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Rewiring of Th-memory-associated gene co-expression networks underlie immunotherapy-induced changes in symptom expression in mite-sensitised atopics

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Funding sources

Supported by funding from ALK Abello, Horsholm, Denmark, and the National Health and Medical Research Council of Australia. AB is supported by a Fellowship from the Simon Lee Foundation. NMT is supported by Asthma Australia Mickie Hardy PhD Scholarship.

Conflicts of interest

The authors declare no conflict of interest.

Words 3491

ABSTRACT

Background

Multiple regulatory mechanisms have been identified employing conventional hypothesis-driven approaches as contributing to allergen-specific immunotherapy outcomes, but understanding of how these integrate to maintain immunological homeostasis is incomplete.

Objective

To explore the potential for unbiased systems-level gene co-expression network analysis to advance understanding of immunotherapy mechanisms.

Methods

We profiled genome-wide allergen-specific Th-memory responses prospectively across 24mths of subcutaneous immunotherapy (SCIT) in 25 rhinitics, documenting changes in immunoinflammatory pathways and associated co-expression networks and their relationships to symptom scores to 36mths.

Results

Prior to immunotherapy, mite-specific Th-memory response networks involved multiple discrete co-expression modules including those related to Th2-, Type1-IFN-, Inflammation-, and FOXP3/IL2-associated signalling. A signature comprising 109 genes correlated with symptom scores, and these mapped to cytokine signalling/T-cell activation-associated pathways, with upstream drivers including hallmark Th1/Th2- and inflammation-associated genes. Reanalysis after 3.5mths SCIT updosing detected minimal changes to pathway/upstream regulator profiles despite 32.5% reduction in symptoms, however network analysis revealed underlying merging of FOXP3/IL2- with Inflammation- and Th2-associated modules. By 12mths on SCIT, symptoms had reduced by 41% without further significant changes to pathway/upstream regulator or network profiles. Continuing SCIT to 24mths

stabilised symptoms at 47% of baseline, accompanied by upregulation of the Type1-IFN-associated network module and its merging into the Th2/FOXP3/IL2/Inflammation module.

Conclusions

SCIT stimulates progressive integration of Th-memory-associated Th2-,FOXP3/IL2-, Inflammation-, and Type1-IFN-signalling subnetworks, forming a single highly integrated co-expression network module, maximising potential for stable homeostatic control of allergen-specific Th2 responses via cross-regulation. Th2-anatogonistic Type1-IFN signalling may play a key role in stabilising clinical effects of SCIT.

Clinical Implication

Stabilisation of the clinical effectiveness of SCIT involves recruitment of Th2-antagonistic Type 1 IFN-dependent signalling into the overall gene co-expression network underlying the allergen-specific Th-memory response, and this does not occur until the 2nd year of treatment.

Capsule summary

SCIT-induced rewiring of the gene network governing allergen-specific Th2-memory, as opposed to selective upregulation of genes associated with regulatory functions, underlies the clinical effectiveness of immunotherapy.

Key words

Immunotherapy, $CD4^+$ Th-memory cells, inhalant allergy, rhinitis, network analysis

Abbreviations

Subcutaneous immunotherapy (SCIT), specific immunotherapy (SIT), peripheral blood mononuclear cells (PBMC)

INTRODUCTION

The primary aim of specific immunotherapy (SIT) for atopic diseases is attenuation of the downstream inflammation resulting from allergen-specific $CD4^+$Th-memory cell activation. Multiple regulatory mechanisms are believed to contribute to SIT-induced modulation of allergen-specific Th-memory-dependent inflammatory symptoms[1-4], but the understanding of precisely how these operate individually/collectively is relatively scant. Traditional approaches to this question have emphasised hypothesis-driven studies on candidate targets selected based on *a priori* knowledge, focusing on relatively limited numbers of effector/regulatory mechanisms relevant to immunoinflammatory processes. However, it is now recognised that immune responses, including those induced by allergens[5,6], involve thousands of genes functioning within complex networks, and hence the application of systems-level analytical methodology which can capture and deconvolute some of this complexity may prove useful in elucidating SIT mechanisms.

In this regard, our group has previously employed genome wide expression profiling of allergen-activated $CD4^+$T-cells to define a highly inter-correlated Th2-associated gene subnetwork that is operative in atopic children at risk of allergic disease[5-7]. In the analyses below we have extended this systems-level approach, to identify the full spectrum of functionally coherent gene subnetworks operating within aeroallergen-specific Th-memory responses in highly symptomatic adult atopics, and to map changes in their architecture and interconnectivity during two years of SIT, in particular changes associated with reduction in Th2-associated allergy symptoms.

METHODS

Study population

House dust mite (HDM) sensitised subjects with perennial rhinitis (n=25; **Table E1**) were recruited at Fremantle Hospital, Western Australia, prior to commencement of a standard 3yr course of HDM-specific subcutaneous immunotherapy (SCIT). The SCIT protocol and mechanistic study design was approved by the institutional ethics committee, and written consent obtained from all subjects.

Immunotherapy protocol

The protocol was based on Blumberga[8], utilising Alutard® SQ *Dermataphagoidies pteronyssinus* (ALK, Horsholm, Denmark). Treatment included 15-week updosing (up to 100,000 SQ-U) and 3-year maintenance with injection intervals of 6±2 weeks; patients recorded daily respiratory symptoms and medication use, the details of which were utilised to generate symptom scores at the end of designated treatment periods[9].

PBMC processing

Peripheral blood was collected prior to immunotherapy (Visit 1/V1), and at 3.5mths (V2), 12mths (V4) and 24mths into treatment (V5; **Figure E1**). PBMC were processed and cryopreserved as described[10].

Study aims and rationale

This exploratory study aimed to evaluate the potential of systems-level network analytic methodology to elucidate short-/long-term effects of SCIT on allergen-specific T-cell memory. Our approach involved stimulation of HDM-responsive $CD4^{+}$Th-memory cells within PBMC

for 24hrs, enabling interactions to occur between the latter and bystander cells, prior to purifying $CD4^+$T-cells for transcriptomic profiling. Microarray analysis was used to compute T-cell differentially expressed gene (DEG) signatures, followed by pathway analysis to identify functionally coherent pathways that map to these signatures (IL2-signalling; Th2 etc). This was carried out in conjunction with upstream regulator analysis to identify "driver" genes that are (statistically) highly likely to have contributed at an earlier stage of culture to the expression patterns seen at 24hrs, including genes triggered within bystander populations in PBMC (myeloid cells, allergen-unresponsive T-cells etc). Independently, network analysis was performed based on measurements of gene-gene connectivity across the unique genes at each visit, enabling eventual identification of both discrete and interconnected co-expression "modules" which summate to the overall "Th-memory associated co-expression network". A subset of the subjects also received grass allergens in the SCIT treatment mix, and sub-analysis of study outcome samples after stratification by treatment did not reveal significant differences in HDM-specific DEG profiles.

In vitro cell cultures and $CD4^+$T cell isolation

PBMC from visits V1-V4 were thawed and cultured together employing the same batch of LPS^{low} HDM extract(10ug/ml) or medium control for 24h[10], whilst V5 was cultured as a stand-alone experiment employing the same reagent batches. $CD4^+$T cells were harvested from cultures using Dynabeads(ThermoFisher) for total RNA extraction with TRIzol(Ambion, Life Technologies) followed by RNAeasy MinElute(Qiagen, Hilde, Germany), and subsequent microarray profiling(Affymetrix PrimeView).

Antibody measurement

HDM-specific IgE and IgG4 levels were quantified by ImmunoCAP(ThermoFisher)[10].

Microarray data pre-processing and differential expression analysis

The raw microarray data are available from the Gene Expression Omnibus repository(GEO Accession #GSE122290). The gene expression data were analysed in R statistical computing software environment(version 3.4.4). A custom chip description file with updated genome information (primeviewhsentrezgcdf, version 22) was utilised to annotate probe sets to genes[11]. Quality control(QC) of raw microarray data was performed with *ArrayQualityMetric*s[12]; one case/control subject at V5 was removed based on QC plots. The robust multi-array average algorithm (*RMA*[13]), was used for background correction and quantile normalisation. Non-informative probe sets were filtered out using the proportion of variation accounted for by the first principal component algorithm (*PVAC*>0.4[14]), resulting in 3264 probe sets(V1, V2 and V4) and 3825 probe sets(V5) for downstream statistical analysis.

DEG were identified using *limma*[15], with a false discovery rate control for multiple testing. Quality weights were included in the linear model with the *arrayWeights()*[15] function. Paired samples were taken into account with the *duplicateCorrelation()*[15] function and the model estimates were adjusted for batch effects. We tested whether response to stimulation is associated with respiratory symptoms at each visit by including both covariates and their interaction in the linear model.

Network analysis

Prior to network construction, the raw expression data were pre-processed separately for each visit with *RMA*[13] and batch variation was removed with *ComBat*[16]. The *PVAC*-filtered data resulted in 4027, 3042, 3912 and 3831 probe sets at V1/V2/V4/V5 respectively, and unique genes from each visit were combined resulting in 5265 genes for network analysis. Co-

expression networks were constructed employing *WGCNA*[17] with the following network parameters: signed networks, softpower = 11, Pearson correlation, minimum module size = 50, deepsplit = 0, merge cut height = 0.1. Principal component analysis was performed at each visit to determine the relatedness of the modules. Modules were examined for enrichment with DEG obtained with *limma* analysis. Network wiring diagrams were constructed utilising the top-weighted 800 network edges (pairwise gene-gene correlations), which were extracted from the adjacency matrix from the expression data. The networks were graphically depicted using Cytoscape[18] (version 3.6.1).

Pathways analysis

Pathway enrichment analysis was performed with InnateDB version 5.4[19] based on a hypergeometric distribution and Benjamini & Hochberg corrected *P-values*≤0.05.

Upstream regulator analysis

Putative regulators of the gene expression patterns were identified employing Ingenuity Systems Upstream Regulator Analysis[20]. Molecular drivers with Benjamini & Hochberg adjusted *P-values*≤0.05 and absolute *Z-scores*≥2.0 were deemed significant. The overlap *P-value* measures enrichment and the *Z-score* measures activation/inhibition of the regulator.

RESULTS

Evolution of symptoms over time during SCIT

Figure 1 illustrates symptom scores(mean±95% CI) at each sampling. The largest drop in symptom scores had occurred by the end of the updosing phase (32.5% reduction; V2/3.5mths) at which point maintenance therapy commenced. The mean symptom score decline reached 37% by V4(12mths) and 51% by V5(24mths), remaining at this level to the end of treatment at V6(36mths). Consistent with the literature[1-3], reductions in symptoms across the first year of SCIT were accompanied by significant increases in serum HDM-specific IgE and IgG4 (**Figure E2A/B**).

$CD4^+$T cell immune responses pre-treatment

The $CD4^+$T-cell responses to HDM prior to mite allergen-specific immunotherapy(V1) comprised 1715 DEG (**Figure 2A/Table E2**). Pathways analysis demonstrated enrichment for genes associated with a number of functionally coherent pathways, in particular involving cytokine/chemokine receptor interactions, antigen processing/presentation and transcriptional activation of T-cells (**Figure 2B**). Upstream driver analysis revealed that the top-ranking putative drivers (ranked in **Figure 2C** by activation *Z-scores* and adjusted *P-values*) were dominated by those associated with Th1/Th2 immunity (including respectively IFNG/IL12; IL4/IL5), inflammation (TNF, IL1B, CSF2, STAT3) and T cell activation (CD40 ligand, CD3/TCR, CD28).

Association of respiratory symptoms with gene expression pre-treatment

We tested whether allergen-driven T-cell response patterns were associated with respiratory symptom scores at each visit. At pre-treatment(V1) we identified a gene signature comprising

109 DEG as significantly associated with symptoms (**Figure 2D, Table E3**). Pathways analysis showed that symptom-associated genes were enriched for cytokine/cytokine-receptor interactions, Th1-/Th2-mediated signalling, calcineurin-regulated NFAT-dependent transcription in lymphocytes, whilst downregulated genes were enriched for cell-cell communication/transmembrane transport (**Figure 2E**). Upstream regulator analysis demonstrated that the top-ranking drivers were associated with T-cell activation (TCR and CD3 complexes, NFATC2, CD40LG), Th1 immunity (IL12 complex/IFNG), Th2 immunity (IL4/IL5), common gamma chain signalling (IL2, IL4, IL7, IL15, IL21), and inflammation (TNF, STAT3, STAT6, IL1B, IL18; **Figure 2F**).

We then replicated the analysis from **Figure 2D**, in this case seeking to identify gene expression patterns that were associated cross-sectionally with attenuated post-treatment respiratory symptom expression scores at V2, V4 and V5 respectively. No significant associations were detected at these later time points, indicating that the contribution of HDM-specific immunoinflammatory responses to the overall expression of clinical symptoms was significantly diminished from V2 onwards relative to pre-treatment.

Impact of SCIT on HDM-specific Th-memory response profiles

We assessed HDM-induced T-cell response patterns at V2, V4 and V5. In excess of 1800 DEG were detected at each of these sampling times **(Figure 3A, Tables E4-6**). The list of up/downregulated pathways and their putative drivers at the different visits during SCIT (**Figure 3B,C**) closely resembled that identified above for V1 (**Figure 2B,C**); the notable exception was the presence of an operational/upregulated type1/2 interferon signalling pathway at V5 (**Figure 3B/C**) that were not active earlier in the time course. As illustrated in **Table I,** which shows activation *Z-scores* at each visit for the V5 upstream drivers, scores for

the IFN-associated drivers are uniformly elevated at V5, in contrast to the stable pattern for other drivers including those related to Th2 inflammation.

Network analysis of the HDM-specific T cell responses

We next constructed weighted gene co-expression networks at each sampling time employing *WGCNA* to obtain a systems-level understanding of how T-cell responses evolved over the course of allergen-specific immunotherapy. First, we calculated ranked expression levels and ranked network connectivity, and compared the data at V1 with the subsequent visits. As illustrated in **Figure E3**, the data showed that variations in network connectivity were most striking at V2 and V5. Notably, variations in network connectivity were also evident at V4, even though no differentially expressed genes were identified between the respective responses at V1 and V4. We next examined the evolution of modular architecture of the coexpression networks over the course of SCIT. To illustrate this, we superimposed the network connectivity patterns for each visit over the modular architecture from V1 (**Figure 4A**). The data showed that V2 and V5 were characterised by the presence of strong correlation patterns between modules. Moreover, this was also reflected by changes in the modular architecture from V1 to V5 (indicated by the colours below **Figure 4B**, left panel), together with a roadmap showing the progressive re-wiring of the modules (denoted A-N) at ensuing visits (**Figure 4B**, right panel). The co-expression networks were organised into 9, 7, 10 and 6 modules respectively at V1, V2, V4 and V5 (**Figure 4B**). We then interrogated the modules for functional coherence and examined them for enrichment of DEG (**Figure 4C**). At V1 prior to immunotherapy, the modules denoted C, F, G and I, relating respectively to metabolism of proteins/Th2 immunity/inflammation/IL2-signalling, were upregulated. Of note, type1 interferon-associated genes formed a discrete module(A), however, these were not differentially expressed. Following completion of updosing at V2, the topological architecture had become restructured

and the modules F, G and I amalgamated forming one upregulated network (module J, **Figure 4B,C**), whilst the type1 interferon network remained unchanged, and lysosome/phagosome/antigen-processing-presentation module C and TCR signalling module D were downregulated.

At V4, the IL2 signalling and Th2 network remained fused (module L) while the inflammation/lysosome/phagosome/antigen processing-presentation (G), metabolism of proteins (C), and type1 interferon-associated (A) modules remained as discrete networks (**Figure 4B,C**). Finally at V5, the original subnetworks from V1 (F, G and I) had consolidated into a single large upregulated co-expression network module (N, **Figure 4B,C**), which included re-wiring with the now upregulated interferon-associated genes which were originally within the (then quiescent) module A at V1 (**Figure 4B,C**).

