
# TACK Molecules Kill HIV-Infected Cells Through Inflammasome Activation

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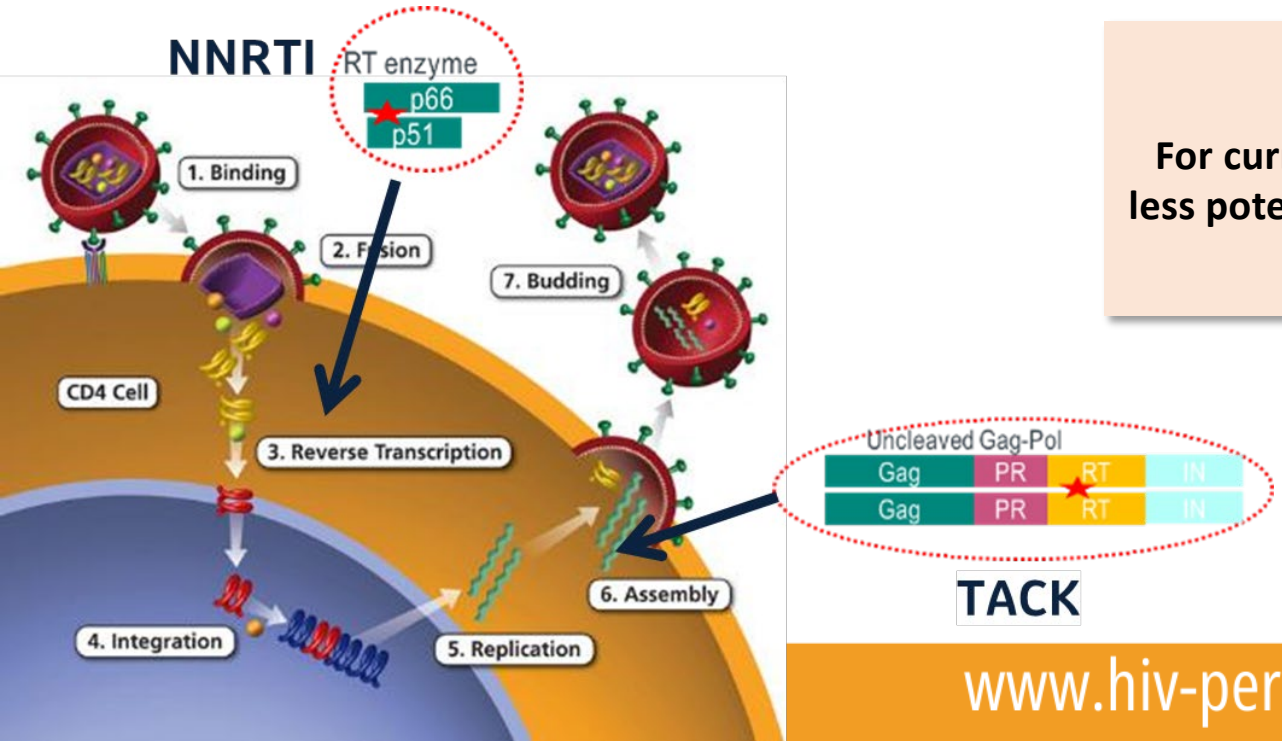
# CONFLICTS OF INTEREST

Paul Zuck is an employee of Merck & Co. Inc., Rahway, NJ, USA

# Eliminating HIV-1 infected cells – A step towards achieving Cure

- Antiretroviral therapy *primarily* blocks viral replication and prevents viral spread to healthy cells
  - ➢ Maintains, but does not reduce, the HIV infected cell reservoir
- Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) inhibit reverse transcriptase (RT) and target early stages of infection
- A few NNRTIs also interfere with late-stage virus replication by enhancing gag-pol processing

→ Early HIV protease activation inside cell (vs maturing, budding virion) →Death of infected cells







**TACK - Targeted Activator of Cell Kill**

**For currently approved NNRTIs, TACK activity is orders of magnitude less potent than NNRTI activity → infected cell death unlikely observed at clinically approved doses.**

Tachedjian G et al. FEBS Letters. 2005
Figueiredo A et al. PLOS Pathogens. 2006
Jochmans D et al. Retrovirology. 2010
Sudo S et al. Journal of Virology 2013
Zerbato J et al. Antimicrobial Agents Chemother 2017
Trinité B et al. Retrovirology. 2019
Wang Q et al. Science 2021

# NNRTI-triggered "**Targeted Activator of Cell Kill** (TACK)" Mechanism

Tachedjian G et al. FEBS Letters. 2005
Figueiredo A et al. PLOS Pathogens. 2006
Jochmans D et al. Retrovirology. 2010
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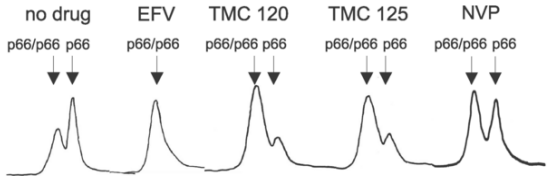
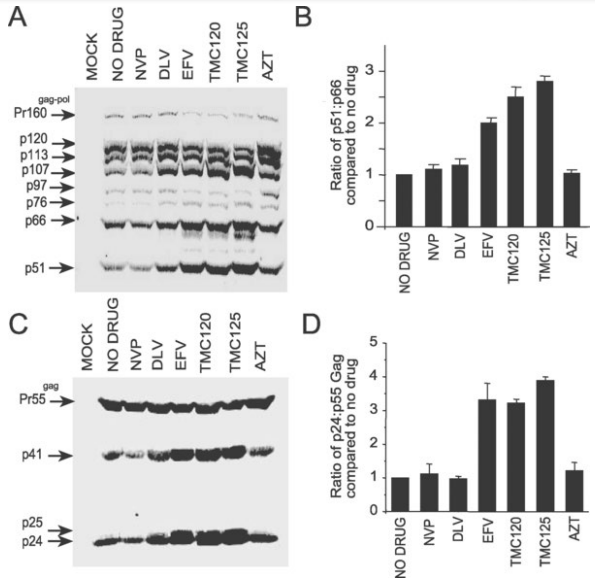
## NNRTI Enhancement of p66 Homodimer Formation



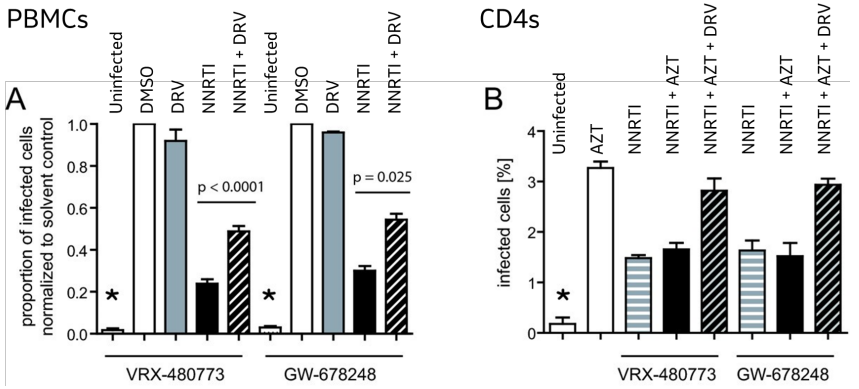

Size exclusion chromatography with purified p66 in the presence or absence of NNRTIs

