Supplementary Materials for

Impairment of immunity to Candida and Mycobacterium in humans with bi-allelic *RORC* mutations

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Case Reports

P1 and P2 were born, in 2002 and 2008 respectively, to consanguineous parents from Israel (Fig 1A, Kindred A). P1 was vaccinated with BCG in early infancy and displayed left upper lobe opacity at the age of 10 months. She developed an enlarged liver at 19 months old. The patient developed recurrent generalized atonic seizures due to multiple granuloma in the brain at 23 months old. She displayed oral thrush and a cystic region in the posterior aspect of the anterior mediastinum on the left side. Examination of oxidative burst in P1 cells was normal. Acid-fast smear was positive from the contents of the patient's abdominal cyst. Histopathological examination of lung tissue showed granuloma. She developed lymphopenia and hypogammaglobulinemia. The patient died due to BCG meningoencephalitis when she was 6 years old. P2 was vaccinated with BCG at the early infancy. He suffered from persistent oral thrush as a young child. He was hospitalized to perform an immunological examination at 2 years old. Physical examination suggested that there were no palpable peripheral lymph nodes. Liver and spleen were palpated of normal size. The patient's lung exam was compatible with mild bronchitis. *Mycobacterium tuberculosis* complex was detected in culture from gastric juice at age 2. P3 is the first cousin of P1 and P2 and was born to consanguineous parents in 2011. P3 was vaccinated with BCG in early infancy. He presented a probable mycobacterial abscess of the thigh at the age of 10 months, and then pneumonia of suspected mycobacterial origin, at the age of five years. Physical examination of the patient revealed an absence of peripheral lymphoid tissue.

P4 was born in 2009 to consanguineous parents from Chile (Fig 1A, Kindred B). She received BCG immunization in early infancy. She had several episodes of oral candidiasis and onychomycosis with positive culture for *Candida albicans*. She presented pulmonary BCG infection, as determined by reverse hybridization from gastric aspiration, at the age of 16 months. The patient also had hepatosplenomegaly. The computer tomography (CT) and echography studies showed diffuse opacification in the upper right lobe and adenopathies in peri-aortic and cava region. Laboratory findings showed normal oxidative burst, and moderate T-cell lymphopenia. *Mycobacterium bovis*-BCG was identified from the biopsy of the paravertebral abscess. The patient showed granuloma in the brain which was detected by MRI at the age of 44 months.

P5, P6 and P7 were born to consanguineous parents from Saudi Arabia (Fig 1A, Kindred C) in 2005, 2009 and 2012, respectively. All three patients received BCG vaccination in infancy. P5 presented with a 2x2cm cervical mass with acid-fast bacilli at age 9 years, and was diagnosed with *Mycobacterium tuberculosis* infection. She was treated with isoniazid, rifampicin and pyrazinamide, and pyrazinamide was discontinued after the first 2 months of treatment. P6 presented abdominal distention due to hepatosplenomegaly at the age of 9 months. Abdominal ultrasound and CT scans showed hepatosplenomegaly, necrotic para-aortic lymphadenopathy and mesenteric lymphadenopathy. *Mycobacterium bovis* was identified in the gastric juice aspirate. Thus, she was given a diagnosis of disseminated BCG. Her symptoms improved in response to anti-TB drug treatment, but hepatomegaly did not resolve. She also suffered from recurrent upper respiratory tract infections. P7 presented with splenomegaly at the age of 3 months. He also showed inflammation at the site of BCG vaccination. Acid-fast bacilli culture was positive for *Mycobacterium bovis*-BCG.

Materials and Methods:

Genome-wide linkage analysis:

Genome-wide linkage analysis was performed as previously described (*38*), with minor modifications. Eight members of Kindred A were genotyped with the Affymetrix Genome-wide Human Mapping 250K array and two members were genotyped with the Genome-Wide Human SNP Array 6.0. One member of Kindred B was genotyped with the Affymetrix Genome-wide Human Mapping 250K array and another was genotyped with the Genome-Wide Human SNP Array 6.0. Five members of Kindred C were genotyped with the Affymetrix Genome-wide Human Mapping 250K array. Genotype calling was achieved with the Affymetrix Power Tools Software Package (http://www.affymetrix.com/estore/partners_programs/programs/developer/tools/powertools.affx). SNPs presenting more than one Mendelian inconsistency were discarded. SNPs were further filtered with population-based filters (*39*). We then used 562,679 SNP markers to carry out linkage analysis, assuming autosomal recessive inheritance with complete penetrance, and a damaging allele frequency of 10^{-4}. Parametric multipoint linkage analysis was carried out with the Merlin program (*40*), considering founders as second-degree relatives. The family founders and HapMap CEU trios were used to estimate allele frequencies and to define linkage clusters, with an r^2 threshold of 0.4.

Whole-exome sequencing:

The method used for whole-exome sequencing (WES) has been described elsewhere (*3,41*). Briefly, genomic DNA extracted from the patients' blood cells was sheared with a Covaris S2 Ultrasonicator (Covaris). An adapter-ligated library was prepared with the Paired-End Sample Prep kit V1 (Illumina). Exome capture was performed with the SureSelect Human All Exon kit (37 or 71 Mb version - Agilent Technologies). Single-end (for P1) or paired-end (P4 and P6) sequencing was performed on an Illumina Genome Analyzer IIx (Illumina), generating 72- or 100-base reads. We used BWA-MEM aligner (*42*) to align the sequences with the human genome reference sequence (hg19 build). Downstream processing was carried out with the Genome analysis toolkit (GATK) (*43*) SAMtools (*44*) and Picard Tools (http://picard.sourceforge.net). Substitution calls were made with GATK UnifiedGenotyper, whereas indel calls were made with SomaticIndelDetectorV2. All calls with a read coverage <2x and a Phred-scaled SNP quality of <20 were filtered out. Single nucleotide variants (SNV) were filtered on the basis of dbSNP135 (http://www.ncbi.nlm.nih.gov/SNP/) and 1000 Genomes (http://browser.1000genomes.org/index.html). All variants were annotated with ANNOVAR (*45*). All *RORC* mutations identified by WES were confirmed by Sanger sequencing. The sequence data are available at Sequence Read Archive (SRA) website (http://www.ncbi.nlm.nih.gov/sra) with following accession numbers (SRS964935, SRS965039, SRS965040, and SRS965042).

Immunoblotting and electrophoretic mobility shift assay (EMSA):

The mutant RORγ or RORγT expression plasmids were generated by site-directed mutagenesis of the pCMV6-RORC-Myc-DDK vector (Origene) and then used to transfect HEK293T cells, in the presence of Lipofectamine LTX (Life Technologies), according to the manufacturer's protocol. The transfectants were incubated with or

without PMA (25 ng/ml) and ionomycin (1 μg/ml) for 4 hours and subjected to immunoblot analysis or nuclear extraction for EMSA. Nuclear and cytoplasmic extracts were prepared with NE-PER Nuclear and Cytoplasmic Extraction Regents (Thermo Scientific), used according to the manufacturer's protocol. Immunoblot analysis was carried out as previously described (*46, 47*). Immunoblotting was performed with antibodies directed against amino acids 131-320 of RORγ (H-190: Santa Cruz), DDK (4C5: Origene), lamin A/C (H-110: Santa Cruz), β-tubulin (T5293: Sigma-Aldrich), β-actin (A5316: Sigma-Aldrich) and GAPDH (G-9: Santa Cruz). EMSA was carried out as previously described (*46*). We incubated 20 μg of nuclear extract with ^{32}P-labeled (a-dATP) RORE-1 probe (5'-gat cAT TCT TCT ATG ACC TCA TTG GGG G-3') or RORE-2 probe (5'- gat cAC CAA AAT GGT GTC ACC CCT GAA C -3'), designed on the basis of the conserved sequences of the *IL17A* promoter region (*48*), for 30 min.

Luciferase reporter assay:

Reporter assays were performed as previously described (*36*). The *IL17A* reporter plasmid, which contains 1125 bp upstream from the start codon of the *IL17A* gene (*49*), was kindly provided by George C. Tsokos (Harvard Medical School). Briefly, *IL17A* reporter plasmids (100 ng/well for a 96-well plate), pRL-SV40 vector (2 ng/well) and WT or mutant pCMV6-RORC-Myc-DDK plasmid (100 ng/well) were used to transfect HEK293T cells in the presence of Lipofectamine LTX. Twenty-four hours after transfection, cells were incubated with or without PMA (25 ng/ml) and ionomycin (1 μg/ml) for 4 hours and subjected to luciferase assays. Experiments were performed in triplicate and *IL17A* promoter activity is expressed as fold-induction relative to mock-transfected cells.

High-throughput Sequencing of *TRA/TRD* and *TRG* loci:

DNA was extracted from peripheral blood mononuclear cells obtained from healthy controls and *RORC*$^{-/-}$ patients. The CDR3 regions were amplified by multiplex PCR, using DNA as the template (Adaptive Biotechnologies/ImmunoSEQ, Seattle, WA) as previously described (*50-53*). Separate PCR reactions were used to amplify the *TRA/TRD* and *TRG* CDR3 regions. The amplicons were sequenced using the Illumina HiSeq platform. In this assay, primer concentrations and computational algorithms are used to correct for the primer bias common to multiplex PCR reactions. Raw sequence data was filtered for errors and aligned to the reference genome based on the TCRα/δ/γ V, D and J gene definitions provided by the IMGT database (www.imgt.org). The assay is quantitative, and the frequency of a given sequence is representative of the frequency of that clonotype in the original sample. Data was analyzed using the ImmunoSEQ analyzer toolset.

qPCR for *TRAV* usage:

Total RNA was prepared from the PBMCs of patients homozygous for *RORC* mutations, and heterozygous or WT family members. RNA was prepared from a total of 200,000 cells, with the RNeasy Micro kit, used according to the manufacturer's instructions (Qiagen). A mixture of random and OligodT primers was used with SuperScript reverse transcriptase (Life Technologies), to generate cDNA. Quantitative PCR was performed with the Light Cycler 480 Probes Master mix (Roche), the following

primers and a *C-alpha* FAM probe. Data were analyzed by the ΔΔCt method, with normalization of the results with respect to *C-alpha*. Primers: Vα18 conventional T cells *TRAV24* (CAC TCT TAA TAC CAA GGA GGG TTA C), Vα24 NKT cells *TRAV10* (CTG GAT GCA GAC ACA AAG CAA AGC), Vα7.2 MAIT cells *TRAV1.2* (CCT CCT TTT GAA GGA GCT CCA GAT G), Vα27 conventional T cells *TRAV39* (CCG TCT CAG CAC CCT CCA CA), Vα19 conventional T cells *TRAV41* (ACA CTG GCT GCA ACA GCA TCC AGG), *C-alpha* forward primer (ACC CTG ACC CTG CCG TGT), *C-alpha* reverse primer (CCT GCC CTG GGG AAG AAG GTGT TCT TC), *C-alpha* FAM probe (ACA AAA CTG TGC TGG ACA TGA GGT CTA TGG).

Antibodies:

PE-anti-CD4, Pacific Blue-anti-CD4, peridinin chlorophyll protein complex (PerCP)/cyanine 5.5-anti-CD45RA, Alexa-647-anti-IFNγ, PECy7-anti-CD117, and FITC-anti-CD45RA antibodies were purchased from eBiosciences. Alexa-647-anti-CXCR5, allophycocyanin-anti-CD38, FITC-anti-CD20, PE-anti-CD4, PerCP-anti-CD3 mAb, PerCP-anti-TCRVδ2, PE-anti-CD27 and streptavidin-PerCP were purchased from Becton Dickinson. Allophycocyanin-anti-CD4 antibody was purchased from Caltag, and FITC-anti-CCR7 antibody was purchased from R&D Systems. FITC-anti-human Lineage cocktail reagent (containing anti-CD3/14/19/20/56), Pacific Blue-anti-CD45, and APCCy7-anti human CRTh2 were purchased from BioLegend.

Ex vivo naïve and effector/memory $CD4^+$ T-cell stimulation:

$CD4^+$ T cells were isolated as previously described (*54*). Briefly, cells were labeled with anti-CD4, anti-CD45RA, and anti-CCR7 antibodies, and naive (defined as $CD45RA^+CCR7^+CD4^+$) T cells or effector/memory (defined as $CD45RA^-CCR7^-CD4^+$) were isolated (> 98% purity) with a FACS Aria (BD Biosciences). Purified naïve or effector/memory $CD4^+$ cells were cultured with T-cell activation and expansion beads (anti-CD2/CD3/CD28; Miltenyi Biotec) for 5 days, and culture supernatants were then used to assess the secretion of the cytokine indicated, by ELISA (IL-22) or cytometric bead array (all other cytokines).

In vitro differentiation of naïve $CD4^+$ T cells:

Naïve $CD4^+$ T cells (defined as $CD45RA^+CCR7^+$ $CD4^+$) were isolated (> 98% purity) using a FACS Aria (BD Biosciences) from healthy controls or patients. They were then cultured under polarizing conditions, as previously described (*54*). Briefly, cells were cultured with T-cell activation and expansion beads (anti-CD2/CD3/CD28; Miltenyi Biotec) alone or under Th1 (IL-12 [20 ng/ml; R&D systems]) or Th17 (TGFβ, IL-1β [20 ng/ml; Peprotech], IL-6 [50 ng/ml; PeproTech], IL-21 [50 ng/ml; PeproTech], IL-23 [20 ng/ml; eBioscience], anti-IL-4 [5 μg/ml], and anti-IFN-γ [5 μg/ml; eBioscience]) polarizing conditions. After 5 days, culture supernatants were used to assess secretion of the cytokines indicated, by ELISA (IL-22) or cytometric bead array (all other cytokines).

