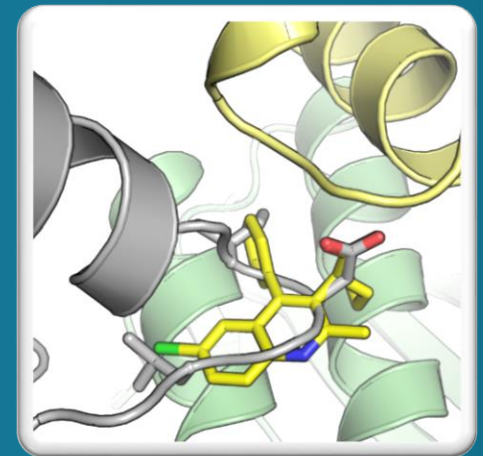


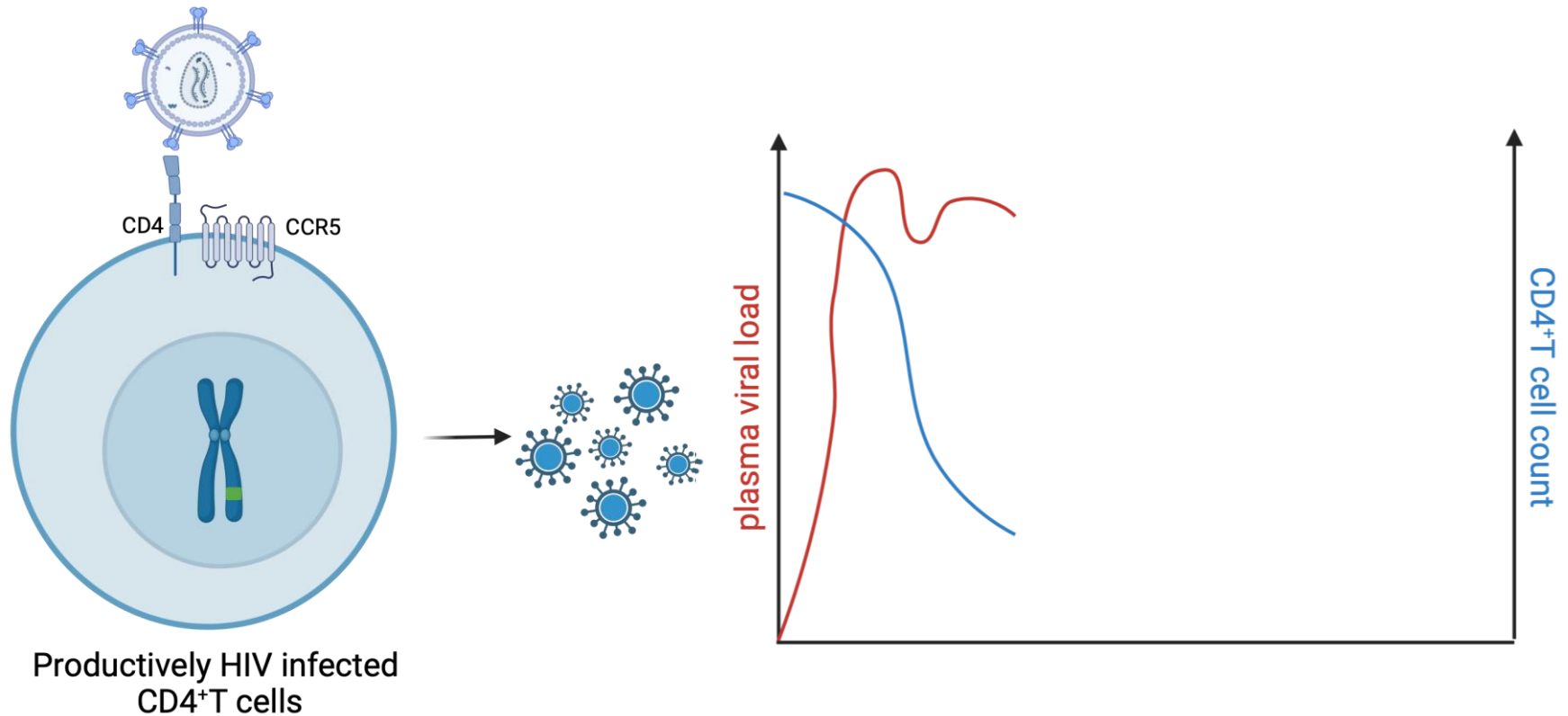


Towards a Cure of HIV infection: to shock or to lock, that is the question.

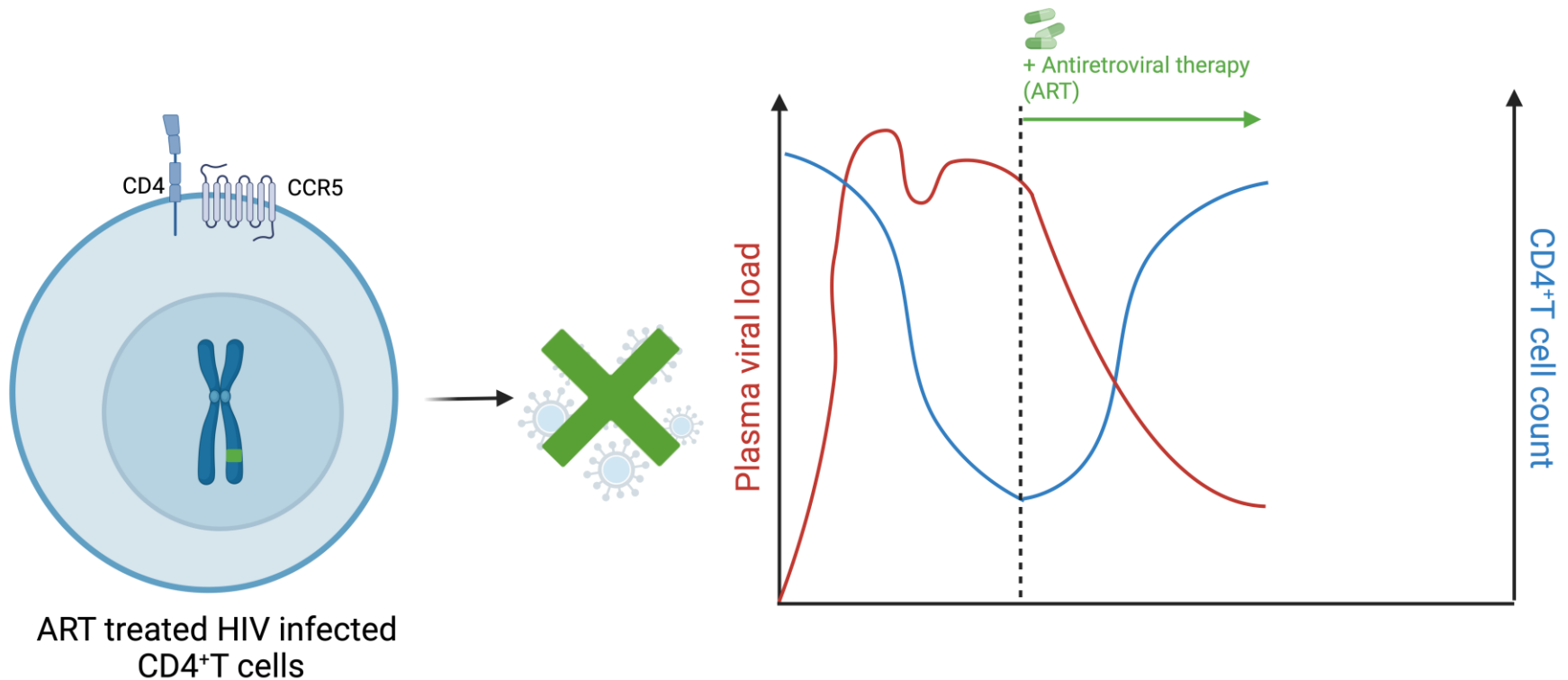
Zeger Debyser MD PhD
 KU Leuven



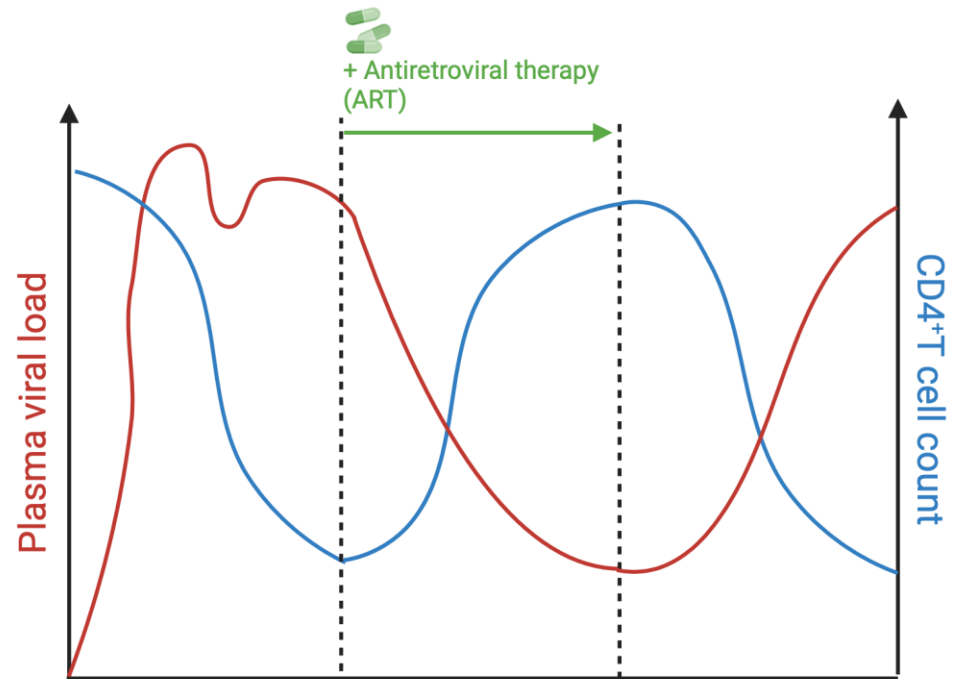
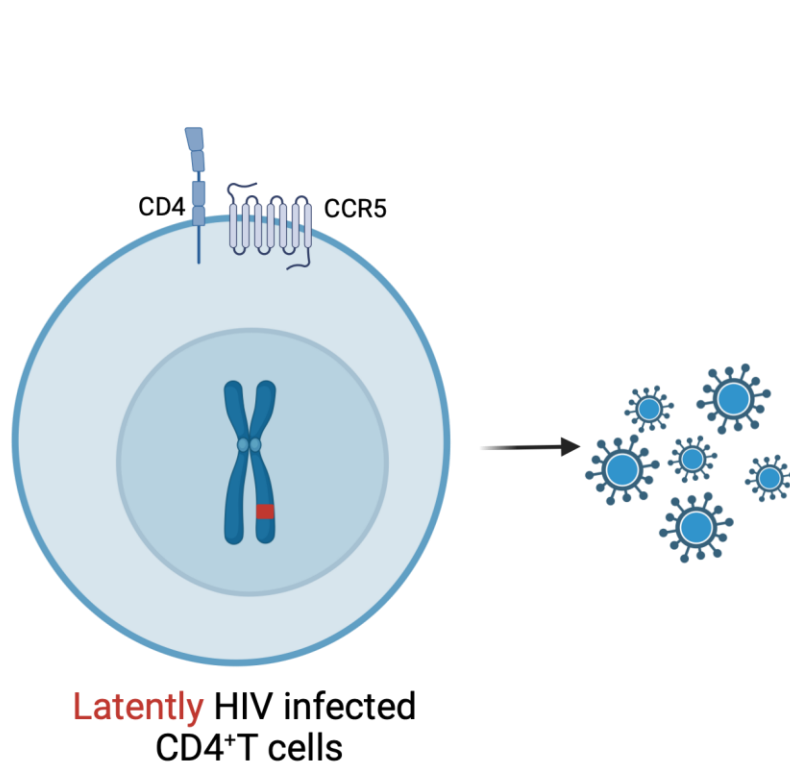
HIV latency as primary hurdle towards an HIV cure



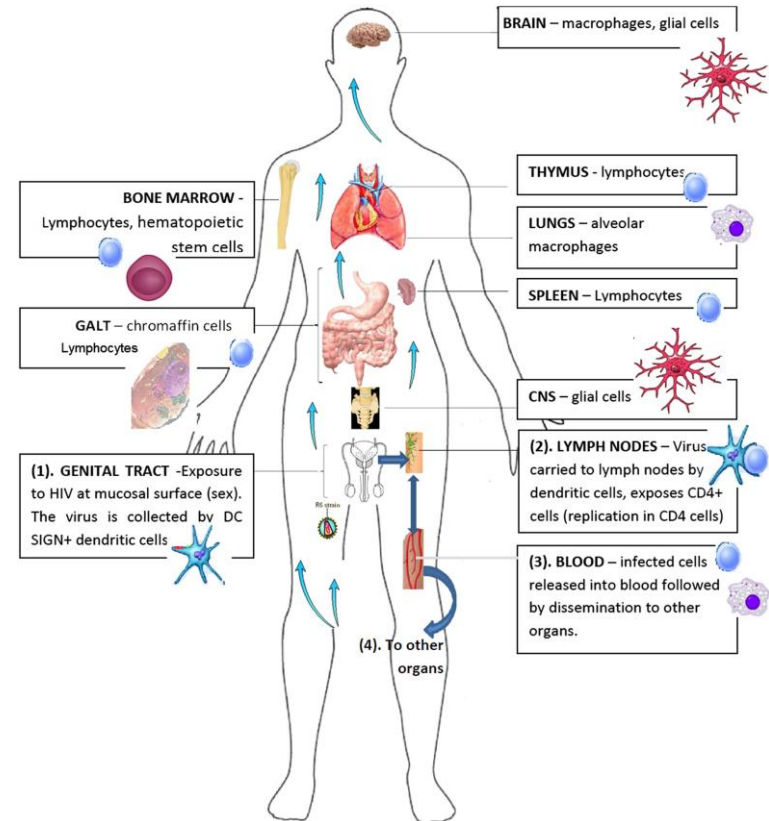
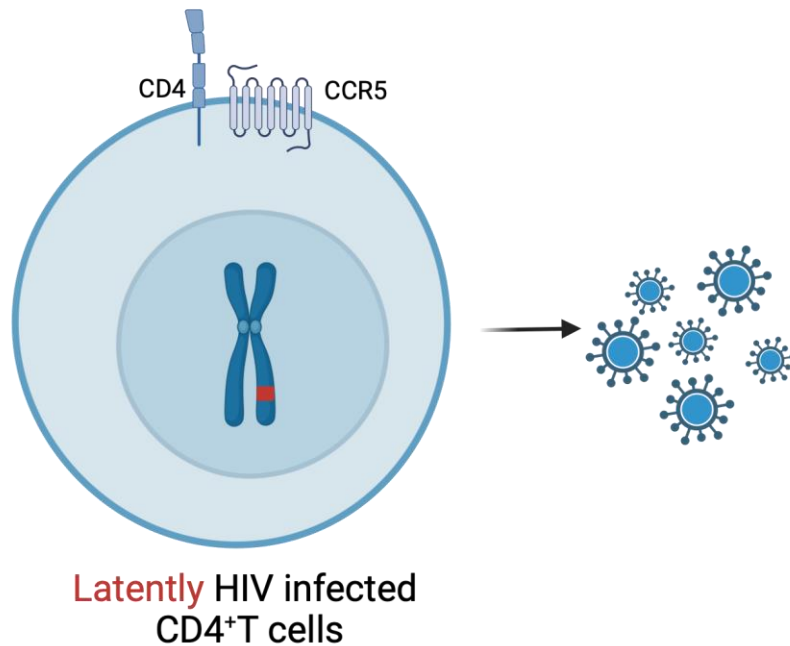
HIV latency as primary hurdle towards an HIV cure



