Comprehensive Analysis of Neutrophil Immunomodulatory Properties and FcR Dynamics in Health and HIV-1 Infection and Therapy

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Abstract

Neutrophils are innate immune cells with key immunomodulatory functions. In a murine retroviral model, we previously showed their essential role in promoting protective immunity during antiviral antibody therapy via Fc–Fcγ receptor (FcγR) interactions. Here, we investigated the immunomodulatory properties of neutrophils in the context of HIV-1 infection and therapy through a comprehensive analysis of their functional activation and the regulation of FcγR expression. Neutrophils from healthy donors (HD) and people living with HIV-1 (PLWH) were stimulated with TLR ligands, free HIV-1, immune complexes (ICs) formed with broadly neutralizing antibodies (bNAbs), or pro-inflammatory cytokines (TNFα, IFNγ). In response, they secreted various cytokines and chemokines that can recruit and activate immune cells in a stimulus-dependent manner. Compared to TLR agonist and cytokine activation, HD neutrophils showed limited cytokine production in response to free HIV-1 or ICs alone as well as a minimal FcγR modulation. However, PLWH neutrophils showed heightened responsiveness to microbial stimuli linked to HIV-1 pathogenesis, secreting higher levels of IFNγ, CXCL1, CCL2, CCL3, and CCL4. They also expressed higher levels of two activating FcγRs (FcγRI and FcγRIIIb), as well as CD11b, CD63, CXCR4, and PD-L1, indicating an altered activation state. These findings highlight the influence of the inflammatory milieu on neutrophil function and FcγR regulation in HIV-1 infection and mAb-based therapies.

Introduction

Neutrophils are the most abundant population of circulating white blood cells and are massively and rapidly recruited following infection. Through diverse mechanisms such as phagocytosis, releasing of reactive-oxygen species (ROS), trapping of the pathogenic agent by neutrophil extracellular traps (NET) (known as NETosis) (1), neutrophils can limit and counteract invading pathogens. Moreover, immunomodulatory properties of neutrophils have been described in recent years, showing their ability to shape the adaptive immune response (2,3). Neutrophils migrate from the site of infection to draining lymph nodes where they interact with multiple immune cells through the production of several cytokines and chemokines (3), making neutrophils key cells in the orchestration of innate and adaptive immunity (3,4). However, the immunomodulatory role of neutrophils in antiviral immunity is still ill-understood, including in the context of persistent viral infections such as HIV-1, as well as in the frame of antiviral monoclonal antibody (mAb)-based immunotherapies. Noteworthy, it has been reported that the functional activation of neutrophils is compromised upon HIV-1 infection which leads to impaired effector functions (i.e. phagocytosis, ROS release, …) and increased apoptosis (5,6). In addition, more recent studies show that neutrophils in people living with HIV-1 (PLWH) are highly activated and may contribute to ongoing chronic inflammation in HIV (7). However, much less is known on the effect of HIV-1 infection on their cytokine and chemokine secretion profile, as well as on the modulation of Fcγ receptors (FcγRs) expressed at their surface, which is a key point in the context of antibody therapy.

The discovery of potent broadly neutralizing antibodies (bNAbs) against HIV-1 has made passive immunotherapy a potential strategy for the prevention and treatment of HIV-1 infection (8). Importantly, recent clinical data have demonstrated the efficacy of several anti-HIV-1 bNAbs to control viremia when administered to PLWH, as well as to induce adaptive immune responses (vaccinal effect) (reviewed in 9). This has given strong support to the idea that bNAbs could broaden the therapeutic arsenal against HIV-1 infection (10–14). Beyond their neutralization capacity through the binding of their Fab fragment to viral antigens, the biological activity of mAbs is also mediated by the Fc moiety upon interaction with the complement system and FcγRs expressed by many cells of the immune system (15,16). This allows to clear free virions from the blood as well as to guide host immune effector cells to kill infected cells by several Fc-mediated mechanisms (i.e. antibody-dependent cellular phagocytosis (ADCP), antibody-dependent cell-mediated cytotoxicity (ADCC), …). Furthermore, mAbs can form immune complexes (ICs) *via* binding with either virions or

83 infected cells, and the subsequent engagement of FcγRs by ICs has immunomodulatory effects
84 leading to the induction of protective immunity. We have previously identified different FcγR-
85 expressing immune cells involved in the vaccinal response during mAb-based therapies
86 (reviewed in 17,18). By using a mouse model of retroviral infection, we have shown a key role
87 for ICs in enhancing antiviral immune responses by multiple innate immune cells such as
88 dendritic cells (DC) (19,20), neutrophils (21,22), monocytes (22) and NK cells (23) in a FcγR-
89 dependent manner. Notably, our findings revealed a key immunomodulatory role for murine
90 neutrophils in the induction of protective immunity upon mAb-therapy (21,22). This occurs
91 through the secretion of multiple cytokines and chemokines upon activation by free virions and
92 ICs. With the aim of extending our observations to the context of HIV-1 infection, here we
93 addressed the immunomodulatory properties of human neutrophils upon activation by different
94 microbial stimuli associated with HIV-1 infection and mAb-based therapy.
95
96 Studying the functional properties of neutrophils presents significant challenges due to their
97 short half-life, fragility, and susceptibility to activation during the purification process. Most
98 studies on their immunomodulatory properties focus on a limited set of immune parameters,
99 often relying on gene expression data and lacking a comprehensive assessment of their
100 functional activation and FcγR modulation. Furthermore, existing data are fragmented and
101 difficult to compare, as variations in purification methods can influence neutrophil activation
102 status. Additionally, variations in pathophysiological conditions (e.g., healthy state or
103 infections caused by viruses, bacteria, or fungi) or therapeutic interventions affecting neutrophil
104 donors introduce further variability, complicating the interpretation and comparison of findings.
105 Here, we conducted a comprehensive and systematic investigation of neutrophil activation,
106 analyzing multiple immune receptors and a broad panel of chemokines and cytokines in
107 neutrophils isolated from HD and PLWH upon activation by various stimuli associated with
108 HIV-1 infection. We observed that neutrophils exhibited a limited capacity to secrete cytokines
109 and chemokines in response to *bona fide* HIV-1 (free virus or ICs) compared to virus-
110 mimicking compounds such as TLR agonists, commonly used as viral surrogates. Our findings
111 also reveal distinct phenotypic and functional differences in neutrophils from PLWH compared
112 to those from HD, including heightened responsiveness to microbial/inflammatory stimuli and
113 upregulated expression of activating FcγRs. These alterations may influence the effectiveness
114 to bNAb therapies. By providing a comprehensive analysis of neutrophil activation and FcγR
115 modulation in response to various HIV-1–related stimuli, this study offers valuable insights into
116 neutrophil-mediated mechanisms that could influence the outcomes of antibody-based

117 therapeutic strategies—either alone or in combination with TLR agonists and/or compounds
118 targeting neutrophil immunomodulatory functions.
119

120 **Results**

121

122 ***Different cytokine/chemokine secretion profile and FcγR cell surface levels in neutrophils***
123 ***stimulated by TLR agonists or pro-inflammatory cytokines***

124 First, we assessed the effect of TLR agonists and inflammatory cytokines (TNF-α and IFN-γ)
125 on the phenotypic and functional activation of human neutrophils isolated from HD. This aimed
126 to determine whether and how the microenvironment resulting from infection might be
127 determinant for modulating the immune response, both in terms of chemokine/cytokine
128 secretion profile and of FcγR expression pattern, as observed in our mouse studies (22). As for
129 TLR agonists, we mainly focused on TLR4 (lipopolysaccharide (LPS)) and TLR7/8 agonists
130 and Resiquimod (R848), due to their role in immune activation associated with viral sensing
131 (TLR7/8) and to the bacterial translocation observed in PLWH (i.e. LPS binding to TLR4). To
132 assess the phenotypic and functional activation of neutrophils we used the following read-out:
133 (i) the upregulation of the cell surface markers CD11b and CD66b, (ii) the modulation of the
134 expression of the main FcγRs constitutively expressed on neutrophils: FcγRIIa (CD32a) and
135 FcγRIIIb (CD16b), and (iii) the cytokine and chemokine secretion profile (**Figure 1**). Flow
136 cytometry analysis was performed on the viable fraction of $CD15^+/CD11b^+$ cells (**Figure 1A**).
137 While life span of neutrophils has been reported to be short (only few hours), specific culture
138 conditions allow extending it. Upon TLR4 and TLR7/8 agonist stimulation during 16-20h,
139 neutrophil survival was around 65 and 75% in average, respectively (**Figure 1B**). TLR7/8
140 agonist led to significant upregulation of CD11b as compared to unstimulated neutrophils and
141 TLR4 agonist stimulation, while TLR4 agonist led to significant upregulation of CD66b as
142 compared to unstimulated neutrophils, with no significant differences between TLR agonists
143 (**Figure 1C**). As for FcγR expression, neutrophil stimulation by either TLR agonist led to
144 decreased expression of the CD32a receptor, as well as a significant decrease in CD16b cell
145 surface levels by TLR4 activation, as compared to unstimulated neutrophils (**Figure 1C**). We
146 next assessed the functional activation of TLR-stimulated neutrophils. Secretion of soluble
147 cytokines (IL-1β, Il-6, TNF-α, IFN-λ1, IL-8, IL-12p70, IFN-α2, IFN-λ2/3, GM-CSF, IFN-β,
148 IL-10, IFN-γ) and chemokines (CXCL10, CCL11, CCL17, CCL2, CCL5, CCL3, CXCL9,
149 CXCL5, CCL20, CXCL1, CXCL11, CCL4) were assayed from cell-free culture supernatants
150 of *in vitro* stimulated neutrophils using bead-based immunoassays. The assessment of
151 neutrophil secretome revealed that both stimuli triggered the secretion of IL-8 and several
152 chemokines (i.e. CCL3, CCL4, CXCL1, CXCL10 and CCL2) albeit to a different extent

