Functional interplay between ACOT8 and Nef enhances HIV-1 infectivity and remodels viral envelope

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BACKGROUND

ACOT8, an acyl-CoA thioesterase localized to peroxisomes, contributes to intracellular lipid homeostasis by catalyzing the hydrolysis of acyl-CoA thioesters [1,2]. Recent evidence has identified ACOT8 as a host factor involved in the HIV-1 replication cycle, mediated in part by its interaction with Nef [3,4]. Nef is a multifunctional accessory protein that reprograms the host cellular environment to promote viral replication and immune evasion [5,6]. Because the lipid composition of the HIV-1 envelope is a key determinant of membrane biophysical properties, fusion competence, and viral infectivity [7], we hypothesized that ACOT8-dependent peroxisomal lipid metabolism may influence the lipidomic architecture of the viral envelope and, consequently, the infectious potential of HIV-1 particles. To investigate this hypothesis, we generated ACOT8 knockout cells and assessed the resulting changes in HIV-1 envelope lipid composition and viral infectivity.

MATERIALS AND METHODS

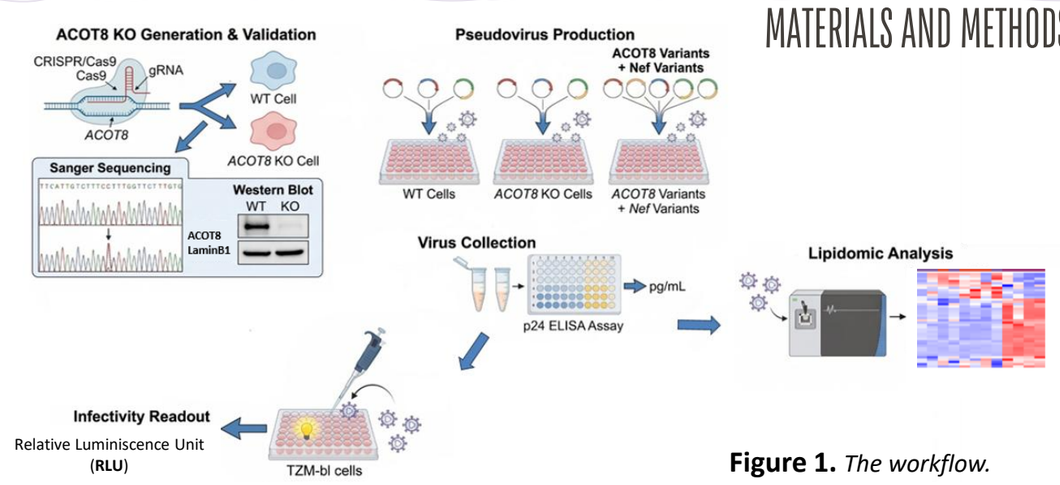

Figure 1. *The workflow.*

RESULTS

Figure 2. *ACOT8 is a host factor required for HIV-1 infectivity.*

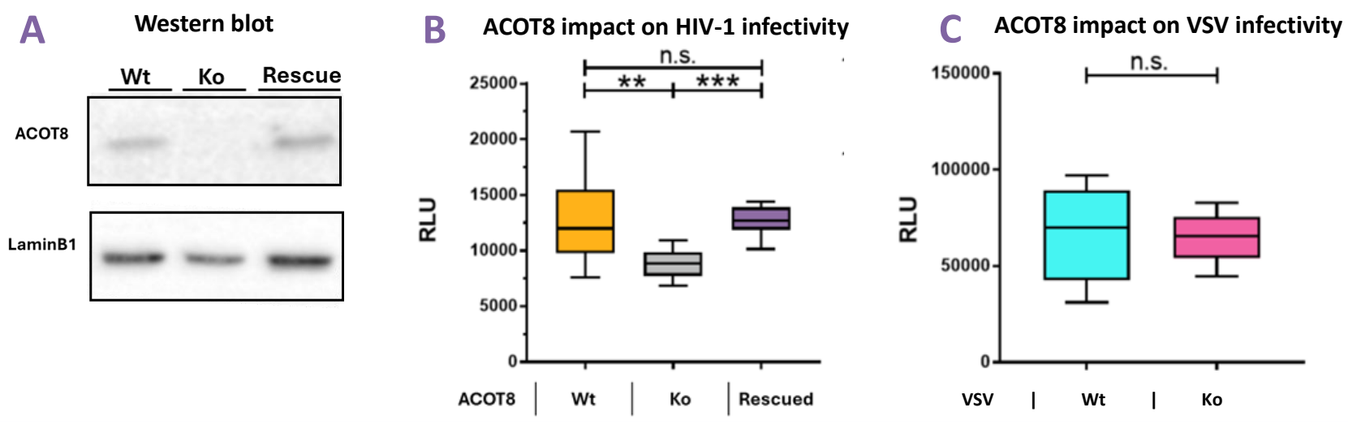

Figure 2. *ACOT8 depletion selectively impairs HIV-1 infectivity.*
(A) Western blot analysis validating the generation of ACOT8 knockout (Ko) cells and the rescue of ACOT8 expression. ACOT8 was detected in wild-type (Wt) cells, absent in Ko cells, and restored in rescued (R) cells. Lamin B1 was used as a loading control. (B) HIV-1 infectivity, measured as relative light units (RLU), in Wt, Ko, and R cells. ACOT8 knockout significantly reduced HIV-1 infectivity compared with Wt cells, whereas ACOT8 re-expression restored infectivity. (C) VSV infectivity was not altered by ACOT8 knockout, indicating that the proviral effect of ACOT8 is selective for HIV-1 and does not reflect a generalized antiviral state. Statistical analyses were performed using the Kruskal-Wallis test (B) and Mann-Whitney test (C).

Figure 3. *ACOT8 deficiency altered the lipid composition of the HIV-1 envelope.*

