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Research Article

Immune profile and mitochondrial alterations driven by age and HIV infection: Associations with T-cell senescence in people with HIV receiving suppressive antiretroviral therapy

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ABSTRACT

Antiretroviral therapy (ART) has significantly extended the life expectancy of people with HIV (PWH), rendering population ageing and immunosenescence prominent clinical priorities. T-cell senescence is linked to mitochondrial dysfunction and drives age-related immune remodelling, yet how HIV infection and ageing jointly shape $CD4^+$ and $CD8^+$ T-cell immunophenotypes and mitochondrial remodelling remains unclear. This cross-sectional study included 61 PWH on suppressive ART for ≥ 12 months and 61 age- and sex-matched HIV-negative men who have sex with men, stratified into younger (≤ 35 years) and older (≥ 50 years) groups. Multiparameter flow cytometry was used to profile $CD4^+$ and $CD8^+$ T-cell differentiation, stemness, activation/exhaustion, and metabolic phenotypes, together with mitochondrial mass and membrane potential. We found that ageing and HIV infection were associated with T-cell remodelling, characterized by expanded late-differentiated phenotypes and reduced stem-like, homeostatic and costimulatory $CD4^+$ and $CD8^+$ T-cell subsets, as indicated by upregulated CD57 and CX3CR1 and downregulated $CD45RA^+CD31^+$, FOXO1, and CD28. Notably, younger PWH had an ageing-like $CD4^+$ T-cell profile, with higher CD57, CX3CR1 and TIGIT expression than younger HIV-negative individuals. In contrast, HIV-related $CD8^+$ T-cell perturbations (KLRG1, CXCR3, NKG2C and CD95) were more pronounced in older PWH. PWH exhibited increased mitochondrial mass and membrane potential in both total and senescent-like $CD4^+$ and $CD8^+$ T cells, particularly in $CD8^+$ T cells from older PWH. In $CD4^+$ T cells, KLRG1 and CX3CR1 expression correlated positively with age, and inversely with $CD4^+$ T-cell counts and CD4/CD8 ratio. By contrast, FOXO1 expression in $CD8^+$ T cells was inversely associated with age, late-differentiation markers, and ART duration in PWH. Overall, age is a major driver of T-cell immunosenescence, and HIV infection modulates and exacerbates these alterations. Mitochondrial stress, FOXO1 downregulation and immune network remodelling support a multifaceted model of HIV-associated immune ageing that may contribute to heterogeneous immune reconstitution in PWH, highlighting potential targets to mitigate immune ageing.

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INTRODUCTION

Antiretroviral therapy (ART) and early intervention have significantly increased the life expectancy of people with HIV (PWH), transforming HIV infection into a manageable chronic condition (Kaplan-Lewis et al., 2017; Teeraananchai et al., 2017; Chan et al., 2025). Consequently, the ageing of PWH is becoming increasingly evident, with the proportion of individuals aged 50 years and older steadily increasing (Sánchez-Conde et al., 2019). In high-income countries, this proportion has already exceeded 50%, and it is steadily rising in low- and middle-income countries (Siedner, 2019; Eke and Eke, 2025). In this context, age-related immune alterations have received increasing attention, with immunosenescence emerging as a critical issue and a central challenge for older PWH (Chauvin and Sauce, 2022). Immunosenescence is a key hallmark of ageing, and its complex role in the ageing process is well established. Immunosenescence not only represents an inevitable consequence of ageing but also accelerates functional decline, increasing susceptibility to infectious diseases, age-related chronic conditions, and malignancies while diminishing vaccine efficacy (Rodrigues et al., 2021; Liu et al., 2023).

T cells are key components of the immune system and are among the most significantly affected immune cells during ageing (Mittelbrunn and Kroemer, 2021). With age, T-cell function progressively declines and is considered a major driver of immunosenescence. Under the combined effects of chronic antigenic stimulation and persistent inflammation, T cells acquire a senescence-like phenotype characterized by the loss of CD27 and CD28 and increased CD57 and KLRG1 expression, ultimately leading to impaired immune function, tissue damage, and immune dysregulation (Brenchley et al., 2003; Rodriguez et al., 2020). Furthermore, metabolic dysregulation and mitochondrial dysfunction are key hallmarks of T-cell immunosenescence (Mittelbrunn and Kroemer, 2021; Choi et al., 2025). In the context of ageing or chronic viral infection, such as chronic HIV infection, T cells develop mitochondrial dysfunction, altered mitochondrial membrane potential, and elevated reactive oxygen species (ROS) levels, suggesting the reprogramming of energy metabolism (Quinn et al., 2019). Even when viral replication is effectively suppressed by antiretroviral therapy, persistent low-grade immune activation and ongoing exposure to antiretroviral drugs can still lead to mitochondrial dysfunction in T cells (Samuels et al., 2017; Rinaldi et al., 2022). However, the relationships between T-cell immunosenescence phenotypes and mitochondrial function in PWH across different age groups have yet to be systematically evaluated.

To better understand the mechanisms underlying the interplay among T cells, ageing, and HIV, this study employed multiparameter flow cytometry to assess T-cell phenotypic changes in younger and older PWH on effective antiretroviral therapy, as well as in age-matched HIV-negative controls. The analysis included the expression of classical senescence markers, activating and inhibitory receptors, and selected molecules related to metabolism and stemness, and changes in mitochondrial mass and mitochondrial membrane potential were further evaluated. This study aims to characterize the perturbations in T cells induced by HIV infection and ageing, elucidate their roles in T-cell phenotypic remodelling, and provide a foundation for identifying potential ageing biomarkers and developing intervention strategies in PWH.

RESULTS

$CD4^+$ T-cell phenotypic alterations across age and HIV infection status

We first systematically compared the immune phenotypic characteristics of $CD4^+$ T cells in older and younger participants from both healthy controls and HIV-1-infected individuals, with a particular focus on the differences in the proportions of cells expressing molecules associated with differentiation (CD57, KLRG1, CX3CR1, and T-bet), stemness (coexpression of CD45RA and CD31, FOXO1, and TCF1), migration (CXCR3), metabolic regulation (CD71, CD98, CD36, and CD73), activation and proliferation (CD38 and Ki67), and exhaustion (TIGIT and Helios) (Fig. 1). In terms of cell differentiation-related phenotypes, the proportions of $CD57^+CD4^+$ and $CX3CR1^+CD4^+$ T cells were increased in the healthy older adults (HO), older people with HIV (PO) and younger people with HIV (PY) compared with the healthy younger adults (HY), whereas these cell frequencies in the PO group were comparable to those in the PY group (Fig. 1A). The frequencies of $KLRG1^+CD4^+$ and $T\text{-}bet^+CD4^+$ T cells were significantly greater in the HO and PO groups compared with the HY group, whereas no significant differences were observed between the PO and PY groups (Fig. 1A). With respect to markers linked to stemness and migration, the proportions of $CD45RA^+CD31^+CD4^+$ and $FOXO1^+CD4^+$ T cells were significantly lower in the PO group compared with the PY and HY groups. In contrast, the frequency of $TCF1^+CD4^+$ T cells in the PY group was comparable to that in the PO group but significantly lower than that in the HY group. Compared with the HO group, the proportion of $CXCR3^+CD4^+$ T cells was increased in the PO group (Fig. 1B).

With respect to metabolic regulatory molecules (Fig. 1C), the proportions of $CD71^+CD4^+$ T cells in the PO group and $CD98^+CD4^+$ T cells in the PY group were greater than those in the HO group, whereas CD36 expression did not markedly differ across the four groups. Strikingly, the proportions of $CD73^+CD4^+$ T cells in the PO and PY groups were lower than those in the HO and HY groups. With respect to activation- and exhaustion-related molecules (Fig. 1D), regardless of HIV-1 infection status, $CD38^+CD4^+$ T cells were more frequent in younger individuals (HY and PY) than in older individuals (HO and PO). $Ki\text{-}67^+CD4^+$ and $TIGIT^+CD4^+$ T cells were present at lower frequencies in the HY group compared with the other three groups. $Helios^+CD4^+$ T cells were more frequent in the PO group than in the HY group, with no significant difference between the PO and PY groups or between the HO and HY groups. Taken together, these findings suggest that both age and HIV infection may be associated with multidimensional phenotypic alterations in $CD4^+$ T cells, with partial overlap with immunosenescence-related phenotypes.

GLM analysis of alterations in $CD4^+$ T-cell immune profiles influenced by age and HIV infection

We employed a generalized linear model (GLM) with bootstrap resampling to systematically evaluate changes in the log odds ratios of $CD4^+$ T-cell expression across age groups (young vs. old) and disease states (HC vs. PWH), and the results are presented as forest plots (Fig. 2). In the HC group, the proportions of $CD4^+$ T cells expressing CD57, KLRG1, CX3CR1, and T-bet, which are associated with terminal differentiation, were significantly greater in the HO group than in the HY group. TIGIT and Ki-67 expression in $CD4^+$ T cells was likewise more frequent in the HO group. In contrast, the expression of CD38, CD31, and CD73, which are involved in activation, stemness, and adenosine generation, was significantly greater in the HY group than in the HO group (Fig. 2A). Among PWH, $CD45RA^+CD31^+$, FOXO1, and CD38 were significantly enriched in the PY group compared with the PO, suggesting that younger PWH retain more prominent stemness-related features and exhibit a greater degree of $CD4^+$ T-cell activation compared with older PWH (Fig. 2B). These findings reveal that differential immune profiles are linked to ageing between healthy subjects and HIV-1-infected individuals.

