**UNIVERSITY OF TORONTO**

# Targeting HIV-1 Nef to Improve the Recognition of HIV-1 Infected Cells

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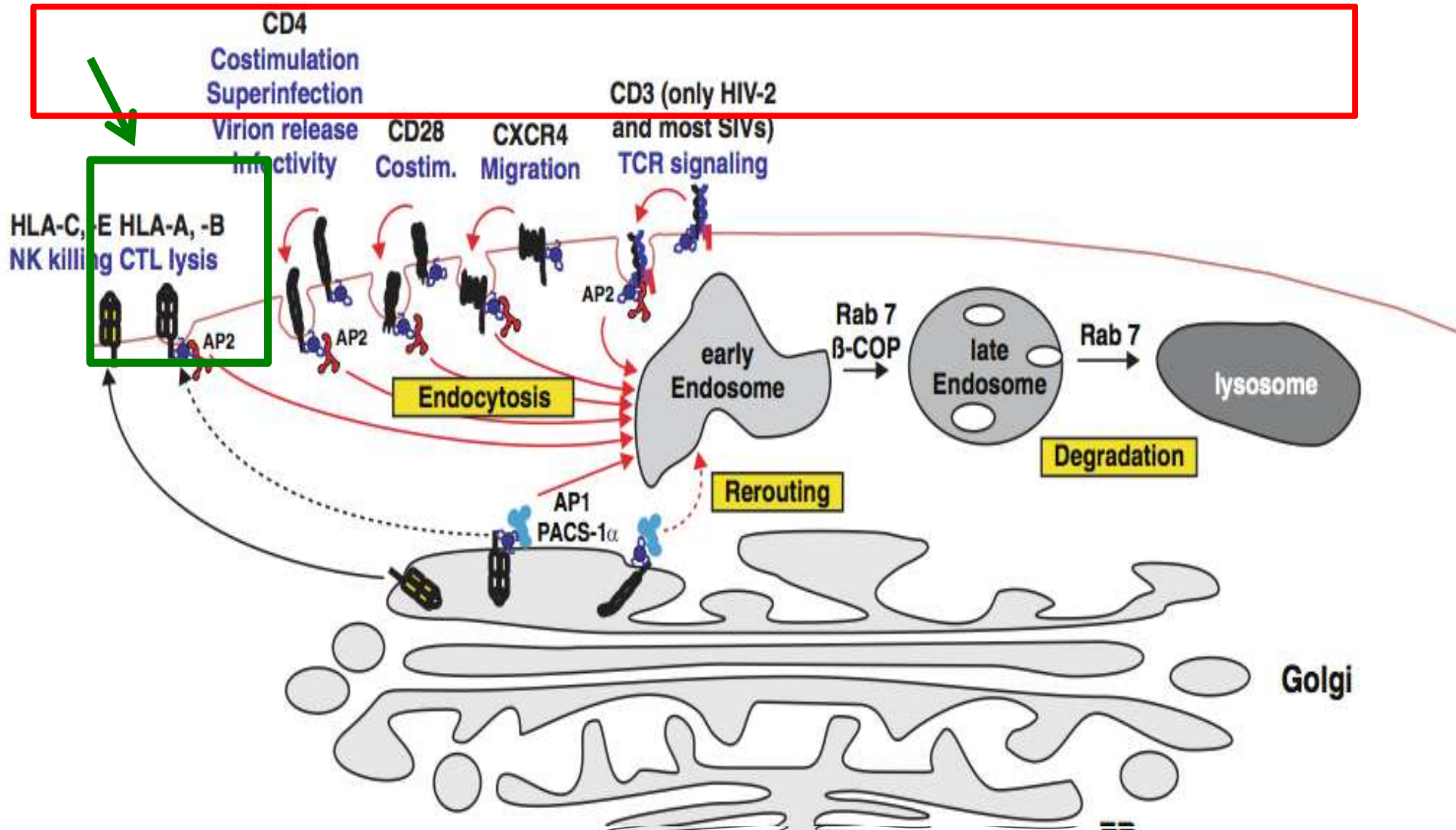
Mario Ostrowski

OHTN 2013
RESEARCH
CONFERENCE

NOVEMBER 17-19, 2013

**CHANGING THE COURSE** OF THE
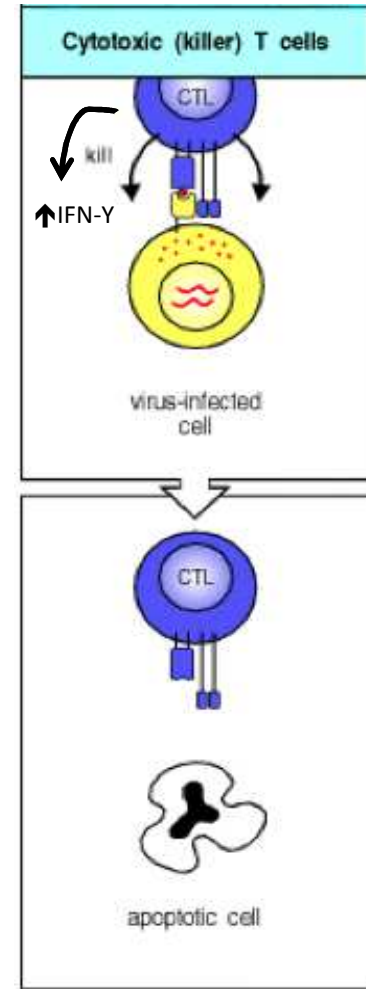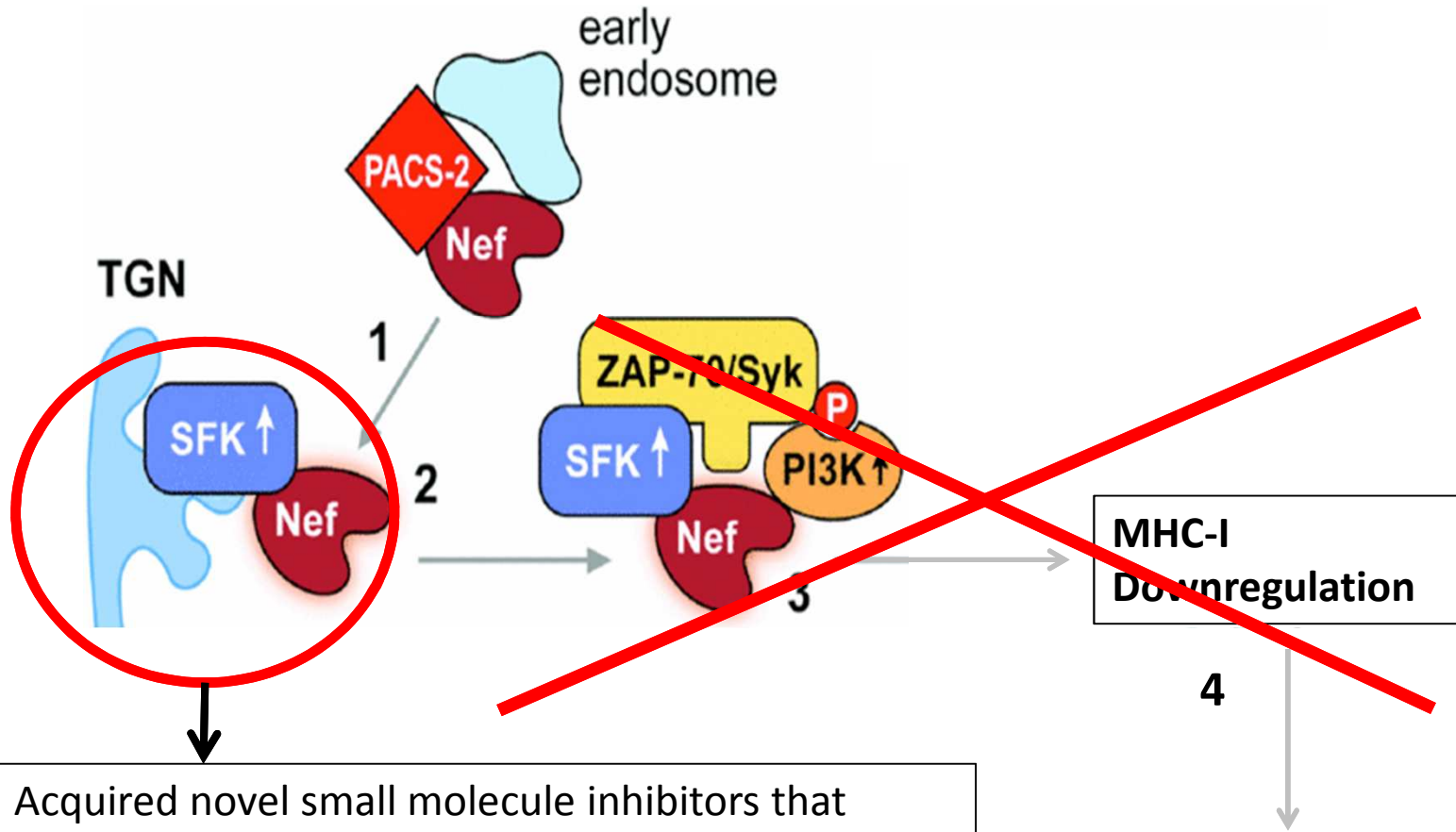**HIV PREVENTION, ENGAGEMENT** AND
**TREATMENT CASCADE**

