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HIV Controllers Have Low Inflammation Associated with a Strong HIV-Specific Immune Response in Blood

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1 **HIV controllers have low inflammation associated with a strong HIV-specific**
2 **immune response in blood**

3

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24 Running Head: Immune response signature of HIV controllers.

25

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31 **Abstract**

32 HIV-Controllers (HIC) maintain control of HIV replication without combined
33 antiretroviral treatment (cART). The mechanisms leading to virus control are not fully
34 known. We used gene expression and cellular analyses to compare HIC and HIV-1
35 infected individuals under cART. In the blood, HIC are characterized by a low
36 inflammation, a down modulation of NK inhibitory cell signaling and an up regulation
37 of T-cell activation gene expression. This balance that persists following stimulation of
38 cells with HIV antigens, was consistent with functional analyses showing a bias towards
39 a Th1 and cytotoxic T cell response and a lower production of inflammatory cytokines.
40 Taking advantage of the characterization of HIC based upon their CD8+ T lymphocyte
41 capacity to suppress HIV-infection, we show that unsupervised analysis of differentially
42 expressed genes fits clearly with this cytotoxic activity allowing the characterization of
43 a specific signature of HIC. These results reveal significant features of HIC making the
44 bridge between cellular function, gene signatures and the regulation of inflammation
45 and killing capacity of HIV-specific CD8+T cells. Moreover, these genetic profiles are
46 consistent through analyses performed from blood to PBMC and T-cells. HIV
47 controllers maintain strong HIV-specific immune responses with low levels of
48 inflammation. Our findings may pave the way for new immunotherapeutic approaches
49 leading to strong HIV-1-specific immune responses while minimizing inflammation.

50 **Importance**

51 A small minority of HIV infected patients, called “HIV Controllers” (HIC) maintains
52 spontaneous control of HIV replication. It is therefore important to identify mechanisms
53 that contribute to the control of HIV replication that may have implications for vaccine
54 design. We observed a low inflammation, a down modulation of natural killer inhibitory
55 cell signaling and an up regulation of T-cell activation gene expression in blood of HIC
56 compared to patients under combined antiretroviral treatment. This profile persists
57 following in vitro stimulation of peripheral blood mononuclear cells with HIV antigens,
58 and was consistent with functional analyses showing a Th1 and cytotoxic T cell
59 response and a lower production of inflammatory cytokines. These results reveal
60 significant features of HIV controllers that maintain strong HIV-specific immune
61 responses with low levels of inflammation. These findings define the immune status of
62 HIC that is probably associated with the control of viral load.

63 **Introduction**

64 If left untreated, HIV-1 infection is characterized by a detectable HIV replication and a
65 rapid decline in CD4+ T lymphocytes leading to AIDS, whereas a small minority of
66 patients, called “HIV Controllers” (HIC) maintains spontaneous control of HIV
67 replication (1-3). Although, this population is heterogeneous and several mechanisms
68 leading to the control of HIV replication contribute to this outcome (4, 5), an efficient
69 HIV-specific CD8+ T cell response appears to be a key factors associated with the
70 control of viremia. HIC maintain polyfunctional CD8+ T cell responses to HIV-1
71 antigens (6, 7) in particular to gag polypeptide (8). A population of HIC exhibiting
72 strong functional HIV-specific cytotoxic CD8+ T cell responses (2) has been
73 characterized (9). Indeed, primary CD8+ T cells from many HIC are able to suppress
74 HIV-1 replication *ex vivo* by efficient granzyme B and perforin mediated killing of
75 infected T cells (10). In previous reports (9, 11), we have defined two sub-groups of
76 HIC in function of the capacity of their CD8+ T cells to *ex vivo* suppress HIV-1
77 infection in autologous CD4+ T lymphocytes (12). Strong responders HIC (SRHIC)
78 exhibit a higher CD8+ T cell HIV-suppressive capacity than weak responders HIC
79 (WRHIC). It was also observed that WRHIC maintain a large pool of HIV Gag-specific
80 central memory T cells that are highly functional and readily expandable upon antigen
81 stimulation, able to reach functions and high frequency similar to those observed in
82 SRHIC (13). A negative correlation between expandable Gag-specific memory T cell
83 responses and residual viremia suggest that these cells actively contribute to the
84 sustained suppression of virus replication (14).

85 In order to identify mechanisms that may contribute to the spontaneous control of HIV
86 replication in HIC, we hypothesized that comparison of blood gene expression profiles

87 of HIC and chronically HIV-infected patients, with high CD4+ T cells counts and
88 suppressed plasma HIV viral load while on cART, might help to identify features of
89 spontaneous HIV control. In a second approach, cellular and genetic analyses of PBMC
90 of these patients stimulated in vitro with HIV antigens were performed. Finally, in order
91 to further characterize the SRHIC and WRHIC, we compared gene profiles of purified
92 CD4+ and CD8+ T lymphocytes. Globally, our results identified key profiles of
93 immune control of viral replication delineating implications for the design of strategies
94 aimed to a sustained remission of HIV infection.

95

96 **Results**

97 **Characteristics of the study population**

98 The blood samples of the cohort comprised 53 HIC subjects and 27 cART treated
99 patients. Clinical characteristics of the two groups are shown in Table 1. No statistically
100 differences were observed between the two groups in terms of age (median of 47 vs 52
101 years old), viral load (1.6 vs 1.3 RNA log₁₀ copies/ml), CD4⁺ T (689 vs 588 cells/mm³)
102 and CD8⁺ T (829 vs 725 cells/mm³) cell counts. No statistically differences were also
103 observed for these parameters between the 10 SRHIC and 9 WRHIC subjects used for
104 purification of CD4 and CD8 T cells, and between HIC and cART used for PBMC
105 purification.

106 **HIC are characterized by an increase in T cell activation and a down modulation** 107 **of inflammatory genes in the blood.**

108 Gene expression profile analysis of whole blood of HIC (n=53) and cART patients
109 (n=27) showed that 1244 genes differentially expressed. Globally, these genes belong to
110 pathways involved in innate immunity and NK signalling, T-cell activation and
111 inflammation. HIC were clearly characterized by a down modulation of genes related to
112 inflammation response with a down regulation of TLRs and TREM1 pathways (TLR1 (-
113 1.73), *TLR4* (-1.91), *TLR6* (-1.87), *TLR8* (-2.61), *CD14* (-1.66), *TREM1* (-2.12) and
114 *TYROBP* (-1.62) and of many pro-inflammatory genes including neutrophils
115 chemotactic factor *IL-8/CXCL8* (-8.14) and its receptors *CXCR1* (-3.12) and *CXCR2* (-
116 4.09) (Fig 1A). More precise analysis revealed also a down modulation in HIC of
117 receptors for the Fc portion of immunoglobulin (*FCGR3A/FCGR3B* (-7.65), *FCER1G*
118 (-1.94) including the CD32A gene (*FCGR2A*) (-2.77) as well as killer cell

119 immunoglobulin-like receptors (*KIR2DL1/KIR2DL3* (-1.85), *KIR3DL1* (-1.82),
120 *KIR2DL4* (-1.7), *KIR2DL5A* (-1.25)) and killer cell lectin like receptors *KLRD1* (-1.76),
121 *KLRC3* (-1.96) and *KLRC2* (-1.69) (Fig. 1A). This result contrasts with an upregulation
122 in HIC of the expression of Src family kinases, *FYN* (+1.66) and *ZAP70* tyrosine kinase
123 (+1.73), *IFNG* (+1.51) and *STAT1* genes (+1.54) (Fig. 1B). Interestingly, the low
124 inflammatory profile in HIC is consistent with the down modulation of inflammation
125 regulatory pathways mitogen-activated protein kinase 1 (*MAPK1*) (-1.59) and PI3-
126 kinase *PIK3CG* (-1.52) and *PIK3CB* (-1.61) (Fig. 1B), a critical regulatory factor that
127 connect immune stimulation and suppression during inflammation (15, 16). Globally, as
128 illustrated in Fig. 1B, analysis revealed significant direct interactions between these
129 pathways linking the down modulation of *PIK3CG* with an increase of T-cell activation
130 (*ZAP70/FYN*) and a decrease of innate cell inhibitory signaling of NK cells (*KIRs*).

131 We have also looked for immunometabolism pathways that play important role in the
132 modulation of the immune system. In whole blood, we have observed an enrichment of
133 gluconeogenesis and lipid metabolism pathways. In that respect, we observed a down
134 regulation in HIC compared to cART, of *ALDOA* (aldolase, fructose-bisphosphate A) (-
135 1.57), *BPGM* (bisphosphoglycerate mutase) (-2.31), *ME2* (malic enzyme 2) (-1.61),
136 *PGAM1* (phosphoglycerate mutase 1) (-1.54) and *PGAM4* (phosphoglycerate mutase 4)
137 (-1.51) and an up-regulation of *ENO3* (enolase 3) (+1.64). In lipid metabolism, there
138 was a down modulation of *PTGS2* (prostaglandin-endoperoxide synthase 2) (-2.88),
139 *CD36* (-2.24), *ACSL1* and 4 (acyl-CoA synthetase long chain family members) (-2.97
140 and -1.56), *SIPRI* and *SIPR3* (sphingosine-1-phosphate receptors) (-1.62 and -1.70),
141 *PCTP* (phosphatidylcholine transfer protein) (-2.13) and *PTGS2* (prostaglandin-
142 endoperoxide synthase 2) (-2.88). In contrast, *PTGR2* (prostaglandin reductase 2)

143 (+2.15), *PLA2G2D* (phospholipase A2 group IID) (+2.18), *SREBF1* (sterol regulatory
144 element binding transcription factor 1) (+1.55) were upregulated in HIC compared to
145 cART. However, the modulated genes did not allow to predict an activation or
146 inhibition of these pathways in HIC compared to cART.

147 **HIC cellular responses to HIV peptides are associated with a low inflammatory**
148 **gene expression associated with Th1 and cytotoxic profiles**

149 We analyzed differences in gene expression of PBMC isolated from HIC (n=25) and
150 cART (n=15) patients, before and after in vitro stimulation with pools of HIV peptides.