To visualise these progressive changes in network architecture during the course of SCIT we plotted the top 800 network edges/connectivity patterns from *WGCNA* employing Cytoscape, focusing initially on the components of the key modules A/F/G/I from V1. **Figure 5/Figure E4** illustrates the major structural changes that occurred in network connectivity between V1 to V5, notably the merging of the initially discrete Th2-, IL2-, and Interferon-signalling modules into a single interconnected coexpression module by the end of 2yrs treatment. As shown this transition appears to involve two stages, with initial coalescing of the Th2- and IL2-signalling modules evident by the end of SCIT updosing(V2), while final merging with the Interferon module was not observed until the second year of SCIT(V5). We also calculated network summary statistics to identify hubs and bottleneck nodes[21] that are thought to play central roles in network stabilisation. With respect to the key V5 module N, 10 of the top 20 hub/bottleneck genes identified via Cytoscape based on the betweenness centrality and degree

metrics[21] were associated with IL2/FOXP3-signalling and 4 with Type1 IFN-signalling (**Table E7**), consistent with their likely contributions to eventual consolidation of the SCIT-induced low responder phenotype.

DISCUSSION

Successful SCIT against aeroallergens is typically a biphasic process, in which symptom improvement commences in responsive subjects within a few months and peaks within the first treatment year, however "consolidation" of the clinical effects of SCIT requires a 2nd (sometimes 3rd) year of maintenance treatment to prevent slow reversion to pre-treatment aeroallergen-responder status[22,23]. Current understanding of the mechanism(s) underlying desensitisation derive principally from studies focusing on the early phase of SCIT, which suggest important roles for T-regulatory cells, allergen-specific IgG4, and a broad range of other negative control mechanisms[1-3]. How these multiple mechanisms interact to control allergen-induced responses in this early phase of SCIT is incompletely understood, and it is not known whether the same range of mechanisms are responsible for eventual stabilisation of the SCIT-induced low-allergen-responder phenotype, or alternatively whether additional control mechanisms are induced/recruited via more prolonged treatment. In this regard it is pertinent to note that (as in **Figure E2**) specific IgE production is maintained or transiently boosted during the early period of SCIT, and declines only slowly with continuing therapy, even in subjects with markedly reduced symptoms[1,2,24], inferring that Th2-polarised immunological memory against the target allergen is not completely abrogated by SCIT, but instead its downstream immunoinflammatory effects are attenuated.

Our principal aim in this study was to test whether unbiased systems-level analyses of HDM-specific Th-memory response profiles spanning the early and later(consolidation) phases of SCIT, could provide mechanistic insight into this biphasic process. Our study yielded two sets of complimentary findings, as follows.

In untreated/highly symptomatic HDM-sensitised subjects, the HDM-induced Th-memory signature is enriched for multiple genes that map to pathways associated with transcriptional activation and cytokine/cytokine-receptor interactions, and the molecular drivers of this response are dominated by hallmark Th1/Th2- and inflammation-associated genes (**Figure 2A-C**), which in turn are correlated with symptom scores (**Figure 2D-F**). This correlation was lost by the end of SCIT updosing(at 3.5mths), but analysis of the accompanying "early post-treatment" HDM-specific DEG signature revealed minimal accompanying changes in underlying pathway/upstream driver profiles, and moreover this pattern did not change substantially throughout the initial course of ensuing maintenance therapy to 12mths (V4; **Figure 3B,C**), despite a further decline in symptom scores (**Figure 1**). Continuation of maintenance therapy for a second 12mth period resulted in stabilisation of symptom scores by 24mths at 51%(mean) below baseline(V5; **Figure 1**), and the appearance in the HDM-specific response profile of upregulated IFN-signalling (**Figure 3B**; **Table I**) and Type1-IFN-associated transcriptional regulators (particularly STAT1 and IRF7; **Figure 3C**).

Additional systems-level gene coexpression analyses based on network connectivity patterns provide a holistic view of the overall HDM-specific Th-memory response which is key to interpretation of these findings. As illustrated in **Figures 4/5/E4**, the use of *WCGNA* enables construction of complex networks comprising both discrete and interconnected modules, each encompassing genes which are highly correlated with each other on the basis of co-expression; exemplars in **Figure 5** are modules designated respectively "Th2 " which includes the core IL4/IL4R/IL5/IL9/IL13 gene signature, and "IL2-signalling" encompassing IL2/IL2R/FOXP3 and related genes. Tracking the connectivity patterns between these and other functionally coherent co-expression modules within the network across the time-course of SCIT suggests that the influence of therapy on the overall allergen-induced DEG response extends beyond

quantitative effects on transcription, and importantly includes coordination/synchronisation of the expression of key sets of genes within the Th-memory response.

Thus at V1(pre-treatment) at which time prominent drivers of symptoms include Th2 effectors exemplified by IL4/IL5 and archetypal inflammatory mediators such as TNF/IL1B/1L18, the network modules associated with Th2-, inflammation-, and IL2/FOXP3-signalling were isolated in the network topology, suggesting they operate relatively independently (modules F,G,I in **Figure 4B**), resulting in a low-level of coordination between the activity of inflammatory and regulatory networks in highly symptomatic subjects. However overall network topology is radically altered during the updosing phase of SCIT such that by V2 the Th2 and IL2/FOXP3 modules together with much of the inflammation subnetwork have coalesced into a single entity (module J/**Figure 4B**), suggesting enhanced coordination of expression of symptom-associated and regulatory genes/pathways, and this coincides with initial symptom decline and termination of the nexus between symptom scores and the overall Th-memory-induced DEG signature. The early SCIT-induced merging of these subnetworks persists in the face of further changes in network topology as therapy continues, culminating during the 2nd year of treatment with additional merging by V5 with the now upregulated IFN-signalling subnetwork, to form the single highly integrated module N (**Figure 4B**) encompassing coordinated Th2-, IL2/FOXP3-, inflammation-, and IFN-associated signalling. In contrast, modules B, C and D encompassing the key “infrastructural” activities (antigen processing/presentation, TCR activation, translation) which underpin the overall Th-memory response, remain as distinct entities throughout.

We performed additional analyses to visualise changes in network wiring across time, with particular emphasis on gene-gene connections in network module N/V5 which encompasses

the main inflammatory effector/regulatory pathways following 2yrs SCIT (**Figure 5)**. As noted in Results, subsidiary analyses (**Table E7**) highlighted the prominence of genes associated with IL2/FOXP3-signalling in stabilising this network and hence consolidation of the clinical effects of SCIT. However additional findings in **Table E7** together with **Table I/Figures 4,5** suggest that other factors related to network topology are also involved. In particular, the upregulation during the 2nd treatment year of the initially quiescent Type1-IFN module and its "wiring" into the aeroallergen-specific Th-memory-associated co-expression network at V5, may also play a key role in this stabilisation process.

In this regard, previous studies have identified the presence of Type1-IFN-associated DEG signatures in both PBMC and sputum-derived cellular response profiles in a variety of settings relevant to asthma-related immunophenotypes[24, 25], with the perceived role of IFNs generally interpreted in the context of host responses to microbial triggers of acute exacerbations. However, we[25] and others have also shown[26, 27] that Type1-IFNs negatively regulate many of the downstream pro-inflammatory effects of Th2 cytokines, including IL4/IL13-induced stimulation the expression of receptors governing the endocytic, trafficking, and IgE-binding properties of myeloid cells[25]. It is thus conceivable that this late-stage rewiring of the network during prolonged maintenance SCIT, resulting in the introduction of coordinated Type1-IFN signalling into the aeroallergen-specific Th-memory response profile, may provide a last-line-of-defence to stabilise immunotherapy via targeting key functions of activated Th2-memory cells that evade control via T-regulatory cells and related mechanisms. Given that the primary source of Type1-IFNs are myeloid cells (particularly DC), the findings above suggest that some of the long-term effects of SCIT may include modulation of the capacity of allergen-specific-Th-memory-cells to chemoattract/cluster-with/stimulate myeloid cells that are present within the microenvironment of the Th-memory cells during their interaction with aeroallergen. There

are several earlier studies reporting SIT-associated effects on myeloid functions, including Type1 IFN response capacity of dendritic cells[28-30]. It is also relevant to note the parallel findings from our earlier studies[31] contrasting the allergen-specific Th2-memory-associated co-expression network in HDM-sensitised atopic children with its counterpart underlying childhood responses to the highly Th2-polarising Diphtheria-tetanus-acellular-pertussis(DTaP) vaccine, the administration of which does not provoke Th2-associated allergic inflammation despite stimulation of high levels of vaccine-antigen-specific IgE in some recipients[32]. Mirroring the post SCIT-findings above, the clinically benign Th2-polarised DTaP-specific Th-memory response also included upregulation of a prominent Th1-IFN-associated gene subnetwork, which was not observed in the corresponding allergen-specific network response[31].

This exploratory study has limitations including sample size, lack of a placebo control group, and non-availability of data on Th-memory profiles in the 3rd year during SCIT, and these deficiencies require addressing in follow-up studies. Importantly, the same network analytic approach needs to be applied to characterisation of the effects of other immunotherapeutic treatments on allergen-specific Th-memory, as SCIT may not be representative of all forms of desensitisation. Notwithstanding these limitations, the study for the first time introduces the concept that remodelling of overall allergen-specific Th-memory response network topology, as opposed to simply quantitative effects on expression of key regulatory and effector genes within the response network, may be central to the efficacy of immunotherapeutic treatment modalities targeting allergy. Rapidly emerging single-cell RNA-Seq technology provides an avenue for elucidation of these network interactions with higher precision than was possible in this study, and their application may open up new possibilities for therapeutic design relevant to allergy control.

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FIGURE LEGENDS

Figure 1. Evolution of symptoms over time during SCIT.

Normalised respiratory symptom scores were plotted (as mean ± 95%CI) for pre-(V1) and post-SCIT treatment (V2, V4, V5 and V6) sampling points.

Figure 2. CD4+ T-cell immune responses pre-treatment (V1) were correlated with respiratory symptoms.

A) Volcano plot showing HDM-specific DEG at V1 (dashed horizontal line=adjusted *p-value*<0.05, red=upregulated genes, blue=downregulated genes). B) Pathways analysis of DEG was carried out with InnateDB (red=upregulated pathways, blue=downregulated pathways). C) Predicted upstream regulators of the DEG (red=activated drivers, blue=inhibited drivers). D) Correlation of respiratory symptoms with CD4+T cell responses at V1; E) corresponding up-and-downregulated pathways and F) upstream regulators.

Figure 3. CD4+ T-cell responses over the course of SCIT treatment (V2, V4 and V5).

A) Volcano plots showing HDM-specific CD4+ T-cell responses at V2, V4 and V5 respectively. The dashed horizontal line indicates an adjusted *p-value*<0.05. Upregulated genes are denoted in red, downregulated genes are denoted in blue. B) Pathways analysis of DEG was carried out with InnateDB (red=upregulated pathways, blue=downregulated pathways). C) Predicted upstream regulators of the DEG (red=activated drivers, blue= inhibited drivers).

Figure 4. Network analysis of the HDM-induced T cell responses.

Co-expression networks were constructed employing *WGCNA*. A) Network connectivity patterns(V2/V4/V5) were superimposed over V1 modular architecture. B) Comparison of V1

modular architecture with the module assignments V2/V4/V5 (left panel). Roadmap showing progressive re-wiring of the modules (A-N) over the course of SCIT(right panel; black circles/lines=differentially expressed, grey=not differentially expressed. C) Box-and-whisker plots showing modules with DEG. Median adjusted *p-values:* *=0.05,**=0.01,***=0.001,****=0.0001.

Figure 5. Reconstruction of the network wiring diagram at V1/V5 reveals changes in network connectivity.

Co-expression networks were constructed employing *WGCNA* and visualised in Cytosape. The top-800 network edges/pairwise connections were utilised for network reconstruction. The node size reflects the number of connections per gene. Node colour spectrum in key (red-blue) designates positive-to-negative log2FC; note module A is not upregulated until V5.

Supplementary Figure E1. SCIT treatment/sample collection schedule.

Treatment included a 15-week updosing (up to 100,000 SQ-U) and a 2-year maintenance with injection intervals of 6±2 weeks, and patients recorded daily symptoms and medication use, the details of which were utilised to generate symptom scores at the end of designated treatment periods.

Supplementary Figure E2. Serum titres of HDM-specific IgE and IgG4.

House dust mite specific (*Dermatophagoides pteronyssinus*) antibody levels were detected by ImmunoCAP, measuring in A) *HDM*- specific IgE and in B) HDM-specific IgG4-specific.

Supplementary Figure E3. Comparison of expression ranks and connectivity ranks at V1 versus subsequent visits.

Supplementary Figure E4. Reconstruction of the network wiring diagram at V2/V4. Co-expression networks were constructed employing *WGCNA* and the top-800 pairwise (gene-gene) connections (extracted from the adjacency matrix) were utilised to reconstruct the network wiring diagram of modules A, F, G and I at V2 and V4 utilising Cytoscape, as per Figure 5. Node colour spectrum in key (red-blue) designates positive-to-negative log2FC.

Table I. Activation Z-scores at each visit for the V5 upstream drivers.

Upstream regulators	V1	V2	V4	V5
NFkB (complex)	5.80	5.85	5.84	7.05
IL1B	6.08	5.80	5.87	6.87
CD40LG	5.74	5.94	6.04	6.50
TCR (complex)	6.66	5.85	6.61	6.28
IL2	6.79	6.66	6.69	6.20
IL7	5.32	5.78	5.27	6.14
TNF	5.58	5.12	5.32	6.10
MYC	5.97	5.75	6.36	6.07
IFNA2	1.65	1.79	0.55	**5.99**
IRF7	0.60	1.95	-0.16	**5.85**
IFNG	3.48	3.93	2.60	**5.78**
IFNA group	2.92	3.36	2.21	**5.63**
TLR4	4.71	4.80	4.11	5.62
IL15	5.24	5.29	5.62	5.54
MYCN	4.02	4.41	4.65	5.35
TLR9	4.76	4.69	3.81	5.00
STAT1	2.27	3.04	1.86	**4.87**
IL18	4.53	4.56	4.46	4.78
CSF2	4.71	4.12	4.32	4.25
IL5	4.22	4.15	4.17	4.02
Immunoglobulin (complex)	2.85	3.37	3.71	3.96
CD3 (complex)	3.52	3.62	3.68	3.96
IL3	3.67	3.63	3.39	3.86
STAT3	3.32	3.37	3.04	2.65
IL27	1.96	1.79	1.00	2.28
IL4	2.49	2.44	2.53	2.19
IL10	-2.23	-2.48	-2.81	-2.85
CD28	-5.38	-4.97	-5.14	-4.49
BCL6	-4.91	-4.93	-4.82	-4.94
RICTOR	-3.64	-4.95	-4.35	-5.14

Putative regulators of the DEG were identified at each visit employing Ingenuity Systems Upstream Regulator Analysis. Molecular drivers with Benjamini & Hochberg adjusted *P-values*≤0.05 and absolute *Z-scores*≥2.0 were deemed significant (positive *Z-scores*=activation, negative *Z-scores*=inhibition).

Supplementary Tables E1-7

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Supplementary Tables E1-7

Rewiring of Th-memory-associated gene co-expression networks underlie immunotherapy-induced changes in symptom expression in mite-sensitised atopics

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Supplementary Table E1. Subject characteristics at recruitment.

Subject characteristics (n=25)	
Age in years (median/range)	28.9 (19-56.8)
Female (%)	80
Current rhinitis (%)	100
Current wheeze (%)	56
HDM sensitised (%)	100
Grass sensitised (%)	72

Supplementary Table E2. Top 100 differentially expressed genes (up/down) in CD4+ T cells prior to SCIT (V1).

