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

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Abstract:

If an expression quantitative trait locus (eQTL) effect is modulated by an environmental stimulus, such as drug exposure or disease status, it can point to key regulatory mediators. In a clinical trial for anti-IL-6 in 157 patients with systemic lupus erythematosus we measured cell counts, interferon (IFN) status, drug exposure and genome-wide gene expression at three time points. First, we confirmed an increase in power using repeat transcriptomic measurements. Then, after detecting 4,976 *cis* eQTLs, we discovered that 154, 185 and 126 had evidence of significant eQTL interactions with T cell proportion, IFN status and anti-IL-6 drug exposure respectively. Next, we found an enrichment of transcription factor binding motifs interrupted by eQTL interaction SNPs, pointing to regulatory mediators of these environmental stimuli and therefore potential therapeutic targets for autoimmune diseases. In particular, IFN interactions are enriched for IRF1 binding site motifs, while anti-IL-6 interactions are enriched for IRF4 motifs. Finally, we used the drug-eQTL interactions to define an informative drug exposure score, reflecting a drug's effect in an individual patient, thus highlighting the potential for utilizing drug-eQTL interactions in a pharmacogenetic framework.

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].

Here, we predicted that if RNA is queried at multiple time points under different exposure states, repeat measurements could increase power to not only detect eQTLs, but also their interactions with environment.

Clinical trials, with their structured study design, may be the ideal setting to detect eQTL interactions with drugs or other environmental perturbations. In clinical trials, it is becoming increasingly common to collect transcriptional and genetic data alongside clinical and physiological data. This extensive phenotyping of the same individuals at multiple time points across conditions can be leveraged to identify eQTL interactions. The profiling of an individual both before and after exposure to a drug provides a unique opportunity to identify *in vivo* drug-eQTL interactions.

As a proof of principle, we examined the modulation of eQTL effects by environmental factors, including cell count, interferon (IFN) status and drug exposure, using data from a phase II clinical trial to evaluate the safety and efficacy of a neutralizing IL-6 monoclonal antibody (PF-04236921) in 157 systemic lupus erythematosus (SLE) patients[19] (**Methods**). IL-6 is a cytokine elevated in SLE and other autoimmune diseases such as rheumatoid arthritis (RA). In SLE, IL-6 is thought to play a role in the observed B cell hyperactivity and autoantibody production[20]. The IL-6 receptor has been successfully targeted with tocilizumab in RA[21] and has shown promise in a phase I trial for SLE[22]. IL-6 itself has been targeted with siltuximab for the treatment of Castleman's disease[23] and this has led to the development of other biologics targeting IL-6, such as PF-04236921. While this drug was not significantly different from placebo for the

primary efficacy endpoint (proportion of patients achieving the SLE Responder Index (SRI-4) at week 24), biologically it effectively reduced free IL-6 protein levels. Given the key role of IL-6 in a range of diseases, IL-6 blockade and mechanism of action are of great interest to study.

In this study, we leverage the power of repeat transcriptional and environmental measurements from a lupus clinical trial to identify eQTL interactions. We discover eQTL interactions with cell count, IFN status and drug exposure. We find the eQTL interaction SNPs are enriched for transcription factor binding motifs (such as IRF1 and IRF4 for IFN and drug exposure respectively) highlighting regulatory mediators of these interactions and potential therapeutic targets.

Results

We conducted whole blood high-depth RNA-seq profiling at 0, 12, and 24 weeks in anti-IL-6 exposed and unexposed individuals with the Illumina TruSeq protocol. We observed and quantified 20,253 gene features and genotyped 608,017 variants genome-wide (**Methods**). Along with each RNA-seq assay, we documented drug exposure and quantified cell counts with FACS and IFN signature status with real-time PCR.

Mapping eQTL in SLE patients

We first mapped *cis* eQTLs (SNPs within 250kb of the transcription start site of the gene) and then tested those eQTLs for interactions with cell counts, IFN status and drug

exposure. eQTL interactions can be explored using our interactive visualization tool (http://baohongz.github.io/Lupus_eQTL).

We used a linear mixed model, including repeat measurements with up to three RNA-seq assays per patient (**Fig. 1A,** 379 samples from 157 patients, **Methods**). To maximize power, we adjusted for 5 population and 25 gene expression principal components (**Methods**). To ensure a set of highly confident eQTLs, we applied a stringent multiple hypothesis testing correction and identified 4,976 *cis* eQTL genes with $p_{eqtl}<2.3x10^{-8}$ (0.05/2,177,889 tests, **Fig. 1B,1C, Supplementary Table 1**).

Repeat measurements increase power to detect eQTL

We observed that repeat samples increased our power by detecting 63% more *cis* eQTLs compared to using a single sample per individual (3,061 genes **Fig. 1B**). Our results are highly concordant with the BIOS cohort, a much larger dataset of 2,166 healthy individuals[1]. 85.4% of our SLE eQTL SNP-gene pairs are reported as eQTLs in BIOS (FDR<0.05). Of these, 99.1% showed consistent direction of effect ($p<5x10^{-16}$, binomial test, **Fig. 1D**). For each of the 4,976 *cis* eQTL genes, we tested the most significantly associated SNP for environmental interactions.

eQTL interactions with T cell proportions

We first tested a type of eQTL interaction that has been examined previously: cell counts[24,25]. We obtained FACS data for 320 samples for which we had contemporaneous RNA-seq profiles (n=152 subjects). We determined the percentage of total lymphocytes that were T cells by gating (**Fig. 2A**). We found 154 T cell-eQTL

interactions with nominal evidence ($p_{interact}$<0.01, **Supplementary Table 1**), whereas from 4,976 tests we would expect ~50 from chance alone. To ensure that our statistics were not inflated, we conducted 1,000 stringent permutations, where we reassigned T cell percentages across samples and retested. This permutation preserved the main eQTL effect, while disrupting interactions that might be present in the data. In no instance did we observe 154 or more interactions at $p_{interact}$<0.01 (maximum=133), suggesting that the number of observed interactions is highly unlikely to have happened by chance (**Fig. 2B**, $p_{permute}$~0/1,000 = <0.001).

