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1 **Macrophage migrating inhibitory factor expression is associated with *Trypanosoma***
2 ***brucei gambiense* infection and is controlled by trans-acting expression quantitative trait**
3 **loci in the Guinean population**

4 Justin Windingoudi Kaboré¹, Oumou Camara², Hamidou Ilboudo³, Paul Capewell⁴, Caroline
5 Clucas⁴, Anneli Cooper⁴, Jacques Kaboré^{1,5}, Mamadou Camara², Vincent Jamonneau⁶,
6 Christiane Hertz-Fowler⁷, Adrien Marie Gaston Bélem⁵, Enock Matovu⁸, Annette Macleod⁴,
7 Issa Sidibé¹, Harry Noyes^{9¶} and Bruno Bucheton^{2,6*¶}, For the TrypanoGEN Research Group, as
8 member of the H3Africa Consortium.

9 ¹Centre International de Recherche-Développement sur l'Élevage en zone Subhumide
10 (CIRDES), Unité des Maladies à Vecteurs et Biodiversités (UMaVeB), Bobo-Dioulasso,
11 Burkina Faso; ²Ministère de la Santé et de l'Hygiène Publique, Programme National de Lutte
12 contre la Trypanosomiase Humaine Africaine (PNLTHA), Conakry, Guinée; ³Institut de
13 Recherche en Sciences de la Santé (IRSS), Unité de Recherche Clinique de Nanoro (URCN),
14 Nanoro, Burkina Faso; ⁴University of Glasgow, Wellcome Trust Centre for Molecular
15 Parasitology, Glasgow, United Kingdom; ⁵Université Nazi Boni (UNB), Bobo-Dioulasso,
16 Burkina Faso; ⁶Institut de Recherche pour le Développement (IRD), UMR IRD-CIRAD 177
17 INTERTRYP, Montpellier, France; ⁷University of Liverpool, Institute of Integrative Biology,
18 Liverpool, United Kingdom; ⁸Makerere University, College of Veterinary Medicine Animal
19 Resources and Biosecurity, Kampala, Uganda; ⁹University of Liverpool, Centre for Genomic
20 Research, Liverpool, United Kingdom.

21

22 * Corresponding author

23 E-mail: bruno.bucheton@ird.fr

24 ¶ These authors contributed equally to this work.

25 **Abstract**

26 Infection by *Trypanosoma brucei gambiense* is characterized by a wide array of clinical
27 outcomes, ranging from asymptomatic to acute disease and even spontaneous cure. In this
28 study, we investigated the association between macrophage migrating inhibitory factor (*MIF*),
29 an important pro-inflammatory cytokine that plays a central role in both innate and acquired
30 immunity, and disease outcome during *T. b. gambiense* infection. A comparative expression
31 analysis of patients, individuals with latent infection and controls found that *MIF* had
32 significantly higher expression in patients ($n = 141$; 1.25 ± 0.07 ; $p < 0.0001$) and latent infections
33 ($n = 25$; 1.23 ± 0.13 ; $p = 0.0005$) relative to controls ($n = 46$; 0.94 ± 0.11). Furthermore, expression
34 decreased significantly after treatment (patients before treatment $n = 33$; 1.40 ± 0.18 versus
35 patients after treatment $n = 33$; 0.99 ± 0.10 , $p = 0.0001$). We conducted a genome wide eQTL
36 analysis on 29 controls, 128 cases and 15 latently infected individuals for whom expression and
37 genotype data were both available. Four loci, including one containing the chemokine CXCL13,
38 were found to associate with *MIF* expression. Genes at these loci are candidate regulators of
39 increased expression of *MIF* after infection. Our study is the first data demonstrating that *MIF*
40 expression is elevated in *T. b. gambiense*-infected human hosts but does not appear to contribute
41 to pathology.

42 **1. Introduction**

43 Found in sub-Saharan countries, human African trypanosomiasis (HAT), or sleeping
44 sickness, is a neglected tropical disease. It is caused by *Trypanosoma brucei gambiense* in
45 western and central Africa, and *T. b. rhodesiense* in eastern Africa. The two forms of the disease
46 are considered lethal without treatment and both are transmitted by tsetse flies of the genus
47 *Glossina* (order *Diptera*) (Buscher et al., 2017; Simarro et al., 2010). *T. b. gambiense* causes
48 97% of all HAT cases and is classically described as a chronic disease with an early
49 haemolymphatic phase (stage 1) characterized by non-specific signs. This is followed by a
50 meningoencephalitic phase (stage 2) in which the parasites penetrate the blood brain barrier,
51 leading to neurological disorders and more specific signs characterised by sleep disorder from
52 which the name sleeping sickness is derived, consists of daytime somnolence and sudden
53 overwhelming sleep urges, and nocturnal insomnia (Buscher et al., 2017; Checchi et al., 2008).
54 However, findings show that *T. b. gambiense* infection can lead to a wide array of clinical
55 outcomes, ranging from acute disease that progresses rapidly to the second stage (Garcia et al.,
56 2000; Truc et al., 1997) to spontaneous self-cures (Checchi et al., 2008; Jamonneau et al., 2012)

57 and even latent infections with very low blood parasitaemia that, in most cases, are not
58 detectable by microscopy. Long-term follow up has shown that this latter type of latent infection
59 may persist for several years (Jamonneau et al., 2012).

60 The pathogenesis of *T. b. gambiense* HAT is poorly understood as disease development is
61 dependent on the host immune response, the genetic variability of the parasites and the host-
62 parasite interactions (Garcia et al., 2006). To address this, a candidate gene study was recently
63 performed in Guinea to explore the association between genes involved in host immunity and
64 the disease. This study found significant associations in the *APOLI* gene a component on the
65 trypanosome lytic factor (TLF) that confers resistance to *T. b. brucei* infection and also with
66 rs1818879 of interleukin-6 (*IL6*) (Cooper et al., 2017; Kabore et al., 2017; Vanhamme et al.,
67 2003). Several suggestive associations were observed at the macrophage migration inhibitory
68 factor (*MIF*) gene locus, although these did not remain significant after Bonferroni correction
69 (Kabore et al., 2017). Although we did not observe significant genetic association at the *MIF*
70 locus in our Guinean HAT cohort, *MIF* is nevertheless known as an important pro-inflammatory
71 cytokine that plays a central role in the control of both innate and acquired immunity in a
72 number of diseases (Calandra, 2003; Renner et al., 2005). Elevated serum levels of *MIF* have
73 been found in patients with malaria (McDevitt et al., 2006), viral hepatitis (Kimura et al., 2006),
74 HIV infection (Regis et al., 2010), rheumatoid arthritis (Morand et al., 2002), sepsis (Bozza et
75 al., 2004), vasculopathy (Zernecke et al., 2008), and Chagas disease (Cutrullis et al., 2013).
76 Importantly, it was also shown that *MIF* mediates the pathogenic inflammatory immune
77 response and increases the recruitment of inflammatory monocytes and neutrophils promoting
78 the most prominent pathological features of experimental trypanosome infections (Stijlemans
79 et al., 2014). Regarding *Trypanosoma cruzi*, *MIF* was also shown to play an important role in
80 the host defence against acute infections, favouring the production of the pro-inflammatory
81 cytokines during the early phase of infection (Reyes et al., 2006). These studies have prompted
82 us to evaluate *MIF* expression in human *T. b. gambiense* infections.

