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# Human T-bet governs innate and innate-like adaptive IFN- $\gamma$ immunity against mycobacteria

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## Summary

Inborn errors of human IFN- $\gamma$  immunity underlie mycobacterial disease. We report a patient with mycobacterial disease due to inherited deficiency of the transcription factor T-bet. The patient has extremely low counts of circulating *Mycobacterium*-reactive NK, invariant NKT (iNKT), mucosal-associated invariant T (MAIT), and V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T lymphocytes, and of *Mycobacterium*-non reactive classic T<sub>H</sub>1 lymphocytes, with the residual populations of these cells also producing abnormally small amounts of IFN- $\gamma$ . Other lymphocyte subsets develop normally, but produce low levels of IFN- $\gamma$ , with the exception of CD8<sup>+</sup>  $\alpha\beta$  T and non-classic CD4<sup>+</sup>  $\alpha\beta$  T<sub>H</sub>1\* lymphocytes, which produce IFN- $\gamma$  normally in response to mycobacterial antigens. Human T-bet deficiency thus underlies mycobacterial disease by preventing the development of innate (NK) and innate-like adaptive lymphocytes (iNKT, MAIT, and V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells) and IFN- $\gamma$  production by them, with mycobacterium-specific, IFN- $\gamma$ -producing, purely adaptive CD8<sup>+</sup>  $\alpha\beta$  T and CD4<sup>+</sup>  $\alpha\beta$  T<sub>H</sub>1\* cells unable to compensate for this deficit.

## Introduction

In the course of primary infection, life-threatening disease in otherwise healthy children, adolescents, and even adults, can result from monogenic inborn errors of immunity (IEI), which display genetic heterogeneity and physiological homogeneity (Casanova, 2015a, 2015b). Mendelian susceptibility to mycobacterial disease (MSMD) is characterized by a selective, inherited predisposition to clinical disease caused by weakly virulent mycobacteria, such as *Mycobacterium bovis* Bacille Calmette-Guérin (BCG) vaccines and environmental mycobacteria (EM) (Bustamante, 2020; Kerner et al., 2020). Patients are also vulnerable to *bona fide* tuberculosis. Patients with typical, “isolated” MSMD are rarely prone to other infectious agents, with the exception of *Salmonella* and occasionally other intra-macrophagic bacteria, fungi, and parasites. Patients with atypical, “syndromic” MSMD often display other clinical phenotypes, infectious or otherwise. MSMD, both “isolated” and “syndromic”, displays a high level of genetic heterogeneity, with causal mutations in 16 genes, and additional allelic heterogeneity, resulting in 31 different disorders. However, there is also physiological homogeneity, as all known genetic causes of MSMD affect interferon gamma (IFN- $\gamma$ )-dependent immunity (Bustamante, 2020; Kerner et al., 2020; Rosain et al., 2019). Mutations of *IFNG*, *IL12B*, *IL12RB1*, *IL12RB2*, *IL23R*, *TYK2*, *ISG15*, *RORC*, *IKBKG* (NEMO), *IRF8*, and *SPPL2A* impede IFN- $\gamma$  production by innate and adaptive immune cells, whereas mutations of *IFNGR1*, *IFNGR2*, *STAT1*, *JAK1*, and *CYBB* impair cellular responses to IFN- $\gamma$  (**Fig. S1A**). The clinical penetrance and severity of MSMD depend strongly on genetic etiology and increase with decreasing levels of IFN- $\gamma$  activity (Dupuis et al., 2000). Seven etiologies also result in an impairment of immunological circuits other than the IFN- $\gamma$  circuit, accounting for “syndromic” MSMD in the corresponding patients (Bustamante, 2020). Collectively, these studies revealed the crucial role

1 of human IFN- $\gamma$  in antimycobacterial immunity and its redundancy for immunity against many  
2 other pathogens.

3         The cellular basis of MSMD in patients with impaired responses to IFN- $\gamma$  involves  
4 mononuclear phagocytes. The ability of these cells to contain the ingested mycobacteria depends  
5 on their activation by IFN- $\gamma$  (Nathan et al., 1983). The cellular basis of MSMD in patients with  
6 impaired IFN- $\gamma$  production is poorly understood, as most types of lymphocytes can produce IFN-  
7  $\gamma$  (Schoenborn and Wilson, 2007). Some genetic etiologies of MSMD affect some lymphocyte  
8 subsets more than others. ISG15 deficiency preferentially impairs the production of IFN- $\gamma$  by NK  
9 cells (Bogunovic et al., 2012; Zhang et al., 2015). IL-12R $\beta$ 2 deficiency preferentially impairs the  
10 production of IFN- $\gamma$  by NK, B,  $\gamma\delta$  T, classic  $\alpha\beta$  T cells, type 1 innate lymphoid cells (ILC1s),  
11 and ILC2s, whereas IL-23R deficiency preferentially impairs the production of this cytokine by  
12 invariant NKT (iNKT) and mucosal-associated invariant T (MAIT) cells, and both IL-12R $\beta$ 1 and  
13 TYK2 deficiencies impair both the IL-12- and IL-23-dependent subsets (Boisson-Dupuis et al.,  
14 2018; Martínez-Barricarte et al., 2018). SPPL2a and IRF8 deficiencies selectively impair the  
15 production of IFN- $\gamma$  by CD4<sup>+</sup> CCR6<sup>+</sup> T<sub>H</sub>1 (T<sub>H</sub>1\*) cells (Hambleton et al., 2011; Kong et al.,  
16 2018), a T<sub>H</sub>1 cell subset enriched in *Mycobacterium*-specific effector cells, whereas CCR6<sup>-</sup> T<sub>H</sub>1  
17 cells do not respond to mycobacteria (Acosta-Rodriguez et al., 2007). ROR $\gamma$ /ROR $\gamma$ T deficiency  
18 impairs the development of iNKT and MAIT cells, and also compromises the production of IFN-  
19  $\gamma$  by  $\gamma\delta$  T and  $\alpha\beta$  T<sub>H</sub>1\* cells (Okada et al., 2015). Interestingly, although the lack of both  $\alpha\beta$  T  
20 and  $\gamma\delta$  T cells in patients with severe combined immunodeficiency (SCID) underlies BCG  
21 disease (Casanova et al., 1995), most, if not all other deficits of antigen-specific  $\alpha\beta$  T-cell  
22 responses, whether affecting only CD4<sup>+</sup> or CD8<sup>+</sup> T cells, such as MHC class II and MHC class I  
23 deficiencies, respectively, typically do not (Aluri et al., 2018; Ardeniz et al., 2015; Dimitrova et

1 al., 2014; Elhasid and Etzioni, 1996; Hanalioglu et al., 2017; Hanna and Etzioni, 2014; De La  
2 Salle et al., 1994; Lum et al., 2019, 2020; Morgan et al., 2011; Saleem et al., 2000; Zimmer et al.,  
3 2005). Moreover, selective deficiencies of NK or iNKT cells do not confer a predisposition to  
4 mycobacterial disease (Casey et al., 2012; Cottineau et al., 2017; Hughes et al., 2012; Latour,  
5 2007; Locci et al., 2009; Tangye et al., 2017). The nature of the IFN- $\gamma$ -producing innate, innate-  
6 like adaptive, and purely adaptive lymphocyte subsets indispensable for antimycobacterial  
7 immunity, either alone or in combination, therefore remains largely unknown. No genetic cause  
8 has yet been identified for half the MSMD patients. We therefore sought to discover a new  
9 genetic etiology of MSMD that would expand the molecular circuit controlling human IFN- $\gamma$   
10 immunity while better delineating the cellular network involved.

## Results

### Identification of an MSMD patient homozygous for an indel variant of *TBX21*

We studied a three-year-old boy (“P”, II.2) born to first-cousin Moroccan parents (**Fig. 1A**). He suffered from disseminated BCG disease (BCG-osis) following vaccination at the age of three months. He also had persistent reactive airway disease (RAD), but was otherwise healthy (see **STAR Methods - Case Report**). He did not suffer from any other severe clinical infectious diseases despite documented (VirScan) infection with various viruses, including Epstein-Barr virus (EBV), human cytomegalovirus (CMV), roseola virus, adenoviruses A, B, C and D, influenza virus A, rhinovirus A, and bacteria, such as *Streptococcus pneumoniae* and *Staphylococcus aureus* (**Fig. S1B**). We hypothesized that P had an autosomal recessive (AR) defect. We performed whole-exome sequencing (WES) on P, his unaffected brother, and both parents. Genome-wide linkage (GWL) analysis revealed 32 linked regions (LOD score >1.3 and size >500 kb) under a model of complete penetrance (**Supplemental Data S1**). In these linked regions, there were 15 rare homozygous non-synonymous or essential splicing variants of 15 different genes (minor allele frequency, MAF < 0.003 in gnomAD v2.1 and 1000 Genomes Project, including for each major ancestry) with a combined annotation-dependent depletion (CADD) score above their mutation significance cutoffs (MSC) (Auton et al., 2015; Itan et al., 2015; Karczewski et al., 2020; Kircher et al., 2014; Zhang et al., 2018) (**Table S1**). After the exclusion of genes with other predicted loss-of-function (LOF) variants with a frequency greater than 0.5% in gnomAD and of genes not expressed in leukocytes, only four candidate genes (*TBX21*, *PSMD9*, *ARHGAP27* and *ERN1*) remained. The c.466\_471delGAGATGinsAGTTTA insertion and/or deletion (indel) variant of *TBX21* (T-box protein 21, or T-box, expressed in T cells, T-bet) was the candidate variant predicted to be the most damaging (Kircher et al., 2014).



Moreover, based on connectivity to *IFNG*, the central gene of the entire network of all known MSMD-causing genes (Itan et al., 2013, 2014), *TBX21* was the most plausible candidate gene (**Table S1**). T-bet is a transcription factor that governs the development or function of several IFN- $\gamma$ -producing lymphocytes in mice, including T<sub>H</sub>1 cells (Lazarevic et al., 2013; Szabo et al., 2000, 2002). These findings suggested that homozygosity for the rare indel variant c.466\_471delGAGATGinsAGTTTA of *TBX21* causes MSMD. We investigated this variant according to the guidelines for genetic studies of single patients (Casanova et al., 2014). Sanger sequencing confirmed that P was homozygous for the indel variant of exon 1 of *TBX21*, whereas his unaffected brother was homozygous wild-type (WT/WT) and both parents were heterozygous (WT/M) (**Fig. S1C**). A closely juxtaposed 12-nucleotide (nt) region identical to this variant sequence was detected 8-nt upstream from the variant, and may have served as a template for its generation (**Fig. S1D**). The variant did not alter the exon 1-exon 2 junction of the *TBX21* mRNA in EBV-transformed B (EBV-B) cells or peripheral blood mononuclear cells (PBMCs) (**Supplemental Data S1**). The variant present in P thus resulted in the replacement of E156 and M157, two amino acids that are highly conserved across different species and among other paralogs of T-box transcription factors, with S156 and L157 (**Fig. 1B** and **Fig. S1E**).

### **Overexpressed mutant T-bet is LOF**

We investigated the expression of the mutant allele (Mut), by overexpressing an empty vector (EV), or vectors containing the WT or Mut allele, or negative controls with the T-box domain deleted (T-del) or K314R, the human ortholog of a known LOF mouse mutant, in human embryonic kidney (HEK) 293T cells (Jang et al., 2013). The production and nuclear translocation of Mut T-bet were impaired (**Fig. 1C** and **Fig. S1F**). An assessment of its binding

1 to consensus T-box regulatory elements (TBRE) in DNA revealed that nuclear proteins from  
2 WT-transfected cells bound the WT-TBRE, but not the Mut-TBRE. Furthermore, this specific  
3 complex was super-shifted by an anti-T-bet antibody (Ab) and inhibited by a competitor probe  
4 (CP). However, Mut T-bet did not bind WT-TBRE DNA (**Fig. 1D**). We also assessed the ability  
5 of WT and Mut T-bet to induce a luciferase transgene under the control of the TBRE or human  
6 *IFNG* proximal promoter (Janesick et al., 2012; Soutto et al., 2002). WT T-bet induced high  
7 levels of luciferase activity with WT-TBRE but not with Mut-TBRE. Mut T-bet and the negative  
8 controls (T-del and K314R) did not induce luciferase activity (**Fig. 1E**). Mut T-bet was also LOF  
9 for transactivation of the *IFNG* promoter, whereas the negative control, K314R, was markedly  
10 hypomorphic (**Fig. 1F**). We investigated the amino-acid substitution responsible for the abolition  
11 of transcriptional activity. We tested the effects of the WT and Mut forms of T-bet and of T-bet  
12 forms with single-residue substitutions (E156S and M157L), or alanine substitutions (E156A and  
13 M157A) on T-bet protein production and transcriptional activity. The loss of E156 (E156S and  
14 E156A) abolished transcriptional activity, but the production of the T-bet protein was unaffected.  
15 By contrast, a loss of methionine residues (M157L and M157A) preserved transcriptional  
16 activity but decreased the levels of T-bet protein (**Fig. S1G and H**). T-bet is required for optimal  
17 IFN- $\gamma$  production in NK, ILCs,  $\gamma\delta$ , and CD4<sup>+</sup> T cells in mice and is sufficient for IFN- $\gamma$   
18 production in NK and CD4<sup>+</sup> T cells in humans (Chen et al., 2007; Lazarevic et al., 2013; Powell  
19 et al., 2012; Szabo et al., 2002; Yu et al., 2006). We investigated the impact of the T-bet  
20 mutation on the induction of *IFNG* expression, by generating CRISPR/Cas9 gene-edited human  
21 NK-92 cell lines lacking *TBX21* (**Supplemental Data S1**). Upon stimulation with IL-12 + IL-18,  
22 these *TBX21* knockout (KO) NK-92 cells displayed a strong impairment of IFN- $\gamma$  production  
23 (**Fig. 1G and Fig. S1I**). We re-introduced the WT or Mut *TBX21*-containing plasmid into *TBX21*

1 KO NK-92 cells. We found that the WT *TBX21* rescued IFN- $\gamma$  production, whereas the Mut  
2 *TBX21* did not (**Fig. 1H** and **Fig. S1J**). Finally, the overexpression of WT, but not of Mut T-bet  
3 increased IFN- $\gamma$  levels to values above those for endogenous production in expanding naïve  
4 CD4<sup>+</sup> T cells from healthy donors (**Fig. 1I** and **J**, **Fig. S1K**). Thus, overexpression of Mut T-bet  
5 abolished DNA binding, and the mutant protein had no transactivation activity and could not  
6 induce IFN- $\gamma$  production in an NK cell line or CD4<sup>+</sup> T cells. It can, therefore, reasonably be  
7 considered a LOF allele.

#### 9 ***TBX21* variants in the general population are functional**

10 The *TBX21* indel variant in P was not found in the gnomAD v2.1.1, v3, Bravo, or Middle  
11 Eastern cohort databases, or our in-house database of more than 8,000 exomes, including > 1,000  
12 for individuals of North African origin (Karczewski et al., 2020; Scott et al., 2016; Taliun et al.,  
13 2019). The CADD score of 34 obtained for this allele is well above the MSC of 3.313 (**Fig. 1K**)  
14 (Itan et al., 2016; Kircher et al., 2014; Zhang et al., 2018). The *TBX21* gene has a low tolerance  
15 of deleterious variations, with a low gene damage index (GDI) score of 3.493 (Itan et al., 2015)  
16 and a low residual variation intolerance score (RVIS: -0.74) (Petrovski et al., 2015). Moreover,  
17 only two predicted LOF variants (variants: 17:47745045 A/AGCTG, and 17:47744913 C/G)  
18 were found in the heterozygous state in gnomAD. As their MAFs were <5 $\times$ 10<sup>-6</sup>, homozygosity  
19 rates for any of these three variants are well below the prevalence of MSMD (about 1/50,000). In  
20 the general population covered by the gnomAD database, seven variants have been identified in  
21 the homozygous state: A109G has a low CADD score, below the MSC, whereas H33Q, I339V,  
22 Y395C, M331I, R218H, and A29P have CADD scores above the MSC (**Fig. 1K** and  
23 **Supplemental Data S1**). The mouse ortholog of H33Q (H32Q) has been shown to be

functionally neutral (Tantisira et al., 2004). None of these seven alleles affected transactivation of the WT-TBRE promoter (**Fig. 1L**). Thus, all the *TBX21* variants present in the homozygous state in gnomAD are functionally neutral. In our in-house cohort of >8,000 exomes from patients with various infectious phenotypes, P is the only patient carrying a rare biallelic variant at the *TBX21* locus. We investigated whether any of the other 36 non-synonymous variants of *TBX21* in our in-house database could underlie infections in the heterozygous state, by testing each of them experimentally (**Supplemental Data S1**). None had any functional impact on transcriptional activity (**Fig. S1L and M**). Therefore, the data for P and his family, our in-house cohort, and the general population suggest that inherited T-bet deficiency, whether complete or partial, is exceedingly rare in the general population ( $< 5.8 \times 10^{-8}$ ). These findings also suggest that homozygosity for the Mut LOF variant of *TBX21* is responsible for MSMD in P.

### **Homozygosity for the *TBX21* mutation underlies complete T-bet deficiency**

We investigated the production and function of endogenous Mut T-bet in *Herpes saimiri* virus-transformed T cells (HVS-T) and primary CD4<sup>+</sup> T cells from P. Levels of *TBX21* mRNA were normal, but endogenous T-bet protein levels were low in P's cells (**Fig. 2A and B, Fig. S2A and B**). Together with the observation of low levels of Mut T-bet protein on overexpression (**Fig. S1F**), these findings suggest that the *TBX21* mutation decreases T-bet protein levels by a post-transcriptional mechanism. T-bet transactivates *IFNG* and *TNF* by directly binding to their regulatory promoter or enhancer (Garrett et al., 2007; Kanhere et al., 2012; Soutto et al., 2002; Szabo et al., 2000; Tong et al., 2005). Levels of spontaneous IFN- $\gamma$  and TNF- $\alpha$  production by P's HVS-T cells were much lower than those for HVS-T cells from all the healthy donors and heterozygous relatives tested (**Fig. 2C - F**). This cytokine production defect in *TBX21* mutant

HVS-T cells was rescued by WT T-bet complementation (**Fig. 2G** and **Fig. S2C**). We investigated whether homozygous variants of *PSMD9*, *ARHGAP27*, or *ERN1* contributed to the low levels of IFN- $\gamma$  production, by transducing HVS-T cells from P with the WT cDNAs of *PSMD9*, *ARHGAP27*, or *ERN1*. Overexpression of the WT *PSMD9*, *ARHGAP27*, or *ERN1* did not rescue the IFN- $\gamma$  deficit (**Fig. S2D – G**). Moreover, the overexpression of these genes in HVS-T cells from P that had already been transduced with WT *TBX21* did not further enhance IFN- $\gamma$  production. These data suggest that *PSMD9*, *ARHGAP27*, and *ERN1* variants make no significant contribution to the patient's immunological and clinical phenotype (**Fig. S2H and I**).

#### **T-bet deficiency alters the expression of downstream target genes**

The functional impact of the T-bet mutation was also investigated in primary CD4<sup>+</sup> T cells. Upon stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin (P/I), IFN- $\gamma$  production was almost entirely abolished in P's expanded T<sub>H</sub>0 cells and TNF- $\alpha$  production was impaired; the production of both these molecules was rescued by WT T-bet (**Fig. 2H and I, Fig. S2J and K**). In T<sub>H</sub>1 conditions, exogenous IL-12 bypassed T-bet and induced moderate IFN- $\gamma$  production by P's cells. However, the levels of IFN- $\gamma$  were still ~60-70% lower than those in healthy donors (**Fig. 2H and I**). We investigated other T-bet-dependent transcriptional targets, by performing RNA-seq to compare T<sub>H</sub>0 cells from 4 healthy donors, P, and P's cells complemented with WT T-bet after incubation with anti-CD3/28 Ab beads. We found that 415 genes were downregulated and 489 genes were upregulated in P's cells relative to healthy donors (**Supplemental Data S1, Table S2**). The complementation of P's cells with WT T-bet reversed the differential expression of 99 of the downregulated and 161 of the upregulated genes (**Supplemental Data S1**). Thus, only a subset of differentially regulated genes were rescued,

possibly due to differences in genetic variants other than *TBX21* between controls and P. Alternatively, T-bet may control the differential expression of some genes early in activation, and the genetic rescue may have occurred too late to reverse this effect. These targets were enriched in cytokine signaling pathway genes (**Fig. 2J**). We therefore decided to focus on genes involved in immunological signaling. Only 32 such genes were upregulated or downregulated in P's cells, but these differences in expression relative to controls were reversed by WT T-bet (**Fig. 2K**). Known T-bet-dependent targets, such as *IFNG*, *CCL3* and *CXCR3*, were downregulated in CD4<sup>+</sup> T cells from the T-bet-deficient patient, whereas *IFNGR2* expression was upregulated (**Fig. 2L**) (Iwata et al., 2017; Jenner et al., 2009). A set of new T-bet target genes was also identified, including *CCL1*, *CCL13*, *CCL4*, *CSF2*, *CXCR5*, *GZMM*, *CD40*, *CD86*, *IL7R*, *IL10*, *LTB*, *ITGA5*, and *ITGB5* (**Fig. 2L**). Collectively, our data indicate that the patient had AR complete T-bet deficiency, affecting the expression of a set of T-bet-dependent target genes, including *IFNG*.

#### **T-bet induces permissive chromatin accessibility and CpG methylation in *IFNG***

We analyzed the molecular mechanisms by which T-bet controls transcription. Epigenetically, T-bet is known to induce a permissive environment for transcription at the *IFNG* promoter through histone modifications and the suppression of CpG methylation (Lewis et al., 2007; Miller and Weinmann, 2010; Tong et al., 2005). However, it remains unknown whether T-bet directly regulates chromatin accessibility in mice or humans. The regulation of CpG methylation at the genome-wide scale by T-bet has never been studied. We performed omni-ATAC-seq analysis and EPIC DNA CpG methylation array analysis with T<sub>H</sub>0 cells derived from P and controls. In P's cells, a gain of chromatin accessibility was observed at 1,661 loci and a loss of chromatin accessibility was observed at 3,675 loci (**Fig. 3A and B, Supplemental Data**

1 **S1, Table S3**). We found that 662 and 1,642 of these loci, respectively, were subject to strict  
2 regulation by T-bet, as their gains and losses of chromatin accessibility were reversed by WT T-  
3 bet (**Fig. 3B, Table S3**). The chromatin regions opened up by T-bet were heavily occupied by  
4 bound T-bet, whereas those closed by T-bet did not typically require T-bet binding (**Fig. 3C**)  
5 (Kanhere et al., 2012). An enrichment in T-box binding elements was observed in loci for which  
6 chromatin accessibility was increased by T-bet, whereas an enrichment in Forkhead box  
7 elements was observed at loci for which chromatin accessibility was decreased by T-bet (**Fig. 3D**  
8 and **E**). The chromatin accessibility of 181 immunological genes (247 loci), including *IFNG*,  
9 *CYBB*, and *TYK2*, three known MSMD-causing genes, was increased by T-bet, whereas that of  
10 73 immunological genes (82 loci) was decreased by T-bet (**Fig. 3F, Supplemental Data S1, and**  
11 **Table S3**). Three known T-bet-dependent targets, *IFNG*, *TNF* and *CXCR3*, were among the top  
12 hits for the differentially regulated loci. The transcription start sites (TSS) of *IFNG* and *TNF*, the  
13 proximal promoter of *IFNG*, and the enhancers of *CXCR3* were inaccessible in T-bet-deficient  
14 cells, and this inaccessibility was rescued by WT T-bet (**Fig. 3G - I**). The EPIC DNA CpG  
15 methylation array analysis identified 644,236 CpG sites that were differentially regulated (**Table**  
16 **S3**). Three CpG loci within *IFNG* were hypermethylated in conditions of T-bet deficiency,  
17 whereas their methylation was reduced to levels similar to those in controls on complementation  
18 with WT T-bet (**Fig. 3J and K**). *NR5A2*, *TIMD4*, *ATXN2*, *ZAK*, *SLAMF8*, *TBKBPI*, *CD247*,  
19 *HDAC4* and several other genes were also regulated in a similar manner (**Fig. 3J and Table S3**).  
20 Interestingly, the methylation of six CpG loci within *ENTPDI* not previously linked to T-bet also  
21 increased in a T-bet-dependent manner (**Fig. 3J and L**). By contrast, *IL10* was a top target for  
22 which CpG methylation was drastically reduced in T-bet deficiency but rescued by WT T-bet  
23 (**Fig. 3M**). Taken together, these results demonstrate that T-bet orchestrates the expression of

target genes by modulating both their chromatin accessibility and CpG methylation. Genome-wide omni-ATAC-seq and CpG methylation array analyses identified new epigenetic targets of T-bet (**Table S3**). They also showed that chromatin accessibility at *IFNG* was increased by T-bet at both the TSS and promoter sites, whereas the CpG methylation of *IFNG* was decreased by T-bet at three different positions.

### **T-bet deficiency impairs NK cell maturation**

We then investigated the role of T-bet in the development of leukocyte lineages. Complete blood counts for fresh samples from P showed that the numbers of lymphocytes, neutrophils, and monocytes were normal. We studied the PBMC subsets of P after recovery from mycobacterial disease, by mass cytometry (cytometry by time-of-flight, CyTOF). A simultaneous analysis of the expression of 38 different cell surface markers was performed to compare the leukocyte subsets of P and his parents, healthy donors, and patients with IL-12R $\beta$ 1 deficiency, the most common genetic etiology of MSMD, as controls (**Fig. 4A** and **Fig. S3A**) (Van Gassen et al., 2015; Korsunsky et al., 2019). The frequencies of the plasmacytoid DC (pDC) and conventional DC 1 and 2 (cDC1 and cDC2) subsets were not affected by human T-bet deficiency (**Fig. S3B**). All major myeloid lineages were normal in P. We therefore focused on the development of lymphoid lineages. Total NK cells (defined as Lin<sup>-</sup>CD7<sup>+</sup>CD16<sup>+</sup> or CD94<sup>+</sup>) were present in normal numbers in P (**Fig. S3C**). However, CD16<sup>+</sup> and CD56<sup>bright</sup> NK cells levels were much lower in P than in controls (**Fig. 4A** and **B**). Moreover, P had an abnormally high frequency of CD56<sup>-</sup>CD127<sup>-</sup> NK cells (**Fig. S3D**); this NK cell subset has low levels of cytotoxicity and is rare in healthy and normal individuals (Björkström et al., 2010). The frequencies of ILC precursors (ILCPs) and ILC2s in P were similar to those in healthy donors



1 and IL-12R $\beta$ 1-deficient patients (**Fig. S3E and F**). In stringent analyses, ILC1 and ILC3 are too  
2 rare for quantification in human peripheral blood (Lim et al., 2017). Thus, human T-bet is  
3 required for the correct development or maturation of NK cells, but not monocytes, DCs, ILC2  
4 or ILCP.

