Rab7+ Vesicles are Involved in HIV-1 Gag Repositioning to Virus-Containing Compartments (VCC) in Macrophages

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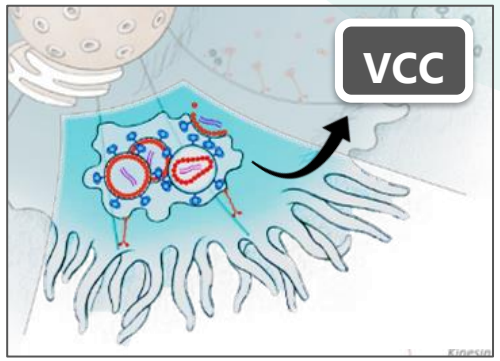
Introduction

- Different from T cells, in macrophages (Mϕ) HIV-1 buds in intracellular compartments termed VCC[1].



- VCCs protect HIV-1 particles from the host humoral response[2] and play a role in Mϕ-to-T cell transmission[3].

- We previously showed in Hela cells that HIV-1 Gag co-traffics with Late endosomes/Lysosomes (LELs), and also that their movement disruption affected HIV-1 release[4,5].

- Further studies in Hela cells demonstrated that the downregulation of LELs-related proteins supresses HIV-1 release, suggesting that LELs direct Gag to membrane assembly sites[6].

We hypothesise that LELs are essential for HIV-1 assembly at VCCs in infected macrophages.

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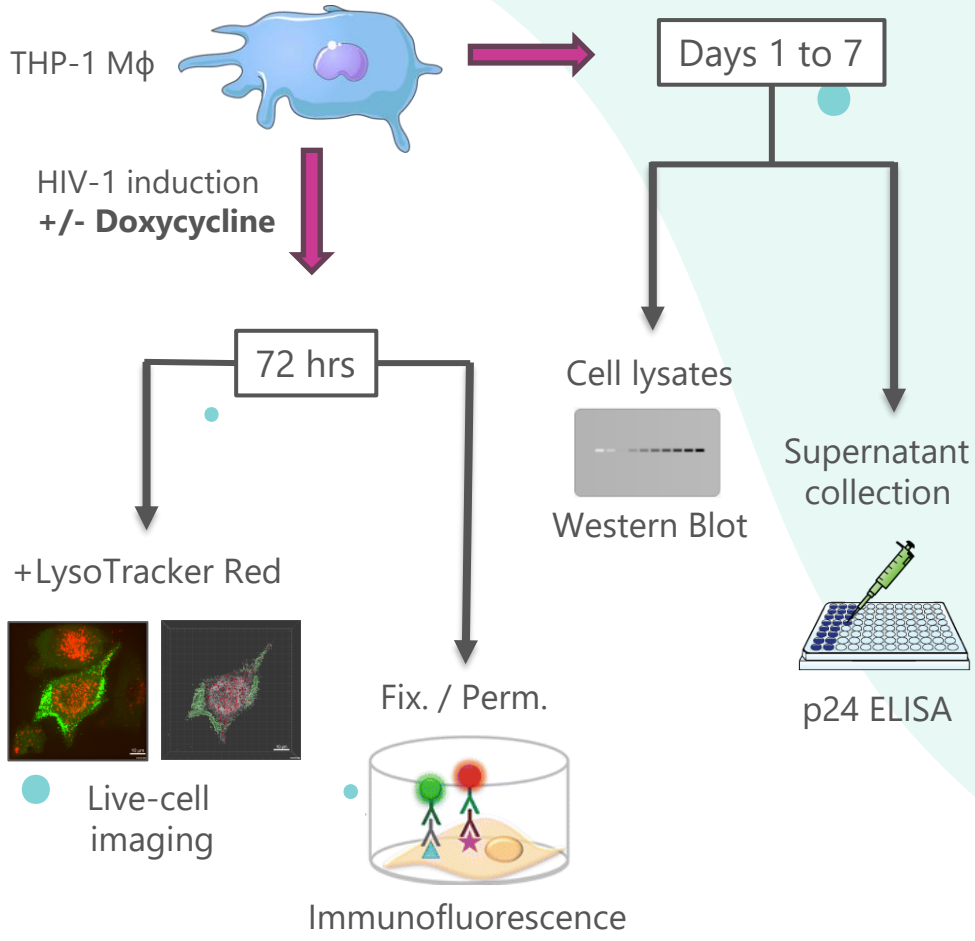
Methodology