## EFV, TMC120, and TMC125 Enhance Intracellular Gag and Gag-Pol Processing



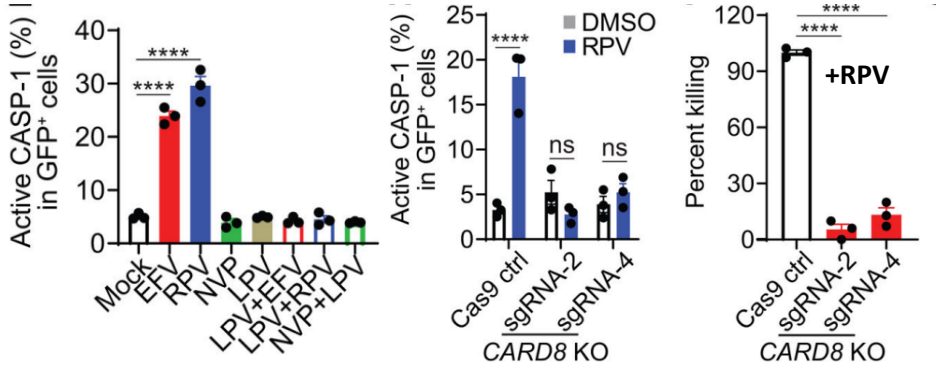

Processing of Gag-Pol and Gag in Infected Cells

## Selective Protease-Dependent Killing of HIV Infected Cells



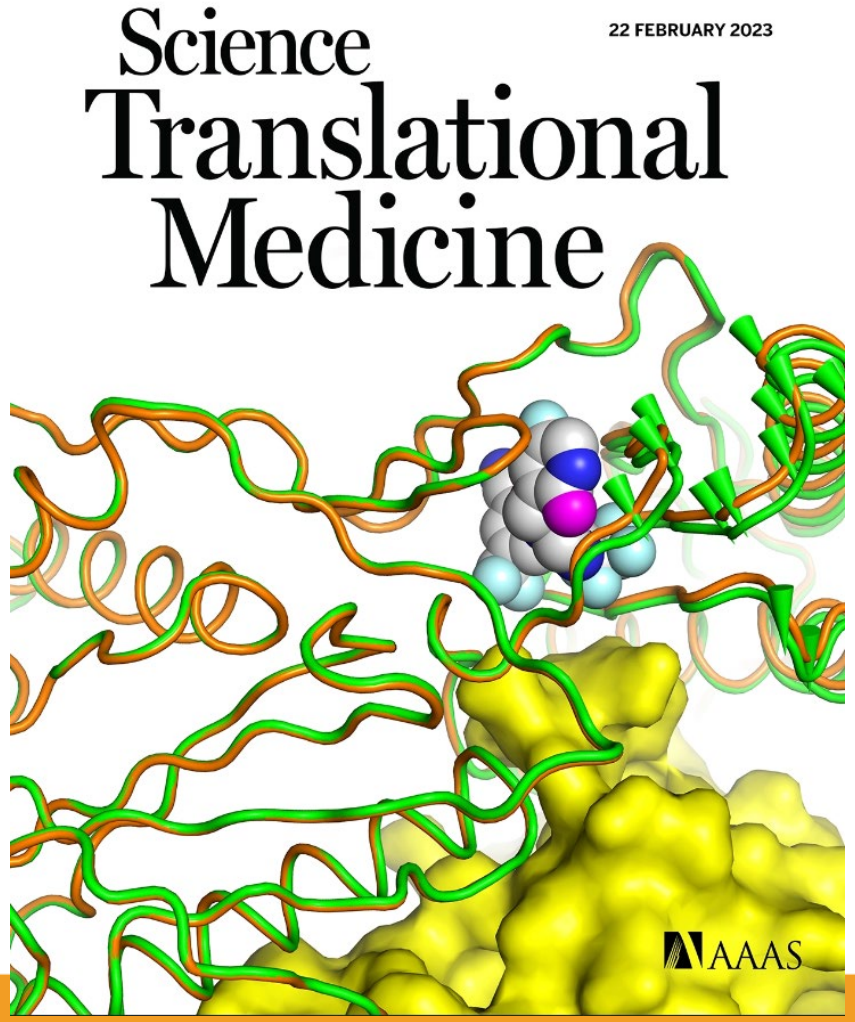

Effects of treatment on the number of infected cells (GFP+) in PBMCs or CD4 T cells infected with HIV-GFP in vitro

## HIV-1 Protease Induces CARD8 Inflammasome Activation→Pyroptosis→Cell Death




Analysis of CASP-1 activation or cell killing in primary CD4+ cells infected with HIV-GFP in vitro.

# NNRTI induction of cell death is optimizable - The TACK Effect




SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

HIV

## Potent targeted activator of cell kill molecules eliminate cells expressing HIV-1

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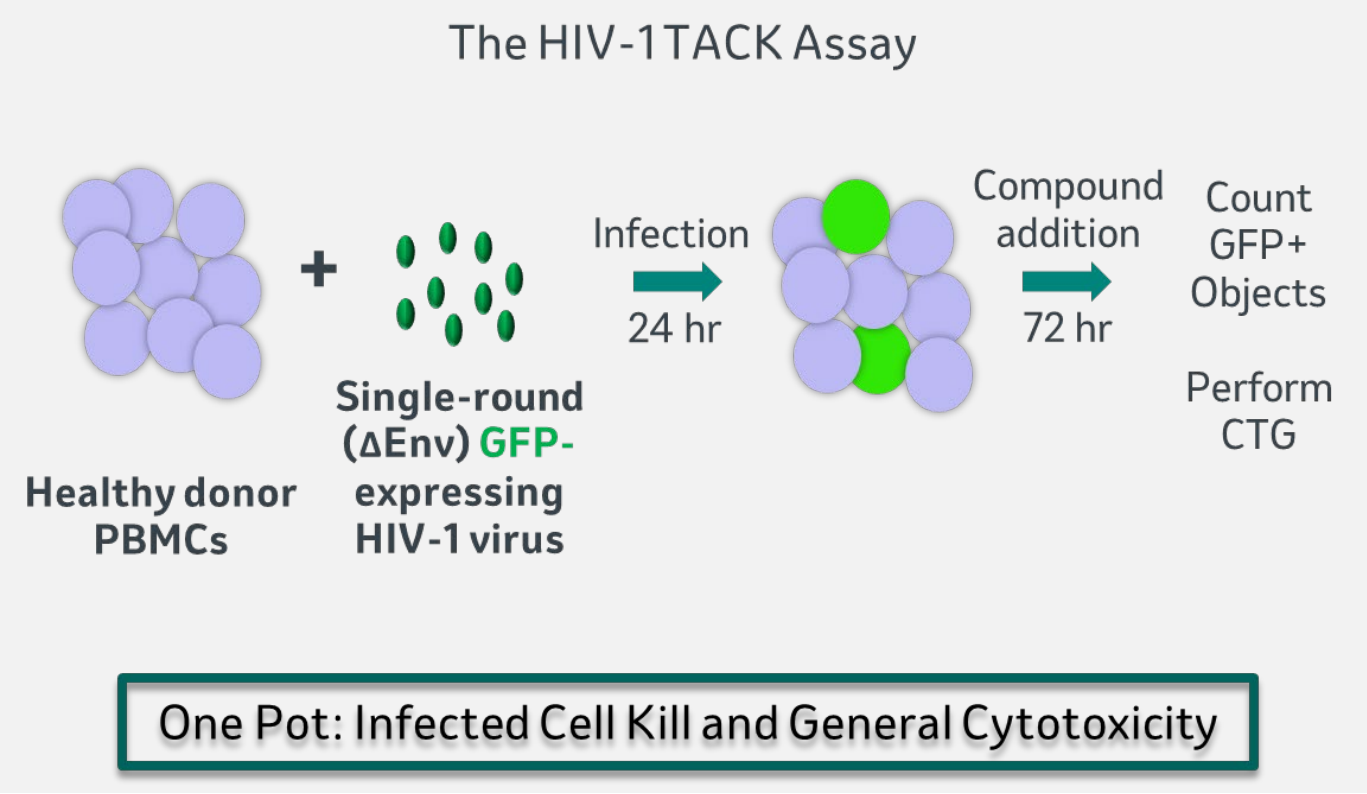
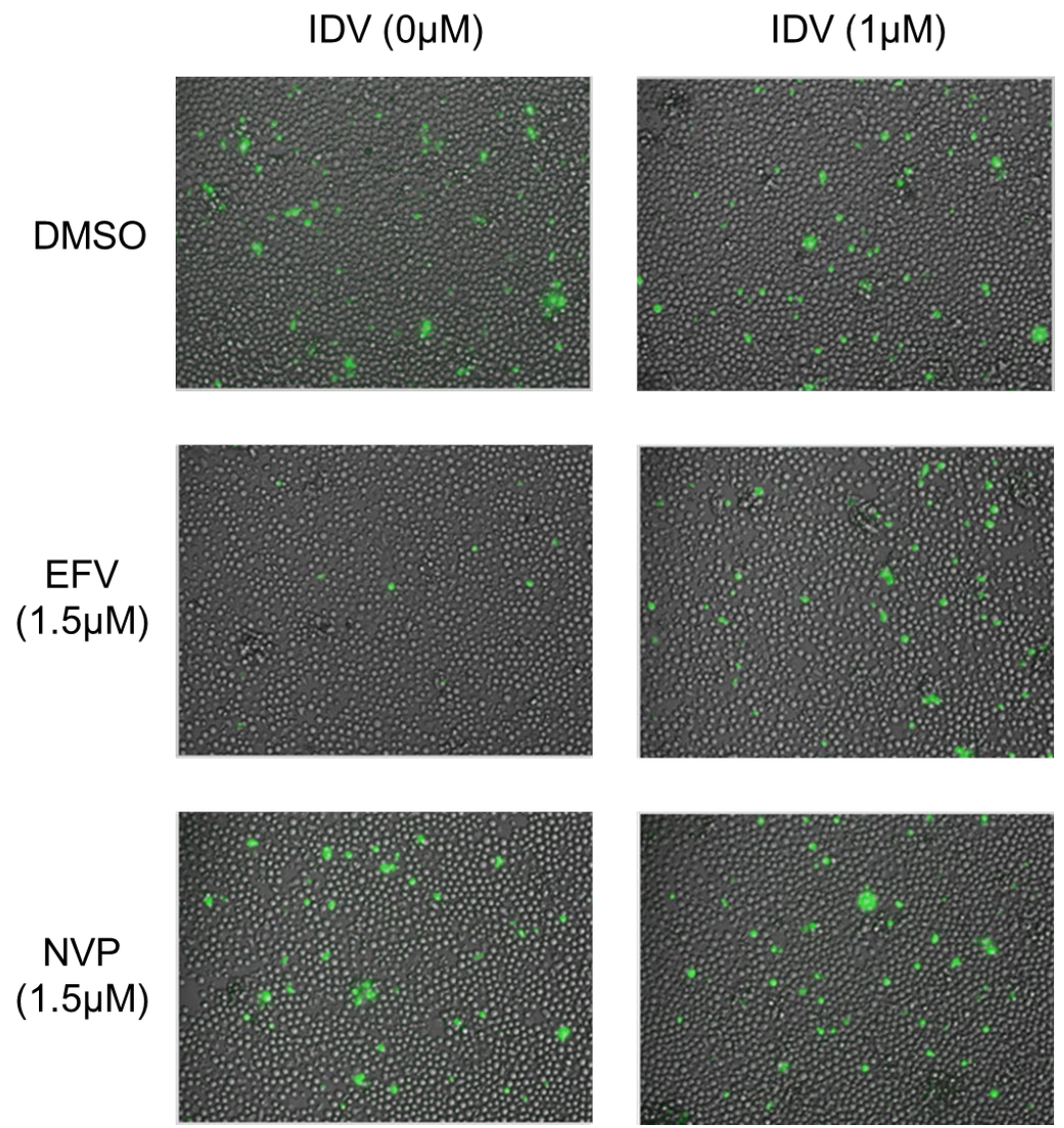
Antiretroviral therapy inhibits HIV-1 replication but is not curative due to establishment of a persistent reservoir after virus integration into the host genome. Reservoir reduction is therefore an important HIV-1 cure strategy. Some HIV-1 nonnucleoside reverse transcriptase inhibitors induce HIV-1 selective cytotoxicity in vitro but require concentrations far exceeding approved dosages. Focusing on this secondary activity, we found bifunctional compounds with HIV-1–infected cell kill potency at clinically achievable concentrations. These targeted activator of cell kill (TACK) molecules bind the reverse transcriptase–p66 domain of monomeric Gag-Pol and act as allosteric modulators to accelerate dimerization, resulting in HIV-1$^+$ cell death through premature intracellular viral protease activation. TACK molecules retain potent antiviral activity and selectively eliminate infected CD4$^+$ T cells isolated from people living with HIV-1, supporting an immune-independent clearance strategy.

