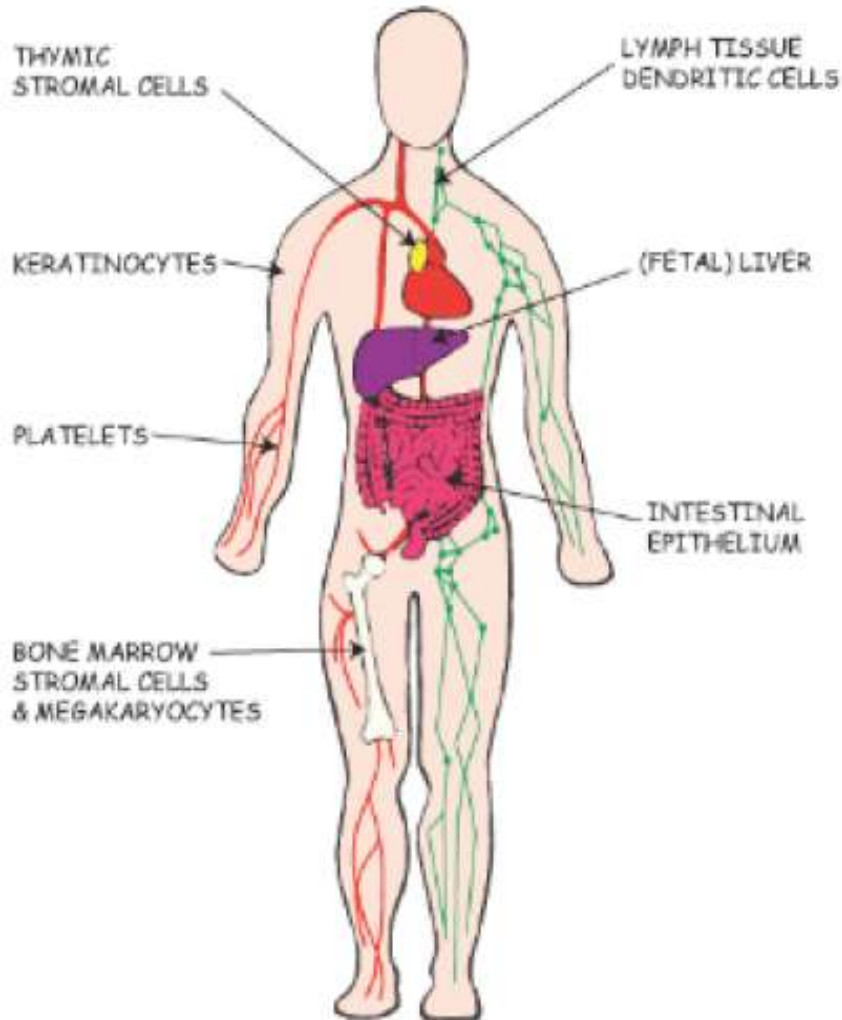

The IL-7 Receptor

A Key Factor in HIV Pathogenesis

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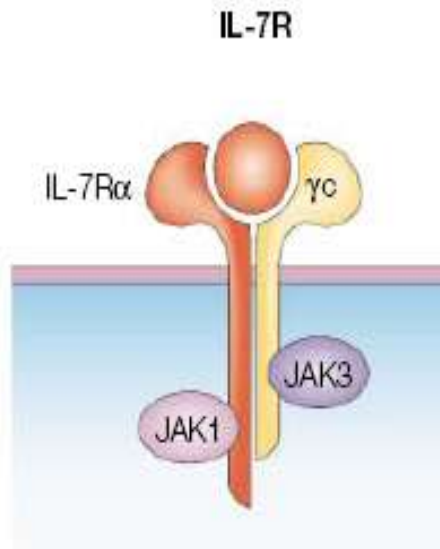


Interleukin-7



- 25 kdal glycoprotein
- Produced by stromal epithelial cells, dendritic cells, intestinal epithelium, vascular endothelium, hepatocytes
- Provides communication to T-cell

Interleukin-7 Receptor



Schluns et al., Nature Reviews-Immunology 2003

IL-7 Receptor:

- **α -chain (CD127)**
- **γ -chain (CD132)** Common to receptors for IL-2, IL-4, IL-7, IL-9, IL-15, IL-21
- Expressed on common lymphoid progenitors, thymocytes, naïve and memory T-cells

IL-7 and T-Lymphocytes

IL-7 is essential to T-cell development and function and plays important roles throughout the life-span of a T-cell:

- Differentiation from stem cells in the bone marrow
 - Differentiation and selection of thymocytes
 - T-cell survival and homeostasis
 - Establishment of memory T-cells
-

IL-7 and CD8 T-Cell Activation



CD8 T-cells and HIV

Cell Mediated Immunity and HIV Infection

- CD8 T-cells remain in the circulation even in patients with advanced disease.
 - CD8 T-cells from HIV-infected patients respond poorly to antigens. Do not proliferate or make perforin in response to antigen and demonstrate weak cytolytic activity.
-

Interleukin-7 and HIV Infection

- Interleukin-7 signaling is essential for optimal CD8 T-cell proliferation and cytotoxic activity
 - Diminished cytotoxic CD8 T-cell activity is a central feature in HIV disease
-

**Does interleukin-7 play a role in
immune dysregulation during
HIV infection?**

Hypothesis:

HIV infection is associated with a down regulation of the IL-7 receptor α -chain (CD127) on CD8 T-cells

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HIV infection is associated with a down regulation of the IL-7 receptor α -chain (CD127) on CD8 T-cells

Corollary:

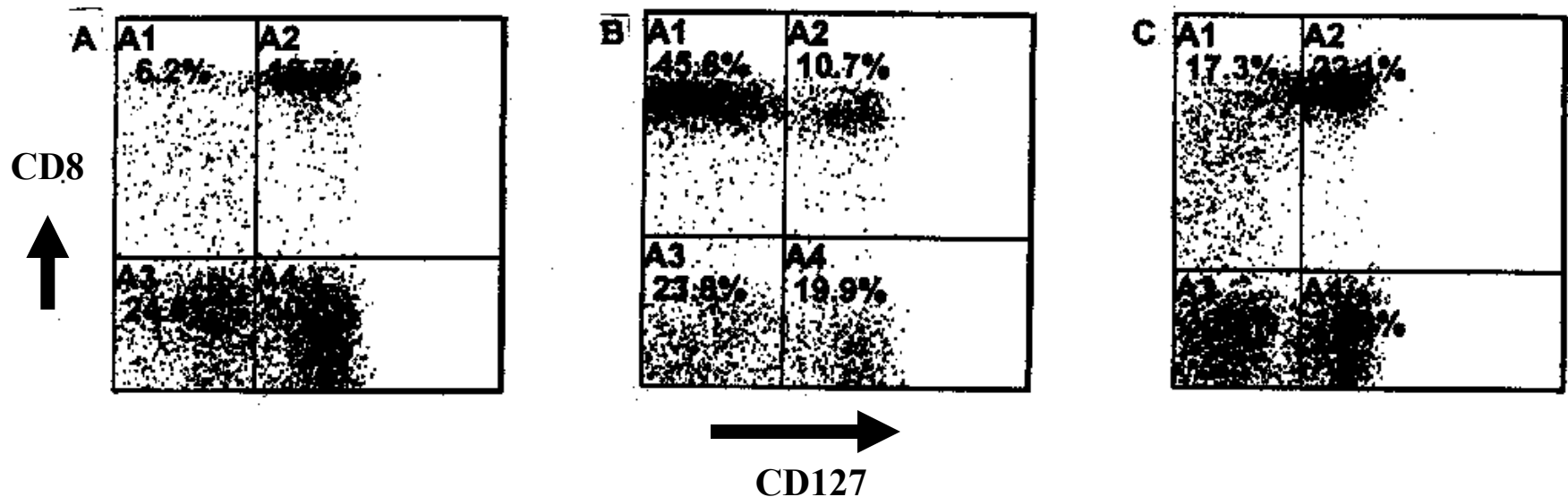
Recovery of CD127 is associated with immune recovery following suppression of HIV replication with antiretroviral therapy.

Representative FACS analysis from three individuals gating on total lymphocytes.

(A) Healthy seronegative control

(B) HIV+ patient naïve to antiretroviral therapy with a CD4 count of 278 and a viral load of 6,028 copies/ml

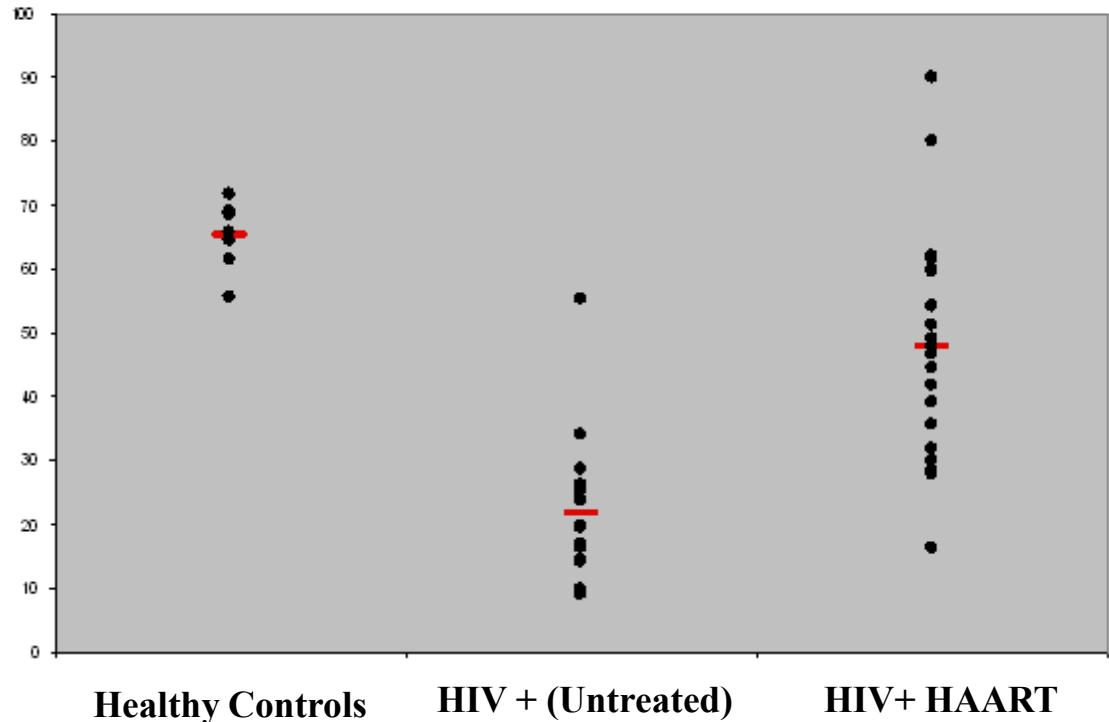
(C) HIV+ patient on antiretroviral therapy. Baseline CD4 count was 108. At the time of analysis, the viral load had been maintained at <50 copies/ml for 41 months and the CD4 counts was 694.



IL-7R and HIV Infection

- 67% reduction in CD8 T-cells expressing IL-7R α in HIV+ patients.
- Partial recovery with sustained HIV suppression on cART.
- Magnitude of recovery proportional to time on suppressive treatment.

CD127 Expression on CD8 T Cells



MacPherson et al., JAIDS 2001

Summary

Active HIV replication is associated with a marked decrease in the level of expression of CD127 on CD8 T-cells.

