

Role of BRD4-modulators in a block-and-lock functional cure of HIV-1

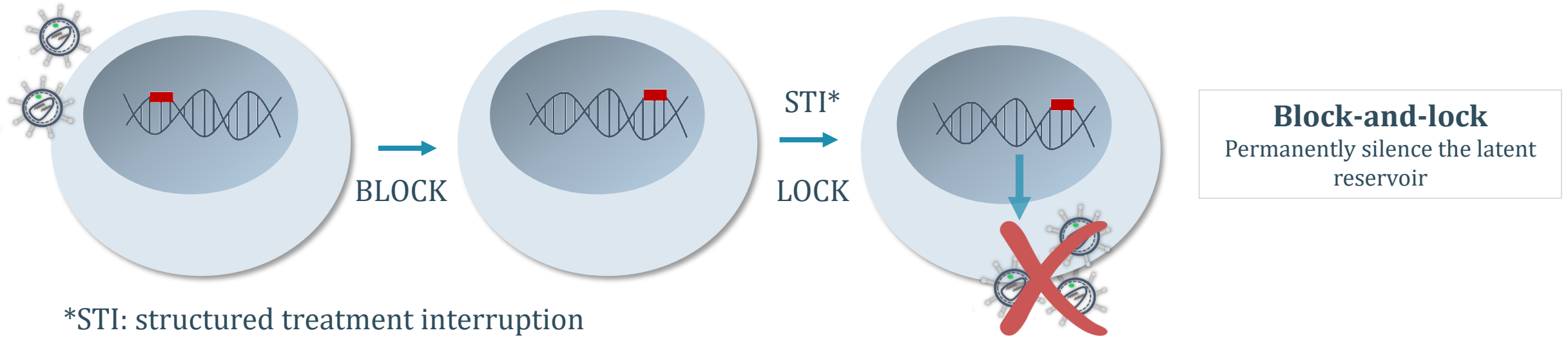
Eline Pellaers, Lore Wils, Anayat Bhat, Julie Janssens, Zhang Peng, Frauke Christ, Zeger Debyser
promotor: Prof. Dr. Debyser Zeger

10th BREACH symposium, Poster contest

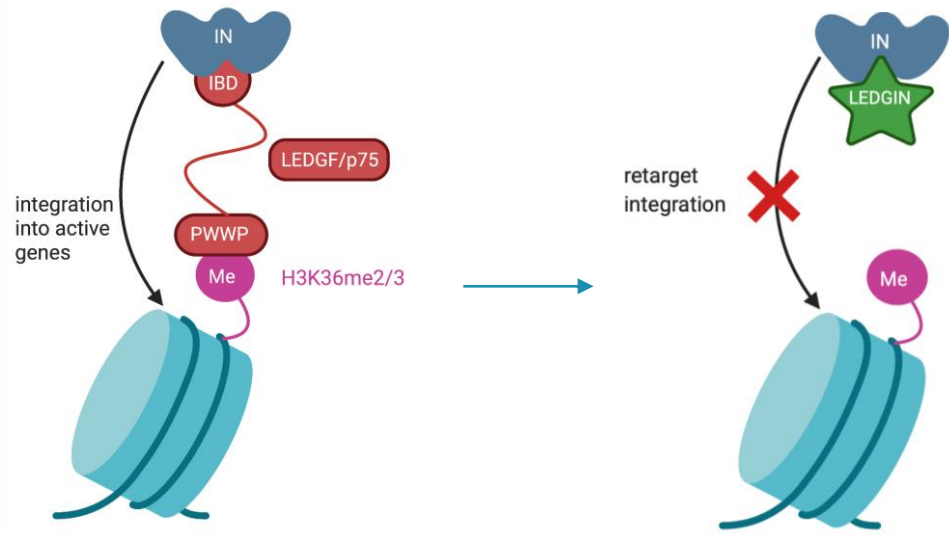
November 23, 2022



Towards a block-and-lock functional cure strategy



*STI: structured treatment interruption



LEDGINs: inhibitors of LEDGF/p75-IN interaction

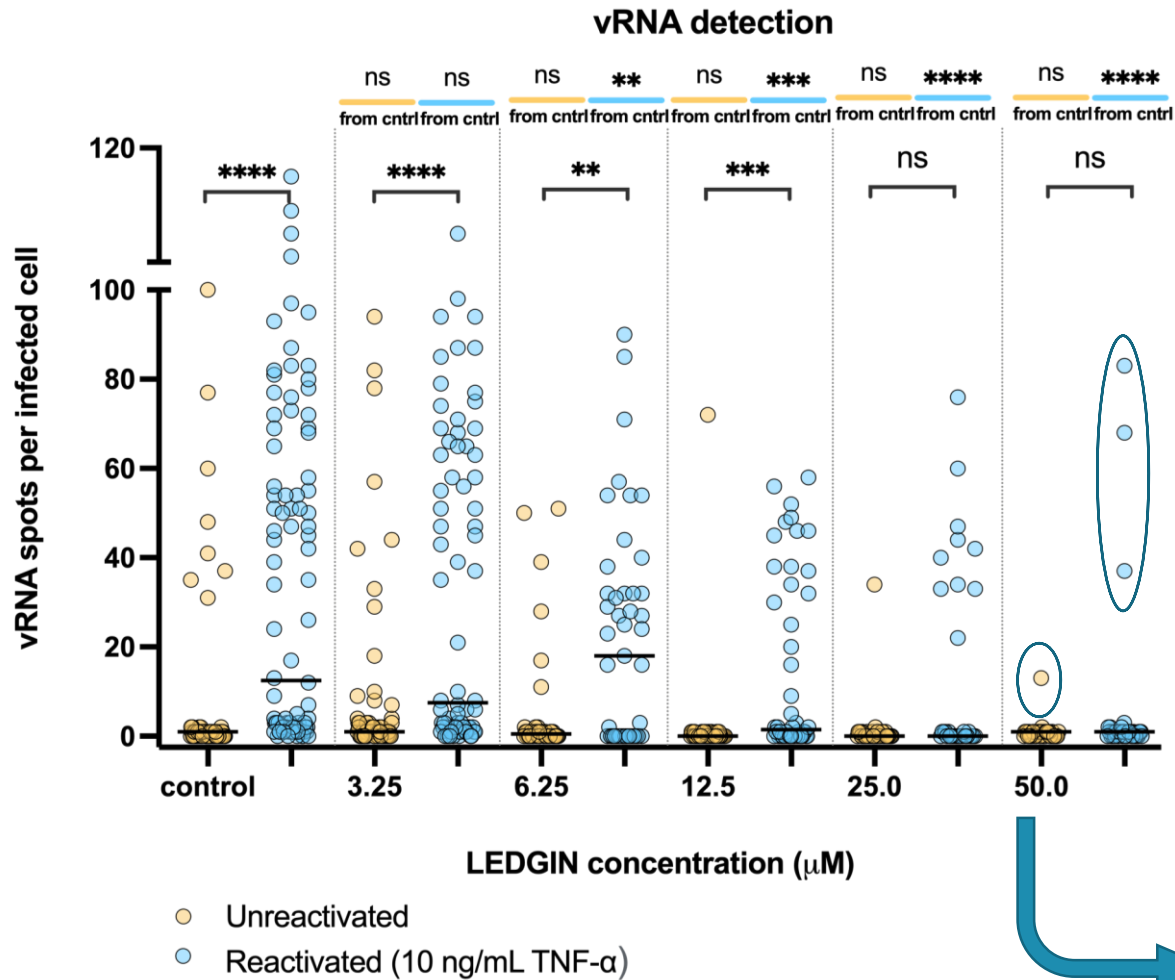
- ✓ Retarget integration
- ✓ Reduce HIV-1 transcription
- ✓ Reduce HIV-1 reactivation

