1 **Title: Discovering *in vivo* eQTL interactions with interferon status and**
2 **drug exposure from a lupus clinical trial**

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5 **Authors:** Emma E. Davenport,[1,2,3] Tiffany Amariuta,[1,2,3,4] Maria Gutierrez-Arcelus,[1,2,3]
6 Kamil Slowikowski,[1,2,3,4] Harm-Jan Westra,[1,2,3] Ying Zhang,[5] Stephen Pearson,[6] David
7 von Schack,[5] Jean S. Beebe,[5] Nan Bing,[5] Michael S. Vincent,[5] Baohong Zhang[5] and
8 Soumya Raychaudhuri[1,2,3,4,7*]

9

10 **Affiliations:**
11 [1]Divisions of Genetics and Rheumatology, Department of Medicine, Brigham and
12 Women's Hospital, Harvard Medical School, Boston, MA, 02115, USA.
13 [2]Partners Center for Personalized Genetic Medicine, Boston, MA, 02115, USA.
14 [3]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard,
15 Cambridge, MA, 02142, USA.
16 [4]Department of Biomedical Informatics, Harvard Medical School, Boston, MA, 02115,
17 USA.
18 [5]Pfizer Inc., Cambridge, MA, 02139, USA.
19 [6]Pfizer New Haven Clinical Research Unit, New Haven, CT, 06511, USA.
20 [7]Faculty of Medical and Human Sciences, University of Manchester, Manchester, M13
21 9PL, UK.

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23

24

25 *Correspondence to:
26 Soumya Raychaudhuri
27 77 Avenue Louis Pasteur
28 Harvard New Research Building, Suite 250D
29 Boston, MA 02446, USA.
30 soumya@broadinstitute.org; 617-525-4484 (tel); 617-525-4488 (fax)

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42 **Abstract:**

43 **If an expression quantitative trait locus (eQTL) effect is modulated by an**

44 **environmental stimulus, such as drug exposure or disease status, it can point to**

45 **key regulatory mediators. In a clinical trial for anti-IL-6 in 157 patients with**

46 **systemic lupus erythematosus we measured cell counts, interferon (IFN) status,**

47 **drug exposure and genome-wide gene expression at three time points. First, we**

48 **confirmed an increase in power using repeat transcriptomic measurements.**

49 **Then, after detecting 4,976 *cis* eQTLs, we discovered that 154, 185 and 126 had**

50 **evidence of significant eQTL interactions with T cell proportion, IFN status and**

51 **anti-IL-6 drug exposure respectively. Next, we found an enrichment of**

52 **transcription factor binding motifs interrupted by eQTL interaction SNPs, pointing**

53 **to regulatory mediators of these environmental stimuli and therefore potential**

54 **therapeutic targets for autoimmune diseases. In particular, IFN interactions are**

55 **enriched for IRF1 binding site motifs, while anti-IL-6 interactions are enriched for**

56 **IRF4 motifs. Finally, we used the drug-eQTL interactions to define an informative**

57 **drug exposure score, reflecting a drug's effect in an individual patient, thus**

58 **highlighting the potential for utilizing drug-eQTL interactions in a**

59 **pharmacogenetic framework.**

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Main Text:

A *cis* expression quantitative trait locus (eQTL) contains a genetic variant that alters expression of a nearby gene. *Cis* eQTLs are ubiquitous across the genome[1] and while most are stable across tissues and conditions, environmental perturbations can alter the effects of some of them[2–8]. If a perturbation disrupts upstream regulatory mechanisms for a gene then it could magnify or dampen an eQTL effect, resulting in a genetic by environment interaction. Therefore, observing a set of eQTL interactions due to a perturbagen (such as drug exposure or disease status) can identify shared upstream regulatory mechanisms. These may represent key transcription factors and affected pathways that inform our understanding of both disease and drug mechanisms. Defining the molecular effects of a drug's action is particularly critical as these effects could help to classify likely non-responders, indicate off-target effects, predict the toxicities of the medication, find more accurate biomarkers and identify diseases for which a drug might be repurposed.

However, *cis* eQTL interactions with environmental factors in humans have been challenging to discover *in vivo*[9–13] even with large cohorts[1,7]. Success at finding *cis* eQTL interactions has largely been found in studies using model organisms[14,15] or treating cells *in vitro* with non-physiologic conditions[16]. Thus far, these studies might be limited in power since they either map eQTLs separately across conditions and fail to exploit the power of repeat measurements[17]. Or they test for genetic variants associated with differential expression and miss information about the magnitude of the eQTL effect in a specific condition[18].

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89   Here, we predicted that if RNA is queried at multiple time points under different

90   exposure states, repeat measurements could increase power to not only detect eQTLs,

91   but also their interactions with environment.

92

93   Clinical trials, with their structured study design, may be the ideal setting to detect eQTL

94   interactions with drugs or other environmental perturbations. In clinical trials, it is

95   becoming increasingly common to collect transcriptional and genetic data alongside

96   clinical and physiological data. This extensive phenotyping of the same individuals at

97   multiple time points across conditions can be leveraged to identify eQTL interactions.

98   The profiling of an individual both before and after exposure to a drug provides a unique

99   opportunity to identify *in vivo* drug-eQTL interactions.

100

101   As a proof of principle, we examined the modulation of eQTL effects by environmental

102   factors, including cell count, interferon (IFN) status and drug exposure, using data from

103   a phase II clinical trial to evaluate the safety and efficacy of a neutralizing IL-6

104   monoclonal antibody (PF-04236921) in 157 systemic lupus erythematosus (SLE)

105   patients[19] (**Methods**). IL-6 is a cytokine elevated in SLE and other autoimmune

106   diseases such as rheumatoid arthritis (RA). In SLE, IL-6 is thought to play a role in the

107   observed B cell hyperactivity and autoantibody production[20]. The IL-6 receptor has been

108   successfully targeted with tocilizumab in RA[21] and has shown promise in a phase I trial

109   for SLE[22]. IL-6 itself has been targeted with siltuximab for the treatment of Castleman's

110   disease[23] and this has led to the development of other biologics targeting IL-6, such as

111   PF-04236921. While this drug was not significantly different from placebo for the

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112   primary efficacy endpoint (proportion of patients achieving the SLE Responder Index

113   (SRI-4) at week 24), biologically it effectively reduced free IL-6 protein levels. Given the

114   key role of IL-6 in a range of diseases, IL-6 blockade and mechanism of action are of

115   great interest to study.

116

117   In this study, we leverage the power of repeat transcriptional and environmental

118   measurements from a lupus clinical trial to identify eQTL interactions. We discover

119   eQTL interactions with cell count, IFN status and drug exposure. We find the eQTL

120   interaction SNPs are enriched for transcription factor binding motifs (such as IRF1 and

121   IRF4 for IFN and drug exposure respectively) highlighting regulatory mediators of these

122   interactions and potential therapeutic targets.

