Interception of HIV-1 replication by membrane trafficking network proteins

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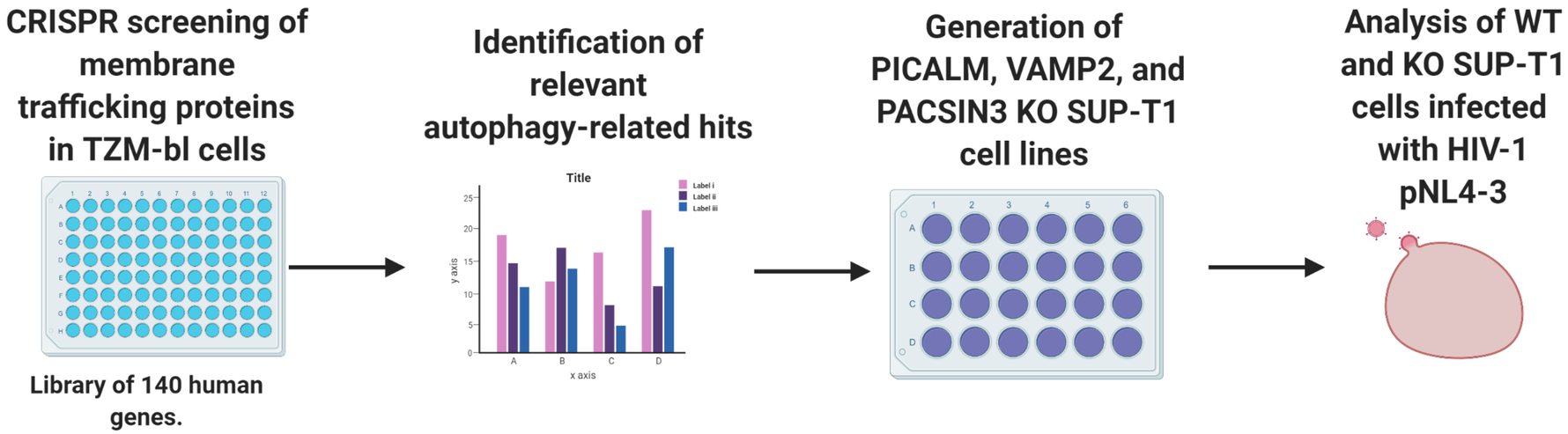
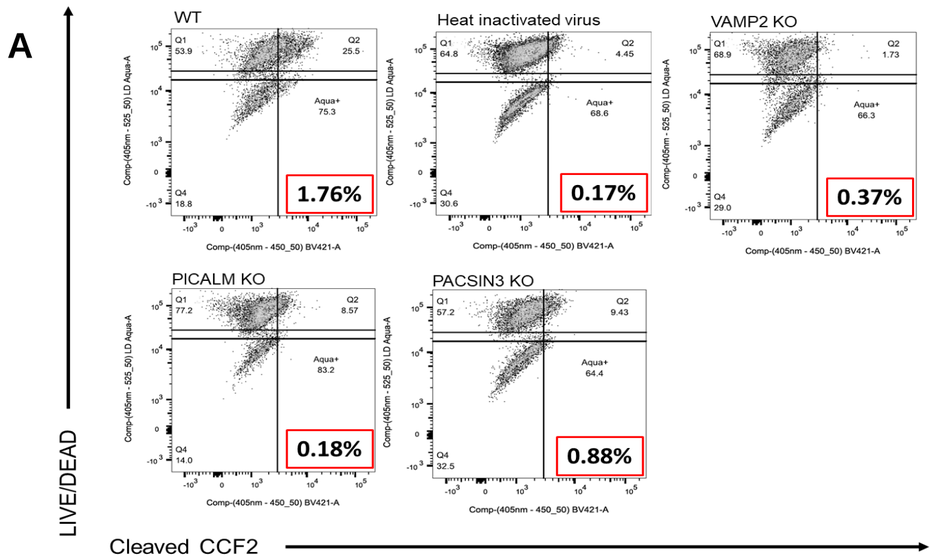
Introduction

- HIV-1 hijacks host proteins at multiple steps, including membrane trafficking proteins involved in autophagy.
- Only a few of these host proteins have been functionally characterized.
- To elucidate their functions, we performed a CRISPR-Cas9 screen of 140 membrane trafficking proteins.
- We identified three host autophagy-related genes (PICALM, VAMP2, and PACSIN3) whose knockout (KO) led to a significant decrease in HIV-1 infectivity.
- We edited these genes in SUP-T1 T cells to generate monoclonal KO cell lines to study their effects on HIV-1 infection.

