

# Impact of epitranscriptomic modifications on HIV life cycle

## Introduction:

Throughout the human immunodeficiency virus (HIV) replication cycle, complex host-pathogen interactions take place in the infected cell, leading to the successful production of new viral particles. To do so, HIV modulates the host cellular machinery in order to support its life cycle, while counteracting intracellular defense mechanisms. The cellular proteins involved in these processes are called HIV dependency factors (HDF) and HIV inhibitory factors (HIF) respectively.

Epitranscriptomics explores post-transcriptional modifications that occur on RNA molecules and represents a new layer of regulation involved in multiple cellular processes, including RNA splicing, export, stability, and translation. We focused on two mRNA epitranscriptomic modifications, m6A and m5C, which are methylation of A and C residues respectively, and that can be found on mRNAs. We investigated the dynamic host response to HIV infection by systematically measuring these two epitranscriptomic expression changes in infected and uninfected SupT1 CD4+ T cells at three timepoints of the viral replication process. We identified genes that are differentially methylated (DM) and hypothesize that these DM genes may represent novel HDF or HIF candidates. Very interestingly, we also identified a m5C hotspot in the viral RNA that may impact viral translation and thus viral replication.

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## Aim of the project:

The present project contains two main objectives : The first one aims at further characterizing the impact of m5C methylation on viral replication, and in particular on viral translation, This analysis will be carried out in part in a cell line depleted for NSUN2, the enzyme responsible for m5C addition. The second one aims at further characterizing selected candidate genes to assess their putative role on HIV replication. Depending on time available, this will include (i) generating KO cells of the candidate, (ii) testing and confirming the impact of the KO candidate on HIV infection, (iii) confirming the effect of the candidate(s) in primary CD4+ T cells, (iv) further characterizing the candidate(s) (endogenous expression in different cell lines and primary cells, response to immunological stimuli, identification of cellular partners, mechanism of action).

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## Experimental approach:

We will generate a CRISPR-Cas9 KO of top HDF/HIF candidate genes using the lentiCRISPRv2 system. We will then transduce the SupT1 T cell line to generate KO cells and infect them with an HIVeGFP-based vector. Cell permissiveness to HIVeGFP will be assessed by the proportion of successfully infected cells expressing GFP, and any alteration compared to the control will indicate an effective impact of the KO candidate gene on HIV replication.

For confirmed HDF or HIF candidates, further characterization will be performed according to the time available, i.e. confirmation of the effect, confirmation of the KO, identification of the viral step impacted, assessment of direct interaction with a viral protein, protein mutations, protein interactome.

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## Significance:

Uncovering the role of m5C methylation on viral translation and identifying novel cellular factors modulating HIV replication cycle and understanding their mechanism of action will improve understanding of HIV biology and uncover novel potential drug targets.