



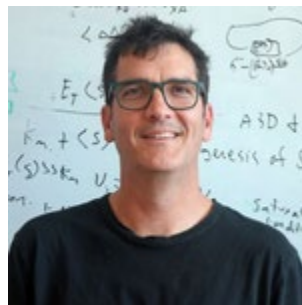
Seminar

From Structure to Function: The HIV Accessory and Regulatory Complexes Center

Monday, February 13, 2023



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Assistant Professor of Medicine
Division of Infectious Diseases
*Northwestern University
Feinberg School of Medicine*



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Professor of Pharmaceutical Chemistry
*UC San Francisco
School of Pharmacy*

Harnessing Tat-dependent Transcriptional Rewiring to Develop Dual-acting Latency Reversing and Promoting Agents

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Assistant Professor, Division of Infectious Diseases

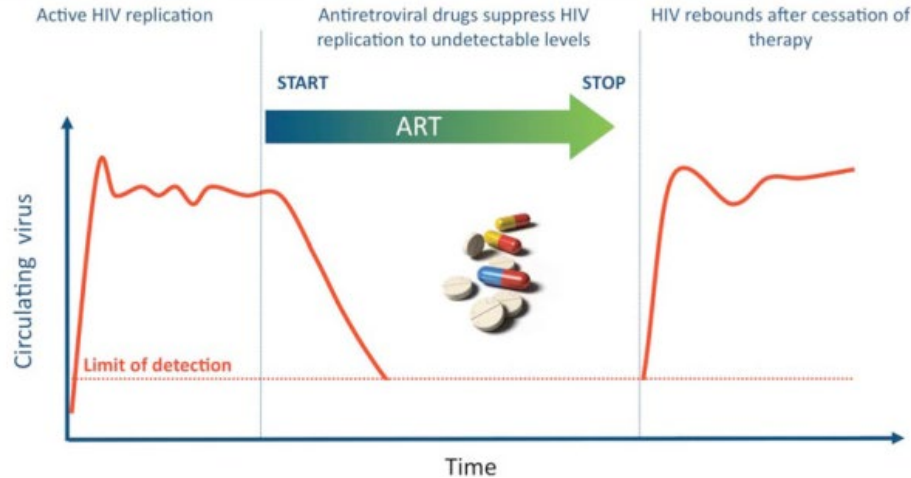
Associate Director, Center for Pathogen Genomics and Microbial Evolution

Director, Emerging and Re-emerging Pathogens Program

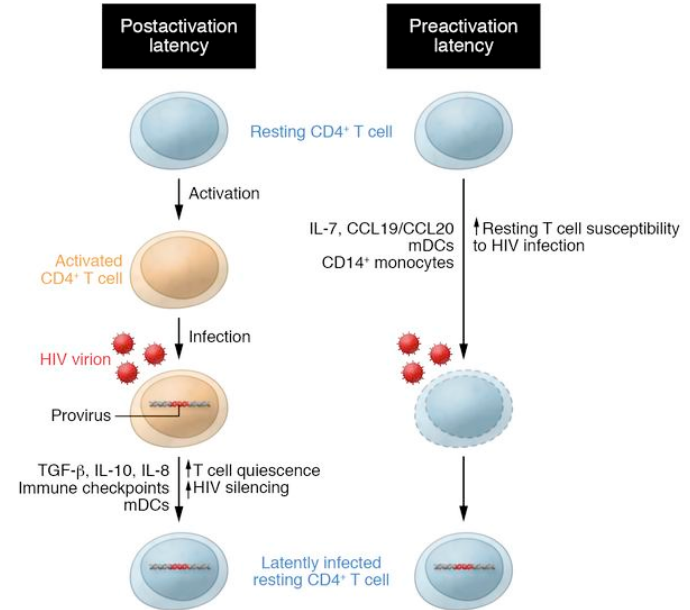
Northwestern University

Third Coast CFAR Seminar Series: February 13, 2023

Persistence of the HIV latent reservoir is the major barrier to the development of a functional cure



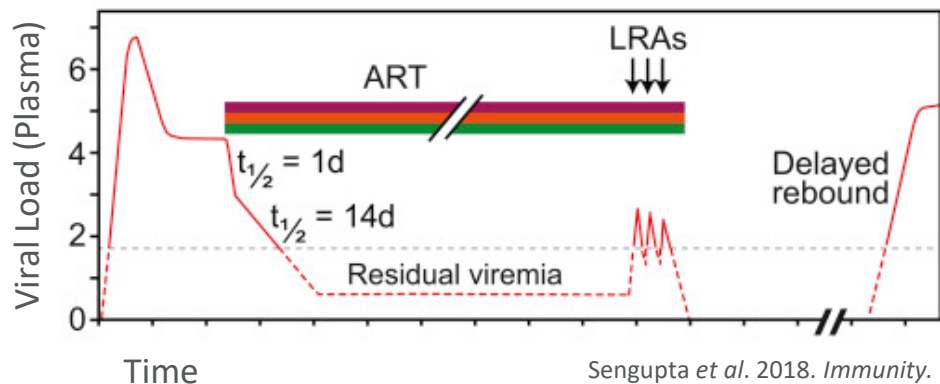
Kulpa & Chomont. 2015. *J Virus Erad.*



Dufour *et al.* 2020. *JCI.*

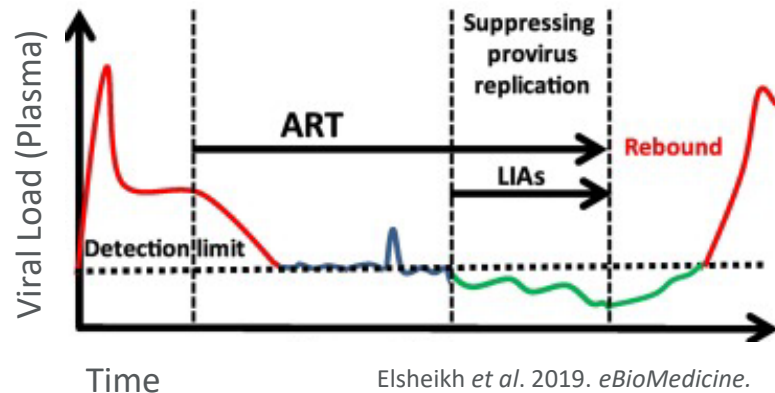
Different strategies to deplete the reservoir and delay rebound following ART cessation

“Shock and Kill”



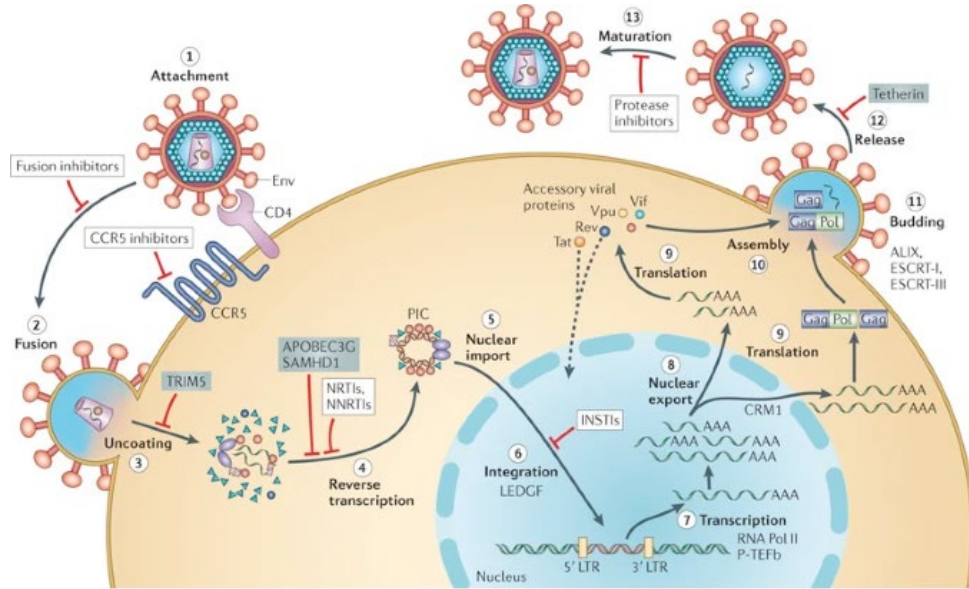
Latency Reversing Agents (LRAs)

“Block and Lock”

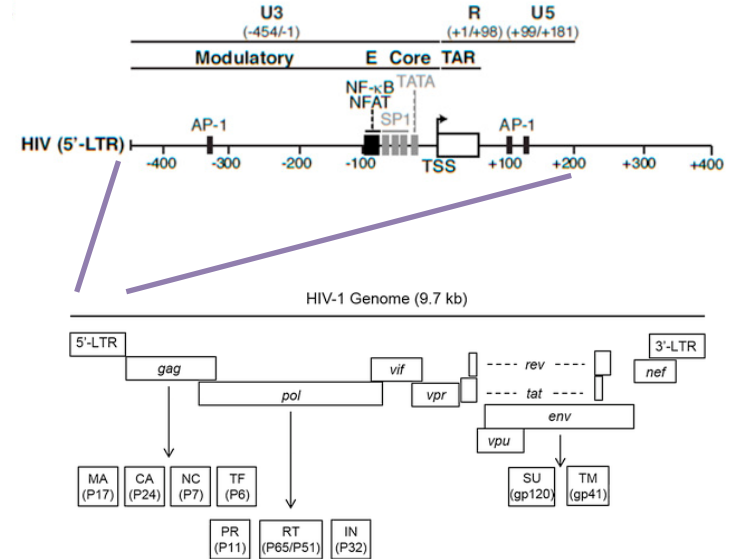


Latency Promoting Agents (LPAs)

Integrated HIV proviruses are transcribed by RNA Pol II and are subject to proximal promoter pausing



