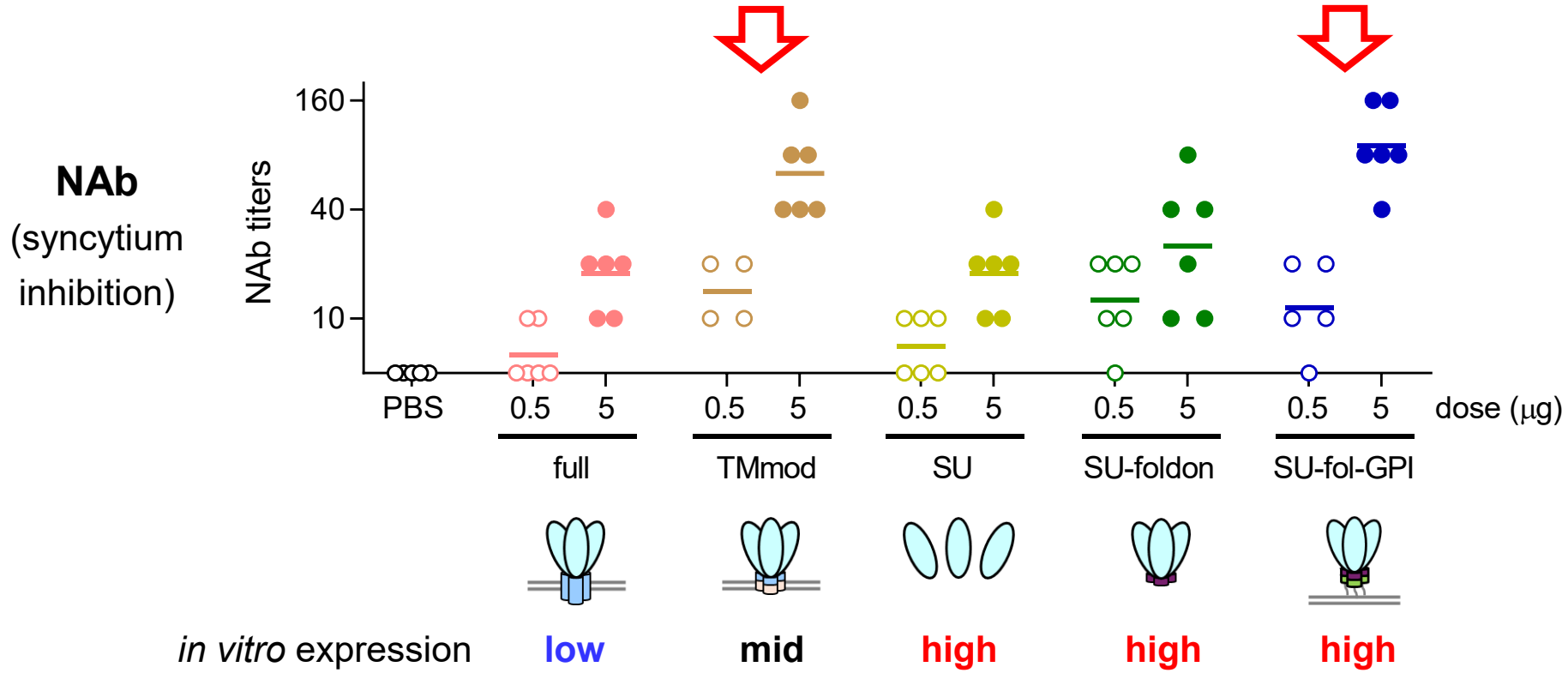
- Anti-gp46 binding antibodies were efficiently induced in mice immunized with LNP-mRNA expressing Env with high *in vitro* expression (SU, SU-foldon, and SU-foldon-GPI).

ELISA
(gp46 IgG)



Mouse experiment to investigate LNP-mRNA-Env immunogenicity

- Anti-HTLV-1 NAb titers were higher in mice immunized with LNP-mRNA expressing membrane-anchored modified Env (TMmod and SU-foldon-GPI).



Summary

- Native full-length HTLV-1 Env expression by mRNA is poor, whereas expression of SU-based immunogens is efficient.
- Mice immunized with LNP-mRNA efficiently expressing Env antigens *in vitro* induced higher levels of anti-gp46 binding antibodies.
- Immunization with LNP-mRNA expressing **TM-modified Env** and **SU-foldon-GPI** efficiently induces anti-HTLV-1 NAb responses in mice.

✓ **LNP-mRNA expressing membrane-anchored Env could be a promising candidate for efficient anti-HTLV-1 NAb induction.**

Collaborators

National Institute of Infectious Diseases (NIID), Japan Institute for Health Security (JIHS)

Department of Latent Infection

Midori Nakamura-Hoshi
Theodore Asigbee Worlanyo
Yasuhiro Hayashi
Nozomi Kuse
Midori Okazaki
Hiroyuki Yamamoto
Tetsuro Matano

Department of Pathology

Rena Sakamoto
Akira Ainai

Department of Bioinformatics and Integrative Omics

Yasuyoshi Hatayama
Akihide Ryo

Research Center for Biosafety, Laboratory Animal and Pathogen Bank

Kenzo Yonemitsu
Yasushi Ami

NIID NATIONAL INSTITUTE OF INFECTIOUS DISEASES
JIHS Japan Institute for Health Security

NHMRC-AMED ASPIRE project ~ Control of endemic HTLV-1 to prevent silent global spread ~

NIID, JIHS

Tetsuro Matano

Hokkaido University

Yorifumi Satou

University of Tokyo

Makoto Yamagishi

University of Melbourne

Damian Purcell
Natasha Jansz
Lloyd Einsiedel

Walter and Eliza Hall Institute

Marcel Doerflinger

University of New South Wales

Chantelle Ahlenstiel
Anthony Kelleher
David Jacques
Pall Thordarson

Queensland Uni of Technology

Kevin Morris

National Institutes of Health

Genoveffa Franchini

ASPIRE HTLV-1 RESEARCH AUSTRALIA-JAPAN

Thomas Jefferson University | Drexel University | Drexel Medicine

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

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

Combination antiretroviral therapy and MCL-1 inhibition mitigate HTLV-1 infection *in vivo*

Marcel Doerflinger,

Walter and Eliza Hall Institute for Medical Research (WEHI)

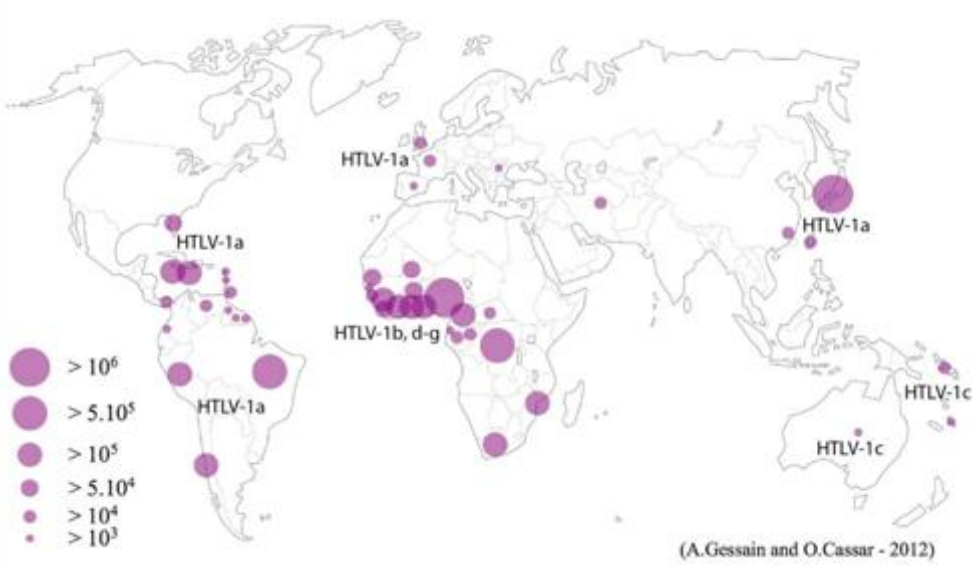
Melbourne, Australia

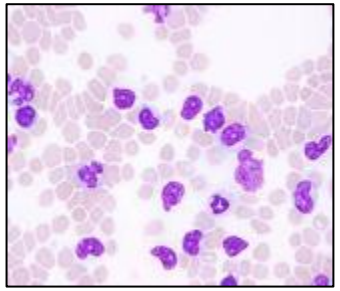
doerflinger.m@wehi.edu.au

Conflicts of interest

The Walter and Eliza Hall Institute receives milestone and royalty payments related to venetoclax.

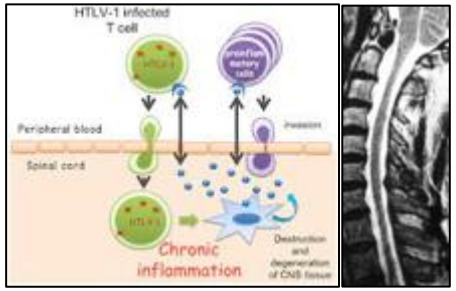
HTLV-1 is strictly human tropic





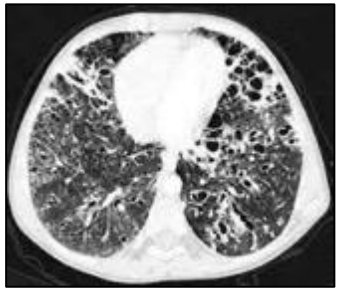
ATLL

aggressive adult T-cell leukaemia/lymphoma



HAM/TSP

A chronic inflammatory disease of the central nervous system, progressive spastic weakness of the lower limbs

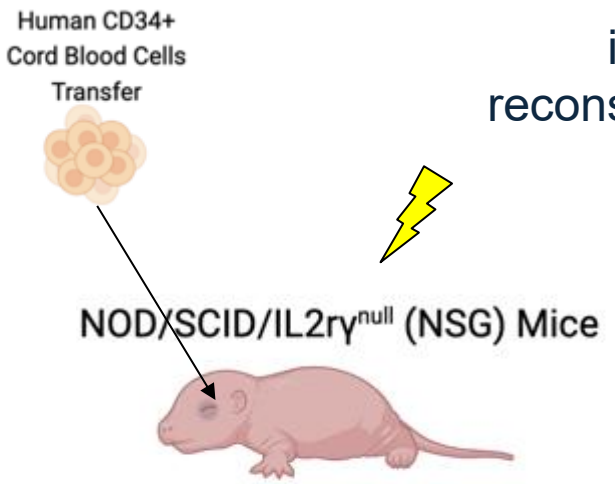


Bronchiectasis

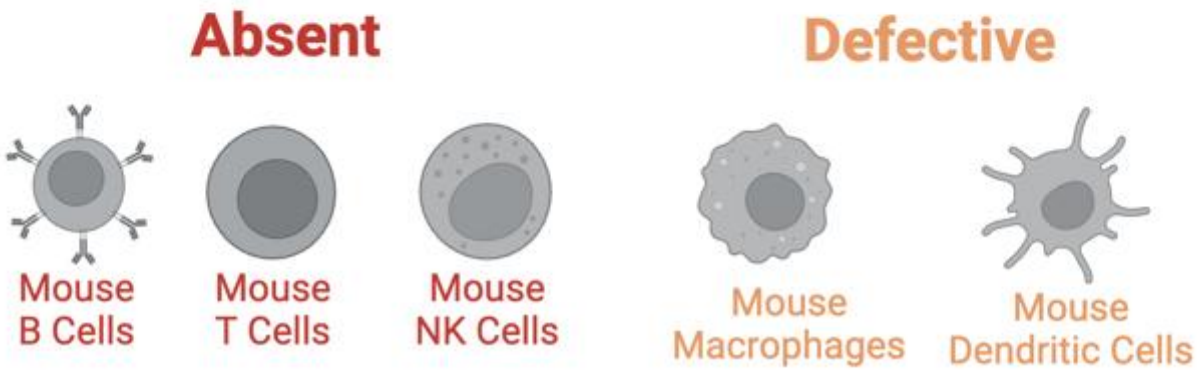
permanent airway damage and inflammation

human tropic

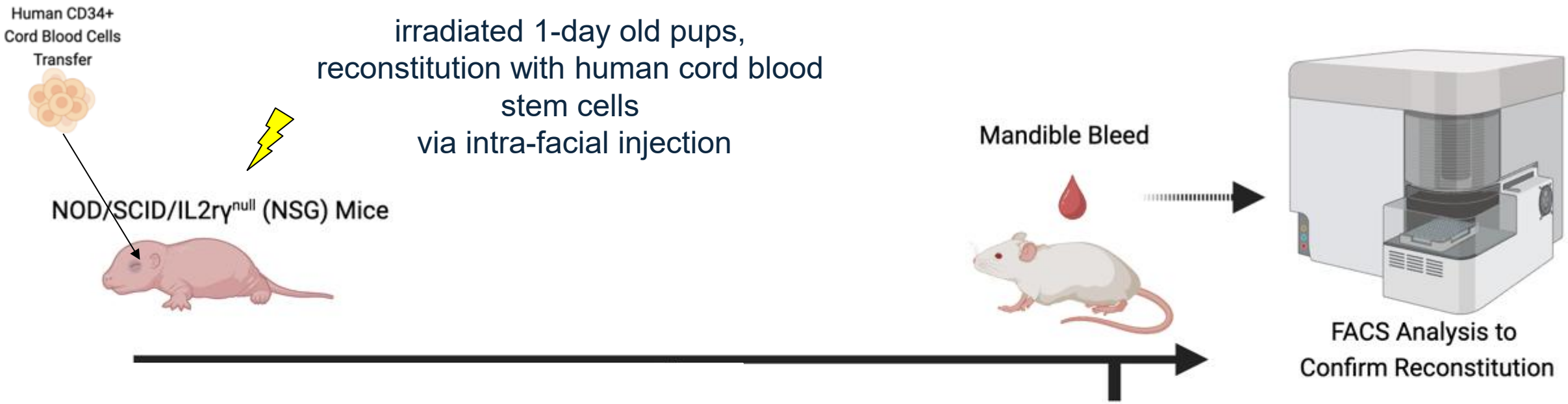
Human Immune System mice

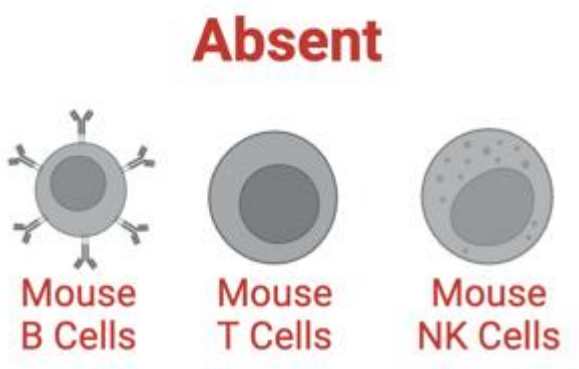


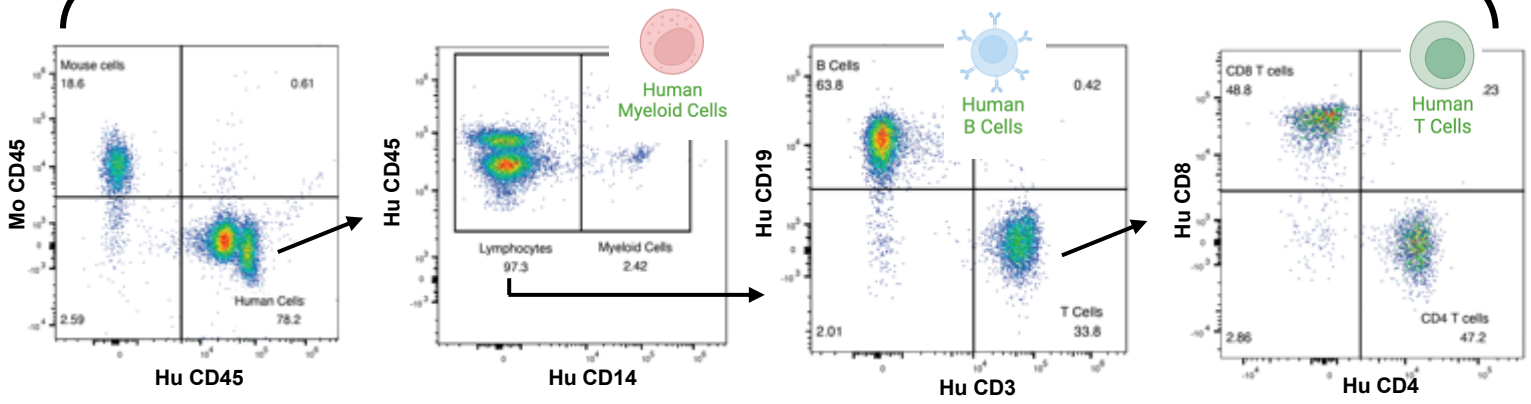
irradiated 1-day old pups,
reconstitution with human cord blood stem cells
via intra-facial injection



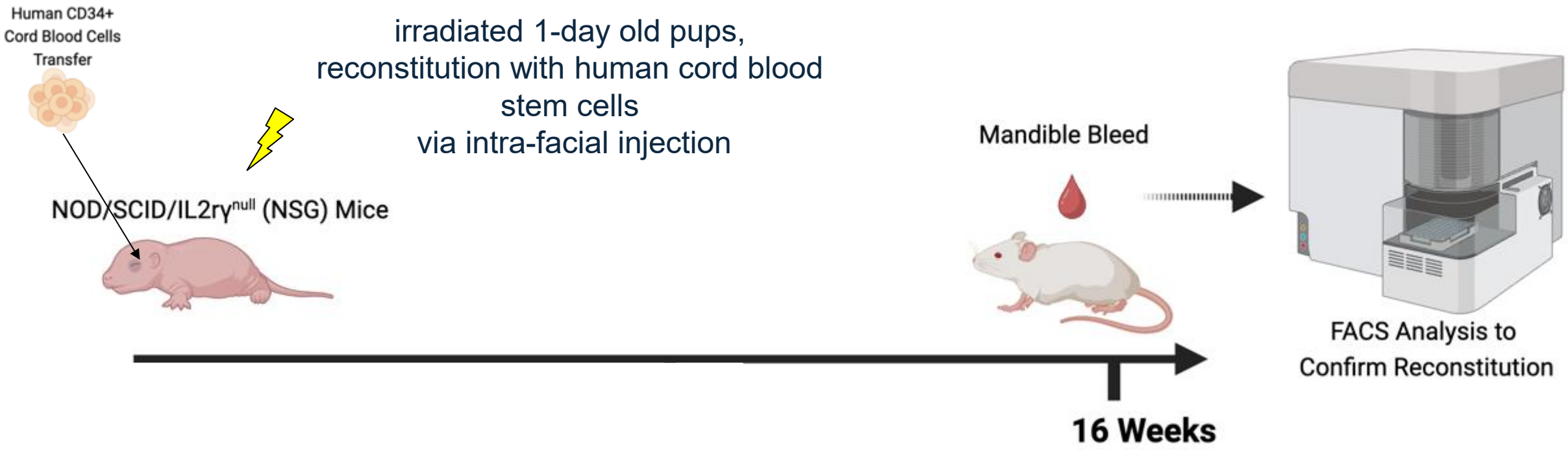
Human Immune System mice



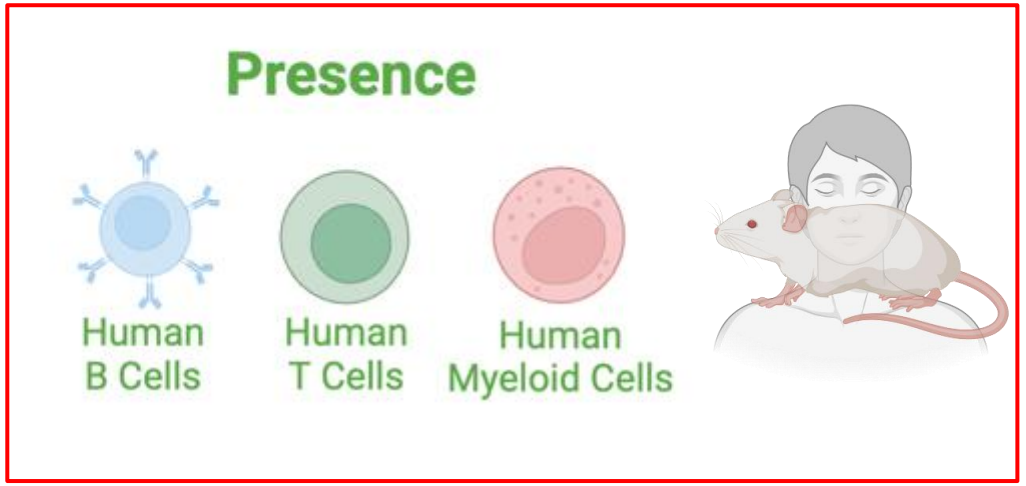




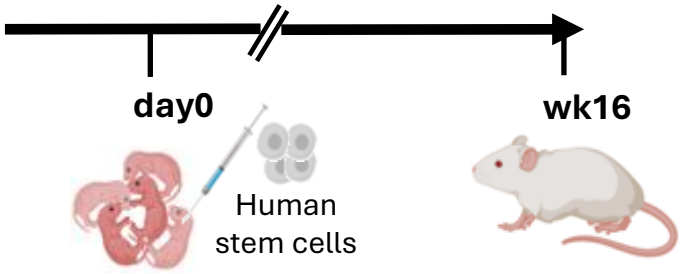
Human Immune System mice



→ Allows infection with strictly human tropic pathogens



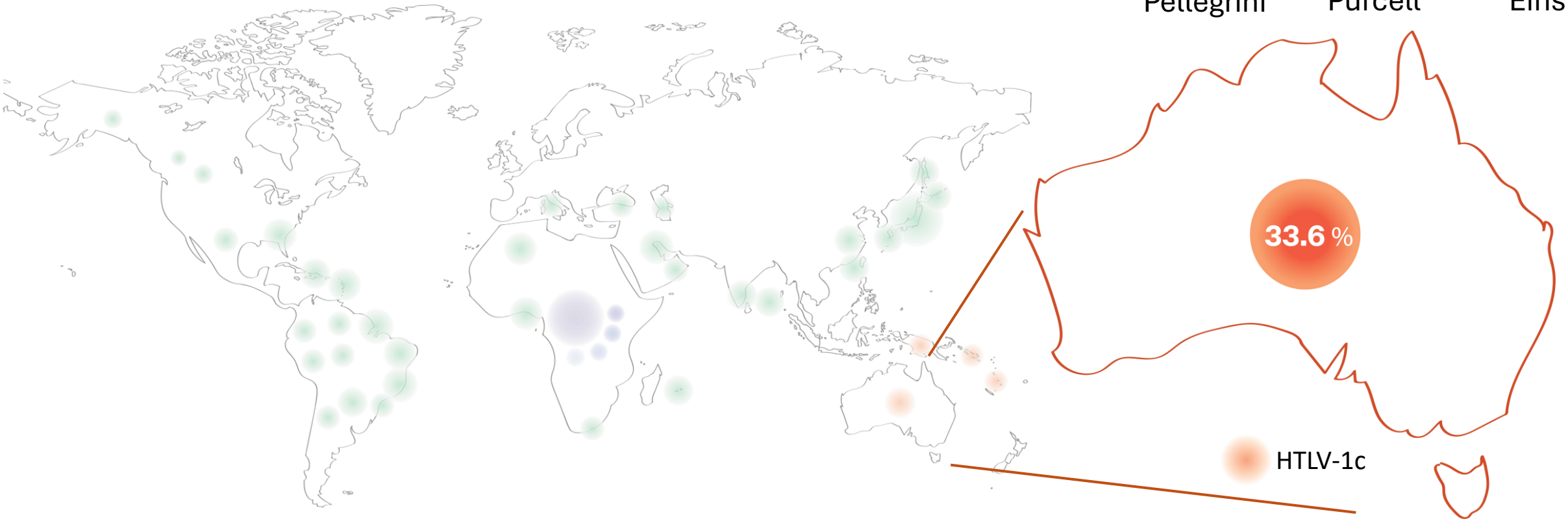
Establishing unique HTLV-1 *in vivo* models



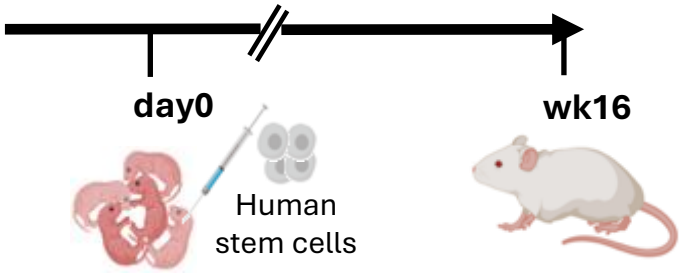
Marc Pellegrini

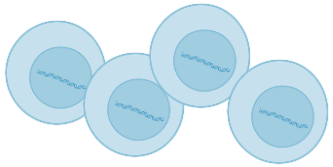
Damian Purcell

Lloyd Einsiedel



Establishing unique HTLV-1 *in vivo* models



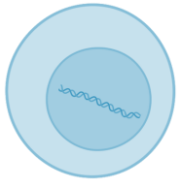


patient derived HTLV-1c-infected cells

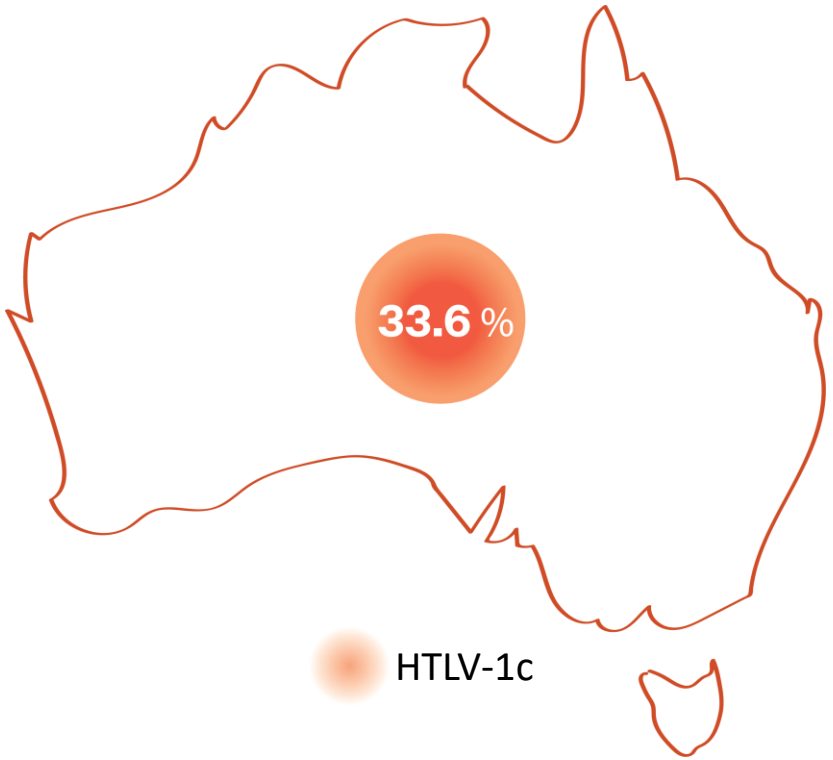
Marc Pellegrini

Damian Purcell

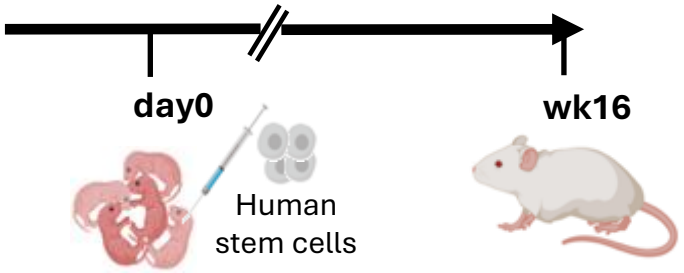
Lloyd Einsiedel

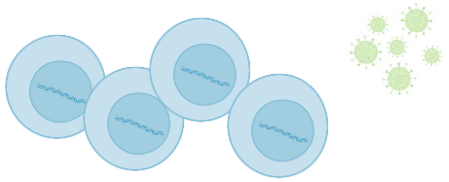


patient derived HTLV-1c-infected cells

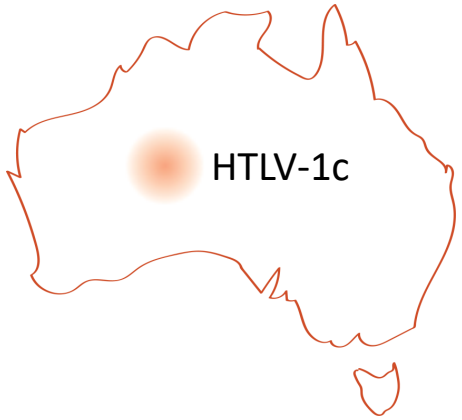


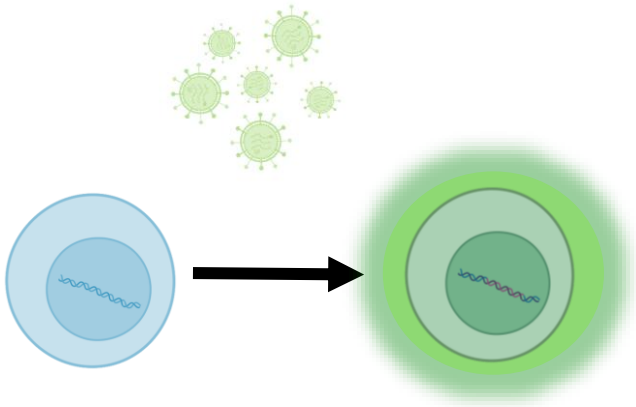
Establishing unique HTLV-1 *in vivo* models



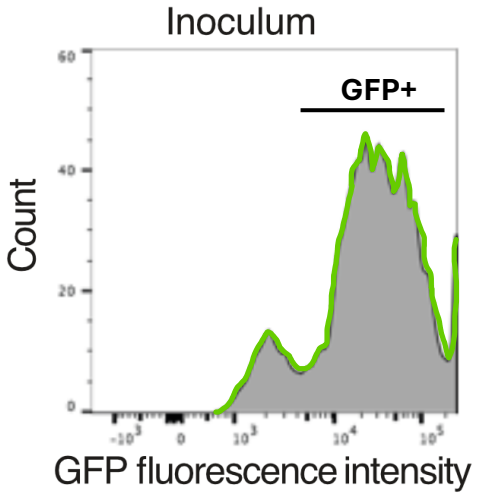


patient derived HTLV-1c-infected cells transduced with a **GFP** reporter lentivirus



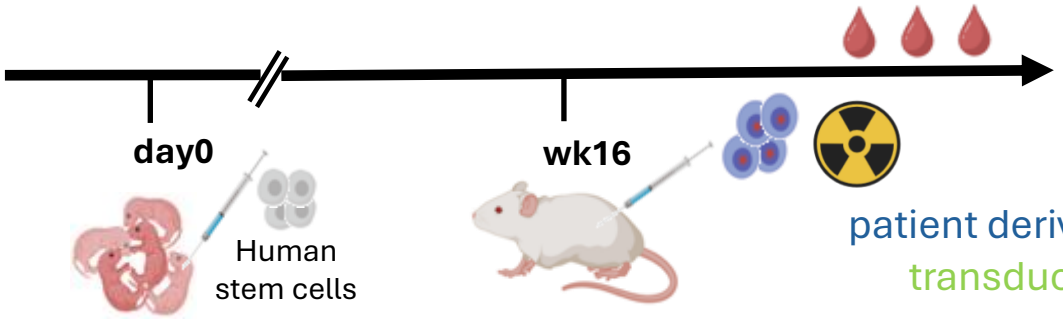


patient derived HTLV-1c-infected cells

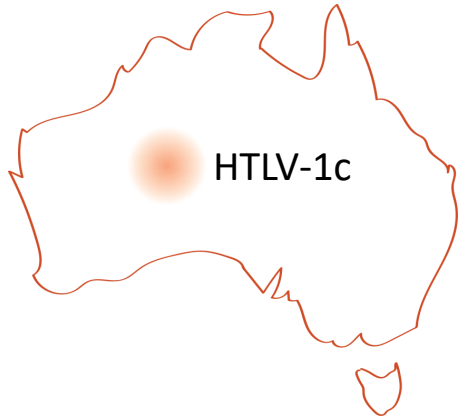


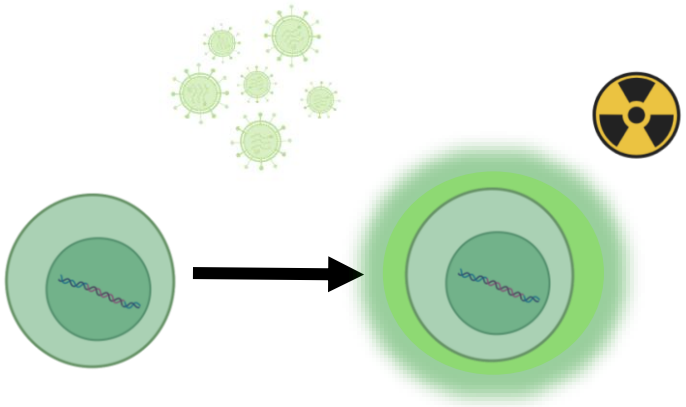
Cooney et. al. *Cell 2025*

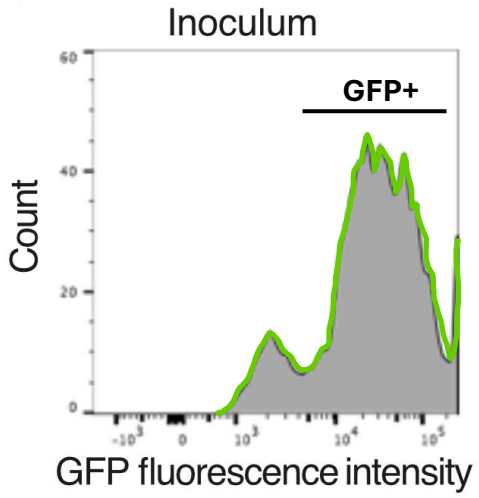
Establishing unique HTLV-1 *in vivo* models

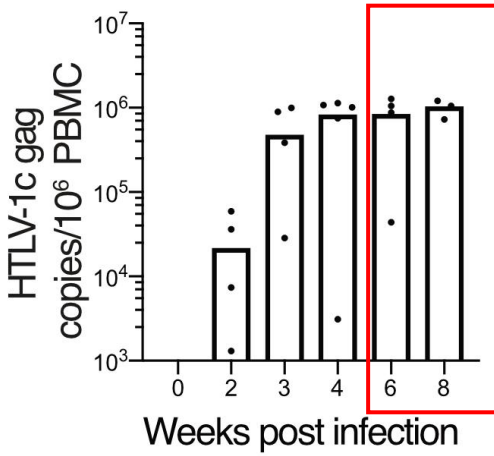


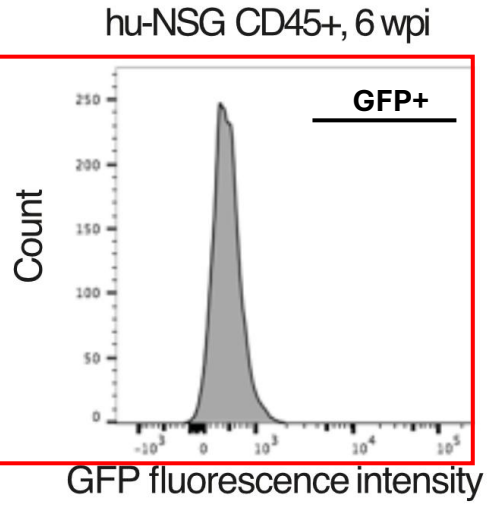
irradiated
patient derived HTLV-1c-infected cells
transduced with a **GFP** reporter
lentivirus







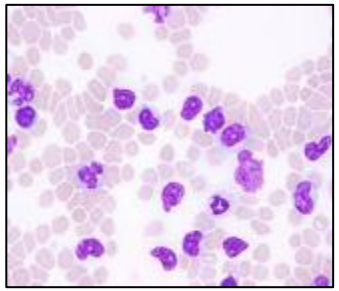




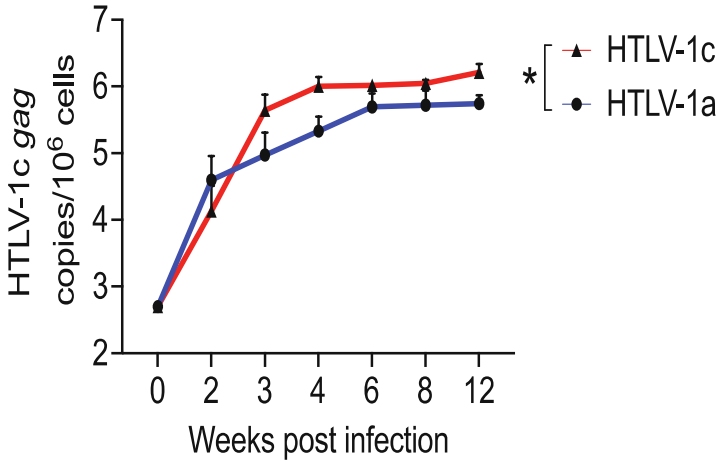
→ ***De novo* infection occurs in human immune system mice**

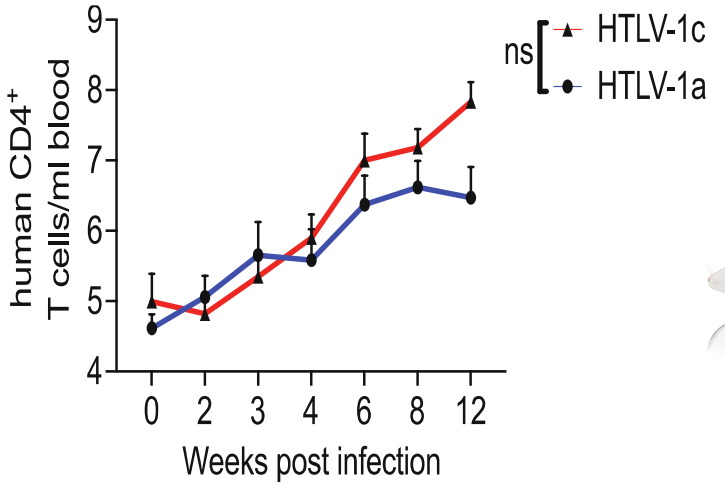
Cooney et. al. *Cell 2025*

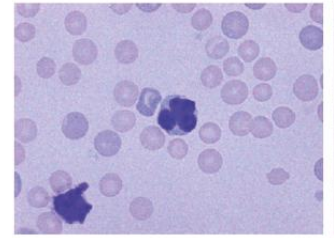
Humanized mice develop HTLV-1 associated disease: ATLL