*Debyser *et al.*, *Viruses*, 2019, 11, 1–12.

2 *Vranckx *et al.*, *EBioMedicine*, 2016, 8, 248–264.

*Christ *et al.*, *Antimicrob. Agents Chemother.*, 2012, 56, 4365–4374.

Role of BRD4 in transcriptional regulation of HIV-1



LEDGINS: inhibitors of LEDGF/p75-IN interaction

- ✓ Retarget integration
- ✓ Reduce HIV-1 transcription
- ✓ Reduce HIV-1 reactivation
- ✗ No complete block of HIV-1 transcription/reactivation

Mechanism of residual high vRNA expression ?

- LEDGINS don't influence proximity of integration sites to enhancers
- HIV transcription stimulated by integration in proximity to enhancers

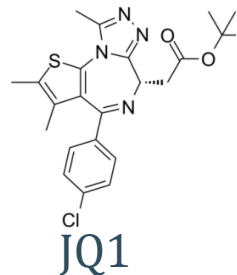
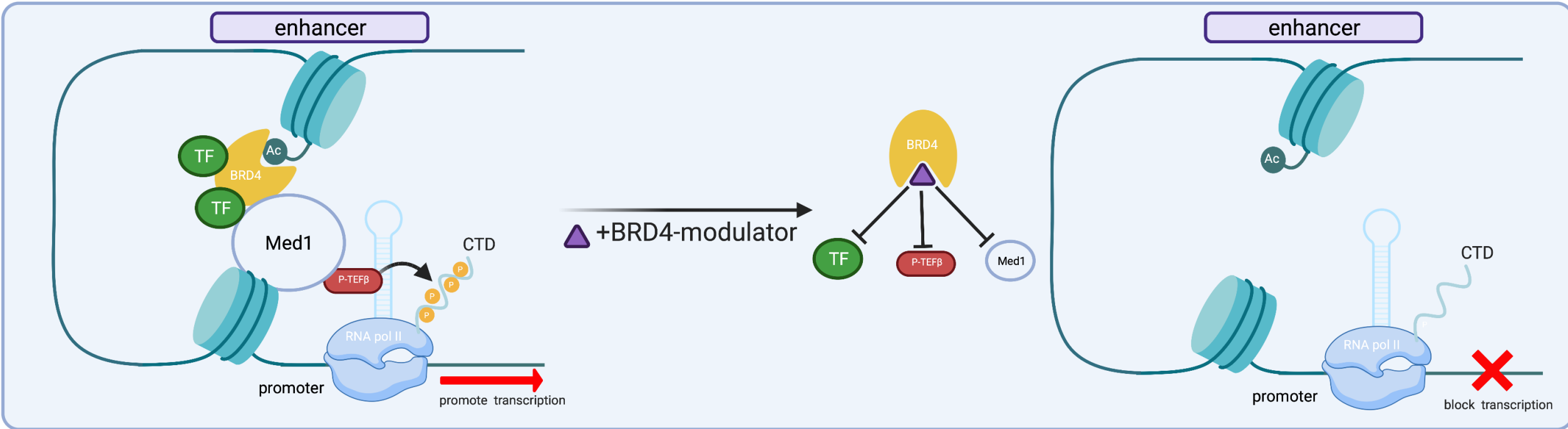
Residual high vRNA expressors due to integration near enhancer regions

*Janssens *et al.*, *Mbio*, 2022.

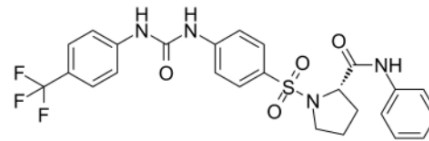
*Vansant *et al.*, *Nucleic Acids Res.*, 2022, 48, 7801-7817.

*Chen *et al.* *Nat. Struct. Mol. Biol.*, 2017, 24, 47-54.

Role of BRD4 in enhancer-dependent transcription



(Li *et al.*, *Nucleic Acids Res.*, 2013, 41(1), 277–287.)



(Niu *et al.*, *J. Clin. Invest.*, 2019, 129, 3361–3373.)

