

11<sup>TH</sup> EDITION

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# HIV PERSISTENCE DURING THERAPY

Reservoirs & Eradication Strategies Workshop



Persistent HIV-1 unintegrated linear DNA can integrate and lead to viral replication after integrase inhibitor treatment removal

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Institut Cochin

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Team « Retrovirus, Infection and Latency »



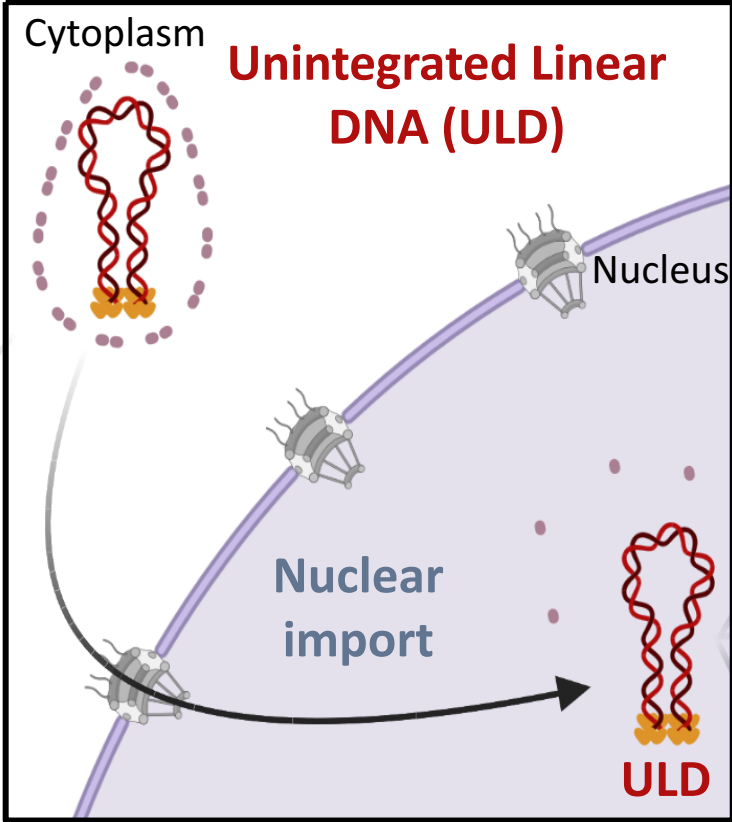
[www.hiv-persistence.com](http://www.hiv-persistence.com)

# CONFLICTS OF INTEREST

The authors declare no conflict of interest

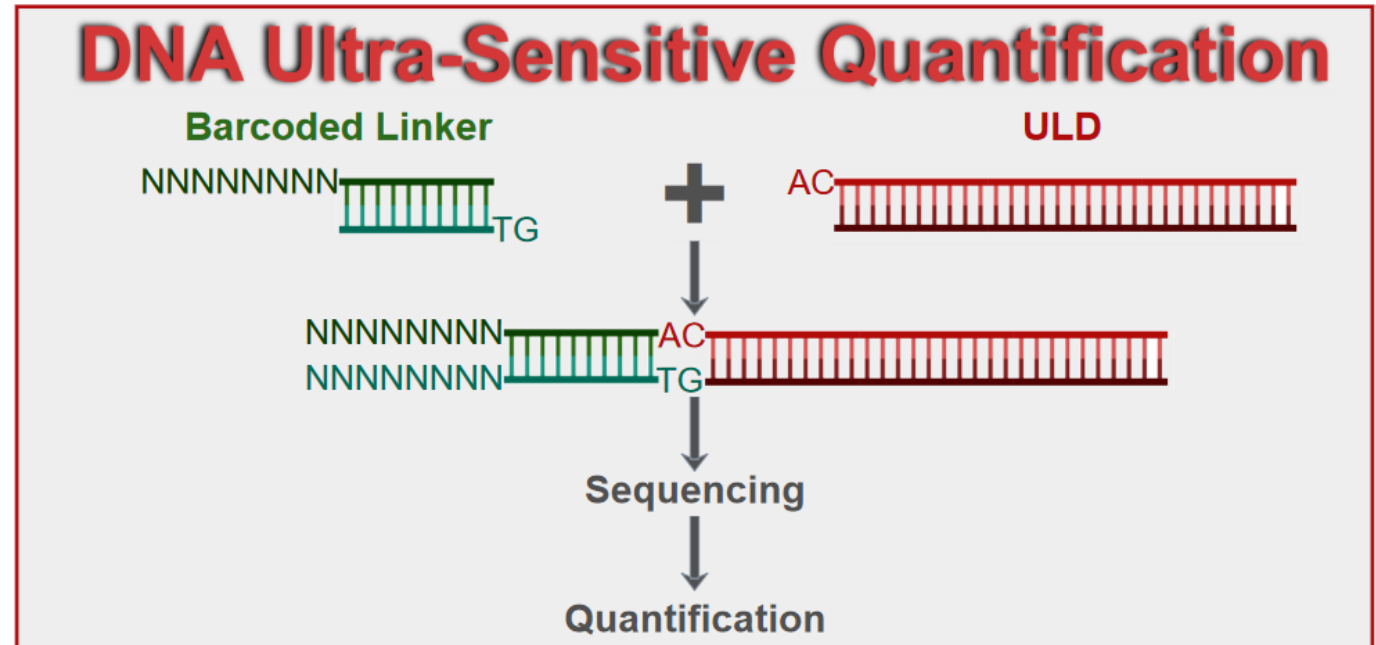
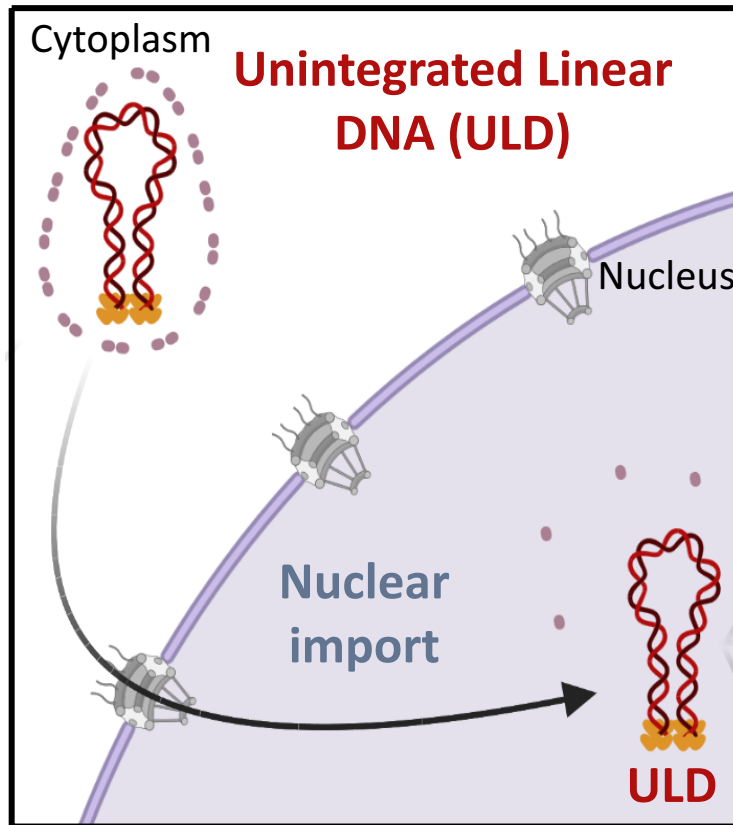
# HIV pre-integrative latency

Only competent integration substrate  
Delay of integration → ULD persistence  
→ Pre-integrative latency



# DNA Ultra Sensitive Quantification to follow ULD persistence

Only competent integration substrate  
Delay of integration → ULD persistence  
→ Pre-integrative latency