Methodology



KO cell lines inhibit viral entry and internalization of CD4



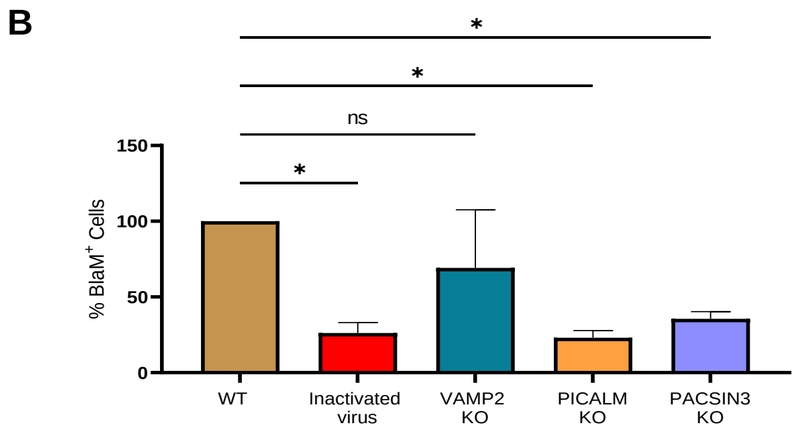
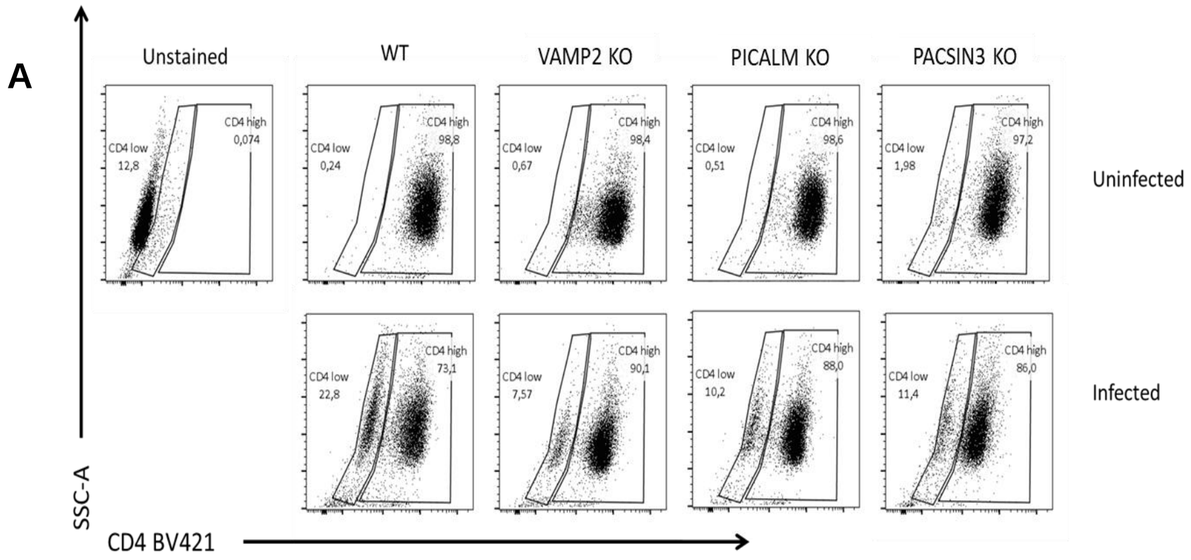


Figure 1. Effects of KOs on HIV-1 entry in human SUP-T1 T cells. (A) Sup-T1 cells were infected with heat treated or non-treated HIV-1 NL4.3 virions containing BlaM-Vpr at an MOI of 1. The fusion assay was analyzed by flow cytometry using a violet laser to excite CCF2. Percentages in each panel are of cells displaying blue fluorescence (virus fusion positive cells) **(B)** Levels of fusion in WT and KO cell lines (MOI = 1; n = 3; error bars, SD).



