

Optimization of Lipid Nanoparticle-based CRISPR-activation to Improve HIV Latency Reversal

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Background

- We previously harnessed the inherent sequence specificity of **CRISPR activation (CRISPRa)** to induce HIV transcription ex vivo but observed **incomplete proviral reactivation**.
- This may be related to **poor expression of the CRISPRa** construct in **primary CD4+ T cells**.
- Here, we **optimised CRISPRa in a lipid nanoparticle (LNP)-based** delivery system to **improve its activity**.

1. Altering mRNA translation directly or indirectly does not improve dCas9 expression in resting CD4+ T cells.

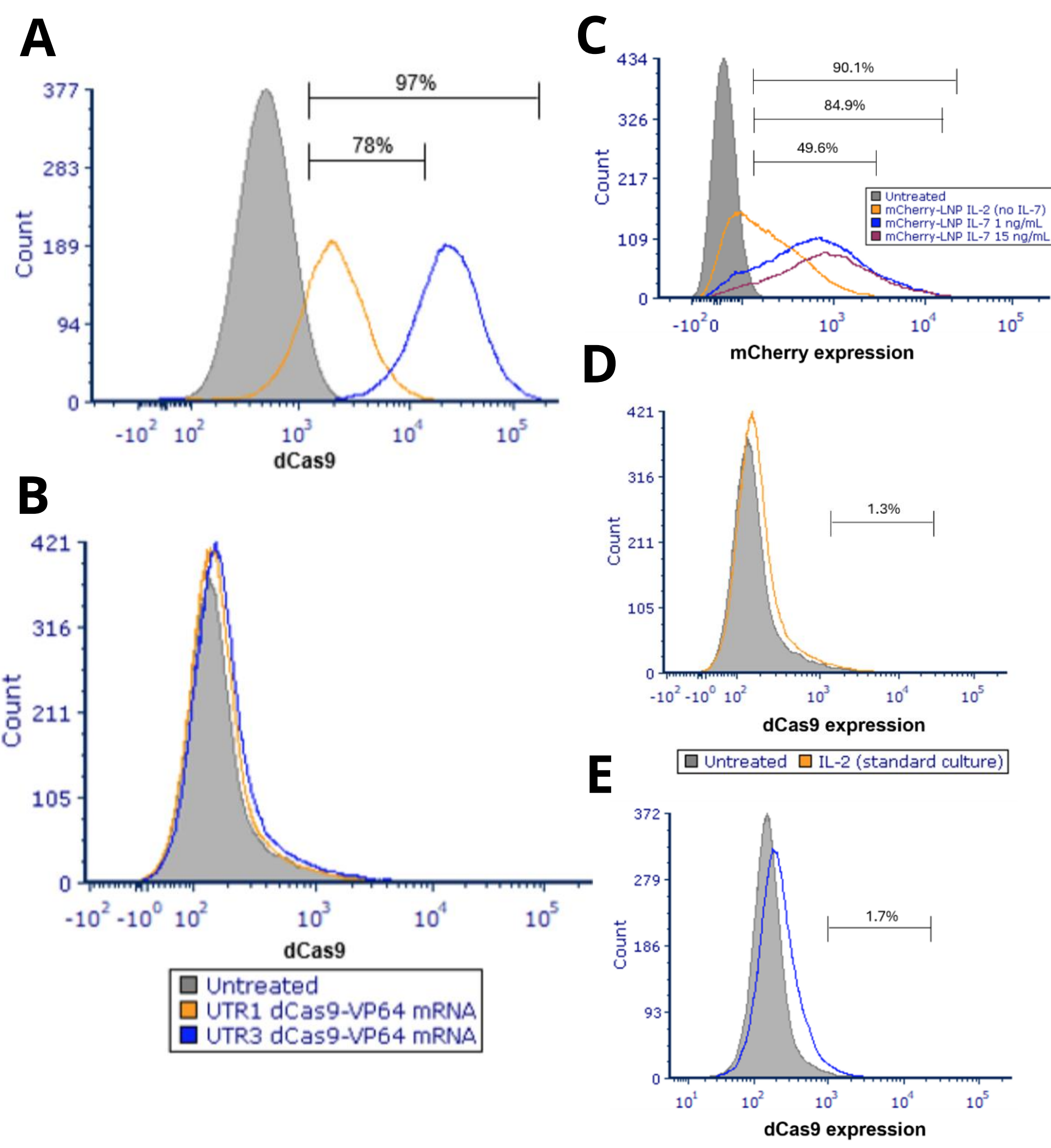


Figure1. (A) dCas9 expression in Jurkat cells (24h) and (B) resting primary CD4+ T cells (72h) following transfection with CRISPRa SAM LNP encapsulating dCas9-VP64 mRNA with either UTR3 or UTR1. (C) mCherry expression in unstimulated CD4+ T cells either 1ng/mL or 15ng/mL of IL-7 culture (72h). (D, E) dCas9 UTR3 expression in IL2 or 1ng/mL of IL7 culture (72h). Expression determined by intracellular staining and flow cytometry.

Larger mRNA size might be the cause of reduced mRNA expression possibly related to mRNA delivery.

