

Impaired IL-23 signalling and Th17 dysfunction in HIV infection

Jason R. Fernandes, Ashok Kumar, Jonathan B. Angel

Fighting the Virus : The Immune Response to HIV Infection

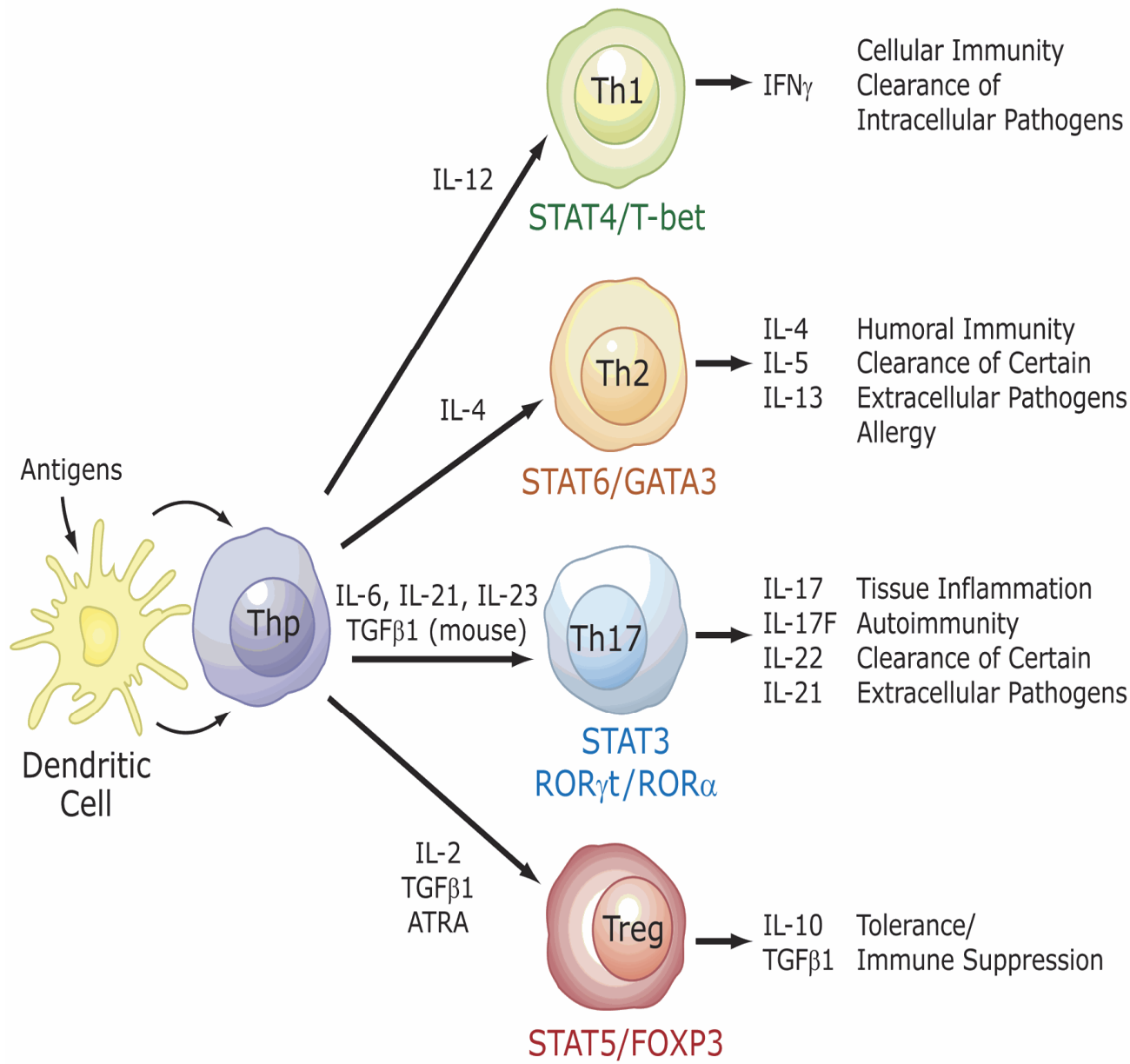
November 18, 2013 – 10:00am



OHTN 2013
RESEARCH
CONFERENCE

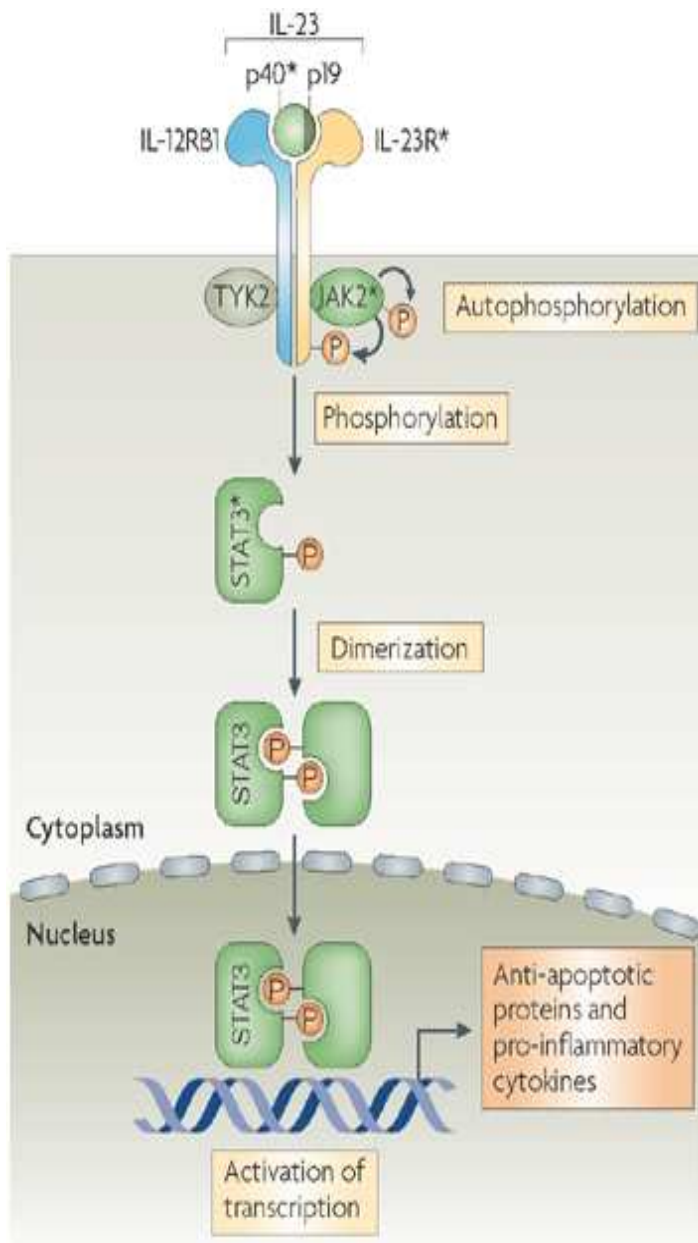
NOVEMBER 17-19, 2013

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



* IL-17:

