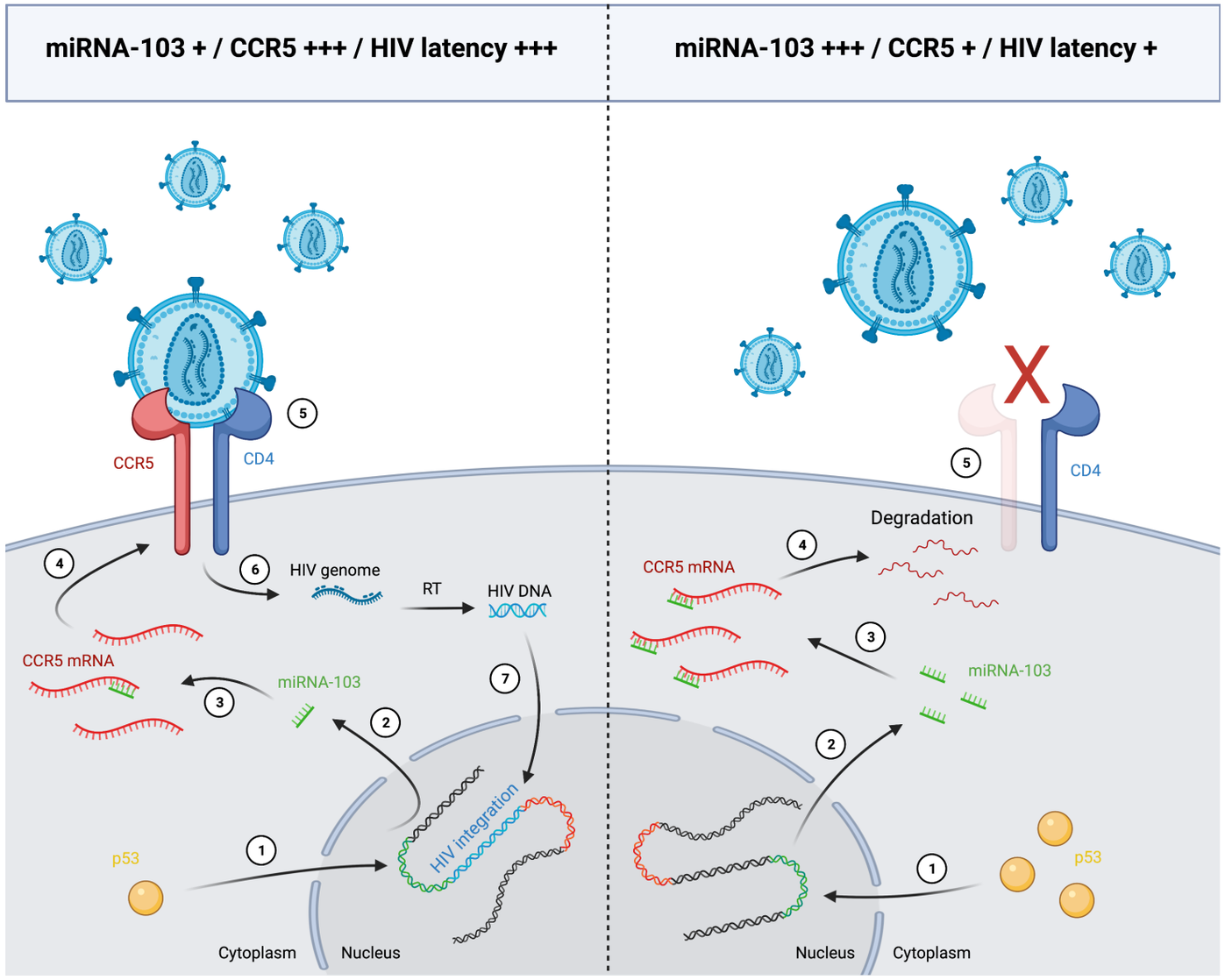
INVESTIGATING THE ROLE OF miRNAs DURING HIV-1 INFECTION OF CD4+ T LYMPHOCYTES



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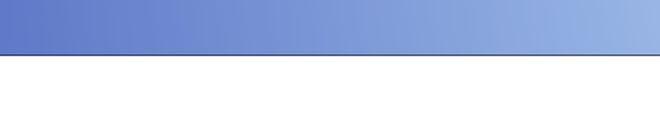
Investigating the role of miRNAs during HIV-1 infection of CD4+ T lymphocytes

