

11TH EDITION

DECEMBER 10-13, 2024

HIV PERSISTENCE DURING THERAPY

Reservoirs & Eradication Strategies Workshop

Fort
Lauderdale

RasGRP1 agonists induce Cyclin T1 translation to reverse HIV-1 latency

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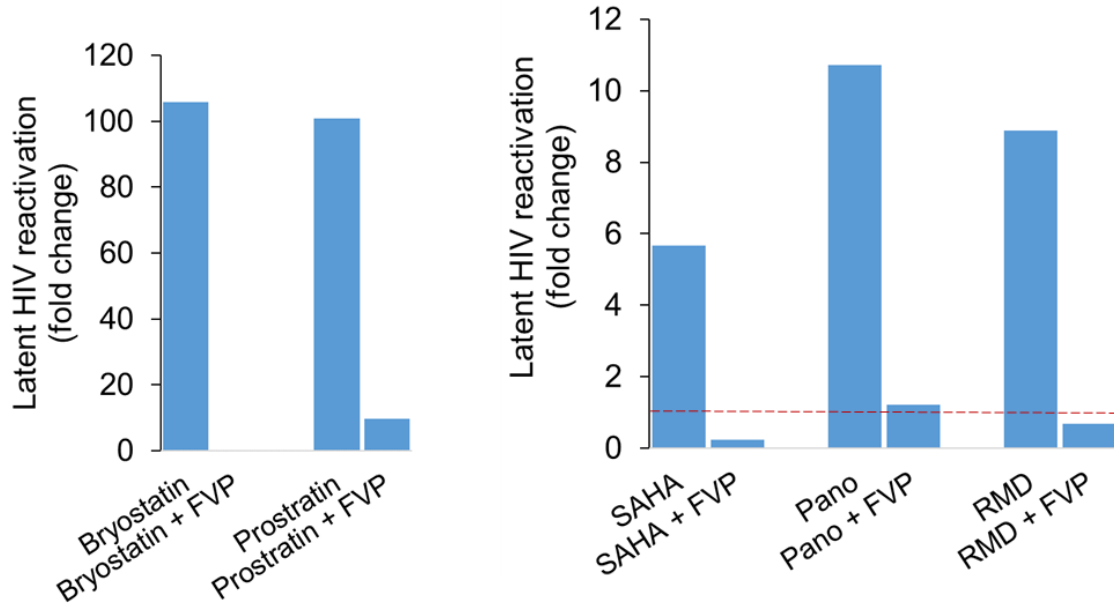
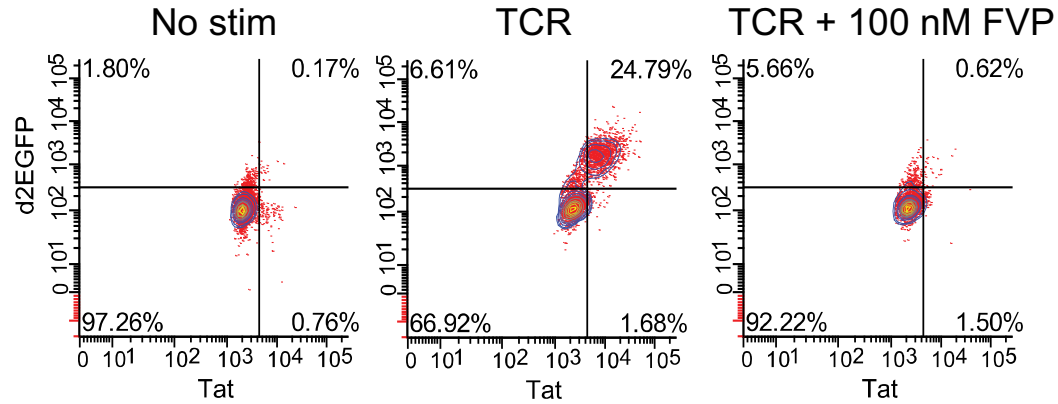
www.hiv-persistence.com

CONFLICTS OF INTEREST

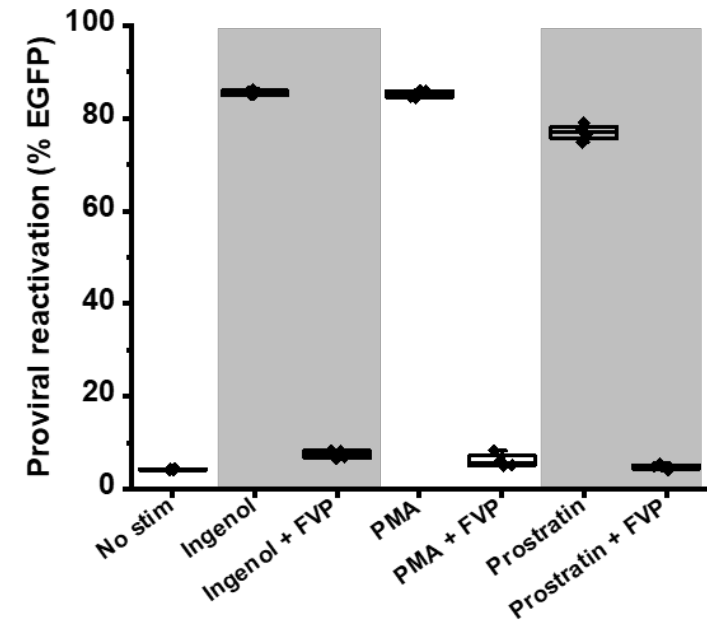
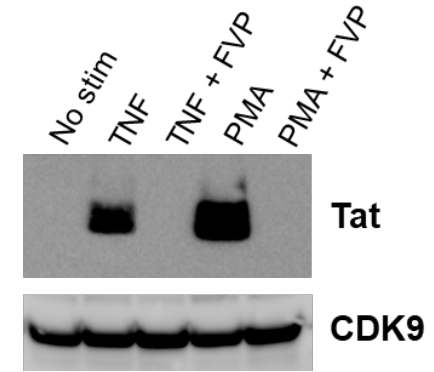
No conflicts of interest to declare

Without P-TEFb, HIV cannot emerge from latency

Ex vivo primary CD4⁺ T-cell model (P-TEFb is inducibly expressed)

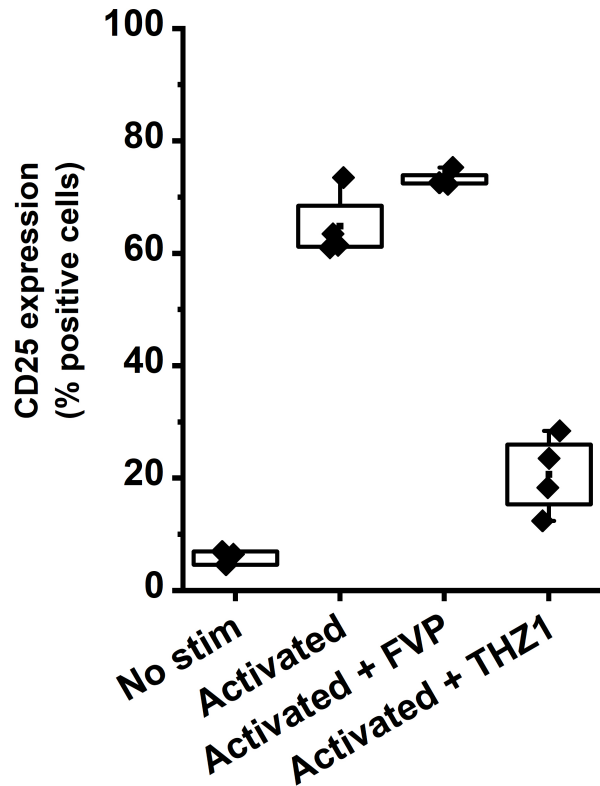


Jurkat cell line model (P-TEFb is constitutively expressed)



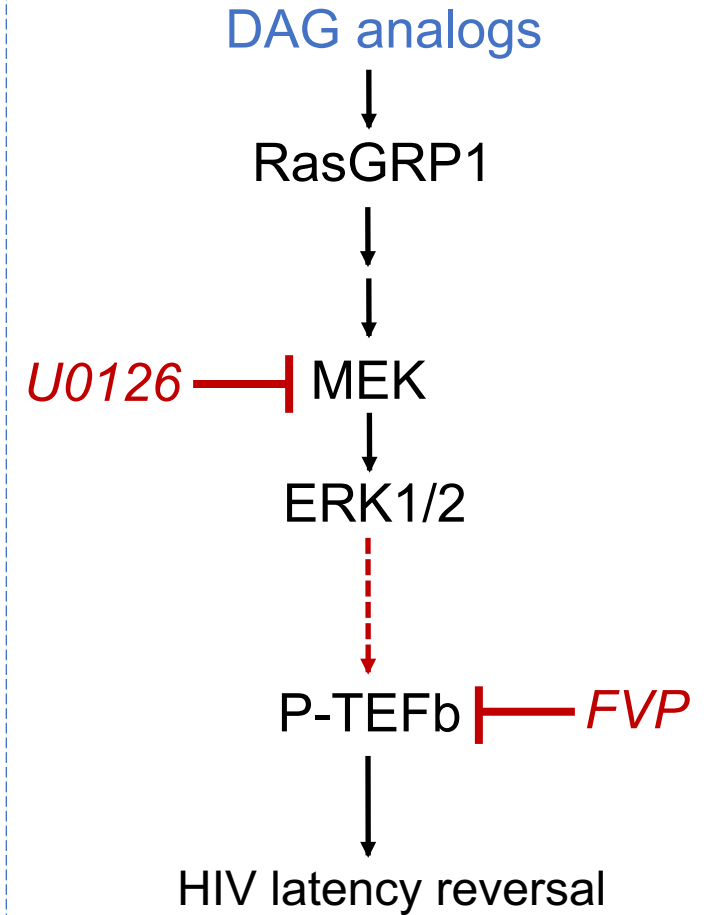
HIV is highly sensitive to inhibition of P-TEFb kinase activity