- * Prodⁿ of IL-6, IL-1 β , TNF- α , IL-8, MCP-1
- * Antimicrobial peptides
- * Wound healing
- * Vital role in maintaining mucosal barrier integrity



Responsible for “fixing” Th17 phenotype
Key outcomes:

- STAT3 phosphorylation (pSTAT3)
- STAT3/RORC transcription (RORa/γt)
- IL17a transcription

HIV and Th17 cells

- Profound GALT Th17 depletion early in infection
- HAART normalizes peripheral CD4⁺ T cell numbers,
 - Th17 cell number/function remains impaired
 - Leads to altered mucosal immune responses
- Loss of Th17 cells drives persistent

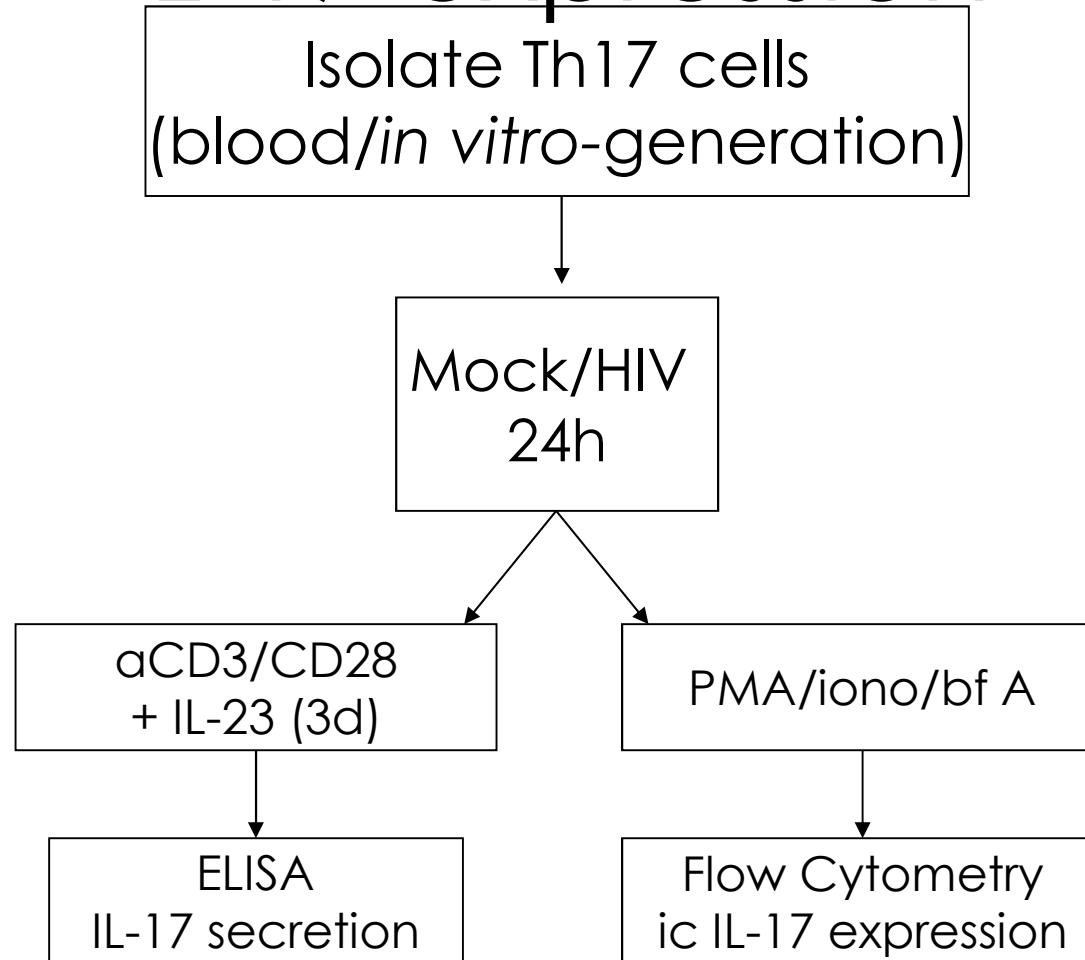
Hypothesis

HIV and its regulatory proteins disturb Th17 function by modulating IL-23-activated intracellular signalling pathways

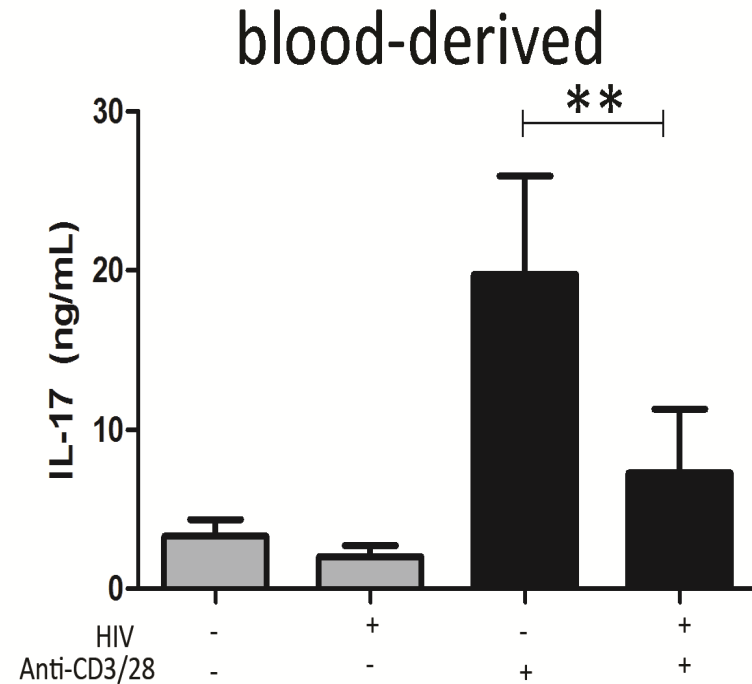
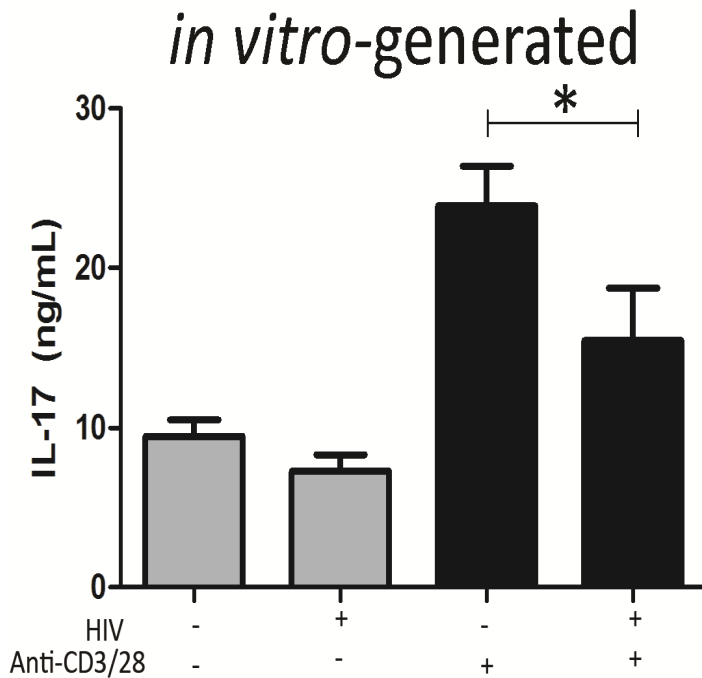
Objectives

1. To determine effects of *in vitro* HIV infection on function of human Th17 cells
2. To determine effects of *in vitro* HIV infection on IL-23 signalling in human Th17 cells
3. To compare blood Th17 cell responses to IL-23 from healthy controls, untreated, and HAART-treated HIV-infected individuals.

