Figure Legends

Fig 1. Characterization of the *JAK3* mutation. (A) Pedigree. **(B)** Immunoblot of lysates from BLCLs derived from patients and controls (left). JAK3 expression relative to controls (right). ***p<0.001, ns=not significant (one-way ANOVA). **(C)** Percentage of $pSTAT5^+$ BLCLs after stimulation for 10 minutes with IL-2 or IL-15. ****p<0.0001 (two-way ANOVA). **(D)** Sanger sequencing of c.2652C>T variant. **(E)** RT-PCR amplification of *JAK3* mRNA encompassing the patient mutation site. **(F)** Diagram of mRNA splice variants induced by mutation. For B and C n=2 for controls, n=2 for patients; data pooled from 2 independent experiments. Columns/lines and bars represent means and SEM.

Extended Figure Legends

Fig E1. Rash on forearm and hand of Patient 2.

Fig E2. H&E staining of biopsy of a skin lesion from Patient 2 at 10x (A) and 20x (B) magnification, showing granulomatous infiltrate. Scale bars = 200 μm.

Fig E3: Quantitative analysis of the TCR Vβ repertoire of T lymphocytes from patient 2. PBMCs were labeled using the IOTest Beta Mark TCR V beta repertoire kit. Analysis of $CD4^+$ and $CD8^+$ T cells was performed by flow cytometry.

Table E1. *In silico* splicing prediction scores for JAK3 c.2652C>T:pV884V

	WT allele C	Mutant allele T
1. MaxEntScan score	-5.24	2.51
2. Human Splicing Finder score	39.6	66.67

1. MaxEntScan: http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html

Score range -20 to +20. A higher score indicates a greater potential for splice site. +3 is the threshold for a likely splice site.

2. Human Splicing Finder: http://www.umd.be/HSF3/index.html

Score range 0–100. A higher score indicates a greater potential for splice site. 65 is the threshold for a likely splice site. If the WT score is under the threshold and the score variation is above +10%, the mutation is likely to create a new splice site. In this case, the difference is +67%, supporting the presence of new donor splice site.

Table E2. Summary of clinical history and treatment outcomes for Patient 1, Patient 2 and their siblings.

Family Member	Sex	Genotype	Presenting History	Treatment Outcome
Patient 1	Female	c.2652C>T homozygous	$T^-B^+NK^{low}$ SCID detected by flow cytometry immediately after birth given clinical history of Affected Sister (see below).	Underwent hematopoietic stem cell transplantation (HSCT) with a 9/10 HLA-A mismatched unrelated donor after myeloablative conditioning. Post transplant course was complicated by severe gut GVHD, which was well controlled by pulse dose steroids. She is currently alive and well.
Patient 2	Male	c.2652C>T homozygous	Granulomatous skin disease starting at 2 years of age.	Currently undergoing evaluation for HSCT. Continues to have no history of recurrent or opportunistic infections. He has been started on trimethoprim/sulfamethoxazole and IVIG.
Healthy Brother	Male	c.2652C>T heterozygous	No significant past medical history.	n/a
Affected Sister	Female	Presumed c.2652C>T homozygous	Hospitalized with pneumonia and disseminated BCG at 6 months of age.	Died at 19 months of age from complications of HSCT.

Supplemental Methods:

Immunoblotting. Cell lysates were immunoblotted using a mAb raised against the N-terminus of JAK3 (clone D7B12, Cell Signaling) or anti-actin (clone ACTN05 (C4), Abcam) followed by HRP-conjugated goat anti–rabbit antibody or goat anti–mouse antibody conjugated to horseradish peroxidase. Densitometry was performed using ImageJ (NIH). JAK3:actin ratios were calculated and normalized to the JAK3:actin ratios of control EBV lines from healthy donors.

STAT5 Phosphorylation. PBMCs from Patient 1, Patient 2 and two healthy controls were infected with EBV in the presence of cyclosporine A to generate BLCLs. BLCLs were stimulated with the indicated concentrations of human IL-2 (Peprotech) or IL-15 (Peprotech) for 10 min at 37^{o}C, before fixation using Lyse/Fix Phosflow buffer (BD Biosciences) and permeabilization using BD PhosflowTM Perm Buffer III according to the manufacturer's instructions. Cells were stained with anti-phospho-STAT5 (Y694-PE) (clone SRBCZX, eBiosciences) and analyzed using a LSRFortessa (BD Biosciences).