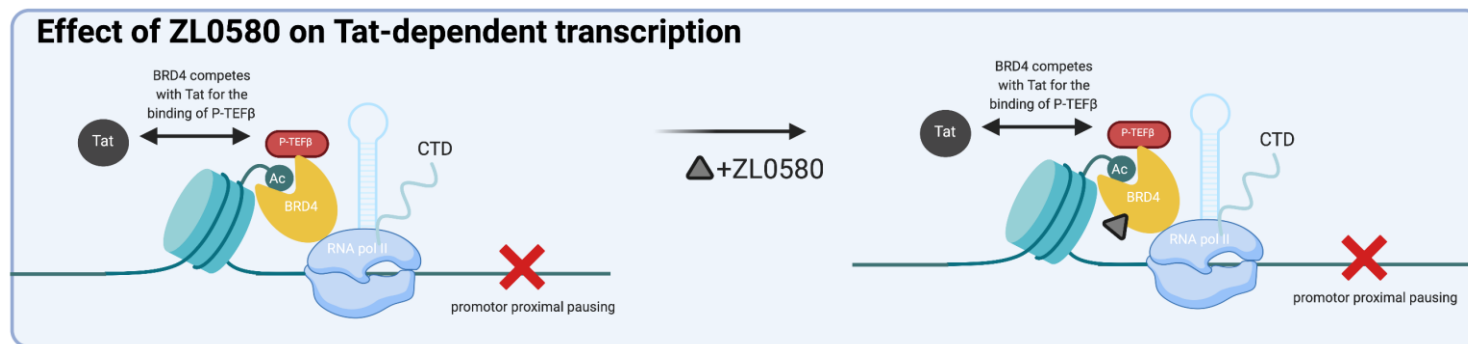
BRD4-modulators

Role of BRD4 in transcriptional regulation of HIV-1

→ JQ1 promotes HIV-1 transcription



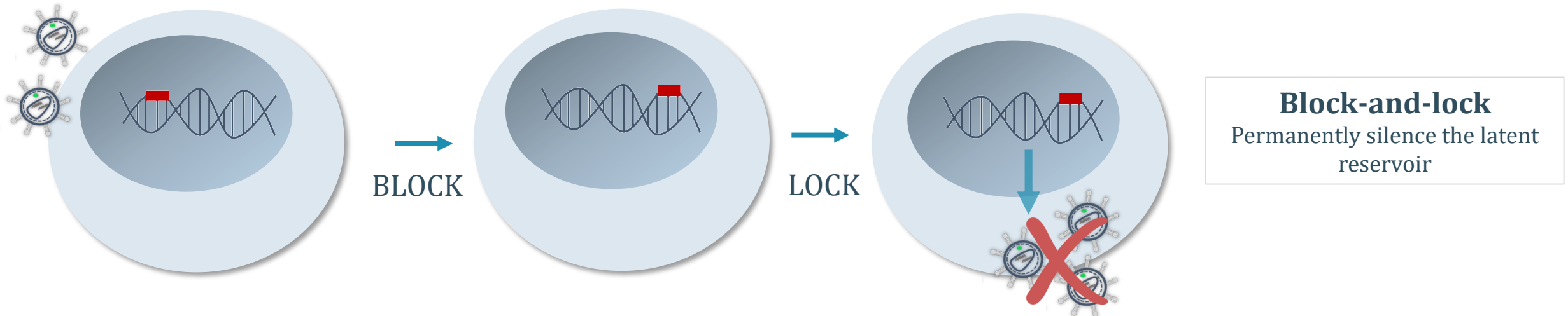
→ ZL0580 inhibits HIV-1 transcription



different binding mode to BRD4

Objectives

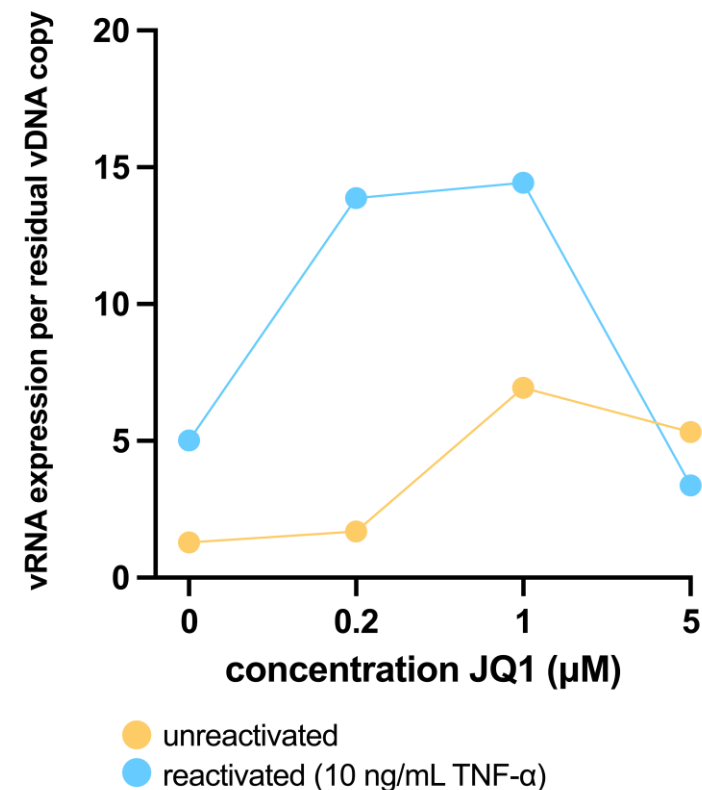
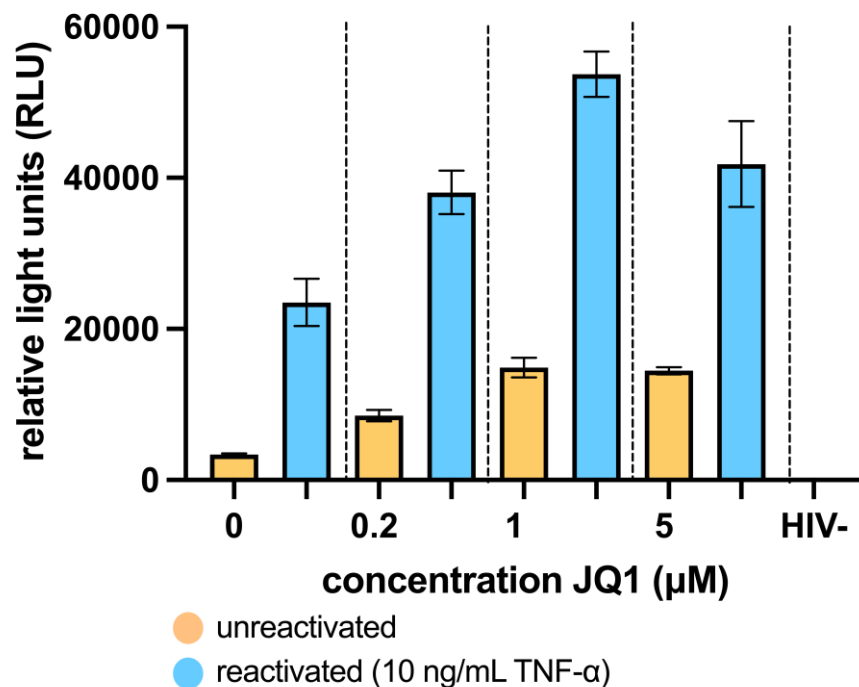
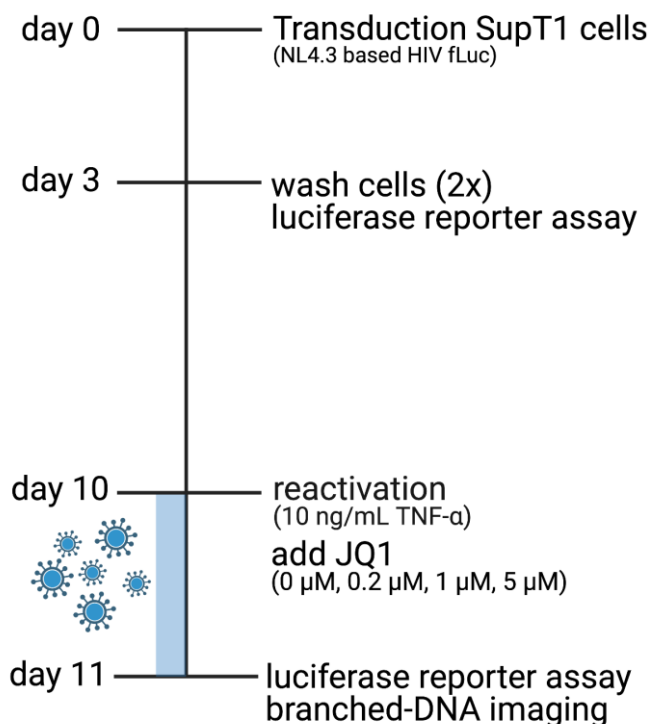
- Objective 1: Effect of JQ1 on basal HIV-1 transcription and reactivation in the absence of LEDGINs
- Objective 2: Effect of ZL0580 on basal HIV-1 transcription and reactivation in the absence of LEDGINs
- Objective 3: Mechanism of action of JQ1 and ZL0580
- Objective 4: Role of JQ1/ZL0580 in a block-and-lock phenotype after LEDGIN-mediated retargeting