- We have no conflicts to report

# HIV Nef

- HIV regulatory protein, Nef = negative effector. Nef is expressed early in the viral life cycle. Has multiple roles



Arhel and Kirchhoff, *Current topics in microbiology and immunology* (2009)

# Nef-mediated HLA-A/B ↓



early endosome

TGN

PACS-2

Nef

1

2

ZAP-70/Syk

SFK ↑

SFK ↑

PI3K ↑

P

Nef

Nef

3

MHC-I Downregulation

4

- Acquired novel small molecule inhibitors that prevent Nef-mediated SFK activation (Dr. Smithgall, U Pittsburgh).

- These drugs have been characterized on cell lines (H9 etc.) and shown o be effective on Hck only (not present in T cells.)

**Evasion of CD8 T cell response → persistence of infected cell**

Cytotoxic (killer) T cells

CTL

kill

↑IFN-Y

virus-infected cell

CTL

apoptotic cell

Janeway *et al. Immunobiology 6th Ed*

# Hypothesis

Nef protects HIV-1 infected cells from CD8 T cell mediated recognition and killing by downmodulating MHC **Repressing this role of Nef could enhance CTL responses** directed against the infected cell, thereby eliminating it.
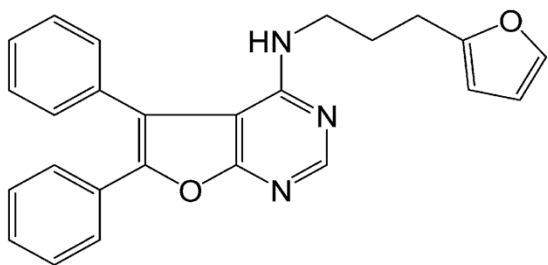
# Nef inhibitors – Two separate mechanisms
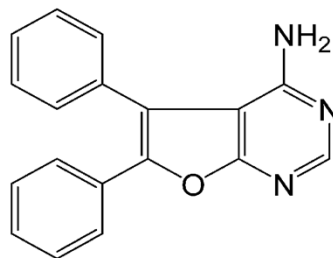


**Diphenylfuropyrimidines – SFK Inhibitors**
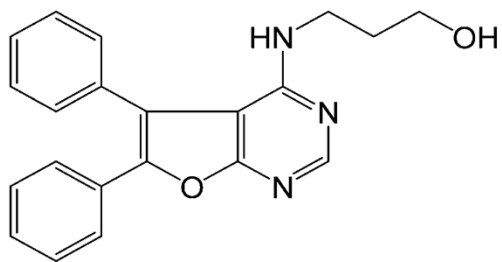
Diphenylfuropyrimidine-4-aminopropanol
DFP-4AP
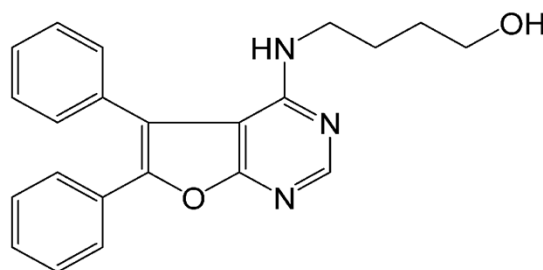
Diphenylfuropyrimidine-4-aminobutanol
DFP-4AB

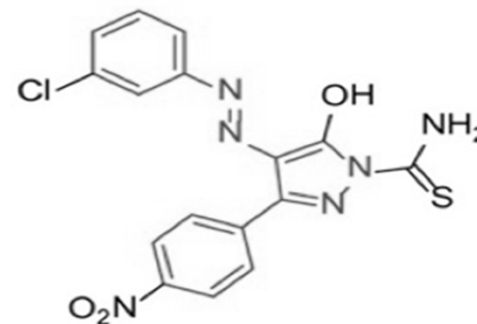Diphenylfuropyrimidine-4-aminopropylfuran
DFP-4APF

Diphenylfuropyrimidine-4-amine
DFP-4A

**Diphenylpyrazole– Nef Binding**

(E)-4-((3-chlorophenyl)diazenyl)-5-hydroxy-3-(4-nitro-phenyl)-1H-pyrazole-1-carbothioamide
B9

Directly bind Nef, Asn126, preventing Nef interaction with SFK

Bind to the ATP-binding site, Thr338, of Hck and Lyn preventing SFK phosphorylation. Shown to be Nef dependent.

Emert-Sedlak *et al. ACS Chem Biol.* (2009)
Emert-Sedlak *et al. Cell Chem. Biol.* (2013)

# Outline of Experiments

1.  Characterize the Nef inhibitors on primary CD4 T cells: **Can they prevent MHC-I downregulation in infected cells?**

2.  **Functional role:** Autologous T cell recognition assays to determine **enhancement of HIV-1 specific CD8 T cell function** cocultured with Nef inhibited CD4 T cells:
    - Readout of assay: **IFN-γ** production and **CD107a** degranulation

# Methods - Timeline

Isolate and prime
CD4 T cells

Chronic HIV-1 on
HAART
(VL<50copies)

Isolate
autologous
CD8 T cells.
Rested
overnight.

**Expt. 1:** Stain
for MHC-I
levels in
infected cells

Analysis of
data. All done
by flow
cytometry.

D=0      2      5      6      7

Infection of
CD4 T cells.
Three sets:
**mock**
(uninfected),
**NL4-3 ΔNef**,
and **NL4-3**

[Cultured with
drugs]

