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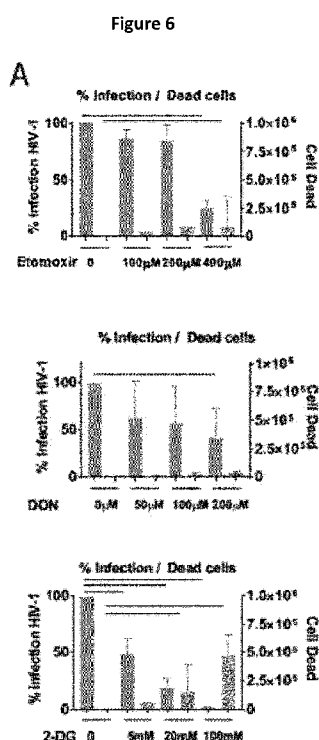
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(54) Title: CELLULAR METABOLISM OF HIV-1 RESERVOIR SEEDING IN CD4+ T CELLS



(57) Abstract: HIV persists in long-lived infected cells that are not affected by antiretroviral treatment. These HIV reservoirs are mainly located in CD4+ T-cells, but their distribution is variable in the different subsets. Susceptibility to HIV-1 increases with CD4+ T-cell differentiation. We evaluated whether the metabolic programming that supports the differentiation and function of CD4+ T-cells affected their susceptibility to HIV-1. We found that differences in HIV- susceptibility between naïve and more differentiated subsets were associated with the metabolic activity of the cells. Indeed, HIV-1 selectively infected CD4+ T-cells with high oxidative phosphorylation and glycolysis, independent of their activation phenotype. Moreover, partial inhibition of glycolysis (i) impaired HIV-1 infection in vitro in all CD4+ T-cell subsets, (ii) 10 decreased the viability of pre-infected cells, and (iii) precluded HIV-1 reactivation in cells from HIV-infected individuals. Our results elucidate the link between cell metabolism and HIV- infection and identify a vulnerability to tackle HIV reservoirs and infections with other pathogens.

CELLULAR METABOLISM OF HIV-1 RESERVOIR SEEDING IN CD4+ T CELLS

FIELD OF THE INVENTION

The present invention relates to compounds and methods for partially inhibiting of cellular metabolism, and particularly glycolysis, in cells targeted by pathogens, such as human immunodeficiency viruses.

BACKGROUND TO THE INVENTION

Combination antiretroviral treatment (cART) blocks HIV-1 replication but does not eliminate infected cells. Replication competent HIV-1 persists in cellular reservoirs that are the origin of rapid viral rebound when treatment is interrupted (Finzi et al., 1997). Identifying the factors underlying the seeding and survival of HIV-infected cells is a priority in the search for an HIV cure (Deeks et al., 2016). CD4+ T-cells are the major target for HIV-1 infection and are thought to constitute most of the HIV-1 reservoir. However, not all CD4+ T-cells contribute equally to the pool of persistently infected cells during cART. The composition of CD4+ T-cells that remain infected is mainly determined by the susceptibility of CD4+ T-cell subsets to HIV infection, their resistance to HIV-induced apoptosis and their life span and turnover potential (Barton et al., 2016). Naïve CD4+ T-cells are highly resistant to HIV-1 infection, while HIV-1 susceptibility increases in more differentiated cell subsets (Roederer et al., 1997; Schnittman et al., 1990). Accordingly, there is a minimal contribution of naïve CD4+ T-cells to the HIV reservoir during cART, which is mainly restricted to the memory cell subsets (Chomont et al., 2009). The susceptibility of CD4+ T-cells to HIV-1 infection depends on the relative abundance of cell factors required by the virus to complete its replication cycle and of cellular restriction factors that counteract infection (Lever and Jeang, 2011). T-cell activation sharply increases the expression of HIV dependency factors and thereby cell susceptibility to HIV-1 infection (Pan et al., 2013; Stevenson et al., 1990), despite the concomitant presence of some restriction factors that the virus can most often circumvent. However, responsiveness to TCR activation (Byrne et al., 1988; Roederer et al., 1997) and susceptibility to HIV infection are not homogeneous across or within CD4+ T-cell subsets. This discrepancy in infection efficacy suggests that HIV-1 has

adapted to infect CD4+ T-cells with a specific cellular program (Cleret-Buhot et al., 2015). The cellular processes orchestrating the optimal conditions for the establishment of HIV-1 infection remain unclear.

Numerous studies have demonstrated the role of cellular metabolism in T-cell immunity (Pearce et al., 2013; Waickman and Powell, 2012). Naïve T-cells circulate in a quiescent state, relying essentially on oxidative phosphorylation (OXPHOS). Upon T-cell activation and after receiving appropriate cues (costimulation, cytokines), naïve T-cells undergo metabolic reprogramming, strongly increasing OXPHOS and, especially, glycolysis, to cope with the energy demands of immune function and rapid proliferation (Pearce et al., 2013). The biomass accumulation that accompanies enhanced cellular metabolism may provide viruses with the abundance of factors that are necessary for their replication. It is worth noting that several retroviruses have evolved to use metabolite transporters as cellular receptors. The glucose transporter 1 (GLUT) is the main receptor for HTLV-1 (Manel et al., 2003); phosphate transporters PiT1 and PiT2 have been reported as surface receptors for koala retrovirus, feline leukemia virus and murine leukemia viruses (Oliveira et al., 2006; Takeuchi et al., 1992; von Laer et al., 1998); and the amino acid transporters ASCT1 and ASCT2 are the receptors for the feline RD-114 endogenous retrovirus (Shimode et al., 2013). Although HIV-1 does not use metabolite transporters as its main receptors, GLUT1 expression is necessary for the post entry steps of HIV-1 replication in CD4+ T-cells (Loisel-Meyer et al., 2012). Moreover, the metabolism of nucleotides is critical for HIV-1 reverse transcription (Amie et al., 2013).

BRIEF SUMMARY OF THE INVENTION

The invention encompasses compositions comprising metabolic inhibitors and methods of using these compositions.

In one embodiment, the invention encompasses a method of reducing HIV production from a T cell comprising contacting a population of cells comprising HIV-infected CD4+ T cells with 2-deoxy-glucose (2-DG) at a concentration that reduces the production of HIV from said HIV-infected CD4+ T cells. In one embodiment, the cells are exposed to a concentration of 1-25 mM 2-DG. In one embodiment, the cells are exposed to a concentration of 3-10 mM 2-DG. In one embodiment, the cells are exposed to a concentration of 5 mM 2-DG.

In one embodiment, the method further comprises contacting the population of cells with a metabolic inhibitor that blocks fatty acid transport to mitochondria. In one embodiment, the metabolic inhibitor is Etomoxir.

5 In one embodiment, the cells are contacted with the metabolic inhibitor *in vitro*. In one embodiment, the cells are contacted with the metabolic inhibitor *in vivo*. In one embodiment, the cells are contacted with an additional metabolic inhibitor of metabolic function in CD4+ T cells.

10 In one embodiment, the population of cells has received cART treatment. The cART can comprise Combivir, Kaletra, Trizivir, Epzicom, Kivexa, Truvada, Atripla, Complera, Eviplera, Stribild, Triumeq, Evotaz, Prezcobix, Dutrebis, Genvoya, or Descovy. The cART can comprise at least 2 or 3 of any of the following compounds: lamivudine; zidovudine; lopinavir; ritonavir; abacavir; tenofovir disoproxil fumarate; emtricitabine; efavirenz; rilpivirine; elvitegravir; cobicistat; dolutegravir; atazanavir; cobicistat; darunavir; and raltegravir.

15 In one embodiment, the invention encompasses a method of reducing virus or bacterial pathogen production from a cell comprising contacting a population of cells pathogen-infected cells with at least 2 metabolic inhibitors of different metabolic functions in the cells at a concentration that reduces the production of the pathogen from said pathogen-infected cells.

20 In one embodiment, one of the metabolic inhibitors blocks glycolysis. In one embodiment, one of the metabolic inhibitors blocks fatty acid transport to mitochondria. In one embodiment, both of the metabolic inhibitors are selected from Table 1. In one embodiment, two of the metabolic inhibitors are DON and 2DG. In one embodiment, three of the metabolic inhibitors are DON and etomoxir and 2DG. In one embodiment, concentration of the metabolic inhibitor(s) is a suboptimal concentration.

In one embodiment, the pathogen is HIV-1.

25 In one embodiment, the invention encompasses a method for measuring the effect of a metabolic inhibitor of metabolic function in cells in a pathogen-infected human comprising: administering at least one dose of a metabolic inhibitor of metabolic function in cells to the human; and measuring the level of pathogen infection in the pathogen-infected human.

In one embodiment, measuring the level of pathogen infection in the pathogen-infected human comprises measuring the level of plasma HIV RNA in an HIV-infected human. In one embodiment, measuring the level of plasma HIV RNA in the HIV-infected human is performed by a reverse transcription and amplification reaction. In one embodiment, the level of HIV infection in the human is measured at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 times.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-D depict CD4+ T-cells subsets have different susceptibilities to HIV-1 infection. Figure 1A depicts representative example of the proportion of 5-days activated CD4+ T cells expressing GFP in the absence of infection (top) or 72 h after challenge with HIV-1_{GFP}-VSV (bottom). Figure 1B depicts relative distribution of CD4+ T-cell subsets in non-activated (NA) and activated (aCD3 5d) cells before HIV challenge and in activated cells not expressing GFP (aCD3 5d GFP-) or expressing GFP (aCD3 5d GFP+) 72 h post challenge. The pie charts (top) represent the median values (n=3 donors). The bottom panels represent the fold change in the CD4+ T-cells subset contribution relative to the non-activated condition (NA). *p<0.05; ** p<0.01. In a different set of experiments, sorted CD4+ T-cell subsets were cultured under NA or activated conditions for 3 (3d) or 5 days (5d) and challenged with HIV-1_{GFP}-VSV. Figure 1C depicts representative example of infection levels in T_n, T_{cm}, T_{tm} and T_{em} cells from a donor in the different conditions analyzed. Figure 1D depicts Medians and IQR values for experiments with cells from 6 donors. Symbols represent the individual data points. Significant differences between experimental conditions are shown for each T-cell subset as horizontal lines. The median infection level in NA T_n cells is displayed as a reference dashed line to facilitate comparison between T-cell subsets.

Figure 2A-B depict HIV-1 infection levels in CD4+ T-cell subsets correlate with the expression levels of genes related to cell metabolism. Figure 2A shows Heat maps displaying the genes differentially expressed (p<0.05) between the CD4+ T-cell subsets (T_n, T_{cm}, T_{tm} and T_{em}) (n= 6 donors) in the absence of activation or after 3 or 5 days of activation with soluble anti-CD3 (i.e., at the time of HIV challenge) (green = downregulation, red = upregulation). Variables are ordered by hierarchical clustering and samples by CD4+ T-cell subsets. Figure 2B shows Spearman's correlation between the levels of gene expression at the time of HIV-1 challenge

and HIV-1 infection levels 72 h after challenge. Only significant correlations ($p < 0.05$) are represented in the graphs (green bars). Genes highlighted in red show the group of genes that correlated with infection levels in all conditions.

Figure 3A-C depict CD4+ T-cell subsets have different metabolic profiles that coincide with their susceptibility to HIV-1 infection OCR and ECAR in non-activated (NA), 3-day activation (3d) and 5-day activation (5d) CD4+ T-cell subsets. Figure 3A depicts Median values of the metabolic variables obtained for the CD4+ T-cell subsets from the 6 donors in the different conditions analyzed. Figure 3B depicts Median and IQR basal OCR (left panel) and ECAR (right panel). Figure 3B depicts basal ECAR/OCR ratio for CD4+ T-cell subsets in different activation states (0d, 3d, 5d). Median values in NA Tn cells are indicated by dashed lines as a reference. Symbols represent independent experiments ($n=6$). Figure 3C shows a Summary of correlations between metabolic parameters at the time of infection in NA, 3d and 5d activated CD4+ T-cell subsets and the % of infected cells 72 h post infection. The green color indicates $p < 0.05$. The size of the circle represents Spearman's coefficients.

Figure 4A-C depict HIV-1-infected CD4+ T-cells are characterized by higher metabolic activity levels. Figure 4A depicts Metabolic activity (OCR and ECAR) of sorted HIV-infected GFP+ and non-infected GFP- CD4+ T-cells ($n=3$). The bioenergetic (XF) phenotypes of GFP+ and GFP- cells (right panel) were determined by the basal OCR and ECAR values. The symbols represent independent experiments ($n=3$ donors). Figure 4B depicts In a different set of experiments, CD4+ T-cells were sorted 72 h after HIV challenge based first on their activation levels (high activation, CD25+/HLA-DR+ or low activation, CD25-/HLA-DR-) and then on the level of GFP expression (GFP- or GFP+ cells). The gating strategy is shown on the left panels. Pie charts (right) represent the median ($n=4$ donors) distribution of the CD4+ T-cell subsets (determined by flow cytometry) for each sorted cell fraction as follows: high activation and GFP+, high activation and GFP-, low activation and GFP+ and low activation and GFP- ($n=4$). Figure 4C depicts Representative analyses of OCR and ECAR (measured as above) for each cell fraction (left) and the median and IQR basal OCR and ECAR for 6 (high activation) and 4 (low activation) donors (right).

Figure 5A-B depicts rate of glucose uptake by CD4+ T-cell subsets is associated with their susceptibility to HIV-1 infection CD4+ T cells were sorted based on their differentiation status (Tn or Tcm) and their rate of 2NBDG uptake. Sorted cells were then challenged with HIV-1_{GFP}-VSV. Figure 5A depicts Representative example of 2NBDG content after sorting (top panels) and the levels of GFP expression 72 h after challenge in CD4+ T-cell fractions exposed (HIV-1) or not (control) to the virus. Figure 5B depicts Percentage of GFP-positive cells among CD4+ T-cell fractions (Tn and Tcm sorted depending on their pre-infection 2-NBDG uptake). Symbols represent individual values (n=3, Tn; n=5, Tcm) donors. Medians and IQR values are represented by horizontal lines.

Figure 6A-F depict inhibition of cell metabolic pathways blocks HIV-1 infection of CD4+ T-cells. Figure 6A depicts Relative level of infection (blue bars) and cell death (purple bars) compared to the control conditions in 5-days activated CD4+ T-cells infected in the absence or presence of increasing amounts of Etomoxir, DON, or 2-DG (median and IQR, n=3 donors). Figure 6B depicts Infection and cell death in CD4+ T-cells exposed to HIV-1 in glucose-containing medium in the absence or presence of 2-DG (5 mM) or in culture medium without glucose (starvation) (left) or in the absence or presence of UK5099 (25 μ M) (right). Figure 6C depicts Relative number of U5-Gag copies in CD4+ T-cells at 6h, 15h or 72h after infection with HIV-1_{GFP}-VSV in the absence or presence of 2-DG. Individual values (symbols), medians and IQRs (horizontal lines) for five different donors are shown. Figure 6D depicts Infection levels and number of U5-Gag copies 72h after challenge with HIV-1_{GFP}-VSV in the absence or presence of 2-DG added at the time of challenge, 4h or 8h post challenge. Values represent the relative levels of infection compared to the control condition (median and IQR, n=3 donors). Figure 6E depicts Changes in HIV-1 infection levels in CD4+ T-cell subsets 72h after the infection of bulk CD4+ T-cells in the absence or in presence of 2-DG (orange symbols) or Etomoxir (beige symbols). Medians (n=7 donors) are shown. Figure 6F depicts Percentage of HIV-1 productively (left panel) or latently (right panel) infected cells 72h after the infection of CD4+ T-cells with HIV-1DuoFluo VSVG particles in the presence of 2-DG or etomoxir. Median and IQR values from experiments with 6 donors are shown. Figure 6G depicts p24 production in supernatants from CD4+T-cell cultures 3 and 7 days after infection with HIV-1 BaL in the absence (blue bars) or presence of 2-

DG (5 mM) (orange bars). Means and standard deviations for three replicates are shown at each time point for experiments done with cells from three different donors.

Figure 7A-D depict suboptimal inhibition of glucose metabolism selectively eliminates preinfected CD4+ T-cells and inhibits HIV-1 amplification from reservoirs. Figure 7A depicts cell viability in sorted pre-infected GFP+ (green) or noninfected GFP- (red) CD4+ T cells cultured for 48 h in the absence or presence of 2-DG. One representative example is shown. Figure 7B depicts relative survival of 2-DG treated cells (circles) was compared to that of nontreated cells (squares) at 24 h and 48 h. Figure 7C depicts changes in the CD4+ T-cell subset distribution 48h after the treatment of infected bulk CD4+ T-cells with 2-DG when compared with the distribution in the control condition. Median values and IQR are shown (n=3 donors). Figure 7D depicts HIV-1 reactivation from CD4+ T-cells from six individuals on cART upon PHA/IL-2 stimulation in the absence (blue line/symbols) or presence of 2-DG (5 mM) (orange line/symbols) (mean and SD, 3 replicates). Mean p24 values in the absence or presence of 2-DG on day 14 post stimulation are shown for all six experiments (right panel).

Figure 8 depicts treatment of CD4+ T cells from the same donor with various metabolic inhibitors at the indicated concentrations, alone or in combination, and shows the synergistic effect of suboptimal amounts of 2DG, DON and ETOMOXIR on the extent of HIV-1 infection.

Figure 9, related to STAR methods

A representative example of the flow cytometry gating strategy used to sort CD4+ T cell subsets. Naïve (Tn; CD4-CD45RA+, CCR7+, CD27+, CD95-), central memory (Tcm; CD4-CD45RA-, CCR7+, CD27+), transitional memory (Ttm; CD4-CD45RA-, CCR7-, CD27+) or effector memory (Tem; CD4-CD45RA-, CCR7-, CD27-) displayed as standard pseudocolor dot plots. These sorted CD4+ T cell subsets were used in the gene expression analyses, metabolic profile assays and HIV-1 susceptibility assays. Table with gender, age and relative CD4+ T cell subset distribution ex vivo for each of the donors studied in the gene expression analyses, metabolic profile assays and HIV-1 susceptibility assays.

Figure 10, related to Figure 2

Spearman's correlation between the levels of expression of all genes analyzed at the time of HIV-1 challenge and HIV-1 infection levels 72 h after challenge for nonactivated (NA), 3-

day activation (3d) or 5-day activation (5d) CD4⁺ T cells. Significant correlations ($p < 0.05$) are shown in green. Venn diagram and table showing the genes correlating with infection in the different activation conditions.

Figure 11, related to Figure 3

5 A) Representative example of flow cytometry results at the end of the glucose uptake assay showing levels of 2-NBDG in nonactivated and 5-day activation CD4⁺ T cells. We defined the following 3 gates based on 2-NBDG capture levels: low, medium and high (upper panels). The levels of 2-NBDG captured by the different CD4⁺ T cell subsets are shown in the overlapping histogram (middle panels). The relative contributions of CD4⁺ T cell subsets to the low, medium and high 2-NBDG cell fractions are displayed in the cumulative bar chart (lower panels). B) A
10 representative example of GLUT1 surface expression (left panel) and glucose uptake (right panel) (2-NBDG assay) in the CD4⁺ T cell subsets studied here after 5 days of suboptimal activation. C) Correlation between metabolic parameters (basal OCR, maximal respiratory capacity, basal and maximal ECAR measure with a Seahorse analyzer) at the time of
15 infection in NA, 3d and 5d activated CD4⁺ T cell subsets and the % of infected cells 72 h post infection. Each symbol represents values for values for one CD4⁺ T cell subset from one individual. The p values of Spearman correlation analyses are shown. Lines depict linear regression of analyzed data.

Figure 12, related to Figure 4

20 A) Representative example of the flow cytometry gating strategy used to sort noninfected GFP⁻ and infected GFP⁺ CD4⁺ T cells 72 h after challenge with single-cycle HIV-1 GFP particles and GFP expression in the purified populations. Representations are displayed as standard pseudocolor dot plots (left). Representative example of the glycolytic activity of GFP⁺ and GFP⁻ sorted cells analyzed before and after the addition of glucose,
25 oligomycin and 2-DG (right). B) Percentage of cells among each CD4⁺ T cell subset expressing HLA-DR and CD25 five days after suboptimal activation with aCD3 (upper panel). Black horizontal lines above the symbols denote statistically significant differences subsets. C) Representative example of expression levels of CD25 and HLA-DR for CD4⁺ T cell fractions sorted as high activation and GFP⁺, high activation and GFP⁻, low activation and GFP⁺ and

low activation and GFP-. D) Changes in the relative contribution of CD4+ T cell subsets to the GFP+ fractions when compared to their GFP- counterparts. Asterisks represent statistically significant differences (* $p < 0.05$; ** $p < 0.01$).

Figure 13, related to STAR methods and Figure 5

5 A) Flow cytometry gating strategy used to sort CD4+ Tn and Tcm cells according to their glucose (2- NBDG) uptake levels and the 2-NBDG content of the CD4+ T cell fractions after sorting. Representations are displayed as standard pseudocolor dot plots. Sorted cells were used for HIV-1 susceptibility assays. B) Representative example (left) and summary (median and IQR, $n=3$ donors) (right) of the glycolytic activity of sorted Tcm cells
10 with high glucose uptake (TcmHGlu) and low glucose uptake (TcmLGlu).

Figure 14, related to Figure 6

Representative example of the metabolic profile of five-day activation CD4+ T cells after being treated with increased concentrations of the metabolic inhibitors Etomoxir (top panel) and 2-DG (middle panel). OCR/ECAR basal ratio after the
15 administration of 2-DG and Etomoxir (Lower panel).

Figure 15, related to Figure 6 A) A representative example of GFP and mCherry expression in noninfected CD4+ T cells or in CD4+ T cells 72 h post infection with the HIV Duo-Fluo I virus. As described in (Calvanese et al., 2013), cells that expressed GFP alone or in combination with mCherry were considered productively infected (I), cells that expressed
20 only mCherry were considered latently infected (L), and cells that lacked expression of both fluorescent markers were considered noninfected (NI). Pie chart diagrams show the median ($n=5$ donors) contribution of CD4+ T cell subsets to the pool of HIV-1 productively infected (upper panel) or latently infected cells (lower panel) 72 h after infection of nonactivated (NA), 3-day activation (3d) or 5-day activation (5d) CD4+ T cells
25 challenged with the HIV-1 DuoFluo I virus. B) Relative infection levels (with respect to naive cells) in 5 days-activated CD4+ T cells from donors ($n=9$) 72h after challenge with HIV Bal or VSVG pseudotyped NL4.3AenvGFP particles. Infection levels were determined by flow cytometry quantification of intracellular p24 and GFP respectively (top panels). Changes in the distribution of cell subsets in HIV+ cells in relation to non- infected cells (bottom panels).

The asterisks represent statistically significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

C) Infection levels in 5 days

activated CD4⁺ T cells 72 h post challenge with HIV-1 Bal in the absence or presence of 2-DG added at the time of challenge, 4h or 8h post challenge. Blue bars represent the relative level of infection (determined by intracellular p24 staining) compared to the control conditions (median and IQR, $n = 3$ donors). Horizontal lines indicate statistical differences.

DETAILED DESCRIPTION OF THE INVENTION

In the present study, we undertook the analysis of the conditions determining the intracellular susceptibility of CD4⁺ T-cell subsets to HIV-1 infection. In particular, we analyzed whether the metabolic program is distinct according to the differentiation of CD4⁺ T-cell subsets and if this determines their susceptibility to HIV-1 infection. We show that cellular metabolism is a central factor driving the HIV-1 infection of CD4⁺ T-cells and that it may be an important target for new therapies against HIV-1.

In this study, we performed a detailed characterization of the bioenergetics of CD4⁺ T_N, T_{CM}, T_{EM} and T_{TM} cells. Upon potent TCR activation, naïve and memory cells have been shown to strongly upregulate their metabolism and acquire effector functions (van der Windt et al., 2013). Here, we show important metabolic differences among the three memory cell populations studied, even in the absence of stimulation. Upon anti-CD3 activation, all CD4⁺ T-cell subsets enhanced their metabolic activity but essentially maintained their distinctive metabolic programs, which matched the requirements for their expected rapid reaction to antigenic stimulation (T_{EM} >> T_{TM} > T_{CM} >> T_N). The metabolic activity of the T-cell subsets overlapped with their susceptibility to HIV-1 infection (Figures 1C and 3B), supporting that the extent of HIV-1 infection in CD4⁺ T-cell subsets was affected by the metabolic environment within the target cells.

Transcript profiling at the time of infection showed that among the CD4⁺ T-cell subsets, there were positive correlations between the frequencies of HIV-infected cells and the expression levels of multiple genes related to cell metabolism. Negative correlations were found between the susceptibility of CD4⁺ T-cells to HIV-1 infection and the expression of SAMHD1, an

efficient HIV-1 restriction factor that also plays an important role in the regulation of cell metabolism (Descours et al., 2012; Mathews, 2015). Surprisingly, strong positive correlations were found between the levels of HIV-infected cells and the expression of a cluster of genes related to the interferon response. Although this point was not specifically explored in the present study, increasing evidence has revealed the interrelationships between cell metabolism and the interferon response (Burke et al., 2014; Zhao et al., 2015). Some type 1 interferons might enhance glycolysis (Fritsch and Weichhart, 2016), and interferon regulatory factors play a key role during the metabolic reprogramming that follows TCR-mediated activation of T-cells (Man et al., 2013). The interaction between the interferon response and cell metabolism may somewhat explain the dichotomy between antiviral and viral-enhancing interferon-stimulated genes (Schoggins and Rice, 2011; Seo et al., 2011). Tem cells, which were the most susceptible to HIV infection in our assay, expressed the strongest levels of several restriction factors such as SLFN11 or APOBEC3G. Our results thus indicate that HIV-1 exploits the metabolic environment that most favors the completion of its replication cycle, and this might be one of the factors underlying the adaptation of HIV-1 to evade some restriction factors.

We further confirmed the association between T-cell metabolism and HIV infection in a series of functional analyses. First, we showed that HIV-infected CD4⁺ T-cells had higher levels of metabolic activity and metabolic potential than HIV-exposed but noninfected cells. This was not solely the consequence of the preferential infection of cells with higher activation levels; when we sorted CD4⁺ T-cells that were matched for the expression of common activation markers, we still found that HIV-infected cells had higher metabolic activity levels than noninfected CD4⁺ T-cells. Although there are well-established links between T-cell activation and cellular metabolism, it is increasingly clear that T-cell functions, including proliferation, the secretion of cytokines and cell survival, are supported through different engagements of the various metabolic pathways (Jones and Bianchi, 2015). This may explain the partial dichotomy between T-cell activation and cell metabolism in HIV infection that we observed in our experiments. Additionally, we found Tn cells expressing high levels of activation markers upon anti-CD3 stimulation, but these cells remained mostly resistant to HIV-1 infection. In contrast, the frequency of infected Tn cells sharply increased when we challenged highly glycolytic Tn

cells. This is in agreement with previous results that showed that expression of GLUT1 is necessary for HIV-1 infection of CD4+ T-cells (Loisel-Meyer et al., 2012) and that, in vitro, HIV preferentially infects CD4+ T-cells expressing GLUT1 and OX40 (Palmer et al.). Overall, our results demonstrate that cells that had higher metabolic activity levels were more susceptible to HIV infection.

In our experimental conditions, we could detect virtually no infected cells when we challenged cells with low metabolic activity levels. Thus, any potential change in cell metabolism that might have been induced directly by HIV particles was not sufficient to promote infection in cells that had low metabolic activity levels at the time of viral challenge. However, it is important to note that because we were interested in understanding the factors modulating HIV infection beyond the expression of HIV receptors, we used single-cycle particles devoid of HIV envelope and pseudotyped with VSV-G in this set of experiments. It is possible that fully replication-competent viruses have a stronger effect on modulating CD4+ T-cell metabolism. CCL5 engagement with CCR5 has been described as increasing glycolysis in T-cells (Chan et al., 2012), and it is possible that gp120 triggers a similar effect. Moreover, HIV infection has been shown to induce increased expression of several glucose transporters in in vitro experiments (Kavanagh Williamson et al., 2018; Sorbara et al., 1996). Overall, viruses appear to possess different mechanisms to enhance cell metabolism to favor viral replication (Goodwin et al., 2015; Sanchez and Lagunoff, 2015), and this deserves additional exploration in the context of HIV infection.

Suboptimal inhibition of glycolysis impaired HIV replication, and this was observed with single-cycle VSVG pseudotyped particles and replication-competent HIV-1 Bal and for all CD4+ T-cell subsets, although the effects were more pronounced in more energetic cells. Inhibition of glycolysis, including several hours after viral entry, severely reduced the accumulation of HIV reverse transcripts and impaired the establishment of both productive and latent infections. Our results thus point to critical steps early during the viral replication cycle (in particular reverse transcription) that are influenced by glycolysis, which agrees with a previous report (Loisel-Meyer et al., 2012). Along these lines, the synthesis of deoxynucleotides, the level of which is a limiting factor for HIV reverse transcription, is very energy demanding and requires

substrates that are provided by different metabolic pathways, such as the pentose phosphate pathway (PPP) that is parallel to glycolysis (Lane and Fan, 2015). Although, unfortunately, genes involved in the PPP were not included in our gene expression panel, we found important differences between CD4+ T-cell subsets and strong correlations with infection levels for several genes such as TP53, ESF1 and RRM2, which play critical roles in the de novo synthesis of dNTPs. In particular we have recently shown that changes in the expression of RRM2 impact HIV-1 replication in macrophages and dendritic cells by modifying the pools of dNTPS (Allouch et al., 2013; Valle-Casuso et al., 2017). Moreover, SAMHD1, the expression levels of which were negatively correlated with infection in our analysis, is a deoxynucleoside triphosphohydrolase that contributes to control the intracellular dNTP concentration during cell-cycle (Mathews, 2015). Our results therefore suggest that metabolically active cells offer an environment with positive synthesis (RRM2) vs degradation (SAMHD1) of dNTP pools that favors HIV-1 reverse transcription. However, other steps of the viral replication cycle may also depend on cell metabolism. The inhibition of glycolysis has been shown to decrease the production of HIV-1 particles (Hegedus et al., 2014), and mTOR, a key regulator of cellular metabolism (Waickman and Powell, 2012), appears to be involved in the establishment of HIV-1 latency in CD4+ T-cells (Besnard et al., 2016).

In our functional experiments we mostly focused on assessing the impact of glycolysis on HIV infection. Our results showing that inhibition of pyruvate transport to the mitochondria with UK5099 blocked HIV infection suggests that glucose oxidation is important for HIV-1 infection. However the relative contribution of aerobic vs oxidative glycolysis remains to be determined. It is likely that other metabolic functions are also important for HIV-1 infection. The inhibition of fatty acid oxidation with Etomoxir had a limited effect on HIV replication in suboptimal conditions, mostly in Tem cells, but it strongly inhibited infection at higher concentrations. However, caution is needed when interpreting results obtained with Etomoxir as it has been shown to produce off target effects at such high concentrations (O'Connor et al., 2018; Yao et al., 2018). A recent report suggested that fatty acid metabolism may also participate in the late steps of viral replication (Kulkarni et al., 2017). Our results with the glutamine antagonist DON suggest that glutamine metabolism may also be necessary for the

optimal infection of CD4+ T-cells. In general, the association between HIV infection and cell metabolism can be exploited to impair HIV-1 replication.

Cell survival is another process regulated by cell metabolism that could be critically relevant for the persistence of infected cells. We found that suboptimal inhibition of glycolysis induced the selective death of cells that had been preinfected in vitro, and this affected all CD4+ T-cell memory subsets. We also show here that the partial inhibition of glycolysis in CD4+ T-cells from HIV-infected individuals on cART potently blocked viral reactivation and spread. Based on our results, this could be the result of a combination of both the elimination of infected cells and the blocking of new cycles of viral amplification by 2-DG. Overall our results point to the potential modulation of cell metabolism as a strategy to combat HIV infection.

Therapies targeting cellular metabolism are gaining interest in the cancer field (Zhao et al., 2013). Metabolic reprogramming observed in tumor cells closely resembles the metabolic profile of HIV-infected T-cells that we describe here. In the context of the physiopathology of HIV infection, high glucose consumption by infected CD4+ T-cells could have additional implications for immune responses. We recently found that while HIV-specific CD8+ T-cells from rare individuals naturally controlling HIV infection are characterized by metabolic plasticity, HIV-specific CD8+ T-cells from most HIV-infected subjects heavily rely on glycolysis to exert their functions (Angin *et al*, submitted). High levels of glucose consumption by CD4+ T-cells at the sites of viral replication might severely limit glucose availability for these CD8+ T-cells and impair their effector function. In addition, lactic acid, which is a product of glycolysis, inhibits effector functions in cytotoxic T-cells (Mendler et al., 2012). Therefore, the metabolic characteristics of HIV-infected CD4+ T-cells may provide the virus with additional mechanisms to mediate immune evasion, as has also been described for tumors (Sugiura and Rathmell, 2018). Because exploiting the host cell metabolic machinery appears to be a common strategy for invading pathogens, including viruses, bacteria and parasites, therapies targeting cell metabolism could affect a large spectrum of infections. Obviously, cell metabolism regulates critical physiological events, including immune responses, and it is necessary to develop a better understanding of the links between cell metabolism and acute and chronic infections. Overall, our study shows that cellular metabolism is a central factor that drives the HIV-1 infection of CD4+ T-cells more

strongly than does the state of differentiation and/or activation, and cellular metabolism may be an important target for new therapies against HIV-1.

CD4+ T-cells expressing PD-1 and other immune checkpoints are enriched in HIV in HIV-infected individuals receiving cART (Banga et al., 2016; Chomont et al., 2009; Fromentin et al., 2016). Interestingly, these immune checkpoints appear to mediate their inhibitory activities through the metabolic reprogramming of the cells (Lim et al., 2017; Patsoukis et al., 2015). This suggests that the metabolic requirements of HIV-1 replication might enduringly imprint the infected cells.

Based on these finding, the invention provides compositions, methods and uses of a compound for inhibiting and treating viral and bacterial pathogen infections (e.g. HIV infections) and methods for assessing the effects of these compounds on pathogen infections. Compounds that inhibit the metabolic function cells can be used for inhibiting and treating pathogen infections *in vitro* and *in vivo*. In one embodiment, compounds that inhibit the metabolic function of CD4+ cells can be used for inhibiting and treating HIV infections *in vitro* and *in vivo*.

Screening Methods

The invention encompasses various screening methods for determining the effect of a compound that inhibits the metabolic function of cells in a pathogen-infected human and *in vitro*.

Within the scope of this invention, “metabolism” refers to chemical transformations within living cells that serve three main purposes: the conversion of food or fuel to energy to run cellular processes, the conversion of food or fuel to building blocks for proteins, lipids, nucleic acids and carbohydrates, and the elimination of waste.

Within the scope of this invention, “metabolic function” means a defined metabolism pathway, not including upstream or downstream effects. Preferably, the metabolic function(s) is(are) selected from those listed in Table 1 and/or Table 2.

Within the scope of this invention, “metabolic enzyme” means an enzyme within a defined metabolism pathway. More preferably, the targeted metabolic enzyme(s) is(are) selected from those listed in Table 1.

Within the scope of this invention, “metabolic inhibitor” means an inhibitor of metabolic process or enzyme within a defined metabolism pathway. Most preferably, the metabolic

inhibitor(s) is(are) selected from those listed in Table 1. In preferred embodiments, the metabolic inhibitor (metabolic pathway/enzyme) is selected from I-DON (Glutaminolysis), 2DG (Glycolysis), etomoxir (fatty acid transport/ Carnitine palmitoyltransferase-1), UK5099 (mitochondrial pyruvate carrier (MPC)), BPTES (Glutaminolysis), Orlistat (fatty acid synthesis),
5 Metformin (fatty acid oxydation), and Rapamycin (mTOR). Preferably, the metabolic inhibitor is active in human CD4+ cells and/or macrophages.

In the present invention, I-DON or DON refers to 6-Diazo-5-oxo-L-norleucine, also named as 6-diazo-5-oxo-l-norleucine or (L)-DON.

All possible combinations of 1, 2, 3, 4, or 5 of the metabolic inhibitors set forth herein
10 (e.g., Table 1 & Table 2) are specifically contemplated. For example, preferred combinations of inhibitors are DON+2DG, DON+etomoxir+2DG, BPTES+UK5099, BPTES+Etomoxir, Etomoxir+UK5099, Etomoxir+2DG, and DON+2DG+ Etomoxir. In addition, all possible combinations of 1, 2, 3, 4, or 5 of the metabolic function targets and metabolic enzyme targets set forth herein (e.g., Table 1, Table 2, and the Examples) are specifically contemplated.

15 In one embodiment, the invention encompasses various screening methods for determining the effect of a metabolic inhibitor that inhibits the metabolic function of cells, preferably CD4+ T cells, in a human, preferably a Human Immunodeficiency Virus-infected human, and *in vitro*.

In one embodiment, the method comprises administering at least one dose of the
20 metabolic inhibitor to a human or to cells *in vitro*; and measuring the level of pathogen (e.g., HIV) infection in the human or cells. Preferably, the metabolic inhibitor is active in human CD4+ cells.

In one embodiment, the method comprises administering at least one dose of the metabolic inhibitor to the human or cells; and measuring the level of plasma HIV RNA in the
25 human or in the cell supernatant. In one embodiment, the method comprises administering at least one dose of the metabolic inhibitor to the human; and measuring the level of HIV-infected reservoir cells in the human.

In various embodiments, the metabolic inhibitor reduces glycolysis or blocks fatty acid transport to mitochondria. In a preferred embodiment, the metabolic inhibitor is administered a

concentration that reduces the production of HIV from HIV-infected CD4+ T cells. In a preferred embodiment, the metabolic inhibitor is 2-deoxy-glucose (2-DG). Preferably, the cells are exposed to a concentration of 1-25 mM 2-DG, 3-10 mM 2-DG, or 5 mM 2-DG. In another preferred embodiment, the metabolic inhibitor is Etomoxir.

5 The metabolic inhibitor can be used by itself, or preferably, in combination with additional metabolic inhibitor, preferably one that affects a different metabolic function. Preferably, the metabolic inhibitor targets one or more of the metabolic functions or enzymes listed in Table 1 and/or Table 2. The metabolic inhibitors listed in Table 1 and Table 2, and modified versions thereof, are particularly preferred.

<i>Targeted metabolic function</i>	<i>Targeted metabolic enzymes</i>	<i>Metabolic metabolic inhibitors (activators*)</i>
Glycolysis	Glucose transporter 1	Phloretin
		WZB117
	Glucose transporter 4	Ritonavir
	Hexokinase	2-Deoxy-D-glucose
		3-Bromopyruvic acid
		LND
	Pyruvate kinase isozymes M2	shRNA
Citric acid cycle		TEPP-46*
	Lactate Dehydrogenase A	FX11
	Monocarboxylate transporters	Oxamate
	Hypoxia-inducible factor 1- α	4-CIN/ α CHC
Fatty acid synthesis	Pyruvate Dehydrogenase Kinase	2-methoxyestradiol
		siRNA
	Pyruvate transporter	DCA
Fatty acid oxydation	Fatty acid synthase	UK5099
		Ceruleinin
		C75
	Acetyl CoA carboxylase	Orlistat
Oxidative Phosphorylation	Carnitine palmitoyl transferase I	TOFA
	AMP Kinase	Etomoxir
		AICAR*
Glutaminolysis	Complex I	Metformin*
	ATP synthase	Rotenone
	Glutaminase	Olygomycin
		BPTES
		I-DON

Table 1. Small molecules targeting cellular metabolism

Drug	Target
2DG	Glucose
Lonidamine	HK
Dichloroacetate (DCA)	PDK
Polyphenon E	LDH-A
FK866	GAPDH
AT-101	LDH-A
PEG-ADI	Arginase 1
Indoximod	LAT1
Acivicin	Glutamine
6-Diazo-5-oxo-L-norleucine (DON)	Glutamine
Orlistat	FASN
Metformin	GPDH
NVP-BEZ235	PI3K/mTOR
GDC-0980 (apitolisib)	PI3K/mTOR
SF1126	PI3K
BYL719	P110a
MLN1117	P110a
AZD8186	p110 β
GSK2636771	p110 β
SAR260301	p110 β
Perifosine	Akt
MK-2206	Akt
GSK690693	Akt
GDC-0068	Akt
BEZ235	PI3K/mTOR
XL765	PI3K/mTOR
GDC0890	PI3K/mTOR
GSK1059615	PI3K/mTOR
AZD8055	mTORC1/2
AZD2014	mTORC1/2

Table 2. Overview of drugs, currently in clinical development (from van der Mijn et al. Cancer & Metabolism (2016) 4:14)

The measurement can provide for a comparison to another infected individual that does not receive the metabolic inhibitor or to a prior measurement from that same infected individual, preferably before treatment with the metabolic inhibitor. Preferably, the measurement of the level of HIV infection in the human is performed at least twice. In some
5 embodiments, the measurement is taken 3, 4, 5, 6, 7, 8, 9, or 10 times. In this way, the measurements can provide for a comparison over time within that infected individual, most preferably with a measurement taken before treatment with the metabolic inhibitor

For example, the level of HIV infection can be assessed by different techniques known to the skilled artisan. For example, the level of HIV infection in the human can be determined by
10 measuring the level of plasma HIV RNA in the human.

For example, the level of plasma HIV RNA in the human can be measured by a reverse transcription and amplification reaction. For example, reverse transcription of the RNA of an HIV can be performed with a “reverse primer” specific for HIV. A “reverse primer” is one that, based on its 5’-3’ orientation, can bind to a single-stranded RNA and serve to initiate generation of a
15 complementary DNA (cDNA) copy of the RNA. The reverse transcription can be accomplished using well known and routine methods. The reaction mix for reverse transcription contains the reagents for the reaction, for example, a reverse primer, dNTPs (dATP, dCTP, dGTP and dTTP), a buffer, and a reverse transcriptase. Exemplary reaction conditions are set forth in the examples.

Amplification of the cDNA copy of an HIV generated by reverse transcription can be
20 performed with a “forward primer” specific for HIV. A “forward primer” is one that, based on its 5’-3’ orientation, can bind to a single-stranded antisense cDNA copy of an RNA generated by reverse transcription and serve to initiate generation of a double-stranded DNA copy of the RNA. The amplification can be accomplished using well known and routine methods. The reagent mix for amplification contains the reagents for the reaction, for example a forward
25 primer, a reverse primer, dNTPs, a buffer, and a DNA polymerase.