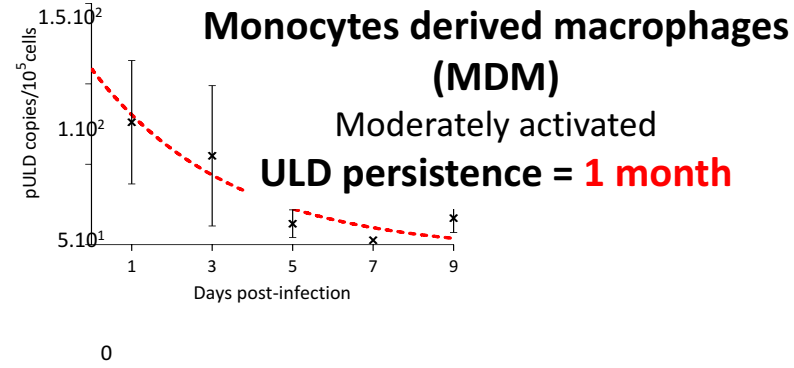
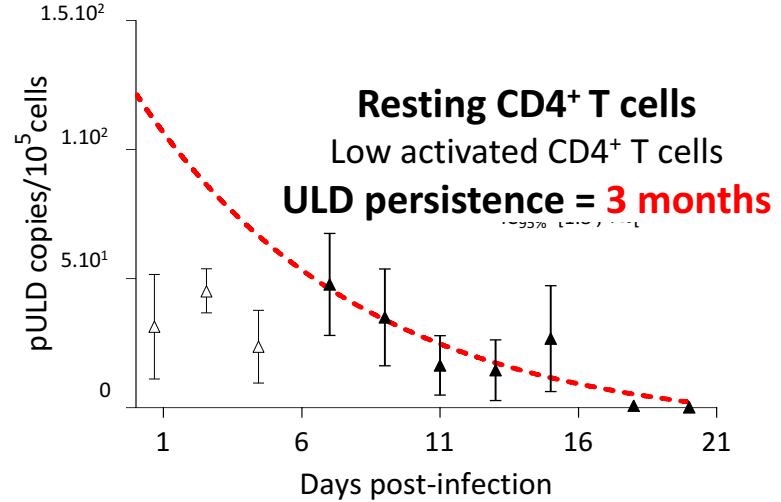
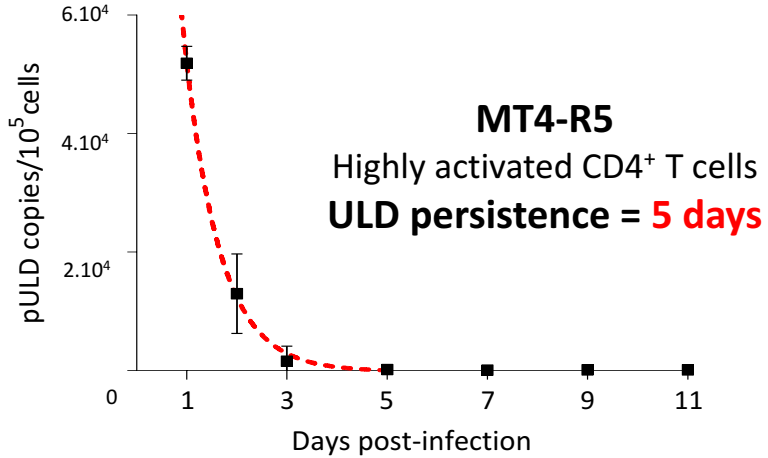
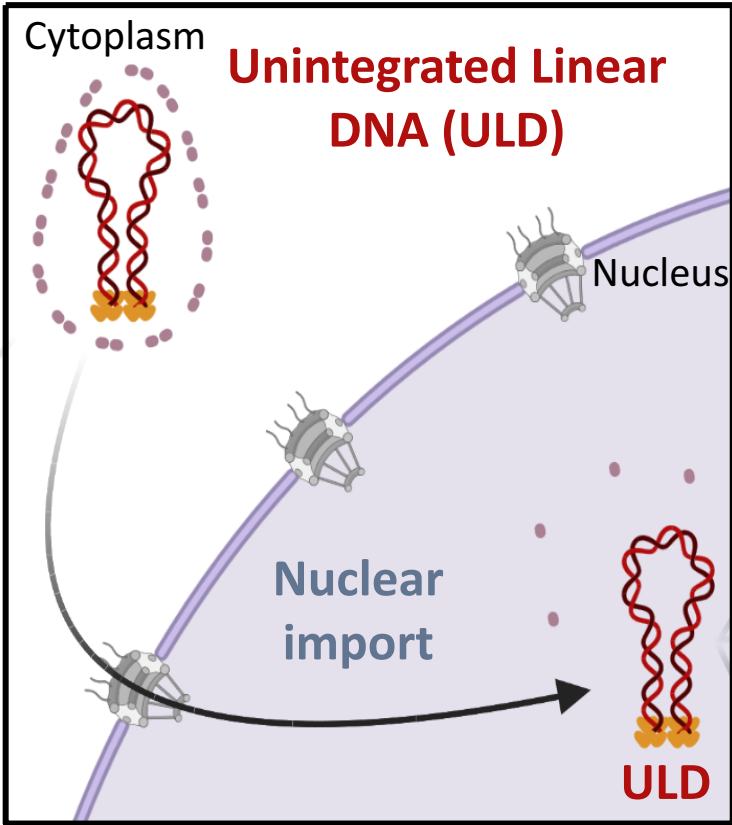
→ Specific to processed ULDs  
→ Ultra-sensitive (up to 1 copy/10<sup>6</sup> cells)

Roux H. M. *et al.*, Cell Reports Methods, 2023  
(HIV persistence during therapy congress 2022 Y1 2.7)



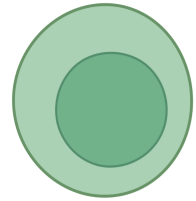
# ULD persistence time is cell-type dependent *in vitro*

Only competent integration substrate  
 Delay of integration → ULD persistence  
 → Pre-integrative latency

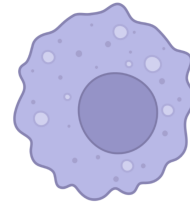


Roux H. M. *et al.*, Cell Reports Methods, 2023  
 (HIV persistence during therapy congress 2022 YI 2.7)

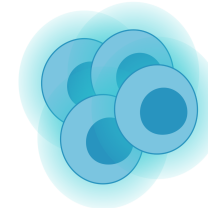
# Persistent ULD localization/integration protocol



Resting CD4<sup>+</sup> T cells

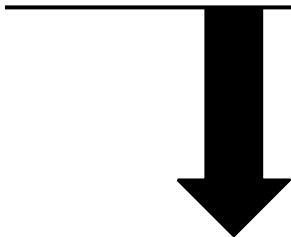


Macrophages



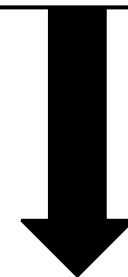
Activated  
CD4<sup>+</sup> T cells

Parameters driving difference of cell-type dependent pre-integrative latency?  
→ ULD Localization



*In situ* hybridization +  
Immunofluorescence

Role of pre-integrative latency in viral persistence?  
→ ULD Integration potential

