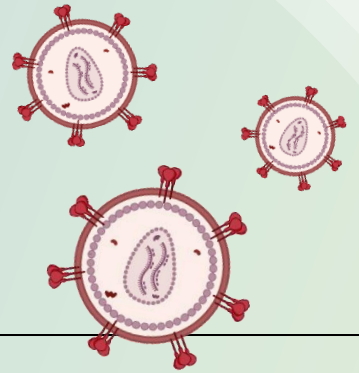

Potent latency reversal by Tat RNA-containing nanoparticle enables multi-omic analysis of the HIV-1 reservoir

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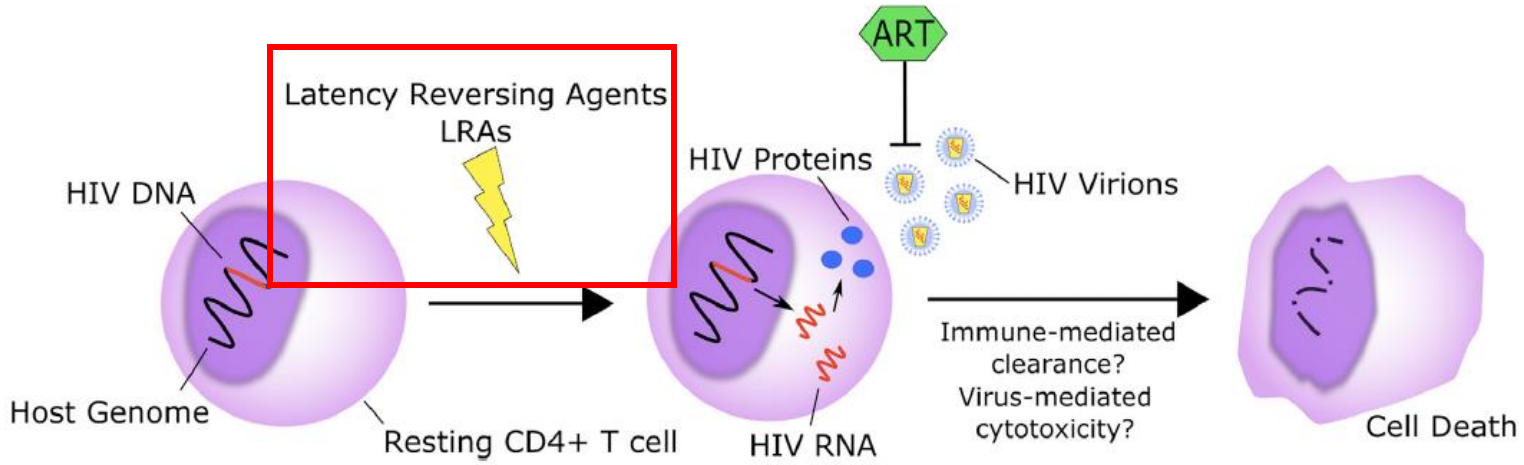
Nature Communications volume 14, Article number: 8397 (December 2023) doi: 10.1038/s41467-023-44020-5

