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SUPPORTING INFORMATION

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Type I interferon suppresses memory Th2 cell cytokine secretion from allergic subjects

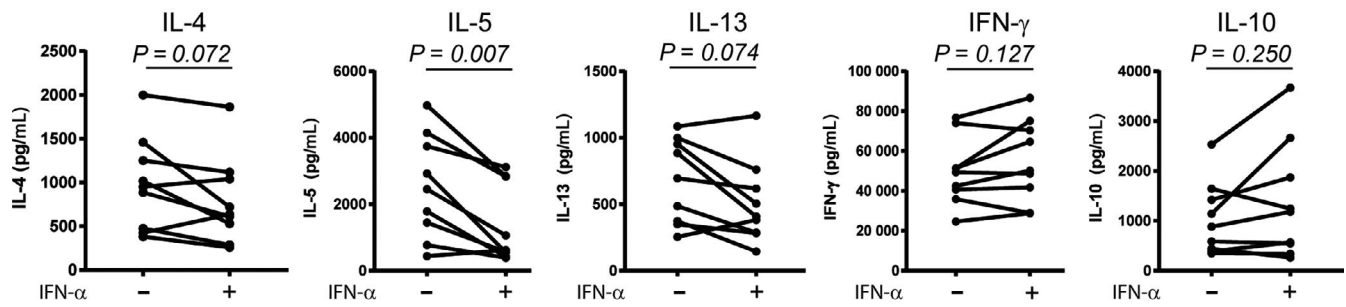
To the Editor,

Upper respiratory viral infections, such as rhinovirus and RSV, are major etiologic agents of allergic airway diseases and drive exacerbations of allergic asthma.¹ Viral infections elicit a variety of innate immune responses from both immune cells and nonimmune cells, and the secretion of the antiviral cytokine IFN- α/β represents the main line of innate protection. Further, IFN- α/β significantly impacts the adaptive response through effects on both T and B cells.² The immune hallmark of allergic diseases is aberrant allergen-induced type 2 inflammation, orchestrated by memory CD4⁺ T cells that produce type 2 cytokines. Our previous studies demonstrated that IFN- α could suppress the development and stability of Th2 cells

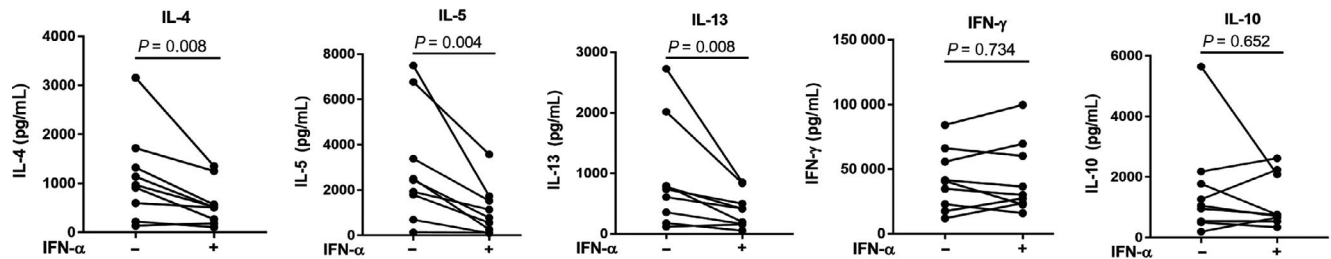
isolated from healthy nonallergic subjects.^{3,4} This observation would suggest that, under normal circumstances, IFN- α/β blocks the Th2 pathway, and this pathway may be defective in the context of the allergic state. However, the role of IFN- α in directly suppressing either Th2 development or stability in CD4⁺ T cells from allergic subjects has not been investigated. In this study, we sought to determine whether IFN- α could suppress allergic memory Th2 cells and explore the transcriptional profiles in this process by RNA sequencing.

A total of 25 subjects (age ≥ 18 years and ≤ 65 years), including 12 allergic rhinitis patients (three comorbid with asthma), four atopic asymptomatic disease-free subjects, and nine healthy subjects were enrolled. Subjects were included in the "allergic" group if they had a