#### 6 **Low levels of the iNKT, MAIT, and V $\delta$ 2<sup>+</sup> $\gamma\delta$ T-cell lineages in T-bet deficiency**

7 iNKT, MAIT, and  $\gamma\delta$  T cells are “innate-like” adaptive T-cell lineages with less T-cell  
8 receptor (TCR) diversity than conventional, “purely” adaptive  $\alpha\beta$  T cells (Chien et al., 2014;  
9 Crosby and Kronenberg, 2018; Godfrey et al., 2019). iNKT cells constitute a group of T cells  
10 with invariant TCRs combining properties from both T cells and NK cells (Crosby and  
11 Kronenberg, 2018). The iNKT cell levels of P were low (present at a level ~27-fold lower than  
12 that in controls) (**Fig. 4C, Fig. S3G and H**). MAIT cells express invariant V $\alpha$ 7.2-J $\alpha$ 33 TCR $\alpha$   
13 restricted by a monomorphic class I-related MHC molecule, along with ligands derived from  
14 vitamin B synthesis (Kjer-Nielsen et al., 2012; Xiao and Cai, 2017). P also had a lower  
15 frequency of MAIT cells than healthy donors (~15-fold) (**Fig. 4D and Fig. S3I**). Total  $\gamma\delta$  T-cell  
16 frequency was normal in P. However, the frequency of the V $\delta$ 2<sup>+</sup> subset, a group of  $\gamma\delta$  T cells that  
17 recognize phosphoantigen (pAgs) (Gu et al., 2018; Harly et al., 2012; Vavassori et al., 2013),  
18 was low (~6-fold lower than control levels) in P, whereas the levels of the V $\delta$ 1<sup>+</sup> subset of  $\gamma\delta$  T  
19 cells seemed to be slightly high (**Fig. 4E, Fig. S3J and K**). Mild abnormalities of B-cell  
20 development and antibody production unrelated to the patient’s mycobacterial disease were  
21 observed and will be reported in a separate study (Yang R, in preparation). CD4<sup>+</sup> and CD8<sup>+</sup>  $\alpha\beta$  T  
22 cells are the two most prevalent blood lineages of adaptive lymphocytes expressing a highly  
23 diverse  $\alpha\beta$  TCR repertoire. Antigen-driven CD8<sup>+</sup> T-cell effector responses and the optimal

1 induction of memory CD8<sup>+</sup> T cells in mice are controlled by T-bet (Bettelli et al., 2004; Juedes et  
2 al., 2004; Sullivan et al., 2003). In the T-bet-deficient patient, total CD8<sup>+</sup> T cells and the  
3 composition of naïve, central memory, effector memory and T<sub>EMRA</sub> cells were normal (**Fig. S4A**).  
4 We further investigated CD8<sup>+</sup> T cells in an unbiased manner, by automatic viSNE clustering  
5 with a panel of surface markers, including chemokine receptors (Amir el et al., 2013). We found  
6 no apparent difference between the memory CD8<sup>+</sup> T cells of P and controls. However, a small  
7 subset of naïve CD8<sup>+</sup> T cells (CD45RA<sup>+</sup>CD38<sup>int</sup>CXCR3<sup>int</sup>CCR6<sup>-</sup>CCR5<sup>-</sup>CD27<sup>high</sup>CD127<sup>high</sup>) was  
8 absent from P (**Fig. S4B**). Thus, the development of iNKT, MAIT cells, Vδ2<sup>+</sup> γδ T cells, and a  
9 small subset of naïve CD8<sup>+</sup> T cells is impaired in T-bet deficiency.

#### 11 **Selective depletion of CCR6<sup>-</sup> T<sub>H</sub>1 cells from CD4<sup>+</sup> T cells in T-bet deficiency**

12 Both P and his heterozygous parents had normal distributions of naïve and memory CD4<sup>+</sup>  
13 T cells (**Fig. S4C**). We further analyzed individual CD4<sup>+</sup> T cell subsets by viSNE clustering on  
14 antigen-experienced memory cells. Several memory CD4<sup>+</sup> T cell subsets typically present in  
15 healthy donors were missing in P. In particular, most of the CXCR3<sup>+</sup> cells, corresponding to T<sub>H</sub>1  
16 cells in humans, and CCR5<sup>+</sup> cells (Groom and Luster, 2011; Loetscher et al., 1998; Sallusto et al.,  
17 1998), were missing in P (**Fig. 4F, Fig. S4D and E**). The frequency of classic CXCR3<sup>+</sup>CCR6<sup>-</sup>  
18 T<sub>H</sub>1 cells was lower than that in all healthy adult and age-matched donors examined, whereas the  
19 frequency of CXCR3<sup>+</sup>CCR6<sup>+</sup> non-classic T<sub>H</sub>1\* cells, which are known to be mostly  
20 *Mycobacterium*-specific, was comparable to that of age-matched healthy donors (**Fig. 4G and H**).  
21 The frequencies of human T<sub>H</sub>2, T<sub>H</sub>17, and follicular helper (T<sub>FH</sub>) cells in peripheral blood were  
22 normal (**Fig. S4F - H**). However, levels of CXCR3<sup>+</sup> T<sub>FH</sub> cells, a group of T<sub>H</sub>1-biased T<sub>FH</sub> cells  
23 that produce IFN-γ together with IL-21 in germinal centers (Ma et al., 2015; Velu et al., 2016;

Zhang et al., 2019a), were low in P (**Fig. S4H**). CXCR3<sup>+</sup> regulatory T cells (Tregs), a group of TH1-skewed Tregs (Koch et al., 2009; Levine et al., 2017; Tan et al., 2016) were also present at abnormally low levels, but the proportion of total Tregs was normal (**Fig. S4I and J**). Thus, human T-bet deficiency selectively impairs development of the classic CXCR3<sup>+</sup>CCR6<sup>-</sup> TH1, CXCR3<sup>+</sup> T<sub>FH</sub>, and CXCR3<sup>+</sup> Treg CD4<sup>+</sup> T-cell subsets, but has no effect on the T<sub>FH</sub>, TH2, TH17, CCR6<sup>+</sup> TH1\*, and total Treg subsets, as shown by CyTOF and flow cytometry.

### **Single-cell transcriptomic profile *in vivo* is altered by T-bet deficiency**

We investigated the development and phenotype of leukocyte subsets in the patient further, by performing single-cell RNA-seq (scRNA-seq) with PBMCs from P and his father. The clustering of the various immune subsets yielded eight distinct major subsets: NK cells, pDCs, monocytes, B cells, CD8<sup>+</sup> cytotoxic T lymphocytes (CD8<sup>+</sup> CTLs), CD8<sup>+</sup> naïve, CD4<sup>+</sup> naïve and CD4<sup>+</sup> effector/memory T (T<sub>EM</sub>) cells (Becht et al., 2019). Consistent with the CyTOF results, normal frequencies of pDC, CD8<sup>+</sup> CTLs, CD4<sup>+</sup> naïve, and T<sub>EM</sub> cells, were obtained, together with a low frequency of NK cells, on scRNA-seq (**Supplemental Data S1**). We investigated the transcriptomic changes at single-cell level associated with T-bet deficiency, by filtering to select all genes with expression detected in > 5% of cells in at least one cluster, with at least a four-fold change in expression. We identified 34 genes as differentially regulated in T-bet-deficient cells relative to a heterozygous control. As for the RNA-seq data, some targets with expression known to be dependent on T-bet, including *CXCR3* in T<sub>EM</sub>, CD8<sup>+</sup> CTLs, and NK cells, and *CCL4* and *CCL3* in all cell types, were downregulated in T-bet-deficient cells (**Fig. 4I**). The expression of *XCL1*, *STAT4*, *SOX4*, *LMNA* and *ANXA1* was also impaired in at least one subset of T-bet-deficient cells (**Fig. 4I**). In humans and mice, XCL1, STAT4, SOX4, LMNA and

ANXA1 are known to be involved in T<sub>H</sub>1 immunity (Dorner et al., 2002, 2003, 2004; Gavins and Hickey, 2012; Kroczeck and Henn, 2012; Nishikomori et al., 2002; Thieu et al., 2008; Toribio-Fernández et al., 2018; Yoshitomi et al., 2018). NKG7 is involved in the initiation of human T<sub>H</sub>1 commitment and its genetic locus is tightly occupied by T-bet (Jenner et al., 2009; Kanhere et al., 2012; Lund et al., 2005). *NKG7* expression in naive CD4<sup>+</sup>, CD8<sup>+</sup> and B cells was dependent on functional T-bet, whereas *PRDM1* was downregulated in CD4<sup>+</sup> T and CD8<sup>+</sup> CTLs cells from P (**Fig. 4I**). The *IFNG* gene was weakly expressed across lymphocyte populations, as shown by scRNAseq, and its expression did not seem to be dependent on T-bet in basal conditions (data not shown). In addition, the expression of several genes not previously linked to T-bet was also altered in at least one cell subset in P (**Fig. 4I**). Thus, in addition to *CXCR3*, *NKG7*, *CCL3* and *CCL4*, which were weakly expressed in at least one leukocyte subset from this patient with human T-bet deficiency, consistent with the findings of RNA-seq and omni-ATAC-seq, the expression of a set of previously unknown target genes in immune subsets is also controlled by T-bet (**Fig. 4I**).

#### **Impaired IFN- $\gamma$ production by NK, MAIT, V $\delta$ 2<sup>+</sup> $\gamma\delta$ T, and CD4<sup>+</sup> T cells**

Human IFN- $\gamma$  is essential for antimycobacterial immunity, as all 31 known genetic etiologies of MSMD affect IFN- $\gamma$ -dependent immunity. The *in vivo* development of NK, iNKT, MAIT, V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T, and classic T<sub>H</sub>1 cells was found to be impaired in P, but it remained possible that the IFN- $\gamma$  production capacity of the remaining lymphocytes could compensate, thereby contributing to antimycobacterial immunity. We assessed the response of P's PBMCs to *ex vivo* stimulation with P/I. They secreted significantly less IFN- $\gamma$  secretion than control cells (**Fig. 5A**). The frequencies of IFN- $\gamma$ - or TNF- $\alpha$ -producing total lymphocytes were also low in P (**Fig. 5B** -

D). We assessed the IFN- $\gamma$  and TNF- $\alpha$  production of lymphocyte subsets by spectral flow cytometry (**Fig. S5A**). Upon P/I stimulation, the frequencies of IFN- $\gamma$ - or TNF- $\alpha$ -producing CD56<sup>+</sup> NK cells were low in P (**Fig. 5E, Fig. S5B and C**). We then assessed of the response of P's NK cells to *ex vivo* stimulation with IL-12, IL-15, and IL-18. Total NK cells from P displayed impaired degranulation, with low levels of CD107a expression, and almost no IFN- $\gamma$  production (**Fig. 5F, Fig. S5D and E**). T-bet deficiency not only reduced the overall frequencies of iNKT, MAIT, and V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells, but also compromised the production of IFN- $\gamma$  and TNF- $\alpha$  *ex vivo* by the residual activated iNKT, MAIT, and V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells detected in P (**Fig. 5G - K, Fig. S5F**). Despite an increase in the frequency of V $\delta$ 1<sup>+</sup>  $\gamma\delta$  T cells *in vivo*, the levels of IFN- $\gamma$ - or TNF- $\alpha$ -producing-V $\delta$ 1<sup>+</sup>  $\gamma\delta$  T cells were also low *ex vivo* (**Fig. 5G, Fig. 5L and M**). By contrast, we observed no difference in IFN- $\gamma$  and TNF- $\alpha$  production by V $\delta$ 1<sup>-</sup>V $\delta$ 2<sup>-</sup>  $\gamma\delta$  T cells between P and controls (**Fig. 5G, Fig. S5G and H**). IFN- $\gamma$ -producing cells from the various lymphocyte subsets examined in healthy donors expressed high levels of T-bet, but all the residual IFN- $\gamma$ -producing cells from the various subsets in P tested negative for T-bet (**Fig. 5G**). We also analyzed the response of  $\alpha\beta$  T cells to P/I. CD8<sup>+</sup> T cells secrete substantial amounts of IFN- $\gamma$  upon microbial challenge (Schoenborn and Wilson, 2007). The frequencies of CD8<sup>+</sup> T cells producing IFN- $\gamma$  or TNF- $\alpha$  in response to P/I did not differ between P and age-matched healthy donors (**Fig. S5I and J**). By contrast, the frequencies of IFN- $\gamma$ - or TNF- $\alpha$ -producing CD4<sup>+</sup> T cells were slightly low in P (**Fig. 5N and O**). Thus, among the residual circulating lymphocytes of P, NK, iNKT, MAIT, V $\delta$ 2<sup>+</sup>, V $\delta$ 1<sup>+</sup>  $\gamma\delta$  T, and CD4<sup>+</sup> T cells had impaired IFN- $\gamma$  production *ex vivo* in response to stimulation with IL-12, IL-15, IL-18 or P/I, whereas V $\delta$ 1<sup>-</sup>V $\delta$ 2<sup>-</sup>  $\gamma\delta$  and CD8<sup>+</sup>  $\alpha\beta$  T cells did not.

## Selective impairment of IFN- $\gamma$ production by T<sub>H</sub> cells in T-bet deficiency

T-bet was first discovered and has been most extensively studied in CD4<sup>+</sup> T cells in the context of mouse T<sub>H</sub>1 cells (Szabo et al., 2000, 2002). This discovery, together with that of GATA3 (Zheng and Flavell, 1997), revealed the molecular determinism of T<sub>H</sub>1/T<sub>H</sub>2 CD4<sup>+</sup> T-cell differentiation and paved the way for an understanding of T<sub>H</sub>17, iTreg, T<sub>H</sub>22, T<sub>FH</sub>, and T<sub>H</sub>9 cell lineage determination (Finotto et al., 2002; Zhu et al., 2010). We therefore investigated the impact of T-bet deficiency on primary CD4<sup>+</sup> T cells. IFN- $\gamma$  production by memory CD4<sup>+</sup> T cells was impaired by T-bet deficiency (**Fig. 5P** and **Q**). Another T<sub>H</sub>1 cytokine, TNF- $\alpha$ , was also produced in smaller amounts by memory CD4<sup>+</sup> T cells from P than by those of most of the healthy donors (**Fig. 5R** and **S**). By contrast, the memory CD4<sup>+</sup> T cells of P produced larger amounts of the T<sub>H</sub>17 effector cytokines IL-17A and IL-22 than did those of healthy donors (**Fig. 5T** and **U**, **Fig. S5K**). Unlike previous studies (Gökmen et al., 2013; Zhang et al., 2019b), we found that T-bet deficiency had no effect on IL-9 production *ex vivo* (**Fig. S5L** and **M**). Surprisingly, *ex vivo* T<sub>H</sub>2 cytokines from memory CD4<sup>+</sup> T cells were also not affected by human T-bet deficiency (**Fig. S5N - Q**). P's memory CD4<sup>+</sup> cells produced less IL-21 *ex vivo* than the memory CD4<sup>+</sup> cells of most of the healthy donors (**Fig. S5R**). We then investigated the role of T-bet in human T<sub>H</sub> cell differentiation *in vitro*. Naïve CD4<sup>+</sup> T cells from P or healthy donors were allowed to differentiate in T<sub>H</sub>0, T<sub>H</sub>1, T<sub>H</sub>2, T<sub>H</sub>9, or T<sub>H</sub>17 conditions. The induction of IFN- $\gamma$  production in naïve CD4<sup>+</sup> T cells was abolished by T-bet deficiency under T<sub>H</sub>1 conditions (**Fig. 5V** and **W**). Similarly, T-bet-deficient naïve CD4<sup>+</sup> T cells produced less TNF- $\alpha$  than the corresponding cells from most controls (**Fig. 5X** and **Y**). Furthermore, the induction of IL-9 under various conditions *in vitro* was weaker in naïve CD4<sup>+</sup> T cells from P than in the corresponding cells from most controls (**Fig. S5S** and **T**). *In vitro*-induced T<sub>H</sub>2 cells from P

1 produced more IL-10, but not IL-13, than control cells (**Fig. S5U and V**). Even memory CD4<sup>+</sup> T  
2 cells from P displayed impaired IFN- $\gamma$  and TNF- $\alpha$  production under T<sub>H</sub>1 polarizing conditions  
3 (**Fig. 5Z**). Thus, AR T-bet deficiency leads not only to defective IFN- $\gamma$  and TNF- $\alpha$  production *ex*  
4 *vivo* and *in vitro*, but also to a moderate upregulation of the production of IL-17A and IL-22, two  
5 cytokines characteristic of T<sub>H</sub>17 cells (Lazarevic et al., 2011).

### 6 7 **Poor cellular response to BCG infection *in vitro* in T-bet deficiency**

8 We investigated the molecular and cellular basis of BCG disease in P, by identifying the  
9 leukocyte subsets producing the largest amounts of IFN- $\gamma$  in a T-bet-dependent manner during  
10 acute BCG infection *in vitro*. The infection of PBMCs with BCG induced IFN- $\gamma$  production,  
11 which was further increased by stimulation with exogenous IL-12 (**Fig. 6A**). PBMCs from P had  
12 low levels of IFN- $\gamma$  and TNF- $\alpha$  production, but the level of IL-5 production in response to BCG  
13 infection was higher than that in most of the controls (**Fig. 6A, Fig. S6A and B**). Almost all the  
14 IFN- $\gamma$ -producing cells in controls had high levels of T-bet expression (**Fig. S6C**). Thus, T-bet<sup>+</sup>  
15 IFN- $\gamma$ <sup>+</sup> double-positive (DP) cells were the major *Mycobacterium*-responsive cells, with a  
16 function potentially dependent on T-bet. However, T-bet<sup>+</sup> IFN- $\gamma$  DP cell levels were low during  
17 acute infection in P (**Fig. 6B, Fig. S6C**). T-bet<sup>+</sup> IFN- $\gamma$  DP cells induced by exogenous IL-12  
18 alone comprised predominantly NK cells, whereas those induced by IL-23 alone also contained a  
19 large proportion of MAIT cells, in addition to NK cells (**Fig. S6D**). Among the T-bet<sup>+</sup> IFN- $\gamma$ <sup>+</sup>  
20 cells of healthy donors, NK, MAIT, V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T, CD4<sup>+</sup> and CD8<sup>+</sup>  $\alpha\beta$  T cells were the dominant  
21 responders during BCG infection, whereas iNKT, V $\delta$ 1<sup>+</sup>  $\gamma\delta$  T, V $\delta$ 1<sup>-</sup>V $\delta$ 2<sup>-</sup>  $\gamma\delta$  T and B cells were  
22 minority responders (**Fig. 6C and D**). However, these subsets of T-bet<sup>+</sup> IFN- $\gamma$ <sup>+</sup> cells were almost  
23 entirely depleted from P's PBMCs following BCG infection (**Fig. 6D**). We then investigated

each leukocyte subset separately (**Supplemental Data S1**). Fewer than 2% of V $\delta$ 1<sup>+</sup>  $\gamma\delta$  T cells, V $\delta$ 1-V $\delta$ 2<sup>-</sup>  $\gamma\delta$  T cells, B cells, CD4<sup>+</sup> or CD8<sup>+</sup>  $\alpha\beta$  T cells became T-bet<sup>+</sup> IFN- $\gamma$ <sup>+</sup> during BCG infection. However, among innate or innate-like lymphocytes, up to 10% of NK cells, 40% of iNKT, and 50% of V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells from healthy donors, but not from P, became T-bet<sup>+</sup> IFN- $\gamma$ <sup>+</sup> in response to BCG infection, and the frequency of these cells was further increased by exogenous IL-12 (**Fig. 6E - G, Fig. S6E and F**). Up to 50% of MAIT cells became T-bet<sup>+</sup> IFN- $\gamma$ <sup>+</sup> during BCG infection, but no difference was observed between age-matched healthy donors and P (**Fig. 6E and Fig. S6E**). Upon acute BCG infection *in vitro*, despite a less robust activation of CD4<sup>+</sup> T cells than of innate or innate-like lymphocytes, the frequency of T-bet<sup>+</sup> IFN- $\gamma$ <sup>+</sup> cells was low among CD4<sup>+</sup> but not among CD8<sup>+</sup>  $\alpha\beta$  T cells of P (**Fig. 6H, Fig. S6G and H**). Thus, the IFN- $\gamma$  production controlled by T-bet during acute BCG infection *in vitro* takes place mostly in NK, iNKT, V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T and CD4<sup>+</sup>  $\alpha\beta$  T cells, but not in V $\delta$ 1<sup>+</sup>  $\gamma\delta$  T cells, V $\delta$ 1-V $\delta$ 2<sup>-</sup>  $\gamma\delta$  T cells, or CD8<sup>+</sup>  $\alpha\beta$  T cells. The development of MAIT cells is also dependent on T-bet, whereas the IFN- $\gamma$  production of these cells during BCG infection is not. These experimental findings *in vitro* do not exclude a contribution of other subsets *in vivo*. NK, iNKT, MAIT, and V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells responded robustly to acute BCG infection *in vitro*, but these subsets were absent or functionally deficient in the patient with inherited T-bet deficiency.

### **Defective prolonged anti-BCG immunity mediated by V $\delta$ 2<sup>+</sup> $\gamma\delta$ T cells**

The stimulation of PBMCs *in vitro* with live BCG involves both antigens specifically recognized by *Mycobacterium*-specific cognate  $\alpha\beta$  and  $\gamma\delta$  T cells and many other stimuli. BCG infection *in vitro* mimics acute infection *in vivo*, but may not be robust enough for investigations of the antigen-specific adaptive immune response, particularly as concerns prolonged adaptive



immunity. We therefore studied V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells and CD4<sup>+</sup>  $\alpha\beta$  T cells, two adaptive immune lymphocyte subsets that produced substantial amounts of IFN- $\gamma$  during BCG infection *in vitro* and are known to function in an antigen-specific manner. In PBMCs from healthy donors, V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells responded most robustly during BCG infection (**Fig. 6E**). V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells, a major subset of  $\gamma\delta$  T cells recognizing pAg from microbial sources (Gu et al., 2018; Harly et al., 2012; Vavassori et al., 2013), are also known to play an essential role in the recall response to mycobacterial re-infection in humans and non-human primates (Chen, 2005; Shen et al., 2002). V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells proliferate vigorously in response to mycobacterial infection *in vivo* and can expand robustly in response to pAg-rich lysates of mycobacterial species *in vitro* (Hoft et al., 1998; Modlin et al., 1989; Panchamoorthy et al., 1991; Parker et al., 1990; Tsukaguchi et al., 1995). We investigated whether the function of V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells was affected in the patient with T-bet deficiency, as these cells represented a small, but important proportion (~ 0.2%) of P's peripheral lymphocytes. The populations of V $\delta$ 2<sup>+</sup> T cells from all controls and relatives of P expanded vigorously following prolonged stimulation with BCG lysates. By contrast, no such expansion was observed for T-bet-deficient V $\delta$ 2<sup>+</sup> T cells (**Fig. 6I and J, Fig. S6I**). After two weeks of expansion, the levels of IFN- $\gamma$  production by T-bet-deficient cells were lower than those for healthy donors' cells (**Fig. 6K**).

#### **Redundant role of T-bet in IFN- $\gamma$ production by BCG-specific cognate T<sub>H</sub>1\* cells**

IFN- $\gamma$  production by CD4<sup>+</sup>  $\alpha\beta$  T cells upon BCG infection *ex vivo* was subtly affected by T-bet deficiency, but it remains unclear whether there is a difference in the mycobacterial antigen-specific CD4<sup>+</sup>  $\alpha\beta$  T-cell response. It also remains unclear whether the memory adaptive immunity to mycobacteria elicited by antigen-specific CD4<sup>+</sup> T cells is dependent on T-bet. We

1 addressed both these unresolved issues by analyzing *Mycobacterium*-responsive CD4<sup>+</sup> T cells  
2 expanded through prolonged stimulation with BCG-lysates or tuberculin purified protein  
3 derivative (PPD) (**Supplemental Data S1**). Like those of BCG-vaccinated healthy donors, P's  
4 CD4<sup>+</sup> T cells proliferated equally well in response to both BCG-lysates and PPD (**Supplemental**  
5 **Data S1**). No difference in IFN- $\gamma$  production from PPD-responsive CD4<sup>+</sup> T cells was observed  
6 between healthy donors and P (**Fig. 6L** and **Fig. S6J**). Unlike PPD, which activated only CD4<sup>+</sup> T  
7 cells, BCG-lysates activated both CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells, albeit to a lesser extent  
8 (**Supplemental Data S1**). Like PPD stimulation, IFN- $\gamma$  production from both BCG-lysate-  
9 responsive CD4<sup>+</sup> and CD8<sup>+</sup> T cells was similar between controls and P (**Fig. 6M** and **N**, **Fig.**  
10 **S6K and L**). We confirmed these findings by screening antigen-reactive T-cell libraries  
11 established from the CD4<sup>+</sup>CCR6<sup>-</sup> (containing classic T<sub>H</sub>1 cells) and CD4<sup>+</sup>CCR6<sup>+</sup> (containing  
12 T<sub>H</sub>1\* *Mycobacterium*-responsive cells) memory subsets (Geiger et al., 2009). Consistent with  
13 our *in vivo* findings, the T cells in the CD4<sup>+</sup>CCR6<sup>-</sup> and CD4<sup>+</sup>CCR6<sup>+</sup> libraries had low levels of  
14 CXCR3 or IFN- $\gamma$  (**Supplemental Data S1**). P's CD4<sup>+</sup>CCR6<sup>+</sup> T-cell library responded robustly to  
15 BCG, tetanus toxoid and *C. albicans*, and his CD4<sup>+</sup>CCR6<sup>-</sup> T-cell library responded normally to  
16 influenza virus, CMV, and EBV. Despite intact proliferation, IFN- $\gamma$  production from T-bet-  
17 deficient T cells responding to influenza virus, EBV, tetanus toxoid, and *C. albicans* was weak.  
18 However, P's CCR6<sup>+</sup> T cells, consisting almost entirely of *Mycobacterium*-specific memory  
19 T<sub>H</sub>1\* cells (Acosta-Rodriguez et al., 2007; Becattini et al., 2015), proliferated robustly in  
20 response to BCG peptides. Moreover, their IFN- $\gamma$  production was normal, and their levels of IL-  
21 10 production were slightly higher (**Fig. 6O** and **Supplemental Data S1**). The normal levels of  
22 IFN- $\gamma$  production could not be attributed to cells with a revertant genotype, as reported in other  
23 T-cell primary immunodeficiency diseases (PIDs) (Davis et al., 2008; Revy et al., 2019),

1 because the IFN- $\gamma$ <sup>+</sup> BCG-specific T-cell clones still carried the *TBX21* indel variant. Thus, the  
2 prolonged immunity to BCG infection mediated by V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T and memory  $\alpha\beta$  T cells was  
3 divergently controlled by T-bet, as T-bet was required for the generation of long-term immunity  
4 due to V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells, but redundant for IFN- $\gamma$  production by BCG-specific cognate CD4<sup>+</sup> T<sub>H</sub>1\*  
5 cells or *Mycobacterium*-responsive CD8<sup>+</sup>  $\alpha\beta$  T cells (**Table S4**).

## Discussion

We report here the identification and study of the first patient discovered to have inherited, complete T-bet deficiency resulting clinically in MSMD. Key observations made in T-bet-deficient mice were validated in this human patient with T-bet deficiency: 1) the development of  $T_H1$  cells and their production of effector cytokines, including IFN- $\gamma$  in particular, requires T-bet (Szabo et al., 2000, 2002); 2) the development of NK and iNKT cells is dependent on T-bet (Townsend et al., 2004); and 3) the regulation of T-bet-dependent targets, including *CXCR3*, *TNF* and *IFNG*, involves both direct transactivation and epigenetic modulation (Miller and Weinmann, 2010). Accordingly, T-bet-deficient mice are highly vulnerable to mycobacteria, including *M. tuberculosis* and *M. avium* (Matsuyama et al., 2014; Sullivan et al., 2005), like mice deficient for other genes that govern IFN- $\gamma$  immunity (Casanova, 1999). By contrast, despite the requirement of T-bet for immunity to a broad spectrum of pathogens following experimental inoculation in mice (Bakshi et al., 2006; Harms Pritchard et al., 2015; Hultgren et al., 2004; Kao et al., 2011; Lebrun et al., 2015; Oakley et al., 2013; Ravindran et al., 2005; Rosas et al., 2006; Svensson et al., 2005; Szabo et al., 2002), the only apparent infectious phenotype of the T-bet-deficient patient is MSMD. Our study provides compelling evidence that inherited T-bet deficiency is a genetic etiology of MSMD due to the disruption of IFN- $\gamma$  immunity (Casanova et al., 2014). This experiment of nature suggests that T-bet is required for protective immunity to intramacrophagic mycobacteria but largely redundant for immunity to most intracellular pathogens, including viruses in particular. This is at odds with findings in mice, but consistent with other genetic etiologies of MSMD, all of which are inborn errors of IFN- $\gamma$  immunity (Bustamante, 2020; Kerner et al., 2020). Our observation further suggests that the functions of T-bet unrelated to IFN- $\gamma$  are redundant in humans. The

1 identification of additional T-bet-deficient patients is required to draw firm conclusions.  
2 However, it is striking that humans genetically deprived of key immunological molecules other  
3 than T-bet or IFN- $\gamma$  often show a much greater redundancy than the corresponding mutant mice  
4 (Casanova and Abel, 2004, 2018).

