HIV-1 Vpu downregulates Tim-3 from the surface of infected CD4+ T cells

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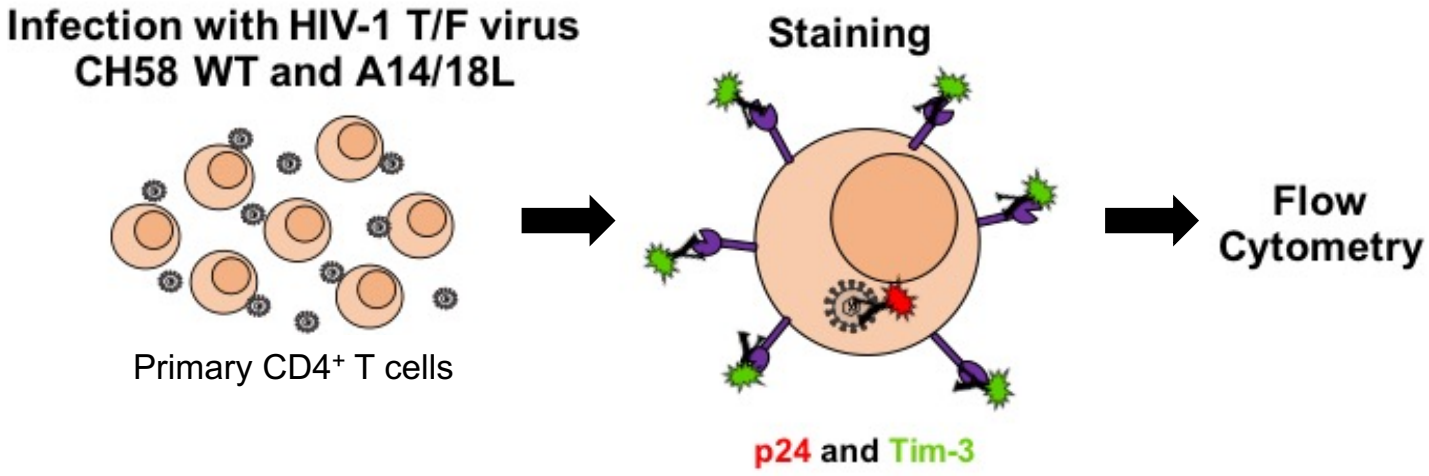
Introduction

- The HIV-1 accessory proteins Vpu and Nef downregulate cell surface molecules by altering their intracellular trafficking
- Vpu enhances viral egress by downregulating tetherin from the surface of infected cells via an interaction through its transmembrane domain ($A_{14/18}$) and induces its sequestration within the trans-Golgi network (TGN)
- The T cell immunoglobulin and mucin domain-containing protein 3 (Tim-3) inhibits viral egress by binding phosphatidylserine (PtdSer) on the surface of virions
- We **hypothesized** that Vpu downregulates cell surface Tim-3 in primary $CD4^+$ T cells

