CPSF6 Regulates Cellular Permissivity to HIV-1 Infection Through Alternative Polyadenylation

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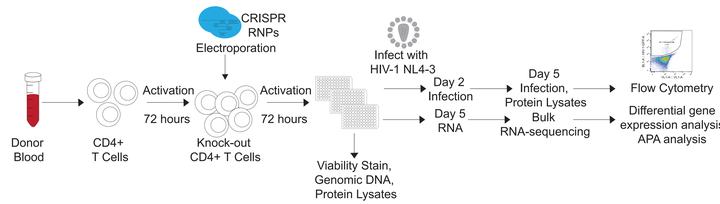
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Background

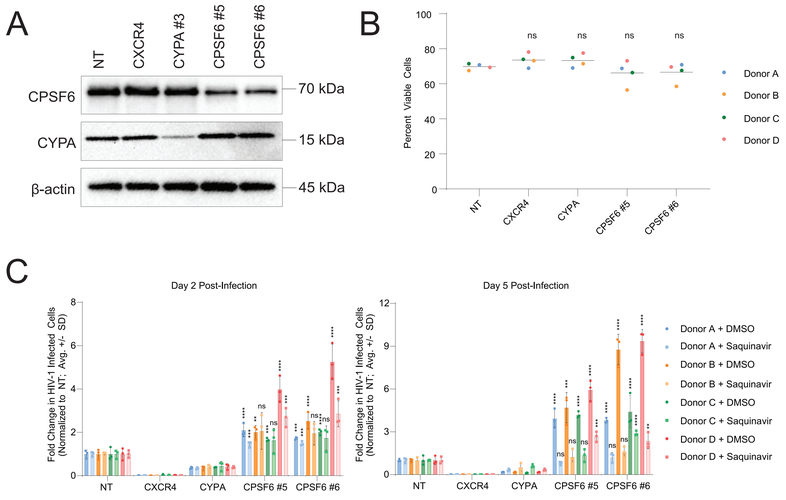
Cleavage and polyadenylation specificity factor 6 (CPSF6) is a host factor that is recruited to HIV-1 capsid cores and plays a multifaceted role in the viral replication cycle[1]. *CPSF6* knock-out phenotypes in cell lines are highly variable, leading to continued debate surrounding the role of CPSF6 in HIV-1 infection. Outside of HIV-1 infection, CPSF6 plays a role in mRNA processing and poly(A) site selection as a member of the CFIm complex[2]. Disruption of CPSF6 function leads to altered poly(A) site selection through a process known as alternative polyadenylation (APA), resulting in usage of proximal poly(A) sites and shortening of 3' UTRs[2]. Changes in APA have been linked to regulation of the antiviral response to other viruses, but the role of APA in HIV-1 infection remains unknown[3]. Many previously described roles of CPSF6 in HIV-1 infection are dependent upon CPSF6 binding to HIV-1 capsid cores. Several studies have shown that this interaction can lead to relocalization of CPSF6 to nuclear speckles in some cell types[4]. We hypothesize that disruption of CPSF6 function, either through knock-out or recruitment of CPSF6 by HIV-1 capsid cores, leads to changes in APA and altered cellular permissivity to HIV-1 infection.

Methods

To explore the role of CPSF6 in HIV-1 infection, we leveraged CRISPR-Cas9 gene editing to knock out *CPSF6* in primary CD4+ T cells. Edited cells were used in spreading infection assays with HIV-1 NL4-3. We assessed the impact of *CPSF6* knock-out on gene expression in primary CD4+ T cells using bulk RNA-sequencing. We analyzed RNA-sequencing data from *CPSF6* knock-out and HIV-1 infected cells using an algorithm to determine changes in poly(A) site usage[5].