5 Our study also suggests unexpected immunological abnormalities not documented in T-  
6 bet-deficient mice that also contribute to the development of MSMD: 1) T-bet was apparently  
7 required for the optimal development of two innate-like adaptive lineages of immune cells,  
8 MAIT and V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells; 2) T-bet was also apparently required for the optimal production of  
9 IFN- $\gamma$  by the few NK, iNKT, MAIT, and V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T lymphocytes that were able to develop  
10 (Bettelli et al., 2004; Daussy et al., 2014; Garrido-Mesa et al., 2019; Intlekofer et al., 2007;  
11 Lugo-Villarino et al., 2003; Pearce et al., 2003; Rankin et al., 2013; Sullivan et al., 2003; Tang et  
12 al., 2010). Unexpectedly, IFN- $\gamma$  production by cognate purely adaptive *Mycobacterium*-specific  
13 T<sub>H</sub>1\* CD4<sup>+</sup> T cells, and by PPD-, or BCG-lysate-responsive CD4<sup>+</sup> or CD8<sup>+</sup>  $\alpha\beta$  T cells, was  
14 unaffected by T-bet deficiency. The compensatory mechanisms underlying intact IFN- $\gamma$   
15 production by these antigen-specific CD4<sup>+</sup> T cells remain to be discovered. Taken together,  
16 impaired IFN- $\gamma$  production by NK and iNKT cells, as in mice, and by MAIT and V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells,  
17 as shown here, accounts for MSMD in this patient with T-bet deficiency, despite normal T<sub>H</sub>1\*  
18 development and function. By contrast, inborn errors of immunity that disrupt IFN- $\gamma$  production  
19 by the selective depletion of NK, iNKT, CD4<sup>+</sup>, or CD8<sup>+</sup>  $\alpha\beta$ T cells do not underlie mycobacterial  
20 disease, because of the compensation provided by other subsets. Conversely, the loss of all T-cell  
21 subsets in SCID patients does result in predisposition to mycobacterial disease. Interestingly, a  
22 different combination of deficits accounts for MSMD in patients with ROR $\gamma$ T deficiency, who  
23 lack iNKT and MAIT cells and whose  $\gamma\delta$  T and T<sub>H</sub>1\* cells do not produce IFN- $\gamma$ , and whose NK

1 cells are unable to compensate (Okada et al., 2015). T-bet and ROR $\gamma$ T deficiencies are  
2 characterized by iNKT, MAIT, and  $\gamma\delta$  T-cell deficiencies, whereas an NK deficit is observed  
3 only in T-bet deficiency and a deficit of T<sub>H</sub>1\* cells is observed only in ROR $\gamma$ T deficiency. We  
4 found that human T-bet was essential for both innate (NK cells) and innate-like (iNKT, MAIT,  
5 and V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells) adaptive immunity to mycobacteria, but, surprisingly, redundant for  
6 classical, purely adaptive immunity (CD4<sup>+</sup> T<sub>H</sub>1\* and CD8<sup>+</sup>  $\alpha\beta$  T cells) to mycobacteria.

7

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**Author contributions**

R.Y., J.B. and J.-L.C. conceptualized the study, designed the experiments, interpreted the data, and wrote the manuscript. R.Y., J.R., Dav.L., M.B., P.G., L.W, C.S.M., S.G.T., J.L.M., M.S.K., F.M., D.J., M.W., Dan.L., F.S., C.A.C., J.-M.D., J.P.D., J.M., Yic.S., J.H., M.O., C.G., D.B., F.A.A., Mah.R., T.K., N.M., C.O-Q., Man.R., and J.B. conducted experiments and analyzed data. R.Y., P.Z., L.T.H., L.Q.M., Yoa.S., G.K., L.A and T.C. analyzed high-throughput sequencing data. M.J., F.B., and F.R. performed clinical serological tests. H.E., I.B., A.E., F.A., A.B. and J.B. took care of the patient. J.E.B. contributed North African exomes. S.B-D., A.P., Q,P-H, L.H.G., P.G., S.G.T., F.S., J. B., and J.-L.C. supervised the study. All authors provided input on the final manuscript.



1    **Declaration of Interests**

2    Dr. Glimcher serves on the Board of Directors of GlaxoSmithKline Pharmaceutical Company  
3    and the Analog Device Corporation and formerly served on the Boards of Bristol Myers Squibb  
4    Pharmaceutical Company and the Waters Corporation. She is also on the Scientific Advisory  
5    Boards of Abpro, Kaleido, and Repare biotechnology companies. Dr. Casanova serves on the  
6    Scientific Advisory Boards of ADMA Biologics Inc., Celgene and Kymera Therapeutics, Inc..  
7    He also consults for Elixiron Immunotherapeutics. Other authors declare no competing interests.

## MAIN Figure Titles and Legends

**Figure 1. Discovery of an MSMD patient with a homozygous *TBX21* variant and the corresponding molecular characterization.** (A) Pedigree of a consanguineous family with the mutant *TBX21* allele. (B) Schematic representation of the mutation. (C) Nuclear and cytoplasmic fractions from HEK 293T cells transfected with indicated *TBX21* cDNA-containing vectors subjected to immunoblotting against indicated proteins. (D) EMSA on nuclear extracts of HEK293T cells transfected with empty vector (EV), WT or Mut *TBX21* alleles. (E) TBRE-reporter luciferase assay testing WT or Mut T-bet in HEK 293T cells. (F) Human *IFNG* reporter luciferase assay testing HEK 293T cells transfected with WT or Mut *TBX21* cDNA-containing vectors, at the indicated concentrations. (G) IFN- $\gamma$  production in response to stimulation with IL-12 and IL-18 in T-bet knock-out (KO) NK-92 cells. (H) IFN- $\gamma$  production in response to stimulation with IL-12 and IL-18 in T-bet KO NK-92 cells complemented with EV, WT, K314R or Mut *TBX21*-containing plasmids. (I) Intracellular IFN- $\gamma$  production in response to P/I in expanding T<sub>H</sub>0 cells transduced with EV, WT or Mut *TBX21* cDNA. (J) IFN- $\gamma$  production from cells transduced as in (I) in response to stimulation with anti-CD3/CD28 Ab. (K) Graph of CADD score against minor allele frequency (MAF), for *TBX21* variants reported in the gnomAD. Mutation significance cutoff (MSC) was shown. (L) TBRE-reporter luciferase assay testing indicated variants of *TBX21* as in (E). **See also Figure S1, Table S1, and Supplemental Data S1.**

In Fig. 1E – J and L, bars represent the mean and standard deviation. Dots represent individual samples or technical replicates. Two-way ANOVA was used for analysis in (E – I). One-way ANOVA was used for analysis in (J and L). In (E – J and L), \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ , ns = not significant.

**Figure 2. The T-bet variant in patient-derived cells is loss-of-function.** (A) Whole-cell lysates from expanding CD4<sup>+</sup> T cells from healthy donors (CTL), a heterozygous parent (WT/M) and the patient (M/M) subjected to immunoblotting. (B) Immunoblotting against indicated proteins of whole-cell lysates of HVS-T cells derived from CTL, M/M and WT/M. (C and D) Spontaneous production of IFN- $\gamma$  (C) and TNF- $\alpha$  (D) from cultures of HVS-T cells from CTL, M/M and WT/M. (E and F) The *IFNG* (E) and *TNF* (F) expression of HVS-T cells, as in (C), was assessed by RT-qPCR. (G) HVS-T cells from CTL, M/M and WT/M were rescued with EV or WT *TBX21*. The percentage of intracellular IFN- $\gamma$ -producing cells with or without P/I stimulation is shown. (H) CD4<sup>+</sup> T cells from CTL, M/M and WT/M were expanded under T<sub>H</sub>0 or T<sub>H</sub>1 conditions. M/M cells were transduced as described in (G). Cells were subjected to ICS for IFN- $\gamma$  and TNF- $\alpha$  in response to P/I. (I) Percentage of IFN- $\gamma$ - and TNF- $\alpha$ -producing cells in (H). (J) Transduced T<sub>H</sub>0 cells, as in (H), were isolated and restimulated, and were subjected to RNA-seq, along with non-transduced cells. Pathway enrichment among differentially regulated genes is shown. (K) The numbers of T-bet-dependent differentially expressed immune genes in RNA-seq are shown. (L) Heat-map of selected T-bet-dependent downstream genes, as in (K). **See also Figure S2, Table S2, and Supplemental Data S1.**

In Fig. 2C – G and I, bars represent the mean and the standard error of the mean. Dots represent individual samples. One-way ANOVA was used for analysis in (C and D). Mann-Whitney tests were used for analysis in (E and F), comparing WT/M or M/M to CTL. In (C - F), \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ , and ns = not significant.

**Figure 3. An altered spectrum of epigenetic regulation in CD4<sup>+</sup> T cells in T-bet deficiency.**

(A) Expanded CD4<sup>+</sup> T<sub>H</sub>0 cells from CTL, IL-12Rβ1-deficient MSMD patients (*IL12RB1* M/M), WT/M, M/M, and M/M cells complemented with empty vector (M/M +EV) or with WT T-bet (M/M +WT) were subjected to omni-ATAC-seq. PCA analysis of the peaks called in the various samples, plotted in ggplot2. (B) Number of called peaks differentially regulated as indicated. (C) Heatmap of all loci opening or closing in a T-bet-dependent manner as in (B). (D and E) The most significantly enriched DNA binding motifs at loci displaying T-bet-dependent increases (D) and loci displaying T-bet-dependent decreases, as in (B and C). (F) Chromatin accessibility heatmap for all the loci displaying the most significant differential regulation of immune genes (CTL – M/M, adjusted *P*-value < 1 x 10<sup>-5</sup>), abolished by WT T-bet (M/M+EV - M/M+WT, adjusted *P*-value < 1 x 10<sup>-5</sup>). (G - I) Chromatin accessibility of transcription start site (TSS), proximal promoter and distal enhancer regions of *IFNG*, *TNF* and *CXCR3*. (J) The differential methylation (beta-value) of CpG sites between M/M and CTL or between M/M+EV and M/M+WT is shown. (K - M) CpG methylation status of CpG sites in *IFNG* (K), *ENTPD1* (L) and *IL10* (M). See also Table S3 and Supplemental Data S1.

**Figure 4. Impaired *in vivo* development of NK, invariant NKT, MAIT, Vδ2<sup>+</sup> γδ T, and T<sub>H</sub>1**

**cells in T-bet deficiency.** (A) UMAP representation shows immunophenotyping of 40,000 CD45<sup>+</sup>CD66b<sup>+</sup>DNA<sup>+</sup> live PBMCs from age-matched controls (Age CTL) and M/M, by CyTOF. (B) Frequencies of CD56<sup>bright</sup> NK cells and CD56<sup>dim</sup>CD16<sup>+</sup> NK cells by CyTOF. (C - E) Percentages of iNKT (C), MAIT (D), and Vδ2<sup>+</sup> γδ T cells (E) among live single cells from indicated individuals, including P's healthy brother (WT/WT), by flow cytometry. (F) viSNE map of 10,000 CD45RA<sup>+</sup> memory CD4<sup>+</sup> T cells from indicated individuals, with expression of

CXCR3 and CCR5 shown. **(G and H)** Frequencies of CCR6<sup>-</sup> T<sub>H</sub>1 (CCR6<sup>-</sup>CXCR3<sup>+</sup>CCR4<sup>-</sup>) (G) and CCR6<sup>+</sup> T<sub>H</sub>1\* (CCR6<sup>+</sup>CXCR3<sup>+</sup>CCR4<sup>-</sup>) cells (H) among live CD45<sup>+</sup>CD66b<sup>-</sup> cells are shown. **(I)** A bubble graph is presented to show genes for which the proportion of cells displaying expression is altered in the M/M cells in comparison with PBMCs from the *TBX21* WT/M father, as a control, by scRNA-seq. The size of the bubble indicates the proportion of cells expressing the gene and the color scale indicates the log2-transformed ratios. The genes highlighted in red were identified as T-bet-regulated by ATAC-seq and/or RNA-seq. LD (low level of detection). ND (not detected). **See also Figure S3 – S4 and Supplemental Data S1.**

In Fig. 4B – E, G and H, bars represent the mean and the standard error of the mean. Dots represent individual samples or technical replicates. Mann-Whitney nonparametric tests were used for analysis in (C – E). In (C – E), \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ , ns = not significant.

**Figure 5. Impaired IFN- $\gamma$  *ex vivo* production from the remaining lymphocytes of the T-bet-deficient patient.** **(A)** IFN- $\gamma$  production in culture supernatants of PBMCs from indicated individuals in response to stimulation with P/I is shown. **(B)** Dot-plots showing the expression of T-bet and IFN- $\gamma$  in total live lymphocytes as in (A). **(C and D)** Frequency of IFN- $\gamma$  (C) or TNF- $\alpha$ -producing (D) cells in response to P/I as in (B). **(E)** Frequency of IFN- $\gamma$ -producing cells among NK cells in response to P/I. **(F)** PBMCs were stimulated with IL-12, IL-15 and IL-18. The expression of CD107a and IFN- $\gamma$  among NK cells were assessed by flow cytometry. **(G)** Dot-plots showing the expression of T-bet and IFN- $\gamma$  by indicated subsets in response to P/I. **(H – O)** Frequency of IFN- $\gamma$ - (H, J, L, N) or TNF- $\alpha$  (I, K, M, O)-producing cells among MAIT cells, V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells, V1<sup>+</sup>  $\gamma\delta$  T cells and CD4<sup>+</sup> T cells in response to P/I. **(P – U)** FACS sorted naïve

and memory CD4<sup>+</sup> T cells activated/expanded. The production of IFN- $\gamma$  was assessed intracellularly (P) and in culture supernatants (Q). The production of TNF- $\alpha$  was assessed intracellularly (R) and in culture supernatants (S). The production of IL-22 (T) and IL-17A (U) was assessed in culture supernatants. (V - Y) Naïve CD4<sup>+</sup> T cells were subjected to activation under T<sub>H</sub>0 or T<sub>H</sub>1 conditions. The production of IFN- $\gamma$  (V and W) and TNF- $\alpha$  (X and Y) was assessed. (Z) Memory CD4<sup>+</sup> T cells were activated under T<sub>H</sub>0 or T<sub>H</sub>1 conditions. The production of IFN- $\gamma$  and TNF- $\alpha$  was assessed. **See also Figure S5.**

In Fig. 5A, C – F, H - Z, bars represent the mean and the standard error of the mean. Dots represent individual samples or technical replicates. Mann-Whitney nonparametric tests were used for analysis in (A, C – E, H - O). In (A, C – E, H - O), \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ , ns = not significant.

**Figure 6. T-bet deficiency leads to defective NK, iNKT and V $\delta$ 2<sup>+</sup>  $\gamma\delta$ T cells, resulting in susceptibility to mycobacteria.** (A) IFN- $\gamma$  secretion by PBMCs from indicated individuals stimulated with and without live *M. bovis* BCG in the presence and absence of IL-12 and IL-23. (B) Percentages of T-bet<sup>+</sup> IFN- $\gamma$ <sup>+</sup> double-positive (DP) cells in the indicated samples, cultured as in (A). (C) Percentages of indicated immune subsets among the DP cells of both CTL and Age CTL individuals. (D) viSNE plots showing different clusters of immune cells among DP cells. (E) Percentages of DP cells within each immune subset, as indicated. (F and G) Dot-plots showing the expression of T-bet and IFN- $\gamma$  in NK (F) and V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells (G), cultured as in (A). (H) Percentages of DP cells within each indicated adaptive immune subset. (I and J) PBMCs from indicated individuals were stimulated with lysates of *M. bovis* BCG (BCG-lysates) for 14 days. Dot plots of V $\gamma$ 9 $\delta$ 2  $\gamma\delta$ T cells (I) and the total number of V $\gamma$ 9 $\delta$ 2  $\gamma\delta$ T cells (J) are shown. (K) The

level of IFN- $\gamma$  in culture supernatants from (J) is shown. **(L - N)** The intracellular IFN- $\gamma$  production of antigen-specific CD4<sup>+</sup> or CD8<sup>+</sup> T cells expanded from PPD or BCG-lysates was measured. The percentages of IFN- $\gamma$ -producing cells among PPD-responsive CD4<sup>+</sup> T (L), BCG-lysate-responsive CD4<sup>+</sup> (M) and CD8<sup>+</sup> T (N) cells are shown. **(O)** Memory CCR6<sup>+</sup>CD4<sup>+</sup> T-cell clones responding to the peptide pool from *M. bovis* BCG were identified. IFN- $\gamma$  production in culture supernatants from these BCG-specific clones was shown. **See also Figure S6, Table S4, and Supplemental Data S1.**

In Fig. 6A, B, E, H, K, L, M and N, bars represent the mean and the standard error of the mean. Dots represent individual samples and technical replicates. In (O), bars represent the mean and the standard deviation. Dots represent individual T-cell clones. In (C), the numbers indicate the mean and the standard error of the mean. Mann-Whitney nonparametric tests were used for analysis in (A, B, E and H). In (A, B, E and H), \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ , and ns = not significant.

## Supplemental figure titles and legends

### **Figure S1. Clinical and genetic features of a patient with MSMD carrying a homozygous *TBX21* variant leading to the substitution of two amino acids, and the characterization of this variant, Related to Figure 1.**

**(A)** Schematic view of the 16 MSMD-causing genes within myeloid and lymphoid cells in the context of host antimycobacterial immunity. **(B)** We tested for serum IgG against various viral peptides or peptides derived from bacteria or other pathogens by phage immunoprecipitation-sequencing. Serum samples from an unrelated healthy three-year-old boy (CTL1), two healthy adults (CTL2 and CTL3), and a *TBX21* M/M P, and IgG-depleted serum and intravenous immunoglobulin (IVIg) were assayed. The heatmap (left) shows the counts of peptides significantly enriched for a given species with less than a continuous seven-residue subsequence, the estimated size of a linear epitope, in common. The species for which the patient had a score greater than our set threshold ( $>3$ ) are shown. Count values above the set threshold are shown on a color scale from green ( $\geq 4$ ) to red ( $\geq 40$ ) and dark-red ( $\geq 80$ ). **(C)** Genomic DNA from P (M/M) and his parents (WT/M) was subjected to Sanger sequencing to confirm the *TBX21* variant. **(D)** Comparison between the WT and Mut sequences of the region in which the variant is located within exon 1 of *TBX21*. An alignment of the WT and Mut sequences revealed a juxtaposed region with a sequence identical to that of the variant sequence. **(E)** Alignment of peptide fragments from human T-box domain-containing transcription factors. **(F)** pCMV6 plasmids containing EV, WT T-bet (WT), the K314R variant of T-bet (K314R), T-bet with a deletion of the T-box domain (T-del), and mutant T-bet (Mut) were used to transfect HEK293T cells. T-bet expression was characterized by immunoblotting with anti-T-bet 4B10 monoclonal Ab and anti-Flag Ab. Anti-GAPDH antibody was used as an endogenous control. **(G)**



1 Mutations of individual residues in the T-bet cDNA (E156S, M157L, E156A, and E157A) were  
2 generated in pCMV6 plasmids by site-directed mutagenesis. The plasmids were used to transfect  
3 HEK293T cells for overexpression. T-bet levels were measured by immunoblotting with anti-T-  
4 bet 4B10 Ab and anti-Flag Ab. Anti-GAPDH Ab was used as endogenous control. **(H)** The  
5 plasmids described in (G) were used to transfect HEK293T cells, together with the *IFNG*  
6 reporter plasmids and *Renilla* luciferase pRL-SV40 plasmids. After 3 days, the luciferase signal  
7 was assessed in a Dual-Glo assay. **(I)** Single-cell CRISPR/Cas9-edited NK-92 clones were  
8 stimulated with 50 pg/mL IL-12 and 10 ng/mL IL-18 or were left untreated (NS). Intracellular  
9 IFN- $\gamma$  production was determined by ICS flow cytometry. **(J)** T-bet-deficient or pLenti-Crispr-  
10 V2-EV-transduced NK-92 single-cell clones were transduced with retroviruses generated from  
11 pLZRS-ires- $\Delta$ NGFR plasmids containing no insert (EV), or the WT, K314R or Mut *TBX21*  
12 cDNA with a C-terminal Flag-tag. They were stimulated with 50 pg/mL IL-12 and 10 ng/mL IL-  
13 18 or were left untreated. Intracellular IFN- $\gamma$  production was determined by ICS flow cytometry.  
14 **(K)** Naïve CD4<sup>+</sup> T cells were expanded under T<sub>H</sub>0 conditions, and were then transduced with  
15 retroviruses generated from pLZRS-ires- $\Delta$ NGFR plasmids containing no insert (EV), or the WT,  
16 or Mut *TBX21* cDNA with a C-terminal Flag-tag. Transduced cells were positive for the  
17 bicistronic marker CD271. Cells were surface-stained for CD271 and intracellularly stained for  
18 IFN- $\gamma$  and TNF- $\alpha$  and subjected to flow cytometry analysis. **(L)** All variants (including the only  
19 homozygous variant – Mut, and 36 heterozygous variants, as indicated) from the HGID in-house  
20 database are represented on the schematic illustration. **(M)** All in-house *TBX21* variants, as in (L),  
21 were tested for transcriptional activity in the human *IFNG* luciferase reporter assay. pCMV6  
22 plasmids containing variants were used to transfect cells, together with the human *IFNG* reporter

plasmids or the control EV pGL4.10-EV luciferase reporter plasmid, and the pRL-SV40 *Renilla* plasmid for luciferase activity measurement.

In (H, I and M), bars represent the mean and the standard deviation. Dots represent individual samples or technical replicates. One-way ANOVA was used for analysis in (H). Two-way ANOVA was used for analysis in (I). A non-parametric analysis for comparison with pGL4.10-p*IFNG* + pCMV6-WT *TBX21* transfection condition was performed in (M). In (H, I and M), \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ .

**Figure S2. The patient has autosomal recessive complete T-bet deficiency, Related to**

**Figure 2.** (A) CD4<sup>+</sup> T cells from healthy donors (CTL), the heterozygous father (WT/M) or P (M/M) were expanded with anti-CD3/CD28 antibody-coated beads. They were subjected to RT-qPCR for *TBX21* with two different probes. Data are displayed as  $2^{-\Delta\Delta C_t}$  after normalization relative to *GUS* (endogenous control) expression, using the control mean as a calibrator. (B) *TBX21* expression was assessed in immortalized T cells (HVS-T) from healthy donors (CTL), the heterozygous father (WT/M) and the patient (M/M), by RT-qPCR, as in (A). (C) P-derived HVS-T cells (M/M) were retrovirally transduced with EV or WT *TBX21* containing a bicistronic CD271 surface marker. Intracellular T-bet and IFN- $\gamma$  production by these cells and by CTL and heterozygous *TBX21* WT/M HVS-T cells was assessed by flow cytometry in the presence and absence of P/I stimulation. (D) Expression of PSMD9 and ARHGAP27 (SH3 domain-containing 20 protein) in HEK293T cells transfected with pTRIP-mCherry-2A-*PSMD9* or *ARHGAP27*-V5 plasmids along with VSVG and Pax2 plasmids, as assessed by immunoblotting with an anti-V5 tag Ab. (E) Expression of ERN1 in HEK293T cells transfected with pLenti-EV or WT-*ERN1*-Cter-GFP plasmids along with VSVG and Pax2 plasmids, as assessed by immunoblotting with

anti-GFP or anti-ERN1Ab. **(F)** Flow cytometry analysis of the intracellular production of IFN- $\gamma$  in control (CTL) HVS-T cells, M/M HVS-T cells from P, and in M/M HVS-T cells transduced with pTRIP-mCherry-2A-plasmids containing WT *PSMD9* or WT *ARHGAP27*, or empty vector. **(G)** Flow cytometry analysis of IFN- $\gamma$  intracellular production in control (CTL) cells, P's HVS-T cells (M/M), and P's HVS-T cells transduced with pLenti-Cter-GFP-2A-puro containing the WT *ERN1* or empty vector. **(H and I)** Flow cytometry analysis of intracellular IFN- $\gamma$  production in control (CTL) cells, M/M HVS-T cells from P, or M/M HVS-T cells retrovirally transduced with EV or WT T-bet (MM+EV or MM+WT) or MM+WT HVS-T cells lentivirally transduced with the cDNA of EV or WT *PSMD9*, *ARHGAP27* (H) and *ERN1* (I). **(J)** Isolated CD4<sup>+</sup> T cells from a healthy donor, a *TBX21* WT/M parent and the *TBX21* homozygous M/M P were expanded with anti-CD3/CD28 antibody-coated beads under T<sub>H</sub>0 or T<sub>H</sub>1 conditions. After 7d, EV or WT T-bet plasmids were used to transduce P's cells in the presence of a bicistronic CD271 surface reporter. Cells were subjected to ICS for IFN- $\gamma$  and TNF- $\alpha$  in response to P/I restimulation. The gating strategy for the experiment is shown. **(K)** Cells transduced as in (J) were isolated by MACS with anti-CD271 antibody-coated beads. Together with non-transduced cells, they were re-stimulated with anti-CD3/CD28 antibody-coated beads, and their culture supernatants were subjected to ELISA for IFN- $\gamma$  and TNF- $\alpha$ .

In (A and B), bars represent the mean and the standard deviation; in (K), bars represent the mean and the standard error of the mean. Dots represent individual samples. One-way ANOVA was used for analysis in (B), comparing WT/M or M/M to CTL. ns = not significant.

**Figure S3. T-bet deficiency results in a normal distribution of dendritic cell subsets, ILCs, and V $\delta$ 1<sup>+</sup>  $\gamma\delta$  T cells, but alters the development of NK cells, iNKT, MAIT and V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells, Related to Figure 4.** (A) PBMCs were immunophenotyped with CyTOF. The gating

1 strategy for different immune subsets in a healthy control is indicated. **(B)** Percentages of pDCs,  
2 cDC1 cells and cDC2 cells among adult healthy donors (CTL), age-matched controls (Age-  
3 matched CTL), IL-12R $\beta$ 1-deficient MSMD patients (*IL12RB1* M/M) and P (*TBX21* M/M), as  
4 indicated in (A). **(C)** PBMCs from P and a healthy control were subjected to the FACS  
5 immunophenotyping of innate lymphoid cells (ILCs) and NK cells. CD117<sup>+</sup> ILCs, consisting  
6 principally of ILC precursors (ILCP) and type 2 ILC (ILC2) cells, were gated manually on  
7 conventional flow cytometry. The gating strategy is shown. **(D)** Total NK cells (CD16<sup>+</sup> or  
8 CD94<sup>+</sup>Lin<sup>-</sup>CD7<sup>+</sup> cells) were plotted with CD56 against CD127 for manual gating on different  
9 NK cell subsets. **(E)** Percentage of ILC precursor and ILC2 cells in adult healthy donors (CTL),  
10 age-matched controls (Age CTL), IL-12R $\beta$ 1-deficient MSMD patients (*IL12RB1* M/M) and P  
11 (*TBX21* M/M), summarized from CyTOF data, as in (A). **(F)** Immunophenotyping of ILC2 and  
12 ILCP cells, analyzed by fluorescence flow cytometry. Plot of ILC2 and ILCP, and the percentage  
13 of ILC2 and ILCP (within live CD45<sup>+</sup> cells) in a healthy donor (CTL), the T-bet-deficient patient  
14 (M/M) and his age/ethnicity-matched healthy brother (WT/WT bro). **(G)** PBMCs from a CTL,  
15 IL-12R $\beta$ 1-deficient MSMD patients (*IL12RB1* M/M), the *TBX21* WT/M parents (WT/M mo or  
16 fa), the T-bet-deficient P (M/M) and his age/ethnicity-matched healthy brother (WT/WT bro)  
17 were stained with surface markers for lymphocytes involved innate immunity (MAIT and iNKT  
18 cells) or both adaptive and innate immunity ( $\gamma\delta$  T cells, in particular V $\delta$ 1 and V $\delta$ 2  $\gamma\delta$  T cells).  
19 The gating strategy for flow cytometry is shown. **(H - J)** iNKT (H), MAIT (I) and V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T (J)  
20 cells are shown for P and his relatives. **(K)** Percentages of V $\delta$ 1<sup>+</sup>  $\gamma\delta$  T cells in healthy adult  
21 donors (CTL), age-matched controls (Age CTL), IL-12R $\beta$ 1-deficient MSMD patients (*IL12RB1*  
22 M/M) and P (*TBX21* M/M), his age/ethnicity-matched healthy brother (*TBX21* WT/WT) and his  
23 healthy parents (*TBX21* WT/M).

