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Cellular Metabolism Is a Major Determinant of HIV-1 Reservoir Seeding in CD4+ T Cells and Offers an Opportunity to Tackle Infection.

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1 **Cellular metabolism is a major determinant of HIV-1 reservoir seeding in CD4+ T cells and**
2 **offers an opportunity to tackle infection**

3

4 José Carlos Valle-Casuso¹, Mathieu Angin¹, Stevann Volant², Caroline Passaes¹, Valérie
5 Monceaux¹, Anastassia Mikhailova¹, Katia Bourdic³, Véronique Avettand-Fenoel^{4,5}, Faroudy
6 Boufassa⁶, Marc Sitbon⁷, Olivier Lambotte^{3,8}, Maria-Isabel Thoulouze⁹, Michaela Müller-
7 Trutwin¹, Nicolas Chomont¹⁰ and Asier Sáez-Cirión¹

8

9 ¹Institut Pasteur, Unité HIV Inflammation et Persistance, Paris, France

10 ²Institut Pasteur, Hub Bioinformatique et Biostatistique – C3BI, USR 3756 IP CNRS – Paris, France

11 ³Assistance Publique Hôpitaux de Paris, Hôpital Bicêtre, Service de Médecine Interne et Immunologie
12 clinique, 94275 Le Kremlin-Bicêtre, France

13 ⁴ Université Paris Descartes, Sorbonne Paris Cité, EA7327, Paris, France.

14 ⁵ Assistance Publique Hôpitaux de Paris, CHU Necker-Enfants, Laboratoire de Virologie, Malades,
15 Paris, France

16 ⁶INSERM U1018, Centre de recherche en Epidémiologie et Santé des Populations, Université Paris
17 Sud, Le Kremlin Bicêtre, France

18 ⁷ Institut de Génétique Moléculaire de Montpellier, University of Montpellier, CNRS, Montpellier,
19 France

20 ⁸CEA, Université Paris Sud, INSERM U1184, Immunology of Viral Infections and Autoimmune Diseases
21 (IMVA), IDMIT Department / IBFJ, Fontenay-aux-Roses, France

22 ⁹Institut Pasteur, Unité Virologie Structurale, Paris, France

23 ¹⁰Centre de Recherche du CHUM and Department of microbiology, infectiology and immunology,
24 Université de Montréal, H2X0A9, Canada

25

26

27 **Lead contact:**

28 **Asier Sáez-Cirión:** Unité HIV Inflammation et Persistance, Institut Pasteur, 28 rue du Docteur Roux,
29 75724 Paris Cedex 15, France. Tel.: 33-145-688-768. Fax: 33-145-688-957.

30 E-mail: asier.saez-cirion@pasteur.fr

31 **ABSTRACT**

32 HIV persists in long-lived infected cells that are not affected by antiretroviral treatment. These HIV
33 reservoirs are mainly located in CD4+ T-cells, but their distribution is variable in the different subsets.
34 Susceptibility to HIV-1 increases with CD4+ T-cell differentiation. We evaluated whether the
35 metabolic programming that supports the differentiation and function of CD4+ T-cells affected their
36 susceptibility to HIV-1. We found that differences in HIV-1 susceptibility between naïve and more
37 differentiated subsets were associated with the metabolic activity of the cells. Indeed, HIV-1
38 selectively infected CD4+ T-cells with high oxidative phosphorylation and glycolysis, independent of
39 their activation phenotype. Moreover, partial inhibition of glycolysis (i) impaired HIV-1 infection in
40 vitro in all CD4+ T-cell subsets, (ii) decreased the viability of pre-infected cells, and (iii) precluded HIV-
41 1 reactivation in cells from HIV-infected individuals. Our results elucidate the link between cell
42 metabolism and HIV-1 infection and identify a vulnerability to tackle HIV reservoirs.

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44 **Keywords:** HIV-1, CD4+ T-cell, T cell differentiation, HIV reservoir, susceptibility to HIV-1, glycolysis,
45 oxidative phosphorylation, cellular metabolism, metabolic inhibitors, 2-DG, DON, UK5099

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49 INTRODUCTION

50 Combination antiretroviral treatment (cART) blocks HIV-1 replication but does not eliminate infected
51 cells. Replication competent HIV-1 persists in cellular reservoirs that are the origin of rapid viral
52 rebound when treatment is interrupted (Finzi et al., 1997). Identifying the factors underlying the
53 seeding and survival of HIV-infected cells is a priority in the search for an HIV cure (Deeks et al.,
54 2016). CD4+ T-cells are the major target for HIV-1 infection and are thought to constitute most of the
55 HIV-1 reservoir. However, not all CD4+ T-cells contribute equally to the pool of persistently infected
56 cells during cART. The composition of CD4+ T-cells that remain infected is mainly determined by the
57 susceptibility of CD4+ T-cell subsets to HIV infection, their resistance to HIV-induced apoptosis and
58 their life span and turnover potential (Barton et al., 2016). Naïve CD4+ T-cells are highly resistant to
59 HIV-1 infection, while HIV-1 susceptibility increases in more differentiated cell subsets (Roederer et
60 al., 1997; Schnittman et al., 1990). Accordingly, there is a minimal contribution of naïve CD4+ T-cells
61 to the HIV reservoir during cART, which is mainly restricted to the memory cell subsets (Chomont et
62 al., 2009). The susceptibility of CD4+ T-cells to HIV-1 infection depends on the relative abundance of
63 cell factors required by the virus to complete its replication cycle and of cellular restriction factors
64 that counteract infection (Lever and Jeang, 2011). T-cell activation sharply increases the expression
65 of HIV dependency factors and thereby cell susceptibility to HIV-1 infection (Pan et al., 2013;
66 Stevenson et al., 1990), despite the concomitant presence of some restriction factors that the virus
67 can most often circumvent. However, responsiveness to TCR activation (Byrne et al., 1988; Roederer
68 et al., 1997) and susceptibility to HIV infection are not homogeneous across or within CD4+ T-cell
69 subsets. This discrepancy in infection efficacy suggests that HIV-1 has adapted to infect CD4+ T-cells
70 with a specific cellular program (Cleret-Buhot et al., 2015). The cellular processes orchestrating the
71 optimal conditions for the establishment of HIV-1 infection remain unclear.

72

73 Numerous studies have demonstrated the role of cellular metabolism in T-cell immunity (Pearce et
74 al., 2013; Waickman and Powell, 2012). Naïve T-cells circulate in a quiescent state, relying essentially

75 on oxidative phosphorylation (OXPHOS). Upon T-cell activation and after receiving appropriate cues
76 (costimulation, cytokines), naïve T-cells undergo metabolic reprogramming, strongly increasing
77 OXPHOS and, especially, glycolysis, to cope with the energy demands of immune function and rapid
78 proliferation (Pearce et al., 2013). The biomass accumulation that accompanies enhanced cellular
79 metabolism may provide viruses with the abundance of factors that are necessary for their
80 replication. It is worth noting that several retroviruses have evolved to use metabolite transporters
81 as cellular receptors. The glucose transporter 1 (GLUT) is the main receptor for HTLV-1 (Manel et al.,
82 2003); phosphate transporters PiT1 and PiT2 have been reported as surface receptors for koala
83 retrovirus, feline leukemia virus and murine leukemia viruses (Oliveira et al., 2006; Takeuchi et al.,
84 1992; von Laer et al., 1998); and the amino acid transporters ASCT1 and ASCT2 are the receptors for
85 the feline RD-114 endogenous retrovirus (Shimode et al., 2013). Although HIV-1 does not use
86 metabolite transporters as its main receptors, GLUT1 expression is necessary for the postentry steps
87 of HIV-1 replication in CD4+ T-cells (Loisel-Meyer et al., 2012). Moreover, the metabolism of
88 nucleotides is critical for HIV-1 reverse transcription (Amie et al., 2013).

89
90 In the present study, we undertook the analysis of the conditions determining the intracellular
91 susceptibility of CD4+ T-cell subsets to HIV-1 infection. In particular, we analyzed whether the
92 metabolic program is distinct according to the differentiation of CD4+ T-cell subsets and if this
93 determines their susceptibility to HIV-1 infection. We show that cellular metabolism is a central
94 factor driving the HIV-1 infection of CD4+ T-cells and that it may be an important target for new
95 therapies against HIV-1.

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97

98 RESULTS

99 CD4+ T-cell subsets have heterogeneous susceptibility to HIV-1 infection

100 We first assessed the relative intrinsic susceptibility of primary CD4+ T-cell subsets (naïve, T_n; central
101 memory, T_{cm}; transitional memory, T_{tm}; and effector memory, T_{em}) to HIV-1 infection. We used
102 single-cycle NL4.3ΔenvGFP particles pseudotyped with VSV-G envelope protein (HIV-1_{GFP}-VSV) to
103 circumvent differences in the surface expression of CCR5 across CD4+ T-cell subsets. We activated
104 CD4+ T-cells with soluble anti-CD3. This ‘suboptimal’ activation protocol has allowed us to expose
105 differences in the susceptibility to HIV-1 of CD4+ T-cells from different individuals that were masked
106 using more potent stimulation protocols (Saez-Cirion et al., 2011). Activation enhanced the
107 susceptibility to HIV-1 without altering the relative contribution of each CD4+ T-cell subset (Figure
108 1A, B). After infection, the relative frequencies of T_n, T_{cm}, T_{tm} and T_{em} cells among GFP-negative
109 (GFP-) cells was identical to that among noninfected CD4+ T-cells (Figure 1B). In contrast, the
110 composition of HIV-infected GFP-positive (GFP+) cells was different from that of noninfected cells,
111 with a significant exclusion of T_n cells and strong enrichment of T_{em} cells. T_{cm} cells were also slightly
112 underrepresented, and T_{tm} cells were overrepresented among GFP+ CD4+ T-cells when compared to
113 the control condition (Figure 1B). These results suggested different susceptibilities to HIV-1 infection
114 of CD4+ T-cell subsets, with T_{em} cells being the most susceptible, followed by T_{tm} and T_{cm} cells, and
115 with T_n cells being strongly resistant to infection.

116
117 To study if these differences were related to the inherent program of each CD4+ T-cell subset, we
118 isolated quiescent CD4+ T_n, T_{cm}, T_{tm} and T_{em} cells (n=6 donors, Figure S1), and we analyzed their
119 susceptibility to HIV-1 with or without activation. Activation enhanced the susceptibility of all CD4+
120 T-cell subsets to HIV-1 infection (Figure 1C). However, this effect was variable according to the
121 subset. There was a tendency for T_{em} cells to be more susceptible than other subsets (p=0.06) in the
122 absence of activation, and this difference became more pronounced after three (p=0.0004, all
123 comparisons) or five days of activation (p=0.012, T_{em} vs T_n and T_{tm} and T_{cm} vs T_n and T_{tm}). Overall,

124 our results recapitulated previous observations showing an inherent hierarchy in the susceptibility of
125 CD4+ T-cell subsets to HIV-1 infection (Buzon et al., 2014; Tabler et al., 2014).

126

127 **Levels of HIV infection are related to the molecular program of CD4+ T-cell subsets**

128 To determine if a molecular program was associated with the susceptibility of CD4+ T-cell subsets to
129 HIV infection, we analyzed the expression of a panel of 96 genes (related to T-cell activation, survival,
130 differentiation and function as well as known viral restriction or HIV facilitating factors, Table S1) in
131 each CD4+ T-cell subpopulation at the time of infection. Nonactivated CD4+ T-cell subsets showed
132 distinct transcriptional profiles that were further enhanced after activation (e.g., 34 genes and 49
133 genes differently expressed between CD4+ T-cell subsets without activation and after 3 days of anti-
134 CD3 treatment, respectively, Figure 2A). These genes were mostly related to signal transduction and
135 the response to stimulus, which could be related to the previously described different susceptibility
136 to CD3 activation of the CD4+ T-cell subsets (Croft et al., 1994; Kumar et al., 2011). The level of HIV-
137 infected cells correlated with the expression of several genes at the time of infection in the different
138 conditions studied (Figure 2B and Figure S2). SAMHD1 showed a negative association with infection.
139 In contrast, positive correlations were observed between infection levels and other antiviral factors
140 (such as APOBEC3G or SLFN11)(Li et al., 2012; Sheehy et al., 2002) as well as several genes involved
141 in the interferon response (IFI6, IFI16, EIF2AK2, and OAS1) (Kane et al., 2016). Significant positive
142 correlations were also observed between the level of HIV infection and the gene expression levels of
143 transcription factors (STAT3, E2F1, and PRDM1), genes that have been proposed to facilitate HIV-1
144 infection (RRM2, HSP90AA, CFL1, and DYNC1H1)(Allouch et al., 2013; Franke and Luban, 1996; Lukic
145 et al., 2014; Roesch et al., 2012) and multiple genes involved in T-cell metabolism (SLC2A3, SLC2A1,
146 SLC2A5, CASP3, FAS, GAPDH, and GUSB). Taken together, these results suggest that, with the
147 exception of SAMHD1, the antiviral restriction factors analyzed did not decisively influence the cell
148 susceptibility to HIV-1, which is in line with the results of previous reports (Jia et al., 2015). Our data
149 indicate that metabolically active cells may offer favorable conditions for HIV infection.

