November 8th, 2022
Palau Macaya, Barcelona, Spain

Transcriptional mechanisms of HIV-1 latency: implications for innovative therapeutic strategies

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November 8th, 2022

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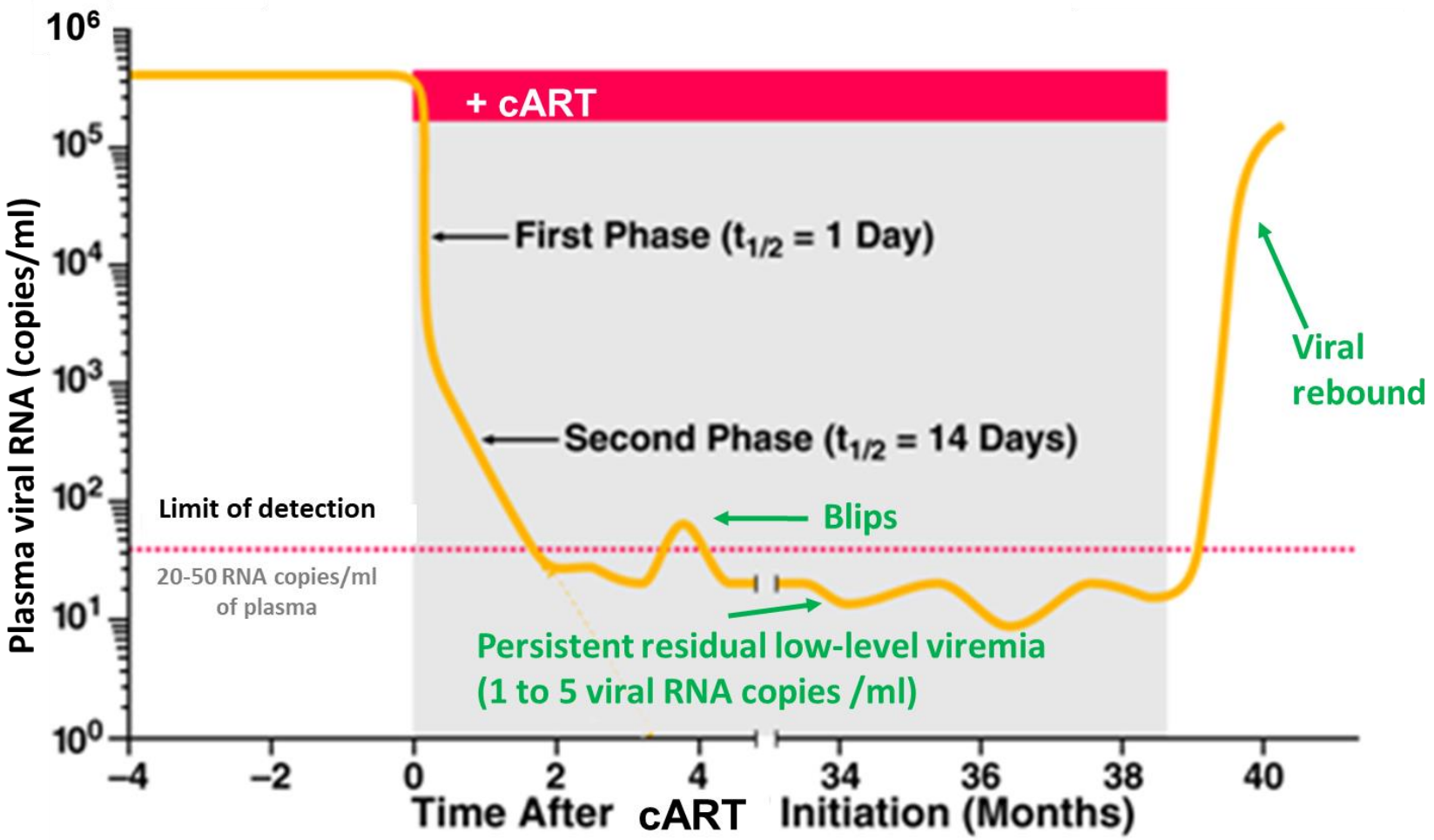
Research Director FRS-FNRS
Director of the Service of Molecular Virology

Combination antiretroviral therapy (cART) is potent and life-prolonging but does not eradicate HIV infection



In presence of cART, a low-level viremia persists which can lead, after interruption of treatment, to a rapid viral rebound due notably to reactivation of HIV-1 expression from latently-infected cells.
While most of the latently-infected cells contains defective proviruses, the low number of replication-competent proviruses forms a very stable reservoir.

Heterogeneous composition of the HIV-1 reservoirs

The main reservoir resides in a small and stable population of latently-infected long-lived resting CD4+ memory T cells However, many cells may contribute to the latent reservoirs including:

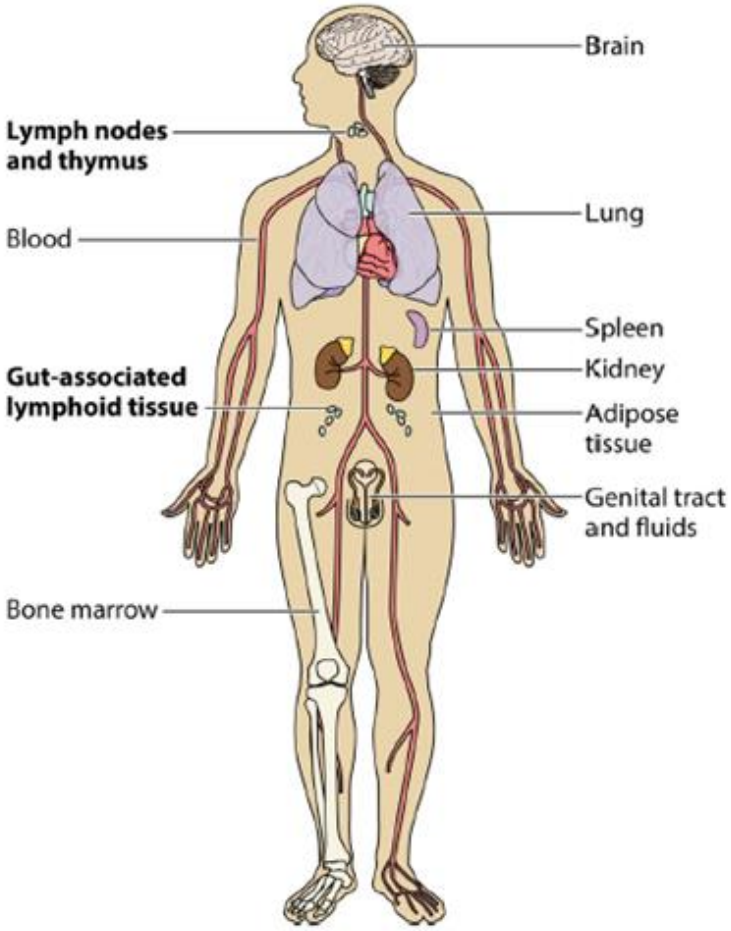
1- Naive CD4+ T cells (Zerbato et al. CID. 2019; Venanzi Rullo E et al. CID 2019)

2- CD4+ "T memory stem cells" (TSCM) (Buzon et al. Nat. Med. 2014; Gattinoni et al., 2011 Nat Med)

3- Distinct lymphocyte populations within tissues, such as T follicular helper (Tfh) cells (Banga et al. 2016 Nat. Med)

4- Active CD4+ T cells as shown by Sarah Palmer (Cell Reports, 2017) **and CD32+CD4+ T cells as recently shown by Alexander Pasternak and Ben Berkhout** (Darcis et al., Cell Reports 2020 - CD32+ CD4+ T cells are highly enriched for HIV DNA)

5- Non-T cells from the myeloid lineage (Kumar et al., Viruses 2014; Honeycutt J. B. et al., Nat. Med. 2017), **including, among others, monocytes, macrophages, dendritic cells (DCs) and tissue macrophages such as microglial cells** (Avalos CR et al., Mbio 2017), **adipose tissue macrophages** (Damouche A. et al., PloS Pathog. 2015) **and urethral macrophages (constitute a principal tissue, replication-competent LPS-inducible HIV-1 reservoir** (Y. Ganor et al., Nat Microbiol. 2019)).



Avettand V et al. Clin Microbiol Rev. 2016

Rebounding viruses can originate from all these heterogeneous reservoirs (M.-A. De Scheerder et al., Cell Host Microbe, 2019).

Major HIV cure strategies aimed at achieveing a remission

1) LIMIT THE SIZE OF THE RESERVOIRS

Early treatment (as highlighted by the Visconti study)

2) DESTROY/INACTIVATE THE RESERVOIRS

Gene therapy

3) SILENCE THE RESERVOIRS

Impede reactivation (the « block-and-lock » strategy) (reinforce latency by suppressing transcription, latency-promoting agents (LPAs))