123

124   **Results**

125   We conducted whole blood high-depth RNA-seq profiling at 0, 12, and 24 weeks in anti-

126   IL-6 exposed and unexposed individuals with the Illumina TruSeq protocol. We

127   observed and quantified 20,253 gene features and genotyped 608,017 variants

128   genome-wide (**Methods**). Along with each RNA-seq assay, we documented drug

129   exposure and quantified cell counts with FACS and IFN signature status with real-time

130   PCR.

131

132   *Mapping eQTL in SLE patients*

133   We first mapped *cis* eQTLs (SNPs within 250kb of the transcription start site of the

134   gene) and then tested those eQTLs for interactions with cell counts, IFN status and drug

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135   exposure. eQTL interactions can be explored using our interactive visualization tool

136   (http://baohongz.github.io/Lupus_eQTL).

137

138   We used a linear mixed model, including repeat measurements with up to three RNA-

139   seq assays per patient (**Fig. 1A,** 379 samples from 157 patients, **Methods**). To

140   maximize power, we adjusted for 5 population and 25 gene expression principal

141   components (**Methods**). To ensure a set of highly confident eQTLs, we applied a

142   stringent multiple hypothesis testing correction and identified 4,976 *cis* eQTL genes with

143     $p_{eqtl} < 2.3 \times 10^{-8}$ (0.05/2,177,889 tests, **Fig. 1B,1C, Supplementary Table 1**).

144

145   *Repeat measurements increase power to detect eQTL*

146   We observed that repeat samples increased our power by detecting 63% more *cis*

147   eQTLs compared to using a single sample per individual (3,061 genes **Fig. 1B**). Our

148   results are highly concordant with the BIOS cohort, a much larger dataset of 2,166

149   healthy individuals[1]. 85.4% of our SLE eQTL SNP-gene pairs are reported as eQTLs in

150   BIOS (FDR<0.05). Of these, 99.1% showed consistent direction of effect ($p < 5 \times 10^{-16}$,

151   binomial test, **Fig. 1D**). For each of the 4,976 *cis* eQTL genes, we tested the most

152   significantly associated SNP for environmental interactions.

153

154   *eQTL interactions with T cell proportions*

155   We first tested a type of eQTL interaction that has been examined previously: cell

156   counts[24,25]. We obtained FACS data for 320 samples for which we had

157   contemporaneous RNA-seq profiles (n=152 subjects). We determined the percentage of

158   total lymphocytes that were T cells by gating (**Fig. 2A**). We found 154 T cell-eQTL

159   interactions with nominal evidence ($p_{interact}$<0.01, **Supplementary Table 1**), whereas

160   from 4,976 tests we would expect ~50 from chance alone. To ensure that our statistics

161   were not inflated, we conducted 1,000 stringent permutations, where we reassigned T

162   cell percentages across samples and retested. This permutation preserved the main

163   eQTL effect, while disrupting interactions that might be present in the data. In no

164   instance did we observe 154 or more interactions at $p_{interact}$<0.01 (maximum=133),

165   suggesting that the number of observed interactions is highly unlikely to have happened

166   by chance (**Fig. 2B**, $p_{permute}$~0/1,000 = <0.001).

167

168   Interactions can be divided into magnifiers, where environmental exposure and eQTL

169   effects are in the same direction, and dampeners where the effects work in the opposite

170   direction (**Fig. 2C, Supplementary Fig. 1**). The *NOD2* rs1981760 eQTL is an example

171   of an interaction that is dampened by increased T cell count (**Fig. 2D**, $p_{interact}$=6.5x10^{-5}),

172   and has separately been shown to vary across cell types[24,25]. For each 10% increase in

173   T cell proportion, the eQTL effect is reduced by about 7%.

174

175   *IFN status eQTL interactions*

176   Many patients with SLE exhibit high levels of genes induced by IFN alpha; these genes,

177   known as the IFN signature, are a marker of disease severity[26,27] and a pathogenic

178   feature of SLE. We explored the influence of IFN alpha on gene regulation after

179   determining the IFN status of every patient at each time point using real-time PCR of 11

180   IFN-inducible genes[28] (**Methods, Fig. 3A**). We identified 185 IFN-eQTL interactions

181   with $p_{interact}$<0.01 (**Supplementary Table 1**). Following the same permutation

182   procedures as above, our observed interactions were unlikely to have occurred by

183   chance (maximum permutation interactions=112, $p_{permute}$<0.001) (**Supplementary Fig.**

184   **2**). We note that interactions with a proxy gene for IFN status have been described[1] and

185   we find overlap of genes with those reported interactions (**Supplementary Fig. 3**). For

186   example, *SLFN5* expression is influenced by the rs12945522 SNP ($p_{interact}$ =1.3×10^{-10},

187   **Fig. 3B**). This effect is dampened in IFN low samples.

188

189   To define transcription factors that drive the response to IFN alpha, we sought to

190   identify motifs that explain the differences between magnifier (n=75) and dampener

191   (n=110) eQTLs (**Supplementary Fig. 4**). We applied HOMER[29] to assess overlap

192   between transcription factor binding motifs and the eQTL interaction SNPs (and SNPs

193   in high linkage disequilibrium (LD, r^2>0.8) in the *cis* window) (**Methods**). We found

194   significant enrichment of motifs for key transcription factors involved in IFN signaling

195   including the IRF1 motif. IRF1 motif disruption occurred for 11 genes with an eQTL

196   dampened in IFN low samples but for only 2 genes with an eQTL magnified (HOMER

197   p=0.001, permutation p<0.048, **Methods, Fig. 3C**, **Supplementary Table 2**). An

198   example is the *GTF2A2* rs2306355 eQTL ($p_{interact}$=8.7×10^{-3}, **Fig. 3D**); rs2306355 is in

199   tight LD (r^2=0.85 in Europeans) with rs6494127, which interrupts the GAAA core of the

200   IRF1 motif (**Fig. 3C**), and likely disrupts IRF1 binding[30]. We observe greater expression

201   of *GTF2A2* in individuals with the rs2306355 A allele compared to G; this difference is

202   dampened in IFN low individuals (**Fig. 3D**).

