1 # Exaggerated in vivo IL-17 responses discriminate recall responses in active TB

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17 ## Running title

18 IL-17 responses in active TB disease

19 ## Manuscript word count

20 3405 words

21 ## Online data

22 This article has an online data supplement, which includes:

23 - Supplementary figures 1-8
24 - Supplementary tables E1-8
25 - Supplementary methods

26

Support

The work presented in this manuscript received funding from the Wellcome Trust (WT101766/Z/13/Z to GP and 207511/Z/17/Z to MN), Medical Research Council (MR_N007727_1 to GST) and National Institute for Health Research Biomedical Research Centre at University College London Hospitals.

Author contributions

Substantial contributions to the conception or design of the work;	GP, CT, GST, LCB LFP, TM, CU, DAM, BMC, MN
Substantial contributions to the acquisition, analysis, or interpretation of data for the work;	GP, CT, GST, LCB, AK, LFP, AF, AA, CV, TB, FLR, TM, BMC, MN
Drafting the work or revising it critically for important intellectual content;	GP, CT, GST, LCB, BMC, MN
Final approval of the version submitted for publication	All authors
Accountability for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved	GP, MN

34 # Abstract

35 Host immune responses at the site of *Mycobacterium tuberculosis* (Mtb) infection serve to contain the
36 pathogen, but also mediate the pathogenesis of tuberculosis (TB) and onward transmission of
37 infection. Interferon gamma (IFNγ) responses do not discriminate between protection and
38 pathogenicity, but IL-17A/F responses, known to drive pathology in diverse chronic inflammatory
39 diseases, have also been associated with TB pathogenesis in animal models. At the site of in vivo
40 immune recall responses to Mtb modelled by the tuberculin skin test, we show for the first time that
41 active TB in humans is also associated with exaggerated IL-17A/F expression, accumulation of Th17
42 cells and IL-17A/F bioactivity, including increased neutrophil recruitment and matrix
43 metalloproteinase-1 expression directly implicated in TB pathogenesis. These features discriminate
44 recall responses in patients with active TB from those with cured or latent infection and are also
45 evident at the site of TB disease. Our data support targeting of this pathway in host-directed therapy
46 for TB.

47

48 ## Abstract word count

49 156 words

Introduction

Mycobacterium tuberculosis (Mtb) infection results in a spectrum of clinical outcomes, from asymptomatic latent infection to symptomatic disease. The focus of host-pathogen interactions is characterised histologically by granuloma formation, a chronic inflammatory process that may contain the infection, but can also result in tissue damage that promotes transmission of infection to other individuals (1, 2). The distinctions that tip the balance between protective and pathogenic immune responses remain a fundamental question in tuberculosis research. This knowledge is expected to inform rational vaccine design and development of host-directed therapies (3).

Chronic inflammatory pathology at the site of human tuberculosis has been the subject of extensive descriptive studies, but discriminating between protective and pathological immune responses has been limited to comparing leucocyte phenotype and function in blood from Mtb-exposed patients with and without active disease (1). We have shown that genome-wide transcriptional profiling of biopsies of the tuberculin skin test (TST) can be used to make comprehensive molecular and systems level assessment of in vivo immune responses at the site of standardised host-pathogen interactions (4–7). Importantly, the transcripts enriched within the TST reflect the genome-wide variation in molecular pathology at the site of tuberculosis (TB) disease (5, 7), suggesting the TST represents a valuable surrogate for assessing TB immunopathogenesis in vivo.

On the premise that active TB disease is predominantly a manifestation of immunopathology, in this study we aimed to test the hypothesis that immune responses at the site of host-pathogen interactions, modelled by the TST, would reveal enrichment of immunopathologic responses in patients with active TB that were absent in individuals with equivalent immune memory for Mtb but without disease.

Results

Immune responses at the site of TST in active and cured TB

The TST has been most extensively used to identify patients with T cell memory for mycobacterial antigens, but the clinical response does not differentiate infected individuals with and without active disease (1). We sought to test the hypothesis that molecular profiling of the TST may identify elements of the recall response which are specifically associated with disease. We undertook 48-hour TSTs in patients with microbiologically-confirmed TB disease within the first month of treatment ('active TB') to identify disease associated responses, and compared these to TST responses in patients within one year of curative TB treatment ('cured TB') to identify non-disease associated recall responses (table

81 E1). Age, gender and site of TB disease were comparable between the two groups (table E2). As
82 expected, clinical induration in response to the TST was not significantly different between the two
83 groups (fig 1A), hitherto interpreted to reflect comparable cell mediated immune memory.

84 In comparison to skin biopsies from the site of control saline injection, 1910 genes were significantly
85 enriched in response to the TST in at least one study group. Of these, 1251 were enriched in both
86 groups (fig 1B). Bioinformatic systems level assessment of the shared response revealed many
87 prototypic cell mediated immune responses which we had previously described in the TST (fig 1C) (5).
88 Pairwise assessment of the integrated list of transcripts that were enriched in either group revealed
89 statistically significant covariance, consistent with the hypothesis that the majority of responses do
90 not discriminate between the two groups (fig 1D).

91 Differential gene expression in the TST in active TB

92 A proportion of genes were differentially enriched between the two groups (fig 1D & tables E3-4). 44
93 genes were expressed significantly more in patients with active TB (table E3) compared to patients
94 with cured TB. Amongst these, pathway analysis identified statistically significant enrichment of
95 transcripts involved in extracellular matrix (ECM) remodelling, such as matrix metalloproteinase 1
96 (MMP-1), and beta defensins that both exert antimicrobial functions and also provide a chemotactic
97 gradient for CCR2-expressing cells, including neutrophils (8) (figs 2A & E1A). MMP-1, previously
98 implicated in pathogenic degradation of the ECM in TB (9), was the most over-expressed gene in
99 active TB compared to cured TB (fig 2B & table E3). This difference was validated at protein level by
100 immunohistochemistry, which also revealed that the differences in MMP-1 expression between
101 patients with active and cured TB was restricted to the inflammatory infiltrates within the TST (fig 2C-
102 E).

103 Elevated IL-17 responses in active TB

104 We hypothesised that the genes over-expressed in active TB were regulated by common upstream
105 signals in the tissue environment. To test this hypothesis, we compared the predicted upstream
106 regulators of differentially expressed transcripts in the TST of active and cured TB patients using
107 Ingenuity Pathway Analysis (6). This analysis suggested that IL-17A induced the expression of genes
108 over-expressed in active TB and not those over-expressed in cured TB (fig 3A). In contrast, IFNγ was
109 predicted to be an upstream signal for gene expression enriched in both active and cured TB (fig 3A).
110 IFNγ responses, largely attributed to T helper (Th)-1 polarised CD4+ T cells are necessary for
111 immunological protection against Mtb (10), but they are insufficient and do not discriminate between
112 people who do and do not develop disease (1). The role of IL-17A in TB is less clear. This cytokine

113 belongs to a family of six structurally related cytokines and shares greatest sequence homology with
114 IL-17F. These bind the same receptor, and consequently exert the same functions, particularly
115 increased neutrophil recruitment via upregulation of chemokine expression (11).