Shock and kill strategy

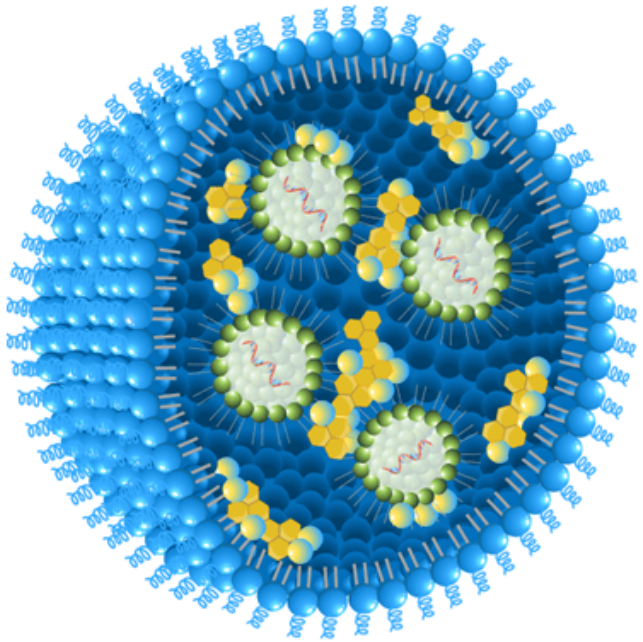


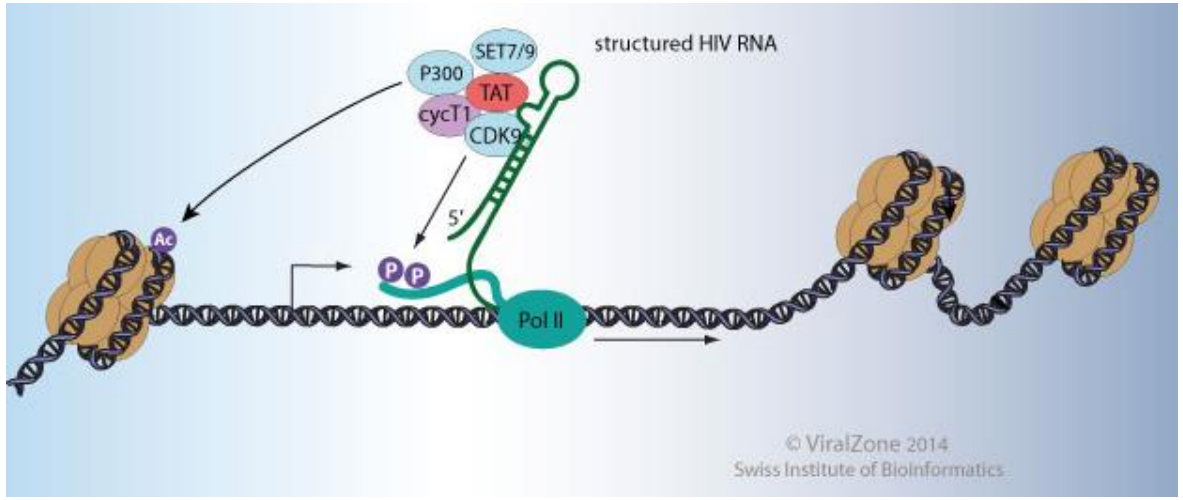
Mitogens	Other classes of LRAs
PMA, PHA, CD3/CD28	HDACi, PKC agonists, etc
Gold standard for *in vitro* assays	Not as potent as mitogens to reactivate HIV
Highly toxic → Not in the clinic	Under evaluation in clinical trials
Induces global T cell activation	Some classes do not induce global activation

Identifying compounds that reactivate HIV efficiently **without modifying the phenotype of the cells** is of interest to study the profile of latently infected cells

Tat-LNP

Tat mRNA containing nanoparticle

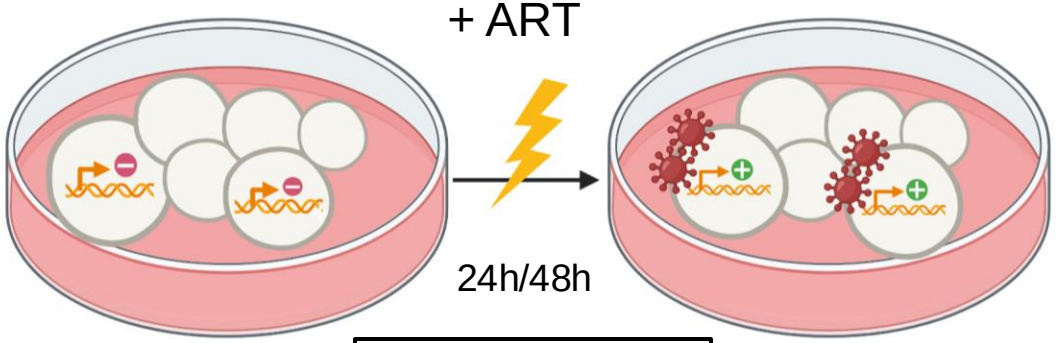




- Regulates HIV transcription
- Mediating the switch between active and latent infection

Hypothesis: delivering exogenous Tat via Tat mRNA-containing nanoparticles (Tat-LNP) could specifically and robustly reactivate latent HIV while preserving the cellular phenotype

Assessing the reactivation capacity of Tat-LNP



+ ART

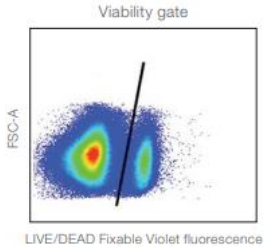
24h/48h

Tat-LNP:1.4nM
PNB: 50nM
Tat-LNP/PNB

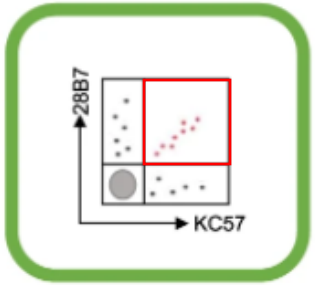
PMA 162nM/
Iono 1ug/mL

PNB = Panobinostat
(HDAC inhibitor)