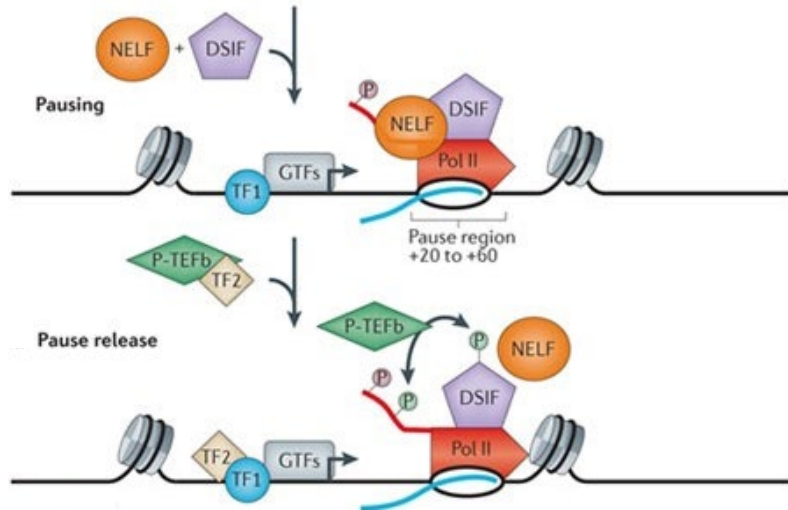
Nature Reviews | Microbiology



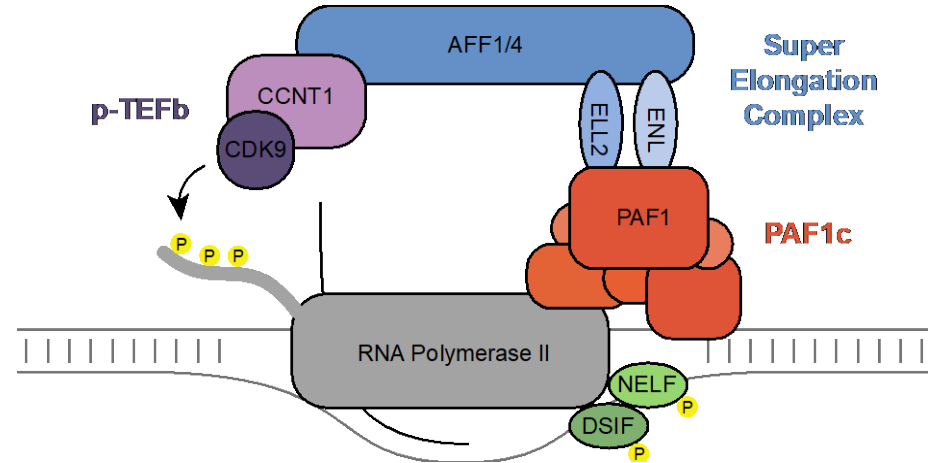
Engelman & Cherepanov. 2012. *Nat. Rev. Microbiol.*

Shukla et al. 2020. *Viruses.*

Licensing of transcriptional elongation is governed by p-TEFb, which is recruited in a highly regulated fashion

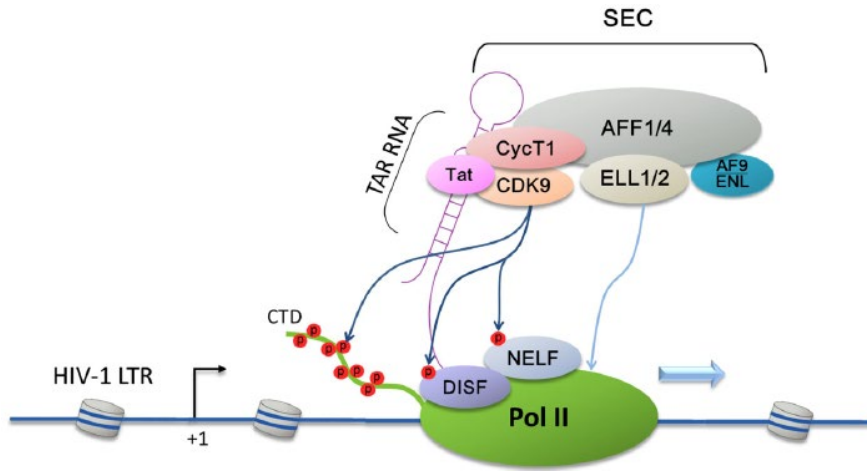


Adelman *et al.* 2012 *Nat. Rev. Gen.*

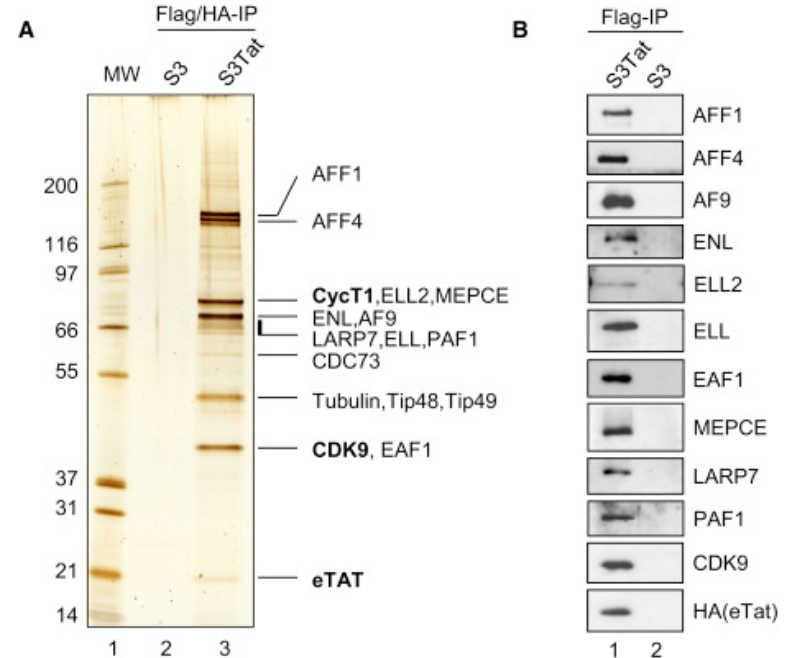


Adapted from: Chou *et al.* 2012 *PNAS*.

HIV overcomes this block by directly recruiting p-TEFb to sites of nascent transcription via the viral Tat protein

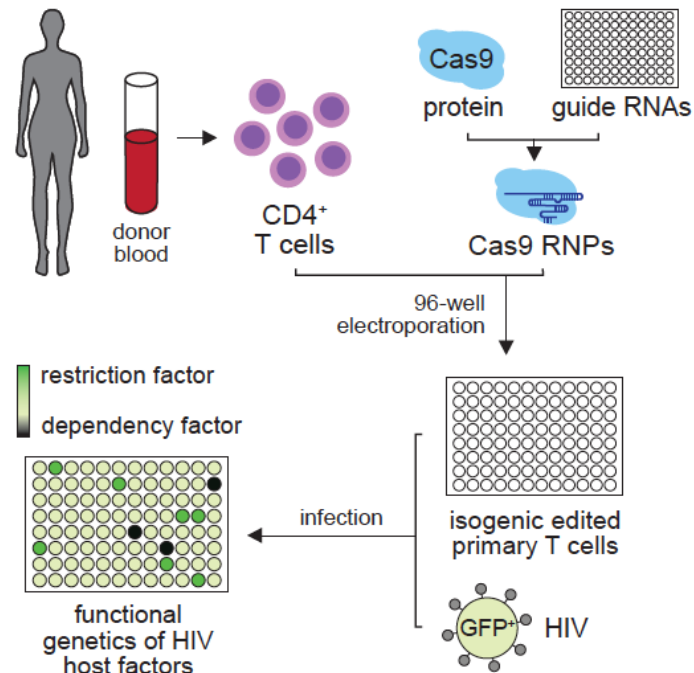
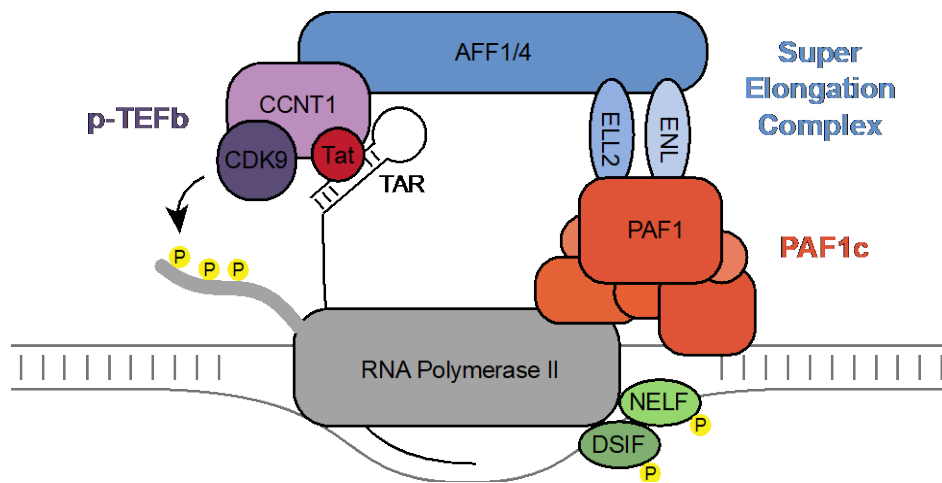


Liu et al. 2014 JZU Sci.



Sobhian et al. 2010 Mol. Cell

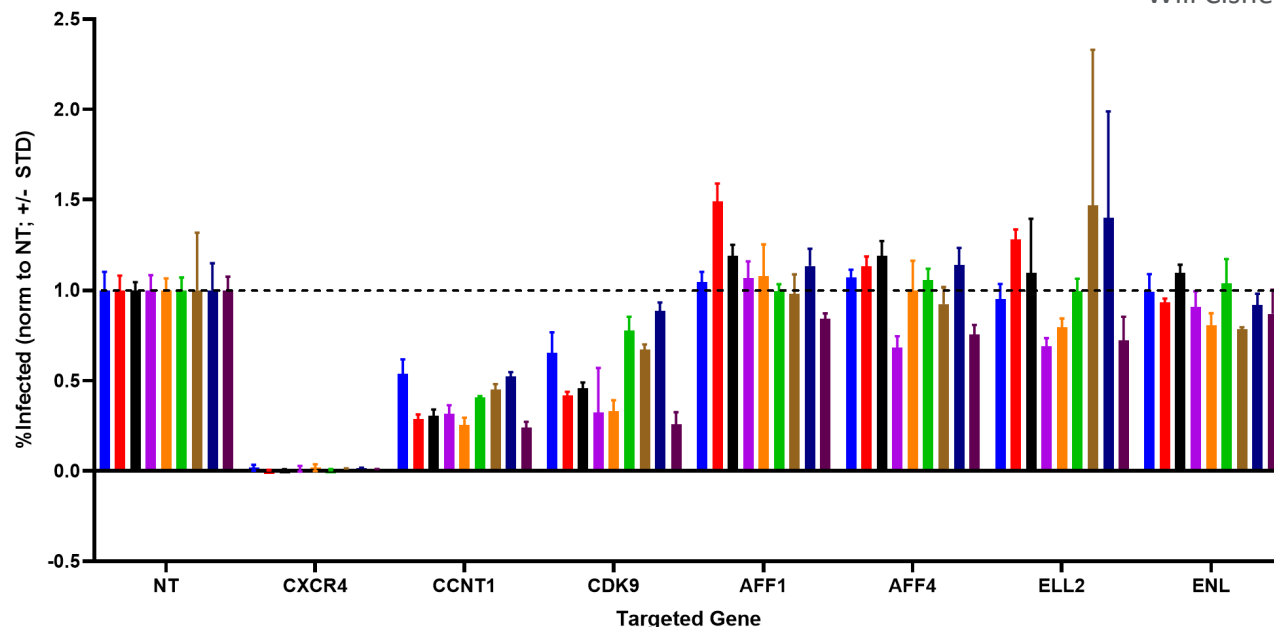
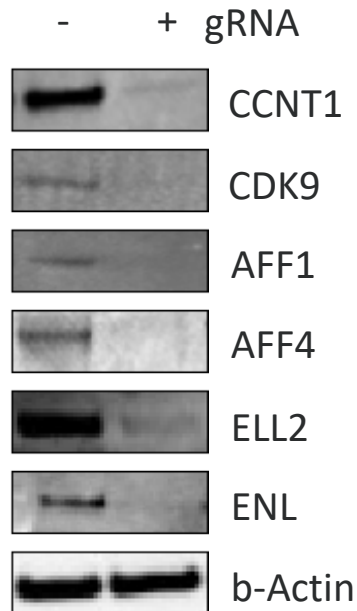
What is the role of the Super Elongation Complex in HIV replication in primary CD4+ T cells?



