

Increased expression of IRF4 and ETS1 in CD4⁺ cells from patients with intermittent allergic rhinitis

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Abstract

Background: The transcription factor (TF) IRF4 is involved in the regulation of Th1, Th2, Th9, and Th17 cells, and animal studies have indicated an important role in allergy. However, IRF4 and its target genes have not been examined in human allergy.

Methods: IRF4 and its target genes were examined in allergen-challenged CD4⁺ cells from patients with IAR, using combined gene expression microarrays and chromatin immunoprecipitation chips (ChIP-chips), computational target prediction, and RNAi knockdowns.

Results: *IRF4* increased in allergen-challenged CD4⁺ cells from patients with IAR, and functional studies supported its role in Th2 cell activation. IRF4 ChIP-chip showed that IRF4 regulated a large number of genes relevant to Th cell differentiation. However, neither Th1 nor Th2 cytokines were the direct targets of IRF4. To examine whether IRF4 induced Th2 cytokines via one or more downstream TFs, we combined gene expression microarrays, ChIP-chips, and computational target prediction and found a putative intermediary TF, namely ETS1 in allergen-challenged CD4⁺ cells from allergic patients. *ETS1* increased significantly in allergen-challenged CD4⁺ cells from patients compared to controls. Gene expression microarrays before and after *ETS1* RNAi knockdown showed that ETS1 induced Th2 cytokines as well as disease-related pathways.

Conclusions: Increased expression of IRF4 in allergen-challenged CD4⁺ cells from patients with intermittent allergic rhinitis leads to activation of a complex transcriptional program, including Th2 cytokines.

The transcription factor (TF) interferon regulatory factor 4 (IRF4) has a critical role in Th2 cell regulation, by controlling cytokine release and apoptosis (1–4). Interferon regulatory factor 4 is also involved in the regulation of Th1, Th9, Th17, and T regulatory cells (5–10). This pleiotropy indicates that IRF4 induces different pathways in different cell types and contexts (4). An important implication is that IRF4 could be used to trace context-dependent pathways in, for example, different diseases. Indeed, IRF4 has been implicated in both malignant and inflammatory diseases (11). To our

knowledge, IRF4 has not been studied in allergic patients, but its potential relevance is supported by studies of animal models (12). In this study, we aimed to analyze IRF4 and IRF4-induced pathways in allergen-challenged CD4⁺ cells from patients with intermittent allergic rhinitis (IAR). For this purpose, we combined chromatin immunoprecipitation chips (ChIP-chips), gene expression microarrays, and novel computational methods with short interfering RNA (siRNA) knockdowns. Neither ChIP-chip technology nor the analytical principles have been previously applied to allergy

research. However, the principles are likely to be generally applicable to elucidate disease-relevant pathways in allergy, as well as the TFs that regulate those pathways. Detailed descriptions of the methods are beyond the size restrictions of this journal and have been given elsewhere (13). Briefly, to determine which genes were regulated by IRF4, we performed IRF4- and RNA polymerase II ChIP-chip analyses of allergen-challenged CD4⁺ cells. The IRF4 ChIP-chip was used to determine IRF4-binding sites and the RNA polymerase II ChIP-chip to determine which of those sites were associated with active transcription. We found that IRF4 was involved in activation or inhibition of some 400 genes, but those genes did not include Th1 and Th2 cytokines. This indicated that IRF4 induced Th2 cytokine release via one or more downstream TFs. To find such TFs, we developed a novel analytical method, which combined gene expression microarrays and ChIP-chip with sequence-based predictions. This resulted in the identification of ETS1 as an IRF4-induced TF. This TF increased greatly in allergen-challenged CD4⁺ cells. Short interfering RNA-mediated knockdown of *ETS1* resulted in decreased levels of Th2 cytokines, and gene expression microarrays before and after *ETS1* knockdown showed altered expression of several pathways of relevance for allergy. Those pathways included novel candidate genes in allergy, which were validated in an independent gene expression microarray study of allergen-challenged CD4⁺ cells from patients with IAR.

Methods

Patients

Blood samples were gathered from 35 asymptomatic patients with grass pollen-induced allergic rhinitis outside of the season. CD4⁺ cells from these patients were used for gene expression profiling ($n = 23$), quantitative real-time PCR ($n = 6$), and ChIP-chip analyses ($n = 6$). Patient characteristics, inclusion criteria, *in vitro* stimulation of allergen-challenged CD4⁺ cells, Q-PCR, flow cytometry, CHIP-chip, sequence-based computational methods, immunoblotting, and statistical methods are described in the Supporting information.

Gene expression microarray analysis of CD4⁺ cells

Allergen- and diluent-challenged CD4⁺ cells from 20 of the 23 patients, as well as Th2-polarized cells, were analyzed with Illumina's Sentrix[®] Human-6 Expression BeadChips (Illumina Inc., San Diego, CA, USA). CD4⁺ cells from the remaining three patients were analyzed with Affymetrix HuGe U133A (Affymetrix Inc., Santa Clara, CA, USA) (14).

Small interfering RNA (siRNA)-mediated gene knockdown of *ETS1*

CD4⁺ Th cells and memory CD4⁺ Th cells were isolated from PBMCs from healthy blood donors, using isolation kits from Miltenyi (Bergisch Gladbach, Germany). Isolated cells

were nucleofected with nucleofection buffer, 1 μ M human on target plus SMARTpool siRNA against *ETS1* (Dharmacon, Lafayette, CO, USA), or nontargeting siRNA (Dharmacon) using the AMAXA nucleofection program U-014. Six hours after the nucleofection cells were washed, activated, and polarized toward either Th1 or Th2 cells (see Supporting information for details).

Results

Analysis of IRF4 in allergen-challenged or Th2-polarized CD4⁺ cells

Q-PCR analysis showed a 27.1 ± 5.6 -fold increase of *IRF4* in allergen-challenged CD4⁺ cells from patients with IAR, compared with that in diluent-challenged CD4⁺ cells ($P < 0.01$). This increase was much higher than in controls, which showed a 5.3 ± 1.7 -fold increase compared to the diluent, $P < 0.05$ (Fig. 1A, B). The increase in IRF4 was associated with significantly increased release of IL-5. IL-5 increased from 15.6 ± 0.8 to 54.4 ± 3.9 pg/mL ($P < 0.001$; Fig. 1C). In healthy controls, however, no significant change in IL-5 was found. As glucocorticoids (GCs) are known to have a beneficial effect on allergic inflammation, we examined whether GCs would downregulate *IRF4* and IL-5. Indeed, Q-PCR showed that GC treatment resulted in a significant decrease in *IRF4* expression level in the allergen-stimulated CD4⁺ cells ($P < 0.01$; Fig. 1B). This was associated with a decrease in IL-5, to 23.4 ± 4.4 pg/mL ($P < 0.001$; Fig. 1C).

The protein expression of IRF4 in CD4⁺ cells was studied by flow cytometry under the following conditions: CD4⁺ cells stimulated with diluent, anti-CD3⁺ anti-CD28, and Th2-polarizing conditions. This showed that IRF4 is induced by the activation of the CD4⁺ cells in both Th2-polarizing and anti-CD3⁺ anti-CD28 culture conditions (see Fig. S1).

Combined IRF4 ChIP-chip and gene expression microarray analysis to find putatively IRF4-regulated genes in allergen-challenged CD4⁺ cells from patients with IAR

Putatively IRF4-regulated genes were identified by ChIP-chip analysis after validation of the anti-IRF4 antibody using immunoprecipitation and Q-PCR of two known IRF4 targets and two negative control targets (Fig. S2). The ChIP-chip analysis resulted in the identification of 2861 binding sites for IRF4. To find actively transcribed sites, RNA polymerase II ChIP-chip experiments were carried out. This showed that 1595 of the 2861 sites were active binding sites for IRF4. These genes were compared with independent gene expression microarray data from allergen- or diluent-challenged CD4⁺ cells from patients with IAR to identify IRF4-induced or IRF4-repressed target genes. Genes were considered IRF4 induced if they (i) bound IRF4 and RNA polymerase II in the ChIP-chip analyses, (ii) showed significant increase in expression, and (iii) were positively correlated with IRF4 in the gene expression microarray data from allergen-chal-

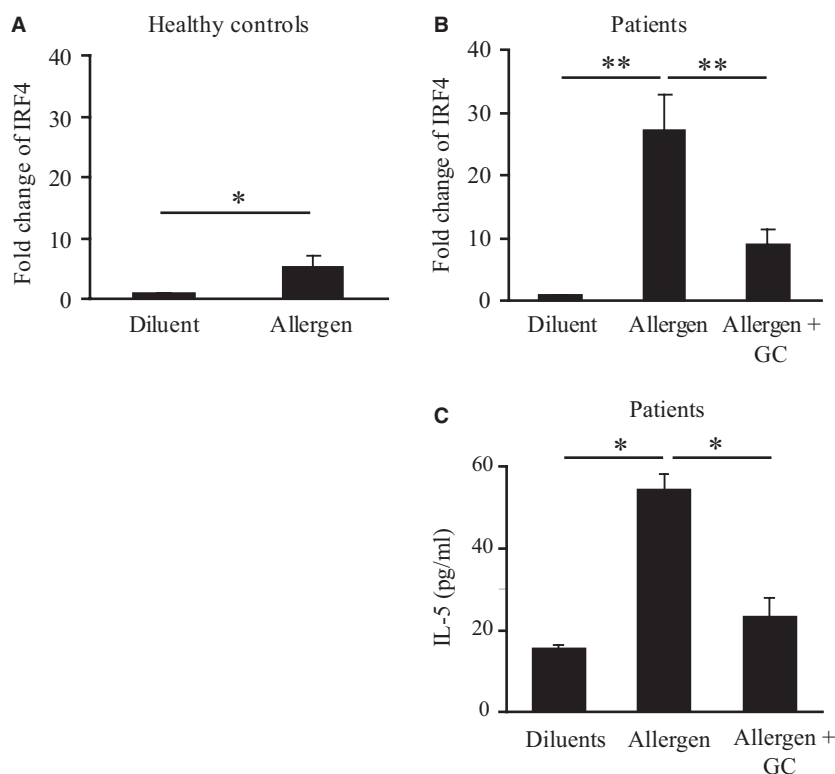


Figure 1 (A) Q-PCR analysis of *IRF4* in $CD4^+$ cells from patients with IAR ($n = 12$) and (B) healthy controls ($n = 3$) treated with diluent or allergen for 1 week. The cells from the patients were also treated with allergen and glucocorticoids (GC). The bar graph illustrates the mean $\pm$ SEM fold changes of gene expression

between allergen- and diluent-challenged cells. * $P < 0.05$, ** $P < 0.005$. (C) The release of IL-5 was measured in supernatants from patients with IAR ($n = 12$) treated for 1 week with diluent, allergen, and allergen in combination with GC (mean $\pm$ SEM, * $P < 0.05$).

lenged $CD4^+$ cells ($n = 296$, Table S1). Conversely, genes were considered IRF4 repressed if they (i) bound IRF4 but not RNA polymerase II, (ii) were differentially expressed and negatively correlated with IRF4 in allergen-challenged $CD4^+$ cells ($n = 91$, Table S2). The IRF4-induced or IRF4-repressed genes were analyzed for pathway enrichment using the Ingenuity Pathway Analysis tool (Fig. 2). This showed that the most significant pathways were involved in Th cell differentiation ($P < 0.001$) and T-cell receptor signaling ($P < 0.01$). IRF4 also regulated a wide range of other cellular functions, including proliferation, metabolism, and DNA methylation. Three genes from the T-cell pathways, *GATA3*, *IL2RA*, and *IL2RG*, were validated with Q-PCR. All three increased in expression, following allergen challenge, and decreased after treatment with GC (Fig. S3). While the Th cell differentiation pathway included the Th2-promoting genes, IL4R and GATA3, none of the IRF4-regulated genes were Th1 or Th2 cytokines. This suggested that IRF4 interacted with one or more other TFs to induce Th2 cytokine production rather than directly regulating this production. We found that the IRF4-induced genes included 28 TFs, of which only GATA3 had previously been described as IRF4 induced. Several of those TFs had been reported to induce Th2 cytokines (ETS1, HIF1A, MAF, MYB, RORA, and

STAT5B (15–22)). Another TF, PRDM1, was known to inhibit Th1 cytokines (23). IRF4, however, also induced the Th1-promoting TF STAT4 (this is commented upon in the Discussion).

