SUPPORTING INFORMATION

Follicular helper T cell signature of replicative exhaustion, apoptosis and senescence in common variable immunodeficiency

Giulia Milardi¹, Biagio Di Lorenzo¹, Jolanda Gerosa¹, Federica Barzaghi²,³, Gigliola Di Matteo⁴,⁵, Maryam Omrani³,⁶, Tatiana Jofra¹, Ivan Merelli³,⁷, Matteo Barcella⁵, Matteo Filippini¹, Anastasia Conti³, Francesca Ferrua²,³, Francesco Pozzo Giuffrida²,³, Francesca Dionisio³, Patrizia Rovere-Querini⁸, Sarah Marktel⁹, Andrea Assanelli⁹, Simona Piemontese⁹, Immacolata Brigida³, Matteo Zoccolillo³, Emilia Cirillo¹⁰, Giuliana Giardino¹⁰, Maria Giovanna Danieli¹¹, Fernando Specchia¹², Lucia Pacillo⁴,⁵, Silvia Di Cesare⁴,⁵, Carmela Giancotta⁴,⁵, Francesca Romano²,³, Alessandro Matarese¹³, Alfredo Antonio Chetta¹⁴, Matteo Trimarchi¹⁵,²³, Andrea Laurenzi¹, Maurizio De Pellegrin¹⁶, Silvia Darin², Davide Montin¹⁷, Maddalena Marinoni¹⁸, Rosa Maria Dellepiane¹⁹, Valeria Sordi¹, Vassilios Lougaris²⁰, Angelo Vacca²¹, Raffaella Melzi¹, Rita Nano¹, Chiara Azzari²², Lucia Bongiovanni²³, Claudio Pignata¹⁰, Caterina Cancrini⁴,⁵, Alessandro Plebani²⁰, Lorenzo Piemonti¹,²⁴, Constantinos Petrovas²⁵, Raffaella Di Micco³, Maurilio Ponzoni²,³,²⁴, Alessandro Aiuti²,³,²⁴*, Maria Pia Cicatele²,³*, and Georgia Fousterti¹⁵*

1. Division of Immunology, Transplantation, and Infectious Diseases, Diabetes Research Institute, IRCCS San Raffaele Hospital, Milan, Italy
2. Pediatric Immunohematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Hospital, Milan, Italy
3. San Raffaele Telethon Institute for Gene Therapy, Sr-TIGET, IRCCS San Raffaele Hospital, Milan, Italy
4. Department of Systems Medicine University of Rome Tor Vergata, Rome, Italy
5. Immune and Infectious Diseases Division, Research Unit of Primary Immunodeficiencies, Academic Department of Pediatrics, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy
6. Department of Computer Science, Systems and Communication University of Milano-Bicocca, Milan, Italy
7. Institute for Biomedical Technologies, National Research Council, Segrate, Italy
8. Department of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Hospital, Milan, Italy
9. Hematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Hospital, Milan, Italy
10. Department of Translational Medical Sciences, Section of Pediatrics, Federico II University of Naples, Italy
11. Marche Polytechnic University of Ancona, Clinica Medica, Ancona, Italy
12. Department of Pediatrics, S. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy
13. Santi Antonio, Biagio and Cesare Arrigo Hospital, Alessandria, Italy
14. Department of Medicine and Surgery, Respiratory Disease and Lung Function Unit, University of Parma, Parma, Italy
15. Otorhinolaryngology Unit, Head and Neck Department, IRCCS San Raffaele Scientific Institute, Milan, Italy.
16. Unit of Orthopaedics, IRCCS San Raffaele Scientific Institute, Milan, Italy
17. Regina Margherita Hospital, Turin, Italy
18. Pediatric Unit, Ospedale "F. Del Ponte", Varese, Italy
19. Department of Pediatrics, Fondazione IRCCS Cà Grande Ospedale Maggiore Policlinico, University of Milan, Milan, Italy
20. Department of Clinical and Experimental Sciences, Pediatrics Clinic and Institute for Molecular Medicine A. Nocivelli, University of Brescia, Brescia, Italy
21. Department of Biomedical Sciences and Human Oncology, University of Bari Medical School, Bari, Italy

22. Pediatric Immunology Division, Department of Pediatrics, Anna Meyer Children's University Hospital, Florence, Italy
23. Pathology Unit, IRCCS San Raffaele Hospital, Milan, Italy
24. University Vita-Salute San Raffaele, Milan, Italy
25. Tissue Analysis Core, Immunology Laboratory, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA

*corresponding authors
Correspondence should be addressed to fousteri.georgia@hsr.it, aiuti.alessandro@hsr.it and cicalese.mariapia@hsr.it.
Materials and Methods

Multiplexing protein level measurements on a single Luminex platform

Secreted protein levels in sera were detected using the Invitrogen™ ProcartaPlex™ Human 65-plex panel kit (Thermo Fisher Scientific Cat. No. EPX650-10065-901). Samples were assayed according to the manufacturer’s instructions (1), and the plates were read on a Luminex xMAP instrument (BioRad). The acquisition and analysis of the samples were performed with the Bio-Plex Manager 6.0 software (BioRad).