116 There is unequivocal evidence that IL-17A/F contribute to host defence against bacterial and fungal
117 pathogens (12). Importantly however, they are also strongly indicated in the immunopathology of
118 chronic inflammatory diseases (12, 13). This includes evidence for IL-17A dependent neutrophil
119 mediated pathology in mouse models of Mtb infection (14–16). Our bioinformatics analysis suggested
120 increased enrichment of transcripts in the human in vivo recall response of patients with active TB
121 may be driven by IL-17A/F activity. Therefore, we sought to test the hypothesis that IL-17A/F activity
122 is exaggerated in active TB. Consistent with this hypothesis the expression of both IL-17A and IL-17F
123 were enriched in the TST of people with active TB compared to cured TB (fig 3B). In contrast, IFNγ
124 transcript levels representing the prototypic molecule in cell mediated immune recall responses was
125 not significantly different (fig 3B). Interestingly, the expression of IL-22, a cytokine with closely related
126 biological function to IL-17A and IL-17F (17), was also not elevated in active TB (fig 3B). The differences
127 revealed at the transcriptional level were also reflected by increased immunofluorescence of IL-17F
128 protein in the TST of people with active TB (fig 3C & 3D).

129 In order to test the functional significance of the differences in IL-17A/F expression between active
130 and cured TB, we evaluated differences in the bioactivity of IL-17A between these groups. We
131 addressed this by generating cellular response modules from the transcriptomes of
132 cytokine-stimulated keratinocytes (KC) (18, 19). We confirmed that these modules were both sensitive
133 and specific for their cognate stimuli by assessing their expression in other independent datasets (fig
134 E2 & table E6). We then compared the geometric mean expression of these cytokine-specific
135 transcriptional modules in the TST transcriptomes. The IL-17A-induced gene module was significantly
136 increased in the TST of people with active TB compared to that of cured TB, but expression of IFNγ,
137 type I IFN or TNF-inducible gene modules was not significantly different between the two groups (fig
138 4A). We extended our approach to evaluating the functional bioactivity of other specific cytokines
139 using transcriptional modules for IL-10- and IL-4/IL-13-inducible gene expression, described in a
140 previous report (5). Neither of these were significantly different in the TST of people with active and
141 cured TB (fig E3A). The IL-4/IL-13 bioactivity module was used as a measure of Th2 responses normally
142 associated with allergy and immune responses to helminths. Another member of the IL-17 family, IL-
143 17E contributes to Th2 responses (11). We found no enrichment of IL-17E expression in the TST of
144 people with active TB consistent with IL-4/IL-13 bioactivity and distinct from IL-17A/F bioactivity (fig
145 E3B).

146 Focusing on mechanisms that may contribute to pathogenesis, enrichment of MMP-1 expression in
147 the TST of patients with active TB (fig 2) can also be attributed to increased IL-17A/F bioactivity,
148 through induction of MMP expression by stromal cells (20). Neutrophils can also contribute to the
149 immunopathology of TB (14–16, 21, 22), and a key function of IL-17A/F is to promote neutrophil
150 recruitment via induction of neutrophil chemokines (12). Therefore, we tested the hypothesis that the
151 TST in active TB will also reveal increased neutrophil recruitment, compared to that of patients with
152 cured TB. We compared the expression of two independently derived transcriptional modules that
153 have been extensively validated to reflect neutrophil frequency in tissues including the TST (23, 24).
154 We found significantly higher expression of the neutrophil modules in people with active TB compared
155 to cured TB (fig 4B). These differences were mirrored by gene expression levels of IL-17A-inducible
156 CXCL1, CXCL8 and S100A9 that drive neutrophil recruitment to sites of inflammation (11, 15) (fig 4C).
157 In contrast, accumulation of monocytes and T cells assessed by their respective gene expression
158 modules (23), and the expression of the monocyte chemoattractant, CCL2, did not differ in the TST of
159 people with active and cured TB (fig 4B&C).

160 Increased frequency of Th17 cells in active TB TST responses

161 IL-17A/F are predominantly produced by Th17 cells and neutrophils (11). Immunohistochemistry
162 revealed that in the TST of people with active TB, IL-17F, originated predominantly from mononuclear
163 cells, rather than from polymorphonuclear cells (fig 5A). Therefore, we tested the hypothesis that
164 Th17 cells were enriched in the TST of people with active TB compared to that of cured TB. We derived
165 transcriptional modules specific for differentially polarised CD4+ Th subsets from a published dataset
166 (25) and demonstrated their specificity in an independent dataset (26) (fig E4A & table E7). We further
167 validated the Th17 module by showing that this correlated closely with the expression of the IL-17A/F
168 bioactivity module within skin biopsies of patients with psoriasis vulgaris, representing an alternative
169 Th17-mediated inflammatory condition (27) (fig E4B). The expression of the Th1- and Th2-associated
170 transcriptional modules in the TST was comparable between patients with active and cured TB, but
171 expression of the Th17 associated module was significantly increased in patients with active TB (figs
172 5B & E3).

173 In order to investigate the mechanism for increased Th17 responses in the TST of patients with active
174 TB, we tested the hypothesis that active TB was also associated with increased circulating Th17 cells.
175 We assessed the expression of the Th subset modules in the transcriptome of PPD-stimulated PBMC
176 from an independent cohort of individuals with active TB disease and latent TB infection (28). In
177 contrast to that observed in the TST, patients with active TB revealed enrichment for the
178 Th1-associated gene expression module in PBMC but showed no difference in the Th17-associated

179 module (fig 5C). As a result, we explored the alternative hypothesis that the inflammatory
180 environment generated at the site of TST promotes the Th17 differentiation observed in patients with
181 active TB. We investigated the expression of cytokines implicated in Th17 differentiation, IL-1β, IL-6,
182 IL-23 and TGFβ (11). The expression of each of these was significantly correlated with enrichment for
183 Th17 cells in the TST of active TB. In contrast there was no correlation with IL-12A and IL-4 expression
184 that drive Th1 and Th2 cell differentiation respectively (fig 5D). To test the hypothesis that conditions
185 favouring Th17 differentiation were present in vivo, we generated and validated a transcriptional
186 response module for IL-1 activity in human fibroblasts (fig E5 and table E8) in order to quantify
187 functional IL-1 activity in the TST. This analysis confirmed increased expression of IL-1 inducible genes
188 in the TST of patients with active TB (fig 5E). Of note, this finding was consistent with the bioinformatic
189 prediction of IL1 signalling upstream of some transcripts that were differentially enriched
190 predominantly in active TB (fig 3A). Overall, these data support a model in which the local tissue
191 microenvironment, in part driven by elevated IL-1 activity, may promote Th17 differentiation within
192 tissues in active TB.

193 IL-17 activity is not a feature of latent TB and is not confounded by demographic background, 194 extrapulmonary disease or time on treatment

195 In order to validate our findings, we compared the TST transcriptome of a second independent cohort
196 of people with active TB with that of individuals with latent TB infection. Consistent with our previous
197 results, this comparison revealed significant enrichment for IL-17A/F bioactivity and neutrophil
198 infiltration in people with active TB (fig 6A). Furthermore, Th17 cells were enriched in those with active
199 TB despite there being no overall difference in T cell numbers compared to the TST in individuals with
200 latent TB (fig 6A). Of note, the comparison to latent TB in this analysis, also excluded the possibility
201 that lower levels of IL-17A/F bioactivity in the TST of people with cured TB compared to active TB was
202 an off-target effect of the antimicrobial treatment for active TB.