Background

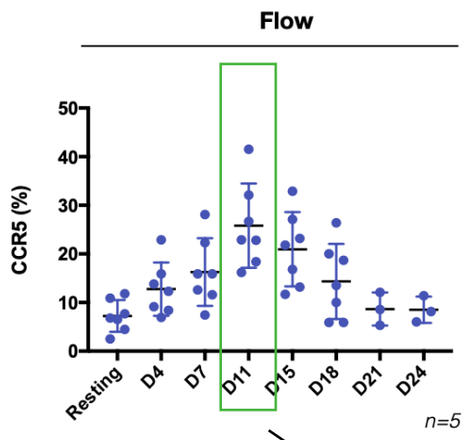
- Shan et al. (Immunity, 2017) suggests that latency in lymphocytes is a consequence of CD4+ T cells infected in a very narrow window following their activation. These effector-to-memory transitioning lymphocytes (CD4+ EMT T cells) are unique in that they have a transient increase in CCR5 expression, but possess a transcriptional state not conducive to HIV-1 expression.
- MiRNAs are small regulatory RNAs which decrease mRNA stability or inhibit mRNA translation.
- We recently identified miRNA-103 as modulators of CCR5 expression in macrophages (Lodge et al. 2020).

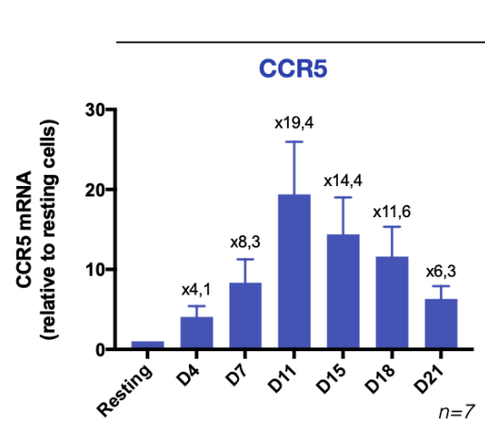
1 - Modulation of CCR5 and miRNA-103 in primary CD4+ T cells

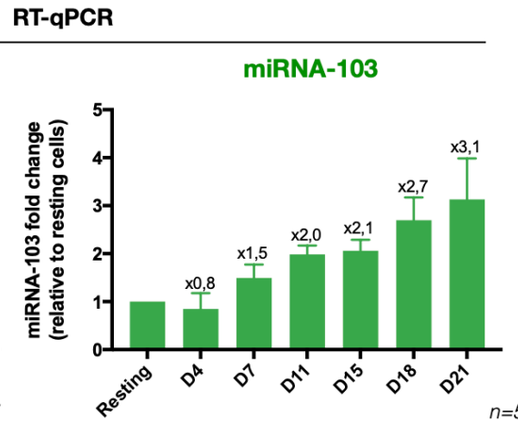
Strategy



Every 3-4 days :
• CCR5 expression
• miRNA-103 expression

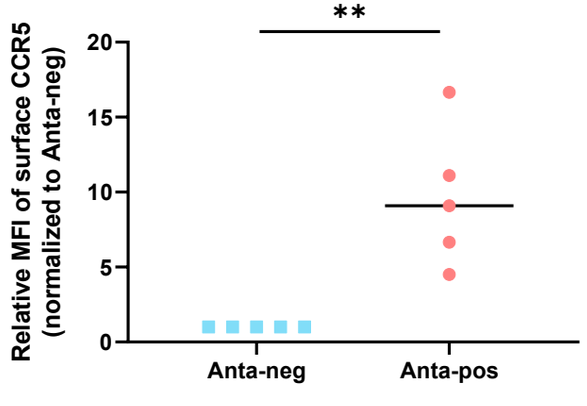






➠ Transfection of miRNA-103 antagomir into CD4+ EMT T cells (D11) prevents the decrease in CCR5, showing that this miRNA plays a role in the modulation of CCR5.