In one embodiment, the method of the invention is performed using a single RT-PCR reagent mix containing the reagents for the reverse transcription and amplification reactions. Preferably, the reverse primer used for the reverse transcription reaction is also used for the amplification reaction.

Preferably, the reverse transcription and amplification reactions are performed in a plastic or glass container, most preferably in the same container.

Amplification methods known in the art include RCA, MDA, NASBA, TMA, SDA, LCR, b-DNA, PCR (all forms including RT-PCR), RAM, LAMP, ICAN, SPIA, QB-replicase, or Invader. A preferred amplification method is the polymerase chain reaction (PCR) amplification. See, e.g., PCR Technology: Principles and Applications for DNA Amplification (Ed. H. A. Erlich, Freeman Press, NY, N.Y., 1992); PCR Protocols: A Guide to Methods and Applications (Eds. Linis, et al., Academic Press, San Diego, Calif., 1990); Mattila et al., Nucleic Acids Res. 19, 4967 (1991); Eckert et al., PCR Methods and Applications 1, 17 (1991); PCR (Eds. McPherson et al., IRL Press, Oxford); and U.S. Pat. Nos. 4,683,202, 4,683,195, 4,800,159, 4,965,188, and 5,333,675. More preferred PCR methods is real-time PCR, PCR-HRM (High-Resolution DNA Melting) (see Andriantsoanirina et al. Journal of Microbiological Methods, 78: 165 (2009)) and PCR coupled to ligase detection reaction based on fluorescent microsphere (Luminex® microspheres).

Amplification techniques include in particular isothermal methods and PCR-based techniques. Isothermal techniques include such methods as nucleic acid sequence-based amplification (NASBA), loop-mediated isothermal amplification (LAMP), helicase-dependent amplification (HDA), rolling circle amplification (RCA), and strand displacement amplification (SDA), exponential amplification reaction (EXPAR), isothermal and chimeric primer-initiated amplification of nucleic acids (ICANs), signal-mediated amplification of RNA technology (SMART) and others (see e.g. Asciello and Baeumner, Lab Chip; 11(8): 1420-1430, 2011).

Preferably, the PCR technique quantitatively measures starting amounts of DNA, cDNA, or RNA. Examples of PCR-based techniques according to the invention include techniques such as, but not limited to, quantitative PCR (Q-PCR), reverse-transcriptase polymerase chain reaction (RT-PCR), quantitative reverse-transcriptase PCR (QRT-PCR), or digital PCR. These techniques are well known and easily available technologies for those skilled in the art.

Preferably, the method is a one-step real-time RT-PCR assay, for example, as described in the Examples. Most preferably, the method is a one-step real-time RT-PCR assay based on TAQMAN probe technology capable of detecting the recently described African E and F

genogroups and including a competitive RNA internal control (IC), for example, as described in the Examples.

Preferably, a probe is used to detect the amplified product. The probe can be labeled with a fluorescent, radioactive, or enzymatic label. The amplified product can be detected with a specific detection chemistry such as fluorescence resonance energy transfer (FRET) probes, TAQMAN probes, molecular beacons, scorpion probes, fluorescently labeled (or other labeled) primers, lightup probes or a dye-based chemistry, DNA, PNA, LNA, or RNA including modified bases that bind to the amplified product to detect the sequence of interest.

Detection of the amplified products can be real-time (during the amplification process) or endpoint (after the amplification process). The invention allows for detection of the amplification products in the same vessel as amplification occurs.

Preferably, a DNA internal control is used to monitor the amplification reaction.

Preferably, a RNA internal control is used to monitor the reverse transcription and amplification reactions.

In some embodiments, the metabolic inhibitor (e.g., 2-DG) is administered in at least one administration of 1-200 mg/kg/day 5-160 mg/kg/day, 10-80 mg/kg/day, 20-70 mg/kg/day, 30-60 mg/kg/day, or 20-40 mg/kg/day. Preferably, the administration is at least 1-5, 5-10, 10-20, 20-40, 40-60, 60-80, 80-100, 100-120, 120-140, or 140-160 mg/kg/day. Preferably, the administration is at least 1, 5, 10, 20, 40, 60, 80, 100, 120, 140, or 160 mg/kg/day of the metabolic inhibitor. Most preferably, the administration is at least 1, 5, 10, 20, 40, 60, 80, 100, 120, 140, or 160 mg/kg/day of 2-DG. Although not specifically enumerated, all values and subranges within the above and below ranges are specifically included as if explicitly written out. The administration of the metabolic inhibitor can be by many methods known in the art, most preferably intravenous, intra-arterial, subcutaneous, intramuscular, sublingual, transmucosal, or oral.

In some embodiments, multiple administrations are given. In various embodiments, at least 1-100, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10-20, 20-50, or 50-100, administrations are given. In various embodiments, the administration is at least twice/ day, twice/week, once/day,

once/week, three times/week, or once/every 2 days. The administration can be given continuously (e.g., with a pump).

In various embodiments, at least .01, .05, 0.1, 0.5, 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 100, 120, 160 mg/kg/day of a metabolic inhibitor is administered for
 5 at least 1, 2, 3, 4, 5, 6, 7 days, 1, 2, 3, 4, 5, 6 weeks, or 1, 2, 3, 4, 5, 6, etc. months.

In various embodiments, at least .01, .05, 0.1, 0.5, 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 100, 120, 160 mg/kg/day is administered every 2 days or 3 times/week for at least 2, 3, 4, 5, 6, 7 days, 1, 2, 3, 4, 5, 6 weeks, or 1, 2, 3, 4, 5, 6, etc. months.

Pathogens

10 Pathogens within the scope of the invention include:

Acinetobacter baumannii, Anaplasma genus, Anaplasma phagocytophilum, Ancylostoma
 braziliense, Ancylostoma duodenale, Arcanobacterium haemolyticum, Ascaris lumbricoides,
 Aspergillus genus, Astroviridae, Babesia genus, Bacillus anthracis, Bacillus cereus, Bartonella
 henselae, BK virus, Blastocystis hominis, Blastomyces dermatitidis, Bordetella pertussis, Borrelia
 burgdorferi, Borrelia genus, Borrelia spp, Brucella genus, Brugia malayi, Bunyaviridae family,
 15 Burkholderia cepacia and other Burkholderia species, Burkholderia mallei, Burkholderia
 pseudomallei, Caliciviridae family, Campylobacter genus, Candida albicans, Candida spp,
 Chlamydia trachomatis, Chlamydophila pneumoniae, Chlamydophila psittaci, CJD prion,
 Clonorchis sinensis, Clostridium botulinum, Clostridium difficile, Clostridium perfringens,
 20 Clostridium perfringens, Clostridium spp, Clostridium tetani, Coccidioides spp, coronaviruses,
 Corynebacterium diphtheriae, Coxiella burnetii, Crimean-Congo hemorrhagic fever virus,
 Cryptococcus neoformans, Cryptosporidium genus, Cytomegalovirus (CMV), Dengue viruses
 (DEN-1, DEN-2, DEN-3 and DEN-4), Dientamoeba fragilis, Ebolavirus (EBOV), Echinococcus
 genus, Ehrlichia chaffeensis, Ehrlichia ewingii, Ehrlichia genus, Entamoeba histolytica,
 25 Enterococcus genus, Enterovirus genus, Enteroviruses, mainly Coxsackie A virus and Enterovirus
 71 (EV71), Epidermophyton spp, Epstein-Barr Virus (EBV), Escherichia coli O157:H7, O111 and
 O104:H4, Fasciola hepatica and Fasciola gigantica, FFI prion, Filarioidea superfamily,
 Flaviviruses, Francisella tularensis, Fusobacterium genus, Geotrichum candidum, Giardia
 intestinalis, Gnathostoma spp, GSS prion, Guanarito virus, Haemophilus ducreyi, Haemophilus

influenzae, *Helicobacter pylori*, *Henipavirus* (Hendra virus Nipah virus), Hepatitis A Virus, Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Hepatitis D Virus, Hepatitis E Virus, Herpes simplex virus 1 and 2 (HSV-1 and HSV-2), *Histoplasma capsulatum*, HIV (Human immunodeficiency virus, particularly HIV-1 and HIV-2), *Hortaea werneckii*, Human bocavirus (HBoV), Human herpesvirus 6 (HHV-6) and Human herpesvirus 7 (HHV-7), Human metapneumovirus (hMPV), Human papillomavirus (HPV), Human parainfluenza viruses (HPIV), Japanese encephalitis virus, JC virus, Junin virus, *Kingella kingae*, *Klebsiella granulomatis*, Kuru prion, Lassa virus, *Legionella pneumophila*, *Leishmania* genus, *Leptospira* genus, *Listeria monocytogenes*, Lymphocytic choriomeningitis virus (LCMV), Machupo virus, *Malassezia* spp, Marburg virus, Measles virus, *Metagonimus yokagawai*, *Microsporidia* phylum, *Molluscum contagiosum* virus (MCV), Mumps virus, *Mycobacterium leprae* and *Mycobacterium lepromatosis*, *Mycobacterium tuberculosis*, *Mycobacterium ulcerans*, *Mycoplasma pneumoniae*, *Naegleria fowleri*, *Necator americanus*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Nocardia* spp, *Onchocerca volvulus*, *Orientia tsutsugamushi*, *Orthomyxoviridae* family (Influenza), *Paracoccidioides brasiliensis*, *Paragonimus* spp, *Paragonimus westermani*, Parvovirus B19, *Pasteurella* genus, *Plasmodium* genus, *Pneumocystis jirovecii*, Poliovirus, Rabies virus, Respiratory syncytial virus (RSV), Rhinovirus, rhinoviruses, *Rickettsia akari*, *Rickettsia* genus, *Rickettsia prowazekii*, *Rickettsia rickettsii*, *Rickettsia typhi*, Rift Valley fever virus, Rotavirus, Rubella virus, Sabia virus, *Salmonella* genus, *Sarcoptes scabiei*, SARS coronavirus, Schistosoma genus, *Shigella* genus, Sin Nombre virus, Hantavirus, *Sporothrix schenckii*, *Staphylococcus* genus, *Staphylococcus* genus, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Strongyloides stercoralis*, *Taenia* genus, *Taenia solium*, Tick-borne encephalitis virus (TBEV), *Toxocara canis* or *Toxocara cati*, *Toxoplasma gondii*, *Treponema pallidum*, *Trichinella spiralis*, *Trichomonas vaginalis*, *Trichophyton* spp, *Trichuris trichiura*, *Trypanosoma brucei*, *Trypanosoma cruzi*, *Ureaplasma urealyticum*, Varicella zoster virus (VZV), Varicella zoster virus (VZV), Variola major or Variola minor, vCJD prion, Venezuelan equine encephalitis virus, *Vibrio cholerae*, West Nile virus, Western equine encephalitis virus, *Wuchereria bancrofti*, Yellow fever virus, *Yersinia enterocolitica*, *Yersinia pestis*, *Yersinia pseudotuberculosis*, and Zika virus.

Treatment Methods and Uses

The invention encompasses methods of treatment using the metabolic inhibitors described above and the use of compositions comprising these metabolic inhibitors in the treatment of a pathogen infection in a human patient.

5 In one embodiment, the method comprises administering at least one metabolic inhibitor to a pathogen-infected human at a concentration that reduces the production of the pathogen from said pathogen-infected cells. In one embodiment, the method comprises administering at least 2 metabolic inhibitors of different metabolic functions in the cells at concentrations that reduce the production of the pathogen from said pathogen-infected cells.

10 Preferably, a suboptimal dosage of the metabolic inhibitor is administered. Within the scope of this invention, the term “suboptimal” refers to a dose at which the metabolic inhibitor does not have on its own maximum effects either on the metabolic pathways, but also do not cause more than 10% cell death of the corresponding uninfected cells (e.g. uninfected CD4+ T cells).

15 In one embodiment, the method comprises administering an effective amount of at least one metabolic inhibitor to an HIV-infected human. An effective amount is an amount of the metabolic inhibitor(s) that reduces the level of detectable pathogen (e.g., plasma HIV RNA in an HIV-infected patient) at least 2-fold. In some embodiments, the administration of metabolic inhibitor(s) reduces the level of detectable pathogen (e.g., plasma HIV RNA in an HIV-infected
20 patient) at least 2-, 4-, 10-, 30-, 50-, or 100-fold.

In some embodiments, the administration of the metabolic inhibitor reduces the pathogen (e.g., viral or bacterial) load in the patient at least 2-, 4-, 10-, 30-, 50-, or 100-fold. In some embodiments, the administration of the metabolic inhibitor reduces the number of HIV-1 infected reservoir cells at least 2-, 4-, 10-, 30-, 50-, or 100-fold. In some embodiments, the
25 administration of the metabolic inhibitor reduces active viral or bacterial replication at least 2-, 4-, 10-, 30-, 50-, or 100-fold. .

The above reductions can be determined by routine techniques in the art, such as by comparing the levels in the patient before and after administration of the metabolic inhibitor, for example by standard PCR amplification methods with patient plasma samples.

The reduction can be assessed at various times after administration, for example at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 20, 40, or 52 weeks after administration of the metabolic inhibitor.

5 In some embodiments, the metabolic inhibitor is administered in at least one administration of 1-200 mg, 5-160 mg, 10-80 mg, or 20-40 mg. Preferably, the administration is at least .01-0.1, 0.1-1, 1-5, 5-10, 10-20, 20-40, 40-60, 60-80, 80-100, 100-120, 120-140, or 140-160 mg. Preferably, the administration is at least .01, .05, 0.1, 0.5, 1, 5, 10, 20, 40, 60, 80, 100, 120, 140, or 160 mg of the metabolic inhibitor. Although not specifically enumerated, all values and subranges within the above and below ranges are specifically included as if explicitly written
10 out.

The administration of the metabolic inhibitor can be by many methods known in the art, most preferably intravenous, intra-arterial, subcutaneous, intramuscular, sublingual, transmucosal, or oral.

15 In some embodiments, multiple administrations are given. In various embodiments, at least 1-100, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10-20, 20-50, or 50-100, administrations are given. In various embodiments, the administration is at least twice/ day, twice/week, once/day, once/week, three times/week, or once/every 2 days.

In various embodiments, at least .01, .05, 0.1, 0.5, 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 100, 120, 160 mg/kg/day is administered for at least 1, 2, 3, 4, 5, 6, 7
20 days, 1, 2, 3, 4, 5, 6 weeks, or 1, 2, 3, 4, 5, 6, etc. months.

In various embodiments, at least 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 100, 120, 160 mg is administered every 2 days or 3 times/week for at least 2, 3, 4, 5, 6, 7 days, 1, 2, 3, 4, 5, 6 weeks, or 1, 2, 3, 4, 5, 6, etc. months.

HIV-infected Patients

25 The methods, uses, and compositions of this invention can be used with HIV-infected patients. In one embodiment, the patient is infected with Human Immunodeficiency Virus type 1 (HIV-1). In one embodiment, the patient is infected with Human Immunodeficiency Virus type 2 (HIV-2).

In one embodiment, the HIV-1 infected patient is acutely infected with HIV. In one embodiment, the HIV-1 infected patient is chronically infected with HIV.

In one embodiment, the HIV-1 infected patient is undergoing cART. In one embodiment, the HIV-1 infected patient has never initiated cART. In various embodiments, the HIV-1 infected patient has previously undergone cART, and either ceases or continues cART.

Pharmaceutical Compositions

The invention encompasses pharmaceutical compositions comprising one or more metabolic inhibitors. The compositions are preferably for the treatment of a pathogen infection in a human, particularly, preferably comprising a combination of at least two, three, or four metabolic inhibitors. The invention further encompasses the use of these compositions in the manufacture of a medicament for the treatment of a pathogen infection and the use of these compositions in the treatment of a pathogen infection.

In various embodiments, the composition contains 1-200 mg, 5-160 mg, 10-80 mg, or 20-40 mg of one or more metabolic inhibitor. Preferably, the composition contains at least 1-5, 5-10, 10-20, 20-40, 40-60, 60-80, 80-100, 100-120, 120-140, or 140-160 mg of one or more metabolic inhibitor. Although not specifically enumerated, all values and subranges within the above ranges are specifically included as if explicitly written out.

The metabolic inhibitors may also be advantageously administered for therapeutic purposes together with other metabolic inhibitors, such as HIV inhibitors, particularly cART, known in the general art to be of value in treating HIV infection. Particularly preferred combinations contain at least one, two, three, or four of the HIV inhibitors listed below. Most preferably, the combination contains at least one of the combination antiretroviral therapies listed below.

Effective concentrations or amounts of a metabolic inhibitor can be mixed with a suitable pharmaceutical carrier or vehicle for systemic, topical or local administration to form pharmaceutical compositions. The metabolic inhibitor is included in an amount effective for treating the pathogen (e.g., HIV) infection. The concentration of active agent in the composition will depend on absorption, inactivation, excretion rates of the active agent, the dosage

schedule, amount administered, particular formulation as well as other factors known to those of skill in the art.

The compositions are intended to be administered by a suitable route, including by way of example and without limitation orally, parenterally, rectally, topically and locally. For oral
5 administration, capsules and tablets can be used. The compositions are in liquid, semi-liquid or solid form and are formulated in a manner suitable for each route of administration.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include any of the following components, in any combination: a sterile diluent, including by way of example without limitation, water for injection, saline solution, fixed oil,
10 polyethylene glycol, glycerine, propylene glycol or other synthetic solvent; antimicrobial agents, such as benzyl alcohol and methyl parabens; antioxidants, such as ascorbic acid and sodium bisulfite; chelating agents, such as ethylenediaminetetraacetic acid (EDTA); buffers, such as acetates, citrates and phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose. Parenteral preparations can be enclosed in ampoules, disposable syringes
15 or single or multiple dose vials made of glass, plastic or other suitable material.

In instances in which the agents exhibit insufficient solubility, methods for solubilizing agents may be used. Such methods are known to those of skill in this art, and include, but are not limited to, using co-solvents, such as dimethylsulfoxide (DMSO), using surfactants, such as TWEEN®, or dissolution in aqueous sodium bicarbonate. Pharmaceutically acceptable
20 derivatives of the agents may also be used in formulating effective pharmaceutical compositions.

Upon mixing or addition of the agent(s), the resulting mixture may be a solution, suspension, emulsion or the like. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the agent in the
25 selected carrier or vehicle. The effective concentration is sufficient for treating one or more symptoms of at least one disease state.

The pharmaceutical compositions are provided for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, sterile parenteral solutions or suspensions, and oral solutions or suspensions, and oil-water emulsions

containing suitable quantities of the agents or pharmaceutically acceptable derivatives thereof. The pharmaceutically therapeutically active agents and derivatives thereof are typically formulated and administered in unit-dosage forms or multiple-dosage forms. Unit-dose forms as used herein refers to physically discrete units suitable for human and animal subjects and packaged individually as is known in the art. Each unit-dose contains a predetermined quantity of the therapeutically active agent sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carrier, vehicle or diluent. Examples of unit-dose forms include ampoules and syringes and individually packaged tablets or capsules. Unit-dose forms may be administered in fractions or multiples thereof. A multiple-dose form is a plurality of identical unit-dosage forms packaged in a single container to be administered in segregated unit-dose form. Examples of multiple-dose forms include vials, bottles of tablets or capsules or bottles of pints or gallons. Hence, multiple dose form is a multiple of unit-doses which are not segregated in packaging.

The composition can contain along with the active agent, for example and without limitation: a diluent such as lactose, sucrose, dicalcium phosphate, or carboxymethylcellulose; a lubricant, such as magnesium stearate, calcium stearate and talc; and a binder such as starch, natural gums, such as gum acacia gelatin, glucose, molasses, polyvinylpyrrolidone, celluloses and derivatives thereof, povidone, crospovidones and other such binders known to those of skill in the art. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, or otherwise mixing an active agent as defined above and optional pharmaceutical adjuvants in a carrier, such as, by way of example and without limitation, water, saline, aqueous dextrose, glycerol, glycols, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting agents, emulsifying agents, or solubilizing agents, pH buffering agents and the like, such as, by way of example and without limitation, acetate, sodium citrate, cyclodextrin derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and other such agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton,

Pa., 15th Edition, 1975. The composition or formulation to be administered will, in any event, contain a quantity of the active agent in an amount sufficient to alleviate the symptoms of the treated subject.

Dosage forms or compositions containing active agent in the range of 0.005% to 100% with the balance made up from non-toxic carrier may be prepared. For oral administration, a pharmaceutically acceptable non-toxic composition is formed by the incorporation of any of the normally employed excipients, such as, for example and without limitation, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, talcum, cellulose derivatives, sodium crosscarmellose, glucose, sucrose, magnesium carbonate or sodium saccharin. Such compositions include solutions, suspensions, tablets, capsules, powders and sustained release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as collagen, ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others. Methods for preparation of these compositions are known to those skilled in the art. The contemplated compositions may contain 0.001%-100% active agent, such as 0.1-85%, or such as 75-95%. The active agents or pharmaceutically acceptable derivatives may be prepared with carriers that protect the agent against rapid elimination from the body, such as time release formulations or coatings. The compositions may include other active agents to obtain desired combinations of properties.

Oral pharmaceutical dosage forms include, by way of example and without limitation, solid, gel and liquid. Solid dosage forms include tablets, capsules, granules, and bulk powders. Oral tablets include compressed, chewable lozenges and tablets which may be enteric-coated, sugar-coated or film-coated. Capsules may be hard or soft gelatin capsules, while granules and powders may be provided in non-effervescent or effervescent forms with the combination of other ingredients known to those skilled in the art.

In some embodiments, the formulations are solid dosage forms, such as capsules or tablets. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or agents of a similar nature: a binder; a diluent; a disintegrating agent; a lubricant; a glidant; a sweetening agent; and a flavoring agent.

Examples of binders include, by way of example and without limitation, microcrystalline cellulose, gum tragacanth, glucose solution, acacia mucilage, gelatin solution, sucrose, and starch paste. Lubricants include, by way of example and without limitation, talc, starch, magnesium or calcium stearate, lycopodium and stearic acid. Diluents include, by way of
5 example and without limitation, lactose, sucrose, starch, kaolin, salt, mannitol, and dicalcium phosphate. Glidants include, by way of example and without limitation, colloidal silicon dioxide. Disintegrating agents include, by way of example and without limitation, crosscarmellose sodium, sodium starch glycolate, alginic acid, corn starch, potato starch, bentonite, methylcellulose, agar and carboxymethylcellulose. Coloring agents include, by way of example
10 and without limitation, any of the approved certified water soluble FI) and C dyes, mixtures thereof; and water insoluble ID and C dyes suspended on alumina hydrate. Sweetening agents include, by way of example and without limitation, sucrose, lactose, mannitol and artificial sweetening agents such as saccharin, and any number of spray dried flavors. Flavoring agents include, by way of example and without limitation, natural flavors extracted from plants such as
15 fruits and synthetic blends of agents which produce a pleasant sensation, such as, but not limited to peppermint and methyl salicylate. Wetting agents include, by way of example and without limitation, propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate, and polyoxyethylene laural ether. Emetic-coatings include, by way of example and without limitation, fatty acids, fats, waxes, shellac, ammoniated shellac and cellulose acetate
20 phthalates. Film coatings include, by way of example and without limitation, hydroxyethylcellulose, sodium carboxymethylcellulose, polyethylene glycol 4000 and cellulose acetate phthalate.

If oral administration is desired, the agent could be provided in a composition that protects it from the acidic environment of the stomach. For example, the composition can be
25 formulated in an enteric coating that maintains its integrity in the stomach and releases the active agent in the intestine. The composition may also be formulated in combination with an antacid or other such ingredient.

When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various

other materials which modify the physical form of the dosage unit, for example, coatings of sugar and other enteric agents. The agents can also be administered as a component of an elixir, suspension, syrup, wafer, sprinkle, chewing gum or the like. A syrup may contain, in addition to the active agents, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

The active materials can also be mixed with other active materials which do not impair the desired action, or with materials that supplement the desired action, such as antacids, H₂ blockers, and diuretics.

Pharmaceutically acceptable carriers included in tablets are binders, lubricants, diluents, disintegrating agents, coloring agents, flavoring agents, and wetting agents. Enteric-coated tablets, because of the enteric-coating, resist the action of stomach acid and dissolve or disintegrate in the neutral or alkaline intestines. Sugar-coated tablets are compressed tablets to which different layers of pharmaceutically acceptable substances are applied. Film-coated tablets are compressed tablets which have been coated with a polymer or other suitable coating. Multiple compressed tablets are compressed tablets made by more than one compression cycle utilizing the pharmaceutically acceptable substances previously mentioned. Coloring agents may also be used in the above dosage forms. Flavoring and sweetening agents are used in compressed tablets, sugar-coated, multiple compressed and chewable tablets. Flavoring and sweetening agents are useful in the formation of chewable tablets and lozenges.

Liquid oral dosage forms include aqueous solutions, emulsions, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules and effervescent preparations reconstituted from effervescent granules. Aqueous solutions include, for example, elixirs and syrups. Emulsions are either oil-in-water or water-in-oil.

Elixirs are clear, sweetened, hydroalcoholic preparations. Pharmaceutically acceptable carriers used in elixirs include solvents. Syrups are concentrated aqueous solutions of a sugar, for example, sucrose, and may contain a preservative. An emulsion is a two-phase system in which one liquid is dispersed in the form of small globules throughout another liquid. Pharmaceutically acceptable carriers used in emulsions are non-aqueous liquids, emulsifying agents and preservatives. Suspensions use pharmaceutically acceptable suspending agents and

preservatives. Pharmaceutically acceptable substances used in non-effervescent granules, to be reconstituted into a liquid oral dosage form, include diluents, sweeteners and wetting agents. Pharmaceutically acceptable substances used in effervescent granules, to be reconstituted into a liquid oral dosage form, include organic acids and a source of carbon dioxide. Coloring and
5 flavoring agents may be used in any of the above dosage forms.

Solvents, include by way of example and without limitation, glycerin, sorbitol, ethyl alcohol and syrup. Examples of preservatives include without limitation glycerin, methyl and propylparaben, benzoic acid, sodium benzoate and alcohol. Non-aqueous liquids utilized in emulsions, include by way of example and without limitation, mineral oil and cottonseed oil.
10 Emulsifying agents, include by way of example and without limitation, gelatin, acacia, tragacanth, bentonite, and surfactants such as polyoxyethylene sorbitan monooleate. Suspending agents include, by way of example and without limitation, sodium carboxymethylcellulose, pectin, tragacanth, Veegum and acacia. Diluents include, by way of example and without limitation, lactose and sucrose. Sweetening agents include, by way of
15 example and without limitation, sucrose, syrups, glycerin and artificial sweetening agents such as saccharin. Wetting agents, include by way of example and without limitation, propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate, and polyoxyethylene lauryl ether. Organic acids include, by way of example and without limitation, citric and tartaric acid. Sources of carbon dioxide include, by way of example and without limitation, sodium
20 bicarbonate and sodium carbonate. Coloring agents include, by way of example and without limitation, any of the approved certified water soluble FD and C dyes, and mixtures thereof. Flavoring agents include, by way of example and without limitation, natural flavors extracted from plants such fruits, and synthetic blends of agents which produce a pleasant taste sensation.

25 For a solid dosage form, the solution or suspension, in for example propylene carbonate, vegetable oils or triglycerides, is encapsulated in a gelatin capsule. Such solutions, and the preparation and encapsulation thereof, are disclosed in U.S. Patent Nos. 4,328,245; 4,409,239; and 4,410,545. For a liquid dosage form, the solution, for example in a polyethylene glycol, may

be diluted with a sufficient quantity of a pharmaceutically acceptable liquid carrier, e.g., water, to be easily measured for administration.

Alternatively, liquid or semi-solid oral formulations may be prepared by dissolving or dispersing the active agent or salt in vegetable oils, glycols, triglycerides, propylene glycol esters (e.g., propylene carbonate) and other such carriers, and encapsulating these solutions or suspensions in hard or soft gelatin capsule shells. Other useful formulations include those set forth in U.S. Patent Nos. Re 28,819 and 4,358,603. Briefly, such formulations include, but are not limited to, those containing an agent provided herein, a dialkylated mono- or poly-alkylene glycol, including, but not limited to, 1,2-dimethoxymethane, diglyme, triglyme, tetraglyme, polyethylene glycol-350-dimethyl ether, polyethylene glycol-550-dimethyl ether, polyethylene glycol-750-dimethyl ether wherein 350, 550 and 750 refer to the approximate average molecular weight of the polyethylene glycol, and one or more antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate, vitamin E, hydroquinone, hydroxycoumarins, ethanolamine, lecithin, cephalin, ascorbic acid, malic acid, sorbitol, phosphoric acid, thiodipropionic acid and its esters, and dithiocarbamates.

Other formulations include, but are not limited to, aqueous alcoholic solutions including a pharmaceutically acceptable acetal. Alcohols used in these formulations are any pharmaceutically acceptable water-miscible solvents having one or more hydroxyl groups, including, but not limited to, propylene glycol and ethanol. Acetals include, but are not limited to, di(lower alkyl) acetals of lower alkyl aldehydes such as acetaldehyde diethyl acetal.

Tablets and capsules formulations may be coated as known by those of skill in the art in order to modify or sustain dissolution of the active ingredient. Thus, for example and without limitation, they may be coated with a conventional enterically digestible coating, such as phenylsalicylate, waxes and cellulose acetate phthalate.

Parenteral administration, generally characterized by injection, either subcutaneously, intramuscularly or intravenously is also contemplated herein. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients, include by way of example and without limitation, water, saline, dextrose, glycerol or ethanol. In addition, if

desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, and other such agents, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate and cyclodextrins.

5 Implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained (see, e.g., U.S. Patent No. 3,710,795) is also contemplated herein. Briefly, the metabolic inhibitor can be dispersed in a solid inner matrix, e.g., polymethylmethacrylate, polybutylmethacrylate, plasticized or unplasticized polyvinylchloride, plasticized nylon, plasticized polyethyleneterephthalate, natural rubber, polyisoprene, polyisobutylene,
10 polybutadiene, polyethylene, ethylene-vinylacetate copolymers, silicone rubbers, polydimethylsiloxanes, silicone carbonate copolymers, hydrophilic polymers such as hydrogels of esters of acrylic and methacrylic acid, collagen, cross-linked polyvinylalcohol and cross-linked partially hydrolyzed polyvinyl acetate, that is surrounded by an outer polymeric membrane, e.g., polyethylene, polypropylene, ethylene/propylene copolymers, ethylene/ethyl acrylate
15 copolymers, ethylene/vinylacetate copolymers, silicone rubbers, polydimethyl siloxanes, neoprene rubber, chlorinated polyethylene, polyvinylchloride, vinylchloride copolymers with vinyl acetate, vinylidene chloride, ethylene and propylene, ionomer polyethylene terephthalate, butyl rubber epichlorohydrin rubbers, ethylene/vinyl alcohol copolymer, ethylene/vinyl acetate/vinyl alcohol terpolymer, and ethylene/vinyloxyethanol copolymer, that is insoluble in
20 body fluids. The agent diffuses through the outer polymeric membrane in a release rate controlling step. The percentage of active agent contained in such parenteral compositions is highly dependent on the specific nature thereof, as well as the activity of the agent and the needs of the subject.

 Parenteral administration includes intravenous, subcutaneous and intramuscular
25 administrations. Preparations for parenteral administration include sterile solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use and sterile emulsions. The solutions may be either aqueous or nonaqueous.

If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof.

Pharmaceutically acceptable carriers used in parenteral preparations include aqueous
5 vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances.

Aqueous vehicles include, by way of example and without limitation, Sodium Chloride Injection, Ringers Injection, Isotonic Dextrose Injection, Sterile Water Injection, Dextrose and
10 Lactated Ringers Injection. Nonaqueous parenteral vehicles include, by way of example and without limitation, fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil. Antimicrobial agents in bacteriostatic or fungistatic concentrations must be added to parenteral preparations packaged in multiple-dose containers which include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters,
15 thimerosal, benzalkonium chloride and benzethonium chloride. Isotonic agents include, by way of example and without limitation, sodium chloride and dextrose. Buffers include phosphate and citrate. Antioxidants include sodium bisulfate. Local anesthetics include procaine hydrochloride. Suspending and dispersing agents include sodium carboxymethylcellulose, hydroxypropyl methylcellulose and polyvinylpyrrolidone. Emulsifying agents include Polysorbate
20 80 (TWEEN® 80). A sequestering or chelating agent of metal ions include EDTA. Pharmaceutical carriers also include, by way of example and without limitation, ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment.

The concentration of the pharmaceutically active agent is adjusted so that an injection
25 provides an effective amount to produce the desired pharmacological effect. The exact dose depends on the age, weight and condition of the patient or animal as is known in the art.

The unit-dose parenteral preparations are packaged in an ampoule, a vial or a syringe with a needle. Preparations for parenteral administration should be sterile, as is known and practiced in the art.

Illustratively, intravenous or intra-arterial infusion of a sterile aqueous solution containing an active agent is an effective mode of administration. Another embodiment is a sterile aqueous or oily solution or suspension containing an active agent injected as necessary to produce the desired pharmacological effect.

5 Injectables are designed for local and systemic administration. Typically a therapeutically effective dosage is formulated to contain a concentration of at least about 0.1% w/w up to about 90% w/w or more, such as more than 1% w/w of the active agent to the treated tissue(s). The active agent may be administered at once, or may be divided into a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and
10 duration of treatment is a function of the tissue being treated and may be determined empirically using known testing protocols or by extrapolation from in vivo or in vitro test data. It is to be noted that concentrations and dosage values may also vary with the age of the individual treated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional
15 judgment of the person administering or supervising the administration of the formulations, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed formulations.

 The agent may be suspended in micronized or other suitable form or may be derivatized, e.g., to produce a more soluble active product or to produce a prodrug or other
20 pharmaceutically acceptable derivative. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the agent in the selected carrier or vehicle. The effective concentration is sufficient for ameliorating the symptoms of the condition and may be empirically determined.

 Lyophilized powders can be reconstituted for administration as solutions, emulsions, and
25 other mixtures or formulated as solids or gels.

 The sterile, lyophilized powder is prepared by dissolving an agent provided herein, or a pharmaceutically acceptable derivative thereof, in a suitable solvent. The solvent may contain an excipient which improves the stability or other pharmacological component of the powder or reconstituted solution, prepared from the powder. Excipients that may be used include, but are

not limited to, dextrose, sorbitol, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent. The solvent may also contain a buffer, such as citrate, sodium or potassium phosphate or other such buffer known to those of skill in the art at, typically, about neutral pH. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions
5 known to those of skill in the art provides the desired formulation. Generally, the resulting solution will be apportioned into vials for lyophilization. Each vial will contain, by way of example and without limitation, a single dosage (10-1000 mg, such as 100-500 mg) or multiple dosages of the agent. The lyophilized powder can be stored under appropriate conditions, such as at about 4°C to room temperature.

10 Reconstitution of this lyophilized powder with water for injection provides a formulation for use in parenteral administration. For reconstitution, about 1-50 mg, such as about 5-35 mg, for example, about 9-30 mg of lyophilized powder, is added per mL of sterile water or other suitable carrier. The precise amount depends upon the selected agent. Such amount can be empirically determined.

15 Topical mixtures are prepared as described for the local and systemic administration. The resulting mixture may be a solution, suspension, emulsions or the like and are formulated as creams, gels, ointments, emulsions, solutions, elixirs, lotions, suspensions, tinctures, pastes, foams, aerosols, irrigations, sprays, suppositories, bandages, dermal patches or any other formulations suitable for topical administration.

20 The agents or pharmaceutically acceptable derivatives thereof may be formulated as aerosols for topical application, such as by inhalation (see, e.g., U.S. Patent Nos. 4,044,126, 4,414,209, and 4,364,923, which describe aerosols for delivery of a steroid useful for treatment of inflammatory diseases, particularly asthma). These formulations for administration to the respiratory tract can be in the form of an aerosol or solution for a nebulizer, or as a microfine
25 powder for insufflation, alone or in combination with an inert carrier such as lactose. In such a case, the particles of the formulation will, by way of example and without limitation, have diameters of less than about 50 microns, such as less than about 10 microns.

The agents may be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams,

and lotions and for application to the eye or for intracisternal or intraspinal application. Topical administration is contemplated for transdermal delivery and also for administration to the eyes or mucosa, or for inhalation therapies. Nasal solutions of the active agent alone or in combination with other pharmaceutically acceptable excipients can also be administered.

5 These solutions, particularly those intended for ophthalmic use, may be formulated, by way of example and without limitation, as about 0.01% to about 10% isotonic solutions, pH about 5-7, with appropriate salts.

Other routes of administration, such as transdermal patches, and rectal administration are also contemplated herein.

10 Transdermal patches, including iontophoretic and electrophoretic devices, are well known to those of skill in the art. For example, such patches are disclosed in U.S. Patent Nos. 6,267,983, 6,261,595, 6,256,533, 6,167,301, 6,024,975, 6,010,715, 5,985,317, 5,983,134, 5,948,433, and 5,860,957.

Pharmaceutical dosage forms for rectal administration are rectal suppositories, capsules
15 and tablets for systemic effect. Rectal suppositories are used herein mean solid bodies for insertion into the rectum which melt or soften at body temperature releasing one or more pharmacologically or therapeutically active ingredients. Pharmaceutically acceptable substances utilized in rectal suppositories are bases or vehicles and agents to raise the melting point. Examples of bases include cocoa butter (theobroma oil), glycerin-gelatin, carbowax
20 (polyoxyethylene glycol) and appropriate mixtures of mono-, di- and triglycerides of fatty acids. Combinations of the various bases may be used. Agents to raise the melting point of suppositories include spermaceti and wax. Rectal suppositories may be prepared either by the compressed method or by molding. The typical weight of a rectal suppository is, by way of example and without limitation, about 2 to 3 gm.

25 Tablets and capsules for rectal administration are manufactured using the same pharmaceutically acceptable substance and by the same methods as for formulations for oral administration.

Kit of Parts

The invention includes a kit of parts for simultaneous, separate, sequential administration to a pathogen-infected patient. The kit can comprise any combination of the metabolic inhibitors of the invention in pharmaceutical compositions together.

The kit of parts can contain at least 1-200 mg, 5-160 mg, 10-80 mg, or 20-40 mg of two or more metabolic inhibitors. Preferably, the kit of parts contains at least 1-5, 5-10, 10-20, 20-40, 40-60, 60-80, 80-100, 100-120, 120-140, or 140-160 mg of two or more metabolic inhibitors. Preferably, the kit of parts contains at least 1, 5, 10, 20, 40, 60, 80, 100, 120, 140, or 160 mg of 2-DG. Although not specifically enumerated, all values and subranges within the above ranges are specifically included as if explicitly written out.

Preferably, the kit of parts additionally contains at least one, two, three, or four of the HIV inhibitors listed below. Most preferably, the kit of parts contains at least one of the combination antiretroviral therapies listed below.

HIV Inhibitors

Entry inhibitors (or fusion inhibitors) interfere with binding, fusion and entry of HIV-1 to the host cell by blocking one of several targets (Wikipedia). Maraviroc works by targeting CCR5, a co-receptor located on human helper T-cells. Enfuvirtide is a peptide drug that must be injected and acts by interacting with the N-terminal heptad repeat of gp41 of HIV to form an inactive hetero six-helix bundle, therefore preventing infection of host cells.

Nucleoside reverse transcriptase inhibitors (NRTI) and nucleotide reverse transcriptase inhibitors (NtRTI) are nucleoside and nucleotide analogues which inhibit reverse transcription. Examples of NRTIs include zidovudine, abacavir, lamivudine, emtricitabine, and tenofovir.

Non-Nucleoside reverse transcriptase inhibitors (NNRTI) inhibit reverse transcriptase by binding to an allosteric site of the enzyme. 1st generation NNRTIs include nevirapine and efavirenz. 2nd generation NNRTIs include etravirine and rilpivirine.

Integrase inhibitors (also known as integrase nuclear strand transfer inhibitors or INSTIs) inhibit the viral enzyme integrase. Integrase inhibitors include raltegravir, elvitegravir, and dolutegravir.

Protease inhibitors block the viral protease enzyme necessary to produce mature virions upon budding from the host membrane. Examples of HIV protease inhibitors are lopinavir, indinavir, nelfinavir, amprenavir, ritonavir, darunavir, and atazanavir.

Maturation inhibitors have a similar effect by binding to gag, and include bevirimat and
 5 vivecon.

Combination antiretroviral therapy (cART) is a mixture of at least two, and preferably three or more different classes of antiretroviral therapy. All different combinations of the antiretroviral therapies specified herein are specifically contemplated. Examples of cART include:

10 Combivir: lamivudine + zidovudine.

Kaletra: lopinavir + ritonavir

Trizivir: abacavir + lamivudine + zidovudine

Epzicom (in USA)/ Kivexa (in Europe and Russia): abacavir + lamivudine.

Truvada: tenofovir disoproxil fumarate + emtricitabine.

15 Atripla: emtricitabine + tenofovir disoproxil fumarate + efavirenz

Complera (in USA)/ Eviplera (in Europe and Russia): emtricitabine + rilpivirine + tenofovir disoproxil fumarate.

Stribild: elvitegravir + cobicistat + emtricitabine + tenofovir disoproxil fumarate.

Triumeq: abacavir + dolutegravir + lamivudine.

20 Evotaz: atazanavir + cobicistat.

Prezcobix: darunavir + cobicistat.

Dutrebis: lamivudine + raltegravir.

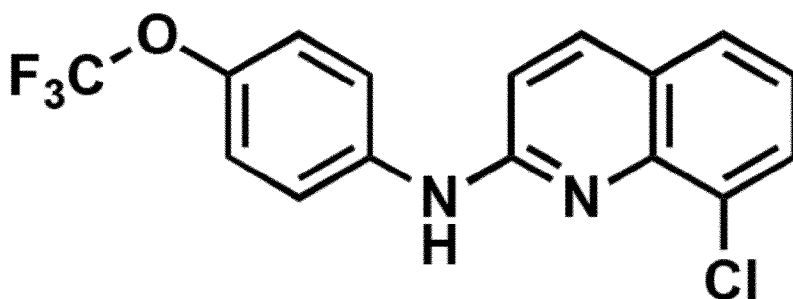
Genvoya: elvitegravir + cobicistat + emtricitabine + tenofovir alafenamide fumarate.

Descovy: emtricitabine + tenofovir alafenamide fumarate.

