

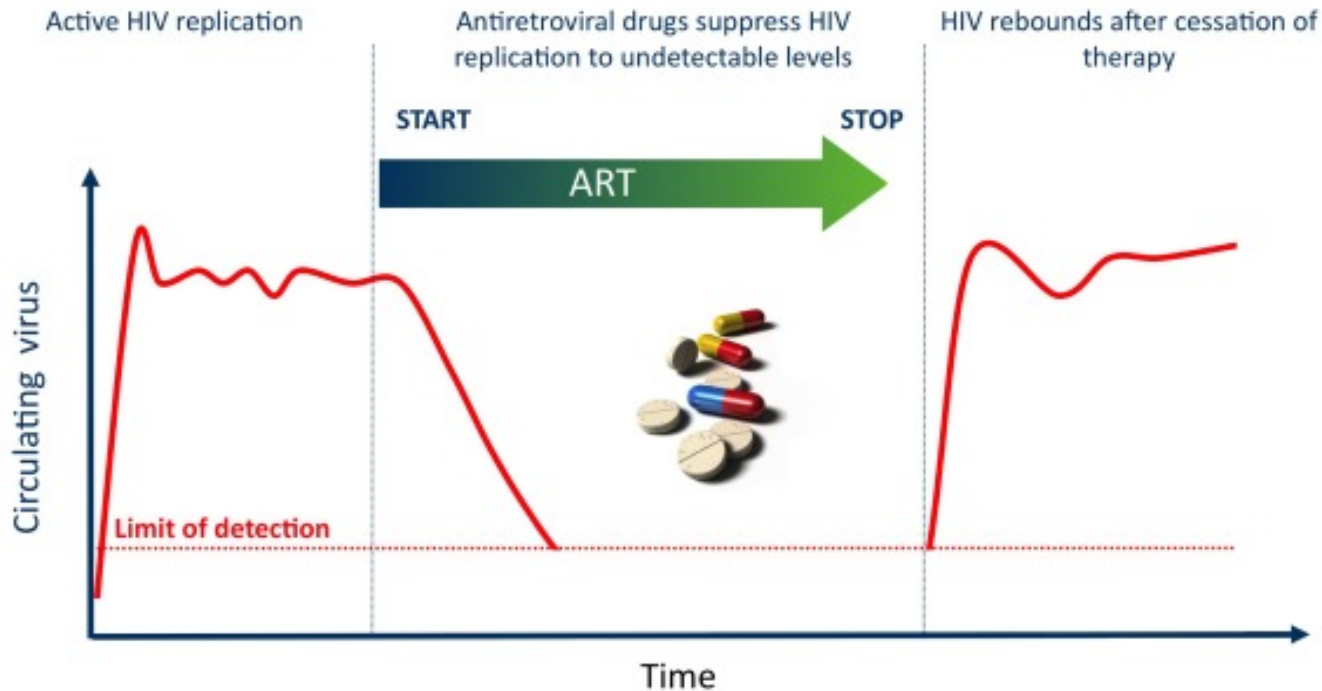


Investigating the role of HIV-1 Nef-mediated exosome modulation during viral reactivation from latency

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HIV persists in latent reservoirs



Kulpa et al., 2015, J Virus Erad.

- ❖ HIV ability to establish a latent reservoir remains one of the greatest challenges towards cure.
- ❖ Several strategies to eliminate viral reservoirs have been developed but none has been fully effective.
- ❖ A better understanding of factors that contribute to HIV latency and reactivation is thus required to improve clinical strategies to eliminate viral reservoirs.

Nef containing exosomes and viral reactivation from latency

- ❖ **HIV-1 Nef promotes the formation of exosomes that contribute to autocrine and paracrine cell signaling.**
- ❖ **Exosomes containing Nef and cellular ADAM17 can trigger CD4 T cell activation, leading to secretion of TNF- α and HIV reactivation in cell culture; however, Nef's role in modulating latency remains understudied.**
- ❖ **Nef is highly diverse, whether naturally arising Nef alleles differs for their ability to mediate viral reactivation from latency remain unexplored.**
- ❖ **Here, we examined Nef alleles for differential ability to mediate latency reactivation.**

