

IMMUNODEFICIENCIES

A recessive form of hyper-IgE syndrome by disruption of ZNF341-dependent STAT3 transcription and activity

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Heterozygosity for human *signal transducer and activator of transcription 3* (STAT3) dominant-negative (DN) mutations underlies an autosomal dominant form of hyper-immunoglobulin E syndrome (HIES). We describe patients with an autosomal recessive form of HIES due to loss-of-function mutations of a previously uncharacterized gene, *ZNF341*. *ZNF341* is a transcription factor that resides in the nucleus, where it binds a specific DNA motif present in various genes, including the *STAT3* promoter. The patients' cells have low basal levels of *STAT3* mRNA and protein. The autoinduction of *STAT3* production, activation, and function by *STAT3*-activating cytokines is strongly impaired. Like patients with *STAT3* DN mutations, *ZNF341*-deficient patients lack T helper 17 (T_H17) cells, have an excess of T_H2 cells, and have low memory B cells due to the tight dependence of *STAT3* activity on *ZNF341* in lymphocytes. Their milder extra-hematopoietic manifestations and stronger inflammatory responses reflect the lower *ZNF341* dependence of *STAT3* activity in other cell types. Human *ZNF341* is essential for the *STAT3* transcription-dependent autoinduction and sustained activity of *STAT3*.

INTRODUCTION

Hyper-immunoglobulin E (IgE) syndrome (HIES) is a relatively common primary immunodeficiency (PID; Online Mendelian Inheritance in Man #147060), first described as Job's syndrome by Wedgwood in 1966 (1) and renamed HIES by Buckley in 1972 (2). It was subsequently shown to typically display autosomal dominant (AD) inheritance, with variable expressivity (3). AD-HIES is characterized by bacterial infections, including, in particular, various staphylococcal diseases, and by fungal infections, such as chronic mucocutaneous candidiasis (CMC) in particular. In the course of infection, clinical and biological signs of inflammation are paradoxically weak in these patients. Patients also display cutaneous and systemic manifestations of allergy (in the broad sense of the term), along with high serum concentrations of total and allergen-specific IgE, and extrahematopoietic features, including facial dysmorphism, deciduous tooth retention, osteopenia, hyperextensibility, and vascular abnormalities (3, 4). They also have B cell and antibody (Ab) deficiencies (5). In 2007, Minegishi *et al.* (6) identified heterozygous, dominant-negative (DN) mutations of the gene encoding signal transducer and activator of transcription 3 (*STAT3*) as responsible for AD-HIES. Most, if not all, cases of AD-HIES are caused by *STAT3* DN mutations (7–9).

Some nonhematopoietic features of AD-HIES were explained by the discovery of patients with overlapping phenotypes, carrying bi-allelic mutations of genes encoding leukemia inhibitory factor receptor (LIFR), interleukin-11 receptor (IL-11R), and the IL-6ST/gp130

common subunit of the IL-6 receptor family, which signal via *STAT3* in various extrahematopoietic cells (10–12). Myeloid cell development is essentially normal in AD-HIES, but lymphocyte development is severely affected, with low frequencies of CD4⁺ and CD8⁺ central memory T cells, T helper 17 (T_H17) cells, T follicular helper (T_{FH}) cells, mucosal-associated invariant T (MAIT) cells, natural killer T (NKT) cells, and memory B cells (5, 7, 13–17). Patients with inborn errors of receptors or cytokines upstream from *STAT3* display overlapping syndromes. Memory B cell deficiency has been detected in IL-6ST-deficient patients and in IL-21R-deficient patients, who also have low frequencies of central memory CD8⁺ T cells, T_{FH} cells, and NKT cells (12, 13, 15, 17–19).

Some leukocyte functions are also abnormal in AD-HIES patients, as shown by studies in vitro. The patients' naïve CD4⁺ T cells display impaired T_H17 differentiation upon stimulation under T_H17-polarizing conditions in vitro, providing a mechanism for the patients' CMC, as seen in patients with inborn errors of IL-17 immunity (14, 20, 21). CD4⁺ T cells are also biased toward the T_H2 lineage, accounting for some of the patients' allergic manifestations (22), whereas the inability of their naïve B cells to differentiate into plasma cells upon stimulation with CD40L and IL-21 underlies Ab deficiency, which is also observed in patients with mutations in *IL21* or *IL21R* (5, 18, 19). IL-10 does not inhibit the response of the patients' myeloid cells to lipopolysaccharide (LPS) (6, 23). Nevertheless, these patients do not display the early-onset colitis observed in patients with IL-10, IL-10R1,

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and IL-10R2 deficiencies (24). Last, poor responses of myeloid cells to IL-6 and related cytokines probably account for the patients' low levels of inflammation, as inferred from the patient with IL-6ST deficiency (12).

In this context, we investigated patients with an autosomal recessive (AR) form of HIES—including CMC, staphylococcal infections, severe allergy, and high serum IgE levels—but apparently with stronger inflammatory responses and fewer extrahematopoietic manifestations than patients with AD-HIES. Their phenotype more closely resembled that of patients with *STAT3* DN mutations than that of patients with other PIDs involving high serum IgE levels often referred to as AR forms of HIES, such as *DOCK8* (dedicator of cytokinesis 8) deficiency (25–28) and *PGM3* (phosphoglucomutase 3) deficiency (29, 30). Patients with *DOCK8* deficiency present none of the extrahematopoietic features of AD-HIES but are highly vulnerable to skin-tropic viral infections. Likewise, patients with *PGM3* deficiency display different extrahematopoietic manifestations, autoimmunity, and a broader susceptibility to infections. We thus tested the hypothesis that the patients studied suffered from a previously undescribed AR inborn error of immunity, closely related to the AD form of HIES. Given the clinical similarity of the AD and AR forms of HIES, we hypothesized that the disease-causing gene underlying the AR form would encode a protein physiologically related to *STAT3*.

RESULTS

The patients are homozygous for truncating mutations of *ZNF341*

We investigated eight patients from six unrelated families, of Moroccan (kindred A), Afro-Caribbean (kindred B), Iranian (kindred C), Turkish (kindreds D and E), and Lebanese (kindred F) descent (Fig. 1, A and B; fig. S1, A to J; tables S1 and S2; and the “Case reports” section). Four

families were known to be consanguineous, whereas the other two families were shown to be consanguineous by whole-exome sequencing (WES), which revealed a high percentage of homozygosity in the patients (fig. S1K) (31). We performed genome-wide linkage analysis on the three living patients from kindreds A and B (P2, P3, and P4), testing the hypothesis of a shared AR trait with full penetrance (fig. S1, L and M). A single 16.8-Mb region on chromosome 20 provided a significant cumulative log of odds score of 4.8 (fig. S1M). We also performed WES for these three patients (fig. S1N). Within the linked region containing 162 protein-coding genes, only *ZNF341*, a gene of unknown function, displayed homozygosity for a rare variant (table S3). This variant (*c.904C>T*) was the same in both families tested and caused replacement of the Arg³⁰² codon with a premature stop codon (R302X). By WES (P5, P6, P7, and P8), we showed that the other four unrelated patients from kindreds C to F also carried homozygous mutations of *ZNF341*. Kindred C displayed the same *c.904C>T* mutation (R302X), whereas kindred D had a frameshift deletion (*c.1062delG*) leading to a premature stop codon (K355fs), kindred E had a nonsense mutation (*c.1626C>G*) replacing the Tyr⁵⁴² codon with a premature stop codon (Y542X), and kindred F had a nonsense mutation (*c.583C>T*) replacing the Gln¹⁹⁵ codon with a premature stop codon (Q195X). Sanger sequencing confirmed all the mutations identified by WES. The segregation of the four mutant alleles of *ZNF341* in the six families was consistent with a fully penetrant AR trait (Fig. 1A and fig. S1, O to T). The K355fs, Y542X, and Q195X mutations were private to kindreds D, E, and F, respectively. There were only two R302X heterozygotes in the ExAC database. Kindreds A, B, and C, which carried R302X, belonged to three different ethnic groups, as confirmed by principal components analysis (fig. S1U) (31). The mutation was recurrent due to a hotspot rather than a founder effect, because the haplotypes encompassing *ZNF341* differed between the three families (Fig. 1C). The four *ZNF341*

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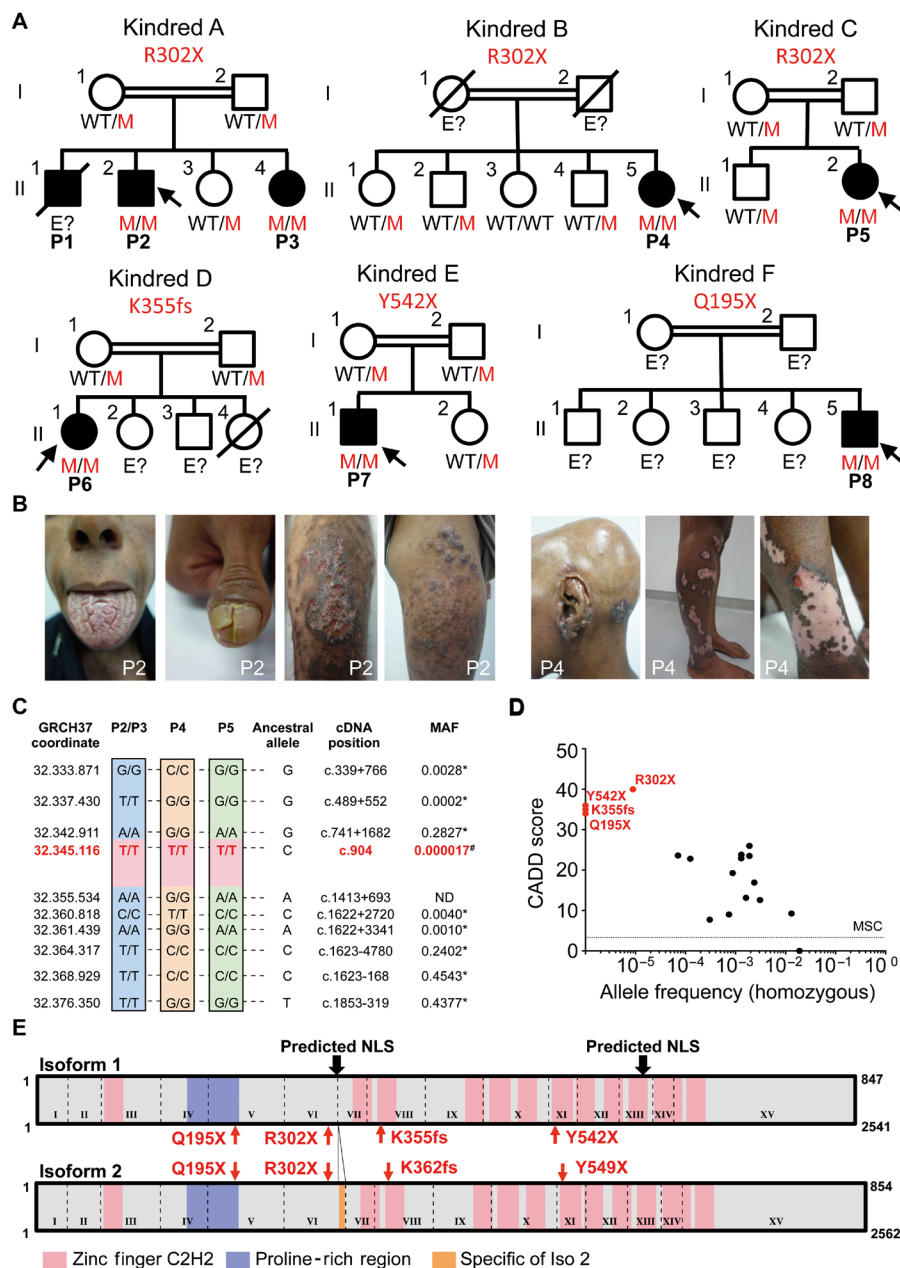


Fig. 1. Autosomal recessive *ZNF341* deficiency. (A) Pedigrees of the six unrelated families, showing familial segregation of the *c.904C>T* (p.R302X) mutant *ZNF341* allele in kindreds A to C and of alleles *c.1062delG* (p.K355fs), *c.1647C>G* (p.Y542X), and *c.583C>T* (p.Q195X) in kindreds D to F, respectively. Generations are designated by Roman numerals (I and II). P1 to P8 are represented by black symbols; the probands are indicated by arrows. Individuals of unknown genotype are labeled with "E?" (B) Representative images of the cutaneous phenotypes of P2 and P4, with tongue and thumb candidiasis, eczematous lesions of the thighs, excoriated and lichenified lesions (P2), and atrophic hypopigmented scars (P4). (C) Comparison of the patients' haplotypes with ancestral alleles. MAF, minor allele frequency. Stars and hashtag indicate frequencies extracted from dbSNP and ExAC, respectively. (D) Frequency and CADD score for all homozygous variants reported in the ExAC database. The dotted line corresponds to the mutation significance cutoff (MSC). The CADD scores of 34, 35, 36, and 40 for the Q195X, K355fs/K362fs, Y542X/Y549X, and R302X mutations, respectively, are well above the MSC of 3.31 for *ZNF341* (33). The *ZNF341* gene has intermediate gene damage index and neutrality index scores of 3.72 and 0.30, respectively, suggesting that *ZNF341* is not under strong purifying selection, consistent with the segregation of *ZNF341* deficiency as an AR disorder. (E) Schematic representation of the *ZNF341* protein. *ZNF341* has two main isoforms, isoform 1 and isoform 2, differing by 21 in-frame nucleotides at the 3' end of exon 6 of the gene, resulting in proteins of 847 and 854 amino acids in length, respectively. Exons are designated by Roman numerals. Exon boundaries are depicted with dashed lines. Predicted ZNF domains (C2H2) and proline-rich regions are shown as light red and blue boxes, respectively. Red arrows indicate the mutations. The predicted NLSs are indicated with thick black arrows.