**Expt. 2:** CD8 T
cell coculture.
6h assay to
measure CD8 T
cell effector
function. **IFN-γ**
and **CD107a**

# HIV-1 infection and MHC-I ⬇



MOCK  NL4-3 ΔNef  NL4-3 WT
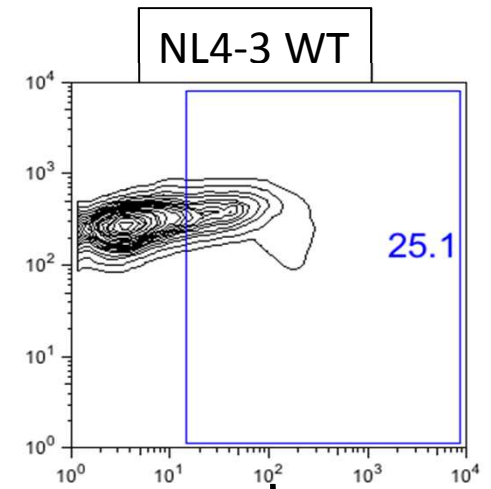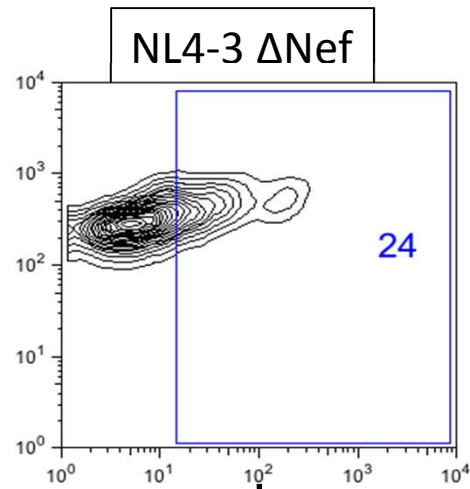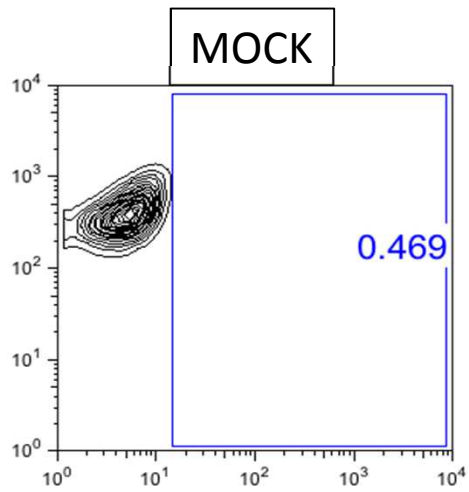
% of MHC-I downregulated cells within the HIV infected subset
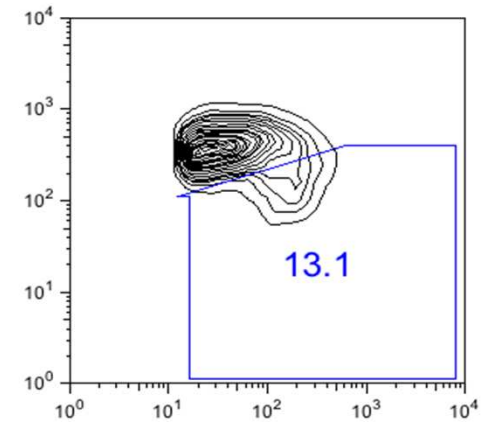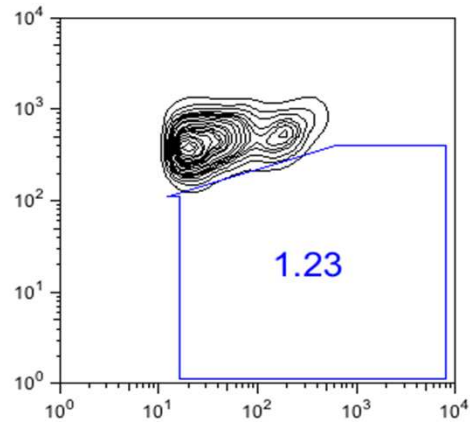
MHC-I

HIV Gag

# Representative Example with each of the drugs
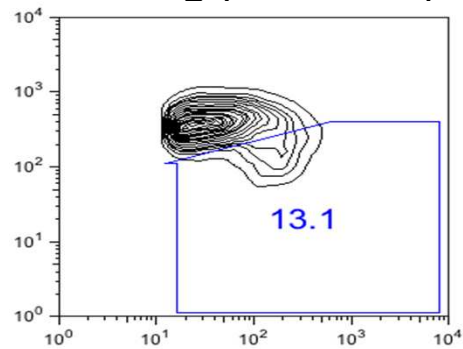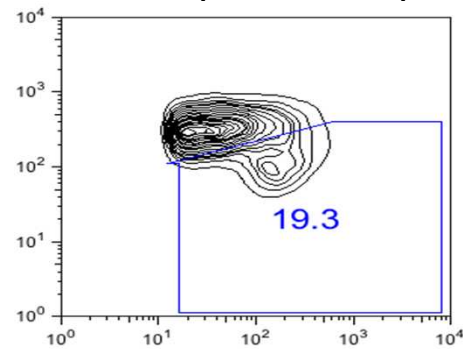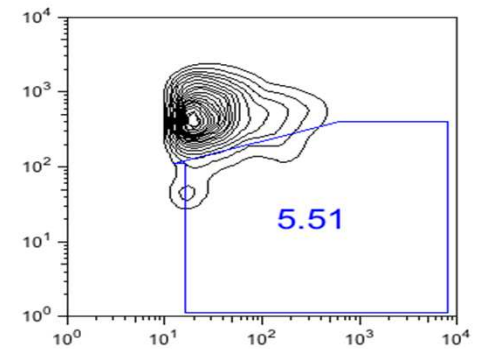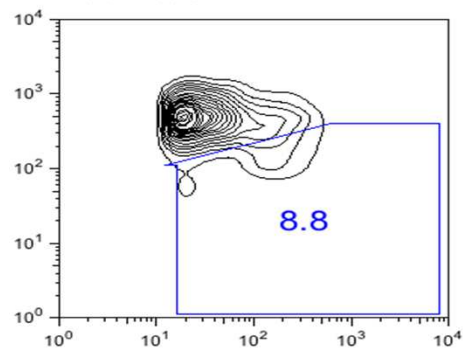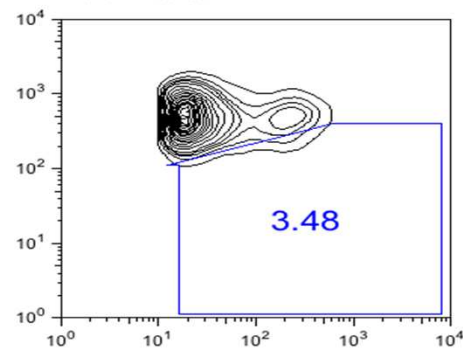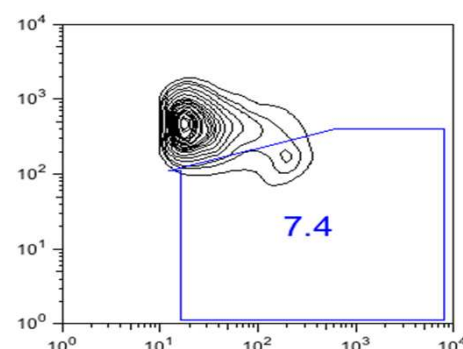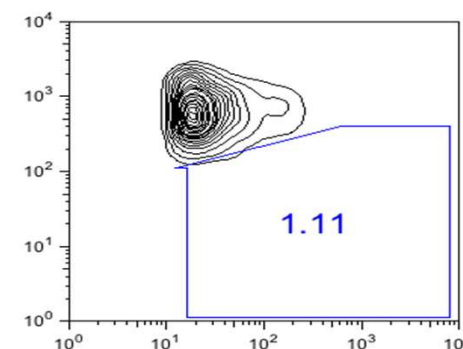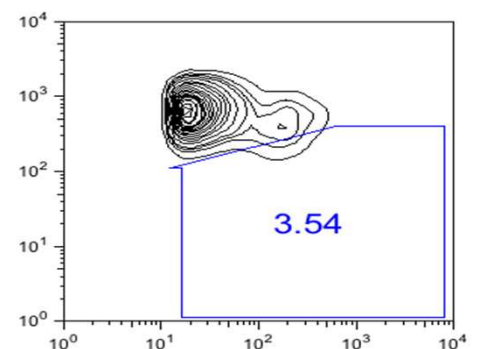
# %MHC-I Nef-SFK inhibitors (DFP)

## DFP-4A

NS; p = 0.0621

## DFP-4AB

**; p = 0.0038

## DFP-4AP

**; p = 0.0020

## DFP-4AF

**; p = 0.0018

# %MHC-I Nef-interacting Drugs

## TS2



NS; p = 0.7988

% MHC downregulated cells

80 — 60 — 40 — 20 — 0

No Drug — TS2

## B9



*; p = 0.0280

% MHC downregulated cells

50 — 40 — 30 — 20 — 10 — 0

No Drug — B9

## 2C



NS; p = 0.7271
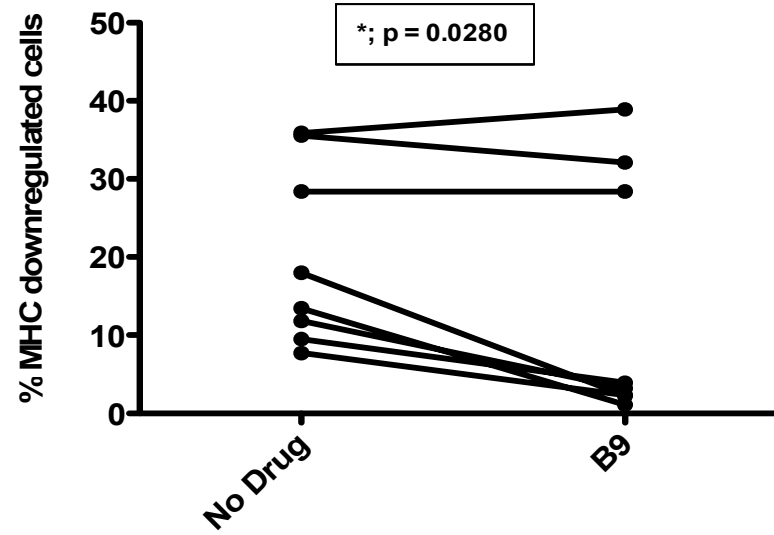(*p = 0.0306 outlier)