Effect of *in vitro* HIV infection on IL-17 expression



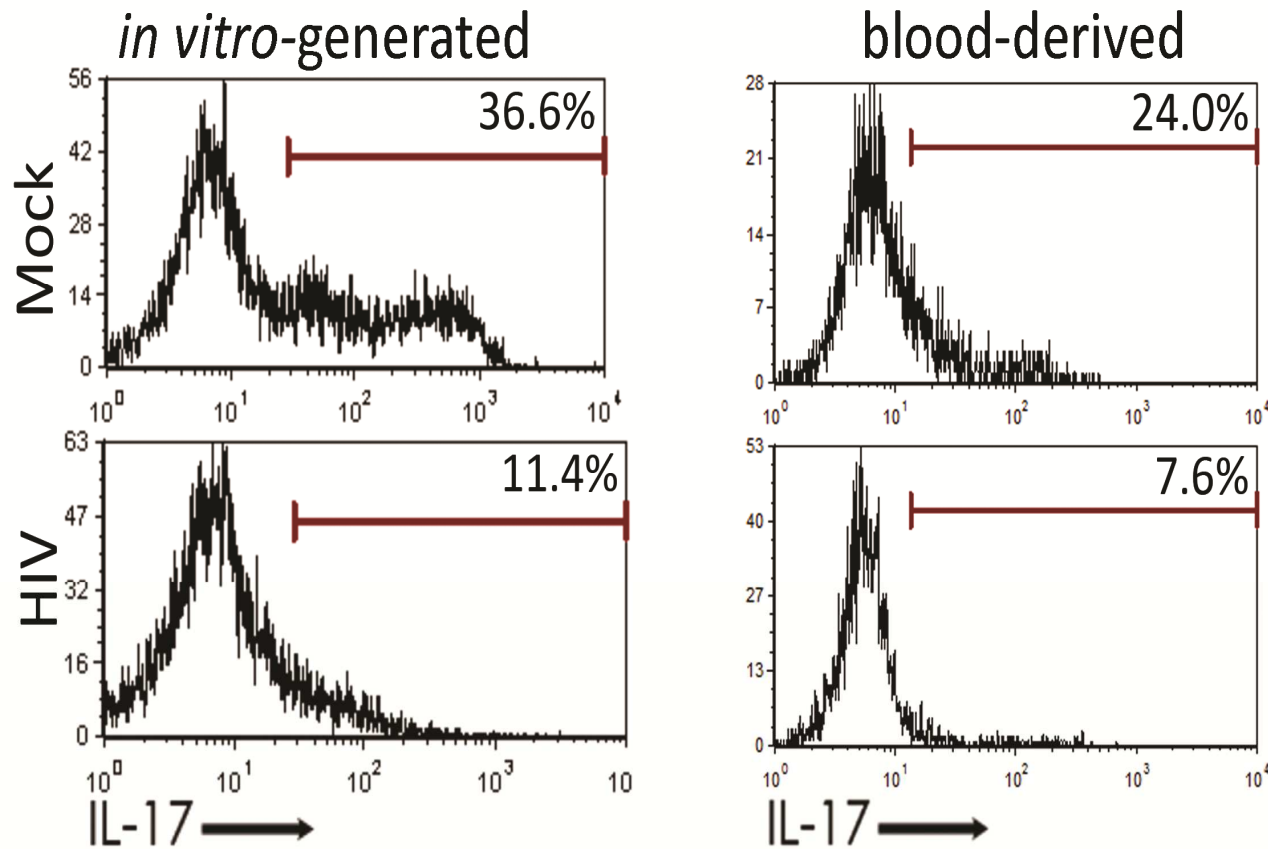
In vitro HIV infection **inhibits** IL-17 secretion



- *In vitro* Th17 cells
- α CD3/28, IL-23 (3d)
- Mean \pm SEM
- * = $p = 0.045$, $n = 9$

- blood Th17 cells
- α CD3/28, IL-23 (3d)
- Mean \pm SEM
- ** = $p < 0.001$, $n = 9$

In vitro HIV infection **inhibits** IL-17 expression

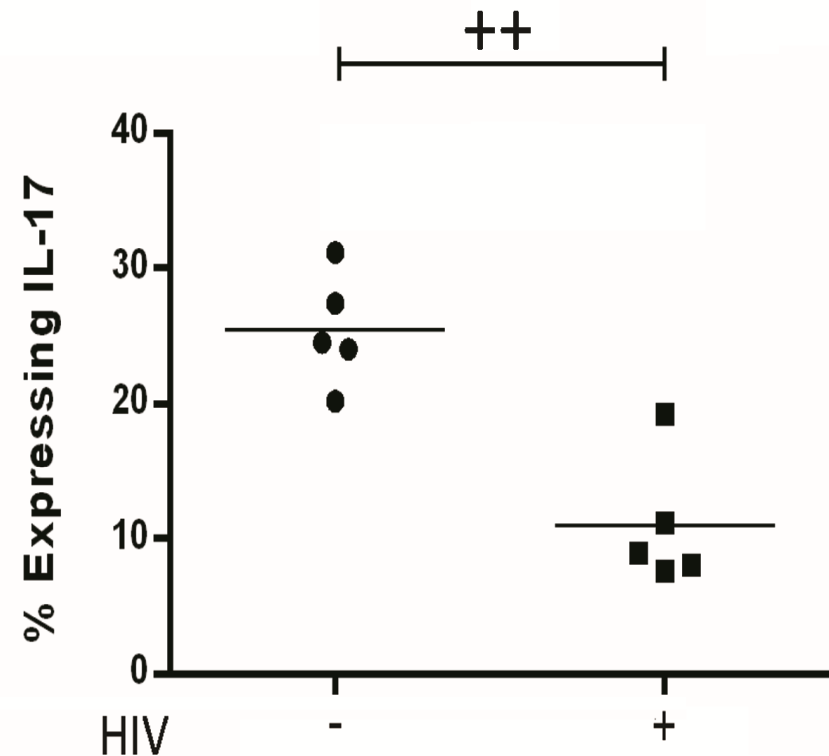
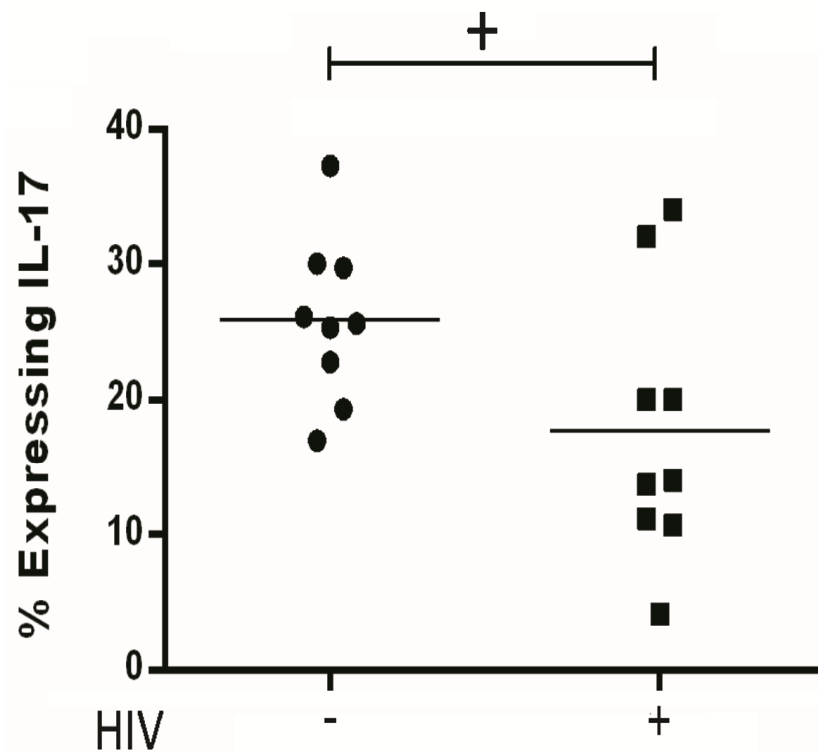