Combined ChIP-chip, gene expression microarrays, and computational target prediction to find IRF4-induced transcription factors that regulate Th2 cytokine release

To determine whether any of the IRF4-induced TFs directly regulated Th2 cytokine release, we combined the IRF4 ChIP-chip and the gene expression microarrays of allergen-challenged $CD4^+$ cells with computational target gene prediction. Briefly, we used sequence-based target prediction to define IRF4-containing *cis*-regulatory modules (CRMs) within a 200-bp window around IRF4-binding sites. This resulted in 57 CRMs, which were used to find modules of putatively co-regulated target gene sets (henceforth called transcriptional modules [TMs]), using gene expression data (Fig. 3). This resulted in 25 TMs, $P < 0.0001$, compared to randomly generated gene sets (Fig. S4 and Table S3–S5). The 25 TMs contained 11 TFs, all of which were differentially expressed (Fig. S5). To find which of those 11 TFs that were most likely to regulate Th2 cytokine release, the promoter regions

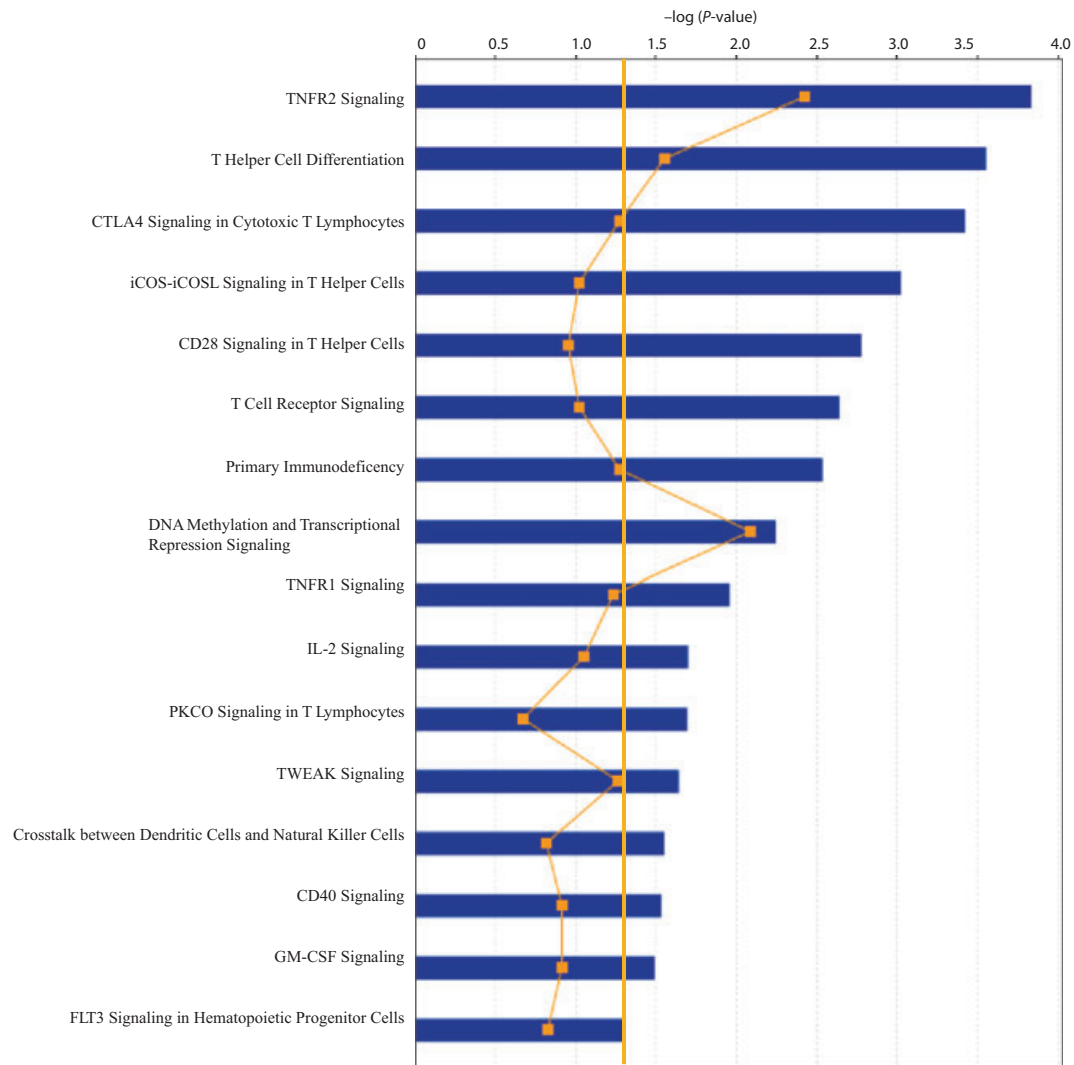


Figure 2 Pathway analysis of IRF4-induced or IRF4-repressed genes using the Ingenuity Pathway Analysis tool. The figure represents

significant pathways with a $P < 0.05$ (represented by the orange line).

of the Th2 cytokines IL-4, IL-5, and IL-13 as well as the Th1 cytokine IFN- γ were analyzed to find predicted TF-binding sites (TFBSs) for the 11 TFs. TFs that had predicted TFBS in the promoter regions of the Th2 cytokines, but not in the promoter region of the Th1 cytokine were considered as likely Th2 regulators. The TF that best matched this criterion was ETS1. This TF had previously been implicated in Th2 cytokine regulation (19–22) but had not been described in human allergy.

Analysis of the role of ETS1 in allergen-challenged CD4 cells from patients with intermittent allergic rhinitis

The mRNA expression of *ETS1* in allergen-challenged CD4⁺ cells from patients increased significantly compared with that in diluent treated cells. *ETS1* also increased in controls although this was less pronounced (Fig. 4). Short interfering RNA-mediated knockdown of *ETS1* in

Th2-polarized cells resulted in significant decreases in *ETS1* expression (Figs 5A and S6). To check for off-target effects, we analyzed the Th2-polarized cells with gene expression microarrays at two time points after knockdown and found no significant changes in genes that may be induced by small RNAs (24). In addition, Q-PCR analysis showed no decrease in *IRF4* and *GATA3*, following knockdown (Fig. S6). However, the *ETS1* knockdown did result in significant decreases in IL-5 and IL-13 protein levels (Fig. 5 B, C).

To identify ETS1-induced pathways, we performed pathway analysis of the gene expression microarray data from Th2-polarized cells at two time points, following knockdown of *ETS1*. The most significant pathways were found at the first time point and included *Role of Cytokines in Mediating Communication Between Immune Cells* ($P < 0.0001$), *T-cell receptor signaling* ($P < 0.001$), and *Airway inflammation in asthma* ($P < 0.01$; Fig. S7). We examined whether the genes

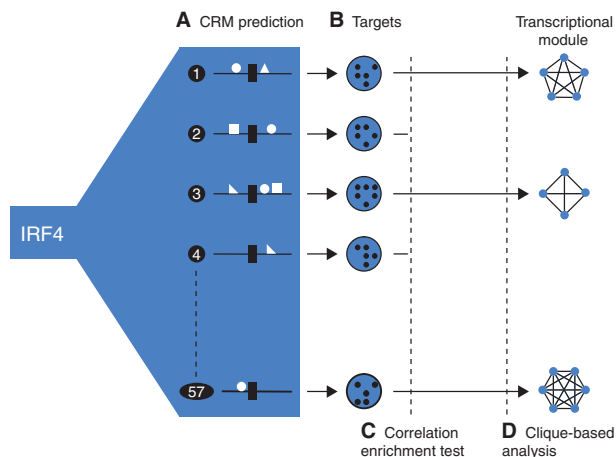


Figure 3 Identification of IRF4-induced transcriptional modules. (A) Prediction of IRF4-containing cis-regulatory modules (CRMs) based on sequence in a 200-bp window around IRF4-binding sites. (B) Identification of putative target genes for each CRM. (C) Selection of modules with correlated putative target genes. (D) Clique-based analysis to extract highly correlated transcriptional modules (TMs).

that were differentially expressed in Th2-polarized cells following *ETS1* knockdown were also differentially expressed in allergen-challenged cells from patients with IAR. We found a significant overlap between the two data sets ($P < 0.01$, Table S7).

Discussion

Allergic inflammation is a highly complex process that involves multiple interacting pathways and hundreds of genes (25–27). The comprehensive mapping of those pathways is therefore a formidable challenge. One approach to address this problem may be to use TFs as tracers to define disease-causing pathways (28). In this study, we applied this principle to allergen-challenged CD4⁺ cells from patients with IAR. We combined ChIP-chip, gene expression microarrays, computational predictions, and siRNA knockdowns. Chromatin immunoprecipitation chip technology has not previously been

used in allergy research, and we also developed novel computational methods to interpret the data. We focused on IRF4, which has an important role in regulating Th2 cells and other T cells but has not been examined in human allergy. We found that IRF4 regulated almost 400 genes. This number is similar to that found in a study of IRF4-induced genes in myeloma cells (29). The genes found in our study were involved in several different pathways, of which the most significant included T helper cell differentiation and T-cell receptor signaling. Interferon regulatory factor 4 also regulated pathways related to cellular proliferation, metabolism, and DNA methylation. This indicates that IRF4 has an important role in activating transcriptional programs that integrate immune responses with general cellular activation processes. However, neither Th1 nor Th2 cytokines were the direct targets of IRF4. This suggested that IRF4 interacted with other TFs to regulate these cytokines. Indeed, the IRF4-induced genes included several TFs previously described to regulate Th2 cytokines, namely *ETS1*, *GATA3*, *HIF1A*, *MAF*, *MYB*, *RORA*, and *STAT5B* (15–22). Another TF, *PRDM1*, inhibited Th1, while *STAT4* is an important Th1 cell activator. Of these TFs, only *GATA3* has previously been described as IRF4 regulated. The increase in *STAT4* does not agree with the paradigm that Th1 cells have a counter-regulatory role in allergy. This novel finding could, however, explain recent studies reporting concurrent increases in Th1 and Th2 cytokines in allergic inflammation (14, 25, 26, 30, 31).

Limitations of the study include that gene expression microarrays do not detect low-abundance genes and that poorly annotated genes may not be included in the pathway analysis. Chromatin immunoprecipitation chip technology detects TF bound to the promoter regions, but such TF do not necessarily regulate transcription. To identify genes that were regulated by IRF4, we combined RNA polymerase II ChIP-chip and gene expression microarrays. The rationale was that an active binding site associated with a differentially expressed gene would indicate that IRF4 regulated that gene. Another problem was to identify which IRF4 induced TFs that directly regulated Th2 cytokines. We therefore developed a novel computational method that integrated the ChIP-chip and gene expression microarray data with sequence-based

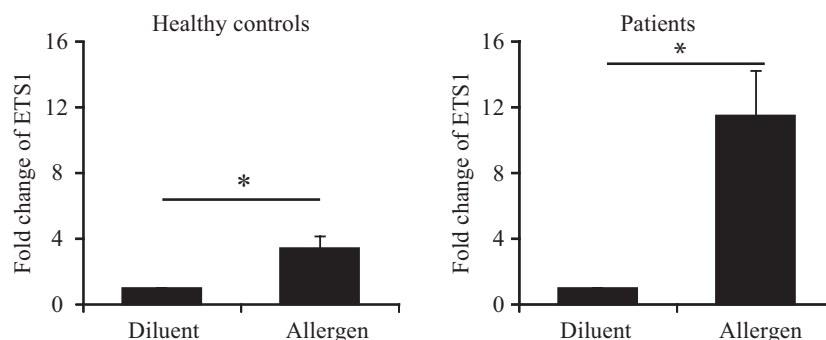


Figure 4 Q-PCR analysis of *ETS1* in CD4⁺ cells from patients with IAR ($n = 6$) and healthy controls ($n = 6$). The bar graph illustrates

the mean $\pm$ SEM fold changes of gene expression between allergen- and diluent-challenged cells. * $P < 0.05$.

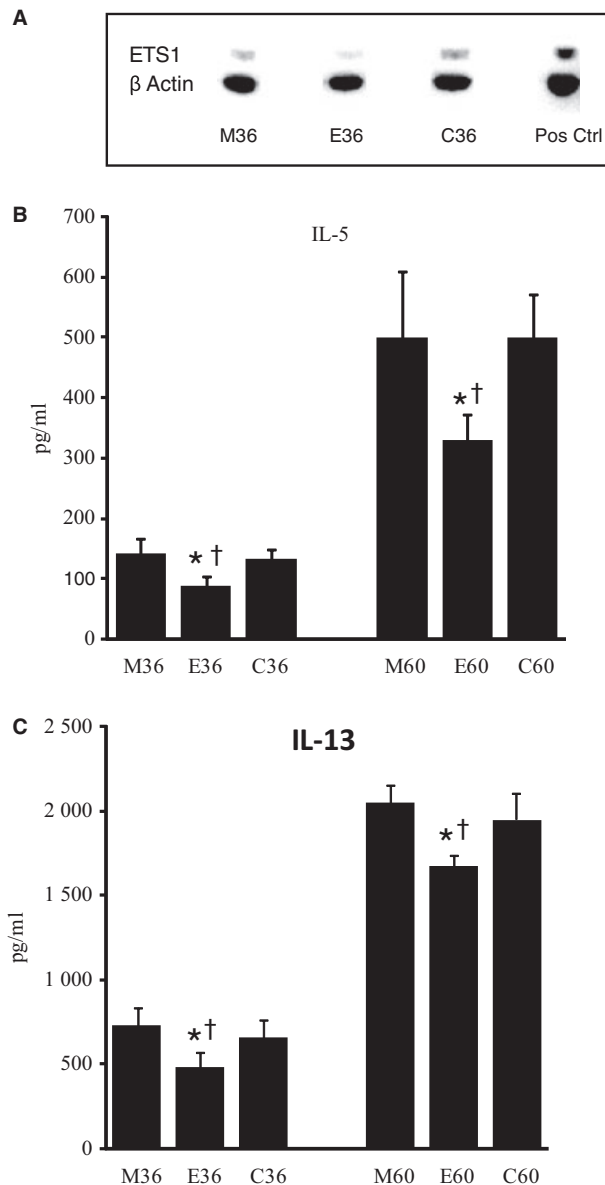


Figure 5 Short interfering RNA (siRNA)-mediated knockdown of *ETS1* in polarized Th2 cells affects Th2 cytokine production. Polarized Th2 cells were transfected with either diluent (M) *ETS1*-specific siRNA pool (E) or nontargeting control siRNA pool (C). (A) The expression of *ETS1* protein in polarized Th2 cells 36 h after transfection with either diluent (M) *ETS1*-specific siRNA (E) or nontargeting control siRNA (C). The positive control (pos Ctrl) shows the expression of the *ETS1* in nontransfected, polarized Th2 cells (one representative experiment of five). (B, C) the levels of the Th2 cytokines IL-5 and IL-13 in cell supernatants after 36 and 60 h after ($n = 5$; mean $\pm$ SEM, * $P < 0.05$, compared with the nontargeting control siRNA and † $P < 0.05$, compared with the diluent).