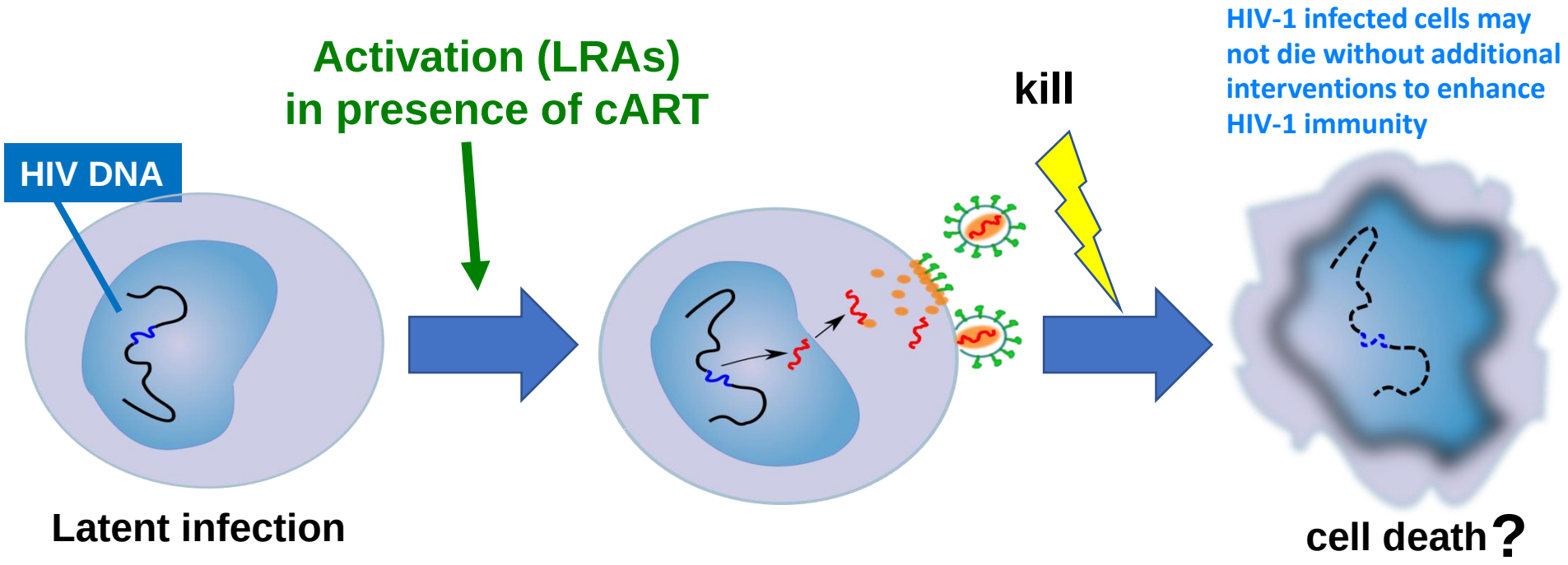
4) SHOCK THE RESERVOIRS

« Flush out » or « kick out » the latent proviruses (latency-reversing agents (LRAs))

5) CONTROL THE RESERVOIRS

Vaccines/immunotherapies

The « shock-and-kill » strategy for purging latent viral reservoirs is one of the most explored approaches in reaching a cure for HIV



The "shock and kill" strategy is based on HIV reactivation in latently-infected cells using LRAs ("shock" phase), while maintaining cART in order to prevent spreading of the infection. This kind of strategy would allow the "kill" phase during which latently-infected cells would then die from viral cytopathic effects or host cytolytic effector mechanisms.

HIV cure strategies

Multiple strategies aimed at achieving a remission are intensively explored.

1) LIMIT THE SIZE OF THE RESERVOIRS

Early treatment (as highlighted by the Visconti study)

2) DESTROY/INACTIVATE THE RESERVOIRS

Gene therapy

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The « block-and-lock » strategy for long-lasting epigenetic silencing of HIV-1 transcription in absence of cART

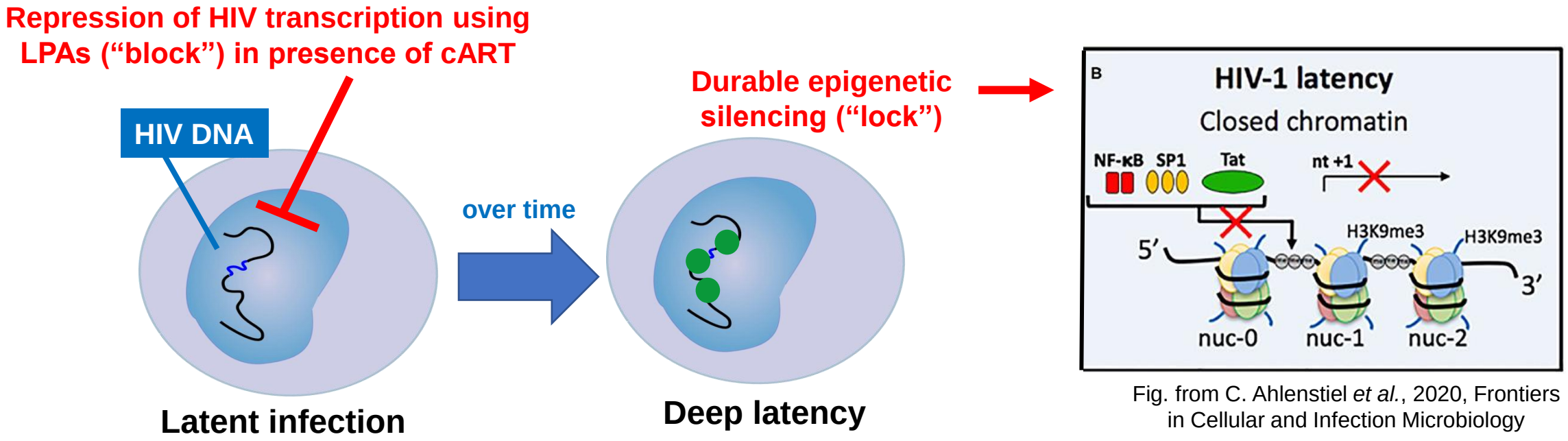
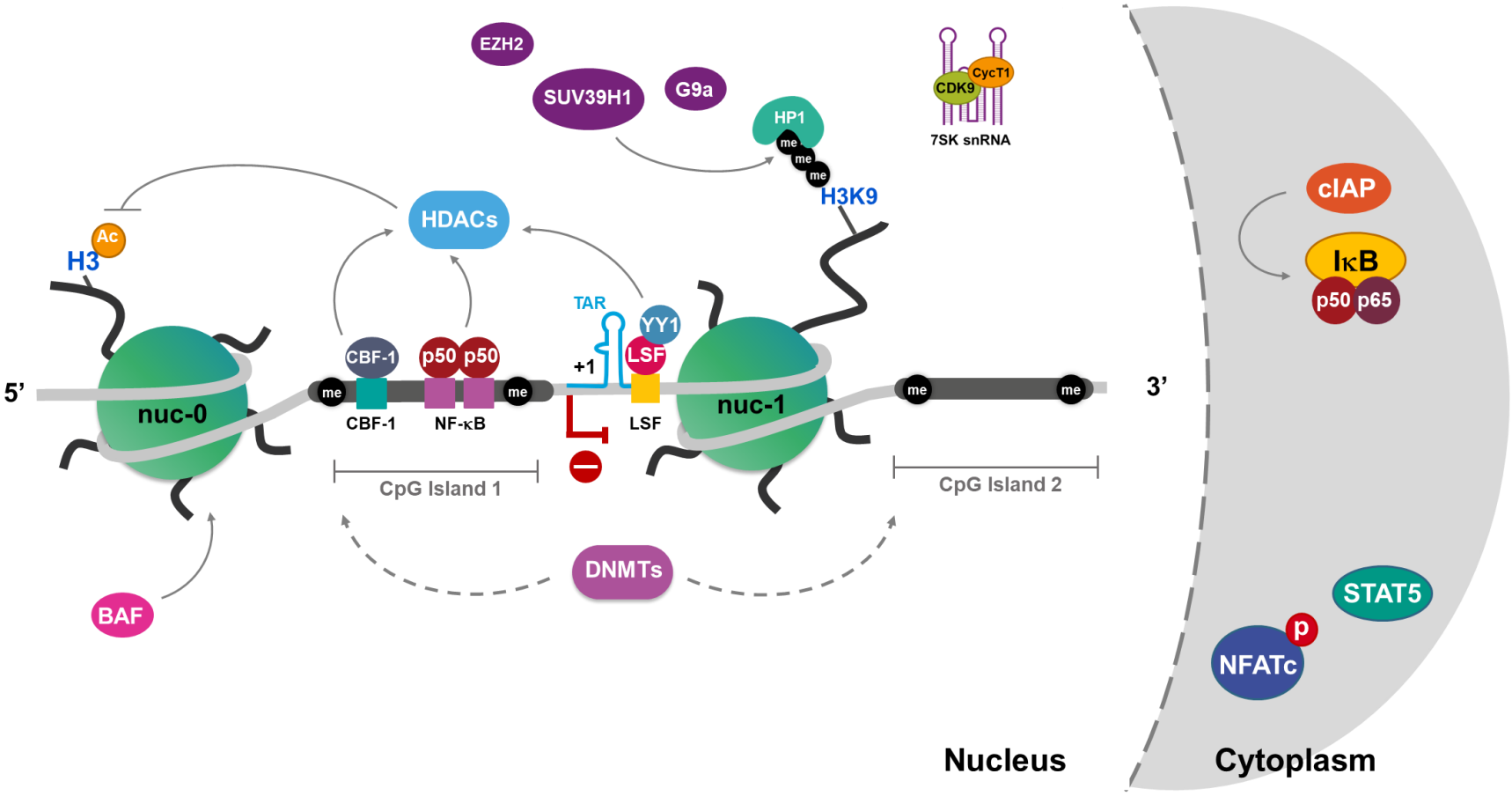


Fig. from C. Ahlenstiel *et al.*, 2020, Frontiers in Cellular and Infection Microbiology

The addition of latency-promoting agents (LPAs), such as HIV Tat inhibitors of HIV-specific transcriptional inhibitors, to an ART regimen, suppresses HIV transcription (“block”), so that a durable epigenetic silencing (“lock”) may be established over time through the accumulation of repressive epigenetic marks at the HIV promoter. This permanent control of the HIV-1 promoter would mean that cART is no longer required.

Heterogeneity and multiplicity of the molecular mechanisms involved in HIV-1 post-integration latency



Epigenetic blocks

- DNA methylation
- Nucleosme positioning
- Histone PTMs (methylation, deacetylation)

Transcription initiation blocks

- Poor availability of positively acting TFs
- Abundance of negatively acting TFs

Transcription elongation blocks

- Negative elongation F
- Absence of Tat
- Sequestration of active P-TEFb

Post-transcriptional blocks

- Blocks to splicing
- Block to export
- Inhibitory miRNAs
- Translation blocks

Studying the mechanisms that drive HIV into latency is critical for targeted HIV cure efforts aiming at either reactivating or blocking viral expression.