153 (**Figure 1D)**. Due to high donor-dependent variability of chemokine secretion, no statistical
154 differences were observed between TLR4 and TLR7/8 activation (**Figure 1D**). However, TLR4
155 stimulation led to the secretion of IFN-λ2/3 in 3 out of 7 samples, while TLR7/8 stimulation
156 did not. No detectable release of the other cytokines or chemokines was observed under our
157 experimental conditions.
158
159 We also assessed the viability, phenotype, and functional activation of human neutrophils
160 stimulated with TNF-α and IFN-γ (**Figure 2**). These cytokines were chosen based on evidence
161 linking virus-driven inflammation, including TNF-α/IFN-γ signatures, to the development of
162 improved antiviral responses in spontaneous HIV controllers (24). At the concentrations used,
163 TNF-α led to a similar neutrophil survival than IFN-γ (75% and 80%, respectively) (**Figure**
164 **2A**). TNF-α induced a significant upregulation of CD66b as well as a significant decrease of
165 CD32a and CD16b cell surface levels. In contrast, the expression levels of these activating
166 markers and FcγRs were not significantly altered in neutrophils activated by IFN-γ (**Figure**
167 **2B**). Notably, IFN-γ stimulation led to a significant increase in the surface expression of the
168 inducible FcγRI (CD64) (15), an effect not observed with TNF-α (**Supplementary Figure 1)**.
169 Thus, significant differences between IFN-γ and TNF-α activation were observed for most of
170 the cell surface markers evaluated. The assessment of neutrophil's secretome revealed that
171 TNF-α activation induced the secretion of several cytokines (IL-8, IL-1ß, IFN-λ2/3, IFN-λ1
172 and IFN-γ) and chemokines (CCL3, CCL4 and CXCL1) (**Figure 2C**). The secretion of IL-8,
173 CCL3, CCL4, and CXCL1 was significantly higher compared to unstimulated neutrophils.
174 Increased levels of IL-1β, IFN-λ2/3, IFN-γ, and CCL2 were also observed in approximately
175 half of the samples tested, although these changes did not reach statistical significance (**Figure**
176 **2C**). In contrast, IFN-γ activation did not induce detectable cytokine or chemokine release,
177 except for a low level of IL-8 secretion.
178
179 Overall, these results highlight a stimulus-specific effect on neutrophil activation, both
180 phenotypically (FcγR and activation marker levels) and functionally (secretome). Furthermore,
181 by studying multiple parameters, these results enabled to establish a comprehensive and
182 comparative landscape of neutrophil activation by these stimuli (some of them used as a
183 surrogate of viral activation) as a basis for comparison with neutrophil activation by HIV-1 and
184 ICs. This is important to highlight as neutrophil activation by TLR agonists and cytokines has

185 been partially addressed in previous studies (25–29). However, the available data are scattered,
186 limited to a narrow set of parameters, and derived from diverse experimental conditions.
187
188 ***Neutrophil FcγR Expression is maintained upon HIV-1 stimulation***
189 We next examined the modulation of the expression of FcγRs in presence of HIV-1 virions,
190 free or in the form of ICs. This is important to assess, as in the context of HIV-1 infection and
191 antibody therapy, the expression of FcγRs might affect the activation of neutrophils by
192 therapeutic antibodies. ICs were made using the anti-HIV-1 bNAb 10-1074, which is currently
193 being tested in clinical trials and has been shown to induce vaccinal effects (9,30). The 10-1074
194 bNAb was chosen due to its higher affinity to the FcγRs CD16b and CD32a as compared to
195 another bNAbs also used in the clinic (3BNC117) (**Supplementary Figure 2**). Activation by
196 HIV-1 virions, ICs and free bNAb had no significant effect on neutrophil survival, which was
197 maintained around 85%, similarly to unstimulated neutrophils (**Figure 3A**). The stimulation
198 with HIV-1 virions and ICs led to mild but significant upregulation of CD11b and CD66b as
199 compared to unstimulated neutrophils (**Figure 3B**). Both stimuli induced comparable
200 neutrophil activation — as evidenced by increased expression of activation markers (**Figure**
201 **3B**) and cytokine/chemokine secretion (**Figure 3C**). However, no significant downregulation
202 of CD16b expression was observed in cells stimulated with HIV-1 or ICs compared to
203 unstimulated cells. A significant decrease in CD32a expression was observed in IC-activated
204 neutrophils (**Figure 3B**), which may reflect a specific interaction between ICs and this FcγR
205 receptor. Importantly, these findings demonstrate that despite neutrophil activation by HIV-1
206 and ICs, the expression of FcγRs—particularly CD16b—is largely preserved. This limited
207 modulation of FcγRs suggests that neutrophil effector and immunomodulatory functions
208 mediated through antibody-dependent mechanisms remain intact, highlighting a potentially
209 favorable immunological landscape for Fc-mediated immunity and therapeutic strategies
210 against HIV-1 infection.
211
212 ***HIV-1 activation of neutrophils induces IL-8, CXCL1, and CCL4 secretion***
213 In parallel, we assessed the functional activation of neutrophils isolated from HD by HIV-1
214 virions, free or in the form of ICs. Free 10-1074 bNAb was used as control and showed no
215 effect on either the expression of cell surface markers or the secretome of neutrophils. Upon
216 exposure to HIV-1 and ICs, neutrophils displayed a measurable activation profile, characterized
217 by the secretion of specific inflammatory mediators, although to a lesser extent as compared to
218 TLR mimicking viral stimuli (TLR7/8). Notably, IL-8, CXCL1, and CCL4 were significantly

219 upregulated compared to unstimulated cells (**Figure 3C**), indicating a preserved capacity to
220 initiate chemotactic and antiviral responses. Additional cytokines and chemokines, including
221 IL-1β, IFN-λ1, IFN-λ2/3, CCL2, and CCL3, were also detectable in a subset of donors,
222 reflecting a variable but inducible immune responsiveness. Importantly, the overall secretome
223 profiles elicited by HIV-1 and ICs were comparable, supporting the notion that neutrophils
224 retain functional responsiveness to both types of stimuli. Collectively, these results highlight a
225 selective and functionally preserved proinflammatory profile of neutrophils upon HIV-1
226 encounter, characterized by a balanced and targeted chemokine release without excessive
227 activation, including in the context of antibody therapy.
228
229 ***Neutrophils from PLWH exhibit increased release of specific cytokines and chemokines***
230 ***upon stimulation with danger signals associated with HIV-1 infection***
231 Neutrophil effector functions are known to be impaired during HIV-1 infection (5,6). Notably,
232 the restoration of these functions with ART has also been evaluated (31). However, the impact
233 of HIV-1 infection on neutrophil cytokine and chemokine secretion profiles remains
234 underexplored, with most studies focusing on individuals at the AIDS stage (32) rather than
235 ART-treated PLWH. Thus, it is important to investigate how HIV-1 infection and ART
236 treatment affect their immunomodulatory potential. To this end, we evaluated the activation of
237 neutrophils isolated from ART-treated PLWH (**Supplementary Figure 3A–D**) in response to
238 TLR agonists, pro-inflammatory cytokines, HIV-1, or ICs, and compared their responses to
239 neutrophils isolated from HD, including newly collected samples. Importantly, no significant
240 differences were observed in neutrophil frequency in the blood of PLWH compared to HD
241 (**Supplementary Figure 3E**) regardless of the time since diagnosis (**Supplementary Figure**
242 **3F**). Our findings reveal that neutrophils from ART-treated PLWH not only retain their capacity
243 to secrete cytokines and chemokines in response to various danger signals —including
244 bacterial- and virus-like stimuli and pro-inflammatory cytokines— but also exhibit an enhanced
245 release of specific mediators compared to HD (**Figure 4**). Notably, upon TLR4 stimulation,
246 neutrophils from PLWH showed increased secretion of CXCL1 and CCL3, while TLR7/8
247 activation led to elevated levels of CCL3 and IFN-γ (**Figure 4A, 4B**). Interestingly, this new
248 set of samples confirmed that TLR4-activated neutrophils can secrete IFN-λ2/3, as observed in
249 the majority of samples from both HD and PLWH (**Figure 4B**). Pro-inflammatory cytokines
250 differentially modulated the secretion profiles of neutrophils from PLWH compared to those
251 from HD (**Figure 4C, 4D**). Specifically, TNF-α stimulation enhanced CCL2 secretion but
252 reduced IFN-λ1 and IFN-λ2/3production by PLWH neutrophils. Furthermore, IFN-γ

