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Permalink

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Journal

JAIDS Journal of Acquired Immune Deficiency Syndromes, 82(2)

ISSN

1525-4135

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Publication Date

2019-10-01

DOI

10.1097/qai.0000000000002121

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Peer reviewed

Some Aspects of CD8+ T Cell Exhaustion are Associated with Altered T Cell Mitochondrial Features and ROS content in HIV Infection

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Abstract

Background—Reversing or preventing T cell exhaustion is an important treatment goal in the context of HIV disease; however, the mechanisms that regulate HIV-specific CD8+ T cell exhaustion are incompletely understood. Since mitochondrial mass (MM), mitochondrial membrane potential (MMP), and cellular reactive oxygen species (ROS) content are altered in exhausted CD8+ T cells in other settings, we hypothesized that similar lesions may arise in HIV infection.

Methods—We sampled cryopreserved PBMCs from HIV-uninfected (n=10) and -infected participants with varying levels and mechanisms of viral control: viremic (VL>2,000 copies/mL; n=8), or aviremic (VL<40 copies/mL) due to ART (n=11) or natural control (n=9). We characterized the MM, MMP, and ROS content of bulk CD8+ T cells and MHC Class I tetramer+ HIV-specific CD8+ T cells by flow cytometry.

Results—We observed higher MM, MMP, and ROS content across bulk effector-memory CD8+ T cell subsets in HIV-infected compared to -uninfected participants. Amongst HIV-specific CD8+ T cells, these features did not vary by the extent or mechanism of viral control but were

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significantly altered in cells displaying characteristics associated with exhaustion (e.g., high PD-1 expression, low CD127 expression, impaired proliferative capacity).

Conclusion—While we did not find that control of HIV replication *in vivo* correlates with the CD8+ T cell MM, MMP, or ROS content, we did find that some features of CD8+ T cell exhaustion are associated with alterations in mitochondrial state. Our findings support further studies to probe the relationship between mitochondrial dynamics and CD8+ T cell functionality in HIV infection.

Keywords

CD8+ T cell; exhaustion; mitochondrial features; ROS content; HIV

Introduction

In most HIV-infected individuals, HIV-specific CD8+ T cells become exhausted.[1] CD8+ T cell exhaustion is marked by impaired T cell effector functions[2-4] and high expression of co-inhibitory proteins such as PD-1.[5-8] T cell exhaustion is observed in settings of chronic T cell stimulation, such as other chronic infections (e.g., hepatitis B and C) and some cancers.[9] Our group and others have proposed strategies to overcome HIV-specific CD8+ T cell exhaustion as a means to harness these cells to achieve a durable remission, or potentially eradication, of HIV.[10,11] However, in order to design effective interventions to target exhaustion, it is essential to understand the molecular pathways that support T cell dysfunction in the context of HIV.

Recently, there has been increasing recognition of the relationship between mitochondrial state, oxidative stress, and T cell function.[12-16] After naïve T cells encounter antigen, effector and memory cell differentiation requires metabolic reprogramming that is associated with changes in mitochondrial features and oxidative stress responses.[17-21] However, when antigen-specific CD8+ T cells lose function and become exhausted in the setting of some cancers and chronic infections (e.g., chronic lymphocytic choriomeningitis virus [LCMV] infection in mice and hepatitis B virus [HBV] infection in humans), they have signs of a broadly dysregulated metabolic state, high mitochondrial mass (MM), altered mitochondrial membrane potential (MMP), and evidence of oxidative stress as measured by the accumulation of reactive oxygen species (ROS).[22-25] Thus, efforts to overcome exhaustion in cancers and chronic infections by directly targeting mitochondrial dysregulation and oxidative stress pathways are being actively explored.[24,26,27]

Some alterations in T cell mitochondrial features and ROS content in HIV-infected individuals have been described.[16,28] In particular, HIV-specific CD8+ T cells isolated from viremic individuals have a higher mitochondrial mass compared to CD8+ T cells isolated from the same individuals that recognize cytomegalovirus (CMV).[29] Additionally, mitochondrial stress can be detected in HIV-specific and activated total CD8+ T cells early in the course of HIV infection.[3,30] Finally, subsets of bulk CD8+ T cells isolated from untreated (viremic) HIV-infected compared to HIV-uninfected individuals have elevated MM, MMP, and ROS content that may persist even after suppression of HIV viremia with antiretroviral therapy (ART).[12,31]

While these studies suggest that HIV infection perturbs the mitochondrial state and ROS content of CD8+ T cells, it remains unclear specifically how these cellular measures are related to CD8+ T cell exhaustion in the setting of HIV infection. We hypothesized that HIV-specific CD8+ T cell populations and bulk CD8+ T cell subsets that have more features of exhaustion (e.g., cells isolated from viremic or ART-suppressed individuals compared to individuals who naturally control HIV infection to undetectable levels in the blood [controllers],[2,32-34] that express high levels of PD-1, or antigen-specific CD8+ T cells that have poor proliferative capacity) would have alterations in mitochondrial features and ROS content similar to what has been observed in exhausted CD8+ T cells in other chronic infections (i.e., high MM, MMP, and ROS content).[22-24] To test this hypothesis, we related phenotypic and functional features of HIV-specific and bulk naïve and effector-memory CD8+ T cell subsets to their MM, MMP, and ROS content in peripheral blood samples from HIV-uninfected compared to HIV-infected participants in three different clinical groups: Viremic individuals with untreated HIV infection, ART-treated HIV+ individuals with durable viral suppression, and HIV controllers.

Methods

Study participants and samples

Cryopreserved peripheral blood mononuclear cell (PBMC) samples (>90% viability by trypan blue staining) were obtained from HIV-infected participants enrolled in the San Francisco-based SCOPE and Options cohorts who were divided into three groups: viremic (individuals with HIV viral load > 2000 copies/mL, not on ART); ART-suppressed (individuals infected for at least two years and on suppressive ART for at least two years with HIV viral load < 40 copies/mL); controller (individuals considered to be "elite controllers" i.e., infected with HIV for > one year, off ART, and with HIV viral loads < 40 copies/mL measured at least twice/year). A control group of HIV-uninfected SCOPE participants with similar demographic features was also included. All groups were matched, as much as possible, according to age and CD4+ T cell counts during the time of sampling (see Table, Supplemental Digital Content 1, which shows participant clinical data). The UCSF Committee on Human Research approved this study, and participants gave informed, written consent before enrollment. PBMCs from these participants were isolated from whole blood through Ficoll density gradient centrifugation, cryopreserved, thawed, and re-suspended in R10 media (RPMI, penicillin/streptomycin, L-glutamine, 10% fetal bovine serum [FBS]) using standard protocols.[35,36]

HIV peptide-MHC Class I tetramers

Biotinylated HIV peptide-MHC Class I monomers provided by the laboratory of Dr. Rafick Pierre-Sékaly were tetramerized via conjugation with streptavidin-BV421 (Biolegend, San Diego, CA) or streptavidin-PE (ProZyme, Hayward, CA).[37]

