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HIV Cure Center
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Strategies for the depletion of HIV reservoir by activation of ISR signaling

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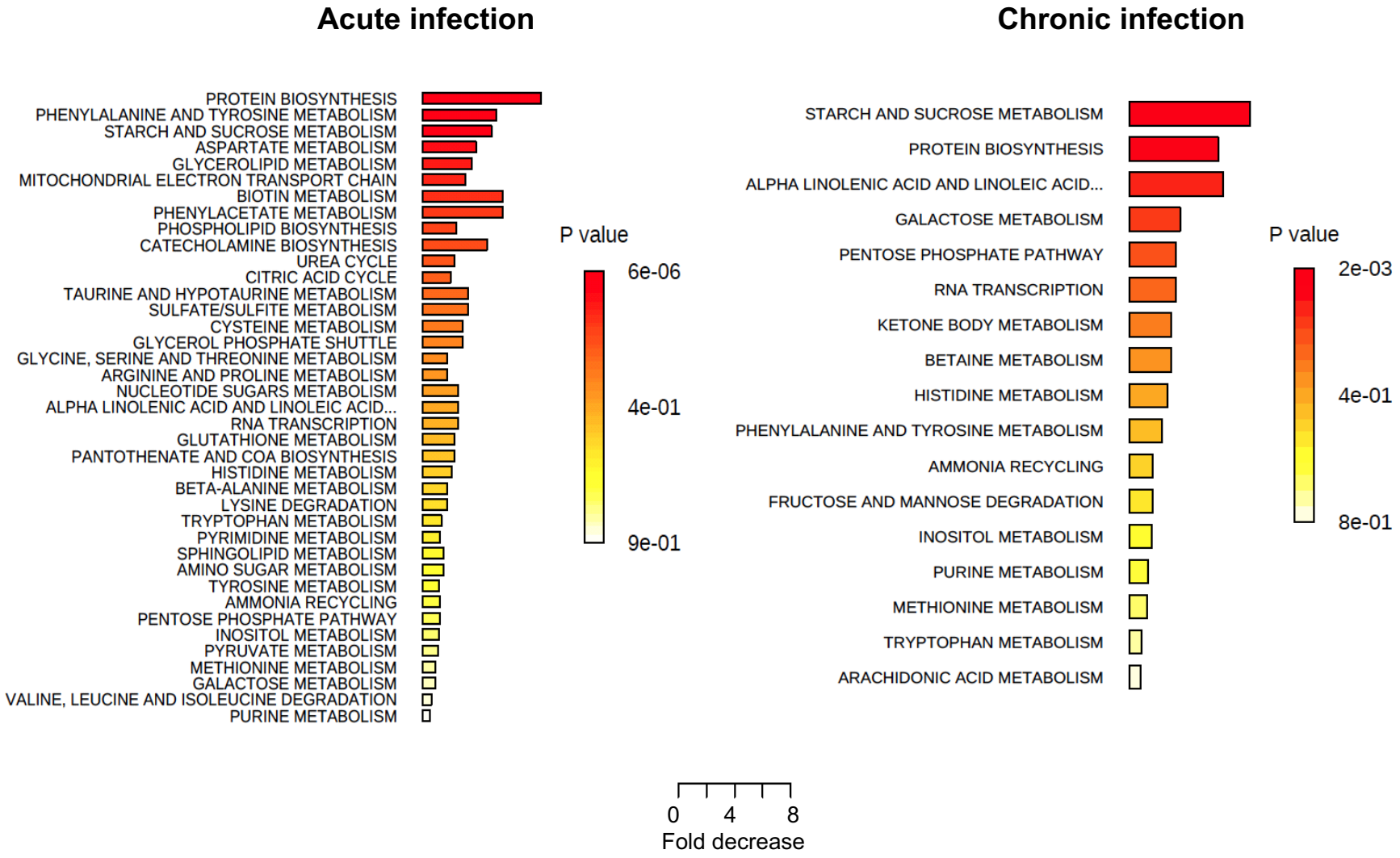
UNC-Chape Hill

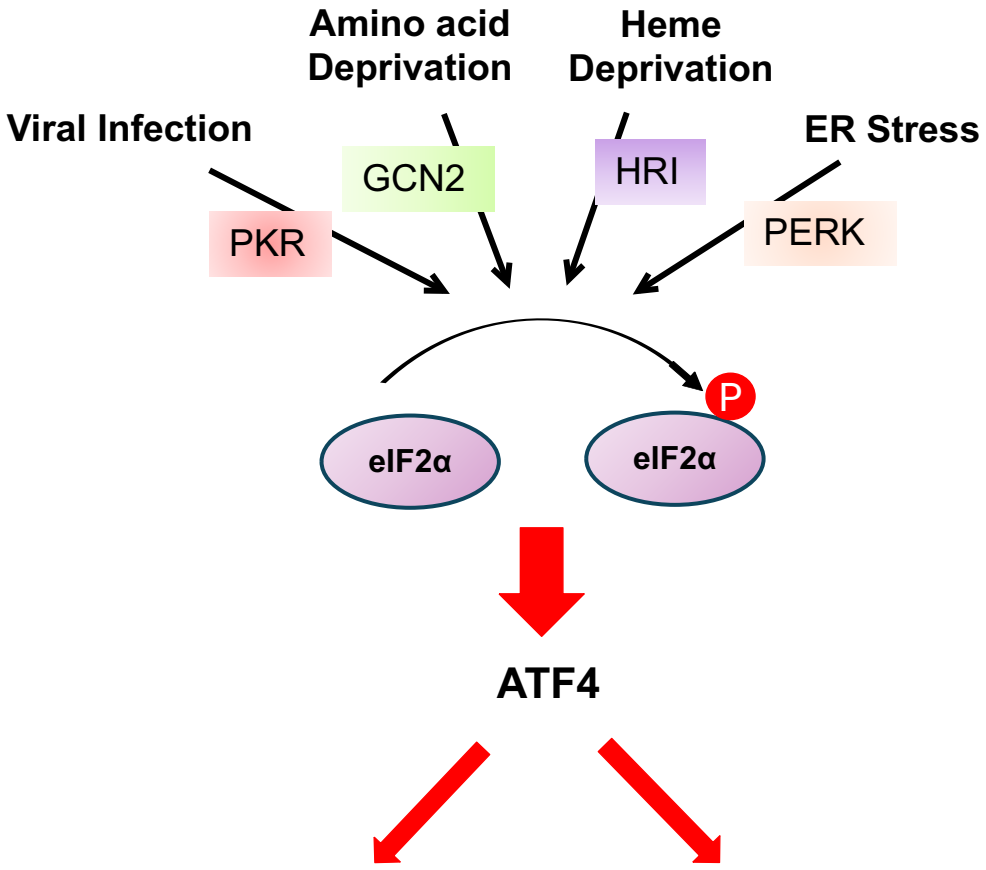
NIMH Satellite Session/HIV Persistence During Therapy, 12/10/2024

SIV infection decreases many amid acids and reduces protein synthesis in the gut in vivo

