A previously unidentified residue within the dileucine motif is required for HIV-1 Nef antagonism of the SERINC5 restriction factor

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Background

Background

- **H**uman **I**mmunodeficiency **V**irus Type **1** (HIV-1) is a global health burden affecting 37 million individuals.
- A major factor contributing to HIV-1 persistence within the human population is its ability to evade both innate and adaptive branches of the host immune system.
- The recently identified host restriction factor **Ser**ine **Inc**orporator **5** (SERINC5) potently restricts HIV-1 virion infectivity.
- SERINC5 mediates its anti-viral functions by readily incorporating into the host-derived outer lipid membrane of egressing virions (Figure 1).
- The HIV-1 accessory protein Nef counters this selective pressure by triggering the endocytosis and internalization of SERINC5 from the cell surface.
- Absence of SERINC5 on the cell surface restores virion infectivity during egress.
- The functional motifs implicated in Nef-mediated SERINC5 antagonism, thus far, are additionally required by Nef to downregulate the **C**luster of **D**ifferentiation **4** (CD4) cell surface receptor.
- Nef is known to "link" SERINC5 and CD4 to the endocytic network by engaging the **A**daptor **P**rotein **2** (AP-2) complex.

Methods

- We sought to characterize how two primary Nef isolates acquired from acutely HIV-1 infected women in Zimbabwe, termed 2410 and 2391, function to antagonize the SERINC5 restriction factor.
- Novel flow cytometry-based Nef-mediated CD4 and SERINC5 downregulation assays were designed.

Preliminary Results

- Isolates 2410 and 2391 both robustly downregulated CD4 from the cell surface.
- Isolate 2410, but not 2391, retained the ability to downregulate SERINC5 from the cell surface.
- Isolate 2391 preserves all motifs required for CD4 downregulation, suggesting a novel Nef functional motif is required for SERINC5 antagonism.

Model for SERINC5-mediated HIV-1 restriction

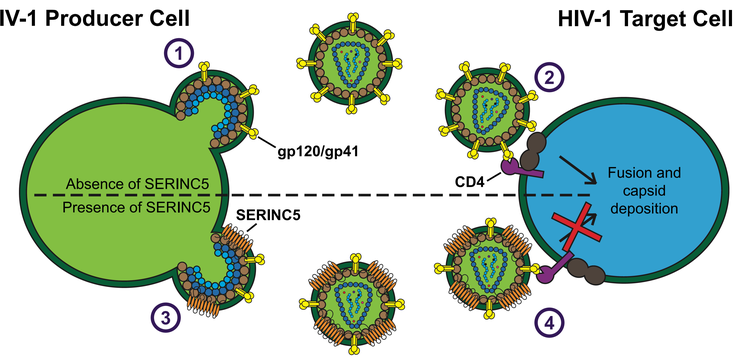
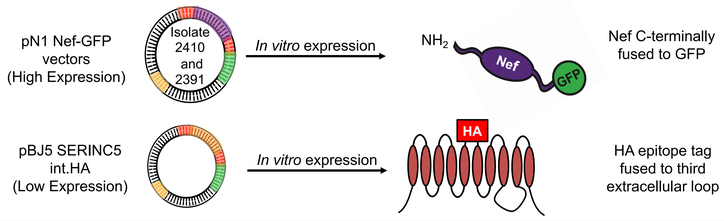


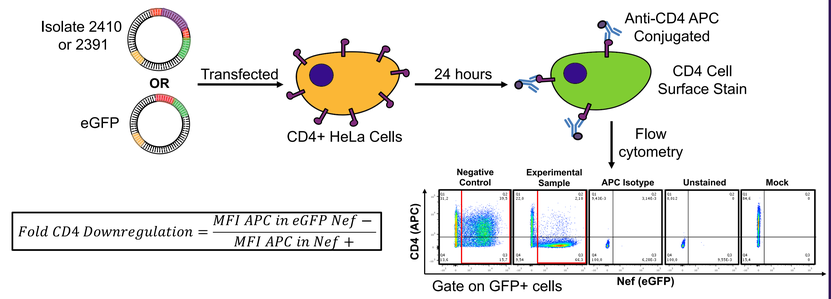
Fig 1. SERINC5 potently restricts HIV-1 virion infectivity. (**1** and **2**) In the absence of SERINC5, (**1**) HIV-1 virions acquire the host-derived outer lipid membrane during viral egress. (**2**) Target cells are infected by engaging CD4. Following membrane fusion, the viral capsid is deposited in the host cytosol, thereby permitting viral replication. (**3**-**4**) In the presence of SERINC5, (**3**) SERINC5 is readily incorporated into egressing virions. (**4**) Virions infect target cells by engaging the CD4. The presence of SERINC5 inhibits viral fusion by increasing the energy barrier required for fusion pore enlargement, thus effectively inhibiting downstream viral replication.