Transfection - Antagomir miRNA-103



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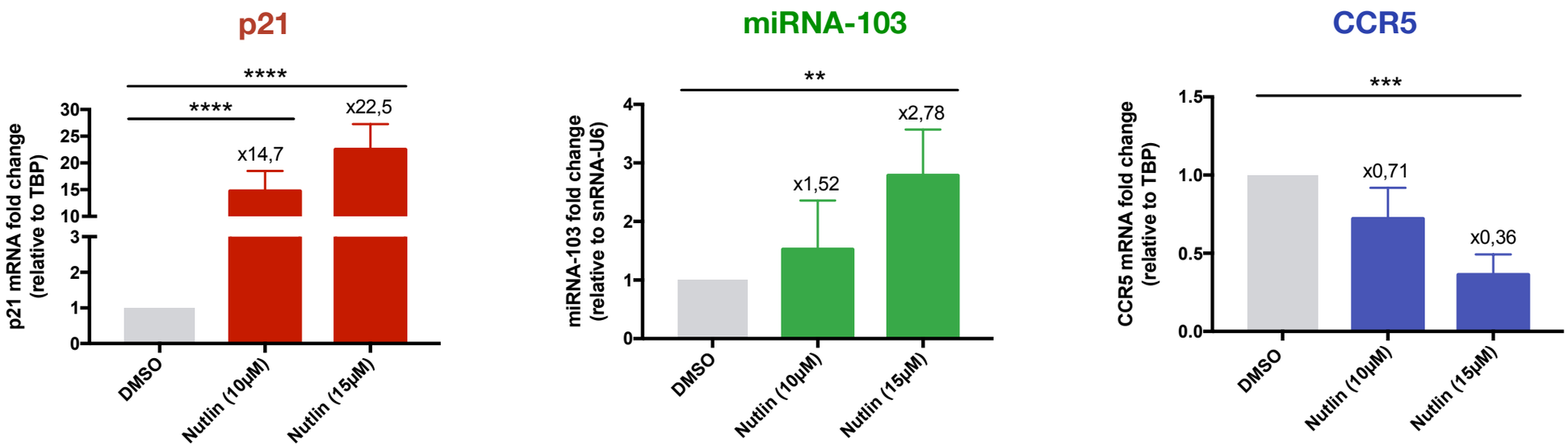
