CONFERENCE

CAHR 2022

CRISPR-Cas9 screen reveals role of PICALM in HIV-1 pathogenesis: interactions between the endolysosomal and immunity pathways

PAOLA GUIZAR[1,2], KRISTIN DAVIS[1,2], ANNE MONETTE[1], ANA LUIZA ABDALLA[1,2], MEIJUAN NIU[1], ANDREW J. MOULAND[1,2,3]

1- LADY DAVIS INSTITUTE AT THE JEWISH GENERAL HOSPITAL

2- DEPARTMENT OF MICROBIOLOGY & IMMUNOLOGY, MCGILL UNIVERSITY

3- DEPARTMENT OF MEDICINE, MCGILL UNIVERSITY

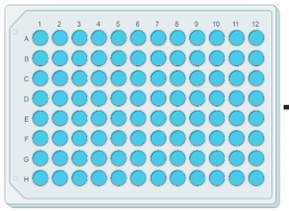
norma.guizaramador@mail.mcgill.ca

Introduction

- HIV-1 hijacks host proteins at multiple steps of infection, including membrane trafficking proteins.
- 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 phosphatidylinositol-binding clathrin assembly protein (PICALM), as a modulator of viral entry, autophagic flux, and immune control during HIV-1 infection in SUP-T1 CD4+ T cells.

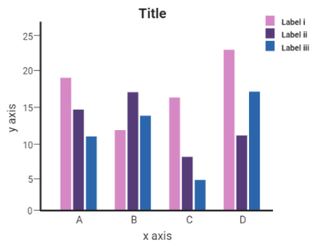
Methodology

CRISPR screening of membrane trafficking proteins in TZM-bl cells

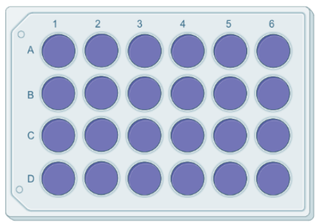


Library of 140 human genes.

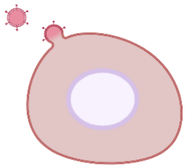
Identification of PICALM as a possible relevant hit