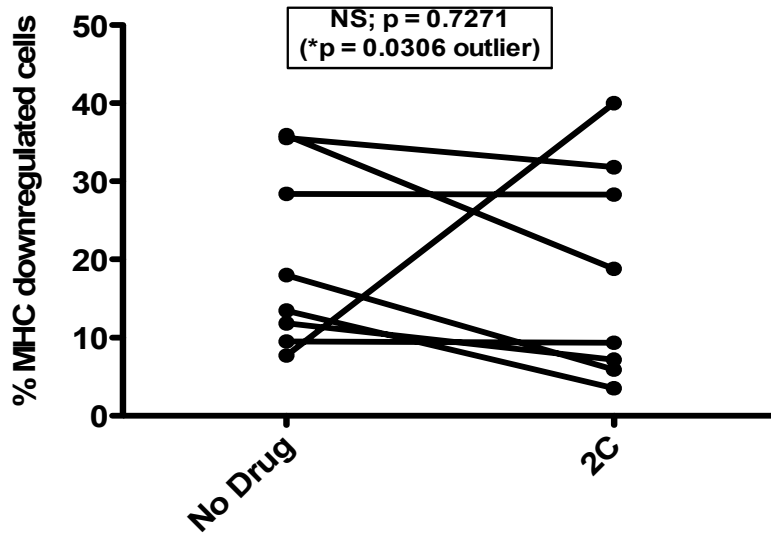% MHC downregulated cells

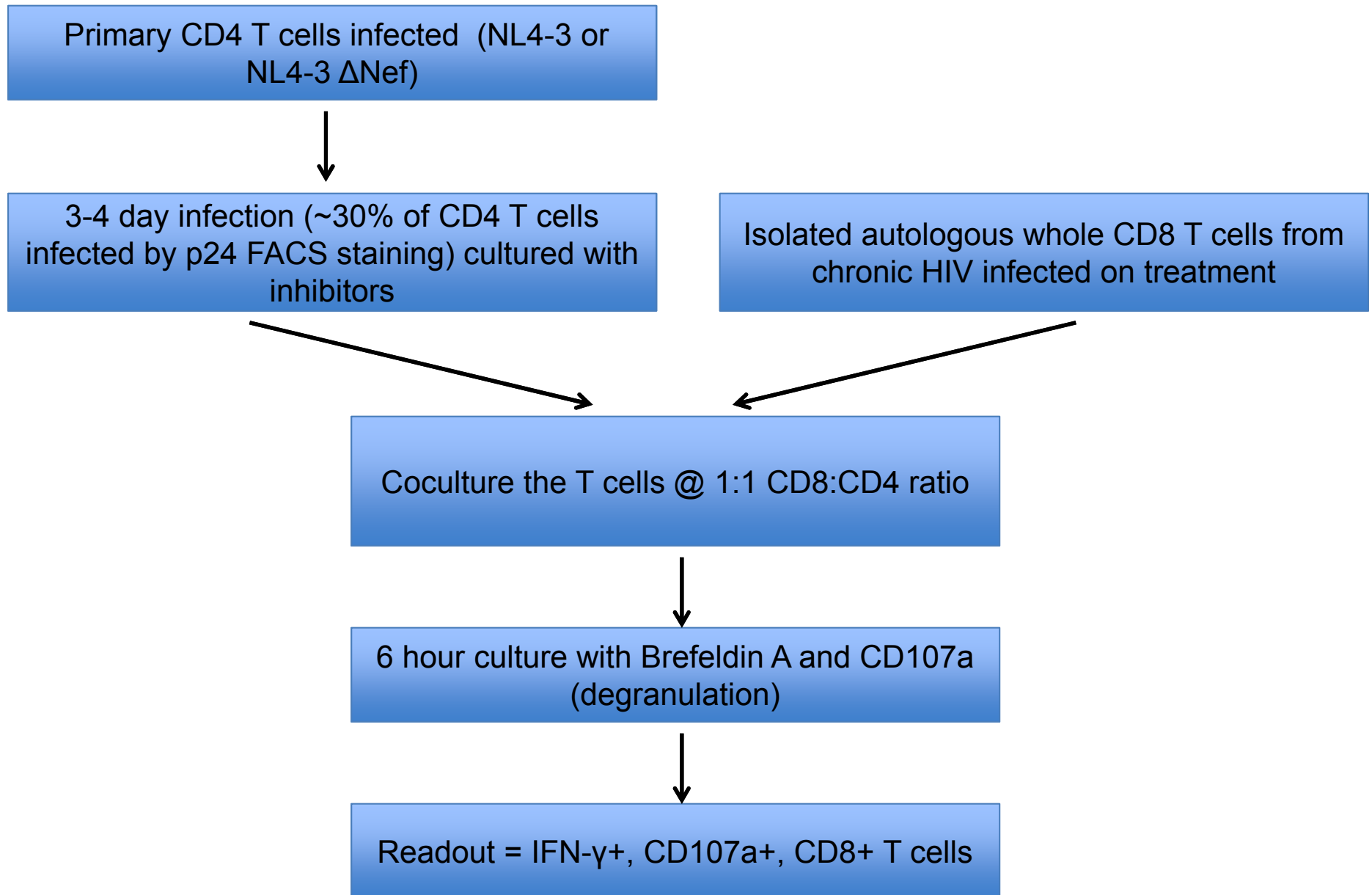50 — 40 — 30 — 20 — 10 — 0
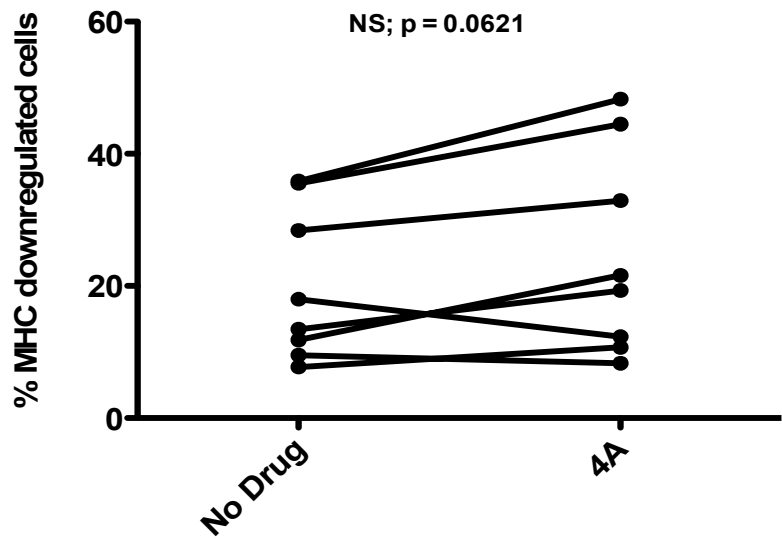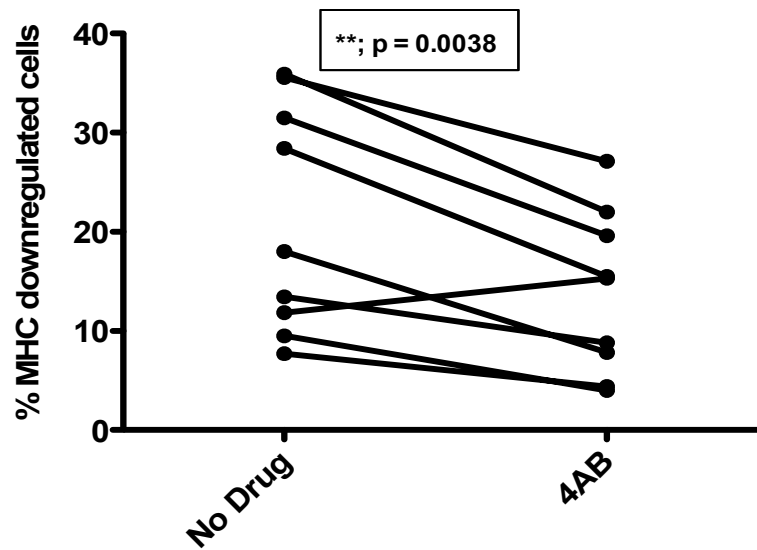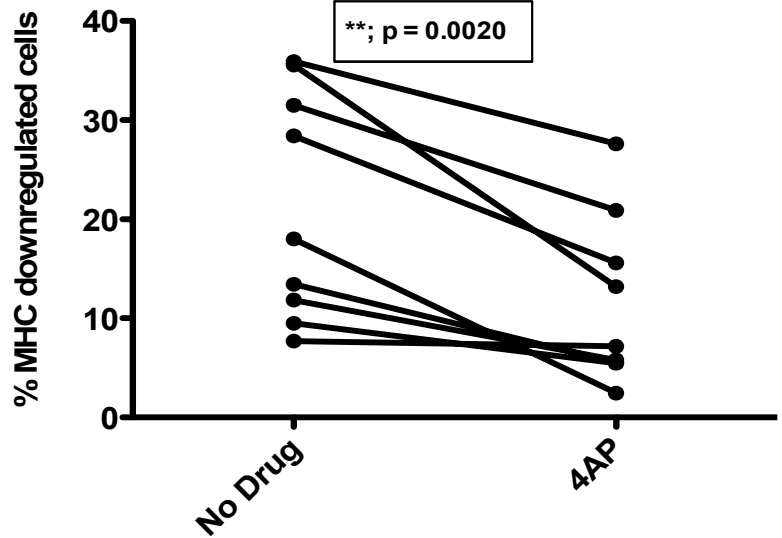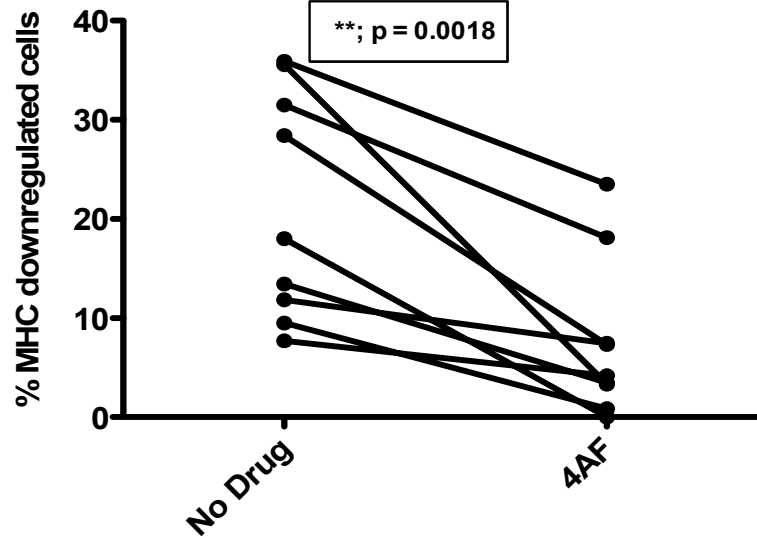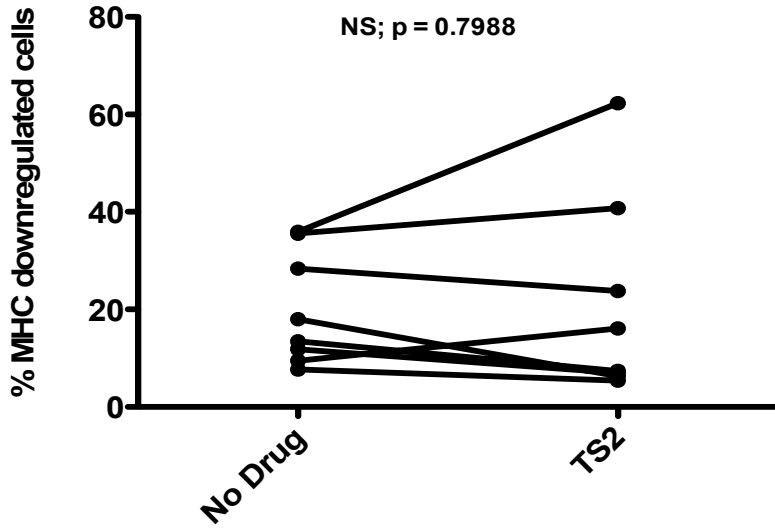
No Drug — 2C

