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1 **Human T-bet governs innate and innate-like adaptive**
2 **IFN- γ immunity against mycobacteria**
3

4 Rui Yang^{1,*}, Federico Mele^{2,3,7}, Lisa Worley^{3,4,37}, David Langlais^{5,6,37}, Jérémie Rosain^{7,8,37},
5 Ibithal Benhsaien^{9,10,38}, Houda Elarabi^{11,38}, Carys A. Croft^{12,13,14,38}, Jean-Marc Doisne^{12,13,38},
6 Peng Zhang^{1,38}, Marc Weisshaar^{15,38}, David Jarrossay², Daniela Latorre¹⁵, Yichao Shen¹,
7 Jing Han¹, Masato Ogishi¹, Conor Gruber^{16,17,18}, Janet Markle¹, Fatima Al Ali¹⁹, Mahbuba
8 Rahman¹⁹, Taushif Khan¹⁹, Yoann Seeleuthner^{7,8}, Gaspard Kerner^{7,8}, Lucas T. Husquin²⁰,
9 Julia L. Maclsaac²¹, Mohamed Jeljeli^{14,22}, Abderrahmane Errami⁹, Fatima Ailal^{9,10}, Michael S.
10 Kobor²¹, Carmen Oleaga-Quintas^{7,8}, Manon Roynard^{7,8}, Mathieu Bourgey^{6,23}, Jamila El
11 Baghdadi²⁴, Stéphanie Boisson-Dupuis^{1,7,8}, Anne Puel^{1,7,8}, Frédéric Batteux^{14,22}, Flore
12 Rozenberg^{14,25}, Nico Marr^{19,26}, Qiang Pan-Hammarström²⁷, Dusan Bogunovic^{16,17,18}, Lluís
13 Quintana-Murci^{20,28}, Thomas Carroll²⁹, Cindy S Ma^{3,4}, Laurent Abel^{1,7,8,39}, Aziz Bousfiha^{9,10,39},
14 James P. Di Santo^{12,13,39}, Laurie H Glimcher^{30,31,32,40}, Philippe Gros^{6,33,40}, Stuart G Tangye^{3,4,40},
15 Federica Sallusto^{2,15,40}, Jacinta Bustamante^{1,7,8,34,41} and Jean-Laurent Casanova^{1,7,8,35,36,41,42,*}
16

17 ¹St Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, Rockefeller
18 University, New York, NY 10065, USA

19 ²Center of Medical Immunology, Institute for Research in Biomedicine, Faculty of Biomedical
20 Sciences, University of Italian Switzerland (USI), CH-6500 Bellinzona, Switzerland.

21 ³Garvan Institute of Medical Research, Darlinghurst 2050, NSW Australia

22 ⁴St Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, Darlinghurst 2050,
23 Australia

24 ⁵Department of Human Genetics, Department of Microbiology and Immunology, McGill
25 University, Montreal, QC H3A 0G1, Canada

26 ⁶McGill University Genome Center, McGill Research Centre on Complex Traits, Montreal, QC
27 H3A 0G1, Canada

28 ⁷Laboratory of Human Genetics of Infectious Diseases, Necker Branch, INSERM UMR 1163,
29 Necker Hospital for Sick Children, 75015 Paris, France, EU

30 ⁸University of Paris, Imagine Institute, 75015 Paris, France, EU

31 ⁹Laboratory of Clinical Immunology, Inflammation and Allergy, Faculty of Medicine and
32 Pharmacy of Casablanca, King Hassan II University, 20460 Casablanca, Morocco

33 ¹⁰Clinical Immunology Unit, Department of Pediatric Infectious Diseases, Children's Hospital,
34 CHU Averroes, 20460 Casablanca, Morocco

35 ¹¹Pediatrics Department, Hassan II Hospital, 80030 Dakhla, Morocco

36 ¹²Innate Immunity Unit, Institut Pasteur, 75724 Paris, France, EU

37 ¹³INSERM U1223, 75015 Paris, France, EU

38 ¹⁴University of Paris, 75006 Paris, France, EU

39 ¹⁵Institute of Microbiology, ETH Zurich, CH-8093 Zurich, Switzerland

40 ¹⁶Department of Microbiology, Icahn School of Medicine at Mount Sinai, NY 10029, USA

41 ¹⁷Department of Pediatrics, Icahn School of Medicine at Mount Sinai, NY 10029, USA

42 ¹⁸The Mindich Child Health and Development Institute, Icahn School of Medicine at Mount
43 Sinai, NY 10029, USA

44 ¹⁹Research Branch, Sidra Medicine, Doha, PO 26999, Qatar

45 ²⁰Human Evolutionary Genetics Unit, CNRS UMR2000, Institut Pasteur, 75015 Paris, France,
46 EU

1 ²¹BC Children's Hospital Research Institute, Department of Medical Genetics, University of
2 British Columbia, Vancouver, BC V5Z 4H4, Canada
3 ²²Immunology Laboratory, Cochin Hospital, AH-HP, 75014 Paris, France, EU
4 ²³Canadian Centre for Computational Genomics, Montreal, QC H3A 0G1, Canada
5 ²⁴Genetics Unit, Military Hospital Mohamed V, 10100 Rabat, Morocco
6 ²⁵Virology Laboratory, Cochin Hospital, AH-HP, 75014 Paris, France, EU
7 ²⁶College of Health and Life Sciences, Hamad Bin Khalifa University, Doha, PO 34110, Qatar
8 ²⁷Department of Biosciences and Nutrition, Karolinska Institutet, 17177 Stockholm, Sweden,
9 EU
10 ²⁸Chair of Human Genomics and Evolution, Collège de France, 75005 Paris, France, EU
11 ²⁹Bioinformatics Resource Center, The Rockefeller University, New York, NY 10065, USA
12 ³⁰Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute, Boston, MA
13 02215, USA
14 ³¹Department of Medicine, Brigham and Women's Hospital, Boston, MA 02115, USA
15 ³²Department of Immunology, Harvard Medical School, Boston, MA 02115, USA
16 ³³Department of Biochemistry, McGill University, Montreal, QC H3G 1Y6, Canada
17 ³⁴Study Center for Primary Immunodeficiencies, Necker Children Hospital, AP-HP, 75015 Paris,
18 France, EU
19 ³⁵Pediatric Hematology-Immunology Unit, Necker Hospital for Sick Children, AP-HP, 75015
20 Paris, France, EU
21 ³⁶Howard Hughes Medical Institute, New York, NY, USA
22 ³⁷These authors contributed equally
23 ³⁸These authors contributed equally
24 ³⁹These authors contributed equally
25 ⁴⁰These authors contributed equally
26 ⁴¹These authors contributed equally
27 ⁴²Lead Contact
28 *Correspondence: ryang@rockefeller.edu and casanova@rockefeller.edu
29

1 **Summary**

2 Inborn errors of human IFN- γ immunity underlie mycobacterial disease. We report a
3 patient with mycobacterial disease due to inherited deficiency of the transcription factor T-bet.
4 The patient has extremely low counts of circulating *Mycobacterium*-reactive NK, invariant NKT
5 (iNKT), mucosal-associated invariant T (MAIT), and V δ 2⁺ $\gamma\delta$ T lymphocytes, and of
6 *Mycobacterium*-non reactive classic T_H1 lymphocytes, with the residual populations of these
7 cells also producing abnormally small amounts of IFN- γ . Other lymphocyte subsets develop
8 normally, but produce low levels of IFN- γ , with the exception of CD8⁺ $\alpha\beta$ T and non-classic
9 CD4⁺ $\alpha\beta$ T_H1* lymphocytes, which produce IFN- γ normally in response to mycobacterial
10 antigens. Human T-bet deficiency thus underlies mycobacterial disease by preventing the
11 development of innate (NK) and innate-like adaptive lymphocytes (iNKT, MAIT, and V δ 2⁺ $\gamma\delta$ T
12 cells) and IFN- γ production by them, with mycobacterium-specific, IFN- γ -producing, purely
13 adaptive CD8⁺ $\alpha\beta$ T and CD4⁺ $\alpha\beta$ T_H1* cells unable to compensate for this deficit.

1 **Introduction**

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

1 of human IFN- γ 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- γ involves
4 mononuclear phagocytes. The ability of these cells to contain the ingested mycobacteria depends
5 on their activation by IFN- γ (Nathan et al., 1983). The cellular basis of MSMD in patients with
6 impaired IFN- γ production is poorly understood, as most types of lymphocytes can produce IFN-
7 γ (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- γ by NK
9 cells (Bogunovic et al., 2012; Zhang et al., 2015). IL-12R β 2 deficiency preferentially impairs the
10 production of IFN- γ 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 β 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- γ by CD4⁺ CCR6⁺ T_H1 (T_H1*) cells (Hambleton et al., 2011; Kong et al.,
16 2018), a T_H1 cell subset enriched in *Mycobacterium*-specific effector cells, whereas CCR6⁻ T_H1
17 cells do not respond to mycobacteria (Acosta-Rodriguez et al., 2007). ROR γ /ROR γ T deficiency
18 impairs the development of iNKT and MAIT cells, and also compromises the production of IFN-
19 γ by $\gamma\delta$ T and $\alpha\beta$ T_H1* 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⁺ or CD8⁺ 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- γ -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- γ
10 immunity while better delineating the cellular network involved.

11

1 **Results**

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

3 We studied a three-year-old boy (“P”, II.2) born to first-cousin Moroccan parents (**Fig.**
4 **1A**). He suffered from disseminated BCG disease (BCG-osis) following vaccination at the age of
5 three months. He also had persistent reactive airway disease (RAD), but was otherwise healthy
6 (see **STAR Methods - Case Report**). He did not suffer from any other severe clinical infectious
7 diseases despite documented (VirScan) infection with various viruses, including Epstein-Barr
8 virus (EBV), human cytomegalovirus (CMV), roseola virus, adenoviruses A, B, C and D,
9 influenza virus A, rhinovirus A, and bacteria, such as *Streptococcus pneumoniae* and
10 *Staphylococcus aureus* (**Fig. S1B**). We hypothesized that P had an autosomal recessive (AR)
11 defect. We performed whole-exome sequencing (WES) on P, his unaffected brother, and both
12 parents. Genome-wide linkage (GWL) analysis revealed 32 linked regions (LOD score >1.3 and
13 size >500 kb) under a model of complete penetrance (**Supplemental Data S1**). In these linked
14 regions, there were 15 rare homozygous non-synonymous or essential splicing variants of 15
15 different genes (minor allele frequency, MAF < 0.003 in gnomAD v2.1 and 1000 Genomes
16 Project, including for each major ancestry) with a combined annotation-dependent depletion
17 (CADD) score above their mutation significance cutoffs (MSC) (Auton et al., 2015; Itan et al.,
18 2015; Karczewski et al., 2020; Kircher et al., 2014; Zhang et al., 2018) (**Table S1**). After the
19 exclusion of genes with other predicted loss-of-function (LOF) variants with a frequency greater
20 than 0.5% in gnomAD and of genes not expressed in leukocytes, only four candidate genes
21 (*TBX21*, *PSMD9*, *ARHGAP27* and *ERN1*) remained. The c.466_471delGAGATGinsAGTTTA
22 insertion and/or deletion (indel) variant of *TBX21* (T-box protein 21, or T-box, expressed in T
23 cells, T-bet) was the candidate variant predicted to be the most damaging (Kircher et al., 2014).

1 Moreover, based on connectivity to *IFNG*, the central gene of the entire network of all known
2 MSMD-causing genes (Itan et al., 2013, 2014), *TBX21* was the most plausible candidate gene
3 (**Table S1**). T-bet is a transcription factor that governs the development or function of several
4 IFN- γ -producing lymphocytes in mice, including T_H1 cells (Lazarevic et al., 2013; Szabo et al.,
5 2000, 2002). These findings suggested that homozygosity for the rare indel variant
6 c.466_471delGAGATGinsAGTTTA of *TBX21* causes MSMD. We investigated this variant
7 according to the guidelines for genetic studies of single patients (Casanova et al., 2014). Sanger
8 sequencing confirmed that P was homozygous for the indel variant of exon 1 of *TBX21*, whereas
9 his unaffected brother was homozygous wild-type (WT/WT) and both parents were heterozygous
10 (WT/M) (**Fig. S1C**). A closely juxtaposed 12-nucleotide (nt) region identical to this variant
11 sequence was detected 8-nt upstream from the variant, and may have served as a template for its
12 generation (**Fig. S1D**). The variant did not alter the exon 1-exon 2 junction of the *TBX21* mRNA
13 in EBV-transformed B (EBV-B) cells or peripheral blood mononuclear cells (PBMCs)
14 (**Supplemental Data S1**). The variant present in P thus resulted in the replacement of E156 and
15 M157, two amino acids that are highly conserved across different species and among other
16 paralogs of T-box transcription factors, with S156 and L157 (**Fig. 1B** and **Fig. S1E**).

