M. tuberculosis antigen-responsive IL17^{+} CD4 T cells are disproportionately spared in ART-suppressed HIV

Paul Ogongo[1,2], Anthony Tran[1], Florence Marzan[3], David Gingrich[3], Melissa Krone[4], Francesca Aweeka[3], Cecilia S Lindestam Arlehamn[5,6], Jeffrey N. Martin[4], Steven G. Deeks[7], Peter W. Hunt[1#*] and Joel D. Ernst[1#*]

[1]Division of Experimental Medicine, University of California, San Francisco, CA, USA

[2]Department of Tropical and Infectious Diseases, Institute of Primate Research, Nairobi, Kenya

[3]Drug Research Unit, Department of Clinical Pharmacy, School of Pharmacy, University of California, San Francisco, CA, USA

[4]Department of Epidemiology and Biostatistics, University of California, San Francisco, CA, USA

[5]Center for Infectious Disease and Vaccine Research, La Jolla Institute for Immunology, La Jolla, CA 92037, USA

[6]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, USA

[7]Division of HIV, Infectious Diseases, and Global Medicine, University of California, San Francisco, CA, USA

[#] Joint senior authors

* Correspondence

Running title: Mtb-responsive Th17 cells during HIV-ART

Abstract

Background. Interleukin 17 producing CD4 T cells contribute to control of *Mycobacterium tuberculosis (Mtb)* infection in humans; whether infection with Human Immunodeficiency Virus (HIV) disproportionately affects distinct Th17 cell subsets that respond to Mtb are incompletely defined.

Methods. We performed high-definition characterization of circulating Mtb-specific Th17 cells by spectral flow cytometry in people with latent TB and treated HIV (HIV-ART). We also measured kynurenine pathway activity in plasma by LC/MS and tested the hypothesis that tryptophan catabolism influences Th17 cell differentiation in this context.

Results. We identified two categories of Th17 cells: T_H17 ($CD4^+V\alpha7.2^-CD161^+CD26^+$) and T17 ($CD4^+V\alpha7.2^-CCR6^+CXCR3^-$) cells that were disproportionately reduced in LTBI with HIV-ART, yet Mtb-responsive IL17-producing CD4 T cells were preserved; we found that IL17-producing CD4 T cells dominate the response to Mtb antigen but not CMV antigen or staphylococcal enterotoxin B (SEB); and tryptophan catabolism negatively correlates with T_H17 and T1T17 but not T17 cell frequencies.

Conclusions. We found differential effects of ART-suppressed HIV on distinct categories of Th17 cells, that IL17-producing CD4 T cells dominate responses to Mtb but not CMV antigen or SEB, and that kynurenine pathway activity is associated with selective decreases of circulating Th17 cells that may contribute to tuberculosis immunity.

Key words: Interleukin 17; CD4 T cells; antigen-responsive; immunity; Tuberculosis; ART-suppressed HIV; kynurenine pathway

51

52 **Introduction**

53 Tuberculosis (TB) remains the leading cause of death among people with HIV (PWH) [1] at
54 least in part because of CD4 T cell depletion [2]. CD4 T cells are critical for immunity to TB,
55 although the CD4 T cell phenotypes, functions, and antigen specificity that contribute to TB
56 immunity are incompletely understood. Importantly, the risk of TB in PWH remains higher
57 than in those without HIV, even after reconstitution by antiretroviral therapy (ART) [3,4],
58 indicating that the effects of HIV on TB immunity are not simply because of loss of bulk CD4
59 T cells. Indeed, HIV may preferentially infect Mtb-specific CD4 T cells [5,6], and one or more
60 defects in Mtb-specific CD4 T cells might be incompletely restored by ART.

61

62 Increasing evidence indicates that CD4 T cells that produce interleukin 17 (IL17), broadly
63 termed T helper 17 (Th17) cells, contribute to control of TB. In Mtb-infected adolescents, IL17
64 transcriptional signatures decreased in blood of those who progressed to active TB compared
65 to non-progressors [7]; another study found a subset of CD4 T cells that produce IL-17 in
66 response to Mtb antigens that is less abundant in TB progressors than in non-progressors [8].
67 Mtb-responsive $CD4^+$ T cells producing IL17 are enriched in human lungs compared to
68 matched blood and inversely correlated with plasma IL-1β, suggesting a role in control of Mtb
69 [9]. IL17 producing CD4 T cells are depleted in HIV infection and their depletion contributes
70 to progression to AIDS through breakdown in intestinal mucosal barrier function [10,11].

71

72 Several approaches are used to identify T helper 17 cells (herein denoted 'Th17' cells to include all
73 subsets regardless of criteria used). IL17 production upon stimulation is the canonical signature of
74 Th17 cells [12,13]. Although this identifies Th17 cells, it does not classify all cells in the Th17
75 lineage, and other cells can produce IL17. In blood of healthy donors and synovial fluid of

76 rheumatoid arthritis patients, a CCR6^{+} subset contained all IL17-producing T cells expressing
77 *RORC* mRNA [14], the transcription factor that supports Th17 differentiation in humans. Use of
78 CCR6 as a marker of Th17 cells has been replicated in naïve cord blood [15], inflammatory
79 diseases [16] and infections like TB [17]. The cell surface markers CD26 [18,19] and CD161
80 [20,21] have also been used to identify Th17 cells. CD26^{hi} CD4^{+} T cells were demonstrated to co-
81 express CD161 and CCR6 and enriched for production of IL17 [18]. In transcriptomic studies,
82 Th17 cells are identified by expression of *RORC*, *IL23R* (supports expansion of IL17-producing
83 cells) and *IL17* mRNA.
84
85 Host metabolism impacts immune responses [22] by driving cell differentiation. One example
86 is the kynurenine pathway of tryptophan catabolism whose products, collectively called
87 kynurenines, inhibit Th17 while promoting Treg development. [23–26]. In TB, circulating
88 tryptophan concentrations ([Trp]) decline in persons with LTBI progressing to active TB [27]
89 and [Trp] increases with TB treatment [23]. Similarly, HIV infected persons have low
90 circulating [Trp] with a link to pathogenesis [28]. Plasma kynurenine/tryptophan ratios (K/T)
91 can distinguish humans with active TB from those without and are significantly higher in
92 MDR-TB patients with or without HIV coinfection compared to controls [23]. Plasma K/T is
93 also elevated in PWH compared to controls, especially in those who have progressed to
94 AIDS[25,29]. While a link between K/T and Treg/Th17 ratio has been established in HIV,
95 similar data are lacking in TB.
96
97 Here, we characterized subsets of Th17 cells defined by distinct criteria in participants with
98 ART-suppressed HIV (HIV-ART), to determine whether IL17-producing CD4 T cells that
99 respond to Mtb antigens are altered in HIV-ART, and to test the hypothesis that indoleamine