Intracellular cytokine analysis

100 uL of peripheral whole blood (EDTA) were cultured in 96-well V bottom plates and stimulated with 12-O-Tetradecanoylphorbol-13-acetate (TPA; 20 ng/mL) and Ionomycin (1µg/mL) (both from Sigma-Aldrich) in the presence of Brefeldin A (1µg/mL) (Sigma-Aldrich), and antibody anti-CXCR5 Brilliant Violet 421 (J252D4, BioLegend) was added during the cell activation. Cultures were maintained 3 h at 37°C in a 5% CO₂ atmosphere. Surface staining was performed with mAbs including: CD4-PE (REA623, Miltenyi Biotec), CD3-APC-Vio770 (BW264/56, Miltenyi Biotec), CD45-Brilliant Violet 510 (HI30, BioLegend). Cytokines were analyzed performing an intracellular staining with the BD Cytofix/Cytoperm Fixation/Permeabilization Kit (BD Biosciences) and with two panels of mAbs (Table E2, panel G-H). The first panel consisted of: IFNγ-Alexa Fluor 488 (4S.B3, BioLegend), IL-17-PE (eBio64CAP17, Invitrogen), IL-21-Alexa Fluor 647 (3A3-N2, BioLegend) (Table E2, panel G). The second one was: IFNγ-Alexa Fluor 488 (4S.B3, BioLegend), IL-10-PE (JES3-9D7, BD Biosciences), IL-4-APC (8D4-8, Invitrogen) (Table E2, panel H). Cells were acquired on FACSCantoII (BD) and analyzed with FlowJo (Tree Star) software.

Supplementary Figures
**FIGURE E1.** Representative gating strategy for (A) cTfh (CXCR5+FoxP3−), cTfr (CXCR5+FOXP3+, cTreg (CXCR5−FOXP3+) cells, the activation marker (PD-1), (B) Tfh subsets and (C) Highly Functional cTfh (PD-1+CXCR3+) cells.
FIGURE E2. Phenotypic analysis of circulating B cells and their subsets in CVID patients. (A) Phenotypic characterization of peripheral blood B cells and B-cell subsets. Gating strategy to identify naïve, memory and switch memory B cells based on expression of CD24, CD38, CD27, and (B) IgH isotypes. (C) Flow cytometric analysis of B cells and B-cell subpopulations isolated from spleen of CVID003 patient with RTEL1 mutation compared to age-matched HC. Frequency of CD19+ B cells, (D) naïve (CD19+CD27−) and (E) memory (CD19+CD27+) B cells, (F) transitional B cells (CD38hiCD24hi), (G) switched memory (CD27+IgM+) B cells and (H-I) their 2 subclasses CD27+IgG+ and CD27+IgA+. (J) Frequencies of mature memory B cells (CD27+IgM+), (K) CD21lo B cells (IgM+IgD+) and (L) plasma cells (CD24−CD38+). In all graphs, red dots represent individual donors and
asterisks indicate statistical significance as calculated by Mann Whitney test. Black bars: median with interquantile range. *p<0.05; **p<0.005; ***p<0.001; ****p<0.0001.

FIGURE E3. Expression analysis of several gene targets in CVID Group A patients (red dots) vs CVID Group B (blue dots). The ProcartaPlex 65-plex data was analyzed using the Bio-Plex Manager Software 6.1.
FIGURE E4. Transcriptomic analysis of sorted CD4+CXCR5+CD25− cTfh cells. (A) Principal component analysis (PCA) on the entire transcriptomic profile, showing Group A and B based on 1000 top differentially expressed genes. Volcano plot showing the transcriptomic analysis of sorted cTfh cells in total CVID patient vs. HC (B) and in Group B patients vs. HC (C). In the volcano plots, red color represent a higher gene expression compare to green color which is for genes low expressed.
FIGURE E5. Cytokine production by group A CVID cTfh cells. (A) Representative flow cytometry gating strategy. (B) Percentages of cTfh expressing pro-inflammatory, activation and Tfh cytokines compared to age-matched HC. In all graphs, dots represent individual donors. Statistical significance was calculated by Mann Whitney test.
FIGURE E6. Telomere length analysis in three group A CVID patients. Nomogram of Telomere Length (TL) in CD45RA+ naive T cells, CD45RA- memory T cells, CD20+ B cells and CD57+ NK from CVID003 (A), CVID010 (B), CVID028 (C)). Percentile lines as annotated. Black dot represents CVID patients. Red, green, and blue curves representing expected telomere length for the indicated proportion of HC.
FIGURE E7. XBP1 and Bcl-6 expression analysis in sorted tonsillar GC Tfh cells, centroblasts (CBs) and centrocytes (CCs) from control donors by ddPCR. (A). Representative gating strategy for Tfh cells, CBs, and CCs sorting. Cells were sorted as follow; Tfh cells as CD3+CD20-, CD4+, PD-1+CXCR5+; CCs as CD20+CD3-, CD27+CD38dim, CXCR4+; CBs as CD20+CD3-, CD27CD38dim, CXCR4+. The complete antibody mix is described in Supplementary Table E3, panel E. (B). XBP1 and Bcl-6 expression was assessed in sorted Tfh cells, CBs and CCs (n = 3). XBP1 was included as quality indicator for CCs and CBs sorting, since evidences from published dataset (http://biogps.org/dataset/E-GEOID-15271/) indicates that XBP1 expression in CCs is 2x increased compared to CBs. The average for technical duplicates was estimated, normalized on HPRT as housekeeping gene, and represented as dark circles; HPRT expression (set at 1) is represented by the dotted line; mean and SD are also shown.
FIGURE E8. Spleen histopathology of CVID003 patient detected in formalin-fixed paraffin-embedded sections by haematoxylin and eosin (H&E) staining (above) or by immunohistochemistry staining (below) showing a reduced white pulp (WP) and down sized germinal centers (GC) while the red pulp (RP) retained a normal appearance. On immunohistochemistry a reduced marginal zone is evident (IgD); sparse plasma cells can also be detected (CD38). Original magnification: H&E 100X; IgD and CD38 200X. GC is highlighted with a dotted circle.
**FIGURE E9.** Phenotypic characterization of B cells and B-cell subsets isolated from spleen of CVID003 patient. (A) Gating strategy to identify naïve, memory and switch memory B cells based on expression of CD24, CD38, CD27, and (B) IgH isotypes.
**FIGURE E10.** Flow cytometric analysis of (A) CD4⁺ T cells, Tfh (CD4⁺CXCR5⁺), PD-1⁺ and CD57⁺ Tfh and germinal center’s markers (Ki67 and Bcl-6) on the spleen of CVID003 compared to age-matched HC. (B) Activated CD8⁺ T cells were also investigated.
Supplementary Tables