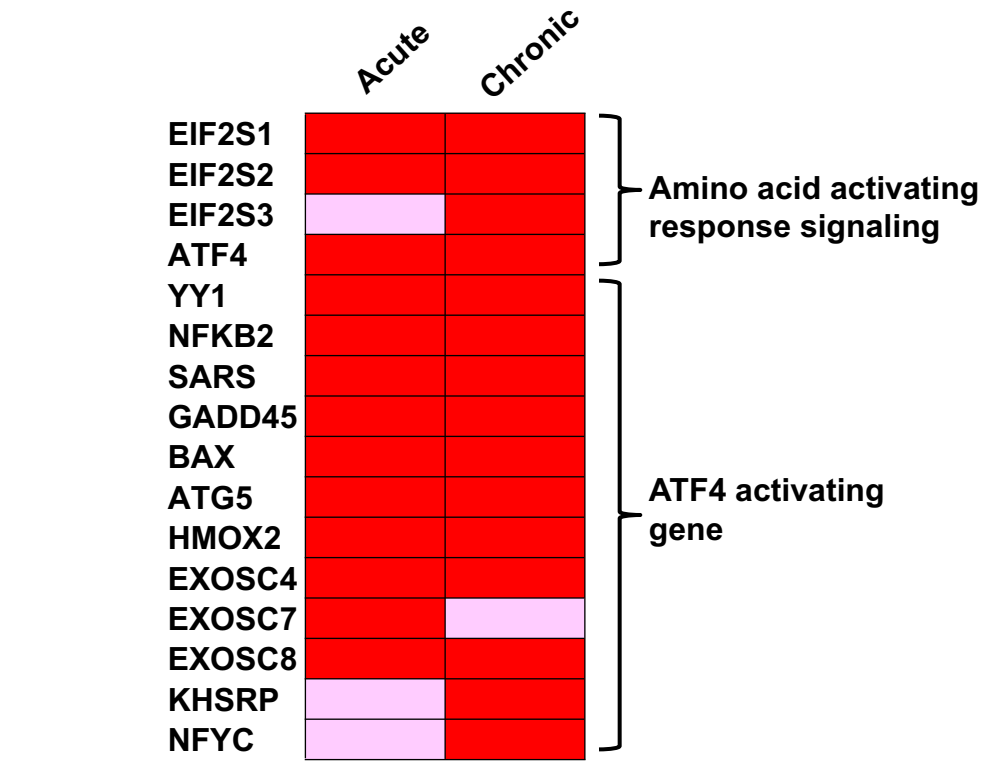


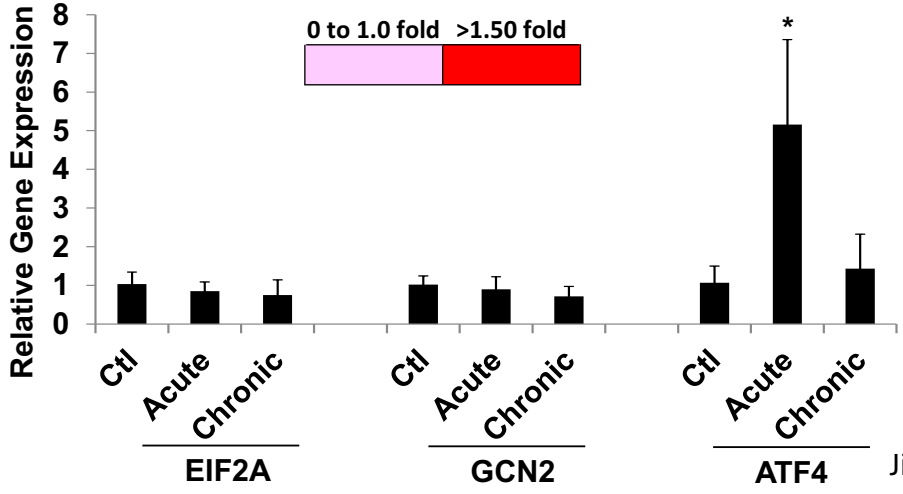
Jiang G, et al., mBio, 2017

Integrated Stress Response (ISR)



ATF4-transactivated downstream gene transcription and signaling





Jiang G, et al., mBio, 2017

Amino acid starvation induces reactivation of silenced transgenes and latent HIV-1 provirus via down-regulation of histone deacetylase 4 (HDAC4)

Ilaria Palmisano[a], Giulia Della Chiara[b], Rosa Lucia D'Ambrosio[a], Claudia Huichalaf[c], Paola Brambilla[d], Silvia Corbetta[b], Michela Riba[a], Rosanna Piccirillo[e], Sergio Valente[f], Giorgio Casari[a,g], Antonello Mai[f], Filippo Martinelli Boneschi[d], Davide Gabellini[c], Guido Poli[b,g,1], and Maria Vittoria Schiaffino[a,1]

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Edited* by Robert C. Gallo, Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD, and approved June 26, 2012 (received for review February 10, 2012)

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 28, Number 8, 2012

DOI: 10.1089/aid.2011.0252

Short Communication
Activating Transcription Factor 4 (ATF4) Promotes HIV Type 1 Activation

Elisabetta Caselli, Sabrina Benedetti, Valentina Gentili, Jessica Grigolato, and Dario Di Luca

➢ Our published study and others showed that ISR/ATF4 activation disrupts latent HIV while ISR/ATF4 suppression is involved in HIV latency

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HIV Exploits Antiviral Host Innate GCN2-ATF4 Signaling for Establishing Viral Replication Early in Infection

Guochun Jiang,[a] Clarissa Santos Rocha,[a] Lauren A. Hirao,[a] Erica A. Mendes,[a] Yuyang Tang,[b] George R. Thompson III,[a] Joseph K. Wong,[c] Satya Dandekar[a]

Department of Medical Microbiology and Immunology, University of California, Davis, California, USA[a]; Department of Molecular and Cellular Biology, University of California, Davis, California, USA[b]; Department of Medicine, University of California, San Francisco, and San Francisco VAMC, San Francisco, California, USA[c]

ABSTRACT Antiviral innate host defenses against acute viral infections include suppression of host protein synthesis to restrict viral protein production. Less is known about mechanisms by which viral pathogens subvert host antiviral innate responses for establishing their replication and dissemination. We investigated early innate defense against human immunodeficiency virus (HIV) infection and viral evasion by utilizing human CD4$^+$ T cell cultures *in vitro* and a simian immunodeficiency virus (SIV) model of AIDS *in vivo*. Our data showed that early host innate defense against the viral infection involves GCN2-ATF4 signaling-mediated suppression of global protein synthesis, which is exploited by the virus for supporting its own replication during early viral infection and dissemination in the gut mucosa. Suppression of protein synthesis and induction of protein kinase GCN2-ATF4 signaling were detected in the gut during acute SIV infection. These changes diminished during chronic viral infection. HIV replication induced by serum deprivation in CD4$^+$ T cells was linked to the induction of ATF4 that was recruited to the HIV long terminal repeat (LTR) to promote viral transcription. Experimental inhibition of GCN2-ATF4 signaling either by a specific inhibitor or by amino acid supplementation suppressed the induction of HIV expression. Enhancing ATF4 expression through selenium administration resulted in reactivation of latent HIV *in vitro* as well as *ex vivo* in the primary CD4$^+$ T cells isolated from patients receiving suppressive antiretroviral therapy (ART). In summary, HIV/SIV exploits the early host antiviral response through GCN2-ATF4 signaling by utilizing ATF4 for activating the viral LTR transcription to establish initial viral replication and is a potential target for HIV prevention and therapy.