Results: Vpu downregulates Tim-3 via its transmembrane domain



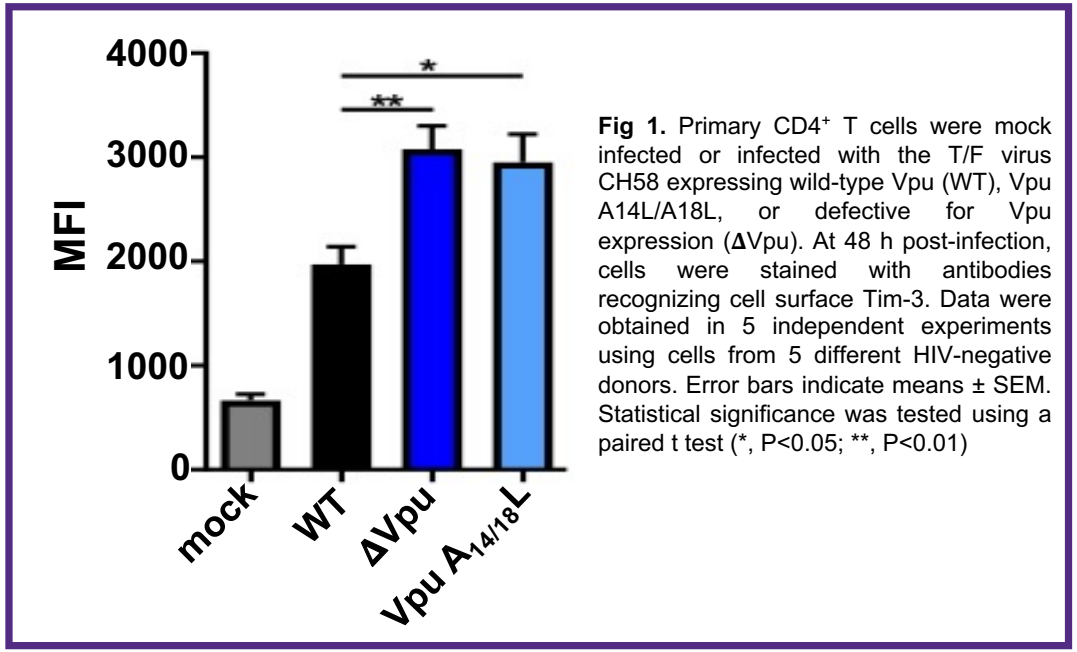
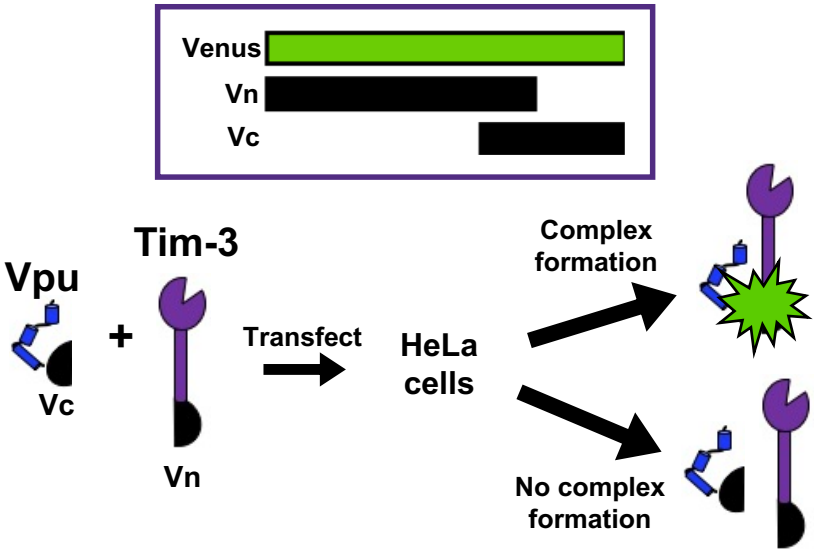


Fig 1. Primary $CD4^+$ T cells were mock infected or infected with the T/F virus CH58 expressing wild-type Vpu (WT), Vpu A14L/A18L, or defective for Vpu expression (ΔVpu). At 48 h post-infection, cells were stained with antibodies recognizing cell surface Tim-3. Data were obtained in 5 independent experiments using cells from 5 different HIV-negative donors. Error bars indicate means ± SEM. Statistical significance was tested using a paired t test (*, P<0.05; **, P<0.01)