25 Rev inhibitors interfere with the biogenesis of viral RNA required for the replication of HIV. Rev inhibitor can function through binding to the Cap Binding Complex at the 5' end of the mRNA coding for 3 structural proteins of the virus. By promoting HIV RNA splicing, these inhibitors can reduce the level of genomic RNA and inhibit HIV replication.

Preferred compounds can be found in U.S. Patents 9,145,367 and 9,061,999, which are hereby incorporated by reference. Particularly preferred compounds are 10-chloro-2,6-dimethyl-2H-pyrido [3',4':4,5]pyrrolo[2,3-g]isoquinoline (IDC16), 8-chloro-N-(4-(trifluoromethoxy)phenyl)quinolin-2-amine (ABX464) and 8-chloro-N-glucuronide-N-(4-(trifluoromethoxy)phenyl)quinolin-2-amine (ABX464-N-glucuronide) compounds, as set forth in Campos et al. Retrovirology (2015) 12:30, which is hereby incorporated by reference.

A particularly preferred compound has the formula:



EXAMPLES**EXAMPLE 1. Isolation and culture of CD4+ T-cells**

CD4+ T-cells were purified (>90%) from freshly isolated PBMCs by negative selection with antibody-coated magnetic beads (EasySep™ Human CD4+ T-cell Enrichment Kit Ref.19052) in a Robosep instrument (Stem Cell Technology).

Purified CD4+ cells (10^6 cell/mL) were cultured in RPMI 1640 containing GlutaMAX, 10% FCS, penicillin (10 IU/mL) and streptomycin (10 µg/mL) in the presence of IL-2 (Miltenyi) at 50 IU/mL (Culture media). Depending on the experiment, cells were left unstimulated or were stimulated for 3 or 5 days with 0.5 µg/mL soluble antiCD3 (BioLegend, Ref.300414, Clone UCHT1) in the absence of CD28 co-stimulation as previously described (Saez-Cirion et al., 2011). Different compounds that target metabolic pathways [2-deoxy-glucose, 2-DG (Seahorse Biotechnologies); (+)-Etomoxir sodium salt hydrate (Sigma, Ref. E1905); UK5099 (Sigma, Ref. PZ0160); 6-diazo-5-oxo-L-norleucine (DON)(Sigma, Ref. D2141); glucose (Seahorse Biotechnologies); oligomycin (Seahorse Biotechnologies) or carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone FCCP (Seahorse Biotechnologies)] were added to the culture media at different times and concentrations depending on the protocol conditions. A glucose-free culture media was used in some infection experiments and is described in the results section [RPMI non-glucose, GlutaMAX, containing 10% FCS, penicillin (10 IU/mL) and streptomycin (10 µg/mL) in the presence of IL-2 (Miltenyi) at 50 IU/mL (culture media)]. After culture, living cells were counted with an automatic Countess cell counter (Invitrogen) based on size and non-staining with trypan blue. The number of living cells was then normalized before analysis.

EXAMPLE 2. HIV infection in vitro

Single-round infections were performed with HIV-1 NL4.3ΔenvΔnef/GFP (Amara et al., 2003) and HIV-1-DuoFluoΔenv(R7GEmC) (provided by Professor Eric Verdin and Dr. Calvanese, NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: Cat# 12595 DuoFluo (R7GEmC)) (Calvanese et al., 2013). Both viruses were pseudotyped with the VSV-G envelope protein by transiently cotransfecting (SuperFect; Qiagen) 293T cells with the proviral vectors and the VSV-G expression vector pMD2.G. Nonactivated or activated CD4 + T-cells were infected in triplicate

(5×10^4 cells/well, 200 μ L) with 35 ng/ 1×10^6 HIV-1 NL4.3 Δ nef/GFP/VSV-G and with 70 ng of HIV-1-DuoFluo(R7GEmC)/VSVg per million cells. Active HIV-1 infection was estimated by flow cytometry (BD LSRII, BD bioscience) as the percentage of GFP-expressing CD4+ T-cells 72 h after infection. Latent HIV infection was estimated by flow cytometry as the percentage of mCherry+GFP-CD4+ T-cells 72 h after infection with HIV-1-DuoFluo(R7GEmC) particles.

HIV-1 reverse transcripts (U5-Gag) were quantified by real-time PCR with an Applied Biosystems 7500 Real-Time PCR System 6, 16 and 72 h after infection of CD4+ T-cells with VSV-G-pseudotyped HIV-1 particles as described in (David et al., 2006). Briefly, total DNA was extracted with the NucleoSpin 8/96 Tissue Core kit (Macherey-Nagel, Ref. 740453.4) and 100 ng of template DNA were used per reaction. DNA loading was controlled by concurrently amplifying the albumin gene by real-time PCR and quantifying with reference to a control human genomic DNA (Roche). The reaction mixture contained 1 $\times$ TaqMan Universal PCR master mix, 300 nM of primers and 200 nM of the fluorogenic probe, in a final volume of 30 μ L. PCR cycle conditions were: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. Copy numbers of U5-Gag were determined with reference to a standard curve prepared by concurrent amplification of serial dilutions of 8E5 cells containing one integrated copy of HIV-1 per cell.

Productive HIV-1 infection in vitro was studied in suboptimally activated CD4+ T-cells (10^6 cells/mL in triplicate) exposed to the HIV-1 BaL strain (R5) (10 ng p24/mL). The cells were cultured in 96-U-well plates for 14 days in the presence or absence of 2-DG (5 mM). Every 3-4 days, the culture supernatants were removed and replaced with fresh culture medium with or without 2-DG. Viral replication was monitored in the supernatants by p24 enzyme-linked immunosorbent assay (ELISA) (XpressBio) or at day 3 by intracellular p24 staining (p24-FITC (clone KC57, Coulter) (Saez-Cirion et al., 2010).

EXAMPLE 3. Flow-assisted sorting of CD4 + T-cell subsets

Cells were first selected based on size and structure to eliminate cellular debris. Then cell singlets and living cells (not stained with LIVE/DEAD Fixable Aqua Dead Cell Stain Kit, Thermofisher) are gated before proceeding with further selection based on phenotypical or functional markers (Figures 9, 12, 13).

Resting (CD25⁻, CD69⁻, HLA-DR⁻) CD4⁺ T-cell subsets [naïve (**T_n**; CD3⁺, CD4⁺, CD45RA⁺, CCR7⁺, CD27⁺, CD95⁻), central memory (**T_{cm}**; CD3⁺, CD4⁺, CD45RA⁻, CCR7⁺, CD27⁺), transitional memory (**T_{tm}**; CD3⁺, CD4⁺, CD45RA⁻, CCR7⁻, CD27⁺) or effector memory (**T_{em}**; CD3⁺, CD4⁺, CD45RA⁻, CCR7⁻, CD27⁻)] were sorted on a FACS ARIA III cell sorter (BD) using the following antibody panel: CD3-eFLuor450 (eBioscience), CD4-alexaFluor700 (BD), CD45RA-ECD (BC), CCR7-PE_Cy7 (BioLegend), CD27-APC (Miltenyi), CD95-PE (Miltenyi), CD25-FITC (BD), CD69-FITC (eBioscience) and HLA-DR-FITC (BD). The gating strategy is depicted in Figure 9. The number of sorted cells varied from 0.5 to 5 million cells depending on the CD4⁺ T-cell subset and the donor. The purity of the sorted subset was greater than 98%.

GFP⁺ and GFP⁻ CD4⁺ T-cells were sorted 72 h after infection with VSV-G pseudotyped NL4.3ΔenvΔnef/GFP particles (Figure 12A). For some experiments, GFP⁺ and GFP⁻ cells were also sorted into the following categories based on their expression of activation markers (CD25-ECD, HLA-DR_PerCyP5.5) (Figure 4 and Figure 12B): high activation GFP⁺ [**H/+** (GFP⁺, CD25⁺, HLA-DR⁺)]; high activation GFP⁻ [**H/-** (GFP⁻, CD25⁺, HLA-DR⁺)]; low activation GFP⁺ [**L/+** (GFP⁺, CD25⁻, HLA-DR⁻)]; and low activation GFP⁻ [**L/-** (GFP⁻, CD25⁻, HLA-DR⁻)].

For some experiments, CD4⁺ T_n and T_{cm} cells were sorted based on their level of glucose uptake after 5 days of stimulation with anti-CD3 (Figure 13A). The cells were washed and incubated with 2-NBDG (2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose) (Thermo Fisher, Ref. N13195) at 75 μM in PBS for 30 min at 37°C. After 3 washes of 10 min each with fresh PBS, the cells were stained with antibodies (CD3-eFLuor450 (eBioscience), CD4-alexaFluor700 (BD), CD45RA-ECD (BC), CCR7-PE_Cy7 (BioLegend), and CD27-APC (Miltenyi)) and sorted as follows: **T_n H_{glu}** (CD3⁺, CD4⁺, CD45RA⁺, CCR7⁺, CD27⁺, 2NBDG⁺); **T_n L_{glu}** (CD3⁺, CD4⁺, CD45RA⁺, CCR7⁺, CD27⁺, 2NBDG⁻); **T_{cm} H_{glu}** (CD3⁺, CD4⁺, CD45RA⁻, CCR7⁺, CD27⁺, 2NBDG⁺); and **T_{cm} L_{glu}** (CD3⁺, CD4⁺, CD45RA⁻, CCR7⁺, CD27⁺, 2NBDG⁻).

EXAMPLE 4. Surface GLUT1 staining

CD4⁺ T-cells were stained with HRBD-rFc, a recombinant fusion protein that specifically binds GLUT1 (Metafora-biosystems, Paris, France), and a secondary goat-anti-Mouse Alexa Fluor 647 antibody (Thermofisher). HRBD is derived from the receptor-binding domain of the human T-cell leukemia virus envelope glycoprotein that binds the extracellular domain of GLUT1 (Manel et

al., 2005). The following antibody panel was used to determine CD4⁺T cell subsets: CD3-eFluor450 (eBioscience), CD4-alexaFluor700 (BD), CD45RA-ECD (BC), CCR7-PE_Cy7 (BioLegend), CD27-PE (BD bioscience).

EXAMPLE 5. Quantitative RT-PCR arrays

5 The expression levels of an array of 96 genes in the CD4⁺ T-cell subsets were quantified by RT-qPCR with a Biomark HQ system. Total RNA was extracted from 5×10^4 CD4⁺ T-cells with an RNA trace kit (Macherey-Nagel, Ref. 740731.4) and treated with DNase, following the manufacturer's instructions. Twenty microliters of RNA (> 10 ng) was reverse transcribed with Reverse Transcription Master Mix (Fluidigm, 100-6298) (5 minutes at 25°C, 30 minutes at 42°C, 10 and 5 minutes at 85°C). A specific target preamplification (STA) was performed by adding PreAmp Master Mix, 96 Primers Mix and EDTA to the cDNA, followed by STA cycling (95°C: 2 min, 18 cycles of [96°C: 5 s, 60°C 4 min]). The sample was then treated with exonuclease I (New England Biolabs) (37°C: 30 min, 80°C: 15 min). Sample premix (SsoFast EvaGreen Supermix with Low ROX (Biorad), DNA Binding Dye (Fluidigm), preamplified Exo 1-treated sample) and assay 15 mix (assay loading reagent (Fluidigm), Delta Gene primers (Fluidigm)) were then loaded on primed 96.96 Dynamic Array chips (Fluidigm). The chips were transferred into a Biomark HQ device (Fluidigm) for thermocycling, and fluorescence was acquired with the GE 96×96 PCR+Melt v2 program. Linear derivative mode baseline correction was applied. We used the Normfinder algorithm (Aarhus University Hospital, Denmark) (Andersen et al., 2004) to identify 20 the optimal normalization gene among the assayed candidates for our experimental conditions. BENC1 was thus identified as the optimal normalization gene based on expression stability in the analyzed samples (Table 5), and the gene expression values were plotted as $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = \Delta Ct_{\text{SAMPLE}} - \Delta Ct_{\text{CONTROL}}$, and $\Delta Ct = Ct_{\text{TARGET GENE}} - Ct_{\text{BENC1}}$.

EXAMPLE 6. Measurement of oxygen consumption and extracellular acidification rates

25 The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured using a Seahorse XF96 metabolic analyzer following the procedure recommended by the manufacturer. Briefly, for all the experiments, different CD4⁺ T-cell populations were seeded at a concentration of 2×10^5 cells per well on XF96 plates (Seahorse Bioscience) precoated with 0.5 mg/ml Cell Tack (Corning, Ref. 354240) immediately before adding Seahorse

XF culture media to each well. Cells were incubated for 50 min in a CO₂-free incubator at 37°C before loading the plate in the Seahorse analyzer. Different programs were run on the Seahorse analyzer depending on the assay. Drug Panel A (1) XFmedia 2) oligomycin (2.5 µM), 3) FCCP (0.9 µM) and 4) rotenone (1 µM) and antimycin A (1 µM)) was injected through ports A, B, C and D, respectively, for the mitochondrial stress test. Drug Panel B (1) XFmedia 2) glucose (10 mM) 3) oligomycin (2.65 µM), and 4) 2-DG (100 mM)) was used for the glycolysis stress test.

EXAMPLE 7. Phenotyping after sorting

In some experiments, sorted GFP+/- CD4+ T-cells subset or CD4+ T bulk cells previously infected with NL4.3Δnef/GFP/VSV-G with or without 2-DG or Etomoxir were incubated with CD3-eFluor450 (eBioscience), CD4-alexaFluor700 (BD Biosciences), CD45RA-ECD (BC), CCR7-PE_Cy7 (BioLegend) and CD27-APC (Miltenyi) to determine the CD4+ T-cell subset distribution. In addition, the activation levels of sorted CD4+ T-cell subsets were assessed with CD25-ECD (BD Biosciences) and HLA-DR-FITC (BD Biosciences). For both protocols, cells were incubated with the antibodies for 25 minutes and then washed in PBS plus 1% FCS and fixed in 4% paraformaldehyde for flow cytometry on an LSRII device (BD Biosciences). The data were analyzed with Kaluza software (Beckman Coulter).

EXAMPLE 8. HIV-1 reactivation in CD4+ T-cells from HIV-1-infected individuals.

Freshly isolated CD4+T-cells (negative selection kit, Stem Cell) from HIV-individuals undergoing successful cART were seeded in 48-well plates (1x10⁶ cells/well, in triplicate) and stimulated with phytohemagglutinin-L (PHA-L, Roche, 1 µg/mL) and IL-2 (Miltenyi) 100UI with or without 2-DG (5 mM). The culture supernatants were collected every 3 to 4 days, and fresh medium +/- 2-DG was added to the cultures. Supernatants were stored at -80°C, and HIV-1 p24 was analyzed later by ultrasensitive digital ELISA (Simoa, Quanterix) (Passaes et al., 2017).

EXAMPLE 9. Differential gene expression

For each gene, we implemented a mixed effects model to detect differential expression between cell types (Tn, Tcm, Ttm and Tem). We defined a model that included the type of cells as a fixed effect and the patient as a random effect. A p-value was then obtained by implementing a likelihood ratio test between the full model and a reduced model without the fixed effect. Heat maps were generated by K-means clustering. Data were filtered by variance

($\delta/\delta_{\max}=0.2$) to reduce background noise. Gene expression data were centered to a mean value of zero and scaled to unit variance.

EXAMPLE 10. Correlation between gene expression, metabolic parameters and HIV-1 susceptibility

5 We computed Spearman's correlation coefficient and tested for significance.

Values are presented in the graphs as medians and interquartile ranges. Statistical analyses were performed using SigmaPlot (Systat Software). The asterisks represent statistically significant differences (* $p<0.05$; ** $p<0.01$). Differences between CD4+ T-cell subsets in different conditions were analyzed with nonparametric signed ANOVA and the multiple
10 comparison Student-Newman-Keuls method. Differences between GFP+ and GFP- CD4+ T-cells or control vs treatment culture conditions were analyzed with paired t-tests. When multiple treatment conditions were tested, ANOVA analyses and the Holm-Sidak method for multiple comparisons versus control group were used, and significant differences between experimental conditions were shown as horizontal lines.

15 **EXAMPLE 11. CD4+ T-cell subsets have heterogeneous susceptibility to HIV-1 infection**

We first assessed the relative intrinsic susceptibility of primary CD4+ T-cell subsets (naïve, T_n; central memory, T_{cm}; transitional memory, T_{tm}; and effector memory, T_{em}) to HIV-1 infection. We used single-cycle NL4.3ΔenvGFP particles pseudotyped with VSV-G envelope protein (HIV-1_{GFP}-VSV) to circumvent differences in the surface expression of CCR5 across CD4+
20 T-cell subsets. We activated CD4+ T-cells with soluble anti-CD3. This 'suboptimal' activation protocol has allowed us to expose differences in the susceptibility to HIV-1 of CD4+ T-cells from different individuals that were masked using more potent stimulation protocols (Saez-Cirion et al., 2011). Activation enhanced the susceptibility to HIV-1 without altering the relative contribution of each CD4+ T-cell subset (Figure 1A, B). After infection, the relative frequencies of
25 T_n, T_{cm}, T_{tm} and T_{em} cells among GFP-negative (GFP-) cells was identical to that among noninfected CD4+ T-cells (Figure 1B). In contrast, the composition of HIV-infected GFP-positive (GFP+) cells was different from that of noninfected cells, with a significant exclusion of T_n cells and strong enrichment of T_{em} cells. T_{cm} cells were also slightly underrepresented, and T_{tm} cells were overrepresented among GFP+ CD4+ T-cells when compared to the control condition

(Figure 1B). These results suggested different susceptibilities to HIV-1 infection of CD4+ T-cell subsets, with Tem cells being the most susceptible, followed by Ttm and Tcm cells, and with Tn cells being strongly resistant to infection.

To study if these differences were related to the inherent program of each CD4+ T-cell subset, we isolated quiescent CD4+ Tn, Tcm, Ttm and Tem cells (n=6 donors, Figure 9), and we analyzed their susceptibility to HIV-1 with or without activation. Activation enhanced the susceptibility of all CD4+ T-cell subsets to HIV-1 infection (Figure 1C). However, this effect was variable according to the subset. There was a tendency for Tem cells to be more susceptible than other subsets ($p=0.06$) in the absence of activation, and this difference became more pronounced after three ($p=0.0004$, all comparisons) or five days of activation ($p=0.012$, Tem vs Tn and Ttm and Tcm vs Tn and Ttm). Overall, our results recapitulated previous observations showing an inherent hierarchy in the susceptibility of CD4+ T-cell subsets to HIV-1 infection (Buzon et al., 2014; Tabler et al., 2014).

EXAMPLE 12. Levels of HIV infection are related to the molecular program of CD4+ T-cell subsets

To determine if a molecular program was associated with the susceptibility of CD4+ T-cell subsets to HIV infection, we analyzed the expression of a panel of 96 genes (related to T-cell activation, survival, differentiation and function as well as known viral restriction or HIV facilitating factors, Table 3) in each CD4+ T-cell subpopulation at the time of infection.

Nonactivated CD4+ T-cell subsets showed distinct transcriptional profiles that were further enhanced after activation (e.g., 34 genes and 49 genes differently expressed between CD4+ T-cell subsets without activation and after 3 days of anti-CD3 treatment, respectively, Figure 2A). These genes were mostly related to signal transduction and the response to stimulus, which could be related to the previously described different susceptibility to CD3 activation of the CD4+ T-cell subsets (Croft et al., 1994; Kumar et al., 2011). The level of HIV-infected cells correlated with the expression of several genes at the time of infection in the different conditions studied (Figure 2B and Figure 10). SAMHD1 showed a negative association with infection. In contrast, positive correlations were observed between infection levels and other antiviral factors (such as APOBEC3G or SLFN11)(Li et al., 2012; Sheehy et al., 2002) as well as

several genes involved in the interferon response (IFI6, IFI16, EIF2AK2, and OAS1) (Kane et al., 2016). Significant positive correlations were also observed between the level of HIV infection and the gene expression levels of transcription factors (STAT3, E2F1, and PRDM1), genes that have been proposed to facilitate HIV-1 infection (RRM2, HSP90AA, CFL1, and DYNC1H1)(Allouch et al., 2013; Franke and Luban, 1996; Lukic et al., 2014; Roesch et al., 2012) and multiple genes involved in T-cell metabolism (SLC2A3, SLC2A1, SLC2A5, CASP3, FAS, GAPDH, and GUSB). Taken together, these results suggest that, with the exception of SAMHD1, the antiviral restriction factors analyzed did not decisively influence the cell susceptibility to HIV-1, which is in line with the results of previous reports (Jia et al., 2015). Our data indicate that metabolically active cells may offer favorable conditions for HIV infection.

EXAMPLE 13. Hierarchy of susceptibility to HIV infection matches metabolic activity of CD4+ T-cell subsets

To explore the possible association between HIV infection and cell metabolism, we determined the metabolic activity of the CD4+ T-cell subsets at the time of infection. We used a cell flux analyzer to measure, in different conditions, the oxygen consumption rate (OCR) and the extracellular acidification rate (ECAR) as indicators of oxidative phosphorylation (OXPHOS) and glycolysis, respectively (Zhang et al., 2012). In the absence of activation in vitro and in agreement with their quiescent nature, all sorted CD4+ T-cell subsets had low levels of metabolic activity (Figure 3A). Nonetheless, small differences between subsets were noted; basal metabolism and metabolic potential were highest in Tem cells and lowest in Tn cells, while Ttm and Tcm cells presented similar intermediate levels (Figures 3A, B). These differences were more pronounced after activation, with all memory cell subsets increasing mitochondrial function and glycolysis to different extents and with different kinetics. The highest metabolic activity was measured in Tem cells, peaking on day 3 after activation and decreasing on day 5. The metabolism of Ttm and Tcm cells increased after 3 days of activation and then remained stable in Ttm cells while continuing to increase in Tcm cells. In contrast, Tn cells showed a modest increase only in mitochondrial function and not in glycolysis and only after 5 days of activation, when their metabolism was heavily relying on OXPHOS (Figure 3C). Accordingly, important differences were also found between CD4+ T-cell subsets regarding their capacity to

uptake glucose and their levels of the surface expression of the GLUT1 receptor, which were lowest in Tn cells and highest in Tem cells (Figures 11A, B). The relative metabolic activity levels of the different cell subsets matched their relative susceptibility to HIV-1 infection (Figure 1C), and we found positive correlations between HIV infection levels and multiple metabolic functions in cells that had been activated (Figure 3D, Figure 11C). These results further point to an influence of the metabolic activity of CD4+ T-cells on their susceptibility to HIV-1.

EXAMPLE 14. HIV-infected CD4+ T-cells are characterized by higher levels of metabolic activity independent of cell activation levels

To analyze if there was a direct link between cell metabolism and HIV-1 infection, we challenged 5-day activated bulk CD4+ T-cells with HIV-1_{gfp}-VSV, and we sorted three days later noninfected GFP- and infected GFP+ cells. HIV-infected CD4+ T-cells had higher levels of basal metabolism and metabolic potential and, overall, a more energetic profile than noninfected cells (Figures 4A and S4A). Although we could detect infected cells among cells with low activation levels (Figure 4B), we found higher proportions of GFP+ cells among CD4+ T-cells expressing activation markers. We therefore evaluated whether differences in the metabolic activity of infected and noninfected CD4+ T-cells were just a consequence of the selective infection of CD4+ T-cells with higher activation levels. We sorted CD4+ T-cells first based on their expression of either high or low levels of both HLA-DR and CD25 and then based on whether they were GFP+ or GFP- (Figures 4B and S4). After 5 days of stimulation, the CD4+ T-cell subsets expressed different levels of activation markers (Figure 12B), which were highest in Tem cells and lowest in Tn cells. This was translated to different contributions of CD4+ T-cell subpopulations in the high- and low-activation sorted cell fractions (Figure 4B). Nevertheless, Tn cells were more frequently found in the GFP- fraction, both in high and low-activated cell populations, whereas the GFP+ fraction was enriched with Tem cells. These results matched the hierarchy of infection that we observed before (Figure 1) and further supported that the susceptibility of CD4+ T-cell subsets to HIV-1 depends on the intrinsic characteristics of these cells independent of their activation status. In this regard, we found that infected GFP+ cells in both the high- and low-activation fractions, had higher basal metabolisms (OCR and ECAR) than

noninfected GFP- cells (Figure 4C). These results demonstrated that HIV-infected CD4+ T-cells were characterized by higher metabolic activity levels.

EXAMPLE 15. HIV-1 infection is preferentially established in CD4+ T-cells with high metabolic activity levels

5 Our results suggest that HIV-1 infection is favored in the environment provided by CD4+ T-cells with high metabolic activity levels. We analyzed if this was due to a selective infection of CD4+ T-cells with the highest metabolic activity levels or if it was HIV-infection that increased the metabolic activity of the cells. We activated CD4+ T-cells and sorted Tn and Tcm cells based on their capacity to uptake high or low levels of the fluorescent glucose analogue 2NBDG (Figure 10 13A), which corresponded to weakly and strongly glycolytic cells respectively (Figure 13B). We infected these purified cell fractions with HIV-1_{gfp}-VSV. Three days after infection, infected GFP+ CD4+ T-cells were only observed among highly glycolytic Tn and Tcm cells, while weakly glycolytic cells were strongly resistant to infection (65x [41x-206x], median [IQR] fold increase in the proportion of GFP+ cells in HGlu vs LGlu cell subsets, p=0.008) (Figure 5). Overall these 15 results confirmed that, in our conditions, the high metabolic activity of infected CD4+ T-cells was one of the causes rather than a consequence of HIV infection.

EXAMPLE 16. Suboptimal inhibition of glucose metabolism blocks HIV-1 replication in CD4+ T-cells

The above results indicate that HIV-1 infection of CD4+ T-cells required high levels of 20 metabolic activity. Therefore, we analyzed if HIV-1 replication could be blocked with metabolic inhibitors. We infected activated CD4+ T-cells with HIV-1_{gfp}-VSV in the presence of increasing amounts of etomoxir, an inhibitor of fatty acid oxidation (FAO), 6-diazo-5-oxo-l-norleucine (DON), a glutamine antagonist, or 2-deoxy glucose (2-DG), a competitive inhibitor of glycolysis. Etomoxir was able to reduce HIV infection but only at high concentrations, well above the levels 25 needed to reduce mitochondrial respiration (Figure 6A and Figure 14). DON reduced HIV infection without inducing cell death, although the extent of the inhibition was heterogeneous. Suboptimal amounts of 2-DG (5 mM), which were enough to significantly reduce glycolysis (Figure 14), decreased HIV-1 infection of CD4+ T-cells with minimal cell toxicity (Figure 6A). These results suggested a higher impact of glucose and glutamine metabolism than FAO on HIV-

1 replication. The role of glucose metabolism was further confirmed in different sets of experiments in which the frequency of HIV-1-infected CD4⁺ T-cells was reduced when the infections were performed in conditions of glucose starvation or in presence of UK5099, a molecule that inhibits the transport of pyruvate, an end product of glycolysis, to the mitochondria (Figure 6B). The presence of 2-DG impaired the accumulation of HIV-1 reverse transcribed products overtime pointing to an early block of viral replication (Figure 6C). 2-DG was able to reduce infection and reverse transcript levels to a similar extent whether it was added to the culture at the time of the challenge or up to 8h later (Figure 6D), indicating that 2-DG was affecting post-entry steps of viral replication. Overall, these results show that a glycolytic environment was necessary for HIV-1 to complete reverse transcription.

2-DG blocked HIV-1 infection in all CD4⁺ T-cell subsets, although the differences were more pronounced in more differentiated (more glycolytic) cells (Figure 6E). Interestingly, Etomoxir slightly reduced viral replication in Tem cells but not in other T-cell subsets, which could be related to the overall highly energetic nature of these cells. We then used VSV-G pseudotyped NL4.3Δenv Duo-Fluo I particles that allow HIV-1 latently and productively infected cells to be distinguished from each other (Calvanese et al., 2013) (Figure 15A). Interestingly, latent infection was more prominent among Tn and Tcm CD4⁺ T-cells, while productive infection was predominantly observed among Tem cells (Figure 15A). Overall, the presence of 2-DG significantly reduced the global number of both latently and productively infected CD4⁺ T-cells (Figure 6F), which agreed with the need for a glycolytic environment for HIV-1 to complete the preintegration steps of its replication cycle.

We then analyzed the impact of inhibition of glycolysis on the infection of CD4⁺ T-cells with a R5 wild-type replication competent virus (HIV-1 Bal). We first confirmed that the hierarchy of infection of CD4⁺ T-cell subsets that we observed with VSV-G single cycle particles (Tn<Tcm<Ttm<Tem) coincided with the hierarchy of infection when we used replication competent HIV-1 Bal (Figure 15B). We found that 2-DG was also able to efficiently blocked infection of CD4⁺ T-cells with HIV-1 Bal (Figure 6G), independently of whether it was added at the time of challenge or 4h/8h after challenge (Figure 15C). All together, these results show that

the inhibition of metabolic activity blocked HIV-1 replication, corroborating that CD4+ T-cell metabolism is an important determinant of HIV-1 infection.

EXAMPLE 17. Suboptimal inhibition of glycolysis eliminates HIV-1 infected cells and impairs HIV amplification from CD4+ T-cell reservoirs

5 We next studied if the preferential establishment of HIV-1 infection in highly glycolytic cells could be used to target HIV-1 reservoirs. First, we analyzed if suboptimal inhibition of glycolysis could selectively eliminate CD4+ T-cells that had been preinfected in vitro. We infected CD4+ T-cells with HIV-1_{gfp}-VSV and sorted infected GFP+ from noninfected GFP- cells (Figure 12A) and cultured them in the absence or presence of 2-DG to inhibit glycolysis. The
10 presence of 2-DG induced higher levels of cell death among infected GFP+ cells than among GFP- cells (Figure 7A and B), affecting all memory T-cell subpopulations (Figure 7C).

As 2-DG was able to both block infection and eliminate infected cells, we wondered whether 2-DG could block HIV spread upon activation of CD4+ T-cells from HIV-infected individuals receiving cART. We isolated CD4+ T-cells from 6 individuals receiving cART (Table 4)
15 and activated the cells with PHA in the absence or presence of 2-DG. In all cases, 2-DG potently blocked HIV-1 amplification, as measured by ultrasensitive analyses of p24 production (Figure 7D). Therefore, the need of HIV for highly glycolytic cells reveals a vulnerability that can be exploited to tackle infection.

EXAMPLE 18. Synergism of multiple metabolic inhibitors

20 CD4+ T cells from a same donor were treated with various metabolic inhibitors, alone or in combination. The results are shown in Figure 8, which shows the synergistic effect of suboptimal amounts of 2DG, DON and ETOMOXIR.

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Tables

Table 3, related to STAR methods and Figure 2 Gene symbols, RefSeq, full gene names and primers used in the transcriptomic q-PCR analysis

Gene	Design RefSeq	FP	RP	Gene Full Name
ACTB	NM_001101.3	CCAACCCGCGAAGATGAC	TAGCACAGCCTGGATAGCAA	actin, beta
AKT1	NM_005163.2	CACACACTCACCGAGAACC	TCGTGGGTCTGGAAGAGTA	v-akt murine thymoma viral oncogene homolog 1
API5	NM_006595.3	AGTTGGCCGAAAACCTTCCA	AGGCCCCGTGCAAAAGTAC	apoptosis inhibitor 5
BCL11B	NM_138576.2	CAACCCGCGAGCACTTGTC	CCTCGTCTTCTCGAGGATGG	B-cell CLL/lymphoma 11B (zinc finger protein)
BCL2	NM_000633.2	ATGTGTGTGGAGAGCGTCAA	GTGCCGGTTCAGGTACTCA	B-cell CLL/lymphoma 2
BCL2L1	NM_138578.1	CTGCCTGCCTTTGCCTAA	CCAAAACACCTGCTCACTCA	BCL2-like 1
BECN1	NM_003766.3	GGTTGAGAAAGGCGAGACAC	ACTGCCCTCTGTGCTCTCAA	beclin 1, autophagy related
CASP3	NM_004346.3	AGGCCGACTTCTTGTATGCA	AACCAGGAGCCATCCTTTGAA	caspase 3, apoptosis-related cysteine peptidase
CASP8	NM_001080124.1	GGAATCTCTCAAAATGCAAACTGG	CAGGATGACCCCTCTTCTCCAT	caspase 8, apoptosis-related cysteine peptidase
CCNT1	NM_001240.3	GATTCAGTCTTACACAGTTCC	TTTTGGGTGCTCTCTCCA	cyclin T1
CDK9	NM_001261.3	GCCAGAAAGTGGCTCTGAA	GATCTCCCGCAAGGCTGTAA	cyclin-dependent kinase 9
CDKN1A	NM_078467.1	TGGAGACTCTCAGGGTTCGAAA	CGGCGTTTGGAGTGGTAGAA	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
CDKN1B	NM_004064.3	GCAATGCCGCGAATAAGGAA	TTGGGGAACCGTCTGAAACA	cyclin-dependent kinase inhibitor 1B (p27, Kip1)
CFL1	NM_005507.2	GCTCCAAGGACGCCATCA	TCCTTGACCTCCTCGTAGCA	cofilin 1 (non-muscle)
CUL4A	NM_003589.2	TGCCGAAAGGCCAAAGGTTAA	CTCCCTCTCTCTAAGCGTTTAC	cullin 4A
DBP1	NM_001923.3	TTGCCCTACAAGCCCATGGAA	CAAGATTTCCACAGCACTCATCC	damage-specific DNA binding protein 1, 127kDa
DDX58	NM_014314.3	GACTGGACGTGGCAAAACA	CTCCACTGGCTTTGAAATGCA	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58
DNAJB1	NM_006145.1	AAGGAGATCGCTGAGGCCTA	TGGGGCCACTCCCCCTTTA	DnaJ (Hsp40) homolog, subfamily B, member 1
DYNC1H1	NM_001376.4	GTTCCGCTGGTGCTGTTTAA	CAAGTGGCCTTGAGGTTGAC	dynein, cytoplasmic 1, heavy chain 1
E2F1	NM_005225.2	AGCTCATTTGCCAAGAAGTCCAA	TCCTGGGTCAACCCCTCAA	E2F transcription factor 1
EIF2AK2	NM_002759.2	TGCCTAATTCAGGACCTCCA	TCACCTTCTGGAATCTCTTCC	eukaryotic translation initiation factor 2-alpha kinase 2
ESRRA	NM_004451.3	GGAGCGAGAGGAGTATGTTCTAC	CTTCTCGCAGCTGCTCCA	estrogen-related receptor alpha
FAS	NM_000043.4	GCATCTGGACCCCTCCTACC	CCTTGGAGTTGATGTCAGTCAC	Fas cell surface death receptor
FOXO3	NM_001455.3	CGCTCTCTCCGCTCGAA	TTTGACGGGGCCACGTA	forkhead box O3
FOXP3	NM_014009.3	TGTGGGGTAGCCATGGAAA	GGGTGCGATGTTGTGGAA	forkhead box P3

GAPDH	NM_002046.4	GAACGGGAAGCTTGTGCATCAA	ATGCCCCCACTTGATTTTGG	glyceraldehyde-3-phosphate dehydrogenase
HIF1A	NM_001530.3	CAGTCGACACAGCCTGGATA	TTCTTCTGGCTCATATCCCATCAA	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
HK2	NM_000189.4	CTGCTGAAGGAAGCGATCCA	TAGTCCGACTGTGTCGTTCCAC	hexokinase 2
HMGA1	NM_002131.3	CCGACCAAAAGGGAAGCAAAA	GTCGCCCCCTTGGTTTCC	high mobility group AT-hook 1
HSP90AA1	NM_001017963.2	ACTGCACCAAGAAATGAAGGAGAA	CCATGTTTCCGAAAGACGTTCC	heat shock protein 90kDa alpha (cytosolic), class A member 1
HSP90AB1	NM_007355.2	TCTCGCATGAAGGAGACACA	CGCACTCGCTCCACAAAA	heat shock protein 90kDa alpha (cytosolic), class B member 1
HSPA1A	NM_005345.5	AGGCTTCCCAGAGCGAAC	GAGAAGAGCTCGGTCCTTCC	heat shock 70kDa protein 1A
HSPA1L	NM_005527.3	TGCGTAATCTGGACGTTTCCA	GGCGATTCCCTTGGCAGTA	heat shock 70kDa protein 1-like
HSPB1	NM_001540.3	AGCTGACGGTCAAGACCAA	GTGTATTTCCGCGTGAAGCA	heat shock 27kDa protein 1
HSPH1	NM_006644.2	TCGGAGTTGCAGCCAAAA	TGAATGCTCGGCCATGAAA	heat shock 105kDa/110kDa protein 1
IFI16	NM_005531.2	GTGAATGGGGTGTGAGGTAC	CACCACCTCCATCTTCCCTGTA	interferon, gamma-inducible protein 16
IFI6	NM_002038.3	TGCTACCTGCTGCTCTTCA	TCAGGGCTTCCAGAACC	interferon, alpha-inducible protein 6
IFNA1	NM_024013.2	TGACTCATAACACCAGGTCC	CAGGGGTGAGAGTCTTTGAA	interferon, alpha 1
IFNB1	NM_002176.2	ATGAGCAGTCTGCACCTGAA	GACTGTACTCCTTGGCCTTCA	interferon, beta 1, fibroblast
IFNG	NM_000619.2	ACTGCCAGGACCCATATGTAA	GTCCATTATCCGCTACATCTGAA	interferon, gamma
IL7R	NM_002185.2	GGAGAAAGTGGCTATGCTCAA	CTGCGATCCATTACACTTCCA	interleukin 7 receptor
IRF1	NM_002198.2	AACAAGGATGCGCTGTTGTCC	TGGATCTGGCTCCTTTTCC	interferon regulatory factor 1
IRF3	NM_001197123.1	ACCAATGGTGGAGGCAGTAC	TGGGCCAACACCATGTTA	interferon regulatory factor 3
IRF4	NM_002480.3	CACCATGACAACGCCCTTACC	CGAGGGTGGCATCATGTA	interferon regulatory factor 4
IRF7	NM_004031.2	GGCAGAGCCGTACCTGTCA	ACCGTGGCGCCCTTGTGA	interferon regulatory factor 7
KLF2	NM_016270.2	ATCCTGCCGCTCCTTCTCCA	CCATGGACAGGATGAAGTCC	Kruppel-like factor 2
MAPK1	NM_002745.4	TTGGTACAGGGCTCCAGAAA	TCTGCCAGAATGCAGCCTA	mitogen-activated protein kinase 1
MAPK3	NM_001040056.1	GCTACACGCGAGTTGCAGTACA	GTCTTGGCGACGTGGTCATA	mitogen-activated protein kinase 3
MKI67	NM_001145966.1	AGAGTAACGCGGAGTGTC	CTTGACACACACATTGTCCCTCA	marker of proliferation Ki-67
MTOR	NM_004958.3	CCAAACCCAGGTGTGATCAA	TCCTCATTTCCAGGCCACTA	mechanistic target of rapamycin (serine/threonine kinase)
MX1	NM_001144925.1	ATGCTACTGTGGCCCCAGAAA	GGCGCACCTTCTCCTCATA	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)
MX2	NM_002463.1	CGACTGGCAGAAAGACTTACC	CTTCTGGTGGCTCTCCTCTTA	myxovirus (influenza virus) resistance 2 (mouse)
MYC	NM_002467.4	CCTGGTGCTCCATGAGGA	CCTGCCTCTTTTCCACAGAAA	v-myc avian myelocytomatosis viral oncogene homolog

MYD88	NM_001172567.1	CTGCAGAGCAAGGAATGTGAC	TGCTGGGGAACCTCTTTCTTCA	myeloid differentiation primary response 88
NFATC1	NM_172390.1	TCCTCTCCAAACACCAAGTCC	AGGATTCGGGCACACAGTCAA	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1
NFATC2	NM_012340.3	TGGAAGCCACCGGTGGATA	TGTGCGGATATGCTTGTTC	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2
NFKB1	NM_001165412.1	CTACCTGGTGCCTCTAGTGAAA	ACCTTTGCTGGTCCCACATA	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
OAS1	NM_016816.2	TACCCTGTGTGTGTGTCCAA	AGAGGACTGAGGAAGACAACC	2'-5'-oligoadenylate synthetase 1, 40/46kDa
PDE4A	NM_001111307.1	ATCACCCCTGGGGTCTCCAA	GAGCACCGACTCATCGTTGTA	phosphodiesterase 4A, cAMP-specific
PIK3CB	NM_006219.1	GCCCTTCTGAACCTGGCTTAA	CCAGCACAGGACAGTGTA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit beta
PML	NM_033238.2	CCAACAACATCTTCTGCTCCA	TTGGAACATCCTCGGCAGTA	promyelocytic leukemia
PPIA	NM_021130.3	TCGTGCCGTTTTGCAGAC	ACGGCAATGTCGAAGAACAC	peptidylprolyl isomerase A (cyclophilin A)
PRDM1	NM_001198.3	CCTGGTACACACGGGAGAAAA	TTGAGATTGCTGGTGCTGCTA	PR domain containing 1, with ZNF domain
PRKAA1	NM_206907.3	CCAACTATGCTGCACCCAGAA	AGAATAACCCCACTGCTCCA	protein kinase, AMP-activated, alpha 1 catalytic subunit
PRKCA	NM_002737.2	ACCATCCGCTCCACACTAAA	AGTCGTGGTCTTTGTCTGAA	protein kinase C, alpha
PSIP1	NM_021144.3	GGTTATGGGAGATAGATAACAATCCA	CATGGTCGGTATCTTCTTTGAA	PC4 and SFRS1 interacting protein 1
RAB7A	NM_004637.5	GTGGATGACAGGCTAGTCACA	ACCTCTGTAGAAGGCCACAC	RAB7A, member RAS oncogene family
RB1	NM_000321.2	TGCGCTCTTGAGGTTGTAA	TCAGAATCCATGGGAAAGACAA	retinoblastoma 1
RHOA	NM_001664.2	GTGCCACACAGTGTGAGAA	TGTGTCCCACAAAGCCAAC	ras homolog family member A
RICTOR	NM_152756.3	CTCCGCTGTCGGAGGTTTCATA	ACACAGCCCTCTGCTTCTTCA	RPTOR independent companion of MTOR, complex 2
RPS6KB1	NM_003161.3	AGACCTGAAGCCGGAGAAATA	AGTCCAAAAGTCTGTAGTTTCACA	ribosomal protein S6 kinase, 70kDa, polypeptide 1
RPTOR	NM_020761.2	GCTCAGAGCTGGAGGATGAA	AGGGTCCACACCAACATTCA	regulatory associated protein of MTOR, complex 1
RRM2	NM_001165931.1	GCAGCAAGCGATGGCATA	GAAACAGCGGGCTTCTGTAA	ribonucleotide reductase M2
SLC1A5	NM_001145145.1	CTCTACCACCTATGAAGAGAGGAA	CAGGATGTTTCATCCCCTCCA	solute carrier family 1 (neutral amino acid transporter), member 5
SLC2A1	NM_006516.2	ATTGTGGCATGTGCTTCC	AGAACCAGGAGCACAGTGAA	solute carrier family 2 (facilitated glucose transporter), member 1
SLFN11	NM_152270.3	TCCAAAGAAAGGCCCTGGAACA	TCTGAGATCAGGTCCCTCCA	schlafen family member 11

SMARCB1	NM_003073.3	CTCCACAACCATCAACAGGAAC	AGCTGGGTCATGGTCATCAA	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1
STAT1	NM_007315.3	ATGCTGGCACCAAGAACGAA	GCTGGCACAAATTGGGTTTCAA	signal transducer and activator of transcription 1, 91kDa
STAT3	NM_003150.3	GGAAATAATGGTGAAGGTGCTGAAC	CCGAGGTCAAACTCCATGTCAAA	signal transducer and activator of transcription 3 (acute-phase response factor)
STAT5B	NM_012448.3	AACAGAGGTTGGTCCGAGAA	GTTTCTGGGACATGGCATCA	signal transducer and activator of transcription 5B
TLR3	NM_003265.2	TCTCATGTCCAACTCAATCCA	CAGCTGAACCTGAGTTCCTA	toll-like receptor 3
TLR7	NM_016562.3	TCTTCAACCAAGACCTCTACATTCC	AGCCCCAAGGAGTTTGGAAC	toll-like receptor 7
TLR8	NM_138636.4	CTGCTGCAAGTTACGGAATGAA	GCGCATAACTCACAGGAACC	toll-like receptor 8
TLR9	NM_017442.3	TGCAACTGGCTGTTCCCTGAA	ACAAGGAAAGGCTGGTGACA	toll-like receptor 9
TP53	NM_000546.5	GACTGTACCACCATCCACTACA	AAAGCTGTTCCGTCGCCAGTA	tumor protein p53
TRAF6	NM_004620.2	TGGCAAATGTCATCTGTGAATAC	GCTGTAGGGCAGTCTAGATCA	TNF receptor-associated factor 6, E3 ubiquitin protein ligase
UNG	NM_080911.2	TGGACCCAGATGTGTGACATAA	AGAGCCCGTGAGCTTGATTA	uracil-DNA glycosylase
VPRBP	NM_014703.2	TGGATGGCGGATGCTTTGATA	TTCAATTGGCTTCCCGGAACA	Vpr (HIV-1) binding protein
APOBEC3F	NM_145298.5	AGCCTGGAGCAGAAAAGTGAA	ATCCTTGGCCGGCTAGTCC	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3F
APOBEC3G	NM_021822.3	CGCAGCCTGTGTCAGAAAA	TTGCTCCAAACAGTGTGAA	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G
GUSB	NM_000181.3	CATCGATGACATCACCGTCAC	ACAGGTTACTGCCCTTGACA	glucuronidase, beta
KLF4	NM_004235.4	CTGCGGCAAAACCTACACAA	CGTCCCAGTCACAGTGGTAA	Kruppel-like factor 4 (gut)
SAMHD1	NM_015474.3	CCCGTGCTGTGAAGTAGACAA	TGCGAGTGTGGAACATGTCA	SAM domain and HD domain 1
SLC2A3	NM_006931.2	CCGTCGGACTCTTCGTCAA	TAAAGCAGCCACCAGTGACA	solute carrier family 2 (facilitated glucose transporter), member 3
TREX1	NM_033629.3	ACCATCTGCTGTACAAACCA	TCCTCCAAAGGCTGGGACTA	three prime repair exonuclease 1
TRIM5	NM_033092.2	AGAAGCAGCAGGAAGCTGAA	GCCAAAGACGTTGGTTTGTCA	tripartite motif containing 5

Code	Gender	Age	Viral load (RNA copies/ml plasma)	Months with undetectable viral load on ART	CD4 T cell count (cells/ μ l blood)	Cell- associated HIV DNA (copies/ 10^6 CD4)
034001	M	57	<40	77	858	3805
034004	F	66	<40	110	593	3605
034005	M	50	<40	39	971	2797
034006	M	61	<40	77	750	8715
034008	M	33	<40	36	1119	4891
L34001	M	55	<40	195	693	NA

M, male; F, female; NA, not available.