(A) Nonallergic



(B) Allergic rhinitis



(C) Suppression ratio

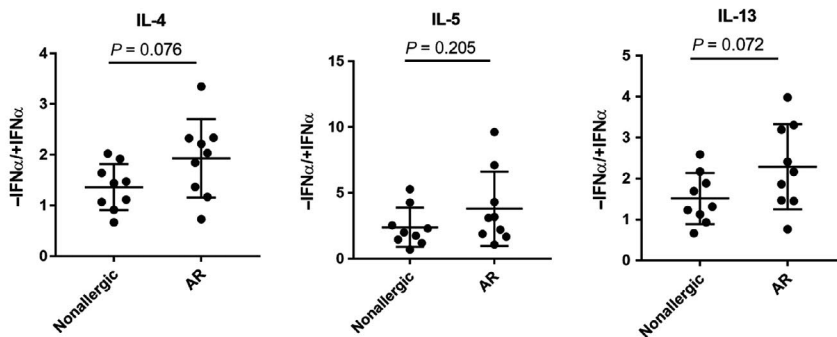


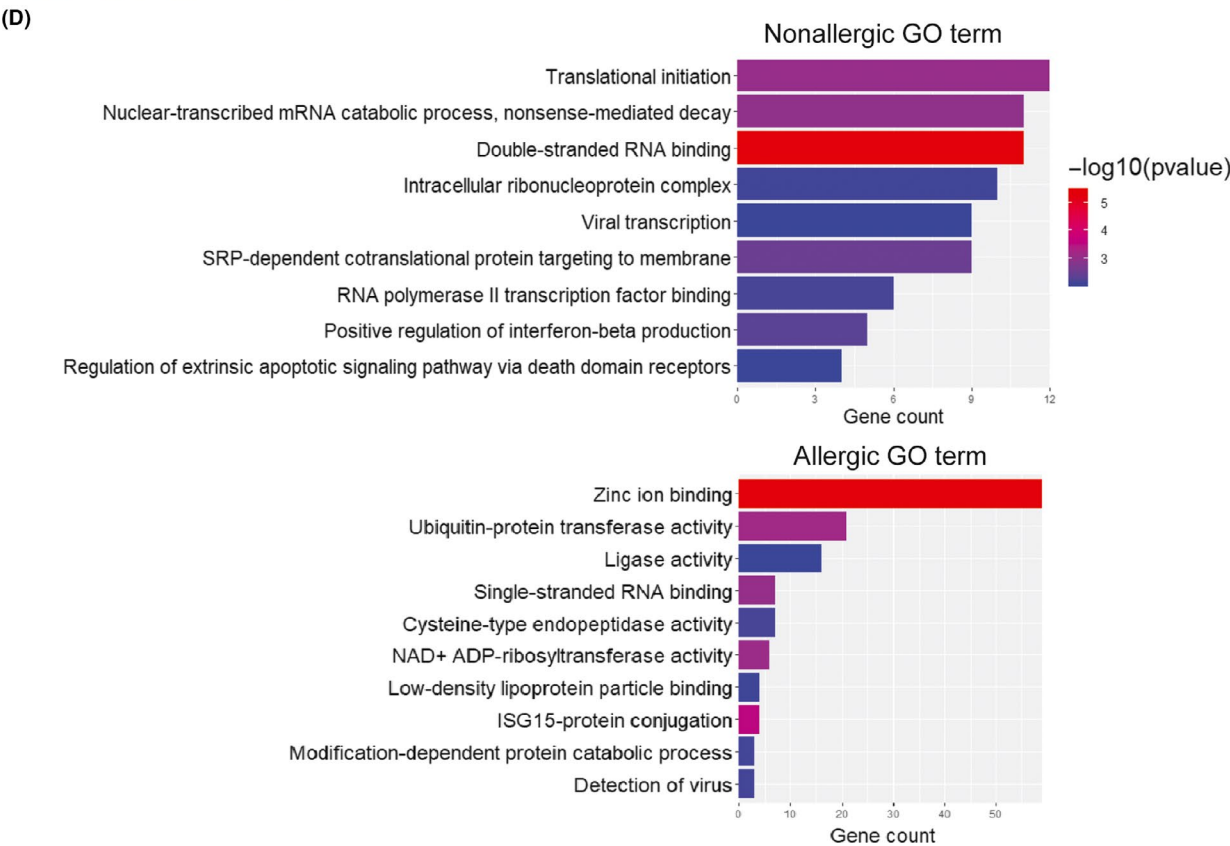
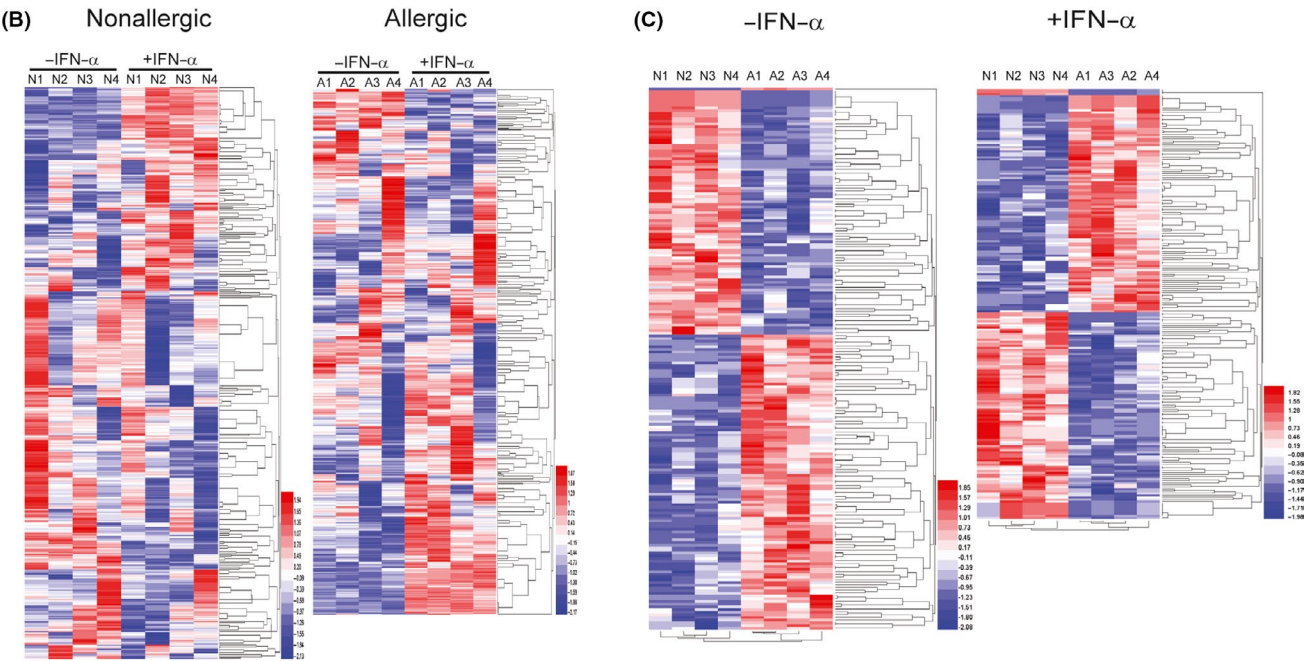
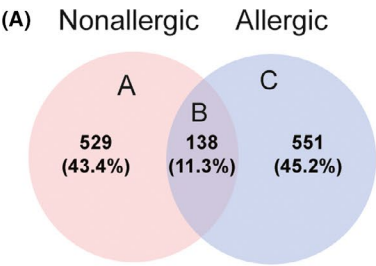
FIGURE 1 IFN- α suppresses IL4, IL5, and IL13, but not IFN- γ and IL10 secretion in human allergic memory T cells (CD4 + CD45RO+). (A) Nonallergic and (B) allergic rhinitis human memory T cells were stimulated with anti-hCD3/CD28 $\pm$ rhIFN- α for 7 d. The concentration of cytokines in cell supernatants was measured by multiplex electrochemiluminescence assay (Meso Scale Discovery [MSD]). Data are shown as mean $\pm$ SD. Each line represents an individual experiment (Nonallergic, $n = 9$; Allergic rhinitis, $n = 9$). (C) Comparison of suppression ratio between nonallergic and allergic subjects. Suppression ratio is defined as the cytokine level in the absence of IFN- α divided by the cytokine level in the presence of IFN- α . Each dot represents an individual subject. Paired Wilcoxon test (A and B) and unpaired t test for suppression ratio (C)

current diagnosis of symptomatic allergic rhinitis without concurrent asthma ($n = 9$, Figure 1B). Subject information is listed in Table S1.