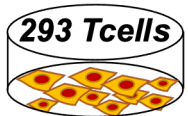
Approach

Exosome generation

pSelect-Nef



Transfection



72 hrs incubation

Collect supernatant

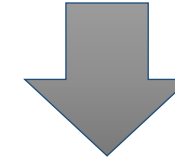


Exosome Isolation and quantification



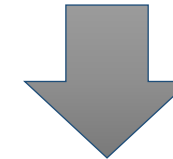
- Exosome Isolation with Invitrogen kit
- Quantification then done with Acetylcholinesterase activity (AChE) kit, Sigma

Exosome containing Nef



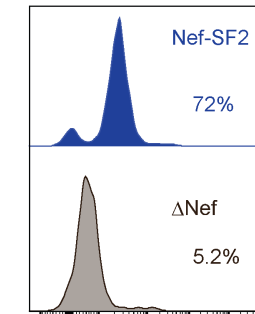
Treatment onto latent cells

HIV-infected CEM T cell clone with single integrated provirus lacking Nef (C-Lat-Nef-KO)



24hrs incubation

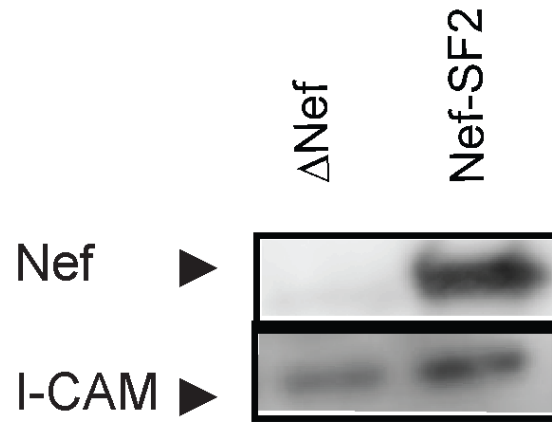
Flow cytometry to detect Gag-p24



Gag-p24 expressing cells

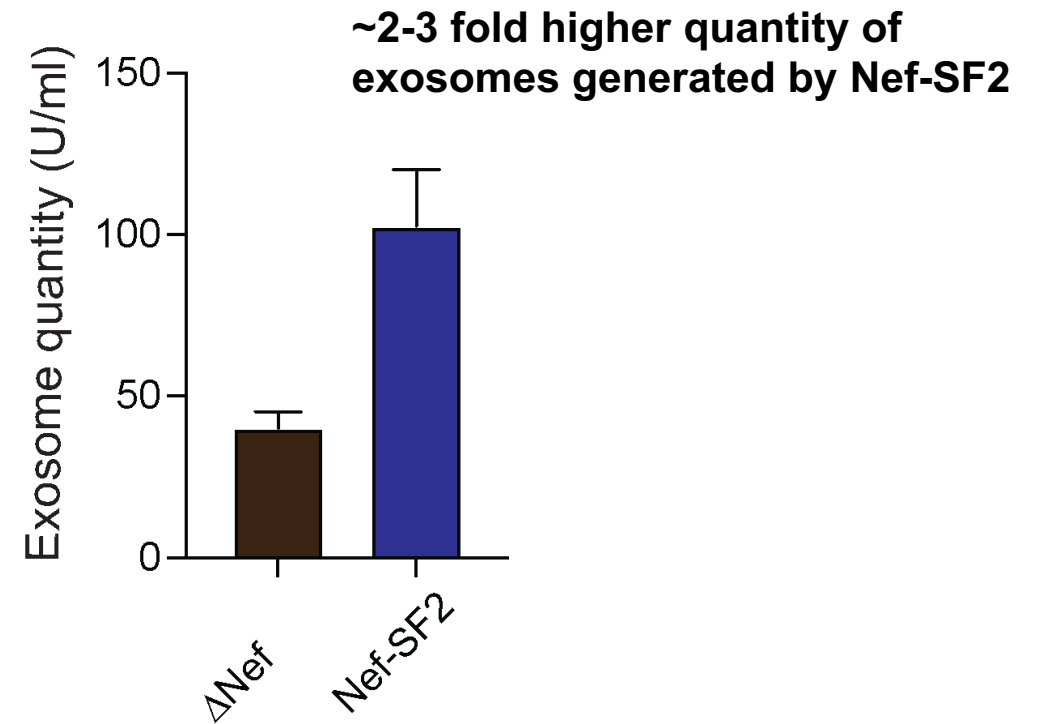
1. Exosome isolation and quantification validation