100 2,3-dioxygenase (IDO) activity and generation of kynurenines influences Th17 differentiation
101 in HIV-ART.
102
103 **Materials and Methods**
104 *Participants and sample collection*
105 PBMC and plasma were obtained from adults with or without HIV enrolled in the UCSF
106 SCOPE cohort [25]. All participants gave written informed consent using protocols approved
107 by the UCSF Instititutional Review Board. Blood was collected into EDTA-containing tubes
108 for cell counts and into ACD-containing tubes for purification of plasma and PBMCs.
109 Processed plasma was stored at -80ºC while PBMC were cryopreserved in liquid nitrogen.
110 Demographic characteristics of the participants are in Tables 1 and 2.
111
112 *Plasma Tryptophan and Kynurenine measurement*
113 Tryptophan and Kynurenine concentrations were measured by liquid chromatography-mass
114 spectrometry as previously described [25,29].
115
116 *PBMC antigen stimulation and Intracellular cytokine staining*
117 Cryopreserved PBMCs were thawed in a 37ºC water bath and transferred into pre-warmed R10
118 media (RPMI 1640 containing L-glutamine with 10% FBS, 1%PenStrep and 1% Hepes). Cells
119 were centrifuged at 2000rpm for 5 minutes at room temperature, supernatants discarded, and
120 cells resuspended before adding 5ml of warm R10 and transfer to a 6-well culture plate and
121 resting overnight in 37ºC/5%CO_2 incubator. After resting, 1x10^6 live cells resuspended in
122 200µL R10 were transferred to each well of a 96-well round bottom plate and duplicate wells
123 were stimulated with Mtb peptide mega pool (Mtb300) [30](2µg/ml), CMV pp65 peptide
124 (1µg/ml) or Staphyloccal enterotoxin B (SEB) positive control (1µg/ml) in the presence of

125 costimulating antibodies anti CD28 (1µg/ml) and anti CD49d (1µg/ml) (BD). Negative
126 controls received no stimulation. After two hours, GolgiStop and GolgiPlug (BD) were added
127 to one well of each of the stimulation conditions, and the cells were incubated for additional
128 18 hours.
129
130 After 20-hour total stimulation, cells were washed, stained with Live/Dead Fixable Blue Dead
131 Cell stain kit (Invitrogen), washed again, then surface antibody cocktail (αCD3 BV510 clone
132 UCHT1, αCD8 BV570 clone RPA-T8, αCCR7 BV785 clone G043H7, αCD95 Alexa Fluor700
133 clone DX2, αCCR6 FITC clone G034E3, αCXCR3 BV 605 clone G025H7, αCD161 APC-
134 Fire750 clone HP-3G10, αCD69 BV650 clone FN50, αCD137 BV711 clone 4B4-1, αOX40
135 PE-Cyanine7 clone (Ber-ACT35) and αTRAV1-2 (Vα7.2) BV421 clone 3C10 (all from
136 BioLegend), αCD4 BUV496 clone SK3, αCD45RA BUV395 clone 5H9, αCD27 BUV615
137 clone L128, αCD25 BUV563 clone 2A3, αCD39 BUV737 clone TU66 and αCD26 BUV805
138 clone M-A261 (all BD)) diluted in Brilliant Violet buffer (BD) was added then kept in the dark
139 for 20 minutes at room temperature., After washing, cells were fixed and permeabilized using
140 eBioscience FOXP3/Transcription Factor staining kit (Invitrogen). Cells were washed twice
141 with 1X eBioscience Perm diluent, then resuspended in intracellular antibody mix (αRORγT
142 Alexa Fluor 647 Clone Q21-559, αIFNγ BB700 clone 2B7 (both from BD Biosciences), αIL17
143 PE clone BL168, αT-bet PE-Dazzle 594 clone 4B10 (both from BioLegend) and αFoxP3 PE-
144 Cyanine5.5 clone PCH101 (Thermofisher)) in eBiosciences perm diluent. Cells were then
145 washed twice with 1X eBioscience Perm diluent and fixed in 2% PFA. Data acquired on a 5-
146 Laser Aurora Spectral Flow cytometer (Cytek).
147
148 *Data and statistical analysis*

149 Spectral flow fcs files were initially analysed using SpectroFlo v3.0 (Cytek) for unmixing and
150 autofluorescence correction. Identification of T cell subsets from unmixed fcs files was
151 performed using Flowjo v10 (Flowjo LLC). Antigen specific cytokine levels are reported after
152 subtraction of values of unstimulated cells for each participant. K/T ratio was determined from
153 the concentrations of tryptophan and kynurenines after quality control checks. Non-parametric
154 tests were used for comparison between two groups for paired (Wilcoxon test) or unpaired
155 (Mann-Whitney test) and $p < 0.05$ considered significant. Adjusted comparisons between
156 groups in continuous variables were performed with linear regression, transforming outcome
157 variables to satisfy model assumptions. Spearman correlation was used to test the association
158 between parameters with a $p < 0.05$ considered significant. All statistical analyses used Prism
159 9.0 (GraphPad).
160
161 **Results**
162
163 ***Subsets of circulating T helper 17 cells are disproportionately reduced in LTBI with treated***
164 ***HIV***
165 To study the effects of LTBI and ART-treated HIV (HIV-ART) on Th17 cells defined by
166 different criteria, we designed a spectral flow cytometry panel encompassing cytokines,
167 chemokine receptors, transcription factors and cell surface markers (Figure 1A&C). We used
168 the following nomenclature: (a) T_H17 are $CD26^+CD161^+$ [18,19]; (b) T17 are $CCR6^+CXCR3^-$
169 [14,17]; and (c) T1T17 are $CCR6^+$ $CXCR3^+$ [17,31]. We first gated out $V\alpha7.2^+$ $CD4^+$ T cells
170 to exclude MAIT cells that share certain markers with Th17 cells [32], since MAITs can
171 produce IL17 [33].
172

173 We also stained for RORγT, the Th17 lineage defining transcription factor [34] and used
174 unsupervised clustering and t-distributed stochastic neighbor embedding (tSNE) to determine
175 whether T_H17 and T17 cells express RORγT and/or produce IL17 upon stimulation with an
176 Mtb peptide mega pool (Mtb300) (Figure 1C). To identify T1T17 (also termed Th1*) cells
177 that respond to Mtb antigens [17], we included T-bet, the Th1 lineage transcription factor [35].
178 We found a positive and significant association ($r = 0.4472$, $p = 0.0002$) (Figure 1B) between
179 T_H17 and T17 cells, and found that cells in both populations express RORγT and produce IL17
180 (Figure 1C). Therefore, T_H17 and T17 cells express other properties of bona fide Th17 cell
181 populations. T-bet expressing cells were present within T_H17 and T17 cell populations
182 suggesting a Th1* population that expresses both CCR6 and CXCR3 and likely produce both
183 IFNγ and IL17 (Figure 1C).
184
185 As expected, circulating CD4 T cells were reduced in HIV-ART compared with HIV-
186 uninfected participants, (Figure 2A). We first phenotyped $CD4^+$ T cell populations based on
187 CD45RA and CCR7 [9] to identify naïve, central memory, effector memory and terminally
188 differentiated T cells (Figure S1 A, left). The predominant populations were naïve and central
189 memory T cells and there was no difference in the frequency of cells in these memory
190 populations in participants with or without HIV (Figure 2B). $CD4^+CD45RA^+CCR7^+$ (naïve T
191 cells) are a heterogenous population, including cells that produce cytokines upon short term
192 stimulation [36], so we characterised the naïve T cells further based on co-expression of CD95
193 and CD27 to identify cells with a stem cell-like memory phenotype (Figure S1 A, right). We
194 found no difference in frequencies of $CD95^+CD27^+$ stem cell-like memory cells or $CD95^-$
195 $CD27^+$ 'true naïve' cells in those with or without HIV (Figure S1 B). Thus, whereas individuals
196 with treated HIV have lower overall frequencies of circulating $CD4^+$ T cells than those without
197 HIV (Tables 1 and 2, Figure 2A), their memory states do not differ.

