# Characterization of the Role of Host Cell Decapping Activators DDX6, LSm1-7 and PatL1 in HIV-1 Replication

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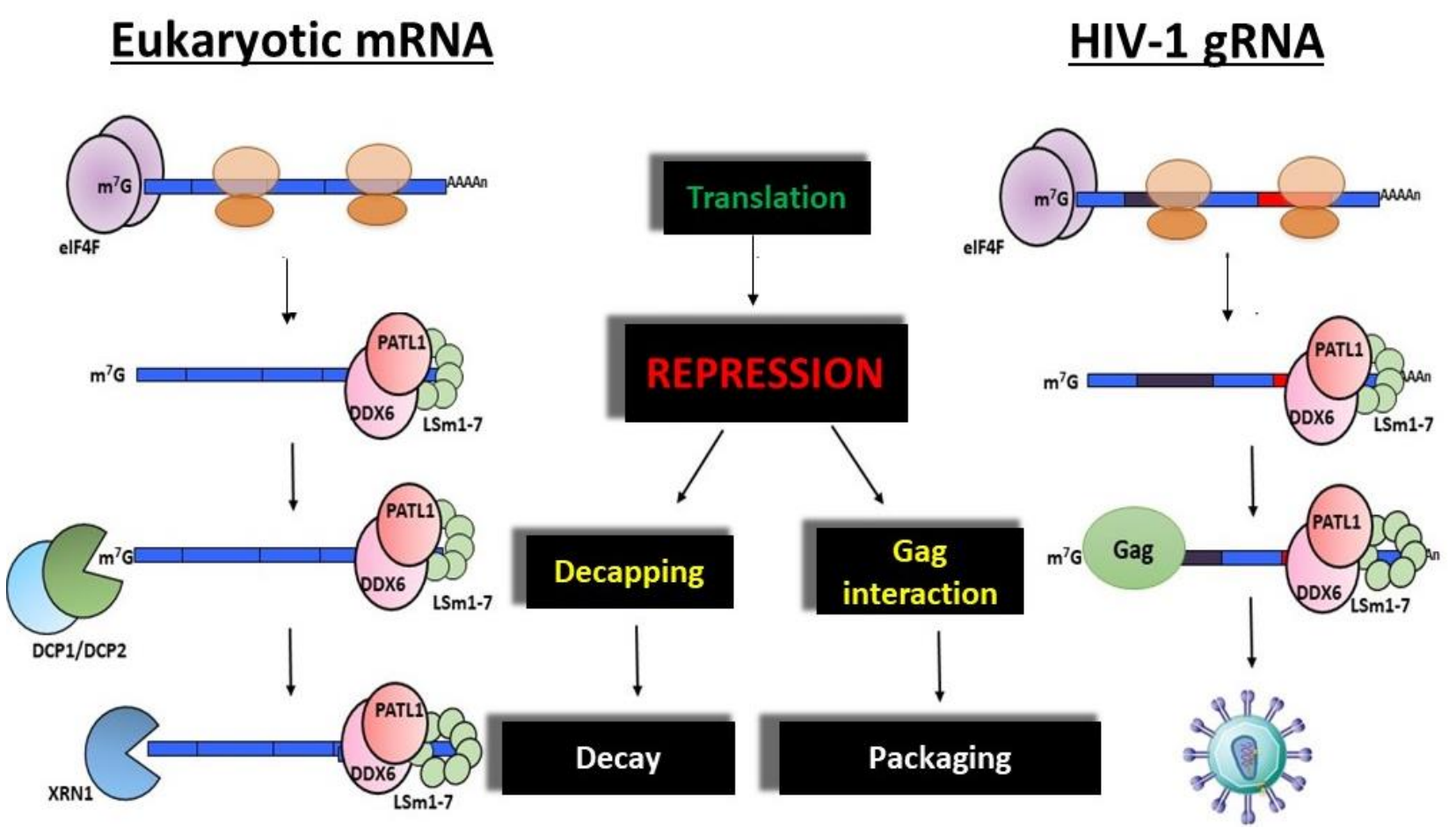