# Screening for TACK Molecules With Improved Potency




**Key:**
IDV – indinavir (protease inhibitor)
EFV – efavirenz (NNRTI)
NVP – nevirapine (NNRTI)

# Identifying potent TACK molecules

***6628 NNRTI Related Analogues Library***

↓

TACK
0.3 µM and 3 µM

↓ *> 50% effect at 3 µM*

TACK Dose-Response Titration | Cell-Titer Glo Dose-Response Titration

***One pot assay with active protease***

↓ *TACK < 1 µM, TACK/CTG > 20*

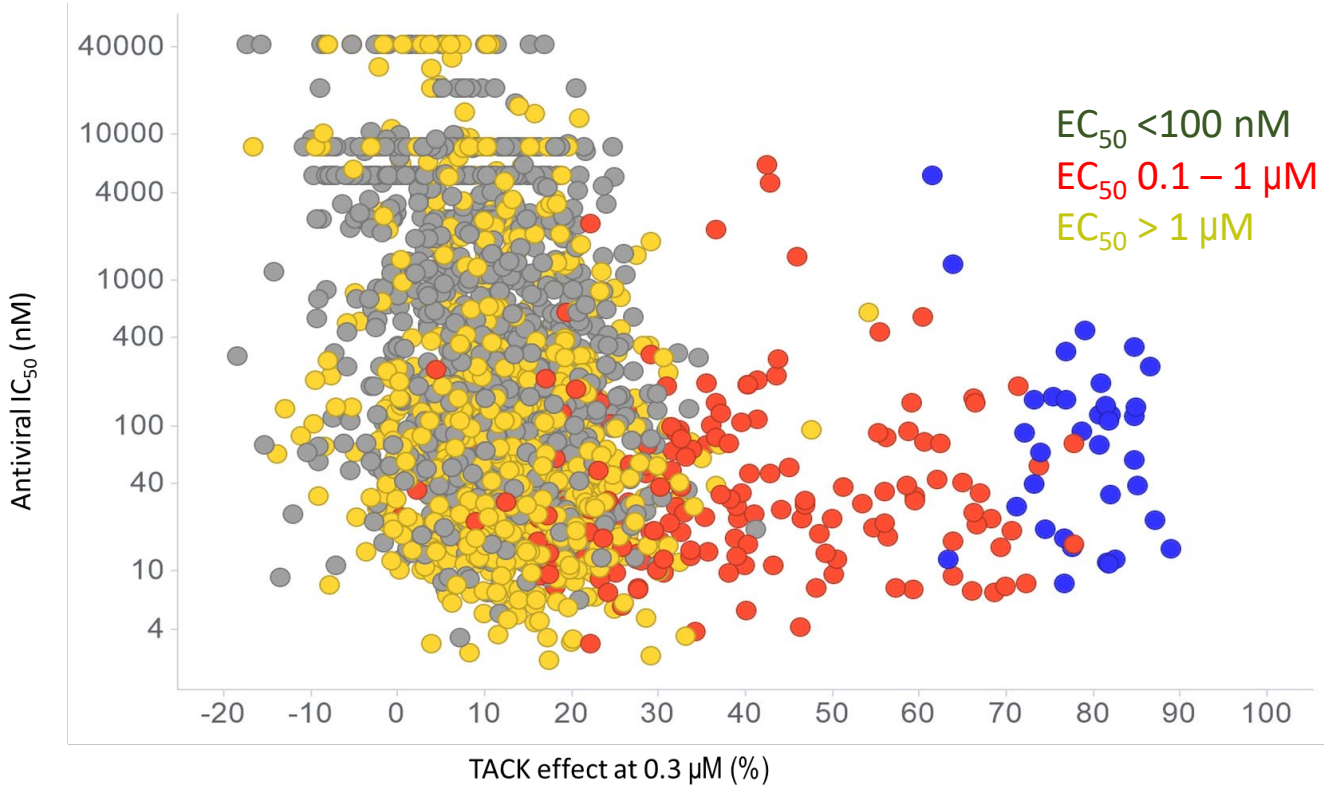
TACK Dose-Response Titration with Protease inhibitor

↓

Select series for further optimization/characterization

110 (1.7%) ≥ 50% effect at 0.3 µM
774 (11%) ≥ 50% effect at 3.0 µM

TACK activity was rare among NNRTIs and TACK-active compounds were generally potent antivirals but not vice versa



# Pyrimidones identified as a promising compound class that could be optimized for TACK activity

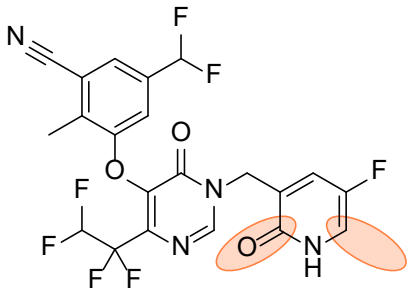
| Compound | Pyr01 | Pyr02 | EFV | NVP |
|---|---|---|---|---|
| TACK WT $EC_{50}$ in PBMCs (nM) | 27.5 ± 12.0 (n=4) | 34400 ± 2820 (n=2) | 1550 ± 618 (n=256) | >40500 (n=2) |
| TACK WT $EC_{50}$ in CD4+ T-cells (nM) | 38.4 ± 3.6 (n=3) | >40500 (n=3) | 4006 ± 171 (n=3) | >40500 (n=3) |
| Antiviral $IC_{50}$ (nM) | 39.7 ± 6.2 (n=6) | 131 ± 38.0 (n=7) | 34.1 ± 8.6 (n=295) | 219 ± 28.4 (n=5) |
| Cytotoxicity $CC_{50}$ (nM) | >40000 (n=3) | >40000 (n=3) | >40000 (n=3) | >40000 (n=3) |
| TACK K103N $EC_{50}$ (nM) | 23.9 ± 3.4 (n=2) | NT | 20100 ± 3600 (n=11) | >42000 (n=1) |
| TACK Y181C $EC_{50}$ (nM) | 21.1 ± 5.1 (n=2) | NT | 1750 ± 756 (n=21) | >42000 (n=1) |

WT=wild-type; NT=not tested

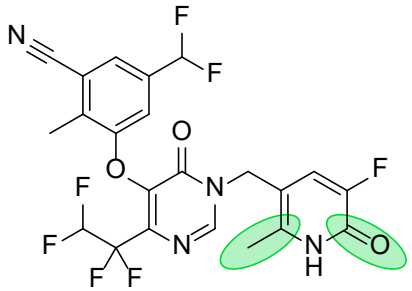
<u>Key Features of Pyr01</u>

- Similar antiviral and TACK potency
- Large window between TACK activity and cytotoxicity
- Minimal effect of common NNRTI RAMs