With respect to HIV infection status, Ki-67, CX3CR1, and TIGIT were upregulated and preferentially associated with the PY group, whereas CD73 and TCF1 were downregulated and preferentially associated with the HY group (Fig. 2C). In contrast, no significant differences in HIV infection-related markers were detected between the HO and PO groups, suggesting that any potential effects of HIV infection on $CD4^+$ T-cell phenotypes in older individuals might be

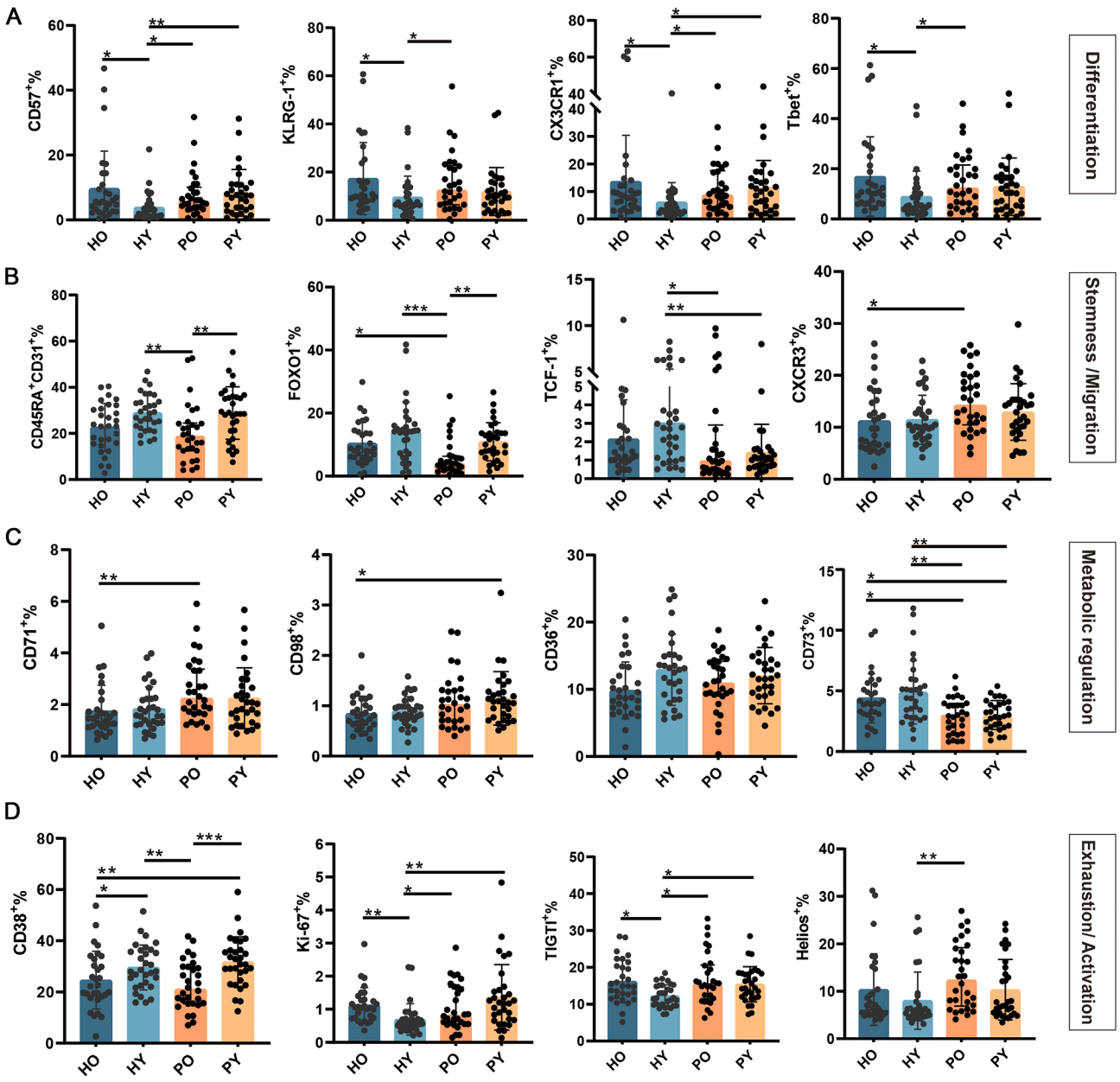

Fig. 1. Phenotypic profiling of CD4$^+$ T cells according to age groups and HIV status. Differentiation-related markers (**A**), stemness/migration-related markers (**B**), metabolic regulatory markers (**C**), and exhaustion/activation markers (**D**) were quantified in healthy younger adults (HY), healthy older adults (HO), younger people with HIV (PY) and older people with HIV (PO). The bars show the mean ± SD, and the dots denote individual participants (HY: $n = 31$; HO: $n = 30$; PY: $n = 31$; PO: $n = 30$). *P* values were calculated using one-way ANOVA with Tukey's multiple-comparison test or the Kruskal-Wallis test with Dunn's post hoc test. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

difficult to distinguish from HIV infection-induced alterations. Overall, the model-based estimates in Fig. 2 were largely consistent with the distributions of the raw proportions shown in Fig. 1 and were compatible with the CD4$^+$ T-cell phenotypic changes associated with features of immunosenescence across different age groups and HIV infection states.

CD8$^+$ T-cell phenotypic alterations across age and HIV infection status

We also systematically compared peripheral blood CD8$^+$ T-cell phenotypes across the different groups, focusing on molecules related to differentiation (CD57, KLRG1, CX3CR1, T-bet, and CD28), stemness (FOXO1, TCF1, and CD31), migration (CCR7 and CXCR3), activation and proliferation (CD38, NKG2C, and Ki67), metabolic regulation (CD36 and CD73), apoptosis (CD95 and Granzyme B), and inhibition/exhaustion (CD85J, PD-1, and TIGIT) (Fig. 3). Among the molecules associated with cell differentiation, the proportions of CD57$^+$CD8$^+$, KLRG1$^+$CD8$^+$, and CX3CR1$^+$CD8$^+$ T cells were greater in the HO group compared with the HY and PY groups as well as the PO group compared with the PY and HY groups (Fig. 3A). The frequency of T-bet$^+$CD8$^+$ T cells was also greater in the HO group compared with the HY group, and this frequency remained relatively high in the PO and PY groups. In contrast, CD28$^+$CD8$^+$ T cells were most frequent in the HY group and decreased in the HO and PO groups (Fig. 3A). With respect to molecules related to stemness, the frequency of FOXO1$^+$CD8$^+$ T cells was generally