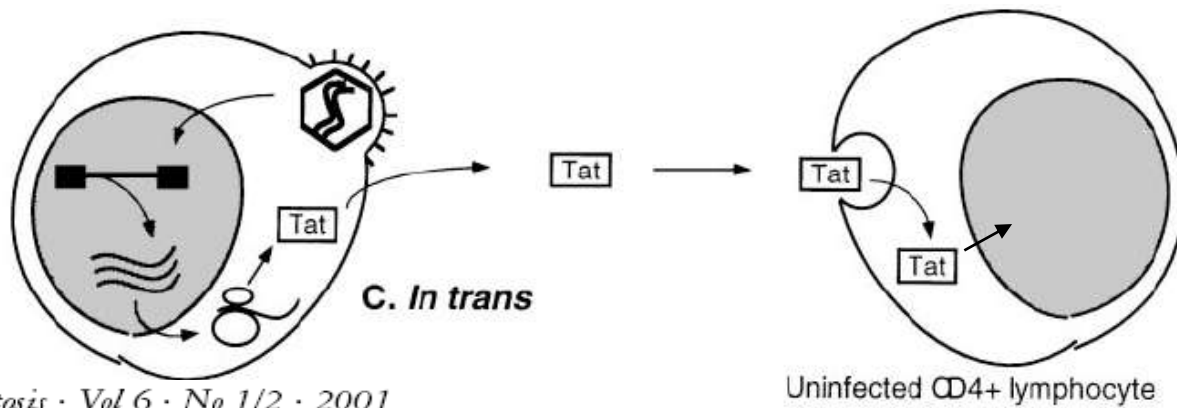
Suppression of HIV replication with effective antiretroviral therapy is associated with an apparent recovery of CD127 on CD8 T-cells.

Given that CD127 (IL-7R α) is necessary for optimal CD8 T-cell proliferation and cytolytic activity, this may explain in part the reduced CTL activity seen in HIV infection and its recovery on antiretroviral therapy

**How does HIV infection lead to a
down regulation of the IL-7
Receptor α -chain on CD8 T-cells?**

Viral factor: HIV-1 Tat

- 14 kDal Protein
- Secreted by HIV-infected cells and can be detected in serum of HIV+ patients.
- Taken up by uninfected T-cells through heparin sulfate receptors and clathrin-dependent endocytosis.
- Soluble Tat suppresses T-cell activity in vitro
- Anti-Tat antibodies are associated with low viral loads and slower disease progression in HIV+ patients



Hypothesis

The decrease in IL-7 Receptor α -chain expression on CD8 T-cells is mediated by soluble HIV Tat protein

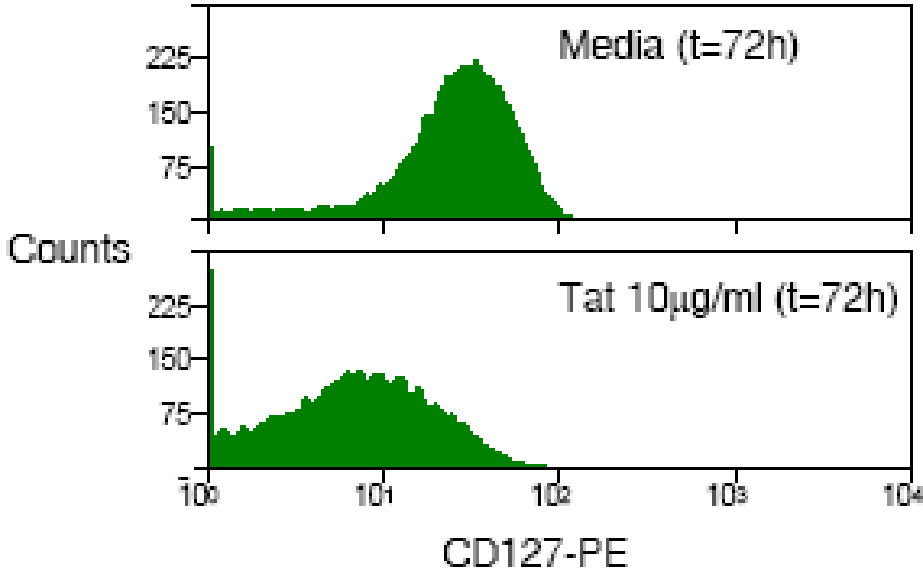
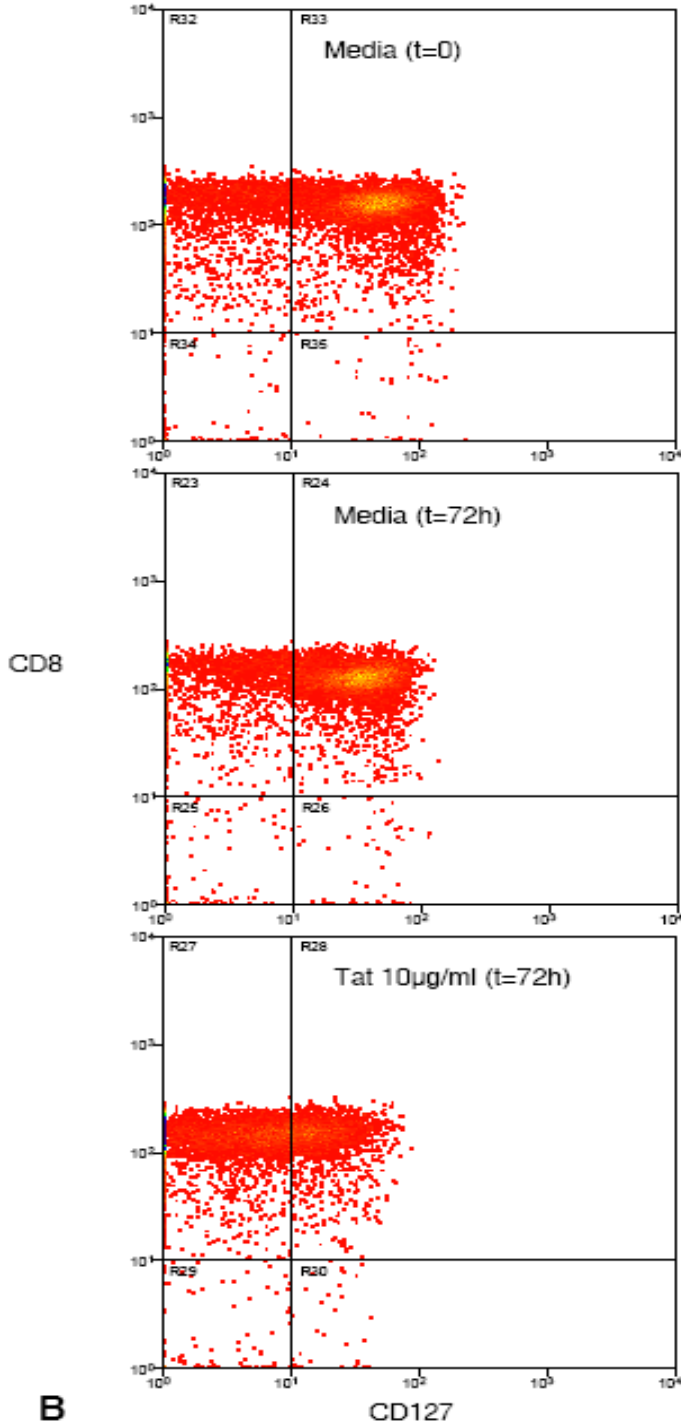
CD8 T-cells isolated from healthy donors

- PBMC were isolated over Ficoll
- CD8+ cells were purified using the AutoMACS magnetic bead cell isolation system

