**Supplementary Fig. 1**. **Genetic and clinical analysis of index patients.** (**a**) Full pedigree of the index family showing five generations. Squares denote male family members, circles female family members, and slashes deceased family members. (**b**) Endoscopic pictures of gastrointestinal lesions in P2. Upper panels and lower left panel, aphthous lesions in stomach and duodenum (yellow arrowhead). Lower right panel, hemorrhagic lesions in antrum (red arrowhead). (**c**) Pedigree of the index families with chromatograms from capillary sequencing displaying the missense mutation detected in *MAP3K14* (c. C1694G; p. Pro565Arg) in P1 and P2, demonstrating perfect segregation of the variant under the assumption of autosomal recessive inheritance.
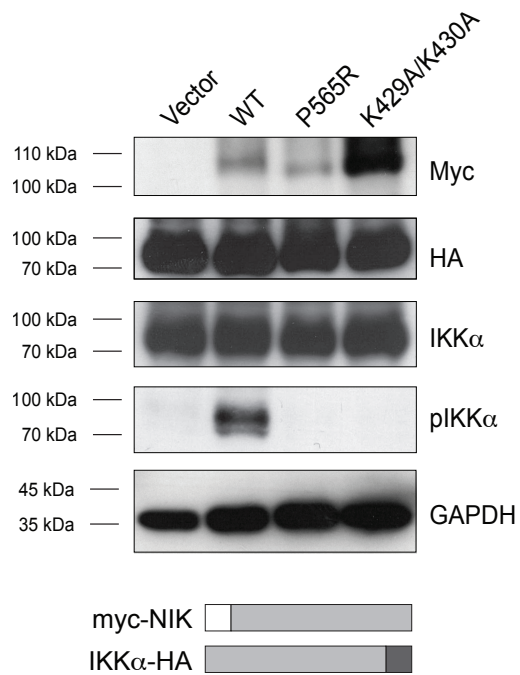
Supplementary Fig. 2. Analysis of kinase activity of NIK variants expressed in HEK293 cells. MYC-tagged NIK[wildtype], NIK[Pro565Arg] or the catalytically inactive mutant NIK[Lys429Arg/Lys430Arg] were transfected into HEK293 cells along with HA-tagged IKKα. Immunoblots were probed with anti-MYC (detecting MYC-NIK), anti-HA (detecting