HIV latency as primary hurdle towards an HIV cure

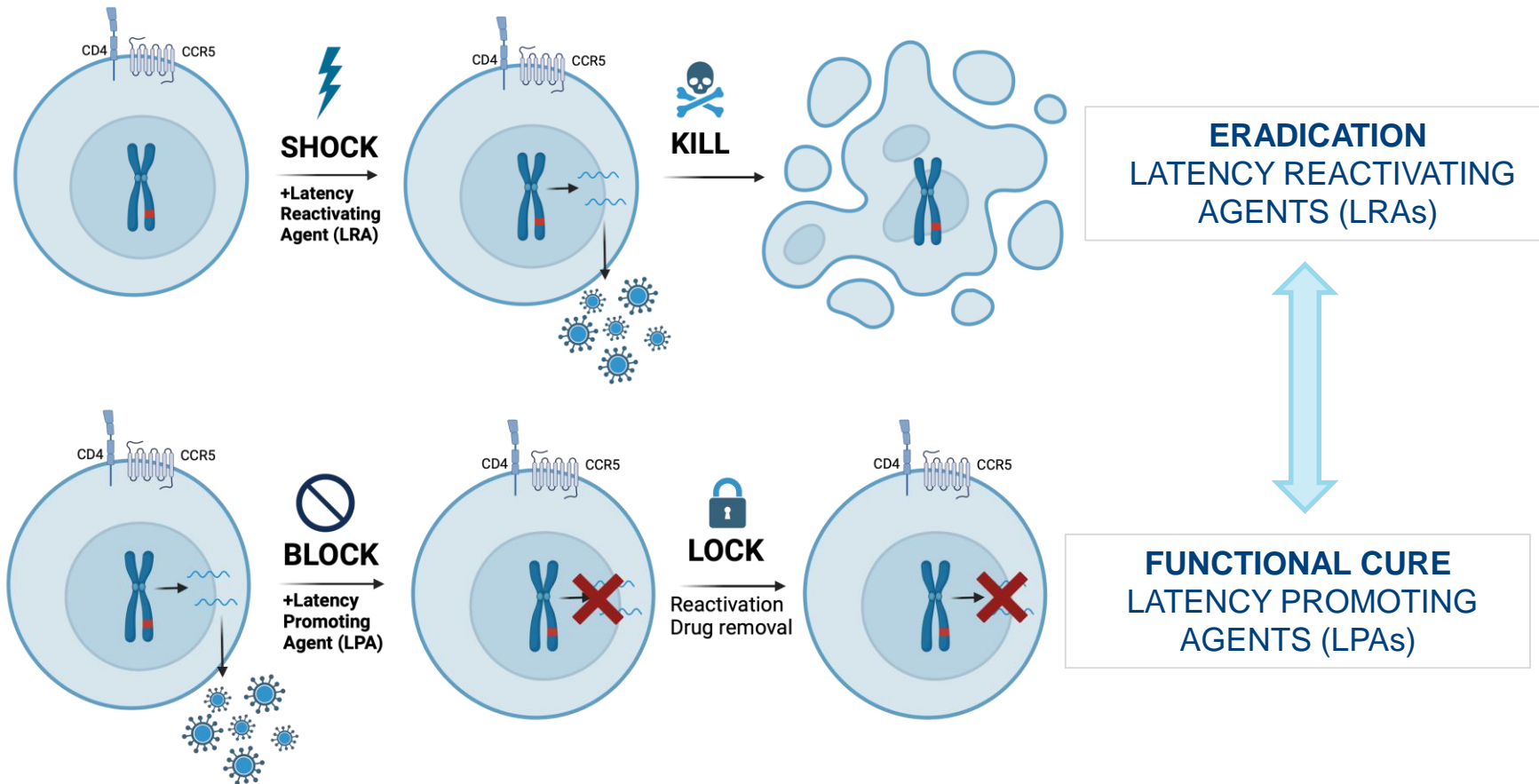


HIV latency as primary hurdle towards an HIV cure



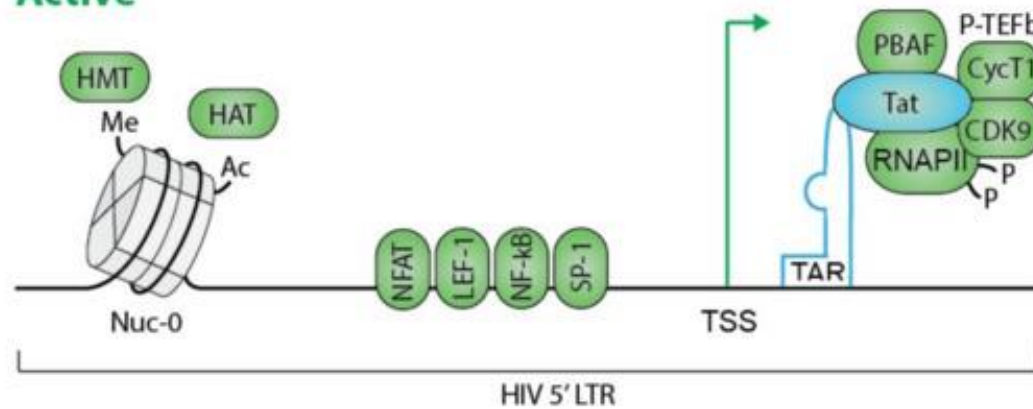
*Mzingwane ML et al., *Rev Med Virol.*, 2017.

How can we cure HIV infection?

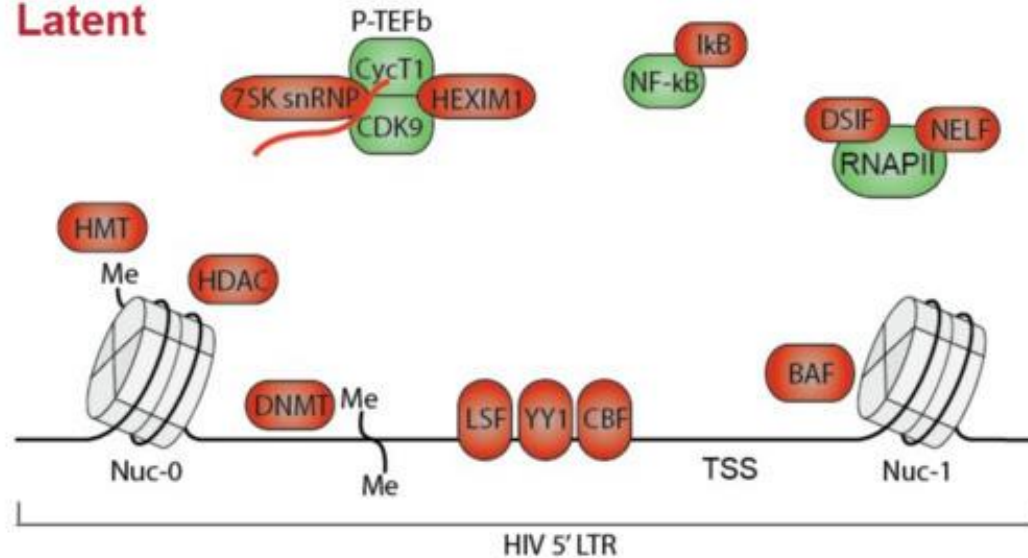


Regulation of transcriptional activity

(a) Active



(b) Latent



Translational evidence for a block-and-lock cure

Elite controllers

- HIV infected patients who control viral replication without ART
- 0.5 % of HIV population

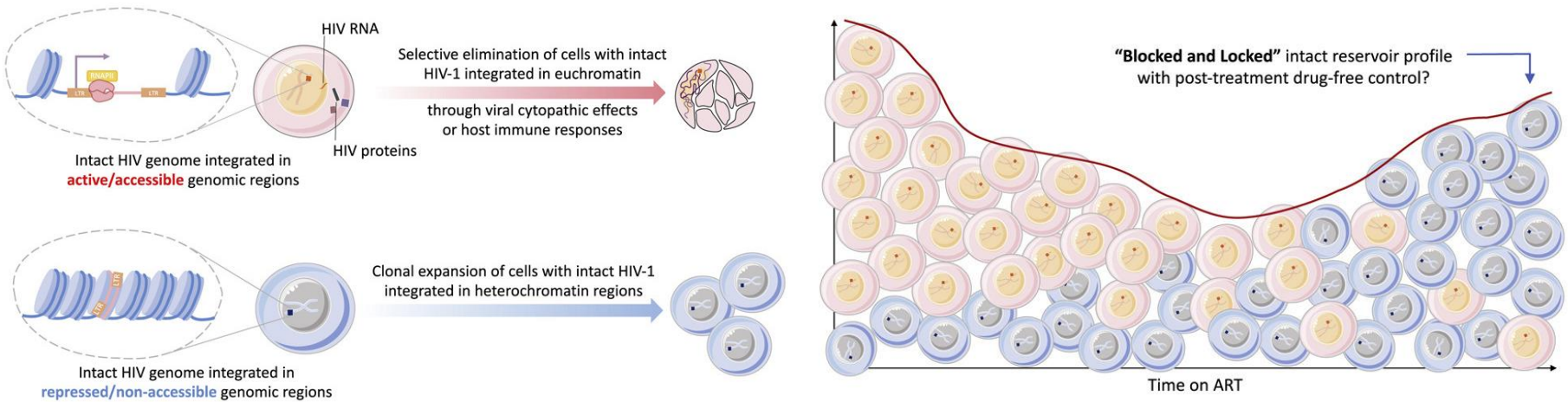
Post-treatment controllers

- Long-term ART treated individuals who control viral replication after treatment-interruption
- 7 % of HIV population

*Jiang *et al.*, *Nature*, 2020.

*Einkauf *et al.*, *J Clin Invest*, 2019.

Translational evidence for a block-and-lock cure



*Lian et al., *Cell Host Microb*, 2022.

News & views

Virology

Deep-sleeping HIV genomes under control

Nicolas Chomont

In a few people living with HIV, the virus remains under control without antiretroviral therapy. It emerges that, in these people, the viral DNA that is integrated into the host genome is in a deeply transcriptionally repressed state. **See p.261**

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- Integration site and RNA expression
- Selection
 - Immune control
 - Toxicity
 - Clonal expansion

Article


Distinct viral reservoirs in individuals with spontaneous control of HIV-1

<https://doi.org/10.1038/s41586-020-2651-8>

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 Check for updates

Chenyang Jiang^{1,2,3}, Xiaodong Lian^{1,2,3}, Ce Gao^{1,2}, Xiaoming Sun¹, Kevin B. Einkauf^{1,2}, Joshua M. Chevalier^{1,2}, Samantha M. Y. Chen¹, Stephane Hua¹, Ben Rhee^{1,2}, Kaylee Chang¹, Jane E. Blackmer¹, Matthew Osborn¹, Michael J. Peluso², Rebecca Hoh², Ma Somsouk², Jeffrey Milush², Lynn N. Bertagnoli⁴, Sarah E. Sweet⁴, Joseph A. Varriale⁴, Peter D. Burbelo⁵, Tae-Wook Chun⁶, Gregory M. Laird⁷, Erik Serrao^{8,9}, Alan N. Engelman^{8,9}, Mary Carrington^{1,10}, Robert F. Siliciano^{4,11}, Janet M. Siliciano^{4,11}, Steven G. Deeks², Bruce D. Walker^{1,11,12,13}, Mathias Lichterfeld^{12,14} & Xu G. Yu^{1,2,12}

Block-and-lock approaches

- **Tat Inhibition by Didehydro-Cortistatin A**

Kessing, C.F et al. In vivo suppression of HIV rebound by didehydro-Cortistatin A, a 'block-and-lock' strategy for HIV-1 cure. *Cell Rep.* **2017**, 21, 600–611

- **LEDGINS retarget integration**

Vranckx L.S. et al. LEDGIN-mediated inhibition of integrase-LEDGF/p75 interaction reduces reactivation of residual provirus. *EBioMedicine.* **2016** Jun;8:248-264.

- **FACT Inhibition by Curaxin CBL0100**

- **RNA-Induced Epigenetic Silencing**

- **HSP90 Inhibitors**

- **Jak-STAT Inhibitors**

- **BRD4 Modulators**

- **mTOR Inhibitors**

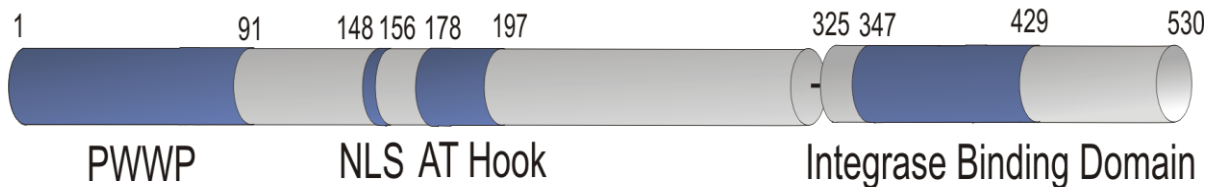
- **Kinase Inhibitors**

- **Triptolide**

Lens Epithelium-Derived Growth Factor

- Cellular stress response
- Transcriptional co-activator (Ge et al., EMBO J. 1998)