Interactions can be divided into magnifiers, where environmental exposure and eQTL effects are in the same direction, and dampeners where the effects work in the opposite direction (**Fig. 2C, Supplementary Fig. 1**). The *NOD2* rs1981760 eQTL is an example of an interaction that is dampened by increased T cell count (**Fig. 2D**, $p_{interact}=6.5x10^{-5}$), and has separately been shown to vary across cell types[24,25]. For each 10% increase in T cell proportion, the eQTL effect is reduced by about 7%.

IFN status eQTL interactions

Many patients with SLE exhibit high levels of genes induced by IFN alpha; these genes, known as the IFN signature, are a marker of disease severity[26,27] and a pathogenic feature of SLE. We explored the influence of IFN alpha on gene regulation after determining the IFN status of every patient at each time point using real-time PCR of 11 IFN-inducible genes[28] (**Methods, Fig. 3A**). We identified 185 IFN-eQTL interactions with $p_{interact}$<0.01 (**Supplementary Table 1**). Following the same permutation procedures as above, our observed interactions were unlikely to have occurred by

chance (maximum permutation interactions=112, $p_{permute}$<0.001) (**Supplementary Fig. 2**). We note that interactions with a proxy gene for IFN status have been described[1] and we find overlap of genes with those reported interactions (**Supplementary Fig. 3**). For example, *SLFN5* expression is influenced by the rs12945522 SNP ($p_{interact}$ =1.3x10^{-10}, **Fig. 3B**). This effect is dampened in IFN low samples.

To define transcription factors that drive the response to IFN alpha, we sought to identify motifs that explain the differences between magnifier (n=75) and dampener (n=110) eQTLs (**Supplementary Fig. 4**). We applied HOMER[29] to assess overlap between transcription factor binding motifs and the eQTL interaction SNPs (and SNPs in high linkage disequilibrium (LD, r^2>0.8) in the *cis* window) (**Methods**). We found significant enrichment of motifs for key transcription factors involved in IFN signaling including the IRF1 motif. IRF1 motif disruption occurred for 11 genes with an eQTL dampened in IFN low samples but for only 2 genes with an eQTL magnified (HOMER p=0.001, permutation p<0.048, **Methods, Fig. 3C**, **Supplementary Table 2**). An example is the *GTF2A2* rs2306355 eQTL ($p_{interact}$=8.7x10^{-3}, **Fig. 3D**); rs2306355 is in tight LD (r^2=0.85 in Europeans) with rs6494127, which interrupts the GAAA core of the IRF1 motif (**Fig. 3C**), and likely disrupts IRF1 binding[30]. We observe greater expression of *GTF2A2* in individuals with the rs2306355 A allele compared to G; this difference is dampened in IFN low individuals (**Fig. 3D**).

Discovery of eQTL interactions with drug exposure

We then examined whether IL-6 blockade alters the relationship between genomic variation and gene expression and induces drug-eQTL interactions. We observed 126

drug-eQTL interactions with $p_{interact}<0.01$ (**Supplementary Table 1**). Following the same permutation strategy as above, we found a median of 77 interactions with $p_{interact}<0.01$ (maximum=117) from 1,000 permutations. This suggests that about half of our drug-eQTL interactions likely represent real biological phenomena, and not statistical artifact (**Supplementary Fig. 5**). These drug-eQTL interactions showed little overlap with the interactions observed for T cell count or IFN status (**Supplementary Fig. 6**). We note biologically relevant drug-eQTL interactions for *IL10* (**Supplementary Fig. 7**), an anti-inflammatory cytokine, *CLEC4C* which has previously been associated in *trans* with an SLE risk allele[31] and *CLEC18A* (**Fig. 4A**) another member of the C-type lectin domain family.

Again, the drug-eQTL interactions can be divided into magnifiers and dampeners (**Supplementary Fig. 8**) and a similar approach as described above can be applied to define transcription factors driving the response to IL-6 blockade. The most enriched motif for eQTLs magnified after drug treatment was IRF4. Strikingly, the IRF4 motif disruption occurred for 16 genes, including *CLEC18A*, with an eQTL magnified after drug treatment compared to 6 genes with an eQTL dampened ($p=1x10^{-5}$, permutation $p<0.01$, **Fig. 4B**, **Methods**, **Supplementary Table 3**).

Searching for transcription factor binding motifs in DNA regions of interest may not be the only way of identifying downstream mediators of IL-6. From chromatin immunoprecipitation sequencing (ChIP-seq), our drug-eQTL interaction SNP (rs2270843) for *CLEC18A* lies in a MAFK binding site. SNPs in high LD ($r^2>0.8$) in the

cis window also lie in JUND, CTCF, RAD21, SMC3 and ZNF143 binding sites. Hence one or more of these factors could also be acting to translate changes in serum IL-6 levels to regulation of *CLEC18A* gene expression.

