# Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Tsai M, Thauland TJ, Huang AY, et al. Disseminated coccidioidomycosis treated with interferon-γ and dupilumab. N Engl J Med 2020;382:2337-43. DOI: 10.1056/NEJMoa2000024

# Disseminated coccidioidomycosis treated with IFN-γ and blockade of IL-4/IL-13

## Supplemental Material

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## Methods

**Approvals**. Written informed consent was obtained for human subjects research, as approved by the UCLA Institutional Review Board. Healthy donors were drawn from the donor pool of the UCLA Blood Bank, utterly deidentified, and purchased as whole blood through a service offered by the UCLA Virology Core.

**Reagents and antibodies.** Cells were grown and assayed in complete T-cell media, consisting of RPMI 1640 with L-glutamine (Gibco #11875) supplemented with 10% fetal bovine serum (Gibco #26140), 10 mM HEPES (Gibco #15630), 1X Pen/Strep (Gibco #15140), 1 mM sodium pyruvate (Gibco #11360) and 55 $\mu$M 2-mercaptoethanol (Gibco #21985). FACS buffer consisted of 1X DPBS (Gibco #14901) supplemented with 2% fetal bovine serum and 1 mM EDTA (ThermoFisher #15575). The following antibodies were used: anti-CD3ε (clone OKT3), anti-CD28 (clone CD28.2), anti-CD4 Brilliant Violet 421 and Alexa Fluor 647 (clone RPA-T4), anti-CD14 Brilliant Violet 421 (clone HCD14), anti-IFN-γ PE (clone 4S.B3), and Human TruStain FcX (Cat# 422302) from Biolegend; anti-CD20 Brilliant Violet 421 (clone H1), anti-phospho-Stat1 (Tyr701) Alexa Fluor 488 (clone 4a), anti-phospho-Stat3 (Tyr705) Alexaxf Fluor 647 (clone 4/P-Stat3), anti-phospho-Stat4 (Tyr693) Alexa Fluor 647 (clone 38/P-Stat4), Mouse IgG2a Isotype Control Alexa Fluor 488 and Alexa Fluor 647 (clone MOPC-173), and anti-IL-4 PE-Cy7 (clone 8D4-8) from BD Biosciences. All antibodies were used at manufacturer-recommended dilutions in FACS experiments. Recombinant human IL-12 p70 (Cat# 200-12), IL-21 (Cat# 200-21) and IFN-γ (Cat# 300-02) were from Peprotech. Recombinant human IFN-α1 was from Cell Signaling Technology (Cat# 8927). Anti-IL-4Ra (Dupilumab) for *in vitro* testing was obtained from the UCLA hospital pharmacy. Phorbol 12-myristate 13-acetate (PMA; Cat# P1585) and lipopolysaccharide (LPS; Cat# L4391) were from Sigma Aldrich. Ionomycin was from EMD Millipore (Cat# 407953).

**T-cell purification, activation and differentiation**. CD4+ cells were purified from heparinized whole blood with the EasySep Direct Human CD4+ T Cell Isolation Kit (StemCell, Cat# 19662). Twelve well plates were pre-coated with 1 $\mu$g/mL anti-CD3ε in PBS for 2 hr at 37 °C. Cells were plated at 1 million per well in 1 mL complete T cell

media supplemented with 2 μg/mL anti-CD28. For Th1 differentiation, 10 ng/mL IL-12 p70 was included in the culture. On day 3, CD4+ cells were removed from the anti-CD3ε coated wells and transferred to 6 well plates. Additional media supplemented with 100 U/mL IL-2 (and 10 ng/mL IL-12 p70 for the Th1 condition) was added to the wells. In some experiments, 50 μg/mL anti-IL-4Rα (dupilumab) was included in the cultures. On day 7, cells were harvested and assayed for cytokine production and pSTAT4 induction.