JQ1 promotes HIV-1 transcription and reactivation with an optimal concentration of 1 μM

Luciferase reporter assay

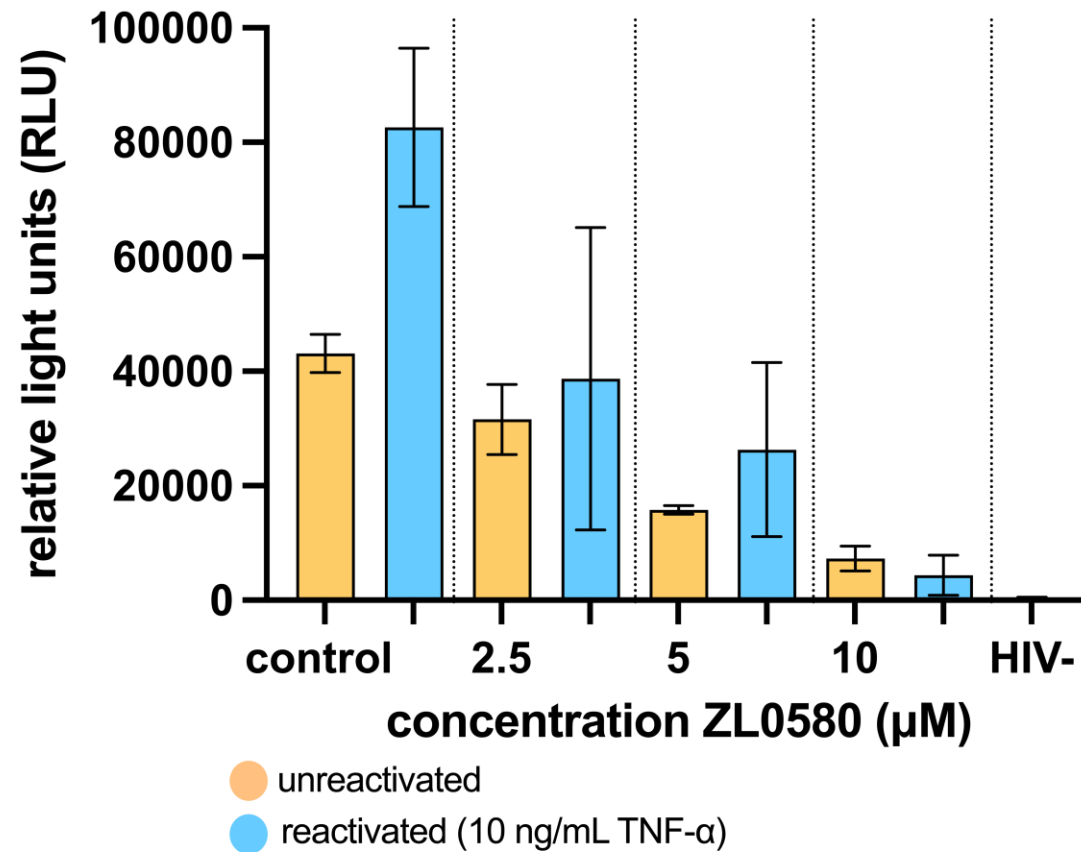
Branched-DNA imaging vRNA expression per residual vDNA copy



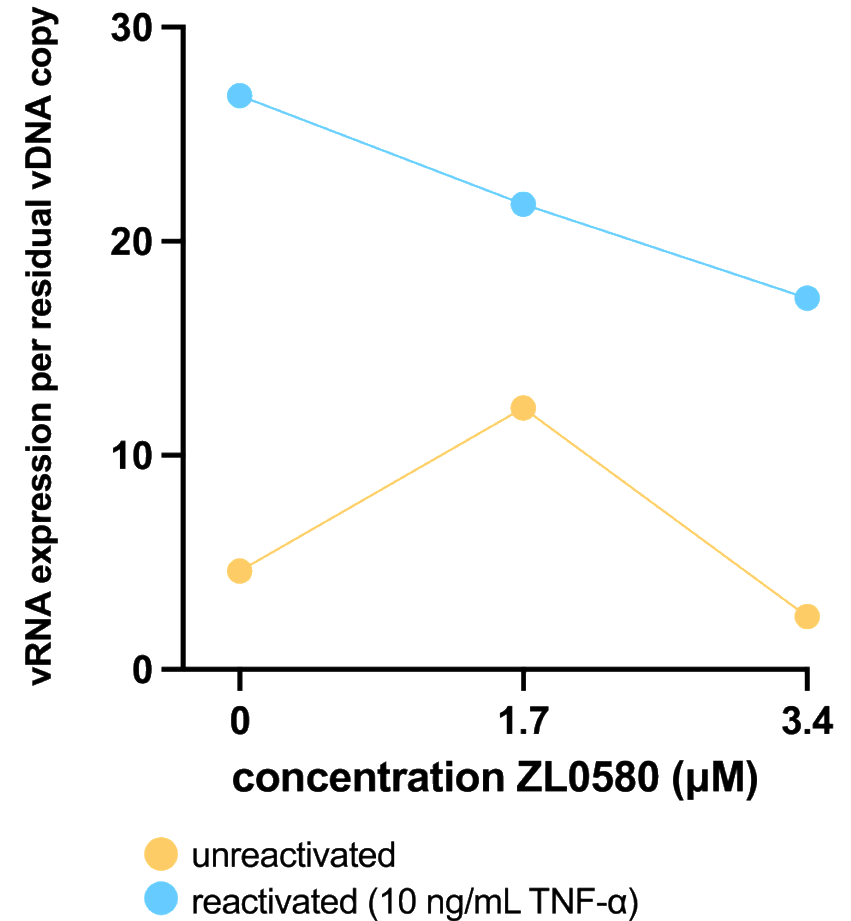
7 vRNA expression per residual vDNA copy = $\frac{\text{Total vRNA spots}}{\text{Total vDNA spots}}$