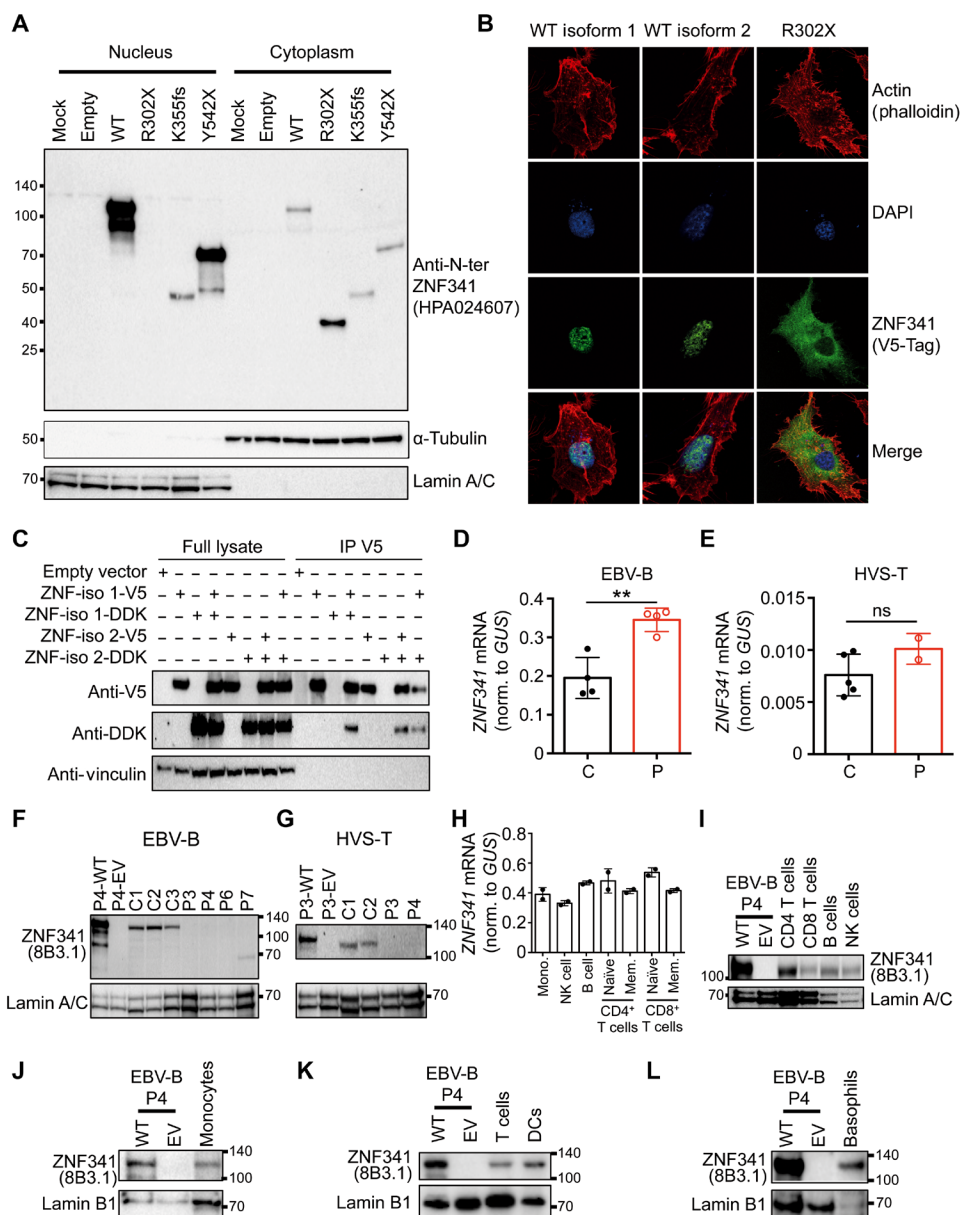
mutations are located in four different exons scattered across the gene. They are the only known mutations of this gene predicted to be loss of function and found in the homozygous state in public and in-house databases. Moreover, these mutations also have the four highest damage prediction scores [combined annotation-dependent depletion (CADD)] of all the variants found to be homozygous (Fig. 1D) (32, 33). Together, these findings strongly suggest that the homozygous *ZNF341* mutations identified in these patients are both deleterious and disease-causing.

The mutant *ZNF341* alleles do not encode full-length isoforms

Nothing is known about the biology of *ZNF341*. The human gene has 15 exons and encodes two main transcripts, differing by 21 in-frame nucleotides due to alternative splicing involving different acceptor sites at the 3' end of exon 6 (Fig. 1E), and yielding proteins of 847 (isoform 1) or 854 (isoform 2) amino acids. The stop codons created by Q195X and R302X are upstream from these 21 nucleotides, unlike those created by K355fs and Y542X. The encoded protein has 12 predicted DNA binding C2H2 zinc finger (ZNF) domains and two predicted nuclear localization sequences (NLSs), suggesting that it is a transcription factor (Fig. 1E) (34, 35). The stop codons created by Q195X and R302X are upstream from the two NLSs, whereas those created by K355fs and Y542X are located between them. We transfected human embryonic kidney (HEK) 293T cells with complementary DNAs (cDNAs) encoding wild-type (WT) *ZNF341* (isoforms 1 and 2), Q195X or R302X (both of which encode a single isoform), K355fs (isoform 1), K362fs (isoform 2), Y542X (isoform 1), or Y549X (isoform 2) and analyzed the products by Western blotting with a polyclonal Ab (pAb) against the N-terminal segment of *ZNF341* (HPA024607; Fig. 2A and fig. S2A). Isoforms encoded by the WT [predicted molecular weight (M_w) values of ~92 and 93 kDa], K355fs/K362fs (~40 and ~41 kDa), and Y542X/Y549X (~58 and ~59 kDa) cDNAs were detected in the nucleus, whereas those encoded by the Q195X (~20 kDa) and R302X (~31 kDa) cDNAs were retained in the cytoplasm. We transfected SV40-transformed fibroblasts from P4 (R302X/R302X) with cDNAs encoding C-terminally V5-tagged

Fig. 2. Molecular characterization of ZNF341 mutations and their expression in leukocyte subsets.

(A) HEK293T cells were transfected with an empty pcDNA plasmid or with pcDNA plasmids encoding the WT, R302X, K355fs, or Y542X ZNF341 isoform 1. Cytoplasmic and nuclear fractions were separated and subjected to immunoblotting with a pAb against the N-terminal segment of ZNF341 (HPA024607). α -Tubulin and lamin A/C were used as controls for the cytoplasmic and nuclear fractions, respectively. Western blots representative of three different experiments are shown. **(B)** SV40 fibroblasts from P4 were transfected with constructs encoding the two WT ZNF341 isoforms or the R302X mutant isoform, with a V5 tag at their C terminus. Cells were analyzed by confocal microscopy 24 hours after transfection. WT and mutant ZNF341 isoforms were detected with an anti-V5 Ab. The cytoplasm and nucleus were identified by phalloidin and 4',6-diamidino-2-phenylindole (DAPI) staining, respectively. An example of staining representative of three independent experiments is shown. **(C)** HEK293T cells were cotransfected with empty plasmid or plasmids containing cDNAs encoding WT isoform 1 and/or 2 of ZNF341, each tagged C-terminally with either V5 or Myc/DDK. Whole-cell lysates (left) or anti-V5 immunoprecipitates (right) are shown. Vinculin was used as a loading control. Results representative of three independent experiments are shown. **(D and E)** RNAs extracted from (D) the EBV-B cells of four controls and P3, P4, P6, and P7 and (E) HVS-T cells from five controls, P3, and P4 were subjected to RT-qPCR for total ZNF341. Data are displayed as $2^{-\Delta\Delta C_t}$ after normalization relative to *GUS* (endogenous control) expression (ΔC_t). Results representative of three independent experiments are shown. Bars represent the mean and the SD. Dots represent the mean of technical duplicates. **(F and G)** Western blot of nuclear protein extracts (50 μ g) obtained from (F) EBV-B cells from P4 stably transduced with an empty vector (EV) or WT ZNF341, three controls, and four patients (P3, P4, P6, and P7) and (G) HVS-T cells from P3 stably transduced with an EV or WT ZNF341, two controls, and two patients (P3 and P4), with a mouse anti-ZNF341 mAb (8B3.1, raised against ZNF341 C-terminal residues 366 to 468). Lamin A/C was used as a loading control. Results representative of three independent Western blots are shown. **(H)** RNA was extracted from the indicated leukocyte subsets from two healthy controls and subjected to RT-qPCR for total ZNF341. Data are displayed as $2^{-\Delta\Delta C_t}$ after normalization relative to *GUS* (endogenous control) expression (ΔC_t). Results representative of three independent experiments are shown. Bars represent the mean and the SD. Dots represent the mean of technical triplicates. **(I)** Nuclear protein extracts (50 μ g) were obtained from the indicated leukocyte subsets from a healthy control and subjected to Western blotting with a mouse anti-ZNF341 mAb (8B3.1). Lamin A/C was used as a loading control. Results representative of three independent experiments are shown. **(J to L)** Nuclear protein extracts were obtained from monocytes (J), CD3⁺ T cells (K), total dendritic cells (K), and basophils (L) from a healthy control and were subjected to Western blotting with the mouse anti-ZNF341 mAb (8B3.1). We used 100, 50, and 25 μ g of nuclear protein extracts in (J), (K), and (L), respectively. Lamin B1 was used as a loading control. Nuclear extracts from P4 EBV-B cells stably transduced with an EV or WT ZNF341 served as negative and positive controls, respectively, for ZNF341 expression in (I) to (L). Results representative of three independent experiments are shown.



proteins. We then analyzed the subcellular distribution of these proteins by confocal microscopy with a V5-specific monoclonal Ab (mAb; Fig. 2B and fig. S2B). Both isoforms of the WT and the K355fs, K362fs, Y542X, and Y549X proteins were localized in the nucleus, whereas the R302X (and by inference R195X) proteins were retained in the cytoplasm. We coexpressed cDNAs encoding WT isoform 1, WT isoform 2, R302X, K355fs, and Y542X, each tagged with V5 or DDK, in HEK293T

cells. Immunoprecipitation and immunoblotting suggested that both WT ZNF341 isoforms could homo- or hetero-oligomerize (Fig. 2C). Homo- and hetero-oligomerization of the mutant proteins was impaired but not abolished (fig. S2, C and D). In this system, we detected no interaction between WT ZNF341 and STAT3, even upon stimulation (fig. S2E). Thus, none of the four mutant alleles encodes a full-length ZNF341 isoform; their products are truncated and oligomerize

poorly, and two of the mutant proteins (Q195X and R302X) are retained in the cytoplasm.

Full-length ZNF341 isoforms are not detected in the patients' cells

Public databases (FANTOM5, HPA, and GTEx) suggest that *ZNF341* is ubiquitously transcribed. We studied the expression of endogenous *ZNF341* in cell lines from healthy controls and patients. Polymerase chain reaction (PCR) and sequencing showed that both full-length transcripts were present in Epstein-Barr virus (EBV)-transformed B cells from P4 (fig. S2, F to I) and in herpes virus saimiri (HVS)-transformed T cells from P3 and P4 (fig. S2J). Moreover, total *ZNF341* mRNA levels, as determined by reverse transcription quantitative PCR (RT-qPCR), were significantly higher in EBV-B cells and slightly higher in HVS-T cells from all patients tested (P3, P4, P6, and P7) than in controls, suggesting that these mutations did not provoke nonsense-mediated mRNA decay in these cells and that WT *ZNF341* down-regulates its own transcription (Fig. 2, D and E). We also detected *ZNF341* mRNA in primary human umbilical vein endothelial cells (HUVECs), in three hematopoietic and nine nonhematopoietic cancer cell lines tested, and in control SV40-transformed fibroblasts and primary keratinocytes (fig. S2, K to M). We performed Western blotting with a ZNF341-specific pAb (HPA067108) or an in-house mAb (8B3.1), both raised against C-terminal residues 366 to 468 (downstream from Q195, R302, and K355 but upstream from Y542 in isoform 1). We detected ZNF341 ($M_w \sim 100$ kDa) in the nuclei of all nine cancer cell lines, in primary HUVECs, primary and SV40-transformed fibroblasts, and primary keratinocytes from healthy donors (fig. S2, N to P). By contrast, ZNF341 was not detected in primary and SV40 fibroblasts or in keratinocytes from P2, P3, and P4 (fig. S2, O and P). Similarly, we detected full-length ZNF341 ($M_w \sim 100$ kDa) in the nuclei of six hematopoietic cell lines (RAJI, Jurkat, K562, U937, HL-60, and clone 15 HL-60; fig. S2N) and EBV-B and HVS-T cells from healthy controls but not in any of the four patients tested (corresponding to three mutations; Fig. 2, F and G). A protein of lower M_w (~ 70 kDa) was observed in the nuclei of EBV-B cells from P7, whose Y542X mutation is predicted to preserve the 8B3.1 mAb epitope (Fig. 2F). Because the pAb (HPA024607) recognizing the N-terminal segment failed to detect endogenous ZNF341 in control cells, we cannot exclude the possibility that low levels of the truncated R302X (and Q195X) and K355fs proteins were also present in the cytoplasm and nucleus, respectively, of the corresponding patients' cells. Last, production of the full-length ZNF341 protein was rescued in the EBV-B cells of P4 (R302X) and HVS-T cells of P3 (R302X) by stable transduction with WT *ZNF341* (isoform 1; Fig. 2, F and G). Together, these data indicate that both full-length ZNF341 isoforms are absent from the nuclei of primary keratinocytes, primary and SV40 fibroblasts, EBV-B cells, and HVS-T cells derived from patients with various mutations of *ZNF341*.