Table 4, related to STAR methods and Figure 2

Clinical characteristics of HIV infected individuals whose CD4+ T cells were used in the study

Table 5, related to STAR methods and Figure 7

Ranking of studied genes after analysis with the Normfinder algorithm (Aarhus University Hospital, Denmark). BECN1 was the most stable gene present in our panel and was used to normalize our RT- qPCR data

Gene name	Stability value
BECN1	0,250549233
PRKAA1	0,288508569
RAB7A	0,30946967
TRAF6	0,319334695
TP53	0,328343002
VPRBP	0,365991082
API5	0,367327819
STAT3	0,370608212
EIF2AK2	0,384103082
HIF1A	0,391907917
TREX1	0,39253953
AKT1	0,394023857
RICTOR	0,395377783
MAPK1	0,396649613
PSIP1	0,408881136
CDKN1B	0,413957015
RPS6KB1	0,416878613
CCNT1	0,417659018
CDK9	0,426303822
MTOR	0,434251173
HMGA1	0,436534261
CUL4A	0,454108452
MYC	0,457005393
MYD88	0,461562789
NFATC1	0,463277429
SAMHD1	0,467104121
CASP8	0,467443456
NFKB1	0,472349996
NFATC2	0,476133255
PRKCA	0,47821775
DDB1	0,482170786
PIK3CB	0,487302561
DDX58	0,496549071
BCL11B	0,498962754
PPIA	0,499844178
BCL2	0,502606509
MAPK3	0,51179285
HSPB1	0,511905741
RHOA	0,515493522
STAT5B	0,516400376
CASP3	0,536630196
HSP90AB1	0,538067061
IRF3	0,546799681
SMARCB1	0,552440933
APOBEC3F	0,55660686
IFI16	0,570759333
IRF1	0,572196628
HSPH1	0,587874332
MX2	0,590279919
SLC2A3	0,601232687

Better stability value
(Best House Keeping gene)

IFI6	0,602522338
IRF4	0,610056472
MX1	0,613977716
CFL1	0,620028452
IL7R	0,64055187
HSP90AA1	0,662468904
HSPA1A	0,665415349
SLFN11	0,689147876
RB1	0,697664041
STAT1	0,702260041
FAS	0,703247399
DNAJB1	0,708604559
TRIM5	0,725969121
IRF7	0,734412588
FOXP3	0,739563525
SLC2A1	0,746229633
RPTOR	0,747416766
ACTB	0,759726907
GAPDH	0,768315184
DYNC1H1	0,77543431
APOBEC3G	0,776517129
HK2	0,794980726
GUSB	0,821595116
PRDM1	0,844574596
BCL2L1	0,867143178
UNG	0,89218868
CDKN1A	0,909445507
FOXO3	0,936183284
IFNB1	0,945277953
E2F1	0,993140533
RRM2	0,993363254
SLC1A5	1,128540312
ESRRA	1,228239862
MKI67	1,236841231
TLR9	1,23828716
IFNG	1,417604718
OAS1	1,614650004
KLF2	1,622314129
PDE4A	1,772367033
TLR8	1,903574476
IFNA1	2,132211213
TLR3	2,263468774
PML	2,39732875
TLR7	2,584686641
KLF4	2,652965521
HSPA1L	3,063617743

Worse stability value
(Worse House Keeping gene)

CLAIMS

1. A method of reducing HIV production from a T cell comprising contacting a population of cells comprising HIV-infected CD4+ T cells with 2-deoxy-glucose (2-DG) at a concentration that reduces the production of HIV from said HIV-infected CD4+ T cells.
- 5 2. The method of claim 1, wherein the cells are exposed to a concentration of 1-25 mM 2-DG.
3. The method of claim 2, wherein the cells are exposed to a concentration of 3-10 mM 2-DG.
- 10 4. The method of claim 3, wherein the cells are exposed to a concentration of 5 mM 2-DG.
5. The method of claim 1, further comprising contacting the population of cells with a metabolic inhibitor that blocks fatty acid transport to mitochondria.
6. The method of claim 5, wherein the metabolic inhibitor is Etomoxir.
- 15 7. The method of any of claims 1-6, wherein the population of cells has received cART treatment.
8. The method of any of claim 1-7, wherein the cells are contacted with the metabolic inhibitor *in vitro*.
9. The method of any of claim 1-7, wherein the cells are contacted with the metabolic inhibitor *in vivo*.
- 20 10. The method of any of claims 1-9, wherein the cells are contacted with an additional metabolic inhibitor of metabolic function in CD4+ T cells.
11. The method of claim 10, wherein the cART comprises Combivir, Kaletra, Trizivir, Epzicom, Kivexa, Truvada, Atripla, Complera, Eviplera, Stribild, Triumeq, Evotaz, Prezcoibix, Dutrebis, Genvoya, or Descovy.

12. The method of claim 10, wherein the cART comprises at least 2 or 3 of any of the following compounds:

lamivudine; zidovudine; lopinavir; ritonavir; abacavir; tenofovir disoproxil fumarate; emtricitabine; efavirenz; rilpivirine; elvitegravir; cobicistat; dolutegravir; atazanavir;
5 cobicistat; darunavir; and raltegravir.

13. A method of reducing virus or bacterial pathogen production from a cell comprising contacting a population of cells pathogen-infected cells with at least 2 metabolic inhibitors of different metabolic functions in the cells at a concentration that reduces the production of the pathogen from said pathogen-infected cells.

10 14. The method of claim 13, wherein one of the metabolic inhibitors blocks glycolysis.

15. The method of claim 13 or 14, wherein one of the metabolic inhibitors blocks fatty acid transport to mitochondria.

16. The method of any of claims 13-15, wherein both of the metabolic inhibitors are selected from Table 1 or Table 2.

15 17. The method of any of claims 13-16, wherein two of the metabolic inhibitors are 6-Diazo-5-oxo-L-norleucine (DON) and (2-Deoxy-D-glucose) 2DG.

18. The method of any of claims 13-16, wherein three of the metabolic inhibitors are DON and etomoxir and 2DG.

19. The method of any of claims 13-18, wherein the pathogen is HIV-1.

20 20. The method of any of claims 13-19, wherein the concentration of each of the metabolic inhibitors is at a suboptimal concentration.

21. A method for measuring the effect of a metabolic inhibitor of metabolic function in cells in a pathogen-infected human comprising:

administering at least one dose of a metabolic inhibitor of metabolic function in cells to

the human; and

measuring the level of pathogen infection in the pathogen-infected human.

22. The method of claim 20, wherein measuring the level of pathogen infection in the pathogen-infected human comprises measuring the level of plasma HIV RNA in an HIV-infected human.

23. The method of claim 21, wherein measuring the level of plasma HIV RNA in the HIV-infected human is performed by a reverse transcription and amplification reaction.

24. The method of any of claims 21-23, wherein the level of HIV infection in the human is measured at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 times.

25. A composition for use in the treatment of a pathogen infection in a human, comprising a combination of at least two metabolic inhibitors at a concentration that reduces the production of the pathogen from the pathogen-infected cells.

26. The composition for use according to claim 25, wherein said at least two metabolic inhibitors are of different metabolic functions in the cells.

27. The composition for use according to claim 25 or 26, wherein one of the metabolic inhibitors blocks glycolysis.

28. The composition for use according to any one of claims 25 to 27, wherein one of the metabolic inhibitors blocks fatty acid transport to mitochondria.

29. The composition for use according to any one claims 25 to 28, wherein both of the metabolic inhibitors are selected from Table 1 or Table 2.

30. The composition for use according to any one of claims 25 to 29, wherein two of the metabolic inhibitors are 6-Diazo-5-oxo-L-norleucine (DON) and 2-Deoxy-D-glucose (2DG).

31. The composition for use according to any one of claims 25 to 30, wherein three of the metabolic inhibitors are DON and etomoxir and 2DG.

32. The composition for use according to any one of claims 25 to 31, wherein the concentration of each of the metabolic inhibitors is at a suboptimal concentration.

33. The composition for use according to any one of claims 25 to 32, wherein the pathogen is HIV-1.

5 34. The composition for use according to any one of claims 25 to 33, wherein the combination of metabolic inhibitors is administered with at least one HIV inhibitor.

35. The composition for use according to claim 34, wherein the combination of metabolic inhibitors is administered with combination antiretroviral therapy.

10 36. A kit of parts for simultaneous, separate or sequential use in the treatment of a pathogen infection in a human, comprising a combination of metabolic inhibitors as defined in any one of claims 25 to 32.

37. The kit of parts according to claim 36, further comprising at least one HIV inhibitor.

15 38. The kit of parts according to claim 37, further comprising at least one combination antiretroviral therapy.

Figure 1

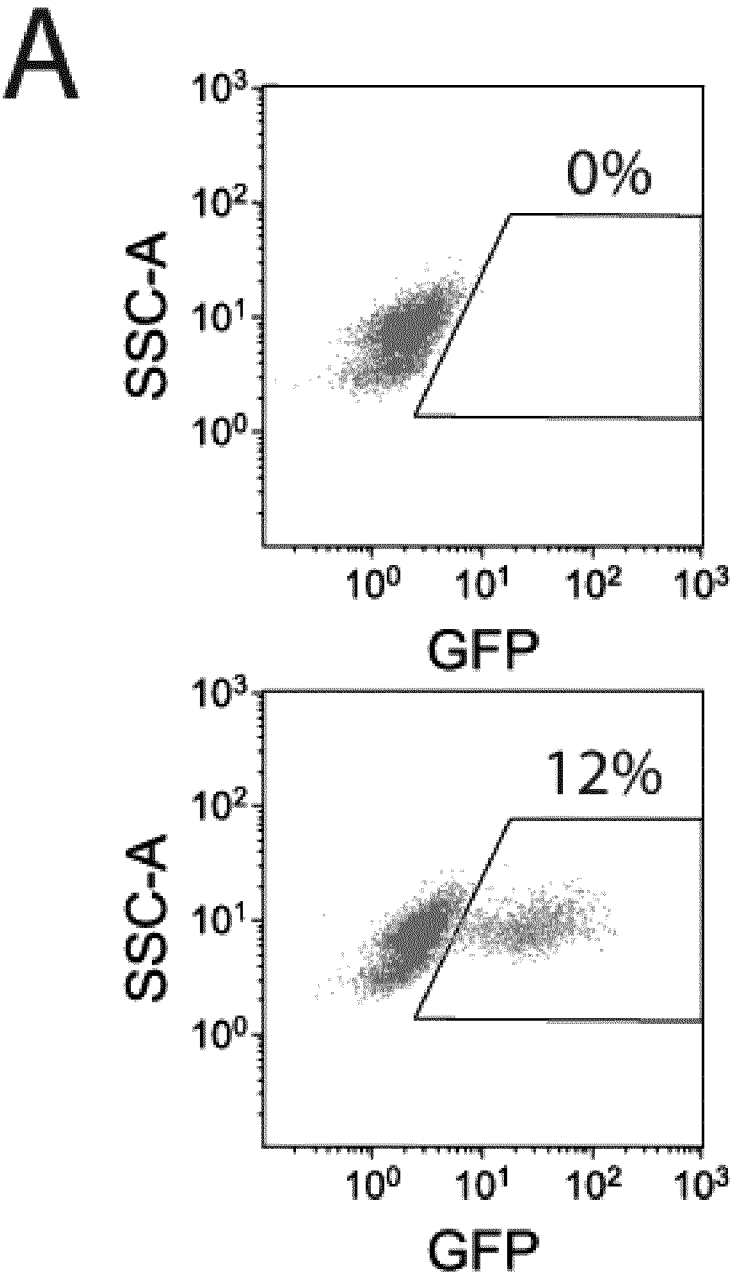


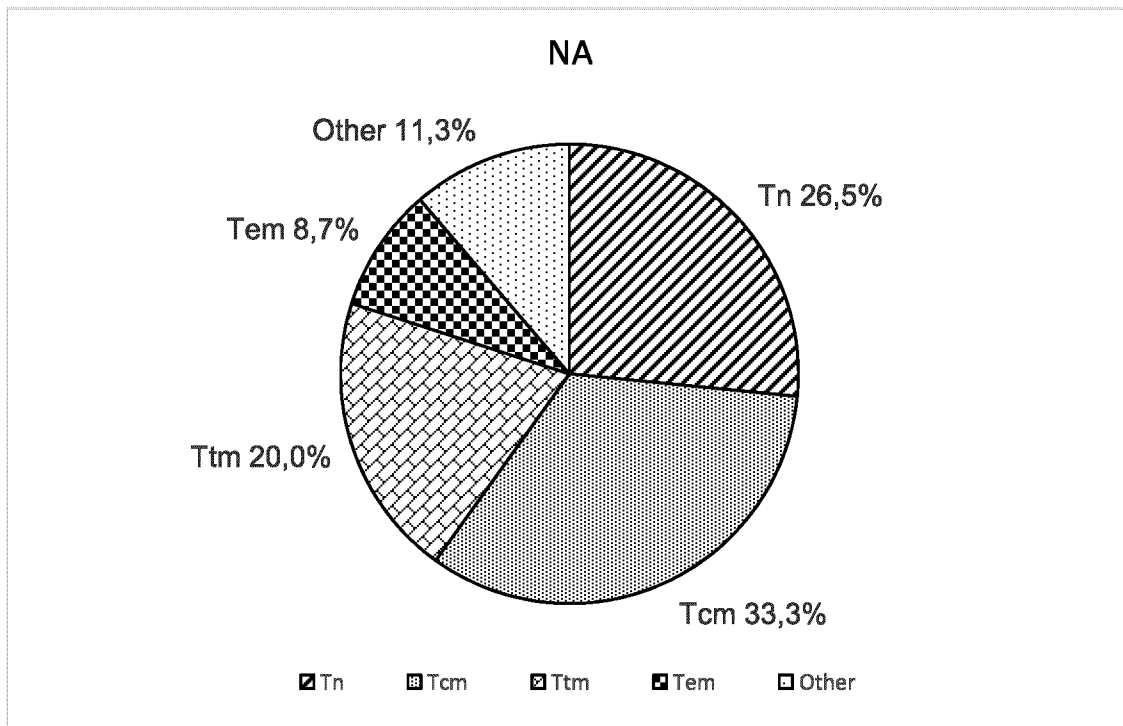
Figure 1 (continuation)**B**

Figure 1 (continuation)

B

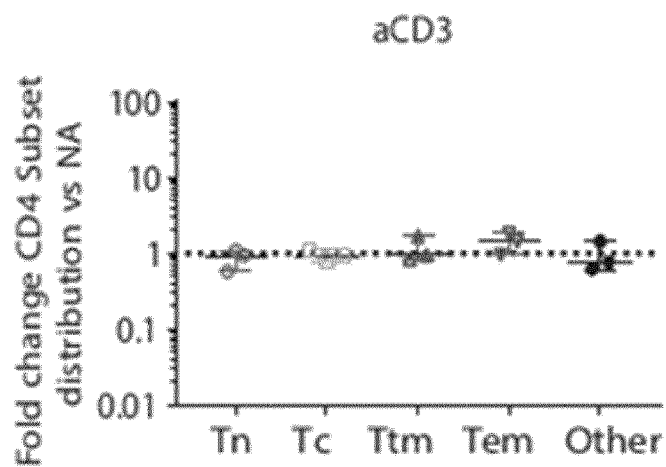
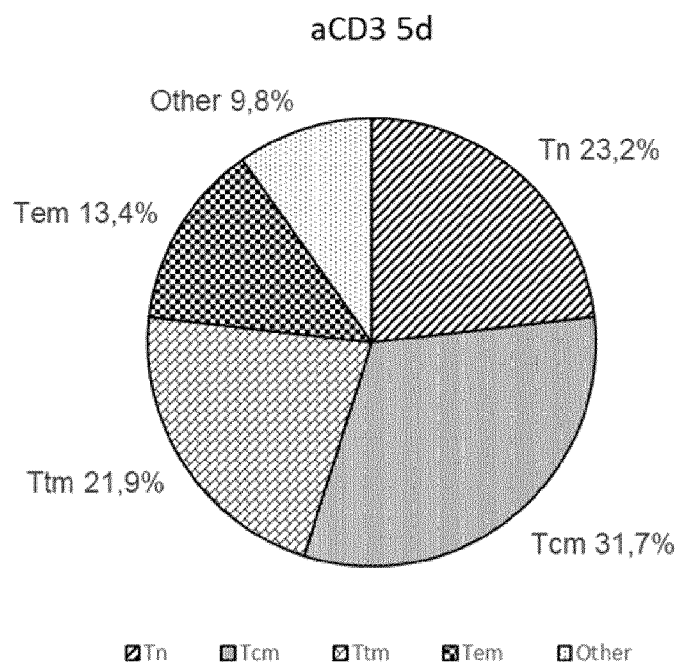


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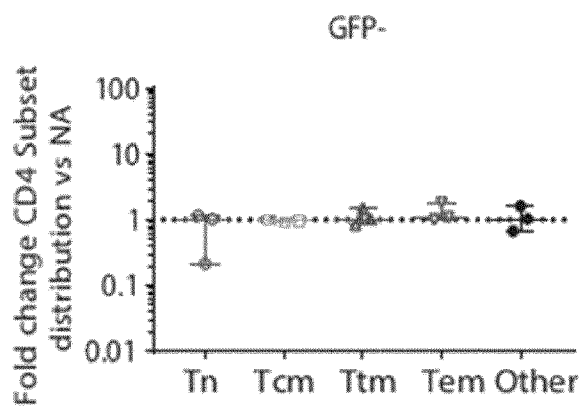
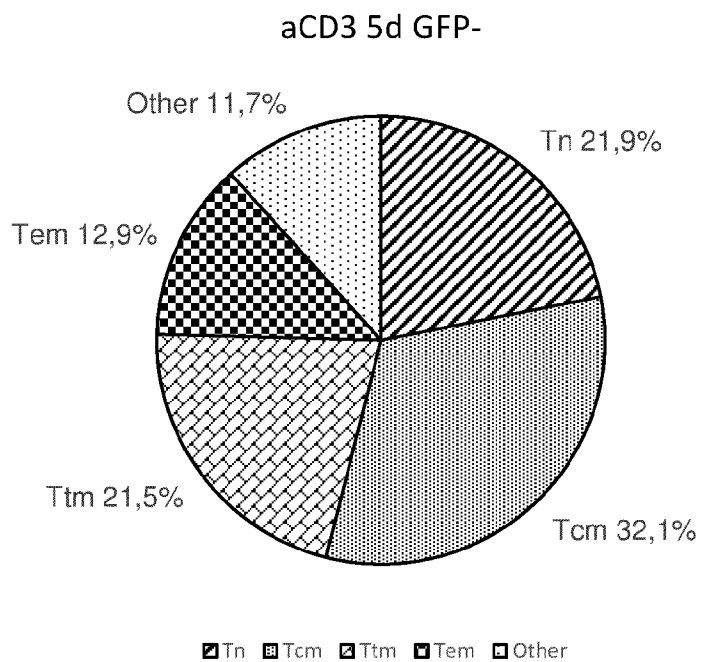
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Figure 1 (continuation)

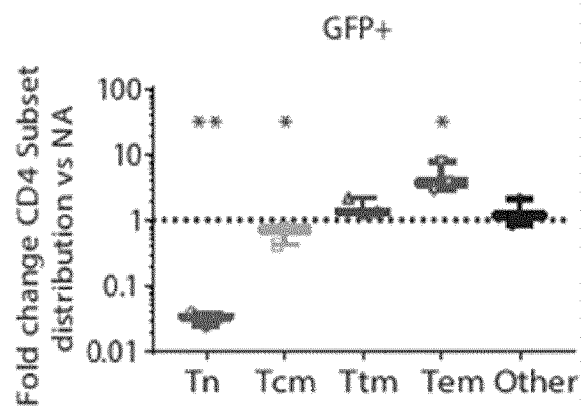
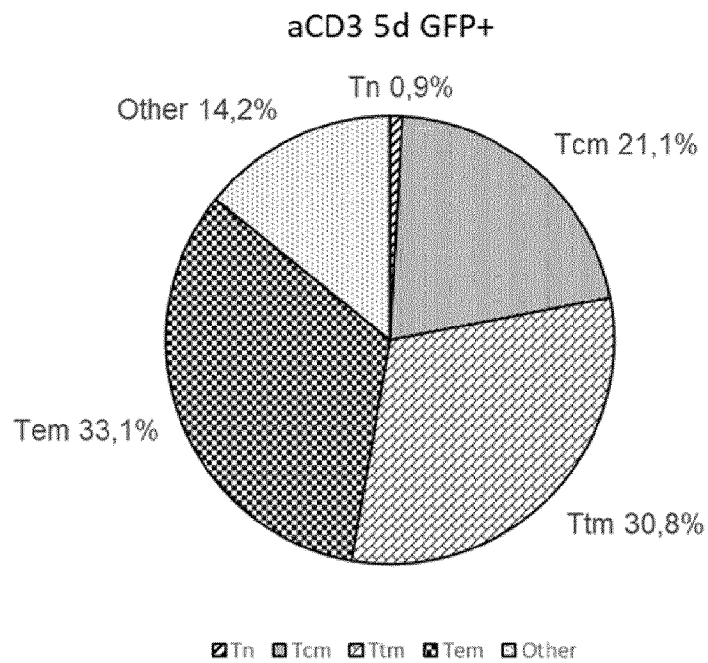
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Figure 1 (continuation)

C

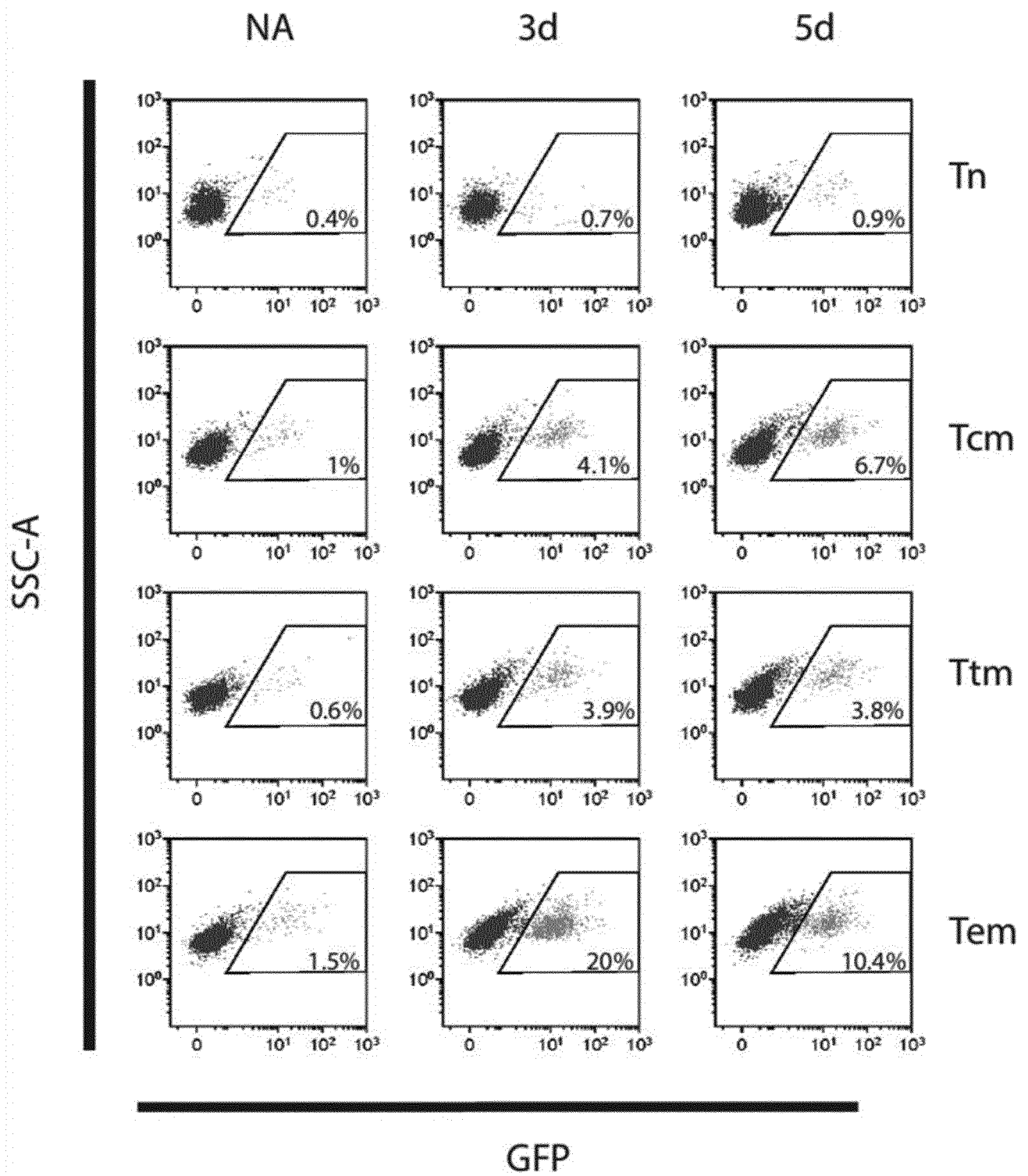


Figure 1 (continuation)

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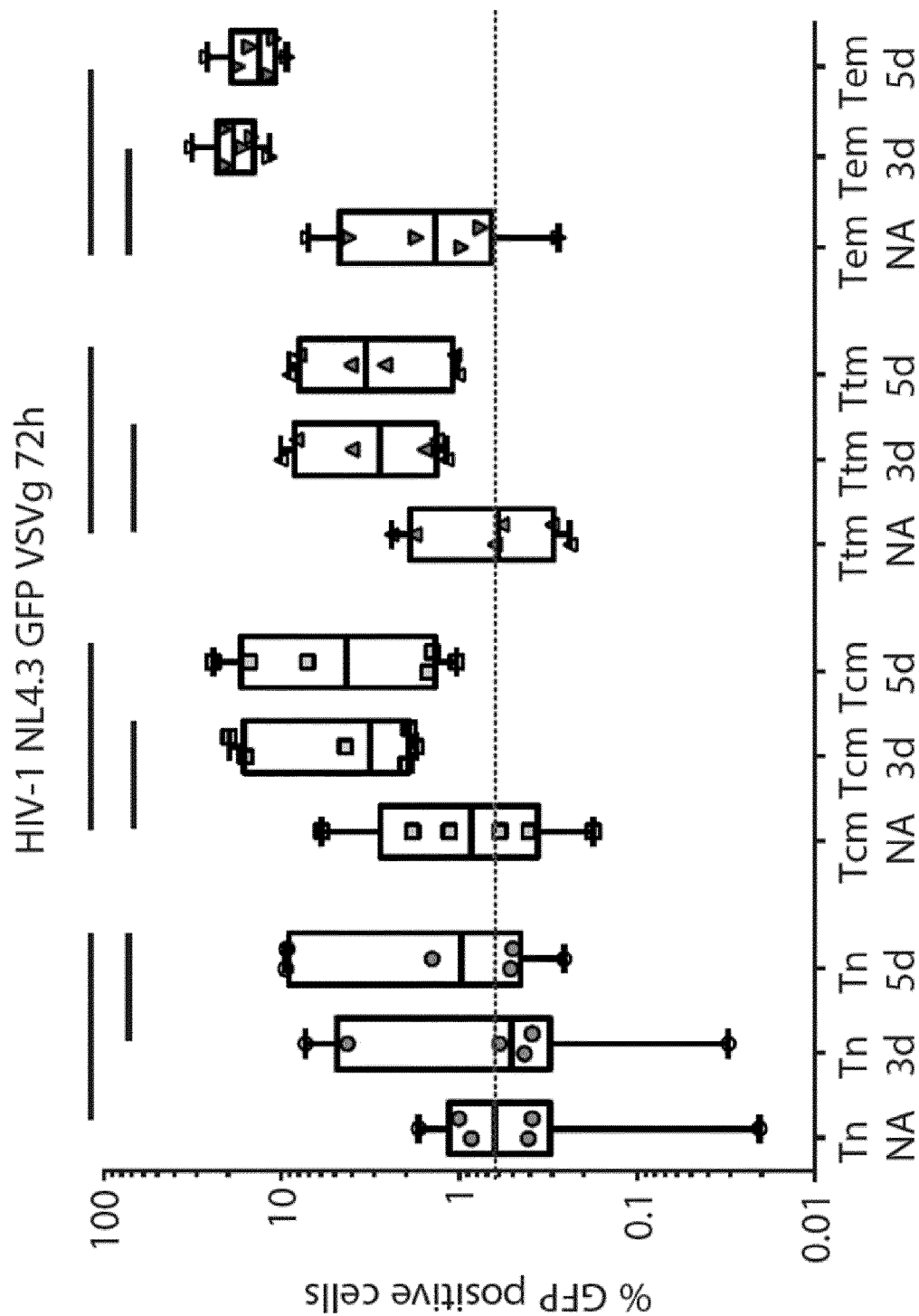


Figure 2

A

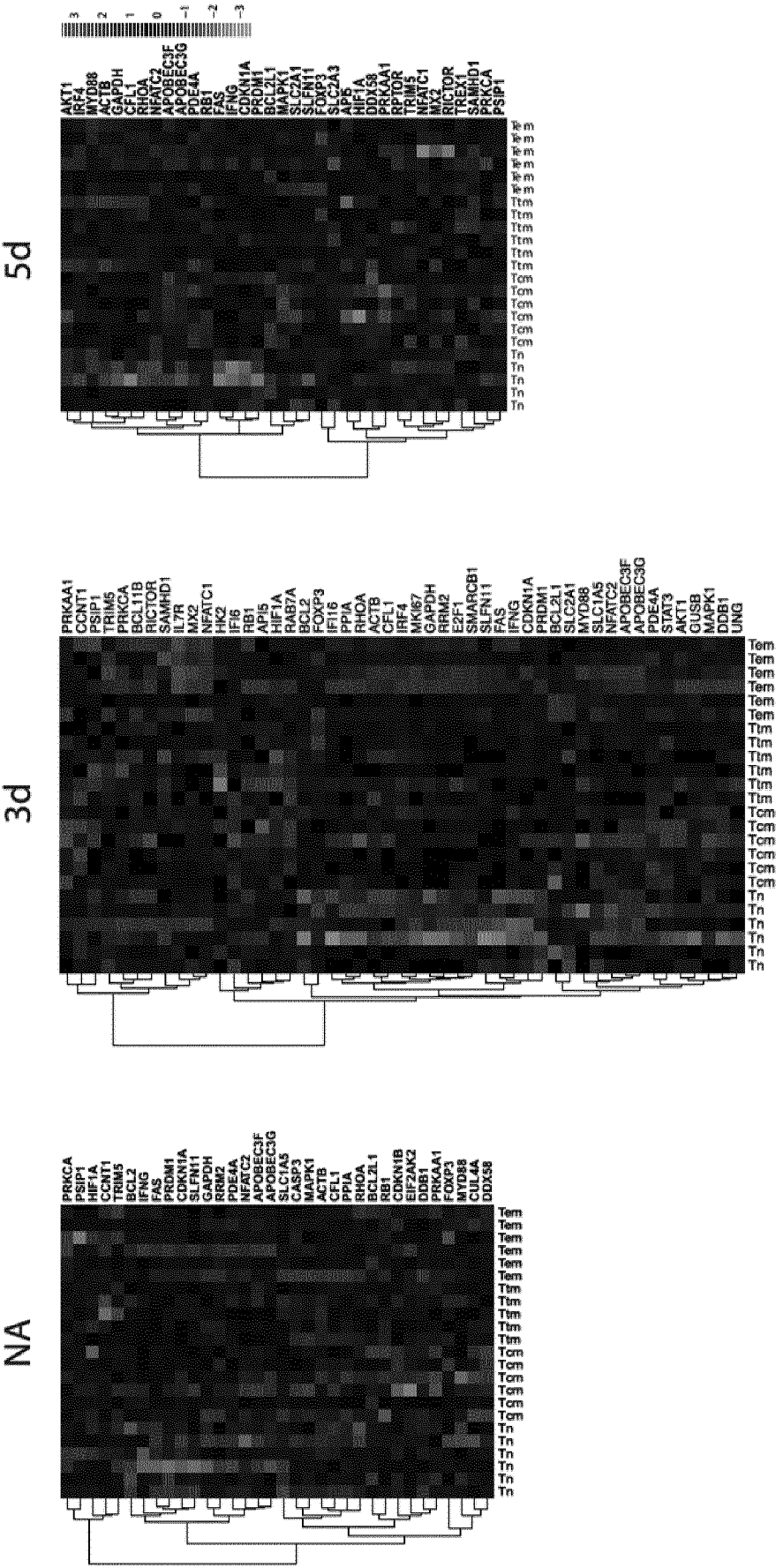


Figure 2 (continuation)