253 stimulation induced the secretion of CCL3 and CCL4 exclusively in neutrophils from PLWH
254 (**Figure 4B, 4D)**. This heightened and selective responsiveness to TLR agonists and pro-
255 inflammatory cytokines suggests a functionally primed state, likely resulting from chronic
256 immune activation or low-grade inflammation associated with HIV-1 infection. In contrast,
257 neutrophils from PLWH had similar secretion profiles than those from HD upon stimulation
258 with free HIV-1 virions or in the form of ICs, both qualitatively (type of cytokine/chemokine)
259 and quantitatively (amount secreted), with the exception of a trend toward reduced CCL4
260 production in response to HIV-1 and IC stimulation (**Supplementary Figure 4)**. These results
261 highlight a distinct neutrophil secretory profile in ART-treated PLWH, particularly under
262 certain stimulatory conditions and/or in specific donor samples. This secretory profile is
263 characterized by both preserved and selectively (stimulus-dependent) heightened
264 immunomodulatory responses. This nuanced cytokine and chemokine landscape suggests a
265 complex yet functionally relevant adaptation of neutrophils during HIV-1 infection, even under
266 effective ART, with potential implications for host defense and inflammation regulation.
267
268 ***Altered FcγR expression dynamics in neutrophils of PLWH***
269 We analyzed FcγR expression on neutrophils from PLWH in response to various danger signals
270 associated with HIV-1 infection, which is particularly relevant in the context of bNAb-based
271 therapies. At baseline, neutrophils from PLWH exhibited significantly higher surface
272 expression of CD16b compared to those from HD (**Supplementary Figure 5A**), while CD32
273 levels showed no significant difference between the two groups (**Supplementary Figure 5B**).
274 Following stimulation with TLR agonists, pro-inflammatory cytokines, HIV-1 virions, or ICs,
275 overall surface expression levels of CD16b and CD32a were comparable between the two
276 groups (**Supplementary Figure 5A-B**). However, when assessing the modulation of FcγR
277 expression relative to baseline levels, distinct differences emerged. Upon TLR4 activation,
278 neutrophils from PLWH exhibited a significantly greater reduction in surface CD16b and
279 CD32a expression compared to HD (**Figure 5A**). In contrast, TLR7/8 stimulation did not
280 induce significant differences in receptor downmodulation between the groups (**Figure 5A**).
281 Similarly to TLR4, TNF-α stimulation caused a more pronounced decrease in CD16b and
282 CD32a expression on neutrophils from PLWH, while IFN-γ selectively reduced CD16b
283 expression (**Figure 5B**). Stimulation with free HIV-1 virions or ICs did not result in any notable
284 differences in FcγR modulation between PLWH and HD (**Figure 5C**). In summary, neutrophils
285 from PLWH exhibit elevated baseline levels of CD16b and show enhanced downmodulation of
286 CD16b and CD32a in response to TLR4 and inflammatory cytokine stimulation. However, this

287 heightened FcγR downregulation was not observed following exposure to viral stimuli such as
288 TLR7/8 agonists or HIV-1 virions. These findings reveal distinct FcγR regulatory dynamics in
289 neutrophils from PLWH, shaped by both the inflammatory environment and the nature of the
290 activating stimulus.
291
292 ***Neutrophils from PLWH display enhanced expression of activating FcγRs, CD63, CXCR4***
293 ***and PD-L1 as well as reduced expression of CD11b and CD49d.***
294 To further evaluate how HIV-1 infection impacts neutrophil properties, we conducted a
295 phenotypic analysis of neutrophils isolated from PLWH and HD. We measured the expression
296 of 18 cell surface markers under basal conditions, focusing on markers relevant to HIV-1
297 infection (CXCR4), neutrophil activation (CD11b, CD66b, CD63, CD62L), maturity (CD10),
298 immunosuppressive properties (PD-L1), and Fc receptors (CD16, CD32, CD64, CD89), among
299 others. Notably, we confirmed the elevated expression of the activating FcγR CD16b and
300 observed a significantly higher expression of the inducible activating FcγR CD64 in neutrophils
301 from PLWH. Additionally, we identified increased expression of the degranulation marker
302 CD63, as well as increased levels of CXCR4 and PD-L1. Conversely, decreased expression of
303 CD49d was detected in PLWH neutrophils (**Figure 6**). These findings demonstrate distinct
304 phenotypic differences in neutrophils from PLWH compared to HD, which may underlay
305 altered functional properties, consistent with the heightened responsiveness to inflammatory
306 stimuli observed in this study.
307

308 **Discussion**

309 We previously demonstrated in a mouse model of retroviral infection the crucial
310 immunomodulatory role of neutrophils in inducing protective (21,22). Here, we provide new
311 insights into the immunomodulatory properties of neutrophils in the context of HIV-1 infection
312 and antiviral antibody therapy, providing a detailed analysis of their secretome and immune
313 receptors expression in a stimulus-specific manner, comparing HD and PLWH. Our work
314 shows that neutrophil from ART-treated PLWH retain their capacity to secrete cytokines and
315 chemokines in response to danger signals and, upon certain stimuli, exhibit enhanced secretion
316 of specific soluble mediators compared to neutrophils from HD, suggesting the effect of HIV-
317 1 infection in neutrophil properties. In addition, notable differences in phenotypic features
318 distinguish them from neutrophils of HD origin. Noteworthy, most studies on
319 immunomodulatory functions of neutrophils have mostly focused on bacterial activation (i.e.
320 TLR4 activation), while the effect of virus-dependent activation is understudied, often relying
321 on TLR-based surrogate models. Our findings fill this knowledge gap by revealing how HIV-1
322 and/or HIV-ICs influence the immunomodulatory functions of neutrophils, and suggest that
323 neutrophils in PLWH maintain functional plasticity and may play a role in shaping immune
324 responses.

325

326 Our data show that both TLR4 and TLR7/8 agonists modulate FcγR expression on neutrophils.
327 Specifically, the TLR4 agonist significantly downregulates CD16b and CD32a, while the
328 TLR7/8 agonist selectively reduces CD32a expression, as previously reported (33). These
329 findings suggest that bacterial signals exert a more pronounced regulatory effect on FcγR
330 expression—particularly on CD16b—compared to viral stimuli. Consistently, exposure to free
331 HIV-1 or ICs has minimal impact on CD16b levels. Additionally, TNF-α, but not IFN-γ,
332 markedly decreases CD16b expression and modestly reduces CD32a. The reduction in FcγR
333 cell surface levels can occur through various mechanisms, including receptor internalization
334 and shedding of the extracellular portion of the FcγR (33,34). Worthy of note, the decrease in
335 FcγR expression following cell activation may serve as a regulatory mechanism, modulating
336 receptor availability on the cell surface and influencing FcγR-mediated cell activation. This
337 stimulus-dependent and pathology-dependent modulation of FcγRs (i.e. increased CD16b and
338 CD64 basal level observed in PLWH neutrophils) might have biological consequences and
339 should be considered in the context of HIV-1 infection and bNAb-based immunotherapies,
340 whether alone or in combinatorial therapeutic approaches involving TLR agonists (14,35–39)

341 as it could influence their therapeutic efficacy. This might also be relevant to consider it in the
342 context of bacterial co-infections in PLWH (40).
343
344 Our work also allowed to dissect at the protein level the chemokine/cytokine secretion profile
345 of neutrophils in a stimulus-dependent manner under both physiological (HD) and pathological
346 (PLWH) conditions. This is important to point out as multiple studies assessing the secretion
347 profile of neutrophils upon different stimuli were based on transcriptomic approaches instead
348 of *bona fide* cytokine/chemokine secretion. Consistent with prior findings, we have shown that
349 neutrophils from HD secrete IL-8, CXCL1, CCL3, and CCL4 upon LPS, R848, or TNF-α
350 activation (25–27,29), while IFN-γ only induced a low secretion of IL-8. Additionally, our work
351 finely describes the secretome of HIV-1-and IC-activated neutrophils, thus far unreported. The
352 secreted cytokines and chemokines, IL-8, CXCL1 and CCL4, play roles in neutrophil activation
353 and recruitment, as well as in recruiting NK cells, monocytes, and other immune cells to sites
354 of inflammation or tissue damage (41). These effects could either amplify inflammation through
355 the recruitment of myeloid cells or enhance viral control through the activation of diverse
356 effector cells. Interestingly, the secretion of different types IFN-λ by neutrophils upon LPS,
357 TNF-α and HIV-1 stimulation, though observed in only a subset of samples, is a novel finding.
358 Worthy of note, IFN-λ1 and IFN-λ2/3 secretion was significatively reduced in TNF-α-activated
359 neutrophils from PLWH, suggesting that the inflammatory environment associated with HIV-
360 1 infection might downregulate IFN-λ secretion by neutrophils. This family of cytokines have
361 been shown to modulate antiviral immune response (42), but its role in HIV-1 infection is still
362 unclear. Further research is needed to confirm IFN-λ secretion by neutrophils under different
363 stimuli and to understand its biological implications in neutrophil function and HIV-1 infection.
364 In this regard, it has been shown that IFN-λ (i) diminishes the production of ROS and
365 degranulation in neutrophils (43) and (ii) IFN-λ3 inhibits HIV-1 infection of macrophages (44).
366
367 Globally, our findings underline that neutrophils isolated from ART-treated PLWH exhibit
368 similar responses to HIV-1 and ICs as neutrophils from HD but exhibit heightened reactivity to
369 TLR agonists and pro-inflammatory cytokines. Notably, the neutrophil secretome in the context
370 of long-term ART has remained largely uncharacterized until now. Our data contrast with
371 earlier reports describing impaired cytokine secretion by LPS-stimulated neutrophils from
372 PLWH who had progressed to AIDS and had been on ART for less than two years (32). These
373 discrepancies suggest that in advanced disease stages, neutrophil responsiveness to pathogen-
374 associated signals may be compromised, potentially contributing to disease progression. In