**Phospho-Stat assays.** *For whole blood:* 20 μL of 10X IL-21, IFN-γ, or IFN-α1 in PBS (or PBS only control) was added to 180 μL of blood in a 5 mL FACS tube to achieve a final concentration of 10 ng/mL. Tubes were incubated at 37 °C for 20 min, at which point 4 mL of pre-warmed 1X Lyse/Fix buffer (BD, Cat# 558049) was added. Cells were fixed for 10 min at 37 °C, centrifuged and washed twice with FACS buffer. *For cultured cells*: 50 μL of 10X IL-12 p70 in media (or media only control) was added to 1 million cells in 450 μL of complete T cell media to achieve a final concentration of 10 ng/mL. The cells were incubated at 37 °C for 20 min, and then an equal volume of pre-warmed Cytofix buffer (BD, Cat# 554655) was added. Cells were fixed for 12 min at 37 °C, centrifuged and washed twice with FACS buffer. *Staining and permeabilization*: Fc receptors were blocked for 5 min at RT, followed by a 20 min stain on ice with anti-CD4, anti-CD14 or anti-CD19. Cells were washed with FACS buffer and permeabilized for 30 min on ice with 1 mL Phosflow Perm Buffer III (BD, Cat# 558050) that had been pre-cooled to -20 °C. After permeabilization, two mL FACS buffer was added and the samples were centrifuged. After three additional washes, the cells were stained with anti-pStat4 or an isotype control for 30 min at RT. Samples were washed three times and data were collected on a Cytek DxP10 flow cytometer. Data were analyzed with FlowJo software.

**Stimulation and intracellular cytokine staining.** To stimulate cytokine production, 1 million CD4+ T cells were incubated in 1 mL complete T-cell media with or without 40 ng/mL PMA and 1 μM ionomycin for 5 hr at 37 °C. For the final 4 hours, 1X Golgiplug (BD, Cat# 555029) was added to all wells. Cells were harvested from the wells, washed with FACS buffer and fixed in 1 mL PBS/2% paraformaldehyde for 30

min at RT. After washing with FACS buffer, cells were permeabilized with FACS buffer containing 0.5% saponin. After blocking Fc receptors, the cells were stained with anti-cytokine antibodies for 30 min at RT and washed three times, all in the presence of saponin. Data were collected and analyzed as above.

For stimulation with Cocci antigen, frozen aliquots of PBMC were thawed and incubated for 24 hrs with 100 ng/mL LPS and 10 µg/mL T27K Cocci antigen. Golgiplug was included for the final 4 hrs of culture. Cells were harvested from the wells, stained for CD4, and then processed as above.

**RNA sequencing.** RNA was extracted from whole blood using the PAXgene Blood RNA Kit (Qiagen). Quantification and quality were assessed using Qubit 3.0 Fluorometer and Agilent bioanalyzer. 1 µg of total RNA was submitted to the UCLA Neuroscience Genomics Core (UNGC) for library construction and RNA sequencing. Sequencing libraries were generated using Illumina TruSeq Stranded Total RNA with Ribo-Zero Globin. Sequencing was performed to generate >65 million 120 base paired-end reads on the Illumina HiSeq 4000. FASTQ files were aligned to GRCh37 using STAR-2.5.2b with Gencode v19 annotation. Quality was assessed using RNA-SeQC v1.1.8. BAM files were analyzed in IGV to generate a sashimi plot of splice alterations.

***IL12RB1* Isoforms.** Splice junction data were downloaded from GTEx V7 (GTEx_Analysis_2016-01-15_v7_STARv2.4.2a_junctions.gct) or from an internal dataset of the IPH. Counts of the exon-exon junctions corresponding to both the long and short isoform for IL12RB1 were extracted (Junction IDs 19_18180524_18182921 and 19_18182144_18182921, respectively) for all whole blood samples (n = 407). Twenty samples with a total read count of less than 10 across both junctions were excluded.