(A. Ait Ammar *et al.,* Front. Microbiol 2020; R. Verdikt *et al.,* Vaccines 2021; A. Rodari *et al*., Annu. Rev. Virol. 2021 ; A.Kula *et al*., Semin. Immunol. 2021)

The impact of the chromatin environment on HIV-1 expression, persistence and clonal expansion dynamics

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Article

Parallel analysis of transcription, integration, and sequence of single HIV-1 proviruses

Kevin B. Einkauf,[1,2,6] Matthew R. Osborn,[1,2,6] Ce Gao,[2,6] Weiwei Sun,[2,6] Xiaoming Sun,[2,5] Xiaodong Lian,[1,2] Elizabeth M. Parsons,[1,2] Gregory T. Gladkov,[2] Kyra W. Seiger,[1,2] Jane E. Blackmer,[1,2] Chenyang Jiang,[1,2] Steven A. Yukl,[3] Eric S. Rosenberg,[4] Xu G. Yu,[1,2] and Mathias Lichterfeld[1,2,7,*]

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Leading Edge

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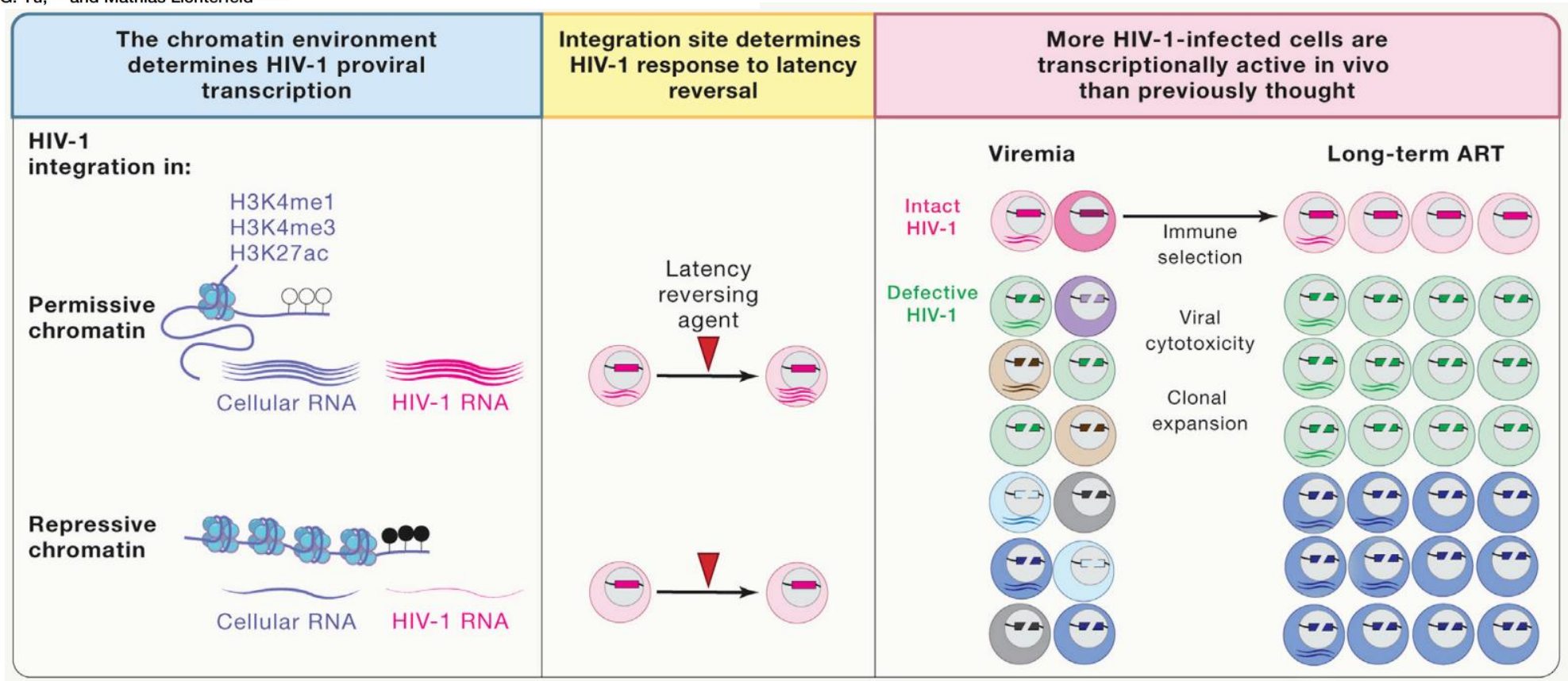
Previews

The loud minority: Transcriptionally active HIV-1-infected cells survive, proliferate, and persist

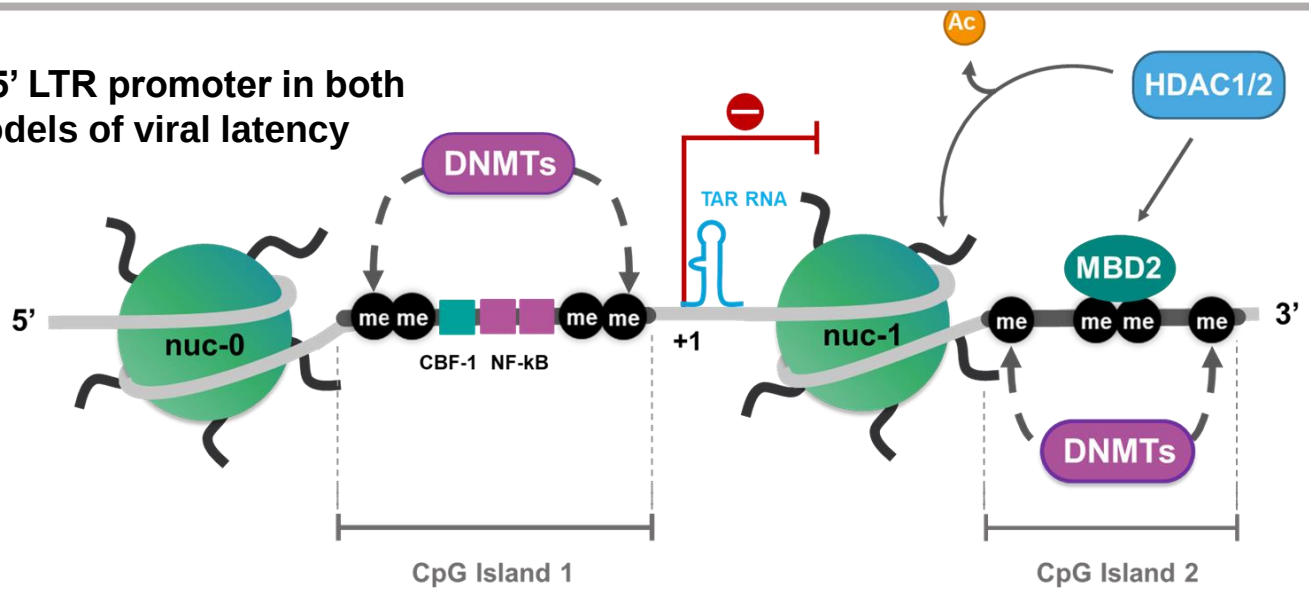
Jack A. Collora[1] and Ya-Chi Ho[1,*]



(Previews from J. A. Collora and Y.-C. Ho, Cell 2022 of Einkauf et al. Cell 2022)

DNA methylation participates to HIV-1 transcriptional silencing

Hypermethylation of the HIV-1 5' LTR promoter in both cell line and primary cell models of viral latency



Kauder et al., Plos Pathogens 2009
Blaskova et al., Plos Pathogens 2009
Blaskova et al., J. Virol. 2012
Palacios et al., J. Virol. 2012
Ho et al. Cell 2013
Trejbalova et al., Clin Epigenetics 2016

Conflicting data in cells from ART-treated aviremic patients

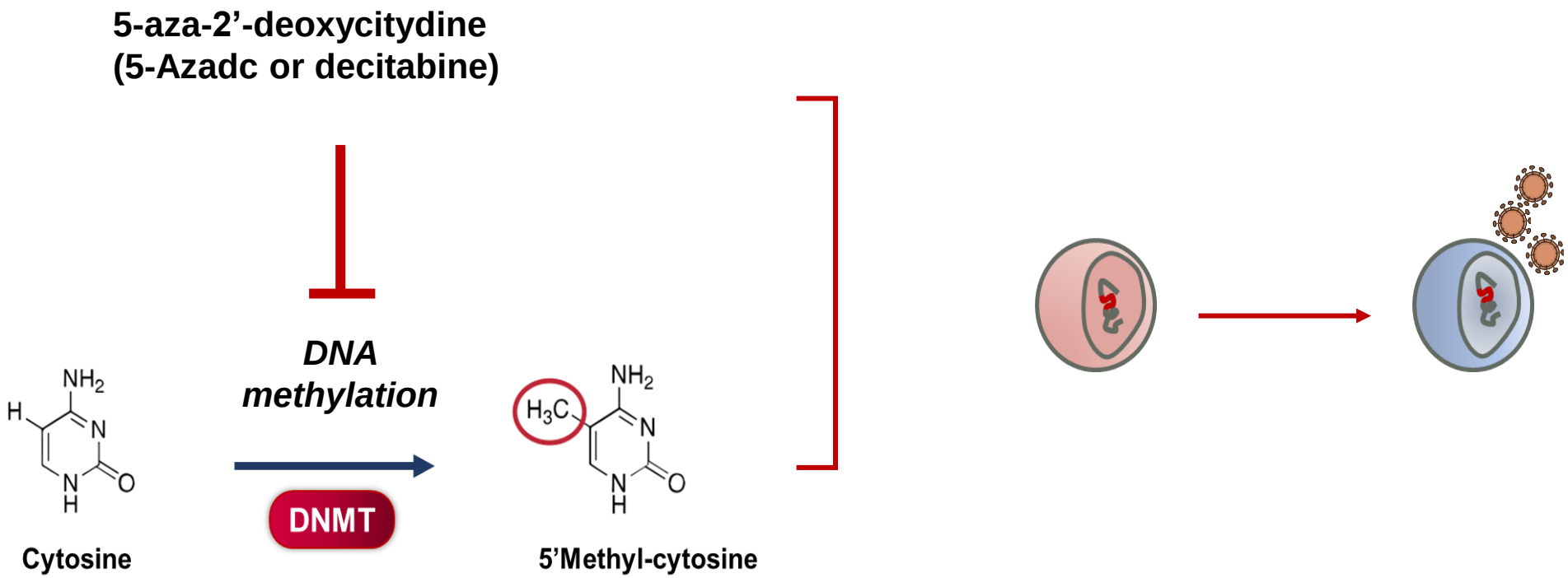
Presence	Absence
Blaskova *et al*., PLoS Path. 2009	Blaskova *et al*., J. Virol. 2012
Palacios *et al*., J. Virol. 2012	Ho *et al*., Cell 2013
Trejbalova *et al*., Clin Epigenetics 2016.	Weber *et al.*, Virology 2014
Cortés-Rubio *et al*., Clin. Epigenetics 2019	Kint *et al*., Clin Epigenetics 2020
Nguyen K. et al., PLoS Pathogens 2021	Boltz *et al*., Viruses 2021

