SCUOLA DI SPECIALIZZAZIONE IN MALATTIE INFETTIVE E TROPICALI

PANDORA ID - 22

TIME TO WEBINAR IN INFECTIOUS DISEASES

PROGETTO DI FORMAZIONE

15 Settembre 2022

IL RESERVOIR DI HIV: CREAZIONE, SELEZIONE E MANTENIMENTO

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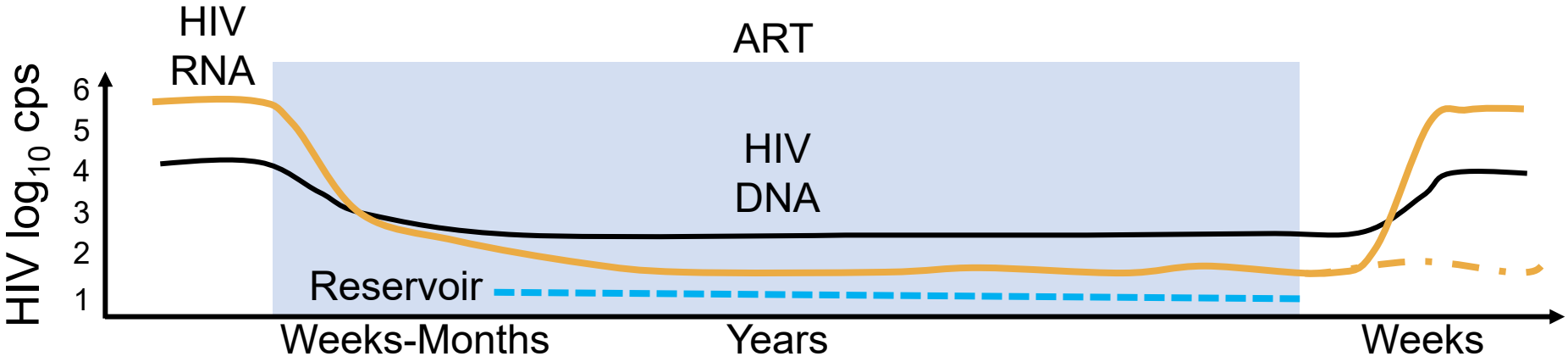
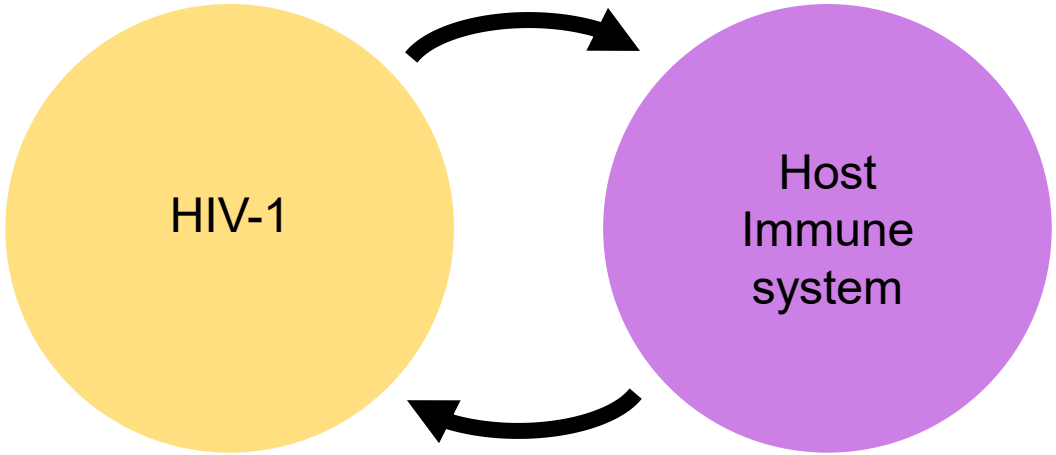
Selection pressures take place before, during, and post ART



10^8 newly infected cells daily



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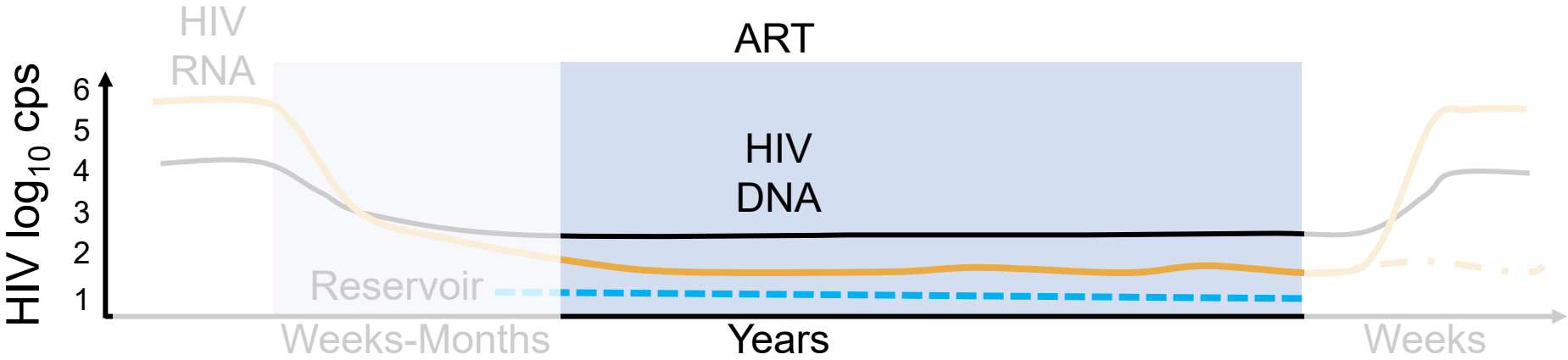
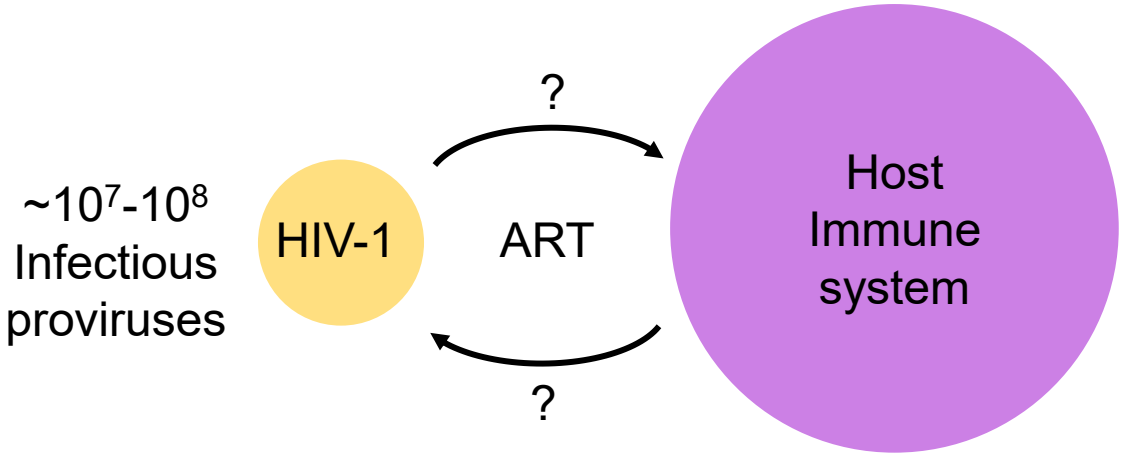
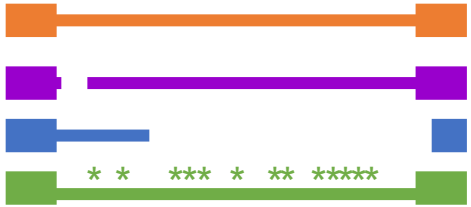




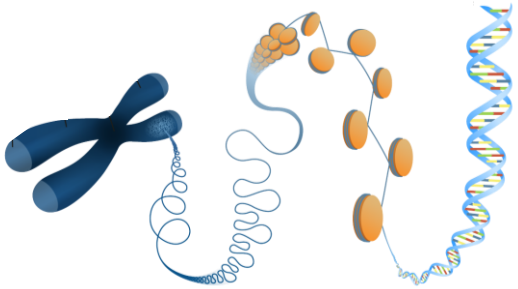
Multi-layered factors shaping HIV-1 persistence