Peripheral blood mononuclear cell (PBMC) activation experiments:

PBMCs were isolated from buffy coats and used at a density of 10^6/ml in RPMI medium supplemented with 10% FBS. Cells were treated with either recombinant (rh)IL-

12 (20 ng/ml; R&D Systems), live BCG (*M. bovis* BCG, Pasteur substrain) at a MOI of 20 BCG cells/leukocyte, or with BCG plus IL-12. ELISA for IFN-γ was performed 48 h after stimulation.

Whole-blood activation experiments:

Venous blood samples were collected into heparin-containing tubes. They were diluted 1:2 in RPMI 1640 (GibcoBRL) supplemented with 100 U/ml penicillin and 100 µg/ml streptomycin (GibcoBRL). Six ml of the diluted blood samples were dispensed into each of 4 wells (1.5 ml/well) of a 48-well plate (Nunc). These samples were incubated for 48 hours at 37°C, under an atmosphere containing 5% CO_2/95% air, and under three different sets of activation conditions: with medium alone, with live BCG (*M. bovis*-BCG, Pasteur substrain) at a MOI of 20 BCG cells/leukocyte, and with BCG plus recombinant (rh)IL-12 (20 ng/ml; R&D Systems). All venous blood samples were collected in accordance with IRB protocols.

Quantitative RT-PCR:

RNA was isolated from PBMC or *Herpesvirus saimiri*-transformed T cells using TRIzol, according to manufacturer's protocol (Invitrogen, Carlsbad, CA). The RNA was DNase-treated (Roche, Mannheim, Germany) and cleaned by passage through RNeasy column (Qiagen). Reverse transcriptase PCR was performed using oligo-dT primers according to the manufacturer's protocol (Applied Biosystems, Foster City, CA). Quantitative real-time PCR (qPCR) was performed with Applied Biosystems Taqman assays using exon-spanning probes, and normalized to the β-glucuronidase (GUS), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), or 18S housekeeping genes.

Retrovirus production and transduction of T cell lines:

T cell lines were generated by infecting healthy control or patient PBMC with *Herpesvirus saimiri*, as previously described (*55*). retroviral vectors pLZRS-IRES-ΔNGFR (empty vector) pLZRS-IRES-*RORCiso2*-ΔNGFR (encoding WT *RORC* isoform 2), both including a purocycim resistance cassette, were generated as previously described (*56, 57*). The ΔNGFR ORF encodes a truncated nerve growth factor receptor (NGFR, also known as CD271) protein that cannot transduce signal, and in this system serves as a cell surface tag. Briefly, 10µg of vector pLZRS-IRES-ΔNGFR or pLZRS-IRES-*RORCiso2*-ΔNGFR were transfected into Phoenix-A packaging cells using Roche X-treme gene 9 (Life Science) following manufacturer specifications. Positively transfected cells were selected with puromycin (Gibco) at a concentration of 2µg/ml until all of them where positive for the surface expression of ΔNGFR as assessed by FACS with PE-anti-NGFR staining (BD Pharmingen). Phoenix-A were then split in two flasks, and media was replaced. After 24 hours, supernatant was collected and retroviral particles were concentrated using Retro-X concentrator (Clontech) following manufacturer instructions. One million *herpesvirus saimiri*-T cells were mixed with retrovirus-containing supernatant in a total volume of 2ml. 24h after, 200µl of FCS were added and the cells allowed to grow for 4 days. Transduced cells were purified by MACS using magnetic bead-conjugated anti-NGFR antibody (Miltenyi Biotec) following manufacturer's protocol.

Amplified T-cell libraries:

Three subsets of $CD45RA^{-}CD25^{-}CD19^{-}CD8^{-}CD4^{+}$ memory T cells were sorted from total PBMCs with a FACS Aria (BD Biosciences), excluding $CD45RA^{+}CCR7^{+}$ naïve cells, and on the basis of CCR6, CXCR3 and CCR4 expression. The $CCR6^{+}$ subset including T cells producing IL-17 and IFN-γ (*25*) were collected. The $CCR6^{-}$ cells were further divided to obtain a $CXCR3^{+}CCR4^{-}$ subset (enriched in cells phenotypically resembling Th1), and a $CCR4^{+}CXC3R3^{-}$ subset (enriched in Th2 cells). The cells were cultured in RPMI 1640 medium supplemented with 2 mM glutamine, 1% (vol/vol) non-essential amino acids, 1% (vol/vol) sodium pyruvate, penicillin (50 U/ml), streptomycin (50 µg/ml) (all from Invitrogen) and 5% heat-inactivated human serum (Swiss Red Cross). The sorted memory T cells (500 cells/well) were polyclonally stimulated with 1 µg/ml PHA (Remel) in the presence of irradiated (45Gy) allogeneic feeder cells (2.5×10^4 per well) and IL-2 (500 IU/ml), in a 96-well plate, and T cell lines were expanded as previously described (*58*). Library screening was performed 14-21 days after initial stimulation, by culturing thoroughly washed T cells (2.5×10^5/well) with autologous irradiated B cells (2.5×10^4), with or without a three-hour pulse with different antigens, including the *M. tuberculosis* peptide pool (0.5 µg/ml/peptide, from A. Sette's laboratory, LJI, comprising 207 peptides), the BCG peptide pool (0.5 µg/ml/peptide, from A. Sette's laboratory, LJI, comprising 211 peptides), *Candida albicans* lysate (5 µg/ml from F. Sallusto's laboratory), or tetanus toxoid (TT, 5 µg/ml, from Novartis). Proliferation was assessed on day 4, after incubation for 16 h with 1 µCi/ml [^{3}H]-thymidine (GE Healthcare). Cytokine concentrations in the culture supernatants were measured after 48 h of stimulation, with Cytometric Bead Arrays (eBioscience). Precursor frequencies were calculated from the number of negative wells, assuming a Poisson distribution, and are expressed per million cells.

Mouse infections:

Male mice lacking the capacity to make RorγT as a result of the insertion of an EGFP coding sequence into the alternative first exon (exon 1γt) of the *Rorc* locus, (*14*) herein referred to as $Rorc^{-/-}$ mice, were a kind gift of Dr Dan Littman; $Rorc^{-/-}$, $Rorc^{+/-}$ and C57BL/6J mice were bred at Trudeau Institute, Inc. $Rorc^{+/-}$ and C57BL/6J mice had similar results and were combined and referred to as control mice. Mice were infected via the aerosol route with either *M. tuberculosis* H37Rv (dose of approx 450 CFU) or *M. bovis*-BCG (Pasteur) (dose of approx 1220) as previously described (*59*). Mice were monitored for weight and health and euthanized when 20% weight loss occurred. Some mice were euthanized and the bacterial burden, in colony forming units (CFU), in the lung and spleen determined as previously described (*59*).

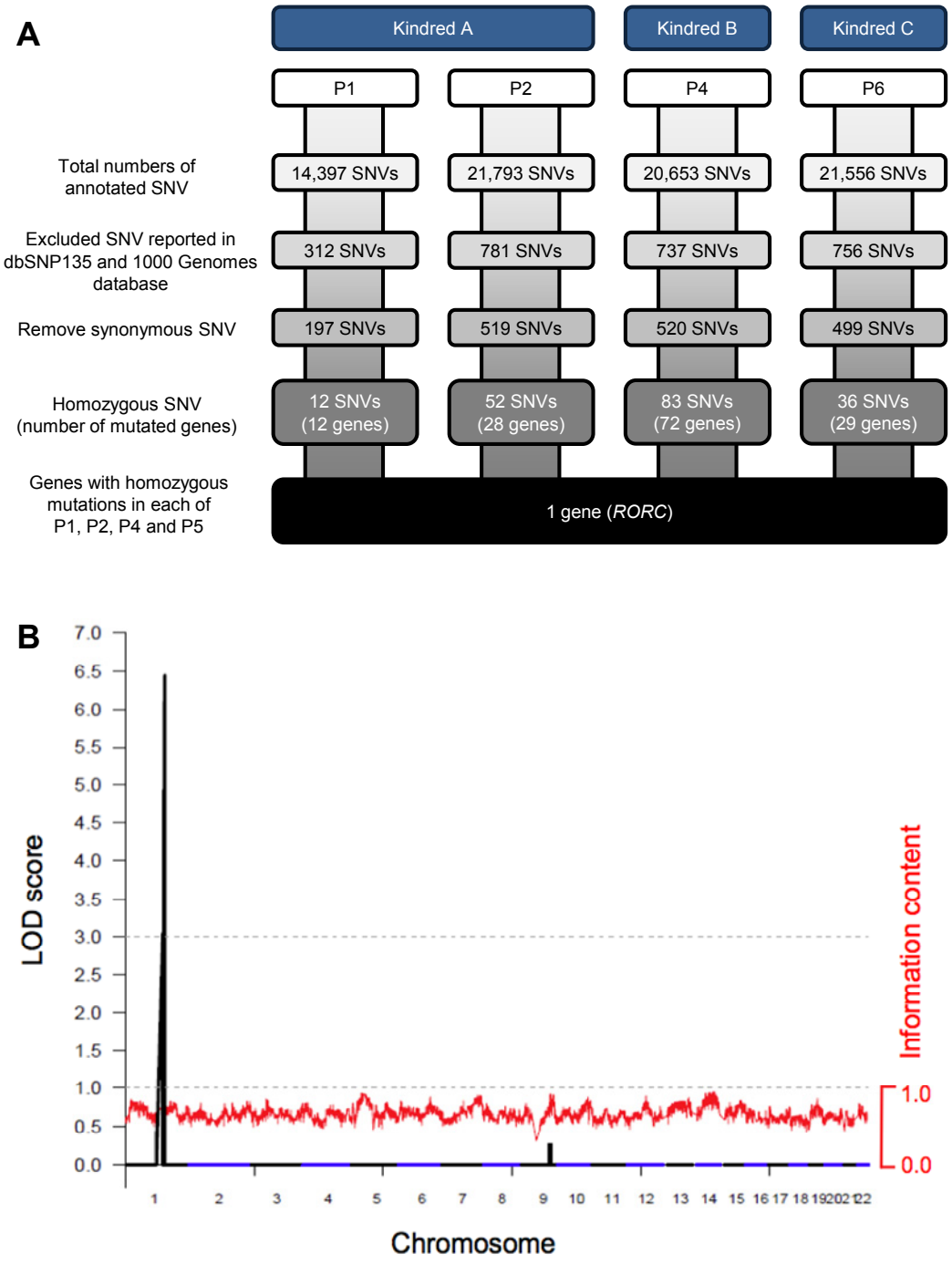

Figure S1. Genome-wide analyses by exome sequencing and linkage.

(A) An analysis of whole-exome sequencing (WES) data identified *RORC* as the only gene carrying homozygous coding mutations in each of P1, P2, P4, and P6. (B) Genome-wide linkage analysis of Kindreds A, B and C. SNP genotyping was performed on DNA

from 10 members of Kindred A, 2 members of Kindred B, and 5 members from Kindred C. After filtering, 562,679 SNP markers were used for parametric multipoint linkage analysis, assuming an autosomal recessive genetic etiology of CMC and MSMD, with complete penetrance, and a damaging allele frequency of 10^{-4}. LOD scores are shown in black and blue for alternate chromosomes, and information content is shown as a red trace. The linkage region with the greatest LOD score was on chromosome 1, and contained *RORC*.

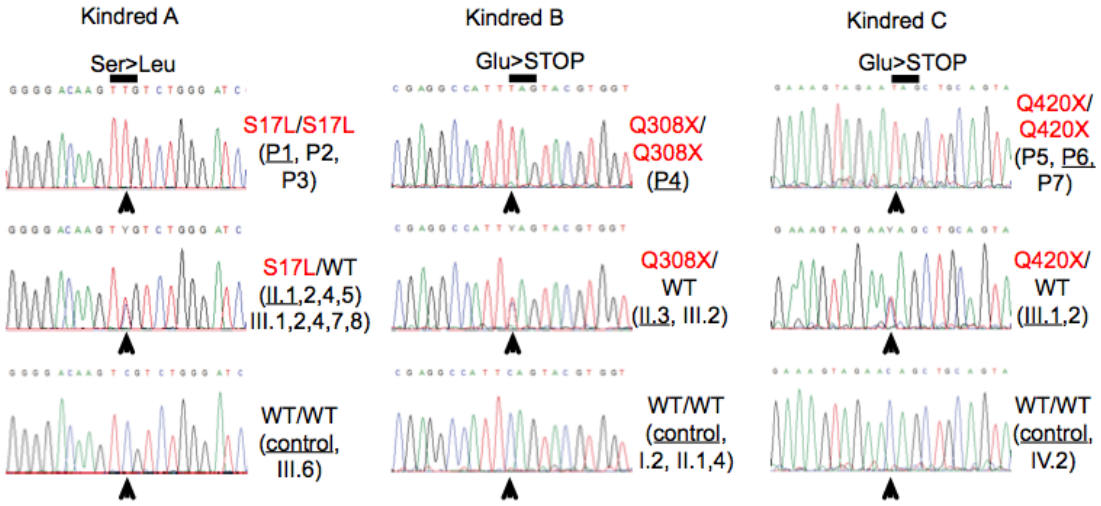

Figure S2. Sanger sequencing of *RORC* mutations in 3 kindreds.