17

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

19 We investigated the expression of the mutant allele (Mut), by overexpressing an empty
20 vector (EV), or vectors containing the WT or Mut allele, or negative controls with the T-box
21 domain deleted (T-del) or K314R, the human ortholog of a known LOF mouse mutant, in human
22 embryonic kidney (HEK) 293T cells (Jang et al., 2013). The production and nuclear
23 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- γ production in NK, ILCs, $\gamma\delta$, and CD4⁺ T cells in mice and is sufficient for IFN- γ
18 production in NK and CD4⁺ 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- γ 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- γ 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- γ levels to values above those for endogenous production in expanding naïve
4 CD4⁺ 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- γ production in an NK cell line or CD4⁺ T cells. It can, therefore, reasonably be
7 considered a LOF allele.

8

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 <5x10⁻⁶, 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

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

12

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

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

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

9

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

11 The functional impact of the T-bet mutation was also investigated in primary CD4⁺ T
12 cells. Upon stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin (P/I), IFN- γ
13 production was almost entirely abolished in P's expanded T_{H0} cells and TNF- α production was
14 impaired; the production of both these molecules was rescued by WT T-bet (**Fig. 2H** and **I**, **Fig.**
15 **S2J** and **K**). In T_{H1} conditions, exogenous IL-12 bypassed T-bet and induced moderate IFN- γ
16 production by P's cells. However, the levels of IFN- γ were still ~60-70% lower than those in
17 healthy donors (**Fig. 2H** and **I**). We investigated other T-bet-dependent transcriptional targets,
18 by performing RNA-seq to compare T_{H0} cells from 4 healthy donors, P, and P's cells
19 complemented with WT T-bet after incubation with anti-CD3/28 Ab beads. We found that 415
20 genes were downregulated and 489 genes were upregulated in P's cells relative to healthy donors
21 (**Supplemental Data S1**, **Table S2**). The complementation of P's cells with WT T-bet reversed
22 the differential expression of 99 of the downregulated and 161 of the upregulated genes
23 (**Supplemental Data S1**). Thus, only a subset of differentially regulated genes were rescued,

1 possibly due to differences in genetic variants other than *TBX21* between controls and P.
2 Alternatively, T-bet may control the differential expression of some genes early in activation,
3 and the genetic rescue may have occurred too late to reverse this effect. These targets were
4 enriched in cytokine signaling pathway genes (**Fig. 2J**). We therefore decided to focus on genes
5 involved in immunological signaling. Only 32 such genes were upregulated or downregulated in
6 P's cells, but these differences in expression relative to controls were reversed by WT T-bet (**Fig.**
7 **2K**). Known T-bet-dependent targets, such as *IFNG*, *CCL3* and *CXCR3*, were downregulated in
8 CD4⁺ T cells from the T-bet-deficient patient, whereas *IFNGR2* expression was upregulated (**Fig.**
9 **2L**) (Iwata et al., 2017; Jenner et al., 2009). A set of new T-bet target genes was also identified,
10 including *CCLI*, *CCL13*, *CCL4*, *CSF2*, *CXCR5*, *GZMM*, *CD40*, *CD86*, *IL7R*, *IL10*, *LTB*, *ITGA5*,
11 and *ITGB5* (**Fig. 2L**). Collectively, our data indicate that the patient had AR complete T-bet
12 deficiency, affecting the expression of a set of T-bet-dependent target genes, including *IFNG*.

13

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

15 We analyzed the molecular mechanisms by which T-bet controls transcription.
16 Epigenetically, T-bet is known to induce a permissive environment for transcription at the *IFNG*
17 promoter through histone modifications and the suppression of CpG methylation (Lewis et al.,
18 2007; Miller and Weinmann, 2010; Tong et al., 2005). However, it remains unknown whether T-
19 bet directly regulates chromatin accessibility in mice or humans. The regulation of CpG
20 methylation at the genome-wide scale by T-bet has never been studied. We performed omni-
21 ATAC-seq analysis and EPIC DNA CpG methylation array analysis with T_H0 cells derived from
22 P and controls. In P's cells, a gain of chromatin accessibility was observed at 1,661 loci and a
23 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*, *TBKBP1*, *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

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

6

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

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

1 and IL-12R β 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.

5

6 **Low levels of the iNKT, MAIT, and V δ 2⁺ $\gamma\delta$ 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 α 7.2-J α 33 TCR α
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 δ 2⁺ 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 δ 1⁺ 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⁺ and CD8⁺ $\alpha\beta$ 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⁺ T-cell effector responses and the optimal

1 induction of memory CD8⁺ 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⁺ T cells and the
3 composition of naïve, central memory, effector memory and T_{EMRA} cells were normal (**Fig. S4A**).
4 We further investigated CD8⁺ 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⁺ T cells of P and controls. However, a small
7 subset of naïve CD8⁺ T cells (CD45RA⁺CD38^{int}CXCR3^{int}CCR6⁻CCR5⁻CD27^{high}CD127^{high}) was
8 absent from P (**Fig. S4B**). Thus, the development of iNKT, MAIT cells, Vδ2⁺ γδ T cells, and a
9 small subset of naïve CD8⁺ T cells is impaired in T-bet deficiency.

10

11 **Selective depletion of CCR6⁻ T_{H1} cells from CD4⁺ T cells in T-bet deficiency**

12 Both P and his heterozygous parents had normal distributions of naïve and memory CD4⁺
13 T cells (**Fig. S4C**). We further analyzed individual CD4⁺ T cell subsets by viSNE clustering on
14 antigen-experienced memory cells. Several memory CD4⁺ T cell subsets typically present in
15 healthy donors were missing in P. In particular, most of the CXCR3⁺ cells, corresponding to T_{H1}
16 cells in humans, and CCR5⁺ 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⁺CCR6⁻
18 T_{H1} cells was lower than that in all healthy adult and age-matched donors examined, whereas the
19 frequency of CXCR3⁺CCR6⁺ non-classic T_{H1}* 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_{H2}, T_{H17}, and follicular helper (T_{FH}) cells in peripheral blood were
22 normal (**Fig. S4F - H**). However, levels of CXCR3⁺ T_{FH} cells, a group of T_{H1}-biased T_{FH} cells
23 that produce IFN-γ together with IL-21 in germinal centers (Ma et al., 2015; Velu et al., 2016;

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

7

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

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

1 ANXA1 are known to be involved in T_H1 immunity (Dorner et al., 2002, 2003, 2004; Gavins and
2 Hickey, 2012; Kroczek and Henn, 2012; Nishikomori et al., 2002; Thieu et al., 2008; Toribio-
3 Fernández et al., 2018; Yoshitomi et al., 2018). NKG7 is involved in the initiation of human T_H1
4 commitment and its genetic locus is tightly occupied by T-bet (Jenner et al., 2009; Kanhere et al.,
5 2012; Lund et al., 2005). *NKG7* expression in naive CD4⁺, CD8⁺ and B cells was dependent on
6 functional T-bet, whereas *PRDM1* was downregulated in CD4⁺ T and CD8⁺ CTLs cells from P
7 (**Fig. 4I**). The *IFNG* gene was weakly expressed across lymphocyte populations, as shown by
8 scRNAseq, and its expression did not seem to be dependent on T-bet in basal conditions (data
9 not shown). In addition, the expression of several genes not previously linked to T-bet was also
10 altered in at least one cell subset in P (**Fig. 4I**). Thus, in addition to *CXCR3*, *NKG7*, *CCL3* and
11 *CCLA*, which were weakly expressed in at least one leukocyte subset from this patient with
12 human T-bet deficiency, consistent with the findings of RNA-seq and omni-ATAC-seq, the
13 expression of a set of previously unknown target genes in immune subsets is also controlled by
14 T-bet (**Fig. 4I**).

15

16 **Impaired IFN- γ production by NK, MAIT, V δ 2⁺ $\gamma\delta$ T, and CD4⁺ T cells**

17 Human IFN- γ is essential for antimycobacterial immunity, as all 31 known genetic
18 etiologies of MSMD affect IFN- γ -dependent immunity. The *in vivo* development of NK, iNKT,
19 MAIT, V δ 2⁺ $\gamma\delta$ T, and classic T_H1 cells was found to be impaired in P, but it remained possible
20 that the IFN- γ production capacity of the remaining lymphocytes could compensate, thereby
21 contributing to antimycobacterial immunity. We assessed the response of P's PBMCs to *ex vivo*
22 stimulation with P/I. They secreted significantly less IFN- γ secretion than control cells (**Fig. 5A**).
23 The frequencies of IFN- γ - or TNF- α -producing total lymphocytes were also low in P (**Fig. 5B** -

1 **D**). We assessed the IFN- γ and TNF- α production of lymphocyte subsets by spectral flow
2 cytometry (**Fig. S5A**). Upon P/I stimulation, the frequencies of IFN- γ - or TNF- α -producing
3 CD56⁺ NK cells were low in P (**Fig. 5E, Fig. S5B and C**). We then assessed of the response of
4 P's NK cells to *ex vivo* stimulation with IL-12, IL-15, and IL-18. Total NK cells from P
5 displayed impaired degranulation, with low levels of CD107a expression, and almost no IFN- γ
6 production (**Fig. 5F, Fig. S5D and E**). T-bet deficiency not only reduced the overall frequencies
7 of iNKT, MAIT, and V δ 2⁺ $\gamma\delta$ T cells, but also compromised the production of IFN- γ and TNF- α
8 *ex vivo* by the residual activated iNKT, MAIT, and V δ 2⁺ $\gamma\delta$ T cells detected in P (**Fig. 5G - K,**
9 **Fig. S5F**). Despite an increase in the frequency of V δ 1⁺ $\gamma\delta$ T cells *in vivo*, the levels of IFN- γ - or
10 TNF- α -producing-V δ 1⁺ $\gamma\delta$ T cells were also low *ex vivo* (**Fig. 5G, Fig. 5L and M**). By contrast,
11 we observed no difference in IFN- γ and TNF- α production by V δ 1⁻V δ 2⁻ $\gamma\delta$ T cells between P
12 and controls (**Fig. 5G, Fig. S5G and H**). IFN- γ -producing cells from the various lymphocyte
13 subsets examined in healthy donors expressed high levels of T-bet, but all the residual IFN- γ -
14 producing cells from the various subsets in P tested negative for T-bet (**Fig. 5G**). We also
15 analyzed the response of $\alpha\beta$ T cells to P/I. CD8⁺ T cells secrete substantial amounts of IFN- γ
16 upon microbial challenge (Schoenborn and Wilson, 2007). The frequencies of CD8⁺ T cells
17 producing IFN- γ or TNF- α in response to P/I did not differ between P and age-matched healthy
18 donors (**Fig. S5I and J**). By contrast, the frequencies of IFN- γ - or TNF- α -producing CD4⁺ T
19 cells were slightly low in P (**Fig. 5N and O**). Thus, among the residual circulating lymphocytes
20 of P, NK, iNKT, MAIT, V δ 2⁺, V δ 1⁺ $\gamma\delta$ T, and CD4⁺ T cells had impaired IFN- γ production *ex*
21 *vivo* in response to stimulation with IL-12, IL-15, IL-18 or P/I, whereas V δ 1⁻V δ 2⁻ $\gamma\delta$ and CD8⁺
22 $\alpha\beta$ T cells did not.