Intact/Defective

Immune escape

Tropism

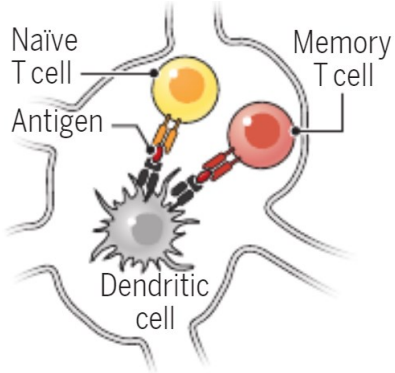


Gene

Epigenetic context

Chromosome

Nuclear architecture



T cell subset

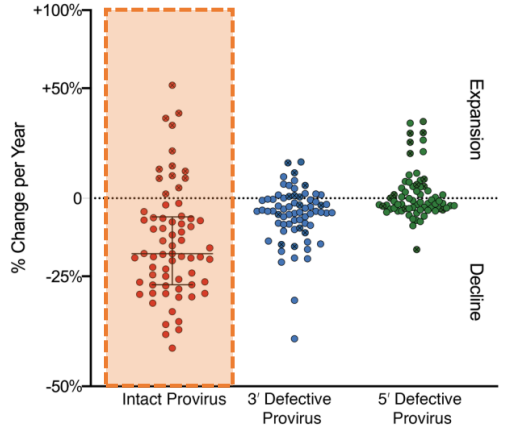
Antigen specificity

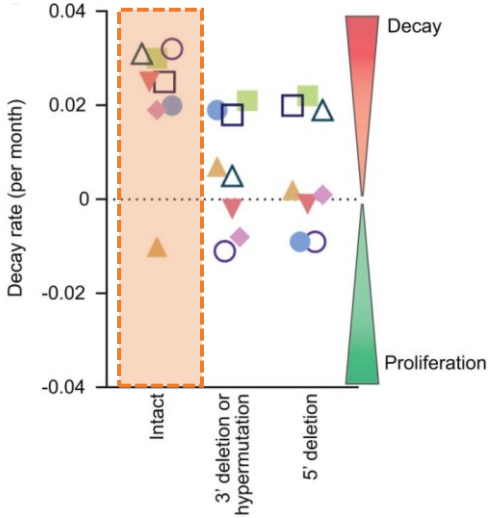
Proliferation

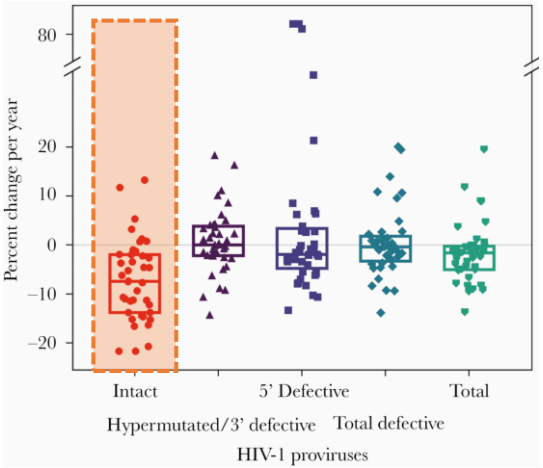
Resistance

Cell program

Intact proviruses (~5%) decay faster than defective ones







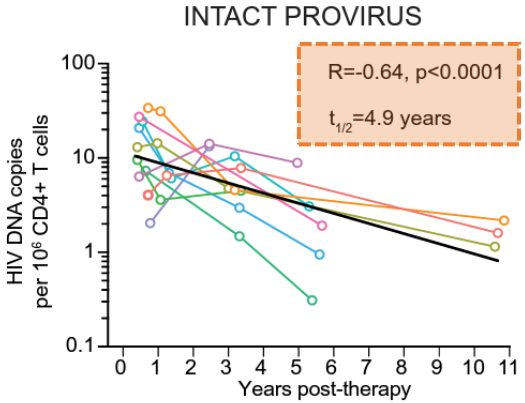
Peluso, 2020

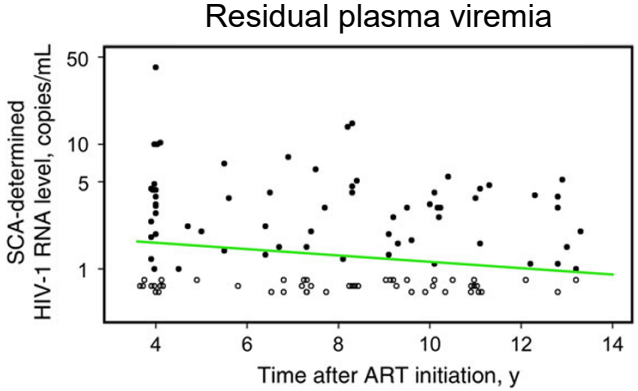
Antar, 2020

Gandhi, 2020

Pinzone, 2019

Cho, 2022

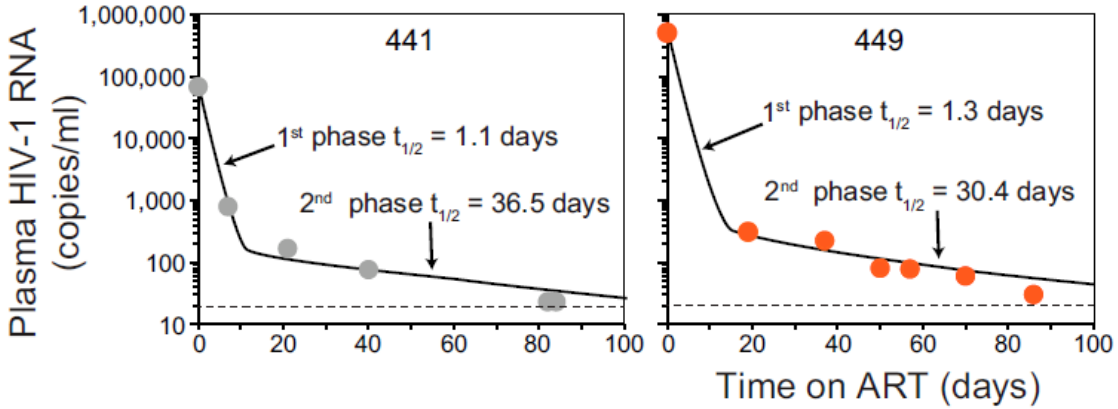


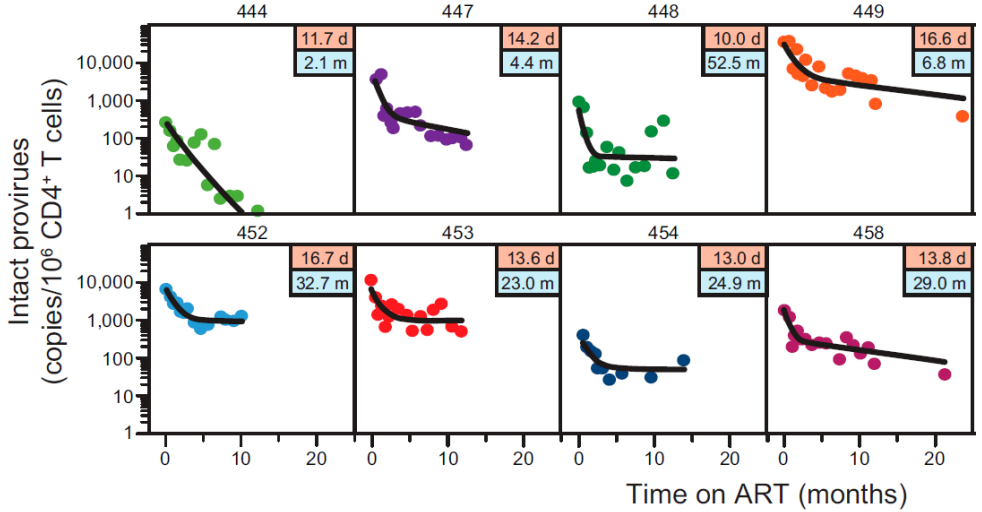


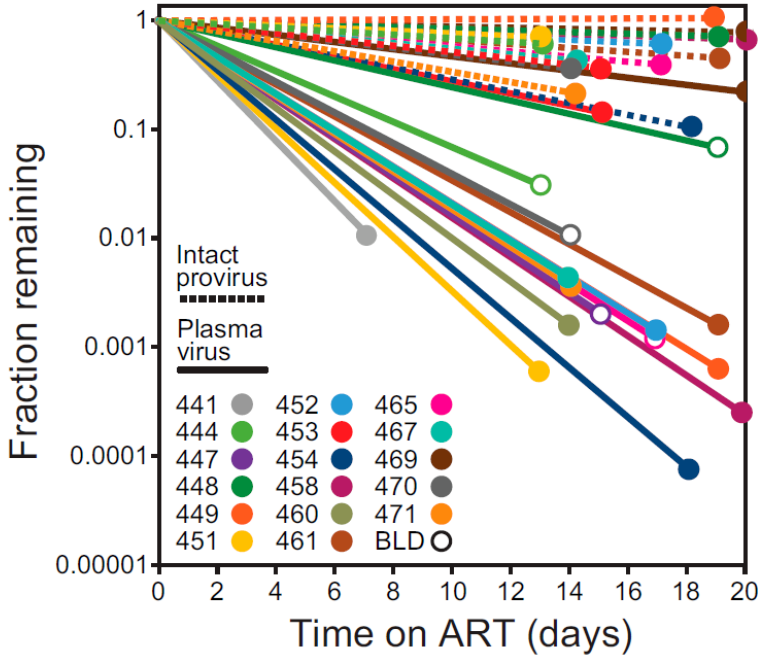
Siliciano, 2003

Riddler, 2016

Intact proviruses in blood have a biphasic but slower decay

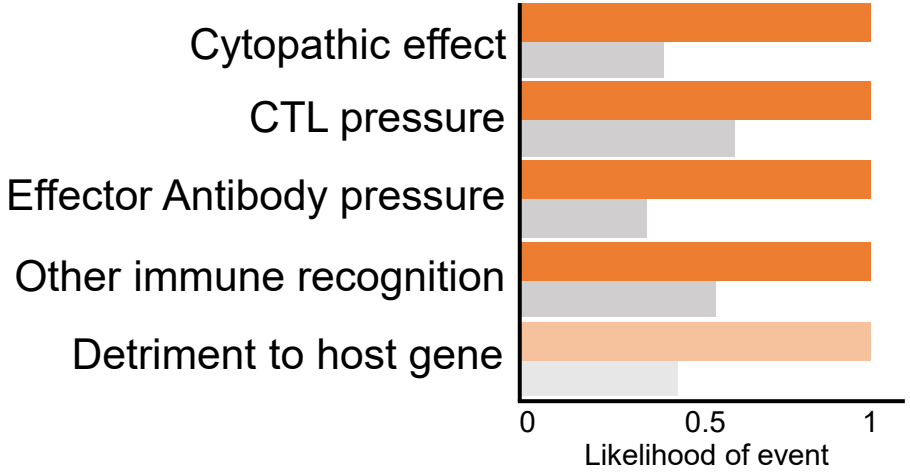


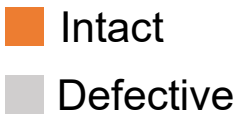


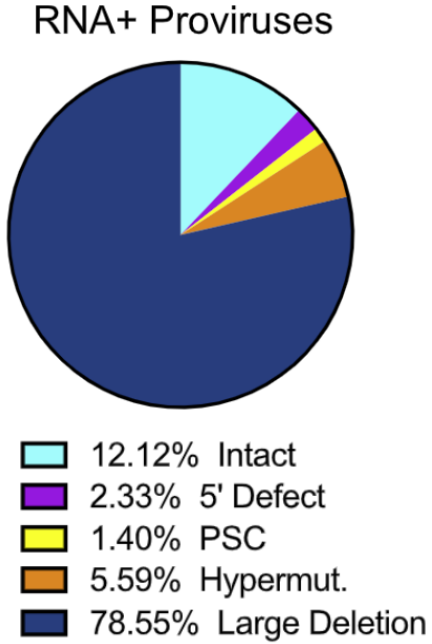


White, 2022

Why intact and defective proviruses undergo different selection?







Defective proviruses can produce RNA and proteins

Defective proviruses can be recognized by CTL

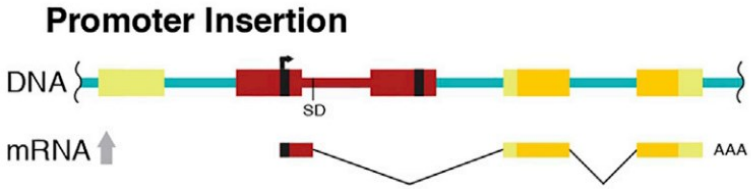
RNA expression is often not enough to produce proteins/virions

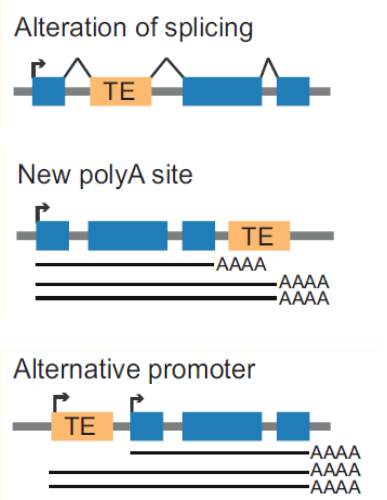
Little signal for ongoing CTL selection on ART

Pollack, 2017 | Imamichi, 2017 | Einkauf, 2022 | Sannier, 2021 | Antar, 2020 | Cho, 2022

Genomic context: HIV-1 integration site

HIV-1 affecting surrounding gene expression



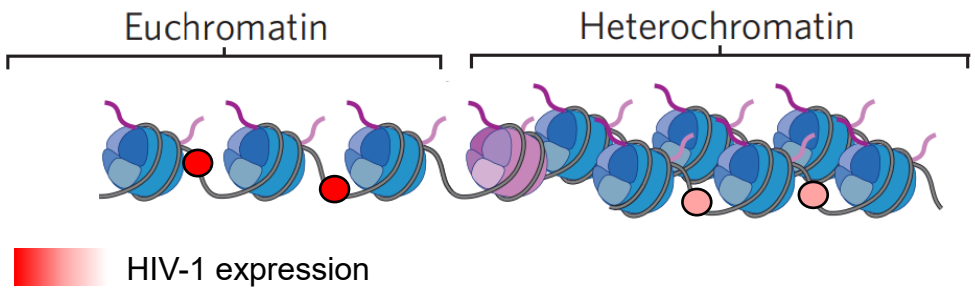


Negative selection

>>Same orientation of highly expressed genes

Positive selection

Surrounding genome affecting HIV-1 expression



Bushman, 2020	Liu, 2020
Yoon, 2020	Mellors, 2021
Lewinski, 2005	Burdick, 2022
Jiang, 2021	Einkauf, 2022

Role of HIV-1 integration: what is the gold standard?

Acute infection *in vivo* or *in vitro*

Long-term ART

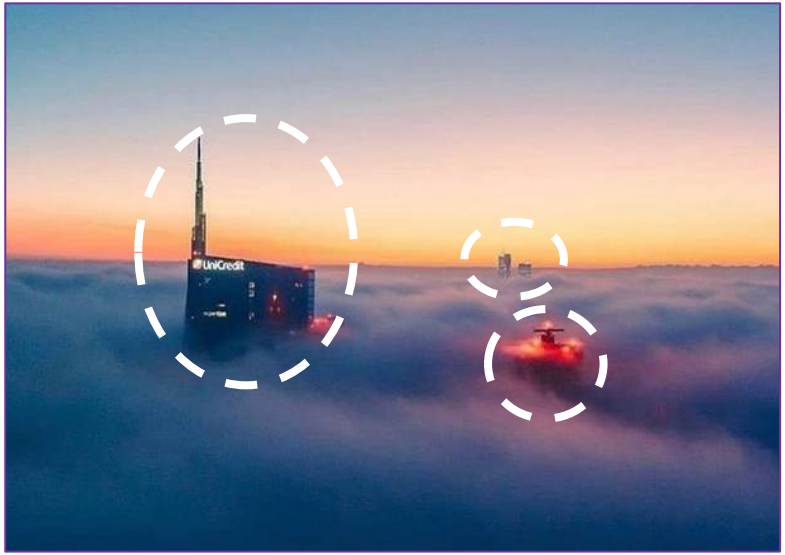
Initial distribution

Result of selection

Coffin and Hughes, 2021

Challenges in detecting HIV-infected clones

Sampled population

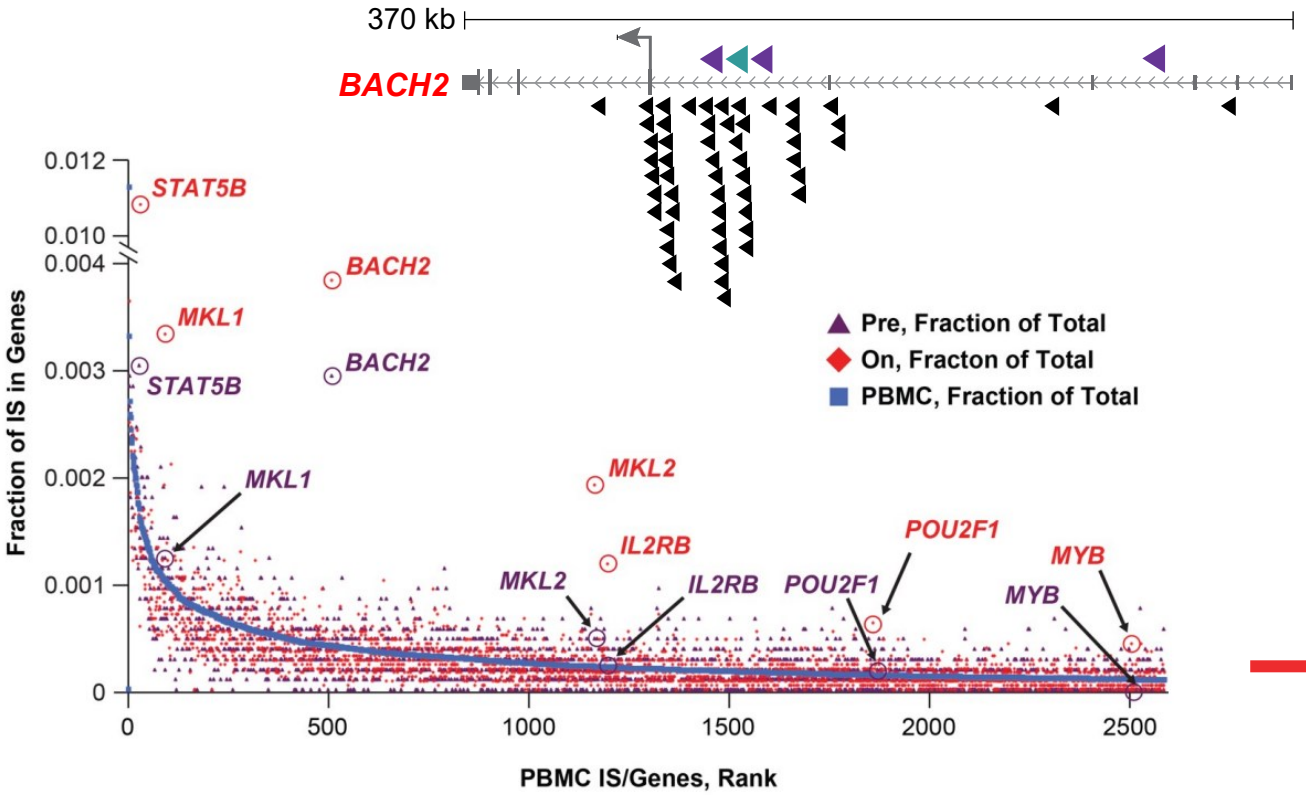


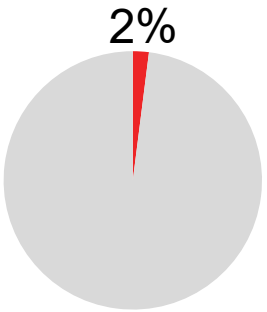
Actual population

If 1000 integration sites are recovered from one individual only clones >10^5 can be detected as clonally expanded.