What is the role of the Super Elongation Complex in HIV replication in primary CD4+ T cells?



Will Cisneros

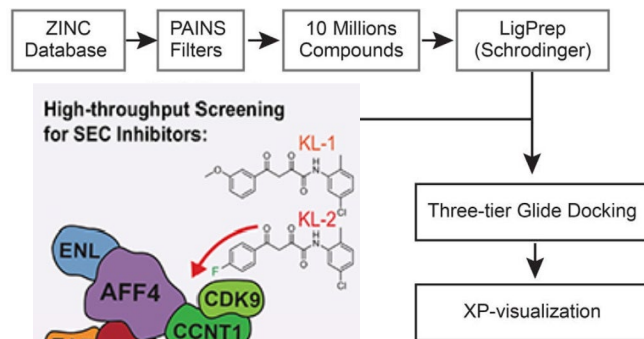


A small molecule inhibitor of the SEC also does NOT inhibit HIV replication in primary CD4+ T cells

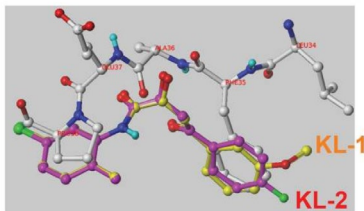


Ali Shilatifard

Ligand Preparation

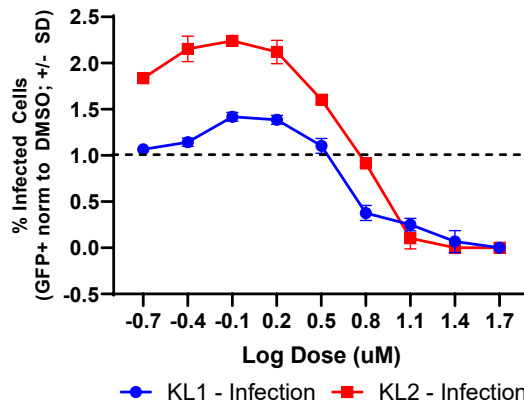


Overlay of compounds with AFF4 (LFAEP) peptide

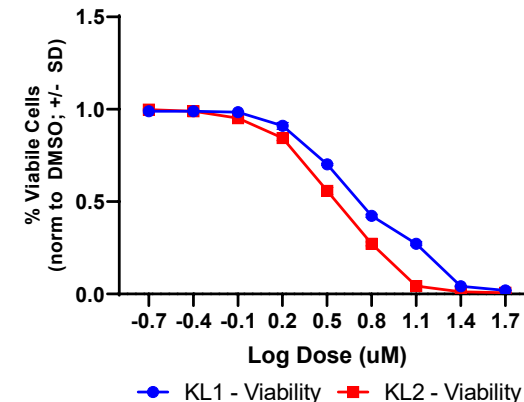


Liang *et al.* 2018 *Cell*.

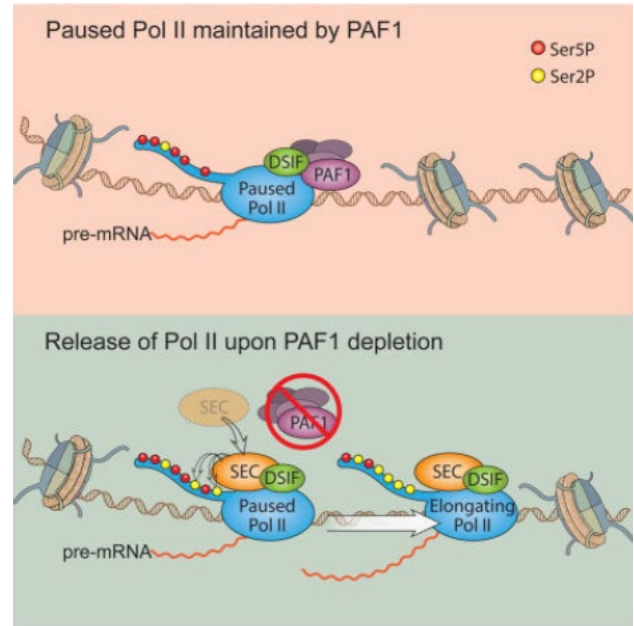
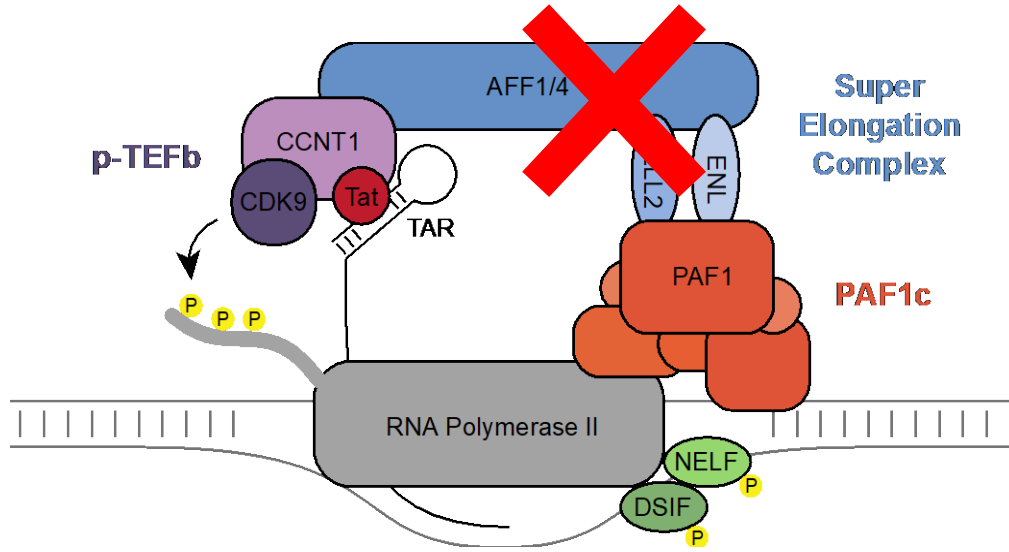
CD4+ T cell Infection - D2



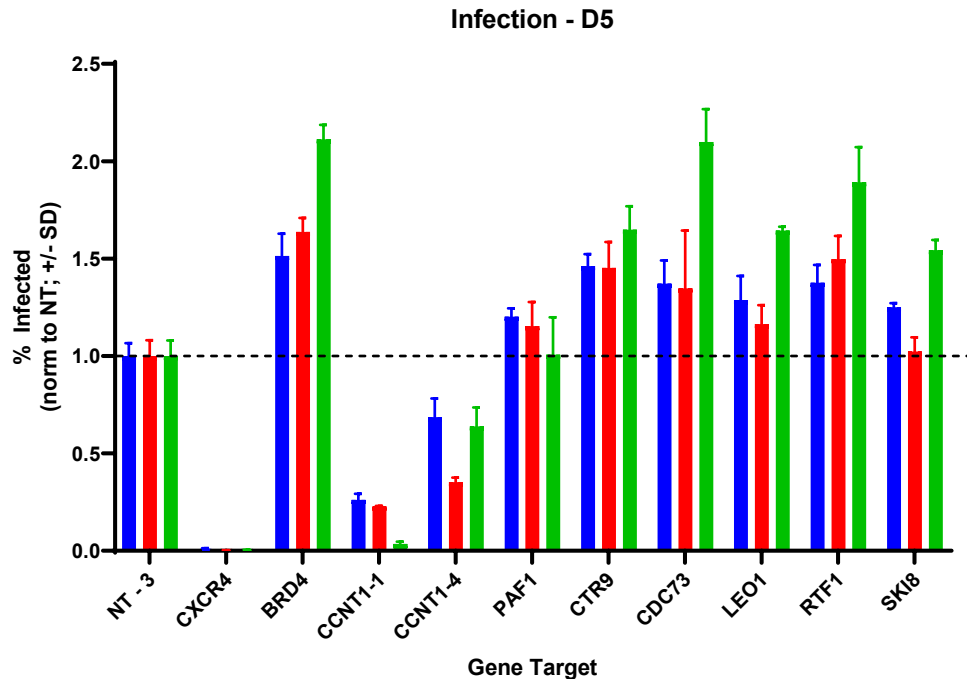
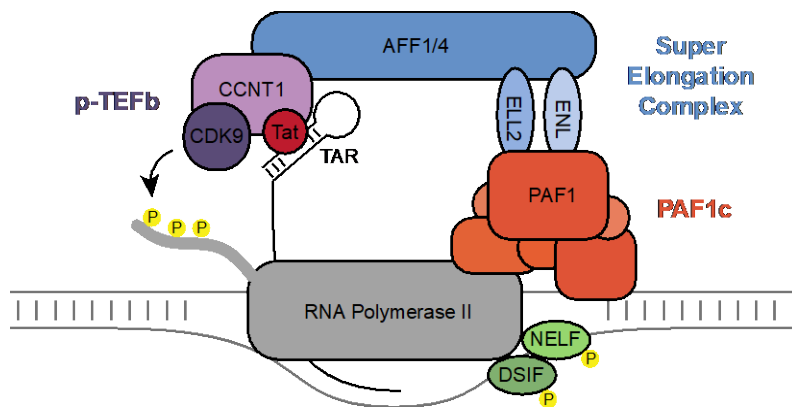
CD4+ T cell Viability - D2



If the SEC is not required for active replication, it implies that the PAF1 complex is ALSO not required for SEC recruitment...