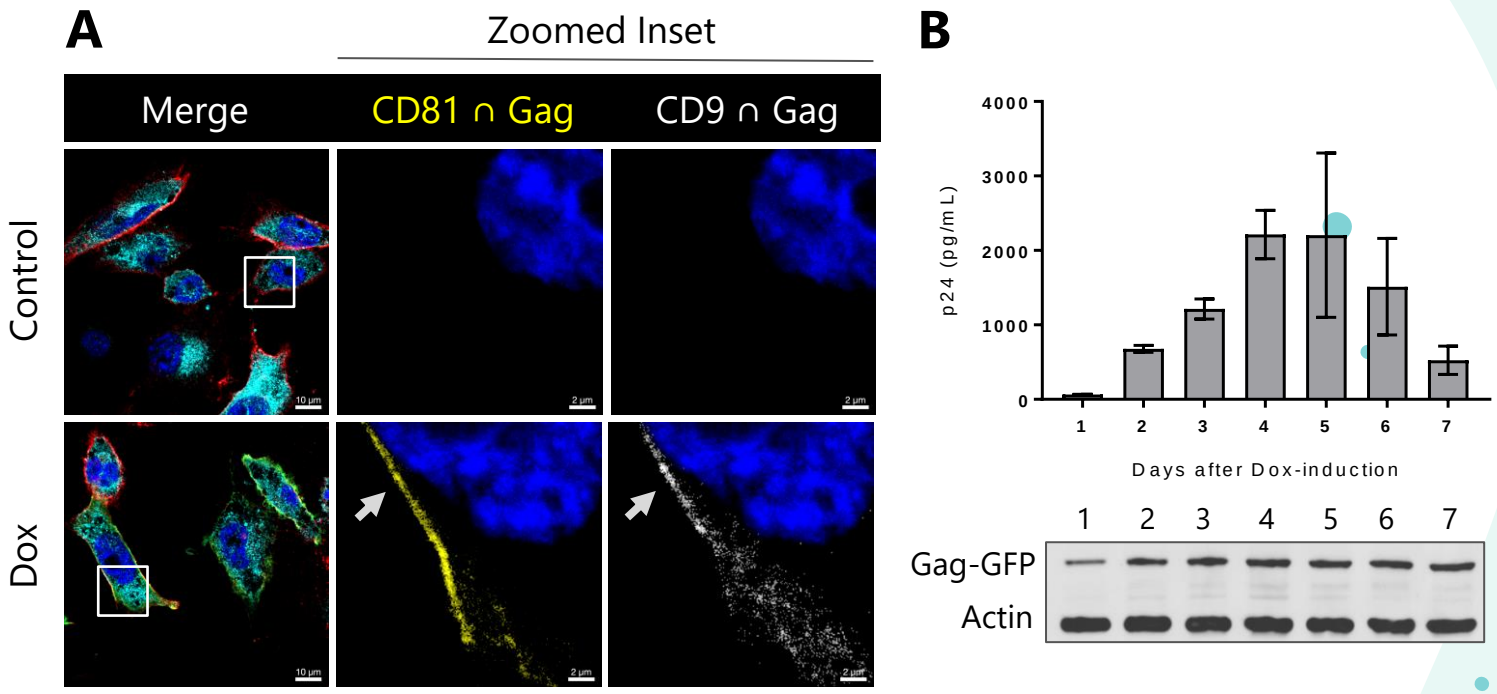
- We used the THP-1 GagZip monocytic cell line, which was transduced with a doxycycline-inducible HIV-1 genome where Gag is fused to GFP. Monocytes were differentiated into Mϕ by the addition of PMA during 48 hrs.



Results

Figure 1. VCCs in HIV-1-inducible THP-1 Mϕ resembles those in human Mϕ.