203 Taken together our data show that active TB is associated with exaggerated Th17 recall responses and
204 IL-17 bioactivity within the tissue microenvironment of host-pathogen interactions. Our findings were
205 replicated using two different methods for transcriptional profiling (microarray figs 4-5 and RNA-Seq
206 fig 6), and were consistent in geographically distinct cohorts (fig E6A). Significantly exaggerated Th17
207 recall and IL-17A/F bioactivity in people with active TB compared to either cured or latent TB were
208 preserved in analyses including only UK cohorts (figs E6B & E6C), confirming that the differences were
209 not due to confounding by other variables in the different cohorts. In addition, we found no difference
210 in these responses between people with pulmonary and extrapulmonary TB disease (fig E7). Of note,
211 people with active TB were assessed at different time points within the first month of treatment

212 (median 11 days, IQR 5-28 days). Rapid changes in the peripheral blood transcriptome associated with
213 active TB have been reported (29, 30), but we found no diminution of the exaggerated Th17 recall
214 responses and IL-17 bioactivity invoked by the TST challenge in this time frame (fig E8), suggesting that
215 the mechanisms that drive these responses in active are not swiftly reversed by treatment.

216 IL-17 activity is present at the site of TB disease

217 Having established that IL-17A/F production by T cells was a prominent feature of the in vivo tissue
218 recall response to Mtb stimulation, we sought to determine whether this was also evident at the site
219 of TB disease. The transcriptome of human TB granuloma (31) showed an enrichment of T cells, Th17
220 cells and IL-17A bioactivity compared to normal lung tissue (fig 6B). In addition, we examined the
221 transcriptome of human Mtb-infected lymphadenitis compared to other 'reactive' causes of
222 lymphadenopathy devoid of granulomatous inflammation or malignancy (32). Interestingly, despite
223 the fact that reactive lymph nodes were enriched for the T cell transcriptional module, the Th17 and
224 IL-17A/F bioactivity modules were enriched within Mtb infected lymph nodes (fig 6C), further
225 confirming enrichment for IL-17A/F bioactivity to be a feature at the site of TB disease.

226 Discussion

227 Active TB disease is characterised by chronic inflammation that can result in significant tissue
228 destruction, necessary for the onward transmission of Mtb (2). Identifying the processes that govern
229 this immunopathology offers the opportunity to intervene therapeutically, limiting tissue damage and
230 transmission of infection. Pathogenic immune pathways have been difficult to identify because most
231 components of the immune response to Mtb do not discriminate between different clinical outcomes
232 of infection (1). The present study provides compelling new evidence that IL-17A/F responses may
233 mediate immunopathology in active TB. The inclusion of multiple cohorts and diverse demographic
234 backgrounds increased the generalisability of our findings and circumvented the limitations of our
235 cross-sectional study design. The findings are consistent with the well-established role of IL-17A/F in
236 the immunopathology of chronic inflammatory human disease exemplified by psoriasis, for which
237 blockade of the IL-17A/F axis provides an effective treatment (13).

238 Importantly, the primary physiological role of IL-17A/F is to promote host defence against bacterial
239 and fungal infection, cogently demonstrated by mice deficient for IL-17A/F or IL-17 receptors, and by
240 humans with inborn errors of IL-17 immunity (12). Mouse models also suggest a protective role for IL-
241 17A/F following BCG vaccination (33) and in the early stages of Mtb infection, particularly in the
242 context of more virulent Mtb strains (34, 35), through localising T cells near Mtb-infected
243 macrophages (34) and by preventing formation of necrotic granuloma (36). However, in mice

244 rendered susceptible to TB disease as a result of IFNγ deficiency or through receiving repeated BCG
245 vaccinations, IL-17A/F responses drive neutrophil-mediated pathology (14–16, 21). We infer from
246 these data that IL-17A/F responses may play a dichotomous role in TB by contributing to protection in
247 early infection, but to the pathology of disease if early infection is not controlled and multibacillary
248 bacterial replication and chronic immune activation ensues. Additional support for this model is
249 evident in IL27R deficient mice, which exhibit exaggerated Th17 responses because they lack IL-27
250 inhibition of RORγT (37). These mice show enhanced clearance of Mtb, but also increased
251 immunopathology dependent on IL-17A. Interestingly, a human candidate gene study identified host
252 genetic variation associated with increased secretion of IL-17A to be correlated with both protection
253 from incident TB but more severe TB disease (38). Taken together, we hypothesise that exaggerated
254 IL-17A/F recall responses are the consequence of chronic multibacillary infection, but in turn mediate
255 increased pathology in established disease. Of the other IL-17 family members that signal via
256 alternative receptors, much less is known about the functional role of IL-17B, IL-17C and IL-17D (11).
257 These cytokine responses were not specifically tested in the present study. IL-17E, known to promote
258 Th2 responses (11), was not differentially enriched in active TB. Equally, we found no evidence of
259 elevated IL-10 responses in active TB, indicating an uncoupling of IL-17A/F activity from regulatory
260 responses, driving immunopathology rather than the control of Mtb replication (39). Another
261 cytokine, IL-22, does have functional overlap with IL-17A/F (17), but did not discriminate between
262 people with and without disease in our study.

263 Our data support the hypothesis that exaggerated IL-17A/F responses arise from Th17 cells, but they
264 do not unequivocally exclude other cellular sources. Our immunohistochemical analysis did not show
265 any clear evidence for IL-17F production by polymorphonuclear cells in the TST, but we were not able
266 to test whether other lymphocyte populations, such as γδ T cells, made a significant contribution (35,
267 40, 41). Future studies will require single cell resolution to definitively confirm the source of
268 exaggerated IL-17A/F responses in this model, as well as to determine whether active TB shows
269 enrichment for 'pathogenic' Th17 cells that express both IFNg and IL-17A/F (42). Consistent with
270 previous studies (40, 43), we found no evidence of increased circulating Th17 cells in active TB,
271 although this was measured in a separate active TB cohort to the ones that underwent TST
272 assessment. Nevertheless, this observation underscores the unique value of molecular level
273 assessment of immune responses in the tissue microenvironment at the site of host-pathogen
274 interactions, modelled by the TST. Importantly, it also suggests the model that differential Th17
275 responses within the TST are governed by immune signalling networks within the tissue
276 microenvironment after T cell recruitment. In active TB patients, the tissue expression of cytokines
277 that promote Th17 differentiation correlated with the transcriptional module for Th17 cells, and

278 elevated IL-1 activity was present in the TST. The source of these cytokines is likely to be myeloid cells
279 (11, 13, 44). Although we cannot prove a causal relationship at this stage, our data are consistent with
280 a model in which infiltrating monocytes cells from patients with active TB secrete elevated levels of
281 cytokines that promote the differentiation of recruited T cells to a Th17 phenotype. Of note, active TB
282 patients have higher frequency of circulating CD14+ CD16+ non-classical monocytes (45), which can
283 potentiate the differentiation of CD4 T cells to a Th17 phenotype in patients with chronic inflammatory
284 conditions (46).