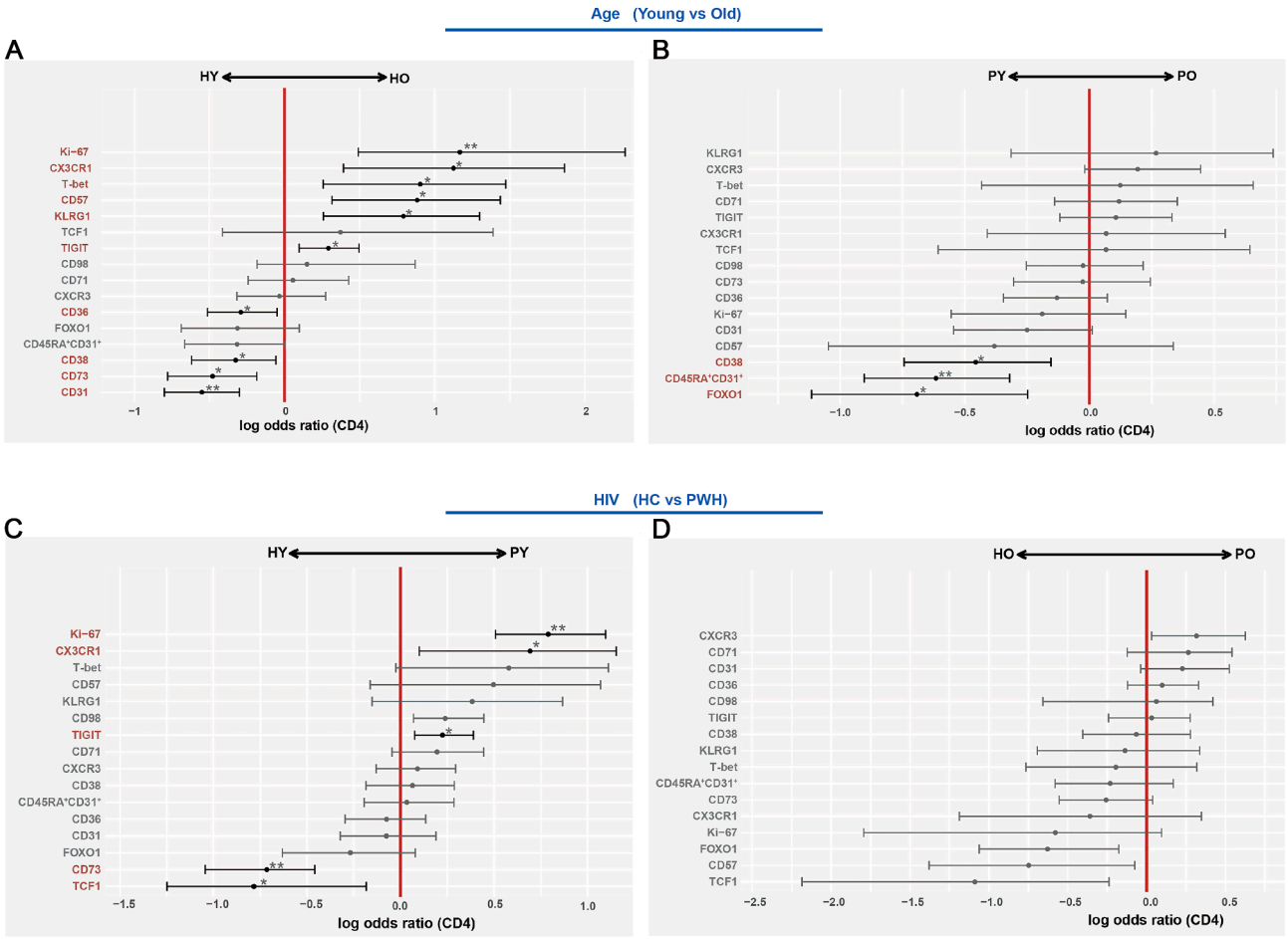

Fig. 2. GLM analysis of CD4$^+$ T-cell–associated marker expression profiles according to age groups and HIV status. Binomial generalized linear models with bootstrap resampling were used to estimate log odds ratios (logORs) for CD4$^+$ T-cell marker expression in comparisons of (**A**) HY vs. HO, (**B**) PY vs. PO, (**C**) HY vs. PY and (**D**) HO vs. PO. Forest plots show logORs (dots) with 95% confidence intervals (horizontal bars). Positive logORs indicate higher odds of marker positivity in the group on the right, and negative logORs indicate higher odds in the group on the left. *P* values were adjusted for multiple testing using the Benjamini-Hochberg method; *$q < 0.05$, **$q < 0.01$. Healthy younger adults (HY, $n = 31$), healthy older adults (HO, $n = 30$), younger people with HIV (PY, $n = 31$), older people with HIV (PO, $n = 30$).

lower in older PWH, with the PO group showing significantly lower levels than the other three groups (Fig. 3B). With respect to other stemness markers, the frequency of TCF1$^+$CD8$^+$ T cells in the HO and HY groups was significantly greater than that in the PO and PY groups (Fig. 3B). CCR7$^+$CD8$^+$ T cells were more frequent in the HY group compared with the HO and PO groups; similarly, their frequency was greater in the PY group compared with the HO and PO groups (Fig. 3B). Compared with that in the HO group, CXCR3 and CD31 expression was significantly greater in the HY, PO, and PY groups (Fig. 3B).

We further evaluated the expression of cell markers related to activation. The frequency of CD38$^+$CD8$^+$ T cells was significantly higher in the PY group than in the healthy controls of the HY and HO groups, whereas the proportion of Ki-67$^+$CD8$^+$ T cells was lower in the HO group compared with the HY group. This situation was not observed in PWH (Fig. 3C). Additionally, NKG2C$^+$CD8$^+$ T cells were more frequent in the PO group than in the healthy controls of the HY and HO groups, with an insignificant difference noted between the PO and PY groups (Fig. 3C). With respect to metabolic molecules, CD36$^+$CD8$^+$ T cells were more frequently observed in the PY group than in the HY group. CD73$^+$CD8$^+$ T cells were most abundant in the HY group and lowest in the PO group, with higher frequencies observed in the HY group than in both the HO and PO groups as well as the PY group compared with the PO group (Fig. 3C). CD8$^+$ T cells from older individuals (HO and PO) exhibit higher CD95 expression than those from younger HY individuals, with the PO group showing further upregulation of CD95 expression relative to that in the PY group. In the HO group, the proportion of Granzyme B$^+$CD8$^+$ T cells was significantly increased compared with that in the HY, PO, and PY groups. Compared with the PY group, the proportions of PD-1$^+$CD8$^+$, CD85J$^+$CD8$^+$, and TIGIT$^+$CD8$^+$ T cells were greater in the HO group, and the latter two types of cells were more frequent in the HO group than in the HY group (Fig. 3D). Taken together, these data reveal systematic differences in the expression of multiple functional markers in CD8$^+$ T cells across age groups and HIV infection states.

GLM analysis of alterations in CD8$^+$ T-cell immune profiles influenced by age and HIV infection

GLM-based analysis of age- and HIV-associated phenotypic alterations in CD8$^+$ T cells was performed. We further applied a GLM with bootstrap resampling to evaluate changes in the log odds ratios of CD8$^+$ T-cell expression across age groups (HY and HO) and HIV infection states (HC and PWH). The results are presented as forest plots (Fig. 4). In healthy controls, T-bet, Granzyme B, CX3CR1, CD57, CD85J, TIGIT, and CD95 expression was significantly enriched in the HO group, whereas CD28, CCR7, CXCR3, and CD73 expression was enriched in the HY group (Fig. 4A). Among PWH,

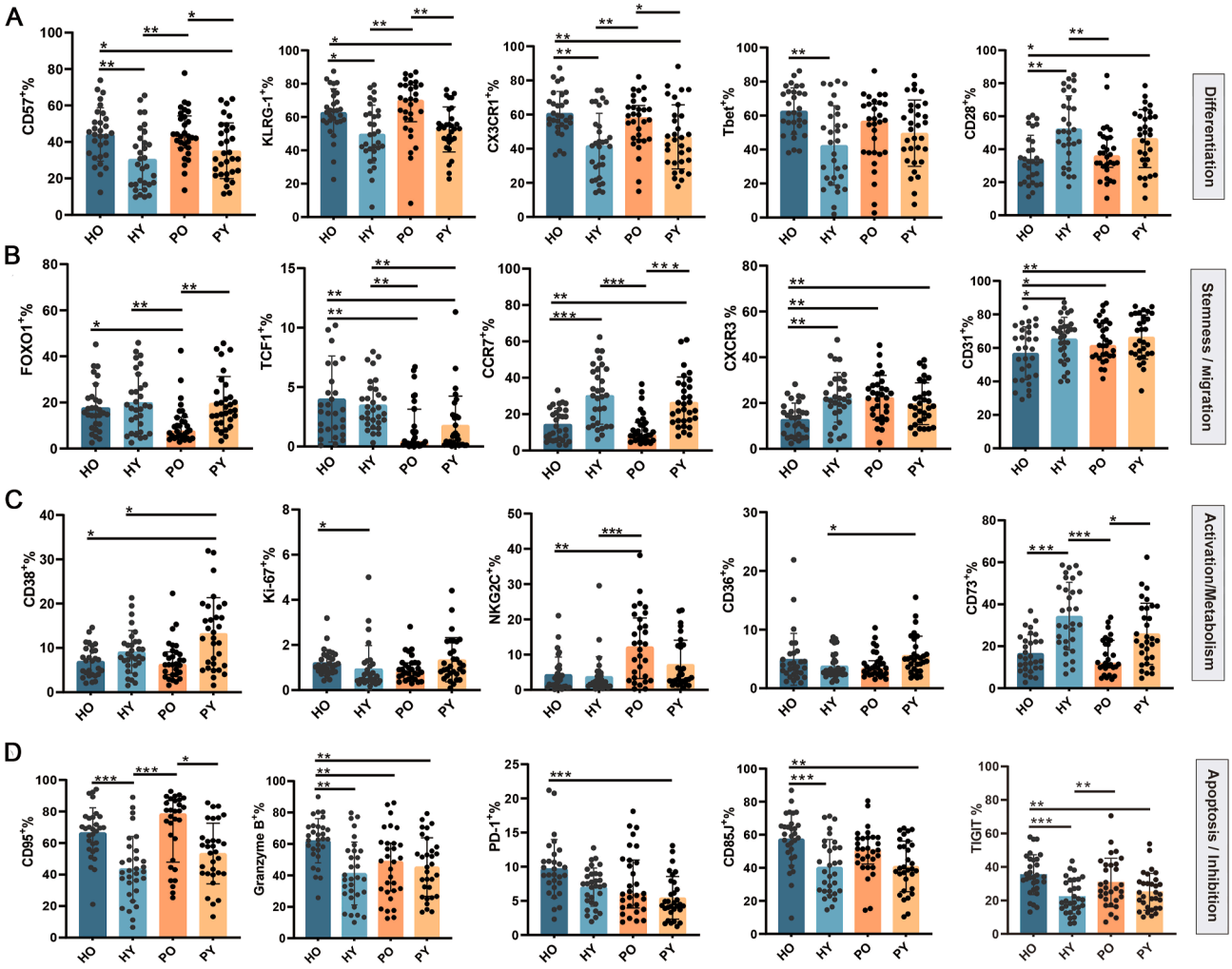

Fig. 3. Phenotypic profiling of CD8$^+$ T cells according to age groups and HIV status. Differentiation (**A**), stemness/migration (**B**), activation/metabolism (**C**), and apoptosis/inhibitory marker expression (**D**) in CD8$^+$ T cells was quantified in the HY, HO, PY and PO groups. The bars show the mean ± SD; the dots denote individual participants. Healthy younger adults (HY, $n = 31$), healthy older adults (HO, $n = 30$), younger people with HIV (PY, $n = 31$), older people with HIV (PO, $n = 30$). *P* values were calculated using one-way ANOVA with Tukey's multiple-comparison test or the Kruskal-Wallis test with Dunn's post hoc test. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

comparisons between the PY and PO groups revealed that KLRG1 and CD95 were significantly enriched in the PO group, whereas FOXO1, CCR7, CD73, and CD38 were enriched in the PY group (Fig. 4B).