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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 S3A,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 S3C). These results further point to an influence of the metabolic activity of CD4+ T-cells on their susceptibility to HIV-1.

175 **HIV-infected CD4+ T-cells are characterized by higher levels of metabolic activity independent of**
176 **cell activation levels**

177 To analyze if there was a direct link between cell metabolism and HIV-1 infection, we challenged 5-
178 day activated bulk CD4+ T-cells with HIV-1_{gfp}-VSV, and we sorted three days later noninfected GFP-
179 and infected GFP+ cells. HIV-infected CD4+ T-cells had higher levels of basal metabolism and
180 metabolic potential and, overall, a more energetic profile than noninfected cells (Figures 4A and
181 S4A). Although we could detect infected cells among cells with low activation levels (Figure 4B), we
182 found higher proportions of GFP+ cells among CD4+ T-cells expressing activation markers. We
183 therefore evaluated whether differences in the metabolic activity of infected and noninfected CD4+
184 T-cells were just a consequence of the selective infection of CD4+ T-cells with higher activation levels.
185 We sorted CD4+ T-cells first based on their expression of either high or low levels of both HLA-DR and
186 CD25 and then based on whether they were GFP+ or GFP- (Figures 4B and S4). After 5 days of
187 stimulation, the CD4+ T-cell subsets expressed different levels of activation markers (Figure S4B),
188 which were highest in Tem cells and lowest in Tn cells. This was translated to different contributions
189 of CD4+ T-cell subpopulations in the high- and low-activation sorted cell fractions (Figure 4B).
190 Nevertheless, Tn cells were more frequently found in the GFP- fraction, both in high and low-
191 activated cell populations, whereas the GFP+ fraction was enriched with Tem cells. These results
192 matched the hierarchy of infection that we observed before (Figure 1) and further supported that
193 the susceptibility of CD4+ T-cell subsets to HIV-1 depends on the intrinsic characteristics of these
194 cells independent of their activation status. In this regard, we found that infected GFP+ cells in both
195 the high- and low-activation fractions, had higher basal metabolisms (OCR and ECAR) than
196 noninfected GFP- cells (Figure 4C). These results demonstrated that HIV-infected CD4+ T-cells were
197 characterized by higher metabolic activity levels.

198

199 **HIV-1 infection is preferentially established in CD4+ T-cells with high metabolic activity levels**

200 Our results suggest that HIV-1 infection is favored in the environment provided by CD4+ T-cells with
201 high metabolic activity levels. We analyzed if this was due to a selective infection of CD4+ T-cells with
202 the highest metabolic activity levels or if it was HIV-infection that increased the metabolic activity of
203 the cells. We activated CD4+ T-cells and sorted Tn and Tcm cells based on their capacity to uptake
204 high or low levels of the fluorescent glucose analogue 2NBDG (Figure S5A), which corresponded to
205 weakly and strongly glycolytic cells respectively (Figure S5B). We infected these purified cell fractions
206 with HIV-1_{gfp}-VSV. Three days after infection, infected GFP+ CD4+ T-cells were only observed among
207 highly glycolytic Tn and Tcm cells, while weakly glycolytic cells were strongly resistant to infection
208 (65x [41x-206x], median [IQR] fold increase in the proportion of GFP+ cells in HGlu vs LGlu cell
209 subsets, p=0.008) (Figure 5). Overall these results confirmed that, in our conditions, the high
210 metabolic activity of infected CD4+ T-cells was one of the causes rather than a consequence of HIV
211 infection.

212

213 **Suboptimal inhibition of glucose metabolism blocks HIV-1 replication in CD4+ T-cells**

214 The above results indicate that HIV-1 infection of CD4+ T-cells required high levels of metabolic
215 activity. Therefore, we analyzed if HIV-1 replication could be blocked with metabolic inhibitors. We
216 infected activated CD4+ T-cells with HIV-1_{gfp}-VSV in the presence of increasing amounts of etomoxir,
217 an inhibitor of fatty acid oxidation (FAO), 6-diazo-5-oxo-l-norleucine (DON), a glutamine antagonist,
218 or 2-deoxy glucose (2-DG), a competitive inhibitor of glycolysis. Etomoxir was able to reduce HIV
219 infection but only at high concentrations, well above the levels needed to reduce mitochondrial
220 respiration (Figure 6A and Figure S6). DON reduced HIV infection without inducing cell death,
221 although the extent of the inhibition was heterogeneous. Suboptimal amounts of 2-DG (5 mM),
222 which were enough to significantly reduce glycolysis (Figure S6), decreased HIV-1 infection of CD4+ T-
223 cells with minimal cell toxicity (Figure 6A). These results suggested a higher impact of glucose and
224 glutamine metabolism than FAO on HIV-1 replication. The role of glucose metabolism was further
225 confirmed in different sets of experiments in which the frequency of HIV-1-infected CD4+ T-cells was

226 reduced when the infections were performed in conditions of glucose starvation or in presence of
227 UK5099, a molecule that inhibits the transport of pyruvate, an end product of glycolysis, to the
228 mitochondria (Figure 6B). The presence of 2-DG impaired the accumulation of HIV-1 reverse
229 transcribed products overtime pointing to an early block of viral replication (Figure 6C). 2-DG was
230 able to reduce infection and reverse transcript levels to a similar extent whether it was added to the
231 culture at the time of the challenge or up to 8h later (Figure 6D), indicating that 2-DG was affecting
232 post-entry steps of viral replication. Overall, these results show that a glycolytic environment was
233 necessary for HIV-1 to complete reverse transcription.

234

235 2-DG blocked HIV-1 infection in all CD4+ T-cell subsets, although the differences were more
236 pronounced in more differentiated (more glycolytic) cells (Figure 6E). Interestingly, Etomoxir slightly
237 reduced viral replication in Tem cells but not in other T-cell subsets, which could be related to the
238 overall highly energetic nature of these cells. We then used VSV-G pseudotyped NL4.3Δenv Duo-Fluo
239 I particles that allow HIV-1 latently and productively infected cells to be distinguished from each
240 other (Calvanese et al., 2013) (Figure S7A). Interestingly, latent infection was more prominent among
241 Tn and Tcm CD4+ T-cells, while productive infection was predominantly observed among Tem cells
242 (Figure S7A). Overall, the presence of 2-DG significantly reduced the global number of both latently
243 and productively infected CD4+ T-cells (Figure 6F), which agreed with the need for a glycolytic
244 environment for HIV-1 to complete the preintegration steps of its replication cycle.

245

246 We then analyzed the impact of inhibition of glycolysis on the infection of CD4+ T-cells with a R5
247 wild-type replication competent virus (HIV-1 Bal). We first confirmed that the hierarchy of infection
248 of CD4+ T-cell subsets that we observed with VSV-G single cycle particles (Tn<Tcm<Ttm<Tem)
249 coincided with the hierarchy of infection when we used replication competent HIV-1 Bal (Figure S7B).
250 We found that 2-DG was also able to efficiently blocked infection of CD4+ T-cells with HIV-1 Bal
251 (Figure 6G), independently of whether it was added at the time of challenge or 4h/8h after challenge

252 (Figure S7C). All together, these results show that the inhibition of metabolic activity blocked HIV-1
253 replication, corroborating that CD4+ T-cell metabolism is an important determinant of HIV-1
254 infection.

255

256 **Suboptimal inhibition of glycolysis eliminates HIV-1 infected cells and impairs HIV amplification**
257 **from CD4+ T-cell reservoirs**

258 We next studied if the preferential establishment of HIV-1 infection in highly glycolytic cells could be
259 used to target HIV-1 reservoirs. First, we analyzed if suboptimal inhibition of glycolysis could
260 selectively eliminate CD4+ T-cells that had been preinfected in vitro. We infected CD4+ T-cells with
261 HIV-1_{gfp}-VSV and sorted infected GFP+ from noninfected GFP- cells (Figure S4A) and cultured them in
262 the absence or presence of 2-DG to inhibit glycolysis. The presence of 2-DG induced higher levels of
263 cell death among infected GFP+ cells than among GFP- cells (Figure 7A and B), affecting all memory T-
264 cell subpopulations (Figure 7C).

265

266 As 2-DG was able to both block infection and eliminate infected cells, we wondered whether 2-DG
267 could block HIV spread upon activation of CD4+ T-cells from HIV-infected individuals receiving cART.
268 We isolated CD4+ T-cells from 6 individuals receiving cART (Table S2) and activated the cells with PHA
269 in the absence or presence of 2-DG. In all cases, 2-DG potently blocked HIV-1 amplification, as
270 measured by ultrasensitive analyses of p24 production (Figure 7D). Therefore, the need of HIV for
271 highly glycolytic cells reveals a vulnerability that can be exploited to tackle infection.

272

273 **DISCUSSION**

274 In this study, we performed a detailed characterization of the bioenergetics of CD4+ Tn, Tcm, Ttm
275 and Tem cells. Upon potent TCR activation, naïve and memory cells have been shown to strongly
276 upregulate their metabolism and acquire effector functions (van der Windt et al., 2013). Here, we
277 show important metabolic differences among the three memory cell populations studied, even in the
278 absence of stimulation. Upon anti-CD3 activation, all CD4+ T-cell subsets enhanced their metabolic
279 activity but essentially maintained their distinctive metabolic programs, which matched the
280 requirements for their expected rapid reaction to antigenic stimulation (Tem>>Ttm>Tcm>>Tn). The
281 metabolic activity of the T-cell subsets overlapped with their susceptibility to HIV-1 infection (Figures
282 1C and 3B), supporting that the extent of HIV-1 infection in CD4+ T-cell subsets was affected by the
283 metabolic environment within the target cells.

284

285 Transcript profiling at the time of infection showed that among the CD4+ T-cell subsets, there were
286 positive correlations between the frequencies of HIV-infected cells and the expression levels of
287 multiple genes related to cell metabolism. Negative correlations were found between the
288 susceptibility of CD4+ T-cells to HIV-1 infection and the expression of SAMHD1, an efficient HIV-1
289 restriction factor that also plays an important role in the regulation of cell metabolism (Descours et
290 al., 2012; Mathews, 2015). Surprisingly, strong positive correlations were found between the levels
291 of HIV-infected cells and the expression of a cluster of genes related to the interferon response.
292 Although this point was not specifically explored in the present study, increasing evidence has
293 revealed the interrelationships between cell metabolism and the interferon response (Burke et al.,
294 2014; Zhao et al., 2015). Some type 1 interferons might enhance glycolysis (Fritsch and Weichhart,
295 2016), and interferon regulatory factors play a key role during the metabolic reprogramming that
296 follows TCR-mediated activation of T-cells (Man et al., 2013). The interaction between the interferon
297 response and cell metabolism may somewhat explain the dichotomy between antiviral and viral-
298 enhancing interferon-stimulated genes (Schoggins and Rice, 2011; Seo et al., 2011). Tem cells, which

299 were the most susceptible to HIV infection in our assay, expressed the strongest levels of several
300 restriction factors such as SLFN11 or APOBEC3G. Our results thus indicate that HIV-1 exploits the
301 metabolic environment that most favors the completion of its replication cycle, and this might be
302 one of the factors underlying the adaptation of HIV-1 to evade some restriction factors.