ARTICLES
https://doi.org/10.1038/s41564-020-0742-9
nature microbiology
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FOXO1 promotes HIV latency by suppressing ER stress in T cells

Albert Vallejo-Gracia[1,2], Irene P. Chen[1,2,6], Rosalba Perrone[3,6], Emilie Besnard[3], Daniela Boehm[1,2], Emilie Battivelli[3], Tugsan Tezil[3], Karsten Krey[1,4], Kyle A. Raymond[5], Philip A. Hull[1], Marius Walter[3], Ireneusz Habrylo[1,2], Andrew Cruz[3], Steven Deeks[2], Satish Pillai[2,5], Eric Verdin[2,3] and Melanie Ott[1,2]✉

Quiescence is a hallmark of CD4$^+$ T cells latently infected with human immunodeficiency virus 1 (HIV-1). While reversing this quiescence is an effective approach to reactivate latent HIV from T cells in culture, it can cause deleterious cytokine dysregulation in patients. As a key regulator of T-cell quiescence, FOXO1 promotes latency and suppresses productive HIV infection. We report that, in resting T cells, FOXO1 inhibition impaired autophagy and induced endoplasmic reticulum (ER) stress, thereby activating two associated transcription factors: activating transcription factor 4 (ATF4) and nuclear factor of activated T cells (NFAT). Both factors associate with HIV chromatin and are necessary for HIV reactivation. Indeed, inhibition of protein kinase R-like ER kinase, an ER stress sensor that can mediate the induction of ATF4, and calcineurin, a calcium-dependent regulator of NFAT, synergistically suppressed HIV reactivation induced by FOXO1 inhibition. Thus, our studies uncover a link of FOXO1, ER stress and HIV infection that could be therapeutically exploited to selectively reverse T-cell quiescence and reduce the size of the latent viral reservoir.

BMB Reports

BMB Rep. 2018; 51(8): 388-393
www.bmbreports.org

Understanding of the functional role(s) of the Activating Transcription Factor 4(ATF4) in HIV regulation and production

Seong-Deok Lee[1], Kyung-Lee Yu[1], Seong-Hyun Park[1], Yu-Mi Jung[1], Min-Jeong Kim[2] & Ji-Chang You[1,2,]*

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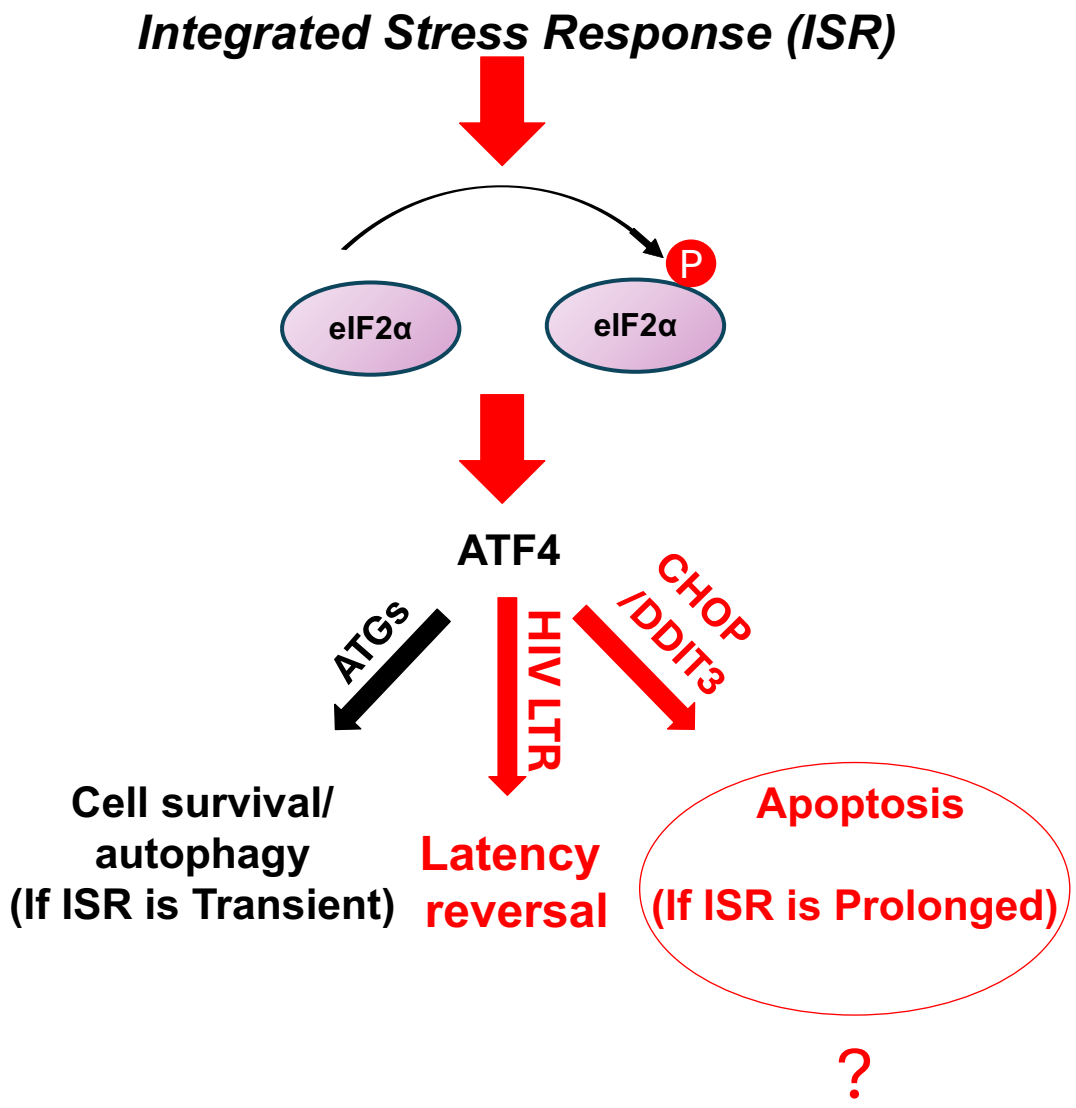
The activating transcription factor (ATF) 4 belongs to the ATF/CREB (cAMP Response Element Binding bZIP [Basic Leucine Zipper]) transcription factor family, and plays a central role in the UPR (Unfolded Protein Response) process in cells. The induction of ATF4 expression has previously been shown to increase the replication of HIV-1. However, the detailed mechanism underlying this effect and the factors involved in the regulation of ATF4 function are still unknown. Here, we demonstrate first that knocking out ATF4 using siRNA shows a strong negative effect on HIV-1 production, indicating that ATF4 is a functional positive cellular factor in HIV-1 production. To determine the mechanism by which ATF4 regulates the HIV-1 life cycle, we assessed the effect of the overexpression of wild type ATF4 and its various derivatives on HIV-1 LTR-mediated transcriptional activation and the production of HIV-1 particles. This effect was studied through co-transfection experiments with either reporter vectors or proviral DNA. We found that the N-terminal domains of ATF4 are involved in HIV-1 LTR-mediated transcriptional activation, and thus in HIV-1 production. [BMB Reports 2018; 51(8): 388-393]

mediates the adaptation of cells to stressors such as endoplasmic reticulum (ER) stress and nutrient deprivation (3). These stressors induce ATF4 expression, resulting in the activation of the unfolded protein response (UPR) pathway, which in turn increases the translation of ATF4 but reduces global protein synthesis (1, 4, 5). ATF4 is capable of forming both homodimers and heterodimers (6). The N-Terminus of ATF4, which is called the p300 interaction site, functions as a transcriptional activation domain and is implicated in protein stability as well (7-9). A basic-leucine zipper (bZIP) domain is located in the C-Terminus of ATF4 and is known to interact with DNA (6, 10). The leucine zipper of the bZIP domain contains alpha helices with leucine residues that mediate dimerization with a parallel leucine zipper domain (11). ATF4 can be localized to the nucleus by a nuclear localization signal sequence KKLKK (amino acids 280 to 284) in the basic region (12). The expression of ATF4 could be stimulated or blocked by viral infection, causing either an increase or decrease in viral production (13-21). Infection with the human cytomegalovirus (HCMV) activates the UPR pathway, and the HCMV proteins activate ATF4 expression (13, 14). The