Results: Vpu and Tim-3 form a complex within cells



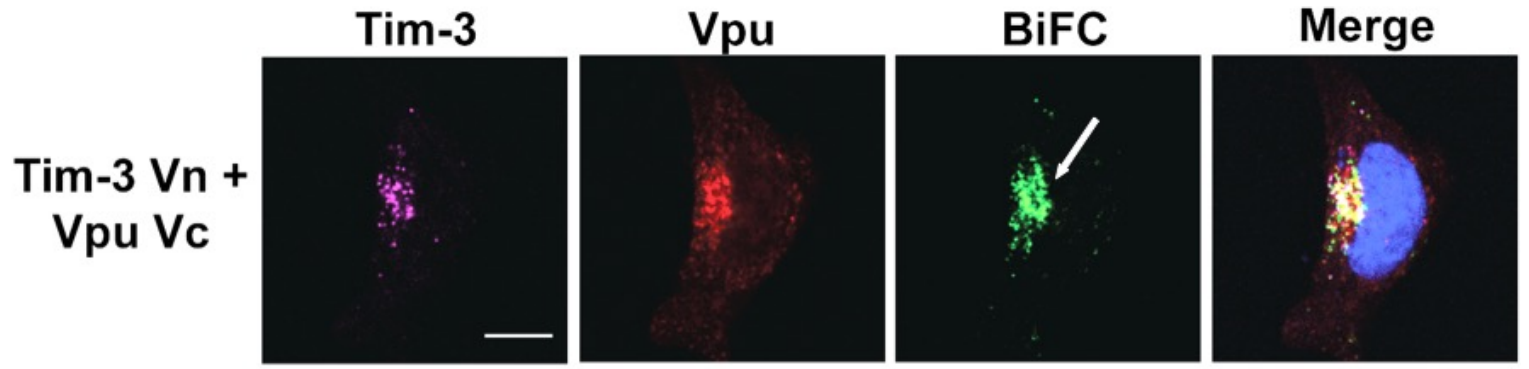
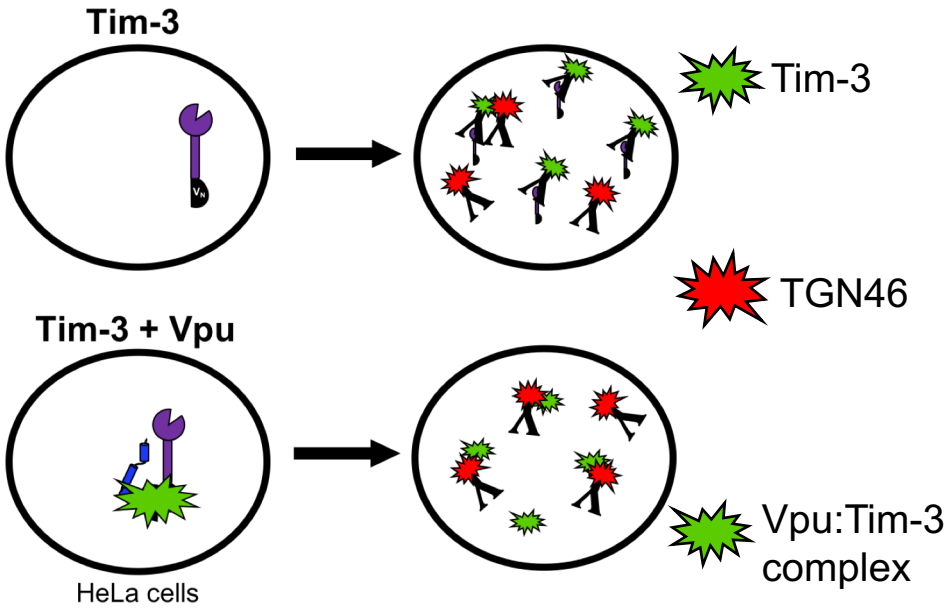


Fig 2. Tim-3-FLAG-Vn and Vpu-Vc were co-transfected into CD4⁺ HeLa cells. Cells were fixed, permeabilized, stained with anti-FLAG antibody (magenta) and Vpu antiserum (red), and imaged at 63x magnification using a confocal microscope with settings optimized for each fluorescent signal. Cells were mounted on ProLong Diamond antifade mountant with DAPI for nuclear staining (blue). BiFC appears green. Representative images from 5 independent experiments are shown. Scale bar is 10 μm.