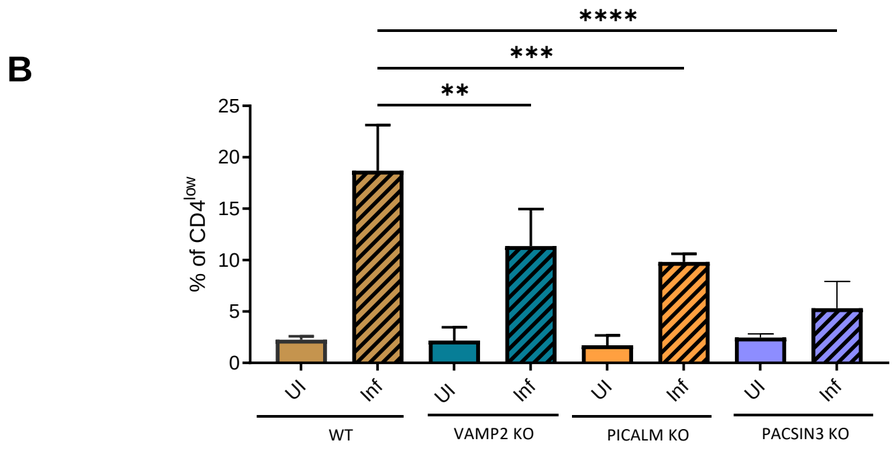


Figure 2. Effects of KOs on CD4 internalization in SUP-T1 T cells. (A) To examine if the KO proteins play a role in the internalization of CD4 during infection, cells were infected and expression of CD4 was examined by flow cytometry 48hrs later. Cells were surface-stained for CD4 (BV421) **(B)** Percentage of cells presenting a low expression of CD4 ($CD4^{low}$) in infected (inf) or uninfected (UI) cells. A significant difference displayed by KO cells suggests that they are likely implicated in CD4 internalization (MOI=5; n= 3; error bars, SD).