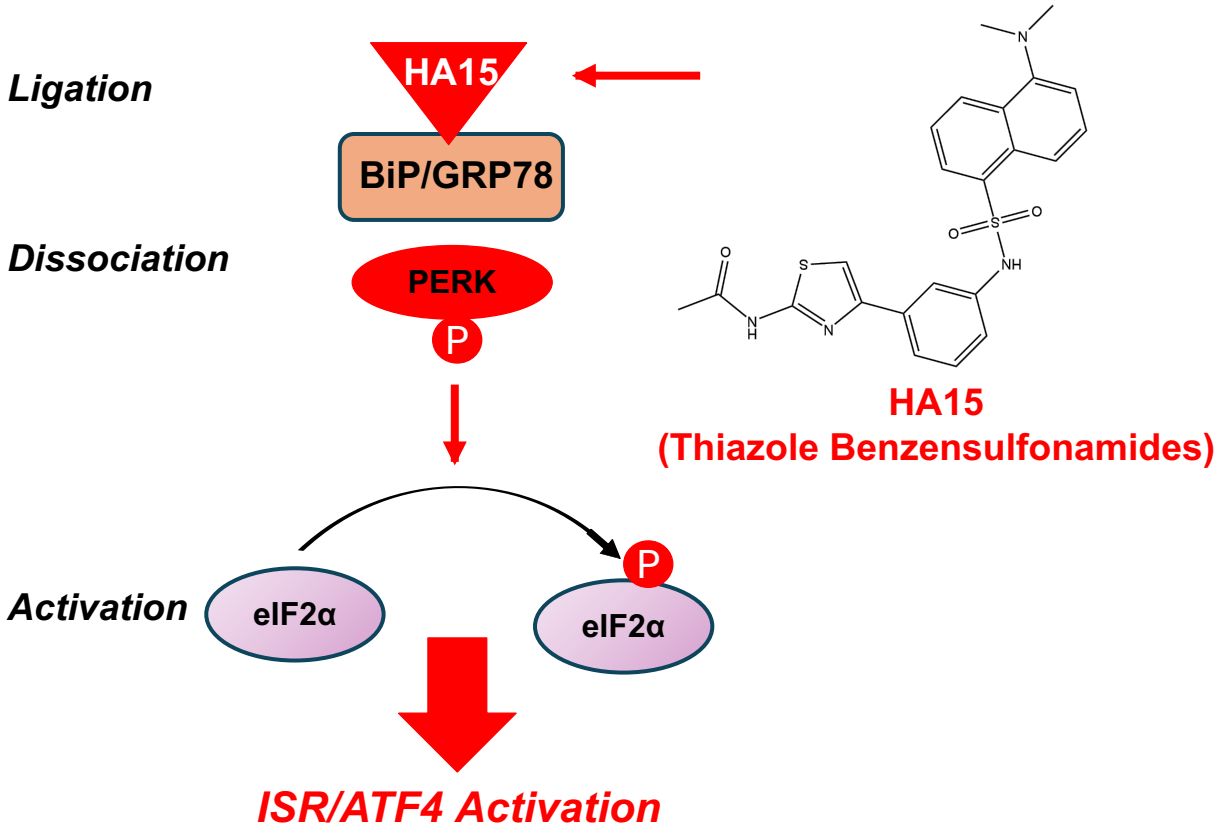


- **We hypothesized that ISR/ATF4 signaling activation may deplete HIV reservoirs**

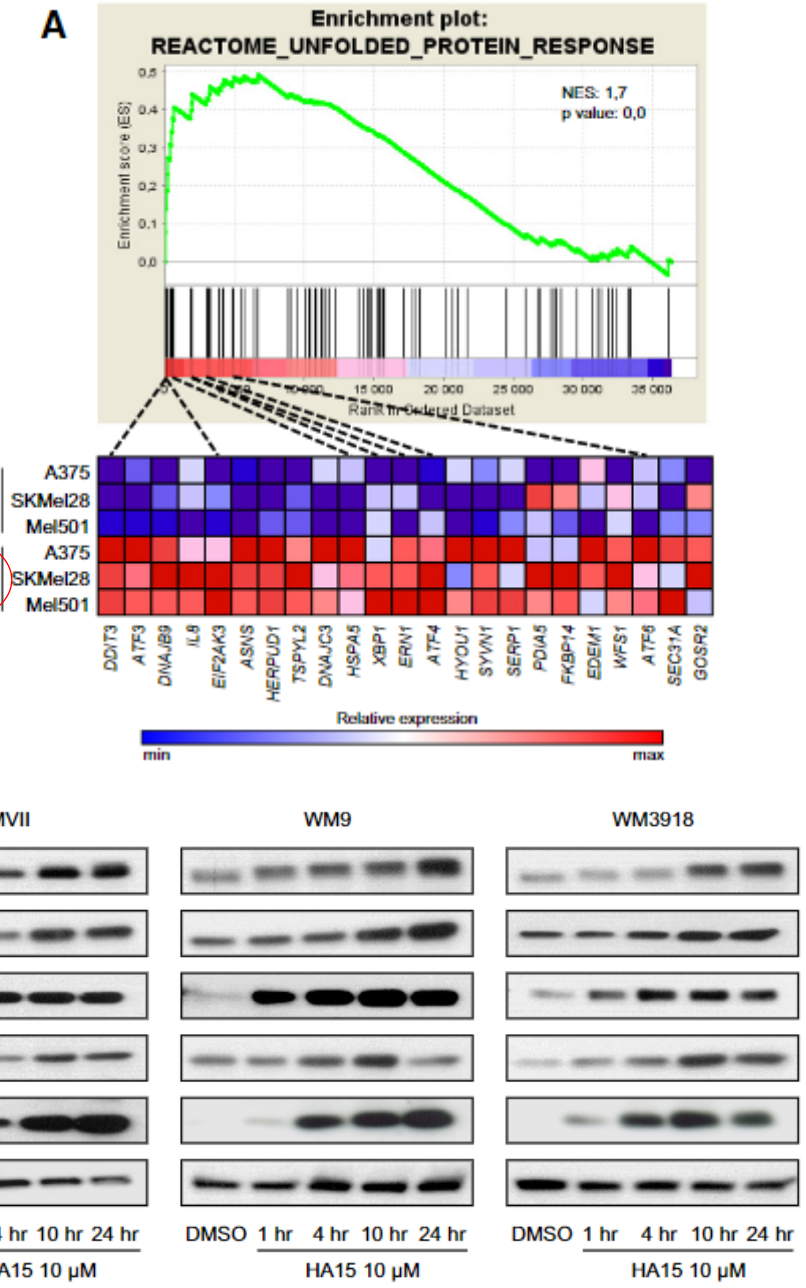
Cancer Cell
Article

Compounds Triggering ER Stress Exert Anti-Melanoma Effects and Overcome BRAF Inhibitor Resistance