B

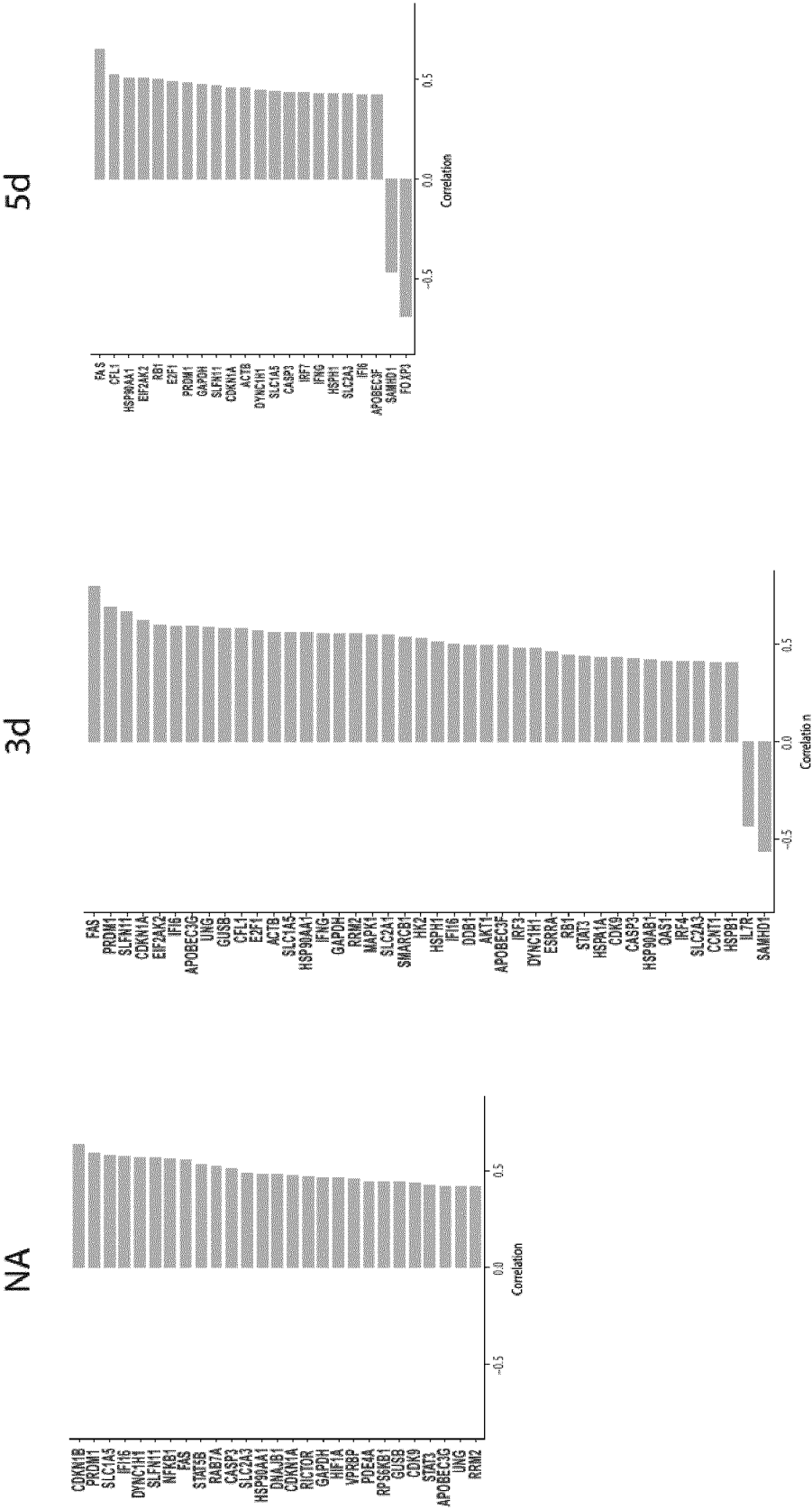


Figure 3

A

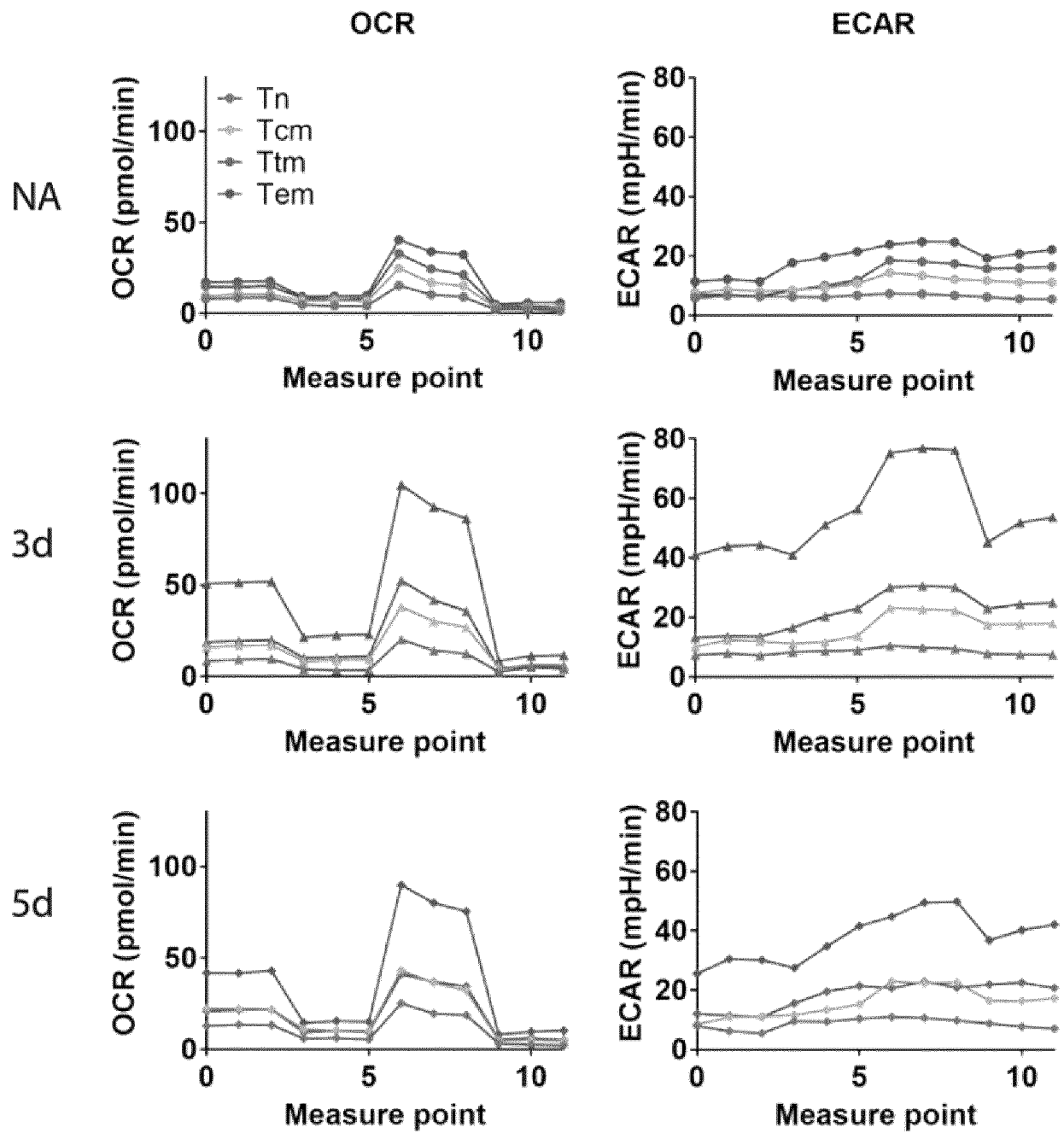


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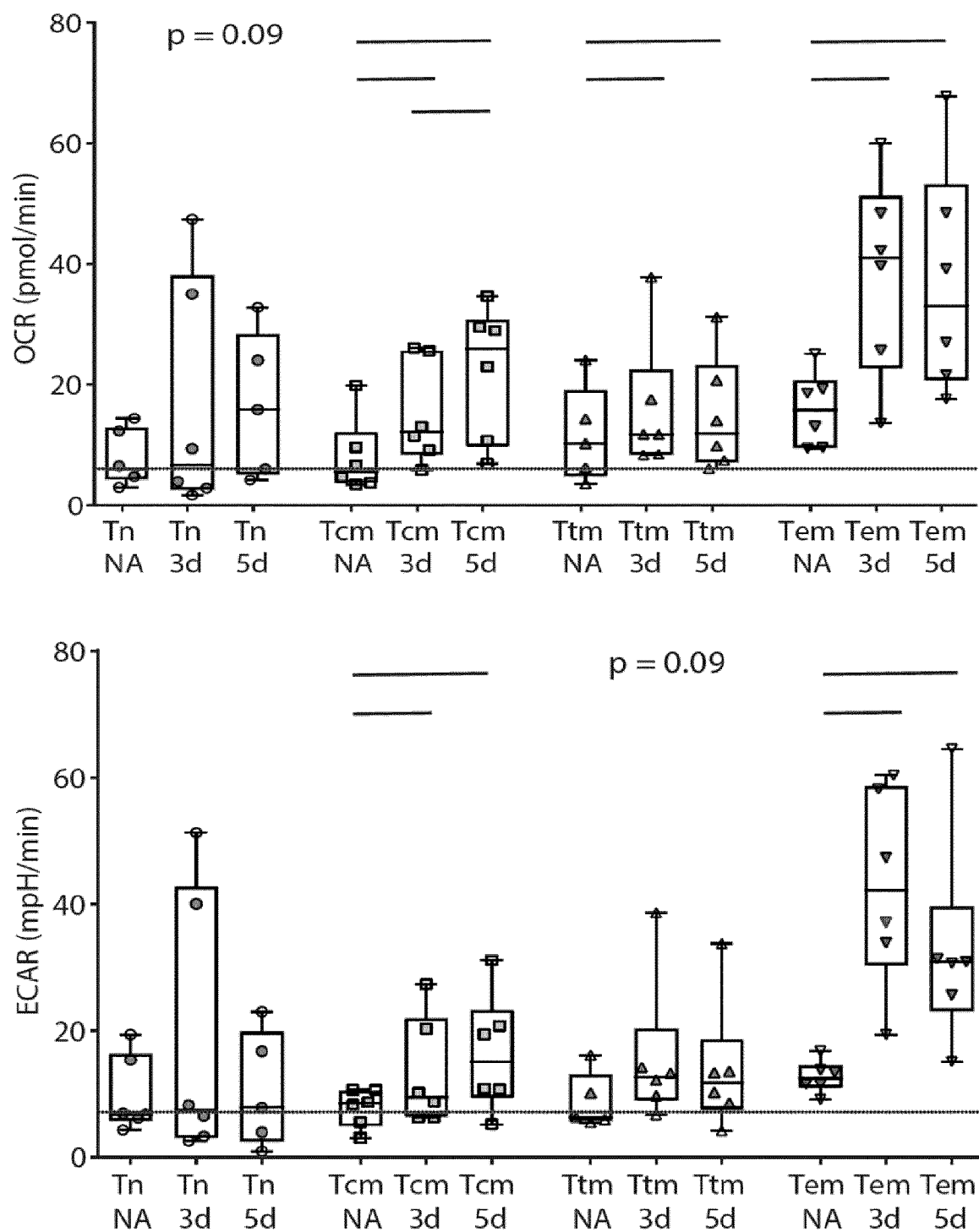
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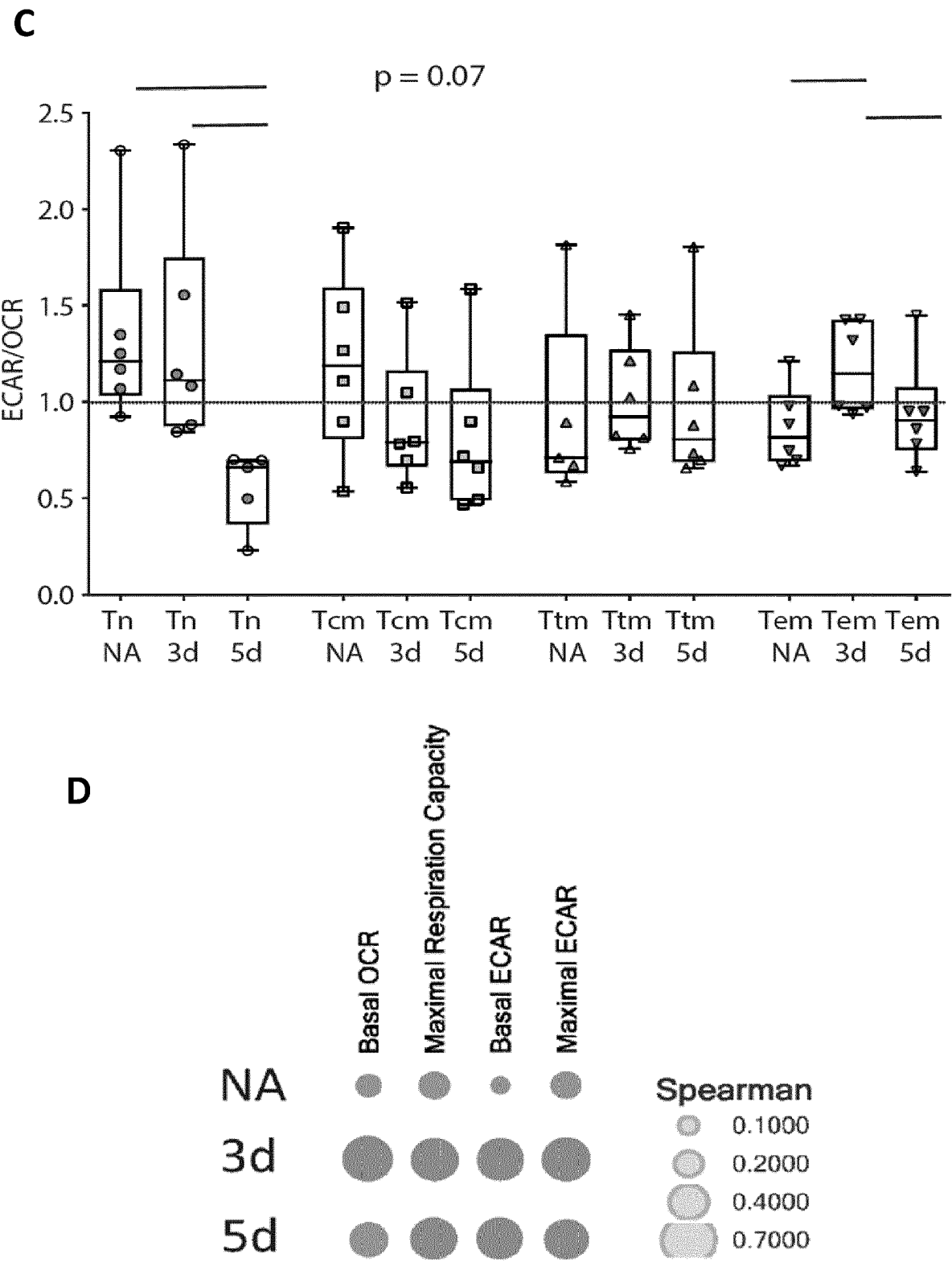


Figure 4
A

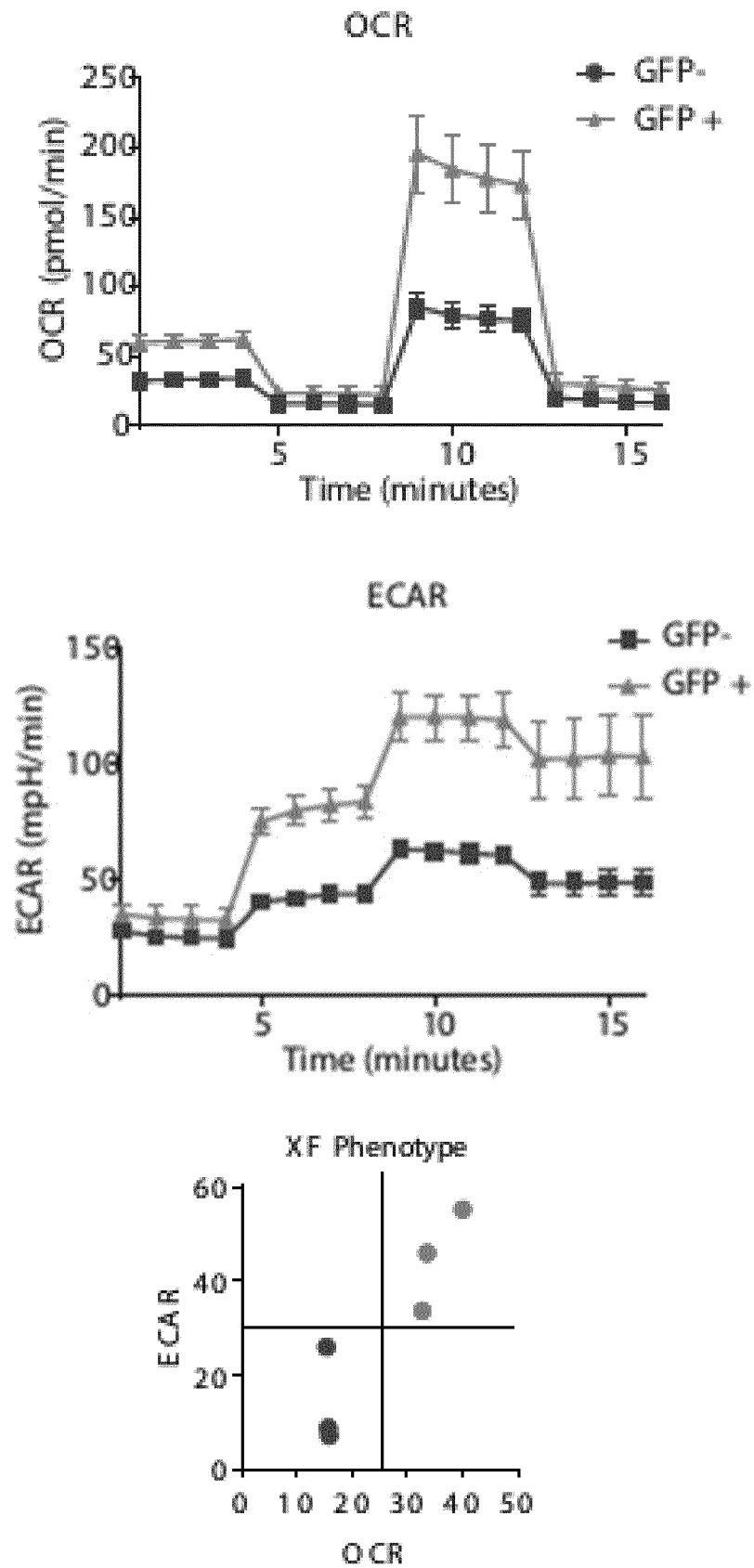


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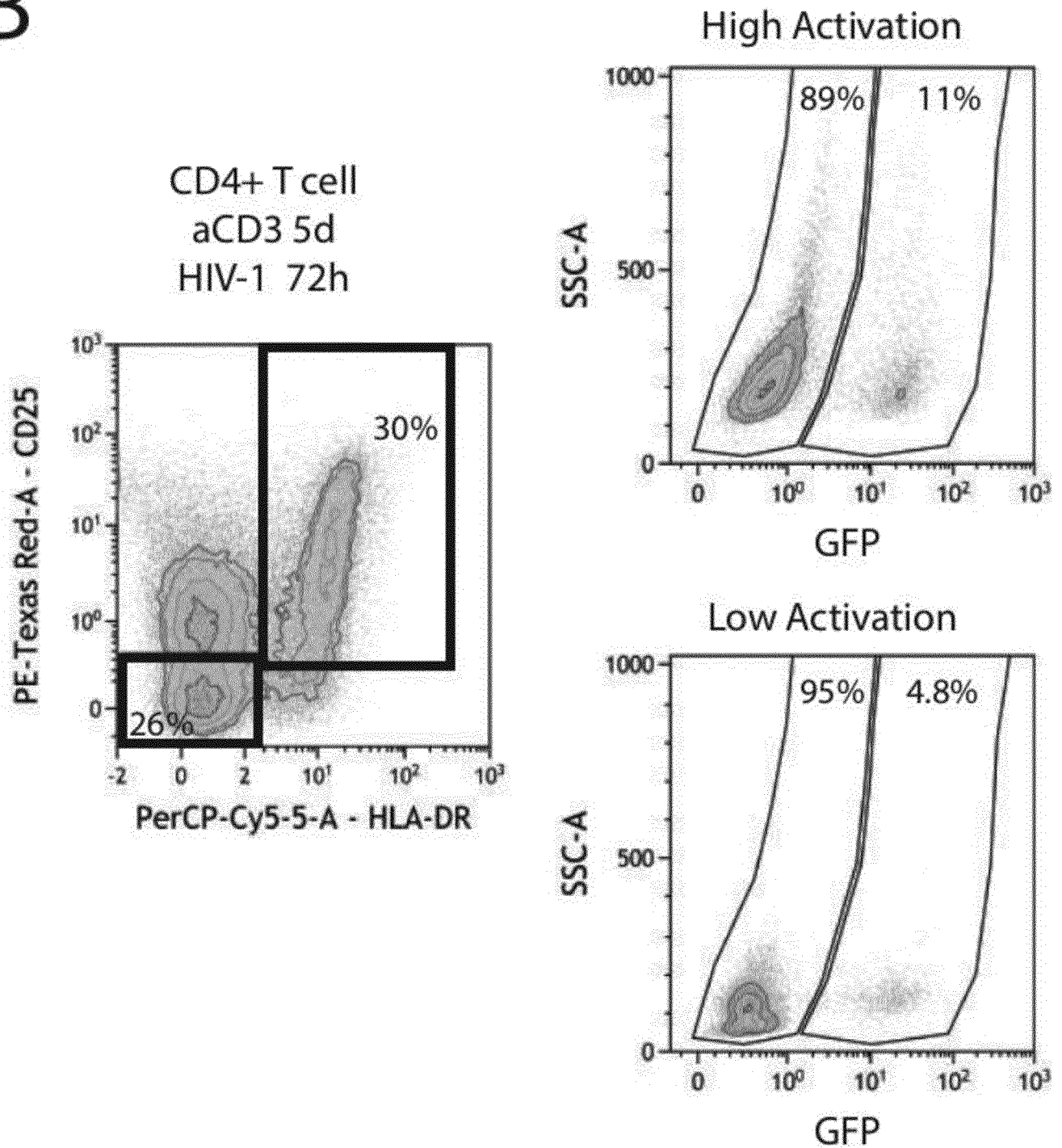
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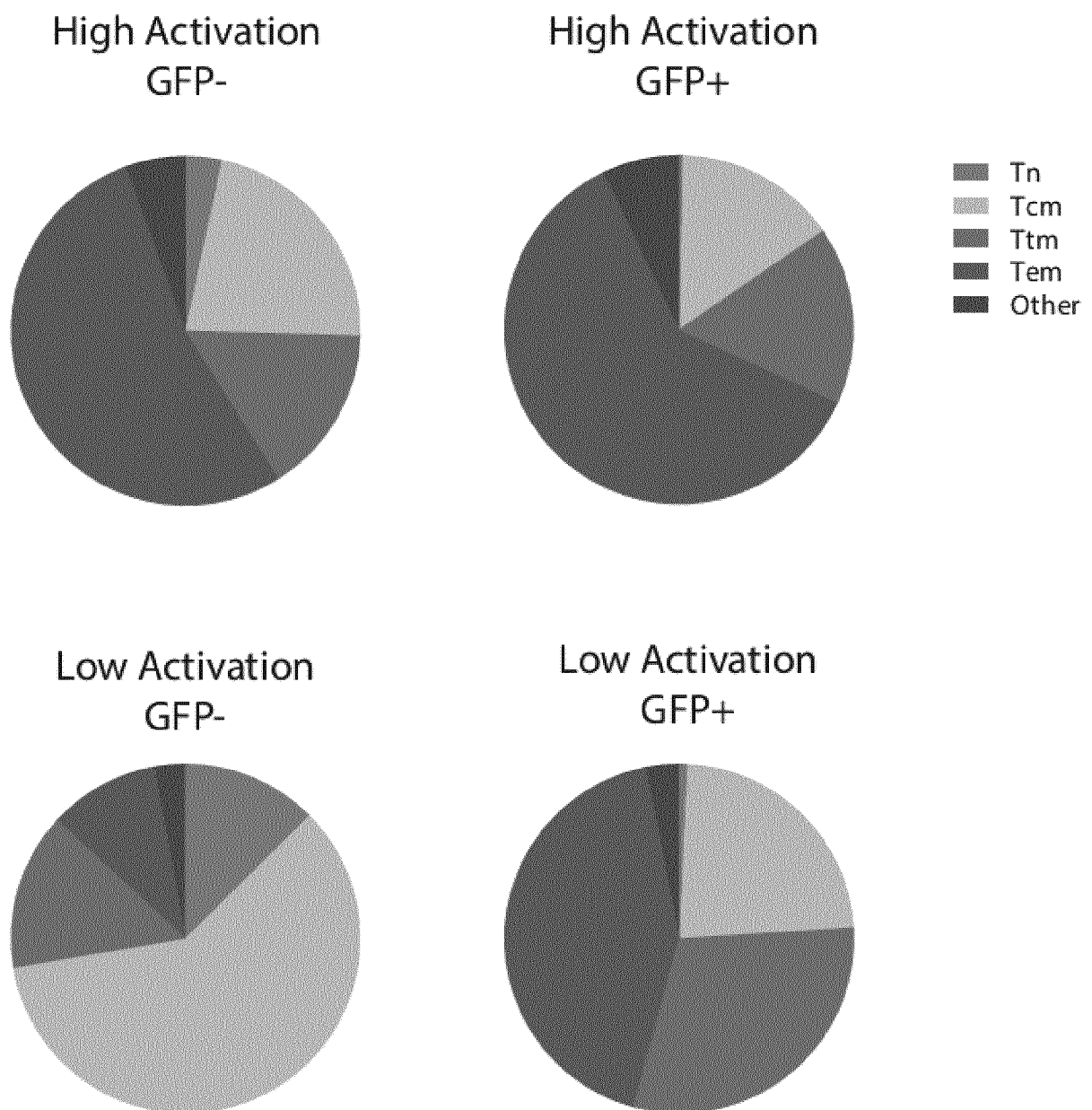
Figure 4 (continuation)**B**

Figure 4 (continuation)

C

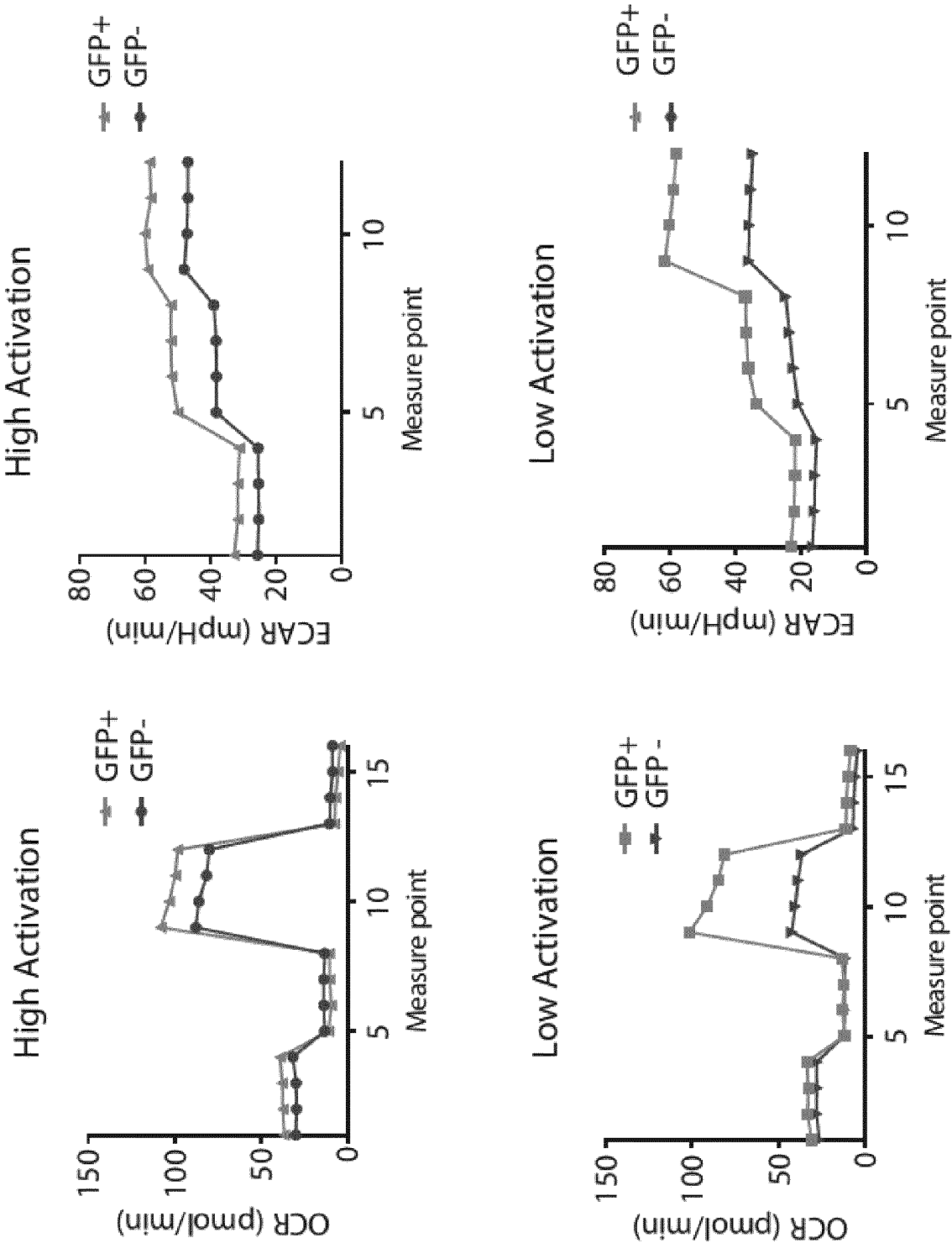


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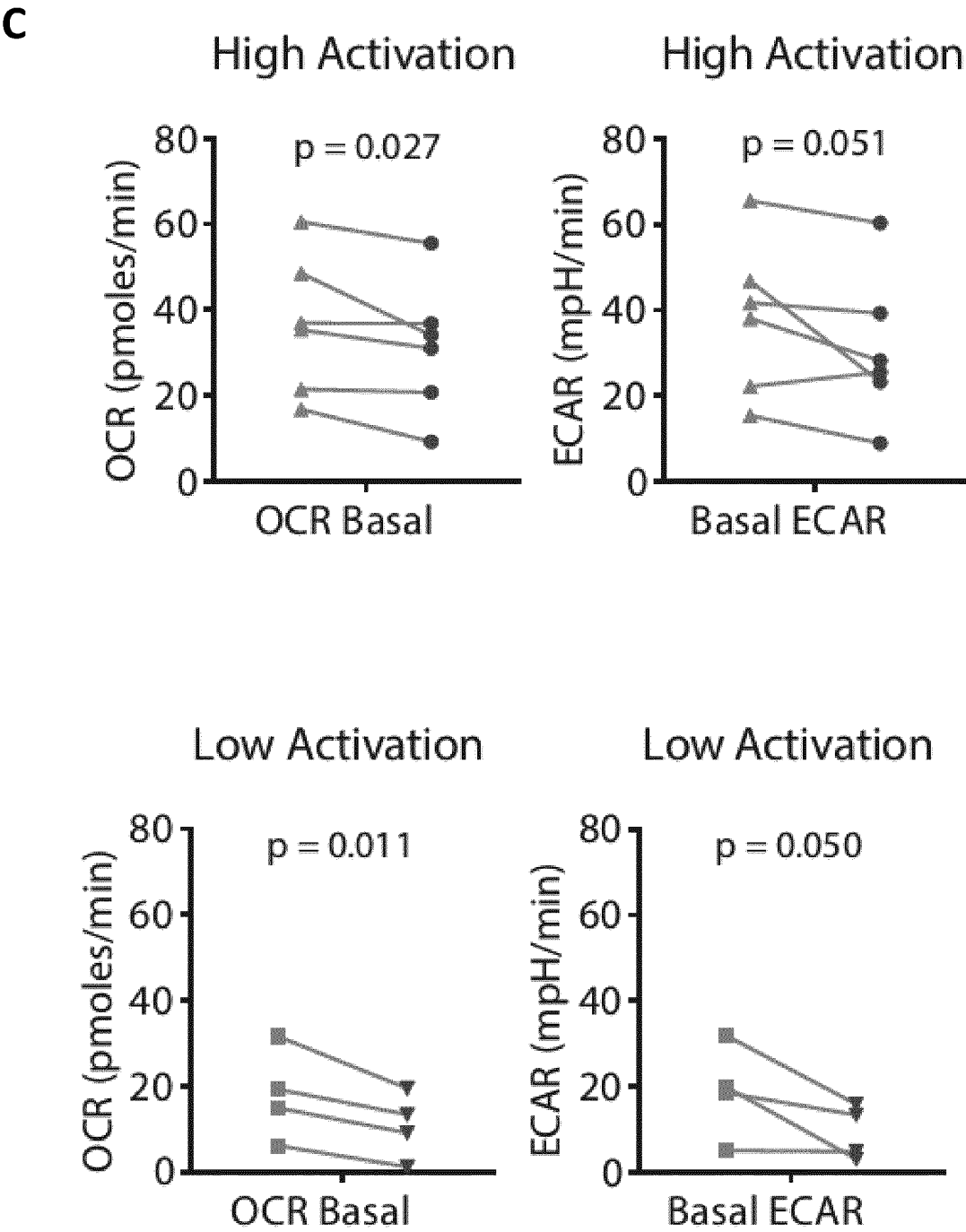


Figure 5

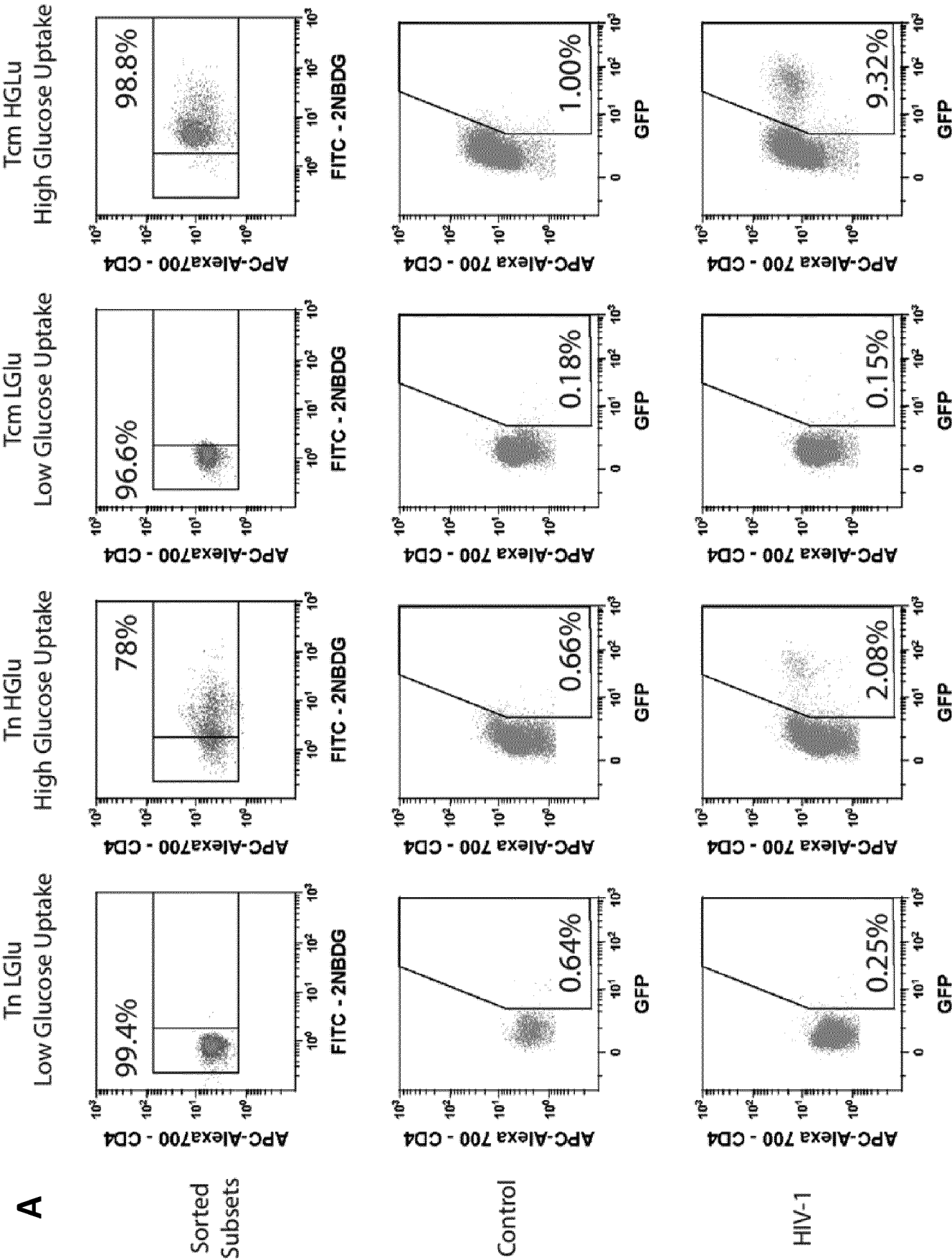


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B

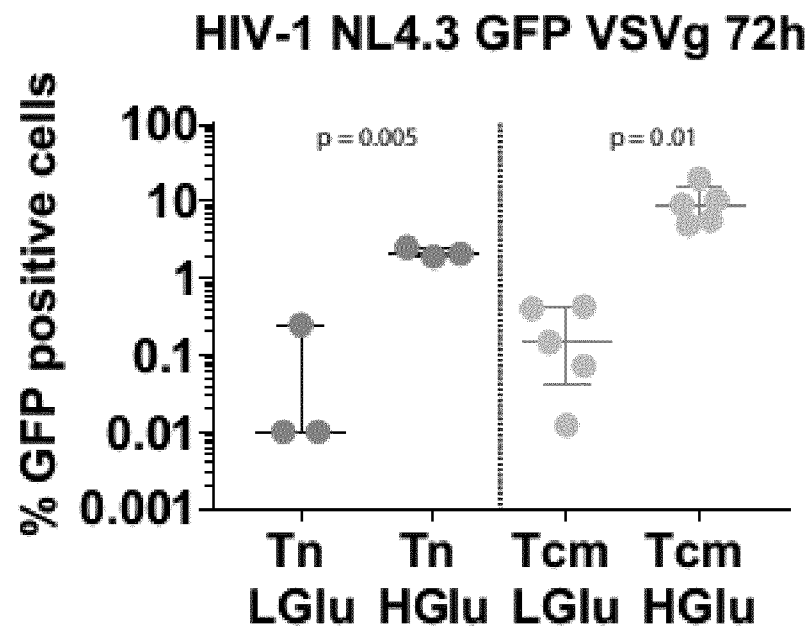


Figure 6

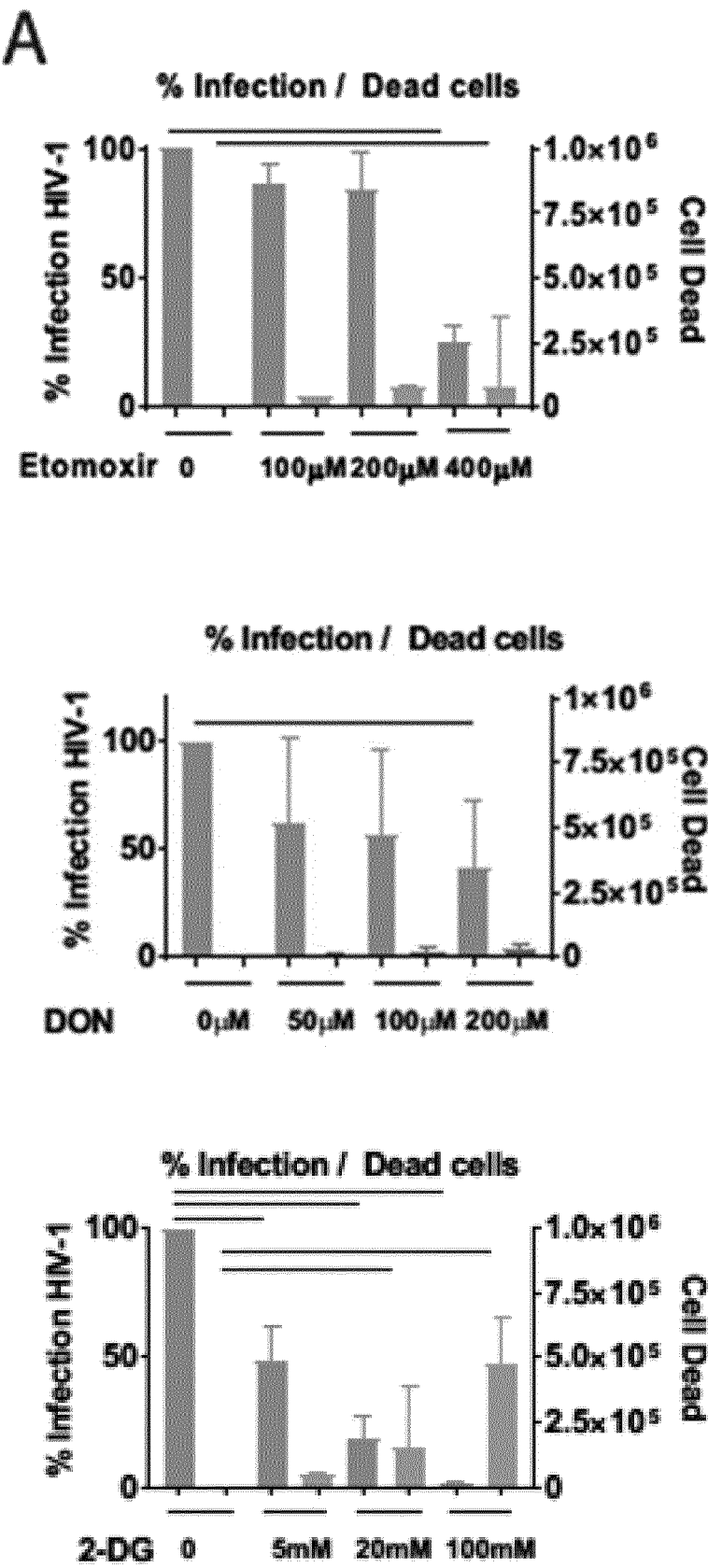


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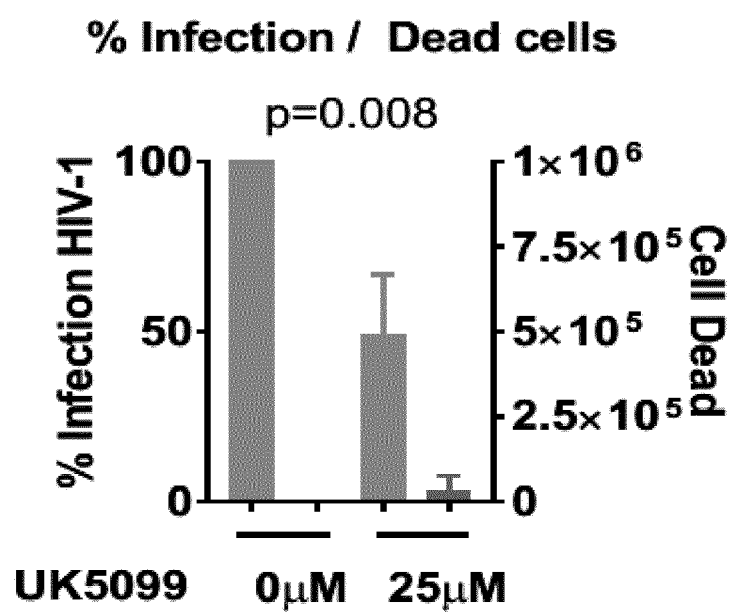
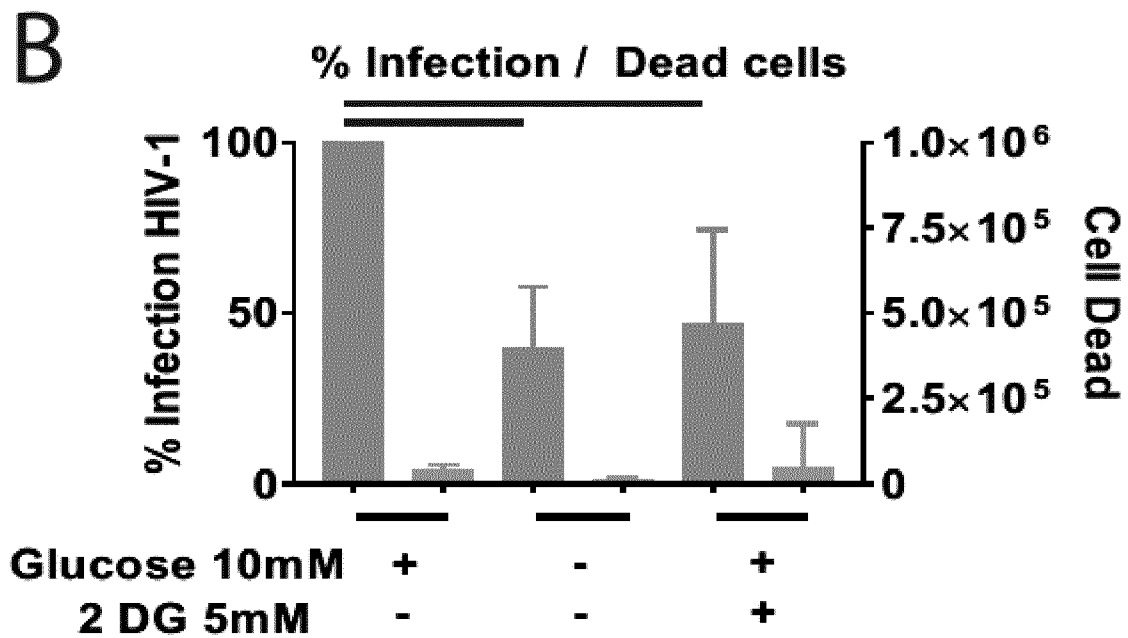


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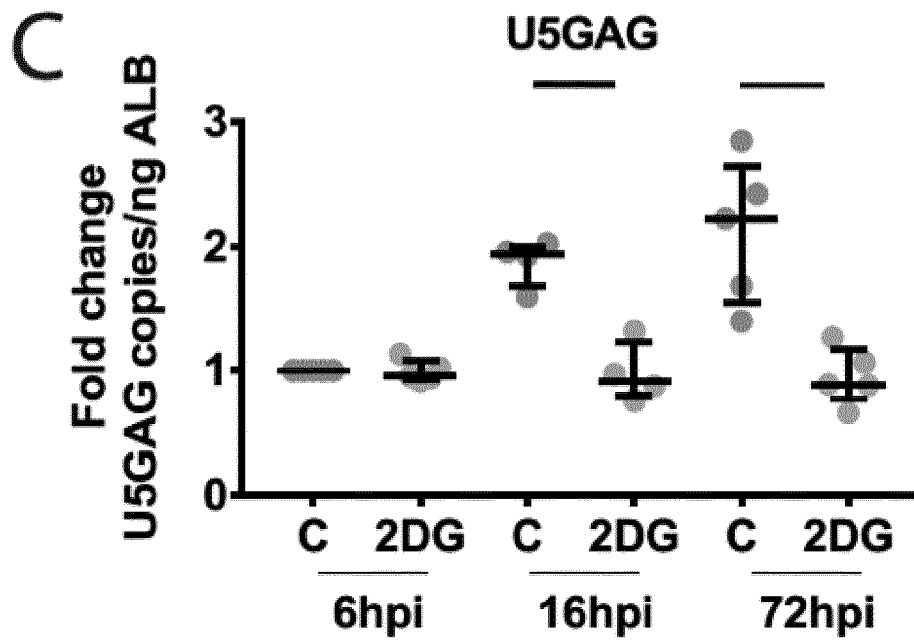