IKKα-HA), anti-IKKα, anti-phospho-IKKα and anti-GAPDH (loading control) antibodies, respectively. Grey bars represent recombinant NIK and IKKα constructs. All uncropped blots can be seen in Supplementary Fig. 9.

a CD40L + IL4

b CD40L + IL21

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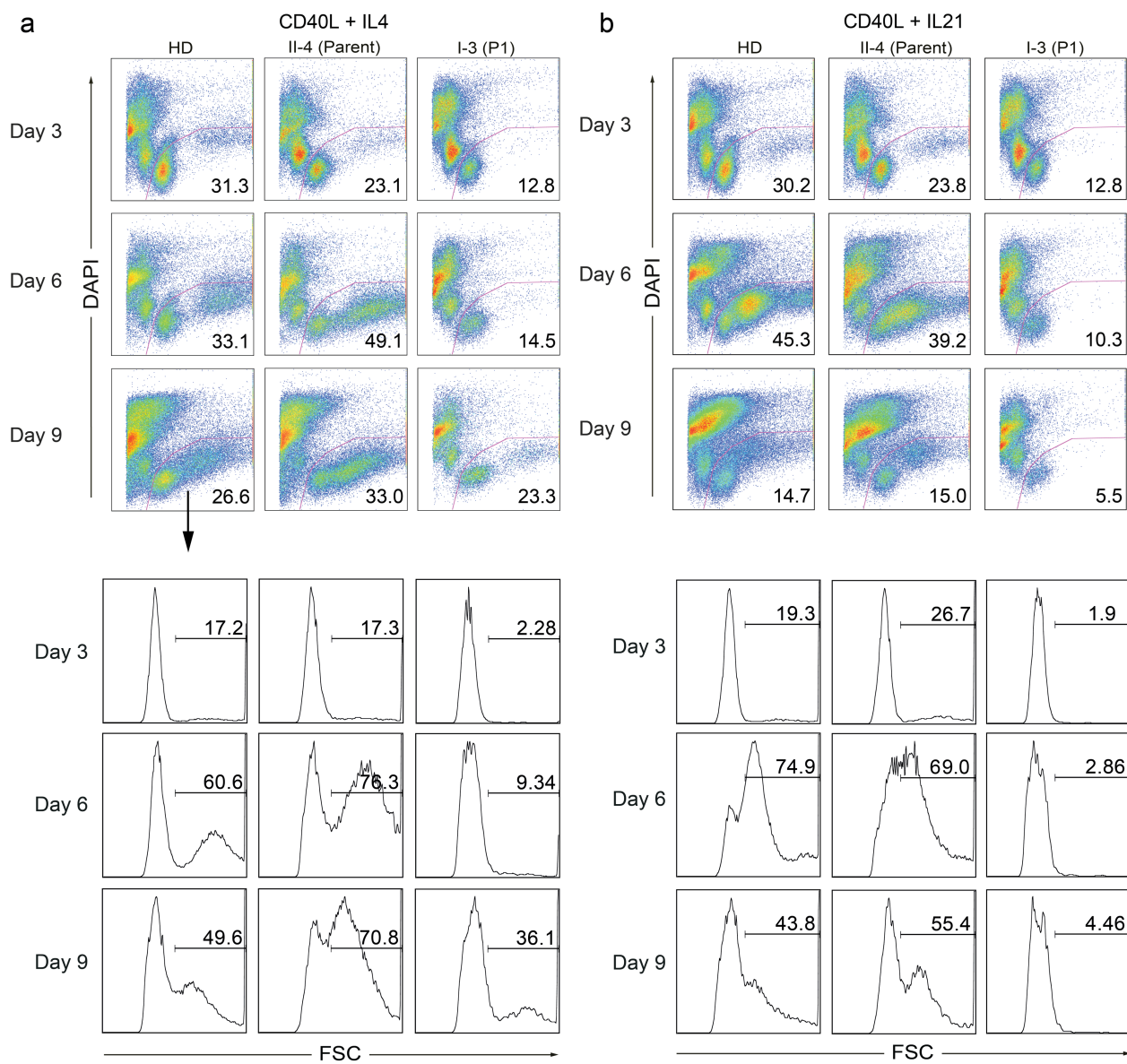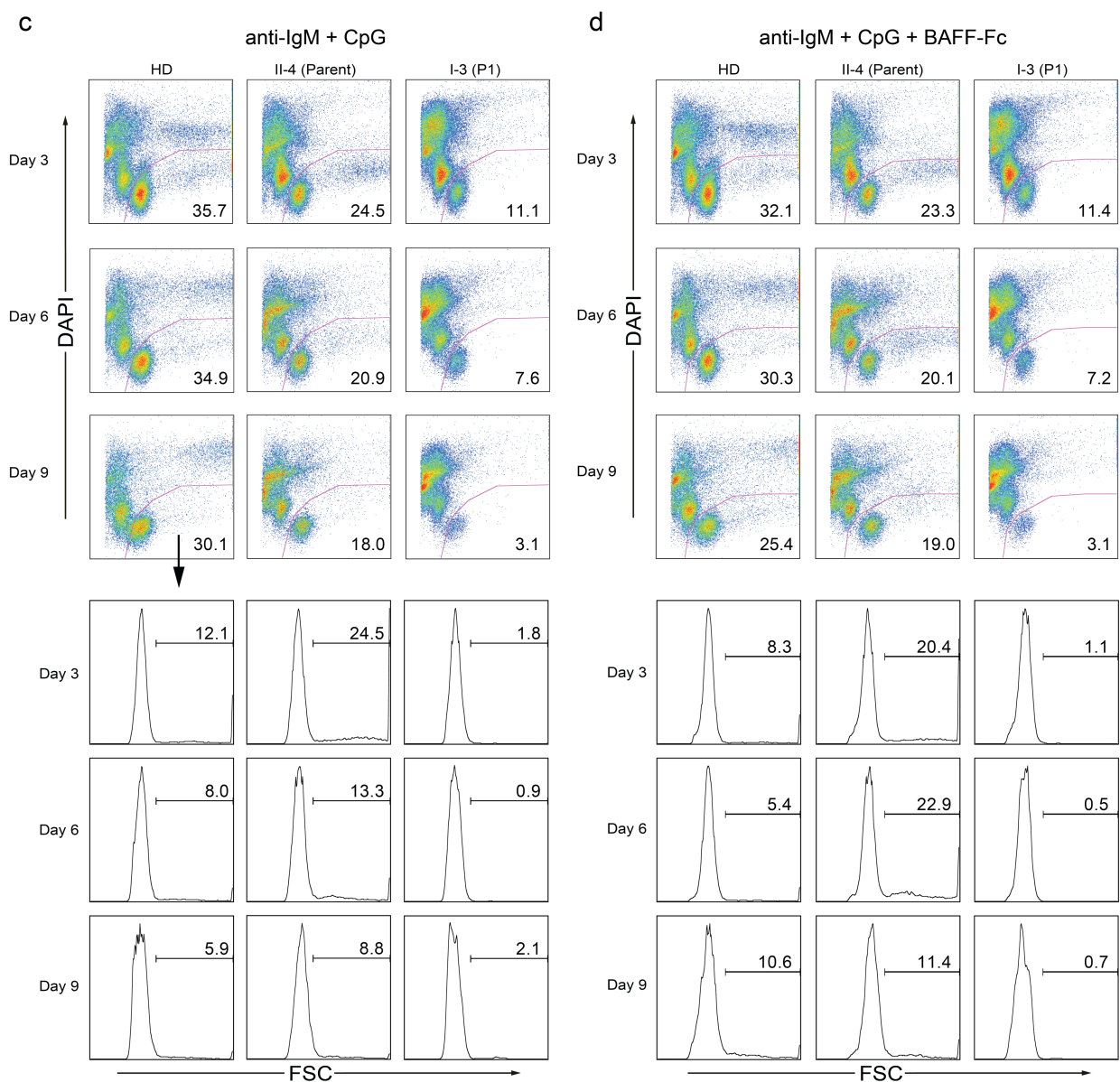
Supplementary Fig. 3. *In vitro* **stimulation of peripheral blood mononuclear cells.** (**a**) Flow cytometry analysis of primary lymphocytes after stimulation with CD40L and IL4. Upper panel: Percentage of DAPI⁻ cells from parents or patient P1 after 3, 6 and 9 days in culture. Lower panel: Forward scatter gate indicating percentages of proliferating blasts, gated on DAPI⁻ cells. (**b**) Flow cytometry analysis of primary lymphocytes after stimulation with CD40L and IL21. Upper panel: Percentage of DAPI⁻ cells from parents or patient P1 after 3, 6 and 9 days in culture. Lower panel: Forward scatter gate indicating percentages of proliferating blasts, gated on DAPI⁻ cells. (**c**) Flow cytometry analysis of primary lymphocytes after stimulation with anti-IgM and CpG. Upper panel: Percentage of DAPI⁻ cells from parents or patient P1 after 3, 6 and 9 days in culture. Lower panel: Forward scatter gate indicating percentages of proliferating blasts, gated on DAPI⁻ cells. (**d**) Flow cytometry analysis of primary lymphocytes after stimulation with anti-IgM, CpG and BAFF-Fc. Upper panel: Percentage of DAPI⁻ cells from parents or patient P1 after 3, 6 and 9 days in culture. Lower panel: Forward scatter gate indicating percentages of proliferating blasts, gated on DAPI⁻ cells.
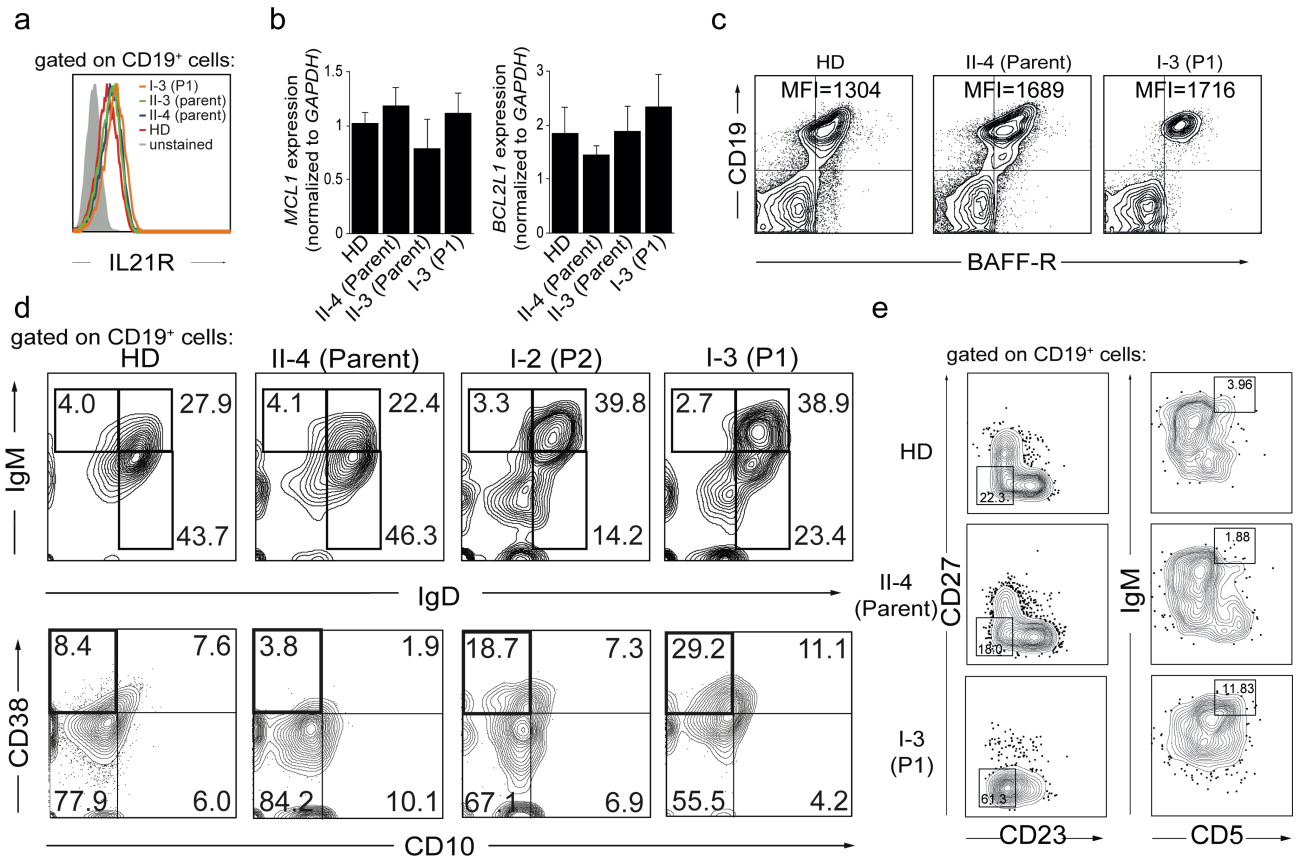
Supplementary Fig. 4. Extended B-cell immunophenotype. (**a**) Expression of IL21R in peripheral blood CD19$^+$ cells of a healthy donor (HD), parents (II-4 and II-3) and patient (I-3) (**b**) Real-time qPCR analysis of *MCL1* and *BCL2L1* expression in sorted peripheral blood naïve B-cells. Transcript expression was normalized to *GAPDH* expression. Mean fold enrichment from one experiment is shown. Error bars denote ±s.d. from three technical replicates. (**c**) Two-dimensional analysis of BAFF-R and CD19 expression on primary lymphocytes after *in vitro* stimulation with CD40L and IL4 for 9 days. MFI – mean