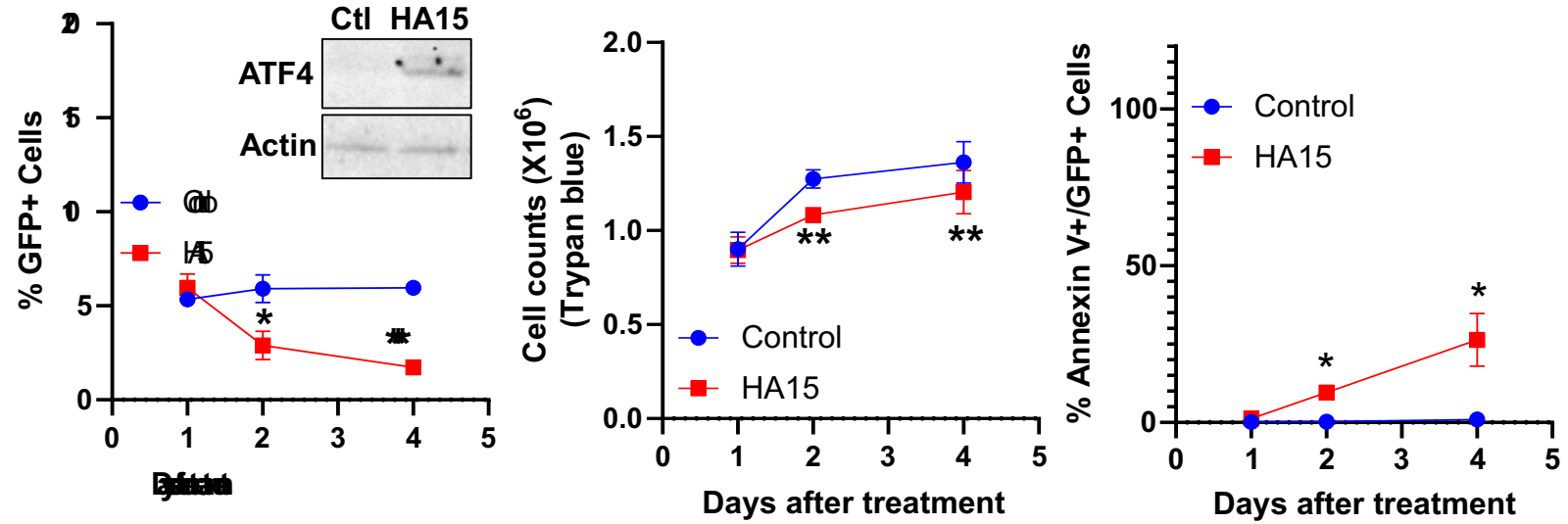
Michaël Cerezo,[1,2,14] Abdelali Lehraiki,[1,2,14] Antoine Millet,[3,14] Florian Rouaud,[1,2] Magali Plaisant,[1,2] Emilie Jaune,[1,2] Thomas Botton,[1,2] Cyril Ronco,[3] Patricia Abbe,[1,2] Hella Amdouni,[3] Thierry Passeron,[1,2,4] Veronique Hofman,[2,5,6] Baharia Mograbi,[2,5] Anne-Sophie Dabert-Gay,[2,7] Delphine Debayle,[2,7] Damien Alcor,[1,2] Nabil Rabhi,[8] Jean-Sébastien Annicotte,[8] Laurent Héliot,[9] Mariano Gonzalez-Pisfil,[9] Caroline Robert,[10] Solange Moréra,[11] Armelle Vigouroux,[11] Philippe Gual,[12] Maruf M.U. Ali,[13] Corine Bertolotto,[1,2,4] Paul Hofman,[2,5,6] Robert Ballotti,[1,2,4] Rachid Benhida,[3,*] and Stéphane Rocchi[1,2,4,*]



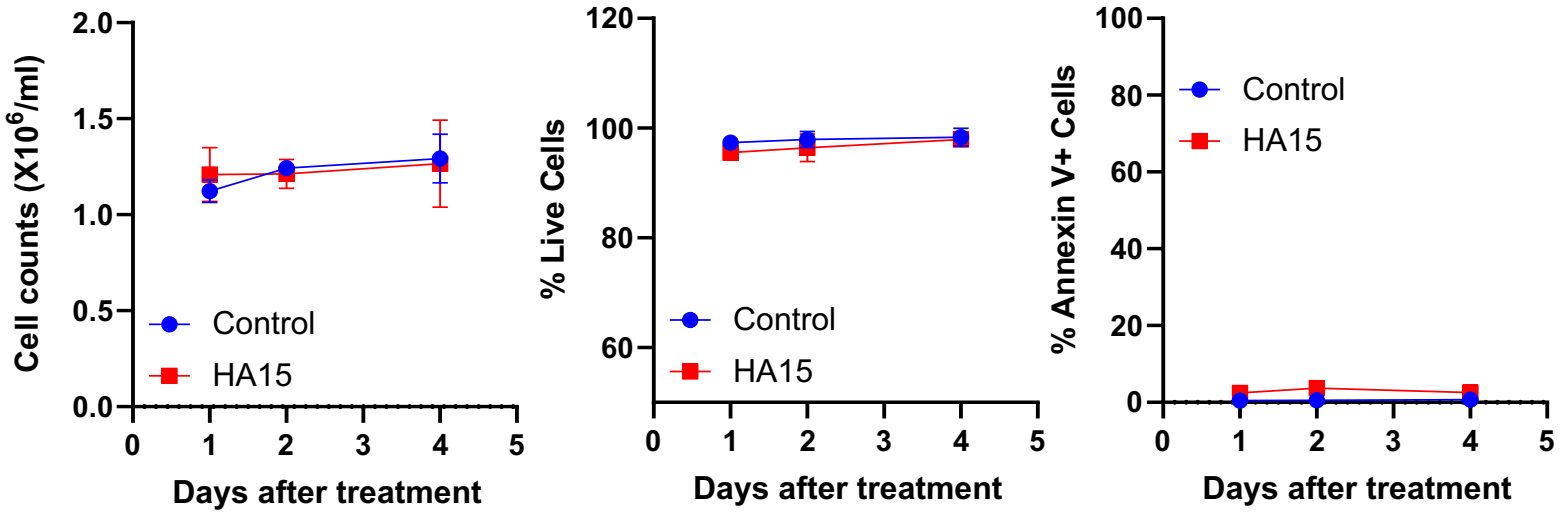
HA15
(Thiazole Benzensulfonamides)



➢ HA15 is a selective ISR/ATF4 signaling agonist by disruption of BiP/GRP78 binding to PERK