ProbeID	EntrezID	Symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
6347_at	6347	CCL2	3.62	7.76	11.58	2.08E-22	1.18E-20	40.30
6354_at	6354	CCL7	3.37	6.62	11.96	2.11E-23	1.32E-21	42.57
5055_at	5055	SERPINB2	3.24	7.42	12.15	6.40E-24	4.45E-22	43.75
3553_at	3553	IL1B	3.00	8.34	10.60	7.82E-20	2.97E-18	34.43
6374_at	6374	CXCL5	2.75	10.79	11.70	9.83E-23	5.73E-21	41.05
2919_at	2919	CXCL1	2.26	7.97	10.16	1.15E-18	3.45E-17	31.77
2921_at	2921	CXCL3	2.26	6.43	10.50	1.50E-19	5.11E-18	33.79
1154_at	1154	CISH	2.10	7.68	15.19	7.66E-32	3.12E-29	61.82
3552_at	3552	IL1A	2.06	5.66	8.32	5.56E-14	7.25E-13	21.11
3558_at	3558	IL2	2.04	4.57	12.33	2.13E-24	1.55E-22	44.84
2791_at	2791	GNG11	1.70	5.78	9.30	1.89E-16	3.95E-15	26.73
4316_at	4316	MMP7	1.66	5.60	9.02	1.01E-15	1.82E-14	25.07
3559_at	3559	IL2RA	1.65	6.83	14.84	6.05E-31	1.57E-28	59.78
4495_at	4495	MT1G	1.63	7.62	8.10	1.97E-13	2.32E-12	19.87
3576_at	3576	CXCL8	1.59	9.94	8.17	1.37E-13	1.68E-12	20.22
6372_at	6372	CXCL6	1.58	3.96	6.86	1.78E-10	1.32E-09	13.17
8835_at	8835	SOCS2	1.56	5.65	10.86	1.65E-20	6.73E-19	35.97
55022_at	55022	PID1	1.48	6.03	8.19	1.20E-13	1.48E-12	20.36
54602_at	54602	NDFIP2	1.43	4.93	8.55	1.50E-14	2.29E-13	22.41
718_at	718	C3	1.38	5.54	9.33	1.57E-16	3.33E-15	26.91
4496_at	4496	MT1H	1.35	7.64	7.64	2.60E-12	2.53E-11	17.33
6364_at	6364	CCL20	1.33	6.61	8.68	7.32E-15	1.17E-13	23.11
6348_at	6348	CCL3	1.32	7.63	6.16	6.76E-09	4.03E-08	9.61
79413_at	79413	ZBED2	1.32	4.70	10.73	3.59E-20	1.43E-18	35.20
51339_at	51339	DACT1	1.31	6.09	7.17	3.41E-11	2.76E-10	14.79
3578_at	3578	IL9	1.31	4.31	4.47	1.59E-05	5.63E-05	2.09
2920_at	2920	CXCL2	1.29	8.02	6.89	1.58E-10	1.18E-09	13.29
7980_at	7980	TFPI2	1.28	4.41	8.14	1.62E-13	1.97E-12	20.06
6367_at	6367	CCL22	1.26	9.06	10.68	5.00E-20	1.94E-18	34.87
8651_at	8651	SOCS1	1.22	6.17	13.59	1.04E-27	1.26E-25	52.40
5473_at	5473	PPBP	1.16	12.17	6.49	1.25E-09	8.24E-09	11.26
199675_at	199675	MCEMP1	1.11	6.65	7.62	2.89E-12	2.77E-11	17.22
3002_at	3002	GZMB	1.09	6.84	5.67	7.27E-08	3.74E-07	7.30
170691_at	170691	ADAMTS17	1.09	5.21	13.45	2.46E-27	2.59E-25	51.54
84830_at	84830	ADTRP	1.08	6.19	13.54	1.46E-27	1.70E-25	52.06
1462_at	1462	VCAN	1.05	4.05	7.45	7.34E-12	6.62E-11	16.30
6447_at	6447	SCG5	1.05	5.08	7.00	8.69E-11	6.74E-10	13.88
114801_at	114801	TMEM200A	1.03	4.99	10.16	1.13E-18	3.42E-17	31.79
5732_at	5732	PTGER2	1.03	8.32	9.13	5.07E-16	9.74E-15	25.75

ProbeID	EntrezID	Symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
3557_at	3557	IL1RN	1.02	6.94	5.65	8.33E-08	4.23E-07	7.17
5292_at	5292	PIM1	1.02	8.90	17.87	1.40E-38	4.58E-35	77.17
4603_at	4603	MYBL1	0.98	8.36	9.27	2.26E-16	4.67E-15	26.55
705_at	705	BYSL	0.98	6.53	14.38	9.35E-30	1.70E-27	57.06
1847_at	1847	DUSP5	0.96	7.01	8.47	2.35E-14	3.44E-13	21.96
3562_at	3562	IL3	0.95	3.28	4.63	8.01E-06	3.01E-05	2.75
10148_at	10148	EBI3	0.95	5.53	9.21	3.26E-16	6.57E-15	26.19
6351_at	6351	CCL4	0.94	9.19	5.54	1.39E-07	6.91E-07	6.67
27074_at	27074	LAMP3	0.94	6.31	7.31	1.60E-11	1.36E-10	15.54
25902_at	25902	MTHFD1L	0.94	7.03	17.58	7.26E-38	1.18E-34	75.55
645784_at	645784	ANKRD36BP2	0.93	5.12	4.77	4.36E-06	1.70E-05	3.34
22797_at	22797	TFEC	-0.76	5.45	-6.10	9.19E-09	5.38E-08	9.31
5836_at	5836	PYGL	-0.78	5.39	-5.79	4.09E-08	2.20E-07	7.86
1675_at	1675	CFD	-0.79	4.75	-8.58	1.26E-14	1.95E-13	22.58
3597_at	3597	IL13RA1	-0.80	5.44	-6.34	2.70E-09	1.69E-08	10.51
7305_at	7305	TYROBP	-0.81	10.08	-5.71	6.15E-08	3.23E-07	7.46
51313_at	51313	FAM198B	-0.81	3.58	-9.82	8.77E-18	2.31E-16	29.76
7850_at	7850	IL1R2	-0.82	6.73	-4.19	4.84E-05	1.58E-04	1.03
2681_at	2681	GGTA1P	-0.82	3.58	-8.60	1.13E-14	1.77E-13	22.68
4286_at	4286	MITF	-0.83	6.07	-7.46	7.21E-12	6.52E-11	16.32
6614_at	6614	SIGLEC1	-0.83	5.61	-6.01	1.43E-08	8.23E-08	8.88
54674_at	54674	LRRN3	-0.84	10.54	-6.59	7.51E-10	5.07E-09	11.76
1536_at	1536	CYBB	-0.84	7.54	-5.92	2.26E-08	1.27E-07	8.44
10288_at	10288	LILRB2	-0.87	6.24	-4.81	3.68E-06	1.45E-05	3.50
10410_at	10410	IFITM3	-0.88	6.59	-4.94	2.10E-06	8.58E-06	4.04
928_at	928	CD9	-0.88	8.26	-7.62	2.90E-12	2.77E-11	17.22
2359_at	2359	FPR3	-0.89	6.59	-6.02	1.35E-08	7.79E-08	8.94
120892_at	120892	LRRK2	-0.89	5.13	-7.94	4.87E-13	5.39E-12	18.98
7941_at	7941	PLA2G7	-0.90	8.92	-5.67	7.43E-08	3.82E-07	7.28
1050_at	1050	CEBPA	-0.90	6.74	-7.78	1.24E-12	1.28E-11	18.06
6252_at	6252	RTN1	-0.90	5.83	-8.78	4.02E-15	6.72E-14	23.71
3109_at	3109	HLA-DMB	-0.91	6.84	-9.02	9.85E-16	1.80E-14	25.10
84417_at	84417	C2orf40	-0.92	6.13	-10.28	5.48E-19	1.74E-17	32.50
6369_at	6369	CCL24	-0.96	9.65	-5.30	4.15E-07	1.91E-06	5.61
2662_at	2662	GDF10	-0.97	3.92	-10.50	1.50E-19	5.11E-18	33.78
5166_at	5166	PDK4	-0.99	4.20	-9.85	7.30E-18	1.95E-16	29.94
54209_at	54209	TREM2	-1.00	5.13	-9.25	2.49E-16	5.09E-15	26.45
3627_at	3627	CXCL10	-1.00	5.05	-3.54	5.41E-04	1.44E-03	-1.25
1823_at	1823	DSC1	-1.02	5.50	-9.49	6.18E-17	1.39E-15	27.83
6039_at	6039	RNASE6	-1.03	6.97	-7.77	1.29E-12	1.32E-11	18.02
54_at	54	ACP5	-1.05	8.58	-10.43	2.27E-19	7.35E-18	33.37
200315_at	200315	APOBEC3A	-1.07	4.78	-4.93	2.24E-06	9.10E-06	3.98

ProbeID	EntrezID	Symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
2214_at	2214	FCGR3A	-1.08	5.99	-4.63	8.01E-06	3.01E-05	2.75
26253_at	26253	CLEC4E	-1.10	6.17	-8.86	2.53E-15	4.28E-14	24.16
55016_at	55016	MARCH1	-1.11	4.41	-10.22	8.06E-19	2.48E-17	32.12
2219_at	2219	FCN1	-1.17	5.88	-6.82	2.28E-10	1.66E-09	12.93
51311_at	51311	TLR8	-1.17	5.23	-8.13	1.66E-13	2.01E-12	20.04
10170_at	10170	DHRS9	-1.18	5.56	-10.58	9.06E-20	3.36E-18	34.29
216_at	216	ALDH1A1	-1.19	3.62	-10.54	1.14E-19	4.04E-18	34.06
4069_at	4069	LYZ	-1.25	10.88	-8.21	1.07E-13	1.34E-12	20.47
10457_at	10457	GPNMB	-1.30	6.47	-7.86	7.55E-13	8.05E-12	18.54
54504_at	54504	CPVL	-1.30	3.93	-8.96	1.38E-15	2.43E-14	24.76
4332_at	4332	MNDA	-1.43	5.49	-7.97	4.06E-13	4.56E-12	19.15
2167_at	2167	FABP4	-1.48	6.90	-7.22	2.62E-11	2.14E-10	15.05
4199_at	4199	ME1	-1.58	5.39	-12.55	5.64E-25	4.38E-23	46.16
3957_at	3957	LGALS2	-1.59	5.49	-5.83	3.50E-08	1.89E-07	8.01
948_at	948	CD36	-1.61	5.46	-10.60	8.18E-20	3.07E-18	34.39
10875_at	10875	FGL2	-1.61	8.18	-10.34	3.91E-19	1.25E-17	32.84
7045_at	7045	TGFBI	-1.65	7.64	-9.80	9.90E-18	2.59E-16	29.64
6035_at	6035	RNASE1	-1.86	6.30	-9.10	6.03E-16	1.14E-14	25.58
79887_at	79887	PLBD1	-2.07	6.48	-14.53	3.83E-30	7.82E-28	57.95

Differentially expressed genes were identified with *limma* contrasting HDM versus CTRL in CD4+ T cells prior to SCIT (V1). ProbeID = , EntrezID = , logFC = log base 2 fold change, AveExpr = average log2-expression level for that gene across all the arrays, t = moderated t-statistic, P.value = the associated p-value, adj.P.Val = the Benjamini & Hochberg adjusted P.value for multiple testing, B = the B-statistic is the log-odds that the gene is differentially expressed.

Supplementary Table E3. Association of respiratory symptoms with gene expression prior to SCIT (V1).

ProbeID	EntrezID	Symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
3578_at	3578	IL9	0.11	4.06	5.86	4.32E-07	5.83E-04	4.94
3567_at	3567	IL5	0.07	3.33	4.64	2.73E-05	3.82E-03	0.85
386653_at	386653	IL31	0.07	3.70	5.51	1.46E-06	7.93E-04	3.74
54602_at	54602	NDFIP2	0.06	4.88	4.79	1.67E-05	3.59E-03	1.33
3596_at	3596	IL13	0.05	4.59	5.10	5.87E-06	1.90E-03	2.36
3458_at	3458	IFNG	0.05	4.45	4.59	3.30E-05	4.14E-03	0.67
3002_at	3002	GZMB	0.05	6.61	3.52	9.64E-04	3.50E-02	-2.60
1154_at	1154	CISH	0.04	7.50	3.69	5.74E-04	2.57E-02	-2.10
3566_at	3566	IL4R	0.04	7.30	3.76	4.61E-04	2.24E-02	-1.89
5996_at	5996	RGS1	0.04	7.94	4.70	2.27E-05	3.59E-03	1.03
387496_at	387496	RASL11A	0.04	4.24	4.09	1.64E-04	1.37E-02	-0.90
8651_at	8651	SOCS1	0.04	6.05	6.04	2.23E-07	5.83E-04	5.60
7293_at	7293	TNFRSF4	0.04	6.86	4.62	2.92E-05	3.82E-03	0.79
55509_at	55509	BATF3	0.03	5.51	4.72	2.10E-05	3.59E-03	1.11
5732_at	5732	PTGER2	0.03	8.25	3.48	1.09E-03	3.69E-02	-2.72
115362_at	115362	GBP5	0.03	8.94	3.62	7.15E-04	2.92E-02	-2.31
84255_at	84255	SLC37A3	0.03	6.28	5.11	5.73E-06	1.90E-03	2.39
3642_at	3642	INSM1	0.03	3.43	3.73	5.10E-04	2.41E-02	-1.99
4783_at	4783	NFIL3	0.03	6.21	4.69	2.31E-05	3.59E-03	1.02
3559_at	3559	IL2RA	0.03	6.80	3.75	4.82E-04	2.32E-02	-1.94
817_at	817	CAMK2D	0.03	7.35	3.82	3.83E-04	2.05E-02	-1.71
2672_at	2672	GFI1	0.03	7.45	4.16	1.32E-04	1.17E-02	-0.69
27314_at	27314	RAB30	0.03	5.40	5.08	6.39E-06	1.90E-03	2.28
170691_at	170691	ADAMTS17	0.02	5.24	3.87	3.32E-04	1.86E-02	-1.58
94120_at	94120	SYTL3	0.02	6.68	3.77	4.51E-04	2.23E-02	-1.87
3565_at	3565	IL4	0.02	3.73	3.58	7.93E-04	3.12E-02	-2.41
197370_at	197370	NSMCE1	0.02	9.45	4.29	8.65E-05	9.11E-03	-0.27
3662_at	3662	IRF4	0.02	6.36	5.36	2.44E-06	1.14E-03	3.23
64859_at	64859	NABP1	0.02	7.82	5.57	1.17E-06	7.63E-04	3.96
4118_at	4118	MAL	0.02	10.93	3.59	7.79E-04	3.10E-02	-2.40
115361_at	115361	GBP4	0.02	6.97	3.47	1.11E-03	3.69E-02	-2.74
3659_at	3659	IRF1	0.02	9.00	3.47	1.12E-03	3.69E-02	-2.74
1803_at	1803	DPP4	0.02	8.90	4.02	2.05E-04	1.49E-02	-1.11
7494_at	7494	XBP1	0.02	9.20	3.98	2.37E-04	1.54E-02	-1.25
604_at	604	BCL6	0.02	8.05	4.43	5.49E-05	6.17E-03	0.17
6875_at	6875	TAF4B	0.02	6.89	4.89	1.21E-05	3.30E-03	1.65
355_at	355	FAS	0.02	7.76	4.41	5.98E-05	6.51E-03	0.09
6004_at	6004	RGS16	0.02	4.34	3.67	6.22E-04	2.61E-02	-2.18
6361_at	6361	CCL17	0.02	6.26	4.11	1.55E-04	1.34E-02	-0.84