**Supplementary Table E1.**

Gene pipeline for the discovery of causative mutations

| SCIDA3: AG18A1A3: AG17A3: AA3:AD20 | CID | CID with associated dysmorphic / dermatological / neurological features | Prevalent Ab deficiency |
|-------------------------------------|-----|-----------------------------------------------------------------------|------------------------|
| CD3D ADA                            | TTC7 | ZAP7 | ATM | CHD7 | ACTB | AID | CD79 | PRKCD | CD40 | PMS2 | IL36RN | NIK/ MAP3 K14 | IgG |
| CD3E AK2               | CARD1 | TRAC | IKBA | BLM | DKC1 | FOXN1 | BLNK | CXC4 | TNFRSF13B | CD40 | RNF168 | TCF3 | IGH | IL21 |
| CORONINA DCLRE1 C         | CD4  | CD3G | IKBKG | MRE11 | RTEI | IKAROS | BTK | ICOS | TNFRSF13C | LRBA | HOIP | CD20 | CD21 | CD21 |
| IL2RG DNAPK              | CD81 | ITK  | UNCI19 | NBS1 | TINF2 | MST1/ST4 | CD1 | PIK3CD | UNG | IL21R | HOIL | TWEAK | IGLL1 | CXC4 |
| IL7R RAG1 CD8A IL21R DOCK2 | RNF1 | 68 | NHP2 | POLE1 | CD7 | 9A | PIK3R1 | PLCg2 | IKAROS | INOS | 0 | NFKB2 | LRRC8 |
| JAK3 RAG2 TAP1 ORAI1 TBX1 SPIN | NOP10 | TBX1  | TERT | POLE2 | CD3Z |
| PTPRC LIG4 TAP2 MAGT1 | MST1/ST4 | RAD50 | TERC | |
| CERNUN NOS TAPB MALT1 | TPP2 | LIG4 | TERT | CD3Z |
| B2M MCM4/PRKDC | RHOH | PMS2 | COH1 | ORA11 |
| CIITA FOXN1 BCL10 | PNP | CTLA4 | CERNUN NOS |
| RFX5 PNP | CTPS1 | STAT3 | TTC7A | IKBA |
| RFXANK IKAROS GFI1 STIM1 LYST | IKBKG |
| RFXAP IKBK | PGM3 | PLCg2 | RAB27A | PGM3 |
## Autoimmunity and lymphoproliferation