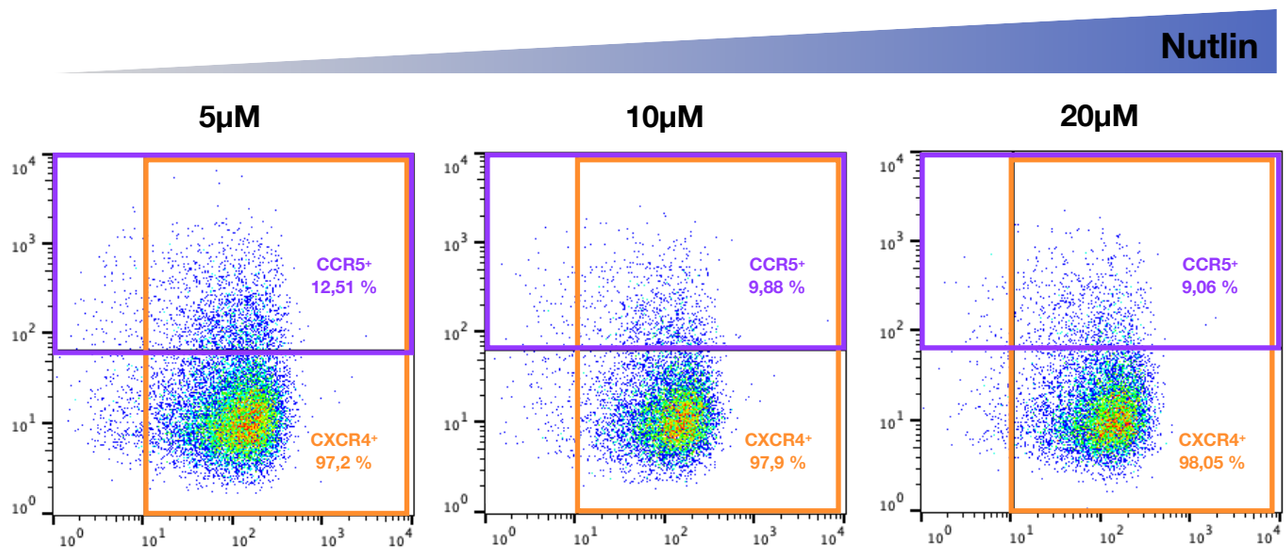
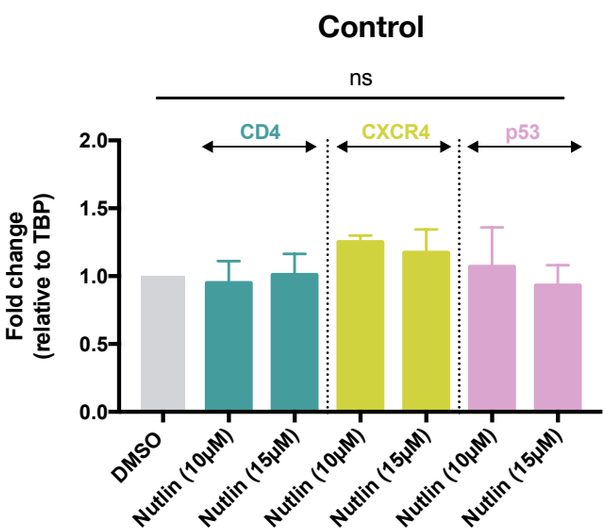
2 - miRNA-103 is modulated by p53 in CD4+ T cells