At the level of HIV infection status, the GLM for the HY group versus the PY group did not reveal any surface molecules with significant HIV infection-related effects (Fig. 4C). In contrast, in the comparison between the HO and PO groups, the inflammatory chemokine receptor CXCR3 and the apoptosis-related molecule CD95 were significantly enriched in the PO group, whereas FOXO1, granzyme B, and TCF1 were enriched in the HO group (Fig. 4D). Overall, the model-based estimates in Fig. 4 were largely consistent with the distributions of the raw proportions shown in Fig. 3 and were compatible with the CD8$^+$ T-cell phenotypic changes associated with features of immunosenescence across different age groups and HIV infection states.

Mitochondrial alterations in age-related T cells in PWH

Mitochondrial dysfunction is a recognized feature of T-cell immunosenescence (Mittelbrunn and Kroemer, 2021). To compare mitochondrial mass and quality in CD4$^+$ and CD8$^+$ T cells across groups, PBMCs were stained with MitoTracker Green FM (MG), and MG fluorescence intensity was quantified in gated T cells (Fig. 5A). Quantitative analysis further revealed that the MG-MFI was significantly greater in CD4$^+$ T cells than in CD8$^+$ T cells across all the study groups (Fig. 5B). When stratified by HIV infection status, the MG-MFI of both CD4$^+$ and CD8$^+$ T cells was significantly greater in PWH than in HCs. Moreover, across the stratified groups, both CD4$^+$ T cells and CD8$^+$ T cells from the PO group had a significantly higher MG-MFI compared with that noted in the HO and HY groups (Fig. 5C and D).

We subsequently analysed the proportion of T-cell subsets associated with ageing (characterized by CD27$^-$CD57$^+$) and their mitochondrial mass. The frequencies of CD27$^-$CD57$^+$ cells among total CD4$^+$ and CD8$^+$ T cells were significantly higher in both the HO and PO groups than in the HY group, consistent with age-related immunosenescence. Moreover, the MG-MFI intensity of CD27$^-$CD57$^+$CD4$^+$ and CD27$^-$CD57$^+$CD8$^+$ T cells was significantly greater in HIV-infected individuals of the PO and PY groups compared with older healthy controls (Fig. 5E and F). In the PY group, the frequency of CD27$^-$CD57$^+$ cells among total CD4$^+$ T cells was significantly higher than that in the HY group, whereas the frequency of CD27$^-$CD57$^+$ cells among total CD8$^+$ T cells was comparable between the PY and HY groups. In contrast, MG-MFI was significantly higher in CD27$^-$CD57$^+$ CD8$^+$ T cells, but not in CD27$^-$CD57$^+$ CD4$^+$ T cells, in the PY group compared with the HY group (Fig. 5E and F). Collectively, old HIV-infected individuals showed higher mitochondrial mass in total CD4$^+$ and CD8$^+$ T cells than old and young healthy controls and also displayed higher mitochondrial mass in

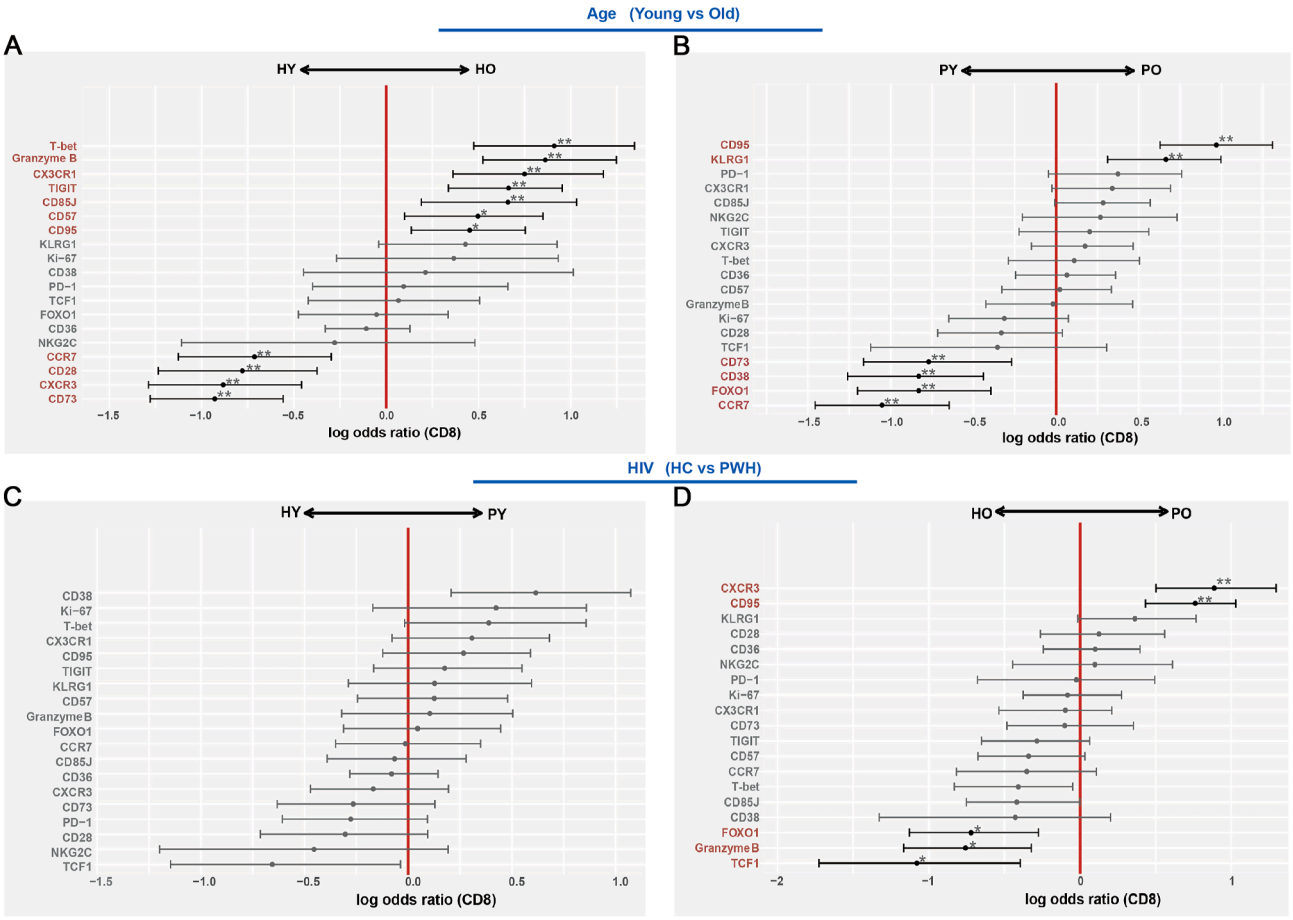

Fig. 4. GLM analysis of CD8$^+$ T-cell–associated marker expression profiles according to age groups and HIV status. Binomial generalized linear models with bootstrap resampling were used to estimate log odds ratios (logORs) for CD8$^+$ T-cell surface marker expression in comparisons of (**A**) HY vs. HO, (**B**) PY vs. PO, (**C**) HY vs. PY and (**D**) HO vs. PO. Forest plots show logORs (dots) with 95% confidence intervals (horizontal bars). Positive logORs indicate higher odds of marker positivity in the group on the right, and negative logORs indicate higher odds in the group on the left. *P* values were adjusted for multiple testing using the Benjamini-Hochberg method; *$q < 0.05$, **$q < 0.01$. Healthy younger adults (HY, $n = 31$), healthy older adults (HO, $n = 30$), younger people with HIV (PY, $n = 31$), older people with HIV (PO, $n = 30$).

senescent-like CD4$^+$ T and CD8$^+$ T cells. These findings indicate altered mitochondrial accumulation in the context of ageing and HIV infection.

Altered mitochondrial membrane potential in T cells in PWH

To assess the impact of HIV infection on T-cell mitochondrial membrane potential (ΔΨm), we measured the ΔΨm in CD4$^+$ and CD8$^+$ T cells from PBMCs using JC-1 staining. The ratio of JC-1 aggregates to monomers (PE/FITC ratio) was used as an indicator of ΔΨm (Fig. 6A and B). Compared with those from HCs, CD4$^+$ T cells from PWH had a significantly greater JC-1 PE/FITC ratio (Fig. 6C). When the different age and infection status groups were compared, the PE/FITC ratio was significantly greater in the PO group compared with the HY group, whereas no significant difference was observed between the PY and HY groups (Fig. 6E). In CD8$^+$ T cells, a similar pattern was observed in which the JC-1 PE/FITC ratio was significantly greater in PWH than in HCs (Fig. 6D). Subgroup analysis revealed that the mitochondrial membrane potential in the PO group was significantly higher than in both the HO and HY groups, and the PY groups also had higher ΔΨm values compared with the HO and HY groups (Fig. 6F). This pattern is consistent with higher mitochondrial membrane potential in CD8$^+$ T cells from PWH, particularly in older individuals.