fluorescence intensity of BAFF-R expression on CD19$^+$ B-cells. (**d**) Flow cytometric analysis of transitional B-cells gated on CD19$^+$ cells. Relative percentages of transitional B-cell populations defined as total transitional B-cells (IgM^{hi} IgD^{hi}), T1 transitional B-cells (CD38$^+$ CD10$^+$), and T2 transitional B-cells (CD38$^+$ CD10$^-$) in peripheral blood of a healthy donor (HD), parent (II-4) and patients (I-2 and I-3) (**e**) Flow cytometric analysis of transitional B-cells gated on CD19$^+$ cells, displaying decreased CD27$^+$CD23$^-$ cells in P1 cells compared to a healthy donor and increase in transitional CD19$^+$CD23$^-$ CD27$^-$CD5$^+$IgM^{hi} cells in the patient compared to controls.
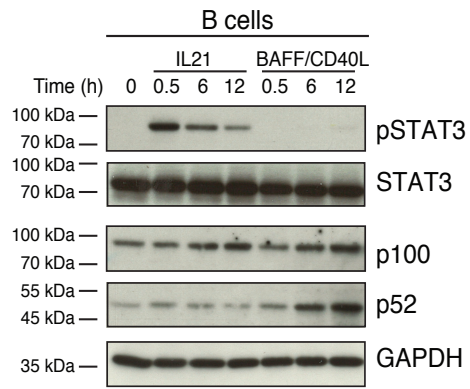
Supplementary Fig. 5. Immunoblot analysis of IL21 stimulated B-cells. Immunoblot analysis of whole cell lysates from healthy donor B-cells sorted from PBMCs after stimulation with IL21 or BAFF and CD40L. Blots were probed for STAT3 and phospho-STAT3 as well as non-canonical NF-κB pathway component p100/p52 and GAPDH as loading control. All uncropped blots can be seen in Supplementary Fig. 9.