ZNF341 deficiency alters the development of lymphoid, but not myeloid, subsets

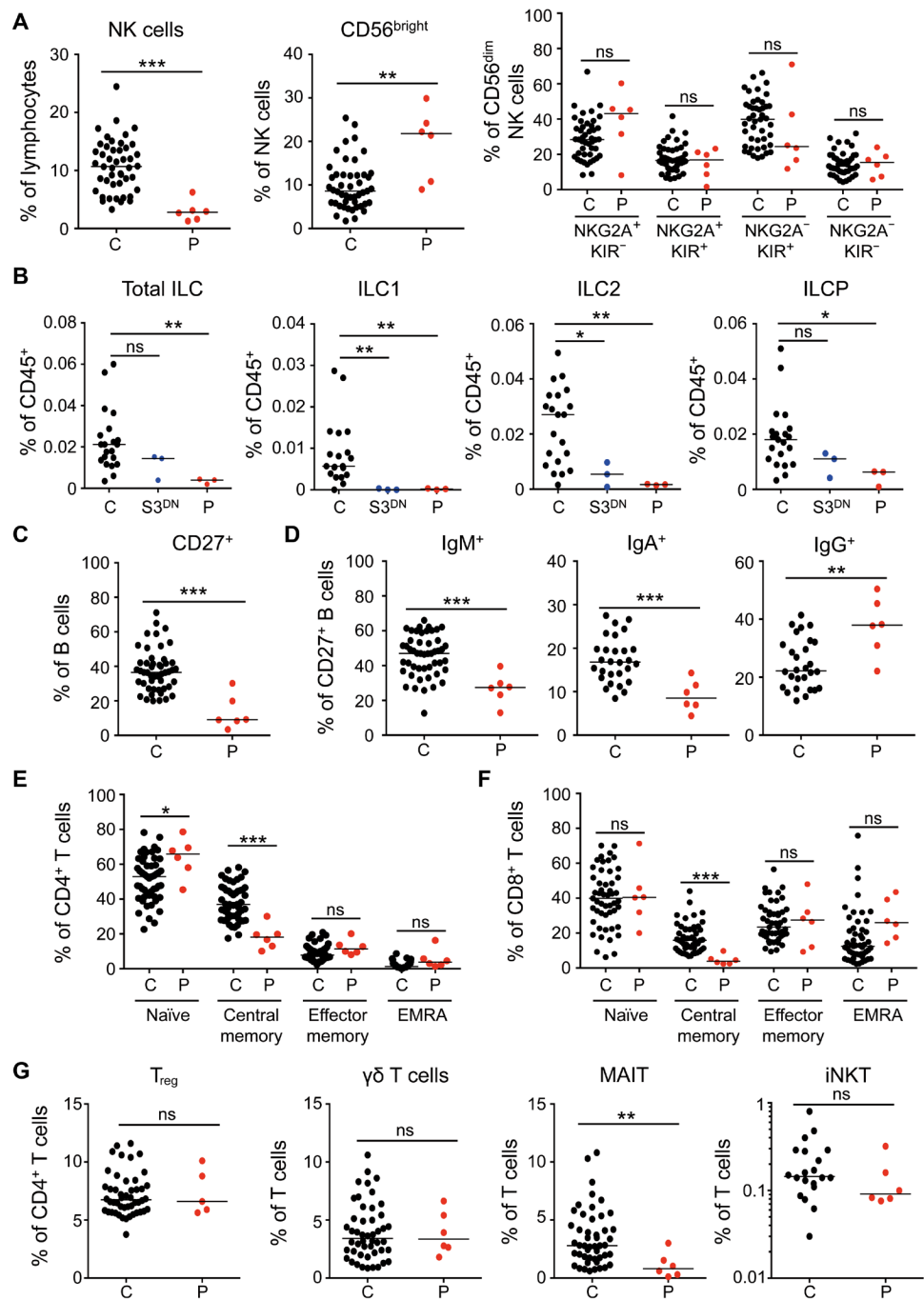
We analyzed the pattern of *ZNF341* expression in leukocyte subsets from healthy controls. *ZNF341* transcripts for both isoforms were detected, in similar amounts, in monocytes, NK, B, CD4⁺, and CD8⁺ T cells, by RT-qPCR and RT-PCR (Fig. 2H and fig. S2Q). Accordingly, a single protein corresponding to either or both isoforms was detected in the nucleus of T, B, NK, monocytes (including both CD14⁺ and CD16⁺ subsets), basophils, and dendritic cells (including

pDCs, cDC1, and cDC2; Fig. 2, I to L). ZNF341 was also detected in the HL-60 and clone 15 HL-60 cell lines (fig. S2N), two acute promyelocytic leukemia cell lines. These lines can be differentiated into neutrophil-like and eosinophil-like cells in vitro, respectively, suggesting that primary neutrophils and eosinophils may express ZNF341, like basophils (Fig. 2L). In this context, we analyzed the distribution of leukocyte subsets in the patients, by flow cytometry. The seven patients tested (P2 to P8) had normal counts of circulating neutrophils and basophils, monocytes, B cells, and T cells but had low counts of NK cells (table S2 and the "Case reports" section). Eosinophil counts were high in three patients (table S2 and the "Case reports" section). The proportions of myeloid (cDC1 and cDC2) and plasmacytoid (pDC) dendritic cells (fig. S3A) and of monocyte subsets (fig. S3B) were normal. CD56^{bright} NK cells were more abundant among NK cells, but their maturation profile was otherwise normal (Fig. 3A). The proportions of innate lymphoid cells (ILCs) among peripheral blood mononuclear cells (PBMCs) were also low, particularly for ILC1 and ILC2 (Fig. 3B). The global proportion of circulating memory B cells was low (Fig. 3C), with low (IgM⁺, IgA⁺) and high (IgG⁺) proportions of Ig isotype-specific memory B cells (Fig. 3D), consistent with the patients' high serum concentrations of IgG1 and IgG4 (table S2). The patients had normal or subnormal serum titers of antigen-specific Abs after infection with common pathogens (table S2), and P4 displayed a normal response to a vaccine booster injection (table S2). The patients' T cells proliferated normally in response to mitogens and antigens in vitro (table S2). The patients had a higher proportion of naïve CD4⁺ T cells and lower proportions of central memory CD4⁺ and CD8⁺ T cells, and of MAIT cells, than controls, but had normal proportions of regulatory T (T_{reg}) cells, $\gamma\delta$ T cells, and invariant NKT (iNKT) cells (Fig. 3, E to G). This distribution of leukocyte subsets very closely resembles that of patients with HIES due to *STAT3* DN mutations, who also have high frequencies of naïve CD4⁺ T cells and low frequencies of CD4⁺ and CD8⁺ central memory T cells, memory B cells, MAIT cells (5, 7, 13, 15–17), and ILC1 and ILC2 cells (Fig. 3B). However, ZNF341-deficient patients also have low NK cell counts (table S2 and Fig. 3A).

ZNF341 is a transcription factor that binds the *STAT3* promoter in T and B cells

We performed chromatin immunoprecipitation sequencing (ChIP-seq) analysis with the anti-ZNF341 mAb 8B3.1 to determine whether ZNF341 bound DNA in vivo and to identify its binding sites throughout the genome. In EBV-B cells from P4 transduced with cDNAs encoding WT ZNF341 isoforms 1 and 2, we found 5842 and 6570 ZNF341-binding DNA regions, respectively, 5003 of which were common to both isoforms. We also analyzed healthy control T cells activated with plate-bound anti-CD3 and soluble anti-CD28 Abs: we identified 1457 binding regions, only 229 of which were common to EBV-B cells. Computational analysis revealed two top-ranked motifs: a ZNF-like binding motif, GGAAC/GA/GGC ($P = 5 \times 10^{-437}$), and an Sp1-like binding motif, GGGAGG ($P = 3.7 \times 10^{-44}$; Fig. 4A). The ZNF-like and Sp1-like motifs have not been described before, and the closest known motifs, for ZNF263 and Sp1, respectively, are markedly different (fig. S4, A and B). The strongest ZNF341-binding site in both EBV-B cells and T cells was located in the *STAT3* promoter ($P = 10^{-310}$ being the most significant P value of all binding sites). Strong ZNF341 binding was also observed for the *STAT1* promoter and *ZNF341* intron 1 ($P = 10^{-242}$ and 10^{-310} ,

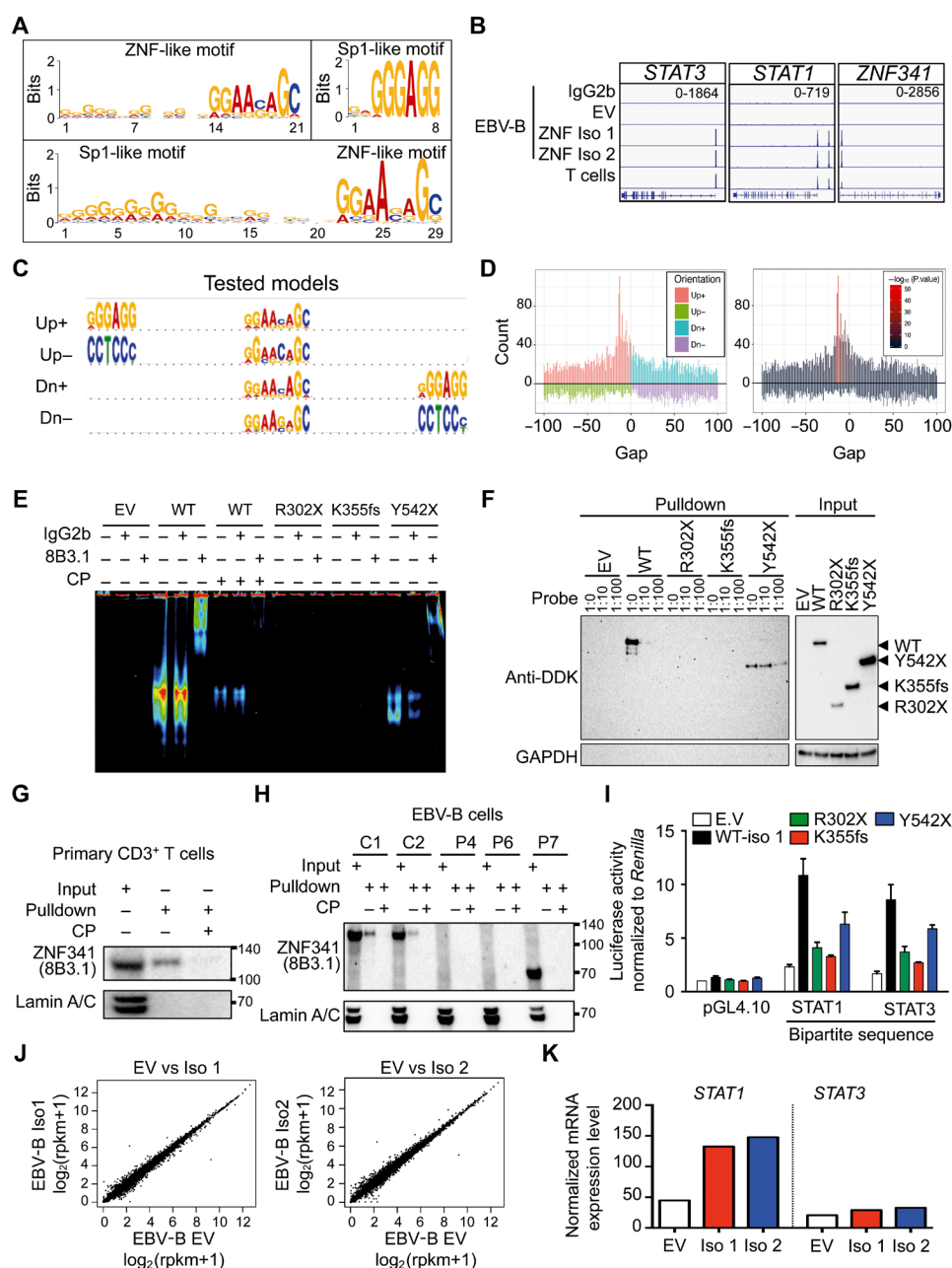
Fig. 3. NK, ILC, B, and T cell subpopulation immunophenotyping. (A) NK cell immunophenotyping for controls (C) ($n = 44$) and patients (P) ($n = 6$, P2 to P7), showing the total NK cell ($CD3^+CD56^+$) frequency in lymphocytes (left), the frequency of $CD56^{bright}$ cells within the NK cell compartment (middle), and the terminal differentiation profile of the $CD56^{dim}$ compartment. (B) ILC phenotyping, showing the frequencies of total ILCs ($Lin^-CD7^+CD56^-CD127^+$), ILC1 ($EOMES^+IFN-\gamma^+$), ILC2 ($GATA3^+IL-13^+$), and ILC precursors ($CD117^+$) among the $CD45^+$ PBMCs of controls ($n = 21$), ZNF341-deficient patients ($n = 3$, P2 to P4), and patients with $STAT3$ DN mutations ($S3^{DN}$, $n = 3$). All analyses were conducted after the exclusion of dead cells. (C) Frequency of $CD27^+$ memory cells within the B cell compartment of patients ($n = 6$) and controls ($n = 47$). (D) Frequency of IgM^+ , IgA^+ , and IgG^+ cells within the memory B cell compartment of patients ($n = 6$) and controls ($n = 26$ to 47). (E and F) Frequency of naïve ($CD45RA^+CCR7^+$), central memory ($CD45RA^+CCR7^+$), effector memory ($CD45RA^+CCR7^-$), and T_{EMRA} ($CD45RA^+CCR7^-$) cells among the $CD4^+$ (E) and $CD8^+$ (F) T cells of patients (P2 to P7) and controls ($n = 51$). (G) T cell subset immunophenotyping. Frequency of T_{reg} ($CD3^+CD4^+CD25^{hi}FoxP3^+$) cells in the $CD4^+$ T cell compartment and frequency of $\gamma\delta$ T ($CD3^+TCR-\gamma\delta^+$), MAIT ($CD3^+CD161^+TCR-\alpha\gamma^+$), and iNKT ($CD3^+TCR-iNKT^+$) cells among the T cells of patients ($n = 5$ or $n = 6$, P2 to P7) and controls ($n = 18$ to 46). In all panels, Mann-Whitney tests were used for comparisons.