In (B and E), bars represent the mean and the standard deviation. In (K), bars represent the mean and the standard error of the mean. Dots represent individual samples. Mann-Whitney nonparametric tests were used for analysis in (K). In (K), \*\*  $p < 0.01$ .

**Figure S4. Preserved T<sub>H</sub>2, T<sub>H</sub>17 and circulating T<sub>FH</sub> memory CD4<sup>+</sup> T cells in T-bet**

**deficiency, Related to Figure 4.** (A) Naïve (CD45RA<sup>+</sup>CCR7<sup>+</sup>), central memory (CM, CD45RA<sup>-</sup>CCR7<sup>+</sup>), effector memory (EM, CD45RA<sup>-</sup>CCR7<sup>-</sup>) and T<sub>EMRA</sub> (CD45RA<sup>+</sup>CCR7<sup>-</sup>) cells were manually gated from CD8<sup>+</sup> T cells on CyTOF data. The percentages of these cells are shown. (B) 10,000 total CD8<sup>+</sup> T cells were clustered by viSNE, based on a selection of surface markers. Manual gating of naïve and memory subsets overlaid on clusters of CD8<sup>+</sup> T cells. CXCR3 and CD38 expression are indicated on this plot. (C) Naïve (CD45RA<sup>+</sup>CCR7<sup>+</sup>), central memory (CM, CD45RA<sup>-</sup>CCR7<sup>+</sup>), effector memory (EM, CD45RA<sup>-</sup>CCR7<sup>-</sup>) and T<sub>EMRA</sub> (CD45RA<sup>+</sup>CCR7<sup>-</sup>) cells were manually gated from CD4<sup>+</sup> T cells on the basis of CyTOF data. The percentages of these cells are shown. (D) The gating strategy for the CyTOF analysis of memory CD4<sup>+</sup> T cells and T<sub>H</sub>1, T<sub>H</sub>2, T<sub>H</sub>17 and T<sub>H</sub>1\* cells is shown. (E) Memory CD4<sup>+</sup> T cells (CD45RA<sup>-</sup>) from indicated individuals were gated out manually, and further subjected to viSNE deconvolution with T-cell chemokine markers for 10,000 randomly selected events. The expression of CXCR3, CCR5, CCR6 and CCR4 is shown. (F and G) Percentages of T<sub>H</sub>2 (CCR6<sup>-</sup>CXCR3<sup>-</sup>CCR4<sup>+</sup>) (F) and T<sub>H</sub>17 cells (CCR6<sup>+</sup>CXCR3<sup>-</sup>CCR4<sup>+</sup>) (G) among memory CD4<sup>+</sup> T cells. (H) Frequencies of the circulating follicular T helper (cT<sub>FH</sub>) cells and their subsets (CXCR3<sup>+</sup>, double-negative as DN, CCR6<sup>+</sup>, or CXCR3<sup>+</sup>CCR6<sup>+</sup> T<sub>FH</sub>) from healthy donors (CTL) and the T-bet-deficient patient (M/M) gated from conventional flow cytometry data. (I) Percentages of Treg cells gated from CyTOF data. (J) Percentages of CXCR3<sup>+</sup> and CCR5<sup>+</sup>CXCR3<sup>-</sup> Tregs gated from CyTOF data.

In (A, C, F, G, H, I and J), bars represent the mean and the standard error of the mean. Dots represent individual samples.

**Figure S5. *Ex vivo* and *in vitro* non-mycobacterial stimulation of the residual lymphocytes**

**from the patient with T-bet deficiency, Related to Figure 5. (A)** Gating strategy for the

surface and intracellular staining of PBMCs *ex vivo* stimulated with P/I, for an age-matched

control sample. **(B)** Intracellular staining of IFN- $\gamma$  and T-bet in NK cells, as shown in (A), from

an age-matched control (Age CTL), P (M/M) and P's healthy brother (WT/WT) stimulated with

P/I. **(C)** Frequencies of TNF- $\alpha$ -producing cells among CD56<sup>+</sup> NK cells, as gated in (A), in the

indicated individuals. **(D)** The gating strategy for gating total NK cells from a travel control

(CTL), P (M/M), P's brother (WT/WT) and P's heterozygous mother (WT/M), in the presence

and absence of stimulation with IL-12, IL-15 and IL-18, is shown. **(E)** Expression of CD107a

and IFN- $\gamma$  among total NK cells, as in (D). **(F)** Frequencies of IFN- $\gamma$ -producing cells among

iNKT cells, as gated in (A) for the indicated individuals. **(G and H)** V $\delta$ 1<sup>+</sup>V $\delta$ 2<sup>-</sup>  $\gamma\delta$  T cells were

gated from PBMCs prepared as shown in (A). Intracellular staining for IFN- $\gamma$  (G) and TNF- $\alpha$  (H)

is shown. **(I and J)** CD8<sup>+</sup> T cells were gated from PBMCs prepared as shown in (A). Intracellular

staining for IFN- $\gamma$  (I) and TNF- $\alpha$  (J) is shown. **(K - R)** Naïve and memory CD4<sup>+</sup> T cells from

controls (CTL) and P (M/M) were sorted by FACS and activated/expanded. Intracellular staining

of IL-22 (K), IL-9 (L) and IL-21 (R). Production of IL-9 (M), IL-4 (N), IL-5 (O), IL-10 (P) and

IL-13 (Q), as measured in culture supernatants. **(S and T)** Naïve CD4<sup>+</sup> T cells were isolated by

FACS and subjected to activation under T<sub>H</sub>0, T<sub>H</sub>9, T<sub>H</sub>17 or T<sub>H</sub>9 conditions combined with T<sub>H</sub>17

conditions. The production of IL-9 was assessed intracellularly (S) and in culture supernatants

(T). **(U)** Naïve CD4<sup>+</sup> T cells were isolated by FACS and subjected to activation under T<sub>H</sub>0, T<sub>H</sub>1

or T<sub>H</sub>2 conditions. IL-10 production, determined with culture supernatants, is shown. (V) Naïve CD4<sup>+</sup> T cells were isolated by FACS and subjected to activation under T<sub>H</sub>0, T<sub>H</sub>2 or T<sub>H</sub>9 conditions. IL-13 production, as measured on culture supernatants.

In (C, F - V), bars represent the mean and the standard error of the mean. Dots represent individual samples. Mann-Whitney non-parametric tests were used for analysis in (C, F - J). In (C, F - J), \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ , ns = not significant.

**Figure S6. T-bet deficiency alters immunity to *M. bovis* BCG, Related to Figure 6. (A and B)**

PBMCs from adult controls (CTL), age-matched controls (Age CTL), P (M/M), P's healthy brother (WT/WT), and P's healthy parents (WT/M) were left unstimulated or stimulated with live *M. bovis* BCG in the presence and absence of IL-12 and IL-23. The production of TNF- $\alpha$  (A) and IL-5 (B) in culture supernatants was assessed with Legendplex cytometric bead arrays. (C) PBMCs from P (M/M), P's healthy brother (WT/WT) and one of P's healthy parents (WT/M), stimulated as in (A), were analyzed for the intracellular expression of T-bet and IFN- $\gamma$  by flow cytometry. Percentages of T-bet<sup>+</sup> IFN- $\gamma$ <sup>+</sup> cells within live single lymphocytes are highlighted. (D) Pie-charts of the percentages of the indicated immune subsets in PBMCs from healthy controls stimulated with IL-12 or IL-23 alone. (E and F) PBMCs from adult controls (CTL), age-matched controls (Age CTL), P (M/M), P's healthy brother (WT/WT), and P's healthy parents (WT/M) were left unstimulated or were stimulated with live *M. bovis* BCG in the presence and absence of IL-12 and IL-23. Frequencies of T-bet<sup>+</sup> IFN- $\gamma$ <sup>+</sup> cells gated on each individual immune subset, including NK, iNKT, MAIT, V $\delta$ 2<sup>+</sup>  $\gamma$  $\delta$  T (Vd2), V $\delta$ 1<sup>+</sup>  $\gamma$  $\delta$  T (Vd1) and V $\delta$ 1<sup>+</sup> V $\delta$ 2<sup>+</sup>  $\gamma$  $\delta$  T (Vd1-Vd1-gdT) cells from different groups of individuals, stimulated with BCG (E) and BCG + IL-23 (F), are shown. (G) Frequencies of T-bet<sup>+</sup> IFN- $\gamma$ <sup>+</sup> cells gated on adaptive immune subsets, including

1 B, CD4<sup>+</sup> T and CD8<sup>+</sup> T cells from different groups of individuals, stimulated with BCG + IL-23.

2 **(H)** Dot-plots showing the expression of intracellular T-bet and IFN- $\gamma$  gated on CD4<sup>+</sup> T cells

3 with and without BCG stimulation in the presence and absence of IL-12 and IL-23. **(I)**

4 Frequency of V $\delta$ 2<sup>+</sup>  $\gamma\delta$  T cells in PBMCs from different groups of individuals expanded with

5 BCG-lysates for 14 d. **(J)** Dot-plots showing intracellular T-bet and IFN- $\gamma$  levels in non-

6 responsive and PPD-responsive CD4<sup>+</sup> T cells following PPD stimulation. **(K and L)** Dot-plots

7 showing intracellular T-bet and IFN- $\gamma$  levels in non-responsive and BCG-lysates-responsive

8 CD4<sup>+</sup> T **(K)** and CD8<sup>+</sup> T cells **(L)**.

9 In (A, B, E, F, G and I), bars represent the mean and the standard error of the mean. In (D),

10 numbers represent the mean and the standard deviation. Dots represent individual samples.

11 Mann-Whitney nonparametric tests were used for analysis in (A, B, E, F and G), \*  $p < 0.05$ , \*\*  $p$

12  $< 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ , ns = not significant.

13



## **STAR Methods**

## **RESOURCE AVAILABILITY**

### **Lead Contact**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Jean-Laurent Casanova (casanova@mail.rockefeller.edu).

### **Materials Availability**

All unique/stable reagents generated in this study are available from the Lead Contact with a completed Materials Transfer Agreement with Inserm or the Rockefeller University.

### **Data and Code Availability**

The whole-exome sequencing, RNA-seq and omni-ATAC-seq datasets generated during this study are available at the Sequence Read Archive (SRA) (PRJNA641463). The scRNA-seq datasets generated during this study are available at the EGA European Genome-Phenome Archive (EGAS00001004504). This study did not generate any unique code. Any other piece of data will be available upon reasonable request.

## 1 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

### 2 **Human subjects**

3       The patient and the relatives studied here were living in and followed up in Morocco. The  
4 study was approved by and performed in accordance with the requirements of the institutional  
5 ethics committee of Necker Hospital for Sick Children, Paris, France, and the Rockefeller  
6 University, New York, USA. Informed consent was obtained for the patient, his relatives, and  
7 healthy control volunteers enrolled in the study. This study was also approved by the Sydney  
8 Local Health District RPAH Zone Human Research Ethics Committee and Research Governance  
9 Office, Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia (protocol X16-  
10 0210/LNR/16/RPAH/257). Written informed consent was obtained from participants or their  
11 guardians. Informed consent from participants in Switzerland was approved by the local ethical  
12 committee (CE3428 authorized by Comitato Etico Cantonale, [www.ti.ch/CE](http://www.ti.ch/CE)). Experiments using  
13 samples from human subjects were conducted in the United States, France, Australia and  
14 Switzerland, in accordance with local regulations and with the approval of the IRBs of  
15 corresponding institutions. Plasma samples from unrelated healthy subjects used as controls for  
16 antibody profiling by phage immunoprecipitation-sequencing (PhIP-Seq) were collected at Sidra  
17 Medicine in accordance with a study protocol approved by the Clinical Research Ethics Board of  
18 Sidra Medicine.

19       Case report of the patient (P) - P was born in 2015 to Moroccan first-cousin parents. He  
20 was vaccinated with BCG at the age of three months. After vaccination, he developed fever and  
21 left axillary lymphadenopathy accompanied by a cutaneous eruption. He received amoxicillin for  
22 10 days, resulting in clinical improvement. At the age of six months, he was hospitalized for  
23 persistent fever, weight loss, cutaneous erythema, ear drainage and persistent oral thrush.

1 Axillary adenopathy was present, with fistulation associated with hepatosplenomegaly and  
2 multiple abdominal adenopathies. The patient was treated with four antimycobacterial drugs; he  
3 displayed a good clinical response to 18 months of treatment and has been off antibiotics for 15  
4 months. He was also found to have mild cytomegalovirus (CMV) viremia at the time (CMV PCR  
5 3.74 log/mL, threshold < 2.4 log/mL), and was treated with ganciclovir. Serological PCR tests  
6 for CMV were negative at the age of two years, but weakly positive at the age of three years  
7 (IgM anti-CMV weakly positive, CMV PCR 3.62 log/mL, threshold < 2.4 log/mL). However,  
8 the patient showed no characteristic features of CMV infection and did not present clinically  
9 confirmed CMV disease. At the age of 11 months, he was hospitalized for four days due to  
10 airway hyperresponsiveness requiring treatment with inhaled steroids and albuterol. The patient  
11 had a high blood eosinophil count, documented since the age of since months (1,460/mm<sup>3</sup>). By  
12 the age of 17 months, he had persistent upper respiratory tract inflammation, with manifestations  
13 of persistent rhinorrhea, difficult respiration, wheezing and coughing that improved on treatment  
14 with oral steroid and salbutamol. The patient has since been treated with inhaled steroids for  
15 persistent upper respiratory tract inflammation. The clinical manifestations affecting the upper  
16 respiratory tract persisted at the age of three years. No trigger was identified for these symptoms  
17 and signs, which were not triggered by exercise, activity, feeding or parasitic infections. A serum  
18 IgE allergen screen was negative for more than 250 allergens tested. The patient requires inhaled  
19 steroid therapy (fluticasone spray) every morning and evening. He also frequently visits the  
20 emergency room, and is hospitalized about once per month for exacerbations requiring oral  
21 steroid (beta-methasone) and inhaled salbutamol. The patient has been vaccinated for *Tetanus*,  
22 *Diphtheria*, and *Haemophilus B*, for all of which positive serum IgG result have been obtained.  
23 He also tested positive for anti-HSV-1 IgG, anti-EBV IgG, anti-CMV IgG, anti-mumps IgG and

anti-parainfluenza IgG in clinical serology examinations. No anatomical abnormalities of the airways were noted. The patient has a brother who is healthy, with no history of severe infectious disease or airway hyperresponsiveness. The genetic cause of the patient's airway hyperresponsiveness will be studied separately.

## **Cell lines**

HEK293T and Phoenix A retroviral packaging cells were cultured in IMDM (Gibco) supplemented with 10% fetal bovine serum (FBS). B cells from the patient or controls were immortalized in-house with Epstein Barr virus (EBV-B cells) and cultured in RPMI 1640 (Gibco) supplemented with 10% FBS. NK-92 cells were cultured in RPMI 1640 (Gibco) supplemented with 10% FBS, 1 mM sodium pyruvate, 200 U/mL recombinant proleukin IL-2 (PROMETHEUS). *Herpesvirus saimiri*-transformed T (HVS-T) cells were generated with either *H. saimiri* strain C488 for transformation or the TERT transformation system (Wang et al., 2016). HVS-T cells were cultured in Panserin/RPMI 1640 (ratio 1:1) supplemented with 20% FBS, L-glutamine, gentamycin and 20 U/mL human rIL-2 (Roche). Isolated CD4<sup>+</sup> T cells were cultured in X-vivo 15/gentamycin/L-glutamine (Lonza) supplemented with human AB Serum (GemCell). Peripheral blood mononuclear cells (PBMCs) were cultured in RPMI 1640 supplemented with 10% FBS for stimulation experiments. All cell lines used tested negative for mycoplasma. HEK293T, Phoenix A and NK-92 cells were purchased from the ATCC.

## METHOD DETAILS

### Phage immunoprecipitation-sequencing (PhIP-Seq)

Patient serum and control samples were analyzed in duplicate by phage immunoprecipitation-sequencing (Hernandez et al., 2018; Xu et al., 2015). We used 10% liquid IVIg from pooled human plasma (Privigen® CSL Behring AG), human IgG-depleted serum (Supplier No HPLASERGFA5ML, Molecular Innovations, Inc.), and plasma samples from two unrelated healthy adult subjects and one unrelated healthy three-year-old boy for comparison. PhIP-Seq was carried out as previously described (Xu et al., 2015), but with the following modifications. We determined levels of total IgG in the serum or plasma samples with the Human IgG total ELISA Ready-SET-Go kit (Thermo Fisher Scientific) and incubated diluted samples containing approximately 4 µg of total IgG at 4°C overnight with  $2 \times 10^{10}$  plaque-forming units (PFUs) of a modified version of the original VirScan phage library (Xu et al., 2015). Specifically, the T7 phage library used here for peptide display contained the same viral peptides as the original VirScan phage library plus additional peptides derived from protein sequences of various microbial B-cell antigens available from the IEDB ([www.iedb.org](http://www.iedb.org)). For the computational analysis and background correction, we also sequenced the phage library before (input library sample) and after immunoprecipitation with beads alone (mock IP). We performed a number ( $n = 46$ ) pf technical repeats. Single-end sequencing was performed with the NextSeq500 system (Illumina), to generate approximately two million reads per sample and ~20 million reads for the input library samples. As previously described (Xu et al., 2015), reads were mapped onto the original library sequences with Bowtie 2 and read counts were adjusted according to library size. A zero-inflated generalized Poisson model was used to estimate the  $p$ -values, to reflect enrichment for each of the peptides. We considered peptides to be significantly

enriched only if the  $-\log_{10} p$ -value was at least 2.3 in all replicates. Species-specific score values were computed for each serum or plasma sample, by counting the significantly enriched peptides for a given species with less than a continuous seven-residue subsequence, the estimated size of a linear epitope, in common. We corrected for unspecific binding of peptides to the capture matrix, by also calculating species-specific background score values by counting the peptides displaying enrichment to the 90th percentile of the mock IP samples. These peptides were used for background subtraction.

#### **Whole-exome sequencing (WES), genotyping and genome-wide linkage (GWL) analysis**

All four members of the family tested were subjected to genomic DNA extraction followed by WES. Exome capture was performed with the SureSelect Human All Exon V4+UTR and SureSelect Human All Exon V6 (Agilent Technologies). Paired-end sequencing was performed on a HiSeq 2000 (Illumina), generating 100-base reads. We used the Genome Analysis Software Kit (GATK) (version 3.4–46) best-practice pipeline to analyze our WES data (Li et al., 2009; McKenna et al., 2010). Reads were aligned with the human reference genome hg19, with BWA (Li and Durbin, 2009). PCR duplicates were removed with Picard tools ([picard.sourceforge.net/](http://picard.sourceforge.net/)). The GATK base quality score recalibrator was applied to correct sequencing artifacts. The GATK HaplotypeCaller was used to identify variant calls (Depristo et al., 2011; McKenna et al., 2010). Variants were annotated with SnpEff ([snpeff.sourceforge.net/](http://snpeff.sourceforge.net/)) (Cingolani et al., 2012).

All four members of the family were genotyped with Genome-Wide Human SNP Array 6.0 data. Genotype calling was achieved with the Affymetrix Power Tools Software Package (<https://www.affymetrix.com/support/developer/powertools/changelog/index.html>). Parametric

1 multipoint linkage analysis was performed with MERLIN 1.1.2 software (Abecasis et al., 2002),  
2 assuming autosomal recessive inheritance with complete penetrance and a damaging allele  
3 frequency of  $1 \times 10^{-4}$ . Allele frequencies were estimated for 562,685 SNP markers, with family  
4 founders and HapMap CEU trios. Markers were clustered using an  $r^2$  threshold (--rsq parameter)  
5 of 0.4. The genetic variant of interest was confirmed by PCR amplification of the region  
6 (forward primer: 5'-gtgaggactacgcgctacc-3', and reverse primer: 5'-cagaagcattgtcgagccag-3')  
7 followed by Sanger sequencing.

### 9 **cDNA sequencing**

10 We used cDNA sequencing to characterize the consequences of the patients' variant. In  
11 brief, total RNA was extracted from EBV-B cells or PBMCs from the patient and healthy donors  
12 (Qiagen). We synthesized cDNA from mRNA, using Superscript III (Thermo Fisher Scientific).  
13 A region spanning the mutation site was amplified by PCR with the forward (5'-  
14 tagaagccaggcgtcagagc-3') and reverse R4 (5'-ctcggcattctggtaggcag-3'), R5 (5'-  
15 gcaatgaactgggtttcttgg-3'), or R6 (5'-gactcaaagttctcccggatcc-3') primers. PCR amplicons were  
16 sequenced with the BigDye Terminator Cycle Sequencing Kit (Thermo Fisher Scientific).  
17 Alternatively, cDNA generated from patient or control PBMCs was amplified by PCR with the  
18 forward primer 5'-tctacactctttccctacacgacgctcttccgatctgtgaggactacgcgctacc-3' and reverse  
19 primer 5'-gtgactggagttcagacgtgtgctcttccgatctgcaatgaactgggtttcttgg-3', to amplify a region  
20 spanning the mutation in a first round of PCR. A second round of PCR was then performed on  
21 the PCR products from the first PCR as a template. We used the forward primer 5'-  
22 aatgatacggcgaccaccgagatctacactctttccctacacgac-3' and the reverse primers 5'-  
23 caagcagaagacggcatcacgagatcgattcgggtgactggagttcagacgtgtg-3', 5'-

1 caagcagaagacggcatacagatctcctagagtgactggagttcagacgtgtg -3', and 5' -

2 caagcagaagacggcatacagattagttgcggtgactggagttcagacgtgtg -3', to barcode two healthy control  
3 cDNAs and the patient's cDNA. Adaptor and barcoded PCR amplicons were mixed and purified,  
4 and used for next-generation sequencing by Nano Format 350 paired-end MiSeq sequencing  
5 (illumina). We aligned MiSeq data to the reference genome with RNA-STAR aligner (Afgan et  
6 al., 2016; Dobin et al., 2013). The aligned sequencing data were then viewed with Integrative  
7 Genome Browser (Robinson et al., 2011).

#### 9 **Reverse transcription-quantitative PCR (RT-qPCR)**

10 Total RNA was extracted from cells with the RNeasy Kit (Qiagen) or the Quick-RNA kit  
11 (Zymo Research). Reverse transcription was performed with the SuperScript III enzyme  
12 (Thermo Fisher Scientific). Messenger RNAs were quantified with the cDNA as a template and  
13 the *TBX21* (Probe 1: Hs00894393 and Probe 2: Hs00894391), *IFNG* (Hs99999041), *TNF*  
14 (Hs01113624), *IL5* (Hs01548712) (Thermo Fisher), *GUSB* (Thermo Fisher) probes.

#### 16 **T-bet/*TBX21* overexpression**

17 A wild-type (WT) *TBX21* plasmid with a pCMV6 backbone was purchased from Origene  
18 (RC207902). The genetic variants of *TBX21* studied here, and the variants described in gnomAD  
19 (<http://gnomad.broadinstitute.org/>) or in our in-house database were introduced into pCMV6-WT  
20 *TBX21* by site-directed mutagenesis PCR with the CloneAmp HiFi PCR Premix (Takara Bio).  
21 *TBX21* WT plasmids or plasmids containing variants were used to transfect HEK293T cells in  
22 the presence of Lipofectamine 2000 reagent (Thermo Fisher Scientific) to achieve  
23 overexpression. The WT or mutant (Mut) *TBX21* was overexpressed in *in vitro*-expanded T cells,



HVS-T cells, or NK-92 cells, as previously described (Martínez-Barricarte et al., 2016). In brief, the *TBX21* plasmid was subcloned into pLZRSires-ΔNGFR (Addgene) (Van De Vosse et al., 2009). We then used pLZRS containing the WT or Mut *TBX21* to transfect Phoenix A cells, and retroviruses were produced and concentrated with Retro-X (Takara Bio). Concentrated retrovirus preparations were used for retroviral transduction.

## **Immunoblotting**

Proteins were solubilized in RIPA buffer containing 10 mM Tris-Cl (pH 8.0), 1 mM EDTA, 0.5 mM EGTA, 1% Triton X100, 0.1% sodium deoxycholate, 0.1% SDS and 150 mM NaCl supplemented with protease-inhibitor cocktail (Complete mini, Roche). Alternatively, nuclear proteins from HEK293T transfectants were purified with the NE-PER Nuclear Extraction kit in accordance with the manufacturer's procedures (Thermo Fisher Scientific). Proteins were quantified with the Bradford assay. Equal amounts of lysate (5 µg to 50 µg of) were mixed with SDS sample buffer and boiled for 5min at 95°C before being subjected to electrophoresis in 12% acrylamide SDS-PAGE gels. The following antibodies were used for immunoblotting: mouse anti-human T-bet 4B10 monoclonal Ab (BioxCell), rabbit anti-human T-bet polyclonal Ab (Proteintech), anti-Flag-M2 HRP-conjugated Ab (Sigma-Aldrich), anti-α-tubulin HRP-conjugated Ab (Proteintech), anti-lamin A/C Ab (Santa Cruz), anti-GAPDH HRP-conjugated Ab (Santa Cruz), anti-STAT1 monoclonal Ab (BD Biosciences), sheep ECL anti-mouse IgG HRP-conjugated Ab (GE Healthcare), and ECL anti-rabbit IgG HRP-conjugated Ab (GE Healthcare).

## **Electrophoretic mobility shift assays (EMSA)**

HEK293T cells were transfected with empty plasmid (EV), WT or mutant *TBX21* pCMV6 plasmids. After 2 days, nuclear extracts were obtained from the cells with the NE-PER Nuclear Extraction Kit (Thermo Fisher Scientific). EMSA was performed as previously described (Liu et al., 2011). In brief, <sup>32</sup>P was used to label consensus T-box responsive element (TBRE) duplexes of DNA oligos (WT oligo: 5'-gataatttcacacctaggtgtgaaatt-3', Mut Oligo as control: 5'-gataatttcacgtctaggtacgaaatt-3' and 5'-gataatttcgtacctagacgtgaaatt-3'). Unlabeled WT TBRE duplex was used as non-radioactive competitive probe. We mixed 10 µg of nuclear extract from each condition or 80 µg (8x) of nuclear extract from Mut *TBX21* transfectants with or without unlabeled probe, 2 µg mouse IgG1 control (BioxCell) or 2 µg mouse anti-T-bet 4B10 monoclonal Ab (BioxCell) and incubated for 30 min. <sup>32</sup>P-labeled WT or Mut TBRE duplex was then added and the mixture was incubated for 30 min on ice in the presence of poly-dIdC (Sigma-Aldrich). Samples were subjected to polyacrylamide gel electrophoresis. The gel was dried and placed against X-ray film for 2 days, after which the radioactive signal was read on a Typhoon platform.

## **Luciferase reporter assays**

HEK293T cells were transfected with the indicated expression plasmids, firefly luciferase plasmids under the control of WT or Mut *TBRE* (Janesick et al., 2012), as previously described, or with human *IFNG* promoters in a pGL4.10 backbone (-565 - +85 of *IFNG*), and with a constitutively expressed *Renilla* luciferase plasmid for normalization (pRL-SV40). Cells were transfected in the presence of Lipofectamine 2000 (Thermo Fisher Scientific) for 3 days. Luciferase levels were measured with Dual-Glo reagent, according to the manufacturer's protocol (Promega). Firefly luciferase values were normalized against

*Renilla* luciferase values, and fold-induction is reported relative to controls transfected with empty plasmid.