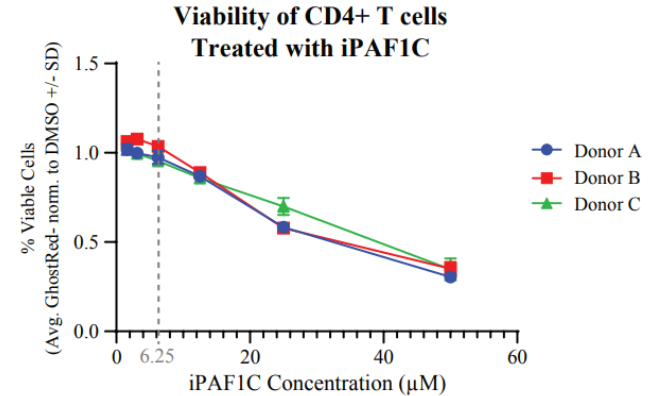
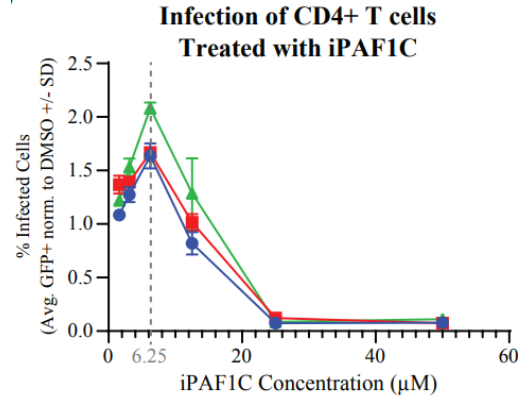
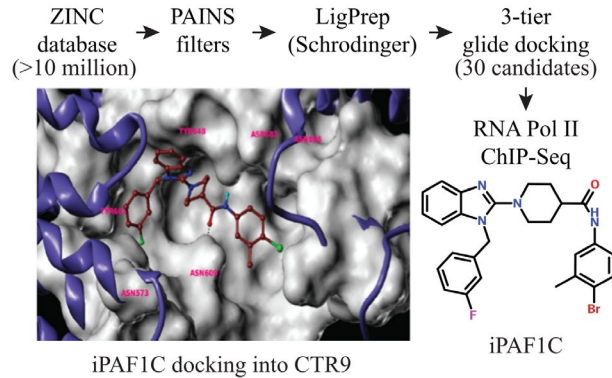
Knockout of PAF1c members increases HIV infection in primary CD4+ T cells



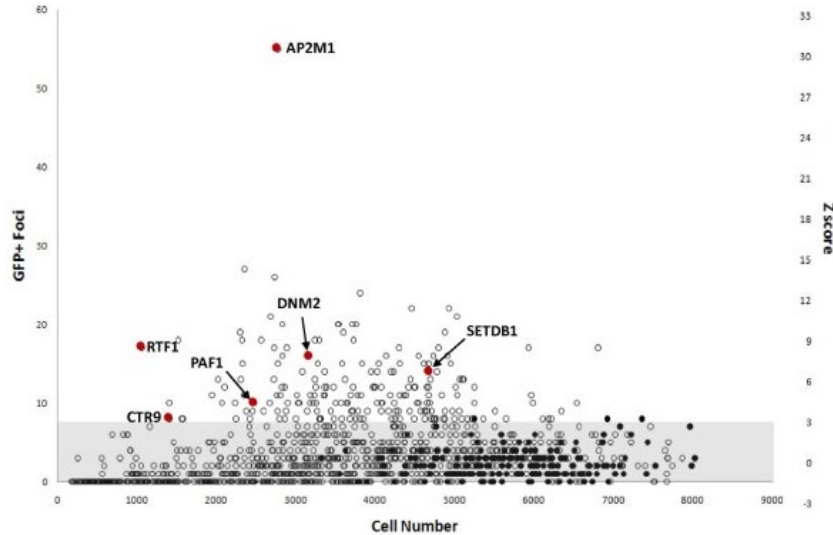
A novel small molecule inhibitor of the PAF1 complex also does increases HIV replication in primary CD4+ T cells



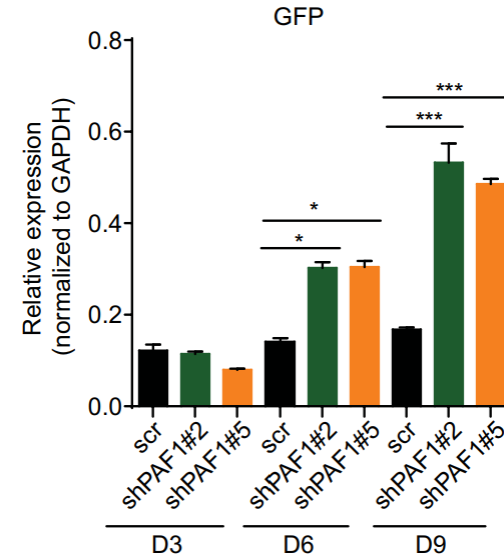
Shimaa Soliman



This is consistent with previous data showing that the PAF1 complex acts as a negative regulator of HIV transcription

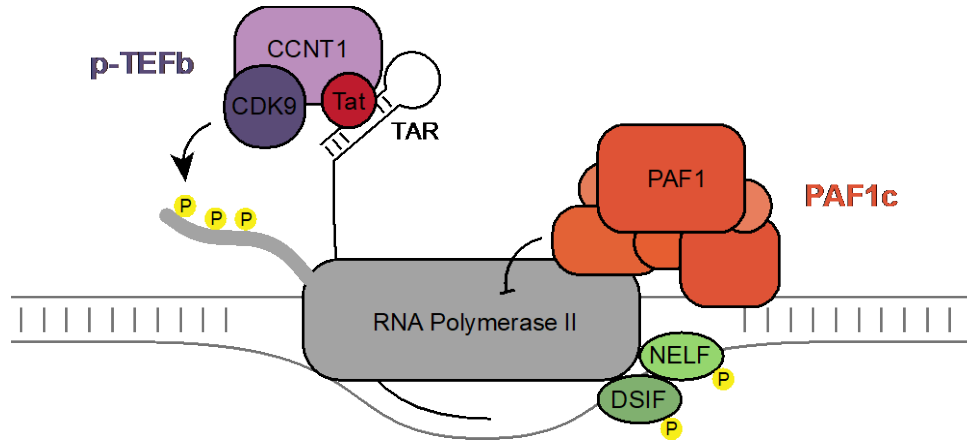


Liu et al. 2011 *Retrovirology*.



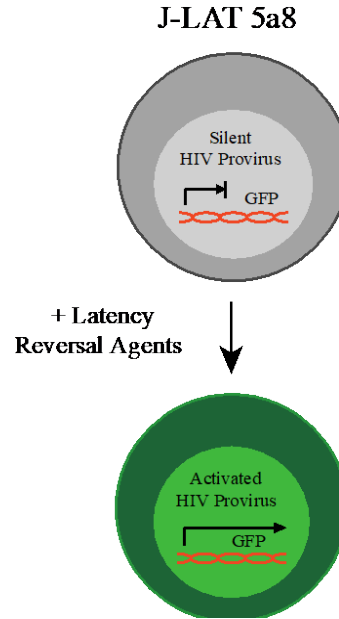
Gao et al. 2020 *Sci. Adv.*

These data suggest that PAF1C inhibitors should act as novel LRAs while SEC inhibitors should have no effect

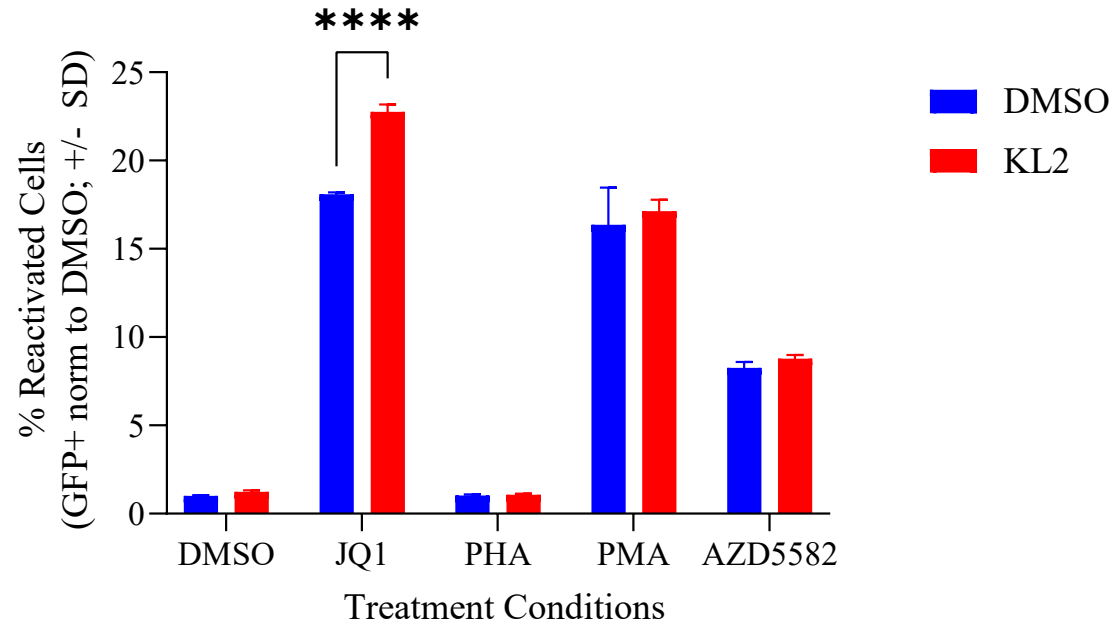


	Function	Inhibitor effect on Latency?
Super Elongation Complex	Not necessary	No effect
PAF1 Complex	Restrictive Factor	Latency reversing