respectively; Fig. 4B). Furthermore, in the 229 motifs common to both control T cells and patient EBV-B cells transduced with ZNF341, we also identified bipartite binding sites containing the Sp1-like motif GGGAGG upstream from the ZNF-like motif GGAAC/GA/GGC ($P < 5.3 \times 10^{-172}$; Fig. 4A). We found that there was a preferential spacing of 13 to 14 nucleotides between the two motifs (Fig. 4, C and D), associated with a stronger binding intensity, as assessed by determining peak intensity (fig. S4C). We then incubated nuclear extracts of HEK293T cells transfected with an empty vector, the C-terminal DDK-tagged WT, R302X, K355fs, or Y542X ZNF341 cDNA with a 5'-tagged fluorescent DNA probe containing the putative bipartite ZNF341-binding motif from the *STAT3* promoter (Fig. 4E and fig. S4D). In electrophoretic mobility shift assays (EMSAs), nuclear proteins from WT-transfected cells bound the probe, whereas complex formation was inhibited by an unlabeled specific probe [competitor probe (CP)], and super-shifting of the complex was observed with the specific anti-ZNF341 8B3.1 or anti-DDK mAb but not with an isotype control mAb. By

contrast, nuclear proteins from R302X- and K355fs-transfected cells did not bind the probe, whereas Y542X-transfected cells displayed weak binding (Fig. 4E and fig. S4D). Incubation of the same nuclear extracts with a 5'-biotinylated DNA probe resulted in the pulldown, with streptavidin-coupled magnetic beads, of complexes consistent with the EMSA results (Fig. 4F). Pulldown experiments with nuclear extracts from healthy control resting primary $CD3^+$ T cells or EBV-B cells showed that endogenous WT ZNF341 bound efficiently to the bipartite DNA binding motif (Fig. 4, G and H). By contrast, the truncated Y542X ZNF341 protein was not pulled down in this system, in experiments with nuclear extracts from P7 EBV-B

Fig. 4. Transcriptional activity of ZNF341. (A) Motif analysis for the top 500 ZNF341-binding sites in P4 EBV-B cells transduced with WT ZNF341 isoform 1, as compared with empty vector (EV). (B) ChIP-seq profile of IgG, EV, and ZNF341 (isoform 1 or isoform 2) or control CD3⁺ T cells after 2 days of stimulation with plate-bound anti-CD3 and soluble anti-CD28 mAb for the genomic loci corresponding to *STAT3*, *STAT1*, and *ZNF341*. The scale of the y axis, corresponding to the number of reads, is indicated at the top right corner of each plot. (C) Models tested to determine the orientation and spacing preferences of the ZNF-like core motif and the Sp1-like motif. (D) Distribution of the observed spacing counts between the Sp1-like motif and the ZNF-like core motif in EBV-B cells from P4 stably transduced with WT ZNF341. Counts are displayed relative to the model tested (left), and *P* values are calculated for a given spacing (right). (E) EMSA of nuclear extracts of HEK293T cells transfected with an EV, the C-terminal DDK-tagged WT, R302X, K355fs, or Y542X ZNF341 alleles. Extracts were incubated with a 5' fluorescent DNA probe containing the putative bipartite ZNF341-binding motif from the *STAT3* promoter in the presence or absence of a control isotype (IgG2b) or an anti-ZNF341 mAb (8B3.1) for supershift experiments. An untagged CP of similar sequence but with a concentration 10 times higher was used to test binding specificity. Results representative of three independent experiments are shown. (F) Pulldown of the 5'-biotinylated DNA probe containing the putative bipartite ZNF341-binding motif from the *STAT3* promoter after incubation with nuclear extracts of HEK293T cells transfected with an EV, the C-terminal DDK-tagged WT, R302X, K355fs, or Y542X ZNF341 alleles. Extracts were incubated in the presence or absence of various doses of a CP to test binding specificity (1:0, 1:10, and 1:100 are the ratios of specific biotinylated probe to CP). The presence or absence of ZNF341 constructs in the pulldown fraction (left) or in the input (right) was assessed by immunoblotting for the C-terminal DDK tag. Results representative of three independent experiments are shown. (G and H) Pulldown of the 5'-biotinylated DNA probe containing the putative bipartite ZNF341-binding motif from the *STAT3* promoter after incubation with nuclear extracts of negatively sorted primary CD3⁺ T cells of one control (G) or of EBV-B cell lines from two controls, P4, P6, and P7 (H). Extracts were incubated in the presence or absence of a CP at a concentration 10 times higher than that of the biotinylated DNA probe, to test binding specificity. The presence or absence of ZNF341 in the pulldown fraction was assessed by immunoblotting with an anti-ZNF341 mAb (8B3.1). Lamin A/C was used as a loading control. Results representative of three independent experiments are shown. (I) Luciferase activity of HEK293T cells cotransfected with WT or mutant ZNF341 isoform 1 plus a pGL4.10 reporter plasmid encoding the luciferase cDNA downstream from the bipartite ZNF341-binding motif of the promoters of *STAT1* and *STAT3*. The results shown are the mean and SD of three independent experiments. (J) Total RNA sequencing data for the EBV-B cells of P4 stably transduced with WT ZNF341 isoform 1 (Iso 1), isoform 2 (Iso 2), or an EV. Scatter dot plots comparing mRNA levels in cells transduced with ZNF341 isoform 1 or 2 with those in cells transduced with the EV. (K) *STAT1* and *STAT3* mRNA levels extracted from total RNA sequencing data.



cells (Fig. 4H). Thus, WT ZNF341 specifically binds a bipartite consensus DNA motif that is present in the promoter of *STAT3*, whereas the three mutant ZNF341 proteins tested (and, by inference, Q195X), including K355fs and Y542X, which can translocate to the nucleus, do not.

ZNF-like and Sp1-like motifs cooperate to enhance ZNF341 DNA binding

We characterized the ZNF341-binding motif in more detail by testing WT ZNF341 in pulldown experiments with probes either corresponding to the bipartite DNA binding motif or containing various

systematic mutations (fig. S4, E and F). We deleted the Sp1-like (Δ Sp1 probe) or the ZNF-like (Δ ZNF probe) motif or introduced single-nucleotide ($n = 8$; probes #1 to #8) or multiple-nucleotide mutations ($n = 4$; #4#7, aA, aG, and aZ probes) at various positions in the ZNF-like motif. Nuclear extracts from HEK293T cells transfected with WT *ZNF341* isoform 1 cDNA or an empty vector were used as positive and negative controls, respectively. The deletion of the Sp1-like (74%) or ZNF-like (94%) motif decreased ZNF341 binding relative to the consensus sequence (WT probe), and deletion of the ZNF-like motif had the strongest impact (fig. S4E). This result is consistent with our ChIP-seq data, showing that the ZNF-like motif is present in all 500 top peaks, whereas the Sp1-like motif is present in only 45% of these peaks. In a similar pulldown experiment, single-nucleotide mutagenesis of the ZNF-like motif (GGAACAGC) only modestly decreased ZNF341 binding relative to the WT consensus DNA sequence (fig. S4F). However, mutations of one of the most (#3) and one of the least conserved nucleotides (#5) within the ZNF-like motif were associated with the largest (82%) and smallest (40%) decreases in DNA binding, respectively. Replacements of multiple nucleotides within the ZNF-like motif (aZ, aA, and aG probes) decreased DNA binding by about 94%, to levels similar to those observed with a probe lacking the ZNF-like motif (Δ ZNF probe). Overall, although either motif within the bipartite sequence is sufficient for at least some detectable binding of ZNF341 to DNA, the ZNF-like motif is more important and acts in synergy with the Sp1-like motif to ensure strong binding of ZNF341 to DNA.

ZNF341 overexpression drives the induction of STAT3

We assessed the ability of ZNF341 to induce transcription from the *STAT1* and *STAT3* promoters (44 base pairs of each promoter containing the canonical bipartite motif) in a luciferase reporter assay in HEK293T cells (Fig. 4I and fig. S4, G and H). Both WT isoforms induced expression from the *STAT1* and *STAT3* promoters. Three of the five mutant isoforms tested induced no luciferase activity, confirming that the R302X and K355fs/K362fs mutant alleles (and, by inference, Q195X) were loss of function. By contrast, the Y542X/Y549X mutant, which bound the canonical motif on EMSA and in pulldown experiments in the overexpression system, but not in EBV-B cells from P7, yielded intermediate levels of luciferase activity, suggesting that it is hypomorphic, at least when overexpressed. The luciferase *STAT1* and *STAT3* constructs containing the Sp1-like or the ZNF-like motif alone failed to induce luciferase activity in the presence of WT ZNF341, demonstrating the requirement of both motifs for ZNF341 activity (fig. S4, G and H). Cotransfection with the WT *ZNF341* cDNA together with an *Sp1* cDNA did not further enhance luciferase activity from *STAT3* or *STAT1* bipartite canonical sequences over that observed for WT *ZNF341* alone (fig. S4I). Consistently, after the overexpression of Sp1 and ZNF341 in HEK293T cells, no interaction was detected between these two proteins in immunoprecipitation experiments (fig. S4J). In conclusion, ZNF341 induces the transcription of *STAT1* and *STAT3* by binding to the bipartite consensus sites in their promoters. Two of the three mutant alleles tested are loss of function (R302X and K355fs), the third being at least severely hypomorphic (Y542X), and the fourth is predicted to be loss of function (Q195X). In light of the clinical and immunological similarities between patients with *ZNF341* and *STAT3* mutations, these findings strongly suggest that ZNF341 may be essential for the transcription of *STAT3*, at least in cells expressing ZNF341, the pattern of expression of which is apparently as broad as that of *STAT3*.