A more common strategy to determine the effect of a perturbagen is to use differential gene expression. For differential expression following drug treatment, we identified 1,161 genes with nominal statistical evidence ($p<0.01$) but modest effects (max fold change=1.3, **Supplementary Fig. 9**). Only 8/126 drug-eQTL interaction genes also show evidence of differential gene expression. This suggests that eQTL interactions offer independent information from differential expression, which might contribute to defining mechanisms.

Concordance of drug-eQTL interactions with protein level interactions

To validate these interactions, we hypothesized that interactions due to drug exposure are likely driven by free IL-6 cytokine levels (our key clinical biomarker of interest). If this is the case, for eQTLs dampened by drug exposure, an increase in free IL-6 should elicit an opposite interaction effect and result in eQTL magnification. We assessed whether eQTL interactions with free IL-6 protein levels measured in the patient serum samples were consistent with those following IL-6 blockade. We observed enrichment in the overlap between cytokine interactions and drug interactions (91/126 interactions in consistent direction, **Fig. 4C,** $p=3.2\times10^{-7}$, binomial test). We were concerned that free IL-6 and drug exposure are not independent in this dataset, and that this concordance might be in part due to the connection between free IL-6 protein levels and IL-6 blockade (**Supplementary Fig. 10**). To assess whether free IL-6 offers independent

interaction effects that were consistent after accounting for drug effect, we first modeled free IL-6 levels to account for the presence or absence of drug, and then we assessed interactions with residual IL-6 levels. Again, we observed a significant number of interactions in a consistent direction with residual IL-6 levels, which are independent of drug exposure (p=0.03, **Supplementary Fig. 11**).

Drug score for assessing drug exposure

We speculated that these drug-eQTL interactions could be used in a clinical pharmacogenetic context to assess effective drug exposure for patients. We defined a simple drug exposure score using the 126 drug-eQTL interactions (**Methods**). For each RNA-seq sample, we assessed whether the expression of the interaction gene was more consistent with the drug exposed or unexposed state for the corresponding interaction SNP genotype. Samples more consistent with the drug-exposed state are assigned a larger drug exposure score. Unsurprisingly, we found a difference in drug exposure score between the unexposed and exposed samples (**Supplementary Fig. 12**) (r_s=0.79, p=6.9x10^{-81}); these differences reflect the fact that the eQTLs were themselves identified by examining samples with and without drug exposure. However, while we did not utilize the administered drug dose to identify drug-eQTL interactions, we found a significant correlation between drug dose (10, 50 or 200mg) and drug exposure score (r_s=0.16, p=0.018) in the drug-exposed samples (**Fig. 4D**).

Discussion

Here, we mapped eQTLs in a cohort of SLE patients and discovered interactions with physiological (T cell abundance) and clinical (IFN status) data, but most importantly,

with drug exposure. We hypothesized that the eQTL effects modulated following treatment with anti-IL-6 were likely being driven by changes in the free IL-6 protein levels. Remarkably, we were able to replicate 91/126 of our drug-eQTL interactions at the protein level, providing additional support that the vast majority of interactions are biologically relevant.

Our ability to identify eQTL interactions in humans *in vivo* stems from the use of a structured study design with repeat measurements of gene expression across different conditions in the same individual. By allowing the same individual to be assessed under different circumstances, the noise in transcriptomic measurements caused by non-genetic factors is reduced. This is apparent in our increased power to detect main eQTL effects with repeat measurements. We observed 4,976 eQTL genes with repeat measurements versus 3,061 with only single measures.

Observing the same individuals when they are both unexposed and exposed to an environmental perturbagen further increases power to find interactions. Structured study designs such as clinical trials provide this design setup and therefore offer excellent opportunities to identify interactions to drug exposure, thereby potentially illuminating mechanisms. This strategy may be particularly informative for research conducted in a clinical setting where the size of a cohort can be limited by the ability to recruit suitable patients.

eQTL interactions with drug interventions or other environmental stimuli are important because they indicate mechanisms of action by highlighting key regulatory factors, such as transcription factors or subclasses of enhancers. These regulatory factors act downstream of the environmental condition of interest and drive groups of eQTL interactions. The IFN status eQTL interactions we identified provide support for this approach. By making use of the direction of effect for the eQTL interaction, we were able to identify an enrichment of dampening eQTL interaction SNPs interrupting the binding sites of transcription factors known to be important in the response to IFN, such as IRF1.

We were intrigued to discover an enrichment of magnifying drug-eQTL interaction SNPs interrupting the binding site of IRF4. It has been suggested that IRF4 works downstream of IL-6 by binding BATF and coordinately regulating the production of IL10 and other genes[32]. Consistent with this, we observed that the *IL10* eQTL does indeed interact with presence of anti-IL-6 (**Supplementary Fig. 7**). Previous studies have highlighted a role for IRF4 in the pathogenesis of autoimmune diseases in mouse and humans. For example in a murine model of SLE, *IRF4* knockout mice did not develop lupus nephritis[33]. In humans, IRF4 is associated with RA[34], a disease in which anti-IL-6 treatment has been successful[21]. Our findings provide further support that IRF4 could be a potential therapeutic target for autoimmune diseases such as RA where anti-IL-6 is effective[35]. As the regulatory genome continues to be mapped[36,37] and available binding sites for different regulator proteins are specifically defined, these approaches to define environmental mechanisms will become more potent. For example, it will become easier

to connect groups of interaction eQTLs being driven by specific transcription factors or a subset of regulatory elements.