From Coffin and Hughes

Host gene misexpression leading to positive selection is rare

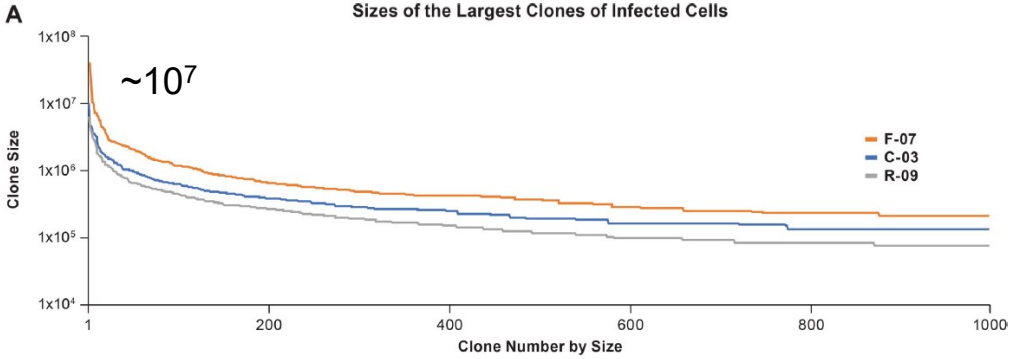


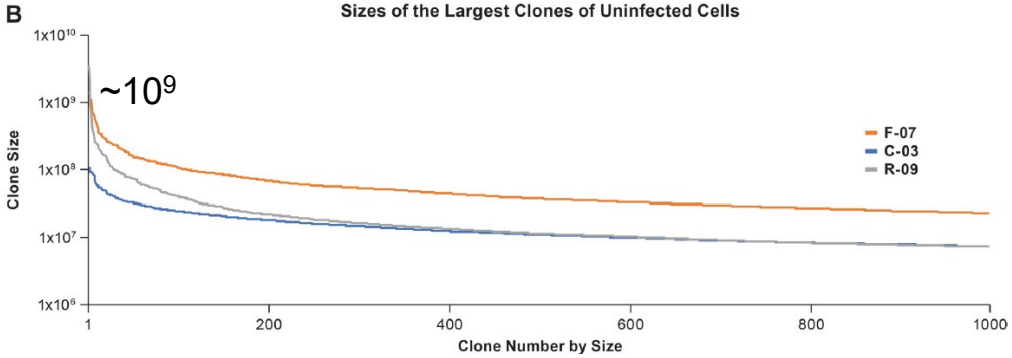


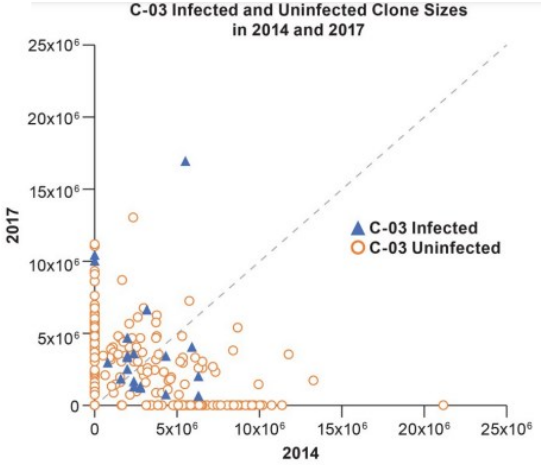
Most proviruses in these genes are likely defective

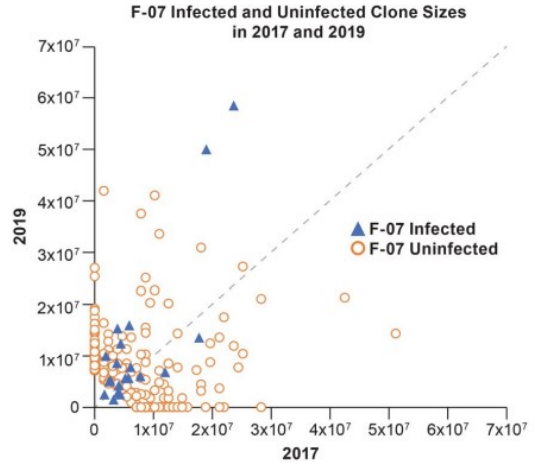
Maldarelli, 2014 | Wagner, 2014 | Cesana, 2017 | Liu, 2020 | Coffin, 2021

Clones of infected T cells are more stable, compared to all T cell clones



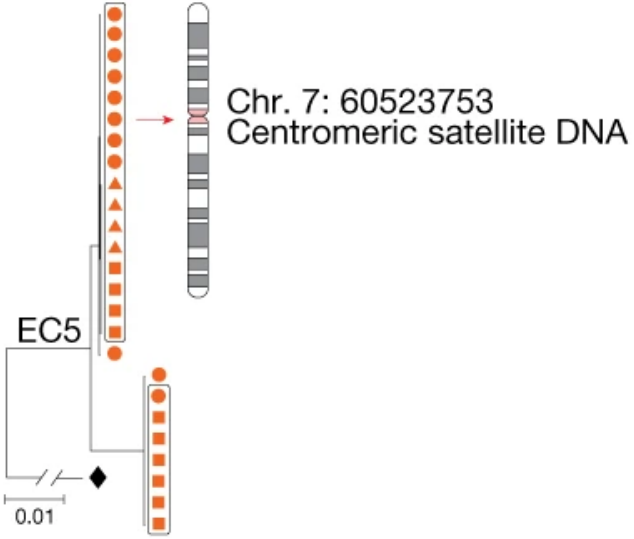


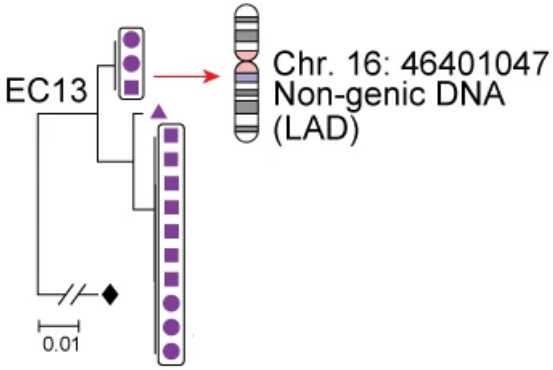




Guo, 2022

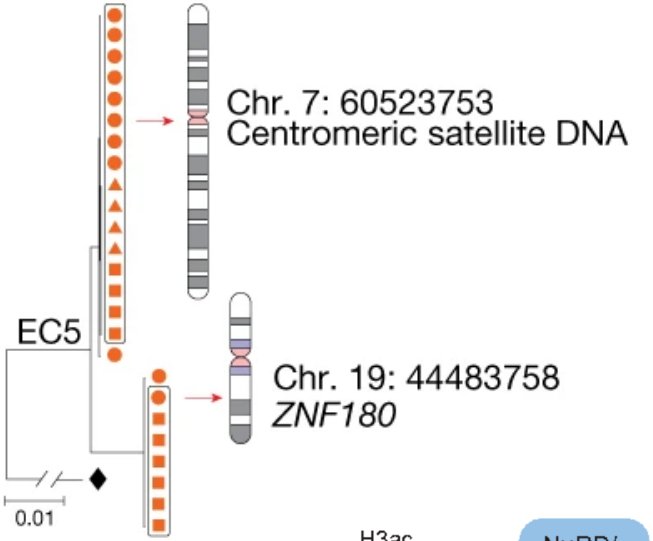
Selection of intact proviruses in loci associated with deeper latency

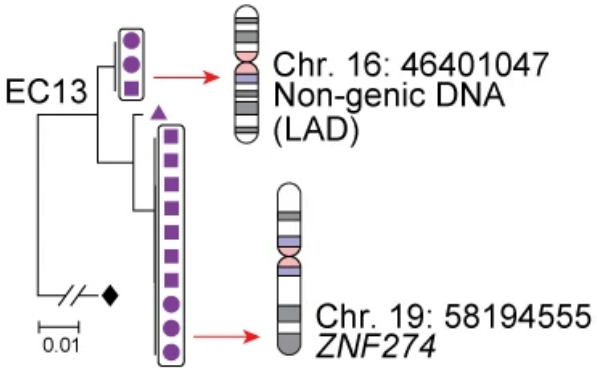


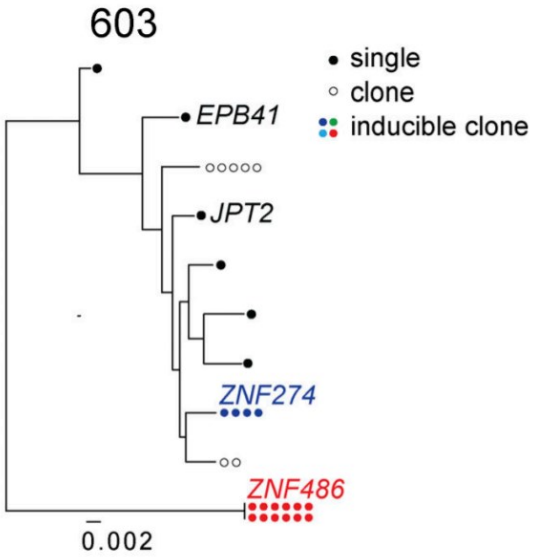


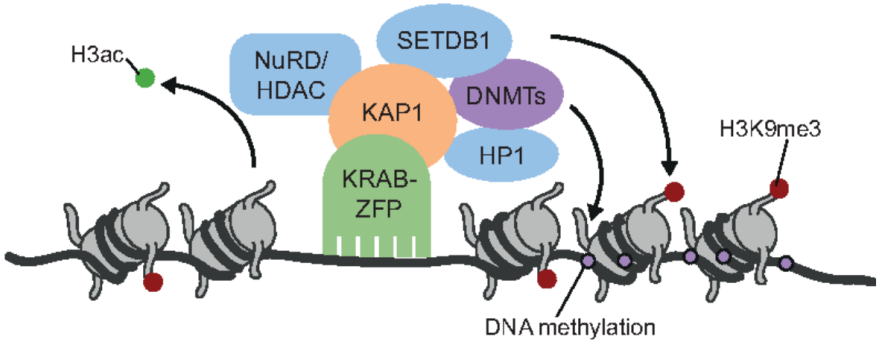
Jiang, 2021 | Huang, 2021 | Lian, 2021 | Einkauf, 2022 | Seiger, 68

Selection of intact proviruses in loci associated with deeper latency





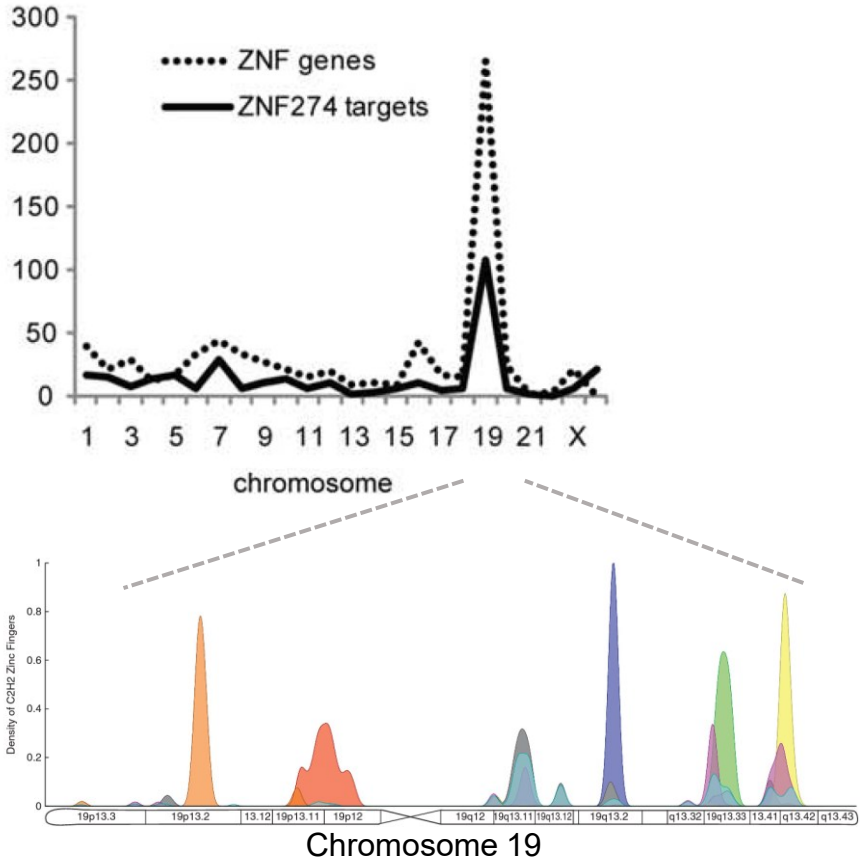




Jiang, 2021 | Huang, 2021 | Lian, 2021 | Einkauf, 2022 | Seiger, 68 | Jakobsson, 2022

Recurring ZNF genes with integrations of intact proviruses

ZNF gene	Overlap	Chr.	Provirus	Paper
ZNF140		12	intact	Huang, 2021
ZNF274		19	intact	
ZNF486		19	intact	
ZNF721		4	intact	
ZNF460		19	intact	
ZNF557		19	intact	
ZNF141		4	intact	Cole, 2021
ZNF274		19	intact	
ZNF268		12	intact	Halvas, 2020
ZNF721		4	intact	
ZNF721*	ABCA11P	4	intact	
ZNF721	ABCA11P	4	intact	Einkauf, 2019
ZNF140		12	intact	
ZNF84		12	intact	
ZNF274		19	intact	
ZNF140		12	intact	Einkauf, 2022
ZNF180		19	intact	Jiang, 2021
ZNF225		19	intact	
ZNF721	ABCA11P	4	intact	
ZNF407		18	intact	
ZNF274		19	intact	
ZNF350	ZNF350-AS1	19	intact	
ZNF470		19	not known	Veenhuis, 2019



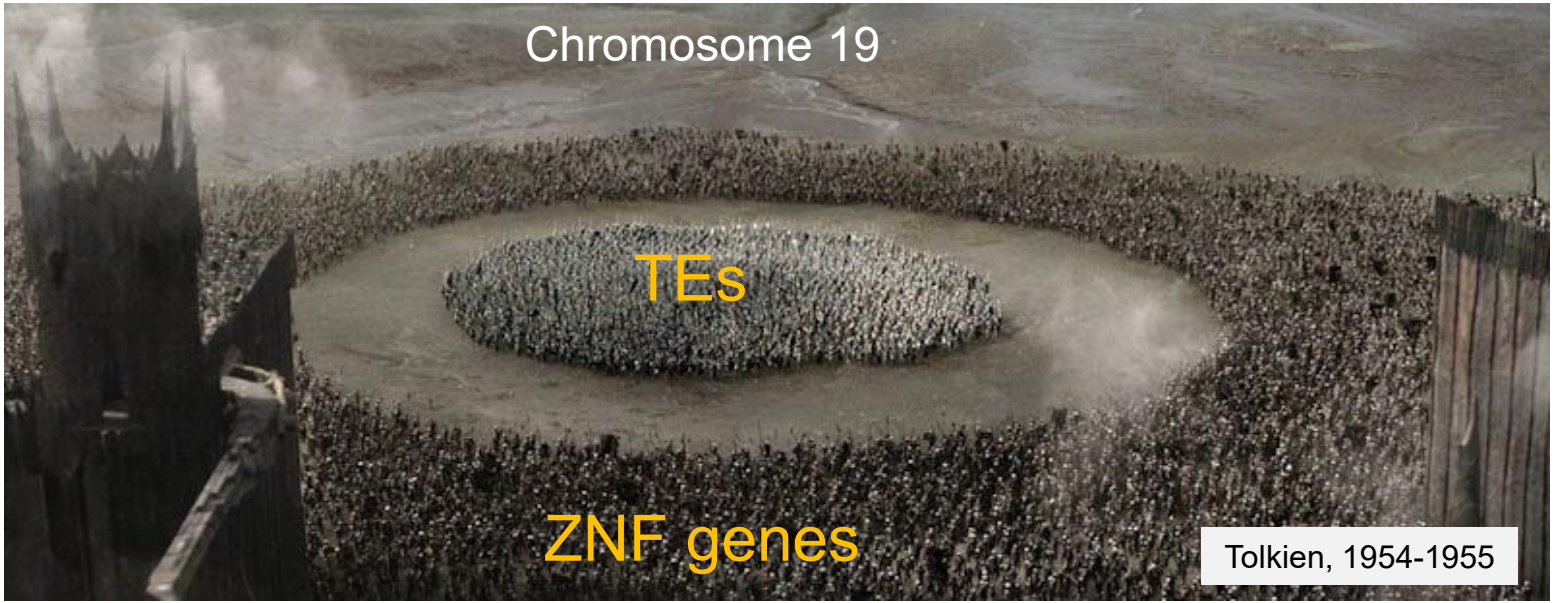
Chromosome 19

Elite controllers On ART

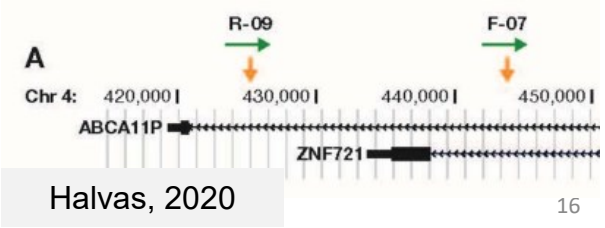
Freitze, 2010 Jakobsson, 2022 Lukic, 2013

Selection of intact proviruses in a state of deeper latency

Do KRAB-ZNF interact directly with intact proviruses? Or they are simply “trapped”, casualties of an ancient arm race?