23

1 **Selective impairment of IFN- γ production by T_H cells in T-bet deficiency**

2 T-bet was first discovered and has been most extensively studied in CD4⁺ T cells in the
3 context of mouse T_{H1} cells (Szabo et al., 2000, 2002). This discovery, together with that of
4 GATA3 (Zheng and Flavell, 1997), revealed the molecular determinism of T_{H1}/T_{H2} CD4⁺ T-cell
5 differentiation and paved the way for an understanding of T_{H17}, iTreg, T_{H22}, T_{FH}, and T_{H9} cell
6 lineage determination (Finotto et al., 2002; Zhu et al., 2010). We therefore investigated the
7 impact of T-bet deficiency on primary CD4⁺ T cells. IFN- γ production by memory CD4⁺ T cells
8 was impaired by T-bet deficiency (**Fig. 5P** and **Q**). Another T_{H1} cytokine, TNF- α , was also
9 produced in smaller amounts by memory CD4⁺ T cells from P than by those of most of the
10 healthy donors (**Fig. 5R** and **S**). By contrast, the memory CD4⁺ T cells of P produced larger
11 amounts of the T_{H17} effector cytokines IL-17A and IL-22 than did those of healthy donors (**Fig.**
12 **5T** and **U**, **Fig. S5K**). Unlike previous studies (Gökmen et al., 2013; Zhang et al., 2019b), we
13 found that T-bet deficiency had no effect on IL-9 production *ex vivo* (**Fig. S5L** and **M**).
14 Surprisingly, *ex vivo* T_{H2} cytokines from memory CD4⁺ T cells were also not affected by human
15 T-bet deficiency (**Fig. S5N - Q**). P's memory CD4⁺ cells produced less IL-21 *ex vivo* than the
16 memory CD4⁺ cells of most of the healthy donors (**Fig. S5R**). We then investigated the role of
17 T-bet in human T_H cell differentiation *in vitro*. Naïve CD4⁺ T cells from P or healthy donors
18 were allowed to differentiate in T_{H0}, T_{H1}, T_{H2}, T_{H9}, or T_{H17} conditions. The induction of IFN- γ
19 production in naïve CD4⁺ T cells was abolished by T-bet deficiency under T_{H1} conditions (**Fig.**
20 **5V** and **W**). Similarly, T-bet-deficient naïve CD4⁺ T cells produced less TNF- α than the
21 corresponding cells from most controls (**Fig. 5X** and **Y**). Furthermore, the induction of IL-9
22 under various conditions *in vitro* was weaker in naïve CD4⁺ T cells from P than in the
23 corresponding cells from most controls (**Fig. S5S** and **T**). *In vitro*-induced T_{H2} cells from P

1 produced more IL-10, but not IL-13, than control cells (**Fig. S5U and V**). Even memory CD4⁺ T
2 cells from P displayed impaired IFN- γ and TNF- α production under T_H1 polarizing conditions
3 (**Fig. 5Z**). Thus, AR T-bet deficiency leads not only to defective IFN- γ and TNF- α 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_H17 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- γ in a T-bet-dependent manner during
10 acute BCG infection *in vitro*. The infection of PBMCs with BCG induced IFN- γ production,
11 which was further increased by stimulation with exogenous IL-12 (**Fig. 6A**). PBMCs from P had
12 low levels of IFN- γ and TNF- α 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- γ -producing cells in controls had high levels of T-bet expression (**Fig. S6C**). Thus, T-bet⁺
15 IFN- γ ⁺ double-positive (DP) cells were the major *Mycobacterium*-responsive cells, with a
16 function potentially dependent on T-bet. However, T-bet⁺ IFN- γ DP cell levels were low during
17 acute infection in P (**Fig. 6B, Fig. S6C**). T-bet⁺ IFN- γ 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⁺ IFN- γ ⁺
20 cells of healthy donors, NK, MAIT, V δ 2⁺ $\gamma\delta$ T, CD4⁺ and CD8⁺ $\alpha\beta$ T cells were the dominant
21 responders during BCG infection, whereas iNKT, V δ 1⁺ $\gamma\delta$ T, V δ 1⁻V δ 2⁻ $\gamma\delta$ T and B cells were
22 minority responders (**Fig. 6C and D**). However, these subsets of T-bet⁺ IFN- γ ⁺ cells were almost
23 entirely depleted from P's PBMCs following BCG infection (**Fig. 6D**). We then investigated

1 each leukocyte subset separately (**Supplemental Data S1**). Fewer than 2% of V δ 1⁺ $\gamma\delta$ T cells,
2 V δ 1-V δ 2⁻ $\gamma\delta$ T cells, B cells, CD4⁺ or CD8⁺ $\alpha\beta$ T cells became T-bet⁺ IFN- γ ⁺ during BCG
3 infection. However, among innate or innate-like lymphocytes, up to 10% of NK cells, 40% of
4 iNKT, and 50% of V δ 2⁺ $\gamma\delta$ T cells from healthy donors, but not from P, became T-bet⁺ IFN- γ ⁺ in
5 response to BCG infection, and the frequency of these cells was further increased by exogenous
6 IL-12 (**Fig. 6E - G, Fig. S6E and F**). Up to 50% of MAIT cells became T-bet⁺ IFN- γ ⁺ during
7 BCG infection, but no difference was observed between age-matched healthy donors and P (**Fig.**
8 **6E and Fig. S6E**). Upon acute BCG infection *in vitro*, despite a less robust activation of CD4⁺ T
9 cells than of innate or innate-like lymphocytes, the frequency of T-bet⁺ IFN- γ ⁺ cells was low
10 among CD4⁺ but not among CD8⁺ $\alpha\beta$ T cells of P (**Fig. 6H, Fig. S6G and H**). Thus, the IFN- γ
11 production controlled by T-bet during acute BCG infection *in vitro* takes place mostly in NK,
12 iNKT, V δ 2⁺ $\gamma\delta$ T and CD4⁺ $\alpha\beta$ T cells, but not in V δ 1⁺ $\gamma\delta$ T cells, V δ 1-V δ 2⁻ $\gamma\delta$ T cells, or CD8⁺
13 $\alpha\beta$ T cells. The development of MAIT cells is also dependent on T-bet, whereas the IFN- γ
14 production of these cells during BCG infection is not. These experimental findings *in vitro* do
15 not exclude a contribution of other subsets *in vivo*. NK, iNKT, MAIT, and V δ 2⁺ $\gamma\delta$ T cells
16 responded robustly to acute BCG infection *in vitro*, but these subsets were absent or functionally
17 deficient in the patient with inherited T-bet deficiency.

18

19 **Defective prolonged anti-BCG immunity mediated by V δ 2⁺ $\gamma\delta$ T cells**

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

1 immunity. We therefore studied V δ 2⁺ $\gamma\delta$ T cells and CD4⁺ $\alpha\beta$ T cells, two adaptive immune
2 lymphocyte subsets that produced substantial amounts of IFN- γ during BCG infection *in vitro*
3 and are known to function in an antigen-specific manner. In PBMCs from healthy donors, V δ 2⁺
4 $\gamma\delta$ T cells responded most robustly during BCG infection (**Fig. 6E**). V δ 2⁺ $\gamma\delta$ T cells, a major
5 subset of $\gamma\delta$ T cells recognizing pAg from microbial sources (Gu et al., 2018; Harly et al., 2012;
6 Vavassori et al., 2013), are also known to play an essential role in the recall response to
7 mycobacterial re-infection in humans and non-human primates (Chen, 2005; Shen et al., 2002).
8 V δ 2⁺ $\gamma\delta$ T cells proliferate vigorously in response to mycobacterial infection *in vivo* and can
9 expand robustly in response to pAg-rich lysates of mycobacterial species *in vitro* (Hoft et al.,
10 1998; Modlin et al., 1989; Panchamoorthy et al., 1991; Parker et al., 1990; Tsukaguchi et al.,
11 1995). We investigated whether the function of V δ 2⁺ $\gamma\delta$ T cells was affected in the patient with
12 T-bet deficiency, as these cells represented a small, but important proportion (~ 0.2%) of P's
13 peripheral lymphocytes. The populations of V δ 2⁺ T cells from all controls and relatives of P
14 expanded vigorously following prolonged stimulation with BCG lysates. By contrast, no such
15 expansion was observed for T-bet-deficient V δ 2⁺ T cells (**Fig. 6I** and **J**, **Fig. S6I**). After two
16 weeks of expansion, the levels of IFN- γ production by T-bet-deficient cells were lower than
17 those for healthy donors' cells (**Fig. 6K**).

18

19 **Redundant role of T-bet in IFN- γ production by BCG-specific cognate T_H1* cells**

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

1 addressed both these unresolved issues by analyzing *Mycobacterium*-responsive CD4⁺ 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⁺ T cells proliferated equally well in response to both BCG-lysates and PPD (**Supplemental**
5 **Data S1**). No difference in IFN- γ production from PPD-responsive CD4⁺ T cells was observed
6 between healthy donors and P (**Fig. 6L** and **Fig. S6J**). Unlike PPD, which activated only CD4⁺ T
7 cells, BCG-lysates activated both CD4⁺ T cells and CD8⁺ T cells, albeit to a lesser extent
8 (**Supplemental Data S1**). Like PPD stimulation, IFN- γ production from both BCG-lysate-
9 responsive CD4⁺ and CD8⁺ 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⁺CCR6⁻ (containing classic T_{H1} cells) and CD4⁺CCR6⁺ (containing
12 T_{H1}* *Mycobacterium*-responsive cells) memory subsets (Geiger et al., 2009). Consistent with
13 our *in vivo* findings, the T cells in the CD4⁺CCR6⁻ and CD4⁺CCR6⁺ libraries had low levels of
14 CXCR3 or IFN- γ (**Supplemental Data S1**). P's CD4⁺CCR6⁺ T-cell library responded robustly to
15 BCG, tetanus toxoid and *C. albicans*, and his CD4⁺CCR6⁻ T-cell library responded normally to
16 influenza virus, CMV, and EBV. Despite intact proliferation, IFN- γ 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⁺ T cells, consisting almost entirely of *Mycobacterium*-specific memory
19 T_{H1}* cells (Acosta-Rodriguez et al., 2007; Becattini et al., 2015), proliferated robustly in
20 response to BCG peptides. Moreover, their IFN- γ 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- γ 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- γ ⁺ BCG-specific T-cell clones still carried the *TBX21* indel variant. Thus, the
2 prolonged immunity to BCG infection mediated by V δ 2⁺ $\gamma\delta$ 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 δ 2⁺ $\gamma\delta$ T cells, but redundant for IFN- γ production by BCG-specific cognate CD4⁺ T_H1*
5 cells or *Mycobacterium*-responsive CD8⁺ $\alpha\beta$ T cells (**Table S4**).

1 **Discussion**

2 We report here the identification and study of the first patient discovered to have
3 inherited, complete T-bet deficiency resulting clinically in MSMD. Key observations made in T-
4 bet-deficient mice were validated in this human patient with T-bet deficiency: 1) the
5 development of T_H1 cells and their production of effector cytokines, including IFN- γ in
6 particular, requires T-bet (Szabo et al., 2000, 2002); 2) the development of NK and iNKT cells is
7 dependent on T-bet (Townsend et al., 2004); and 3) the regulation of T-bet-dependent targets,
8 including *CXCR3*, *TNF* and *IFNG*, involves both direct transactivation and epigenetic
9 modulation (Miller and Weinmann, 2010). Accordingly, T-bet-deficient mice are highly
10 vulnerable to mycobacteria, including *M. tuberculosis* and *M. avium* (Matsuyama et al., 2014;
11 Sullivan et al., 2005), like mice deficient for other genes that govern IFN- γ immunity (Casanova,
12 1999). By contrast, despite the requirement of T-bet for immunity to a broad spectrum of
13 pathogens following experimental inoculation in mice (Bakshi et al., 2006; Harms Pritchard et al.,
14 2015; Hultgren et al., 2004; Kao et al., 2011; Lebrun et al., 2015; Oakley et al., 2013; Ravindran
15 et al., 2005; Rosas et al., 2006; Svensson et al., 2005; Szabo et al., 2002), the only apparent
16 infectious phenotype of the T-bet-deficient patient is MSMD. Our study provides compelling
17 evidence that inherited T-bet deficiency is a genetic etiology of MSMD due to the disruption of
18 IFN- γ immunity (Casanova et al., 2014). This experiment of nature suggests that T-bet is
19 required for protective immunity to intramacrophagic mycobacteria but largely redundant for
20 immunity to most intracellular pathogens, including viruses in particular. This is at odds with
21 findings in mice, but consistent with other genetic etiologies of MSMD, all of which are inborn
22 errors of IFN- γ immunity (Bustamante, 2020; Kerner et al., 2020). Our observation further
23 suggests that the functions of T-bet unrelated to IFN- γ 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- γ 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 δ 2⁺ $\gamma\delta$ T cells; 2) T-bet was also apparently required for the optimal production of
9 IFN- γ by the few NK, iNKT, MAIT, and V δ 2⁺ $\gamma\delta$ 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- γ production by cognate purely adaptive *Mycobacterium*-specific
13 T_H1* CD4⁺ T cells, and by PPD-, or BCG-lysate-responsive CD4⁺ or CD8⁺ $\alpha\beta$ T cells, was
14 unaffected by T-bet deficiency. The compensatory mechanisms underlying intact IFN- γ
15 production by these antigen-specific CD4⁺ T cells remain to be discovered. Taken together,
16 impaired IFN- γ production by NK and iNKT cells, as in mice, and by MAIT and V δ 2⁺ $\gamma\delta$ T cells,
17 as shown here, accounts for MSMD in this patient with T-bet deficiency, despite normal T_H1*
18 development and function. By contrast, inborn errors of immunity that disrupt IFN- γ production
19 by the selective depletion of NK, iNKT, CD4⁺, or CD8⁺ $\alpha\beta$ 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 γ T deficiency, who
23 lack iNKT and MAIT cells and whose $\gamma\delta$ T and T_H1* cells do not produce IFN- γ , and whose NK

1 cells are unable to compensate (Okada et al., 2015). T-bet and ROR γ 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_{H1}* cells is observed only in ROR γ T deficiency. We
4 found that human T-bet was essential for both innate (NK cells) and innate-like (iNKT, MAIT,
5 and V δ 2⁺ $\gamma\delta$ T cells) adaptive immunity to mycobacteria, but, surprisingly, redundant for
6 classical, purely adaptive immunity (CD4⁺ T_{H1}* and CD8⁺ $\alpha\beta$ T cells) to mycobacteria.