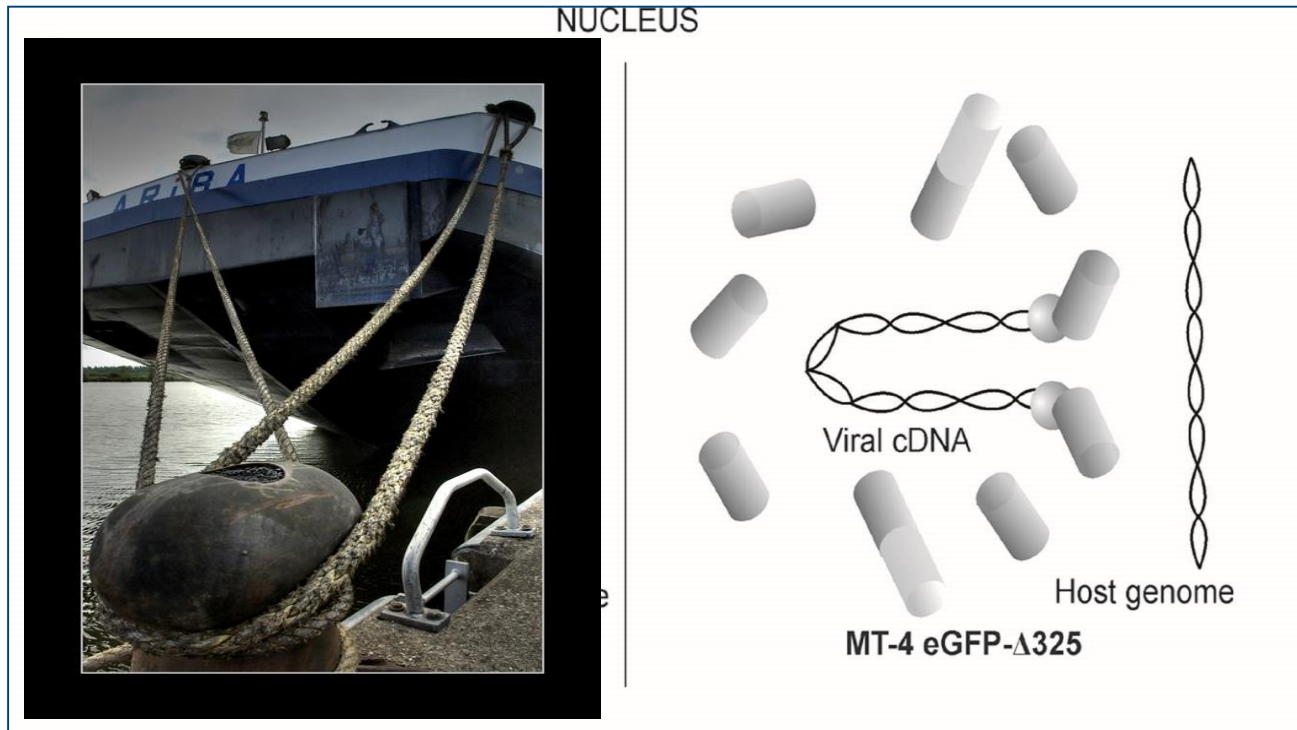
LEDGF/p75



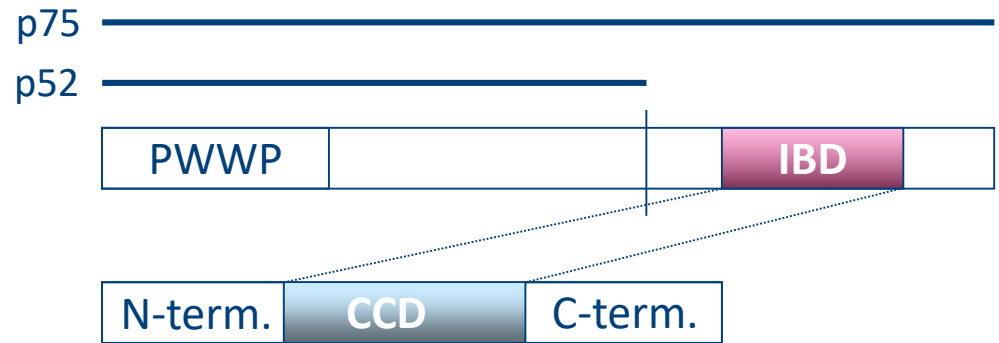
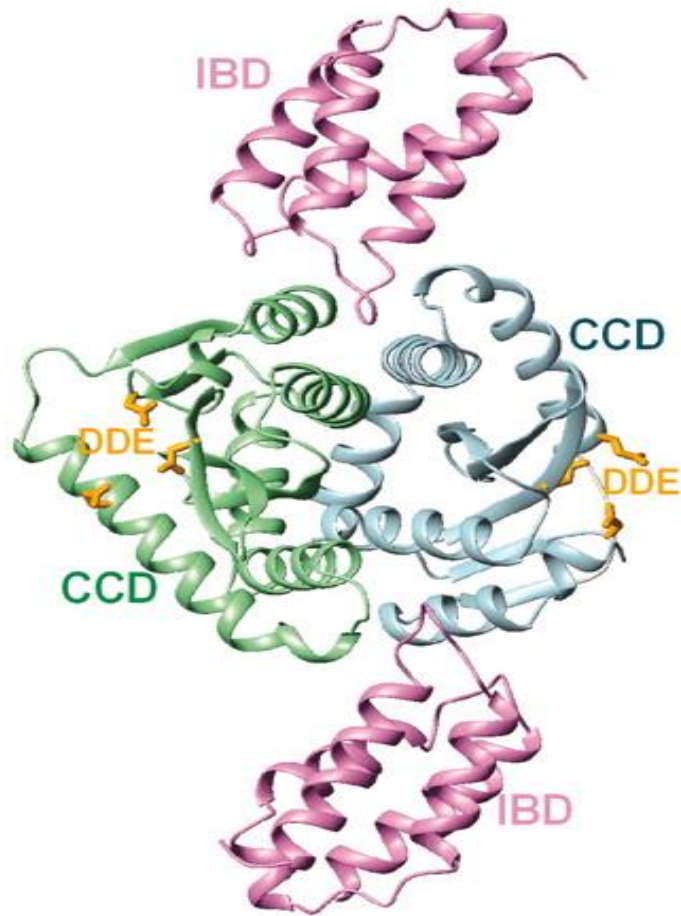
p52



A molecular tether for the HIV PIC



Interaction of LEDGF/p75 and HIV-IN



IBD: integrase binding domain
CCD: catalytic core domain

LEDGINS as novel first-in-class non-catalytic integrase inhibitors (2010)

ARTICLE

PUBLISHED ONLINE: 16 MAY 2010 | DOI: 10.1038/NCHEMIBIO.370

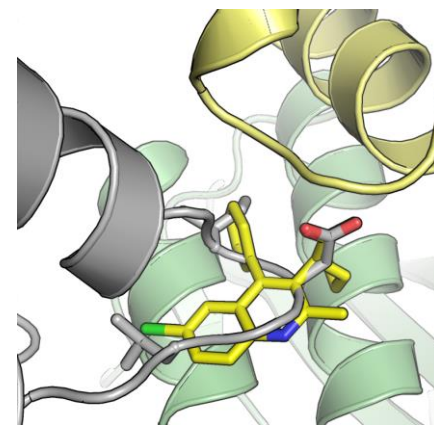
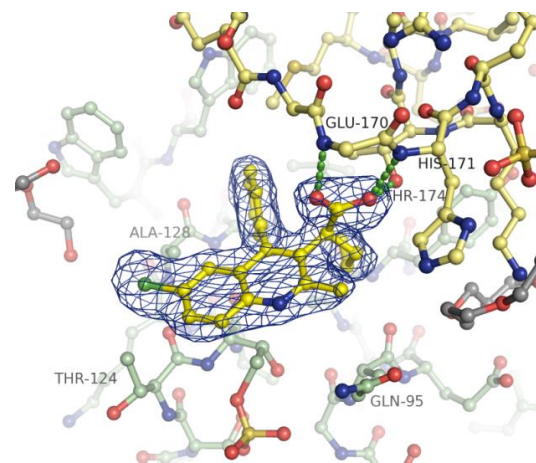
nature
chemical biology

Rational design of small-molecule inhibitors of the LEDGF/p75-integrase interaction and HIV replication

Frauke Christ^{1,7}, Arnout Voet^{2,7}, Arnaud Marchand^{5,7}, Stefan Nicolet^{3,6}, Belete A Desimie¹, Damien Marchand⁵, Dorothée Bardiot⁵, Nam Joo Van der Veken¹, Barbara Van Remoortel¹, Sergei V Strelkov³, Marc De Maeyer², Patrick Chaltin^{4,5} & Zeger Debyser^{1*}

Lens epithellum-derived growth factor (LEDGF/p75) is a cellular cofactor of HIV-1 integrase that promotes viral integration by tethering the preintegration complex to the chromatin. By virtue of its crucial role in the early steps of HIV replication, the interaction between LEDGF/p75 and integrase represents an attractive target for antiviral therapy. We have rationally designed a series of 2-(quinolin-3-yl)acetic acid derivatives (LEDGINS) that act as potent inhibitors of the LEDGF/p75-integrase interaction and HIV-1 replication at submicromolar concentration by blocking the integration step. A 1.84-Å resolution crystal structure corroborates the binding of the inhibitor in the LEDGF/p75-binding pocket of integrase. Together with the lack of cross-resistance with two clinical integrase inhibitors, these findings define the 2-(quinolin-3-yl)acetic acid derivatives as the first genuine allosteric HIV-1 integrase inhibitors. Our work demonstrates the feasibility of rational design of small molecules inhibiting the protein-protein interaction between a viral protein and a cellular host factor.

CX04328 binds into a small molecule binding pocket in the dimer interface different from the strand transfer inhibitor binding pocket. CX04328 does not alter the overall shape of the IN structure and competes with LEDGF (gray) for the binding to integrase.



KU LEUVEN

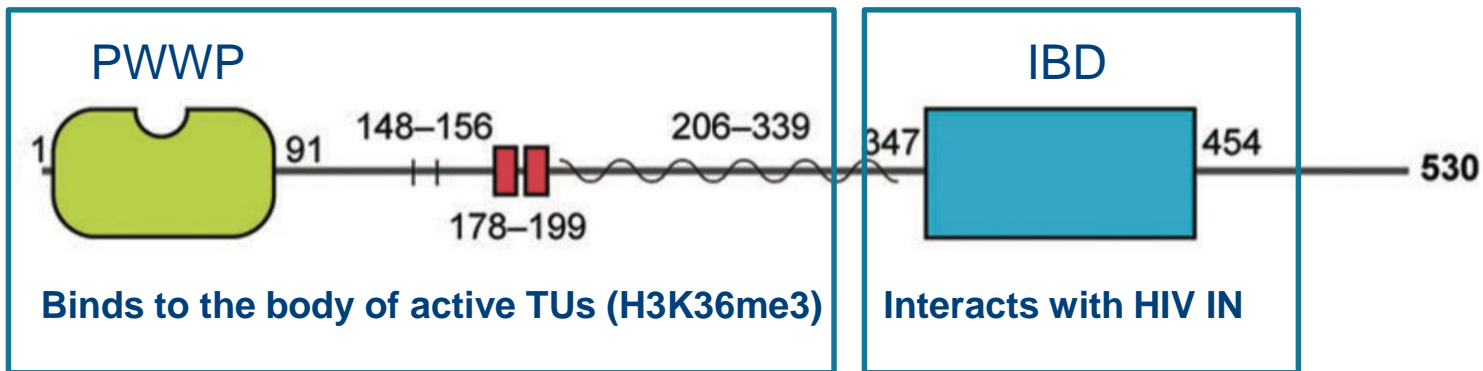
LEDGF/p75



→ LEDGF/p75 directs integration to body of active transcription units

Chromatin Binding Domain (CBD)

Protein Binding Domain (PBD)



→ LEDGF/p75 depletion shifts integration out of TUs

LEDGINs as tool to study this hypothesis

1. Inhibition of the LEDGF/p75-IN interaction

(Christ *et al.* Nat. Chem. Biol., 2010)

2. Allosteric IN inhibition (EARLY)

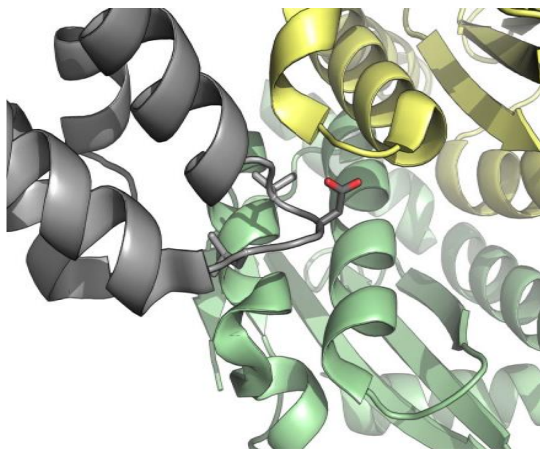
(Christ *et al.* AAC, 2012; Kessl *et al.* J. Biol. Chem., 2012; Tsiang *et al.* J. Biol. Chem., 2012)

3. Dysfunctional IN multimerisation/assembly (LATE)

(Jurado *et al.* PNAS, 2013 ; Desimmie *et al.* Retrovirol., 2013; Balakrishnan *et al.*, PLoS ONE 2013)

4. Retargeting integration + block and lock (EARLY/LATE)

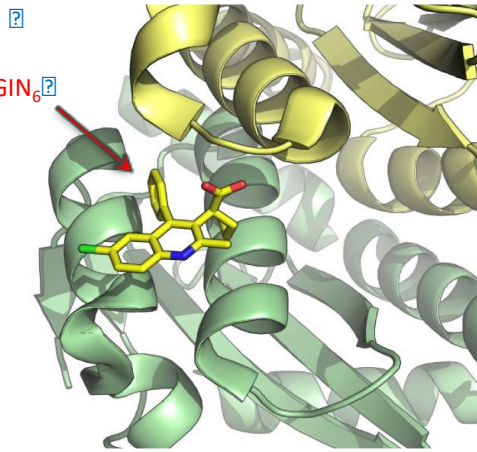
(Vranckx *et al.* EBiomedecine 2016)



LEDGF/p75BD

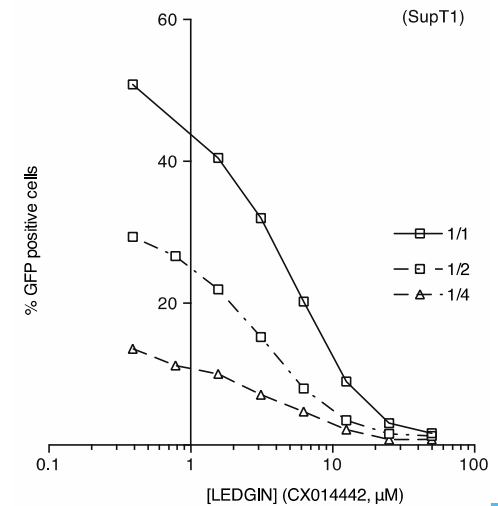
HIV-IN CCD dimer

LEDGIN₆



HIV-IN CCD dimer

Dose-response

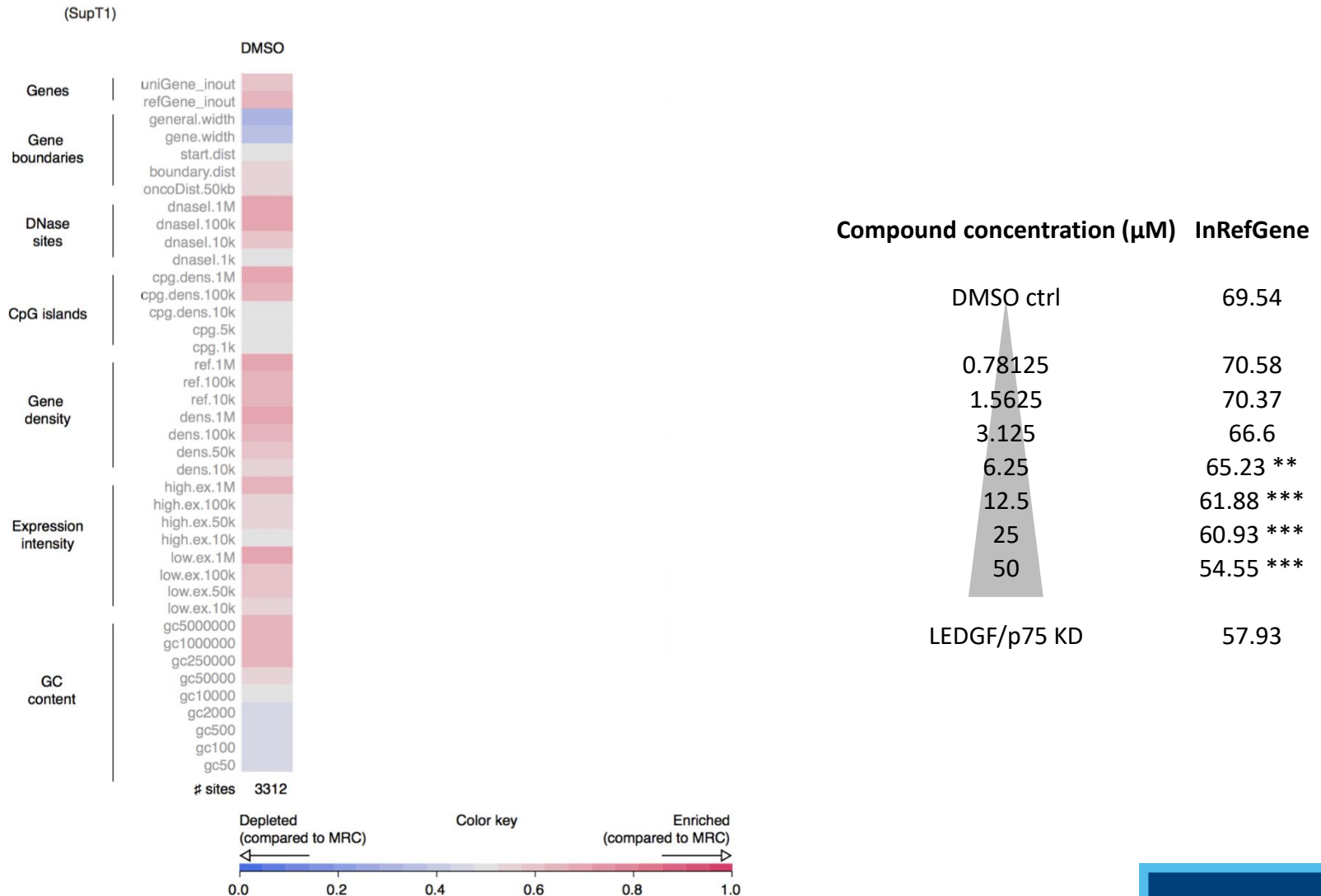


How does inhibition with the integrase-LEDGF/p75 interaction interfere with the lentiviral integration process?