THP-1 Mϕ were seeded and HIV-1 Gag-GFP expression was induced by the addition of doxycycline (Dox). **A** Confocal microscopy of Mϕ 72 hrs after Dox-induction labeled against Gag-GFP (green), and the VCC markers CD81 (red) and CD9 (cyan). A representative image is shown in Merge. Zoomed insets depict the colocalized pixels between CD81-Gag (yellow), and CD9-Gag (white). Gag-GFP colocalizes with both VCC markers (arrowheads). Scale bars represents 10 µm in merge and 2 µm in inset pictures. **B** Viral release quantification was done replacing media 24 hrs before each sampling. HIV-1 p24 concentration in supernatants was determined during 7 days (upper panel). HIV-1 accumulates in Dox-induced THP-1 Mϕ, observing a decrease in release after 4 days, but no change in intracellular concentration determined by western blot against Gag (bottom panel).



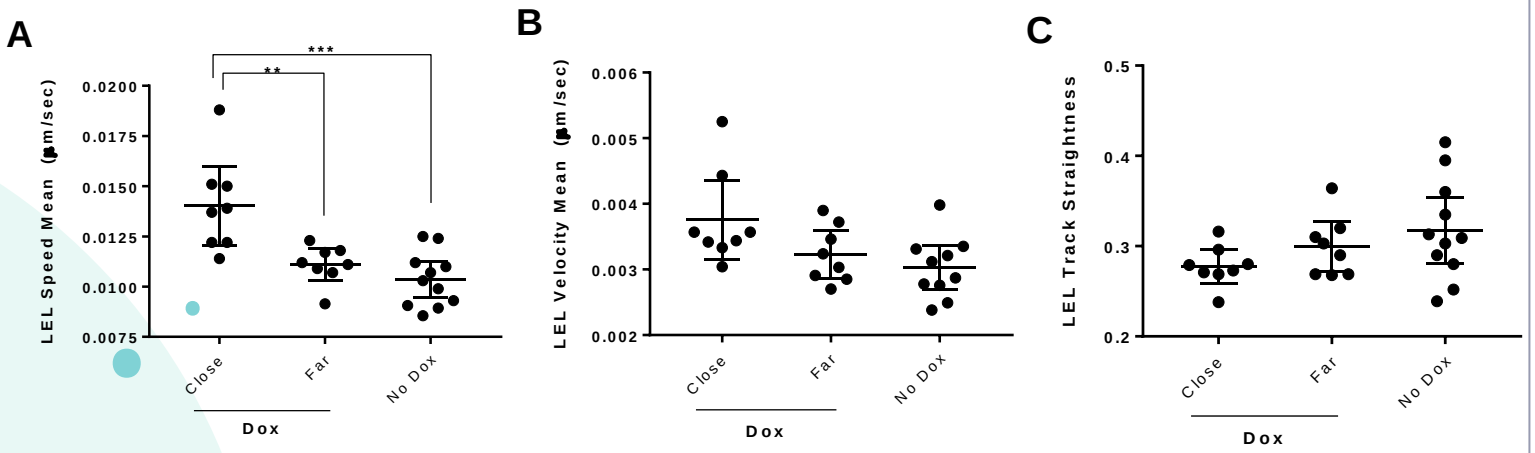


Figure 2. HIV-1 Gag expression in Mϕ increased late endosomes / lysosomes (LELs) mobility.

72 hrs after Dox-induction, THP-1 Mϕ were incubated 30 minutes with LysoTracker and then recorded during 30. LELs tracks were sorted and analysed as close (less than 0.1 µm) or far (more than 0.1 µm) from Gag-GFP VCCs. Tracks speed **(A)** and velocity **(B)** (length and displacement, respectively) were determined together with tracks straightness **(C)**. LELs that are in close proximity to VCCs have a significant increase in the length of their tracks over time. Each circle represent the data from one cell. Statistical analysis was performed using one-way ANOVA. ** $p < 0{,}005$; *** $p < 0{,}0005$.