➤ Nef expression in exosomes



❖ Nef can be detected in our exosome preparation

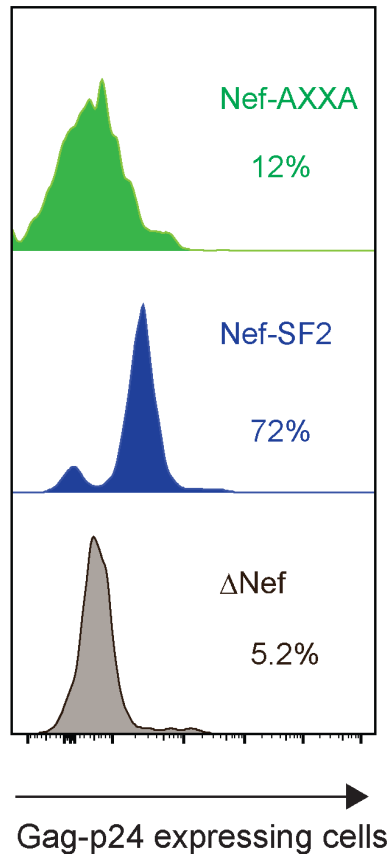
➤ Quantification with Acetylcholinesterase activity (AChE) kit, Sigma



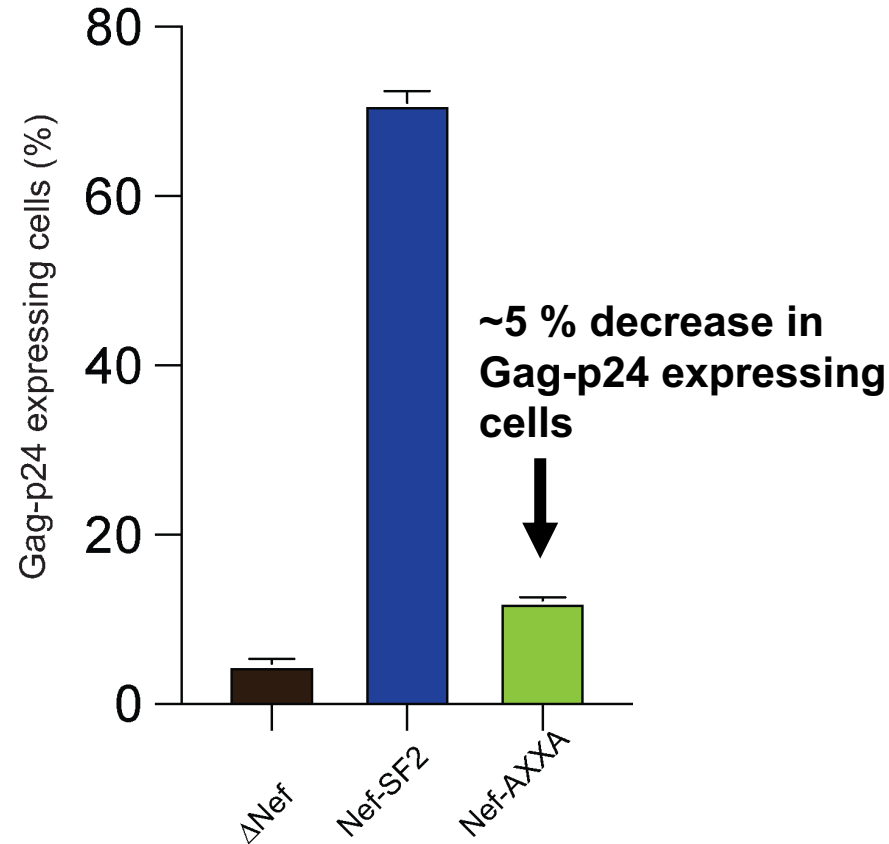
❖ Quantification of exosomes echoes previous observation