In addition to defining drug interactions, the ability to focus on interactions with specific patient phenotypes might point to key targets for disease intervention. For example, our discovery of many IFN interactions was likely enabled by the prevalence of this key immunophenotype in SLE patients compared to healthy controls[26,27]. Our study largely replicates the IFN interactions discovered in a much larger study of healthy controls[1]. The IFN status immunophenotype is now a target for therapy. A recent phase II clinical trial has shown that an antagonist to the type I IFN receptor, acting upstream of IRF1, reduced severity of symptoms in SLE. Interestingly, the antagonist was more effective in the patients with a high baseline IFN status[38]. This example provides a compelling case study for how understanding master regulators of key disease phenotypes might lead to promising new therapeutic strategies. We speculate that this provides a mechanism for stratified medicine for future studies, which may be applicable to other diseases.

Finally, we argue that drug-eQTL interactions can augment pharmacogenetic strategies and may be informative for patient response. For many biologic medications, predictive pharmacogenetics has been challenging; for example, studies to define genetic or non-genetic biomarkers of anti-TNF response have not been successful[39,40]. Our drug exposure score, based on a novel approach using SNP-gene pairs from drug-eQTL interactions, might reflect the biological activity that a medication is having upon an 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*

We collected peripheral venous blood samples in PAXgene Blood RNA tubes (PreAnalytiX GmbH, BD Biosciences) for high-depth RNA-seq profiling at 0, 12, and 24 weeks. We extracted total RNA from blood samples using the PAXgene Blood RNA kit (Qiagen) at a contract lab using a customized automation method. We assessed the yield and quality of the isolated RNA using Quant-iT™ RiboGreen® RNA Assay Kit (ThermoFisher Scientific) and Agilent 2100 Bioanalyzer (Agilent Technologies), respectively. Following quality assessment, we processed an aliquot of 500-1000 ng of each RNA with a GlobinClear-Human kit, (ThermoFisher Scientific) to remove globin mRNA. We then converted RNA samples to cDNA libraries using TruSeq RNA Sample Prep Kit v2 (Illumina) and sequenced using Illumina HiSeq 2000 sequencers. We generated an average of 40M 100bp pair-end reads per sample for downstream analysis.

We successfully obtained 468 RNA-seq profiles from 180 patients. We aligned reads to the reference genome and quantified gene expression using Subread[41] and featureCounts[42] respectively. We included genes with at least 10 reads (CPM>0.38) in at least 32 samples (minimum number of patients with both unexposed and exposed RNA-seq assays in a drug group) prior to normalization. Following quality control (QC), we removed 4 samples as outliers. We then normalized 20,253 transcripts using the trimmed mean of M-values method and the edgeR R package[43]. Expression levels are presented as $\log_2$(cpm +1).

Genotyping

We genotyped 160 individuals across 964,193 variants genome-wide with the Illumina HumanOmniExpressExome-8v1.2 beadchip. We removed SNPs if they deviated from Hardy-Weinberg Equilibrium ($p < 1x10^{-7}$), had a minor allele frequency <5%, missingness >2% or a heterozygosity rate greater than 3 standard deviations from the mean (PLINK[44,45]). For mapping eQTLs, we removed SNPs on the Y chromosome. Following QC, we used 608,017 variants for further analysis. We removed one sample with high missingness and outlying heterozygosity rate from further analysis.

Cell counts

We collected blood samples for cytometry analysis at weeks 0, 12 and 24. Samples were subjected to flow cytometry for T cell immunophenotyping. We counted T cells (CD3+) as a percentage of lymphocytes (CD45+). FACS data were available for 320 samples from 152 subjects.

Interferon status

We classified the interferon (IFN) status of each sample at each time point from the expression of 11 IFN response genes (*HERC5*, *IFI27*, *IRF7*, *ISG15*, *LY6E*, *MX1*, *OAS2*, *OAS3*, *RSAD2*, *USP18*, *GBP5*)[28] using TaqMan Low Density Arrays. We classified samples as high or low IFN based on the first PCA score for the expression of these genes. IFN status was available for 376 samples from 157 subjects.

Drug exposure

Samples were assigned as unexposed (placebo or week 0 samples) or drug exposed (week 12 and week 24 samples in the drug groups).

Free IL-6 protein levels

We determined free IL-6 protein levels from serum using a commercial sandwich ELISA selected for binding only free IL-6. The assay was validated according to FDA biomarker and fit-for purpose guidelines. Free IL-6 protein levels were available for 311 samples from 145 subjects. Since IL6 levels were highly nonparametric, we ranked samples in order of IL-6 protein levels and included in the model to identify cytokine-eQTL interactions.

Statistical analysis

eQTL and interaction analysis

In total, 157 patients (with 379 RNA-seq samples) had good quality gene expression and genotyping data for eQTL analysis. All statistical analyses were carried out in R[46].

We defined a *cis* eQTL as the SNP within 250kb either side of the GENCODE[47] transcription start site of the gene. We first applied a linear model for the first available time point to identify each eQTL using the first 25 principal components of gene expression and the first 5 principal components of genotyping as covariates. SNPs were encoded as 0, 1 and 2. To adjust for multiple testing during eQTL discovery we used a stringent Bonferroni corrected *p*-value threshold of 2.3×10^{-8} (0.05/ 2,177,889 tests).