# TACK active Pyr01 and inactive Pyr02 bind comparably to RT heterodimer

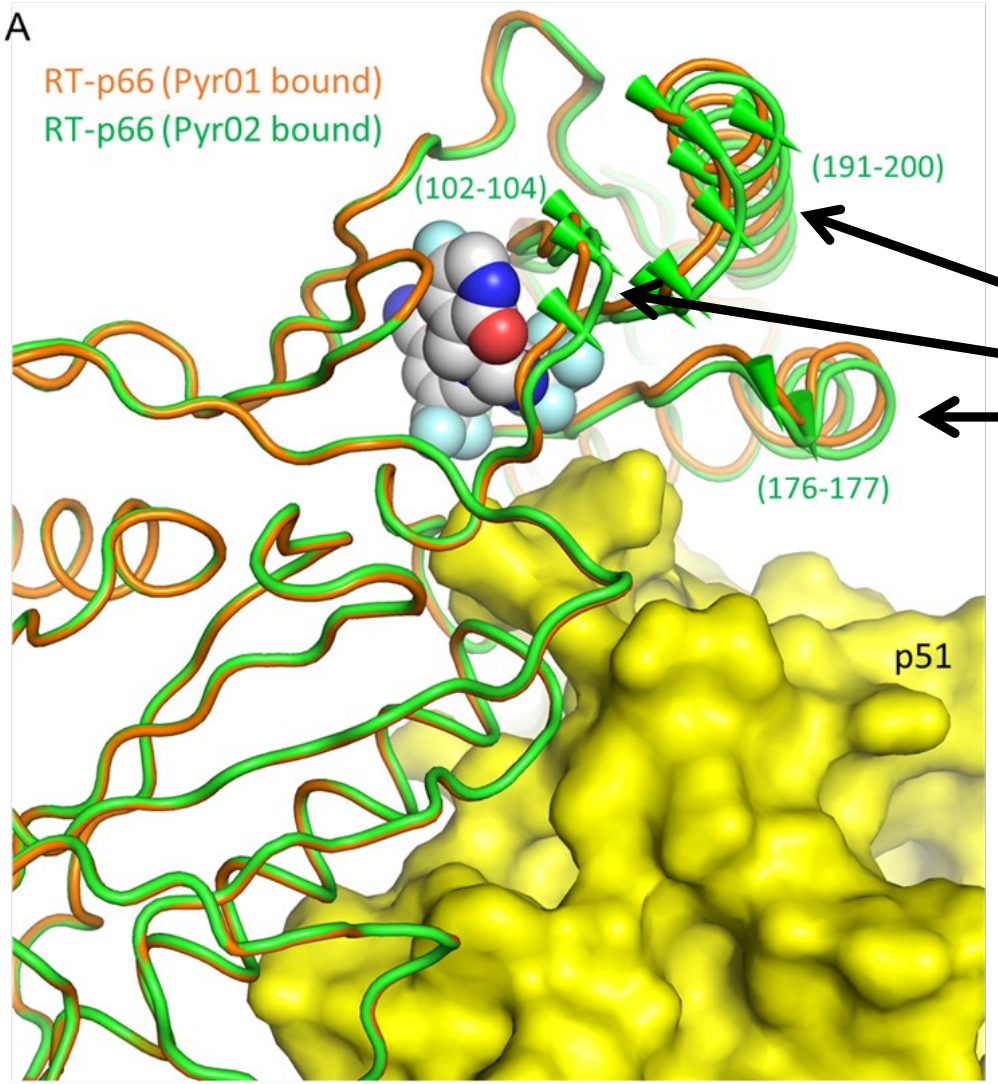


Pyr01



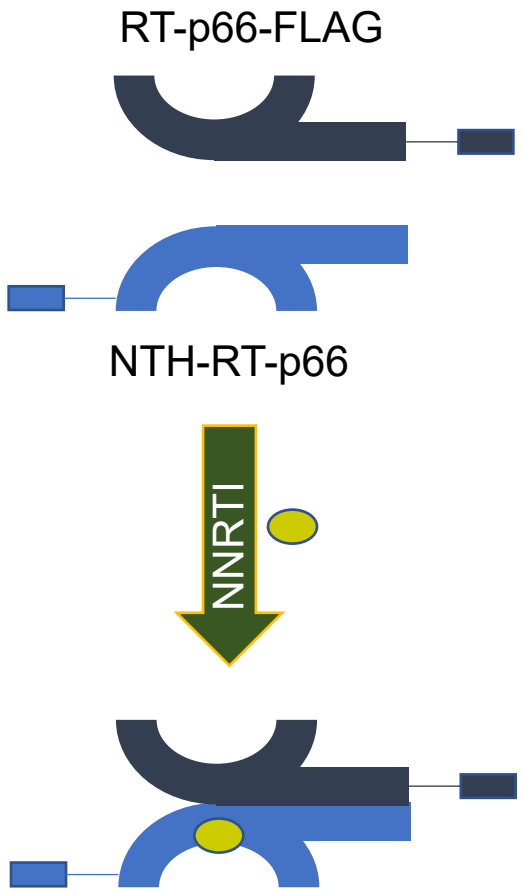
Pyr02

An all-atom overlay of crystal structures of HIV-1 RT (p66/p51) bound with TACK-active Pyr01 and TACK-inactive Pyr02 (76% Tanimoto similarity) reveals little structural difference

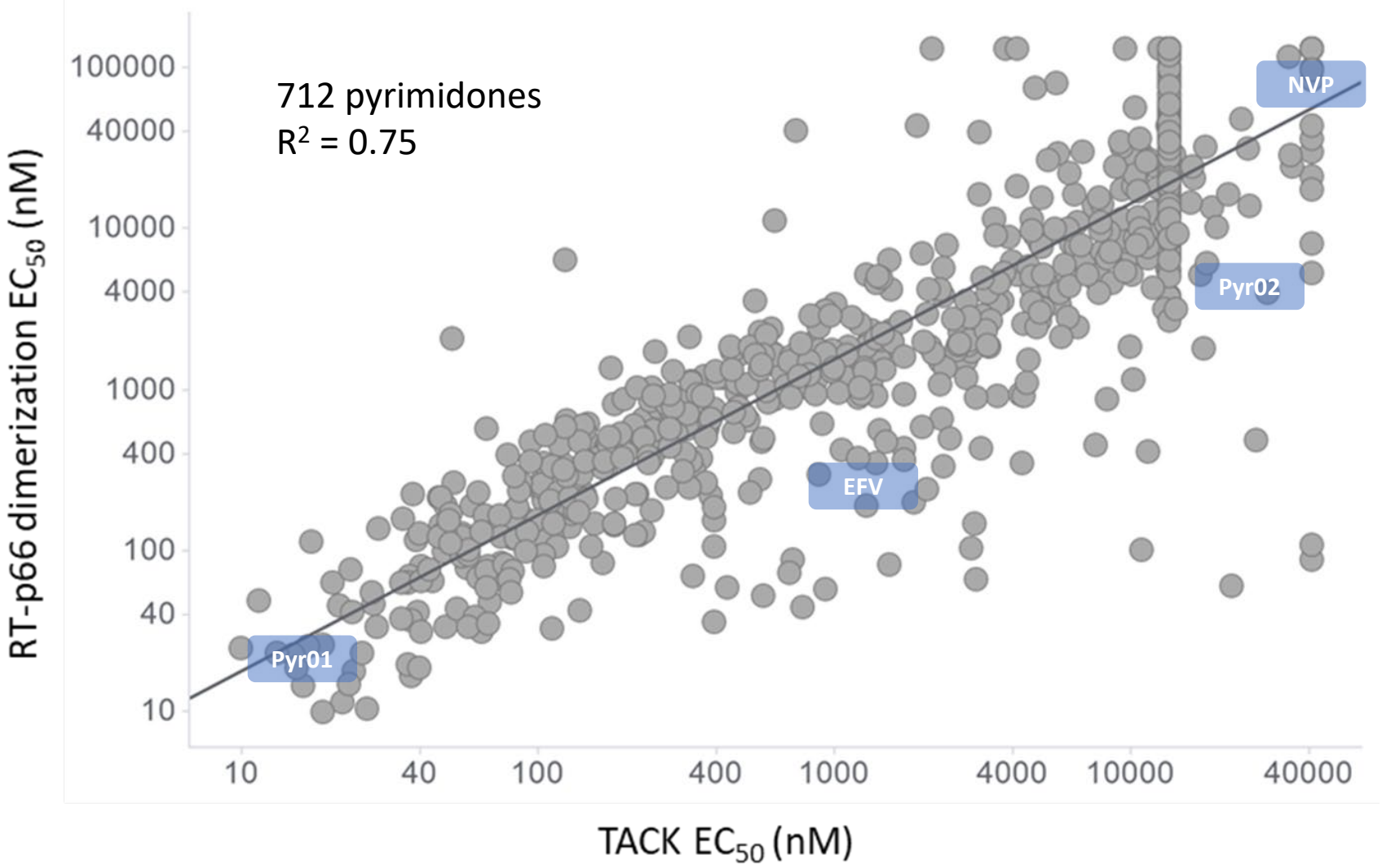



Only regions with backbone shifts greater than 1 Å

# Strong correlation between TACK and RT-p66 dimerization activities




**Detect NNRTI-induced RT dimer using HTRF**



# Competition binding experiments reveal that TACK effect is likely mediated by the ability of NNRTIs to bind monomeric p66-RT