Primary CD4+ T cells were treated with Nutlin, a p53 stabilizer.

By qPCR







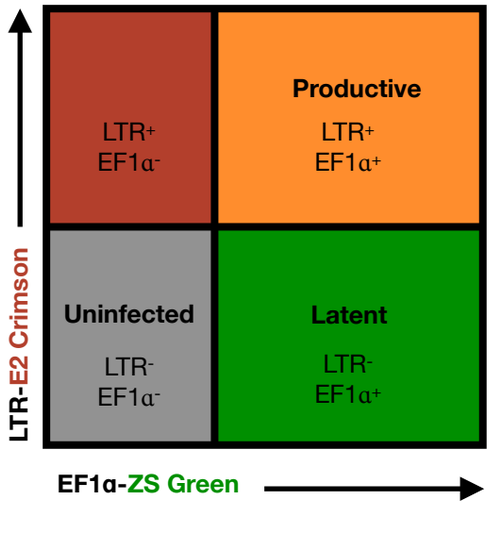
For all n=4

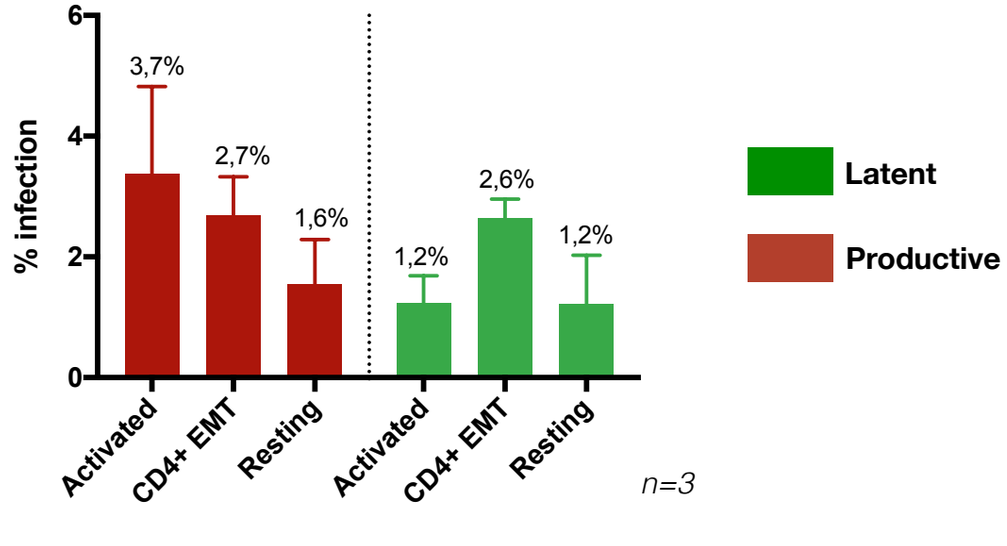
➠ ***Treatment with Nutlin increases miRNA-103 and decreases the CCR5 co-receptor.***

Investigating the role of miRNAs during HIV-1 infection of CD4+ T lymphocytes

3 - CD4+ EMT T cells are more susceptible to establishing latency

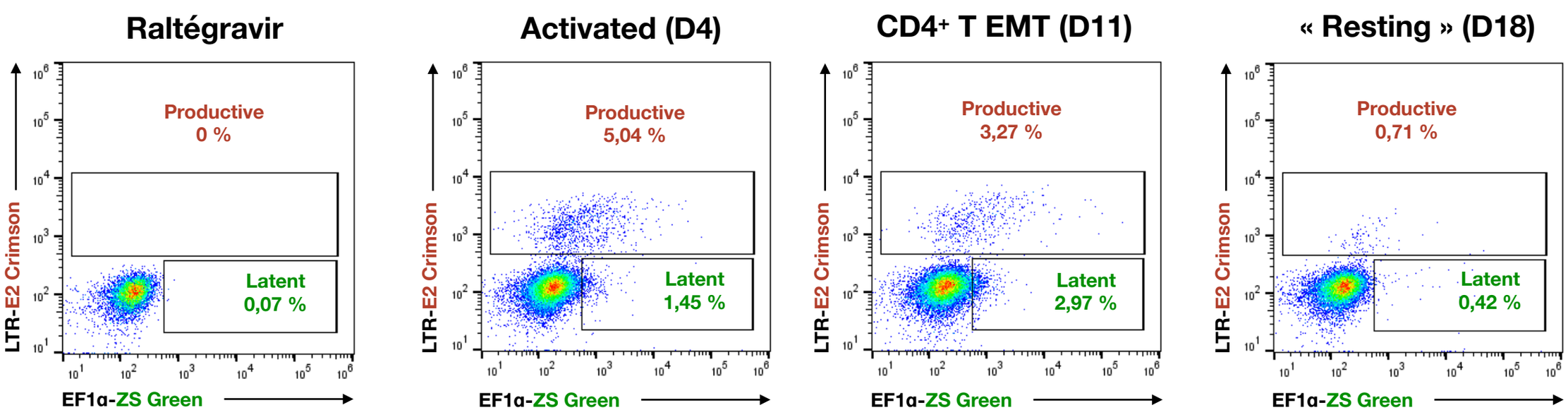
CD4+ T cells at different stages were infected with a dual reporter virus. HIV_{CRMZ} contains E2-Crimson under the control of the HIV-1 specific promoter (LTR), and ZS-Green under the control of the constitutive EF1α promoter.