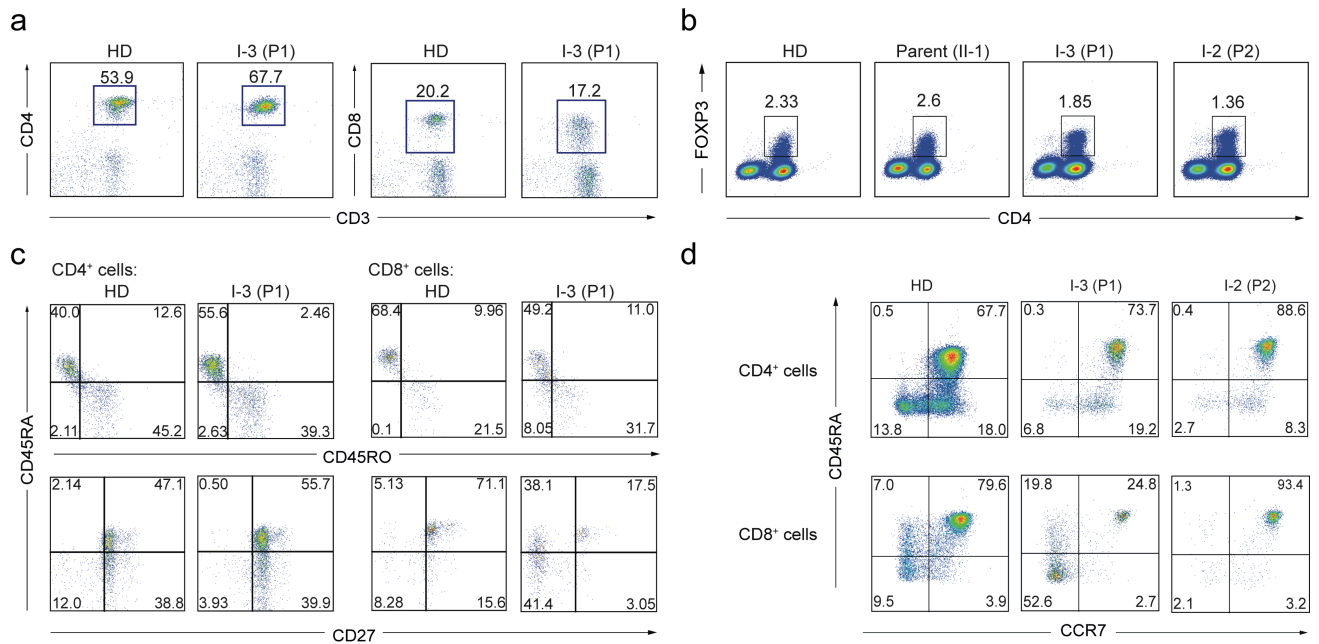
Supplementary Fig. 6. Extended T-cell immunophenotype. (**a**) T helper (CD3⁺CD4⁺) and T cytotoxic (CD3⁺CD8⁺) cell subsets shown for P1 and healthy donor (**b**) P1 and P2 Foxp3 expression (gated on CD3⁺ cells), indicating regulatory T-cells. (**c**) T-cell memory populations represented as T_{naive} CD45RA⁺CD27⁺ and CD45RA⁺CD45RO⁻, T_{CM} (T central memory) CD45RA⁻CD27⁺, T_{EM} (T effector memory) CD45RA⁻CD27⁻, gated on T helper CD3⁺CD4⁺ cells (left) and T cytotoxic CD3+CD8+ cells (right). (**d**) P1, P2 and healthy donor T cell memory populations using the markers CD45RA and CCR7. T_{naive} defined as CD45RA⁺CCR7⁺, T_{CM} defined as CD45RA⁻CCR7⁺, T_{EM} defined as CD45RA⁻CCR7⁻, gated on T helper CD3⁺CD4⁺ cells (upper) and T cytotoxic CD3+CD8+ cells (lower).