Purity by flow cytometry

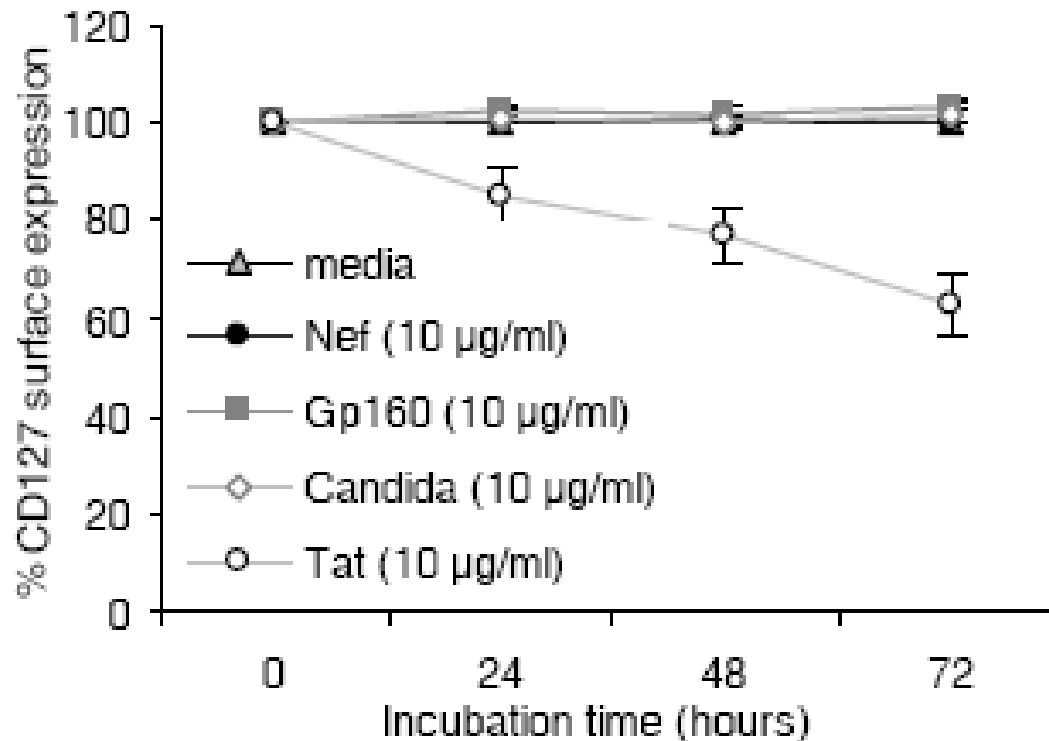
- 95-99% pure CD8+
-

Tat down regulates CD127 on CD8 T-cells

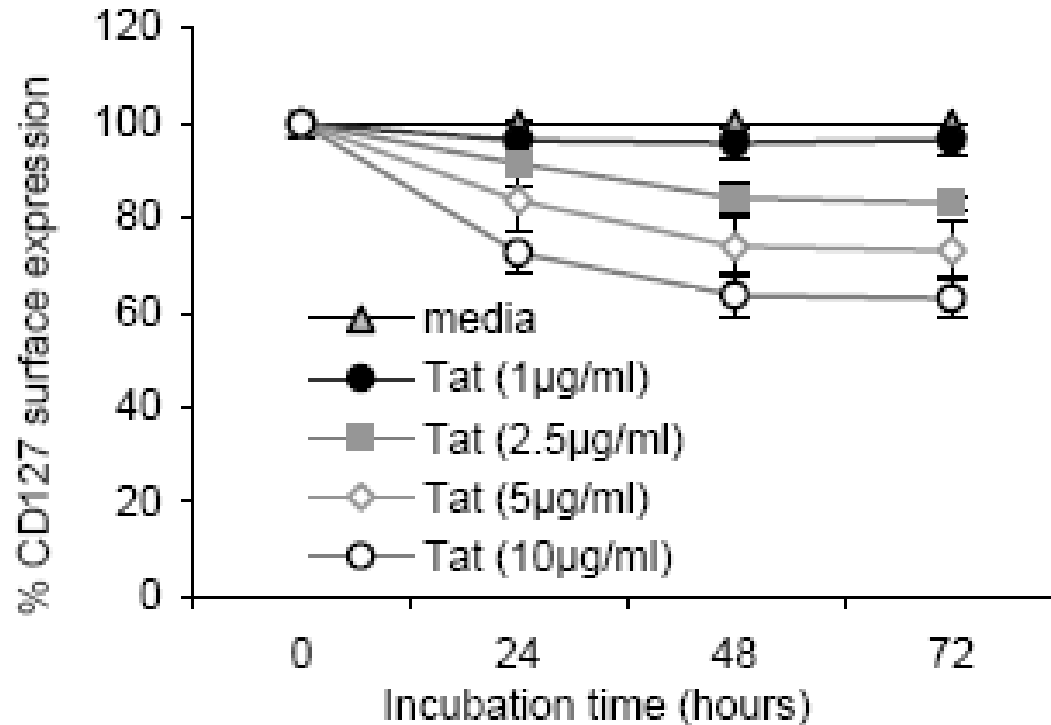


B

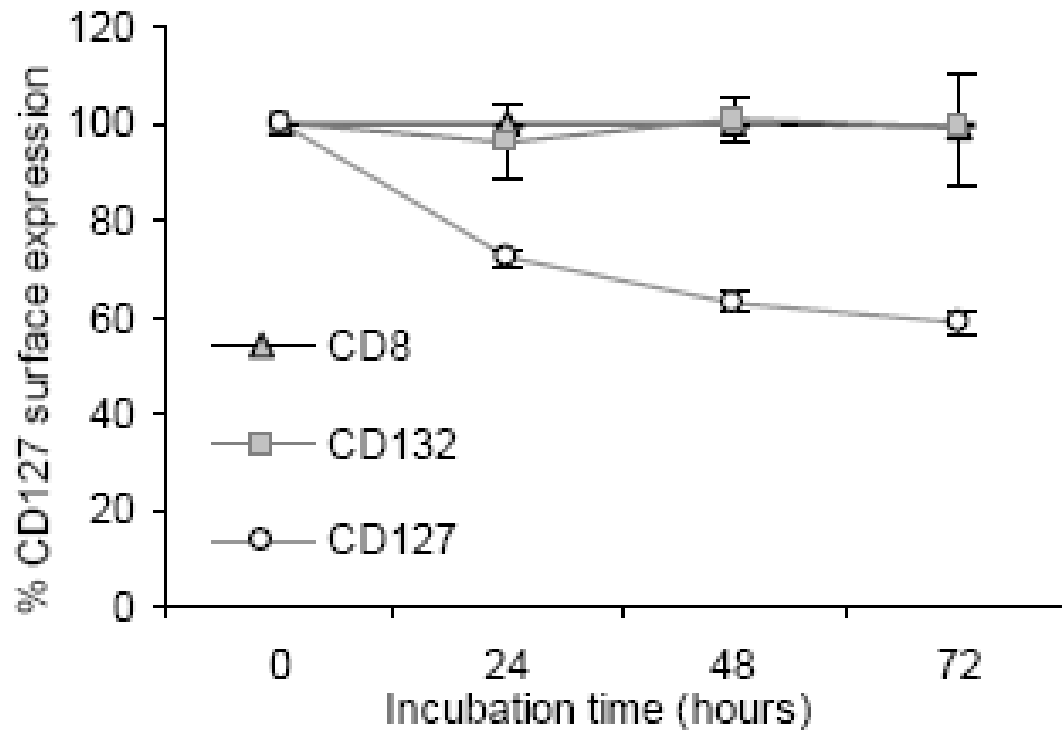
CD127 is not down regulated by other HIV proteins



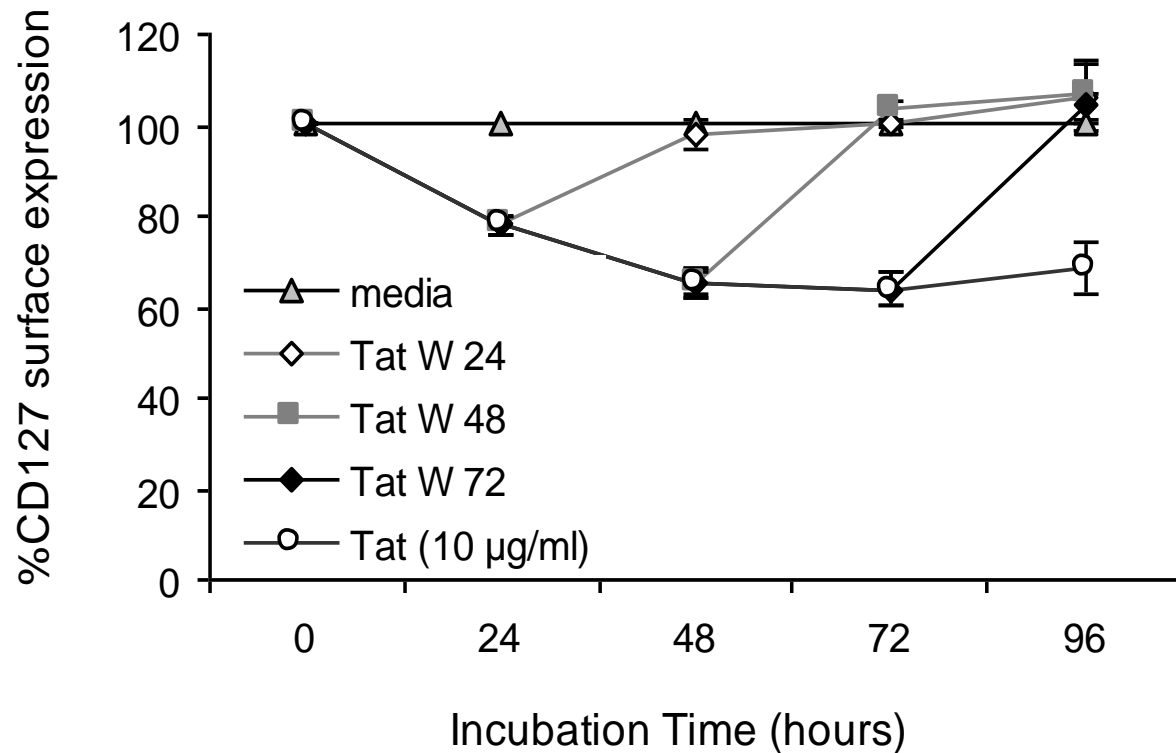
HIV Tat induces a time and dose dependant decrease in CD127 expression



Tat does not effect IL-7R γ -chain



Effect of Tat on CD127 expression is reversible



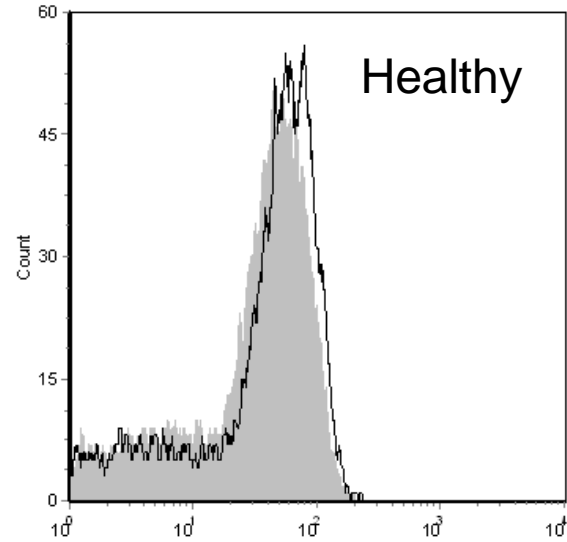
Tat induces a down-regulation of IL7R α expression on CD8 T-cells:

- Dose and Time Dependant
 - Specific for CD127
 - Effect is blocked with anti-Tat antibodies
 - Effects both naïve and memory cells
 - Reversible: requires continuous presence of Tat
 - Down regulation is not due to cell stimulation or apoptosis
-

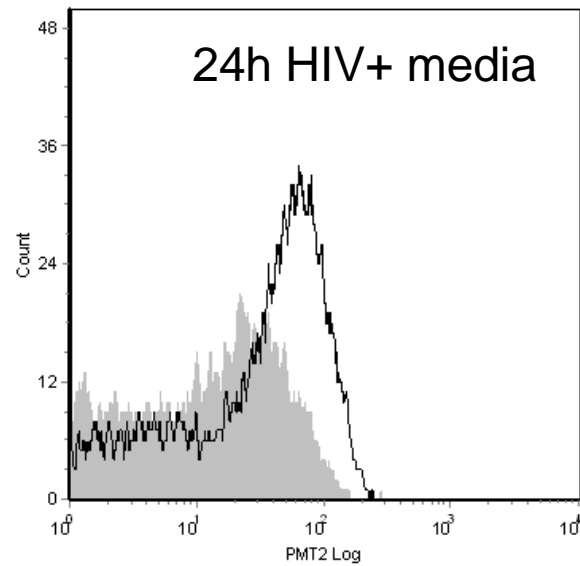
**Tat down regulates surface expression of
CD127 on CD8 T-Cells in vitro...**

Is this reflected in vivo?