**Figure 1**. Retroviral genomic RNA (gRNA) has a dual role in the replicative cycle: (i) it is used as a template for viral proteins synthesis, and (ii) must be encapsidated as a genome to generate new infectious viral particles. The different models proposed to explain how the HIV-1 gRNA is engaged towards packaging and/or translation are controversial and the mechanisms that regulate this crucial stage in the formation of new viral particles are still poorly understood. It is known that HIV-1 co-opts the cellular RNA helicase DDX6 in complexes that promote the assembly of viral particles, however its role in these complexes is unknown. Interestingly, DDX6 promotes decapping during mRNA decay through its activity as a translational repressor. From this evidence arises the question: Does other activators of decapping, such as LSm1-7 or PatL1, participate in HIV-1 replication? Cellular decapping activators DDX6, LSm1-7 and PatL1 act in a crucial step of the cellular mRNA decay pathway: translational repression. By displacing ribosomes and eukaryotic initiation factors, these proteins allow the decay of an mRNA (left). We propose that LSm1-7, PatL1 and DDX6 could inhibit translation of HIV-1 genomic RNA (HIV-1 gRNA), allowing its interaction with Gag and avoiding decay to ensure packaging and efficient viral particle production (right).

# DDX6, LSm1 and PatL1 act as a complex to repress HIV-1 gRNA translation

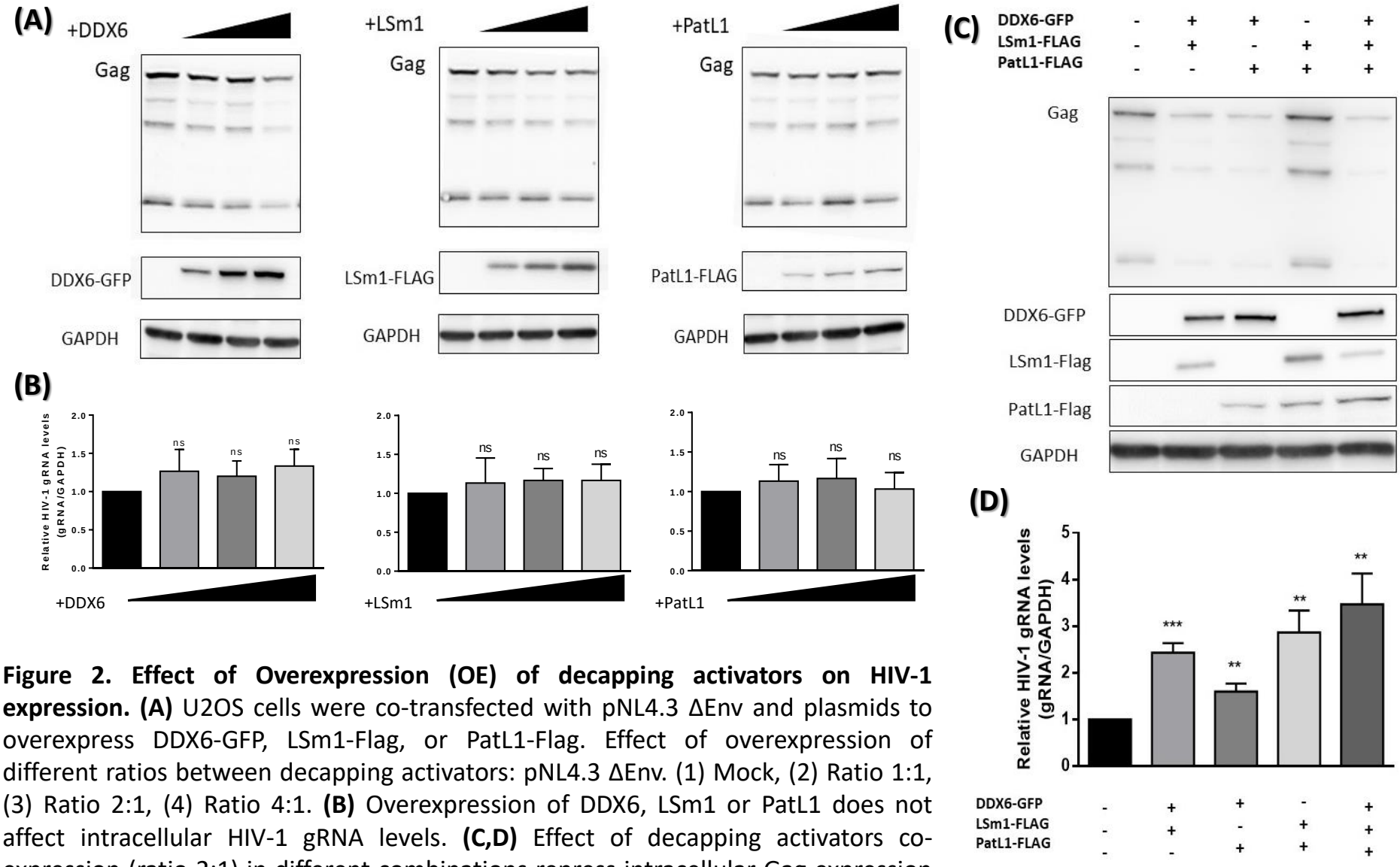