Correlations of alterations in T-cell immune profiles with age and HIV infection-related clinical parameters

Building on the results of the preceding phenotypic analyses, we examined the correlations between the levels of various markers expressed by CD4$^+$ and CD8$^+$ T cells and age, as well as their mutual relationships across total healthy controls and HIV-infected individuals stratified by CD4$^+$ and CD8$^+$ T cells, to identify coordinated patterns of variation in immunosenescence (Fig. 7). In CD4$^+$ T cells from HCs, the expression levels of markers associated with late cell differentiation, including CD57, KLRG1, CX3CR1, T-bet, and the CD27$^-$CD57$^+$CD4$^+$ subset, were positively correlated with one another and with increasing age. Conversely, their proportions were inversely correlated with those of CD45RA$^+$CD31$^+$CD4$^+$ and CD38$^+$CD4$^+$ T cells, which were negatively correlated with age (Fig. 7A). In addition, CD73 was positively correlated with PD-1, but inversely correlated with CX3CR1 and the CD27$^-$CD57$^+$CD4$^+$ T-cell subset. In HCs, it showed a weak (non-significant) negative association with age (Fig. 7A). However, this correlational pattern was not fully recapitulated in CD4$^+$ T cells from PWH. Specifically, positive correlations between the proportions of cells expressing CD57, KLRG1, CX3CR1, and T-bet and the CD27$^-$CD57$^+$CD4$^+$ subset were retained, but these cells did not exhibit positive correlations with age. Additionally, no inverse correlation was

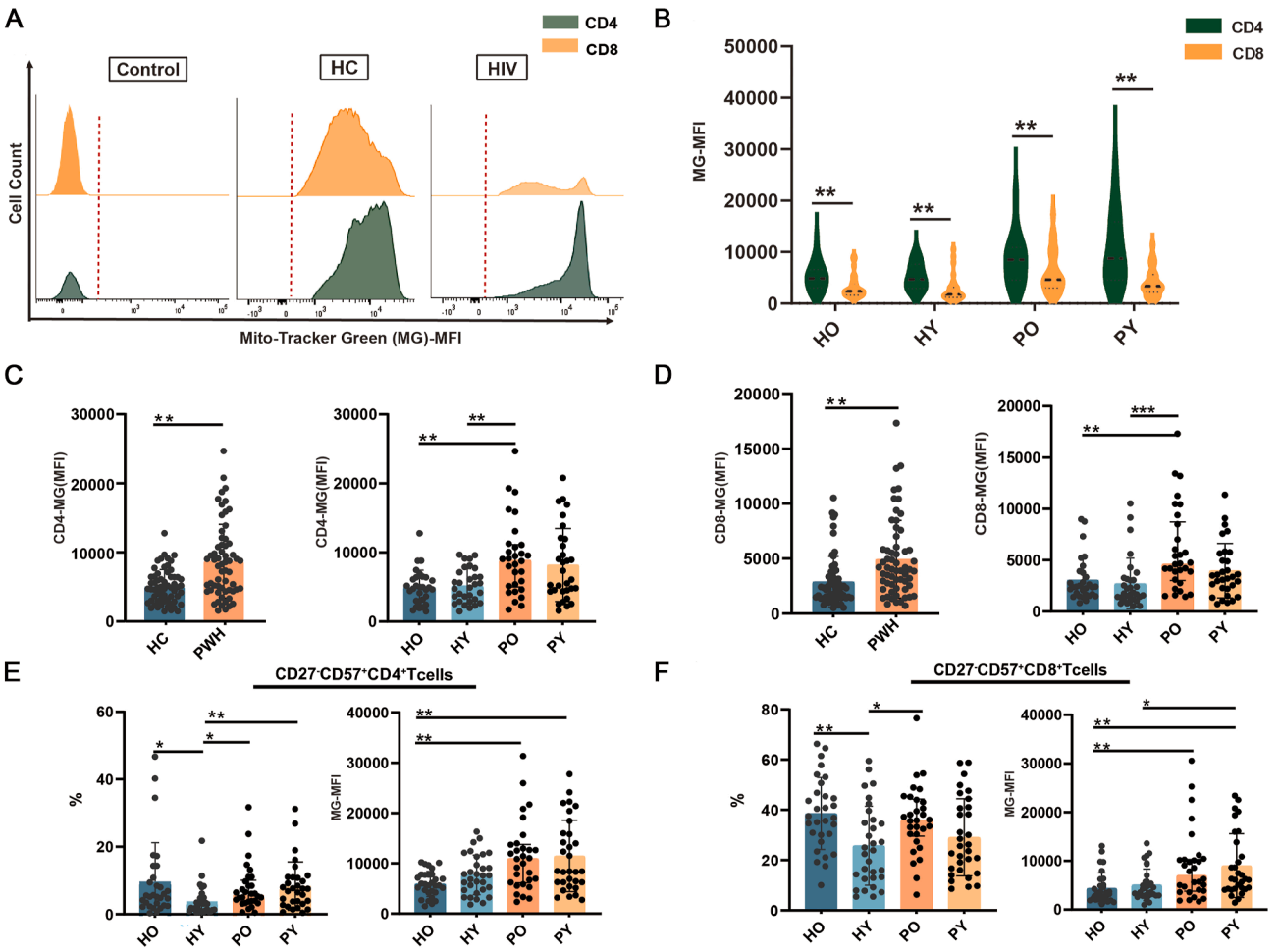

Fig. 5. Mitochondrial mass in $CD4^+$ and $CD8^+$ T cells according to age groups and HIV status. **A** Representative flow cytometry histograms of MitoTracker Green mean fluorescence intensity (MFI) in $CD4^+$ and $CD8^+$ T cells from HCs and PWH. **B** MG-MFI in total $CD4^+$ and $CD8^+$ T cells from the HY, HO, PY and PO groups. **C, D** MG-MFI of $CD4^+$ (**C**) and $CD8^+$ (**D**) T cells in HCs vs. PWH (left) and across the HY, HO, PY and PO groups (right). **E, F** Frequencies of $CD27^-CD57^+$ cells among total $CD4^+$ T cells (**E**) or total $CD8^+$ T cells (**F**), respectively, and MitoTracker Green MFI within these subsets across the four groups. Violin and bar plots show individual participants and group means ± SDs. *P* values were calculated using one-way ANOVA with Tukey's multiple-comparison test or the Kruskal-Wallis test with Dunn's post hoc test. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Healthy younger adults (HY, $n = 31$), healthy older adults (HO, $n = 30$), younger people with HIV (PY, $n = 31$), older people with HIV (PO, $n = 30$).

observed between the expression levels of CX3CR1 and CD38 on $CD4^+$ T cells (Fig. 7B).

The expression levels of CD57, KLRG1, CX3CR1, T-bet; the $CD27^-CD57^+CD8^+$ subset; and the expression of the inhibitory or apoptosis-related molecules CD95 and CD85J were strongly positively correlated with one another and positively correlated with age in $CD8^+$ T cells from HCs, whereas their expression levels were clearly negatively correlated with phenotypes related to early differentiation and migration, such as those of CD28 and CCR7 Similarly, CD73 was positively correlated with CCR7, CD28, and FOXO1, but inversely correlated with the late-differentiation/inhibitory module, and showed a negative association with age (Fig. 7C). This opposing correlation pattern is consistent with two relatively distinct phenotypic modules within the $CD8^+$ T-cell compartment, namely, one enriched for early or stem-like costimulatory features and the other for late differentiated or senescent/inhibitory features. These correlational patterns were similar in $CD8^+$ T cells from PWH; however, markedly inverse correlations between their expression levels and that of CCR7 were not observed (Fig. 7D). Additionally, CD73 was positively correlated with CCR7, CD28, and FOXO1, while showing inverse correlations with late-differentiation markers such as CX3CR1 and CD57, as well as a negative association with age. Although the overall correlation patterns were broadly similar between HCs and PWH, FOXO1 expression in $CD4^+$ and $CD8^+$ T cells was not associated with age in HCs but was markedly negatively correlated with age in PWH (Fig. 7D).

To further investigate the associations between key immune phenotypes and clinical parameters, we assessed the correlations among the indicated markers and $CD4^+$ T-cell counts, the CD4/CD8 ratio, and ART duration (Fig. 7E–L). The results revealed that the frequencies of $FOXO1^+$ cells among both $CD4^+$ and $CD8^+$ T cells were significantly negatively correlated with ART duration, indicating a lower representation of $FOXO1^+$ stem-like/memory T-cell subsets in individuals who received longer-term therapy (Fig. 7E and F). An overall inverse relationship was observed between the CD4/CD8 ratio and the proportions of $T\text{-}bet^+CD4^+$ and $T\text{-}bet^+CD8^+$ T cells (Fig. 7G and H). A lower CD4/CD8 ratio was associated with increased frequencies of T-bet expression in T lymphocytes, which exhibited effector and inflammatory features. In addition, late-differentiated phenotypes, such as $CX3CR1^+CD4^+$ and $KLRG1^+CD4^+$ T cells, were negatively correlated with indices of immune reconstitution, including the CD4/CD8 ratio and $CD4^+$ T-cell counts (Fig. 7I–L), suggesting an association between the accumulation of late differentiation phenotypes and suboptimal immune recovery.