## Figure S1

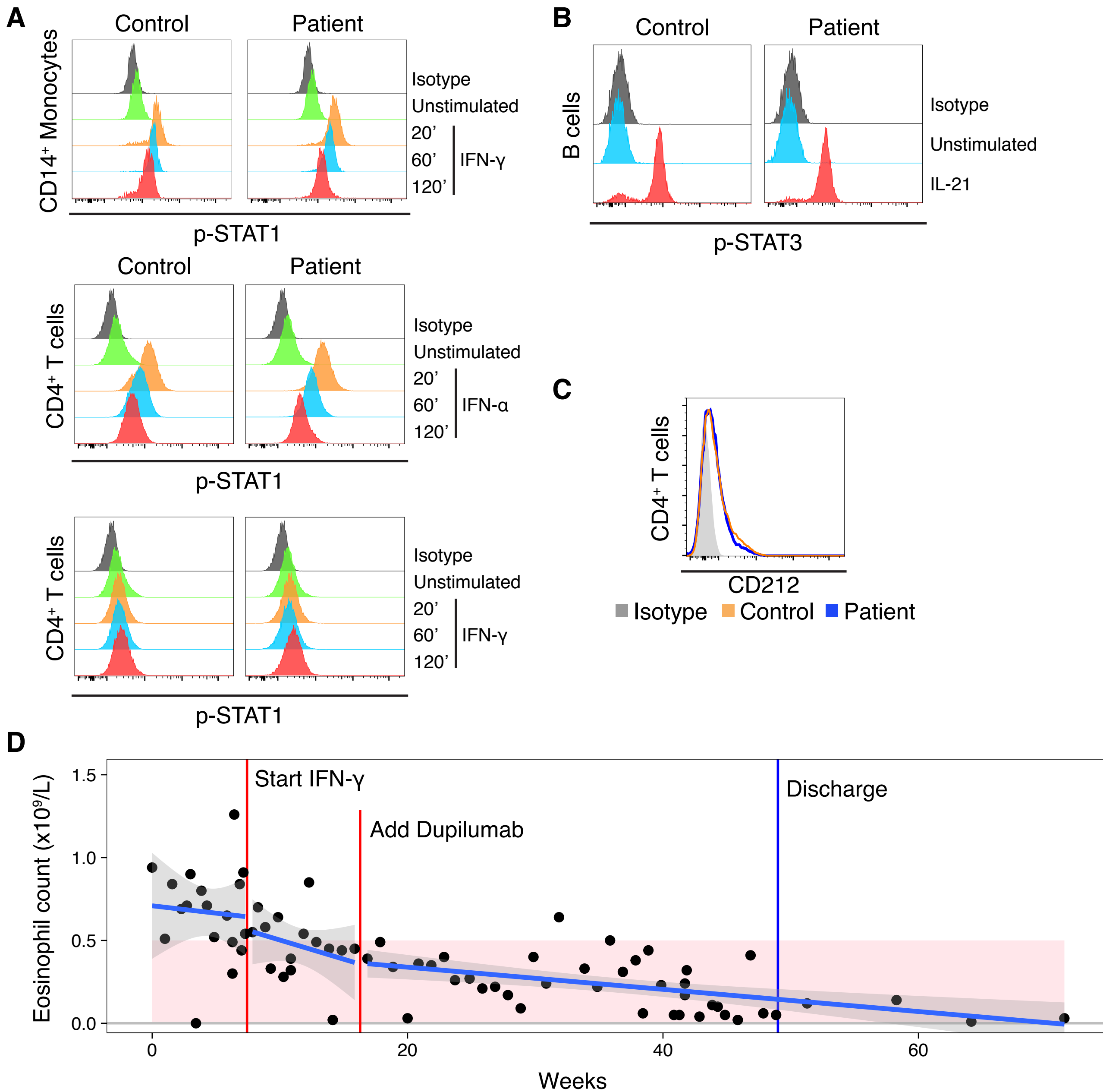


**Fig. S1. Normal responses of STAT1 and STAT3 in the proband.** (A) Peripheral blood cells were stimulated with IFN-α and IFN-γ and phosphorylation of STAT1 evaluated by flow cytometry as a function of time. (B) Peripheral blood B cells from the patient or a healthy control were stimulated with IL-21 and phosphorylation of STAT3 evaluated by flow cytometry. (C) Expression of CD212 (IL12RB1) by flow cytometry. (D) Patient's absolute eosinophil counts over time.