Sanger sequencing of the *RORC* mutations responsible for the S17L, Q308X, and Q420X forms of the RORγT protein, from patients and healthy relatives in Kindreds A, B, and C, respectively (refer to Fig. 1A for individual identifiers).

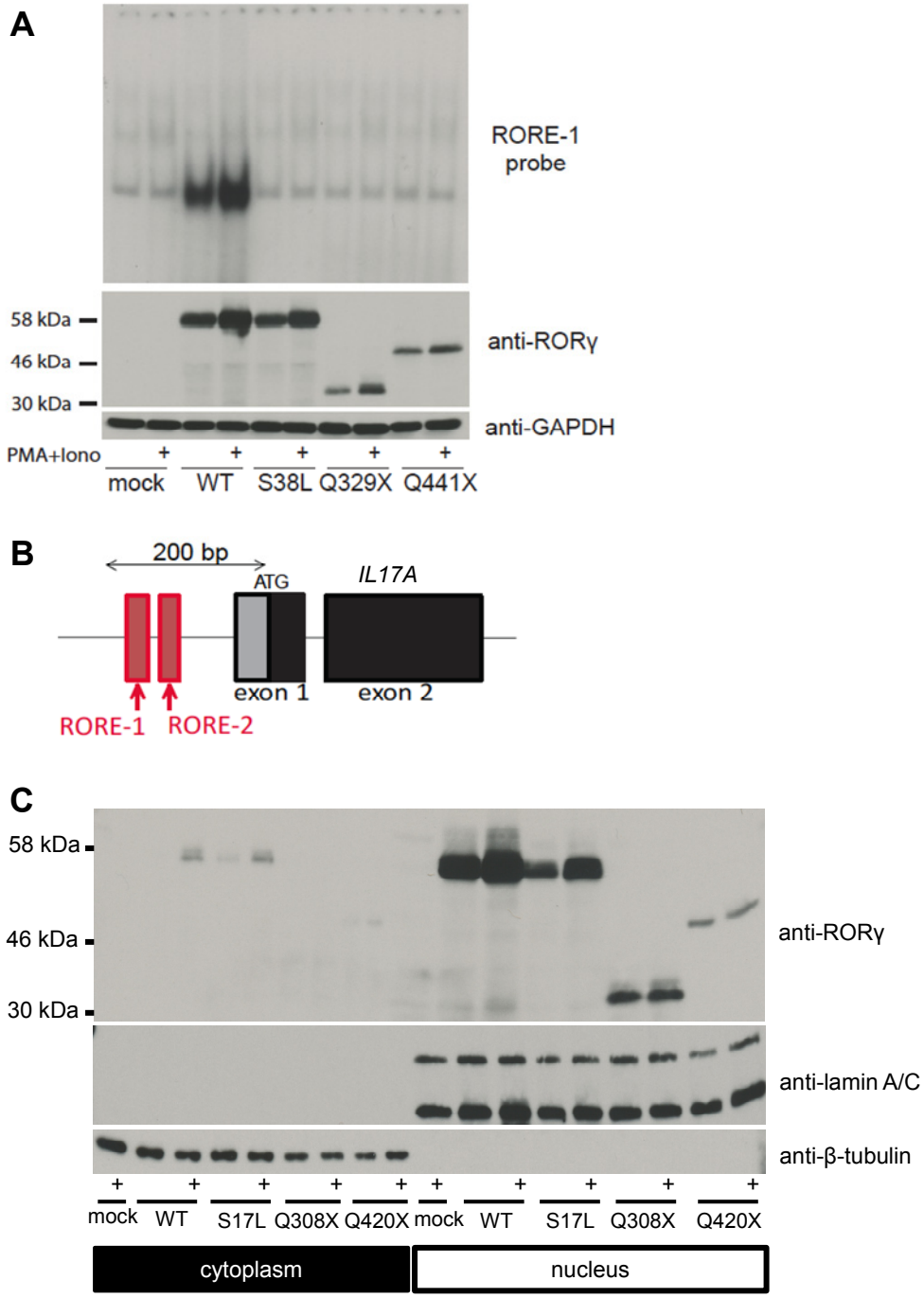

Figure S3. Analysis of RORγ protein expression and function.

(**A**) HEK293T cells were either mock-transfected or transfected with plasmids encoding the WT, S38L, Q329X, or Q441X forms of RORγ. After 24 hours, cells were either left untreated or were stimulated with PMA (25 ng/ml) and ionomycin (1 μg/ml) for 4 hours. Whole-cell lysates were subjected to western blotting (**lower panel**) and nuclear lysates

were subjected to EMSA with the ^{32}P-labeled RORE-1 probe derived from the *IL17A* promoter sequence (**upper panel**). (**B**) Graphical representation of the location of the RORE-1 and RORE-2 sequences used to generate probes for EMSA. (**C**) HEK293T cells were either mock-transfected or transfected with plasmids encoding the WT, S17L, Q308X, or Q420X forms of RORγT. After 24 hours, cells were either left untreated or were stimulated with PMA (25 ng/ml) and ionomycin (1 μg/ml) for 4 hours, and nuclear and cytoplasmic extracts were obtained for immunoblotting.

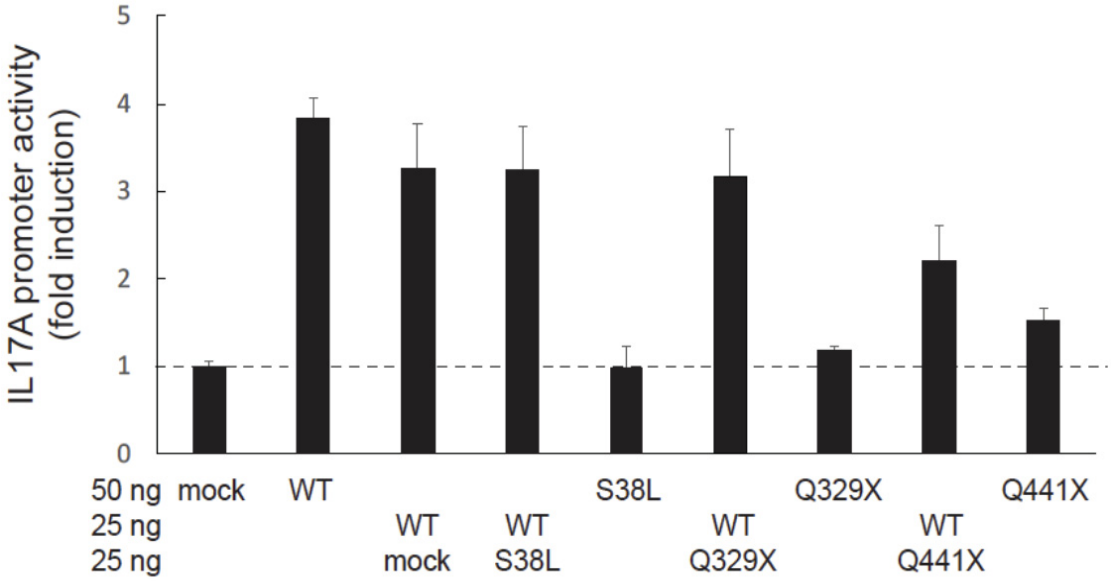

Figure S4. RORγ proteins encoded by mutant alleles are loss of function.

Luciferase reporter assays were performed with the *IL17A* reporter plasmid. *IL17A* reporter plasmids (100 ng/well), pRL-SV40 vector (2 ng/well) and plasmids encoding the S38L, Q329X, or Q441X forms of RORγ (50 ng/well) were used to transfect HEK293T cells. After 24 hours, cells were stimulated with PMA (25 ng/ml) and ionomycin (1 μg/ml) for 4 hours, then subjected to luciferase assays. Experiments were performed in triplicate, and *IL17A* promoter activity is expressed as fold-induction relative to mock-transfected cells. Error bars represent SEM.

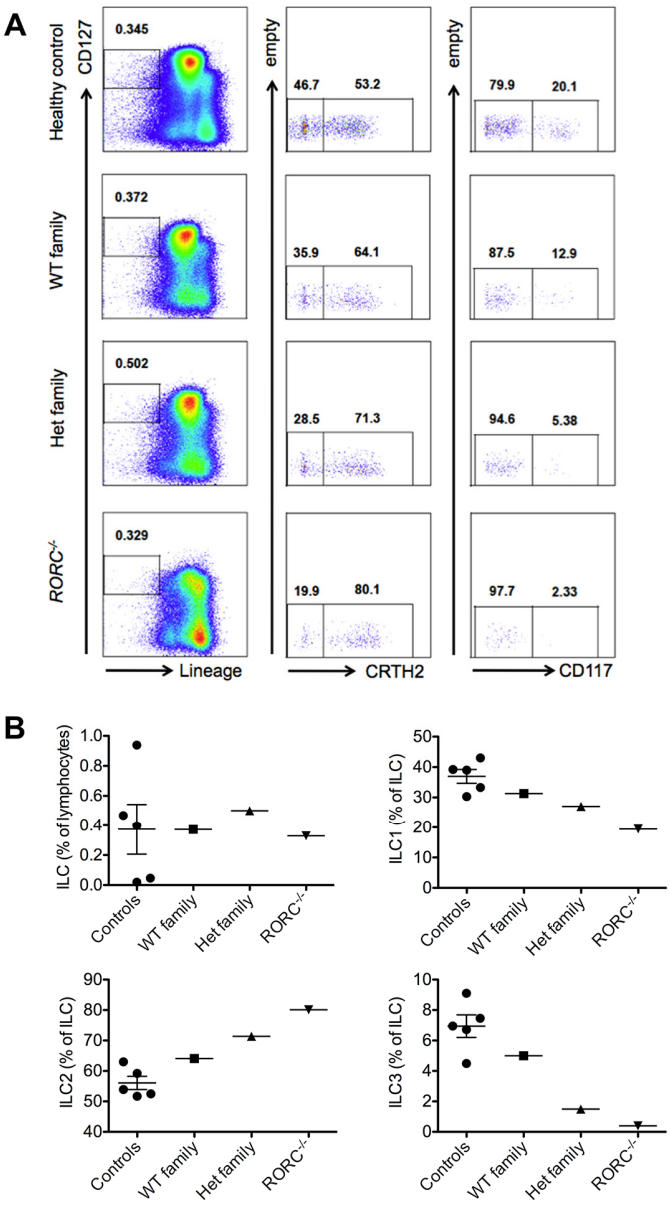

Figure S5. RORγT deficient patients show barely detectable ILC3.

(**A**) Flow cytometric analysis of innate lymphoid cell (ILC) populations in healthy donor, WT family, heterozygous family, and *RORC*$^{-/-}$ patients' PBMC. Total PBMC were gated on the exclusion of a cell viability dye, forward and side scatter properties, and CD45+. A lineage antibody cocktail and CD127 were used to define total ILC. Within this gate, CRTH2+ cells were considered ILC2 and CRTH2- cells were further subdivided into ILC1 and ILC3 subsets by expression of CD117. Plots for WT family, Het family and *RORC*$^{-/-}$ represent the 2 independent samples, concatenated following acquisition to enable visualization of very rare populations. (**B**) Frequency of ILC in *RORC*$^{-/-}$ patients relative to controls. Total ILC as well as ILC subsets were defined by flow cytometry as in (A). The frequency of total ILC, and the percentage of ILC consistent with the surface

phenotypes for ILC1, ILC2 and ILC3 are shown. Results for WT family, Het family and *RORC*$^{-/-}$ represent the 2 independent samples, concatenated following acquisition to enable accurate determination of the frequency of very rare populations.