83 To establish a potential role for *MIF* in determining *T. b. gambiense* clinical diversity, we
84 examined *MIF* transcript expression in a Guinean cohort. For this study, *MIF* mRNA expression
85 levels were determined by qPCR in HAT patients before and after treatment, individuals
86 harbouring long term latent infections and endemic uninfected controls. In addition, the
87 availability of genome wide SNP data generated within the framework of the TrypanoGEN
88 network for some of these individuals (Ilboudo et al., 2017) made it possible to identify
89 expression quantitative trait loci (eQTLs) potentially involved in the regulation of *MIF*

90 expression. The identification of these loci contributes to our understanding of how MIF
91 expression is controlled and may have relevance to a wide range of infectious and immune-
92 pathophysiological disease.

93 **2. Materials and methods**

94 *2.1. Ethics statement*

95 Samples were collected during medical surveys conducted by the national control program
96 (NCP) of the Republic of Guinea according to the national HAT diagnostic procedures
97 approved by the Ministry of Health in Guinea. All participants were informed of the objective
98 of the study in their own language and signed an informed consent form. For participants less
99 than 18 years of age, informed consent was obtained from their parents. Approval for this study
100 was obtained from the « comité consultative de déontologie et d'éthique » (CCDE) of the
101 « Institut de Recherche pour le Développement » (1-22/04/2013). This study is part of the
102 TrypanoGEN project that aims to understand the genetic basis of human susceptibility to
103 trypanosomiasis. Samples were archived in the TrypanoGen Biobank at CIRDES (Ilboudo et
104 al., 2017).

105 *2.2. Study population*

106 All individuals participating in this study were identified during medical surveys performed
107 between 2007 and 2011 by the NCP of the Republic of Guinea. Identification and sampling of
108 study participants were performed in three active HAT foci (Forecariah, Dubreka and Boffa)
109 located along the Guinean coast according to standard procedures describe previously (Ilboudo
110 et al., 2011). For each participant, 5 ml of blood was taken in anticoagulant blood collection
111 tubes to collect plasma samples and buffy coats for further DNA extraction. Two additional ml
112 of blood were also taken with PAXgene Blood RNA tubes (PreAnalytiX). All samples were
113 frozen in a portable freezer at -20°C in the field and were stored in the lab at -80°C. For all
114 participants, the Card Agglutination Test for Trypanosomiasis (CATT) was performed. Direct
115 microscopic examination of the buffy coat and/or the lymph node aspirate (if swollen lymph
116 nodes were present) were performed if an individual was CATT positive, (Camara et al., 2010;
117 Magnus et al., 1978). The immune trypanolysis test was performed at CIRDES on all plasma
118 samples, as previously described (Jamonneau et al., 2010). For this study, 212 individuals were
119 selected and three categories of phenotype were defined according to the following criteria:

120 *i.* Cases or HAT patients ($n=141$): Parasitologically confirmed patients, with CATT
121 plasma dilution end titers $\geq 1/4$ and trypanolysis test (TL) positive. All HAT patients diagnosed
122 within this study were treated according to the NCP procedures. Patients with HAT stage 1
123 disease (CSF white cell counts, 5) were treated by daily injection of pentamidine for 8
124 consecutive days. Early stage 2 patients (CSF white cell counts 6-20) were treated with one
125 injection of pentamidine every 2 days for 20 days (10 total injections). Late stage 2 patients
126 (CSF white cell count, > 20) were treated with three cycles of melarsoprol injections (one-third
127 of the dose on the 1st day, two-thirds of the dose on the 2nd day and a full dose on days 3 and 4)
128 administered 10 days apart. Of the 141 patients, 33 were sampled again 6 months after treatment
129 at their first follow-up and were included in this study to examine *MIF* expression before and
130 after treatment.

131 *ii.* Latent infections or Seropositive ($n=25$): individuals with CATT plasma dilution end
132 titers $\geq 1/4$, TL positive, parasitology negative (no trypanosomes detected by mAECT and/or
133 by examination of cervical lymph node aspirate when cervical adenopathies were present) and
134 who maintained this phenotype for least two years.

135 *iii.* Endemic controls ($n=46$): Individuals with negative CATT results and negative TL,
136 living in the same villages as a HAT patient and/or a seropositive subject.
137 The age and gender characteristics of the different study groups is given in supplementary Table
138 S1.

139 2.3. *mRNA preparation and cDNA synthesis*

140 Total mRNA from blood was extracted with the PAXgene Blood RNA kit (PreAnalytiX) and
141 quantified with a NanoDrop spectrophotometer (NanoDrop Technologies). The mRNA quality
142 was checked using the RNA Integrity Number (RIN) on an Agilent 2100 Bioanalyzer with the
143 RNA 600 Nano LabChip (Agilent Technologies). Total mRNA was reverse transcribed
144 according to the manufacturer's instructions, using High Capacity cDNA Reverse Transcription
145 kit, Applied Biosystems and RNase inhibitor (Applied Biosystems) at a final concentration of
146 1.0 U/ml. The cDNAs were stored at -20°C and diluted to 1:5 with RNase-free water for use as
147 a template in the real-time PCR analysis.

148 2.4. *MIF expression assays by RT-qPCR*

149 The real-time quantitative PCR analysis was conducted using the AriaMx machine (Agilent
150 Technologies). All of the TaqMan RT-qPCR reagents, including the primers and probes, were

151 purchased from Applied Biosystems. The RT-qPCR analysis was conducted using predesigned
152 and optimized Assays on Demand (Applied Biosystems). The following assays were used: MIF
153 (ID: Hs00236988_g1) and TBP (ID: Hs00427621_m1). The reaction parameters were a 2min
154 at 50°C hold and a 5min at 95°C hold, followed by 45 cycles of 15s at 95°C for a melting, and
155 1min at 60°C for annealing and extension. All of the measurements were performed in triplicate.
156 A relative quantitation was conducted using *TBP* as a reference gene (Ledderose et al., 2011).
157 The parameter cycle threshold (C_t) was defined as the cycle number at which the fluorescence
158 intensity exceeds a fixed threshold. Relative amounts of mRNA for target genes were calculated
159 using the comparative C_t method ($2^{-\Delta\Delta C_t}$). As the assays were optimized for PCR efficiency by
160 the manufacturer, the mRNA-expression levels were estimated according to the delta-Ct values.

