Supplementary Materials

Supplementary Text

Section S1. Case reports for families A to D

Patients Family A-C

Eight patients belong to three Arab families from a single village (total population ~14,000 residents) in Israel. Some of these families immigrated from Sudan and Egypt in the 19th century. They settled in a swamp area, and until recent decades were isolated from other villages. This encouraged consanguineous marriages within the village. All our patients were known to be the offspring of consanguineous couples who were first cousins.

Seven of the eight patients described here were initially diagnosed with severe atopic dermatitis, and later in life, with hyper-IgE syndrome. The immunologic work-up was mostly normal (n=7), except for highly elevated IgE in six patients. One patient with an inverted CD4/CD8 lymphocyte ratio had normal serum IgE levels. Mild intellectual disability was diagnosed in six children.

FAMILY A includes three affected boys out of eight siblings:

Patient A.II.1 is a 24-year-old man with severe dermatitis. He has had multiple admissions for the drainage of cold abscesses, several lung infections, and recurrent otitis. He also had bacteremia due to methicillin-resistant *Staphylococcus aureus* (MRSA). At the age of 17 years, he developed multifocal osteomyelitis involving the lumbar vertebrae and iliac bone. He receives continuous antibiotics and antihistamine treatment. The National Institutes of Health (NIH) hyper-IgE score (*47*) was 62.

Patient A.II.2 is the brother of patient A.II.1, a 23-year-old male with chronic severe dermatitis, recurrent pneumonia, abscesses, and severe glossitis due to moniliasis. At the age of 12 years, he

was admitted with necrotizing pneumonia caused by MRSA. At age 22 years, the patient had a laminectomy for spinal stenosis at the level of C1/C2 which had caused spinal cord pressure. He is mentally challenged and lives in a boarding school for disabled children. The NIH-hyper-IgE score was 55. Serum IgE levels measured at 45,000 IU/ml. He is on continuous antibiotics treatment.

Patient A.II.3, a brother of patient A.II.1 and A.II.2, is a 21-year-old male with chronic dermatitis, involving the legs and lower back, mild oral thrush, and abscesses, including one perianal abscess. He also is mentally challenged and lives together with his brother (patient A.II.2) in a boarding school for disabled children. He has been treated with continuous antibiotics and antihistamine medication. The NIH-hyper-IgE score was 58. His immunology testing revealed undetectable serum IgE levels (<4 IU/ml – upon repeated testing); IgG 2,400 mg/dl; IgA 195 mg/dl; IgM 48 mg/dl. Lymphocyte subsets: CD3 83%; CD4 31%; CD8 46%; CD4/CD8-ratio 0.67; CD20 15%; CD16 2%; CD56 2%. He had occasional eosinophilia of 15%-18% (AEC 700-1100 mm^3).

FAMILY B includes three affected girls out of seven siblings.

Patient B.II.1 is an 18-year-old girl with severe dermatitis involving the greater part of her body, including the limbs, back, and scalp. She was diagnosed with severe atopic dermatitis until 12 years of age and later with hyper-IgE syndrome. She has intense pruritus and occasional skin abscesses, cutaneous infections with candida mainly on the scalp, and lung and ear infections. Her height is 150 cm. She has an intellectual disability and cannot read or write, except for signing her name. The parents attributed this to her poor school attendance caused by the immunological disease. She was admitted to the hospital several times for intravenous antibiotic treatment and she is a chronic carrier of MRSA. She receives continuous antibiotics and

antihistamine treatment. The NIH-hyper-IgE score was 33. Serum IgE values measured at 54,000 IU/ml.

Patient B.II.4

This 12-year-old girl, a sister of patient B.II.1, has severe dermatitis, short stature, and intellectual disability. The dermatitis involves mainly the limbs and her back. She was diagnosed with atopic dermatitis until the age of 6 years, when she was diagnosed with hyper-IgE syndrome. She has chronic glossitis and oral thrush, and frequent ear infections. She has severe pruritus leading to bleeding lesions and occasional cutaneous candida infections mainly affecting the scalp. She has been admitted several times for intravenous antibiotic treatment and is a chronic MRSA carrier. Her height was 126 cm at the age of 12 years (-4SD for age); weight was 30 kg (third percentile for age). She refused to attend school and cannot read or write. She receives continuous treatment with antibiotics and antihistamine medication. The NIH-hyper-IgE score was 30. Serum IgE measured at 29,000 IU/ml.

Patient B.II.6

This one-year-old girl is a sister of patients B.II.1 and B.II.4. At the age of 6 months, she was admitted to an intensive care unit with RSV bronchiolitis, pneumonia and oral thrush. In addition, she has mild dermatitis. Serum IgE at this time was 150 IU/ml (moderately elevated for age). The NIH-hyper-IgE score was 12.

FAMILY C includes two affected males out of six siblings.

Patient C.II.1 is a 35-year-old man with chronic dermatitis and multiple recurrent cold abscesses. At birth, he was diagnosed with atrial septal defect and pulmonary stenosis, which were surgically corrected. He has had numerous admissions for the drainage of cold abscesses, including a giant subcutaneous skin tumor measuring 10 cm in diameter, which was a collection of pus. Abscess cultures grew coagulase-positive *Staphylococcus aureus*. He has an intellectual

disability, including difficulties in reading and writing, and no arithmetic knowledge. Serum IgE levels measured at 23,300 IU/ml. NIH- hyper-IgE score was 36.

Patient C.II.6, the brother of patient C.II.1, is a 14-year-old boy with dry skin, and mild dermatitis involving the face, neck, and elbows. There were no infections except for otitis. He was never admitted to hospital. He has a mild developmental delay. He had transient IgA deficiency (IgA <6.5 mg/dl until the age of 10 years, and 220 mg/dl at the age of 13 years). He had occasional mild eosinophilia (AEC 1200/ml). Serum IgE levels measured at 7,640 IU/ml. The NIH-hyper-IgE score was 18.

FAMILY D includes three affected females.

Patient D.II.1 is a 36-year-old woman suffering from recurrent severe skin abscesses. She has hearing loss because of recurrent otitis, using a hearing aid device. She has been suffering from oral moniliasis since her infancy period. She had an iliopsoas abscess due to *S. aureus*. Salmonella enteritis was treated with ciprofloxacin at the age of 27 years. Nasopharyngeal carcinoma developed five years ago and the patient was cured by surgery, chemotherapy (cyclophosphamide, doxorubicin, vincristine), and radiotherapy. She developed papillary cell carcinoma of the left thyroid gland in 2017. She is currently being treated with IVIG every three weeks. Serum IgE levels measured at 13,916 IU/ml. Her NIH-hyper-IgE score was 60.

Patient D.II.2, the sister of patient D.II.1, is a 30-year-old woman with chronic severe dermatitis and cold abscesses. She also has recurrent sinusitis. Serum IgE levels measured at 2,507 IU/ml. The NIH-hyper-IgE score was 28.

Patient D.II.4, the sister of patients D.II.1 and D.II.2, is a 18-year-old female with chronic severe dermatitis and cold abscesses. In addition, she had recurrent otitis and sinusitis. Serum IgE levels measured at 1,825 IU/ml. Her NIH hyper-IgE score was 26.

Section S2. Defective B cell development and T_H17 cell differentiation in HIES patients with *ZNF341* nonsense mutations

Patients with *ZNF341* mutations had normal CD19 lymphocyte counts, but an increased percentage of naïve B cells (IgD^+CD27^-), and reduced memory B cells ($CD27^+$). This observation is in line with what has been observed in AD-HIES patients with dominant-negative mutations in *STAT3* (*48*). Consistently, all memory B cell subpopulations including IgG^+, IgA^+, IgM^+ were significantly reduced in the affected individuals (fig. S1A). Patients had normal counts for naïve and memory $CD4^+$ and $CD8^+$ T cells, and the $CD4^+$ subsets called Th1, Th1*, and Th2 (fig. S1B). STAT3-dependent CCR6 expression (*23*) was significantly reduced in memory $CD4^+$ T helper cells in patients (mean of $CCR6^+$ 16.6%, SD 6.35 for patients vs 32.7%, SD 5.54 for controls). Patient-derived PBMCs failed to differentiate into IL-17-producing $CD4^+$ T cells using IL-1β and IL-6 in combination with T cell activation/expansion (Fig. 2F). In addition, we tested an alternative cytokine stimulation (IL-21 and TGF-β). Again patients´ PBMCs did not differentiate into IL-17-producing $CD4^+$ T cells (fig. S1C). Thus, Th17 cell differentiation is affected in patients with *ZNF341* mutations. In addition to the reduced percentage of IL-17^+ T cells, patient-derived PBMCs also showed reduced numbers of IL-22^+ T cells after stimulation with PMA/ ionomycin (fig. S1D).

In addition to the elevated IgE, serum IgG was also high in all patients. In both patients B.II.1 and C.II.1, specific antibody responses to vaccination showed a normal response to *tetanus-toxoid* vaccination, but only a weak response to *diphtheria toxin*. Additionally, pneumococcal IgG titers after Pneumovax-23 vaccination were low for patient C.II.1 (table S1).