203

204   *Discovery of eQTL interactions with drug exposure*

205   We then examined whether IL-6 blockade alters the relationship between genomic

206   variation and gene expression and induces drug-eQTL interactions. We observed 126

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207   drug-eQTL interactions with $p_{interact}$<0.01 (**Supplementary Table 1**). Following the same

208   permutation strategy as above, we found a median of 77 interactions with $p_{interact}$<0.01

209   (maximum=117) from 1,000 permutations. This suggests that about half of our drug-

210   eQTL interactions likely represent real biological phenomena, and not statistical artifact

211   (**Supplementary Fig. 5**). These drug-eQTL interactions showed little overlap with the

212   interactions observed for T cell count or IFN status (**Supplementary Fig. 6**). We note

213   biologically relevant drug-eQTL interactions for *IL10* (**Supplementary Fig. 7**), an anti-

214   inflammatory cytokine, *CLEC4C* which has previously been associated in *trans* with an

215   SLE risk allele[31] and *CLEC18A* (**Fig. 4A**) another member of the C-type lectin domain

216   family.

217

218   Again, the drug-eQTL interactions can be divided into magnifiers and dampeners

219   (**Supplementary Fig. 8**) and a similar approach as described above can be applied to

220   define transcription factors driving the response to IL-6 blockade. The most enriched

221   motif for eQTLs magnified after drug treatment was IRF4. Strikingly, the IRF4 motif

222   disruption occurred for 16 genes, including *CLEC18A*, with an eQTL magnified after

223   drug treatment compared to 6 genes with an eQTL dampened ($p=1 \times 10^{-5}$, permutation

224   p<0.01, **Fig. 4B**, **Methods**, **Supplementary Table 3**).

225

226   Searching for transcription factor binding motifs in DNA regions of interest may not be

227   the only way of identifying downstream mediators of IL-6. From chromatin

228   immunoprecipitation sequencing (ChIP-seq), our drug-eQTL interaction SNP

229   (rs2270843) for *CLEC18A* lies in a MAFK binding site. SNPs in high LD (r^2>0.8) in the

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230   *cis* window also lie in JUND, CTCF, RAD21, SMC3 and ZNF143 binding sites. Hence

231   one or more of these factors could also be acting to translate changes in serum IL-6

232   levels to regulation of *CLEC18A* gene expression.

233

234   A more common strategy to determine the effect of a perturbagen is to use differential

235   gene expression. For differential expression following drug treatment, we identified

236   1,161 genes with nominal statistical evidence (p<0.01) but modest effects (max fold

237   change=1.3, **Supplementary Fig. 9**). Only 8/126 drug-eQTL interaction genes also

238   show evidence of differential gene expression. This suggests that eQTL interactions

239   offer independent information from differential expression, which might contribute to

240   defining mechanisms.

241

242   *Concordance of drug-eQTL interactions with protein level interactions*

243   To validate these interactions, we hypothesized that interactions due to drug exposure

244   are likely driven by free IL-6 cytokine levels (our key clinical biomarker of interest). If this

245   is the case, for eQTLs dampened by drug exposure, an increase in free IL-6 should

246   elicit an opposite interaction effect and result in eQTL magnification. We assessed

247   whether eQTL interactions with free IL-6 protein levels measured in the patient serum

248   samples were consistent with those following IL-6 blockade. We observed enrichment in

249   the overlap between cytokine interactions and drug interactions (91/126 interactions in

250   consistent direction, **Fig. 4C,** p=3.2x10^{-7}, binomial test). We were concerned that free

251   IL-6 and drug exposure are not independent in this dataset, and that this concordance

252   might be in part due to the connection between free IL-6 protein levels and IL-6

253   blockade (**Supplementary Fig. 10**). To assess whether free IL-6 offers independent

10

254 interaction effects that were consistent after accounting for drug effect, we first modeled

255 free IL-6 levels to account for the presence or absence of drug, and then we assessed

256 interactions with residual IL-6 levels. Again, we observed a significant number of

257 interactions in a consistent direction with residual IL-6 levels, which are independent of

258 drug exposure (p=0.03, **Supplementary Fig. 11**).

259

260 *Drug score for assessing drug exposure*

261 We speculated that these drug-eQTL interactions could be used in a clinical

262 pharmacogenetic context to assess effective drug exposure for patients. We defined a

263 simple drug exposure score using the 126 drug-eQTL interactions (**Methods**). For each

264 RNA-seq sample, we assessed whether the expression of the interaction gene was

265 more consistent with the drug exposed or unexposed state for the corresponding

266 interaction SNP genotype. Samples more consistent with the drug-exposed state are

267 assigned a larger drug exposure score. Unsurprisingly, we found a difference in drug

268 exposure score between the unexposed and exposed samples (**Supplementary Fig.**

269 **12**) (r_s=0.79, p=6.9x10^{-81}); these differences reflect the fact that the eQTLs were

270 themselves identified by examining samples with and without drug exposure. However,

271 while we did not utilize the administered drug dose to identify drug-eQTL interactions,

272 we found a significant correlation between drug dose (10, 50 or 200mg) and drug

273 exposure score (r_s=0.16, p=0.018) in the drug-exposed samples (**Fig. 4D**).

274

275 **Discussion**

276 Here, we mapped eQTLs in a cohort of SLE patients and discovered interactions with

277 physiological (T cell abundance) and clinical (IFN status) data, but most importantly,

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278   with drug exposure. We hypothesized that the eQTL effects modulated following

279   treatment with anti-IL-6 were likely being driven by changes in the free IL-6 protein

280   levels. Remarkably, we were able to replicate 91/126 of our drug-eQTL interactions at

281   the protein level, providing additional support that the vast majority of interactions are

282   biologically relevant.

283

284   Our ability to identify eQTL interactions in humans *in vivo* stems from the use of a

285   structured study design with repeat measurements of gene expression across different

286   conditions in the same individual. By allowing the same individual to be assessed under

287   different circumstances, the noise in transcriptomic measurements caused by non-

288   genetic factors is reduced. This is apparent in our increased power to detect main eQTL

289   effects with repeat measurements. We observed 4,976 eQTL genes with repeat

290   measurements versus 3,061 with only single measures.

291

292   Observing the same individuals when they are both unexposed and exposed to an

293   environmental perturbagen further increases power to find interactions. Structured study

294   designs such as clinical trials provide this design setup and therefore offer excellent

295   opportunities to identify interactions to drug exposure, thereby potentially illuminating

296   mechanisms. This strategy may be particularly informative for research conducted in a

297   clinical setting where the size of a cohort can be limited by the ability to recruit suitable

298   patients.

299

300   eQTL interactions with drug interventions or other environmental stimuli are important

301   because they indicate mechanisms of action by highlighting key regulatory factors, such

302   as transcription factors or subclasses of enhancers. These regulatory factors act

303   downstream of the environmental condition of interest and drive groups of eQTL

304   interactions. The IFN status eQTL interactions we identified provide support for this

305   approach. By making use of the direction of effect for the eQTL interaction, we were

306   able to identify an enrichment of dampening eQTL interaction SNPs interrupting the

307   binding sites of transcription factors known to be important in the response to IFN, such

308   as IRF1.