285 Our findings challenge the long-established view that curative treatment of TB does not lead to
286 contemporaneous changes to immunological recall responses (47). Our data are consistent with the
287 hypothesis that IL-17A-inducible neutrophil chemotaxis and expression of MMP-1 represents a key
288 mediator of immunopathology in TB by promoting granuloma formation, bacterial replication and
289 matrix degradation that can lead to cavitation and onward transmission (9, 48). Our results support
290 future studies to evaluate the impact of modulating IL-17A/F activity to ameliorate the pathology
291 associated with chronic TB disease. The availability of therapies that block the IL-17A/F cytokine axis,
292 developed and licensed for chronic inflammatory diseases (13), offers invaluable opportunities to
293 transition from proof of concept pre-clinical studies, for example in non-human primate models, to
294 first in man experiments.

295

296 Methods

297 Study populations

298 The cohorts of patients recruited to the study are described in detail in Supplementary Methods.
299 Inclusion and exclusion criteria are in table E1 and the demographic, clinical and laboratory data for
300 each study group is summarised in table E2.

301 Study approval

302 Recruitment of patients with cured and latent TB was approved by UK National Research Ethics
303 Committee (reference number: 14/LO/0505) and Universidad Peruana Cayetano Heredia Institutional
304 Ethics Committee (reference number: 62349). Recruitment of patients for the validation active TB
305 cohort was approved UK National Research Ethics Committee (reference number: 16/LO/0776).

306 Study schedule and sampling

307 On recruitment to the study, all participants received 0.1 mL intradermal injection of two units
308 tuberculin (Serum Statens Institute) or saline in the volar aspect of one forearm, and this site was
309 marked with indelible ink. Measurement of the TST site, biopsy collection and sample storage was
310 performed as previously described (4) and is expanded in more detail in Supplementary Methods.

311 Sample processing

312 The TST transcriptome from active TB patients in the discovery cohort was derived directly from the
313 data repository E-MTAB-3254 (ArrayExpress - https://www.ebi.ac.uk/arrayexpress/). Skin samples
314 from all other participants were processed for RNA extraction and transcriptional profiling by either
315 microarrays or RNA-Seq as described in Supplementary Methods.

316 Whole genome transcriptional profiling and analysis software

317 Raw microarray data was processed and normalised as previously described (49). Raw RNASeq counts
318 were normalised within-sample into TPM (transcripts per million) to remove feature-length and
319 library-size effects (50). Log2 transformed TPM were used for further analysis, including
320 transcriptional module derivation and expression that is described in further detail in Supplementary
321 Methods.

322 Transcriptomic data repositories

323 All transcriptional datasets used in this study are described in table E5. Accession numbers refer to
324 datasets in the ArrayExpress repository (https://www.ebi.ac.uk/arrayexpress/). The TST
325 transcriptome of the discovery cohort of patients with active TB was derived from dataset E-MTAB-
326 3254, the cured TB TST transcriptome was derived from dataset E-MTAB-6815, and the validation
327 active TB and latent TB TST transcriptomes were derived from dataset E-MTAB-6816.

328 Immunostaining

329 Immunostaining of IL-17F was performed on 10 μm sections as previously described (51).
330 Immunostaining of MMP-1 was performed as previously described (52). More detailed methods for
331 both these targets, as well as approaches to quantify staining intensity, is available in Supplementary
332 Methods.

333

334 ## Acknowledgements

335 We thank our team of research nurses, in particular Cristina F Turienzo and Victoria Dean, for their
336 assistance in recruiting patients and collecting samples, and the UCL-UCLH Pathogen Genomics Unit
337 for RNASeq sample processing (https://www.ucl.ac.uk/infection-immunity/pathogen-genomics-unit).

338 None of the authors have commercial affiliations or consultancies, stock or equity interests, or patent-
339 licensing arrangements that could be considered a conflict of interest regarding the submitted
340 manuscript.

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514
515

516 # Figure legends

517 **Figure 1. TST transcriptome in patients with active and cured TB disease.** A) Induration at the site of
518 TST was recorded by routine clinical assessments in both populations (mm) and was not different
519 between the two groups (Mann-Whitney test). B) Venn diagram depicting genes significantly
520 upregulated 2-fold in the TST of patients with active or cured TB relative to control saline injection.
521 ($p<0.01$ by t-test with no multiple testing correction). C) TST transcriptome common to both active
522 and cured TB summarised as a network diagram. Purple nodes represent Reactome database
523 functional pathways, yellow nodes represent genes and edges reflect relationship between pathways
524 and genes. Pathway node diameters are proportional to the respective pathway –log10 p value
525 enrichment statistic. D) Pairwise dot-plot of 1910 gene integrated TST signature in patients with either
526 active or cured TB. Dotted line reflects line of perfect covariance. p value derived from linear
527 regression model between the two variables. Red and blue dots represent genes >2 fold differentially
528 expressed ($p<0.01$ by t-test with no multiple testing correction) between active and cured TB.

529

530 **Figure 2. MMP-1 over-expression in active TB.** A) Network diagram of genes and Reactome pathways
531 over-expressed in the TST of patients with active TB compared to cured TB. Purple nodes represent
532 Reactome database functional pathways, yellow nodes represent genes and edges reflect relationship
533 between pathways and genes. Pathway node diameters are proportional to the respective pathway –
534 log10 p value enrichment statistic. B) mRNA expression in TST of patients with active and cured TB of
535 MMP1 gene. C) Representative MMP-1 immunohistochemistry staining in inflammatory infiltrates
536 with TST samples from patients with active and cured TB. D) Differential MMP-1 staining intensity for
537 18 cellular infiltrates in each group of patients relative to adjacent zones of skin with no cellular
538 infiltration. E) MMP-1 staining intensity in TST zones outside inflammatory infiltrates. * = $p<0.01$ by
539 Mann-Whitney test.

540

541 **Figure 3. IL-17 is over expressed in active TB.** A) Network diagram depicting upstream cytokine
542 analysis of genes differentially expressed in TST of patients with active and cured TB. Red and blue
543 nodes represent significant genes overexpressed in active and cured TB respectively as identified in
544 fig 1D. Green nodes represent predicted cytokines regulating the gene expression of red and blue
545 nodes. Edges depict relationship between upstream regulators and differentially expressed genes. B)
546 Expression of selected cytokine transcript within the TSTs of active and cured TB patients. C)
547 Expression of IL-17F by immunofluorescence in TST of patients with active TB. Left panel = phase
548 contrast image, right panel = IL-17F positivity (white). D) Quantification of IL-17F staining throughout

549 TST sections from patients with active and cured TB, determined by pixel intensity as a proportion of
550 area sampled. Each dot represents IL-17F expression in the cross-section of an entire TST biopsy from
551 one patient. * p<0.01 by Mann-Whitney test.

552

553 **Figure 4. TST challenge in active TB is characterised by enrichment of IL-17 responses.** Enrichment in
554 TST relative to saline injection of A) keratinocyte response modules of cytokine bioactivity, B) immune
555 cell modules and C) CXCL1, CXCL8, S100A9 and CCL2 genes. Neutrophil (Neut.) modules M37.1 and
556 ImSig validated for sensitivity and specificity in references (23, 24) respectively. * = p<0.01 by Mann-
557 Whitney test.