ZL0580 inhibits HIV-1 transcription and reactivation

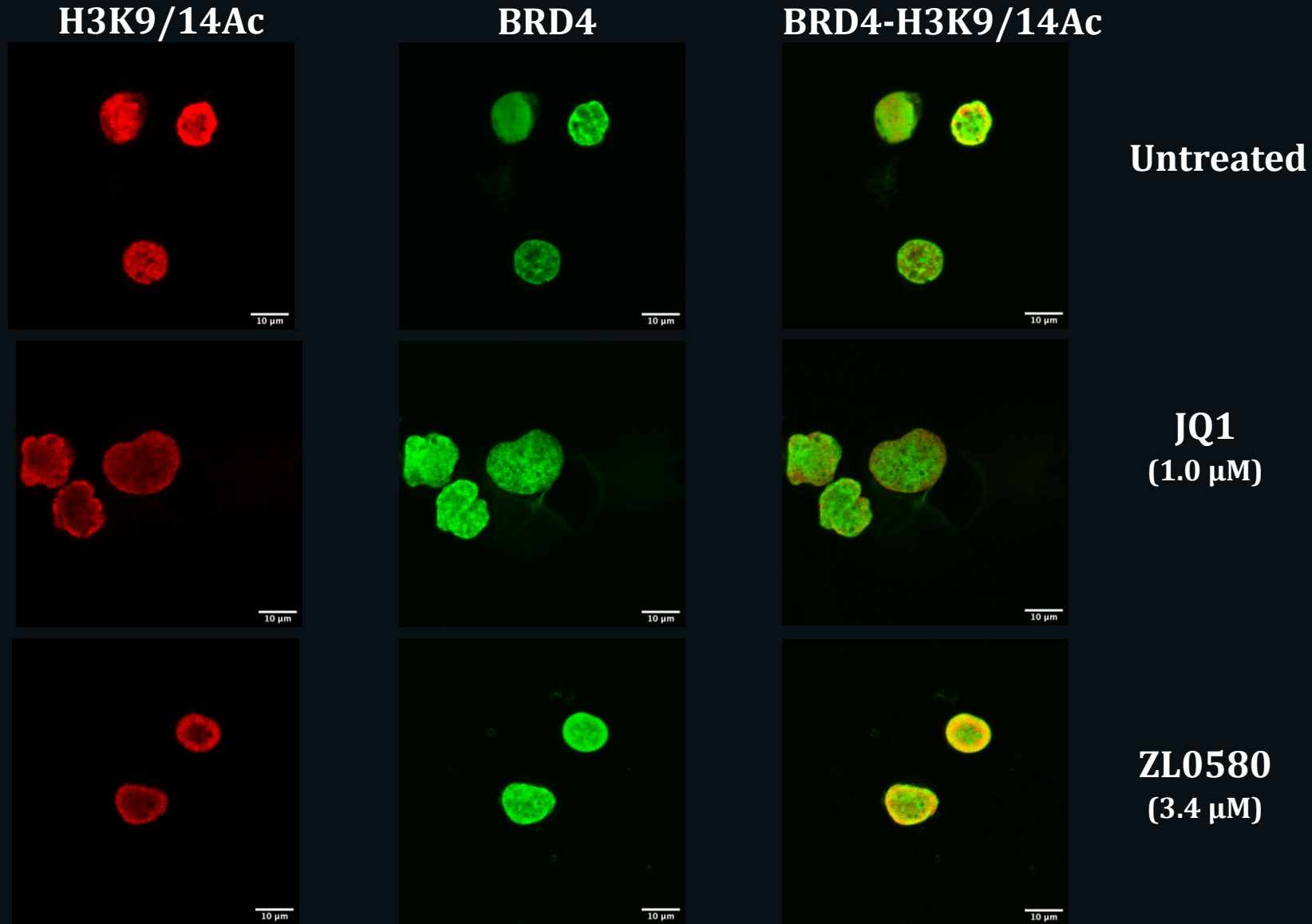
Luciferase reporter assay



Branched-DNA imaging vRNA expression per residual vDNA copy



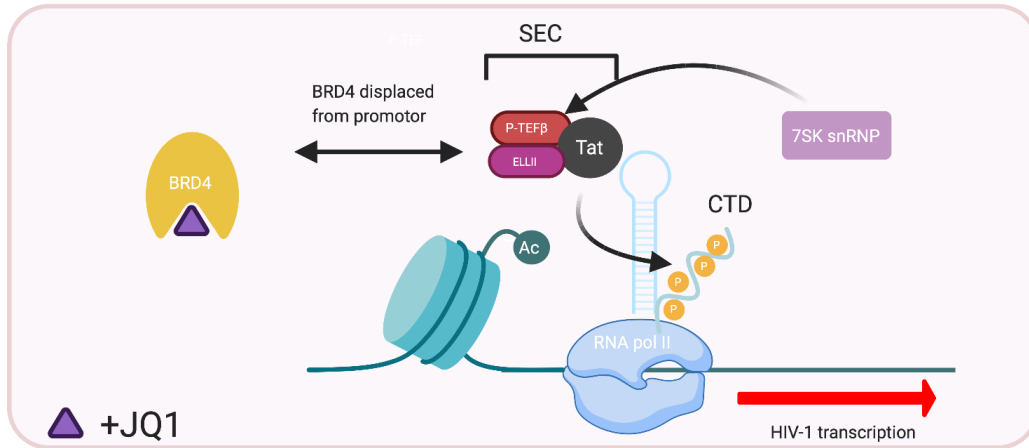
Co-localization of BRD4 with H3K9/14Ac



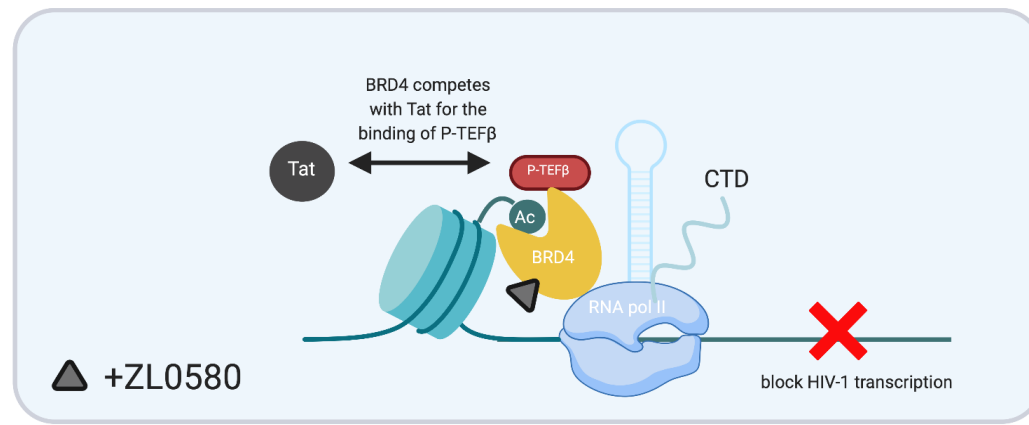
scalebar: 10 μm

Mechanism of action of JQ1/ ZL0580

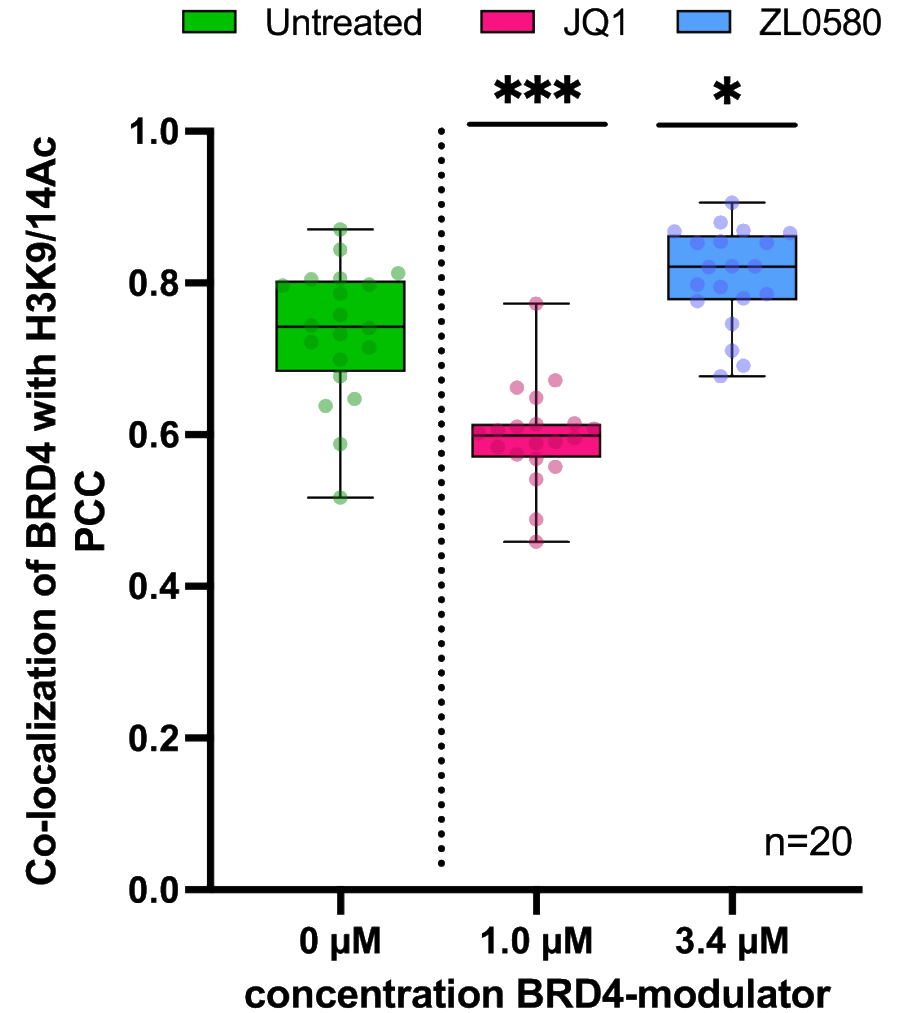