predictions. This method was based on identifying groups of genes with shared combinations of TF-binding sites (TFBS) adjacent to the ChIP-chip-defined IRF4-binding sites, whose

expression levels were correlated in the allergen-challenged $CD4^+$ cells. This resulted in the identification of several groups of genes, which we refer to as TMs. Finally, those TMs were searched to find TFs with predicted binding sites in the promoter regions of Th2, but not Th1 cytokines. This resulted in the identification of *ETS1*. This TF has previously been demonstrated to regulate differentiation of Th2 and Th17 cells (19–22), but has not been described in allergy. We found a significant increase in *ETS1* in allergen-challenged $CD4^+$ cells from patients compared to controls. In Th2-polarized cells, siRNA-mediated knockdown of *ETS1* resulted in decreased release of Th2 cytokines. This decrease was modest, but statistically significant. A likely explanation for the modest effects was the involvement of several TFs other than *ETS1* in the regulation of Th2 cytokines (24). A possible confounding factor could be off-target effects of the siRNA knockdown (24). However, gene expression microarray and Q-PCR analysis did not indicate any such effects. By contrast, pathway analysis of the gene expression microarray data showed that several pathways relevant to allergy were significantly affected by the *ETS1* knockdown. Those pathways were involved in Th2 cell differentiation, as well as in Th2 cell regulation of effector cells like eosinophils. Moreover, airway inflammation in asthma was one of the most significant pathways. We found a significant overlap between the genes that were differentially expressed after *ETS1* knockdown and those found in allergen-challenged $CD4^+$ cells.

For future studies, we propose that the analytical principles in this article can be applied to comprehensively map disease-associated pathways in allergy, as well as the TFs that regulate those pathways. The increasing availability of gene expression microarray and ChIP-chip data in the public domain will facilitate such mapping.

Acknowledgments

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Conflict of interest

None.

Supporting Information

Additional Supporting Information may be found in the online version of this article found at: <http://www.wileyonlinelibrary.com>

Figure S1. Expression of IRF4 in $CD4^+$ T cells. $CD4^+$ T cells were cultured with diluents, or with anti-CD3 and anti-CD28, or under Th2 polarization condition (anti-CD3 and

anti-CD28, IL-4 and anti-IL-12) for 48 h. The shaded histogram represents isotype control and the solid line represents IRF4.

Figure S2. Validation of immunoprecipitating anti-human IRF4 antibodies in allergen-challenged CD4⁺ cells from allergic patients ($n = 3$). The number of bindings events/1000 cells for known IRF4 targets (IRF4 and TLR4) were compared with two negative controls (Untr12 and Untr20).

Figure S3. Q-PCR analysis of *GATA3*, *IL2RA* and *IL2RG* in CD4⁺ cells from patients with IAR ($n = 20$). The bar graph illustrates the mean $\pm$ SEM fold changes of gene expression between allergen- and diluent -challenged cells ($**P < 0.01$), as well as between allergen- challenged and hydrocortisone treated cells ($^{\S}P < 0.01$).

Figure S4. Histogram showing the distribution of P values from the correlation-based enrichment analysis. The black line shows the number of unique predicted TF binding sites in each interval.

Figure S5. Hierarchical cluster analysis of microarray data profiling all TFs in IRF4-regulated TMs. Each row represents a TF and each column a sample. The scale represents log base 2 expression values.

Figure S6. Q-PCR analysis after ETS1-knock down. Polarized Th2 cells were transfected with either diluent (M) *ETS1*-

specific siRNA pool (E) or non-targeting control siRNA pool ($n = 5$). The figure shows the expression levels of *ETS1*, *IRF4* and *GATA3* 0 and 12 h after the activation (6 and 18 h after the nucleofection).

Figure S7. Pathway analysis after ETS1 knock-down ETS1 knock-down was performed in Th2 polarized CD4⁺ cells. Differentially expressed genes after the knock-down were submitted to Ingenuity Pathway Analysis (IPA). The figure represents significant pathways with a P value < 0.05 (represented by the orange line).

Table S1. List of the IRF4-induced genes.

Table S2. List of the IRF4-reduced genes.

Table S3. CRM-Matrix associations.

Table S4. Matrix-TF associations.

Table S5. CRM/Target associations.

Table S6. Genes affected by ETS1 block.

Table S7. Genes that are affected by ETS1 block and differentially expressed in allergen-challenged CD4⁺ cells.

Data S1. Methods.

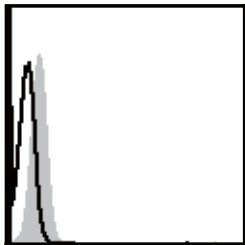
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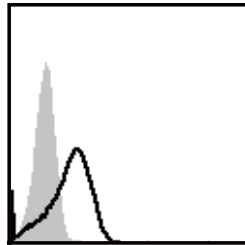
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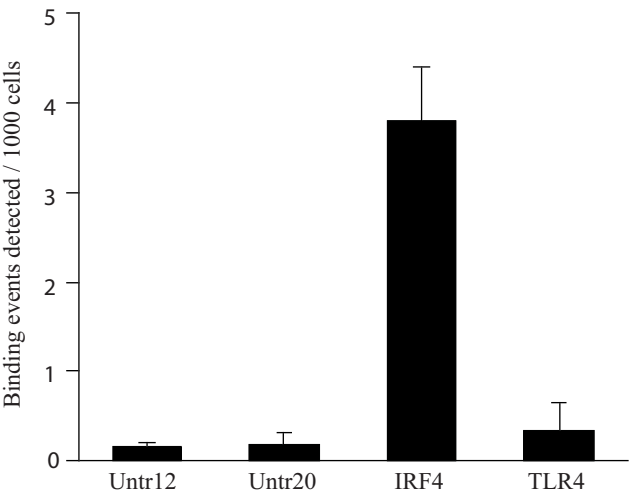
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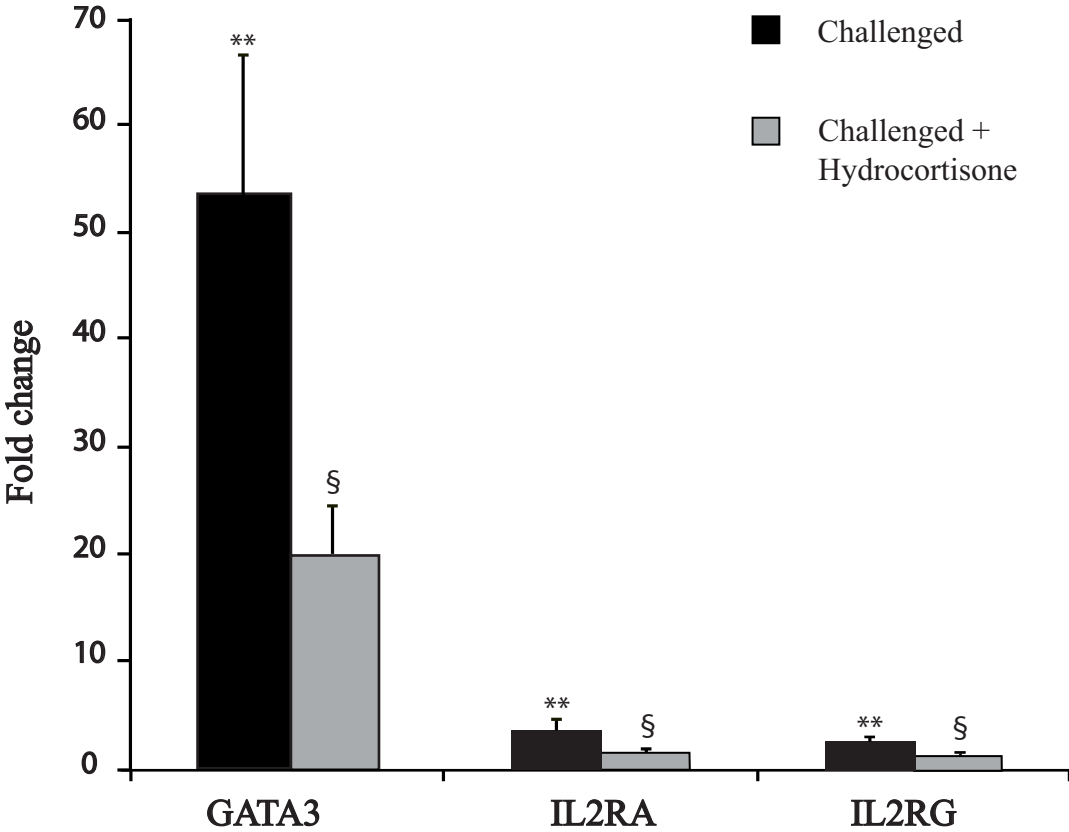


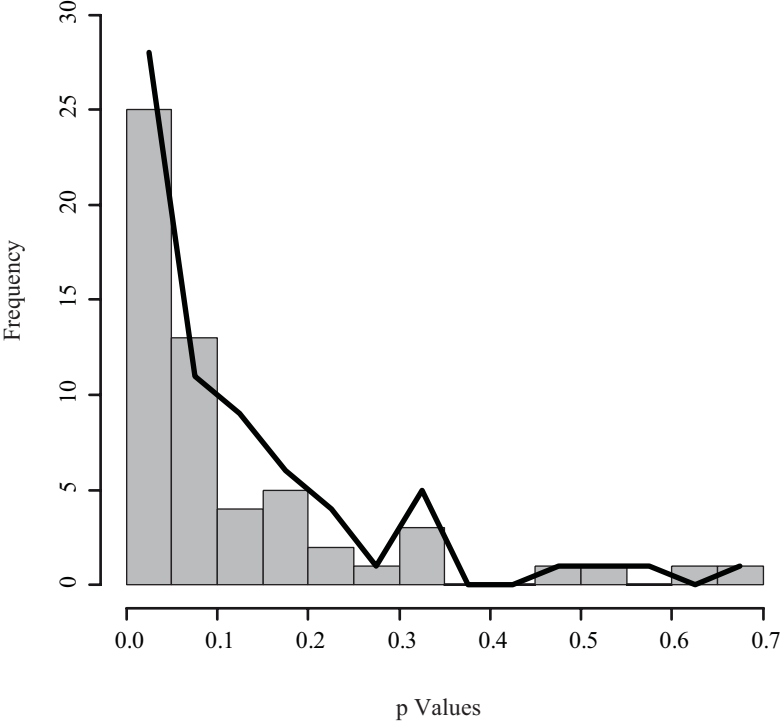
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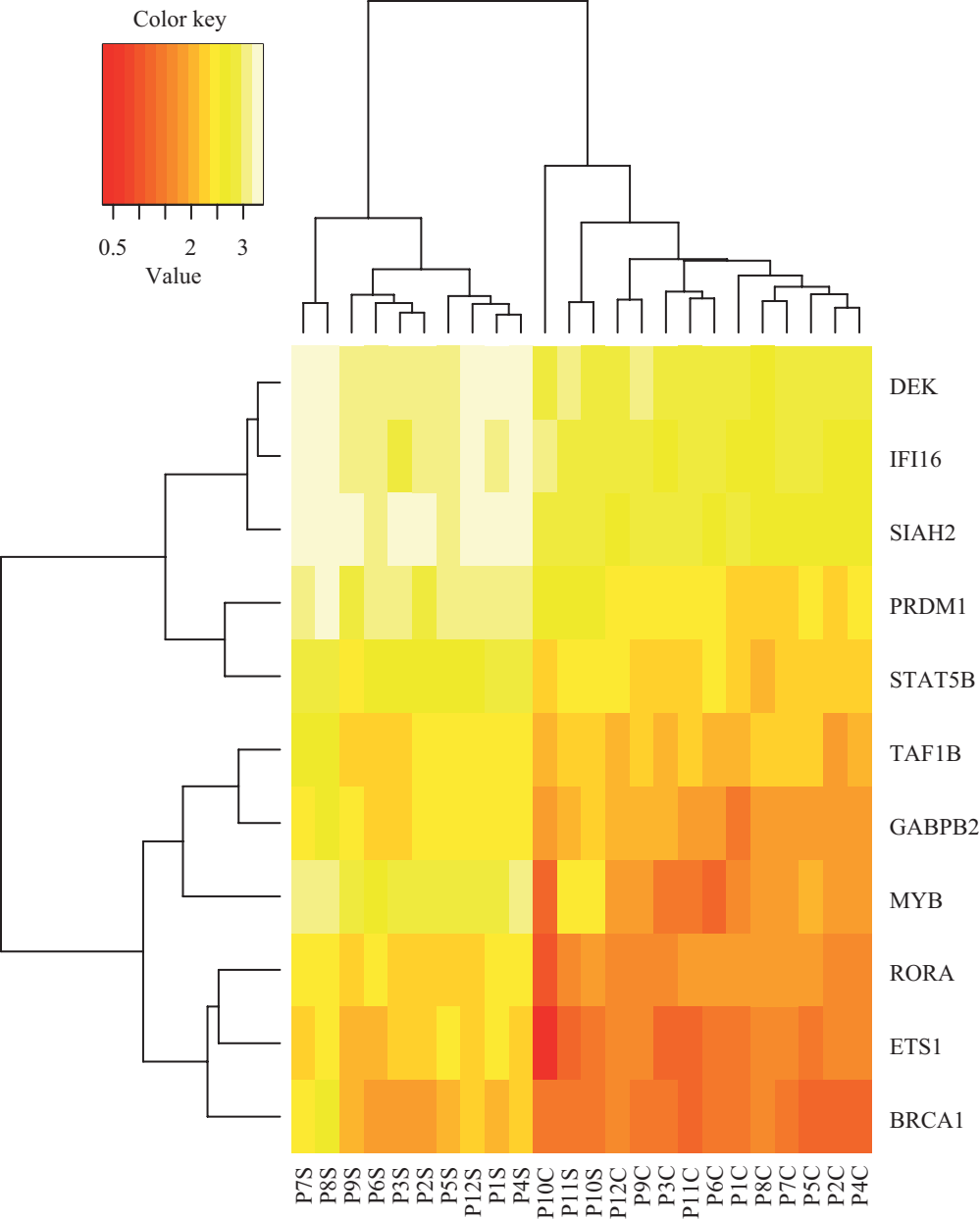