Nef inhibitors effectively prevents MHC-I ⬇ compared to NL4-3 infection in the absence of drugs.
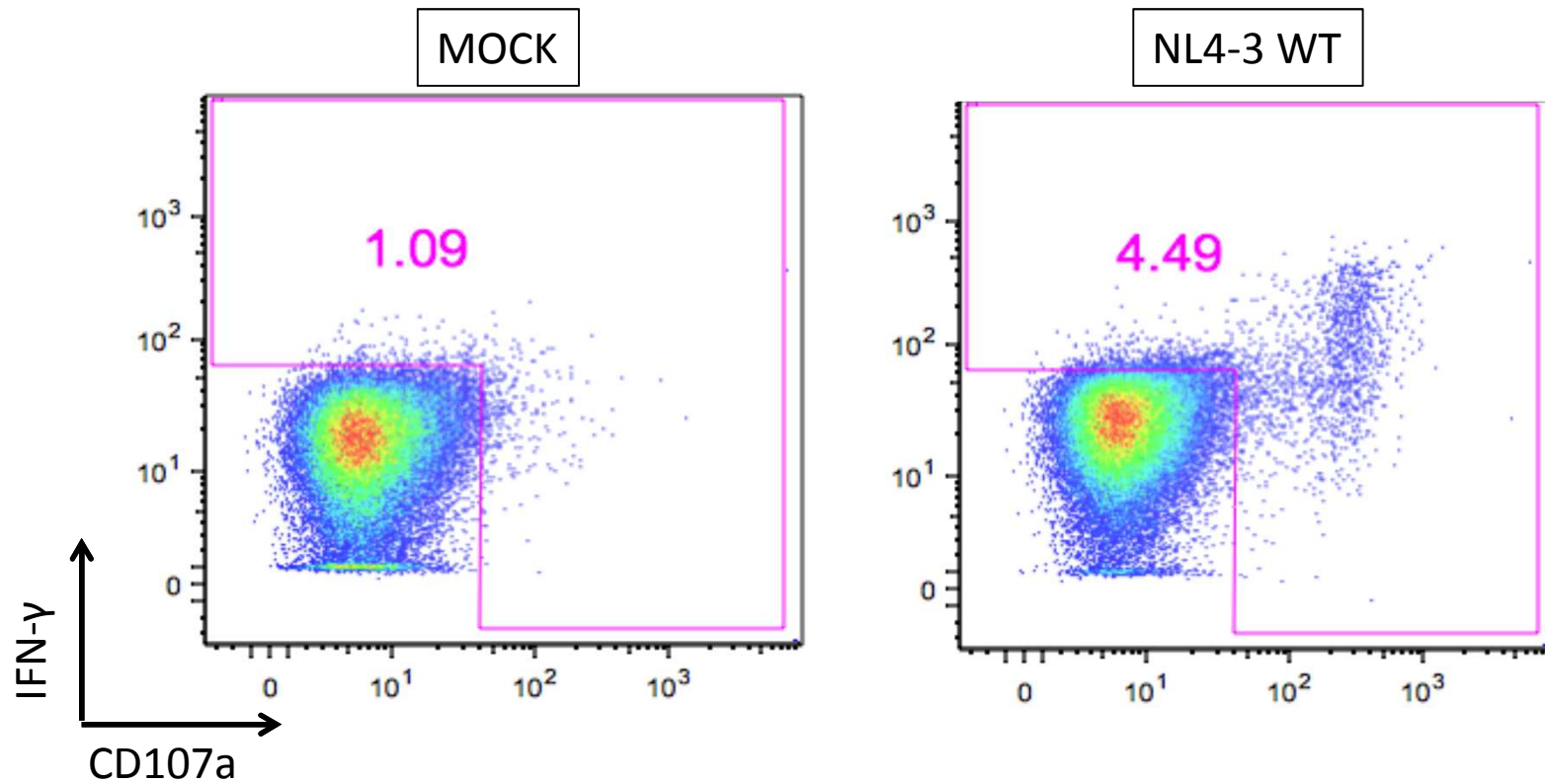
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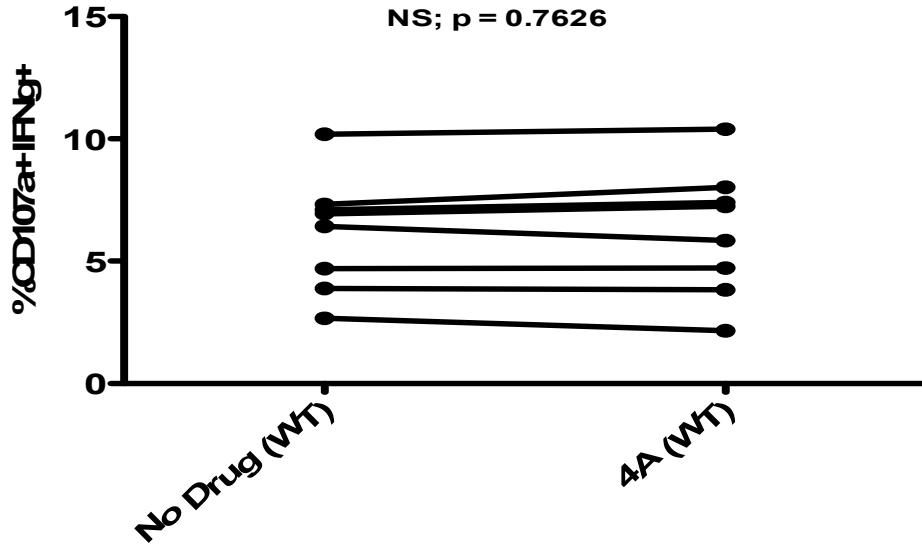
# T Cell Recognition Assay

