# 179-LOXL3 REGULATES HIV-1 INFECTION IN HUMAN PRIMARY MACROPHAGES

HANY, LAURENT[1]; Ouellet, Michel[1]; Tremblay, Michel.J[1,2]

[1] Centre de recherche du CHU de Québec-Université Laval, Pavillon CHUL, 2705 boul. Laurier, local RC709, Québec, QC, G1V 4G2
[2] Département de microbiologie-infectiologie et immunologie, Faculté de médecine, Université Laval, Québec, QC, G1V 0A6

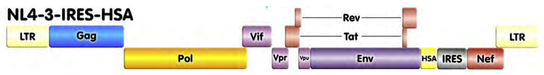
## Background

Despite therapeutic progresses and with our present knowledge, an HIV cure is still not within our reach. One of the suspected reason for HIV persistence is the constitution of a viral reservoir inside target cells such as macrophages. In vitro, only a small percentage of macrophages are infected and can therefore contribute to the establishment of a reservoir. To better understand molecular mechanisms of macrophages permissiveness, we performed a transcriptomic analysis of infected and uninfected bystander cells. Precise mapping of the interactions between HIV-1 and host signaling pathways identified several new potential host-encoded regulators of HIV-1 replication in macrophages.
One of these was the Lysyl-Oxidase-like-3 (LOXL3), a member of the lysyl oxidase family regulating collagen/elastin crosslinking and possibly modulating transcription.

## Material and Methods

### Sorting of HIV-1-infected cells

We developed an experimental model based on the identification and isolation of infected monocyte-derived macrophages (MDMs) within a heterogeneous population. MDMs are exposed to a fully replicative R5-tropic virus coexpressing the Heat Stable Antigen (HSA). Immuno-magnetic sorting was then performed to separate HSA-positive cells from the uninfected/bystander population.




Schematic representation of the NL4.3-Bal-Env-IRES-HSA

The murine Heat Stable Antigen (HSA) is transcriptionally fused to *nef* gene through an IRES sequence into HIV-1 NL4.3 molecular clone (described in Ref 1). The SalI–BamHI fragment that contains most of the *env* gene from NL4-3Balenv (described in Ref 2) was swapped in place of the native NL4-3 *env* gene in NL4-3-IRES-HSA.

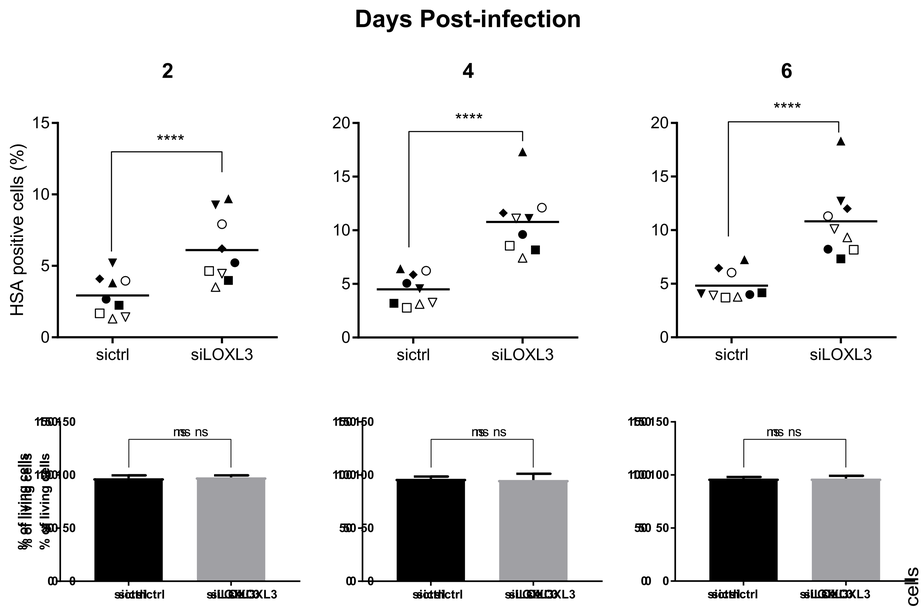
### Flow cytometry studies

MDMs were transfected or left untransfected (NT) for 72h with control or indicated gene-specific siRNA. Cells were then assayed for membranous CD4 detection or infected with the complete NL4.3-Bal-Env-IRES-HSA virus or a VSV-G pseudotyped envelope-deleted virus (NL4.3 Δenv IRES-HSA + VSV-G) called VSV-G-HSA hereafter. Infection was monitored after the indicated days post infection (dpi) as the percentage of $HSA^+$ cells by flow cytometry. For some experiments cell death was assayed using fluorescent Fixable Viability Dye.