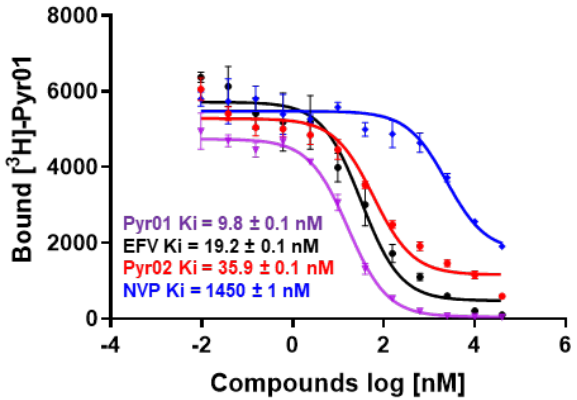
Protein

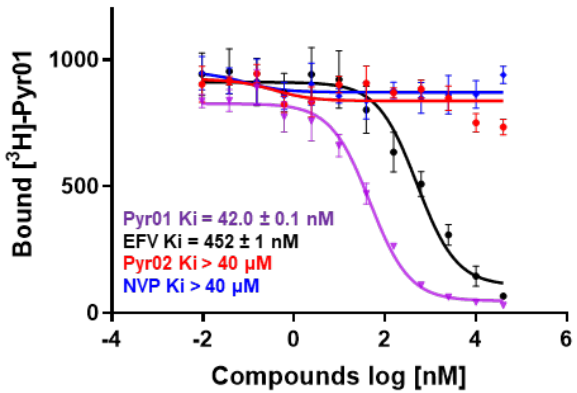
**WT RT-p66**

**W401A RT-p66**
(monomeric)

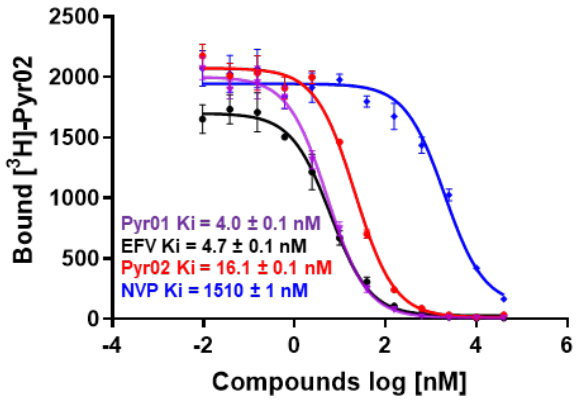
## Pre-Bound Compound

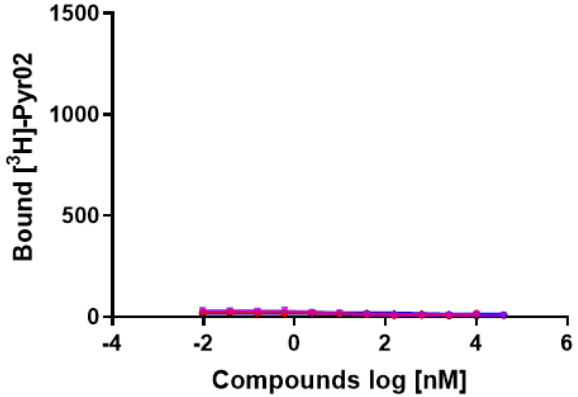
**[$^3$H]-Pyr01**




**[$^3$H]-Pyr02**




Competing Compound

**TACK-Active**
Pyr01 — EFV

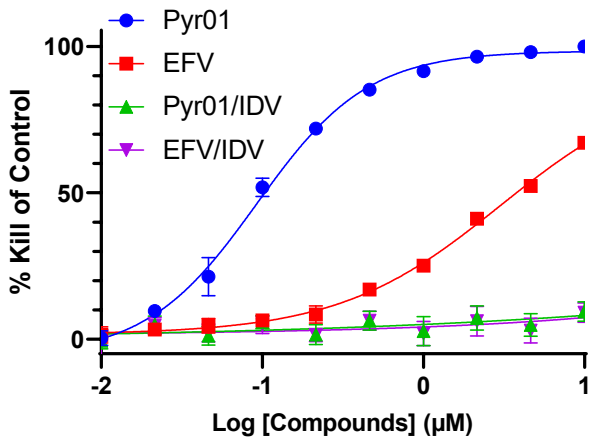
**TACK-Inactive**
Pyr02 — NVP

- All compounds bind and can compete for binding on dimeric RT
- Only TACK-active compounds bind and compete for binding on monomeric RT

# TACK occurs via caspase 1 induced pyroptosis

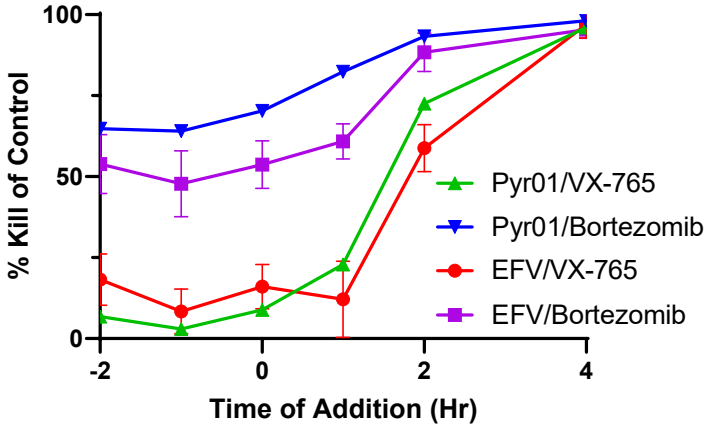
## TACK is HIV-1 Protease dependent

+/- IDV (Protease Inhibitor)

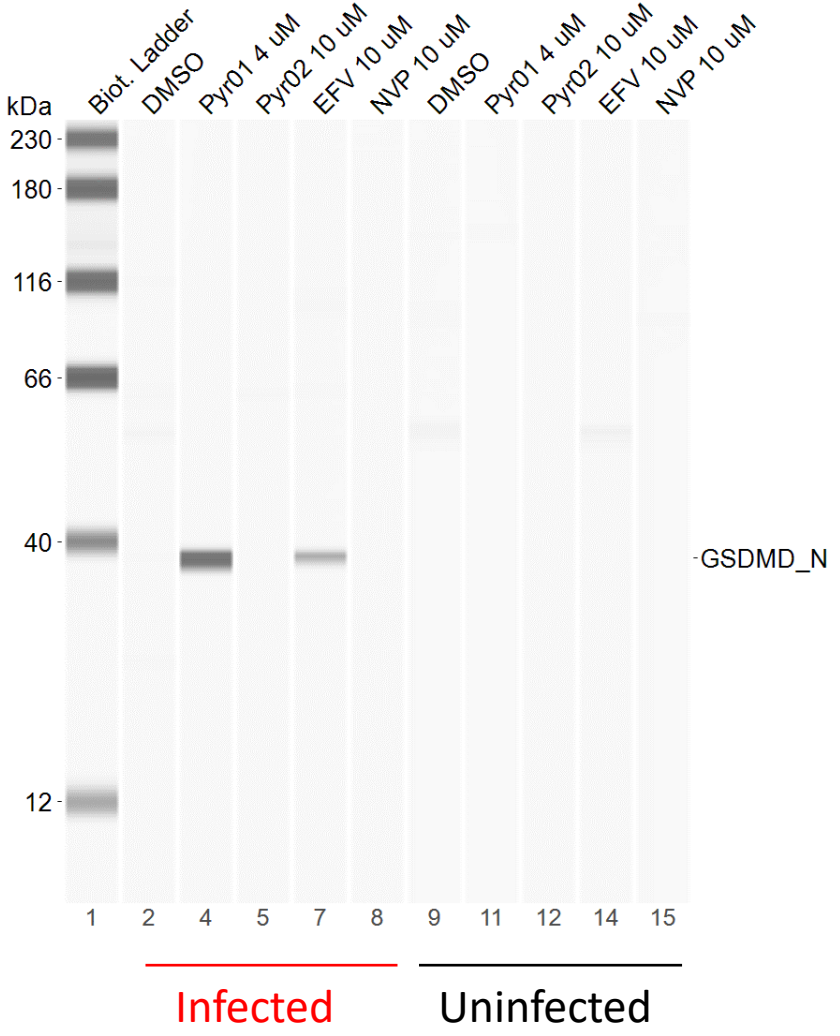



## TACK is Caspase 1 and Proteasome dependent

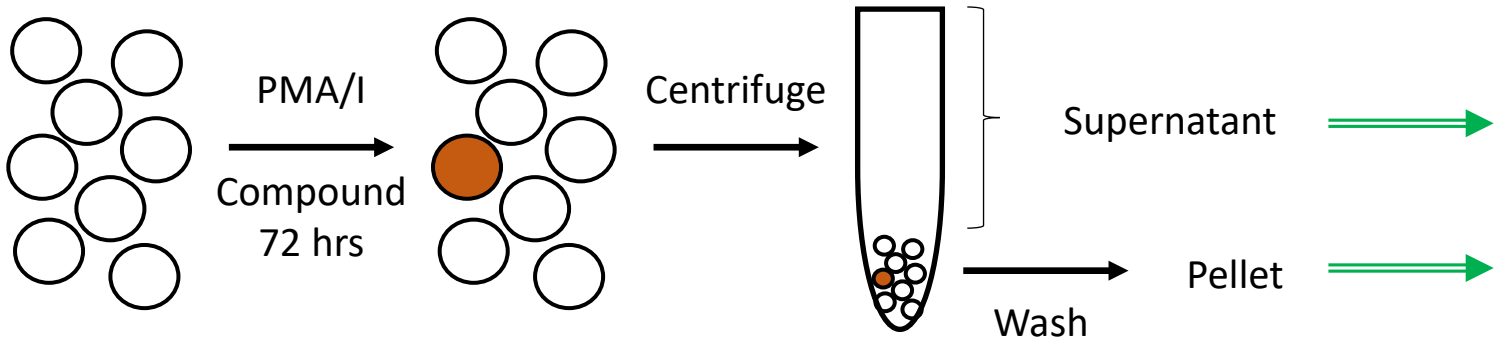
+/- VX-765 (Casp1 Inhibitor) or Bortezomib (Proteasome Inhibitor)



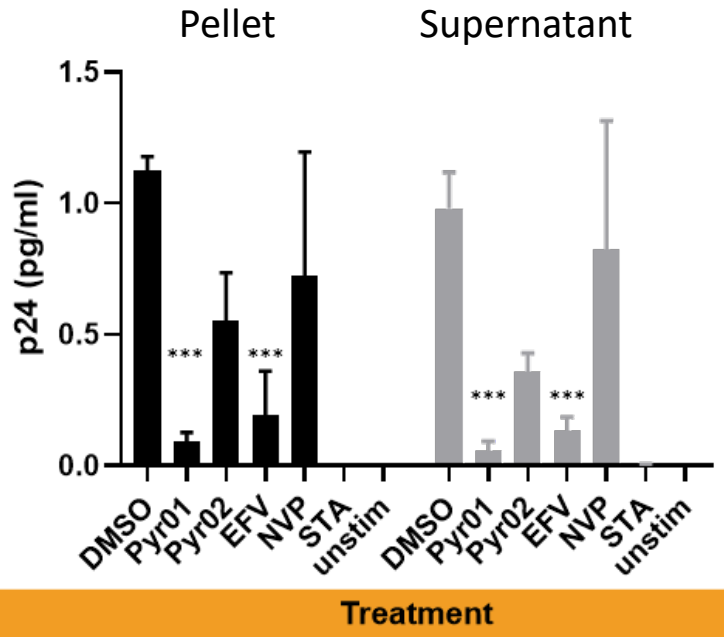

## TACK results in Gasdermin D cleavage in CD4 T-cells



# Assessing TACK effect in CD4+ T-cells from ART treated PLWH




p24 Digital Enzyme-linked Immunosorbent Assay (ELISA) Using Single Molecule Array (Simoa) Technology