Healthy controls

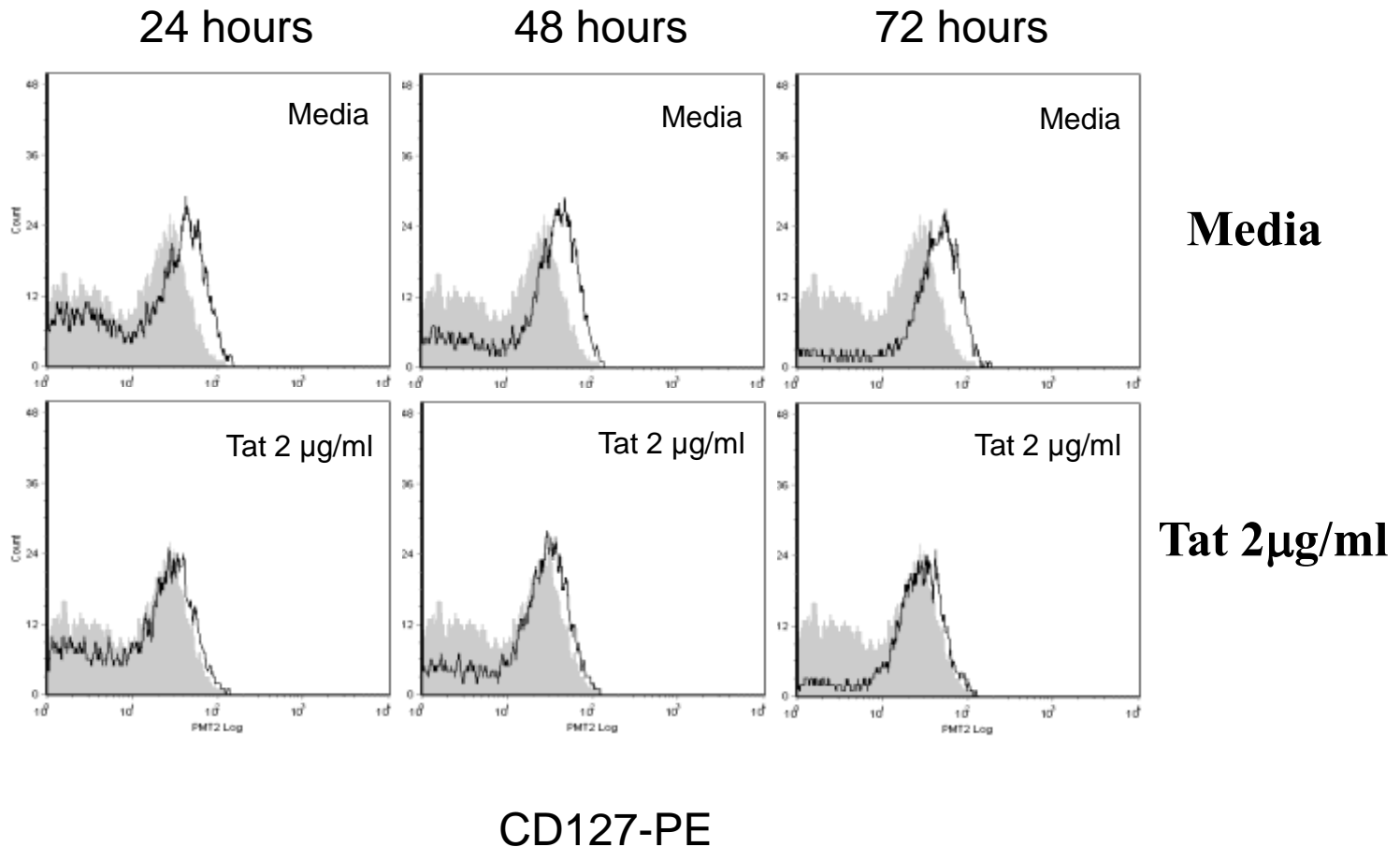


HIV+ patients
recover CD127
on the surface of
CD8 T-cells



CD127-PE

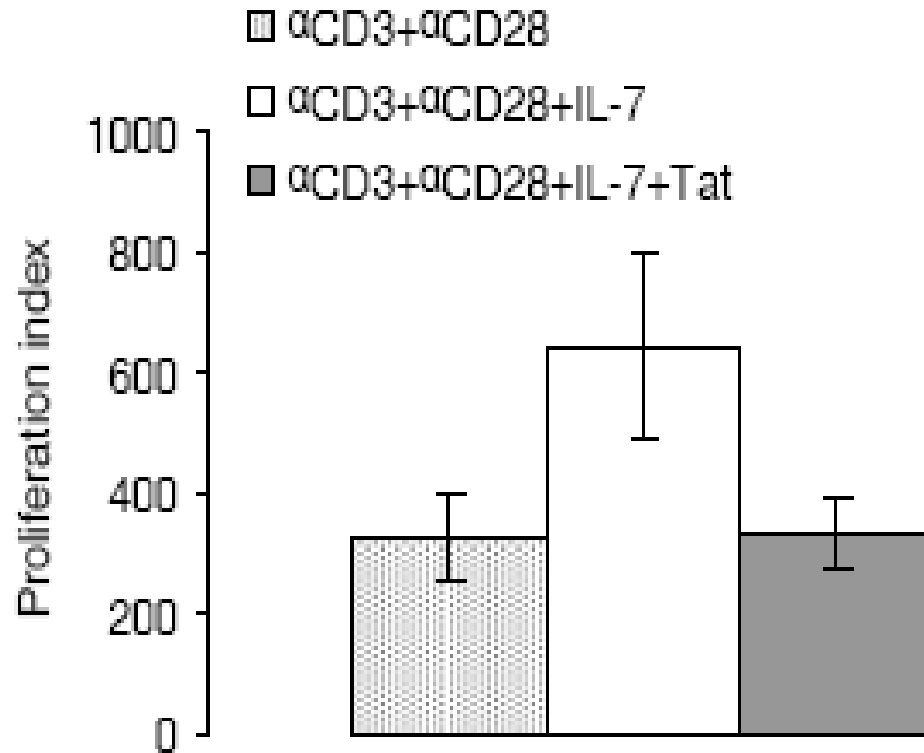
Tat protein inhibits recovery of CD127 on CD8 T-Cells isolated from HIV+ patients



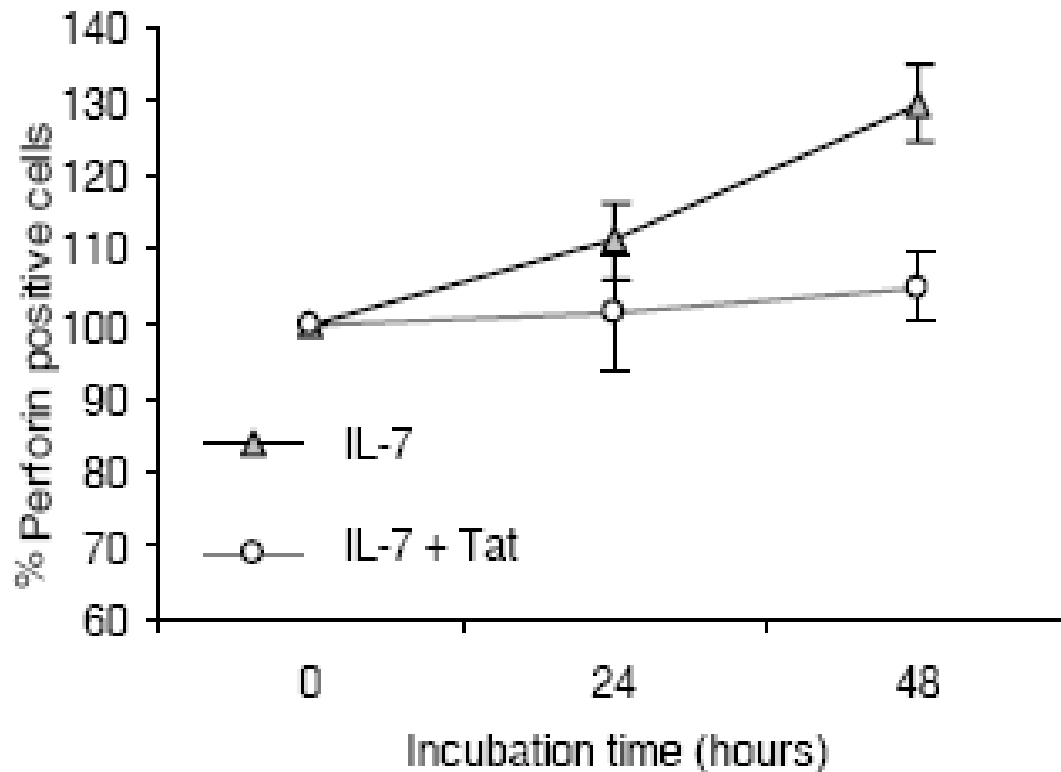
**Tat down regulates surface expression of
CD127 on CD8 T-Cells...**

**Is there any functional relevance to this
decreased expression of IL-7R α
on CD8 T-cells?**

Tat inhibits IL-7 induced proliferation



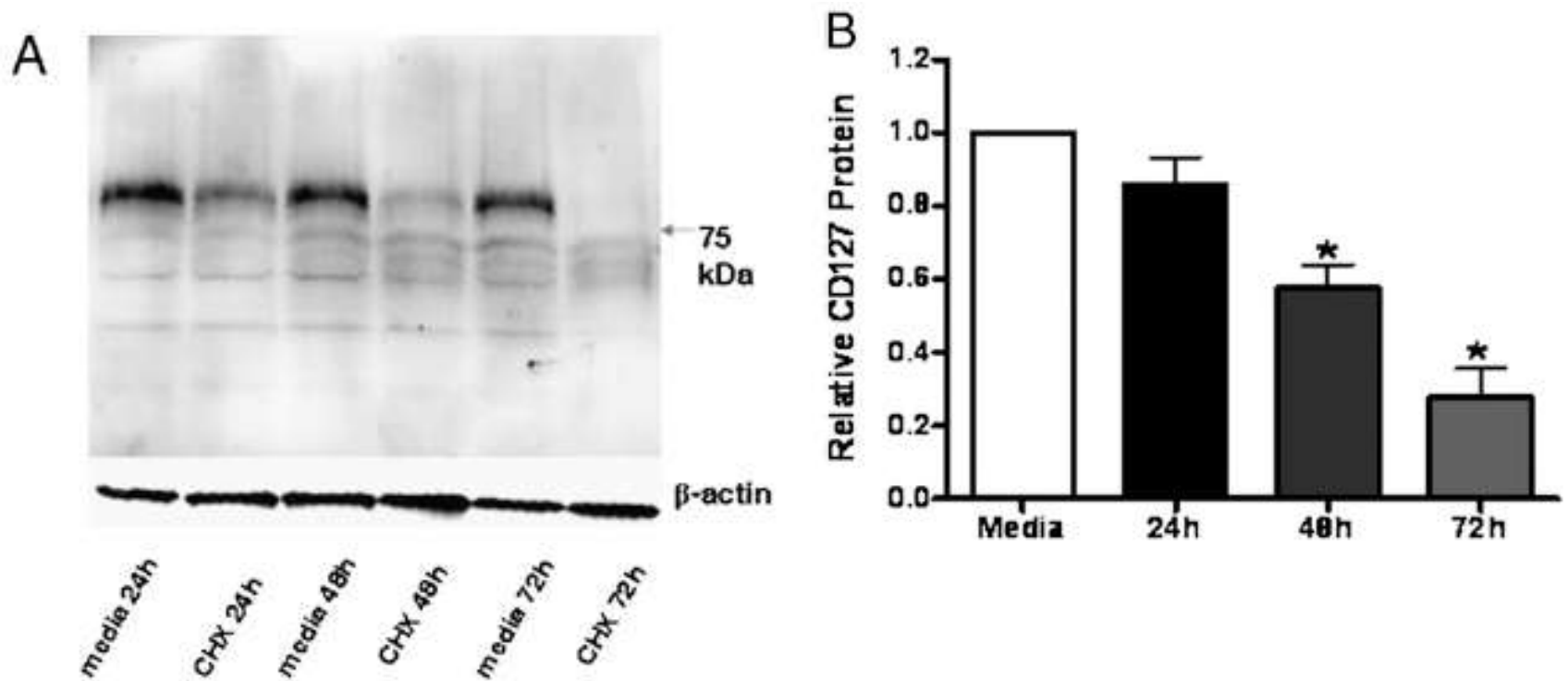
Tat inhibits IL-7 induced Perforin production