Memory CD4⁺ T cells



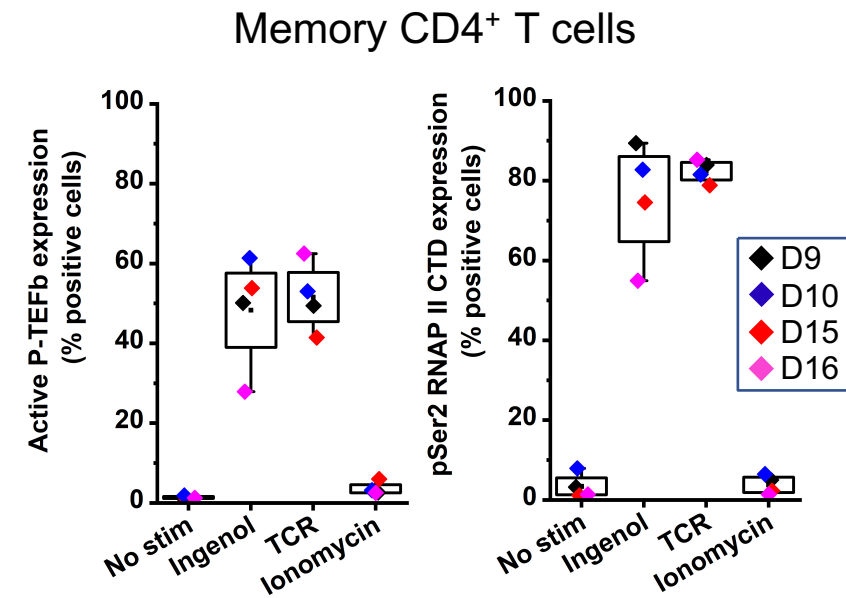
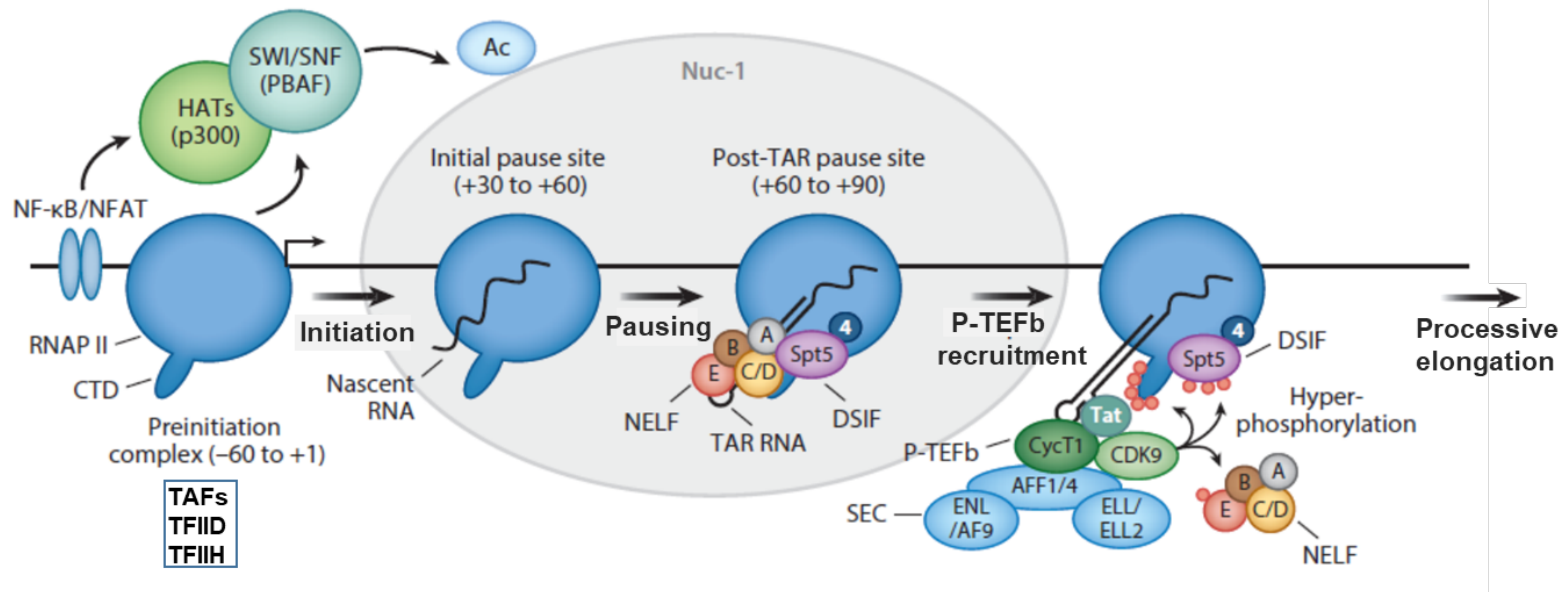
- FVP – P-TEFb kinase inhibitor
- THZ1 – Transcription initiation inhibitor of CDK7 (TFIIH) kinase

- HIV transcription is much more sensitive to P-TEFb inhibition than homeostatic cellular transcription (by ~15X) [Chao and Price (2001) JBC]
- In contrast to most cellular genes, HIV is highly activated by the depletion of a negative elongation factor (NELF-E) that is regulated by P-TEFb [Jadlowski et al (2014) MCB]



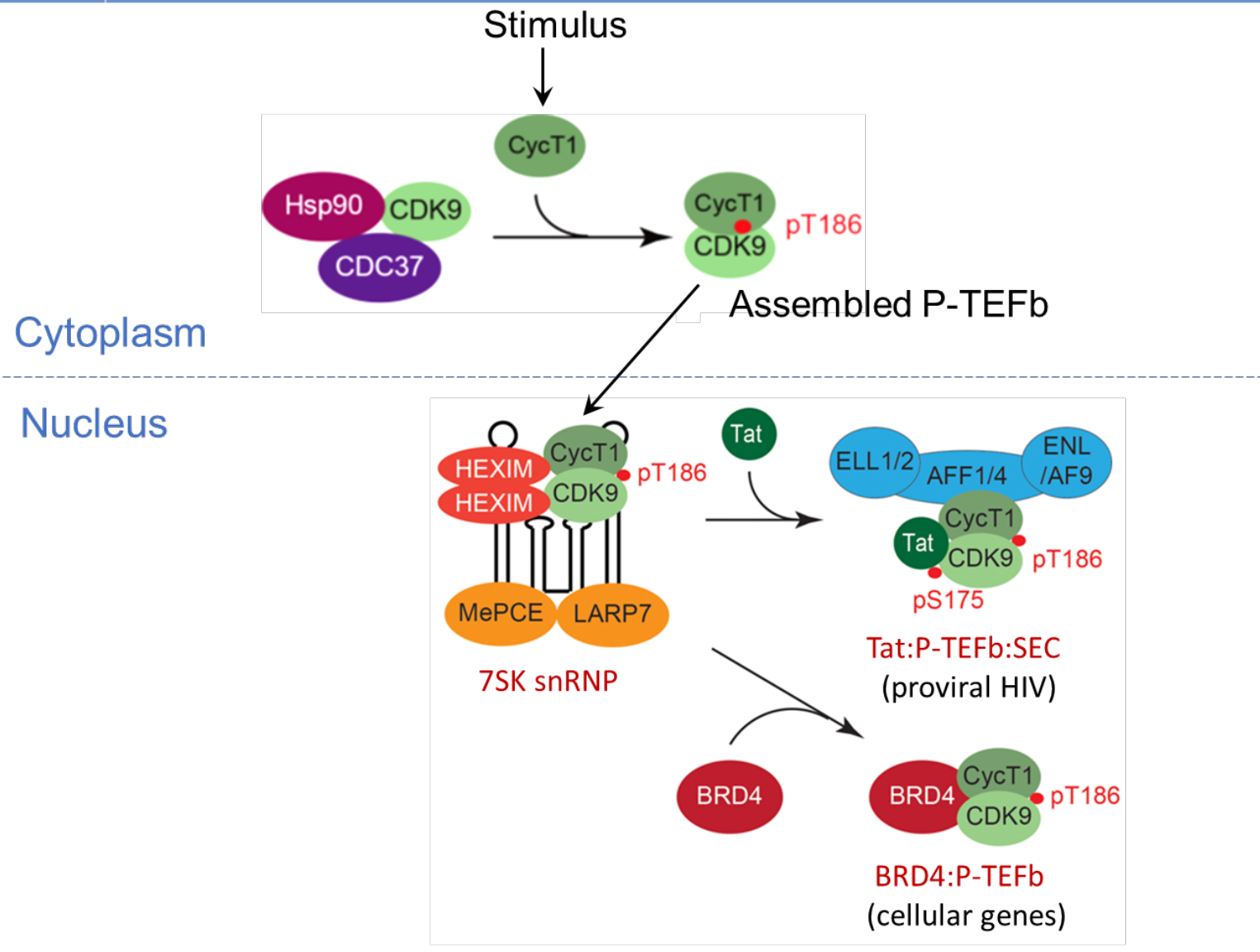
Alternatively, inhibition of P-TEFb activity is a potential effective route to 'Blocking and Locking' HIV transcription

P-TEFb stimulates efficient HIV RNA synthesis

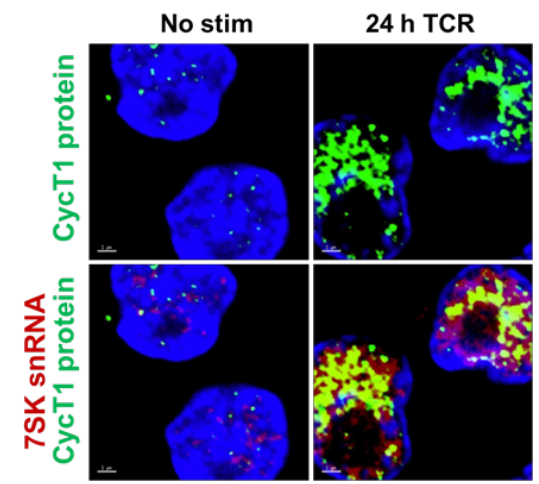
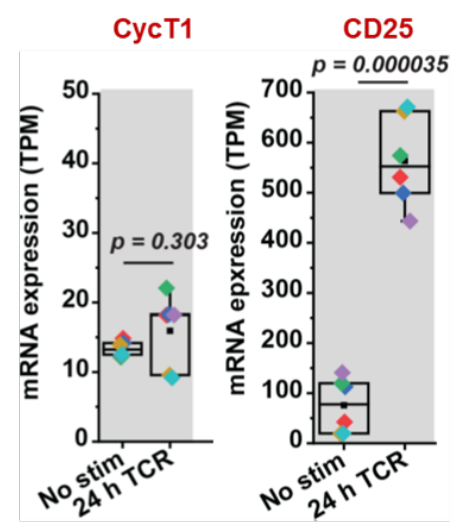


A two-pronged LRA strategy that induces the biogenesis of P-TEFb and overcomes the epigenetic repression at the proviral promoter will provide the synergy required to reverse HIV latency regardless of viral subtype

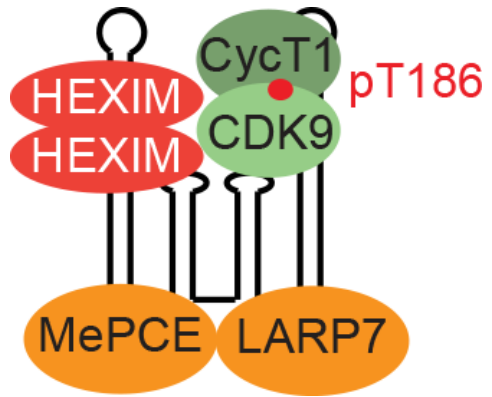
P-TEFb biogenesis precedes the reactivation of latent HIV