Section S3. Exclusion of mutations in *STAT3* in the etiology of the HIES phenotype in family A

In Family A, mutations in all coding exons and intron-exon boundaries of *STAT3* were excluded by next generation sequencing (NGS) of patients A.II.1 and A.II.3. A wild-type *STAT3* cDNA sequence was confirmed by Sanger sequencing of long range RT-PCR products derived from stimulated PBMCs of patient A.II.1 and the healthy sibling A.II.5. Mutations in the *STAT3* promoter region (chr20:40541608-40538416; GRCh37) were excluded by NGS or Sanger sequencing of genomic DNA. A large deletion in the *STAT3* genomic region was excluded by a comparative genomic hybridization (CGH) array for patients A.II.1 and C.II.1.

Section S4. Identification of *ZNF341* as an AR-HIES–associated gene in a linkage region on chromosome 20 by WES

To detect the underlying genetic defect in the index Family A, we first excluded mutations in known HIES- and candidiasis-related genes by targeted panel sequencing. Subsequently, a genome-wide homozygosity mapping was done by SNP chip for Family A. A single large interval consistent with linkage was identified on chromosome 20 (chr20). Homozygosity was confirmed and the boundaries were clarified with microsatellite markers yielding a maximal interval of 17.9 - 38.8 Mbp and a minimal interval of 19.8 - 36.4 Mbp (flanked by microsatellite markers D20S471 and D20S859). Some of the same microsatellites were genotyped in Family D, which was also consistent with linkage to chr20, but with a different disease-associated haplotype from that of Family A (suggesting a different mutation in the same chr20 region).

Since the linkage region on chr20 contained hundreds of genes, we performed WES on two affected individuals and a healthy sibling of Family A. Within the minimal linkage interval of Family A, three sequence variants were detected with only the nonsense mutation in *ZNF341* affecting the coding sequence and being absent from dbSNP at that time.

Section S5. Expression of various *ZNF341* mRNA variants in multiple cell lines and PBMCs

The expression pattern of *ZNF341* transcripts (table S2) was evaluated in various cell types by reverse transcription (RT)-PCR with primer pair 1. A 574bp fragment, corresponding to transcript for isoform 1 and/or isoform 2 (ENST00000375200, ENST00000342427) was observed in all cell lines tested (Jurkat, HaCaT, EBV-transformed B cells, Ramos, HEK293T, HT1080, Colon205, HeLa, U2OS, JK6L, U266, and THP1). A weaker 377 bp fragment corresponding to transcript ENST00000497876 (predicted to undergo nonsense-mediated decay) and/or transcript for isoform 3 was obtained with Ramos, HEK293T and Colon205 cell lines (fig. S2A). Isoform 1 and/or isoform 2 were detected in PBMCs, monocytes, $CD3^+$ T cells, and granulocytes, whereas transcript ENST00000497876 and/or isoform 3 were only present in monocytes (fig. S2B).

Section S6. Supplementary Materials and Methods

Antibodies for immunophenotyping

For immunophenotyping, PBMCs were stained for cell surface markers with the following fluorochrome-conjugated antibodies: F(ab')2 Fragment Goat Anti-Human Serum IgA-FITC and F(ab')2 Fragment Goat Anti-Human IgG- Alexa Fluor 647 (both from JacksonImmunoResearch), CD27-PE (M-T271), CCR6-PE (11A9), CCR4-PeCy7 (1G1), CD3-APC-H7 (SK7), and CD45RA-PeCy7 (HI100) (all from BD Biosciences, Heidelberg, Germany), IgM-PerCp-Cy5.5 (MHM-88), IgD-PeCy7 (IA6-2), CD19-APC-Cy7 (HIB19), CD27-BV421 (M-T271), CXCR3-Alexa Flour 647 (G025H7), CCR7-BV421 (G043H7), CD25-Alexa Flour 647 (M-A251) and Zombie Aqua (all from BioLegend, London United Kingdom), and CD45RA-FITC (JS-83), CD4-PerCp-Cy5.5 (RPA-T4), and CD127-FITC (eBioRDR5) (all from eBioscience, Frankfurt, Germany).

Intracellular IL-22 staining

For intracellular IL-22 staining, fresh or frozen PBMCs were stimulated for 6 or 15 hours with 50ng/ml PMA and 1µg/ml ionomycin (both from Sigma) in the presence of 5µg/ml Brefeldin A (BD Biosciences). Cells were stained for surface markers with anti-CD4 FITC (clone RPA-T4, BD Biosciences), anti-CD45RO PE-Cy7 (clone UCHL1, eBiosciences) and anti-CD3 APC H7 (clone SK7, BD Bioscience), fixed and permeabilized using Cytofix kit (BD Biosciences). For intracellular staining anti-IL-22 PerCP-Cy5.5 (clone 2G12A41; isotype control IgG2a κ; Biolegend) and anti-IL-17 PE (Clone eBio64DEC17, eBioscience) were used. Fixable viability dye eFluor 506 (eBioscience, Frankfurt, Germany) was used according to the manufacturer´s instructions.

Gating strategies for flow cytometry analysis are shown in figure S8-S11.

Genetic linkage analysis

The genomic region containing the gene mutated in Family A was identified in 2007-2008 using a low-density SNP genotyping chip, microsatellite markers on chromosome 20 for fine mapping, and homozygosity mapping using previously described methods (*49, 50*). For genetic linkage analysis, LOD scores were computed using FASTLINK (*51–53*) assuming a fully-penetrant recessive disease with a low-disease allele frequency of 0.001.

Whole exome sequencing (WES)

Genomic DNA was isolated from granulocyte pellets by alcohol precipitation according to standard procedures. WES was performed on gDNA of two patients and one healthy sibling of family A and an in-house bioinformatics WES data analysis pipeline was used for variant discovery.

Next Generation Sequencing (NGS) and Sanger sequencing

Sequencing data were generated by targeted panel re-sequencing. A customized gene panel containing all exons, including splice site regions of known HIES and CMC genes (including *STAT3* and the *STAT3* promoter region) and *ZNF341* (for the identification of the mutation in family D), was designed with the help of Agilent's web-based SureDesign application. Digestion of the DNA samples, hybridization of the restriction fragments, capturing of the target DNA, closing the circular fragments through a ligation reaction, and purification of the amplified target libraries was accomplished by following the manufacturer's instructions (Agilent, Waldbronn, Germany). Enrichment was validated using a 2100 Bioanalyzer and samples were pooled in equimolar amounts for multiplexed sequencing using Illumina v2 kit reagents. After denaturation with NaOH, the libraries were diluted to a final concentration of 8-12pM and processed for

sequencing on an Illumina MiSeq following the manufacturer`s guidelines. Data were analyzed with Agilent's SureCall software.

The identified nonsense mutations in exon 6 and exon 8 of *ZNF341* isoform 1 were confirmed by standard Sanger sequencing (GATC Konstanz, Germany) with the primers ex6-fw 5'-GTG CCA AAC CAG TGT GTG-3', ex6-rs 5'-GTG CCA AAC CAG TGT GTG-3' and ex8-fw 5'-ATG TTC TTT CTG GGC CTT CC-3` and ex8-rs 5'- CAG AGA GGT CAT GGG GTT TG-3`. The sequence of the *STAT3* promoter was either analyzed by NGS covering the genomic region chr17:40,541,608-40,538,416 (GRCh37) or by Sanger sequencing (primers upon request), whereby a 19 bp sized region (GRCh37: chr17: 40,540,741- 40,540,759) was not analyzed.

CGH Array

Comparative genomic hybridization (CGH) was performed by the lab of Dr. Michael Leipold at the Institute of Human Genetics, Faculty of Medicine, University of Freiburg, by using a CytoSureConstitutional (DDD) v3 180K-Chip from Oxford Gene Technology (ogt).

Transcriptome study

Expression Profiling on HumanHT-12 v4 Expression BeadChip (Illumina) was performed at the microarray unit of the DKFZ, Genomics and Proteomics Core Facility, Heidelberg, Germany, following manufacturer's protocols.

With the Illumina TotalPrep RNA Amplification Kit (life technologies) single-stranded cRNA (labelled with biotin) was generated from input amounts of 60ng total RNA. For hybridization the "Standard Illumina Hybridization Protocol" Part # 11322355 (Whole-Genome Gene Expression Direct Hybridization Assay Guide) was followed: 500 ng of cRNA were hybridized for 17 hours at 58°C on the microarray. After staining with Cy3-Streptavidin against biotin the

microarray was scanned with Illumina iScan-Scanner according to "Standard Illumina Scanning Protocol" Part # 11322355 (Whole-Genome Gene Expression Direct Hybridization Assay Guide). Raw data was quantile normalized with R and Bioconductor package "preprocessCore". As test for significance the student's t-test was used on the bead expression values of each sample and for comparing the two samples, additionally the Benjamini-Hochberg correction was applied.

