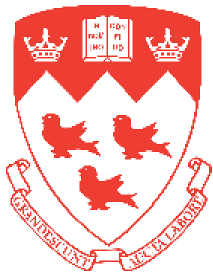

HIV-1 Repositions Late Endosomes / Lysosomes and Alters Their Motility to Direct Gag to Virus-Containing Compartments (VCC) in Macrophages

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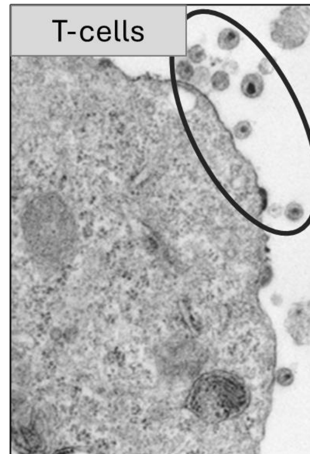
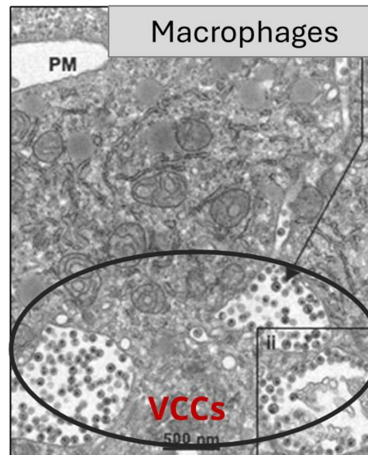
Background

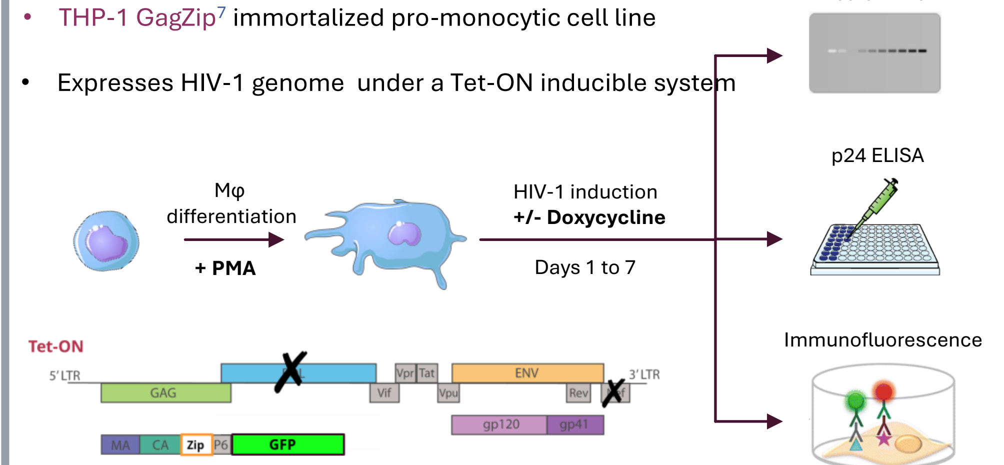
- Different from T cells, in macrophages (Mφ) HIV-1 buds in intracellular compartments termed VCC[1].
- VCCs protect HIV-1 particles from the host humoral response[2] and play a role in Mφ-to-T cell transmission[3].
- We previously showed in Hela cells – resembling T cells assembly model - that HIV-1 Gag co-traffics with Late endosomes/Lysosomes (LELs). Likewise, modulation of LEL movement affected HIV-1 release[4,5].
- Also, downregulation of LELs-related proteins supresses HIV-1 release, suggesting that LELs play a role in Gag trafficking to assembly sites[6].