161 2.5. Genotype data

162 DNA was extracted from buffy coat (BC) samples using a Qiagen DNA extraction kit
163 (QIAamp DNA Blood Midi Kit) following the manufacturer's instructions. DNA extracts were
164 stored at -20°C. All DNA samples were quantified on a NanoDrop spectro-photometer, and
165 shipped to Illumina, San Diego, California where they were genotyped on the H3Africa 2.5
166 million SNP chip. Genotypes were extracted from the Illumina genotype file and converted to
167 Plink format using the H3ABioNet Nextflow topbottom.nf workflow in the H3ABioNet GWAS
168 pipeline (<https://github.com/h3abionet/h3agwas>).

169 2.6. Statistical analysis

170 2.6.1. Differential Gene Expression analysis.

171 The $2^{-\Delta\Delta C_t}$ method was applied to determine gene expression levels for each individual (Livak
172 and Schmittgen, 2001) and results used to compare the relative expression of *MIF* between
173 HAT phenotypes. The Shapiro-Wilk test was initially used to test the null hypothesis (H_0) that
174 the level of expression followed a normal distribution (p -value > 0.05). As *MIF* expression
175 levels did not follow a normal distribution, we used the non-parametric Wilcoxon-test to
176 perform the intergroup comparisons. This tested the H_0 that there was no difference in the mean
177 expression of *MIF* between the phenotypes. We also used a paired Wilcoxon-test to compare
178 *MIF* expression level in the same patient before and after treatment. Association of *MIF*
179 expression levels with recorded covariates (gender, age, disease focus and HAT phenotypes)
180 were also analysed using univariate and multivariate linear regression models. All data are
181 presented as means \pm CI₉₅ and pairs of conditions and p -values < 0.05 were considered

182 significant. All analyses were performed with R 3.4.4 software (R Development Core Team,
183 2018).

184 2.6.2. *Expression QTL analysis.*

185 A genome wide association study was undertaken in Plink to identify SNP associated with *MIF*
186 expression levels. Quality control of the genotyped data was performed using Plink 1.9. Closely
187 related samples were identified and one of each pair of close relatives were removed. We
188 removed duplicate samples and samples with missing data > 5% and loci with minor allele
189 frequencies < 5% and loci which were not in Hardy-Weinberg equilibrium ($p < 0.001$). We
190 created a Plink phenotype file (Sample ID, sex, age, expression of *MIF*) containing 172 samples
191 constituted of 29 controls, 128 cases and 15 individuals with latent infections for whom both
192 expression and genotype data were available. We used linear regression in Plink 1.9 to perform
193 eQTL analysis using the “*linear*” function, including the disease status as a covariate. Loci
194 where significant eQTL were detected were reanalysed using a much denser set of SNP markers
195 obtained by imputation from our genotype data and 1000 genomes reference data. Imputation
196 was done by H3ABioNet at the University of Capetown using 1000 genome project genotypes
197 as reference (Genomes Project et al., 2015).

198 3. Results

199 3.1. *MIF expression in HAT patients, individuals with latent infections and endemic* 200 *controls*

201 MIF mRNA quantification was conducted on 212 samples that consisted of healthy
202 endemic controls $n = 46$ (21.70%) and *T. b. gambiense*-infected individuals that were
203 subdivided into two phenotypes: 1) patients with active HAT $n = 141$ (66.51%) of whom 33
204 (23.40%) were sampled again after treatment, and 2) individuals with latent infections $n = 25$
205 (11.79%) who tested positive in serology, but were negative upon microscopic examination and
206 exhibited few or no symptoms. These individuals were followed for a period of at least two
207 years and none developed detectable blood parasitaemia during their follow up despite
208 remaining positive in serology. The sex ratio (male: female) of the study population was 1.41
209 (124/88) and the mean age (range) was 32.76 (5–85) years. Detailed characteristics of the study
210 cohort are provided in Table S1.

211 *MIF* expression levels were assayed by RT-qPCR and compared between groups of
212 individuals. As shown in Fig 1 and Table S2, *MIF* expression levels were found to be
213 significantly elevated in patients (1.25 ± 0.07 ; $p < 0.0001$) and individuals with a latent infection

214 (1.23±0.13; $p = 0.0005$) when compared to controls (0.94±0.11). No expression differences
215 were observed between HAT cases and individuals with latent infections ($p = 0.809$). As shown
216 in Table 1 the HAT status was the main factor explaining *MIF* expression levels in a
217 multivariate linear regression model including gender, age and disease focus. These results
218 suggest that *MIF* expression is induced during *T. b. gambiense* infection in humans but that
219 expression levels are not correlated with the clinical status. *MIF* mean expression levels
220 decreased significantly in patients after treatment (1.40±0.18 before treatment versus 0.99±0.10
221 after treatment; $p = 0.0001$) and were similar to expression levels observed in controls
222 (0.94±0.11; $p = 0.255$).

223 3.2. Localisation of *MIF* expression quantitative trait loci

224 We next performed an eQTL analysis using our *MIF* expression data and genotype data
225 available for a subset of samples to identify SNP loci that were associated with *MIF* expression.
226 In all e-QTLs analysis of *MIF* expression, the infection status was included as a covariate in the
227 regression analysis. Samples were genotyped on the H3Africa Illumina SNP chip that contains
228 2.5 million SNP loci and is enriched for common African SNPs. We found SNPs at four loci,
229 located on chromosomes 1, 3, 4 and 10 respectively, that had significant associations with *MIF*
230 expression ($p < 10^{-7}$) (Fig 2, Table 2). We did not observe any association signal on
231 chromosome 22 at the *MIF* locus. SNPs were annotated with the closest gene(s) using Genome
232 Reference Consortium Human Build 37 (GRCh37/hg19) data (Table 3). In particular, the
233 chemokine (C-X-C motif) ligand 13 (*CXCL13*) gene, involved in both inflammatory and B-cell
234 activation, was found to be proximal to the index SNP on chromosome 4 ($p=7.12 \times 10^{-8}$). Genetic
235 association at the four identified loci were then further explored by the mean of imputation to
236 increase the number of SNPs in these chromosomal regions (Fig 2b, 2c, 2d, 2e). At each
237 candidate loci, additional SNPs were found to be significantly associated with *MIF* expression
238 levels thus supporting the genome wide association results (Table S3). A sharp peak of
239 association was observed for the eQTL on chromosome 3 whereas association signals were
240 broader for the eQTLs on chromosome 1, 4 and 10.