Western blotting

Fresh or frozen PBMCs, EBV transformed B cells, PSF, or transfected HEK293T cells were washed with PBS, pelleted and lysed in lysis buffer (50mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1mM EDTA, 1% NP40), containing EDTA-free Protease Inhibitor Cocktail (Roche) and Phosphatase Inhibitor Cocktail 2 and 3 (Sigma-Aldrich). Protein concentrations were determined with the Pierce BCA Protein Assay Kit (Thermo Scientific). Protein lysates were separated by 10% SDS-PAGE gels and transferred to PVDF membranes, followed by 5% nonfat milk (Bio-Rad Lab) blocking. Membranes were probed with primary antibodies separately for STAT3 (MAB1799; R&D Systems), ZNF341 (monoclonal customized antibody or polyclonal Atlas Antibody HPA024607), GAPDH (G9295; Sigma), or beta-actin (NB 600-532; Novus Biologicals), followed by HRP-conjugated anti-mouse/rabbit secondary antibodies (sc-2096 and sc-2077; Santa Cruz Biotech, Heidelberg Germany). Signals were detected with ECL substrates LumiGLO or SignalFire Plus (Cell Signalling) on a Fusion Fx7 imaging system (PeqLab, Erlangen Germany) and quantified with Image J software.

Primers for ChIP-Seq

ChIP primers were as follow STAT3: 5'-GGA GAG CAG CTA GGA GAA AGG-3' and 5'-CAG GAC ATT CCG GTC ATC TT-3'; UNTR5: 5'-CTG TAC CTG GGG TTC ATT CAT T-3'

and 5’-CAG TAA GCC GTT CAC TCT CAC A-3’; ActB: 5’-AGA AGT CGC AGG ACC ACA CT-3’ and 5’-CAG CTC CAG GGT AAA AGG TG-3’.

Isolation of monocytes, granulocytes and CD3$^+$ T cells

CD14$^+$ Monocytes were isolated with the CD14 MicroBeads human kit from MACS Miltenyi Biotec according to the manufacturer’s protocol. Polymorphprep was used for isolation of granulocytes by density gradient centrifugation. CD3$^+$ T cells were isolated by fluorescence-activated cell sorting with the antibody CD3-FITC (UCHT1) from BD Biosciences.

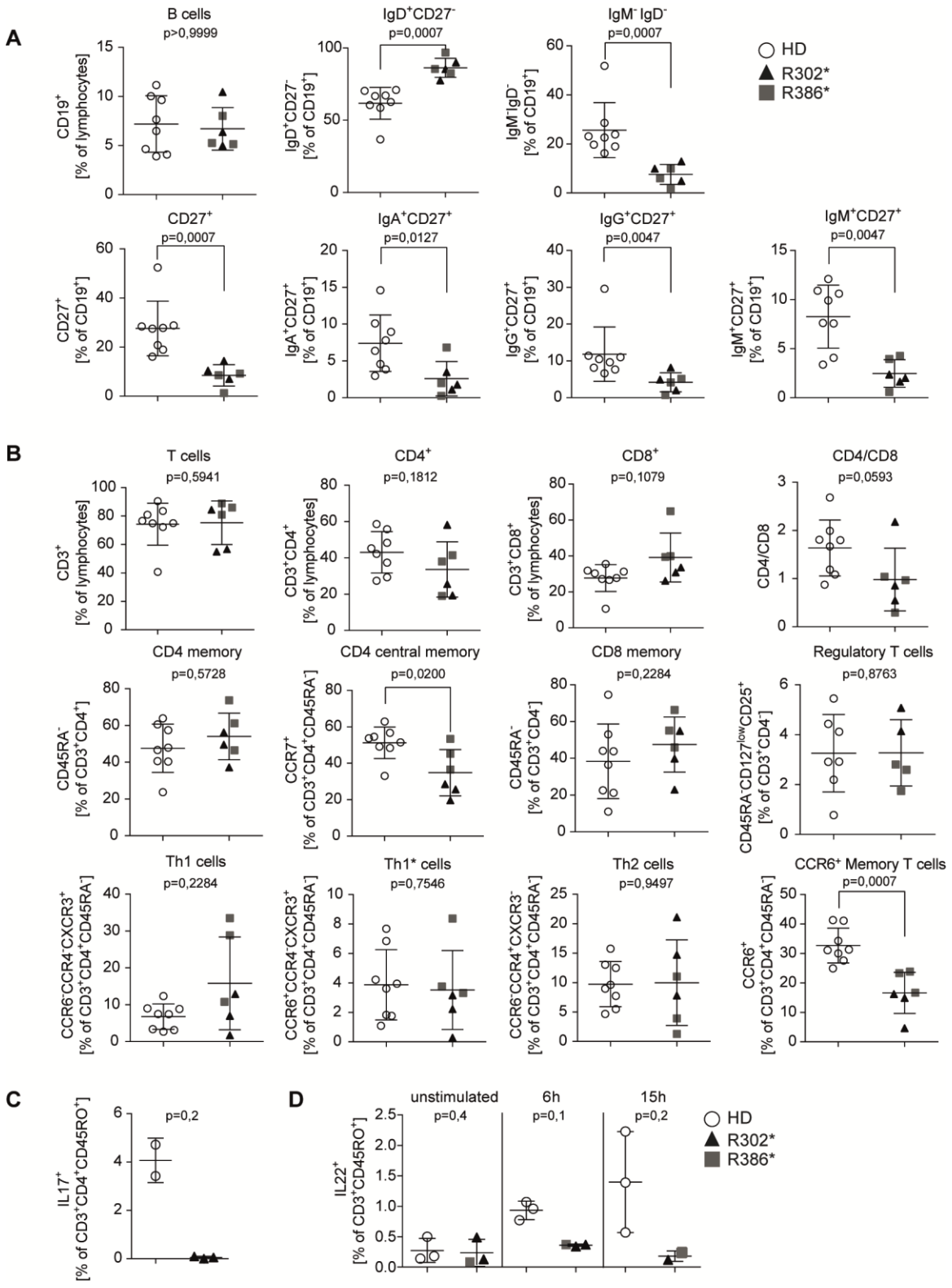

Fig. S1. B and T cell subsets in patient-derived and HD PBMCs.

(**A**) Flow cytometry of PBMCs from healthy donors (HD) and patients with homozygous *ZNF341* nonsense mutations demonstrates increased numbers of naïve IgD^+ CD19-positive B cells but a reduced naïve class switched (IgM^-IgD^-) subpopulation (upper panels) as well as reduced memory B cells ($CD27^+$) and IgA/IgG/IgM-positive class switched memory B cells (lower panels). (**B**) In patients with homozygous *ZNF341* mutations the CD3-positive T cells had normal subpopulations of naïve $CD4^+$ or $CD8^+$ (upper panels) and memory $CD4^+$ ($CD45RA^-$), slightly reduced central memory $CD4^+$ cells ($CD45RA^-$ $CCR7^+$), and normal memory $CD8^+$ ($CD45RA^-$) and Tregs ($CD45RA^-CD127^{low}CD25^+$) (middle panels). The subpopulations of $CD3^+CD4^+$ lymphocytes showed normal distributions of Th1 ($CD45RA^-CCR6^-CCR4^-CXCR3^+$), Th1* ($CD45RA^-CCR6^+CCR4^-CXCR3^+$) and Th2 ($CD45RA^-CCR6^-CCR4^+CXCR3^-$), and weakly reduced $CCR6^+$ memory cells ($CCR6^+CD3^+CD4^+CD45RA^-$ T cells) (lower panels). (HD, n=8, n=7 for Tregs; patients n=6, n=5 for Tregs). **(C)** Flow cytometric analyses of PBMCs demonstrate impaired Th17 differentiation upon *in vitro* stimulation (d4) with Th17 polarizing cytokines TGF-β and IL-21 in combination with T cell activation/expansion (HD, n=2, patient n=3). (**D**) Reduced percentages of IL-22^+ T cells upon *in vitro* stimulation with PMA/ionomycin (HD, n=3; patient n=3, n=2 for 15h). Open circles, healthy donor controls (HD); triangles, patients with R302* mutation; squares, patients with R386* mutation. Significance was determined using Mann-Whitney test, p values are indicated.