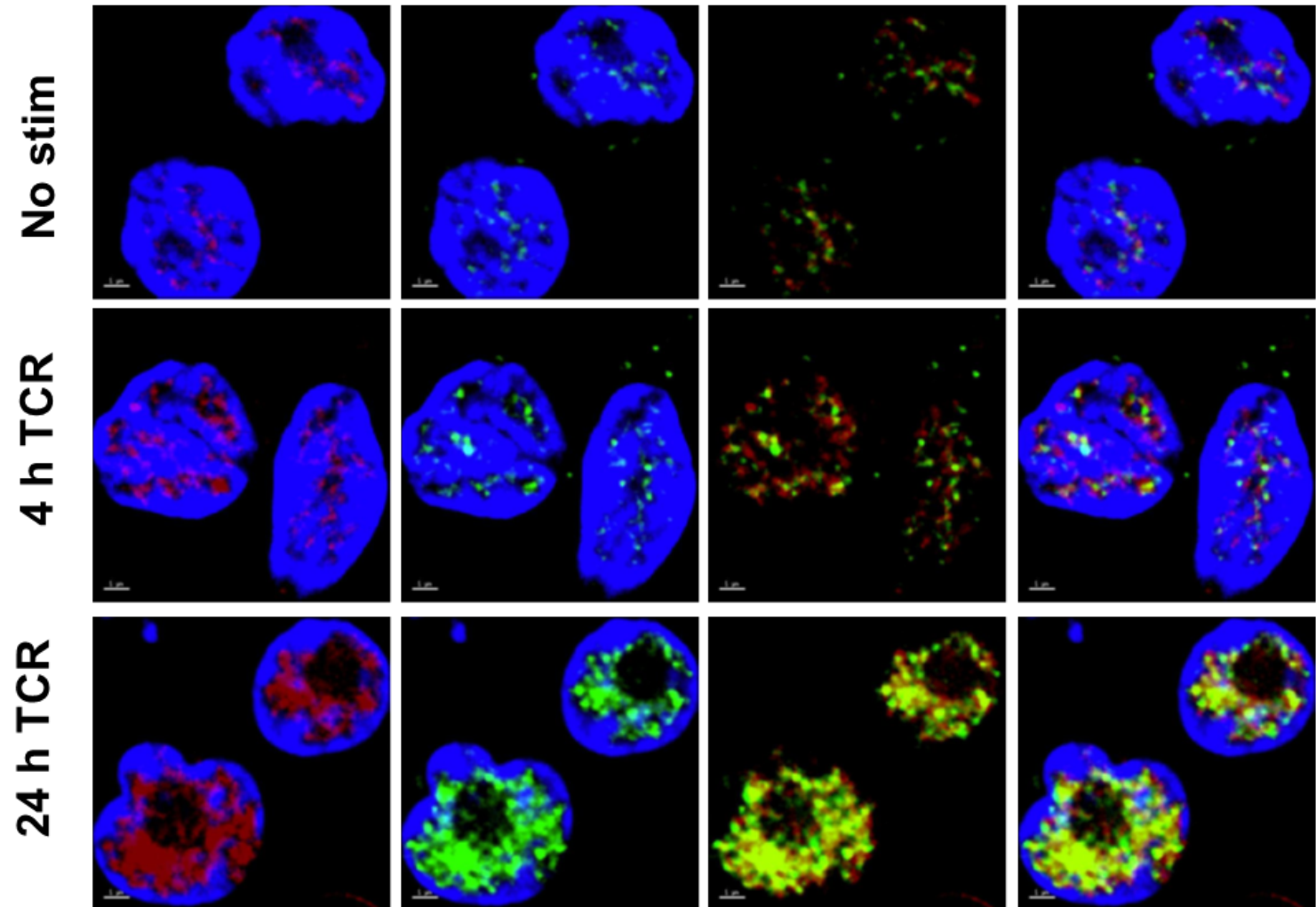
Memory CD4⁺ T cells



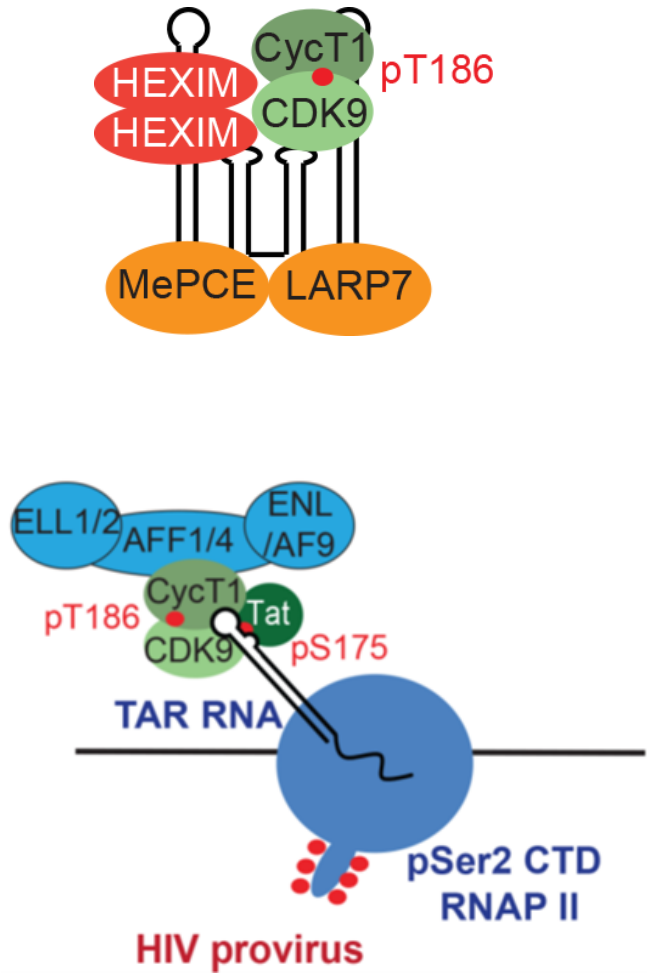
7SK snRNP assembly in activated memory CD4⁺ T cells



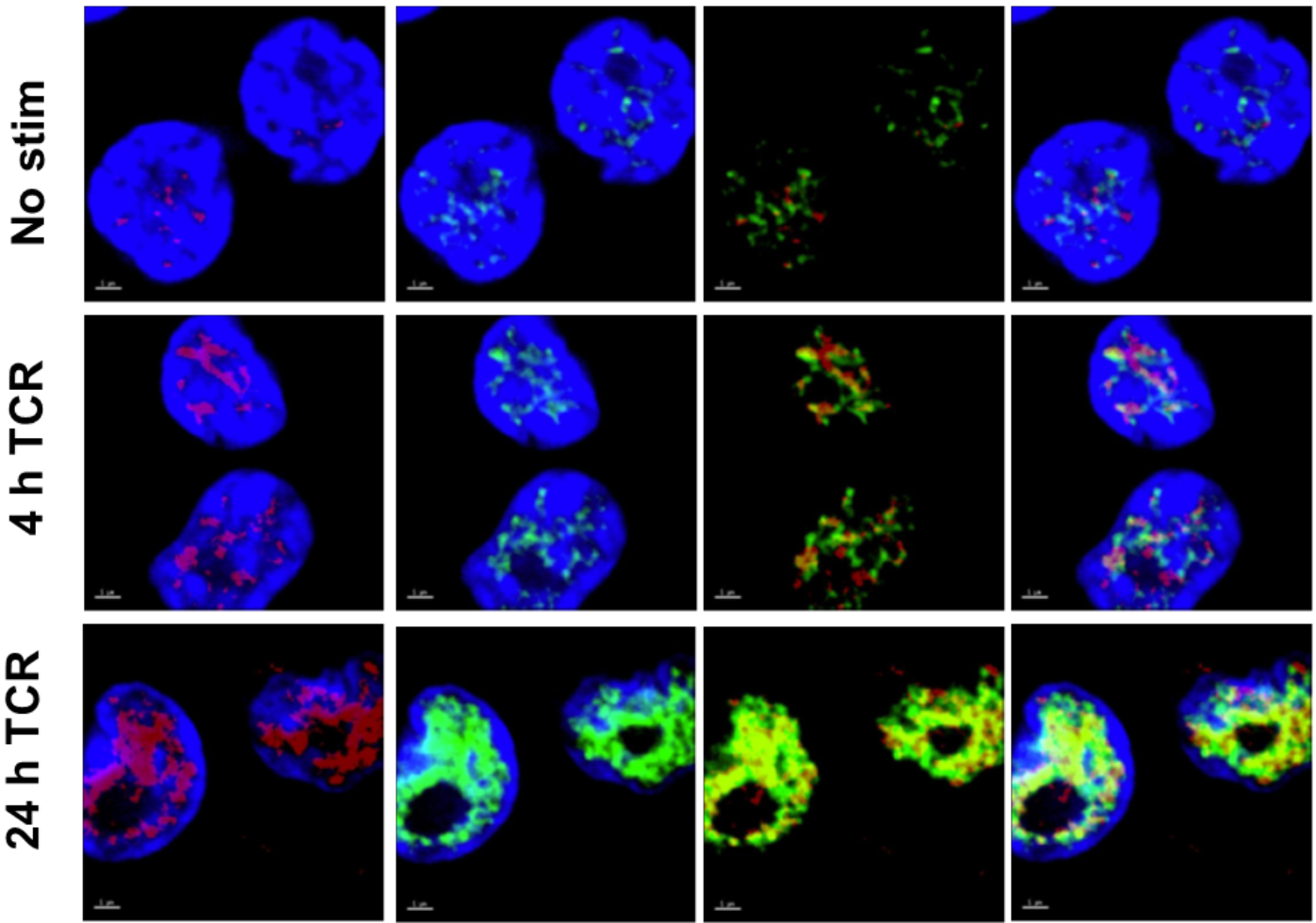
7SK snRNA/LARP7/DNA



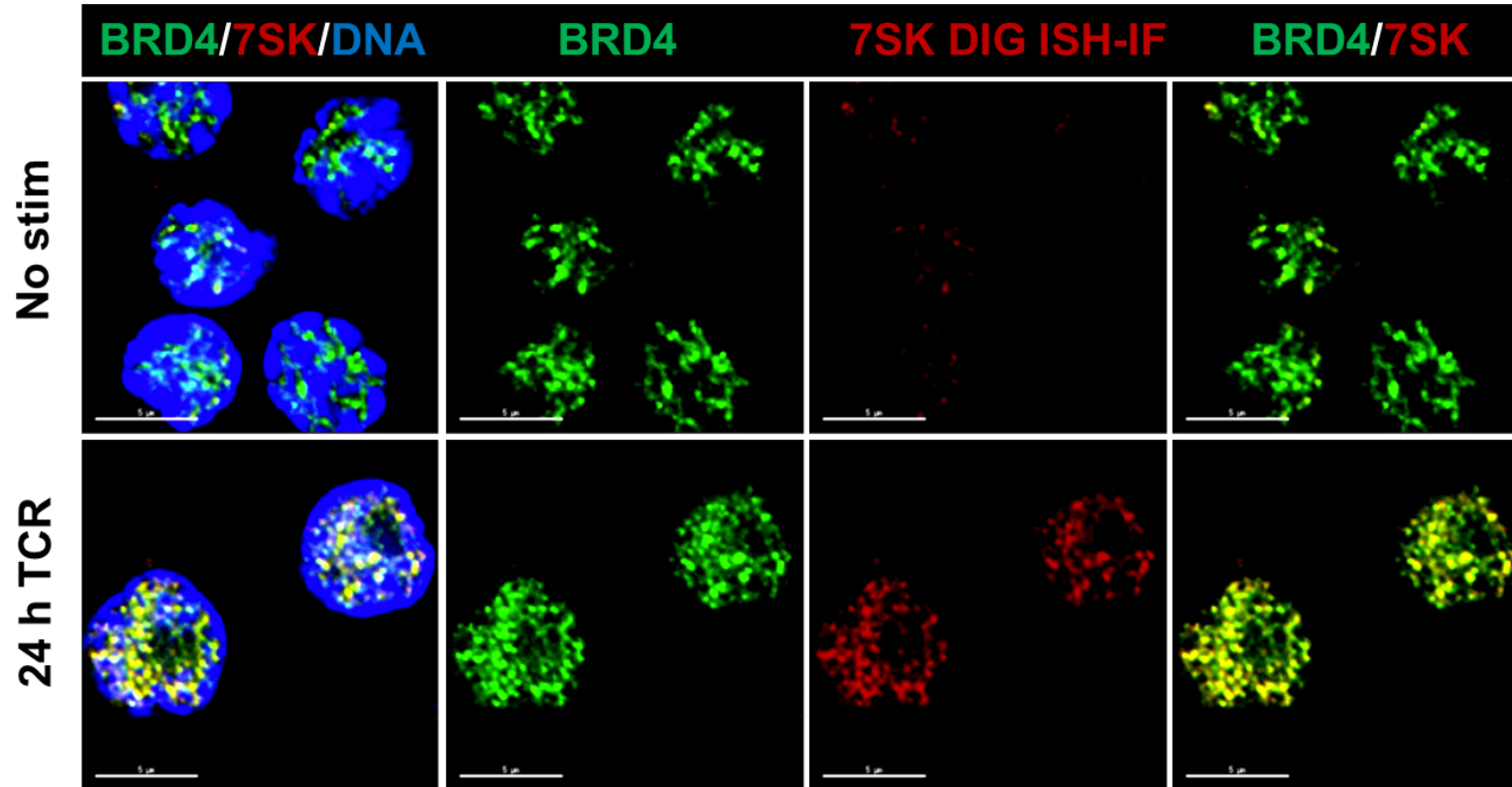
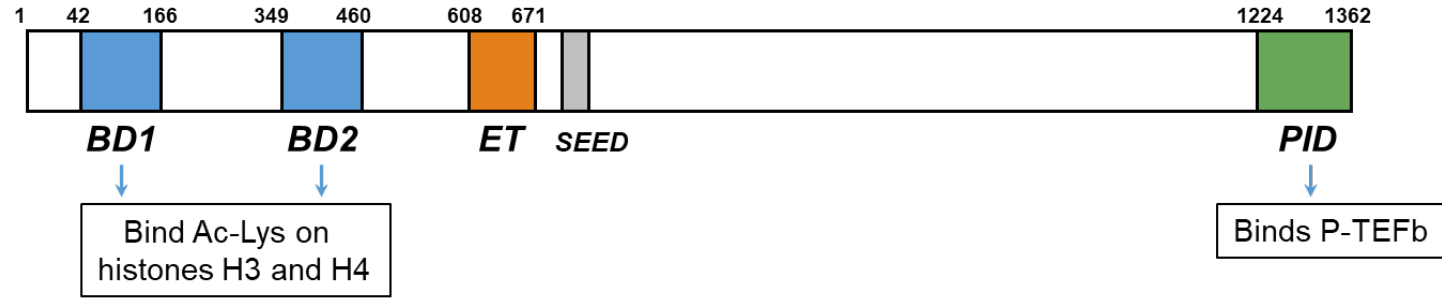
Assembled 7SK snRNP colocalizes with transcribing RNAP II in activated memory T cells



7SK snRNA/pSer2 CTD RNAP II/DNA

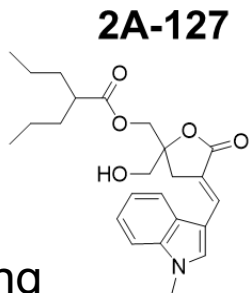


BRD4 is constitutively expressed and resident on chromatin in memory CD4⁺ T cells