Experimental Tools



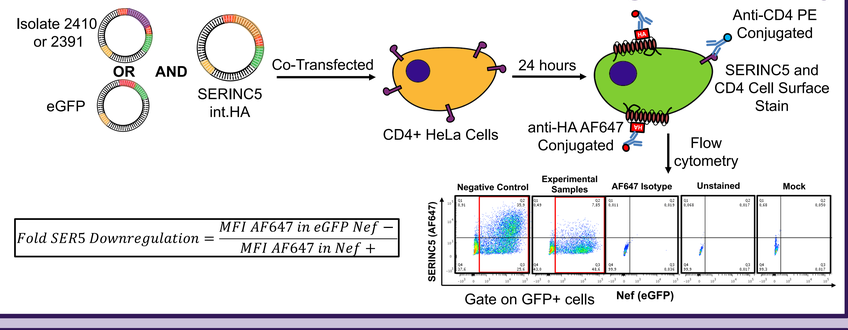
Methods

Nef-mediated cell-surface CD4 Downregulation Assay



$$Fold\ CD4\ Downregulation = \frac{MFI\ APC\ in\ eGFP\ Nef\ -}{MFI\ APC\ in\ Nef\ +}$$

Nef-mediated cell-surface SERINC5 Downregulation Assay



$$Fold\ SER5\ Downregulation = \frac{MFI\ AF647\ in\ eGFP\ Nef\ -}{MFI\ AF647\ in\ Nef\ +}$$

Results

1. Swapping N-terminal Nef regions does not significantly alter CD4 or SERINC5 downregulation ability

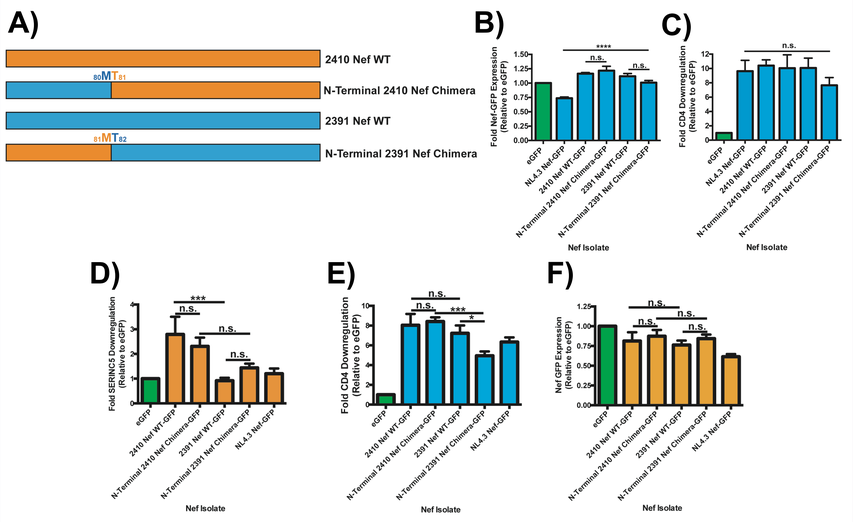


Fig 2. (**A**) Scheme of Nef chimeras. (**B** and **C**) CD4 downregulation assay. CD4+ HeLa cells expressing Nef isolates were surface stained for CD4, analyzed by flow cytometry and then quantified for Nef expression levels (**B**) and Nef-mediated cell-surface CD4 downregulation (**C**). (**D** to **F**) SERINC5 downregulation assay. CD4+ HeLa cells expressing Nef isolates and SERINC5 were surface stained for CD4 and SERINC5, analyzed by flow cytometry, and the extent of Nef-mediated downregulation of SERINC5 (**D**) and CD4 (**E**), as well as Nef expression levels *in vitro* (**F**) were quantified. (WT; wildtype, n.s.; non-significant, * p < 0.05, *** p < 0.001, **** p < 0.0001, n=3)