To map eQTLs using multiple samples for each individual, we applied a random intercept linear mixed model using the first 25 principal components of gene expression and the first 5 principal components of genotyping as covariates and patient as a random effect:

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

Where $E_{i,j}$ is gene expression for the i^{th} sample from the j^{th} subject, θ is the intercept, β_{geno} is the genotype effect (eQTL), $(\kappa_j|i)$ is the random effect for the i^{th} sample from the j^{th} subject, $pc_{i,l}$ is principal component l of gene expression for sample i, $pc_{j,m}$ is principal component m of genotyping for subject j.

We used the most significant SNP (with $p<2.3 \times 10^{-8}$) from the 4,976 identified eQTL genes to explore eQTL interactions. For each environmental interaction analysis, we further filtered these eQTLs to include only those with at least two individuals homozygous for the minor allele of the SNP being tested in each of the environmental factor groups. For example we required two of these individuals in each of the drug exposed and drug unexposed groups. To identify eQTL interactions, we added an additional covariate to the model for example drug exposure, and an interaction term between this covariate and the genotype of the SNP:

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 i^{th} sample from the j^{th} 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 jth 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 ($p<2.3x10^{-8}$). 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

(FDR<0.05). After removing 60 SNPs, which could not be mapped to a strand 4,154/4,190 (99.1%) had a *z*-score (eQTL effect) in a consistent direction.

Magnifiers and Dampeners

An eQTL interaction can either magnify or dampen the original eQTL effect. We multiplied the interaction *z*-score by the sign of the original eQTL effect (genotype beta) and defined magnifiers as interactions with an adjusted *z*-score > 0 and dampeners as interactions with an adjusted *z*-score < 0.

Differential gene expression analysis

To identify differentially expressed genes following drug exposure, we applied a random intercept linear mixed model using the first 25 principal components of gene expression and the first 5 principal components of genotyping as covariates and patient as a random effect.

Conditional analysis for IL-6 protein levels

We modeled the relationship between free IL-6 protein levels and drug exposure using a linear model. We used the residuals from this model in our interaction linear mixed model to identify IL-6 protein interactions independent of drug exposure.

Drug exposure score

We used linear discriminant analysis to assign a drug exposure score for each sample. A score was calculated for each gene (see equation below) and then the final drug exposure score is the average across the 126 drug-eQTL genes.

503

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

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

motifs that were significantly enriched in the foreground relative to background. Motifs were plotted using the SeqLogo R library[49].

We determined permutation p values for enrichment of the IRF1 and IRF4 transcription factor binding sites as follows. For IRF1, the motif is interrupted by interaction SNPs (or SNPs in LD) corresponding to 11 dampening genes and 2 magnifying genes. We permuted which genes were labeled as magnifiers or dampeners 10,000 times and counted the number of genes in each category with an IRF1 motif interrupted. We found 478 occurrences from 10,000 trials with at least 11 dampening genes ($p<0.0479$). For IRF4 the motif is interrupted by SNPs corresponding to 16 magnifying genes and 6 dampening genes. Using the same permutation approach, we found 133 occurrences from 10,000 trials with at least 16 magnifying genes ($p<0.0134$).

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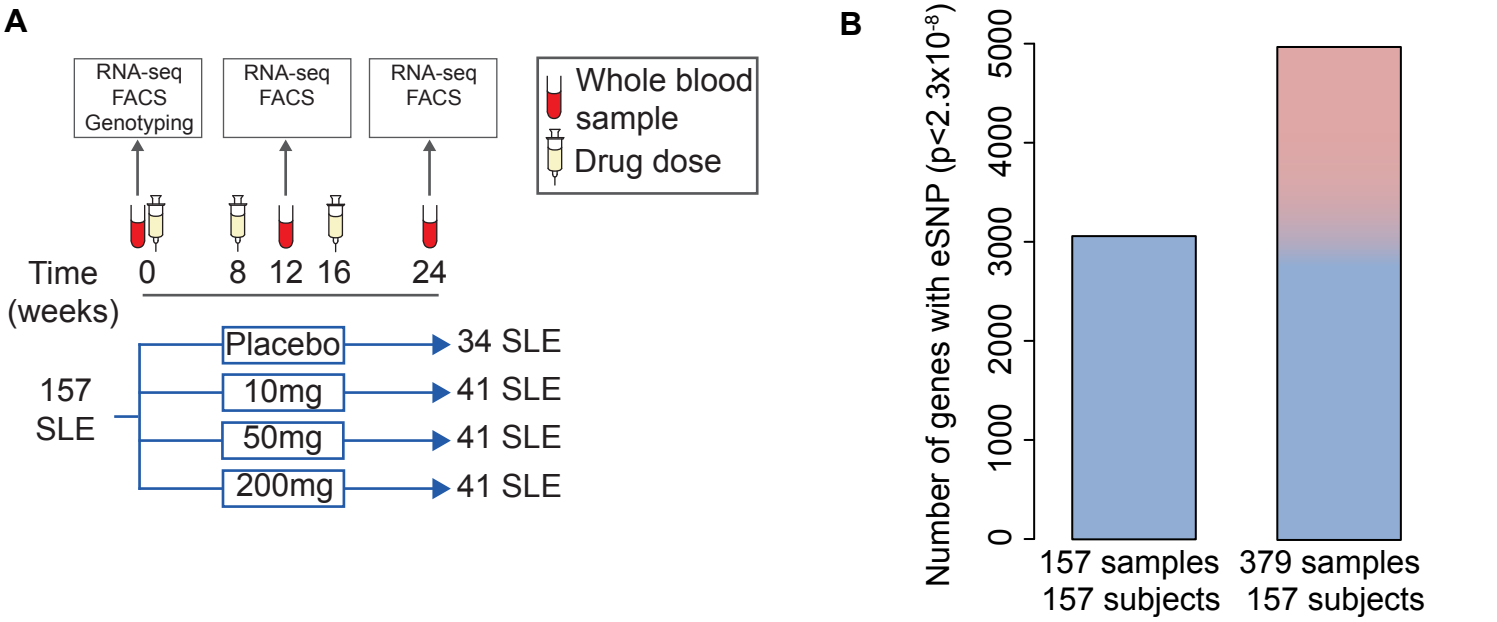
Author Contributions