151 Gene expression analysis before HIV peptides stimulation revealed that 113 genes were
152 differentially expressed. Analysis on the Ingenuity Pathway software showed that these
153 genes are significantly involved in inflammation with a down regulations of many genes
154 such as *IL1A* (-2.28) and *IL1B* (-7.02), *IL6* (-5.71), *CXCL5* (-6.89), *CXCL13* (-1.97),
155 *CCL23* (-1.68), *CXCL1* (-4.19), *TREM1* (-1.66) and *CD14* (-2.86) (Fig. 2A). Some of
156 these genes are also related to granulocytes adhesion and diapedesis (*IL1A*, *IL1B*,
157 *CXCL5*, *CXCL1*, *FPR1*, *FPR2*, *CCL22*, *CXCL13*, *CCL19*, *CCL23*) and to IL6, HMGB
158 and TREM1 signaling (*IL1B*, *IL6*, *CD14*, *IL1A*, *FOS*, *LAT2*, *RHOA*, *TREM1*). We also
159 observed a down regulation of genes involved in iron homeostasis pathway such as
160 *HBA1/HBA2* (-12.93), *HBB* (-12.3), *HBG1* (-8.14), *HBG2* (-7.58), *IL6* (-5.71), *ALAS2* (-
161 4.52), *SLC11A1* (-2.24) and *SLC25A37* (-1.88). Likewise, gene expression analysis of
162 HIV peptides stimulated PBMC between HIC and cART revealed that 144 annotated
163 genes were differentially expressed. Pathway analyses showed, as for unstimulated
164 cells, a down regulation of genes belonging to inflammatory immune response,
165 including *CD14* (-5.12), *CXCL8* (-1.84), *TREM1* (-1.71) and *IL6* (-7.78), as well as
166 *CXCL5* (-7.87), *IL1B* (-5.45), *IL1A* (-4.74), *CCL3L1* (-4.17), *CXCL1* (-3.82) and *CCL24*

167 (-3.55) (Fig 2B). We further observed a significant up regulation of genes related to the
168 interferon pathway such as *IFIT1* (+3.54), *IFI44L* (+2.50), *IFI44* (+1.94), *MX1* (+2.02),
169 *OAS3* (+1.91) (Fig 2C).

170 These genetic characteristics were found to be consistent with the profile of cytokine
171 production of in vitro stimulated PBMC from HIC (20 samples) and cART (15
172 samples), as shown in Fig. 3. We observed a lower production of IL1 β and a higher
173 production of IP10, TNF α and MIP-1 β in HIC compared to cART as measured by
174 Luminex (Fig. 3A). This result was confirmed by ICS analysis after PBMC stimulation
175 with HIV peptides showing a higher frequency of CD8+ T cell producing TNF α , MIP-
176 1 β and IFN γ in HIC patients compared to cART (p=0.0127, Mann-Whitney test) (Fig.
177 3B). In contrast, no difference was observed in the profile of cytokine production for
178 CD4 T cells of HIC and cART (Fig. 3C).

179 **Genetic and functional analyses of CD8+ T cells from SRHIC and WRHIC reveal**
180 **specific signatures.**

181 We analysed cytokine patterns of in vitro stimulated PBMC and gene expression
182 profiles of purified CD4 and CD8+ lymphocytes from SRHIC and WRHIC. PBMC
183 stimulation with HIV peptides led to a significant higher production of IL-2, IP-10,
184 Granzyme A, Perforin and MIP-1 β in SRHIC as compared to WRHIC (Fig. 4A), which
185 is consistent with a stronger Th1- and T effector-cytokines response in SRHIC subjects
186 (Fig. 4B). Phenotypic analyses in ICS assay confirmed a higher frequency of CD8+ T
187 cells producing cytokines in SRHIC compared to WRHIC group (p=0.031, Mann-
188 Whitney test), specially MIP-1 β (p=0.024) (Fig. 5A). CD8+ T cells from SRHIC and
189 WRHIC were highly polyfunctional (55 to 60% of cells exhibit 2 or 3 cytokines) in both
190 groups. Although CD4+ T cells from both SRHIC and WRHIC patients were highly

191 polyfunctional (60 to 75% of the cells exhibit 2 or 3 cytokines), no differences were
192 observed between groups in terms of cytokine production following HIV peptide
193 stimulation (Fig. 5B).

194 We then compared gene expression profiles of *ex vivo* CD8⁺ and CD4⁺ T lymphocytes
195 purified from SRHIC and WRHIC. In contrast to CD4⁺ T lymphocytes (Fig. 6A),
196 unsupervised hierarchical clustering analysis of CD8⁺ T lymphocytes showed a perfect
197 clustering of SRHIC and WRHIC groups (Fig. 6B). We found 804 annotated
198 differentially expressed genes between SRHIC and WRHIC CD8 cells. Analysis of
199 gene expression profiles of CD8⁺ T lymphocytes showed an up regulation in SRHIC of
200 genes involved in the IFN γ pathway (Fig. 7), while proinflammatory genes such as
201 *CXCL8* (-3.53), *IL1B* (-2.28), *IRAK3* (-1.61), *TYROBP* (-3.13) and *FCER1G* (-3.37)
202 were down regulated. CD8⁺ T lymphocytes from SRHIC exhibited also a significant
203 upregulation of *CX3CRI* (+2.21) gene expression, a marker of CD8 effector memory
204 cells (17).

205 Among 804 genes differentially expressed between CD8⁺ T cells from SRHIC and
206 WRHIC, 133 were also part of those identified in blood gene expression differences
207 between HIC and cART (Fig. 8A). These genes are mainly associated with a down
208 modulation of inflammation. Among the 671 genes differentially expressed specifically
209 between CD8⁺ T cells from WRHIC and SRHIC (excluding the 133 genes
210 differentiating blood gene expression of HIC from cART), four main functions were
211 identified: three were predicted as activated (T cell response, cytotoxicity of leukocytes
212 and killing Natural Killer cells) and one was predicted as inhibited (activation of
213 leukocytes). The down regulation of genes such as *NFKB1* was consistent with the
214 decrease of leukocyte activation and increase of leukocyte toxicity (Fig. 8B).

215 These data reveal significant features of HIC making the bridge between HIV-specific
216 cellular function; i.e polyfunctionality, low proinflammatory responses, cytotoxic
217 activity and gene signatures. Interestingly enough, these genetic profiles are consistent
218 through the analyses of *ex vivo* whole blood and PBMC to analyses performed at the
219 cellular population levels.

220 **Discussion**

221 We report here results of extensive functional and gene expression analyses performed
222 in whole blood and at cellular level through PBMC and purified CD4 and CD8 T cells
223 in a cohort of HIC. Globally, these analyses performed through the different
224 compartments were consistent. They show that HIC individuals, as compared to
225 chronically HIV-1 infected individuals under cART have a low inflammatory
226 background which contrasts with activation of adaptive immune response pathways.
227 Interestingly this balance persists following *in vitro* stimulation of cells with HIV
228 antigens. This genetic profile was also consistent with functional analyses as assessed
229 by the production and cellular expression of cytokines. Finally, taking advantage of the
230 characterization of HIC based upon their *in vitro* CD8+ T lymphocyte capacity of
231 killing HIV-infected cells, we show clearly that unsupervised genetic analysis of
232 differentially expressed genes fits clearly with this cytotoxic activity. Here again we
233 found a balance between low activation and the commitment of genes associated with
234 cytotoxicity and T cell response.

235 Although cART has significantly improved the prognosis of HIV infected individuals,
236 they remain at increased risk of morbidity and mortality (18, 19). These clinical events
237 are supposed to be related to residual immune activation and inflammation in cART-
238 treated patients. The immune activation is also associated to the poor HIV-specific
239 response in chronically infected patients (20). Several studies have shown that HIC
240 exhibited cellular and serological markers of immune activation and inflammation
241 despite a spontaneous control of HIV replication (21-24). However, no evidence of
242 persistent inflammation was observed when HIC were defined using stringent criteria in
243 relation to the cutoff level of viremia (≤ 50 copies/mL) and a minimum follow-up time

244 of >5 years, compared to HIV uninfected subjects (25). We found here, as compared to
245 cART patients, that the level of inflammatory gene expression remains still dramatically
246 reduced in HIC with a significant down regulation of TLRs, TRIM1 and CXCL8/IL8.
247 This result extends several observations showing that HIC have significantly lower
248 levels of IL-8 mRNA when PBMCs were exposed to exogenous HIV-1 compared to
249 HIV-progressors, cART treated or not, and HIV uninfected control (26). It was also
250 observed a higher expression of CXCL8 in untreated HIV-1 infected progressors and
251 cART nonresponders when compared to LTNPs (long term non progressors) and cART
252 responders, respectively. Furthermore, a negative correlation of plasma levels of
253 CXCL8 with CD4 counts was found in HIV-1 infected cART naïve subjects, while the
254 CXCL8 levels positively correlated with viral load in the cART treated children (27).
255 These observations suggest a strong link between CXCL8 through its proinflammatory
256 action, to viral replication and disease progression. On the other hand, El-Far M et al.
257 (28) underlined the role of proinflammatory IL-32 cytokine in the failure of virus
258 replication control in HIC. We did not find any differences between HIC and cART
259 patients in the expression of IL-32 gene in our study where there was no failure to
260 control viral replication, neither in HIC nor in cART patients. Beside the down
261 regulation of inflammatory genes, HIC down regulated many genes belonging to the
262 natural killer cell signaling pathway such as receptors for the Fc portion of
263 immunoglobulin, inhibitory killer cell immunoglobulin-like receptors and killer cell
264 lectin like receptors. Interestingly, studies on HIV slow progressors linked the
265 protective effect of NK cells with certain killer immunoglobulin-like receptors and their
266 ligands the human leukocyte antigen-class I molecules (HLA) on the target cells (29,
267 30). The responsiveness of NK cells varies depending on the number of inhibitory

268 receptors (iKIR) expressed in particular KIR2DL1/KIR2DL3 (29, 31, 32). Interestingly,
269 expansion of the activating KIR3DS1+ and the inhibitory KIR3DL1+ NK cells are
270 increased in patients with acute HIV-1 infection in the presence of HLA-B Bw480I.
271 However, it was not associated with reduction in HIV levels in the blood. Engagement
272 of the inhibitory KIR3DL1 receptor on these NK cells with its ligand on the target HIV
273 infected cells could lead to the inhibition of NK cell cytotoxicity. Similarly, studies
274 have shown that CD56⁻ CD16⁺ NK cells, which are expanded in HIV-viremic
275 individuals, have impaired function and high expression of inhibitory KIR2DL2 and
276 KIR2DL3 receptors, which would explain their defective lytic capability toward HIV-
277 infected cells (33). Although we did not evaluate the functional capacity of NK cells in
278 HIC, one can hypothesis that the down regulation of iKIR, observed in HIC may result
279 in strong NK cell activation leading to viral load control.