To determine the suppressive effects of IFN- α on allergic and nonallergic memory CD4 + CD45RO+ T cells (Tm), we stimulated purified Tm in vitro for 7 days in the absence or presence of IFN- α (2a) (1000 U/mL). Cells were then washed extensively and restimulated

with anti-CD3/anti-CD28 activation beads for 24 hours. Cytokines secreted into the supernatants were assessed by Meso Scale multiplex ELISA assays. As expected, IFN- α suppressed Th2 cytokine expression in Tm from nonallergic subjects, with IL5 being significantly inhibited in all subjects, while IL4 and IL13 were suppressed in the majority of subjects (7/9, Figure 1A). Further, we found that all three

FIGURE 2 Differentially expressed genes (DEGs) induced by IFN- α in nonallergic and allergic subjects. Memory T cells (CD4 + CD45RO+) were isolated from peripheral blood of allergic individuals, then stimulated with anti-hCD3/CD28 $\pm$ rhIFN- α for 7 days. (A) Venn diagram showing exclusive and shared DEGs in nonallergic and allergic subjects (DESeq2 analysis, Benjamini-Hochberg method FDR < 0.01 and $P < .01$). (B) Heat map showing expression value (Z-score on DESeq2 value) for each DEG induced by IFN- α in nonallergic and allergic groups. Paired RNA-Seq data from each individual are shown in the columns. (C) Heat map showing expression value (Z-score on DESeq2 value) for each DEG in nonallergic and allergic subjects within the same treatment condition ($\pm$ rhIFN- α). RNA-Seq data from each individual are shown in the columns. (D) Enrichment pathways induced by IFN- α in nonallergic and allergic subjects and exclusive gene ontology (GO) terms in nonallergic and allergic subjects



Th2 cytokines (IL4, IL5, and IL13) were significantly suppressed by IFN- α in allergic Tm ($P < .001$, Figure 1B). Moreover, this suppression was specific to Th2 cytokines as neither IFN- γ nor IL10 were significantly altered in Tm from either nonallergic or allergic subjects. The suppressive effects of IFN- α on Th2 cytokines IL4 and IL5 were confirmed by standard ELISA assays (Figure S1). Finally, the overall magnitude of suppression was compared between allergic and nonallergic Tm. We found no differences in the suppression ratio of IL4, IL5, and IL13 in allergic and nonallergic Tm (Figure 1C), demonstrating a dominant role for IFN- α in suppressing Th2 cytokine expression in Tm in both allergic and nonallergic subjects.

To verify the effect of IFN- α on intracellular cytokine expression, we quantified Th1 and Th2 cytokine levels by intracellular staining and flow cytometry. In agreement with our ELISA data, we found the frequencies of IL4 + IL5+ cells, IL4 + IL13+ cells, and IL5 + IL13+ cells were significantly inhibited by IFN- α in both allergic and nonallergic Tm (Figures S2-S3). While we found that IFN- α/β could suppress Th2 cytokine secretion from cells isolated from both nonallergic and allergic subjects, the racial makeup of the nonallergic and allergic cohorts was different, with no African-American subjects enrolled in the nonallergic group. It is possible that there exist differences in responses between racial subsets, which the current study could not measure due to the small sample size.

In order to gain a more comprehensive view of potential intrinsic differences in response to IFN- α between nonallergic and allergic subjects, purified Tm cells were cultured for 7 days in the absence or presence of IFN- α (2a) (1000 U/mL). Then RNA was extracted from Tm without restimulation. RNA sequencing was performed to identify unique IFN- α -regulated transcriptional pathways that were stably expressed in these cells following a period of rest. Although the magnitude of differential gene expression was small, we set a strict false discovery rate (Q value) at 0.01 and $P < .01$, in order to identify genes that were regulated by IFN- α in all subjects in each cohort.

We found that IFN- α regulated a unique set of genes that differed significantly between nonallergic and allergic subjects. RNA-Seq revealed 551 differentially expressed genes (DEGs) in allergic and 529 DEGs in nonallergic individuals that were exclusively regulated in each cohort, with only 138 sharing DEGs in both the groups (Figure 2A). Cluster analysis (Figure 2B-C) revealed consistent magnitudes of expression in the paired samples from each donor and significant differences in transcriptional profiles between nonallergic and allergic subjects. Enrichment analysis showed Gene Ontology (GO) term zinc ion binding, ubiquitin-protein transferase activity, and interferon-stimulated gene (ISG)15 protein conjugation were exclusively regulated in the allergic group (Figure 2D).

We identified several specific genes and pathways that could have significant impact on antiviral response. Our data showed 40 DEGs were related to ubiquitin conjugation in allergic Tm. Among them, 26 out of 40 were exclusively differentially expressed in allergic Tm (Figure S3a). We confirmed suppressor of cytokine signaling 1 (SOCS1) and tumor necrosis factor receptor-associated factor 2 (TRAF2) were exclusively upregulated by IFN- α in allergic but not in nonallergic subjects by qRT-PCR (Figure S4b). A SOCS1

polymorphism was found to be associated with allergic asthma,⁵ which potentially implicates the IFN- α pathway in atopic regulation. Further, Glimcher et al⁶ revealed that TRAF2 repressed the Th2 cytokine IL4, and this effect was mediated through interaction with nuclear factor of activated T cells (NFAT)-interacting protein 45 (NIP45). Although we found that IFN- α/β could suppress Th2 cytokines in T cells from allergic subjects, this was only one aspect of T-cell function that was measured. There may be other aspects of T-cell development, function, and phenotype that may be differentially regulated by IFN- α/β in allergic T cells, and the transcriptomics data presented here would be useful for later interpretation.

Viral infections, which induce IFN- α secretion, pose a significant threat in early childhood, synergizing with the allergic march and promoting allergic airway exacerbations.⁷ How IFN- α influences early T-cell responses in the context of the allergic state is unclear. Our findings are important as we show that memory Th2 cells can be suppressed by IFN- α , suggesting that there is no intrinsic block to their suppression. Rather, the allergic state may effectively suppress IFN- α secretion through IgE-mediated signaling in innate cells.⁸ In support of this, recent clinical studies found that lowering of overall IgE levels with omalizumab restored viral-induced IFN- α responses in innate cells.⁹

Collectively, this is the first study revealing the suppressive effect of IFN- α on memory Th2 cells from allergic subjects. Moreover, we found ubiquitination conjugation enzymes might be involved in this process. Understanding the overall impact of IFN- α signaling on T-cell function will help to open new avenues of therapy to elicit permanent tolerance in allergic T cells.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Circulating neutrophil and eosinophil extracellular traps are markers of severe asthma