- Blood/*in vitro* Th17 cells, n = 5/9
- PMA/iono/Bfa (6h)

In vitro HIV infection **inhibits** IL-17 expression

in vitro-generated

blood-derived



- Blood/*in vitro* Th17 cells, n = 5/9
- PMA/iono/Bfa (6h)

Effect of *in vitro* HIV infection on IL-23 signalling

Isolate blood Th17 cells

Mock/HIV
24h

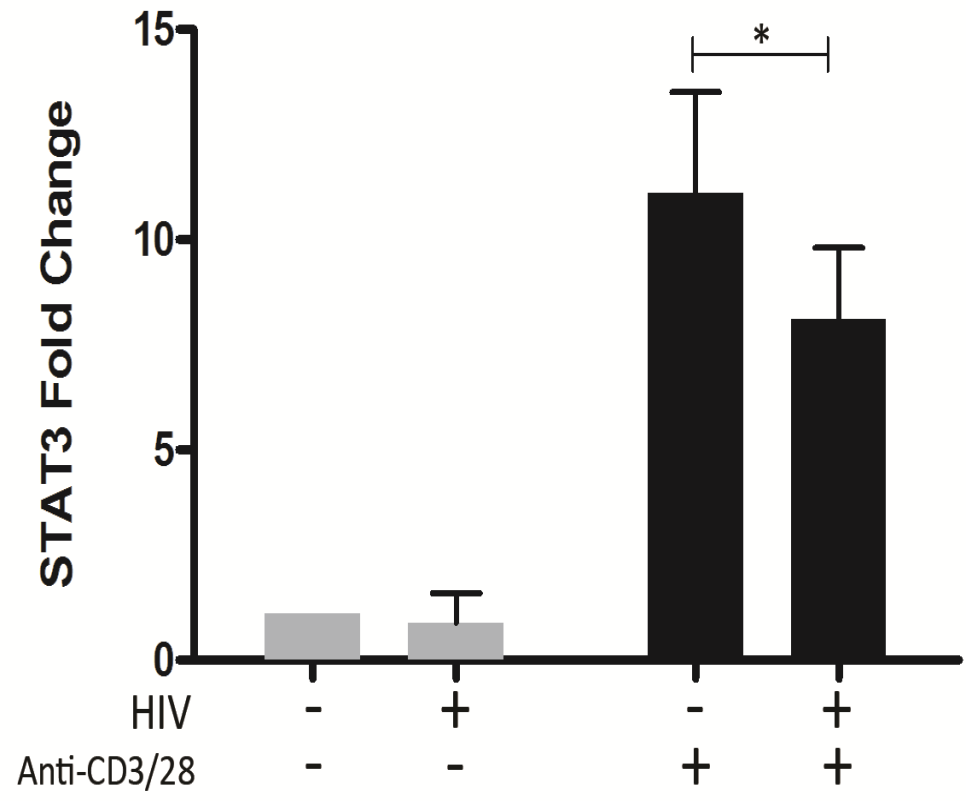
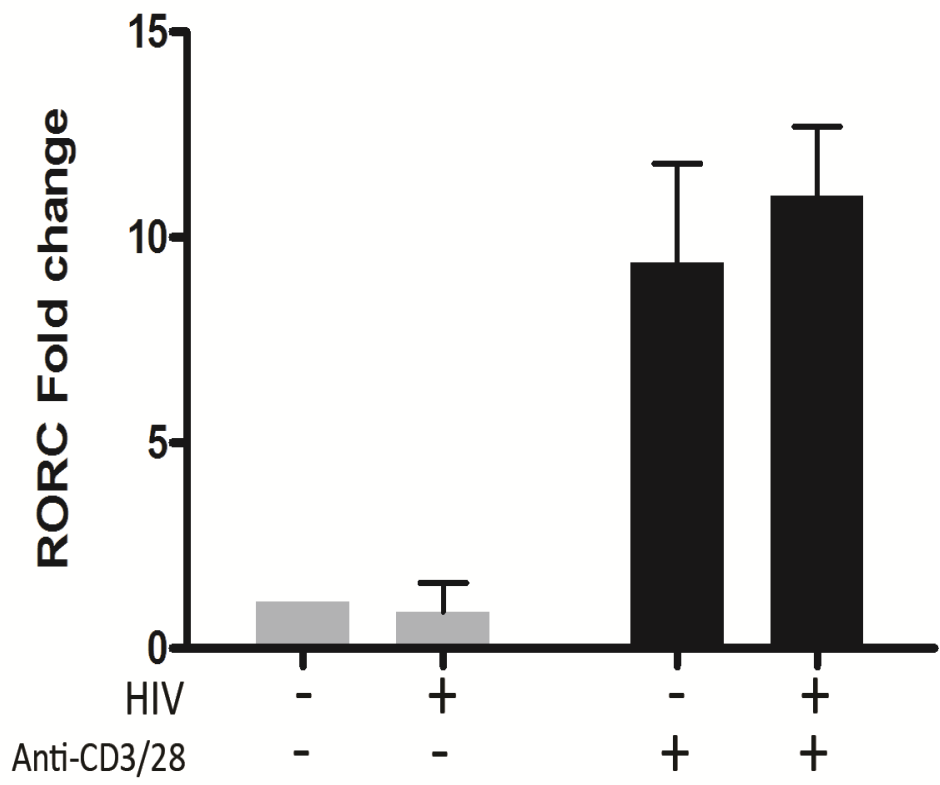
αCD3/CD28
+ IL-23