309

310   We were intrigued to discover an enrichment of magnifying drug-eQTL interaction SNPs

311   interrupting the binding site of IRF4. It has been suggested that IRF4 works downstream

312   of IL-6 by binding BATF and coordinately regulating the production of IL10 and other

313   genes[32]. Consistent with this, we observed that the *IL10* eQTL does indeed interact with

314   presence of anti-IL-6 (**Supplementary Fig. 7**). Previous studies have highlighted a role

315   for IRF4 in the pathogenesis of autoimmune diseases in mouse and humans. For

316   example in a murine model of SLE, *IRF4* knockout mice did not develop lupus

317   nephritis[33]. In humans, IRF4 is associated with RA[34], a disease in which anti-IL-6

318   treatment has been successful[21]. Our findings provide further support that IRF4 could

319   be a potential therapeutic target for autoimmune diseases such as RA where anti-IL-6 is

320   effective[35]. As the regulatory genome continues to be mapped[36,37] and available binding

321   sites for different regulator proteins are specifically defined, these approaches to define

322   environmental mechanisms will become more potent. For example, it will become easier

323   to connect groups of interaction eQTLs being driven by specific transcription factors or a

324   subset of regulatory elements.

325

326   In addition to defining drug interactions, the ability to focus on interactions with specific

327   patient phenotypes might point to key targets for disease intervention. For example, our

328   discovery of many IFN interactions was likely enabled by the prevalence of this key

329   immunophenotype in SLE patients compared to healthy controls[26,27]. Our study largely

330   replicates the IFN interactions discovered in a much larger study of healthy controls[1].

331   The IFN status immunophenotype is now a target for therapy. A recent phase II clinical

332   trial has shown that an antagonist to the type I IFN receptor, acting upstream of IRF1,

333   reduced severity of symptoms in SLE. Interestingly, the antagonist was more effective in

334   the patients with a high baseline IFN status[38]. This example provides a compelling case

335   study for how understanding master regulators of key disease phenotypes might lead to

336   promising new therapeutic strategies. We speculate that this provides a mechanism for

337   stratified medicine for future studies, which may be applicable to other diseases.

338

339   Finally, we argue that drug-eQTL interactions can augment pharmacogenetic strategies

340   and may be informative for patient response. For many biologic medications, predictive

341   pharmacogenetics has been challenging; for example, studies to define genetic or non-

342   genetic biomarkers of anti-TNF response have not been successful[39,40]. Our drug

343   exposure score, based on a novel approach using SNP-gene pairs from drug-eQTL

344   interactions, might reflect the biological activity that a medication is having upon an

345   individual, and may be modeling an effective medication activity level. This score may

346 therefore be helpful in stratifying individuals when assessing response to a medication

347 for example those with a higher drug exposure score may have a better response to

348 treatment. We note a limitation of this study is that the drug itself did not achieve its

349 primary efficacy endpoint of improving SLE outcomes. Hence, while the drug exposure

350 score for this study tracked with the biological effect of the drug (reducing free IL-6

351 protein levels), it might not be useful for SLE specifically. However, such a scoring

352 system could be implemented easily in most phase III trials for a broad range of

353 therapeutics, where the numbers of samples are far in excess of this phase II trial,

354 ensuring better powered and more accurate eQTL-interaction mapping.

355

356 **Methods**

357 *Study design*

358 The objectives of this study were to map eQTLs in a cohort of lupus patients and

359 identify eQTL interactions with environmental perturbations such as drug treatment to

360 shed light on drug and disease mechanisms. SLE patients were recruited to a phase II

361 clinical trial to test the efficacy and safety of an IL-6 monoclonal antibody (PF-

362 04236921). The patient population recruited to this trial have been detailed extensively

363 by Wallace et al.[19] 183 patients (forming a multiethnic cohort) were randomized to

364 receive three doses of drug (10, 50 or 200mg) or placebo at three time points during the

365 trial (weeks 0, 8 and 16).

366

367 *RNA-sequencing*

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368   We collected peripheral venous blood samples in PAXgene Blood RNA tubes

369   (PreAnalytiX GmbH, BD Biosciences) for high-depth RNA-seq profiling at 0, 12, and 24

370   weeks. We extracted total RNA from blood samples using the PAXgene Blood RNA kit

371   (Qiagen) at a contract lab using a customized automation method. We assessed the

372   yield and quality of the isolated RNA using Quant-iT™ RiboGreen® RNA Assay Kit

373   (ThermoFisher Scientific) and Agilent 2100 Bioanalyzer (Agilent Technologies),

374   respectively. Following quality assessment, we processed an aliquot of 500-1000 ng of

375   each RNA with a GlobinClear-Human kit, (ThermoFisher Scientific) to remove globin

376   mRNA. We then converted RNA samples to cDNA libraries using TruSeq RNA Sample

377   Prep Kit v2 (Illumina) and sequenced using Illumina HiSeq 2000 sequencers. We

378   generated an average of 40M 100bp pair-end reads per sample for downstream

379   analysis.

380

381   We successfully obtained 468 RNA-seq profiles from 180 patients. We aligned reads to

382   the reference genome and quantified gene expression using Subread[41] and

383   featureCounts[42] respectively. We included genes with at least 10 reads (CPM>0.38) in

384   at least 32 samples (minimum number of patients with both unexposed and exposed

385   RNA-seq assays in a drug group) prior to normalization. Following quality control (QC),

386   we removed 4 samples as outliers. We then normalized 20,253 transcripts using the

387   trimmed mean of M-values method and the edgeR R package[43]. Expression levels are

388   presented as $\log_2(\text{cpm} +1)$.

389

390   *Genotyping*

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391 We genotyped 160 individuals across 964,193 variants genome-wide with the Illumina

392 HumanOmniExpressExome-8v1.2 beadchip. We removed SNPs if they deviated from

393 Hardy-Weinberg Equilibrium ($p < 1 \times 10^{-7}$), had a minor allele frequency <5%,

394 missingness >2% or a heterozygosity rate greater than 3 standard deviations from the

395 mean (PLINK[44,45]). For mapping eQTLs, we removed SNPs on the Y chromosome.

396 Following QC, we used 608,017 variants for further analysis. We removed one sample

397 with high missingness and outlying heterozygosity rate from further analysis.

398

399 *Cell counts*

400 We collected blood samples for cytometry analysis at weeks 0, 12 and 24. Samples

401 were subjected to flow cytometry for T cell immunophenotyping. We counted T cells

402 (CD3+) as a percentage of lymphocytes (CD45+). FACS data were available for 320

403 samples from 152 subjects.