198

199 Next, we quantitated Th17 cells in those with LTBI without HIV or with HIV-ART and found
200 significantly lower frequencies of T_H17 ($CD4^+V\alpha7.2^-CD26^+CD161^+$) and T1T17 ($CD4^+V\alpha7.2^-$
201 $CCR6^+CXCR3^+$) cells (expressed as % of $CD4^+$ T cells) in HIV-ART than in those without
202 HIV (Figure 2C). Although T17 cells ($CD4^+V\alpha7.2^-CCR6^+CXCR3^-$) and Th1 ($CD4^+V\alpha7.2^-$
203 $CCR6^-CXCR3^+$) cells were also present at lower frequencies in HIV-ART, the difference was
204 not significant (Figure 2C). These data demonstrate that certain circulating Th17 cell subsets
205 are disproportionately depleted in HIV-ART, while others are relatively preserved.

206

207 Th17 and regulatory T cells (Tregs) develop under related but distinct cytokine environments
208 [37]. Tregs contribute to immune homeostasis by maintaining unresponsiveness to self-
209 antigens, suppressing exaggerated immune responses, and promoting epithelial tissue integrity
210 [38]. Human Tregs are identified by the expression of high levels of CD25 and the transcription
211 factor FoxP3 [39]. Since the kynurenine pathway has differential effects on Th17 and Treg
212 differentiation, we characterised Treg cells (Figure S2A, left) and found that the frequency of
213 circulating Treg was similar in HIV-ART and those without HIV (Figure S2B). We also found
214 that the frequency of activated ($CD39^+$) Tregs did not differ in the two groups (Figure S2A,
215 right) although there was considerable variation in $CD39^+$ Treg, especially in HIV-ART
216 (Figure S2 C). We also compared the ratio of T_H17 and T17 cells to Tregs between the groups
217 and found a trend toward a lower T_H17/Treg in HIV-ART than in those without HIV (Figure
218 S2D), but the difference was not significant, and T17/Treg ratios were similar in HIV-ART
219 and those without HIV (Figure S2E). Taken together, we found that certain circulating Th17
220 cell subsets are disproportionately reduced in LTBI with treated HIV, suggesting that even
221 effective treatment of HIV does not reconstitute all Th17 cell subsets equally. We did not find
222 differences in Treg by HIV status.

223

224 ***Mtb antigen-responsive IL17-producing cells are enriched in T_H17 compared with T17***
225 ***populations in HIV-ART***

226 Having established that $CD26^+CD161^+$ T_H17 and $CCR6^+$ T17 cells are bona fide Th17
227 populations, we determined whether T_H17 and T17 cells differ in IL17 production after
228 stimulation. HIV-ART participants with LTBI had significantly more $IL17^+$ cells in T_H17 cells
229 than in T17 cells upon stimulation with the Mtb300 peptide pool (Figure 3A, left). All except
230 one participant had $IL17^+$ cells in T_H17 cells compared with 11 of 21 participants with $IL17^+$
231 cells in T17 cells (Figure 3A, left). We did not have enough cells from participants without
232 HIV (cohort 1) for cytokine analysis. We confirmed this observation in cohort 2; regardless of
233 the sampling time point, there were significantly more $IL17^+$ cells in T_H17 cells than in T17
234 cells (Figure 3A, right). We performed a similar analysis after stimulation with SEB and found
235 an opposite pattern: IL17 production was significantly higher in T17 than T_H17 cells (Figure
236 3B). Additionally, CMV peptide stimulation induced equivalent production of IL17 by T_H17
237 and T17 cells, although there was a trend towards higher responses in T17 cells (Figure S3A).
238 These data reveal enrichment of IL17 production to Mtb antigens by a subset of Th17 cells
239 marked by expression of CD26 and CD161 (T_H17) which are distinct from IL17 producing
240 cells that are marked by expression of chemokine receptor CCR6 (T17) and those that produce
241 IL17 in response to SEB or CMV.

242

243 ***Mtb antigen-responsive IL17-producing $CD4^+$ T cells are relatively preserved in people***
244 ***with LTBI and HIV-ART***

245 Next, we assessed IFNγ and IL17 production after Mtb300 stimulation. In HIV-ART, only 4
246 of 21 (19%) had detectable IFNγ responses compared with 11 of 21 (52%) who had detectable
247 IL17 responses ($p = 0.0516$, Fisher's exact test). Additionally, the median IL17 response was

248 higher than the median IFNγ response although this did not reach significance (Figure 3C, left).
249 Similar IL-17 responses to Mtb antigen stimulation were observed in people with HIV before
250 and after LTBI diagnosis and treatment. Treatment of LTBI did not change either IFNγ or IL17
251 responses although the response magnitudes varied by participant (Figure 3C, right). Similarly,
252 more participants had detectable IL17 responses than detectable IFNγ responses at all 3
253 sampling times: prior to being TST positive, and at INH initiation and treatment completion:
254 2/11 vs 10/11, 3/11 vs 9/11 and 6/14 vs 12/14, respectively (Figure 3C, right).
255
256 To determine whether predominant production of IL17 over IFNγ was specific to Mtb antigens,
257 we used a CMV peptide pool to stimulate PBMC from participants (Tables 1 and 2) who had
258 sufficient cells (Figure 3D). This revealed a pattern distinct from that with Mtb300: participants
259 with LTBI and HIV-ART (cohort 1) exhibited higher magnitude and higher prevalence of IFNγ
260 than IL17 responses (IFNγ: 11/16 (69%) vs IL17: 5/16 (31%)) (Figure 3D, left) ($p = 0.0756$,
261 Fisher's exact test). Similarly, in Cohort 2 there was a higher magnitude of IFNγ responses
262 than IL17 responses at all time points, reaching statistical significance at baseline (prior to
263 TST^{+}) timepoint (Figure 3D – right). As with CMV and in contrast to Mtb300, SEB induced
264 higher magnitude IFNγ than IL17 responses (Figure S3B). Together, these data demonstrate
265 that in LTBI and HIV-ART, IL17 responses to Mtb are relatively preserved compared with
266 IFNγ responses, and this is distinct compared to CMV and SEB responses. This is also contrary
267 to the commonly held impression that IFNγ is the predominant response to Mtb.
268
269 ***IDO activity is increased in people with LTBI and HIV-ART, and negatively correlates with***
270 ***circulating Th17 cells***
271 We quantitated tryptophan and kynurenine concentrations in plasma of participants with LTBI
272 with or without HIV-ART and inferred IDO activity based on the K/T ratio. We found

273 significantly lower plasma tryptophan concentrations in participants with HIV-ART compared
274 with those without HIV (Figure 4A) and kynurenine concentrations were higher in HIV-ART
275 but did not achieve statistical significance when compared with the HIV uninfected group
276 (Figure 4B). Finally, there was a significantly higher K/T ratio in people with HIV-ART
277 compared to those without HIV (Figure 4C). Although we cannot ascertain the contribution of
278 LTBI to the observed IDO activity because we did not have participants who were not exposed
279 to Mtb, these observations agree with prior reports showing that IDO-1 mediated tryptophan
280 catabolism is elevated in PWH, including those initiated on early treatment and ART-
281 suppressed viral loads [29].
282
283 Since kynurenines favour development of Tregs while suppressing Th17 generation [25], we
284 determined the correlation of plasma K/T and Th17 cells, Tregs, and Th17/Treg ratios in
285 Cohort 1 participants. This revealed a significant inverse relationship between K/T and
286 circulating T_H17 and T17 (Figure 4D-E), while there was no correlation between K/T and Tregs
287 (Figure 4D, middle). We also found significant inverse correlations between K/T and
288 T_H17/Treg and T17/Treg (Figure 4D-E). Importantly, we found no correlation between K/T
289 and T1T17, Th1, or $IL17^+$ cell frequencies (Fig. S4A-B and data not shown). Although we
290 confirmed high IDO activity (Figure 4) and found reduction in circulating Th17 (especially
291 T_H17) cells in HIV-ART compared to HIV uninfected people (Figure 2), Tregs and the
292 Th17/Treg ratio were similar in the two groups (Figure S2). Thus, increased IDO activity is
293 associated with a reduction in circulating Th17 cell subsets, but the frequency of IL17
294 producing CD4 T cells does not correlate with IDO activity.
295
296 **Discussion**