Primary CD4 T cells infected  (NL4-3 or NL4-3 ΔNef)

↓

3-4 day infection (~30% of CD4 T cells infected by p24 FACS staining) cultured with inhibitors

Isolated autologous whole CD8 T cells from chronic HIV infected on treatment

Coculture the T cells @ 1:1 CD8:CD4 ratio

↓

6 hour culture with Brefeldin A and CD107a (degranulation)

↓

Readout = IFN-γ+, CD107a+, CD8+ T cells

# T Cell Recognition Assay



Gated on CD8 T cells

# CD8 Response – Nef-SFK (DFP)



**DFP-4A**

NS; p = 0.7626

%CD107a+IFNg+

No Drug (WT) · 4A (WT)

**DFP-4A (dNef)**

NS; p = 0.6336

%CD107a+IFNg+

No Drug (dNef) · 4A (dNef)

**DFP-4AP**

\*, p = 0.0231

%CD107a+IFNg+

No Drug · 4AP

**DFP-4AP (dNef)**

NS; p = 0.0620

%CD107a+IFNg+

No Drug (dNef) · 4AP (dNef)
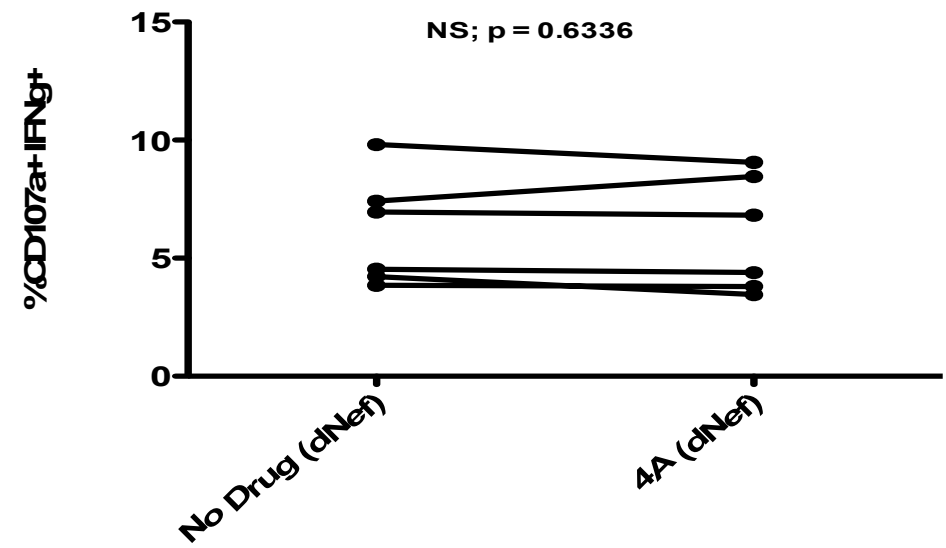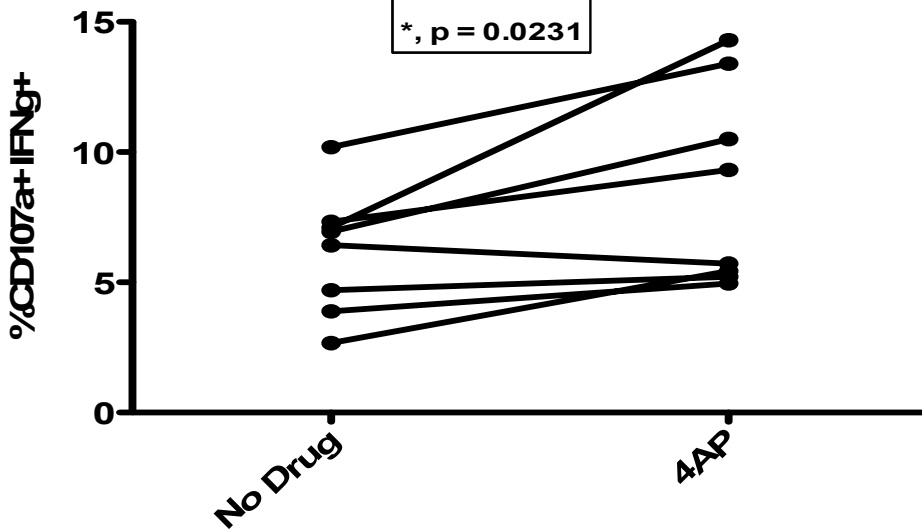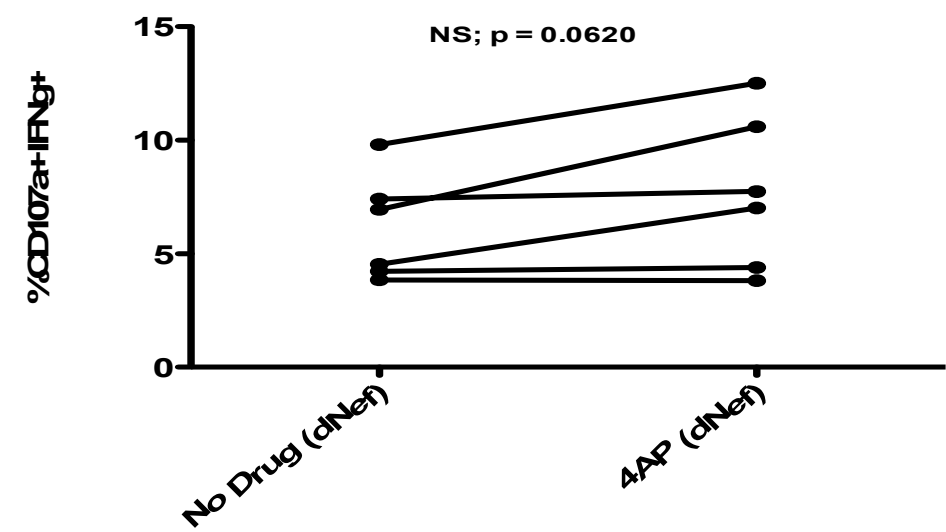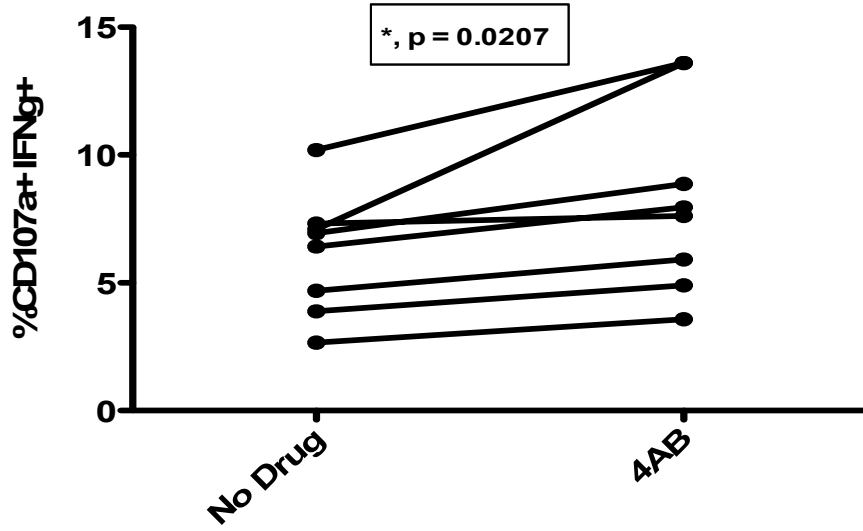
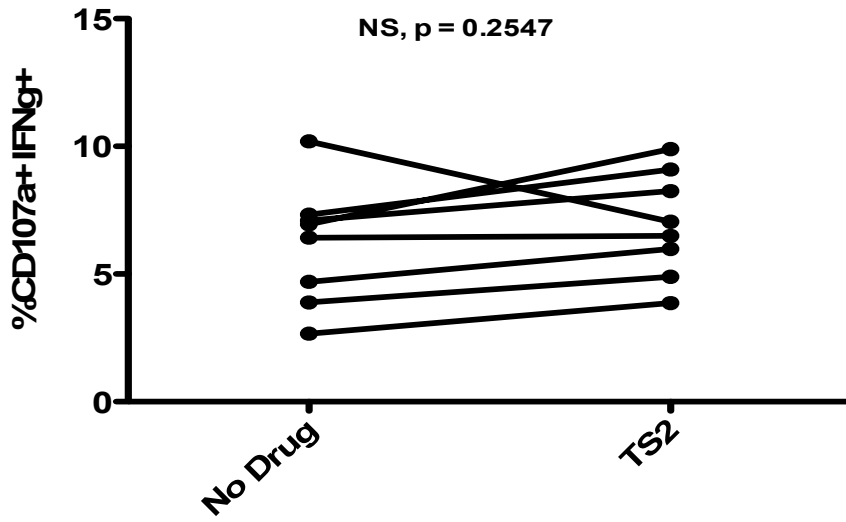**DFP-4AB**