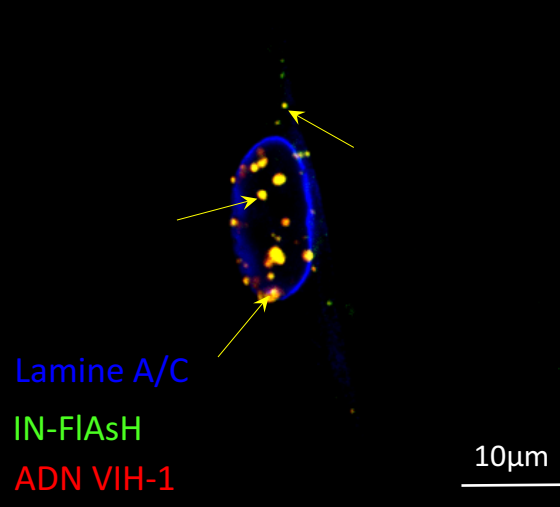


INSTI removal + adapted qVOA

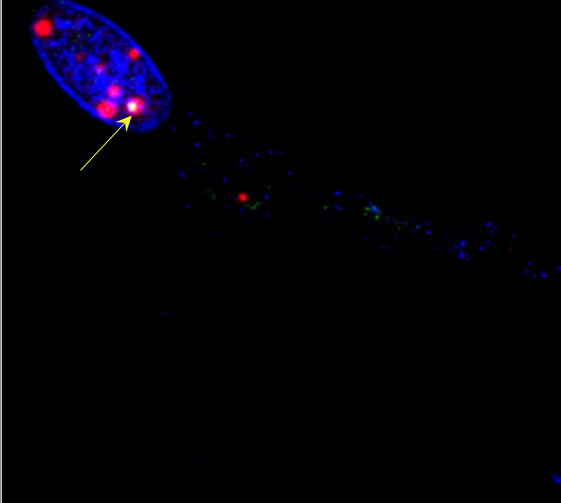
# ULD Localization in cells

MDM  
 $t_{1/2} = 3$   
days

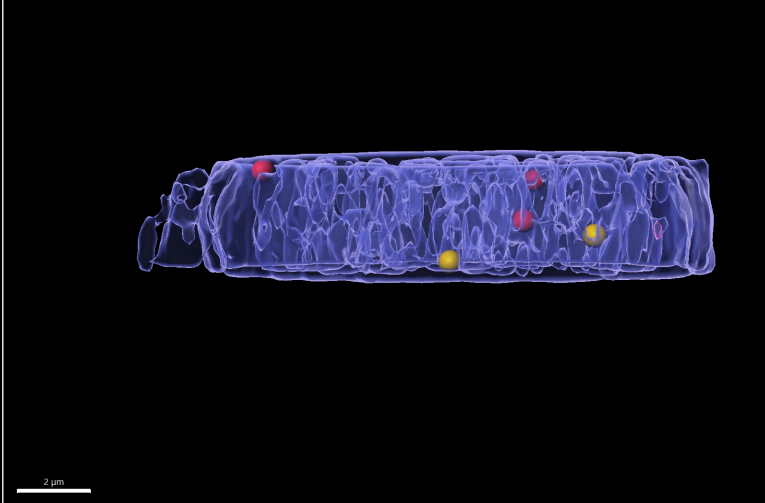
**0,5  $t_{1/2}$  (=D1)**



**2  $t_{1/2}$  (=D7)**



**2  $t_{1/2}$  (=D7)**



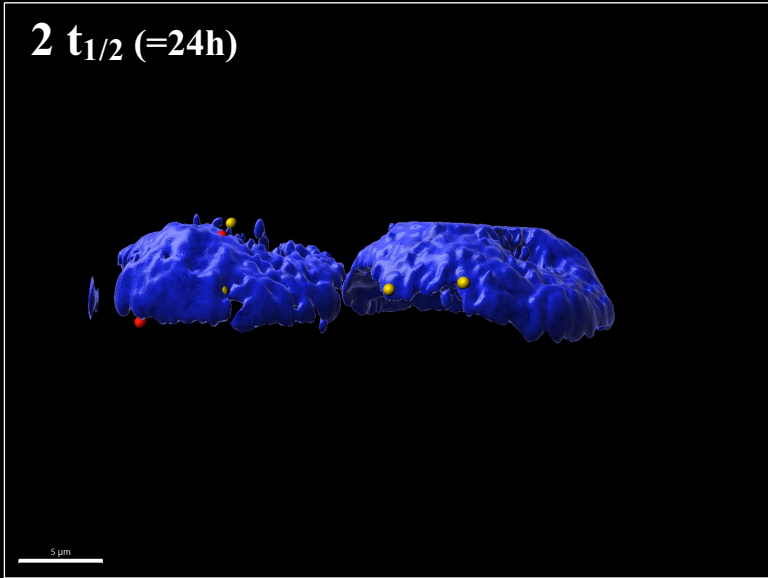
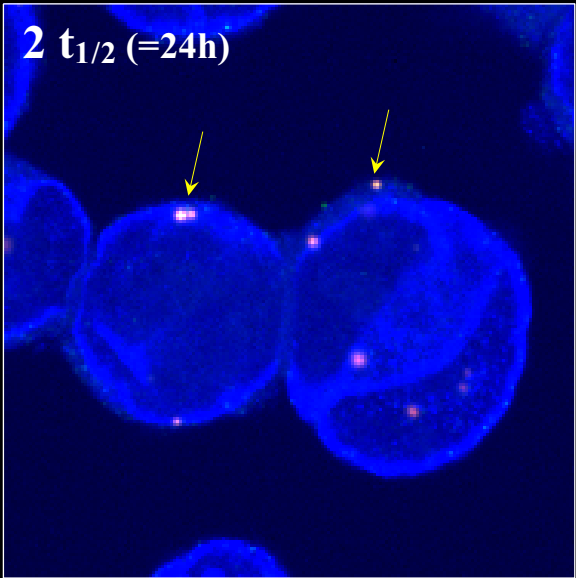
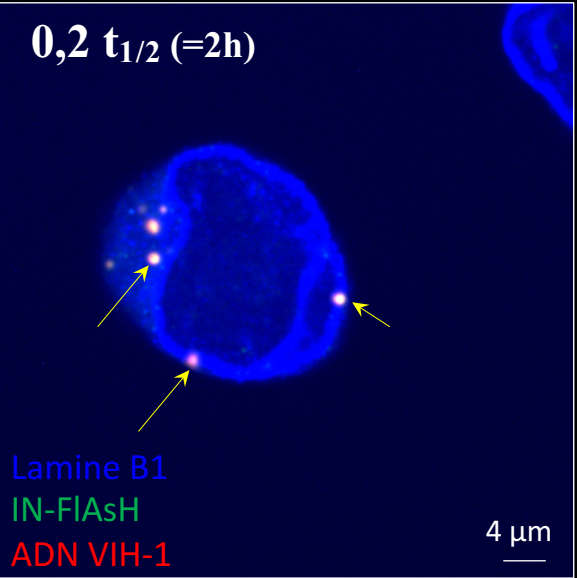
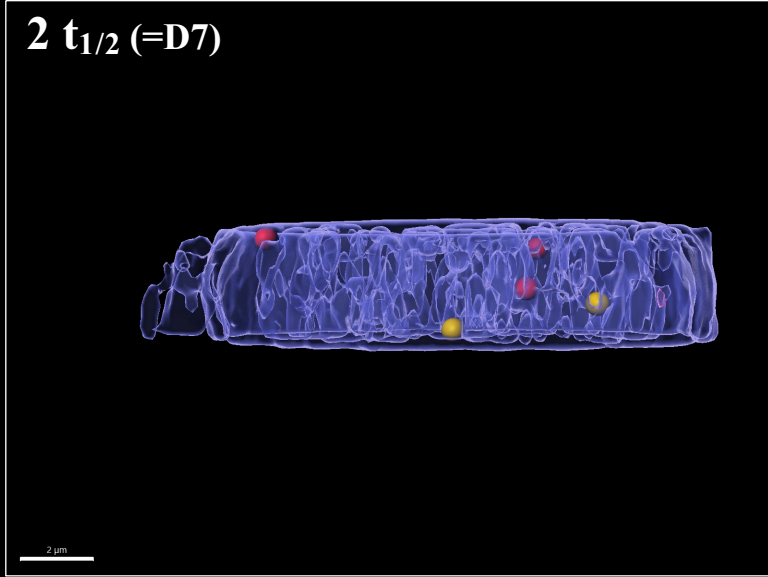
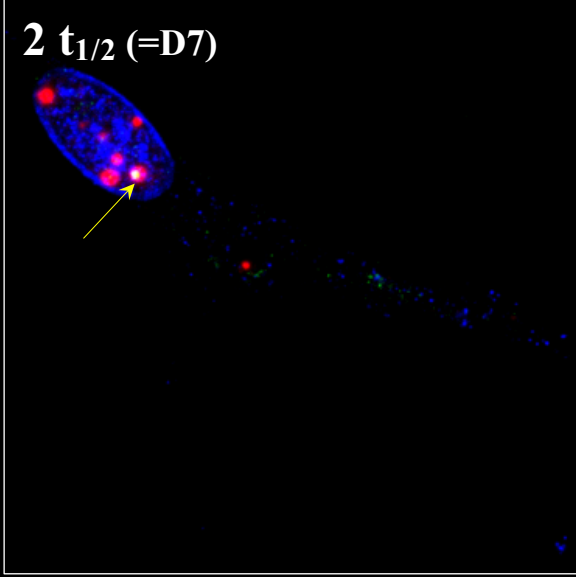
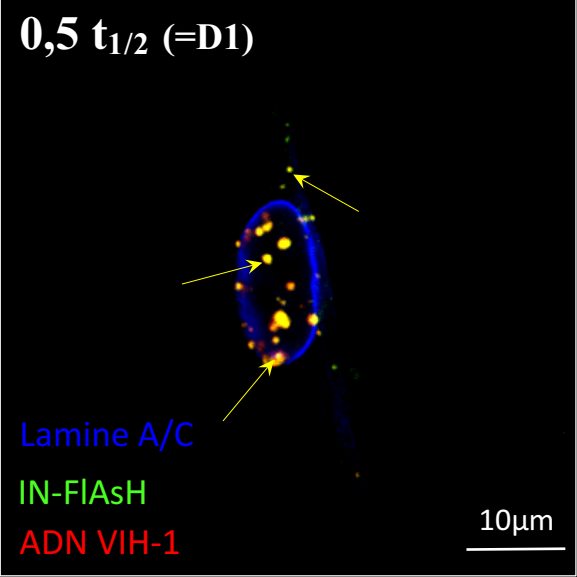
Integrase  
ULD  
Viral DNA (Circles)

# ULD Localization in cells

MDM  
 $t_{1/2} = 3$   
 days

Integrase  
 ULD  
 Viral DNA (Circles)

