CRISPR Screening Reveals Mitochondrial Dysfunction as a Novel Mechanism of HIV Reactivation

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BACKGROUND

HIV persists in people living with HIV by establishing a latent reservoir in CD4⁺ T cells, where the virus remains transcriptionally silent and is not eliminated by antiretroviral therapy. This latent reservoir is the major barrier to HIV cure, and identifying host cellular mechanisms that maintain latency is essential for developing effective latency-reversing or eradication strategies. CRISPR-based functional genomic screening provides a powerful and unbiased approach to systematically identify host genes that regulate HIV latency. In this study, we used the dual-reporter HIVGKO system, which enables precise identification of latently infected and reactivated cells through differential expression of GFP and mKO2, to perform a genome-wide CRISPR screen aimed at uncovering novel host factors controlling HIV latency.

METHODS

Jurkat T cells were infected with HIVGKO to generate Jurkat GKO Latent (JGL) cells, and 5 million mKO2-positive latently infected cells were isolated by FACS on day 5 post-infection. A genome-wide CRISPR library containing approximately 120,000 guide RNAs targeting all protein-coding genes was transduced into JGL cells. On day 7, GFP-positive reactivated cells were collected by FACS sorting. Genomic DNA was extracted, next-generation sequencing was performed, and guide RNA abundance was compared with pre-sort cells to identify genes involved in latency maintenance. Candidate genes were individually knocked out in JGL and J-Lat clones, and HIV reactivation was evaluated by flow cytometry, RT-qPCR, and Western blotting. Autophagy was assessed by LC3 Western blotting, mitophagy was measured using mitochondrial Keima (mt-Keima) reporter flow cytometry, and autophagy-related chemical compounds were tested for their effects on HIV reactivation.

RESULTS

Fig. 2

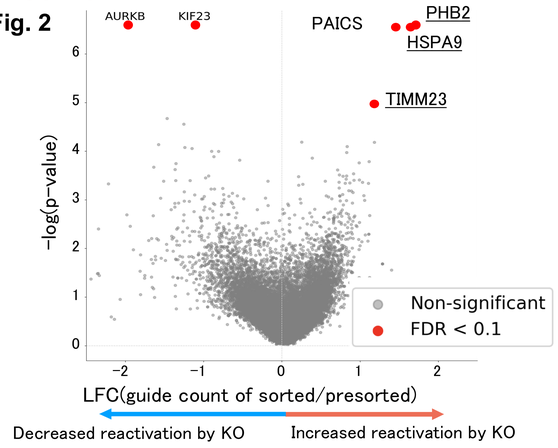


Fig. 2 Volcano plot of the CRISPR screening. Four genes involved in maintaining HIV latent infection were identified, and three of them encoded mitochondrial proteins (underline).

Fig. 5

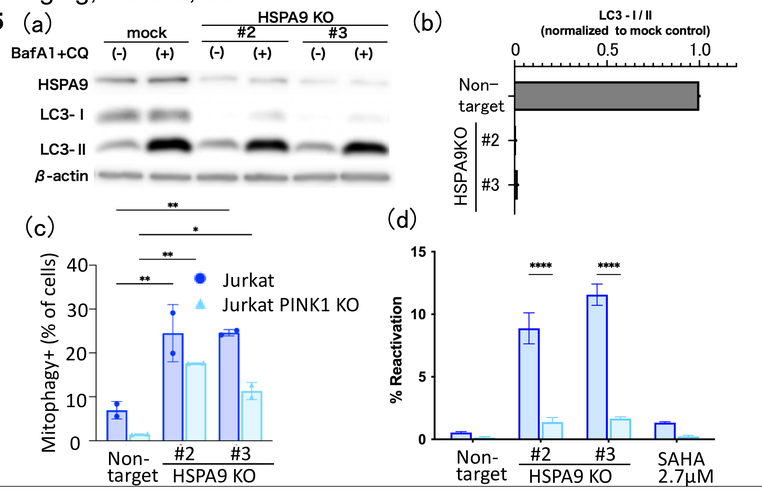


Fig. 4 Knock Out of HSPA9 Induced Autophagy and Mitophagy via Parkin/PINK1 Pathway. (a) Western blotting for LC3, (b) LC3 I/II ratio quantified using Fiji, (c) Mitophagy and (d) HIV Reactivation induced by knockout of HSPA9 depending on Parkin/PINK1pathway. Knockout of PINK1 decreased HSPA9 knockout-induced mitophagy and HIV-1 reactivation.

Fig. 6

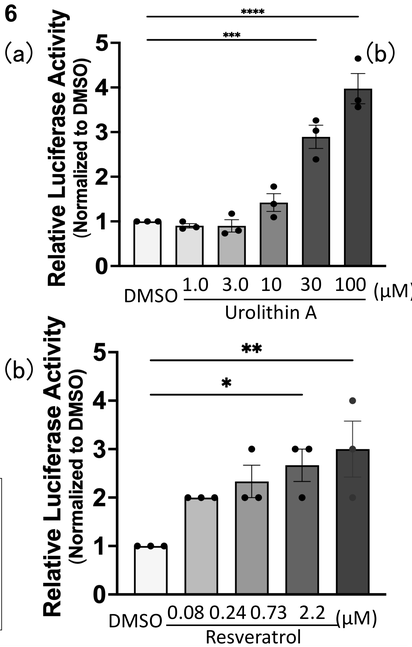


Fig.6 Mitophagy inducer, Urolithin A (a) and Resveratrol (b), induced HIV-LTR promoter-driven transcription evaluated by HIV-LTR Luc assay in HEK293T.