Temporal parameters duration of :

cART **infection**

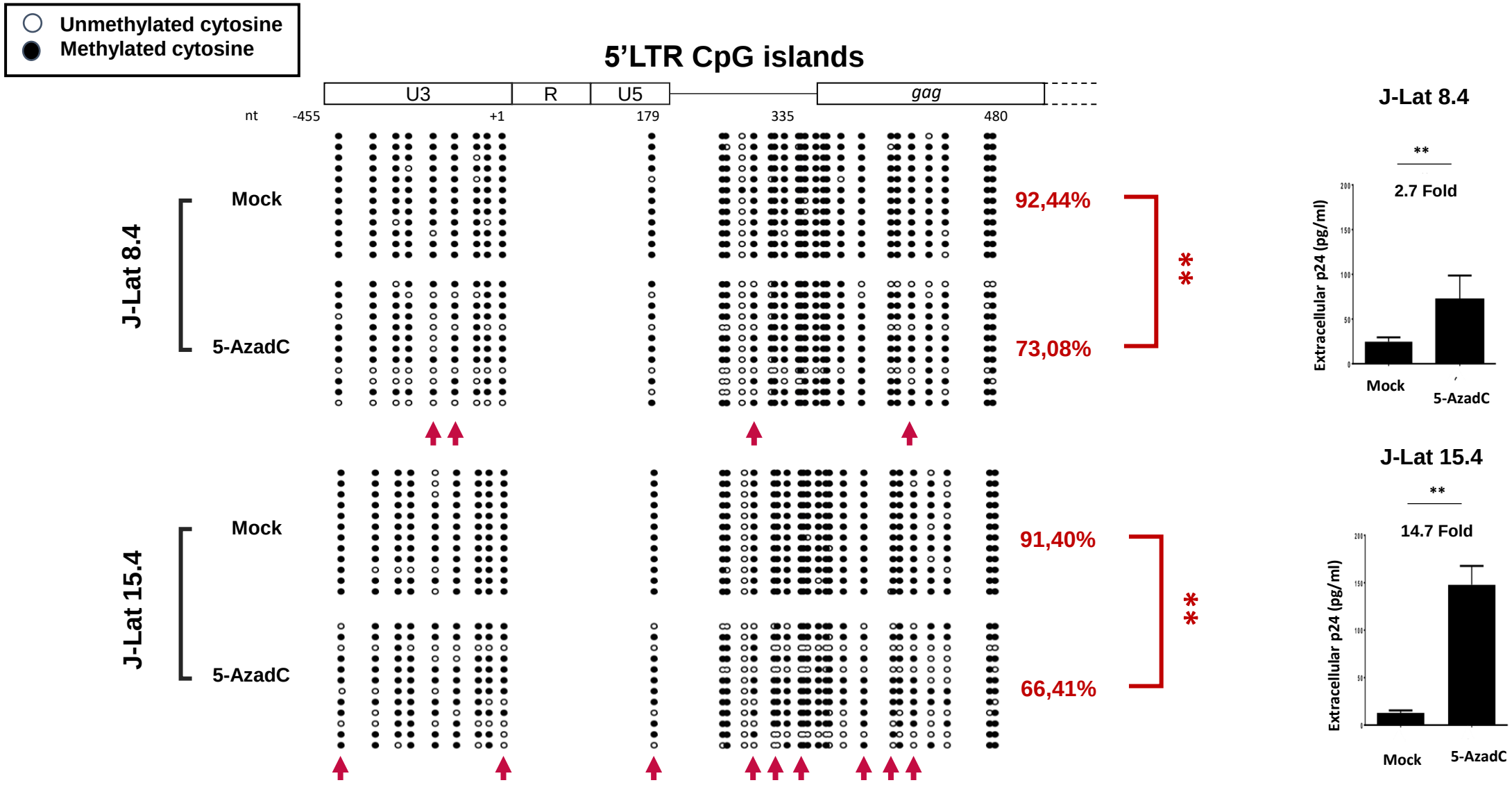
Accordingly, M. Lichterfield demonstrated a progressive longitudinal accumulation of proviruses integrated in chromosomal regions with hypermethylated cytosine residues, suggesting a role of DNA methylation in proviral silencing during prolonged ART (Einkauf *et al*., Cell 2022).

The DNA methylation inhibitor 5-aza-2'-deoxycitydine (5-Azadc or decitabine) reactivates HIV-1 from latency in cell lines and in patient cells



Kauder et al., Plos Pathogens 2009
Blaskova et al., Plos Pathogens 2009
Bouchat et al., EMBO Mol Med 2016
He et al. 2020 J. Virol.
Verdikt et al., eBioMedicine 2022

5-AzadC induced different extents of 5'LTR DNA demethylation and of HIV-1 reactivation depending on the J-Lat cell line studied



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A statistical approach enables the identification of significant 5-AzadC-induced differentially demethylated positions (DDMPs)

Cell line	CpG position†	Probability of 5-AzadC demethylation	p-value††	Significance	Location†††	DDMPs
J-Lat 8.4	-[119, 120]	0.64	0.0045	**	CRE site	5
	-[96, 97]	0.33	0.0466	*	NF-κB site	6
	+[183, 184]	0.33	0.0466	*	N/A	
	+[205, 206]	0.33	0.0466	*	Interferon-Stimulated Response Element	14
	+[231, 232]	0.45	0.0320	*	N/A	
	+[360, 361]	0.33	0.0466	*	Coding sequence of $p17^{Gag}$	
J-Lat 15.4	-[217, 218]	0.33	0.0466	*	N/A	
	-[47, 48]	0.42	0.0186	*	Sp1 site	9
	+[109, 110]	0.33	0.0466	*	U5 interacting with ψ	
	+[205, 206]	0.42	0.0186	*	Interferon-Stimulated Response Element	14
	+[231, 232]	0.42	0.0186	*	N/A	
	+[234, 235]	0.33	0.0466	*	Stem loop 1 (SL1) of ψ	
	+[243, 244]	0.33	0.0466	*	Zinc Knuckles in $p7^{Gag}$ binding to SL1	
	+[295, 296]	0.50	0.0069	*	SL2 of ψ	
	+[314, 315]	0.33	0.0466	*	SL3 of ψ	
	+[341, 342]	0.42	0.0186	*	SL4 of ψ	
	+[347, 348]	0.33	0.0466	*	SL4 of ψ	
	+[360, 361]	0.36	0.0129	*	Coding sequence of $p17^{Gag}$	

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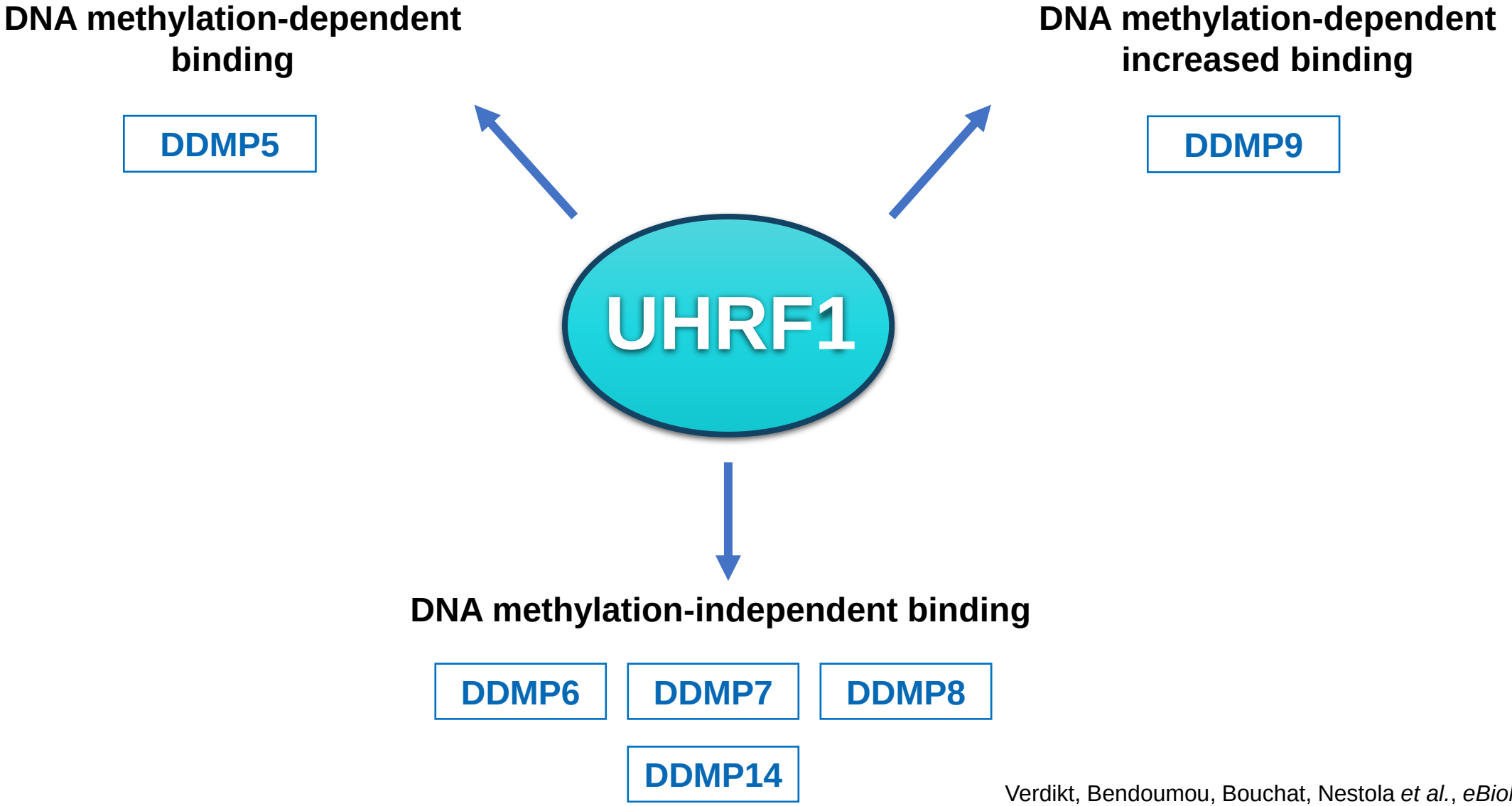
UHRF1 binds *in vitro* to multiple DDMPs within the HIV-1 promoter through different binding modalities