375 contrast, our results highlight that ART not only preserves neutrophil effector functions (6,45),
376 but also their cytokine/chemokine secretion capacity. Additionally, our work sheds new light
377 on the phenotypic characteristics of neutrophils in PLWH. First, we observed an increased basal
378 expression of two activating FcγRs, CD16b and CD64, while CD32a levels remained
379 unchanged. Notably, CD64 expression on neutrophils is inducible by IFN-γ (46,47)
380 (**Supplementary Figure 1**) , supporting a model in which IFN-γ–mediated conditioning alters
381 neutrophil properties in PLWH. This IFN-γ–mediated conditioning is further consistent with
382 the elevated expression of PD-L1 observed on neutrophils from PLWH. The upregulation of
383 CD16b and CD64 could potentially enhance neutrophil binding to viral ICs. However, this
384 increase did not correlate with enhanced cytokine or chemokine secretion upon IC stimulation,
385 suggesting that these two FcγR are not the primary drivers of cytokine/chemokine induction in
386 response to HIV-ICs. Instead, this points toward a potential role for CD32a—which remained
387 unchanged—in mediating these Fc-dependent responses. This interpretation aligns with the
388 known functional specialization of FcγRs: CD32a is a potent activator of phagocytosis and
389 inflammatory signaling, while CD16b mainly promotes β1 integrin activation, contributing to
390 a pro-adhesive phenotype and the formation of NETs (15). Further investigations are required
391 to determine the potential FcγR-dependent enhancement of cytokines/chemokine by HIV-ICs
392 as well as the FcγRs involved. Additionally, the upregulation of FcγRs could play a role in
393 clearing infected cells through ADCC (48,49) but also in promoting inflammatory immune
394 responses such as reactive oxygen species (ROS) production (50) and NET formation (51).
395 These phenomena can be key in antiviral immune defense but, if exacerbated, they can induce
396 pathological inflammation. In this regard, upregulation of these FcγRs could point to a role for
397 neutrophils in promoting/maintaining chronic inflammation in PLWH. Second, we observed
398 increased expression of CD63, a marker of degranulation that is associated with the release of
399 granules containing enzymes, antimicrobial peptides, and other effector molecules (52,53)
400 suggesting that neutrophils from PLWH might be actively degranulating, possibly in response
401 to infection or inflammation. Third, our results also show an enhanced expression of CXCR4
402 and PD-L1. Interestingly, the upregulation of these two cell surface molecules has recently been
403 associated with neutrophil aging in PLWH (54). However, in contrast to our study, the
404 assessment of CXCR4 and PD-L1 expression was made using samples from ART-naive
405 PLWH. Elevated CXCR4 expression may enhance chemotaxis towards inflammatory sites,
406 potentially aiding immune surveillance but also raising concerns about facilitating HIV-1
407 dissemination (55). Worthy of note, enhanced expression of CXCR4 was observed at any

408 clinical stage of PLWH (**Supplementary Figure 6**). The concurrent decrease in CD49d
409 expression, an integrin subunit essential for adhesion and migration, could indicate altered
410 migratory behaviors, favoring CXCR4-mediated pathways. Globally, the enhanced expression
411 of FcγRs, CD63, CXCR4 as well as the decreased expression of CD49d suggests that
412 neutrophils from PLWH show a state of heightened activation, readiness, and targeted
413 migration of neutrophils. It may suggest an adaptive response for rapid pathogen clearance and
414 active participation in acute inflammatory responses. However, it could also imply a risk of
415 pathological inflammation or tissue damage if not properly controlled. Finally, we confirmed
416 the upregulation of PD-L1 in neutrophils from PLWH, consistent with previous studies
417 describing its expression across various neutrophil subsets (54,56,57) and irrespective of ART
418 (54,56) in PLWH. PD-L1 expression has been associated with T-cell immunosuppressive
419 effects that can be counteracted by blocking the PD-1/PD-L1 axis (54,56), suggesting a
420 potential immunosuppressive role for neutrophils in PLWH.
421
422 In conclusion, our work dissects the phenotypic and functional properties of PLWH neutrophils.
423 While these cells retain their capacity to secrete cytokines and chemokines, they also exhibit
424 immunosuppressive features, such as PD-L1 expression. Our findings also provide new insights
425 on the modulation of FcγR expression by pro-inflammatory cytokines and pathogen-associated
426 danger signals relevant to HIV-1 infection—factors that should be considered in the context of
427 antibody-based therapies. In addition to providing a comprehensive analysis of neutrophil
428 immunomodulatory properties and FcγR dynamics in both health and HIV-1 infection, these
429 findings have important implications for the development of tailored antiviral therapies for
430 PLWH, particularly those involving bNAbs, either alone or in combination with TLR agonists.
431 Moreover, these insights could inform future combinatorial approaches designed to enhance
432 immune responses by targeting neutrophil-mediated regulatory mechanisms—extending
433 beyond bNAb-based interventions— to include strategies such as PD-1/PD-L1 blockade to
434 counteract immunosuppression (54,56) or advanced therapies aimed at limiting potential
435 neutrophil-driven pathological inflammation.
436

437 **Material & Methods**

438 *Human blood samples*

439 Blood samples from anonymized HD were provided by the French establishment for blood
440 donations (Etablissement Français du Sang, EFS, Montpellier, France) according to the
441 agreement between this establishment and INSERM (21PLER2018-0069). EFS provided 5mL
442 of fresh whole blood in EDTA for each healthy donor. The use of blood samples from
443 anonymized PLWH under ART was approved by the Centre for Biological Resources
444 (DRI#305-2019-NC and amendment DRI_2022-69-SR). The samples were collected and
445 provided by the Virology laboratory of the Centre Hospitalier Universitaire (CHU) Lapeyronie
446 of Montpellier (France). Median age was 57 years old ([22;76]) for 31 PLWH donors (19M;
447 13F). All donors were under successful ART with no detectable viral load. 2 to 3mL of whole
448 blood in EDTA were provided for each PLWH donors.

449

450 *Human neutrophil purification*

451 Whole blood sample was diluted to three times its volume with a solution of PBS 1X (Gibco,
452 cat #10010-015) supplemented with EDTA at 0,2 mM (Invitrogen, cat #15575-038) and loaded
453 on Histopaque-1119 (Sigma-Aldrich, cat #11191). Density gradient was performed by 800g
454 centrifugation with slow acceleration and no brake (acceleration 4, brake 0) for 20 minutes.
455 Granulocytes phase was collected, washed with PBS containing EDTA (0,2 mM) and 2% FBS
456 (FBS, Eurobio, REF#CVFSVF00-01) and neutrophils were purified with CD15-positive
457 selection kit (Stemcell technologies, CD15+ positive selection REF#18651) as previously
458 described in (57). Purity assessed by flow cytometry was > 98%.

459

460 *Cell culture*

461 Purified neutrophils were plated at a concentration of 2 million cells/mL in 96 U-bottom wells
462 plate (3 x 10^5 /well) and cultured in RPMI 1640 supplemented with GlutaMAX^{TM} medium
463 (Gibco, cat #61870-010), 10% fetal bovine serum (FBS, Eurobio, REF#CVFSVF00-01), 1%
464 penicillin/streptomycin (Gibco, cat #15140-122) and 10 ng/mL of G-CSF (R&D, cat #214-CS-
465 025).

466

467 *In vitro cell stimulation*

468 Cells were stimulated overnight using TLR agonist, inflammatory cytokines or viral
469 determinants under the following conditions: (i) TLR4 agonist (LPS ; Sigma-Aldrich, cat
470 #L4524) or TLR7/8 agonist (R848 ; Invivogen, cat #tlrl-r848) at 0,5 µg/mL each; (ii) IFN-γ

471 (R&D, cat #285-IF-100) at 50ng/mL or TNF-α (Peprotech, cat #AF-300-01A) at 1ng/mL; (iii)
472 free HIV-1 virions (Ad8 strain, 3.10^4 viral particles) or ICs made with HIV-1 Ad8 and the 10-
473 1074 bNAb (as described below) or the bNAb alone (at 1µg/mL). Dose-response experiments
474 were previously conducted to determine the optimal stimulation conditions.
475
476 *Virus production, purification and titration*
477 2.5 million human embryonic kidney cells (293THEK cell) were seeded in 10 ml of growth
478 media 1 day before transfection. At 50-70% confluence, cells were transfected with calcium
479 phosphate precipitate method, with 10 µg total quantity of HIV-1 molecular clone expressing
480 plasmids, as in (58). At 12 hours post transfection, cells were washed and fresh media
481 supplemented with 20mM Hepes was added on the cells. Cell culture supernatant containing
482 viral particles was collected 36 hours post-transfection, pooled and filtrated through 0.45 µm
483 and then purified by ultracentrifugation on a cushion of 25% sucrose in TNE buffer (10 mM
484 Tris-HCl [pH 7.4], 100 mM NaCl, 1 mM EDTA) at 100000xg, for 1 hour 30 minutes at 4°C,
485 in a SW32Ti Beckman Coulter rotor. Dry pellet was resuspended with RPMI without serum, at
486 4°C overnight. The viral particles were quantified using Videodrop system (Myriade, France).
487
488 *Recombinant* HIV-1 *bNAb production*
489 Recombinant human HIV-1 IgG1 antibodies, anti-CD4bs 3BNC117 (59) and anti-V3-glycan
490 10-1074 (60), were produced by co-transfection of Freestyle 293-F cells (Thermo Fisher
491 Scientific) using the polyethyleneimine (PEI)-based precipitation method as previously
492 described (61), and purified by affinity chromatography using protein G Sepharose 4 fast flow
493 beads according to the manufacturer's instructions (Cytiva). Purified IgG antibodies were
494 dialyzed against PBS using Slide-A-Lyzer Cassettes (10K MWCO; Thermo Fisher Scientific),
495 and quantified using NanoDrop™ ONE instrument (Thermo Fisher Scientific). Both bNAbs
496 are of IgG1 isotype and were used at a concentration of 1µg/mL to form ICs.
497
498 *Immune complexe formation*
499 Viruses (3.10^4 viral particles) and bNAb (1µg/mL) were diluted in culture medium and mixed
500 together on a 1,5mL Eppendorf, then mixed on a rotative agitator for 20 minutes. ICs were
501 gently plated with cells in 96 U-bottom wells plate.
502
503 *Flow cytometry immunophenotyping of neutrophils*