Results

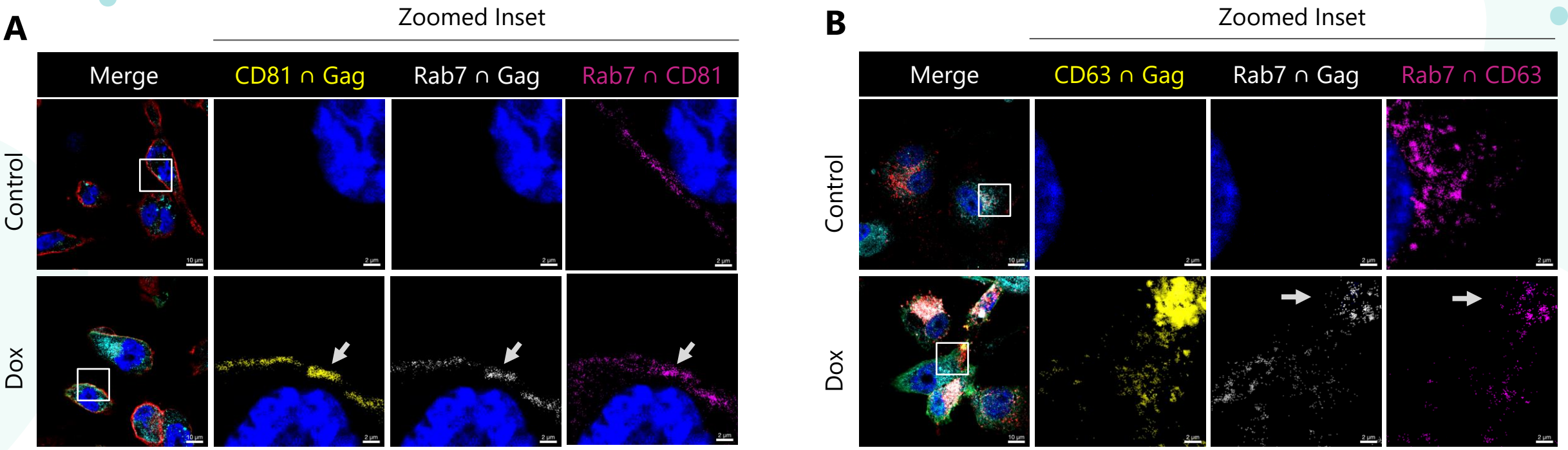


Figure 3. HIV-1 Gag colocalizes with Rab7+ vesicles at VCCs.

THP-1 Mϕ were seeded and HIV-1 Gag-GFP expression was induced by the addition of doxycycline (Dox). 72 hrs after induction cells were fixed and observed by confocal microscopy. **A** THP-1 Mϕ were labeled against Gag-GFP (green), the VCC marker CD81 (red), and the LEL protein Rab7 (cyan). A representative image is shown in Merge. Zoomed insets depict the colocalized pixels between CD81-Gag (yellow), Rab7-Gag (white) and Rab7-CD81 (magenta). Rab7 colocalizes with Gag-GFP and CD81 at the same region where VCCs are formed (arrowheads). Because Rab7 is present inside cells as free in the cytoplasm or anchored to membranes, in **B** THP-1 Mϕ were labeled as before but staining against CD63 (red), a marker for intracellular membranes. A representative image is shown in Merge. Zoomed insets depict the colocalized pixels between CD63-Gag (yellow), Rab7-Gag (white) and Rab7-CD63 (magenta). A population of Gag-GFP colocalizes with Rab7 at vesicles membranes (arrowheads). Scale bars represents 10 μm in merge and 2 μm in inset pictures.

Conclusions

- VCCs observed in HIV-1-inducible THP-1 Mϕ resemble those in human monocyte-derived Mϕ.
- Upon induction of HIV-1 Gag expression in Mϕ, LELs exhibited increased speed when in close proximity to VCC, in comparison to distal LELs or non-induced control Mϕ.
- Membrane-bound Rab7 colocalized with Gag within VCCs.
- These results demonstrate that HIV-1 modulates LELs positioning in Mϕ, thereby promoting Gag assembly and trafficking to VCCs.

Acknowledgements

- Meijuan Niu (Technical support)
- Christian Young (Imaging facility)

Funding

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

(Grant)

Agencia Nacional de Investigación y Desarrollo
Ministerio de Ciencia, Tecnología, Conocimiento e Innovación
Gobierno de Chile

(Scholarship)

(Experimental Medicine Travel Award)