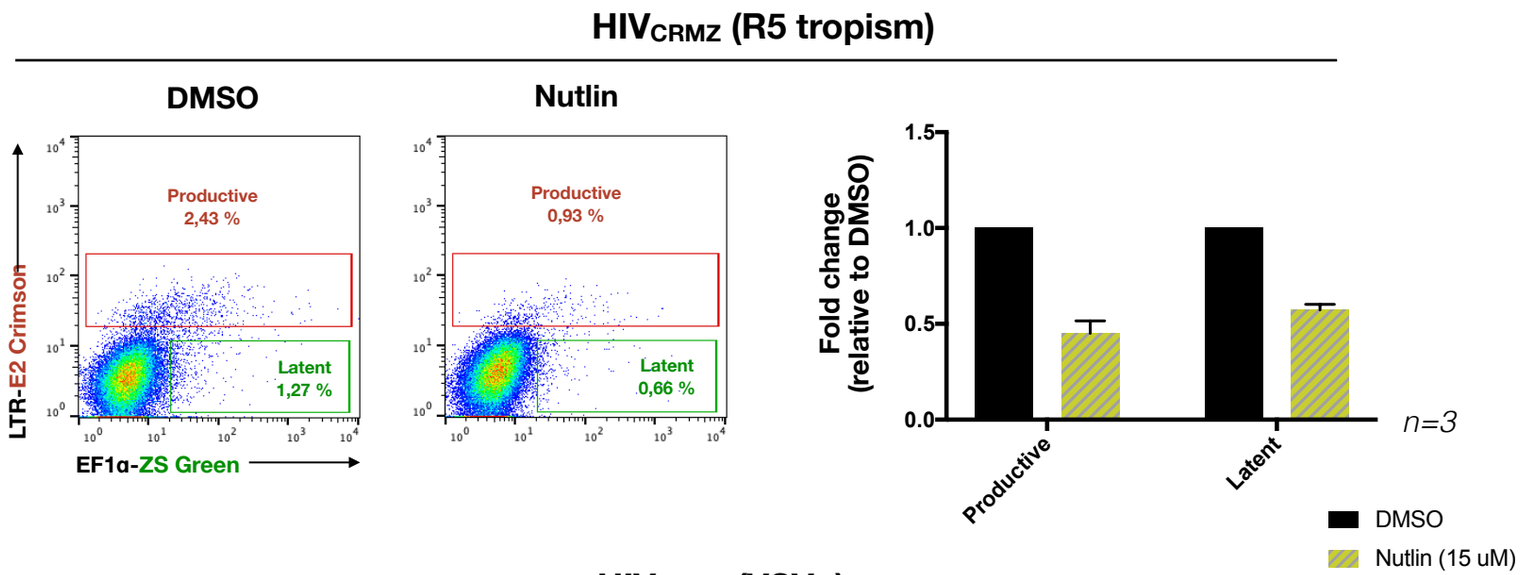
Representative donor :