What is the impact of LEDGINs on:

1. Integration site distribution?
2. Establishment of HIV latency?
3. Reactivation from HIV latency?

1. LEDGINs (CX014442) shift HIV integration out of TUs

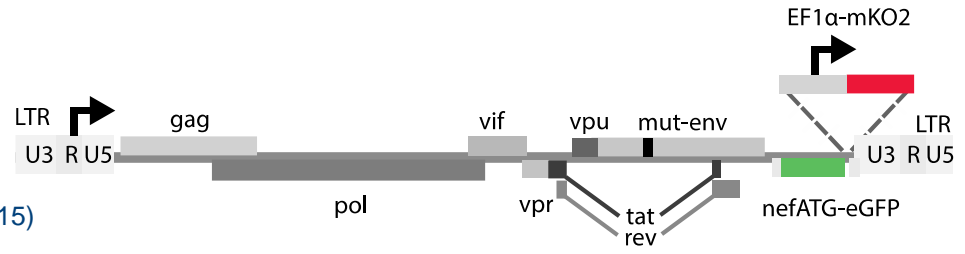


HIV Double reporter virus

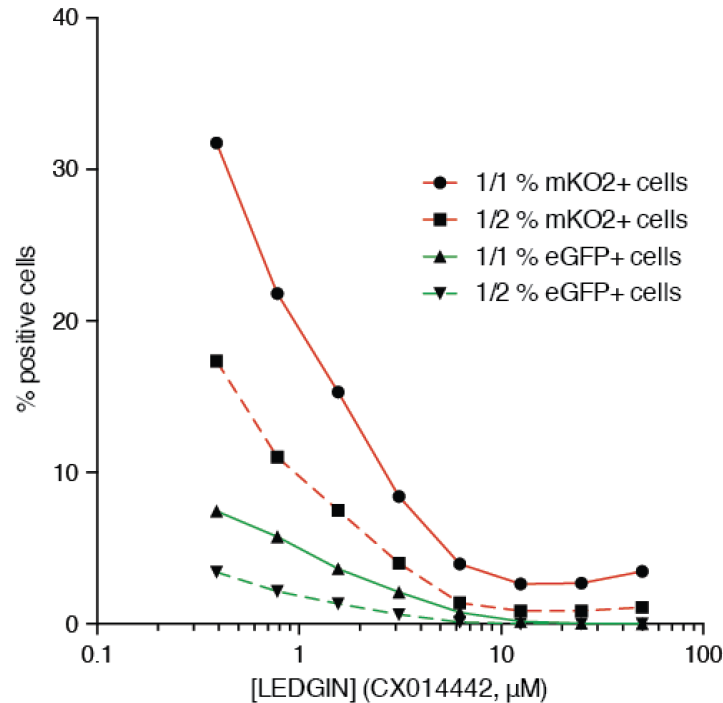
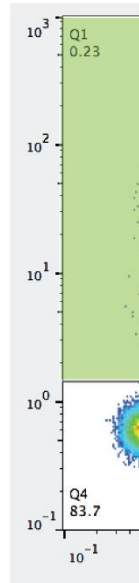
HIV LAI-OGH (VSV-G)

Collaboration with Eric Verdin (Emilie Battivelli)

(Calvanese *et al.* Virol., 2013; Chavez *et al.* PLoS Pathog., 2015)



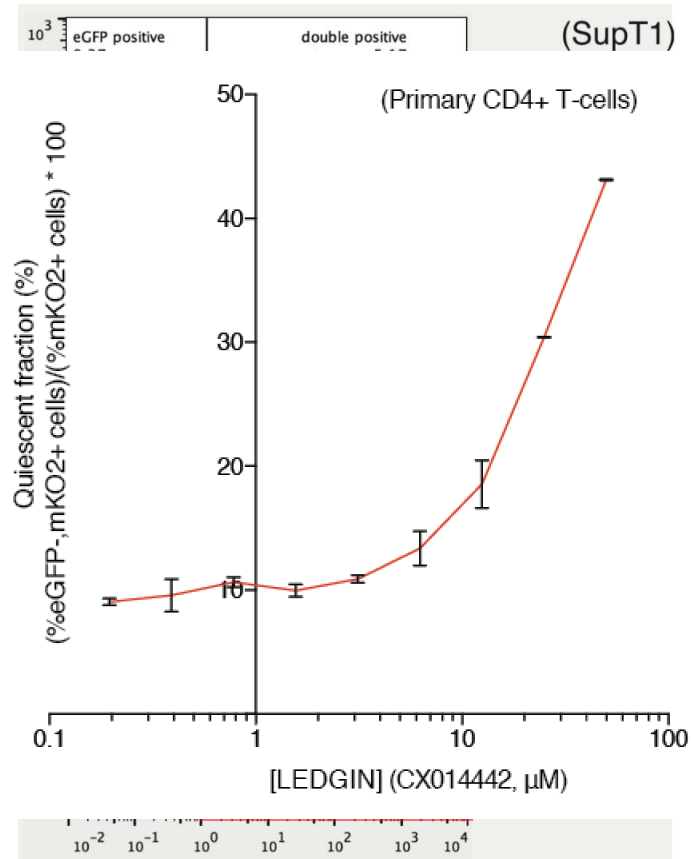
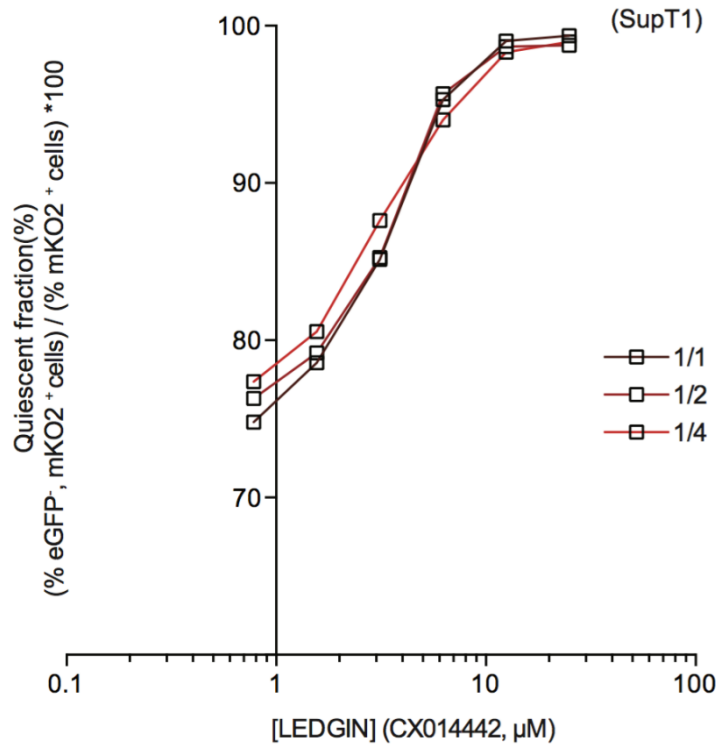
eGFP



Highly infected

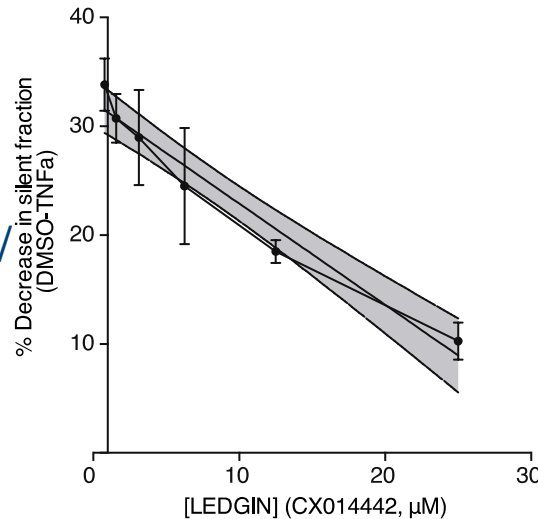
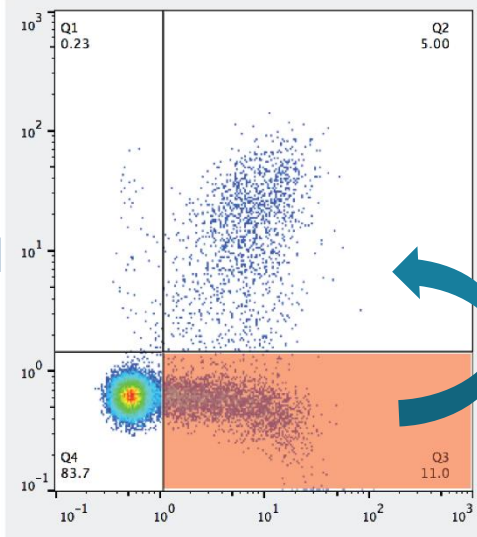
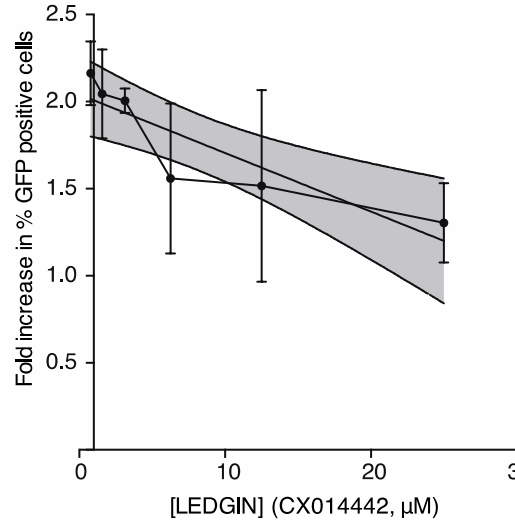
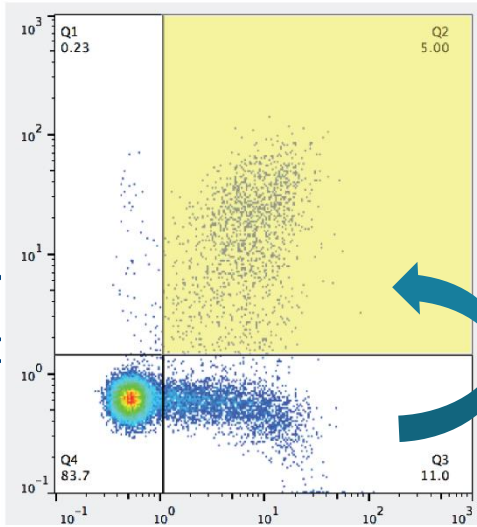
LEDGINs inhibit HIV infection
Highly infected

2. LEDGIN treatment during infection increases the quiescent fraction



Quiescently
infected

3. LEDGIN treatment induces a residual silent reservoir refractory to HIV reactivation



LEDGINs

Dose-dependent reduction in reactivation from quiescence

SupT1, 11D p.i.
TNFalpha -DMSO

2.

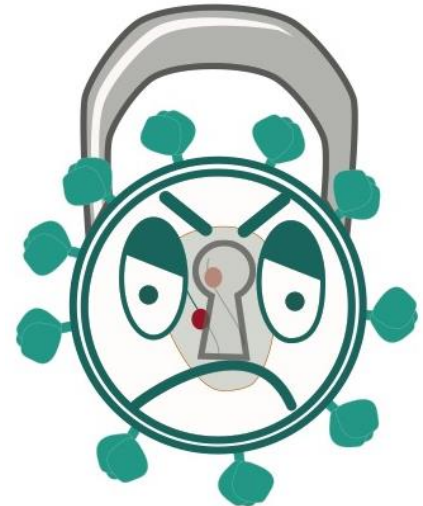
Conclusion : LEDGINs as a potential CURE strategy

LEDGINs function as potent ANTI-RETROVIRALS

With **RESIDUAL** integrated provirus:

- Shifted out of transcription units
- Shifted towards the inner nucleus
- Transcriptionally silent
- Refractory to reactivation from latency

SHOCK & KILL
↕
BLOCK & LOCK



The chromatin landscape at the HIV-1 integration site determines viral expression

The chromatin landscape at the HIV-1 provirus integration site determines viral expression (barcoded viruses)

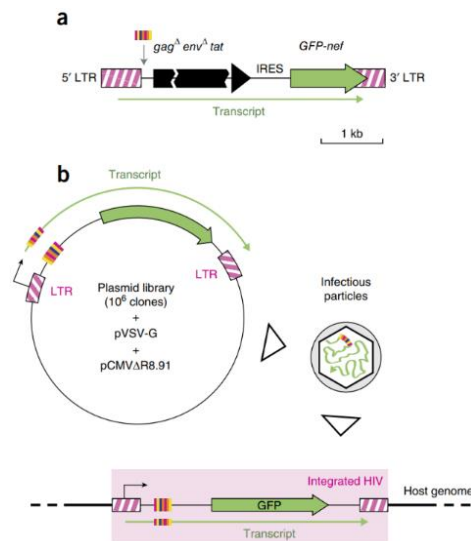
Gerlinde Vansant, Heng-Chang Chen, Eduard Zorita, Katerina Trejbalová, Dalibor Miklík, Guillaume Filion, Zeger Debyser

Nucleic Acids Research, Volume 48, Issue 14, 2020, Pages 7801–7817,



The molecular mechanism underlying LEDGIN block and lock

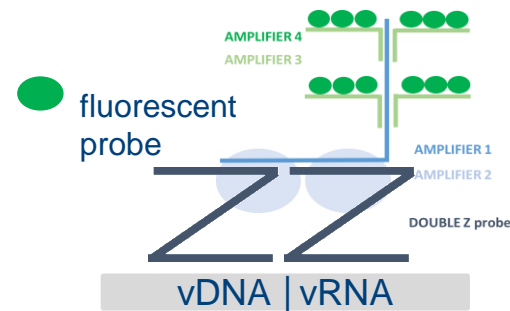
B-HIVE (bar-coded HIV)



Advantage

- Measure integration sites
- LinkRNA transcription to individual integration site and its epigenetic features

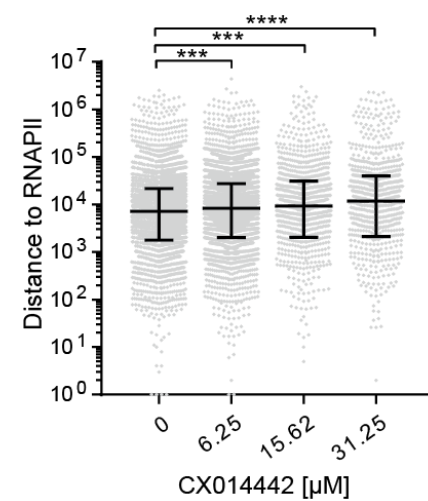
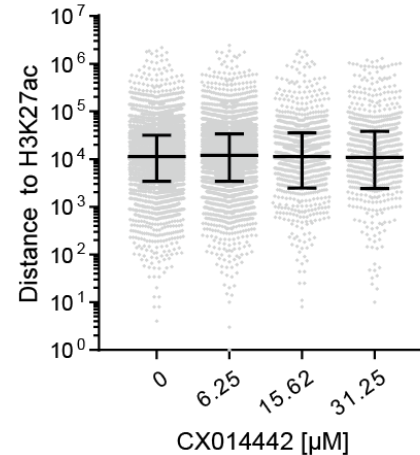
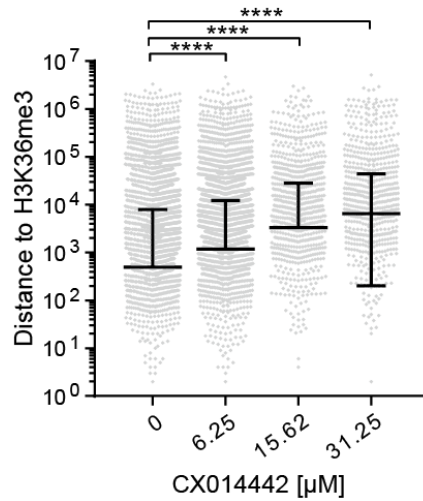
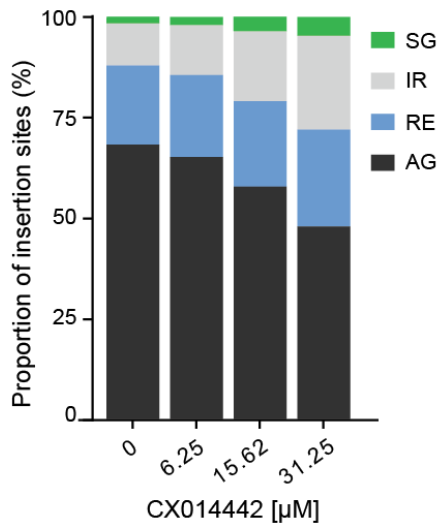
B-DNA imaging (sensitive FISH)