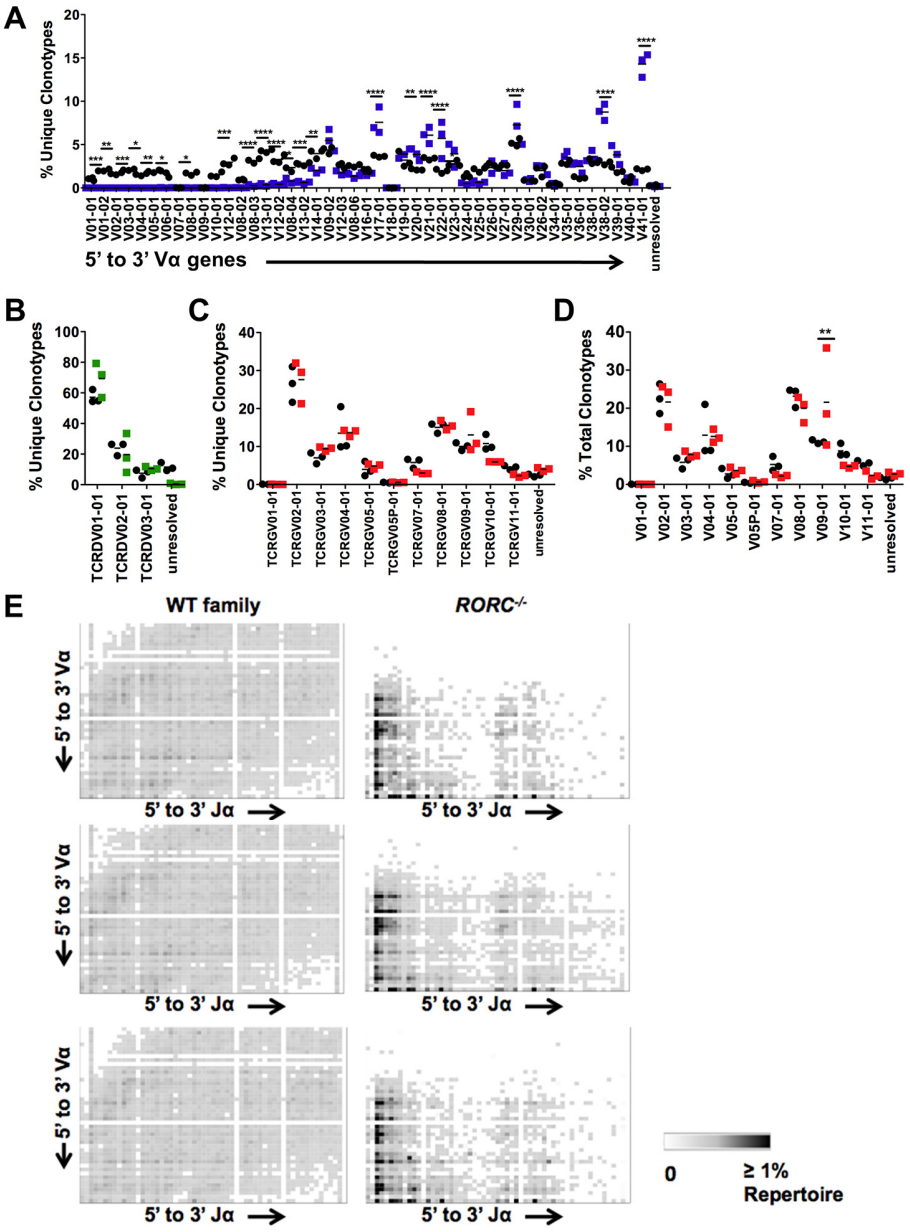

Figure S6. RORγT deficient patients show abnormal TCR repertoires.

(**A**) Impaired 5' Vα usage in *RORC*$^{-/-}$ patients. The percentage of unique clonotypes expressing each *TRAV* gene is plotted respectively for WT family members (n=3, black symbols) and *RORC*$^{-/-}$ patients (n=3, blue symbols). (**B**) The percentage of unique clonotypes expressing each *TRDV* gene is plotted respectively for WT family members (n=3, black symbols) and *RORC*$^{-/-}$ patients (n=3, green symbols). (**C**) The percentage of unique clonotypes expressing each *TRGV* gene is plotted respectively for WT family members (n=3, black symbols) and *RORC*$^{-/-}$ patients (n=3, red symbols). (**D**) *TRGV* usage is skewed in *RORC*$^{-/-}$ patients with overuse of *TRGV9*. The percentage of total clonotypes

expressing each *TRGV* gene family is plotted respectively for WT family members (n=3, black symbols) and *RORC*$^{-/-}$ patients (n=3, red symbols). (**E**) Impaired 5' Vα and 3' Jα usage and pairing in RORγT deficiency. DNA was extracted from PBMC of 3 WT family members and 3 *RORC*$^{-/-}$ patients. Separate PCR reactions were used to amplify the *TRA/TRD* and *TRG* CDR3 regions, and the amplicons were sequenced as described in Materials and Methods. Vα and Jα pairing in unique clonotypes of a healthy control and patient with RORγT deficiency are depicted in the heat maps. White represents an absence of a given Vα and Jα pairing. Light gray reflects a low frequency while black represents a higher frequency of use. Vα, Vδ and Vγ usage in controls and patients was compared with 2-way ANOVA with Bonferroni correction for multiple comparisons, *p ≤0.05, ** p ≤ 0.01, *** p ≤ 0.001, ****p ≤ 0.0001.

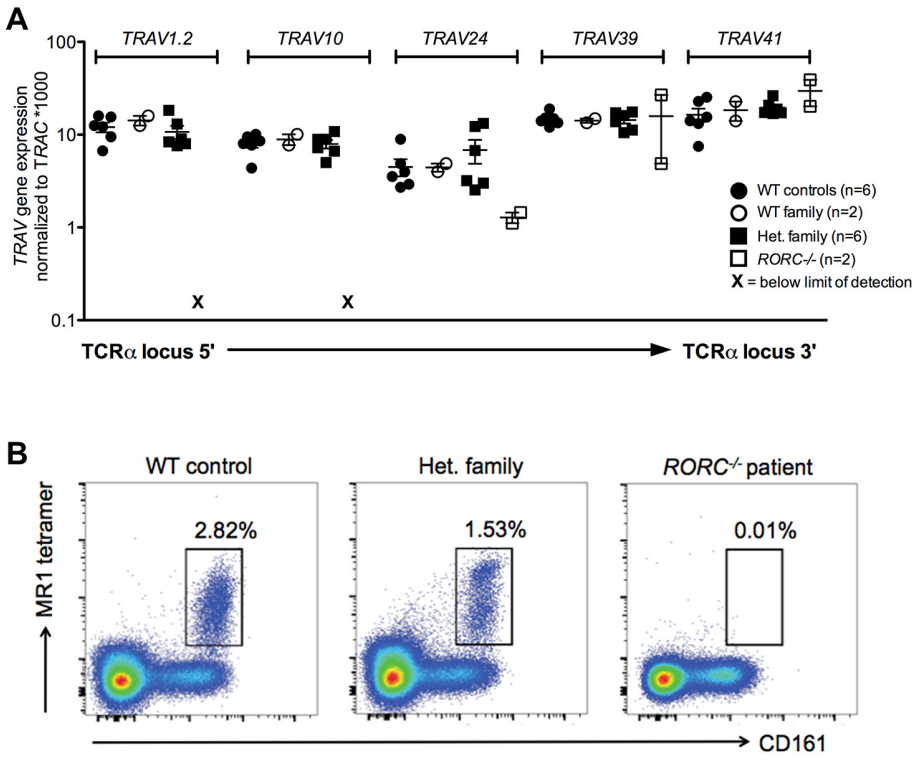

Figure S7. qPCR and FACS validation of absent MAIT cells in *RORC*$^{-/-}$ patients. (**A**) Quantitative PCR of TCR Vα segments, *TRAV1.2, TRAV10*, *TRAV24*, *TRAV39* and *TRAV41* normalized with respect to the level of *TRAC* (encoding Cα) in PBMCs from healthy controls (*n*=6), WT family members (*n*=2), heterozygous family members (*n*=8) and *RORC*$^{-/-}$ patients (*n*=2). (**B**) MAIT cells recognize vitamin B metabolites bound to MHC-related molecule 1 (MR1) (*60*) and can be identified more specifically with tetramers of MR1 complexed with 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil 5-OP-RU (*61, 62*). An analysis with tetramers confirmed the absence of MAIT cells in *RORC*$^{-/-}$ patients. PBMC were gated on FSC and SSC profile and CD3 expression, then MAIT cells were identified with modified human major histocompatibility complex (MHC)-related class I-like molecule (MR1) tetramers loaded with 5-OP-RU (*62*). Tetramer staining showed the presence of MAIT cells in a WT control and a heterozygous family member, but not in an *RORC*$^{-/-}$ patient.

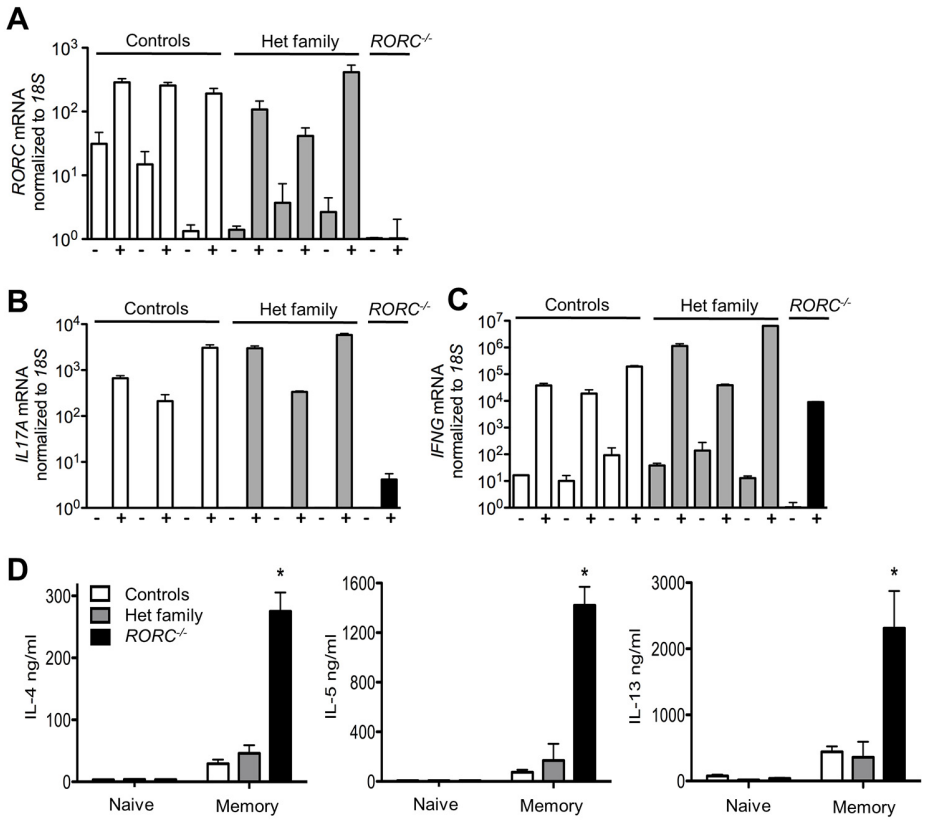

Figure S8. PBMC of *RORC*$^{-/-}$ patients show intact induction of *IFNG* but diminished *IL17A* in response to a polyclonal stimulus.

(**A**) PBMC of 3 WT controls (white bars), 3 heterozygous family members (grey bars) and 1 *RORC*$^{-/-}$ patient (black bars) were stimulated (+) or not (-) with PMA and ionomycin for 6 hours, then total RNA was extracted and used for qRT-PCR for total *RORC*, as described in Materials and Methods. Total *RORC* expression was normalized to 18S. (**B**) Expression of *IL17A* was assessed, as in (A). (**C**) *IFNG* expression was tested as in (A). (**D**) Cytokine production by CD4^{+} T cells from *RORC*$^{-/-}$ patients. Naïve and memory CD4^{+} T cells from WT controls (n=7), heterozygous family members (n=2) and *RORC*$^{-/-}$ patients (n=3) were purified (>98% purity) by FACS, cultured with TAE beads (anti-CD2/CD3/CD28) for 5 days, and the culture supernatants were then used to evaluate the secretion of the cytokine indicated, with cytometric bead arrays.*p<0.05 versus WT controls, in two-tailed Mann-Whitney tests.

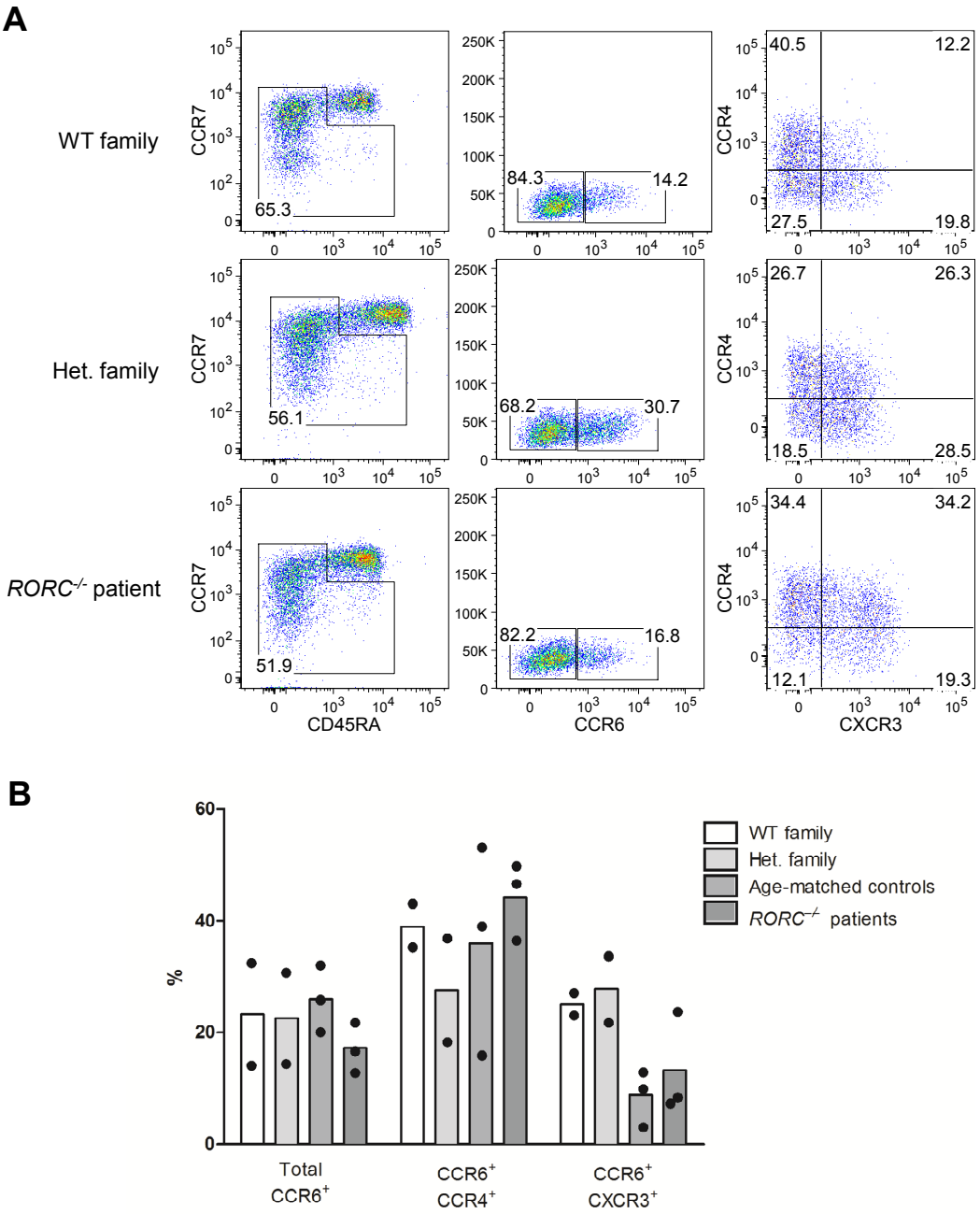

Figure S9. Sorting of CD4$^+$ memory subsets for proliferation assays.