| Autoinflammatory disorders (includes periodic fevers) | Defects of Phagocytes counts / function | Cytopenias | Lymphoproliferation | Enteropathy |
|------------------------------------------------------|----------------------------------------|------------|-------------------|------------|
| **Autoimmunity and lymphoproliferation** | **Defects of Phagocytes counts / function** | **Cytopenias** | **Lymphoproliferation** | **Enteropathy** |
| AIRE | PRF1 | CASP10 | ZAP70 | WASL | LCK | IL1RN | TNFRSF1A | ELANE | SBDS | CSF3R | CARD9 | ALPS genes | MAGT1 | WAS |
| FOX3 | UNC13D | CASP8 | PLCg2 | WIP1 | ORAI1 | LPIN2 | PLCg2 | GFI1 | ITGB2 (CD18) | MPO | IL17F | STK4 | ITK | WASL |
| IL2RA | STX1 | FADD | PIK3CD | WAS | PIK3CD | MEFV | CECR1 | HAX1 | SLC35C1 | CEBPE | IL17RA | PNP | PRKCD | FOXP3 |
| STAT1 | STXB2P2 | FAS | TYK2 | IL10 | CD27 | MVK | HOIP | LAMTOR2 | KINDLIN3 | CXCR4 | IL17RC | IKAROS | CTPS1 | CD25 |
| STAT3 | SH2D1A | FASL | STIM1 | IL10RA | TPP2 | NLRP12 | HOIL | G6PC3 | CYBA | MST1/STK4 | IRAK4 | TPP2 | CD27 | XIAP |
| CTLA4 | XIAP | NRAM1 | STAT5B | IL10RB | NFAT5 | CIA1 | PRKCDB | GFI1 | CYBB | ACTB | MYD88 | GATA2 | RHOH | ITCH |
| ITCH | LYST | KRAF2 | SH2D1A | CD27 | IKBA | NOD2 | IL36RN | RAC2 | NCF1 | IFNGR1 | IFNGR2 | ICOS | STK4 | TRAC |
| LRB4 | RAB27A | PRKCD | TRAC | IKBKG | PTPPIP1 | ROBL3 | NCF2 | IFNGR2 | | | | LYST | LYST | CD40 |
| | | | | | | | | | | | | RAB27A | RAB27A | CD40L |
| | | | | | | | | | | | | TWEAK | TPP2 | IKBA |
| | | | | | | | | | | | | CD40 | PN5L | NFAT5 |
| | | | | | | | | | | | | CD40L | NOD2 | |
| | | | | | | | | | | | | LRBA | IL21R | |
| | | | | | | | | | | | | FOXP3 | IL21R | |
| | | | | | | | | | | | | WAS | IL10R | |
| | | | | | | | | | | | | | | IL10 |

### Genes

- **AIRE**
- **PRF1**
- **CASP10**
- **ZAP70**
- **WASL**
- **LCK**
- **IL1RN**
- **TNFRSF1A**
- **ELANE**
- **SBDS**
- **CSF3R**
- **CARD9**
- **ALPS genes**
- **MAGT1**
- **WAS**
- **FOX3**
- **UNC13D**
- **CASP8**
- **PLCg2**
- **WIP1**
- **ORAI1**
- **LPIN2**
- **PLCg2**
- **GFI1**
- **ITGB2 (CD18)**
- **MPO**
- **IL17F**
- **STK4**
- **ITK**
- **WASL**
- **IL2RA**
- **STX1**
- **FADD**
- **PIK3CD**
- **WAS**
- **PIK3CD**
- **MEFV**
- **CECR1**
- **HAX1**
- **SLC35C1**
- **CEBPE**
- **IL17RA**
- **PNP**
- **PRKCD**
- **FOXP3**
- **STAT1**
- **STXB2P2**
- **FAS**
- **TYK2**
- **IL10**
- **CD27**
- **MVK**
- **HOIP**
- **LAMTOR2**
- **KINDLIN3**
- **CXCR4**
- **IL17RC**
- **IKAROS**
- **CTPS1**
- **CD25**
- **STAT3**
- **SH2D1A**
- **FASL**
- **STIM1**
- **IL10RA**
- **TPP2**
- **NLRP12**
- **HOIL**
- **G6PC3**
- **CYBA**
- **MST1/STK4**
- **IRAK4**
- **TPP2**
- **CD27**
- **XIAP**
- **CTLA4**
- **XIAP**
- **NRAF1**
- **STAT5B**
- **IL10RB**
- **NFAT5**
- **CIA1**
- **PRKCDB**
- **GFI1**
- **CYBB**
- **ACTB**
- **MYD88**
- **GATA2**
- **RHOH**
- **ITCH**
- **ITCH**
- **LYST**
- **KRAF2**
- **SH2D1A**
- **CD27**
- **IKBA**
- **NOD2**
- **IL36RN**
- **RAC2**
- **NCF1**
- **IFNGR1**
- **ICOS**
- **STK4**
- **TRAC**
- **LRBA**
- **RAB27A**
- **PRKCD**
- **TRAC**
- **DOC8K**
- **IKBKG**
- **PTPIP1**
- **ROBL3**
- **NCF2**
- **IFNGR2**
- **LOT**
- **LYST**
- **CD40**
- **CD40L**
- **TWEAK**
- **TPP2**
- **IKBA**
- **CD40**
- **PN5L**
- **NFAT5**
- **CD40L**
- **NOD2**
- **LRBA**
- **IL21R**
- **FOXP3**
- **IL21R**
- **WAS**
- **IL10R**
- **IL10**
- **JAGN1**
- **NCF4**
- **GATA2**
- **PGM3**
### Therapeutic and clinical features of CVID patients of group A and B