The project was conceived and designed by EED, MSV, BZ and SR. Statistical analysis was conducted by EED, TA, MG-A, KS and H-JW. Molecular data was obtained and organized by YZ, SP, DvS, JSB, NB, MSV and BZ. The initial manuscript was written by EED and SR. All authors edited and approved the manuscript.



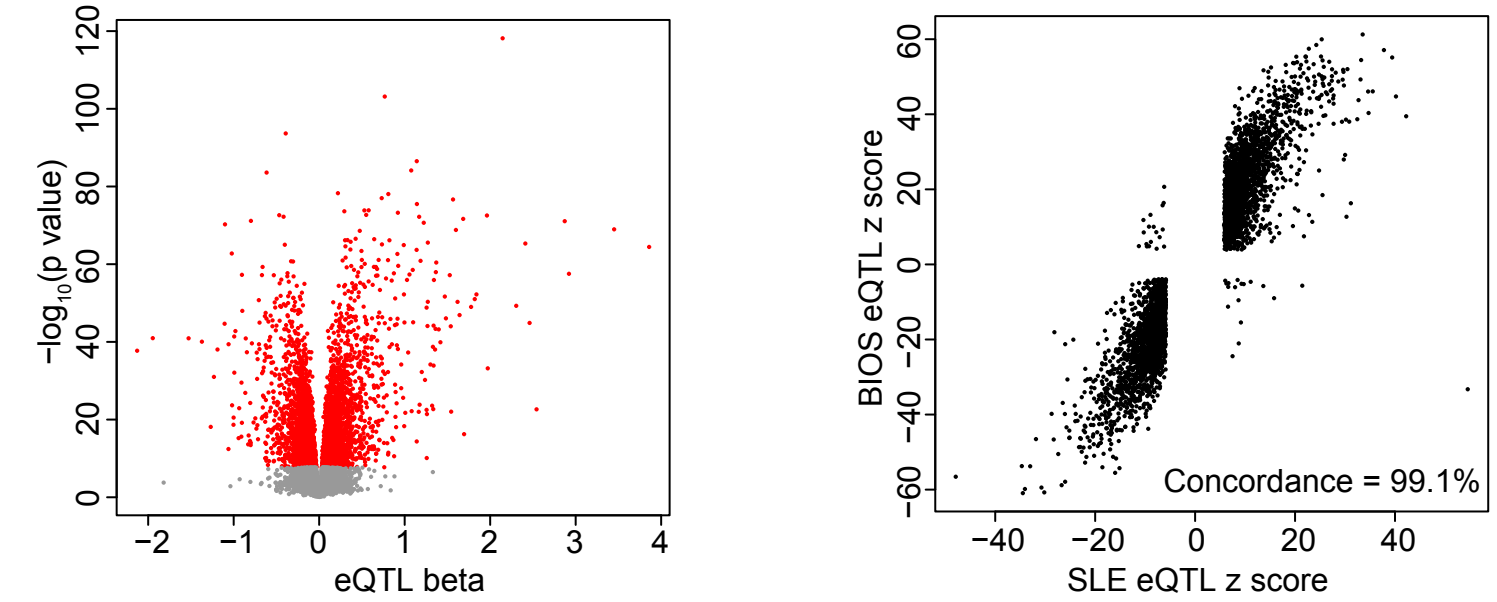


Figure 1

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 $p<2.3x10^{-8}$). (**D**) Concordance of SLE eQTL effects ($p<2.3x10^{-8}$) 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.