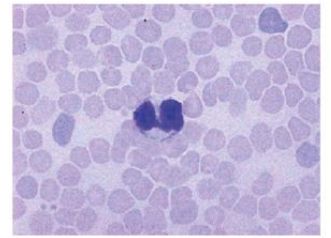
ATLL



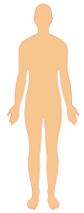


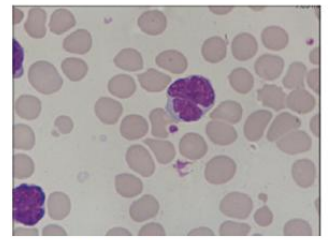


HTLV-1c-infected

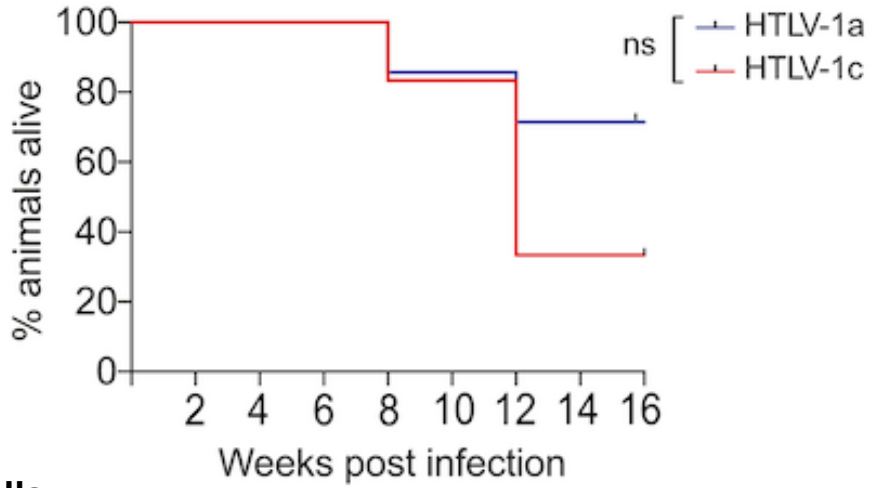


HTLV-1a-infected





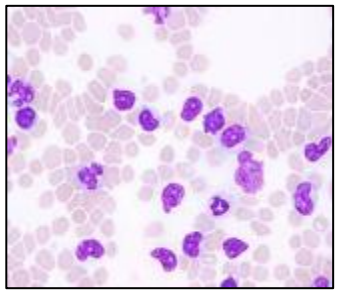
ATL human sample reference



HTLV-1c: patient PBMC derived HIS mouse cells
HTLV-1a: MT2-derived HIS mouse cells

Cooney et. al. *Cell 2025*

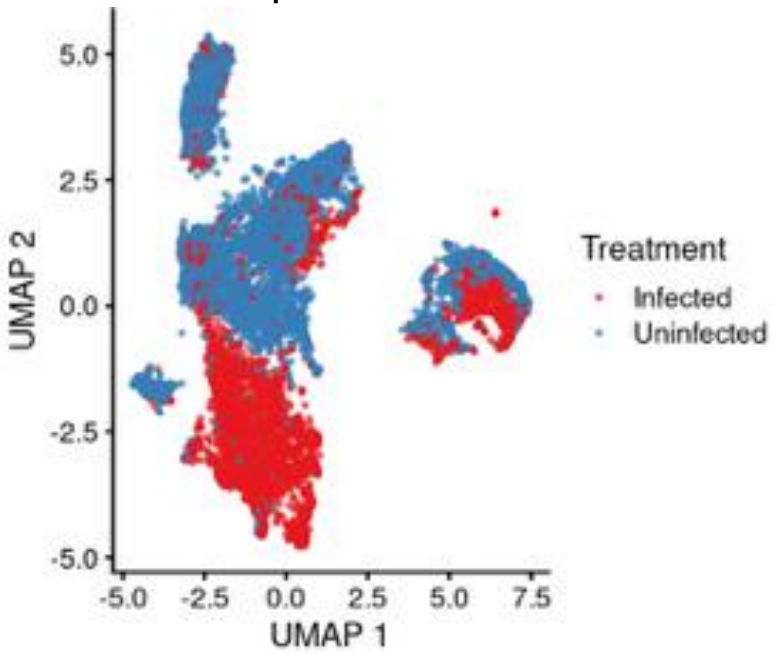
Humanized mice develop HTLV-1 associated disease: ATLL



ATLL

HTLV-1c

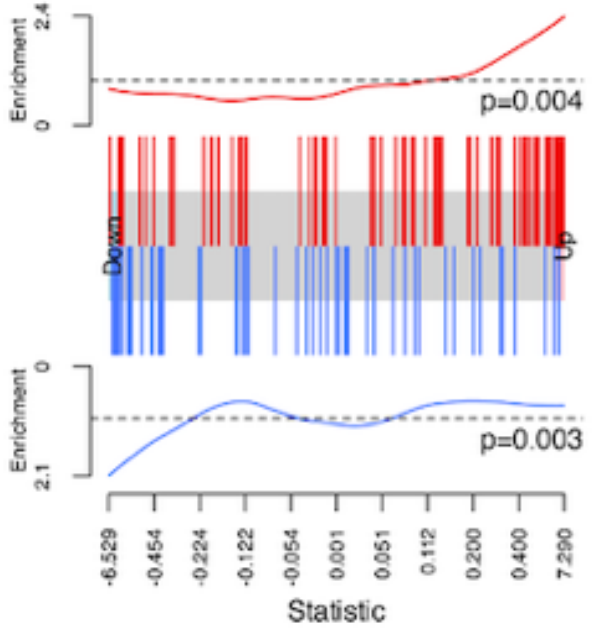
scRNAseq – CD4 T cells



HTLV-1c vs

ATLL (HTLV-1a)

Differentially expressed genes in ATL

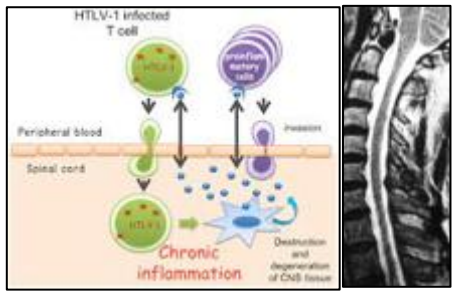


Natasha Jansz

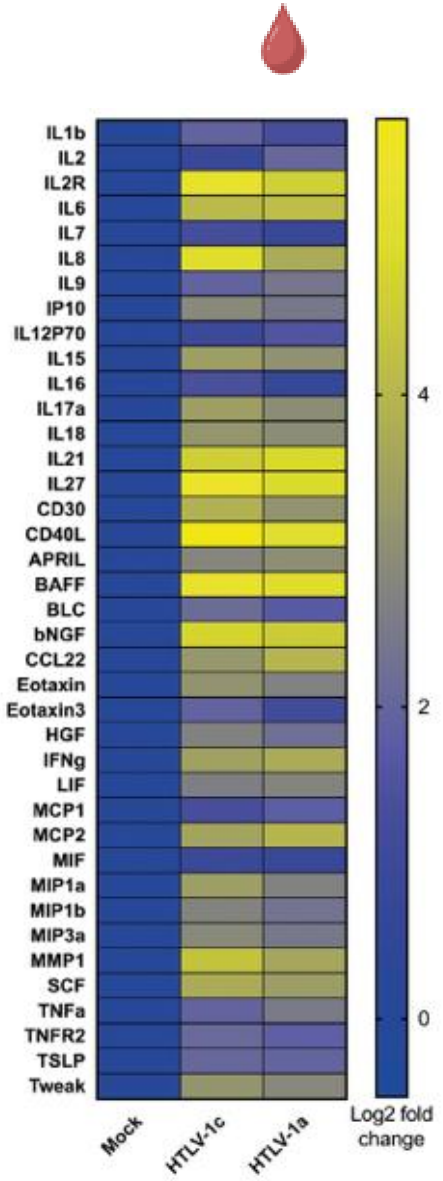
(comparison with: Yamagishi, M et al Nature Comms 2021)

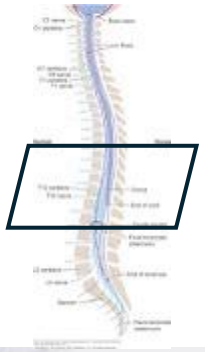
Cooney et. al. *Cell 2025*

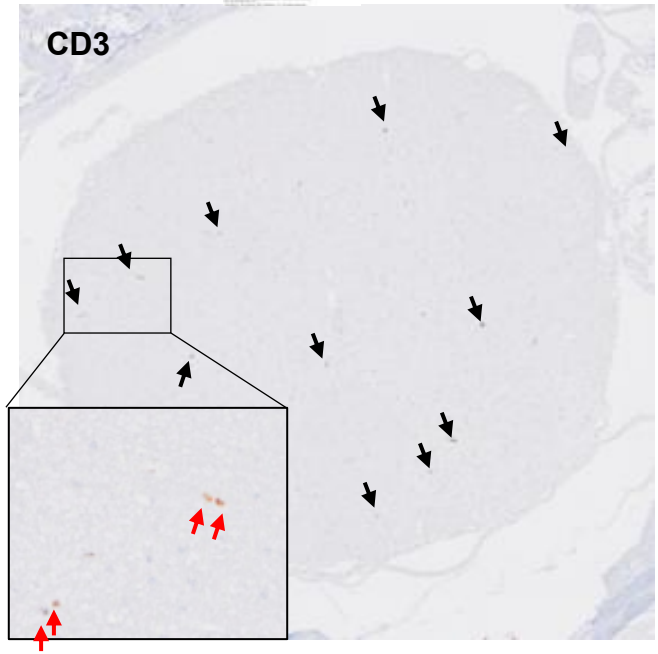
Systemic inflammation, spinal cord T cell infiltration and brain microgliosis



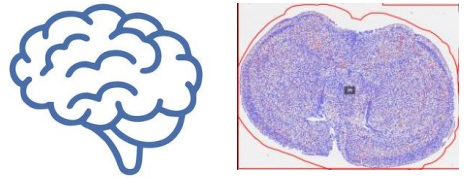
HAM

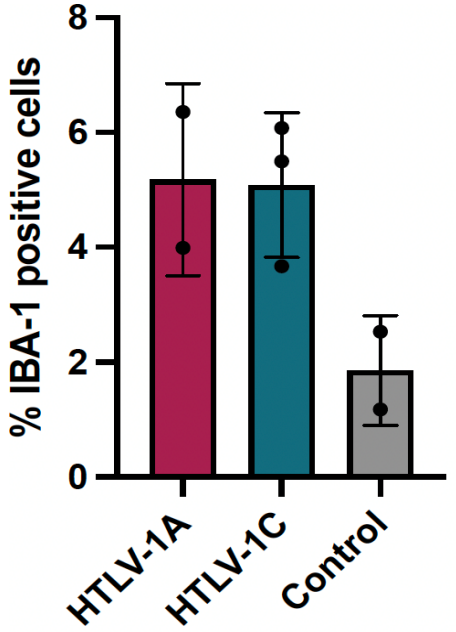






HTLV-1c infected
(8 wpi)

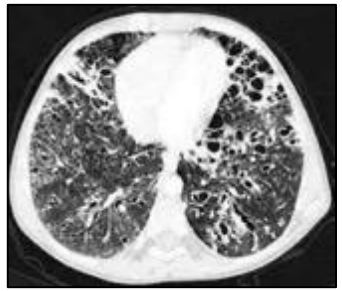




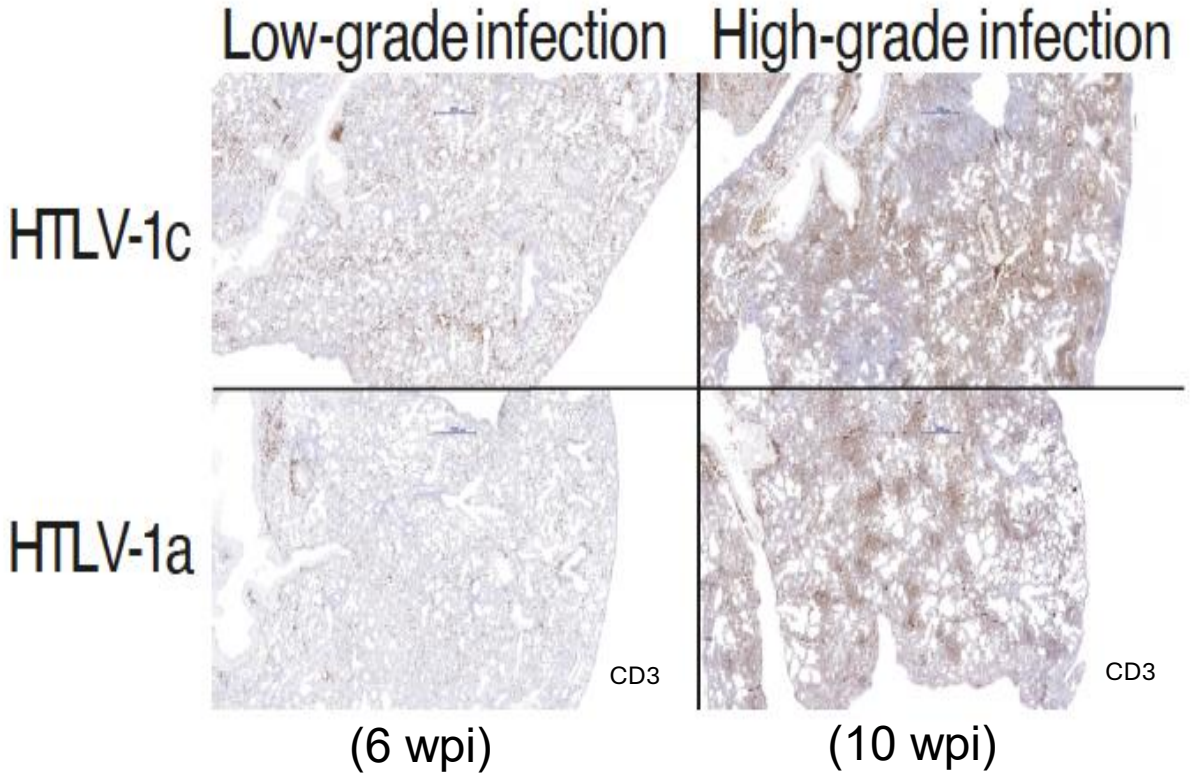
(8 wpi)

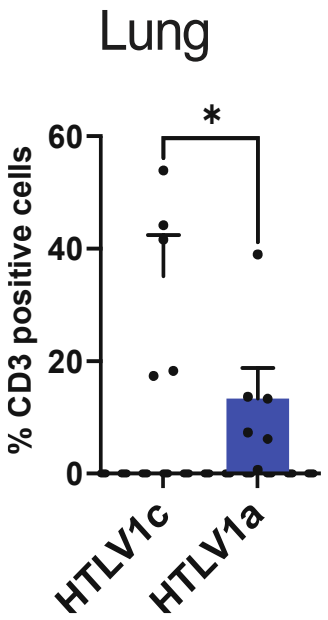
Cooney et. al. *Cell 2025*
Doerflinger lab, unpublished

Lung infiltration and inflammation in HTLV-1 infected HIS mice



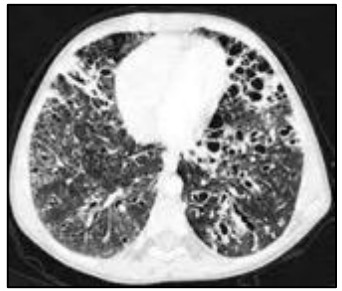
Lung Disease





Cooney et. al. *Cell 2025*

Cross-species spatial transcriptomics in vivo discovery biology



Lung Disease

Human: Immune, effector and signalling (218)

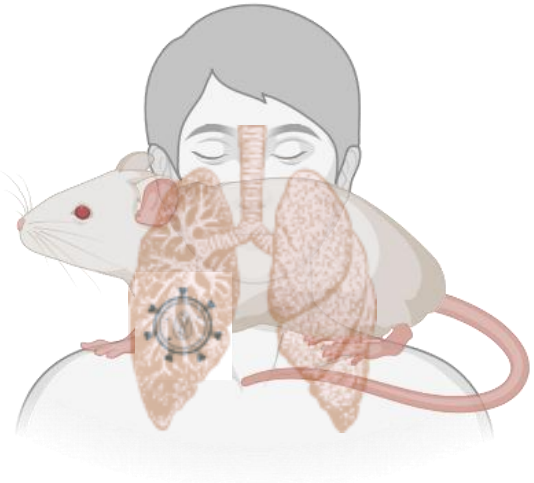
Mouse: Lung cell-specific genes (68)

HTLV-1: 16 gene “groups” covering canonical genes, subtypes A and C (separately), transcripts from both genome strands (LTR, gag, pol, env, pro, tax region, hbz, pol(-))

10x XENIUM spatial

3-species

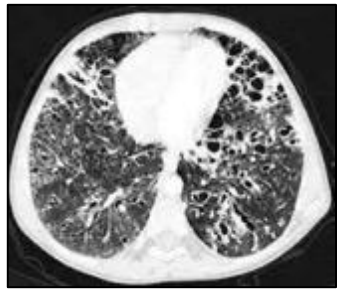
300-gene custom panel



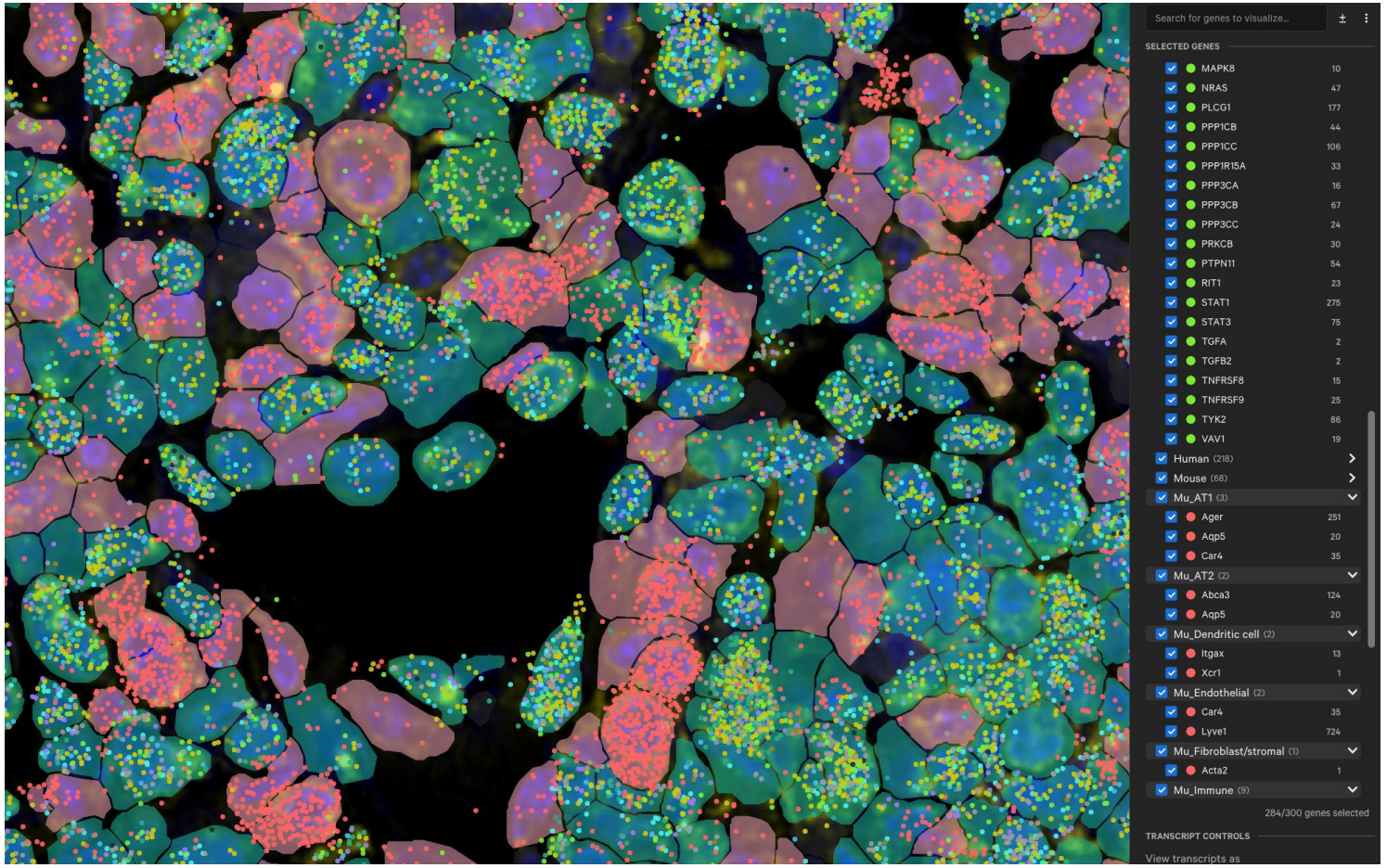
Doerflinger lab, unpublished

Cross-species spatial transcriptomics in vivo discovery biology

HTLV-1a (8 weeks post infection)

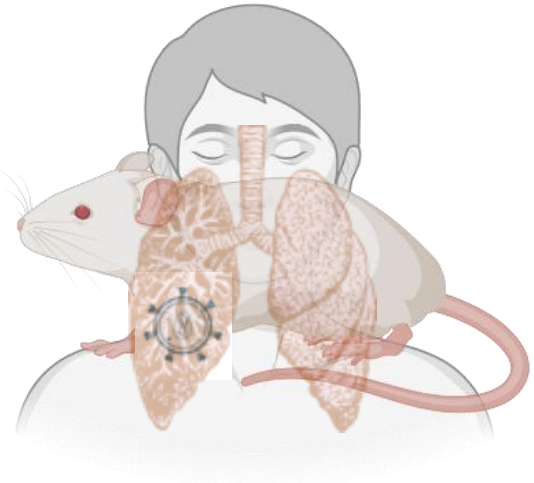


Lung Disease



pink cells – mouse
green cells – human

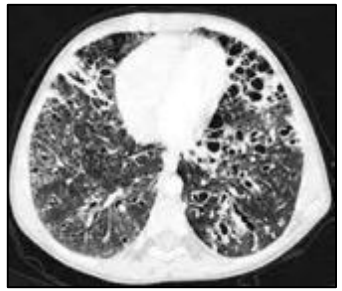
10x XENIUM spatial
3-species
300-gene custom panel



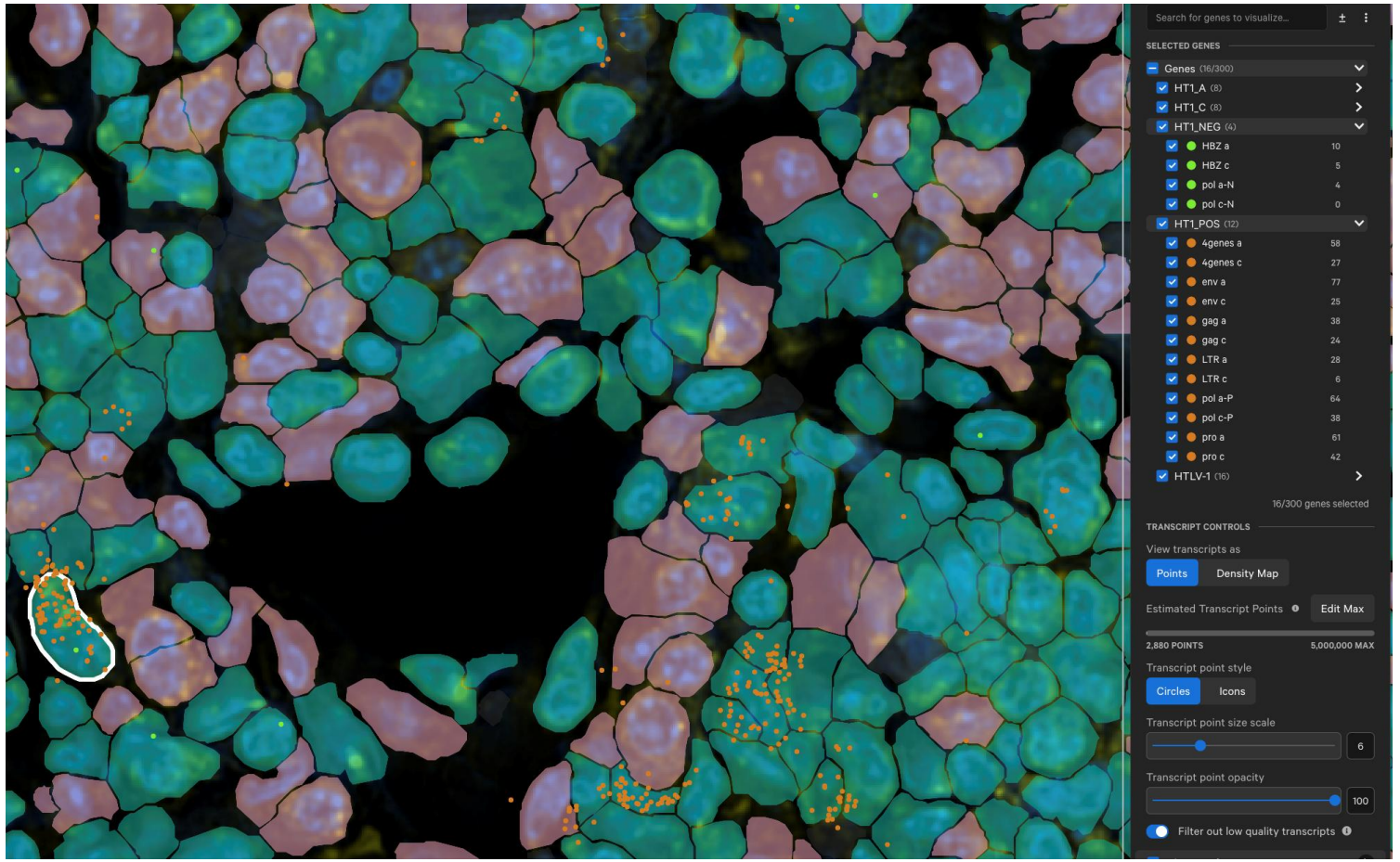
Doerflinger lab, unpublished

Cross-species spatial transcriptomics in vivo discovery biology

HTLV-1a (8 weeks post infection)

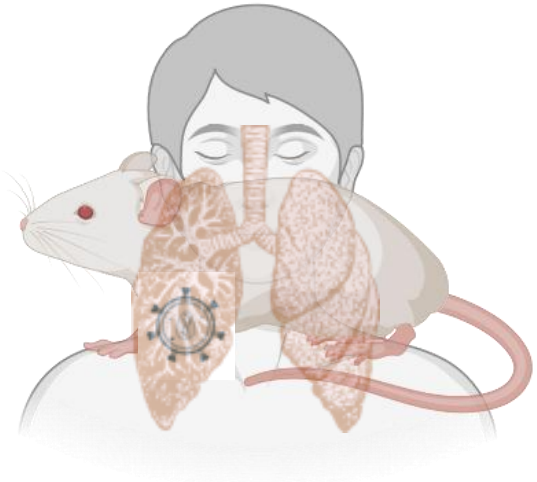


Lung Disease



pink cells – mouse
green cells – human
orange – HTLV-1

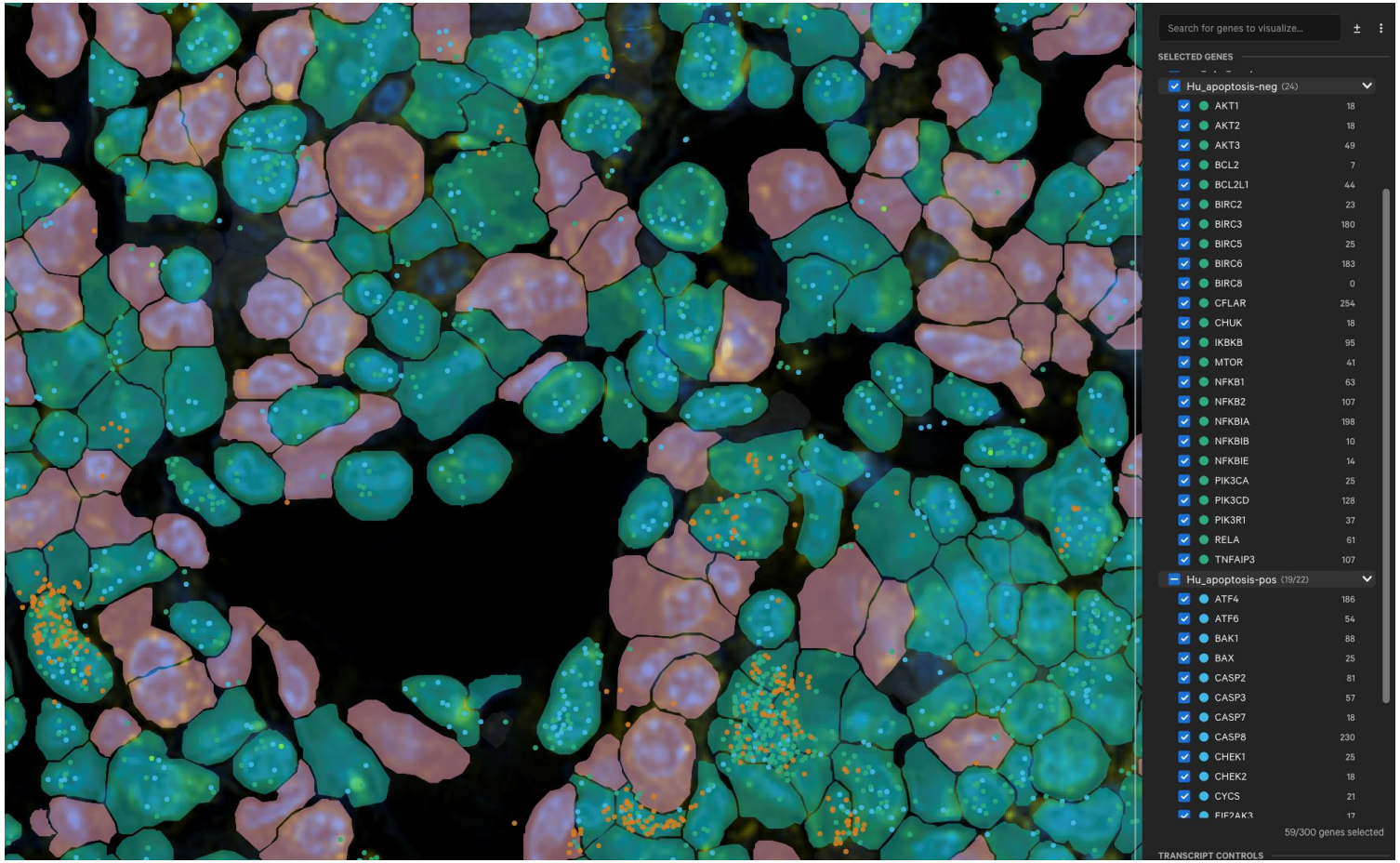
10x XENIUM spatial
3-species
300-gene custom panel

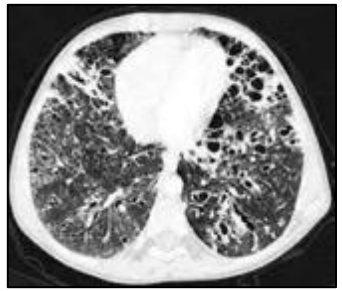


Doerflinger lab, unpublished

Cross-species spatial transcriptomics in vivo discovery biology

HTLV-1a (8 weeks post infection)



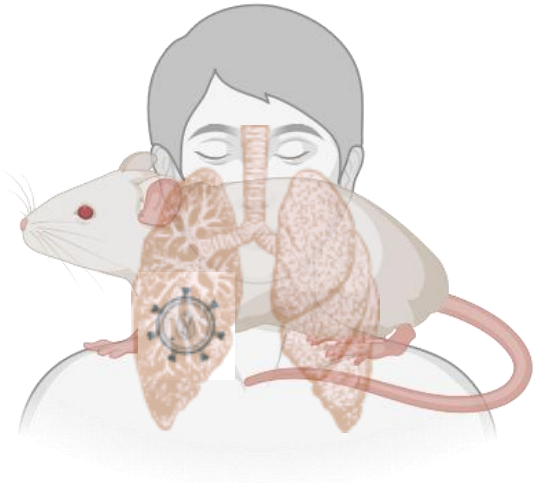


Lung Disease

10x XENIUM spatial

3-species

300-gene custom panel



pink cells – mouse
green cells – human
orange – HTLV-1

light green → anti-apoptotic
light blue → pro-apoptotic

→ Cells undergoing replicative infection have anti-apoptotic signature.

Doerflinger lab, unpublished

Humanised mouse models of HTLV-1

- **study biology *in vivo***

-biomarkers and (early) drivers of disease

-differences in virus subtypes

- **pre-clinical evaluation of**

  - vaccines

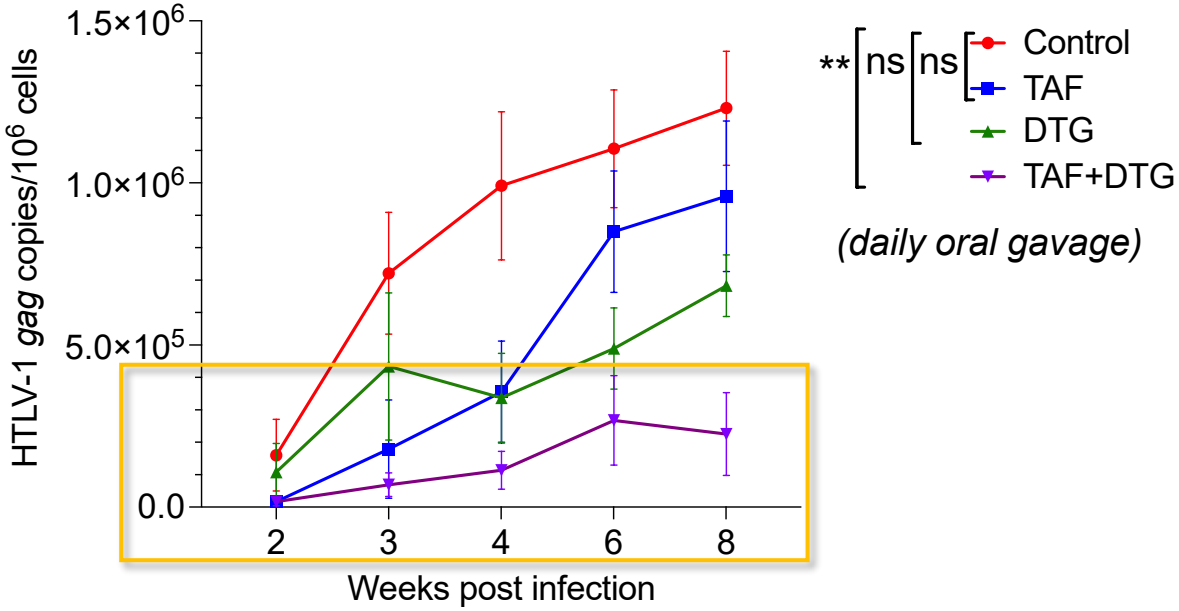
  - antivirals

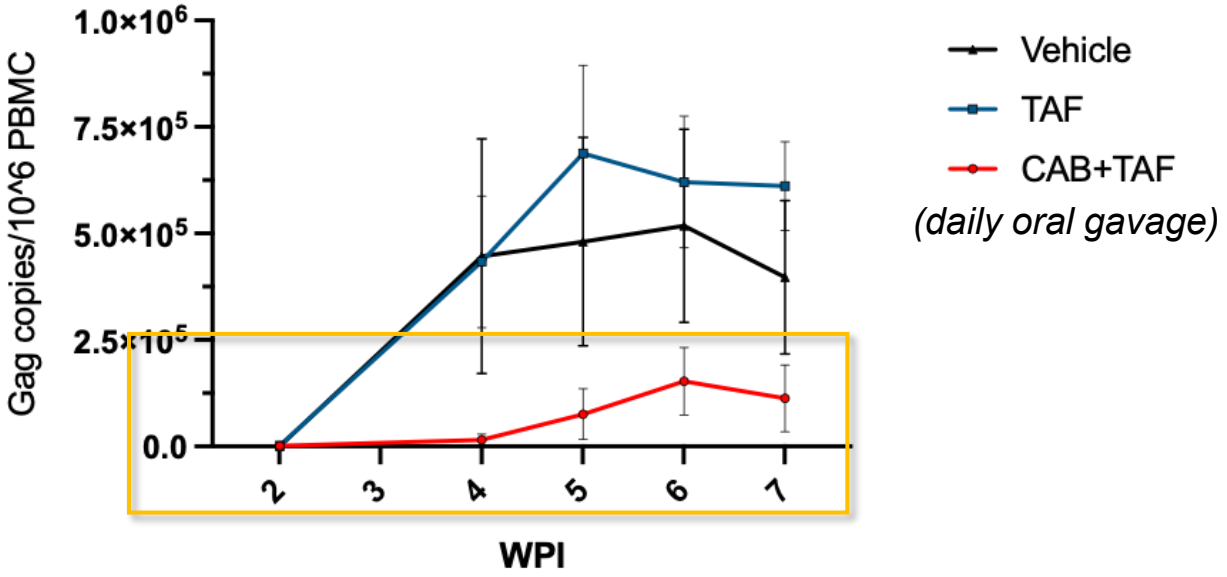
  - therapeutics

HIV combination ART attenuates proviral expansion *in vivo* - potential as (long acting) PrEP and PEP for HTLV-1

- ***nucleotide reverse transcriptase inhibitor:*** **TAF:** *tenofovir alafenamide*
- ***integrase strand transfer inhibitor:***
  DTG: *dolutegravir*

CAB: *cabotegravir*
(part of Cabuvena slow-release injectable)