Results: Vpu induces the sequestration of Tim-3 within the TGN



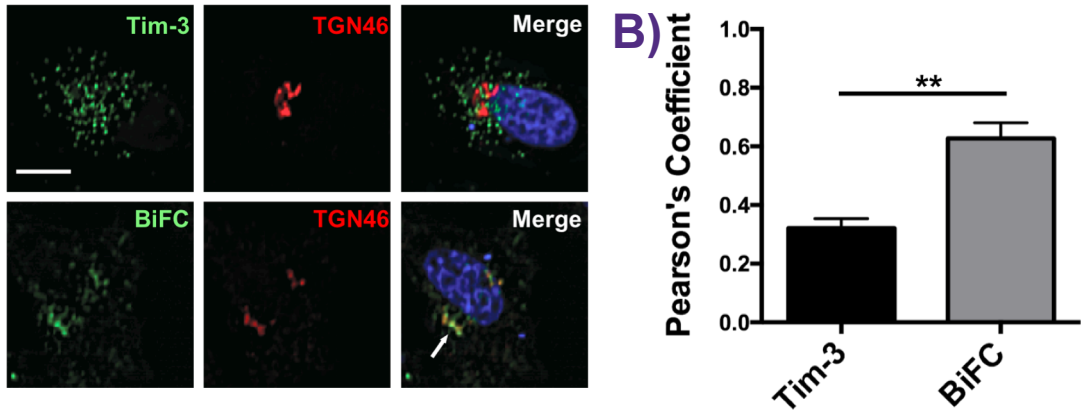
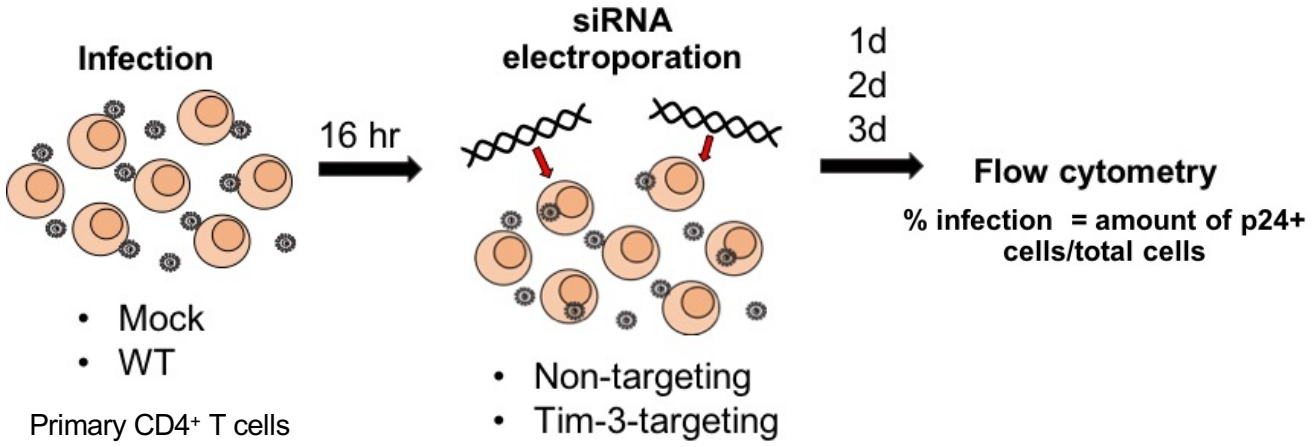


Fig 3. Tim-3-FLAG-VN was transfected into CD4⁺ HeLa cells alone or was cotransfected with Vpu-VC. Cells were fixed, permeabilized, and stained with anti-FLAG and anti-TGN46 antibodies. Cells were subsequently imaged using a wide-field microscope. BiFC appears green, FLAG staining was imaged under the far-red channel (modified postimaging to appear green), and staining of the TGN appears red. Cells were mounted on DAPI-Fluoromount G for nuclear staining (blue).