Co-localization of BRD4 with H3K9/14Ac



↓ co-localization

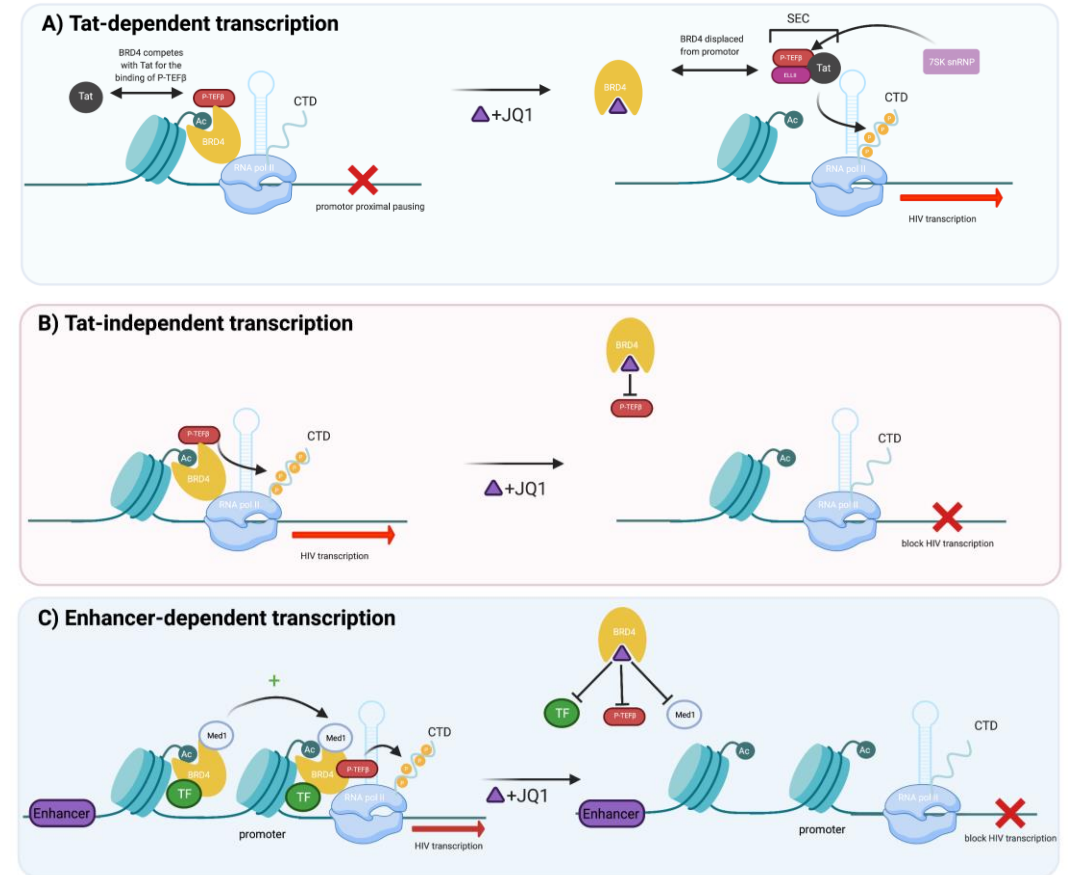
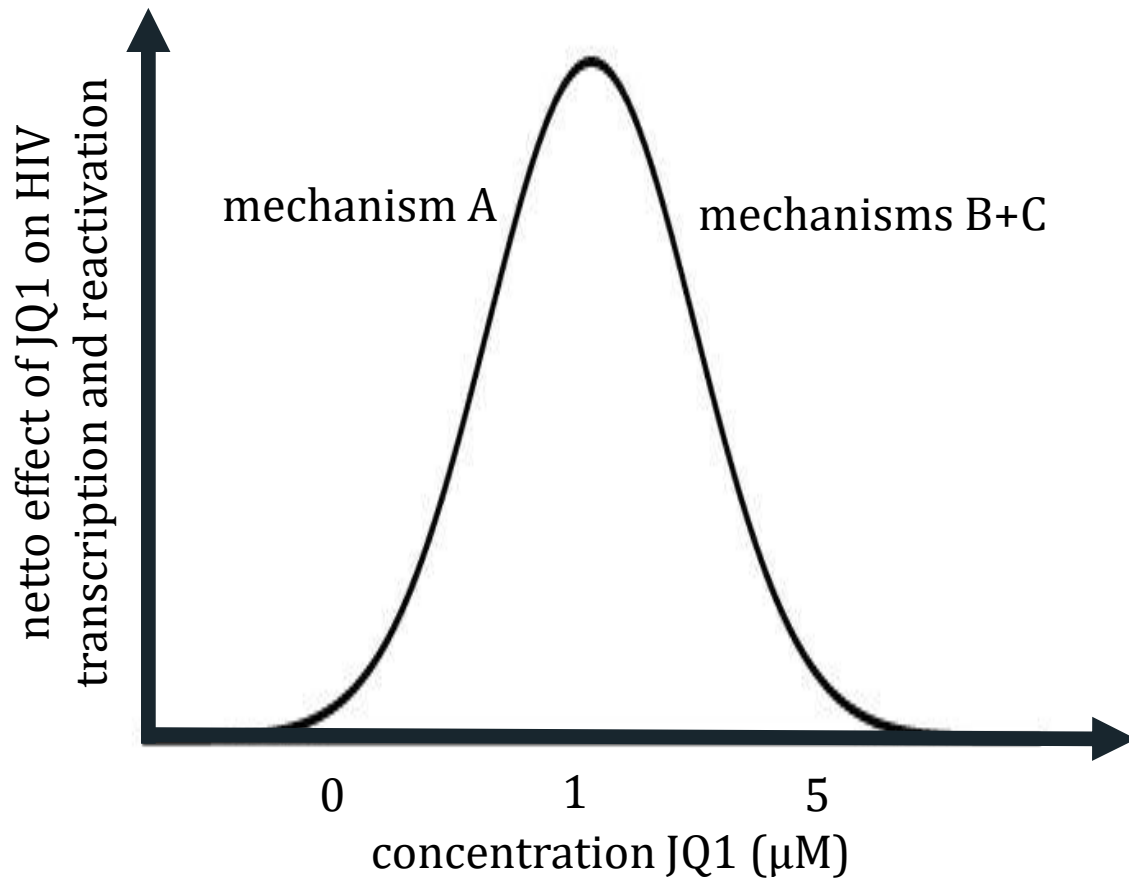


↑ co-localization



Conclusions

JQ1 promotes basal transcription and reactivation of HIV-1 with an optimal concentration of 1 μM



*Li *et al.*, *Nucleic Acids Res.*, 2013, 41(1), 277-287..

*Moon *et al.*, *Mol Cell.*, 2005, 19(4), 523-534.