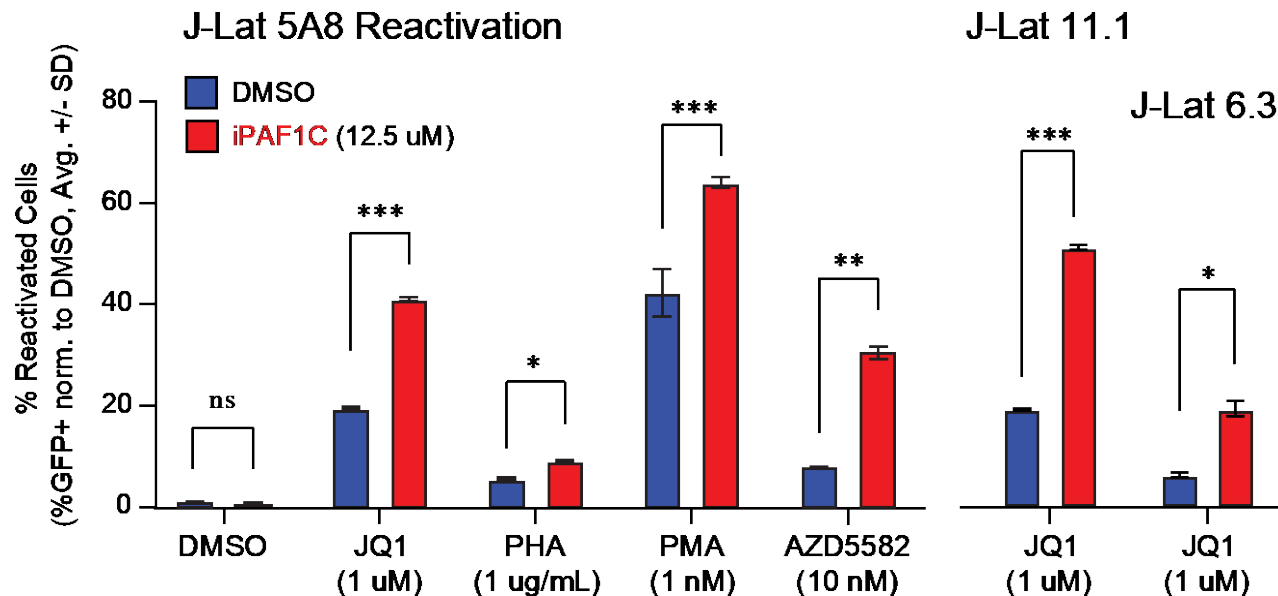
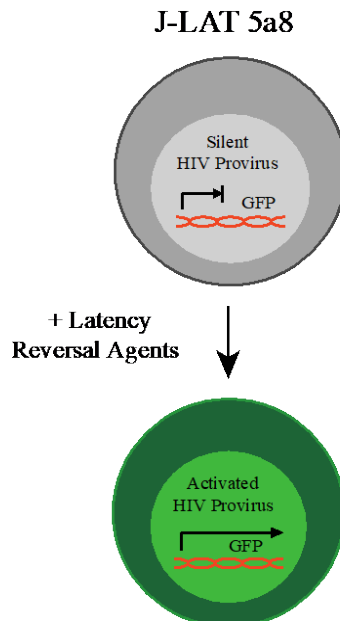
SEC inhibitors do not impact latency reactivation in J-Lat models



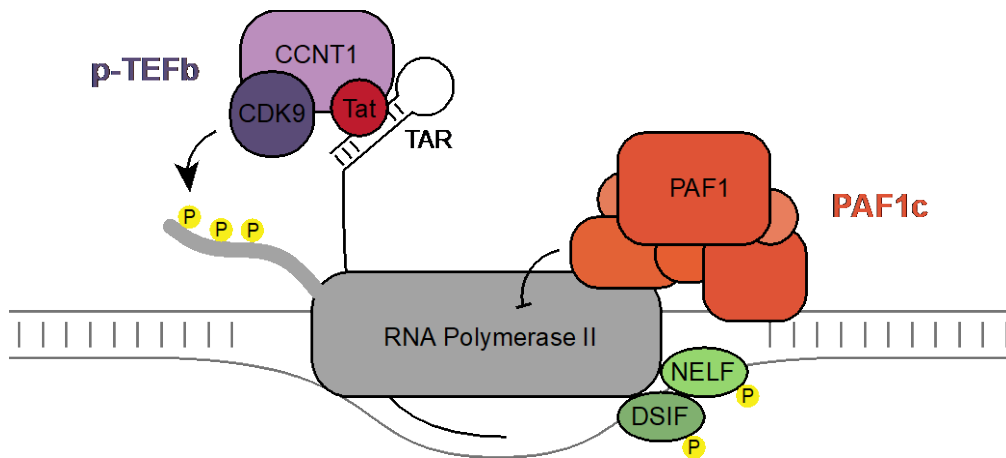
J-Lat 5A8 Reactivation



PAF1c inhibitors synergize with latency reversing agents to increase latency reactivation in J-Lat cells

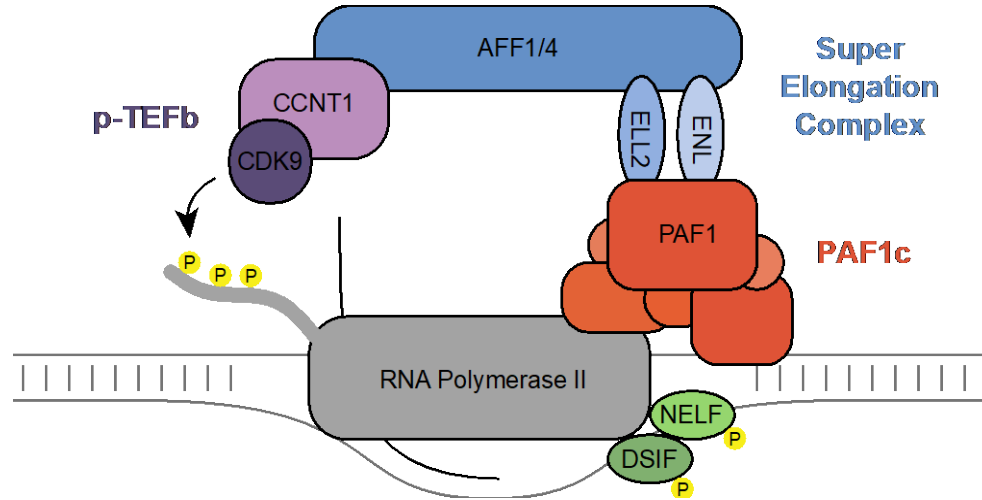


But what happens in the ABSENCE of Tat?



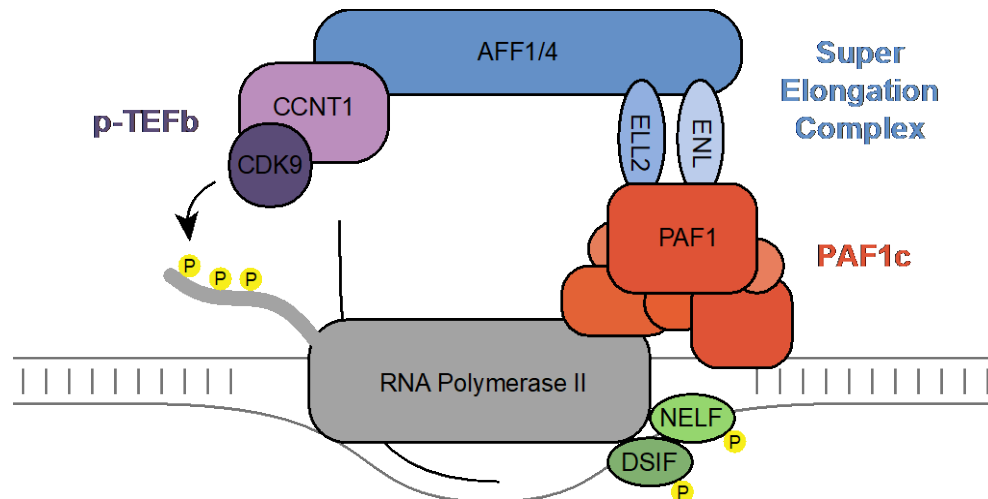
	+ Tat/TAR
Super Elongation Complex	Not necessary
PAF1 Complex	Restrictive Factor

But what happens in the ABSENCE of Tat?



	+ Tat/TAR	- Tat/TAR
Super Elongation Complex	Not necessary	Dependency Factor
PAF1 Complex	Restrictive Factor	Dependency Factor

But what happens in the ABSENCE of Tat?

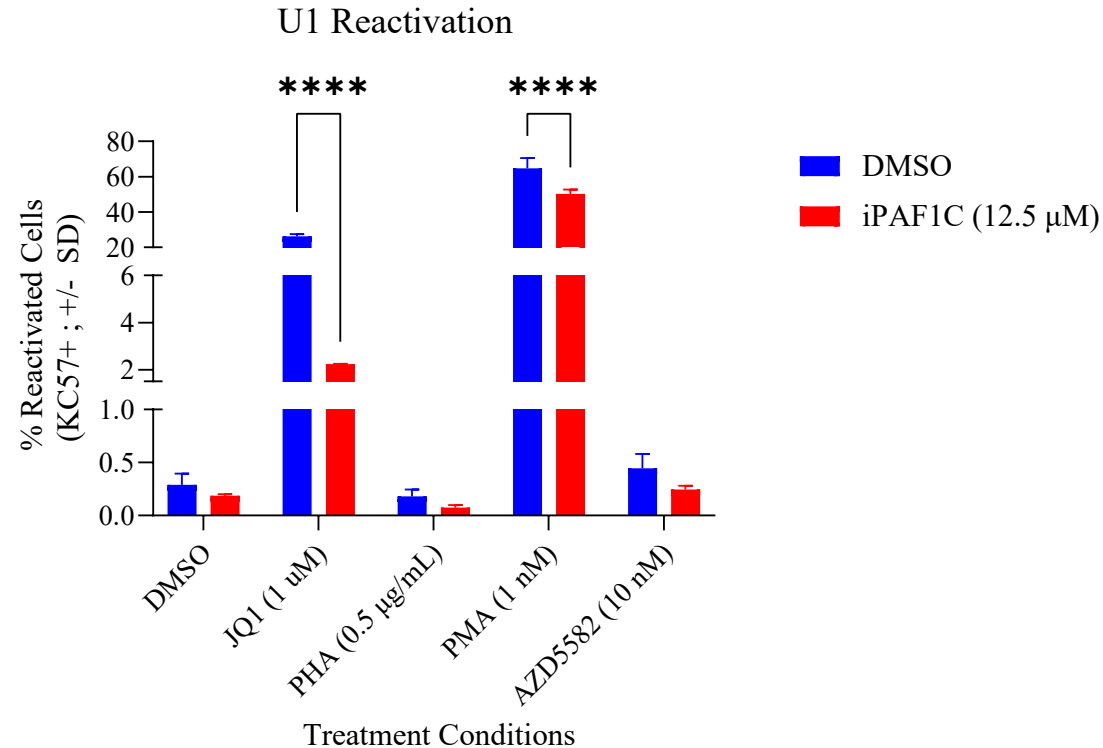


Inhibitor	+ Tat/TAR	- Tat/TAR
Super Elongation Complex	n/a	Latency Promoting
PAF1 Complex	Latency Reversing	Latency Promoting