Cooney et. al. *Cell 2025*
Doerflinger lab, unpublished

Humanised mouse models of HTLV-1

- **study biology *in vivo***

-biomarkers and (early) drivers of disease

-differences in virus subtypes

- **pre-clinical evaluation of**

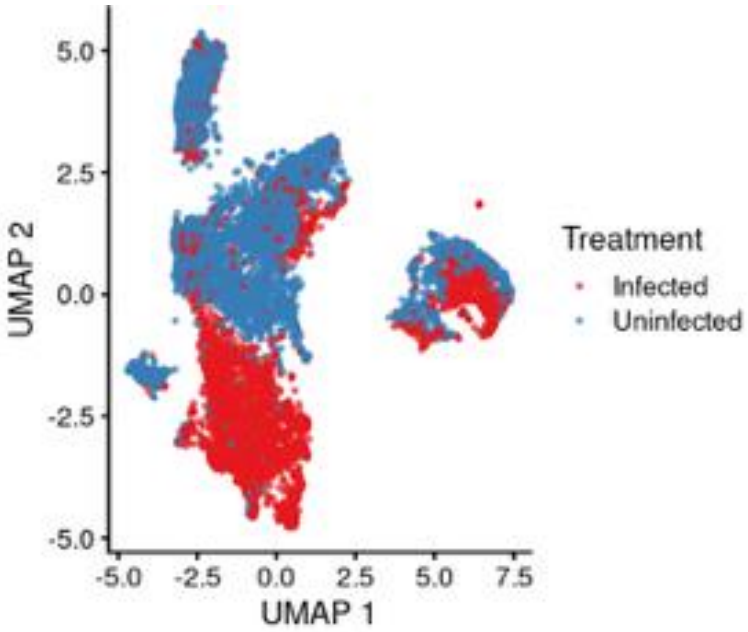
  - vaccines

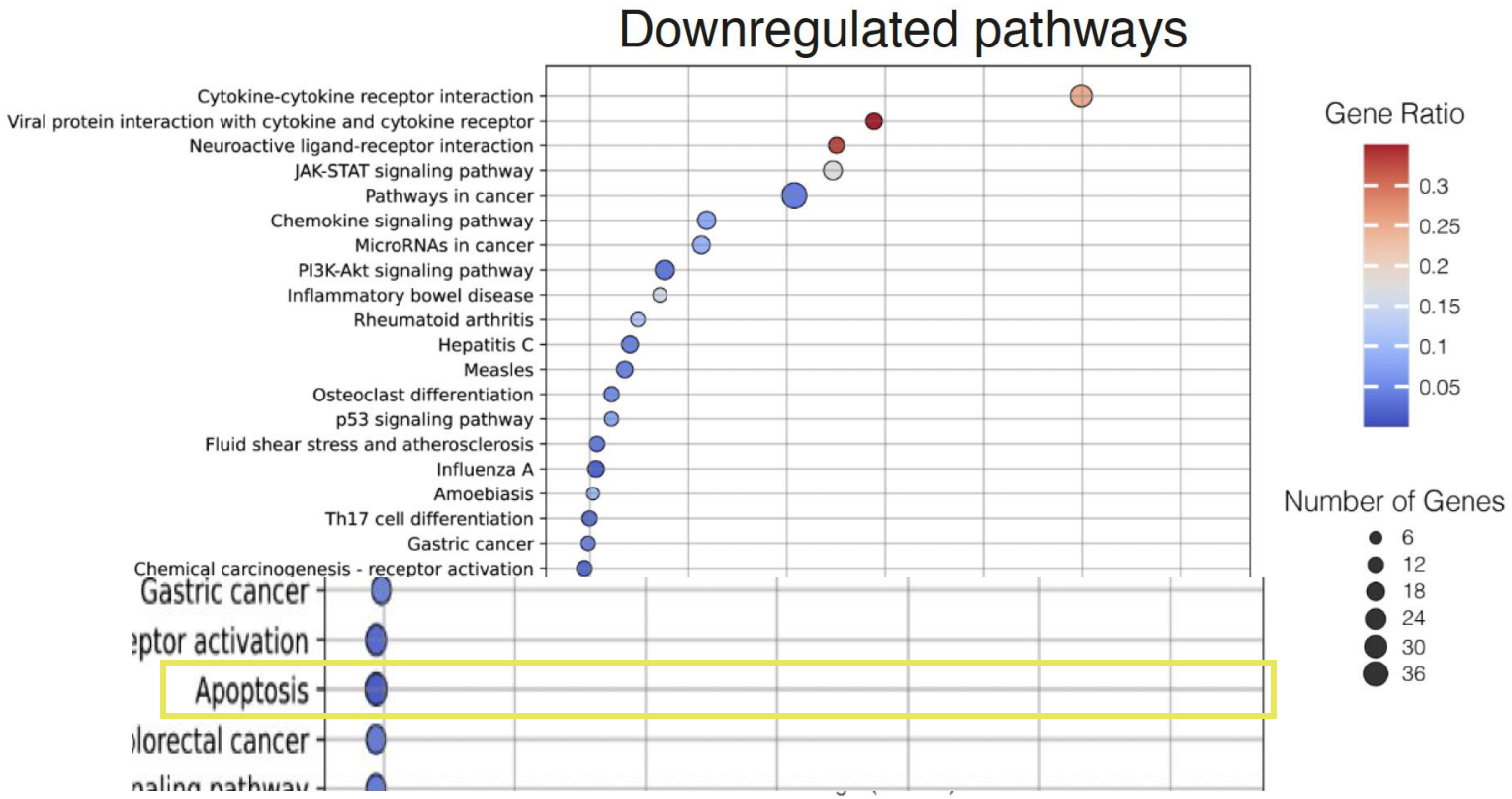
  - antivirals

  - therapeutics

Discovery Biology to identify host targets for HTLV-1 therapy

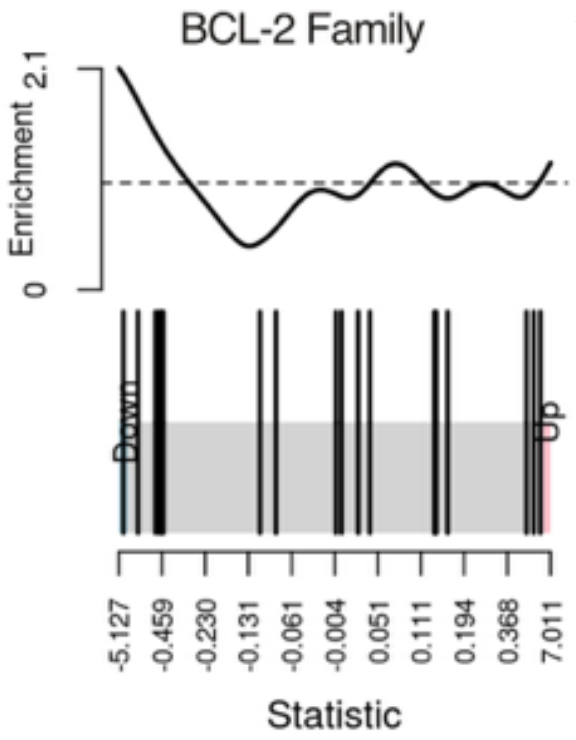
scRNAseq – CD4 T cells

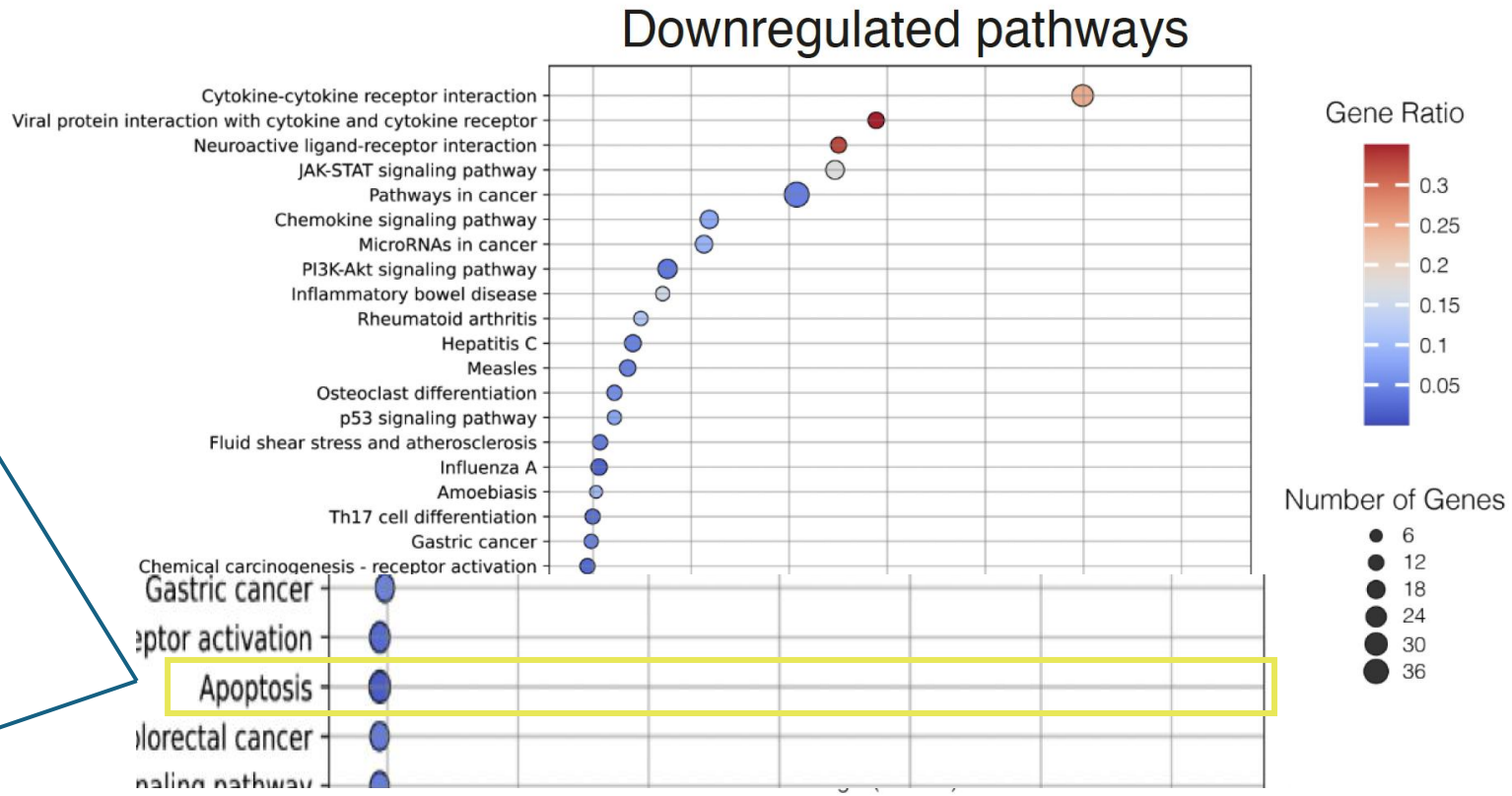




Cooney et. al. *Cell 2025*

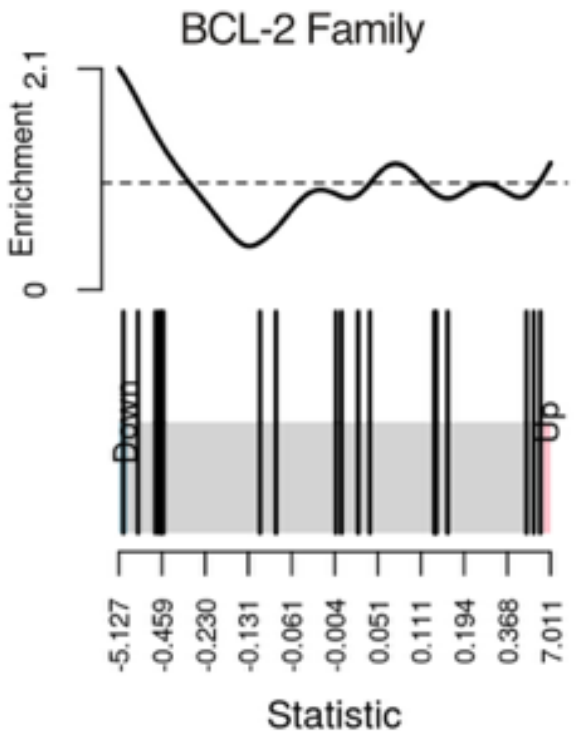
Intrinsic apoptosis is dysregulated in HTLV-1 infected cells

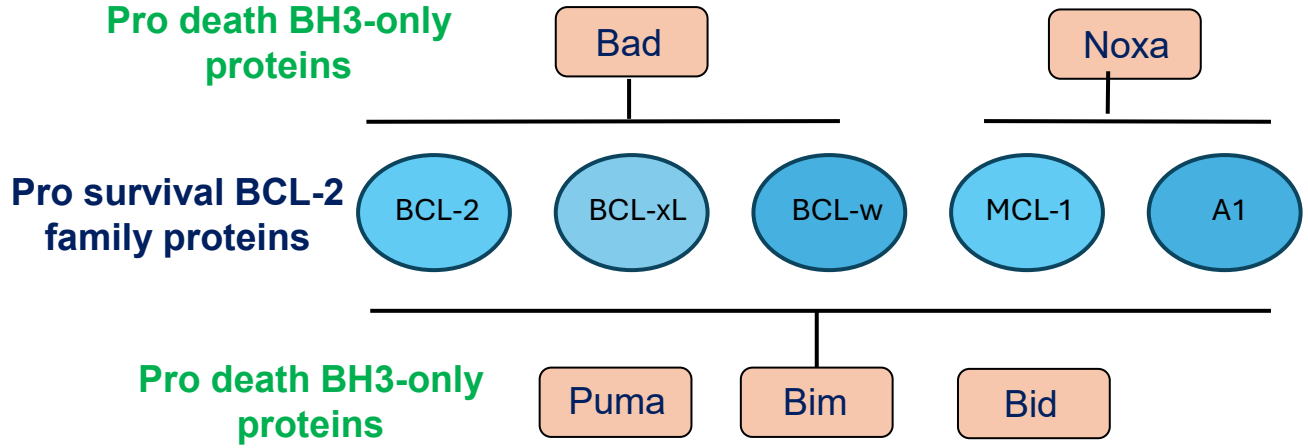




Cooney et. al. *Cell 2025*

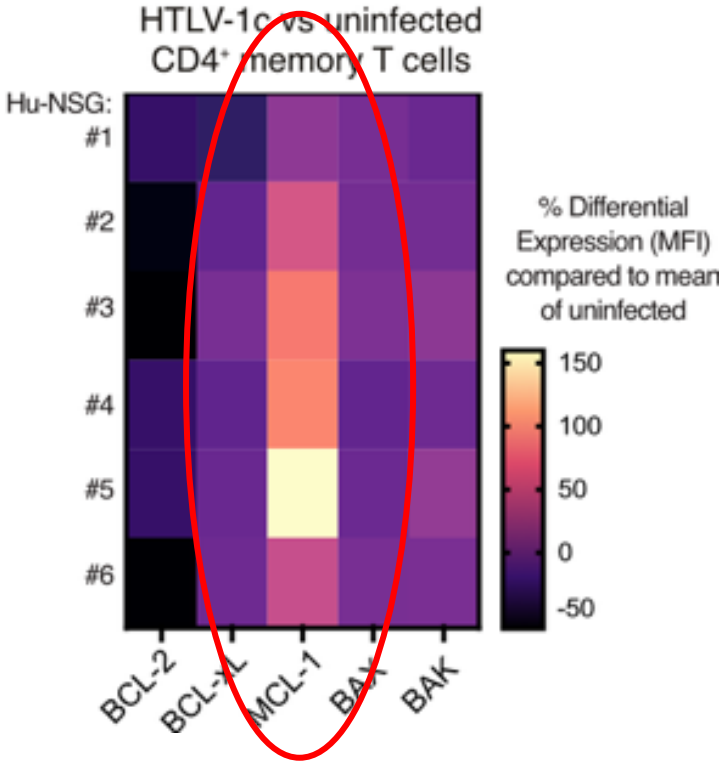
Intrinsic apoptosis is dysregulated in HTLV-1 infected cells

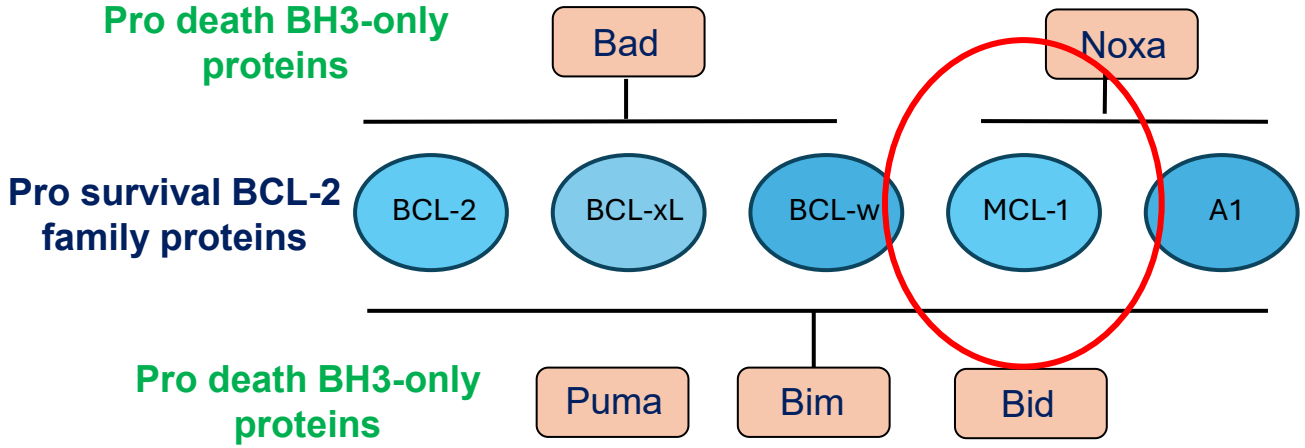




Cooney et. al. *Cell 2025*

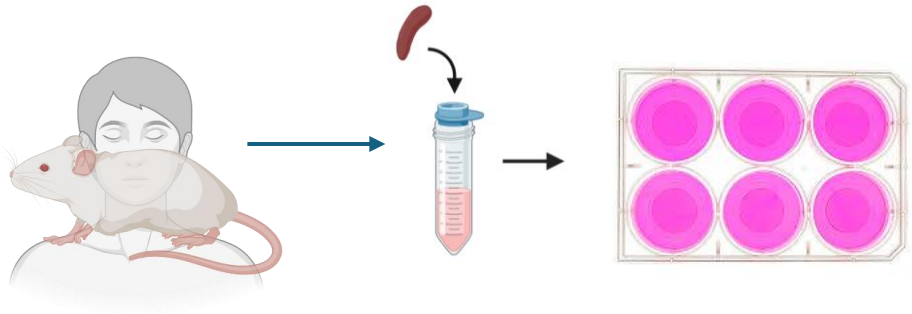
Pro-survival protein MCL-1 prevents apoptosis in HTLV-1

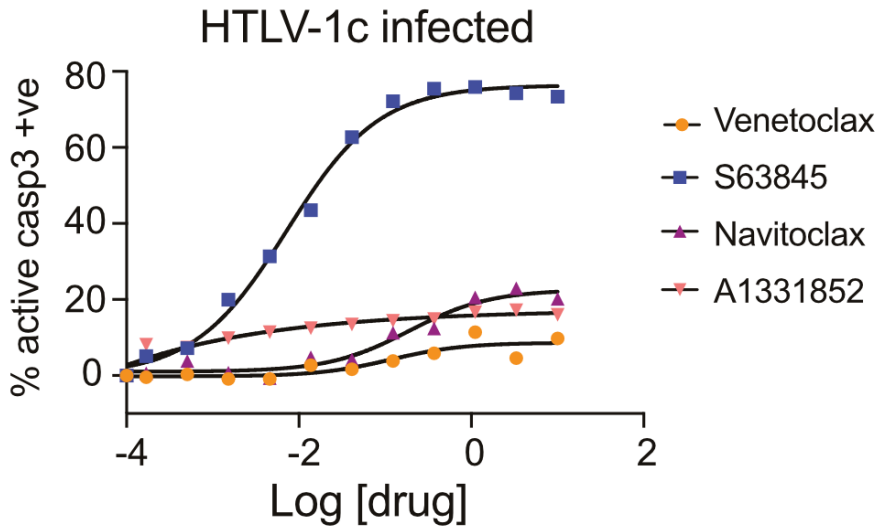


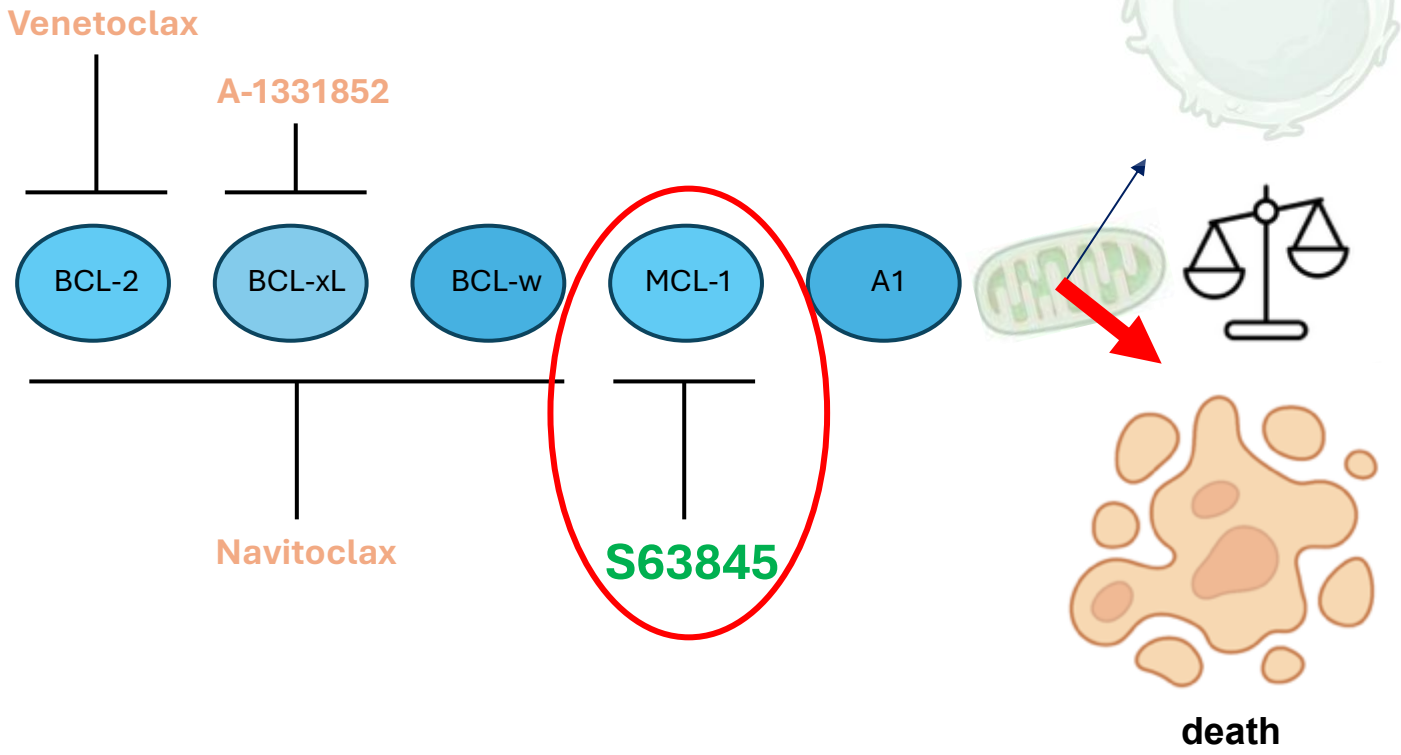


Cooney et. al. *Cell 2025*

BH3 mimetic MCL-1 inhibitor can induce intrinsic apoptosis

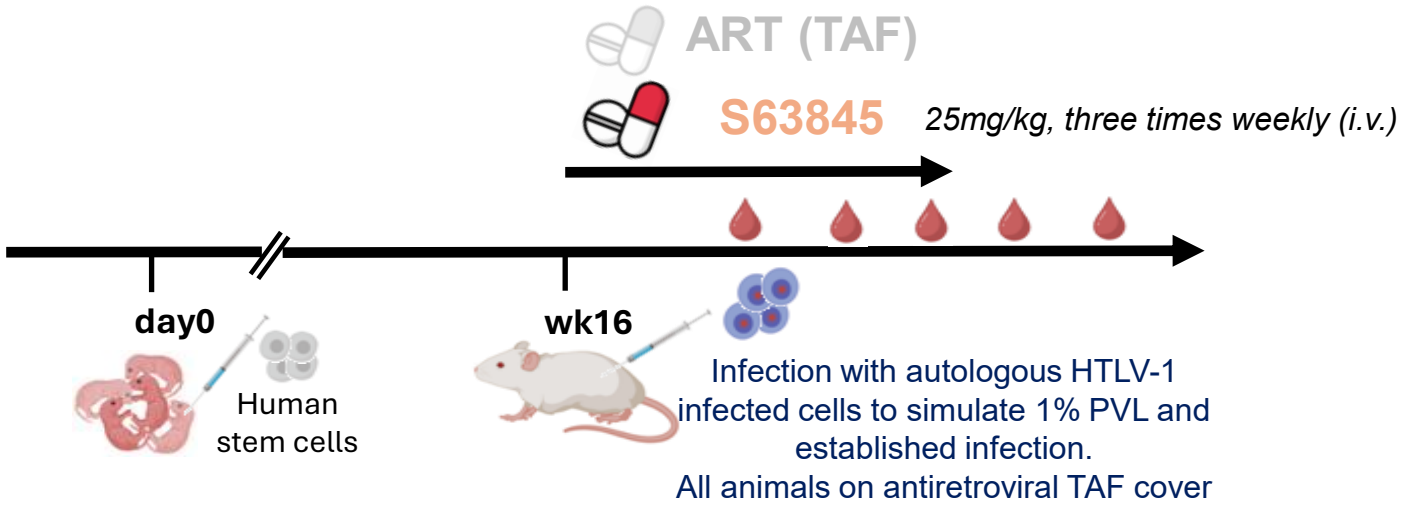


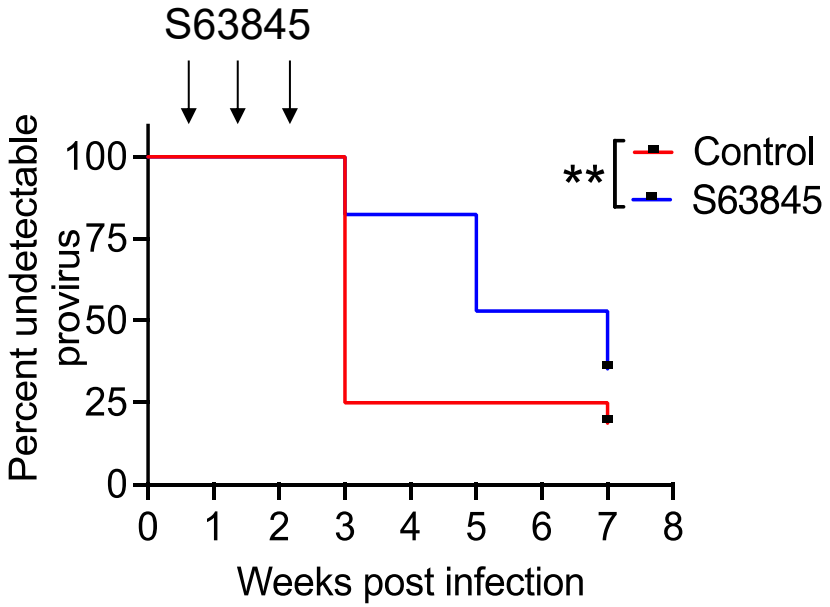


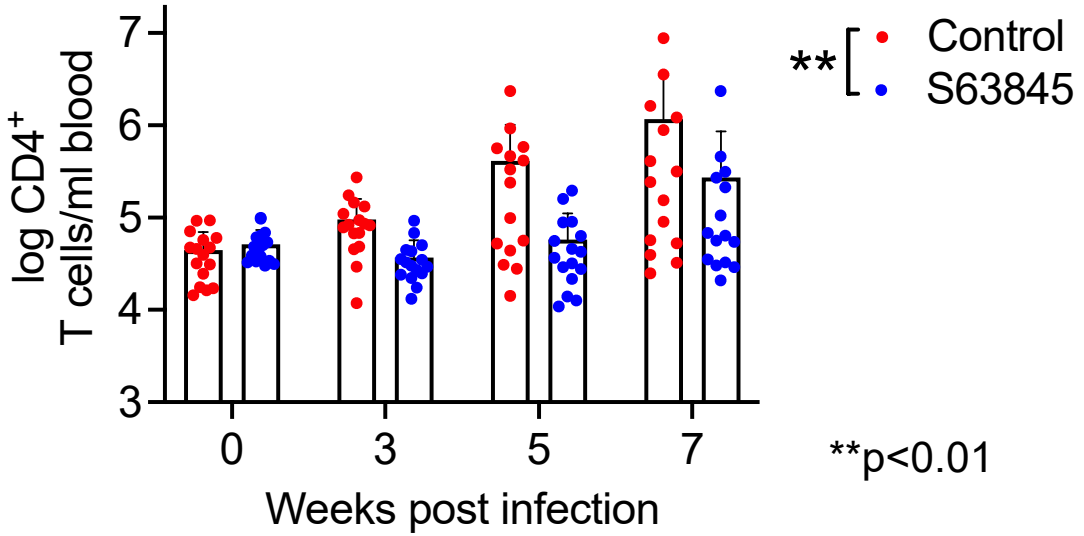


Cooney et. al. *Cell 2025*

Inhibition of MCL-1 mitigates disease progression *in vivo*

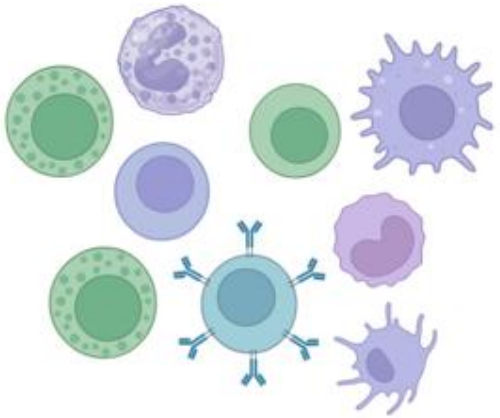




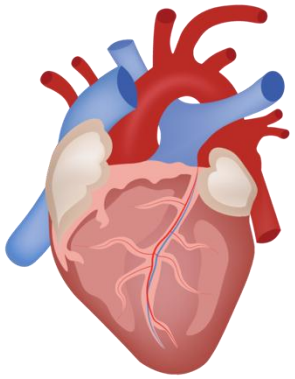


Cooney et. al. *Cell 2025*

Systemic small molecule MCL-1 inhibition – a dead end?

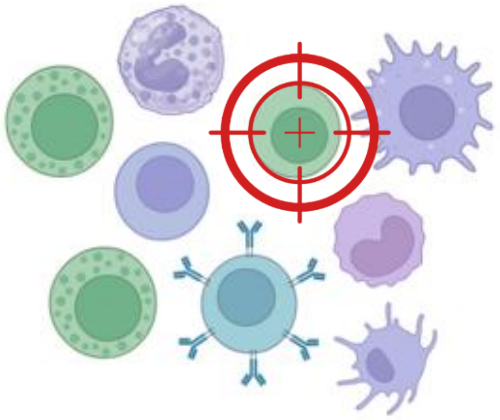


MCL-1 is necessary for the survival of many cells *beyond* T/B lymphocytes, such as cells of the nervous system or cardiomyocytes

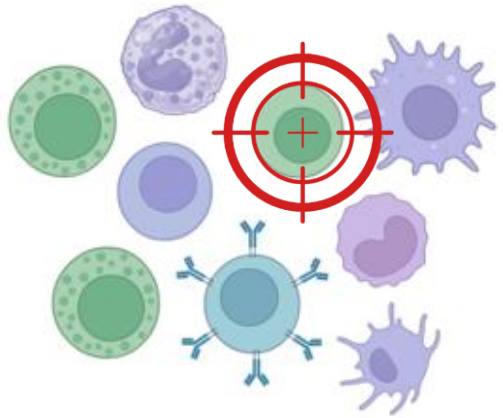


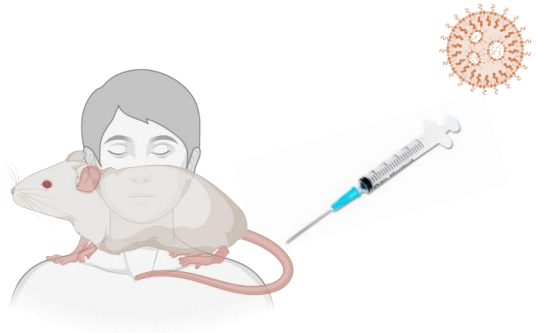
Targeting MCL-1 affects the survival and function of human cardiomyocytes
Due to safety signals of cardiotoxicity, a phase 1 dose escalation clinical trial has been suspended (NCT 03465540)

Precision targeting to T cells via LNPs



Precision targeting to T cells via LNPs





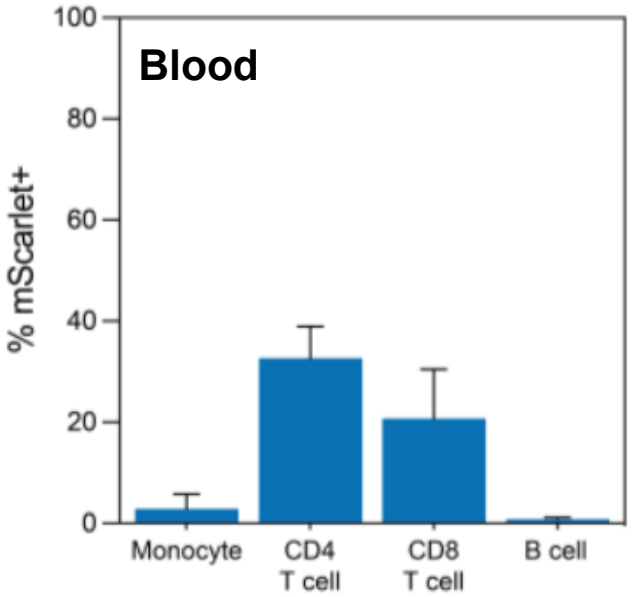
T-cell targeting LNPs,
loaded with mScarlet reporter RNA,
1x i.v. injection,
tissue analysis 16h

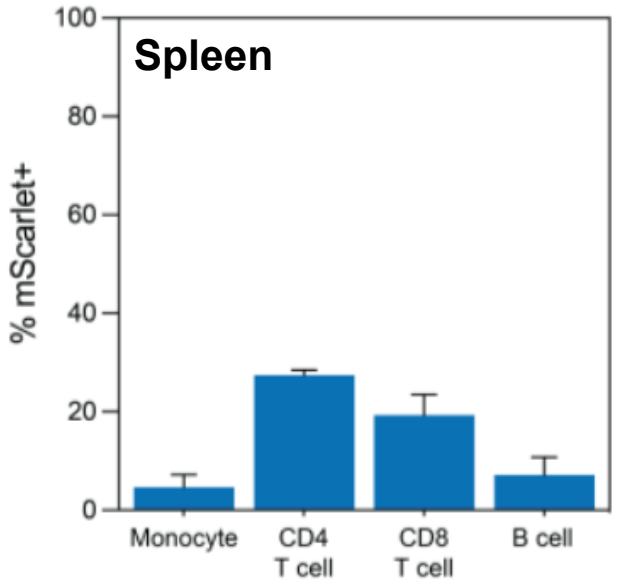
T- cell targeting LNPs:

Doherty Institute

MONASH University | MONASH INSTITUTE OF PHARMACEUTICAL SCIENCES

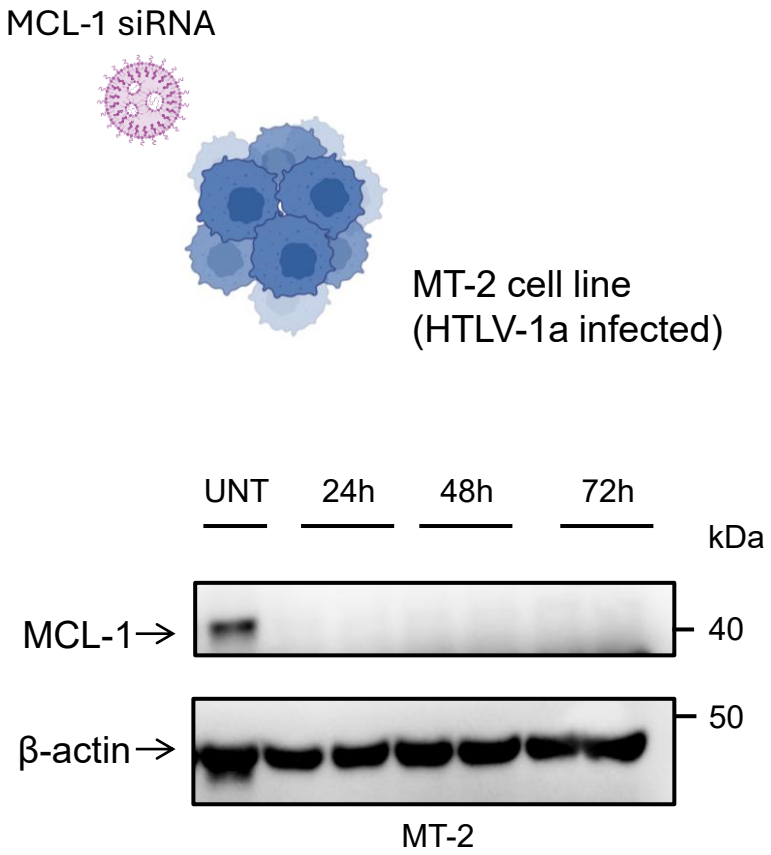
Sharon Lewin
Paula Cevaal
Angus Johnston

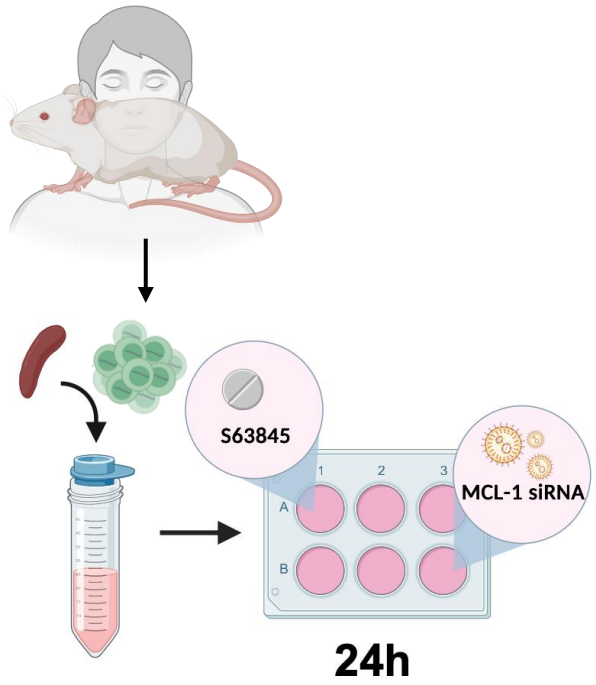


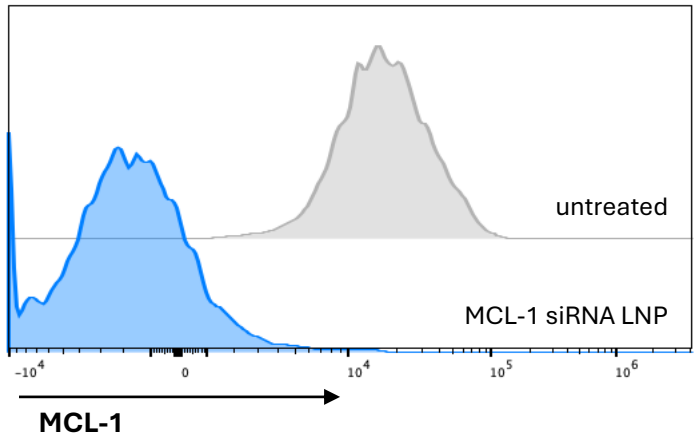


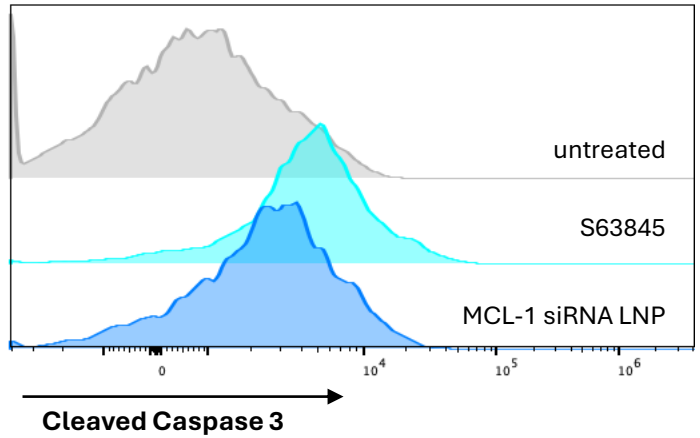
Cevaal et al, Nature Comms 2026

Targeting MCL-1 2.0 – RNAi kills HTLV-1 infected cells *ex vivo*





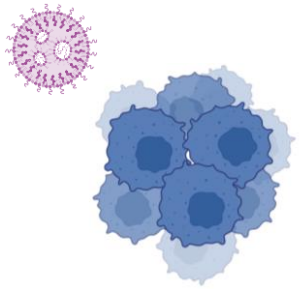




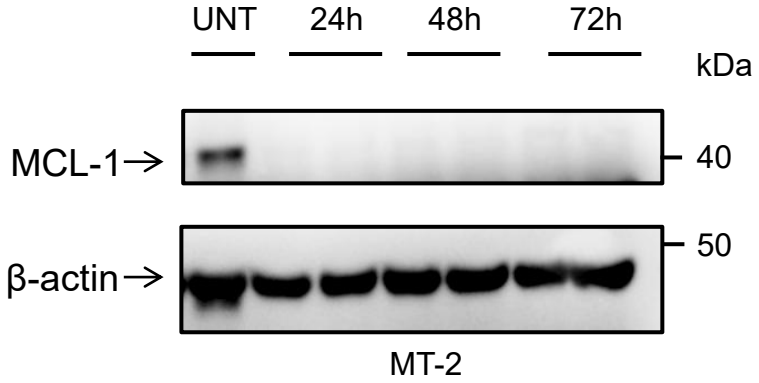
Doerflinger lab, unpublished

Targeting MCL-1 2.0 – RNAi kills HTLV-1 infected cells *ex vivo*

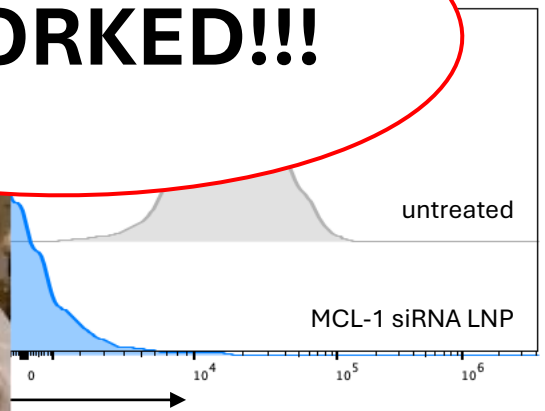
MCL-1 siRNA

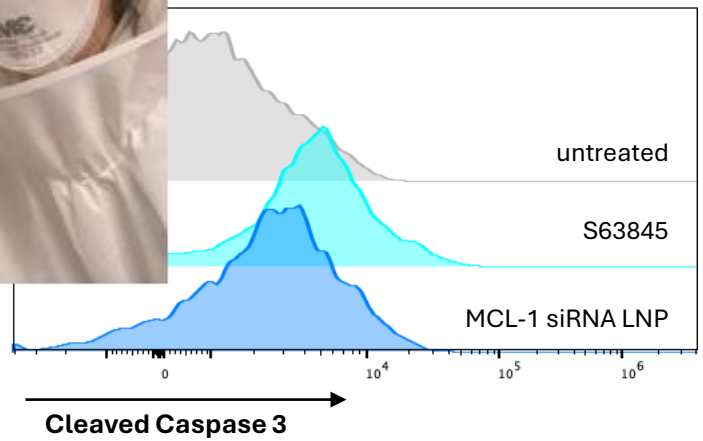


MT-2 cell line
(HTLV-1a infected)



IT WORKED!!!