Immunophenotyping by flow cytometry

PBMCs were evaluated by three flow cytometry staining panels (see Table, Supplemental Digital Content 2, which has information about the antibodies). $2–4 \times 10^6$ PBMCs from each participant were stained with their corresponding HIV peptide-MHC Class I tetramer at

37°C and 5% CO_2 for 20 minutes. After washing, the cells were stained with fixable cell viability dye (LIVE/DEAD Fixable Aqua Stain, Thermo Fisher Scientific, Waltham, MA). For staining panels 1 and 2, this was followed by staining with the mitochondrial/ROS dyes (see below) and then staining with surface marker antibodies at room temperature for 25 minutes. For staining panel 3, after proliferation assay was completed (see below), surface marker staining was performed, followed by fixation and permeabilization using the Foxp3 Transcription Factor Staining Buffer Kit (Thermo Fisher) and intracellular staining with Granzyme B and Perforin. Multiparametric flow cytometry analysis was accomplished immediately after staining using an LSRII flow cytometer (BD Biosciences, San Jose, CA). Data were analyzed using FlowJo v10.3 software (Tree Star, Ashland, OR).

Mitochondrial marker and ROS content analysis

MM and total ROS content of cells were evaluated by staining with 12.5nM MitoTracker Green (MitoGREEN) and 1.25μM CellROX Deep Red (CellROX; both from Thermo Fisher), respectively, according to the manufacturer's instructions (staining panel 1; see Figure 1 for gating strategy). In order to evaluate MMP, PBMCs were first stained with their corresponding HIV peptide-MHC Class I tetramer and then stimulated for five hours at 37°C with 5% CO_2 with 1μg/mL HIV peptide pools comprised of peptides derived from the Gag, Nef, or Pol HIV proteins (depending on and including the relevant tetramer peptide; NIH AIDS Reagent Program) or R10 media as an unstimulated control. A previous study that utilized JC-1 staining to assess the MMP of CD8+ T cells stimulated these cells with anti-CD3 antibody.[24] For more physiologic conditions, we stimulated with HIV peptide pools. MMP was evaluated via staining with 0.25μg/mL JC-1 according to the manufacturer's instructions (Thermo Fisher; staining panel 2). Cells treated with 50μM carbonyl cyanide 3-chlorophenylhydrazone (CCCP; R&D Systems, Minneapolis, MN) to induce mitochondrial membrane depolarization were included for JC-1 stain gating purposes.

Proliferation assay

Thawed PBMCs in R10 media were rested overnight at 37°C and 5% CO_2. Cells were then labeled with CellTrace Violet (CTV; Thermo Fisher) according to the manufacturer's instructions. 1.0×10^6 CTV-labeled cells were incubated for 6 days at 37°C with 5% CO_2 in R10 media alone or R10 containing 0.2μg/mL HIV peptide pools (NIH AIDS Reagent Program). The cells were then stained with staining panel 3.

Statistical analysis

Statistical analysis was performed using GraphPad Prism v7 (GraphPad Software, La Jolla, CA) and STATA v14 (Stata Corp., College Station, TX). Pairwise comparisons between groups were assessed with Wilcoxon rank sum tests and correlations between continuous variables were assessed with Spearman's rank correlations. Adjusted differences between groups were assessed with linear regression, log_{10}-transforming outcome variables when necessary to satisfy normality assumptions.

Results

MM, MMP, and ROS content of bulk CD8+ T cells vary according to differentiation state and HIV infection status

To assess the mitochondrial state of bulk CD8+ T cells in HIV-infected and -uninfected participants, we evaluated their MM, MMP, and ROS content. We first assessed these features in bulk naïve and effector-memory CD8+ T cell subsets. As others have described in part,[12,38] we found that the MM, MMP, and ROS content of bulk CD8+ T cells vary according to their effector-memory differentiation state (see Figure, Supplemental Digital Content 3, which shows bulk CD8+ T cell gating strategy: TN=naïve CD45RA+CCR7+, TCM=central memory CD45RA-CCR7+, TTM=transitional memory CD45RA-CCR7-CD27+, TEM=effector memory CD45RA-CCR7-CD27-, TEMRA=effector memory RA CD45R+CCR7-). Amongst both HIV-uninfected participants as well as across our cohort of HIV-infected participants, we observed that TN cells have the lowest median MM, MMP, and ROS content (see Fig. 1A-C and Tables, Supplemental Digital Contents 4-5, which show the comparison of these measures in bulk CD8+ T cell subsets from HIV-uninfected and -infected participants).

Next, we sought to evaluate how these measures are impacted by the presence of HIV infection. Compared to HIV-uninfected participants, HIV-infected participants had a significantly higher median MM in all subsets except for TCM cells, a higher median MMP in the TEM and TEMRA cells, and a higher median ROS content in TTM and TEM CD8+ T cell subsets (Fig. 1A-C). All significantly different measurements remained statistically significant after adjustment for age. Elevated MM, MMP, and ROS content were apparent in bulk CD8+ T cells from HIV-infected participants regardless of the degree or mechanism of HIV viral control *in vivo*, although these differences did not always reach statistical significance when bulk CD8+ T cells from controllers were compared to the HIV-uninfected group (see Table, Supplemental Digital Content 6, which shows the comparison of these features in CD8+ T cell subsets from HIV-infected versus -uninfected participants).

HIV clinical status does not alter the MM, MMP, or ROS content of HIV-specific CD8+ T cells

After establishing that HIV infection broadly impacts the mitochondrial state and ROS content of bulk effector-memory CD8+ T cells across clinical groups, we next evaluated the MM, MMP, and ROS content of HIV-specific CD8+ T cells isolated from viremic, ART-suppressed, and controller participants. Based on previous studies showing enhanced functional capacity of HIV-specific CD8+ T cells from controllers, we expected that these features would be different in HIV-specific CD8+ T cells from controllers compared viremic and ART-suppressed individuals.[2,22-24,32,33] In fact, the MM, MMP, and ROS content of HIV-specific CD8+ T cells did not vary according to the extent or mechanism of viral control (Fig. 1D-F). Additionally, MMP did not change significantly with five-hour *in vitro* peptide stimulation (Fig. 1E; all other MMP graphs are therefore shown with unstimulated cells only). We did note that the MM, MMP, and ROS content of HIV-specific CD8+ T cells from all three HIV-infected groups (Fig. 1D-F) closely resemble these features in bulk CD8+ T cell TTM and TEM populations (i.e., high MM, MMP, and ROS content; Fig. 1A-C), which likely reflects the fact that HIV-specific CD8+ T cells mostly fall within the

TTM/TEM phenotypes (see Figure, Supplemental Digital Content 7, which shows the effector-memory phenotypes of tetramer+ HIV-specific CD8+ T cells). Interestingly, while we did not observe a significant correlation between the three markers we evaluated within HIV-specific CD8+ T cells, some markers did positively correlate with each other in the bulk TTM and TEM CD8+ T cells (e.g., TTM cells with higher MM also have higher ROS accumulation and MMP; see Figure, Supplemental Digital Content 8, which shows correlation plots).