STAT3 function is normal in ZNF341-deficient immortalized cell lines

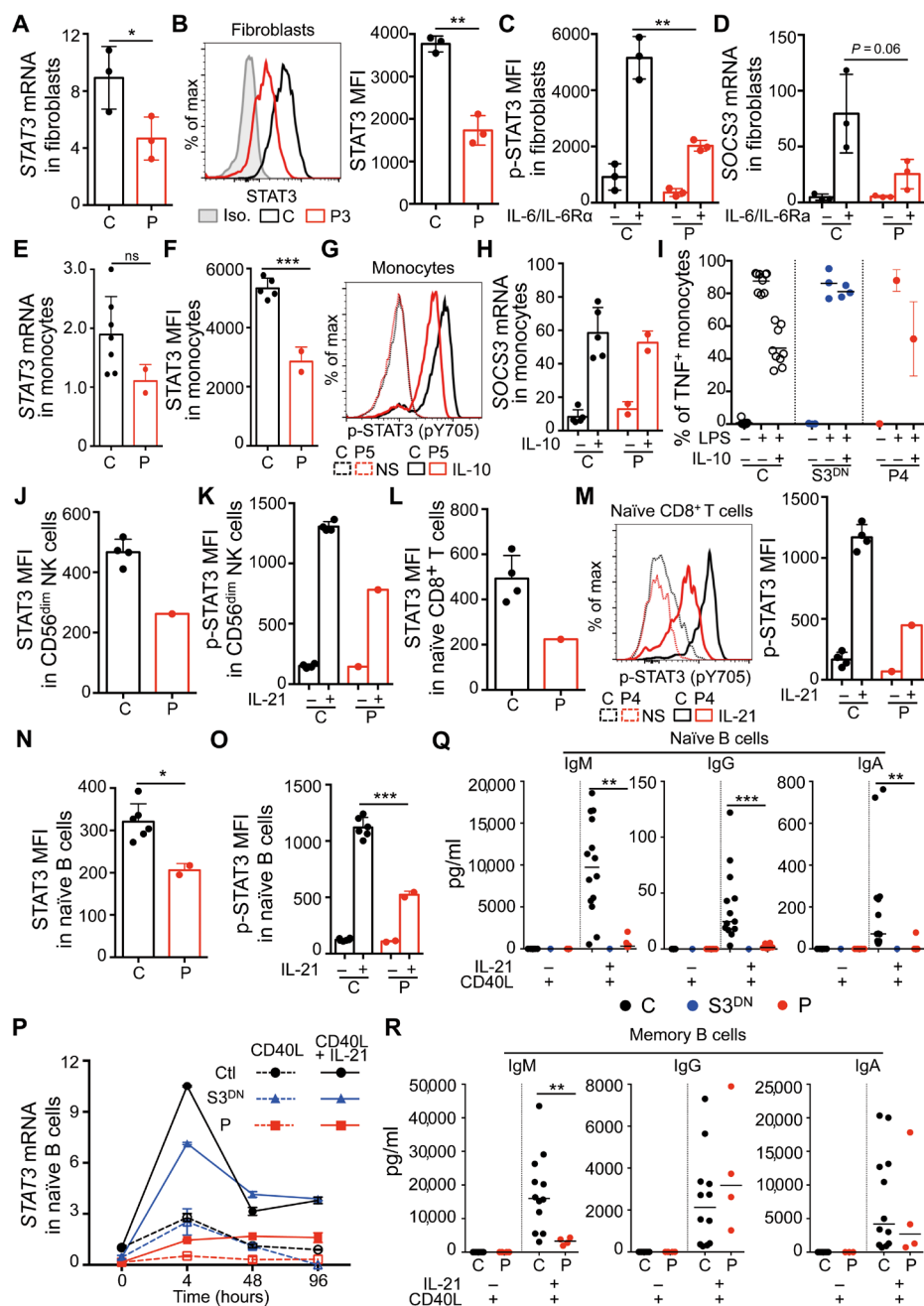
We performed total RNA sequencing (RNA-seq) to determine whether ZNF341 controlled the transcription of target genes, including *STAT3* in particular, in immortalized lymphoid cell lines. *ZNF341* overexpression had very little impact on the transcriptome of P4 EBV-B cells (Fig. 4J). We found that, after transduction with WT *ZNF341* isoform 1 or 2, mRNA levels differed significantly ($P < 0.01$) from those in cells transduced with an empty vector for only 28 and 52 mRNAs (tables S4 and S5), respectively, including *STAT1* [fold change (FC) = 3.0 and 3.3], but not *STAT3* (FC = 1.4 and 1.6; Fig. 4K). Thus, the binding of ZNF341 to the promoters of *STAT3* and other genes does not strongly drive their transcription in EBV-B lines. We then analyzed *STAT1* and *STAT3* mRNA and protein levels in cell lines from patients and controls (fig. S4, K to O). We found that *STAT1* mRNA levels were in the lower part of the control range in EBV-B cells, HVS-T cells, and SV40-immortalized fibroblasts from patients, as determined by RT-qPCR (Fig. S4K). *STAT1* protein levels were also lower (about two- to threefold) in the patients' EBV-B cells, HVS-T cells, and SV40 fibroblasts than in controls, as shown by Western blotting and flow cytometry (fig. S4, L to O). The complementation of HVS-T cells from P3 and EBV-B cells from P4 with WT *ZNF341* increased *STAT1* mRNA and protein levels, as shown by comparison with cells transduced with an empty vector (fig. S4, K to O). By contrast, *STAT3* mRNA and protein levels were within or near the normal range in EBV-B cells from the patients (fig. S4, K, L, and O). These levels were slightly lower in patient HVS-T cells and SV40 fibroblasts (fig. S4, K and M to O). The transduction of HVS-T cells from P3 and EBV-B cells from P4 with WT *ZNF341* increased *STAT3* mRNA and protein levels relative to those in cells transduced with an empty vector (fig. S4, K to M and O). Last, the amounts of phosphorylated *STAT1* and *STAT3* were within the normal range in EBV-B cells stimulated with interferon- α (IFN- α) and IL-21, respectively (fig. S4P). Accordingly, the induction of target genes (*CXCL10* and *SOC3*) in patients' EBV-B cells stimulated with IFN- α or IL-21 was normal (fig. S4Q), as was that in patients' SV40 fibroblasts stimulated with IFN- α or IL-6/IL-6R α (fig. S4R). Overall, immortalized cell lines from the patients displayed no major *STAT3* phenotype, with the possible exception of slightly low levels of *STAT3* mRNA and protein in HVS-T cells and SV40 fibroblasts.

STAT3 function is impaired in ZNF341-deficient primary fibroblasts

Some ZNF341-deficient patients displayed nonhematopoietic features seen in AD-HIES patients, such as facial dysmorphism (P6 and P7), joint hyperextensibility (P6 and P8), recurrent bone fractures (P7), jaw and tooth abnormalities (P3 and P5), and a high palate (P4, P5, P6, and P7). We therefore analyzed the impact of ZNF341 deficiency in primary fibroblast cell lines. We evaluated *STAT3* mRNA levels by RT-qPCR and protein levels by flow cytometry and Western blotting. We found that *STAT3* levels were about 50% lower in the patients' fibroblasts ($n = 3$) than in fibroblasts from healthy controls (Fig. 5, A and B, and fig. S5A). We then assessed the impact of the *STAT3*-activating cytokine IL-6. After 30 min of costimulation with IL-6 and soluble IL-6R α (hereafter referred to as IL-6/IL-6R α), *STAT3* phosphorylation was ~60% lower in ZNF341-deficient fibroblasts than in WT fibroblasts (Fig. 5C). Low levels of *STAT3* phosphorylation were associated with weaker ($P = 0.06$) *SOC3* mRNA induction after 2 hours of stimulation (Fig. 5D). The activation of *STAT3*

Fig. 5. STAT3 production and function in primary cells.

(A to D) STAT3 production and function in primary fibroblasts. *STAT3* mRNA levels (A) as evaluated by RT-qPCR after RNA extraction from the primary fibroblasts of three controls and three patients (P2 to P4). Data are displayed as $2^{-\Delta\Delta C_t}$ after normalization relative to *GUS* (endogenous control) expression (ΔC_t). Data representative of two independent experiments are shown. (B) STAT3 levels, as evaluated by flow cytometry, in primary fibroblasts. (Left) Representative image of STAT3 expression in fibroblasts from P3 and a healthy control, as well as the isotopic control. (Right) Recapitulative graph showing the mean fluorescence intensity (MFI) of STAT3, as measured by flow cytometry, in three controls and three patients (P2 and P4). Data representative of two independent experiments are shown. (C) Recapitulative graph of the MFI of pY705-STAT3, as evaluated by flow cytometry, in primary fibroblasts from three controls and three patients (P2 and P4), left unstimulated or after 30 min of stimulation with IL6/IL-6Ra. (D) *SOC33* mRNA levels, as evaluated by RT-qPCR after RNA extraction from the primary fibroblasts of three controls and three patients (P2 and P4), with or without 2 hours of stimulation with IL6/IL-6Ra. Data are displayed as $2^{-\Delta\Delta C_t}$ after normalization relative to *GUS* (endogenous control) expression (ΔC_t). Data representative of two independent experiments are shown. **(E to I)** STAT3 production and function in monocytes. *STAT3* mRNA levels (E), as evaluated by RT-qPCR after RNA extraction from the primary monocytes of three controls, P4, and P5. Data are displayed as $2^{-\Delta\Delta C_t}$ after normalization relative to *GUS* (endogenous control) expression (ΔC_t). (F) STAT3 expression, as measured by flow cytometry in primary monocytes. Recapitulative graph of the MFI of STAT3, as measured by flow cytometry, in three controls, P4, and P5. (G) STAT3 phosphorylation (pY705), evaluated by flow cytometry, in monocytes from P5 and a healthy control after 30 min of stimulation with IL-10. Representative image for two patients tested. (H) *SOC33* mRNA levels, as evaluated by RT-qPCR after RNA extraction from the primary monocytes of five controls and two patients (P4 and P5), with or without 2 hours of stimulation with IL-10. Data are displayed as $2^{-\Delta\Delta C_t}$ after normalization relative to *GUS* (endogenous control) expression (ΔC_t). (I) Percentage of TNF⁺ monocytes for nine controls, P4 (tested twice), and three STAT3-DN patients after 4 hours of stimulation with LPS in the presence or absence of IL-10, as evaluated by flow cytometry. **(J)** Flow cytometry quantification of STAT3 levels in primary NK cells. Recapitulative graph of the MFI of STAT3 measured in four controls and P4. **(K)** Graph of the MFI of pY705-STAT3, as evaluated by flow cytometry, in primary CD56^{dim} NK cells from four controls and P4, left unstimulated or after 30 min of stimulation with IL-21. **(L)** Graph of the MFI of STAT3 in primary naïve CD8⁺ T cells, measured in four controls and P4. **(M)** Representation of the phosphorylation of STAT3 (pY705) in primary naïve CD8⁺ T cells from one control and P4, evaluated by flow cytometry, without stimulation or after 30 min of stimulation with IL-21 (left). (Right) Graph of the MFI of pY705-STAT3, as evaluated by flow cytometry, in primary naïve CD8⁺ T cells from four controls and P4, left unstimulated or after 20 min of stimulation with IL-21. **(J to M)** Data are representative of two independent experiments (P4 and P8). **(N)** Flow cytometry quantification of STAT3 levels in primary B cells. Recapitulative graph of the MFI of STAT3, as measured by flow cytometry, in six controls, P4, and P6. **(O)** Quantification of the phosphorylation of STAT3 (pY705) in primary B cells from six controls, P4, and P6, evaluated by flow cytometry, with the cells left unstimulated or after 30 min of stimulation with IL-21. **(P)** Levels of *STAT3* mRNA in naïve B cells from one healthy individual, one STAT3 DN patient, and one ZNF341-deficient patient (P8) after 0, 4, 48, and 96 hours of stimulation with the indicated combinations of CD40L and IL-21. Data are displayed as $2^{-\Delta\Delta C_t}$ after normalization relative to *GUS* (endogenous control) expression (ΔC_t) and nonstimulated cells of the control ($\Delta\Delta C_t$). **(Q)** Sorted naïve B cells from controls ($n = 14$), one STAT3-DN patient, and six ZNF341-deficient patients (P2 to P4, P6, P7, and P8) were cultured in the presence of CD40L (200 ng/ml), with or without IL-21 (50 ng/ml), for 7 days. The production (pg/ml) of IgM, IgG, and IgA was then assessed by Ig heavy chain-specific ELISA on cell culture supernatants. **(R)** Sorted memory B cells from controls ($n = 12$) and four ZNF341-deficient patients (P2 to P4, and P8) were cultured in the presence of CD40L (200 ng/ml), with or without IL-21 (50 ng/ml), for 7 days. The production (pg/ml) of IgM, IgG, and IgA was then assessed by Ig heavy chain-specific ELISA on cell culture supernatants.



by IL-6 in murine cells or the human HepG2 cell line up-regulates expression of the *STAT3* gene itself (36, 37). After IL-6/IL-6R α treatment, we observed a weak induction of *STAT3* mRNA in patients' cells relative to control cells (fig. S5B). These data indicate that ZNF341 deficiency results in low baseline levels of *STAT3* mRNA and protein, together with impaired *STAT3* phosphorylation and transcriptional activity, in primary fibroblasts and, by inference, in other nonhematopoietic cell types. These experiments provide a mechanism of action of ZNF341, and a mechanism of disease in the studied AR-HIES patients, by functionally connecting the ZNF341 deficit with *STAT3*, which is mutated in patients with AD-HIES. These data also explain the extrahematopoietic features observed in some patients. The somewhat rarer and milder nonhematopoietic features of ZNF341-deficient patients than of patients with DN *STAT3* mutations may reflect a certain level of redundancy of ZNF341 in some cell types.

STAT3 function is normal in ZNF341-deficient monocytes

Another particular feature of AR ZNF341 deficiency, differentiating this condition from AD *STAT3* deficiency, is the apparently stronger inflammatory response during infection. Because monocytes from healthy donors contain the ZNF341 protein (Fig. 2J), we analyzed the impact of ZNF341 deficiency in primary monocytes. We found low levels of *STAT3* mRNA and protein in the monocytes of ZNF341-deficient patients, as shown by RT-qPCR and flow cytometry (Fig. 5, E and F). Moreover, *STAT3* phosphorylation levels were ~50% lower in ZNF341-deficient monocytes after stimulation with IL-10, a *STAT3*-activating cytokine, than in control cells (Fig. 5G). Monocyte IL-10 signaling is impaired in *STAT3*-DN patients (6). We therefore measured *SOCS3* mRNA induction in monocytes from five controls, P4, and P5 after 2 hours of IL-10 stimulation. Levels of *SOCS3* mRNA induction were found to be normal in the patients (Fig. 5H). We then studied tumor necrosis factor (TNF) production by fresh monocytes from healthy controls ($n = 9$), *STAT3*-DN patients ($n = 3$), and P4 in response to LPS stimulation, alone or in the presence of IL-10 (Fig. 5I). As expected, IL-10 inhibited TNF production upon LPS activation in monocytes from controls but not in monocytes from the *STAT3*-DN patients. By contrast, the IL-10-mediated inhibition of TNF production in P4 monocytes upon LPS stimulation was reproducibly intact, suggesting that ZNF341 deficiency had no effect on *STAT3*-dependent responses to IL-10, and, by inference, to other cytokines, in these cells, as shown for IL-6 (fig. S5C). These findings indicate that low baseline levels of *STAT3* do not necessarily lead to low levels of *STAT3* activity in ZNF341-deficient cells, although monocytes and fibroblasts differ in this respect. This experiment also provided an explanation for the stronger inflammation observed in ZNF341-deficient patients than in *STAT3*-DN patients.