504 For monitoring phenotypic activation, cells were stained at room temperature with a viability
505 dye (ZombieRed™, Biolegend #423109) for 15 minutes then washed and stained for 20
506 minutes at room temperature with fluorochrome-conjugated antibodies. Antibodies for flow
507 cytometry were PerCPCy5-5-CD15 (HI98, #301922), AF488-CD11b (ICRF44, #301317),
508 AF647-CD66b (G10F5, #305110), PC7-CD16a/b (3G8, #302016), PE-CD32a/b (FUN-2,
509 #303206) and PE-CD64 (10.1, #305008) from BioLegend. Finally, cells were washed and
510 resuspended with PBS1X. Data acquisition was performed on a Novocyte (Agilent
511 Technologies) flow cytometer.
512
513 For immunophenotyping of HD's and PLWH's neutrophils the following antibodies were used:
514 PerCPCy5-5-CD15 (HI98, #301922), PE-LOX-1 (15C4, #358604), BV510-CD11b (ICRF44,
515 #301334), AF647-CD66b (G10F5, #305110), BV605-CD62L (DREG-52, #304834), BV650-
516 HLA-DR (L243, #307650), BV711-CD63 (H5C6, #353042), PC7-CD16a/b (3G8, #302016),
517 PE-CF594-CD64 (10.1, #565389), APC-Fire750-CD32a/b (FUN-2, #303220), APC-CD177
518 (MEM-166, #315808), BV605-CD49d (9F10, #304324), BV711-CD31 (WM59, #303136),
519 PE-CF594-CD10 (HI10a, #312227), PE-CD89 (A59, #555686), PC7-CXCR4 (12G5,
520 #306514) from BioLegend. Additionally, PE-PD-1 (3.13, Miltenyi #130-177-384), PC7-PD-
521 L1 (MIH1, BD #558017) and APC-CD172α (15-414, eBiosciences #17-1729-42). The viability
522 staining was made by resuspending the cells with 1mL DAPI (Biolegend #422801) diluted at
523 3μM with an incubation of 15 minutes at room temperature. After the washing of fluorescent
524 antibodies, cells were fixed with 100 µL PFA4% for 10 minutes at room temperature. Finally,
525 cells were washed and resuspended with PBS1X. Data collection was carried out using a BD
526 LSRFortessa flow cytometer from BD Bioscience.
527
528 Forward scatter area and forward scatter height as well as side scatter area and height were used
529 to remove doublets from flow cytometry analyses. Gating is done on live cells as assessed by
530 viability dye and on CD15-positive cells. Data were analyzed using FlowJo software version
531 10.5.3 (TreeStar).
532
533 *Cytokines and chemokines secretion quantification*
534 Secretion of soluble cytokines and chemokines were assayed from cell-free culture supernatants
535 of *in vitro* stimulated neutrophils using bead-based immunoassays (LEGENDplex™,
536 Biolegend). Two assays were used allowing the quantification of IL-1β, Il-6, TNF-α, IP-10
537 (CXCL10), IFN-λ1, IL-8 (CXCL8), IL-12p70, IFN-α2, IFN-λ2/3, GM-CSF, IFN-β, IL-10,

538 IFN-γ (LegendPlex Human anti-virus response panel, Biolegend #740390) and CCL11,
539 CCL17, CCL2, CCL5, CCL3, CXCL9, CXCL5, CCL20, CXCL1, CXCL11, CCL4
540 (LegendPlex Human proinflammatory chemokines panel, Biolegend #740985). Immunoassays
541 plates were read on a Novocyte (Agilent Technologies) flow cytometer and data were analyzed
542 with the appropriate Biolegend LEGENDplex™ software (https://legendplex.qognit.com).
543
544 *Surface Plasmon resonance (SPR)*
545 Affinity of bNAbs for FcγRs was assessed by SPR experiments performed on a Biacore T200
546 (GE Healthcare). SPR experiments were performed on a T200 apparatus at 25°C in PBS
547 containing 0,05 % P20 surfactant (Cytiva). Anti-histidine antibody (R&D Systems) was
548 covalently immobilized on a CM5-S sensor chip flowcell (Fc2) by amine coupling according
549 to the manufacturer's instructions (Cytiva). A control reference surface (flowcell Fc1) was
550 prepared using the same chemical treatment but without anti-His antibody. All kinetic
551 measurements in Fc1 and Fc2 were performed by single-cycle titration at 100µl/min. Each
552 human FcγR (R&D Systems) was captured on immobilized anti-His antibodies at 100-200 RU
553 level. Five increasing concentrations (3,6, 11, 33, 100, 300 nM) of antibody were injected
554 (injection time = 60s) at 100µl/min on captured receptors. After a dissociation step of 600s in
555 running buffer, sensor surfaces were regenerated using 10µl of glycine-HCl pH1.5. All the
556 sensorgrams were corrected by subtracting the low signal from the control reference surface
557 and buffer blank injections. Kinetic parameters were evaluated from the sensorgrams using a
558 two-states or a steady-state models from the T200 evaluation software.
559
560 *Statistics*
561 Statistical analyses were performed using Prism software version 9.5.1 (GraphPad). Simple
562 group comparisons were performed using the Wilcoxon signed rank test for paired data or the
563 Mann–Whitney test for unpaired data. Multiple group comparisons were performed using
564 Friedmann test for paired data or using Kruskal-Wallis test for unpaired data with additional
565 multiple comparisons Dunn test. Significance was assigned as follows: $*p < 0.05$, $**p < 0.01$,
566 $***p < 0.001$, and $****p < 0.0001$.

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Authors contribution

Soledad Marsile-Medun (SM-M), Mar Naranjo-Gomez (MN-G) and Mireia Pelegrin (MP) defined the research program; SM-M, Manon Souchard (MS), Daouda Abba Mousssa (DAM), Giang Ngo (GN), Martine Pugnière (MPu,) and MN-G, performed the experiments; Valerie Lorin (VL) and Hugo Mouquet (HM) provided advice on and generated bNAbs; Elisa Reynaud (ER) and Edouard Tuaillon (ET) managed and provided PLWH blood samples. Rayane Dibsy (RD) and Delphine Muriaux (DM) provided advice on and performed HIV-1 production, purification and quantification. SM-M, MS, MN-G and MP carried out the data analyses. SM-M, MN-G and MP wrote the manuscript. Grants to SM-M, MN-G and MP funded the study. All authors discussed, commented on and approved the manuscript in its final form.

Conflict of interests

None

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752

753 **Figure Legends**
754
755 **Figure 1. Activation of human neutrophils stimulated *in vitro* by Toll-like receptor (TLR) agonists.**
756 Neutrophils from HD were activated overnight by TLR4 (LPS) or TLR7/8 (R848) agonists. Activated
757 neutrophils and corresponding cell culture supernatants were collected to evaluate neutrophil viability
758 and phenotypic activation via flow cytometry, as well as to analyze their secretome using bead-based
759 immunoassays. (A). Gating strategy used for all experiments. (B-C) Viability (B) and phenotypic
760 activation (C) by monitoring cell surface markers using flow cytometry. (D) Cytokines and chemokines
761 secretion profile. Secretion of soluble cytokines (IL-1β, Il-6, TNF-α, CXCL10, IFN-λ1, IL-8, IL-12p70,
762 IFN-α2, IFN-λ2/3, GM-CSF, IFN-β, IL-10, IFN-γ) and chemokines (IL-8, CXCL10, CCL11, CCL17,
763 CCL2, CCL5, CCL3, CXCL9, CXCL5, CCL20, CXCL1, CXCL11, CCL4) was assayed from cell-free
764 cell culture supernatants. Only cytokines/chemokines detected in cell culture supernatants are
765 represented. Values are mean +/- SD of 14 (B-C) and 8 (D) independent measurements. Significance
766 was assigned as follows: $*p < 0.05$, $**p <0,01$, $***p < 0.001$ and $****p < 0.0001$ NS: non-stimulated.
767
768 **Figure 2. Activation of human neutrophils stimulated *in vitro* by TNF-α or IFN-γ.**
769 Neutrophils from HD were activated overnight by TNF-α at 1 ng/mL or IFN-γ at 50 ng/ml. Activated
770 neutrophils and corresponding cell culture supernatants were collected to evaluate neutrophil viability
771 and phenotypic activation via flow cytometry, as well as to analyze their secretome using bead-based
772 immunoassays. (A-B) Viability (A) and phenotypic (B) activation assessed by monitoring cell surface
773 markers. (C) Cytokines and chemokines secretion profile was performed as in **Figure 1** and is
774 represented for each cytokine/chemokine detected upon TNF-α. IFN-γ activation did not induce
775 detectable cytokine or chemokine release, except for a low level of IL-8 secretion, and is therefore not
776 shown in panel C. Values are mean +/- SD of 8 independent measurements. Significance was assigned
777 as follows: $*p < 0.05$, and $**p < 0.01$. NS: non-stimulated.
778
779 **Figure 3. Activation of human neutrophils stimulated *in vitro* by HIV-1 virions, ICs or free bNAb.**
780 Neutrophils from HD were activated overnight by HIV-1 virions, ICs or free bNAb. Activated
781 neutrophils and corresponding cell culture supernatants were collected to evaluate neutrophil viability
782 and phenotypic activation via flow cytometry, as well as to analyze their secretome using bead-based
783 immunoassays. (A-B) Viability (A) and phenotypic activation (B) assessed by monitoring cell surface
784 markers. (C) Cytokines and chemokines secretion profile was performed as in **Figure 1** and is
785 represented for each cytokine/chemokine detected. Values are mean +/- SD of at least 15 (A-B) and 11
786 (C) independent measurements. Several cytokines/chemokines were detected only in a fraction of
787 neutrophil samples assayed. Significance was assigned as follows: $*p < 0.05$, $**p < 0.01$ and $***p <$
788 0.001, NS: non-stimulated.
789
790 **Figure 4. Activation of neutrophils isolated from HD or PLWH stimulated *in vitro* by TLR**
791 **agonists LPS (TLR4) and R848 (TLR7/8) or inflammatory cytokines (TNF-α and IFN-γ).**
792 Neutrophils from HD were activated overnight by TLR agonists or pro-inflammatory cytokines. Cell
793 culture supernatants of activated neutrophils were collected to analyze their secretome using bead-based
794 immunoassays, as for **Figure 1**. (A-B) Cytokines and chemokines secretion profile upon activation by
795 TLR agonists, represented as a heatmap (A) and histograms (B) for each cytokine/chemokine assessed.
796 (C-D) Cytokines and chemokines secretion profile upon activation by inflammatory cytokines,
797 represented as a heatmap (C) and histograms (D) for each cytokine/chemokine assessed. Values are
798 mean +/- SD of at least 9 independent measurements. Several cytokines and chemokines were only
799 detected in a fraction of samples assayed.
800
801 **Figure 5. Modulation of the expression of FcγR on neutrophils isolated from HD or PLWH**
802 **stimulated *in vitro* by TLR agonists, inflammatory cytokines, HIV-1 virions, ICs or free bNAb.**
803 (A-C) Modulation of the cell surface levels of CD16 and CD32 upon activation by TLR agonists (A),
804 cytokines (B), and HIV-1 virions, ICs or free bNAb (C). Fold change is measured versus unstimulated
805 cells (dashed lines). It is represented as the ratio mean fluorescence intensity (MFI) stimulated cells/MFI
806 unstimulated cells. Values are mean +/- SD of at least 14 (A), 8 (B) and 5 (C) independent
807 measurements. Significance was assigned as follows: $*p < 0.05$ and $**p < 0.01$

Figure 6. Phenotypic characterization of neutrophils isolated from PLWH and HD blood samples. Neutrophils purified from whole blood of HD and PLWH were immunophenotyped by assessing the expression of 18 cell surface markers using multiparametric flow cytomety. Data include at least 19 PLWH blood samples and 12 HD samples. Statistical analyses were done using unpaired Mann-Whitney test. Significance was assessed as follows: $*p < 0.05$, $**p < 0.01$, and $****p < 0.0001$.