Expression of PD-1 and CD127 identifies CD8+ T cells with distinct mitochondrial states

Although the mitochondrial features of HIV-specific CD8+ T cells did not vary significantly based on the ability to control HIV replication *in vivo*, we asked whether cellular markers that distinguish CD8+ T cells with different functional states might mark cells with different MM, MMP, and/or ROS content. Therefore, we next assessed the differences in these features within HIV-specific and bulk CD8+ T cells distinguished by the expression of PD-1 and CD127, which are both proteins used to identify antigen-specific CD8+ T cells with different functional capacity. In the context of HIV and other infections, higher PD-1 expression is associated with increased functional exhaustion of antigen-specific CD8+ T cells[5,6,39] while high CD127 expression is associated with the survival of functional memory CD8+ T cells.[40-43] Compared to HIV-specific CD8+ T cells that do not express PD-1, HIV-specific CD8+ T cells that are PD-1+ have a higher median MM and ROS content (Fig. 2A-C). In contrast, compared to HIV-specific CD8+ T cells that do not express CD127, CD127+ HIV-specific CD8+ T cells have a lower median MM and ROS content (Fig. 2D-F).

We also found a similar pattern in bulk TEM CD8+ T cells from HIV-infected participants (see Figure, Supplemental Digital Content 9A-F, which shows MM, MMP, and ROS content of TEM CD8+ T cells according to PD-1 or CD127 expression). When we explored subsets of cells that have different combinations of high versus low expression of PD-1 and CD127, we found that bulk TEM CD8+ T cells that have a phenotype typically associated with a greater degree of functional exhaustion (i.e., cells that express PD-1 but not CD127 [PD-1+CD127-]) also have the highest median MM, MMP, and ROS content (see Fig. 3 for HIV-infected participants and Figure, Supplemental Digital Content 9G-I, for HIV-uninfected participants). Due to small numbers of cells in each gated population, we could not reliably perform this same analysis of sub-populations of cells defined by both PD-1 and CD127 expression in HIV-specific CD8+ T cells.

The mitochondrial mass of HIV-specific CD8+ T cells inversely correlates with their proliferative capacity

Finally, we evaluated how the MM, MMP, and ROS content HIV-specific CD8+ T cells correlate with their function in terms of expression of the cytolytic molecules, Granzyme B and Perforin, and proliferative capacity after 6-day *in vitro* peptide stimulation (see Figure, Supplemental Digital Content 10, which shows the gating strategy for proliferating cells). We found that these measures did not correlate with the expression of Granzyme B or Perforin in the HIV-specific CD8+ T cells (with or without peptide stimulation, data not shown). However, MM of the unstimulated tetramer+ HIV-specific CD8+ T cell population was associated with a decreased capacity of these cells to proliferate after peptide

stimulation (Fig. 4). Using linear regression, this relationship remained significant even after adjusting for clinical group (p=0.048) as well as the proportion of HIV-specific CD8+ T cells that were either PD-1+ (p=0.03) or CD127+ (p=0.03) prior to stimulation.

Discussion

Although dysregulation of mitochondrial state and oxidative stress (as measured by the accumulation of ROS) has been tied to CD8+ T cell exhaustion in other chronic infections and some cancers,[22-24] this relationship has not been clearly defined in the context of HIV infection.[12,29,44] Here, we evaluated the MM, MMP, and ROS content of CD8+ T cells in the peripheral blood of HIV-infected and -uninfected participants, and specifically asked how these features relate to aspects of T cell exhaustion in this infection. We found that bulk and HIV-specific CD8+ T cells exhibit evidence of mitochondrial and oxidative stress during HIV infection regardless of whether or not viremia is controlled. This observation held true despite the fact that the individuals in the viremic group were, on average, younger individuals whose T cells might otherwise be expected to show fewer signs of T cell mitochondrial stress.[45] Although we did not observe significant differences in the mitochondrial state of CD8+ T cells between participants from different HIV-infected clinical groups, we did find that bulk and HIV-specific CD8+ T cells with phenotypic and functional evidence of exhaustion (e.g., high PD-1 expression, low CD127 expression, and/or reduced proliferative capacity) had evidence of higher MM (or potentially oxidative stress; as measured by increased MitoGREEN staining),[46] MMP, and ROS content. Our results extend previous studies describing mitochondrial features of CD8+ T cells in HIV infection in three important ways: first, instead of focusing on a single measure of mitochondrial state,[29] we characterized a total of three distinct features (MM, MMP, and ROS content) in the same CD8+ T cell populations; second, we compared three clinical groups of HIV-infected participants to ask whether the mitochondrial state and ROS content of bulk and HIV-specific CD8+ T cells varies depending on the presence and mechanism of viral control; and third, we related the mitochondrial state and ROS content of CD8+ T cells to their phenotypic and functional features.

Our results provide new insights into how HIV infection impacts the mitochondrial state and ROS content of bulk CD8+ T cell effector-memory subsets.[12] We found that TEM and TTM CD8+ T cells generally display higher MM, MMP and ROS content compared to naïve CD8+ T cells, and that these alterations are more pronounced in HIV-infected participants in all three clinical groups. We also noted that even naïve CD8+ T cells from viremic and ART-suppressed participants have a higher MM and ROS content compared to naïve CD8+ T cells isolated from uninfected participants. This difference in antigen-inexperienced naïve CD8+ T cells could suggest an explanation for the altered adaptive immune responses to heterologous stimuli that is observed in HIV-infected individuals, even after ART suppression.[47-51] We also found that, compared to HIV-uninfected individuals, ART-suppressed participants have a higher ROS content across most CD8+ T cell subsets, which may relate to the effects of residual infection and/or the observation that antiretroviral therapy itself can impact T cell metabolism.[52,53]

As we anticipated based on prior studies,[12,29] we found that HIV-specific CD8+ T cells have higher median MM, MMP, and ROS content compared to naïve CD8+ T cells. Furthermore, we observed that these phenotypes are similar to bulk CD8+ T cells that share the same effector-memory phenotype (i.e., TTM/TEM). Because the proliferative and killing capacity of HIV-specific CD8+ T cells in controllers tend to be enhanced compared to viremic or ART-suppressed individuals,[2,32] we had hypothesized that these cells from controllers would have a mitochondrial and ROS content profile similar to functional virus-specific CD8+ T cells described in other settings (i.e., low MM and ROS content).[22-24] We were surprised to find that these features in HIV-specific CD8+ T cells did not differ significantly between HIV clinical groups. Our results suggest that the parameters we assessed are not correlated with the different *in vitro* (and theoretical *in vivo*) functional potential of HIV-specific CD8+ T cells isolated in different states of viral suppression. The similarity between the groups could indicate that the influence of chronic inflammation from the persistent infection is a dominant influence on these parameters regardless of the presence of viremia or the mechanism of viral control. Indeed, T cells in all three groups are exposed to high levels of inflammatory cytokines,[54-56] and cytokine exposure can impact T cell metabolism independent of antigen exposure.[21,57] The relationship between T cell MM, MMP, and ROS content and chronic inflammation could be further explored by future studies evaluating these measures in antigen-specific CD8+ T cells of a different specificity (e.g., CMV-specific CD8+ T cells) or comparing these measures to levels of soluble markers of chronic inflammation (e.g., sCD14, LPS or FABP).[58,59] Furthermore, because mitochondrial phenotypes and cellular ROS content represent only one dimension of cellular metabolic health and do not directly test mitochondrial or metabolic function, it is possible that evaluation of other features of metabolic state could reveal differences between the clinical groups. It is also possible that differences between the groups may only be observed after stimulation or that we did not have had the power to detect subtle differences between groups because of the relatively small number of participants in our study.