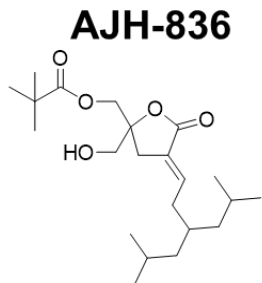


Synthetic DAG indololactones preferentially bind RasGRP over PKC

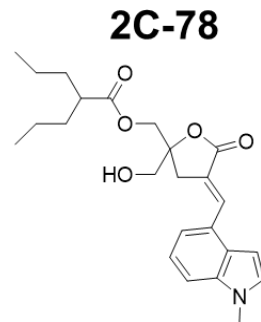
In vitro binding
 affinities (nM):



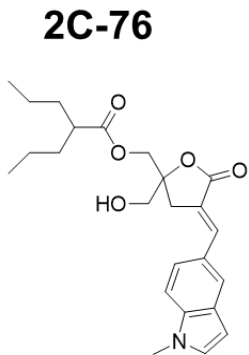
16.2 ± 1.0 (PKCα)
 0.33 ± 0.06 (RasGRP3)
 0.25 ± 0.10 (RasGRP1-C1)



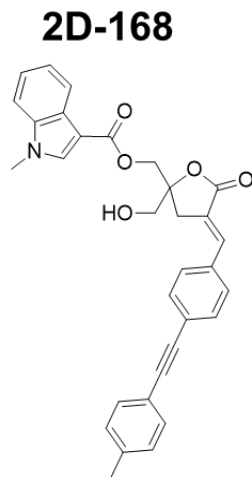
4.51 ± 0.5 (PKCα)



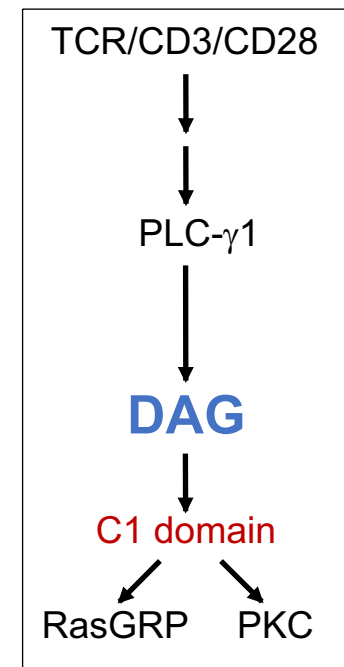
17.8 ± 2.0 (PKCα)
 1.63 ± 0.18 (RasGRP3)
 1.55 ± 0.10 (RasGRP1-C1)



8.25 ± 0.88 (PKCα)
 0.34 ± 0.01 (RasGRP3)
 0.41 ± 0.10 (RasGRP1-C1)



116 ± 18 (PKCα)
 22.6 ± 1.8 (RasGRP1)



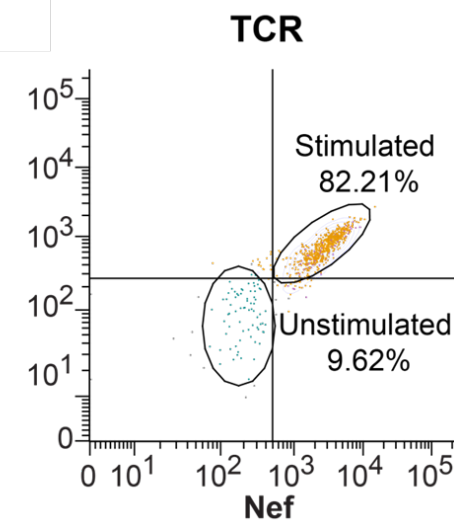
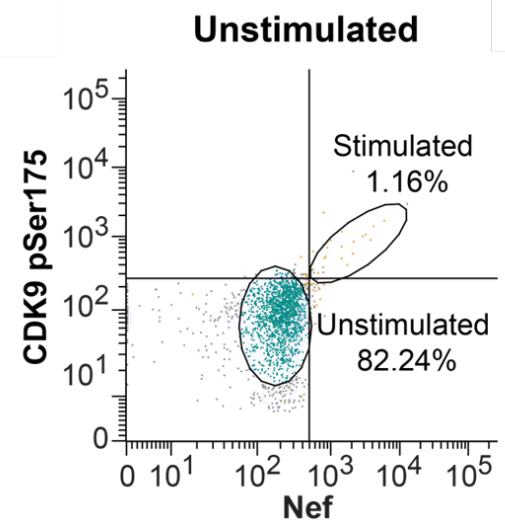
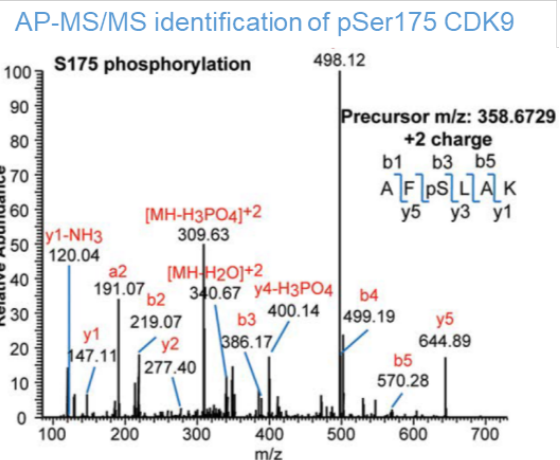
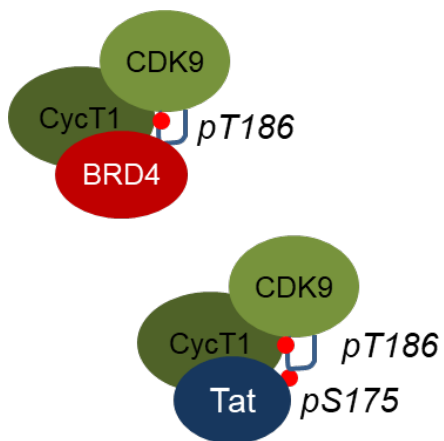
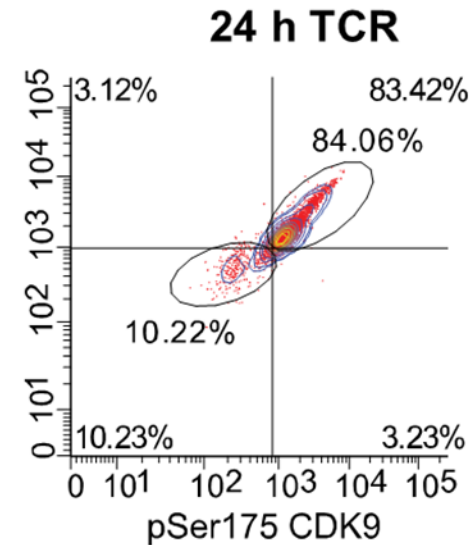
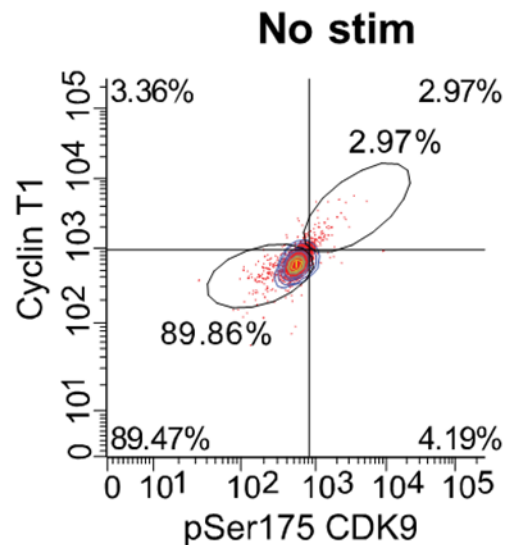
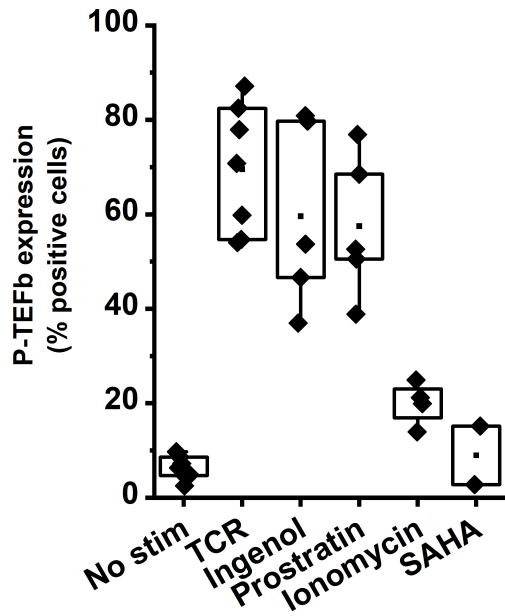
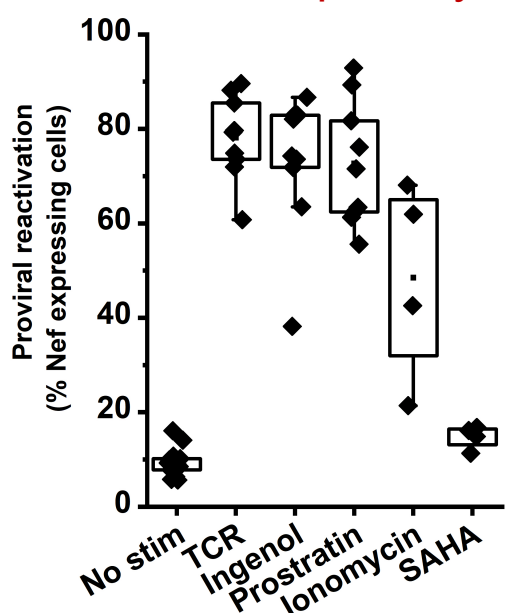
PKC agonists

- Ingenol
- Prostratin
- Bryostatins

Elhalem et al (2021) J. Med. Chem.
 Elhalem et al (2017) Bioorg. Med. Chem.
 Garcia et al (2014) Bioorg Med. Chem.

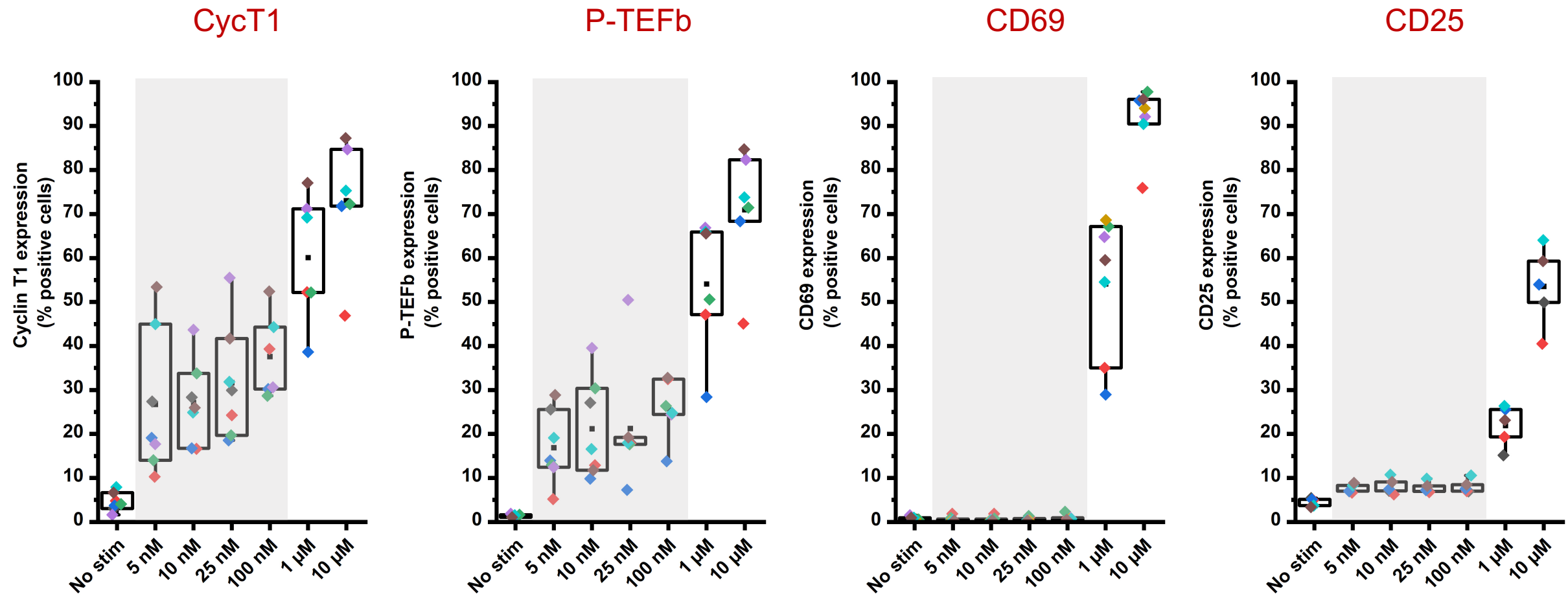
Latent HIV reactivation tracks with inducible P-TEFb expression