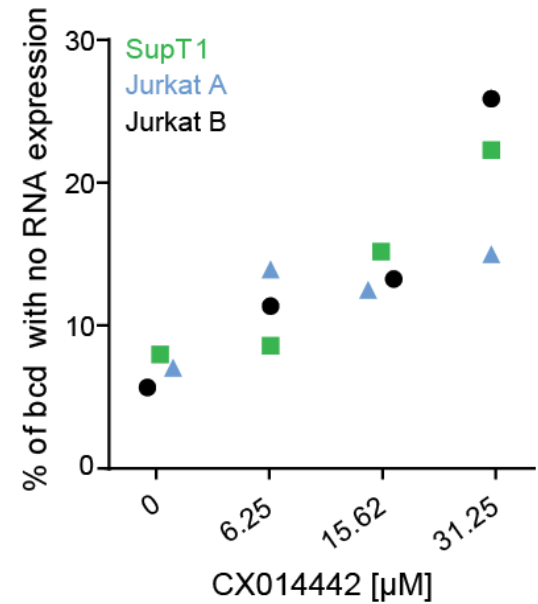
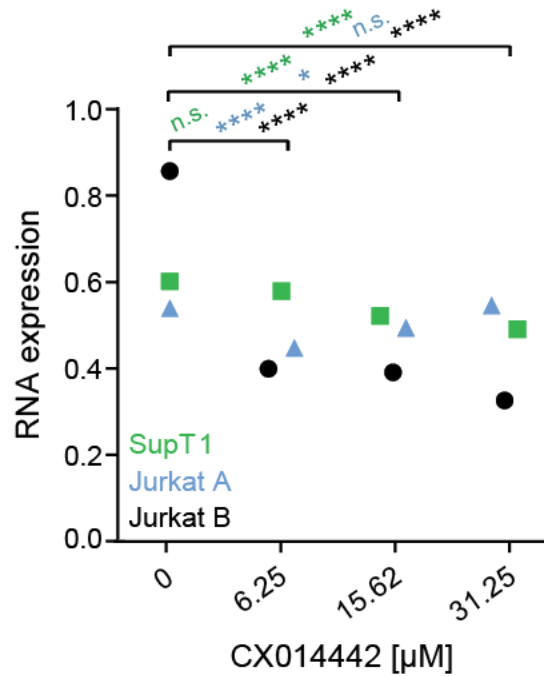
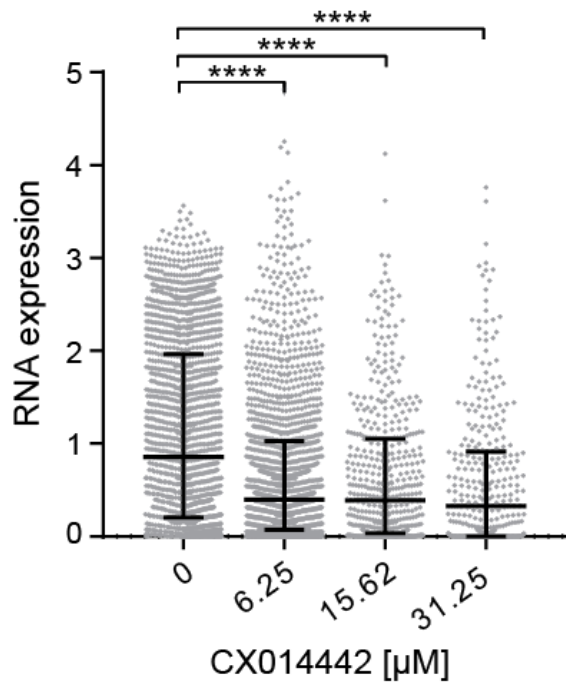
Advantage

- Image RNA transcription per DNA copy
- Image location of DNA copy in the nucleus

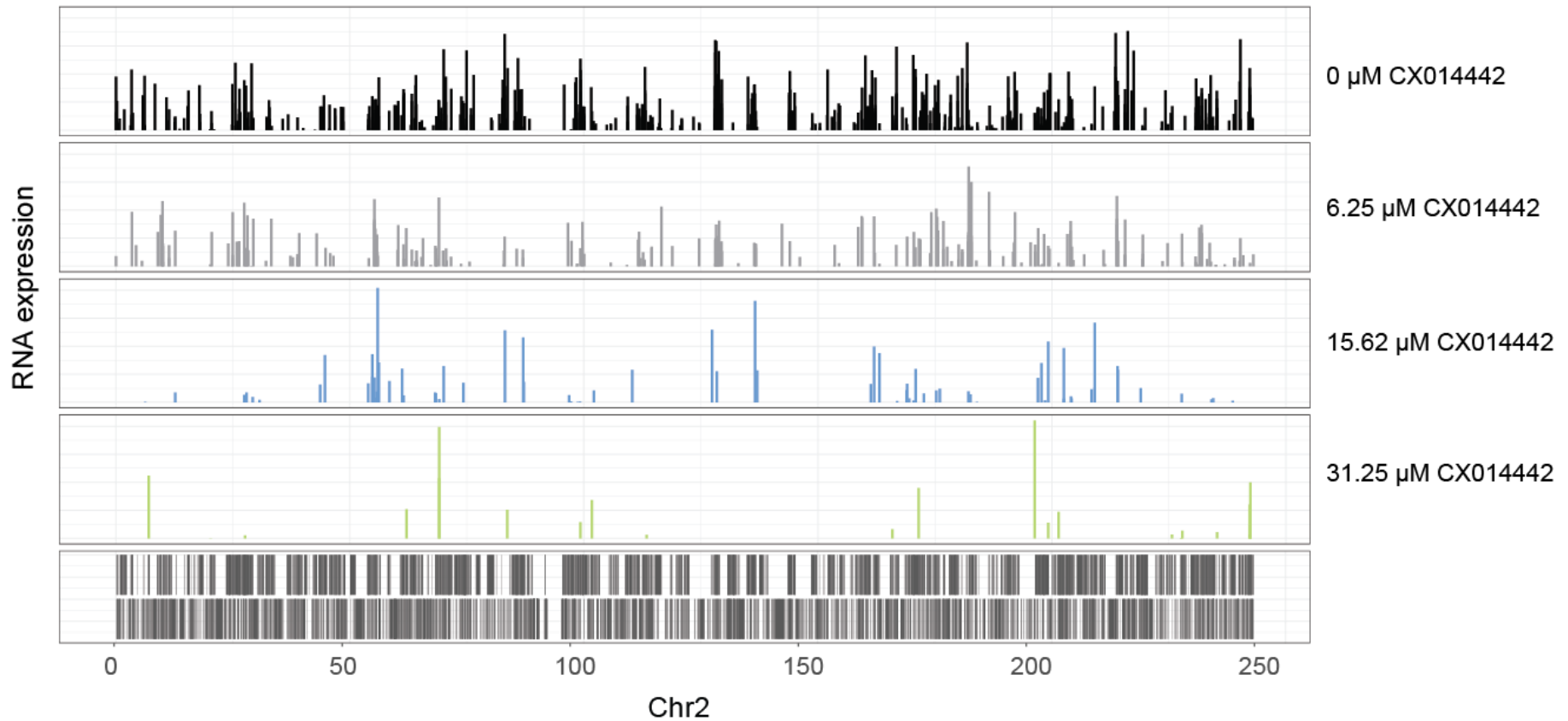
LEDGINs retarget HIV-1 integration



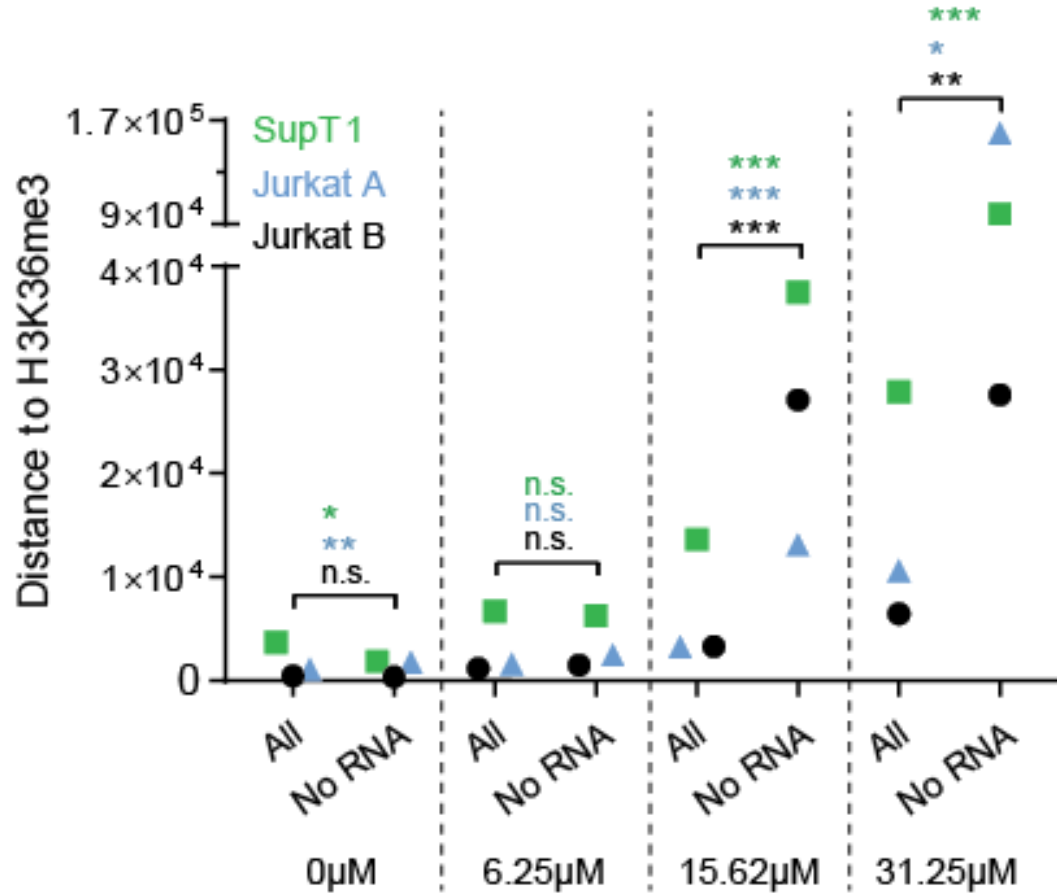
LEDGINs reduce RNA expression



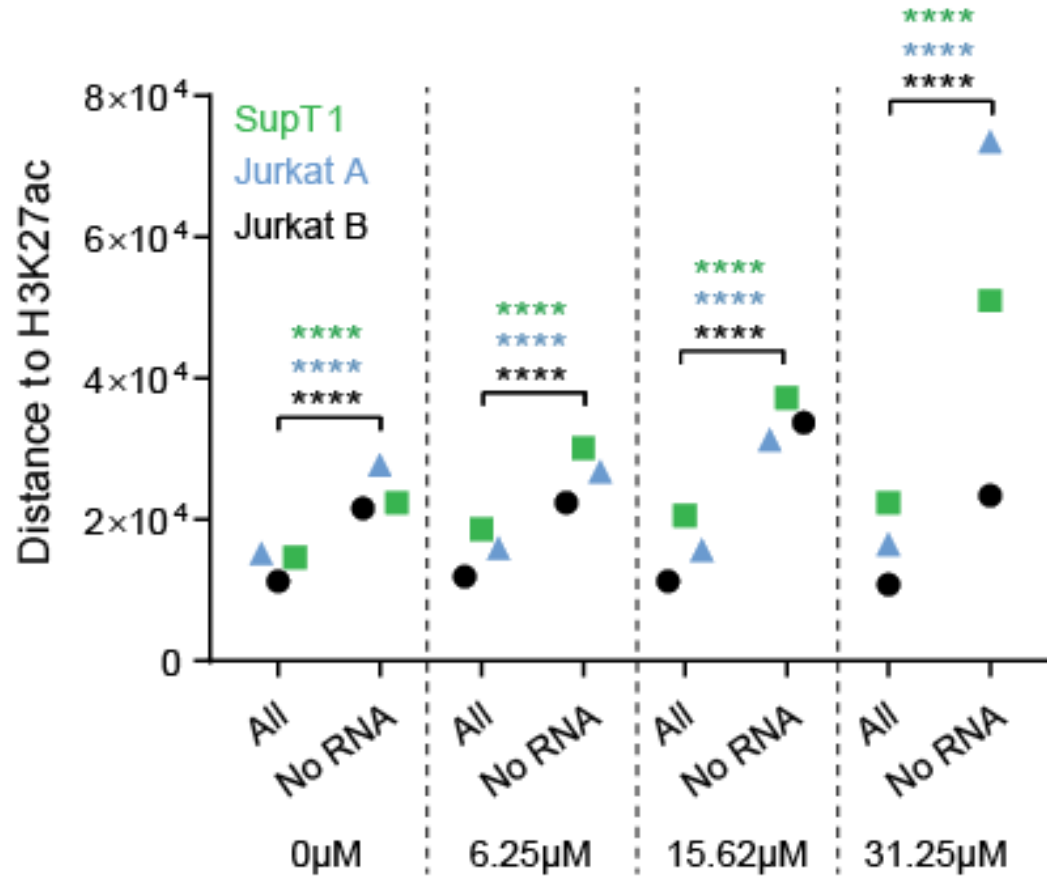
Reduction in RNA expression per chromosome



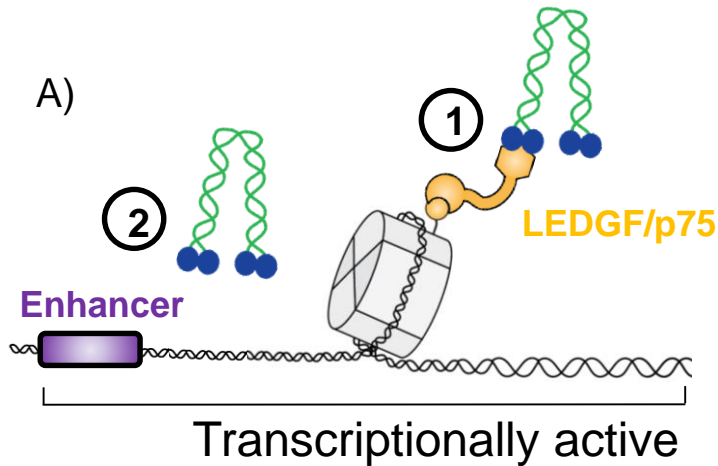
After LEDGIN treatment no-expressors are further away from H3K36me3 (LEDGF mark)