404

405 *Interferon status*

406 We classified the interferon (IFN) status of each sample at each time point from the

407 expression of 11 IFN response genes (*HERC5*, *IFI27*, *IRF7*, *ISG15*, *LY6E*, *MX1*, *OAS2*,

408 *OAS3*, *RSAD2*, *USP18*, *GBP5*)[28] using TaqMan Low Density Arrays. We classified

409 samples as high or low IFN based on the first PCA score for the expression of these

410 genes. IFN status was available for 376 samples from 157 subjects.

411

412 *Drug exposure*

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413   Samples were assigned as unexposed (placebo or week 0 samples) or drug exposed

414   (week 12 and week 24 samples in the drug groups).

415

416   *Free IL-6 protein levels*

417   We determined free IL-6 protein levels from serum using a commercial sandwich ELISA

418   selected for binding only free IL-6. The assay was validated according to FDA

419   biomarker and fit-for purpose guidelines. Free IL-6 protein levels were available for 311

420   samples from 145 subjects. Since IL6 levels were highly nonparametric, we ranked

421   samples in order of IL-6 protein levels and included in the model to identify cytokine-

422   eQTL interactions.

423

424   *Statistical analysis*

425   *eQTL and interaction analysis*

426   In total, 157 patients (with 379 RNA-seq samples) had good quality gene expression

427   and genotyping data for eQTL analysis. All statistical analyses were carried out in R[46].

428

429   We defined a *cis* eQTL as the SNP within 250kb either side of the GENCODE[47]

430   transcription start site of the gene. We first applied a linear model for the first available

431   time point to identify each eQTL using the first 25 principal components of gene

432   expression and the first 5 principal components of genotyping as covariates. SNPs were

433   encoded as 0, 1 and 2. To adjust for multiple testing during eQTL discovery we used a

434   stringent Bonferroni corrected *p*-value threshold of 2.3×10^{-8} (0.05/ 2,177,889 tests).

435

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436   To map eQTLs using multiple samples for each individual, we applied a random

437   intercept linear mixed model using the first 25 principal components of gene expression

438   and the first 5 principal components of genotyping as covariates and patient as a

439   random effect:

440

441    $$E_{i,j} = \theta + \beta_{geno} \cdot g_j + (\kappa_j | i) + \sum_{l=1}^{25} \phi_l \cdot pc_{i,l} + \sum_{m=1}^{5} \gamma_m \cdot pc_{j,m}$$

442

443   Where $E_{i,j}$ is gene expression for the ith sample from the jth subject, θ is the intercept,

444   β_{geno} is the genotype effect (eQTL), $(\kappa_j | i)$ is the random effect for the ith sample from the

445   j^{th} subject, $pc_{i,l}$ is principal component l of gene expression for sample i, $pc_{j,m}$ is principal

446   component m of genotyping for subject j.

447

448   We used the most significant SNP (with $p < 2.3 \times 10^{-8}$) from the 4,976 identified eQTL

449   genes to explore eQTL interactions. For each environmental interaction analysis, we

450   further filtered these eQTLs to include only those with at least two individuals

451   homozygous for the minor allele of the SNP being tested in each of the environmental

452   factor groups. For example we required two of these individuals in each of the drug

453   exposed and drug unexposed groups. To identify eQTL interactions, we added an

454   additional covariate to the model for example drug exposure, and an interaction term

455   between this covariate and the genotype of the SNP:

456

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457
$$E_{i,j} = \theta + \beta_{geno} \cdot g_j + (\kappa_j | i) + \sum_{l=1}^{25} \phi_l \cdot pc_{i,l} + \sum_{m=1}^{5} \gamma_m \cdot pc_{j,m} +$$
$$\beta_{drug} \cdot d_i + \beta_x \cdot d_i \cdot g_j$$

458

459 Where $E_{i,j}$ is gene expression for the ith sample from the jth subject, θ is the intercept,

460 β_{geno} is the genotype effect (eQTL), $(\kappa_j | i)$ is the random effect for the ith sample from the

461 j^{th} subject, $pc_{i,l}$ is principal component l of gene expression for sample i, $pc_{j,m}$ is principal

462 component m of genotyping for subject j, β_{drug} is the drug effect (differential gene

463 expression) and β_x is the interaction effect.

464

465 We determined the significance of the interaction term with a likelihood ratio test.

466

467 To rigorously confirm the relative enrichment of eQTL interactions, we shuffled the

468 interaction covariate (for example drug exposure) 1,000 times and calculated the

469 number of significant interactions observed in each permutation. For T cell counts and

470 IFN high/low status, we shuffled across all samples. For drug interaction permutation

471 analysis, we maintained the number of individuals in the drug group and the number of

472 samples with exposure to drug.

473

474 *Concordance with an eQTL study in healthy individuals*

475 In the SLE cohort, we classified 4,976 *cis* eQTL genes ($p<2.3 \times 10^{-8}$). The *z*-score for the

476 most associated SNP for each of these genes was compared to the *z*- score from a

477 previously published eQTL dataset from whole blood from 2,166 healthy individuals[1].

478 4,250/4976 SNP-gene pairs (85.4%) were also reported in the BIOS dataset

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479   (FDR<0.05). After removing 60 SNPs, which could not be mapped to a strand

480   4,154/4,190 (99.1%) had a *z*-score (eQTL effect) in a consistent direction.

481

482   *Magnifiers and Dampeners*

483   An eQTL interaction can either magnify or dampen the original eQTL effect. We

484   multiplied the interaction *z*-score by the sign of the original eQTL effect (genotype beta)

485   and defined magnifiers as interactions with an adjusted *z*-score > 0 and dampeners as

486   interactions with an adjusted *z*-score < 0.

487

488   *Differential gene expression analysis*

489   To identify differentially expressed genes following drug exposure, we applied a random

490   intercept linear mixed model using the first 25 principal components of gene expression

491   and the first 5 principal components of genotyping as covariates and patient as a

492   random effect.

493

494   *Conditional analysis for IL-6 protein levels*

495   We modeled the relationship between free IL-6 protein levels and drug exposure using

496   a linear model. We used the residuals from this model in our interaction linear mixed

497   model to identify IL-6 protein interactions independent of drug exposure.

498

499   *Drug exposure score*

500   We used linear discriminant analysis to assign a drug exposure score for each sample.

501   A score was calculated for each gene (see equation below) and then the final drug

502   exposure score is the average across the 126 drug-eQTL genes.

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$$Drug\ exposure\ score\ for\ gene\ =\ \frac{1}{2}(\frac{G\ -\ G_{Unexp}}{SE})^2\ -\ \frac{1}{2}(\frac{G\ -\ G_{Exp}}{SE})^2$$

504

505 Where G is gene expression for a given sample, G_{Unexp} is predicted mean gene

506 expression for unexposed samples of the relevant SNP genotype, G_{Exp} is predicted

507 mean gene expression for exposed samples of the relevant SNP genotype and SE is

508 standard error for the intercept term of the model (unexposed expression for genotype

509 0).