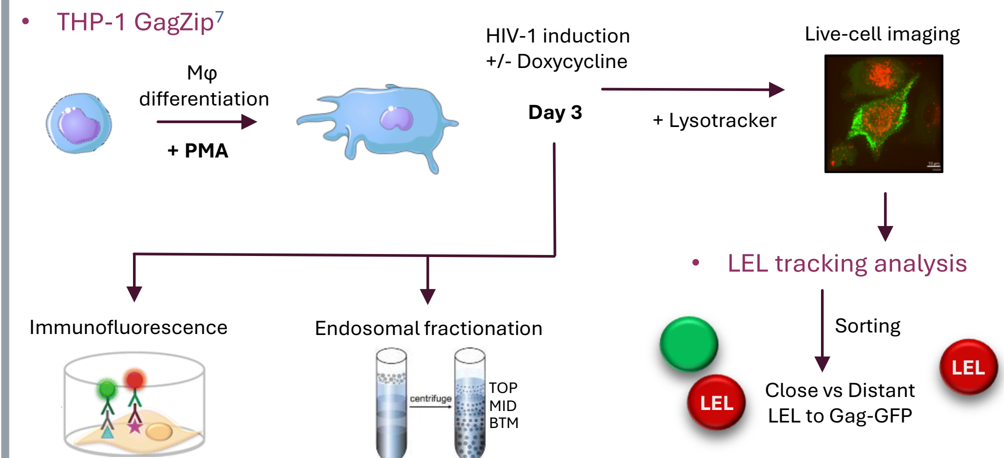


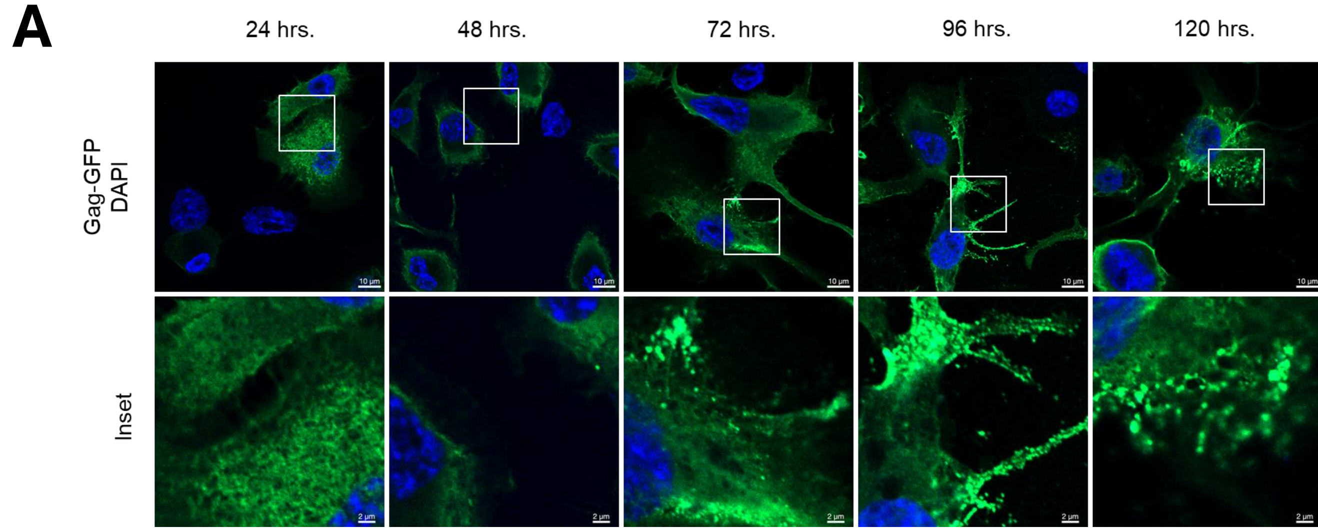
Hypothesis: Gag hijacks LELs trafficking machinery to direct virus assembly to VCCs in HIV-1 infected Macrophages.