303

304 We further confirmed the association between T-cell metabolism and HIV infection in a series of
305 functional analyses. First, we showed that HIV-infected CD4+ T-cells had higher levels of metabolic
306 activity and metabolic potential than HIV-exposed but noninfected cells. This was not solely the
307 consequence of the preferential infection of cells with higher activation levels; when we sorted CD4+
308 T-cells that were matched for the expression of common activation markers, we still found that HIV-
309 infected cells had higher metabolic activity levels than noninfected CD4+ T-cells. Although there are
310 well-established links between T-cell activation and cellular metabolism, it is increasingly clear that T-
311 cell functions, including proliferation, the secretion of cytokines and cell survival, are supported
312 through different engagements of the various metabolic pathways (Jones and Bianchi, 2015). This
313 may explain the partial dichotomy between T-cell activation and cell metabolism in HIV infection that
314 we observed in our experiments. Additionally, we found Tn cells expressing high levels of activation
315 markers upon anti-CD3 stimulation, but these cells remained mostly resistant to HIV-1 infection. In
316 contrast, the frequency of infected Tn cells sharply increased when we challenged highly glycolytic Tn
317 cells. This is in agreement with previous results that showed that expression of GLUT1 is necessary
318 for HIV-1 infection of CD4+ T-cells (Loisel-Meyer et al., 2012) and that, in vitro, HIV preferentially
319 infects CD4+ T-cells expressing GLUT1 and OX40 (Palmer et al.). Overall, our results demonstrate that
320 cells that had higher metabolic activity levels were more susceptible to HIV infection.

321

322 In our experimental conditions, we could detect virtually no infected cells when we challenged cells
323 with low metabolic activity levels. Thus, any potential change in cell metabolism that might have
324 been induced directly by HIV particles was not sufficient to promote infection in cells that had low

325 metabolic activity levels at the time of viral challenge. However, it is important to note that because
326 we were interested in understanding the factors modulating HIV infection beyond the expression of
327 HIV receptors, we used single-cycle particles devoid of HIV envelope and pseudotyped with VSV-G in
328 this set of experiments. It is possible that fully replication-competent viruses have a stronger effect
329 on modulating CD4+ T-cell metabolism. CCL5 engagement with CCR5 has been described as
330 increasing glycolysis in T-cells (Chan et al., 2012), and it is possible that gp120 triggers a similar effect.
331 Moreover, HIV infection has been shown to induce increased expression of several glucose
332 transporters in in vitro experiments (Kavanagh Williamson et al., 2018; Sorbara et al., 1996). Overall,
333 viruses appear to possess different mechanisms to enhance cell metabolism to favor viral replication
334 (Goodwin et al., 2015; Sanchez and Lagunoff, 2015), and this deserves additional exploration in the
335 context of HIV infection.

336

337 Suboptimal inhibition of glycolysis impaired HIV replication, and this was observed with single-cycle
338 VSVG pseudotyped particles and replication-competent HIV-1 Bal and for all CD4+ T-cell subsets,
339 although the effects were more pronounced in more energetic cells. Inhibition of glycolysis, including
340 several hours after viral entry, severely reduced the accumulation of HIV reverse transcripts and
341 impaired the establishment of both productive and latent infections. Our results thus point to critical
342 steps early during the viral replication cycle (in particular reverse transcription) that are influenced by
343 glycolysis, which agrees with a previous report (Loisel-Meyer et al., 2012). Along these lines, the
344 synthesis of deoxynucleotides, the level of which is a limiting factor for HIV reverse transcription, is
345 very energy demanding and requires substrates that are provided by different metabolic pathways,
346 such as the pentose phosphate pathway (PPP) that is parallel to glycolysis (Lane and Fan, 2015).
347 Although, unfortunately, genes involved in the PPP were not included in our gene expression panel,
348 we found important differences between CD4+ T-cell subsets and strong correlations with infection
349 levels for several genes such as TP53, ESF1 and RRM2, which play critical roles in the de novo
350 synthesis of dNTPs. In particular we have recently shown that changes in the expression of RRM2

351 impact HIV-1 replication in macrophages and dendritic cells by modifying the pools of dNTPS (Allouch
352 et al., 2013; Valle-Casuso et al., 2017). Moreover, SAMHD1, the expression levels of which were
353 negatively correlated with infection in our analysis, is a deoxynucleoside triphosphohydrolase that
354 contributes to control the intracellular dNTP concentration during cell-cycle (Mathews, 2015). Our
355 results therefore suggest that metabolically active cells offer an environment with positive synthesis
356 (RRM2) vs degradation (SAMHD1) of dNTP pools that favors HIV-1 reverse transcription. However,
357 other steps of the viral replication cycle may also depend on cell metabolism. The inhibition of
358 glycolysis has been shown to decrease the production of HIV-1 particles (Hegedus et al., 2014), and
359 mTOR, a key regulator of cellular metabolism (Waickman and Powell, 2012), appears to be involved
360 in the establishment of HIV-1 latency in CD4+ T-cells (Besnard et al., 2016).

361
362 In our functional experiments we mostly focused on assessing the impact of glycolysis on HIV
363 infection. Our results showing that inhibition of pyruvate transport to the mitochondria with UK5099
364 blocked HIV infection suggests that glucose oxidation is important for HIV-1 infection. However the
365 relative contribution of aerobic vs oxidative glycolysis remains to be determined. It is likely that other
366 metabolic functions are also important for HIV-1 infection. The inhibition of fatty acid oxidation with
367 Etomoxir had a limited effect on HIV replication in suboptimal conditions, mostly in Tem cells, but it
368 strongly inhibited infection at higher concentrations. However, caution is needed when interpreting
369 results obtained with Etomoxir as it has been shown to produce off target effects at such high
370 concentrations (O'Connor et al., 2018; Yao et al., 2018). A recent report suggested that fatty acid
371 metabolism may also participate in the late steps of viral replication (Kulkarni et al., 2017). Our
372 results with the glutamine antagonist DON suggest that glutamine metabolism may also be necessary
373 for the optimal infection of CD4+ T-cells. In general, the association between HIV infection and cell
374 metabolism can be exploited to impair HIV-1 replication.

375

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

384

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

402 study shows that cellular metabolism is a central factor that drives the HIV-1 infection of CD4+ T-cells
403 more strongly than does the state of differentiation and/or activation, and cellular metabolism may
404 be an important target for new therapies against HIV-1.

405

406 **Limitations of Study**

407 We show here that HIV-1 requires a metabolically rich cellular environment to establish both
408 productive and latent HIV-1 infection in CD4+ T cell subsets. However, our analyses were limited to
409 circulating cells. It remains to be elucidated how this association is affected by the metabolic
410 resources and the particular T-cell programs of the different tissues where HIV-1 replicates. It also
411 remains to be determined if HIV-infected cells sustain the enhanced metabolic activity levels over
412 time, even in the absence of active viral replication, and if this could serve to identify and target
413 persistently infected cells on cART. CD4+ T-cells expressing PD-1 and other immune checkpoints are
414 enriched in HIV in HIV-infected individuals receiving cART (Banga et al., 2016; Chomont et al., 2009;
415 Fromentin et al., 2016). Interestingly, these immune checkpoints appear to mediate their inhibitory
416 activities through the metabolic reprogramming of the cells (Lim et al., 2017; Patsoukis et al., 2015).
417 This suggests that the metabolic requirements of HIV-1 replication might enduringly imprint the
418 infected cells.

419

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424

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432

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434 MS is inventor on patents describing the use of RBD ligands and is the co-founder of METAFORA-
435 biosystems, a start-up company that focuses on metabolite transporters under physiological and
436 pathological conditions. AS-C has received consultancy fees from ViiV healthcare, and speaker fees
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439

440 **AUTHOR CONTRIBUTIONS**

441 JCV-C, VM, MA, CP, AM, VA-F performed experiments; JCV-C, SV and AS-C analyzed the data; KB, FB,
442 MS, M-IT and OL provided key reagents or contributed to the inclusion of study participants and the
443 obtaining and validation of clinical information; JCV-C, NT, MS, MM-T, OL, NC and AS-C contributed
444 to the conception and discussion of the study; JCV-C and AS-C designed the study; AS-C supervised
445 the study; JCV-C and AS-C drafted the article; and all authors critically reviewed the manuscript.

446

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664

665 **FIGURE LEGENDS**

666 **Figure 1. CD4+ T-cells subsets have different susceptibilities to HIV-1 infection**

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

680

681 **Figure 2. HIV-1 infection levels in CD4+ T-cell subsets correlate with the expression levels of genes**
682 **related to cell metabolism**

683 A) Heat maps displaying the genes differentially expressed (p<0.05) between the CD4+ T-cell subsets
684 (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
685 with soluble anti-CD3 (i.e., at the time of HIV challenge) (green = downregulation, red =
686 upregulation). Variables are ordered by hierarchical clustering and samples by CD4+ T-cell subsets. B)
687 Spearman's correlation between the levels of gene expression at the time of HIV-1 challenge and
688 HIV-1 infection levels 72 h after challenge. Only significant correlations (p<0.05) are represented in
689 the graphs (green bars). Genes highlighted in red show the group of genes that correlated with
690 infection levels in all conditions.

691

692 **Figure 3. CD4+ T-cell subsets have different metabolic profiles that coincide with their**
693 **susceptibility to HIV-1 infection**

694 OCR and ECAR in nonactivated (NA), 3-day activation (3d) and 5-day activation (5d) CD4+ T-cell
695 subsets. A) Median values of the metabolic variables obtained for the CD4+ T-cell subsets from the 6
696 donors in the different conditions analyzed. B) Median and IQR basal OCR (left panel) and ECAR (right
697 panel) and C) basal ECAR/OCR ratio for CD4+ T-cell subsets in different activation states (0d, 3d, 5d).
698 Median values in NA Tn cells are indicated by dashed lines as a reference. Symbols represent
699 independent experiments (n=6). D) Summary of correlations between metabolic parameters at the
700 time of infection in NA, 3d and 5d activated CD4+ T-cell subsets and the % of infected cells 72 h post
701 infection. The green color indicates $p < 0.05$. The size of the circle represents Spearman's coefficients.

702

703 **Figure 4. HIV-1-infected CD4+ T-cells are characterized by higher metabolic activity levels**

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

715

716 **Figure 5. Rate of glucose uptake by CD4+ T-cell subsets is associated with their susceptibility to**
717 **HIV-1 infection**

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

725

726 **Figure 6. Inhibition of cell metabolic pathways blocks HIV-1 infection of CD4+ T-cells**

727 A) Relative level of infection (blue bars) and cell death (purple bars) compared to the control
728 conditions in 5-days activated CD4+ T-cells infected in the absence or presence of increasing amounts
729 of Etomoxir, DON, or 2-DG (median and IQR, n=3 donors). B) Infection and cell death in CD4+ T-cells
730 exposed to HIV-1 in glucose-containing medium in the absence or presence of 2-DG (5 mM) or in
731 culture medium without glucose (starvation) (left) or in the absence or presence of UK5099 (25 μM)
732 (right). C) Relative number of U5-Gag copies in CD4+ T-cells at 6h, 15h or 72h after infection with
733 HIV-1_{GFP}-VSV in the absence or presence of 2-DG. Individual values (symbols), medians and IQRs
734 (horizontal lines) for five different donors are shown. D) Infection levels and number of U5-Gag
735 copies 72h after challenge with HIV-1_{GFP}-VSV in the absence or presence of 2-DG added at the time of
736 challenge, 4h or 8h post challenge. Values represent the relative levels of infection compared to the
737 control condition (median and IQR, n=3 donors). E) Changes in HIV-1 infection levels in CD4+ T-cell
738 subsets 72h after the infection of bulk CD4+ T-cells in the absence or in presence of 2-DG (orange
739 symbols) or Etomoxir (beige symbols). Medians (n=7 donors) are shown. F) Percentage of HIV-1
740 productively (left panel) or latently (right panel) infected cells 72h after the infection of CD4+ T-cells
741 with HIV-1DuoFluo VSVG particles in the presence of 2-DG or etomoxir. Median and IQR values from

742 experiments with 6 donors are shown. G) p24 production in supernatants from CD4+T-cell cultures 3
743 and 7 days after infection with HIV-1 BaL in the absence (blue bars) or presence of 2-DG (5 mM)
744 (orange bars). Means and standard deviations for three replicates are shown at each time point for
745 experiments done with cells from three different donors.