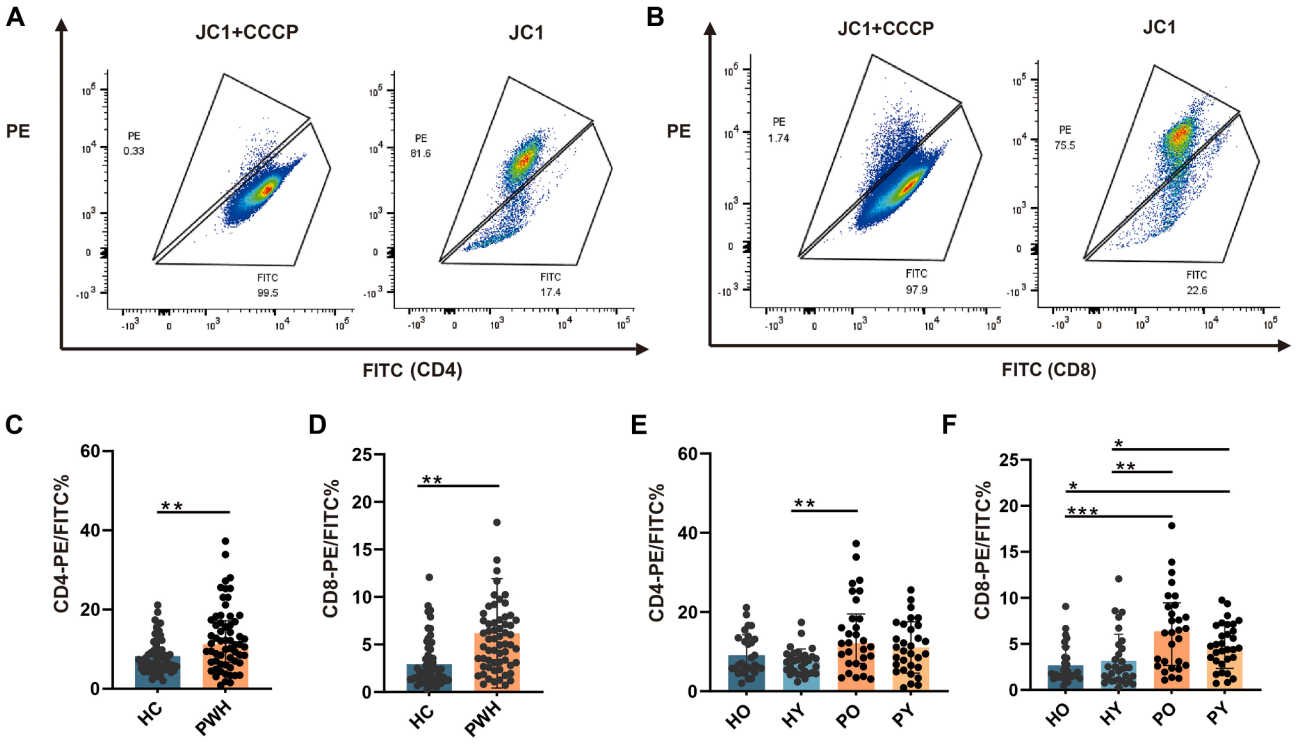

Fig. 6. Mitochondrial membrane potential in $CD4^+$ and $CD8^+$ T cells. **A, B** Representative flow cytometry plots showing JC-1 staining of $CD4^+$ (**A**) and $CD8^+$ (**D**) T cells, with CCCP-treated samples used as depolarized controls. **C, D** JC-1 PE/FITC ratios in $CD4^+$ (**C**) and $CD8^+$ (**D**) T cells from HCs and PWH. **E, F** PE/FITC ratios in $CD4^+$ (**E**) and $CD8^+$ (**F**) T cells across the HY, HO, PY and PO groups. The bars show the mean ± SD, and the dots represent individual participants. Statistical comparisons were performed using one-way ANOVA with Tukey's multiple-comparison test or the Kruskal-Wallis test with Dunn's post hoc test. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Healthy younger adults (HY, $n = 31$), healthy older adults (HO, $n = 30$), younger people with HIV (PY, $n = 31$), older people with HIV (PO, $n = 30$).

DISCUSSION

Senescent T cells accumulate progressively with age (Akbar et al., 2016). In individuals with HIV infection, ageing is a multifactorial process that cannot be adequately captured by any single biomarker; therefore, a composite assessment incorporating multiple biomarkers is preferable (Rodés et al., 2022). Although several studies have investigated the relationship between HIV infection and immune ageing, systematic, multidimensional comparisons of multiple T-cell senescence markers across distinct age groups and HIV infection states remain limited (Lagathu et al., 2017; Li et al., 2025). In the present study, we comprehensively characterized markers of cell differentiation, stemness exhaustion and apoptosis, migration, and metabolic regulation, along with mitochondrial functional parameters, in $CD4^+$ and $CD8^+$ T cells from age-stratified HCs and PWH.

On the basis of the aforementioned multidimensional characterization, our results indicate that both physiological ageing and HIV infection are linked to canonical immunosenescent phenotypes but display distinct patterns of impact on $CD4^+$ and $CD8^+$ T cells. Consistent with previous studies (Rodés et al., 2022; Elias Junior et al., 2024; Zhang et al., 2024), we observed significant age-related immunosenescence characteristics in HCs and PWH. Specifically, regardless of HIV infection status, compared with young healthy controls, elderly individuals exhibited markedly upregulated CD57, KLRG1, CX3CR1, TIGIT, and T-bet expression on both types of $CD4^+$ T cells and increased CD57, KLRG1, CX3CR1, TIGIT, and CD95 expression on $CD8^+$ T cells. Previous studies have demonstrated that these CD57, KLRG1, CX3CR1 and T-bet molecules are closely associated with repeated antigenic stimulation, the accumulation of terminally differentiated T cells, and immunosenescence (Dolfi et al., 2013; Zwijnenburg et al., 2023; Akiyama et al., 2025), suggesting that with advancing age, the T-cell compartment shifts as a whole towards a highly differentiated effector state. Notably, in younger individuals, HIV infection promotes premature enrichment of $CD57^+$ and $CX3CR1^+$ subsets among $CD4^+$ T cells, suggesting that the immunosenescence process may be initiated earlier. This early skewing may arise in the context of HIV-associated immune activation and low-grade inflammation, potentially shaped by gut barrier dysfunction–linked microbial translocation and modulated by frequent coinfections (e.g., CMV). Lifestyle factors, including smoking and alcohol use, as well as comorbidities, may further influence these trajectories by increasing systemic inflammatory burden and ageing-related risk (Rodés et al., 2022; Jin et al., 2025). Further analysis revealed that in people with HIV, the expression of thymic output-related factors of the $CD45RA^+CD31^+$ subset among $CD4^+$ T cells, as well as the expression of stemness-related molecules of FOXO1, decreased progressively with age (Fig. 1B). Given the critical role of these molecules in maintaining T-cell stemness, self-renewal capacity, and functional plasticity and in limiting the development of an exhausted phenotype (Sturmlechner et al., 2023), these findings suggest that in the context of ageing and chronic HIV infection, the regenerative potential and stemness reserve of $CD4^+$ T cells may be particularly vulnerable to depletion.

In contrast, alterations in $CD8^+$ T cells predominantly involved terminal differentiation-related phenotypes and the remodelling of functional regulatory molecules. CD28 deficiency in T cells, a classical indicator of T-cell immunosenescence (Humblin et al., 2023), was reflected by a significant reduction in the proportion of $CD28^+CD8^+$ T cells in older individuals. Moreover, CD73 expression on $CD8^+$ T cells was also significantly decreased, particularly in older PWH, which is consistent with reports linking CD73 expression to T-cell survival and an age-related decrease in its expression (Fang et al., 2021). Consistent with these phenotypic differences, further age-association analyses revealed a clear negative correlation between age and the expression of both CD28 and CD73 on $CD8^+$ T cells in PWH (Fig. 7D and Supplementary Fig. S1). In parallel with these findings, CD95 expression on $CD8^+$ T cells