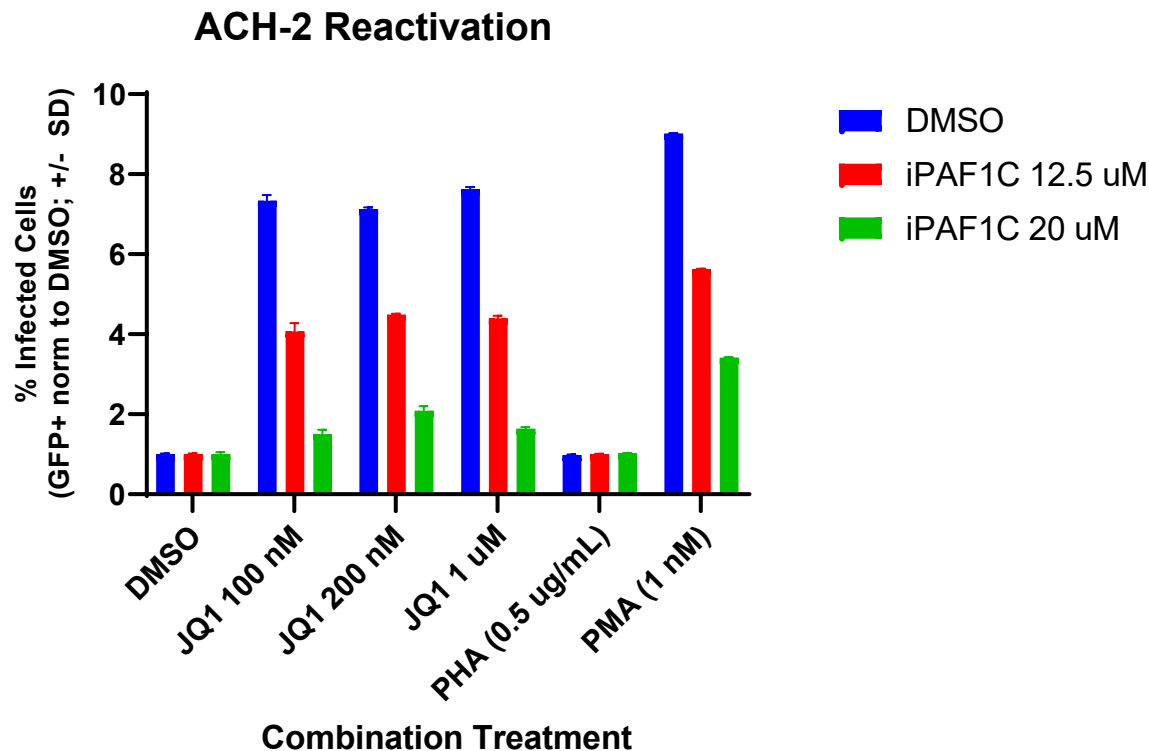
We can use cell line models of HIV latency that differ in Tat functionality

	Cell Line	Notable proviral mutation(s)	Integration Site
Functional Tat/TAR	J-Lat 5a8	FS in Env; Nef-	MAT2a
	J-Lat 6.3	FS in Env; Nef-	undetermined
Non-functional Tat/TAR	ACH-2	TAR mutant	NT5C3A
	U1	Tat mutant	AC079807.4 (X Chr)

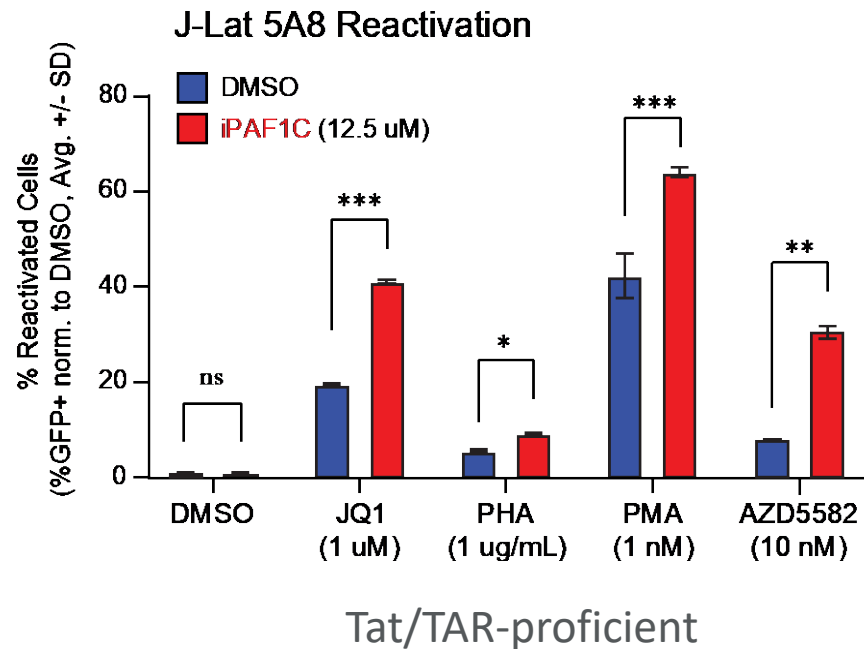
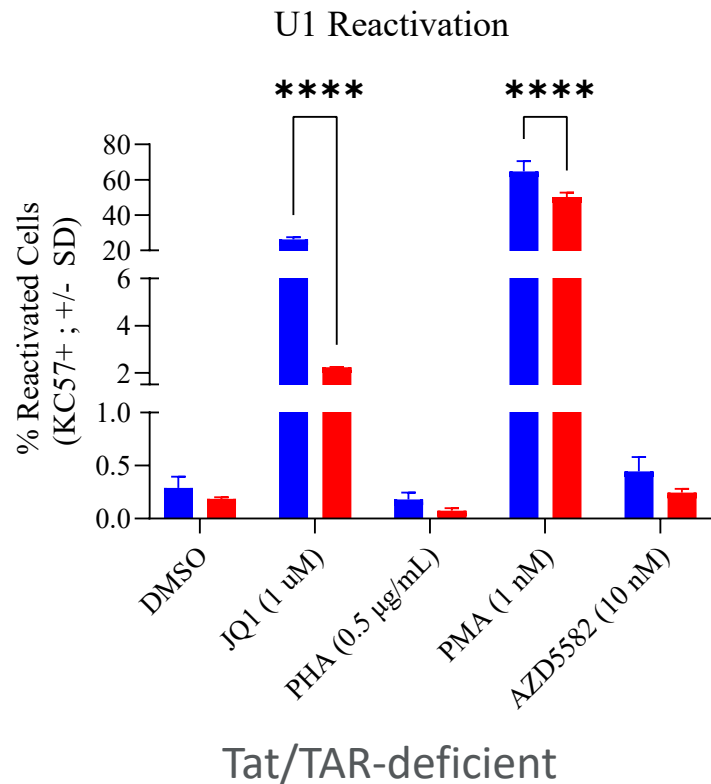
PAF1c inhibitors **decrease** the potency of latency reversing agents in Tat-TAR deficient models (U1)



PAF1c inhibitors **decrease** the potency of latency reversing agents in Tat-TAR deficient models (ACH-2)

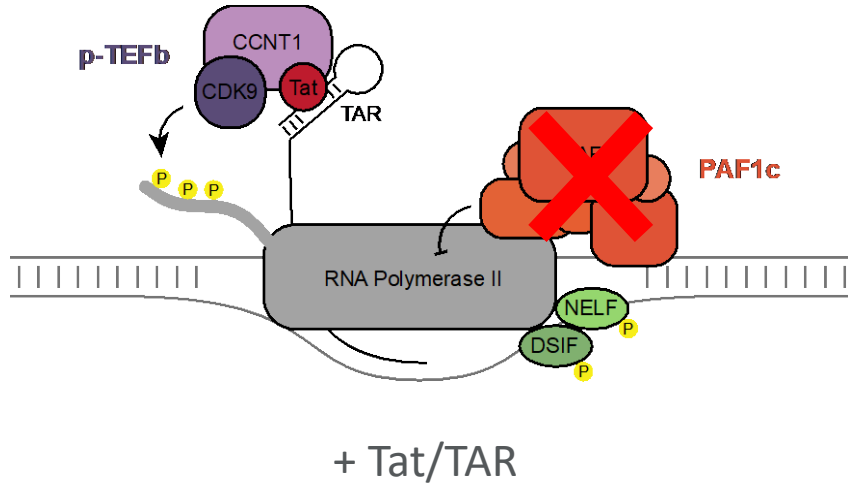


PAF1c inhibitors **decrease** the potency of latency reversing agents in Tat-TAR deficient models (U1)

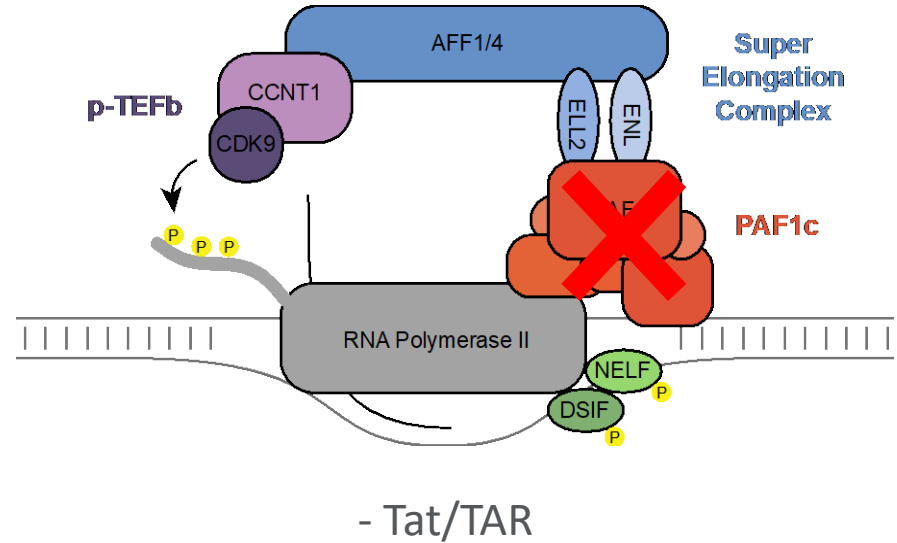


PAF1c inhibitors are dual-acting latency reversing and promoting agents dependent on Tat expression