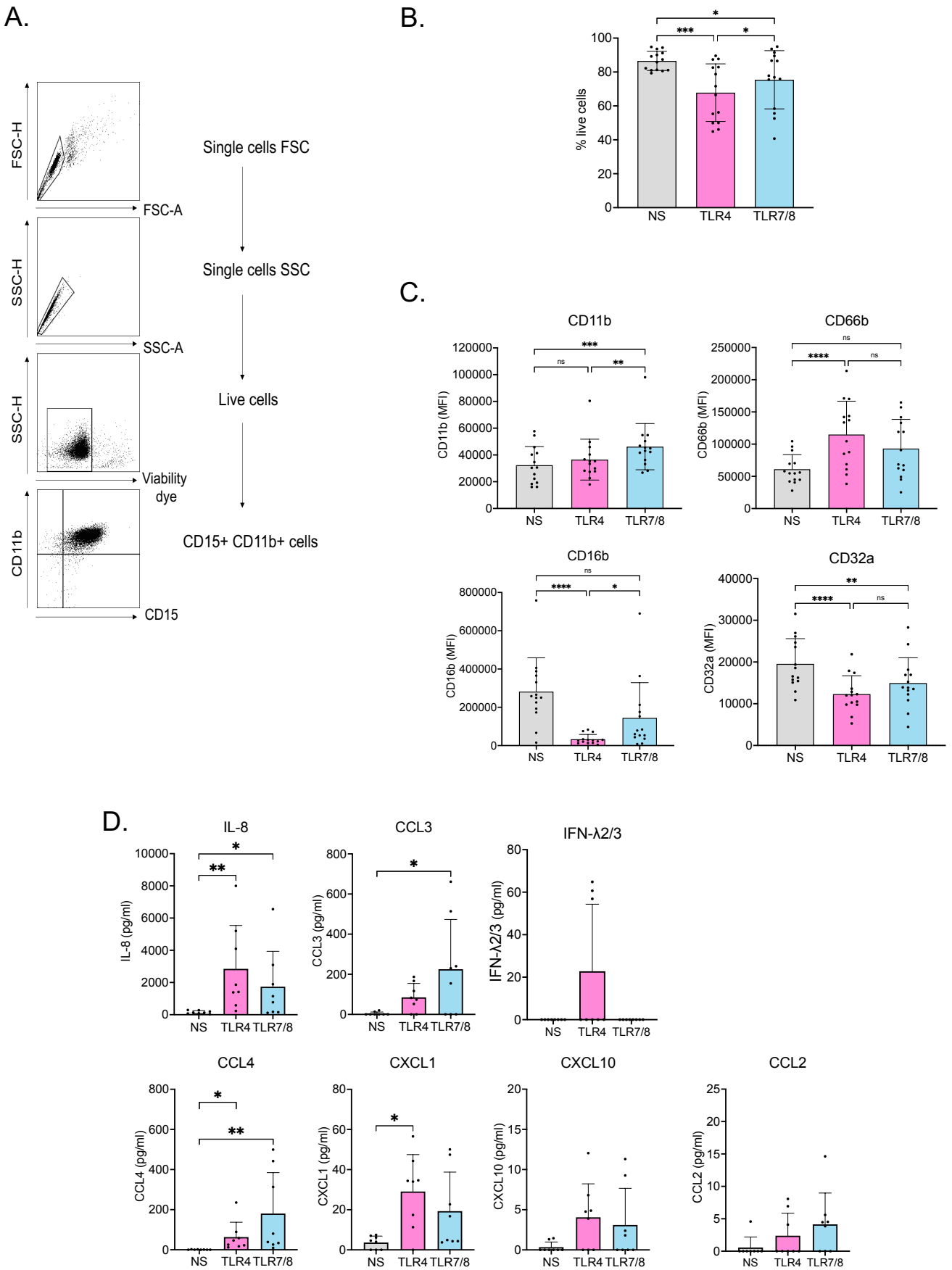
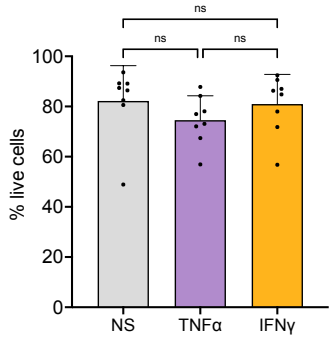
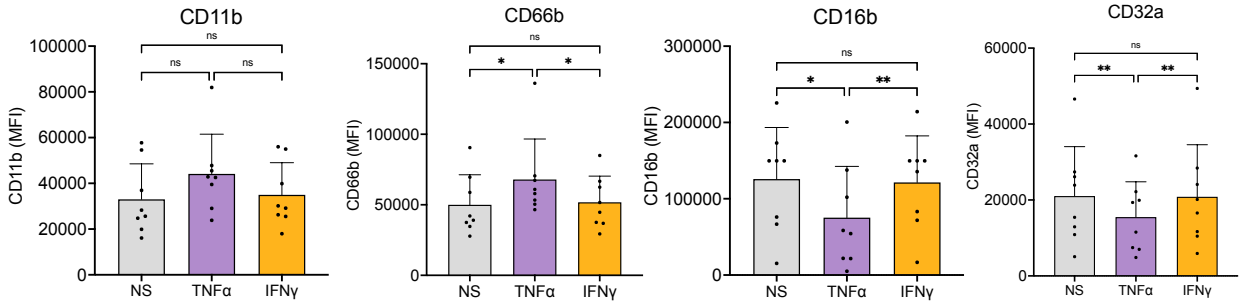

A.
FSC-H
FSC-A
Single cells FSC
SSC-H
SSC-A
Single cells SSC
SSC-H
Viability dye
Live cells
CD11b
CD15
CD15+ CD11b+ cells
B.
% live cells
NS
TLR4
TLR7/8
C.
CD11b
CD11b (MFI)
CD66b
CD66b (MFI)
CD16b
CD16b (MFI)
CD32a
CD32a (MFI)
D.
IL-8
IL-8 (pg/ml)
CCL3
CCL3 (pg/ml)
IFN-λ2/3
IFN-λ2/3 (pg/ml)
CCL4
CCL4 (pg/ml)
CXCL1
CXCL1 (pg/ml)
CXCL10
CXCL10 (pg/ml)
CCL2
CCL2 (pg/ml)

Figure 1

A.


ns
ns
ns
% live cells
100
80
60
40
20
0
NS
TNFα
IFNγ

B.


CD11b
CD11b (MFI)
CD66b
CD66b (MFI)
CD16b
CD16b (MFI)
CD32a
CD32a (MFI)
ns
*
**
NS
TNFα
IFNγ

D.