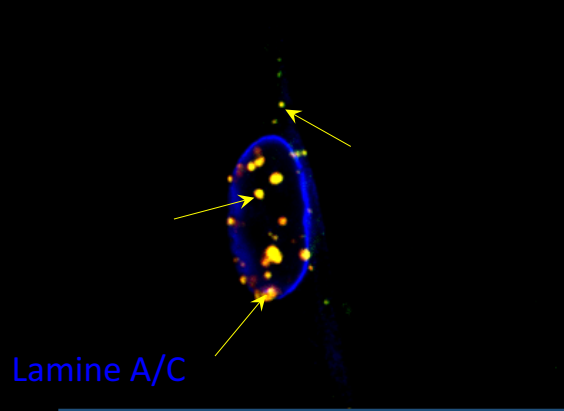
Activated  
 CD4<sup>+</sup> T-cells  
 $t_{1/2} = 0,5$   
 days



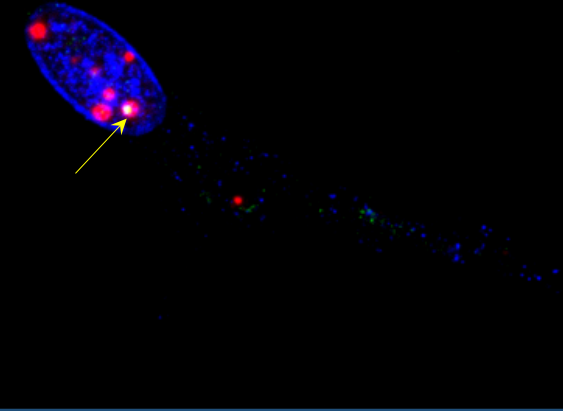
# ULD Localization in cells

MDM  
 $t_{1/2} = 3$   
 days

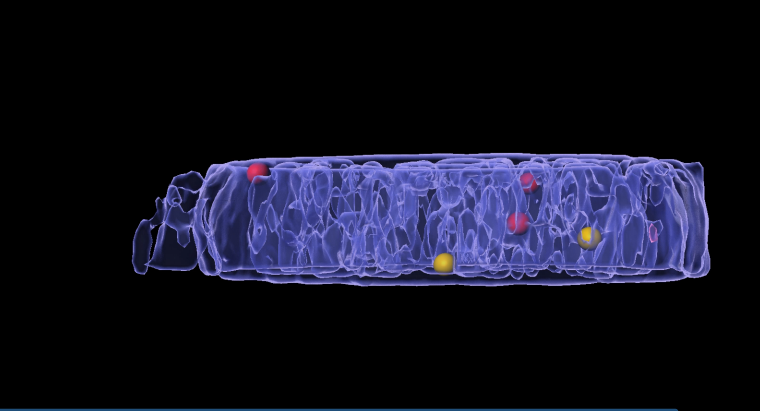
0,5  $t_{1/2}$  (=D1)



2  $t_{1/2}$  (=D7)



2  $t_{1/2}$  (=D7)

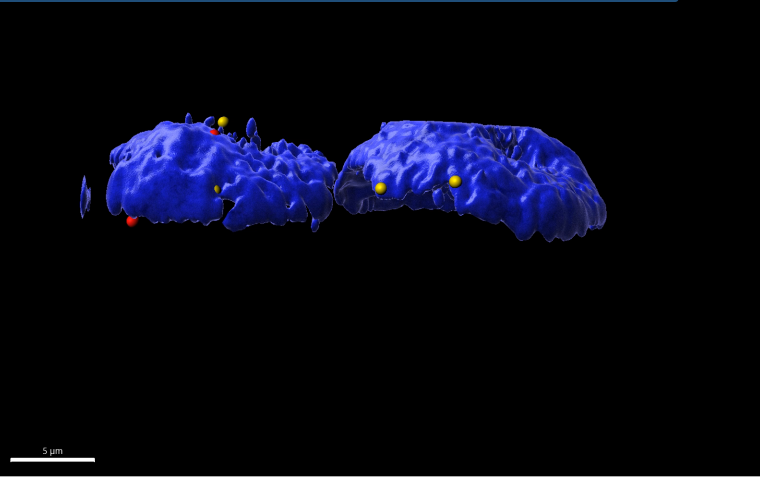
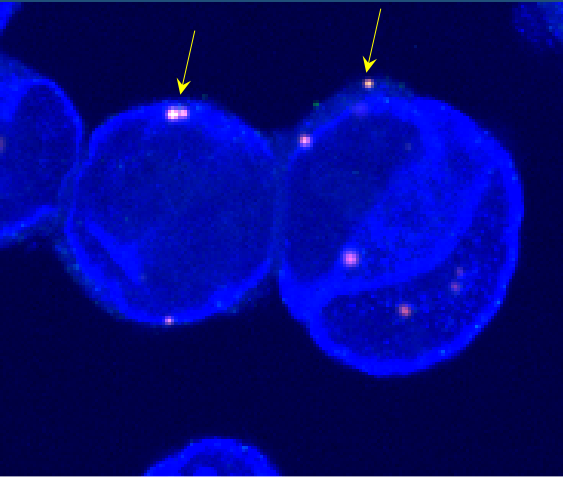
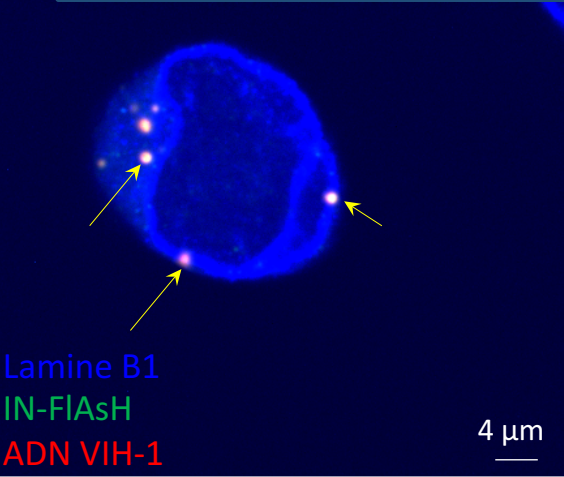


IN-  
 AD

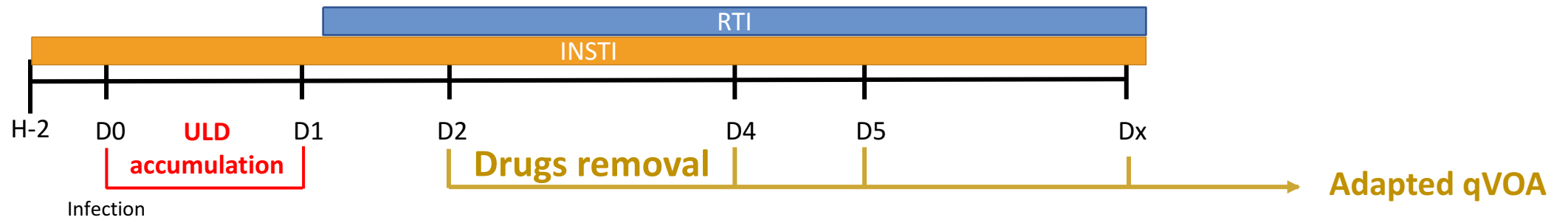
→ ULD localization differs between cell type  
 (nucleus in MDM and perinucleus in CD4<sup>+</sup> T cells)  
 → Integrase detected at late time points : Integration potential conservation?