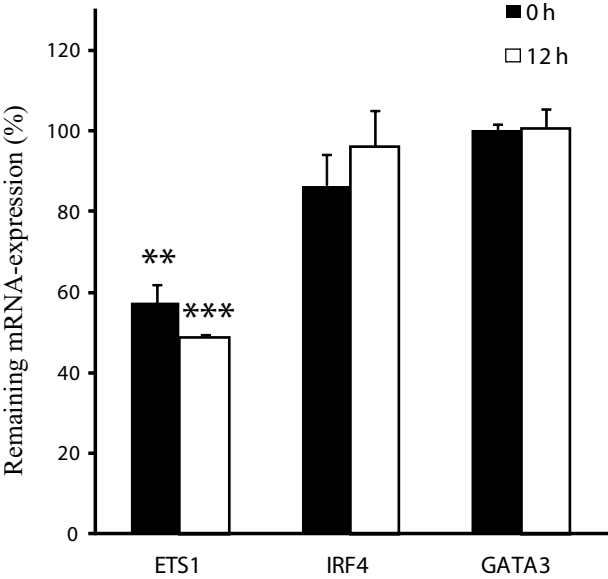
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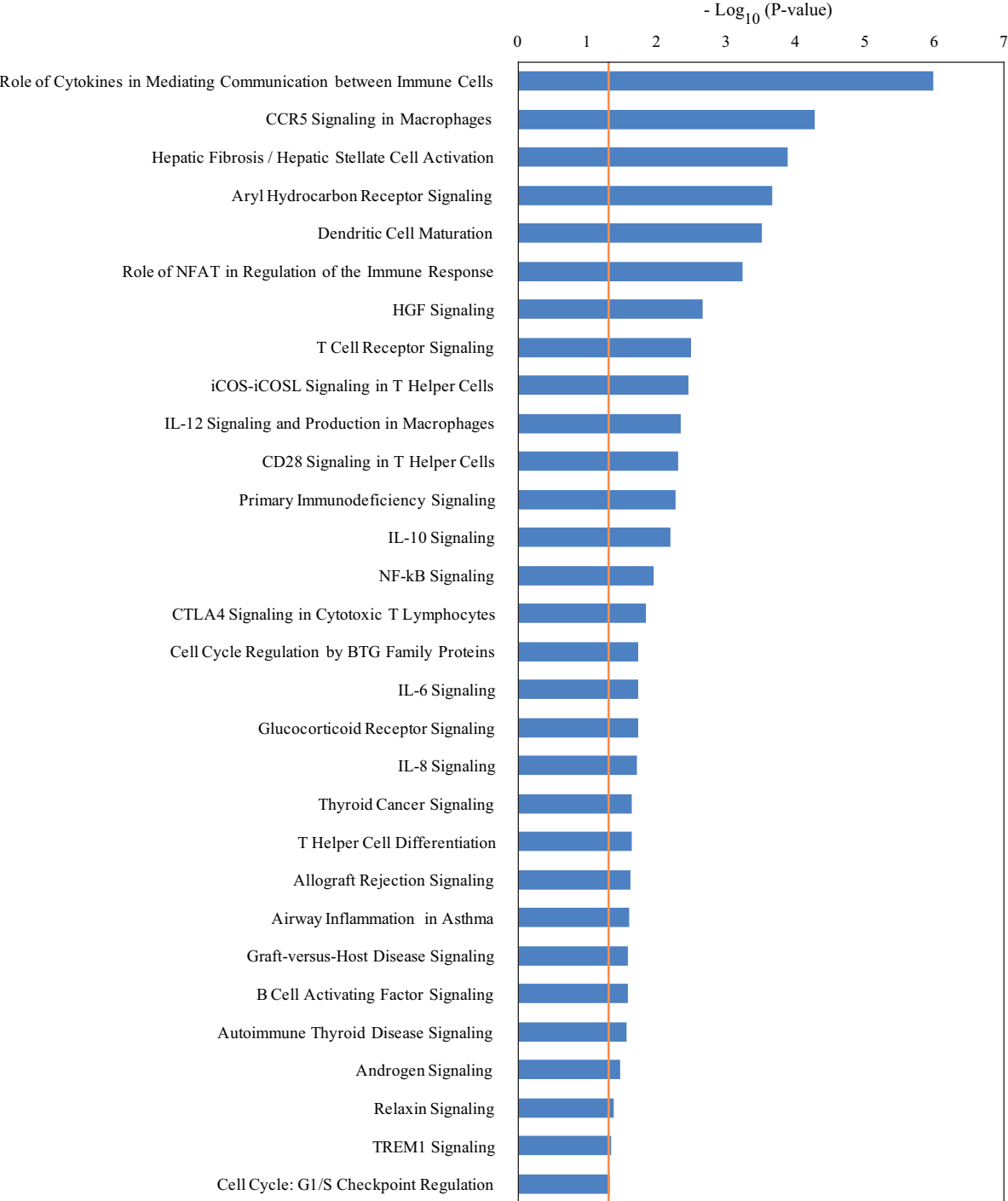












Supplementary information

Increased expression of IRF4 and ETS1 in CD4⁺ cells from patients with intermittent allergic rhinitis

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METHODS

Patient characteristics

The median (range) age of the patients was 25 (22-26) for women and 24 (20-30) for men. Six healthy individuals were analyzed as controls (two females). Their median (range) was 35 (27-37). The diagnosis of grass pollen-induced allergic rhinitis was based on a positive history of grass pollen-induced allergic rhinitis and positive skin prick tests against grass pollen. All patients were seen outside of the pollen season and were asymptomatic at the time of inclusion in the study. None of the patients had received any local or systemic treatment with glucocorticoids or antihistamines within two weeks before samples were taken. Other exclusion criteria included a history of perennial symptoms, positive skin prick test responses against house dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) and molds (*Cladosporium* and *Alternaria* species). Healthy controls had no history of allergic or any other chronic disease, and had negative tests for specific allergens. The study was approved by the Ethics Committee of the Medical Faculty, Göteborg University.

In vitro stimulation of PMBC with allergen

For the quantitative real-time PCR, gene expression microarray and ChIP-chip studies of CD4⁺ cells, PBMCs from patients with grass pollen-induced allergic rhinitis were prepared and challenged with diluent, grass pollen extract (100 µg/mL, ALK-Abello A/S,

Hørsholm, Denmark) at a density of 10^6 cells/mL, or grass pollen extract and hydrocortisone (at a 10^{-7} M concentration) as previously described methods.(1, 2) After seven days of incubation at 37 °C and 5 % CO₂ the CD4⁺ cells were enriched by using anti-CD4-coated paramagnetic microbeads and a MACS system according to the instructions of the manufacturer (Miltenyi Biotech GmbH, Bergisch Gladbach, Germany).

Quantitative real-time polymerase chain reaction (QRT-PCR) analysis

Double-stranded cDNA was prepared from 100 ng RNA by using a High Capacity cDNA Archive Kit with random hexamer primers (Applied Biosystems, Foster City, CA).

Quantification of IRF4 (Hs00180031_m1), GATA3 (Hs00231122_m1), and ETS1 (Hs00428287_m1) transcripts was performed using Taqman gene expression assays (Applied Biosystems, Foster City, CA). Quantitative PCR was performed in triplicate with the ABI7900HT Sequence Detection System using 2 ng cDNA as template. The expression of each gene was normalized against the expression of GUSB (b-glucuronidase; Hs99999908_m1) used as an endogenous control. cDNA samples and TaqMan PCR mastermix (Applied Biosystems) were pipetted in a 384-well plate. The Ct value was calculated using the SDS program version 2.2.1 (Applied Biosystems, Foster City, CA). Reactions were characterized by comparative method (Ct target gene – Ct endogenous control = ΔC_T). The fold change between allergen stimulated or allergen stimulated + cortisone and control subjects was calculated as $2^{-\Delta\Delta C_T}$.

Flow cytometry analysis

CD4⁺ cells were enriched from PBMCs from a healthy individual using a CD4⁺ T Cell Isolation Kit II according to the instructions of the manufacturer (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). *In vitro* differentiation of Th2 cells was performed as previously described.(3) Briefly, 2x10⁶ CD4⁺ cells were incubated for 48 hours in the presence of plate-bound anti-CD3 and soluble anti-CD28 (both from Beckman Coulter, Inc.), IL-4 and anti-IL-12 antibodies (Both from R&D systems Ltd, Minneapolis, MN). In addition, CD4⁺ cells stimulated with diluents, or plate-bound anti-CD3 and soluble anti-CD28 for 48 hours were included. For staining of intracellular IRF4 Cytofix/Cytoperm solution (BD Biosciences, San Jose, CA) was used for simultaneous fixation and permeabilization of cells. The rat anti-IRF4-FITC (3E4) monoclonal antibody and the corresponding isotype control normal rat IgG1-FITC (sc-130921 and sc-2827, Santa Cruz Biotechnology, Santa Cruz, CA) were used. The flow cytometry analysis was performed on a FACSCalibur instrument (BD Biosciences, San Jose, CA). Data analysis was performed using CELLQuest (BDIS) software packages.

ChIP-chip analysis

PBMCs from six patients were stimulated with allergen as described and were treated with 1% formaldehyde for 15 minutes at room temperature. The cross linking reaction was neutralised by the addition of glycine to a final concentration of 125 mM. CD4⁺ cells were isolated and chromatin was isolated by adding lysis buffer, followed by disruption

with a Dounce homogenizer. Lysates were sonicated and the DNA sheared to an average length of 300-500 bp DNA (Input) was prepared by treating aliquots of chromatin with RNase, proteinase K and heat for de-crosslinking, followed by ethanol precipitation. Factor binding was detected using an antibody against IRF4 (sc-6059, Santa Cruz Biotechnology, Santa Cruz, CA) and transcription was detected using an antibody against RNA polymerase II (sc-9001, Santa Cruz Biotechnology, Santa Cruz, CA). Complexes were washed, eluted from the beads with SDS buffer, and subjected to RNase and proteinase K treatment. Crosslinks were reversed by incubation overnight at 65 C, and ChIP DNA was purified by phenol-chloroform extraction and ethanol precipitation. Quantitative PCR (QPCR) reactions were carried out in triplicate on specific genomic regions using SYBR Green Supermix (Bio-Rad, Hercules, CA). The resulting signals were normalized for primer efficiency by carrying out QPCR for each primer pair using Input DNA.

ChIP and Input DNAs were amplified using either random priming or whole-genome amplification (WGA). For random priming, a fixed sequence of 17 bases containing nine random bases at the 3' end was used in four linear amplification reactions using Sequenase (USB). Following purification, the randomly primed ChIP DNA was amplified for 30 cycles using a fixed sequence primer. For WGA, the GenomePlex WGA Kit (Sigma, St Louis, MO) was used. The resulting amplified DNA was purified, quantified, and tested by QPCR at the same specific genomic regions as the original ChIP DNA to assess quality of the amplification reactions. Amplified DNA was fragmented and labelled using the DNA Terminal Labeling Kit (Affymetrix, Inc., Santa Clara, CA)

and then hybridized to Affymetrix GeneChip Human Promoter 1.0R arrays at 45 °C overnight. Arrays were washed and scanned, and the resulting CEL files were analyzed using Affymetrix tiling analysis software, TAS. Thresholds were selected, and the resulting BED files were analyzed using Genpathway ChAS software that provides comprehensive information on genomic annotation, peak metrics and sample comparisons for all peaks (intervals).