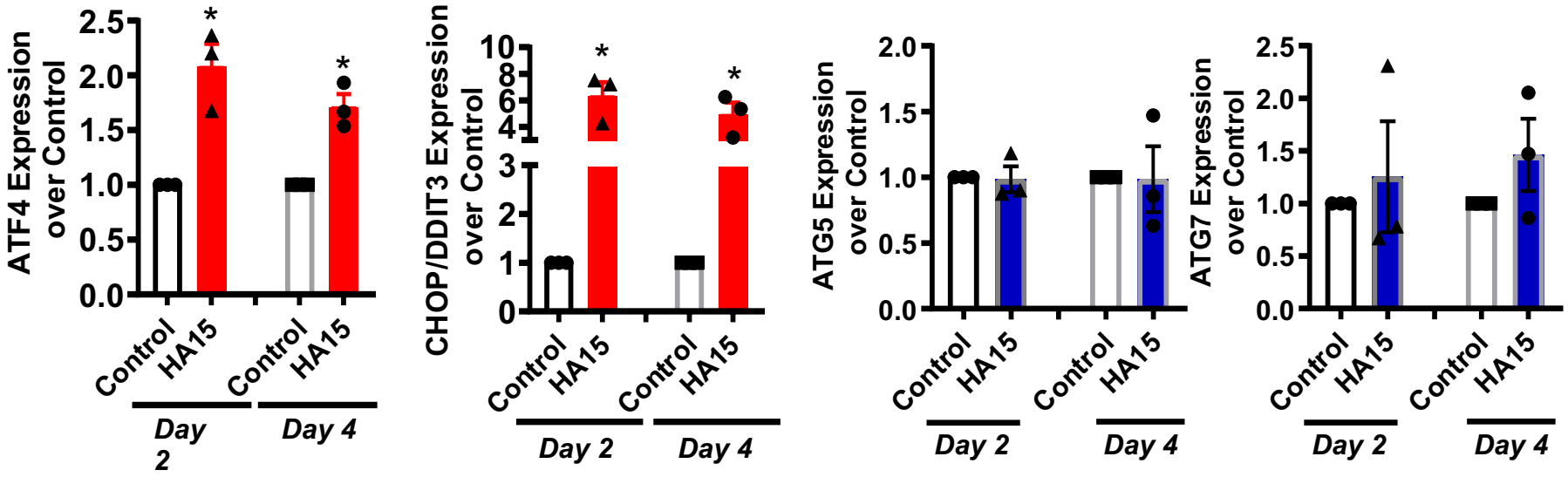


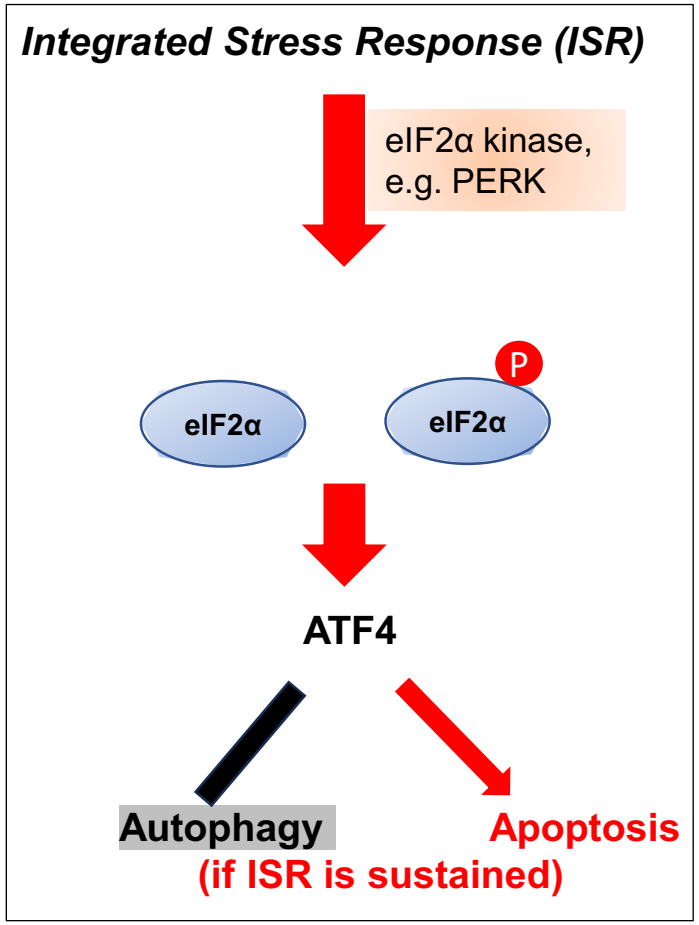
➢ **ISR/ATF4 activation reduces GFP+ (HIV+) cells by apoptosis in primary CD4+ T cell model of HIV latency**



➢ **Of note, induction of cell death by ISR/ATF4 activation does not occur in HIV negative primary CD4+ T cells**

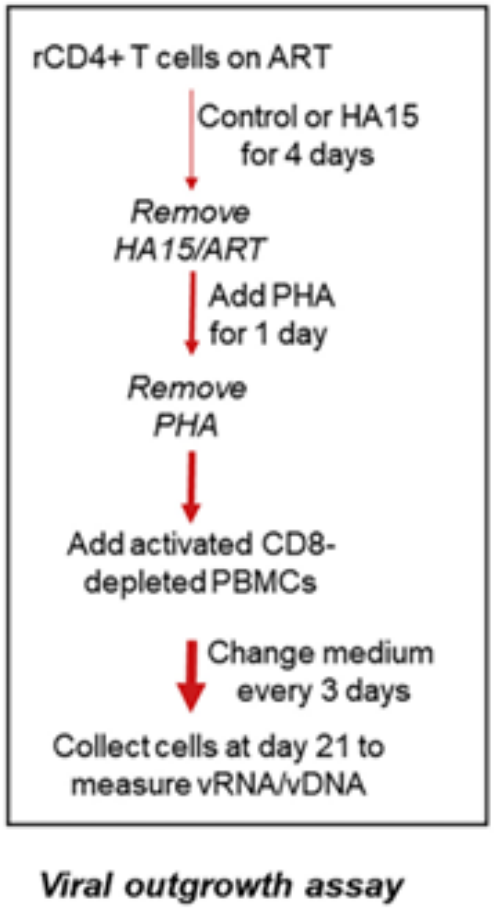
Li D, et al., iScience, 2023





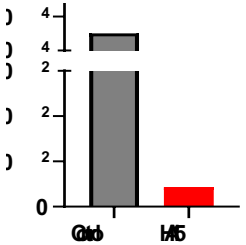
- **ISR/ATF4 activation by HA15 induces cell death gene CHOP rather than autophagy gene ATGs for cell survival, which further supports our hypothesis that ISR/ATF4 signaling can be exploited for selective reservoir depletion.**