Activated  
 CD4<sup>+</sup> T-cells  
 $t_{1/2} = 0,5$   
 days

0,

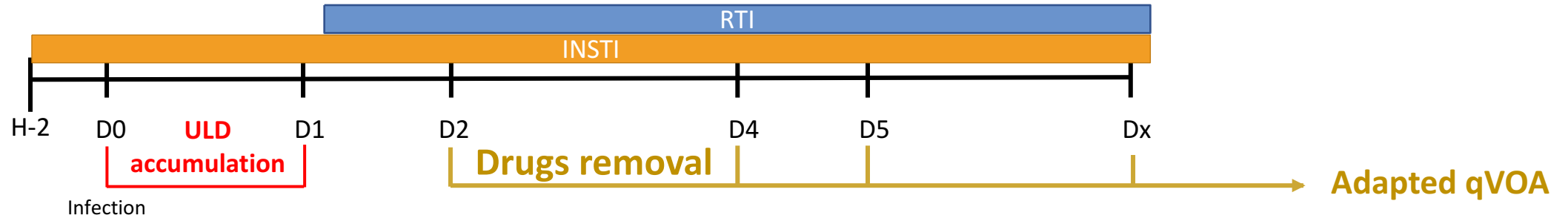


# Persistent ULD can integrate and lead to viral production after INSTI removal

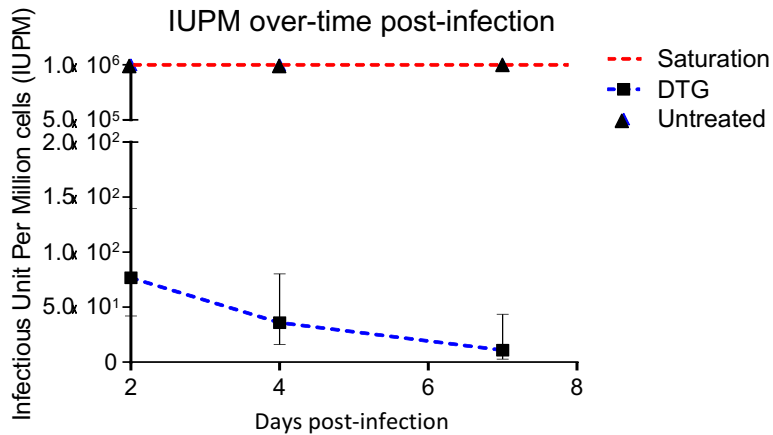




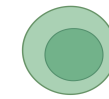
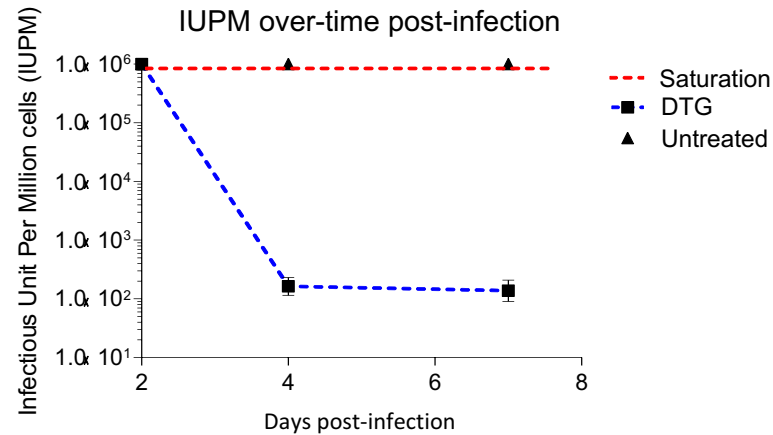
# Persistent ULD can integrate and lead to viral production after INSTI removal



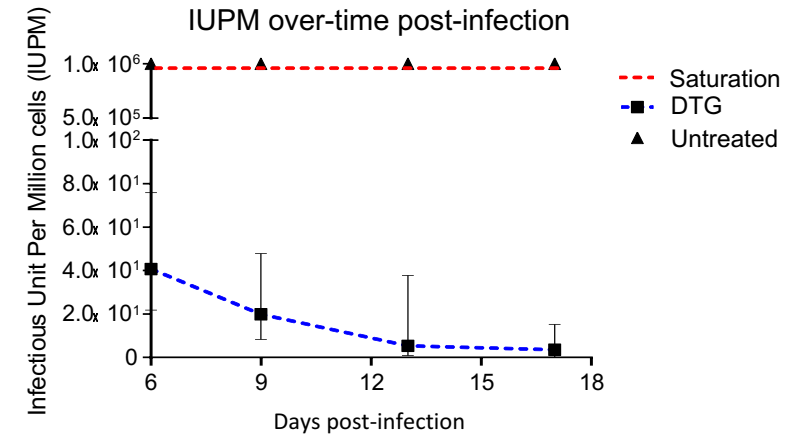
Activated CD4<sup>+</sup> T cells



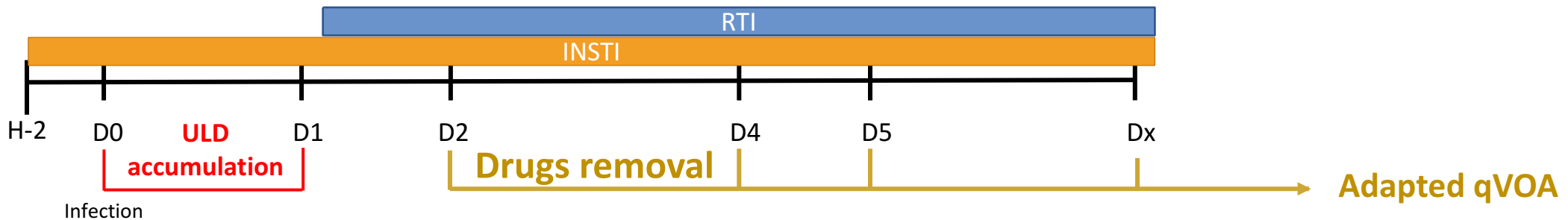
Primary Macrophages



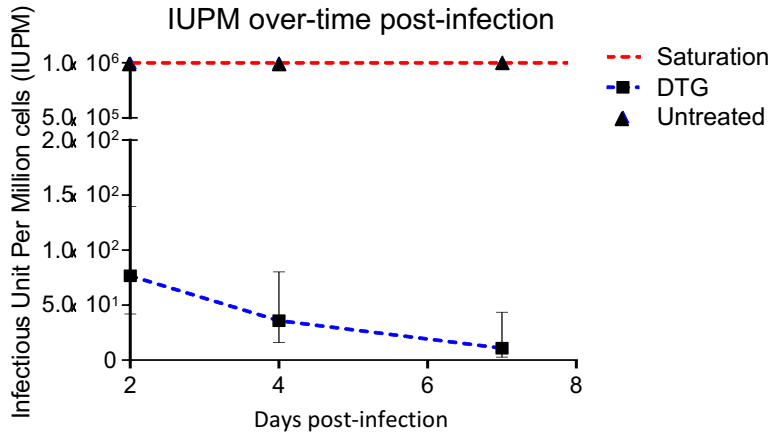
Primary Resting CD4<sup>+</sup> T cells



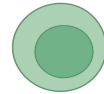
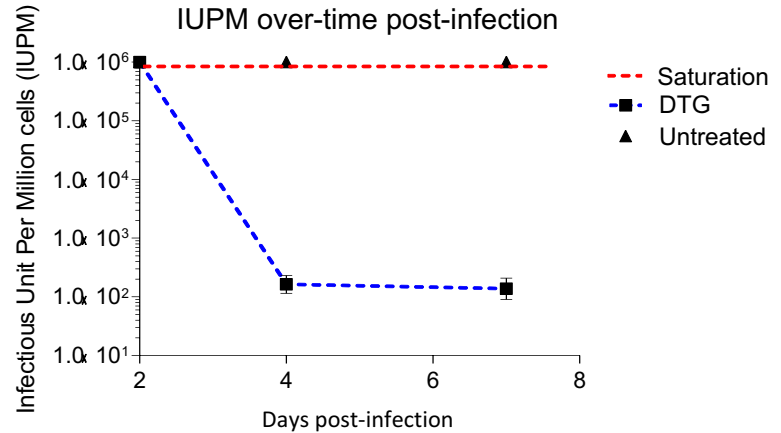
# Persistent ULD can integrate and lead to viral production after INSTI removal



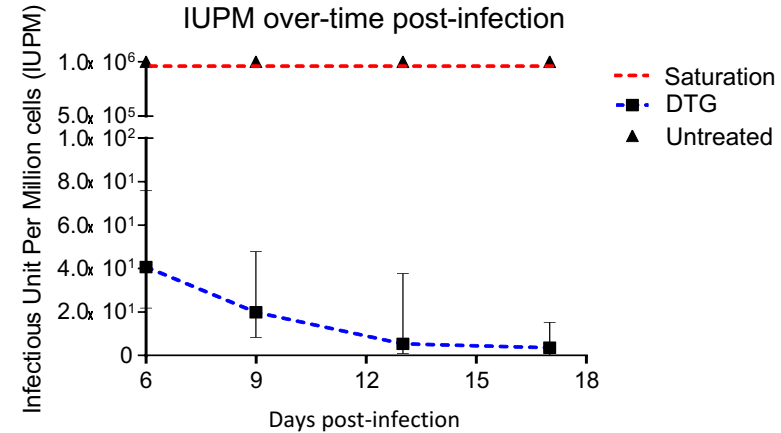
Activated CD4<sup>+</sup> T cells



Primary Macrophages



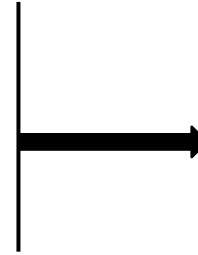
Primary Resting CD4<sup>+</sup> T cells



→ INSTI treatment removal at late ULD persistence time leads to viral replication  
 → Decrease of viral replication after INSTI treatment removal over time

**LEVI syndrom** (Long-acting Early Viral Inhibition) :

- Infection occurred during Cab-La PrEP treatment
- Often associated with drug resistance
- Viral breakthrough can occur months after PrEP interruption