To the Editor,

Asthma is a chronic inflammatory airway disease in which neutrophils and eosinophils are major players.^{1,2} Eosinophilic airway infiltration is a hallmark of type 2 inflammation that affects about 50% of adult asthma patients, while neutrophilic infiltration defines a subgroup of patients with severe and steroid-refractory asthma.² Both cells participate in airway epithelium damage by releasing cytotoxic proteins, reactive oxygen species, and possibly, extracellular traps. Neutrophil and eosinophil extracellular traps (NETs and EETs) are web-like DNA filaments with anti-infectious and pro-inflammatory properties, whose involvement in the pathophysiology of asthma seems likely. In mouse models, NET inhibition was protective against rhinovirus-induced asthma exacerbations in a dust mite allergic asthma model,³ and DNase I therapy improved airway resistance and reduced NET accumulation in another model of ovalbumin-induced asthma.⁴ In humans, NETs and EETs have been observed in lung biopsies of asthma patients, and a link between cell-free DNA in sputum and asthma severity has been recently suggested.^{5,6} However, cell-free DNA can have multiple origins and is not considered a specific marker of netosis.⁷ Moreover, relevance of NETs in serum and BALF of asthma patients has not been evaluated so far. Since DNA-granular protein complexes are considered the best markers of NET remnants to date,⁷ we measured the concentration

of myeloperoxidase (MPO)-DNA and eosinophil major basic protein (EMBP)-DNA complexes in serum and bronchoalveolar lavage fluid (BALF) from patients included in the French national COBRA cohort.⁸ To establish the clinical relevance of EETs and NETs in human asthma, we correlated these levels with clinical data.

Clinical characteristics of the 217 asthma patients are shown in Table 1. BALF samples from 51 patients were also analyzed. Systemic and alveolar inflammation was evaluated by multiplex cytokine measurement. MPO-DNA and EMBP-DNA complexes were measured by ELISA. (See Appendix S1 for detailed methods).

Patients with severe asthma had higher circulating NET level than patients with either mild or moderate asthma (GINA score 4 vs 1-3) or healthy controls ($P = .035$ and $P = .020$, respectively; Figure 1A). NET level was also significantly increased in patients with poor or partial vs good control ($P = .0047$ and $P = .011$, respectively; Figure 1B). Patients with high circulating NET level were significantly younger and had earlier asthma onset than those with low NET level (Table 1, $P = .004$ and $P = .032$, respectively). These results were confirmed by logistic regression analysis showing NET level as an independent predictor of poor control even after adjustment for age, age of asthma onset, smoking status, inhaled corticosteroid (ICS) dose, and sex (OR = 2.43, $P = .013$; Table S1). In contrast to NET level, circulating EET level was not linked to asthma severity (Figure 1C)

Online supplementary material

Type I interferon suppresses memory Th2 cell cytokine secretion from allergic subjects

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Abbreviations list

IFN: Interferon

T_m: CD4⁺CD45RO⁺ memory T cells

GO: Gene Ontology

DEGs: Differential expressed genes

ISG: interferon-stimulated gene

SOCS1: suppressor of cytokine signaling 1

TRAF2: tumor necrosis factor receptor-associated factor 2

NFAT: nuclear factor of activated T cells

NIP45: NFAT-interacting protein 45

METHODS

Study subjects

This study was approved by the University of Texas, Southwestern Medical Center Institutional Review Board (IRB). Written informed consents were obtained from a total of 25 subjects (age ≥ 18 years and ≤ 65 years), including 12 allergic rhinitis patients (3 comorbid with asthma), 4 atopic asymptomatic disease-free subjects, and 9 healthy subjects. The inclusion criterion for asthma were established according to the Global Strategy for Asthma Management and Prevention (GINA): (1) physician-diagnosed asthma; (2) positive bronchial provocation test (fall in FEV1 from baseline of $\geq 20\%$ with standard doses of histamine), or positive bronchial dilation test (fall in FEV1 from baseline of $\geq 12\%$ with standard dose of methacholine), or average daily diurnal PEF variability $> 10\%$; (3) newly diagnosed patients. The diagnosis of allergic rhinitis was based on the criterion of the Initiative on Allergic Rhinitis and its Impact on Asthma (ARIA) should also be satisfied: (1) history of nasal symptoms by nose itching, obstruction, sneezing and rhinorrhea; (2) positive skin test result or specific immunoglobulin E (sIgE). All participants underwent skin prick testing for 14 common indoor and outdoor aeroallergens as previously described¹. Atopy was defined as positive skin test to ≥ 1 allergen without any allergic disease history or symptoms. Patients had not received antihistamines or intranasal steroid treatments 1 week before the skin test, or oral steroids at least 4 weeks prior to the skin test. Subjects were included in the “allergic” group if they had a current diagnosis of allergic rhinitis or allergic asthma and/or atopy. All subjects were free from upper and lower respiratory tract infections for 4 weeks preceding the study. Subjects were excluded if any of the following condition was apply. (1) Past or current infection with HIV, hepatitis, or tuberculosis. (2) History of Guillain-Barre syndrome. (3) Current illicit drug or tobacco user. (4) Recent (past 4 months) infection with influenza or vaccination with LAIV (FluMist). (5) Any vaccinations within 2 weeks prior to consent. (6) Organ transplant recipient or currently on steroids and/or immune-suppressant drugs. (7) Pregnancy or nursing mothers. (8) History of a recent “cold” or respiratory viral infection within the preceding four weeks. Subjects information is listed in Table S1.

Memory T cells isolation and cell culture

Peripheral blood was collected by venipuncture from donors. PBMCs were isolated using Ficoll-Plaque (Corning, USA). Memory CD4⁺CD45RO⁺ T cells were enriched from PBMCs by negative bead kit (EasySep, Stemcell, USA) as previously described². Purity was greater than 90%. Cells were activated with plate-bound anti-CD3 (OKT3, 3 mg/ml), anti-CD28 (3 mg/ml), and IL-2 (50 U/ml) in complete IMDM supplemented with 10% FBS with or without IFN- α (2a) (1000 U/ml). After 7 days, cells were

harvested and washed with PBS, counted and resuspended in 10% FBS. Cells were restimulated with Dynabeads human T-activator CD3/CD28 (ThermoFisher) in a 1:1 ratio for 24 h, and supernatant was collected for electrochemiluminescence assays and ELISA. For flow cytometry, cells were restimulated by phorbol-12-myristate-13-acetate (PMA, 50 ng/ml) and ionomycin (1 µg/ml) for 4 h, and monensin (2 µM) for the last 2 h prior to intracellular staining and FACS analysis.