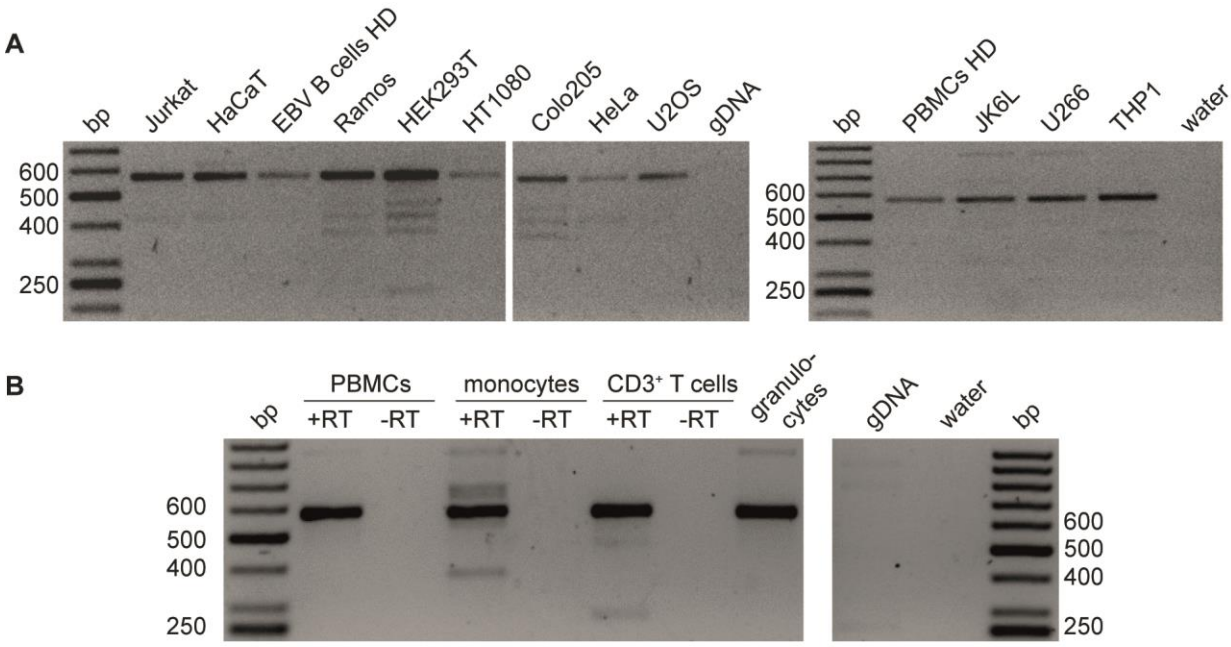

Fig. S2. Ubiquitous expression of *ZNF341* mRNA in various cell types.

(**A**) *ZNF341* transcripts are detectable by reverse transcription (RT)-PCR in all cell lines and cell types tested (as indicated), including healthy donor-derived PBMCs and EBV-transformed B cells. (**B**) *ZNF341* transcripts are detectable in HD PBMCs, monocytes, $CD3^+$ T cells and granulocytes. No RT samples, no template samples (water) and genomic DNA (gDNA) served as controls. DNA size markers are indicated. For primer specification, see the legend of Table S2.

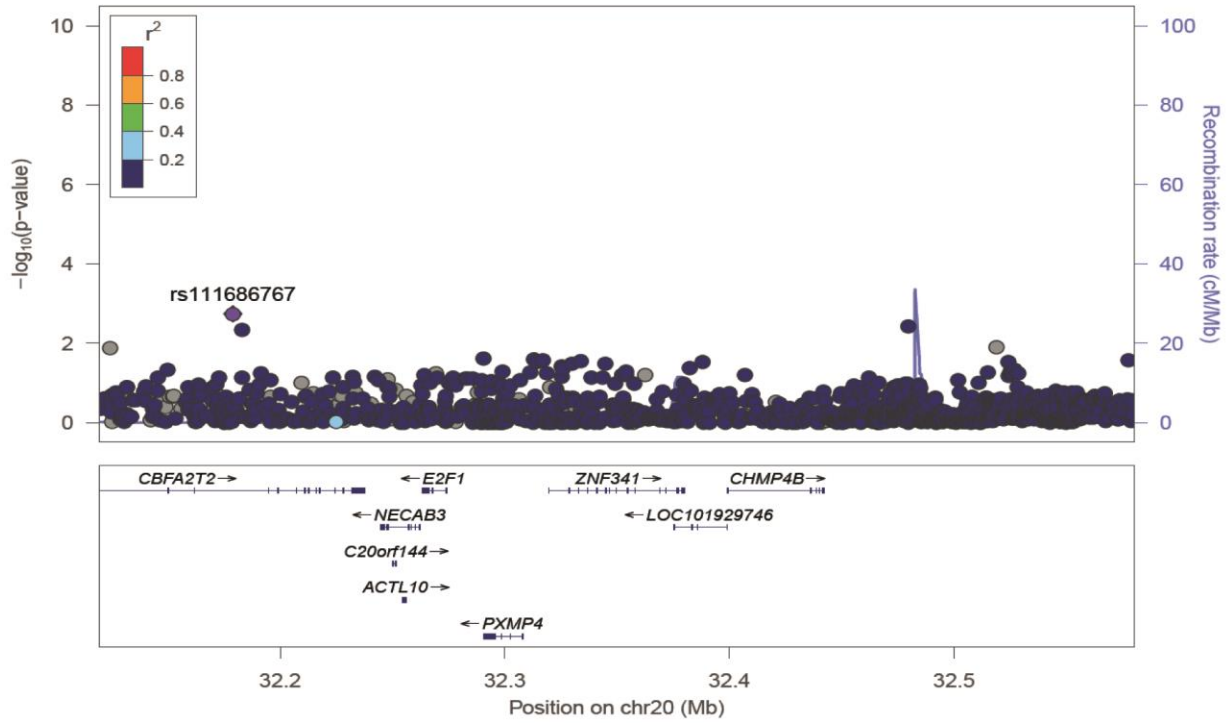

Fig. S3. No association of the genomic region 20q11.22 spanning the *ZNF341* locus with atopy.

(top panel) Regional association plot (from EAGLE Meta-analysis) of the locus 20q11.22 depicting the -log10 P-values with regard to the physical location of each marker. The purple diamond indicates the variant with the lowest p-value (rs111686767; p=0.0018). The color of the filled dots (see color code) corresponds to the strength of linkage disequilibrium (r2) with rs111686767. Blue line: recombination intensity (cM/Mb). (bottom panel) Positions and gene annotations are according to NCBI's build 37 (hg19).

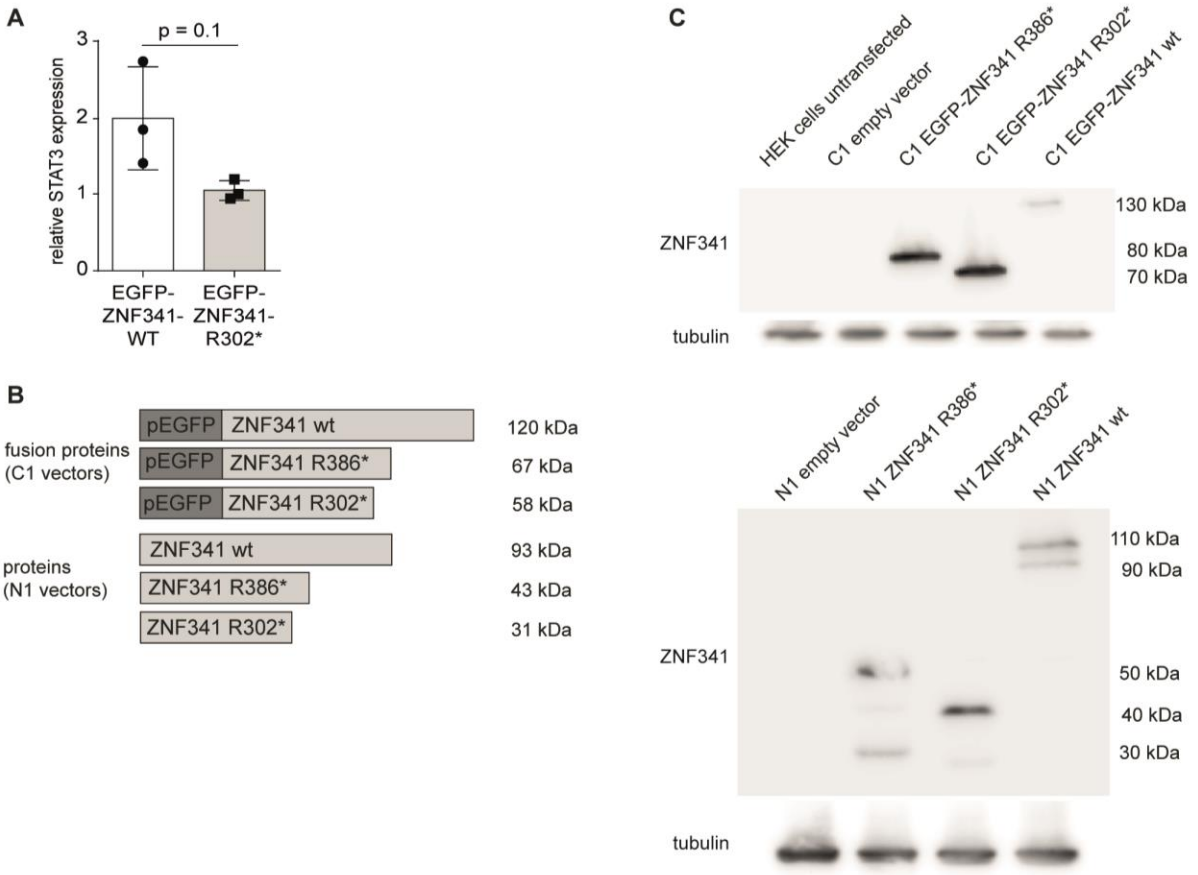

Fig. S4. Ectopic expression of ZNF341 variants in HEK293T cells.