## Figure S2

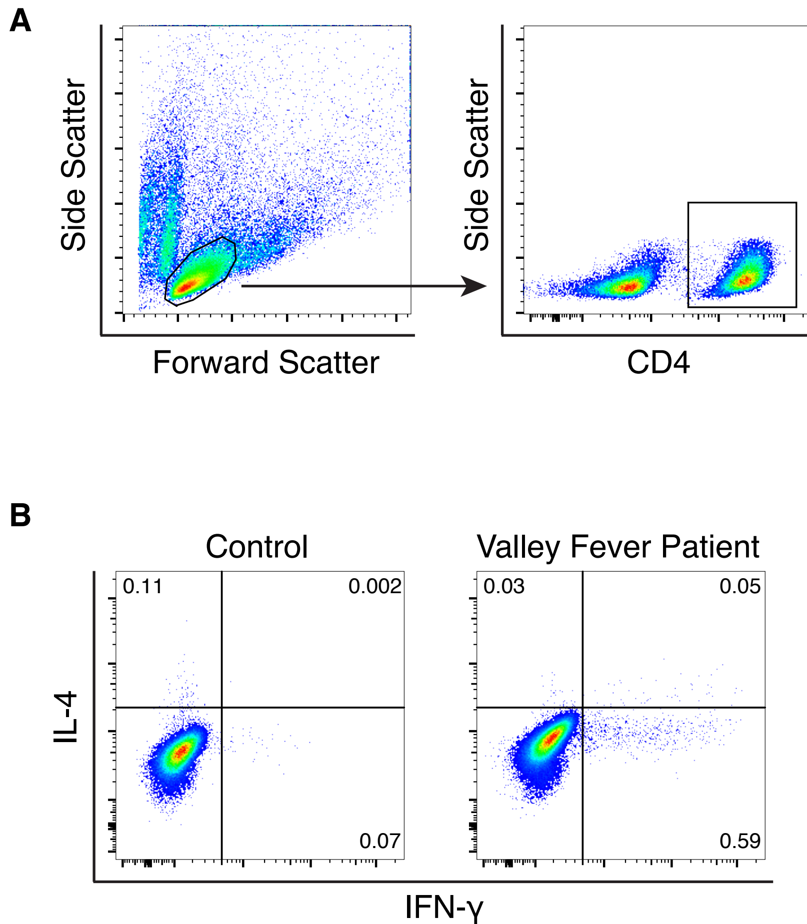


**Fig. S2. Control Valley Fever patient produces a Th1 response to stimulation with Coccidioides antigen. (A)** Gating strategy for experiments where PBMC were stimulated with Coccidioides antigen (see Fig. 1F). **(B)** Stimulation of PBMC from a patient who had recovered from Valley Fever with T27K Coccidioides antigen revealed an exclusively Th1 response.