Despite the fact that we did not observe a difference in HIV-specific CD8+ T cell MM, MMP, or ROS content between different clinical groups of HIV-infected participants, we did find that high PD-1 expression and/or low CD127 expression mark sub-populations of bulk effector-memory and HIV-specific CD8+ T cells with higher MM, MMP, and ROS content. We also found that HIV-specific CD8+ T cells with higher MM had impaired proliferative capacity after peptide stimulation. Therefore, although CD8+ T cell mitochondrial state is not directly correlated with HIV viral control, higher MM, MMP, and ROS content is associated with some phenotypic and functional characteristics of exhausted cells. It is notable that the cells that appear to be more exhausted have evidence of increased mitochondrial activity, which may be consistent with the interpretation that while these cells are unable to proliferate effectively, they are not metabolically inert. These associations mirror patterns described in the murine model of chronic LCMV infection[22] as well as in human hepatitis B-specific CD8+ T cells,[23,24] two examples where the function of exhausted antigen-specific CD8+ T cells can be improved by *in vitro* targeting of mitochondrial metabolic pathways.

In summary, we describe here in multiple settings of HIV viral control the nuanced relationship between the mitochondrial state and ROS content of bulk and HIV-specific

CD8+ T cells, their differentiation state, and some features of T cell exhaustion. Our findings add to a growing body of literature demonstrating the importance of cell-intrinsic factors in the regulation of HIV-specific CD8+ T cell exhaustion.[60-62] These studies provide rationale for further investigation of the relationship between mitochondrial dynamics and T cell function in HIV, and support additional studies to explore whether interventions designed to overcome T cell exhaustion by targeting mitochondrial fitness[24,26,27] might also have some efficacy in improving HIV-specific CD8+ T cell function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

References

1. Kuchroo VK, Anderson AC, Petrovas C. Coinhibitory receptors and CD8 T cell exhaustion in chronic infections. Curr Opin HIV AIDS. 2014;9(5):439–445. [PubMed: 25010894]

2. Migueles SA, Weeks KA, Nou E, et al. Defective human immunodeficiency virus-specific CD8+ T-cell polyfunctionality, proliferation, and cytotoxicity are not restored by antiretroviral therapy. J Virol. 2009;83(22):11876–11889. [PubMed: 19726501]

3. Trautmann L, Mbitikon-Kobo FM, Goulet JP, et al. Profound metabolic, functional, and cytolytic differences characterize HIV-specific CD8 T cells in primary and chronic HIV infection. Blood. 2012;120(17):3466–3477. [PubMed: 22955926]

4. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol. 2015;15(8):486–499. [PubMed: 26205583]

5. Trautmann L, Janbazian L, Chomont N, et al. Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. Nat Med. 2006;12(10):1198–1202. [PubMed: 16917489]

6. Day CL, Kaufmann DE, Kiepiela P, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature. 2006;443(7109):350–354. [PubMed: 16921384]

7. Yamamoto T, Price DA, Casazza JP, et al. Surface expression patterns of negative regulatory molecules identify determinants of virus-specific CD8+ T-cell exhaustion in HIV infection. Blood. 2011;117(18):4805–4815. [PubMed: 21398582]

8. Chew GM, Fujita T, Webb GM, et al. TIGIT Marks Exhausted T Cells, Correlates with Disease Progression, and Serves as a Target for Immune Restoration in HIV and SIV Infection. PLoS Pathog. 2016;12(1):e1005349. [PubMed: 26741490]

9. McKinney EF, Smith KG. T cell exhaustion and immune-mediated disease-the potential for therapeutic exhaustion. Curr Opin Immunol. 2016;43:74–80. [PubMed: 27744240]

10. Barouch DH, Deeks SG. Immunologic strategies for HIV-1 remission and eradication. Science. 2014;345(6193):169–174. [PubMed: 25013067]

11. Bui JK, Mellors JW. Reversal of T-cell exhaustion as a strategy to improve immune control of HIV-1. AIDS. 2015;29(15):1911–1915. [PubMed: 26355569]

12. Masson JJR, Murphy AJ, Lee MKS, Ostrowski M, Crowe SM, Palmer CS. Assessment of metabolic and mitochondrial dynamics in CD4+ and CD8+ T cells in virologically suppressed HIV-positive individuals on combination antiretroviral therapy. PLoS One. 2017;12(8):e0183931. [PubMed: 28854263]

13. Palmer CS, Cherry CL, Sada-Ovalle I, Singh A, Crowe SM. Glucose Metabolism in T Cells and Monocytes: New Perspectives in HIV Pathogenesis. EBioMedicine. 2016;6:31–41. [PubMed: 27211546]

14. Sena LA, Li S, Jairaman A, et al. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. Immunity. 2013;38(2):225–236. [PubMed: 23415911]

15. Patsoukis N, Weaver JD, Strauss L, Herbel C, Seth P, Boussiotis VA. Immunometabolic Regulations Mediated by Coinhibitory Receptors and Their Impact on T Cell Immune Responses. Front Immunol. 2017;8:330. [PubMed: 28443090]

16. Younes SA, Talla A, Pereira Ribeiro S, et al. Cycling CD4+ T cells in HIV-infected immune nonresponders have mitochondrial dysfunction. J Clin Invest. 2018;128(11):5083–5094. [PubMed: 30320604]

17. Pearce EL, Walsh MC, Cejas PJ, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature. 2009;460(7251):103–107. [PubMed: 19494812]

18. van der Windt GJ, Everts B, Chang CH, et al. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. Immunity. 2012;36(1):68–78. [PubMed: 22206904]

19. Pollizzi KN, Patel CH, Sun IH, et al. mTORC1 and mTORC2 selectively regulate CD8(+) T cell differentiation. J Clin Invest. 2015;125(5):2090–2108. [PubMed: 25893604]

20. Buck MD, O'Sullivan D, Klein Geltink RI, et al. Mitochondrial Dynamics Controls T Cell Fate through Metabolic Programming. Cell. 2016;166(1):63–76. [PubMed: 27293185]

21. Almeida L, Lochner M, Berod L, Sparwasser T. Metabolic pathways in T cell activation and lineage differentiation. Semin Immunol. 2016;28(5):514–524. [PubMed: 27825556]

22. Bengsch B, Johnson AL, Kurachi M, et al. Bioenergetic Insufficiencies Due to Metabolic Alterations Regulated by the Inhibitory Receptor PD-1 Are an Early Driver of CD8(+) T Cell Exhaustion. Immunity. 2016;45(2):358–373. [PubMed: 27496729]

23. Schurich A, Pallett LJ, Jajbhay D, et al. Distinct Metabolic Requirements of Exhausted and Functional Virus-Specific CD8 T Cells in the Same Host. Cell Rep. 2016;16(5):1243–1252. [PubMed: 27452473]

24. Fisicaro P, Barili V, Montanini B, et al. Targeting mitochondrial dysfunction can restore antiviral activity of exhausted HBV-specific CD8 T cells in chronic hepatitis B. Nat Med. 2017;23(3):327–336. [PubMed: 28165481]

25. Siska PJ, Beckermann KE, Mason FM, et al. Mitochondrial dysregulation and glycolytic insufficiency functionally impair CD8 T cells infiltrating human renal cell carcinoma. JCI Insight. 2017;2(12).