Acknowledgements

Doerflinger Lab
Lena Scherer
Le Wang
Liana Mackiewicz
Lewis Williams
Merle Dayton
Stefanie Bader
Reet Bhandari
Kathy Davidson
Jan Schaefer
David Romero
William Clow
Phil Pingen
Anne-Dyanna Jayatilake

Pellegrini Lab
Marc Pellegrini
James Cooney
George Ashdown
Simon Preston
Cody Allison
Greg Ebert

Doherty Institute
Damian Purcell
Lloyd Einsiedel
Natasha Jansz
Ashley Hirons
Samantha Grimley

Sharon Lewin
Paula Cevaal
Michael Roche

WEHI Collaborators
James Vince
Andreas Strasser
Maria Tanzer
Rory Bowden
Jaffar Jabari
Shaian Tovakolian
Allan Motyer
Tony Papenfuss
André Sampson
Gemma Kelly

Raymond Yip
Ellen Tsui
Ana Maluenda
Stephen Wilcox

Marco Herold
Smitha Georgy
Charis Teh

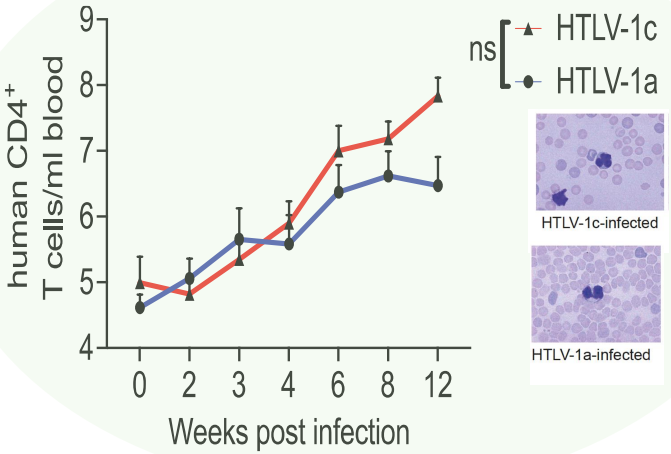
WEHI
Bioservices
Imaging
Histology
Genomics
Vets
FACS
Consumer Program
FSO, Reception
Catering, Events

Those living with HTLV-1

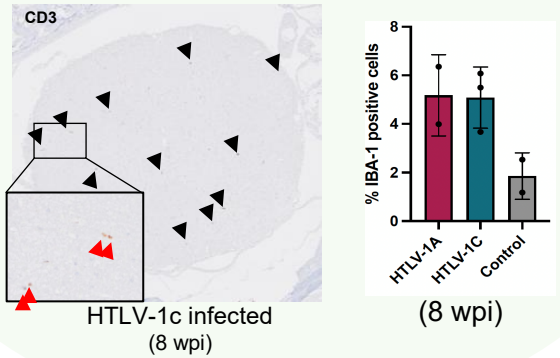
doerflinger.m@wehi.edu.au

Summary

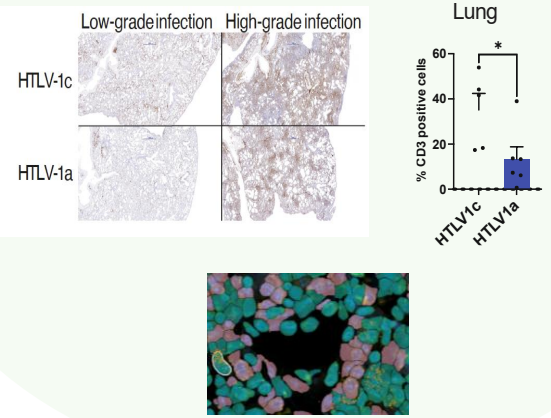
ATL



HAM

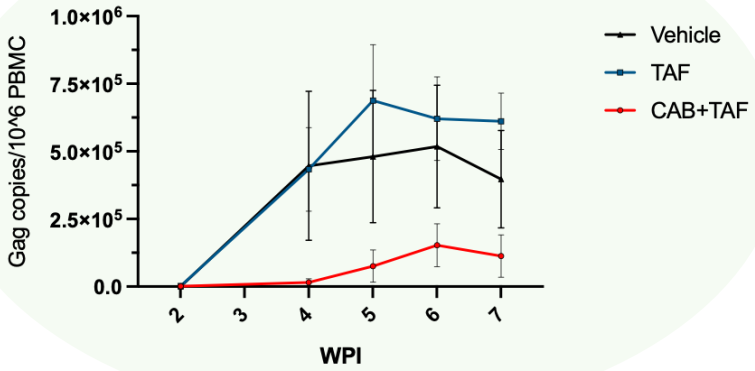


Lung disease

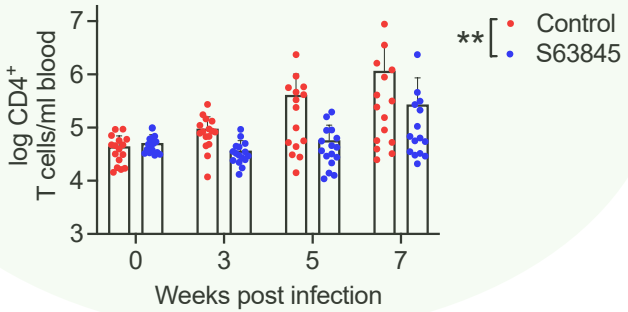


Questions ??

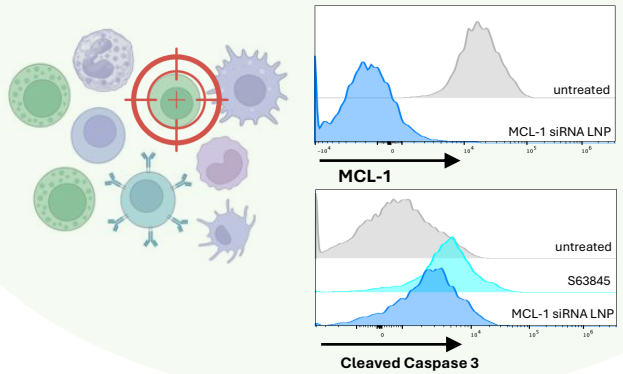
Antivirals



Cell death inducing therapy



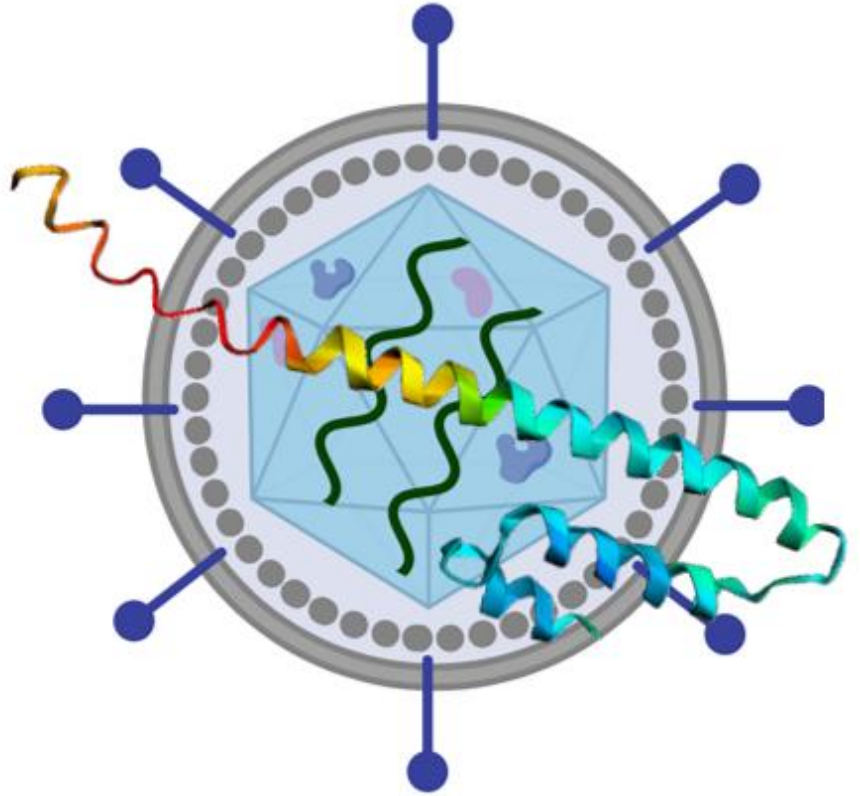
Precision LNP/RNA therapy



doerflinger.m@wehi.edu.au

A small polypeptide encoded by antisense long non-coding RNA mediates BLV replication

Jouant Thomas



LIÈGE université
Gembloux
Agro-Bio Tech

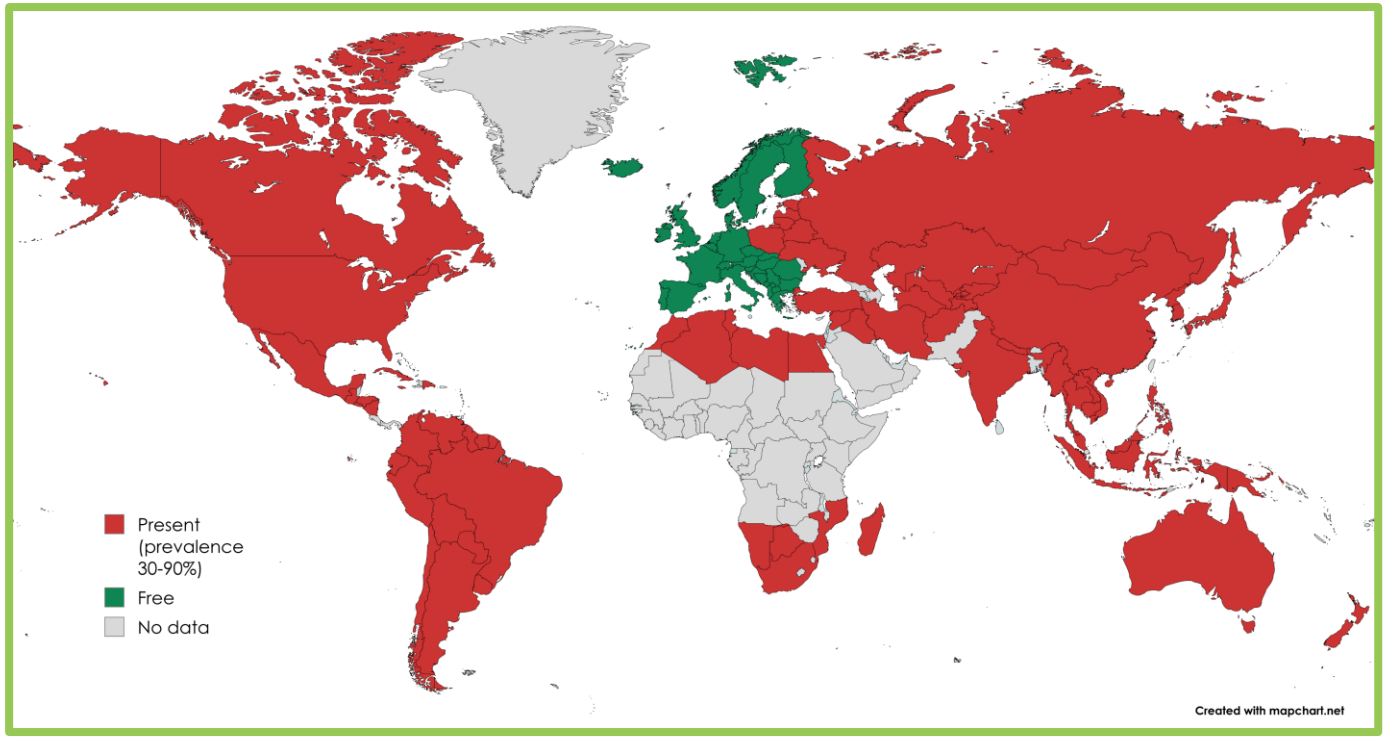
LIÈGE université
GIGA institute

HTLV 2026

5th June 2026

Introduction

Host species: Cattle, zebu, yak, bison and buffalo

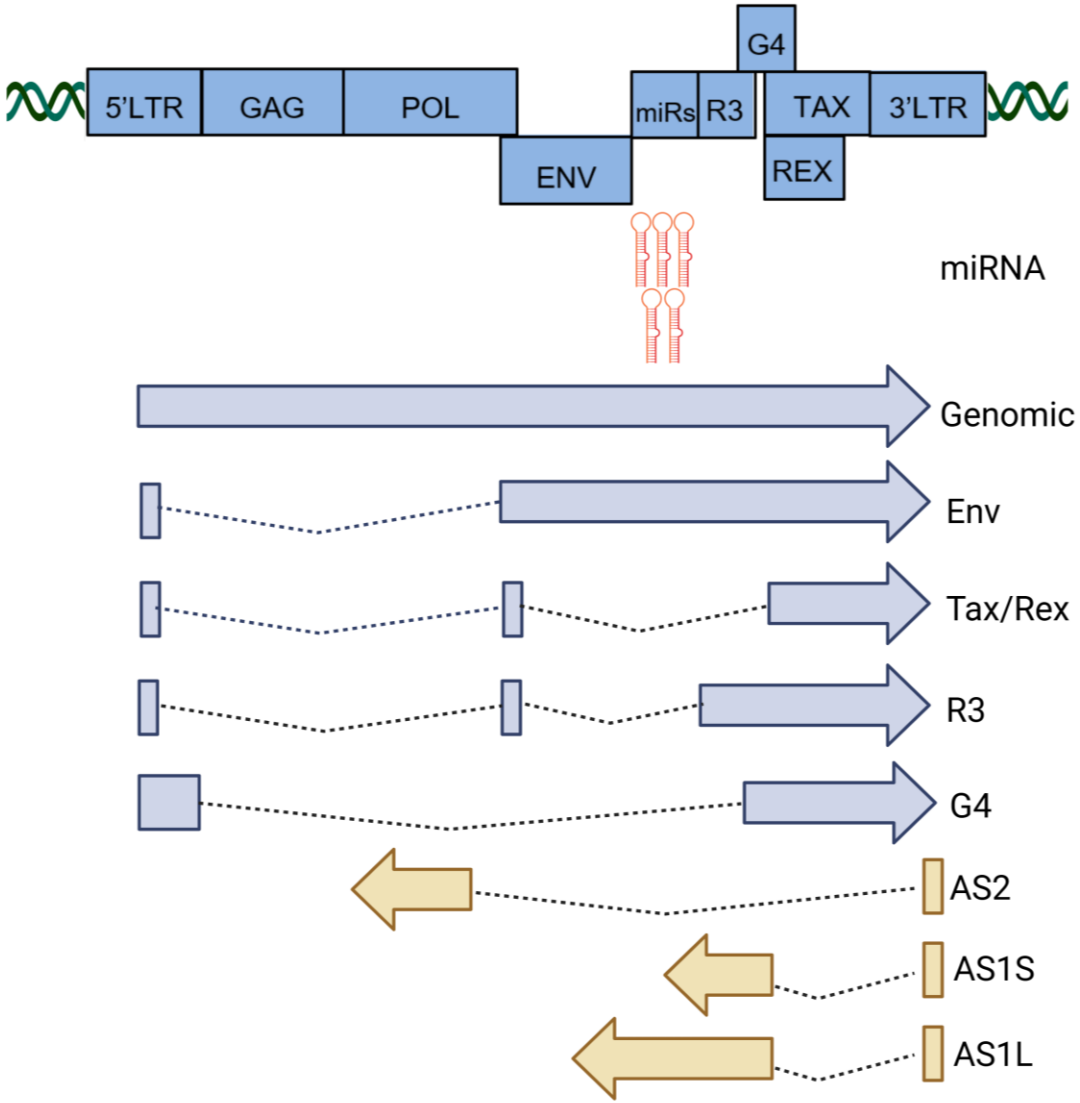


Pathology

- Leukemia (5%)
- Persistent lymphocytosis (30%)

Economic Losses

Shorter lifespan



PLOS | PATHOGENS

RESEARCH ARTICLE

Bovine Leukemia Virus Small Noncoding RNAs Are Functional Elements That Regulate Replication and Contribute to Oncogenesis In Vivo

Nicolas A. Gillet[1,2]*, Malik Hamaidia[1,2], Alix de Brogniez[1,2], Gerónimo Gutiérrez[3], Nathalie Renotte[1,2], Michal Reichert[4], Karina Trono[3], Luc Willems[1,2]*

PLOS PATHOGENS

RESEARCH ARTICLE

Ablation of non-coding RNAs affects bovine leukemia virus B lymphocyte proliferation and abrogates oncogenesis

Roghaiyeh Safari[1,2], Jean-Rock Jacques[1,2], Yves Brostaux[3], Luc Willems[1,2]*

PLOS PATHOGENS

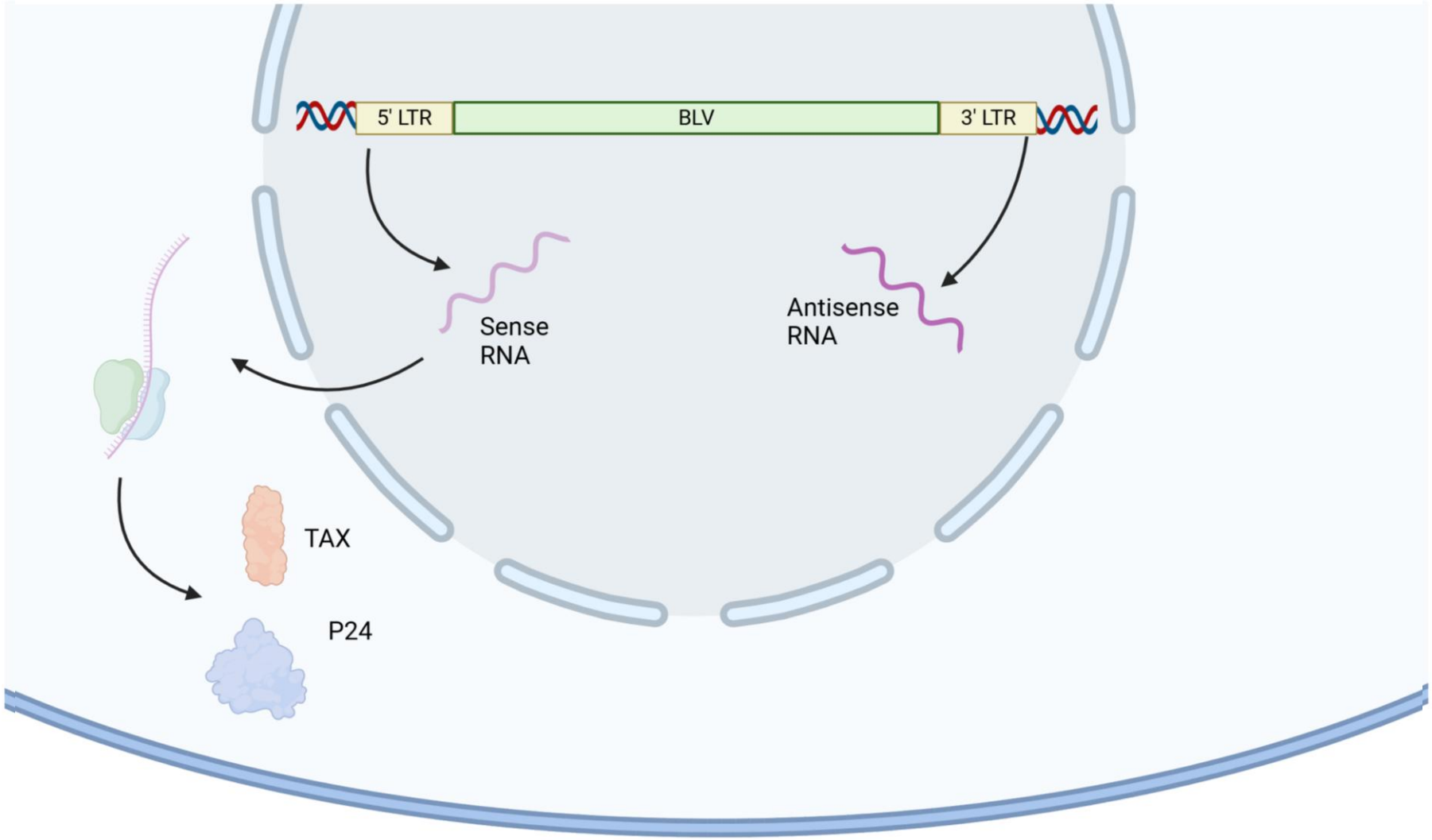
RESEARCH ARTICLE

Reduction of antisense transcription affects bovine leukemia virus replication and oncogenesis

Thomas Joris[1‡], Thomas Jouant[1‡], Jean-Rock Jacques[1], Lorian Gouverneur[1], Xavier Saintmard[1], Lea Vilanova Mañá[1], Majeed Jamakhani[1], Michal Reichert[2], Luc Willems[1]*

Joris, T. (2021) *Encyclopedia of Virology*
Durkin, K. (2016) *Retrovirology*

Aim of the study



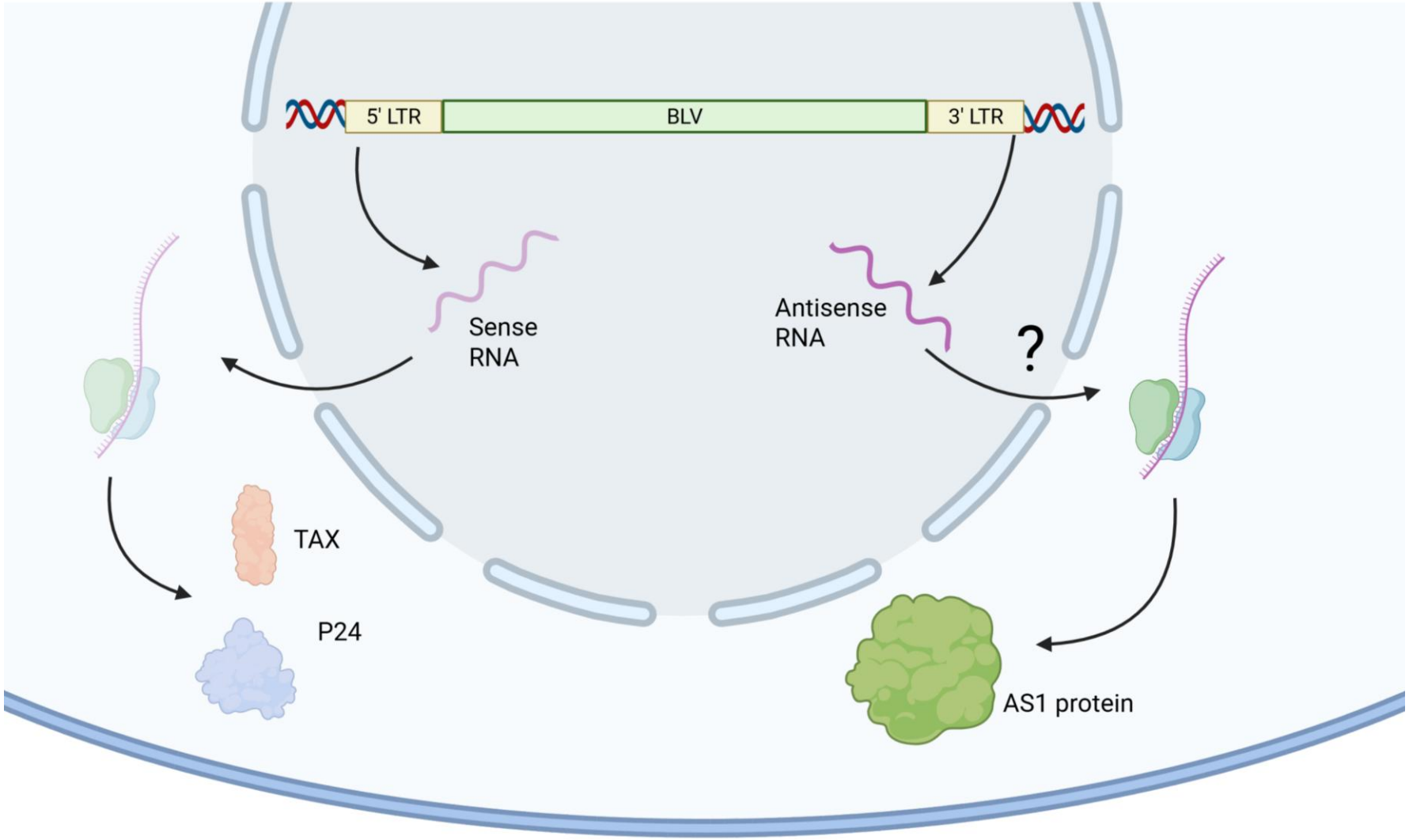
- Decrease in sense transcripts over time
- Antisense RNAs are constitutively expressed

What is the role of antisense RNAs?

- Proviral load ↘
- Pathogenicity ↘

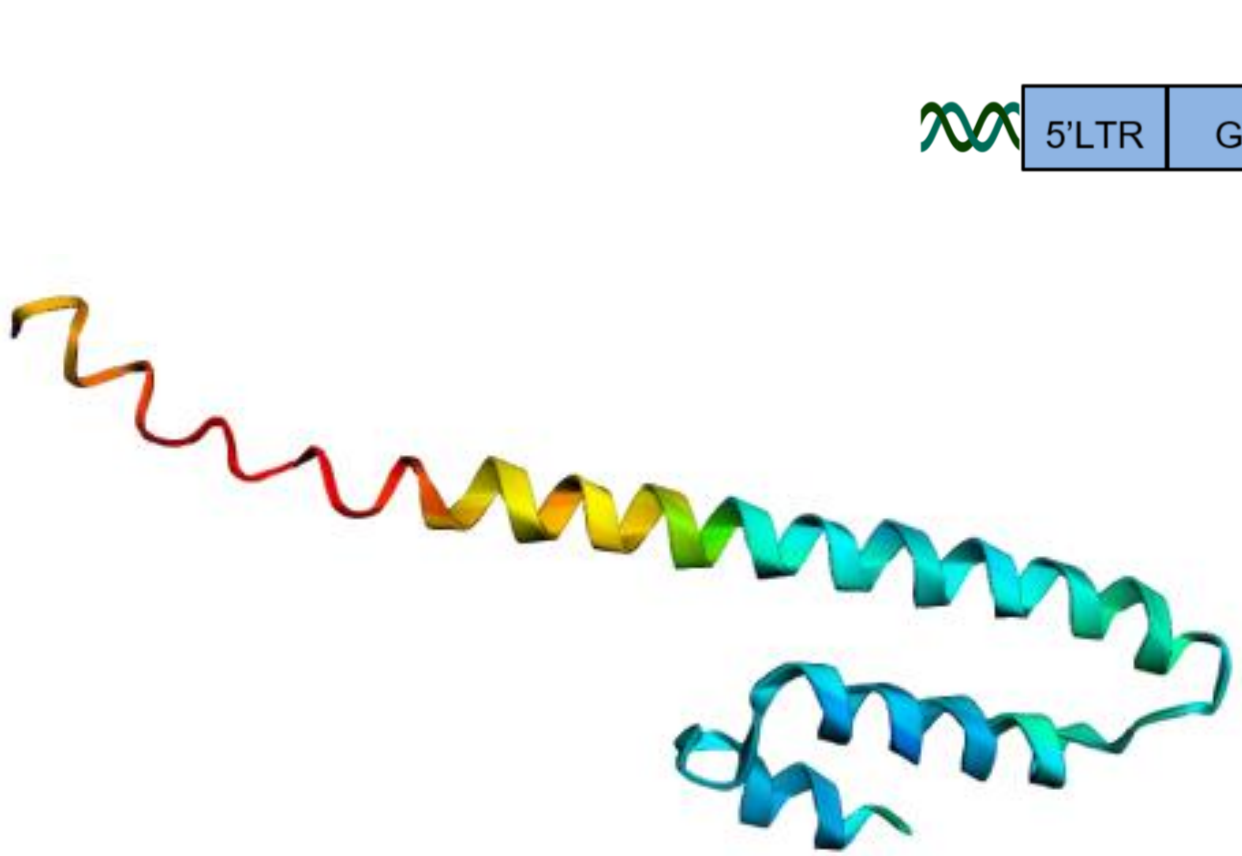
Joris, T. (2024) *PLoS Pathog*

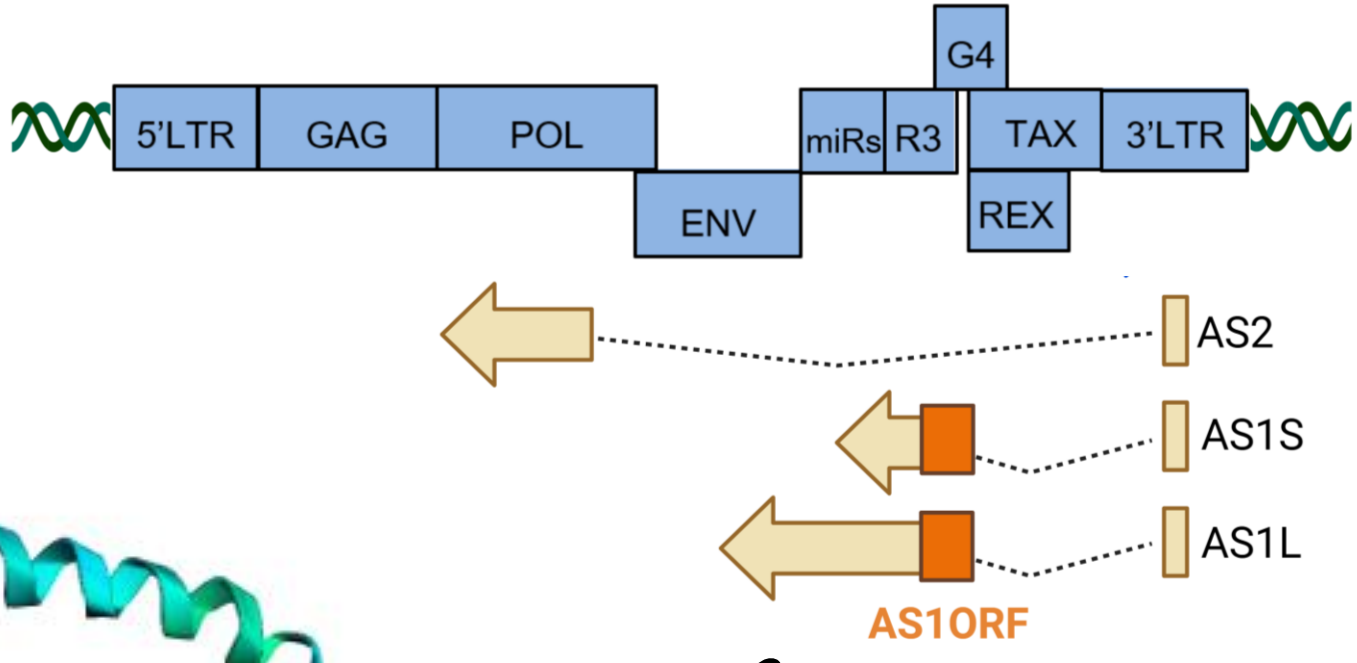
Aim of the study



Antisense RNAs are predicted to be non-coding

The AS1 RNA contains a 264-bp open reading frame



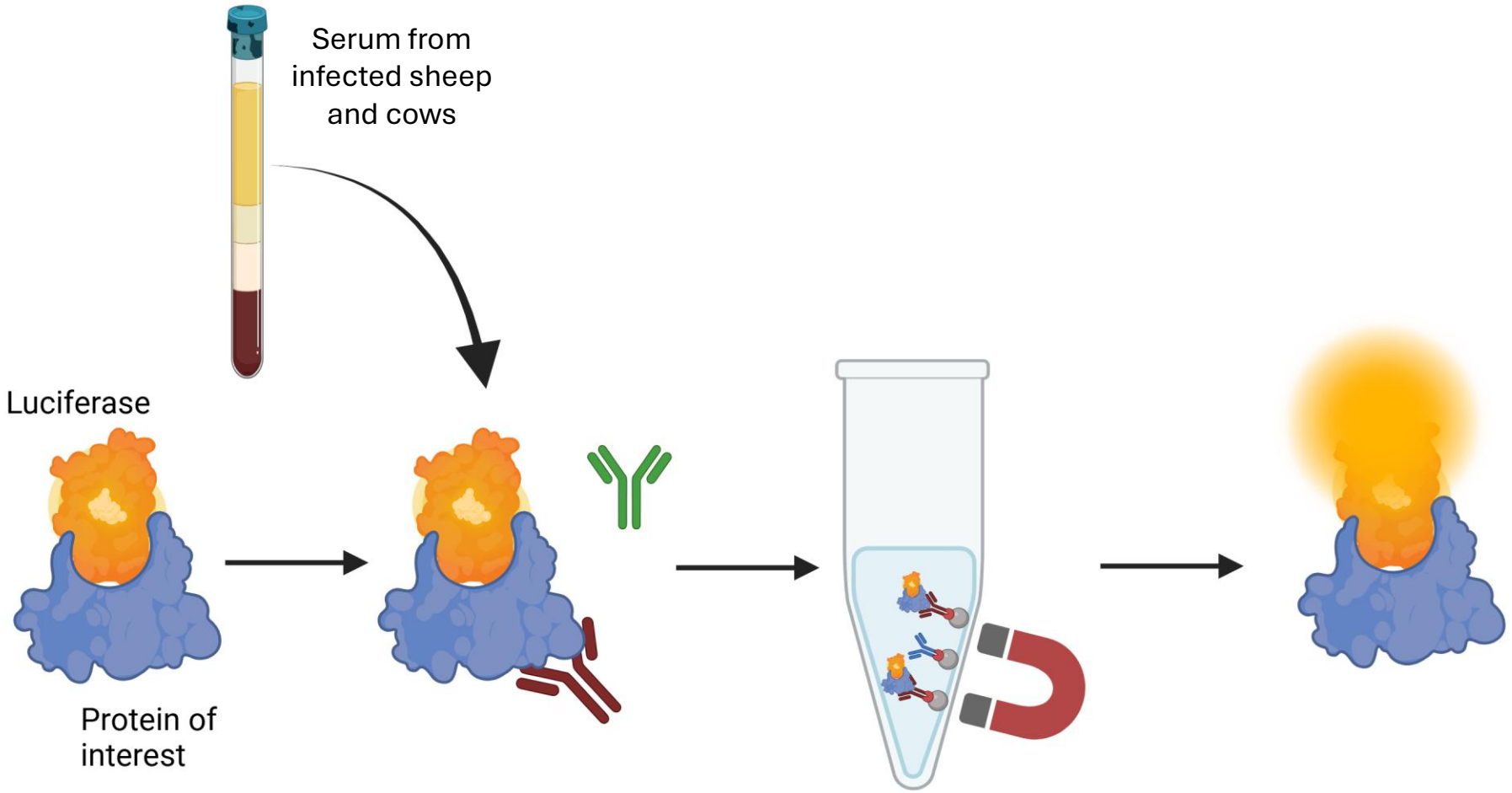


AlphaFold2

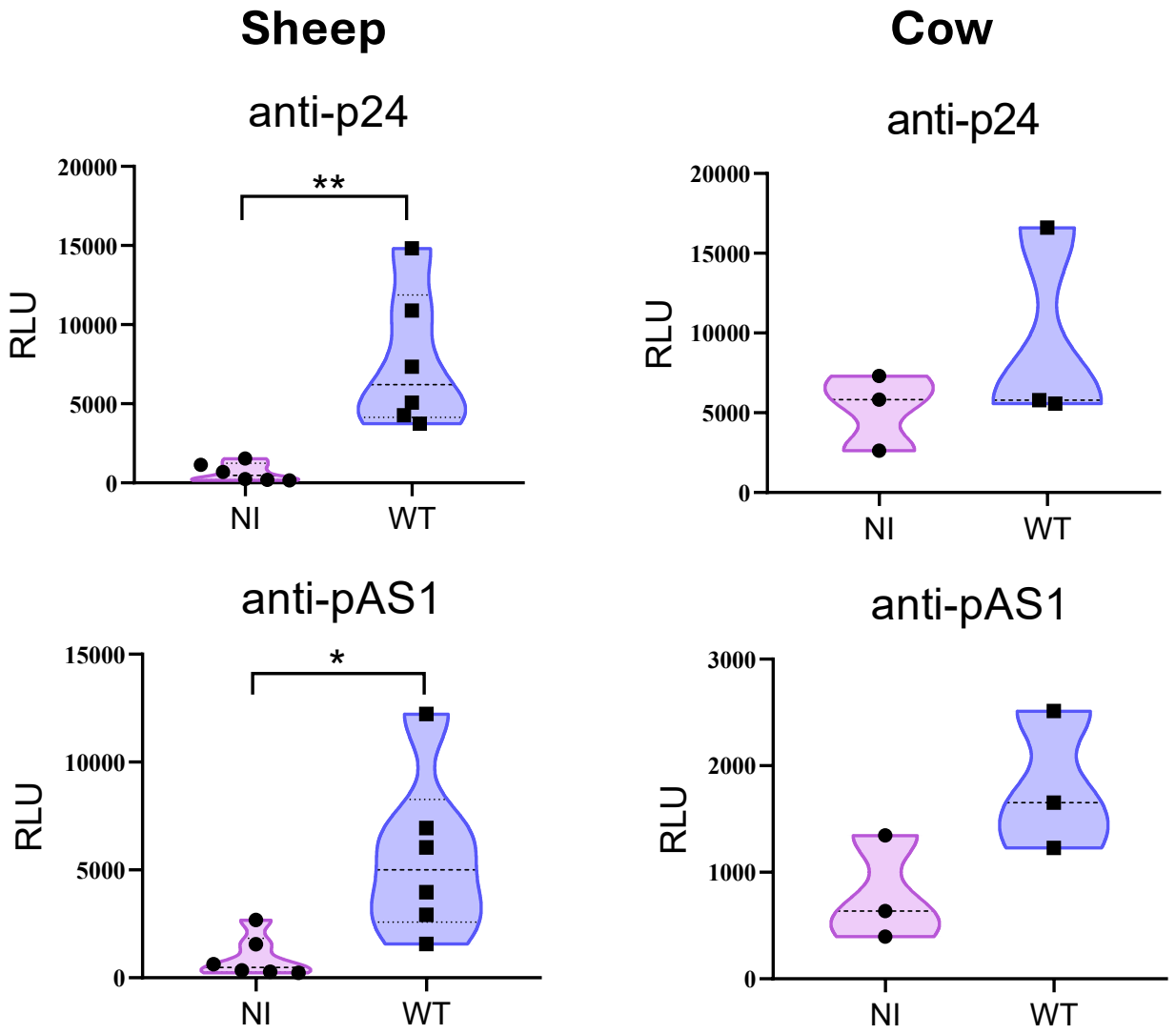
Result

What is the evidence that a protein is translated from AS1ORF ?