2. Nef residues 151-174 are required for SERINC5 downregulation

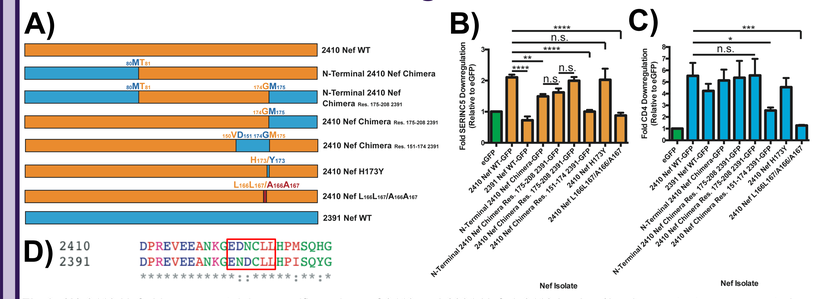


Fig 3. (**A**) 2410 Nef chimeras containing specific regions of 2410 and 2391 Nef. A 2410 $L_{166}L_{167}/A_{166}A_{167}$ mutant was generated as a negative control for CD4 and SERINC5 cell-surface downregulation. (**B** and **C**) SERINC5 and CD4 downregulation assay. CD4+ HeLa cells expressing Nef isolates/mutants and SERINC5 were surface stained for CD4 and SERINC5. Flow cytometry analysis then quantified downregulation of cell surface SERINC5 (**B**) and CD4 (**C**). (**D**) Amino acid sequence alignment comparing residues 151-174 of Nef between isolates 2410 and 2391. Red box indicates Nef dileucine motif. (Res; residue, WT: wildtype, n.s.; non-significant, * p <0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001, n=3)

3. Nef residue D_{164} abrogates SERINC5 downregulation

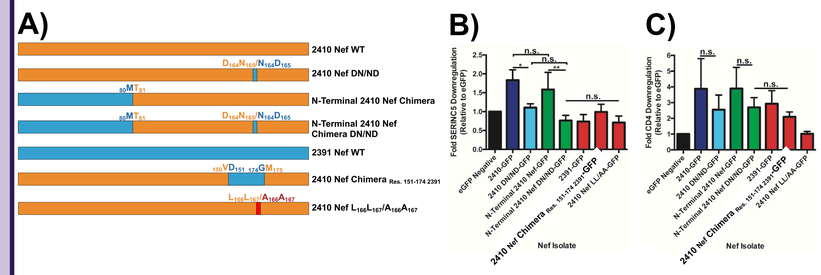


Fig 4. (**A**) Scheme of 2410 Nef chimeras. A 2410 $L_{164}L_{165}/A_{164}A_{165}$ Nef mutant was generated as a negative control for cell-surface CD4 and SERINC5 downregulation. (**B** and **C**) SERINC5 and CD4 downregulation assay in CD4+ HeLa cells as described previously. (Res; residue, WT; wildtype, n.s; non-significant, * p < 0.05, ** p < 0.01, n=3)

Future Directions

- Isolates 2410 and 2391 (along with respective 2410 chimeras/mutants) will be cloned into our proviral pNL4.3 ΔGag/Pol eGFP Nef retroviral vectors
- Infectivity of purified viral particles will be assessed by infecting TZM-bl cells expressing a luciferase reporter under the transcriptional regulation of the HIV-1 LTR.

Conclusions and Significance

- Swapping the N-terminus of 2410 with that of 2391 did not significantly impact cell-surface SERINC5 or CD4 downregulation.
- 2410 Nef Chimera $_{\text{Res. 151-174 2391}}$ suffered impairments in CD4 downregulation, suggesting 2391 Nef must utilize additional motifs to compensate for CD4 downregulation.
- D_{164} within the Nef dileucine motif impairs cell-surface SERINC5 and CD4 downregulation ability.

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