297 Studies that demonstrate Th17 cells contribute to the control of Mtb infection in humans [7–9]
298 used different approaches to identify the cells. We used a combination of cell markers [14,17–
299 21] to broadly characterize Th17 cells and then confirmed that they produce IL17, instead of
300 using IL17 production alone to define Th17 cells [5,9]. Since there are cytokine-independent
301 mechanisms of T cell responses, and since cytokine production can be influenced by
302 stimulation conditions, our approach affords a broader characterization of Th17 cells to
303 determine the impact of HIV coinfection. Indeed, a recent study in macaques showed
304 granulomas that controlled Mtb were enriched for T1T17 (Th1*) cells that did not express IL17
305 transcripts [40]. Furthermore, since Mtb-antigen specific cells must traffic to sites of disease,
306 using chemokine receptor expression alone in blood may underreport the population of these
307 cells. Our finding of disproportionate reduction of specific circulating Th17 cell categories in
308 HIV-ART reveals the importance of using multiple criteria to identify CD4 T cells of interest,
309 and that ART-mediated suppression of HIV does not reconstitute all Th17 cell types equally.
310 Studies to identify CD4 T cell defects that contribute to TB susceptibility in HIV and HIV-
311 ART will benefit from higher resolution characterization of CD4 T cell subsets.
312
313 Our finding of a higher magnitude of IL17- than IFNγ-producing $CD4^+$ T cells in HIV-ART
314 upon Mtb300 stimulation is contrary to the impression that IFNγ production is the dominant
315 response to Mtb infection [41]. This may have implications for TB diagnosis since current tests
316 for detection of responses to Mtb antigens rely on IFNγ production, whose sensitivity is
317 variable and is impacted by HIV infection [42]. Recent studies revealed evidence that people
318 who have been exposed to Mtb but remain negative by IFNγ responses have detectable
319 responses to Mtb antigens when other readouts are used [43]. Until recently, those individuals
320 were thought to remain uninfected by Mtb; our results add weight to the evolving concept that
321 considering IFNγ as the canonical responses to Mtb infection requires reconsideration.

322 Our findings indicate that IL17 responses to Mtb but not CMV antigens or SEB can be
323 preserved in HIV-ART. We hypothesize that IL17-producing cells may be less activated than
324 IFNγ-producing cells and/or express the CCR5 coreceptor at lower levels or at lower
325 frequencies and are thus preserved from HIV infection. The technical challenge of optimal
326 measurement of T cell activation and cytokine production in one condition (detection of
327 cytokines requires protein transport inhibitors which hinder T cell activation marker
328 expression) precluded us from testing this hypothesis.
329
330
331 The K/T ratio is a strong predictor of mortality risk in ART-suppressed PWH [44], does not
332 normalize in PWH initiating ART early, and durably suppressed early-ART initiators have high
333 plasma KT ratios compared with HIV-negative controls [29]. Therefore, the elevated
334 tryptophan catabolism in HIV-ART reported here and previously [25,29] may explain, in part,
335 the persistently increased risk of tuberculosis in treated HIV. Inhibition of IDO-1 with 1-
336 methyl-D-tryptophan (indoximod) in macaques was reported to decrease Mtb burdens and
337 pathology, and increased proliferation of $CD4^+$ and $CD8^+$ memory T cells [45] Together, these
338 results suggest that enhancing Th17 cell responses by IDO inhibition may be beneficial in the
339 context of TB.
340
341 Our study has limitations: 1) the participants were from a study investigating the long-term
342 clinical and immunological consequences of HIV infections and their treatment, therefore, we
343 had few HIV uninfected participants; 2) 10 of 13 PWH initially with a negative TST prior to
344 TST conversion had high viral loads that probably reduced the sensitivity of diagnosis; 3) INH
345 treatment was not given as directly observed therapy (DOT) and we could not ascertain
346 treatment compliance by the study participants. Nevertheless, we found that Th17 cells are a

347 heterogenous population and IL17 responses to Mtb are preserved in HIV-TB coinfected
348 individuals, and we demonstrated the link between IDO activity and circulating Mtb-
349 responsive Th17 cells in humans. With IDO-1 inhibitors in clinical trials [46], management of
350 TB disease might benefit from simultaneous therapeutic inhibition of IDO-1 activity to enhance
351 Th17 cell responses, particularly in PWH where HIV further enhances tryptophan catabolism
352 via the kynurenine pathway.
353

354 **NOTES**

355 **Funding:** This research was supported by a grant from the National Institutes of Health: UCSF-
356 Gladstone Center for AIDS Research (P30 AI027763).

357

358 **Acknowledgments:** We thank the study participants in the SCOPE study for providing
359 samples, UCSF Core Immunology Lab and UCSF-CFAR Specimen Processing & Banking
360 Subcore.

361

362 **Conflict of interest:** The authors have no conflict of interest to declare.

363

364 **Presentations:** This work was presented at the 1st Bay Area Tuberculosis Symposium
365 (BATS) September 9, 2022, San Francisco, CA.

366

367 **Correspondence:** Joel D. Ernst, M.D.
368 Division of Experimental Medicine
369 University of California, San Francisco
370 1001 Potrero Ave, Bldg. 3, Room 601 | San Francisco, CA 94110
371 joel.ernst@ucsf.edu
372 Peter W. Hunt, MD
373 Division of Experimental Medicine
374 Zuckerberg San Francisco General Hospital
375 1001 Potrero Avenue
376 Bldg 3, Room 605B, San Francisco, CA. 94110
377 peter.hunt@ucsf.edu

378 **References**

379 1. Global tuberculosis report 2021. https://www.who.int/publications-detail-
380 redirect/9789240037021.
381 2. Kwan, C. K. & Ernst, J. D. HIV and tuberculosis: a deadly human syndemic. *Clin.*
382 *Microbiol. Rev.* **24**, 351–376 (2011).
383 3. Lawn, S. D., Myer, L., Edwards, D., Bekker, L.-G. & Wood, R. Short-term and long-term
384 risk of tuberculosis associated with CD4 cell recovery during antiretroviral therapy in
385 South Africa. *AIDS* **23**, 1717–1725 (2009).
386 4. Gupta, A., Wood, R., Kaplan, R., Bekker, L.-G. & Lawn, S. D. Tuberculosis incidence rates
387 during 8 years of follow-up of an antiretroviral treatment cohort in South Africa:
388 comparison with rates in the community. *PLoS ONE* **7**, e34156 (2012).
389 5. Scriba, T. J. *et al.* Distinct, specific IL-17- and IL-22-producing CD4+ T cell subsets
390 contribute to the human anti-mycobacterial immune response. *J. Immunol.* **180**, 1962–
391 1970 (2008).
392 6. Geldmacher, C. *et al.* Preferential infection and depletion of Mycobacterium
393 tuberculosis-specific CD4 T cells after HIV-1 infection. *J. Exp. Med.* **207**, 2869–2881
394 (2010).
395 7. Scriba, T. J. *et al.* Sequential inflammatory processes define human progression from
396 M. tuberculosis infection to tuberculosis disease. *PLoS Pathog.* **13**, e1006687 (2017).
397 8. Nathan, A. *et al.* Multimodally profiling memory T cells from a tuberculosis cohort
398 identifies cell state associations with demographics, environment and disease. *Nat*
399 *Immunol* **22**, 781–793 (2021).
400 9. Ogongo, P. *et al.* Tissue-resident-like CD4+ T cells secreting IL-17 control
401 Mycobacterium tuberculosis in the human lung. *J Clin Invest* **131**, 142014 (2021).