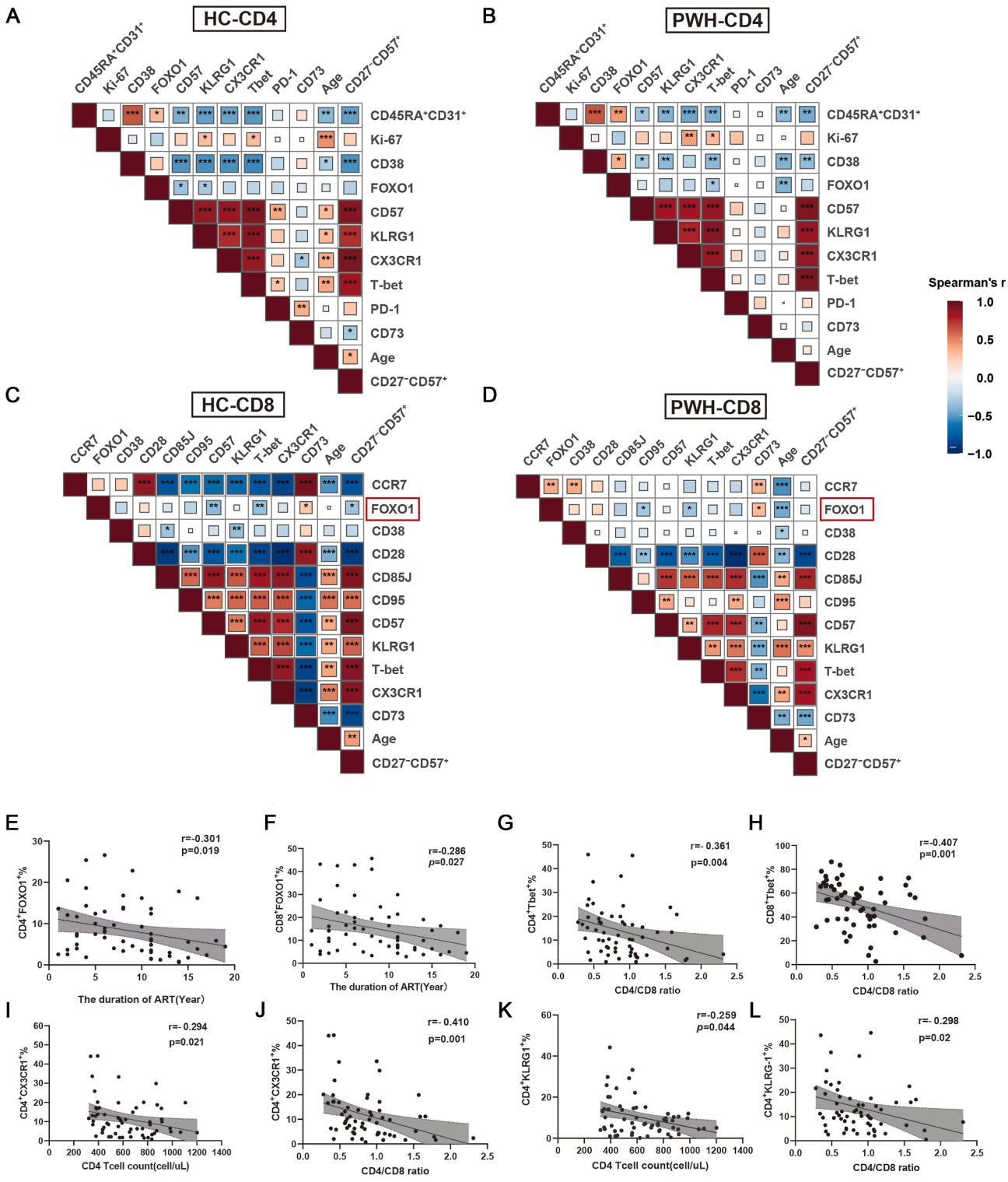

Fig. 7. Correlation structure of $CD4^+$ and $CD8^+$ T-cell phenotypes in HCs and PWH, and their clinical associations. **A–D** Spearman correlation matrices of relevant phenotypic markers in $CD4^+$ and $CD8^+$ T cells from HCs and PWH. The heatmaps show pairwise correlation coefficients (Spearman's r), with red indicating positive correlations and blue indicating negative correlations; only statistically significant correlations are shown (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). **E–L** Spearman correlations between $CD4^+$ or $CD8^+$ T-cell phenotypic markers and clinical parameters in PWH. Each plot shows individual participants with linear regression lines and 95% confidence intervals. Healthy younger adults (HY, $n = 31$), healthy older adults (HO, $n = 30$), younger people with HIV (PY, $n = 31$), older people with HIV (PO, $n = 30$).

increased with age in both PWH and HIV-negative individuals. The enrichment of $CD95^+CD8^+$ T cells in older adults suggests the accumulation of apoptosis-prone subsets, which may impair T-cell homeostasis independent of HIV status. Notably, in PWH the frequency of $CD27^-CD57^+CD4^+$ T cells, although elevated compared with HIV-negative individuals, did not show significant associations with chronological age or current $CD4^+$ T-cell counts in this cohort. This may indicate an HIV-related, relatively age-independent accumulation of this

senescent-like subset, a finding that warrants confirmation in larger studies.

Younger PWH may better preserve mitochondrial quality control and antioxidant defense, characterized by increased expression of CX3CR1, CD57, KLRG1 and T-bet, as well as TIGIT and CD95, accompanied by reduced expression of naïve/stem-like markers ($CD45RA^+CD31^+$, CD28, CD73). Second, HIV infection imposed age-independent perturbations, reflected by the premature enrichment of $CD57^+$, $TIGIT^+$ and $CX3CR1^+$ $CD4^+$ T cells, decreased frequencies of $TCF1^+$ $CD4^+$ and $CD8^+$ T cells in younger PWH versus age-matched HCs, and pronounced mitochondrial hyperpolarization in $CD8^+$ T cells. Third, the marked loss of $FOXO1^+CD4^+$ and $FOXO1^+CD8^+$ T cells was most evident in older PWH, pointing to a synergistic "double hit" of chronological ageing and HIV infection.

Analysis based on GLMs revealed that both age and HIV infection are associated with alterations in the T-cell immune phenotype repertoire, although the patterns of these associations vary across different populations and T-cell subsets. First, among healthy individuals, age-related changes indicate a progressive shift in T cells towards effector- and senescence-like states. Along the "senescence/suppression-proliferation" axis, $CD4^+$ T cells display more pronounced alterations, with advancing age being associated with increased Ki-67, CX3CR1, and TIGIT expression and concomitant decreases in CD28 and CD73 expression. In contrast, $CD8^+$ T cells exhibit a stronger bias towards an "enhanced cytotoxic" profile, characterized by age-related upregulation of Granzyme B, T-bet, and CX3CR1 expression, whereas circulation-associated phenotypes such as CCR7 and CXCR3 are better preserved in younger individuals. In contrast, within the PWH cohort, the age-related gradient observed in healthy individuals was much less apparent. These findings suggest that, in the context of HIV infection, the relationship between age and T-cell phenotypes is likely modulated by multiple factors, including infection-driven immune activation, antiretroviral therapy, coinfections, and interindividual variability (De Francesco et al., 2019; Rodés et al., 2022). As a result, clear age stratification is difficult to demonstrate in cross-sectional analyses.

Clinically, the CD4/CD8 ratio is used as a surrogate marker of immunosenescence in PWH (Sainz et al., 2013; Hove-Skovsgaard et al., 2020; Vassallo et al., 2025). Consistent with these findings, we observed significant inverse correlations between the CD4/CD8 ratio and multiple ageing-related T-cell markers, including CX3CR1, CD57 and KLRG1, supporting a link between these phenotypes and HIV-associated immunosenescence. Consistent with previous reports, FOXO1 has been shown to restrain T-cell senescence and maintain protein homeostasis (Jin et al., 2020; Delpoux et al., 2021). In our cohort, FOXO1 expression was reduced in PWH, especially in older individuals, and GLM analyses revealed a stronger inverse association among FOXO1 levels, age, and senescence-related $CD8^+$ and $CD4^+$ T-cell phenotypes in PWH than in HIV-negative controls. Notably, we observed an inverse association between ART duration and the frequencies of $FOXO1^+CD4^+$ and $FOXO1^+CD8^+$ T cells. In this cross-sectional study, ART duration likely acts as a composite marker that partially reflects disease chronicity—including longer time since infection and cumulative immune perturbation—in addition to treatment exposure. Collectively, these findings suggest that, even under durable virological suppression, the FOXO1-dependent stem-like T-cell compartment may remain vulnerable to ageing-like immune remodelling in PWH. We emphasize that longitudinal studies with regimen-level stratification and adequate covariate adjustment are needed to disentangle HIV- versus ART-related contributions.

In addition to these phenotypic alterations, mitochondrial changes are involved in T-cell ageing. Mitochondrial dysfunction is a hallmark of ageing and contributes to T-cell senescence through its effects on ATP production, calcium homeostasis and apoptosis (Spinelli and Haigis, 2018; Mittelbrunn and Kroemer, 2021). Mechanistically, age-related declines in mitochondrial quality control promote the accumulation of dysfunctional mitochondria and amplify mtROS/mtDNA-associated inflammatory signaling, which restricts the maintenance of long-lived memory/stem-like T-cell pools (Escrig-Larena et al., 2023). In treated PWH, recent studies support a mitochondrial-immune axis in which HIV/ART-associated perturbations of oxidative phosphorylation and mitochondrial danger signaling contribute to persistent immune activation and inflammation despite viral suppression (Jin et al., 2025; Ma et al., 2025). Notably, mitochondrial dysfunction does not invariably manifest as a loss of mitochondrial membrane potential. Under conditions of chronic antigenic stimulation, elevated mitochondrial membrane potential may represent an intermediate stress state associated with increased mtROS production, ultimately leading to depolarization and apoptosis (Weinberg and Chandel, 2025).

In our study, compared with HIV-negative controls, PWH exhibited increased mitochondrial mass and membrane potential in both $CD4^+$ and $CD8^+$ T cells. Notably, these alterations were most pronounced in older PWH, in whom mitochondrial mass and membrane potential reached their highest levels, rather than following a simple linear relationship with age. One possible explanation for our observation that increased mitochondrial mass and hyperpolarization were confined to older PWH is that ageing-related declines in antioxidant defences and mitophagy/mitochondrial turnover, together with prolonged cumulative ART exposure and age-related comorbidities, may promote stress-associated mitochondrial remodelling and the accumulation of hyperpolarized mitochondria under conditions of chronic immune activation (Jin et al., 2025; Luo et al., 2025). Younger PWH may better preserve mitochondrial quality control and antioxidant defences, resulting in milder and more heterogeneous mitochondrial alterations. Consistent with features of cellular ageing, this phenotype likely reflects increased metabolic demand and oxidative stress, accompanied by disrupted mitochondrial homeostasis. Previous studies have demonstrated that mitochondrial dysfunction and oxidative stress are key features of T-cell impairment in HIV infection (Rinaldi et al., 2022; Chan et al., 2025). However, this interpretation remains hypothetical, as ROS, mitophagy flux, and drug levels were not directly measured and thus require further investigation.

Previous work has indicated that $CD8^+$ T cells are more prone to senescent phenotypes, whereas $CD4^+$ T cells tend to exhibit greater mitochondrial mass during ageing (Callender et al., 2020). Similarly, we found that compared with control cells, $CD4^+$ T cells consistently exhibited increased mitochondrial mass across age groups and infection status. This pattern is consistent with reports of mitochondrial imbalance and metabolic reprogramming during ageing and is compatible with the possibility that metabolic dysregulation contributes to T-cell immunosenescence in the context of HIV infection (Akiso et al., 2023). Our findings demonstrating ageing-like T-cell remodelling and mitochondrial alterations in virologically suppressed PWH highlight potential therapeutic avenues. Strategies aimed at reducing residual immune activation and inflammation, including metabolic modulators targeting the immune activation–ageing axis, may help alleviate T-cell ageing and mitochondrial dysfunction (Mo et al., 2023). In addition, lifestyle interventions (e.g., physical activity and smoking cessation) and approaches that preserve FOXO1-mediated T-cell stemness and mitochondrial homeostasis may enhance immune fitness, although these hypotheses warrant validation in longitudinal and interventional studies (Durstenfeld et al., 2024).