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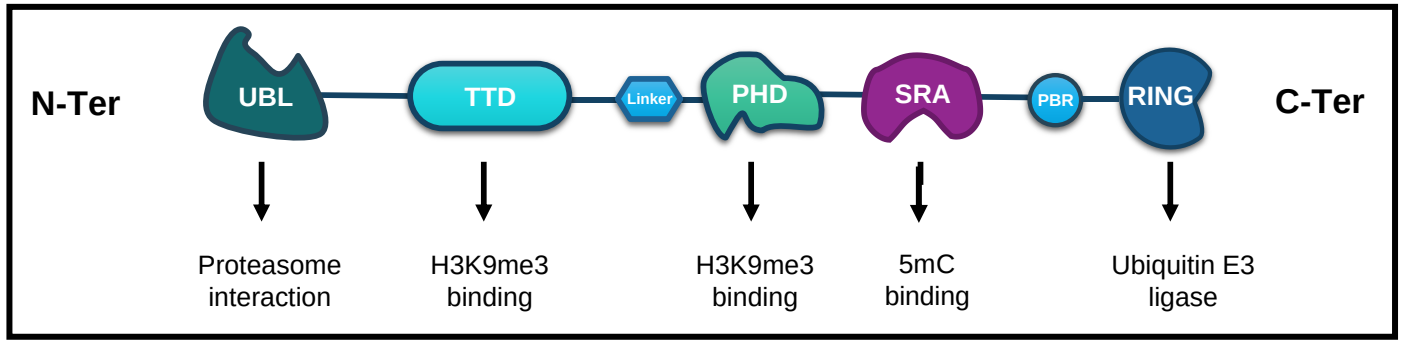
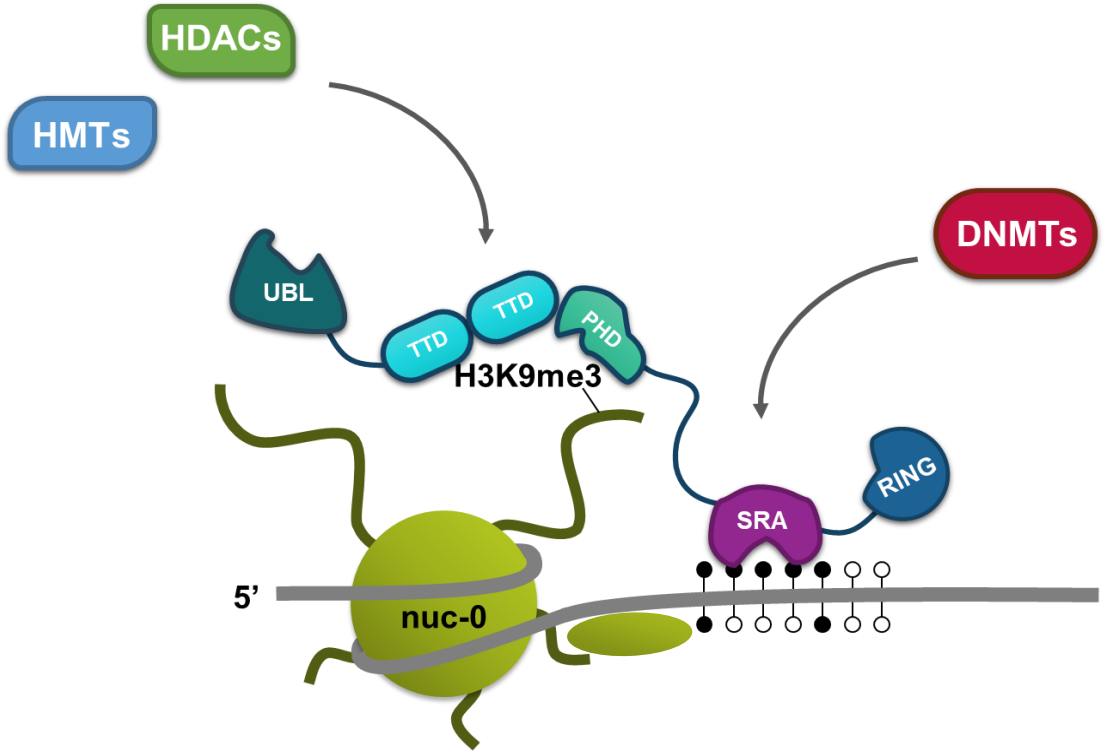
UHRF1 (Ubiquitin-like containing PHD and RING Finger domains 1) : an important epigenetic integrator



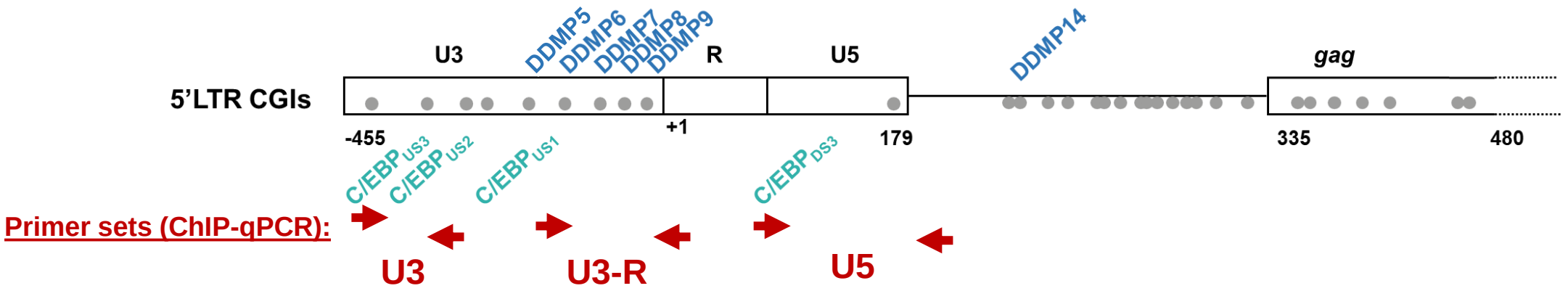


UHRF-1 is a protein known to regulate and maintain heterochromatic equilibrium through action on both DNA methylation and histone modifications, notably through recognizing several epegenetic marks and interacting with enzymes catalyzing these marks (DNMT, HMT, HDAC).

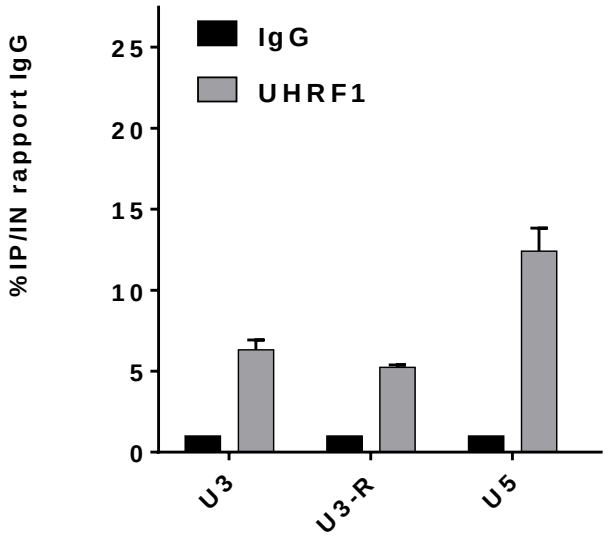
UHRF1 COULD ACT BY MAINTAINING AN HETEROCHROMATIC ENVIRONMENT AT THE HIV-1 5'LTR

(Adapted from Xue *et al.*, Oncotargets and Therapy, 2019)

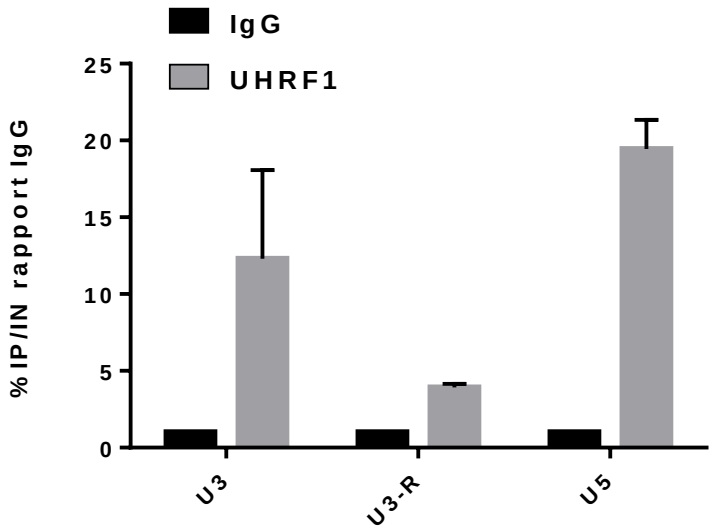
UHRF1 is recruited *in vivo* to the latent HIV-1 promoter