+ IL-23 or IL-6

qRT-PCR
RORC/STAT3

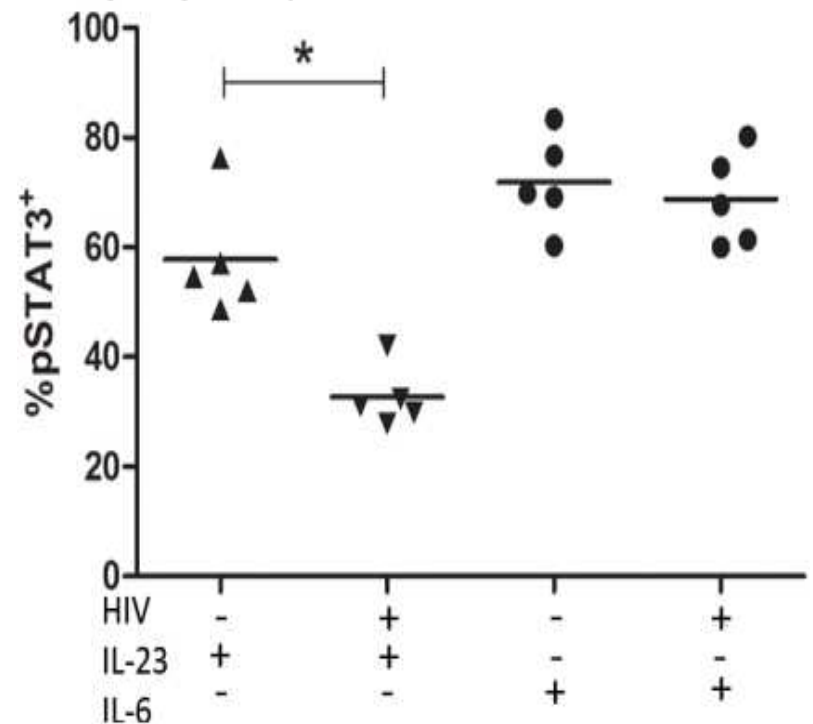
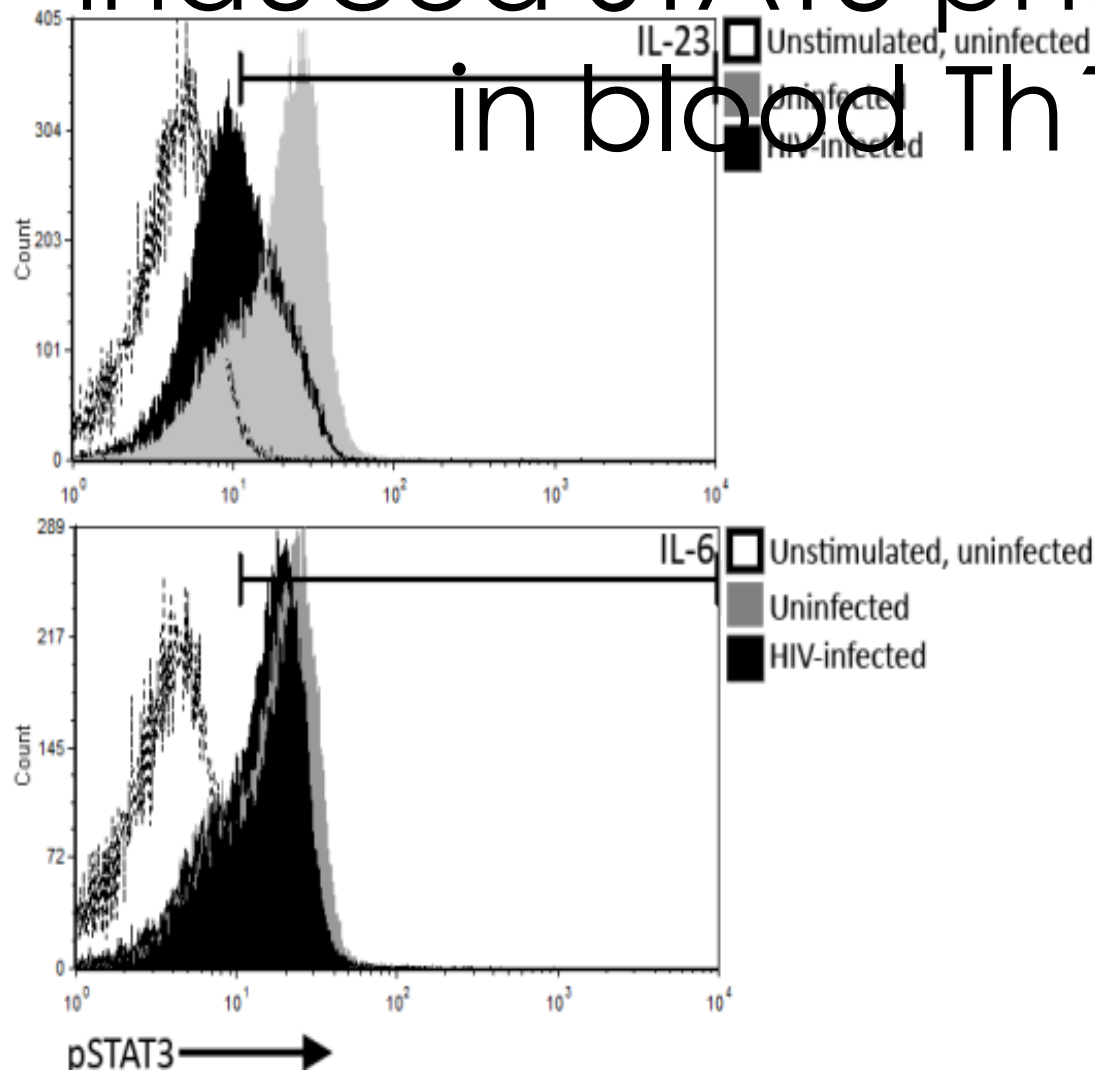
Flow Cytometry
pSTAT3