(**A**) The relative *STAT3* mRNA expression (*STAT3/GUSB*) was two-fold increased 48 hours post transfection of EGFP-fused wild-type ZNF341 in HEK293T cells but remained unchanged with the truncated ZNF341-R302* mutant variant. Bars represent mean +/-SD of three independent transfection and RT-qPCR experiments. Values were normalized to the empty vector control. (**B**) Schematic drawing of wild-type and R302* or R386* mutant EGFP-fused (C1) and non-fused (N1) ZNF341 expression constructs with expected protein sizes. (**C**) HEK293T cells 48h post transfection with the indicated expression vectors were analyzed by Western blotting with the polyclonal anti ZNF341 antibody. Empty vectors and untransfected HEK293T cells served as

controls. Mutant variants gained higher expression levels than wild-type proteins. Representative results are shown (n=3 for R302* and n=1 for R386*).

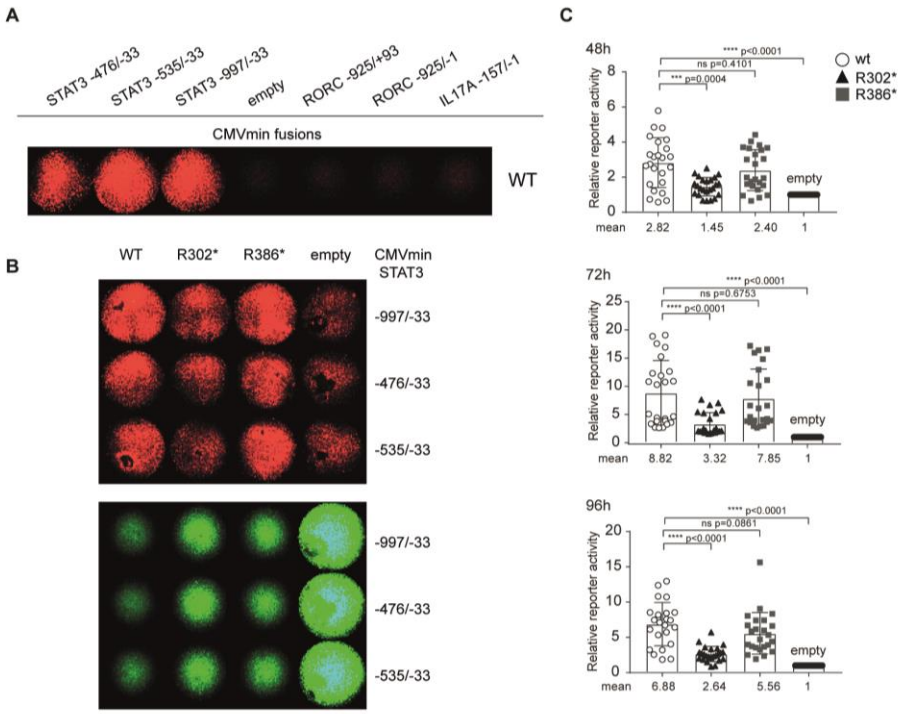

Fig. S5. Ectopically expressed wt ZNF341 binds to the *STAT3* promoter leading to transcriptional activation.

Synthetic promoters with upstream genomic sequences fused to the CMV minimal promoter driving a red fluorescence (tdTomato) reporter were co-transfected with EGFP-ZNF341 variants in HEK293T cells. (**A**) *STAT3* promoters (-997/-33; -535/-33 or -476/-33 relative to the transcription start) are activated by ZNF341 wild-type whereas the CMV minimal promoter alone (empty reporter vector) and *RORC*- and *IL17A*-promoter fusion constructs are silent. (**B**) Robust reporter activity (upper panel) with the wild-type ZNF341 and the much higher expressed R386* variant and marginally increased activation with the R302* mutant compared to baseline (empty expression vector). EGFP fluorescence intensities (lower panel) indicate expression levels of the indicated transfected constructs. Representative results are shown. (**C**) Reporter assays (n=4) were performed in 48 well plates in quadruplicate and relative reporter activity was evaluated

with a Fluorospot reader at variable time points (48h/72h/96h; n=2 each) as indicated. Significance was determined using Mann-Whitney test, p values are indicated.

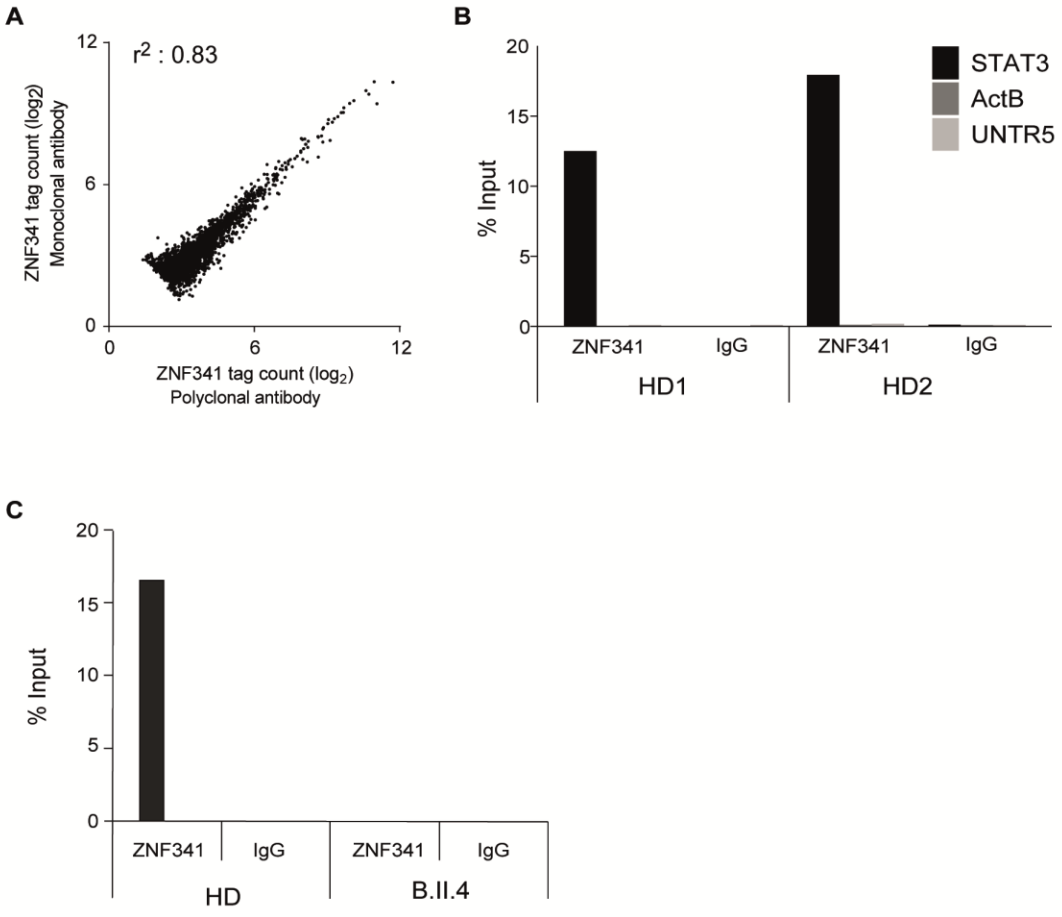

Fig. S6. Occupancy by ZNF341 across the *STAT3* promoter as determined by ChIP.

(**A**) Quantification of tag counts obtained with ZNF341 monoclonal or polyclonal antibody in ZNF341 peaks. Pearson correlation test (r^2 0.83) indicated a high correlation. (**B**) ChIP was performed in EBV cell lines derived from two healthy donors using polyclonal antibody against ZNF341. For HD1, data are representative of two independent experiments. (**C**) ChIP was performed as in B using one healthy donor and patient (B.II.4). Data are representative of two independent experiments. IgG was used as control and the ACTB promoter and a transcriptionally inactive region (UNTR) served as target controls.