280 We also observed a down regulation in HIC of receptors for the Fc portion of
281 immunoglobulin (*FCGR3A/FCGR3B, FCGR2A and FCER1G*). Many studies indicate
282 that antibody-induced effectors responses mediated through FCGR signaling contribute
283 to the control and prevention of HIV-1 infection (34-36). FCGR2A (CD32A) receptor
284 has also been reported as a marker of the CD4⁺ T cell HIV reservoir in HIV-infected
285 patients (37), but more recently contradictory works have shown that CD32 is not a
286 marker of HIV-1 reservoir but of CD4⁺ T cell activation in HIV⁺ individuals (38, 39).
287 Despite that the role of the FC receptors in virus control remains to be thoroughly
288 explored, one can speculate that the down regulation of these receptors could be
289 associated with both the lower activation/inflammation and HIV reservoir observed in
290 HIC compared to cART (40). It was also reported that the quality rather than the
291 number of the FCGR signaling, could be responsible of the wider poly-functional Fc-

292 mediated responses observed in HIC (36, 41). In parallel, there is a down regulation in
293 HIC of mitogen-activated protein kinase 1 (*MAPK1*) and PI 3-kinase (*PIK3CG* and
294 *PIK3CB*), both are critical regulatory factor of immune stimulation and suppression
295 during inflammation (15, 16, 42). In mice, an inhibition of PI3K promotes adaptive
296 immunity and CTL activities (16, 43). Here, we observed a down regulation of PI3K
297 associated to a down regulation of many inflammatory genes including IL-4 especially
298 in HIC presenting a strong viral inhibition capacity. Globally, the observation in HIC of
299 a link between the low expression of *PIK3CG* and both an activation of T-cell signaling
300 and a down modulation of inflammatory pathways is reminiscent to the action of this
301 “switcher” in the balance between immune suppression and inflammation (16). HIC
302 seem to develop an efficient adaptive immune response through a modulation of
303 expression of regulatory molecules of cytoplasmic signal transduction pathways *FYN*,
304 *ZAP70*, *MAPK1*. Indeed, increase in expression of Src family kinases, *FYN* and *ZAP70*
305 tyrosine kinase in HIC are in favor of activation of T cells through the TCR, which
306 allows a specific immune response (44, 45). This specific response was associated to a
307 drastic down regulation of chemoattractive molecules such as *CXCL5*, *IL1B*, *IL1A*,
308 *CCL3L1*, *CXCL1* and *CCL24* in HIV-peptides stimulated PBMC of HIC compared to
309 cART. The same profile was observed with CD8 T lymphocytes of SRHIC compared to
310 WRHIC, that also have less proinflammatory response, through down regulation of
311 mRNA of *CXCL8*, *S100A8*, *S100A9* and *IL1B*, while the *IFNG* response was activated.
312 Immunological and virological aspects in the blood, gut associated lymphoid tissues
313 (GALT) and lymph nodes of HIC and cART showed the crucial role in the virus control
314 of both HIV specific responses and immune activation (44, 45). Our observations
315 highlight only mechanisms involved in the blood of HIC compared to cART patients.

316 Though, HIV infection induces also the expression of different components of the
317 inflammasomes in GALT (46) and both the immune regulation and delayed progression
318 to AIDS were associated with a particular activation phenotype of T cells in GALT
319 from HIV-controllers (47). Furthermore, in HIV infection the immune activation and
320 inflammation were also associated with immunometabolism reprogramming through the
321 use of glucose and fatty-acid (48). In whole blood, we observed an enrichment of
322 gluconeogenesis and lipid metabolism pathways in differentially expressed genes
323 between HIC and cART, but it was not possible to determine if there was activation or
324 inhibition of these pathways.

325 Altogether, we show that HIC associate an anti-inflammatory state and strong adaptive
326 immune response to virus that probably allows for the control of viral loads below the
327 limits of detection. Efficient HIV therapeutic vaccine would mimic such response
328 profiles by inducing strong HIV-specific immune response whereas minimizing
329 inflammation.

330

331 **Materials and Methods**

332 **Patients and samples**

333 Whole blood samples were collected from 53 HIV HIC subjects of the ANRS CO21
334 CODEX cohort and 27 HIV-cART treated patients followed in Henri Mondor Hospital
335 (Créteil, France). HIC individuals were never treated with cART, HIV-infected for at
336 least 5 years and with last five consecutive plasma HIV RNA < 400 HIV-RNA
337 copies/ml (49). Control cART patients exhibited plasma HIV RNA <50 copies HIV-
338 RNA/ml for at least 2 years and CD4 lymphocytes ≥ 500 cells/mm³. CD4 and CD8 T
339 lymphocytes were purified from SRHIC and WRHIC subjects, and Peripheral blood
340 mononuclear cell (PBMC) from HIC and cART patients. The study protocol was
341 approved by the regional investigational review board (Comité de Protection des
342 Personnes Ile-de-France VII and IX) with approval reference 05-22 and 10-023. The
343 study protocol was performed in compliance with the tenets of the Declaration of
344 Helsinki.

345 **RNA isolation and microarray sample preparation**

346 Whole blood RNA was purified using Tempus™ Spin RNA Isolation Kit
347 (ThermoFisher scientific). PBMC, CD4- and CD8-lymphocytes RNA were purified on
348 Qiagen RNeasy Micro Kit. RNA was quantified using a ND-8000 spectrophotometer
349 (NanoDrop Technologies, Fisher Scientific, Illkirch Cedex, France) before being
350 checked for integrity on a 2100 BioAnalyzer (Agilent Technologies, Massy Cedex,
351 France). cDNA was synthesized and biotin-labelled cRNA was generated by an in vitro
352 transcription reaction using Ambion Illumina TotalPrep RNA Amplification Kits

353 (Applied Biosystem/Ambion, Saint-Aubin, France). Labeled cRNA were hybridized on
354 Illumina Human HT-12V4 BeadChips.

355 **CD4 and CD8 T lymphocytes isolation**

356 CD4- and CD8-lymphocytes were isolated only from SRHIC and WRHIC subjects (9).
357 T cells were isolated with an automated Robosep cell separator (STEMCELL) by
358 indirect magnetic cell sorting with a T cell enrichment kit (STEMCELL) customized to
359 also eliminate gamma/delta T cells. CD4+ T cells were subsequently separated by
360 positive selection using anti-CD4 coated beads (STEMCELL) and CD8+ T were
361 recovered in the resulting negative fraction. The purities of CD4 and CD8-T cells were
362 > 95%.

363 **In vitro stimulation of purified PBMC with HIV peptides for gene expression and** 364 **cytokines profile analyses**

365 After resting, 8.10^5 of thawed cells were stimulated for 24 hours in 48-well plates with a
366 HIV peptide pool of 36 peptides (15-mers overlapping by 11 amino acids peptides)
367 covering 5 regions of HIV Gag, Pol and Nef (50). Cells were then pelleted for
368 transcriptomic analysis. In parallel, 5.10^5 cells were cultured in triplicate in 96 deep well
369 plates and stimulated with the same antigens. At day 2, supernatants were collected for
370 Luminex assay. 100 Units/ml IL2 (Miltenyi Biotec) was added in the culture medium at
371 days 2 and 5 for longer stimulation. At day 8, all wells were split in 2, and cells were re-
372 stimulated with the same antigens either for 6 hours in the presence of brefeldin A for
373 ICS assay or for 24h for Luminex assay.
374 For ICS analyses, cells were first stained with surface monoclonal antibodies: anti-CD3
375 Alexa 700, anti-CD4 BV421 (BD Biosciences, Le Pont de Claix, France), anti-CD8

376 eFluor780 (affymetrix/eBioscience, Paris, France) and a viability marker (Live dead
377 fixable Aqua Dead cell stain kit from Life Technologies, Saint Aubin, France),
378 permeabilized and fixed with Cyto fix/Cytoperm Buffer (BD Biosciences). Cells were
379 then stained with intracellular antibodies: anti-IFNG PerCP Cy5.5, anti-TNF α PE-Cy7
380 and anti-MIP1B PE (BD Biosciences). Data were acquired with a LSRII flow cytometer
381 (BD, Le Pont de Claix, France), with a minimum of 100000 events collected in CD3+
382 alive cells, analyzed using FlowJo software, and the specific response has been
383 expressed as the percentage of CD4 or CD8 T cells.

384 For Luminex assay, 14 cytokines have been measured in the supernatants of cell
385 cultures at days 2 and 9 using Millipore reagents (MILLIPLEX Human CD8 T-Cell
386 Panel with IL-2, IL-5, IL-10, IL-13, IFN γ , TNF α , MIP-1 β , Perforin, Granzyme A and
387 Granzyme B; Magnetic beads and antibodies for human IP10, IL-21, IL-17A and IL-1 β ,
388 Millipore, Chicago, USA). Data were acquired with the Bio-Plex 200 systemTM (Bio-
389 Rad, Marnes-la-Coquette, France).

390 **Statistics**

391 Microarray data analyses were performed using R software version 3.2.2 (The R
392 foundation for Statistical Computing, Vienna, Austria). Gene transcription data were
393 pre-processed (51, 52) and corrected for potential batch effect (53). Statistical
394 comparisons between groups were based on empirical Bayes moderated t-statistics (54).
395 An adaptive FDR procedure was used to control for test multiplicity. Unsupervised
396 hierarchical clustering heatmap analysis was performed on raw scaling expression using
397 Euclidean distance matrix and Ward's linkage (55). Canonical pathway and biological
398 function analyses were then carried out using genes differentially expressed between

399 groups with adaptive FDR-adjusted $P \leq 0.05$ and fold-change $|FC| \geq 1.5$. Ingenuity
400 Pathway Analysis software (IPA®, Qiagen, Redwood City,
401 www.qiagen.com/ingenuity) was used for gene pathway and function analyses. Mann
402 Whitney tests have been used to compare cytokine production by T cells and PBMC.

403 **Data availability**

404 All microarray data are MIAME compliant, and the raw and normalized data have been
405 deposited in the MIAME-compliant database Gene Expression Omnibus (GEO) under
406 accession number GSE108297.