Semi-quantitative RT-PCR. Total RNA was prepared from PBMCs cells from Patient 1 or a healthy control using the RNeasy kit (Qiagen). cDNA synthesis and amplification were performed from total RNA using the Superscript III One Step RT-PCR System with Platinum Taq DNA polymerase. Primers used for *JAK3* were: forward primer 5'- GACTTTCAGCGGGAGATTCA-3' (exon 19), and reverse primer 5'- TACTCCATGC CCTTGCAGAT-3' (spans exon 19 and 20).

Figure E1

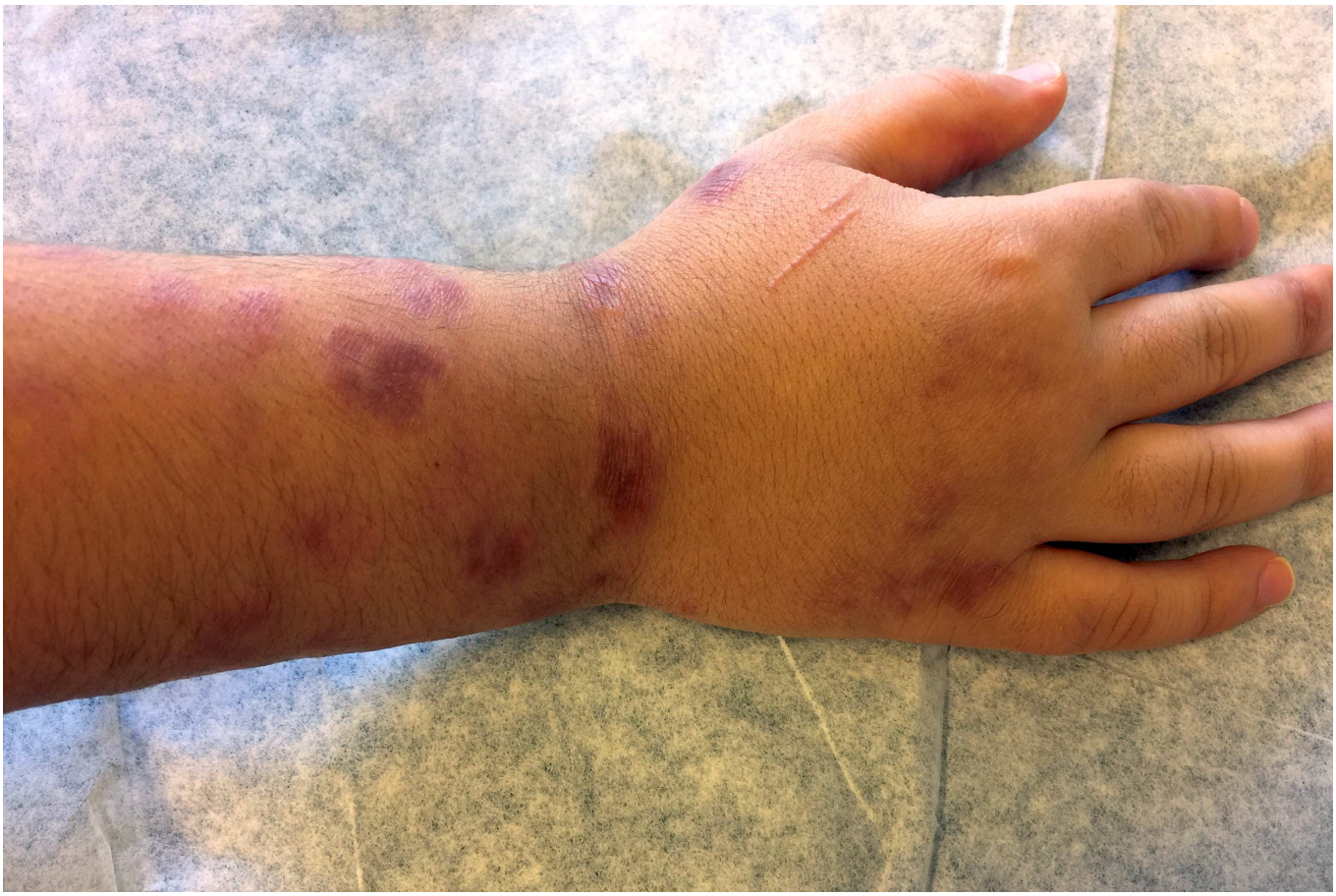
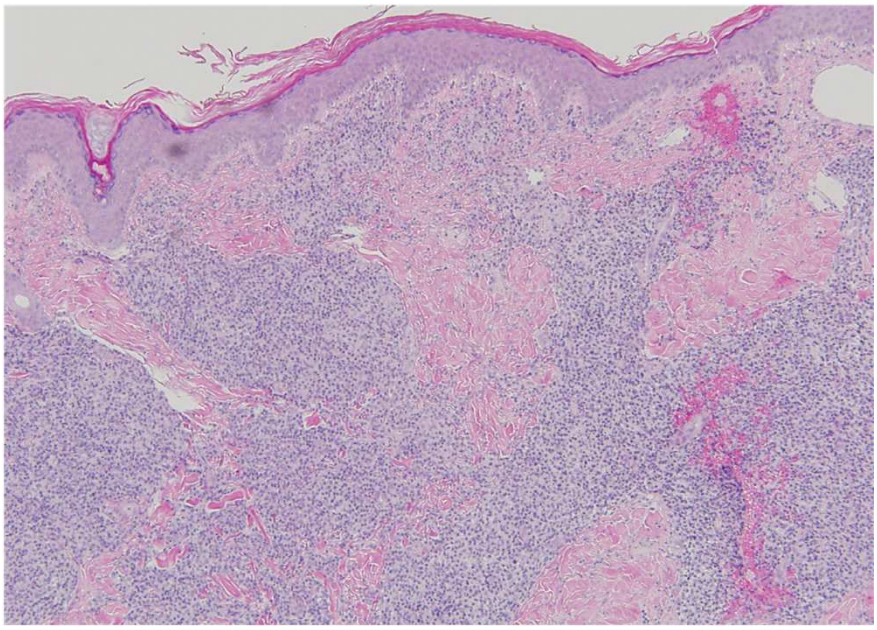