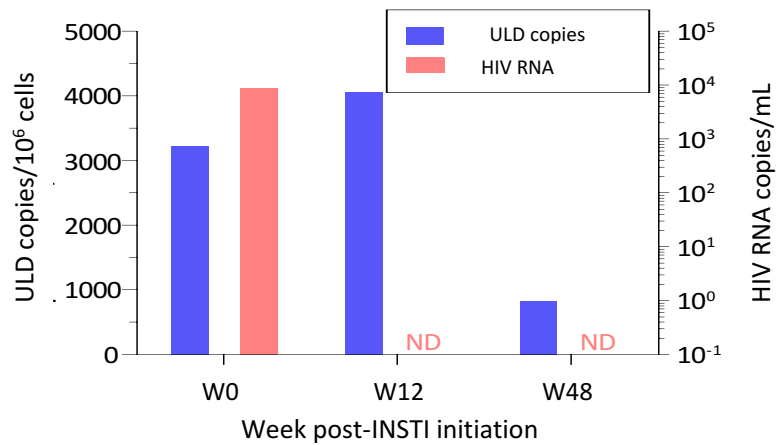
**Importance of pre-integrative latency  
in PrEP strategies?**

# Perspectives and implication in PrEP/treatment strategies

## LEVI syndrome (Long-acting Early Viral Inhibition) :

- Infection occurred during Cab-La PrEP treatment
- Often associated with drug resistance
- Viral breakthrough can occur months after PrEP interruption

→ **Importance of pre-integrative latency in PrEP strategies?**



→ **Role of pre-integrative latency in HIV persistence?**

**ULD detected 1 year after INSTI-based treatment initiation**

**OPTIPRIM-2 cohort**  
*Unpublished data*

## Unintegrated Linear DNA

### Conclusions:

- ULD persistence and localization differ between cell types
- ULD can integrate after INSTI removal and lead to viral replication

### Consequences:

- ULD may participate in HIV persistence/viral rebound?
- ULD may be important in PrEP strategies?

# Acknowledgment



## Team “Dendritic cells, tumoral and viral microenvironment immunostimulation”

Dr Jacques DUTRIEUX  
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Bénédicte CHARMETEAU  
Manon BABISE  
Baptiste BENSOT  
Dr Hélène ROUX  
Dr Rémi CHEYNIER  
Dr Anne COUEDEL COURTEILLE  
Dr Magali RANCEZ

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Dr Roy MATKOVIC  
Dr Caroline CHARRE  
Pr Antoine CHERET  
Elise GARDIENNET  
Adeline DURETZ  
And many others!



Franck LETOURNEUR  
Juliette HAMROUNE  
Lucie ADOUX



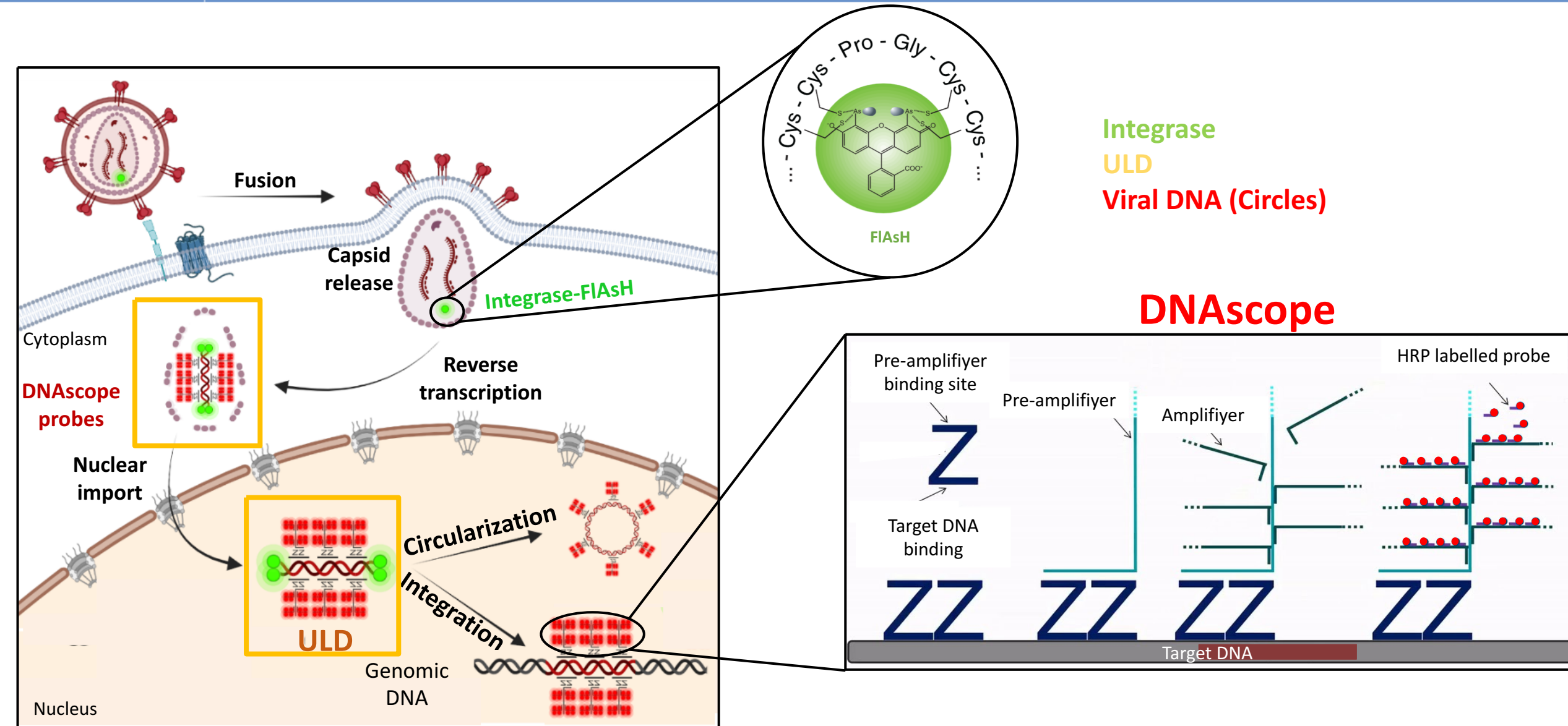
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Dr Thomas GUILBERT  
Julie LESIEUR



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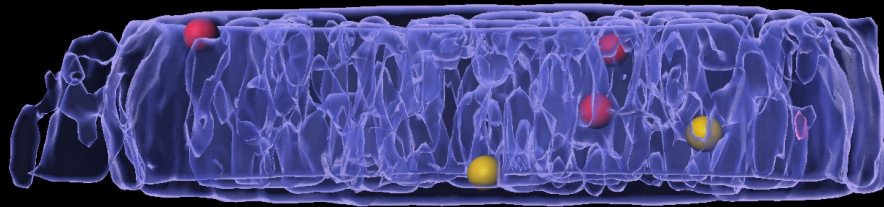


# DNAscope protocol to follow ULD Localization over time

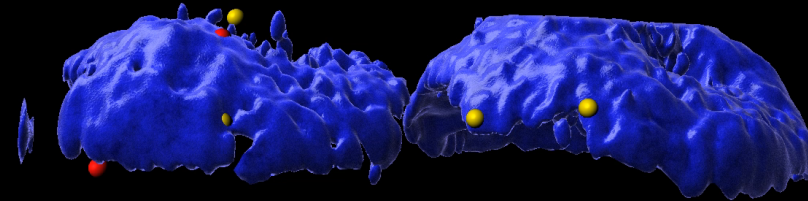


# ULD Localization in cells

MDM  
 $2t_{1/2}$  (=D7)