402 10. Ma, W.-T., Yao, X.-T., Peng, Q. & Chen, D.-K. The protective and pathogenic roles of IL-
403 17 in viral infections: friend or foe? *Open Biol* **9**, 190109 (2019).
404 11. Wacleche, V. S., Landay, A., Routy, J.-P. & Ancuta, P. The Th17 Lineage: From Barrier
405 Surfaces Homeostasis to Autoimmunity, Cancer, and HIV-1 Pathogenesis. *Viruses* **9**,
406 E303 (2017).
407 12. Iwakura, Y., Ishigame, H., Saijo, S. & Nakae, S. Functional specialization of interleukin-
408 17 family members. *Immunity* **34**, 149–162 (2011).
409 13. Shen, H. & Chen, Z. W. The crucial roles of Th17-related cytokines/signal pathways in
410 M. tuberculosis infection. *Cell Mol Immunol* **15**, 216–225 (2018).
411 14. Acosta-Rodriguez, E. V. *et al.* Surface phenotype and antigenic specificity of human
412 interleukin 17-producing T helper memory cells. *Nat. Immunol.* **8**, 639–646 (2007).
413 15. Singh, S. P., Zhang, H. H., Foley, J. F., Hedrick, M. N. & Farber, J. M. Human T cells that
414 are able to produce IL-17 express the chemokine receptor CCR6. *J Immunol* **180**, 214–
415 221 (2008).
416 16. Annunziato, F. *et al.* Phenotypic and functional features of human Th17 cells. *J. Exp.*
417 *Med.* **204**, 1849–1861 (2007).
418 17. Lindestam Arlehamn, C. S. *et al.* Memory T cells in latent Mycobacterium tuberculosis
419 infection are directed against three antigenic islands and largely contained in a
420 CXCR3+CCR6+ Th1 subset. *PLoS Pathog.* **9**, e1003130 (2013).
421 18. Bengsch, B. *et al.* Human Th17 cells express high levels of enzymatically active
422 dipeptidylpeptidase IV (CD26). *J. Immunol.* **188**, 5438–5447 (2012).
423 19. Zhao, X. *et al.* Involvement of CD26 in Differentiation and Functions of Th1 and Th17
424 Subpopulations of T Lymphocytes. *J Immunol Res* **2021**, 6671410 (2021).

425 20. Cosmi, L. *et al.* Human interleukin 17-producing cells originate from a CD161+CD4+ T
426 cell precursor. *J. Exp. Med.* **205**, 1903–1916 (2008).
427 21. Maggi, L. *et al.* CD161 is a marker of all human IL-17-producing T-cell subsets and is
428 induced by RORC. *European Journal of Immunology* **40**, 2174–2181 (2010).
429 22. Li, S. *et al.* Metabolic Phenotypes of Response to Vaccination in Humans. *Cell* **169**, 862-
430 877.e17 (2017).
431 23. Collins, J. M. *et al.* Tryptophan catabolism reflects disease activity in human
432 tuberculosis. *JCI Insight* (2020) doi:10.1172/jci.insight.137131.
433 24. Adu-Gyamfi, C. G. *et al.* Plasma Indoleamine 2, 3-Dioxygenase, a Biomarker for
434 Tuberculosis in Human Immunodeficiency Virus-Infected Patients. *Clin Infect Dis* **65**,
435 1356–1358 (2017).
436 25. Favre, D. *et al.* Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the
437 balance of TH17 to regulatory T cells in HIV disease. *Sci Transl Med* **2**, 32ra36 (2010).
438 26. Desvignes, L. & Ernst, J. D. Interferon-gamma-responsive nonhematopoietic cells
439 regulate the immune response to Mycobacterium tuberculosis. *Immunity* **31**, 974–985
440 (2009).
441 27. Weiner, J. *et al.* Metabolite changes in blood predict the onset of tuberculosis. *Nat*
442 *Commun* **9**, 5208 (2018).
443 28. Murray, M. F. Tryptophan depletion and HIV infection: a metabolic link to
444 pathogenesis. *Lancet Infect Dis* **3**, 644–652 (2003).
445 29. Schnittman, S. R. *et al.* Abnormal Levels of Some Biomarkers of Immune Activation
446 Despite Very Early Treatment of Human Immunodeficiency Virus. *J Infect Dis* **223**,
447 1621–1630 (2021).

448 30. Lindestam Arlehamn, C. S. *et al.* A Quantitative Analysis of Complexity of Human
449 Pathogen-Specific CD4 T Cell Responses in Healthy M. tuberculosis Infected South
450 Africans. *PLoS Pathog* **12**, e1005760 (2016).
451 31. Foreman, T. W. *et al.* CD4 T cells are rapidly depleted from tuberculosis granulomas
452 following acute SIV co-infection. *Cell Rep* **39**, 110896 (2022).
453 32. Wong, E. B., Ndung'u, T. & Kasprowicz, V. O. The role of mucosal-associated invariant T
454 cells in infectious diseases. *Immunology* **150**, 45–54 (2017).
455 33. Lu, B. *et al.* IL-17 production by tissue-resident MAIT cells is locally induced in children
456 with pneumonia. *Mucosal Immunology* **13**, 824–835 (2020).
457 34. Ivanov, I. I. *et al.* The Orphan Nuclear Receptor RORγt Directs the Differentiation
458 Program of Proinflammatory IL-17+ T Helper Cells. *Cell* **126**, 1121–1133 (2006).
459 35. Szabo, S. J. *et al.* A Novel Transcription Factor, T-bet, Directs Th1 Lineage Commitment.
460 *Cell* **100**, 655–669 (2000).
461 36. Mpande, C. A. M. *et al.* Functional, Antigen-Specific Stem Cell Memory (TSCM) CD4+ T
462 Cells Are Induced by Human Mycobacterium tuberculosis Infection. *Front Immunol* **9**,
463 324 (2018).
464 37. Wang, T. *et al.* Regulatory T cells in rheumatoid arthritis showed increased plasticity
465 toward Th17 but retained suppressive function in peripheral blood. *Ann Rheum Dis* **74**,
466 1293–1301 (2015).
467 38. Boothby, I. C., Cohen, J. N. & Rosenblum, M. D. Regulatory T cells in skin injury: At the
468 crossroads of tolerance and tissue repair. *Sci Immunol* **5**, eaaz9631 (2020).
469 39. Fontenot, J. D., Gavin, M. A. & Rudensky, A. Y. Foxp3 programs the development and
470 function of CD4+CD25+ regulatory T cells. *Nat Immunol* **4**, 330–336 (2003).