Ex vivo primary CD4⁺ T-cell model



2A127 stimulates P-TEFb expression with minimal T-cell activation

2A127

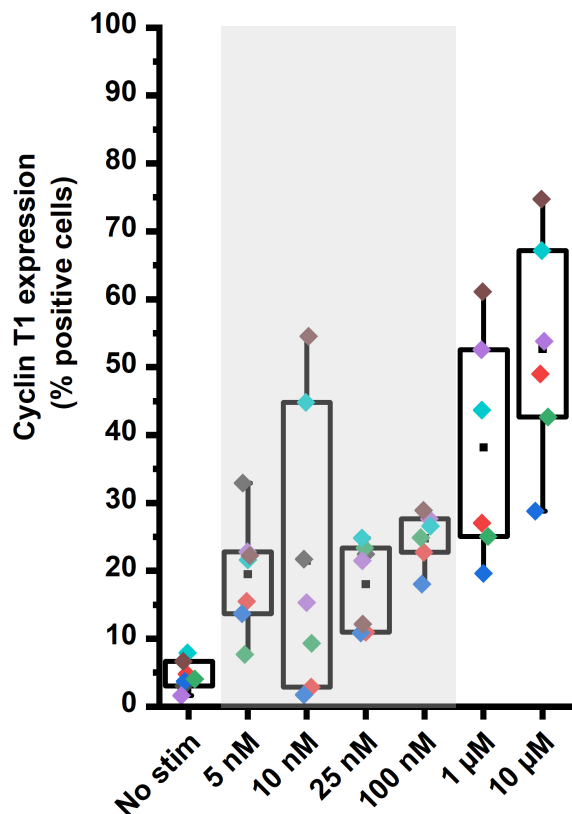


All experiments were done in memory CD4⁺ T cells derived from two healthy adult donors (male and female)

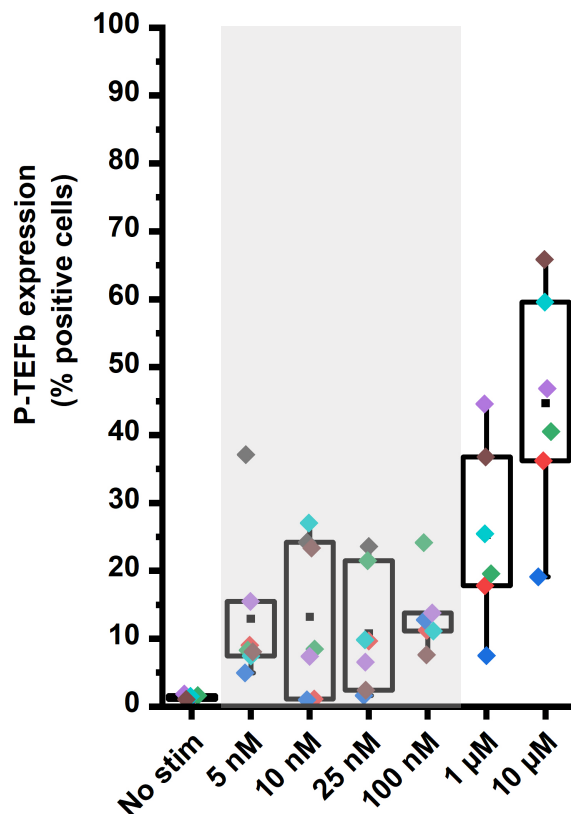
2C78 stimulates P-TEFb expression with minimal T-cell activation