**Figure 2. Effect of Overexpression (OE) of decapping activators on HIV-1 expression. (A)** U2OS cells were co-transfected with pNL4.3 ΔEnv and plasmids to overexpress DDX6-GFP, LSm1-Flag, or PatL1-Flag. Effect of overexpression of different ratios between decapping activators: pNL4.3 ΔEnv. (1) Mock, (2) Ratio 1:1, (3) Ratio 2:1, (4) Ratio 4:1. **(B)** Overexpression of DDX6, LSm1 or PatL1 does not affect intracellular HIV-1 gRNA levels. **(C,D)** Effect of decapping activators co-expression (ratio 2:1) in different combinations repress intracellular Gag expression **(C)** and increase relative gRNA levels compared to the control condition **(D)**.

# Decapping activators relocalize HIV-1 gRNA

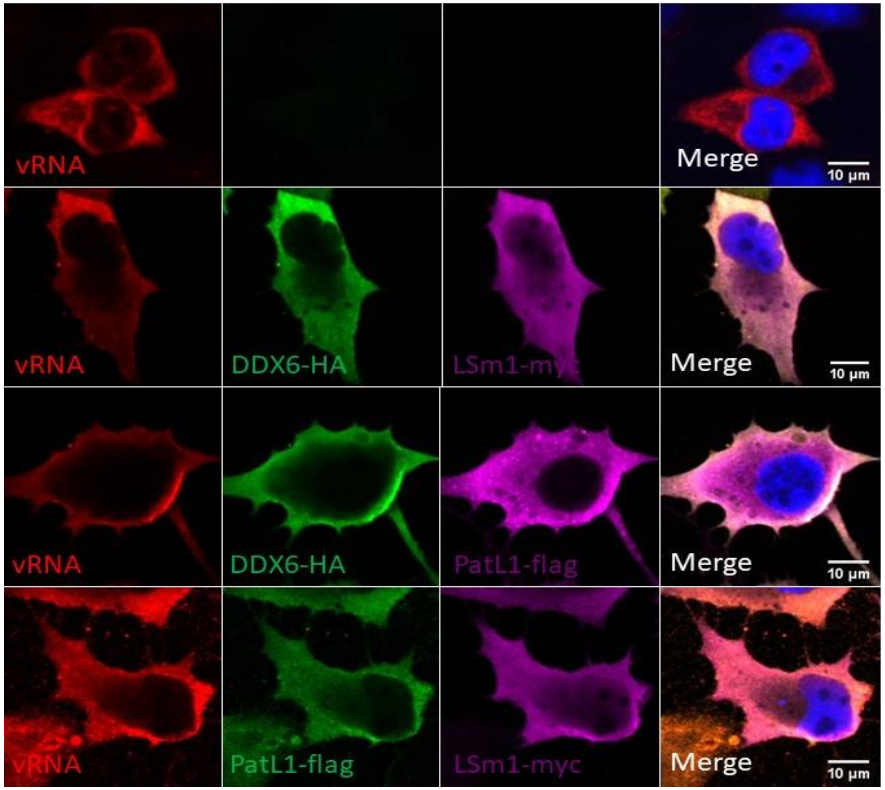


**Figure 3**. U2OS cells were co-transfected with pNL4.3 Δenv and plasmids to overexpress DDX6-HA, LSm1-myc and PatL1-flag in different combinations using a 2:1 ratio (overexpressor:pNL4.3 Δenv). 24 hours post-transfection cells were subjected to FISH to detect HIV-1 gRNA using a specific probe and IF to detect overexpressed proteins. This experiment reveals that HIV-1 gRNA is relocalized when the decapping activators are overexpressed.

