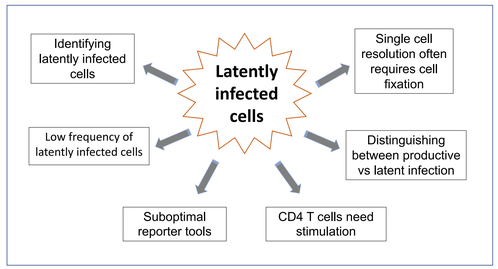
$CD4^+$ T cell subset-specific differences in HIV latency establishment

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BACKGROUND

- HIV integration into genome creates a long-lived reservoir
- Lifelong combination antiretroviral therapy (cART) suppresses replication **but does not eradicate the virus**
- It is unclear if all the $CD4^+$ T cell subpopulations are equally susceptible to latency establishment
- Efficient reporter tools are necessary to identify infected cells to investigate the molecular mechanisms of HIV latency establishment



METHODS

To dissect the molecular mechanisms of latency establishment at the single $CD4^+$ T cell level:

- Infect primary human $CD4^+$ T cells with pMorpheus-V5
- Determine frequency of LTR-active and LTR-silent HIV infection in $CD4^+$ T cell and different CD4+ T cell subsets at a single-cell level by multi-dimensional analysis
- Sort LTR-actively, and LTR-silently infected cells to measure provirus, and transcription by FACS

TAKE HOME MESSAGES

- **pMorpheus-V5 is a Δ*Env* dual-reporter HIV vector encoding all accessory proteins that allows the identification of latency in primary human $CD4^+$ T cell populations.**
- **LTR-silent (latent) infections are as frequent as LTR-active (productive) infections.**
- **T_{EM} and T_{REG} have the highest level of latent infection.**
- **T_N are most prone to harboring latent proviruses**

Fig 2. LTR-silent and LTR-active infection of $CD4^+$ T cells

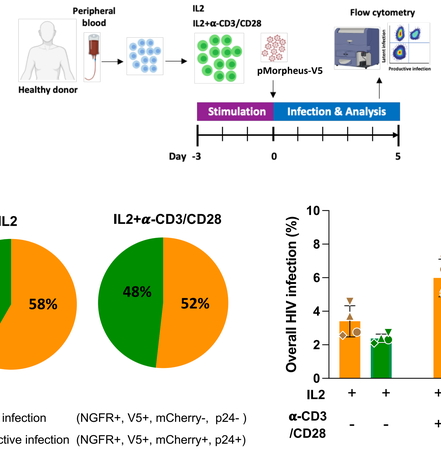


Fig 4. Identify seven $CD4^+$T cell subpopulations by multi-dimensional analysis

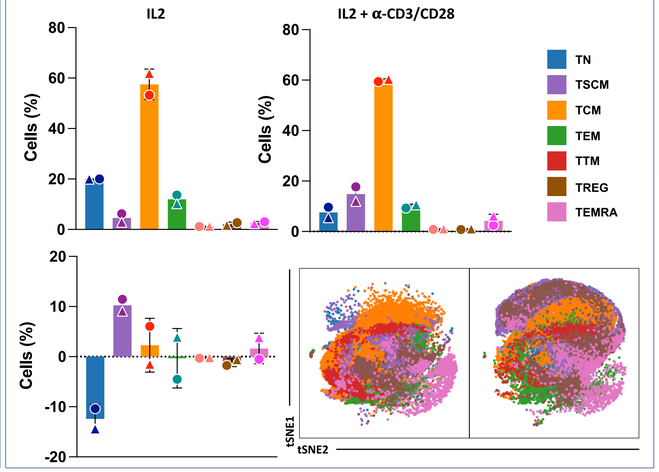


Fig 6. More frequent LTR-active infection of activated T cells

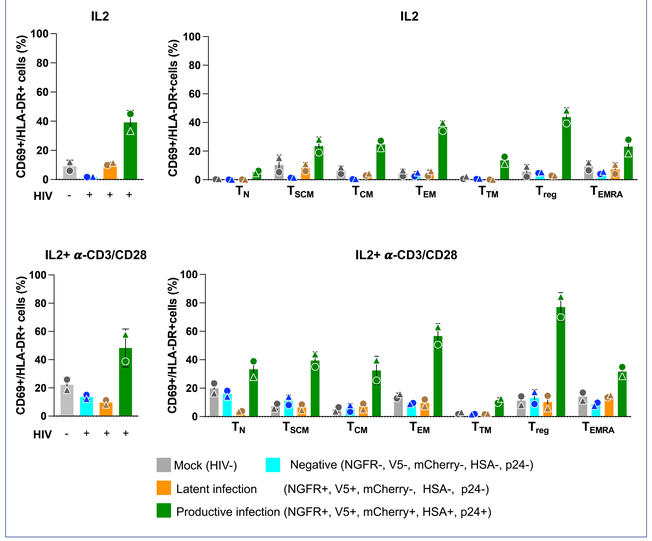


Fig 1. Map of pMorpheus-V5 (pLAI2 V5-NGFR HSA-mCherry)