510

511 *HOMER analysis for transcription factor binding motif enrichment*

512 We used the HOMER software suite[29] to look for enrichment of transcription factor

513 binding motifs in the 185 IFN-eQTL interactions (p<0.01) and the 126 drug-eQTL

514 interactions (p<0.01). Each eQTL interaction was identified using the most highly

515 associated SNP for that eQTL. However, as this SNP is not necessarily the functional

516 SNP, we additionally considered all those with an $r^2 \geq 0.8$ in the 1000 Genomes

517 European population[48] within 250kB of the transcription start site of the gene. We

518 defined our motif search window as 20 bp on either side of each SNP (i.e. 41 bp wide).

519

520 For each environmental factor, we divided the eQTL interactions into magnifiers or

521 dampeners and conducted two separate HOMER analyses: one with magnifiers in the

522 foreground and dampeners in the background; the other with dampeners in the

523 foreground and magnifiers in the background. HOMER reported the transcription factor

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524   motifs that were significantly enriched in the foreground relative to background. Motifs

525   were plotted using the SeqLogo R library[49].

526

527   We determined permutation p values for enrichment of the IRF1 and IRF4 transcription

528   factor binding sites as follows. For IRF1, the motif is interrupted by interaction SNPs (or

529   SNPs in LD) corresponding to 11 dampening genes and 2 magnifying genes. We

530   permuted which genes were labeled as magnifiers or dampeners 10,000 times and

531   counted the number of genes in each category with an IRF1 motif interrupted. We found

532   478 occurrences from 10,000 trials with at least 11 dampening genes ($p<0.0479$). For

533   IRF4 the motif is interrupted by SNPs corresponding to 16 magnifying genes and 6

534   dampening genes. Using the same permutation approach, we found 133 occurrences

535   from 10,000 trials with at least 16 magnifying genes ($p<0.0134$).

536

537

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654
655

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662

663 **Author Contributions**

664 The project was conceived and designed by EED, MSV, BZ and SR. Statistical analysis

665 was conducted by EED, TA, MG-A, KS and H-JW. Molecular data was obtained and

666 organized by YZ, SP, DvS, JSB, NB, MSV and BZ. The initial manuscript was written by

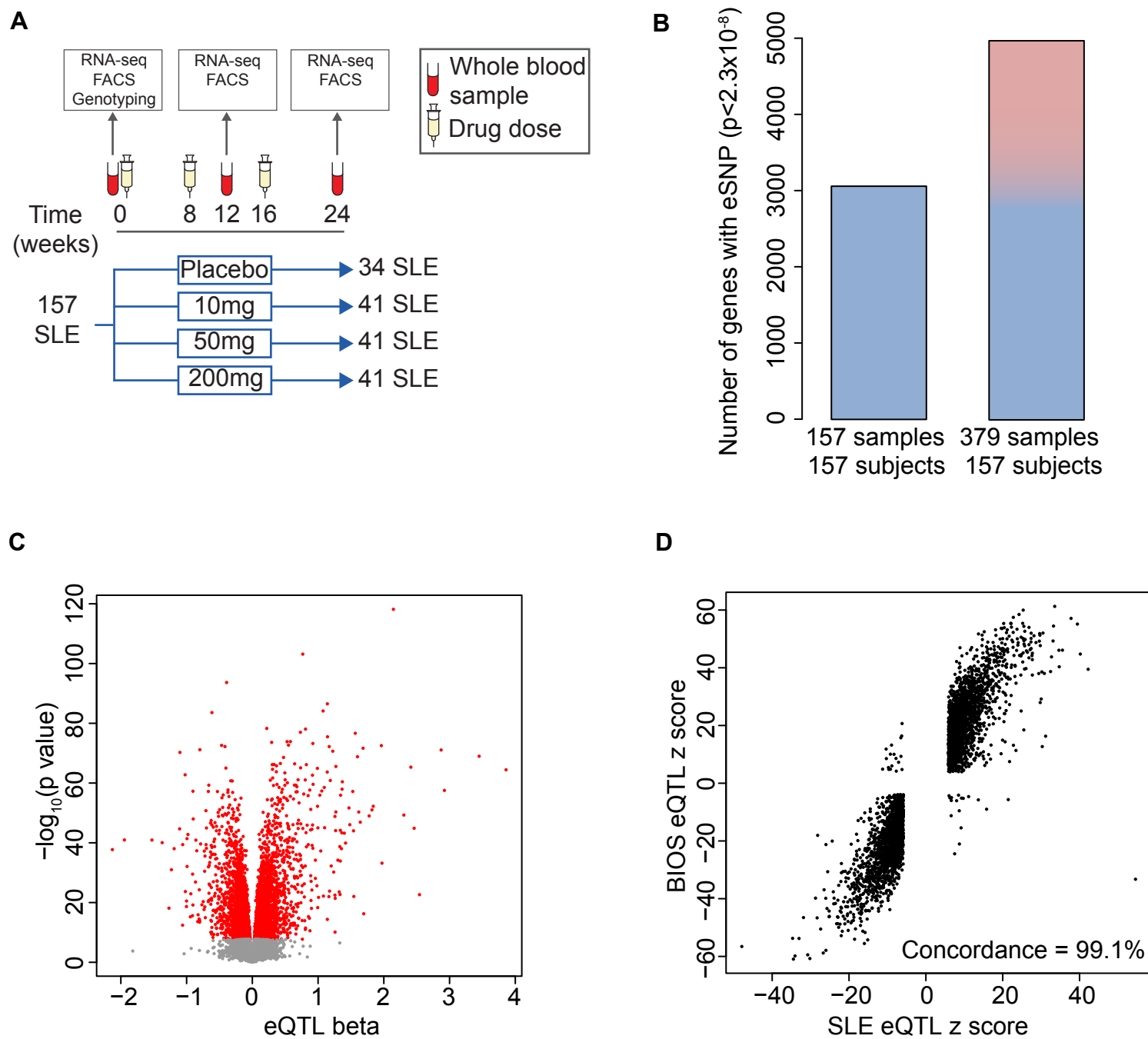667 EED and SR. All authors edited and approved the manuscript.