ProbeID	EntrezID	Symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
83939_at	83939	EIF2A	0.02	6.55	4.03	2.03E-04	1.49E-02	-1.10
50616_at	50616	IL22	0.02	4.56	3.78	4.41E-04	2.21E-02	-1.85
6641_at	6641	SNTB1	0.02	6.34	4.21	1.13E-04	1.06E-02	-0.54
494143_at	494143	CHAC2	0.02	6.37	3.36	1.56E-03	4.72E-02	-3.06
10906_at	10906	TRAFD1	0.02	7.23	5.26	3.45E-06	1.41E-03	2.89
10797_at	10797	MTHFD2	0.02	8.84	4.65	2.67E-05	3.82E-03	0.88
3628_at	3628	INPP1	0.02	6.92	3.73	5.16E-04	2.41E-02	-2.00
9246_at	9246	UBE2L6	0.02	10.17	3.40	1.37E-03	4.25E-02	-2.93
8809_at	8809	IL18R1	0.02	5.96	3.46	1.14E-03	3.72E-02	-2.76
10538_at	10538	BATF	0.02	8.30	4.02	2.05E-04	1.49E-02	-1.11
64332_at	64332	NFKBIZ	0.01	10.35	4.22	1.11E-04	1.06E-02	-0.51
6890_at	6890	TAP1	0.01	10.08	3.67	6.08E-04	2.61E-02	-2.16
10308_at	10308	ZNF267	0.01	8.64	3.98	2.32E-04	1.54E-02	-1.23
10653_at	10653	SPINT2	0.01	8.68	3.99	2.29E-04	1.54E-02	-1.22
5971_at	5971	RELB	0.01	7.98	3.55	8.86E-04	3.29E-02	-2.52
4049_at	4049	LTA	0.01	6.66	3.82	3.93E-04	2.07E-02	-1.74
3560_at	3560	IL2RB	0.01	9.95	3.40	1.36E-03	4.25E-02	-2.93
23102_at	23102	TBC1D2B	0.01	6.89	4.23	1.04E-04	1.03E-02	-0.46
81671_at	81671	VMP1	0.01	8.73	4.01	2.15E-04	1.53E-02	-1.16
23212_at	23212	RRS1	0.01	9.75	4.07	1.79E-04	1.41E-02	-0.98
9262_at	9262	STK17B	0.01	8.59	4.44	5.31E-05	6.17E-03	0.20
5292_at	5292	PIM1	0.01	8.85	3.56	8.53E-04	3.24E-02	-2.48
3702_at	3702	ITK	0.01	9.18	5.72	6.94E-07	5.83E-04	4.48
5236_at	5236	PGM1	0.01	7.65	4.19	1.19E-04	1.08E-02	-0.58
5906_at	5906	RAP1A	0.01	10.27	3.50	1.03E-03	3.69E-02	-2.67
8611_at	8611	PLPP1	0.01	7.56	4.07	1.75E-04	1.41E-02	-0.96
836_at	836	CASP3	0.01	7.32	3.95	2.58E-04	1.65E-02	-1.33
2023_at	2023	ENO1	0.01	12.30	3.67	6.22E-04	2.61E-02	-2.18
10531_at	10531	PITRM1	0.01	7.65	3.94	2.70E-04	1.65E-02	-1.38
7702_at	7702	ZNF143	0.01	7.63	4.57	3.44E-05	4.16E-03	0.63
1503_at	1503	CTPS1	0.01	6.76	3.69	5.83E-04	2.57E-02	-2.12
57559_at	57559	STAMBPL1	0.01	8.70	4.26	9.68E-05	9.88E-03	-0.38
6675_at	6675	UAP1	0.01	7.95	4.63	2.81E-05	3.82E-03	0.82
1615_at	1615	DARS	0.01	10.15	3.87	3.34E-04	1.86E-02	-1.58
6993_at	6993	DYNLT1	0.01	10.32	3.66	6.23E-04	2.61E-02	-2.18
29851_at	29851	ICOS	0.01	8.95	4.80	1.63E-05	3.59E-03	1.36
8509_at	8509	NDST2	0.01	7.37	3.43	1.27E-03	4.05E-02	-2.86
123920_at	123920	CMTM3	0.01	8.87	3.88	3.23E-04	1.86E-02	-1.55
54552_at	54552	GNL3L	0.01	8.68	3.49	1.06E-03	3.69E-02	-2.69
9595_at	9595	CYTIP	0.01	10.47	3.87	3.36E-04	1.86E-02	-1.59
388962_at	388962	BOLA3	0.01	8.74	3.79	4.24E-04	2.16E-02	-1.81
9188_at	9188	DDX21	0.01	10.17	3.94	2.68E-04	1.65E-02	-1.37

ProbeID	EntrezID	Symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
51602_at	51602	NOP58	0.00	10.33	3.48	1.09E-03	3.69E-02	-2.71
55863_at	55863	TMEM126B	0.00	10.27	3.70	5.56E-04	2.52E-02	-2.07
3608_at	3608	ILF2	0.00	10.44	3.61	7.38E-04	2.97E-02	-2.34
6277_at	6277	S100A6	-0.01	11.92	-3.81	4.04E-04	2.09E-02	-1.77
51696_at	51696	HECA	-0.01	11.37	-4.71	2.20E-05	3.59E-03	1.07
83442_at	83442	SH3BGRL3	-0.01	10.01	-3.34	1.65E-03	4.94E-02	-3.11
6281_at	6281	S100A10	-0.01	11.43	-3.37	1.50E-03	4.59E-02	-3.02
6653_at	6653	SORL1	-0.01	11.44	-3.93	2.73E-04	1.65E-02	-1.39
10365_at	10365	KLF2	-0.01	11.67	-3.57	8.23E-04	3.16E-02	-2.45
11329_at	11329	STK38	-0.01	11.07	-3.71	5.38E-04	2.47E-02	-2.04
6095_at	6095	RORA	-0.01	9.66	-4.06	1.81E-04	1.41E-02	-0.99
2533_at	2533	FYB1	-0.01	10.89	-3.99	2.26E-04	1.54E-02	-1.20
64968_at	64968	MRPS6	-0.01	11.35	-3.55	8.74E-04	3.28E-02	-2.51
27106_at	27106	ARRDC2	-0.01	9.65	-3.48	1.07E-03	3.69E-02	-2.70
6932_at	6932	TCF7	-0.01	8.50	-3.37	1.49E-03	4.58E-02	-3.01
51411_at	51411	BIN2	-0.01	8.56	-3.43	1.24E-03	4.02E-02	-2.84
79026_at	79026	AHNAK	-0.01	8.95	-3.83	3.74E-04	2.03E-02	-1.69
3987_at	3987	LIMS1	-0.01	10.26	-3.65	6.48E-04	2.68E-02	-2.22
28984_at	28984	RGCC	-0.01	11.66	-4.72	2.10E-05	3.59E-03	1.11
1043_at	1043	CD52	-0.01	12.61	-5.71	7.14E-07	5.83E-04	4.45
9214_at	9214	FCMR	-0.01	9.65	-3.58	8.12E-04	3.16E-02	-2.44
85478_at	85478	CCDC65	-0.01	7.04	-3.40	1.36E-03	4.25E-02	-2.93
26119_at	26119	LDLRAP1	-0.01	9.40	-3.47	1.10E-03	3.69E-02	-2.73
1831_at	1831	TSC22D3	-0.01	9.15	-4.72	2.14E-05	3.59E-03	1.09
6526_at	6526	SLC5A3	-0.01	11.33	-3.87	3.35E-04	1.86E-02	-1.59
120224_at	120224	TMEM45B	-0.01	6.91	-3.49	1.04E-03	3.69E-02	-2.68
3399_at	3399	ID3	-0.02	9.29	-4.84	1.40E-05	3.51E-03	1.51
10457_at	10457	GPNMB	-0.04	6.81	-3.54	9.08E-04	3.33E-02	-2.54

We tested whether allergen-driven T-cell response patterns were associated with respiratory symptoms scores prior to SCIT (V1) employing *limma*. ProbeID = , EntrezID = , logFC = log base 2 fold change, AveExpr = average log2-expression level for that gene across all the arrays, t = moderated t-statistic, P.value = the associated p-value, adj.P.Val = the Benjamini & Hochberg adjusted P.value for multiple testing, B = the B-statistic is the log-odds that the gene is differentially expressed.

Supplementary Table E4. Top 100 differentially expressed genes (up/down) in CD4+ T cells during SCIT (V2, 3.5mths).

ProbeID	EntrezID	Symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
3553_at	3553	IL1B	4.18	8.34	15.11	1.17E-31	3.56E-30	61.32
5055_at	5055	SERPINB2	4.10	7.42	15.69	3.90E-33	1.45E-31	64.71
6347_at	6347	CCL2	4.03	7.76	13.16	1.46E-26	2.31E-25	49.62
4316_at	4316	MMP7	3.54	5.60	19.68	6.67E-43	8.37E-41	87.12
3552_at	3552	IL1A	3.37	5.66	13.92	1.47E-28	2.86E-27	54.20
6374_at	6374	CXCL5	3.28	10.79	14.22	2.38E-29	4.79E-28	56.02
2919_at	2919	CXCL1	3.26	7.97	14.94	3.30E-31	9.06E-30	60.28
3558_at	3558	IL2	3.18	4.57	19.64	8.38E-43	1.01E-40	86.89
1154_at	1154	CISH	2.98	7.68	22.07	2.29E-48	7.49E-46	99.64
2921_at	2921	CXCL3	2.91	6.43	13.79	3.15E-28	5.97E-27	53.44
6354_at	6354	CCL7	2.89	6.62	10.47	1.70E-19	1.31E-18	33.42
8835_at	8835	SOCS2	2.64	5.65	18.69	1.48E-40	1.34E-38	81.74
3578_at	3578	IL9	2.59	4.31	8.98	1.22E-15	6.52E-15	24.61
3576_at	3576	CXCL8	2.50	9.94	13.09	2.12E-26	3.32E-25	49.25
3559_at	3559	IL2RA	2.35	6.83	21.57	2.98E-47	8.10E-45	97.09
54602_at	54602	NDFIP2	2.34	4.93	14.31	1.42E-29	2.98E-28	56.53
6372_at	6372	CXCL6	2.26	3.96	10.04	2.27E-18	1.57E-17	30.85
6364_at	6364	CCL20	2.22	6.61	14.80	7.53E-31	1.92E-29	59.46
4312_at	4312	MMP1	2.18	3.93	10.31	4.55E-19	3.37E-18	32.45
3620_at	3620	IDO1	2.18	7.00	11.65	1.38E-22	1.42E-21	40.50
79413_at	79413	ZBED2	2.17	4.70	18.09	4.22E-39	3.28E-37	78.40
6348_at	6348	CCL3	2.15	7.63	10.25	6.48E-19	4.71E-18	32.09
51339_at	51339	DACT1	1.99	6.09	11.08	4.39E-21	3.82E-20	37.06
3002_at	3002	GZMB	1.96	6.84	10.40	2.69E-19	2.02E-18	32.97
3562_at	3562	IL3	1.90	3.28	9.46	7.56E-17	4.51E-16	27.37
8651_at	8651	SOCS1	1.85	6.17	21.04	4.88E-46	9.95E-44	94.31
114801_at	114801	TMEM200A	1.78	4.99	17.81	1.98E-38	1.41E-36	76.86
6367_at	6367	CCL22	1.77	9.06	15.35	2.95E-32	9.72E-31	62.69
7293_at	7293	TNFRSF4	1.75	7.04	15.75	2.74E-33	1.08E-31	65.06
2920_at	2920	CXCL2	1.70	8.02	9.29	2.02E-16	1.16E-15	26.39
10148_at	10148	EBI3	1.68	5.53	16.68	1.27E-35	6.28E-34	70.41
1847_at	1847	DUSP5	1.65	7.01	14.82	6.61E-31	1.71E-29	59.59
4495_at	4495	MT1G	1.61	7.62	8.20	1.16E-13	5.32E-13	20.09
6363_at	6363	CCL19	1.60	4.18	12.18	5.40E-24	6.30E-23	43.73
5732_at	5732	PTGER2	1.59	8.32	14.38	8.97E-30	1.98E-28	56.99
27074_at	27074	LAMP3	1.59	6.31	12.60	4.23E-25	5.57E-24	46.27
5996_at	5996	RGS1	1.56	8.12	14.38	8.96E-30	1.98E-28	56.99
112744_at	112744	IL17F	1.53	4.61	10.50	1.47E-19	1.14E-18	33.57
705_at	705	BYSL	1.53	6.53	22.78	6.33E-50	2.58E-47	103.22

ProbeID	EntrezID	Symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
6346_at	6346	CCL1	1.45	5.36	11.14	2.99E-21	2.70E-20	37.44
2791_at	2791	GNG11	1.45	5.78	8.09	2.16E-13	9.59E-13	19.48
50810_at	50810	HDGFL3	1.44	6.47	13.50	1.78E-27	3.17E-26	51.72
8553_at	8553	BHLHE40	1.43	7.04	16.62	1.73E-35	8.30E-34	70.11
3566_at	3566	IL4R	1.42	7.40	12.54	5.99E-25	7.76E-24	45.92
10409_at	10409	BASP1	1.42	7.65	12.85	9.23E-26	1.30E-24	47.78
3604_at	3604	TNFRSF9	1.40	5.40	14.35	1.09E-29	2.38E-28	56.79
4603_at	4603	MYBL1	1.40	8.36	13.49	1.97E-27	3.43E-26	51.62
5292_at	5292	PIM1	1.39	8.90	25.00	1.31E-54	2.13E-51	113.94
414062_at	414062	CCL3L3	1.39	7.18	8.21	1.04E-13	4.82E-13	20.20
6004_at	6004	RGS16	1.38	4.44	19.51	1.66E-42	1.94E-40	86.21
9332_at	9332	CD163	-1.09	4.99	-4.36	2.46E-05	5.53E-05	1.32
3434_at	3434	IFIT1	-1.12	8.38	-5.28	4.65E-07	1.25E-06	5.15
10437_at	10437	IFI30	-1.14	11.13	-8.69	6.69E-15	3.37E-14	22.92
7850_at	7850	IL1R2	-1.14	6.73	-5.93	2.05E-08	6.26E-08	8.20
5836_at	5836	PYGL	-1.15	5.39	-8.71	5.95E-15	3.01E-14	23.04
200315_at	200315	APOBEC3A	-1.16	4.78	-5.46	2.03E-07	5.64E-07	5.96
50856_at	50856	CLEC4A	-1.17	7.81	-9.93	4.38E-18	2.95E-17	30.19
22797_at	22797	TFEC	-1.17	5.45	-9.58	3.68E-17	2.26E-16	28.08
2517_at	2517	FUCA1	-1.17	7.69	-13.95	1.23E-28	2.40E-27	54.38
55016_at	55016	MARCH1	-1.18	4.41	-11.07	4.61E-21	4.00E-20	37.01
26509_at	26509	MYOF	-1.20	6.79	-9.41	9.99E-17	5.88E-16	27.09
10170_at	10170	DHRS9	-1.20	5.56	-10.99	7.40E-21	6.34E-20	36.54
54209_at	54209	TREM2	-1.21	5.13	-11.40	6.20E-22	6.03E-21	39.01
1378_at	1378	CR1	-1.23	5.94	-9.97	3.54E-18	2.40E-17	30.41
6252_at	6252	RTN1	-1.23	5.83	-12.21	4.62E-24	5.42E-23	43.89
26253_at	26253	CLEC4E	-1.23	6.17	-10.09	1.74E-18	1.21E-17	31.11
3109_at	3109	HLA-DMB	-1.23	6.84	-12.49	8.51E-25	1.09E-23	45.57
4286_at	4286	MITF	-1.23	6.07	-11.29	1.23E-21	1.17E-20	38.32
2934_at	2934	GSN	-1.28	8.83	-9.56	4.05E-17	2.48E-16	27.99
54_at	54	ACP5	-1.29	8.58	-12.99	4.10E-26	6.11E-25	48.59
5166_at	5166	PDK4	-1.32	4.20	-13.50	1.86E-27	3.29E-26	51.67
54504_at	54504	CPVL	-1.39	3.93	-9.78	1.12E-17	7.22E-17	29.27
10261_at	10261	IGSF6	-1.40	7.72	-9.75	1.34E-17	8.63E-17	29.09
84417_at	84417	C2orf40	-1.40	6.13	-16.05	4.72E-34	1.99E-32	66.81
1050_at	1050	CEBPA	-1.40	6.74	-12.37	1.71E-24	2.10E-23	44.88
3627_at	3627	CXCL10	-1.41	5.05	-5.06	1.26E-06	3.23E-06	4.19
51311_at	51311	TLR8	-1.48	5.23	-10.49	1.56E-19	1.20E-18	33.51
928_at	928	CD9	-1.52	8.26	-13.48	2.08E-27	3.60E-26	51.56
7305_at	7305	TYROBP	-1.56	10.08	-11.26	1.43E-21	1.34E-20	38.18
6383_at	6383	SDC2	-1.59	8.05	-10.17	1.08E-18	7.67E-18	31.59
2219_at	2219	FCN1	-1.76	5.88	-10.47	1.76E-19	1.35E-18	33.39