Li D, et al., iScience, 2023



Viral outgrowth assay

A *Cell-associated vRNA*



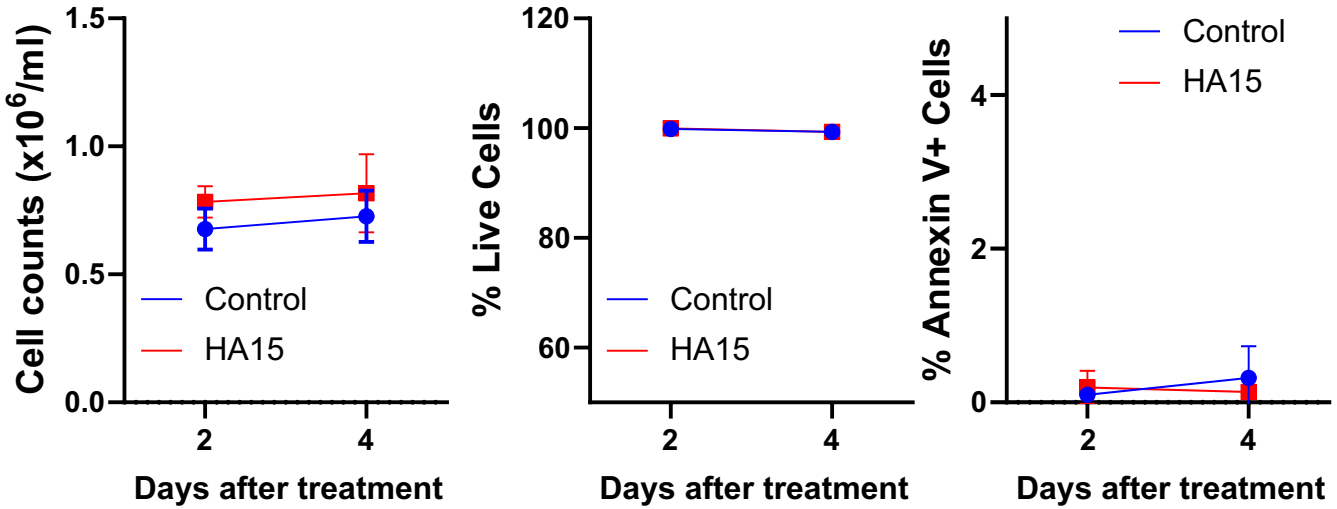
B *Cell-associated vDNA*

C *Cell-free vRNA in the Supernatants*

Pt 4 **Pt 5** **Pt 6** **Pt 7** **Pt 8**

➢ **ISR/ATF4 activation depletes replication-competent HIV in rCD4+ T cells isolated from PWH on ART**

Li D, et al., iScience, 2023



- **Intriguingly, ISR/ATF4 activation by HA15 has minimal impacts on suvival of rCD4+ T cell isolated from PWH on ART, further supporting that ISR/ATF4 can selectively deplete HIV+ cells but not HIVneg cells.**

Li D, et al., iScience, 2023

Can ISR/ATF4 activation disrupt and deplete replication-competent HIV in the brain microglia (MG)?

JCI The Journal of Clinical Investigation

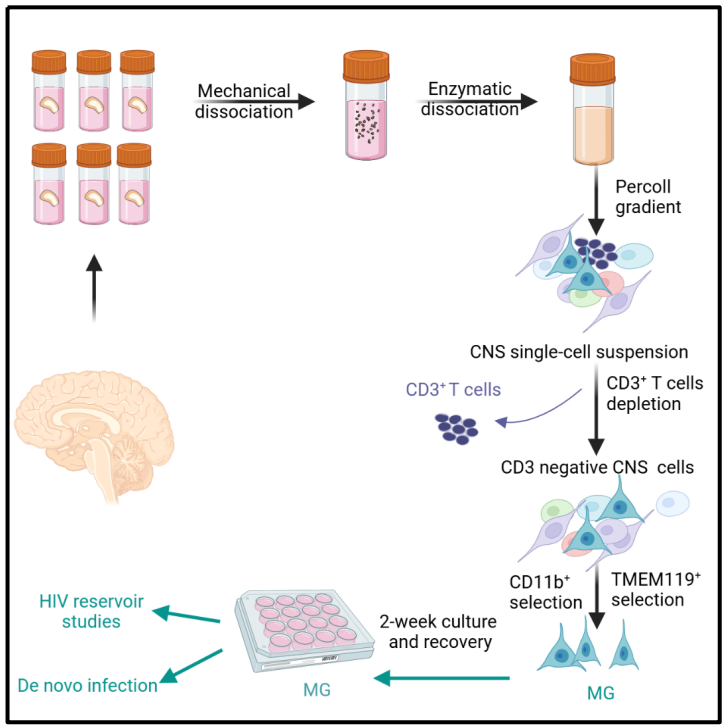
Brain microglia serve as a persistent HIV reservoir despite durable antiretroviral therapy

Yuyang Tang, … , David M. Margolis, Guochun Jiang

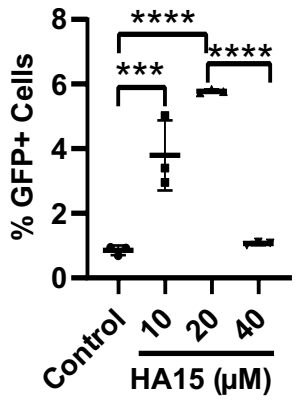
J Clin Invest. 2023;133(12):e167417. https://doi.org/10.1172/JCI167417.

Research Article AIDS/HIV

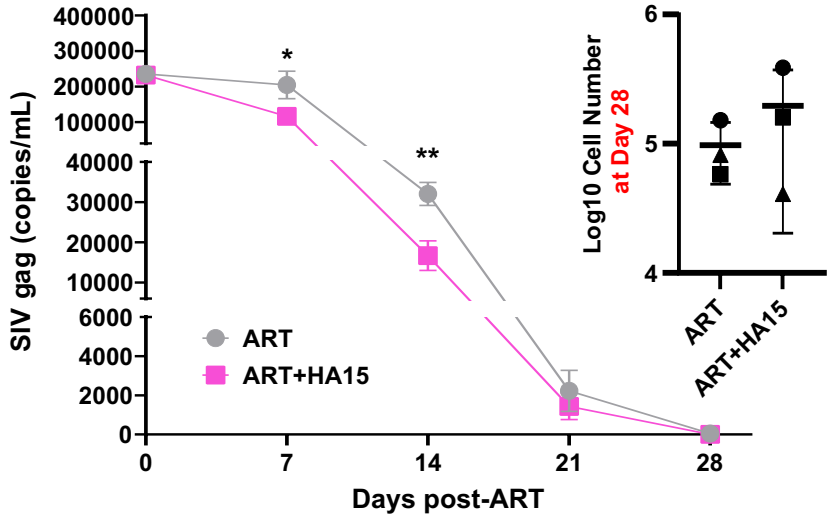
Brain microglia (MG) may serve as a human immunodeficiency virus 1 (HIV) reservoir and ignite rebound viremia following cessation of antiretroviral therapy (ART), but they have yet to be proven to harbor replication-competent HIV. Here, we isolated brain myeloid cells (BrMCs) from nonhuman primates and rapid autopsy of people with HIV (PWH) on ART and sought evidence of persistent viral infection. BrMCs predominantly displayed microglial markers, in which up to 99.9% of the BrMCs were TMEM119$^+$ MG. Total and integrated SIV or HIV DNA was detectable in the MG, with low levels of cell-associated viral RNA. Provirus in MG was highly sensitive to epigenetic inhibition. Outgrowth virus from parietal cortex MG in an individual with HIV productively infected both MG and PBMCs. This inducible, replication-competent virus and virus from basal ganglia proviral DNA were closely related but highly divergent from variants in peripheral compartments. Phenotyping studies characterized brain-derived virus as macrophage tropic based on the ability of the virus to infect cells expressing low levels of CD4. The lack of genetic diversity in virus from the brain suggests that this macrophage-tropic lineage quickly colonized brain regions. These data demonstrate that MG harbor replication-competent HIV and serve as a persistent reservoir in the brain.