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422 **References**

- 423 1. **O'Connell KA, Brennan TP, Bailey JR, Ray SC, Siliciano RF, Blankson JN.**
424 2010. Control of HIV-1 in elite suppressors despite ongoing replication and
425 evolution in plasma virus. *J Virol* **84**:7018-7028.
- 426 2. **Saez-Cirion A, Lacabaratz C, Lambotte O, Versmisse P, Urrutia A, Boufassa**
427 **F, Barre-Sinoussi F, Delfraissy JF, Sinet M, Pancino G, Venet A.** 2007. HIV
428 controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo
429 and peculiar cytotoxic T lymphocyte activation phenotype. *Proc Natl Acad Sci U S*
430 *A* **104**:6776-6781.
- 431 3. **Saksena NK, Rodes B, Wang B, Soriano V.** 2007. Elite HIV controllers: myth or
432 reality? *AIDS Rev* **9**:195-207.
- 433 4. **Saez-Cirion A, Pancino G.** 2013. HIV controllers: a genetically determined or
434 inducible phenotype? *Immunol Rev* **254**:281-294.
- 435 5. **Walker BD, Yu XG.** 2013. Unravelling the mechanisms of durable control of
436 HIV-1. *Nat Rev Immunol* **13**:487-498.
- 437 6. **Betts MR, Nason MC, West SM, De Rosa SC, Migueles SA, Abraham J,**
438 **Lederman MM, Benito JM, Goepfert PA, Connors M, Roederer M, Koup RA.**
439 2006. HIV nonprogressors preferentially maintain highly functional HIV-specific
440 CD8+ T cells. *Blood* **107**:4781-4789.
- 441 7. **Makedonas G, Betts MR.** 2011. Living in a house of cards: re-evaluating CD8(+)
442 T-cell immune correlates against HIV. *Immunol Rev* **239**:109-124.
- 443 8. **Almeida JR, Price DA, Papagno L, Arkoub ZA, Sauce D, Bornstein E, Asher**
444 **TE, Samri A, Schnuriger A, Theodorou I, Costagliola D, Rouzioux C, Agut H,**
445 **Marcelin AG, Douek D, Autran B, Appay V.** 2007. Superior control of HIV-1

- 446 replication by CD8+ T cells is reflected by their avidity, polyfunctionality, and
447 clonal turnover. *J Exp Med* **204**:2473-2485.
- 448 9. **Saez-Cirion A, Sinet M, Shin SY, Urrutia A, Versmisse P, Lacabaratz C,**
449 **Boufassa F, Avettand-Fenoel V, Rouzioux C, Delfraissy JF, Barre-Sinoussi F,**
450 **Lambotte O, Venet A, Pancino G.** 2009. Heterogeneity in HIV suppression by
451 CD8 T cells from HIV controllers: association with Gag-specific CD8 T cell
452 responses. *J Immunol* **182**:7828-7837.
- 453 10. **Migueles SA, Osborne CM, Royce C, Compton AA, Joshi RP, Weeks KA, Rood**
454 **JE, Berkley AM, Sacha JB, Cogliano-Shutta NA, Lloyd M, Roby G, Kwan R,**
455 **McLaughlin M, Stallings S, Rehm C, O'Shea MA, Mican J, Packard BZ,**
456 **Komoriya A, Palmer S, Wiegand AP, Maldarelli F, Coffin JM, Mellors JW,**
457 **Hallahan CW, Follman DA, Connors M.** 2008. Lytic granule loading of CD8+ T
458 cells is required for HIV-infected cell elimination associated with immune control.
459 *Immunity* **29**:1009-1021.
- 460 11. **Lecuroux C, Saez-Cirion A, Girault I, Versmisse P, Boufassa F, Avettand-**
461 **Fenoel V, Rouzioux C, Meyer L, Pancino G, Lambotte O, Sinet M, Venet A.**
462 2014. Both HLA-B*57 and plasma HIV RNA levels contribute to the HIV-specific
463 CD8+ T cell response in HIV controllers. *J Virol* **88**:176-187.
- 464 12. **Saez-Cirion A, Shin SY, Versmisse P, Barre-Sinoussi F, Pancino G.** 2010. Ex
465 vivo T cell-based HIV suppression assay to evaluate HIV-specific CD8+ T-cell
466 responses. *Nat Protoc* **5**:1033-1041.
- 467 13. **Ndhlovu ZM, Proudfoot J, Cesa K, Alvino DM, McMullen A, Vine S,**
468 **Stampouloglou E, Piechocka-Trocha A, Walker BD, Pereyra F.** 2012. Elite

469 controllers with low to absent effector CD8+ T cell responses maintain highly
470 functional, broadly directed central memory responses. *J Virol* **86**:6959-6969.

471 14. **Ndhlovu ZM, Stampouloglou E, Cesa K, Mavrothalassitis O, Alvino DM, Li**
472 **JZ, Wilton S, Karel D, Piechocka-Trocha A, Chen H, Pereyra F, Walker BD.**
473 2015. The Breadth of Expandable Memory CD8+ T Cells Inversely Correlates with
474 Residual Viral Loads in HIV Elite Controllers. *J Virol* **89**:10735-10747.

475 15. **Korhonen R, Moilanen E.** 2014. Mitogen-activated protein kinase phosphatase 1
476 as an inflammatory factor and drug target. *Basic Clin Pharmacol Toxicol* **114**:24-
477 36.

478 16. **Kaneda MM, Messer KS, Ralainirina N, Li H, Leem CJ, Gorjestani S, Woo G,**
479 **Nguyen AV, Figueiredo CC, Foubert P, Schmid MC, Pink M, Winkler DG,**
480 **Rausch M, Palombella VJ, Kutok J, McGovern K, Frazer KA, Wu X, Karin**
481 **M, Sasik R, Cohen EE, Varner JA.** 2016. PI3Kgamma is a molecular switch that
482 controls immune suppression. *Nature* **539**:437-442.

483 17. **Bottcher JP, Beyer M, Meissner F, Abdullah Z, Sander J, Hochst B, Eickhoff S,**
484 **Rieckmann JC, Russo C, Bauer T, Flecken T, Giesen D, Engel D, Jung S,**
485 **Busch DH, Protzer U, Thimme R, Mann M, Kurts C, Schultze JL,**
486 **Kastenmuller W, Knolle PA.** 2015. Functional classification of memory CD8(+)
487 T cells by CX3CR1 expression. *Nat Commun* **6**:8306.

488 18. **Guaraldi G, Orlando G, Zona S, Menozzi M, Carli F, Garlassi E, Berti A, Rossi**
489 **E, Roverato A, Palella F.** 2011. Premature age-related comorbidities among HIV-
490 infected persons compared with the general population. *Clin Infect Dis* **53**:1120-
491 1126.

- 492 19. **Nou E, Lo J, Grinspoon SK.** 2016. Inflammation, immune activation, and
493 cardiovascular disease in HIV. *Aids* **30**:1495-1509.
- 494 20. **Paiardini M, Muller-Trutwin M.** 2013. HIV-associated chronic immune
495 activation. *Immunol Rev* **254**:78-101.
- 496 21. **Owen RE, Heitman JW, Hirschhorn DF, Lanteri MC, Biswas HH, Martin JN,**
497 **Krone MR, Deeks SG, Norris PJ.** 2010. HIV+ elite controllers have low HIV-
498 specific T-cell activation yet maintain strong, polyfunctional T-cell responses. *Aids*
499 **24**:1095-1105.
- 500 22. **Noel N, Boufassa F, Lecuroux C, Saez-Cirion A, Bourgeois C, Dunyach-Remy**
501 **C, Goujard C, Rouzioux C, Meyer L, Pancino G, Venet A, Lambotte O.** 2014.
502 Elevated IP10 levels are associated with immune activation and low CD4(+) T-cell
503 counts in HIV controller patients. *Aids* **28**:467-476.
- 504 23. **Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC,**
505 **Ledergerber B, Lundgren J, Neuhaus J, Nixon D, Paton NI, Neaton JD.** 2008.
506 Inflammatory and coagulation biomarkers and mortality in patients with HIV
507 infection. *PLoS Med* **5**:1496-1508.
- 508 24. **Sereti I, Altfeld M.** 2016. Immune activation and HIV: an enduring relationship.
509 *Curr Opin HIV AIDS* **11**:129-130.
- 510 25. **Cortes FH, de Paula HHS, Bello G, Ribeiro-Alves M, de Azevedo SSD, Caetano**
511 **DG, Teixeira SLM, Hoagland B, Grinsztejn B, Veloso VG, Guimaraes ML,**
512 **Morgado MG.** 2018. Plasmatic Levels of IL-18, IP-10, and Activated CD8(+) T
513 Cells Are Potential Biomarkers to Identify HIV-1 Elite Controllers With a True
514 Functional Cure Profile. *Front Immunol* **9**:1576.

- 515 26. **Sachdeva R, Shilpi RY, Simm M.** 2014. The interplay between the X-DING-CD4,
516 IFN-alpha and IL-8 gene activity in quiescent and mitogen- or HIV-1-exposed
517 PBMCs from HIV-1 elite controllers, AIDS progressors and HIV-negative controls.
518 *Innate Immun* **20**:173-183.
- 519 27. **Pananghat AN, Aggarwal H, Prakash SS, Makhdoomi MA, Singh R, Lodha R,**
520 **Ali S, Srinivas M, Das BK, Pandey RM, Kabra SK, Luthra K.** 2016. IL-8
521 Alterations in HIV-1 Infected Children With Disease Progression. *Medicine*
522 (Baltimore) **95**:e3734.
- 523 28. **El-Far M, Kouassi P, Sylla M, Zhang Y, Fouda A, Fabre T, Goulet JP, van**
524 **Grevenynghé J, Lee T, Singer J, Harris M, Baril JG, Trottier B, Ancuta P,**
525 **Routy JP, Bernard N, Tremblay CL.** 2016. Proinflammatory isoforms of IL-32
526 as novel and robust biomarkers for control failure in HIV-infected slow
527 progressors. *Sci Rep* **6**:22902.
- 528 29. **Hens J, Jennes W, Kestens L.** 2016. The role of NK cells in HIV-1 protection:
529 autologous, allogeneic or both? *AIDS Res Ther* **13**:15.
- 530 30. **Kamya P, Tallon B, Melendez-Pena C, Parsons MS, Migueles SA, Connors M,**
531 **Miconiatis S, Song R, Boulet S, Bruneau J, Tremblay CL, Bernard NF.** 2012.
532 Inhibitory Killer Immunoglobulin-like Receptors to self HLA-B and HLA-C
533 ligands contribute differentially to Natural Killer cell functional potential in HIV
534 infected slow progressors. *Clin Immunol* **143**:246-255.
- 535 31. **Joncker NT, Fernandez NC, Treiner E, Vivier E, Raulet DH.** 2009. NK cell
536 responsiveness is tuned commensurate with the number of inhibitory receptors for
537 self-MHC class I: the rheostat model. *J Immunol* **182**:4572-4580.