Distance to H3K27Ac affects RNA transcription independently from LEDGF/p75



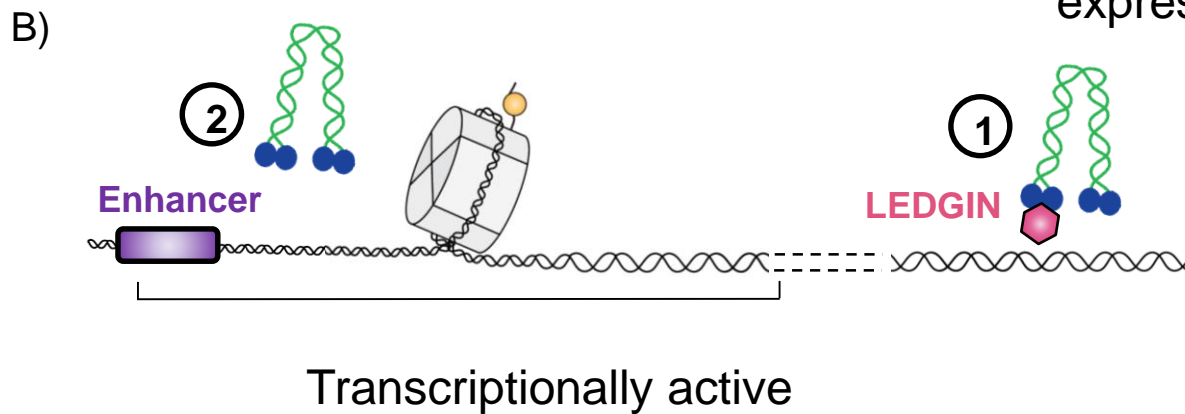
Both LEDGF/p75 and super-enhancers determine transcription levels of HIV provirus



- ① LEDGF/p75-dependent
- ② LEDGF/p75-independent



Remaining high-expressors in presence of LEDGINs



The chromatin landscape at the HIV-1 integration site determines viral expression

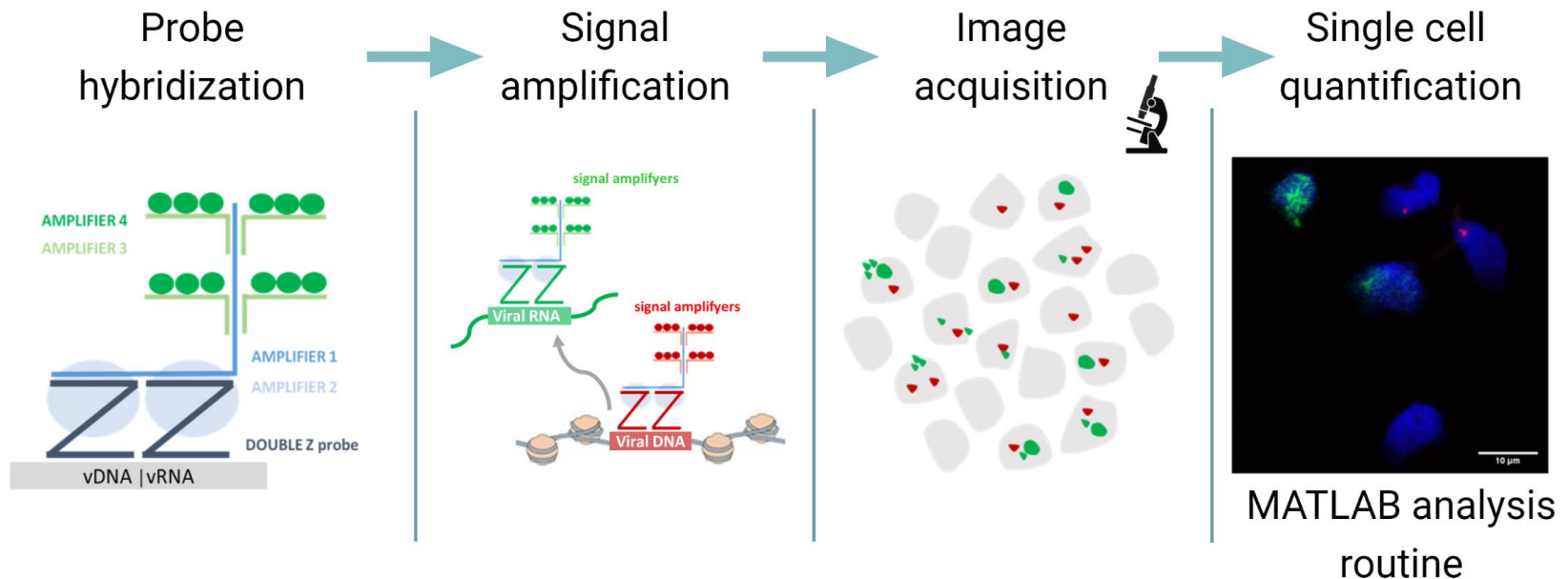
Single-Cell Imaging Shows That the Transcriptional State of the HIV-1 Provirus and Its Reactivation Potential Depend on the Integration Site.

Janssens, J., De Wit, F., Parveen, N., Debyser, Z.
MBIO 2022.



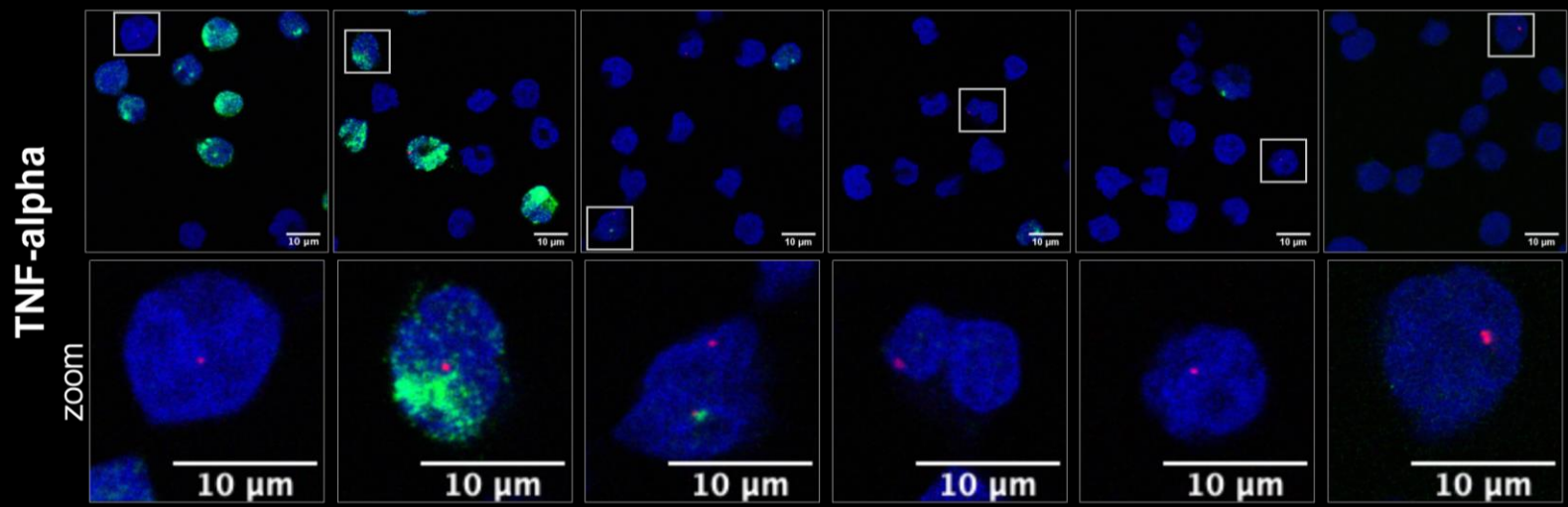
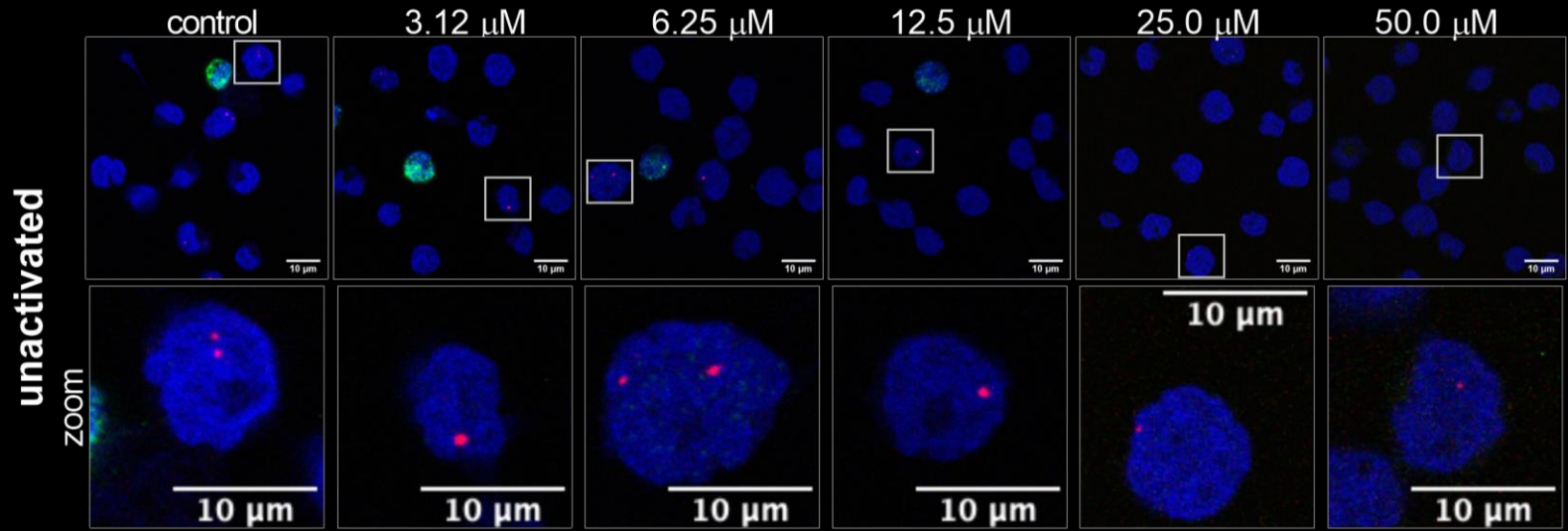
Study the effect of LEDGINs in single cells with bDNA imaging

- Fluorescent in situ hybridization (FISH)
- Simultaneous labelling of viral DNA and vRNA in single cells



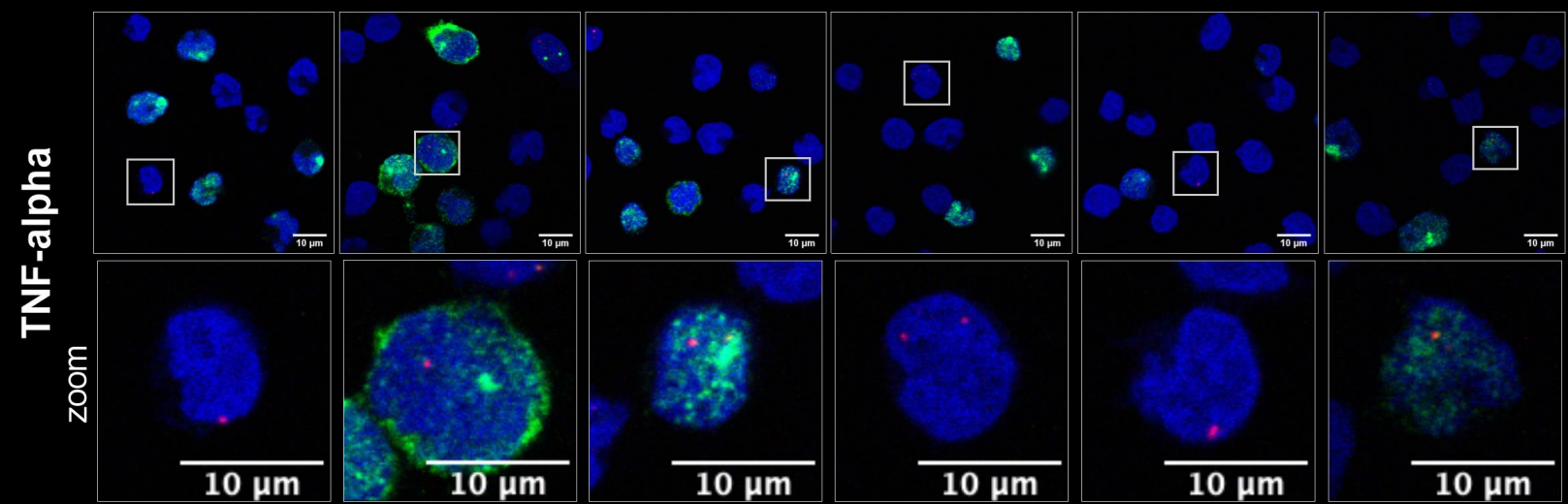
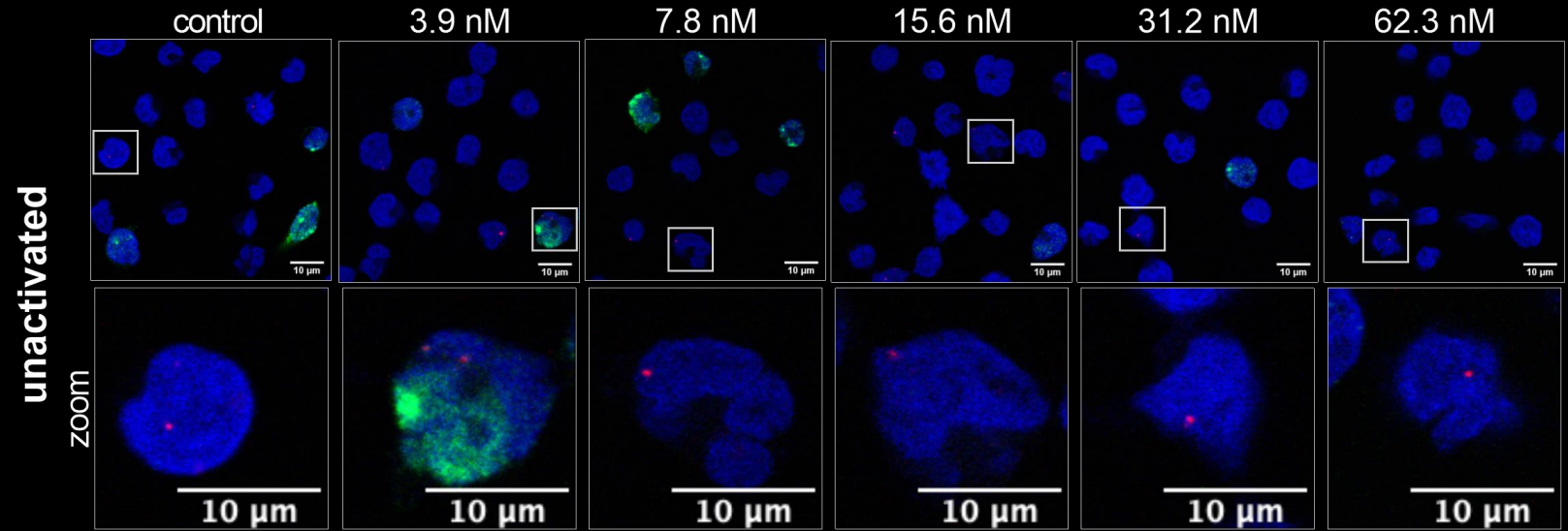
CX014442