26. Shehata HM, Murphy AJ, Lee MKS, et al. Sugar or Fat?-Metabolic Requirements for Immunity to Viral Infections. Front Immunol. 2017;8:1311. [PubMed: 29085369]

27. Bantug GR, Fischer M, Grahlert J, et al. Mitochondria-Endoplasmic Reticulum Contact Sites Function as Immunometabolic Hubs that Orchestrate the Rapid Recall Response of Memory CD8(+) T Cells. Immunity. 2018;48(3):542–555 e546. [PubMed: 29523440]

28. Masson JJR, Cherry CL, Murphy NM, et al. Polymorphism rs1385129 Within Glut1 Gene SLC2A1 Is Linked to Poor CD4+ T Cell Recovery in Antiretroviral-Treated HIV+ Individuals. Front Immunol. 2018;9:900. [PubMed: 29867928]

29. Petrovas C, Mueller YM, Dimitriou ID, et al. Increased mitochondrial mass characterizes the survival defect of HIV-specific CD8(+) T cells. Blood. 2007;109(6):2505–2513. [PubMed: 17095625]

30. Takata H, Buranapraditkun S, Kessing C, et al. Delayed differentiation of potent effector CD8(+) T cells reducing viremia and reservoir seeding in acute HIV infection. Sci Transl Med. 2017;9(377).

31. Yu F, Hao Y, Zhao H, et al. Distinct Mitochondrial Disturbance in CD4+T and CD8+T Cells From HIV-Infected Patients. J Acquir Immune Defic Syndr. 2017;74(2):206–212. [PubMed: 27608061]

32. Migueles SA, Laborico AC, Shupert WL, et al. HIV-specific CD8+ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. Nat Immunol. 2002;3(11):1061–1068. [PubMed: 12368910]

33. Migueles SA, Osborne CM, Royce C, et al. Lytic granule loading of CD8+ T cells is required for HIV-infected cell elimination associated with immune control. Immunity. 2008;29(6):1009–1021. [PubMed: 19062316]

34. Migueles SA, Connors M. Success and failure of the cellular immune response against HIV-1. Nat Immunol. 2015;16(6):563–570. [PubMed: 25988888]

35. Fuss IJ, Kanof ME, Smith PD, Zola H. Isolation of whole mononuclear cells from peripheral blood and cord blood. Curr Protoc Immunol. 2009;Chapter 7:Unit7 1.

36. Owen RE, Sinclair E, Emu B, et al. Loss of T cell responses following long-term cryopreservation. J Immunol Methods. 2007;326(1–2):93–115. [PubMed: 17707394]

37. Class I MHC Tetramer Preparation: Overview. NIH Tetramer Core Facility- Emory University. Production Protocols Web site. http://tetramer.yerkes.emory.edu/support/protocols#10. Published 2005 Accessed2018.

38. van der Windt GJ, O'Sullivan D, Everts B, et al. CD8 memory T cells have a bioenergetic advantage that underlies their rapid recall ability. Proc Natl Acad Sci U S A. 2013;110(35):14336–14341. [PubMed: 23940348]

39. Barber DL, Wherry EJ, Masopust D, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006;439(7077):682–687. [PubMed: 16382236]

40. Schluns KS, Kieper WC, Jameson SC, Lefrancois L. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells in vivo. Nat Immunol. 2000;1(5):426–432. [PubMed: 11062503]

41. Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. Nat Immunol. 2003;4(12):1191–1198. [PubMed: 14625547]

42. van Leeuwen EM, de Bree GJ, Remmerswaal EB, et al. IL-7 receptor alpha chain expression distinguishes functional subsets of virus-specific human CD8+ T cells. Blood. 2005;106(6):2091–2098. [PubMed: 15947093]

43. Sabbaj S, Heath SL, Bansal A, et al. Functionally competent antigen-specific CD127(hi) memory CD8+ T cells are preserved only in HIV-infected individuals receiving early treatment. J Infect Dis. 2007;195(1):108–117. [PubMed: 17152014]

44. Willig AL, Overton ET. Metabolic Complications and Glucose Metabolism in HIV Infection: A Review of the Evidence. Curr HIV/AIDS Rep. 2016;13(5):289–296. [PubMed: 27541600]

45. Lee HCW, Y. H. Mitochondria and Aging In: Scatena R BP, Giardina B, ed. Advances in Mitochondrial Medicine. Advances in Experimental Medicine and Biology. Vol 942 Springer, Dordrecht; 2012.

46. Doherty E, Perl A. Measurement of Mitochondrial Mass by Flow Cytometry during Oxidative Stress. React Oxyg Species (Apex). 2017;4(10):275–283. [PubMed: 29806036]

47. Serrano-Villar S, Perez-Elias MJ, Dronda F, et al. Increased risk of serious non-AIDS-related events in HIV-infected subjects on antiretroviral therapy associated with a low CD4/CD8 ratio. PLoS One. 2014;9(1):e85798. [PubMed: 24497929]

48. Cobos Jimenez V, Wit FW, Joerink M, et al. T-Cell Activation Independently Associates With Immune Senescence in HIV-Infected Recipients of Long-term Antiretroviral Treatment. J Infect Dis. 2016;214(2):216–225. [PubMed: 27073222]

49. Muyanja E, Ssemaganda A, Ngauv P, et al. Immune activation alters cellular and humoral responses to yellow fever 17D vaccine. J Clin Invest. 2014;124(7):3147–3158. [PubMed: 24911151]

50. Avelino-Silva VI, Miyaji KT, Hunt PW, et al. CD4/CD8 Ratio and KT Ratio Predict Yellow Fever Vaccine Immunogenicity in HIV-Infected Patients. PLoS Negl Trop Dis. 2016;10(12):e0005219. [PubMed: 27941965]

51. Colin de Verdiere N, Durier C, Samri A, et al. Immunogenicity and safety of yellow fever vaccine in HIV-1-infected patients. AIDS. 2018;32(16):2291–2299. [PubMed: 30096071]

52. Koczor CA, Lewis W. Nucleoside reverse transcriptase inhibitor toxicity and mitochondrial DNA. Expert Opin Drug Metab Toxicol. 2010;6(12):1493–1504. [PubMed: 20929279]

53. Perrin S, Cremer J, Roll P, et al. HIV-1 infection and first line ART induced differential responses in mitochondria from blood lymphocytes and monocytes: the ANRS EP45 "Aging" study. PLoS One. 2012;7(7):e41129. [PubMed: 22829920]

54. Kamat A, Misra V, Cassol E, et al. A Plasma Biomarker Signature of Immune Activation in HIV Patients on Antiretroviral Therapy. PLOS One. 2012.