746

747 **Figure 7. Suboptimal inhibition of glucose metabolism selectively eliminates preinfected CD4+ T-**
748 **cells and inhibits HIV-1 amplification from reservoirs**

749 A) Cell viability in sorted pre-infected GFP+ (green) or noninfected GFP- (red) CD4+ T cells cultured
750 for 48 h in the absence or presence of 2-DG. A) One representative example is shown. B) Relative
751 survival of 2-DG treated cells (circles) was compared to that of nontreated cells (squares) at 24 h and
752 48 h. C) Changes in the CD4+ T-cell subset distribution 48h after the treatment of infected bulk CD4+
753 T-cells with 2-DG when compared with the distribution in the control condition. Median values and
754 IQR are shown (n=3 donors). D) HIV-1 reactivation from CD4+ T-cells from six individuals on cART
755 upon PHA/IL-2 stimulation in the absence (blue line/symbols) or presence of 2-DG (5 mM) (orange
756 line/symbols) (mean and SD, 3 replicates). Mean p24 values in the absence or presence of 2-DG on
757 day 14 post stimulation are shown for all six experiments (right panel).

758

759

760 **STAR METHODS**

761 **CONTACT FOR REAGENT AND RESOURCE SHARING**

762 Further information and requests for resources and reagents should be directed to the Lead Contact,
763 Asier Saez-Cirion (asier.saez-cirion@pasteur.fr). Request for biological resources will be fulfilled
764 based on availability and upon the establishment of an MTA.

765

766 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

767 Blood samples from non-HIV-infected donors were obtained from the French blood bank
768 (Etablissement Français du Sang) as part of an agreement with the Institut Pasteur (C CPSL UNT,
769 number 15/EFS/023). Fifty-milliliter blood samples were obtained from six HIV-infected individuals
770 on antiretroviral therapy who had HIV plasma viral loads <50 RNA copies/mL from the ANRS
771 TRANSbioHIV study after obtaining written informed consent in accordance with the Declaration of
772 Helsinki (Table S2). The TRANSbioHIV study was approved by the Ethics Review Committee (Comité
773 de protection des personnes) of Île-de-France VII.

774

775 **METHOD DETAILS**

776 **Isolation and culture of CD4+ T-cells**

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

780 Purified CD4+ cells (10^6 cell/mL) were cultured in RPMI 1640 containing GlutaMAX, 10% FCS,
781 penicillin (10 IU/mL) and streptomycin (10 µg/mL) in the presence of IL-2 (Miltenyi) at 50 IU/mL
782 (Culture media). Depending on the experiment, cells were left unstimulated or were stimulated for 3
783 or 5 days with 0.5 µg/mL soluble antiCD3 (BioLegend, Ref.300414, Clone UCHT1) in the absence of
784 CD28 co-stimulation as previously described (Saez-Cirion et al., 2011). Different compounds that
785 target metabolic pathways [2-deoxy-glucose, 2-DG (Seahorse Biotechnologies); (+)-Etomoxir sodium

786 salt hydrate (Sigma, Ref. E1905); UK5099 (Sigma, Ref. PZ0160); 6-diazo-5-oxo-l-norleucine
787 (DON)(Sigma, Ref. D2141); glucose (Seahorse Biotechnologies); oligomycin (Seahorse
788 Biotechnologies) or carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone FCCP (Seahorse
789 Biotechnologies)] were added to the culture media at different times and concentrations depending
790 on the protocol conditions. A glucose-free culture media was used in some infection experiments and
791 is described in the results section [RPMI non-glucose, GlutaMAX, containing 10% FCS, penicillin (10
792 IU/mL) and streptomycin (10 µg/mL) in the presence of IL-2 (Miltenyi) at 50 IU/mL (culture media)].
793 After culture, living cells were counted with an automatic Countess cell counter (Invitrogen) based on
794 size and non-staining with trypan blue. The number of living cells was then normalized before
795 analysis.

796

797 **HIV infection in vitro**

798 Single-round infections were performed with HIV-1 NL4.3ΔenvΔnef/GFP (Amara et al., 2003) and
799 HIV-1-DuoFluoΔenv(R7GEmC) (provided by Professor Eric Verdin and Dr. Calvanese, NIH AIDS
800 Reagent Program, Division of AIDS, NIAID, NIH: Cat# 12595 DuoFluo (R7GEmC)) (Calvanese et al.,
801 2013). Both viruses were pseudotyped with the VSV-G envelope protein by transiently cotransfecting
802 (SuperFect; Qiagen) 293T cells with the proviral vectors and the VSV-G expression vector pMD2.G.
803 Nonactivated or activated CD4 + T-cells were infected in triplicate (5×10^4 cells/well, 200 µL) with 35
804 ng/ 1×10^6 HIV-1 NL4.3Δnef/GFP/VSV-G and with 70 ng of HIV-1-DuoFluo(R7GEmC)/VSVg per million
805 cells. Active HIV-1 infection was estimated by flow cytometry (BD LSRII, BD bioscience) as the
806 percentage of GFP-expressing CD4+ T-cells 72 h after infection. Latent HIV infection was estimated by
807 flow cytometry as the percentage of mCherry+GFP-CD4+ T-cells 72 h after infection with HIV-1-
808 DuoFluo(R7GEmC) particles.

809

810 HIV-1 reverse transcripts (U5-Gag) were quantified by real-time PCR with an Applied Biosystems 7500
811 Real-Time PCR System 6, 16 and 72 h after infection of CD4+ T-cells with VSV-G–pseudotyped HIV-1

812 particles as described in (David et al., 2006). Briefly, total DNA was extracted with the NucleoSpin
813 8/96 Tissue Core kit (Macherey-Nagel, Ref. 740453.4) and 100 ng of template DNA were used per
814 reaction. DNA loading was controlled by concurrently amplifying the albumin gene by real-time PCR
815 and quantifying with reference to a control human genomic DNA (Roche). The reaction mixture
816 contained 1× TaqMan Universal PCR master mix, 300 nM of primers and 200 nM of the fluorogenic
817 probe, in a final volume of 30 µl. PCR cycle conditions were: 50°C for 2 min, 95°C for 10 min, and 40
818 cycles of 95°C for 15 s and 60°C for 1 min. Copy numbers of U5-Gag were determined with reference
819 to a standard curve prepared by concurrent amplification of serial dilutions of 8E5 cells containing
820 one integrated copy of HIV-1 per cell.

821

822 Productive HIV-1 infection in vitro was studied in suboptimally activated CD4⁺ T-cells (10⁶ cells/mL in
823 triplicate) exposed to the HIV-1 BaL strain (R5) (10 ng p24/ml). The cells were cultured in 96-U-well
824 plates for 14 days in the presence or absence of 2-DG (5 mM). Every 3-4 days, the culture
825 supernatants were removed and replaced with fresh culture medium with or without 2-DG. Viral
826 replication was monitored in the supernatants by p24 enzyme-linked immunosorbent assay (ELISA)
827 (XpressBio) or at day 3 by intracellular p24 staining (p24-FITC (clone KC57, Coulter) (Saez-Cirion et al.,
828 2010).

829

830 **Flow-assisted sorting of CD4 + T-cell subsets**

831 Cells were first selected based on size and structure to eliminate cellular debris. Then cell singlets
832 and living cells (not stained with LIVE/DEAD Fixable Aqua Dead Cell Stain Kit, Thermofisher) are gated
833 before proceeding with further selection based on phenotypical or functional markers (Figures S1,
834 S4, S5).

835

836 Resting (CD25⁻, CD69⁻, HLA-DR⁻) CD4⁺ T-cell subsets [naïve (**T_n**; CD3⁺, CD4⁺, CD45RA⁺, CCR7⁺,
837 CD27⁺, CD95⁻), central memory (**T_{cm}**; CD3⁺, CD4⁺, CD45RA⁻, CCR7⁺, CD27⁺), transitional memory

838 (**Ttm**; CD3+, CD4+, CD45RA-, CCR7-, CD27+) or effector memory (**Tem**; CD3+, CD4+, CD45RA-, CCR7-,
839 CD27-)] were sorted on a FACS ARIA III cell sorter (BD) using the following antibody panel: CD3-
840 eFLuor450 (eBioscience), CD4-alexaFluor700 (BD), CD45RA-ECD (BC), CCR7-PE_Cy7 (BioLegend),
841 CD27-APC (Miltenyi), CD95-PE (Miltenyi), CD25-FITC (BD), CD69-FITC (eBioscience) and HLA-DR-FITC
842 (BD). The gating strategy is depicted in Figure S1. The number of sorted cells varied from 0.5 to 5
843 million cells depending on the CD4+ T-cell subset and the donor. The purity of the sorted subset was
844 greater than 98%.

845

846 GFP+ and GFP- CD4+ T-cells were sorted 72 h after infection with VSV-G pseudotyped
847 NL4.3ΔenvΔnef/GFP particles (Figure S4A). For some experiments, GFP+ and GFP- cells were also
848 sorted into the following categories based on their expression of activation markers (CD25-ECD, HLA-
849 DR_PerCyP5.5) (Figure 4 and Figure S4B): high activation GFP+ [**H/+** (GFP+, CD25+,HLA-DR+)]; high
850 activation GFP- [**H/-** (GFP-, CD25+, HLA-DR+)]; low activation GFP+ [**L/+**, (GFP+, CD25-,HLA-DR-)]; and
851 low activation GFP- [**L/-** (GFP-, CD25-,HLA-DR-)].

852

853 For some experiments, CD4+ Tn and Tcm cells were sorted based on their level of glucose uptake
854 after 5 days of stimulation with anti-CD3 (Figure S5A). The cells were washed and incubated with 2-
855 NBDG (2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose) (Thermo Fisher, Ref. N13195)
856 at 75 μM in PBS for 30 min at 37°C. After 3 washes of 10 min each with fresh PBS, the cells were
857 stained with antibodies (CD3-eFLuor450 (eBioscience), CD4-alexaFluor700 (BD), CD45RA-ECD (BC),
858 CCR7-PE_Cy7 (BioLegend), and CD27-APC (Miltenyi)) and sorted as follows: **Tn HGLu** (CD3+, CD4+,
859 CD45RA+, CCR7+, CD27+, 2NBDG+); **Tn LGLu** (CD3+, CD4+, CD45RA+, CCR7+, CD27+, 2NBDG-); **Tcm**
860 **HGLu** (CD3+, CD4+, CD45RA-, CCR7+, CD27+, 2NBDG+); and **Tcm LGLu** (CD3+, CD4+, CD45RA-, CCR7+,
861 CD27+, 2NBDG-).

862

863 **Surface GLUT1 staining**

864 CD4+ T-cells were stained with HRBD-rFc, a recombinant fusion protein that specifically binds GLUT1
865 (Metafora-biosystems, Paris, France), and a secondary goat-anti-Mouse Alexa Fluor 647 antibody
866 (Thermofisher). HRBD is derived from the receptor-binding domain of the human T-cell leukemia
867 virus envelope glycoprotein that binds the extracellular domain of GLUT1 (Manel et al., 2005). The
868 following antibody panel was used to determine CD4+ T cell subsets: CD3-eFluor450 (eBioscience),
869 CD4-alexaFluor700 (BD), CD45RA-ECD (BC), CCR7-PE_Cy7 (BioLegend), CD27-PE (BD bioscience).