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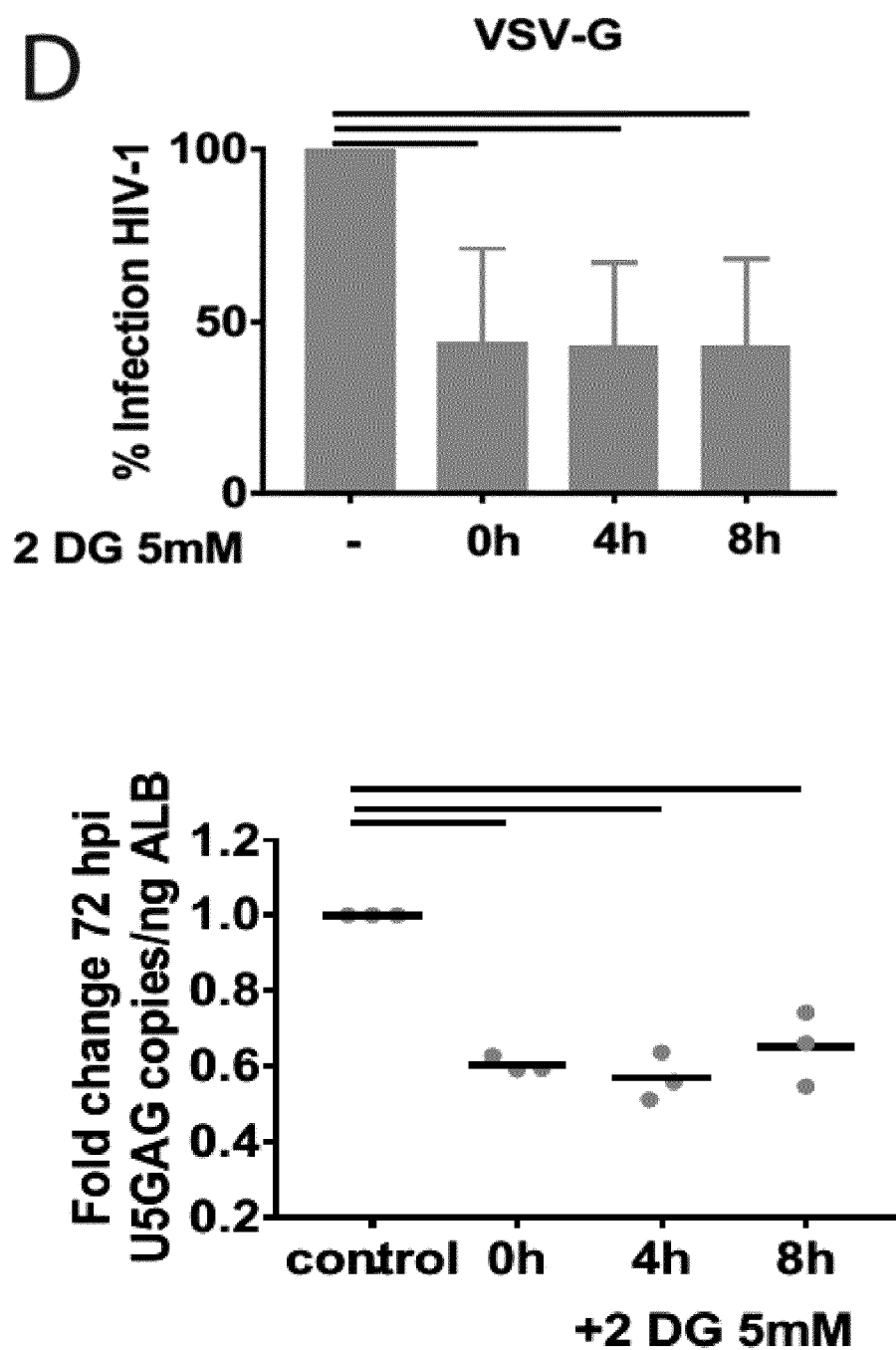
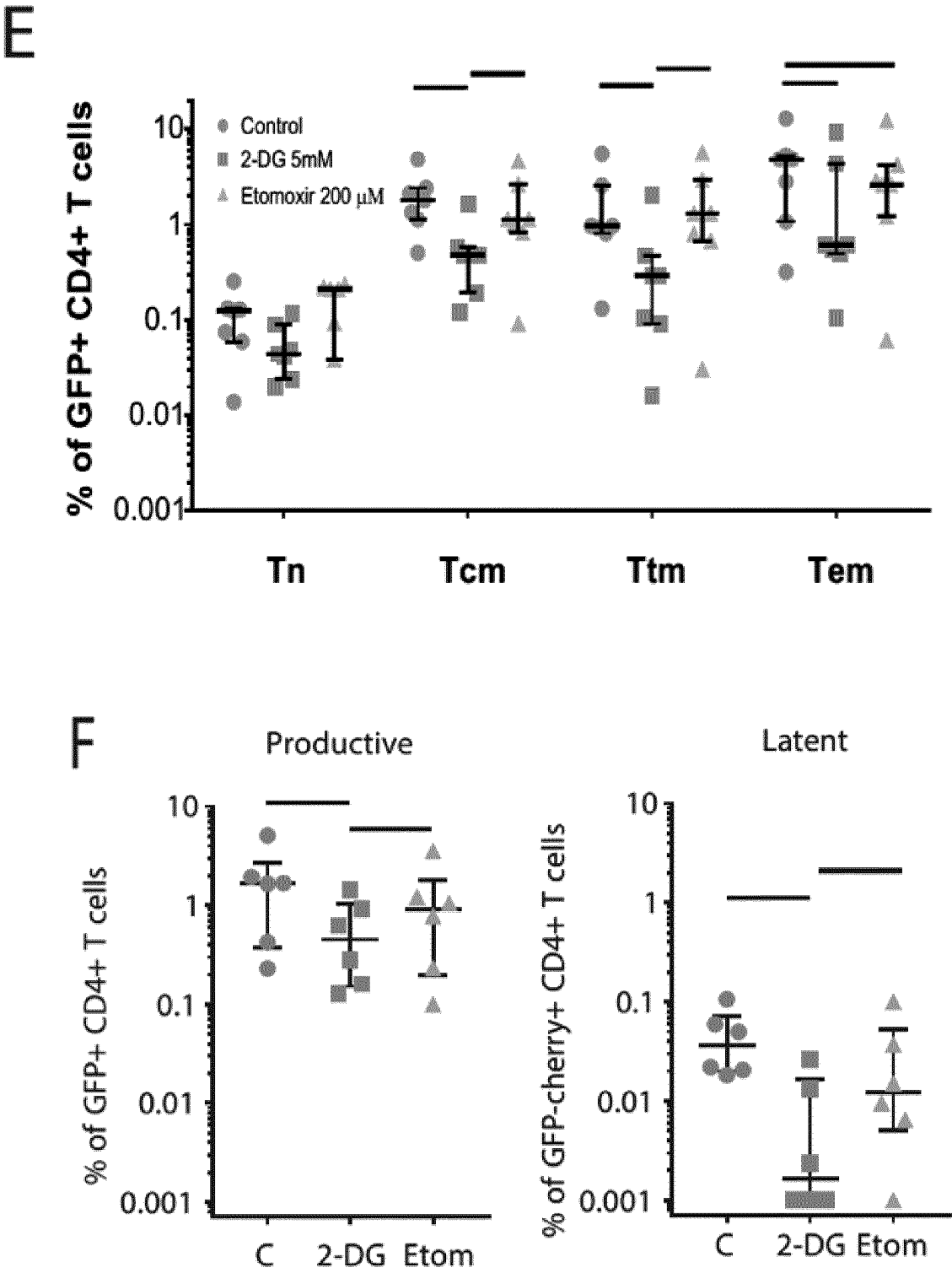


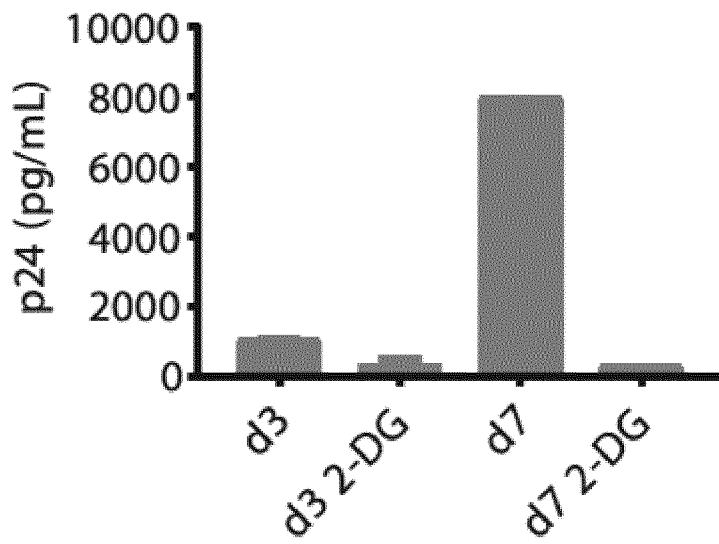
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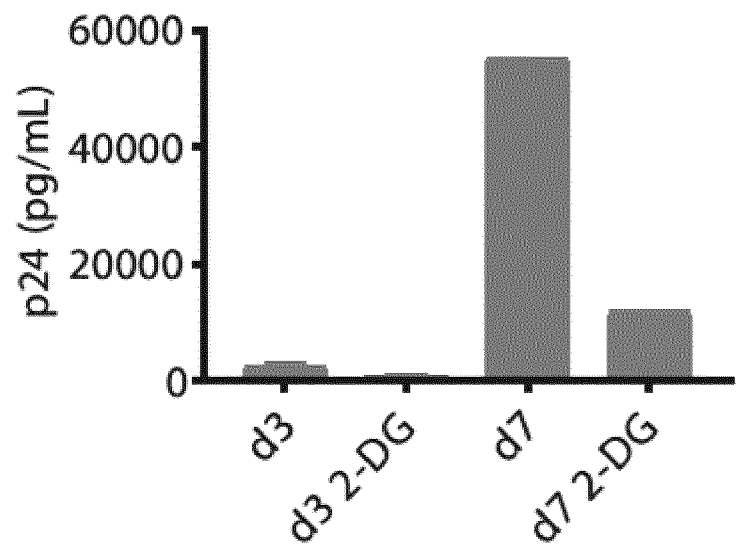
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EFS 543

Figure 6 (continuation)



EFS 560



EFS 559

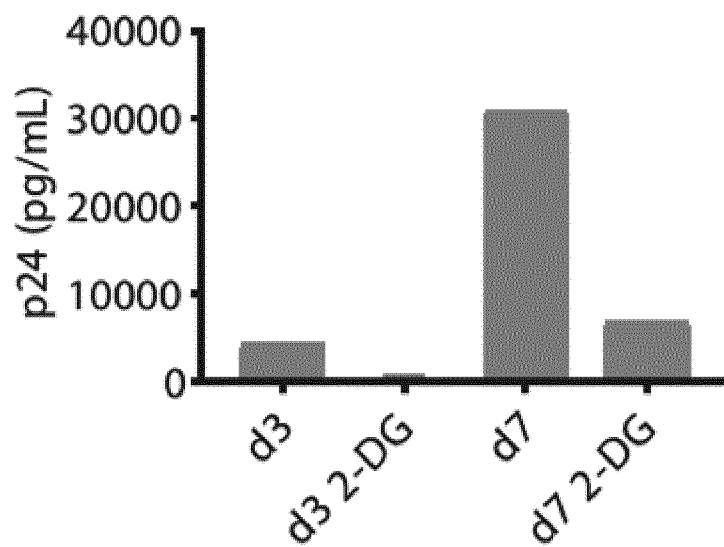


Figure 7

A

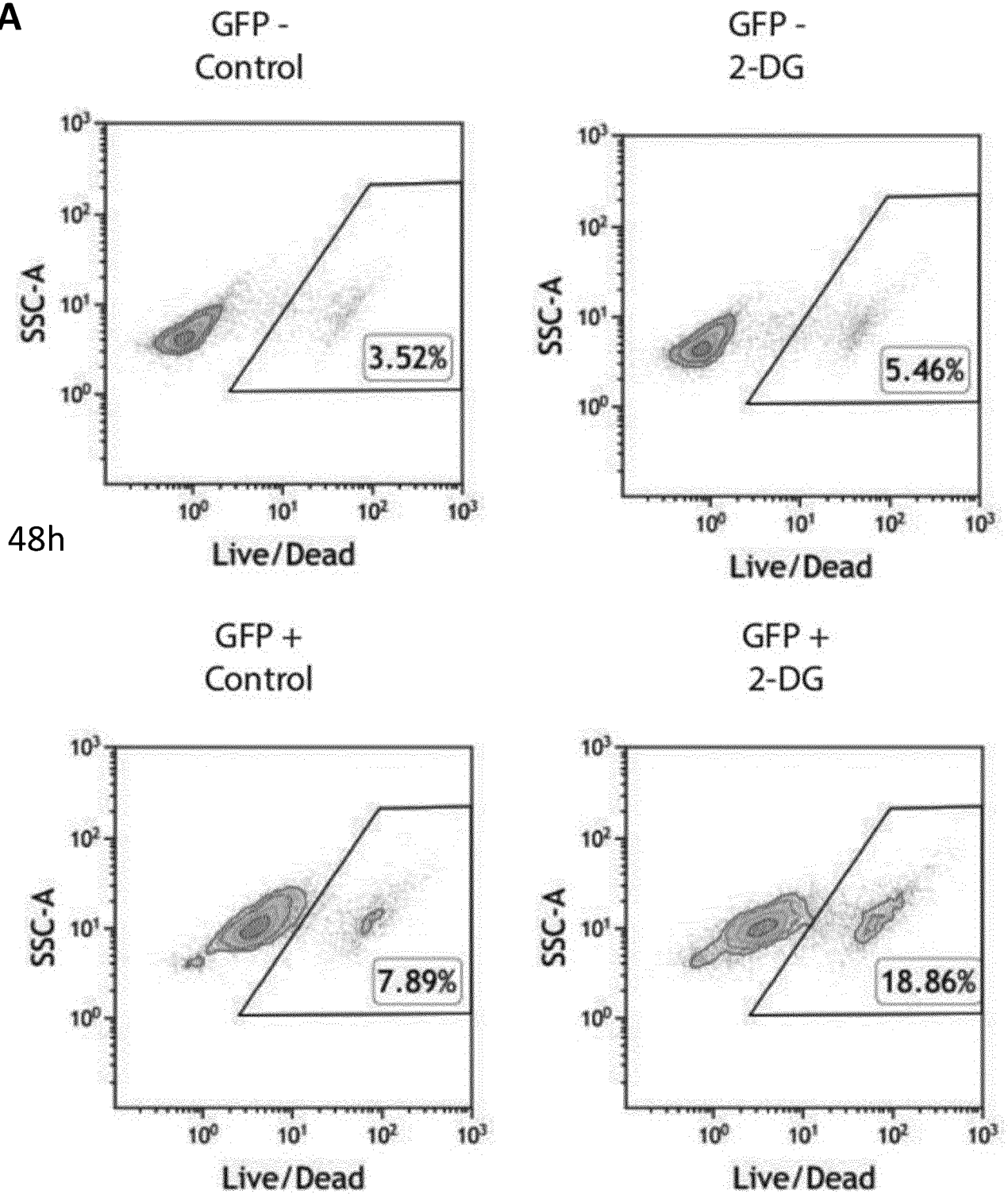
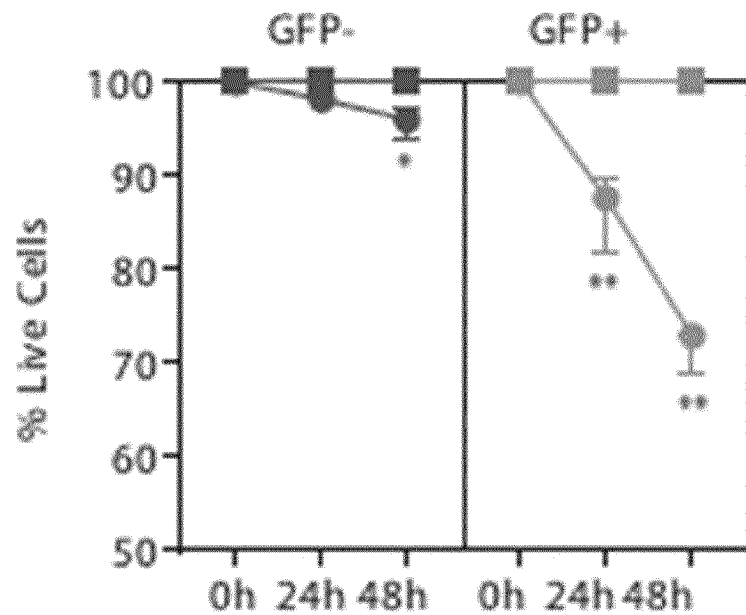


Figure 7 (continuation)

B



C

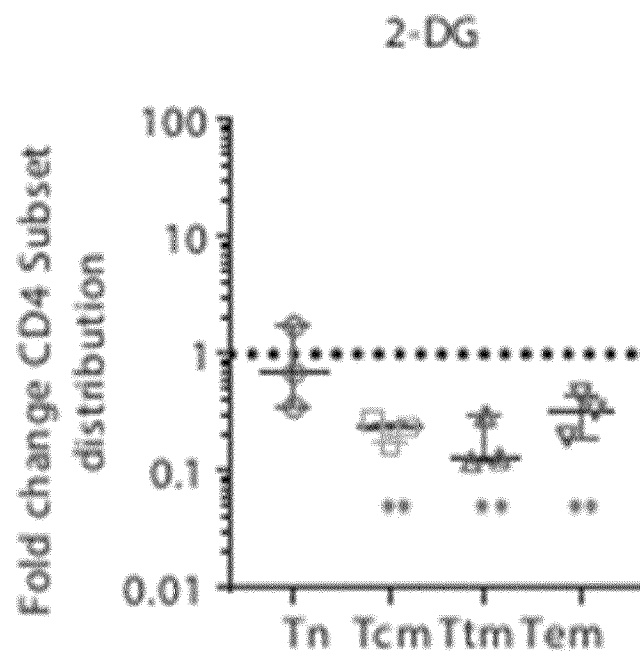


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D

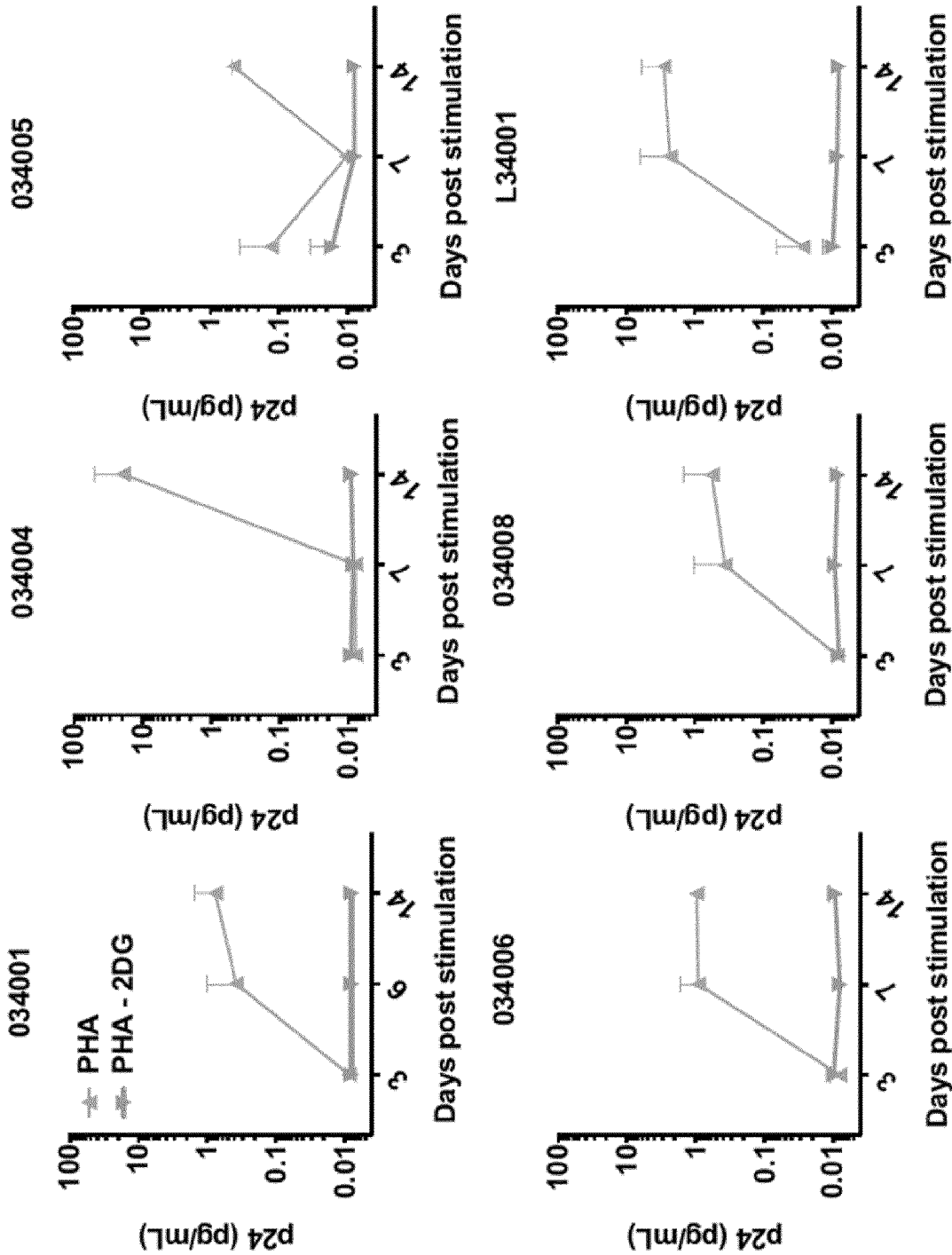


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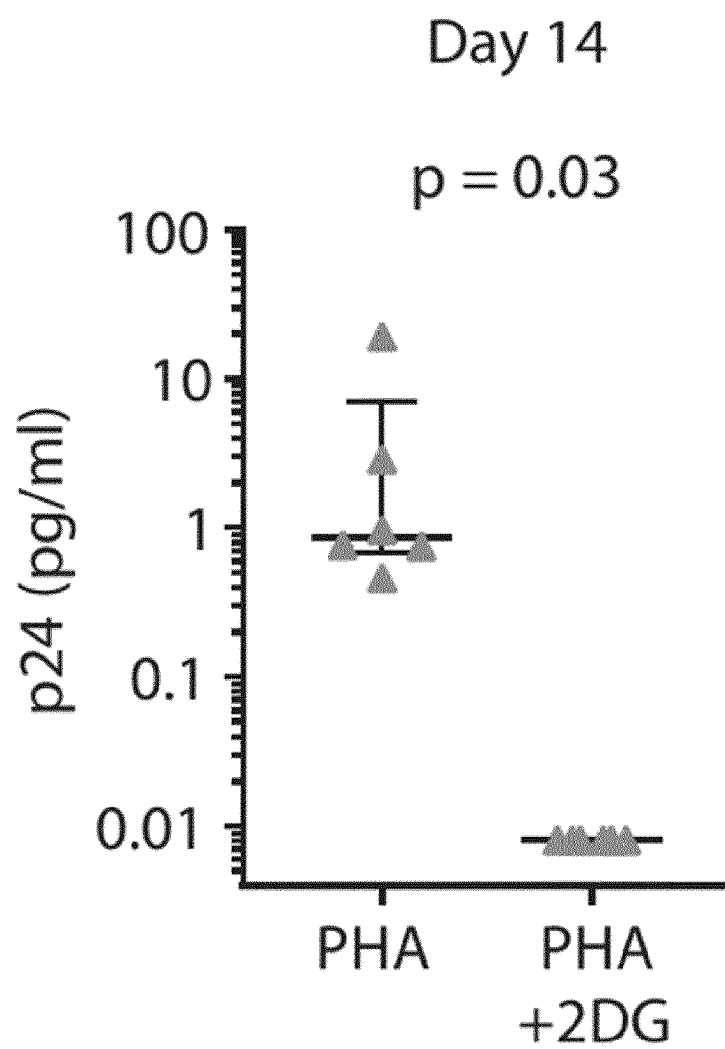


Figure 8

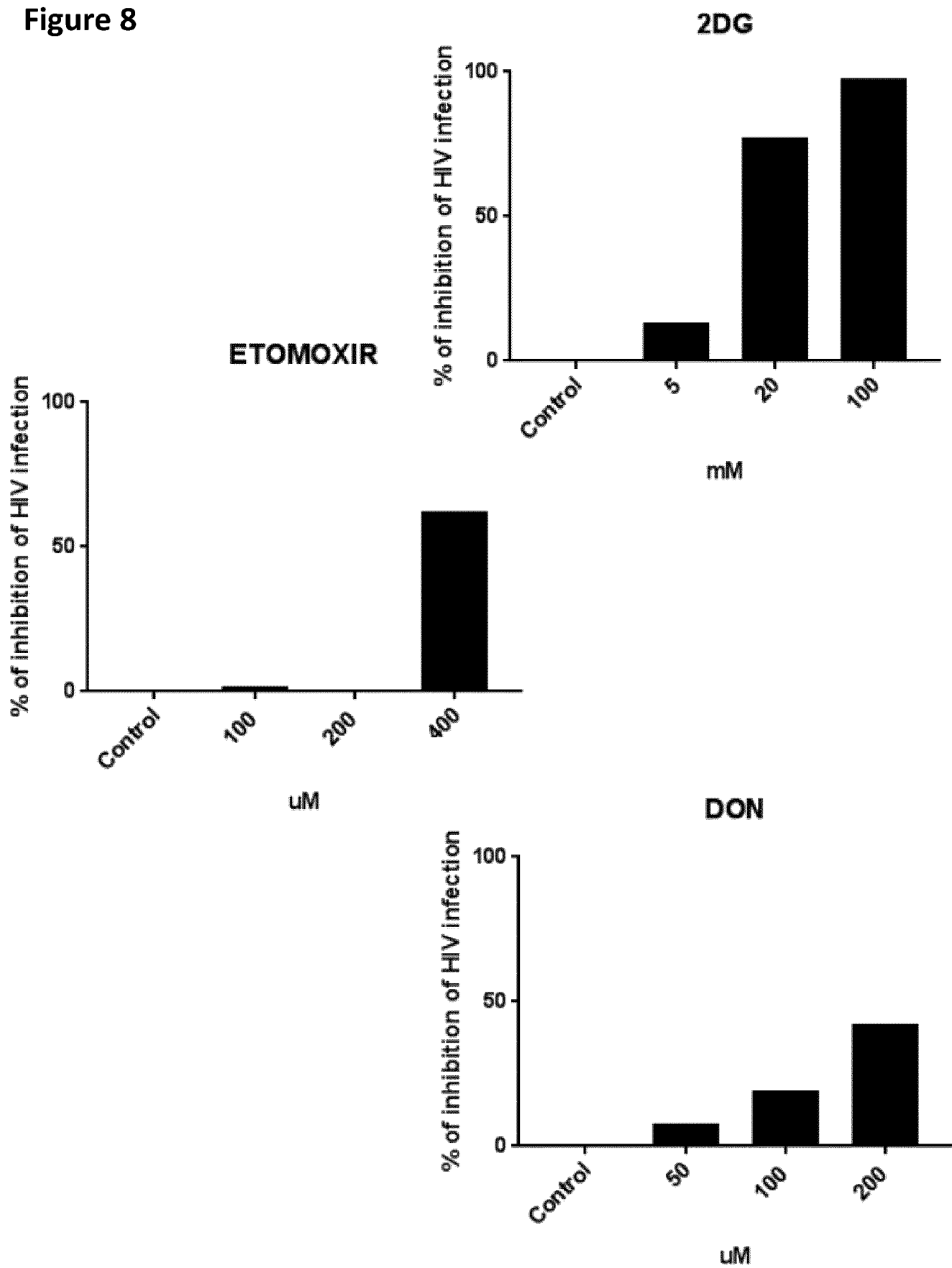


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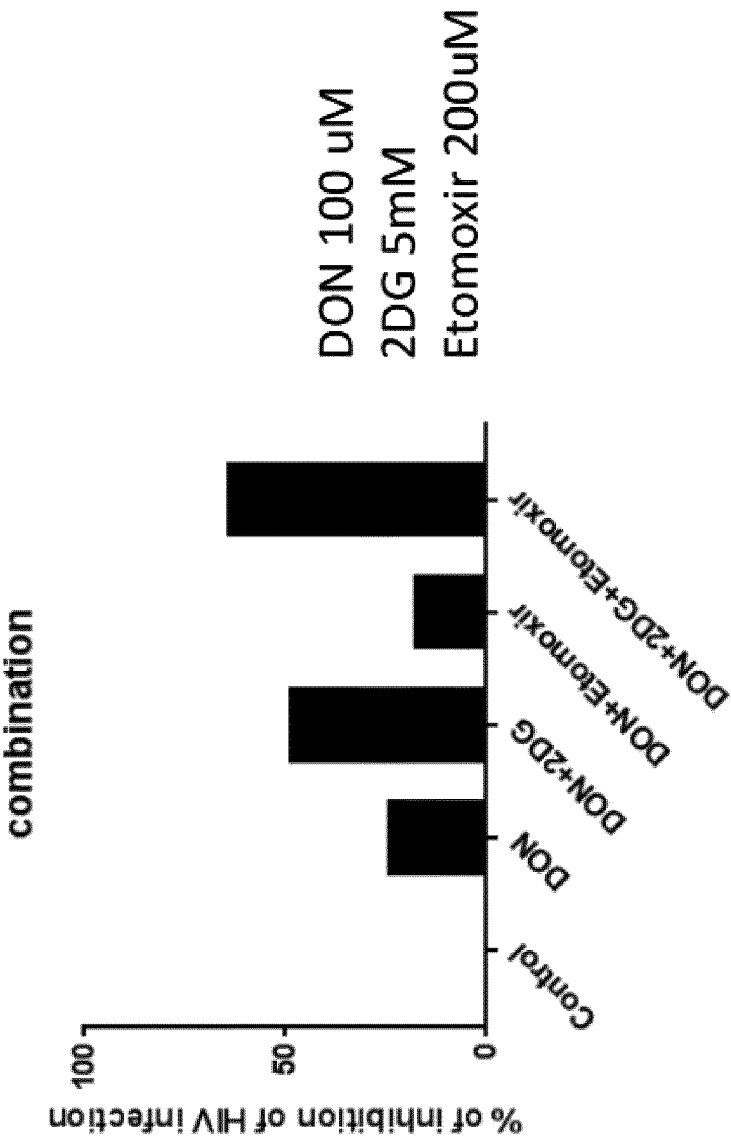


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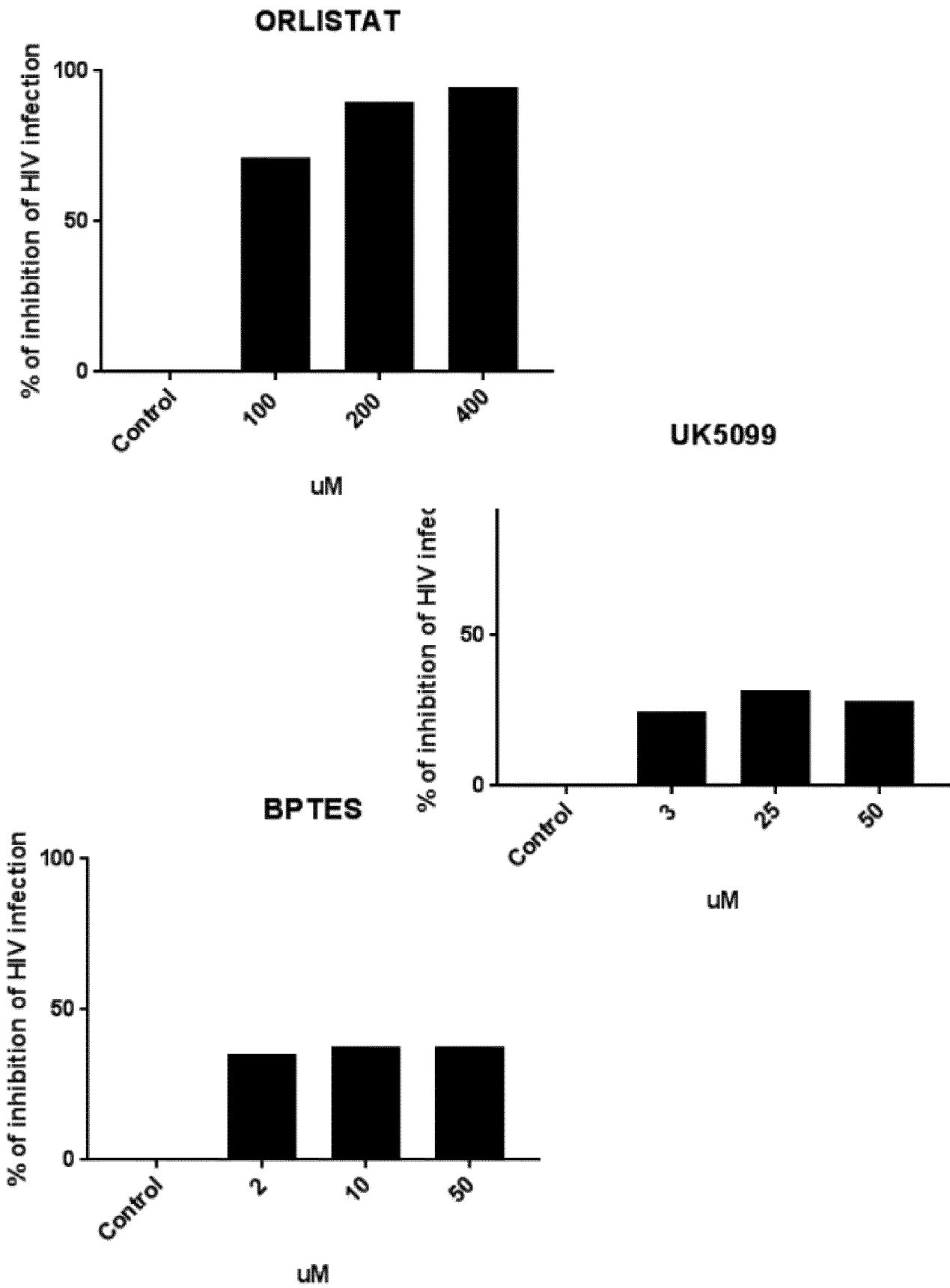


Figure 9

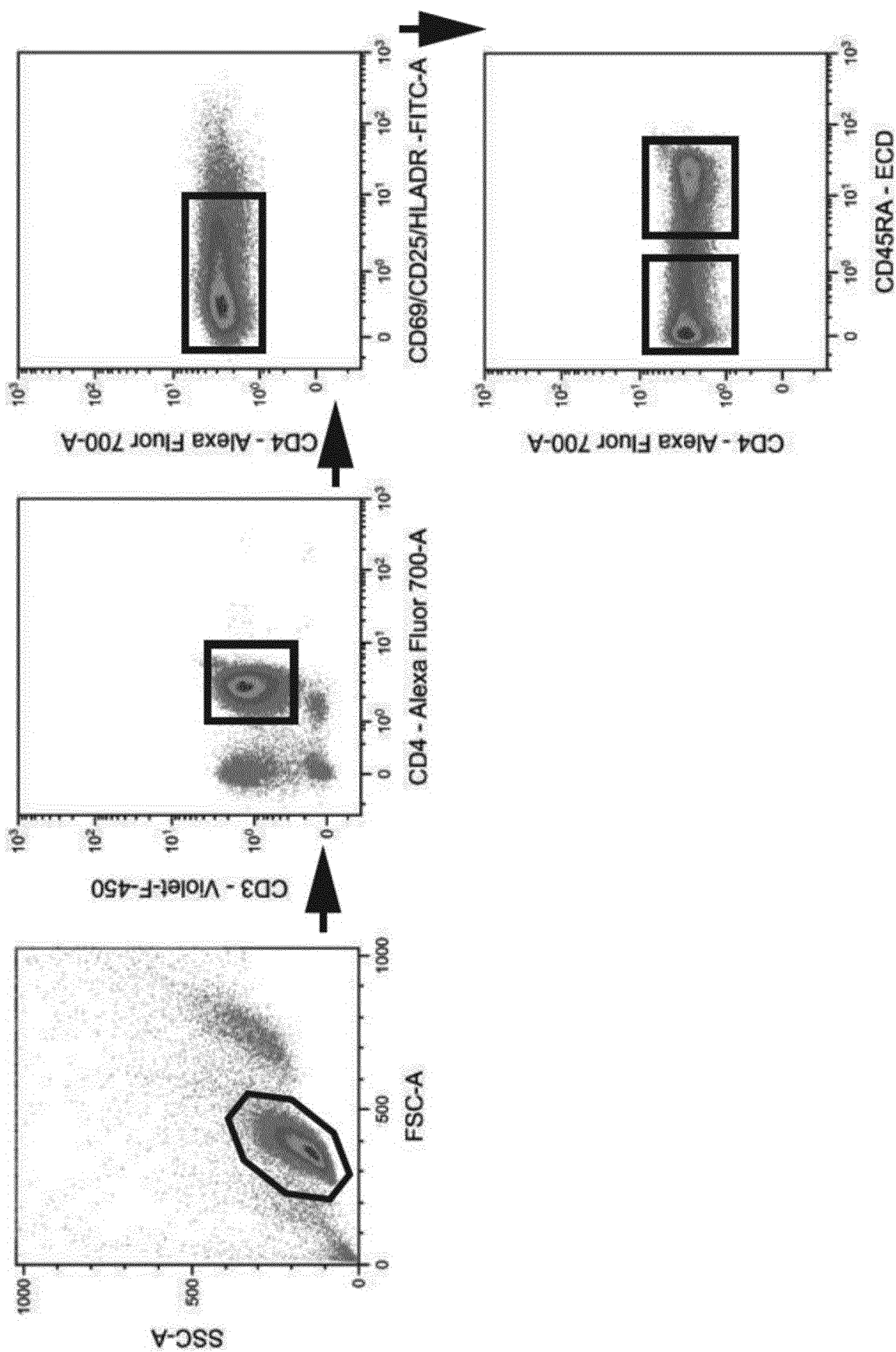
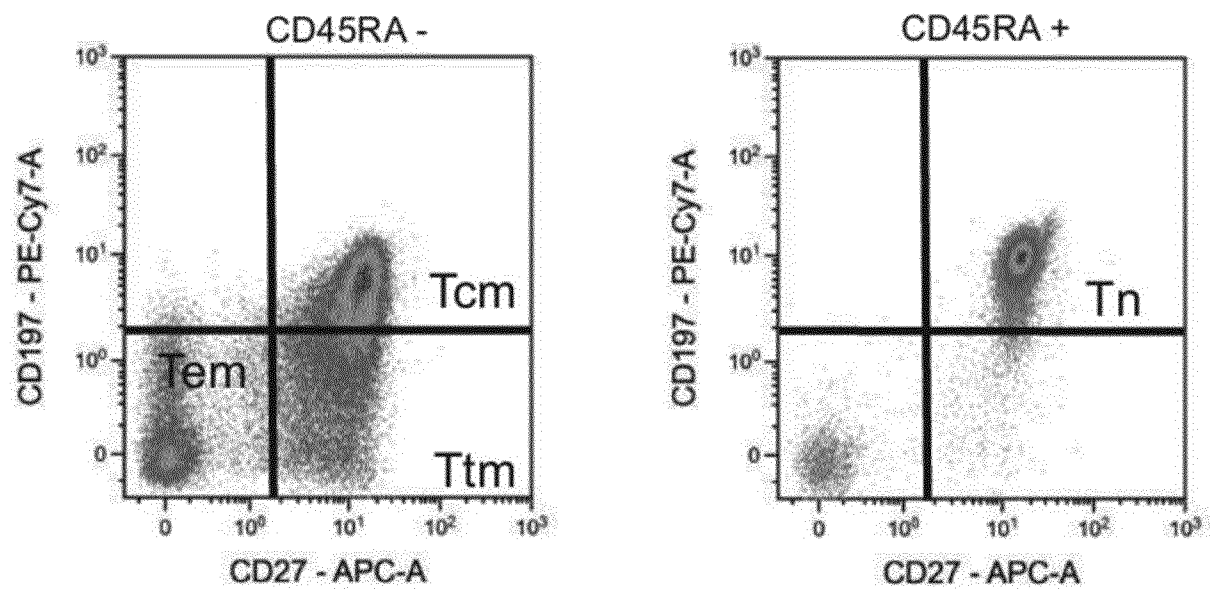


Figure 9 (continuation)



EFSM	459	466	469	470	471	472
Gender	Female	Male	Female	Female	Male	Male
Age (years)	24	18	28	51	52	36
% Tn	41.7	50.7	37	41	43.5	15.5
% Tcm	21.8	17.2	20	24.1	18.9	30.2
% Ttm	15.9	16.8	18.3	14.7	13.4	28.5
% Tem	6.89	3.04	8.11	4.79	3.42	12.9
% Others	13.71	12.26	16.59	15.41	20.78	12.9