55. Noel N, Boufassa F, Lecuroux C, et al. Elevated IP10 levels are associated with immune activation and low CD4$^+$ T-cell counts in HIV controller patients. AIDS. 2014;28(4):467–476. [PubMed: 24378753]

56. Platten M, Jung N, Trapp S, et al. Cytokine and Chemokine Signature in Elite Versus Viremic Controllers Infected with HIV. AIDS Research and Human Retroviruses. 2016;32(6).

57. Buck MD, Sowell RT, Kaech SM, Pearce EL. Metabolic Instruction of Immunity. Cell. 2017;169(4):570–586. [PubMed: 28475890]

58. Tenorio AR, Zheng Y, Bosch RJ, et al. Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. J Infect Dis. 2014;210(8):1248–1259. [PubMed: 24795473]

59. Moro-Garcia MA, Mayo JC, Sainz RM, Alonso-Arias R. Influence of Inflammation in the Process of T Lymphocyte Differentiation: Proliferative, Metabolic, and Oxidative Changes. Front Immunol. 2018;9:339. [PubMed: 29545794]

60. Quigley M, Pereyra F, Nilsson B, et al. Transcriptional analysis of HIV-specific CD8+ T cells shows that PD-1 inhibits T cell function by upregulating BATF. Nat Med. 2010;16(10):1147–1151. [PubMed: 20890291]

61. Gaiha GD, McKim KJ, Woods M, et al. Dysfunctional HIV-specific CD8+ T cell proliferation is associated with increased caspase-8 activity and mediated by necroptosis. Immunity. 2014;41(6): 1001–1012. [PubMed: 25526311]

62. Buggert M, Tauriainen J, Yamamoto T, et al. T-bet and Eomes are differentially linked to the exhausted phenotype of CD8+ T cells in HIV infection. PLoS Pathog. 2014;10(7):e1004251. [PubMed: 25032686]
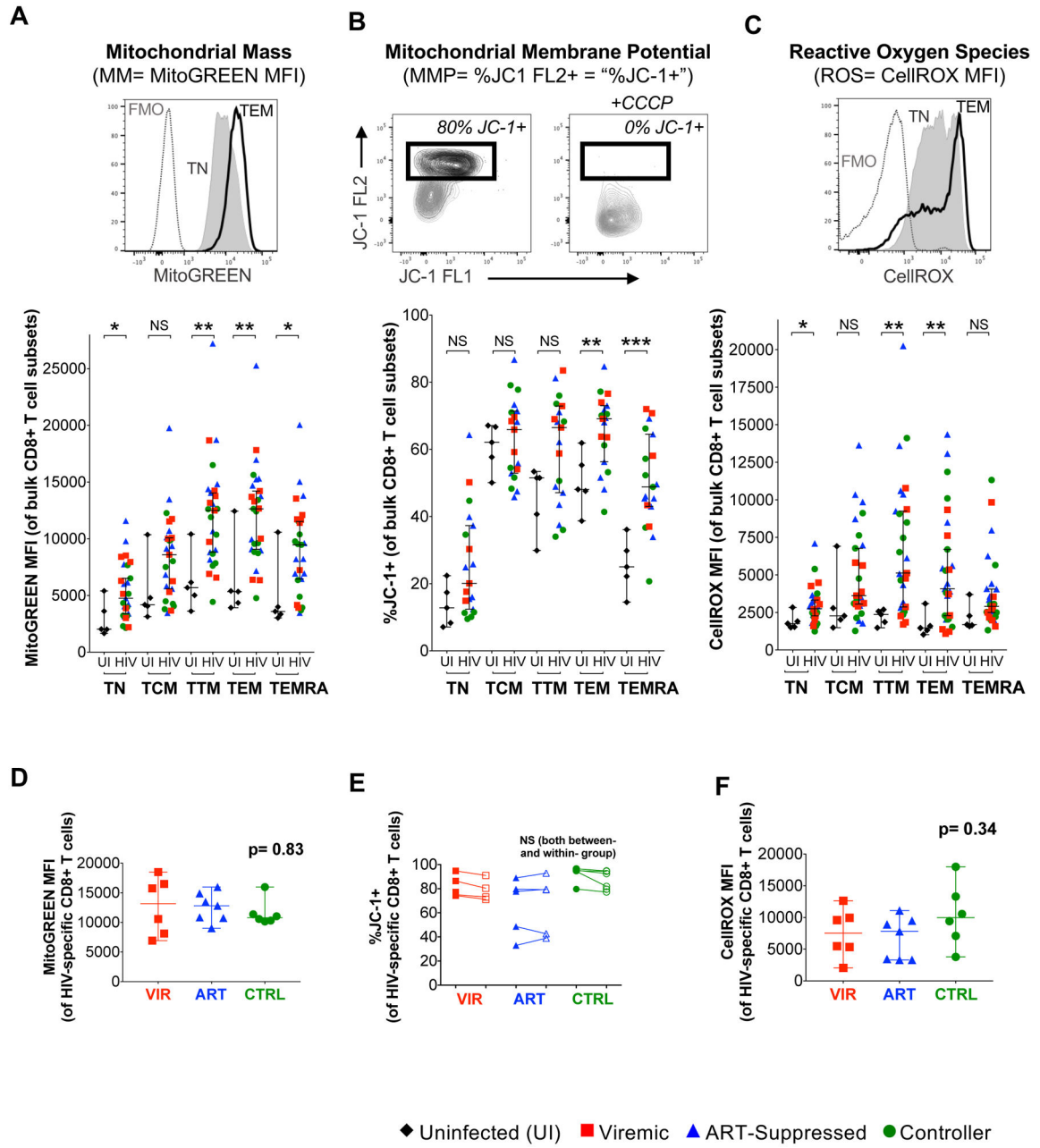
Fig. 1. MM, MMP, and ROS content of bulk and HIV-specific (tetramer+) CD8+ T cells by HIV status.
(A and D) Mitochondrial Mass (MitoGREEN MFI), (B and E) Mitochondrial Membrane Potential (%JC-1+), and (C and F) ROS Content (CellROX MFI) of (A-C) bulk CD8+ T cell subsets (naïve [TN], transitional memory [TTM], central memory [TCM], effector memory [TEM], effector memory RA [TEMRA]) in HIV-infected individuals (marked according to clinical group: Viremic, red squares; ART-suppressed, blue triangles; Controllers, green circles) and HIV-uninfected individuals (black diamonds), or (D-F) of MHC Class I tetramer + HIV-specific CD8+ T cells isolated from HIV+ individuals. %JC-1 measured in unstimulated cells (open symbols) and after 5-hour stimulation with cognate peptide (closed