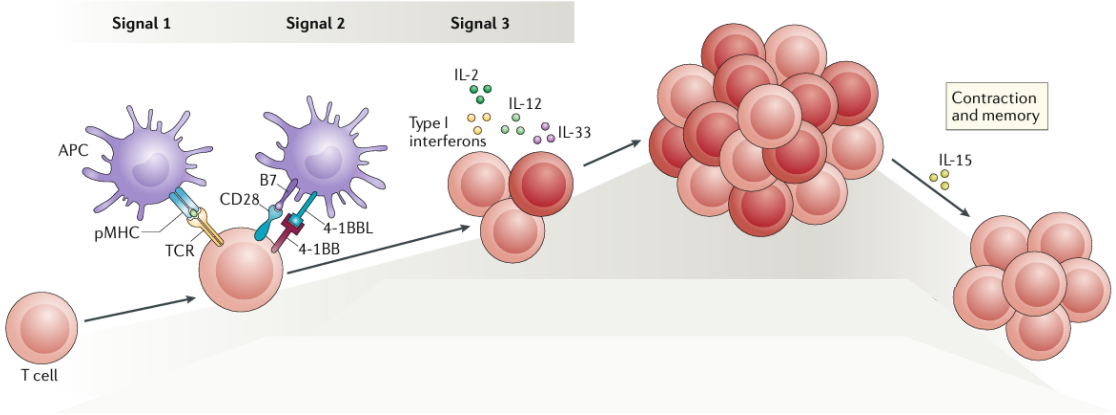


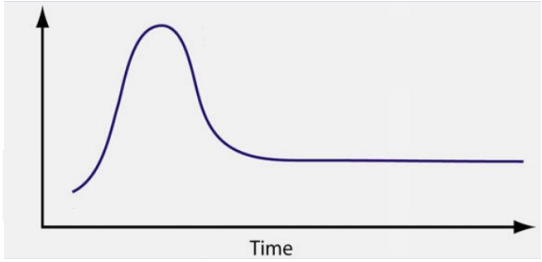
Some intact proviruses in ZNF genes can be induced *ex vivo*
-What about physiological T cell stimulation? (e.g. cognate antigen)
-Can they lead to viral rebound upon treatment interruption?



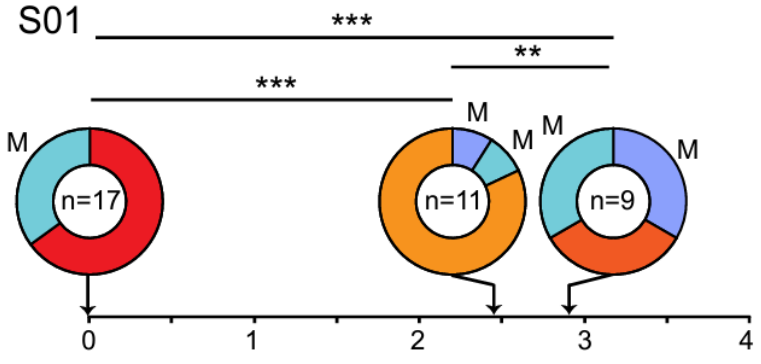
Halvas, 2020

Reservoir fluctuations resemble T cell response dynamics



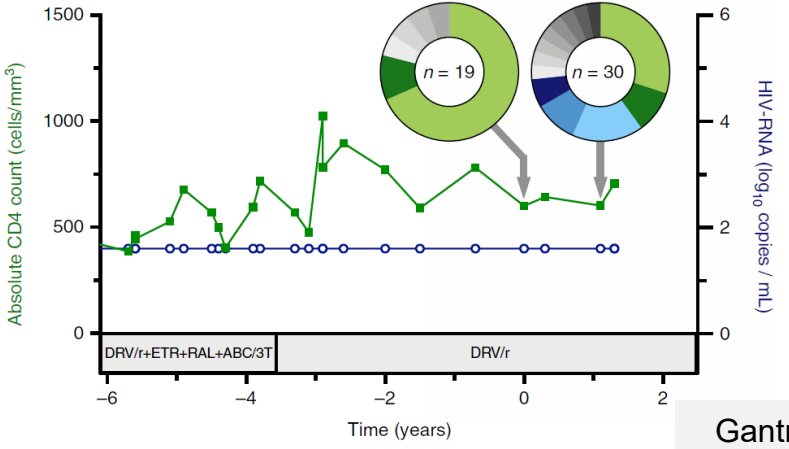


Viral outgrowth



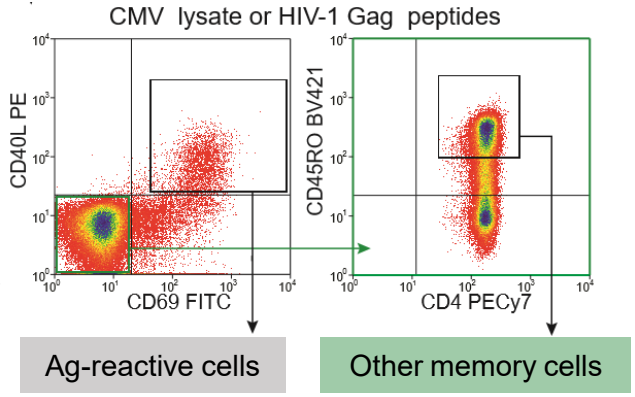
Wang, 2018

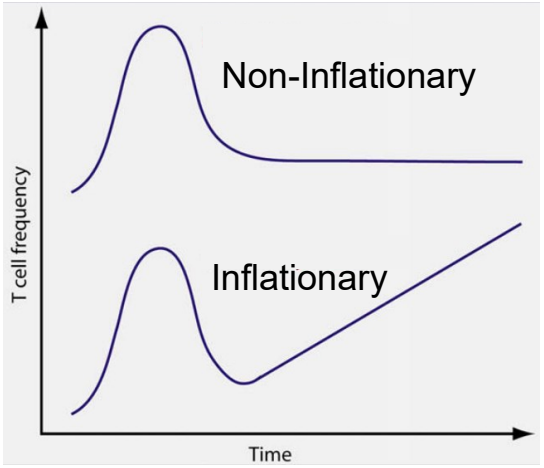
TCR-seq of p24 positive cells

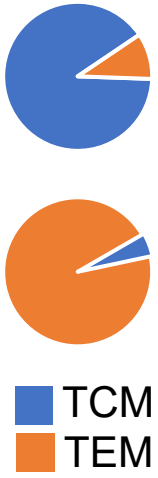


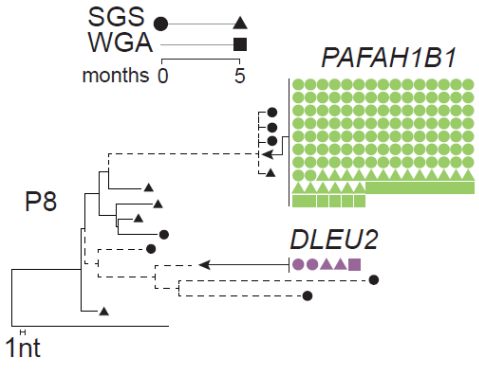
Gantner, 2020

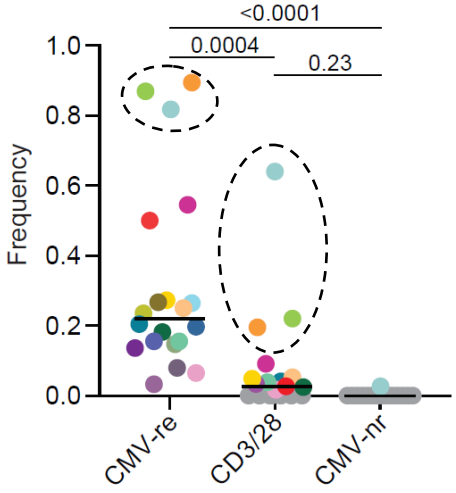
Antigen-driven selection contributes to HIV persistence

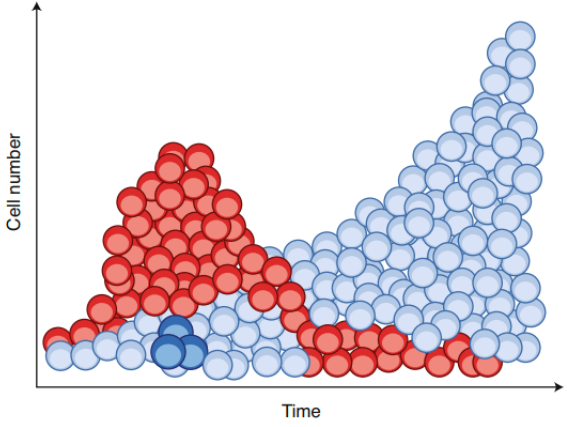






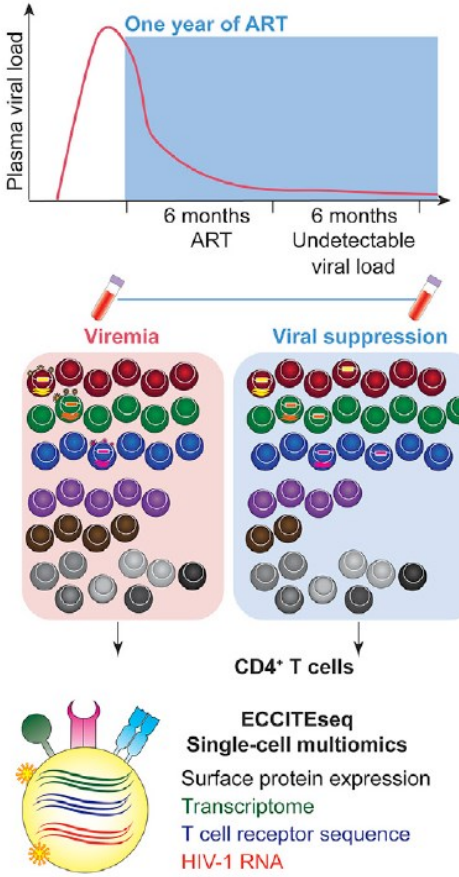


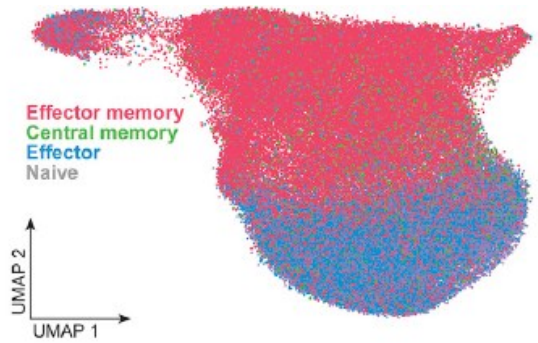


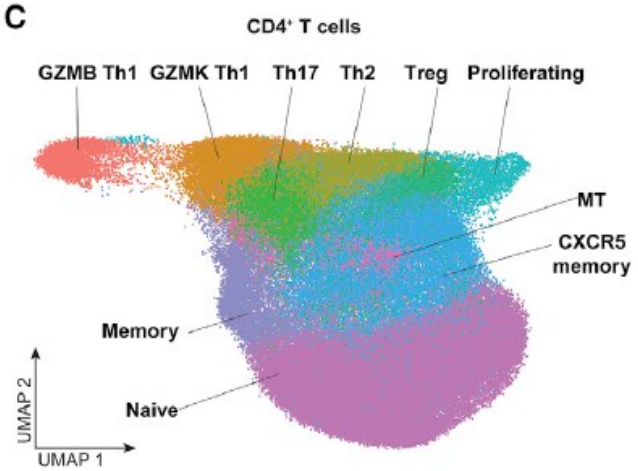


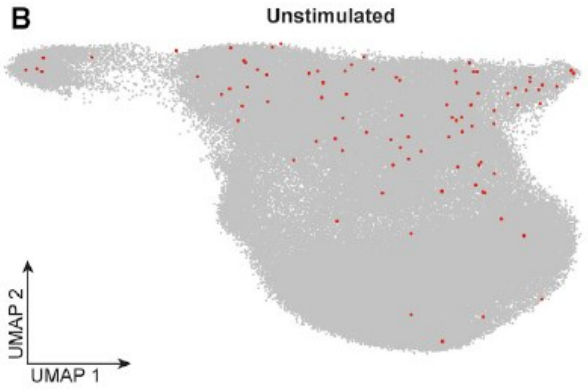
Mendoza, 2020 | Simonetti, 2021 | Klenerman, 2008 | Grassmann, 2020

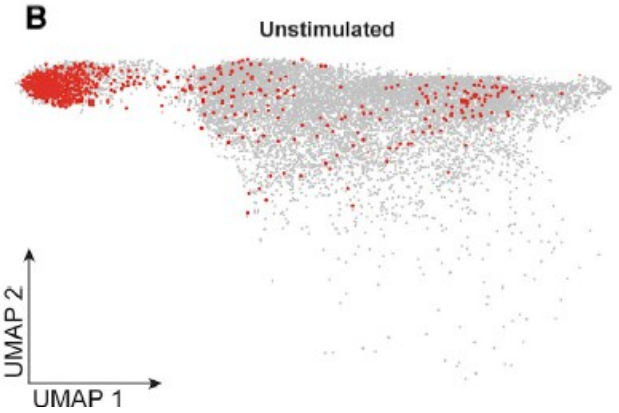
HIV+ expanded clones are enriched in cytotoxic T cells