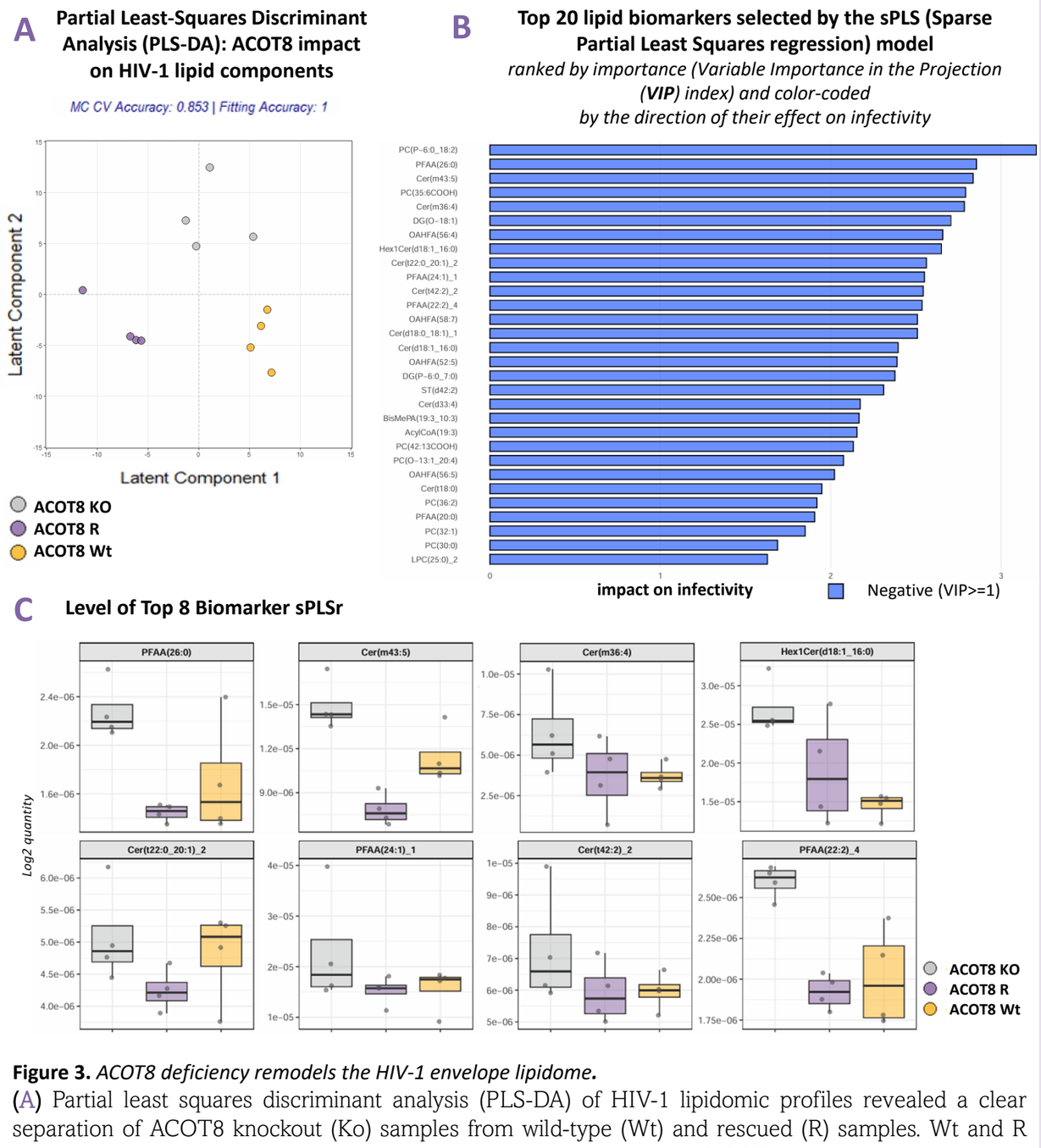

Figure 3. *ACOT8 deficiency remodels the HIV-1 envelope lipidome.*
(A) Partial least squares discriminant analysis (PLS-DA) of HIV-1 lipidomic profiles revealed a clear separation of ACOT8 knockout (Ko) samples from wild-type (Wt) and rescued (R) samples. Wt and R samples displayed a similar spatial distribution along latent component 2 (LC2), consistent with lipidomic rescue following ACOT8 re-expression. (B) Sparse PLS variable importance in projection (VIP) analysis of the ACOT8 Ko model identified lipid species negatively associated with infectivity and selectively enriched in Ko-derived virions. These features included peroxisome-associated fatty acids (PFAAs), ceramides (Cer), O-acyl-ω-hydroxy fatty acids (OAHFAs), and acyl-CoA intermediates, indicating substantial remodeling of the viral envelope lipidome upon ACOT8 loss. (C) Boxplot analysis of the top eight discriminant biomarkers confirmed the marked accumulation of very-long-chain fatty acids (VLCFAs) and ceramide species in Ko-derived HIV-1 particles, with restoration toward Wt levels in the R condition. These findings suggest that ACOT8 regulates the incorporation of peroxisome-derived lipid species into the HIV-1 envelope.

Figure 4. *The Nef–ACOT8 interaction promotes HIV-1 infectivity through selective envelope lipid remodeling.*