2C78

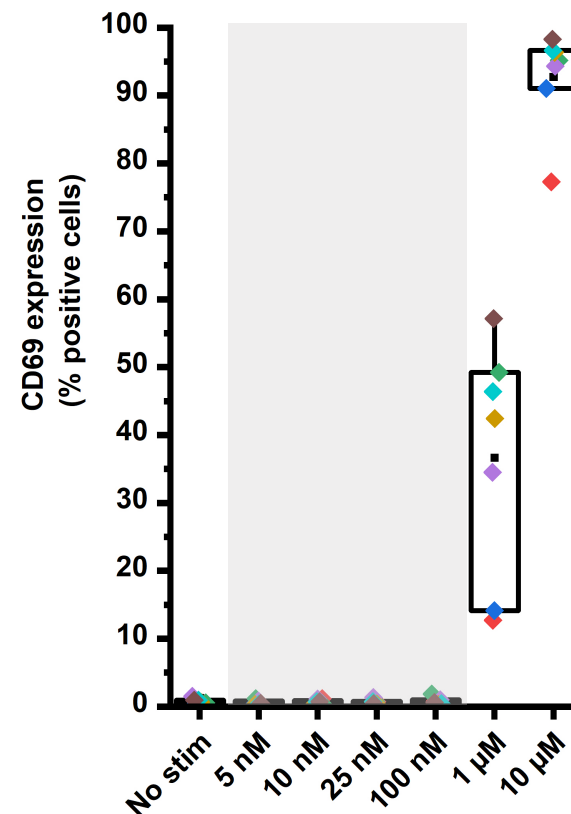
CycT1



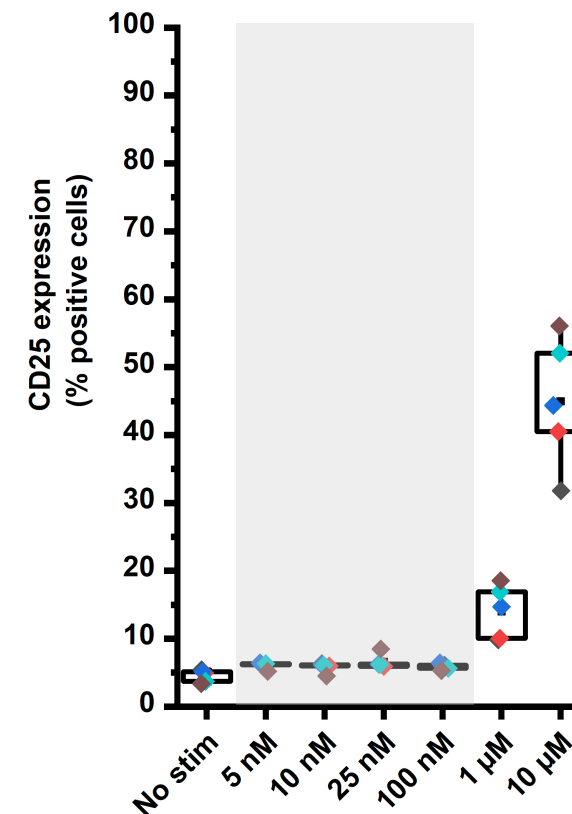
P-TEFb



CD69

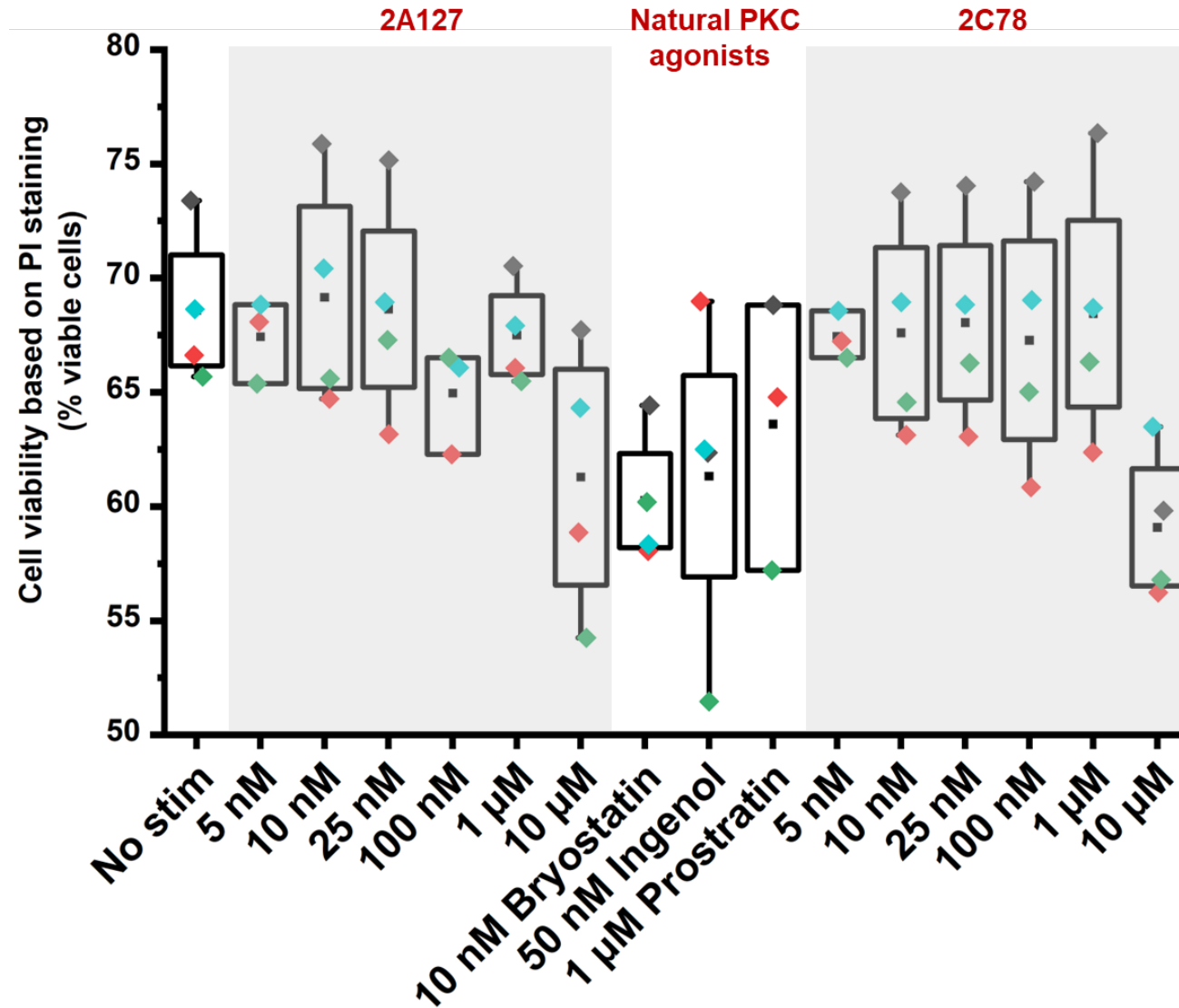


CD25

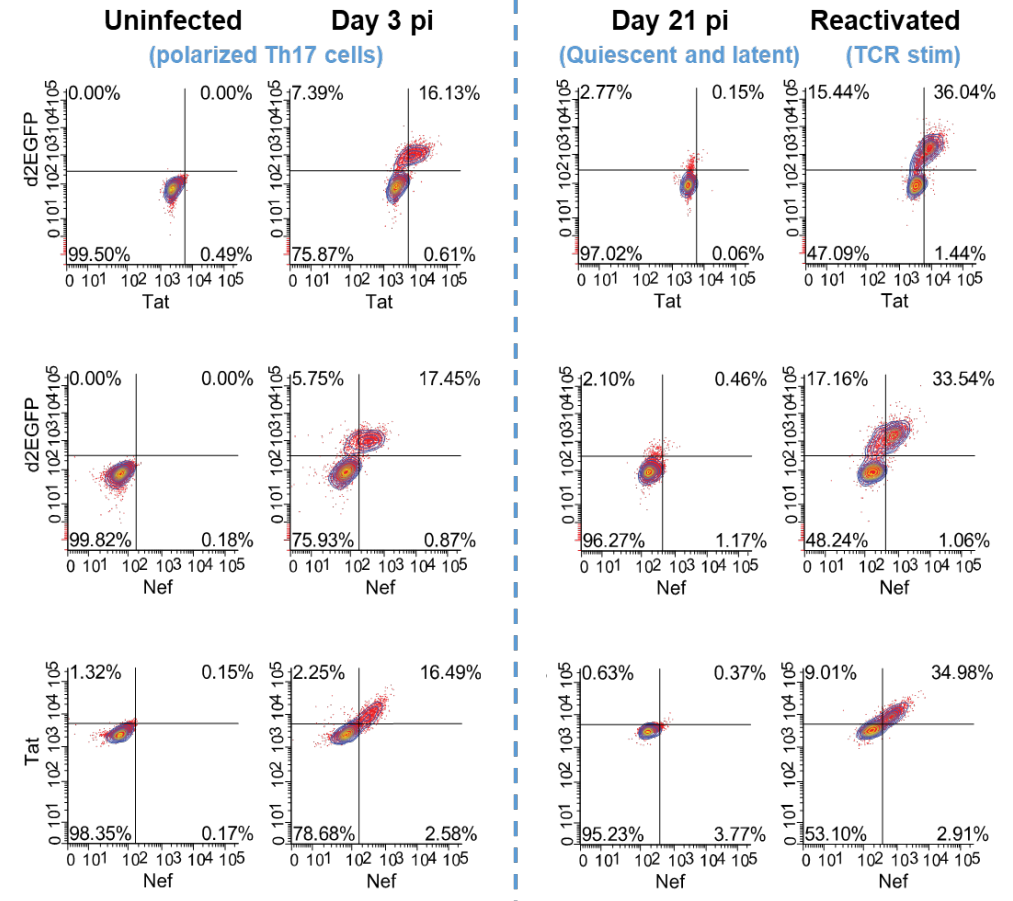
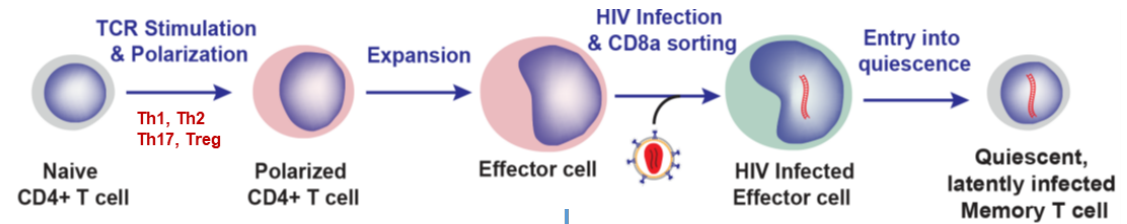


All experiments were done in memory CD4⁺ T cells derived from two healthy adult donors (male and female)

Cell viability measurements in memory CD4⁺ T cells



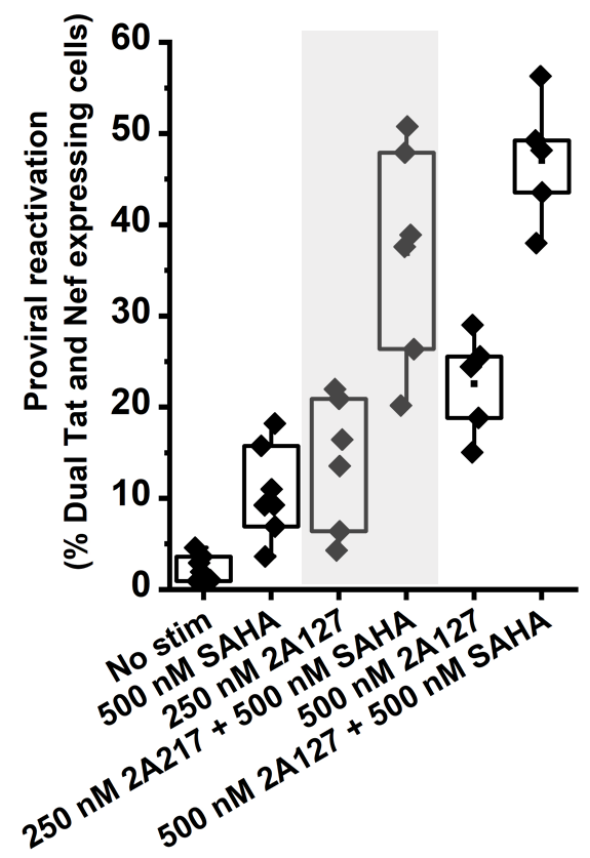
In vitro generation of latently infected primary T cells



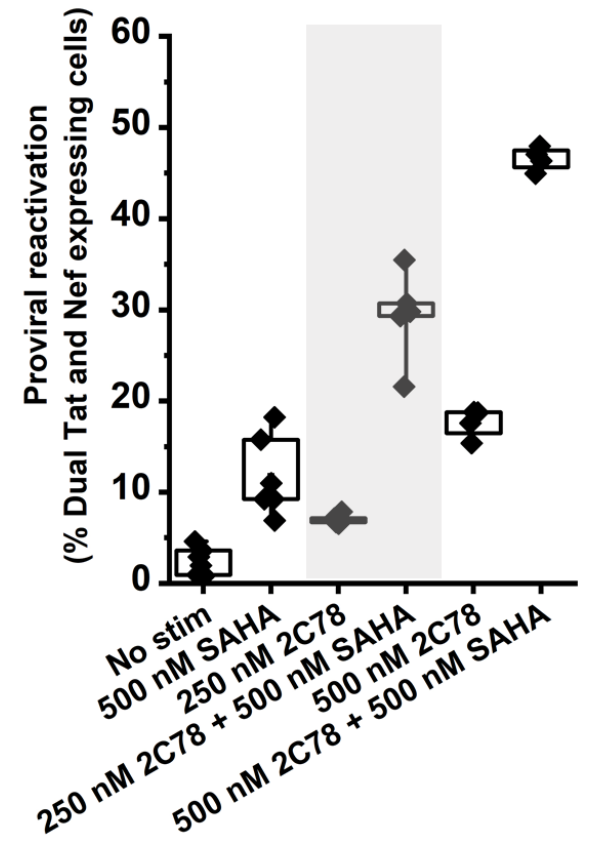
Dobrowolski et al (2019) mBio
Shukla et al (2022) Methods Mol Biol
Mbonye et al (2024) STAR Protocols

DAG indololactones and SAHA show synergy in viral reactivation

2A127 +/- SAHA

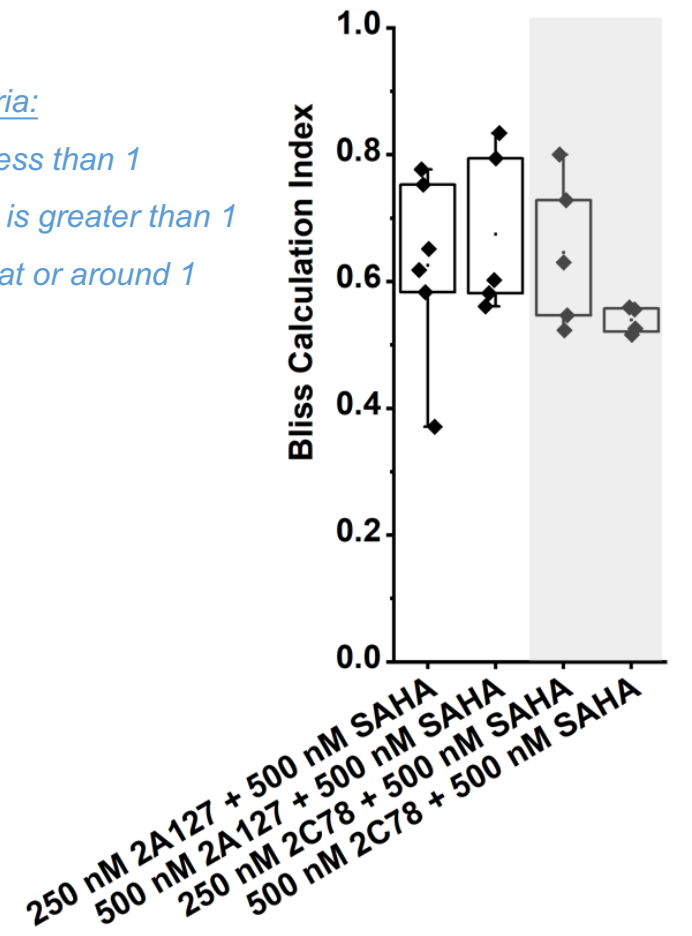


2C78 +/- SAHA

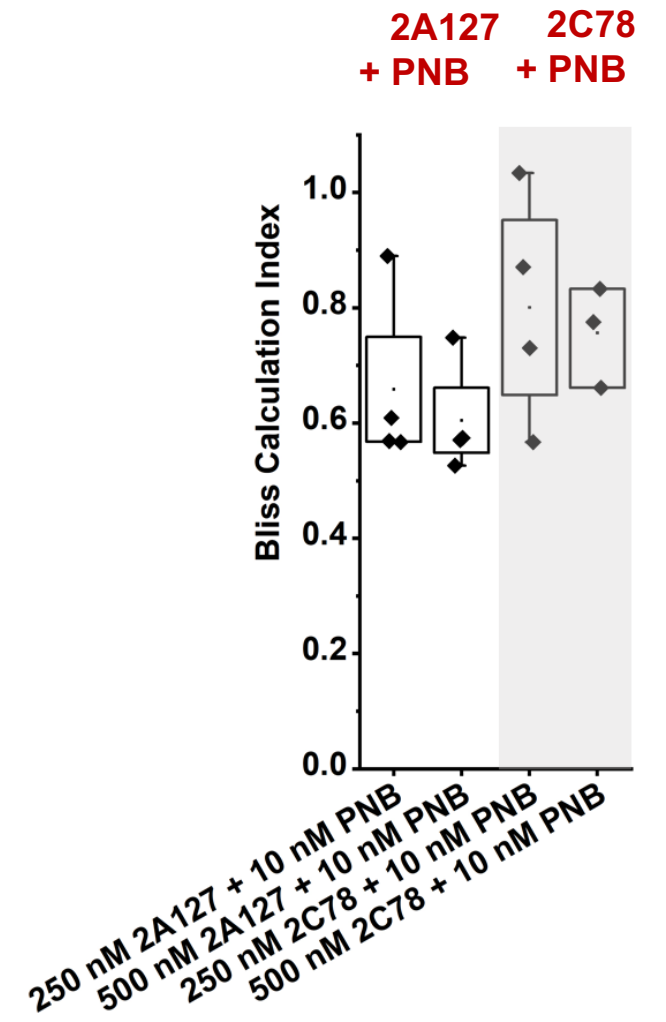
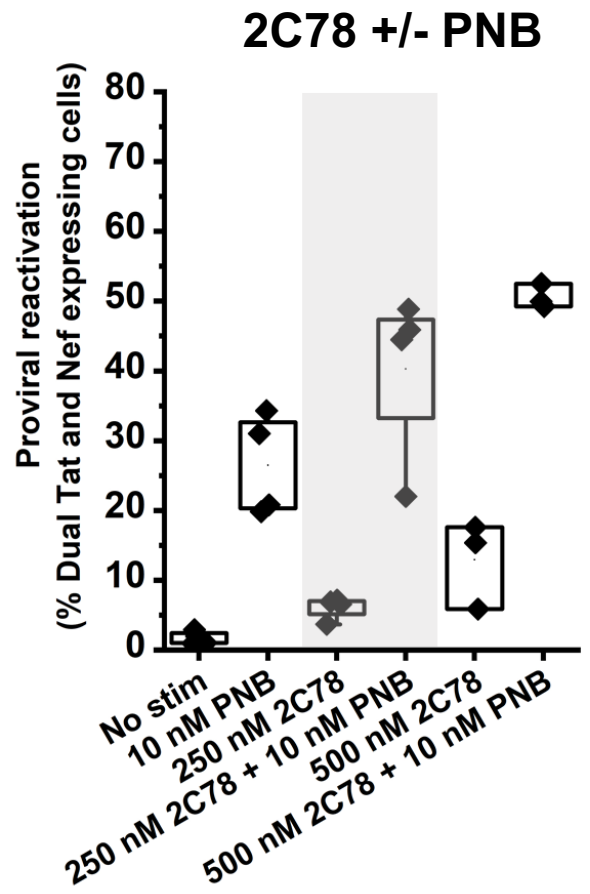
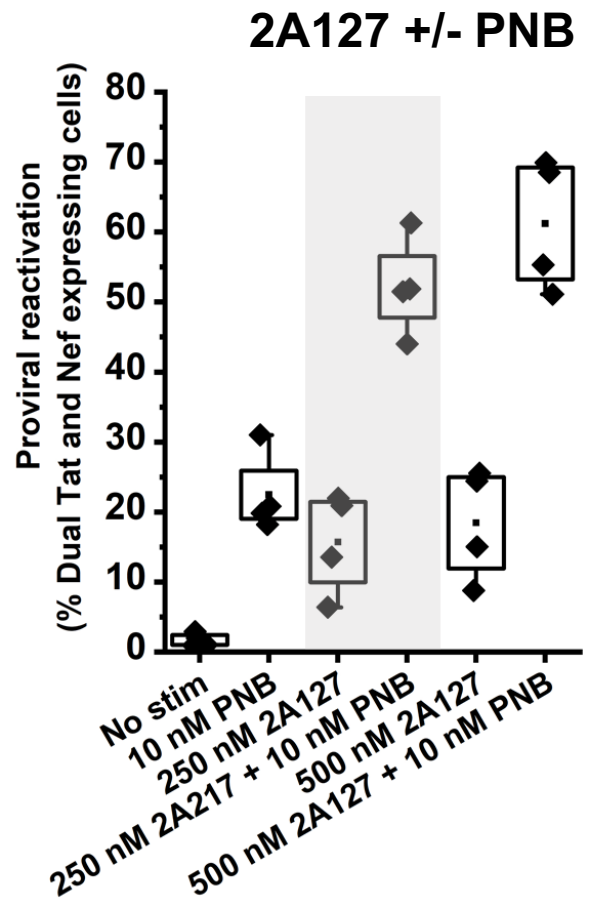