## References

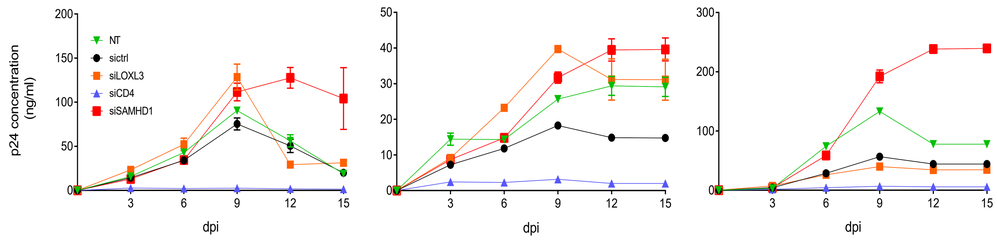
1. **Imbeault**, M., Lodge, R., Ouellet, M. & Tremblay, M. J. (2009) Virology 393, 160–167.
2. **Dornadula** G., Zhang H., Shetty S., Pomerantz R.J. (1999) Virology 253, 10–16

## Results



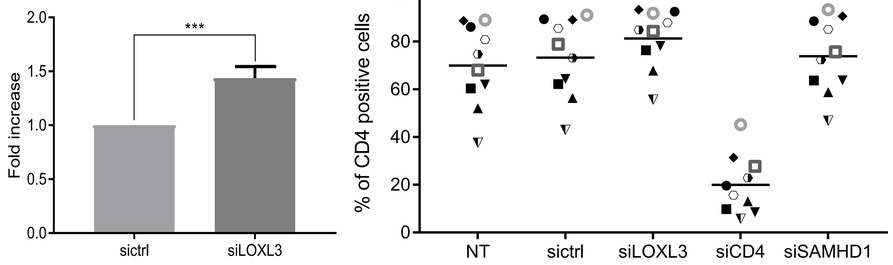

**LOXL3 gene silencing increases infection of MDMs by HIV-1 without affecting cell viability**

MDMs were treated as indicated previously and infected with NL4.3-Bal-Env-IRES-HSA virus.
Infection and cell viability were monitored by flow cytometry (top and bottom panels respectively) after the indicated days post-infection (dpi).
Statistics : Ratio paired t test n = 9.
****P<0.0001, ns = non significant.



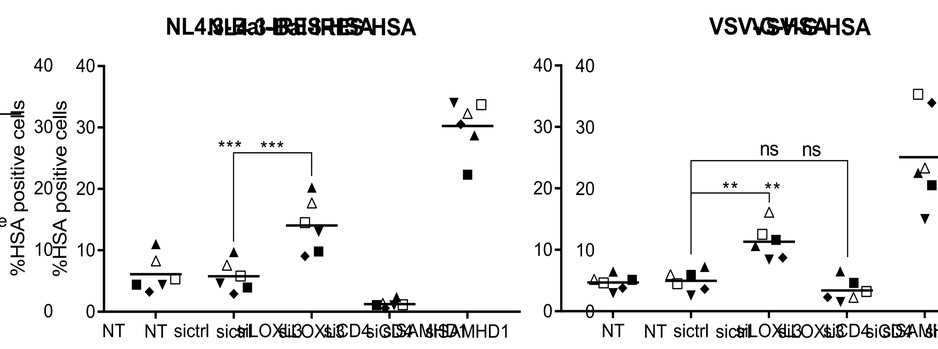

**Silencing of LOXL3 increases HIV-1 production**

MDMs were left untransfected (NT) or transfected using indicated gene-specific siRNA for 72h, then infected with NL4.3-Bal-Env viruses (MOI: 0,1). Medium was collected at 3 to 15 days post infection and viral production was assessed by an in house p24 ELISA. Each point corresponds to the average of p24 concentration of technical duplicates.
Data of these 3 donors are representative of the variability observed among 9 independent donors.




**LOXL3 silencing upregulates CD4 expression**

MDMs were transfected with the indicated siRNA for 72h. CD4 mRNA and its membranous expression were monitored by qRT-PCR (left panel) or by flow cytometry (right panel), respectively.
Statistics : Ratio paired t test (left) n = 4 and One-way ANOVA (right) n = 10.
**P<0.01, ***P<0.001, ns = non significant.




**Increased susceptibility to HIV-1 after silencing of LOXL3 is independent of the virus' entry route**

MDMs were transfected as mentioned previously and infected with the indicated virus.
Percentage of HSA positive cells was monitored after 6 days post infection.
Statistics : One way ANOVA n = 6.
**P<0.01, ***P<0.001, ns = nonsignificant.

## Conclusions

Gene silencing of LOXL3 results in a significant increase in the susceptibility of MDMs to HIV infection and viral production. Therefore LOXL3 could be classified as a negative regulator of HIV-1 replication in macrophages. The increase of CD4 expression at the membrane following the knockdown of LOXL3 could have explained its effect. However, data collected with the VSV-G-pseudotyped HIV-1 instead suggest that control of CD4 expression does not solely account for LOXL3-mediated modulation of macrophage susceptibility to HIV-1 infection. Identification of the mode of action of LOXL3 will broaden our perspective regarding unsuspected cellular factors that play a role in the replicative cycle of HIV-1.
A better understanding of HIV-1 regulation in macrophages will potentially allow the development of new strategies aimed at targeting this understudied reservoir.

## Acknowledgements

This study was supported by The Canadian HIV Cure Enterprise Team Grant HIG-133050 (to MJT) from the CIHR in partnership with CANFAR and IAS.