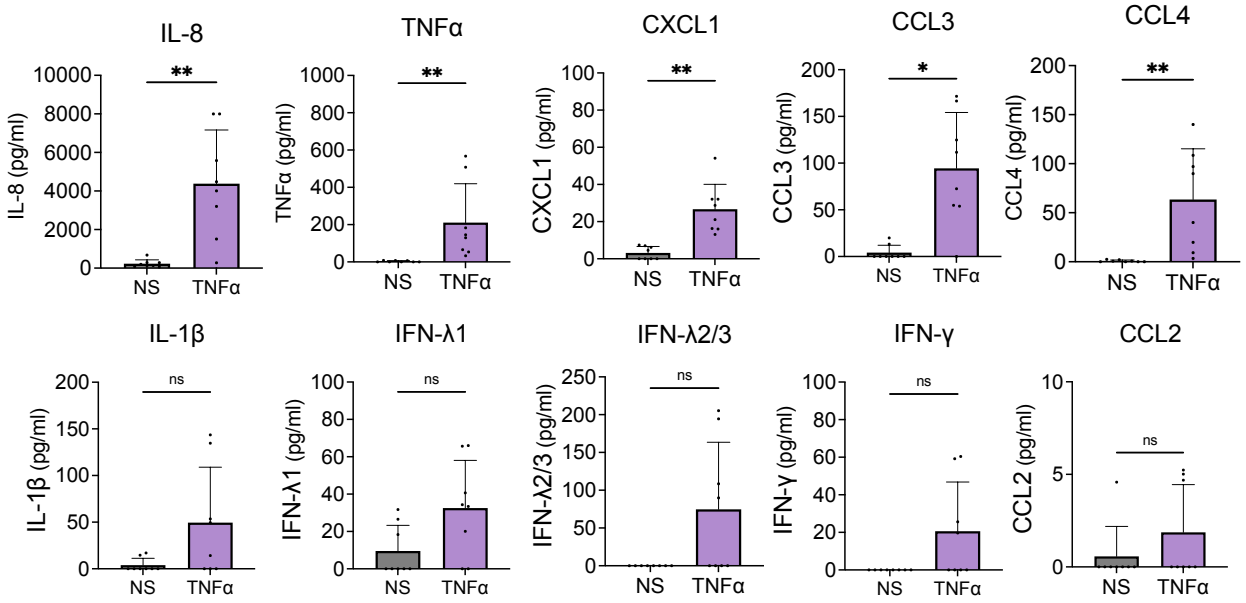
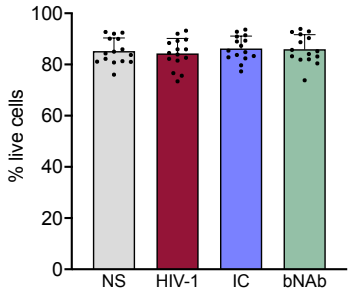
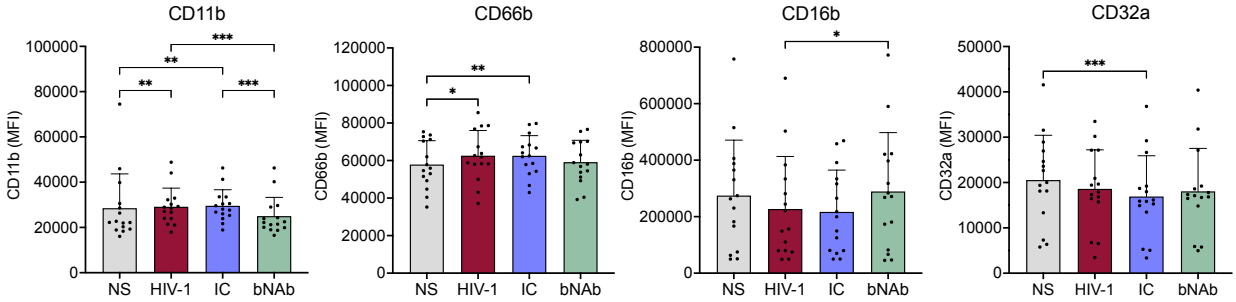

IL-8
IL-8 (pg/ml)
TNFα
TNFα (pg/ml)
CXCL1
CXCL1 (pg/ml)
CCL3
CCL3 (pg/ml)
CCL4
CCL4 (pg/ml)
IL-1β
IL-1β (pg/ml)
IFN-λ1
IFN-λ1 (pg/ml)
IFN-λ2/3
IFN-λ2/3 (pg/ml)
IFN-γ
IFN-γ (pg/ml)
CCL2
CCL2 (pg/ml)
ns
*
**
NS
TNFα

Figure 2

A.


% live cells
100
80
60
40
20
0
NS
HIV-1
IC
bNAb

B.


CD11b
CD66b
CD16b
CD32a
CD11b (MFI)
CD66b (MFI)
CD16b (MFI)
CD32a (MFI)
NS
HIV-1
IC
bNAb

C.

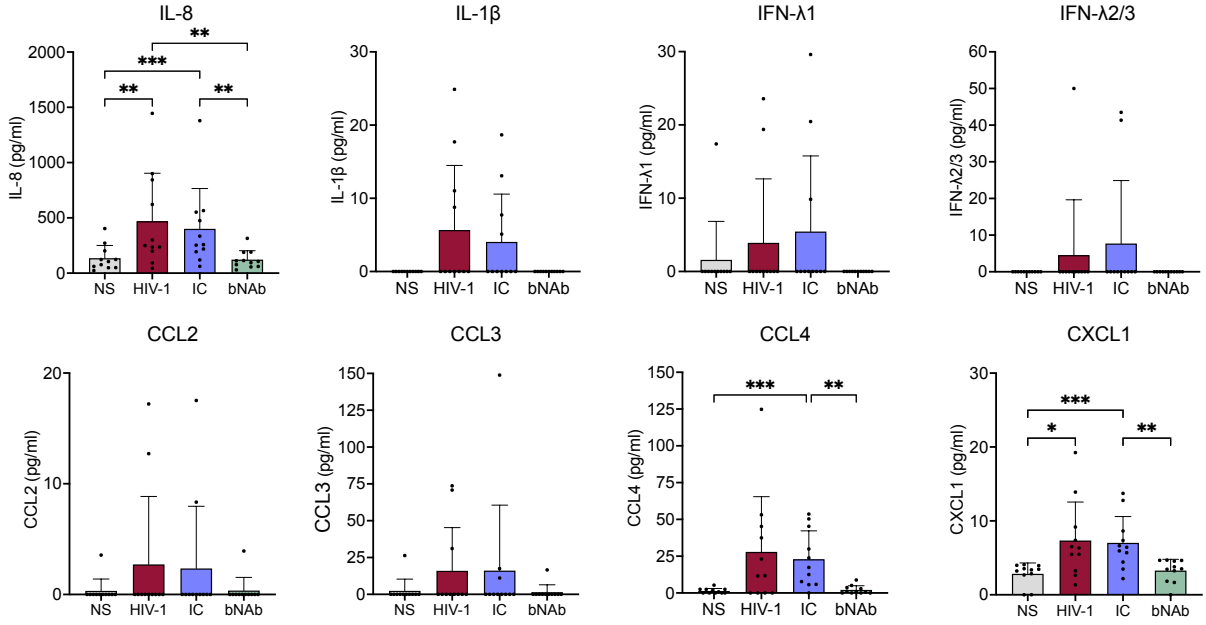

IL-8
IL-1β
IFN-λ1
IFN-λ2/3
CCL2
CCL3
CCL4
CXCL1
IL-8 (pg/ml)
IL-1β (pg/ml)
IFN-λ1 (pg/ml)
IFN-λ2/3 (pg/ml)
CCL2 (pg/ml)
CCL3 (pg/ml)
CCL4 (pg/ml)
CXCL1 (pg/ml)
NS
HIV-1
IC
bNAb