Figure E2

A



B

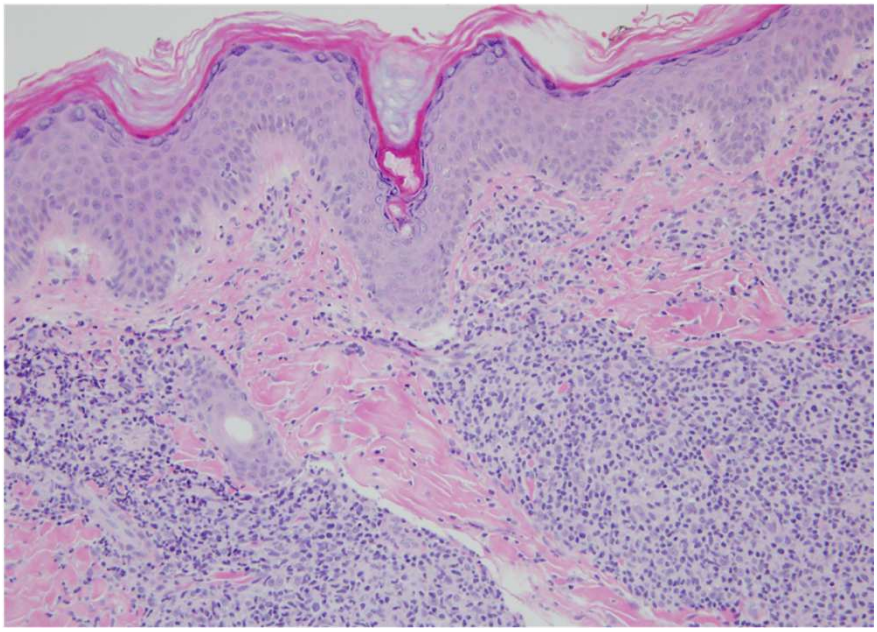
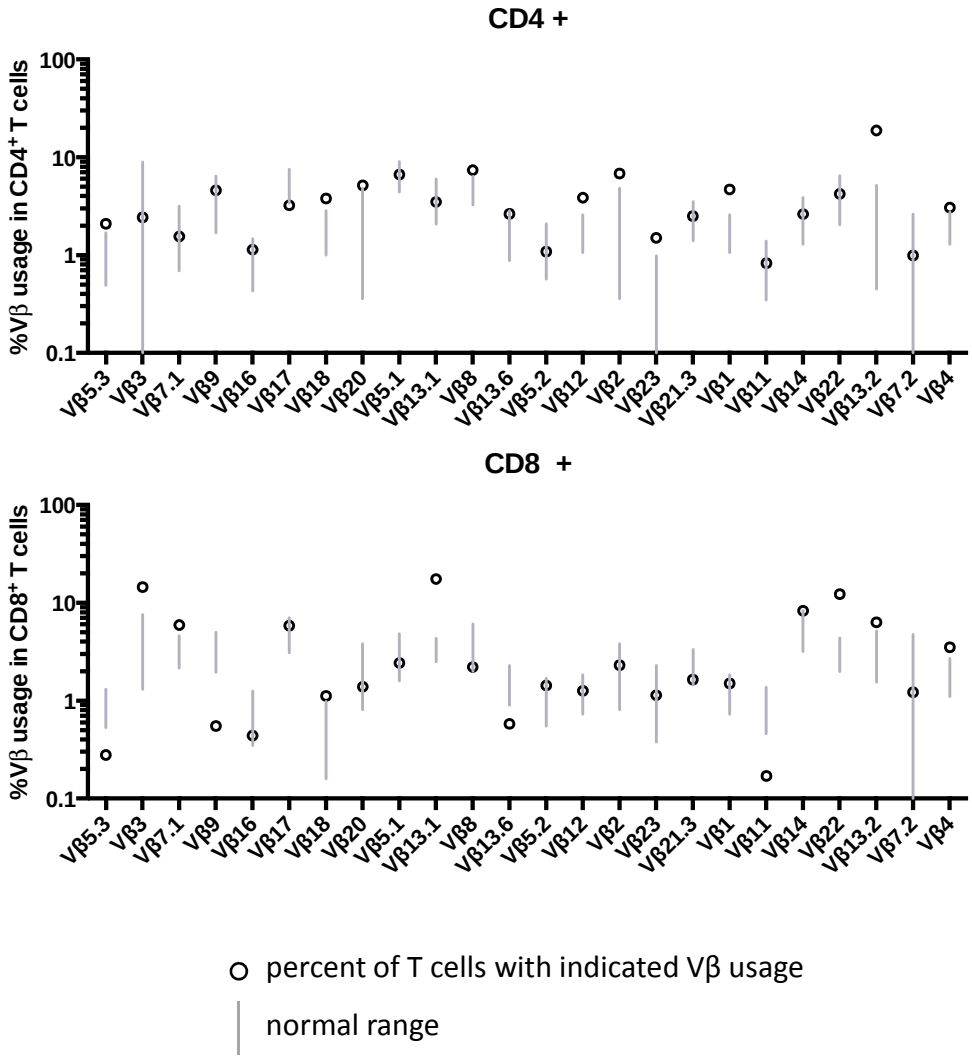


Figure E3


CD4 +
%Vβ usage in CD4+ T cells
100
10
1
0.1
Vβ5.3
Vβ3
Vβ7.1
Vβ9
Vβ16
Vβ17
Vβ18
Vβ20
Vβ5.1
Vβ13.1
Vβ8
Vβ13.6
Vβ5.2
Vβ12
Vβ2
Vβ23
Vβ21.3
Vβ1
Vβ11
Vβ14
Vβ22
Vβ13.2
Vβ7.2
Vβ4
CD8 +
%Vβ usage in CD8+ T cells
100
10
1
0.1
Vβ5.3
Vβ3
Vβ7.1
Vβ9
Vβ16
Vβ17
Vβ18
Vβ20
Vβ5.1
Vβ13.1
Vβ8
Vβ13.6
Vβ5.2
Vβ12
Vβ2
Vβ23
Vβ21.3
Vβ1
Vβ11
Vβ14
Vβ22
Vβ13.2
Vβ7.2
Vβ4
percent of T cells with indicated Vβ usage
normal range