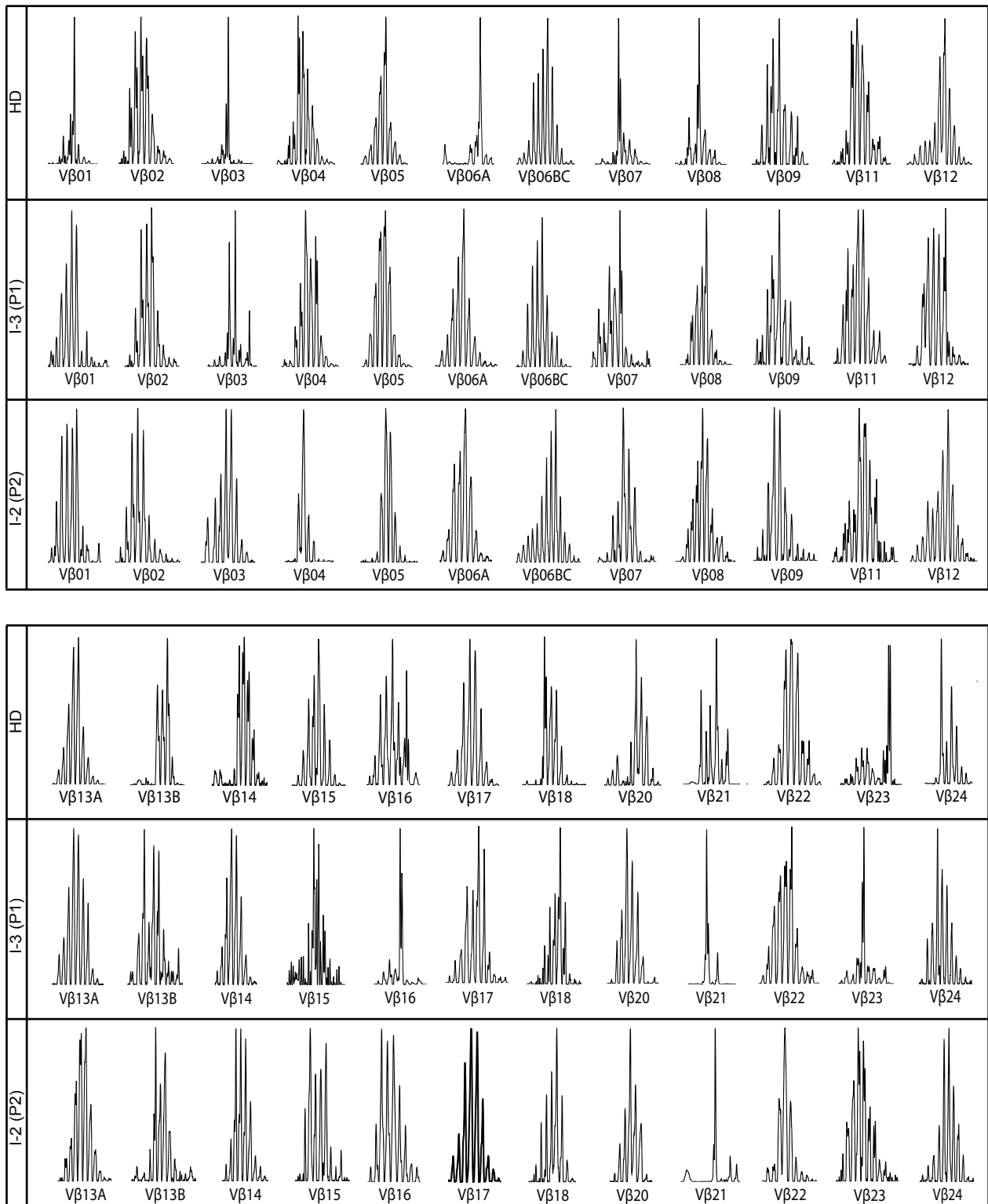
Supplementary Fig. 7. TCR Vβ spectratyping analysis. The analysis of P1 and P2 shows a normal, pseudo-Gaussian usage of the TCR Vβ repertoire comparable to a healthy control. Each Vβ gene segment analyzed is indicated below the respective histogram.