241 In order to assess the importance of *T. b. gambiense* infected individuals in the
242 identification of *MIF* eQTLs, we repeated the analysis for the four identified regions on
243 chromosomes 1, 3, 4 and 10 on several groups of individuals: all, HAT cases and controls and
244 infected individuals only (HAT cases and latent infections). In all cases lower mean p-values
245 were obtained in the analysis excluding controls (Fig 3) although effect sizes were lower in this
246 analysis. Most controls had low *MIF* expression levels and were thus probably not much

247 informative in the analysis. This was particularly true for the e-QTLs on chromosomes 3 and
248 10 for which the strength of genetic association appears highly dependent of *T. b. gambiense*
249 infected individuals.

250 **4. Discussion**

251 Although several studies have investigated the role of *MIF* polymorphisms rs36086171
252 and rs12483859 in HAT resistance/susceptibility (Ahouty et al., 2017; Kabore et al., 2017; Ofon
253 et al., 2017), the present study is the first to look at *MIF* expression levels in a HAT endemic
254 population containing individuals with latent infections. The principal finding was that, as
255 observed for other infectious diseases, *MIF* expression is elevated during *T. b. gambiense*
256 infection. However, levels did not correlate with disease severity as expression was found to be
257 elevated in both HAT patients with active disease and individuals harbouring latent infections.
258 A second major finding was the identification of four eQTLs involved in the control of *MIF*
259 expression that may be relevant in wide array of disease settings. This demonstrates that
260 studying humans infected with pathogens that stimulate an immune response may provide new
261 insights into wider immune regulation mechanisms.

262 *MIF* is known to be a critical upstream mediator of innate immunity and is involved in a
263 number of pathophysiological inflammatory processes such as glomerulonephritis (Yang et al.,
264 1998) and asthma (Mizue et al., 2005). It is a pleiotropic cytokine produced by a variety of
265 immune cells, including lymphocytes, macrophages and pituitary cells (Calandra et al., 1995).
266 *MIF* expression is regulated by several factors, such as exposure to endotoxins, inflammatory
267 cytokines and glucocorticoids, and exhibits a wide range of immune activities. This can include
268 the induction of the Toll-like receptor 4 (*TLR4*) gene and the induction of inflammatory
269 cytokines, such as INFG, TNFA, IL8, IL6 or IL12. *MIF* is also known to sustain macrophage
270 activation and the secretion of microbicidal molecules, including nitric oxide (Rosado and
271 Rodriguez-Sosa, 2011).

272 Up-regulation of *MIF* expression has been reported for many infectious and parasitic
273 diseases, demonstrating that *MIF* can play either a protective or deleterious role in the immune
274 response to different pathogens (Rosado and Rodriguez-Sosa, 2011). For example, the *TLR4*-
275 mediated response to *T. cruzi* has been reported as one of the main pathways involved in the
276 early production of cytokines associated with human innate immunity during the acute stage
277 (Oliveira et al., 2004). Other studies have shown that *MIF* secreted by activated macrophages
278 plays an important role in innate immune defences against *Mycobacterium tuberculosis* by

279 acting in an autocrine fashion to inhibit the growth of virulent mycobacteria (Oddo et al., 2005).
280 Low expression alleles of *MIF* were also shown to confer an increased risk of *M. tuberculosis*
281 disease in some populations (Das et al., 2013). In the present study, we found that *MIF*
282 expression was significantly upregulated during *T. b. gambiense* infections in both patients with
283 active disease and individuals harbouring latent infections. *T. b. gambiense* thus seems to be a
284 potent inducer of MIF in humans but MIF does not appear to control infection outcome, at least
285 in the Guinean population. This is in contrast to a previous study in mice, where MIF expression
286 was found to be increased in susceptible mice infected by *T. brucei*. In this model, MIF was
287 proposed to be a major element promoting the most prominent pathological features of
288 experimental trypanosomiasis (Stijlemans et al., 2014). Results presented here suggest that this
289 may not be the case in our Guinean cohort. Interestingly other proinflammatory cytokines that
290 are modulated by MIF, including IL8 and IL6, have been found to be significantly increased in
291 individuals with latent infections (Gineau et al., 2016; Ilboudo et al., 2014). In contrast,
292 susceptibility to disease was associated with increased levels of immunosuppressive molecules,
293 such as IL-10 and soluble HLA-G (Gineau et al., 2016; Ilboudo et al., 2014). This suggest that
294 although MIF seems to be induced during *T. b. gambiense* infections, control of
295 resistance/susceptibility to HAT occurs downstream of MIF activation in our study population.

296 This study has also identified four trans-acting eQTLs that are associated with the
297 control of *MIF* expression. No signs of association were found at the *MIF* locus on
298 chromosome 22, despite multiple cis-eQTL SNP being reported by the larger study undertaken
299 by GTEx Consortium (GTEx Consortium, 2015). This could be due to the lower coverage of
300 the H3Africa Illumina SNP chip in this region. It is noteworthy however that only suggestive
301 genetic associations were observed between *MIF* polymorphisms and HAT in this Guinean
302 population (Kabore et al., 2017). Another explanation is that *MIF* expression regulatory
303 polymorphisms are too rare in this population for associations to be detected with our sample
304 size. Importantly, most studies examining *MIF* regulation in infectious disease have focused
305 on cis-regulatory elements (Rosado and Rodriguez-Sosa, 2011). Our study suggests that other
306 loci in the genome may also play an important role and should be investigated further to better
307 understand the mechanisms of *MIF* regulation and implication in disease exacerbation or
308 infection control. The eQTL on chromosome 4 encompasses the *CXCL13* gene. Excessive
309 CXCL13 production has been associated with the development of systemic lupus
310 erythematosus nephritis and recombinant CXCL13 has been shown to induce the up-regulation
311 of *MIF* expression in human podocytes *in vitro* (Worthmann et al., 2014). Interestingly two

312 genes coding for RNA regulatory molecules, a small nucleolar RNA (*SNORD2*) and a micro
313 RNA (*MIR3924*), were identified on chromosome 10 in this study, neither of these appeared to
314 have any homology with sequences at the *MIF* locus suggesting that any regulatory effect that
315 they might have on *MIF* is indirect through other genes. However, we only considered genes
316 that either encompassed the significant SNP or were the closest gene when the SNP was located
317 in an intergenic region. As shown with our imputed data (Fig 2), association signals can extend
318 up to a 100kb on chromosomes 3, 4 and 10 suggesting that other candidate genes involved in
319 *MIF* regulation may be located further away from the index SNPs.

320 Furthermore, we found that the most significant eQTLs that may be involved in *MIF*
321 expression regulation were obtained in analyses using infected individuals only (including HAT
322 patients and individuals with latent infections) and excluding controls (Fig 3). This would
323 suggest that *MIF* is actively regulated by trans-acting genes only when there is an inflammatory
324 response, and that this trans-regulation dominates over any cis-regulation. At other times basal
325 expression may be cis-regulated but our sample of 29 controls may have been too small to detect
326 this or there may be no common regulatory variants at *MIF* in this population. This suggests
327 that the *T. b. gambiense* infection has boosted the power to detect genetic loci controlling *MIF*
328 expression. As potent inducers of immune responses, pathogens may thus serve as a valuable
329 tool that may increase the power to unravel regulatory mechanisms involved in the control of
330 critical mediators of immunity in human populations.