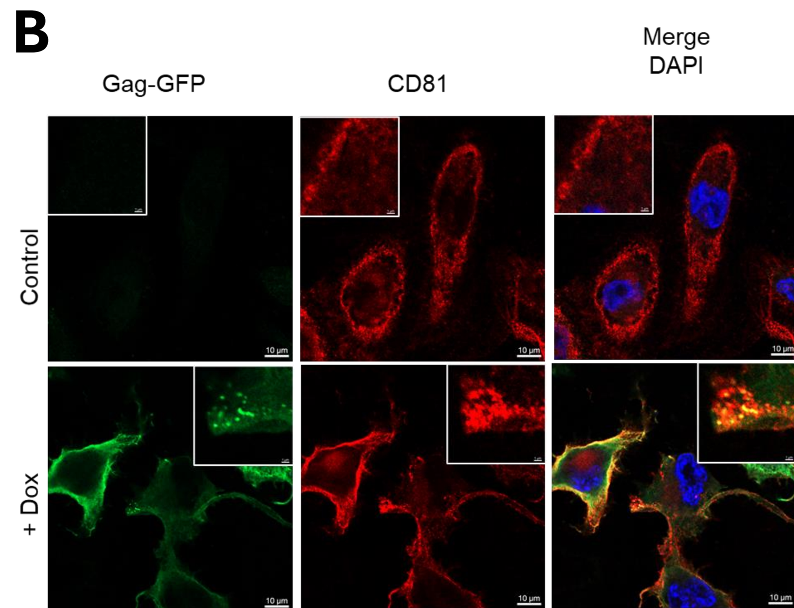
AIM 1. Establishment of a model to study VCC formation in HIV-1 infected macrophages.



AIM 2. Evaluate the relevance of Late endosomes / Lysosomes in HIV-1 Gag trafficking towards VCC.







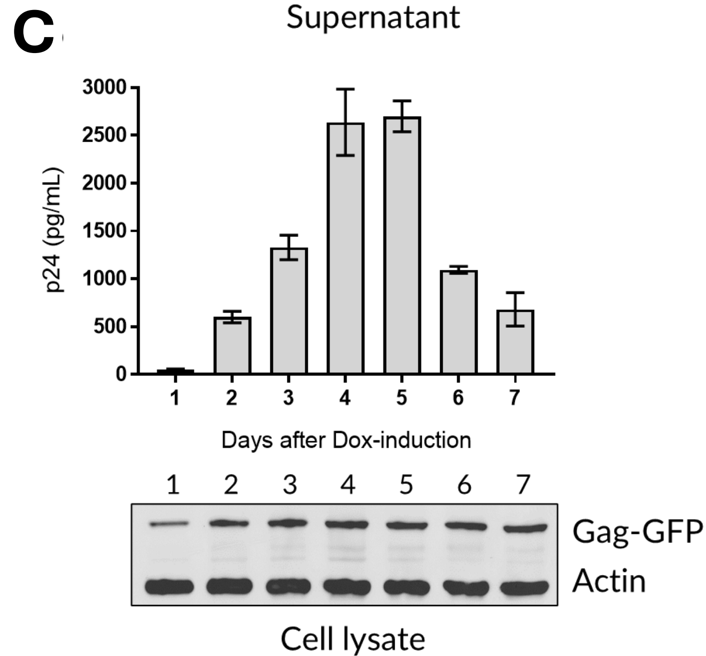
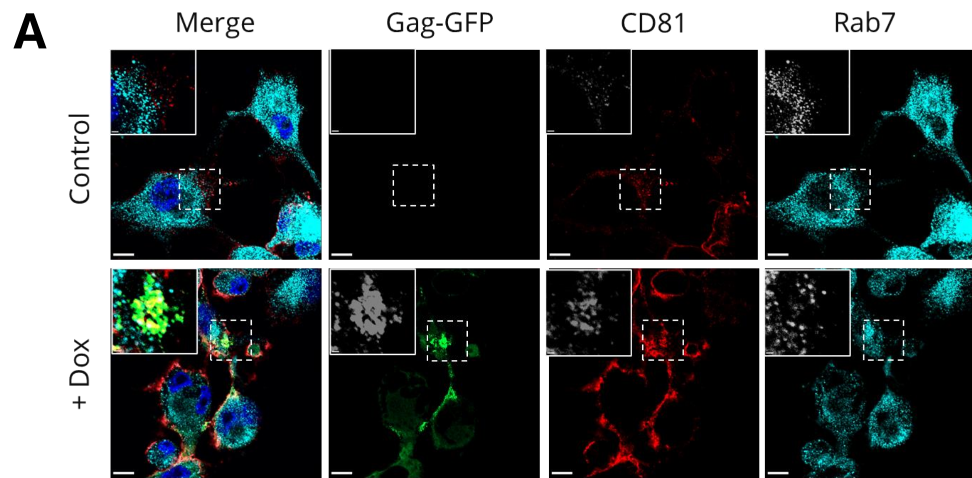


FIG 1. THP-1 GagZip macrophages resembles VCCs phenotype.