PICALM KO cells show impaired autophagy flux and leads to an increase in Gag

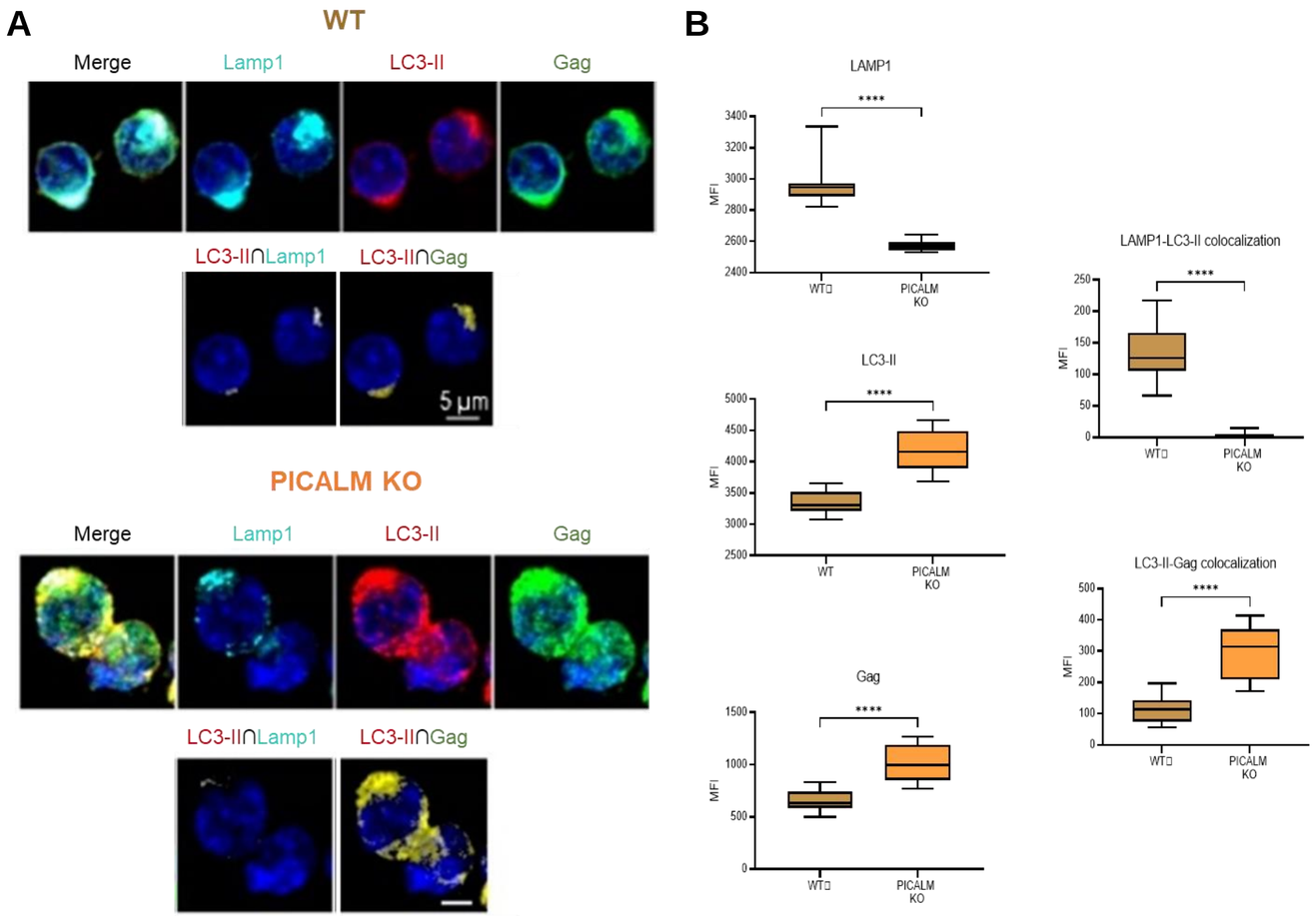


Figure 3. PICALM KO affects the expression and colocalization of HIV-1 Gag and autophagy proteins Lamp1 and LC3-II. WT and KO cells were infected with WT provirus (MOI 5) for 48hrs. **(A-B)** Cells were collected for microscopic analysis by immunofluorescence staining of the HIV-1 Gag protein and host proteins LC3-II and Lamp1. **(C)** Corresponding box plots quantifying mean fluorescence intensity (MFI) demonstrate that PICALM KO caused increased LC3-II expression without LAMP1 colocalization, increased Gag expression, and increased LC3-II-Gag colocalization.

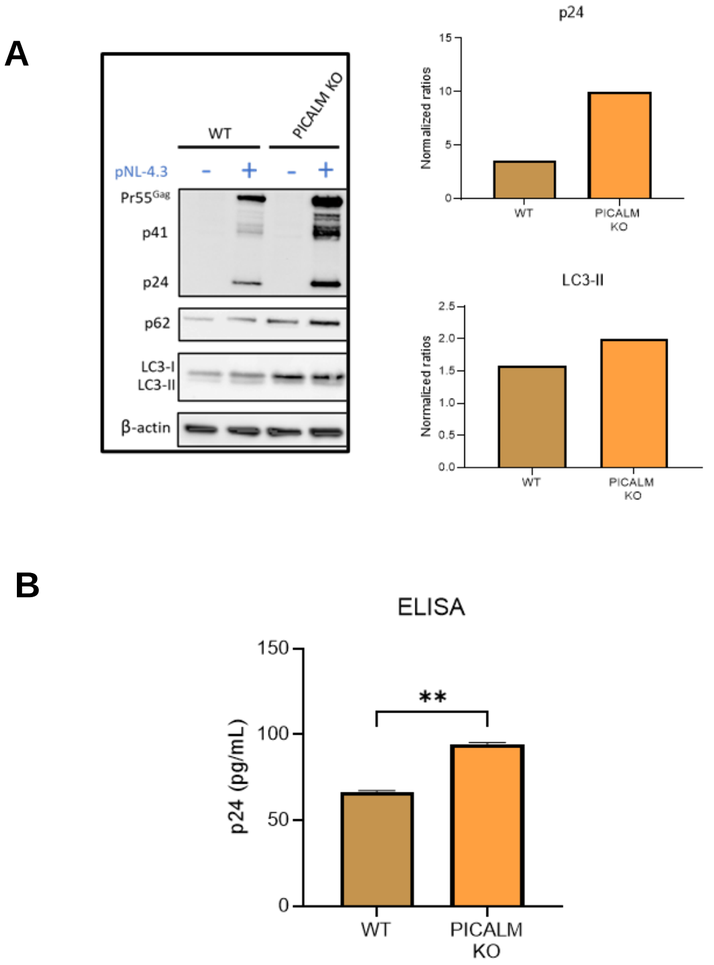
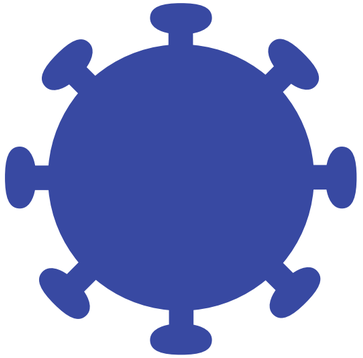


Figure 4. PICALM KO affects the expression and colocalization of HIV-1 Gag and autophagy proteins Lamp1 and LC3-II. (A) Western blots of HIV-1 Gag and LC3-II isoforms. Side panel demonstrates densitometric analysis of LC3-II and Gag blots. **(B)** Virus quantification was performed via enzyme-linked immunosorbent assay on supernatant-derived HIV-1 p24.

Conclusions and perspectives

- Viral entry was inhibited by the KO cell lines. This inhibition suggests that HIV-1 virus entry into SUP-T1 cells is endocytic pathway-mediated.
- HIV-1 infection of PICALM KO led to both increased expression of Gag and LC3-II as well as its colocalization.
- PICALM seems to block or intercept HIV-1 infection through autophagy.
- In addition, the KO cell lines appear to be involved in the internalization of CD4 during HIV-1 infection.
- We are currently working on the KO rescue experiments to confirm their effects on HIV-1 infection.



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*The authors declare no conflicts of interest.