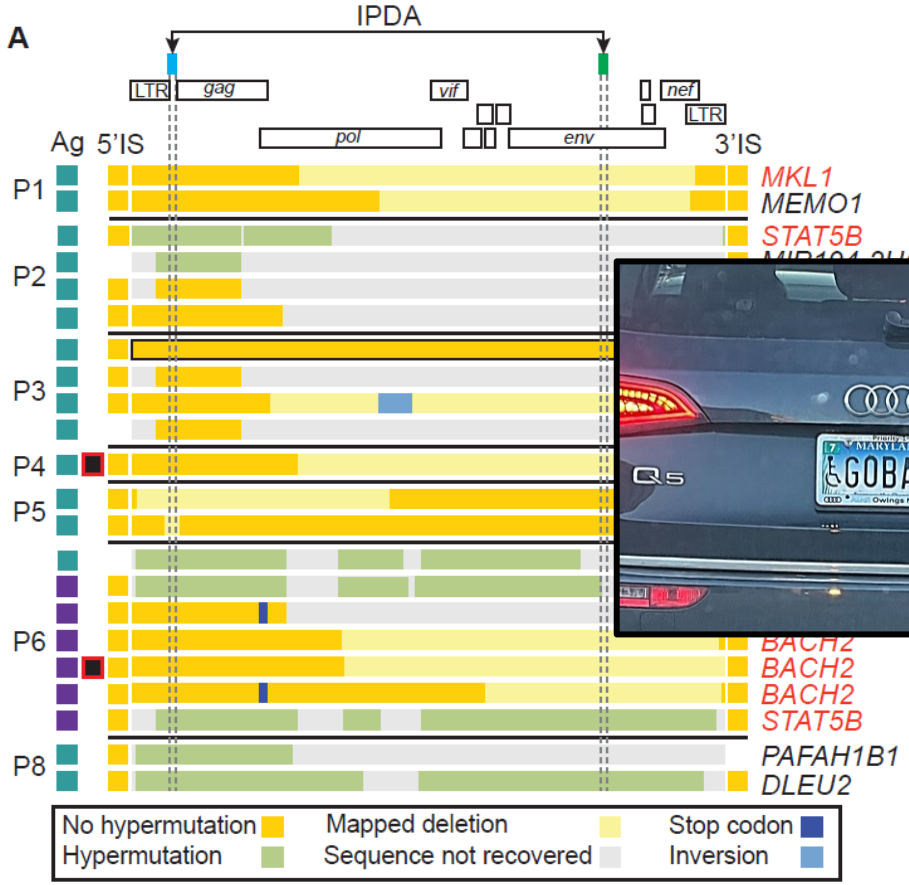


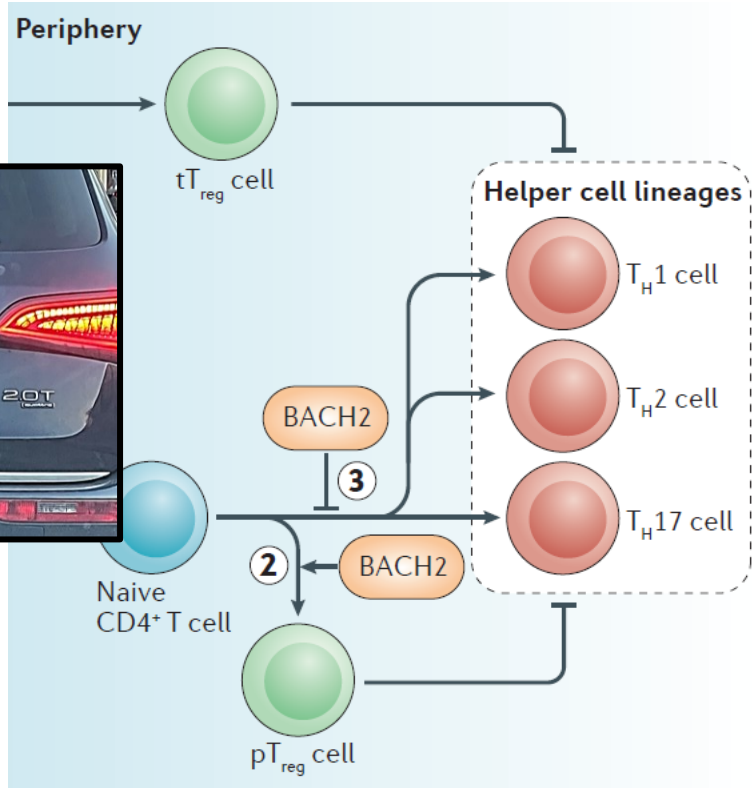




HIV-1 RNA⁺ T cell clones
HIV-1 RNA⁻ T cell clones

Possible synergy between HIV integration and immune selection



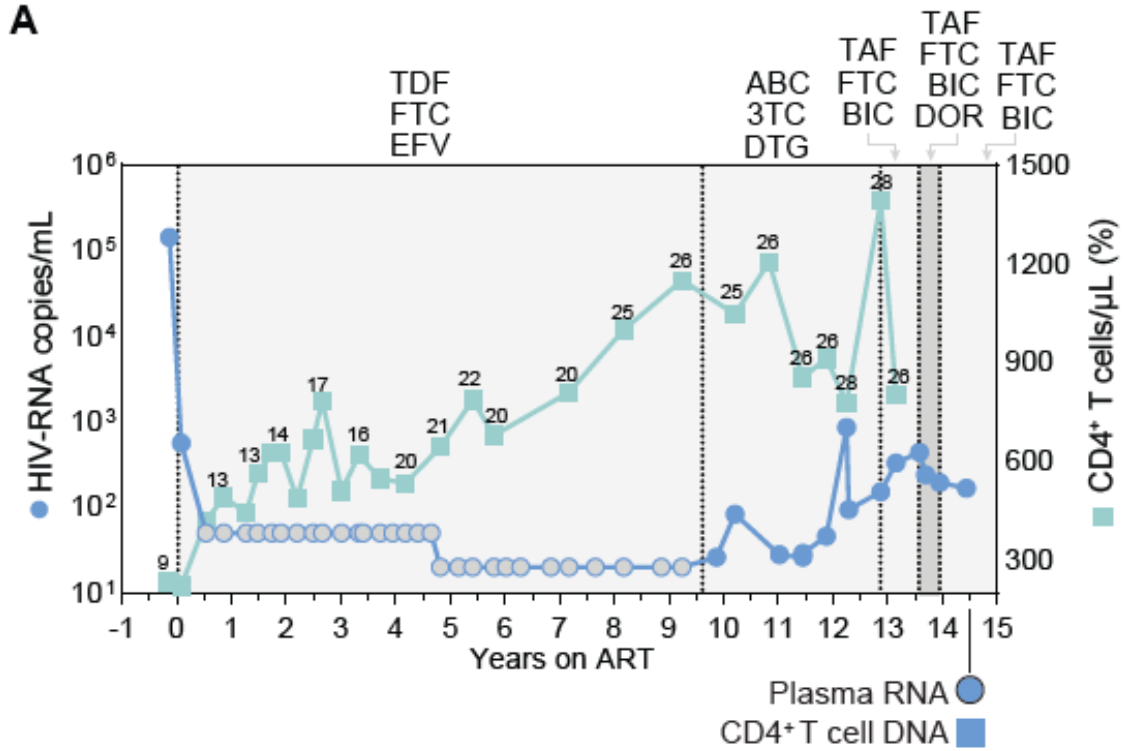


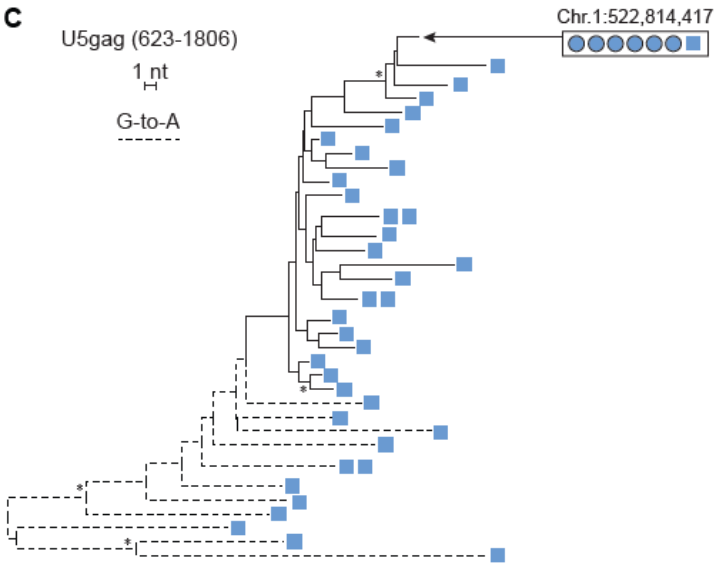
Simonetti, 2021

Igarashi, 2017

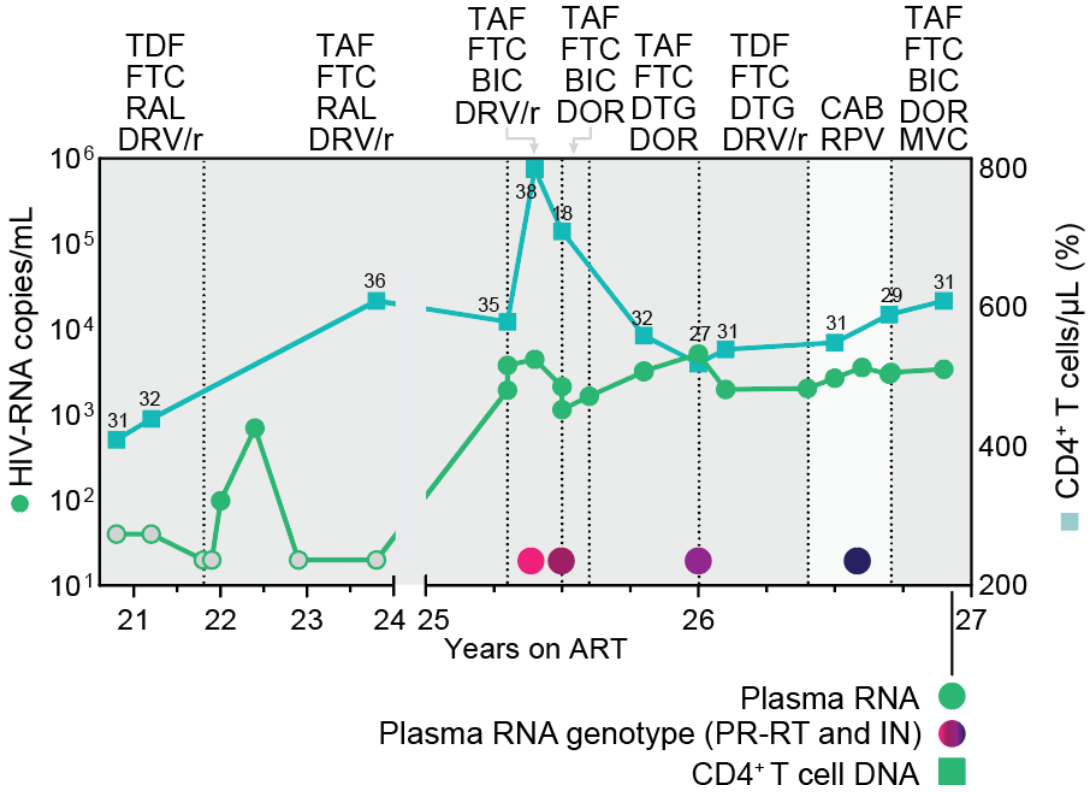
Time for a little digression…

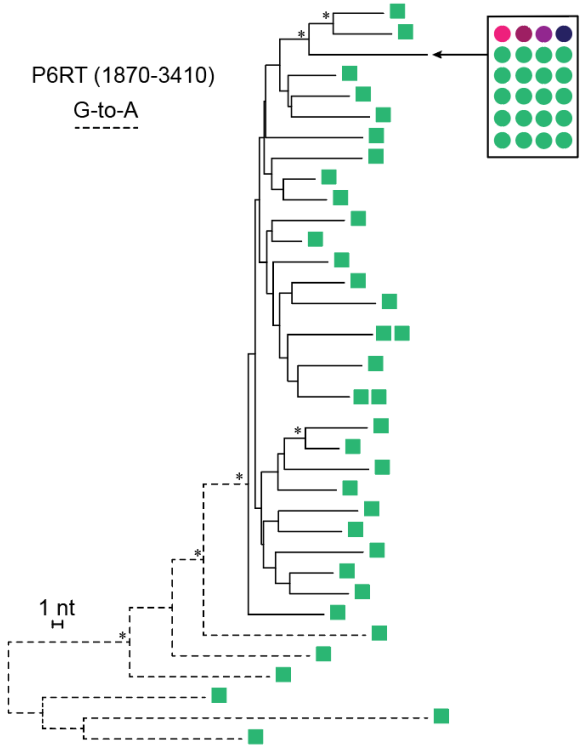
New onset low level viremia is usually caused by a single, rare provirus





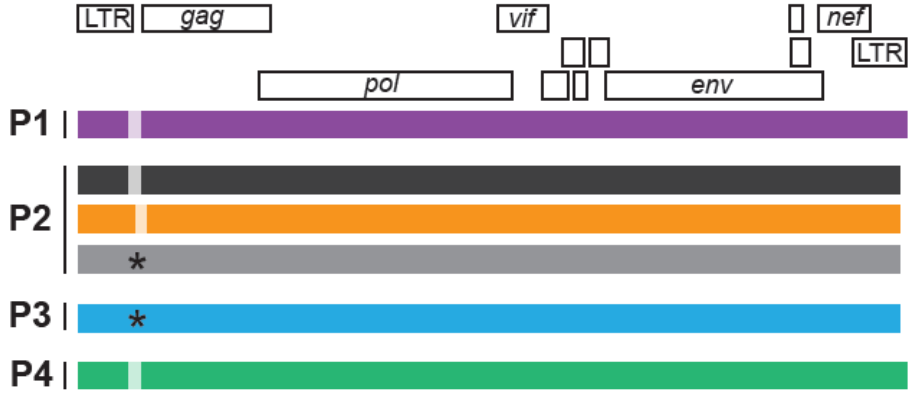
~3000 copies/mL of plasma HIV RNA caused by a single drug-sensitive variant