THP-1 Gag-zip cells were seeded and differentiated into macrophages for subsequent HIV-1 induction by the addition of doxycycline. Cells and supernatant samples were collected every 24 hrs. **(A)** Fixed THP-1 Gag-zip macrophages observed by confocal microscopy. Gag-GFP accumulated in distinctive punctae 72 hrs after HIV-1 induction. **(B)** Fixed THP-1 Gag-zip macrophages stained against CD81 72 hrs after dox-induction. The VCC marker CD81 (red) colocalizes with Gag-GFP punctae. Nuclear staining (blue) using DAPI. Scale bar = 10 μm / 2 μm (Insets). **(C)** Viral release quantification by p24 ELISA from supernatants (top) or Gag-GFP intracellular levels in cell lysates by Western blot (bottom). HIV-1 release decreased over time, while Gag-GFP intracellular levels were constant, suggesting viral accumulation inside macrophages.



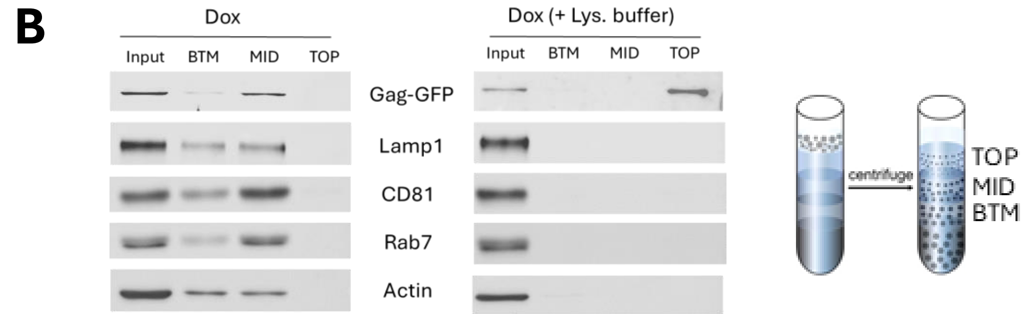
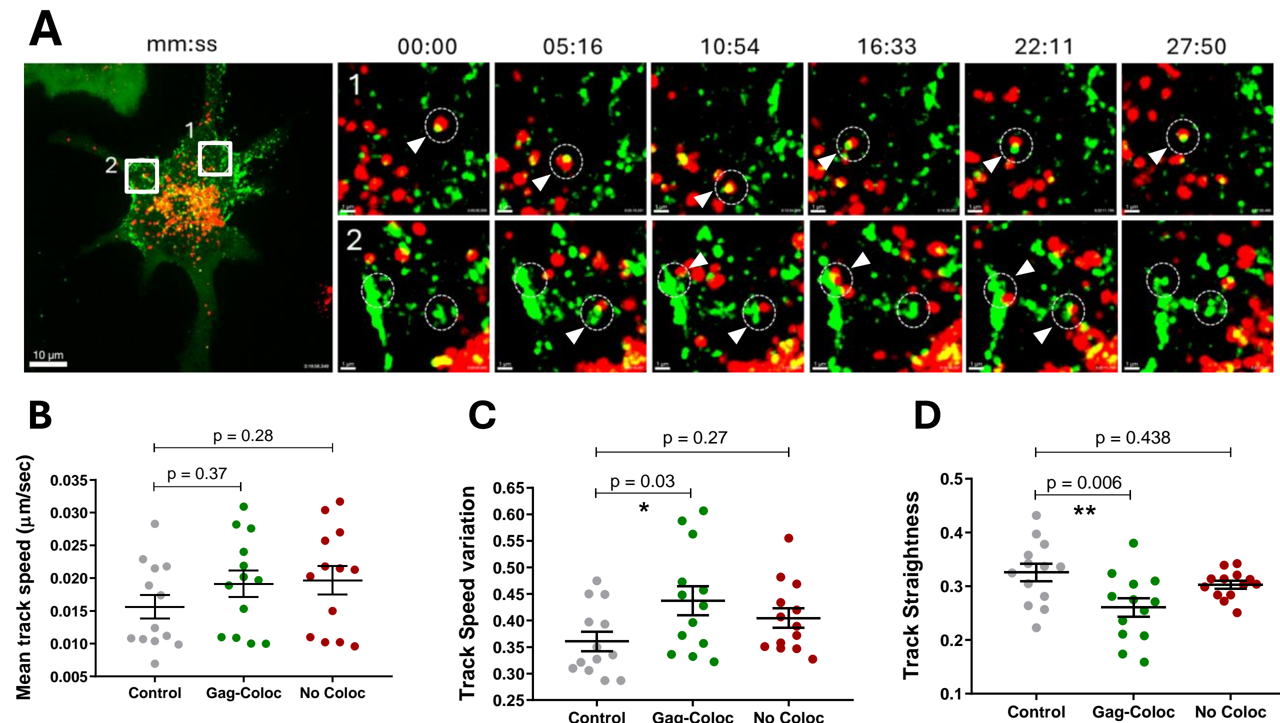