DAPI vDNA vRNA



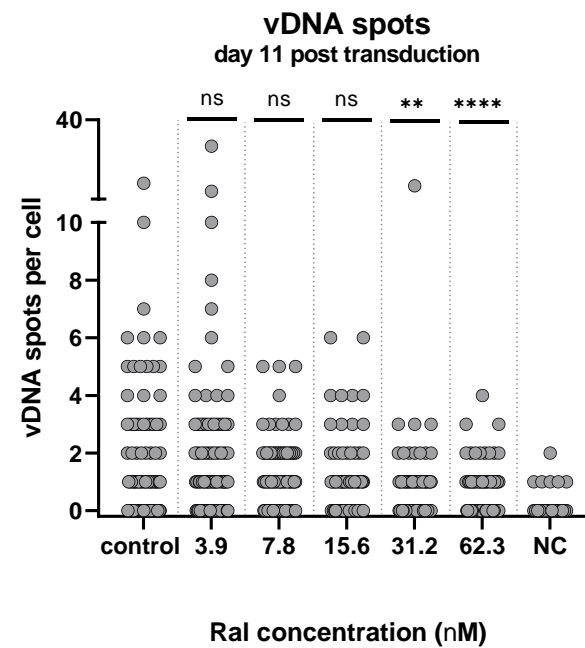
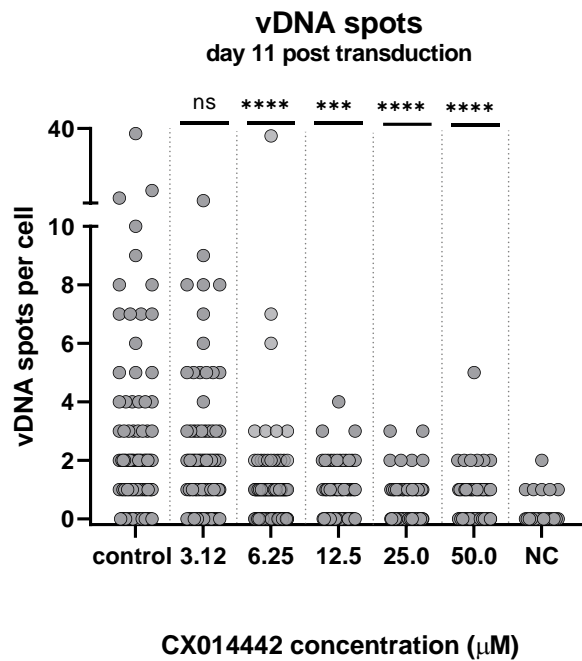
DAPI vDNA vRNA

RAL



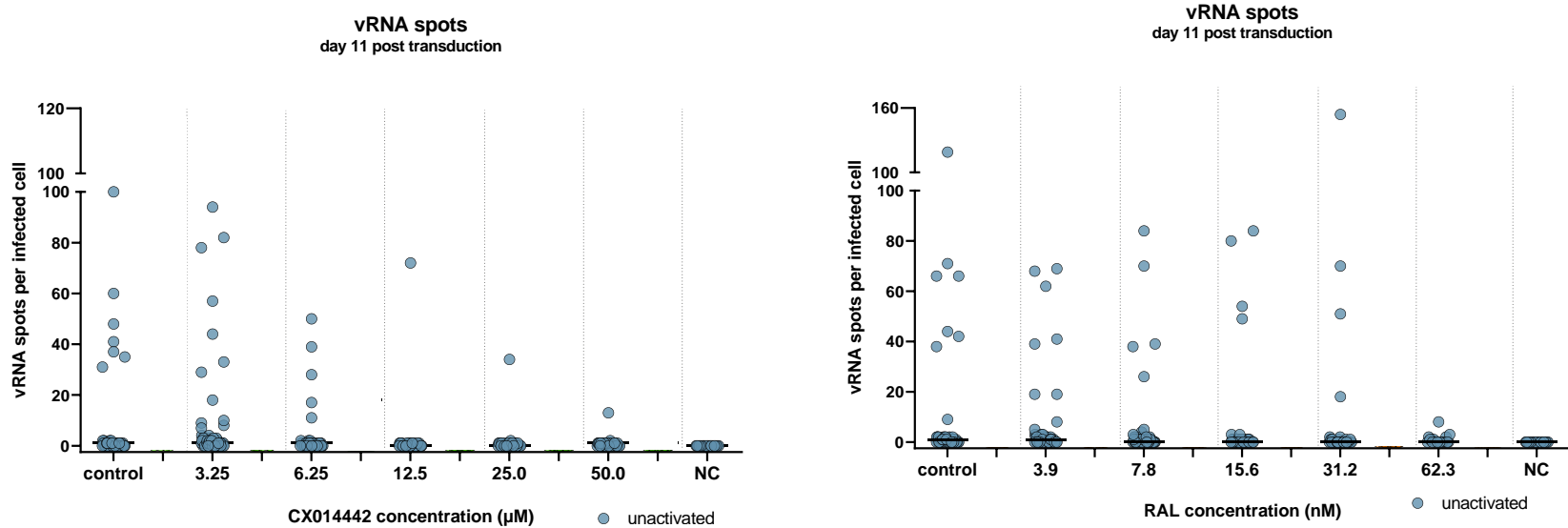
1. LEDGINs reduce HIV-1 integration

HIV DNA (vDNA) spots per cell measure of integrated HIV



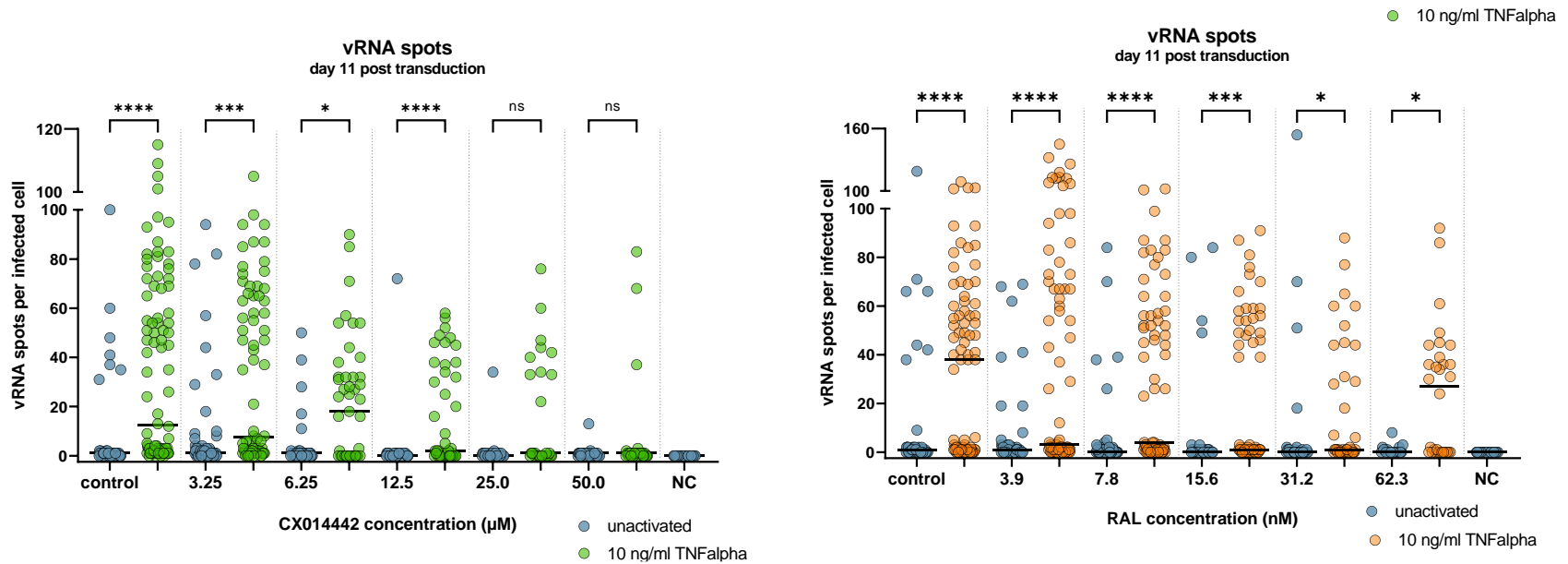
2. LEDGINs reduce HIV-1 integration and viral RNA expression

HIV RNA (vRNA) spots per cell to measure HIV transcription (non-activated) and reactivation (TNFalpha)



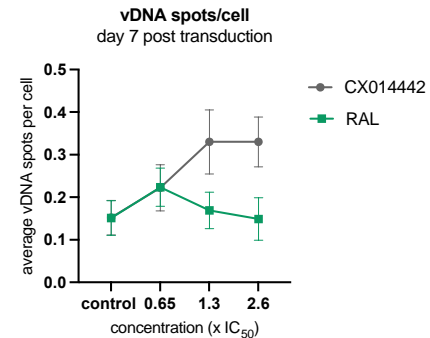
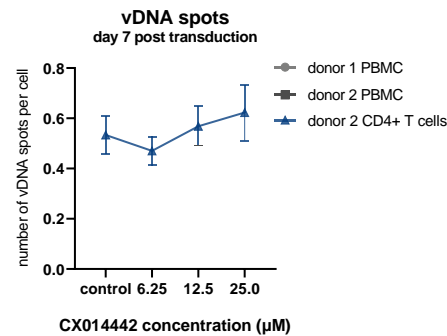
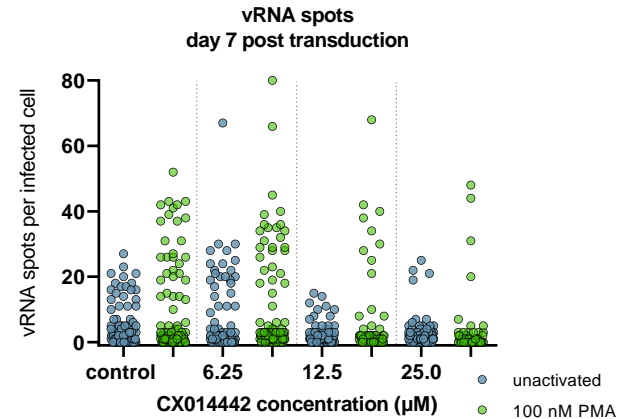
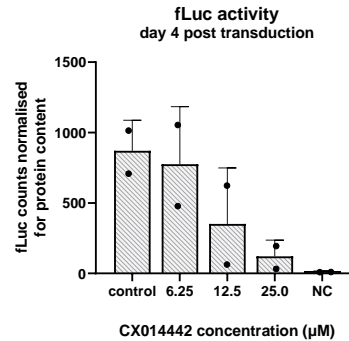
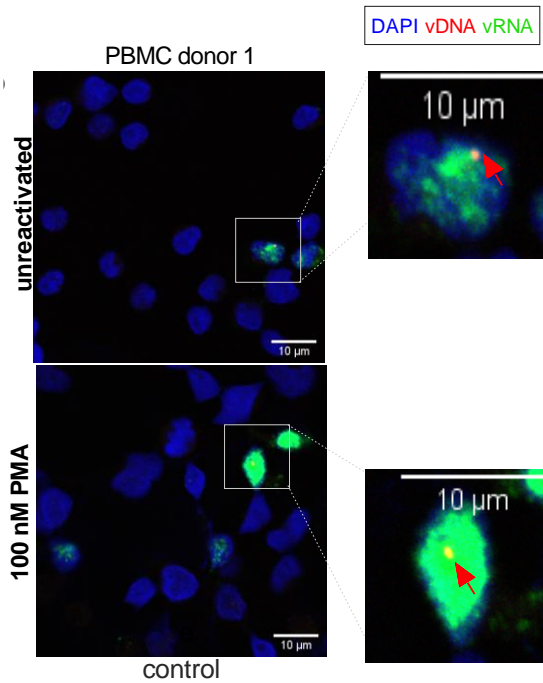
3. LEDGINs reduce HIV-1 reactivation

HIV RNA (vRNA) spots per cell to measure HIV transcription (non-activated) and reactivation (TNFalpha)



Molecular Virology and Gene Therapy

4. LEDGINs reduce viral RNA expression and reactivation in primary cells



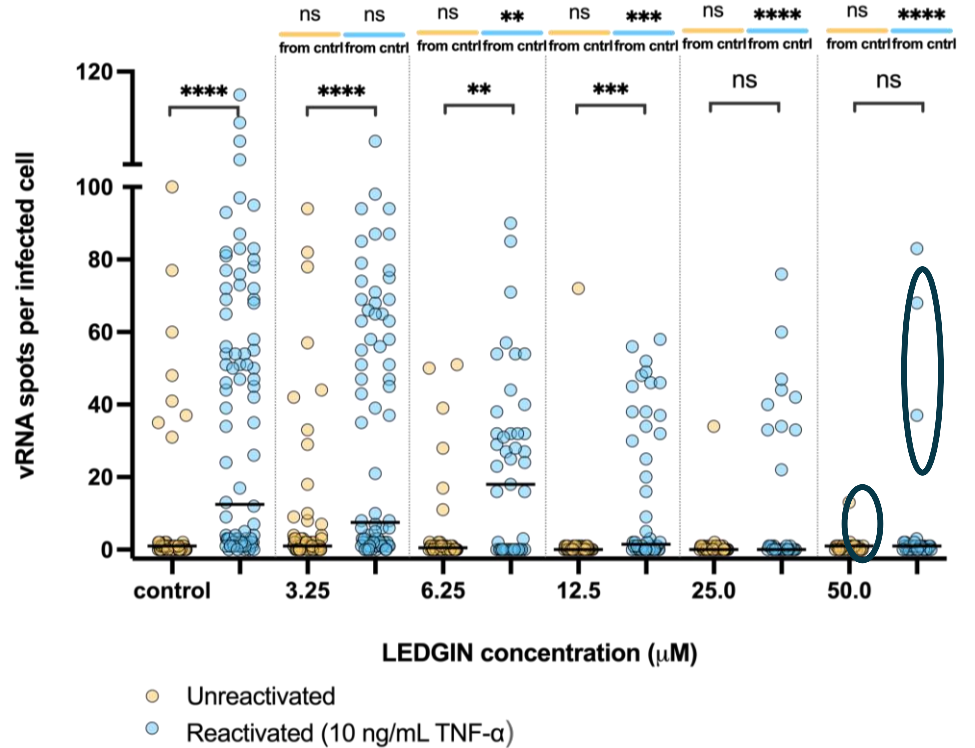
TOWARDS A FUNCTIONAL CURE OF HIV-1 INFECTION: BRD4 MODULATOR ZL0580 AND LEDGINS ADDITIVELY BLOCK AND LOCK HIV-1 TRANSCRIPTION

Eline Pellaers, Julie Janssens, Lore Wils, Alexe Denis, Feng Da, Frauke Christ, Zhang Peng and Zeger Debyser



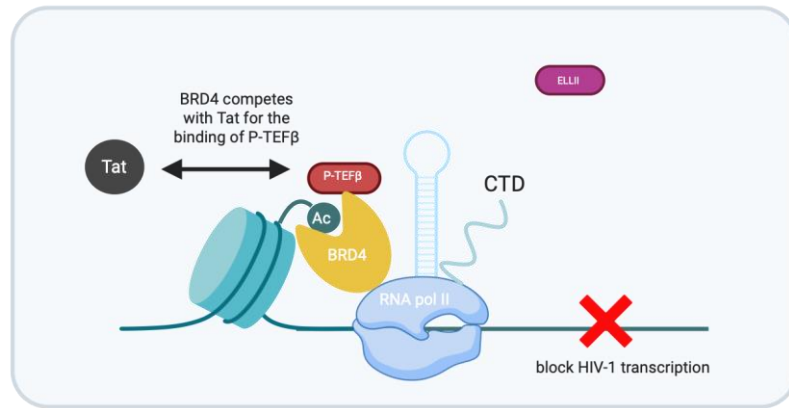
5. Enhanced block-and-lock

How to silence residual high vRNA expression

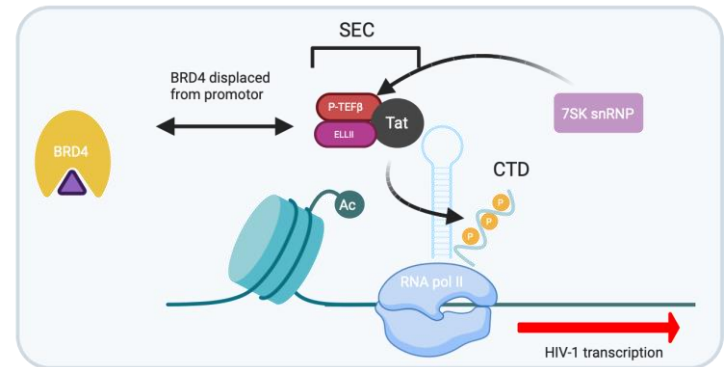


*dot= number of vRNA spots per infected cell
*line= median

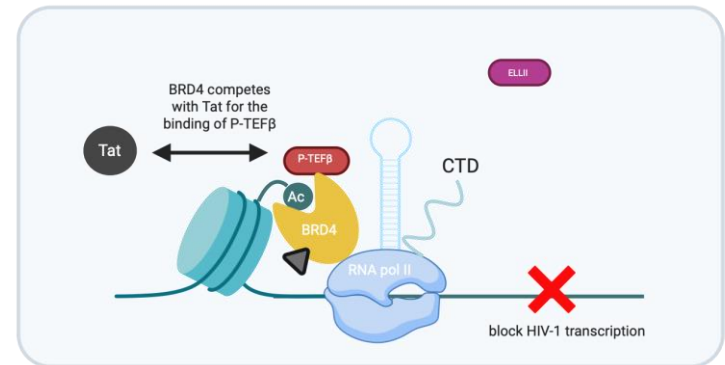
Role of BRD4 in transcriptional regulation of HIV-1