Prediction of IRF4-containing cis-regulatory modules (CRMs) by combined ChIP- and DNA sequence analysis

In order to find TFs that act in a combinatorial manner with IRF4 in gene regulation, we searched for TFs having a large number of binding sites close to our experimentally determined IRF4 binding sites. This was performed by searching for binding sites across a set of compiled 200 bp windows centered around each of the signal peaks of the IRF4 ChIP-chip data.

We used three different sources for TFBS predictions: UCSC conserved sites

[http://genome.ucsc.edu/cgi-](http://genome.ucsc.edu/cgi-bin/hgTrackUi?hgsid=120495366&c=chrX&g=tfbsConsSites)

[bin/hgTrackUi?hgsid=120495366&c=chrX&g=tfbsConsSites](http://genome.ucsc.edu/cgi-bin/hgTrackUi?hgsid=120495366&c=chrX&g=tfbsConsSites), CisRed(4) and Compo(5).

For one of the sources we have also used different parameters (Compo). The results indeed seem complementary, and instead of selecting a single method, we selected multiple predictions across methods and settings.

Both the "UCSC conserved sites" and CisRed are static collections of DNA binding site locations for different TFs. We determined which TFs had binding sites overlapping with, each sequence window around IRF4 and considered the 10 TFs that occurred in the largest number of windows as a prediction (separately for each source).

Compo is a method for discovering CRMs with associated binding sites that are over-represented across an input set of regulatory regions. We used Compo to predict CRMs overrepresented in the IRF4 sequence windows versus a background consisting of the set of all 2kb upstream regions of RefSeq genes not overlapping IRF4-windows. All human-associated position weight matrices (PWMs) of Transfac release 9.4 was used as input TF motifs for CRMs. Compo allows the stringency of PWM scanning to be specified, and we tried runs where stringency was adjusted so that the number of PWM occurrences corresponded to 10 %, 20 %, 50 % or the same as the number of input sequences (IRF4-sites). Compo also allows the size of discovered CRMs to vary, and we tried runs— with 1, 2 and 3 TFs per CRM. For each run, the top ten predicted CRMs were used as predictions.

Finally, for each prediction we compiled a list of genes occurring downstream or around each of the IRF4 sequence windows that the TF occurred in. These gene lists were used for evaluating similarity of gene expression.

Identification of Transcriptional Modules (TMs) using gene expression data

An initial dataset of nearly 47,000 probes and 32 arrays was filtered using information on well-annotated genes and Illumina detection p-values. The resulting dataset had almost 18,000 probes, on which Pearson correlation values were computed. The corresponding correlation histogram showed a slight positive skew, and demonstrated a roughly normal distribution as is typically seen (data not shown). To determine IRF4-containing CRMs that putatively regulate their corresponding target gene sets, a permutation test was performed. For each CRM target gene set, 10,000 random samples of the same size as the

corresponding target gene set were performed. The P values were based on a comparison between mean correlation values of random permutations, and those of the CRM target sets. Target gene sets with an average correlation coefficient significantly higher than expected by chance ($P < .05$) were considered regulated. Random samples were taken from the full set of IRF4 target genes from the ChIP-chip analysis. To validate the predicted CRMs, the process was repeated on 57 sets of 100 random genes. This permutation was also repeated 10 000 times. The predicted CRMs were then compared to this random distribution.

A reduced histogram was computed using gene pairs with shared upstream CRMs and at least ten observations in common. A global correlation threshold was chosen to create a single graph for each enriched CRM target gene set, from which paracliques were extracted.(6) This threshold was set at $|0.82|$, based on maximal clique tripling as previously described.(7) From each graph, the TM was defined as the largest paraclique.

ETS1 gel electrophoresis (SDS-PAGE) and immunoblotting

Electrophoresis and blotting were performed using standard protocols. SDS-PAGE of cell extracts was done in 10% acrylamide Bis-Tris gels (Invitrogen, Carlsbad, CA) with MOPS running buffer. 10 μ g total protein was added to each lane. After electrophoresis cell extract proteins were electro-blotted onto PVDF membranes for further analysis with Western blot. The membrane was blocked in TBS-Tween 0.05%, Milk 5%. Sliced membranes were incubated with mouse anti-human Ets1 (1 μ g/mL, Santa Cruz Biotechnology, Santa Cruz, CA) or mouse anti-human β actin (0.2 μ g/mL, Santa Cruz

Biotechnology, Santa Cruz, CA) antibodies in 2% milk, TBS-T 0.05% over night at 4 °C.

The membrane slices were washed with TBS-T 0.05% and incubated with goat anti-mouse IgG₁-HRP antibody (0.2 µg/mL, Santa Cruz Biotechnology, Santa Cruz, CA) in 2% milk, TBS-Tween 0.05% at room temperature for 1 hour. SuperSignal West Pico chemiluminescent kit (Thermo Scientific, Rockford, IL) was applied for antibody detection according to manufacturer's protocol.

Analysis of IL-5 and IL-13 in supernatants from allergen-challenged PBMCs or polarized CD4⁺ Th cells

Analysis of IL-5 and IL-13 was performed using commercial ELISA kits(R&D Systems Ltd, Minneapolis, MN), according to the manufacturer's instructions.

Statistical analysis

For the gene expression microarray analyses we estimated the effect of allergen stimulation on each gene, using a linear model with a Bayesian smoothing described by Smyth and implemented in the Bioconductor package LIMMA. Finally, the probe sets were ranked according to their evidence of being differentially expressed, allowing a false discovery rate of 5%. *P* values were calculated as a measure of statistical significance of each correlation and adjusted for multiple comparisons using the Bioconductor q-value package.

For values derived from ELISA and Q-PCR Student's t-Tests were performed when the data was found to be normally distributed. Otherwise unpaired comparisons were performed using the non-parametric Mann-Whitney U test (SPSS 15.0 for Windows).

Paired comparisons were performed with the Wilcoxon signed-rank test in Q-PCR validation studies.

Paraclique analysis

Paraclique analysis is a method to find highly connected clusters of nodes in a network. A clique is defined as a set of completely connected nodes. A paraclique is less stringent, allowing the inclusion of nodes that share connections with most nodes in a clique, down to a given threshold. For this analysis, the threshold was 10% of the original cliques' size.(6)

Small interfering RNA (siRNA) mediated gene knockdown of *ETS1*

CD4⁺ Th cells and memory CD4⁺ Th cells were isolated from PBMCs from healthy blood donors using isolation kits from Miltenyi (Bergisch Gladbach, Germany) according to the manufacturer's instruction. Isolated cells were nucleofected either with nucleofection buffer, 1 μ M human on target plus SMARTpool siRNA against *ETS1* (Dharmacon, Lafayette, CO) or non-targeting siRNA (Dharmacon, Lafayette, CO) using the AMAXA nucleofection program U-014. Six hours after the nucleofection cells were washed, activated and polarized either towards Th1 or Th2 as previously described.(3). Briefly. The isolated CD4⁺ cells were activated with plate bound anti-CD3 (500ng/ml for coating), with soluble anti-CD28 (500ng/ml) and with IL-2 (17ng/ml, all purchased from R&D). For Th1 polarization we complemented the medium with anti-IL-4 (5 μ g/ml), IL-

12 (2,5ng/ml) and IL-2 (17ng/ml). Th2 polarization was induced with anti-IL-12 (5µg/ml) and IL-4 (10ng/ml). For RT-PCR and microarray analysis the cells were harvested at 0, 12 or 36 hours of polarization from three biological replicates. For IL-5, IL-13 and IFN-γ cytokine analysis, the supernatant media were collected after 36 and 60 hours.

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Figure legends

Fig S1. Expression of IRF4 in CD4⁺ T cells. CD4⁺ T cells were cultured with diluents, or with anti-CD3 and anti-CD28, or under Th2 polarization condition (anti-CD3 and anti-CD28, IL-4 and anti-IL-12) for 48 hours. The shaded histogram represents isotype control and the solid line represents IRF4.

Fig S2. Validation of immunoprecipitating anti-human IRF4 antibodies in allergen-challenged CD4⁺ cells from allergic patients (n = 3). The number of bindings events / 1000 cells for known IRF4 targets (IRF4 and TLR4) were compared with two negative controls (Untr12 and Untr20).

Fig. S3. Q-PCR analysis of *GATA3*, *IL2RA* and *IL2RG* in CD4⁺ cells from patients with IAR (n = 20). The bar graph illustrates the mean $\pm$ SEM fold changes of gene expression between allergen- and diluent -challenged cells (** $P < .01$), as well as between allergen-challenged and hydrocortisone treated cells ($^{\$}P < .01$).

Fig S4. Histogram showing the distribution of *P* values from the correlation-based enrichment analysis. The black line shows the number of unique predicted TF binding sites in each interval.

Fig S5. Hierarchical cluster analysis of microarray data profiling all TFs in IRF4-regulated TMs. Each row represents a TF and each column a sample. The scale represents log base 2 expression values.

Fig S6. Q-PCR analysis after ETS1-knock down. Polarized Th2 cells were transfected with either diluent (M) *ETS1*-specific siRNA pool (E) or non-targeting control siRNA pool (n = 5). The figure shows the expression levels of *ETS1*, *IRF4* and *GATA3* 0 and 12 hours after the activation (6 and 18 hours after the nucleofection).

Fig S7. Pathway analysis after ETS1 knock-down ETS1 knock-down was performed in Th2 polarized CD4⁺ cells. Differentially expressed genes after the knock-down were submitted to Ingenuity Pathway Analysis (IPA). The figure represents significant pathways with a *P* value < .05 (represented by the orange line).

Table SI

See Supplementary excel file “Table SI.xls”

Table SII

See Supplementary excel file “Table SII.xls”

Table SIII. CRM-Matrix associations

CRM	Matrices
compo_tpf0.1_1comp_3	M00346
compo_tpf0.1_2comp_3	M00346
compo_tpf0.1_3comp_7	M00346
compo_tpf0.2_1comp_2	M00789
compo_tpf0.2_1comp_3	M00346
compo_tpf0.2_1comp_4	M00462
compo_tpf0.2_1comp_7	M00062
compo_tpf0.2_2comp_4	M00747, M00624
compo_tpf0.5_1comp_2	M00346
compo_tpf0.5_1comp_5	M00472
compo_tpf0.5_1comp_8	M00447
compo_tpf0.5_2comp_9	M00032, M00499
compo_tpf0.5_3comp_10	M00478, M00464, M00980
compo_tpf0.5_3comp_3	M00478, M00930, M00616
compo_tpf0.5_3comp_4	M00473, M00489, M00747
compo_tpf1.0_1comp_1	M00346
compo_tpf1.0_1comp_5	M00699
compo_tpf1.0_1comp_7	M00447
compo_tpf1.0_1comp_9	M00640
compo_tpf1.0_2comp_2	M00462, M00493

compo_tpf1.0_2comp_3	M00473, M00462
compo_tpf1.0_2comp_5	M00346, M00493
compo_tpf1.0_2comp_7	M00040, M00493
compo_tpf1.0_3comp_10	M00478, M00472, M00750
compo_tpf1.0_3comp_2	M00040, M00478, M00473

Position weight matrices (PWMs) found in enriched CRMs

Table SIV - Matrix-TF associations

Matrix	Transcription factor
M00004	ATF-2
M00006	aMEF-2, MEF-2A
M00032	NF-AT, NF-AT1, NF-AT1C, NF-AT2, NF-AT3, NF-AT4
M00040	ATF-2
M00062	IRF-1
M00069	YY1
M00093	Oct-1, POU2F1, POU2F1a
M00099	S8
M00148	SRY
M00160	SRY
M00161	Oct-1, POU2F1, POU2F1a
M00216	TBP, TFIID
M00248	Oct-1, POU2F1, POU2F1a, POU2F1b, POU2F1c
M00258	ISGF-3
M00346	GATA-1, GATA-1A
M00424	Nkx6-1
M00447	AR
M00451	Nkx3-1
M00462	GATA-6, GATA-6A, GATA-6B
M00464	POU3F2
M00472	FOXO4

M00473	FOXO1
M00476	FOXO4
M00478	CDC5L
M00489	Nkx6-2
M00493	STAT5A
M00499	STAT5A
M00624	DBP
M00640	HOXA4
M00671	TCF-4
M00699	IRF-8
M00747	IRF-1
M00750	HMG, HMG-Y, HMGI-C
M00789	GATA-1, GATA-1A, GATA-2, GATA-3, GATA-4, GATA-5, GATA-5A, GATA-5B, GATA-6, GATA-6A, GATA-6B
M00795	Brn1, OCA-B, Oct-1, Oct-10, Oct-2, Oct-2.1, Oct-2.2, Oct-2.5, Oct-9, oct-B2, oct-B3, Oct-R, Octa-factor, octamer-binding, POU2F1, POU2F1a, POU2F1b, POU2F1c, POU2F2, POU2F2B, POU2F3, POU3F1, POU3F2, POU4F1, POU4F1(l), POU5F1, POU5F1A, POU5F1B, POU5F1C
M00926	AP-1, c-Fos, c-Jun, FosB, Fra-1, Fra-2, JunB, JunD
M00930	Oct-1, POU2F1, POU2F1a
M01014	Sox-xbb1, Sox10, Sox11, Sox12, Sox13, Sox14, Sox15, Sox18, Sox2, Sox20, Sox21, Sox3, Sox4, Sox5, Sox6-Isoform1, Sox8, Sox9, SRY