FIG 2. HIV-1 Gag-GFP colocalizes with LEL at VCCs.

THP-1 Gag-zip cells differentiated into macrophages and HIV-1 induced by the addition of doxycycline. **(A)** 72 hrs after HIV-1 induction, macrophages were fixed and stained against Gag-GFP, the VCC marker CD81 (red), and the LEL marker Rab7 (cyan). Gag-GFP punctae colocalized with both markers. Nuclear staining using DAPI. Scale bar = 10 µm / 2 µm (Insets). **(B)** Sucrose step gradient fractionation of THP-1 Gag-Zip macrophages 72 hrs after HIV-1 induction. Bottom, middle and top fractions were collected and analyzed by western blot against HIV-1 p17, Lamp1, Rab7, CD81, and Actin. To confirm purified membrane-bound proteins, cells were lysed in the presence of detergent before ultracentrifugation (right panel). Gag-GFP is enriched in the same fraction as LEL markers.

FIG 3. HIV-1 Gag-GFP moves alongside with LEL and its induction alters LEL motility in macrophages.

THP-1 Gag-zip cells were seeded and differentiated into macrophages for subsequent HIV-1 induction by the addition of doxycycline. 72 hrs after, cells were incubated with Lysotracker and recorded during 30 min. **(A)** Time-lapse images from a recorded cell. A subpopulation of LEL moves alongside with Gag-GFP (arrowheads) during several minutes (1), while others come in close contact for shorter periods of time (2). Scale bar = 10 / 1 µm (insets). LEL tracks mean values per cell were analyzed (n=13 per condition). Mean LEL track speed **(B)**, speed variation **(C)**, and straightness **(D)** were analyzed for LEL that were in close contact with Gag-GFP (Gag-coloc. Less than 0.7 µm), distant (No coloc.) or non-induced control macrophages. Colocalizing LEL with Gag showed an increased speed variation and a concomitant decrease in straightness. Each symbol represents the mean value of each parameter per cell, and bars represent SEM. One-way ANOVA statistical analysis, with Dunnett's post-test against non-induced control macrophages.



Conclusions

- THP-1 Gag-zip model resembles VCCs observed in HIV-1-infected human Macrophages.
- HIV-1 Gag-GFP is present at LELs membranes and colocalizes with them at VCCs.
- Upon HIV-1 induction, a subpopulation of LELs moves alongside Gag-GFP, exhibiting increased speed variation and reduced straightness in comparison to distal LELs or control Macrophages.

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