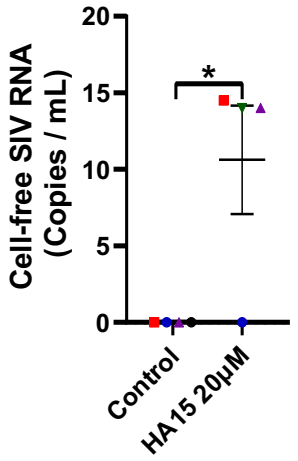
Primary MG model of HIV latency



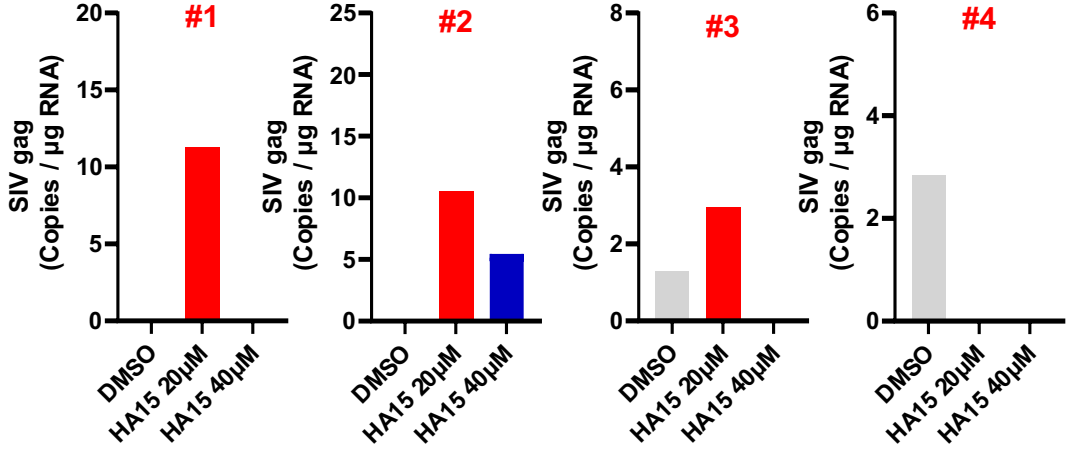
SIV infection in brain MG isolated from NHP



Brain MG from NHP on ART



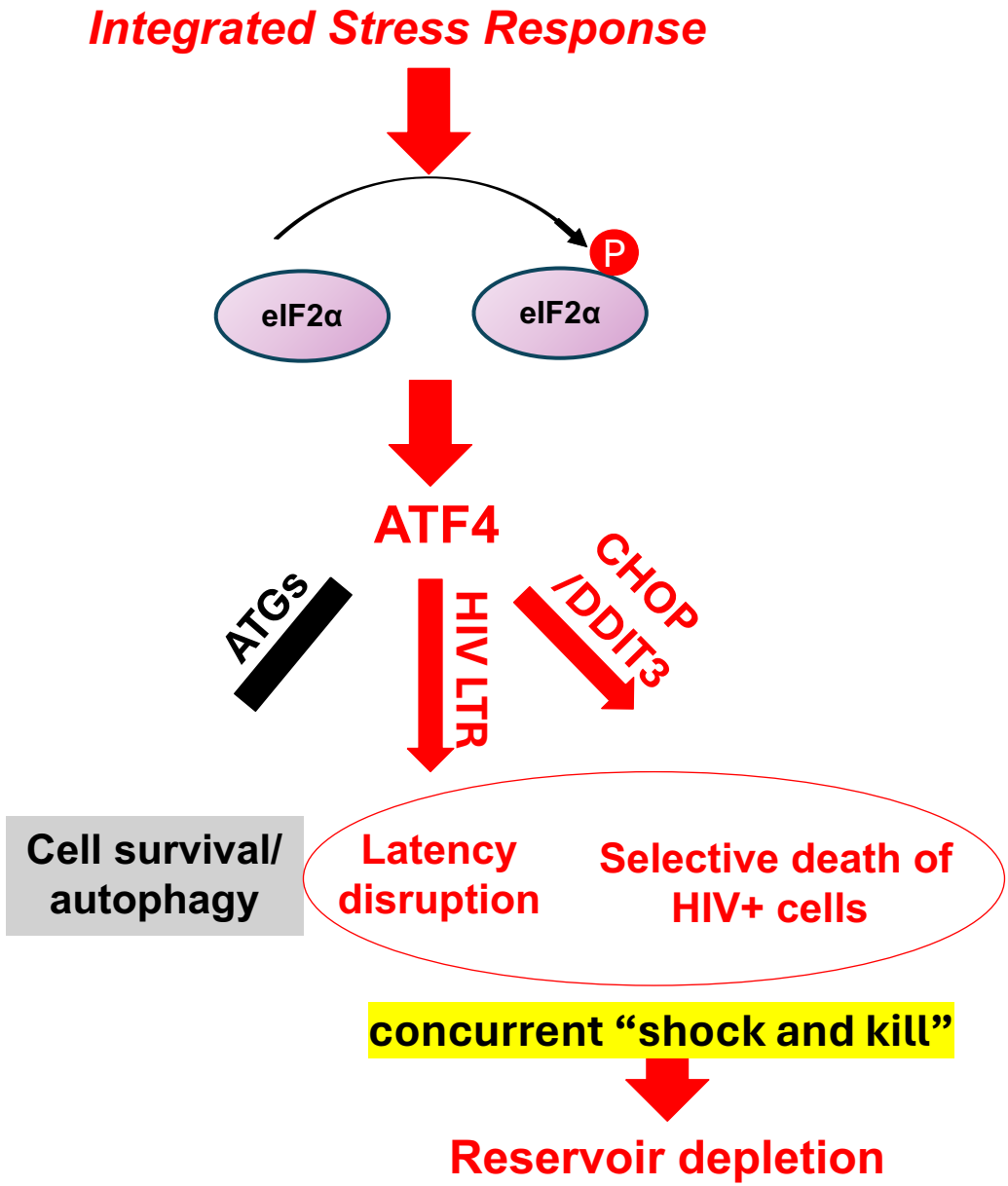
Brain MG from NHP on ART



Unpublished

These data suggest that

- ✓ ISR/ATF4 signaling activation disrupts latent HIV in CD4+ T cells.
- ✓ ISR/ATF4 activation reduces replication-competent HIV by inducing cell death gene CHOP/DDIT3 in rCD4+ T cells with minimal impact on HIVneg cells.
- ✓ ISR/ATF4 activation disrupts latent SIV, reduces viral reservoirs, and facilitate suppressing SIV replication in brain MG isolated from NHPs.
- ✓ Together, ISR/ATF4 activation may deplete HIV reservoirs by a **concurrent "shock and kill"** in both T cells and brain MG, with minimal impact on autophagy and HIVneg cells.



Acknowledgments

Jiang Lab

Dajiang Li
Xiaoyi Li
Yuyang Tang
Hongjie Chen
Waqas Ahmed
Ciniso Shabangu
Nikesh Katuwal
Trevor Shumate

UC San Diego CFAR

Sara Gianella Weibel
Davey Smith
Antoine Chaillon
Caroline Ignacio
Brendon Woodworth

Participants in the "Last Gift" and UNC cohorts

UNC HIV Cure Center

David M. Margolis
Nancie Archin
Ed Browne
Martina Kovarova

UC-Davis and California National Research Primary Center

Satya Dandekar

National Research Primary Centers

Afam Okoye (Oregon)
Woong-Ki Kim and Prasun K Datta (Tulane)