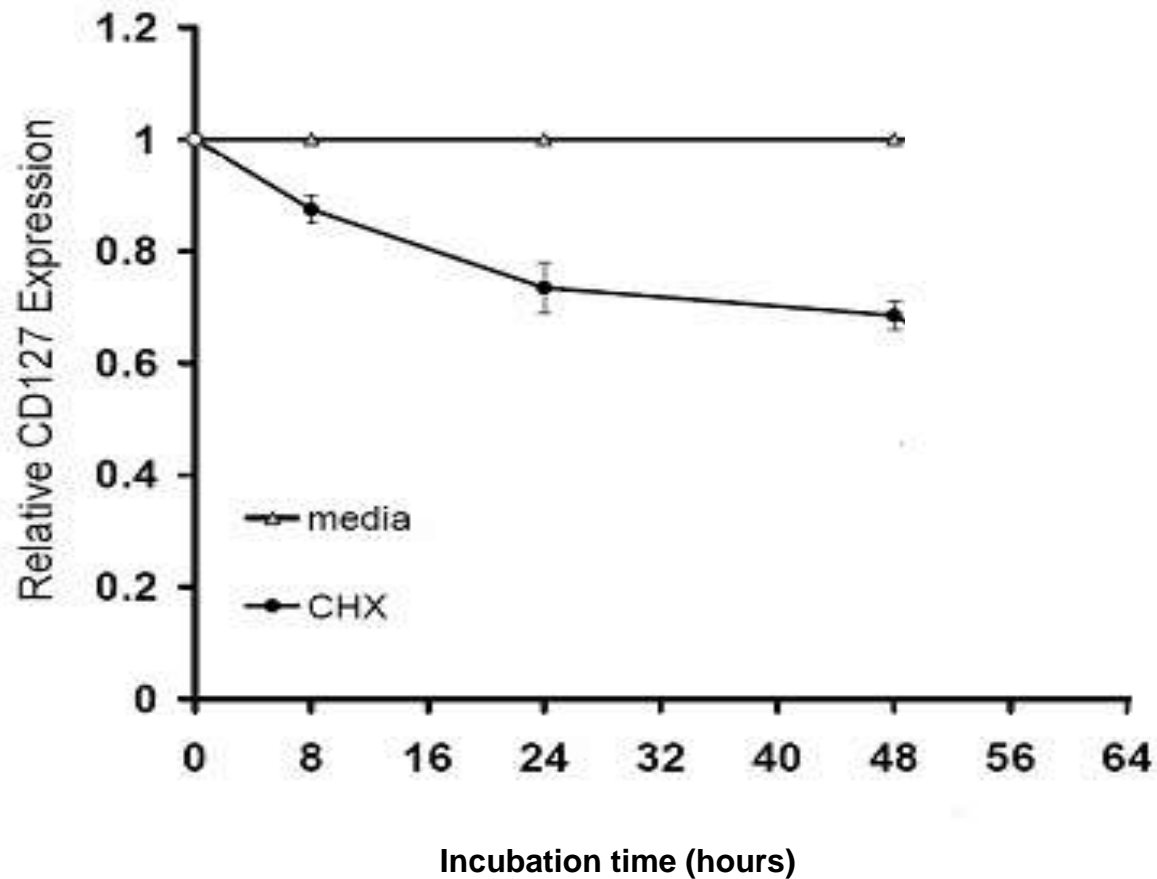
By down regulating the IL-7R at the cell surface, Tat is able to induce anergy in CD8 T-cells.

**How does HIV Tat down regulate
the IL-7 Receptor on CD8 T-cells?**

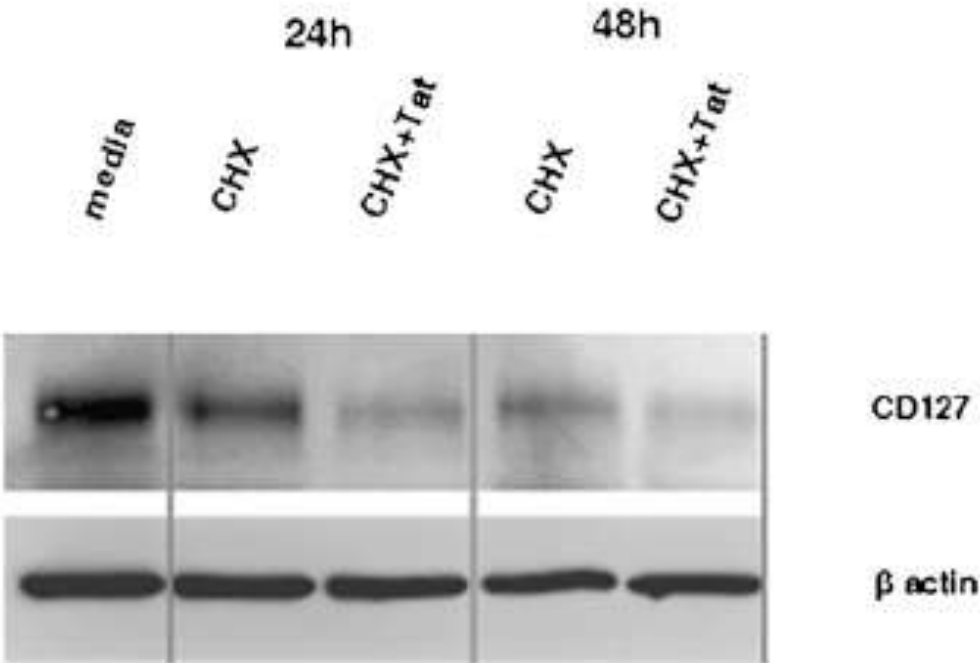
CD127 is stable in resting CD8 T-cells with a half-life of 55 hours



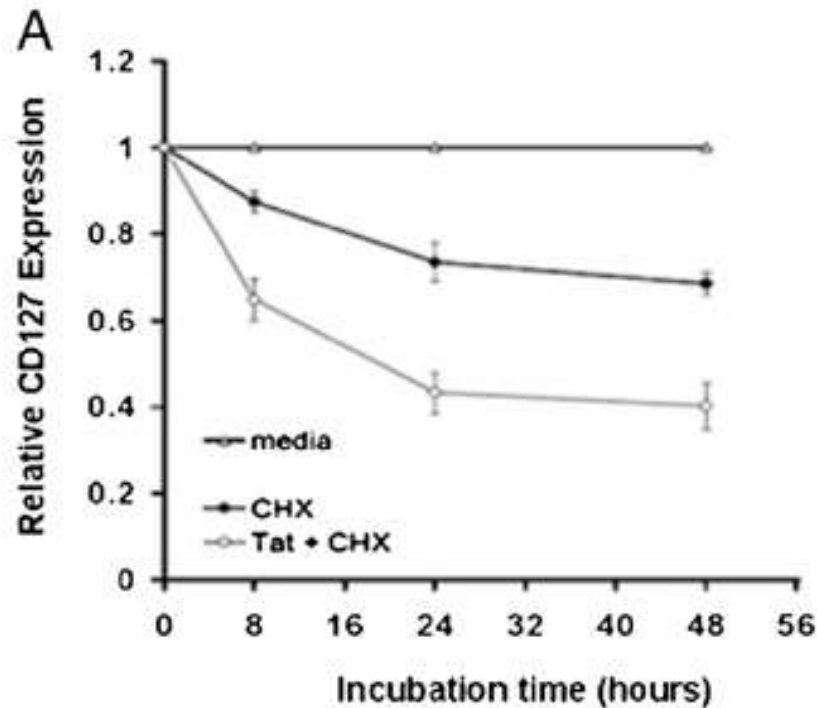
CD127 decays from the surface of resting CD8 T-cells at a slow rate



Tat decreases the half-life of CD127 protein

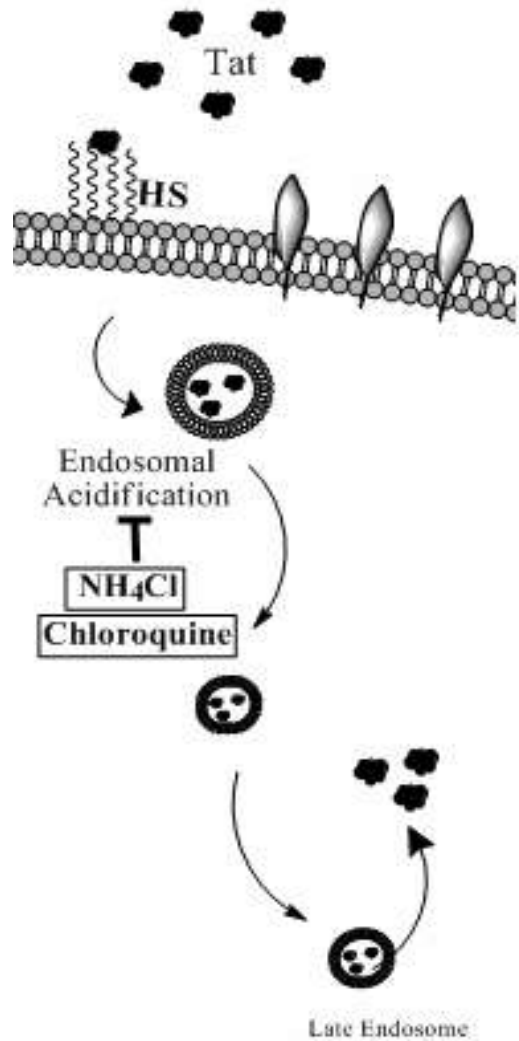


Tat increases the rate of decay of CD127 from the surface of resting CD8 T-cells



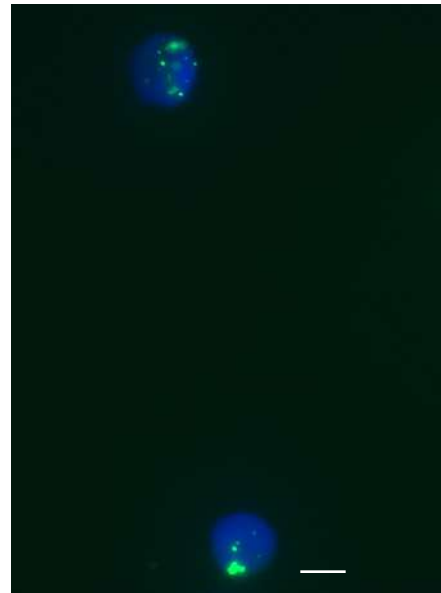
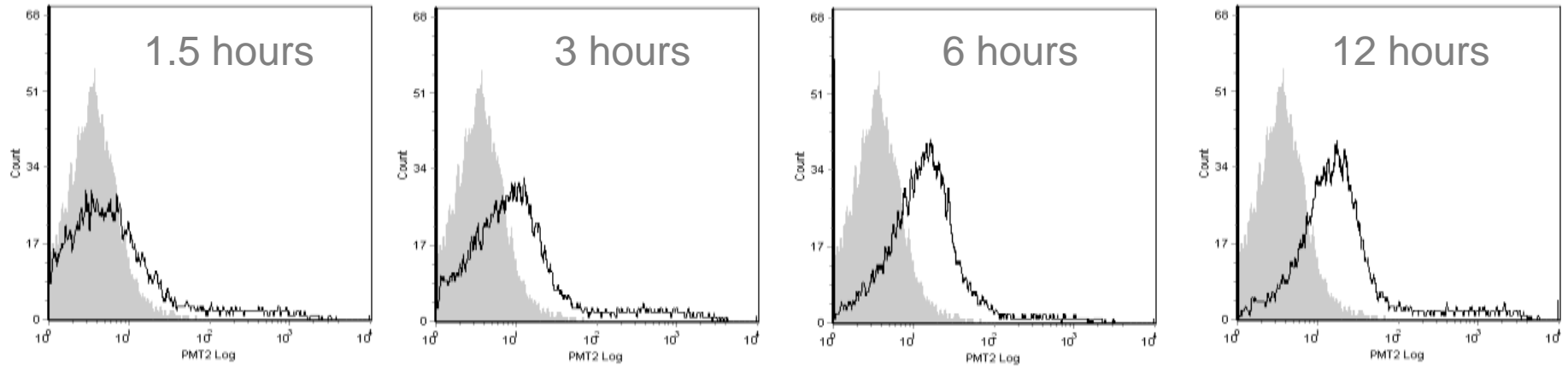
-
- CD127 decays from the surface of resting CD8 T-cells with a half life of 55 hours
 - Tat increases the rate of decay of CD127 from the surface of CD8 T-cells

How does Tat exert its effect?