- 538 32. **Korner C, Granoff ME, Amero MA, Sirignano MN, Vaidya SA, Jost S, Allen**
539 **TM, Rosenberg ES, Altfeld M.** 2014. Increased frequency and function of
540 KIR2DL1-3(+) NK cells in primary HIV-1 infection are determined by HLA-C
541 group haplotypes. *Eur J Immunol* **44**:2938-2948.
- 542 33. **Mavilio D, Lombardo G, Benjamin J, Kim D, Follman D, Marcenaro E,**
543 **O'Shea MA, Kinter A, Kovacs C, Moretta A, Fauci AS.** 2005. Characterization
544 of CD56-/CD16+ natural killer (NK) cells: a highly dysfunctional NK subset
545 expanded in HIV-infected viremic individuals. *Proc Natl Acad Sci U S A*
546 **102**:2886-2891.
- 547 34. **Dugast AS, Tonelli A, Berger CT, Ackerman ME, Sciaranghella G, Liu Q, Sips**
548 **M, Toth I, Piechocka-Trocha A, Ghebremichael M, Alter G.** 2011. Decreased
549 Fc receptor expression on innate immune cells is associated with impaired
550 antibody-mediated cellular phagocytic activity in chronically HIV-1 infected
551 individuals. *Virology* **415**:160-167.
- 552 35. **Lewis GK.** 2014. Role of Fc-mediated antibody function in protective immunity
553 against HIV-1. *Immunology* **142**:46-57.
- 554 36. **Ackerman ME, Mikhailova A, Brown EP, Dowell KG, Walker BD, Bailey-**
555 **Kellogg C, Suscovich TJ, Alter G.** 2016. Polyfunctional HIV-Specific Antibody
556 Responses Are Associated with Spontaneous HIV Control. *PLoS Pathog*
557 **12**:e1005315.
- 558 37. **Descours B, Petitjean G, Lopez-Zaragoza JL, Bruel T, Raffel R, Psomas C,**
559 **Reynes J, Lacabaratz C, Levy Y, Schwartz O, Lelievre JD, Benkirane M.** 2017.
560 CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent
561 proviruses. *Nature* **543**:564-567.

- 562 38. **Badia R, Ballana E, Castellvi M, Garcia-Vidal E, Pujantell M, Clotet B, Prado**
563 **JG, Puig J, Martinez MA, Riveira-Munoz E, Este JA.** 2018. CD32 expression is
564 associated to T-cell activation and is not a marker of the HIV-1 reservoir. *Nat*
565 *Commun* **9**:2739.
- 566 39. **Garcia M, Navarrete-Munoz MA, Ligos JM, Cabello A, Restrepo C, Lopez-**
567 **Bernaldo JC, de la Hera FJ, Barros C, Montoya M, Fernandez-Guerrero M,**
568 **Estrada V, Gorgolas M, Benito JM, Rallon N.** 2018. CD32 Expression is not
569 Associated to HIV-DNA content in CD4 cell subsets of individuals with Different
570 Levels of HIV Control. *Sci Rep* **8**:15541.
- 571 40. **Nissen SK, Christiansen M, Helleberg M, Kjaer K, Jorgensen SE, Gerstoft J,**
572 **Katzenstein TL, Benfield T, Kronborg G, Larsen CS, Laursen A, Pedersen G,**
573 **Jakobsen MR, Tolstrup M, Mogensen TH.** 2018. Whole Exome Sequencing of
574 HIV-1 long-term non-progressors identifies rare variants in genes encoding innate
575 immune sensors and signaling molecules. *Sci Rep* **8**:15253.
- 576 41. **Alvarez RA, Maestre AM, Law K, Durham ND, Barria MI, Ishii-Watabe A,**
577 **Tada M, Kapoor M, Hotta MT, Rodriguez-Caprio G, Fierer DS, Fernandez-**
578 **Sesma A, Simon V, Chen BK.** 2017. Enhanced FCGR2A and FCGR3A signaling
579 by HIV viremic controller IgG. *JCI Insight* **2**:e88226.
- 580 42. **Hawkins PT, Stephens LR.** 2015. PI3K signalling in inflammation. *Biochim*
581 *Biophys Acta* **1851**:882-897.
- 582 43. **De Henau O, Rausch M, Winkler D, Campesato LF, Liu C, Cymerman DH,**
583 **Budhu S, Ghosh A, Pink M, Tchaicha J, Douglas M, Tibbitts T, Sharma S,**
584 **Proctor J, Kosmider N, White K, Stern H, Soglia J, Adams J, Palombella VJ,**
585 **McGovern K, Kutok JL, Wolchok JD, Merghoub T.** 2016. Overcoming

586 resistance to checkpoint blockade therapy by targeting PI3Kgamma in myeloid
587 cells. *Nature* **539**:443-447.

588 44. **Wang H, Kadlec TA, Au-Yeung BB, Goodfellow HE, Hsu LY, Freedman TS,**
589 **Weiss A.** 2010. ZAP-70: an essential kinase in T-cell signaling. *Cold Spring Harb*
590 *Perspect Biol* **2**:a002279.

591 45. **Sharma N, Akhade AS, Qadri A.** 2016. Src kinases central to T-cell receptor
592 signaling regulate TLR-activated innate immune responses from human T cells.
593 *Innate Immun* **22**:238-244.

594 46. **Feria MG, Taborda NA, Hernandez JC, Rugeles MT.** 2018. HIV replication is
595 associated to inflammasomes activation, IL-1beta, IL-18 and caspase-1 expression
596 in GALT and peripheral blood. *PLoS One* **13**:e0192845.

597 47. **Gonzalez SM, Taborda NA, Correa LA, Castro GA, Hernandez JC, Montoya**
598 **CJ, Rugeles MT.** 2016. Particular activation phenotype of T cells expressing HLA-
599 DR but not CD38 in GALT from HIV-controllers is associated with immune
600 regulation and delayed progression to AIDS. *Immunol Res* **64**:765-774.

601 48. **Palmer CS, Palchaudhuri R, Albargy H, Abdel-Mohsen M, Crowe SM.** 2018.
602 Exploiting immune cell metabolic machinery for functional HIV cure and the
603 prevention of inflammaging. *F1000Res* **7**:125.

604 49. **Noel N, Lerolle N, Lecuroux C, Goujard C, Venet A, Saez-Cirion A, Avettand-**
605 **Fenoel V, Meyer L, Boufassa F, Lambotte O.** 2015. Immunologic and Virologic
606 Progression in HIV Controllers: The Role of Viral "Blips" and Immune Activation
607 in the ANRS CO21 CODEX Study. *PLoS One* **10**:e0131922.

608 50. **Richert L, Hue S, Hocini H, Raimbault M, Lacabaratz C, Surenaud M,**
609 **Wiedemann A, Tisserand P, Durier C, Salmon D, Lelievre JD, Chene G,**

610 **Thiebaut R, Levy Y.** 2013. Cytokine and gene transcription profiles of immune
611 responses elicited by HIV lipopeptide vaccine in HIV-negative volunteers. *Aids*
612 **27**:1421-1431.

613 51. **Bolstad BM, Irizarry RA, Astrand M, Speed TP.** 2003. A comparison of
614 normalization methods for high density oligonucleotide array data based on
615 variance and bias. *Bioinformatics* **19**:185-193.

616 52. **Xie Y, Wang X, Story M.** 2009. Statistical methods of background correction for
617 Illumina BeadArray data. *Bioinformatics* **25**:751-757.

618 53. **Johnson WE, Li C, Rabinovic A.** 2007. Adjusting batch effects in microarray
619 expression data using empirical Bayes methods. *Biostatistics* **8**:118-127.

620 54. **Smyth GK.** 2004. Linear models and empirical bayes methods for assessing
621 differential expression in microarray experiments. *Stat Appl Genet Mol Biol*
622 **3**:Article3.

623 55. **Eisen MB, Spellman PT, Brown PO, Botstein D.** 1998. Cluster analysis and
624 display of genome-wide expression patterns. *Proc Natl Acad Sci U S A* **95**:14863-
625 14868.

626

627 **Figure legends**

628 **Fig. 1. Gene expression in HIC and cART in whole blood.** (A) Heatmap of genes
629 belonging to the main pathways associated to the differentially expressed genes in
630 whole blood of HIC and cART patients, including NK cells, TLRs, TREM1 and
631 CXCL8 pathways. (B) Relationships between genes differentially expressed in whole
632 blood of HIC compared to cART patients. Red symbols are overexpressed genes in
633 HIC compared to cART patients, green symbols are underexpressed genes. Solid
634 lines represent direct links between genes and dashed lines represent indirect links
635 (with no more than one gene between the two genes).

636

637 **Fig. 2. Gene expression in HIV peptides unstimulated and stimulated peripheral**
638 **blood mononuclear cell (PBMC) of HIC and cART.** (A) Main differentially
639 expressed genes between HIC and cART patients associated with inflammation
640 before HIV peptides stimulation. (B and C) Differentially expressed genes between
641 HIC and cART patients associated to inflammation and IFN signaling after HIV
642 peptides stimulation of PBMC.

643

644 **Fig. 3. Cytokines profiles of peripheral blood mononuclear cell (PBMC) from HIC**
645 **and cART patients stimulated *in vitro* with HIV peptides covering Gag, Pol and**
646 **Nef antigens.** (A) Cytokine measurements (pg/ml) in supernatants of stimulated
647 PBMC from HIC (n=20) and cART (n=15). Cytokine secretion was measured in
648 supernatants after HIV peptide stimulation of PBMC using Bio-Plex 200 system™
649 (Bio-Rad) at day 2 of stimulation for IP-10 and IL-1β, and at day 9 after a re-
650 stimulation for 24 hours for TNFα and MIP-1β. (B, C) CD8 and CD4 T cell

651 producing cytokines after PBMC stimulation with HIV peptides for 9 days as
652 measured by ICS. **(B)** Frequency of CD8 T cells producing TNF α , IFN α and MIP-
653 1 β (sum of the cytokines or individual cytokines) in 18 HIC and 14 cART patients.
654 **(C)** Frequency of CD4 T cells producing TNF α , IFN γ and MIP-1 β (sum of the
655 cytokines or individual cytokines) in 18 HIC and 14 c-ART patients. Horizontal lines
656 represent the median \pm IQR, and Mann-Whitney test has been used.

657

658 **Fig. 4. Cytokines profiles of peripheral blood mononuclear cell (PBMC) from**
659 **strong (SRHIC) and weak (WRHIC) responders HIC, stimulated in vitro with**
660 **HIV peptides covering Gag, Pol and Nef antigens. (A)** Cytokine measurements
661 (pg/ml) in supernatants of stimulated PBMC from 10 SRHIC and 10 WRHIC.
662 Horizontal lines represent median \pm IQR and Mann-Whitney test has been used to
663 compare cytokines secretion among groups of patients. **(B)** Heatmap of 14 cytokine
664 profiles of SRHIC and WRHIC. Cytokine secretion was measured in supernatants
665 after HIV peptide stimulation of PBMC using Bio-Plex 200 systemTM (Bio-Rad) at
666 day 2 of stimulation for IL-2, IL-1 β and IP10 or at day 9 after a re-stimulation for 24
667 hours for all other cytokines. The white color indicates a very low cytokine
668 concentration (or no detection), and the dark red color indicates a high cytokine
669 concentration.