471 40. Gideon, H. P. *et al.* Multimodal profiling of lung granulomas in macaques reveals
472 cellular correlates of tuberculosis control. *Immunity* **55**, 827-846.e10 (2022).
473 41. Flynn, J. L. & Chan, J. Immune cell interactions in tuberculosis. *Cell* **185**, 4682–4702
474 (2022).
475 42. Chen, H. *et al.* Diagnostic accuracy of the interferon-gamma release assay in acquired
476 immunodeficiency syndrome patients with suspected tuberculosis infection: a meta-
477 analysis. *Infection* **50**, 597–606 (2022).
478 43. Lu, L. L. *et al.* IFN-γ-independent immune markers of Mycobacterium tuberculosis
479 exposure. *Nat. Med.* **25**, 977–987 (2019).
480 44. Byakwaga, H. *et al.* The kynurenine pathway of tryptophan catabolism, CD4+ T-cell
481 recovery, and mortality among HIV-infected Ugandans initiating antiretroviral therapy.
482 *J Infect Dis* **210**, 383–391 (2014).
483 45. Gautam, U. S. *et al.* In vivo inhibition of tryptophan catabolism reorganizes the
484 tuberculoma and augments immune-mediated control of Mycobacterium tuberculosis.
485 *Proc. Natl. Acad. Sci. U.S.A.* **115**, E62–E71 (2018).
486 46. Tang, K., Wu, Y.-H., Song, Y. & Yu, B. Indoleamine 2,3-dioxygenase 1 (IDO1) inhibitors in
487 clinical trials for cancer immunotherapy. *J Hematol Oncol* **14**, 68 (2021).
488
489

Table 1: Cohort 1 (Characteristics of participants with and without HIV with Latent Tuberculosis Infection)			
Characteristic	**HIV negative (n =7)**	**HIV positive (n = 21)**	**P**
Age, median (IQR), y	58 (44 -61)	47 (38 - 58)	0.5395
Sex, Male (%)	7 (100)	21 (100)	
Race/Ethnicity (n, (%))			
Black	6 (86)	8 (38)	
Asian	1 (14)	2 (10)	
White	0	7 (30)	
Middle East	0	1 (5)	
Multiracial	0	2 (10)	
Pacific Islander	0	1 (5)	
Combined antriretroviral therapy (Yes/No)	No	Yes	
CD4 Counts, median (IQR), cells/mm3	1297 (707 -1349)	640 (487 - 906)	0.0082
Plasma viral load (copies/mL)	None	< 40	

Table 2: Cohort 2 (Characteristics of participants with HIV before and after LTBI diagnosis and treatment)				
Characteristic	**TST neg**	**TST+ INH initiation**	**TST+INH completion**	**P**
Age, median (IQR), y	45 (42 - 51)	_	_	
Sex, Male (%)	10 (71)	_	_	
Race/Ethnicity (n, (%))				
Black	5 (36)	_	_	
Asian	1 (7)	_	_	
White	7 (50)	_	_	
Pacific Islander	1 (7)	_	_	
CD4 Counts, median (IQR), cells/mm3	527 (230 - 781)	442 (367 - 588)	464 (361 - 724)	0.9172
HIV infection (n, %)	13 (100)	11 (100)	13 (100)	
Detectable viral load (n; median (IQR), copies/mL)	10; 3455 (614 - 24888)	6; 4895 (497 - 35920)	6; 2980 (170 - 59542)	0.9632
Suppressed viral load (n)	3	5	7	
Combined antriretroviral therapy				
On treatment (n)	7	6	10	
Not on treatment (n)	6	5	3	

490

Figure 1:

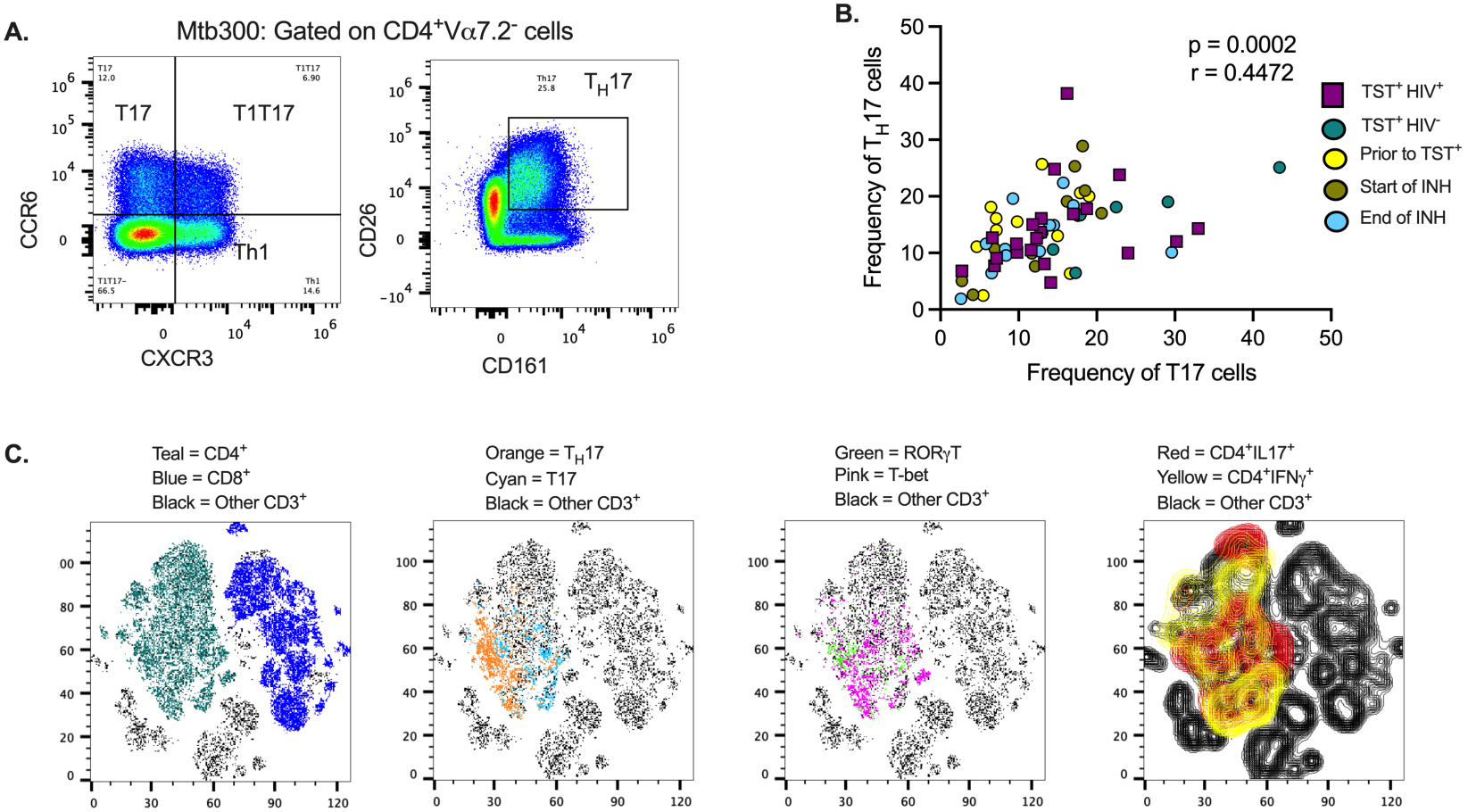

Figure 1. Different definitions of T helper 17 cells reveal high but incomplete concordance. (**A**) Representative flow cytometry plots and gating schemes for definition of T helper 17 cells by published marker criteria independent of cytokine expression. Left panel, chemokine receptor CCR6 and CXCR3 expression used to distinguish T17, T1T17, and Th1 non-MAIT CD4 T cells. Right panel: Use of surface expression of CD26 and CD161 to identify T_H17 non-MAIT CD4 T cells. Cells in both panels were stimulated with the MTB300 antigenic peptide pool. (**B**) High correlation of T_H17 ($CD26^+CD161^+$) and T17 ($CCR6^+CXCR3^-$) non-MAIT CD4 T cells in cell populations from participants with varying TB and HIV status. Spearman correlation p and r values are shown for results pooled from all participants. (**C**) t-stochastic neighbor embedding (t-SNE) analysis reveals incomplete concordance of transcription factor (RORγt and T-bet) and cytokine (IFNγ and IL17) production and surface phenotypes of non-MAIT $CD4^+$ T cells. In this analysis, protein transport inhibitors (golgi stop and golgi plug) were not added to PBMC during antigen stimulation thus weak cytokine signals which are best shown in contour format.