STAT3 production and activation are impaired in ZNF341-deficient cytotoxic lymphocytes

ZNF341-deficient patients have low NK cell counts and low frequencies of central memory CD8⁺ T cells. Both these cell types expressed ZNF341 in healthy controls (Fig. 2I). We analyzed the impact of ZNF341 deficiency in the remaining primary NK cells and in naïve CD8⁺ T cells, analyzing the expression and activation of *STAT3* (Fig. 5, J to M, and fig. S5, D to F). We tested *STAT3* expression by flow cytometry in CD56^{dim} NK cells and in naïve CD8⁺ T cells and found that *STAT3* expression was decreased in the patients by about 50% in both subsets, when compared with healthy controls (Fig. 5, J to L). After IL-21 stimulation, *STAT3* phosphorylation was decreased by ~40 and 60% in

ZNF341-deficient CD56^{dim} NK cells and naïve CD8⁺ T cells, respectively, relative to controls (Fig. 5, K and M). Normal NK cell numbers in *STAT3*-DN patients suggests that low *STAT3* expression and function in ZNF341-deficient NK cells is unlikely to explain their NK cell lymphopenia. In contrast, the similarly low frequency of central memory CD8⁺ T cells in ZNF341-deficient patients and patients with DN *STAT3* mutations suggests that low *STAT3* expression and function is responsible for impaired central memory CD8⁺ T cell differentiation in ZNF341-deficient patients. Collectively, these findings showed that the impact of ZNF341 deficiency, in terms of *STAT3* baseline expression and inducible activation, extended beyond fibroblasts and monocytes to cytotoxic NK and CD8⁺ T cells. However, we did not test *STAT3* activity in these two cell types.

STAT3 activity is impaired in ZNF341-deficient B cells

We next analyzed the impact of ZNF341 deficiency in primary naïve and memory B lymphocytes. Compared with controls, *STAT3* expression was decreased in patients' primary naïve B cell subsets by about 50%, as determined by flow cytometry (Fig. 5N and fig. S5G). After IL-21 stimulation, *STAT3* phosphorylation was decreased by ~50% in naïve B cells from ZNF341-deficient patients, when compared with controls (Fig. 5O and fig. S5H). We assessed *STAT3* induction in naïve B cells from controls, a *STAT3*-DN patient, and a ZNF341-deficient patient (P8), upon stimulation with CD40L with or without IL-21 (Fig. 5P). Stimulation for 4 hours with CD40L alone induced a transient tripling of *STAT3* mRNA levels in controls and patients. Incubation with CD40L plus IL-21 induced a ~10-fold increase in *STAT3* mRNA levels in naïve B cells from controls after 4 hours of stimulation, demonstrating synergy between the CD40L and IL-21 pathways. In contrast, naïve B cells from a ZNF341-deficient patient displayed no such induction after stimulation with CD40L and IL-21. Normal induction was detected with *STAT3*-DN naïve B cells, suggesting that the residual *STAT3* activity in these cells was sufficient to induce *STAT3* mRNA up-regulation upon costimulation with CD40L and IL-21. Because ZNF341-deficient patients have selectively high serum IgG and IgE levels and high levels of allergen-specific IgE (fig. S5I), together with memory B cell deficiency, we investigated the ability of their B cells to secrete Ig in various culture conditions requiring *STAT3* activation. We sorted naïve and memory B cells from controls and patients and cultured them for 7 days in the presence of CD40L, alone or together with IL-21 (Fig. 5, Q and R). The induction of IgM, IgG, and IgA secretion by naïve B cells from the patients in response to stimulation with CD40L and IL-21 was severely impaired (<0.1 to 10% of normal levels; Fig. 5Q). By contrast, the levels of IgM and IgA secretion by ZNF341-deficient memory B cells were 10 to 30% those in controls, whereas the secretion of IgG was unaffected (Fig. 5R). Naïve and memory B cells from the patients produced normal levels of IgM in response to CD40L/CpG/BCR engagement (used as a control; fig. S5J). These data closely resemble published findings for patients with AD-HIES due to DN *STAT3* mutations (5, 18). It strongly suggests that ZNF341 deficiency in circulating naïve B cells affects their function by preventing these cells from responding to IL-21 via *STAT3*. These findings may explain the B cell phenotype observed in both ZNF341- and *STAT3*-mutated patients with HIES. They also suggest that impairment of the autoinduction of *STAT3* (i.e., the induction of *STAT3* mRNA and protein by phosphorylated *STAT3* dimers) is a critical mechanism underlying the pathogenesis of ZNF341 deficiency.

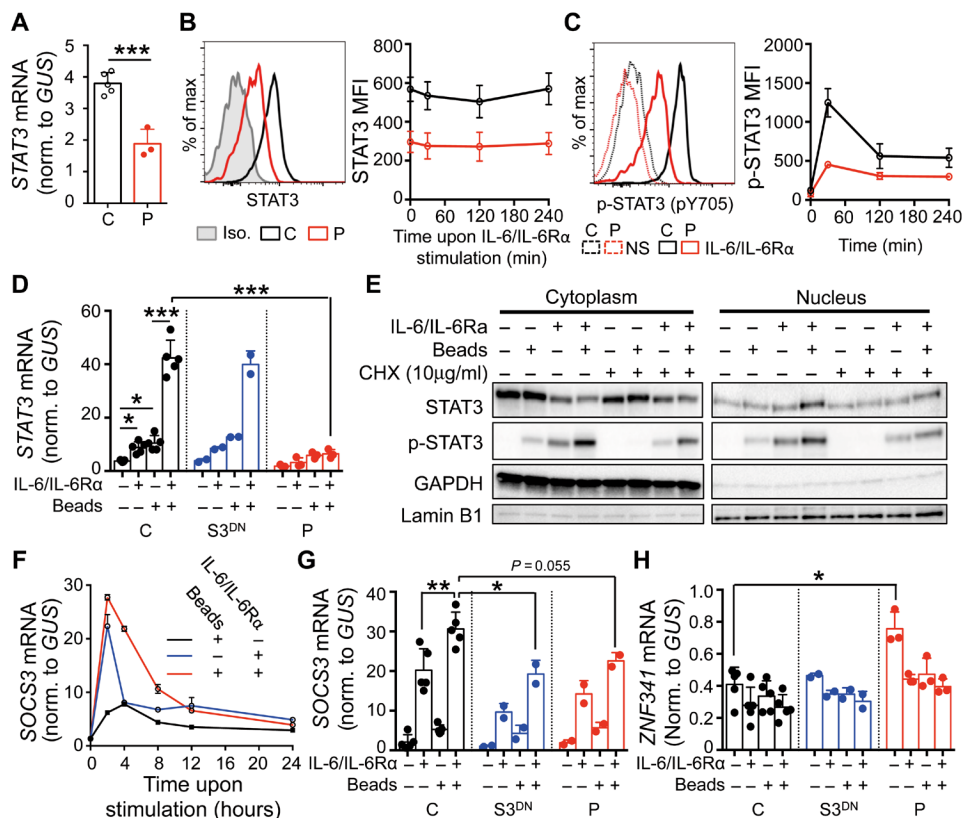
STAT3 autoinduction is impaired in ZNF341-deficient naïve CD4⁺ T cells

We then assessed the impact of ZNF341 deficiency in primary CD4⁺ T lymphocytes. Steady-state endogenous levels of STAT3 mRNA and protein were about 50% lower in the patients' (P2, P3, and P4) naïve CD4⁺ T cells than in those of healthy controls (Fig. 6, A and B). These low levels of STAT3 were associated with lower levels of IL-6/IL-6R α -induced STAT3 phosphorylation (~65%; Fig. 6C). The autoinduction of *Stat3* in mice is observed in T cells, in which it enhances cell proliferation and survival upon CD3 and IL-6 costimulation (37). Furthermore, impaired autoinduction of *Stat3* was reported in mouse protein kinase C- θ (PKC- θ)-deficient naïve CD4⁺ T cells upon T cell receptor (TCR) stimulation with T_H17-polarizing cytokines, disrupting T_H17 cell differentiation (38). We therefore assessed *STAT3* induction in naïve CD4⁺ T cells from controls, STAT3-DN patients, and ZNF341-deficient patients (P4, P5, and P7) upon stimulation with anti-CD2/CD3/CD28 mAb-coated beads (T_H0) with or without IL-6/IL-6R α or T_H17-polarizing cytokines [transforming growth factor- β (TGF- β), IL-1 β , IL-6, IL-21, and IL-23; Fig. 6D and fig. S6A]. Stimulation for 2 or 4 hours with IL-6/IL-6R α , anti-CD2/CD3/CD28 mAb-

coated beads, or T_H17-polarizing cytokines alone induced a transient two- to sixfold increase in *STAT3* mRNA levels, peaking after 2 hours of stimulation in controls (Fig. 6D and fig. S6A). Incubation with anti-CD2/CD3/CD28 mAb-coated beads plus IL-6/IL-6R α or T_H17-polarizing cytokines induced a 10- to 20-fold increase in *STAT3* mRNA levels in naïve CD4⁺ T cells from controls after 2 hours of stimulation, demonstrating a potent synergy between the CD2/CD3/CD28 and IL-6R pathways (Fig. 6D and fig. S6A). By contrast to the four *STAT3*-activating cytokines tested—IL-6 and IL-21 (*STAT3*) and IFN- α and IFN- γ (*STAT1* and *STAT3*)—the IL-2, IL-4, and IL-12 cytokines, which activate *STAT5*, *STAT6*, and *STAT4*, respectively, induced no such synergy (fig. S6B). Naïve CD4⁺ T cells from ZNF341-deficient patients displayed no such synergy after stimulation with anti-CD2/CD3/CD28 mAb-coated beads plus IL-6/IL-6R α or T_H17-polarizing cytokines (Fig. 6D and fig. S6A). Normal synergy was detected with *STAT3*-DN naïve CD4⁺ T cells, suggesting that the residual *STAT3* activity in these cells was sufficient to induce *STAT3* mRNA up-regulation upon costimulation (Fig. 6D and fig. S6A). We compared the transcriptomes of naïve CD4⁺ T cells from four controls and two patients (P4 and P7) on

Fig. 6. Impaired STAT3 production and function in naïve CD4⁺ cells from ZNF341-deficient patients.

(A) *STAT3* mRNA levels, as evaluated by RT-qPCR after RNA extraction from the naïve CD4⁺ T cells of five controls, P4, P5, and P7. Data are displayed as $2^{-\Delta\Delta C_t}$ after normalization relative to *GUS* (endogenous control) expression (ΔC_t). (B) *STAT3* protein levels, as evaluated by flow cytometry, in naïve CD4⁺ T cells. (Left) Representative histogram of *STAT3* levels in P4, one healthy control, and the isotypic control. (Right) Recapitulative graph of the MFI of *STAT3*, as evaluated by flow cytometry, in naïve primary CD4⁺ T cells from five controls, P2, P3, and P4, left unstimulated or after 30, 120, and 240 min of stimulation with IL-6/IL-6R α . (C) Flow cytometry quantification of the phosphorylation of *STAT3* (pY705) in naïve CD4⁺ T cells after stimulation with IL-6/IL-6R α . (Left) Representative flow cytometry histogram for *STAT3* (pY705) in the naïve CD4⁺ T cells of P4 and in those of one healthy control, left unstimulated or after 30 min of stimulation with IL-6/IL-6R α . (Right) Recapitulative graph of the MFI of *STAT3* (pY705) for five controls, P2, P3, and P4 after 0, 30, 120, and 240 min of IL-6/IL-6R α stimulation. Dots and error bars show the means and SDs, respectively. (D) Levels of *STAT3* mRNA in thawed naïve CD4⁺ T cells from healthy individuals ($n = 5$), two *STAT3* DN (*S3*^{DN}) patients, and three ZNF341-deficient patients (P4, P5, and P7) after 2 hours of stimulation with the indicated combinations of IL-6/IL-6R α and beads (anti-CD2/CD3/CD28 mAb-coated beads). Data are displayed as $2^{-\Delta\Delta C_t}$ after normalization relative to *GUS* (endogenous control) expression (ΔC_t). (E) Western blot of cytoplasmic and nuclear protein extracts of naïve CD4⁺ T cells obtained from one healthy control after 4 hours of stimulation with the indicated combinations of IL-6/IL-6R α and beads (anti-CD2/CD3/CD28 mAb-coated beads) in the presence or absence of cycloheximide (CHX) (10 μ g/ml). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and lamin B1 were used as loading controls for the cytoplasmic and nuclear fractions, respectively. Data representative of three independent experiments are shown. (F) Induction of *SOCS3* mRNA in control naïve CD4⁺ T cells after stimulation with IL-6/IL-6R α and/or beads (anti-CD2/CD3/CD28 mAb-coated beads) for 0, 2, 4, 8, 12, and 24 hours. Data are displayed as $2^{-\Delta\Delta C_t}$ after normalization relative to *GUS* (endogenous control) expression (ΔC_t). Dots and error bars represent the mean and SD for two donors. (G and H) Levels of *SOCS3* (G) or *ZNF341* (H) mRNA in thawed naïve CD4⁺ T cells from healthy individuals ($n = 5$), two *STAT3* DN patients (*S3*^{DN}), and two (P4 and P7) or three (P4, P5, and P7) ZNF341-deficient patients after 2 hours of stimulation with the indicated combinations of IL-6/IL-6R α and beads (anti-CD2/CD3/CD28 mAb-coated beads). Data are displayed as $2^{-\Delta\Delta C_t}$ after normalization relative to *GUS* (endogenous control) expression (ΔC_t). Bar graphs and error bars represent the mean and SD.