↳ Luciferase immunoprecipitation system (Lips)



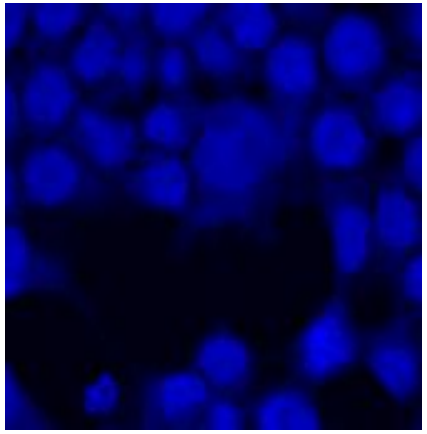
What is the evidence that a protein is translated from AS1ORF ?



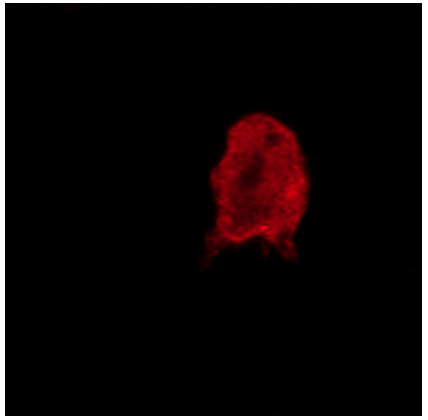
→ Antibodies against AS1 are detected in vivo

Does AS1 protein interact with a viral protein ?

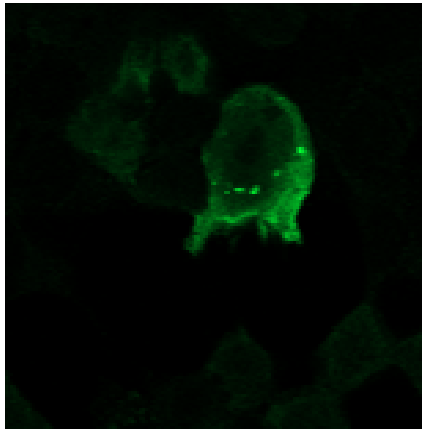
DAPI



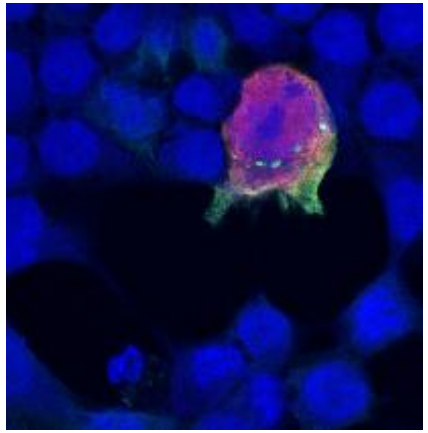
AS1

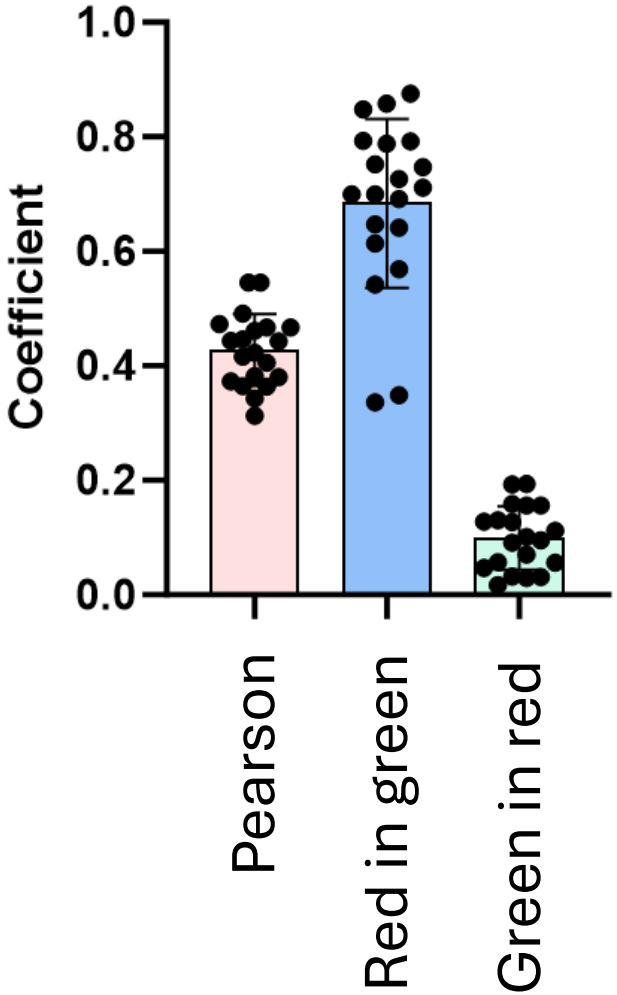


Tax



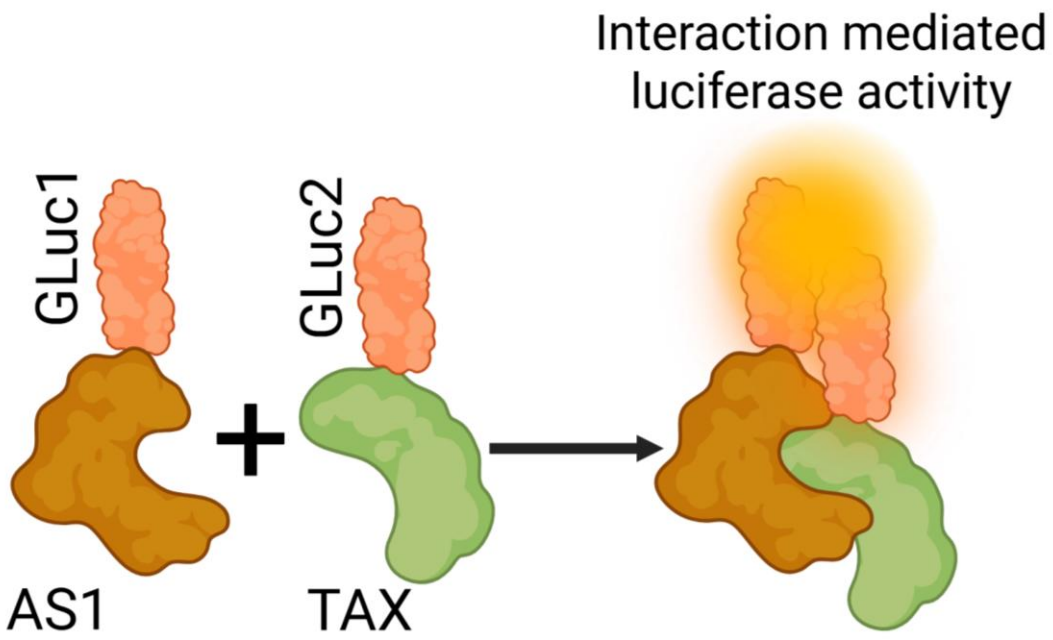
Merge

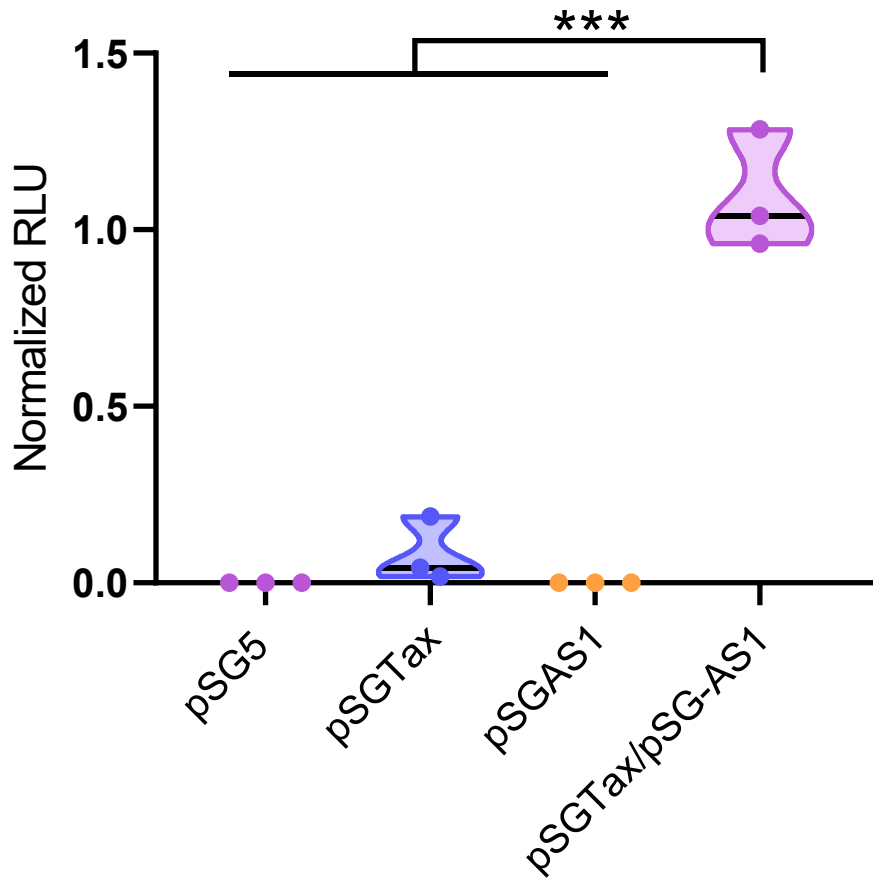




0 → 0,3 low
0,3 → 0,5 moderate
0,5 → 1 strong

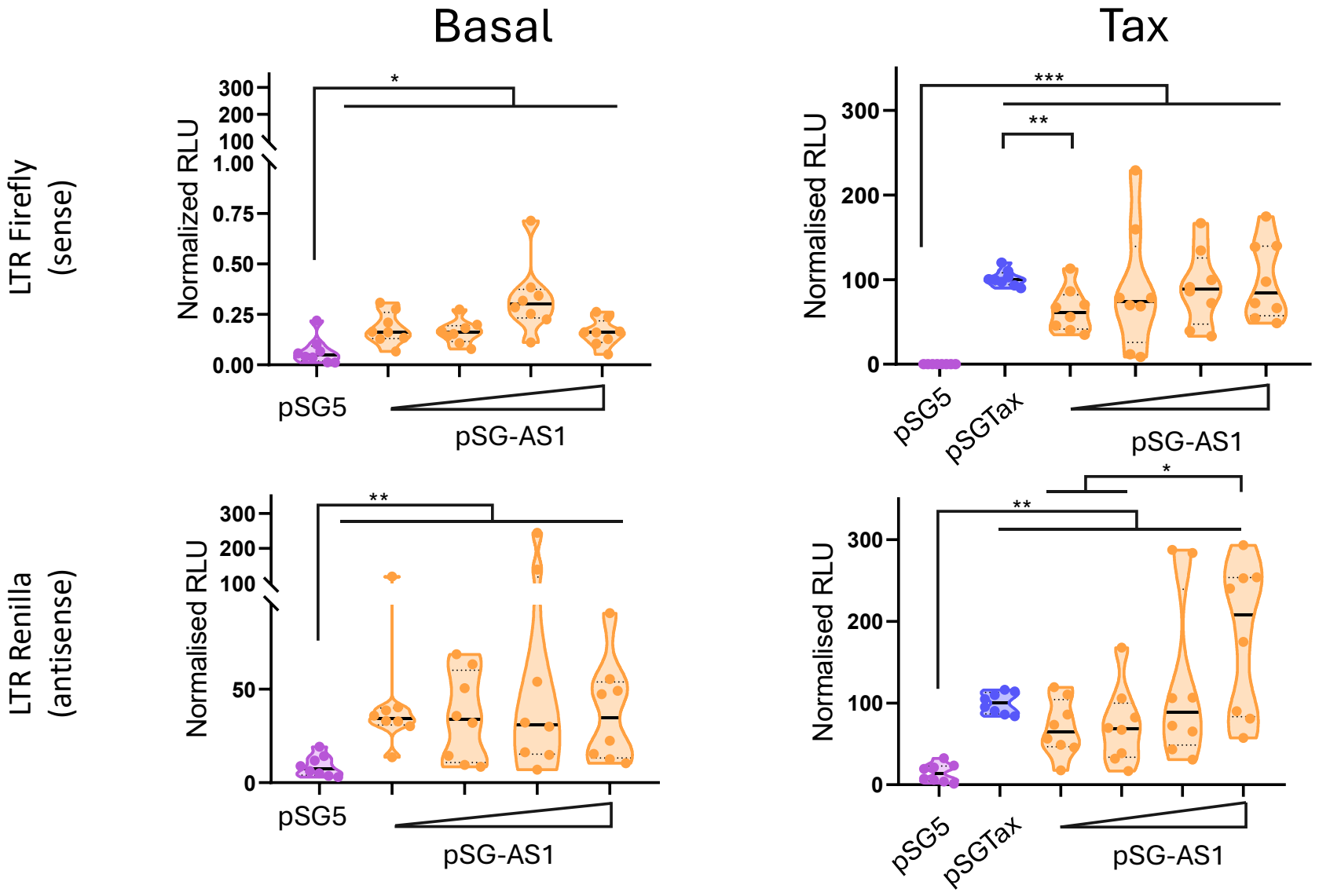
Does AS1 protein interact with a viral protein ?





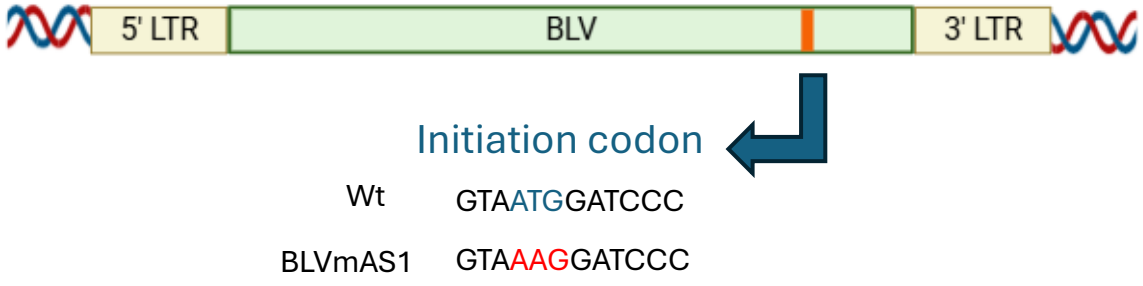
→ The AS1 protein interacts with Tax

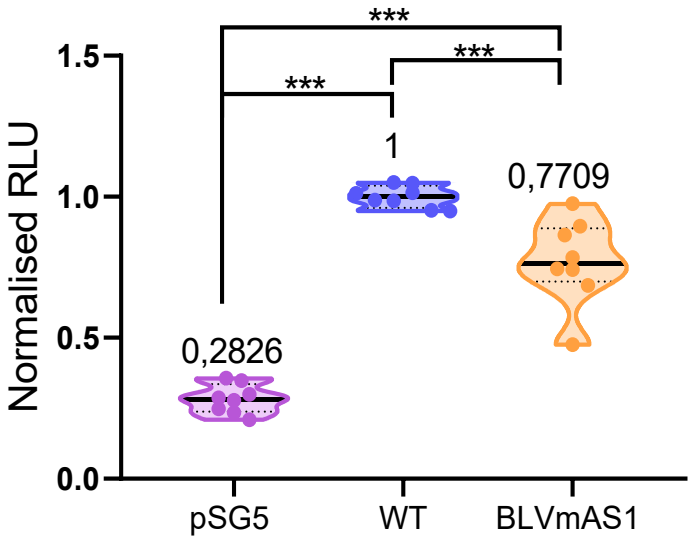
Does AS1 protein influence LTR transcription ?

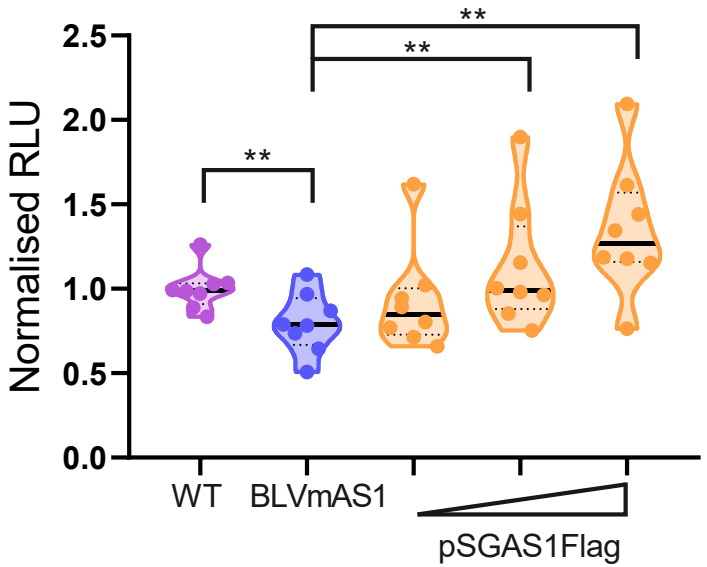


→ The AS1 increases basal transcription directed by the LTR without impacting Tax transactivation

Does AS1ORF impact viral infectivity in cells ?

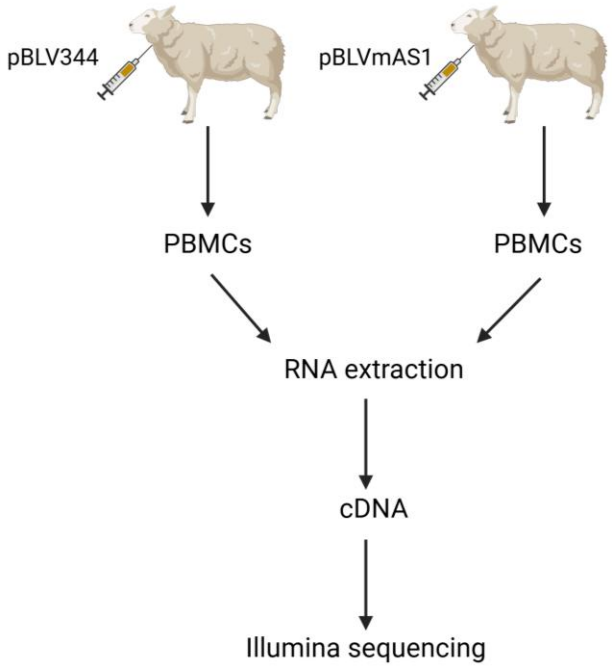


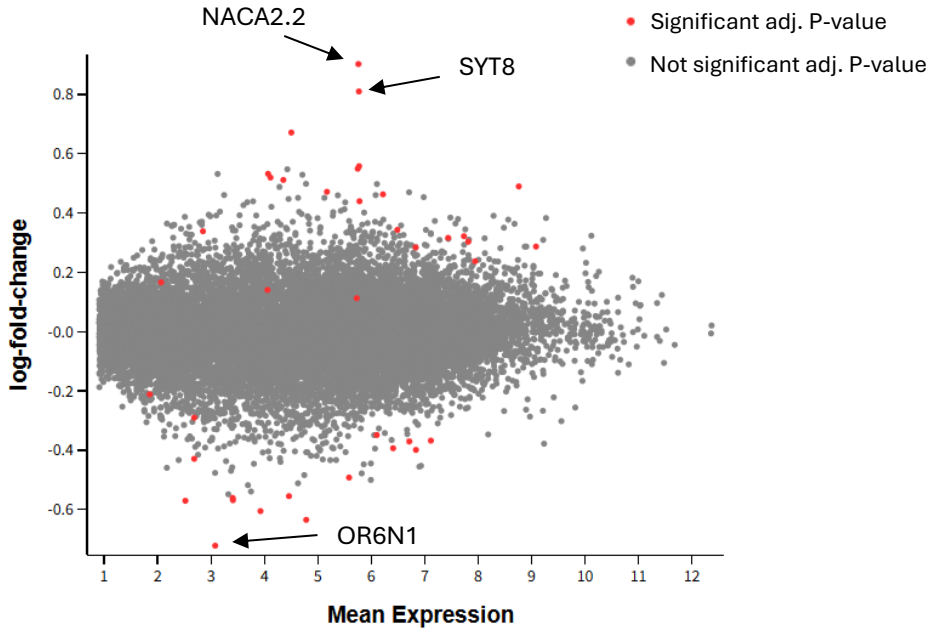


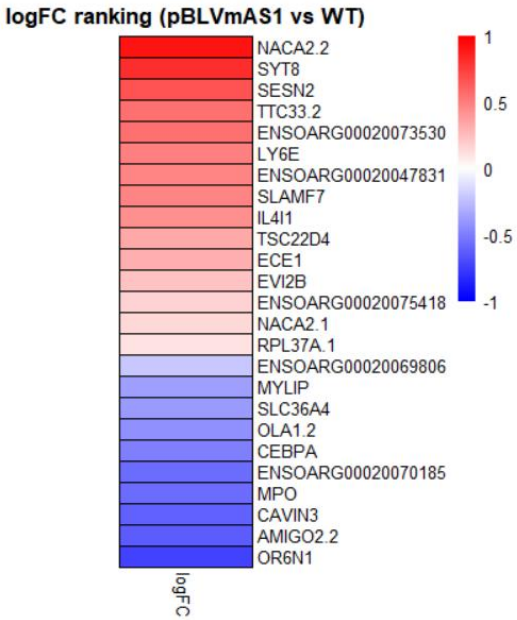


→ Mutation of the AS1ORF decreases infectivity in vitro
The AS1 protein increases the infectivity of the AS1-deficient provirus

What is the effect of AS1ORF in vivo ?

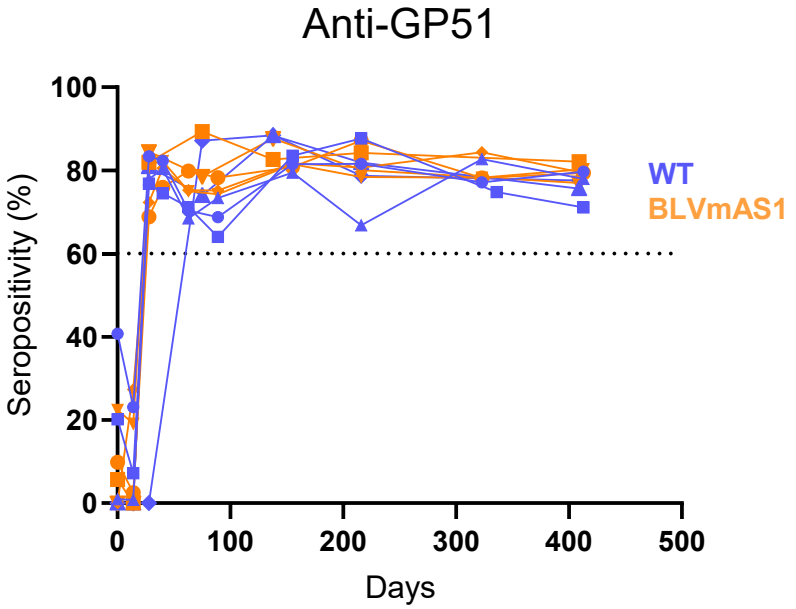


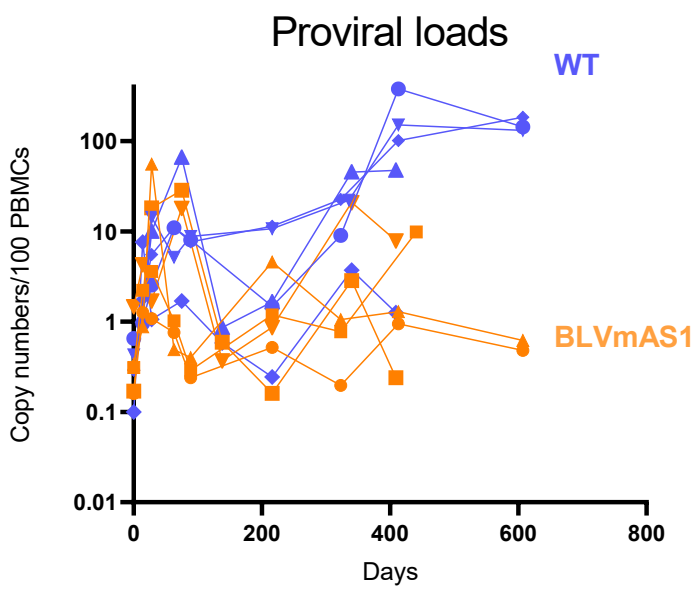


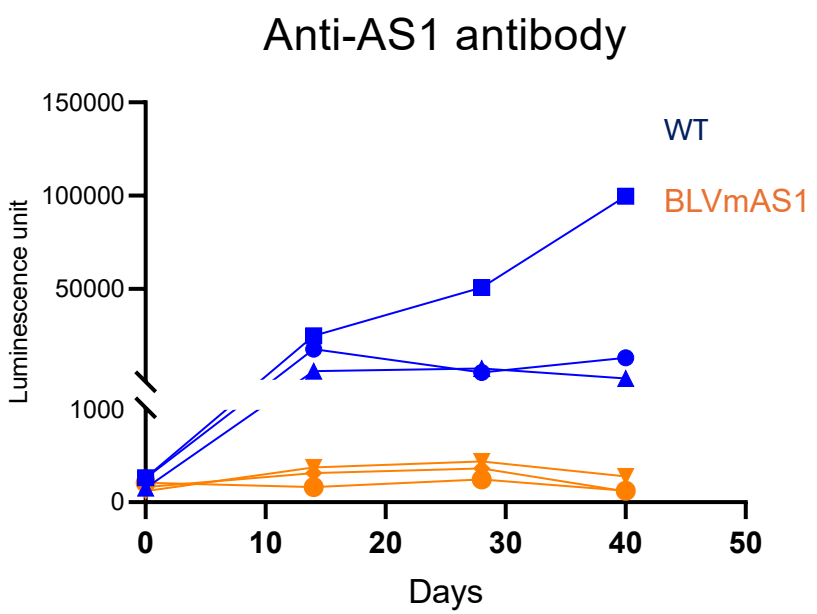


→ Mutation of the AS1ORF modifies the transcriptome of sheep PBMCs

Does AS1ORF impact viral infectivity in sheep ?

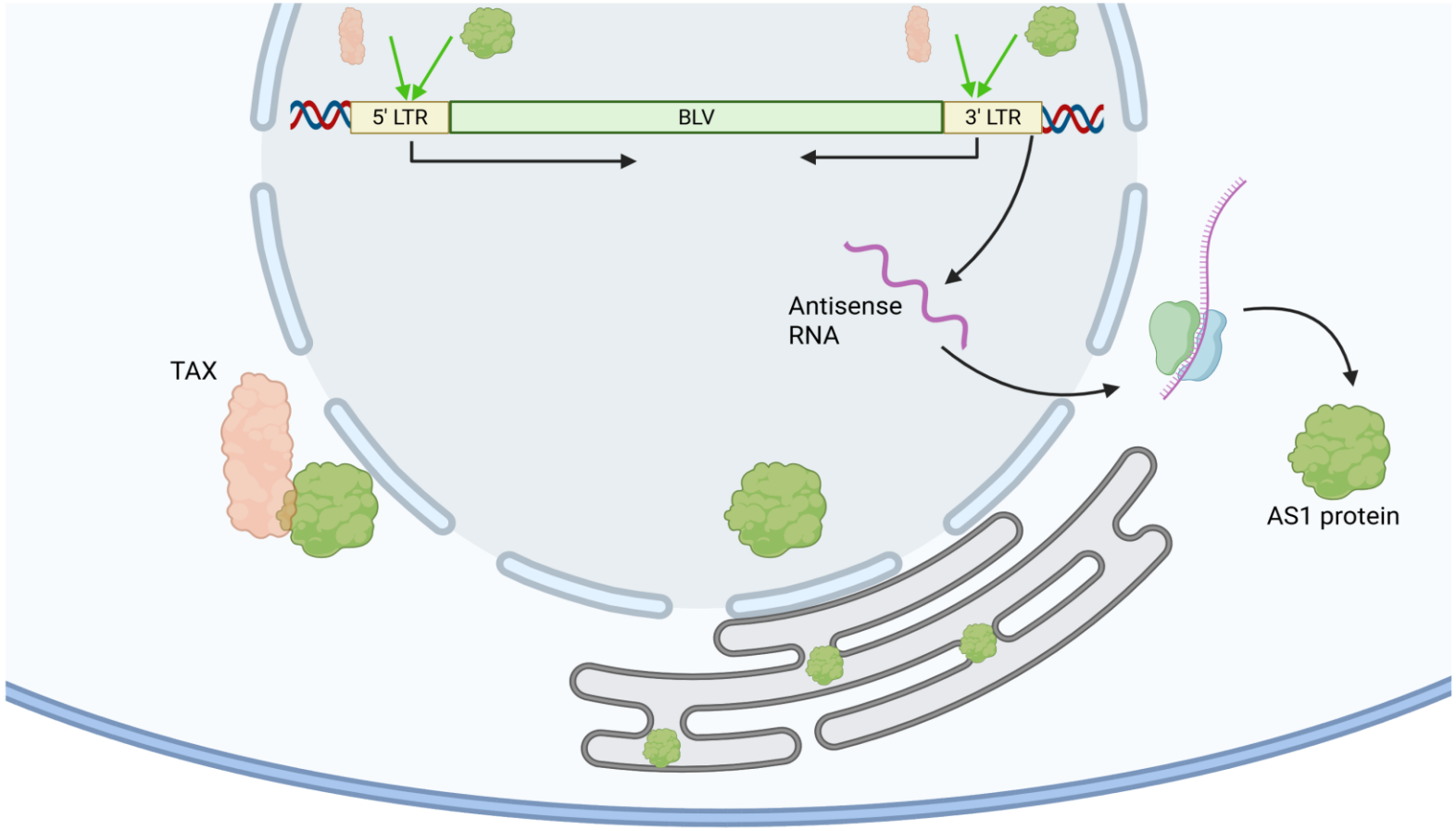






→ The AS1-deficient provirus (pBLVmAS1) is infectious, replicates at reduced proviral load in sheep

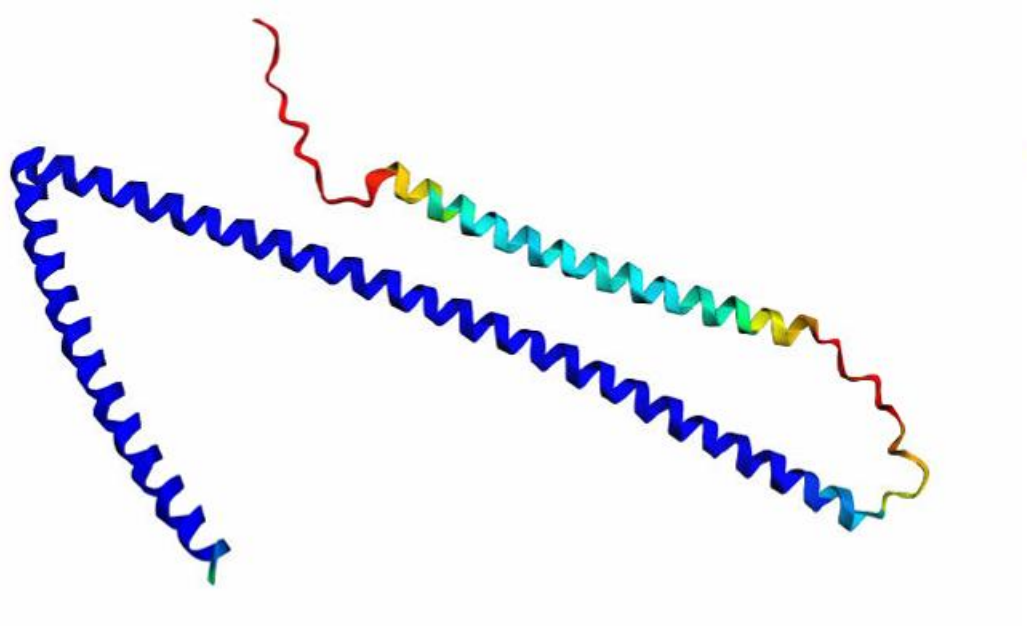
Conclusion



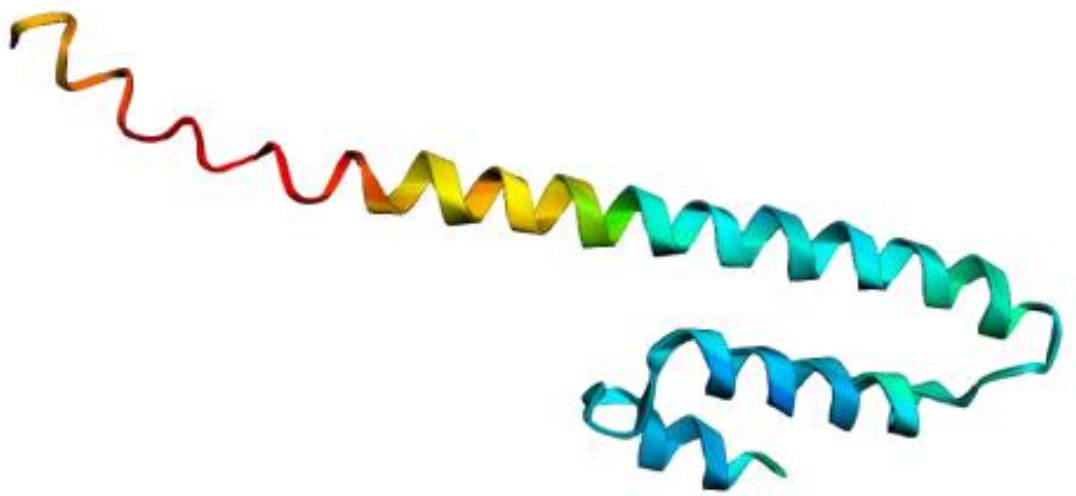
- Evidence of AS1 protein (pAS1) expression in vivo
- pAS1 is localized primarily in the nucleus and the perinuclear region
- pAS1 interacts with Tax
- pAS1 enhances viral infectivity
- A mutation in the translation initiation codon reduces infectivity and proviral load

Conclusion

sHBZ



AS1



Acknowledgements

thomas.jouant@uliege.be

ECM Lab
(Liege University, Belgium)

Luc Willems

Malik Hamaidia

Alexis Fontaine

Thomas Jouant

Xavier Saintmard

Roxane Terres

Florine Doucet

Manon Zwaenepoel

Songkang Qin

Chen Tan

Dandan Ye

Jean-Rock Jaques

Mei Ren

Léa Bulsei

Yunghang Zhang

Animal Genomics Lab
(Liege University, Belgium)

Anne Van den Broeke

Anne-Sophie Reuter

Damien Renette

Bioinformatics platform
(Liege University, Belgium)

Arnaud Lavergne

Institut de recherche en infectiologie
(Montpellier University, France)

Antoine Gross

National Veterinary Research Institute (Pulawy, Poland)