7

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25

1 **Author contributions**

2 R.Y., J.B. and J.-L.C. conceptualized the study, designed the experiments, interpreted the data,
3 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.,
4 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.,
5 F.A.A., Mah.R., T.K., N.M., C.O-Q., Man.R., and J.B. conducted experiments and analyzed
6 data. R.Y., P.Z., L.T.H., L.Q.M., Yoa.S., G.K., L.A and T.C. analyzed high-throughput
7 sequencing data. M.J., F.B., and F.R. performed clinical serological tests. H.E., I.B., A.E., F.A.,
8 A.B. and J.B. took care of the patient. J.E.B. contributed North African exomes. S.B-.D., A.P.,
9 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
10 input on the final manuscript.

11

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.

1 **MAIN Figure Titles and Legends**

2 **Figure 1. Discovery of an MSMD patient with a homozygous *TBX21* variant and the**
3 **corresponding molecular characterization. (A)** Pedigree of a consanguineous family with the
4 mutant *TBX21* allele. **(B)** Schematic representation of the mutation. **(C)** Nuclear and cytoplasmic
5 fractions from HEK 293T cells transfected with indicated *TBX21* cDNA-containing vectors
6 subjected to immunoblotting against indicated proteins. **(D)** EMSA on nuclear extracts of
7 HEK293T cells transfected with empty vector (EV), WT or Mut *TBX21* alleles. **(E)** TBRE-
8 reporter luciferase assay testing WT or Mut T-bet in HEK 293T cells. **(F)** Human *IFNG* reporter
9 luciferase assay testing HEK 293T cells transfected with WT or Mut *TBX21* cDNA-containing
10 vectors, at the indicated concentrations. **(G)** IFN- γ production in response to stimulation with IL-
11 12 and IL-18 in T-bet knock-out (KO) NK-92 cells. **(H)** IFN- γ production in response to
12 stimulation with IL-12 and IL-18 in T-bet KO NK-92 cells complemented with EV, WT, K314R
13 or Mut *TBX21*-containing plasmids. **(I)** Intracellular IFN- γ production in response to P/I in
14 expanding T_H0 cells transduced with EV, WT or Mut *TBX21* cDNA. **(J)** IFN- γ production from
15 cells transduced as in (I) in response to stimulation with anti-CD3/CD28 Ab. **(K)** Graph of
16 CADD score against minor allele frequency (MAF), for *TBX21* variants reported in the gnomAD.
17 Mutation significance cutoff (MSC) was shown. **(L)** TBRE-reporter luciferase assay testing
18 indicated variants of *TBX21* as in (E). **See also Figure S1, Table S1, and Supplemental Data**
19 **S1.**

20 In Fig. 1E – J and L, bars represent the mean and standard deviation. Dots represent individual
21 samples or technical replicates. Two-way ANOVA was used for analysis in (E – I). One-way
22 ANOVA was used for analysis in (J and L). In (E – J and L), * $p < 0.05$, ** $p < 0.01$, *** $p <$
23 0.001 , **** $p < 0.0001$, ns = not significant.

1
2 **Figure 2. The T-bet variant in patient-derived cells is loss-of-function.** (A) Whole-cell lysates
3 from expanding CD4⁺ T cells from healthy donors (CTL), a heterozygous parent (WT/M) and
4 the patient (M/M) subjected to immunoblotting. (B) Immunoblotting against indicated proteins
5 of whole-cell lysates of HVS-T cells derived from CTL, M/M and WT/M. (C and D)
6 Spontaneous production of IFN- γ (C) and TNF- α (D) from cultures of HVS-T cells from CTL,
7 M/M and WT/M. (E and F) The *IFNG* (E) and *TNF* (F) expression of HVS-T cells, as in (C),
8 was assessed by RT-qPCR. (G) HVS-T cells from CTL, M/M and WT/M were rescued with EV
9 or WT *TBX21*. The percentage of intracellular IFN- γ -producing cells with or without P/I
10 stimulation is shown. (H) CD4⁺ T cells from CTL, M/M and WT/M were expanded under T_H0 or
11 T_H1 conditions. M/M cells were transduced as described in (G). Cells were subjected to ICS for
12 IFN- γ and TNF- α in response to P/I. (I) Percentage of IFN- γ - and TNF- α -producing cells in (H).
13 (J) Transduced T_H0 cells, as in (H), were isolated and restimulated, and were subjected to RNA-
14 seq, along with non-transduced cells. Pathway enrichment among differentially regulated genes
15 is shown. (K) The numbers of T-bet-dependent differentially expressed immune genes in RNA-
16 seq are shown. (L) Heat-map of selected T-bet-dependent downstream genes, as in (K). **See also**
17 **Figure S2, Table S2, and Supplemental Data S1.**

18 In Fig. 2C – G and I, bars represent the mean and the standard error of the mean. Dots represent
19 individual samples. One-way ANOVA was used for analysis in (C and D). Mann-Whitney tests
20 were used for analysis in (E and F), comparing WT/M or M/M to CTL. In (C - F), * $p < 0.05$, **
21 $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, and ns = not significant.

22

1 **Figure 3. An altered spectrum of epigenetic regulation in CD4⁺ T cells in T-bet deficiency.**
2 (A) Expanded CD4⁺ T_{H0} cells from CTL, IL-12Rβ1-deficient MSMD patients (*IL12RB1* M/M),
3 WT/M, M/M, and M/M cells complemented with empty vector (M/M +EV) or with WT T-bet
4 (M/M +WT) were subjected to omni-ATAC-seq. PCA analysis of the peaks called in the various
5 samples, plotted in ggplot2. (B) Number of called peaks differentially regulated as indicated. (C)
6 Heatmap of all loci opening or closing in a T-bet-dependent manner as in (B). (D and E) The
7 most significantly enriched DNA binding motifs at loci displaying T-bet-dependent increases (D)
8 and loci displaying T-bet-dependent decreases, as in (B and C). (F) Chromatin accessibility
9 heatmap for all the loci displaying the most significant differential regulation of immune genes
10 (CTL – M/M, adjusted *P*-value < 1 x 10⁻⁵), abolished by WT T-bet (M/M+EV - M/M+WT,
11 adjusted *P*-value < 1 x 10⁻⁵). (G - I) Chromatin accessibility of transcription start site (TSS),
12 proximal promoter and distal enhancer regions of *IFNG*, *TNF* and *CXCR3*. (J) The differential
13 methylation (beta-value) of CpG sites between M/M and CTL or between M/M+EV and
14 M/M+WT is shown. (K - M) CpG methylation status of CpG sites in *IFNG* (K), *ENTPD1* (L)
15 and *IL10* (M). See also Table S3 and Supplemental Data S1.

16
17 **Figure 4. Impaired *in vivo* development of NK, invariant NKT, MAIT, Vδ2⁺ γδ T, and T_{H1}**
18 **cells in T-bet deficiency.** (A) UMAP representation shows immunophenotyping of 40,000
19 CD45⁺CD66b⁻DNA⁺ live PBMCs from age-matched controls (Age CTL) and M/M, by CyTOF.
20 (B) Frequencies of CD56^{bright} NK cells and CD56^{dim}CD16⁺ NK cells by CyTOF. (C - E)
21 Percentages of iNKT (C), MAIT (D), and Vδ2⁺ γδ T cells (E) among live single cells from
22 indicated individuals, including P's healthy brother (WT/WT), by flow cytometry. (F) viSNE
23 map of 10,000 CD45RA⁻ memory CD4⁺ T cells from indicated individuals, with expression of

1 CXCR3 and CCR5 shown. **(G and H)** Frequencies of CCR6⁻ T_H1 (CCR6⁻CXCR3⁺CCR4⁻) (G)
2 and CCR6⁺ T_H1* (CCR6⁺CXCR3⁺CCR4⁻) cells (H) among live CD45⁺CD66b⁻ cells are shown.
3 **(I)** A bubble graph is presented to show genes for which the proportion of cells displaying
4 expression is altered in the M/M cells in comparison with PBMCs from the *TBX21* WT/M father,
5 as a control, by scRNA-seq. The size of the bubble indicates the proportion of cells expressing
6 the gene and the color scale indicates the log₂-transformed ratios. The genes highlighted in red
7 were identified as T-bet-regulated by ATAC-seq and/or RNA-seq. LD (low level of detection).
8 ND (not detected). **See also Figure S3 – S4 and Supplemental Data S1.**
9 In Fig. 4B – E, G and H, bars represent the mean and the standard error of the mean. Dots
10 represent individual samples or technical replicates. Mann-Whitney nonparametric tests were
11 used for analysis in (C – E). In (C - E), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$,
12 ns = not significant.

13
14 **Figure 5. Impaired IFN- γ *ex vivo* production from the remaining lymphocytes of the T-bet-**
15 **deficient patient. (A)** IFN- γ production in culture supernatants of PBMCs from indicated
16 individuals in response to stimulation with P/I is shown. **(B)** Dot-plots showing the expression of
17 T-bet and IFN- γ in total live lymphocytes as in (A). **(C and D)** Frequency of IFN- γ (C) or TNF-
18 α -producing (D) cells in response to P/I as in (B). **(E)** Frequency of IFN- γ -producing cells
19 among NK cells in response to P/I. **(F)** PBMCs were stimulated with IL-12, IL-15 and IL-18.
20 The expression of CD107a and IFN- γ among NK cells were assessed by flow cytometry. **(G)**
21 Dot-plots showing the expression of T-bet and IFN- γ by indicated subsets in response to P/I. **(H -**
22 **O)** Frequency of IFN- γ - (H, J, L, N) or TNF- α (I, K, M, O)-producing cells among MAIT cells,
23 V δ 2⁺ $\gamma\delta$ T cells, V1⁺ $\gamma\delta$ T cells and CD4⁺ T cells in response to P/I. **(P – U)** FACS sorted naïve

1 and memory CD4⁺ T cells activated/expanded. The production of IFN- γ was assessed
2 intracellularly (P) and in culture supernatants (Q). The production of TNF- α was assessed
3 intracellularly (R) and in culture supernatants (S). The production of IL-22 (T) and IL-17A (U)
4 was assessed in culture supernatants. (V - Y) Naïve CD4⁺ T cells were subjected to activation
5 under T_{H0} or T_{H1} conditions. The production of IFN- γ (V and W) and TNF- α (X and Y) was
6 assessed. (Z) Memory CD4⁺ T cells were activated under T_{H0} or T_{H1} conditions. The production
7 of IFN- γ and TNF- α was assessed. **See also Figure S5.**

8 In Fig. 5A, C – F, H - Z, bars represent the mean and the standard error of the mean. Dots
9 represent individual samples or technical replicates. Mann-Whitney nonparametric tests were
10 used for analysis in (A, C – E, H - O). In (A, C – E, H - O), * $p < 0.05$, ** $p < 0.01$, *** $p <$
11 0.001 , **** $p < 0.0001$, ns = not significant.