668

Figure 1

Figure 1. Identifying eQTLs in SLE patients (**A**) Clinical trial structure and sampling strategy. (**B**) Number of eQTL genes identified using a linear model (left) and a linear mixed model (right). (**C**) Volcano plot of eQTL effects for the most significantly associated SNP for each gene (red color indicates $p<2.3\times10^{-8}$). (**D**) Concordance of SLE eQTL effects ($p<2.3\times10^{-8}$) with eQTLs observed in the BIOS cohort[1] of healthy individuals (FDR <0.05). Each point represents the most significant SNP-gene pair for the SLE eQTL.
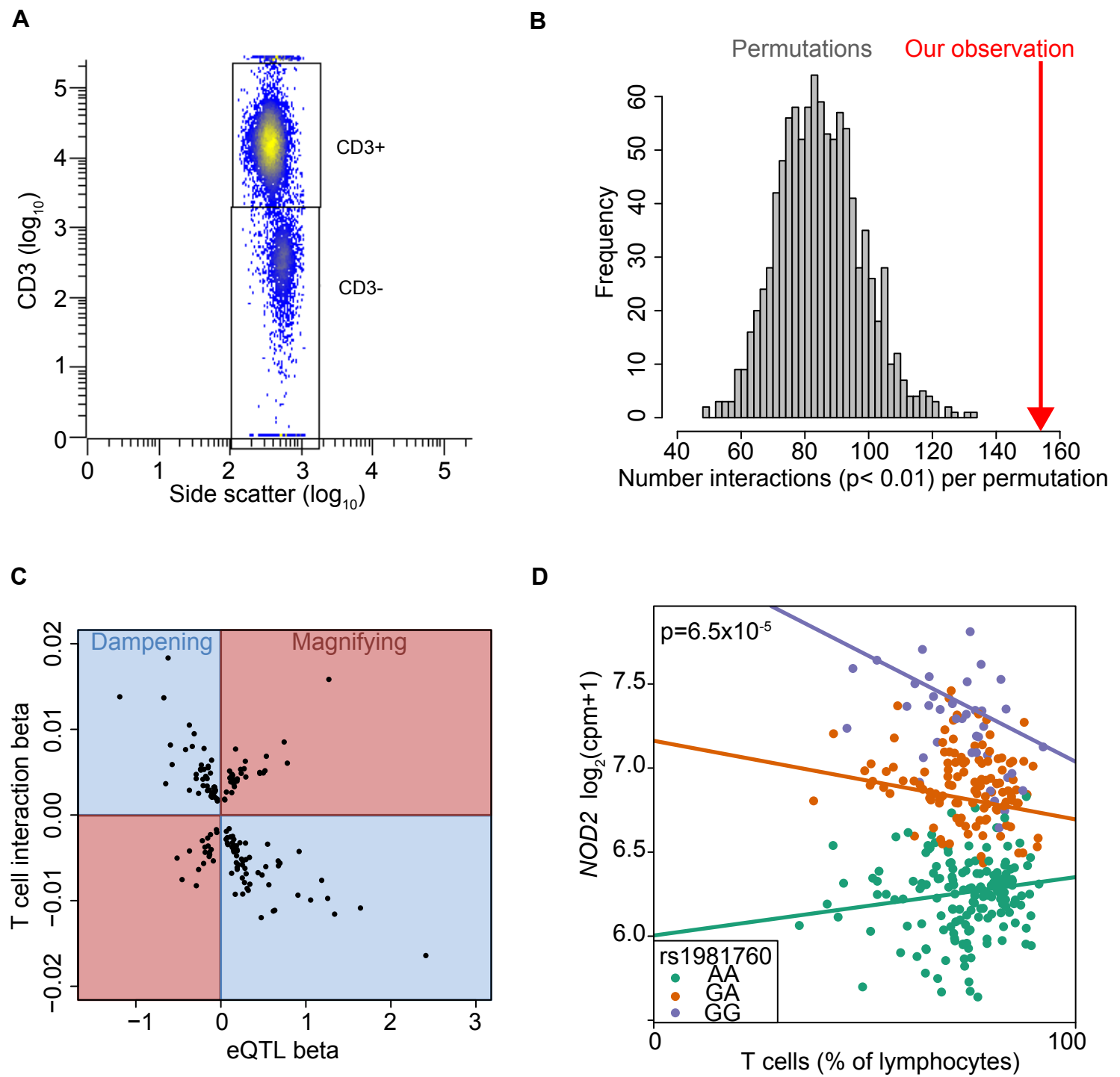
Figure 2

Figure 2. eQTL interactions with T cell percentages (**A**) Flow cytometry gating strategy to determine T cells as a percentage of lymphocytes. (**B**) Number of significant interactions (p<0.01) from 1,000 permutations of T cell counts (median=85). (**C**) Effect of the T cell interaction on the original eQTL effect. (**D**) T cell interaction with the *NOD2* eQTL. The eQTL effect is dampened as T cell percentage increases.
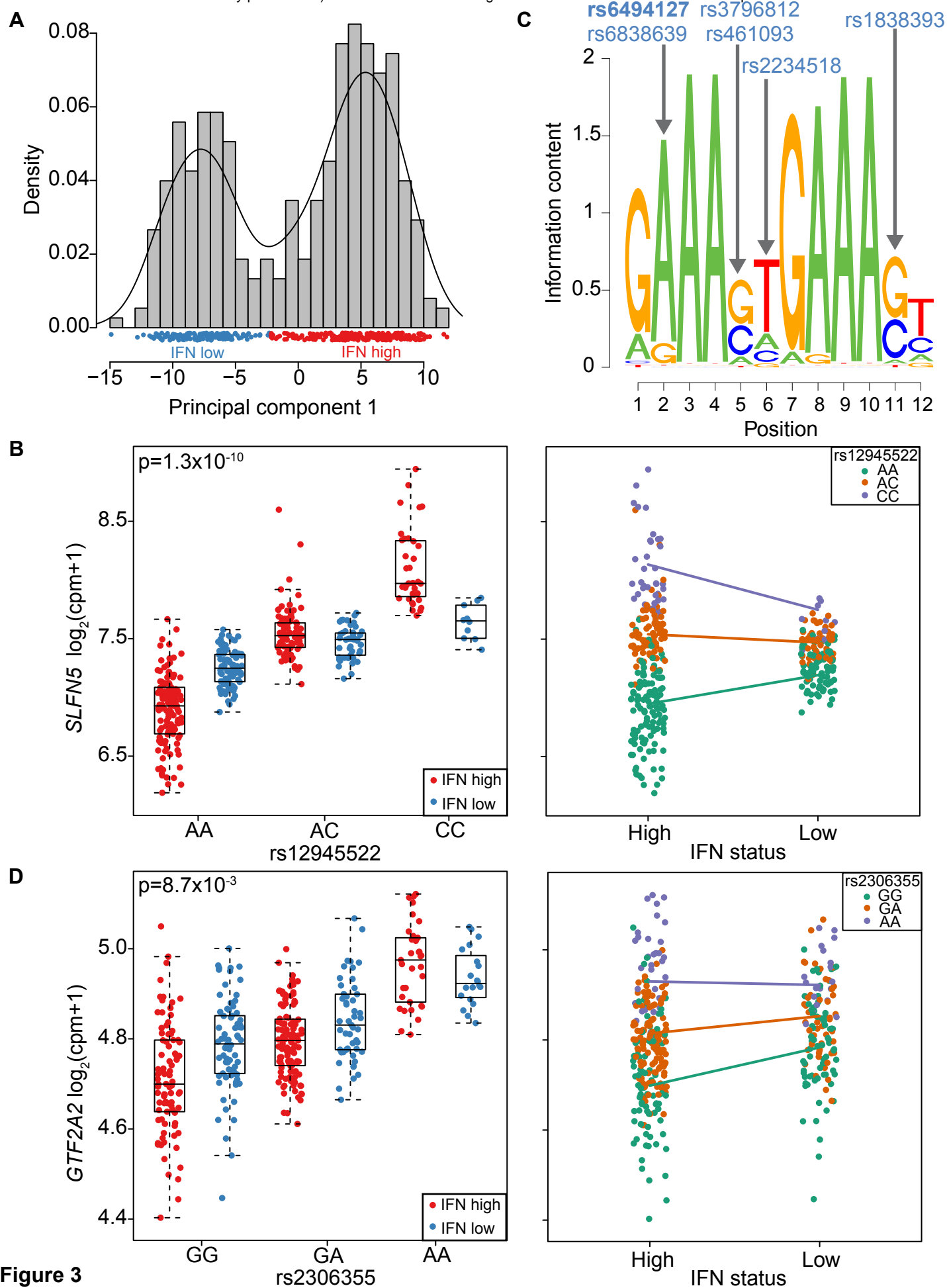
Figure 3