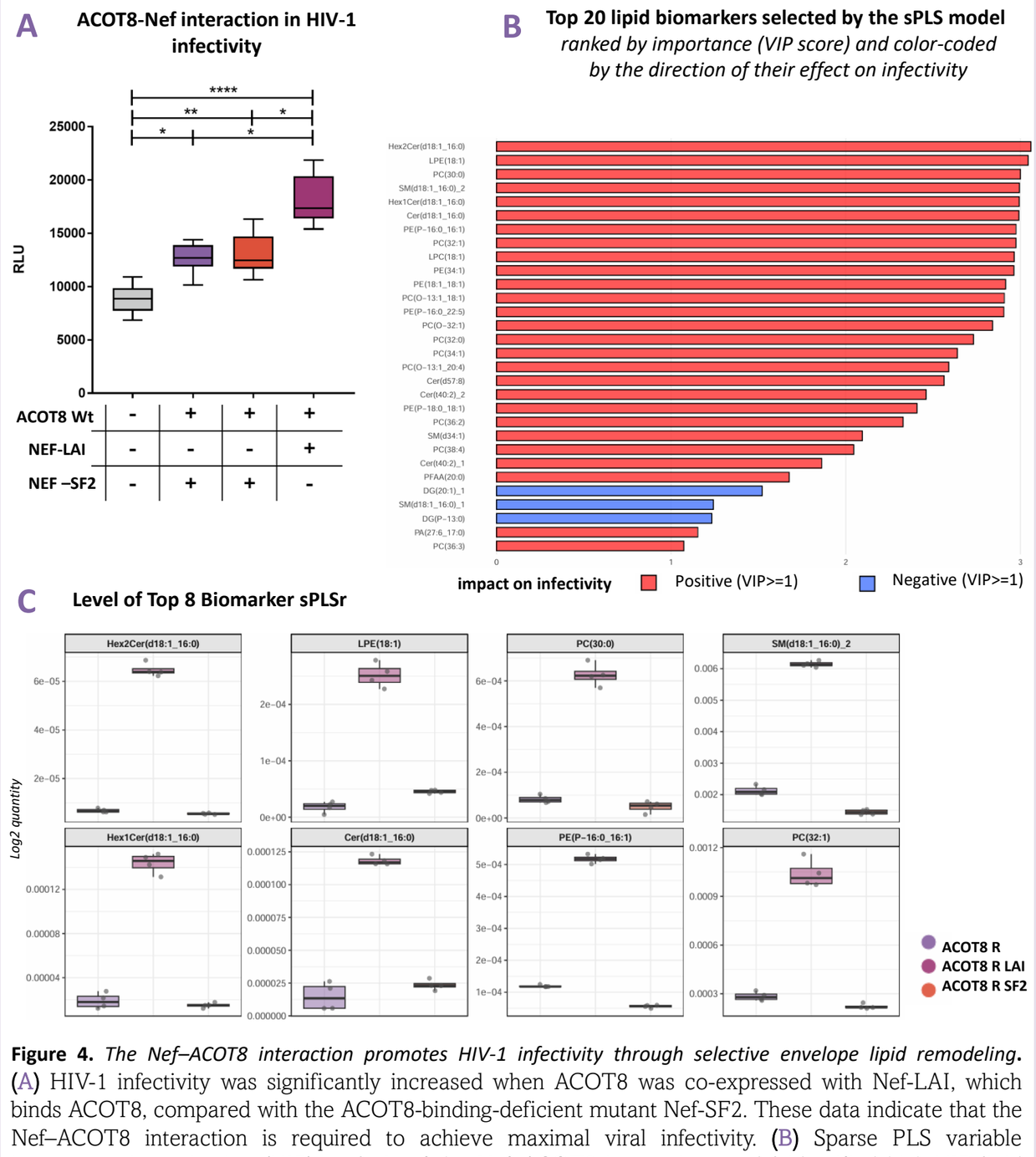

Figure 4. *The Nef–ACOT8 interaction promotes HIV-1 infectivity through selective envelope lipid remodeling.*
(A) HIV-1 infectivity was significantly increased when ACOT8 was co-expressed with Nef-LAI, which binds ACOT8, compared with the ACOT8-binding-deficient mutant Nef-SF2. These data indicate that the Nef–ACOT8 interaction is required to achieve maximal viral infectivity. (B) Sparse PLS variable importance in projection (VIP) analysis of the Nef–ACOT8 interaction model identified high-VIP lipid species predominantly positively associated with infectivity. These included phosphatidylcholines (PC), phosphatidylethanolamines (PE), sphingomyelins (SM), and hexosylceramides, which were enriched in the Nef-LAI condition, consistent with a lipid environment favorable to membrane fusion. A smaller subset of lipids negatively associated with infectivity, including diacylglycerols (DG) and selected SM species, was enriched in the low-infectivity Nef-SF2 condition. (C) Boxplot analysis of the top eight sPLSR biomarkers confirmed the selective enrichment of fusion-associated lipid species in ACOT8-rescued cells expressing Nef-LAI and their depletion in cells expressing Nef-SF2. Collectively, these findings link the Nef–ACOT8 interaction to defined remodeling of the HIV-1 envelope lipidome.

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CONCLUSIONS

- ACOT8 is required for optimal HIV-1 infectivity in an envelope-dependent manner, and re-expression of ACOT8 fully restores viral infectivity.
- The Nef–ACOT8 interaction [3,4] enhances HIV-1 infectivity, as virions produced in the presence of ACOT8-binding Nef-LAI display higher infectious capacity than those carrying the ACOT8-binding-deficient Nef-SF2 variant [5,6].
- ACOT8 regulates the HIV-1 envelope lipidome [1,2]. Loss of ACOT8 results in the accumulation of very-long-chain fatty acids and ceramide species, whereas ACOT8 re-expression restores the lipidomic profile toward wild-type conditions.
- The Nef–ACOT8 axis [3,4] promotes selective enrichment of fusogenic lipid species, including PC, PE, SM, and HexCer, within the viral envelope, supporting the formation of a fusion-competent membrane and thereby increasing HIV-1 infectivity [5,6].

These findings identify ACOT8 as a host lipid-metabolic factor exploited by HIV-1 Nef to remodel the viral envelope lipidome and enhance particle infectivity, highlighting the Nef–ACOT8 axis as a potential target for antiviral intervention [1–6].