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

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

8 In Fig. 6A, B, E, H, K, L, M and N, bars represent the mean and the standard error of the mean.
9 Dots represent individual samples and technical replicates. In (O), bars represent the mean and
10 the standard deviation. Dots represent individual T-cell clones. In (C), the numbers indicate the
11 mean and the standard error of the mean. Mann-Whitney nonparametric tests were used for
12 analysis in (A, B, E and H). In (A, B, E and H), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p <$
13 0.0001 , and ns = not significant.

14

1 **Supplemental figure titles and legends**

2

3 **Figure S1. Clinical and genetic features of a patient with MSMD carrying a homozygous**
4 ***TBX21* variant leading to the substitution of two amino acids, and the characterization of**
5 **this variant, Related to Figure 1. (A)** Schematic view of the 16 MSMD-causing genes within
6 myeloid and lymphoid cells in the context of host antimycobacterial immunity. **(B)** We tested for
7 serum IgG against various viral peptides or peptides derived from bacteria or other pathogens by
8 phage immunoprecipitation-sequencing. Serum samples from an unrelated healthy three-year-old
9 boy (CTL1), two healthy adults (CTL2 and CTL3), and a *TBX21* M/M P, and IgG-depleted
10 serum and intravenous immunoglobulin (IVIg) were assayed. The heatmap (left) shows the
11 counts of peptides significantly enriched for a given species with less than a continuous seven-
12 residue subsequence, the estimated size of a linear epitope, in common. The species for which
13 the patient had a score greater than our set threshold (>3) are shown. Count values above the set
14 threshold are shown on a color scale from green (≥ 4) to red (≥ 40) and dark-red (≥ 80). **(C)**
15 Genomic DNA from P (M/M) and his parents (WT/M) was subjected to Sanger sequencing to
16 confirm the *TBX21* variant. **(D)** Comparison between the WT and Mut sequences of the region in
17 which the variant is located within exon 1 of *TBX21*. An alignment of the WT and Mut
18 sequences revealed a juxtaposed region with a sequence identical to that of the variant sequence.
19 **(E)** Alignment of peptide fragments from human T-box domain-containing transcription factors.
20 **(F)** pCMV6 plasmids containing EV, WT T-bet (WT), the K314R variant of T-bet (K314R), T-
21 bet with a deletion of the T-box domain (T-del), and mutant T-bet (Mut) were used to transfect
22 HEK293T cells. T-bet expression was characterized by immunoblotting with anti-T-bet 4B10
23 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- γ 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- Δ 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- γ production was determined by ICS flow cytometry.
14 **(K)** Naïve CD4⁺ T cells were expanded under T_H0 conditions, and were then transduced with
15 retroviruses generated from pLZRS-ires- Δ 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- γ and TNF- α 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

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

3 In (H, I and M), bars represent the mean and the standard deviation. Dots represent individual
4 samples or technical replicates. One-way ANOVA was used for analysis in (H). Two-way
5 ANOVA was used for analysis in (I). A non-parametric analysis for comparison with pGL4.10-
6 p*IFNG* + pCMV6-WT *TBX21* transfection condition was performed in (M). In (H, I and M), * p
7 < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

8

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

10 **Figure 2.** (A) CD4⁺ T cells from healthy donors (CTL), the heterozygous father (WT/M) or P
11 (M/M) were expanded with anti-CD3/CD28 antibody-coated beads. They were subjected to RT-
12 qPCR for *TBX21* with two different probes. Data are displayed as $2^{-\Delta\Delta C_t}$ after normalization
13 relative to *GUS* (endogenous control) expression, using the control mean as a calibrator. (B)
14 *TBX21* expression was assessed in immortalized T cells (HVS-T) from healthy donors (CTL),
15 the heterozygous father (WT/M) and the patient (M/M), by RT-qPCR, as in (A). (C) P-derived
16 HVS-T cells (M/M) were retrovirally transduced with EV or WT *TBX21* containing a bicistronic
17 CD271 surface marker. Intracellular T-bet and IFN- γ production by these cells and by CTL and
18 heterozygous *TBX21* WT/M HVS-T cells was assessed by flow cytometry in the presence and
19 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*
21 plasmids along with VSVG and Pax2 plasmids, as assessed by immunoblotting with an anti-V5
22 tag Ab. (E) Expression of ERN1 in HEK293T cells transfected with pLenti-EV or WT-*ERN1*-
23 Cter-GFP plasmids along with VSVG and Pax2 plasmids, as assessed by immunoblotting with

1 anti-GFP or anti-ERN1Ab. (F) Flow cytometry analysis of the intracellular production of IFN- γ
2 in control (CTL) HVS-T cells, M/M HVS-T cells from P, and in M/M HVS-T cells transduced
3 with pTRIP-mCherry-2A-plasmids containing WT *PSMD9* or WT *ARHGAP27*, or empty vector.
4 (G) Flow cytometry analysis of IFN- γ intracellular production in control (CTL) cells, P's HVS-T
5 cells (M/M), and P's HVS-T cells transduced with pLenti-Cter-GFP-2A-puro containing the WT
6 *ERN1* or empty vector. (H and I) Flow cytometry analysis of intracellular IFN- γ production in
7 control (CTL) cells, M/M HVS-T cells from P, or M/M HVS-T cells retrovirally transduced
8 with EV or WT T-bet (MM+EV or MM+WT) or MM+WT HVS-T cells lentivirally transduced
9 with the cDNA of EV or WT *PSMD9*, *ARHGAP27* (H) and *ERN1* (I). (J) Isolated CD4⁺ T cells
10 from a healthy donor, a *TBX21* WT/M parent and the *TBX21* homozygous M/M P were
11 expanded with anti-CD3/CD28 antibody-coated beads under T_{H0} or T_{H1} conditions. After 7d,
12 EV or WT T-bet plasmids were used to transduce P's cells in the presence of a bicistronic
13 CD271 surface reporter. Cells were subjected to ICS for IFN- γ and TNF- α in response to P/I
14 restimulation. The gating strategy for the experiment is shown. (K) Cells transduced as in (J)
15 were isolated by MACS with anti-CD271 antibody-coated beads. Together with non-transduced
16 cells, they were re-stimulated with anti-CD3/CD28 antibody-coated beads, and their culture
17 supernatants were subjected to ELISA for IFN- γ and TNF- α .
18 In (A and B), bars represent the mean and the standard deviation; in (K), bars represent the mean
19 and the standard error of the mean. Dots represent individual samples. One-way ANOVA was
20 used for analysis in (B), comparing WT/M or M/M to CTL. ns = not significant.

21
22 **Figure S3. T-bet deficiency results in a normal distribution of dendritic cell subsets, ILCs,**
23 **and V δ 1⁺ $\gamma\delta$ T cells, but alters the development of NK cells, iNKT, MAIT and V δ 2⁺ $\gamma\delta$ T**
24 **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 β 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⁺ 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⁺ or
8 CD94⁺Lin⁻CD7⁺ 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 β 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⁺ 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 β 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 δ 1 and V δ 2 $\gamma\delta$ T cells).
19 The gating strategy for flow cytometry is shown. **(H - J)** iNKT (H), MAIT (I) and V δ 2⁺ $\gamma\delta$ T (J)
20 cells are shown for P and his relatives. **(K)** Percentages of V δ 1⁺ $\gamma\delta$ T cells in healthy adult
21 donors (CTL), age-matched controls (Age CTL), IL-12R β 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).

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

4

5 **Figure S4. Preserved T_{H2} , T_{H17} and circulating T_{FH} memory $CD4^+$ T cells in T-bet**

6 **deficiency, Related to Figure 4. (A)** Naïve ($CD45RA^+CCR7^+$), central memory (CM, $CD45RA^-$

7 $CCR7^+$), effector memory (EM, $CD45RA^-CCR7^-$) and T_{EMRA} ($CD45RA^+CCR7^-$) cells were

8 manually gated from $CD8^+$ T cells on CyTOF data. The percentages of these cells are shown. **(B)**

9 10,000 total $CD8^+$ T cells were clustered by viSNE, based on a selection of surface markers.

10 Manual gating of naïve and memory subsets overlaid on clusters of $CD8^+$ T cells. CXCR3 and

11 CD38 expression are indicated on this plot. **(C)** Naïve ($CD45RA^+CCR7^+$), central memory (CM,

12 $CD45RA^-CCR7^+$), effector memory (EM, $CD45RA^-CCR7^-$) and T_{EMRA} ($CD45RA^+CCR7^-$) cells

13 were manually gated from $CD4^+$ T cells on the basis of CyTOF data. The percentages of these

14 cells are shown. **(D)** The gating strategy for the CyTOF analysis of memory $CD4^+$ T cells and

15 T_{H1} , T_{H2} , T_{H17} and T_{H1}^* cells is shown. **(E)** Memory $CD4^+$ T cells ($CD45RA^-$) from indicated

16 individuals were gated out manually, and further subjected to viSNE deconvolution with T-cell

17 chemokine markers for 10,000 randomly selected events. The expression of CXCR3, CCR5,

18 CCR6 and CCR4 is shown. **(F and G)** Percentages of T_{H2} ($CCR6^-CXCR3^-CCR4^+$) (F) and T_{H17}

19 cells ($CCR6^+CXCR3^-CCR4^+$) (G) among memory $CD4^+$ T cells. **(H)** Frequencies of the

20 circulating follicular T helper (c T_{FH}) cells and their subsets ($CXCR3^+$, double-negative as DN,

21 $CCR6^+$, or $CXCR3^+CCR6^+$ T_{FH}) from healthy donors (CTL) and the T-bet-deficient patient

22 (M/M) gated from conventional flow cytometry data. **(I)** Percentages of Treg cells gated from

23 CyTOF data. **(J)** Percentages of $CXCR3^+$ and $CCR5^+CXCR3^-$ Tregs gated from CyTOF data.

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

3

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

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

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

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

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

9 P/I. **(C)** Frequencies of TNF- α -producing cells among CD56⁺ NK cells, as gated in (A), in the

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

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

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

13 and IFN- γ among total NK cells, as in (D). **(F)** Frequencies of IFN- γ -producing cells among

14 iNKT cells, as gated in (A) for the indicated individuals. **(G and H)** V δ 1⁻V δ 2⁻ γ δ T cells were

15 gated from PBMCs prepared as shown in (A). Intracellular staining for IFN- γ (G) and TNF- α (H)

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

17 staining for IFN- γ (I) and TNF- α (J) is shown. **(K - R)** Naïve and memory CD4⁺ T cells from

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

19 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

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

21 FACS and subjected to activation under T_H0, T_H9, T_H17 or T_H9 conditions combined with T_H17

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

23 (T). **(U)** Naïve CD4⁺ T cells were isolated by FACS and subjected to activation under T_H0, T_H1

1 or T_H2 conditions. IL-10 production, determined with culture supernatants, is shown. (V) Naïve
2 CD4⁺ T cells were isolated by FACS and subjected to activation under T_H0, T_H2 or T_H9
3 conditions. IL-13 production, as measured on culture supernatants.
4 In (C, F - V), bars represent the mean and the standard error of the mean. Dots represent
5 individual samples. Mann-Whitney non-parametric tests were used for analysis in (C, F - J). In
6 (C, F - J), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns = not significant.