Michal Reichert

Instituto Nacional de Tecnología Agropecuaria
(Buenos Aires, Argentina)

Karina Trono

Illustrations Created in BioRender.com

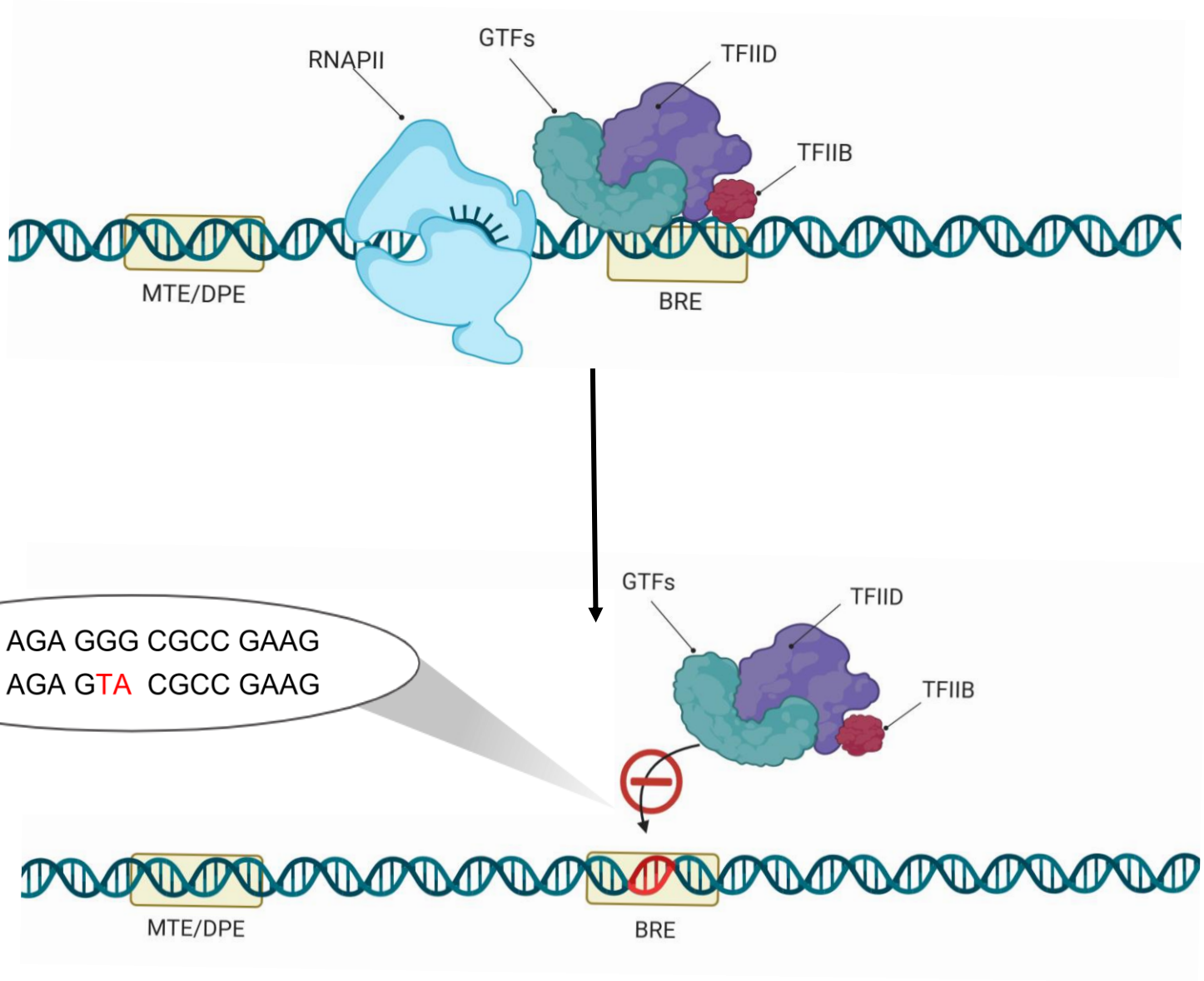
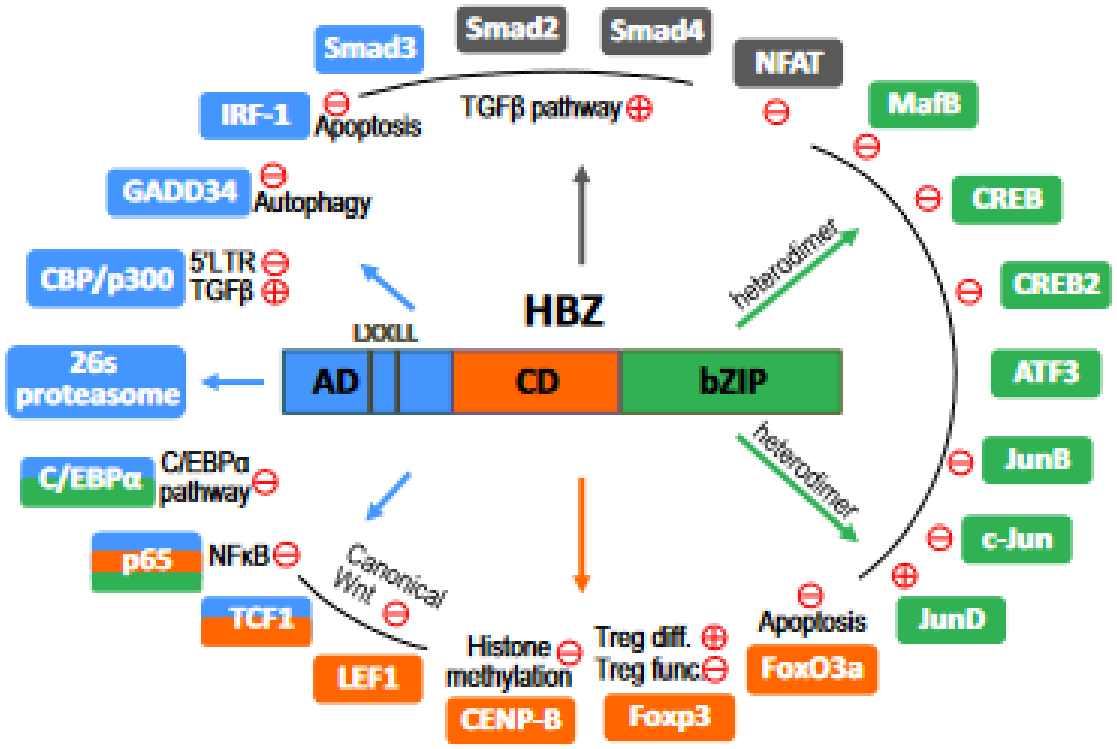
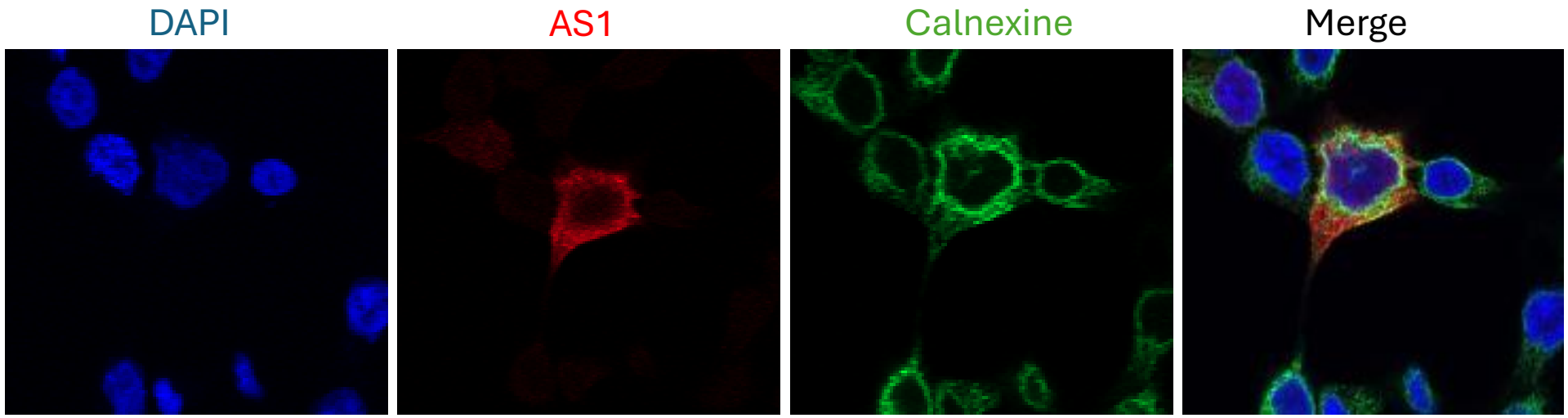

RNAPII
GTFs
TFIID
TFIIB
MTE/DPE
BRE
WT AGA GGG CGCC GAAG
A1 AGA GTA CGCC GAAG
GTFs
TFIID
TFIIB
MTE/DPE
BRE

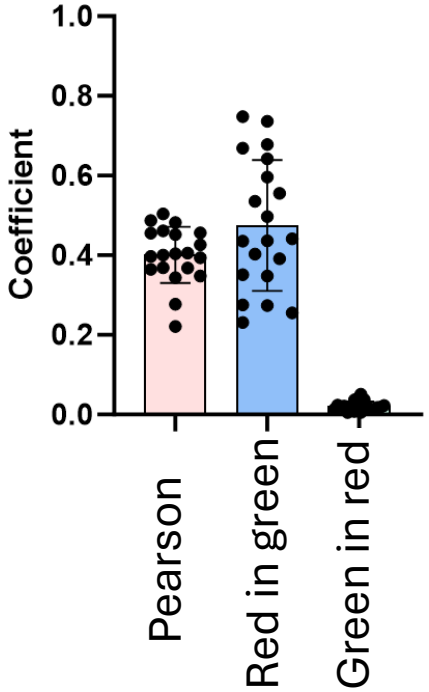
Table 1 | Character and regulatory function of Tax and HBZ.

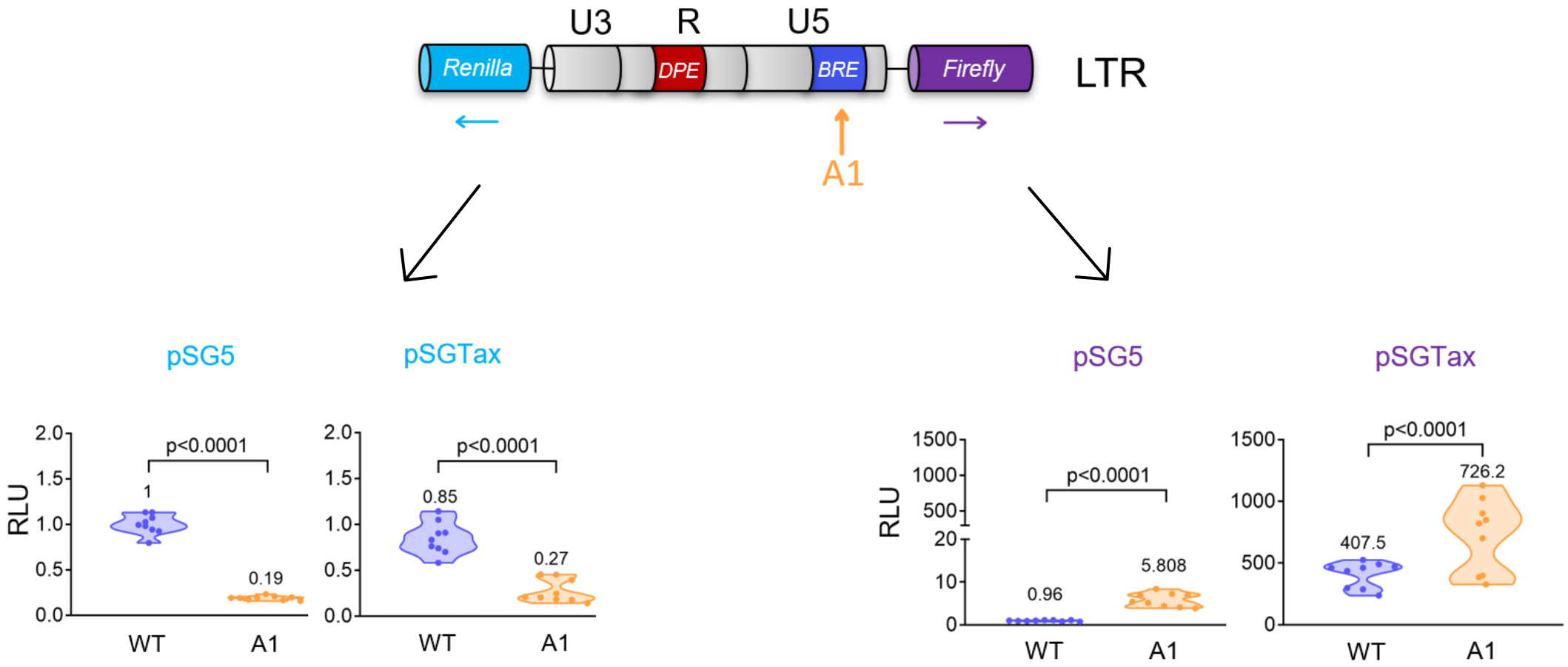
	Tax	HBZ
Expression in ATL cells	~40%	100%
Contain genetic changes	Yes	No
Function as mRNA	ND	Yes
Immortalize T cells	Yes	No
Target by CTL	Yes	No
HTLV-1 5′LTR transcription	Activate	Inhibit
Classical NF-κB pathway	Activate	Inhibit
Alternative NF-κB pathway	Activate	No
AP-1 activity	Activate	Inhibit
JunD transcription	Activate	Activate
T-cell proliferation	Activate	Activate
TGF-β signaling	Inhibit	Activate
Host immune response	Enhance	Suppress



Zhao, T. *et al. Front. Microbiol.* 3, (2012)
Ma, G. *et al. Retrovirology* 13, 16 (2016)

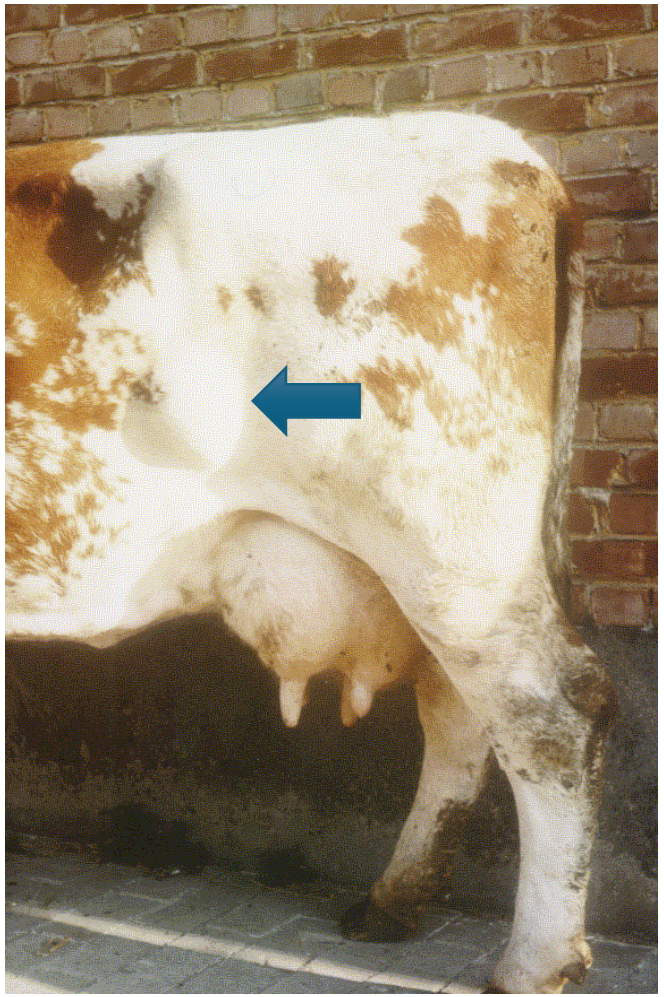

DAPI
AS1
Calnexine
Merge


Coefficient
1.0
0.8
0.6
0.4
0.2
0.0
Pearson
Red in green
Green in red


U3
R
U5
Renilla
DPE
BRE
Firefly
LTR
A1
pSG5
pSGTax
pSG5
pSGTax
RLU
p<0.0001
1
0.19
0.85
0.27
0.96
5.808
407.5
726.2
WT
A1

BLV

- naturally infects the bovine species
- can be experimentally transmitted to sheep
- Present in wild-life: llama, yak, bison, water bufalo, zebu, alpaca (Vicugna pacos)
- one third of cows develop a persistent lymphocytosis
- up to 10 % of acute B-cell leukemia or lymphoma
- weakens immune response (mastitis)
- poor effectiveness of vaccination against other pathogens (BHV-1, rotavirus, coronavirus, L Pomona)
- economic loss: death, reduction in milk production, custom restrictions, first cause of carcass condemnation
- a vaccine is available



Gillet et al Retrovirology **4**: 18; Doucet et al Viruses **17**: 10.3390

Loss of viral enhancer and viral CTCF binding site impairs *Hbz* expression and HTLV-1 persistence

Amanda Panfil, PhD

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

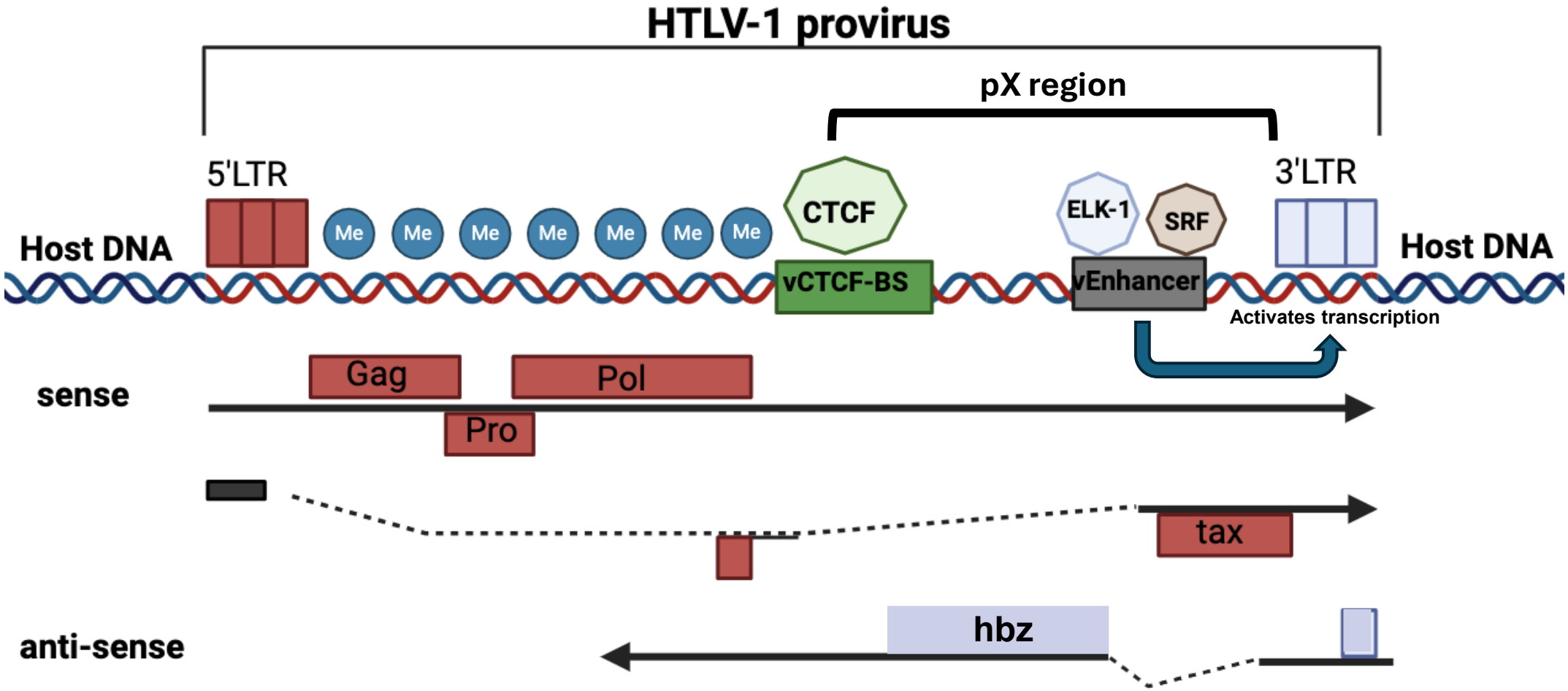
June 3-6, 2026 | Philadelphia, Pennsylvania, USA

THE OHIO STATE UNIVERSITY
CENTER FOR RETROVIRUS RESEARCH

Conflicts of interest:

I have no conflicts of interest.

HTLV-1 proviral genetic and epigenetic elements

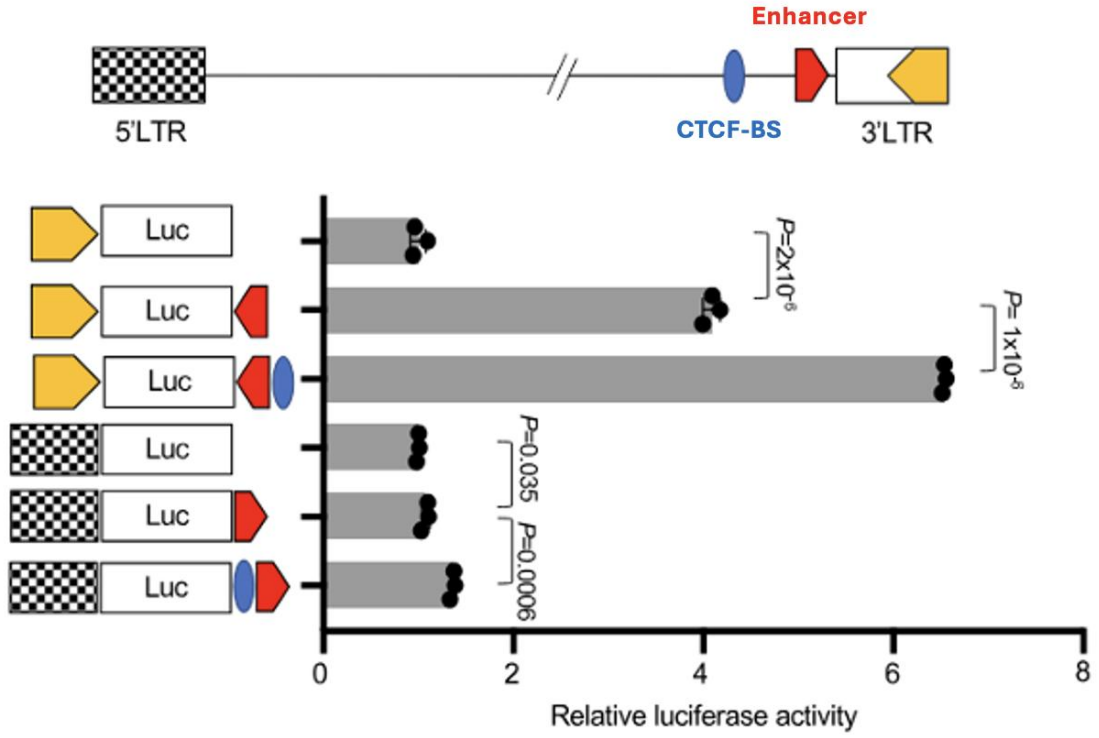


Hbz contributes to viral persistence and disease progression

Satou et al, PNAS (2016)

Cheng et al, PLOS Pathogens (2021)

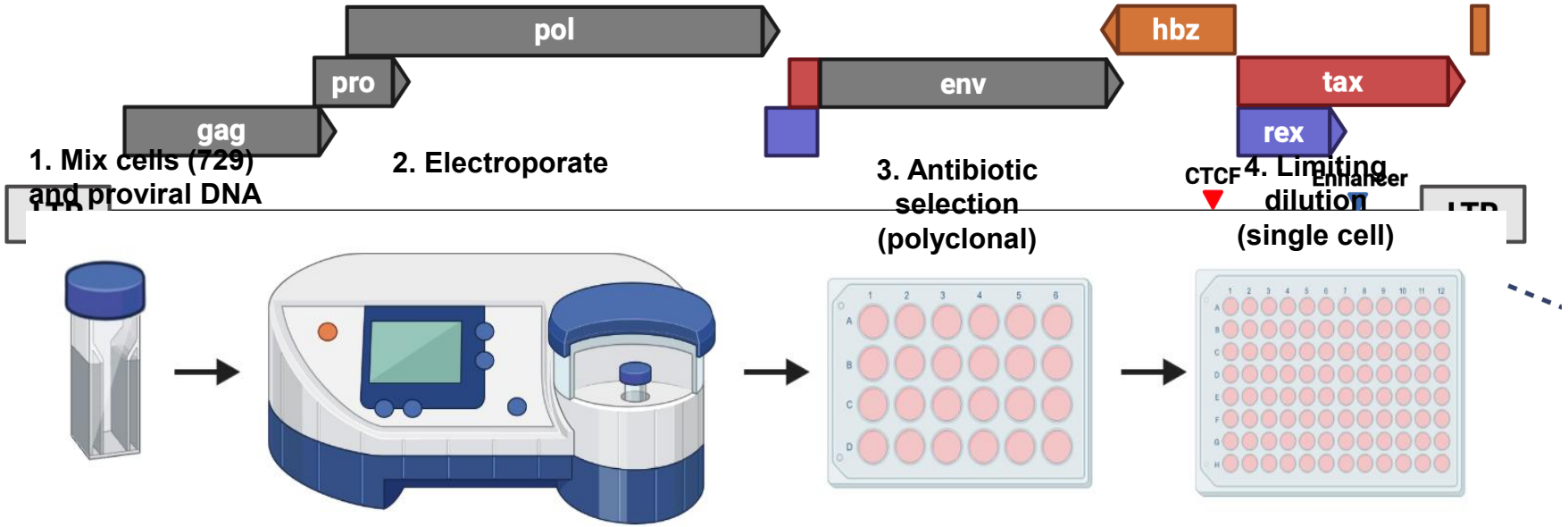
vCTCF-BS increases vEnhancer activity at the 3' LTR promoter

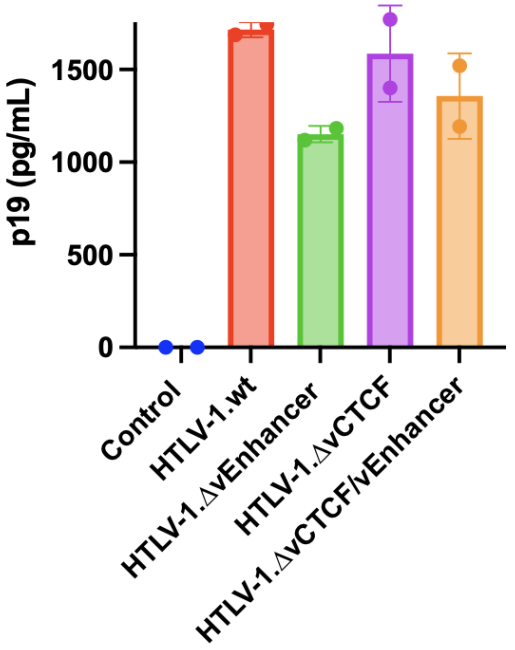


Matsuo, Nature Communications (2022)

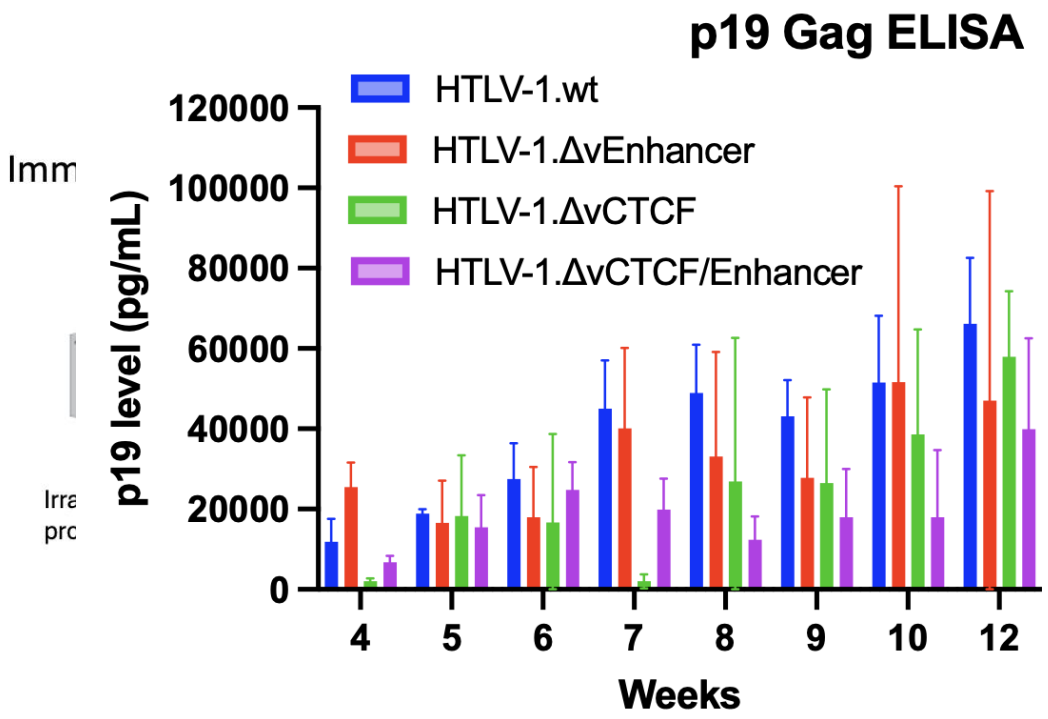
Hypothesis: vCTCF-BS and vEnhancer element cooperate to activate *hbz* gene expression, promoting cellular proliferation, and thus influencing viral persistence and disease progression

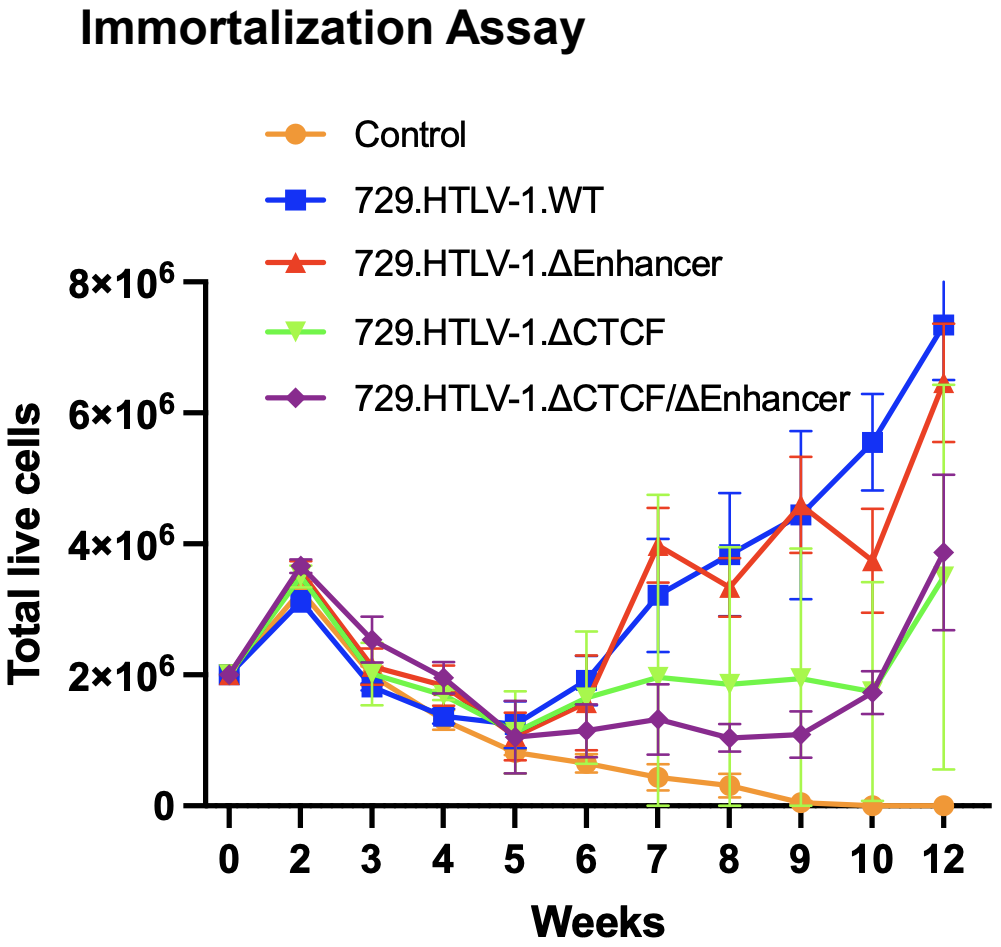
Generation of HTLV-1.△vCTCF/vEnhancer proviral plasmid



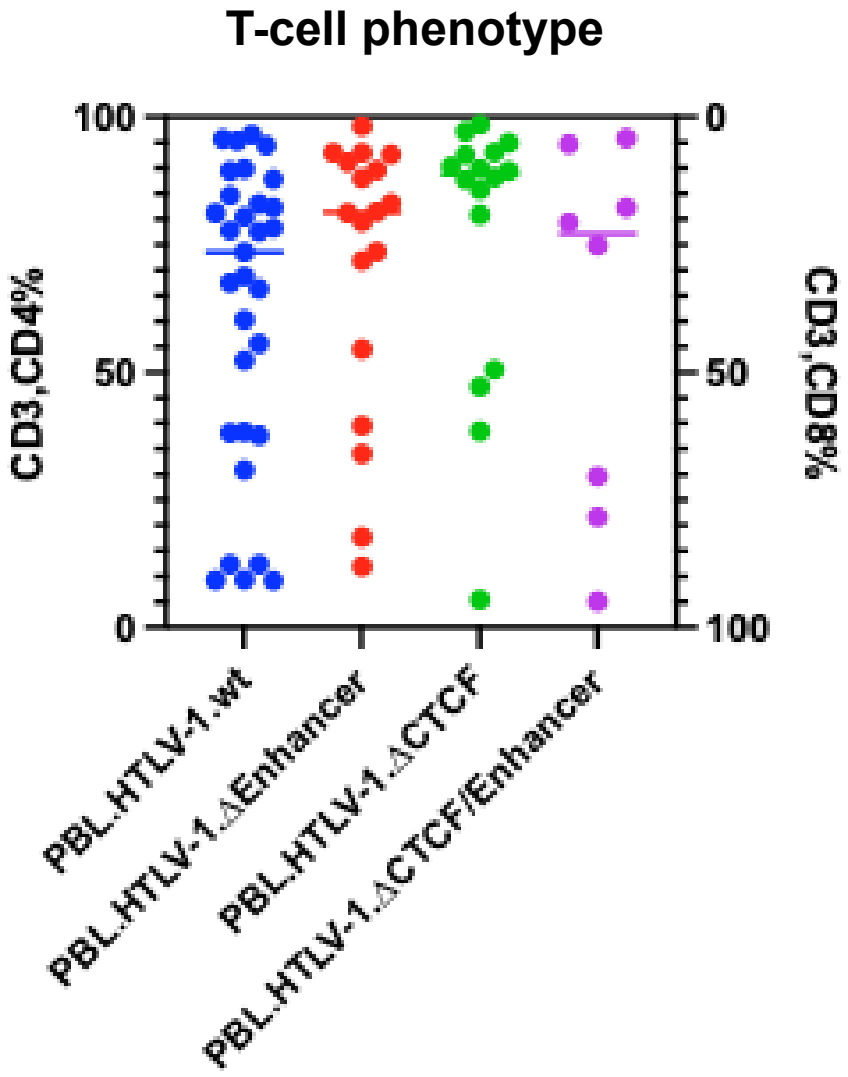


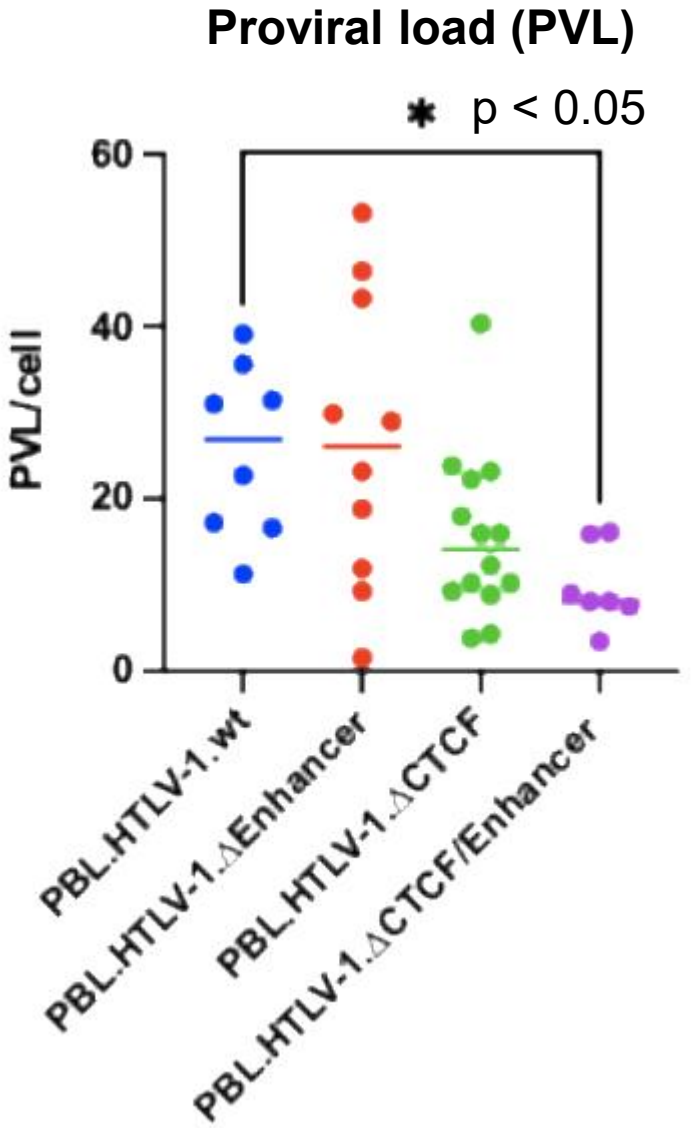
vCTCF-BS and vEnhancer element are dispensable for infection & immortalization