Figure 3. eQTL interactions with IFN status (**A**) Designation of IFN status for each sample from the real-time PCR expression of 11 genes (first principal component). (**B**) IFN status interaction with the *SLFN5* eQTL plotted with respect to rs12945522 genotype (left) and IFN status of the sample (right). (**C**) The IRF1 motif enriched among eQTLs dampened in IFN low samples. Arrows indicate positions of the motif interrupted by interaction SNPs (or SNPs in strong LD). Blue indicates these SNPs correspond to dampened eQTLs. (**D**) IFN status interaction with the *GTF2A2* eQTL plotted with respect to rs2306355 genotype (left) and IFN status of the sample (right).
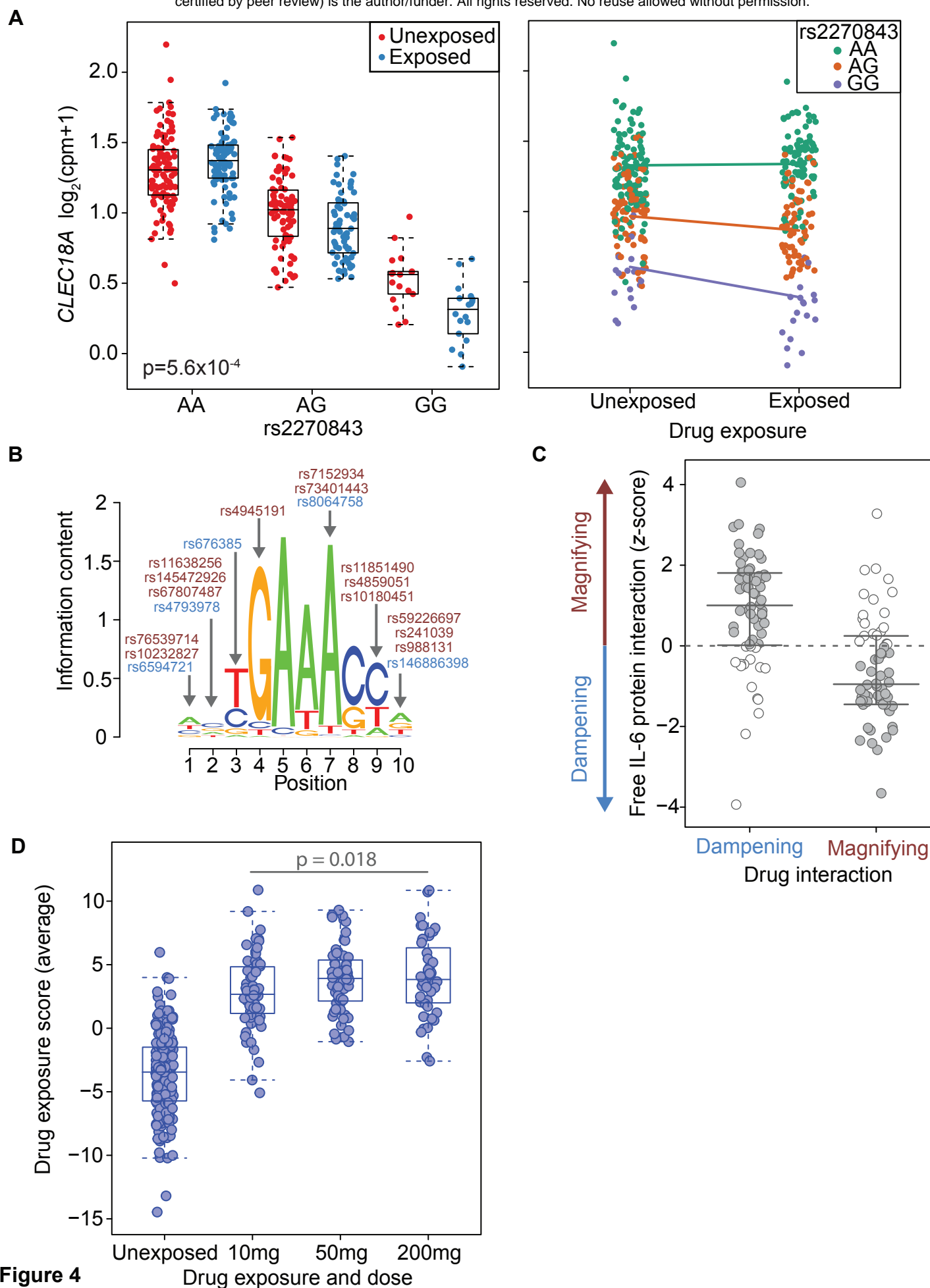
Figure 4

Figure 4. eQTL interactions with drug exposure (**A**) Drug exposure interaction with the *CLEC18A* eQTL plotted with respect to rs2270843 genotype (left) and drug exposure (right). (**B**) The IRF4 motif enriched among eQTLs magnified following drug treatment. Arrows indicate positions of the motif interrupted by interaction SNPs (or SNPs in strong LD). Red and blue indicate SNPs corresponding to magnified and dampened eQTLs respectively. (**C**) Concordance of free IL-6 protein interaction effects with drug exposure interaction effects (grey indicates consistent direction). (**D**) Drug exposure score calculated from 126 drug-eQTL interactions.