Generation of PICALM KO SUP-T1 cell line



Analysis of WT and PICALM KO SUP-T1 cells infected with HIV-1



Results

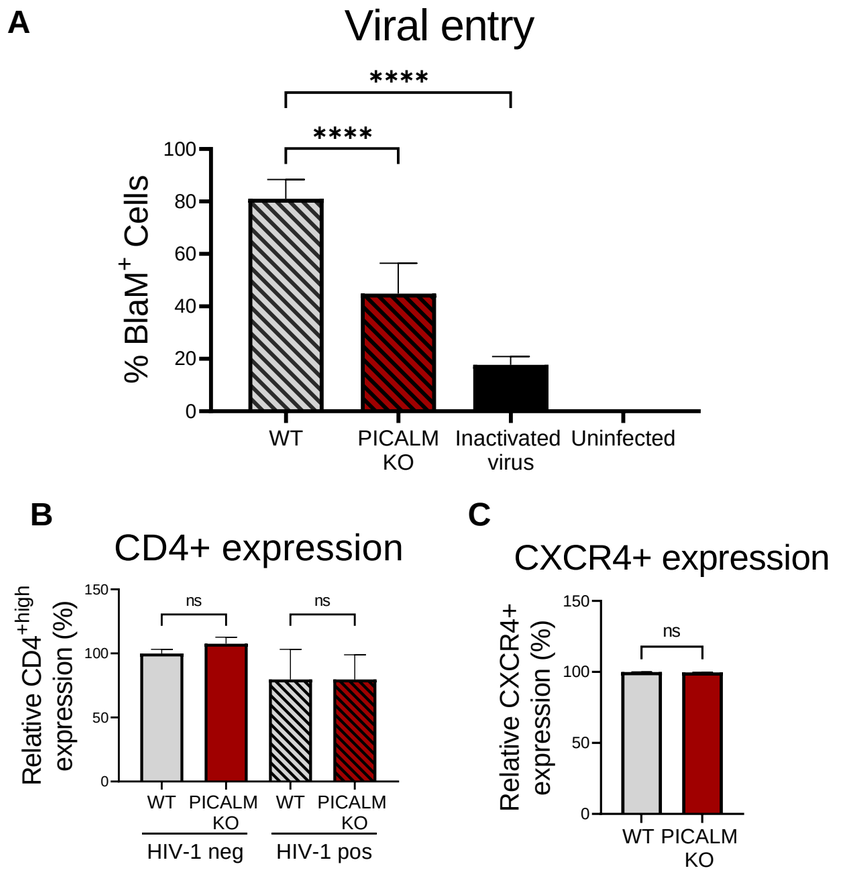
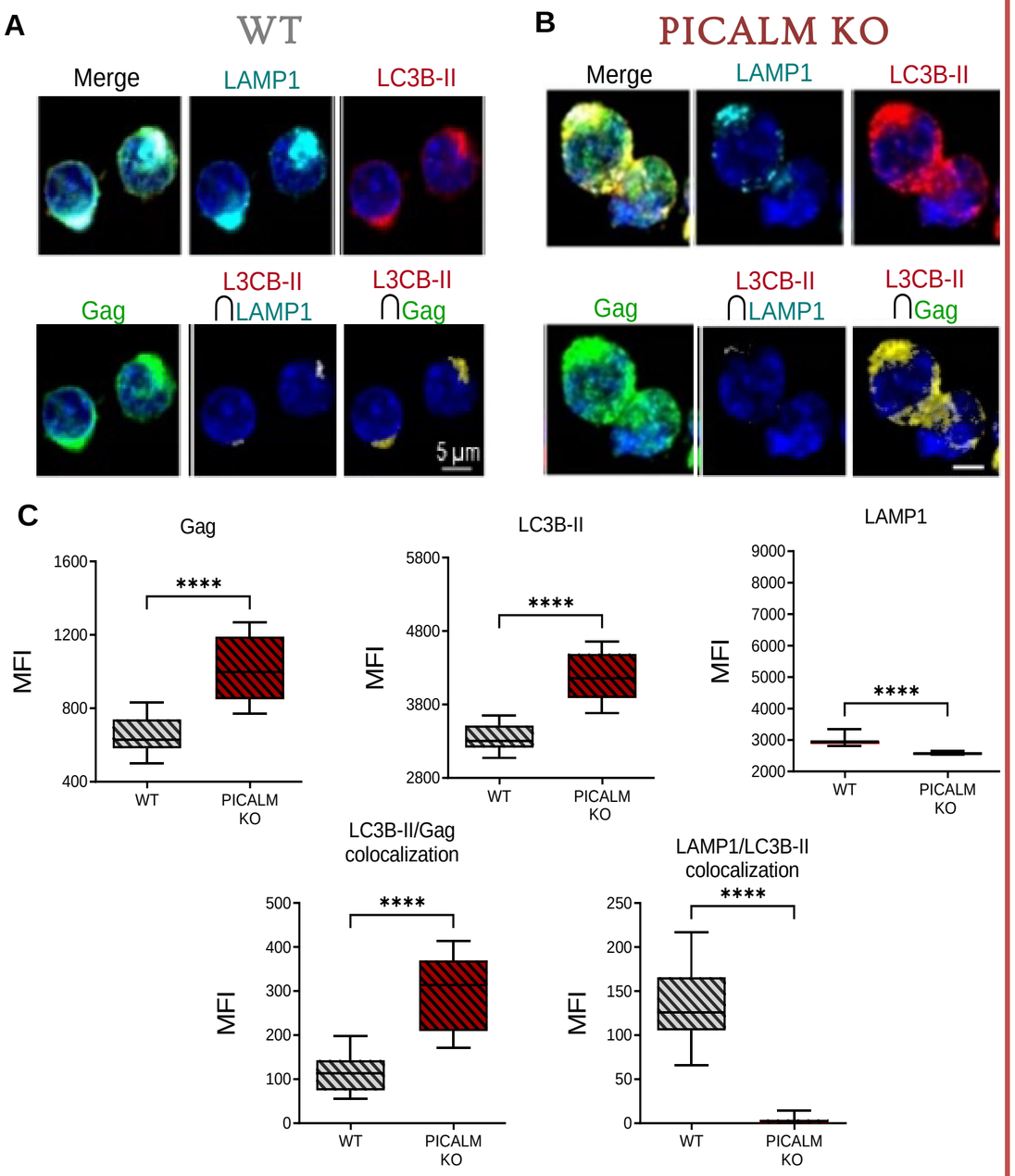


Figure 1. Effects of PICALM KO on HIV-1 entry in SUP-T1 T cells. **(A)** Levels of viral entry in WT and PICALM KO cell lines measured by the BlaM assay (MOI = 1). **(B-C)** To examine if the effects on viral entry was due to a role of PICALM in the internalization of CD4 or CXCR4 during an infection, expression of CD4 and CXCR4 was examined by flow cytometry.

Figure 2. PICALM KO SupT1 cells support a faster replication of HIV-1 through autophagy arrest. WT and PICALM KO cells were infected with WT provirus (MOI 5) for 48hrs. **(A-B)** Cells were collected for immunofluorescence (IF) staining of HIV-1 Gag and host proteins LC3B-II and LAMP1. **(C)** Corresponding box plots quantifying mean fluorescence intensity (MFI) show that PICALM KO caused a decrease in LAMP1-LC3B-II colocalization, increased LC3B-II expression, increased Gag expression, and increased LC3B-II-Gag colocalization.