In vitro HIV infection **inhibits** STAT3 transcription in Th17 cells



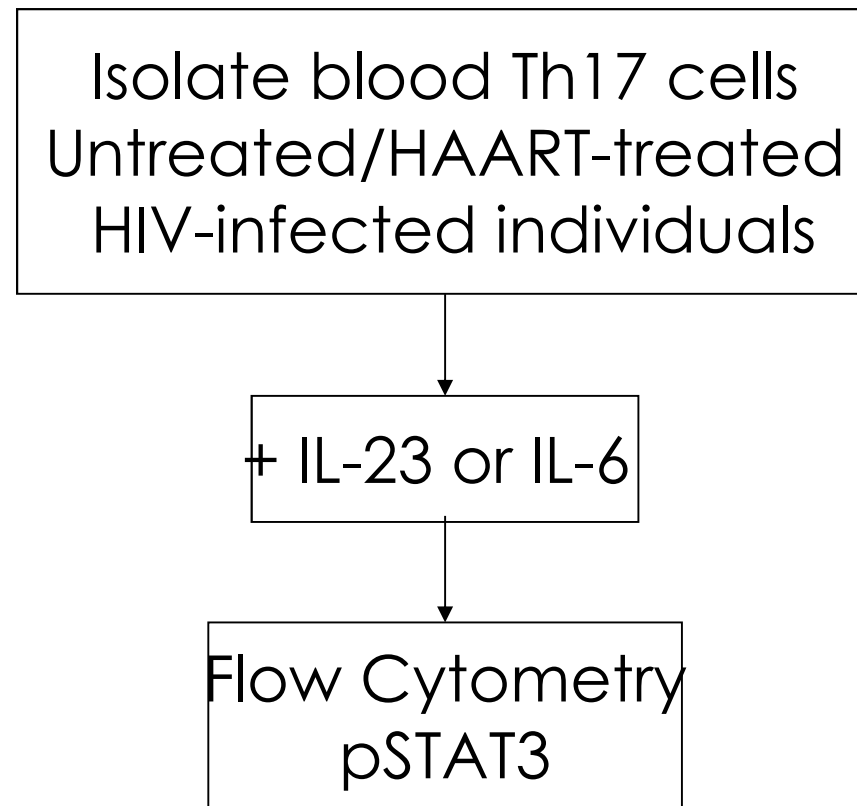
- blood Th17 cells
- αCD3/28, IL-23 (3d)
- ΔΔCt method (semi-q)
- Mean ± SEM
- ns, n = 9

In vitro HIV infection **inhibits** IL-23-induced STAT3 phosphorylation in blood Th17 cells

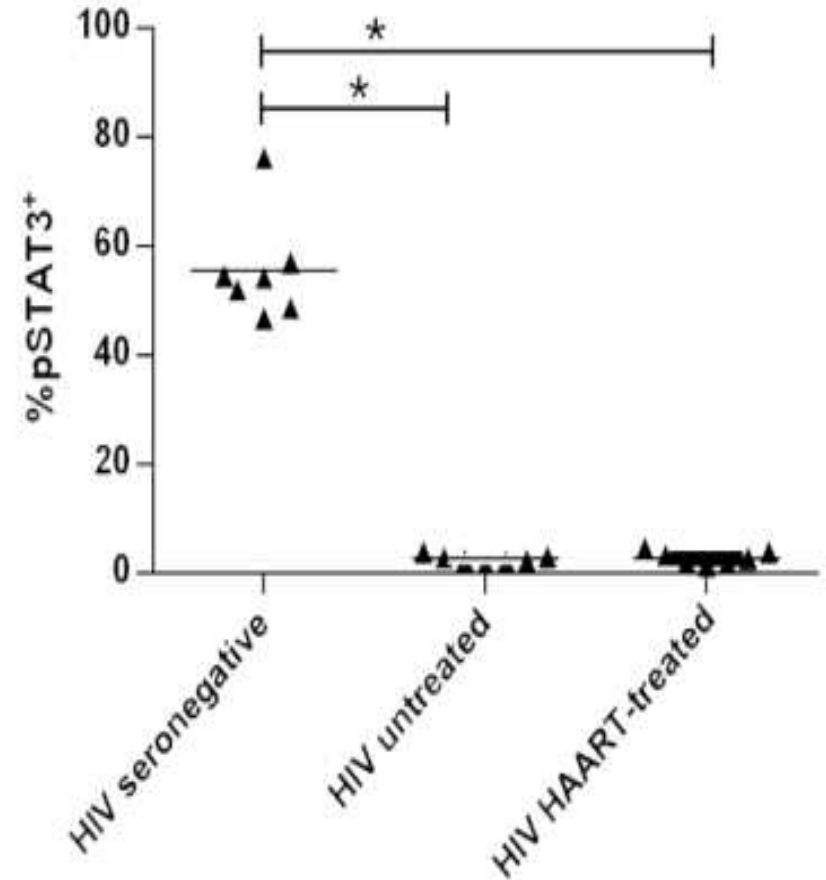
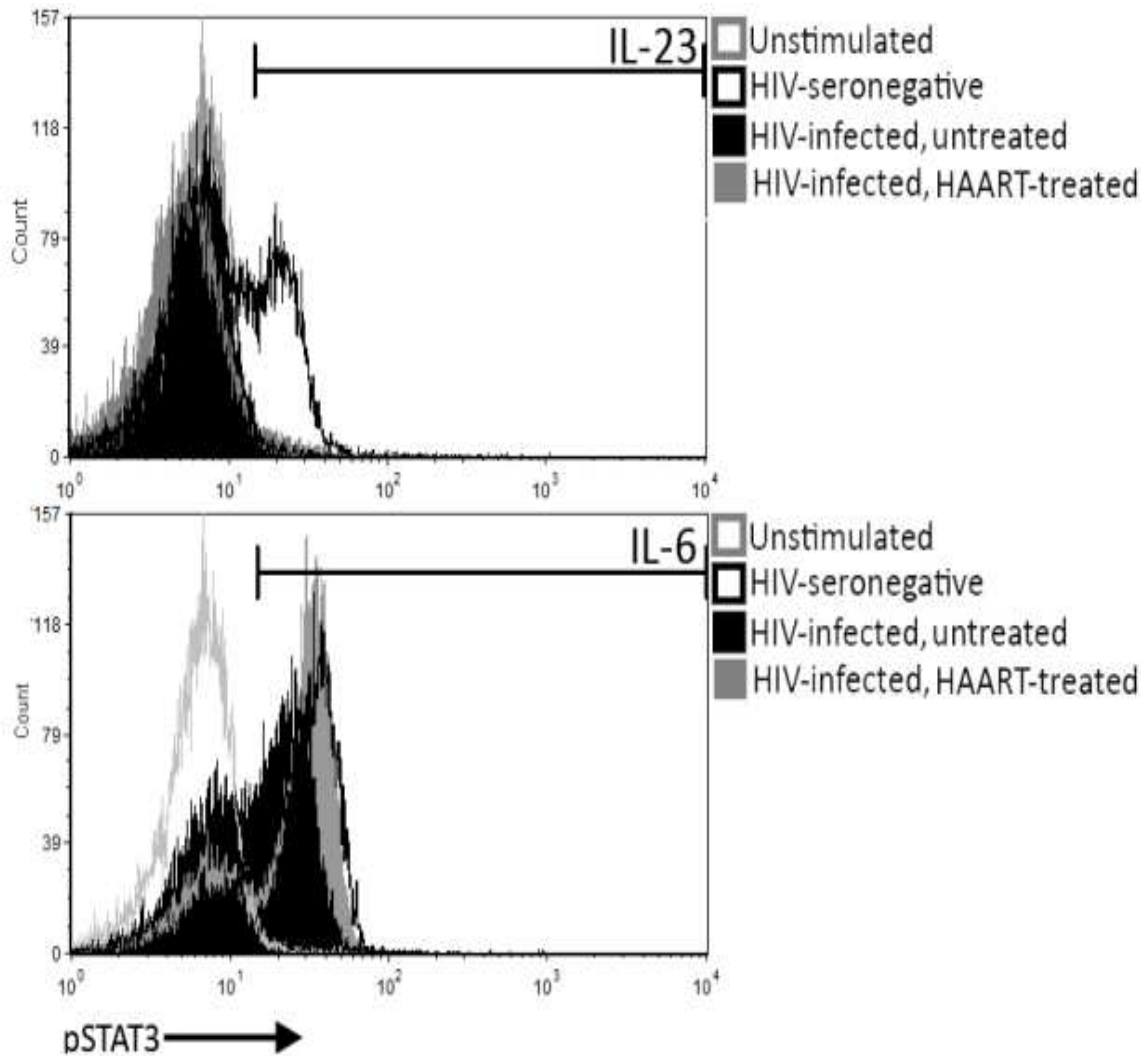