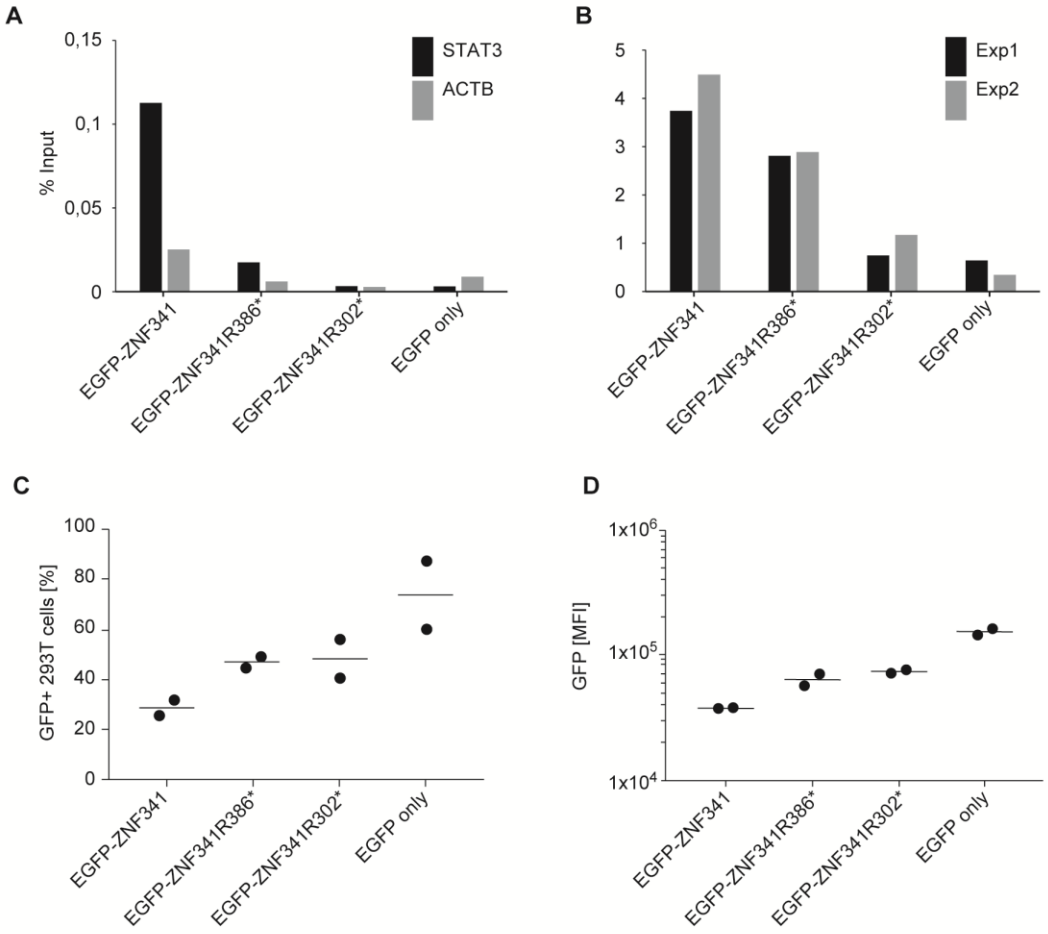

Fig. S7. ZNF341 R386* shows reduced binding to the *STAT3* promoter.

(A+B) GFP-ChIP assay in HEK293T cells overexpressing EGFP-tagged ZNF341 proteins (wt, R386*, R302*) or EGFP only. Binding at *STAT3* promoter (*STAT3*) is shown as normalized to Input **(A)** or to ACTB **(B)**. **(C)** Percentage of EGFP-positive cells after transfection with ZNF341 variants as determined by flow cytometry. **(D)** MFI as a measure of EGFP-tagged ZNF341 protein expression (n= 2).

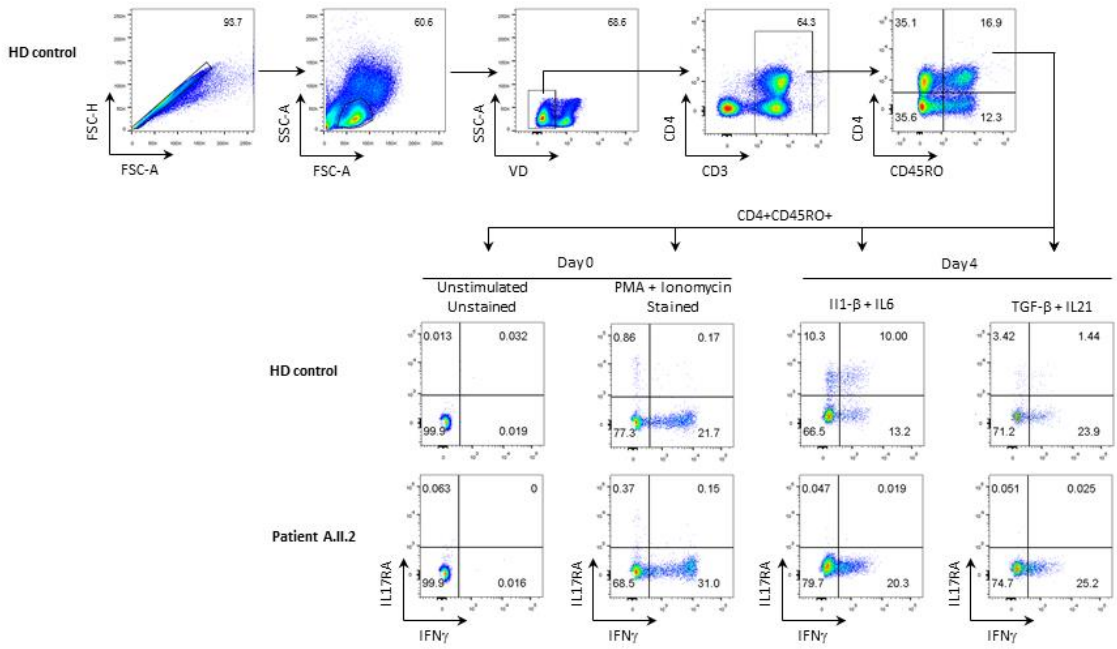

Fig. S8. Flow cytometry gating strategy for in vitro T_H17 cell differentiation assay.

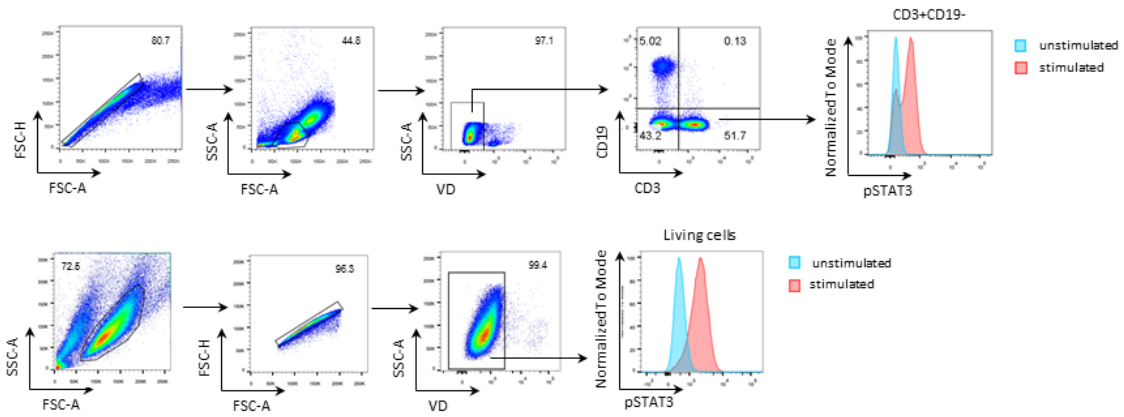

Fig. S9. Flow cytometry gating strategy for Y705 phosphorylation of STAT3.