7

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

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

1 B, CD4⁺ T and CD8⁺ 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- γ gated on CD4⁺ T cells
3 with and without BCG stimulation in the presence and absence of IL-12 and IL-23. **(I)**
4 Frequency of V δ 2⁺ $\gamma\delta$ 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- γ levels in non-
6 responsive and PPD-responsive CD4⁺ T cells following PPD stimulation. **(K and L)** Dot-plots
7 showing intracellular T-bet and IFN- γ levels in non-responsive and BCG-lysates-responsive
8 CD4⁺ T **(K)** and CD8⁺ 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

1 **STAR Methods**

2

3 **RESOURCE AVAILABILITY**

4 **Lead Contact**

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

7

8 **Materials Availability**

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

11

12 **Data and Code Availability**

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

18

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). 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/mm³). 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

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

5

6 **Cell lines**

7 HEK293T and Phoenix A retroviral packaging cells were cultured in IMDM (Gibco)
8 supplemented with 10% fetal bovine serum (FBS). B cells from the patient or controls were
9 immortalized in-house with Epstein Barr virus (EBV-B cells) and cultured in RPMI 1640 (Gibco)
10 supplemented with 10% FBS. NK-92 cells were cultured in RPMI 1640 (Gibco) supplemented
11 with 10% FBS, 1 mM sodium pyruvate, 200 U/mL recombinant proleukin IL-2
12 (PROMETHEUS). *Herpesvirus saimiri*-transformed T (HVS-T) cells were generated with either
13 *H. saimiri* strain C488 for transformation or the TERT transformation system (Wang et al., 2016).
14 HVS-T cells were cultured in Panserin/RPMI 1640 (ratio 1:1) supplemented with 20% FBS, L-
15 glutamine, gentamycin and 20 U/mL human rIL-2 (Roche). Isolated CD4⁺ T cells were cultured
16 in X-vivo 15/gentamycin/L-glutamine (Lonza) supplemented with human AB Serum (GemCell).
17 Peripheral blood mononuclear cells (PBMCs) were cultured in RPMI 1640 supplemented with
18 10% FBS for stimulation experiments. All cell lines used tested negative for mycoplasma.
19 HEK293T, Phoenix A and NK-92 cells were purchased from the ATCC.

20

21

1 **METHOD DETAILS**

2 **Phage immunoprecipitation-sequencing (PhIP-Seq)**

3 Patient serum and control samples were analyzed in duplicate by phage
4 immunoprecipitation-sequencing (Hernandez et al., 2018; Xu et al., 2015). We used 10% liquid
5 IVIg from pooled human plasma (Privigen® CSL Behring AG), human IgG-depleted serum
6 (Supplier No HPLASERGF5ML, Molecular Innovations, Inc.), and plasma samples from two
7 unrelated healthy adult subjects and one unrelated healthy three-year-old boy for comparison.
8 PhIP-Seq was carried out as previously described (Xu et al., 2015), but with the following
9 modifications. We determined levels of total IgG in the serum or plasma samples with the
10 Human IgG total ELISA Ready-SET-Go kit (Thermo Fisher Scientific) and incubated diluted
11 samples containing approximately 4 µg of total IgG at 4°C overnight with 2×10^{10} plaque-
12 forming units (PFUs) of a modified version of the original VirScan phage library (Xu et al.,
13 2015). Specifically, the T7 phage library used here for peptide display contained the same viral
14 peptides as the original VirScan phage library plus additional peptides derived from protein
15 sequences of various microbial B-cell antigens available from the IEDB (www.iedb.org). For the
16 computational analysis and background correction, we also sequenced the phage library before
17 (input library sample) and after immunoprecipitation with beads alone (mock IP). We performed
18 a number ($n = 46$) pf technical repeats. Single-end sequencing was performed with the
19 NextSeq500 system (Illumina), to generate approximately two million reads per sample and ~20
20 million reads for the input library samples. As previously described (Xu et al., 2015), reads were
21 mapped onto the original library sequences with Bowtie 2 and read counts were adjusted
22 according to library size. A zero-inflated generalized Poisson model was used to estimate the p -
23 values, to reflect enrichment for each of the peptides. We considered peptides to be significantly

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

8

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

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

21 All four members of the family were genotyped with Genome-Wide Human SNP Array
22 6.0 data. Genotype calling was achieved with the Affymetrix Power Tools Software Package
23 (<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 1×10^{-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.

8

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'-tctacacttttcctacacgacgctcttccgatctgtgaggactacgcgctacc-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 aatgatacggcgaccaccgagatctacacttttcctacacgac-3' and the reverse primers 5'-
23 caagcagaagacggcatacagatcgtattcgggtgactggagttcagacgtgtg-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).

8

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.

15

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,

1 HVS-T cells, or NK-92 cells, as previously described (Martínez-Barricarte et al., 2016). In brief,
2 the *TBX21* plasmid was subcloned into pLZRSires- Δ NGFR (Addgene) (Van De Vosse et al.,
3 2009). We then used pLZRS containing the WT or Mut *TBX21* to transfect Phoenix A cells, and
4 retroviruses were produced and concentrated with Retro-X (Takara Bio). Concentrated retrovirus
5 preparations were used for retroviral transduction.

6

7 **Immunoblotting**

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

22

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

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

15

16 **Luciferase reporter assays**

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

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

3

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

5 T-bet-deficient NK-92 cells were generated according to a protocol described elsewhere
6 (Sanjana et al., 2014). We used oligonucleotides for gRNA9 and gRNA15 (gRNA9_Forward: 5'-
7 caccgtccaacaatgtgacccaggt-3', gRNA_Reverse: 5'-aacacctgggtcacattgttgac-3';
8 gRNA15_Forward: 5'-caccgccgcactcaccgtcctgct-3', gRNA15_reverse: 5'-
9 aaacagcagggacggtgagtgcggc-3'). DNA duplex was annealed from the oligonucleotides above,
10 and was inserted into pLenti-CRISPR-V2 plasmids (Addgene) (Sanjana et al., 2014). We then
11 used pLenti-CRISPR plasmids, VSV-G envelope, and psPAX2 plasmids to transfect HEK293T
12 cells in the presence of Lipofectamine 2000. Supernatants containing lentiviruses were harvested
13 and concentrated with Lenti-X concentrator (Takara Bio). We resuspended 10⁶ NK-92 cells with
14 20x concentrated lentiviruses for gRNA9, gRNA15 or empty plasmid, in the presence of
15 proleukin IL-2 (200 U/mL) (PROMETHEUS). After incubation for 2 days, we added 3.75
16 µg/mL puromycin to the culture for the selection of stably transduced NK-92 cells. After a
17 further two weeks, cells were harvested for the Surveyor Nuclease Assay (Integrated DNA Tech)
18 and a three-primer PCR-based screening system (Harayama and Riezman, 2017) to confirm
19 successful DNA editing. Cells were plated at a density of 0.7 cells in 200µL per well in a U-
20 bottomed 96-well plate. Two weeks later, single-cell clones were screened with a three-primer
21 PCR-based system, with Phire Direct PCR Master Mix (Thermo Fisher Scientific). The primers
22 used for this screening assay were gRNA9_Forward: 5'- agcccctaccctaattcct-3',
23 gRNA9_Reverse: 5'-ggcatctattcctgggacc-4', gRNA9_Reverse_in: 5'-cttttgaagagcaggtcctacct-3'

1 and gRNA15_Forward: 5'-GCCTGAATATGACCCCGTC-3', gRNA15_Reverse: 5'-
2 caccactaccaccactaaagc-3'; gRNA15_Forward_in: 5'-acagagatgatcatcaccaagca-3'. Single-cell
3 clones with biallelic disruptions were selected for validation by RT-qPCR and western blotting.
4 EV-transduced single-cell clones were randomly picked as controls. Cells were then transduced
5 with retroviruses generated from pLZRS-ires- Δ CD271 plasmids containing EV, WT, Mut or
6 K314R *TBX21*, as described above (Martínez-Barricarte et al., 2016). Stably transduced cells
7 were isolated with anti-CD271 antibody-coated beads (Miltenyi Biotec). Cells were stimulated
8 with 50 pg/mL IL-12 (R&D) and 10 ng/mL IL-18 (InvivoGen), or were left unstimulated, and all
9 cells were incubated for one day. The IFN- γ content of the supernatants was determined by
10 ELISA. Intracellular IFN- γ production was measured with flow cytometry with intracellular
11 staining (ICS). In brief, cells were fixed and permeabilized (BioLegend), and were subjected to
12 ICS staining with FcBlock (Miltenyi Biotec), anti-T-bet BV421 (BioLegend) and anti-IFN- γ PE
13 (BioLegend) before their use for ICS flow cytometry analysis.

14

15 **Cytokine determinations in *TBX21*-transduced primary CD4⁺ T cells**

16 PMBCs from three healthy donors were stained with FcBlock (Miltenyi Biotec), anti-
17 CD4-APC-Cy7, anti-CD45RA-Alexa488, anti-CCR7-Alexa647 and Live-Dead exclusion Aqua.
18 Naïve CD4⁺ T cells were isolated by FACS. Cells were stimulated with anti-CD3/CD28 Ab
19 Dynabeads (Thermo Fisher Scientific) at a 1:1 cell:bead ratio under T_H0 or T_H2 (recombinant
20 human IL-4 12.5 ng/mL, R&D) polarizing conditions, in the presence of 100 U/mL proleukin IL-
21 2 (PROMETHEUS). Fresh medium (X-vivo 15, 5% human AB serum) and cytokines were
22 added every 3 days. After 7 days, cells were restimulated with Dynabeads at a 1:2 ratio
23 (bead:cell). One day after restimulation, cells were transduced with retroviruses generated from

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

11

12 **HVS-T cell analysis**

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

1 (BioLegend), anti-IL-13-APC (BioLegend), anti-IL-10-PE-Dazzle594 (BioLegend) antibodies.
2 ICS analysis was performed on an Aurora cytometer (Cytex).

3 HVS-T cells were also analyzed for IFN- γ production in response to *PSMD9*,
4 *ARHGAP27* and *ERN1* cDNA overexpression. In brief, pTRIP-mCherry-2A-PSMD9 and
5 *ARHGAP27* were generated by subcloning from MGC clones BC004184 and BC067345
6 (transOMIC). A C-terminal V5 tag was added upstream from the stop codons. The pLenti-Cter-
7 GFP-2A-puro empty vector plasmid was obtained from OriGene. The pCMV6-ERN1-Cter-Myc-
8 Flag plasmid was obtained from OriGene. pLenti-ERN1-Cter-GFP (fusion)-2A-puro was
9 generated by the double digestion, with *EcoRI* and *XhoI*, of pCMV6-ERN1 and pLenti-Cter-
10 GFP-2A-puro, followed by the isolation of the ERN1 fragment by gel extraction, and T4 ligation.
11 Lentiviruses containing cDNAs encoding EV, PSMD9, ARHGAP27, ERN1-GFP were generated
12 in HEK293T cells by transfection with pTRIP or pLenti-GFP-2A-puro, VSV.G and Pax2
13 packaging plasmids. Transfected HEK293T cells were analyzed for the expression of PSMD9
14 and ARHGAP27 by assessing the expression of the C-terminal V5 tag and ERN1 by western
15 blotting with anti-GFP Ab or anti-ERN1 Ab (Proteintech). Lentiviruses containing the cDNAs of
16 EV, *PSMD9*, *ARHGAP27* and *ERN1-GFP* were used to transduce HVS-T cells derived from the
17 cells of P (M/M), or M/M HVS-T cells retrovirally transduced with WT T-bet (M/M+WT). After
18 2 – 4 days of transduction, cells were stimulated with 100 ng/mL PMA and 2 μ M ionomycin for
19 8 h and harvested for staining for flow cytometry. ERN1-transduced HVS-T cells and their
20 control HVS-T cells were stained with Zombie NIR Fixable Viability Dye (BioLegend) and
21 surface staining was then performed with FcBlock, anti-CD271-PE/Dazzle594 (BioLegend),
22 anti-CD4-BUV563 (BD Biosciences) and anti-CD8-BUV737 (BD Biosciences) antibodies. Cells
23 were fixed and permeabilized with the FOXP3/Transcription Factor Buffer kit (Thermo Fisher

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

9

10 **Functional analysis of expanded T-bet-deficient CD4⁺ T cells *in vitro***

11 CD4⁺ T cells were isolated from PBMCs from five healthy controls (CTL), IL-12R β 1-
12 deficient (*IL12RB1* M/M) patients, the T-bet WT/M heterozygous father (*TBX21* WT/M), and
13 the T-bet M/M patient (*TBX21* M/M), with anti-CD4 antibody-coated beads (Miltenyi Biotec).
14 They were then expanded with anti-CD3/CD28 antibody-coated Dynabeads (Thermo Fisher
15 Scientific) at a 1:1 (cell:bead) ratio under T_{H0} or T_{H1} (2.5 ng/mL IL-12 R&D and anti-human IL-
16 4 neutralizing Ab InVivoMab, BioXcell) conditions, in the presence of 100 U/mL proleukin IL-2
17 (PROMETHEUS) and anti-human IL-10 neutralizing Ab (Thermo Fisher Scientific). Medium
18 (X-vivo15 5% human AB serum) and cytokine cocktails were refreshed every 3 – 4 days. After 7
19 days, the culture consisted of >95% CD4⁺ T cells. Cells were restimulated with anti-CD3/CD28
20 Ab-coated Dynabeads every 7-8 days. P's CD4⁺ T cells were transduced with retroviruses
21 obtained from pLZRS-ires- Δ CD271 plasmids with or without WT *TBX21* or EV, as previously
22 described (Martínez-Barricarte et al., 2016). Six days after transduction, cells were restimulated
23 with 25 ng/mL PMA and 500 nM ionomycin in the presence of GolgiPlug (BD Biosciences) for