870

871 **Quantitative RT-PCR arrays**

872 The expression levels of an array of 96 genes in the CD4+ T-cell subsets were quantified by RT-qPCR
873 with a Biomark HQ system. Total RNA was extracted from 5×10^4 CD4+ T-cells with an RNA trace kit
874 (Macherey-Nagel, Ref. 740731.4) and treated with DNase, following the manufacturer's instructions.
875 Twenty microliters of RNA (> 10 ng) was reverse transcribed with Reverse Transcription Master Mix
876 (Fluidigm, 100-6298) (5 minutes at 25°C, 30 minutes at 42°C, and 5 minutes at 85°C). A specific target
877 preamplification (STA) was performed by adding PreAmp Master Mix, 96 Primers Mix and EDTA to
878 the cDNA, followed by STA cycling (95°C: 2 min, 18 cycles of [96°C: 5 s, 60°C 4 min]). The sample was
879 then treated with exonuclease I (New England Biolabs) (37°C: 30 min, 80°C: 15 min). Sample premix
880 (SsoFast EvaGreen Supermix with Low ROX (Biorad), DNA Binding Dye (Fluidigm), preamplified Exo 1-
881 treated sample) and assay mix (assay loading reagent (Fluidigm), Delta Gene primers (Fluidigm)) were
882 then loaded on primed 96.96 Dynamic Array chips (Fluidigm). The chips were transferred into a
883 Biomark HQ device (Fluidigm) for thermocycling, and fluorescence was acquired with the GE 96×96
884 PCR+Melt v2 program. Linear derivative mode baseline correction was applied. We used the
885 Normfinder algorithm (Aarhus University Hospital, Denmark) (Andersen et al., 2004) to identify the
886 optimal normalization gene among the assayed candidates for our experimental conditions. BENC1
887 was thus identified as the optimal normalization gene based on expression stability in the analyzed
888 samples (Table S3), and the gene expression values were plotted as $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = \Delta Ct_{\text{SAMPLE}} -$
889 $\Delta Ct_{\text{CONTROL}}$, and $\Delta Ct = Ct_{\text{TARGET GENE}} - Ct_{\text{BENC1}}$.

890

891 **Measurement of oxygen consumption and extracellular acidification rates**

892 The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured using
893 a Seahorse XF96 metabolic analyzer following the procedure recommended by the manufacturer.
894 Briefly, for all the experiments, different CD4⁺ T-cell populations were seeded at a concentration of
895 2×10^5 cells per well on XF96 plates (Seahorse Bioscience) precoated with 0.5 mg/ml Cell Tack
896 (Corning, Ref. 354240) immediately before adding Seahorse XF culture media to each well. Cells were
897 incubated for 50 min in a CO₂-free incubator at 37°C before loading the plate in the Seahorse
898 analyzer. Different programs were run on the Seahorse analyzer depending on the assay. Drug Panel
899 A (1) XFmedia 2) oligomycin (2.5 μM), 3) FCCP (0.9 μM) and 4) rotenone (1 μM) and antimycin A (1
900 μM)) was injected through ports A, B, C and D, respectively, for the mitochondrial stress test. Drug
901 Panel B (1) XFmedia 2) glucose (10 mM) 3) oligomycin (2.65 μM), and 4) 2-DG (100 mM)) was used
902 for the glycolysis stress test.

903

904 **Phenotyping after sorting**

905 In some experiments, sorted GFP⁺/⁻ CD4⁺ T-cells subset or CD4⁺ T bulk cells previously infected with
906 NL4.3Δnef/GFP/VSV-G with or without 2-DG or Etomoxir were incubated with CD3-eFLuor450
907 (eBioscience), CD4-alexaFluor700 (BD Biosciences), CD45RA-ECD (BC), CCR7-PE_Cy7 (BioLegend) and
908 CD27-APC (Miltenyi) to determine the CD4⁺ T-cell subset distribution. In addition, the activation
909 levels of sorted CD4⁺ T-cell subsets were assessed with CD25-ECD (BD Biosciences) and HLA-DR-FITC
910 (BD Biosciences). For both protocols, cells were incubated with the antibodies for 25 minutes and
911 then washed in PBS plus 1% FCS and fixed in 4% paraformaldehyde for flow cytometry on an LSRII
912 device (BD Biosciences). The data were analyzed with Kaluza software (Beckman Coulter).

913

914 **HIV-1 reactivation in CD4⁺ T-cells from HIV-1-infected individuals.**

915 Freshly isolated CD4+T-cells (negative selection kit, Stem Cell) from HIV-individuals undergoing
916 successful cART were seeded in 48-well plates (1×10^6 cells/well, in triplicate) and stimulated with
917 phytohemagglutinin-L (PHA-L, Roche, 1 $\mu\text{g}/\text{mL}$) and IL-2 (Miltenyi) 100UI with or without 2-DG (5
918 mM). The culture supernatants were collected every 3 to 4 days, and fresh medium +/- 2-DG was
919 added to the cultures. Supernatants were stored at -80°C , and HIV-1 p24 was analyzed later by
920 ultrasensitive digital ELISA (Simoa, Quanterix) (Passaes et al., 2017).

921

922 **QUANTIFICATION AND STATISTICAL ANALYSIS**

923 **Differential gene expression**

924 For each gene, we implemented a mixed effects model to detect differential expression between cell
925 types (Tn, Tcm, Ttm and Tem). We defined a model that included the type of cells as a fixed effect
926 and the patient as a random effect. A p-value was then obtained by implementing a likelihood ratio
927 test between the full model and a reduced model without the fixed effect. Heat maps were
928 generated by K-means clustering. Data were filtered by variance ($\delta/\delta_{\text{max}}=0.2$) to reduce background
929 noise. Gene expression data were centered to a mean value of zero and scaled to unit variance.

930

931 **Correlation between gene expression, metabolic parameters and HIV-1 susceptibility**

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

933

934 **Other analyses**

935 Values are presented in the graphs as medians and interquartile ranges. Statistical analyses were
936 performed using SigmaPlot (Systat Software). The asterisks represent statistically significant
937 differences (* $p < 0.05$; ** $p < 0.01$). Differences between CD4+ T-cell subsets in different conditions
938 were analyzed with nonparametric signed ANOVA and the multiple comparison Student-Newman-
939 Keuls method. Differences between GFP+ and GFP- CD4+ T-cells or control vs treatment culture
940 conditions were analyzed with paired t-tests. When multiple treatment conditions were tested,

941 ANOVA analyses and the Holm-Sidak method for multiple comparisons versus control group were
942 used, and significant differences between experimental conditions were shown as horizontal lines.