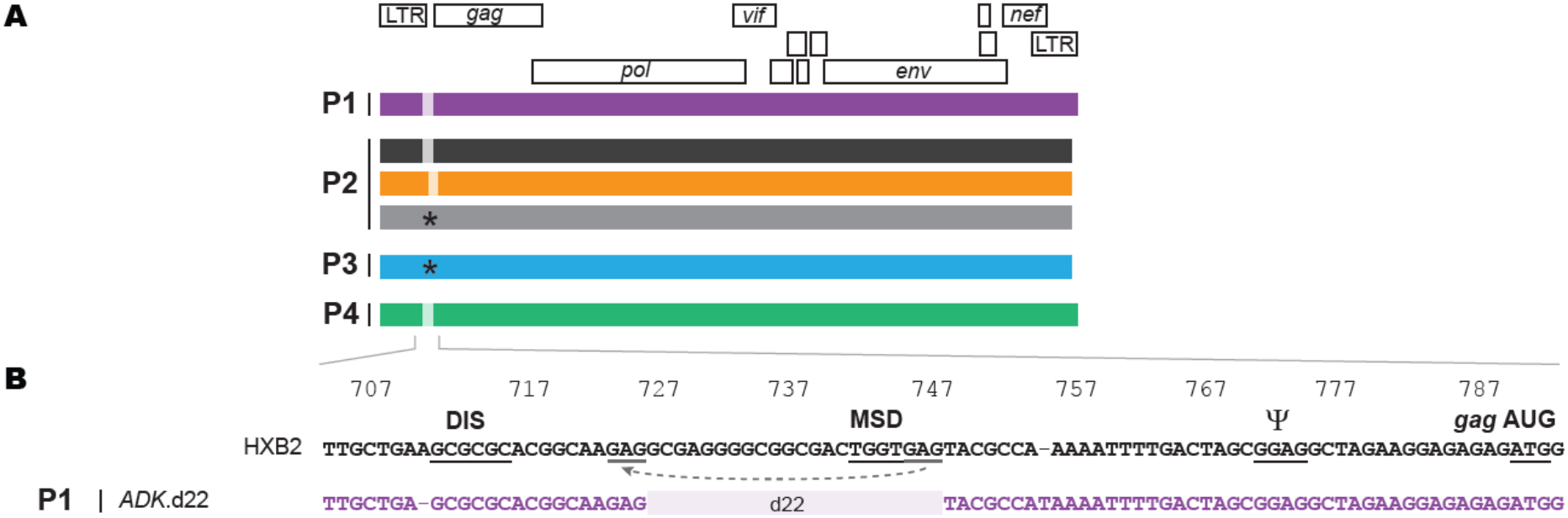


Proviruses cause of NSV show defects in the 5’ leader

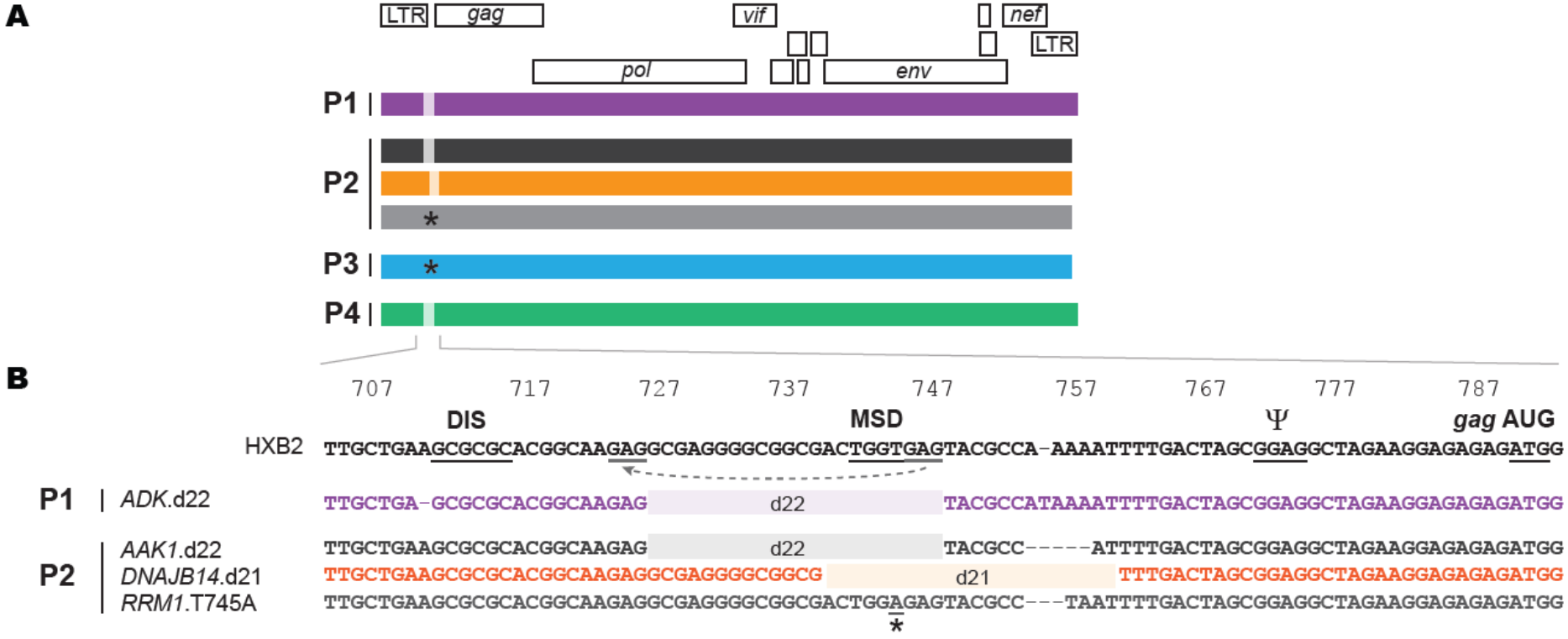
A



Proviruses cause of NSV show defects in the 5’ leader



Proviruses cause of NSV show defects in the 5’ leader



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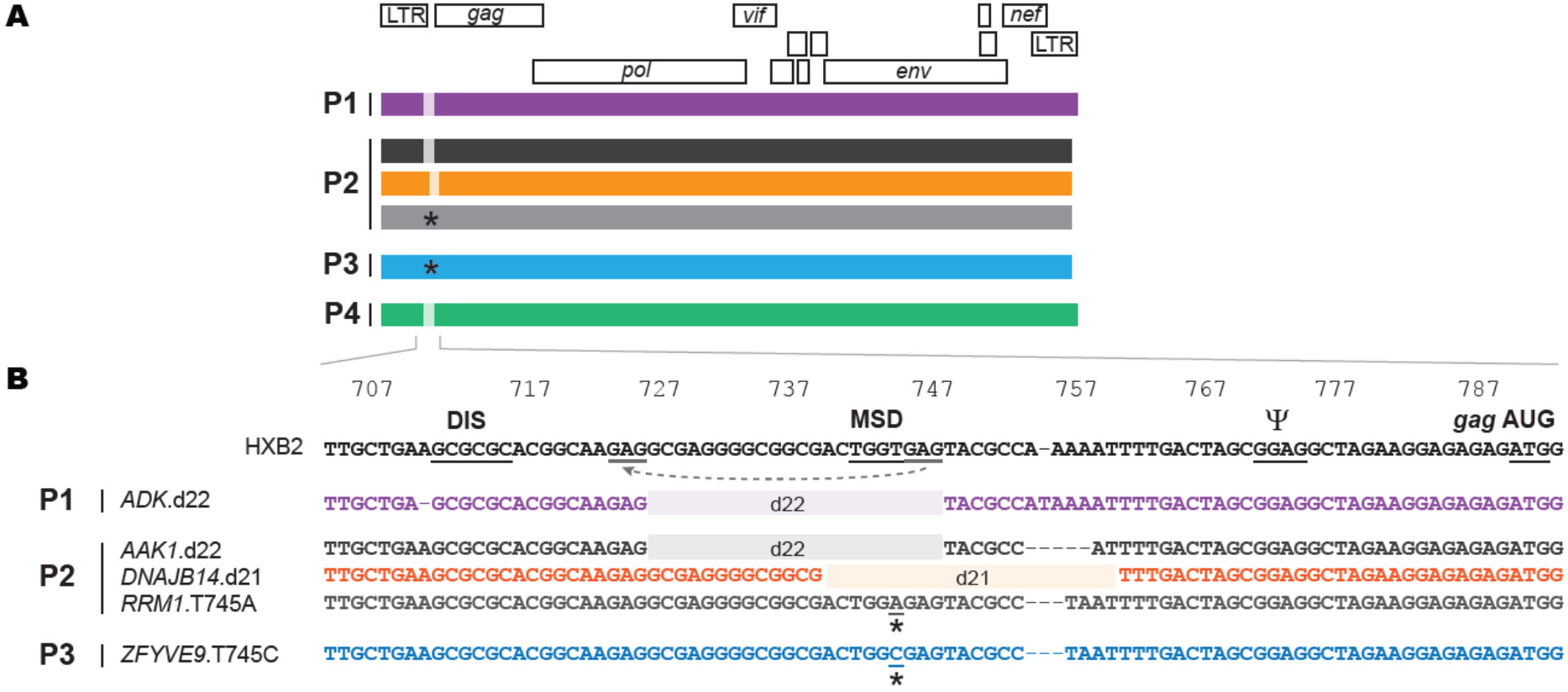
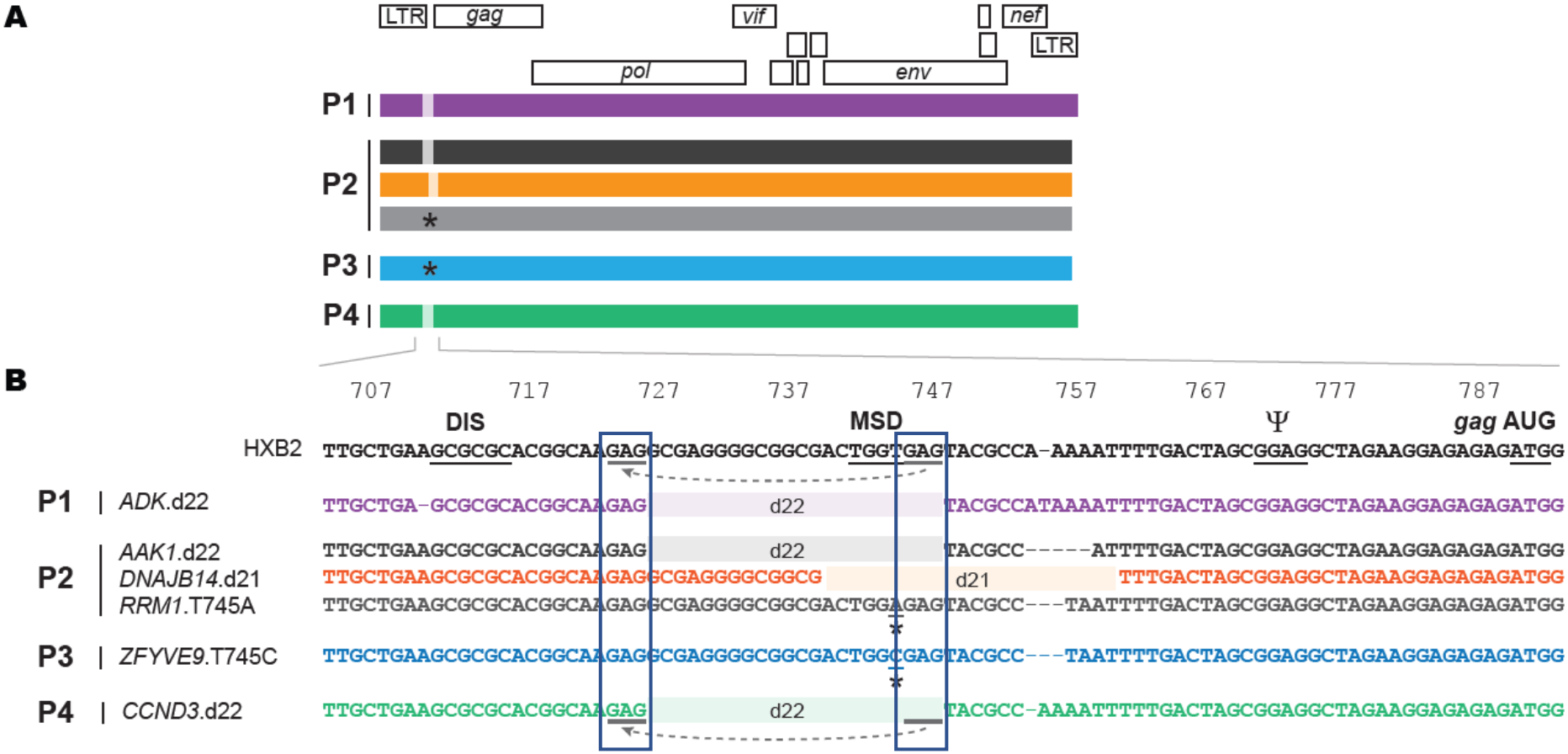


Proviruses cause of NSV show defects in the 5’ leader



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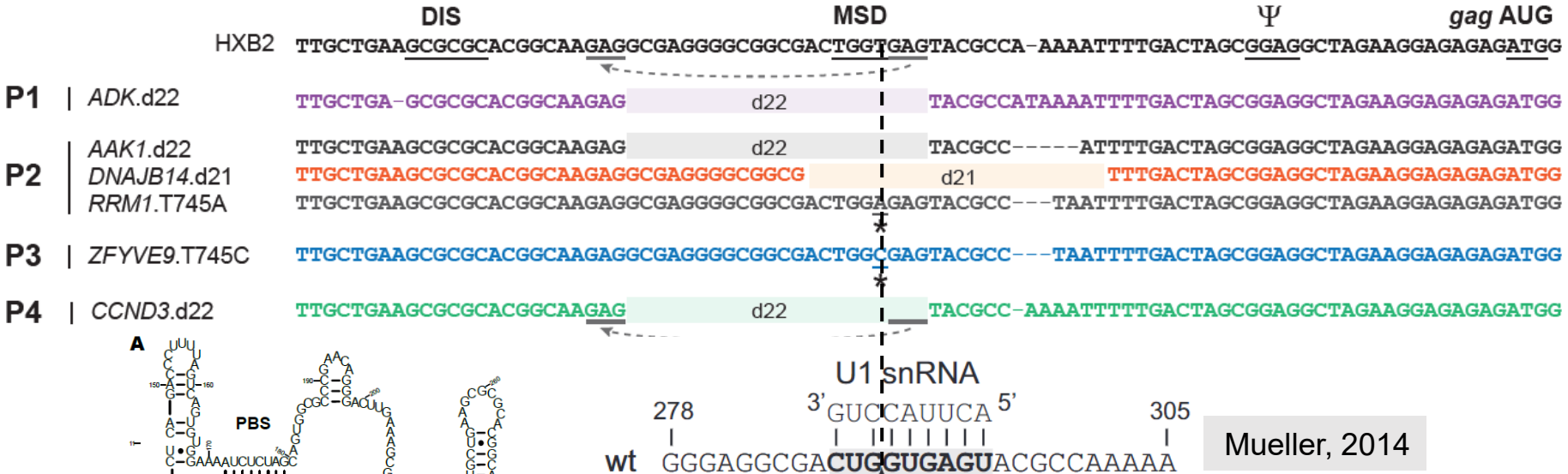
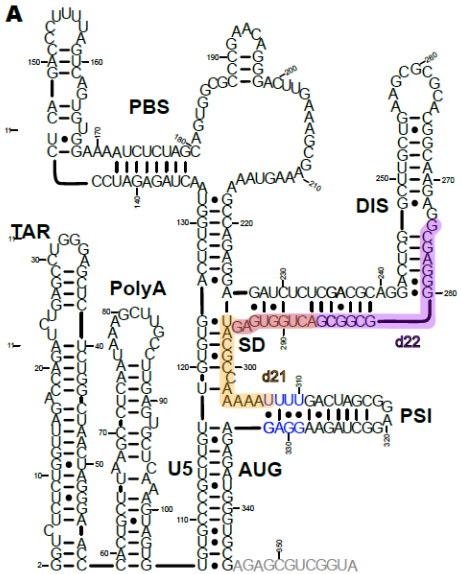
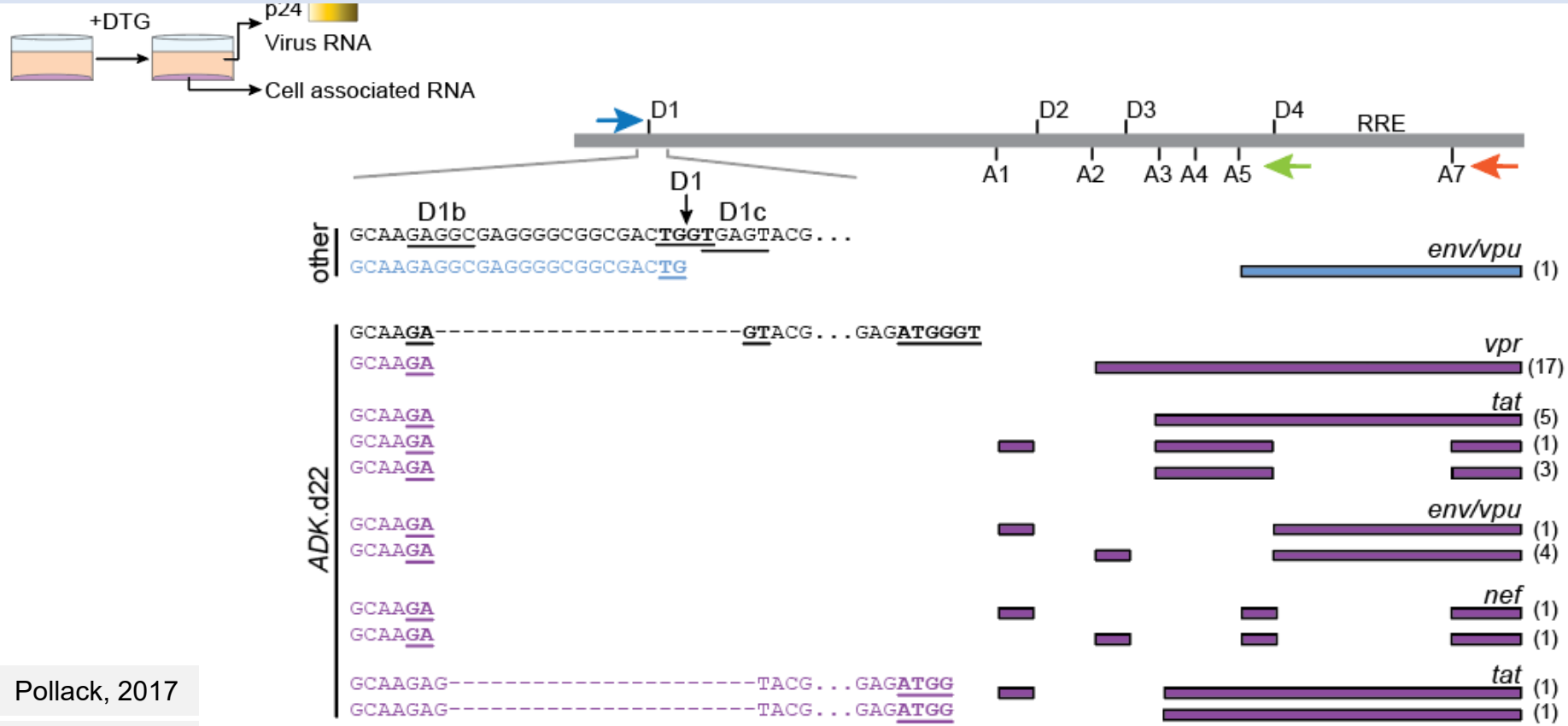