670

671 **Fig. 5. CD4 and CD8 T cell producing cytokines after peripheral blood**
672 **mononuclear cell (PBMC) stimulation with HIV peptides for 9 days measured**
673 **by ICS assay. (A)** Frequency of CD8 T cells producing TNF α , IFN γ and MIP-1 β
674 (sum of the cytokines or individual cytokines) in 9 SRHIC and 9 WRHIC. **(B)**

675 Frequency of CD4 T cells producing TNF α , IFN γ and MIP-1 β (sum of the cytokines
676 or individual cytokines) in 9 SRHIC and 9 WRHIC. Horizontal lines represent the
677 median \pm IQR, and Mann-Whitney test has been used. Pie charts represent the cell
678 poly functionality, ie the relative proportion of CD8 and CD4 T cells producing 1
679 (grey), 2 (dark grey) or 3 (black) cytokines.

680

681 **Fig. 6. Unsupervised hierarchical clustering of differentially expressed genes**
682 **between SRHIC and WRHIC subjects in purified T cell populations. (A)**
683 Unsupervised hierarchical clustering of CD4+ T lymphocytes samples between 8
684 SRHIC and 9 WRHIC subjects. **(B)** Unsupervised clustering of CD8+ T lymphocytes
685 samples between 10 SRHIC and 10 WRHIC subjects.

686

687 **Fig. 7. IFN γ pathway associated with differentially expressed genes between**
688 **SRHIC and WRHIC purified CD8 T cells.** Overexpressed genes with FC \geq 1.5 are
689 represented in red, underexpressed genes in green and genes with a FC $<$ 1.5 and $>$ 1.2
690 in gray.

691

692 **Fig. 8. Summary of genes differentially expressed in the various experiments**
693 **performed in the study. (A)** Commonly differentially expressed genes between HIC
694 and cART PBMC at 6 and 24 hours of stimulation, between SRHIC and WRHIC
695 CD4, between SRHIC and WRHIC CD8 and between HIC and cART in the whole
696 blood (WB). Commonly differentially expressed genes between HIC and cART in
697 WB and between SRHIC and WRHIC CD8+T cells, are indicated by stars. **(B)**
698 Predicted functions committed based on the 671 genes differentially expressed

699 specifically between SRHIC and WRHIC CD8⁺ T lymphocytes, using Ingenuity
700 software. Green symbols are underexpressed genes in SRHIC compared to WRHIC,
701 red symbols are overexpressed genes. Supplementary legends are depicted in the
702 figure.

Table 1. Characteristics of the HIC and cART subjects.

	HIC	cART patients
Number of subjects	53	27
Age in years (Q1/Q3)	47 (20/79)	52 (40/64)
Genders (F; M)	F 24; M 29	F 12; M 15
HIV-1 plasma viral load (RNA copies/ml)		
Mean (sd)	1.6 (0.46)	1.3 (0.16)
Median (Q1/ Q3)	1.4 (1.3/1.9)	1.3 (1.3/1.3)
CD4+ lymphocytes		
Number of subjects	53	27
Count (cells/mm ³)		
Mean (sd)	713 (249)	606 (186)
Median (Q1/ Q3)	689 (502/859)	588 (498/698)
CD8+ lymphocytes		
Number of subjects	50	27
Count (cells/mm ³)		
Mean (sd)	829 (398)	725 (330)
Median (Q1/ Q3)	794 (593/920)	681 (526/852)