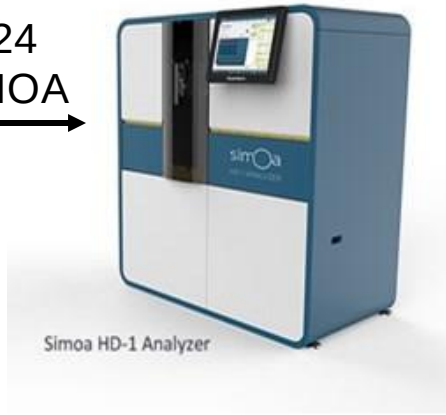
Cell viability



HIV-Flow



P24 SIMOA



Viability of CD4 T cells

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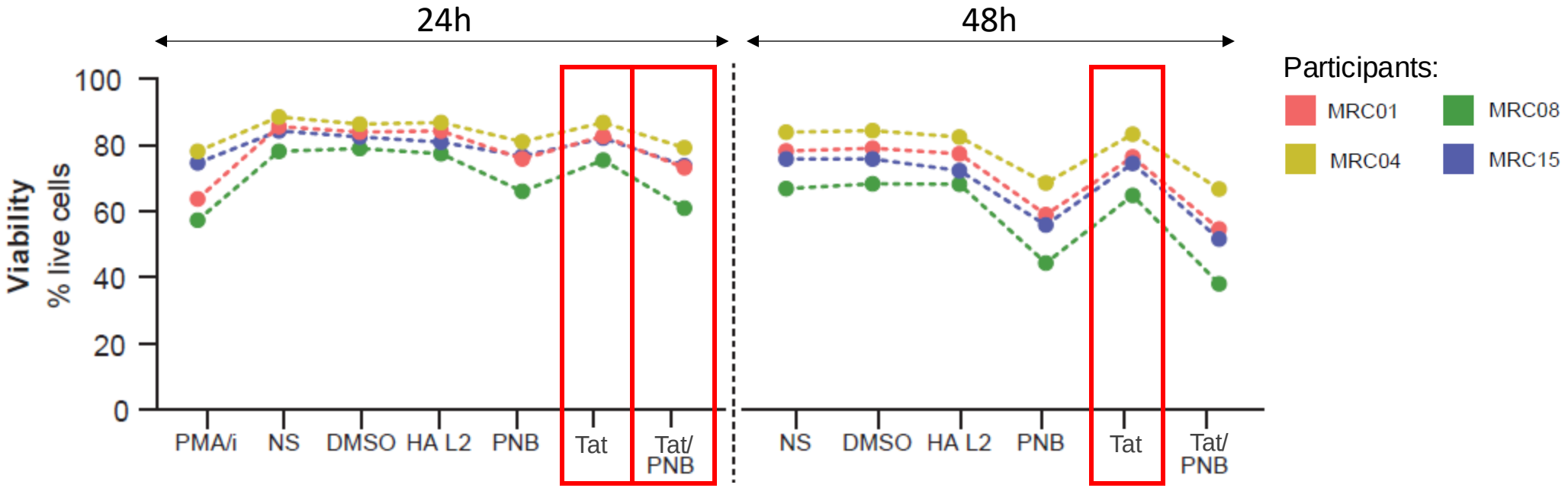

- Tat-LNP does not affect cell viability (both at 24h and 48h)
- Tat-LNP/PNB is associated with limited cell death at 24h post-stimulation

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HIV-Flow: frequency of p24+ cells following latency reversal

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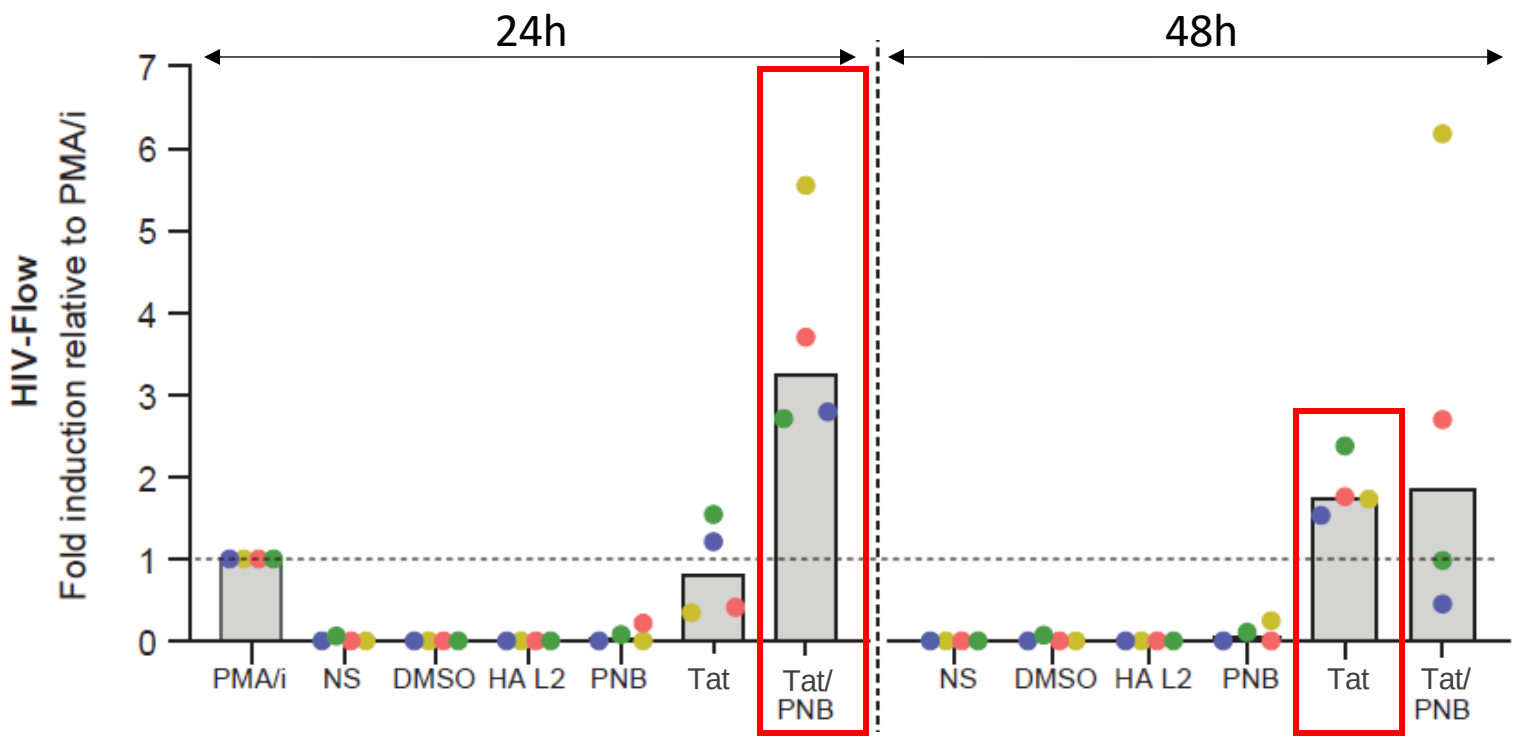
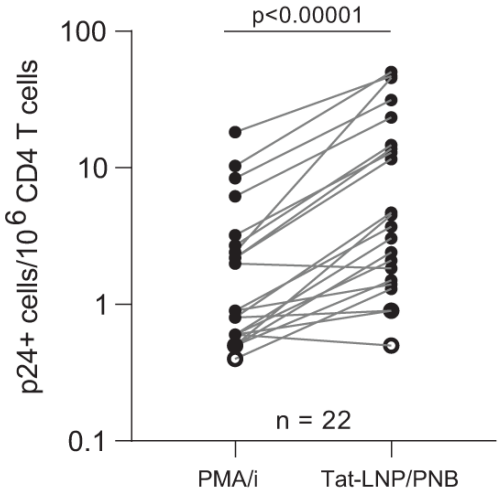