Mueller, 2014

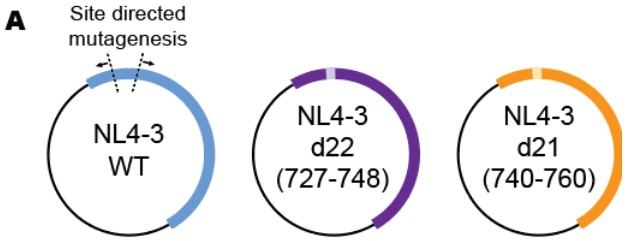
5’L defective proviruses are inducible ex vivo upon T cell activation

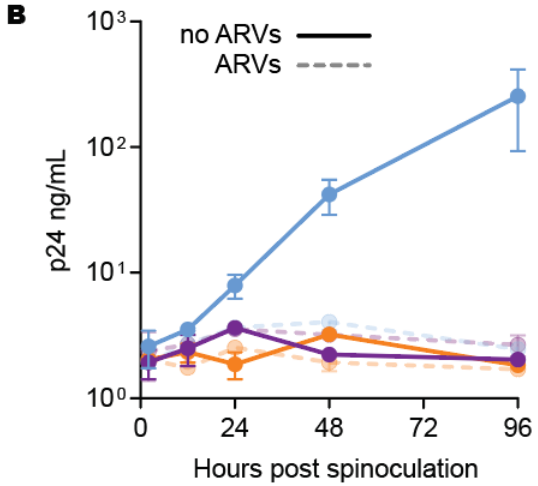


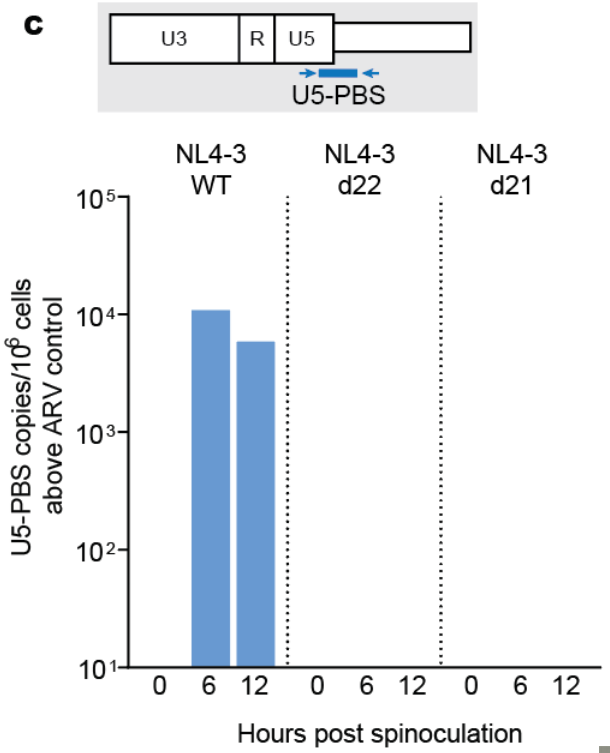
Pollack, 2017

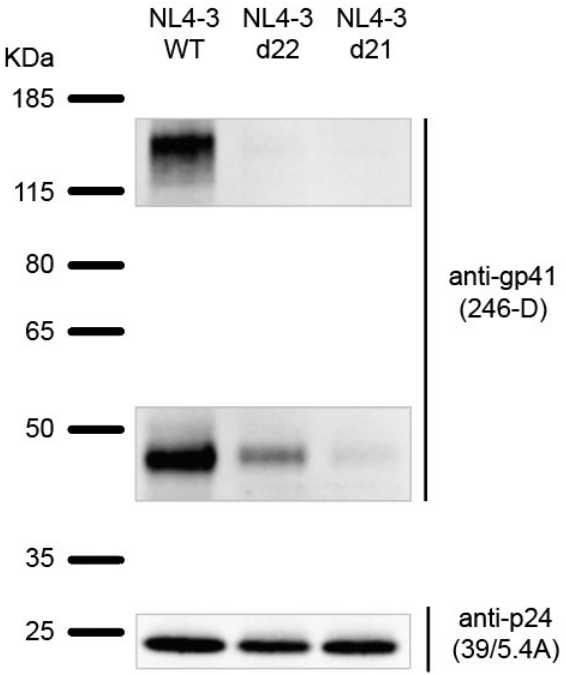
Cole, 2021

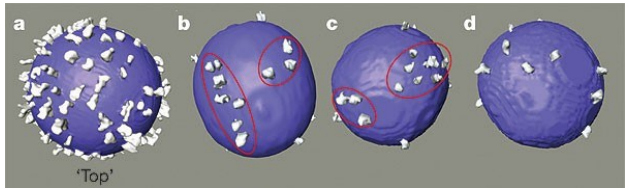
Small 5’ leader deletion lead to noninfectious viral particles



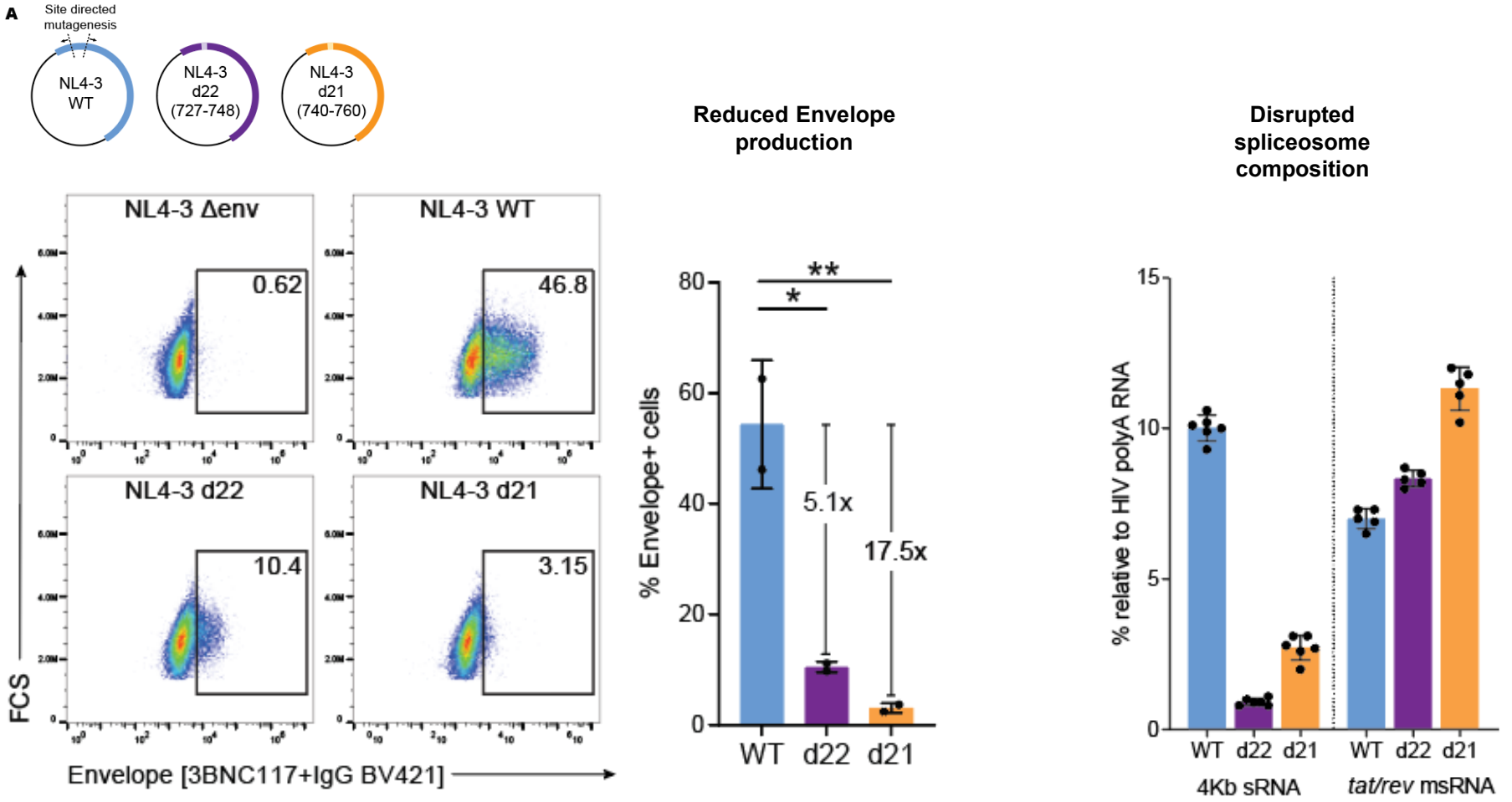




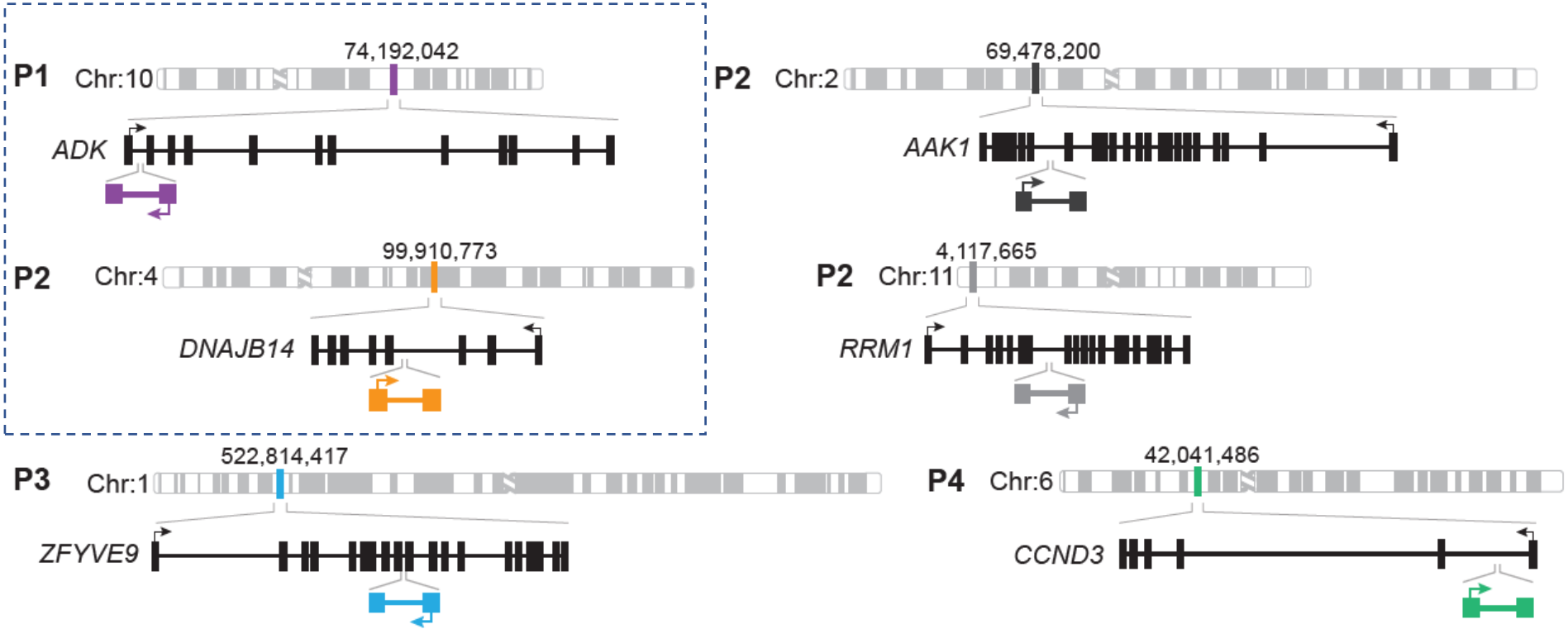




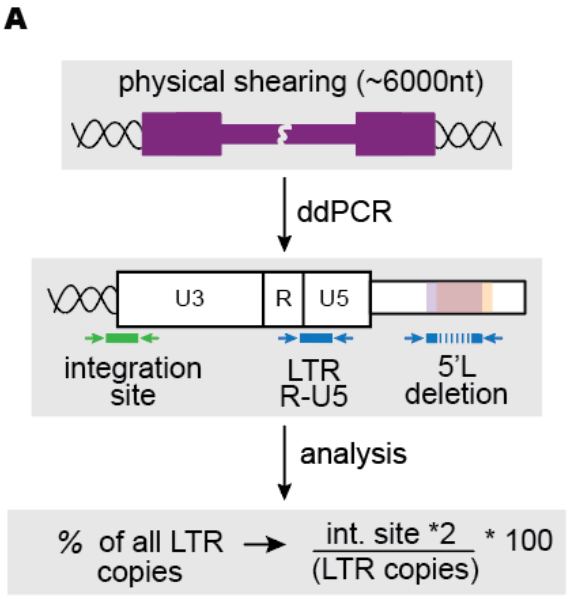
293 cells transfected with 5'L defective vector express lower Envelope