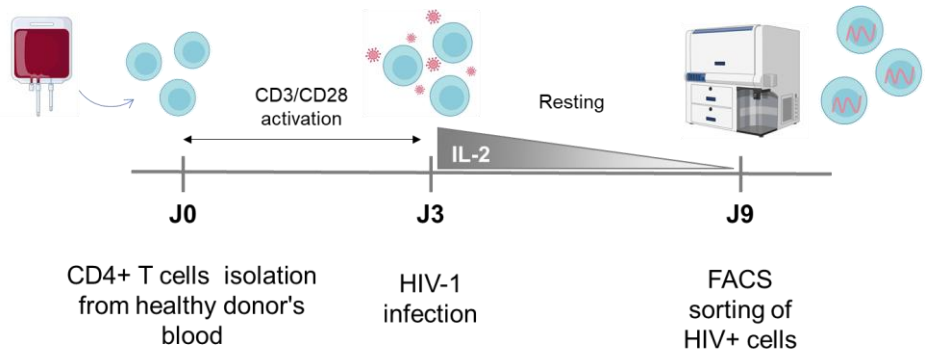


J-Lat 8.4 cell line model



Primary CD4+ T cell model





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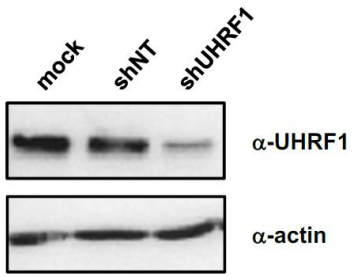
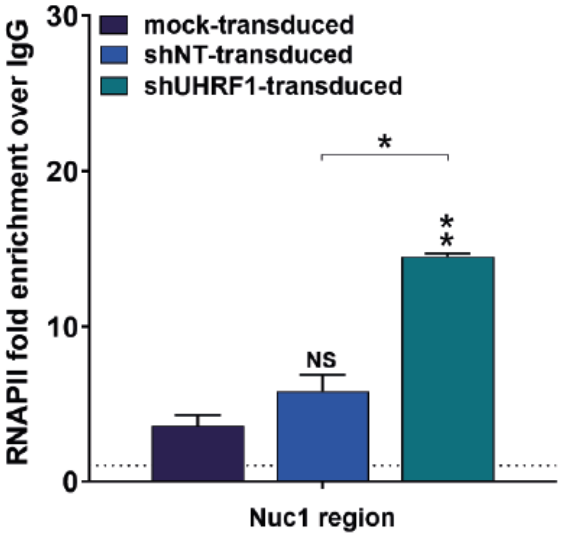
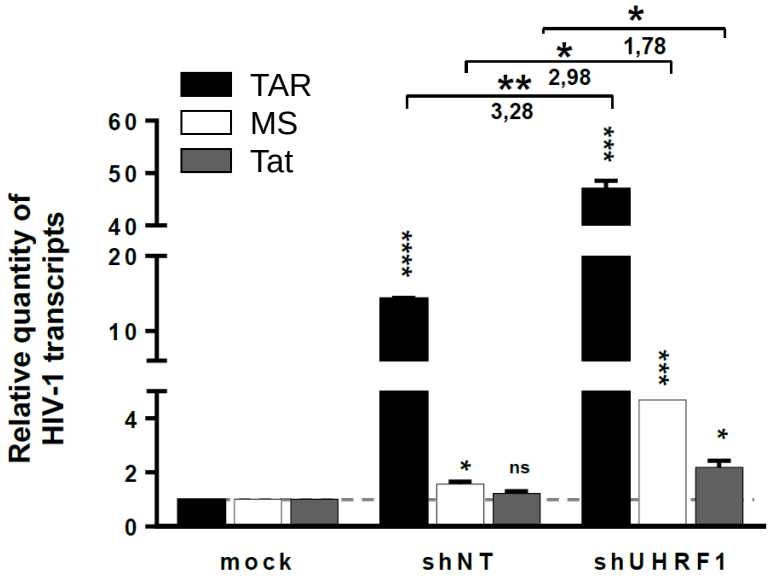
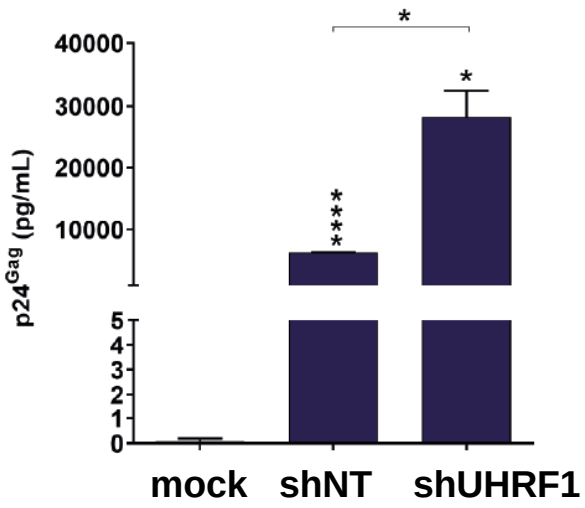
UHRF1 knockdown induces HIV-1 reactivation from latency

J-Lat 8.4 model









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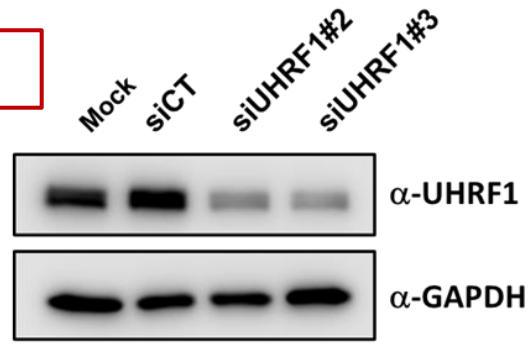