Cytokine measurement

IL4, IL5, IL13, IL10 and IFN-γ concentrations in cell culture supernatants were measured using a multiplex electrochemiluminescence assay (Meso Scale Discovery [MSD], Rockville, Maryland, USA). The detection limits were IFN-γ: 3.21-10000 pg/mL, IL4: 0.15-10000 pg/mL, IL5: 0.18-10000 pg/mL, IL10: 0.61-10000 pg/mL and IL13: 0.46-10000 pg/mL. Data were acquired using a SECTOR S 6000 plate reader (MSD). The levels of IL4, IL5 and IFN-γ in the supernatant of cell culture were confirmed by ELISA (BioLegend, Inc. USA). The detection limits were 3.9 pg/ml for IL4 and IL5, and 7.8 pg/ml for IFN-γ. All values below the detection limits were set at 0.

Flow cytometry

Surface marker and intracellular cytokine staining was performed as previously described². Cells were stained with the following anti-human monoclonal antibodies: anti-IL4-BV421 (BioLegend), anti-IL5-PE (BioLegend), anti-IL13-PerCP-Cy5.5 (BioLegend) for intracellular staining, anti-CD4-BV421 (BioLegend), anti-CD45RO-PE (BD Biosciences) for surface staining. Clone information for antibodies is in Table S2. Cells were first stained for surface antigens after isolation to determine the purity. Following fixation and permeabilization with 2.5% formaldehyde and 0.1% saponin, cells were stained with conjugated Abs to intracellular antigens. Samples were acquired on a BD LSR II flow cytometer (BD Biosciences) and analyzed with the data analysis software FlowJo (FLOWJO, LLC, Ashland, Ore). Fluorescence was determined for all cells in each sample after debris, dead cells, and aggregates were excluded by forward angle and side scatter gating. Gating of cytokine-expressing cells was guided by staining negative control (unstimulated cells) (Figure S2).

RNA-Sequencing and data analysis

Tm were purified from PBMCs of nonallergic (n=4) and allergic rhinitis donors (n=4). Total RNA was isolated (RNeasy Min Kit, QIAGEN, Germany) from cells on Day 0 before culture and from cells activated for 7 days in the absence or presence of IFN- α as described above. Amplified cDNA libraries were constructed using Illumina TruSeq RNA Sample Preparation Kit v2 (Illumina, San Diego, CA). RNA-seq was performed with the Illumina NextSeq 550 system (Illumina, San Diego, CA) with one flow cell for 12 samples. The run number is NextSeq 177 with SE-85.

Sequencing reads were mapped against the human genome (hg19) using the Illumina's BCL2Fastq2. Sequencing read coverage per gene was counted using HTSeq-count. To identify genes differentially expressed between presence and absence of IFN- α conditions in nonallergic and allergic groups, we performed negative binomial tests for pairwise comparisons employing the Bioconductor package DESeq2, disabling the default options for independent filtering and Cooks cutoff. We considered differentially expressed genes (DEGs) between any pairwise comparison when the DESeq2 analysis resulted in a Benjamini-Hochberg-adjusted $P < .01$, false discovery rate (FDR, Q value) $< .01$. We used the DEGs between presence and absence of IFN- α conditions in each group to grow an enrichment pathway analysis by the Database for Annotation, Visualization and Integrated Discovery (DAVID 6.8).

Accession numbers

All sequencing data sets were deposited in the Sequence Read Archive of the National Center for Biotechnology Information (accession number: PRJNA524375).

Statistical analysis

All data are shown as mean $\pm$ SD. Statistical analysis was performed by Wilcoxon paired test with GraphPad Prism software version 7. The P values < 0.05 were considered as significant.

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TABLE S1. Characteristics of subjects

Subject	Sex	Age	Race	Allergy disease	Skin test
1*	Female	35	Asian	No	Neg
2*	Female	23	Caucasian	No	Neg
3*	Female	32	Caucasian	No	Neg
4*	Male	27	Asian	No	Neg
5	Female	28	Caucasian	No	Neg
6	Female	43	Caucasian	No	Neg
7	Female	26	Caucasian	No	Neg
8	Male	29	Asian	No	Neg
9	Female	51	Asian	No	Neg
10*	Female	27	Mix	AR	Elm, Oak mix, Marsh elder, Ragweed, Bermuda, Timothy, Alternaria, Dog and Cat dander
11*	Female	31	Asian	AR	Elm, Mt. Cedar , Oak mix, Marsh elder, Bermuda , Dust mite
12*	Male	32	Asian	AR	Marsh elder, Ragweed, Bermuda
13*	Female	53	African American	AR	Elm, Mt. Cedar, Oak, Marsh Elder, Ragweed, Bermuda, Alternaria, Dust mite, Cockroach, Dog, and Cat
14	Female	46	African American	AR	Elm and Mt. Cedar
15	Female	39	Caucasian	AR	Elm, Oak mix, Ragweed, Bermuda, Timothy, Alternaria, Dust mite
16	Male	54	Asian	AR	Mt. Cedar, Bermuda, Dog dander
17	Female	40	African American	AR	Elm, Mt Cedar, Oak mix, Marsh elder, Ragweed, Bermuda, Timothy, Alternaria, Aspergillus, Hormodendrum, Dust mite, Cockroach, Dog and cat dander
18	Female	37	Caucasian	AR	Elm, Oak mix, March elder, Bermuda, Timothy, Dust mite, Cat dander
19	Female	32	Asian	AR, asthma	Elm, Ragweed, Bermuda
20	Male	42	African American	AR, asthma	Bermuda, Timothy, Alternaria
21	Male	27	African American	AR, asthma	Elm, Mt. Cedar, Bermuda, Timothy, Alternaria, Cockroach, Cat dander
22	Male	25	Mix	No	Elm , Mt. Cedar
23	Female	27	Caucasian	No	Mt. Cedar, Alternaria, Cat dander
24	Female	28	Caucasian	No	Elm, Mt. Cedar, Bermuda, Timothy, Dust mite
25	Male	31	African American	No	Bermuda, Aspergillus, Hormodendrum, Dust mite, Cockroach, Cat dander

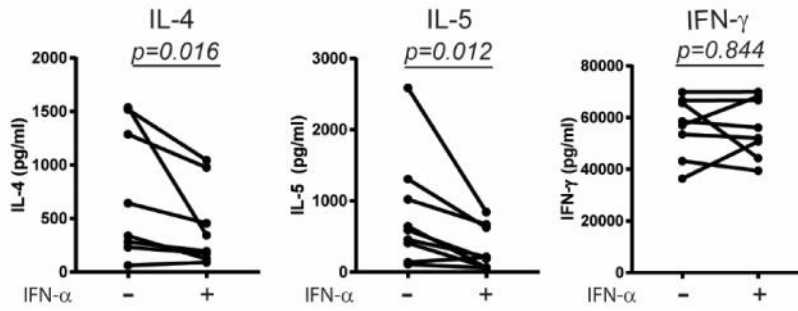
Ragweed, Short ragweed; Mt. Cedar, Mountain cedar; Bermuda, Bermuda grass; Timothy, Timothy grass; Alternaria, Alternaria tenuis; Aspergillus, Aspergillus fumigatus

* indicates the subjects for RNA-Seq.