*Lovén *et al.*, *Cell*, 2013, 153, 320-334

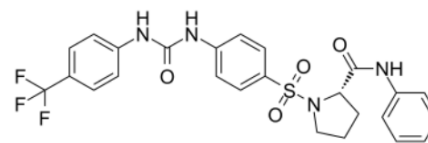
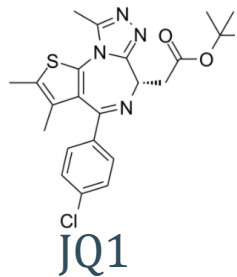
Conclusions

**JQ1 promotes basal transcription and reactivation of HIV-1
with an optimal concentration of 1 μ M**

ZL0580 hampers basal transcription and reactivation of HIV-1

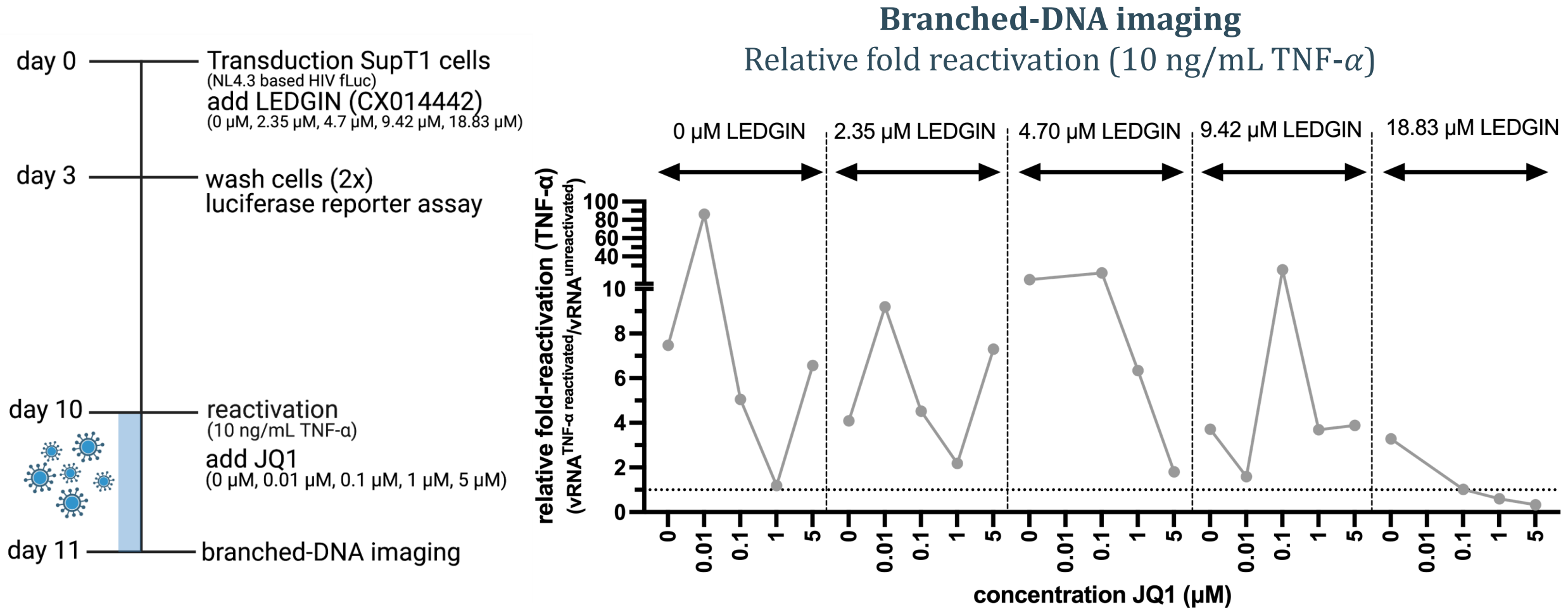
Mechanism of action:

- JQ1 decreases the co-localization of BRD4 with the acetylation marker
- ZL0580 increases the co-localization of BRD4 with the acetylation marker



ZL0580

Combination of JQ1 and LEDGINs results in a complete lock of TNF- α mediated reactivation

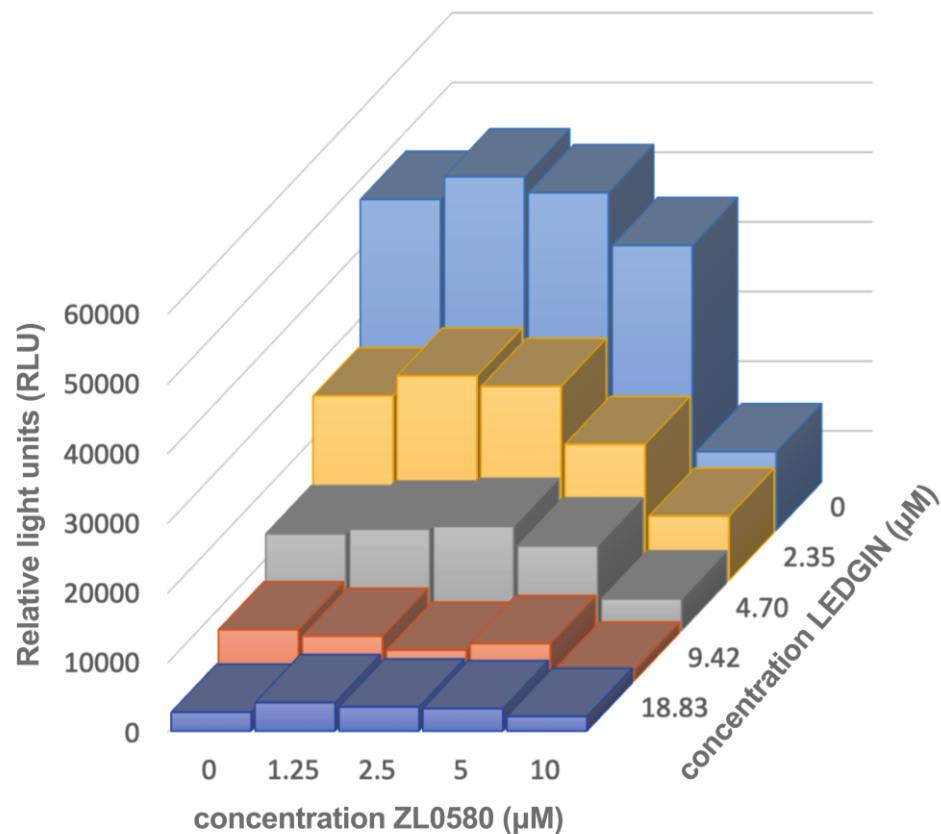


13 Relative fold reactivation (TNF- α) = $\frac{\text{Total vRNA spots in reactivated cells}}{\text{Total vRNA spots in unreactivated cells}}$

ZL0580 and LEDGINs have an additive effect in promoting latency

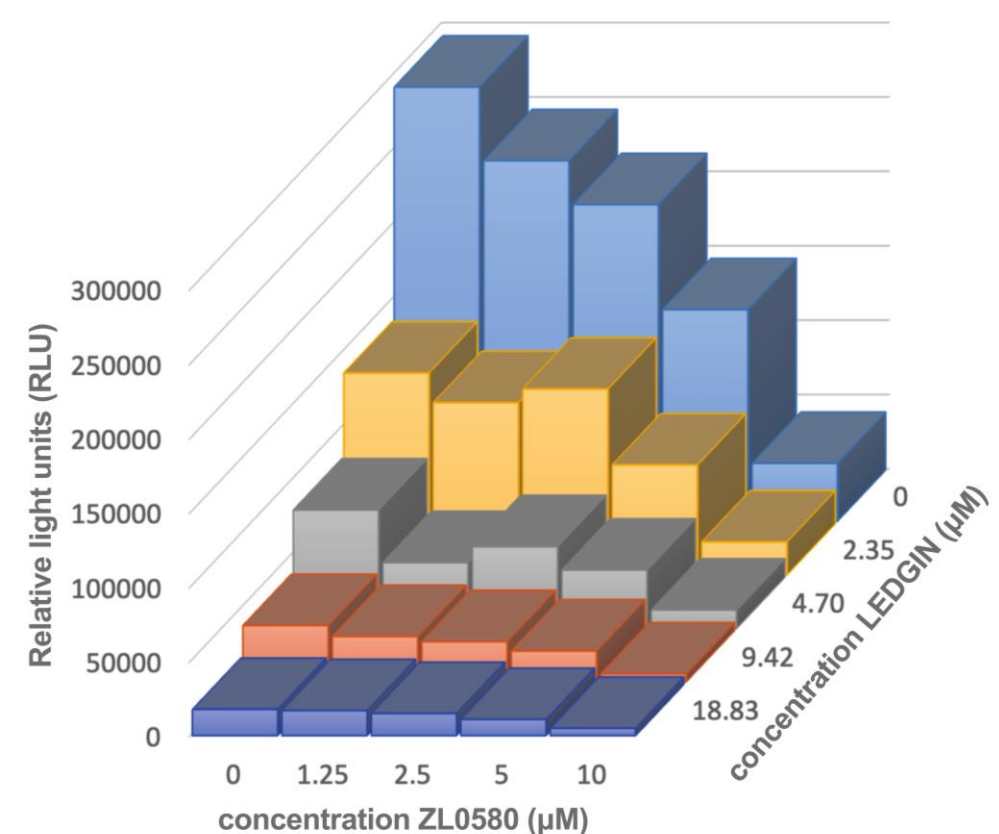
Blocking activity

Luciferase reporter assay of unreactivated cells



Locking activity

Luciferase reporter assay of reactivated cells (10 ng/mL TNF- α)



Conclusions

JQ1 shows additive effect in locking activity with LEDGINs

ZL0580 shows additive effect in blocking and locking activity with LEDGINs