Source: TRANSFAC

Table SV - CRM/Target associations

CRM	Targets
compo_tpf0.1_1comp_3	TANK, POLA2, TAF1B, STK17B, GABPB2, MCL1, NME7, C10orf45, STAT5B, RAD21, CPSF5, LRRFIP2, LAPTM4B, C3orf6, POLE2, KIAA1524
compo_tpf0.1_2comp_3	TANK, POLA2, TAF1B, STK17B, GABPB2, MCL1, NME7, C10orf45, STAT5B, RAD21, CPSF5, LRRFIP2, LAPTM4B, C3orf6, POLE2, KIAA1524
compo_tpf0.1_3comp_7	TANK, POLA2, TAF1B, STK17B, GABPB2, MCL1, NME7, C10orf45, STAT5B, RAD21, CPSF5, LRRFIP2, LAPTM4B, C3orf6, POLE2, KIAA1524
compo_tpf0.2_1comp_2	HNRPC, POLA2, STK17B, GABPB2, MCL1, SIAH2, NME7, STAT5B, EPLIN, RORA, CDCA7, LMNB1, LAPTM4B, AIM2, KIAA1078, C3orf6, POLE2, KIAA1524, KIF23, OIP5, BUB1B
compo_tpf0.2_1comp_3	TANK, POLA2, TAF1B, STK17B, GABPB2, MCL1, NME7, C10orf45, STAT5B, RAD21, CPSF5, LRRFIP2, LMNB1, LAPTM4B, FIGNL1, FLJ20641, C3orf6, POLE2, KIAA1524, KIF23, OIP5, BUB1B
compo_tpf0.2_1comp_4	HNRPC, TANK, POLA2, TAF1B, PSMA5, NPC2, STK17B, GABPB2, SIAH2, NME7, C10orf45, STAT5B, RORA, LMNB1, LAPTM4B, C3orf6, POLE2, KIF23, BUB1B

compo_tpf0.2_1comp_7 XTP3TPA, HNRPC, SP100, VIL2, TRAF1, ICOS, MCM3, PPP1R16B, DC13, CCT8, CBX5, RGS1, AIM2, FLJ20641, KIF2C, MSI2, KIAA1524, KIF23, ASPM, RRM2, TPX2

compo_tpf0.2_2comp_4 MLC1SA, SAMSN1, PSMA5, FLJ10407, UCHL5, TOP2A

compo_tpf0.5_1comp_2 XTP3TPA, HNRPC, TANK, SUPT16H, POLA2, CTLA4, TAF1B, PSMA5, LY9, STK17B, ICOS, GABPB2, MCL1, SIAH2, PPP1R16B, hnRNPA3, INPP5A, ARL3, C10orf45, RAFTLIN, STAT5B, SNRPF, SOCS1, PSMA2, FXR1, CPSF5, CORO1A, CCT8, CBX5, PSCDBP, IFNG, FYN, LMNB1, LAPTM4B, FLJ20641, C3orf6, POLE2, LOC388272, SESN3, KIAA1524, IL2RA, KIF23, OIP5, BUB1, BUB1B

compo_tpf0.5_1comp_5 HNRPC, RECQL, FBXO5, MRPS27, SP100, RPA3, POLA2, C18orf9, RACGAP1, GART, SFRS3, STK17B, PTGER2, GABPB2, MCL1, PRDM1, SIAH2, RAB27A, NME7, ARL3, AUH, SH2D1A, SOCS1, CCT8, PSCDBP, ECT2, CDCA7, FYN, DHFR, PPP2R5C, POLE2, LOC388272, SESN3, POLQ, KIAA1524, IL2RA, KIF23, ASPM, OIP5, BUB1, BUB1B

compo_tpf0.5_1comp_8 PTPN22, FBXO5, MRPS27, SP100, CTLA4, RACGAP1, PSMA5, GART, LY9, NPC2, GBP5, DEK, STK17B, VAMP5, MCM3, MCL1, PRDM1, PPP1R16B, RAB27A, hnRNPA3, ARL3, SRI, C10orf45, STAT5B, LOC400368, SOCS1, FXR1, CCT8, LCP2, IFNG, FYN, LMNB1, STK6, AIM2, SRPK2, C3orf6, BRCA1, MSI2, SESN3, POLQ, IL2RA, ASPM, BUB1,

	KIFC1
compo_tpf0.5_2comp_9	C3orf4, PTPN22, FBXO5, MRPS27, H2AFZ, CTLA4, TAF1B, ICOS, GABPB2, MCL1, INPP5A, ARL3, C10orf45, AUH, PSMA2, CPSF5, PSCDBP, RORA, CDCA7, IL2RA, ASPM, TPX2
compo_tpf0.5_3comp_10	FLJ11712, PTPN22, C10orf45, FXR1, IFI16, CGI-116, PSCDBP, RGS1, ECT2
compo_tpf0.5_3comp_3	PTPN22, PTPRC, STK17B, C10orf45, RBMX, SH2D1A, PSCDBP, PTPN22, KIAA1524, BUB1B
compo_tpf0.5_3comp_4	PELI1, RECQL, FBXO5, SP100, RAD21, PSCDBP, WTAP, PPP2R5C, YWHAZ, P2RY10
compo_tpf1.0_1comp_1	HNRPC, PTPN22, TANK, POLA2, H2AFZ, RACGAP1, TAF1B, PSMA5, LY9, DEK, STK17B, ICOS, GABPB2, MCM3, MCL1, PRDM1, SIAH2, PPP1R16B, hnRNPA3, NME7, INPP5A, ARL3, SRI, C10orf45, SOD1, RAFTLIN, STAT5B, LOC400368, RBMX, SH2D1A, SNRPF, SOCS1, PSMA2, FXR1, CPSF5, CORO1A, CCT8, CBX5, PSCDBP, LRRFIP2, IFNG, RORA, RGS1, FYN, LMNB1, LAPTM4B, STK6, AIM2, PPP2R5C, FLJ20641, C3orf6, KIF2C, POLE2, LOC388272, SESN3, POLQ, KIAA1524, IL2RA, KIF23, ASPM, OIP5, BUB1, BUB1B
compo_tpf1.0_1comp_5	XTP3TPA, HNRPC, FBXO5, MLC1SA, RPA3, KIAA1160, BCL2L12, H2AFZ, C18orf9, CTLA4, RACGAP1, TAF1B,

VIL2, PSMA5, ARHGAP12, NPC2, DEK, STK17B, ICOS,
 GABPB2, MCM3, MCL1, PRDM1, LSM2, SIAH2, PPP1R16B,
 RAB27A, NME7, BID, SNRPB, INPP5A, ARL3, SRI, C10orf45,
 SOD1, DC13, LOC400368, AUH, SOCS1, PSMA2, CPSF5,
 CCT8, CBX5, PSCDBP, RORA, RGS1, CDCA7, LMNB1,
 LAPTM4B, SRPK2, FLJ20641, C3orf6, ETS1, KIF2C, POLE2,
 SESN3, KIAA1524, IL2RA, KIF23, ASPM, RRM2, TPX2

compo_tpf1.0_1comp_7 PTPN22, FBXO5, MRPS27, SP100, CTLA4, RACGAP1,
 PSMA5, GART, LY9, NPC2, GBP5, DEK, STK17B, VAMP5,
 MCM3, MCL1, PRDM1, PPP1R16B, RAB27A, hnRNPA3,
 ARL3, SRI, C10orf45, STAT5B, LOC400368, SOCS1, FXR1,
 CCT8, LCP2, IFNG, FYN, LMNB1, STK6, AIM2, SRPK2,
 C3orf6, BRCA1, MSI2, SESN3, POLQ, IL2RA, ASPM, BUB1,
 KIFC1

compo_tpf1.0_1comp_9 C3orf4, XTP3TPA, FLJ11712, HNRPC, PTPN22, FBXO5,
 MLC1SA, TANK, SP100, RPA3, POLA2, H2AFZ, C18orf9,
 RACGAP1, HSPC138, TAF1B, NUP155, GART, LY9, SFRS3,
 DEK, STK17B, VAMP5, ICOS, GABPB2, PRDM1, LSM2,
 SIAH2, RAB27A, hnRNPA3, FLJ10407, NME7, SNRPB,
 INPP5A, ARL3, SRI, C10orf45, SOD1, DC13, STAT5B, AUH,
 RBMX, PSMA2, PPIH, FXR1, MFAP1, CCT8, CBX5, UCHL5,
 PSCDBP, LRRFIP2, IFNG, RORA, RGS1, FYN, LMNB1,
 LAPTM4B, STK6, AIM2, USP28, FLJ20641, ASB2, C3orf6,

	BRCA1, MSI2, SESN3, POLQ, KIAA1524, IL2RA, KIF23, ASPM, OIP5, BUB1B, RRM2, TPX2
compo_tpf1.0_2comp_2	HNRPC, FBXO5, RPA3, POLA2, C18orf9, CTLA4, RACGAP1, HSPC138, TAF1B, TRAF1, LY9, NPC2, STK17B, VAMP5, ICOS, GABPB2, MCM3, MCL1, PRDM1, SIAH2, PPP1R16B, RAB27A, NME7, INPP5A, P2RY10, SRI, C10orf45, DC13, STAT5B, AUH, SNRPF, PSMA2, CPSF5, CCT8, CBX5, UCHL5, RORA, ECT2, CDCA7, FYN, STK6, AIM2, MYB, DHFR, PPP2R5C, KIAA1078, FLJ20641, CD38, POLE2, LOC388272, SESN3, KIAA1524, IL2RA, KIF23, OIP5, BUB1B, TPX2
compo_tpf1.0_2comp_3	HNRPC, RECQL, TANK, RPA3, POLA2, C18orf9, RACGAP1, TAF1B, LY9, NPC2, STK17B, VAMP5, PTGER2, GABPB2, MCL1, PRDM1, SIAH2, PPP1R16B, ACTR3, NME7, INPP5A, C10orf45, STAT5B, AUH, SH2D1A, SNRPF, PSMA2, IFI16, CPSF5, CCT8, LCP2, CGI-116, C14orf166, LRRFIP2, ECT2, CDCA7, FYN, LMNB1, AIM2, PPP2R5C, POLE2, LOC388272, SESN3, KIAA1524, IL2RA, KIF23, OIP5
compo_tpf1.0_2comp_5	XTP3TPA, HNRPC, POLA2, H2AFZ, CTLA4, RACGAP1, HSPC138, TAF1B, LY9, GBP4, DEK, STK17B, ICOS, GABPB2, MCM3, MCL1, PRDM1, SIAH2, PPP1R16B, NME7, SNRPB, INPP5A, ARL3, SRI, C10orf45, SOD1, STAT5B, SNRPF, SOCS1, PSMA2, CPSF5, CORO1A, CBX5, PSCDBP,

IFNG, RORA, STK6, AIM2, DHFR, PPP2R5C, FLJ20641,
 ETS1, KIF2C, POLE2, LOC388272, SESN3, POLQ, KIAA1524,
 IL2RA, KIF23, OIP5, BUB1B, TPX2

compo_tpf1.0_2comp_7 PTPN22, FBXO5, RPA3, POLA2, H2AFZ, CTLA4, RACGAP1,
 TAF1B, TRAF1, EED, LY9, NPC2, GBP4, STK17B, VAMP5,
 ICOS, GABPB2, MCM3, SIAH2, RAB27A, NME7, C10orf45,
 RAFTLIN, STAT5B, AUH, CPSF5, PSCDBP, RORA, ECT2,
 STK6, AIM2, PPP2R5C, FLJ20641, ASB2, POLE2, SESN3,
 IL2RA, KIF23, BUB1, BUB1B, TPX2

compo_tpf1.0_3comp_10 HNRPC, POLA2, H2AFZ, GART, STK17B, GABPB2, MCM3,
 PRDM1, SIAH2, NME7, ARL3, C10orf45, SH2D1A, FXR1,
 DYRK2, CCT8, ECT2, CDCA7, MSI2, LOC388272, SESN3,
 POLQ, KIAA1524, KIF23, BUB1B, TPX2

Supplementary Table SVI

See Supplementary excel file “Table SVI.xls”

Table SVII. Genes that are affected by ETS1 block and differentially expressed in allergen-challenged CD4⁺ cells

Gene symbols	Fold Change
ETS1	-2.3686
GZMA	-1.64123
CORO1A	-1.45682
LMNA	1.439595
CCL3L1	-1.75359
GPR56	1.423115
PTPRCAP	-1.53456
RASSF2	-1.38622
IL16	-1.4725
IGFBP4	1.518219
IL1A	-1.38169
ADAM19	-1.37063
CD79B	-1.41358
SNTB1	-1.40251
TNFSF13B	-1.48981
SOX8	1.415418
CCR7	1.332962
EMILIN2	-1.33463

EVL	-1.31274
IL24	-1.40477
ALOX5AP	-1.4267
CCR2	-1.2908
SLA	-1.3113
GMFG	-1.54727
CASP4	-1.26298
RRAGD	1.271791
FOS	1.294316
TCF7	1.256613
CCL3	-1.31588
CTSH	-1.26408
IL4I1	-1.25214
IFITM2	-1.37503
TNFRSF18	1.251014
PTPN22	1.253253

Genes that are differentially expressed both in allergen-challenged CD4⁺ cells and Th2 polarized cells after *ETS1* knock-down as described in the results.