**DFP-4AB (dNef)**
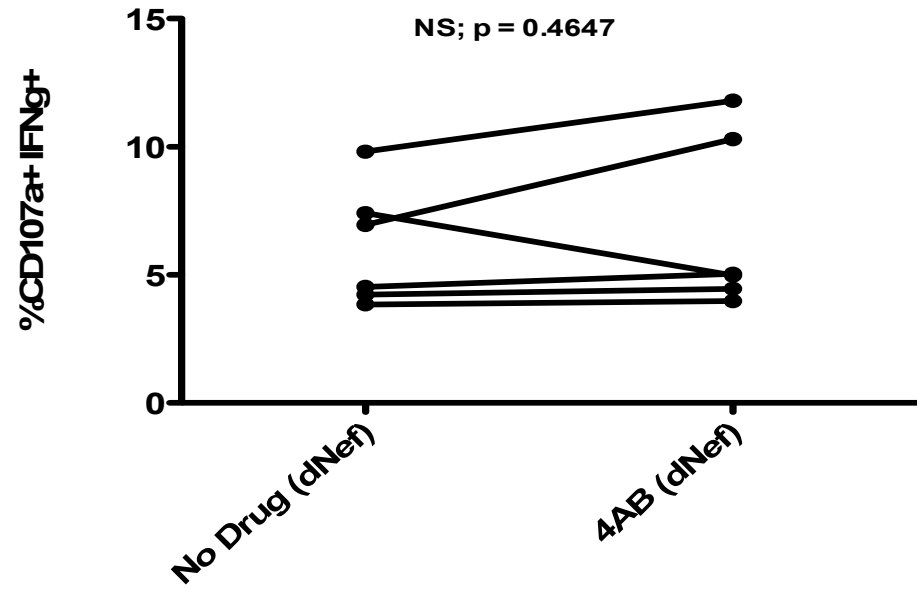
**DFP-4AF**
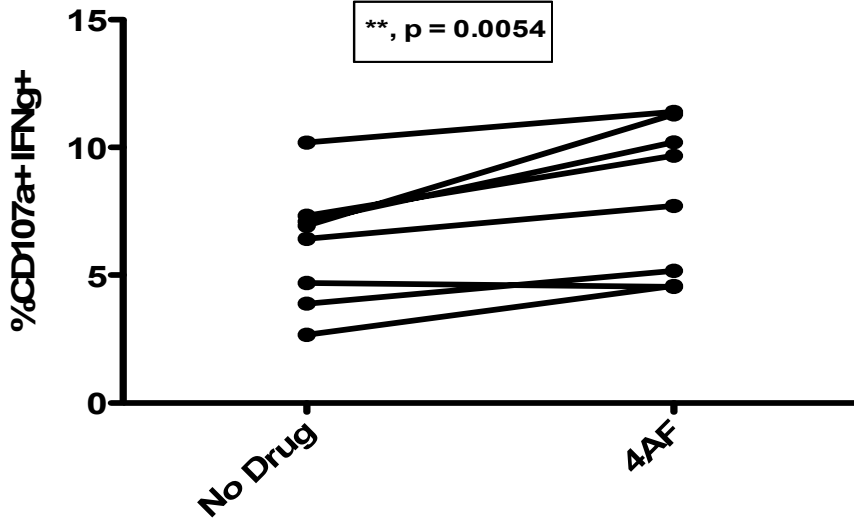
**DFP-4AF (dNef)**
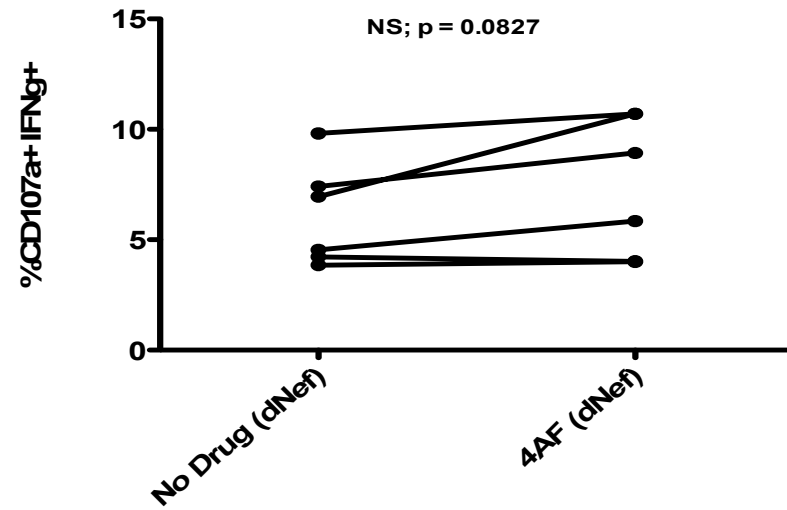
# CD8 Response – Nef-interacting drugs

## TS2



NS, p = 0.2547

%CD107a+ IFNg+

No Drug — TS2

## TS2 (dNef)



NS; p = 0.8578

%CD107a+ IFNg+

No Drug (dNef) — TS2 (dNef)