Figure 3

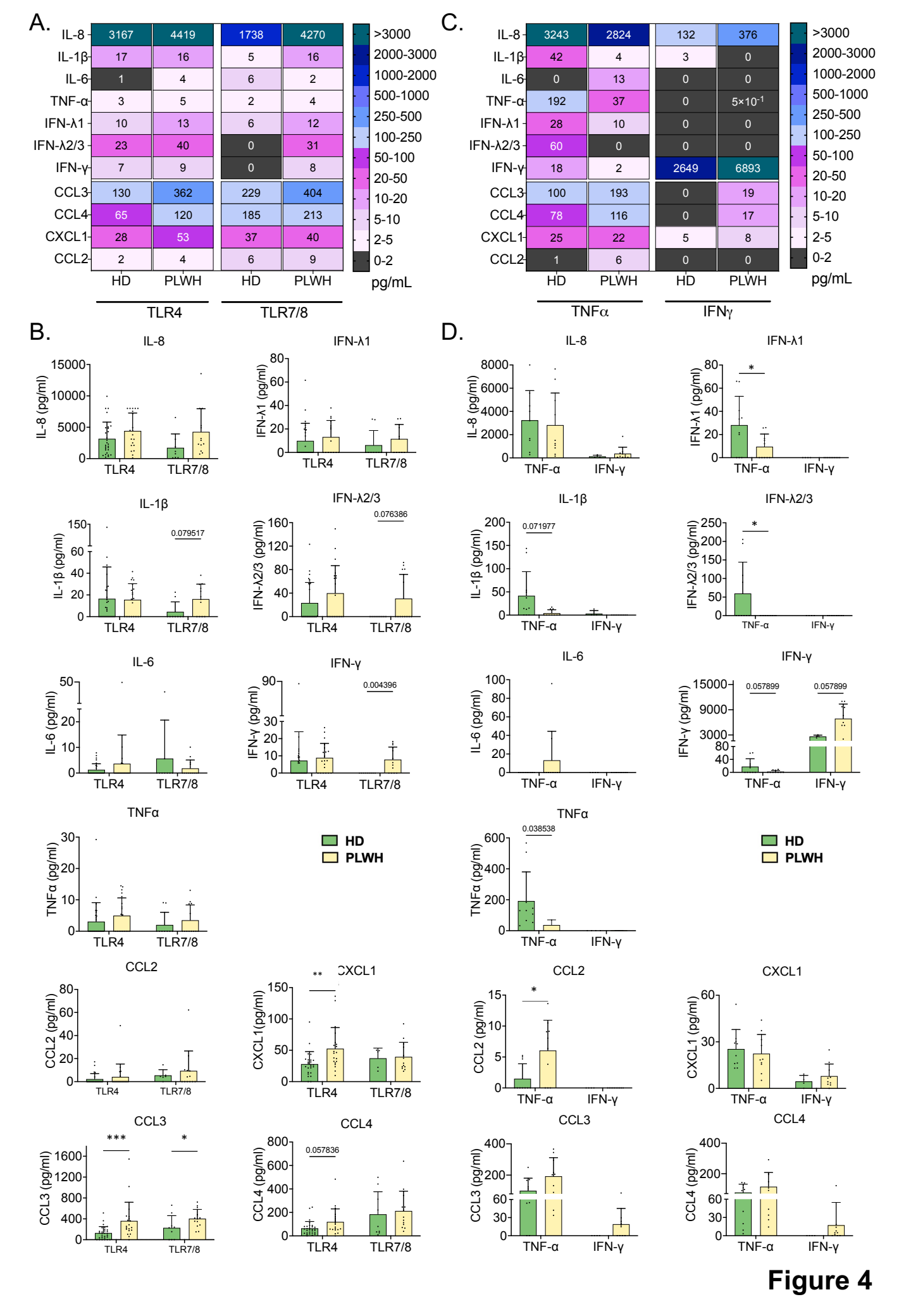

A.
IL-8 3167 4419 1738 4270
IL-1β 17 16 5 16
IL-6 1 4 6 2
TNF-α 3 5 2 4
IFN-λ1 10 13 6 12
IFN-λ2/3 23 40 0 31
IFN-γ 7 9 0 8
CCL3 130 362 229 404
CCL4 65 120 185 213
CXCL1 28 53 37 40
CCL2 2 4 6 9
HD PLWH HD PLWH
TLR4
TLR7/8
>3000
2000-3000
1000-2000
500-1000
250-500
100-250
50-100
20-50
10-20
5-10
2-5
0-2
pg/mL
C.
IL-8 3243 2824 132 376
IL-1β 42 4 3 0
IL-6 0 13 0 0
TNF-α 192 37 0 5×10-1
IFN-λ1 28 10 0 0
IFN-λ2/3 60 0 0 0
IFN-γ 18 2 2649 6893
CCL3 100 193 0 19
CCL4 78 116 0 17
CXCL1 25 22 5 8
CCL2 1 6 0 0
HD PLWH HD PLWH
TNFα
IFNγ
B.
IL-8
IFN-λ1
IL-1β
0.079517
IFN-λ2/3
0.076386
IL-6
IFN-γ
0.004396
TNFα
HD
PLWH
CCL2
CXCL1
**
CCL3
***
*
CCL4
0.057836
TLR4
TLR7/8
D.
IL-8
IFN-λ1
*
IL-1β
0.071977
IFN-λ2/3
*
IL-6
IFN-γ
0.057899
0.057899
TNFα
0.038538
HD
PLWH
CCL2
*
CXCL1
CCL3
CCL4
TNF-α
IFN-γ

Figure 4

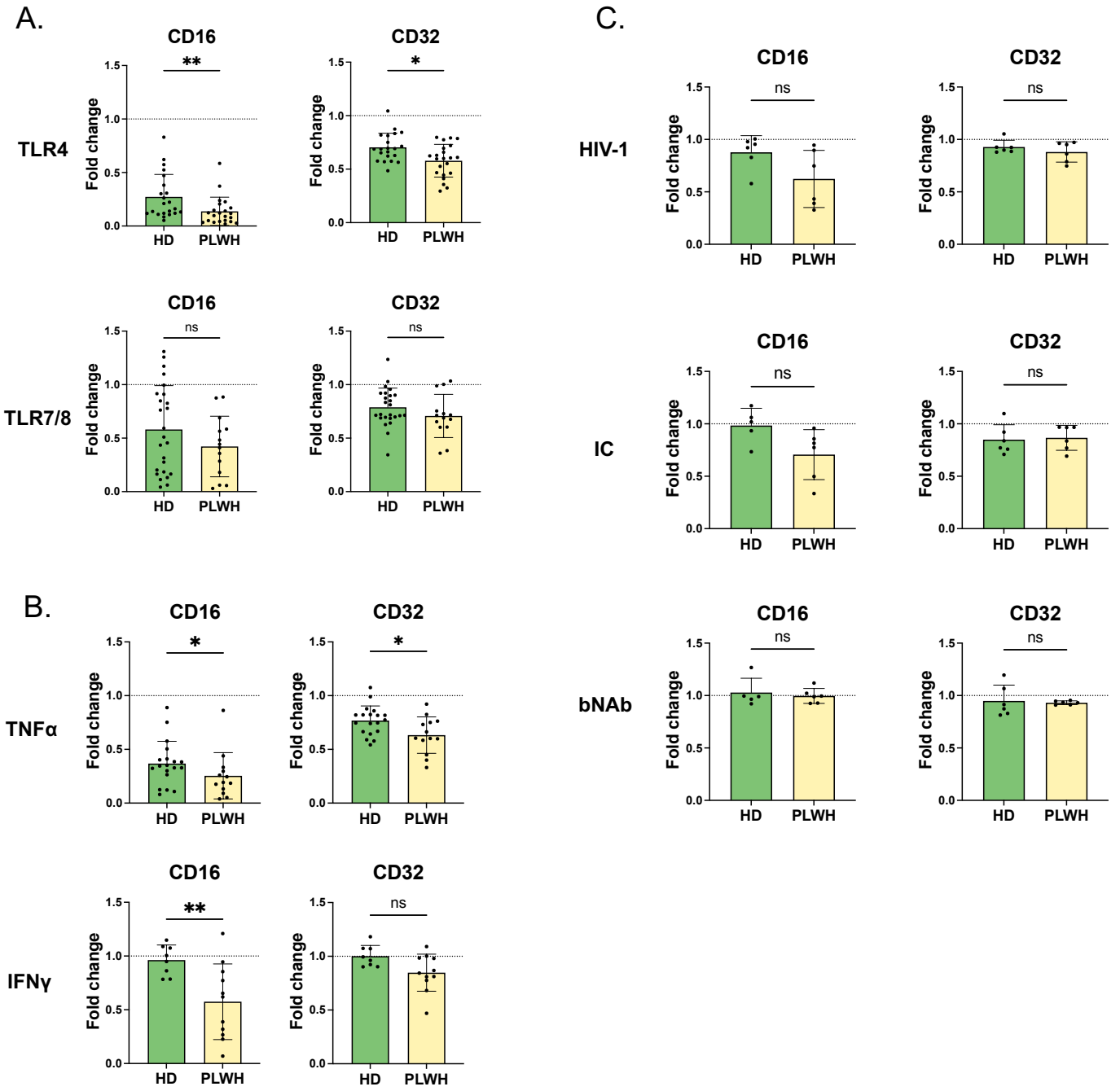

A.
CD16
CD32
TLR4
TLR7/8
Fold change
1.5
1.0
0.5
0.0
HD
PLWH
**
*
ns
B.
TNFα
IFNγ
C.
HIV-1
IC
bNAb

Figure 5

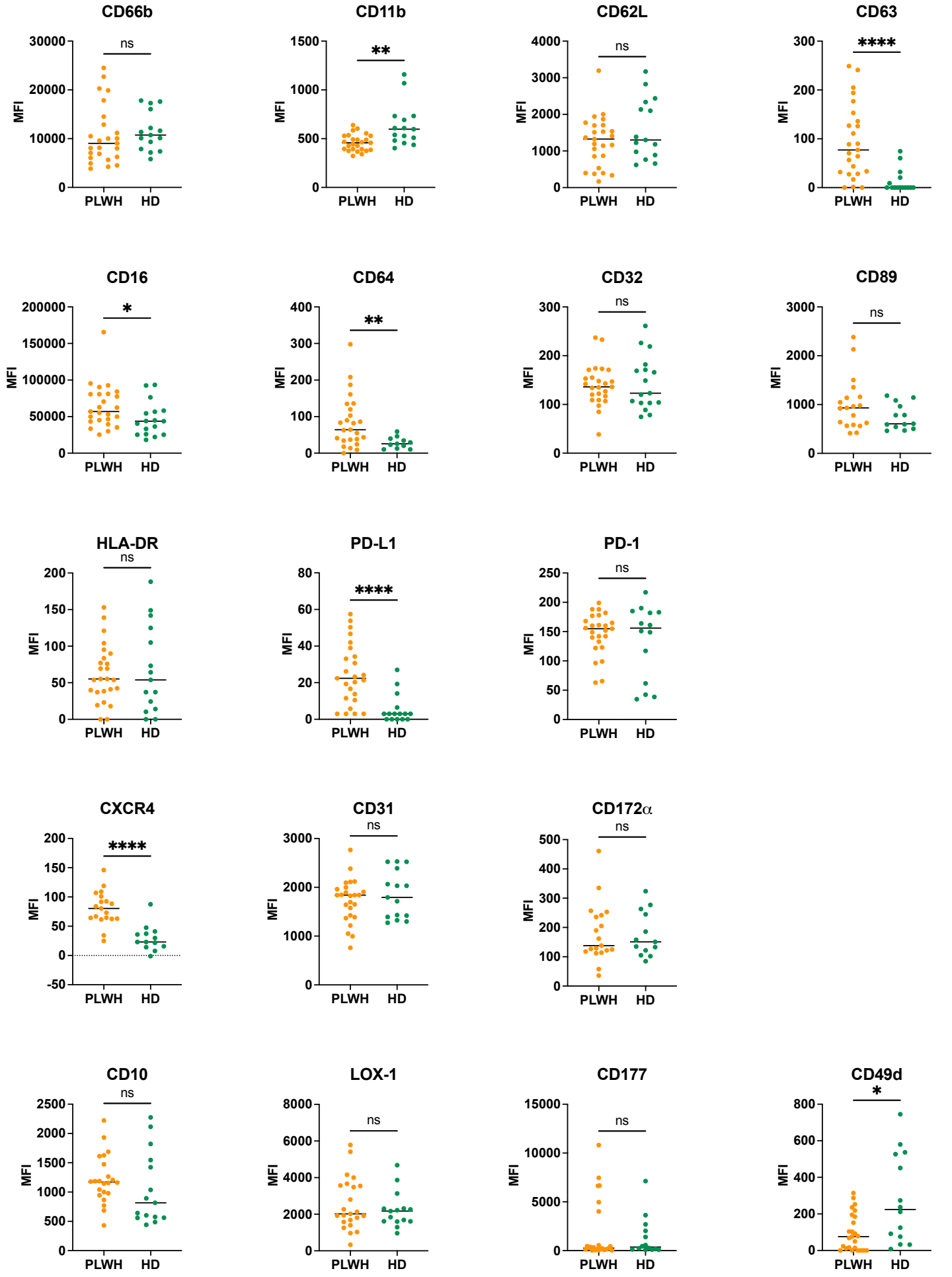

CD66b
ns
CD11b
**
CD62L
ns
CD63
****
CD16
*
CD64
**
CD32
ns
CD89
ns
HLA-DR
ns
PD-L1
****
PD-1
ns
CXCR4
****
CD31
ns
CD172α
ns
CD10
ns
LOX-1
ns
CD177
ns
CD49d
*
MFI
PLWH
HD

Figure 6