- blood Th17 cells
- 50 ng/mL IL-23/IL-6 (15 min)
- * p = 0.001, n = 5

Effect of HIV on IL-23 signalling in HIV-infected individuals



STAT3 responses to IL-23 are **absent** from blood Th17 cells of HIV-infected individuals regardless of treatment status



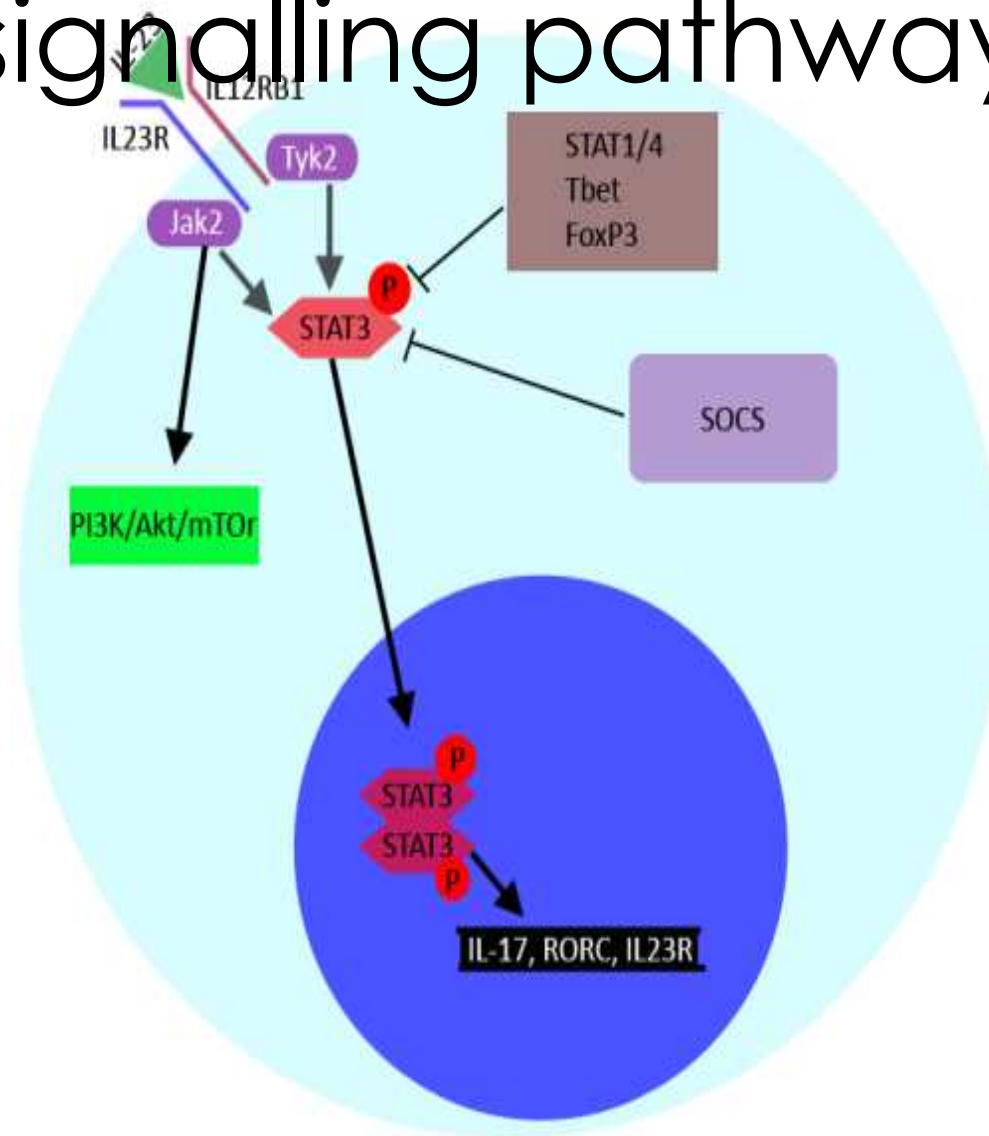
- blood Th17 cells
- 50 ng/mL IL-23/IL-6 (15 min)
- * $p < 0.001$, $n = 7$

Conclusions

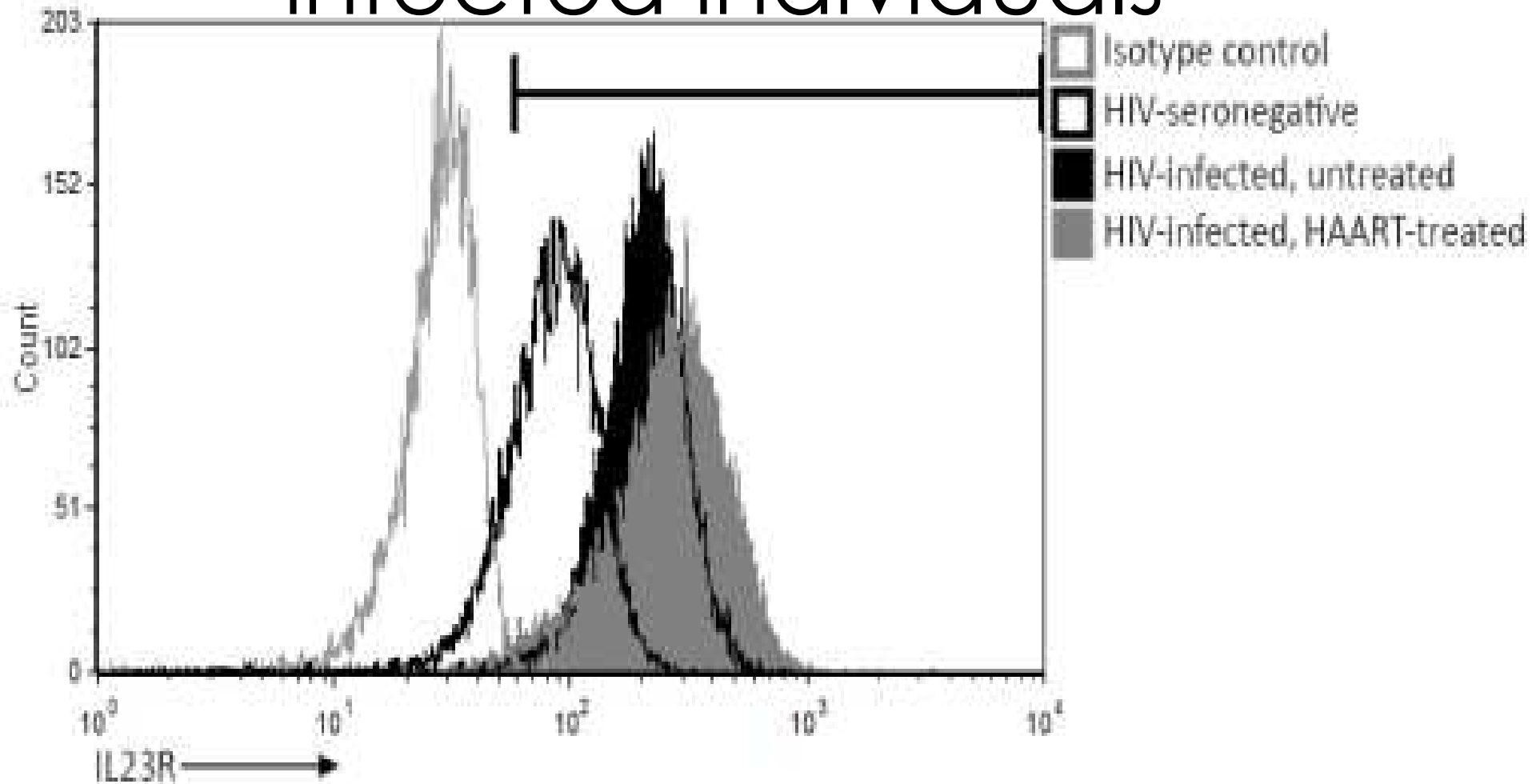
“IL-23 signalling is significantly reduced in Th17 cells infected *in vitro* and is completely absent in Th17 cells from HIV-infected individuals”