Figure 10

NA

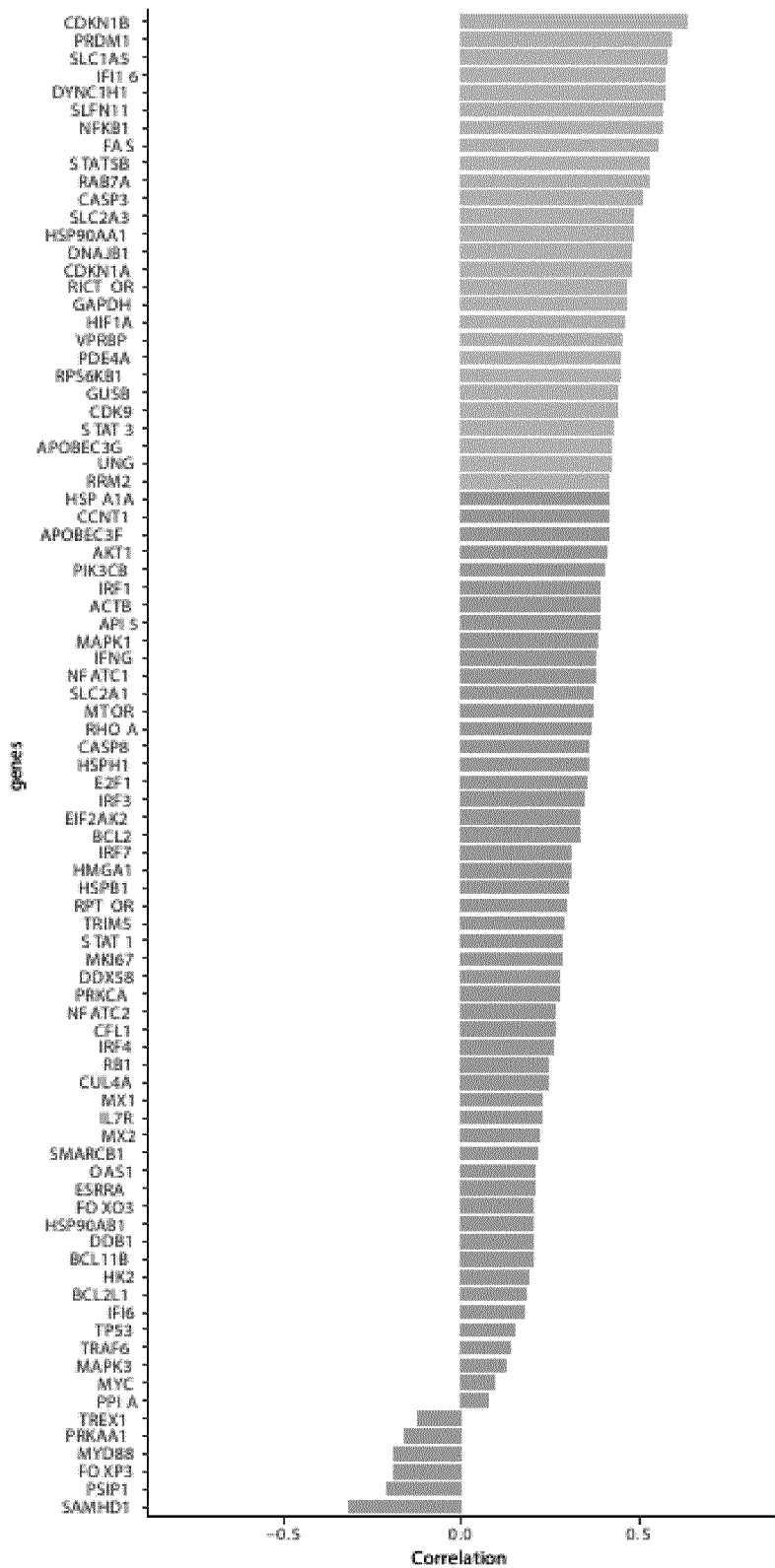


Figure 10
(continuation)

3d

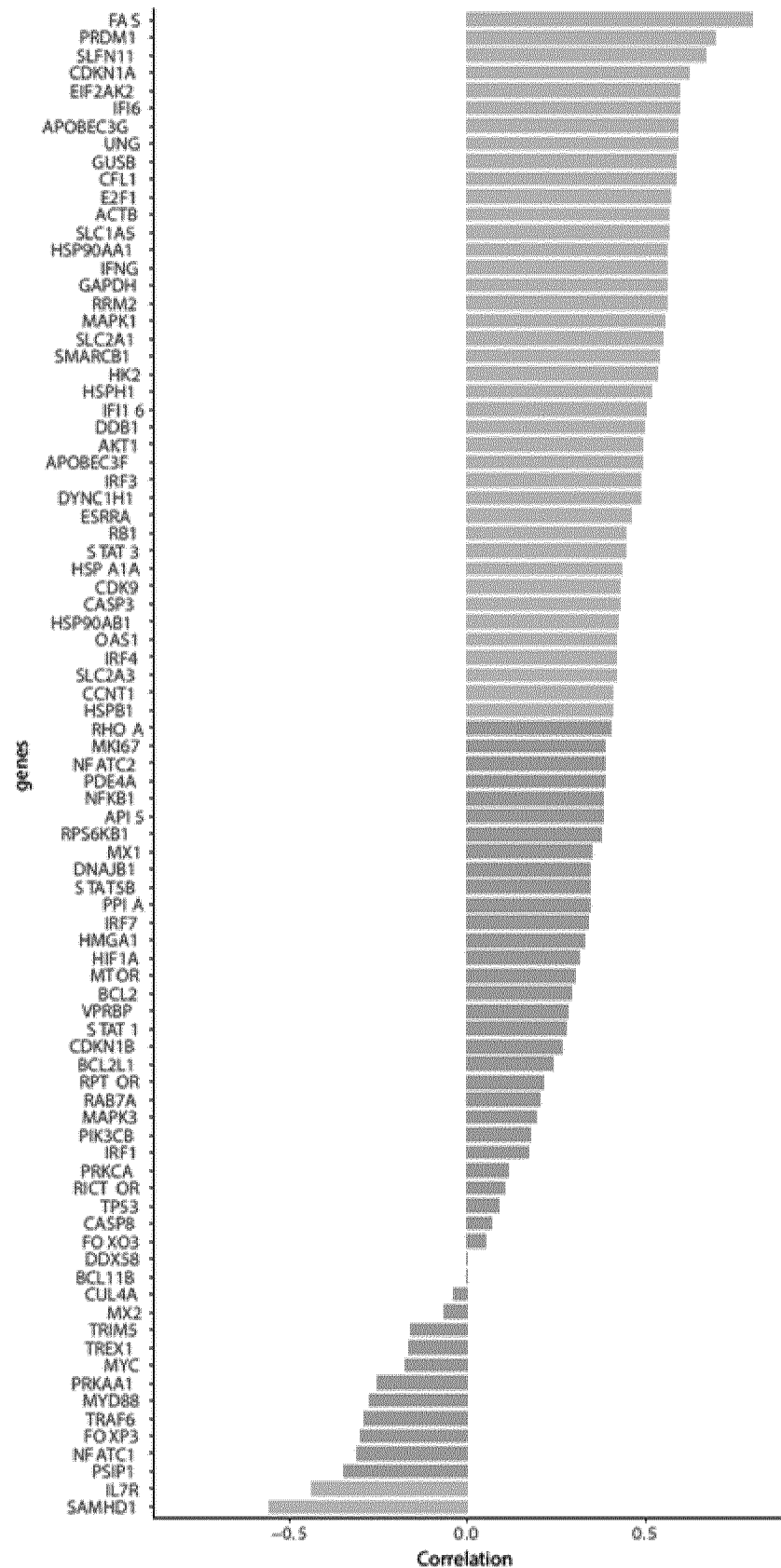


Figure 10
(continuation)

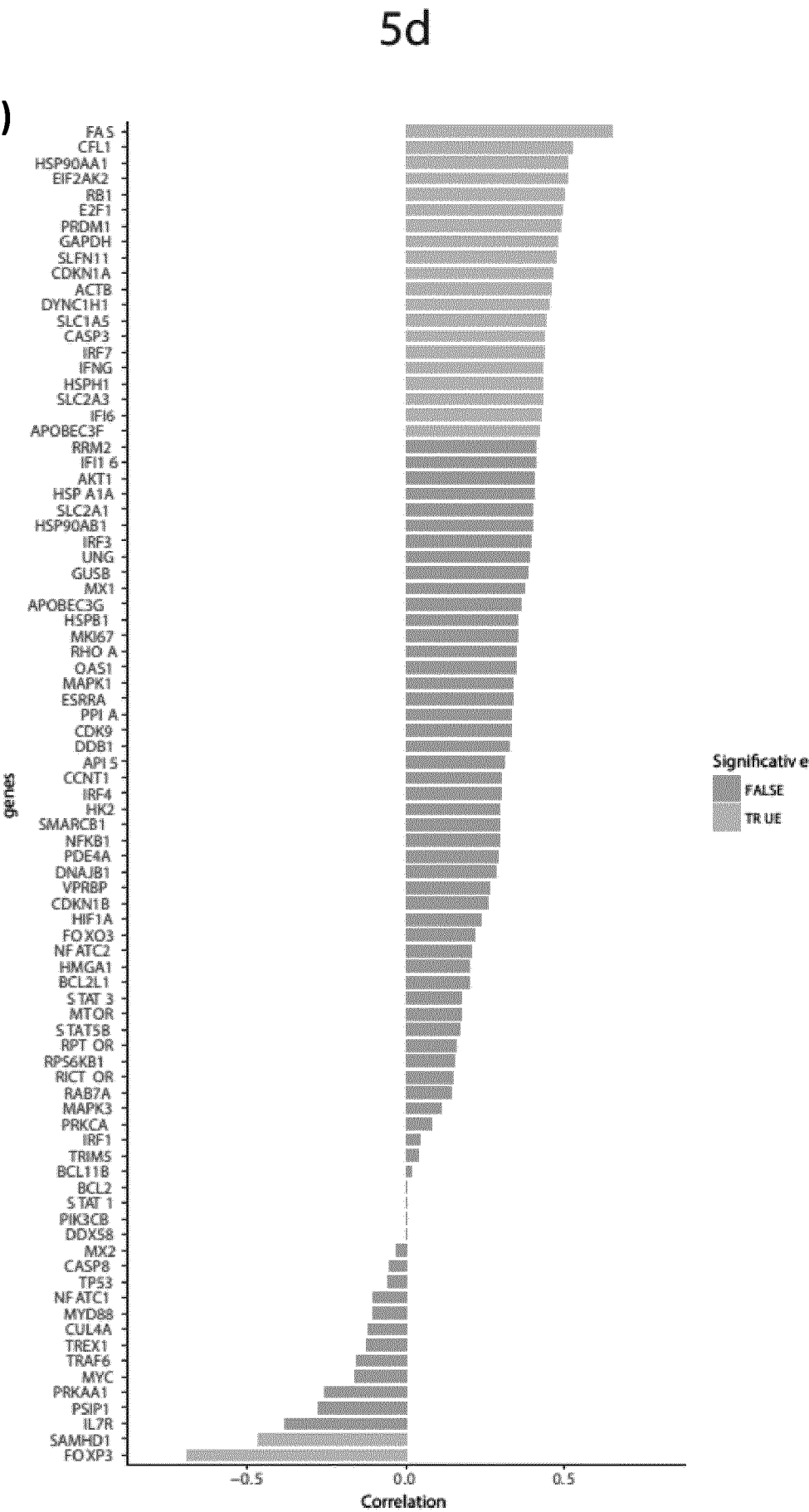


Figure 10
(continuation)

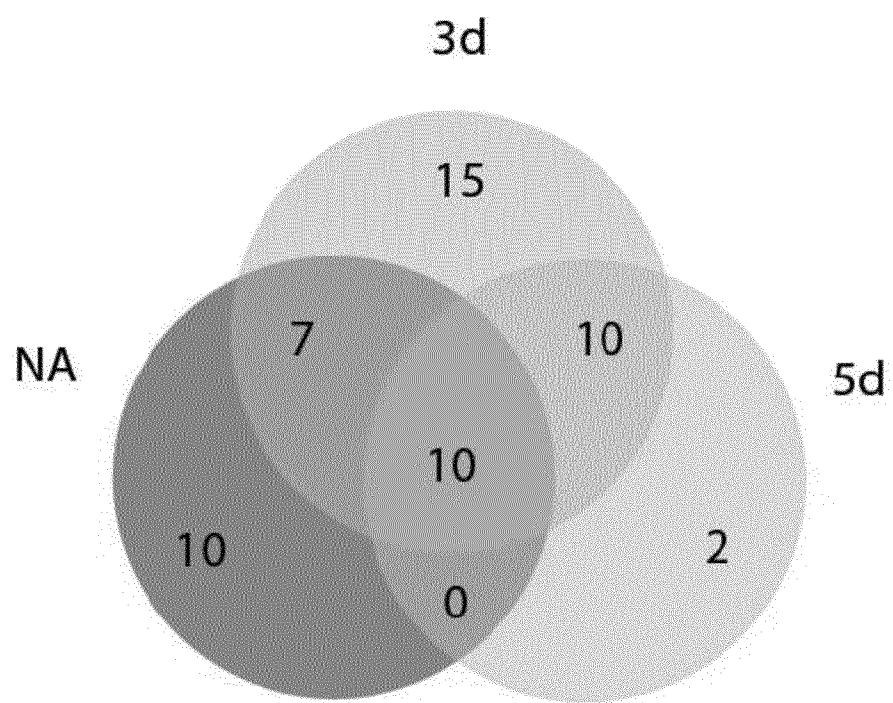


Figure 10
(continuation)

NA	3d	5d	NA & 3d	3d & 5d	NA & 5d	NA & 3d & 5d
RRM2	SAMHD1	FOXP3	RRM2	SAMHD1	GAPDH	GAPDH
UNG	IL7R	SAMHD1	UNG	SLC2A3	CDKN1A	CDKN1A
APOBEC3G	HSPB1	APOBEC3F	APOBEC3G	CASP3	HSP90AA1	HSP90AA1
STAT3	CCNT1	IFI6	STAT3	RB1	SLC2A3	SLC2A3
CDK9	SLC2A3	SLC2A3	CDK9	DYNC1H1	CASP3	CASP3
GUSB	IRF4	HSPH1	GUSB	APOBEC3F	FAS	FAS
RPS6KB1	OAS1	IFNG	GAPDH	HSPH1	SLFN11	SLFN11
PDE4A	HSP90AB1	IRF7	CDKN1A	GAPDH	DYNC1H1	DYNC1H1
VPRBP	CASP3	CASP3	HSP90AA1	IFNG	SLC1A5	SLC1A5
HIF1A	CDK9	SLC1A5	SLC2A3	HSP90AA1	PRDM1	PRDM1
GAPDH	HSPA1A	DYNC1H1	CASP3	SLC1A5		
RICTOR	STAT3	ACTB	FAS	ACTB		
CDKN1A	RB1	CDKN1A	SLFN11	E2F1		
DJB1	ESRRA	SLFN11	DYNC1H1	CFL1		
HSP90AA1	DYNC1H1	GAPDH	IFI16	IFI6		
SLC2A3	IRF3	PRDM1	SLC1A5	EIF2AK2		
CASP3	APOBEC3F	E2F1	PRDM1	CDKN1A		
RAB7A	AKT1	RB1		SLFN11		
STAT5B	DDIT1	EIF2AK2		PRDM1		
FAS	IFI16	HSP90AA1		FAS		
NFKB1	HSPH1	CFL1				
SLFN11	HK2	FAS				
DYNC1H1	SMARCB1					
IFI16	SLC2A1					
SLC1A5	MAPK1					
PRDM1	RRM2					
CDKN1B	GAPDH					
	IFNG					
	HSP90AA1					
	SLC1A5					
	ACTB					
	E2F1					
	CFL1					
	GUSB					
	UNG					
	APOBEC3G					
	IFI6					
	EIF2AK2					
	CDKN1A					
	SLFN11					
	PRDM1					
	FAS					
27	42	22	17	20	10	10

Figure 11

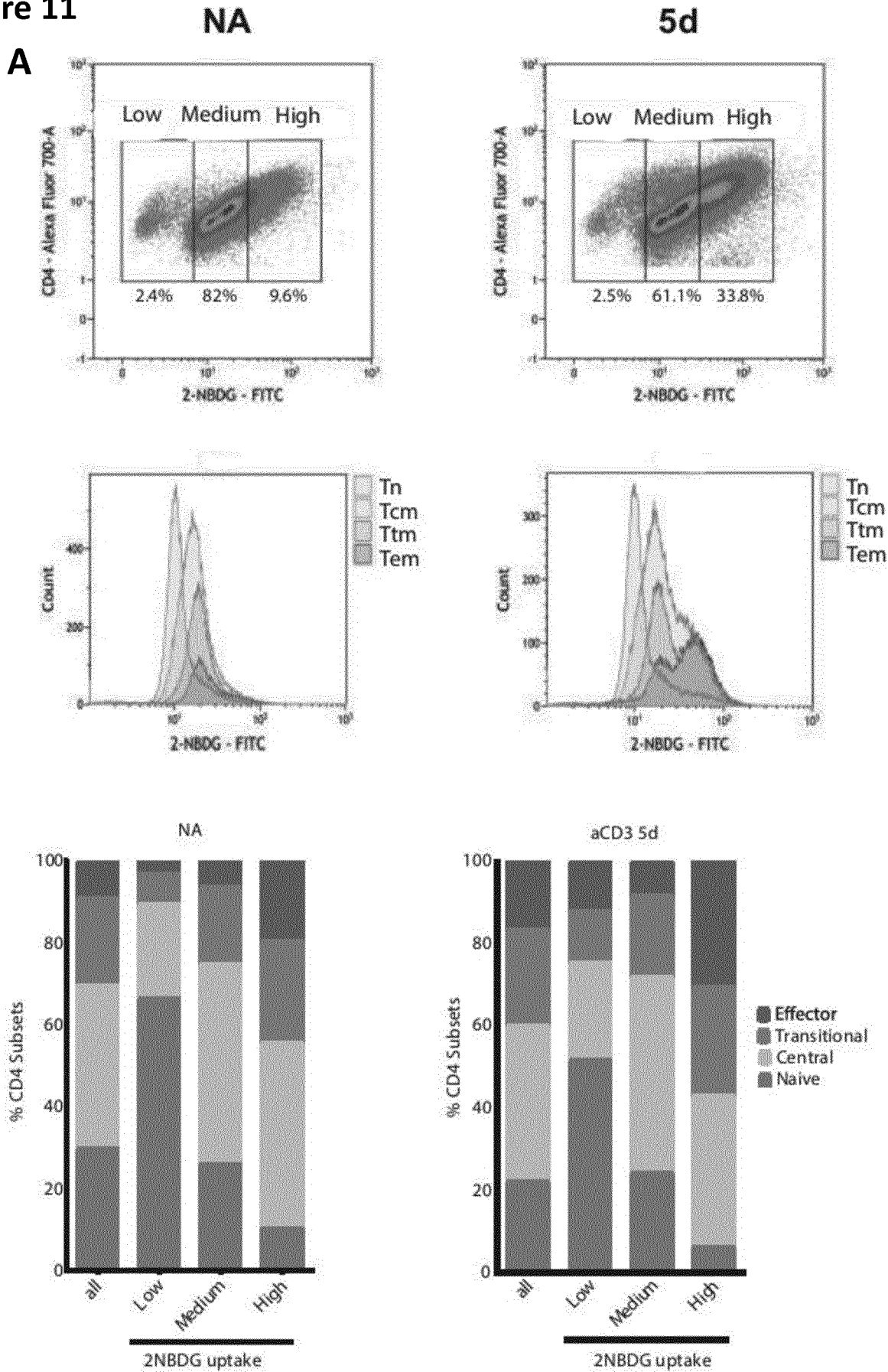
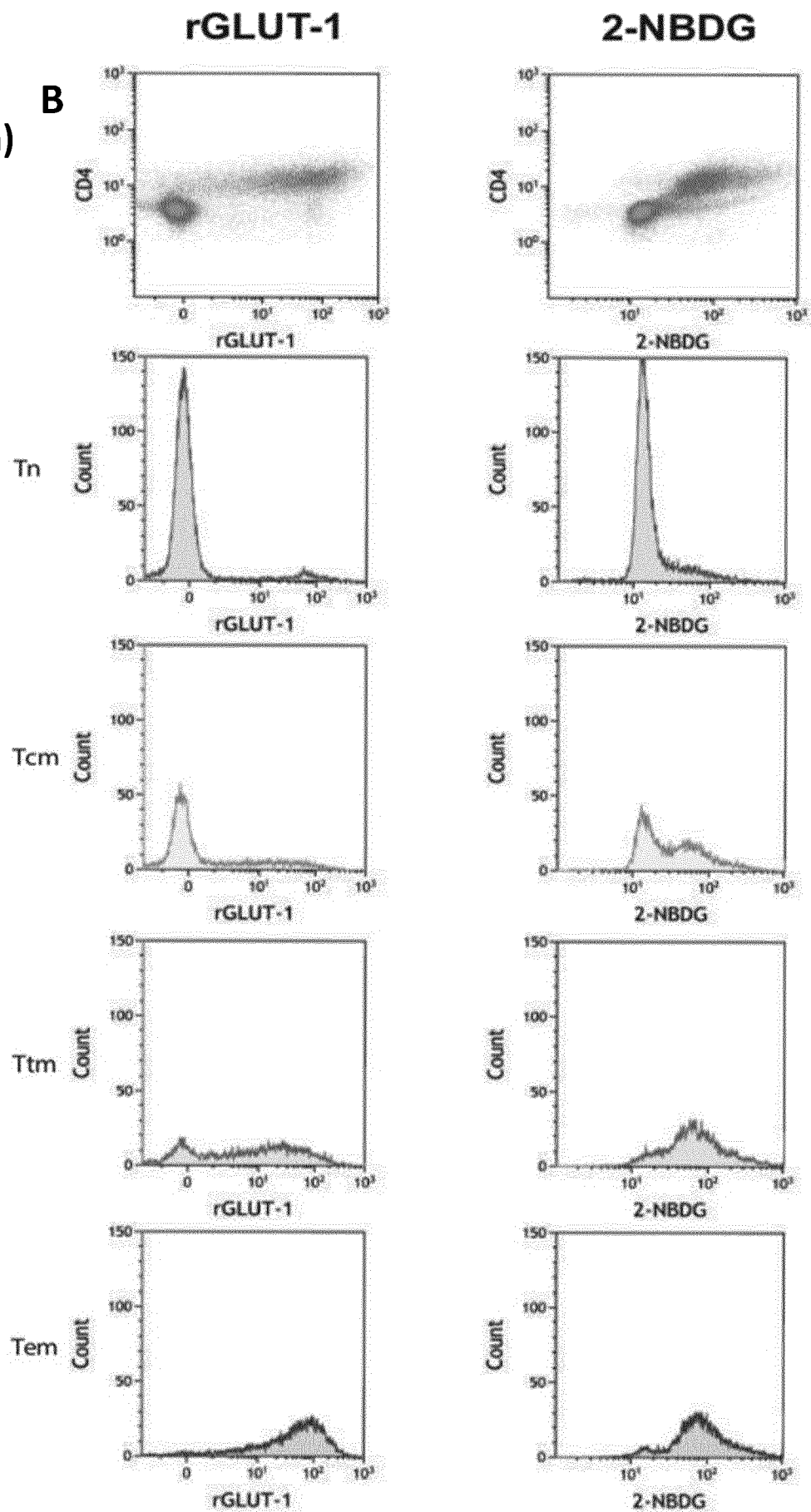


Figure 11
(continuation)



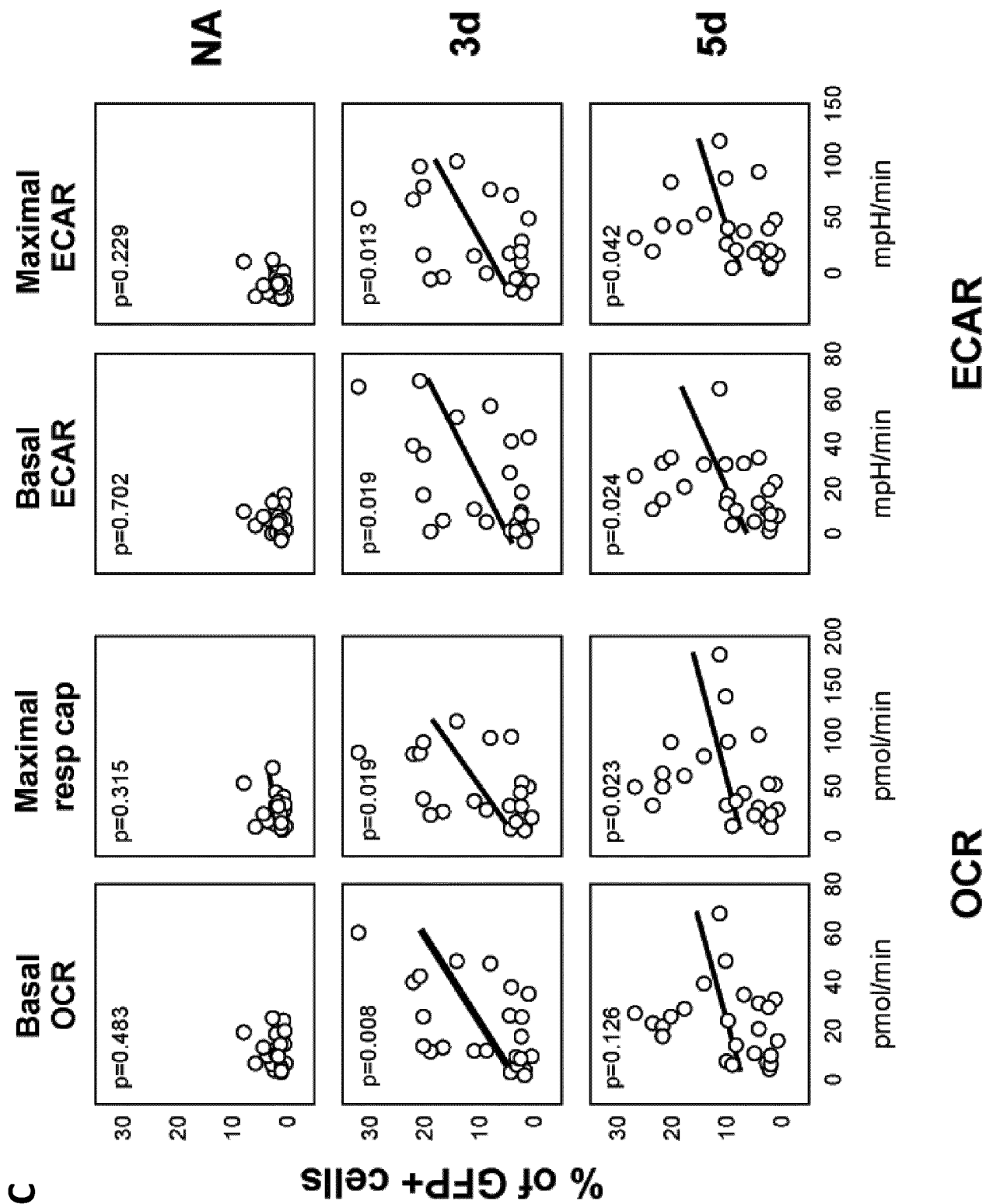


Figure 12

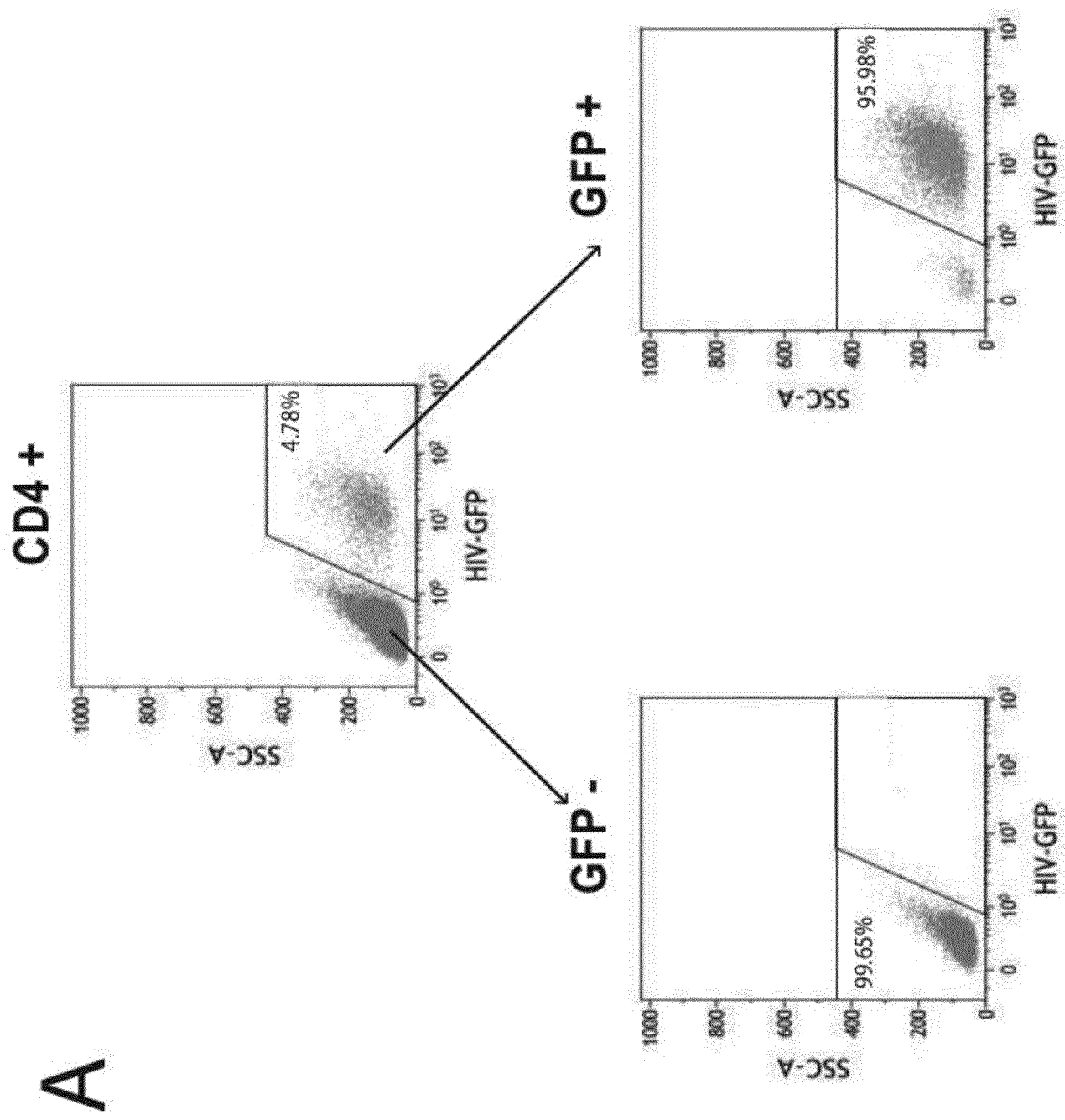


Figure 12
(continuation)

A

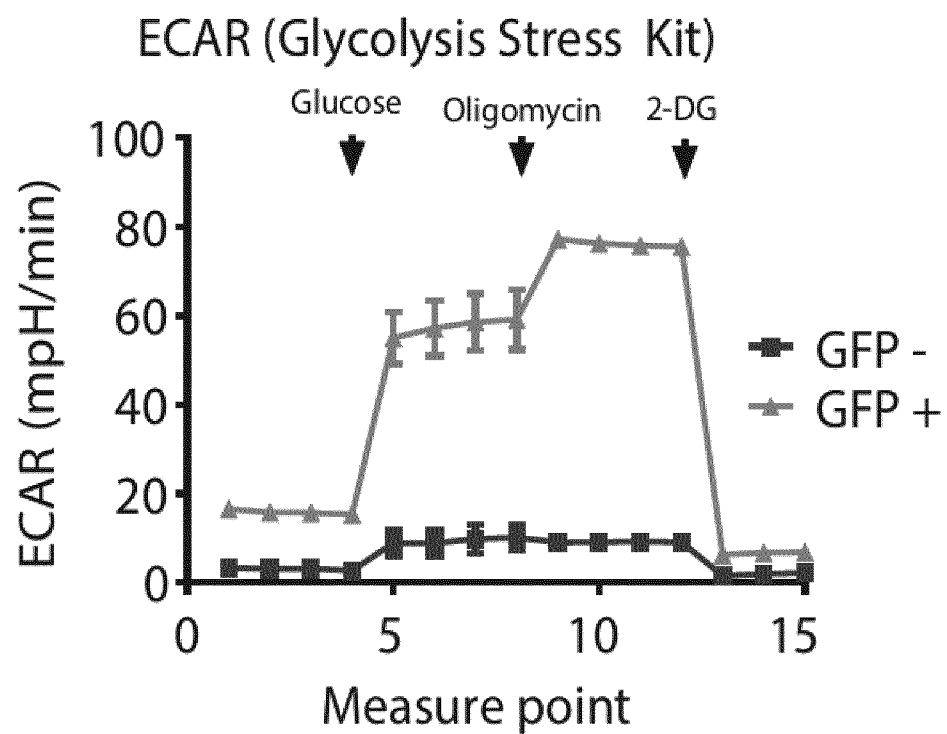


Figure 12
(continuation)

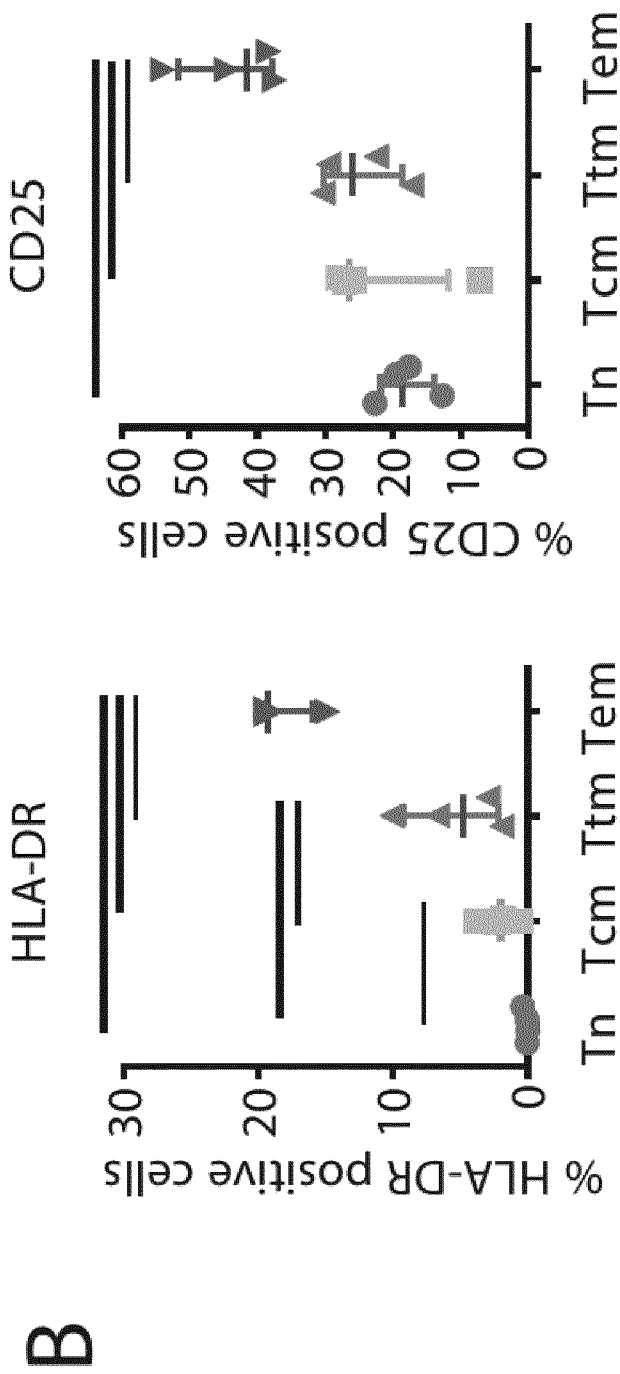


Figure 12
(continuation)

C

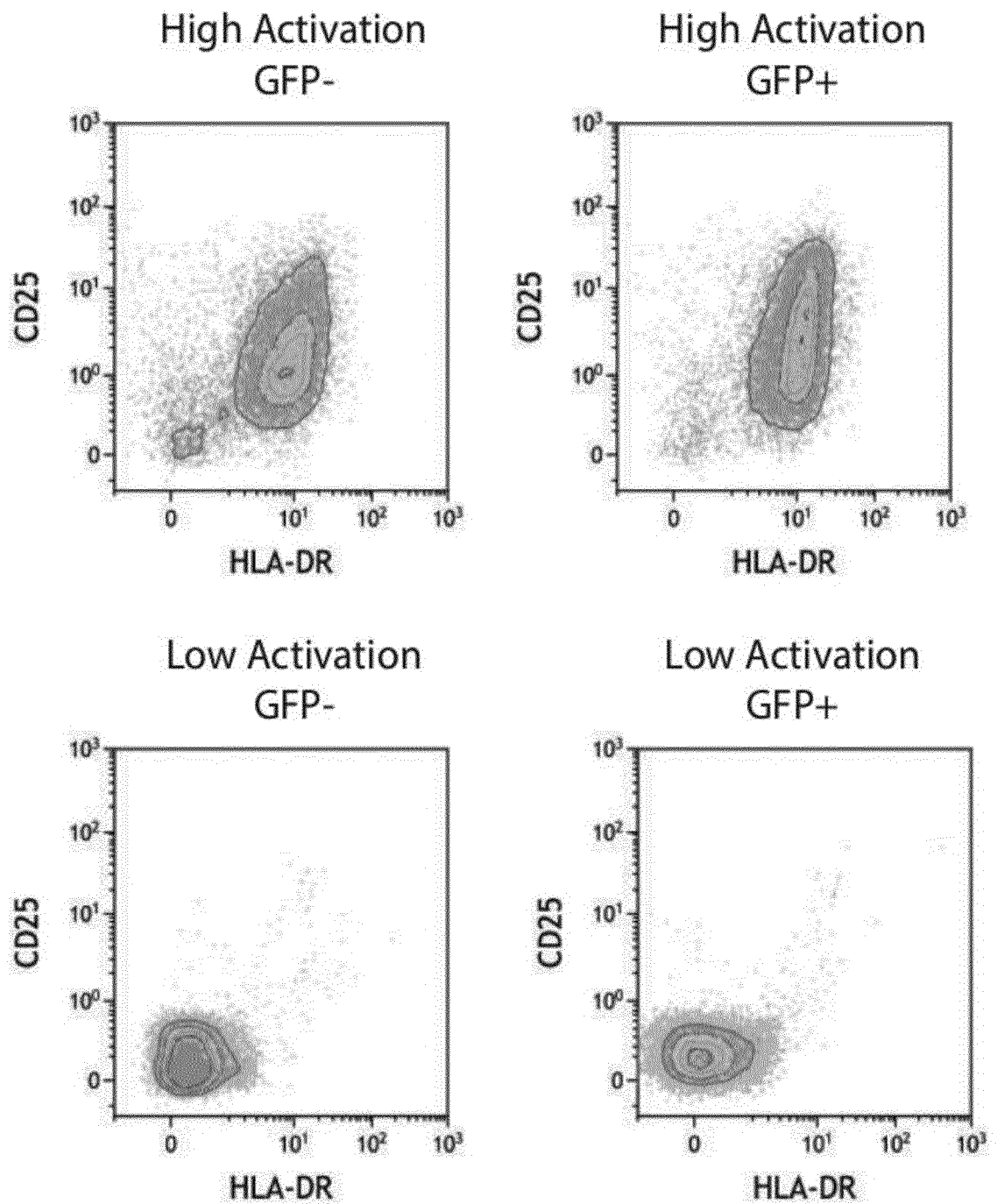


Figure 12
(continuation)