## B9



**, p = 0.0027
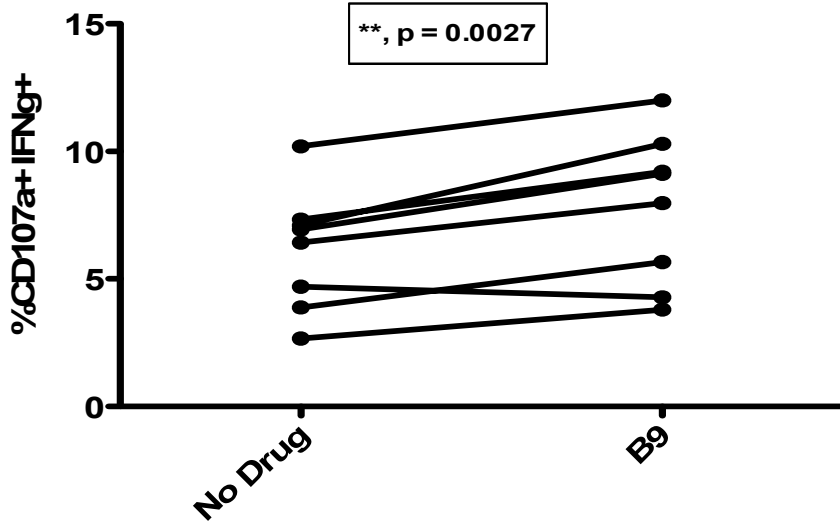
%CD107a+ IFNg+

No Drug — B9
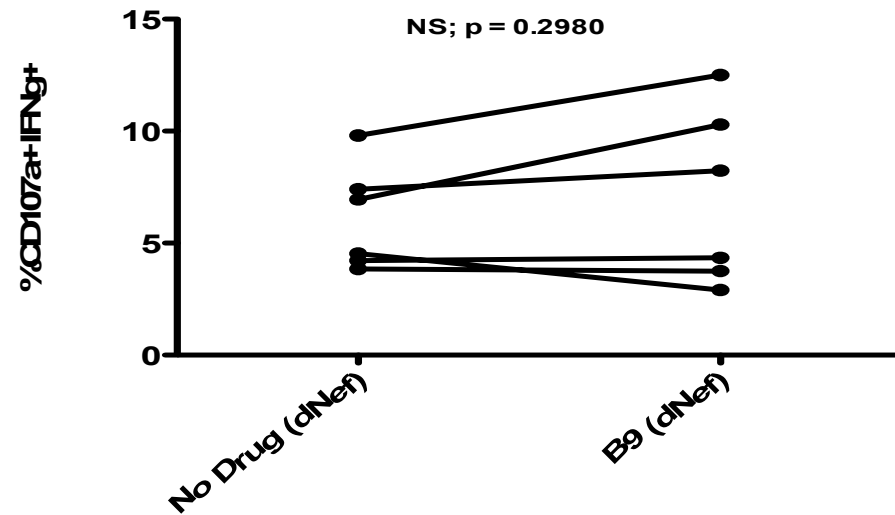
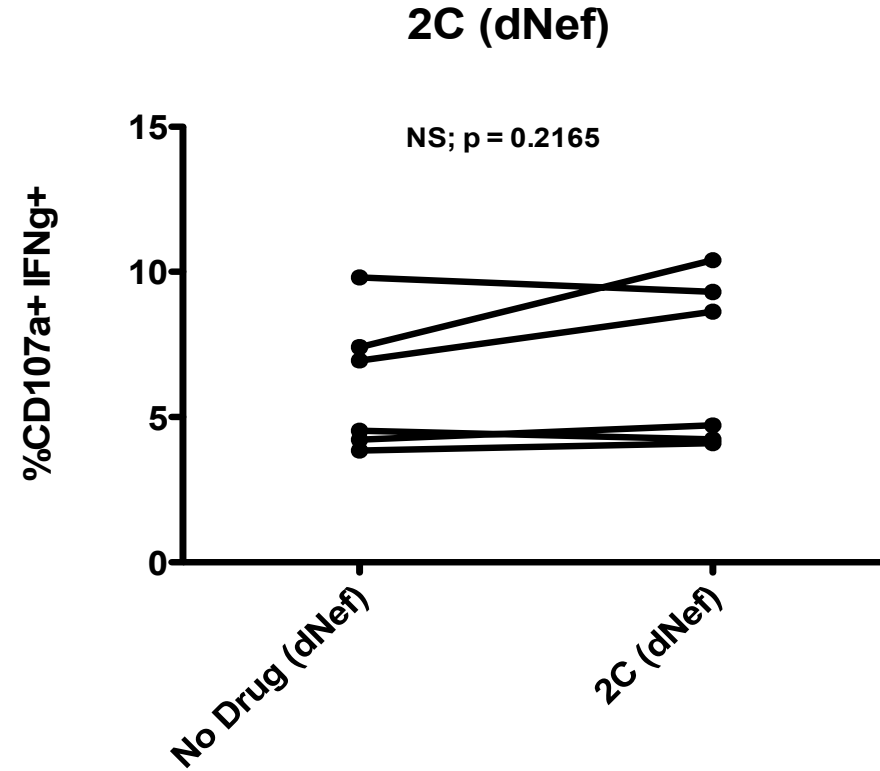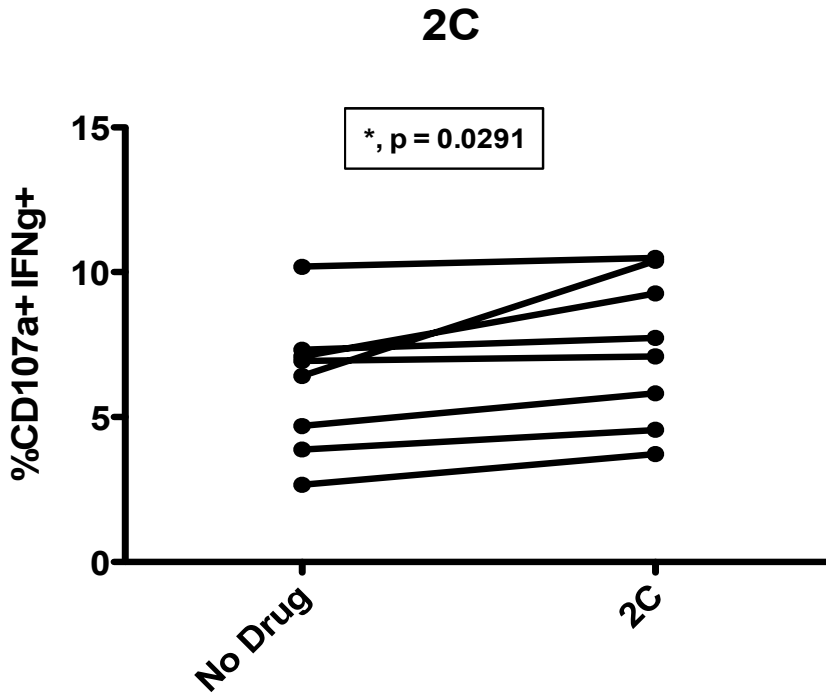## B9 (dNef)



NS; p = 0.2980

%CD107a+ IFNg+

No Drug (dNef) — B9 (dNef)
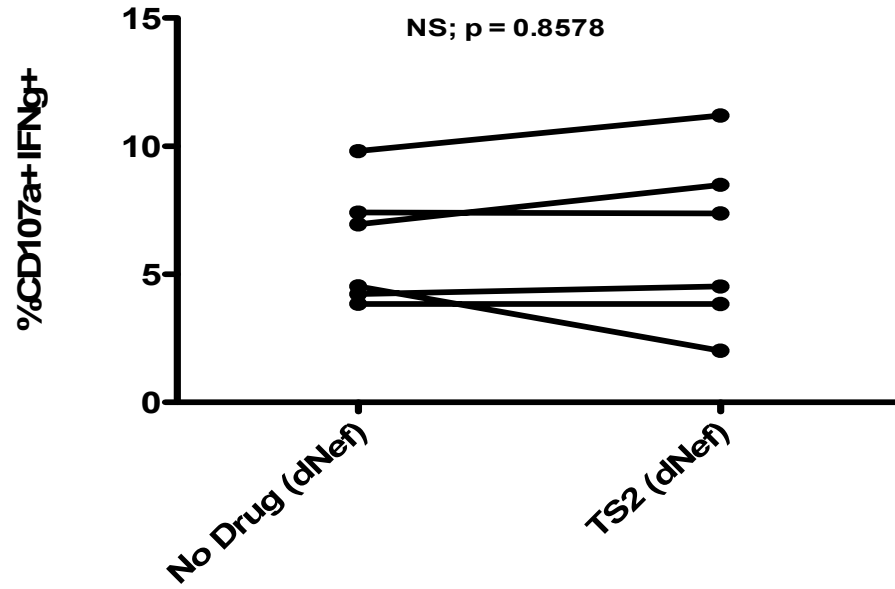
NL4-3 ΔNef virus does not improve CD8 T cell response in the presence of drugs.

Significant, albeit moderate, improvement of CD8 T cell function observed only in the presence of Nef expression.

# Summary

## HIV-1 Nef:

- Novel drugs appear to **prevent MHC-I downregulation** in primary CD4 T cells.

- Suppression of MHC-I downregulation correlates with **improved CD8 T cell effector function** as measured by:
    - ↑**IFN-γ** production; and
    - ↑**CD107a** (degranulation)

- Effects were **Nef dependent** as evident by NL4-3 ΔNef controls.

- First study demonstrating improvement of T cell function with Nef drugs. Suggests another approach towards **HIV-1 eradication.**

- Will establish role for CD8 T cells in elimination assays investigating **long-term killing of HIV-infected cells** *in vitro* in the presence of drugs.

# Acknowledgements

**Ostrowski Lab:**

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