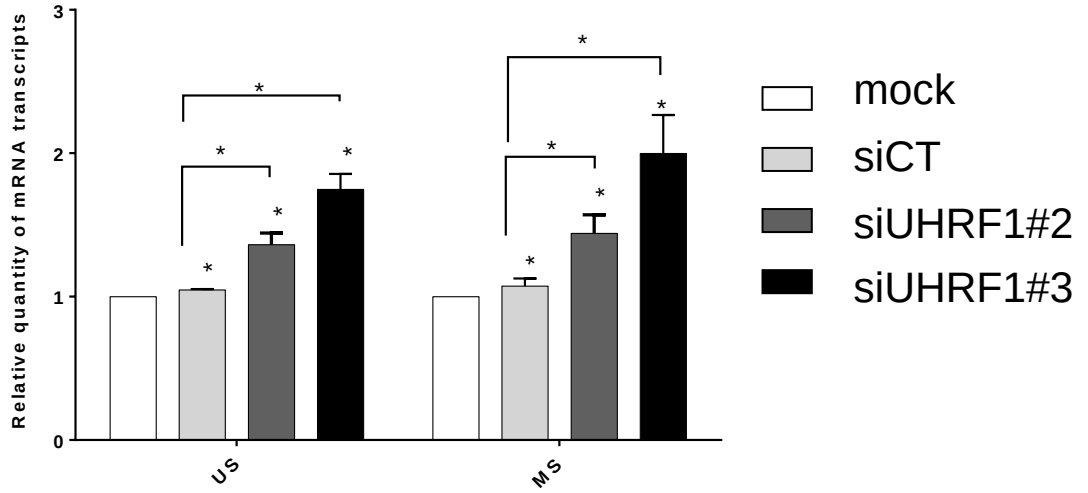

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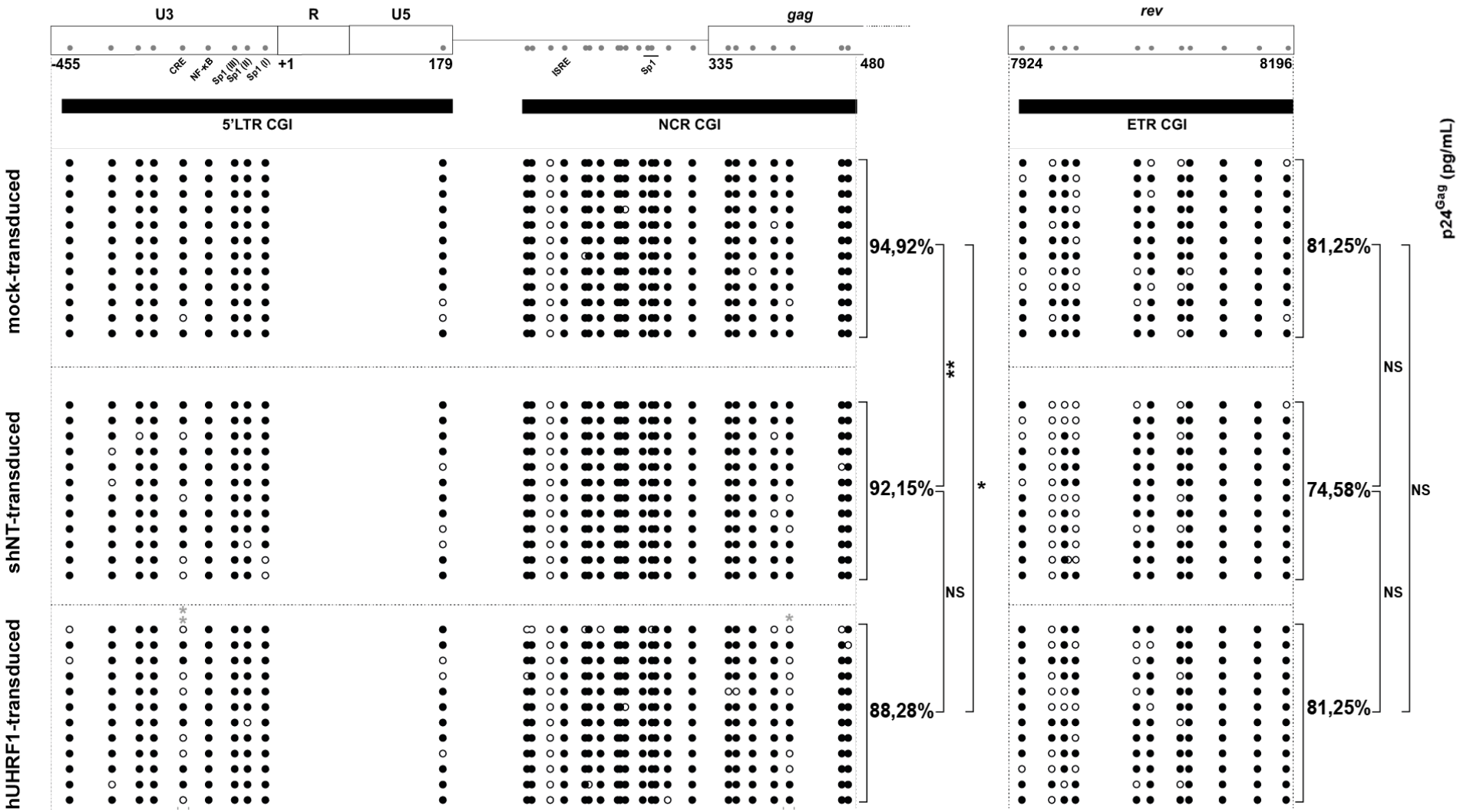
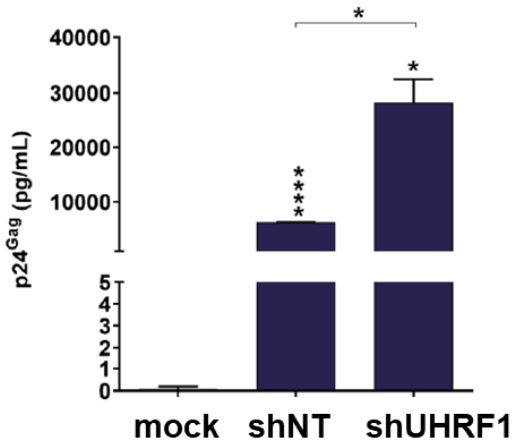
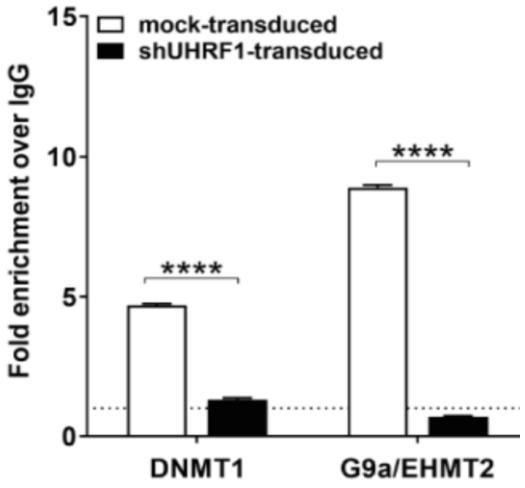
UHRF1 knockdown induces a global 5' LTR demethylation and a decreased *in vivo* recruitment of DNMT1 and G9a to the 5'LTR

J-Lat 8.4 model







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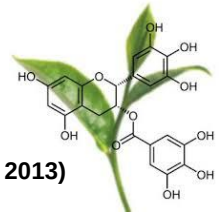
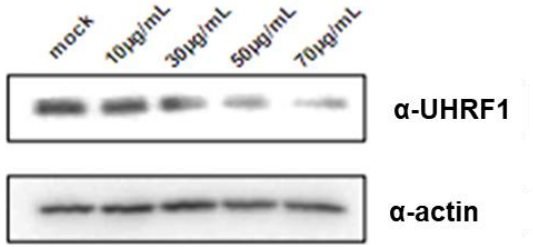
Pharmacological inhibition of UHRF1 induces HIV-1 reactivation in CD8$^+$-depleted PBMCs from cART-treated individuals

UHRF1 ⊣ EpiGalloCatechin-3-Gallate (EGCG)

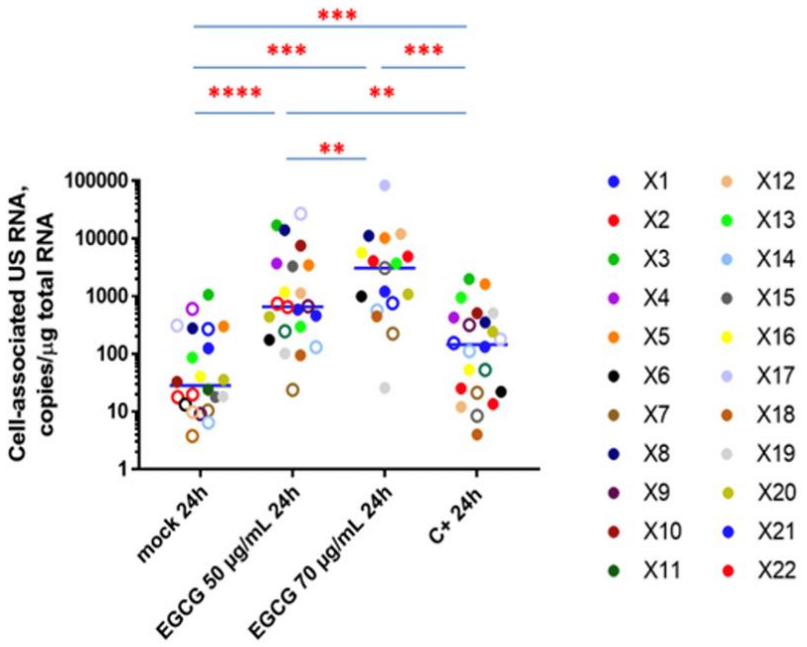


(Achour *et al.*, Biochem. Biophys. Res. Commun. 2013)

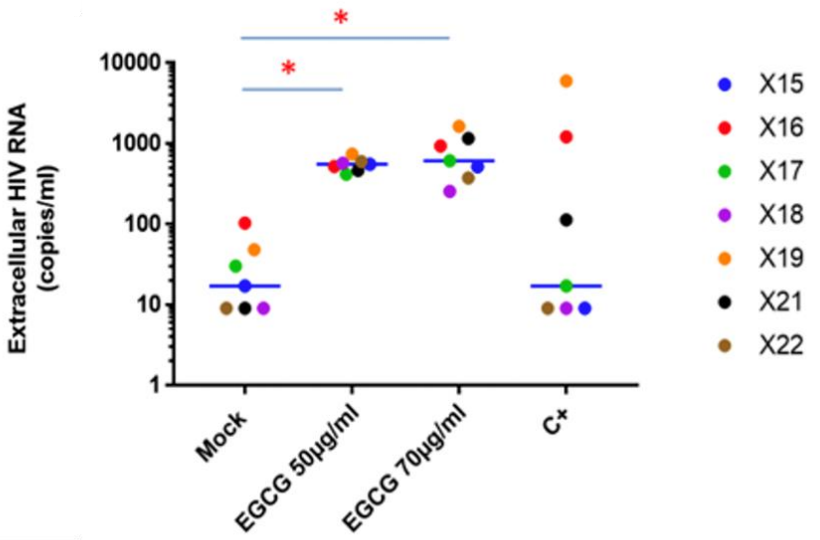
J-LAT 8.4 CELLS



Median level of HIV-1 intracellular US RNAs



Median level of HIV extracellular US RNAs



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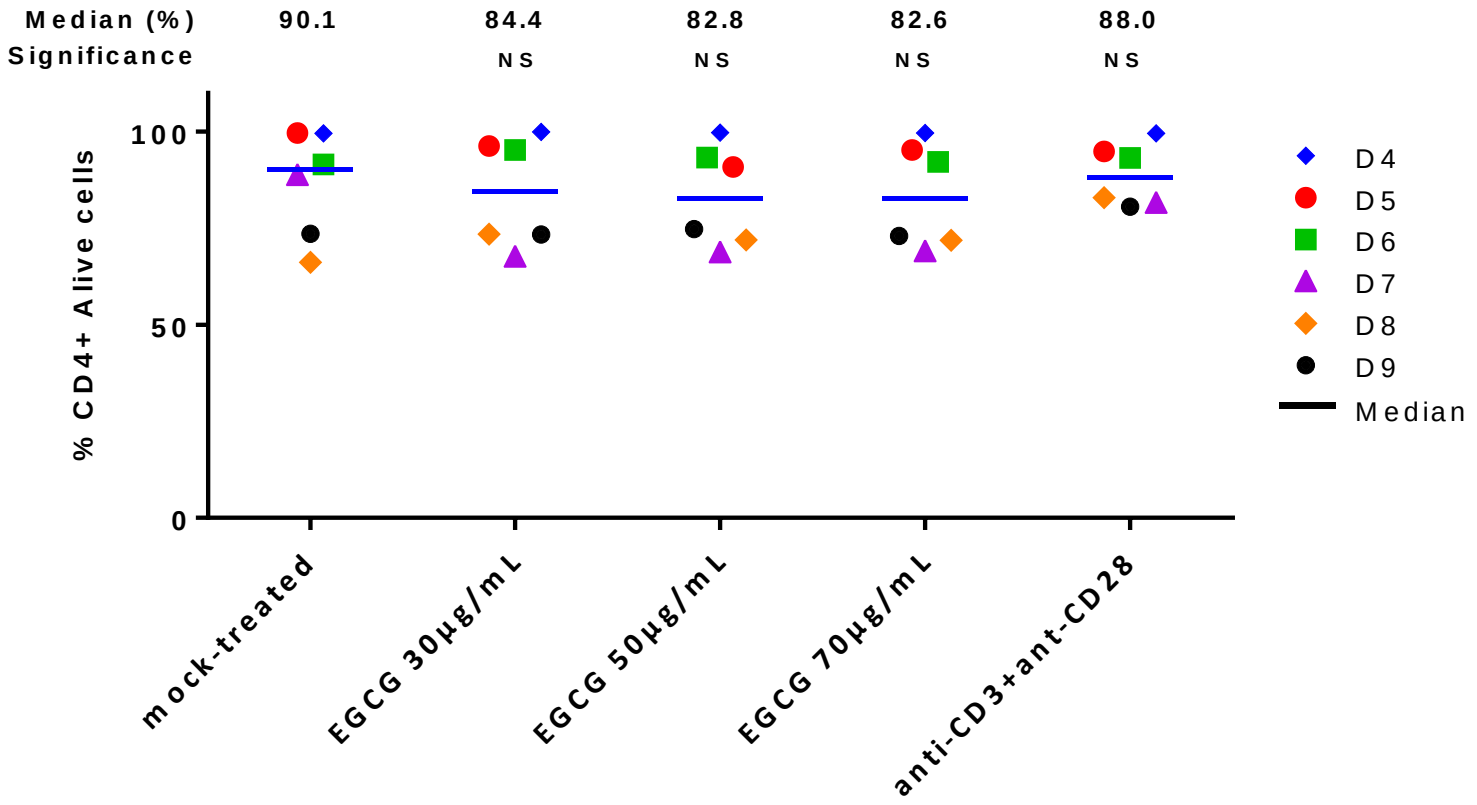
EGCG INDUCES NO EFFECT ON $CD4^+$ CELLS VIABILITY