3. CD7-targeted CRISPRa SAM LNP does not reactivate HIV reservoir ex vivo.

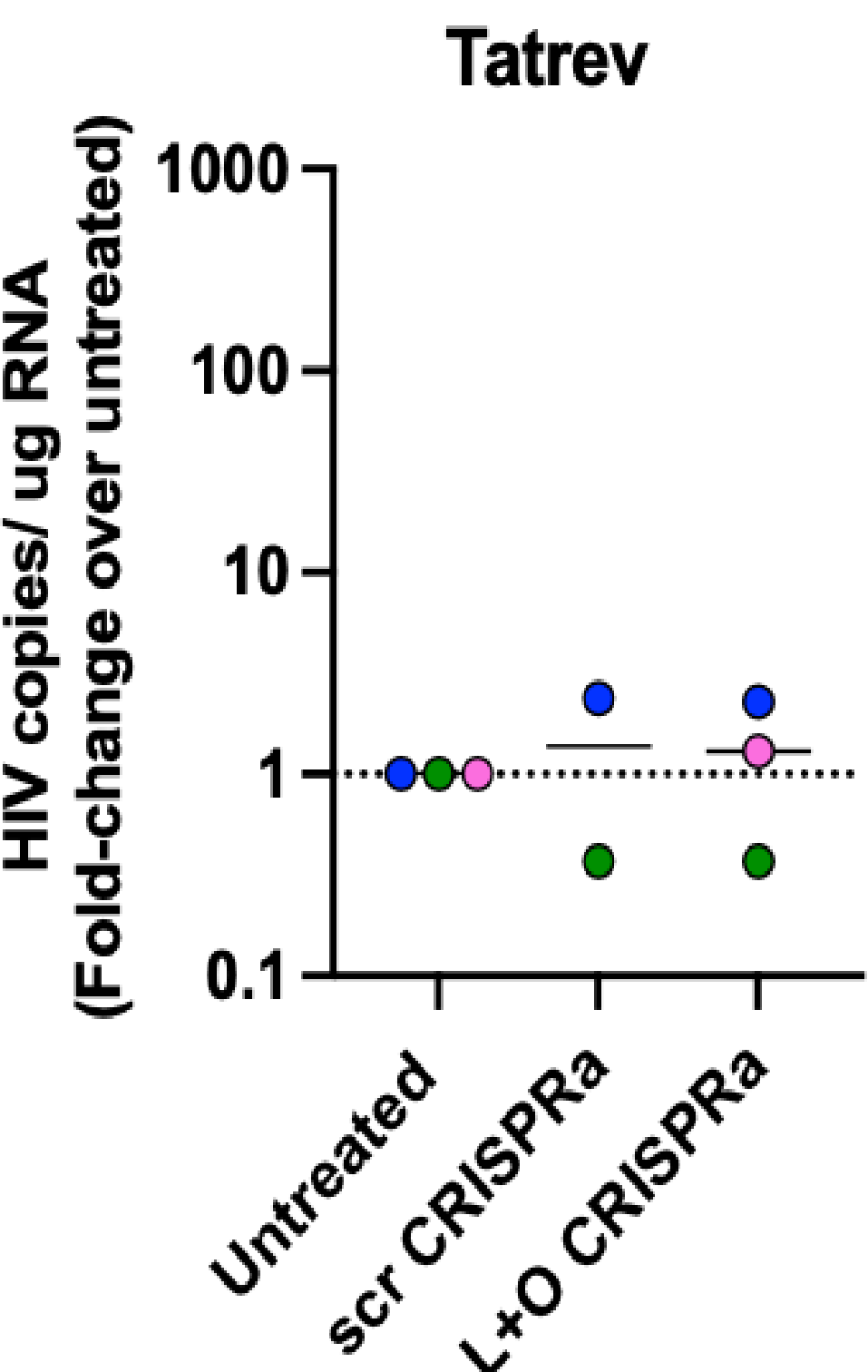
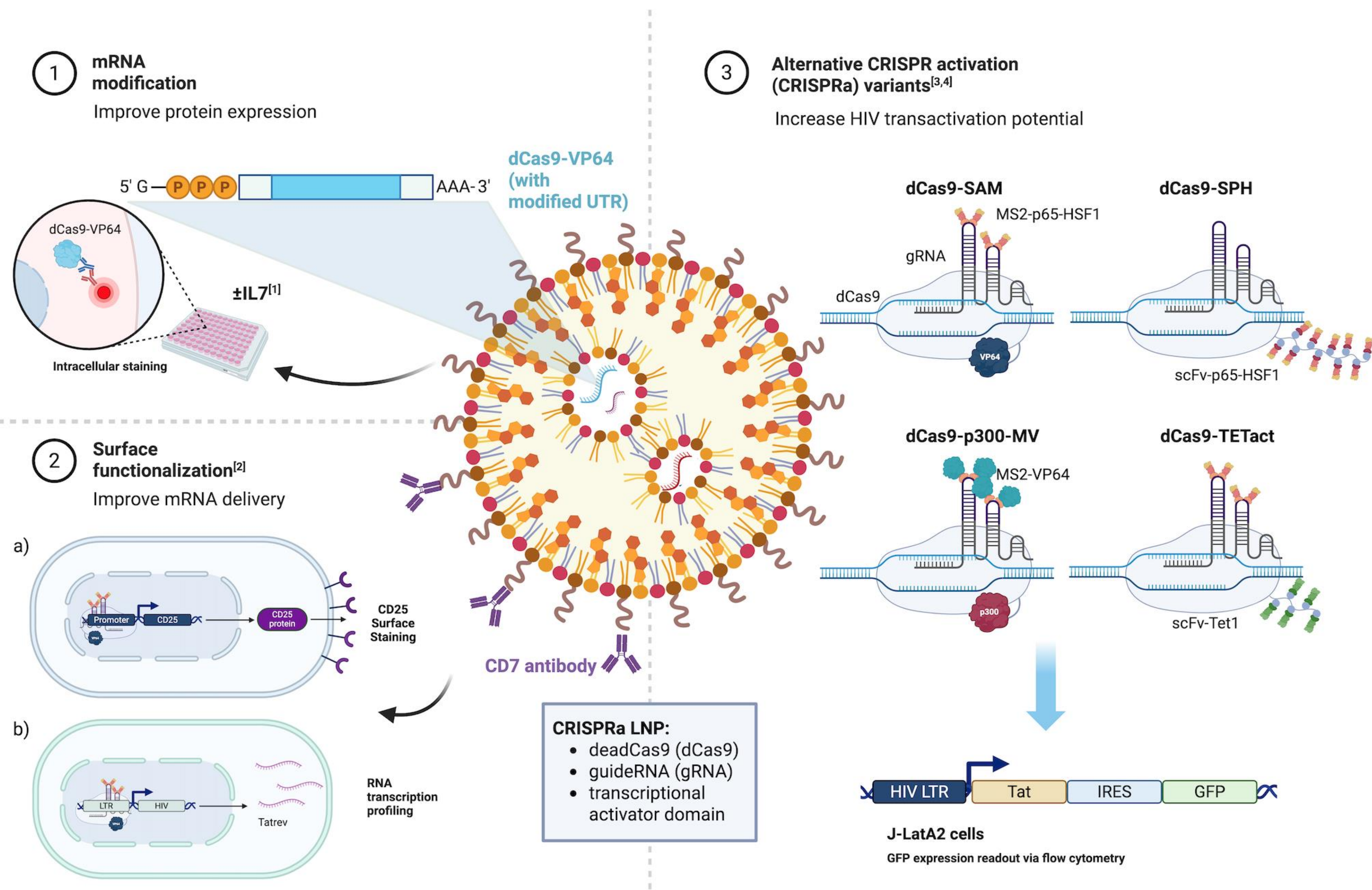