| Patients (Group A) | Gender | Age at diagnosis | Age at analysis | IgG at diagnosis | IgM at diagnosis | Treatment |
|-------------------|--------|------------------|-----------------|------------------|------------------|-----------|
| CVID003           | M      | 22               | 22              | 6.36             | 0.63             | Ig iv; Sirolimus; MMF |
| CVID008           | M      | 35               | 36              | 0.9              | 0.19             | Ig iv |
| CVID010           | M      | 11               | 12              | 4.21             | <0.05            | Ig iv; Sirolimus |
| CVID013           | M      | 16               | 16              | 3.44             | 0.33             | RTX for neuritis; Ig iv |
| CVID017           | F      | 12               | 38              | na               | na               | Ig iv; CyA, then steroids and AZA for Autoimmune Hepatitis |
| CVID022           | M      | 35               | 36              | 3.87             | 0.37             | Ig iv; CCS + Rituximab (for ITP) |
| CVID024           | F      | 28               | 53              | na               | na               | Ig iv; Endoxan + Vincristine + Rituximab for ITP |
| CVID028           | M      | 14               | 16              | 5.21             | 0.26             | Hyqvia CCS for ITP |
| CVID030           | F      | 46               | 52              | 4.75             | <0.18            | Ig iv |

| Patients (Group B) | Gender | Age at diagnosis | Age at analysis | IgG at diagnosis | IgM at diagnosis | Treatment |
|-------------------|--------|------------------|-----------------|------------------|------------------|-----------|
| CVID009           | M      | 15               | 15              | 2.76             | 0.11             | Ig iv; Rituximab for ITP |
| PTPN22            | M      | 20               | 20              | 2.79             | 0.21             | Ig sc |
| CVID011           | M      | 37               | 38              |                 |                 | Ig iv |
| CVID019           | M      | 14               | 15              | 3.88             | 0.46             | none |
| CVID020           | F      | 10               | 14              | 5.4              | 1.17             | none |
| CVID021           | M      | 10               | 11              | 5.13             | 0.15             | Ig iv; CCS + MMF + Eltrombopag + Sirolimus for ITP |
| CVID023           | F      | 29               | 48              | na               | na               | Hyqvia |
| CVID025           | F      | 34               | 35              | na               | na               | Ig sc |
| CVID027           | M      | 26               | 27              | 5.04             | 0.3              | Hyqvia |
| CVID029           | F      | 57               | 63              | 5.45             | 0.34             | Ig iv |
| CVID032           | F      | 5                | 7               | 2.66             | 1.67             | none |
| CVID033           | M      | 10               | 10              | na               | na               | Hizentra |
| CVID034           | M      | 17               | 17              | 3.18             | 4.19             | Hyqvia; CCS, ciclofosfamide, ciclosporina, tacrolimus e micofenolato, Rituximab |
| CVID035           | F      | 13               | 14              | na               | na               | Privigen |
| CVID036           | M      | 24               | 25              | 3.19             | 0.2              | Hizentra |
| CVID037           | M      | 24               | 25              | 4.18             | 0.32             | Hizentra |
| TID197            | F      | 35               | 49              | na               | na               | Ig iv |
| CVID015           | M      | 3                | 37              | na               | na               | Ig iv |

na: not available
CCS: corticosteroids
## Supplementary Table E3.