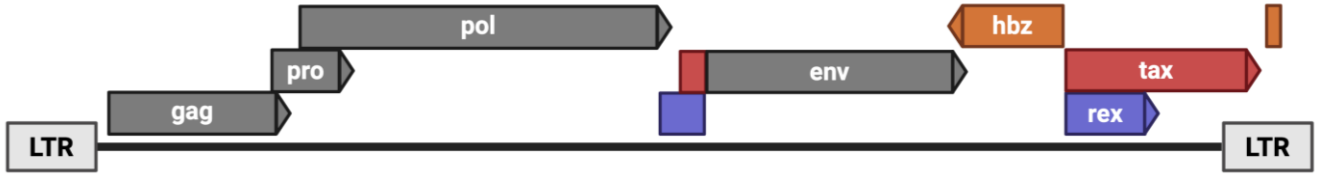


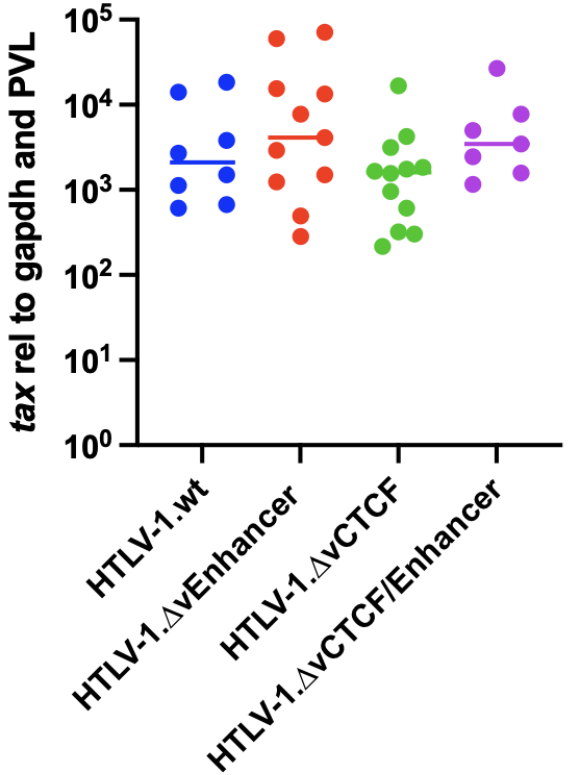
Loss of viral CTCF-BS and vEnhancer element decreases PVL

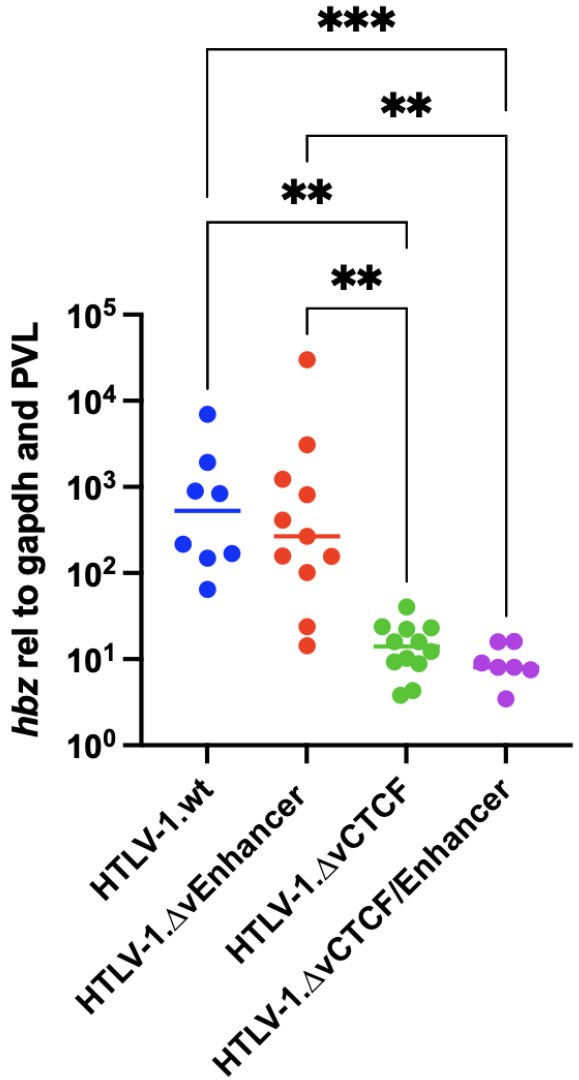




Loss of vCTCF-BS and vEnhancer element decreases hbz transcript abundance in newly immortalized T-cell lines

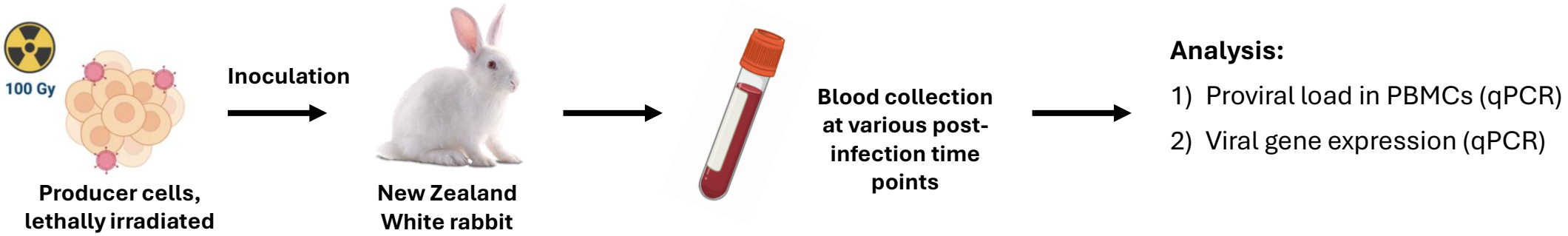


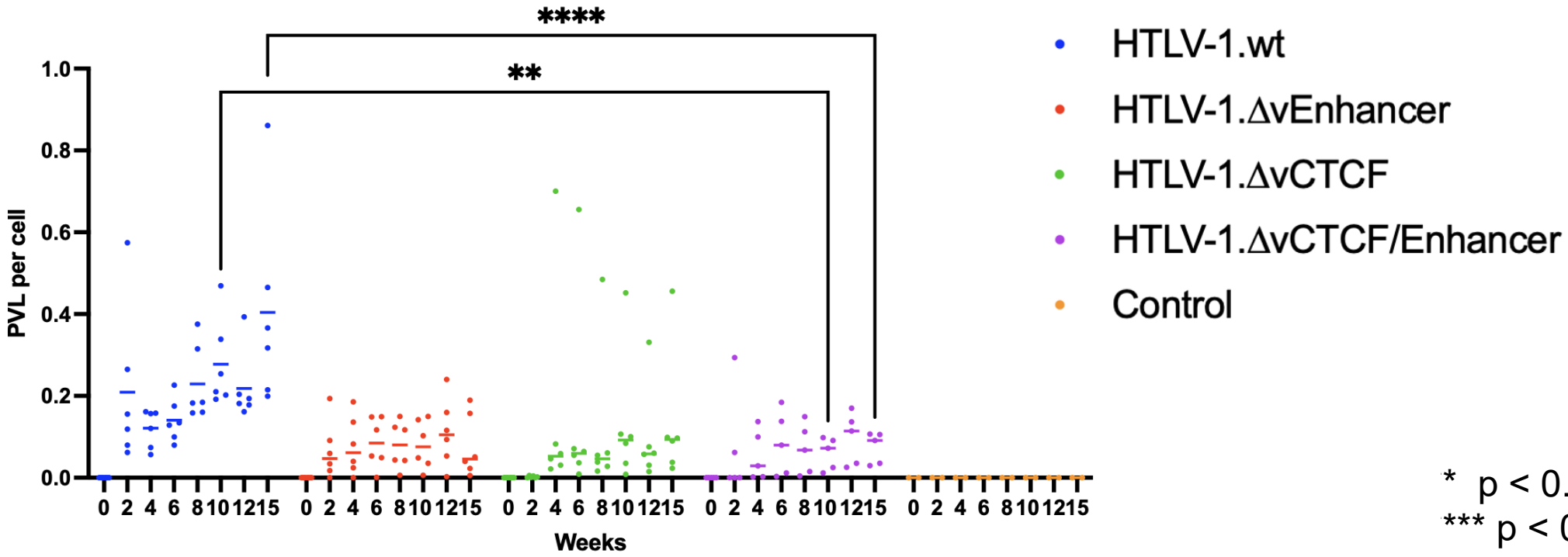




** p < 0.01
*** p < 0.001

Loss of vCTCF-BS and vEnhancer element decreases PVL *in vivo*



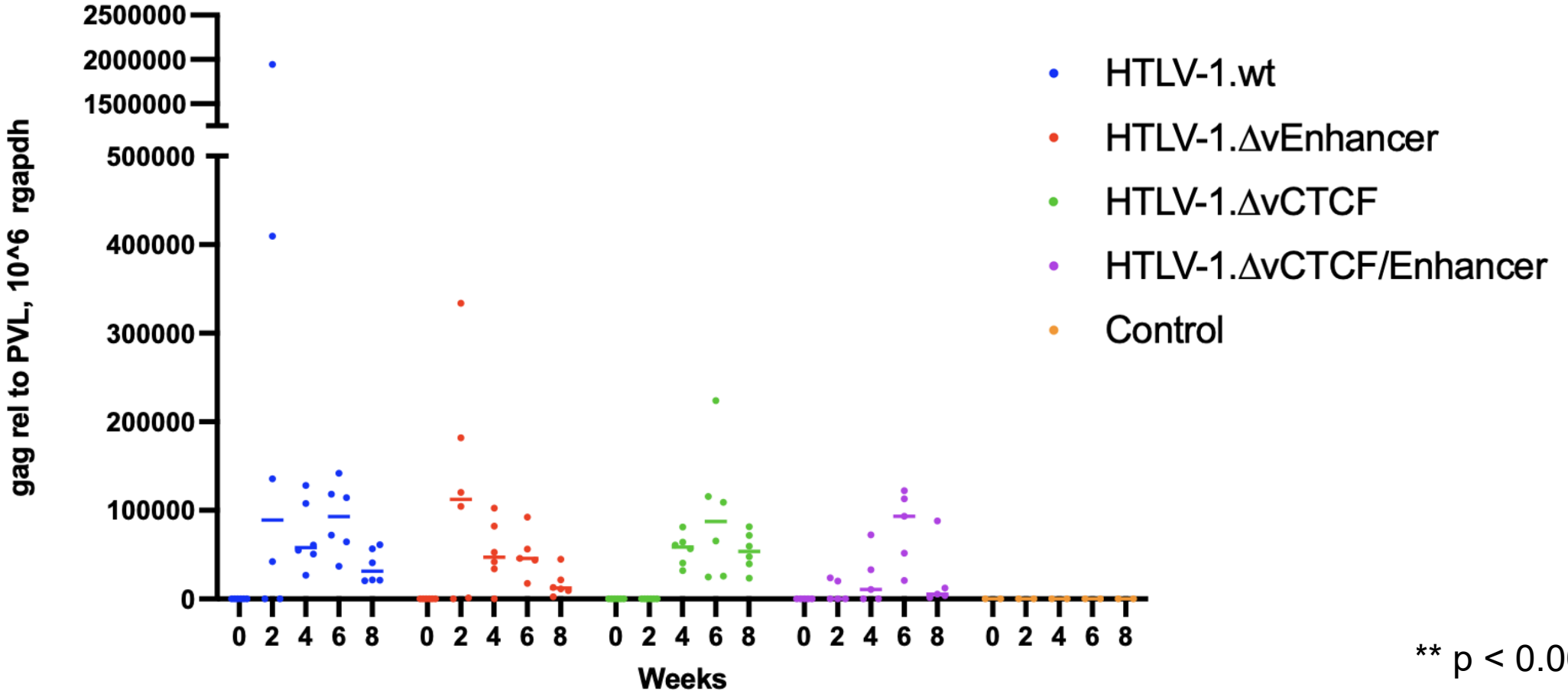


* $p < 0.01$
*** $p < 0.0001$

Loss of vCTCF-BS and vEnhancer element decreases *hbz* gene expression *in vivo*

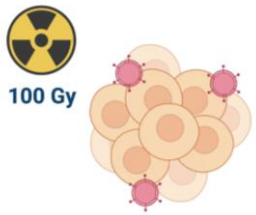
hbz gene expression

gag gene expression



** p < 0.0001

Loss of viral CTCF-BS and vEnhancer element delays disease development *in vivo*



Producer cells, lethally irradiated

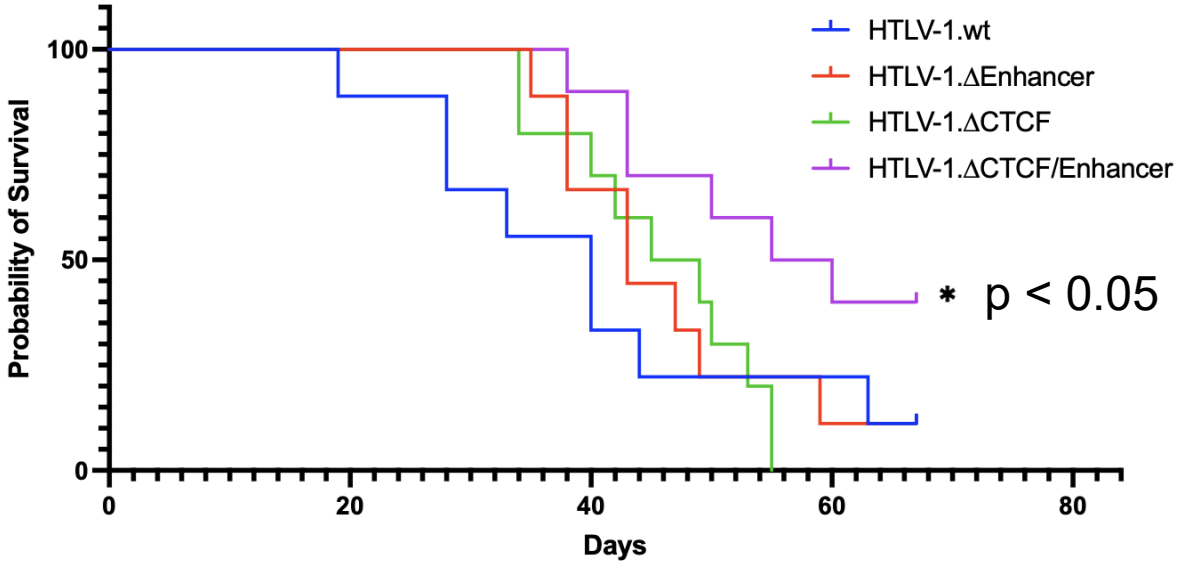
Inoculation

humanized immune system mice

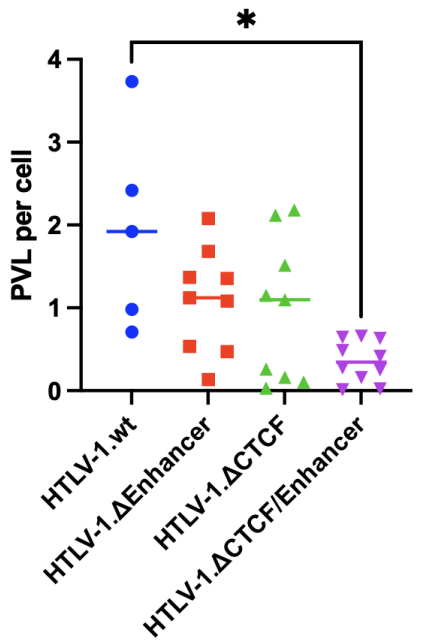
Collect spleen after euthanasia

Analysis:

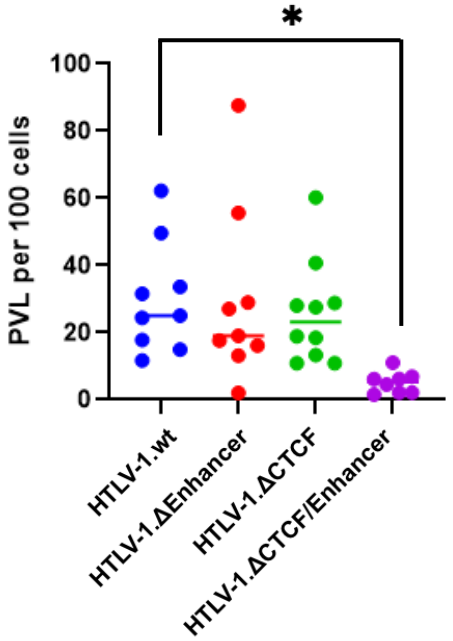
1) Survival
2) Proviral load in PBMCs (qPCR)
3) Viral gene expression in PBMCs (qPCR)



PVL 4 weeks p.i.



PVL necropsy



Summary & Conclusions

- Generated HTLV-1 ΔvCTCF/vEnhancer proviral plasmids and producer cell lines
- vCTCF-BS and vEnhancer
  - not required for infection or T-cell immortalization
- Loss of vCTCF-BS and vEnhancer
  - decreases proviral load and *hbz* gene expression in immortalized T-cell lines
  - decreases viral persistence in NZW rabbits
  - decreases disease development in humanized immune system mice

vCTCF-BS and vEnhancer element cooperate to activate *hbz* gene expression, regulate viral persistence, and promote disease progression

Acknowledgements

Amanda Panfil Lab

- Afia Asamoah
- Anna Childers, MS
- Rani Chunduri, MS PhD
- Amelie Mockbee
- Bethany Pepple, MS
- Cameron Phelps
- **Rocio Zaldivar, MS**

Resources

- OSU CCC
- NCI P01CA100730

Collaborators

Stefan Niewiesk Lab

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

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

HTLV-1c Integration Rewires Host Nuclear Architecture and Enhancer Regulation in Infected T-Lymphocytes

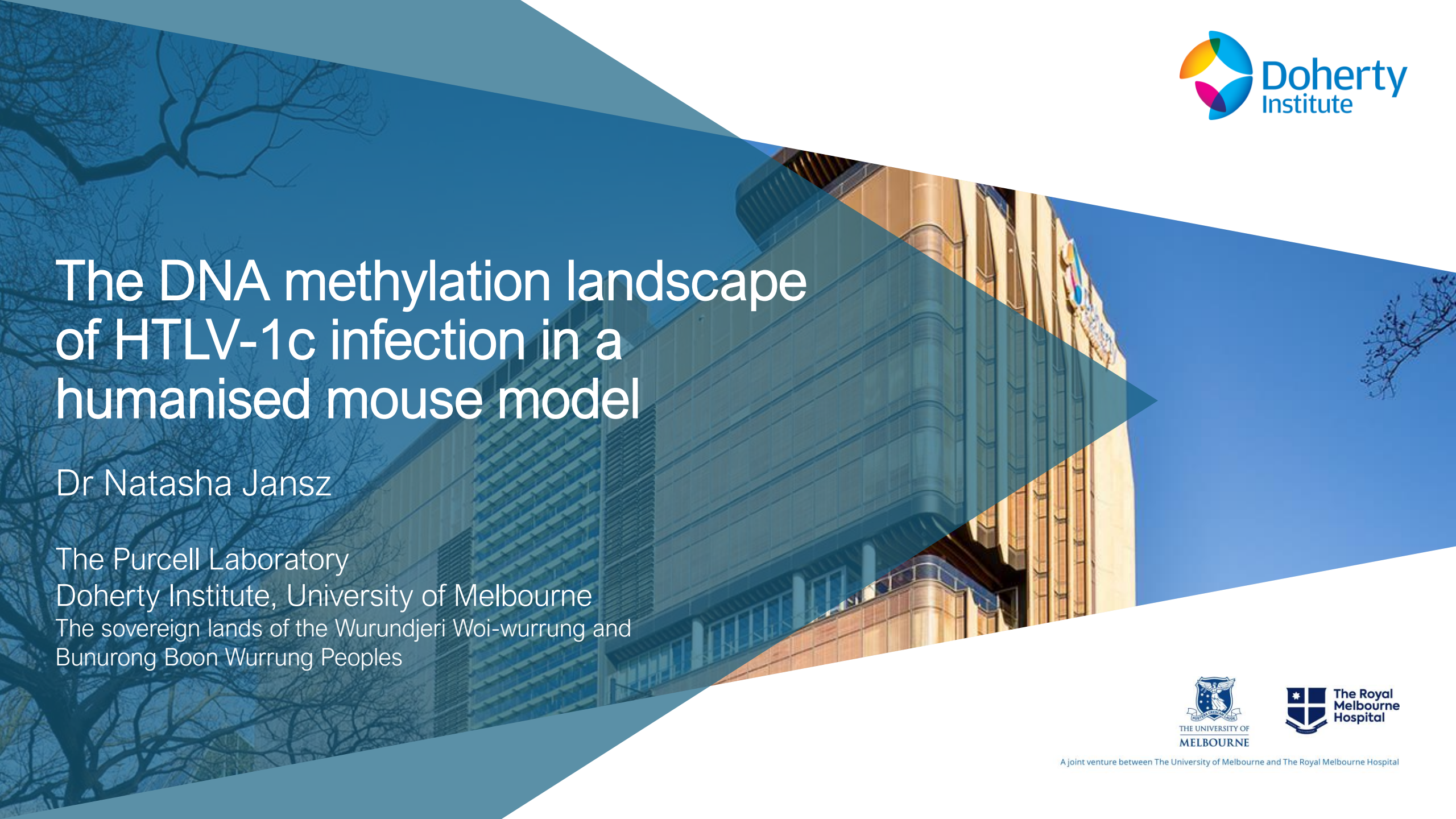
Natasha Jansz
The Doherty Institute - University of Melbourne, Australia

The DNA methylation landscape of HTLV-1c infection in a humanised mouse model

Dr Natasha Jansz

The Purcell Laboratory
Doherty Institute, University of Melbourne

The sovereign lands of the Wurundjeri Woi-wurrung and Bunurong Boon Wurrung Peoples



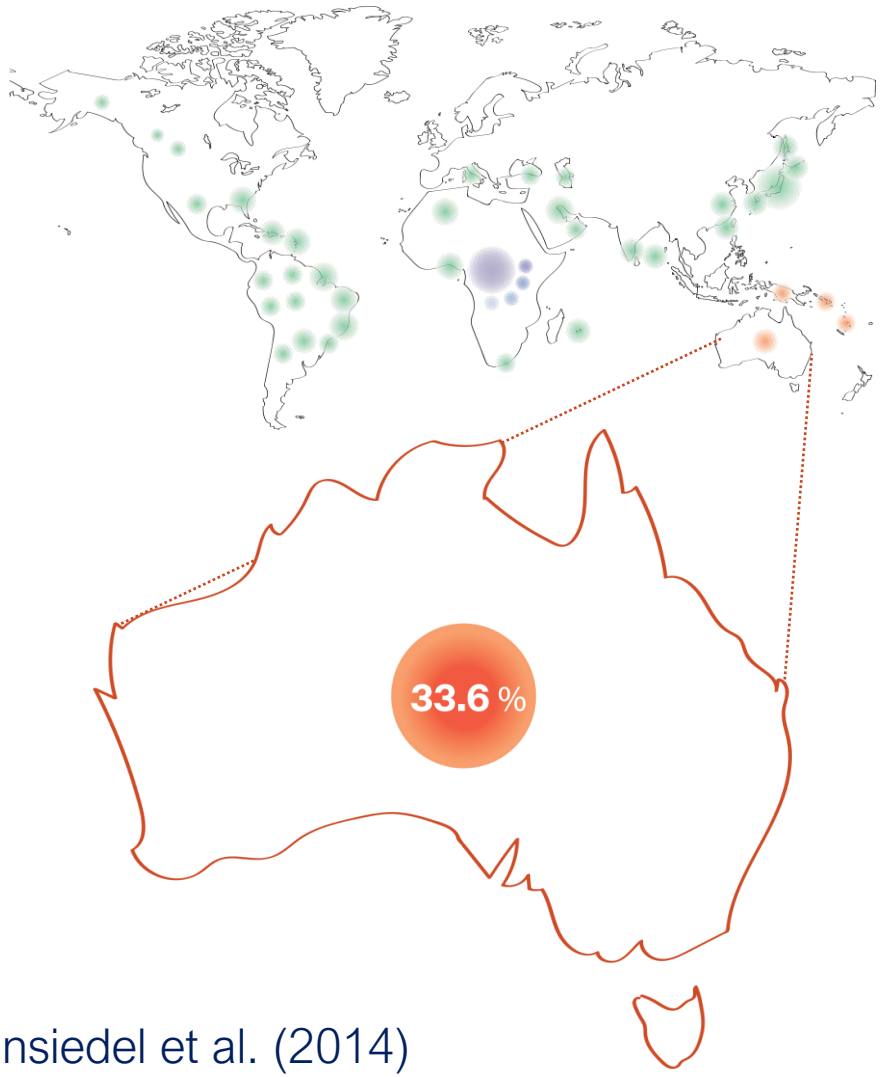
Doherty Institute

THE UNIVERSITY OF MELBOURNE

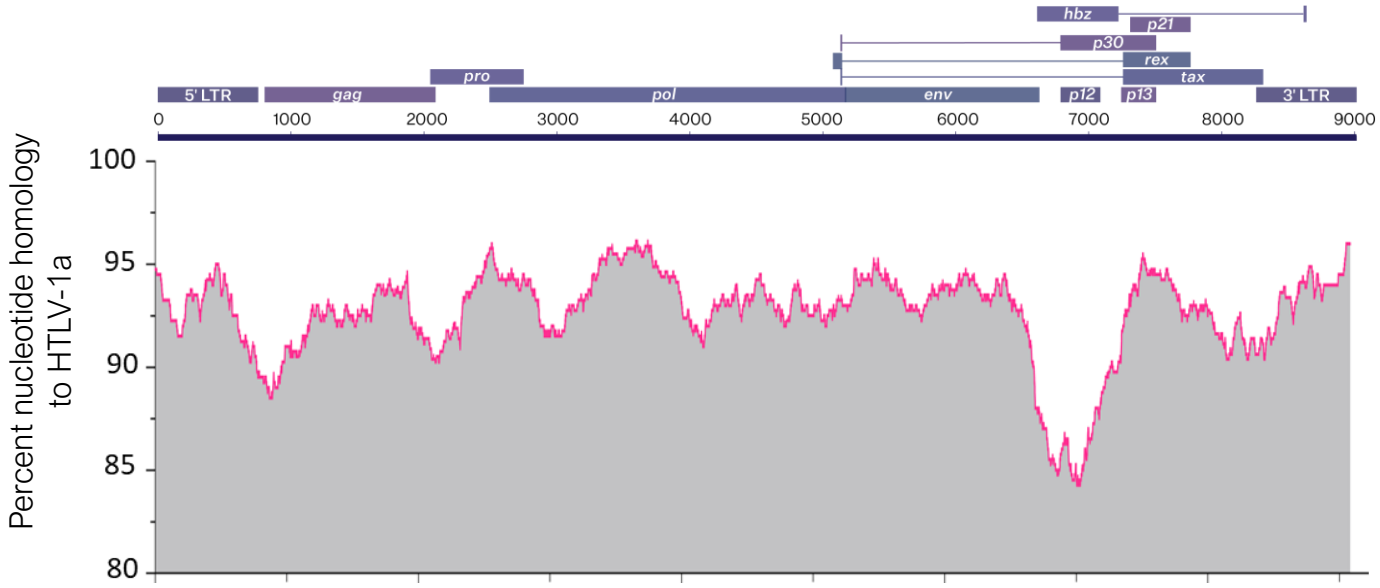
The Royal Melbourne Hospital

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

HTLV-1c is a divergent subtype endemic in Oceania

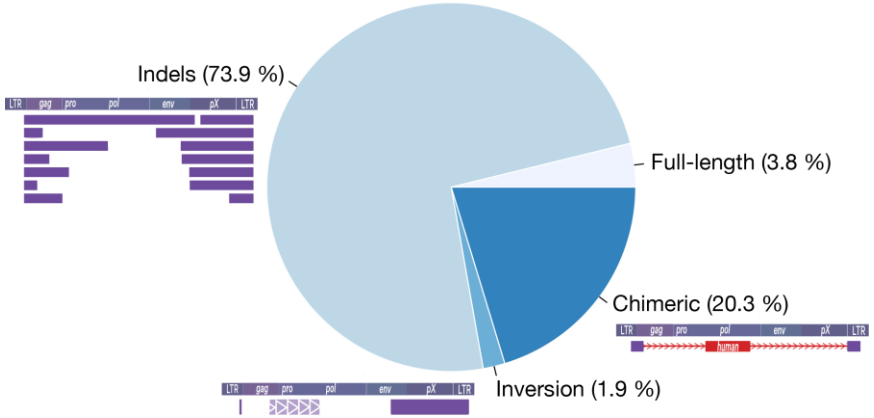


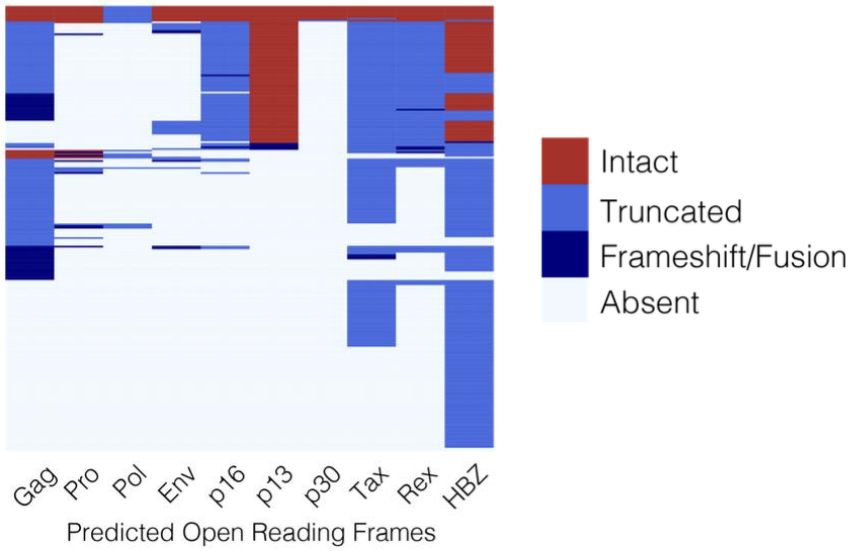
Einsiedel et al. (2014)

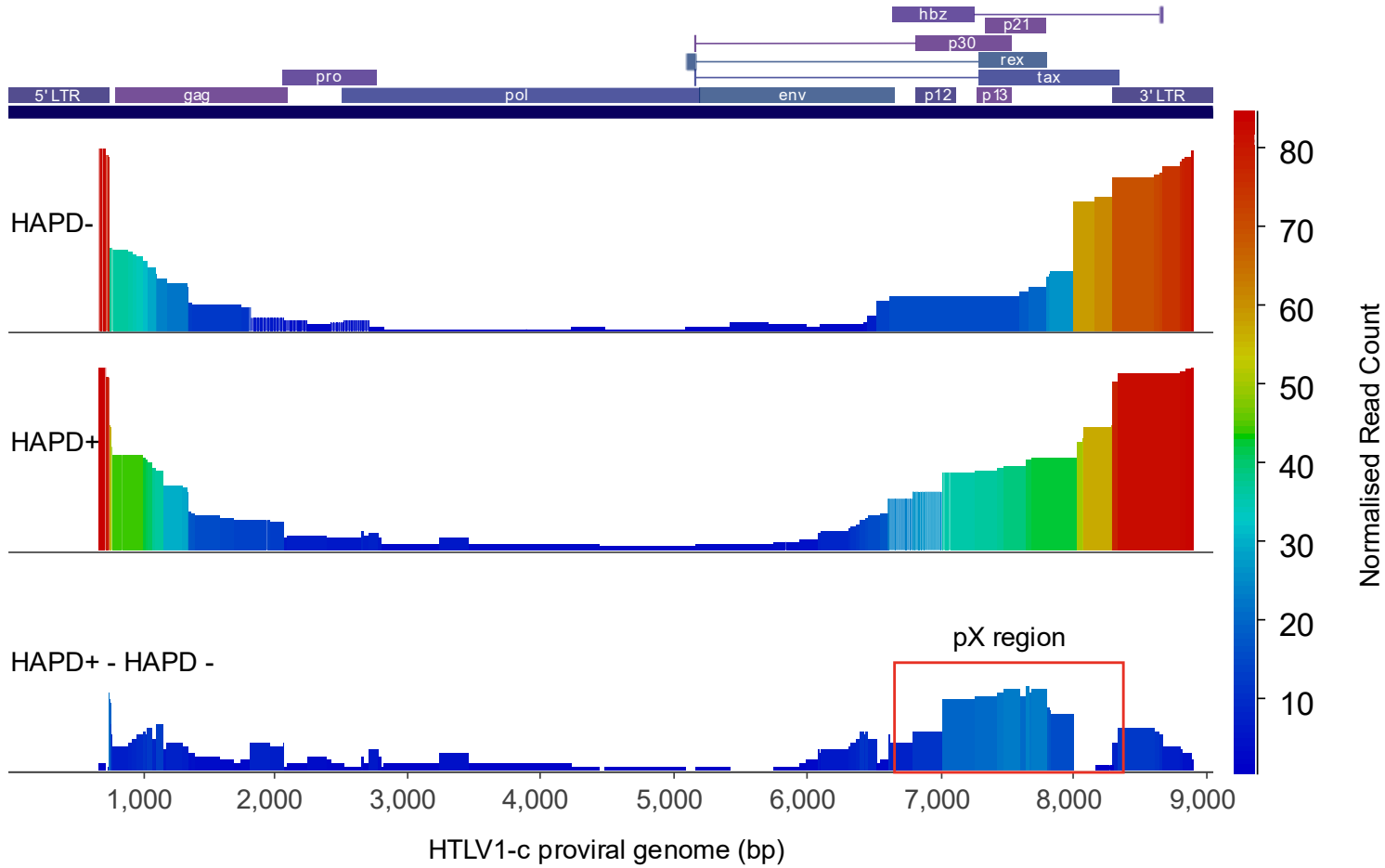


Hirons, Yurick et al. *Retrovirology* (2024)

Haplotype resolved structural variation of HTLV-1c provirus in a Central Australian clinical cohort