558

559 **Figure 5. Active TB is characterised by elevated Th17 cells**. A) IL-17F immunohistochemistry in TST of
560 patients with active TB. Black arrows point to mononuclear cells that express IL-17F and white arrows
561 point to polymorphonuclear cells that do not express IL-17F. B) Th17 and Th1 cell module enrichment
562 in TST relative to saline injection. C) Expression of Th17 and Th1 modules in PPD-stimulated PBMC
563 from patients with active or latent TB (data originated from dataset GSE27984). * = p<0.01 by Mann-
564 Whitney test. D) Relationship between expression of Th17 transcriptional module and individual
565 cytokine gene expression in TST of cytokines implicated in polarising CD4+ T cells to a Th17 phenotype.
566 Data points represent patients with active TB. Spearman rank correlation coefficients (r^2) and p values.
567 D) Enrichment in TST relative to saline injection of IL-1β fibroblast response modules indicative of IL-
568 1 cytokine bioactivity. * = p<0.01 by Mann-Whitney test.

569

570

571 **Figure 6. Th17 cells and IL-17 activity are a feature of both TST reactions and sites of human TB**
572 **disease.** A) Enrichment in TST relative to saline injection of transcriptional modules for IL-17
573 bioactivity, neutrophils, Th17 cells and T cells in TST of independent cohort of patients with active TB
574 and a separate cohort of individuals with latent TB infection. B) Expression of T cells, Th17 cells and IL-
575 17 bioactivity modules from the site of human TB granuloma relative to healthy lung tissue (dataset
576 GSE20050) and C) in human TB infected lymph nodes (LN) relative to reactive lymph nodes that do not
577 display evidence of granulomatous inflammation or cancer (dataset E-MTAB-2547). * = p<0.01 by
578 Mann-Whitney test.

Figure 1

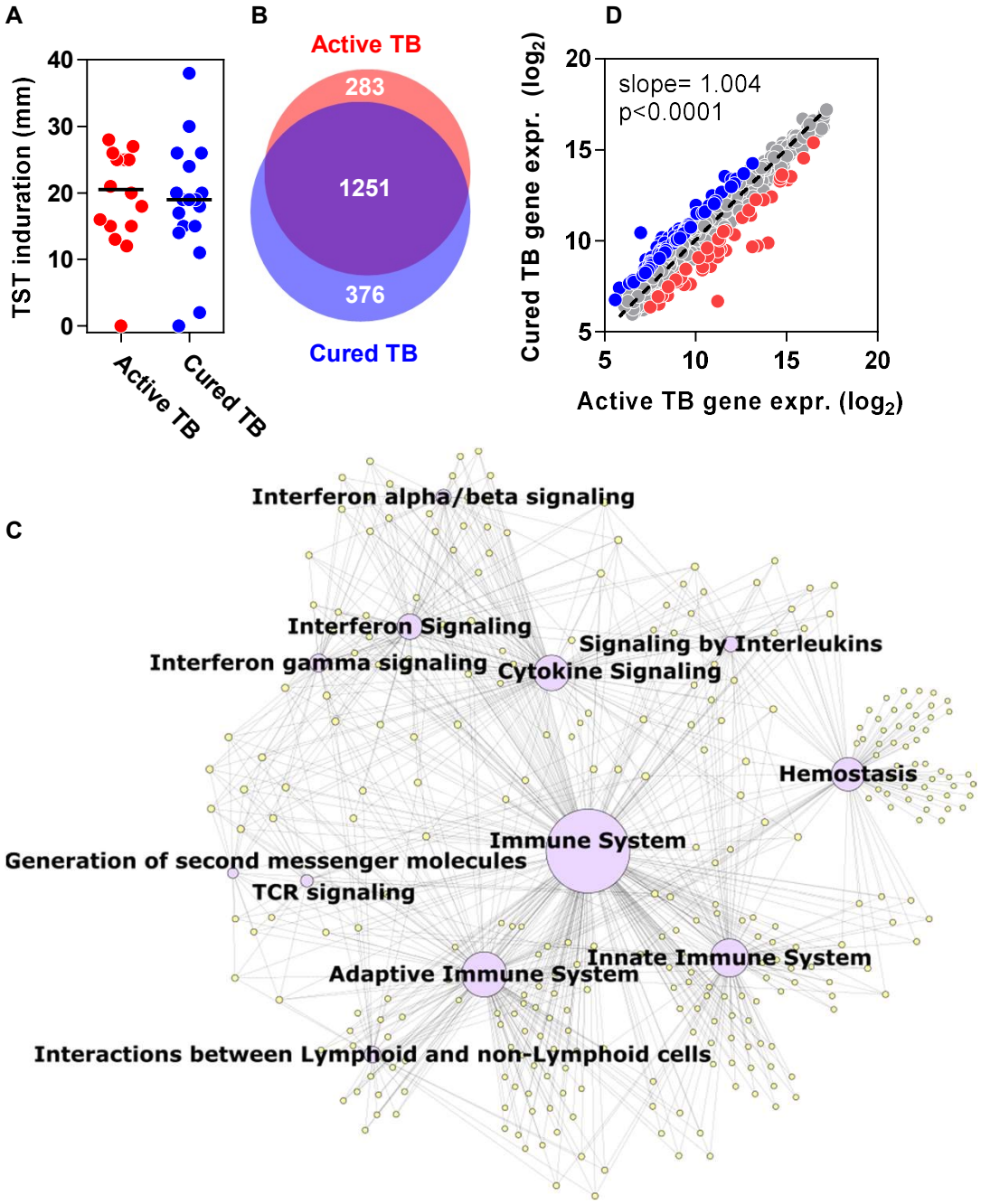

A
TST induration (mm)
40
30
20
10
0
Active TB
Cured TB
B
Active TB
283
1251
376
Cured TB
D
Cured TB gene expr. (log2)
slope= 1.004
p<0.0001
20
15
10
5
5
10
15
20
Active TB gene expr. (log2)
C
Interferon alpha/beta signaling
Interferon Signaling
Signaling by Interleukins
Interferon gamma signaling
Cytokine Signaling
Hemostasis
Immune System
Generation of second messenger molecules
TCR signaling
Innate Immune System
Adaptive Immune System
Interactions between Lymphoid and non-Lymphoid cells