symbols) in antigen-specific cells. (A-C): P-values calculated using Mann-Whitney U test (*p<0.05, **p<0.01; NS, difference is not significant). (D-F): P-values for differences between clinical groups calculated using Kruskal-Wallis H test and for differences within groups (for %JC-1+) using Wilcoxon signed rank test. NS, difference is not significant. Horizontal lines represent median values and error bars represent 95% confidence intervals.
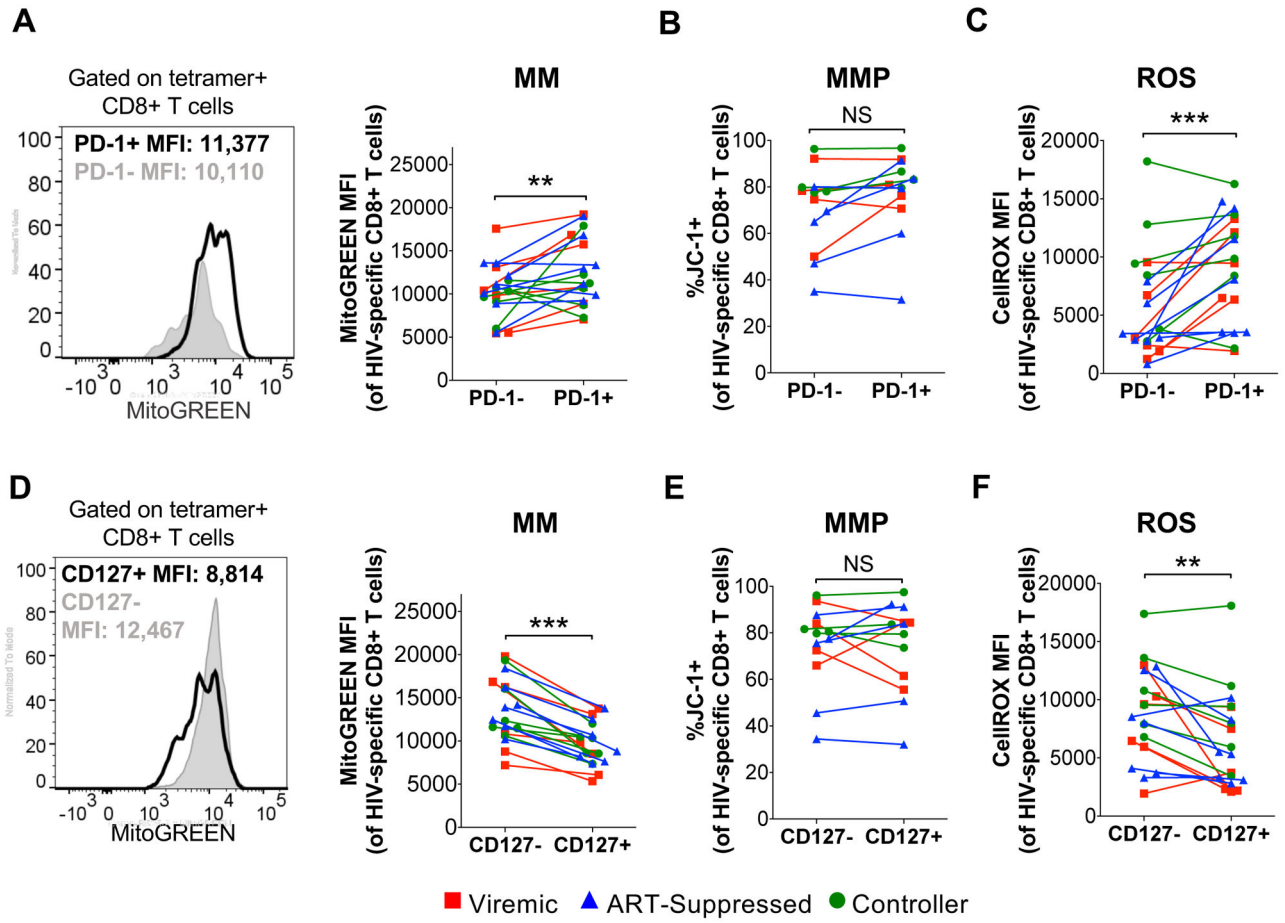
Fig. 2. Expression of PD-1 or CD127 identifies HIV-specific CD8+ T cells with distinct MM, MMP, and ROS content.