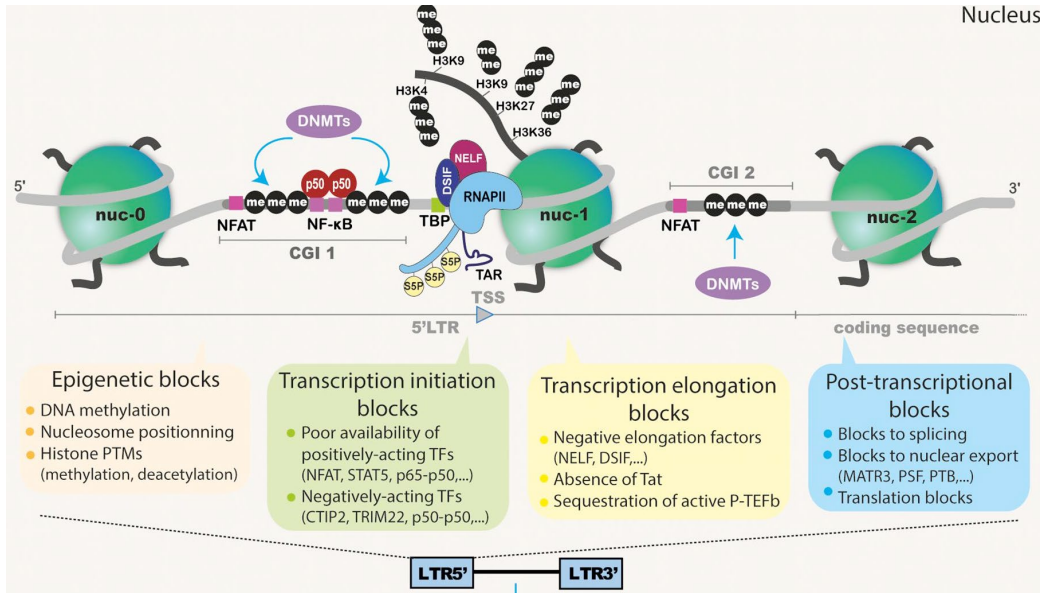
Latency Reversing Agent (LRA)



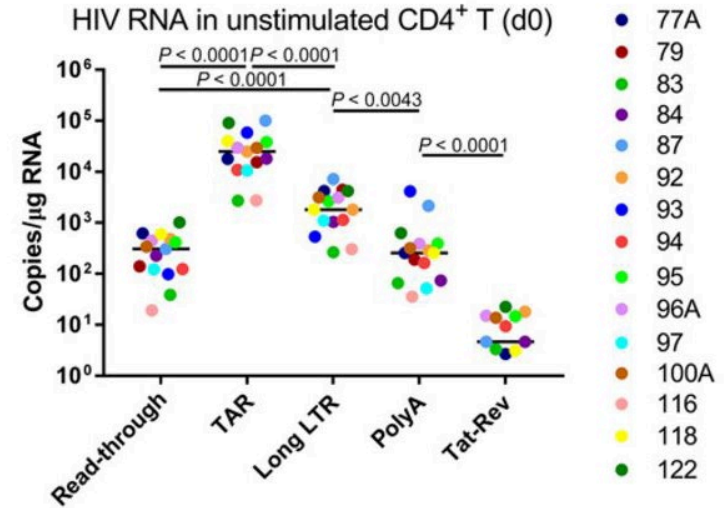
Latency Promoting Agent (LPA)



Transcriptional blocks in latent proviruses are multifaceted



Ait-Ammar et al. 2020. *Front. Microbiol.*



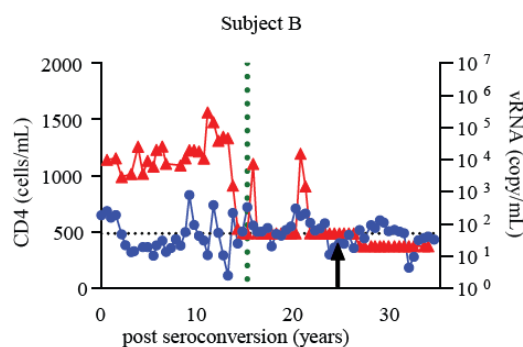
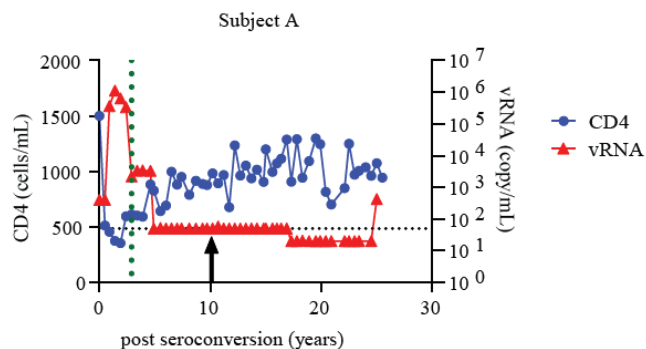
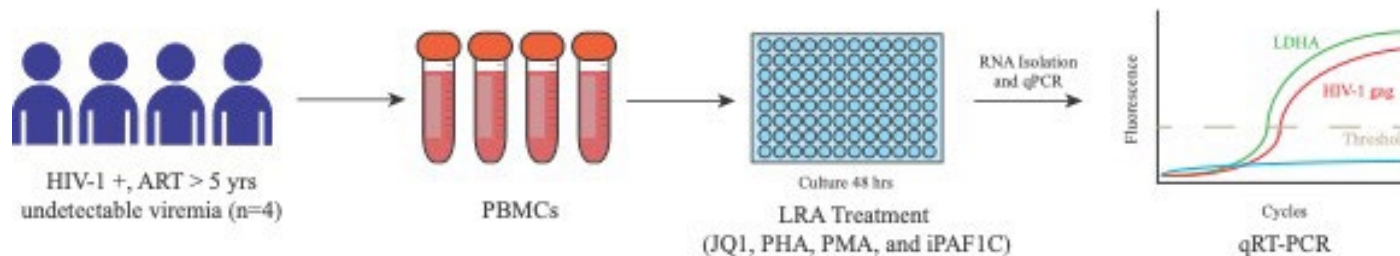
Yukl et al. 2018. *Science Translational Medicine.*

What effect does iPAF1C have in cells from PLWH?



Steve Wolinsky

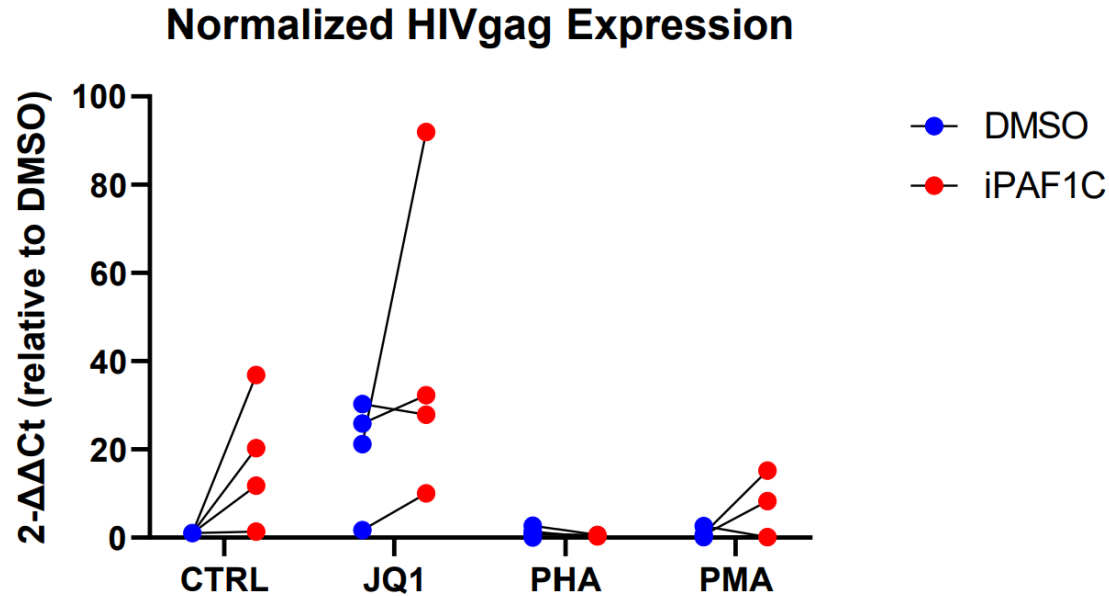
MWCCS
MACS/WIHS COMBINED COHORT STUDY



Experimental Background

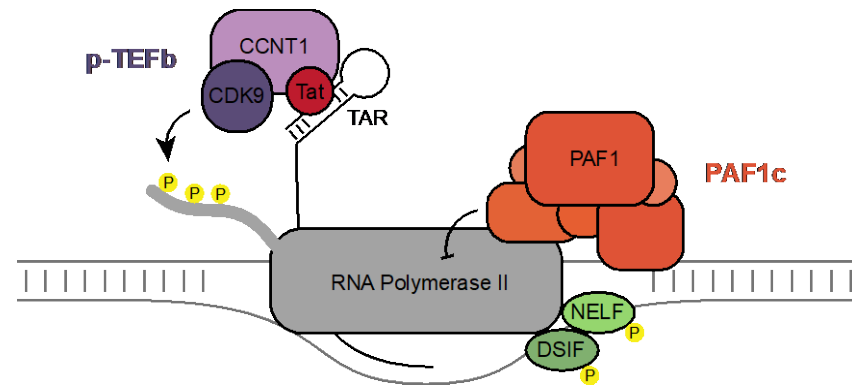
- 4 HIV+ patients in MACS cohort
- 5+ cumulative years of ART
- At time of blood draw, had undetectable viral RNA levels

PAF1c inhibitors increased expression of cell-associated HIV RNA in cells from PLWH



First-in-class PAF1C inhibitors act as dual latency reversing AND latency promoting agents in a Tat-dependent manner

1. How does iPAF1C synergize with canonical LPAs?
2. How can we monitor reactivation as a function of Tat expression at the single cell level in patient cells?
3. Can we improve compound efficiency through medicinal chemistry?
4. Are there other dual-acting transcriptional complexes that can be chemically targeted?