Figure 2

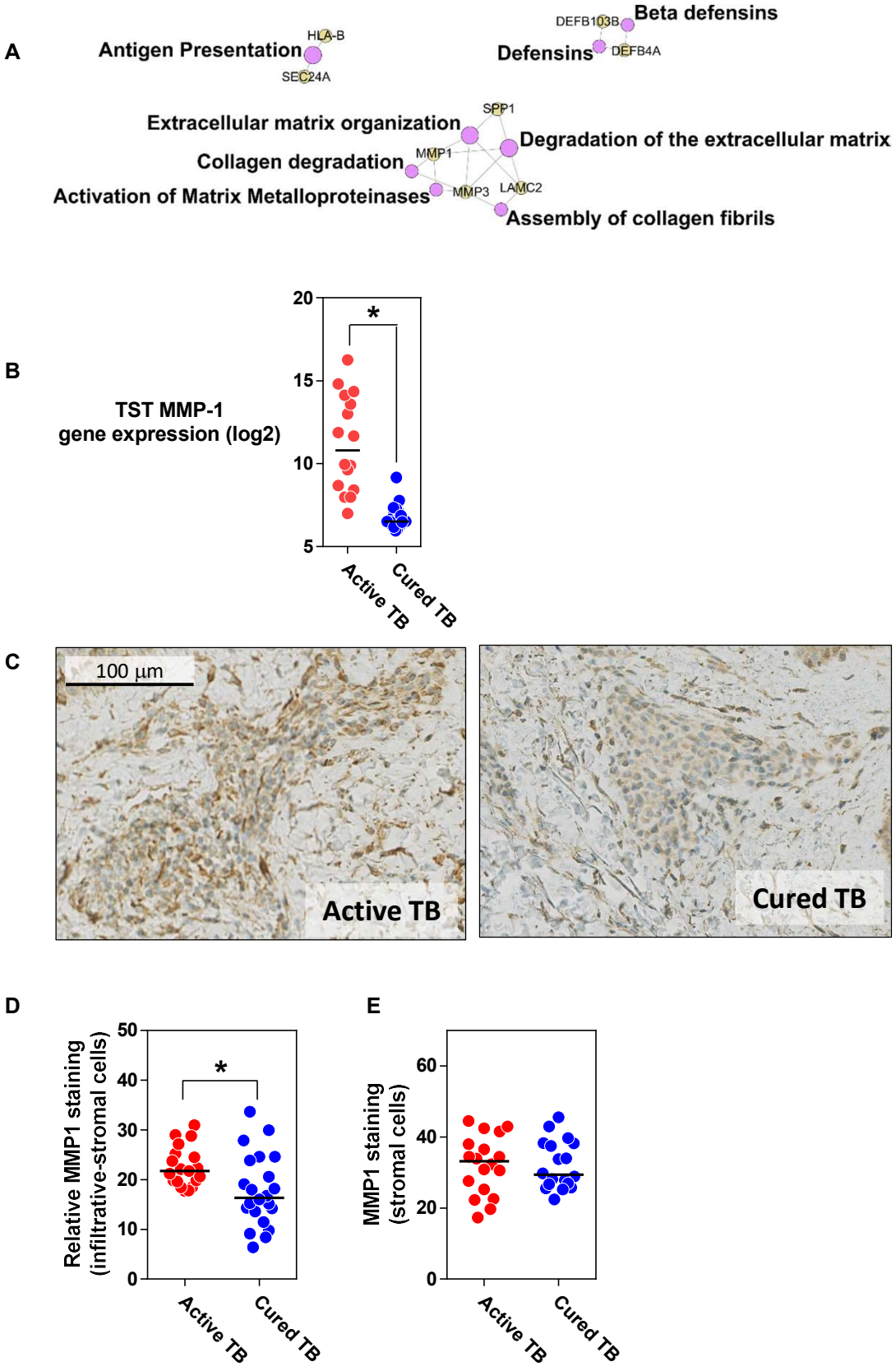

A
Antigen Presentation
HLA-B
SEC24A
Defensins
Beta defensins
DEFB103B
DEFB4A
Extracellular matrix organization
SPP1
Degradation of the extracellular matrix
Collagen degradation
MMP1
Activation of Matrix Metalloproteinases
MMP3
LAMC2
Assembly of collagen fibrils
B
TST MMP-1
gene expression (log2)
20
15
10
5
*
Active TB
Cured TB
C
100 µm
Active TB
Cured TB
D
Relative MMP1 staining
(infiltrative-stromal cells)
50
40
30
20
10
0
*
Active TB
Cured TB
E
MMP1 staining
(stromal cells)
60
40
20
0
Active TB
Cured TB

Figure 3

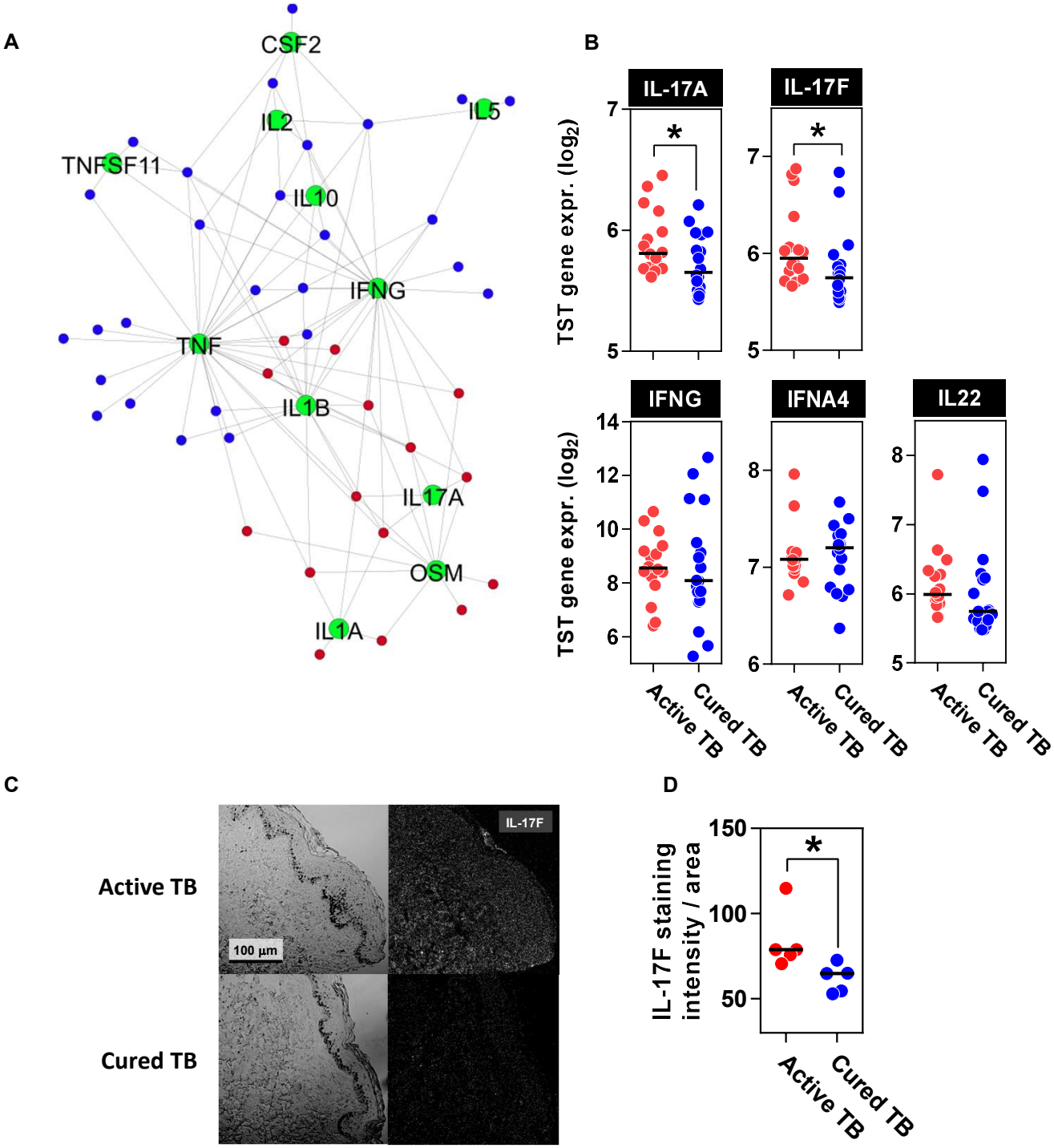

A
CSF2
IL2
IL5
TNFSF11
IL10
IFNG
TNF
IL1B
IL17A
OSM
IL1A
B
IL-17A
IL-17F
IFNG
IFNA4
IL22
TST gene expr. (log2)
Active TB
Cured TB
C
Active TB
Cured TB
IL-17F
100 µm
D
IL-17F staining intensity / area
Active TB
Cured TB