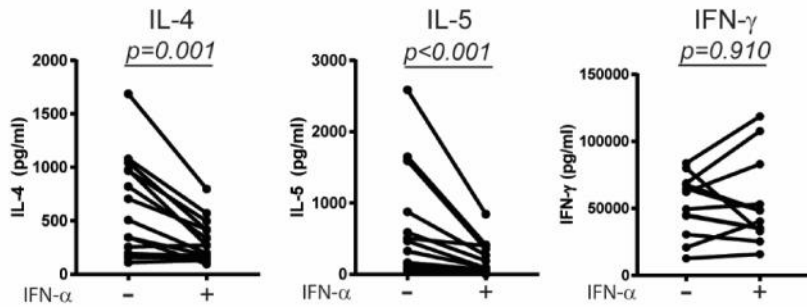
TABLE S2. Antibodies for flow cytometry

Antibody	Vendor	Clone
anti-IL4-BV421	BioLegend	MP4-25D2
anti-IL5-PE	BioLegend	JES1-39D10
anti-IL13-PerCP-Cy5.5	BioLegend	JES10-5A2
anti-CD4-BV421	BioLegend	OKT4
anti-CD45RO-PE	BD Biosciences	UCHL1

A Nonallergic



B Allergic



C IL-4 suppression ratio IL-5 suppression ratio IFN-γ suppression ratio

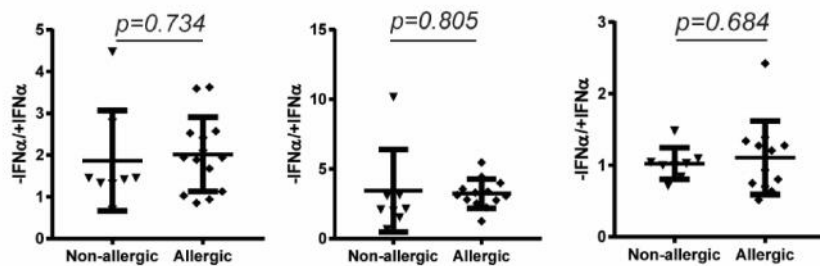


Figure S1. IFN-α suppresses IL-4 and IL-5, but not IFN-γ secretion in human Nonallergic and Allergic memory T cells (CD4+CD45RO+). (A) Nonallergic and (B) allergic human memory T cells were stimulated with anti-hCD3/CD28 ± rhIFN-α for 7 days. The concentration of different cytokines on cell supernatants was measured by ELISA. Data are shown as mean ± SD. Each line represents an individual experiment (n=8-14). (C) Comparison of suppression ratio between nonallergic and allergic subjects. Suppression ratio is defined as the cytokine level in the absence of IFN-α divided by the cytokine level in the presence of IFN-α. Each dot represents an individual experiment. Paired Wilcoxon test (A-B). Unpaired t test for suppression ratio (C).