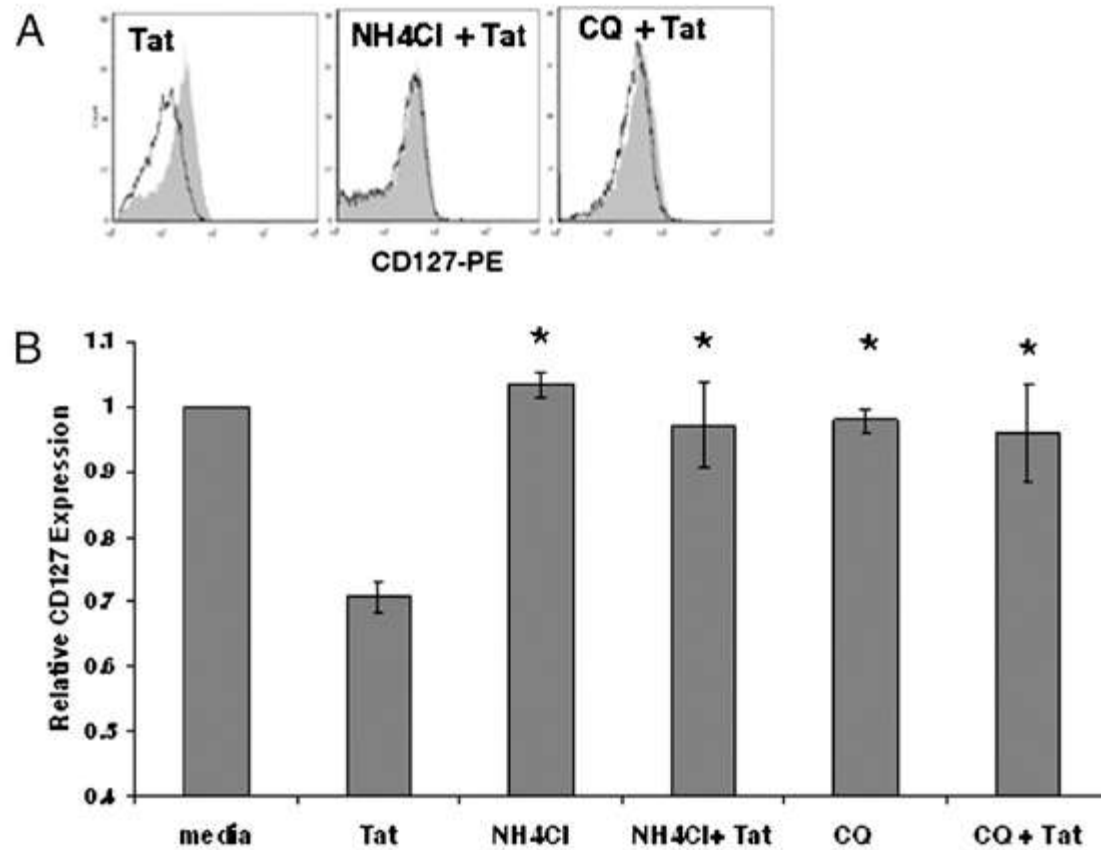


- Tat enters Lymphocytes through receptor mediated endocytosis, followed by exit from late endosomes to exert effect in the cytoplasm (Vendeville et al., Mol. Biol. Cell, 2004)
- endosomal acidification is required for early to late endosomal development and release of Tat into the cytoplasm.

Tat is internalized by CD8 T-cells



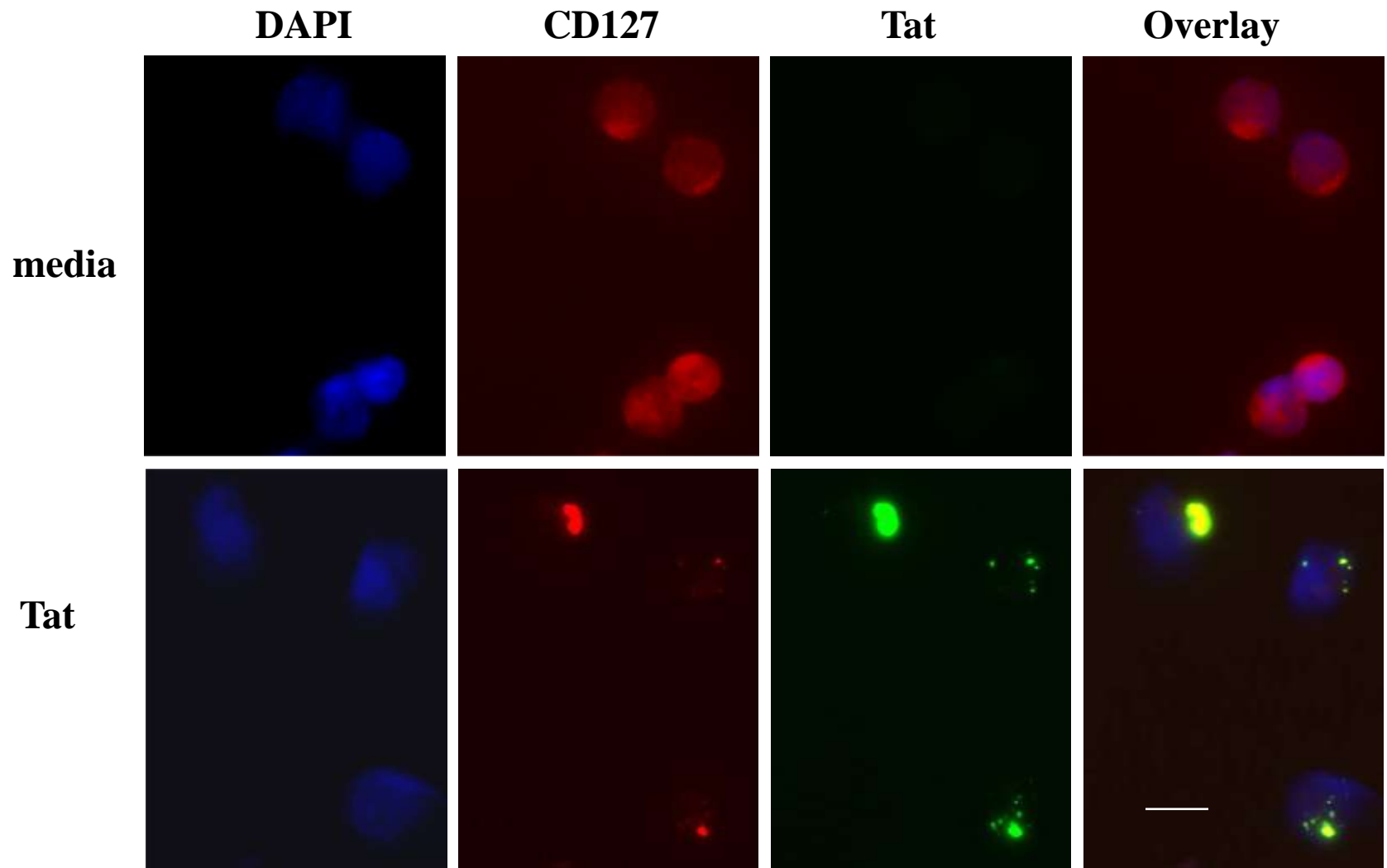
Tat trapped in endocytic vesicles cannot down regulate CD127 at the cell surface



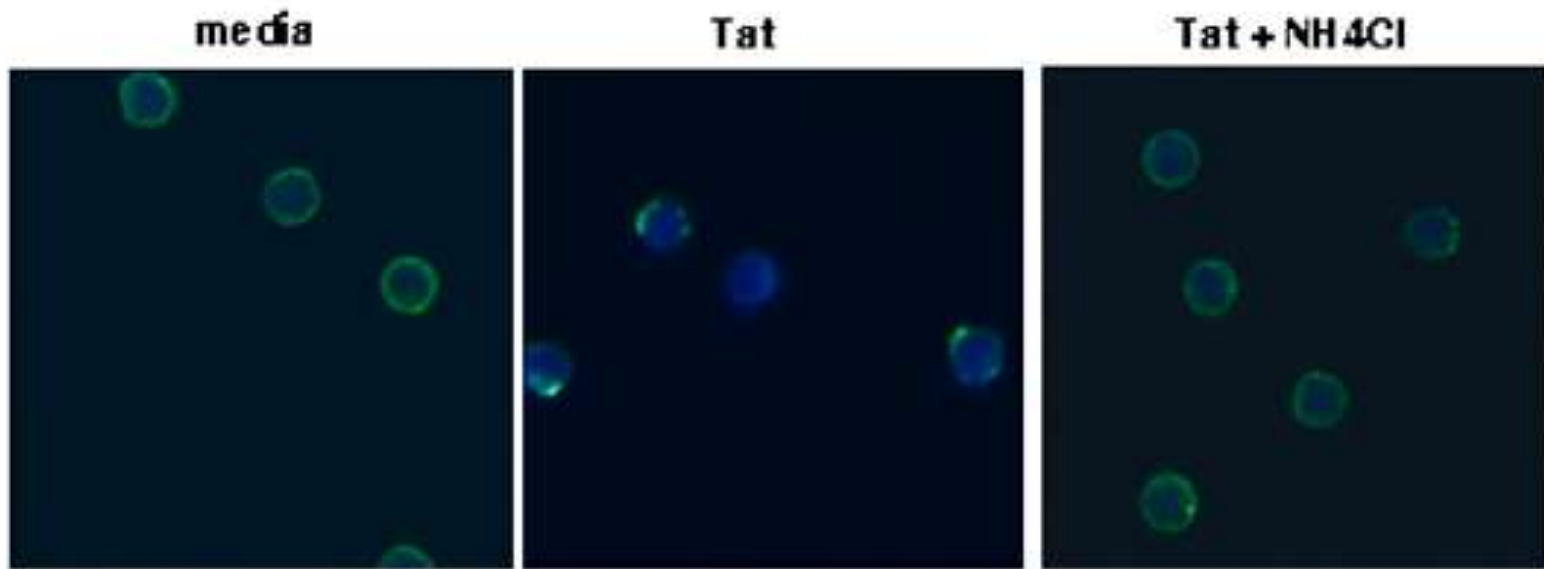
Tat entry into the cell and subsequent exit from late endosomes are required for Tat to exert its effect on CD127.

What happens after Tat enters the Cytosol?