Participants:

MRC01 MRC08

MRC04 MRC15



➢ The highest fold induction relative to PMA/i is observed at 24h post-stim with the combination Tat-LNP/PNB

SIMOA: p24 release in the supernatant following latency reversal

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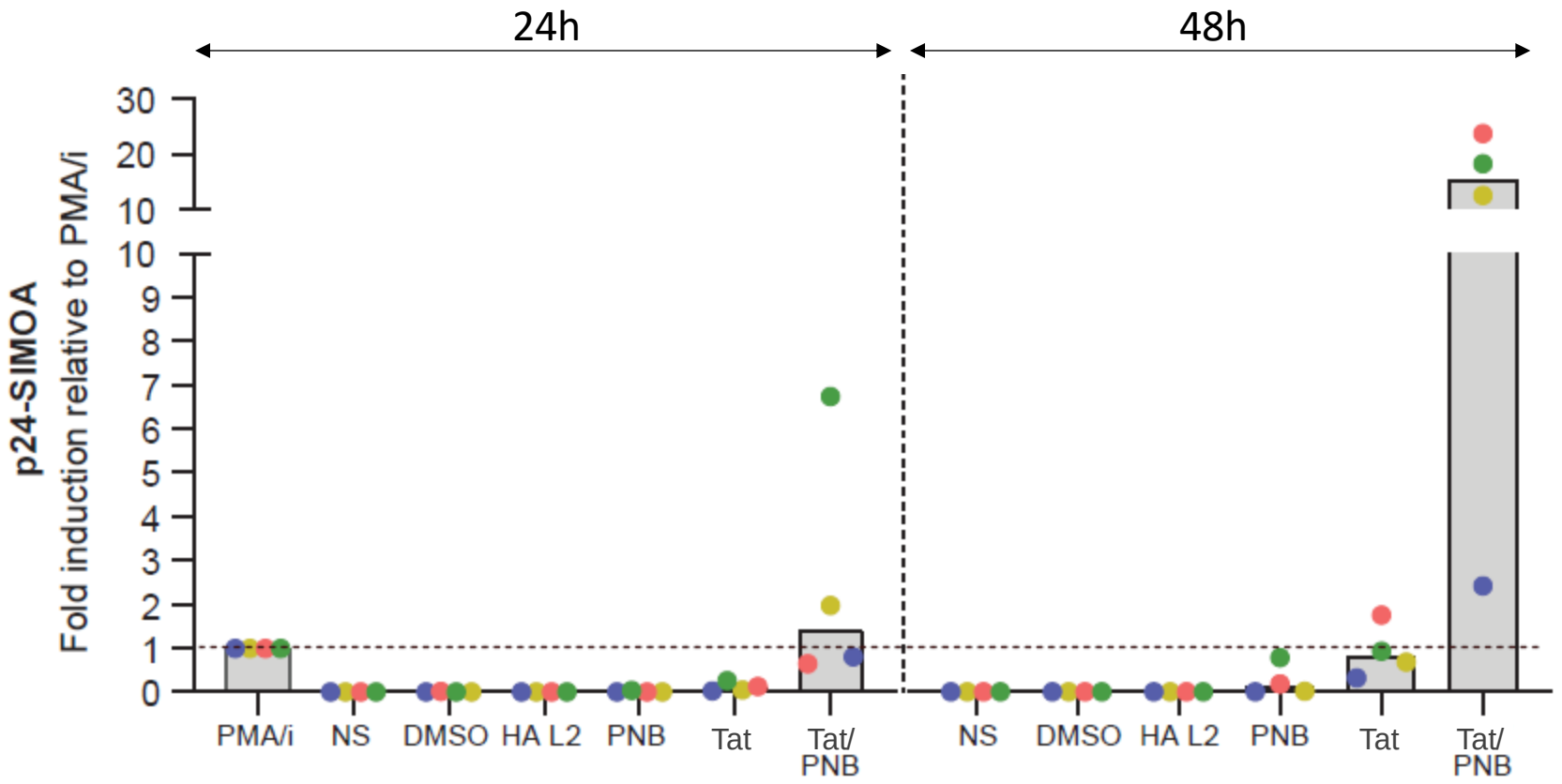