Fig. 1A

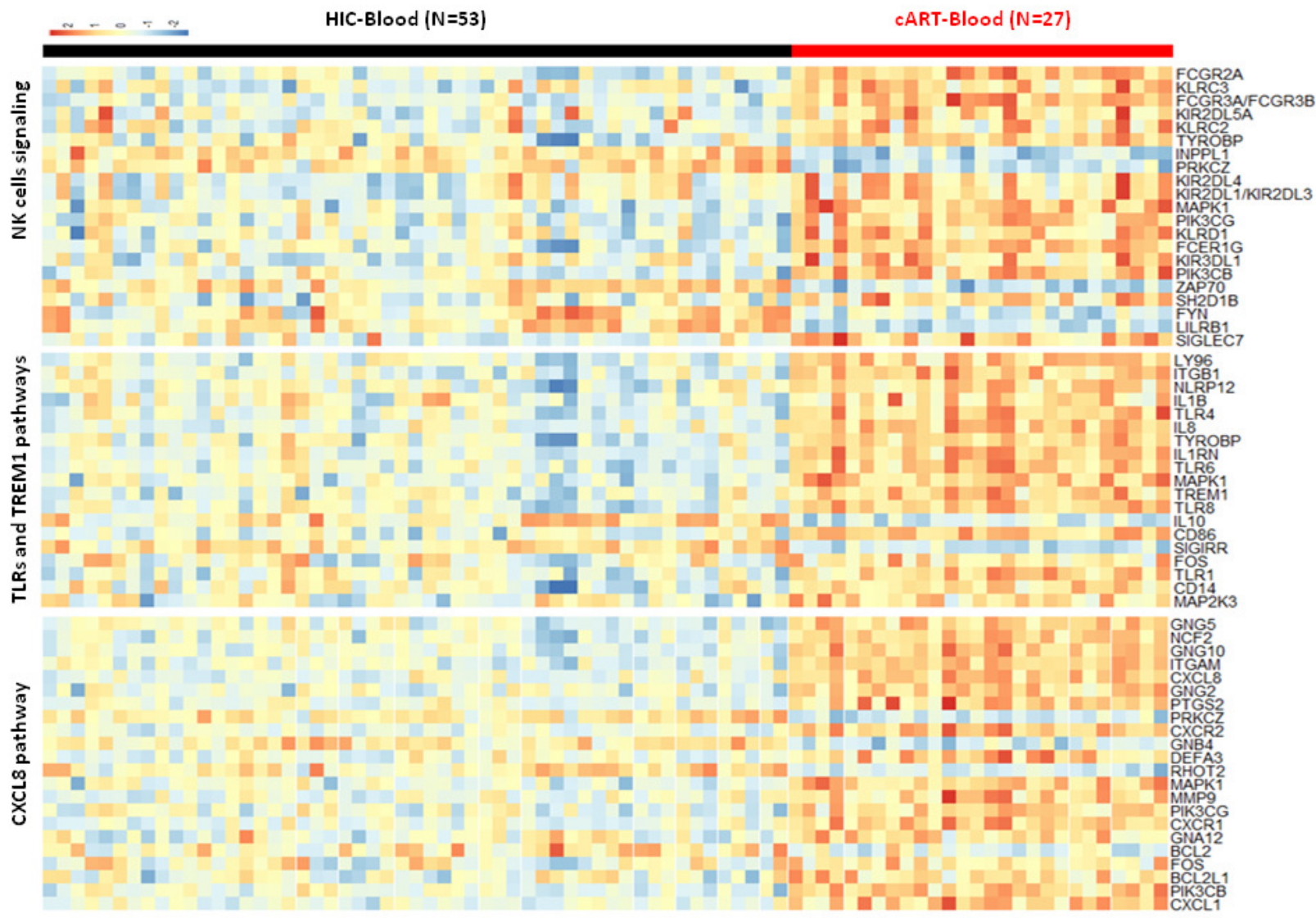


Fig. 1B

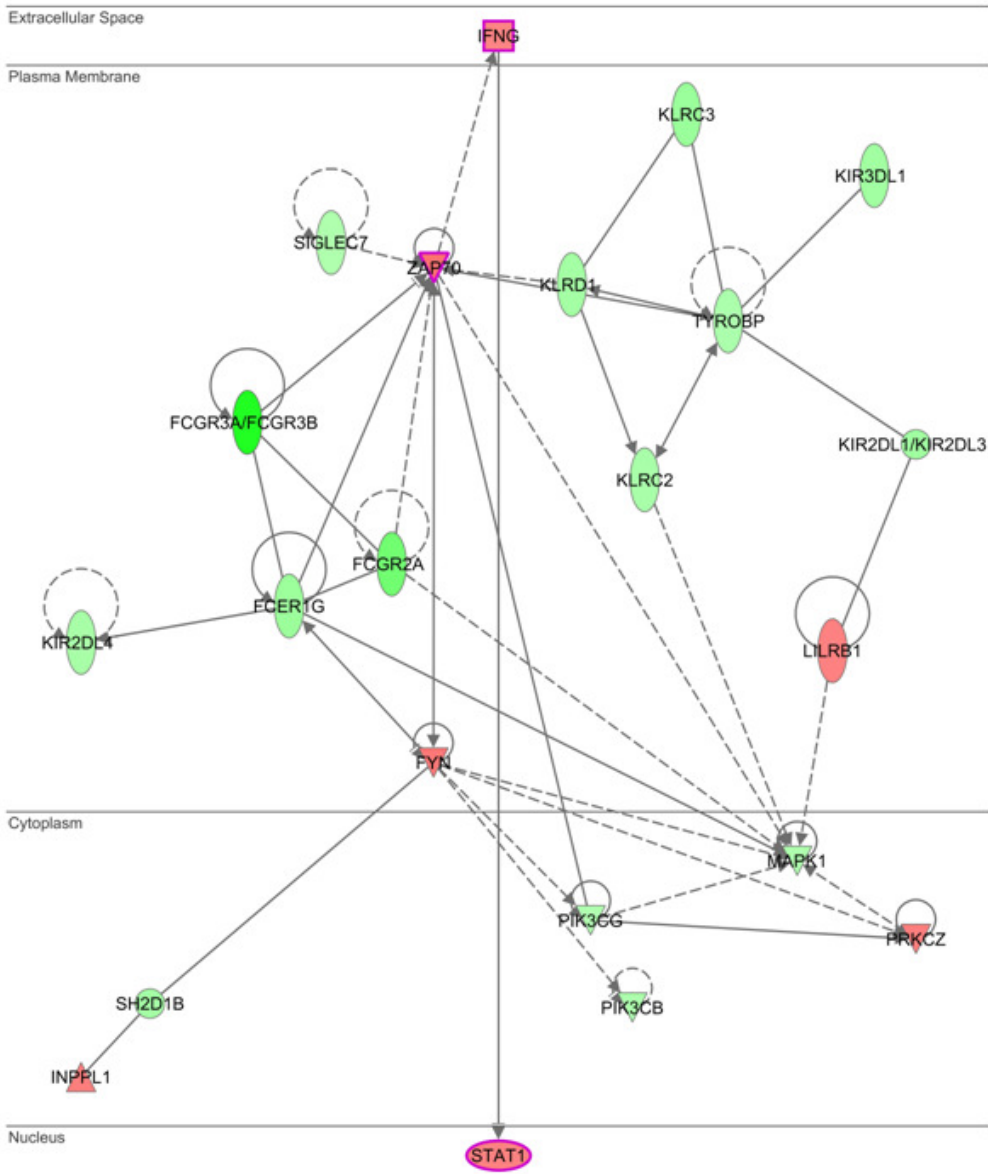


Fig. 2

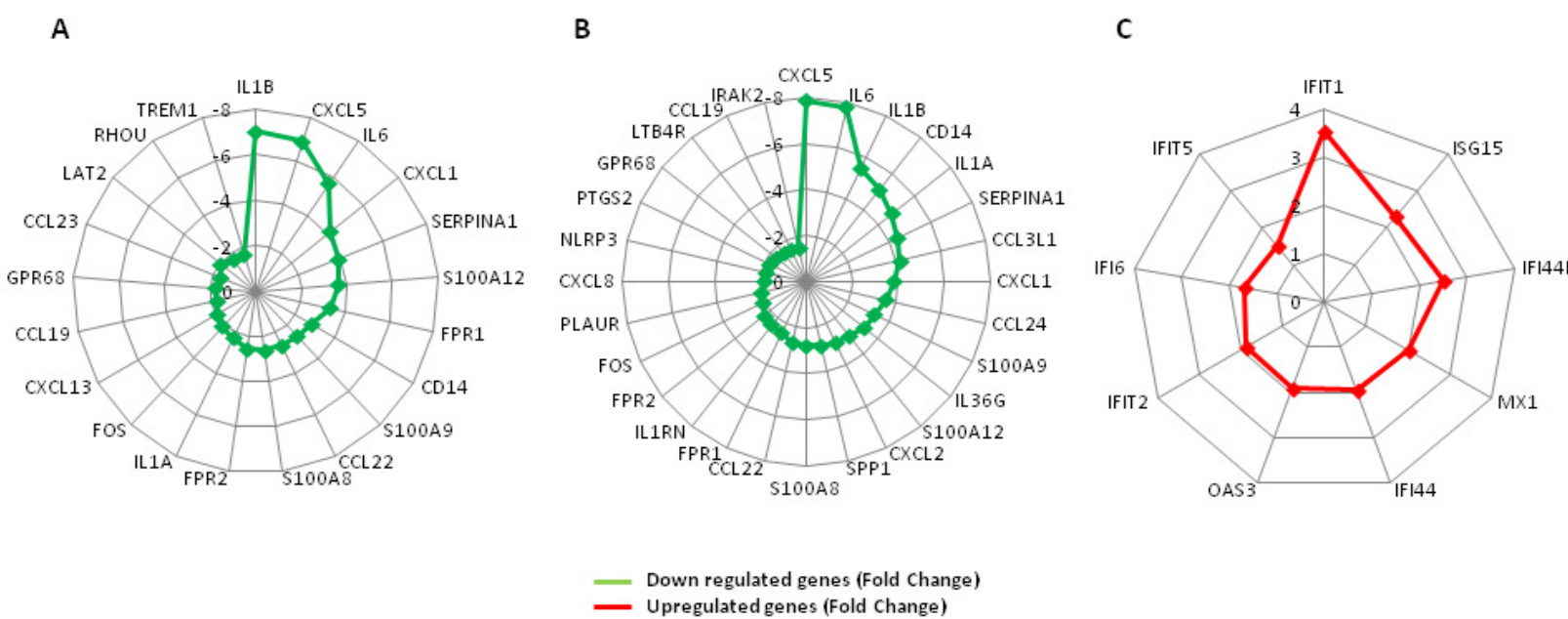
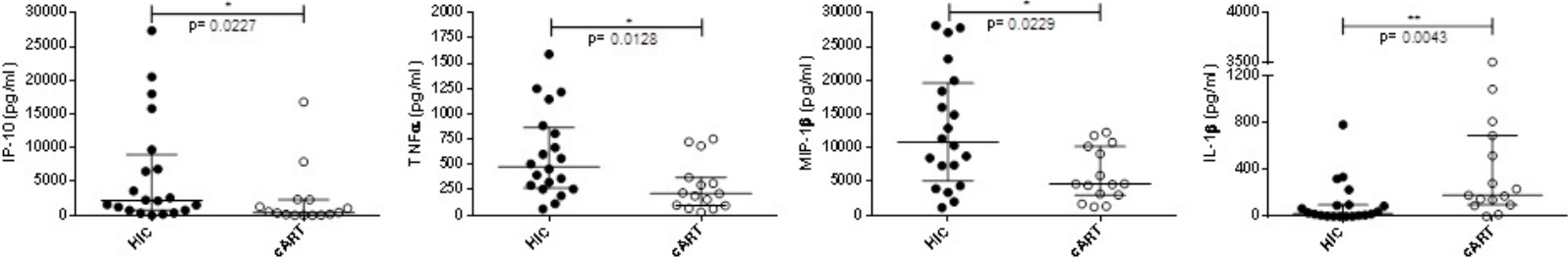
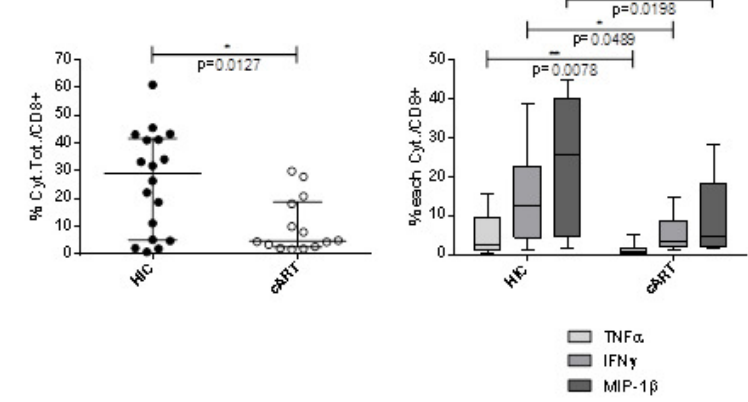


Fig. 3

A



B



C

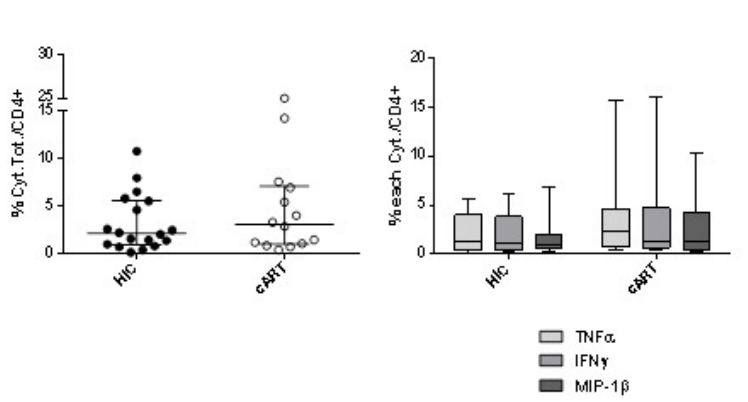


Fig. 4

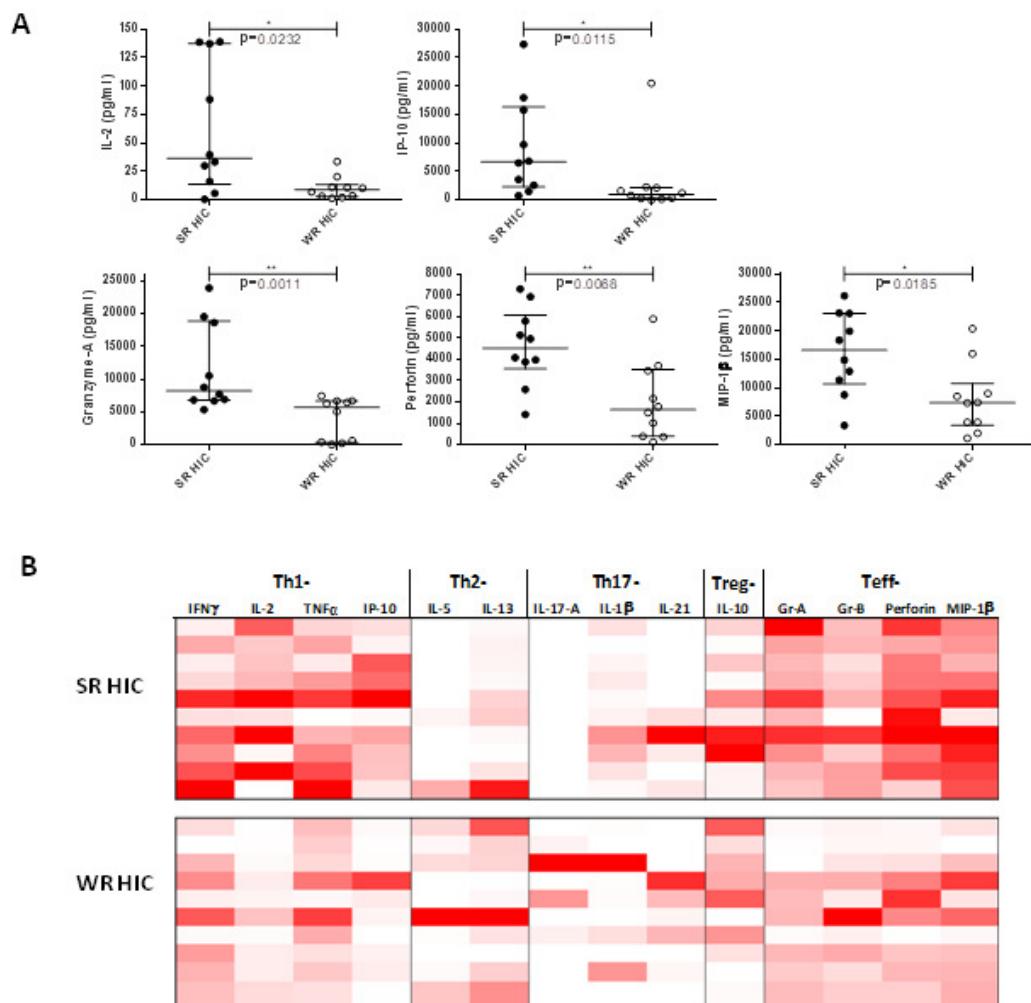
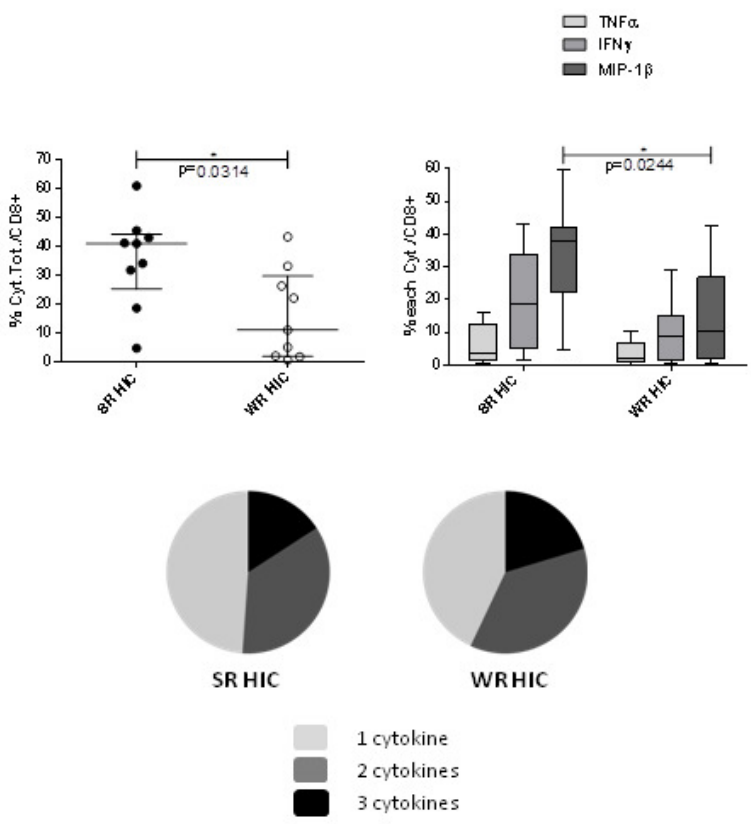