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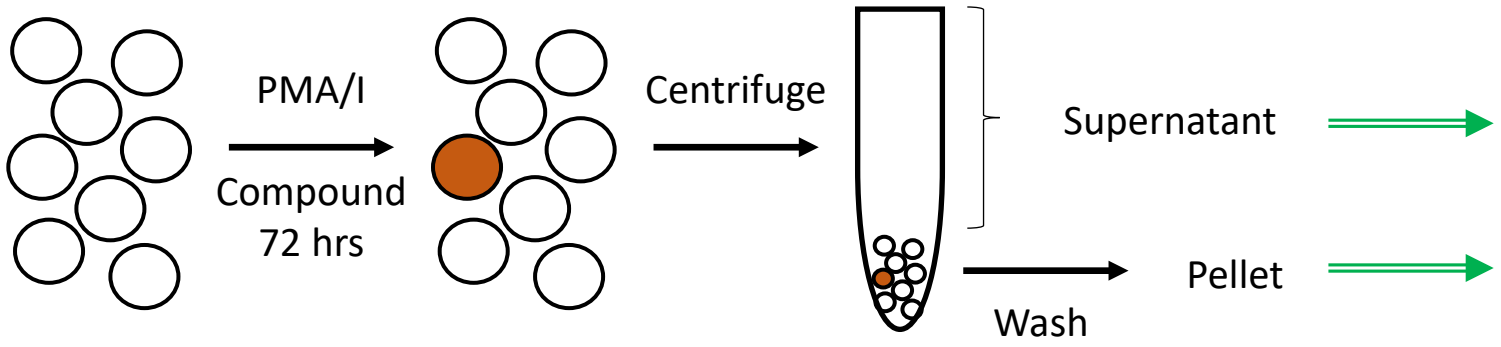


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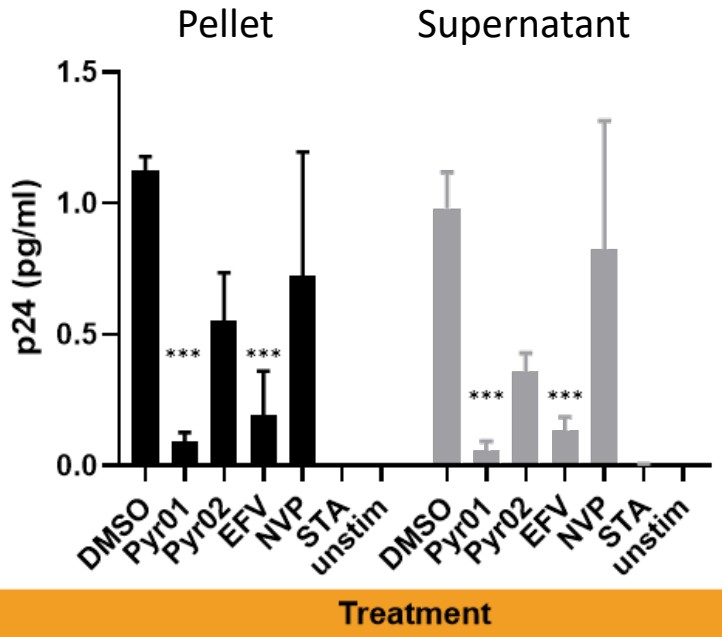
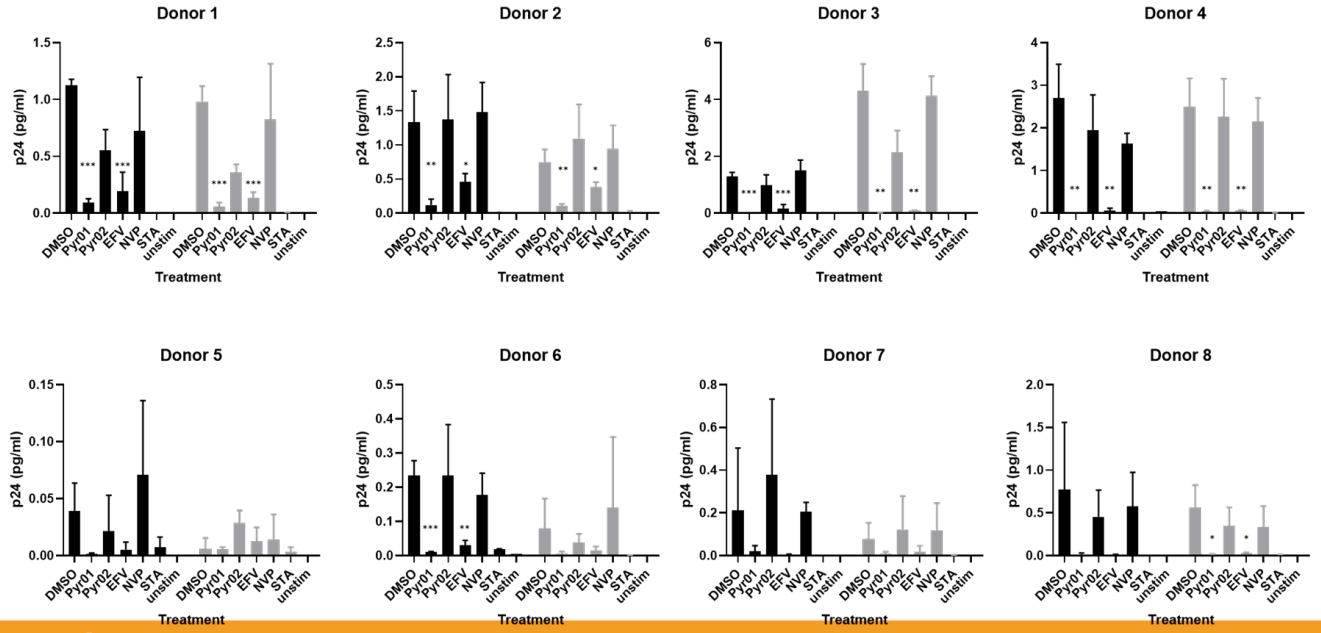
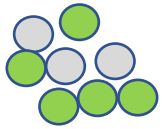


# Use MSD HIV Viremic Mouse Model to Monitor Viral Decay After Treatment With a TACK vs Non-TACK NNRTI

PBMCS from healthy donor

3 days
+PHA



Infect activated PBMCs with wild-type replication competent virus at low MOI

1-2 days

Inject into Immunodeficient NSG-MHC I/II DKO mice (IP)

3 weeks

Monitor pVL weekly and randomize accordingly

Treat with TACK or Non-TACK NNRTI : terminal timepoints on day 0, 1, 3, and 7 for PK and pVL

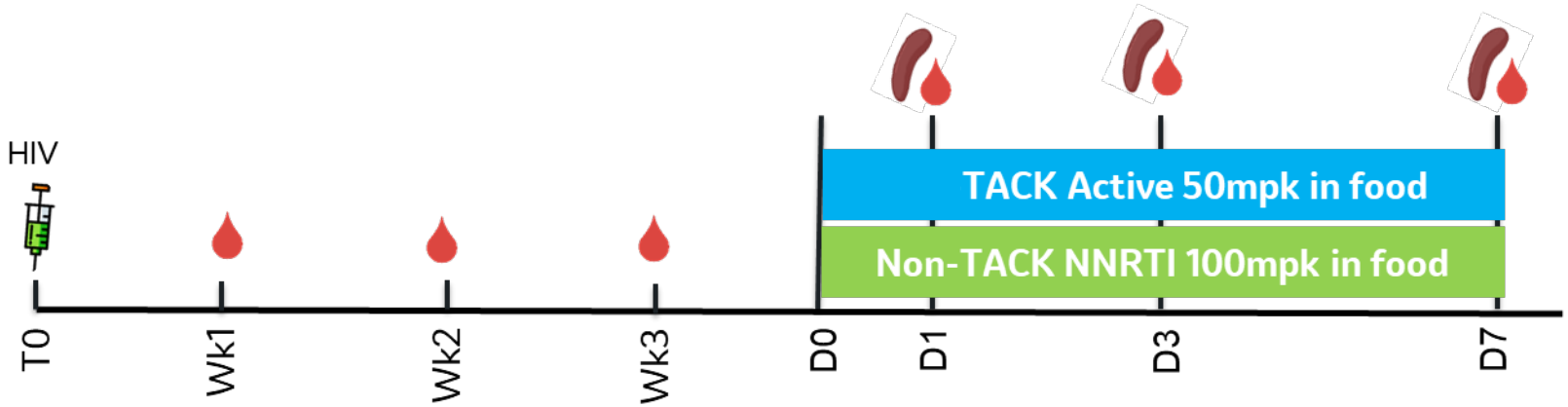
## Potential Biomarkers:

Blood Assays:

- Plasma Viral Load (pVL)
- Plasma pharmacokinetics

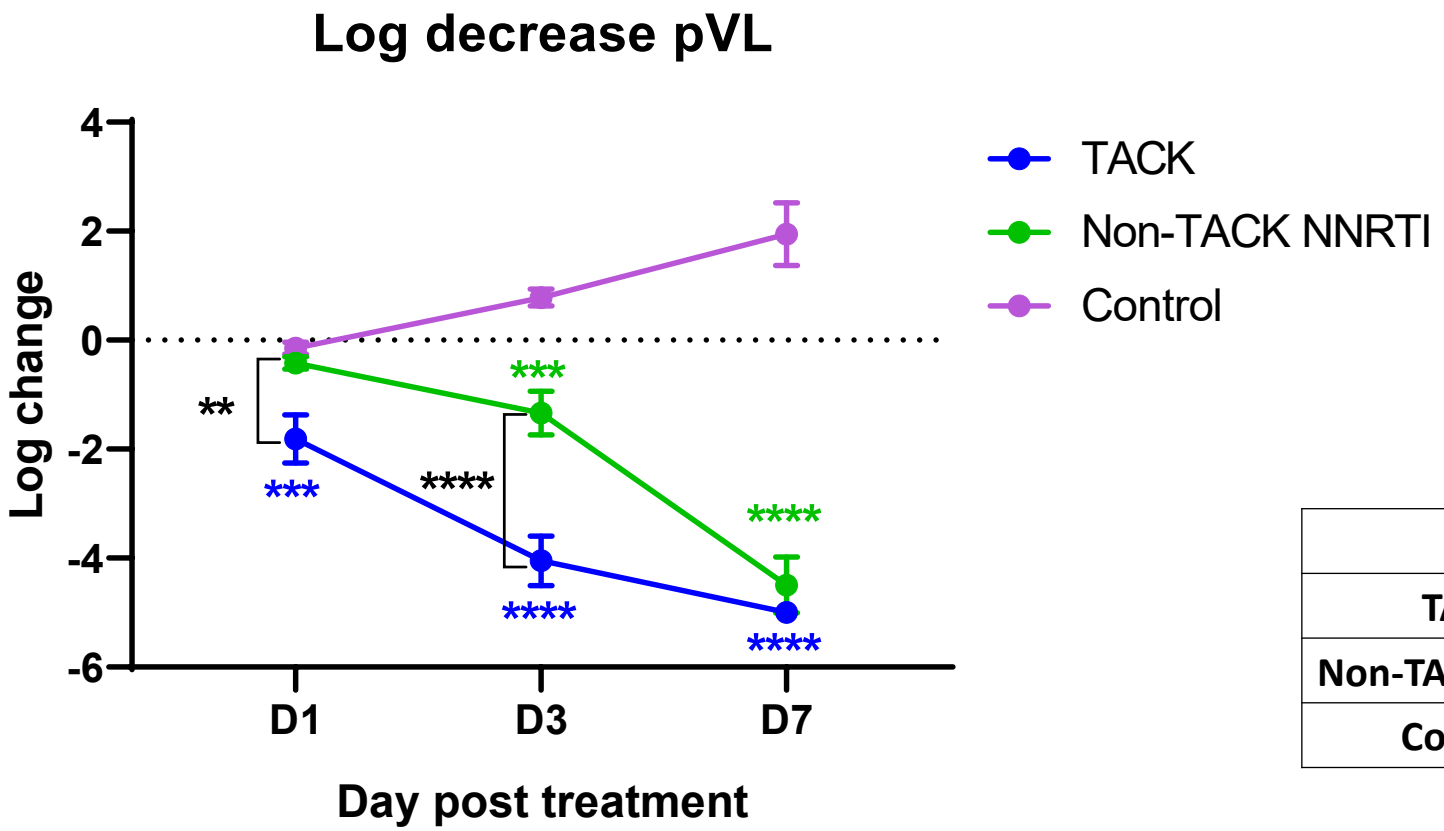
Spleen Assays:

- RNAscope (total HIV RNA)
- p24 Immunohistochemistry (IHC)
- p24 flow cytometry




2022 HIV Persistence Meeting - Maxwell, J et al Journal of Virus Eradication 8S (2022)100158

# Faster decrease in plasma viral load decay with TACK-active




**Pharmacokinetics (D1-D7)**

- Both compounds maintained inhibitory quotients ≥ 50 for NNRTI activity
- Only the TACK compound exceeded levels required for TACK activity

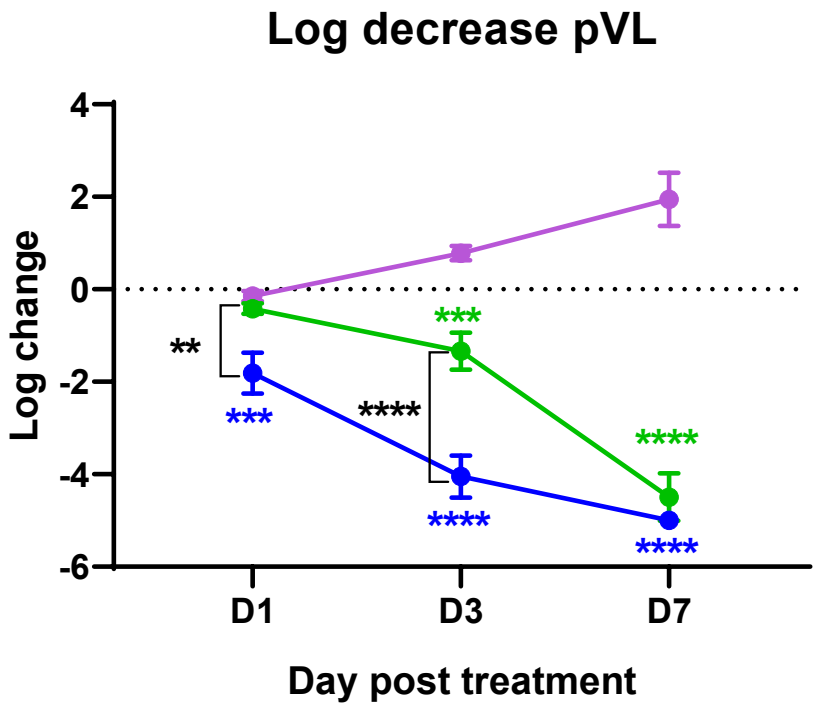
**Undetectable pVL**

| | D1 | D3 | D7 |
|---|---|---|---|
| **TACK** | **2**/10 | **7**/10 | **9**/9 |
| **Non-TACK NNRTI** | 0/10 | **1**/10 | **9**/9 |
| **Control** | 0/10 | 0/10 | 0/10 |

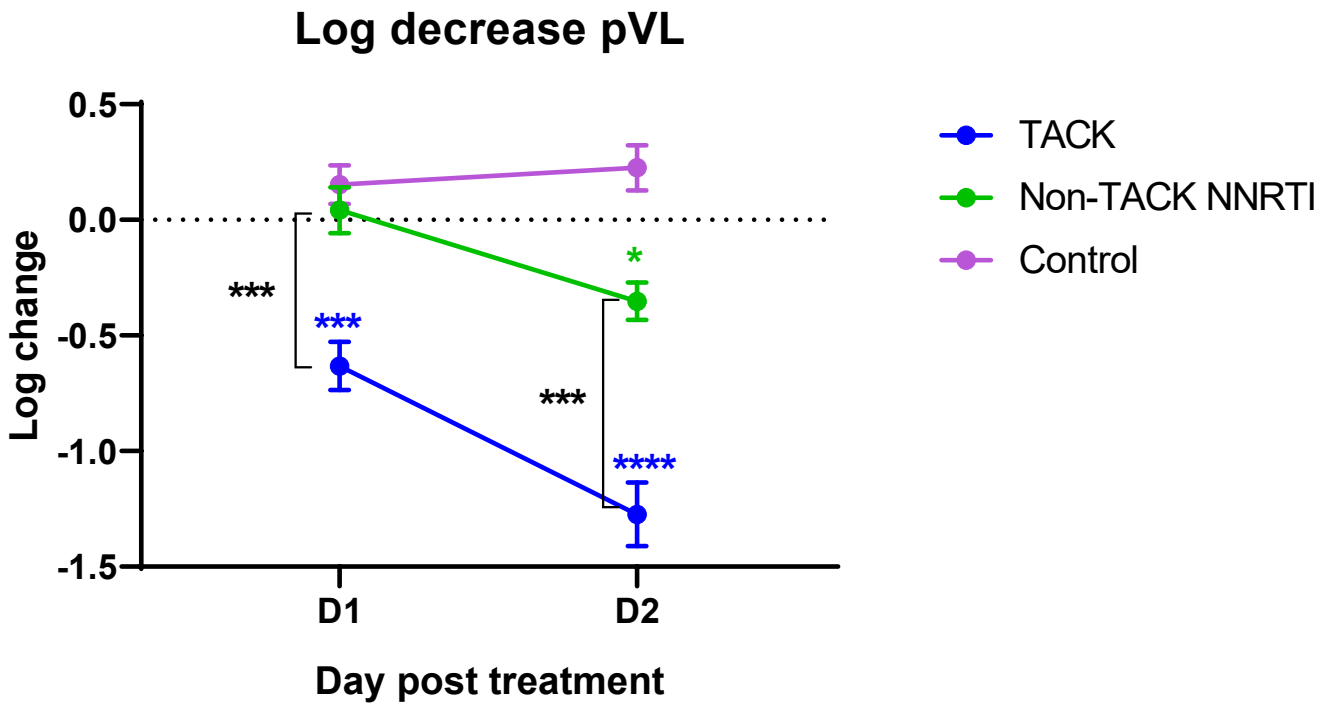
- Significant decrease in pVL with TACK at D1, D3 and D7 when compared to Vehicle Control
- Significant difference in pVL decrease with TACK when compared to non-TACK NNRTI at D1 and D3 indicating faster viral decay with TACK activity.