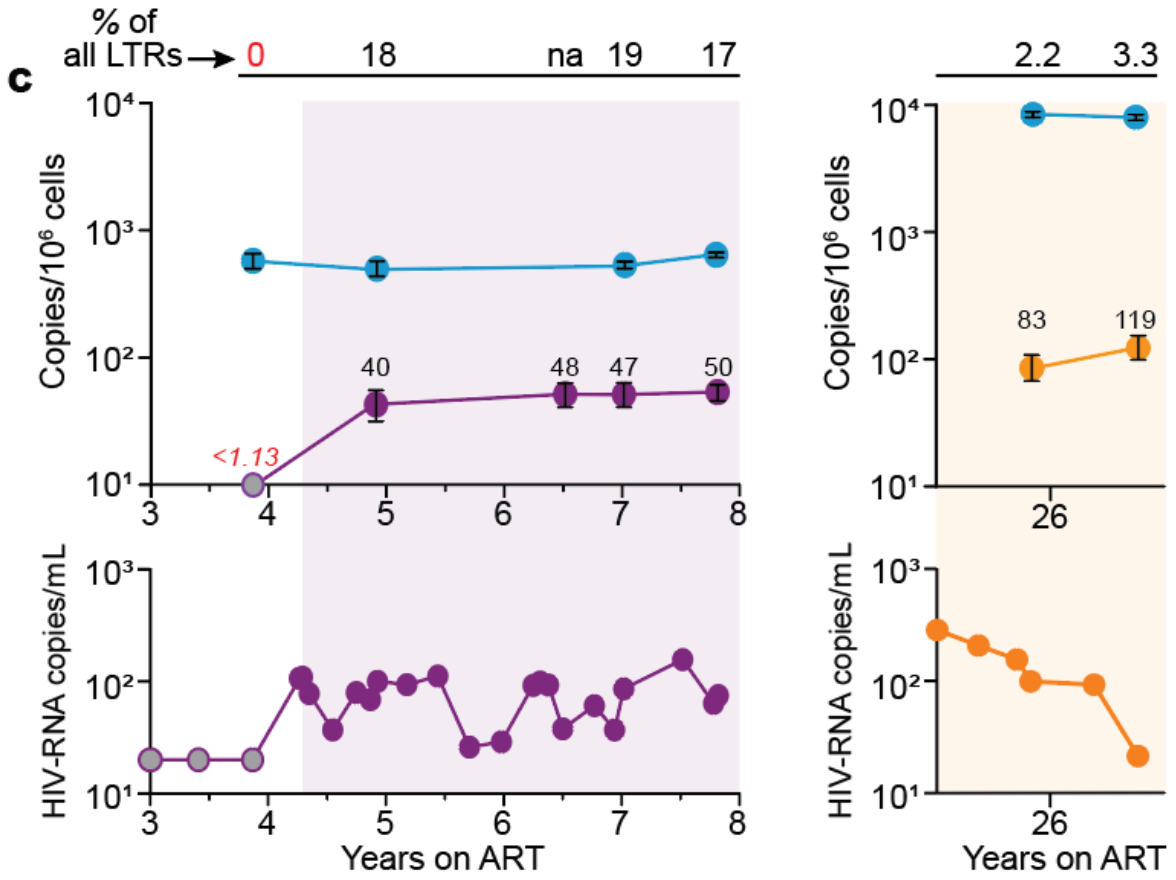


Defective proviruses causing low level viremia are located within genes not linked to HIV insertional mutagenesis



Clones expanded around onset of NSV and are stable over time

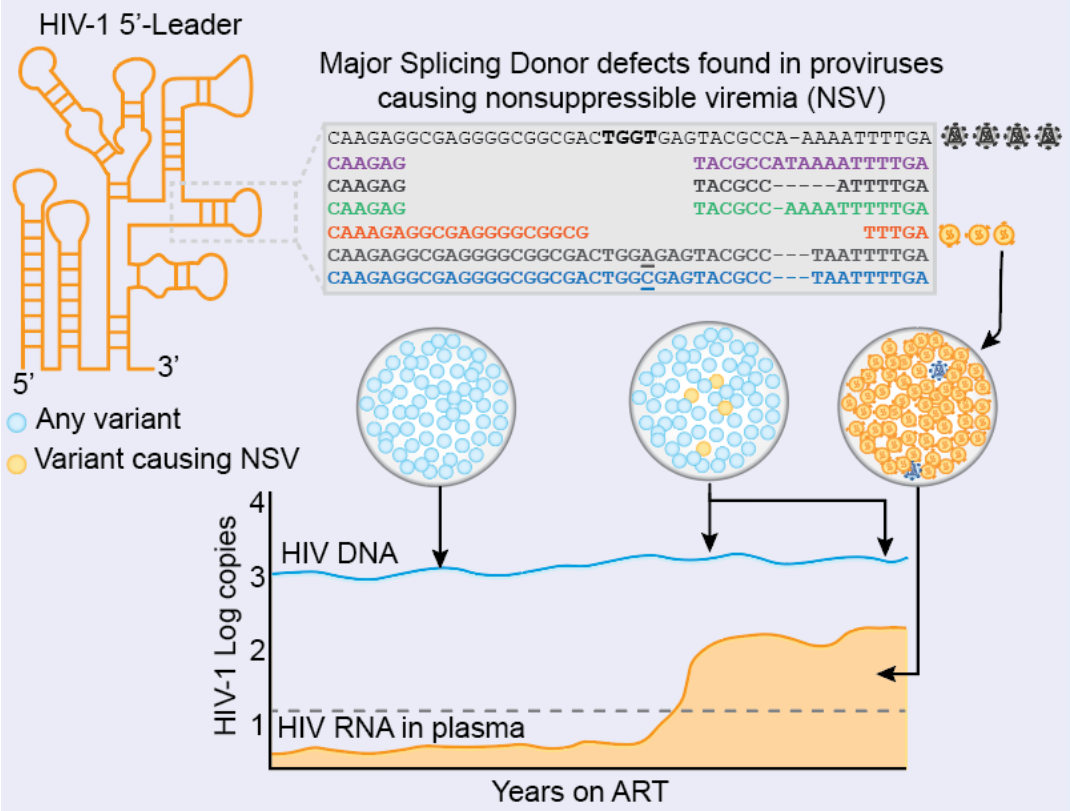




Total LTR
ADK.d22
DNAJB14.d21

ADK.d22 **~10 million** CD4⁺ T cells
DNAJB14.d21 **~32 million** CD4⁺ T cells

Non-infectious viremia is common among those with LLV



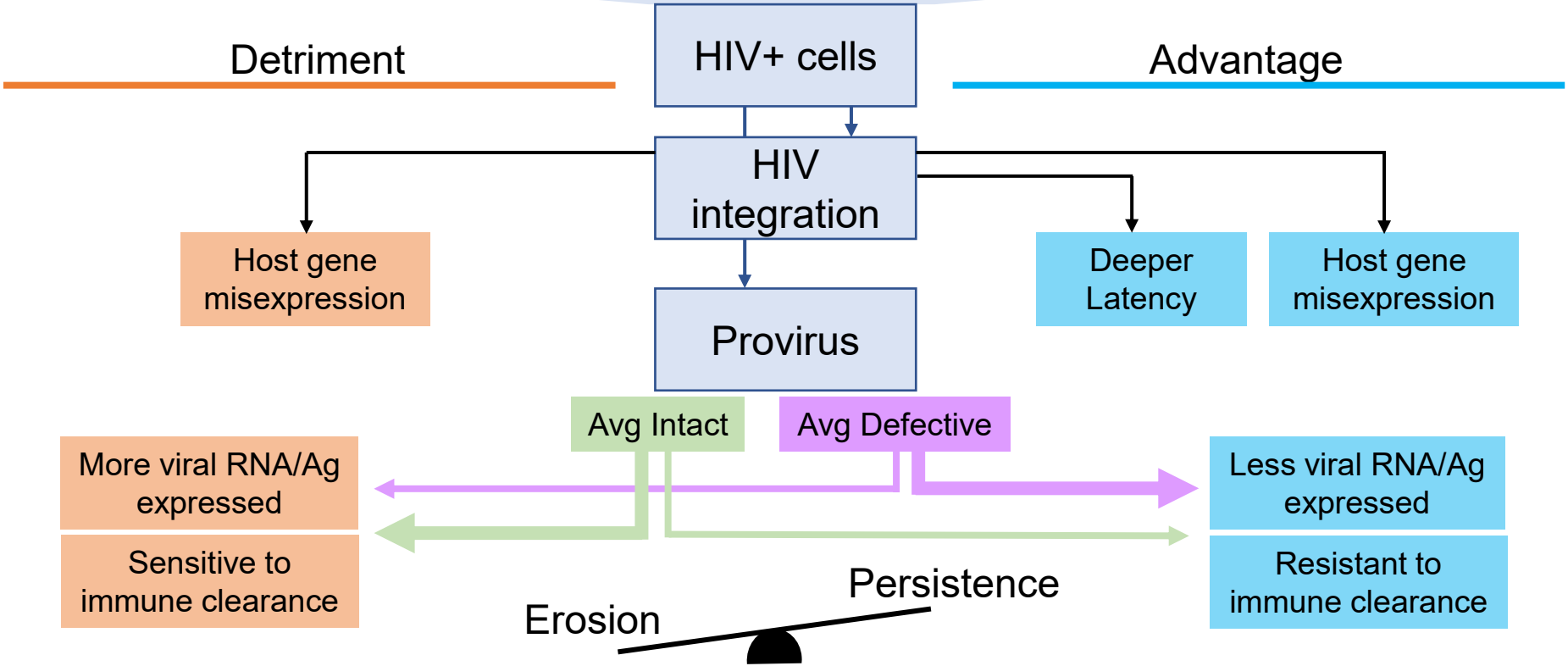
White, Wu, *et al.,* submitted

Clinical ultrasensitive assays should be developed to rule out altogether drug resistance and 5' leader defects

What makes one of these clones constantly produce virus?
-type of stimulation?
-recognition of an antigen that is always present?
-commensal pathogens?
-chronic virus?
-HIV itself?
-self?

Putting everything together…

Proviruses persist in those cells that are meant to persist
(expansion, immune response, stimuli, cell program, resistance to death, …)



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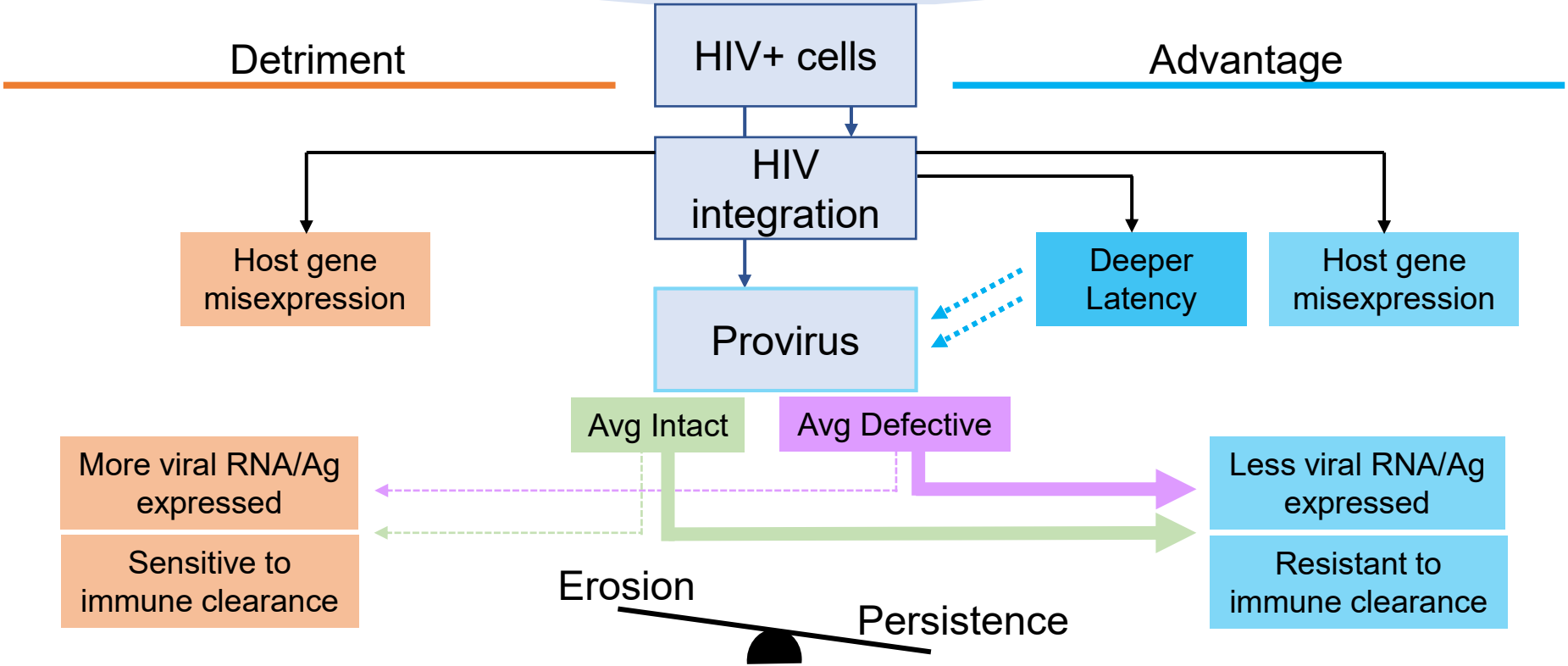
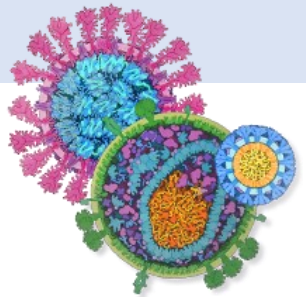


Take home messages



- There are **multiple, layered factors** shaping the HIV reservoir. No single factor can explain the persistence of every HIV-infected cell.
- On average, intact and defective **proviruses are expressed at similar, low frequencies.**
- **Integration** provides a selective advantage in only few infected cells.
- Clonal expansion is mostly driven by **immune stimuli.**
- **Large, sharable, datasets are needed**. Additional data on paired proviral structures and integration sites will help understanding the persistence of intact proviruses caused by specific chromosomal locations (e.g. ZNF genes).
- **Defective proviruses** with specific defects **can cause low-level viremia** and complicate art management. Their role in pathogenesis remains unclear.

Acknowledgments

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MGH
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Xu Yu

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DELANEY COLLABORATORY

Delaney AIDS Research Enterprise
DARE
to find a cure

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