Participants:

MRC01 MRC08

MRC04 MRC15

➢ Stimulation with Tat-LNP alone or in combination with panobinostat leads to viral particles release in the culture supernatant

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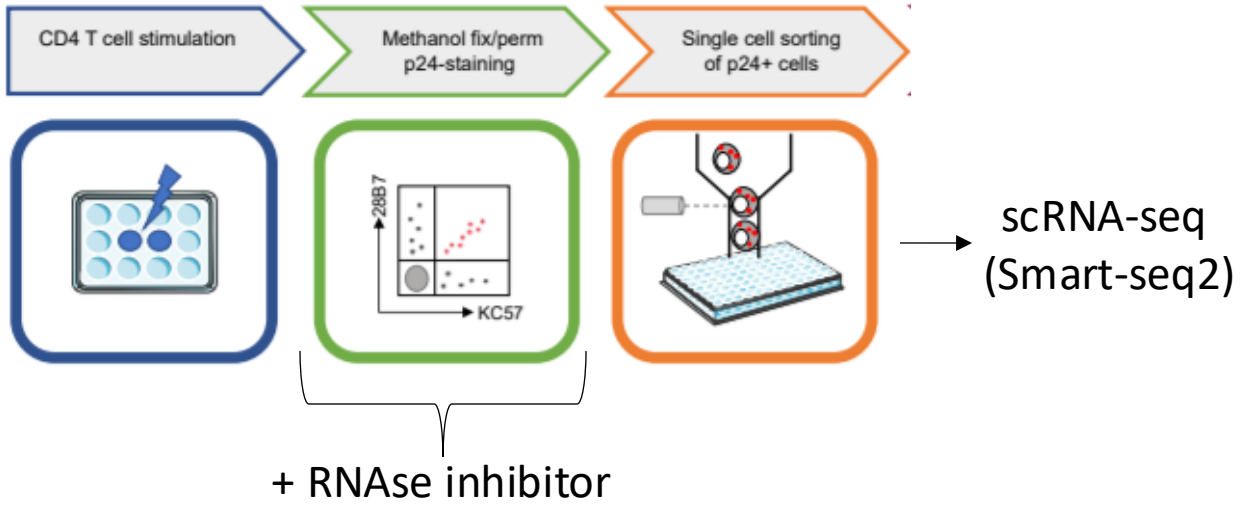
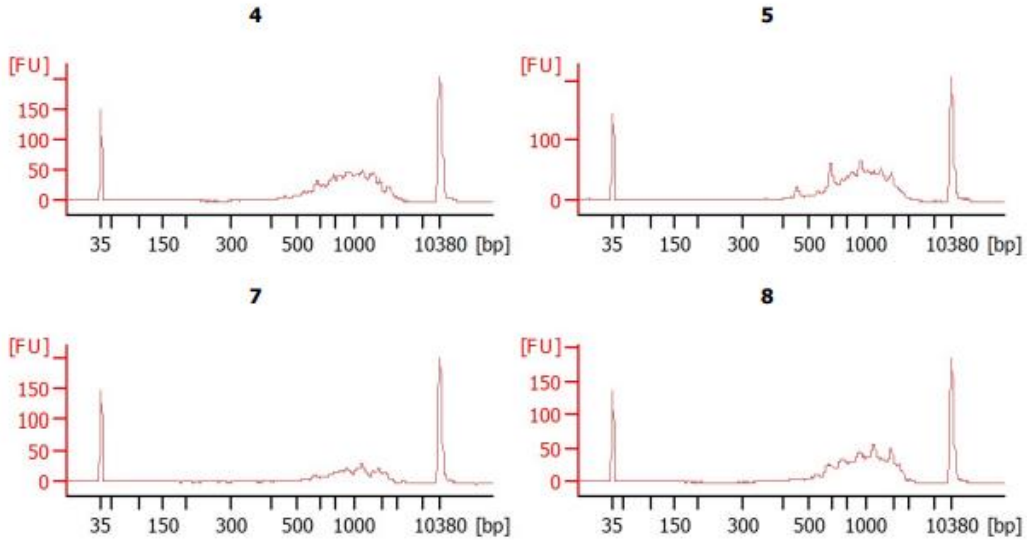
Transcriptomic analyses of p24+ cells