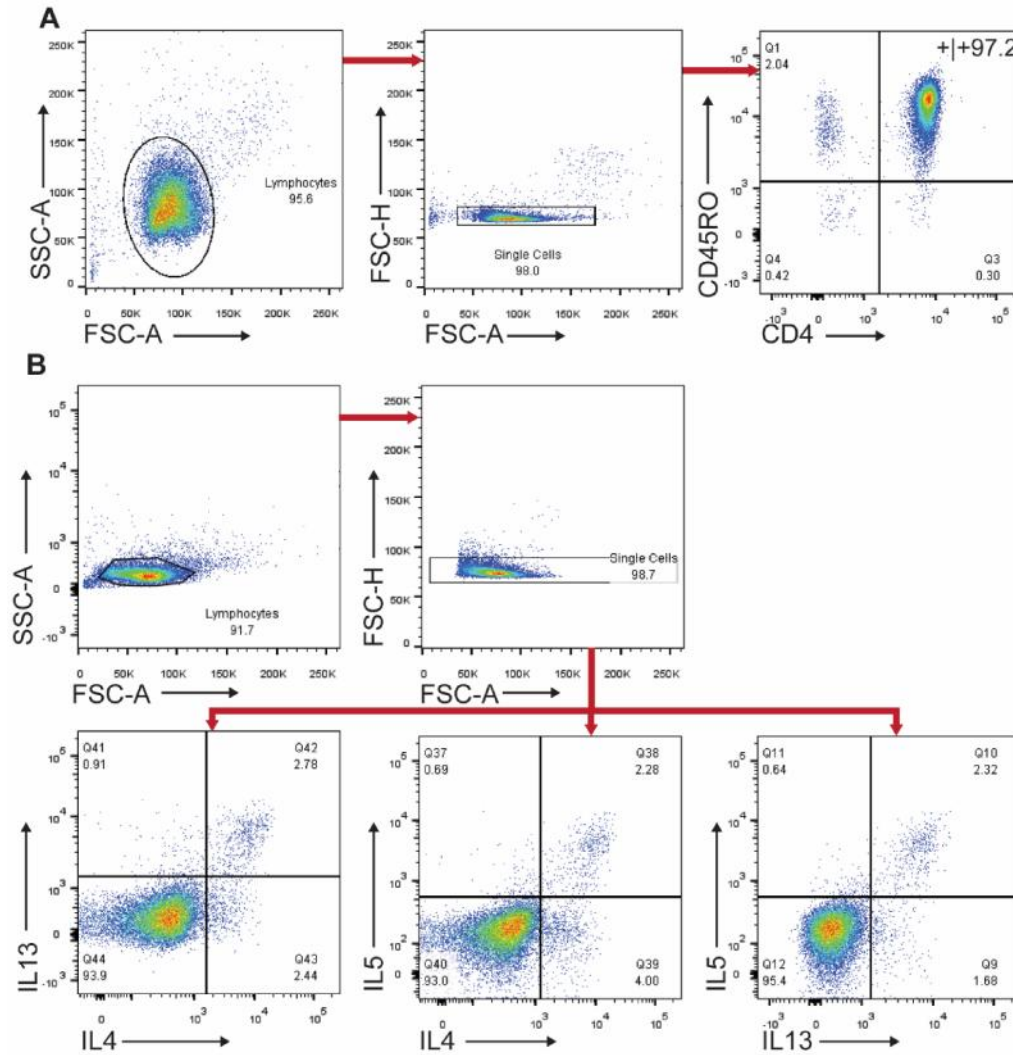
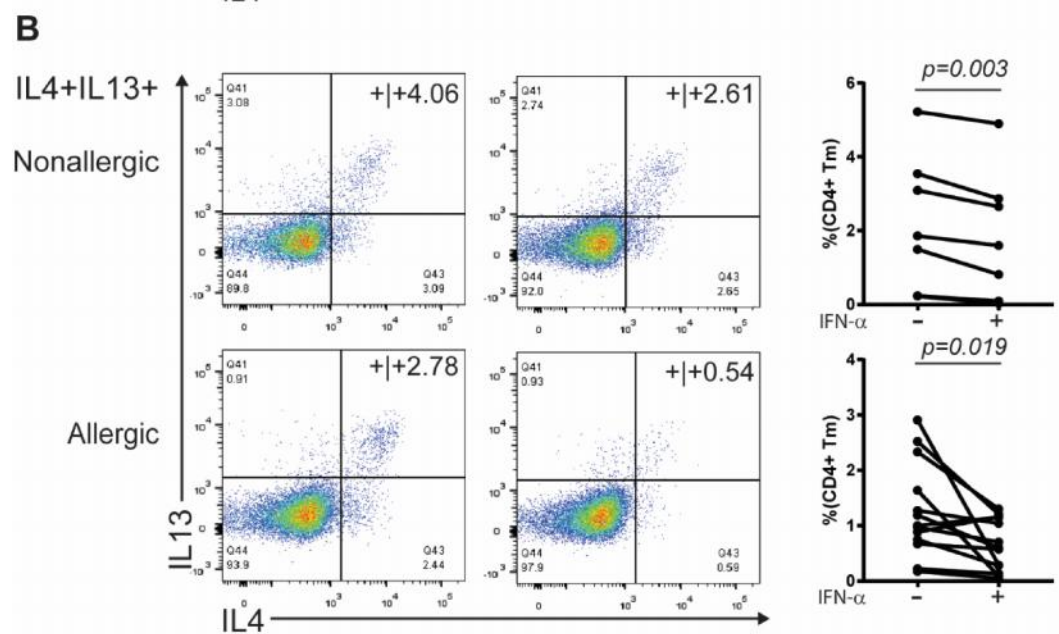
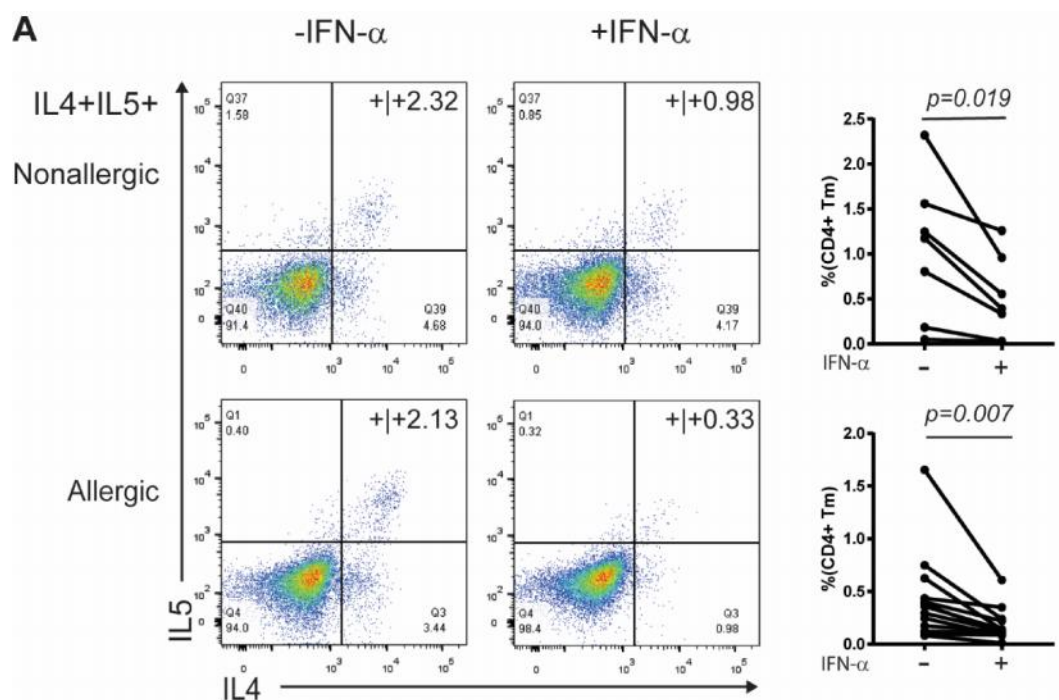


Figure S2. Gating strategy for CD4+CD45RO⁺ memory cells and intracellular cytokines. (A) Gating for purified peripheral CD4+CD45RO⁺ memory cells. The purity was 97.2%. (B) Gating for intracellular cytokine-expressing cells after culture and stimulation. FSC, forward scatter; SSC, side scatter.