Table S1. List of the IRF4-induced genes

Entrez gene ID	Gene symbol	IRF4 Corr. q-value	Diff. exp. q-value
132	ADK	0.019	0.010
158	ADSL	0.042	0.002
330	BIRC3	0.001	0.000
403	ARL3	0.004	0.000
522	ATP5J	0.013	0.022
639	PRDM1	0.001	0.000
753	C18orf1	0.026	0.043
817	CAMK2D	0.012	0.001
894	CCND2	0.002	0.000
914	CD2	0.012	0.000
915	CD3D	0.020	0.000
940	CD28	0.018	0.000
969	CD69	0.003	0.000
1163	CKS1B	0.003	0.000
1452	CSNK1A1	0.001	0.030
1477	CSTF1	0.041	0.000
1479	CSTF3	0.022	0.002
1493	CTLA4	0.003	0.000
1615	DARS	0.032	0.002
1654	DDX3X	0.017	0.002
1719	DHFR	0.001	0.000
2113	ETS1	0.021	0.000
2287	FKBP3	0.000	0.005
2534	FYN	0.007	0.000
2553	GABPB2	0.020	0.000
2618	GART	0.018	0.000
2625	GATA3	0.003	0.000
2935	GSPT1	0.016	0.002
3006	HIST1H1C	0.007	0.000
3014	H2AFX	0.007	0.000
3015	H2AFZ	0.019	0.000
3017	HIST1H2BD	0.021	0.000
3091	HIF1A	0.013	0.000
3094	HINT1	0.020	0.009
3146	HMGB1	0.002	0.000
3178	HNRPA1	0.019	0.000
3181	HNRPA2B1	0.004	0.000
3183	HNRPC	0.023	0.001
3192	HNRPU	0.023	0.002
3398	ID2	0.012	0.000
3428	IFI16	0.001	0.000
3559	IL2RA	0.002	0.000
3561	IL2RG	0.041	0.000
3833	KIFC1	0.007	0.000
4076	M11S1	0.032	0.000
4087	SMAD2	0.003	0.006
4094	MAF	0.015	0.000
4170	MCL1	0.013	0.000

4172	MCM3	0.008	0.000
4236	MFAP1	0.001	0.004
4329	ALDH6A1	0.044	0.006
4676	P1L4	0.041	0.000
4686	NCBP1	0.017	0.002
4714	NDUFB8	0.017	0.022
4750	NEK1	0.001	0.005
5110	PCMT1	0.020	0.001
5111	PC	0.001	0.000
5140	PDE3B	0.027	0.000
5150	PDE7A	0.027	0.006
5216	PFN1	0.015	0.001
5383	PMS2L5	0.031	0.000
5496	PPM1G	0.030	0.001
5527	PPP2R5C	0.001	0.000
5528	PPP2R5D	0.011	0.032
5557	PRIM1	0.025	0.000
5682	PSMA1	0.028	0.000
5683	PSMA2	0.034	0.000
5686	PSMA5	0.025	0.000
5687	PSMA6	0.016	0.037
5696	PSMB8	0.008	0.000
5701	PSMC2	0.006	0.031
5732	PTGER2	0.006	0.000
5781	PTPN11	0.008	0.000
5788	PTPRC	0.002	0.001
5885	RAD21	0.043	0.000
5908	RAP1B	0.019	0.027
5928	RBBP4	0.013	0.000
5984	RFC4	0.007	0.000
5996	RGS1	0.002	0.000
6004	RGS16	0.013	0.001
6095	RORA	0.028	0.000
6164	RPL34	0.006	0.004
6210	RPS15A	0.017	0.014
6241	RRM2	0.038	0.010
6310	SCA1	0.028	0.000
6421	SFPQ	0.000	0.001
6428	SFRS3	0.033	0.000
6434	SFRS10	0.003	0.016
6478	SIAH2	0.014	0.000
6503	SLA	0.001	0.009
6558	SLC12A2	0.027	0.001
6626	SNRPA	0.020	0.003
6647	SOD1	0.024	0.000
6672	SP100	0.006	0.000
6733	SRPK2	0.004	0.000
6775	STAT4	0.006	0.001
6777	STAT5B	0.017	0.000
6890	TAP1	0.037	0.000
7001	PRDX2	0.007	0.000
7128	TNFAIP3	0.008	0.002

7185	TRAF1	0.009	0.000
7385	UQCRC2	0.045	0.011
7430	VIL2	0.026	0.000
7534	YWHAZ	0.032	0.000
7738	ZNF184	0.035	0.041
7903	SIAT8D	0.004	0.007
8364	HIST1H4C	0.004	0.000
8543	LMO4	0.029	0.001
8631	SCAP1	0.003	0.000
8698	EDG6	0.017	0.000
8726	EED	0.001	0.000
8819	SAP30	0.001	0.000
8894	EIF2S2	0.032	0.015
9014	TAF1B	0.004	0.000
9111	NMI	0.008	0.003
9126	CSPG6	0.016	0.001
9158	FIBP	0.036	0.006
9219	MTA2	0.043	0.008
9252	RPS6KA5	0.045	0.001
9256	BZRAP1	0.014	0.000
9262	STK17B	0.004	0.000
9363	RAB33A	0.006	0.000
9459	ARHGEF6	0.005	0.008
9493	KIF23	0.002	0.000
9589	WTAP	0.032	0.000
9595	PSCDBP	0.029	0.000
9631	NUP155	0.015	0.000
9702	KIAA0092	0.023	0.000
9735	KNTC1	0.027	0.000
9788	MTSS1	0.027	0.000
9908	G3BP2	0.001	0.005
9910	HHL	0.003	0.028
9918	CP1	0.009	0.000
9929	KIAA0063	0.032	0.001
9937	DCLRE1A	0.022	0.001
9948	WDR1	0.003	0.005
9991	ROD1	0.006	0.001
10007	GNPDA1	0.021	0.000
10010	TANK	0.014	0.000
10036	CHAF1A	0.008	0.006
10048	RANBP9	0.033	0.000
10069	C21orf6	0.011	0.007
10096	ACTR3	0.005	0.001
10125	RASGRP1	0.001	0.002
10198	MPHOSPH9	0.006	0.000
10225	CD96	0.027	0.026
10240	MRPS31	0.033	0.026
10276	NET1	0.041	0.000
10318	TNIP1	0.001	0.000
10384	BTN3A3	0.026	0.011
10403	KNTC2	0.008	0.000
10465	PPIH	0.015	0.002

10606	PAICS	0.015	0.000
10627	MRCL3	0.030	0.019
10654	PMVK	0.042	0.021
10663	CXCR6	0.026	0.000
10765	JARID1B	0.016	0.001
10802	SEC24A	0.009	0.045
10982	MAPRE2	0.038	0.025
10987	COPS5	0.036	0.027
11004	KIF2C	0.003	0.000
11007	DIPA	0.050	0.021
11040	PIM2	0.019	0.000
11051	CPSF5	0.001	0.000
11100	HNRPUL1	0.036	0.028
11151	CORO1A	0.015	0.000
11331	REA	0.026	0.005
11335	CBX3	0.012	0.001
11339	OIP5	0.003	0.006
22938	SKIIP	0.004	0.003
23043	TNIK	0.015	0.001
23048	FNBP1	0.008	0.007
23077	MYCBP2	0.008	0.001
23118	MAP3K7IP2	0.004	0.004
23180	RAFTLIN	0.019	0.000
23208	SYT11	0.036	0.000
23231	KIAA0746	0.050	0.001
23429	RYBP	0.011	0.048
23468	CBX5	0.006	0.000
23478	SPC18	0.012	0.015
23587	DERP6	0.006	0.003
23649	POLA2	0.013	0.000
25897	RNF19	0.015	0.001
26127	FGFR1OP2	0.026	0.008
26147	PHF19	0.045	0.001
26191	PTPN22	0.001	0.000
26271	FBXO5	0.008	0.000
26512	DDX26	0.031	0.000
27131	SNX5	0.037	0.016
27249	C2orf25	0.004	0.002
27316	RBMX	0.018	0.000
27334	P2RY10	0.008	0.000
27430	MAT2B	0.006	0.001
29093	MRPL22	0.023	0.002
29121	LLT1	0.001	0.000
29127	RACGAP1	0.009	0.000
29851	ICOS	0.003	0.000
29909	GPR171	0.005	0.000
29922	NME7	0.006	0.002
30836	ERBP	0.015	0.027
50808	AK3L1	0.012	0.001
51019	CGI-116	0.009	0.003
51023	MRPS18C	0.018	0.005
51142	CHCHD2	0.010	0.002

51155	HN1	0.007	0.000
51192	CKLF	0.033	0.000
51203	NUSAP1	0.002	0.000
51474	EPLIN	0.001	0.000
51501	HSPC138	0.008	0.004
51602	NOP5/NOP58	0.003	0.002
51603	CGI-01	0.042	0.009
51676	ASB2	0.022	0.000
54165	RP42	0.042	0.001
54499	LOC54499	0.015	0.015
54812	AFTIPHILIN	0.006	0.000
54882	ANKHD1	0.008	0.003
54900	LAX	0.006	0.000
54904	WHSC1L1	0.028	0.003
54931	RG9MTD1	0.044	0.020
55055	FLJ10036	0.004	0.002
55157	FLJ10514	0.013	0.002
55171	FLJ10560	0.013	0.029
55293	UEV3	0.048	0.001
55726	FLJ10637	0.006	0.000
55732	FLJ10706	0.006	0.000
55771	FLJ11029	0.003	0.000
55781	RIOK2	0.049	0.003
55832	TIP120A	0.001	0.003
55835	CENPJ	0.039	0.001
55839	BM039	0.012	0.000
55843	ARHGAP15	0.041	0.011
55863	HT007	0.006	0.008
56650	C3orf4	0.024	0.000
56829	ZC3HAV1	0.004	0.000
56942	DC13	0.032	0.000
57122	NUP107	0.040	0.002
57461	KIAA1160	0.031	0.001
57646	USP28	0.006	0.003
57650	KIAA1524	0.001	0.000
57714	KIAA1618	0.017	0.000
57819	LSM2	0.028	0.001
58516	C12orf14	0.021	0.001
63892	THADA	0.019	0.007
63931	MRPS14	0.042	0.002
64083	GOLPH3	0.016	0.002
64092	SAMSN1	0.027	0.000
64216	TFB2M	0.016	0.012
64332	MAIL	0.036	0.012
64375	ZNFN1A4	0.007	0.001
64431	ACTR6	0.001	0.046
64857	CLG	0.026	0.000
65008	MRPL1	0.018	0.002
79009	DDX50	0.045	0.009
79029	SPATA5L1	0.046	0.032
79568	FLJ22555	0.001	0.002
79621	FLJ11712	0.014	0.002

79670	ZCCHC6	0.003	0.001
79683	ZDHHHC14	0.004	0.000
79968	FLJ12973	0.024	0.000
80008	FLJ23235	0.006	0.005
80010	C9orf76	0.030	0.000
80185	FLJ23263	0.033	0.005
80196	RNF34	0.018	0.018
81539	SLC38A1	0.043	0.006
83461	CDCA3	0.002	0.007
83593	RASSF5	0.039	0.000
83641	C10orf45	0.020	0.000
84231	TRAF7	0.013	0.044
84305	PYM	0.004	0.006
84923	FLJ14775	0.000	0.003
84991	RBM17	0.002	0.018
90701	LOC90701	0.001	0.000
91137	LOC91137	0.018	0.001
91419	KUB3	0.017	0.002
92667	C20orf72	0.044	0.001
92815	HIST3H2A	0.036	0.000
94120	SYTL3	0.004	0.000
115004	C6orf150	0.016	0.009
115106	CCDC5	0.017	0.001
118980	SFXN2	0.027	0.000
124944	MGC49942	0.010	0.000
124995	MRPL10	0.030	0.000
128077	MGC46719	0.042	0.022
134492	LOC134492	0.001	0.001
140465	MLC1SA	0.013	0.000
140691	RNF36	0.016	0.001
143686	SESN3	0.001	0.001
153830	FLJ31951	0.043	0.001
163786	DKFZp761A078	0.004	0.003
196294	FLJ25059	0.006	0.012
200916	LOC200916	0.004	0.001
283237	MGC29649	0.012	0.003
283991	MGC29814	0.013	0.001
285513	LOC285513	0.025	0.000
388272	LOC388272	0.006	0.001
400368	LOC400368	0.029	0.000