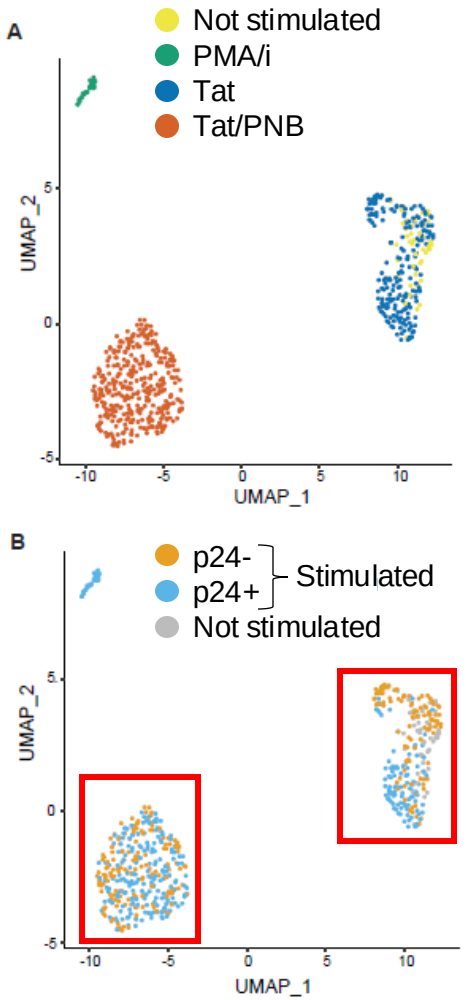
- Tat-LNP: 108 p24+ cells
- Tat-LNP/PNB: 212 p24+ cells
- PMA/i: 28 p24+ cells

348 p24+ cells

- **+ 309 p24- cells (CD45RO+)**

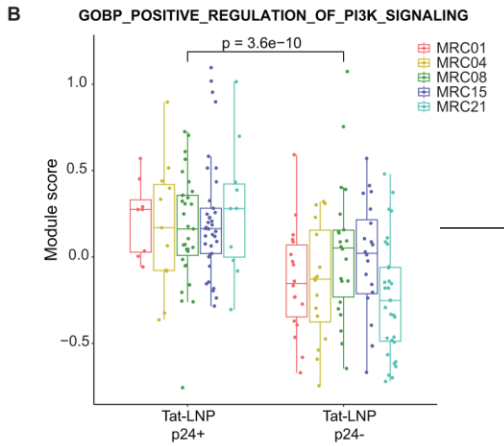
N =7 ART-treated individuals

Transcriptomic analyses of p24+ cells

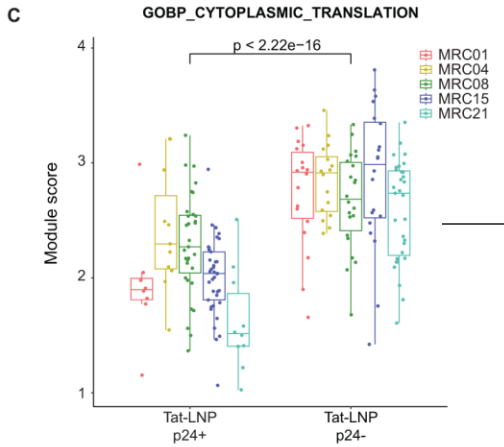


- P24+ cells display a distinct transcriptional landscape compared to p24- cells
- 6 DEG between p24+ and p24- cells: 4 upregulated, 2 downregulated in p24+ vs p24-

Gene set enrichment analysis



→ Favors cell survival in p24+ cells?

