0194: NEF DIMERIZATION REGULATES HIV-1 INFECTIVITY AND SERINC5 INCORPORATION

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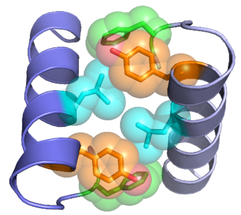
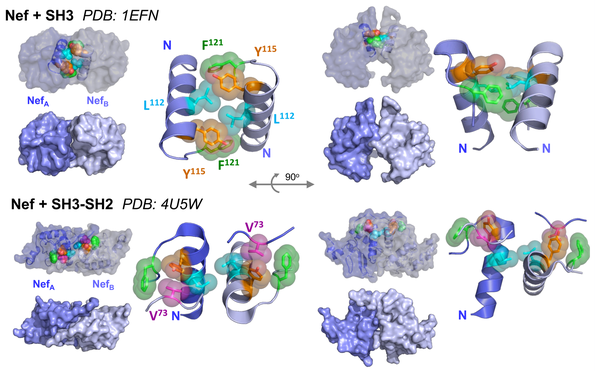
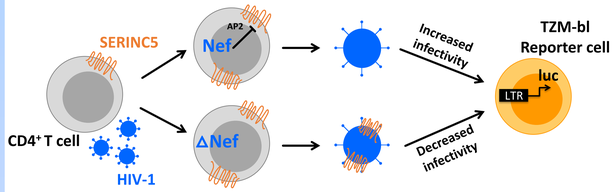


Conserved amino acids important for Nef homodimerization and function



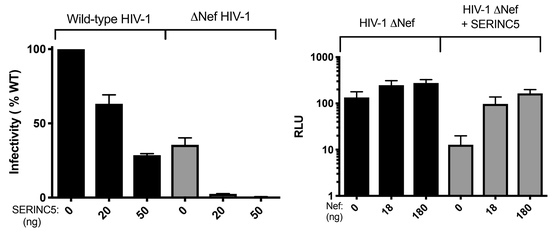
- Nef is an HIV-1 accessory factor critical for viral infectivity replication, immune evasion and AIDS progression
- Nef lacks intrinsic biochemical functions and hijacks host cell signaling and trafficking pathways for the benefit of the virus
- Partner protein and small molecule binding induces Nef homodimerization in multiple X-ray crystal structures
- Conserved Nef residues essential for stable dimer formation include Leu112, Tyr115, and Phe121
- Mutation of these residues disrupts Nef self-association in cells, inhibits Nef enhancement of viral replication, and reverses Nef-mediated downregulation of CD4 and MHC-I

HIV-1 restriction by SERINC5 is antagonized by Nef via downregulation in producer cells



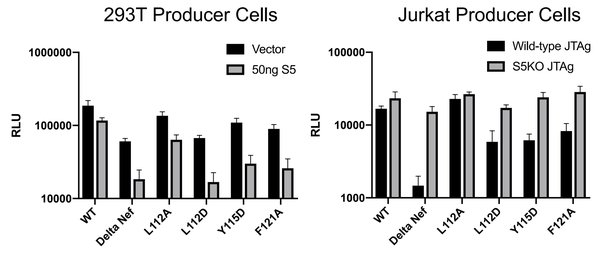
- SERINC5 is a multi-pass transmembrane protein expressed in CD4+ T cells and other host cells for HIV-1
- Incorporation of SERINC5 into the membrane of newly synthesized virions suppresses their infectivity
- Nef counteracts SERINC5 by inducing downregulation of the restriction factor from producer cell membranes to prevent incorporation into budding virions
- Nef antagonism of SERINC5 occurs via an AP-2 endocytic pathway mechanistically similar to Nef-mediated downregulation of CD4