491

Figure 2:

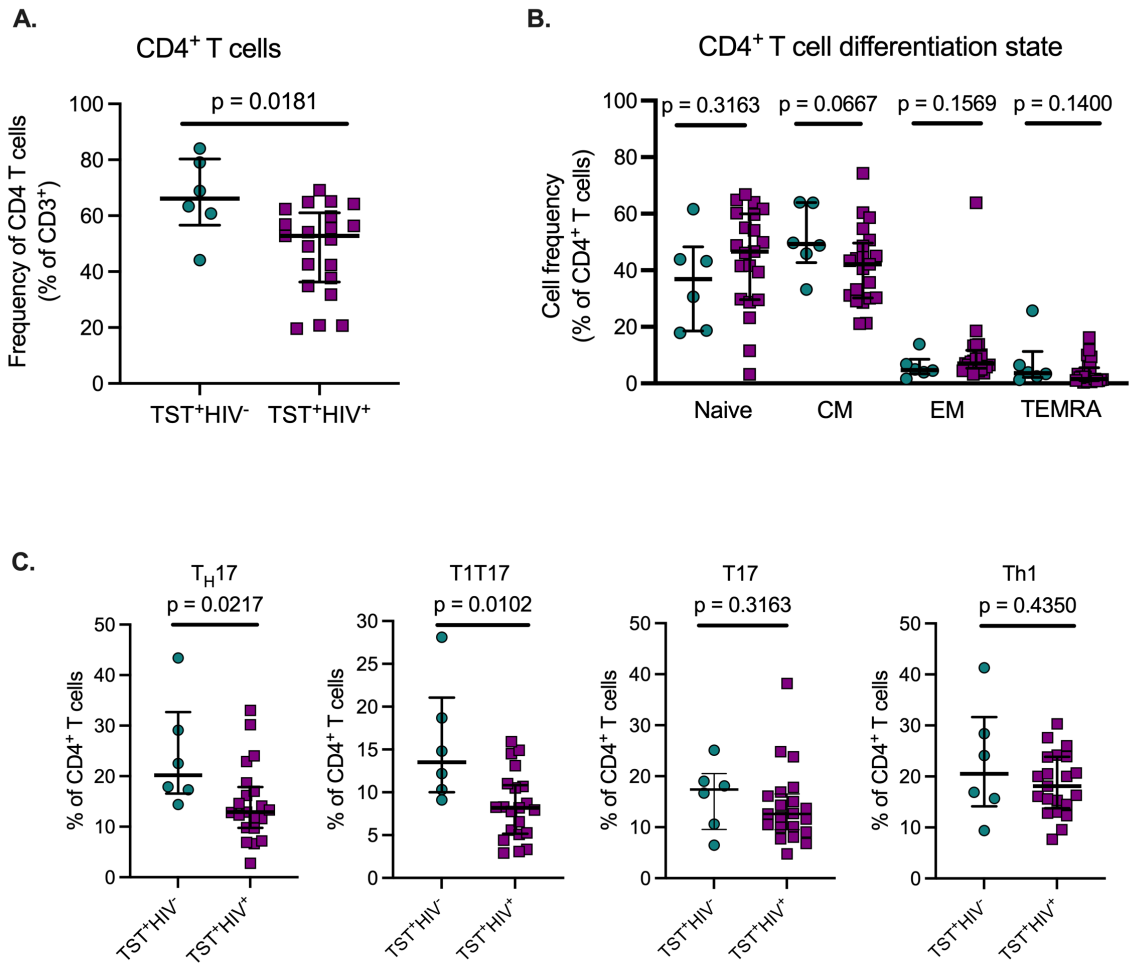

Figure 2. **ART treated HIV infection is associated with disproportionate depletion of distinct subsets of T helper cells. (A)** Reduced frequency of bulk CD4 T cells in HIV$^+$ vs HIV$^-$ participants in Cohort 1. **(B)** ART-treated HIV is not associated with differential distribution of CD4 T cells in memory T cell subsets. Naive: CD45RA$^+$CCR7$^+$; Central Memory (CM): CD45RA$^-$CCR7$^+$); Effector Memory: CD45RA$^-$CCR7$^-$; TEMRA: CD45RA$^+$CCR7$^-$). **(C)** Disproportionate reduction in frequencies of T_H17 and T1T17 but not T17 or Th1 cells in ART-treated HIV. T_H17=CD4$^+$Vα7.2$^-$CD26$^+$CD161$^+$; T1T17 (also termed Th1*): CD4$^+$Vα7.2$^-$CCR6$^+$CXCR3$^+$; T17: CD4$^+$Vα7.2$^-$CCR6$^+$CXCR3$^-$; and Th1: CD4$^+$Vα7.2$^-$CCR6$^-$CXCR3$^+$. Statistical comparisons were by Mann-Whitney test.

492
493

494

Figure 3:

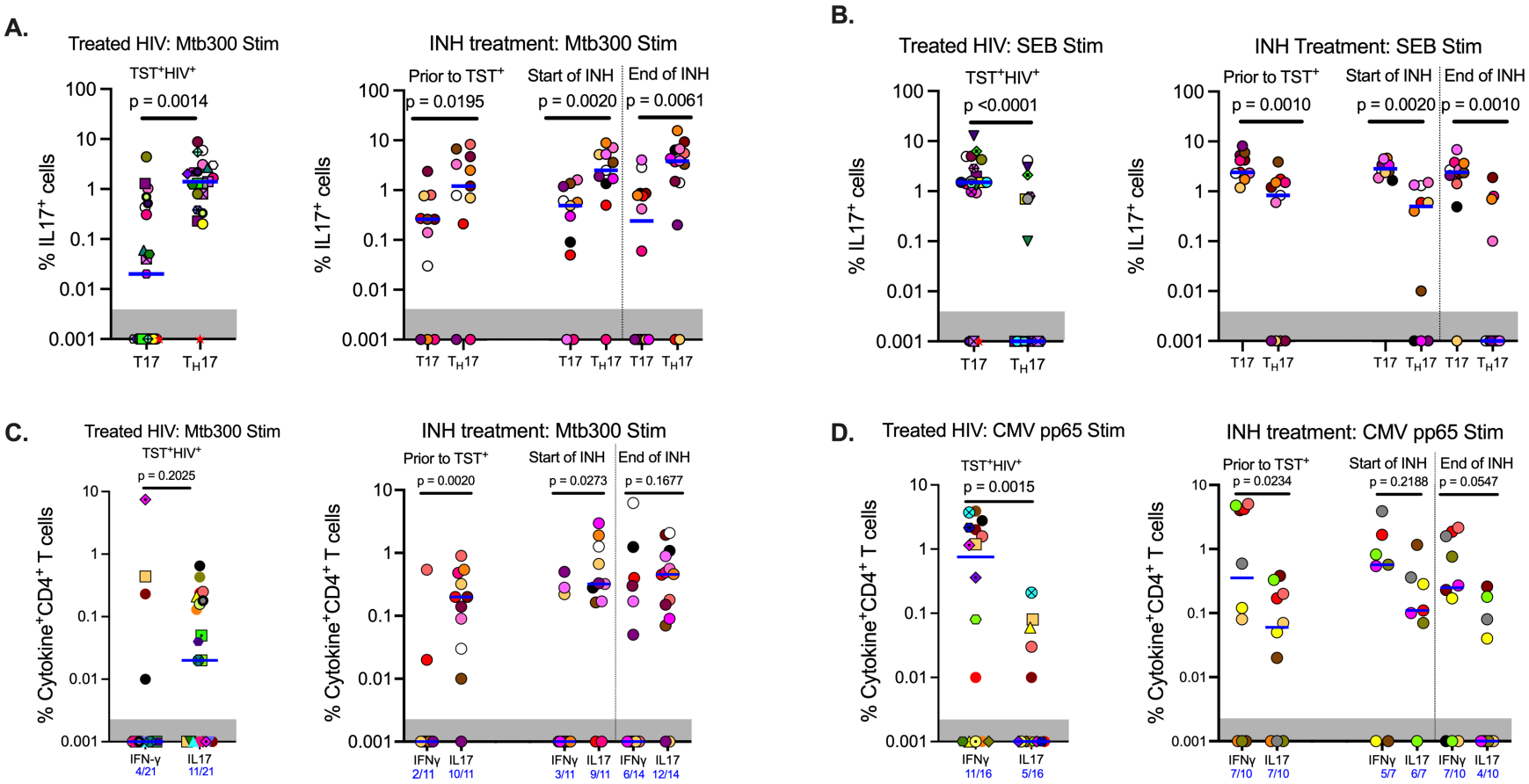

Figure 3. **In contrast to responses to other stimuli, *Mtb* antigen responsive IL17 expression is enriched in CD26$^+$CD161$^+$ T$_H$17 rather than CCR6$^+$CXCR3$^-$ T17 cells. (A)** Left panel (Cohort 1): CD4 T cells that produce IL17 in response to MTB300 are predominantly in the T$_H$17 subset. Right panel (Cohort 2): MTB300-stimulated IL17 production predominates in T$_H$17 rather than T17 cells regardless of TST status and is not altered by INH preventive therapy. **(B)** Left panel (Cohort 1): In contrast to MTB300-responsive cells, IL17 production predominates in T17 cells in response to SEB activation. Right panel (Cohort 2): INH preventive therapy does not alter the pattern of IL17 production by SEB-stimulated T17 or T$_H$17 cells. **(C)** Left panel (Cohort 1): In ART-treated HIV$^+$ participants, CD4 T cells that produce IFNγ in response to MTB300 stimulation are present at low frequencies. Right panel (Cohort 2): **(D)** Left panel (Cohort 1): CMV pp56 antigen stimulation induces higher frequencies of IFNγ than IL17-producing CD4 T cells. Right panel (Cohort 2): INH treatment does not alter CD4 T cell responses to CMV pp65 antigen stimulation.

495

Figure 4:

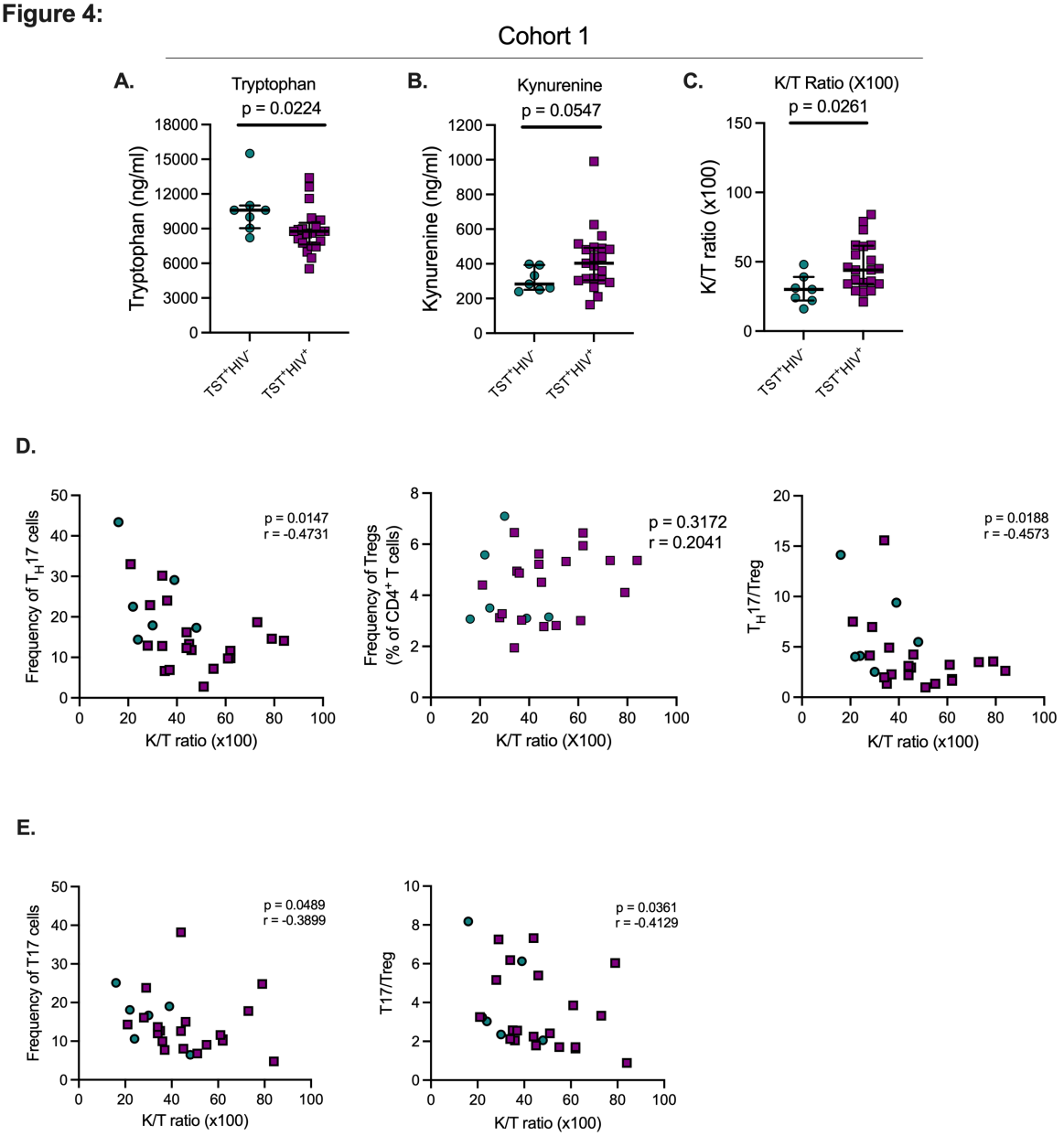

Figure 4. In TST+ participants, HIV infection is associated with increased indoleamine-2,3-dioxygenase (IDO) activity, which negatively correlates with TH17 and T17 cell frequencies. (A) Plasma tryptophan concentrations; **(B)** Plasma kynurenine concentrations; **(C)** Plasma Kynurenine (K)/Tryptophan (T) ratios as a reflection of IDO activity. **(D)** Left panel: correlation of TH17 cell frequencies with plasma K/T ratios; Middle panel: Correlation of regulatory T cells and plasma K/T ratios; Right panel: Correlation of TH17/Treg ratios and plasma K/T ratios. **(E)** Left panel: correlation of T17 cell frequencies with plasma K/T ratio; Right panel: Correlation of T17/Treg ratios and plasma K/T ratios. Statistical analyses were by Mann-Whitney (panels A-C) or Spearman correlation (D and E).