#### **Generation and analysis of T-bet-deficient NK-92 cells**

T-bet-deficient NK-92 cells were generated according to a protocol described elsewhere (Sanjana et al., 2014). We used oligonucleotides for gRNA9 and gRNA15 (gRNA9\_Forward: 5'-caccgtccaacaatgtgacccaggt-3', gRNA9\_Reverse: 5'-aacacctgggtcacattgttgac-3'; gRNA15\_Forward: 5'-caccgccgcactcaccgtcctgct-3', gRNA15\_reverse: 5'-aacacagcaggacggtgagtgcggc-3'). DNA duplex was annealed from the oligonucleotides above, and was inserted into pLenti-CRISPR-V2 plasmids (Addgene) (Sanjana et al., 2014). We then used pLenti-CRISPR plasmids, VSV-G envelope, and psPAX2 plasmids to transfect HEK293T cells in the presence of Lipofectamine 2000. Supernatants containing lentiviruses were harvested and concentrated with Lenti-X concentrator (Takara Bio). We resuspended  $10^6$  NK-92 cells with 20x concentrated lentiviruses for gRNA9, gRNA15 or empty plasmid, in the presence of proleukin IL-2 (200 U/mL) (PROMETHEUS). After incubation for 2 days, we added 3.75  $\mu$ g/mL puromycin to the culture for the selection of stably transduced NK-92 cells. After a further two weeks, cells were harvested for the Surveyor Nuclease Assay (Integrated DNA Tech) and a three-primer PCR-based screening system (Harayama and Riezman, 2017) to confirm successful DNA editing. Cells were plated at a density of 0.7 cells in 200  $\mu$ L per well in a U-bottomed 96-well plate. Two weeks later, single-cell clones were screened with a three-primer PCR-based system, with Phire Direct PCR Master Mix (Thermo Fisher Scientific). The primers used for this screening assay were gRNA9\_Forward: 5'-agcccctaccctaattcct-3', gRNA9\_Reverse: 5'-ggcatctattcctgggacc-4', gRNA9\_Reverse\_in: 5'-cttttgaagagcaggtcctacct-3'

and gRNA15\_Forward: 5'-GCCTGAATATGACCCCGTC-3', gRNA15\_Reverse: 5'-caccactaccaccactaaagc-3'; gRNA15\_Forward\_in: 5'-acagagatgatcatcaccaagca-3'. Single-cell clones with biallelic disruptions were selected for validation by RT-qPCR and western blotting. EV-transduced single-cell clones were randomly picked as controls. Cells were then transduced with retroviruses generated from pLZRS-ires-ΔCD271 plasmids containing EV, WT, Mut or K314R *TBX21*, as described above (Martínez-Barricarte et al., 2016). Stably transduced cells were isolated with anti-CD271 antibody-coated beads (Miltenyi Biotec). Cells were stimulated with 50 pg/mL IL-12 (R&D) and 10 ng/mL IL-18 (InvivoGen), or were left unstimulated, and all cells were incubated for one day. The IFN-γ content of the supernatants was determined by ELISA. Intracellular IFN-γ production was measured with flow cytometry with intracellular staining (ICS). In brief, cells were fixed and permeabilized (BioLegend), and were subjected to ICS staining with FcBlock (Miltenyi Biotec), anti-T-bet BV421 (BioLegend) and anti-IFN-γ PE (BioLegend) before their use for ICS flow cytometry analysis.

#### **Cytokine determinations in *TBX21*-transduced primary CD4<sup>+</sup> T cells**

PMBCs from three healthy donors were stained with FcBlock (Miltenyi Biotec), anti-CD4-APC-Cy7, anti-CD45RA-Alexa488, anti-CCR7-Alexa647 and Live-Dead exclusion Aqua. Naïve CD4<sup>+</sup> T cells were isolated by FACS. Cells were stimulated with anti-CD3/CD28 Ab Dynabeads (Thermo Fisher Scientific) at a 1:1 cell:bead ratio under T<sub>H</sub>0 or T<sub>H</sub>2 (recombinant human IL-4 12.5 ng/mL, R&D) polarizing conditions, in the presence of 100 U/mL proleukin IL-2 (PROMETHEUS). Fresh medium (X-vivo 15, 5% human AB serum) and cytokines were added every 3 days. After 7 days, cells were restimulated with Dynabeads at a 1:2 ratio (bead:cell). One day after restimulation, cells were transduced with retroviruses generated from

pLZRS-ires- $\Delta$ CD271 plasmids containing WT or Mut *TBX21*, as previously described (Martínez-Barricarte et al., 2016). Transduced cells were isolated with anti-CD271 antibody-coated beads (Miltenyi Biotec) 14 days after stimulation. Intracellular cytokine production was determined by ICS. In brief, cells were restimulated with 25 ng/mL phorbol 12-myristate 13-acetate (PMA) and 0.5  $\mu$ M ionomycin for 6 h in the presence of GolgiPlug (BD Biosciences). Cells were stained with Zombie-NIR live-dead exclusion dye (BioLegend), FcBlock (Miltenyi Biotec), and FITC-conjugated anti-CD271 antibody (BioLegend). Cells were then fixed, permeabilized and subjected to ICS staining with anti-TNF- $\alpha$ -BV510 (BioLegend), anti-IFN- $\gamma$ -PERCP/Cy5.5 (BioLegend), anti-IL-4-PE, anti-IL-13-APC antibodies, followed by flow cytometry analysis with an Aurora cytometer (Cytex).

## **HVS-T cell analysis**

We plated 200,000 HVS-T cells from healthy donors or P in a 96-well U-bottomed plate at a density of  $1 \times 10^6$  cells/mL. The plate was incubated for 2 days, and the culture supernatants and cells were harvested for ELISA or RT-qPCR for *IFNG* and *TNF*. HVS-T cells from P were transduced with retroviruses generated from pLZRS-ires- $\Delta$ CD271 plasmids containing WT *TBX21* or EV, as previously described (Martínez-Barricarte et al., 2016). After incubation for 5 days, the HVS-T cells were stimulated with 25 ng/mL PMA and 500 nM ionomycin or were left untreated; they were then incubated in the presence of GolgiPlug (BD Biosciences) for 6-8 h. Cells were stained with Zombie-NIR live-dead dye (BioLegend), FcBlock (Miltenyi Biotec), anti-CD4-BV750 (BD Biosciences), anti-CD3-BV650 (BD Biosciences), anti-CD8a-Pacific Blue (BioLegend), and anti-CD271-FITC (BioLegend) antibodies, fixed and permeabilized for ICS staining with anti-T-bet-PE (BioLegend), anti-IFN- $\gamma$ -BV711 (BioLegend), anti-TNF- $\alpha$ -BV510

(BioLegend), anti-IL-13-APC (BioLegend), anti-IL-10-PE-Dazzle594 (BioLegend) antibodies.

ICS analysis was performed on an Aurora cytometer (Cytex).

HVS-T cells were also analyzed for IFN- $\gamma$  production in response to *PSMD9*, *ARHGAP27* and *ERN1* cDNA overexpression. In brief, pTRIP-mCherry-2A-PSMD9 and ARHGAP27 were generated by subcloning from MGC clones BC004184 and BC067345 (transOMIC). A C-terminal V5 tag was added upstream from the stop codons. The pLenti-Cter-GFP-2A-puro empty vector plasmid was obtained from OriGene. The pCMV6-ERN1-Cter-Myc-Flag plasmid was obtained from OriGene. pLenti-ERN1-Cter-GFP (fusion)-2A-puro was generated by the double digestion, with *EcoRI* and *XhoI*, of pCMV6-ERN1 and pLenti-Cter-GFP-2A-puro, followed by the isolation of the ERN1 fragment by gel extraction, and T4 ligation. Lentiviruses containing cDNAs encoding EV, PSMD9, ARHGAP27, ERN1-GFP were generated in HEK293T cells by transfection with pTRIP or pLenti-GFP-2A-puro, VSV.G and Pax2 packaging plasmids. Transfected HEK293T cells were analyzed for the expression of PSMD9 and ARHGAP27 by assessing the expression of the C-terminal V5 tag and ERN1 by western blotting with anti-GFP Ab or anti-ERN1 Ab (Proteintech). Lentiviruses containing the cDNAs of EV, *PSMD9*, *ARHGAP27* and *ERN1*-GFP were used to transduce HVS-T cells derived from the cells of P (M/M), or M/M HVS-T cells retrovirally transduced with WT T-bet (M/M+WT). After 2 – 4 days of transduction, cells were stimulated with 100 ng/mL PMA and 2  $\mu$ M ionomycin for 8 h and harvested for staining for flow cytometry. ERN1-transduced HVS-T cells and their control HVS-T cells were stained with Zombie NIR Fixable Viability Dye (BioLegend) and surface staining was then performed with FcBlock, anti-CD271-PE/Dazzle594 (BioLegend), anti-CD4-BUV563 (BD Biosciences) and anti-CD8-BUV737 (BD Biosciences) antibodies. Cells were fixed and permeabilized with the FOXP3/Transcription Factor Buffer kit (Thermo Fisher

Scientific) and stained intracellularly with anti-T-bet-PE/Cy7 (BioLegend), anti-IFN- $\gamma$ -BV711 (BioLegend) and anti-TNF- $\alpha$ -BV510 (BioLegend) antibodies. For PSMD9- and ARHGAP27-transduced HVS-T cells and their control HVS-T cells, cells were stained with Zombie NIR Fixable Viability Dye (BioLegend) and then surface stained with FcBlock and anti-CD271-Alexa647 antibody (BD Pharmingen). Cells were fixed with 2.8% PFA and permeabilized with the FOXP3/Transcription Factor Buffer kit (Thermo Fisher Scientific). They were then subjected to intracellular staining with anti-T-bet-PE (BioLegend) and anti-IFN- $\gamma$ -BV711 (BioLegend) antibodies. ICS analysis was performed on an Aurora cytometer (Cytex).

#### **Functional analysis of expanded T-bet-deficient CD4<sup>+</sup> T cells *in vitro***

CD4<sup>+</sup> T cells were isolated from PBMCs from five healthy controls (CTL), IL-12R $\beta$ 1-deficient (*IL12RB1* M/M) patients, the T-bet WT/M heterozygous father (*TBX21* WT/M), and the T-bet M/M patient (*TBX21* M/M), with anti-CD4 antibody-coated beads (Miltenyi Biotec). They were then expanded with anti-CD3/CD28 antibody-coated Dynabeads (Thermo Fisher Scientific) at a 1:1 (cell:bead) ratio under T<sub>H</sub>0 or T<sub>H</sub>1 (2.5 ng/mL IL-12 R&D and anti-human IL-4 neutralizing Ab InVivoMab, BioXcell) conditions, in the presence of 100 U/mL proleukin IL-2 (PROMETHEUS) and anti-human IL-10 neutralizing Ab (Thermo Fisher Scientific). Medium (X-vivo15 5% human AB serum) and cytokine cocktails were refreshed every 3 – 4 days. After 7 days, the culture consisted of >95% CD4<sup>+</sup> T cells. Cells were restimulated with anti-CD3/CD28 Ab-coated Dynabeads every 7-8 days. P's CD4<sup>+</sup> T cells were transduced with retroviruses obtained from pLZRS-ires- $\Delta$ CD271 plasmids with or without WT *TBX21* or EV, as previously described (Martínez-Barricarte et al., 2016). Six days after transduction, cells were restimulated with 25 ng/mL PMA and 500 nM ionomycin in the presence of GolgiPlug (BD Biosciences) for

6 h. Cells were stained with Zombie NIR Live-Dead exclusion reagents (BioLegend), anti-CD271-FITC antibody and were then fixed and permeabilized. They were stained with anti-TNF- $\alpha$ -BV510, anti-IL-13-APC, anti-IL-4-PE, anti-IL-17A-BV785, anti-IL-10-PE-Dazzle594, anti-IL-5-BV421 and anti-IFN- $\gamma$ -PerCp-Cy5.5 antibodies and subjected to flow cytometry in an Aurora cytometer (Cytek). Three weeks after their initial expansion, transduced CD271<sup>+</sup> CD4<sup>+</sup> T cells from P were isolated with anti-CD271 antibody-coated beads (Miltenyi Biotec). The cells isolated, and expanded CD4<sup>+</sup> T<sub>H</sub>0 cells from healthy donors or T-bet wt/m individuals were restimulated with anti-CD3/CD28 antibody-coated beads in the absence of cytokine for 16 h or 48 h. Cytokine levels were determined in 13-Legendplex assays (BioLegend) on culture supernatants from cultures restimulated for 48 h. Total RNA was extracted from cells restimulated for 16 h for RNA-seq analysis (Qiagen). In brief, 100 ng of total RNA was used to generate RNA-Seq libraries with the Illumina TruSeq stranded mRNA LT kit (Cat# RS-122-2101). Libraries prepared with unique barcodes were pooled at equal molar ratios. The pool was denatured and sequenced on an Illumina NextSeq 500 sequencer with high-output V2 reagents and NextSeq Control Software v1.4 to generate 75 bp single reads, according to the manufacturer's protocol (Illumina, Cat# 15048776).

RNA-seq FASTQ files were obtained, and inspected by fastqc for quality control of the raw data. The sequencing reads of each FASTQ file were then mapped to the GENCODE human reference genome GRCh38.p13 (Frankish et al., 2019) with STAR aligner v2.7 (Dobin et al., 2013), and the quality of each mapped alignment in the BAM file was evaluated by RSeQC (Wang et al., 2012). Reads were quantified to generate gene-level read counts from the read alignment, with featureCounts v1.6.0 (Liao et al., 2014), based on GENCODE GRCh38.p13 gene annotation. The gene-level read counts were then normalized and log<sub>2</sub>-transformed by



DESeq2 (Love et al., 2014), to obtain the gene expression value for all genes and all samples. An analysis of the differential expression of all genes and immune genes was performed to compare gene expression in the four TBET samples (*TBX21* WT/M, *TBX21* M/M, M/M+EV, and M/M+WT) with that in five control samples, with each sample having two technical replicates. The 1,984 immune-related genes were identified from the immune system process gene set in the Molecular Signature Database (MSigDB) (Liberzon et al., 2015). By applying the trimmed mean of M values (TMM), normalization and gene-wise generalized linear model regression were performed in edgeR (Robinson et al., 2010). The genes displaying significant differential expression were selected on the basis of a  $p$ -value  $\leq 0.05$  and a FDR  $\leq 0.05$ . The differential gene expression data were plotted as a heatmap by ComplexHeatmap (Gu et al., 2016), and genes and samples were clustered on the basis of complete linkage and Euclidean distance of gene expression values.

#### **Omni-ATAC-seq on CD4<sup>+</sup> T cells and analysis**

CD4<sup>+</sup> T cells derived from healthy controls, *IL12RB1* M/M patients, a *TBX21* WT/M parent and the *TBX21* M/M P were expanded under T<sub>H</sub>0 conditions, as described above. Nine days after the retroviral transduction of CD4<sup>+</sup> T cells from P, as described above, CD271<sup>+</sup> cells were isolated with anti-CD271 antibody-coated beads (Miltenyi Biotec). Omni-ATAC-seq library preparation was performed as previously described (Corces et al., 2017). In brief, 50,000 cells from three healthy donors (CTL), two *IL12RB1* M/M patients, technical duplicates for a *TBX21* WT/M individual, the *TBX21* M/M P and P cells complemented with EV (M/M+EV) or WT (M/M+WT) *TBX21* were harvested, washed with 50  $\mu$ l cold PBS, and centrifuged to obtain a pellet. Pellets were lysed with 50  $\mu$ l cold lysis buffer consisting of 48.5  $\mu$ l resuspension buffer

(10 mM Tris-HCl pH 7.5, 10 mM NaCl, 3 mM MgCl<sub>2</sub> in water), 0.5 µl 10% NP-40, 0.5 µl 10% Tween-20, and 0.5 µl 1% digitonin. Lysates were incubated on ice and then washed with 0.1% Tween-20 resuspension buffer. Lysates were centrifuged and the nuclei in the pellet were subjected to Tn5 transposition with 50 µL of a mixture of 25 µL 2 x TD buffer, 16.5 µL PBS, 0.5 µL 10% Tween-20, 0.5 µL 1% digitonin, 2.5 µL Tn5 transposase, 5 µL nuclease-free water at 37 °C in a thermomixer operating at 1,000 rpm for 30 min. DNA fragments were extracted with the MinElute PCR purification kit and eluted in 30 µl EB buffer (Qiagen). We used 10 µL of eluted DNA for the amplification of DNA fragments with the NEBNext PCR master mix (New England BioLab), over six cycles, with the Ad1\_forward and 15 indexed Ad2\_reverse primers, as previously described (Buenrostro et al., 2013, 2015). The partially amplified library was subjected to quantitative PCR analysis with the Ad1 and Ad2 primers, Sybr Green reagents and NEBNext PCR master mix for 25 cycles. Additional PCR cycles were performed, based on 1/3 the maximal fluorescence for each sample, as previously described (Buenrostro et al., 2013, 2015). A left-sided isolation of PCR products was performed with AMPure beads (Beckman Coulter). DNA was quantified with a Qubit fluorimeter. Equal amounts of each sample were pooled and subjected to paired-end sequencing on a NextSeq high-output sequencer generating 75 bp reads.

The ATAC-seq reads were aligned with the hg38 genome from the BSgenome.Hsapiens.UCSC.hg38 Bioconductor package (version 1.4.1), with Rsubread's alignment method in paired-end mode, with fragments of 1 to 5,000 base pairs in length considered correctly paired (Liao et al., 2014). Normalized, fragment signal bigWigs were created with the rtracklayer package. Peak calls were made with MACS2 software in BAMPE mode (Feng et al., 2012; Zhang et al., 2008) and peak summits were used in MEME-ChIP

software the identification of known and novel motifs (Ma et al., 2014). Differential ATAC-seq signals were identified with the DESeq2 package (Love et al., 2014). Fragment length distribution plots and nucleosome-free/mono-nucleosome meta-TSS plots were produced with the profileplyr package (Carroll and Barrows, 2019). Heatmaps of ChIP-seq and ATAC-seq signals were created from the normalized bigWigs signal, with DeepTools2 software (Ramírez et al., 2016).

T-bet ChIP-seq data (SRR332104) and its input control (SRR332102) were retrieved as unaligned sequences from the European Nucleotide Archive (Kanhare et al., 2012). Sequences were aligned with the hg38 genome from the Bsgenome.Hsapiens.UCSC.hg38 Bioconductor package (version 1.4.1), with Rsubread's alignment method, and predicted fragment lengths calculated with the ChIPQC package (Carroll et al., 2014; Liao et al., 2014; de Santiago and Carroll, 2018). Normalized, fragment-extended bigWigs signals were created with the rtracklayer package.

## **EPIC DNA methylation arrays and quantification of DNA methylation**

CD4<sup>+</sup> T cells derived from healthy controls, a *TBX21* WT/M individual and the *TBX21* M/M P were expanded under T<sub>H</sub>0 conditions, as described above. Seventeen days after the retroviral transduction of CD4<sup>+</sup> T cells from P as described above, CD271<sup>+</sup> cells were isolated with anti-CD271 antibody-coated beads (Miltenyi Biotec). DNA from non-transduced or CD271<sup>+</sup> isolated CD4<sup>+</sup> T cells was extracted with a DNA mini kit (Qiagen). DNA was subjected to bisulfite conversion with the EZ DNA Methylation Kit (ZymoResearch). We quantified the DNA in all samples by performing EPIC arrays (Illumina), following the manufacturer's instructions. Technical replicates were included for several samples during EPIC processin, to

ensure that the measurements were reliable. All microarrays were scanned with the Illumina HiScan system. Data files were processed and converted to beta-values, corresponding to the percentage CpG methylation for a given CpG site. We wrote scripts to filter all loci, retaining those with higher levels of methylation in M/M and M/M+EV than the maximum methylation level in controls, and lower levels of methylation in M/M+WT than in controls (group 1), and those with lower levels of methylation in M/M and M/M+EV than the minimum level of methylation in controls, and higher levels of methylation in M/M+WT than the minimum level in controls (group 2). We then measured and plotted the difference in CpG methylation between the patient and controls, by subtracting the mean methylation level for the five controls from that for M/M and comparing the value obtained with the level of restored CpG methylation by subtracting the level of methylation in M/M+WT from the methylation in M/M+EV. On the plot, red dots represent the loci with higher levels of methylation in the patient than in controls, and green dots represent loci with lower levels of methylation in the patient than in controls. Dot size is proportional to the fold difference in methylation in patients relative to the mean value for controls. The genes of our interest are highlighted on the scatter plot.

## **Immunophenotyping of leukocytes**

In the CyTOF experiment, PBMCs from 26 healthy adults of diverse ethnicities, nine child or adolescent controls (age range: 1 to 17 years, with diverse ancestries, including one age-matched child from Morocco), six IL-12R $\beta$ 1-deficient patients (recruited from previous studies), and the T-bet-deficient patient (M/M). We recruited 26 healthy adult controls locally in New York City. We analyzed the PBMCs of four individuals on two separate dates, as duplicates. We obtained 30 vials of cells from these adult controls, 10 of which were frozen after being allowed

1 to rest at room temperature for 24 – 48 h to mimic travel conditions. The vials of cells from the  
2 six IL-12R $\beta$ 1-deficient patients and the age-matched controls were typically frozen after  
3 centrifugation on Ficoll following the overnight shipment of the blood sample. For this  
4 experiment, the PBMCs of P (M/M) were prepared from blood that had traveled overnight from  
5 Morocco to Paris. Metal-conjugated mAbs were obtained from Fluidigm according to the  
6 manufacturer's instructions. The following antibodies were included in the staining panel: anti-  
7 CD45-89Y, anti-CD57-113In, anti-CD11c-115In, anti-CD33-141Pr, anti-CD19-142Nd, anti-  
8 CD45RA-143Nd, anti-CD141-144Nd, anti-CD4-145Nd, anti-CD8-146Nd, anti-CD20-147Sm,  
9 anti-CD16-148Nd, anti-CD127-149Sm, anti-CD1c-150Nd, anti-CD123-151Eu, anti-CD66b-  
10 152Sm, anti-PD-1-153Eu, anti-CD86-154Sm, anti-CD27-155Gd, anti-CCR5-156Gd, anti-  
11 CD117-158Gd, anti-CD24-159Tb, anti-CD14-160Gd, anti-CD56-161Dy, anti-gdTCR-162Dy,  
12 anti-DRTH2-163Dy, anti-CLEC12A-164Dy, anti-CCR6-165Ho, anti-CD25-166Er, anti-CCR7-  
13 167Er, anti-CD3-168Er, anti-CX3CR1-169Tm, anti-CD38-170Er, anti-CD161-171Yb, anti-  
14 CD209-172Yb, anti-CXCR3-173Yb, anti-HLA-DR-174Yb, anti-CCR4-176Yb, anti-CD11b-  
15 209Bi.

16 For the identification of immunophenotypes unique to the *TBX21* patient, we compared  
17 data from six batches of CyTOF experiments including age-matched pediatric controls. In  
18 addition to manually inspecting data from each batch separately, we also compared  
19 immunophenotypes across batches in an objective and unbiased manner, with a computerized  
20 system (Ogishi et al. Submitted). This method involved a combination of high-dimensional  
21 batch-correction, unsupervised clustering, and batch-specific cell-type classification through  
22 machine learning. We first used a computational approach to normalize batch effects and identify  
23 phenotypes unique to the *TBX21* patient through an unbiased clustering approach (Manuscript in

1 preparation). Each of the FCS files was processed to exclude dead cells and doublets  
2 automatically. Cells from healthy local controls were used for batch correction with *Harmony*  
3 (Korsunsky et al., 2019). Data were down-sampled to 200,000 cells per batch before batch  
4 correction. Unsupervised clustering was then performed with batch-corrected marker expression  
5 values and the *FlowSOM* and *ConsensusClusterPlus* packages in *Bioconductor* (Van Gassen et  
6 al., 2015; Wilkerson and Hayes, 2010). We retained 40 clusters after metaclustering. Each cluster  
7 was manually inspected and annotated on the basis of its marker expression profile. A batch-  
8 specific cell-type classifier was then trained on the uncorrected expression values and cell-type  
9 annotations. We sampled a maximum of 100 cells per cell type at random, to improve  
10 computation efficiency and to alleviate class imbalance issues, as some of the cell types are  
11 much rarer than others. A highly randomized tree algorithm was used, with tuning of the  
12 hyperparameters *mtry* and *numRandomCuts* for each batch (Geurts et al., 2006). Classifiers were  
13 trained through five repeats of 10-fold cross-validation with internal upsampling. Internal  
14 accuracy during cross-validation always exceeded ~95%. Finally, the trained classifier was  
15 applied to the rest of the cells, including the patient's cells, to predict their cell types. With this  
16 approach, any cell could be classified probabilistically as belonging to one of the predefined cell  
17 types, making it possible to avoid a strong skewing of patient data, which would affect the batch-  
18 correction and clustering steps. For the purposes of visualization, batch correction was performed  
19 with *Harmony* on cells from all controls and patients. Data were down-sampled to 40,000 cells  
20 per batch before batch correction. Finally, the trained classifier was applied to all cells, including  
21 the patient's cells, to predict their cell types and, thus, to identify immunophenotypes for each  
22 individual. After harmonization, dimension reduction was performed by Uniform Manifold  
23 Approximation and Projection (UMAP) (Becht et al., 2019). The figures are color-coded on the

basis of the cell type identified by the unsupervised clustering approach described above. All analyses were performed with R ver.3.6.3.

We also deconvoluted 10,000 memory CD4<sup>+</sup> T cells (DNA<sup>+</sup>Live\_CD45<sup>+</sup>CD66b<sup>+</sup>CD123<sup>+</sup>CD3<sup>+</sup>CD20<sup>+</sup>CD56<sup>+</sup>γδTCR<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup>CD45RA<sup>+</sup>) into viSNE plots based on 20 surface markers (113ln\_CD57, 115ln\_CD11c, 149sm\_CD127, 150Nd\_CD1c, 154Sm\_CD86, 155Gd\_CD27, 156Gd\_CCR5, 158Gd\_CD117, 159Tb\_CD24, 163Dy\_CRTH2, 165Ho\_CCR6, 166Er\_CD25, 167Er\_CCR7, 169Tm\_CX3CR1, 170Er\_CD38, 171Yb\_CD161, 173Yb\_CXCR3, 174Yb\_HLA-DR, 176Yb\_CCR4, 209Bi\_CD11b) (Amir el et al., 2013). Analyses were performed by manually gating populations for NK cells, naïve and memory subsets of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, innate lymphoid cells (ILCs), Tregs and dendritic cell subsets. For T helper cell analysis, we included an additional four healthy donors from a separate experiment (same staining panel) in the analysis. Manual gating was used for T<sub>H</sub> analysis. We also deconvoluted 10,000 CD8<sup>+</sup> T cells into viSNE plots based on 113ln\_CD57, 115ln\_CD11c, 143Nd-CD45RA, 144Nd-CD141, 149sm\_CD127, 150Nd\_CD1c, 154Sm\_CD86, 155Gd\_CD27, 156Gd\_CCR5, 158Gd\_CD117, 159Tb\_CD24, 163Dy\_CRTH2, 165Ho\_CCR6, 166Er\_CD25, 167Er\_CCR7, 169Tm\_CX3CR1, 170Er\_CD38, 171Yb\_CD161, 173Yb\_CXCR3, 174Yb\_HLA-DR, 176Yb\_CCR4, 209Bi\_CD11b (Amir el et al., 2013).