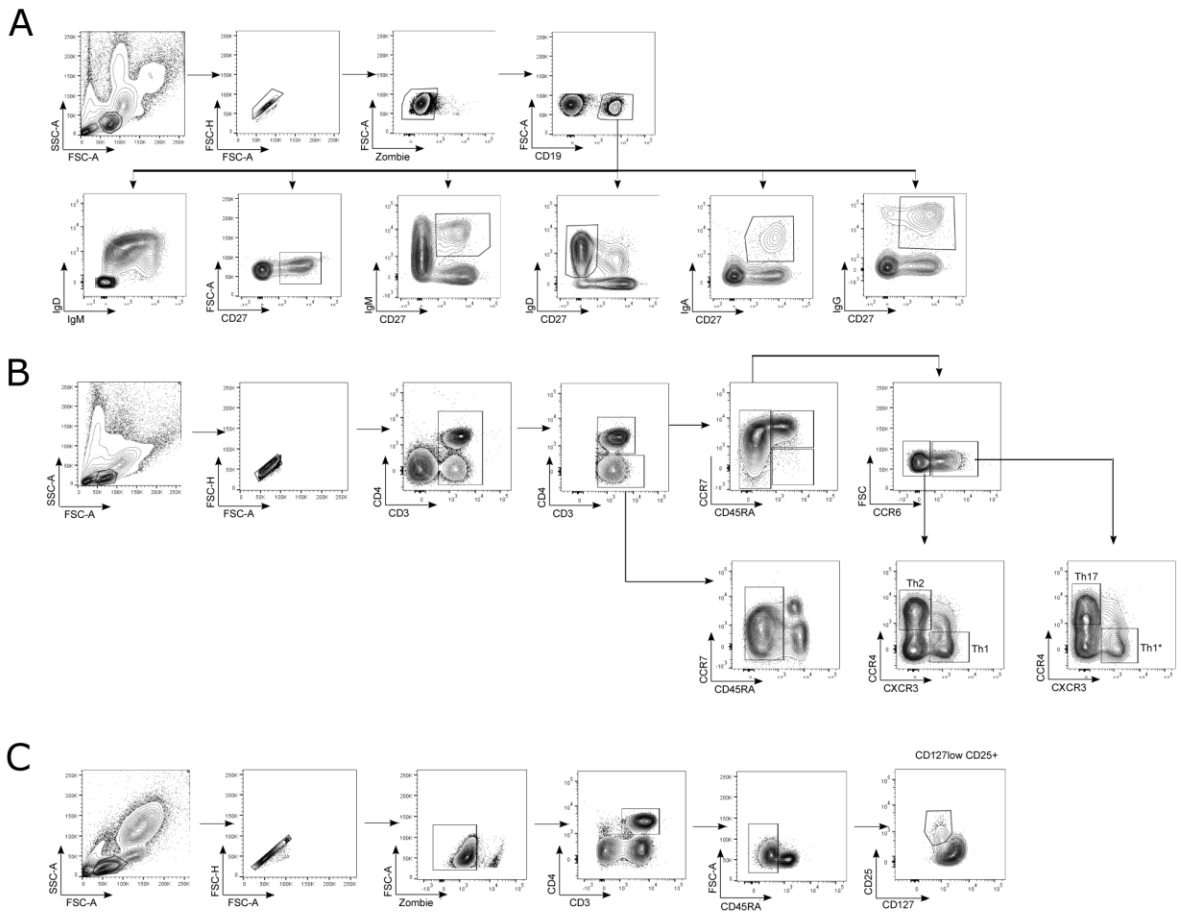

Fig. S10. Flow cytometry gating strategy for immune phenotyping of PBMCs.

(**A**) B cell panel, (**B**) T helper cell staining and (**C**) Regulatory T cell panel.

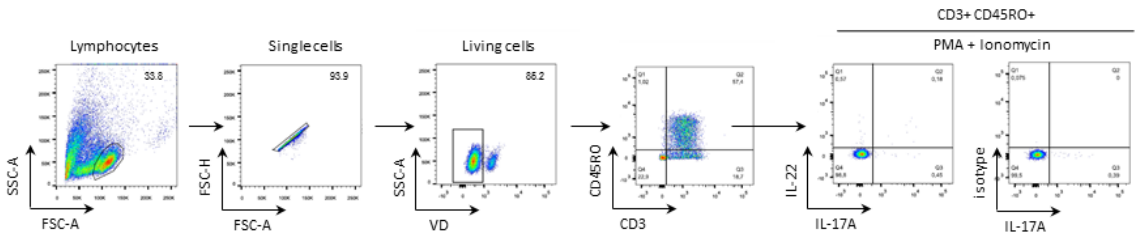

Fig. S11. Flow cytometry gating strategy for IL-22^{+} T cells.

Table S1. Clinical and immunological phenotype of HIES patients with *ZNF341* mutations.

Patient ID	**A.II.1**	**A.II.2**	**A.II.3**	**B.II.1**	**B.II.4**	**B.II.6**	**C.II.1**	**C.II.6**	**D.II.1**	**D.II.2**	**D.II.4**
Year of birth	1992	1993	1994	1998	2004	2015	1981	2002	1981	1986	1999
Sex	m	m	m	f	f	f	m	m	f	f	f
Age of onset	2-5 years	2-5 years	2-5 years	1 year	1 year	6 months	unknown	unknown	<1 year	2-5 years	unknown
Age at evaluation [years]	24	23	22	17	10	6 months	17	13	22	30	17
Ethnicity	Israeli-Arab	Israeli-Arab	Israeli-Arab	Israeli-Arab	Israeli-Arab	Israeli-Arab	Israeli-Arab	Israeli-Arab	Turkish	Turkish	Turkish
Country of Origin	Israel	Israel	Israel	Israel	Israel	Israel	Israel	Israel	Turkey	Turkey	Turkey
Consanguinity	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
First clinical symptom	Atopic dermatitis	Atopic dermatitis	Atopic dermatitis	Atopic dermatitis	Atopic dermatitis	Recurrent infections	Atopic dermatitis	Atopic dermatitis	unknown	unknown	unknown
Current diagnosis	HIES	HIES	HIES	HIES	HIES	Mild dermatitis	HIES	HIES	HIES	HIES	HIES
NIH Score	46 (aged 13) 62 (aged 24)	41 (aged 15) 55 (aged 23)	16 (aged 14) 58 (aged 22)	33	30	12	36	18	60	22 (aged 14) 28 (aged 30)	26
Clinical Features											
Respiratory tract involvement	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Upper respiratory infections (sinusitis/otitis)	1-2 / year	1-2 / year	1-2 / year	1-4/ year	1-2/ year	no	unknown	otitis	>8/ year	>6/ year	>3/year

Lower respiratory infections (pneumonias)	>6	>3	>3	unknown	unknown	1, RSC bronchiolitis	unknown	unknown	>3 (Proteus mirabilis, Staph.aureus)	no	1
Other pulmonary features	Bronchiectasis, Pneumatoceles	Bronchiectasis	Bronchiectasis	no	no	unknown	no	no	Asthma, Bronchiectasis	unknown	unknown
Skin involvement	yes	yes	yes	yes	yes	unknown	yes, severe	yes, severe	yes	yes	yes
Skin infection	yes	yes	yes	yes	yes	no	yes	yes	yes	unknown	unknown
Abscesses (number)	>20	>5	>2	>6	>4	no	>20	>3	>4	3-4	>4
• **Location**	disseminated	disseminated	disseminated	disseminated	disseminated	not applicable	disseminated	disseminated	neck and iliopsoas	disseminated	disseminated
• **Germ**	Staph. Aureus	Staph. aureus	Staph. Aureus	Staph. Aureus	Staph. Aureus	not applicable	Staph. aureus	Staph. aureus	Staph. aureus	unknown	unknown
Eczema	↑↑↑	↑↑↑	↑↑	↑↑↑	↑↑	mild	↑	↑	↑↑↑	↑↑↑	↑↑↑
Candidiasis	fingernail	oral, glossitis	oral, fingernail	yes, scalp	oral, scalp	oral	no	no	ear	no	no
Viral infections	no	no	no	no	no	no	no	no	unknown	unknown	unknown
Molluscum contagiosum	no	no	no	no	no	no	no	no	no	no	no
Herpes	no	no	no	no	no	no	no	no	local herpes simplex	no	no
Warts	no	no	no	no	no	no	no	no	no	no	no
Cutaneous vasculitis	no	no	no	no	no	no	no	no	no	no	no
Newborn rash	unknown	unknown	unknown	unknown	unknown	no	unknown	unknown	yes	no	no

Dry skin	yes	yes	yes	yes	yes	yes	yes	yes	no	no	no
Psoriasis	no	no	no	no	no	no	no	no	no	no	no
Allopecia/ Hair loss	no	no	no	yes	no	no	no	no	no	no	no
Others	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown	iliopsoas abscess	unknown	unknown
Dentition affected	yes	yes	yes	unknown	yes	unknown	unknown	unknown	no	no	no
Retained primary teeth	yes (2 retained teeth)	yes (2 retained teeth)	yes (2 retained teeth)	unknown	yes	unknown	unknown	unknown	no	no	no
Fractures (with minimal trauma)	no	no	no	no	no	no	yes	no	no	no	no
Scoliosis	yes	no	no	no	no	no	no	no	no	no	no
Hyperexten-sibility of joints	no	no	no	no	no	no	no	no	no	no	no
Facial abnormalities	Increased nose width, high palate	Increased nose width, high palate	Increased nose width	no	Increased nose width	unknown	Increased nose width	Increased nose width	High palate, macrognatic, narrow foreland, maxillary hypoplasia	High palate, macrognatic, narrow foreland	High palate
Other Infections	Osteomyelitis, MRSA	MRSA	MRSA	MRSA	MRSA	unknown	unknown	unknown	unknown	unknown	unknown
Autoimmunity	no	no	no	no	no	no	no	no	no	no	no
Allergies	no	no	no	no	no	no	no	no	no	no	no

Lymphoproliferation	no	no	no	no	no	no	no	no	no	no	no
Splenomegaly/ Hepatomegaly	no	no	no	no	no	no	no	no	no	no	no
Lymphadenopathy	no	no	no	yes	yes	no	no	no	no	no	no
Lymphocytic organ infiltration	no	no	no	unknown	unknown	no	no	no	no	no	no
Growth retardation	no	no	no	no	short stature (126 cm aged 12 = -4SD)	unknown	unknown	unknown	size: 10^{th} to 25th percentile; weight: 3^{rd} to 10^{th} percentile	no	no
Mental delay	unknown	yes	yes	yes	yes	no	yes	yes	yes (mild)	no	no