### Healthy controls

| Sample | Age | Date of birth | Date of analysis | Exact Age | Sex |
|--------|-----|---------------|------------------|-----------|-----|
| HC212  | ped | 20/10/2003    | 26/10/2015       | 12        | F   |
| HC213  | ped | 07/10/2003    | 26/10/2015       | 12        | M   |
| HC214  | ped | 20/10/2002    | 26/10/2015       | 13        | M   |
| HC215  | ped | 12/09/2002    | 26/10/2015       | 13        | M   |
| HC216  | ped | 14/09/2001    | 26/10/2015       | 14        | F   |
| HC273  | ped | 22/07/2003    | 15/02/2016       | 12        | F   |
| HC275  | ped | 15/05/1999    | 15/02/2016       | 16        | F   |
| HC277  | ped | 13/04/2003    | 29/02/2016       | 12        | M   |
| HC278  | ped | 15/11/2001    | 29/02/2016       | 14        | F   |
| HC280  | ped | 08/07/2002    | 29/02/2016       | 13        | M   |
| HC307  | ped | 17/05/2004    | 30/05/2016       | 12        | M   |
| HC308  | ped | 08/08/2003    | 30/05/2016       | 12        | M   |
| HC309  | ped | 18/08/2002    | 30/05/2016       | 13        | M   |
| HC310  | ped | 09/04/2005    | 30/05/2016       | 11        | M   |
| HC325  | ped | 09/10/2004    | 14/11/2016       | 12        | F   |
| HC326  | ped | 12/02/2004    | 14/11/2016       | 12        | M   |
| HC327  | ped | 18/11/2002    | 14/11/2016       | 14        | M   |
| HC328  | ped | 09/09/2002    | 14/11/2016       | 14        | F   |
| HC331  | ped | 27/01/2005    | 05/12/2016       | 11        | F   |
| HC333  | ped | 11/03/2003    | 05/12/2016       | 13        | F   |
| HC334  | ped | 06/08/2002    | 05/12/2016       | 14        | F   |
| HC335  | ped | 11/09/2000    | 05/12/2016       | 16        | M   |
| HC336  | ped | 30/09/2004    | 12/12/2016       | 12        | M   |
| HC337  | ped | 28/12/2007    | 12/12/2016       | 8         | M   |
| HC344  | ped | 13/04/2004    | 09/01/2017       | 12        | M   |
| HC346  | ped | 03/03/2003    | 09/01/2017       | 13        | F   |
| HC349  | ped | 13/11/2011    | 25/01/2017       | 5         | M   |
| HC350  | ped | 13/08/2008    | 25/01/2017       | 8         | M   |
| HC396  | ped | 17/06/2009    | 20/09/2017       | 8         | F   |
| HC397  | ped | 27/06/2010    | 21/09/2017       | 7         | F   |
| HC398  | ped | 27/07/2013    | 25/09/2017       | 4         | F   |
| HC400  | ped | 13/06/2005    | 12/10/2017       | 12        | M   |
| HC401  | ped | 28/11/2005    | 12/10/2017       | 11        | M   |
| HC431  | ped | 15/11/2005    | 12/04/2018       | 12        | F   |
| HC462  | ped | 29/10/2009    | 13/05/2019       | 9         | M   |
| ID   | Type | Name | DOB           | DoD           | Age | Gender |
|------|------|------|---------------|---------------|-----|--------|
| HC449| ped  |      | 01/10/2016    | 22/07/2019    | 2   | F      |
| HC409| ped  |      | 31/12/2004    | 22/11/2017    | 12  | M      |
| HC421| ped  |      | 07/11/2008    | 14/02/2018    | 9   | F      |
| HC436| ped  |      | 01/09/2002    | 16/05/2018    | 15  | F      |
| HC359| ad   |      | 12/05/1990    | 03/05/2017    | 27  | F      |
| HC055| ad   |      | 29/08/1982    | 12/10/2017    | 35  | F      |
| HC068| ad   |      | 12/05/1977    | 12/10/2017    | 40  | F      |
| HC145| ad   |      | 04/08/1981    | 10/05/2017    | 35  | F      |
| HC148| ad   |      | 23/04/1976    | 21/03/2016    | 39  | F      |
| HC151| ad   |      | 03/04/1969    | 08/05/2017    | 46  | F      |
| HC159| ad   |      | 04/11/1990    | 08/05/2017    | 26  | M      |
| HC184| ad   |      | 28/04/1963    | 24/03/2016    | 52  | F      |
| HC194| ad   |      | 24/03/1989    | 05/06/2016    | 27  | F      |
| HC198| ad   |      | 22/11/1996    | 08/10/2015    | 18  | F      |
| HC199| ad   |      | 29/07/1996    | 08/10/2015    | 19  | F      |
| HC200| ad   |      | 22/12/1989    | 08/10/2015    | 25  | F      |
| HC211| ad   |      | 05/03/1992    | 14/10/2015    | 23  | M      |
| HC221| ad   |      | 23/05/1995    | 11/12/2015    | 20  | M      |
| HC232| ad   |      | 10/09/1993    | 18/01/2016    | 22  | M      |
| HC233| ad   |      | 11/08/1996    | 18/01/2016    | 19  | F      |
| HC234| ad   |      | 15/08/1996    | 18/01/2016    | 19  | F      |
| HC235| ad   |      | 18/11/1992    | 18/01/2016    | 23  | M      |
| HC236| ad   |      | 17/10/1995    | 18/01/2016    | 20  | M      |
| HC242| ad   |      | 31/05/1996    | 19/01/2016    | 19  | F      |
| HC243| ad   |      | 05/04/1996    | 19/01/2016    | 19  | F      |
| HC247| ad   |      | 13/02/1996    | 21/01/2016    | 20  | F      |
| HC248| ad   |      | 03/08/1996    | 21/01/2016    | 19  | F      |
| HC249| ad   |      | 13/09/1994    | 25/01/2016    | 21  | M      |
| HC250| ad   |      | 21/06/1995    | 25/01/2016    | 20  | M      |
| HC251| ad   |      | 02/11/1993    | 25/01/2016    | 22  | F      |
| HC252| ad   |      | 17/09/1995    | 25/01/2016    | 20  | F      |
| HC253| ad   |      | 21/03/1996    | 25/01/2016    | 19  | F      |
| HC262| ad   |      | 02/10/1996    | 27/01/2016    | 19  | F      |
| HC263| ad   |      | 25/10/1995    | 27/01/2016    | 20  | F      |
| HC268| ad   |      | 06/10/1996    | 28/01/2016    | 19  | F      |
| HC269| ad   |      | 25/06/1988    | 13/04/2016    | 27  | F      |
| HC271| ad   |      | 26/12/1988    | 09/02/2016    | 27  | F      |
| HC276| ad   |      | 15/03/1980    | 16/02/2016    | 35  | F      |
| HC282| ad   |      | 18/07/1980    | 08/03/2016    | 35  | M      |
| HC283| ad   |      | 01/01/1980    | 08/03/2016    | 36  | M      |
### Antibodies and immunostaining panels used for whole blood and PBMC

| Immunostaining panel | Antibody | Fluorochrome | Clone | Manufacturer |
|----------------------|----------|--------------|-------|--------------|
| cITh/cTfr cell panel | FOXP3    | FITC         | 259D  | BioLegend    |
| (PBMC)               |          |              |       |              |
| CD45RA               | PE       | PE           | H1100 | Miltenyi     |
| PD-1                 | PE-Cy7   | PE-Cy7       | J105  | eBioscience  |
| CD4                  | PerCP    | VIT4         |       | Miltenyi     |