Figure 4

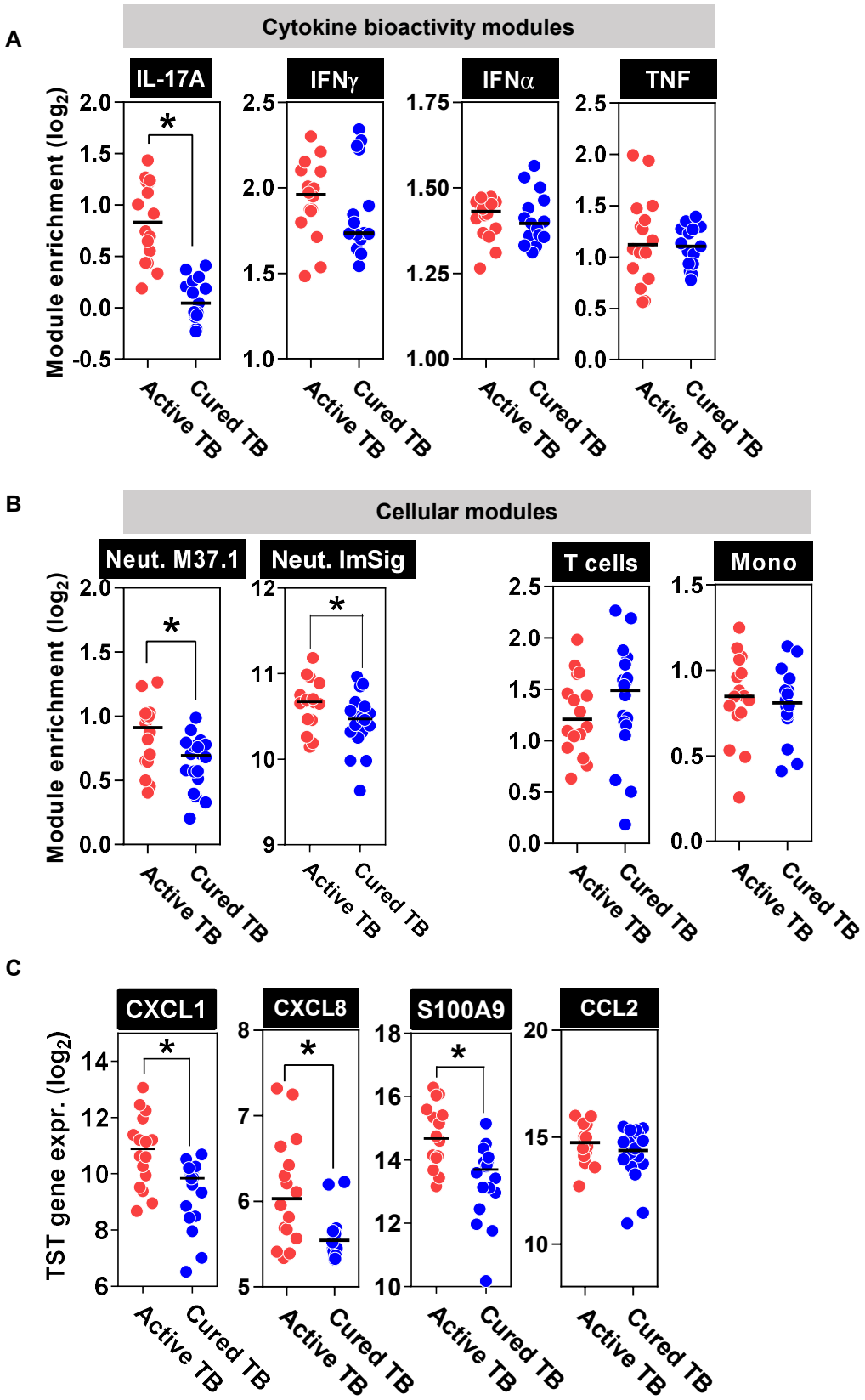
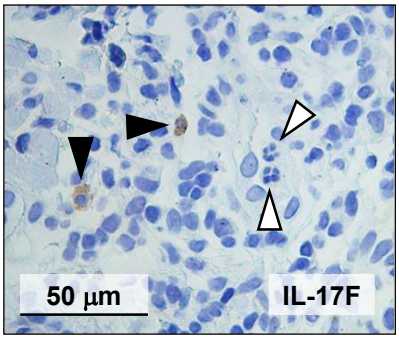
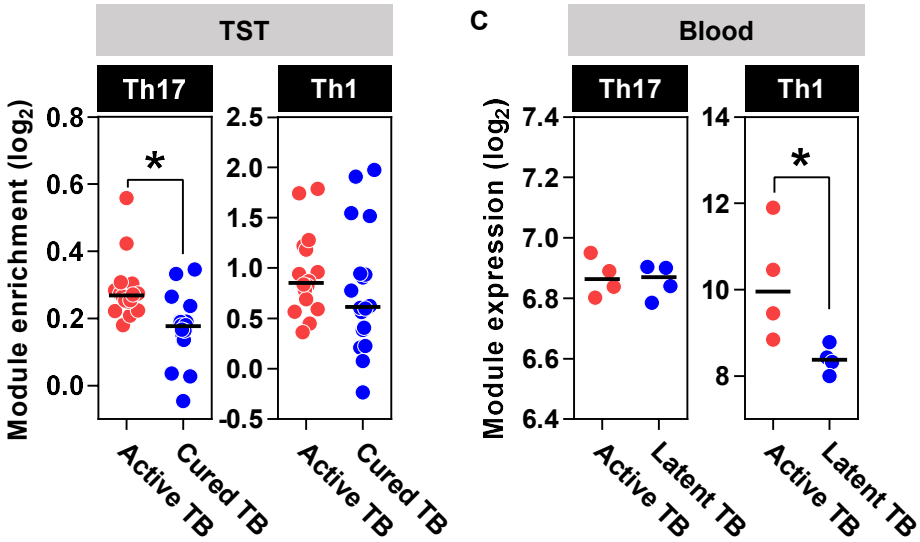
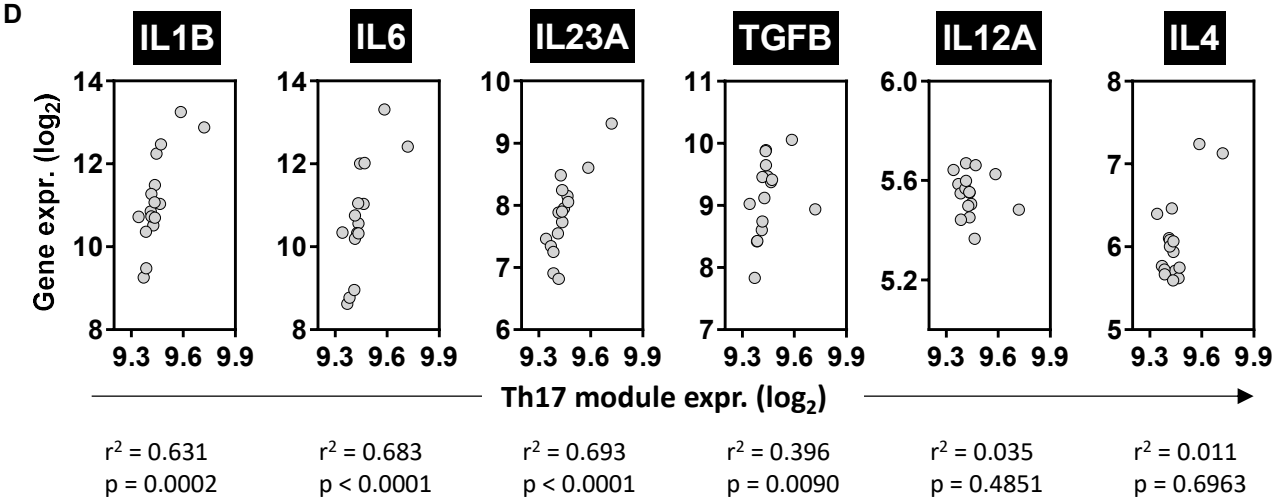