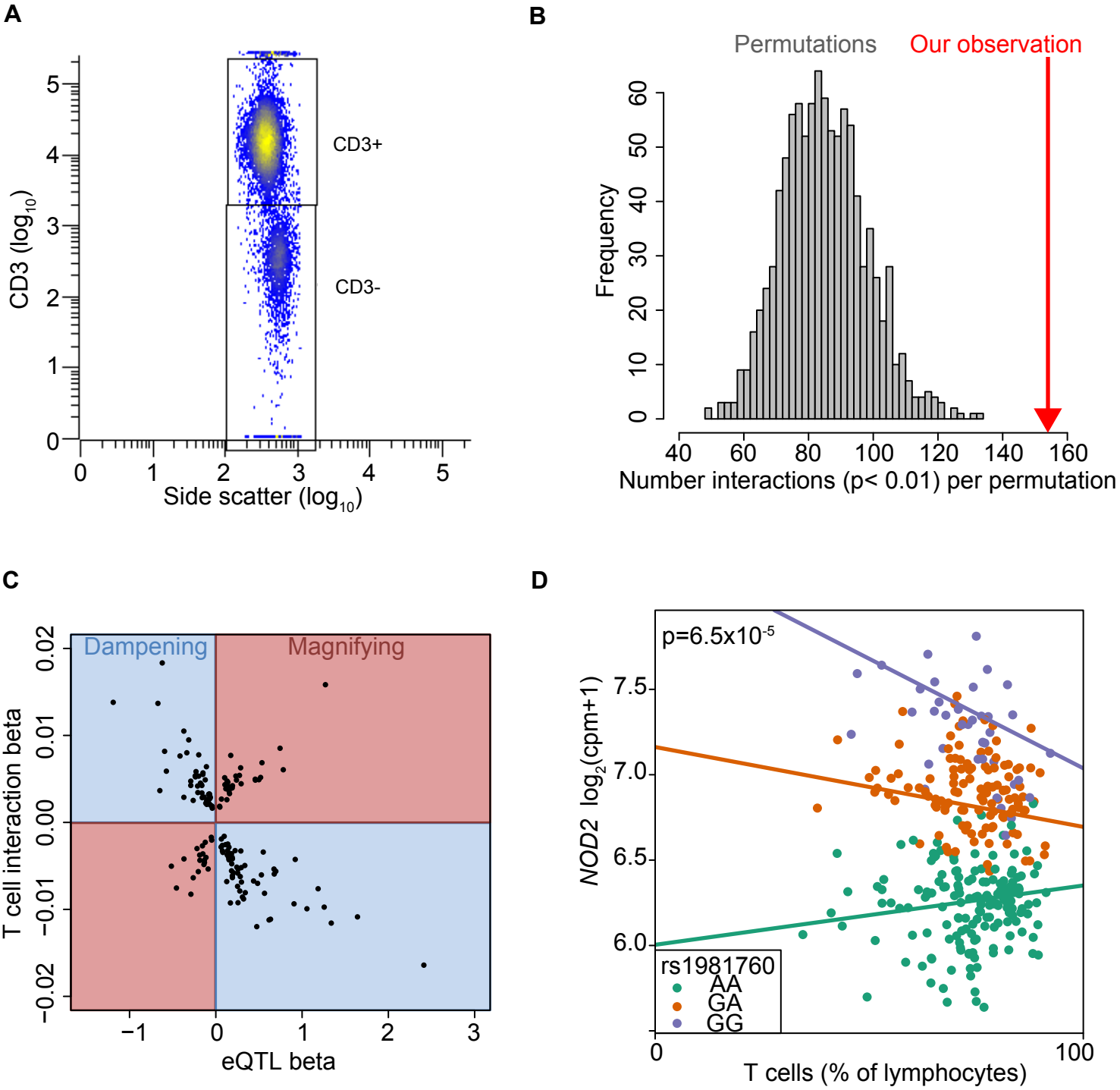


Figure 2

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.