Immunophenotyping for MAIT, invariant NKT cells, Vδ2 and Vδ1 cells was performed in three different batches. In the first batch, PBMCs from 18 healthy controls (CTL), three IL-12Rβ1-deficient individuals (*IL12RB1* M/M), two T-bet-heterozygous parents (*TBX21* WT/M), P (*TBX21* M/M) and P's healthy brother (*TBX21* WT/WT) were included. They were stained with the following panel: FcBlock (Miltenyi Biotec), Zombie-NIR live-dead exclusion dye (BioLegend), anti-CD3-Alexa532 (Thermo Fisher Scientific), anti-γδTCR-FITC (Thermo Fisher

1 Scientific), anti-V $\delta$ 2-APC-Fire750 (BioLegend), anti-CD56-BV605 (BioLegend), anti-CD4-  
2 BV750 (BD Biosciences), anti-CD8a-BV510 (BioLegend), anti-V $\alpha$ 7.2-BV711 (BioLegend),  
3 anti-V $\alpha$ 24-J $\alpha$ 18-PE-Cy7 (BioLegend), anti-V $\delta$ 1-Vioblue (Miltenyi Biotec), anti-CD161-PE  
4 (BioLegend) and anti-V $\beta$ 11-APC (Miltenyi Biotec) antibodies. Cells were analyzed with an  
5 Aurora cytometer (Cytek). In the 2<sup>nd</sup> batch, 31 adult control samples, seven samples from age-  
6 matched controls (age CTL), including a Moroccan child, and samples from P (M/M) and his  
7 mother sample were included. In the 3<sup>rd</sup> batch, 16 adult control samples, five samples from age-  
8 matched controls (age CTL) and a sample from P were included. In the 3<sup>rd</sup> batch, 16 adult  
9 control samples, five samples from age-matched controls (age CTL) and P's sample were  
10 included. For the three batches considered together, we obtained 65 data points for healthy adults,  
11 with PBMCs from 20 healthy adults undergoing two analyses in different batches, PBMCs from  
12 three samples undergoing analyses three times, in different batches and PBMCs from 16  
13 individuals analyzed only once. All 12 age-matched controls were different individuals. Three  
14 vials of PBMCs from P, isolated from fresh blood and frozen in the same batch, were analyzed in  
15 three separate batches. P's parents and P's brother's PBMCs were isolated from fresh blood and  
16 frozen. The age matched controls were of various ethnic backgrounds, and all were aged 1 – 17  
17 years at the time of blood sampling. The PBMCs of most of these age-matched controls were  
18 prepared from blood that had been transported overnight. PBMCs from three IL-12R $\beta$ 1-deficient  
19 individuals were prepared from blood transported overnight. In the second and third batches,  
20 immunophenotyping for MAIT, invariant NKT cells, V $\delta$ 2 and V $\delta$ 1 cells was performed with a  
21 flow cytometry staining panel containing FcBlock (Miltenyi Biotec), Zombie-NIR live-dead  
22 exclusion dye (BioLegend), anti- $\gamma\delta$ TCR-alexa 647 (BioLegend), anti-CD3-V450 (BD  
23 Biosciences), anti-CD56-BV605 (BioLegend), anti-CD4-BUV563 (BD Biosciences), anti-



V $\delta$ 1TCR-FITC (Miltenyi), anti-CD8-BUV737 (BD Biosciences), anti-V $\delta$ 2TCR-APC/Fire750 (BioLegend), anti-CD20-BV785 (BioLegend), anti-V $\alpha$ 7.2-BV711 (BioLegend), anti-CD161-PE (BioLegend), MR1-5-OP-RU-tetramer (NIH tetramer core facility), anti-V $\beta$ 11-APC (Miltenyi), anti-iNKT-BV480 (BD Biosciences) antibodies. Cells were analyzed with an Aurora cytometer (Cytek). Three batches of data were compiled for the final analysis. The percentages of the various immune subsets were determined by manual gating.

Immunophenotyping for innate lymphoid cells and NK cells was confirmed separately, by conventional flow cytometry. In brief, biotinylated anti-human anti-CD1a (HI149), anti-CD3 (OKT3), anti-CD5 (UCHT2), anti-CD14 (61D3), anti-CD19 (HIB19), anti-CD34 (4H11), anti-CD123 (6H6), anti-CD203c (FR3-16A11), anti-CD303 (AC144), anti-TCR $\alpha\beta$  (IP26), anti-TCR $\gamma\delta$  (B1) and anti-Fc $\epsilon$ RI $\alpha$  (AER-37(CRA-1)) antibodies were used in combination with streptavidin BUV661, and conjugated anti-CD62L-FITC (Dreg-56), anti-CD94-PerCP-Vio700 (REA113), anti-TCRV $\alpha$ 24J $\alpha$ 18-PE (6B11), anti-CD26-PE-CF594 (M-A261), anti-CD127-PE-Cy7 (eBioRDR5), anti-CRTH2-Alexa Fluor 647 (BM16), anti-CD161-Alexa Fluor 700 (HP-3G10), anti-EOMES-APC-eFluor 780 (WD1928), anti-NKp46-BV421 (9E2/NKp46), anti-CD45RA-BV570 (HI100), anti-CD117-BV605 (104D2), anti-ROR $\gamma$ t-BV650 (Q21-559), anti-CD7-BV711(M-T701), anti-CD16-BUV496 (3G8), anti-CD25-BUV563 (2A3), anti-CD56-BUV737 (NCAM16.2) and anti-CD45-BUV805 (HI30); anti-T-bet-BV786 (O4-46), anti-GATA3-BUV395 (L50-823) antibodies. Antibodies were purchased from BioLegend, Thermo Fisher Scientific, BD Biosciences, or Miltenyi Biotec. Fc receptors were blocked with IgG from human serum (Sigma-Aldrich). Surface membrane staining was performed in Brilliant Stain Buffer (BD Biosciences). Transcription factors were stained with the Foxp3 staining kit (Thermo Fisher Scientific), according to the manufacturer's instructions. The fixable viability dye eFluor

506 (Thermo Fisher Scientific) was used to exclude dead cells. Samples were acquired on a BD Symphony A5 cytometer (BD Biosciences) with FACSDiva 8 software and were analyzed with FlowJo 10 (BD Biosciences).

Immunophenotyping was confirmed separately for the T- and B-cell subsets by conventional flow cytometry. In brief, PBMCs from healthy controls and P were labeled with mAbs against CD3, CD4, CD8, CD45RA, CCR7, CD127, CD25, CXCR5, CXCR3, CCR6, PD-1, and CD56. The CD4<sup>+</sup> and CD8<sup>+</sup> T-cell subsets were separated into naïve (T<sub>Naive</sub>: CCR7<sup>+</sup>CD45RA<sup>+</sup>), central memory (T<sub>CM</sub>: CCR7<sup>+</sup>CD45RA<sup>-</sup>), effector memory (T<sub>EM</sub>: CCR7<sup>-</sup>CD45RA<sup>-</sup>), and terminally differentiated effector memory cells (T<sub>EMRA</sub>: CCR7<sup>-</sup>CD45RA<sup>+</sup>) after initial gating on CD8<sup>-</sup>CD4<sup>+</sup> or CD8<sup>+</sup>CD4<sup>-</sup> cells. Proportions of regulatory T cells (CD4<sup>+</sup>CD127<sup>lo</sup>CD25<sup>hi</sup>), total memory (CD4<sup>+</sup>CD45RA<sup>-</sup>), and circulating Tfh (cTfh; CD4<sup>+</sup>CD45RA<sup>-</sup>CXCR5<sup>+</sup>) cells, and subsets of total memory and cTfh cells defined on the basis of CXCR3 and CCR6 expression (T<sub>H1</sub>: CCR6<sup>-</sup>CXCR3<sup>+</sup>, T<sub>H17</sub>: CCR6<sup>+</sup>CXCR3<sup>-</sup>, T<sub>H1</sub>\*/T<sub>H17</sub>: CCR6<sup>+</sup>CXCR3<sup>+</sup>, T<sub>H2</sub>: CCR6<sup>-</sup>CXCR3<sup>-</sup>) were also determined.

### Single-cell (sc) RNA-seq and analysis

We performed scRNA-seq on PBMCs obtained from the *TBX21* WT/M heterozygous father and the *TBX21* M/M patient. PBMCs were quickly thawed at 37°C and gently resuspended by serial additions of DMEM + 10% heat-inactivated (HI) FBS, according to the recommendations from the 10x Genomics “Sample preparation Fresh Frozen Human PBMC” protocol Rev C. Cells were centrifuged at 400 x g for 5 min, washed with DMEM + 10% HI-FBS, and cells were then counted and viability was assessed with the LIVE/DEA Viability kit (Thermo Fisher Scientific) according to the manufacturer’s guidelines. Following centrifugation

1 at 400 x *g* for 5 min, cells were resuspended at a concentration of 1000 cells/ $\mu$ l in PBS + 0.04%  
2 BSA and loaded onto a 10 x Genomics Chromium chip for single-cell capture. Reverse  
3 transcription and library preparation were performed with Chromium Single Cell 3' Reagent Kits  
4 (v2) in accordance with the manufacturer's guidelines, and library quality was assessed with a  
5 Bioanalyzer DNA chip. Each library was sequenced on two lanes of an Illumina HiSeq 4000  
6 sequencer in a 28 bp/98 bp paired-end configuration.

7 The sequence-read quality of individual sequencing lanes was assessed with BVAtools  
8 (<https://bitbucket.org/mugqic/bvatools>). Cell Ranger v3.0.1 was used to aggregate the sequences  
9 from two lanes/samples, to map reads to the hg38 human reference genome assembly, for  
10 filtering, and counting barcodes and UMIs; a list of UMI counts was thus obtained for each gene  
11 and each cell. Cells outside the [5%,95%] interval for these metrics or with >10% mitochondrial  
12 genes were excluded. The DoubletFinder package was used to identify and filter out cell  
13 doublets (McGinnis et al., 2019). After the removal of dead cells and doublets, 10,409 cells for  
14 the T-bet wt/m heterozygous father and 11,741 cells for the T-bet m/m P were analyzed together  
15 with the Seurat v3 R package (Stuart et al., 2019), and cell clustering was performed by the  
16 Uniform Manifold Approximation and Projection dimension reduction method (Becht et al.,  
17 2019), using the most variable genes, but excluding mitochondrial and ribosomal protein genes.  
18 This analysis identified eight distinct cell clusters; the marker genes for each cluster were  
19 identified with Seurat and used to determine cell types. Differential expression was analyzed by  
20 comparing UMI gene counts between the patient and the control for each cluster, with the MAST  
21 approach (Finak et al., 2015). Genes with a *p*-value < 10<sup>-25</sup> for differential expression were  
22 analyzed in more detail. For each selected gene, cells in which the expression of the gene was  
23 detectable were counted for the index case and the control in each cluster, to reduce the dropout

bias inherent to droplet scRNA approaches. The number of cells expressing the gene was normalized according to the number of cells in each cluster and the fold-difference in expression was calculated by comparing the index case and the control. For the figures, cluster annotations and the levels of expression of genes of interest were projected onto the UMAP clustering, but with the cells of the patient and the control shown separately.

### **Stimulation of NK cells *ex vivo***

PBMCs from five healthy donors including a travel control, the T-bet WT/M mother, the T-bet WT/WT brother and the T-bet M/M P were left untreated or stimulated with IL-12 (Miltenyi Biotec), IL-15 (Miltenyi Biotec) and IL-18 (MBL/CliniSciences) for 18 h. Cells were treated with GolgiPlug and GolgiStop (BD Biosciences), and stained with anti-CD107a-PE antibody (eBioH4A4, Thermo Fisher Scientific) for 4 h. They were then stained with eFluor506 live-dead reagents (Thermo Fisher Scientific), followed by antibodies binding to surface antigens, including anti-CD1a-FITC (H1149), anti-CD3-FITC (OKT3), anti-CD5-FITC (UCHT2), anti-CD14-FITC (TUK4), anti-CD19-FITC (HIB19), anti-CD34-FITC (581), anti-CD123-FITC (6H6), anti-CD203c (NP4D6), anti-CD303-FITC (201A), anti-Fc $\epsilon$ RI $\alpha$ -FITC (AER-37(CRA-1)), anti-TCR $\alpha\beta$ -FITC (IP26), anti-TCR $\gamma\delta$ -FITC (B1), anti-CD94-PerCP-Vio700 (REA113), anti-CD127-PE-Cy7 (eBioRDR5), anti-NKp46-APC (9E2/NKp46), anti-CD7-BV711 (M-T701), anti-CD56-BV786 (NCAM16.2), anti-CD16-BUV737 (3G8), and anti-CD45-BUV805 (HI30) antibodies. All the markers targeted by FITC-conjugated Abs were considered to be Lineage-specific markers (Lin). Cells were fixed and permeabilized (Fix & Perm; Thermo Fisher Scientific) and subjected to intracellular staining with anti-granzyme B-PE-CF594 (GB11), anti-EOMES-APC-eFluor 780 (WD1928), anti-perforin-BV421 ( $\delta$ G9), anti-TNF- $\alpha$ -BV605 (Mab11)

1 and anti-IFN- $\gamma$ -BUV395 (B27) antibodies. Antibodies were purchased from BioLegend, Thermo  
2 Fisher Scientific, BD Biosciences, or Miltenyi Biotec. We analyzed ICS and surface staining  
3 with a BD LSRFortessa flow cytometer (BD Biosciences).

#### 5 **CD4<sup>+</sup> T-cell isolation and functional characterization *ex vivo* and T<sub>H</sub> differentiation *in vitro***

6 The healthy controls (CTL) for this experiment were adults recruited locally in Australia,  
7 and PBMCs for P (M/M) were prepared from two separate overnight shipments of blood from  
8 Casablanca to Paris and frozen. The two dots in these figure panels represent data from two  
9 independent experiments on two batches. PBMCs from healthy donors and P were incubated  
10 with mAbs against CD4, CD45RA, CCR7, CXCR5, CD127 and CD25. Naïve and memory  
11 CD4<sup>+</sup> T cells were isolated by first excluding Tregs (CD25<sup>hi</sup>CD127<sup>lo</sup>) and then sorting  
12 CD4<sup>+</sup>CD45RA<sup>+</sup>CCR7<sup>+</sup>CXCR5<sup>-</sup> and CD4<sup>+</sup>CD45RA<sup>-</sup>CCR7<sup>±</sup> cells, respectively. Isolated naïve  
13 and memory CD4<sup>+</sup> T cells were then cultured in 96-well round-bottomed well plates (30-40 x10<sup>3</sup>  
14 cells/well) with T-cell activation and expansion (TAE) beads (anti-CD2/CD3/CD28; Miltenyi  
15 Biotech) alone (T<sub>H</sub>0) or under T<sub>H</sub>1 (50 ng/mL IL-12), T<sub>H</sub>2 (100 U/mL, IL-4), T<sub>H</sub>9 (2.5 ng/mL  
16 TGF- $\beta$ , 100 U/mL IL-4), or T<sub>H</sub>17 (2.5 ng/mL TGF- $\beta$ , 50 ng/mL IL-1- $\beta$ , 50 ng/mL IL-6, 50  
17 ng/mL IL-21, 50 ng/mL IL-23, 50 ng/mL PGE2) polarizing conditions. After 5 days,  
18 supernatants were harvested and the production of IL-4, IL-5, IL-9, IL-10, IL-13, IL-17A, IL-  
19 17F, IFN- $\gamma$  and TNF- $\beta$  was assessed with cytometric bead arrays (Becton Dickinson); IL-22  
20 secretion was measured by ELISA (eBioscience). For cytokine expression, activated CD4<sup>+</sup> T  
21 cells were restimulated with PMA (100 ng/mL)/ionomycin (750 ng/mL) for 6 h, with brefeldin A  
22 (10  $\mu$ g/mL) added after two hours. Cells were then fixed and the intracellular expression of IL-4,  
23 IL-9, IL-13, IL-10, IL-17A, IL-17F, IL-22, IL-21 and IFN- $\gamma$  was determined.

## **Stimulation of PBMCs with PMA/ionomycin for intracellular staining and flow cytometry analysis**

This experiment was performed twice, on two different dates. The two batches of data were compiled for the final analysis. Fourteen healthy adult donors were recruited at the Rockefeller University (New York, USA). Two healthy adult controls, four age-matched controls, P and his relatives were recruited at Necker Hospital (Paris, France). PBMCs from two of 16 adult controls were frozen after being allowed to rest at room temperature for 24 – 48 h to mimic transport conditions. The PBMCs from the other 14 adult controls were prepared directly from freshly collected blood and frozen. Two vials of PBMCs from P were used in this experiment: one prepared directly from fresh blood before freezing, and the other prepared and frozen after overnight shipment of the blood samples. The PBMCs of four age-matched controls were prepared from blood that had been shipped overnight. The PBMCs of P's relatives were prepared from blood that had been shipped overnight. PBMCs from six healthy adult controls were stimulated twice, in two separate batches, as technical replicates. The second vial of P's PBMCs was stimulated in two sets of wells, as technical duplicates. The PBMCs of three age-matched controls were stimulated in two sets of wells, as technical duplicates. The PBMCs of P's WT/M mother were stimulated in two sets of wells, as technical duplicates.

In both batches of experiments, we plated 300,000 cells per well in 96-well U-bottomed plates, at a density of  $1.5 \times 10^6$  cells/ml. Cells were cultured for 40 h in RPMI. They were then stimulated with 50 ng/mL PMA and 1  $\mu$ M ionomycin. After 1 h, Golgiplug was added to the culture, in accordance with the manufacturer's protocol. After another 7 h, cells were harvested for staining and flow cytometry. In brief, cells were first stained with Zombie NIR Viability kit

(BioLegend) for 15 min. They were then stained with FcBlock (Miltenyi), anti- $\gamma\delta$ TCR-alexa 647 (BioLegend), anti-CD3-V450 (BD Biosciences), anti-CD56-BV605 (BioLegend), anti-CD4-BUV563 (BD Biosciences), anti-V $\delta$ 1TCR-FITC (Miltenyi), anti-CD8-BUV737 (BD Biosciences), anti-V $\delta$ 2TCR-APC/Fire750 (BioLegend), anti-CD20-BV785 (BioLegend), anti-V $\alpha$ 7.2-Alexa 700 (BioLegend), MR1-5-OP-RU-tetramer (NIH tetramer core facility), anti-V $\beta$ 11-APC (Miltenyi), and anti-iNKT-BV480 (BD Biosciences) antibodies for 30 min. The stained cells were fixed with the FOXP3/Transcription Factor kit (Thermo Fisher Scientific) and stained intracellularly with anti-T-bet-PE/Cy7 (BioLegend), anti-IFN- $\gamma$ -BV711 (BioLegend), anti-TNF- $\alpha$ -BV510 (BioLegend), anti-IL-17A-PERCP/Cy5.5, anti-ROR $\gamma$ T-PE (BD Biosciences), anti-CD3-V450 (BD Biosciences), anti-CD4-BUV563 (BD Biosciences) and anti-CD8-BUV737 (BD Biosciences) antibodies in Perm/Wash buffer. Cells were then analyzed in a CyTek Aurora spectral flow cytometer.

#### **Stimulation of PBMCs with PMA/ionomycin for cytokine secretion**

This experiment was done in three batches, on three separate dates. The data from the three batches were compiled for the final analysis. Thirty healthy adult controls were recruited at the Rockefeller University (New York, USA) and one healthy adult control was recruited at Necker Hospital (Paris, France). PBMCs from eight of the 31 adult controls were frozen after being allowed to rest at room temperature for 24 – 48 h to mimic travel conditions. The PBMCs of all the other 23 adult controls were isolated from fresh blood and frozen. PBMCs from 18 adult controls were analyzed once. Those from 13 adult controls were analyzed twice, in two separate batches. Three vials of PBMCs from P were used in this experiment: two vials of PBMCs isolated from fresh blood and then frozen, and a vial of PBMCs isolated from blood that

had been transported overnight, which were then frozen. These three vials of PBMCs from P were used separately, in three batches. One vial of PBMCs prepared from fresh blood was plated in two sets of wells as technical duplicates. Thus, four data points were measured for P. The PBMCs of P's relatives were prepared from blood that had been shipped overnight. PBMCs from P's healthy brother, mother and father were each analyzed once. Five age-matched controls were recruited from around the world, including one matched for both age and ethnicity from Morocco. PBMCs were isolated from the blood of these patients after overnight shipment and frozen. PBMCs from three age-matched controls were plated in two sets of wells, as technical duplicates. The PBMCs of the age- and ethnicity-matched control were plated in technical triplicates. In three batches of experiments, we plated 300,000 cells per well in 96-well U-bottomed plates, at a density of  $1.5 \times 10^6$  cells/mL. Cells were cultured for 40 h in RPMI. They were then stimulated by incubation with 50 ng/mL PMA and 1  $\mu$ M ionomycin for 8 h. Supernatants were harvested for human T<sub>H</sub> cytokine analysis with the 13-plex LegendPlex kit (BioLegend).

#### **Stimulation of PBMCs with live BCG infection for cytokine production**

This experiment was performed in three batches, on three dates. The data from the three batches were compiled for the final analysis. Thirty healthy adult controls were recruited at the Rockefeller University (New York, USA) and one healthy adult control was recruited at Necker Hospital (Paris, France). PBMCs from eight of the 31 adult controls were frozen after being allowed to rest at room temperature for 24 – 48 h to mimic travel conditions. The PBMCs of all the other 23 adult controls were isolated from fresh blood and frozen. PBMCs from 19 adult controls were analyzed once, and those from 10 adult controls were assayed twice, in two



1 separate batches. PBMCs from one control were plated in three sets, as technical triplicates, in  
2 one batch. PBMCs from one control were assayed five times in three separate batches, as  
3 technical replicates. In total, 47 data points were obtained for adult controls. Five age-matched  
4 controls were recruited globally, including one matched for both age and ethnicity from Morocco.  
5 Their PBMCs were obtained from blood that had been shipped overnight and were frozen.  
6 PBMCs from three age-matched controls were plated in two sets of wells, as technical duplicates.  
7 PBMCs from the other age-matched controls were plated in single sets. Eight data points in total  
8 were obtained for age-matched controls. Three vials of PBMCs from P were used in this  
9 experiment: two vials of PBMCs isolated from fresh blood and then frozen, and a vial of PBMCs  
10 isolated from blood that had been transported overnight, which were then frozen. These three  
11 vials of PBMCs from P were used separately, in three batches. In total three data points were  
12 generated for P. PBMCs from P's relatives were isolated from blood that had been shipped  
13 overnight. PBMCs from P's healthy brother, mother and father were each analyzed once each.

14 In all three batches of experiments, we plated 300,000 cells per well in 96-well U-  
15 bottomed plates, at a density of  $1.5 \times 10^6$  cells/ml. For each sample, cells were plated in the  
16 presence and absence of live *M. bovis*-BCG at a MOI=1 (gift from Carl Nathan), and in the  
17 presence and absence of recombinant IL-12 (5 ng/mL, R&D) or recombinant IL-23 (10 ng/mL,  
18 R&D) (Vogt and Nathan, 2011). Golgiplug (BD Biosciences) was added to each well after 40 h  
19 of stimulation. Eight hours later, supernatants were harvested for cytokine determinations in a  
20 13-plex Legendplex assay (BioLegend), and cells were collected by centrifugation for FACS  
21 staining. In brief, cells were first stained with the Zombie NIR Viability kit (BioLegend) for 15  
22 minutes. They were then stained with FcBlock (Miltenyi Biotec), anti- $\gamma\delta$ TCR-alexa 647  
23 (BioLegend), anti-CD3-V450 (BD Biosciences), anti-CD56-BV605 (BioLegend), anti-CD4-

1 BUV563 (BD Biosciences), anti-V $\delta$ 1TCR-FITC (Miltenyi Biotec), anti-CD8-BUV737 (BD  
2 Biosciences), anti-V $\delta$ 2TCR-APC/Fire750 (BioLegend), anti-CD20-BV785 (BioLegend), anti-  
3 V $\alpha$ 7.2-Alexa 700 (BioLegend), MR1-5-OP-RU-tetramer (NIH tetramer core facility), anti-V $\beta$ 11-  
4 APC (Miltenyi Biotec), and anti-iNKT-BV480 (BD Biosciences) antibodies for 30 minutes. The  
5 stained cells were fixed with the FOXP3/Transcription Factor Buffer set (Thermo Fisher  
6 Scientific) and intracellularly stained with anti-T-bet-PE/Cy7 (BioLegend), anti-IFN- $\gamma$ -BV711  
7 (BioLegend), anti-TNF- $\alpha$ -BV510 (BioLegend), anti-IL-17A-PERCP/Cy5.5 and anti-ROR $\gamma$ T-PE  
8 (BD Biosciences) antibodies in Perm/Wash buffer. Cells were acquired on a CyTek Aurora  
9 spectral flow cytometer and the data were analyzed with Cytobank. T-bet<sup>+</sup> IFN- $\gamma$ <sup>+</sup> double-  
10 positive cells were gated manually from the live single-lymphocyte population. The viSNE  
11 algorithm was then used to cluster these T-bet<sup>+</sup> IFN- $\gamma$ <sup>+</sup> double-positive cells with surface lineage-  
12 determining markers (Amir el et al., 2013). Automated viSNE clusters were plotted with tSNE1  
13 and tSNE2. Clusters were then overlaid on manually gated immune cell subsets for illustration.

#### 15 **V $\delta$ 2<sup>+</sup> $\gamma\delta$ T cell stimulation**

16 Lysates of *M. bovis*-BCG were prepared and provided by Dr. Carl F. Nathan. PBMCs  
17 from adult controls, IL-12R $\beta$ 1 m/m patients, the T-bet M/M P and his relatives were resuspended  
18 at a density of 1x10<sup>6</sup> cells/mL. We plated 200  $\mu$ L cells per well for each condition, in a 96-well  
19 U-bottomed plate. We added BCG lysate to each well at a final concentration of 5  $\mu$ g/mL.  
20 Recombinant IL-2 (Roche) was added to a final concentration of 10 U/mL. Fresh medium and  
21 IL-2 were added to the culture every three to four days. Eleven days after stimulation, the cells  
22 were fully confluent in the 96-well plate. Cells were then transferred to a 48-well plate and each  
23 well was topped up to 500  $\mu$ L per well with medium. After 14 days of stimulation, the cells were

1 stained with FcBlock (Miltenyi Biotec), Zombie-NIR live/dead (BioLegend), anti-CD3-  
2 Alexa532 (Thermo Fisher Scientific), anti- $\gamma\delta$ TCR-FITC (Thermo Fisher Scientific), anti-V $\delta$ 2  
3 TCR-APCFire750 (BioLegend), anti-CD56-BV605 (BioLegend), anti-CD4-BV750 (BD  
4 Biosciences), anti-CD8a-BV510 (BioLegend), anti- $\beta$ 11TCR-APC (Miltenyi Biotec), anti-  
5 CD161-PE (BioLegend), anti- V $\alpha$ 7.2 TCR-BV711 (BioLegend), anti-V $\alpha$ 24-J $\alpha$ 18 (iNKT)-  
6 PE/Cy7 (BioLegend), anti-V $\delta$ 1-Vioblue (Miltenyi Biotec), anti-CD16-BV650 (BioLegend), anti-  
7 CD20-BV785 (BioLegend), anti- $\alpha\beta$ TCR-Alexa700 (BioLegend) antibodies and analyzed by  
8 flow cytometry on an Aurora cytometer (Cytex). Supernatants were harvested for human T<sub>H</sub>  
9 cytokine analysis with the 13-plex LegendPlex kit (BioLegend).

#### 11 **Stimulation of $\alpha\beta$ T cells with PPD and BCG-lysate**

12 PBMCs from four BCG-vaccinated healthy controls and P were stained with the  
13 CellTrace Far-Red Cell Proliferation Kit (Thermo Fisher Scientific) at a dilution of 1:25,000.  
14 Cells were washed and resuspended at a density of  $2 \times 10^6$  cells/mL in RPMI 10% human AB  
15 serum (Gemini). Cells were plated in 96-well U-bottomed plates and stimulated with BCG-lysate  
16 at a final concentration of 5  $\mu$ g/mL or with tuberculin purified protein derivative (PPD) at a final  
17 concentration of 5  $\mu$ g/mL (STATEN SERUM INSTITUT). Cells were cultured for 6 days and  
18 then harvested and stained with the Zombie NIR Viability kit (BioLegend) for 15 minutes. Cells  
19 were then surface-stained with FcBlock, anti-CD3-V450 (BD Biosciences), anti-CD4-BUV563  
20 (BD Biosciences), anti-CD8-BUV737 (BD Biosciences), anti-V $\delta$ 2TCR-APC/Fire750  
21 (BioLegend), anti-CD56-BV605 (BioLegend) and anti-CD20-BV785 (BioLegend) antibodies for  
22 30 minutes. Cells were fixed with the FOXP3/Transcription Factor Buffer kit (Thermo Fisher  
23 Scientific) and stained by overnight incubation with anti-T-bet-PE/Cy7 (BioLegend), anti-IFN- $\gamma$ -

BV711 (BioLegend), anti-TNF- $\alpha$ -BV510 (BioLegend) and anti-human/mouse-Ki-67 (BioLegend) antibodies in Perm/Wash buffer. Data were acquired in a CyTek Aurora spectral flow cytometer.