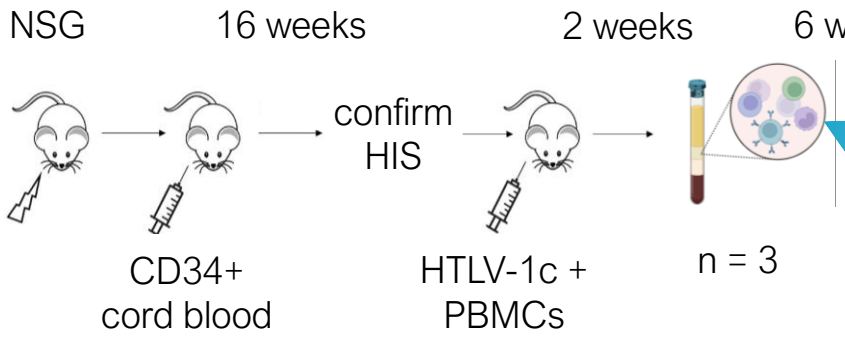




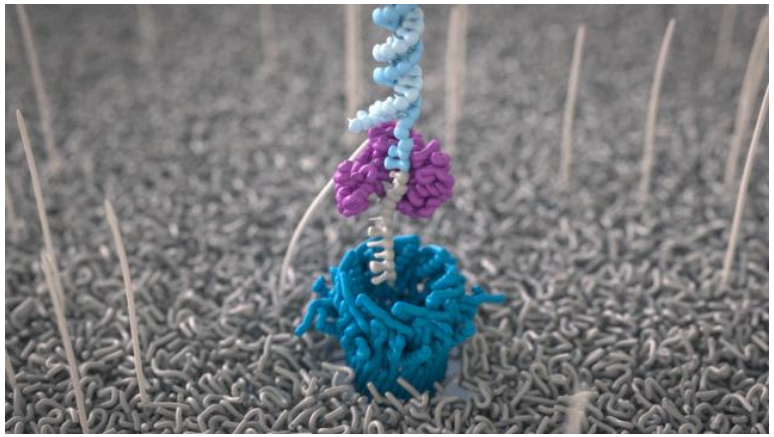


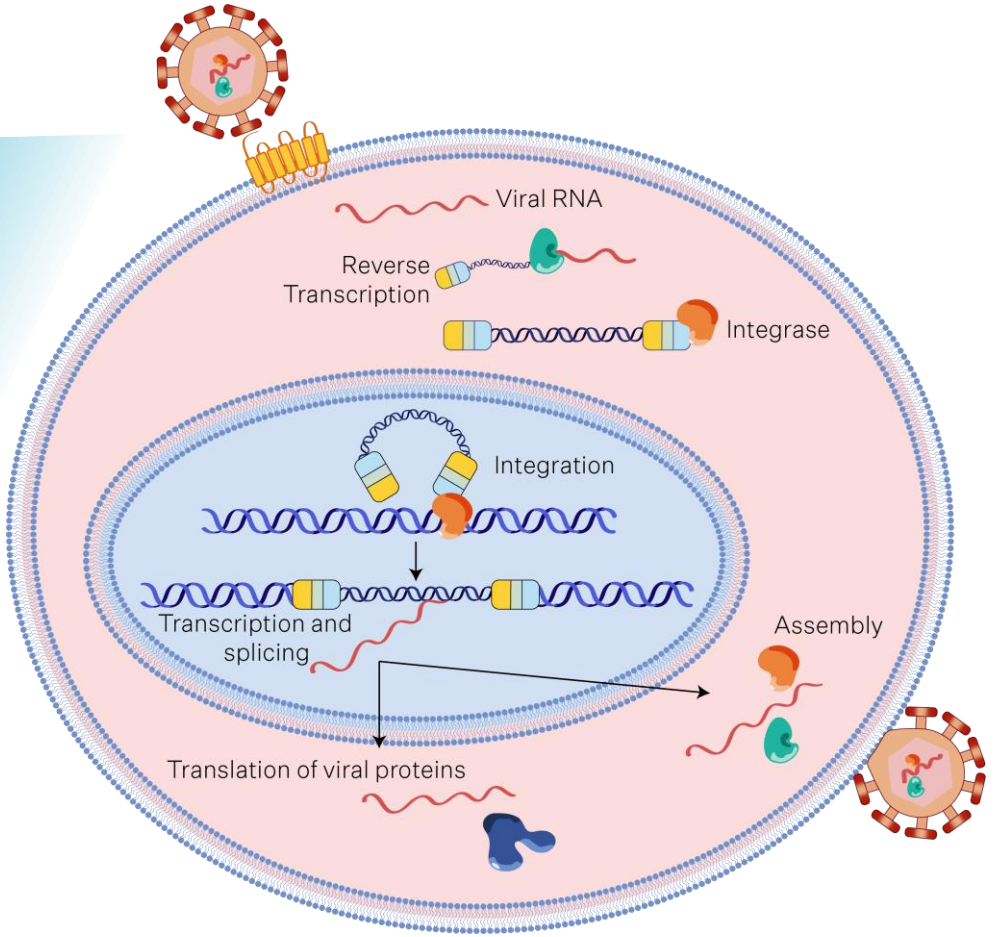
Hirons, Jansz et al. *In revision*

Long-read whole genome sequencing in a hu-NSG mouse model of HTLV-1c infection

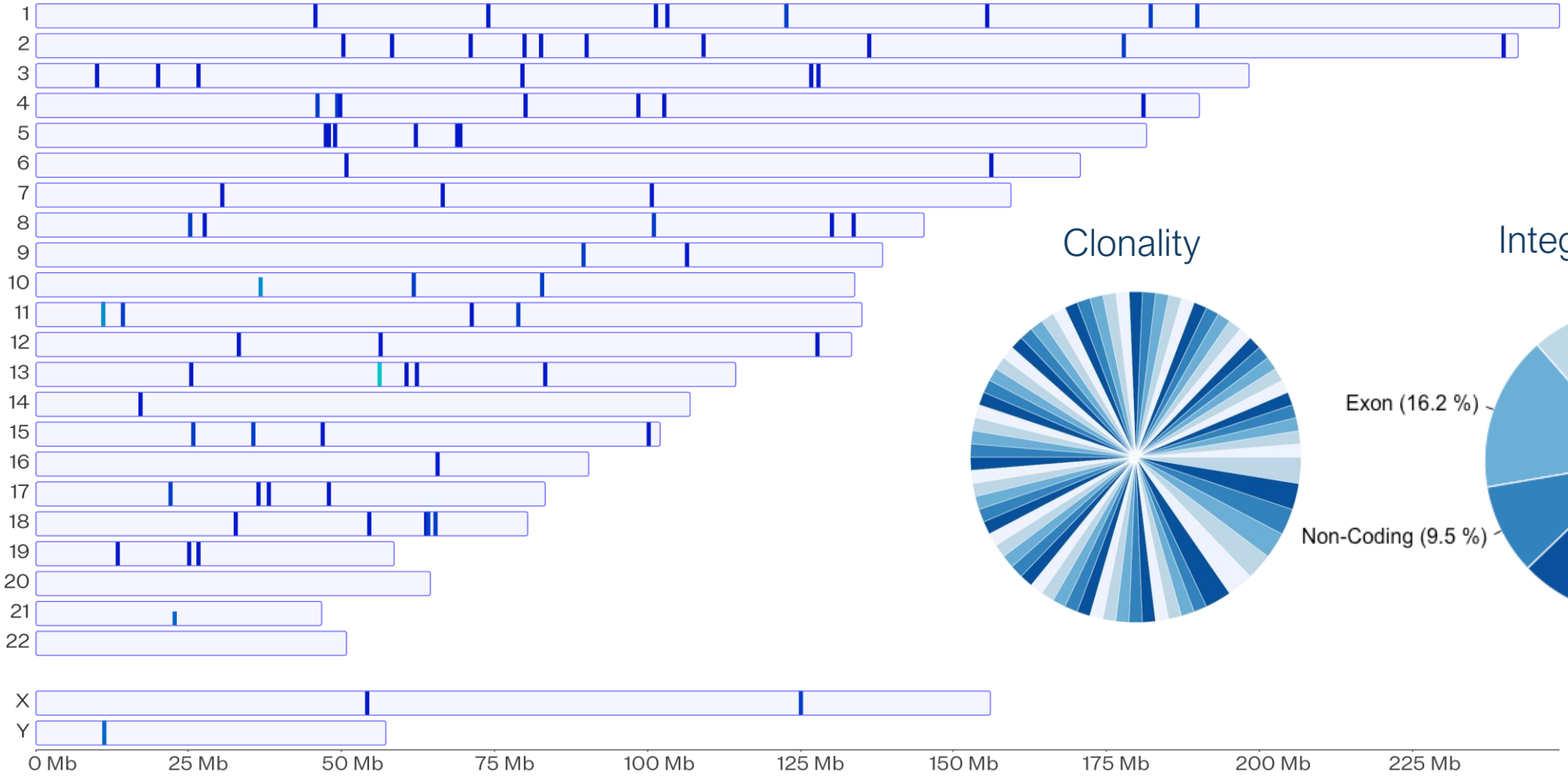


Cooney et al. *Cell* 2025



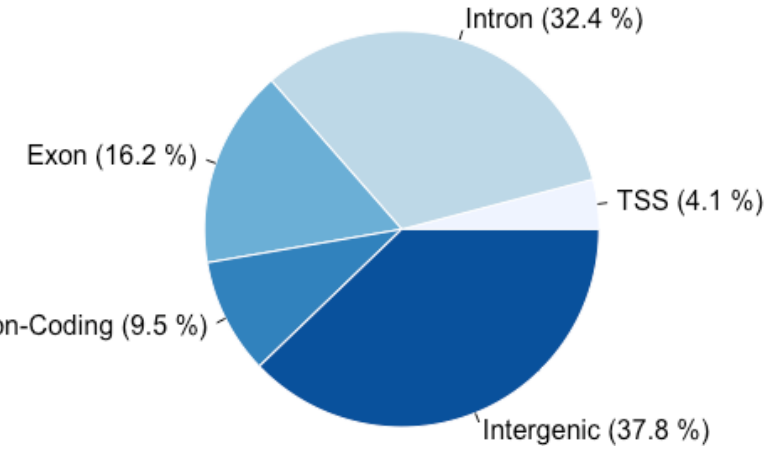


Polyclonal expansion of infected CD4+ and CD8+ T-cells in hu-NSG mice

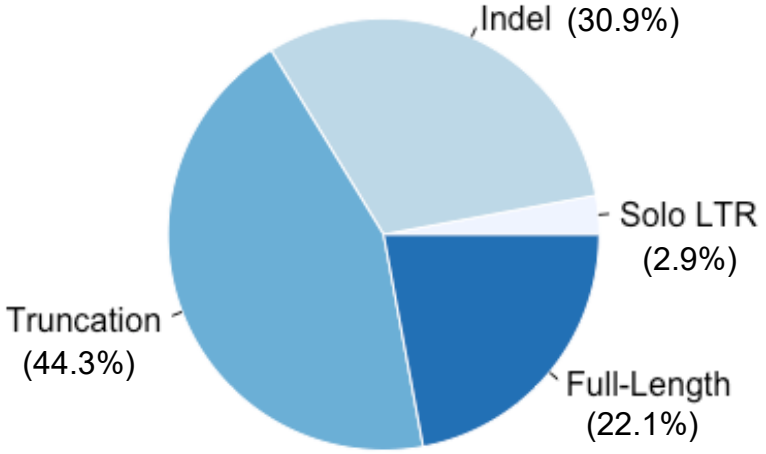


Clonality

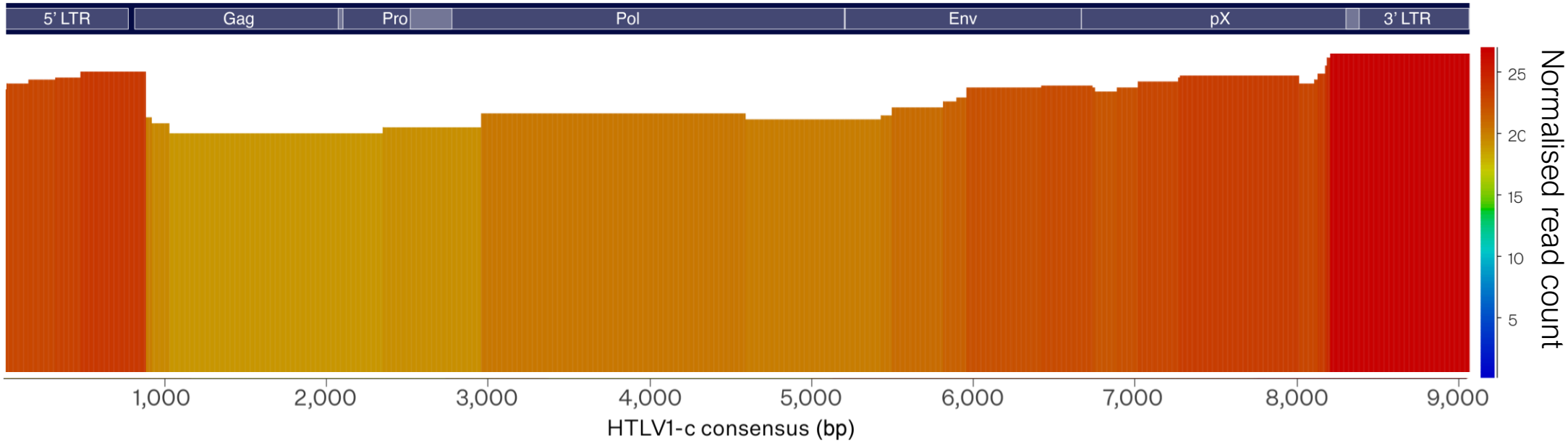
Integration Sites



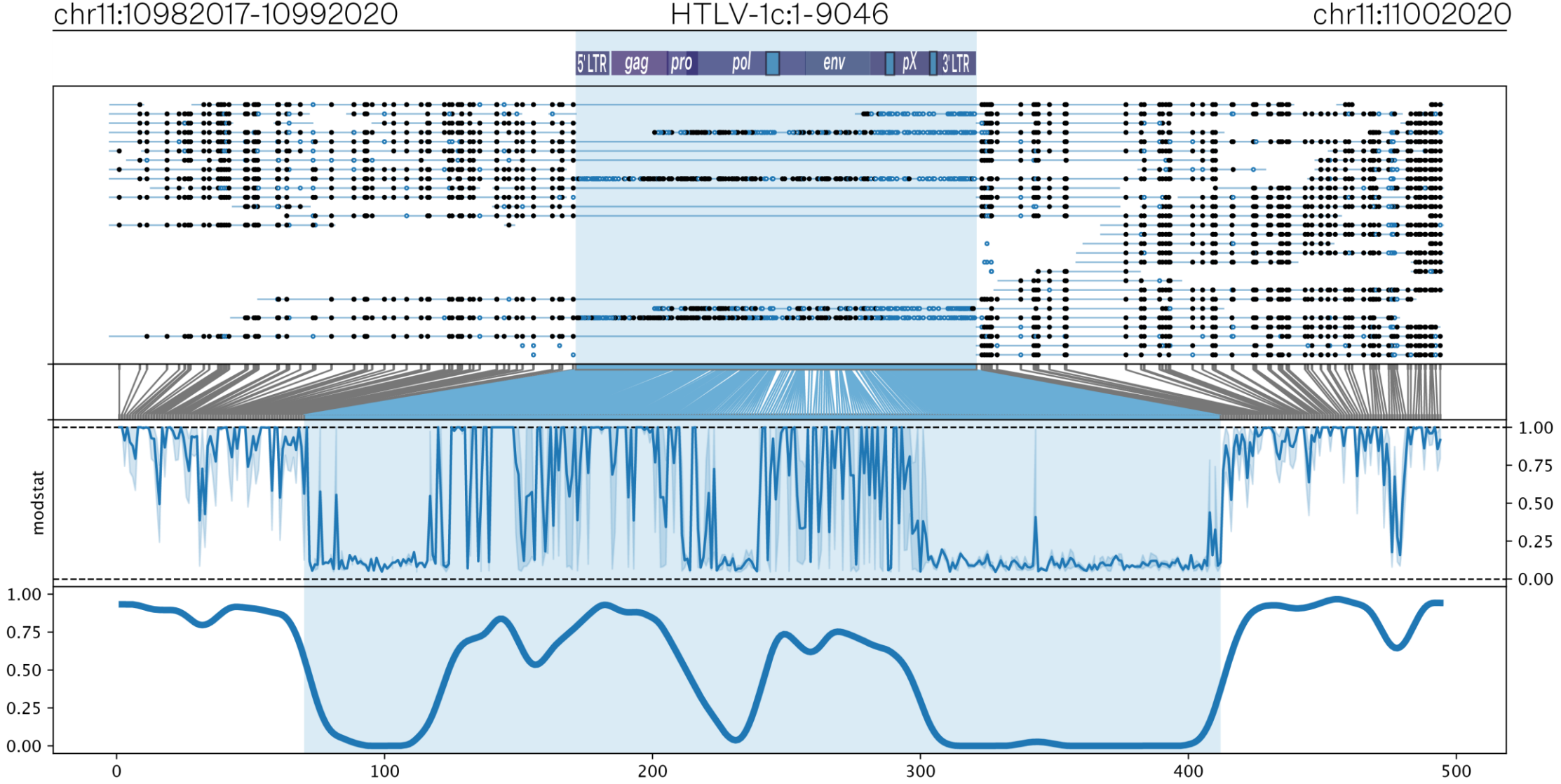
HTLV-1c genomic instability in hu-NSG mice



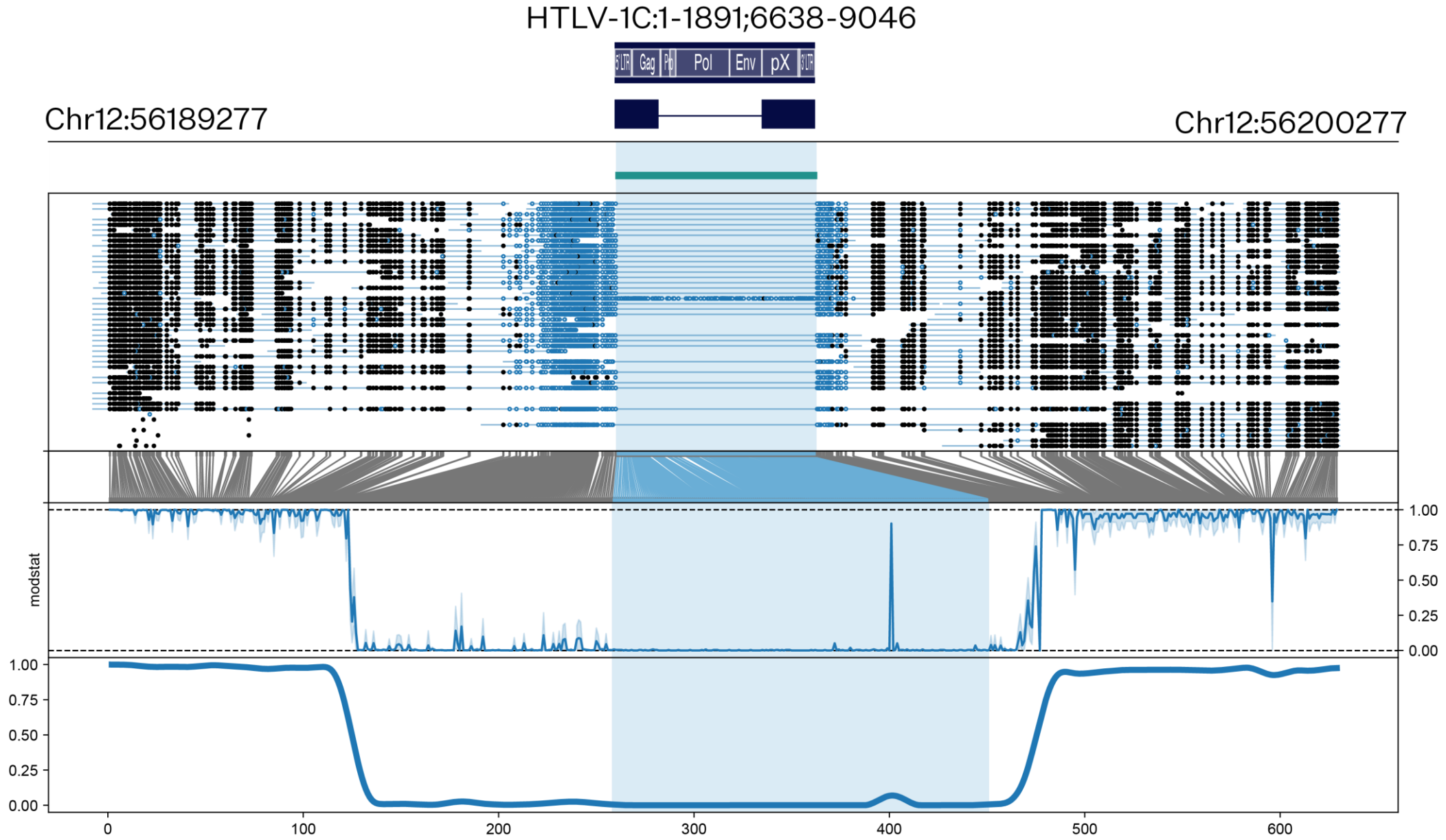
HTLV-1c
6 weeks



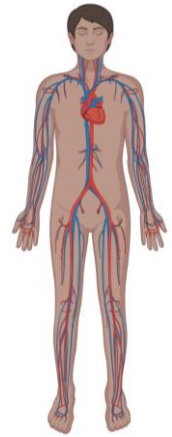
HTLV-1c is methylated over structural genes and doesn't alter local host 5mC

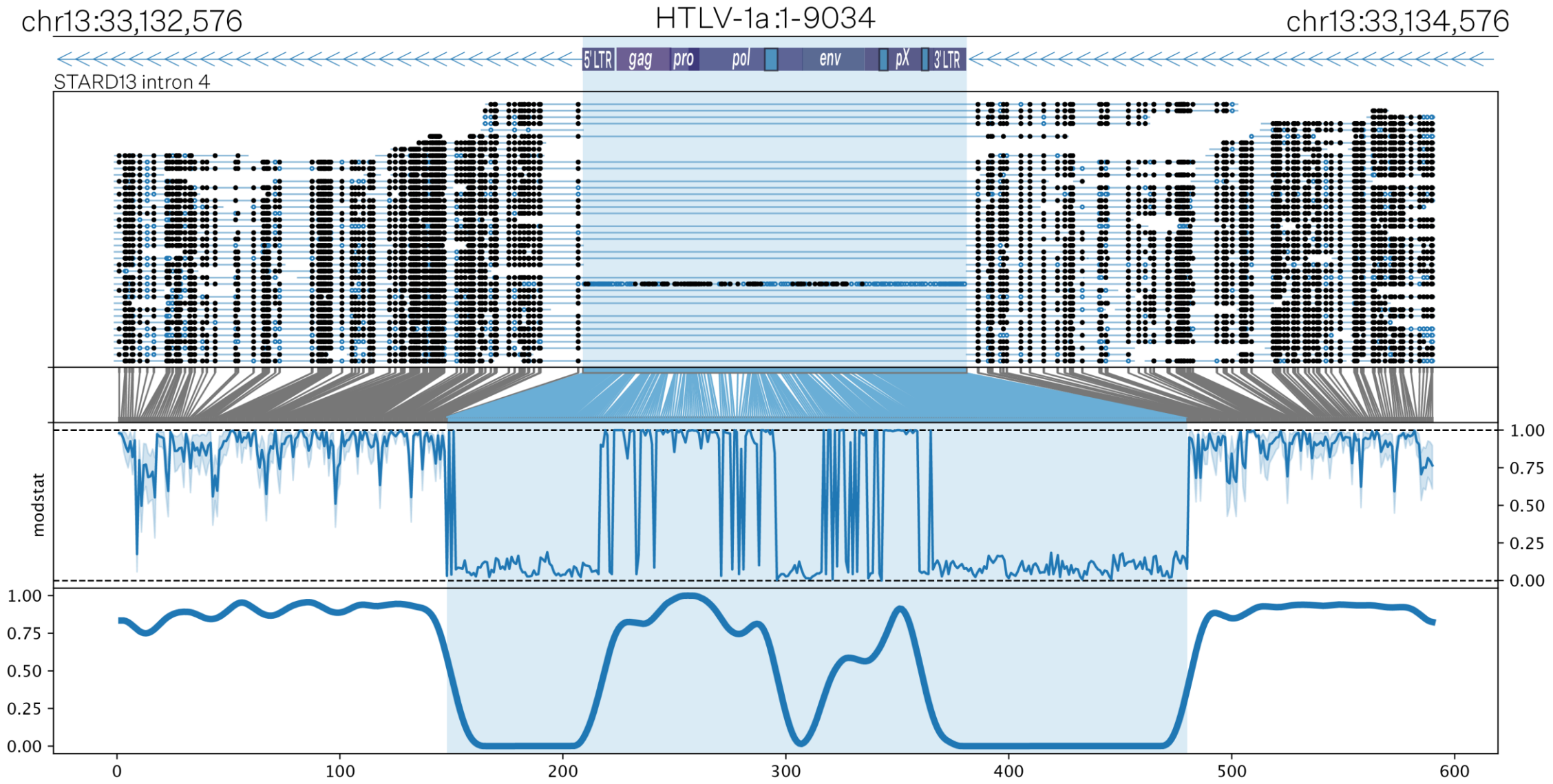


HTLV-1c breakpoints occur at 5mC junctions

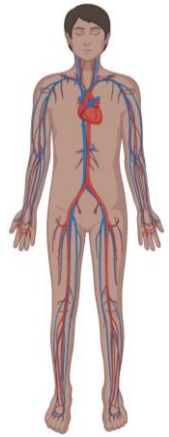


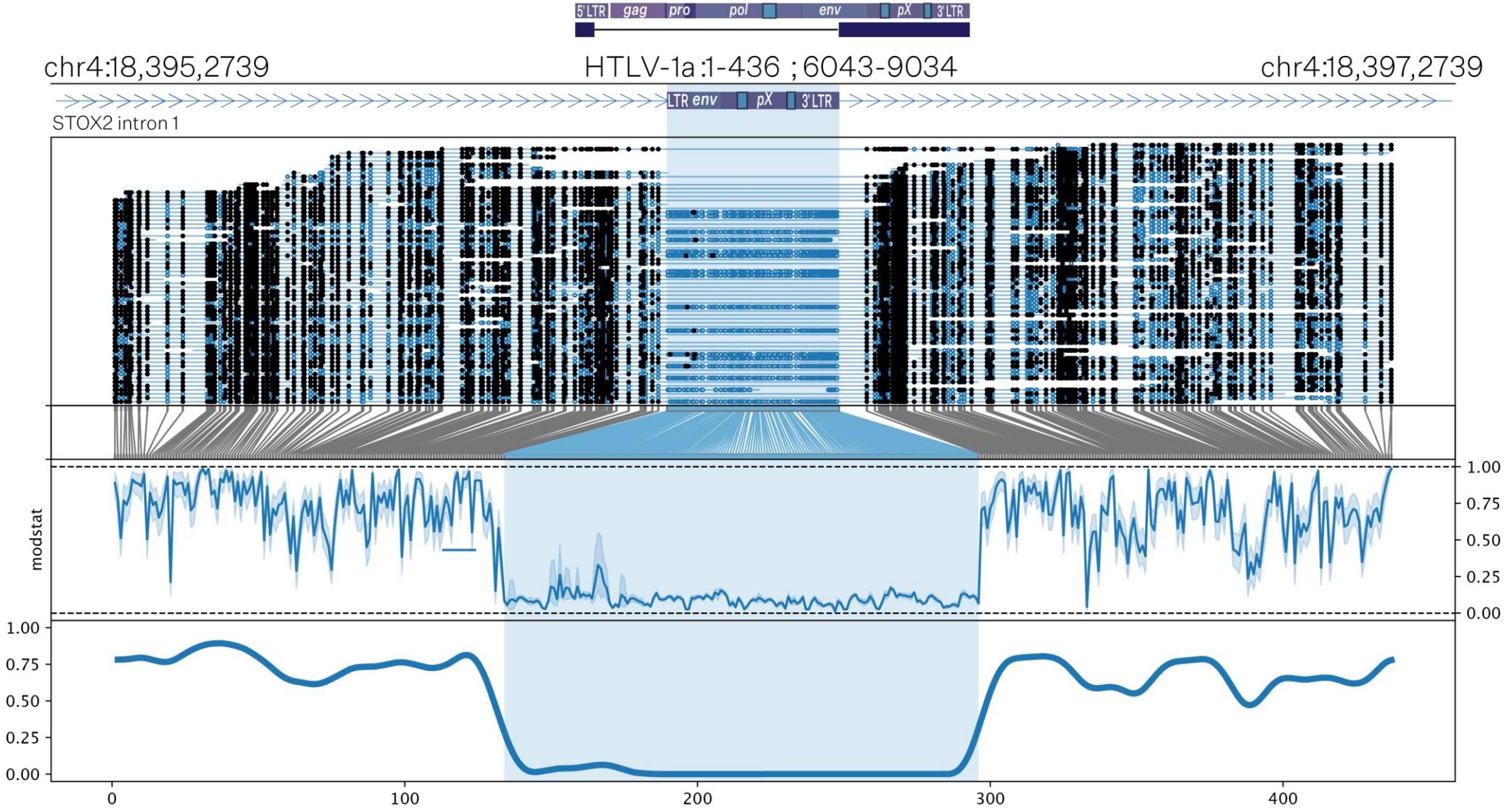
5mC distribution observed in HTLV-1a + ATL samples



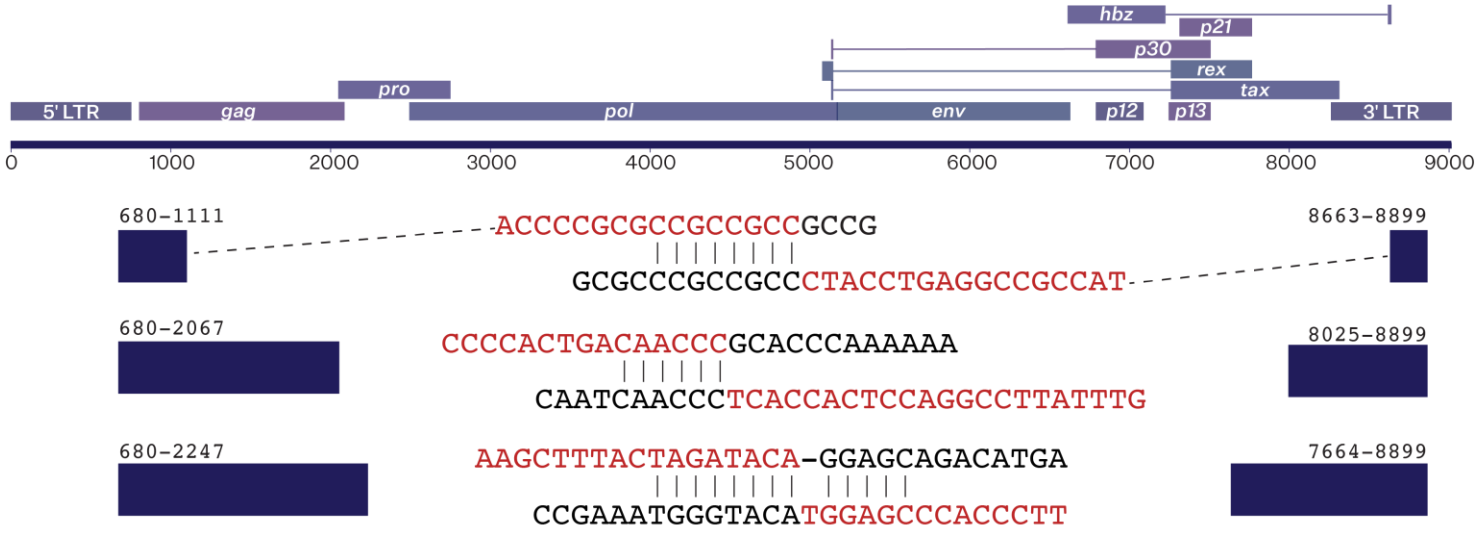


Structural variants in HTLV-1a + ATL samples are hypomethylated



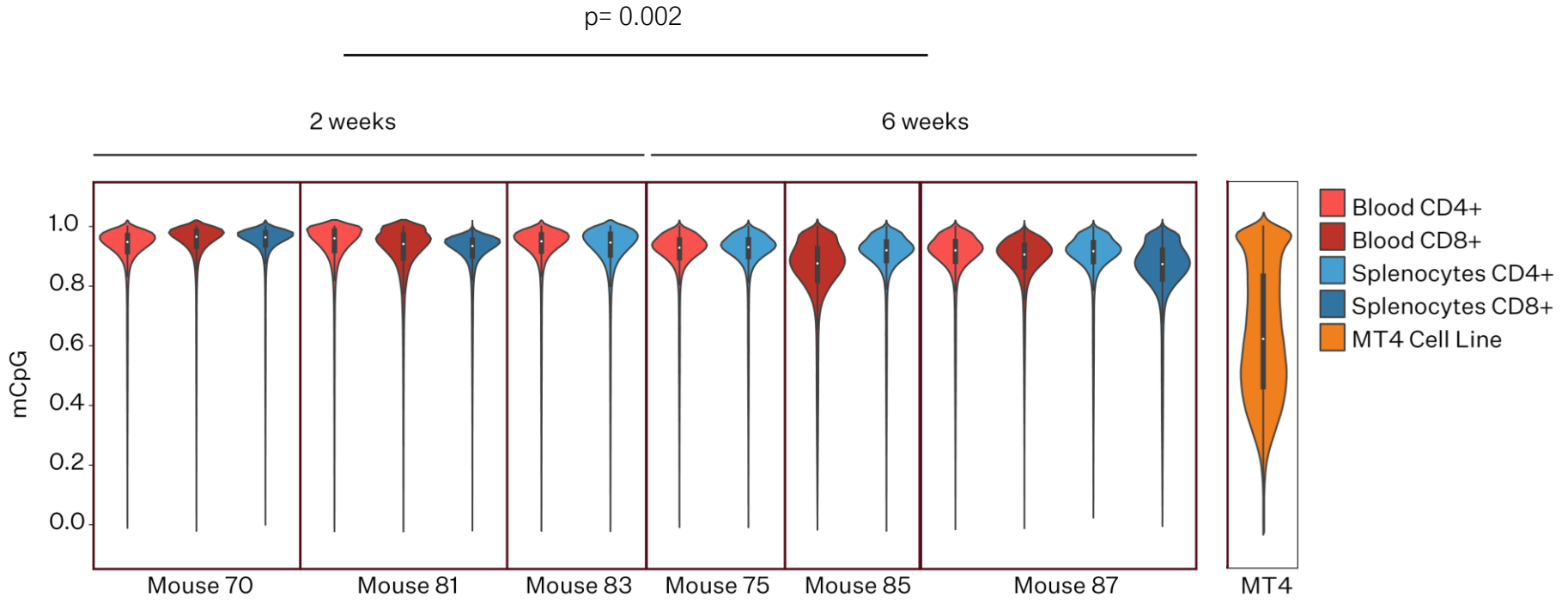


Does DNA methylation of the HTLV-1c provirus function to maintain genomic integrity?



- 5mC often associated with gene silencing, however it is neither necessary nor sufficient for gene silencing
- 5mC plays important roles in transcriptional regulation through altering effector protein binding
- 5mC is important in preventing aberrant homologous recombination
- Defective breakpoint junctions contain regions of microhomology

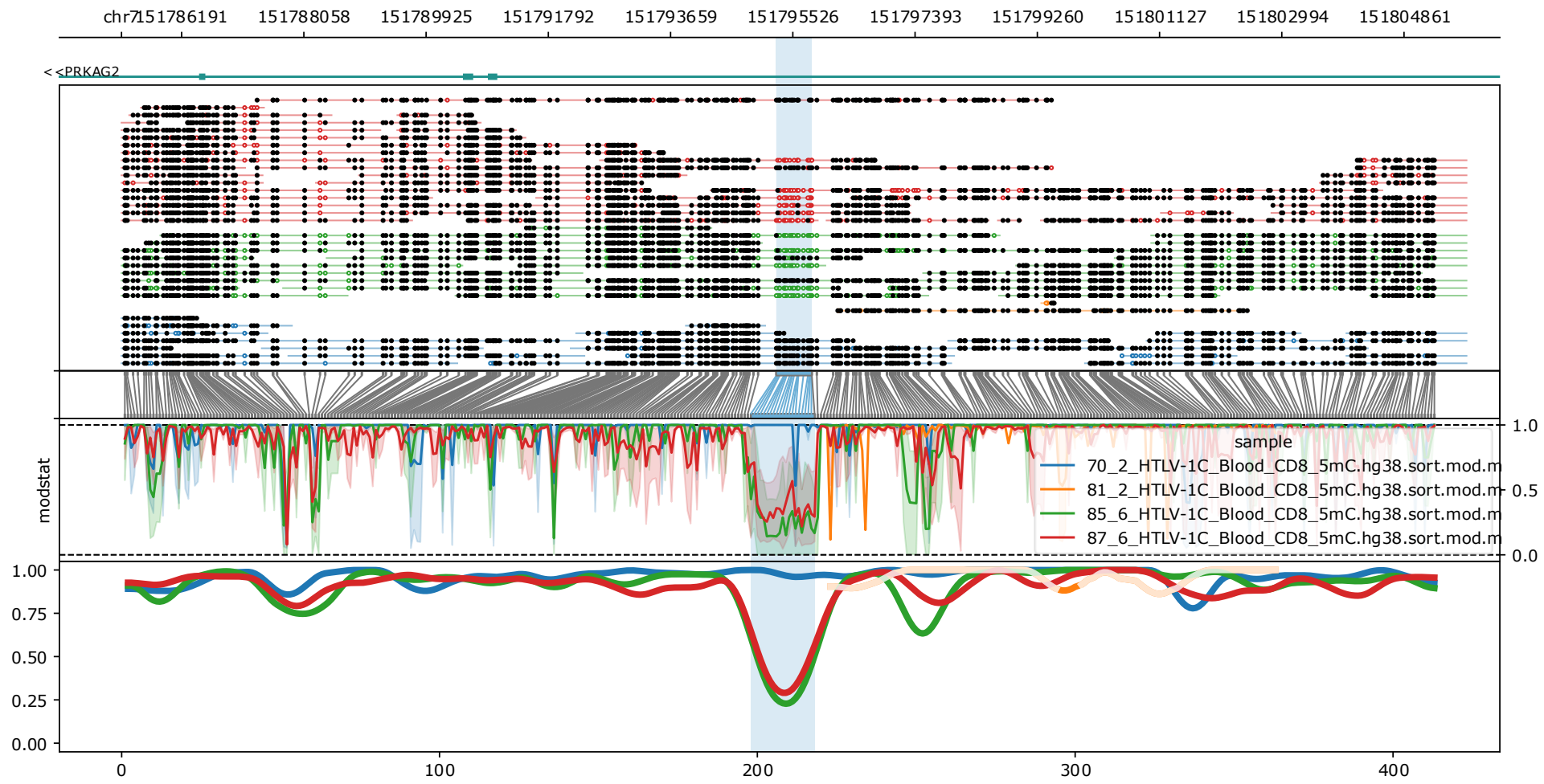
Subtle global reduction in 5mC over time following infection and disease progression



Genome wide differential analysis of 5mC (methylartist and DSS)

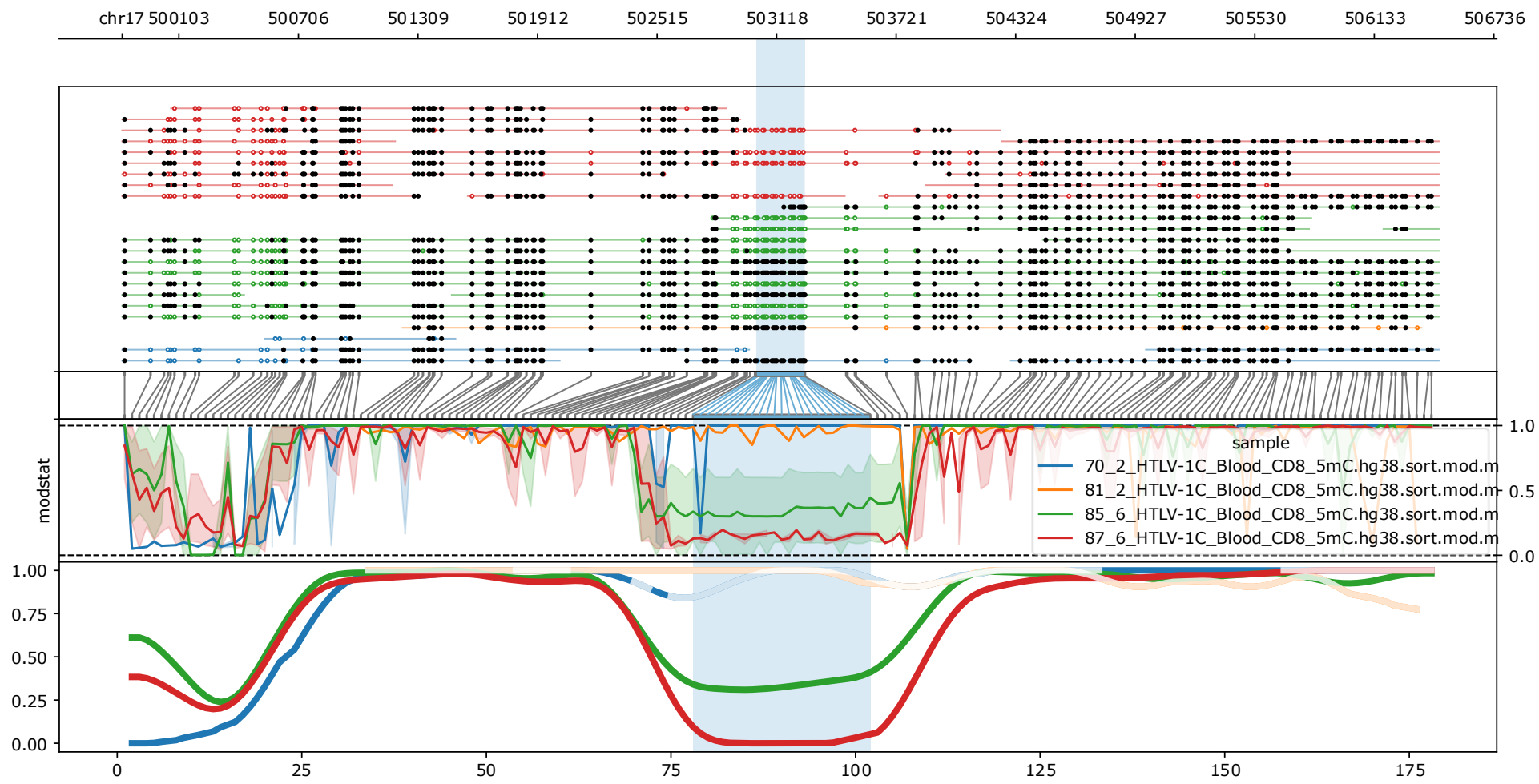
- 91 differentially methylated regions (DMR) FDR < 0.05 detected
- Fold change between 0.5 and 0.8

Hypomethylation of intergenic enhancers at 6wpi



Fold change in 6wpi = -0.64

Hypomethylation of distal enhancers at 6wpi



Fold change in 6wpi = -0.61

Role of enhancer re-wiring in genome regulation of HTLV-1 infection

In hu-NSG mice and clinical samples, integration of provirus does not alter 5mC in cis

- 5mC loss due to exogenous DNA insertion usually seen after a round of meiosis

Changes in enhancer 5mC upon proviral integration suggests changes occur in trans

- Does this predict enhancer activity and transcription?
- Are 5mC changes causal or downstream of effectors?

Acknowledgements

Doherty Institute

Damian Purcell

Lloyd Einsiedel

Ashley Hirons

Sarah Collins

Mater Research - UQ

Geoffrey Faulkner

Nathan Smits

Adam Ewing

Hannah Byrne

Seth Cheetham

WEHI

Marcel Doerflinger

Marc Pellegrini

James Cooney

Lewis Williams

Le Wany

Merle Dayton

Kathryn Davidson

Hokkaido University

Yorifumi Satou

Sharmin Nahar Sithi

Dr Ashley Hirons

Dr James Cooney

Dr Marcel Doerflinger

People living with HTLV-1

NSG mice