Tat co-localizes with CD127



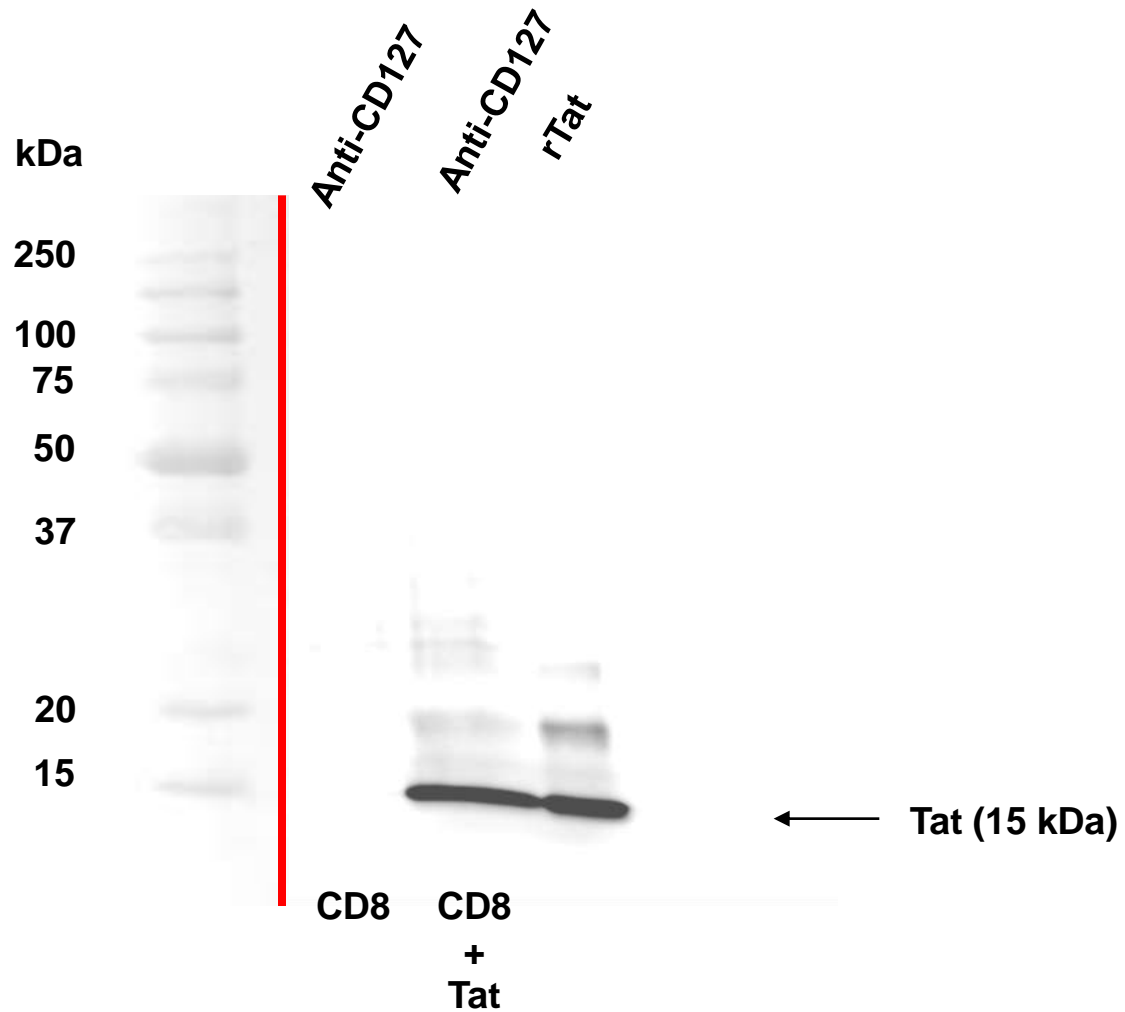
Tat trapped in endocytic vesicles cannot down regulate CD127 at the cell surface



**Tat associates with CD127 and induces
receptor aggregation.**

Do Tat and CD127 interact?

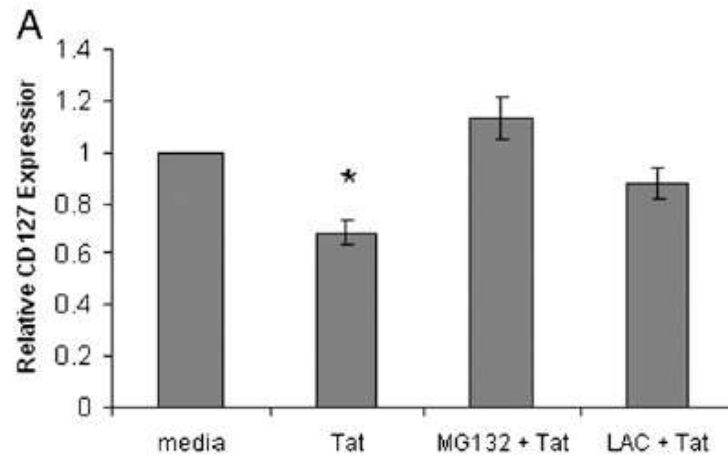
Anti-CD127 antibodies co-immunoprecipitate the HIV Tat Protein



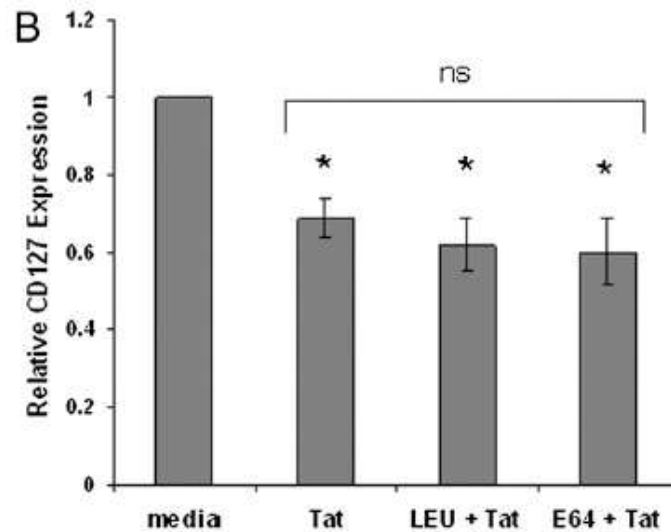
**Tat binds to the cytoplasmic tail of CD127
and induces receptor endocytosis.**

**Once inside the cell, what is the destination
of CD127?**

Proteasome inhibitors block Tat's effect

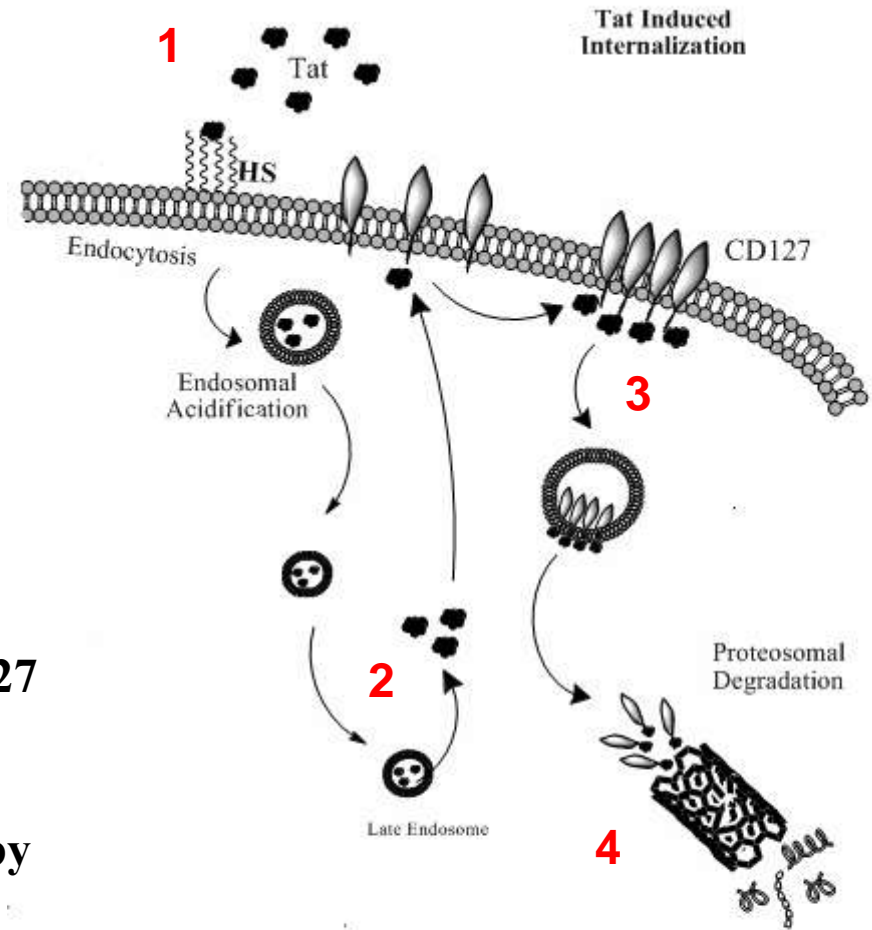


Lysosome inhibitors do not block Tat's effect

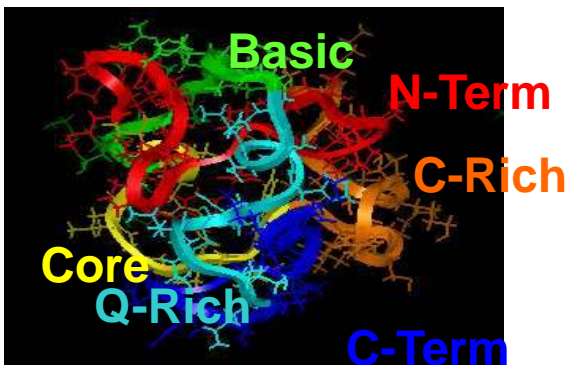
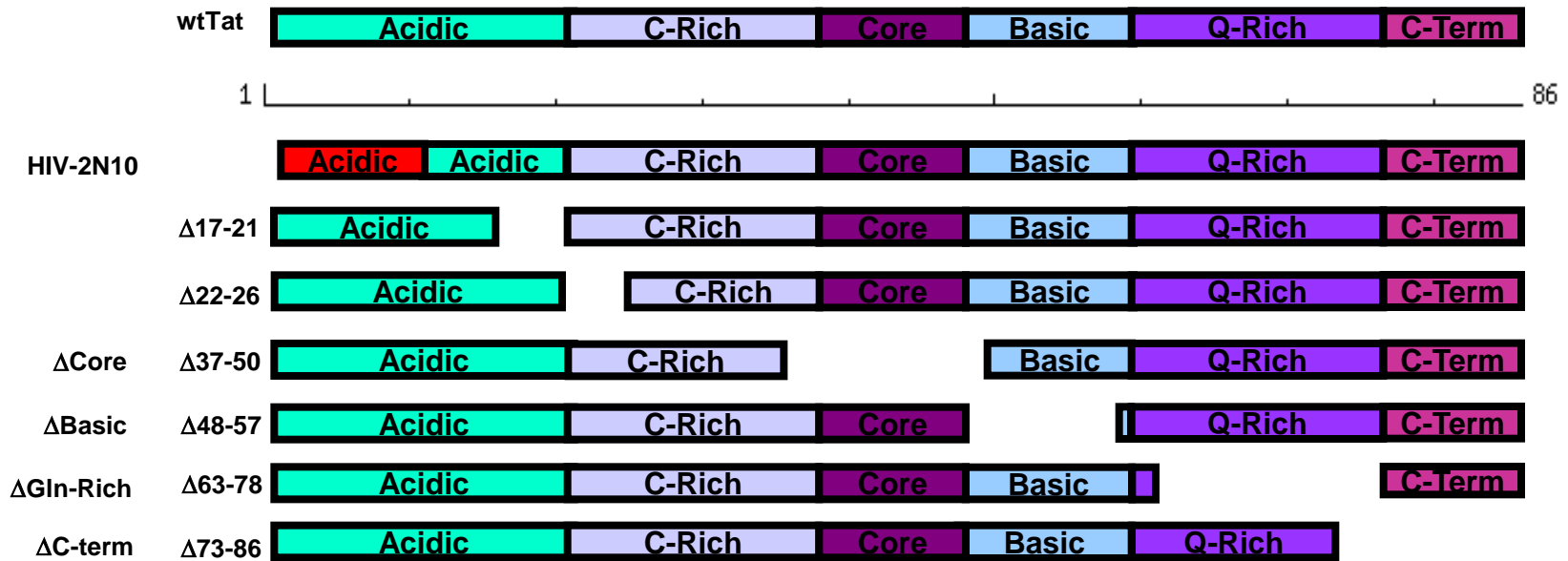


Summary

1. Tat enters the cell by receptor (HS) mediated endocytosis.
2. Tat exits late endosomes to enter the cytosol.
3. Tat binds the cytoplasmic tail of CD127 inducing receptor endocytosis.
4. CD127 is internalized and degraded by the proteasome.



