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The absence of the human thioesterase ACOT8 negatively affects HIV-1 infectivity *in vitro*

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During the replicative cycle of HIV-1, a broad spectrum of interactions between viral and host proteins occurs. Among these, the thioesterase ACOT8 has been reported to interact with the HIV-1 Nef protein. Here we study the effect of ACOT8 depletion on HIV-1 infectivity.
CRISPR/Cas9 was used to knock-out ACOT8 gene in HEK293T cells. Knock-out was verified by Sanger sequencing and Western blot. Off-target analysis was performed by Cas-OFFinder web tool, and the most likely sites were amplified by PCR followed by Sanger sequencing. HIV-1 pseudotyped viruses were produced in wild type and ACOT8 knock-out HEK293T cells and titrated by HIV-1 p24 quantification. The same viral input was used to infect the TZM-bl cell line, allowing quantification of viral infectivity by Luciferase detection. A rescue experiment was performed by transfecting the knock-out cells with a plasmid encoding for ACOT8 to further verify the absence of off-target due to CRISPR/Cas9. The Mann-Whitney test was used to detect statistically significant differences between the viral infectivity in presence or absence of ACOT8.
HEK293T knock-out clones obtained exhibit non-in-frame deletions in the ACOT8 region. The absence of protein expression was verified by Western blot. Knock-out clones show no modifications in the predicted off-target sites.
HIV-1 pseudotyped viruses showed lower infectivity when produced in absence of ACOT8 than when produced in its presence (p-value<0.0001). In contrast, no significant differences were observed using the control envelope of VSV. Pseudotyped virus produced in knock-out cells in which ACOT8 expression was restored showed comparable levels of infectivity to virus produced in HEK293T wild type cells.
Our preliminary data suggest that ACOT8 absence in cells producing HIV-1 pseudotyped virus is associated with reduced viral infectivity. Further experiments are underway to characterize the mechanisms modulating infectivity and other factors involved in this interaction.