This study has several limitations. First, its cross-sectional design limits our ability to assess temporal dynamics and to draw causal inferences regarding T-cell immune phenotypes. Second, our analyses were restricted to peripheral blood T cells and focused primarily on phenotypic markers, without further functional assays or tissue-based validation. Third, detailed information on modifiable lifestyle factors—such as smoking, alcohol consumption, and substance use—was not systematically collected. Furthermore, all participants in this study were men recruited from an MSM cohort. Biological sex can modulate T-cell phenotypes and immune ageing via sex hormone-mediated pathways (Hoffmann et al., 2023). These factors may have introduced

residual lifestyle- and sex-related confounding and may limit the generalizability of our findings to women; thus, residual confounding by these factors cannot be fully excluded. Nevertheless, the use of age-stratified cohorts, comprehensive phenotypic panels and integrated GLM and correlation analyses provides a detailed overview of age- and HIV infection-related T-cell remodelling at the population level.

CONCLUSIONS

In summary, our findings suggest that age is an important determinant of T-cell immunosenescence, with distinct manifestations in $CD4^+$ and $CD8^+$ T-cell subsets and that HIV infection may further modulate, and in some contexts amplify, these age-related immune alterations. Together, mitochondrial stress, FOXO1 downregulation and an imbalance of immune functional networks are consistent with a multifaceted pattern of HIV-associated immune ageing, which may, at least in part, contribute to the heterogeneity of immune reconstitution. Future longitudinal and multiomics studies are warranted to confirm these observations and clarify the key mechanisms involved.

MATERIALS AND METHODS

Subjects

This study was conducted at Beijing Youan Hospital, Capital Medical University. A total of 61 PWH who met the inclusion criteria were included. All participants had received ART for ≥ 12 months and, at enrolment, had plasma HIV-1 RNA levels below the limit of detection, $CD4^+$ T-cell counts ≥ 350 cells/μL, and negative cytomegalovirus (CMV) antibody tests. On the basis of age, PWH were stratified into a younger group (PY; ≤ 35 years; $n = 31$) and an older group (PO; ≥ 50 years; $n = 30$). In parallel, 61 HIV-negative men who have sex with men, matched for age and sex, were recruited as healthy controls (HCs). These participants were similarly stratified into a younger group (HY; ≤ 35 years; $n = 31$) and an older group (HO; ≥ 50 years; $n = 30$). The PWH and HC groups were generally comparable in terms of age and other baseline characteristics, as summarized in Table 1.

$CD4^+$ T-cell counts and HIV-1 RNA quantification

Peripheral blood lymphocyte subsets were counted by flow cytometry using BD Multitest™ CD3-FITC/CD8-PE/CD45-PerCP/CD4-APC reagents (BD Biosciences, San Jose, CA, USA). The plasma HIV-1 viral load (VL, copies/mL) was quantified using real-time PCR (Abbott Molecular, Inc., Des Plaines, IL, USA), with a lower limit of detection of 40 copies/mL.

CMV IgG antibody detection

Plasma samples stored at −80 °C were thawed at 4 °C immediately before testing. CMV-specific IgG antibodies were measured using a commercial ELISA kit (Human Anti-Cytomegalovirus IgG ELISA Kit, Abcam, ab108724) according to the manufacturer's instructions. The plasma samples were diluted 1:100 with the supplied sample diluent and added to CMV antigen-coated microplate wells. After incubation, washing, and colour development, the absorbance was read at 450 nm. The results are expressed as standard units (sample mean absorbance divided by the cut-off value) × 10 and interpreted according to the manufacturer's recommendations as negative (< 9 units), equivocal (9–11 units), or positive (> 11 units).

Flow cytometry and intracellular staining

PBMCs were thawed, resuspended in RPMI 1640 medium, and adjusted to 1×10^6 cells/100 μL. Dead cells were excluded using the Zombie Aqua™ Fixable Viability Kit (BioLegend, cat. no. 423102), and viable cells were stained for surface markers with fluorochrome-conjugated antibodies for 30 min at 4 °C in the dark. After being washed with PBS, the samples were analysed on a BD FACSLyric flow cytometer. For intracellular staining, the cells were fixed and permeabilized using the Foxp3/transcription factor staining buffer set (eBioscience™, cat. no. 00-5523-00), followed by intracellular staining with the corresponding antibodies. Calibration beads (CST) were used to ensure consistency of the fluorescence intensity measurements across the experiments. Flow cytometry data were analysed using FlowJo v10.0 software (Tree Star, Ashland, OR, USA). The gating strategies are shown in Supplementary Figs. S1–S3.

Mitochondrial mass and membrane potential assays

PBMCs were stained immediately after isolation. Mitochondrial mass was assessed by incubating PBMCs with MitoTracker™ Green FM (Invitrogen, Thermo Fisher Scientific, cat. no. M46750) at 37 °C and 5% CO_2 for 40 min in the dark and quantifying the mean fluorescence intensity (MFI) of MitoTracker Green. The mitochondrial membrane potential (ΔΨm) was evaluated by incubating PBMCs with JC-1 dye

Table 1
Baseline characteristics of the study participants.

Characteristics	HC	PWH		*P* value
Number	61	61		N/A
Male/female	61/0	61/0		N/A
Age (years)	35 [31-56]	37 [28-55]		0.73
18~35 [n (%)]	31 (50.8)	31 (50.8)		
≥50 [n (%)]	30 (49.2)	30 (49.2)		
CD4 count (cells/μL)	/	588 [433-820]		N/A
CD8 count (cells/μL)	/	812 [544-1021]		N/A
CD4/CD8	/	0.81 [0.57, 1.07]		N/A
Viral load (copies/mL)	/	TND		N/A
Antiretroviral treatment	/	BIC/FTC/TAF	26 (42.6%)	N/A
		TDF+3 TC + EFV	16 (26.2%)	
		DTG+3 TC	12 (19.7%)	
		Others	7 (11.5%)	
Duration of ART (years)	/	8 [4-11]		N/A

Statistics: values are expressed as n (%) or median [IQR]. *P*-values were calculated using the Mann–Whitney *U* test. Healthy Controls; PWH, People with HIV; NTs, Treatment-Naïve patients. ART: Antiretroviral therapy; BIC, bictegravir; FTC, emtricitabine; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; 3 TC, lamivudine; EFV, efavirenz; DTG, dolutegravir; TND: Undetectable viral load. VL, viral load. The symbol "N/A" indicates that the parameter was not applicable or unavailable in the corresponding group. The percentage of antiretroviral therapy regimens was calculated based on the total number of participants in the HIV-infected individual group ($n = 61$).

(MitoProbe™ JC-1 Assay Kit for Flow Cytometry; Invitrogen, Thermo Fisher Scientific, M34152) at 37 °C and 5% CO_2 for 30 min in the dark, and the results are expressed as the ratio of red (aggregate) to green (monomer) JC-1 fluorescence. After staining, the cells were washed with PBS and then incubated with a viability dye and surface-stained with antibodies against CD3, CD4, CD8, and other markers for 30 min. Samples were immediately acquired on a BD FACSLyric flow cytometer. Flow cytometry data were analysed using FlowJo v10.0 software (Tree Star, Ashland, OR, USA).

Quantification and statistical analysis

Spearman correlation analyses were performed using Prism v5.03 (GraphPad Software, San Diego, CA, USA). Data normality was assessed with the Shapiro-Wilk test. For comparisons among multiple groups, approximately normally distributed data were analysed using one-way ANOVA followed by Tukey's post hoc test, whereas nonnormally distributed data were analysed using the Kruskal-Wallis test with Dunn's multiple comparisons test.

Differences in the proportions of T cells expressing specific phenotypic markers between groups were evaluated in R v4.3.1 using generalized linear models with a binomial distribution (logistic regression), with the number of marker-positive cells over the total number of cells as the response variable and group as the predictor. Effect sizes are reported as log odds ratios (logORs) with 95% confidence intervals (CIs). To obtain more robust inference, model parameters were estimated using a combination of bootstrapping and permutation testing, with 2000 bootstrap resamples and 5000 permutations of group labels. Multiple testing across markers was controlled using the Benjamini-Hochberg procedure to control the false discovery rate (FDR). Benjamini-Hochberg-adjusted P values (q values) were reported, and $q < 0.05$ was considered to indicate statistical significance. Forest plots were generated to display LogORs and their 95% CIs.

DATA AVAILABILITY

Data supporting the findings are available from the corresponding authors upon reasonable request.

ETHICS STATEMENT

All experimental procedures conducted in this study were reviewed and approved by the Research Ethics Committee of Beijing Youan Hospital (Ethics Approval No. [2025−002]). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. All participants provided written informed consent for the collection of information and for clinical samples to be stored and used for research. The methods used conformed to approved guidelines and regulations.

AUTHOR CONTRIBUTIONS

Junyan Jin: methodology, investigation, data curation, writing-original draft, formal analysis, writing-review & editing; Xin Zhang: methodology, supervision, writing-original draft, writing-review & editing; Qianqian Xu: sampling, investigation; Wei Xia: resources, data curation; Hongxia Yan: resources, data curation; Hao Wu: resources, data curation; Christiane Moog: supervision, writing-review & editing; Tong Zhang: conceptualization, supervision, writing-review & editing; Bin Su: conceptualization, supervision, writing-review & editing, funding acquisition.

CONFLICT OF INTEREST

The authors declare no competing financial interests.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2026.05.001.

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