2. Exosomal Nef ability to mediate viral reactivation from latency validated

A



B



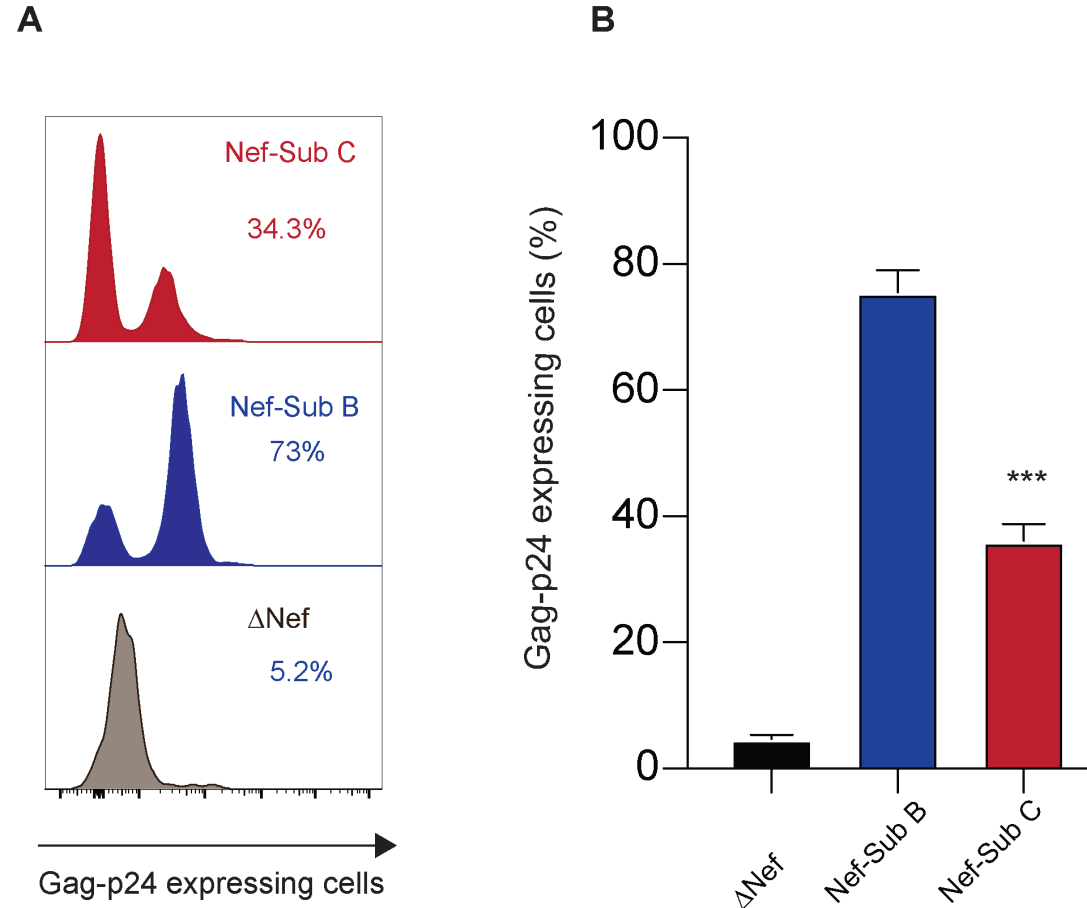
➤ Nef-AXXA is impaired in its ability to mediate ADAM17 activation

✓ 4000 U/ml of exosome quantity used

❖ Nef-AXXA ability to mediate reactivation from latency correlates with its impaired ability to activate ADAM17

3. Exosomal Nef ability to mediate reactivation from latency may differ across viral subtypes

- Two Nef isolates from major HIV-1 pandemic subtypes were analyzed, 4000 U/ml of exosome quantity used



❖ **Nef-subtype B isolate containing exosomes displays significantly higher ability to mediate reactivation from latency compared Nef-subtype C**

4. Summary and future prospects

- ❖ Exosomal Nef ability to mediate viral reactivation from latency differs among natural arising Nef alleles.
- ❖ A larger, more diverse panel of Nef alleles will be examined to cement our observation and possibly illuminate natural arising Nef polymorphisms/motifs that are critical for this function.
- ❖ This works highlight the novel role of Nef in modulating latency which have potential to improve clinical strategies in eliminating viral reservoirs.

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