**Supplementary Table E4.**
| CD25   | APC    | 2A3    | BD Biosciences |
|--------|--------|--------|----------------|
| ICOS   | PE-Cy7 | ISA-3  | eBioscience    |
| CD3    | APC-Cy7| BW264/56| Miltenyi      |
| CXCR5  | BV421  | J252D4 | BioLegend      |
| CD19   | PO     | SJ25C1 | BD Biosciences |
| CD14   | PO     | TUK4   | Miltenyi       |
| CD8    | PO     | BW135/80| Miltenyi      |

**cTfh subsets panel (whole blood)**

| CD45RA | FITC   | T6D11  | Miltenyi      |
|--------|--------|--------|----------------|
| CD4    | PE     | REA623 | Miltenyi      |
| CCR6   | PerCP  | G034E3 | BioLegend      |
| CXCR3  | APC    | IC6    | BD Biosciences |
| ICOS   | PE-Cy7 | ISA-3  | Invitrogen     |
| CD3    | APC-Cy7| BW264/56| Miltenyi      |
| CXCR5  | BV421  | J252D4 | BioLegend      |
| CD45   | PO     | HI30   | BioLegend      |

**Highly functional cTfh cell panel (whole blood)**

| CD45RA | FITC   | T6D11  | Miltenyi      |
|--------|--------|--------|----------------|
| CD4    | PerCP  | VIT4   | Miltenyi      |
| ICOS   | PE-Cy7 | ISA-3  | eBioscience    |
| CXCR3  | APC    | IC6    | BD Biosciences |
| PD-1   | PE     | J43    | ThermoFisher   |
| CD3    | APC-Cy7| BW264/56| Miltenyi      |
| CXCR5  | BV421  | J252D4 | BioLegend      |
| CD45   | PO     | HI30   | BioLegend      |

**Sorting set up (PBMC)**

| CD45RA | FITC   | REA1047| Miltenyi      |
|--------|--------|--------|----------------|
| CD3    | PE     | SK7    | BD Biosciences |
| CD4    | PO     | VIT4   | Miltenyi      |
| CD25   | APC    | BC96   | BioLegend      |
| CXCR5  | BV421  | J252D4 | BioLegend      |

**Apoptosis panel (PBMC)**

| Annexin-V | FITC | BD Biosciences |
|-----------|------|----------------|
| P.I.      | PE   | BD Biosciences |
| CD4       | PerCP| OKT4          | BioLegend       |
| CD3       | APC  | SK7           | BD Biosciences  |
| PD-1      | PE-Cy7| eBioJ105  | Invitrogen      |
| CD45RA    | APC-H7| HI100       | BioLegend       |
| CXCR5     | BV421| J252D4       | BioLegend       |
| CD45      | PO   | HI30         | BioLegend       |

**B cell helper assay panel (PBMC)**

| CD19   | FITC   | 4G7    | BD Biosciences |
|--------|--------|--------|----------------|
| CD27   | PE     | L128   | BD Biosciences |
| CD25   | APC    | 2A3    | BD Biosciences |
| CD4    | PE-Vio770| M-T321 | Miltenyi       |
| CXCR5  | BV421  | J252D4 | BioLegend      |
## Supplementary Table E5.

### Antibodies and immunostaining panels used for PBMC and spleen

| Immunostaining panel | Antibody | Fluorochrome | Clone | Manufacturer          |
|----------------------|----------|--------------|-------|-----------------------|
| B cells (Mix 1)      | IgM      | FITC         | G20-127 | BD Biosciences        |
|                      | CD21     | PE           | B-LY4  | BD Biosciences        |
|                      | CD27     | APC          | M-T271 | BD Biosciences        |
|                      | CD38     | PerCP-Cy5.5  | HIT2   | BD Biosciences        |
|                      | CD19     | PE-Cy7       | SJ 25C1| BD Biosciences        |
|                      | CD24     | PB           | SN3    | EXBIO                 |
|                      | IgD      | BIO          | IA6-2  | BD Biosciences        |
|                      | Streptavidin | PO   | -      | ThermoFisher           |
| B cells (Mix 2)      | IgA      | FITC         | polyclonal | Jackson Immunoresearch |
|                      | CD21     | PE           | B-LY4  | BD Biosciences        |
|                      | CD27     | APC          | M-T271 | BD Biosciences        |
|                      | CD38     | PerCP-Cy5.5  | HIT2   | BD Biosciences        |
|                      | CD19     | PE-Cy7       | SJ 25C1| BD Biosciences        |
|                      | CD24     | PB           | SN3    | EXBIO                 |
|                      | IgD      | BIO          | IA6-2  | BD Biosciences        |
|                      | Streptavidin | PO   | -      | ThermoFisher           |
| B cells (Mix 3)      | IgG      | FITC         | polyclonal | Jackson Immunoresearch |
|                      | CD21     | PE           | B-LY4  | BD Biosciences        |
|                      | CD27     | APC          | M-T271 | BD Biosciences        |
|                      | CD38     | PerCP-Cy5.5  | HIT2   | BD Biosciences        |
|                      | CD19     | PE-Cy7       | SJ 25C1| BD Biosciences        |
|                      | CD24     | PB           | SN3    | EXBIO                 |
### Antibodies and immunostaining panel used for immunohistochemistry