331 **5. Conclusion**

332 This study has shown that *T. b. gambiense* infection induces *MIF* expression in humans and has
333 enabled us to locate four trans-acting eQTLs controlling its expression. As most pathogens are
334 potent inducers of immunological molecules, such as MIF, our study demonstrates that
335 combining clinical phenotypes, whole genome genotypes and expression data in infectious
336 diseases, may contribute to improving our knowledge of immune regulation and susceptibility
337 to other immune-pathophysiological diseases.

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522

523 **Figure legends**

524 **Fig 1: *MIF* expression in HAT patients, individuals carrying latent infections and endemic**
525 **controls.** (A) MIF expression levels according to the clinical status: endemic controls ($n = 46$),
526 HAT patients or cases ($n = 141$), latent infections ($n = 25$). (B) MIF expression levels in cases
527 before treatment ($n = 33$) and the same cases after treatment ($n = 33$). 1_Cases BeT: cases before
528 treatment, 2_Cases AfT: cases after treatment, **** $p < 0.0001$, ** $p < 0.01$, ns: not significant.

529 **Fig 2: Manhattan plots showing the locations of MIF expression eQTL.** The e-QTL analysis
530 was performed by linear regression using the clinical status (HAT patient, latent infection,
531 endemic control) as a covariate. A. eQTL mapping using the whole genome genotype dataset
532 from the Illumina 2.5M SNP chip. Association results after including imputed SNPs at the
533 candidate e-QTLs on chromosome 1 (B.), chromosome 3 (C.), chromosome 4 (D.) and
534 chromosome 10 (E.).

535 **Fig 3: Bar plot representing the mean p-value of significant imputed SNPs at each eQTL**
536 **locus according to the infection status.** For each model the phenotype was used as covariate
537 in the linear regression analysis. Covariate coding: 1-**Infected vs Controls (n=172)**: Controls
538 were coded 1 and both active and latent infections were coded 2; 2- **HAT cases versus Controls**
539 **(n=157)**: Controls and active cases were coded 1 and 2 and latent infections were coded 0
540 (missing) and were excluded; 3-**HAT cases versus Latent (n=143)**: Latent infections were
541 coded 1, active cases were coded 2, controls were coded 0 and were excluded.

542 **Table 1: Analysis of *MIF* expression levels according to recorded covariates**

Covariates			Univariate	Multivariate
	<i>n</i>	<i>MIF</i> ^a	<i>p</i>	<i>p</i>
<i>Sex</i>				
Females	88	1.22 ± 0.10		
Males	124	1.16 ± 0.07	0.3121	0.2250
<i>Age</i>				
≤ 24 years	68	1.15 ± 0.10		
25 – 39 years	77	1.21 ± 0.11	0.2866	0.2614
≥ 40 years	67	1.19 ± 0.09		
<i>Disease focus</i>				
Forecariah	61	1.22 ± 0.13		
Dubreka	106	1.20 ± 0.71	0.0951	0.0395*
Boffa	45	1.27 ± 0.13		
<i>HAT phenotypes</i>				
Controls	46	0.94 ± 0.11	<0.0001	<0.0001***
Latent infections	25	1.23 ± 0.13		
HAT	141	1.25 ± 0.07		

543 ^a Mean fold change ± mean confidence interval

544 **Table 2: SNPs reaching genome wide significance in the *MIF* e-QTL analysis.**

<i>CHR</i>	<i>SNP</i>	<i>BP</i>	<i>AI</i>	<i>TEST</i>	<i>NMISS</i>	<i>BETA</i>	<i>STAT</i>	<i>P</i>
1	kgp15347739	116918691	G	ADD	120	0.5036	5.937	3.04E-08
3	snp-known116300863	115325154	A	ADD	149	0.4603	5.642	8.44E-08
4	kgp20876446	78506066	G	ADD	120	0.5356	5.754	7.12E-08
10	snp-known78014716	58884536	C	ADD	120	0.5346	5.696	9.29E-08

545 CHR: Chromosomes, SNP: single nucleotide polymorphism, AI: minor allele name, BP, base-pair physical
546 position, ADD: additive model. The e-QTL analysis was performed on the whole data set (n=172) using a linear
547 regression model with the clinical status as a covariate.

548 **Table 3: Proximal e-QTLs candidate genes controlling *MIF* expression.**

<i>CHR</i>	<i>SNP</i>	<i>Gene name</i>	<i>Gene stable ID</i>	<i>Gene description</i>
1	kgp15347739	AL136376.1	ENSG00000269279.1	Uncharacterized protein
3	snp-known116300863	RP11-190P13.1	ENSG00000244157.1	eukaryotic translation initiation factor 4E family member 2 pseudogene 2
3	snp-known116300863	GAP43	ENSG00000172020.8	growth associated protein 43
4	kgp20876446	CXCL13	ENSG00000156234.7	chemokine (C-X-C motif) ligand 13
10	snp-known78014716	SNORD2	ENSG00000238707.1	Small nucleolar RNA SNORD2
10	snp-known78014716	MIR3924	ENSG00000264747.1	microRNA 3924