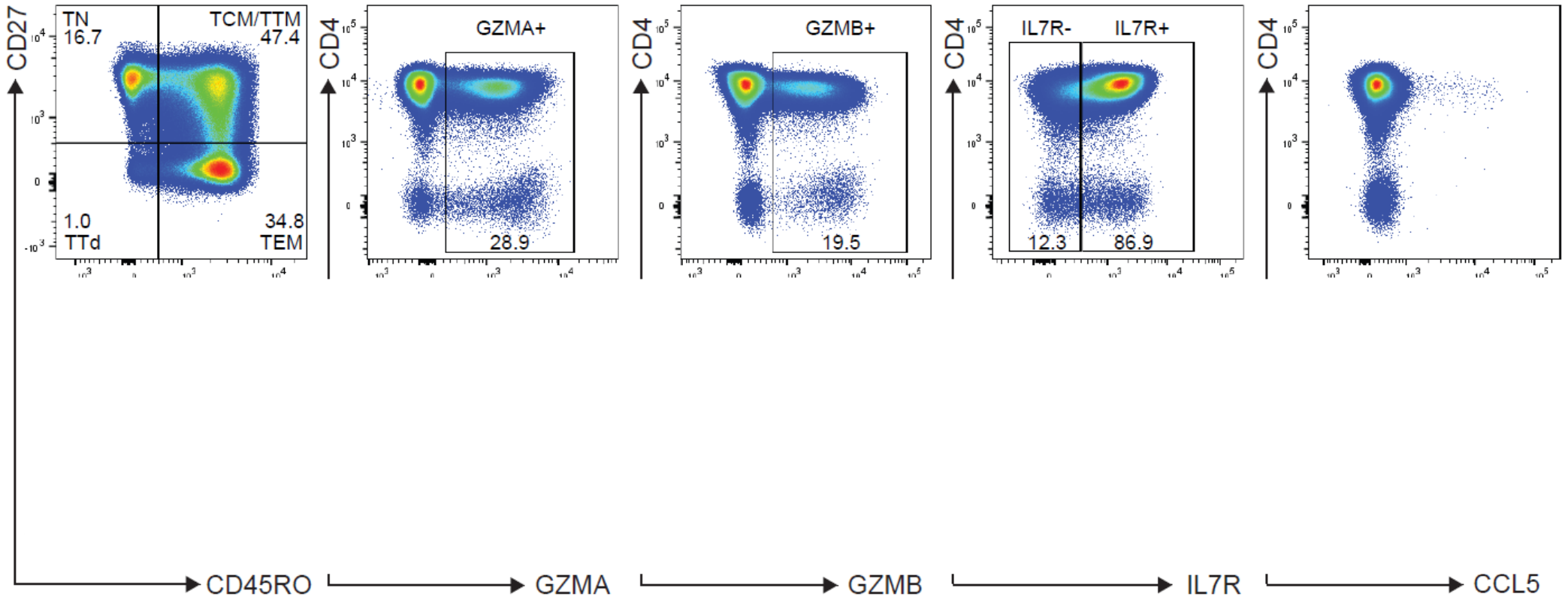


→ Promotes latency in p24+ cells?

- heightened PI3K/Akt signaling, with downregulation of protein translation in p24+ cells

→ HIV-infected cells display distinct signatures that facilitate their long-term persistence

Confirmation of the transcriptomic hits at the protein level



Panel
CD3/CD4
CD45RO
CD27
GZMA
GZMB
IL7R
CCL5
P24 (1)
P24 (2)

➢ Stimulation with Tat-LNP alone does not modify the expression of the markers of interest