| Immunostaining panel | Antibody (Primaries) | Chromogen | Clone | Manufacturer |
|----------------------|-----------------------|------------|-------|--------------|
| B cells              | CD20                  | DAB        | L26   | Ventana      |
|                      | Bcl-6                 | DAB        | GI19E/A8 | Cell Marque |
|                      | Bcl-2                 | DAB        | SP66  | Ventana      |
|                      | Ki-67                 | DAB        | 30-9  | Ventana      |
|                      | IgM                   | DAB        | rabbit polyclonal | Cell Marque |
|                      | IgD                   | DAB        | rabbit polyclonal | Cell Marque |
|                      | CD38                  | DAB        | SP149 | Cell Marque |
| T cells              | CD3                   | DAB        | 2GV6  | Ventana      |
|                      | Bcl-2                 | DAB        | SP66  | Ventana      |
|                      | CD4                   | DAB        | SP35  | Ventana      |
|                      | CD8                   | DAB        | SP57  | Ventana      |
|                      | Ki-67                 | DAB        | 30-9  | Ventana      |
| Plasma cells         | kappa chain           | DAB        | rabbit polyclonal | Ventana |
|                      | lambda chain          | DAB        | rabbit polyclonal | Ventana |
|                      | IgM                   | DAB        | rabbit polyclonal | Cell Marque |
|                      | IgD                   | DAB        | rabbit polyclonal | Cell Marque |
|                      | CD38                  | DAB        | SP149 | Cell Marque |
| Tfh cells            | CD3                   | DAB        | 2GV6  | Ventana      |
### Supplementary Table E7.

Primers used for amplification and sequencing of genomic DNA

| Exon | Forward       | Reverse                | Gene   | Patient     |
|------|---------------|------------------------|--------|-------------|
| 68   | GCTGTGCTAGGCTCAAATCC | TTTGGGCAGGAAGTTTGAAT | PRKDC  | CVID003     |
| 15   | CAGGTTTTCCACTTTGCTC | GAACCACACCGTGCTGT | STXBP2 | CVID003     |
| 9    | GAAATGGGAGCAGACCAGGAG | TAGCATGTACATACCAGGCTCT | NOD2   | CVID019     |
| 2    | CAGTCGTTGCGGATGCTAC | AGGGTGCGGAGCGTGACAGCGAG | PRF1   | CVID003, CVID019 |
| 28   | AGAGGAAAGACTCTGAACTGTG | GGAACCCGAGAGTCAAGCA  | PTPRC  | CVID017     |
| 3    | GAGCTGAATTTGATTTCAGGAC | TGAGTACGGAGCTCTACCC | TNFRSF13C | CVID013    |
| 5    | GCCACCCACACACATACAAA | TGCTTTTAAATGGCCAGCAC | LYST2  | CVID028, CVID017 |
| 26   | CGCAGTGCCATTGGATTCTTT | CTGAGGCTCTGTGCTCCACA | INO80  | CVID013     |
| 24   | TGGATGAGATGAAAGGGGCGAG | TCAGAGGGAAGACAGCTCC | RTEL1  | CVID010     |
| 5    | CAGATTGAAAGTGGTGCCGAG | TCCGGGCATAAGAAACCAGTG | TINF2  | CVID010     |
| 5 | GACCCAGTCTGACAGCTTCT | GGTACCCCTTCCTCCCAACAT | UNC119 | CVID017 |
|---|---------------------|-----------------------|--------|--------|
| 8 | AGAGGGAGGAGGACTGTTAGT | CCTTTACCTGATCTCCCC | NOD2 | CVID011 |
| 4 | TGTCTGGTCAGCTTGCTACA | AGCTGGTTCTCTTGAGGACC | RTEL1 | CVID011 |
| 23 | GTTTGATGGAAAGCGTACACA | AGGCTGGCTTGAGGTTTGTA | STAT1 | CVID019 |
| 2 | TCTCGGAGACCAGATTCTGC | CAGTCTCCGAGTCCCCCTAAC | CASP8 | CVID019 |
| 29 | GAAGCAGGAGTTGAGCCAAG | TTCTGGGTCTCCACTCAGAAA | RTEL1 | CVID003 |
| 8 | TTTGAGCCGCTGTTAACAGCC | TGTCCTCCCACCTATCCCA | PI3KCD | CVID028 |
| 4 | CAGCTTGCTACACCGGACCGACATC | AGCTGGTTCTCTTGAGGACC | RTEL1 | CVID028 |
| 24 | GGTAAGCCGGTCTGGTGA | TCACAGAGGAAGACAGCTCC | RTEL1 | CVID024 |
| 8 | CACAGCCAATACCACCACCTG | CTACCTCTCCCCGCTCAC | MST1 | CVID024 |
| 5 | GTCACCCCTACCTAGTGC | AGAAGCTGCAGGTCTCCAC | TNFRSF13B | CVID024 |