microarrays after 2 hours of costimulation with anti-CD2/CD3/CD28 mAb-coated beads and IL-6/IL-6R α . Relevant cytokine receptor mRNAs (i.e., *IL2RG*, *IL6R*, *IL6ST*, *IL21R*, and *IL23R*) were present at normal levels in the patients' cells, suggesting that the impairment of STAT3 induction was not due to impaired cytokine receptor expression. Only 3 and 65 transcripts showed a fold change in expression (mean mRNA level of patients/mean mRNA level of controls) of less than 0.4 or more than 2.5, respectively (table S6). Of the three genes underexpressed in patients, *STAT3* displayed the largest decrease in expression in patients (FC = 0.34), demonstrating that the lack of ZNF341 resulted primarily in a decrease in *STAT3* mRNA induction in the patients' cells. Conversely, of the 65 genes less strongly expressed in controls than in patients after costimulation, the largest difference in mRNA levels was observed for *IFNG* (FC = 11.6). This finding is consistent with the higher levels of IFN- γ expression observed in naïve CD4 $^{+}$ T cells from STAT3-DN patients than in control cells, after stimulation with anti-CD2/CD3/CD28 mAb-coated beads and T $_{H}1$ -polarizing cytokines (22). We further investigated *STAT3* autoregulation in naïve CD4 $^{+}$ T cells by assessing the impact of 4 hours of stimulation with anti-CD2/CD3/CD28 mAb-coated beads, IL-6, or both on *STAT3* protein production, phosphorylation, and localization (Fig. 6E). *STAT3* and phosphorylated *STAT3* levels were higher in the nucleus of control naïve CD4 $^{+}$ T cells after 4 hours of costimulation than after stimulation with IL-6/IL-6R α or anti-CD2/CD3/CD28 mAb-coated beads alone. This increase was affected by cycloheximide treatment, suggesting that *STAT3* mRNA autoinduction is associated with the de novo synthesis of *STAT3* protein (Fig. 6E). Moreover, costimulation with anti-CD2/CD3/CD28 mAb-coated beads and IL-6/IL-6R α resulted in long-lasting *SOCS3* mRNA induction in naïve control CD4 $^{+}$ T cells (Fig. 6F). Accordingly, the induction of *SOCS3* mRNA was impaired in ZNF341-deficient patients after 2 and 4 hours of costimulation (Fig. 6G and fig. S6C). Last, we found that basal *ZNF341* mRNA levels were normal in STAT3-DN naïve CD4 $^{+}$ T cells but were high in ZNF341-deficient cells (Fig. 6H), further suggesting that ZNF341 autoregulates its own expression (Fig. 2D). Consistent with this finding, we detected a strong ZNF341-binding site in the first intron of *ZNF341* by ChIP-seq (Fig. 4B). Two hours of IL-6/IL-6R α stimulation in ZNF341-deficient naïve CD4 $^{+}$ T cells restored normal *ZNF341* mRNA levels, suggesting that *STAT3* may also regulate *ZNF341*

expression (Fig. 6H). Overall, these data show that ZNF341 is required for baseline *STAT3* production, autoinduction, activation, and sustained activity in primary human T cells.

T $_{H}17$ development is impaired in ZNF341-deficient patients

We investigated the mechanism underlying CMC in ZNF341-deficient patients by analyzing the development of IL-17 $^{+}$ CD4 $^{+}$ T cells. Inborn errors of IL-17 immunity underlie all known forms of isolated or syndromic CMC (21, 39–45), including HIES due to DN *STAT3* mutations (14, 20, 46, 47). The proportions of T $_{H}$ subsets among the circulating memory CD4 $^{+}$ T cells of ZNF341-deficient patients were

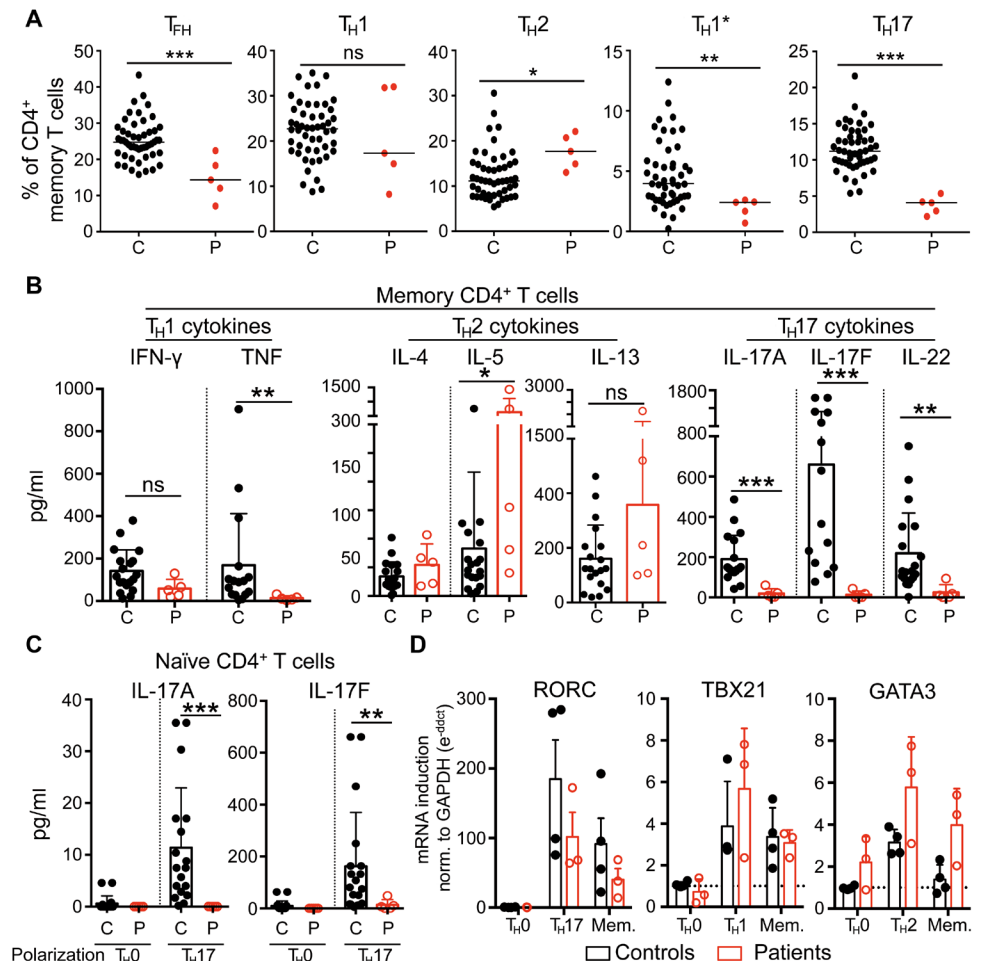


Fig. 7. Impaired T $_{H}17$ differentiation in ZNF341-deficient patients. (A) Frequency of T $_{H}$ subsets within the CD4 $^{+}$ memory compartments of controls (n = 49) and patients (n = 5). Subsets were defined as follows: T $_{FH}$ (CXCR5 $^{+}$), T $_{H}1$ (CXCR5 $^{+}$ CXCR3 $^{+}$ CCR4 $^{+}$ CCR6 $^{-}$), T $_{H}2$ (CXCR5 $^{+}$ CXCR3 $^{+}$ CCR4 $^{+}$ CCR6 $^{-}$), T $_{H}1^{*}$ (CXCR5 $^{+}$ CXCR3 $^{+}$ CCR4 $^{+}$ CCR6 $^{+}$), and T $_{H}17$ (CXCR5 $^{+}$ CXCR3 $^{+}$ CCR4 $^{+}$ CCR6 $^{+}$). Mann-Whitney tests were used for comparisons. (B) Secretion (pg/ml) of T $_{H}1$ (IFN- γ and TNF), T $_{H}2$ (IL-4, IL-5, and IL-13), and T $_{H}17$ (IL-17A, IL-17F, and IL-22) cytokines by memory CD4 $^{+}$ T cells after 5 days of culture under T $_{H}0$ conditions (anti-CD2/CD3/CD28 mAb-coated beads). Mann-Whitney tests were used for comparisons. (C) Secretion (pg/ml) of T $_{H}17$ (IL-17A and IL-17F) cytokines by naïve CD4 $^{+}$ T cells after 5 days of culture under T $_{H}0$ -polarizing conditions (anti-CD2/CD3/CD28 mAb-coated beads) or T $_{H}17$ -polarizing conditions (anti-CD2/CD3/CD28 mAb-coated beads together with IL-1 β , IL-6, IL-21, IL-23, and TGF- β). Mann-Whitney tests were used for comparisons. (D) Expression of *RORC*, *TBX21*, and *GATA3* by naïve CD4 $^{+}$ T cells after 5 days of culture under T $_{H}0$ -, T $_{H}17$ -, or T $_{H}1$ -polarizing conditions (anti-CD2/CD3/CD28 mAb-coated beads together with IL-12) or T $_{H}2$ -polarizing conditions (anti-CD2/CD3/CD28 mAb-coated beads together with IL-4) or by memory (Mem.) CD4 $^{+}$ T cells, as determined by RT-qPCR, relative to *GAPDH*. Results are shown for four controls and three ZNF341-deficient patients (P2 to P4).

normal for T_H1 cells, high for T_H2 cells, and low for T_{FH} , T_H1^* , and T_H17 cells, as shown by the expression of specific surface markers (Fig. 7A), as in STAT3 DN patients (48, 49). We stimulated memory $CD4^+$ T cells with anti-CD2/CD3/CD28 mAb-coated beads (T_H0) for 5 days and measured cytokine production by enzyme-linked immunosorbent assay (ELISA), cytometric bead array, and intracellular staining methods (Fig. 7B and fig. S7, A to C). The production of T_H1 cytokines (TNF and IFN- γ) was low to normal (Fig. 7B and fig. S7A) and that of IL-6, IL-10, and IL-21 was normal (fig. S7, B and C), whereas that of T_H2 cytokines (IL-4, IL-5, and IL-13) was normal or higher than normal (Fig. 7B and fig. S7A). In marked contrast, the production of T_H17 cytokines (IL-17A, IL-17F, and IL-22) by ZNF341-deficient memory $CD4^+$ T cells was much weaker than that by control cells (Fig. 7B and fig. S7A). We then purified naïve $CD4^+$ T cells from the patients and cultured them in vitro under T_H1 - or T_H17 -polarizing conditions. T_H1 polarization induced normal and high levels of TNF and IFN- γ , respectively, in the patients' cells (fig. S7D), consistent with the microarray data (table S6) and similar to the results obtained from STAT3-DN patients (22). By contrast, T_H17 polarization did not lead to the production of detectable amounts of IL-17A and IL-17F (Fig. 7C). Accordingly, *RORC* expression tended to be weaker, although this difference was not statistically significant, in memory $CD4^+$ T cells and naïve $CD4^+$ T cells from patients cultured for 5 days under T_H17 conditions in vitro (Fig. 7D). These data are consistent with the ChIP-seq data for T cells, in which no ZNF341-binding site was detected in the *RORC* gene. By contrast, the expression of *TBX21* and *GATA3* was normal or slightly higher than normal in memory $CD4^+$ T cells and naïve $CD4^+$ T cells from patients cultured under T_H1 - and T_H2 -polarizing conditions, respectively (Fig. 7D). Mutations of *ZNF341* may have had a global effect on T cell responses, impairing not only responses elicited through cytokine receptors but also those elicited through the TCR. We tested this hypothesis by measuring the calcium flux in naïve $CD4^+$ T cells induced by TCR engagement (fig. S7E). The kinetics and magnitude of the TCR-mediated activation of naïve $CD4^+$ T cells from two ZNF341-deficient patients were similar to those of corresponding cells from healthy donors, suggesting that TCR signaling was intact in the absence of ZNF341. Furthermore, ZNF341-deficient naïve and memory $CD4^+$ T cells underwent rounds of normal proliferation in vitro in response to T_H0 or polarizing conditions (fig. S7, F and G). The IL-17 phenotype observed in ZNF341-deficient patients was not, therefore, due to impaired cell division and TCR-induced calcium signaling.