ProbeID	EntrezID	Symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
7941_at	7941	PLA2G7	-1.79	8.92	-11.54	2.62E-22	2.64E-21	39.86
10288_at	10288	LILRB2	-1.79	6.24	-10.14	1.24E-18	8.71E-18	31.45
1536_at	1536	CYBB	-1.79	7.54	-12.85	9.04E-26	1.28E-24	47.80
760_at	760	CA2	-1.83	6.49	-13.57	1.19E-27	2.16E-26	52.11
2359_at	2359	FPR3	-1.87	6.59	-12.99	3.93E-26	5.89E-25	48.63
6039_at	6039	RNASE6	-1.88	6.97	-14.51	4.25E-30	9.90E-29	57.73
2214_at	2214	FCGR3A	-1.92	5.99	-8.39	3.85E-14	1.85E-13	21.19
4199_at	4199	ME1	-1.99	5.39	-16.08	4.08E-34	1.75E-32	66.95
4332_at	4332	MNDA	-2.09	5.49	-11.92	2.69E-23	2.95E-22	42.13
6369_at	6369	CCL24	-2.12	9.65	-11.94	2.37E-23	2.64E-22	42.25
4069_at	4069	LYZ	-2.22	10.88	-14.93	3.43E-31	9.33E-30	60.24
948_at	948	CD36	-2.29	5.46	-15.39	2.29E-32	7.71E-31	62.94
10457_at	10457	GPNMB	-2.29	6.47	-14.20	2.74E-29	5.45E-28	55.88
3957_at	3957	LGALS2	-2.36	5.49	-8.83	2.92E-15	1.51E-14	23.74
10875_at	10875	FGL2	-2.55	8.18	-16.73	9.16E-36	4.60E-34	70.74
79887_at	79887	PLBD1	-2.71	6.48	-19.47	2.01E-42	2.19E-40	86.02
7045_at	7045	TGFBI	-2.77	7.64	-16.85	4.62E-36	2.39E-34	71.42
6035_at	6035	RNASE1	-3.15	6.30	-15.72	3.27E-33	1.26E-31	64.88
2167_at	2167	FABP4	-3.70	6.90	-18.39	7.91E-40	6.80E-38	80.07

Differentially expressed genes were identified with *limma* contrasting HDM versus CTRL in CD4+ T cells during SCIT (V2, 3.5mths). ProbeID = , EntrezID = , logFC = log base 2 fold change, AveExpr = average log2-expression level for that gene across all the arrays, t = moderated t-statistic, P.value = the associated p-value, adj.P.Val = the Benjamini & Hochberg adjusted P.value for multiple testing, B = the B-statistic is the log-odds that the gene is differentially expressed.

Supplementary Table E5. Top 100 differentially expressed genes (up/down) in CD4+ T cells during SCIT (V4, 12mths).

ProbeID	EntrezID	Symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
5055_at	5055	SERPINB2	3.01	7.42	11.19	2.30E-21	1.07E-19	37.91
6347_at	6347	CCL2	3.01	7.76	9.52	5.06E-17	1.04E-15	28.01
6354_at	6354	CCL7	2.93	6.62	10.32	4.26E-19	1.24E-17	32.74
3553_at	3553	IL1B	2.77	8.34	9.69	1.83E-17	3.98E-16	29.01
1154_at	1154	CISH	2.23	7.68	16.00	6.28E-34	3.42E-31	66.59
3558_at	3558	IL2	2.18	4.57	13.05	2.80E-26	2.19E-24	49.12
6374_at	6374	CXCL5	2.10	10.79	8.85	2.69E-15	4.13E-14	24.08
2921_at	2921	CXCL3	2.01	6.43	9.21	3.16E-16	5.52E-15	26.20
3552_at	3552	IL1A	1.98	5.66	7.93	5.27E-13	5.58E-12	18.87
2919_at	2919	CXCL1	1.94	7.97	8.62	9.94E-15	1.37E-13	22.79
8835_at	8835	SOCS2	1.94	5.65	13.34	4.66E-27	5.25E-25	50.90
3559_at	3559	IL2RA	1.77	6.83	15.82	1.84E-33	7.50E-31	65.52
4316_at	4316	MMP7	1.72	5.60	9.26	2.40E-16	4.34E-15	26.47
51339_at	51339	DACT1	1.48	6.09	8.02	3.20E-13	3.55E-12	19.37
54602_at	54602	NDFIP2	1.47	4.93	8.71	5.97E-15	8.52E-14	23.29
6364_at	6364	CCL20	1.46	6.61	9.43	8.71E-17	1.69E-15	27.47
2791_at	2791	GNG11	1.44	5.78	7.80	1.11E-12	1.13E-11	18.14
6372_at	6372	CXCL6	1.41	3.96	6.10	9.10E-09	5.19E-08	9.29
79413_at	79413	ZBED2	1.37	4.70	11.07	4.59E-21	2.03E-19	37.22
3576_at	3576	CXCL8	1.36	9.94	6.90	1.45E-10	1.05E-09	13.35
8651_at	8651	SOCS1	1.31	6.17	14.42	7.44E-30	1.16E-27	57.29
6348_at	6348	CCL3	1.23	7.63	5.70	6.47E-08	3.18E-07	7.38
114801_at	114801	TMEM200A	1.21	4.99	11.79	5.70E-23	3.58E-21	41.57
3562_at	3562	IL3	1.18	3.28	5.69	6.59E-08	3.22E-07	7.37
718_at	718	C3	1.16	5.54	7.77	1.25E-12	1.26E-11	18.02
4495_at	4495	MT1G	1.16	7.62	5.69	6.66E-08	3.25E-07	7.35
3578_at	3578	IL9	1.15	4.31	3.88	1.61E-04	4.67E-04	-0.14
4603_at	4603	MYBL1	1.14	8.36	10.61	7.59E-20	2.55E-18	34.44
3002_at	3002	GZMB	1.13	6.84	5.79	4.12E-08	2.11E-07	7.82
5732_at	5732	PTGER2	1.12	8.32	9.83	8.12E-18	1.85E-16	29.82
2920_at	2920	CXCL2	1.12	8.02	5.91	2.26E-08	1.20E-07	8.41
7980_at	7980	TFPI2	1.11	4.41	6.95	1.13E-10	8.40E-10	13.59
6367_at	6367	CCL22	1.10	9.06	9.20	3.49E-16	6.05E-15	26.10
705_at	705	BYSL	1.08	6.53	15.67	4.46E-33	1.62E-30	64.64
84830_at	84830	ADTRP	1.07	6.19	13.33	5.24E-27	5.53E-25	50.79
5292_at	5292	PIM1	1.05	8.90	18.35	9.86E-40	1.61E-36	79.82
4496_at	4496	MT1H	1.03	7.64	5.76	4.74E-08	2.41E-07	7.69
91523_at	91523	PCED1B	1.02	8.77	16.97	2.29E-36	2.49E-33	72.15
55022_at	55022	PID1	1.02	6.03	5.58	1.14E-07	5.35E-07	6.83

ProbeID	EntrezID	Symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
170691_at	170691	ADAMTS17	1.02	5.21	12.50	7.96E-25	5.65E-23	45.81
4312_at	4312	MMP1	1.01	3.93	4.62	8.32E-06	2.92E-05	2.68
3624_at	3624	INHBA	1.00	6.01	9.28	2.16E-16	3.99E-15	26.57
6447_at	6447	SCG5	1.00	5.08	6.61	6.88E-10	4.64E-09	11.82
25902_at	25902	MTHFD1L	0.99	7.03	18.41	6.91E-40	1.61E-36	80.17
6875_at	6875	TAF4B	0.97	6.83	14.94	3.25E-31	6.24E-29	60.39
1839_at	1839	HBEGF	0.95	4.19	9.85	7.45E-18	1.71E-16	29.90
3566_at	3566	IL4R	0.93	7.40	7.92	5.38E-13	5.68E-12	18.85
1847_at	1847	DUSP5	0.92	7.01	8.01	3.33E-13	3.66E-12	19.32
1462_at	1462	VCAN	0.92	4.05	6.44	1.62E-09	1.04E-08	10.98
7293_at	7293	TNFRSF4	0.89	7.04	7.74	1.48E-12	1.47E-11	17.86
1823_at	1823	DSC1	-0.83	5.50	-7.65	2.46E-12	2.36E-11	17.35
3434_at	3434	IFIT1	-0.83	8.38	-3.82	2.00E-04	5.71E-04	-0.35
7077_at	7077	TIMP2	-0.84	7.27	-8.61	1.07E-14	1.48E-13	22.71
6614_at	6614	SIGLEC1	-0.85	5.61	-6.05	1.16E-08	6.43E-08	9.06
8714_at	8714	ABCC3	-0.86	5.55	-7.41	9.18E-12	8.15E-11	16.06
760_at	760	CA2	-0.86	6.49	-6.16	6.72E-09	3.90E-08	9.59
84417_at	84417	C2orf40	-0.86	6.13	-9.59	3.47E-17	7.25E-16	28.38
3597_at	3597	IL13RA1	-0.89	5.44	-7.07	5.97E-11	4.67E-10	14.22
22797_at	22797	TFEC	-0.90	5.45	-7.16	3.70E-11	2.98E-10	14.69
5836_at	5836	PYGL	-0.95	5.39	-6.95	1.14E-10	8.41E-10	13.59
3109_at	3109	HLA-DMB	-0.95	6.84	-9.30	1.87E-16	3.49E-15	26.71
50856_at	50856	CLEC4A	-0.97	7.81	-7.97	4.28E-13	4.59E-12	19.08
7850_at	7850	IL1R2	-0.97	6.73	-4.91	2.41E-06	9.10E-06	3.88
120892_at	120892	LRRK2	-0.99	5.13	-8.76	4.43E-15	6.43E-14	23.59
4286_at	4286	MITF	-0.99	6.07	-8.81	3.41E-15	5.07E-14	23.85
928_at	928	CD9	-0.99	8.26	-8.55	1.53E-14	2.05E-13	22.37
10410_at	10410	IFITM3	-1.04	6.59	-5.82	3.62E-08	1.87E-07	7.95
7305_at	7305	TYROBP	-1.04	10.08	-7.32	1.57E-11	1.32E-10	15.53
6252_at	6252	RTN1	-1.06	5.83	-10.26	6.06E-19	1.73E-17	32.39
1536_at	1536	CYBB	-1.08	7.54	-7.53	4.77E-12	4.40E-11	16.70
2359_at	2359	FPR3	-1.09	6.59	-7.33	1.44E-11	1.22E-10	15.62
54504_at	54504	CPVL	-1.10	3.93	-7.50	5.82E-12	5.30E-11	16.51
1050_at	1050	CEBPA	-1.10	6.74	-9.40	1.05E-16	2.01E-15	27.29
10288_at	10288	LILRB2	-1.10	6.24	-6.05	1.14E-08	6.30E-08	9.08
55016_at	55016	MARCH1	-1.10	4.41	-10.06	2.04E-18	5.28E-17	31.19
6369_at	6369	CCL24	-1.12	9.65	-6.09	9.29E-09	5.28E-08	9.27
2214_at	2214	FCGR3A	-1.12	5.99	-4.75	4.78E-06	1.73E-05	3.22
216_at	216	ALDH1A1	-1.13	3.62	-9.91	4.95E-18	1.19E-16	30.31
10170_at	10170	DHRS9	-1.13	5.56	-10.06	2.09E-18	5.34E-17	31.16
5166_at	5166	PDK4	-1.14	4.20	-11.29	1.18E-21	5.89E-20	38.57
7941_at	7941	PLA2G7	-1.15	8.92	-7.20	2.93E-11	2.40E-10	14.92

ProbeID	EntrezID	Symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
6039_at	6039	RNASE6	-1.16	6.97	-8.67	7.45E-15	1.05E-13	23.07
26253_at	26253	CLEC4E	-1.16	6.17	-9.25	2.54E-16	4.53E-15	26.41
54_at	54	ACP5	-1.16	8.58	-11.38	6.85E-22	3.55E-20	39.11
54209_at	54209	TREM2	-1.18	5.13	-10.76	3.10E-20	1.11E-18	35.33
51311_at	51311	TLR8	-1.25	5.23	-8.59	1.21E-14	1.65E-13	22.60
3627_at	3627	CXCL10	-1.27	5.05	-4.44	1.76E-05	5.85E-05	1.96
200315_at	200315	APOBEC3A	-1.29	4.78	-5.88	2.65E-08	1.39E-07	8.25
4069_at	4069	LYZ	-1.45	10.88	-9.46	7.25E-17	1.43E-15	27.65
4332_at	4332	MNDA	-1.46	5.49	-8.06	2.49E-13	2.84E-12	19.61
2219_at	2219	FCN1	-1.48	5.88	-8.56	1.44E-14	1.95E-13	22.42
10457_at	10457	GPNMB	-1.48	6.47	-8.91	1.83E-15	2.89E-14	24.46
2167_at	2167	FABP4	-1.52	6.90	-7.32	1.51E-11	1.27E-10	15.57
948_at	948	CD36	-1.69	5.46	-11.01	6.85E-21	2.94E-19	36.83
3957_at	3957	LGALS2	-1.73	5.49	-6.27	3.79E-09	2.29E-08	10.15
4199_at	4199	ME1	-1.76	5.39	-13.80	2.99E-28	3.75E-26	53.63
7045_at	7045	TGFBI	-1.82	7.64	-10.71	4.13E-20	1.45E-18	35.05
10875_at	10875	FGL2	-1.82	8.18	-11.56	2.39E-22	1.30E-20	40.15
6035_at	6035	RNASE1	-1.94	6.30	-9.39	1.09E-16	2.07E-15	27.25
79887_at	79887	PLBD1	-2.23	6.48	-15.53	9.82E-33	2.91E-30	63.86

Differentially expressed genes were identified with *limma* contrasting HDM versus CTRL in CD4+ T cells during SCIT (V4, 12 mths). ProbeID = , EntrezID = , logFC = log base 2 fold change, AveExpr = average log2-expression level for that gene across all the arrays, t = moderated t-statistic, P.value = the associated p-value, adj.P.Val = the Benjamini & Hochberg adjusted P.value for multiple testing, B = the B-statistic is the log-odds that the gene is differentially expressed.