(A) Representative images from 3 independent experiments are shown. Scale bar is 10 μm. (B) Colocalization of Tim-3 or BiFC fluorescence signals (green) with TGN46 fluorescence (red) was quantified using the JACoP plug-in in ImageJ. Results are presented as mean Pearson's coefficients ± SEM. A two-tailed paired t test was used to evaluate differences in colocalization between Tim-3 alone and Tim-3 and Vpu with TGN46. Over 30 cells were quantified over 3 independent experiments. (**, P<0.01).

Results: Tim-3 knockdown increases HIV infection



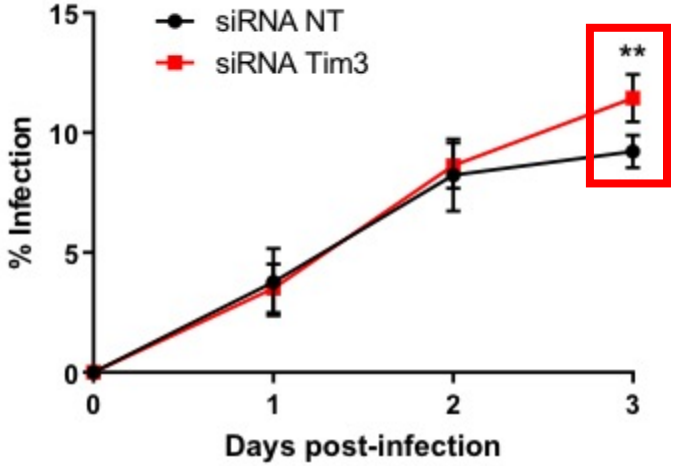
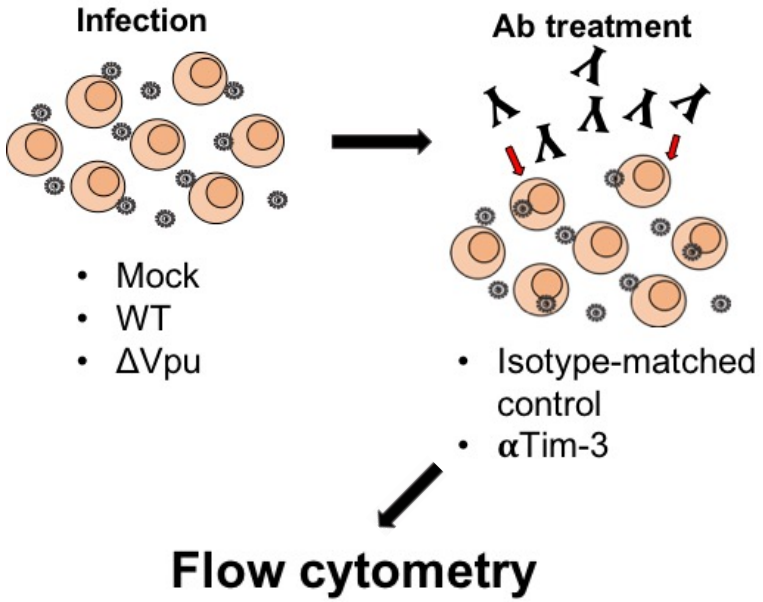


Fig 4. Primary CD4+ T cells were mock infected or infected with CH58 WT. At 16h post-infection, infected cells were electroporated with siRNAs targeting Tim-3 mRNA or nontargeting (NT) siRNAs. The percentage of infected (p24+) cells was evaluated at 0 h, 24 h, 48 h, and 72 h post-infection using flow cytometry. These data were obtained in 5 independent experiments using cells from 5 different HIV-negative donors. Error bars indicate means ± SEM. Statistical significance was tested using a paired t test (**, P<0.01).