A
Cytokine bioactivity modules
IL-17A
IFNγ
IFNα
TNF
Module enrichment (log2)
Active TB
Cured TB
B
Cellular modules
Neut. M37.1
Neut. ImSig
T cells
Mono
C
CXCL1
CXCL8
S100A9
CCL2
TST gene expr. (log2)

Figure 5


A
50 µm
IL-17F


B
TST
Th17
Th1
Module enrichment (log2)
0.8
0.6
0.4
0.2
0.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
*
Active TB
Cured TB
Active TB
Cured TB
C
Blood
Th17
Th1
Module expression (log2)
7.4
7.2
7.0
6.8
6.6
6.4
14
12
10
8
*
Active TB
Latent TB
Active TB
Latent TB


D
IL1B
IL6
IL23A
TGFB
IL12A
IL4
Gene expr. (log2)
14
12
10
8
14
12
10
8
10
9
8
7
6
11
10
9
8
7
6.0
5.6
5.2
8
7
6
5
9.3 9.6 9.9
Th17 module expr. (log2)
r2 = 0.631
p = 0.0002
r2 = 0.683
p < 0.0001
r2 = 0.693
p < 0.0001
r2 = 0.396
p = 0.0090
r2 = 0.035
p = 0.4851
r2 = 0.011
p = 0.6963

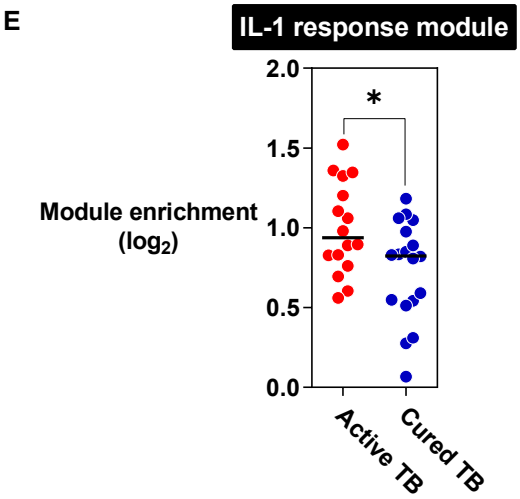
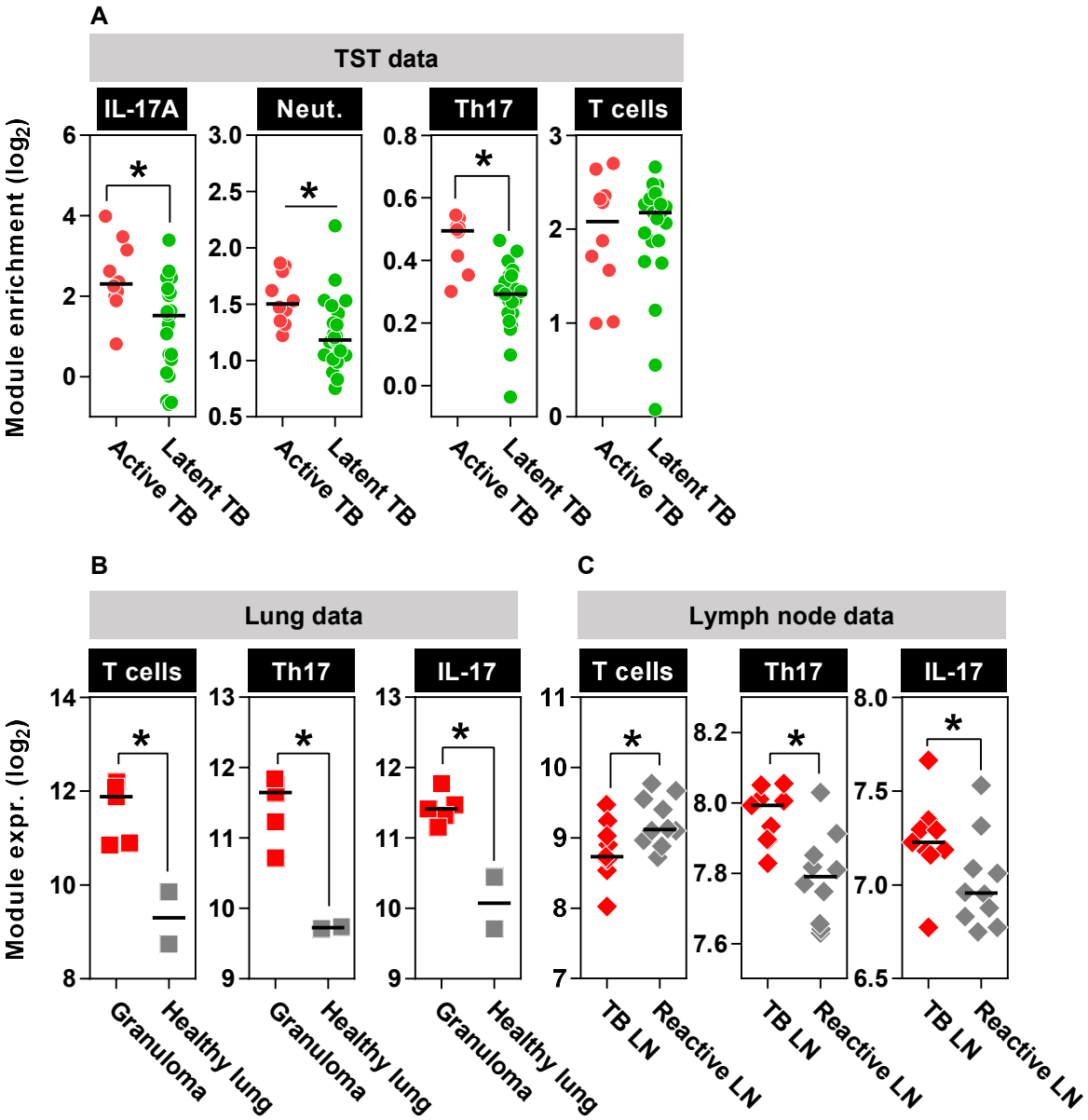

E
IL-1 response module
Module enrichment
(log2)
2.0
1.5
1.0
0.5
0.0
*
Active TB
Cured TB

Figure 6


A
TST data
IL-17A
Neut.
Th17
T cells
Module enrichment (log2)
Active TB
Latent TB
B
Lung data
T cells
Th17
IL-17
Module expr. (log2)
Granuloma
Healthy lung
C
Lymph node data
T cells
Th17
IL-17
TB LN
Reactive LN