LIVE-DEAD STAINING

24h EGCG treatment of $CD8^+$-depleted PBMCs isolated from healthy donors



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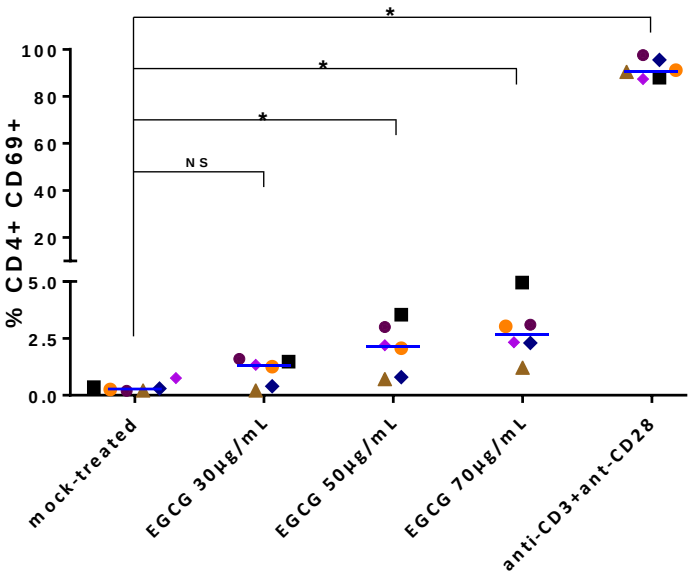
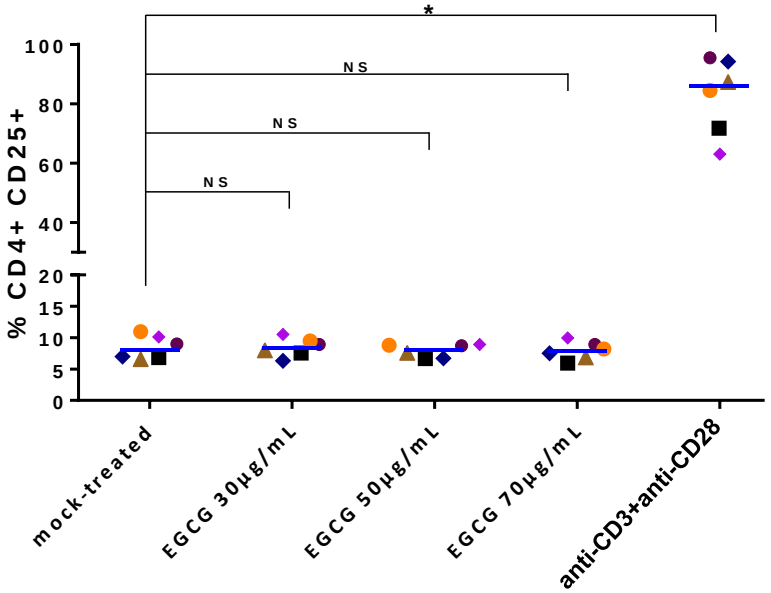
EFFECT OF EGCG ON T CELL SURFACE ACTIVATION MARKERS

24H

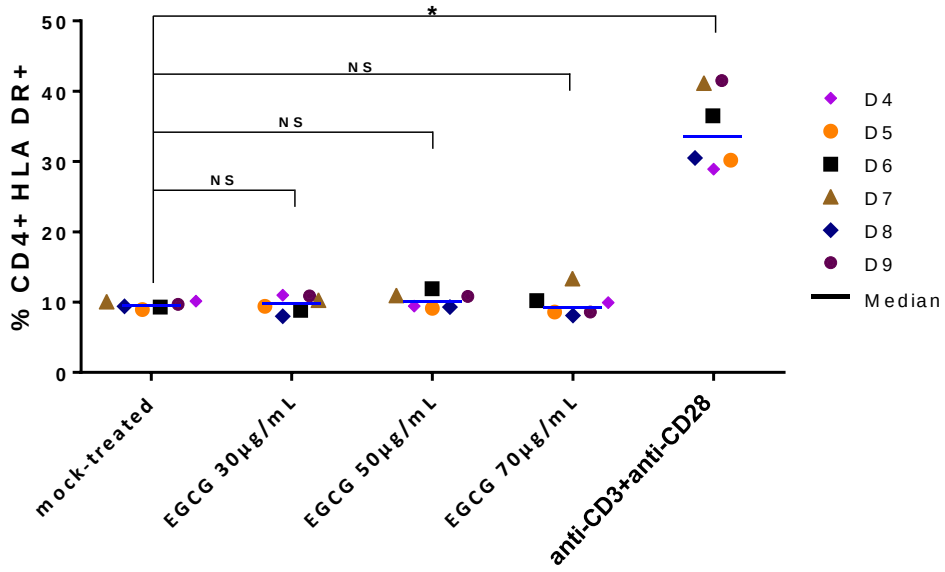
EARLY (CD69)



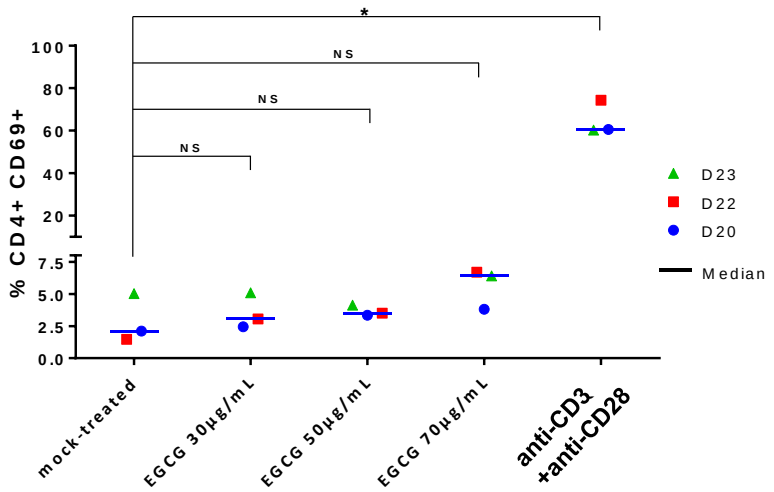
INTERMEDIATE (CD25)



LATE (HLA DR+)



6 Days

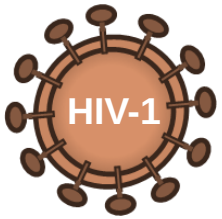


***Ex vivo* EGCG treatment reactivates HIV-1 expression without inducing a strong T cell activation.**

Verdikt, Bendoumou, Bouchat, Nestola *et al.*, *eBioMedicine* 2022

EGCG IS A PROMISING CANDIDATE FOR SHOCK AND KILL STRATEGY

EGCG



EARLY STEPS : RECEPTOR RECOGNITION

(Williamson *et al*, J Allergy Clin Immunol.2006)

REVERSE TRANSCRIPTION

(Li *et al*, Antivir.Chem.Chemother. 2011)

LATE STEPS : TRANSCRIPTION (Yang *et al*, EMBO Rep. 2020)

VIRAL PARTICLE STABILITY

(Yamaguchi *et al*, Antiviral Res. 2002)

REACTIVATES HIV-1 FROM LATENCY

(Verdikt R. *et al.*, eBioMedicine 2022)

THE NATURAL COMPOUND EGCG APPEARS TO BE A PROMISING CANDIDATE FOR A "SHOCK-AND-KILL" STRATEGY, PROVOKING A TRANSCRIPTIONAL AND TRANSLATIONAL WAKE UP OF LATENTLY INFECTED CELLS WHILE MAINTAINING A SUPPRESSION OF VIRUS PRODUCTION AND REPLICATION.

CONCLUSIONS UHRF1

- **We identified UHRF1 as a novel factor involved in the epigenetic repression of HIV-1 transcription through both DNA methylation-dependent and -independent mechanisms, such as histone methylation.**
- **Therefore, UHRF1 could constitute a new therapeutic target for anti-HIV cure strategies.**
- **The pharmacological UHRF1 inhibitor EGCG reactivates HIV-1 gene expression in *ex vivo* $CD8^+$-depleted PBMCs cultures from cART-treated aviremic HIV^+ patients :**
  - **without impacting viability of CD4+ cells.**
  - **without inducing a strong T-cell immune activation.**

+ Antiviral properties

EGCG IS AN ATTRACTIVE LRA CANDIDATE

Acknowledgments

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