Figure3. Measurement of Tatrev multi-spliced transcripts in isolated CD4+ T cells from people living with HIV (PLWH) transfected with CD7-conjugated CRISPRa SAM LNP for 72h.

Comparison of CRISPRa SAM LNP encapsulating either HIV LTR-targeting gRNAs (L and O) or non-targeting gRNA (scr) by RNA profiling assay.

A more potent CRISPRa variant is needed to induce HIV transcription.

Aims & Methods



2. CD7-targeted CRISPRa SAM LNP enhances activity in non-stimulated primary CD4+ T cells.

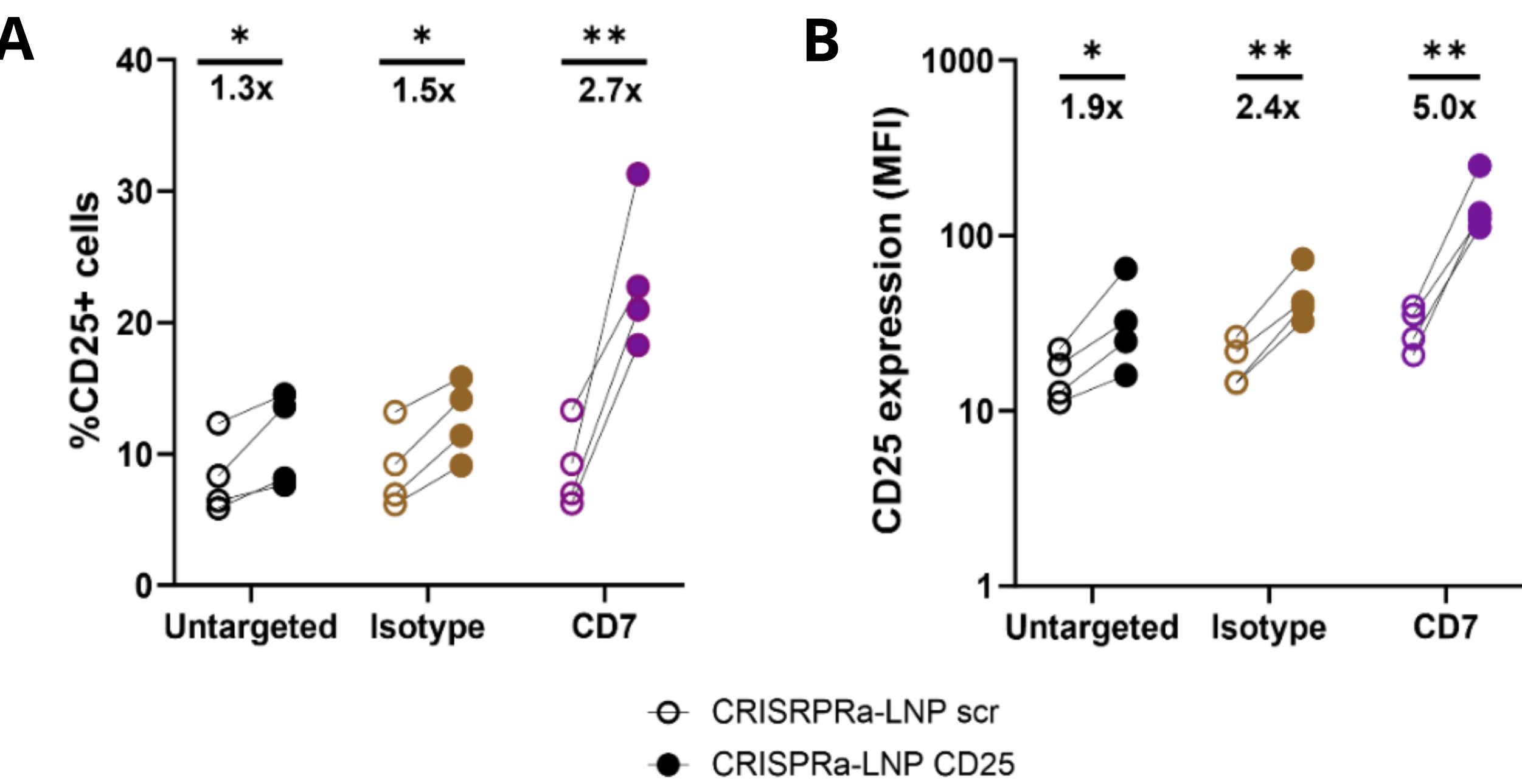


Figure2. (A) Percentage of CD25 surface expression and (B) Median fluorescence intensity (MFI) of CD25 expression in resting primary T cells transfected with either untargeted or isotype or CD7-targeted CRISPRa SAM LNP (6 days). *p<0.05, **p<0.01 by ratio paired t-test (n=4).

Comparison of CRISPRa SAM LNP encapsulating **dCas9-VP64 (UTR3)**, MS2-p65-HSF1 and either CD25-targeting gRNA or non-targeting scr gRNA by surface staining and flow cytometry.

CD7 surface functionalization improve CRISPRa potency hence we want to further test CRISPRa activity ex vivo.