#### **Isolation and screening of CD4<sup>+</sup> T cell lines**

The T-cell clone experiment was performed as previously described (Geiger et al., 2009). In brief, CCR6<sup>+</sup> and CCR6<sup>-</sup> CD4<sup>+</sup> memory T cells were sorted from CD14<sup>-</sup> PBMCs with a FACS Aria (BD Biosciences), excluding CD45RA<sup>+</sup>CCR7<sup>+</sup> naïve cells, and CD25<sup>+</sup>, CD19<sup>+</sup>, and CD8<sup>+</sup> cells. Sorted T cells (500 cells/well) were polyclonally stimulated with 1  $\mu$ g/mL PHA (Remel) in the presence of irradiated (45 Gy) allogeneic feeder cells (10<sup>4</sup> per well) and IL-2 (500 IU/mL) in RPMI complete medium. After expansion for at least 20 days, the T-cell lines were analyzed by surface or intracellular staining with the following monoclonal antibodies: anti-CCR6-PE (BD Biosciences; clone: 11A9); anti-CXCR3-APC (BD Biosciences; clone: 1C6); anti-IFN- $\gamma$ -FITC or anti-IFN- $\gamma$ -PE (BD Biosciences, clone: B27); anti-IL-17A-eFluor660 FITC (eBiosciences, clone: eBio64DEC17); anti-IL-4-PE (BD Biosciences, clone: MP4-25D2); anti-IL-22-PerCP-eFluor-710 (Thermo Fisher Scientific, clone: 22URTI). For identification of the antigen-responding T-cell clones, the T-cell lines were also screened by culturing thoroughly washed cells (2.5 $\times$ 10<sup>5</sup> cells/well) with autologous irradiated B cells (2.5 $\times$ 10<sup>4</sup> cells/well), with or without a three-hour pulse of the following antigens: *M. tuberculosis* peptide pool (0.5  $\mu$ g/mL/peptide, comprising 207 15-mer peptides), BCG peptide pool (0.5  $\mu$ g/mL/peptide, comprising 211 15-mer peptides), HCMV peptide pool (0.5  $\mu$ g/mL/peptide, comprising 76 15-20-mer peptides), EBV peptide pool (0.5  $\mu$ g/mL/peptide, comprising 46 15-20-mer peptides), Influenza virus HA peptide pool (2  $\mu$ g/mL/peptide, comprising 351 15-mer peptides), *Candida albicans* peptide pool (0.5  $\mu$ g/mL/peptide, comprising 252 15-mer peptides) or tetanus toxoid

peptide pool (1  $\mu\text{g/mL}$ /peptide, comprising 125 15-20-mer peptides). These peptide pools were kindly provided by Cecilia Lindestam Arlehamn and Alessandro Sette at the La Jolla Institute for Immunology (Lindestam Arlehamn et al., 2016). Proliferation was assessed on day 4, after incubation for 16 h with 1  $\mu\text{Ci/mL}$  [ $^3\text{H}$ ]-thymidine (GE Healthcare). The concentrations of cytokines in the culture supernatants produced by the antigen-responding T-cell clones were measured after 48 h of stimulation with cytometric bead arrays (eBioscience).

## QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis was performed using PRISM software (Graphpad). All values are expressed as Mean  $\pm$  SD or SEM as indicated in the figure legends. Student's t-test, one-way ANOVA, two-way ANOVA, or non-parametric tests were performed tailored to the hypotheses tested, and were explained in figure legends. Statistical significance was accepted at  $\alpha < 0.05$ .

## References

- Abecasis, G.R., Cherny, S.S., Cookson, W.O., and Cardon, L.R. (2002). Merlin—rapid analysis of dense genetic maps using sparse gene flow trees. *Nat. Genet.* *30*, 97–101.
- Acosta-Rodriguez, E. V, Rivino, L., Geginat, J., Jarrossay, D., Gattorno, M., Lanzavecchia, A., Sallusto, F., and Napolitani, G. (2007). Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nat. Immunol.* *8*, 639–646.
- Afgan, E., Baker, D., van den Beek, M., Blankenberg, D., Bouvier, D., Čech, M., Chilton, J., Clements, D., Coraor, N., Eberhard, C., et al. (2016). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. *Nucleic Acids Res.* *44*, W3–W10.
- Aluri, J., Gupta, M., Dalvi, A., Mhatre, S., Kulkarni, M., Hule, G., Desai, M., Shah, N., Taur, P., Vedam, R., et al. (2018). Clinical, immunological, and molecular findings in five patients with Major histocompatibility complex class II deficiency from India. *Front. Immunol.* *9*, 16.
- Amir el, A.D., Davis, K.L., Tadmor, M.D., Simonds, E.F., Levine, J.H., Bendall, S.C., Shenfeld, D.K., Krishnaswamy, S., Nolan, G.P., and Pe'er, D. (2013). viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. *Nat Biotechnol* *31*, 545–552.
- Ardeniz, Ö., Unger, S., Onay, H., Ammann, S., Keck, C., Cianga, C., Gerçeker, B., Martin, B., Fuchs, I., Salzer, U., et al. (2015).  $\beta$ 2-Microglobulin deficiency causes a complex immunodeficiency of the innate and adaptive immune system. *J. Allergy Clin. Immunol.* *136*, 392–401.
- Auton, A., Abecasis, G.R., Altshuler, D.M., Durbin, R.M., Abecasis, G.R., Bentley, D.R., Chakravarti, A., Clark, A.G., Donnelly, P., Eichler, E.E., et al. (2015). A global reference for human genetic variation. *Nature* *526*, 68–74.
- Bakshi, C.S., Malik, M., Carrico, P.M., and Sellati, T.J. (2006). T-bet deficiency facilitates airway colonization by *Mycoplasma pulmonis* in a murine model of asthma. *J Immunol* *177*, 1786–1795.
- Becattini, S., Latorre, D., Mele, F., Foglierini, M., De Gregorio, C., Cassotta, A., Fernandez, B., Kelderman, S., Schumacher, T.N., Corti, D., et al. (2015). T cell immunity. Functional heterogeneity of human memory CD4(+) T cell clones primed by pathogens or vaccines. *Science* (80-. ). *347*, 400–406.
- Becht, E., McInnes, L., Healy, J., Dutertre, C.-A., Kwok, I.W.H., Ng, L.G., Ginhoux, F., and Newell, E.W. (2019). Dimensionality reduction for visualizing single-cell data using UMAP. *Nat. Biotechnol.* *37*, 38–44.
- Bettelli, E., Sullivan, B., Szabo, S.J., Sobel, R.A., Glimcher, L.H., and Kuchroo, V.K. (2004). Loss of T-bet, but not STAT1, prevents the development of experimental autoimmune encephalomyelitis. *J Exp Med* *200*, 79–87.

1 Björkström, N.K., Ljunggren, H.-G., and Sandberg, J.K. (2010). CD56 negative NK cells: origin,  
2 function, and role in chronic viral disease. *Trends Immunol.* *31*, 401–406.

3 Bogunovic, D., Byun, M., Durfee, L.A., Abhyankar, A., Sanal, O., Mansouri, D., Salem, S.,  
4 Radovanovic, I., Grant, A. V, Adimi, P., et al. (2012). Mycobacterial disease and impaired IFN-  
5 gamma immunity in humans with inherited ISG15 deficiency. *Science* (80-. ). *337*, 1684–1688.

6 Boisson-Dupuis, S., Ramirez-Alejo, N., Li, Z., Patin, E., Rao, G., Kerner, G., Lim, C.K.,  
7 Kremontsov, D.N., Hernandez, N., Ma, C.S., et al. (2018). Tuberculosis and impaired IL-23-  
8 dependent IFN- $\gamma$  immunity in humans homozygous for a common TYK2 missense variant. *Sci.*  
9 *Immunol.* *3*, eaau8714.

10 Buenrostro, J.D., Giresi, P.G., Zaba, L.C., Chang, H.Y., and Greenleaf, W.J. (2013).  
11 Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin,  
12 DNA-binding proteins and nucleosome position. *Nat Methods* *10*, 1213–1218.

13 Buenrostro, J.D., Wu, B., Chang, H.Y., and Greenleaf, W.J. (2015). ATAC-seq: A Method for  
14 Assaying Chromatin Accessibility Genome-Wide. *Curr Protoc Mol Biol* *109*, 21 29 1-9.

15 Bustamante, J. (2020). Mendelian susceptibility to mycobacterial disease: recent discoveries.  
16 *Hum. Genet.* *139*, 993–1000.

17 Carroll, T., and Barrows, D. (2019). profileplyr: Visualization and annotation of read signal over  
18 genomic ranges with profileplyr.

19 Carroll, T.S., Liang, Z., Salama, R., Stark, R., and de Santiago, I. (2014). Impact of artifact  
20 removal on ChIP quality metrics in ChIP-seq and ChIP-exo data. *Front. Genet.* *5*, 75.

21 Casanova, J.L. (1999). IL-12 and IFN- $\gamma$  in host defense against mycobacteria and salmonella in  
22 mice and men. *Curr. Opin. Immunol.* *11*, 346–351.

23 Casanova, J.L. (2015a). Human genetic basis of interindividual variability in the course of  
24 infection. *Proc. Natl. Acad. Sci. U. S. A.* *112*, E7118–E7127.

25 Casanova, J.L. (2015b). Severe infectious diseases of childhood as monogenic inborn errors of  
26 immunity. *Proc. Natl. Acad. Sci. U. S. A.* *112*, E7128–E7137.

27 Casanova, J.L., and Abel, L. (2004). The human model: A genetic dissection of immunity to  
28 infection in natural conditions. *Nat. Rev. Immunol.* *4*, 55–66.

29 Casanova, J.L., and Abel, L. (2018). Human genetics of infectious diseases: Unique insights into  
30 immunological redundancy. *Semin. Immunol.* *36*, 1–12.

31 Casanova, J.-L., Conley, M.E., Seligman, S.J., Abel, L., and Notarangelo, L.D. (2014).  
32 Guidelines for genetic studies in single patients: lessons from primary immunodeficiencies. *J.*  
33 *Exp. Med.* *211*, 2137–2149.

34 Casanova, J.L., Jouanguy, E., Lamhamedi, S., Blanche, S., and Fischer, A. (1995).

1 Immunological conditions of children with BCG disseminated infection. *Lancet* (London,  
2 England) *346*, 581.

3 Casey, J.P., Nobbs, M., McGettigan, P., Lynch, S., and Ennis, S. (2012). Recessive mutations in  
4 MCM4/PRKDC cause a novel syndrome involving a primary immunodeficiency and a disorder  
5 of DNA repair. *J. Med. Genet.* *49*, 242–245.

6 Chen, Z.W. (2005). Immune regulation of gammadelta T cell responses in mycobacterial  
7 infections. *Clin. Immunol.* *116*, 202–207.

8 Chen, L., He, W., Kim, S.T., Tao, J., Gao, Y., Chi, H., Intlekofer, A.M., Harvey, B., Reiner, S.L.,  
9 Yin, Z., et al. (2007). Epigenetic and Transcriptional Programs Lead to Default IFN- $\gamma$  Production  
10 by  $\gamma\delta$  T Cells. *J. Immunol.* *178*, 2730–2736.

11 Chien, Y., Meyer, C., and Bonneville, M. (2014).  $\gamma\delta$  T Cells: First Line of Defense and Beyond.  
12 *Annu. Rev. Immunol.* *32*, 121–155.

13 Cingolani, P., Platts, A., Wang, L.L., Coon, M., Nguyen, T., Wang, L., Land, S.J., Lu, X., and  
14 Ruden, D.M. (2012). A program for annotating and predicting the effects of single nucleotide  
15 polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2;  
16 iso-3. *Fly* (Austin). *6*, 80–92.

17 Corces, M.R., Trevino, A.E., Hamilton, E.G., Greenside, P.G., Sinnott-Armstrong, N.A., Vesuna,  
18 S., Satpathy, A.T., Rubin, A.J., Montine, K.S., Wu, B., et al. (2017). An improved ATAC-seq  
19 protocol reduces background and enables interrogation of frozen tissues. *Nat. Methods* *14*, 959–  
20 962.

21 Cottineau, J., Kottemann, M.C., Lach, F.P., Kang, Y.-H., Vély, F., Deenick, E.K., Lazarov, T.,  
22 Gineau, L., Wang, Y., Farina, A., et al. (2017). Inherited GINS1 deficiency underlies growth  
23 retardation along with neutropenia and NK cell deficiency. *J. Clin. Invest.* *127*, 1991–2006.

24 Crosby, C.M., and Kronenberg, M. (2018). Tissue-specific functions of invariant natural killer T  
25 cells. *Nat. Rev. Immunol.* *18*, 559–574.

26 Daussy, C., Faure, F., Mayol, K., Viel, S., Gasteiger, G., Charrier, E., Bienvenu, J., Henry, T.,  
27 Debien, E., Hasan, U.A., et al. (2014). T-bet and Eomes instruct the development of two distinct  
28 natural killer cell lineages in the liver and in the bone marrow. *J. Exp. Med.* *211*, 563–577.

29 Davis, B.R., Dicola, M.J., Prokopishyn, N.L., Rosenberg, J.B., Moratto, D., Muul, L.M.,  
30 Candotti, F., and Michael Blaese, R. (2008). Unprecedented diversity of genotypic revertants in  
31 lymphocytes of a patient with Wiskott-Aldrich syndrome. *Blood* *111*, 5064–5067.

32 Depristo, M.A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J.R., Hartl, C., Philippakis,  
33 A.A., Del Angel, G., Rivas, M.A., Hanna, M., et al. (2011). A framework for variation discovery  
34 and genotyping using next-generation DNA sequencing data. *Nat. Genet.* *43*, 491–501.

35 Dimitrova, D., Ong, P.Y., O’Gorman, M.R.G., and Church, J.A. (2014). Major  
36 Histocompatibility Complex Class II Deficiency Complicated by *Mycobacterium avium*



1     Complex in a Boy of Mixed Ethnicity. *J. Clin. Immunol.* *34*, 677–680.

2     Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M.,  
3     and Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* *29*, 15–  
4     21.

5     Dorner, B.G., Scheffold, A., Rolph, M.S., Huser, M.B., Kaufmann, S.H.E., Radbruch, A., Flesch,  
6     I.E.A., and Kroczeck, R.A. (2002). MIP-1 , MIP-1 , RANTES, and ATAC/lymphotactin function  
7     together with IFN- as type 1 cytokines. *Proc. Natl. Acad. Sci.* *99*, 6181–6186.

8     Dorner, B.G., Steinbach, S., Hüser, M.B., Kroczeck, R.A., and Scheffold, A. (2003). Single-cell  
9     analysis of the murine chemokines MIP-1alpha, MIP-1beta, RANTES and ATAC/lymphotactin  
10    by flow cytometry. *J. Immunol. Methods* *274*, 83–91.

11    Dorner, B.G., Smith, H.R.C., French, A.R., Kim, S., Poursine-Laurent, J., Beckman, D.L., Pingel,  
12    J.T., Kroczeck, R.A., and Yokoyama, W.M. (2004). Coordinate Expression of Cytokines and  
13    Chemokines by NK Cells during Murine Cytomegalovirus Infection. *J. Immunol.* *172*, 3119–  
14    3131.

15    Dupuis, S., Döffinger, R., Picard, C., Fieschi, C., Altare, F., Jouanguy, E., Abel, L., and  
16    Casanova, J.L. (2000). Human interferon-gamma-mediated immunity is a genetically controlled  
17    continuous trait that determines the outcome of mycobacterial invasion. *Immunol. Rev.* *178*,  
18    129–137.

19    Elhasid, R., and Etzioni, A. (1996). Major histocompatibility complex class II deficiency: a  
20    clinical review. *Blood Rev.* *10*, 242–248.

21    Feng, J., Liu, T., Qin, B., Zhang, Y., and Liu, X.S. (2012). Identifying ChIP-seq enrichment  
22    using MACS. *Nat. Protoc.* *7*, 1728–1740.

23    Finak, G., McDavid, A., Yajima, M., Deng, J., Gersuk, V., Shalek, A.K., Slichter, C.K., Miller,  
24    H.W., McElrath, M.J., Prlic, M., et al. (2015). MAST: a flexible statistical framework for  
25    assessing transcriptional changes and characterizing heterogeneity in single-cell RNA  
26    sequencing data. *Genome Biol.* *16*, 278.

27    Finotto, S., Neurath, M.F., Glickman, J.N., Qin, S., Lehr, H.A., Green, F.H., Ackerman, K.,  
28    Haley, K., Galle, P.R., Szabo, S.J., et al. (2002). Development of spontaneous airway changes  
29    consistent with human asthma in mice lacking T-bet. *Science* (80-. ). *295*, 336–338.

30    Frankish, A., Diekhans, M., Ferreira, A.-M., Johnson, R., Jungreis, I., Loveland, J., Mudge, J.M.,  
31    Sisu, C., Wright, J., Armstrong, J., et al. (2019). GENCODE reference annotation for the human  
32    and mouse genomes. *Nucleic Acids Res.* *47*, D766–D773.

33    Garrett, W.S., Lord, G.M., Punit, S., Lugo-Villarino, G., Mazmanian, S.K., Ito, S., Glickman,  
34    J.N., and Glimcher, L.H. (2007). Communicable ulcerative colitis induced by T-bet deficiency in  
35    the innate immune system. *Cell* *131*, 33–45.

36    Garrido-Mesa, N., Schroeder, J.-H., Stolarczyk, E., Gallagher, A.L., Lo, J.W., Bailey, C.,

1 Campbell, L., Sexl, V., MacDonald, T.T., Howard, J.K., et al. (2019). T-bet controls intestinal  
2 mucosa immune responses via repression of type 2 innate lymphoid cell function. *Mucosal*  
3 *Immunol.* *12*, 51–63.

4 Van Gassen, S., Callebaut, B., Van Helden, M.J., Lambrecht, B.N., Demeester, P., Dhaene, T.,  
5 and Saeys, Y. (2015). FlowSOM: Using self-organizing maps for visualization and interpretation  
6 of cytometry data. *Cytom. Part A* *87*, 636–645.

7 Gavins, F.N.E., and Hickey, M.J. (2012). Annexin A1 and the regulation of innate and adaptive  
8 immunity. *Front. Immunol.* *3*, 354.

9 Geiger, R., Duhon, T., Lanzavecchia, A., and Sallusto, F. (2009). Human naive and memory  
10 CD4+ T cell repertoires specific for naturally processed antigens analyzed using libraries of  
11 amplified T cells. *J. Exp. Med.* *206*, 1525–1534.

12 Geurts, P., Ernst, D., and Wehenkel, L. (2006). Extremely randomized trees. *Mach. Learn.* *63*, 3–  
13 42.

14 Godfrey, D.I., Koay, H.-F., McCluskey, J., and Gherardin, N.A. (2019). The biology and  
15 functional importance of MAIT cells. *Nat. Immunol.* *20*, 1110–1128.

16 Gökmen, M.R., Dong, R., Kanhere, A., Powell, N., Perucha, E., Jackson, I., Howard, J.K.,  
17 Hernandez-Fuentes, M., Jenner, R.G., and Lord, G.M. (2013). Genome-Wide Regulatory  
18 Analysis Reveals That T-bet Controls Th17 Lineage Differentiation through Direct Suppression  
19 of IRF4. *J. Immunol.* *191*, 5925–5932.

20 Groom, J.R., and Luster, A.D. (2011). CXCR3 in T cell function. *Exp. Cell Res.* *317*, 620–631.

21 Gu, S., Borowska, M.T., Boughter, C.T., and Adams, E.J. (2018). Butyrophilin3A proteins and  
22 Vγ9Vδ2 T cell activation. *Semin. Cell Dev. Biol.* *84*, 65–74.

23 Gu, Z., Eils, R., and Schlesner, M. (2016). Complex heatmaps reveal patterns and correlations in  
24 multidimensional genomic data. *Bioinformatics* *32*, 2847–2849.

25 Hambleton, S., Salem, S., Bustamante, J., Bigley, V., Boisson-Dupuis, S., Azevedo, J., Fortin, A.,  
26 Haniffa, M., Ceron-Gutierrez, L., Bacon, C.M., et al. (2011). IRF8 mutations and human  
27 dendritic-cell immunodeficiency. *N. Engl. J. Med.* *365*, 127–138.

28 Hanalioglu, D., Ayvaz, D.C., Ozgur, T.T., van der Burg, M., Sanal, O., and Tezcan, I. (2017). A  
29 novel mutation in TAP1 gene leading to MHC class I deficiency: Report of two cases and review  
30 of the literature. *Clin. Immunol.* *178*, 74–78.

31 Hanna, S., and Etzioni, A. (2014). MHC class I and II deficiencies. *J. Allergy Clin. Immunol.*  
32 *134*, 269–275.

33 Harayama, T., and Riezman, H. (2017). Detection of genome-edited mutant clones by a simple  
34 competition-based PCR method. *PLoS One* *12*, e0179165.

1 Harly, C., Guillaume, Y., Nedellec, S., Peigne, C.M., Monkkonen, H., Monkkonen, J., Li, J.,  
2 Kuball, J., Adams, E.J., Netzer, S., et al. (2012). Key implication of CD277/butyrophilin-3  
3 (BTN3A) in cellular stress sensing by a major human gammadelta T-cell subset. *Blood* *120*,  
4 2269–2279.

5 Harms Pritchard, G., Hall, A.O., Christian, D.A., Wagage, S., Fang, Q., Muallem, G., John, B.,  
6 Glatman Zaretsky, A., Dunn, W.G., Perrigoue, J., et al. (2015). Diverse roles for T-bet in the  
7 effector responses required for resistance to infection. *J Immunol* *194*, 1131–1140.

8 Hernandez, N., Melki, I., Jing, H., Habib, T., Huang, S.S.Y., Danielson, J., Kula, T., Drutman, S.,  
9 Belkaya, S., Rattina, V., et al. (2018). Life-threatening influenza pneumonitis in a child with  
10 inherited IRF9 deficiency. *J. Exp. Med.* *215*, 2567–2585.

11 Hoft, D.F., Brown, R.M., and Roodman, S.T. (1998). Bacille Calmette-Guerin vaccination  
12 enhances human gamma delta T cell responsiveness to mycobacteria suggestive of a memory-  
13 like phenotype. *J Immunol* *161*, 1045–1054.

14 Hughes, C.R., Guasti, L., Meimaridou, E., Chuang, C.H., Schimenti, J.C., King, P.J., Costigan,  
15 C., Clark, A.J.L., and Metherell, L.A. (2012). MCM4 mutation causes adrenal failure, short  
16 stature, and natural killer cell deficiency in humans. *J. Clin. Invest.* *122*, 814–820.

17 Hultgren, O.H., Verdrengh, M., and Tarkowski, A. (2004). T-box transcription-factor-deficient  
18 mice display increased joint pathology and failure of infection control during staphylococcal  
19 arthritis. *Microbes Infect* *6*, 529–535.

20 Intlekofer, A.M., Takemoto, N., Kao, C., Banerjee, A., Schambach, F., Northrop, J.K., Shen, H.,  
21 Wherry, E.J., and Reiner, S.L. (2007). Requirement for T-bet in the aberrant differentiation of  
22 unhelped memory CD8+ T cells. *J Exp Med* *204*, 2015–2021.

23 Itan, Y., Zhang, S.-Y., Vogt, G., Abhyankar, A., Herman, M., Nitschke, P., Fried, D., Quintana-  
24 Murci, L., Abel, L., and Casanova, J.-L. (2013). The human gene connectome as a map of short  
25 cuts for morbid allele discovery. *Proc. Natl. Acad. Sci.* *110*, 5558–5563.

26 Itan, Y., Mazel, M., Mazel, B., Abhyankar, A., Nitschke, P., Quintana-Murci, L., Boisson-  
27 Dupuis, S., Boisson, B., Abel, L., Zhang, S.-Y., et al. (2014). HGCS: an online tool for  
28 prioritizing disease-causing gene variants by biological distance. *BMC Genomics* *15*, 256.

29 Itan, Y., Shang, L., Boisson, B., Patin, E., Bolze, A., Moncada-Velez, M., Scott, E., Ciancanelli,  
30 M.J., Lafaille, F.G., Markle, J.G., et al. (2015). The human gene damage index as a gene-level  
31 approach to prioritizing exome variants. *Proc Natl Acad Sci U S A* *112*, 13615–13620.

32 Itan, Y., Shang, L., Boisson, B., Ciancanelli, M.J., Markle, J.G., Martinez-Barricarte, R., Scott,  
33 E., Shah, I., Stenson, P.D., Gleeson, J., et al. (2016). The mutation significance cutoff: gene-level  
34 thresholds for variant predictions. *Nat Methods* *13*, 109–110.

35 Iwata, S., Mikami, Y., Sun, H.-W., Brooks, S.R., Jankovic, D., Hirahara, K., Onodera, A., Shih,  
36 H.-Y., Kawabe, T., Jiang, K., et al. (2017). The Transcription Factor T-bet Limits Amplification  
37 of Type I IFN Transcriptome and Circuitry in T Helper 1 Cells. *Immunity* *46*, 983-991.e4.

1 Janesick, A., Shiotsugu, J., Taketani, M., and Blumberg, B. (2012). RIPPLY3 is a retinoic acid-  
2 inducible repressor required for setting the borders of the pre-placodal ectoderm. *Development*  
3 *139*, 1213–1224.

4 Jang, E.J., Park, H.R., Hong, J.H., and Hwang, E.S. (2013). Lysine 313 of T-box is crucial for  
5 modulation of protein stability, DNA binding, and threonine phosphorylation of T-bet. *J*  
6 *Immunol* *190*, 5764–5770.

7 Jenner, R.G., Townsend, M.J., Jackson, I., Sun, K., Bouwman, R.D., Young, R.A., Glimcher,  
8 L.H., and Lord, G.M. (2009). The transcription factors T-bet and GATA-3 control alternative  
9 pathways of T-cell differentiation through a shared set of target genes. *Proc. Natl. Acad. Sci. U.*  
10 *S. A.* *106*, 17876–17881.

11 Juedes, A.E., Rodrigo, E., Togher, L., Glimcher, L.H., and von Herrath, M.G. (2004). T-bet  
12 controls autoaggressive CD8 lymphocyte responses in type 1 diabetes. *J Exp Med* *199*, 1153–  
13 1162.

14 Kanhere, A., Hertweck, A., Bhatia, U., Gökmen, M.R., Perucha, E., Jackson, I., Lord, G.M., and  
15 Jenner, R.G. (2012). T-bet and GATA3 orchestrate Th1 and Th2 differentiation through lineage-  
16 specific targeting of distal regulatory elements. *Nat. Commun.* *3*, 1268.

17 Kao, C., Oestreich, K.J., Paley, M.A., Crawford, A., Angelosanto, J.M., Ali, M.A., Intlekofer,  
18 A.M., Boss, J.M., Reiner, S.L., Weinmann, A.S., et al. (2011). Transcription factor T-bet  
19 represses expression of the inhibitory receptor PD-1 and sustains virus-specific CD8+ T cell  
20 responses during chronic infection. *Nat Immunol* *12*, 663–671.

21 Karczewski, K.J., Francioli, L.C., Tiao, G., Cummings, B.B., Alföldi, J., Wang, Q., Collins, R.L.,  
22 Laricchia, K.M., Ganna, A., Birnbaum, D.P., et al. (2020). The mutational constraint spectrum  
23 quantified from variation in 141,456 humans. *Nature* *581*, 434–443.

24 Kerner, G., Rosain, J., Guérin, A., Al-Khabaz, A., Oleaga-Quintas, C., Rapaport, F., Massaad,  
25 M.J., Ding, J.Y., Khan, T., Ali, F. Al, et al. (2020). Inherited human IFN- $\gamma$  deficiency underlies  
26 mycobacterial disease. *J. Clin. Invest.* *130*, 3158–3171.

27 Kircher, M., Witten, D.M., Jain, P., O’Roak, B.J., Cooper, G.M., and Shendure, J. (2014). A  
28 general framework for estimating the relative pathogenicity of human genetic variants. *Nat*  
29 *Genet* *46*, 310–315.

30 Kjer-Nielsen, L., Patel, O., Corbett, A.J., Le Nours, J., Meehan, B., Liu, L., Bhati, M., Chen, Z.,  
31 Kostenko, L., Reantragoon, R., et al. (2012). MR1 presents microbial vitamin B metabolites to  
32 MAIT cells. *Nature* *491*, 717–723.

33 Koch, M.A., Tucker-Heard, G., Perdue, N.R., Killebrew, J.R., Urdahl, K.B., and Campbell, D.J.  
34 (2009). The transcription factor T-bet controls regulatory T cell homeostasis and function during  
35 type 1 inflammation. *Nat. Immunol.* *10*, 595–602.

36 Kong, X.F., Martinez-Barricarte, R., Kennedy, J., Mele, F., Lazarov, T., Deenick, E.K., Ma, C.S.,  
37 Breton, G., Lucero, K.B., Langlais, D., et al. (2018). Disruption of an antimycobacterial circuit

1 between dendritic and helper T cells in human SPPL2a deficiency. *Nat Immunol* 19, 973–985.