Supplementary Table E6. Top 100 differentially expressed genes (up/down) in CD4+ T cells during SCIT (V5, 24mths).

ProbeID	EntrezID	Symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
6374_at	6374	CXCL5	3.39	8.33	11.51	7.02E-12	7.50E-11	16.98
6347_at	6347	CCL2	3.25	5.41	12.77	6.50E-13	1.05E-11	19.40
5055_at	5055	SERPINB2	3.04	5.91	10.50	5.34E-11	4.51E-10	14.92
1154_at	1154	CISH	2.69	7.34	20.71	4.95E-18	8.61E-16	31.28
3553_at	3553	IL1B	2.62	7.23	9.65	3.22E-10	2.18E-09	13.09
3558_at	3558	IL2	2.42	4.19	15.85	3.80E-15	1.69E-13	24.61
3578_at	3578	IL9	2.41	3.89	5.98	2.26E-06	6.56E-06	4.11
8835_at	8835	SOCS2	2.39	4.70	17.80	2.21E-16	1.58E-14	27.47
6354_at	6354	CCL7	2.35	4.62	9.77	2.48E-10	1.74E-09	13.36
4316_at	4316	MMP7	2.13	4.29	10.95	2.12E-11	1.99E-10	15.86
79413_at	79413	ZBED2	2.10	4.09	16.07	2.74E-15	1.33E-13	24.94
3559_at	3559	IL2RA	2.09	5.85	25.81	1.78E-20	2.27E-17	36.84
3552_at	3552	IL1A	2.04	4.74	8.44	4.99E-09	2.50E-08	10.30
2919_at	2919	CXCL1	1.91	6.74	9.06	1.20E-09	6.94E-09	11.76
54602_at	54602	NDFIP2	1.90	4.40	12.36	1.39E-12	1.96E-11	18.63
6364_at	6364	CCL20	1.79	5.76	15.73	4.59E-15	2.00E-13	24.42
3002_at	3002	GZMB	1.74	6.23	7.92	1.72E-08	7.63E-08	9.04
3562_at	3562	IL3	1.73	2.78	6.71	3.47E-07	1.17E-06	6.00
114801_at	114801	TMEM200A	1.70	4.91	17.46	3.55E-16	2.23E-14	27.00
8651_at	8651	SOCS1	1.69	5.77	20.43	6.98E-18	1.11E-15	30.93
2921_at	2921	CXCL3	1.68	4.74	9.56	3.91E-10	2.60E-09	12.89
51339_at	51339	DACT1	1.66	5.45	10.41	6.37E-11	5.25E-10	14.74
3576_at	3576	CXCL8	1.61	8.14	9.04	1.25E-09	7.22E-09	11.71
7293_at	7293	TNFRSF4	1.58	6.75	12.73	6.98E-13	1.12E-11	19.33
1847_at	1847	DUSP5	1.54	6.21	13.24	2.83E-13	5.39E-12	20.24
5732_at	5732	PTGER2	1.52	7.76	18.87	5.17E-17	5.21E-15	28.93
5996_at	5996	RGS1	1.45	7.74	16.77	9.61E-16	5.10E-14	25.99
3620_at	3620	IDO1	1.42	5.76	14.21	5.26E-14	1.36E-12	21.95
6367_at	6367	CCL22	1.41	7.99	16.84	8.73E-16	4.84E-14	26.09
5292_at	5292	PIM1	1.40	8.04	28.56	1.29E-21	4.67E-18	39.40
50810_at	50810	HDGFL3	1.36	5.64	17.79	2.23E-16	1.58E-14	27.46
115362_at	115362	GBP5	1.33	8.56	14.51	3.24E-14	8.98E-13	22.44
112744_at	112744	IL17F	1.32	4.21	5.51	7.86E-06	2.06E-05	2.86
3604_at	3604	TNFRSF9	1.30	4.71	13.28	2.64E-13	5.12E-12	20.31
8553_at	8553	BHLHE40	1.30	6.73	14.49	3.36E-14	9.23E-13	22.40
6348_at	6348	CCL3	1.29	6.06	5.57	6.71E-06	1.77E-05	3.02
27074_at	27074	LAMP3	1.29	5.00	19.11	3.78E-17	4.25E-15	29.25
3458_at	3458	IFNG	1.29	4.45	8.53	4.02E-09	2.06E-08	10.52
25902_at	25902	MTHFD1L	1.26	6.36	24.97	4.20E-20	4.01E-17	36.00

ProbeID	EntrezID	Symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
7291_at	7291	TWIST1	1.25	3.19	11.35	9.56E-12	9.83E-11	16.67
6875_at	6875	TAF4B	1.24	6.28	22.11	9.39E-19	2.63E-16	32.93
705_at	705	BYSL	1.24	6.13	20.76	4.69E-18	8.58E-16	31.33
6004_at	6004	RGS16	1.24	4.03	18.96	4.57E-17	4.99E-15	29.06
4603_at	4603	MYBL1	1.23	7.95	18.74	6.18E-17	5.91E-15	28.75
170691_at	170691	ADAMTS17	1.20	4.91	15.27	9.37E-15	3.35E-13	23.69
3566_at	3566	IL4R	1.20	6.80	11.02	1.86E-11	1.78E-10	15.99
3596_at	3596	IL13	1.17	3.87	6.09	1.72E-06	5.08E-06	4.39
4312_at	4312	MMP1	1.15	3.13	4.81	5.15E-05	1.20E-04	0.98
84830_at	84830	ADTRP	1.12	6.02	19.74	1.67E-17	2.36E-15	30.06
84937_at	84937	ZNRF1	1.11	6.62	19.13	3.68E-17	4.25E-15	29.27
8714_at	8714	ABCC3	-0.98	4.69	-7.76	2.53E-08	1.08E-07	8.66
3108_at	3108	HLA-DMA	-0.99	6.18	-9.21	8.59E-10	5.25E-09	12.09
3597_at	3597	IL13RA1	-0.99	3.85	-7.60	3.68E-08	1.53E-07	8.28
29992_at	29992	PILRA	-0.99	6.18	-8.87	1.85E-09	1.03E-08	11.31
55016_at	55016	MARCH1	-1.01	3.11	-8.12	1.05E-08	4.86E-08	9.55
2517_at	2517	FUCA1	-1.02	6.50	-8.40	5.40E-09	2.67E-08	10.22
140807_at	140807	KRT72	-1.04	6.09	-17.52	3.26E-16	2.15E-14	27.08
120892_at	120892	LRRK2	-1.04	3.55	-7.63	3.48E-08	1.46E-07	8.33
22797_at	22797	TFEC	-1.04	3.70	-7.96	1.54E-08	6.88E-08	9.16
4286_at	4286	MITF	-1.05	4.86	-8.58	3.62E-09	1.88E-08	10.63
5836_at	5836	PYGL	-1.10	3.80	-7.85	2.01E-08	8.81E-08	8.89
50856_at	50856	CLEC4A	-1.12	6.34	-9.91	1.86E-10	1.34E-09	13.65
928_at	928	CD9	-1.12	6.75	-8.15	9.92E-09	4.63E-08	9.61
1471_at	1471	CST3	-1.12	4.78	-8.67	2.89E-09	1.54E-08	10.86
1823_at	1823	DSC1	-1.13	4.72	-14.17	5.66E-14	1.41E-12	21.87
1378_at	1378	CR1	-1.13	5.42	-15.70	4.81E-15	2.07E-13	24.37
10437_at	10437	IFI30	-1.13	9.57	-8.84	1.96E-09	1.09E-08	11.26
6252_at	6252	RTN1	-1.14	4.68	-9.55	4.06E-10	2.68E-09	12.85
2934_at	2934	GSN	-1.16	7.25	-8.02	1.35E-08	6.11E-08	9.29
1050_at	1050	CEBPA	-1.18	5.83	-8.84	1.99E-09	1.10E-08	11.24
5166_at	5166	PDK4	-1.23	3.07	-9.76	2.54E-10	1.77E-09	13.33
84417_at	84417	C2orf40	-1.24	5.53	-17.06	6.28E-16	3.64E-14	26.42
760_at	760	CA2	-1.26	4.80	-7.81	2.22E-08	9.65E-08	8.79
26253_at	26253	CLEC4E	-1.28	4.86	-9.86	2.06E-10	1.47E-09	13.55
10462_at	10462	CLEC10A	-1.29	4.00	-6.90	2.10E-07	7.34E-07	6.51
2359_at	2359	FPR3	-1.29	5.43	-8.88	1.79E-09	1.01E-08	11.35
51311_at	51311	TLR8	-1.30	4.01	-8.33	6.51E-09	3.15E-08	10.03
6383_at	6383	SDC2	-1.31	6.07	-9.10	1.10E-09	6.45E-09	11.84
54504_at	54504	CPVL	-1.36	3.09	-8.01	1.37E-08	6.20E-08	9.28
2214_at	2214	FCGR3A	-1.36	4.06	-7.05	1.44E-07	5.23E-07	6.89
3109_at	3109	HLA-DMB	-1.39	5.27	-10.89	2.39E-11	2.21E-10	15.73

ProbeID	EntrezID	Symbol	logFC	AveExpr	t	P.Value	adj.P.Val	B
4199_at	4199	ME1	-1.40	3.77	-8.63	3.17E-09	1.68E-08	10.76
10261_at	10261	IGSF6	-1.40	5.84	-10.06	1.33E-10	1.01E-09	13.99
6369_at	6369	CCL24	-1.46	7.25	-8.28	7.31E-09	3.51E-08	9.92
10288_at	10288	LILRB2	-1.56	4.50	-9.22	8.39E-10	5.14E-09	12.12
10457_at	10457	GPNMB	-1.63	4.52	-11.55	6.46E-12	7.06E-11	17.06
7305_at	7305	TYROBP	-1.64	8.42	-13.33	2.39E-13	4.72E-12	20.42
1536_at	1536	CYBB	-1.70	5.64	-12.86	5.53E-13	9.27E-12	19.56
7941_at	7941	PLA2G7	-1.73	7.07	-12.37	1.36E-12	1.93E-11	18.65
6039_at	6039	RNASE6	-1.78	5.31	-11.54	6.55E-12	7.12E-11	17.05
2219_at	2219	FCN1	-1.83	5.10	-9.53	4.17E-10	2.74E-09	12.83
948_at	948	CD36	-1.85	4.28	-11.06	1.71E-11	1.64E-10	16.08
4332_at	4332	MNDA	-1.85	3.66	-8.47	4.59E-09	2.33E-08	10.39
10875_at	10875	FGL2	-1.95	6.62	-10.51	5.16E-11	4.38E-10	14.95
7045_at	7045	TGFBI	-2.08	6.21	-12.14	2.10E-12	2.71E-11	18.21
3957_at	3957	LGALS2	-2.25	4.40	-9.94	1.73E-10	1.26E-09	13.72
2167_at	2167	FABP4	-2.30	4.61	-9.39	5.75E-10	3.64E-09	12.50
4069_at	4069	LYZ	-2.34	9.29	-15.34	8.50E-15	3.12E-13	23.79
6035_at	6035	RNASE1	-2.38	5.47	-10.34	7.40E-11	6.01E-10	14.59
79887_at	79887	PLBD1	-2.40	5.07	-11.47	7.55E-12	7.95E-11	16.91

Differentially expressed genes were identified with *limma* contrasting HDM versus CTRL in CD4+ T cells during SCIT (V5, 24mths). ProbeID = , EntrezID = , logFC = log base 2 fold change, AveExpr = average log2-expression level for that gene across all the arrays, t = moderated t-statistic, P.value = the associated p-value, adj.P.Val = the Benjamini & Hochberg adjusted P.value for multiple testing, B = the B-statistic is the log-odds that the gene is differentially expressed.

Supplementary Table E7. Cytoscape metrics for the network at V5.

GeneID	Module assignment V1	BetweennessCentrality	Degree
PRDX4	IL2 signalling (I)	0.1616	20
BATF	IL2 signalling (I)	0.1297	43
CISH	Th2 (F)	0.1243	42
NDFIP2	Th2 (F)	0.1056	20
MTHFD2	Inflammation (G)	0.0899	11
PSMD14	Inflammation (G)	0.0873	7
MVP	IL2 signalling (I)	0.0768	26
SOCS1	IL2 signalling (I)	0.0725	48
DTX3L	Type 1 IFN (A)	0.0564	14
SOCS2	IL2 signalling (I)	0.0508	31
BHLHE40	Th2 (F)	0.0475	26
APOL1	Type 1 IFN (A)	0.0462	12
PSME1	Type 1 IFN (A)	0.0458	17
PARP9	Type 1 IFN (A)	0.0457	16
IL2	IL2 signalling (I)	0.0445	31
PIM1	IL2 signalling (I)	0.0442	37
PSMA3	Inflammation (G)	0.0431	5
ARID5A	IL2 signalling (I)	0.0402	32
IL2RA	IL2 signalling (I)	0.0402	40
ZBED2	IL2 signalling (I)	0.0374	33

Network wiring diagrams were constructed utilising the top-weighted 800 gene-gene interactions extracted from the adjacency matrix from the expression data. BetweennessCentrality = genes in the network that have many "shortest paths" going through them, i.e. connector genes. The closeness centrality measure of each gene to another in the network is a number between 0 and 1; Degree = number of connections with other genes in the network.

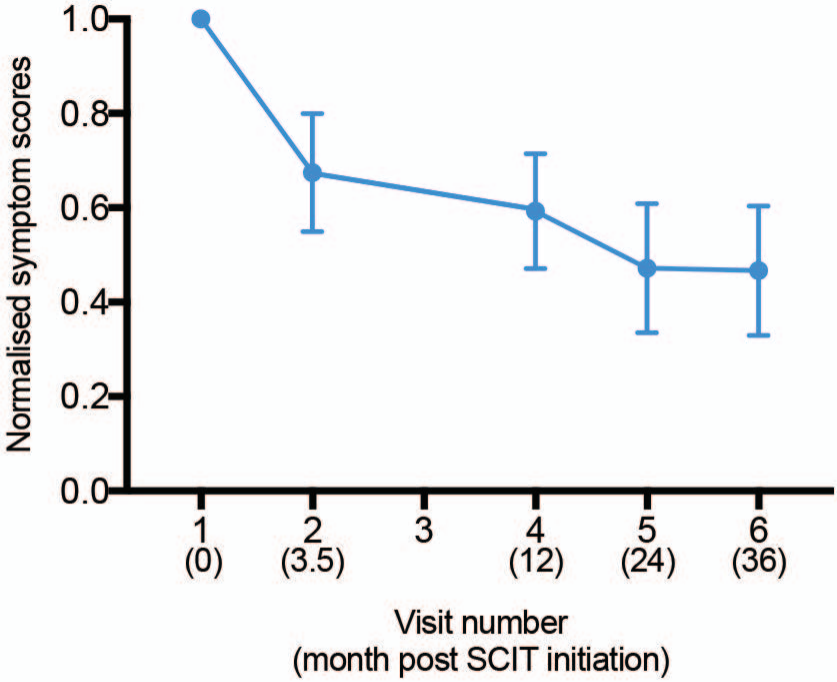

1.0
0.8
0.6
0.4
0.2
0.0
Normalised symptom scores
1
(0)
2
(3.5)
3
4
(12)
5
(24)
6
(36)
Visit number
(month post SCIT initiation)

