A targeted CRISPR screen identifies ETS1 as a regulator of HIV latency

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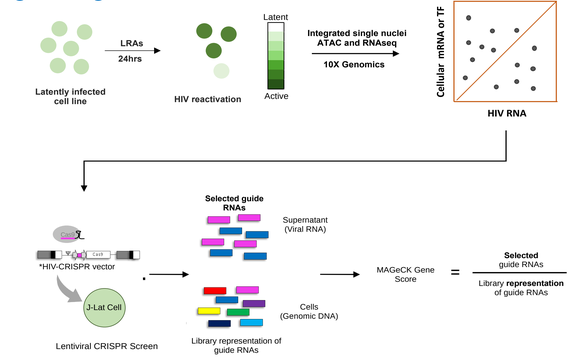
BACKGROUND

Reactivation of latently infected cells with existing potential latency reversing agents (LRAs) is inefficient and is likely due to a combination of stochastic processes that regulate viral gene expression and the existence of multiple layers of repression to HIV gene expression in CD4 T cells.

Lenti-CRISPR transduction and CRISPR nucleofection was employed in J-Lat and primary CD4 cell models to screen the host cell transcripts **that control HIV gene expression in response to hallmark LRAs**

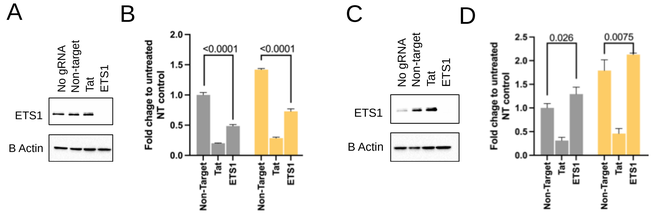
METHODS

Schematic of targeted CRISPR screen for novel latency regulating factors



RESULTS

ETS1 knockout in primary CD4 T cells reveals positive and negative roles in HIV expression



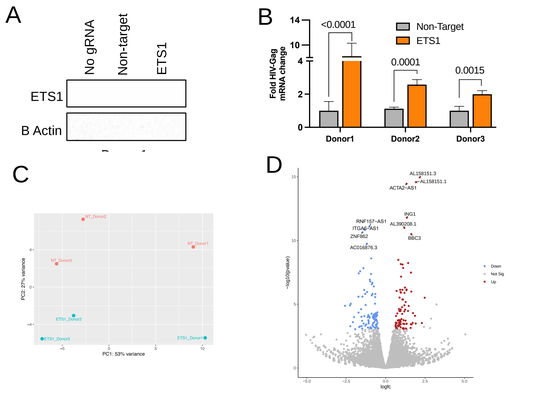
Western blot of the ETS1-targeting or a control Cas9/gRNA nucleofected primary cells for beta actin and ETS1 expression at one **(A)** and two weeks **(C)** post nucleofection. Flow cytometry of infected cells without stimulation (Gray) or after 24 h of stimulation with vorinostat (Yellow) at one **(B)** and two **(D)** week post nucleofection.

ETS1 can play both a positive and negative role in HIV expression. ETS1 maintains transcriptional repression of the clinical HIV reservoir.

Transcriptomic profiling of ETS1-depleted cells displayed increased expression of the long non-coding RNA MALAT1.

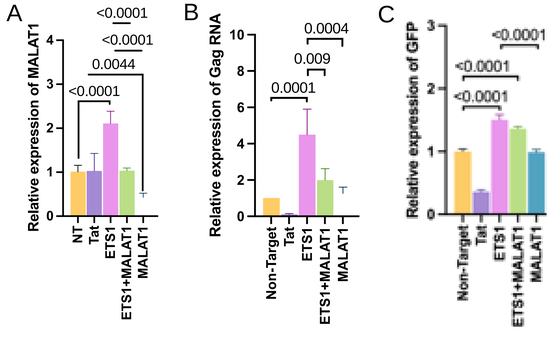
ETS1 knockout resulted enhanced abundance of activating modifications (H3K9Ac, H3K27Ac, H3K4me3) on histones located at the HIV long terminal repeat.

ETS1 knockout reactivates HIV transcription in CD4 T cells from clinical reservoir and found the depletion of ETS1 upregulates the MALAT1



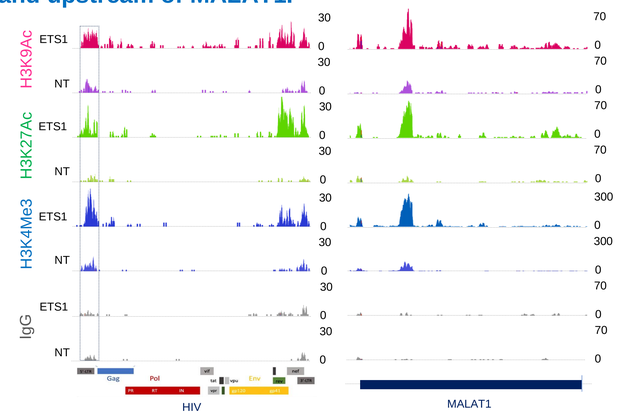
CRISPR nucleofection of ETS1- or NT gRNAs was performed after a 24 hr culture of PWH CD4 T cells followed by quantification of Gag RNA expression at 3 days post nucleofection by quantitative PCR. **(A)** Depletion of ETS1 was analyzed by western blot. **(B)** Relative abundance of Gag viral RNA expression. **(C)** PCA plot of samples based on gene expression data for each sample. **(D)** Volcano plot of overall differentially expressed genes (DEGs) between HIV infected CD4 T cells nucleofected with ETS1 vs NT gRNAs.

Depletion of ETS1 increased MALAT1 RNA and vRNA expression, and depletion of MALAT1 inhibited the vRNA expression within latently infected cells.



In vitro generated primary latent CD4 cells were nucleofected with Cas9/gRNA ribonucleoprotein complexes targeting ETS1, MALAT1, or a combination of ETS1 and MALAT1. Relative abundance of MALAT1 RNA expression (**A**), and Gag RNA (**B**), and HIV protein expression (**C**). MALAT1 and Gag viral RNA expression was normalized to cells nucleofected with NT control.

ETS1 depletion increases the abundance of transcription activating histone marks at the HIV LTR and upstream of MALAT1.



J-Lat 10.6 cells were nucleofected with ETS1 targetting or control non-targetting gRNAs. At 1 week post nucleofection, nucleofected cells were analyzed by Cleavage Under Targets & Release Using Nuclease (CUT&RUN) using antibodies against H3K9ac, H3K27ac and controls H3K4Me3 and IgG. Normalized coverage of sequencing reads across the HIV reference genome and MALAT1 is displayed using Integrative Genomics Viewer version 2.17.

CONCLUSIONS

Overall, these data indicate that **ETS1 is an important regulator of HIV latency** and could represent a useful target for latency reversing strategies.

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