Results: Blocking Tim-3:PtdSer binding increases HIV-1 infection



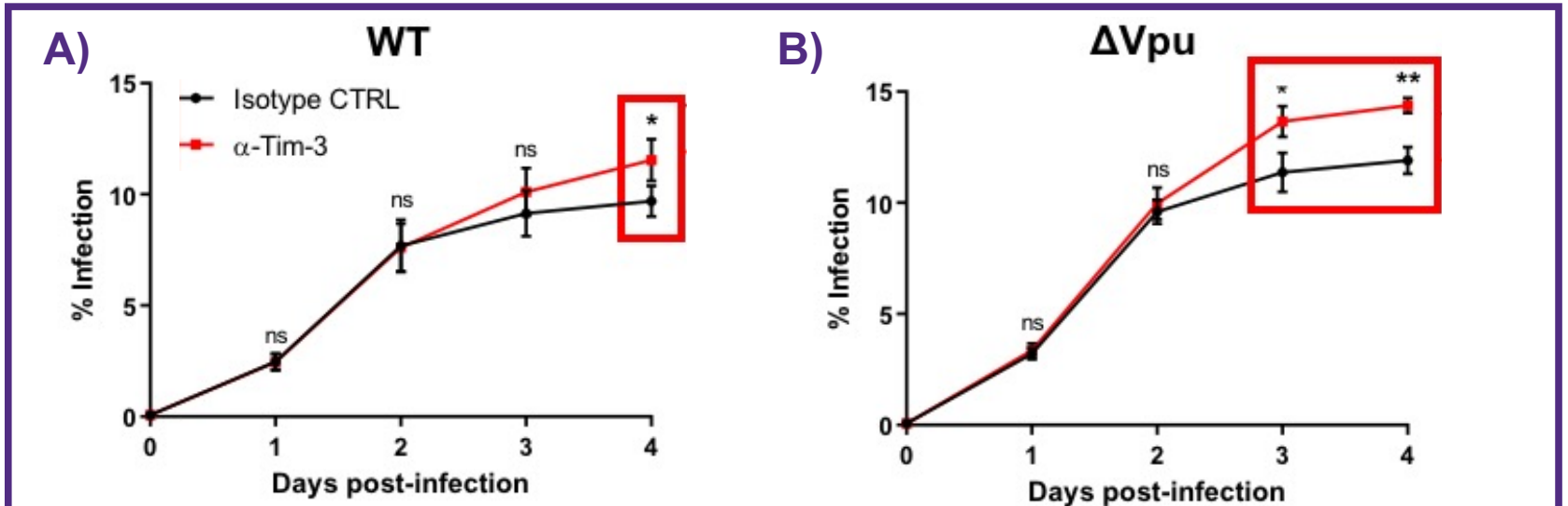


Fig 5. Primary CD4+ T cells were infected with CH58 viruses, either WT (A) or defective for Vpu expression (ΔVpu; B). Following spin infection, cells were treated with a Tim-3-blocking antibody or its matched IgG isotype control .The percentage of infected (p24+) cells was evaluated at 0 h, 24 h, 48 h, 72 h, and 96 h post-infection. These data were obtained in 5 independent experiments using cells from 5 different HIV-negative donors. Error bars indicate means ± SEM. Statistical significance was tested using a paired t test (*, P<0.05; **, P<0.01; ns, nonsignificant).

Conclusions

- Vpu downregulates cell surface levels of Tim-3 on infected $CD4^+$ T cells
- The Vpu transmembrane domain is critical for Tim-3 downregulation
- Vpu and Tim-3 form a complex within cells
- Tim-3 is increasingly localized to the TGN in the presence of Vpu
- Tim-3 inhibits HIV-1 infection and this is, in part, dependent on its ability to bind PtsSer

Significance

- This research provides valuable insight into the mechanisms used by HIV-1 to counter host restriction factors
- As Tim-3 inhibits viral replication and this is counteracted by Vpu, blocking this interaction may be a novel therapeutic target

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