(A) T cells from the PBMCs of one WT family member, one heterozygous family member, and one *RORC*$^{-/-}$ patient were sorted on the basis of a hierarchical gating strategy: exclusion of a live/dead stain, forward and side scatter properties, CD3$^+$CD4$^+$,

followed by exclusion of $CD45RA^{+}CCR7^{+}$, and then sorting on the basis of CCR6 expression. The $CCR6^{+}$ cells were collected altogether. The $CCR6^{-}$ cells were subdivided into a $CXCR3^{+}CCR4^{-}$ subset (enriched in cells phenotypically resembling Th1 cells) and a $CXCR3^{-}CCR4^{+}$ subset (enriched in Th2 cells). (B) The frequencies of memory T cells defined by expression of the chemokine receptors shown in (A) were assessed in WT family members (n=2), heterozygous family members (n=2), age-mathed controls (n=3) and *RORC*$^{-/-}$ patients (n=3).

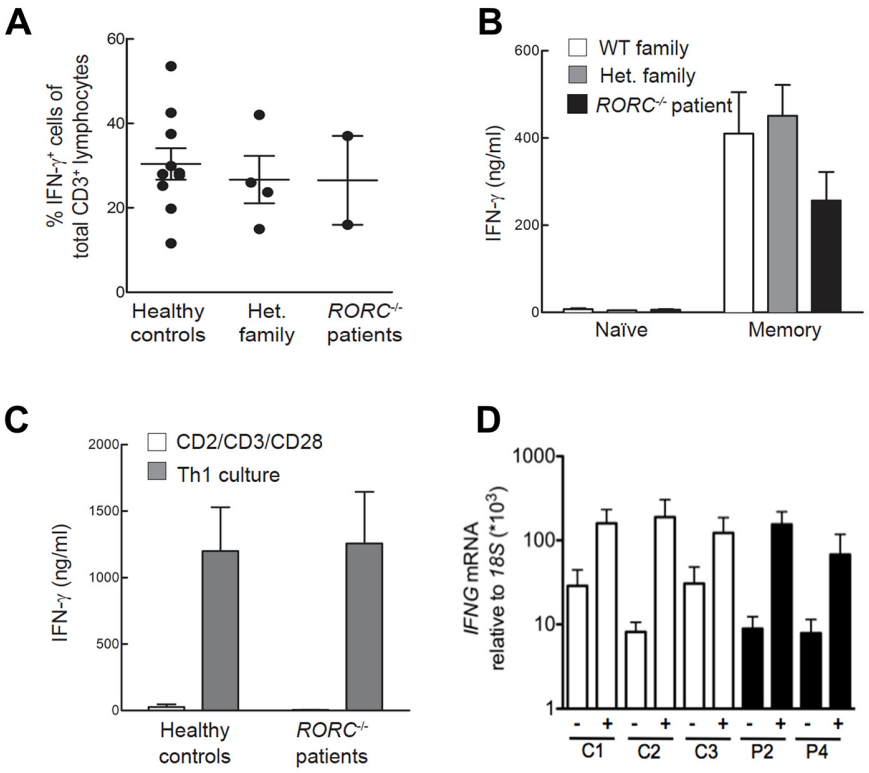

Fig S10. *RORC*$^{-/-}$ patients' T cells respond to polyclonal stimulation with normal IFN-γ production.

(**A**) Whole blood from healthy WT donors, heterozygous family members, or *RORC*$^{-/-}$ patients was activated by incubation with PMA (25 ng/ml) and ionomycin (1 μg/ml), as in Fig. 3A, then assessed by intracellular flow cytometry, for the production of IFN-γ. (**B**) Naïve and memory CD4^{+} T cells from WT controls (*n*=7), heterozygous family members (*n*=2) and *RORC*$^{-/-}$ patients (*n*=3) were purified (>98% purity) by FACS, cultured with TAE beads (anti-CD2/CD3/CD28) for 5 days, and the culture supernatants were then assessed for IFN-γ secretion, with cytometric bead arrays. (**C**) IFN-γ production by *in vitro*-differentiated CD4^{+} T cells from control donors and *RORC*$^{-/-}$ patients. Naïve CD4^{+} T cells were purified from the PBMCs of WT controls (*n*=6) or *RORC*$^{-/-}$ patients P2, P3 and P4, then cultured for 5 days with either TAE beads (anti-CD2/CD3/CD28) alone or with IL-12. After 5 days, culture supernatants were assessed for IFN-γ secretion with cytometric bead arrays. (**D**) *Herpesvirus saimiri*-transformed T cells from healthy donors (white bars) or *RORC*$^{-/-}$ patients (black bars) were cultured in the presence (+) or absence (-) of PMA and ionomycin for 6 hours, then total RNA was extracted and used for qRT-PCR for *IFNG*, as described in Materials and Methods. Total *RORC* expression was normalized to 18S.

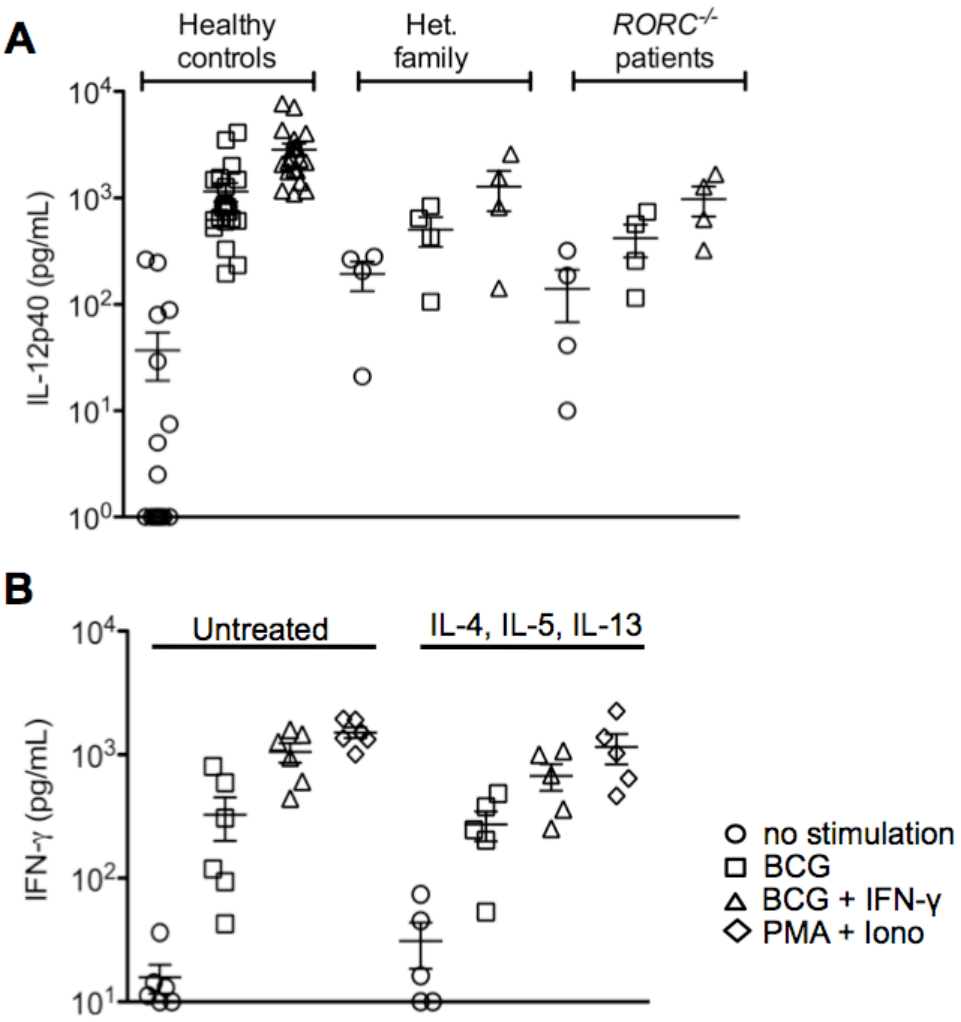

Figure S11. *RORC*$^{-/-}$ patients respond to mycobacterial challenge by producing normal amounts of IL-12p40.

(**A**) Whole-blood cells from healthy controls (*n*=20), heterozygous family members (*n*=4) and *RORC*$^{-/-}$ patients (*n*=4), were left unstimulated or were stimulated with BCG alone or BCG plus IFN-γ for 48 h. ELISA was then used to determine IL-12p40 levels in the culture supernatants. (**B**) IL-4, IL-5 and IL-13 do not impair *in vitro* production of IFN-γ following BCG and BCG + IL-12 stimulation. PBMC from 5 healthy donors were stimulated with BCG, BCG + IL-12 or PMA + ionomycin in the presence or absence of IL-4, IL-5 and IL-13 (10ng/mL each cytokine) and cell culture supernatant was tested by ELISA 48 h later. No differences in IFN-γ production were observed in as a consequence of the addition of IL-4, IL-5, and IL-13.

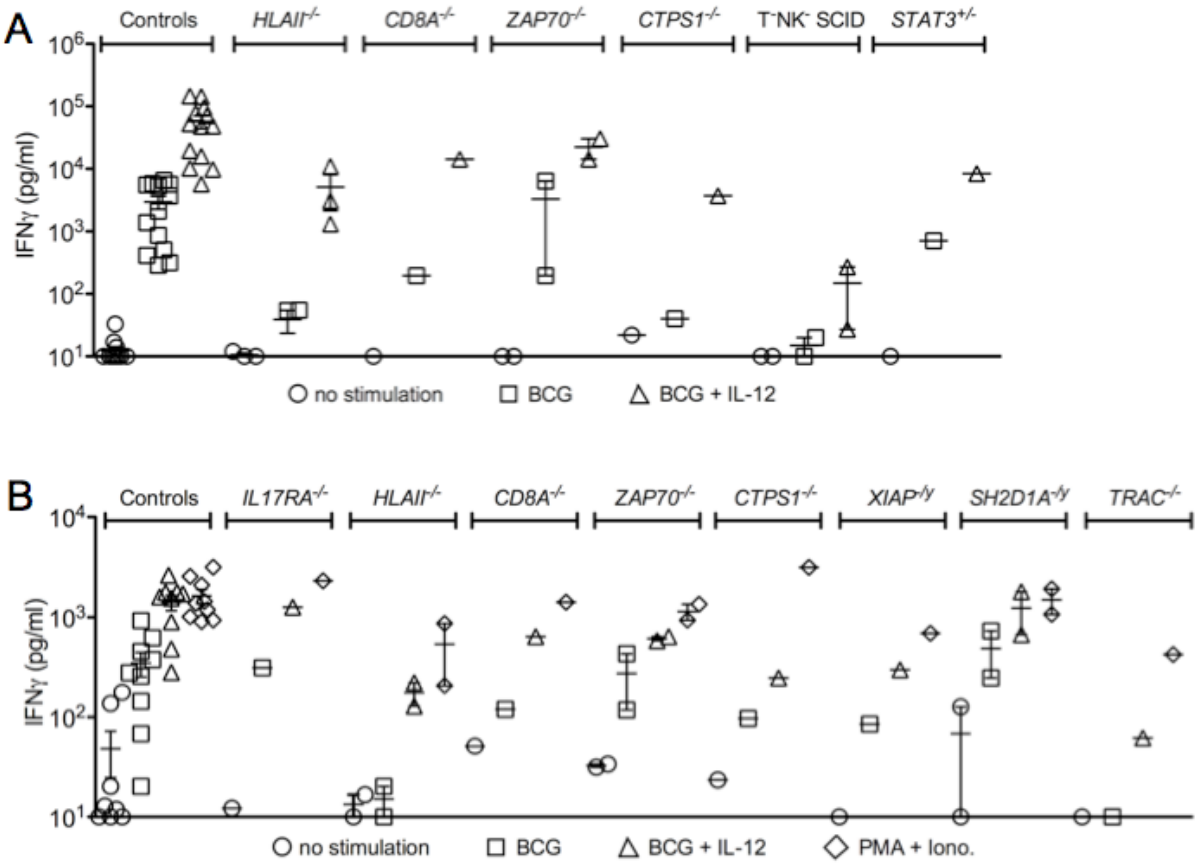

Figure S12. Assessment of IFN-γ response in whole blood or PBMC of patients with other primary immunodeficiencies affecting T cell development or function.