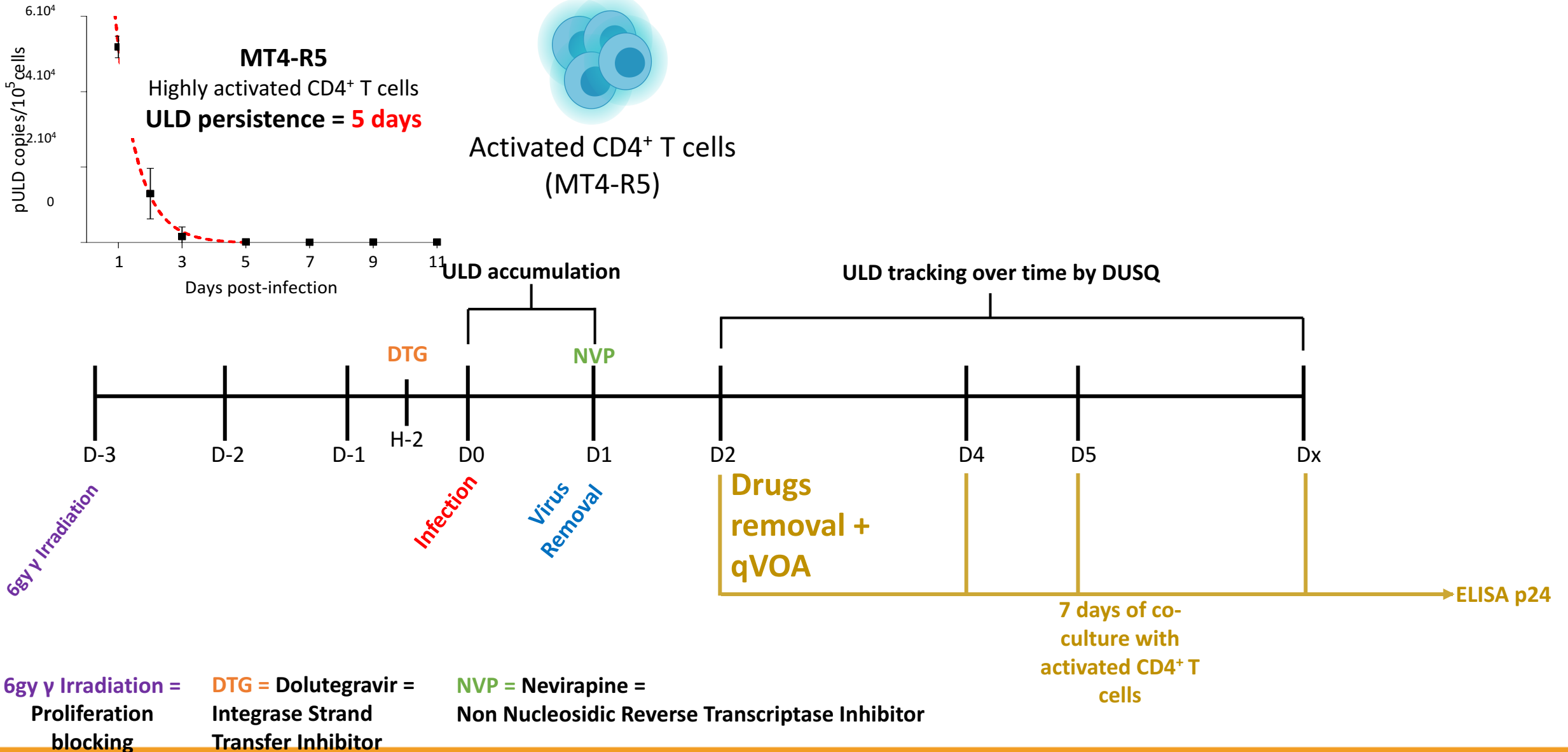
MT4-R5  
 $2 t_{1/2}$  (=24h)



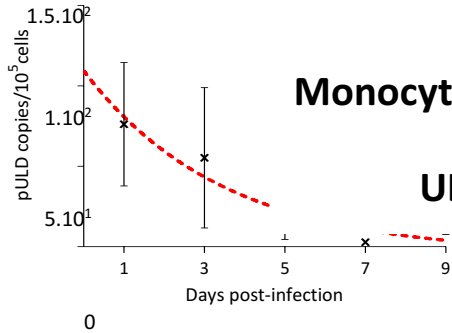
ULD (Integrase + DNA)  
Viral DNA (Circles)  
Nucleus

- ULD localization differs between cell type  
(nucleus in MDM and perinucleus in CD4<sup>+</sup> T cells)
- Integrase detected at late time points : Integration potential conservation?

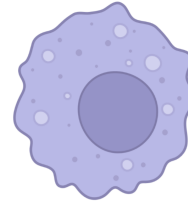
# Experimental protocol in Activated CD4<sup>+</sup> T cells



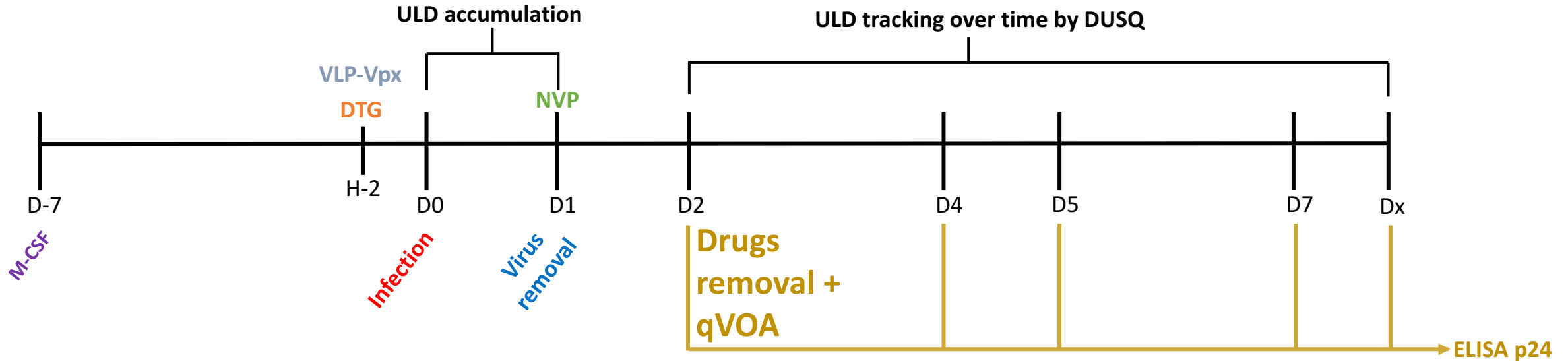
# Experimental protocol in Monocytes Derived Macrophages (MDM)



**Monocytes derived macrophages (MDM)**  
Moderately activated  
**ULD persistence = 1 month**



MDM



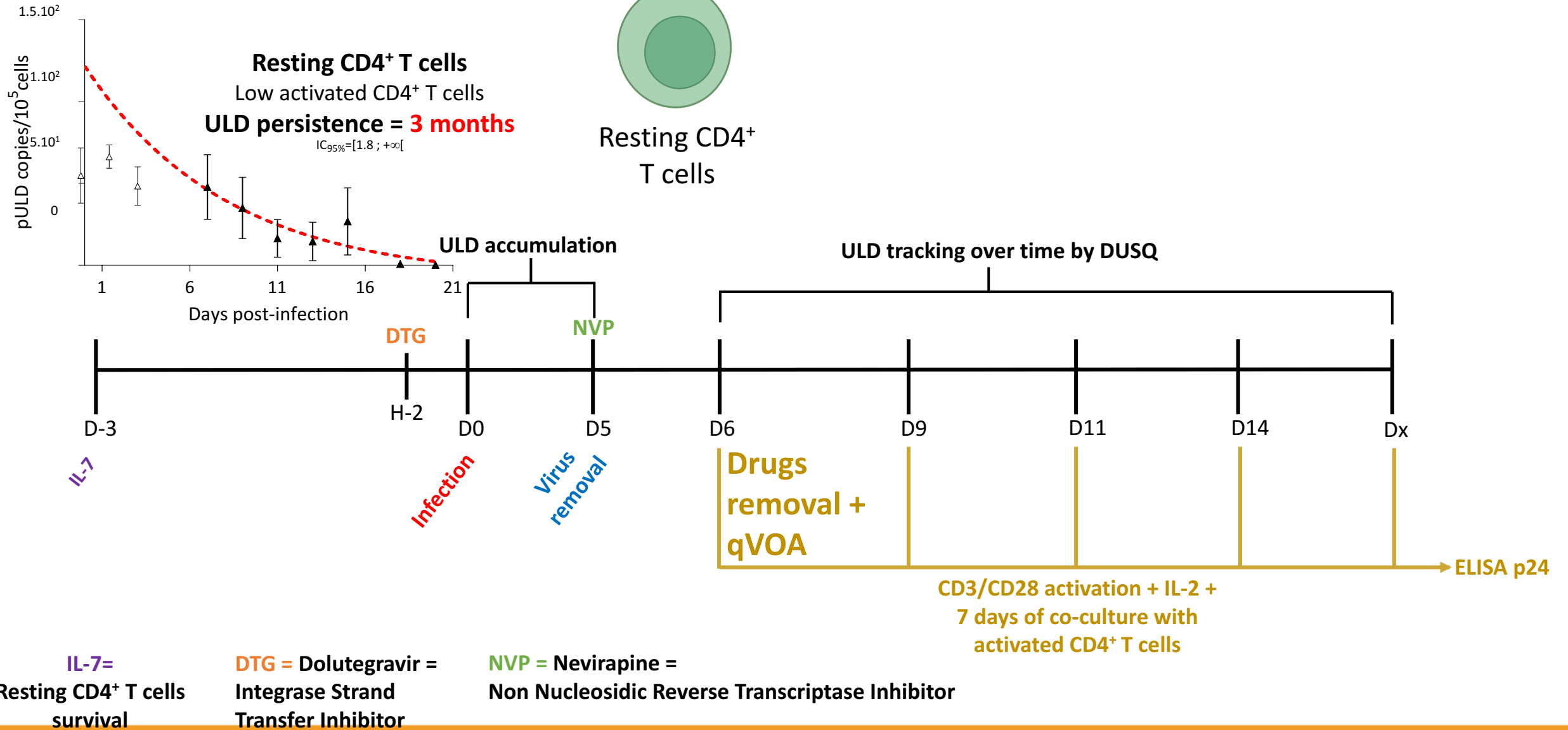
**M-CSF=**  
Monocytes  
differentiation in  
MDM