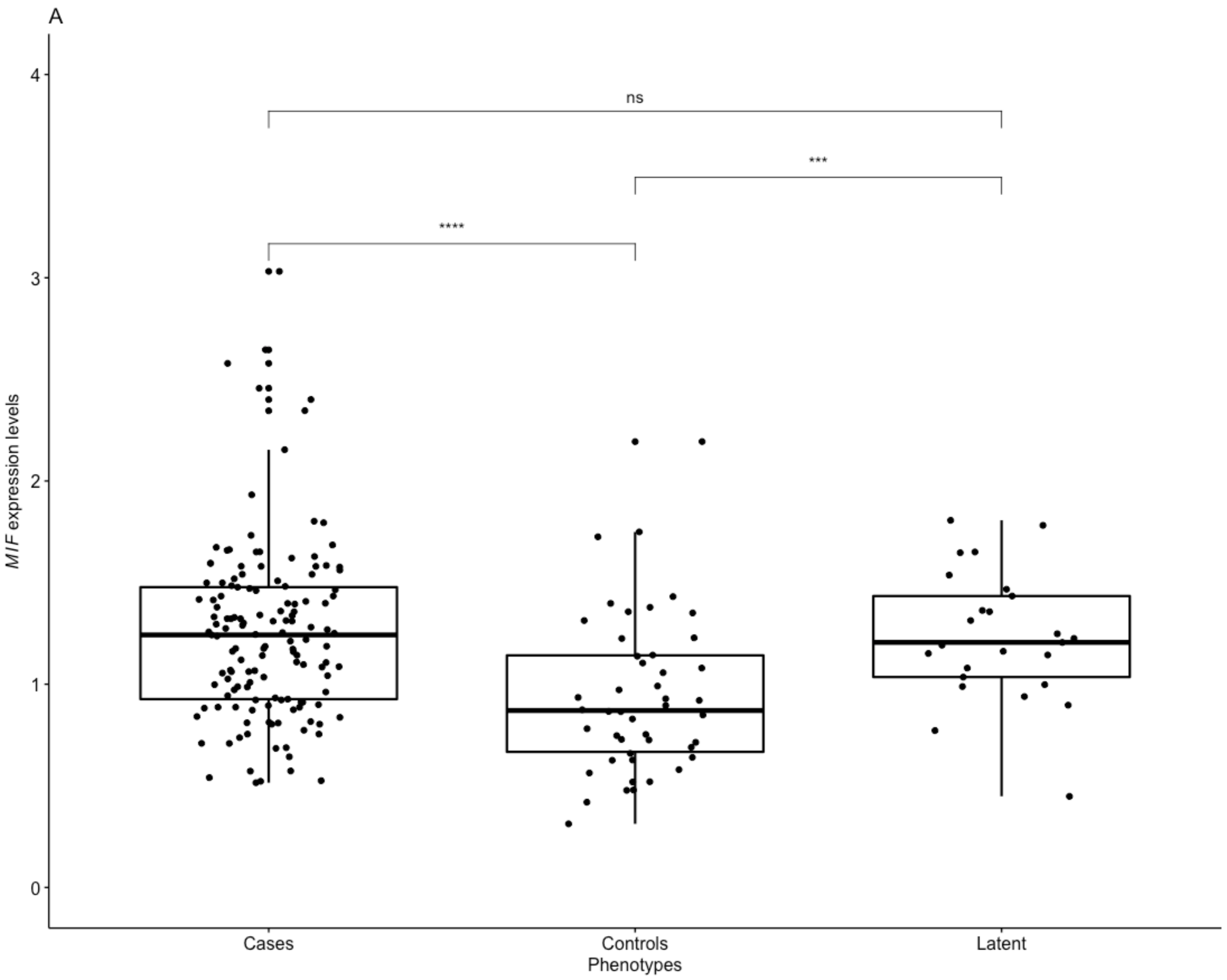
549 CHR: Chromosomes, SNP: single nucleotide polymorphism. Each SNP reaching genome wide association
550 significance was annotated with either the gene they were located in or the genes immediately upstream or
551 downstream for intergenic SNPs.

552 **Supporting Information Legends**

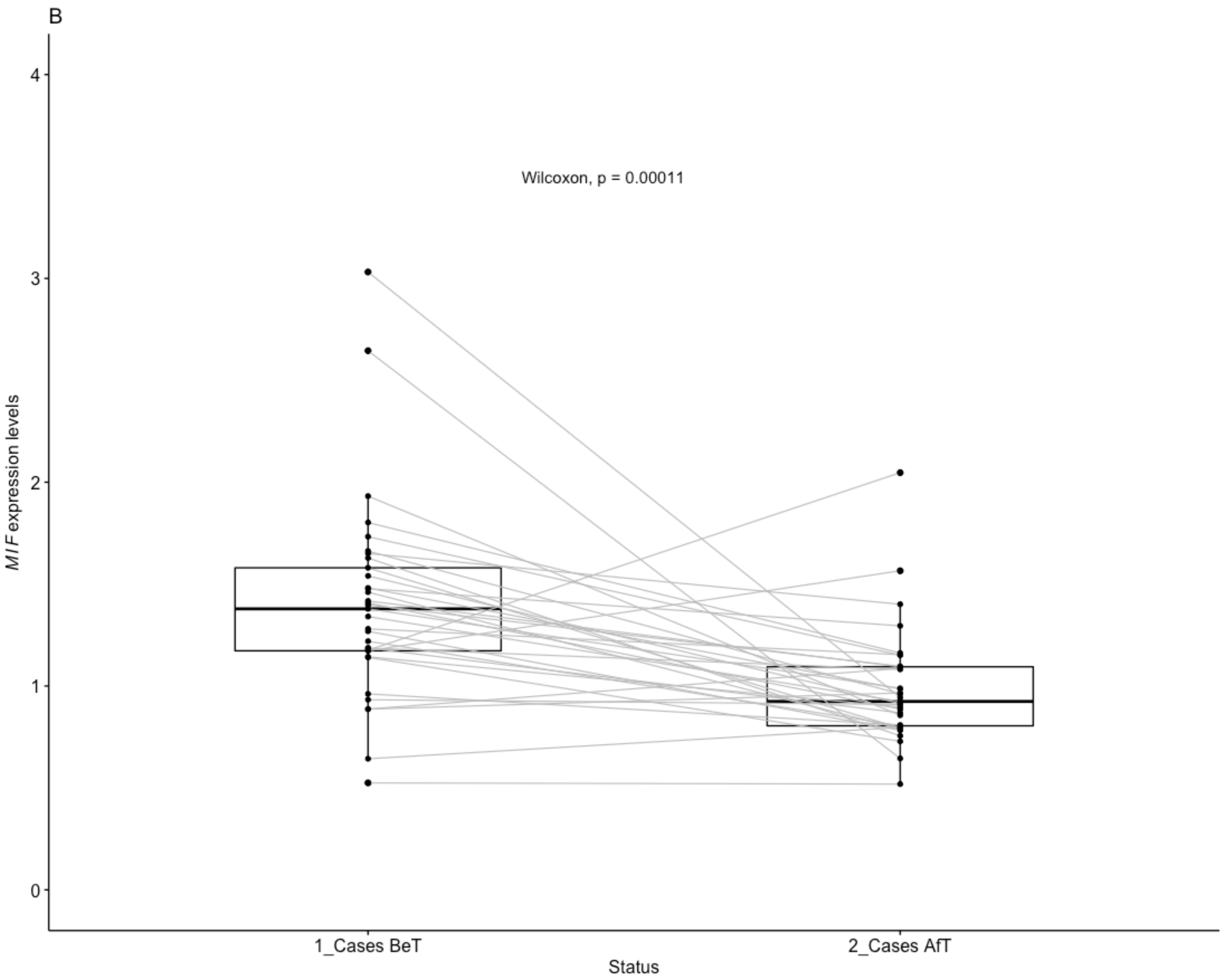
553 **Table S1: The age and gender characteristics of the different study phenotype groups**