4. Alternative CRISPRa SPH variant improve LTR-mediated reactivation in J-LatA2 cells.

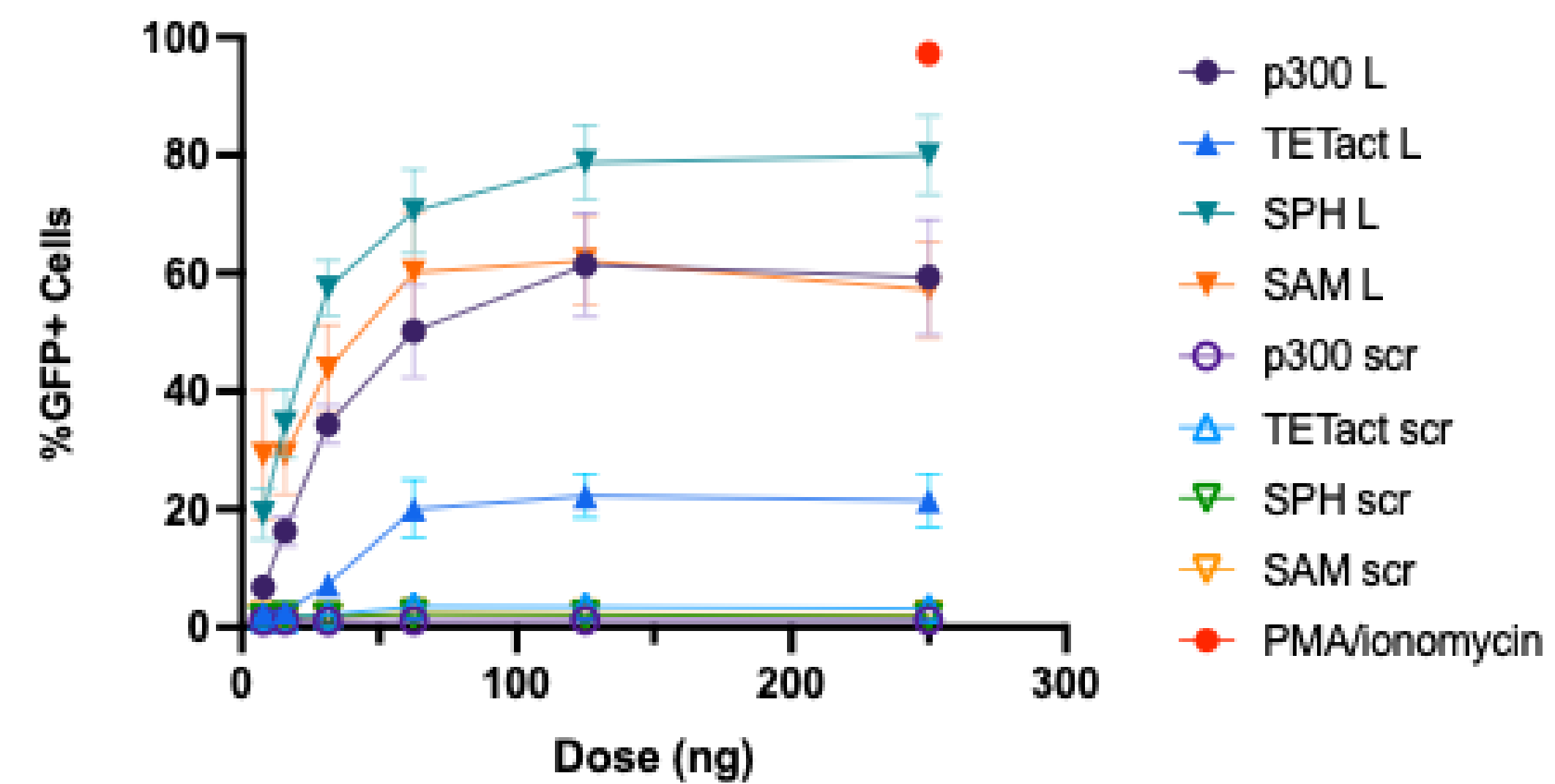


Figure4. HIV reactivation in J-LatA2 cells measured via GFP expression. J-LatA2 cells were treated with CRISPRa LNP (p300 or TETact or SPH or SAM) with HIV LTR-targeting gRNA (indicated as L) or non-targeting gRNA (indicated as scr) for 24h. PMA/ionomycin as positive control. GFP expression determined by flow cytometry.

CRISPRa SPH induce higher level of HIV reactivation possibly due to increase recruitment of transcriptional activators rather than epigenetic modification (p300 or TETact).

Conclusion

CRISPRa SAM activity is improved through alteration in mRNA and use of CD7-targeted LNPs. However, the improved system does not reactivate HIV ex vivo.

CRISPRa variant SPH LNP reactivates HIV better than CRISPRa SAM LNP in cell lines.

Further work to explore:

- Use of **CD7-conjugated CRISPRa SPH LNP** in isolated CD4+ T cells from PLWH for HIV reactivation.
- smaller Cas9 orthologs** from different bacteria species for better translation in primary T cells.



A joint venture between The University of Melbourne and The Royal Melbourne Hospital

References

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