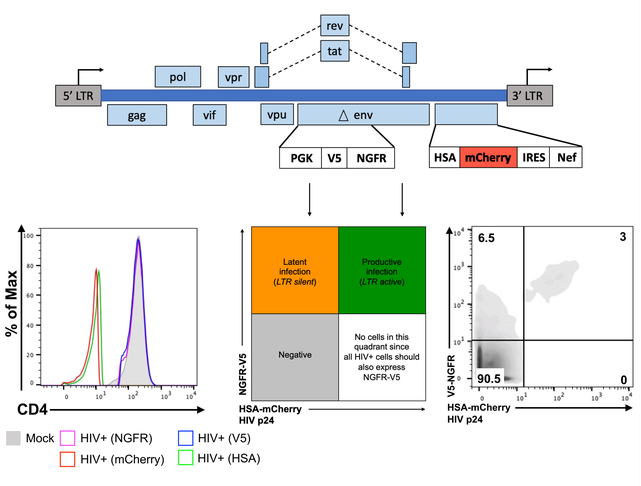


Fig 3. Latently infected cells harbor provirus, but not HIV transcripts

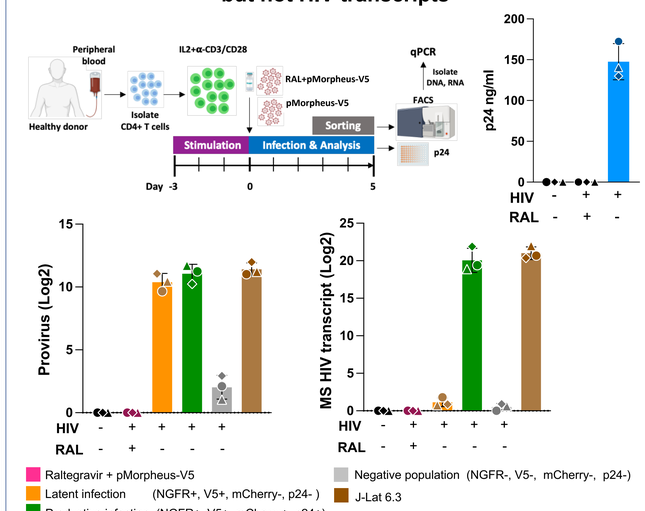
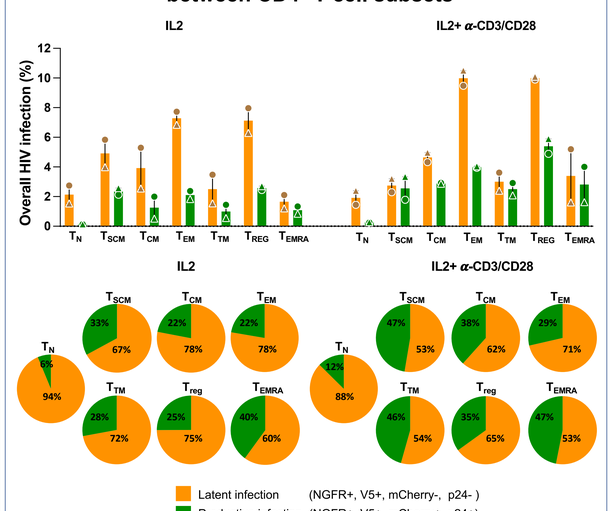


Fig 5. LTR-active or LTR-silent infection differs between $CD4^+$ T cell subsets



CONCLUSIONS

We developed a dual-reporter HIV, pMorpheus-V5

- Minimal promoter interference between LTR-dependent and LTR-independent promoters
- *Nef* down-regulated CD4 expression
- Confirmed LTR-silent infection by two assays
- LTR-silent $CD4^+$ T cells harbor provirus that is not transcribed

pMorpheus-V5 dual-reporter HIV distinguishes latently and productively infected CD4 subsets at the single-cell level

- More than 50% of infected cells harbor LTR-silent proviruses in all $CD4^+$ T cell subpopulations
- T_{EM} and T_{REG} had the highest level of latent infections

Activation alters the distribution of 7 distinct $CD4^+$ T cell subsets from two donors

- T_{SCM} subsets increased from 6% in IL2 to 18% when treated with IL2+α-CD3/CD28
- T_{REG} decreased from 3% in IL2 to 1% in IL2+ α-CD3/CD28

LTR-active infection occurs more frequently in activated T cells