**VLP-Vpx =**  
SAMHD1  
degradation =  
DNA synthesis

**DTG = Dolutegravir =**  
Integrase Strand  
Transfer Inhibitor

**NVP = Nevirapine =**  
Non Nucleosidic Reverse Transcriptase Inhibitor

# Experimental protocol in Resting CD4<sup>+</sup> T cells



# HIV-1 Unintegrated DNA

## CIRCULAR DNA

Auto-integration: truncated/rearranged circles

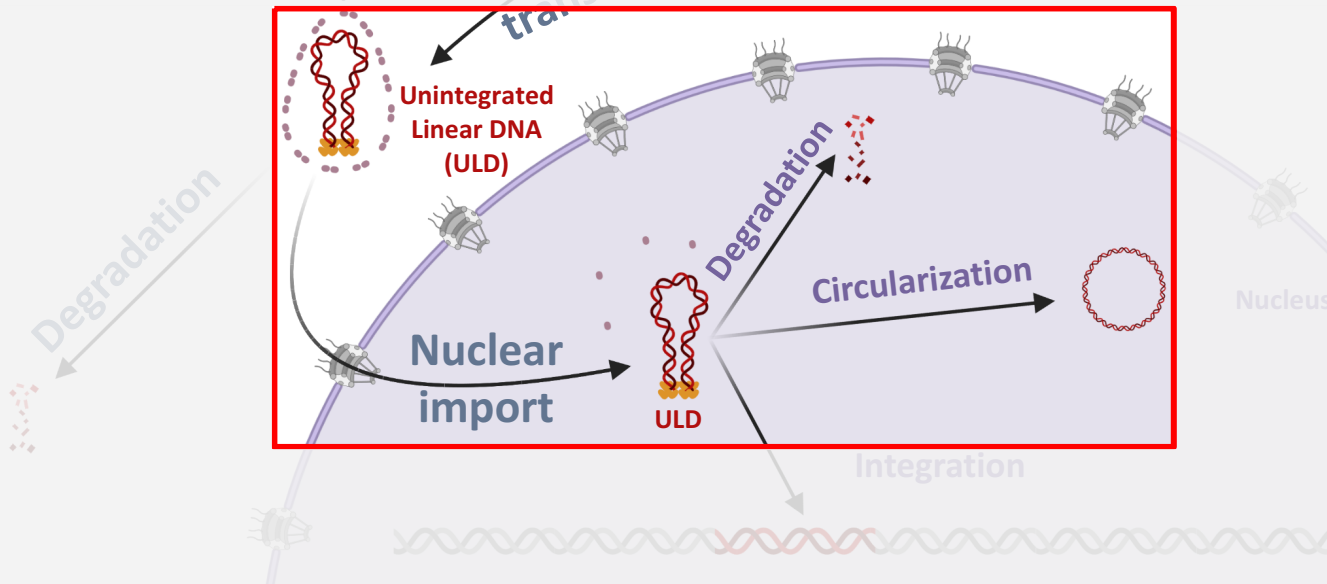
Homologous recombination: 1LTR circles

Repair (Non homologous End Joining): 1 or 2 LTR circles

## LINEAR DNA

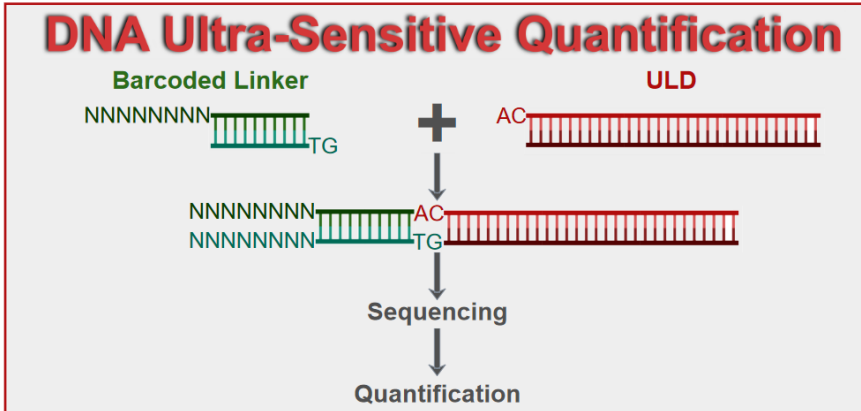
**20-35% of vDNA**

*Orlandi et al, Journal of Translational Medicine, 2020*



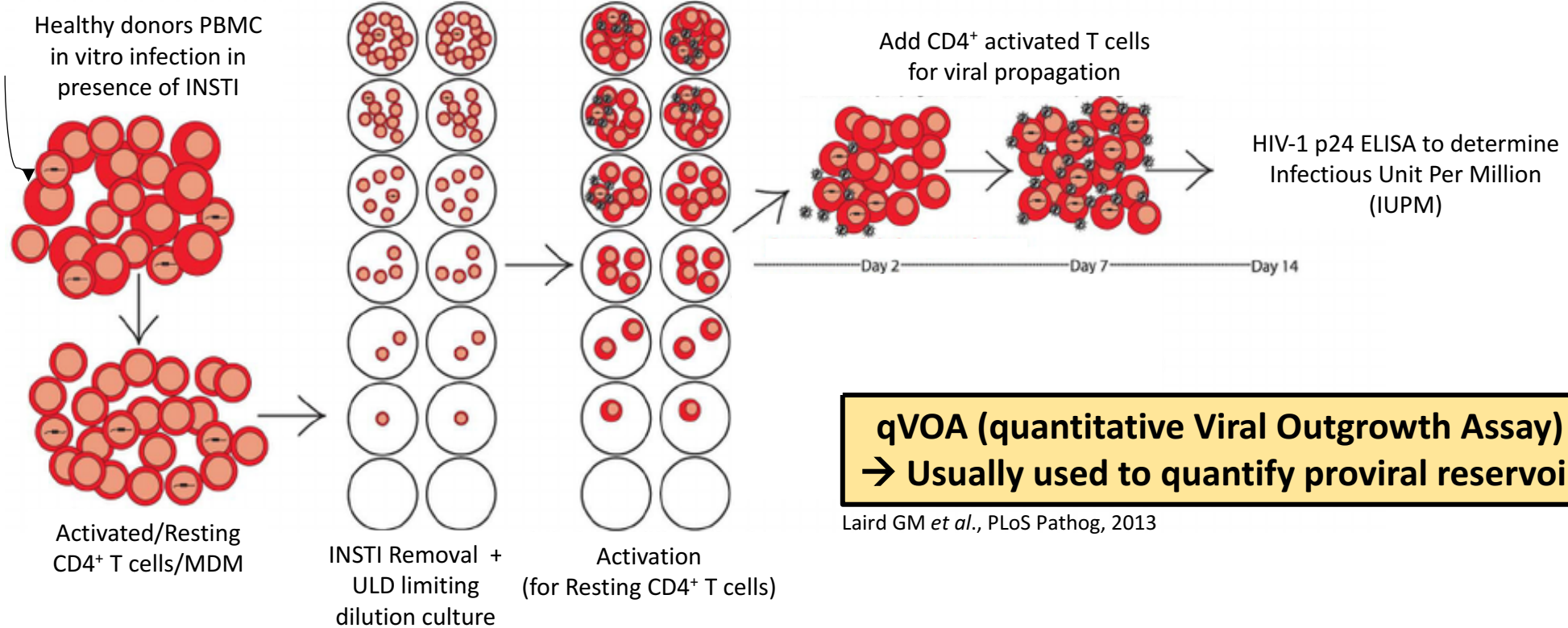


# DUSQ and qVOA



**DUSQ**  
→ Specific  
→ Ultra sensitive (up to 1 copy/10<sup>6</sup> cells)

Roux H. M. *et al.*, Cell Reports Methods, 2023  
(HIV persistence during therapy congress 2022 YI 2.7)



**qVOA (quantitative Viral Outgrowth Assay)**  
→ Usually used to quantify proviral reservoir

Laird GM *et al.*, PLoS Pathog, 2013