Mitochondrial mass (MitoGREEN MFI; A, D), Mitochondrial Membrane Potential (%JC-1+; B, E), and ROS content (CellROX MFI; C, F) of tetramer+ HIV-specific CD8+ T cells gated according to PD-1 or CD127 expression. P-values calculated using Wilcoxon signed-rank test (**p<0.01, ***p<0.001; NS, difference is not significant).
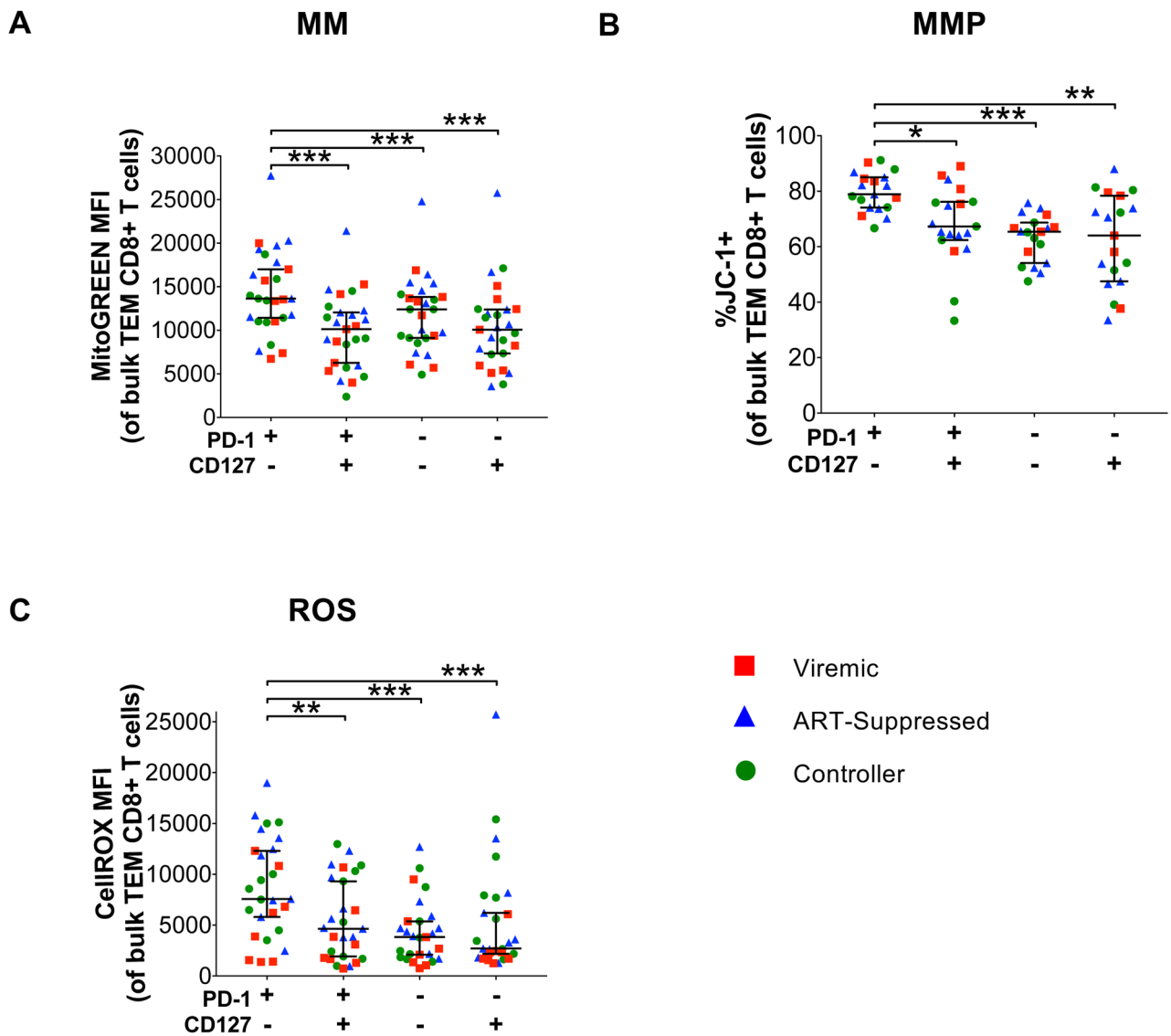
Fig. 3. PD-1+CD127- TEM CD8+ T cells have the highest MM, MMP and ROS content levels.
MM, MMP, and ROS content of sub-populations of bulk effector memory (TEM) CD8+ T cells from HIV-infected individuals defined by the co-expression of PD-1 and CD127 as measured by: (A) Mitochondrial Mass (MitoGREEN MFI), (B) Mitochondrial Membrane Potential (%JC-1+), and (C) ROS content (CellROX MFI). P-values calculated using Friedman test with post-hoc testing by Dunn's multiple comparison test (**p<0.01, ***p<0.001). Horizontal lines represent median values and error bars represent 95% confidence intervals.
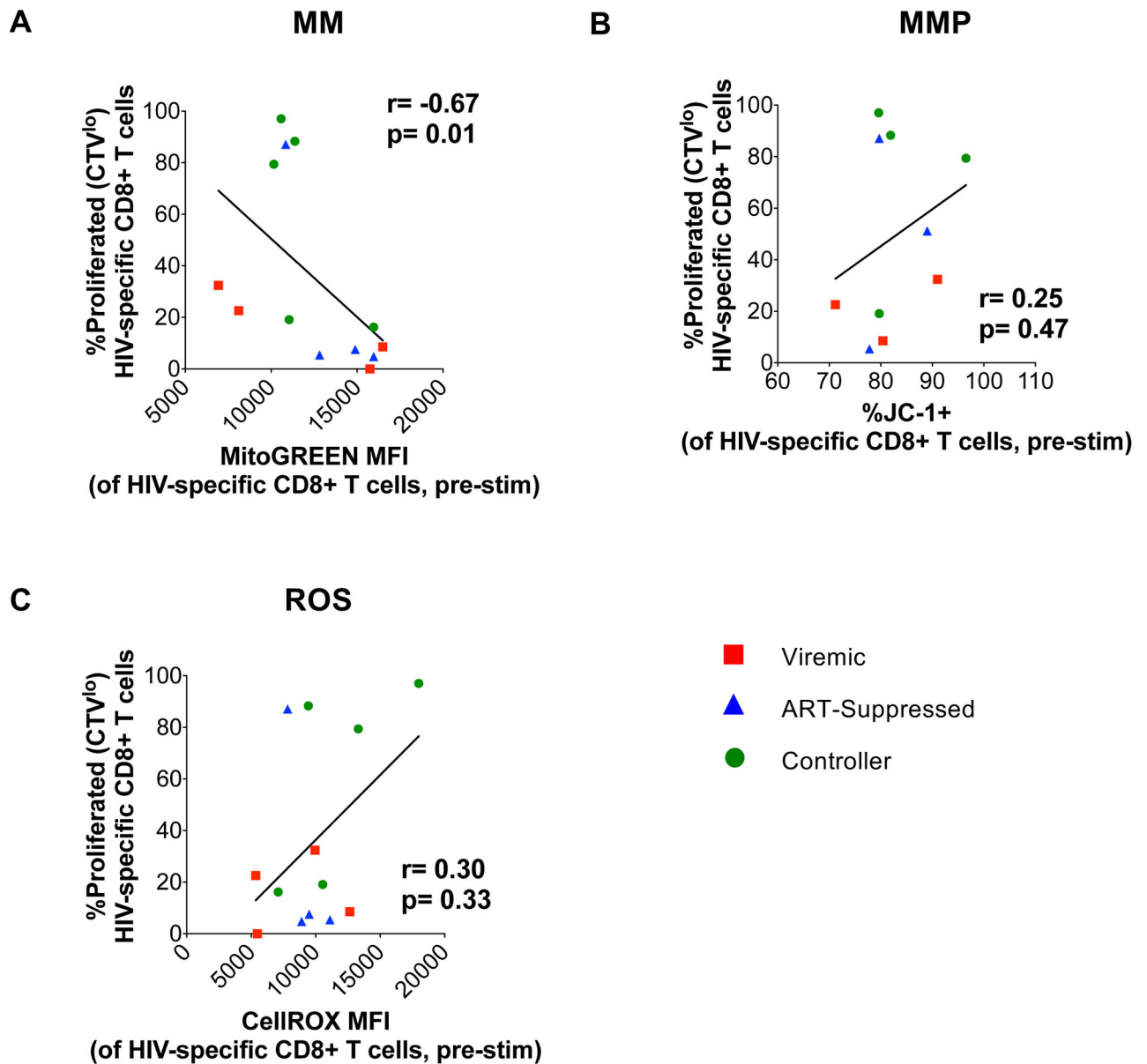
A

MM

r= -0.67
p= 0.01

B

MMP

r= 0.25
p= 0.47

C

ROS

r= 0.30
p= 0.33

■ Viremic

▲ ART-Suppressed

● Controller

Fig. 4. Proliferative capacity of HIV-specific CD8+ T cells is inversely correlated with their mitochondrial mass.
Correlation between the frequency of HIV-specific CD8+ T cells that proliferated after six days of *in vitro* stimulation with cognate peptide, and their pre-stimulation (A) Mitochondrial Mass (MitoGREEN MFI), (B) Mitochondrial Membrane Potential (%JC-1+), and (C) ROS content (CellROX MFI). r and p-values calculated using Spearman's rank correlation test.