Table S2. List of the IRF4-reduced genes

Entrez gene ID	Gene symbol	IRF4 Corr. q-value	Diff. Exp. q-value
172	AFG3L1	0.013	0.005
177	AGER	0.031	0.000
411	ARSB	0.036	0.005
535	ATP6V0A1	0.020	0.001
813	CALU	0.032	0.007
822	CAPG	0.030	0.000
1209	CLPTM1	0.003	0.016
1230	CCR1	0.031	0.000
1500	CTNND1	0.042	0.000
1675	DF	0.036	0.000
1687	DF5	0.010	0.000
1797	DOM3Z	0.024	0.002
2519	FUCA2	0.039	0.004
2745	GLRX	0.014	0.023
2874	GPS2	0.016	0.003
3300	DJB2	0.031	0.005
3586	IL10	0.002	0.050
3603	IL16	0.034	0.019
3632	INPP5A	0.003	0.000
3707	ITPKB	0.050	0.000
3983	ABLIM1	0.016	0.000
4053	LTBP2	0.001	0.000
4063	LY9	0.002	0.000
4125	MAN2B1	0.027	0.000
4296	MAP3K11	0.017	0.001
4668	GA	0.026	0.002
4779	NFE2L1	0.004	0.001
4836	NMT1	0.001	0.006
5937	RBMS1	0.018	0.002
6322	SCML1	0.013	0.001
6482	SIAT4A	0.009	0.000
6641	SNTB1	0.017	0.028
7067	THRA	0.042	0.001
7305	TYROBP	0.014	0.000
7376	NR1H2	0.008	0.027
8445	DYRK2	0.004	0.001
8857	FCGBP	0.050	0.003
8869	SIAT9	0.011	0.018
8985	PLOD3	0.004	0.000
9019	MPZL1	0.008	0.002
9056	SLC7A7	0.020	0.000
9138	ARHGEF1	0.043	0.006
9159	PCSK7	0.020	0.007
9374	PPT2	0.031	0.000
9601	ERP70	0.049	0.001
9641	IKBKE	0.018	0.009
10019	LNK	0.025	0.001
10161	P2RY5	0.016	0.001
10347	ABCA7	0.001	0.000

10577	NPC2	0.014	0.000
10695	TNRC5	0.025	0.000
10948	STARD3	0.006	0.003
23031	MAST3	0.009	0.005
23065	KIAA0090	0.046	0.044
23152	CIC	0.005	0.006
23258	RAB6IP1	0.040	0.000
23265	EXOC7	0.006	0.035
23271	KIAA1078	0.023	0.000
23307	KIAA0674	0.020	0.002
23352	RBAF600	0.034	0.015
23381	EST1B	0.009	0.012
23603	CORO1C	0.018	0.003
23643	LY96	0.022	0.000
25966	C21orf25	0.030	0.001
26019	UPF2	0.032	0.005
28232	SLCO3A1	0.005	0.007
28951	TRIB2	0.014	0.000
51107	APH-1A	0.015	0.005
54455	FBXO42	0.045	0.000
55114	ARHGAP17	0.018	0.019
55707	FLJ10420	0.014	0.026
55785	FGD6	0.040	0.000
55846	MDS028	0.005	0.000
56927	GPR108	0.031	0.021
57728	PWDMP	0.032	0.000
59307	SIGIRR	0.032	0.000
64778	FAD104	0.015	0.000
78994	MGC3121	0.032	0.015
79630	FLJ23221	0.046	0.001
80262	Lin10	0.025	0.046
83440	ADP-GK	0.020	0.001
83548	COG3	0.005	0.002
83871	RAB34	0.016	0.007
84695	LOXL3	0.026	0.003
91039	DPP9	0.009	0.001
91748	C14orf43	0.018	0.002
122773	KLHDC1	0.027	0.000
129303	FLJ90024	0.001	0.000
147179	WIRE	0.007	0.001
256987	C5orf12	0.020	0.002
284058	LOC284058	0.008	0.000

Table SVI. Genes affected by ETS1 block

ID	GeneID	FC	P.Value	FDR
ILMN_2122103	ETS1	-2.61	1.50E-05	7.54E-03
ILMN_1687538	ETS1	-2.37	7.63E-10	9.96E-06
ILMN_2099528	BTLA	-1.82	7.28E-08	2.56E-04
ILMN_1778536	BTLA	-1.77	7.84E-08	2.56E-04
ILMN_1773245	CCL3L1	-1.75	6.95E-06	5.73E-03
ILMN_2121408	HBEGF	-1.75	3.61E-08	2.36E-04
ILMN_2100209	CCL4L1	-1.73	7.60E-05	1.77E-02
ILMN_1779324	GZMA	-1.64	1.37E-06	2.63E-03
ILMN_1669174	IL4	-1.63	5.12E-06	4.78E-03
ILMN_2376204	LTB	-1.60	3.86E-05	1.23E-02
ILMN_1688279	PVRIG	-1.55	9.19E-06	6.00E-03
ILMN_1711617	GMFG	-1.55	1.60E-04	2.75E-02
ILMN_1672417	PTPRCAP	-1.53	7.90E-06	5.73E-03
ILMN_2049536	TRPV2	-1.52	3.72E-06	4.41E-03
ILMN_1798256	UPP1	-1.49	1.79E-05	8.05E-03
ILMN_1758418	TNFSF13B	-1.49	4.22E-05	1.25E-02
ILMN_1712026	NLRP3	-1.48	4.83E-06	4.78E-03
ILMN_2408851	ARHGAP30	-1.48	3.45E-06	4.41E-03
ILMN_1813572	IL16	-1.47	1.60E-05	7.70E-03
ILMN_2290628	IL16	-1.47	1.03E-05	6.04E-03
ILMN_1807925	GNG2	-1.47	1.41E-06	2.63E-03
ILMN_1811272	GPR81	-1.47	1.94E-05	8.27E-03
ILMN_2163819	KIF21B	-1.46	1.28E-05	6.98E-03
ILMN_1713749	CORO1A	-1.46	2.84E-06	4.32E-03
ILMN_2374036	CTSL1	-1.45	4.94E-06	4.78E-03
ILMN_2218856	CCL3L1	-1.45	3.04E-04	4.14E-02
ILMN_1683700	TNFSF12	-1.43	8.34E-06	5.73E-03
ILMN_1723141	OTUD1	-1.43	1.49E-05	7.54E-03
ILMN_1797875	ALOX5AP	-1.43	1.43E-04	2.68E-02
ILMN_1812995	CTSL1	-1.43	3.07E-05	1.11E-02
ILMN_1653708	CORO1B	-1.42	8.62E-05	1.98E-02
ILMN_1659077	CD40LG	-1.42	7.37E-06	5.73E-03
ILMN_2366212	CD79B	-1.41	3.67E-05	1.23E-02
ILMN_1697554	SASH3	-1.41	1.26E-04	2.53E-02
ILMN_2328094	DACT1	-1.41	4.04E-05	1.25E-02
ILMN_1774685	IL24	-1.40	6.77E-05	1.70E-02
ILMN_1793410	SNTB1	-1.40	3.85E-05	1.23E-02
ILMN_1761778	TNFSF8	-1.40	1.32E-04	2.53E-02
ILMN_1775542	FAIM3	-1.39	2.52E-05	9.97E-03
ILMN_1750805	ARHGAP30	-1.39	2.65E-04	3.69E-02
ILMN_1751164	ARHGAP30	-1.39	1.06E-05	6.04E-03
ILMN_2188247	IL17F	-1.39	3.84E-04	4.58E-02
ILMN_2412384	CCNE2	-1.39	3.72E-05	1.23E-02
ILMN_1709683	RASSF2	-1.39	9.91E-06	6.04E-03
ILMN_1801068	DACT1	-1.39	3.00E-05	1.11E-02
ILMN_1768814	KLHL6	-1.38	1.65E-05	7.70E-03
ILMN_1658483	IL1A	-1.38	2.06E-05	8.41E-03
ILMN_1747355	CCL3L1	-1.38	6.65E-05	1.70E-02
ILMN_1738725	LIF	-1.38	4.50E-05	1.31E-02

ILMN_1717334	VAV1	-1.38	7.23E-05	1.75E-02
ILMN_1673352	IFITM2	-1.38	3.78E-04	4.57E-02
ILMN_1699651	IL6	-1.37	6.49E-05	1.70E-02
ILMN_1713751	ADAM19	-1.37	2.83E-05	1.09E-02
ILMN_1651776	FHOD1	-1.37	1.03E-04	2.17E-02
ILMN_1695079	ZNF101	-1.36	3.81E-05	1.23E-02
ILMN_1812618	CENTD3	-1.35	2.08E-04	3.31E-02
ILMN_1712431	FAM113B	-1.35	4.18E-05	1.25E-02
ILMN_1775708	SLC2A3	-1.34	4.52E-04	4.96E-02
ILMN_1710434	TBC1D10C	-1.34	9.84E-05	2.17E-02
ILMN_2105573	CCL3L3	-1.34	1.02E-04	2.17E-02
ILMN_1697268	EMILIN2	-1.33	5.65E-05	1.54E-02
ILMN_1777998	ARHGAP25	-1.33	7.42E-05	1.76E-02
ILMN_1674160	BIN1	-1.33	1.55E-04	2.75E-02
ILMN_1680037	FAM65A	-1.33	1.80E-04	2.98E-02
ILMN_1710017	CD79B	-1.32	1.13E-04	2.34E-02
ILMN_1771987	SLC44A2	-1.32	4.16E-04	4.62E-02
ILMN_1787750	CD200	-1.32	2.49E-04	3.54E-02
ILMN_1671509	CCL3	-1.32	3.07E-04	4.14E-02
ILMN_1730622	EVL	-1.31	6.12E-05	1.63E-02
ILMN_2291954	SLA	-1.31	1.59E-04	2.75E-02
ILMN_1719756	ZAP70	-1.31	2.27E-04	3.44E-02
ILMN_2309245	BIN1	-1.30	1.03E-04	2.17E-02
ILMN_1694757	CTSL1	-1.30	1.86E-04	3.04E-02
ILMN_1757074	GNG10	-1.30	3.02E-04	4.14E-02
ILMN_2227573	GSTO1	-1.30	8.78E-05	1.98E-02
ILMN_1661755	FAM129B	-1.30	4.04E-04	4.62E-02
ILMN_1651365	ZBED2	-1.30	2.52E-04	3.54E-02
ILMN_1667825	MLKL	-1.29	1.29E-04	2.53E-02
ILMN_1671142	GPR68	-1.29	1.14E-04	2.34E-02
ILMN_1774761	CCR2	-1.29	1.44E-04	2.68E-02
ILMN_1718671	GJB6	-1.29	1.76E-04	2.97E-02
ILMN_1715603	IL23A	-1.29	1.58E-04	2.75E-02
ILMN_2345898	SLA	-1.29	2.18E-04	3.42E-02
ILMN_1725814	IL24	-1.29	3.87E-04	4.58E-02
ILMN_2352303	RASSF2	-1.28	3.72E-04	4.57E-02
ILMN_1662799	GPSM3	-1.27	3.69E-04	4.57E-02
ILMN_1671554	LPIN1	-1.27	4.17E-04	4.62E-02
ILMN_1732127	RBKS	-1.27	3.72E-04	4.57E-02
ILMN_1776678	GIMAP7	-1.27	3.93E-04	4.58E-02
ILMN_2342835	P2RY14	-1.27	2.35E-04	3.49E-02
ILMN_2390853	CTSH	-1.26	3.31E-04	4.33E-02
ILMN_1778059	CASP4	-1.26	1.77E-04	2.97E-02
ILMN_1808196	GSTO1	-1.26	3.66E-04	4.57E-02
ILMN_2065690	GRAMD3	-1.26	2.51E-04	3.54E-02
ILMN_1664644	ATG16L2	-1.26	3.89E-04	4.58E-02
ILMN_2211534	TMC8	-1.25	2.24E-04	3.44E-02
ILMN_1659960	IL4I1	-1.25	3.56E-04	4.55E-02
ILMN_1658821	SAMD1	1.24	3.22E-04	4.25E-02
ILMN_2349633	TNFRSF18	1.25	4.13E-04	4.62E-02
ILMN_1715885	PTPN22	1.25	4.18E-04	4.62E-02
ILMN_1676470	TCF7	1.26	2.38E-04	3.49E-02

ILMN_2396948	PSMC3IP	1.26	2.44E-04	3.54E-02
ILMN_1806256	MGC27121	1.26	4.16E-04	4.62E-02
ILMN_1699772	RRAGD	1.27	1.97E-04	3.17E-02
ILMN_1736704	DIXDC1	1.28	3.76E-04	4.57E-02
ILMN_1669523	FOS	1.29	2.37E-04	3.49E-02
ILMN_1717197	CD3G	1.29	3.16E-04	4.21E-02
ILMN_1680579	ATP2B4	1.30	2.23E-04	3.44E-02
ILMN_1813379	TNFRSF9	1.32	6.90E-05	1.70E-02
ILMN_2300695	IKZF3	1.33	3.41E-04	4.41E-02
ILMN_1696749	LMNA	1.33	1.30E-04	2.53E-02
ILMN_1715131	CCR7	1.33	5.57E-05	1.54E-02
ILMN_2384122	GPR56	1.34	1.46E-04	2.68E-02
ILMN_1789244	SOX8	1.42	5.47E-05	1.54E-02
ILMN_2352097	GPR56	1.42	7.67E-06	5.73E-03
ILMN_1737394	LMNA	1.44	2.98E-06	4.32E-03
ILMN_1688480	CCND1	1.50	1.52E-04	2.75E-02
ILMN_1665865	IGFBP4	1.52	1.96E-05	8.27E-03
ILMN_1759766	CTXN1	1.60	8.38E-07	2.19E-03

Genes that are differentially expressed after ETS1 knock-down followed by 12 hours of Th2 polarization compared to non-targeting control siRNA.