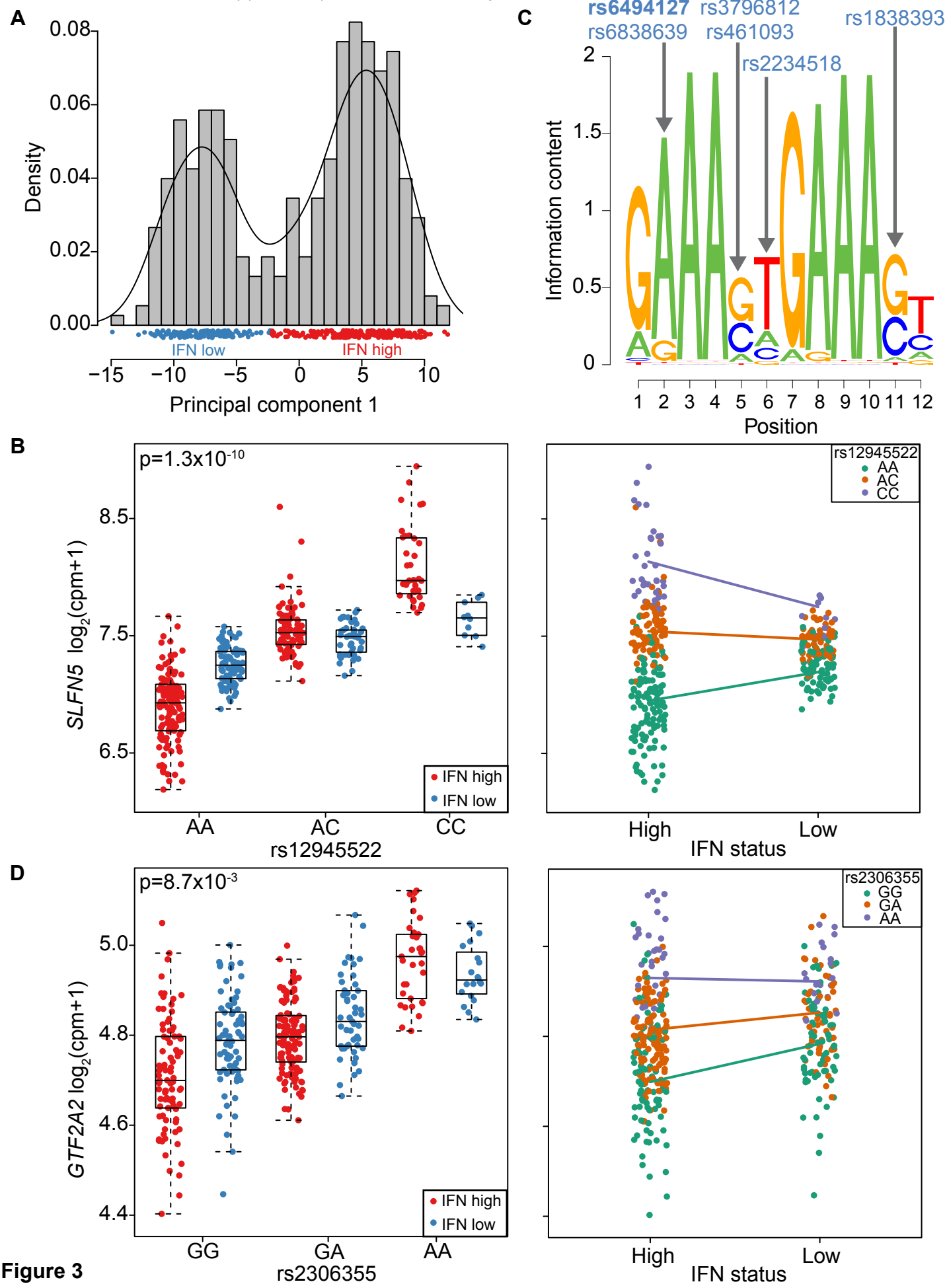


Figure 3

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).

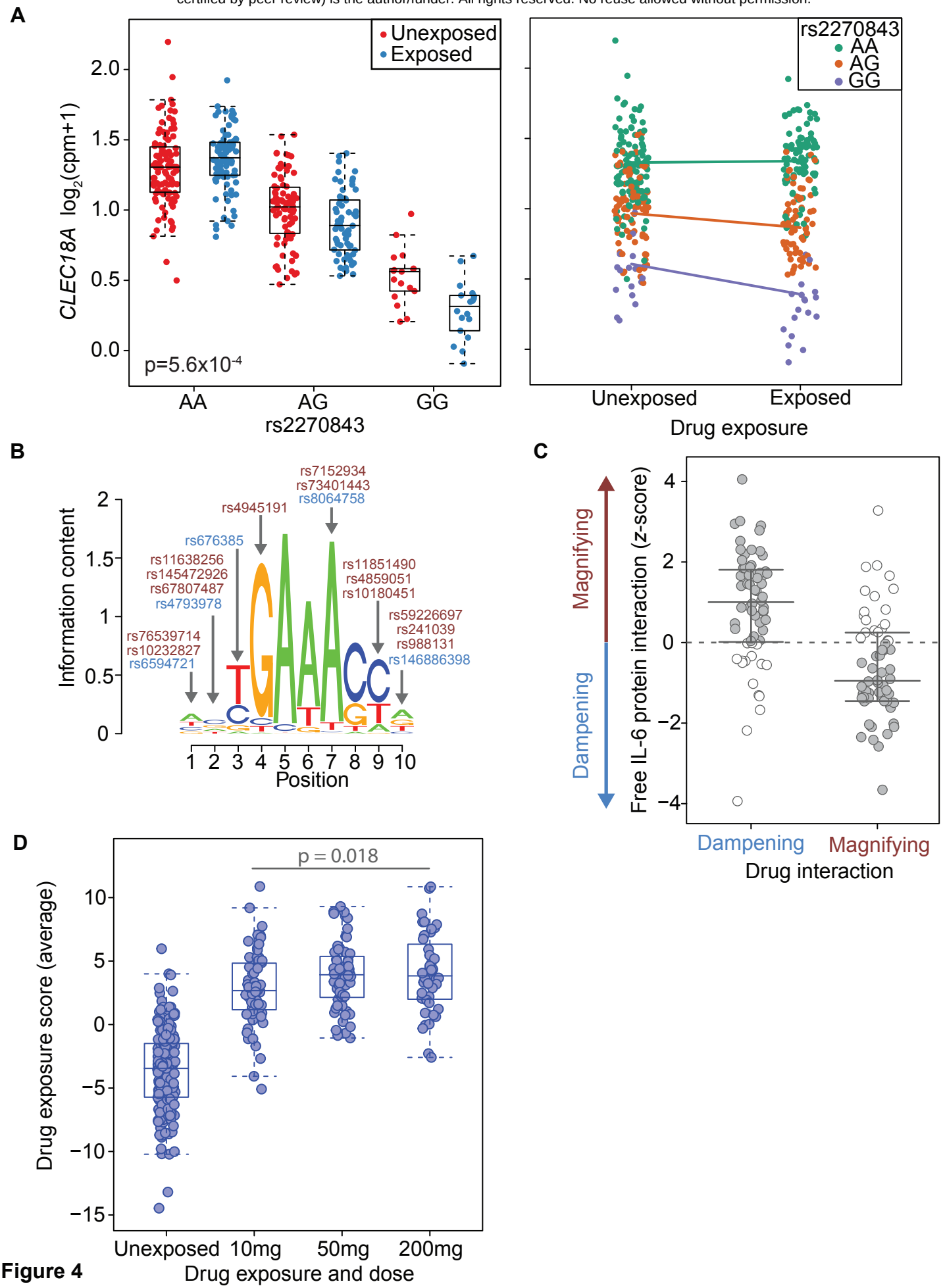


Figure 4

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.