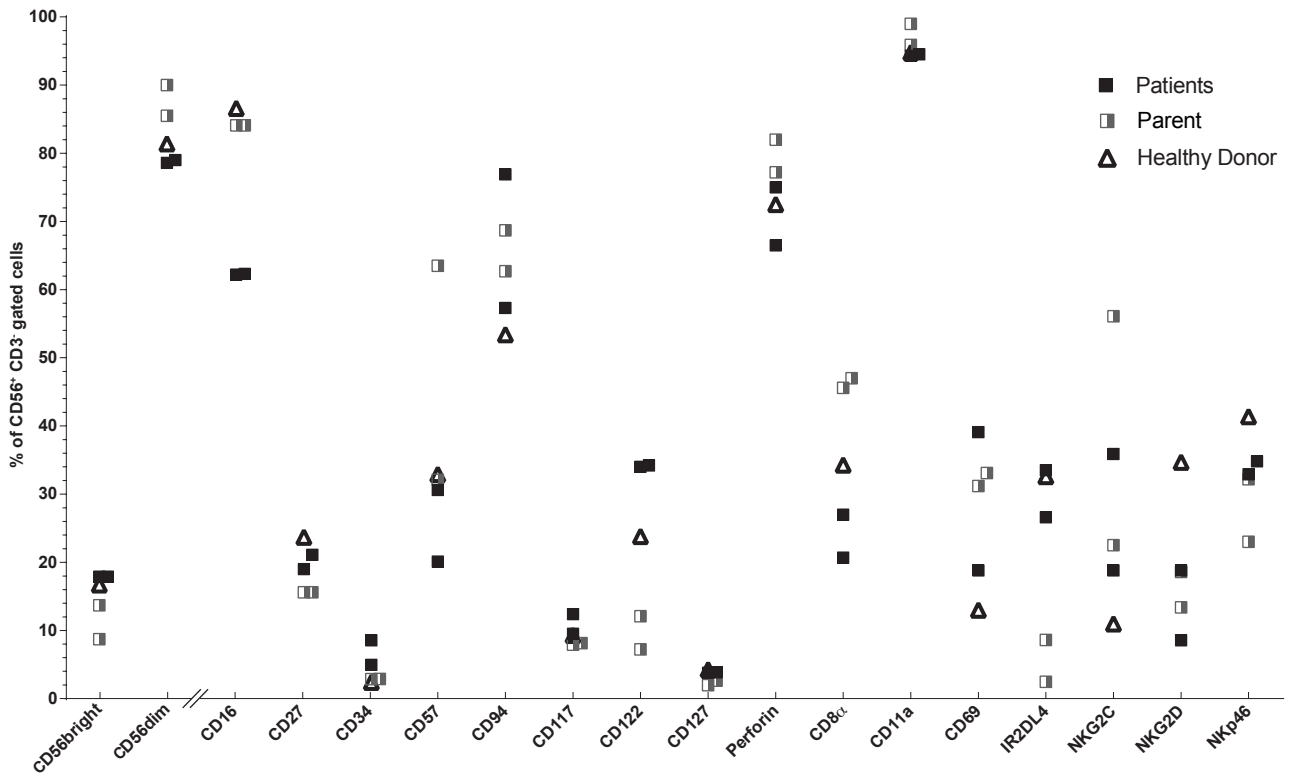
Supplementary Fig. 8. Extended NK immunophenotype. Plots of percentages of NK-cell subsets (all gated on CD3⁻CD56⁺ subsets) in both patients, P1 and P2 (filled squares), parent (half filled squares) and healthy donor control (triangles). NK-cell markers used were CD16, CD27, CD34, CD57, CD94, CD117, CD122, CD127, Perforin, CD8α, CD11a, CD69, KIR2DL4, NKG2C, NKG2D and NKp46 (all in normal range).
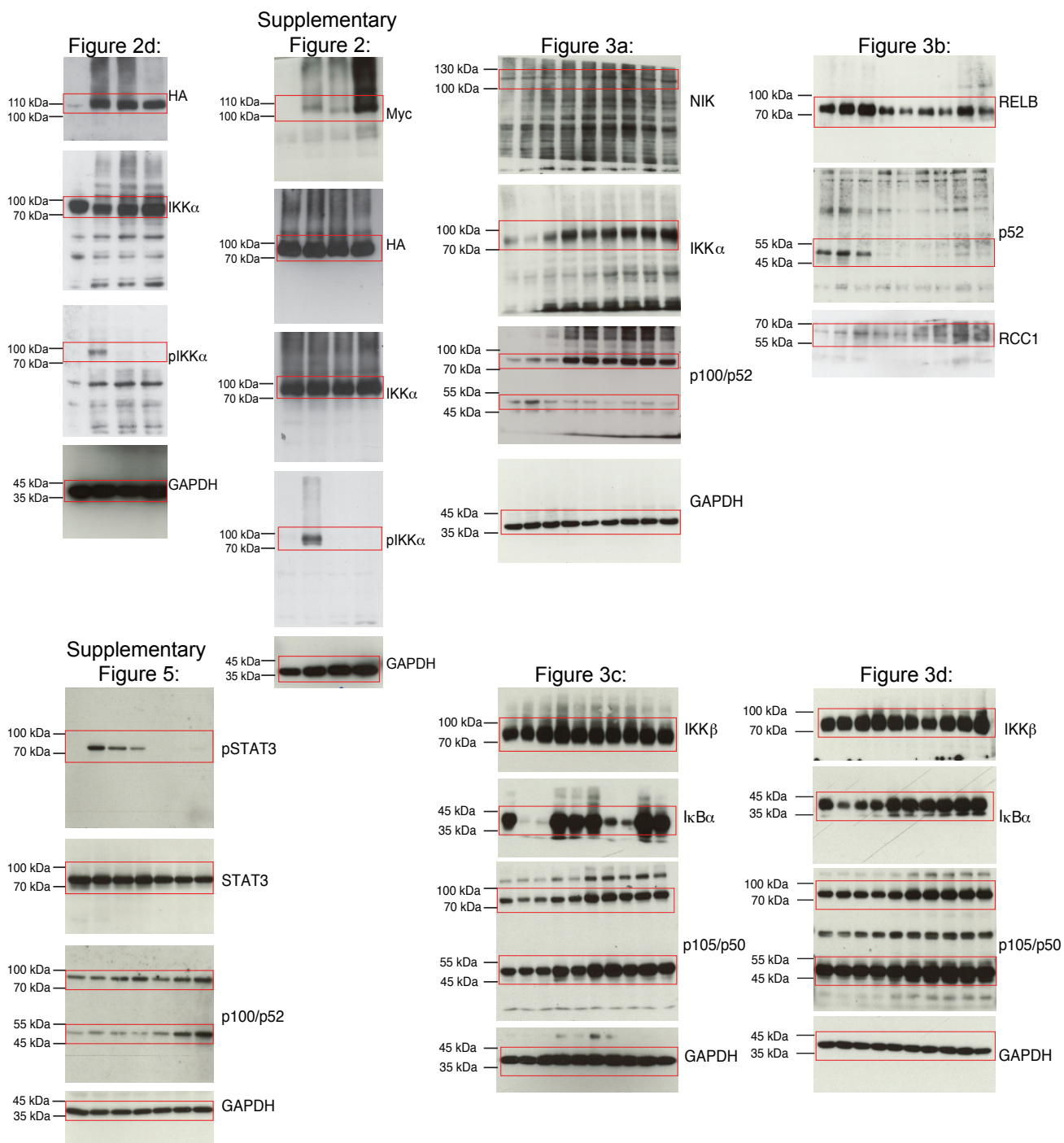
Supplementary Fig. 9. Uncropped immunoblots. Figures corresponding to the blots are indicated above each panel. Red boxes indicate the part of the blot used for cropped presentation.

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Supplementary Tables

Immunoglobulin levels	Patient 1 (I-3)			Patient 2 (I-2)		
Age	10m	7y	9y	1y3m	1y9m	2y5m
IgG (mg/dL)	**120** (300-1000)	914* (600–1300)	1200* (600–1300)	714[?] (350–1000)	1130* (350–1000)	1300* (500-1300)