1 6 h. Cells were stained with Zombie NIR Live-Dead exclusion reagents (BioLegend), anti-
2 CD271-FITC antibody and were then fixed and permeabilized. They were stained with anti-
3 TNF- α -BV510, anti-IL-13-APC, anti-IL-4-PE, anti-IL-17A-BV785, anti-IL-10-PE-Dazzle594,
4 anti-IL-5-BV421 and anti-IFN- γ -PerCp-Cy5.5 antibodies and subjected to flow cytometry in an
5 Aurora cytometer (Cytek). Three weeks after their initial expansion, transduced CD271⁺ CD4⁺ T
6 cells from P were isolated with anti-CD271 antibody-coated beads (Miltenyi Biotec). The cells
7 isolated, and expanded CD4⁺ T_H0 cells from healthy donors or T-bet wt/m individuals were
8 restimulated with anti-CD3/CD28 antibody-coated beads in the absence of cytokine for 16 h or
9 48 h. Cytokine levels were determined in 13-Legendplex assays (BioLegend) on culture
10 supernatants from cultures restimulated for 48 h . Total RNA was extracted from cells
11 restimulated for 16 h for RNA-seq analysis (Qiagen). In brief, 100 ng of total RNA was used to
12 generate RNA-Seq libraries with the Illumina TruSeq stranded mRNA LT kit (Cat# RS-122-
13 2101). Libraries prepared with unique barcodes were pooled at equal molar ratios. The pool was
14 denatured and sequenced on an Illumina NextSeq 500 sequencer with high-output V2 reagents
15 and NextSeq Control Software v1.4 to generate 75 bp single reads, according to the
16 manufacturer's protocol (Illumina, Cat# 15048776).

17 RNA-seq FASTQ files were obtained, and inspected by fastqc for quality control of the
18 raw data. The sequencing reads of each FASTQ file were then mapped to the GENCODE human
19 reference genome GRCh38.p13 (Frankish et al., 2019) with STAR aligner v2.7 (Dobin et al.,
20 2013), and the quality of each mapped alignment in the BAM file was evaluated by RSeQC
21 (Wang et al., 2012). Reads were quantified to generate gene-level read counts from the read
22 alignment, with featureCounts v1.6.0 (Liao et al., 2014), based on GENCODE GRCh38.p13
23 gene annotation. The gene-level read counts were then normalized and log₂-transformed by

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

13

14 **Omni-ATAC-seq on CD4⁺ T cells and analysis**

15 CD4⁺ T cells derived from healthy controls, *IL12RB1* M/M patients, a *TBX21* WT/M
16 parent and the *TBX21* M/M P were expanded under T_H0 conditions, as described above. Nine
17 days after the retroviral transduction of CD4⁺ T cells from P, as described above, CD271⁺ cells
18 were isolated with anti-CD271 antibody-coated beads (Miltenyi Biotec). Omni-ATAC-seq
19 library preparation was performed as previously described (Corces et al., 2017). In brief, 50,000
20 cells from three healthy donors (CTL), two *IL12RB1* M/M patients, technical duplicates for a
21 *TBX21* WT/M individual, the *TBX21* M/M P and P cells complemented with EV (M/M+EV) or
22 WT (M/M+WT) *TBX21* were harvested, washed with 50 μ l cold PBS, and centrifuged to obtain
23 a pellet. Pellets were lysed with 50 μ l cold lysis buffer consisting of 48.5 μ l resuspension buffer

1 (10 mM Tris-HCl pH 7.5, 10 mM NaCl, 3 mM MgCl₂ in water), 0.5 μl 10% NP-40, 0.5 μl 10%
2 Tween-20, and 0.5 μl 1% digitonin. Lysates were incubated on ice and then washed with 0.1%
3 Tween-20 resuspension buffer. Lysates were centrifuged and the nuclei in the pellet were
4 subjected to Tn5 transposition with 50 μL of a mixture of 25 μL 2 x TD buffer, 16.5 μL PBS, 0.5
5 μL 10% Tween-20, 0.5 μL 1% digitonin, 2.5 μL Tn5 transposase, 5 μL nuclease-free water at
6 37 °C in a thermomixer operating at 1,000 rpm for 30 min. DNA fragments were extracted with
7 the MinElute PCR purification kit and eluted in 30 μl EB buffer (Qiagen). We used 10 μL of
8 eluted DNA for the amplification of DNA fragments with the NEBNext PCR master mix (New
9 England BioLab), over six cycles, with the Ad1_forward and 15 indexed Ad2_reverse primers,
10 as previously described (Buenrostro et al., 2013, 2015). The partially amplified library was
11 subjected to quantitative PCR analysis with the Ad1 and Ad2 primers, Sybr Green reagents and
12 NEBNext PCR master mix for 25 cycles. Additional PCR cycles were performed, based on 1/3
13 the maximal fluorescence for each sample, as previously described (Buenrostro et al., 2013,
14 2015). A left-sided isolation of PCR products was performed with AMPure beads (Beckman
15 Coulter). DNA was quantified with a Qubit fluorimeter. Equal amounts of each sample were
16 pooled and subjected to paired-end sequencing on a NextSeq high-output sequencer generating
17 75 bp reads.

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

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

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

14

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

16 CD4⁺ T cells derived from healthy controls, a *TBX21* WT/M individual and the *TBX21*
17 M/M P were expanded under T_H0 conditions, as described above. Seventeen days after the
18 retroviral transduction of CD4⁺ T cells from P as described above, CD271⁺ cells were isolated
19 with anti-CD271 antibody-coated beads (Miltenyi Biotec). DNA from non-transduced or
20 CD271⁺ isolated CD4⁺ T cells was extracted with a DNA mini kit (Qiagen). DNA was subjected
21 to bisulfite conversion with the EZ DNA Methylation Kit (ZymoResearch). We quantified the
22 DNA in all samples by performing EPIC arrays (Illumina), following the manufacturer's
23 instructions. Technical replicates were included for several samples during EPIC processin, to

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

16

17 **Immunophenotyping of leukocytes**

18 In the CyTOF experiment, PBMCs from 26 healthy adults of diverse ethnicities, nine
19 child or adolescent controls (age range: 1 to 17 years, with diverse ancestries, including one age-
20 matched child from Morocco), six IL-12R β 1-deficient patients (recruited from previous studies),
21 and the T-bet-deficient patient (M/M). We recruited 26 healthy adult controls locally in New
22 York City. We analyzed the PBMCs of four individuals on two separate dates, as duplicates. We
23 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 β 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

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

3 We also deconvoluted 10,000 memory CD4⁺ T cells (DNA⁺Live_CD45⁺CD66b⁻CD123⁻
4 CD3⁺CD20⁻CD56⁻γδTCR⁻CD4⁺CD8⁻CD45RA⁻) into viSNE plots based on 20 surface markers
5 (113ln_CD57, 115ln_CD11c, 149sm_CD127, 150Nd_CD1c, 154Sm_CD86, 155Gd_CD27,
6 156Gd_CCR5, 158Gd_CD117, 159Tb_CD24, 163Dy_CRTH2, 165Ho_CCR6, 166Er_CD25,
7 167Er_CCR7, 169Tm_CX3CR1, 170Er_CD38, 171Yb_CD161, 173Yb_CXCR3, 174Yb_HLA-
8 DR, 176Yb_CCR4, 209Bi_CD11b) (Amir el et al., 2013). Analyses were performed by manually
9 gating populations for NK cells, naïve and memory subsets of CD4⁺ and CD8⁺ T cells, innate
10 lymphoid cells (ILCs), Tregs and dendritic cell subsets. For T helper cell analysis, we included
11 an additional four healthy donors from a separate experiment (same staining panel) in the
12 analysis. Manual gating was used for T_H analysis. We also deconvoluted 10,000 CD8⁺ T cells
13 into viSNE plots based on 113ln_CD57, 115ln_CD11c, 143Nd-CD45RA, 144Nd-CD141,
14 149sm_CD127, 150Nd_CD1c, 154Sm_CD86, 155Gd_CD27, 156Gd_CCR5, 158Gd_CD117,
15 159Tb_CD24, 163Dy_CRTH2, 165Ho_CCR6, 166Er_CD25, 167Er_CCR7, 169Tm_CX3CR1,
16 170Er_CD38, 171Yb_CD161, 173Yb_CXCR3, 174Yb_HLA-DR, 176Yb_CCR4, 209Bi_CD11b
17 (Amir el et al., 2013).

18 Immunophenotyping for MAIT, invariant NKT cells, Vδ2 and Vδ1 cells was performed
19 in three different batches. In the first batch, PBMCs from 18 healthy controls (CTL), three IL-
20 12Rβ1-deficient individuals (*IL12RB1* M/M), two T-bet-heterozygous parents (*TBX21* WT/M), P
21 (*TBX21* M/M) and P's healthy brother (*TBX21* WT/WT) were included. They were stained with
22 the following panel: FcBlock (Miltenyi Biotec), Zombie-NIR live-dead exclusion dye
23 (BioLegend), anti-CD3-Alexa532 (Thermo Fisher Scientific), anti-γδTCR-FITC (Thermo Fisher

1 Scientific), anti-V δ 2-APC-Fire750 (BioLegend), anti-CD56-BV605 (BioLegend), anti-CD4-
2 BV750 (BD Biosciences), anti-CD8a-BV510 (BioLegend), anti-V α 7.2-BV711 (BioLegend),
3 anti-V α 24-J α 18-PE-Cy7 (BioLegend), anti-V δ 1-Vioblue (Miltenyi Biotec), anti-CD161-PE
4 (BioLegend) and anti-V β 11-APC (Miltenyi Biotec) antibodies. Cells were analyzed with an
5 Aurora cytometer (Cytex). In the 2nd 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 3rd batch, 16 adult control samples, five samples from age-
8 matched controls (age CTL) and a sample from P were included. In the 3rd 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 β 1-deficient
19 individuals were prepared from blood transported overnight. In the second and third batches,
20 immunophenotyping for MAIT, invariant NKT cells, V δ 2 and V δ 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-

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

7 Immunophenotyping for innate lymphoid cells and NK cells was confirmed separately,
8 by conventional flow cytometry. In brief, biotinylated anti-human anti-CD1a (HI149), anti-CD3
9 (OKT3), anti-CD5 (UCHT2), anti-CD14 (61D3), anti-CD19 (HIB19), anti-CD34 (4H11), anti-
10 CD123 (6H6), anti-CD203c (FR3-16A11), anti-CD303 (AC144), anti-TCR $\alpha\beta$ (IP26), anti-
11 TCR $\gamma\delta$ (B1) and anti-Fc ϵ RI α (AER-37(CRA-1)) antibodies were used in combination with
12 streptavidin BUV661, and conjugated anti-CD62L-FITC (Dreg-56), anti-CD94-PerCP-Vio700
13 (REA113), anti-TCRV α 24J α 18-PE (6B11), anti-CD26-PE-CF594 (M-A261), anti-CD127-PE-
14 Cy7 (eBioRDR5), anti-CRTH2-Alexa Fluor 647 (BM16), anti-CD161-Alexa Fluor 700 (HP-
15 3G10), anti-EOMES-APC-eFluor 780 (WD1928), anti-NKp46-BV421 (9E2/NKp46), anti-
16 CD45RA-BV570 (HI100), anti-CD117-BV605 (104D2), anti-ROR γ t-BV650 (Q21-559), anti-
17 CD7-BV711(M-T701), anti-CD16-BUV496 (3G8), anti-CD25-BUV563 (2A3), anti-CD56-
18 BUV737 (NCAM16.2) and anti-CD45-BUV805 (HI30); anti-T-bet-BV786 (O4-46), anti-
19 GATA3-BUV395 (L50-823) antibodies. Antibodies were purchased from BioLegend, Thermo
20 Fisher Scientific, BD Biosciences, or Miltenyi Biotec. Fc receptors were blocked with IgG from
21 human serum (Sigma-Aldrich). Surface membrane staining was performed in Brilliant Stain
22 Buffer (BD Biosciences). Transcription factors were stained with the Foxp3 staining kit (Thermo
23 Fisher Scientific), according to the manufacturer's instructions. The fixable viability dye eFluor

1 506 (Thermo Fisher Scientific) was used to exclude dead cells. Samples were acquired on a BD
2 Symphony A5 cytometer (BD Biosciences) with FACSDiva 8 software and were analyzed with
3 FlowJo 10 (BD Biosciences).