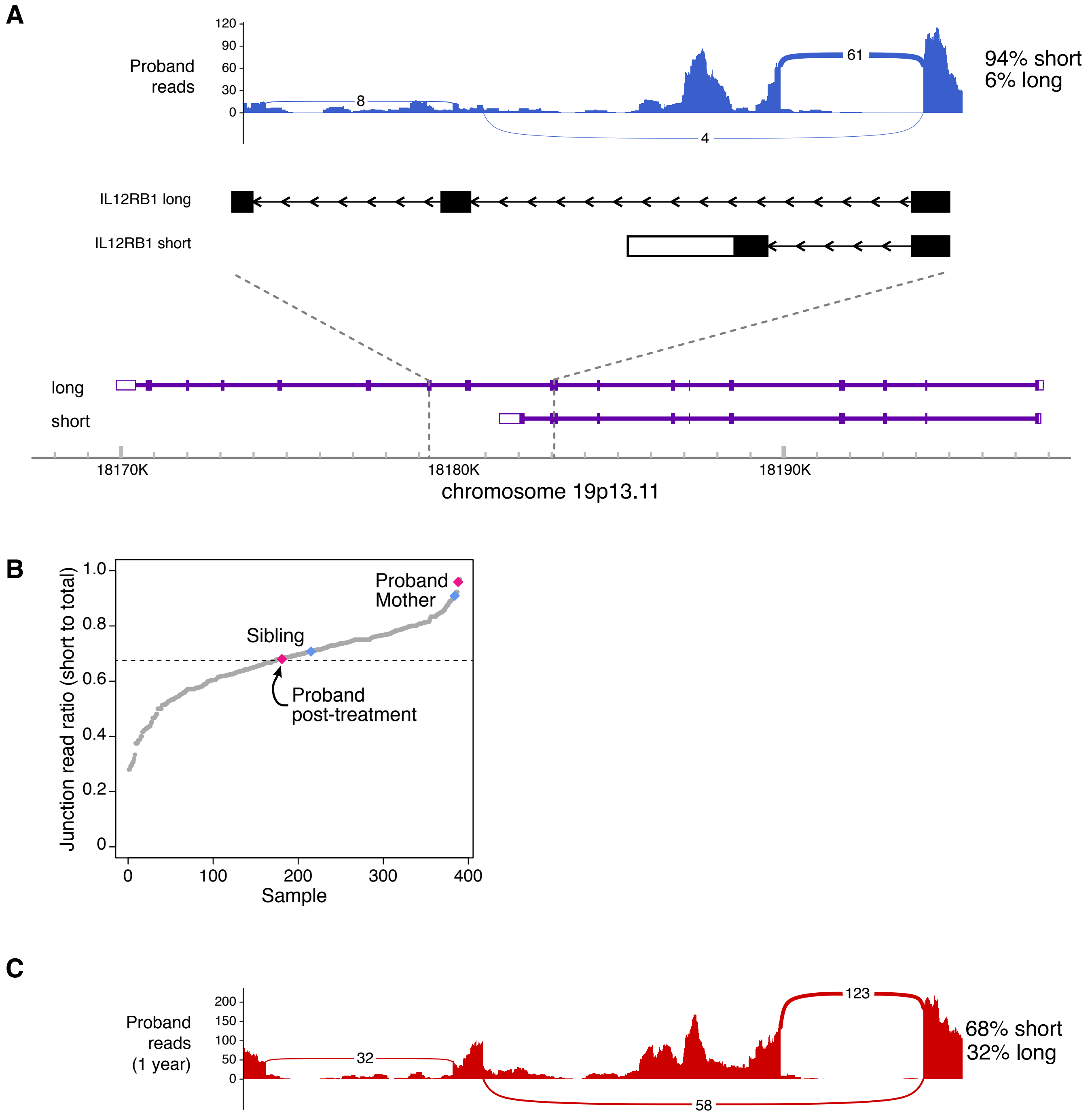


**Fig. S3. Transcriptional differences in the proband. (A)** Sashimi plot showing the two transcripts of the *IL12RB1* gene (short and long). Reads of the proband are shown spanning the exon-exon junctions that form the short or long isoforms. **(B)** Gray dots show the proportion of the *IL12RB1* transcripts of the short isoform compared to total *IL12RB1* transcripts from RNA-sequencing of whole blood cells from 387 non-immunodeficient individuals. Superimposed on the plot is the percentage of short transcripts for the proband. The healthy controls had an average of 67.4% ± 12.8% short isoform (mean ± SD, dashed line), giving the proband's transcript a Z-score of 2.22. **(C)** Sashimi plot showing the proband's whole blood transcripts of *IL12RB1* one year after treatment with IFN-γ and dupilumab, including reads spanning the exon-exon junctions of the short and long isoforms.