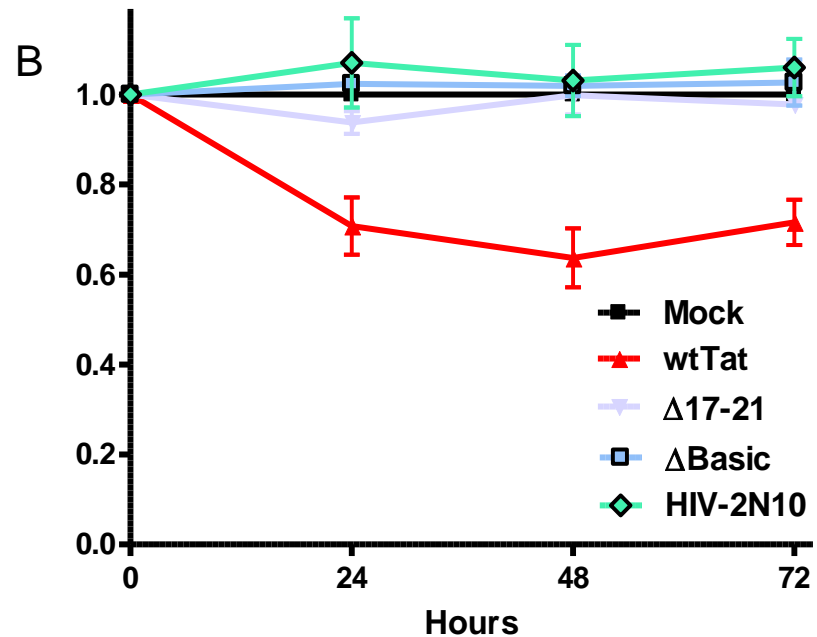
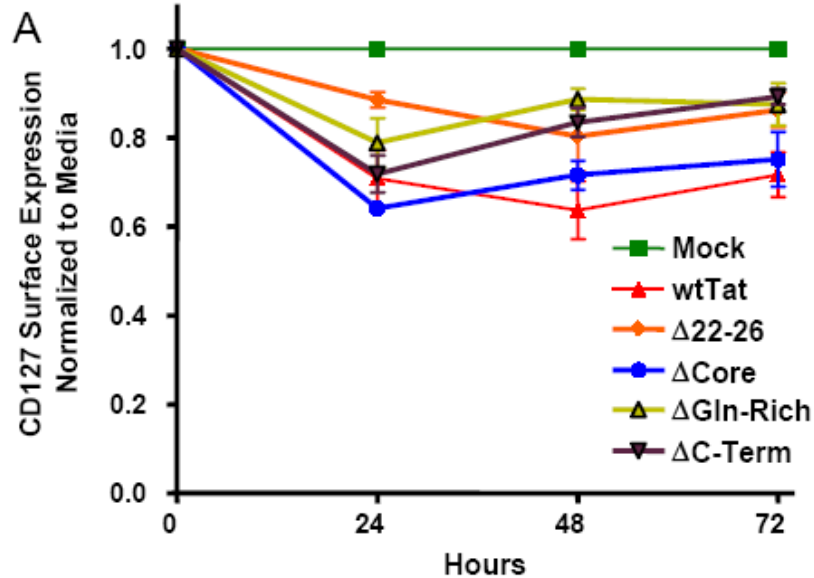
Mutational Analysis of the Tat Protein



HIV-1
HIV-2

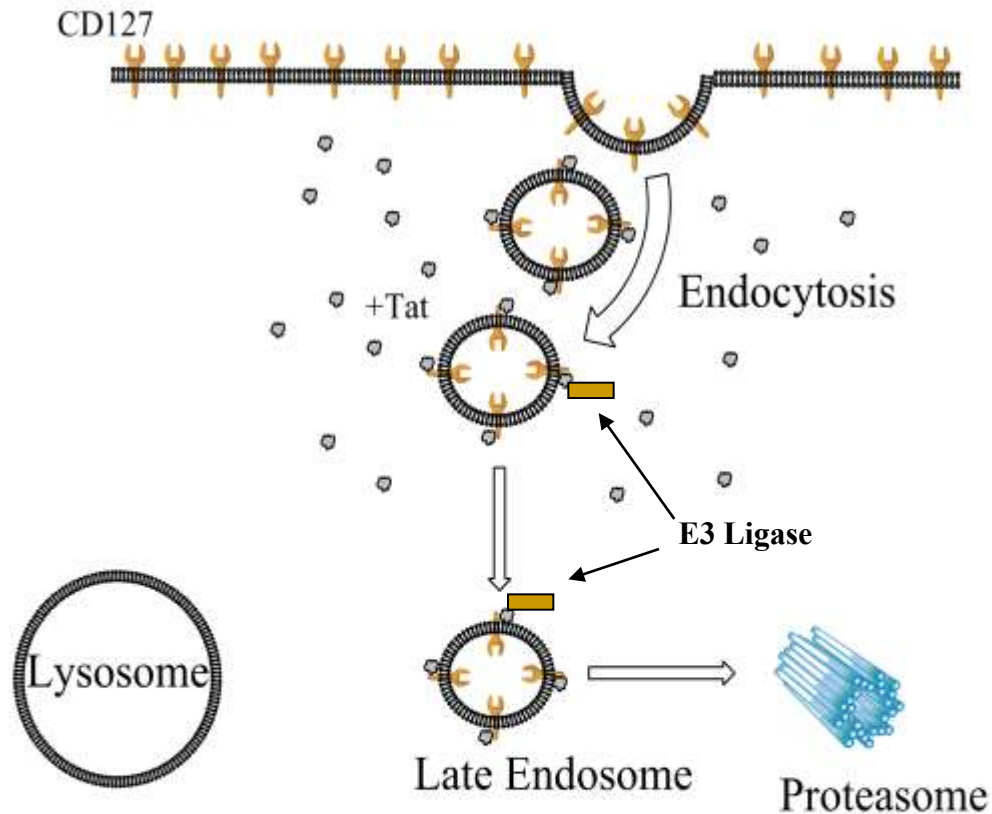
MEPVDPRLEPW
METHLKAPESS

Deletions of amino acids 1-10, 17-21 or the basic domain abolish Tat's ability to down regulate CD127 on the cell surface

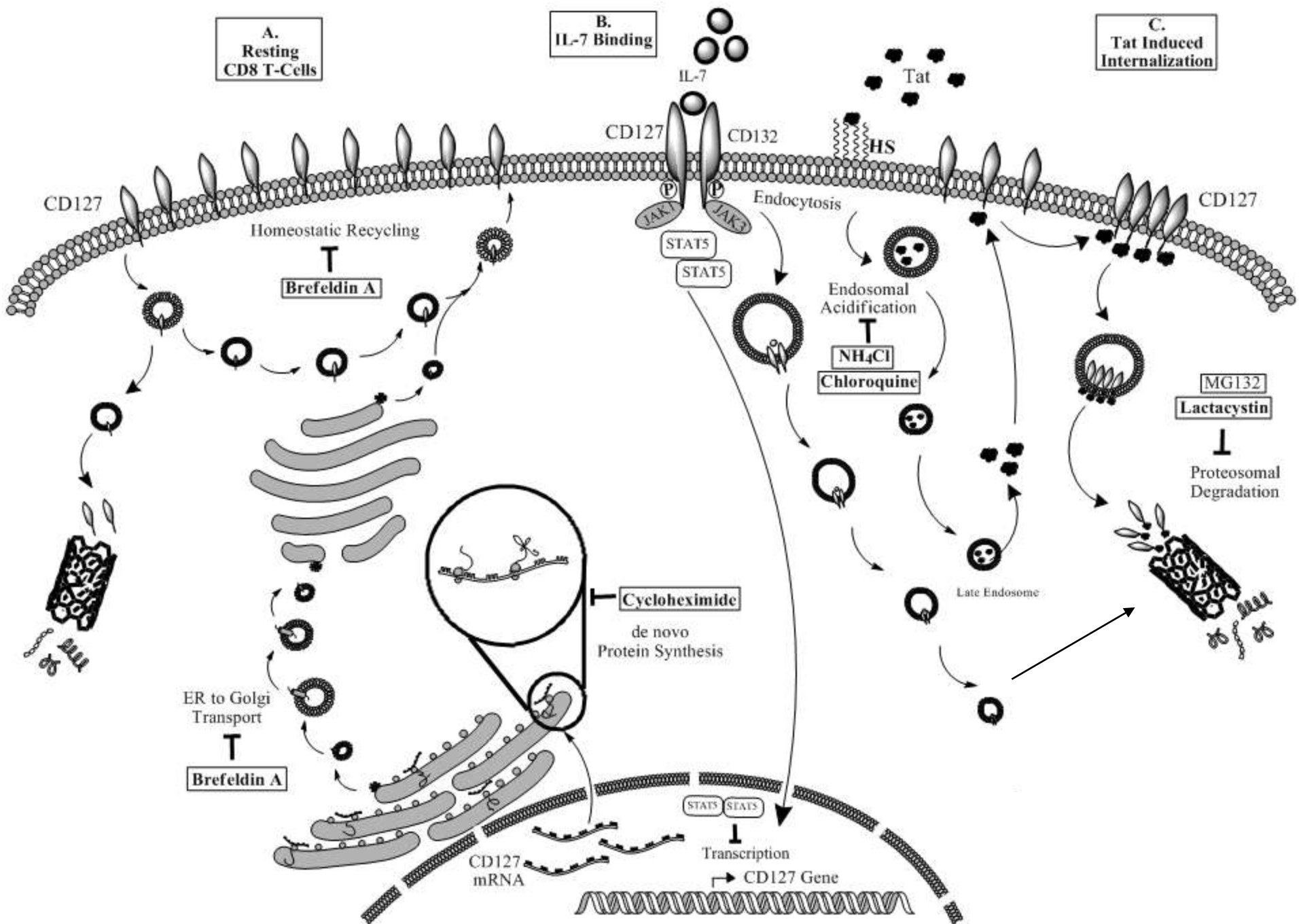


Tat binds via its amino terminal domain to the cytoplasmic tail of CD127 and directs it to the proteasome for degradation.

Current Model



- By binding to the cytoplasmic tail of CD127, Tat recruits an E3-ubiquitin ligase to the receptor thus inducing receptor internalization and degradation.



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