▲ +JQ1
(LRA)



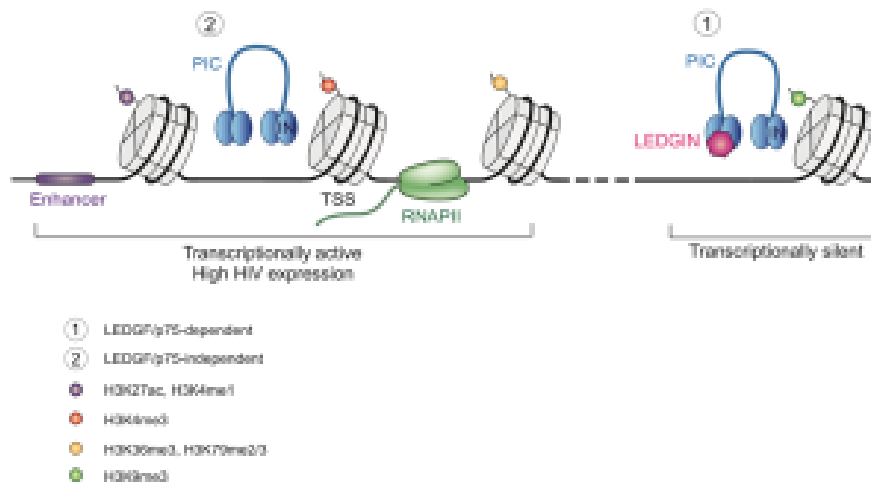
▲ +ZL0580
(LPA)



* Li, Z. *et al.*, *Nucleic Acids Res.*, 2013.

* Niu, Q. *et al.*, *J. Clin. Invest.*, 2019.

Role of enhancers

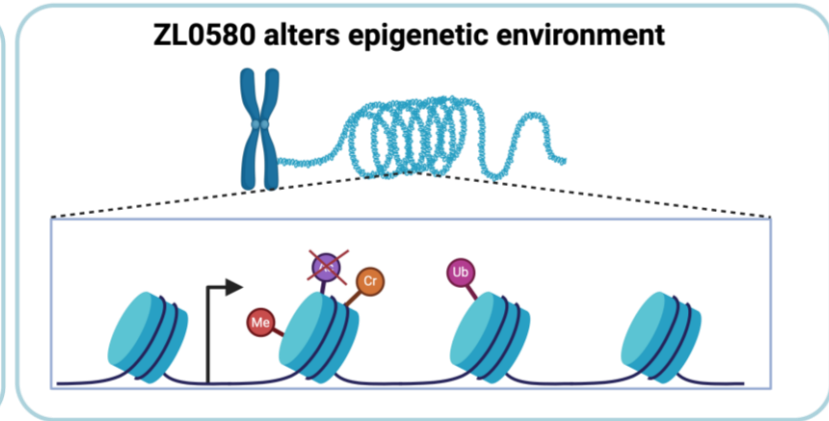
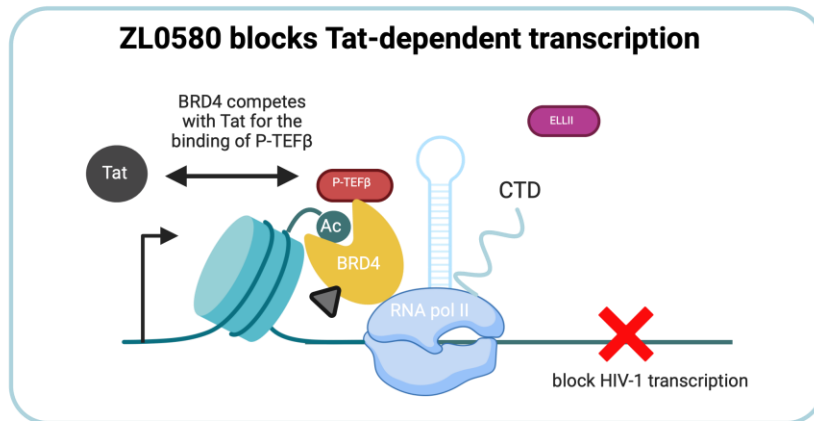
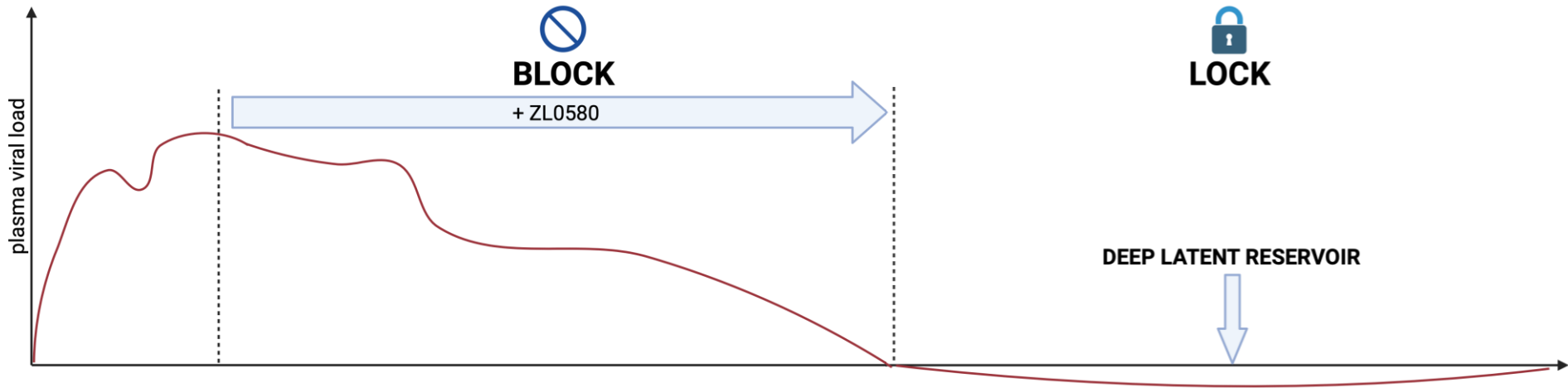


→LEDGINs don't influence proximity of integration sites to enhancers (Vansant *et al.*, *Nucleic Acids Res.*, 2022.)

→HIV transcription is stimulated by integration in proximity to enhancers (Chen *et al.* *Nat. Struct. Mol. Biol.*, 2017.)

↓
Residual high vRNA expression after LEDGIN-treatment due to enhancers ?

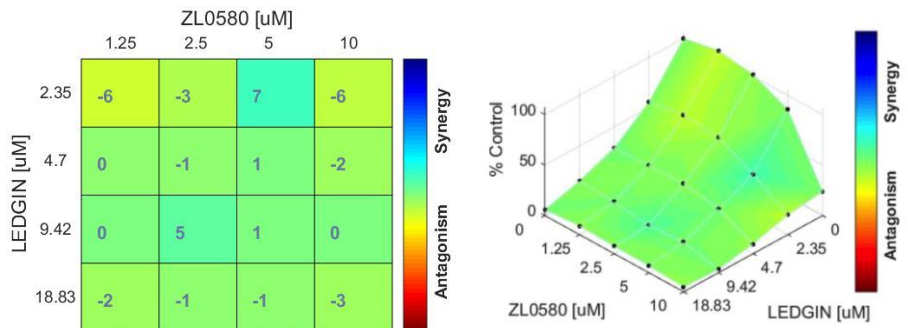
Block-and-lock phenotype



ZL0580 and LEDGINs have an additive effect in promoting latency

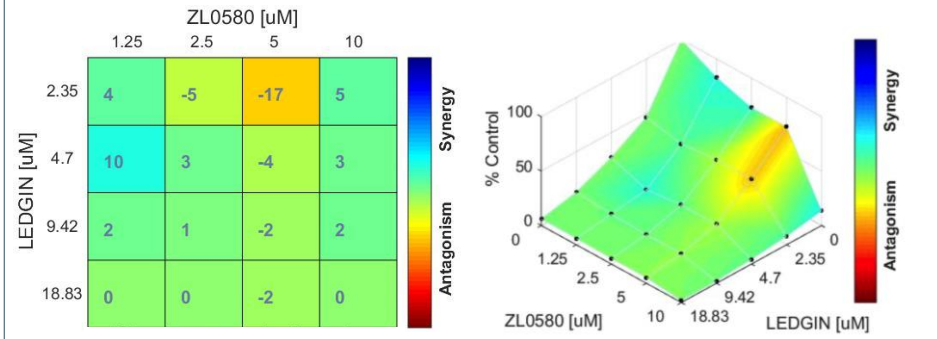
Blocking activity

Synergy scores of non-reactivated cells



Locking activity

Synergy scores of reactivated cells
(10 ng/mL TNF- α)



<-10: *antagonistic*;

-10 to 10: *additive*

>10: *synergistic*

*Results generated with Combobenefit

*Veroli G., et al., *Bioinformatics*, (2016).

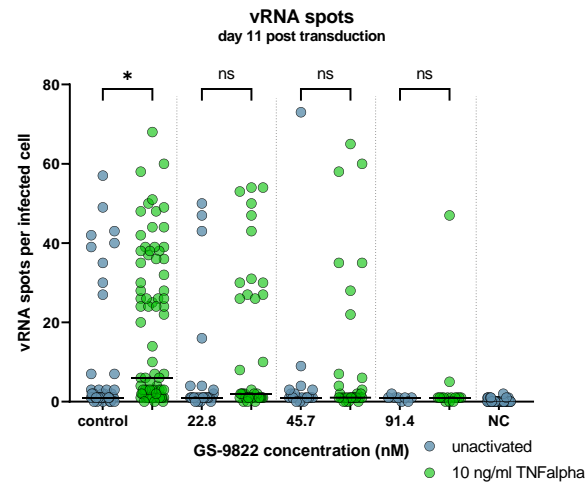
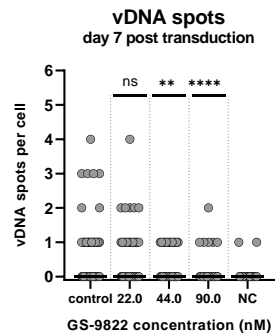
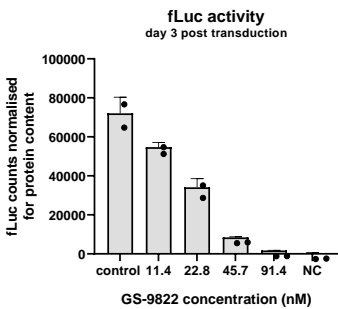
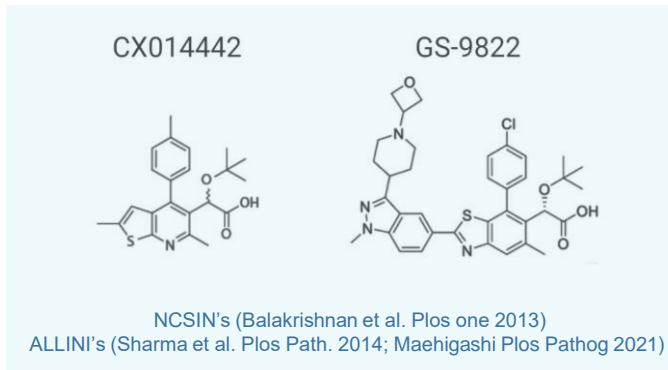
7. Towards the clinic

The block-and-lock phenotype in HIV patients

- Positive selection of proviruses with lower transcriptional activity in patients on prolonged ART (Einkauf et al. 2019, 2022)
- In elite controllers, HIV is integrated in regions associated with deep latency → block-and-lock (Jiang et al. Nature, 2020)

LEDGINs as part of ART to accelerate the natural block-and-lock phenotype that occurs in elite controllers and prolonged ART

LEDGIN GS-9822 reduces viral RNA expression and reactivation at nanomolar range



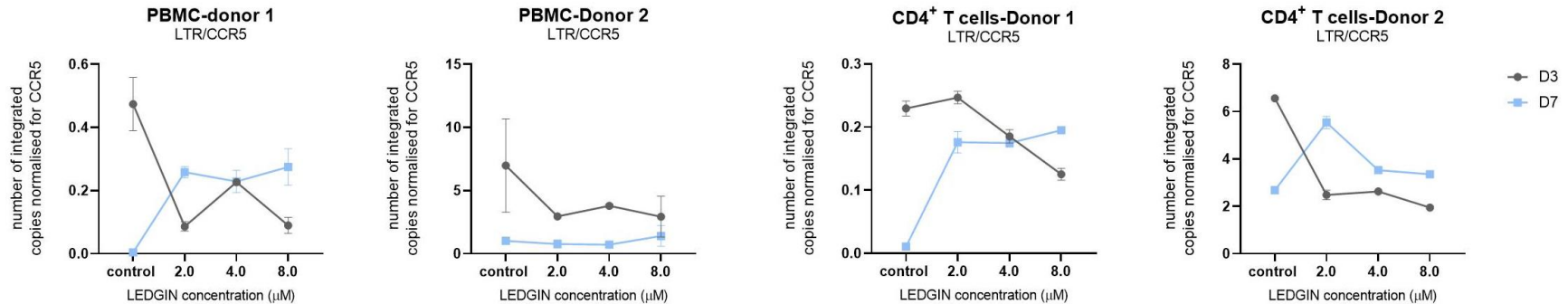
Effect on basal transcription (unactivated cells)

GS-9822 (nM)	total _{vDNA}	total _{vRNA}	vRNA per copy
0 (control)	29	409	14.1
22.8	15	167	11.1
45.9	5	105	21.0
91.4	4	7	1.75

Effect on reactivation (10 ng/ml TNF-alpha)

GS-9822 (nM)	total _{vDNA}	total _{vRNA}	vRNA per copy
0 (control)	14	1422	101.6
22.8	12	547	45.6
45.9	8	342	42.8
91.4	3	62	20.7

LEDGIN-induced selection of deep latent provirus



- Selection of integrated copies **over time**
- Dose-dependent effect after LEDGIN treatment
- Positive selection of proviruses with lower transcriptional activity in patients at specific genomic locations (Einkauf et al. 2019, 2022)

Selection of LEDGIN-retargeted but deep latent provirus

Towards clinical cure trials

- Time line
 - 2003: LEDGF/p75
 - 2010: LEDGINs
 - 2016: block-and-lock
 - Clinical trial?
- How to test?
 - First as antiviral
 - Measure integration sites, QVOA...
 - For cure (remission):
 - Prep
 - Acute infection
 - First line treatment
 - Chronic infection: treatment interruption??

Take home message

- Let a thousand flowers bloom in HIV cure research
- Block-and-lock represents an alternative approach
- that is supported by clinical evidence (the block-and-lock phenotype)
- Goal is to accelerate the natural block-and-lock mechanism
- Clinical trials should provide proof-of-evidence
- Research on
 - Enhanced block-and-lock (combinations)
 - Block-and-shock or shock-and-block



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