# Results are reproducible

## Experiment 1

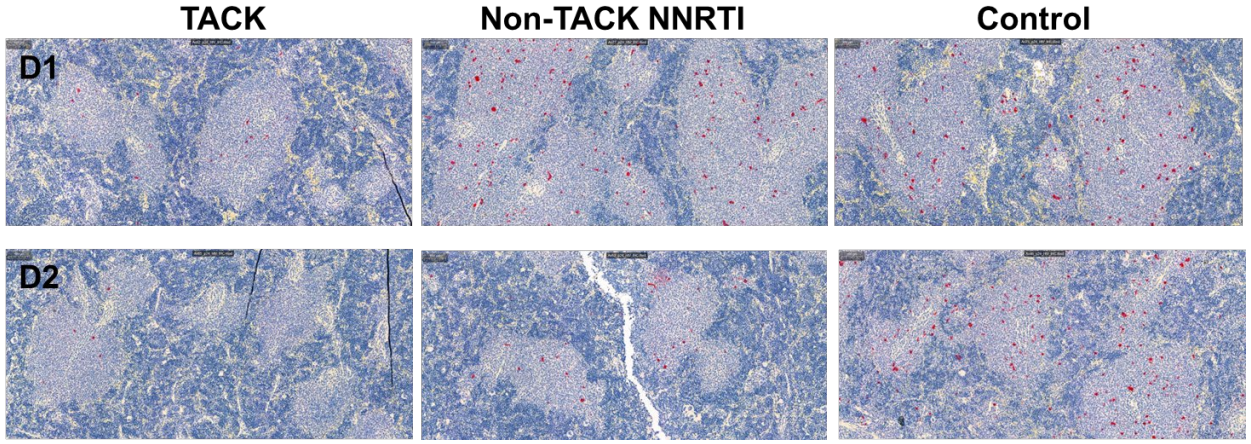


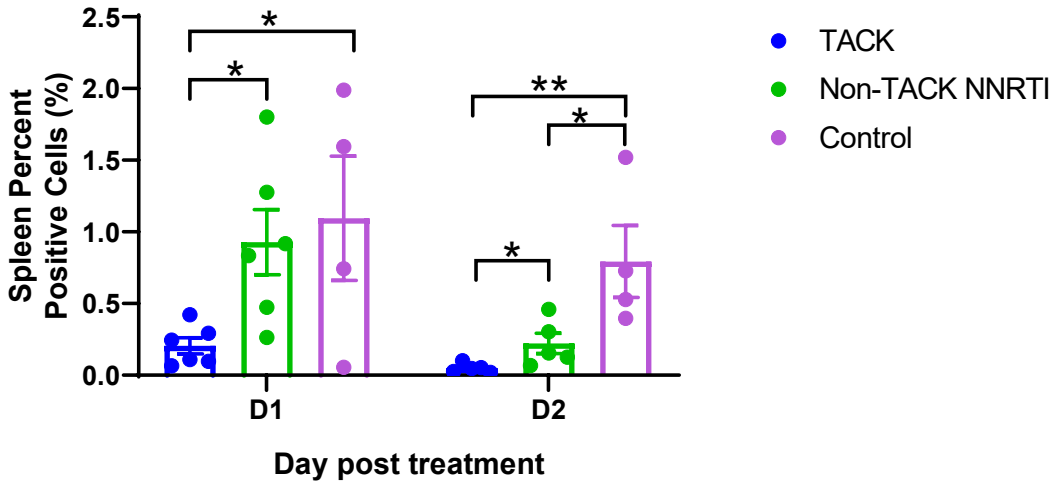

## Experiment 2



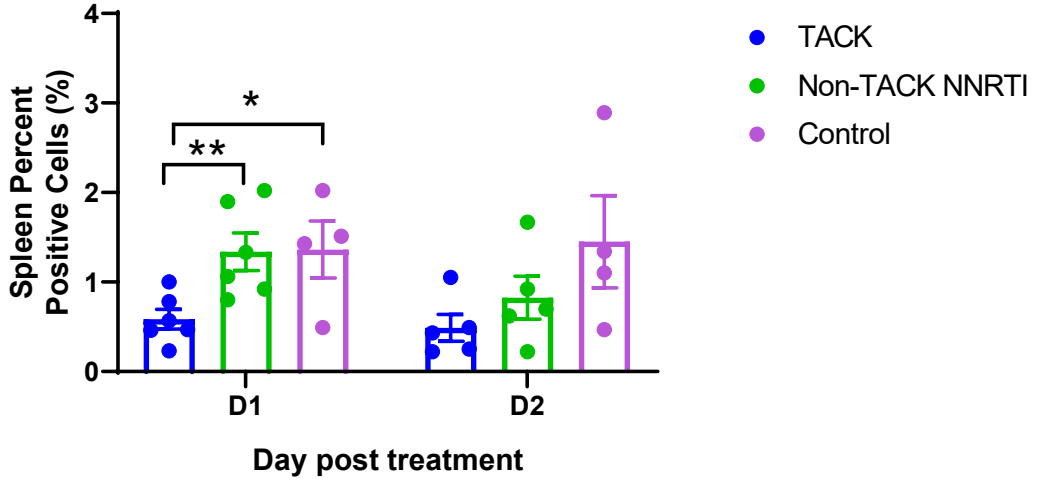
# Similar enhancement in viral decay for TACK vs. Non-TACK NNRTI observed in the spleen via analysis of p24

## Immunohistochemistry



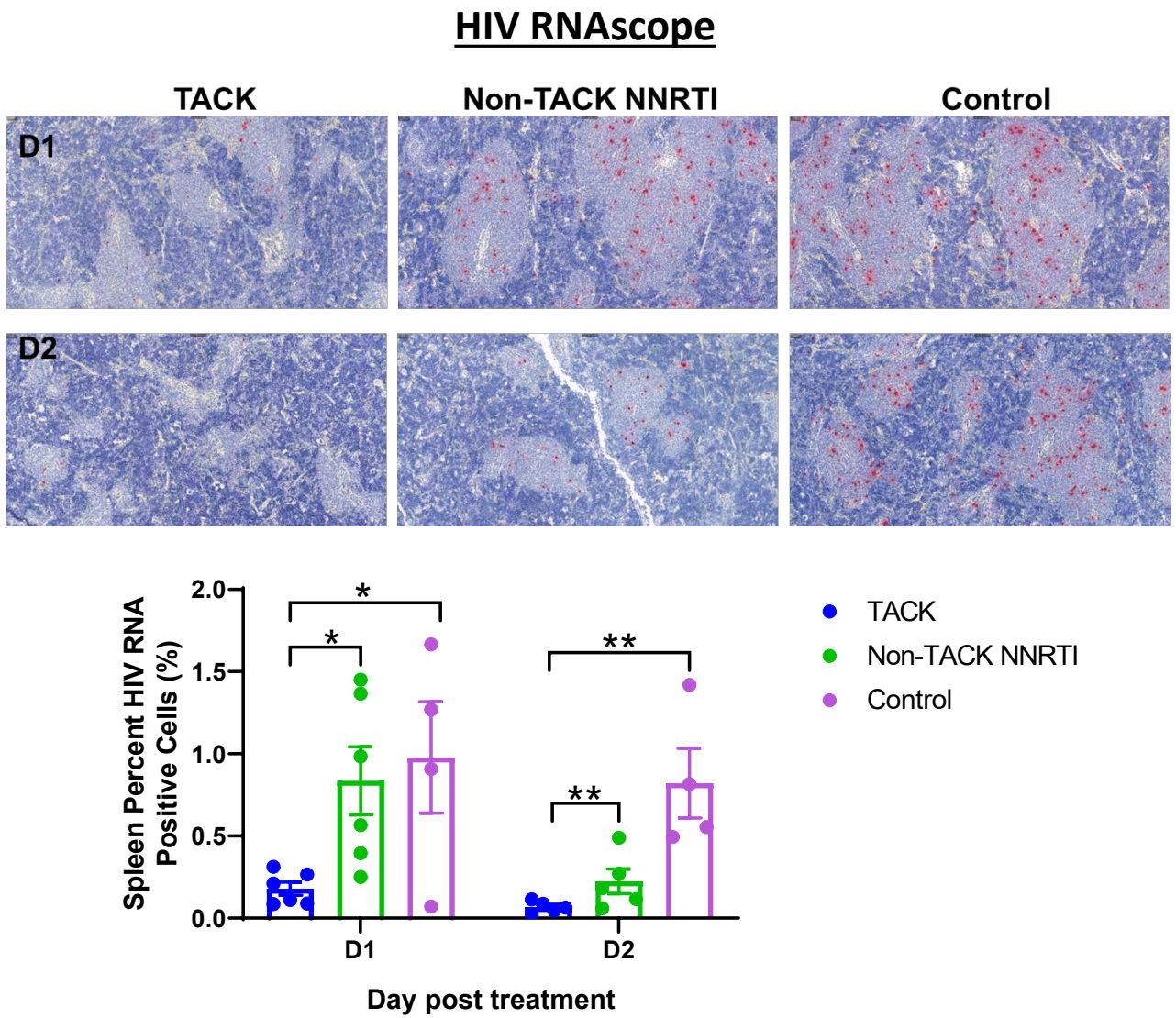




## Flow Cytometry




Unpublished

# Similar enhancement in viral decay for TACK vs. Non-TACK NNRTI observed in the spleen via analysis of RNA




Unpublished

# Summary

- We screened for molecules that can induce selective, intrinsic cell death in HIV-1 infected cells, a property we have termed TACK (Targeted Activator of Cell Kill)
- Focusing on a previously described secondary effect of certain NNRTIs, we have invented extremely potent RT-targeting TACK molecules
    - TACK molecules differentiate from standard NNRTIs by the ability to bind monomeric RT-p66
    - TACK drives enhanced Gag-Pol dimerization, leading to premature intracellular HIV protease activation
    - TACK activity was observed in primary cells and in an HIV Viremic Mouse Model

# Acknowledgements

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Jill Maxwell
Guoxin Wu
Paul Zuck

Quantitative Biosciences
Lei Ba
Carolyn Bahnck-Teets
Marina Bukhtiyarova
Steven S. Carroll
John F. Fay
Shih Lin Goh
Daniel Krosky
Jessica Lewis
Yangsi Ou
Payal Sheth
Min Xu

C&S Chemistry
Daniel J. Klein
Deping Wang
Beata Zamlynny

PPDM
Deborah Dooney
Stacey Polsky-Fisher
Daniel I. S. Rosenbloom

BARDS
Tian Zhao

Evotec Ltd.
Meigang Gu
Matthias Zebisch

…and many others