Bliss synergy criteria:
 Synergy – if CI is less than 1
 Antagonism – if CI is greater than 1
 Additivity – if CI is at or around 1

2A127 + SAHA 2C78 + SAHA

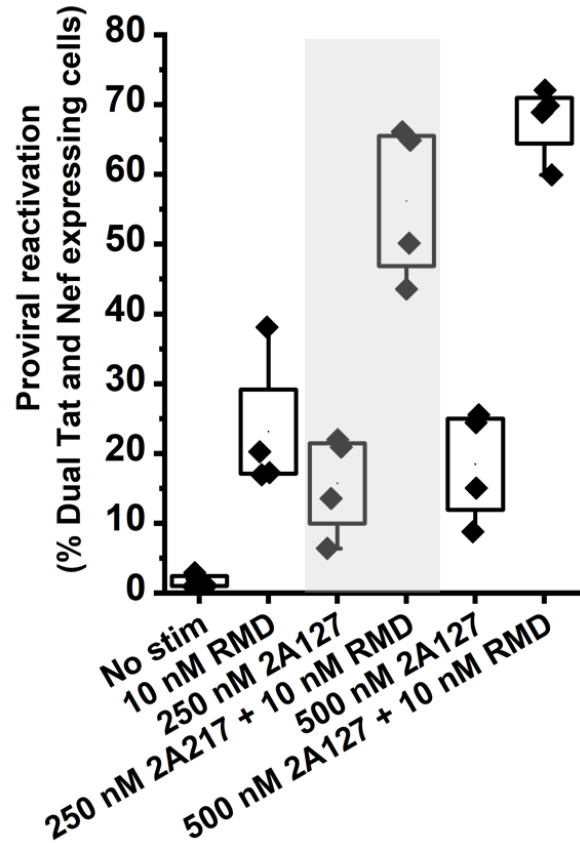


DAG indololactones and Panobinostat show synergy in viral reactivation

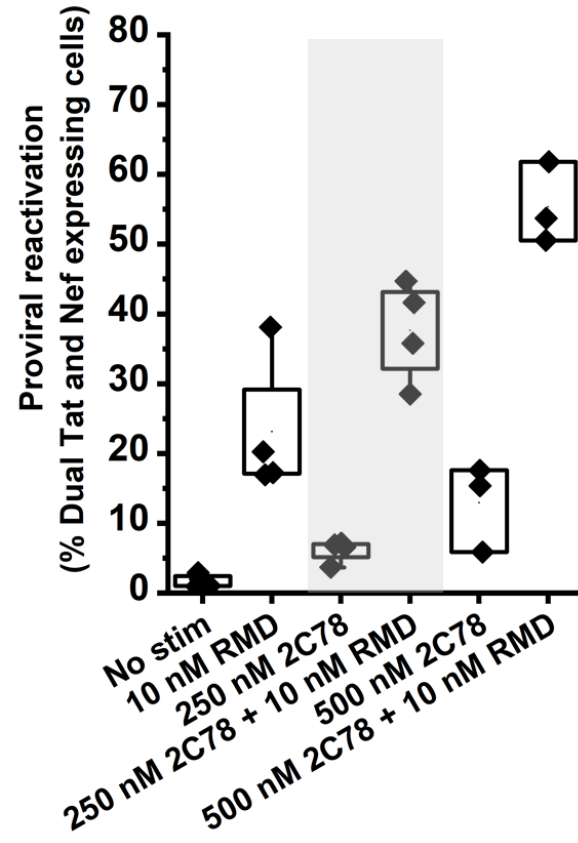


DAG indololactones and Romidepsin show synergy in viral reactivation

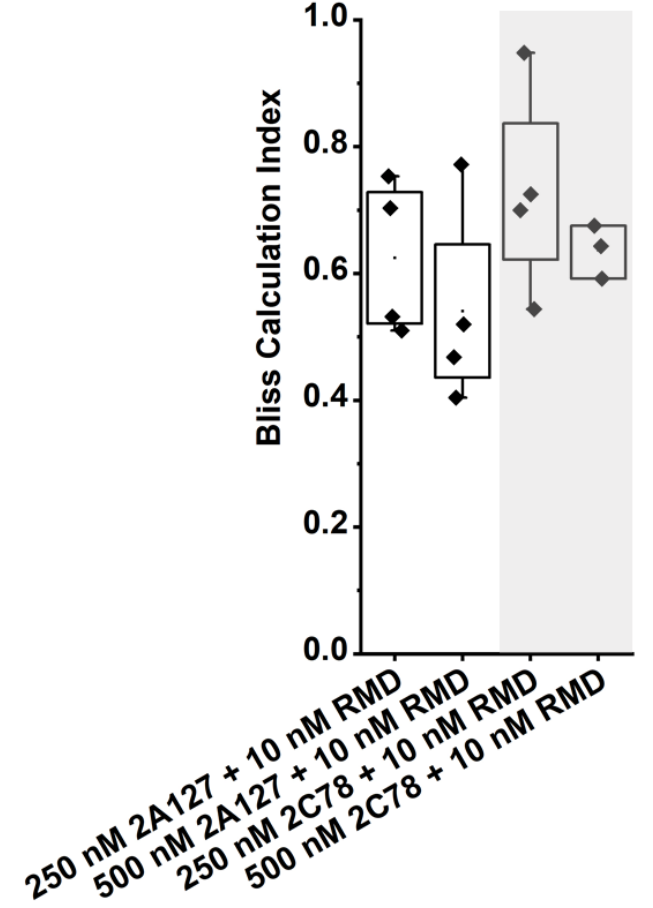
2A127 +/- RMD



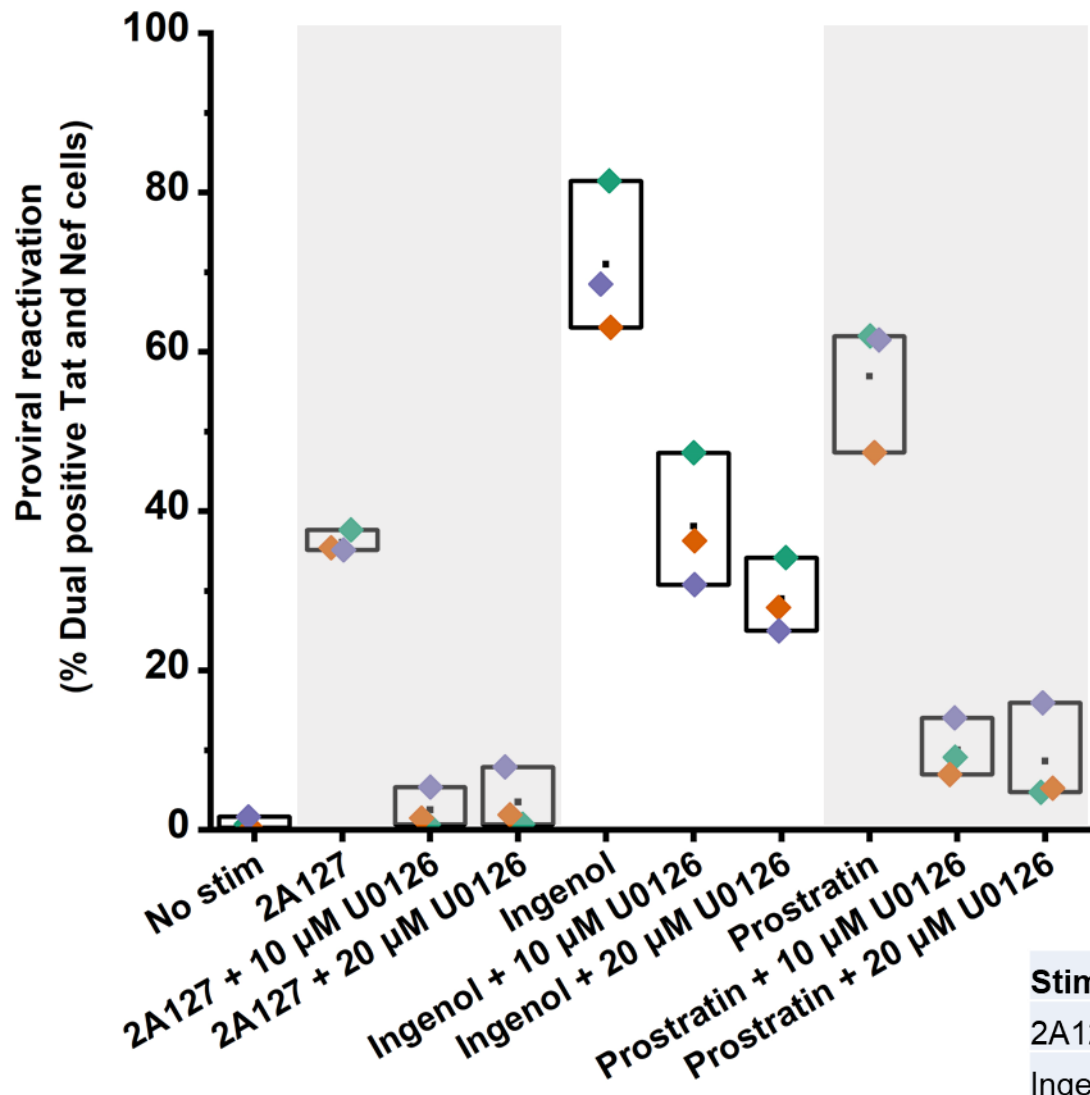
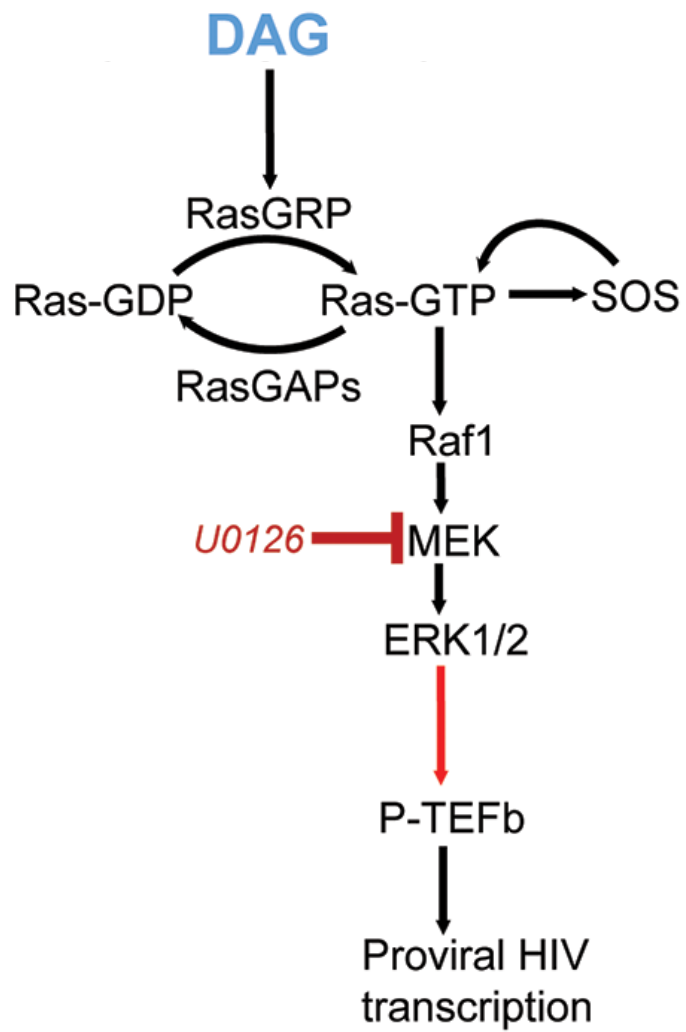
2C78 +/- RMD