D

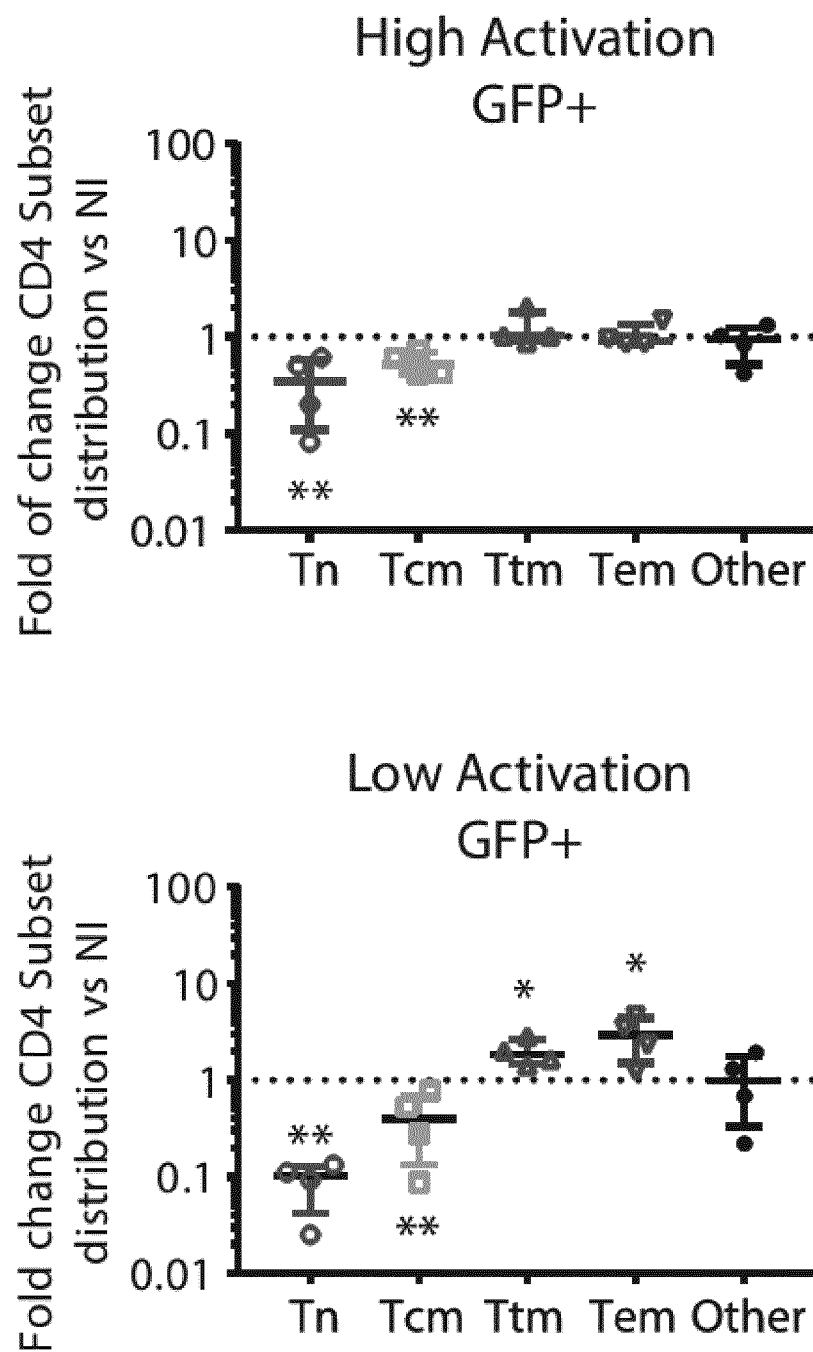


Figure 13

A

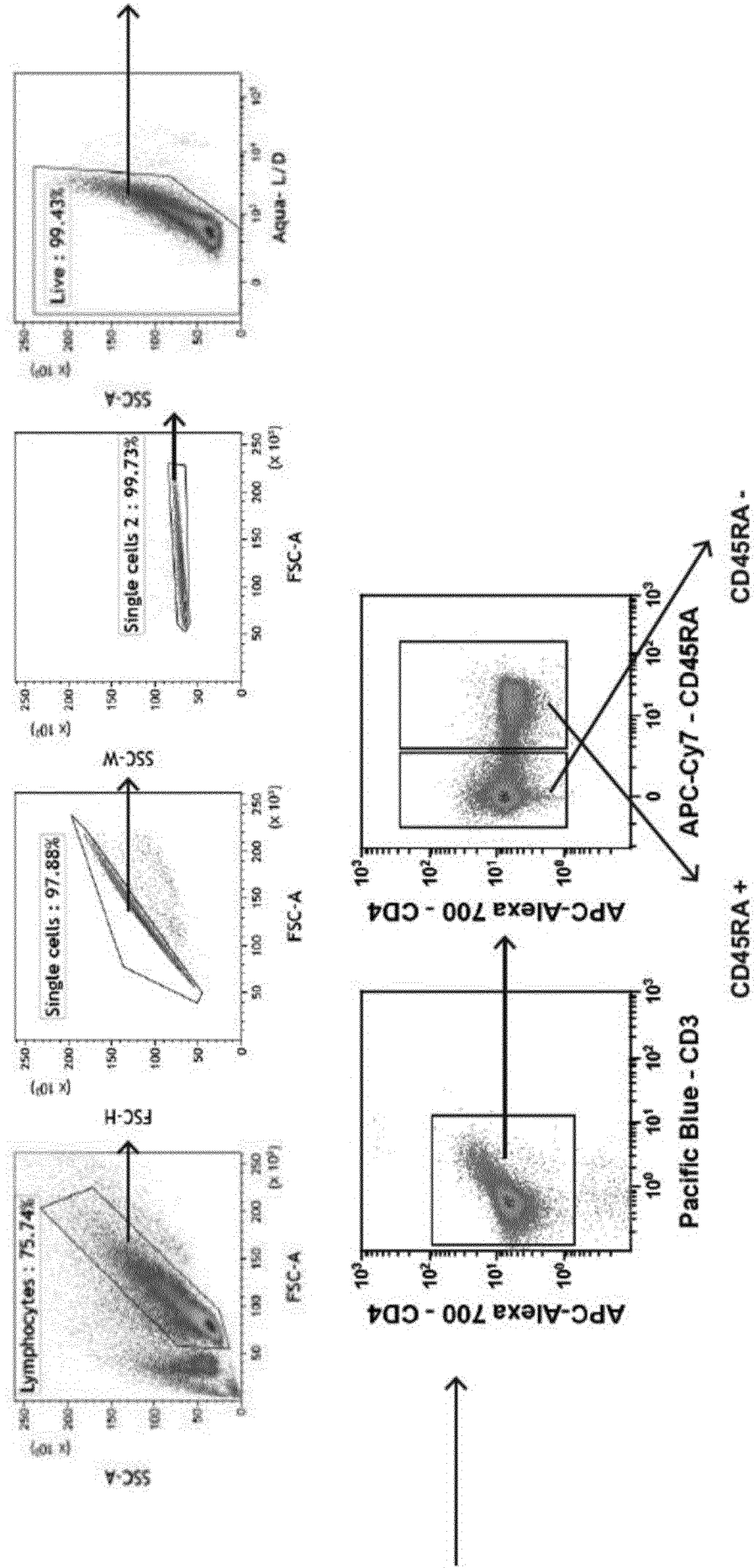


Figure 13
(continuation)

A

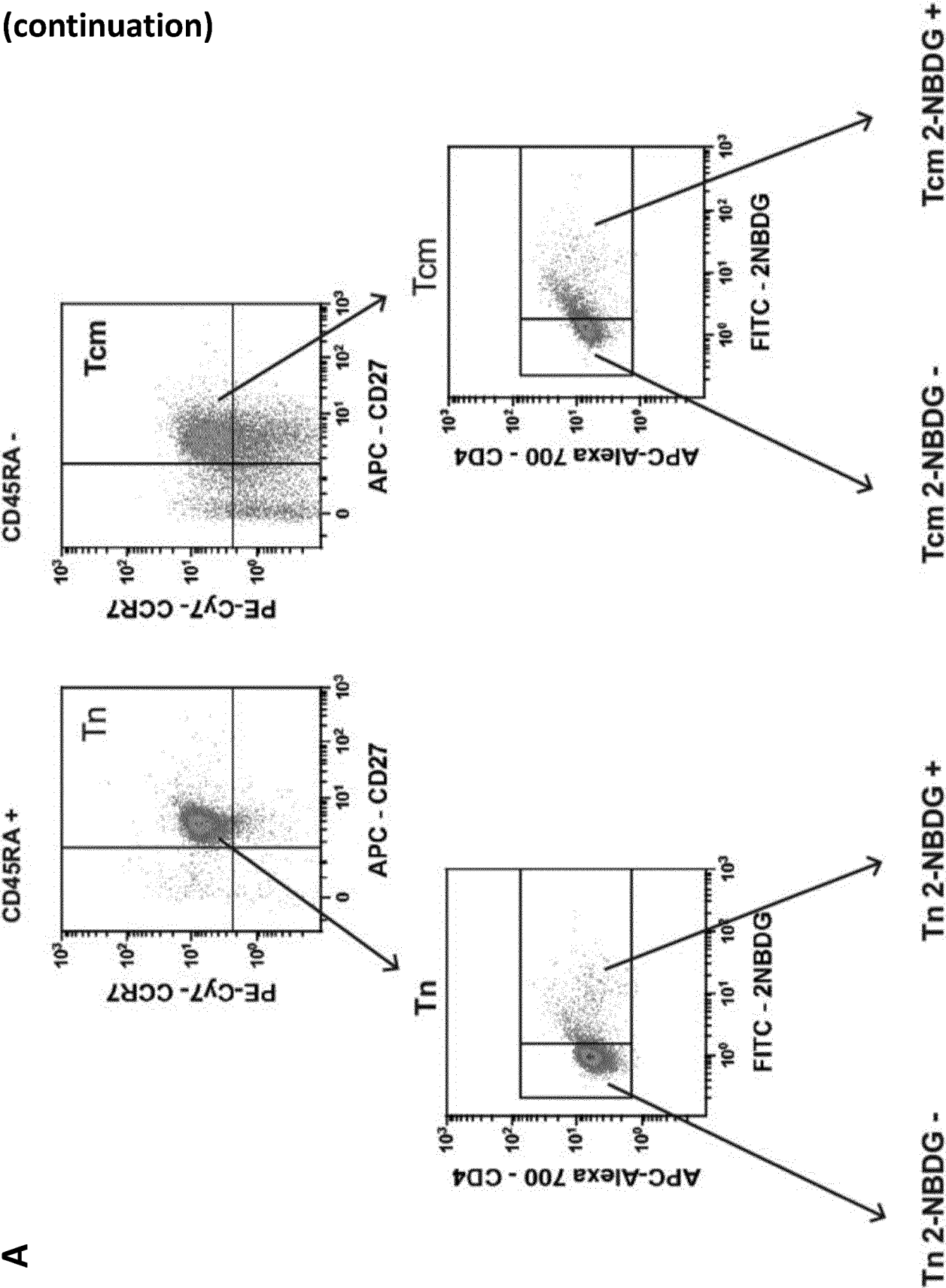


Figure 13
(continuation)

A

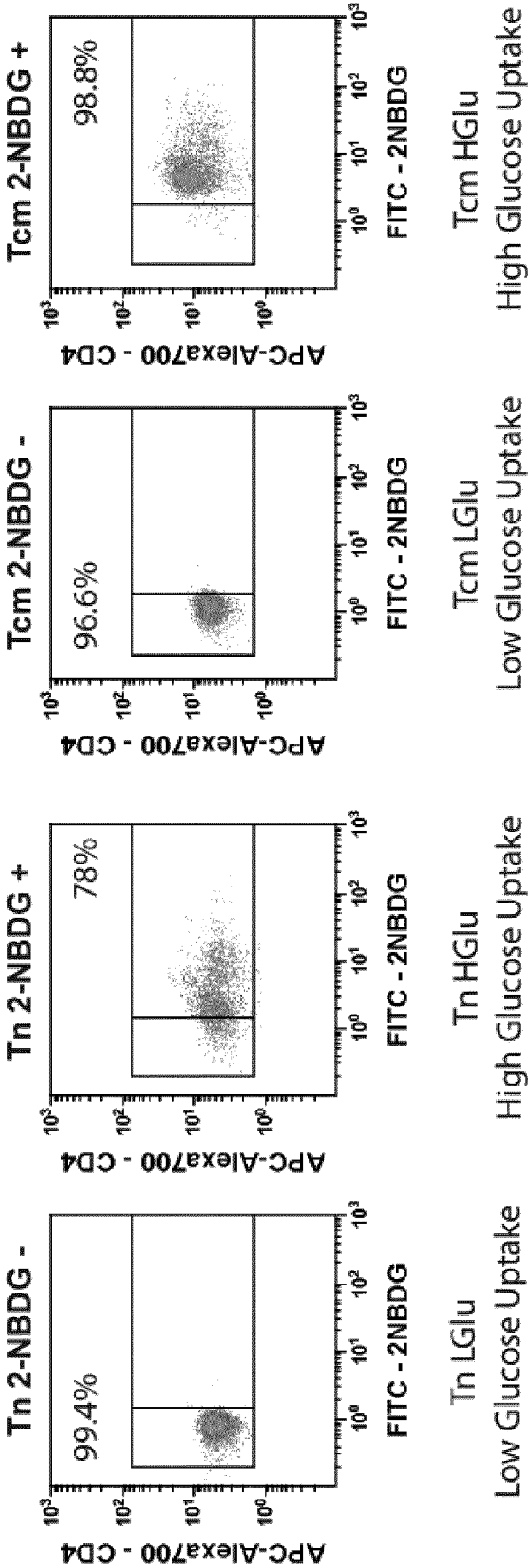


Figure 13
(continuation)

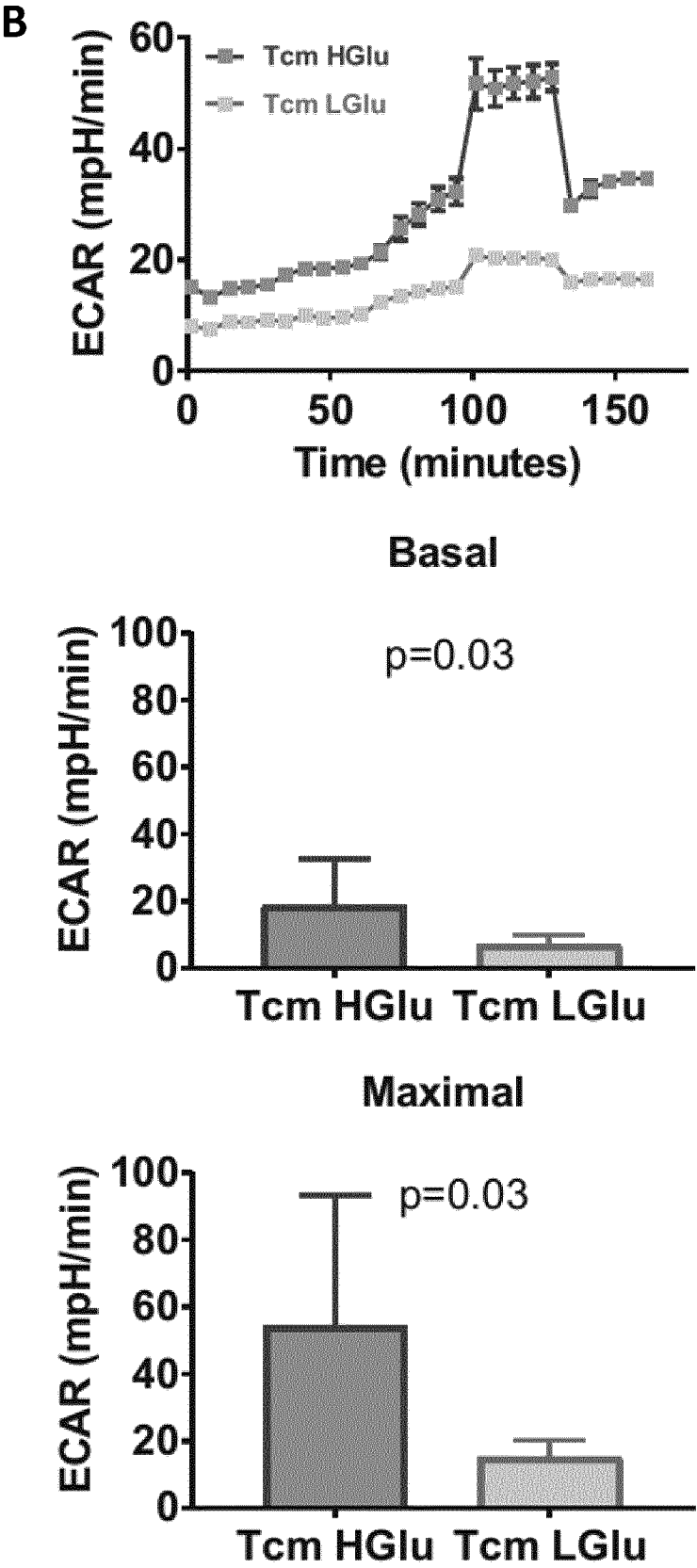


Figure 14

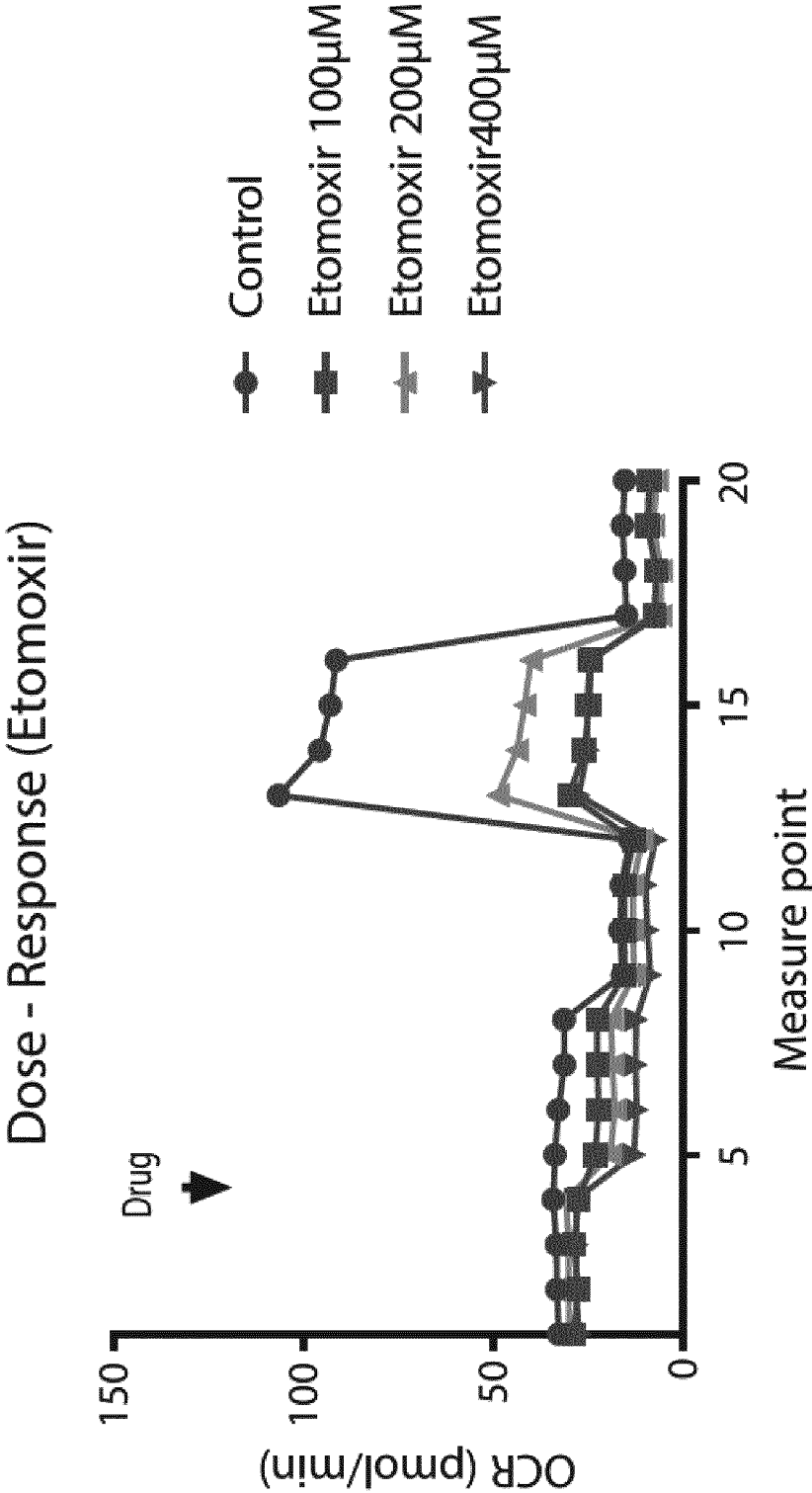


Figure 14
(continuation)

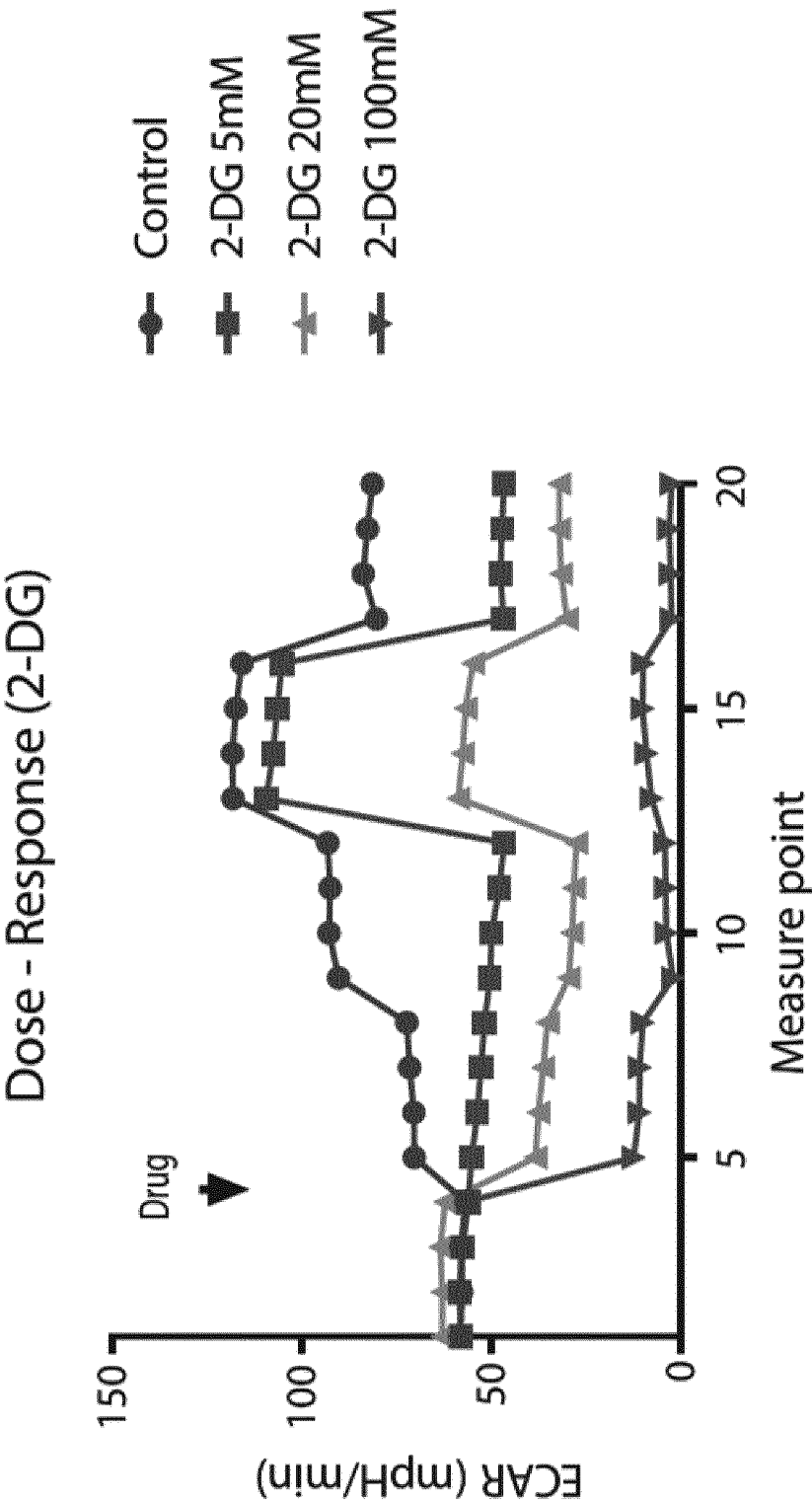


Figure 14
(continuation)

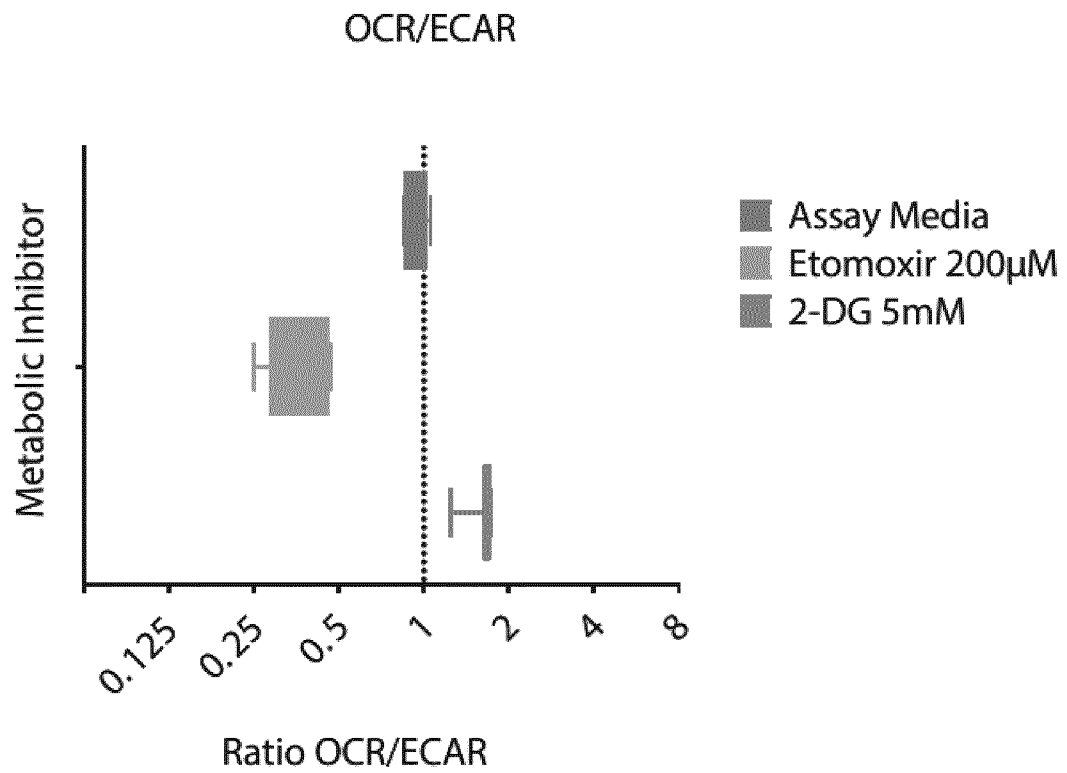


Figure 15

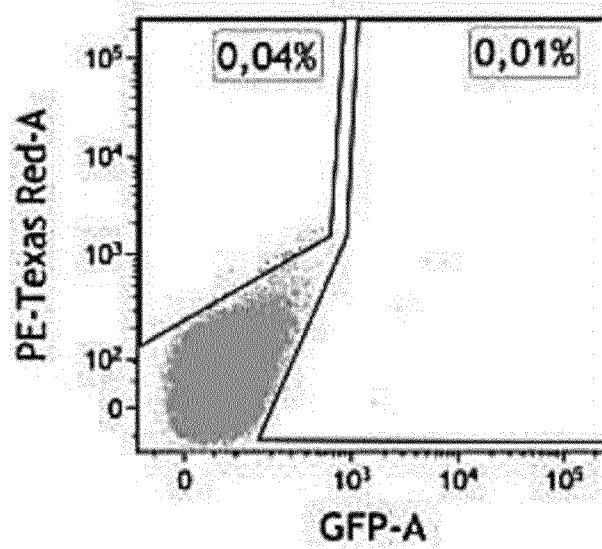
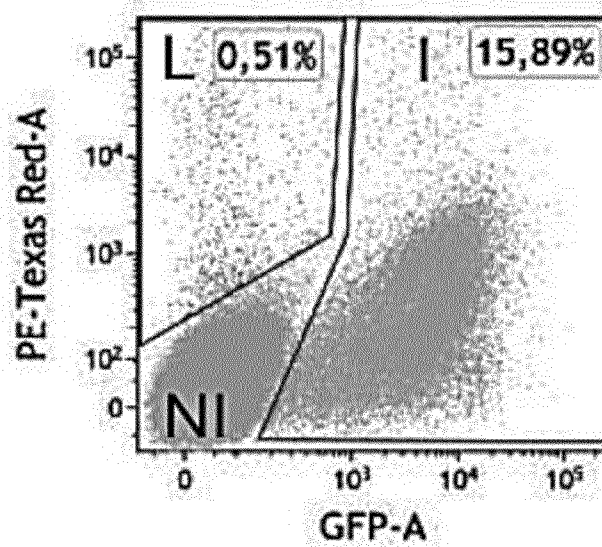
A**Non Infected****Infected**

Figure 15
(continuation)

A

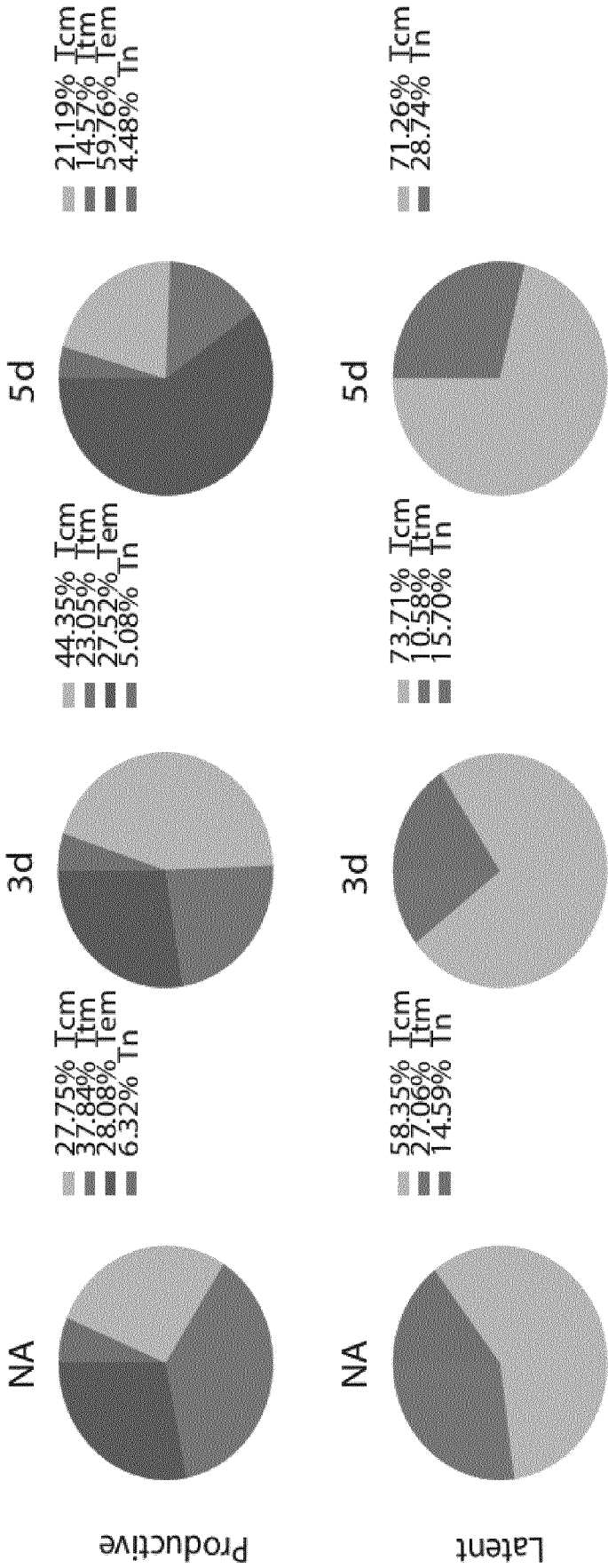


Figure 15
(continuation)

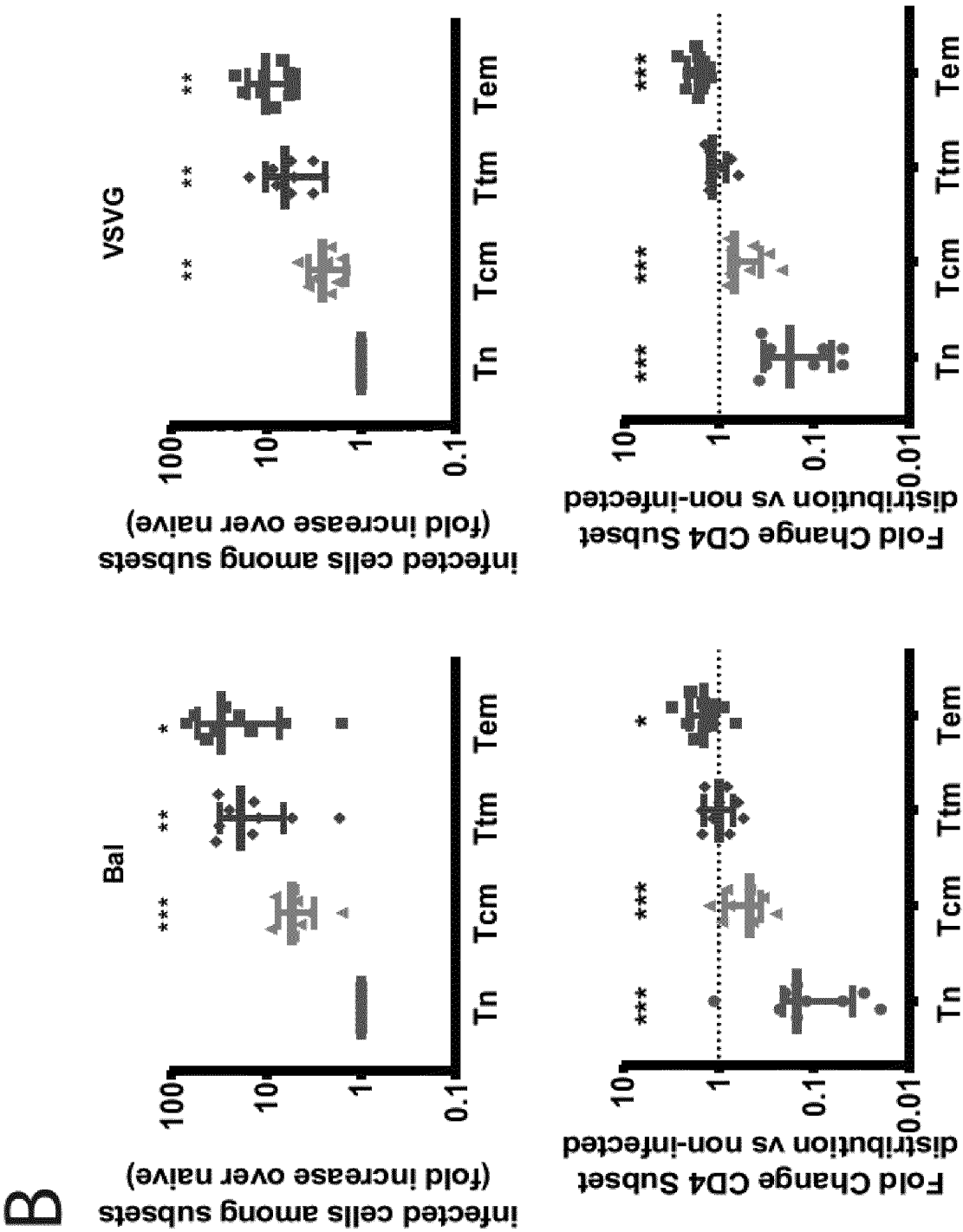
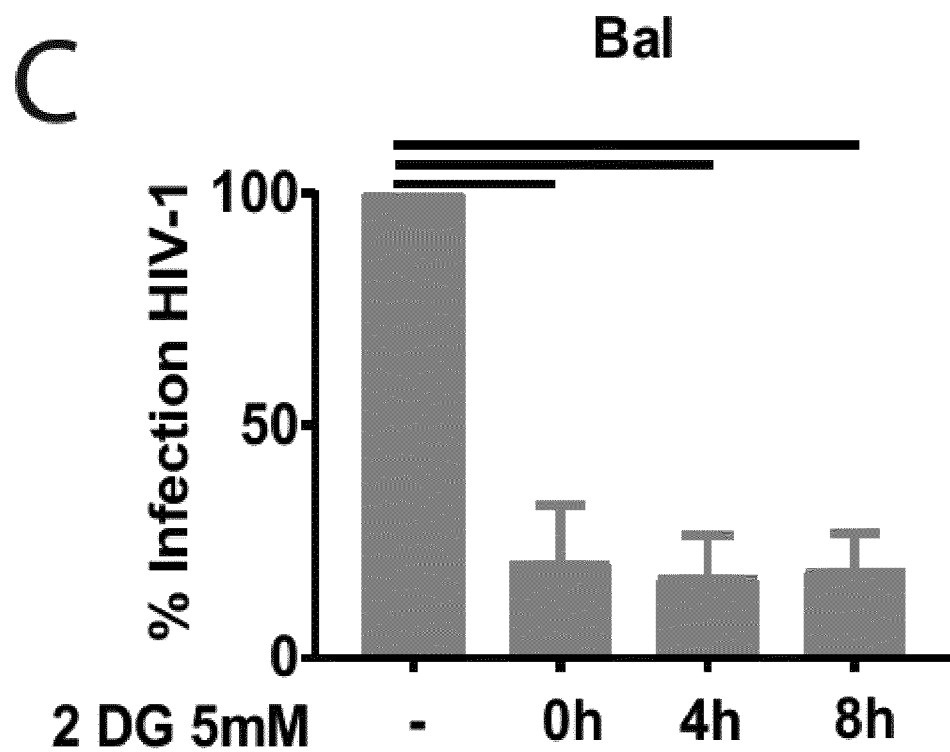


Figure 15
(continuation)



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/086782

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61P31/18 A61K31/055 A61K31/7004 A61K47/34
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61P A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KULKARNI ANURAG ET AL: "Glucose Metabolism and Oxygen Availability Govern Reactivation of the Latent Human Retrovirus HTLV-1", CELL CHEMICAL BIOLOGY, vol. 24, no. 11, 16 November 2017 (2017-11-16), page 1377, XP085251458, ISSN: 2451-9456, DOI: 10.1016/J.CHEMBIOL.2017.08.016 Results, Discussion ----- -/--	1-38



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

15 April 2020

Date of mailing of the international search report

28/04/2020

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Rojo Romeo, Elena

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/086782

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	S. LOISEL-MEYER ET AL: "Glut1-mediated glucose transport regulates HIV infection", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 109, no. 7, 30 January 2012 (2012-01-30), pages 2549-2554, XP055685521, US ISSN: 0027-8424, DOI: 10.1073/pnas.1121427109 Results, Discussion -----	1-38
Y	ANDREA HEGEDUS ET AL: "HIV-1 pathogenicity and virion production are dependent on the metabolic phenotype of activated CD4+ T cells", RETROVIROLOGY, BIOMED CENTRAL LTD., LONDON, GB, vol. 11, no. 1, 25 November 2014 (2014-11-25), page 98, XP021206215, ISSN: 1742-4690, DOI: 10.1186/S12977-014-0098-4 Results, Discussion -----	1-38
Y	MANJUSHA M. KULKARNI ET AL: "Cellular fatty acid synthase is required for late stages of HIV-1 replication", RETROVIROLOGY, vol. 14, no. 1, 29 September 2017 (2017-09-29), XP055685508, DOI: 10.1186/s12977-017-0368-z Results, Discussion -----	1-38
X,P	MATHIEU ANGIN ET AL: "Metabolic plasticity of HIV-specific CD8+ T cells is associated with enhanced antiviral potential and natural control of HIV-1 infection", NATURE METABOLISM, vol. 1, no. 7, 1 July 2019 (2019-07-01), pages 704-716, XP055684776, DOI: 10.1038/s42255-019-0081-4 the whole document ----- -/--	1-38

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/086782

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	JOSÉ CARLOS VALLE-CASUSO ET AL: "Cellular Metabolism Is a Major Determinant of HIV-1 Reservoir Seeding in CD4+ T Cells and Offers an Opportunity to Tackle Infection", CELL METABOLISM, vol. 29, no. 3, 1 March 2019 (2019-03-01), pages 611-626.e5, XP055685531, United States ISSN: 1550-4131, DOI: 10.1016/j.cmet.2018.11.015 the whole document -----	1-38
A	E. J. ARTS ET AL: "HIV-1 Antiretroviral Drug Therapy", COLD SPRING HARBOR PERSPECTIVES IN MEDICINE, vol. 2, no. 4, 25 January 2012 (2012-01-25), pages a007161-a007161, XP055170185, DOI: 10.1101/cshperspect.a007161 the whole document -----	1-38
A	JOSÉ LÓPEZ-ALDEGUER ET AL: "NEW CHALLENGES FOR HIV INFECTION New targets and new drugs in the treatment of HIV", ENFERM INFECC MICROBIOL CLIN, vol. 23 (suppl 2), 1 January 2005 (2005-01-01), pages 33-40, XP055685591, the whole document -----	1-38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2019/086782

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1-38(partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-38

Searched claims (all partially)

1.1. claims: 1-12(partially)

A method of reducing HIV production from a T cell comprising contacting a population of cells comprising HIV-infected CD4+ T cells with 2-deoxy-glucose (2-DG) at a concentration that reduces the production of HIV from said HIV-infected CD4+ T cells; embodiments thereof.

1.2. claims: 13-20, 25-38(all partially)

A method of reducing virus or bacterial pathogen production from a cell comprising contacting a population of cells pathogen-infected cells with at least 2 metabolic inhibitors of different metabolic functions in the cells at a concentration that reduces the production of the pathogen from said pathogen-infected cells; composition for use in the treatment of a pathogen infection in a human, comprising a combination of at least two metabolic inhibitors at a concentration that reduces the production of the pathogen from the pathogen-infected cells; embodiments thereof.

1.3. claims: 21-24(partially)

A method for measuring the effect of a metabolic inhibitor of metabolic function in cells in a pathogen-infected human comprising:administering at least one dose of a metabolic inhibitor of metabolic function in cells to the human; andmeasuring the level of pathogen infection in the pathogen-infected human; embodiments thereof.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 1-38(partially)

Claims 1-38 (partially) are not examined here because no search report was established for the subject-matter of these claims according to Article 17.2(a) PCT (see also ISR).

The method claim 1 and dependent claims 2-12 do not meet the requirements of Art. 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved (at a concentration that reduces the production of HIV from said HIV-infected CD4+ T cells) which merely amounts to a statement of the underlying problem instead of defining the subject-matter in terms of technical features.

The same applies to claim 13 and dependent claims 14-20 ("at a concentration that reduces the production of the pathogen (undetermined) from said pathogen-infected cells"). This is even more a result-to-be-achieved since the skilled person does not know which pathogen is meant and which metabolic inhibitors are to be used.

The same applies to claim 25 and dependent claims 26-38.

Furthermore, present application ONLY supports the use of the tested compounds in the context of HIV.

Finally, it is pointed out that "suboptimal concentrations" of a metabolic inhibitor has no technical meaning. The skilled person is at a loss to know what is meant.

In view of the above the search has been limited to the therapeutic use of the disclosed metabolic inhibitors in the context of HIV and other retroviruses.

Re
Item IV

Lack of unity of invention

According to Rule 13 PCT an application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept, i.e. having at least one common technical feature defining a contribution over the known prior art.

Present independent claims are linked by the common matter of contacting an infected cell with a metabolic inhibitor to reduce the production of the pathogen in said infected cell.

Kulkarni et al./D1 disclose the use of various metabolic inhibitors, e.g. of the glycolysis or of the electron transport chain in mitochondria to inhibit production of the dormant retrovirus HTLV-1 from infected cells. This document finishes with the suggestion to target these pathways to complement existing antiretrovirals and improve treatment efficacy.

This common matter is not new, and thus does not involve special technical features.

The remaining features of the independent claims are:

- claim

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1: the inhibitor is 2-DG and the pathogen is HIV

- claims 13 and 25: any

inhibitors and any virus or bacterial pathogen

- claim 21: none

These

features (or absence of them) are not corresponding.

In the absence of

same or corresponding therapeutic features, there is lack of unity and the claims are deemed to concern 3 groups of inventions. However, in view of the limited support and sufficiency of disclosure provided by present application, no additional search fees were requested for subject-matter which is not supported nor sufficiently disclosed.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.