Fig. 3

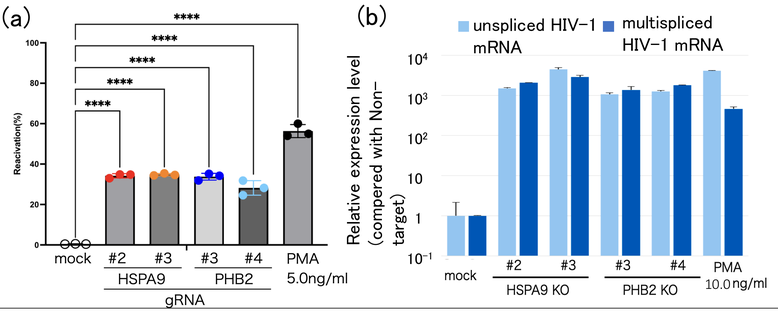


Fig. 3 Knock Out of PHB2 or HSPA9 Reactivated HIV-1 in JGL
(a) Knockout of these genes induced HIV reactivation in JGL cells (18.3%, 20.0%, 6.0%, and 17.3%, respectively (b) RT-qPCR of HIV-1 mRNA transcription normalized by β-actin in JGL cells following PHB2 or HSPA9 knockout. # : gRNA number

Fig. 7

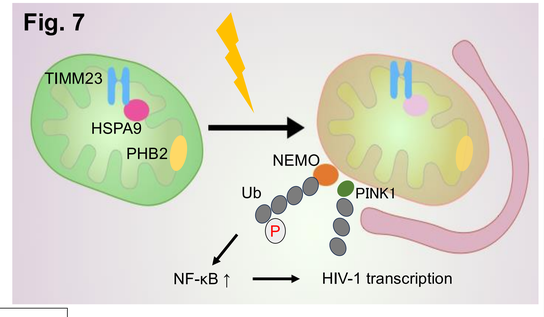


Fig. 7. Working hypothesis.
Mitochondrial dysfunction promotes the recruitment and aggregation of NEMO, leading to activation of NF-κB signaling (Harding et al., *Mol. Cell 2023*). Activated NF-κB enhances transcription from the HIV LTR, thereby inducing viral reactivation.

Fig. 1

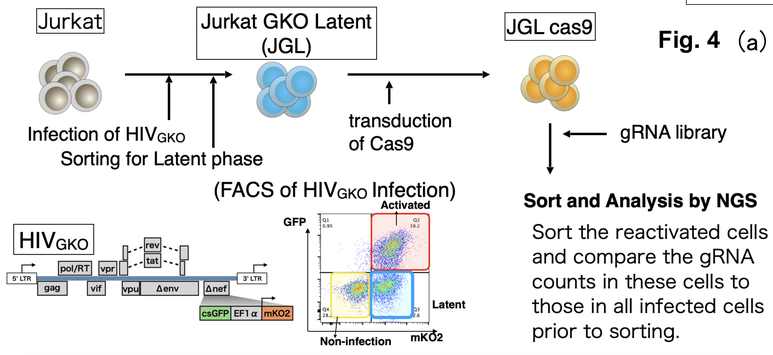


Fig. 1. Schematic of the CRISPR screening strategy and HIV$_{GKO}$

Fig. 4

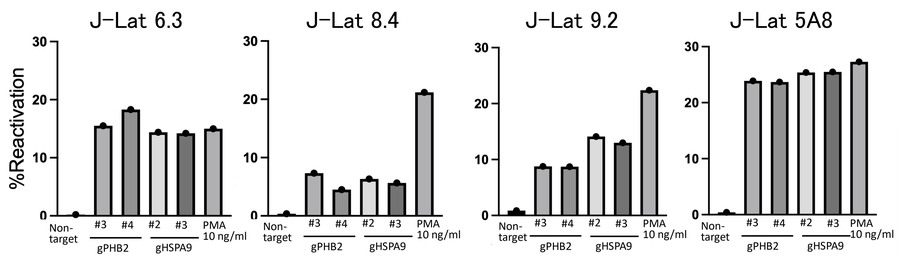


Fig. 4 Knock Out of PHB2 or HSPA9 Reactivated HIV-1 in Various J-Lat Cell Lines (a) %reactivation of Knockout of PHB2 or HSPA9 in J-Lat cell lines. # : gRNA number

CONCLUSIONS

Our genome-wide CRISPR screening identifies mitochondrial-associated genes as key regulators of HIV latency and demonstrates that mitochondrial dysfunction and mitophagy can induce HIV reactivation. These findings uncover a previously unrecognized role of mitochondrial homeostasis in maintaining HIV latency and suggest that targeting mitochondrial pathways may represent a novel therapeutic strategy to eliminate the latent HIV reservoir in HIV cure approaches.

PLAIN LANGUAGE SUMMARY

HIV can remain hidden in immune cells in a silent state that prevents a cure; using genome-wide CRISPR screening, we identified four human genes—three linked to mitochondria—that help maintain this latency, and disrupting them reactivated HIV.
These findings show that mitochondrial dysfunction can trigger HIV to wake up, suggesting that targeting mitochondrial pathways may provide a new strategy to eliminate the hidden HIV reservoir and advance cure research.