Immunoglobulin Levels (date)	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown	07/04/2016	07/04/2016	07/04/2016
IgG [mg/dl]	1,710	2,980	2,420	2,600	2,420	unknown	2,360	unknown	1,680	1,580	1,460
IgG [mg/dl] Subclasses	not done	IgG1= 1,930 IgG2= 220 IgG3= 72 IgG4= 42	IgG1= 1,360 IgG2= 435 IgG3= 47 IgG4= 63	IgG1= 1,640 IgG2= 206 IgG3= 50 IgG4= 1,010	IgG1= 1,300 IgG2= 400 IgG3= 30 IgG4= 450	not done	IgG1= 1,530 IgG2= 240 IgG3= 84 IgG4= 67	not done	IgG1= 1,670 IgG2= 186 IgG3= 54 IgG4= 331	IgG1= 1,180 IgG2= 121 IgG3= 68.8 IgG4= 330	IgG1= 1,090 IgG2= 186 IgG3= 45.3 IgG4= 519
IgM [mg/dl]	115	62	64	150	unknown	unknown	73	unknown	70.5	54.8	85.3
IgA [mg/dl]	170	190	193	150	unknown	unknown	98	unknown	179	371	203
IgE [IU/ml]	33,200 (2008: 210,000)	44,900 (2008: 10,000)	< 4 (2008: 15,000)	54,360	28,900	150 (moderately elevated for age)	23,300	11,000 and 7,640	13,916	2,507	1,825
polyclonal	yes	yes	yes	yes	yes	unknown	unknown	unknown	unknown	unknown	unknown
Specific Antibody responses; Serology/Virology											
Tetanus IgG	not done	not done	not done	yes	not done	not done	yes	not done	unknown	unknown	unknown
Diphtheria IgG	not done	not done	not done	weak	not done	not done	weak	not done	unknown	unknown	unknown
Pneumococcal IgG	not done	not done	not done	not done	not done	not done	weak	not done	unknown	unknown	unknown
pneumovax 23 vaccination	not done	not done	not done	not done	not done	not done	yes	not done	unknown	unknown	unknown

CMV IgG/IgM	not done	not done	not done	negative	not done	not done	not done	not done	IgG-positive IgM-negative	no	IgM-negative
EBV IgG/IgM	not done	not done	not done	IgG-positive IgM-negative	negative	not done	not done	not done	unknown	no	no
Others	no	no	Polio ↑	Pertussis-negative	Pertussis-negative	no	no	no	no	no	no
Parameters of acute phase response											
Infection status at time of evaluation	not done	steady state	steady state	steady state	steady state	steady state	not done	not done	steady state (pneumonia)	not done	not done
CRP [mg/l]	not done	7	<3	<3	<3	5	not done	not done	4.5 (160)	not done	not done
Ferritin [ng/ml]	not done	93	42	46	16	17	not done	not done		not done	not done
SAA [mg/l]	not done	not done	not done	not done	not done	not done	not done	not done	9.34	not done	not done
Mendel Mantoux (PPD)	not done	not done	not done	not done	not done	not done	not done	not done	negative	not done	not done
Blood counts											
RBC [cells/µl]	5 mio	4.9 mio	4.8 mio	4.9 mio	4.7 mio	unknown	5.4 mio	unknown	4.5 mio	4.6 mio	5 mio
WBC [cells/ml]	10,900	7,800	4,700	8,500	6,640	unknown	3,900	unknown	5,250	4,130	4,680
ANC [cells/ml]	5,700	1,500	1,100	5,500	2,200	unknown	1,900	unknown	1,890	2,060	2,770
Platelet count [cells/ml]	460,000	245,000	290,000	270,000	286,000	unknown	240,000	unknown	282,000	374,000	336,000

Lymphocytes absolute [cells/µl]	2,200	1,900	1,900	1,400	3,100	unknown	1,100	unknown	2,060	3,110	3,340
Eosinophils [cells/µl]	2,200	900	1,000 (700-1,100;15-18%)	700	900	unknown	300	1,200	290	220	700
CD3+ [cells/µl] and [%]	not done	1,640 (86%)	1,665 (83%)	1,544 (72%)	2,476 (42%)	not done	not done	not done	2,250.4 (84.8%)	84%	unknown
CD3+CD4+ T cells [cells/µl] and [%]	not done	900 (47%)	618 (31%)	786 (39%)	1,300 (42%)	not done	not done	not done	530.86 (19.4%)	35.5 %	unknown
CD3+CD8+ T cells [cells/µl] and [%]	not done	676 (36%)	928 (46%)	648 (32%)	1,026 (33%)	not done	not done	not done	1,651 (64.1%)	41%	unknown
CD4/CD8 ratio	not done	1.32	0.67	1.2	1.2	not done	not done	not done	0.3	0.87	unknown
CD19+ [cells/µl] and [%]	not done	170 (9.5%)	308 (15.4%)	352 (17.6%)	490 (15.8%)	not done	not done	not done	198.12 (12.9%)	8.5%	unknown
CD56+CD16+ [cells/µl] and [%]	not done	76 (4%)	42 (2.1%)	20 (4.3%)	108 (3.5%)	not done	not done	not done	55.8 (1.3%)	7.7%	unknown
Treatment											
Antifungals	no	Fluconazole	no	Fluconazole	Fluconazole	no	no	no	no	no	no
Antibiotics	yes	yes	yes	yes	yes	no	yes	no	yes	no	no
Prophylactic antibiotics	AUGMENTIN (amoxicillin/ clavulanat	AUGMENTIN (amoxicillin/ clavulanat	AUGMENTIN (amoxicillin/ clavulanat	AUGMENTIN (amoxicillin/ clavulanat	AUGMENTIN (amoxicillin/ clavulanat	no	AUGMENTIN (amoxicillin/ clavulanat	no	Trimethoprim/sulfamethoxazole 5mg/kg/d	no	no

	e)	e) RESPRIM (trimethoprim/sulfamethoxazole)	e) RESPRIM (trimethoprim/sulfamethoxazole)	e)	e) RESPRIM (trimethoprim/sulfamethoxazole)		e)		ay on 3 days a week		
Immunglobulin treatment (s.c./i.v.)	no	no	no	no	no	no	no	no	IVIG 25 gr (454 mg/kg), in 3 weeks intervals	no	no
Bisphosphonates	no	no	no	no	no	no	no	no	no	no	no
Others	antihistamine treatment	no	antihistamine treatment	antihistamine treatment	antihistamine treatment	no	no	no	IVIG since 2008	no	no

unknown (data not available), ↑ indicating the degree of affection

Table S2. *ZNF341* encodes three protein coding isoforms.

Several minor variants with unknown relevance are indicated. Primer pair 1 (fw 5'-CTC CCA CAA TGC TCG CGT AG-3' and rv 5'-ACG GAA TGC ATG TTC AGG CTG-3') spans exon 3 (of isoform 1 and 2) which is not included in transcript ENST00000497876 and isoform 3 and thus produces two different products.

Ensemble Transcript	Biotype	Ensembl exons	Uni Prot	NCBI Ref Seq	NCBI Protein	Protein Size		RT-PCR product with primer pair 1 [bp]
						[aa]	[kDa]	
ENST00000375200	Protein coding	15	Q9BYN7-1	NM_001282933	**Isoform 1 NP_001269862**	854	92.7	574
ENST00000342427	Protein coding	15	Q9BYN7-2	NM_032819	**Isoform 2 NP_116208**	847	92	574
—	—	—	—	NM_001282935	**Isoform 3 NP_001269864**	764	83.6	377
ENST00000483118	Nonsense mediated decay	14	E9PN62		**Isoform 4 (none)**	604	64.2	no amplicon
ENST00000497876	Nonsense mediated decay	14	E9PQQ0	—	—	72	7.4	377
ENST00000493497	Retained intron	3	—	—	—	no protein		no amplicon

Table S3. Transcriptome analysis of patient A.II.1 with gene-specific fold changes in comparison to healthy sibling A.II.5.

Only genes relevant to the main document or for immunodeficiencies are listed.

Gene	Fold change
IL10	1.71
ATF3	1.64
FOXP3	1.19
SMAD3	1.17
IL5	1.16
ATF4	1.14
IL22	1.07
IL23R	1.07
IL17A	1.06
RORC	1.05
SPTBN1	1.05
STAT5B	1.03
STATH	1.01
IL21	0.99
STAT2	0.97
IL23A	0.96
IL21R	0.95
EGF	0.94
ZNF341	0.93
SOCS3	0.92
CTLA4	0.9
STAT4	0.88
ZNF382	0.86
STAT5A	0.85
HMGB1	0.83
STAT6	0.81
IL6	0.73
STAT1	0.65
KAT6A	0.63
JAK1	0.61
STAT3	0.51