Patients' T_H cells show an abnormal transcriptome profile

The patients' $CD4^+$ T cells were further studied globally by gene expression analysis (fig. S7H). Consistent with the skewing toward T_H2 cells, ZNF341-deficient memory $CD4^+$ T cells had higher levels of mRNAs encoding not only *GATA3*, IL-4, IL-5, and IL-13 but also IL-5R, IL-31 (produced by T_H2 cells), and IL-18BP (inhibiting IL-18-induced IFN- γ production, thereby relieving the inhibitory effect of IFN- γ on T_H2 responses). ZNF341-deficient memory $CD4^+$ T cells continued to produce large amounts of *GATA3*, *IL4*, *IL13*, and *IL31* under T_H17 -polarizing conditions. Moreover, ZNF341-deficient memory $CD4^+$ T cells had lower basal levels of *IL17A*, *IL17F*, *IL22*, and *CCR6* expression under T_H0 conditions than control memory $CD4^+$ T cells, and the expression of *IL17A*, *IL17F*, and *CCR6* was not increased by culturing ZNF341-deficient memory $CD4^+$ T cells under T_H17 conditions (fig. S7H). Impaired expression was also observed

for other genes typically associated with T_H17 cells, including *RORA* (encoding ROR- α); *IL26* and *CCL20* (both expressed by human T_H17 cells); and *IL23A*, *IL1A*, *IL1R1*, *IL1R2*, *IL6R*, and *IL23R* (which encode proteins through which IL-1 β , IL-6, and IL-23 signal to induce T_H17 cells). The increase in *RORC*, *CCL20*, *IL1A*, *IL1R1*, *IL1R2*, and *IL23A* expression observed in normal memory $CD4^+$ T cells after culture in T_H17 -polarizing conditions was abolished by *ZNF341* mutations, highlighting the key role of this transcription factor in T_H17 differentiation. Consistently, total RNA sequencing in isolated $CD3^+$ T cells from P4 and a control after 24 and 48 hours of stimulation with anti-CD3 and anti-CD28 mAbs revealed that 6 of the 10 genes for which the most significant decreases in expression were recorded in patients were key markers of IL-17 immunity (*IL17F*, *IL26*, *RORC*, and *CCR6*; table S7). In summary, ZNF341-deficient $CD4^+$ T cells can differentiate in vivo and in vitro into some types of effector cells, but their ability to generate T_H17 cells is selectively and severely impaired. As in patients with DN *STAT3* mutations, this defect accounts for the CMC observed in the patients, as fibroblasts and keratinocytes from P2 to P4 responded normally to IL-17A (fig. S7, I to K) (21, 43–45). The lack of ZNF341 prevents T_H cells from producing sufficient amounts of functional STAT3, and thereby of ROR- γ /ROR- γ T, during T_H17 development (14, 41).

Allergy and hyper-IgE are associated with enhanced T_H2 responses

Unlike the CMC observed in patients and our findings for IL-17, the pathogenesis of severe allergy and hyper-IgE in these patients is more difficult to decipher, because monogenic causes of isolated allergy and hyper-IgE affecting specific cytokine signaling pathways are only just beginning to emerge (50, 51). However, the microarray analysis of $CD4^+$ T cells was instructive, revealing aberrant expression of potential mediators of inflammation in ZNF341-deficient patients (fig. S7H). We detected high levels of expression of the inflammatory chemokine genes *CCL1*, *CCL3*, and *CCL5* and the inflammatory chemokine receptor gene *CXCR3*, which is coexpressed by T_H1 cells. This finding is consistent with the detection of high levels of expression for *IFNG* and *EOMES*, encoding a transcription factor required for the generation of pathogenic proinflammatory IFN- γ -producing $CD4^+$ T cells. The persistently higher levels of expression of many of these inflammatory or T_H2 -type genes in ZNF341-deficient than in control memory $CD4^+$ T cells under conditions of T_H17 polarization also highlight the role of ZNF341 in repressing the expression of gene signatures characteristic of alternative fates of effector $CD4^+$ T cells. The patients thus displayed an enhanced T_H2 bias in multiple assays (Fig. 7B and fig. S7A), with a higher frequency of T_H2 cells and higher levels of *GATA3* mRNA in memory $CD4^+$ T cells (Fig. 7, A and D). Last, we tested the hypothesis that the patients' $CD8^+$ T cells also contributed to their allergic phenotype (52, 53). Genome-wide transcriptome analysis showed that up to 12 mRNAs were up-regulated in the patients' $CD8^+$ HVS-T cells, to levels at least 2.5 times those in $CD8^+$ HVS-T cells from healthy controls (fig. S7L). Two of these genes encoded cytokines known to play a key role in the allergy process: IL-5, which drives the development of eosinophils (54), and IL-9, which drives the development of basophils (55). These data suggest that the allergic features of ZNF341-deficient patients were due to the enhanced production of some, but not all, T_H2 -like cytokines, including IL-5 and IL-9, in particular, by both $CD4^+$ and $CD8^+$ T cells. These T cell abnormalities are similar to those seen in HIES patients with DN mutations of *STAT3* (22, 49).

DISCUSSION

We report ZNF341 deficiency in eight patients from six unrelated kindreds displaying an AR form of HIES, with stronger inflammatory responses and fewer nonhematopoietic manifestations than reported for AD-HIES caused by DN *STAT3* mutations. This experiment of nature indicates that human ZNF341 is required for protective mucocutaneous immunity to *Candida albicans*. The impaired development of IL-17⁺ CD4⁺ T cells accounts for the development of CMC in these patients (7, 14, 20, 39–42, 46, 47). The cellular mechanisms by which ZNF341 regulates allergy are less clear, although several T_H2 cytokines (e.g., IL-5 and IL-9) are produced in excess by the patients' T cells. The molecular mechanism by which ZNF341 operates in T cells involves STAT3. No haploinsufficiency has been observed at the human *STAT3* locus; the HIES-causing *STAT3* mutations studied to date are all DN (6, 56, 57). The mechanism by which ZNF341 deficiency is pathogenic in T cells is based on a 50% decrease in constitutive levels of STAT3 and a >60% decrease in levels of STAT3 phosphorylation upon stimulation with STAT3-activating cytokines. ZNF341 deficiency prevents the induction of *STAT3* transcription by cytokines that activate *STAT3* itself (autoinduction). Together, low baseline *STAT3* mRNA and protein levels, and the impaired autoinduction of *STAT3* itself, result in lower levels of *STAT3* activation (phosphorylation) and transcriptional activity, as low as those in patients with DN *STAT3* mutations. Consequently, *STAT3*-dependent genes, such as *RORC*, *IL17A*, and *IL17F*, are poorly induced. Consistent with these findings, the overexpression of a hyperactive form of *STAT3* in naïve mouse CD4⁺ T cells and *STAT3* gain-of-function mutations in humans were shown to increase T_H17 cell differentiation (58, 59). The same mechanism probably operates in B cells, accounting for the B cell phenotypes of ZNF341-deficient patients being identical to those of HIES patients with DN *STAT3* mutations (5, 18). Given that patients with the various forms of inherited *STAT1* deficiency suffer from mycobacterial and/or viral diseases (60) and that individuals heterozygous for loss-of-function *STAT1* alleles that are not DN are asymptomatic, the lower levels of *STAT1* observed in ZNF341-deficient patients are unlikely to account for or even contribute to their immunological or clinical phenotypes.

Patients with ZNF341 deficiency appear to develop fewer and milder nonhematopoietic developmental phenotypes than those with *STAT3* DN mutations. A possible explanation for this is that IL-11R, LIFR, IL-6ST, and similar receptors in cells responsible for the tooth, skeletal, joint, and vascular phenotypes of AD-HIES patients may not necessarily require as much *STAT3* to function properly as the cytokine receptors tested in primary fibroblasts (10–12). However, these patients do develop such somatic features, consistent with the *STAT3* phenotype detected in the fibroblasts of ZNF341-deficient patients. One of the more distinctive features of ZNF341 deficiency is the somewhat stronger inflammatory response in the course of infection. We detected ZNF341 in all leukocytes tested, including basophils, monocytes, and dendritic cells. However, the expression of this protein in monocytes seems to be functionally redundant for *STAT3* activation, in contrast to the situation in *STAT3*-DN patients, possibly explaining the normal biological and clinical inflammation seen in ZNF341-deficient patients. Conversely, the NK lymphopenia seen in ZNF341-deficient but not *STAT3*-DN patients suggests that ZNF341 may be essential for at least one *STAT3*-independent function. However, an alternative interpretation is that *STAT3* activation is more profoundly impaired in NK progenitors lacking ZNF341 than in those carrying a DN *STAT3* mutation. Further studies will be required to

compare the expression patterns of ZNF341 and *STAT3* proteins across human tissues and cell types. Studies of *Zfp341*, the mouse ortholog of human *ZNF341*, may also facilitate analysis of the *STAT3*-dependent and *STAT3*-independent roles of this transcription factor in different cell types.

The identification of ZNF341 highlights the power of human genetics for discovering previously unknown biochemical and immunological pathways of biological and medical relevance (61–63) and defining the level of redundancy of human genes for host defense in natural conditions (64). Much is already known about the structure and function of *STAT* proteins, but this study reveals the importance of the transcriptional regulation of *STAT* genes (65–68). Previous studies had hinted at the importance of *STAT* transcriptional regulation (36–38). Unlike *STAT3*, which resides in the cytosol and only accumulates in the nucleus upon activation, ZNF341 is constitutively expressed in the nucleus, where it is required for optimal baseline and inducible *STAT3* transcription, and particularly for autoinduction, the lack of which prevents the production of sufficient amounts of *STAT3* protein and the sustained activity of this protein during cell stimulation. Further studies are required to identify potential partners of ZNF341 during *STAT3* autoinduction. In T and B cells, we have further shown that ZNF341 protein deficiency is associated with higher levels of ZNF341 mRNA. Both ZNF341 and *STAT3* are potent inducers of *STAT3* transcription, and they may act in concert to decrease ZNF341 transcription. This would prevent excessive *STAT3*-dependent responses that would be deleterious to the host, as observed in patients with heterozygous gain-of-function mutations of *STAT3* (69–71).

MATERIALS AND METHODS

Study design

We studied eight patients from five kindreds suffering from HIES. Using WES, we found that all living patients carry homozygous truncating mutation in the *ZNF341* gene. No predicted homozygous loss-of-function mutation of *ZNF341* has been described in public databases. ZNF341 function is unknown in mouse and human. The main objective of this study was to characterize in-depth ZNF341 expression and function in human, and the molecular, cellular, and immunological impacts of its disruption in human.

Case reports

Detailed case reports of all eight patients can be found in the Supplementary Materials.

Experimental methods

Information about reagents used and experimental procedures can be found in the Supplementary Materials.

Statistics

Two-tailed Mann-Whitney tests or unpaired *t* tests were used for single comparisons of independent groups. Paired *t* tests were used for single comparisons of paired groups. In the relevant figures, “ns” indicates “not significant.” ****P* < 0.001, ***P* < 0.01, and **P* < 0.05. Analyses were performed with GraphPad software.

SUPPLEMENTARY MATERIALS

immunology.sciencemag.org/cgi/content/full/3/24/eaat4956/DC1

Materials and Methods

Fig. S1. Clinical features, histology, and genetics.

Fig. S2. Molecular characterization of *ZNF341* mutations and endogenous expression.

Fig. S3. Myeloid immunophenotyping.

Fig. S4. ChIP-seq analysis, DNA binding site mutagenesis, luciferase assays, and STAT1 and STAT3 production and function in cell lines derived from the patients' cells.

Fig. S5. STAT3 levels in primary fibroblasts, NK cells, and B cells.

Fig. S6. STAT3 mRNA induction in naïve CD4⁺ T cells.

Fig. S7. Patients' T cell function evaluation and SV40-fibroblast and keratinocyte responses to IL-17 cytokines.

Table S1. Clinical summary of *ZNF341*-deficient patients.

Table S2. Biological parameters of *ZNF341*-deficient patients.

Table S3. Rare coding variants of P2/P3 (top) and P4 (bottom) within the linkage regions of kindreds A and B.

Table S4. RNA-seq data obtained from P4 EBV-B cells transduced with *ZNF341* WT isoform 1 or an empty vector.

Table S5. RNA-seq data obtained from P4 EBV-B cells transduced with *ZNF341* WT isoform 2 or an empty vector.

Table S6. Microarray data obtained from sorted naïve CD4⁺ T cells.

Table S7. RNA-seq data obtained from freshly isolated CD3⁺ T cells.

Table S8. DNA sequences used for luciferase reporter plasmids and as fluorescent probes and biotinylated probes.

Table S9. ChIP-seq and RNA-seq quality control information.

Data file S1

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M.L.-V., C.P., A.H., A.S., A.O., N.R., and T.A.C. provided material from the patients and reagents. J.-X.L., P.L., N.D., and W.J.L. performed and analyzed the ChIP-seq. B.G., J.B., B.B., I.M., J.P.D., L.A., W.J.L., and S.G.T. provided expertise and feedback. J.-L.C., A.P., and V.B. secured funding. All the authors critically reviewed the manuscript. **Competing interests:** J.P.D. is a founder of and shareholder in AXENIS (Paris, France). The other authors declare that they have no competing interests. **Data and materials availability:** The RNA-seq and ChIP-seq data are available at the Gene Expression Omnibus (GEO) under the deposit number GSE113194. The microarray data are available on GEO under the deposit numbers GSE113889 and GSE113945, respectively.

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A recessive form of hyper-IgE syndrome by disruption of ZNF341-dependent STAT3 transcription and activity

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Fingers on the trigger

Hyper-immunoglobulin E syndromes (HIESs) are rare genetic immunodeficiency diseases characterized by bacterial infections, chronic mucocutaneous candidiasis, allergies, and skeletal abnormalities that are associated with excessive T_H2 responses and impaired T_H17 immunity. Béziat *et al.* and Frey-Jakobs *et al.* have studied patients with an autosomal recessive form of HIES and identified mutations in the zinc finger transcription factor ZNF341 as the culprit. Loss-of-function mutations encoding truncated forms of ZNF341 interfered with its ability to recognize a bipartite binding site located in the promoter of STAT3, the transcription factor mutated in most cases of autosomal dominant HIES. ZNF341-supported transcription of STAT3 is a key upstream regulatory step needed to trigger the normal induction of the T_H17 differentiation pathway. These findings reveal a previously unappreciated layer of transcriptional regulation controlling JAK-STAT signaling.

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