- How does HIV inhibit IL-23 signalling?
 - Inhibition of kinases?
 - Induction of negative regulators?
 - Reduced receptor expression?

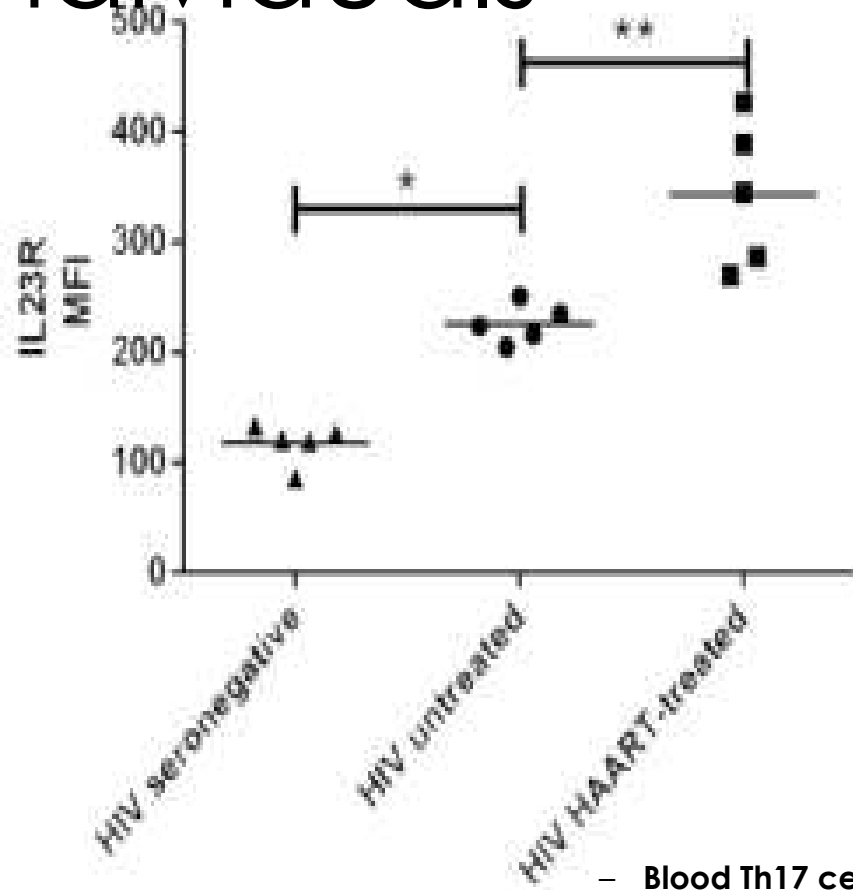
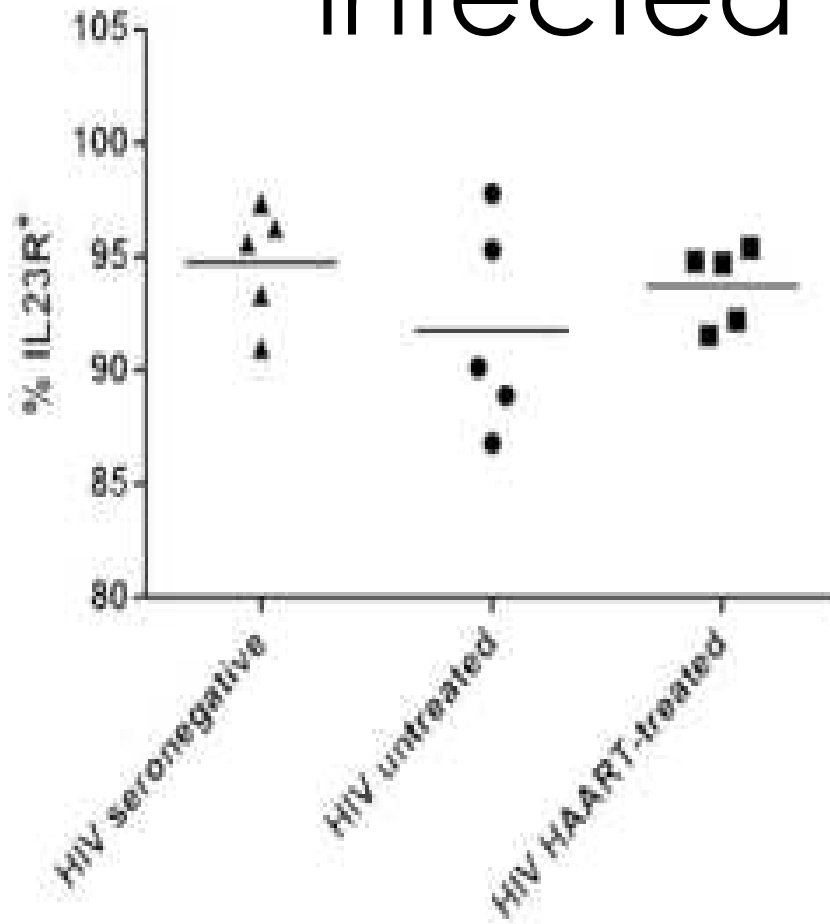
IL-23-responsive signalling pathways



IL23R is not downregulated on blood Th17 cells from HIV-infected individuals



IL23R is not downregulated on blood Th17 cells from HIV-infected individuals



- Blood Th17 cells, ctrls & pt
- * p = 0.004, n = 5
- ** p = 0.002, n = 5

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