(**A**) Whole blood from healthy controls (n=11), HLA-II deficient patients (*HLAII*$^{-/-}$, n=3 (*28*)), a CD8α deficient patient (*CD8A*$^{-/-}$ (*31*)), ZAP70 deficient patients (*ZAP70*$^{-/-}$, n=2, (*34*)), a CTPS1 deficient patient (*CTPS1*$^{-/-}$ (*33*)), patients with T-NK- Severe Combined Immunodeficiency (*30*) and a STAT3 autosomal dominant patient (*STAT3*$^{+/-}$ (*63*)) were stimulated for 48 hours as previously described, and IFN-γ was measured in culture supernatant. (**B**) PBMC from healthy controls (n=11), IL-17RA deficient (*IL17RA*$^{-/-}$ (*1*)), XIAP deficient (*XIAP*$^{-/y}$, n=1, (*64*), SAP deficient (*SH2D1A*$^{-/y}$, n=1, (*65*), TCRα deficient (*TRAC*$^{-/-}$ (*33*)) patients as well as PBMC from patients with several of the primary immunodeficiencies described in (A), (*HLAII*$^{-/-}$, n=2 (*28*); *CD8A*$^{-/-}$ n=1 (*29*)) were stimulated for 48 hours as previously described, and IFN-γ was measured in culture supernatant.

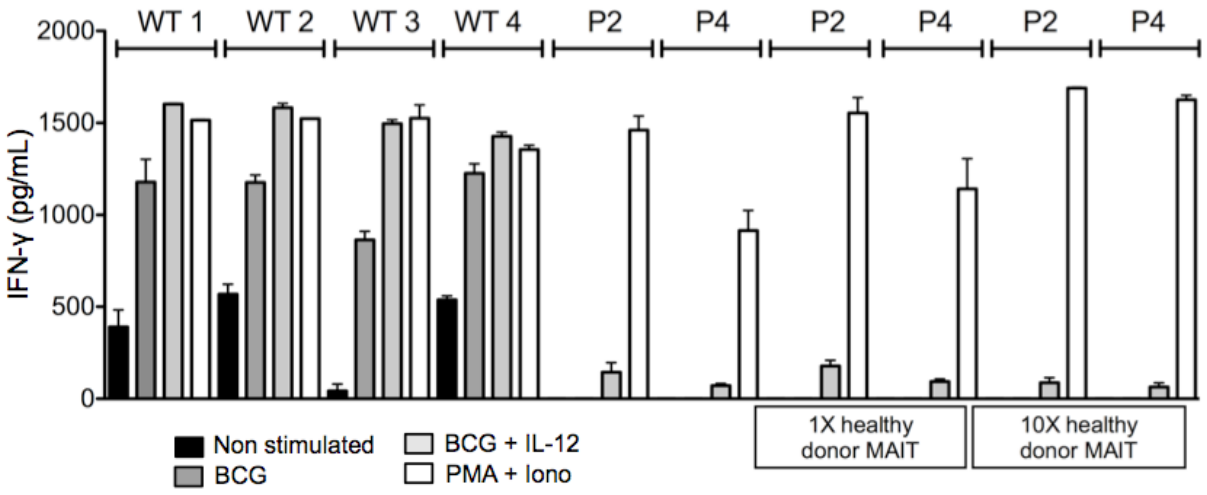

Figure S13. MAIT cells do not rescue the impaired production of IFN-γ by *RORC*$^{-/-}$ PBMCs in response to Mycobacteria.

We assessed the possible contribution of MAIT cells to the observed IFN-γ defect of the PBMCs of patients with *RORC* mutations (Fig. 5B), by stimulating equal numbers of WT healthy control PBMCs (WT 1-4, *n*=4), PBMCs from P2 or P4 alone, or PBMCs from P2 or P4 supplemented with the orthotopic frequency of MAIT (1X MAIT) or 10 times the normal frequency of MAIT (10X MAIT), for 48 hours with BCG, BCG plus IL-12, or PMA and ionomycin. The concentration of IFN-γ in the culture supernatant was determined by ELISA.

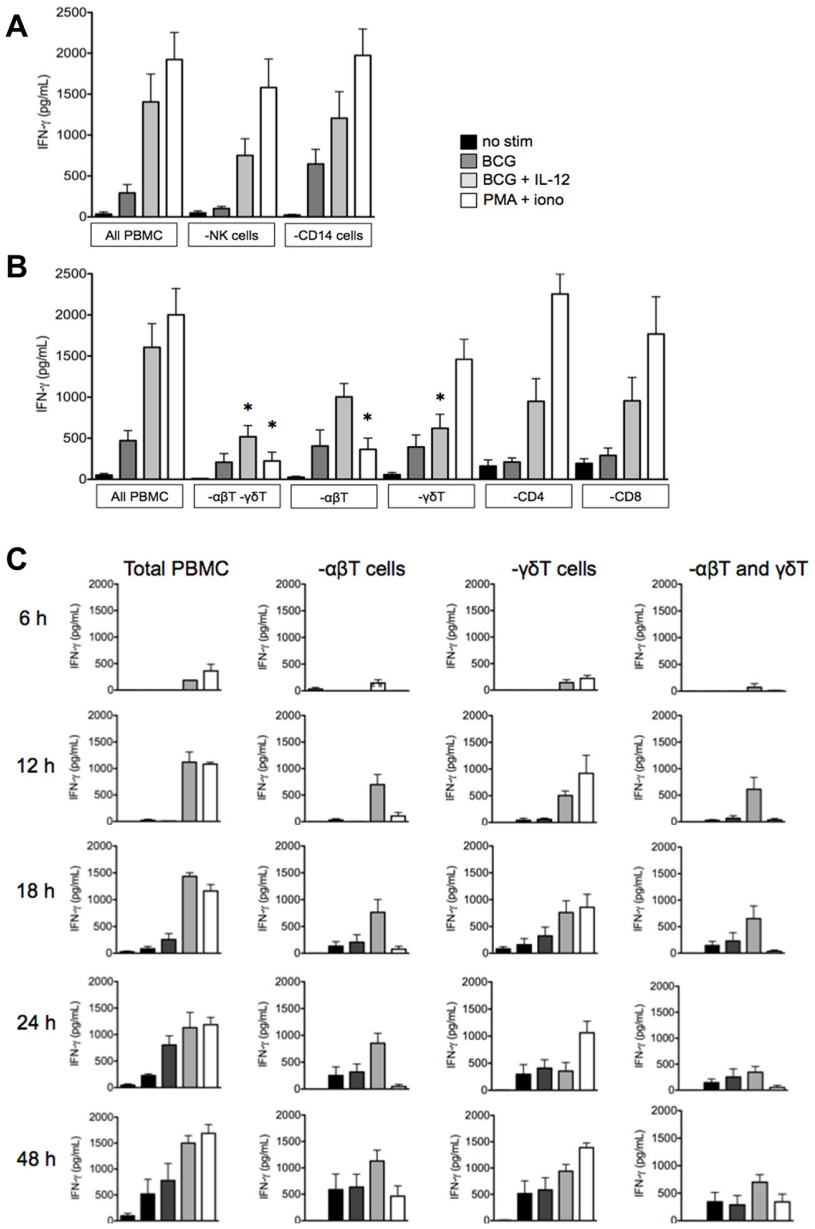

Figure S14. Depletion of αβT cells, or γδT cells, or both leads to decreased IFN-γ production in response to BCG + IL-12.

(**A**) PBMC from healthy controls (n=5-8) were labeled with magnetic bead-conjugated antibodies against CD56 (in the case of NK depletion) or CD14, then separated by magnetic activated cell sorting (MACS). The purity of each population was assessed by flow cytometry with antibodies directed against the same markers, and each population was confirmed to be >96% negative for the marker indicated. 200,000 cells depleted of the indicated population, or unfractionated cells (total PBMC) were cultured in the presence of live BCG, BCG plus IL-12, or PMA plus ionomycin, for 48 hours. IFN-γ levels in culture supernatants were assessed by ELISA. (**B**) PBMC from healthy controls

(n=5-8) were labeled with magnetic bead-conjugated antibodies against αβTCR, γδTCR, CD4, or CD8, or co-stained with antibodies against αβTCR and γδTCR, then separated by magnetic activated cell sorting (MACS). The purity of each population was assessed by flow cytometry with antibodies directed against the same markers, and each population was confirmed to be >96% negative for the marker indicated. 200,000 cells depleted of the indicated population, or unfractionated cells (total PBMC) were cultured in the presence of live BCG, BCG plus IL-12, or PMA plus ionomycin, for 48 hours. IFN-γ levels in culture supernatants were assessed by ELISA. (**C**) PBMC from healthy controls (n=3) were labeled with magnetic bead-conjugated antibodies against αβTCR, γδTCR, or both, then separated by magnetic activated cell sorting (MACS). The purity of each population was assessed by flow cytometry, and cells were stimulated as in (B), for 6, 12, 18, 24 or 48 hours, as indicated. IFN-γ levels in culture supernatants were assessed by ELISA. *$p<0.05$ by Mann-Whitney test, comparison with total PBMC.

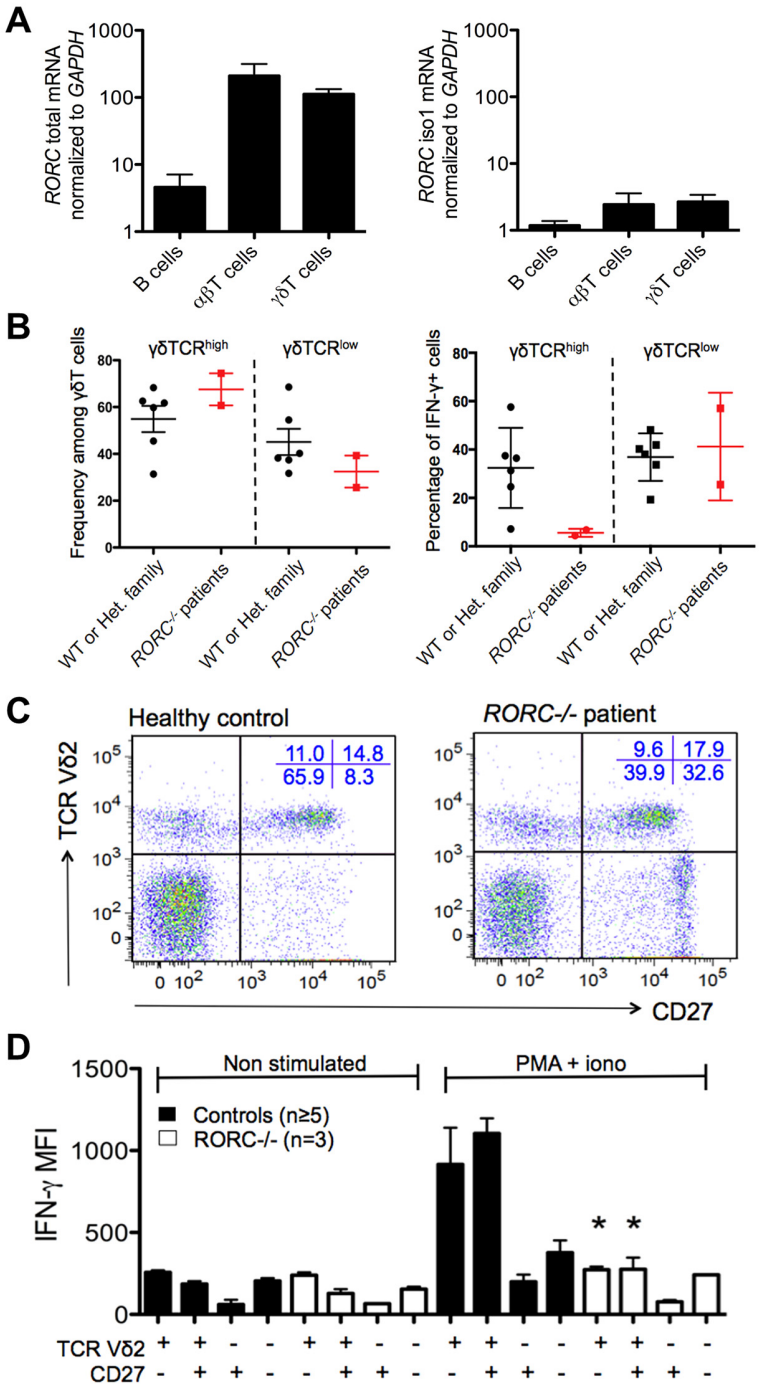

Figure S15. A subset of *RORC*$^{-/-}$ patients' γδT cells show reduced IFN-γ production.