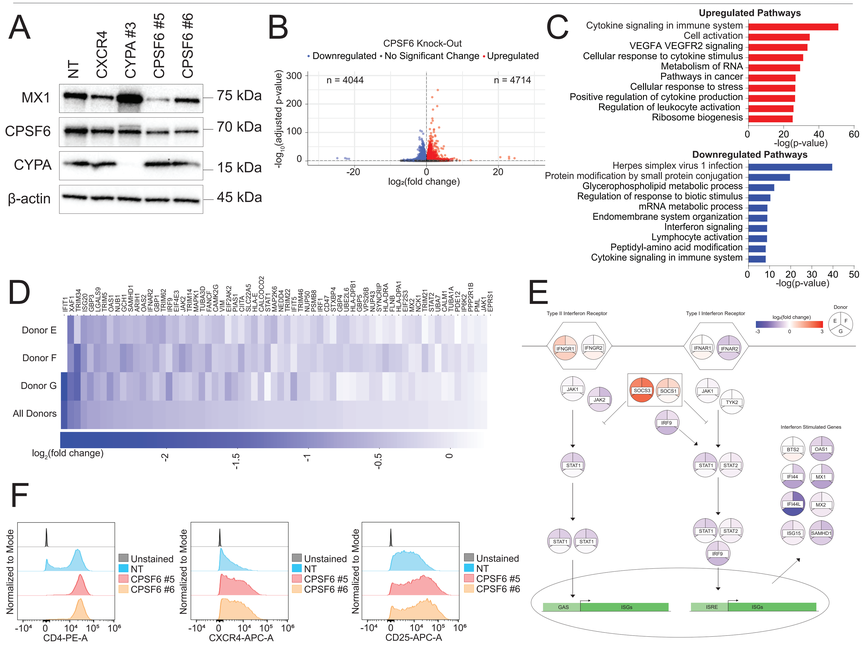


CPSF6 knock-out primary CD4+ T cells are more permissive to HIV-1 infection



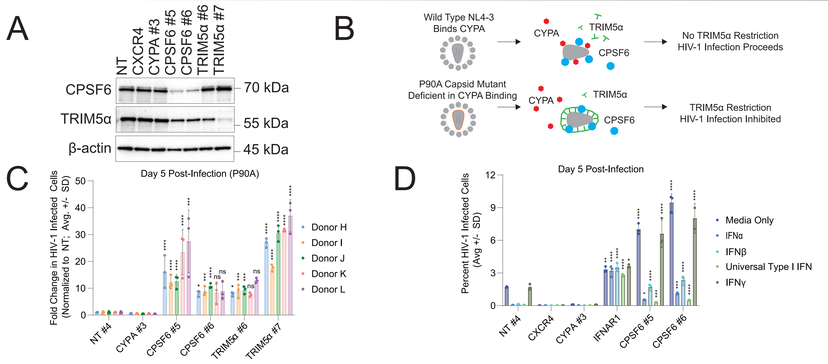
A) Immunoblot shows knock-out of host factors *CPSF6* and Cyclophilin A (*CYPA*) in primary CD4+ T cells lysates harvested at day 4 post-editing. One representative donor shown. B) Cell viability as measured by fluorescent amine dye 4 days post-electroporation in 4 representative donors. C) Bar chart shows HIV-1 infection rates normalized to non-targeting (NT) controls at day 2 (left) and day 5 (right) of spreading infection assay with HIV-1 NL4-3 Nef:IRES:GFP in knock-out primary CD4+ cells as assessed by flow cytometry. Cells were treated with the protease inhibitor Saquinavir or a DMSO control 24 hours post-infection to assess both single-round and spreading infection rates. Four donors shown with average of technical triplicates +/- SD. Statistics calculated using an ANOVA followed by Dunnett's test for multiple comparisons, ns = not significant, * = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001, **** = p ≤ 0.0001.

CPSF6 knock-out in primary CD4+ T cells leads to transcriptional rewiring



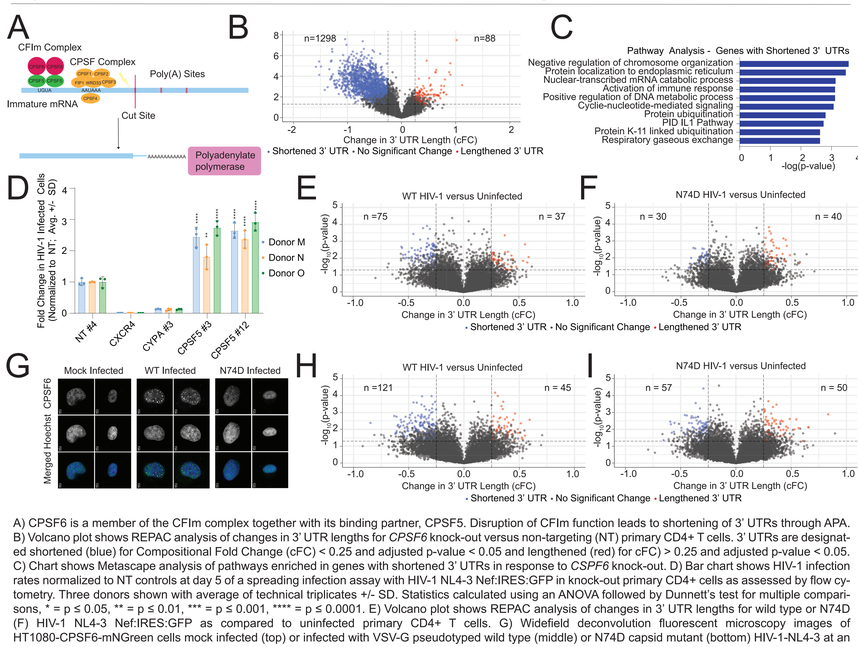
A) Immunoblot shows expression of representative ISG (MX1) in protein lysates harvested from HIV-1-infected primary CD4+ T cells at day 5 post-infection. One representative donor shown. B) Volcano plot shows differentially expressed genes in RNA-seq data from uninfected *CPSF6* knock-out primary CD4+ T cells as compared to non-targeting controls. C) Charts show Metascape pathway analysis of top 10 upregulated (top, red) and downregulated (bottom, blue) pathways in aggregate RNA-seq data from three donors ranked by log(p-value). D) Heatmap shows log(fold change) for three donors overlayed on schematic of signaling events downstream of the type I and type II interferon receptors, including ISGs that are induced by these signaling pathways. E) Heatmap shows differential expression of genes in the Reactome term "Interferon Gamma Pathway Signaling" (R-HSA-877300) as shown by log(fold change) for each indicated gene. F) Histograms show fluorescence intensity of CD4 (left), CXCR4 (middle), and CD25 (right) in primary CD4+ T cells from one representative biological replicate at day five post-editing as measured by immunostaining and flow cytometry. Y-axis is normalized to the mode, histogram visualized using FlowJo version 10.10