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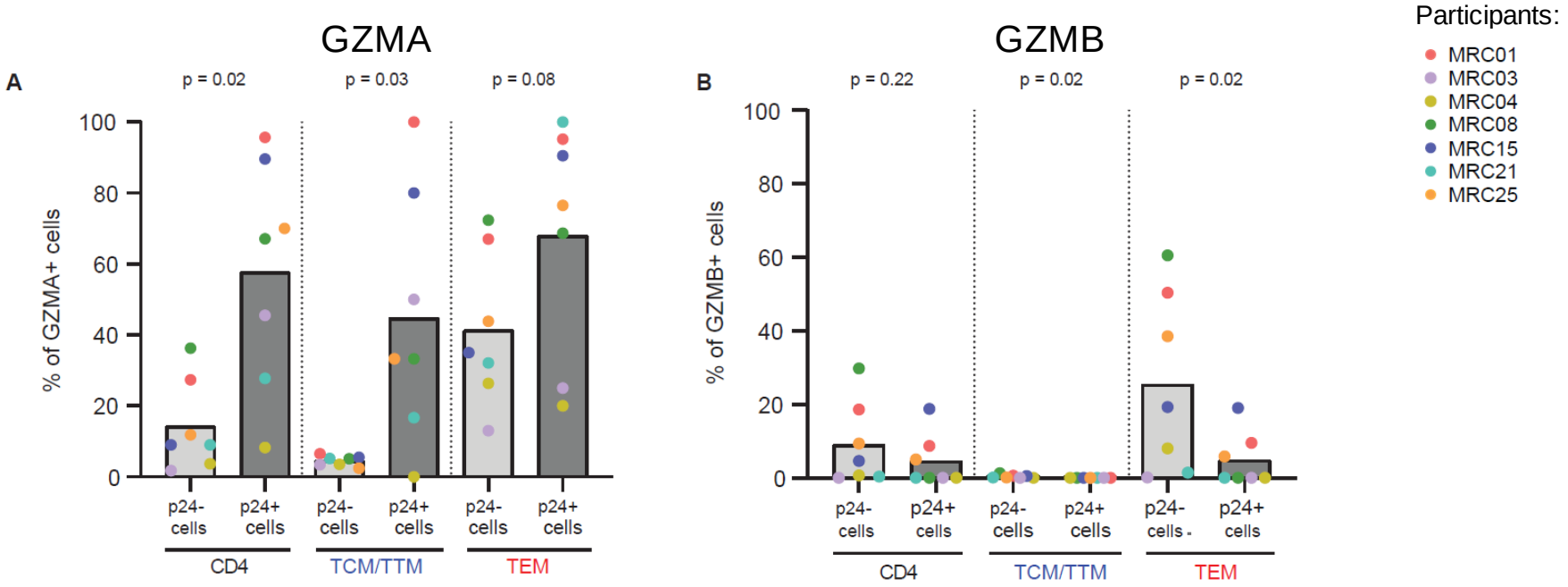
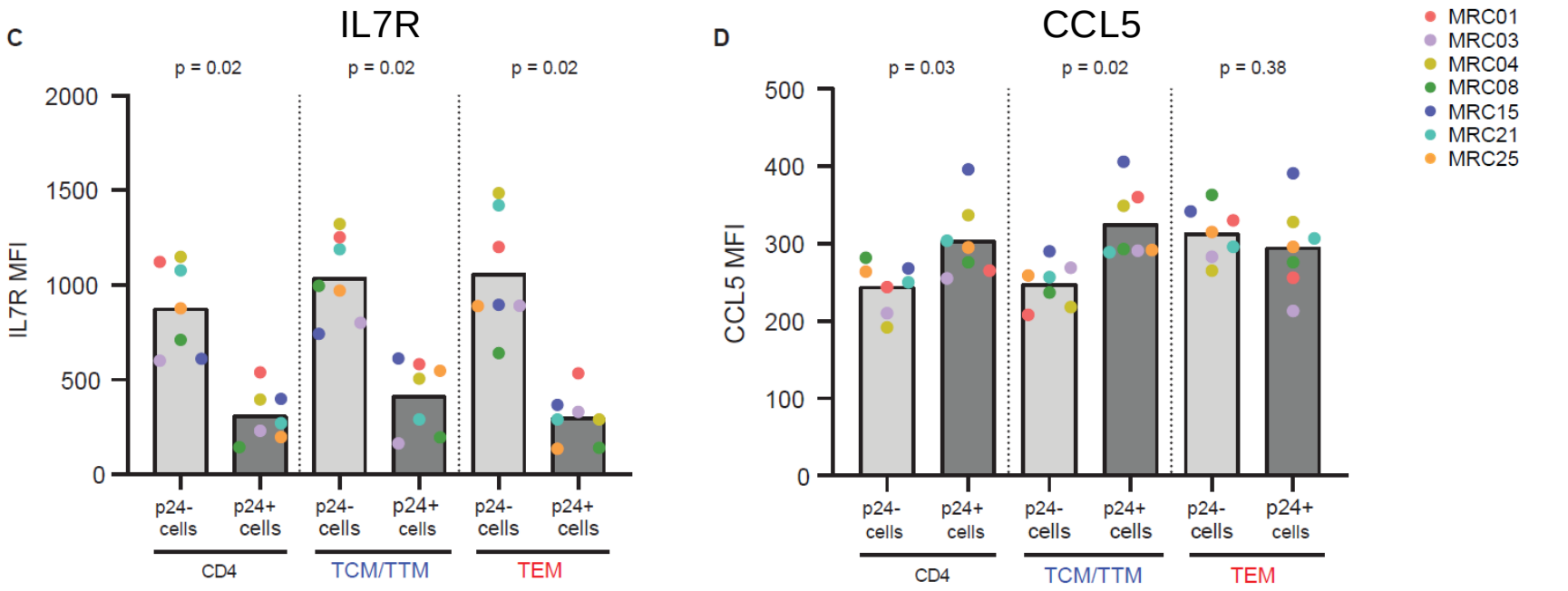
The transcriptomic hits are confirmed at the protein level



- The percentage of GZMA+ cells is higher in p24+ cells compared to p24- cells
- The percentage of GZMB+ cells is lower in p24+ cells compared to p24- cells

The transcriptomic hits are confirmed at the protein level



- IL7R expression is downregulated in p24+ cells compared to p24- cells
- CCL5 expression is upregulated in p24+ cells compared to p24- cells

Conclusions

- Tat-LNP:
  - Reactivates HIV from latency
  - Does not impact cell viability *in vitro*
  - Does not modify the transcriptome of CD4 T cells

- Tat-LNP in combination with panobinostat induces latency reversal in a higher proportion of latently infected cells than PMA/i

- Tat-LNP can be used as a tool to uncover transcriptomic and proteomic features of the translation-competent HIV reservoir
  - Allowed the identification of 6 DEG in p24+ cells compared to p24- cells
  - 3 out of the 6 hits were confirmed at the protein level
  - Identification of potential novel therapeutic targets, such as the long non-coding RNA *LINC02964*

Acknowledgements

HIV Cure Research Center

HCRC
Basiel Cole
Laurens Lambrechts
Sofie Rutsaert
Ytse Noppe
Tine Struyve
Nele de Langhe
Sarah Gerlo
Linos Vandekerchove

Janssen
Filmon Eyassu
Erik Nijs
Ellen Van Gulck
Daniel Boden

Arcturus Therapeutics
Jerel Vega
Jinho Park

Liège university
Anne Van den Broeke
Jerome Wayet

All the participants from the study!

AGENTSCHAP INNOVEREN & ONDERNEMEN

UNIVERSITEIT GENT

Janssen

Flow cytometry and sequencing cores from Ghent and Janssen
Nurses, doctors and leukapheresis core from UZ Ghent