943

944

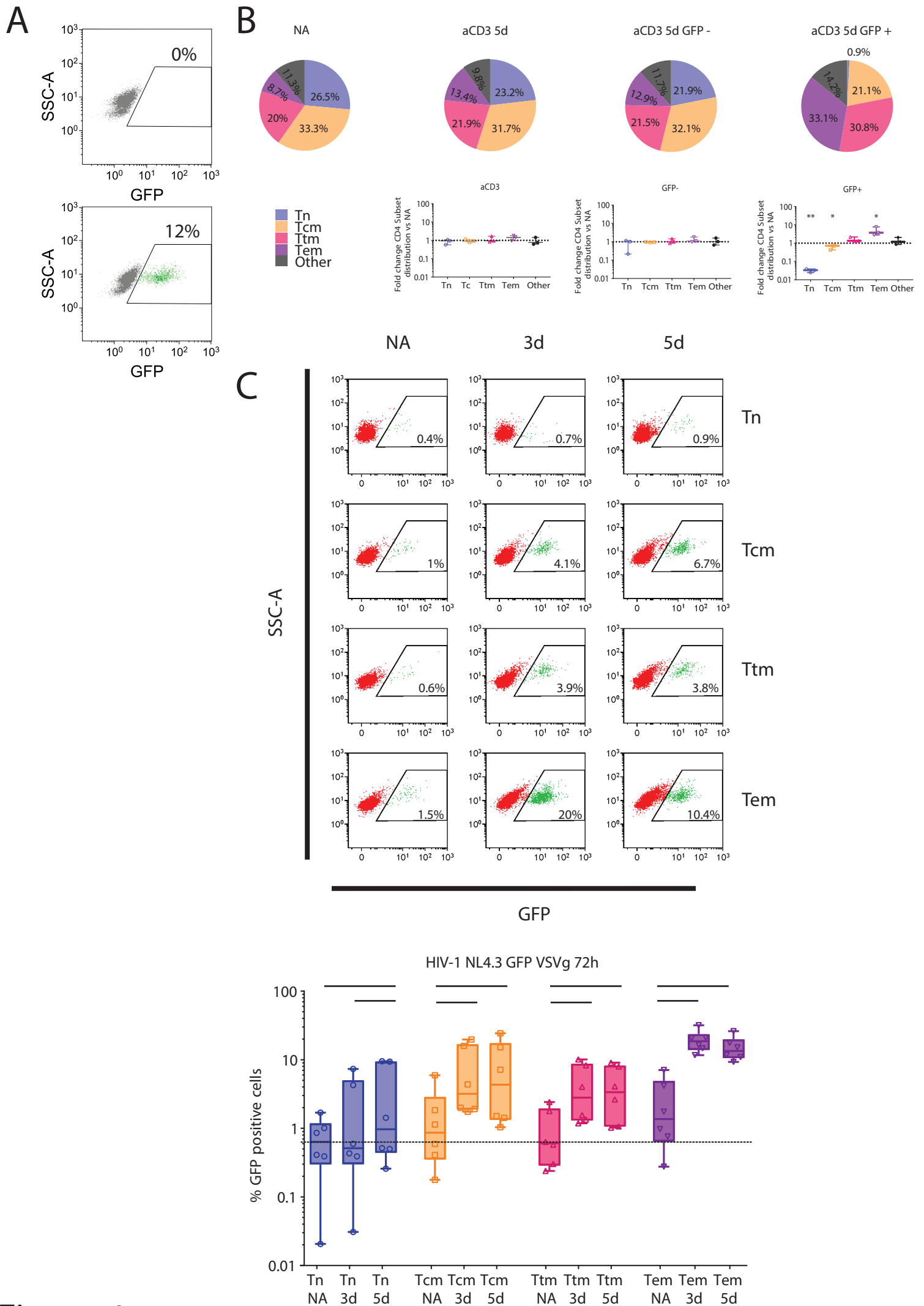


Figure 1

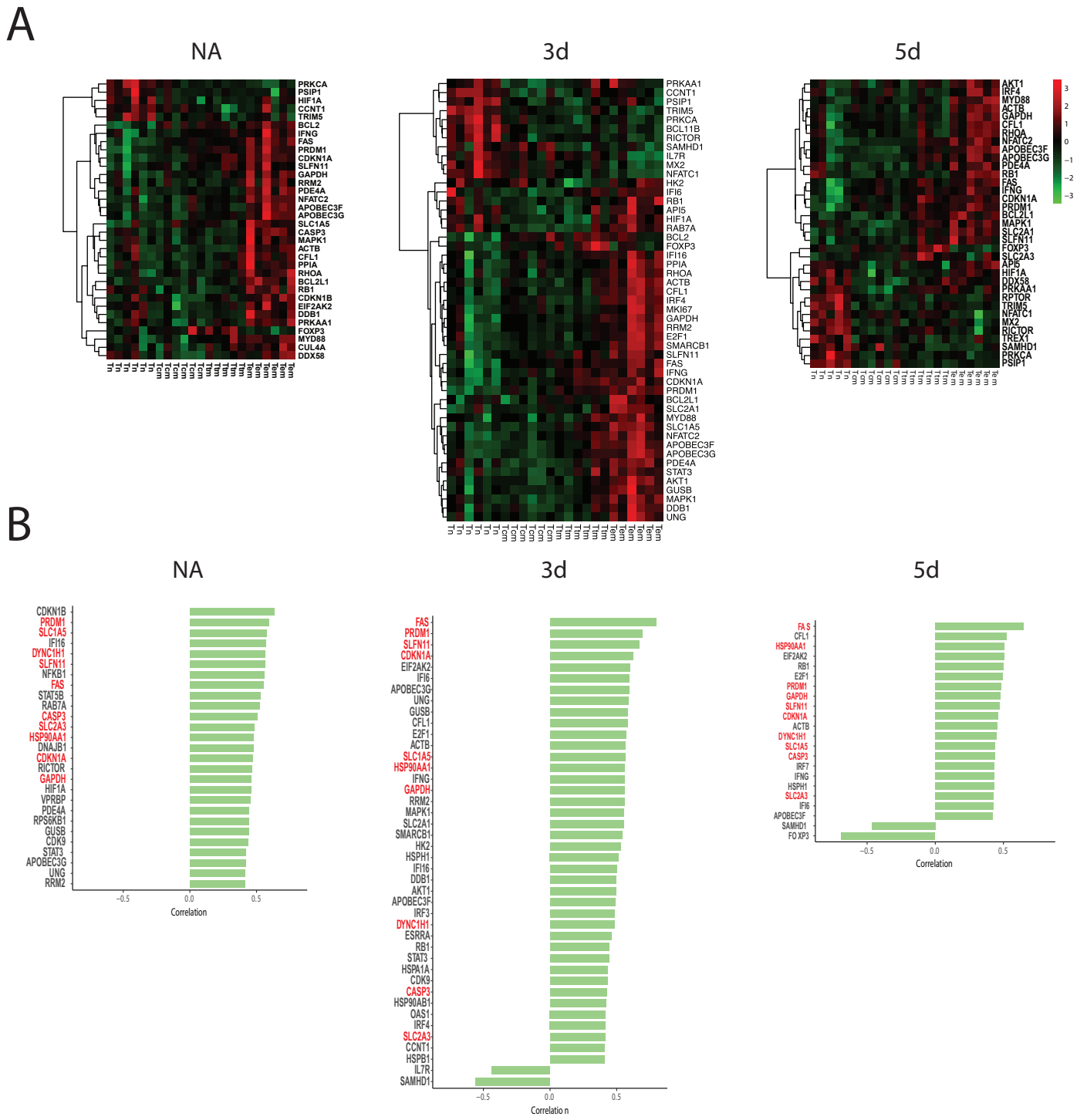
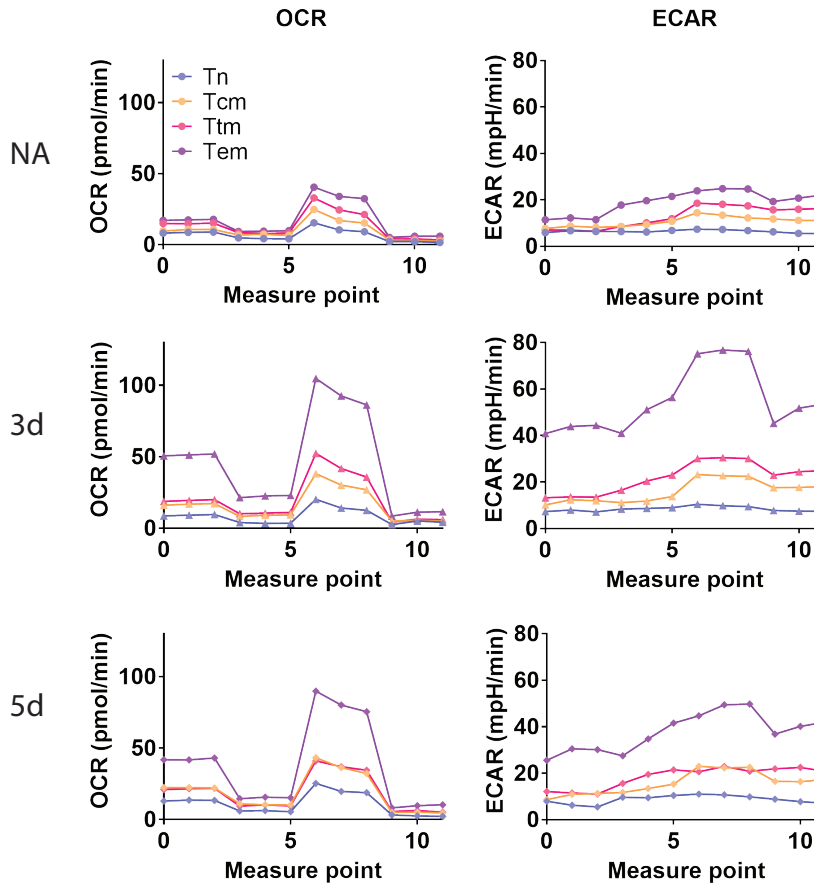
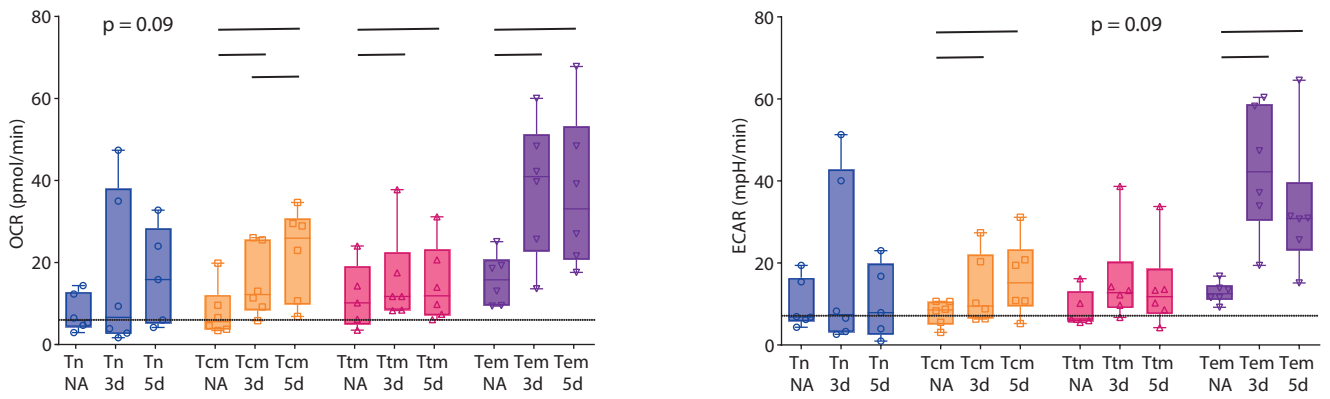


Figure 2

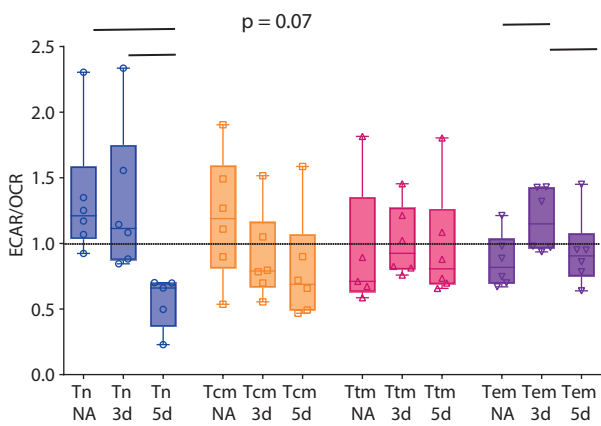
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B