CPSF6 knock-out cells are less sensitive to interferon and more susceptible to TRIM5α-sensitive capsid mutants



A) Immunoblot shows reduction in TRIM5α protein levels in lysates from *CPSF6* and *TRIM5α* knock-out primary CD4+ T cells harvested at day 4 post-editing. B) Wild type HIV-1 NL4-3 capsid binds CYPA, which shields from TRIM5α restriction. The P90A capsid mutant HIV-1 derived from NL4-3 NEF:IRES:GFP is deficient in CYPA binding, leading to TRIM5α-mediated restriction. C) Bar chart shows HIV-1 infection rates normalized to non-targeting (NT) controls at day 5 of spreading infection assay in knock-out primary CD4+ cells infected with P90A HIV-1. Five representative donors shown with average of technical triplicates +/- SD. D) Bar chart shows HIV-1 infection rates at day 5 of spreading infection assay in knock-out primary CD4+ cells pre-treated with 100 U/mL IFNα, IFNβ, IFNγ, Universal Type I IFN, or media only control. One representative donor shown with average of technical triplicates +/- SD. Statistics calculated using an ANOVA followed by Dunnett's test for multiple comparisons, ns = not significant, * = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001, **** = p ≤ 0.0001.

CPSF6 knock-out and recruitment of CPSF6 by HIV-1 cores lead to changes in APA

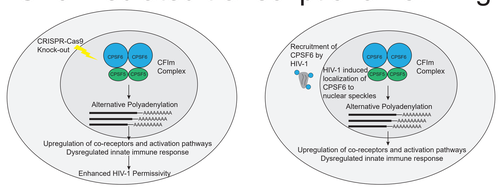


A) CPSF6 is a member of the CFIm complex together with its binding partner, CPSF5. Disruption of CFIm function leads to shortening of 3' UTRs through APA. B) Volcano plot shows REPAC analysis of changes in 3' UTR lengths for *CPSF6* knock-out versus non-targeting (NT) primary CD4+ T cells. 3' UTRs are designated shortened (blue) for Compositional Fold Change (cFC) < 0.25 and adjusted p-value < 0.05 and lengthened (red) for cFC) > 0.25 and adjusted p-value < 0.05. C) Chart shows Metascape analysis of pathways enriched in genes with shortened 3' UTRs in response to *CSPF6* knock-out. D) Bar chart shows HIV-1 infection rates normalized to NT controls at day 5 of a spreading infection assay with HIV-1 NL4-3 Nef:IRES:GFP in knock-out primary CD4+ cells as assessed by flow cytometry. Three donors shown with average of technical triplicates +/- SD. Statistics calculated using an ANOVA followed by Dunnett's test for multiple comparisons, * = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001, **** = p ≤ 0.0001. E) Volcano plot shows REPAC analysis of changes in 3' UTR lengths for wild type or N74D (F) HIV-1 NL4-3 Nef:IRES:GFP as compared to uninfected primary CD4+ T cells. G) Widefield deconvolution fluorescent microscopy images of HT1080-CPSF6-mNGreen cells mock infected (top) or infected with VSV-G pseudotyped wild type (middle) or N74D capsid mutant (bottom) HIV-1-NL4-3 at an MOI of 10 at 6 hours post-infection. CPSF6-mNGreen in green, Hoechst DNA stain in blue. H) Volcano plot shows REPAC analysis of changes in 3' UTR lengths for WT VSV-G-pseudotyped HIV-1-NL4-3-infected or N74D infected (I) versus uninfected HT1080 cells from 3 technical replicates. Cells were infected at an MOI of 10 and RNA was extracted at 6 hours post-infection.

Conclusions and Future Directions

- *CPSF6* knock-out in primary CD4+ T cells enhances cellular permissivity to HIV-1 infection and leads to broad transcriptional rewiring characterized by differential expression of the interferon signaling pathway and known HIV-1 host factors, including TRIM5α
- *CPSF6* knock-out triggered changes in APA and shortening of 3' UTR lengths
- Infection of primary CD4+ T cells with HIV-1 viruses that bind CPSF6 led to changes in APA and shortening of 3' UTRs
- HT1080 cells, which exhibit a greater degree of relocalization of CPSF6 in cell nuclei in response to HIV-1 infection, undergo more extensive shortening of 3' UTRs following HIV-1 infection
- Together, these data suggest that disruption of CPSF6 function leads to transcriptional rewiring driven by changes in APA, resulting in enhanced permissivity to HIV-1 infection in some cell types

Model of CPSF6-mediated transcriptional rewiring in CD4+ T cells



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Acknowledgements

This work was supported by NIH grant 1R01AI165236-01 and HARC grant U54 AI170792.

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