Nef-defective HIV-1 is more sensitive to SERINC5 antagonism of Infectivity



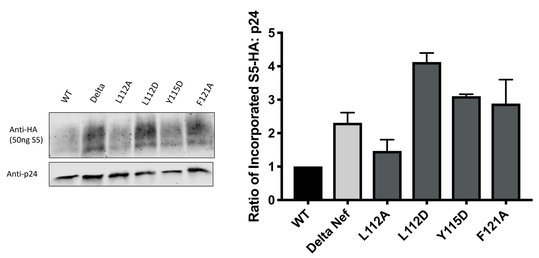
Expression of SERINC5 in HIV-1 producer cells impairs viral infectivity. *Left:* 293T cells were co-transfected with HIV-1NL4-3 proviral DNA (wild-type or ΔNef) along with 0, 20, or 50 ng of SERINC5-HA. Viral supernatants were collected 48 h later, quantified by p24 AlphaLISA, and equivalent p24 titers were used to infect TZM-bl reporter cells. Luciferase activity was normalized to the wild-type control in the absence of SERINC5, and is shown as mean percent control value ± SE. *Right:* Ectopic Nef expression rescues SERINC5 inhibition of HIV-1 ΔNef infectivity. HIV-1 ΔNef was produced in transfected 293T cells in the presence or absence of SERINC5 and Nef expression vectors as indicated. Infectivity of the resulting viral supernatants was measured following infection of TZM-bl cells with equivalent p24 inputs. Results are shown as relative luciferase units (RLU) ± SE (log scale).

Nef dimerization mutants impair HIV-1 infectivity in a SERINC5-dependent manner



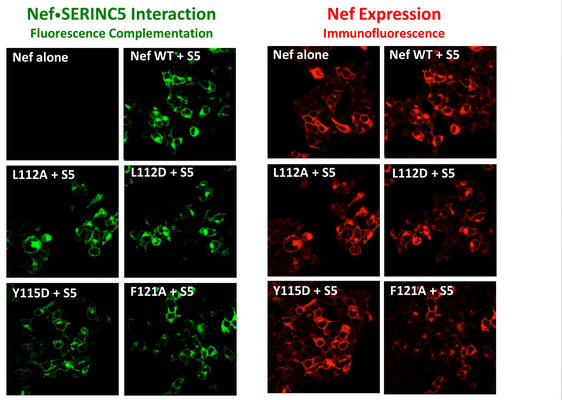
Reduced infectivity of HIV-1 expressing homodimerization-defective mutants of Nef. These experiments used a chimeric form of HIV-1_{NL4-3} expressing wild-type Nef_{SF2}, the dimerization-defective Nef_{SF2} mutants (L112A, L112D, Y115D, F121A) or no Nef (ΔNef). *Left*: Viruses were produced in 293T cells transfected with each provirus in the presence or absence of a SERINC5 expression plasmid. Viral supernatants were quantified via p24 AlphaLISA, and equal p24 titers were used to infect TZM-bl reporter cells. Luciferase activity was measured 48 h later, and results are shown as relative luciferase units (RLU) ± SD. *Right*: Jurkat TAg cells (parent and SERINC5 knock-out; kind gift from Massimo Pizzato) were transfected with the same panel of wild-type and Nef mutant proviruses. Viral supernatants were harvested 72 h later, equivalent p24 titers were used to infect TZM-bl cells, and luciferase activity is reported as per above.