C



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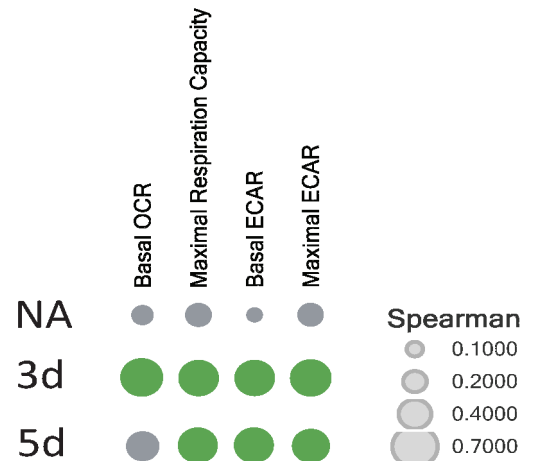
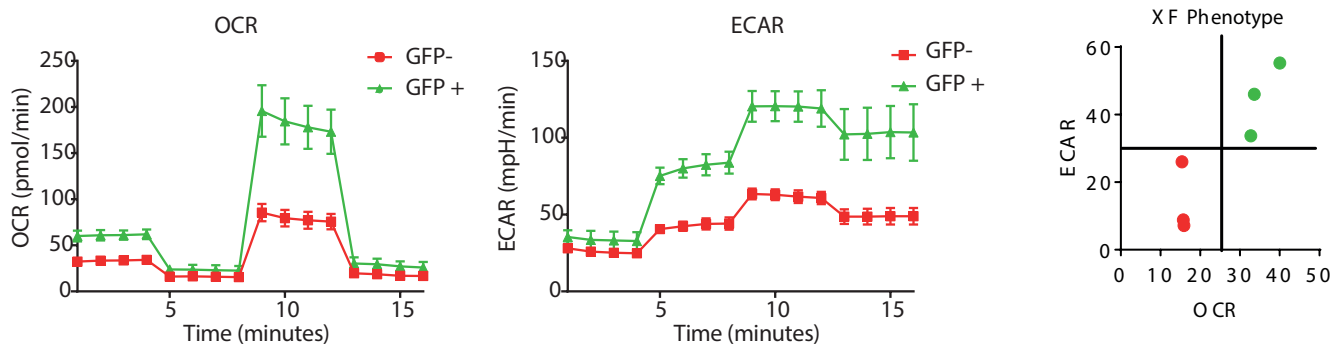
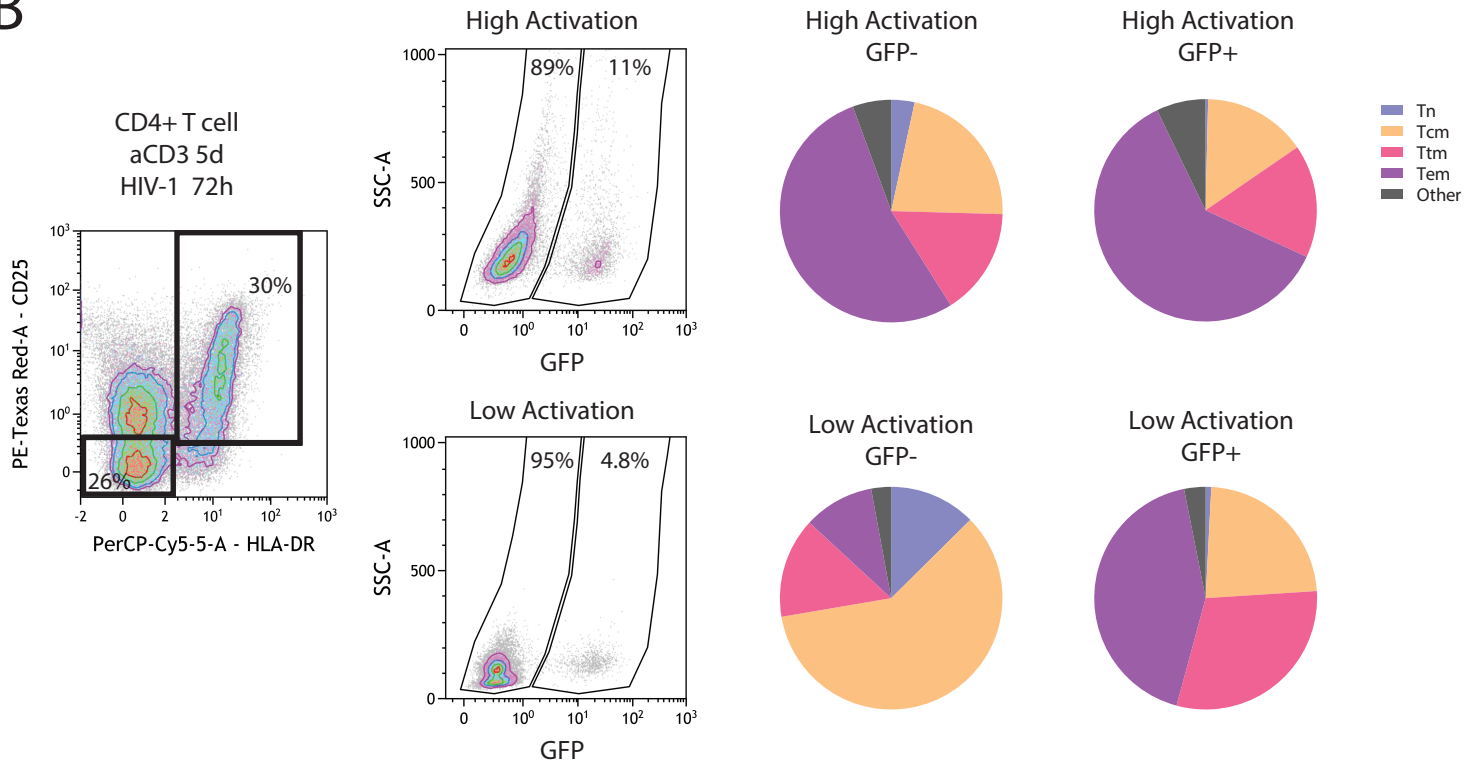
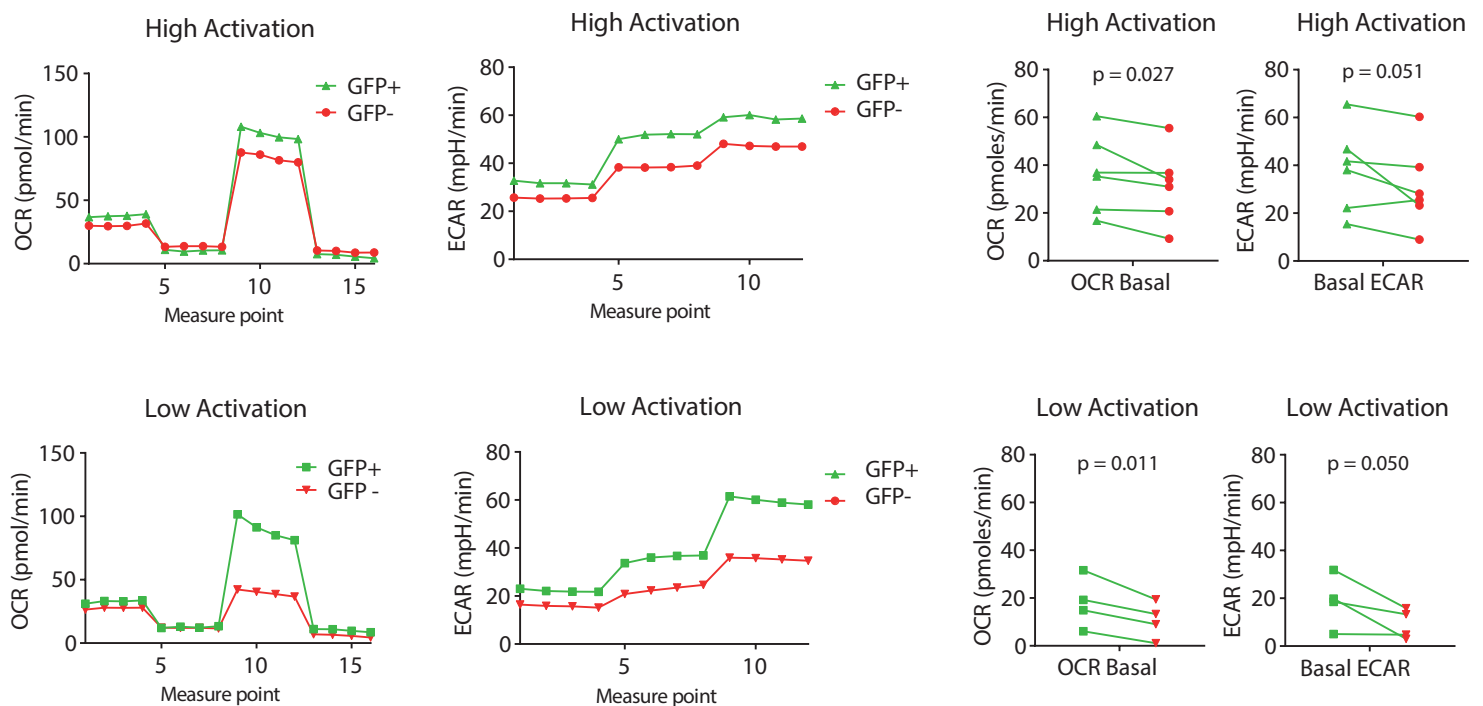
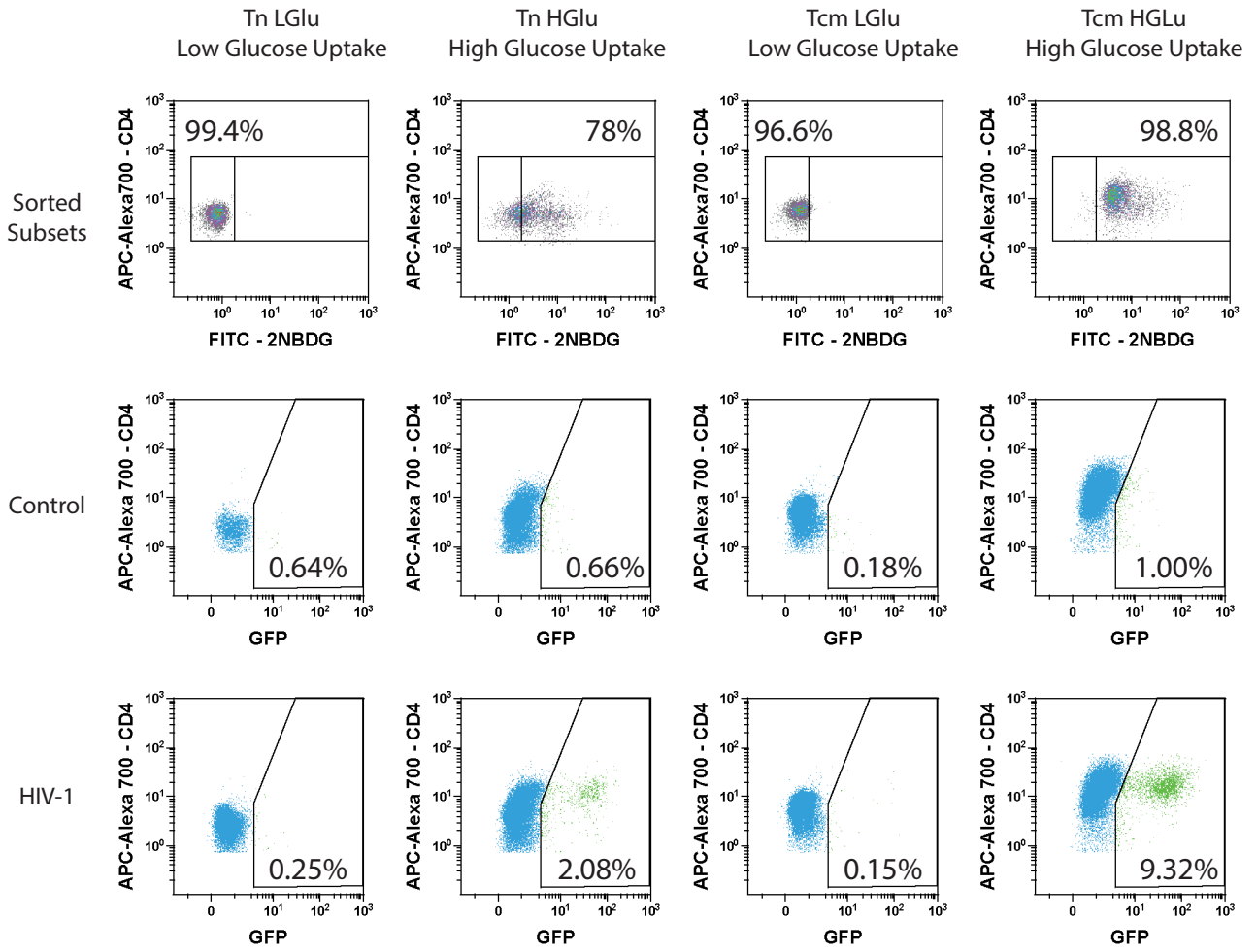


Figure 3

A**B****C****Figure 4**

A



B

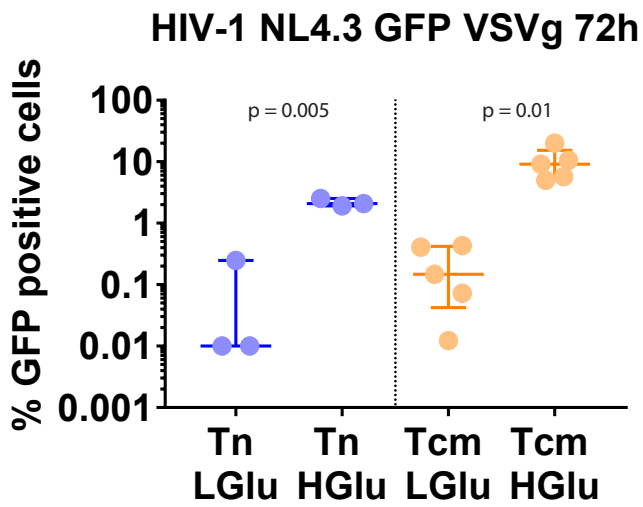
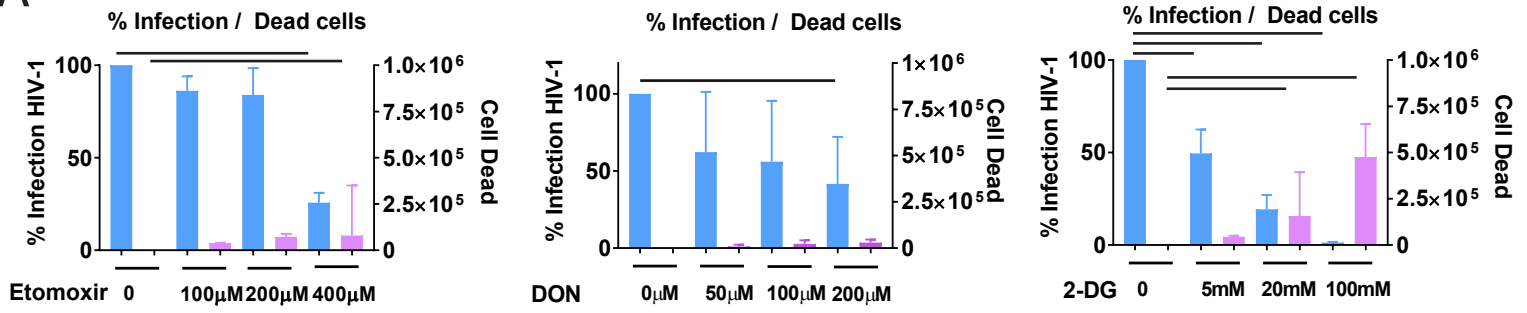
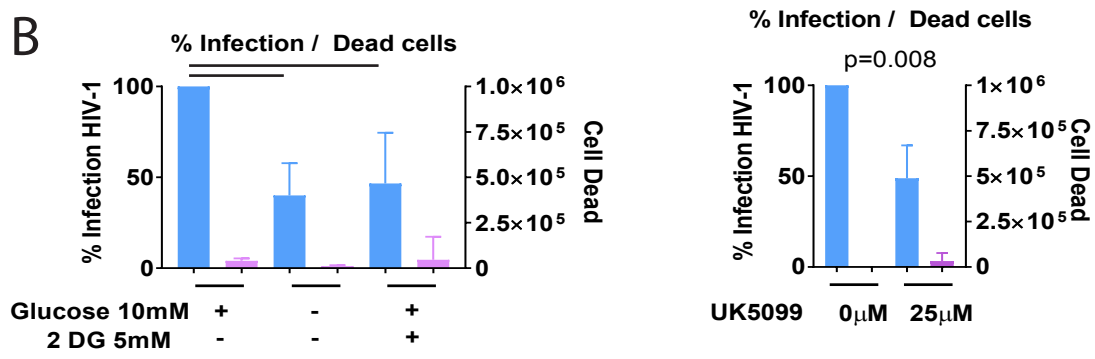