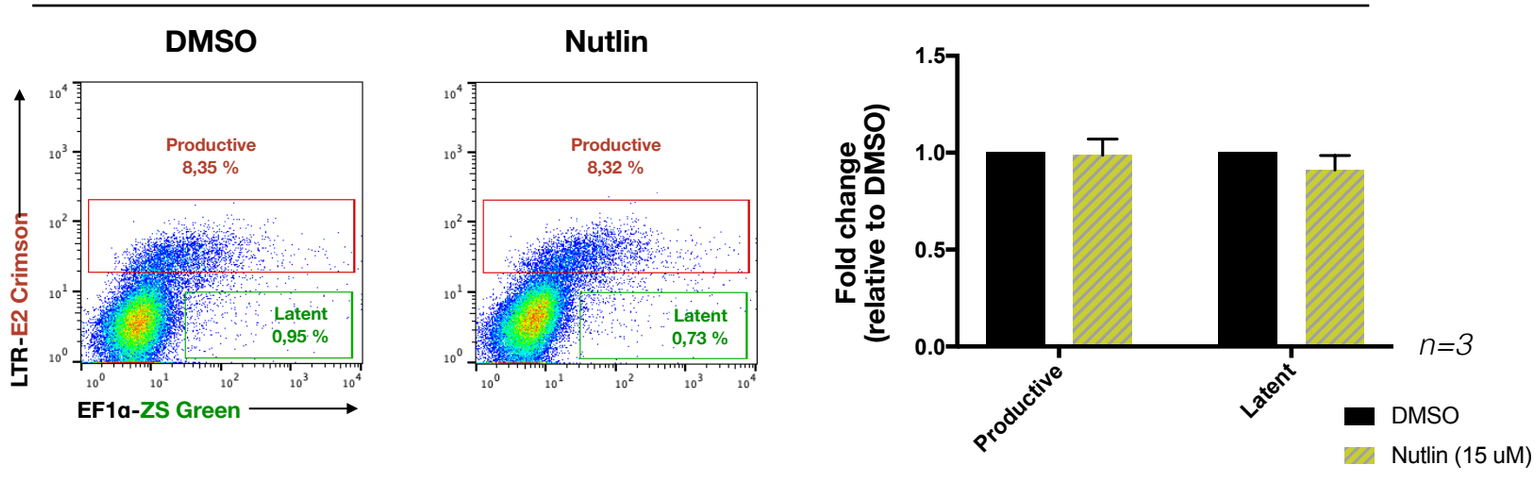
HIV_{CRMZ} (R5 tropism)

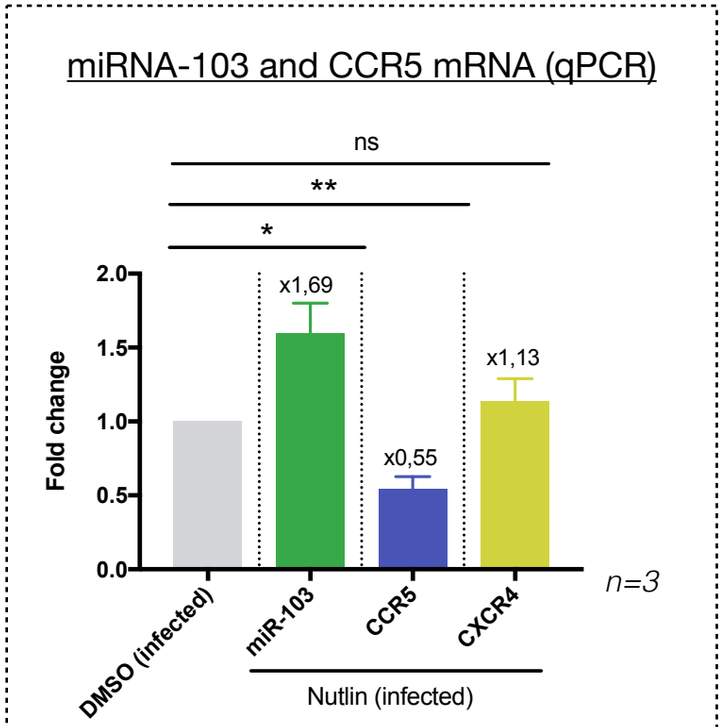


Investigating the role of miRNAs during HIV-1 infection of CD4+ T lymphocytes

4 - Treatment with nutlin decreases the frequency of latent cells in CD4+ EMT T cells







➠ **The effects observed are dependent and specific to miRNA-103 and CCR5.**

Conclusion

- MiRNA-103 is partly responsible for modulation of CCR5 co-receptor
- Using HIV_{CRMZ}, we show that CD4+ EMT T cells are more susceptible to establish HIV-1 latency.
- We show that treatment with Nutlin decreases CCR5 in these cells, thereby inhibiting infection and establishing latency.
- **Relevance for HIV cure** : This study will help us better understand how the modulation of miRNAs and their targeted cellular genes can contribute to the establishment of HIV-1 latency in CD4+ T cells and will provide new insights to counteract the development of HIV-1 reservoirs.