# DDX6, LSm1 and PatL1 interact with HIV-1 Gag

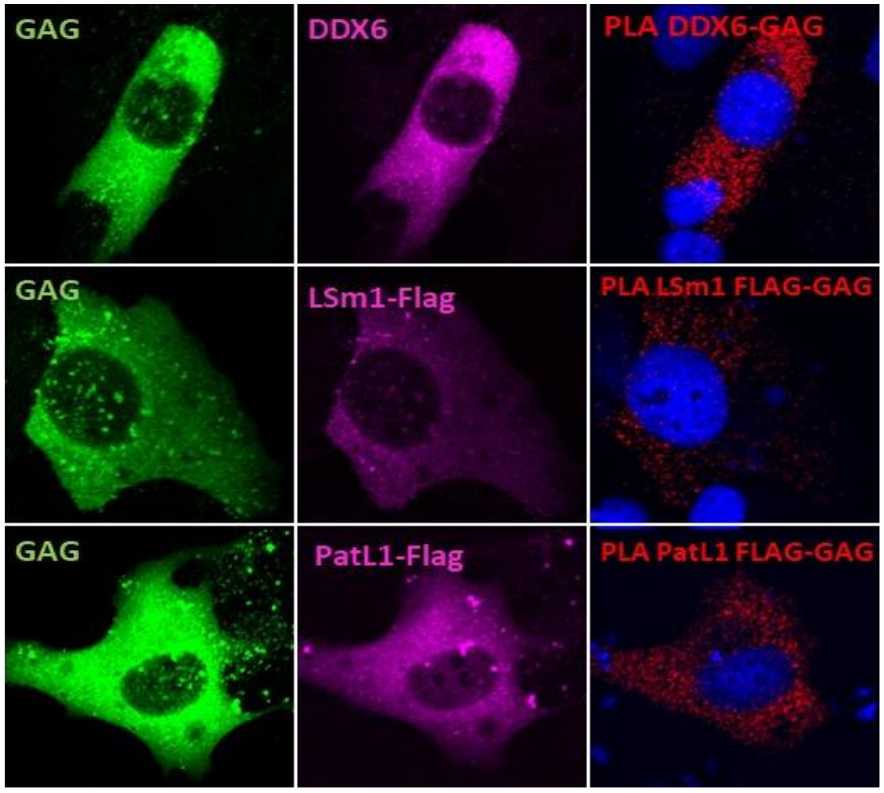


**Figure 4.** Proximity Ligation Assay (PLA) allow the detection of <40 nm proximity between 2 different proteins by confocal microscopy. In this assay we used antibodies against endogenous DDX6 and Flag to detect LSm1-Flag and PatL1-Flag due to the abscence of commercially available antibodies for LSm1 and PatL1 that can be used for immunofluerescence (IF). PLA reveals proximity between all the decapping activators and HIV-1 Gag as can be seen by the presence of red dots in cells expressing HIV-1.

# Conclusions and future perspectives

- Here we show that the overexpression of DDX6 induces a significant decrease in intracellular Gag without affecting gRNA levels, suggesting that DDX6 represses gRNA translation.
- While the overexpression of either LSm1 or PatL1 has little effect on intracellular levels of Gag and gRNA, we show that when these proteins are overexpressed together with DDX6, the repressive role of DDX6 on HIV-1 Gag expression is potentiated, suggesting that they act in complex to repress Gag expression
- The overexpression of the decapping activators in combination results in both a repositioning of the gRNA as well as a significant increase in intracellular levels. These results suggest that these proteins could act stabilizing the viral RNA and allowing its trafficking to the plasma membrane.
- As a future perspective, we will evaluate the effect of these host proteins in HIV-1 gRNA incorporation in viral particles. Given that these proteins have been implicated in the replication cycles of other viruses (e.g., HCV, WNV, BMV), their study represents an additional avenue towards new anti-viral compounds.

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*The authors declare no conflict of interest.