4 Immunophenotyping was confirmed separately for the T- and B-cell subsets by
5 conventional flow cytometry. In brief, PBMCs from healthy controls and P were labeled with
6 mAbs against CD3, CD4, CD8, CD45RA, CCR7, CD127, CD25, CXCR5, CXCR3, CCR6, PD-
7 1, and CD56. The CD4⁺ and CD8⁺ T-cell subsets were separated into naïve (T_{Naive}:
8 CCR7⁺CD45RA⁺), central memory (T_{CM}: CCR7⁺CD45RA⁻), effector memory (T_{EM}: CCR7⁻
9 CD45RA⁻), and terminally differentiated effector memory cells (T_{EMRA}: CCR7⁻CD45RA⁺) after
10 initial gating on CD8⁻CD4⁺ or CD8⁺CD4⁻ cells. Proportions of regulatory T cells
11 (CD4⁺CD127^{lo}CD25^{hi}), total memory (CD4⁺CD45RA⁻), and circulating Tfh (cTfh;
12 CD4⁺CD45RA⁻CXCR5⁺) cells, and subsets of total memory and cTfh cells defined on the basis
13 of CXCR3 and CCR6 expression (T_{H1}: CCR6⁻CXCR3⁺, T_{H17}: CCR6⁺CXCR3⁻, T_{H1}*/T_{H1-17}:
14 CCR6⁺CXCR3⁺, T_{H2}: CCR6⁻CXCR3⁻) were also determined.

15

16 **Single-cell (sc) RNA-seq and analysis**

17 We performed scRNA-seq on PBMCs obtained from the *TBX21* WT/M heterozygous
18 father and the *TBX21* M/M patient. PBMCs were quickly thawed at 37°C and gently resuspended
19 by serial additions of DMEM + 10% heat-inactivated (HI) FBS, according to the
20 recommendations from the 10x Genomics “Sample preparation Fresh Frozen Human PBMC”
21 protocol Rev C. Cells were centrifuged at 400 x g for 5 min, washed with DMEM + 10% HI-
22 FBS, and cells were then counted and viability was assessed with the LIVE/DEA Viability kit
23 (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/ μ 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 < 10⁻²⁵ 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

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

6

7 **Stimulation of NK cells *ex vivo***

8 PBMCs from five healthy donors including a travel control, the T-bet WT/M mother, the
9 T-bet WT/WT brother and the T-bet M/M P were left untreated or stimulated with IL-12
10 (Miltenyi Biotec), IL-15 (Miltenyi Biotec) and IL-18 (MBL/CliniSciences) for 18 h. Cells were
11 treated with GolgiPlug and GolgiStop (BD Biosciences), and stained with anti-CD107a-PE
12 antibody (eBioH4A4, Thermo Fisher Scientific) for 4 h. They were then stained with eFluor506
13 live-dead reagents (Thermo Fisher Scientific), followed by antibodies binding to surface antigens,
14 including anti-CD1a-FITC (H1149), anti-CD3-FITC (OKT3), anti-CD5-FITC (UCHT2), anti-
15 CD14-FITC (TUK4), anti-CD19-FITC (HIB19), anti-CD34-FITC (581), anti-CD123-FITC
16 (6H6), anti-CD203c (NP4D6), anti-CD303-FITC (201A), anti-Fc ϵ RI α -FITC (AER-37(CRA-1)),
17 anti-TCR $\alpha\beta$ -FITC (IP26), anti-TCR $\gamma\delta$ -FITC (B1), anti-CD94-PerCP-Vio700 (REA113), anti-
18 CD127-PE-Cy7 (eBioRDR5), anti-NKp46-APC (9E2/NKp46), anti-CD7-BV711 (M-T701),
19 anti-CD56-BV786 (NCAM16.2), anti-CD16-BUV737 (3G8), and anti-CD45-BUV805 (HI30)
20 antibodies. All the markers targeted by FITC-conjugated Abs were considered to be Lineage-
21 specific markers (Lin). Cells were fixed and permeabilized (Fix & Perm; Thermo Fisher
22 Scientific) and subjected to intracellular staining with anti-granzyme B-PE-CF594 (GB11), anti-
23 EOMES-APC-eFluor 780 (WD1928), anti-perforin-BV421 (δ G9), anti-TNF- α -BV605 (Mab11)

1 and anti-IFN- γ -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).

4

5 **CD4⁺ T-cell isolation and functional characterization *ex vivo* and T_H 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⁺ T cells were isolated by first excluding Tregs (CD25^{hi}CD127^{lo}) and then sorting
12 CD4⁺CD45RA⁺CCR7⁺CXCR5⁻ and CD4⁺CD45RA⁻CCR7[±] cells, respectively. Isolated naïve
13 and memory CD4⁺ T cells were then cultured in 96-well round-bottomed well plates (30-40 x10³
14 cells/well) with T-cell activation and expansion (TAE) beads (anti-CD2/CD3/CD28; Miltenyi
15 Biotech) alone (T_{H0}) or under T_{H1} (50 ng/mL IL-12), T_{H2} (100 U/mL, IL-4), T_{H9} (2.5 ng/mL
16 TGF- β , 100 U/mL IL-4), or T_{H17} (2.5 ng/mL TGF- β , 50 ng/mL IL-1- β , 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- γ and TNF- β was assessed with cytometric bead arrays (Becton Dickinson); IL-22
20 secretion was measured by ELISA (eBioscience). For cytokine expression, activated CD4⁺ T
21 cells were restimulated with PMA (100 ng/mL)/ionomycin (750 ng/mL) for 6 h, with brefeldin A
22 (10 μ 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- γ was determined.

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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 1.5×10^6 cells/ml. Cells were cultured for 40 h in RPMI. They were then stimulated with 50 ng/mL PMA and 1 μ 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

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

13

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

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

1 had been transported overnight, which were then frozen. These three vials of PBMCs from P
2 were used separately, in three batches. One vial of PBMCs prepared from fresh blood was plated
3 in two sets of wells as technical duplicates. Thus, four data points were measured for P. The
4 PBMCs of P's relatives were prepared from blood that had been shipped overnight. PBMCs from
5 P's healthy brother, mother and father were each analyzed once. Five age-matched controls were
6 recruited from around the world, including one matched for both age and ethnicity from
7 Morocco. PBMCs were isolated from the blood of these patients after overnight shipment and
8 frozen. PBMCs from three age-matched controls were plated in two sets of wells, as technical
9 duplicates. The PBMCs of the age- and ethnicity-matched control were plated in technical
10 triplicates. In three batches of experiments, we plated 300,000 cells per well in 96-well U-
11 bottomed plates, at a density of 1.5×10^6 cells/mL. Cells were cultured for 40 h in RPMI. They
12 were then stimulated by incubation with 50 ng/mL PMA and 1 μ M ionomycin for 8 h.
13 Supernatants were harvested for human T_H cytokine analysis with the 13-plex LegendPlex kit
14 (BioLegend).

15

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

17 This experiment was performed in three batches, on three dates. The data from the three
18 batches were compiled for the final analysis. Thirty healthy adult controls were recruited at the
19 Rockefeller University (New York, USA) and one healthy adult control was recruited at Necker
20 Hospital (Paris, France). PBMCs from eight of the 31 adult controls were frozen after being
21 allowed to rest at room temperature for 24 – 48 h to mimic travel conditions. The PBMCs of all
22 the other 23 adult controls were isolated from fresh blood and frozen. PBMCs from 19 adult
23 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 1.5×10^6 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 δ 1TCR-FITC (Miltenyi Biotec), anti-CD8-BUV737 (BD
2 Biosciences), anti-V δ 2TCR-APC/Fire750 (BioLegend), anti-CD20-BV785 (BioLegend), anti-
3 V α 7.2-Alexa 700 (BioLegend), MR1-5-OP-RU-tetramer (NIH tetramer core facility), anti-V β 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- γ -BV711
7 (BioLegend), anti-TNF- α -BV510 (BioLegend), anti-IL-17A-PERCP/Cy5.5 and anti-ROR γ 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⁺ IFN- γ ⁺ 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⁺ IFN- γ ⁺ 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.

14

15 **V δ 2⁺ $\gamma\delta$ 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 β 1 m/m patients, the T-bet M/M P and his relatives were resuspended
18 at a density of 1x10⁶ cells/mL. We plated 200 μ 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 μ 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 μ 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 δ 2
3 TCR-APCFire750 (BioLegend), anti-CD56-BV605 (BioLegend), anti-CD4-BV750 (BD
4 Biosciences), anti-CD8a-BV510 (BioLegend), anti- β 11TCR-APC (Miltenyi Biotec), anti-
5 CD161-PE (BioLegend), anti- V α 7.2 TCR-BV711 (BioLegend), anti-V α 24-J α 18 (iNKT)-
6 PE/Cy7 (BioLegend), anti-V δ 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_H
9 cytokine analysis with the 13-plex LegendPlex kit (BioLegend).

10

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 2×10^6 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 μ g/mL or with tuberculin purified protein derivative (PPD) at a final
17 concentration of 5 μ 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 δ 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- γ -

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

3

4 **Isolation and screening of CD4⁺ T cell lines**

5 The T-cell clone experiment was performed as previously described (Geiger et al., 2009).

6 In brief, CCR6⁺ and CCR6⁻ CD4⁺ memory T cells were sorted from CD14⁻ PBMCs with a
7 FACS Aria (BD Biosciences), excluding CD45RA⁺CCR7⁺ naïve cells, and CD25⁺, CD19⁺, and
8 CD8⁺ cells. Sorted T cells (500 cells/well) were polyclonally stimulated with 1 μ g/mL PHA
9 (Remel) in the presence of irradiated (45 Gy) allogeneic feeder cells (10⁴ per well) and IL-2 (500
10 IU/mL) in RPMI complete medium. After expansion for at least 20 days, the T-cell lines were
11 analyzed by surface or intracellular staining with the following monoclonal antibodies: anti-
12 CCR6-PE (BD Biosciences; clone: 11A9); anti-CXCR3-APC (BD Biosciences; clone: 1C6);
13 anti-IFN- γ -FITC or anti-IFN- γ -PE (BD Biosciences, clone: B27); anti-IL-17A-eFluor660 FITC
14 (eBiosciences, clone: eBio64DEC17); anti-IL-4-PE (BD Biosciences, clone: MP4-25D2); anti-
15 IL-22-PerCP-eFluor-710 (Thermo Fisher Scientific, clone: 22URTI). For identification of the
16 antigen-responding T-cell clones, the T-cell lines were also screened by culturing thoroughly
17 washed cells (2.5 \times 10⁵ cells/well) with autologous irradiated B cells (2.5 \times 10⁴ cells/well), with or
18 without a three-hour pulse of the following antigens: *M. tuberculosis* peptide pool (0.5
19 μ g/mL/peptide, comprising 207 15-mer peptides), BCG peptide pool (0.5 μ g/mL/peptide,
20 comprising 211 15-mer peptides), HCMV peptide pool (0.5 μ g/mL/peptide, comprising 76 15-
21 20-mer peptides), EBV peptide pool (0.5 μ g/mL/peptide, comprising 46 15-20-mer peptides),
22 Influenza virus HA peptide pool (2 μ g/mL/peptide, comprising 351 15-mer peptides), *Candida*
23 *albicans* peptide pool (0.5 μ g mL/peptide, comprising 252 15-mer peptides) or tetanus toxoid

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

7

8 **QUANTIFICATION AND STATISTICAL ANALYSIS**

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

13

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