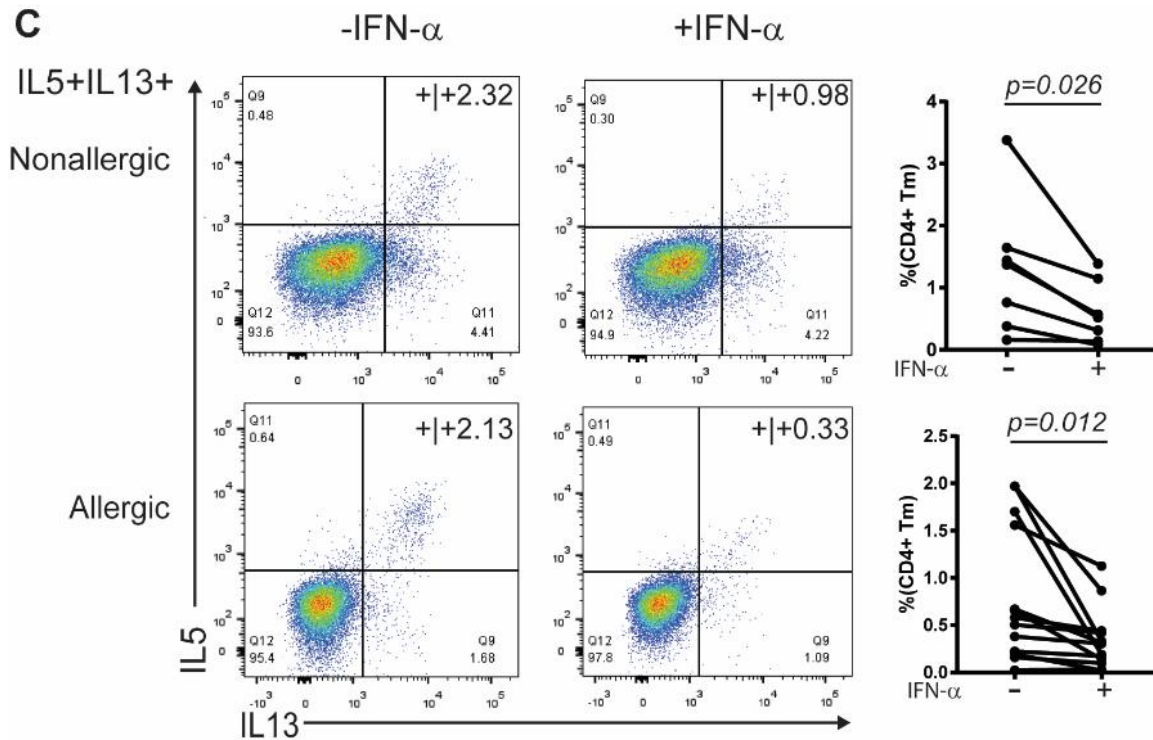


Figure S3. $IFN-\alpha$ suppresses IL4+IL5+, IL4+IL13+ and IL5+IL13+ memory T cells (CD4+CD45RO+) in nonallergic and allergic individuals. Nonallergic and allergic human memory T cells were stimulated with anti-hCD3/CD28 $\pm$ rh $IFN-\alpha$ for 7 days and then restimulation with PMA/Ionomycin and Brefeldin A for 4h. Frequency of double positive intracellular cytokines expressing cells was measured by flow cytometry. Percentages of (A) IL4+IL5+, (B) IL4+IL13+ and (C) IL5+IL13 cells within CD4+T memory cells. Representative flow cytometric plots are shown. Data are shown as mean. Each line represents an individual subject (n=7-13). Paired Wilcoxon test.

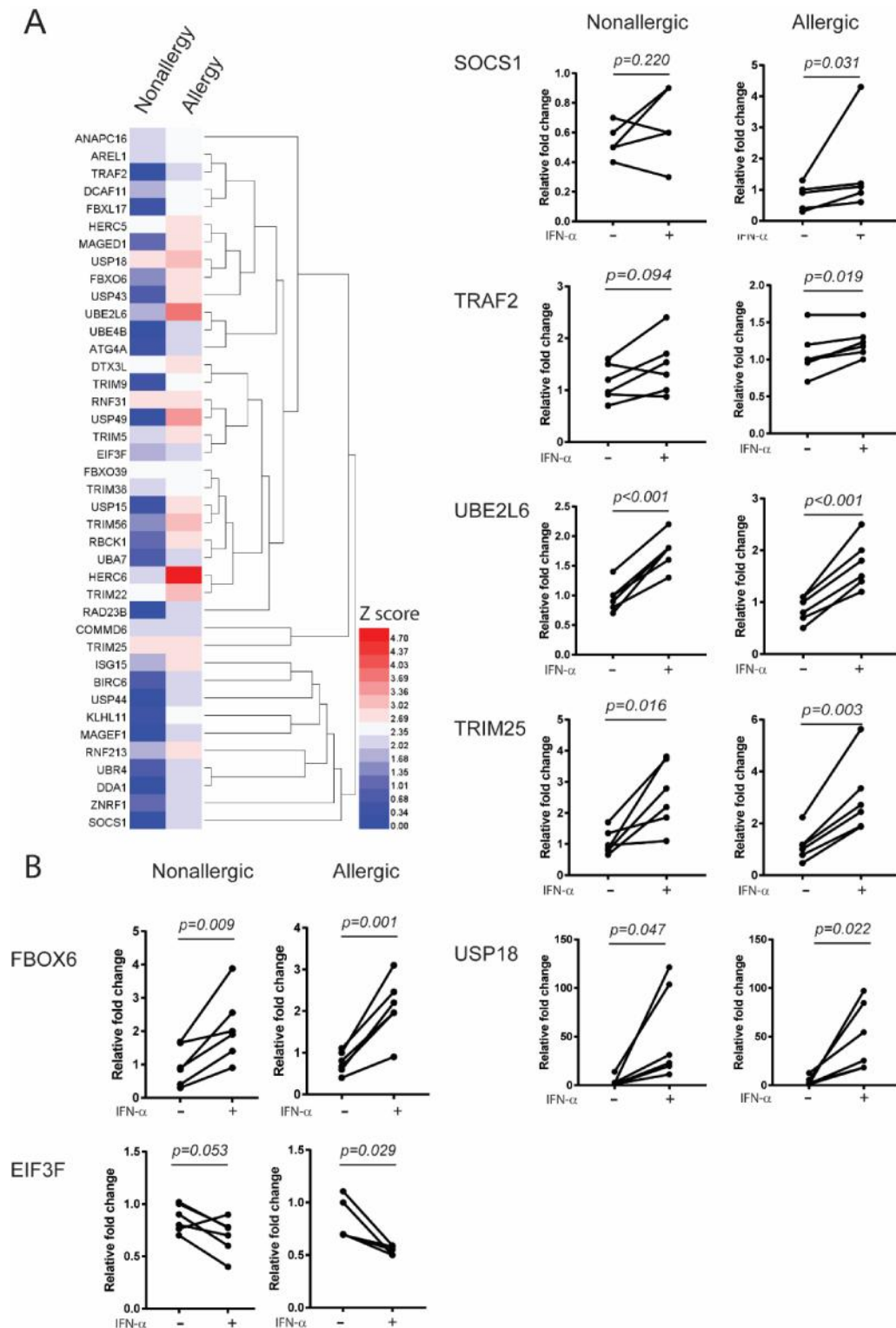


Figure S4. Ubiquitin conjugation pathway induced by IFN- α in nonallergic and allergic subjects.

(A) Heat map showing expression value of Z score on $-\log_{10}$ p value. Paired student t test. (B) mRNA expression of FBXO6, EIF3F, SOCS1, TRAF2, UBE2L6, TRIM25 and USP18 in nonallergic and allergic subjects by qRT-PCR. Paired Wilcoxon test.