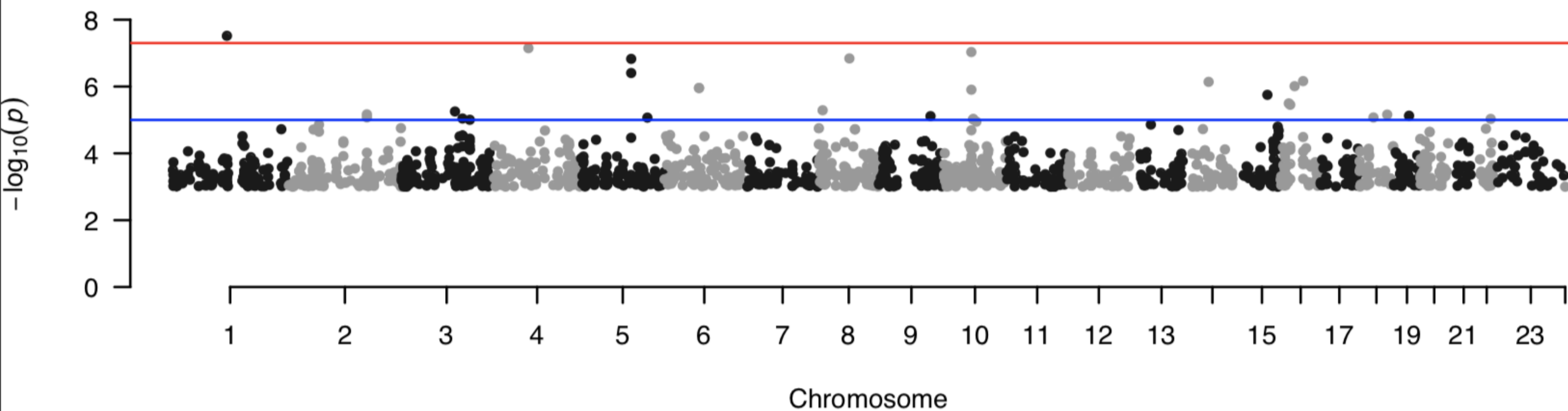
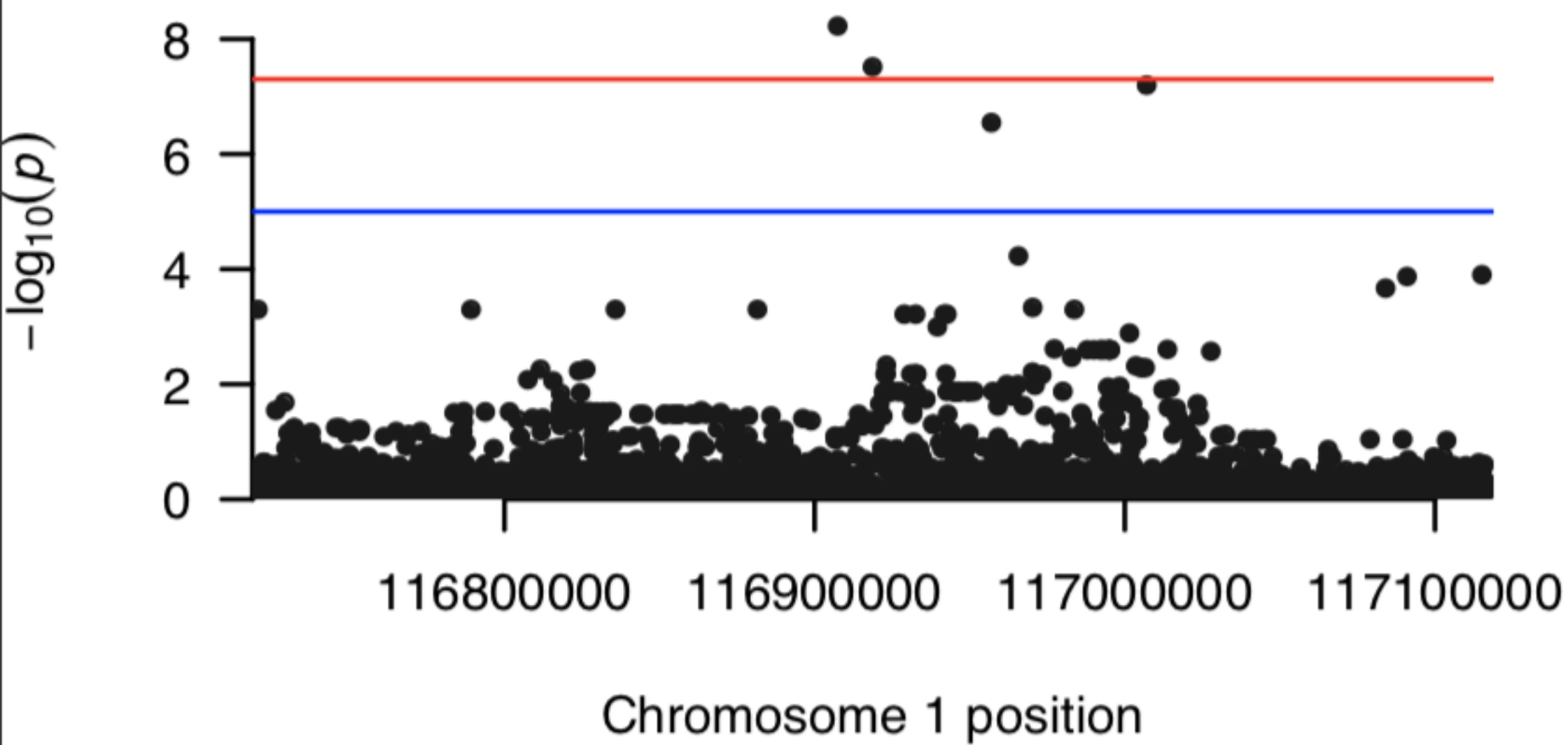
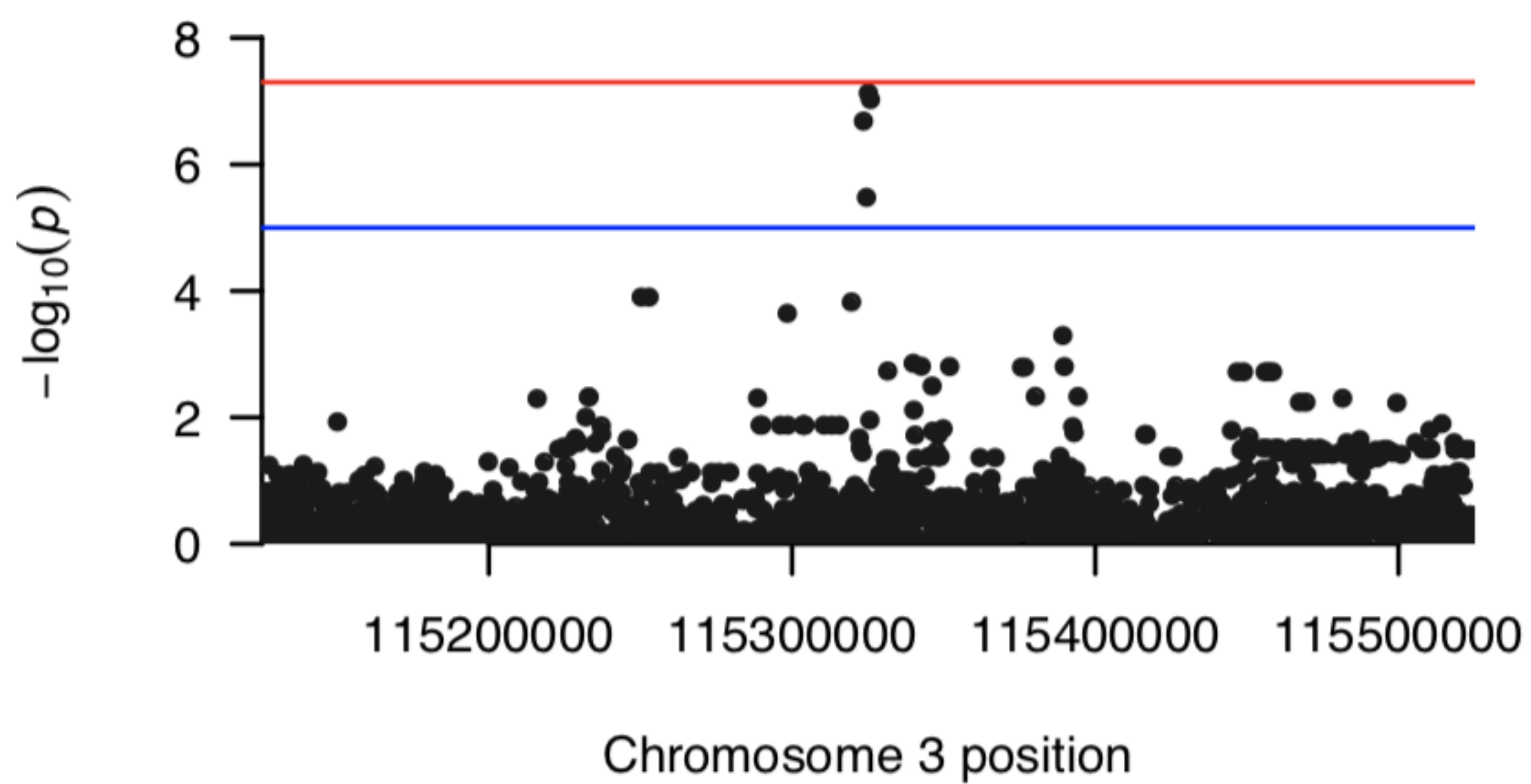
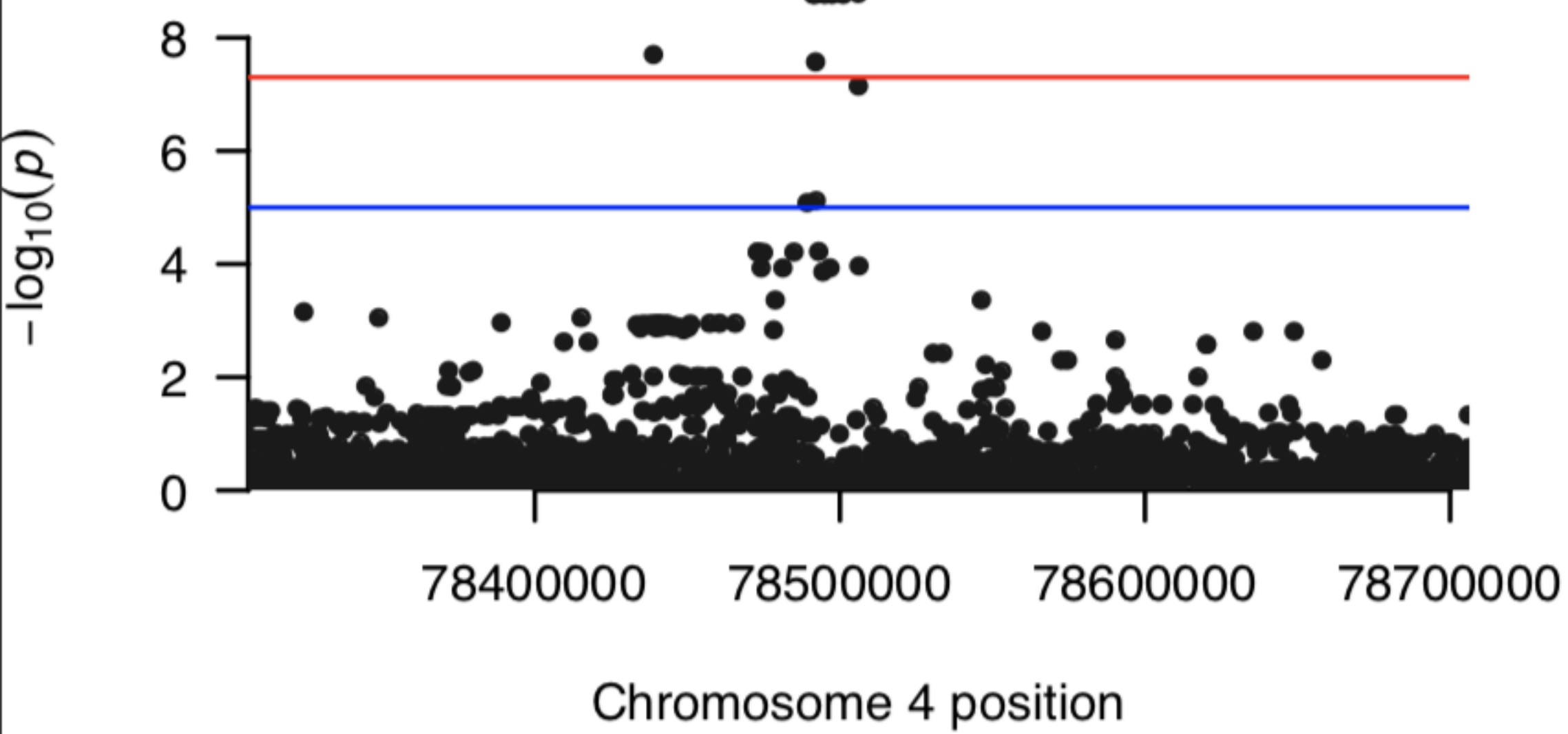
554 **Table S2: *MIF* Expression levels according to the HAT clinical status**

555 **Table S3: SNPs reaching genome wide significance in the *MIF* e-QTL analysis with**
556 **imputed data.**



B



A**B****C****D****E**