Figure 1

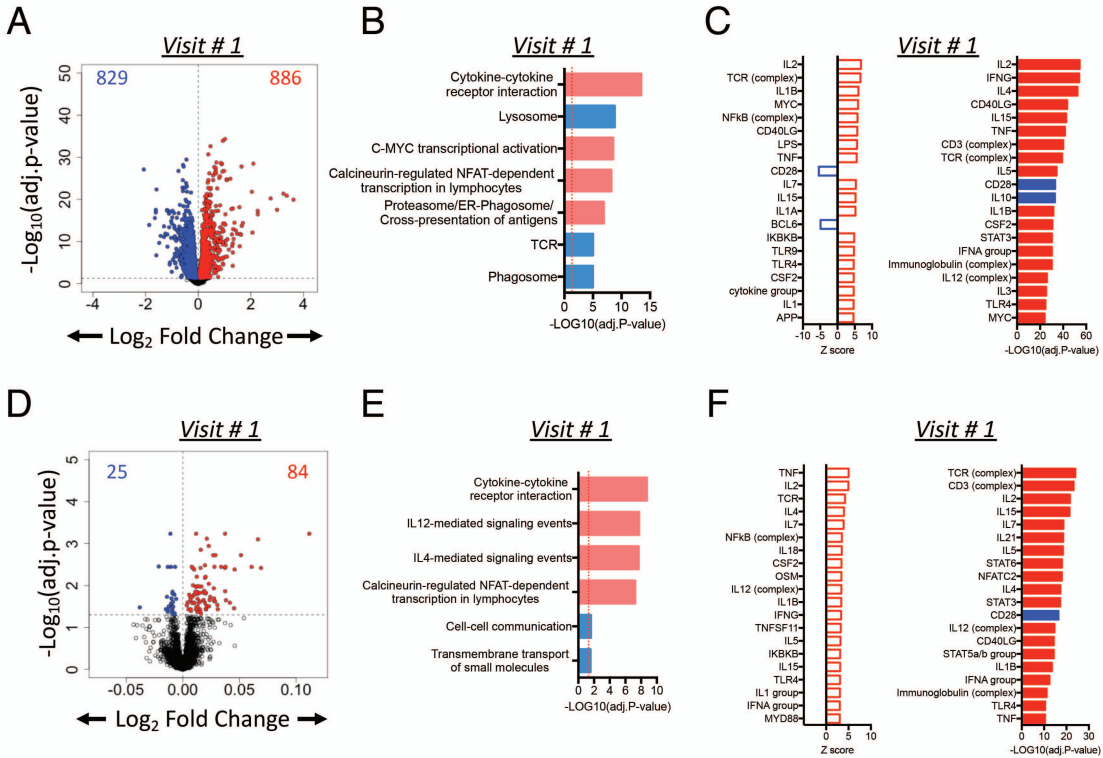

A
Visit # 1
829
886
-Log10(adj.p-value)
Log2 Fold Change
B
Visit # 1
Cytokine-cytokine receptor interaction
Lysosome
C-MYC transcriptional activation
Calcineurin-regulated NFAT-dependent transcription in lymphocytes
Proteasome/ER-Phagosome/ Cross-presentation of antigens
TCR
Phagosome
-LOG10(adj.P-value)
C
Visit # 1
IL2
TCR (complex)
IL1B
MYC
NFkB (complex)
CD40LG
LPS
TNF
CD28
IL7
IL15
IL1A
BCL6
IKBKB
TLR9
TLR4
CSF2
cytokine group
IL1
APP
Z score
IL2
IFNG
IL4
CD40LG
IL15
TNF
CD3 (complex)
TCR (complex)
IL5
CD28
IL10
IL1B
CSF2
STAT3
IFNA group
Immunoglobulin (complex)
IL12 (complex)
IL3
TLR4
MYC
-LOG10(adj.P-value)
D
Visit # 1
25
84
-Log10(adj.p-value)
Log2 Fold Change
E
Visit # 1
Cytokine-cytokine receptor interaction
IL12-mediated signaling events
IL4-mediated signaling events
Calcineurin-regulated NFAT-dependent transcription in lymphocytes
Cell-cell communication
Transmembrane transport of small molecules
-LOG10(adj.P-value)
F
Visit # 1
TNF
IL2
TCR
IL4
IL7
NFkB (complex)
IL18
CSF2
OSM
IL12 (complex)
IL1B
IFNG
TNFSF11
IL5
IKBKB
IL15
TLR4
IL1 group
IFNA group
MYD88
Z score
TCR (complex)
CD3 (complex)
IL2
IL15
IL7
IL21
IL5
STAT6
NFATC2
IL4
STAT3
CD28
IL12 (complex)
CD40LG
STAT5a/b group
IL1B
IFNA group
Immunoglobulin (complex)
TLR4
TNF
-LOG10(adj.P-value)

Figure 2

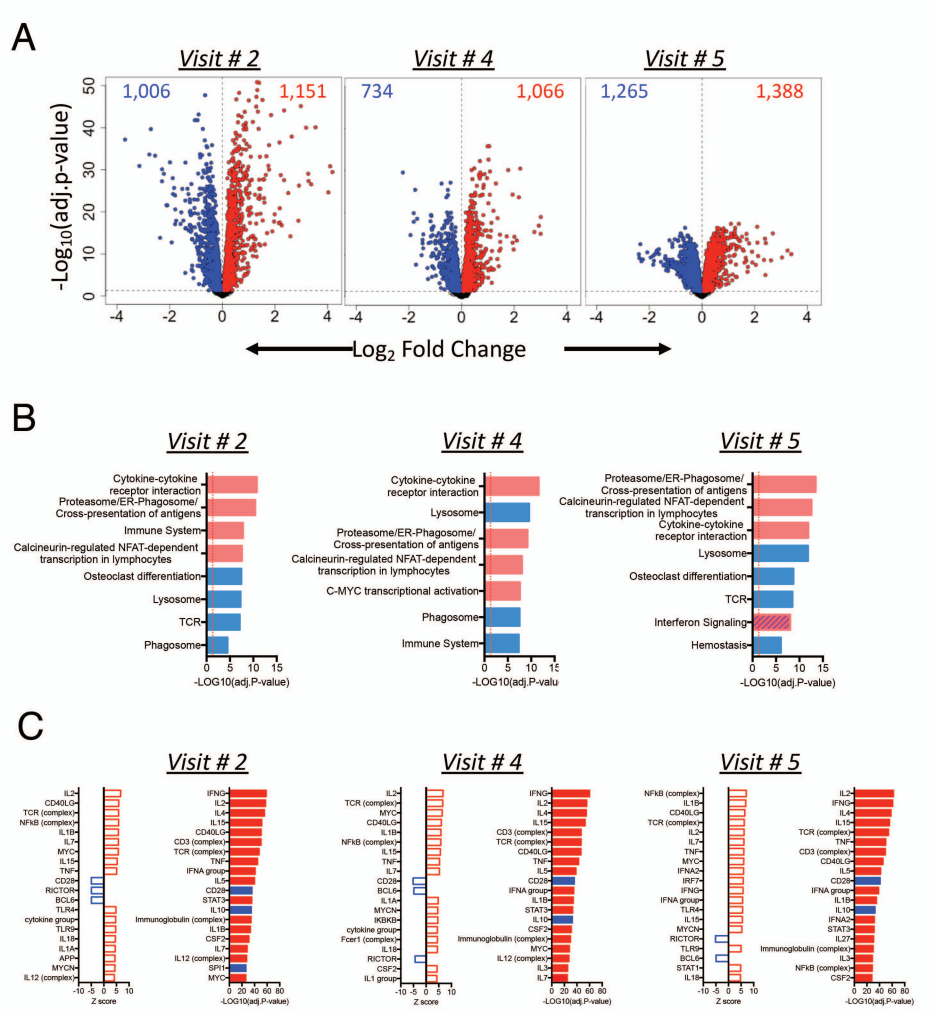
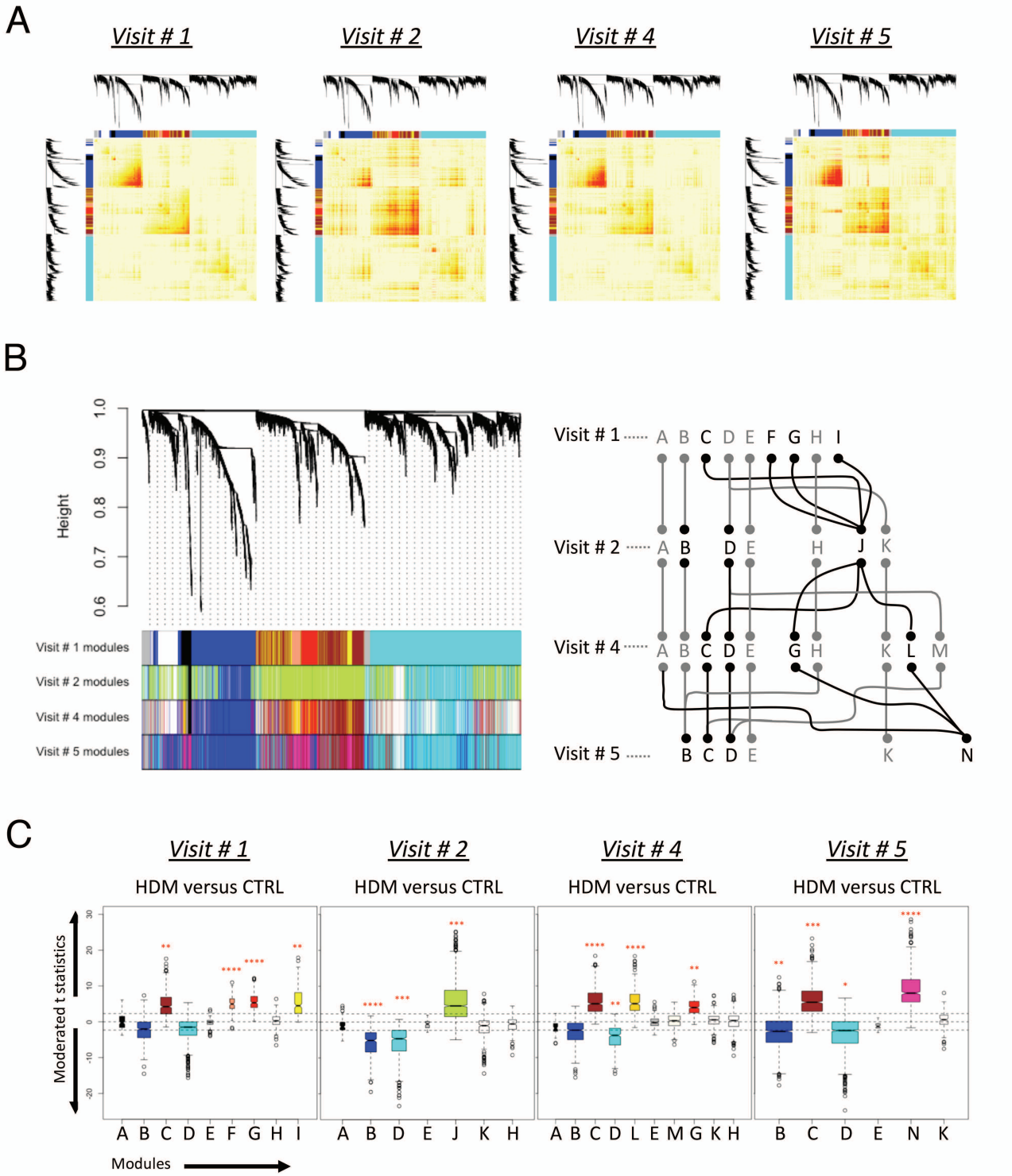

A
Visit # 2
Visit # 4
Visit # 5
1,006
1,151
734
1,066
1,265
1,388
-Log10(adj.p-value)
Log2 Fold Change
B
Visit # 2
Cytokine-cytokine receptor interaction
Proteasome/ER-Phagosome/ Cross-presentation of antigens
Immune System
Calcineurin-regulated NFAT-dependent transcription in lymphocytes
Osteoclast differentiation
Lysosome
TCR
Phagosome
-LOG10(adj.P-value)
Visit # 4
Cytokine-cytokine receptor interaction
Lysosome
Proteasome/ER-Phagosome/ Cross-presentation of antigens
Calcineurin-regulated NFAT-dependent transcription in lymphocytes
C-MYC transcriptional activation
Phagosome
Immune System
-LOG10(adj.P-valu
Visit # 5
Proteasome/ER-Phagosome/ Cross-presentation of antigens
Calcineurin-regulated NFAT-dependent transcription in lymphocytes
Cytokine-cytokine receptor interaction
Lysosome
Osteoclast differentiation
TCR
Interferon Signaling
Hemostasis
-LOG10(adj.P-value)
C
Visit # 2
Z score
-LOG10(adj.P-value)
Visit # 4
Z score
-LOG10(adj.P-value)
Visit # 5
Z score
-LOG10(adj.P-value)

Figure 3



Module Legend			
A	Type 1 interferon – not differentially expressed	**H**	FAS signaling – not differentially expressed
B	Lysosome/phagosome/antigen processing & presentation	**I**	IL2 signaling / Foxp3
C	Ribosome/Translation/Metabolism of proteins	**J**	Th2, inflammation/proteasome/phagosome & cytokine receptor interaction, metabolism of proteins, IL2 signalling/Foxp3,
D	TCR signaling	**K**	Transcription & IL6 signalling – not differentially expressed
E	Unassigned/unclustered genes – not differentially expressed	**L**	Th2, proteasome/phagosome & cytokine receptor interaction, metabolism of proteins, IL2 signalling/Foxp3
F	Th2	**M**	Ribosomal translation – not differentially expressed
G	Inflammation/chemokine & cytokine receptor interactions/phagosome/proteasome	**N**	Th2, inflammation/proteasome/phagosome & cytokine receptor interaction, metabolism of proteins, IL2 signaling/Foxp3, **type 1 interferon**