Fig. 5

A



B

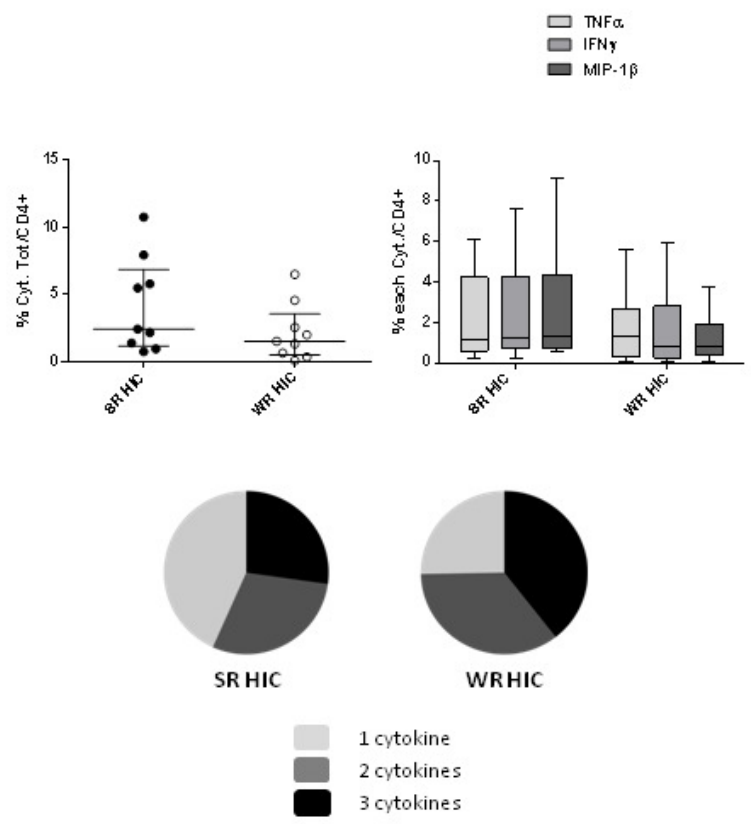


Fig. 6

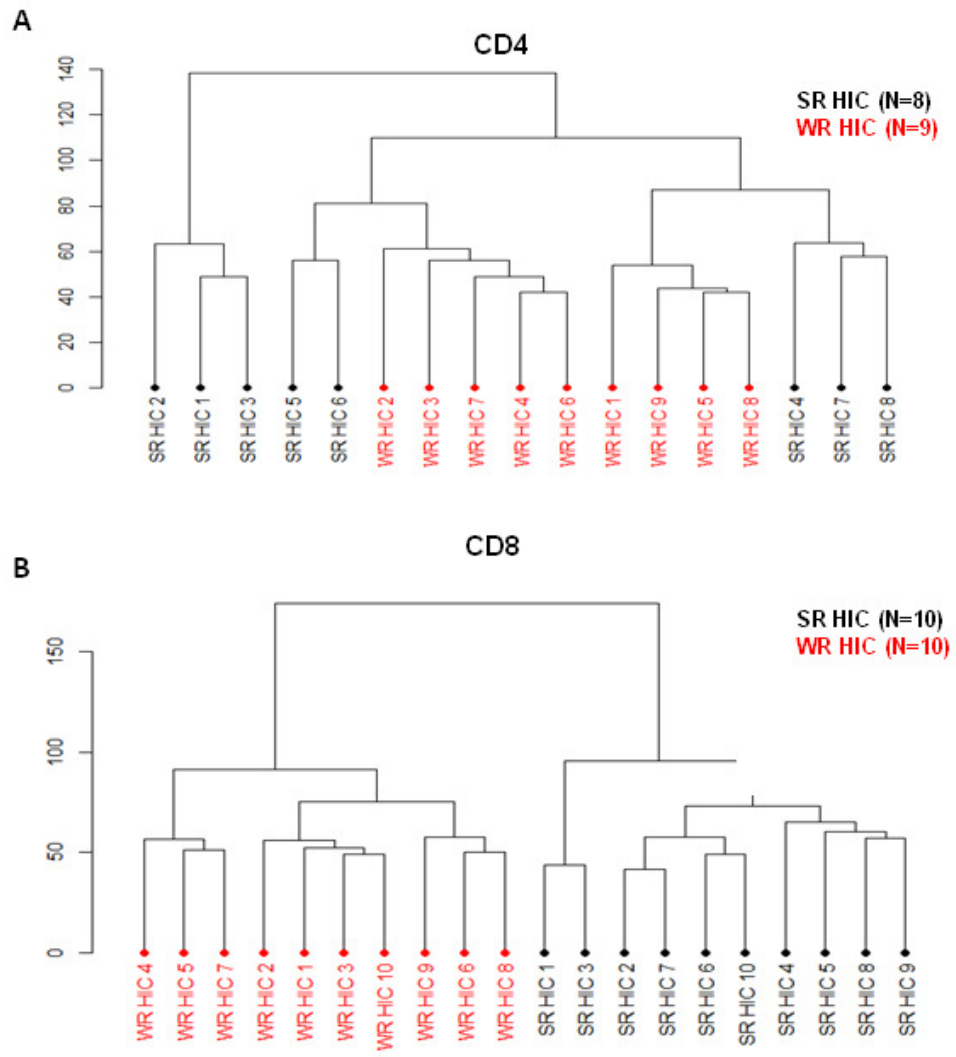


Fig. 7

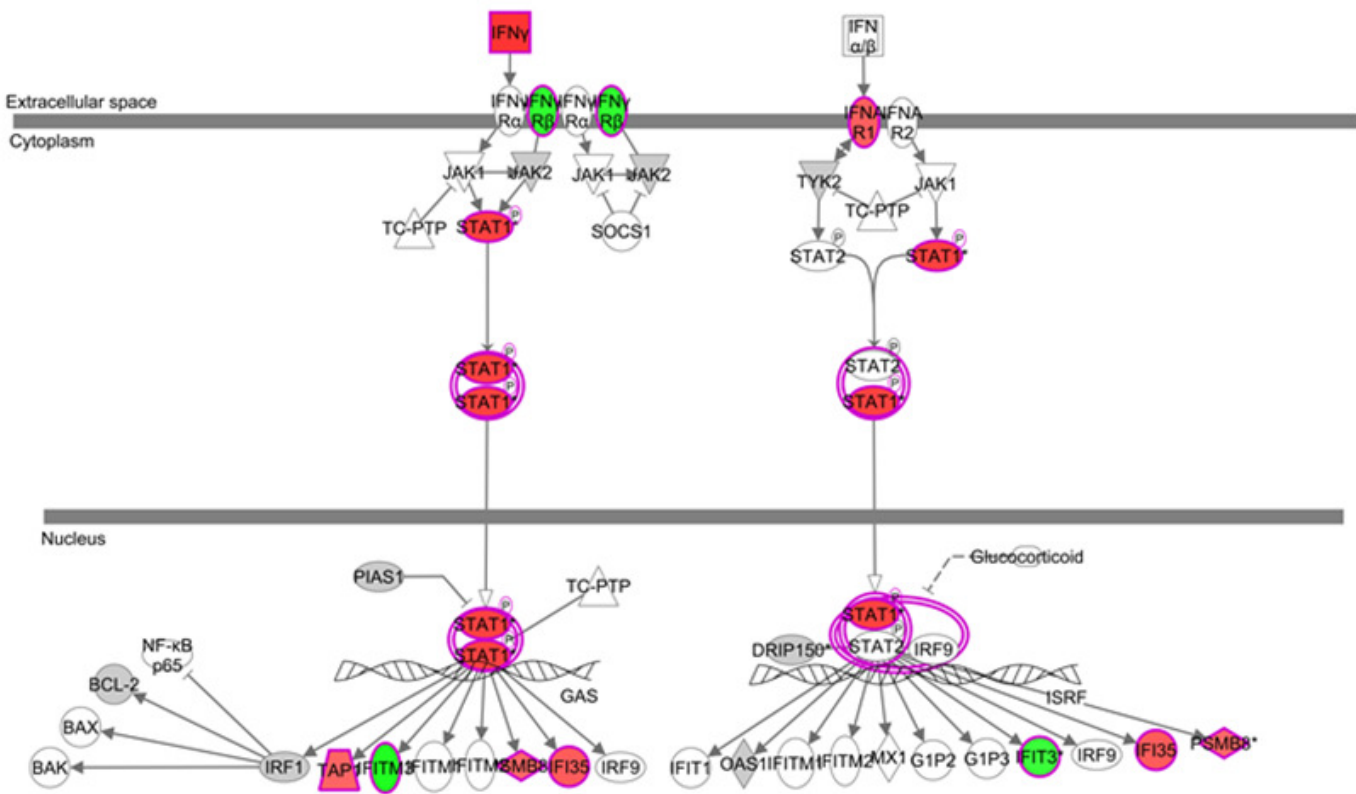


Fig. 8 A