2 Korsunsky, I., Millard, N., Fan, J., Slowikowski, K., Zhang, F., Wei, K., Baglaenko, Y., Brenner,  
3 M., Loh, P. ru, and Raychaudhuri, S. (2019). Fast, sensitive and accurate integration of single-  
4 cell data with Harmony. *Nat. Methods* 16, 1289–1296.

5 Kroczeck, R.A., and Henn, V. (2012). The Role of XCR1 and its Ligand XCL1 in Antigen Cross-  
6 Presentation by Murine and Human Dendritic Cells. *Front. Immunol.* 3, 14.

7 De La Salle, H., Hanau, D., Fricker, D., Urlacher, A., Kelly, A., Salamero, J., Powis, S.H.,  
8 Donato, L., Bausinger, H., Laforet, M., et al. (1994). Homozygous human TAP peptide  
9 transporter mutation in HLA class I deficiency. *Science* (80-. ). 265, 237–241.

10 Latour, S. (2007). Natural killer T cells and X-linked lymphoproliferative syndrome. *Curr. Opin.*  
11 *Allergy Clin. Immunol.* 7, 510–514.

12 Lazarevic, V., Chen, X., Shim, J.H., Hwang, E.S., Jang, E., Bolm, A.N., Oukka, M., Kuchroo,  
13 V.K., and Glimcher, L.H. (2011). T-bet represses TH 17 differentiation by preventing Runx1-  
14 mediated activation of the gene encoding ROR $\gamma$ t. *Nat. Immunol.* 12, 96–104.

15 Lazarevic, V., Glimcher, L.H., and Lord, G.M. (2013). T-bet: a bridge between innate and  
16 adaptive immunity. *Nat Rev Immunol* 13, 777–789.

17 Lebrun, A., Portocarrero, C., Kean, R.B., Barkhouse, D.A., Faber, M., and Hooper, D.C. (2015).  
18 T-bet Is Required for the Rapid Clearance of Attenuated Rabies Virus from Central Nervous  
19 System Tissue. *J Immunol* 195, 4358–4368.

20 Levine, A.G., Mendoza, A., Hemmers, S., Moltedo, B., Niec, R.E., Schizas, M., Hoyos, B.E.,  
21 Putintseva, E. V, Chaudhry, A., Dikiy, S., et al. (2017). Stability and function of regulatory T  
22 cells expressing the transcription factor T-bet. *Nature* 546, 421–425.

23 Lewis, M.D., Miller, S.A., Miazgowicz, M.M., Beima, K.M., and Weinmann, A.S. (2007). T-  
24 bet’s ability to regulate individual target genes requires the conserved T-box domain to recruit  
25 histone methyltransferase activity and a separate family member-specific transactivation domain.  
26 *Mol. Cell. Biol.* 27, 8510–8521.

27 Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler  
28 transform. *Bioinformatics* 25, 1754–1760.

29 Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G.,  
30 Durbin, R., and 1000 Genome Project Data Processing Subgroup (2009). The Sequence  
31 Alignment/Map format and SAMtools. *Bioinformatics* 25, 2078–2079.

32 Liao, Y., Smyth, G.K., and Shi, W. (2014). featureCounts: an efficient general purpose program  
33 for assigning sequence reads to genomic features. *Bioinformatics* 30, 923–930.

34 Liberzon, A., Birger, C., Thorvaldsdóttir, H., Ghandi, M., Mesirov, J.P., and Tamayo, P. (2015).  
35 The Molecular Signatures Database Hallmark Gene Set Collection. *Cell Syst.* 1, 417–425.

1 Lim, A.I., Li, Y., Lopez-Lastra, S., Stadhouders, R., Paul, F., Casrouge, A., Serafini, N., Puel, A.,  
2 Bustamante, J., Surace, L., et al. (2017). Systemic Human ILC Precursors Provide a Substrate for  
3 Tissue ILC Differentiation. *Cell* 168, 1086-1100.e10.

4 Lindestam Arlehamn, C.S., McKinney, D.M., Carpenter, C., Paul, S., Rozot, V., Makgotlho, E.,  
5 Gregg, Y., van Rooyen, M., Ernst, J.D., Hatherill, M., et al. (2016). A Quantitative Analysis of  
6 Complexity of Human Pathogen-Specific CD4 T Cell Responses in Healthy M. tuberculosis  
7 Infected South Africans. *PLOS Pathog.* 12, e1005760.

8 Liu, L., Okada, S., Kong, X.-F., Kreins, A.Y., Cypowyj, S., Abhyankar, A., Toubiana, J., Itan, Y.,  
9 Audry, M., Nitschke, P., et al. (2011). Gain-of-function human STAT1 mutations impair IL-17  
10 immunity and underlie chronic mucocutaneous candidiasis. *J. Exp. Med.* 208, 1635–1648.

11 Locci, M., Ghici, E.D., Marangoni, F., Bosticardo, M., Catucci, M., Aiuti, A., Cancrini, C.,  
12 Marodi, L., Espanol, T., Bredius, R.G.M., et al. (2009). The Wiskott-Aldrich syndrome protein is  
13 required for iNKT cell maturation and function. *J. Exp. Med.* 206, 735–742.

14 Loetscher, P., Uguccioni, M., Bordoli, L., Baggiolini, M., Moser, B., Chizzolini, C., and Dayer,  
15 J.-M. (1998). CCR5 is characteristic of Th1 lymphocytes. *Nature* 391, 344–345.

16 Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and  
17 dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15, 550.

18 Lugo-Villarino, G., Maldonado-Lopez, R., Possemato, R., Penaranda, C., and Glimcher, L.H.  
19 (2003). T-bet is required for optimal production of IFN-gamma and antigen-specific T cell  
20 activation by dendritic cells. *Proc Natl Acad Sci U S A* 100, 7749–7754.

21 Lum, S.H., Neven, B., Slatter, M.A., and Gennery, A.R. (2019). Hematopoietic Cell  
22 Transplantation for MHC Class II Deficiency. *Front. Pediatr.* 7, 516.

23 Lum, S.H., Anderson, C., McNaughton, P., Engelhardt, K.R., MacKenzie, B., Watson, H., Al-  
24 Mousa, H., Al-Herz, W., Al-Saud, B., Mohammed, R., et al. (2020). Improved transplant  
25 survival and long-term disease outcome in children with MHC class II deficiency. *Blood* 135,  
26 954–973.

27 Lund, R., Ahlfors, H., Kainonen, E., Lahesmaa, A.-M., Dixon, C., and Lahesmaa, R. (2005).  
28 Identification of genes involved in the initiation of human Th1 or Th2 cell commitment. *Eur. J.*  
29 *Immunol.* 35, 3307–3319.

30 Ma, C.S., Wong, N., Rao, G., Avery, D.T., Torpy, J., Hambridge, T., Bustamante, J., Okada, S.,  
31 Stoddard, J.L., Deenick, E.K., et al. (2015). Monogenic mutations differentially affect the  
32 quantity and quality of T follicular helper cells in patients with human primary  
33 immunodeficiencies. *J. Allergy Clin. Immunol.* 136, 993-1006.e1.

34 Ma, W., Noble, W.S., and Bailey, T.L. (2014). Motif-based analysis of large nucleotide data sets  
35 using MEME-ChIP. *Nat. Protoc.* 9, 1428–1450.

36 Martínez-Barricarte, R., de Jong, S.J., Markle, J., de Paus, R., Boisson-Dupuis, S., Bustamante,

- 1 J., van de Vosse, E., Fleckenstein, B., and Casanova, J.-L. (2016). Transduction of *Herpesvirus*  
2 *saimiri* -Transformed T Cells with Exogenous Genes of Interest. In Current Protocols in  
3 Immunology, (Hoboken, NJ, USA: John Wiley & Sons, Inc.), pp. 7.21C.1-7.21C.12.
- 4 Martínez-Barricarte, R., Markle, J.G., Ma, C.S., Deenick, E.K., Ramírez-Alejo, N., Mele, F.,  
5 Latorre, D., Mahdavian, S.A., Aytekin, C., Mansouri, D., et al. (2018). Human IFN- immunity  
6 to mycobacteria is governed by both IL-12 and IL-23. *Sci. Immunol.* 3.
- 7 Matsuyama, M., Ishii, Y., Yageta, Y., Ohtsuka, S., Ano, S., Matsuno, Y., Morishima, Y., Yoh,  
8 K., Takahashi, S., Ogawa, K., et al. (2014). Role of Th1/Th17 balance regulated by T-bet in a  
9 mouse model of *Mycobacterium avium* complex disease. *J Immunol* 192, 1707–1717.
- 10 McGinnis, C.S., Murrow, L.M., and Gartner, Z.J. (2019). DoubletFinder: Doublet Detection in  
11 Single-Cell RNA Sequencing Data Using Artificial Nearest Neighbors. *Cell Syst.* 8, 329-337.e4.
- 12 McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella,  
13 K., Altshuler, D., Gabriel, S., Daly, M., et al. (2010). The Genome Analysis Toolkit: A  
14 MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 20,  
15 1297–1303.
- 16 Miller, S.A., and Weinmann, A.S. (2010). Molecular mechanisms by which T-bet regulates T-  
17 helper cell commitment. *Immunol Rev* 238, 233–246.
- 18 Modlin, R.L., Pirmez, C., Hofman, F.M., Torigian, V., Uyemura, K., Rea, T.H., Bloom, B.R.,  
19 and Brenner, M.B. (1989). Lymphocytes bearing antigen-specific  $\gamma\delta$  T-cell receptors accumulate  
20 in human infectious disease lesions. *Nature* 339, 544–548.
- 21 Morgan, N. V., Goddard, S., Cardno, T.S., McDonald, D., Rahman, F., Barge, D., Ciupek, A.,  
22 Straatman-Iwanowska, A., Pasha, S., Guckian, M., et al. (2011). Mutation in the TCR $\alpha$  subunit  
23 constant gene (TRAC) leads to a human immunodeficiency disorder characterized by a lack of  
24 TCR $\alpha\beta$ + T cells. *J. Clin. Invest.* 121, 695–702.
- 25 Nathan, C.F., Murray, H.W., Wiebe, M.E., and Rubin, B.Y. (1983). Identification of interferon-  
26 gamma as the lymphokine that activates human macrophage oxidative metabolism and  
27 antimicrobial activity. *J Exp Med* 158, 670–689.
- 28 Nishikomori, R., Usui, T., Wu, C.-Y., Morinobu, A., O’Shea, J.J., and Strober, W. (2002).  
29 Activated STAT4 has an essential role in Th1 differentiation and proliferation that is  
30 independent of its role in the maintenance of IL-12R beta 2 chain expression and signaling. *J.*  
31 *Immunol.* 169, 4388–4398.
- 32 Oakley, M.S., Sahu, B.R., Lotspeich-Cole, L., Solanki, N.R., Majam, V., Pham, P.T., Banerjee,  
33 R., Kozakai, Y., Derrick, S.C., Kumar, S., et al. (2013). The transcription factor T-bet regulates  
34 parasitemia and promotes pathogenesis during *Plasmodium berghei* ANKA murine malaria. *J*  
35 *Immunol* 191, 4699–4708.
- 36 Okada, S., Markle, J.G., Deenick, E.K., Mele, F., Averbuch, D., Lagos, M., Alzahrani, M., Al-  
37 Muhsen, S., Halwani, R., Ma, C.S., et al. (2015). Impairment of immunity to *Candida* and

1 Mycobacterium in humans with bi-allelic RORC mutations. *Science* (80-. ). *349*, 606–613.

2 Panchamoorthy, G., McLean, J., Modlin, R.L., Morita, C.T., Ishikawa, S., Brenner, M.B., and  
3 Band, H. (1991). A predominance of the T cell receptor V gamma 2/V delta 2 subset in human  
4 mycobacteria-responsive T cells suggests germline gene encoded recognition. *J. Immunol.* *147*,  
5 3360–3369.

6 Parker, C.M., Groh, V., Band, H., Porcelli, S.A., Morita, C., Fabbi, M., Glass, D., Strominger,  
7 J.L., and Brenner, M.B. (1990). Evidence for extrathymic changes in the T cell receptor  
8 gamma/delta repertoire. *J. Exp. Med.* *171*, 1597–1612.

9 Pearce, E.L., Mullen, A.C., Martins, G.A., Krawczyk, C.M., Hutchins, A.S., Zediak, V.P.,  
10 Banica, M., DiCioccio, C.B., Gross, D.A., Mao, C.-A., et al. (2003). Control of Effector CD8+ T  
11 Cell Function by the Transcription Factor Eomesodermin. *Science* (80-. ). *302*, 1041–1043.

12 Petrovski, S., Gussow, A.B., Wang, Q., Halvorsen, M., Han, Y., Weir, W.H., Allen, A.S., and  
13 Goldstein, D.B. (2015). The Intolerance of Regulatory Sequence to Genetic Variation Predicts  
14 Gene Dosage Sensitivity. *PLoS Genet* *11*, e1005492.

15 Powell, N., Walker, A.W., Stolarczyk, E., Canavan, J.B., Gökmen, M.R., Marks, E., Jackson, I.,  
16 Hashim, A., Curtis, M.A., Jenner, R.G., et al. (2012). The Transcription Factor T-bet Regulates  
17 Intestinal Inflammation Mediated by Interleukin-7 Receptor+ Innate Lymphoid Cells. *Immunity*  
18 *37*, 674–684.

19 Ramírez, F., Ryan, D.P., Grüning, B., Bhardwaj, V., Kilpert, F., Richter, A.S., Heyne, S., Dündar,  
20 F., and Manke, T. (2016). deepTools2: a next generation web server for deep-sequencing data  
21 analysis. *Nucleic Acids Res.* *44*, W160–W165.

22 Rankin, L.C., Groom, J.R., Chopin, M., Herold, M.J., Walker, J.A., Mielke, L.A., McKenzie,  
23 A.N.J., Carotta, S., Nutt, S.L., and Belz, G.T. (2013). The transcription factor T-bet is essential  
24 for the development of NKp46+ innate lymphocytes via the Notch pathway. *Nat. Immunol.* *14*,  
25 389–395.

26 Ravindran, R., Foley, J., Stoklasek, T., Glimcher, L.H., and McSorley, S.J. (2005). Expression of  
27 T-bet by CD4 T cells is essential for resistance to Salmonella infection. *J Immunol* *175*, 4603–  
28 4610.

29 Revy, P., Kannengiesser, C., and Fischer, A. (2019). Somatic genetic rescue in Mendelian  
30 haematopoietic diseases. *Nat. Rev. Genet.* *20*, 582–598.

31 Robinson, J.T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E.S., Getz, G., and  
32 Mesirov, J.P. (2011). Integrative genomics viewer. *Nat. Biotechnol.* *29*, 24–26.

33 Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: a Bioconductor package for  
34 differential expression analysis of digital gene expression data. *Bioinformatics* *26*, 139–140.

35 Rosain, J., Kong, X.F., Martinez-Barricarte, R., Oleaga-Quintas, C., Ramirez-Alejo, N., Markle,  
36 J., Okada, S., Boisson-Dupuis, S., Casanova, J.L., and Bustamante, J. (2019). Mendelian



1 susceptibility to mycobacterial disease: 2014–2018 update. *Immunol. Cell Biol.* 97, 360–367.

2 Rosas, L.E., Snider, H.M., Barbi, J., Satoskar, A.A., Lugo-Villarino, G., Keiser, T., Papenfuss, T.,  
3 Durbin, J.E., Radzioch, D., Glimcher, L.H., et al. (2006). Cutting edge: STAT1 and T-bet play  
4 distinct roles in determining outcome of visceral leishmaniasis caused by *Leishmania donovani*.  
5 *J Immunol* 177, 22–25.

6 Saleem, M.A., Arkwright, P.D., Davies, E.G., Cant, A.J., and Veys, P.A. (2000). Clinical course  
7 of patients with major histocompatibility complex class II deficiency. *Arch. Dis. Child.* 83, 356–  
8 359.

9 Sallusto, F., Lenig, D., Mackay, C.R., and Lanzavecchia, A. (1998). Flexible Programs of  
10 Chemokine Receptor Expression on Human Polarized T Helper 1 and 2 Lymphocytes. *J. Exp.*  
11 *Med.* 187, 875–883.

12 Sanjana, N.E., Shalem, O., and Zhang, F. (2014). Improved vectors and genome-wide libraries  
13 for CRISPR screening. *Nat Methods* 11, 783–784.

14 de Santiago, I., and Carroll, T. (2018). Analysis of ChIP-seq Data in R/Bioconductor. pp. 195–  
15 226.

16 Schoenborn, J.R., and Wilson, C.B. (2007). Regulation of Interferon- $\gamma$  During Innate and  
17 Adaptive Immune Responses. *Adv. Immunol.* 96, 41–101.

18 Scott, E.M., Halees, A., Itan, Y., Spencer, E.G., He, Y., Azab, M.A., Gabriel, S.B., Belkadi, A.,  
19 Boisson, B., Abel, L., et al. (2016). Characterization of Greater Middle Eastern genetic variation  
20 for enhanced disease gene discovery. *Nat. Genet.* 48, 1071–1076.

21 Shen, Y., Zhou, D., Qiu, L., Lai, X., Simon, M., Shen, L., Kou, Z., Wang, Q., Jiang, L., Estep, J.,  
22 et al. (2002). Adaptive immune response of Vgamma2Vdelta2+ T cells during mycobacterial  
23 infections. *Science* (80-. ). 295, 2255–2258.

24 Soutto, M., Zhang, F., Enerson, B., Tong, Y., Boothby, M., and Aune, T.M. (2002). A minimal  
25 IFN-gamma promoter confers Th1 selective expression. *J Immunol* 169, 4205–4212.

26 Stuart, T., Butler, A., Hoffman, P., Hafemeister, C., Papalexi, E., Mauck, W.M., Hao, Y.,  
27 Stoeckius, M., Smibert, P., and Satija, R. (2019). Comprehensive Integration of Single-Cell Data.  
28 *Cell* 177, 1888–1902.e21.

29 Sullivan, B.M., Juedes, A., Szabo, S.J., Von Herrath, M., and Glimcher, L.H. (2003). Antigen-  
30 driven effector CD8 T cell function regulated by T-bet. *Proc. Natl. Acad. Sci. U. S. A.* 100,  
31 15818–15823.

32 Sullivan, B.M., Jobe, O., Lazarevic, V., Vasquez, K., Bronson, R., Glimcher, L.H., and Kramnik,  
33 I. (2005). Increased susceptibility of mice lacking T-bet to infection with *Mycobacterium*  
34 *tuberculosis* correlates with increased IL-10 and decreased IFN-gamma production. *J Immunol*  
35 175, 4593–4602.

1 Svensson, A., Nordstrom, I., Sun, J.B., and Eriksson, K. (2005). Protective immunity to genital  
2 herpes simplex [correction of simpex] virus type 2 infection is mediated by T-bet. *J Immunol*  
3 *174*, 6266–6273.

4 Szabo, S.J., Kim, S.T., Costa, G.L., Zhang, X., Fathman, C.G., and Glimcher, L.H. (2000). A  
5 novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* *100*, 655–669.

6 Szabo, S.J., Sullivan, B.M., Stemmann, C., Satoskar, A.R., Sleckman, B.P., and Glimcher, L.H.  
7 (2002). Distinct effects of T-bet in TH1 lineage commitment and IFN-gamma production in CD4  
8 and CD8 T cells. *Science* (80-. ). *295*, 338–342.

9 Taliun, D., Harris, D.N., Kessler, M.D., Carlson, J., Szpiech, Z.A., Torres, R., Taliun, S.A.G.,  
10 Corvelo, A., Gogarten, S.M., Kang, H.M., et al. (2019). Sequencing of 53,831 diverse genomes  
11 from the NHLBI TOPMed Program. *BioRxiv* 563866.

12 Tan, T.G., Mathis, D., and Benoist, C. (2016). Singular role for T-BET+CXCR3+ regulatory T  
13 cells in protection from autoimmune diabetes. *Proc. Natl. Acad. Sci. U. S. A.* *113*, 14103–14108.

14 Tang, Y., Desierto, M.J., Chen, J., and Young, N.S. (2010). The role of the Th1 transcription  
15 factor T-bet in a mouse model of immune-mediated bone-marrow failure. *Blood* *115*, 541–548.

16 Tangye, S.G., Palendira, U., and Edwards, E.S.J. (2017). Human immunity against EBV—  
17 lessons from the clinic. *J. Exp. Med.* *214*, 269–283.

18 Tantisira, K.G., Hwang, E.S., Raby, B.A., Silverman, E.S., Lake, S.L., Richter, B.G., Peng, S.L.,  
19 Drazen, J.M., Glimcher, L.H., and Weiss, S.T. (2004). TBX21: a functional variant predicts  
20 improvement in asthma with the use of inhaled corticosteroids. *Proc Natl Acad Sci U S A* *101*,  
21 18099–18104.

22 Thieu, V.T., Yu, Q., Chang, H.-C., Yeh, N., Nguyen, E.T., Sehra, S., and Kaplan, M.H. (2008).  
23 Signal transducer and activator of transcription 4 is required for the transcription factor T-bet to  
24 promote T helper 1 cell-fate determination. *Immunity* *29*, 679–690.

25 Tong, Y., Aune, T., and Boothby, M. (2005). T-bet antagonizes mSin3a recruitment and  
26 transactivates a fully methylated IFN-gamma promoter via a conserved T-box half-site. *Proc*  
27 *Natl Acad Sci U S A* *102*, 2034–2039.

28 Toribio-Fernández, R., Zorita, V., Rocha-Perugini, V., Iborra, S., Martínez del Hoyo, G., Chevre,  
29 R., Dorado, B., Sancho, D., Sanchez-Madrid, F., Andrés, V., et al. (2018). Lamin A/C augments  
30 Th1 differentiation and response against vaccinia virus and *Leishmania major*. *Cell Death Dis.* *9*,  
31 9.

32 Townsend, M.J., Weinmann, A.S., Matsuda, J.L., Salomon, R., Farnham, P.J., Biron, C.A.,  
33 Gapin, L., and Glimcher, L.H. (2004). T-bet regulates the terminal maturation and homeostasis  
34 of NK and Valpha14i NKT cells. *Immunity* *20*, 477–494.

35 Tsukaguchi, K., Balaji, K.N., and Boom, W.H. (1995). CD4+ alpha beta T cell and gamma delta  
36 T cell responses to *Mycobacterium tuberculosis*. Similarities and differences in Ag recognition,

1 cytotoxic effector function, and cytokine production. *J. Immunol.* *154*, 1786–1796.

2 Vavassori, S., Kumar, A., Wan, G.S., Ramanjaneyulu, G.S., Cavallari, M., El Daker, S., Beddoe,  
3 T., Theodossis, A., Williams, N.K., Gostick, E., et al. (2013). Butyrophilin 3A1 binds  
4 phosphorylated antigens and stimulates human gammadelta T cells. *Nat Immunol* *14*, 908–916.

5 Velu, V., Mylvaganam, G.H., Gangadhara, S., Hong, J.J., Iyer, S.S., Gumber, S., Ibegbu, C.C.,  
6 Villinger, F., and Amara, R.R. (2016). Induction of Th1-Biased T Follicular Helper (Tfh) Cells  
7 in Lymphoid Tissues during Chronic Simian Immunodeficiency Virus Infection Defines  
8 Functionally Distinct Germinal Center Tfh Cells. *J. Immunol.* *197*, 1832–1842.

9 Vogt, G., and Nathan, C. (2011). In vitro differentiation of human macrophages with enhanced  
10 antimycobacterial activity. *J. Clin. Invest.* *121*, 3889–3901.

11 Van De Vosse, E., Verhard, E.M., De Paus, R.A., Platenburg, G.J., Van Deutekom, J.C.T.,  
12 Aartsma-Rus, A., and Van Dissel, J.T. (2009). Antisense-mediated exon skipping to correct IL-  
13 12Rβ1 deficiency in T cells. *Blood* *113*, 4548–4555.

14 Wang, L., Wang, S., and Li, W. (2012). RSeQC: quality control of RNA-seq experiments.  
15 *Bioinformatics* *28*, 2184–2185.

16 Wang, Y., Ma, C.S., Ling, Y., Bousfiha, A., Camcioglu, Y., Jacquot, S., Payne, K., Crestani, E.,  
17 Roncagalli, R., Belkadi, A., et al. (2016). Dual T cell- and B cell-intrinsic deficiency in humans  
18 with biallelic RLTPR mutations. *J. Exp. Med.* *213*, 2413–2435.

19 Wilkerson, M.D., and Hayes, D.N. (2010). ConsensusClusterPlus: A class discovery tool with  
20 confidence assessments and item tracking. *Bioinformatics* *26*, 1572–1573.

21 Xiao, X., and Cai, J. (2017). Mucosal-associated invariant T cells: New insights into antigen  
22 recognition and activation. *Front. Immunol.* *8*, 1.

23 Xu, G.J., Kula, T., Xu, Q., Li, M.Z., Vernon, S.D., Ndung'u, T., Ruxrungtham, K., Sanchez, J.,  
24 Brander, C., Chung, R.T., et al. (2015). Comprehensive serological profiling of human  
25 populations using a synthetic human virome. *Science* (80-. ). *348*, aaa0698–aaa0698.

26 Yoshitomi, H., Kobayashi, S., Miyagawa-Hayashino, A., Okahata, A., Doi, K., Nishitani, K.,  
27 Murata, K., Ito, H., Tsuruyama, T., Haga, H., et al. (2018). Human Sox4 facilitates the  
28 development of CXCL13-producing helper T cells in inflammatory environments. *Nat. Commun.*  
29 *9*, 3762.

30 Yu, J., Wei, M., Becknell, B., Trotta, R., Liu, S., Boyd, Z., Jaung, M.S., Blaser, B.W., Sun, J.,  
31 Benson Jr., D.M., et al. (2006). Pro- and antiinflammatory cytokine signaling: reciprocal  
32 antagonism regulates interferon-gamma production by human natural killer cells. *Immunity* *24*,  
33 575–590.

34 Zhang, J., Liu, W., Wen, B., Xie, T., Tang, P., Hu, Y., Huang, L., Jin, K., Zhang, P., Liu, Z., et al.  
35 (2019a). Circulating CXCR3+ Tfh cells positively correlate with neutralizing antibody responses  
36 in HCV-infected patients. *Sci. Rep.* *9*, 10090.

1 Zhang, P., Bigio, B., Rapaport, F., Zhang, S.-Y., Casanova, J.-L., Abel, L., Boisson, B., and Itan,  
2 Y. (2018). PopViz: a webserver for visualizing minor allele frequencies and damage prediction  
3 scores of human genetic variations. *Bioinformatics* 34, 4307–4309.

4 Zhang, X., Bogunovic, D., Payelle-Brogard, B., Francois-Newton, V., Speer, S.D., Yuan, C.,  
5 Volpi, S., Li, Z., Sanal, O., Mansouri, D., et al. (2015). Human intracellular ISG15 prevents  
6 interferon-alpha/beta over-amplification and auto-inflammation. *Nature* 517, 89–93.

7 Zhang, Y., Liu, T., Meyer, C.A., Eeckhoute, J., Johnson, D.S., Bernstein, B.E., Nussbaum, C.,  
8 Myers, R.M., Brown, M., Li, W., et al. (2008). Model-based Analysis of ChIP-Seq (MACS).  
9 *Genome Biol.* 9, R137.

10 Zhang, Y., Siegel, A.M., Sun, G., Dimaggio, T., Freeman, A.F., and Milner, J.D. (2019b).  
11 Human TH9 differentiation is dependent on signal transducer and activator of transcription  
12 (STAT) 3 to restrain STAT1-mediated inhibition. *J. Allergy Clin. Immunol.* 143, 1108-1118.e4.

13 Zheng, W., and Flavell, R.A. (1997). The transcription factor GATA-3 is necessary and  
14 sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* 89, 587–596.

15 Zhu, J., Yamane, H., and Paul, W.E. (2010). Differentiation of Effector CD4 T Cell Populations.  
16 *Annu. Rev. Immunol.* 28, 445–489.

17 Zimmer, J., Andrès, E., Donato, L., Hanau, D., Hentges, F., and de la Salle, H. (2005). Clinical  
18 and immunological aspects of HLA class I deficiency. *QJM - Mon. J. Assoc. Physicians* 98,  
19 719–727.

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