2A127 + RMD 2C78 + RMD

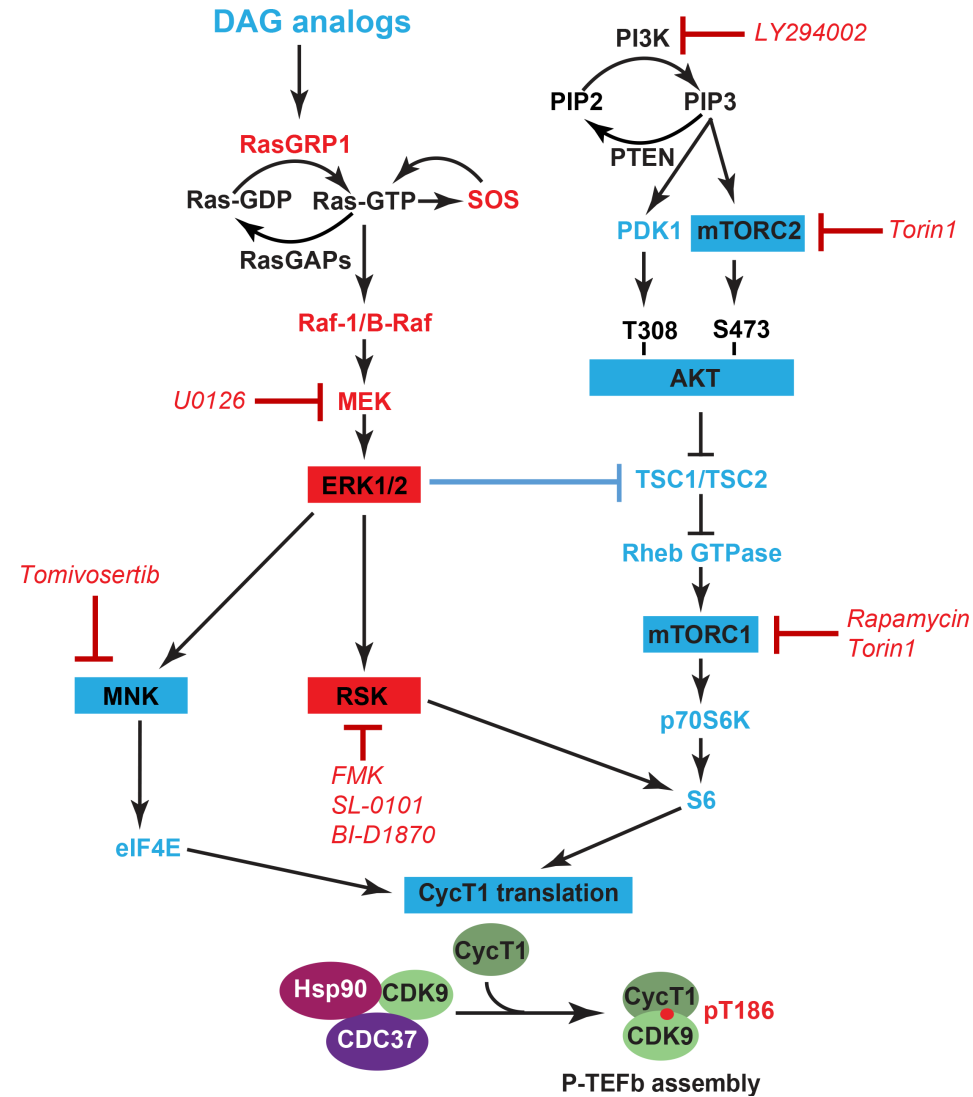


Latent HIV reactivation by 2A127 is wholly mediated by ERK1/2 signaling

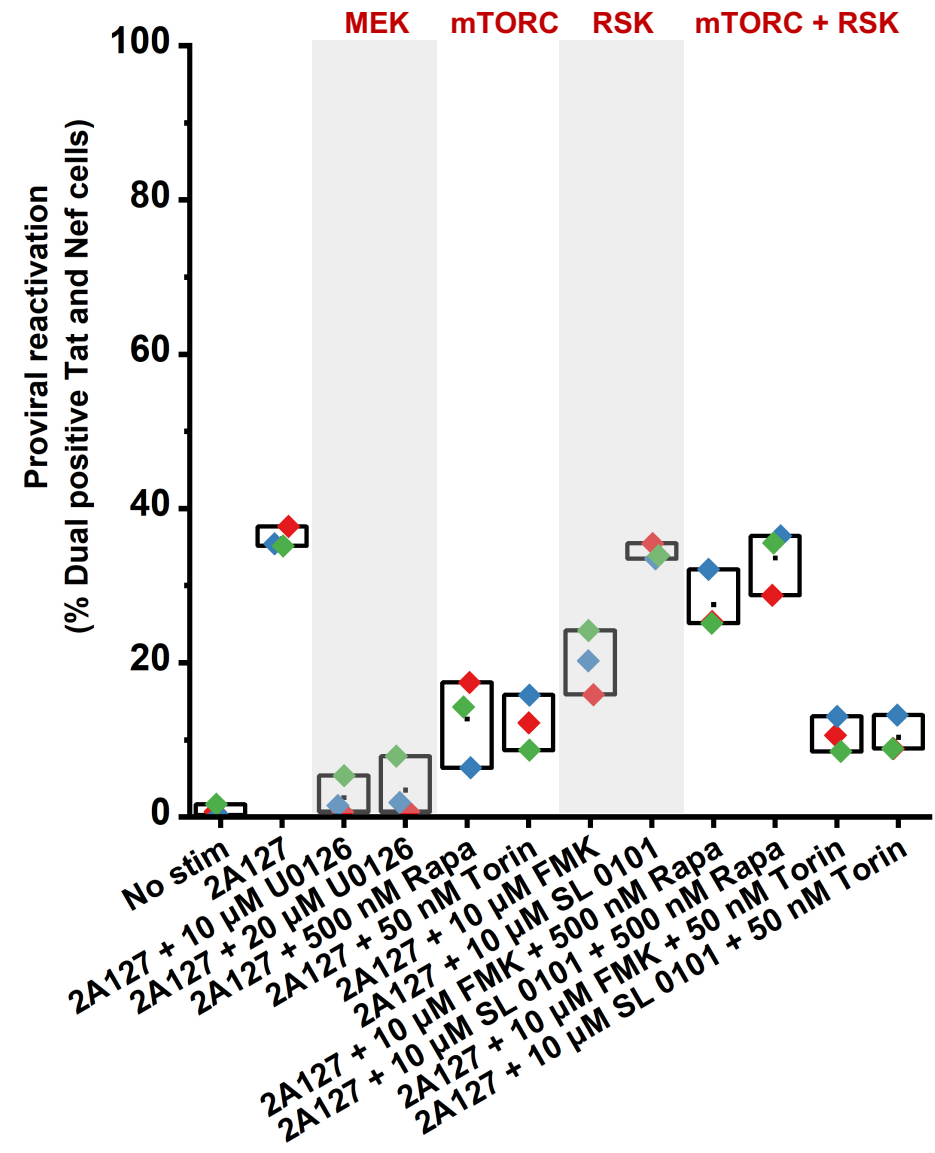
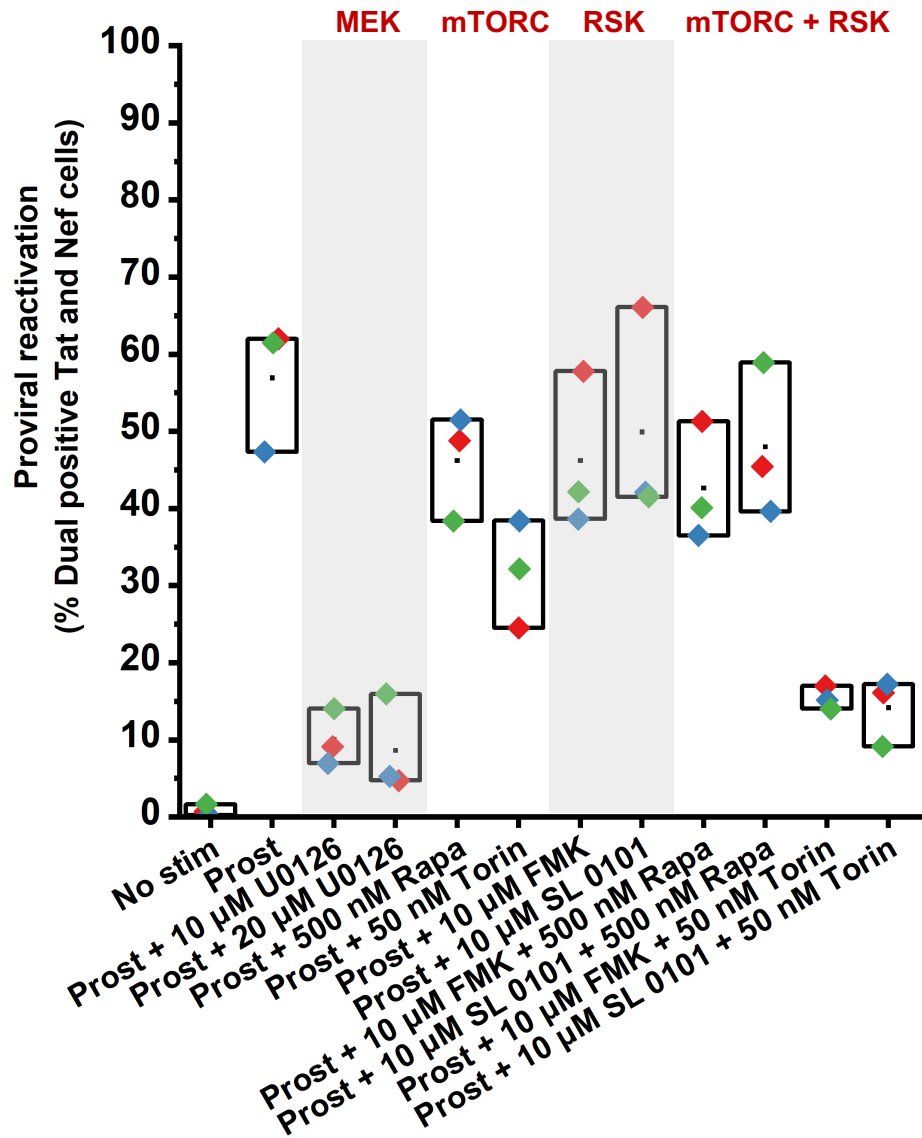


Stimulus	Concentrations used
2A127	1 µM
Ingenol-3-angelate	50 nM
Prostratin	1 µM

Regulation of mRNA translation by ERK signaling



mTORC and RSK inhibitor effects on DAG mimetic-induced proviral reactivation



Summary

- DAG indololactones can induce P-TEFb expression in memory CD4⁺ T cells with minimal T-cell activation
- DAG indololactones synergize with HDAC inhibitors to promote the emergence of HIV from latency in an *ex vivo* primary cell model
- While the RasGRP1-MEK-ERK1/2 pathway is critical for mediating latent HIV reactivation in response to natural C1 domain agonists, it seems to be able to exploit both the mTORC1 and RSK (p90S6K) pathways
- Unlike the natural PKC agonists, 2A127 appears to primarily utilize the signaling of ERK through mTORC1 to reactivate latent HIV