Results

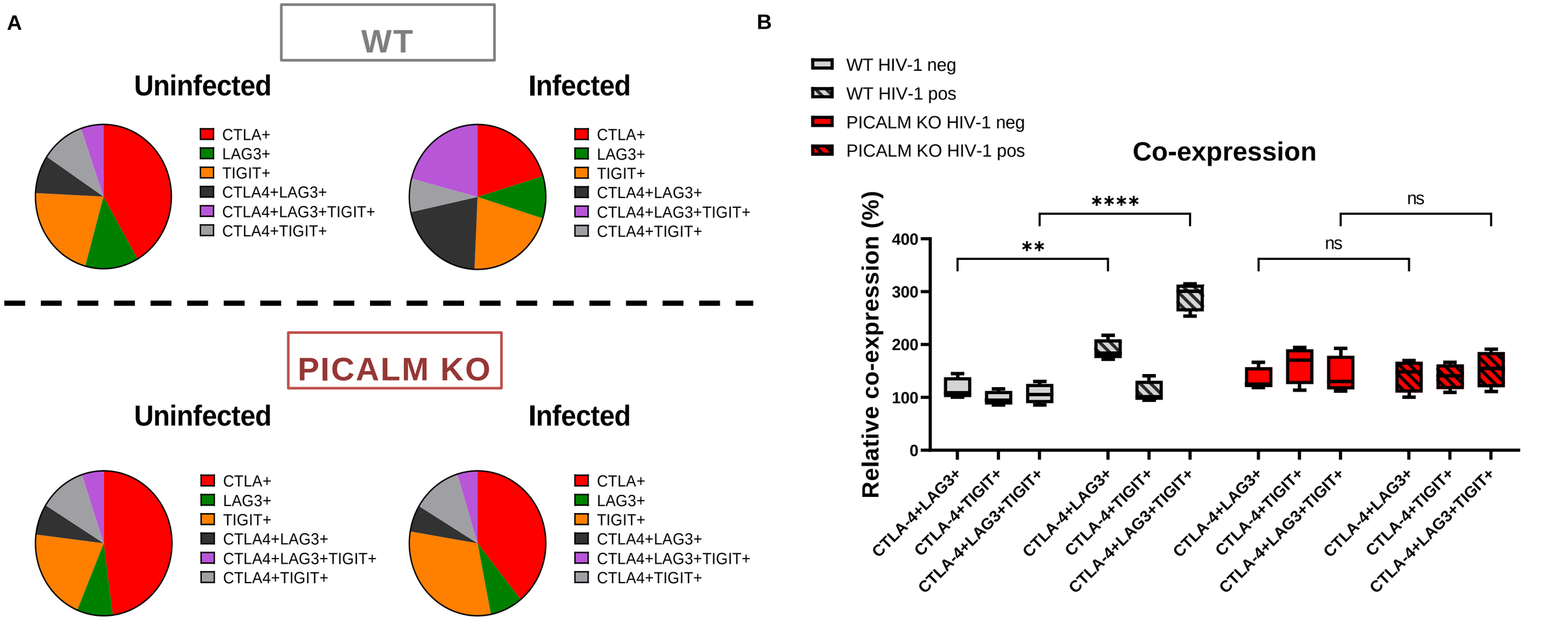


Figure 3. Expression and co-expression of CTLA-4, TIGIT and LAG-3 are affected during HIV-1 infection in PICALM KO cells. WT and PICALM KO cells were infected with WT provirus (MOI 5) for 48hrs. (A) The expression and co-expression of CTLA-4, LAG-3, and TIGIT were measured by multiparametric flow cytometry. **(B)** Corresponding box plots quantifying the relative co-expression show that CTLA-4, LAG-3, and TIGIT in PICALM KO cells show a reduction in co-expression of immune checkpoint molecules (ICs) during HIV-1 infection.

Conclusions

- Viral entry was markedly inhibited by KO of PICALM. This inhibition suggests that HIV-1 virus entry into SUP-T1 cells is endocytic pathway-mediated.

- The diminished efficiency of HIV-1 fusion with KO cells was not due to downregulation of CD4 or CXCR4 in these cells.

- The knockout of PICALM led to a reduced colocalization of LAMP1-LC3B-II, suggesting a defect in autophagosome/lysosome fusion. In addition, HIV-1 infection of PICALM KO led to both increased expression of Gag and its colocalization with LC3B-II, providing a link between autophagy and HIV-1 pathogenesis.

- Co-expression of CTLA-4, LAG-3, and TIGIT, essential ICs that are important for the restriction of viral persistence, show a different pattern of expression during HIV-1 infection in PICALM KO cells compared to the WT, possibly leading to a higher viral titer.

Jewish General Hospital
Lady Davis Institute for Medical Research

McGill UNIVERSITY

CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

GTO Grandeza de México EDUCAFIN

Acknowledgments: This work was supported by a grant from the CIHR (MOP-56974) to AJM, and Mexican EDUCAFIN scholarship to PG.

*The authors declare no conflicts of interest.