Median adj. p-value: * = 0.05, ** = 0.01, *** = 0.001, **** = 0.0001

Figure 4

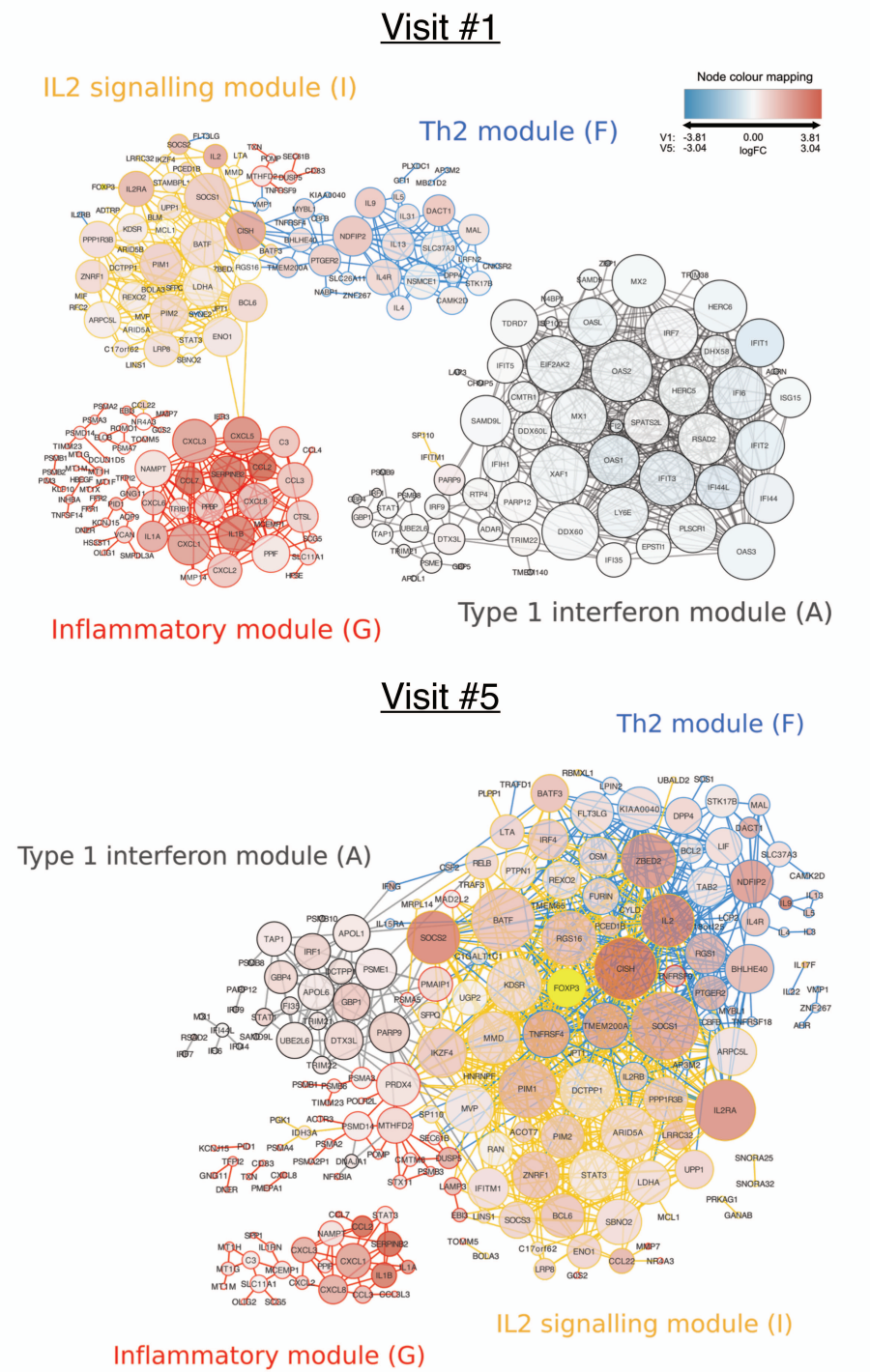
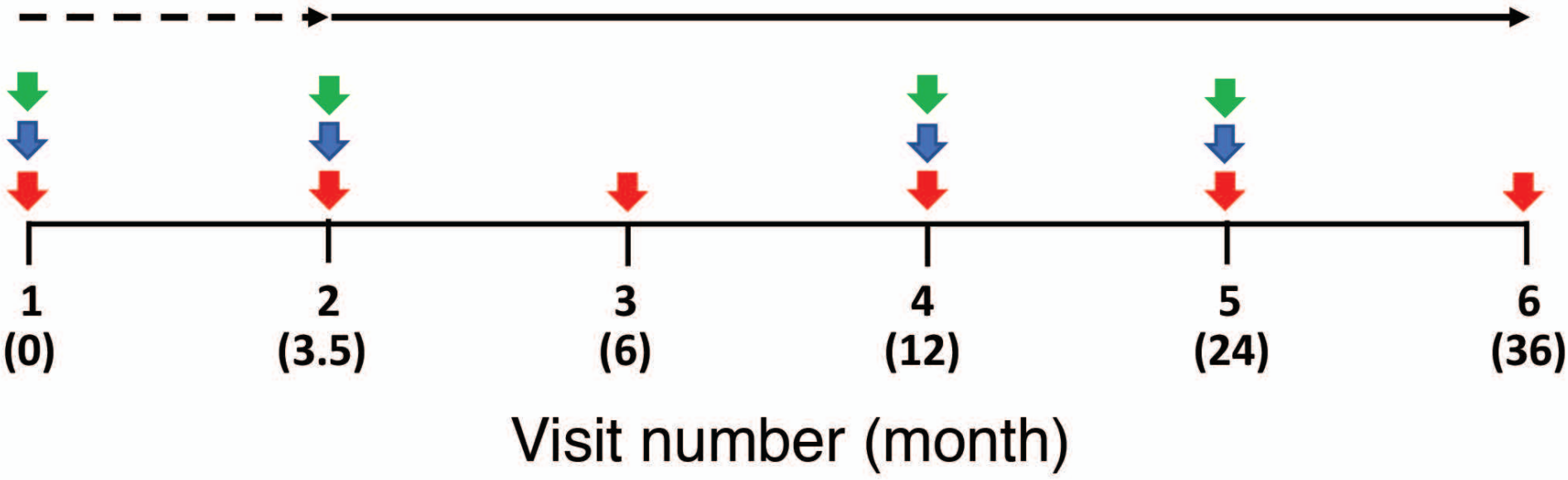

Visit #1
IL2 signalling module (I)
Th2 module (F)
Node colour mapping
V1: -3.81 0.00 3.81
V5: -3.04 logFC 3.04
Inflammatory module (G)
Type 1 interferon module (A)
Visit #5
Th2 module (F)
Type 1 interferon module (A)
IL2 signalling module (I)
Inflammatory module (G)

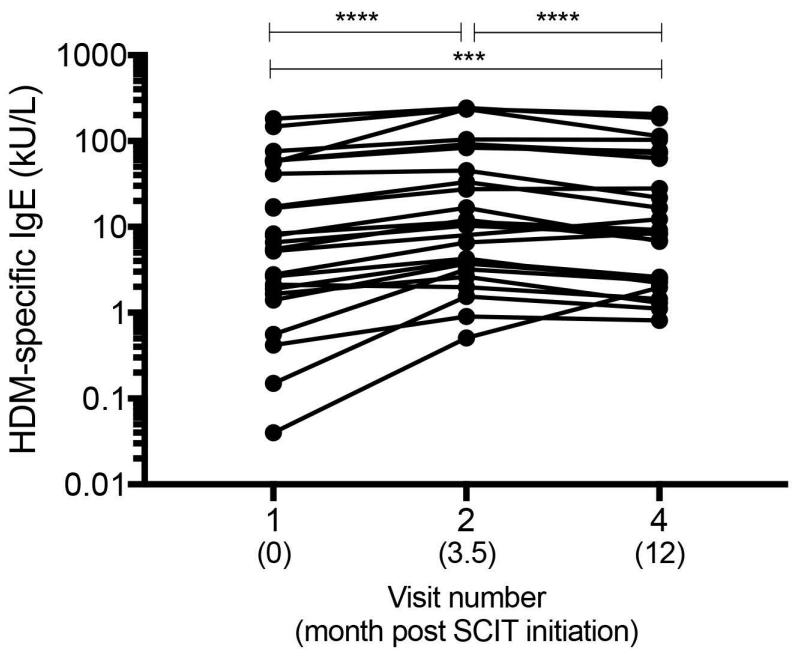
Figure 5

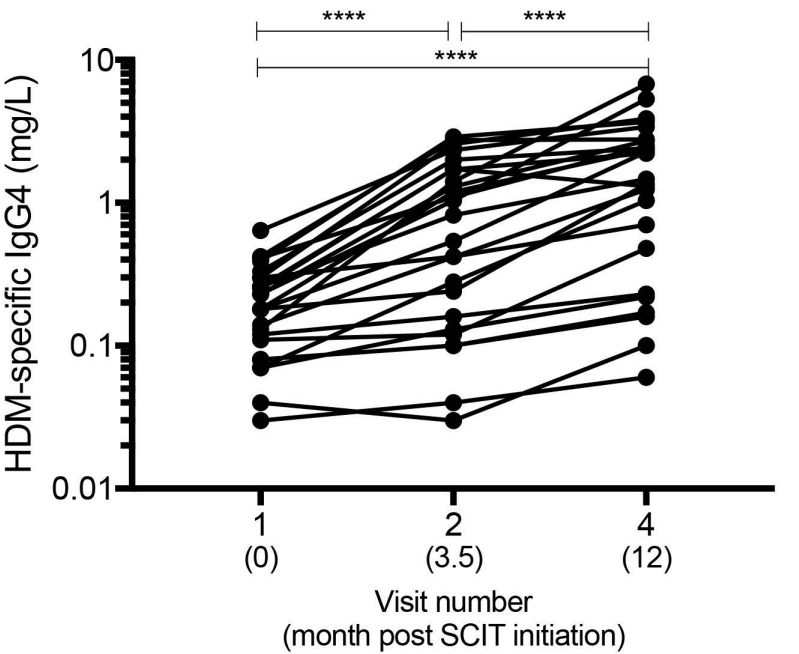
SCIT treatment/sample collection schedule



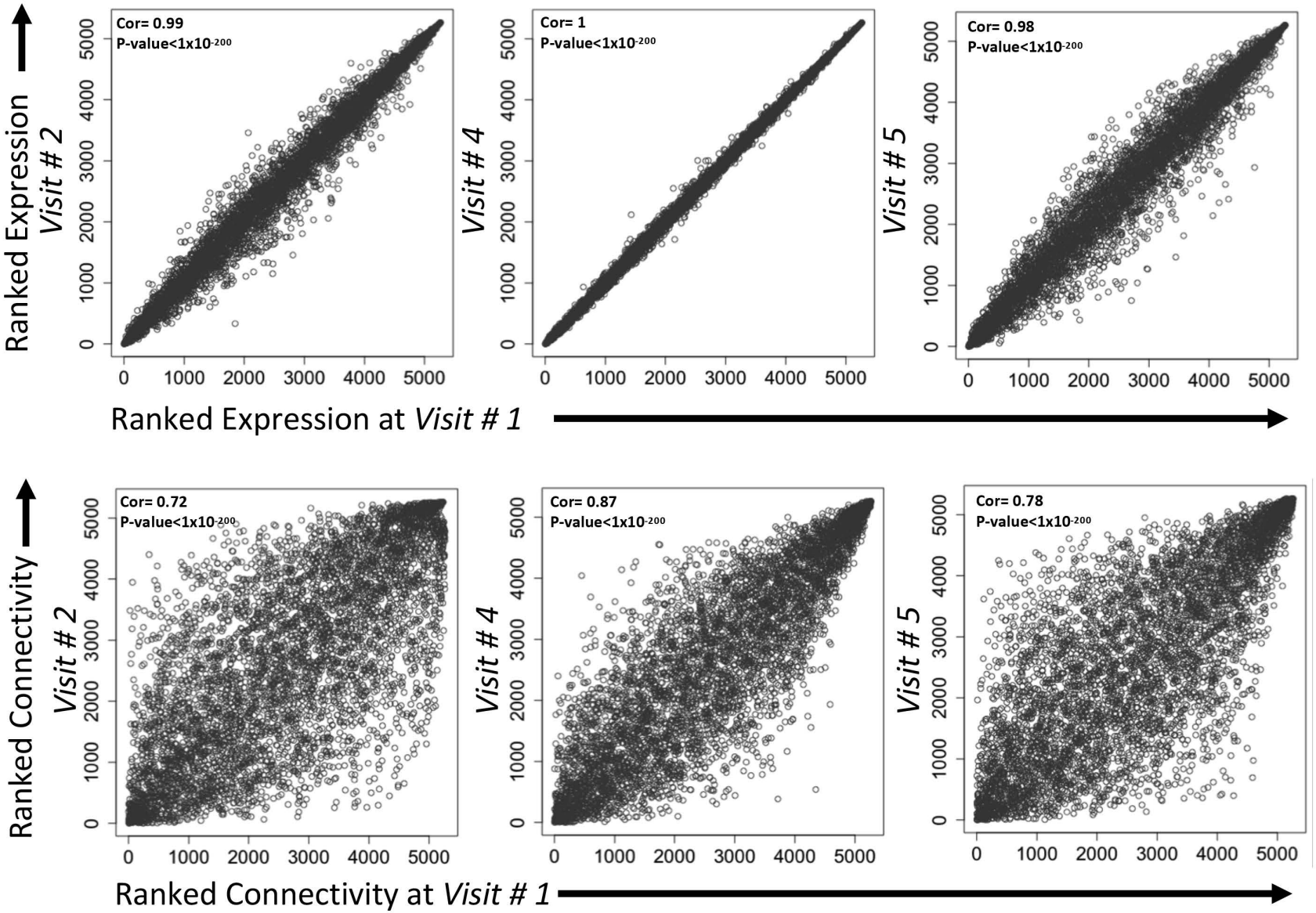
Clinical assessment
Serum for antibody
PBMC for transcriptomics
SCIT up-dosing
SCIT monthly maintenance therapy

Supplementary Figure E1

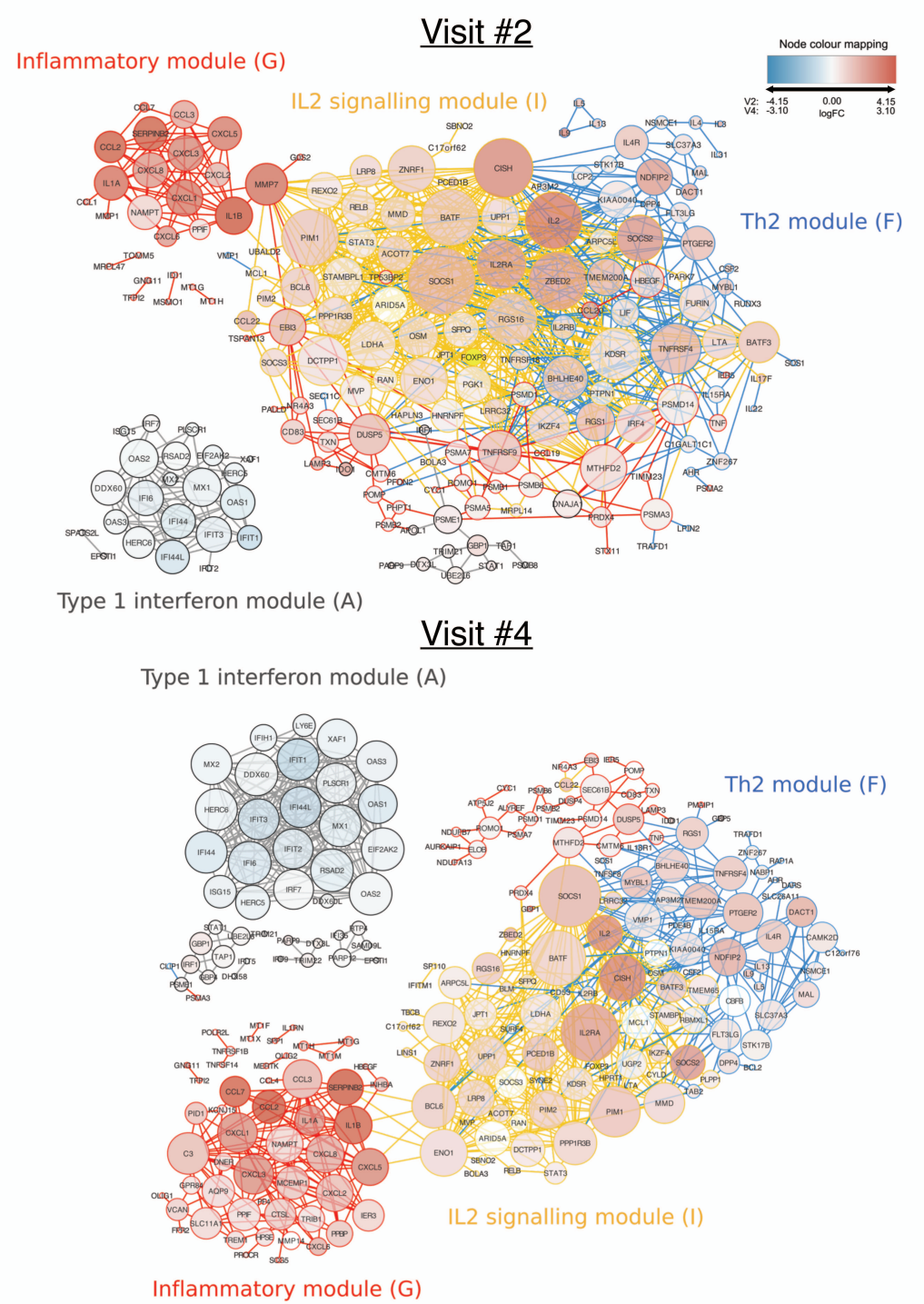




Supplementary Figure E2



Supplementary Figure E3



Supplementary Figure E4