(**A**) *RORC* isoform 2 (RORγT) is expressed by healthy donor αβT and γδT cells in the steady state. PBMC from healthy controls (n=3) were labeled with magnetic bead-conjugated antibodies against CD19 (in the case of B cells), αβTCR or γδTCR, then separated by magnetic activated cell sorting (MACS). Total RNA was extracted and

qRT-PCR was performed as described in Materials and Methods, using probes recognizing both *RORC* isoforms 1 and 2 (*RORC* total) or isoform 1, encoding the RORγ protein, only (*RORC* iso1). Results normalized to GAPDH are presented (arbitrary units). Due to the low abundance of isoform 1 transcript in all cells tested, the elevated total *RORC* transcript detected in αβT and γδT cells is due to the presence of isoform 2, encoding RORγT. (**B**) PBMCs from either WT or heterozygous family members (*n*=6) or from *RORC*$^{-/-}$ patients (*n*=2) were stimulated with PMA and ionomycin for 5 hours in the presence of brefeldin A, stained with a fixable cell viability dye, and for CD3 and γδTCR, then fixed and permeabilized and stained for intracellular IFN-γ and analyzed by flow cytometry. Cells were gated on exclusion of the viability dye, forward and side scatter profile, and CD3^{+}γδTCR^{+} cells. The γδT cells were further gated on the basis of high or low levels of expression of the γδTCR, and the percentage of IFN-γ^{+} cells was then determined within each subset. (**C**) PBMC from healthy controls (n=3) or *RORC*$^{-/-}$ patients (n=3) were labeled with magnetic bead-conjugated antibodies against γδTCR then purified by magnetic activated cell sorting (MACS). Purified γδT cells stimulated with PMA + ionomycin for 6 hours in the presence of brefeldin A. Cells were stained with a fixable cell viability dye, and antibodies directed against CD27 and the TCR Vδ2. Cells were fixed and permeabilized then stained for intracellular IFN-γ. Analysis of CD27 and Vδ2 expression reveals no defect in the percentages of these cells in *RORC*$^{-/-}$ patients. (**D**) Analysis of the median fluorescence intensity (MFI) of intracellular IFN-γ from cells in (C) reveals that healthy donor TCR Vδ2^{+} cells respond with robust IFN-γ production, while Vδ2^{+} cells from *RORC*$^{-/-}$ patients do not.

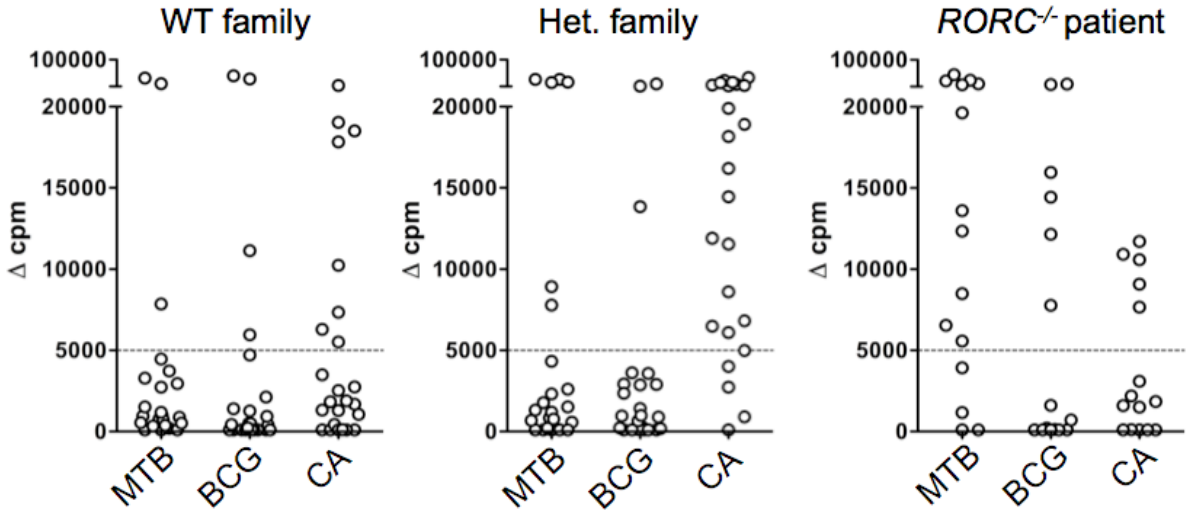

Figure S16. An independent experiment confirmed that CCR6^{+}CD4^{+} memory T cells from *RORC*$^{-/-}$ patients displayed no profound defect in terms of the frequency of *M. tuberculosis*- or *C. albicans*-responsive T cells.

Memory CCR6^{+}CD4^{+} T cells from one WT family member, one heterozygous family member, and one *RORC*$^{-/-}$ patient were purified as in Fig. S9. The sorted memory T cells (500 cells/well, 24 wells in total) were polyclonally stimulated with 1 µg/ml PHA in the presence of irradiated (45Gy) allogeneic feeder cells (2.5×10^4 per well) and IL-2 (500 IU/ml), in a 96-well plate, and T-cell lines were expanded as previously described (*59*). Library screening was performed 14-21 days after initial stimulation, by culturing thoroughly washed T cells (2.5×10^5/well) with autologous irradiated B cells (2.5×10^4), with or without a 3 h pulse with various antigens, including *C. albicans* lysate (CA, 5 µg/ml), *M. tuberculosis* peptide pool (MTB, 0.5 µg/ml/peptide), or *M. bovis*-BCG peptide pool (BCG, 0.5 µg/ml/peptide). Proliferation was assessed by evaluating radiolabel incorporation on day 4, after 16 h of incubation with 1 µCi/ml $^{[3H]}$thymidine, and is expressed as Δcpm values.

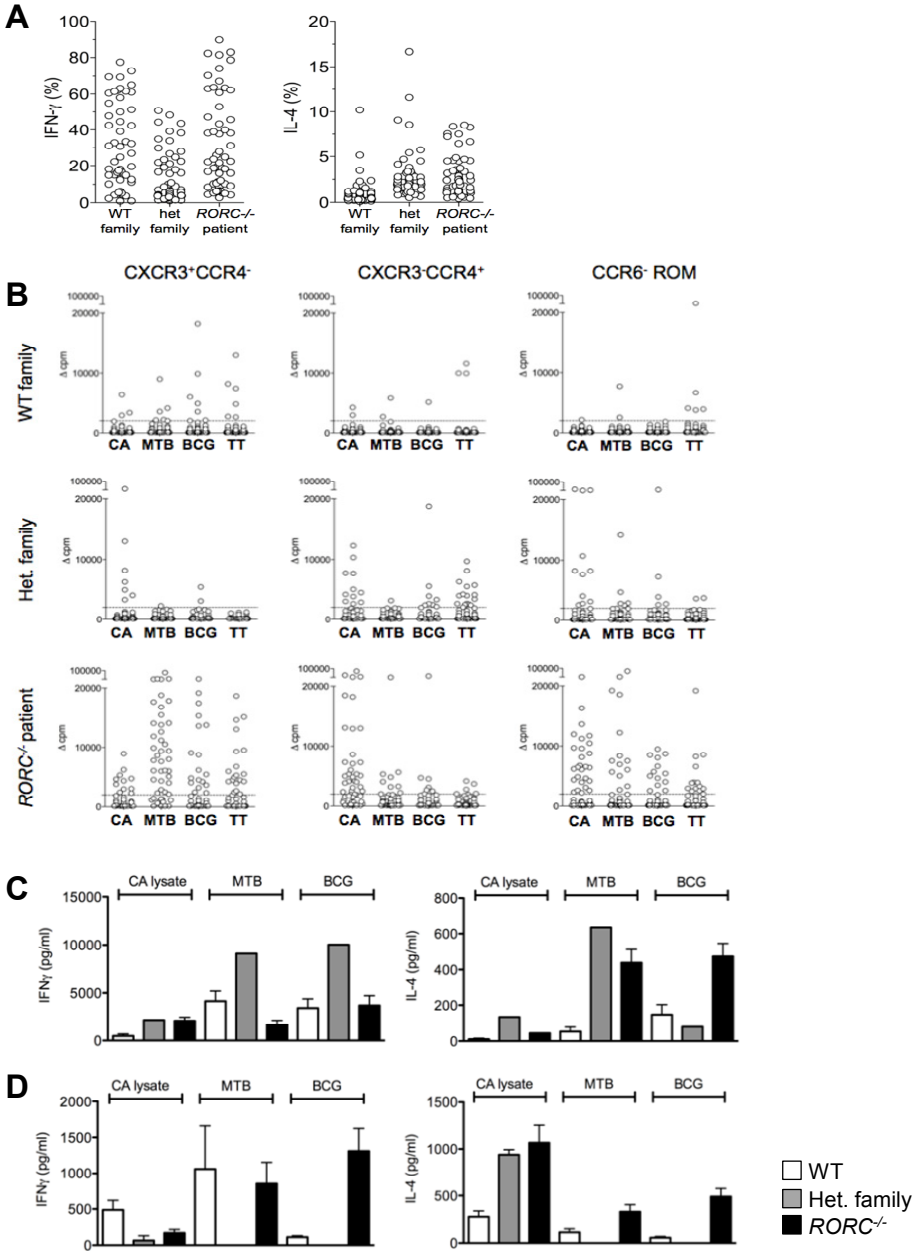

Figure S17. Naive CD4$^+$ T cells from *RORC*$^{-/-}$ patient are able to produce normal levels of IFN-γ in non-polarizing conditions.

(**A**) Naïve CD4$^+$ T cells from one WT family member, one heterozygous family member, and one *RORC*$^{-/-}$ patient were purified, directly cloned by limiting dilution, and polyclonally stimulated with 1 μg/ml PHA in the presence of irradiated (45Gy) allogeneic feeder cells (2.5×10^4 per well) and IL-2 (500 IU/ml), in a 96-well plate in the presence of neutralizing antibodies to IL-4 and IL-12, as described in (*66*). This method measures the cells' default propensity to acquire the capacity to produce IFN-γ or IL-4 in non-polarizing conditions (*66*). All 3 samples produced comparable levels of IFN-γ,

indicating that $CD4^+$ T cells from *RORC*$^{-/-}$ patients do not have a pervasive global defect in IFN-γ production. (**B**) Proliferation of sorted $CD4^+$ memory subsets in response to peptide pools. Subsets of memory $CD4^+$ T cells from one WT family member, one heterozygous family member, and one *RORC*$^{-/-}$ patient were purified as in Fig. S10. The sorted memory T cells (500 cells/well) were polyclonally stimulated with 1 μg/ml PHA in the presence of irradiated (45Gy) allogeneic feeder cells (2.5×10^4 per well) and IL-2 (500 IU/ml) in a 96-well plate, and T-cell lines were expanded as previously described (*58*). Library screening was performed 14-21 days after initial stimulation, by culturing thoroughly washed T cells (2.5×10^5/well) with autologous irradiated B cells (2.5×10^4), with or without a three-hour pulse with various antigens, including 5 μg/ml *Candida albicans* lysate (CA), 0.5 μg/ml/peptide of the *Mycobacterium tuberculosis* peptide pool (MTB), 0.5 μg/ml/peptide of the *Mycobacterium bovis*-BCG peptide pool (BCG), or 5 μg/ml tetanus toxoid (TT). Proliferation was assessed by evaluating radiolabel incorporation on day 4, after 16 h of incubation with 1 μCi/ml $^{[3H]}$thymidine, and is expressed as Δcpm values. (**C**) $CXCR3^+CCR4^-$ (defined as Th1) cells from WT donors (n=4), a heterozygous family member (n=1) and *RORC*$^{-/-}$ patients (n=3) were stimulated with *Candida albicans* lysate (CA), *Mycobacterium tuberculosis* peptides (MTB) or BCG. The cytokines indicated were determined in the culture supernatants from wells with Δcpm values above the cutoff value. (**D**) $CXCR3^-CCR4^+$ (defined as Th2) cells were stimulated and cytokines were assessed in culture supernatants as in (C).