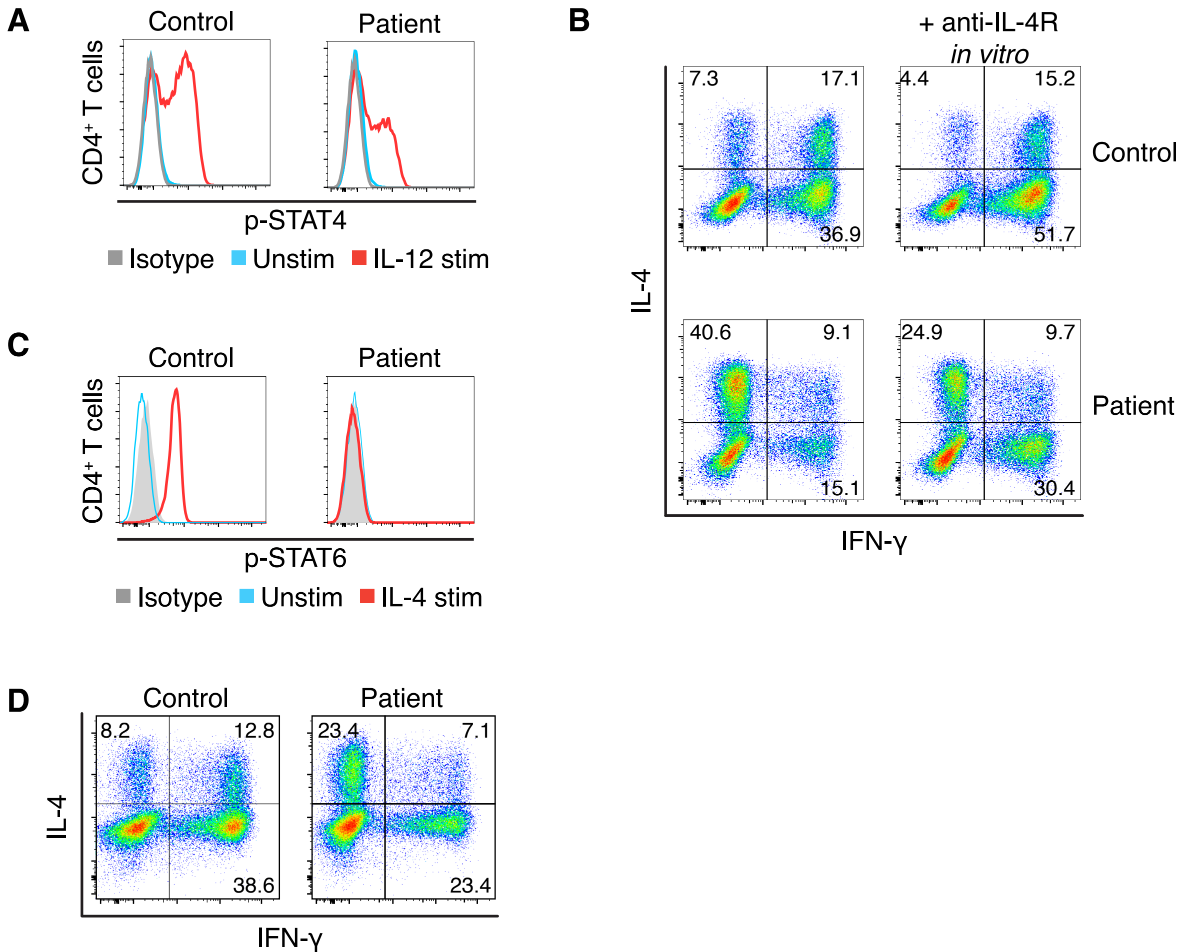


**Fig. S4. Immunological responses to treatment. (A)** Improved response to IL-12 stimulation in CD4+ T cell effectors after initiation of treatment with IFN-γ. **(B)** Enhanced IFN-γ production and decreased IL-4 production in CD4+ T cell effectors cultured with dupilumab *ex vivo*. **(C)** Peripheral blood CD4+ T cells from the patient while on dupilumab or a healthy, untreated control subject were stimulated with IL-4 and phosphorylation of STAT6 shown. **(D)** Normalization of Th1 and Th2 cells after treatment with IFN-γ and dupilumab.

**Table S1. Laboratory data.**

| | **Baseline (admission)** | **Reference Ranges** |
|---|---|---|
| Lymphocyte Counts | | |
| CD3+ T lymphocytes | 2,193 (75%) | 1,400-3,700 (56-75%) |
| CD4+ T-cell helper subset | 1,235 (43%) | 700-2,200 (28-47%) |
| CD8+ Cytotoxic T cell subset | 842 (29%) | 490-1,300 (16-30%) |
| CD19+ B lymphocytes | 545 (19%) | 390-1,400(14-33%) |
| NK lymphocytes | 141 (5%) | 130-720 (4-17%) |
| | | |
| Immunoglobulins | | |
| IgG | 2,060 mg/dL | 540-1,330 mg/dL |
| IgA | 257 mg/dL | 30-160 mg/dL |
| IgM | 89 mg/dL | 40-140 mg/dL |
| IgE | 2,396 kIU/L | <20 kIU/L |
| Neutrophil oxidative burst | 96% positive | >90% positive |

**Table S2. Responses to treatment**

| | **Baseline (admission)** | **Antifungals +IFN-γ** | **Antifungals + IFN-γ + Dupilumab** |
|---|---|---|---|
| IgE (kIU/L) | 2,396 | 354 | 109 |
| IgG (mg/dL) | 2,190 | 2,060 | 1,350 |
| CRP | 9.5 | 3.0 (wk 10) | 1.0 (wk 20) |