Acknowledgements



Northwestern Medicine®
Feinberg School of Medicine



Ali Shilatifard



Steve Wolinsky



Shima Soliman



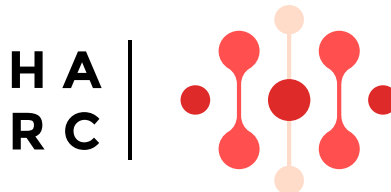
Will Cisneros



U19 AI135964



P30 AI117943



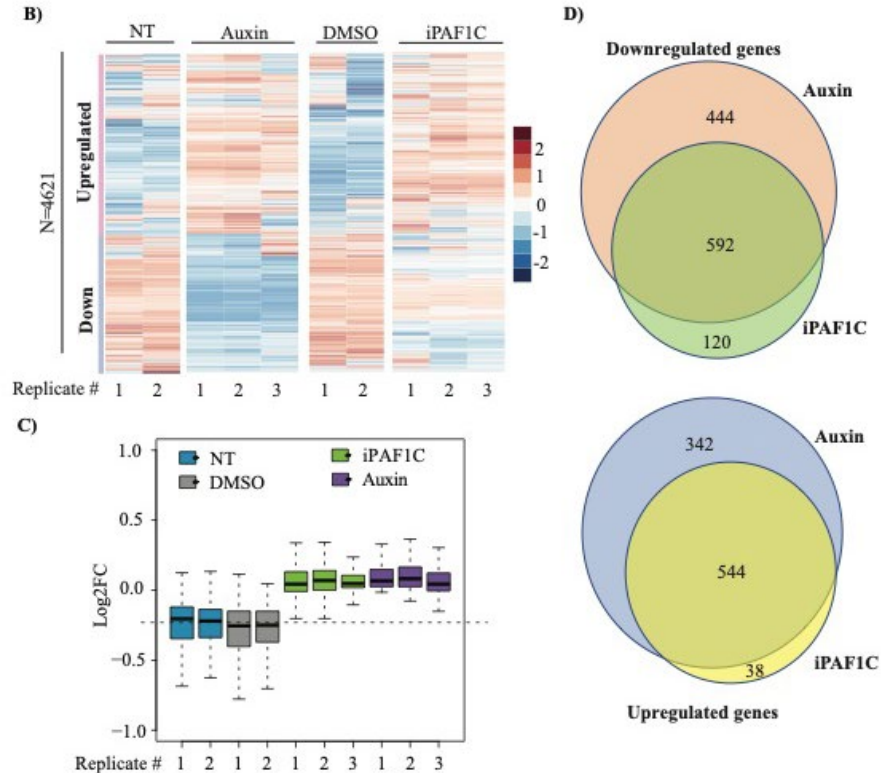
U54 AI170792

R01 AI167778
R01 AI165236
R01 AI150455
R01 AI150998
R21 AI163912

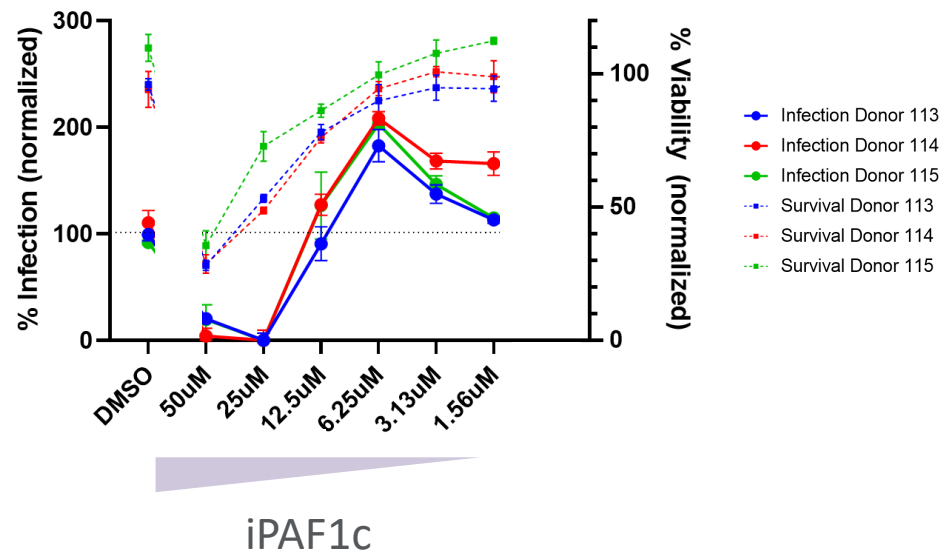
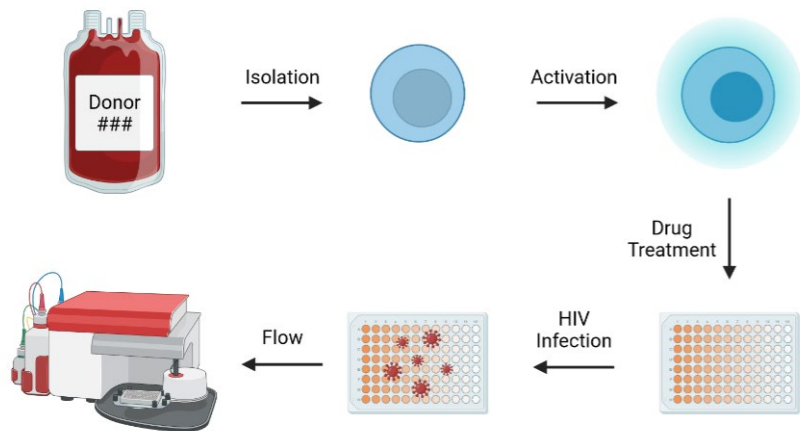


U19 AI171110

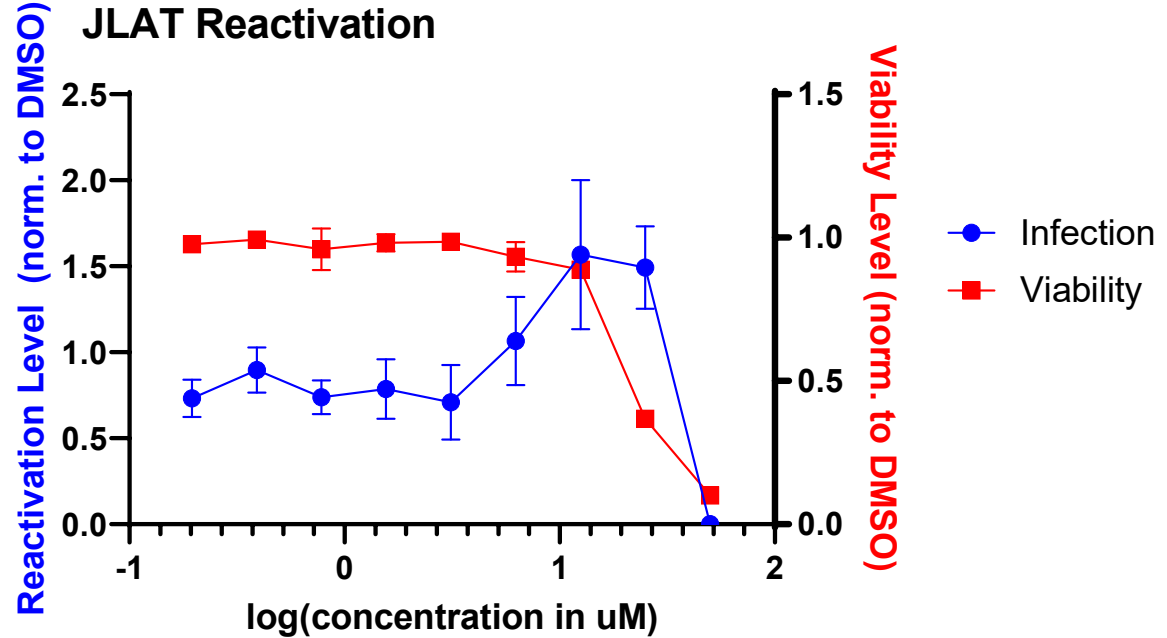
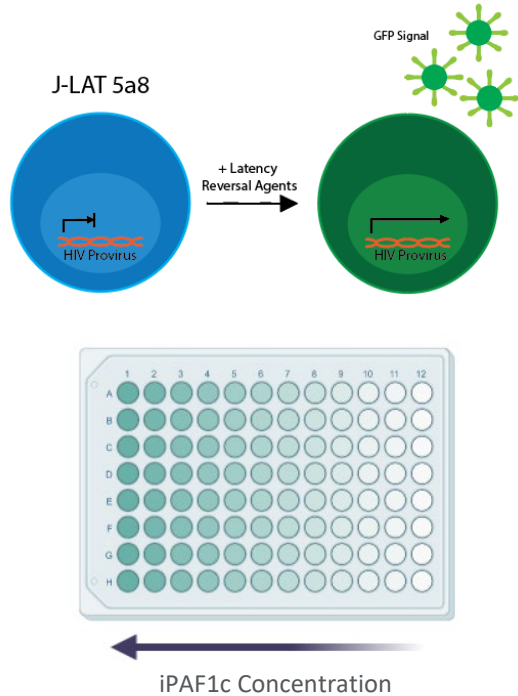
PAF1c inhibitors mimic the action of auxin-inducible PAF1 degradation on RNA transcript abundance



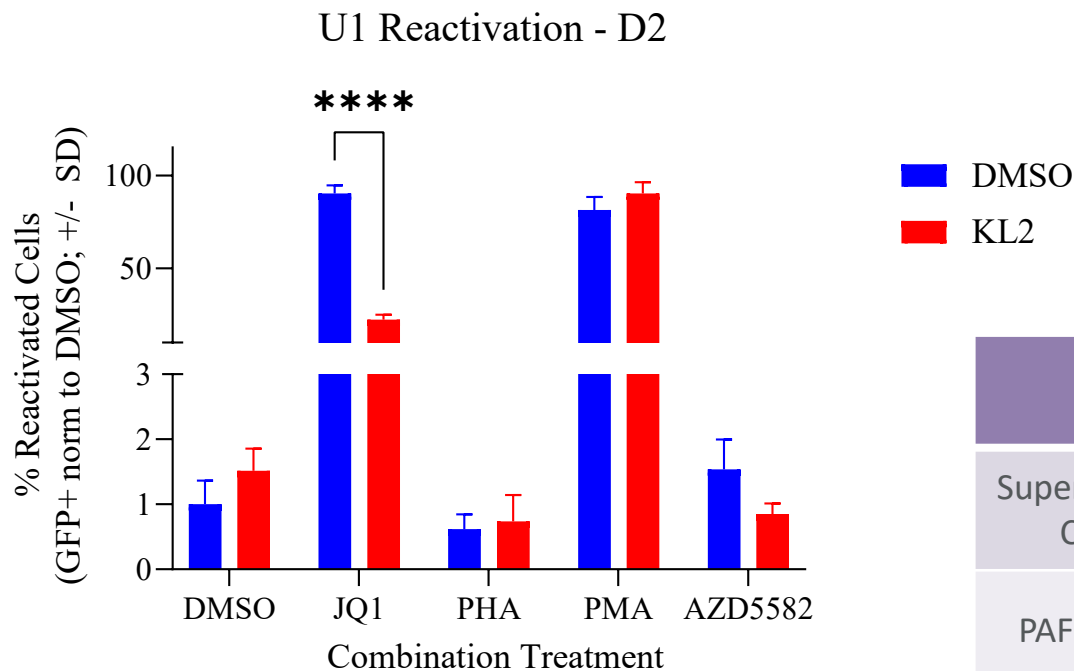
iPAF1c boosts active infection in primary CD4+ T cells



iPAF1c has minimal impact on latency reactivation in J-Lat 5A8 cells as a sole treatment



SEC inhibitors limit latency reactivation in Tat-deficient models (such as U1 cells)



	+ Tat/TAR	- Tat/TAR
Super Elongation Complex	Not necessary	Dependency Factor
PAF1 Complex	Restrictive Factor	Dependency Factor