Mutation of Nef Dimer Interface Residues Increases Virion SERINC5 Incorporation



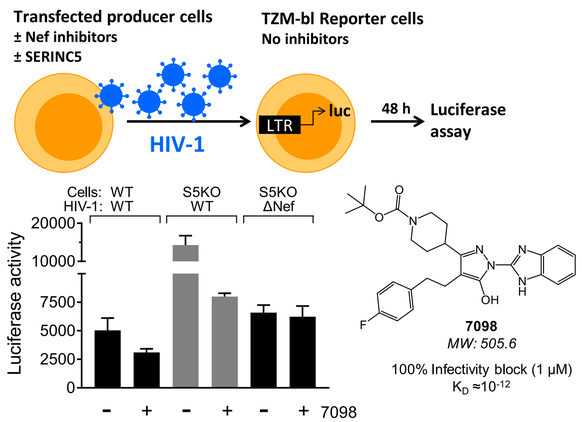
HIV-1 mutants expressing dimerization-defective Nef exhibit impaired SERINC5 exclusion from newly synthesized virions. These experiments used a chimeric form of HIV-1_{NL4-3} expressing wild-type Nef_{SF2}, the dimerization-defective Nef_{SF2} mutants (L112A, L112D, Y115D, F121A) or no Nef (ΔNef). Viruses were produced in 293T cells transfected with each proviral clone and in the presence of a SERINC5-HA expression plasmid (50 ng) over 48 h. Virions were isolated via ultracentrifugation on 20% sucrose cushions, followed by lysis in SDS-PAGE sample buffer supplemented with 50 mM TCEP. Viral proteins were separated via SDS-PAGE and immunoblotted to detect incorporated SERINC5 (via the HA tag) or the HIV-1 capsid protein (p24) as a loading control. Immunoreactive bands were detected and quantified using the Li-Cor infrared imaging system, and SERINC5 signal intensities were normalized to p24 levels. *Left:* Representative immunoblot from one of two independent experiments. *Right:* average SERINC5:p24 ratio from both experiments ± SE.

Nef Dimerization Defective Mutants Interact with SERINC5 in Cells



Dimerization-defective Nef mutants interact with SERINC5 in cells. Bimolecular fluorescence complementation (BiFC) was used to examine Nef interaction with SERINC5 in a cell-based format. Wild-type and mutant forms of Nef were fused to an N-terminal fragment of the YFP variant Venus (Nef-VN) and co-expressed with SERINC5 fused to a complementary C-terminal Venus fragment (S5-VC; kind gift of Yong-Hui Zheng) in 293T cells for 24 h. Cells were fixed, permeabilized, immunostained for Nef, and then imaged on a Olympus FV1000 confocal microscope. Panels above show the fluorescent signal arising from Nef-SERINC5 interaction (BiFC) and Nef expression via immunofluorescence.

Nef Inhibitor 7098 Inhibits Infectivity at the Producer Cell Level



Treatment of Jurkat producer cells with hydroxypyrazole Nef inhibitor 7098 reduces infectivity in a Nef-dependent manner. Wild-type (WT) and SERINC5-knockout (S5KO) Jurkat cells producing WT or ΔNef HIV-1 were treated ± the hydroxypyrazole Nef inhibitor **7098** (100 nM). TZM-bl reporter cells were infected with equivalent p24 titers of newly synthesized virions and luciferase activity was assayed 48 h later; data are expressed as mean luciferase activity ± SE. No cytotoxicity was observed at this inhibitor concentration. For more information on hydroxypyrazole Nef inhibitors, see Poster #0191.

Summary and Conclusions

- Wild-type HIV-1 but not the ΔNef mutant overcomes SERINC5 restriction of viral infectivity
- Mutation of conserved Nef residues essential for homo-dimerization (L112, Y115, F121) reduces Nef enhancement of infectivity from both 293T and Jurkat producer cells in a SERINC5 dependent manner
- SERINC5 incorporation into virions is increased in the presence of Nef dimer interface mutants.
- Nef dimer interface mutants express within cells and interact directly with SERINC5.
- Treatment of Jurkat cells with a hydroxypyrazole Nef inhibitor impairs infectivity at the producer cell level, suggesting an inhibitor effect on dimerization.

References

Cell-based Fluorescence Complementation Reveals a Role for HIV-1 Nef Protein Dimerization in AP-2 Adaptor Recruitment and CD4 Co-receptor Down-regulation. *J Biol Chem* 292, 2670-2678, 2017

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HIV-1 Nef promotes infection by excluding SERINC5 from virion incorporation. *Nature 526, 212-7, 2015*