Figure 5

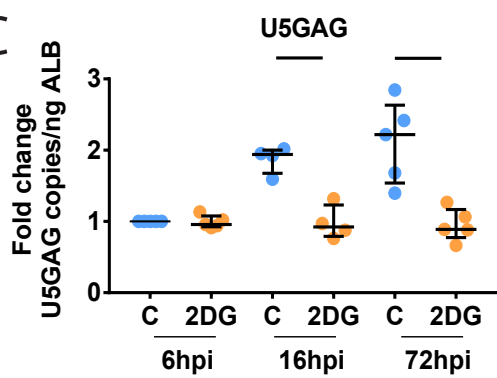
A



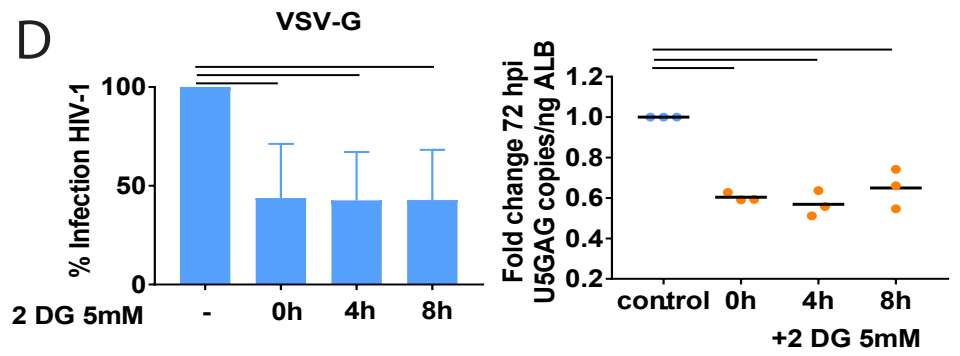
B



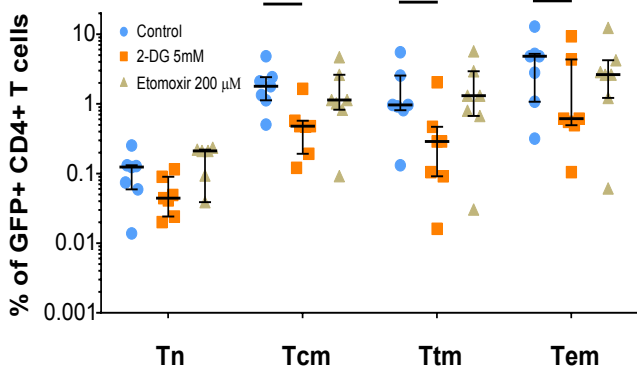
C



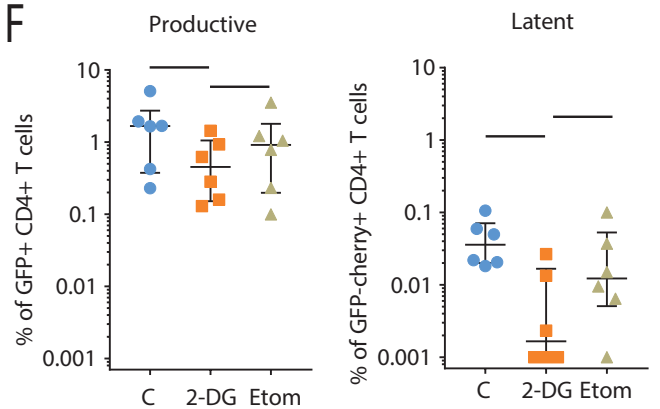
D



E



F



G

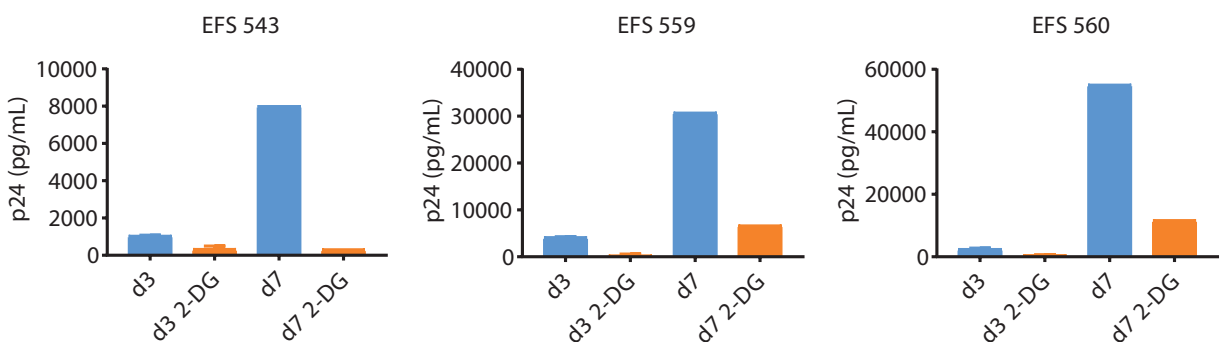


Figure 6

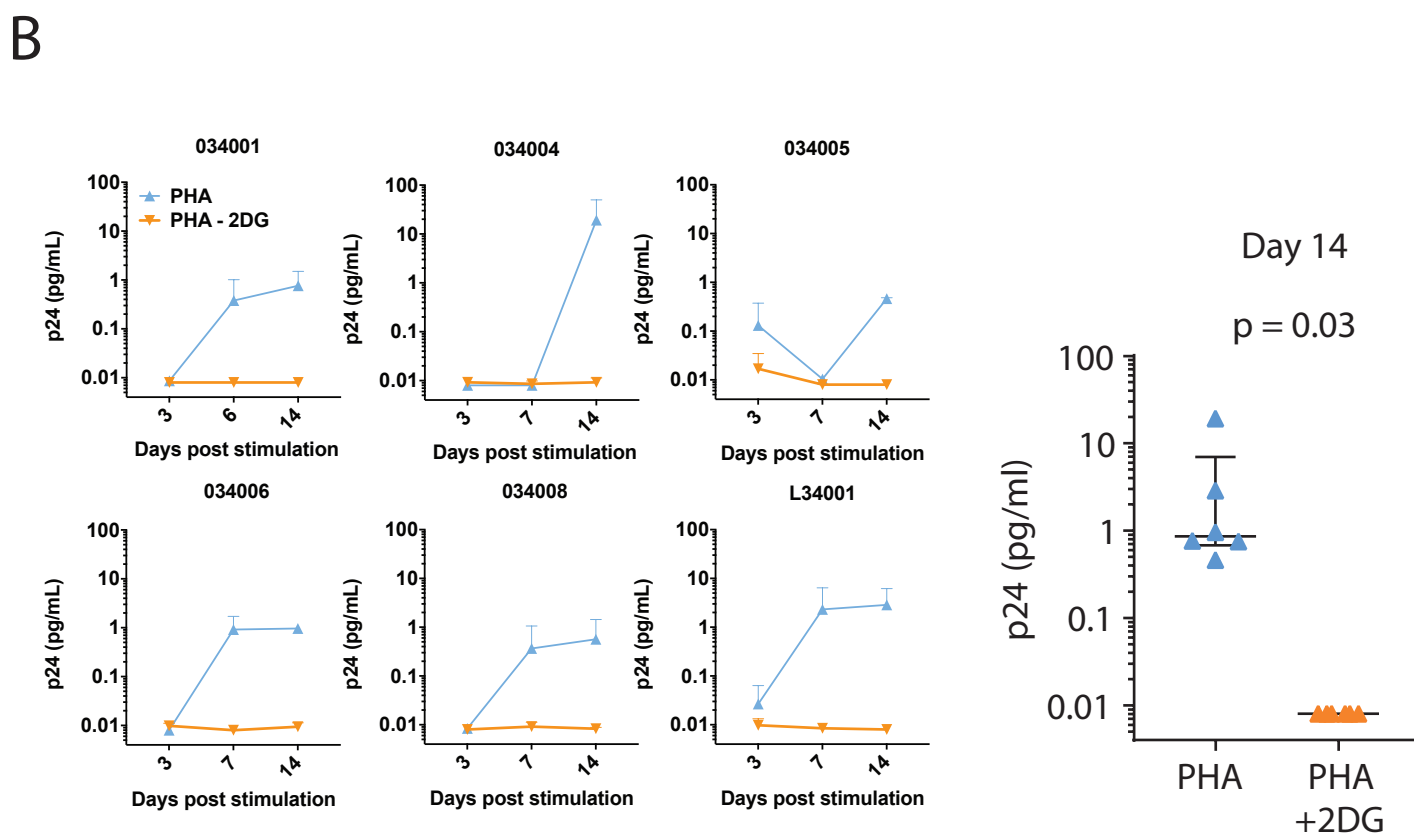
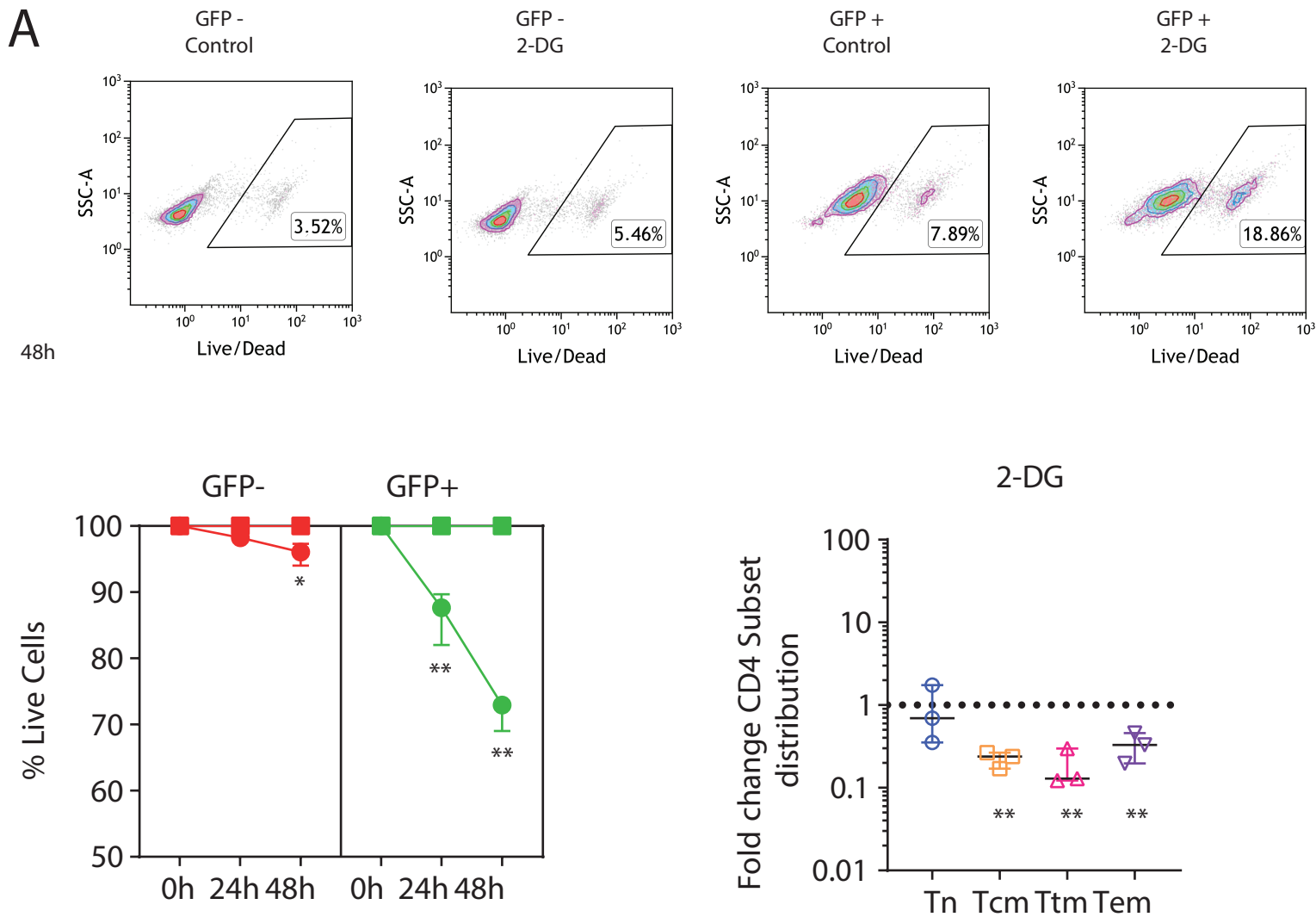


Figure 7

Oxphos
glycolysis