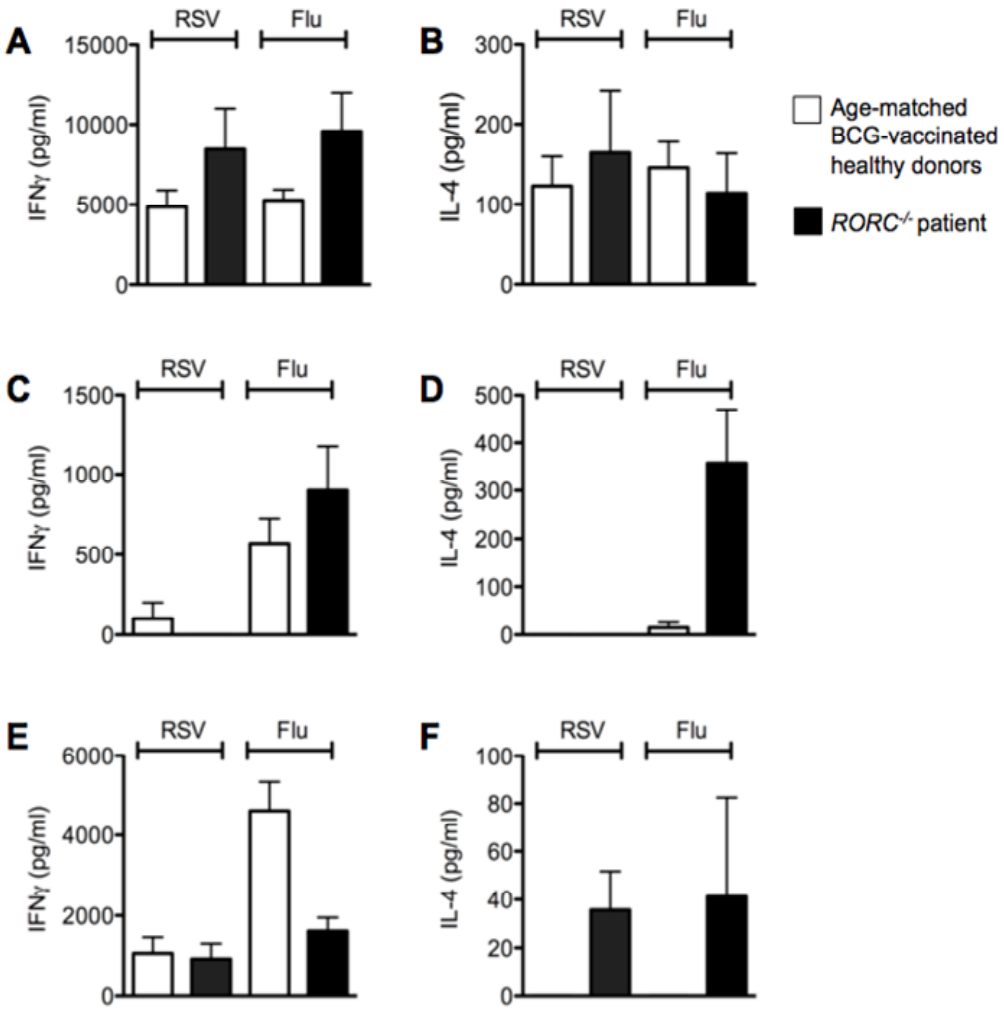

Figure S18. Memory CD4$^+$ T cells from *RORC*$^{-/-}$ patient produce IFN-γ and IL-4 normally in response to viral stimulation.

(**A**, **B**) Sorted CXCR3$^+$CCR4$^-$ memory CD4$^+$ T cells (defined as Th1), from WT age-matched and BCG-vaccinated healthy donors (n=3) and one *RORC*$^{-/-}$ patient, were polyclonally stimulated (500 cells/well) with 1 μg/ml PHA in the presence of irradiated (45Gy) allogeneic feeder cells (2.5×10^4 per well) and IL-2 (500 IU/ml), to generate T-cell libraries. Library screening was performed 14-21 days after initial stimulation, by culturing thoroughly washed T cells (2.5×10^5/well) with autologous irradiated B cells (2.5×10^4), with or without a three-hour pulse with respiratory syncytial virus (RSV) or influenza vaccine 2013-2014 peptide pools. The cytokines indicated were determined in the culture supernatants for wells with Δcpm values above the cutoff value. (**C**, **D**) Purified CXCR3$^-$CCR4$^+$ memory CD4$^+$ T cells (defined as Th2) were stimulated and cultured as in (A, B). (**E, F**) Sorted CCR6$^+$ memory CD4$^+$ T cells were stimulated and cultured as in (A, B).

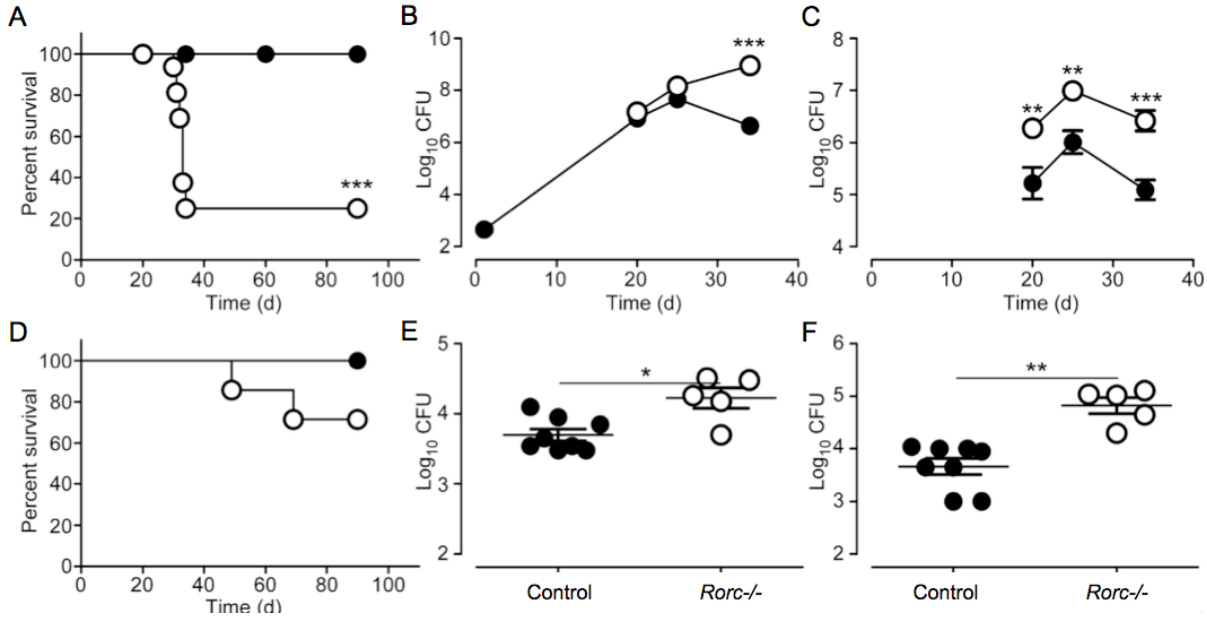

Figure S19. Mice deficient for RorγT are more susceptible to mycobacterial infection than intact mice.

(**A**) Control (closed circle) or *Rorc*$^{-/-}$ (open circles) mice, n>20 per group, were infected via the aerosol route with *M. tuberculosis* and survival was determined over time, ***p<0.0001 by Log-rank (Mantel-Cox) test. (**B, C**) Mice were infected, as in (A), and bacterial burden in the lung (**B**) and spleen (**C**) determined at indicated time points, n=4 mice per group per time point. **p<0.001, ***p<0.0001 by ANOVA followed by Bonferroni's multiple comparison test. Panels show combined data from two independent experiments each showing the same outcome. (**D**) Control (closed circle) or *Rorc*$^{-/-}$ (open circles) mice were infected via the aerosol route with *M. bovis*-BCG and the survival was determined over time, n=7-8 per group. (**E, F**) Mice were infected as in (D), and bacterial burden in the lung (**E**) and spleen (**F**) determined at 90 days post-infection. One experiment (n=5-8 per group) shown, representative of two independent experiments with comparable results. *p<0.05, **p<0.001 by Mann Whitney test.

	P1	P2	P3	P4	P5	P6	P7
Aphtous stomatitis	No	No	No	No	No	Recurrent (7 episodes/year) with partial response	No
Genital mucosa	No	No	No	Recurrent (4 episodes/year) with complete response	No	Recurrent (4 episodes/year) with complete response	No
Intestinal tract	No	No	No	No	No	No	No
Nail	No	No	No	2 nails with complete response	2-3 nails with complete response	2-3 nails with complete response	No
Oral mucosa	Persistent thrush at 2 year with complete response	Persistent	No	Recurrent thrush (7 episodes/year) with complete response	No	Recurrent thrush (12 episodes/year) with complete response	Recurrent thrush (6 episodes/year) with complete response
Perionyxis	No	No	No	No	Recurrent (1-2 episodes/year) with complete response	Recurrent (3 episodes/year) with complete response	No
Scalp	No	No	No	No	No	No	No
Skin	No	No	No	Recurrent intertrigo (5 episodes/year) with complete response	No	Recurrent intertrigo (10 episodes/year) with partial response	Recurrent intertrigo (6 episodes/year) with complete response
Treatment	Unknown	- Oral: Fluconazol	No	- Local: Clotrimazole - Oral: Fluconazol	- Local: Miconazole	- Local: Nystatin, Miconazole, Terbinafine	- Local: Miconazole, Terbinafine
Prophylaxis	No	No	No	Fluconazol	No	No	No

Table S1. Summary of fungal phenotypes of *RORC*$^{-/-}$ patients.

Clinical features of *Candida albicans* infections in 7 *RORC*$^{-/-}$ patients are listed.

	P2	**P3**	**P4**	**Het. Family (*n*=6)**	**Controls (*n*=7-9)**
Total T cells	54.6	67.0	66.1	79.0 ± 1.6	72.1±3.6
$CD4^+$ T cells	18.8; 21.1	28.5	19.5; 26.7	45.7 ± 2.1	49.6 ± 3.6
T_{regs}	7.5; 9.2	8.7	7.4; 12.6	4.7 ± 1.6	7.5 ± 1.0
Total memory $CD4^+$ T cells	60.5; 47.1	51	63; 54.3	30.7 ± 8.4	44.5 ± 9.1
	% of memory $CD4^+$ T cells				
$CD4^+CXCR3^+$ T cells	41.7; 25.8	26.2	27.7; 17.4	18.1 ± 7.7	21.0 ± 2.3
$CD4^+CCR6^+$ T cells	8.6; 7.3	18.7	13.2; 16.3	14.5 ± 3.2	18.5 ± 3.3
$CD4^+CCR6^+CXCR3^+$ T cells	11.2; 7.2	9.0	14.3; 8.2	6.4 ± 1.1	11.2 ± 2.7
$CD8^+$ T cells	4.3	20.4		32.5±2.1	27.7±3.0
	% of total $CD8^+$ T cells				
Naïve ($CD45RA^+CCR7^+$)	28.2	7.0		46.8±5.8	42.6±5.1
Central memory ($CD45RA^-CCR7^+$)	3.3	1.2		5.6±1.2	3.9±0.5
Effector memory ($CD45RA^+CCR7^-$)	20.3	19.8		20.7±3.0	27.0±1.7
T_{EMRA} cells ($CD45RA^-CCR7^-$)	48.2	72.0		26.9±4.9	26.7±4.1

B cells	39.3;	12.7	44.6, 45.0	11.3 ± 4.7	12.7 ± 3.9
Memory B cells	5.6, 3.1	3.7	3.6, 2.5	27.7 ± 17	18.3 ± 2.0
% of $CD3^+$					
γδT cells	52.3; 42.8	29.2, 27.1	21.4	7.5±0.8	5.2±1.7
$\gamma\delta TCR^{hi}$ (% of γδT)	60.7, 55.1	74.4, 68.0	75.6	54.9±5.6	20.6±6.8
$\gamma\delta TCR^{lo}$ (% of γδT)	39.3; 44.9	25.6, 32.0	24.4	45.1±5.6	79.4±6.8
% of $CD3^+$					
NKT cells ($V\alpha24^+V\beta11^+$)	0	0		0.36±0.16	0.17±0.05
MAIT cells ($V\alpha7.2^+CD161^{hi}$)	0.0	0.0	0.0	1.2±0.4	3.0±1.2

Table S2. Immunophenotyping of *RORC*$^{-/-}$ patients' PBMC.

Immunophenotyping of PBMCs from *RORC*$^{-/-}$ patients, heterozygous family members, and healthy controls was performed as described in the Materials and Methods. The values presented for the *RORC*$^{-/-}$ patients are a percentage of the indicated lymphocyte subset, obtained on 1 or 2 separate occasions; the values for the heterozygous relatives are the mean ± SEM of 2-6 individuals; the values for the controls are the mean ± SEM of 7-9 normal healthy donors.

	***RORC*$^{-/-}$ patients**	***Rorc*$^{-/-}$ mice**
Hematological phenotypes	**Cell counts per μl blood**	
$CD3^+$	Low but within the normal range	One sixth normal levels (*67*)
$CD4^+$	Slightly low	One tenth normal levels (*67*)
$CD8^+$	Slightly low	One third normal levels (*67*)
B cells	Normal	One half normal levels (*67*)
NK cells	Normal	Not reported
Type 1 NKT cells	Absent	Absence documented (*18*)
MAIT cells	Absent	Absence is inferred (*12*)
Lymphoid organ size		
Thymus	Smaller than in age-matched controls	20% smaller than in WT controls (*67*)
Spleen	Enlarged	Twice the size of that in WT controls (*67*)
Peripheral lymph nodes	Absent (axillary, cervical, inguinal)	Absent (*67*)
Peyer's patches	Not tested	Absent (*67*)

Table S3. Comparison of immune phenotypes in *RORC*$^{-/-}$ patients and *Rorc*$^{-/-}$ mice.
Patients with biallelic *RORC* mutations display several hematological and lymphoid organ phenotypes similar to those previously reported in *Rorc*$^{-/-}$ mice. Like these mice, *RORC*$^{-/-}$ patients display T-cell lymphopenia, albeit of lower severity than that observed in mice. *Rorc*$^{-/-}$ mice have B-cell frequency defects, whereas *RORC*$^{-/-}$ patients do not. The absence of *RORC/Rorc* results in abnormal TCRα repertoire generation, including an absence of type 1 NKT and MAIT cells in both species. *RORC*$^{-/-}$ patients had a small thymus, an enlarged spleen and no peripheral lymph nodes, as also reported for *Rorc*$^{-/-}$ mice.