IgM (mg/dL)	72.8 (30-100)	**205** (40–160)	**20.4** (40–160)	46.9 (40–140)	**23.8** (40–140)	**20.4** (40–180)
IgA (mg/dL)	**13.5** (30-140)	**24.9** (60–220)	**<0.05** (60–220)	**23.6** (30–120)	**6.3** (30–120)	**16.7** (40–180)

Supplementary table 1. Evaluation of immunoglobulin levels in P1 and P2. Reference values of relative cell counts are given in brackets[1].

*Under IgG replacement

[?]Possibly under IgG replacement

	Patient 1 (I-3)		Patient 2 (I-2)					
Age	7y	9y	1y3m	1y9m	2y5m	2y9m	3y	3y8m
Cryptosporidium (stool)	**Pos.**	**Pos.**	Neg.	Neg.	Neg.	Neg.	**Pos.**	Neg.
Giardia	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Rotavirus	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Adenovirus	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
CMV	**Pos.**	Neg.	Neg.	Neg.	Neg.	Neg.	**Pos.**	Neg.
HIV	ND	ND	ND	ND	ND	ND	Neg.	ND
Anti HB	ND	ND	Neg.	ND	ND	ND	ND	ND
Isohemagglutinin Anti B	**Blood group: AB**	ND	ND	ND	ND	**Anti A: 1/32+, Anti B:1/16+**	ND	ND

Supplementary table 2. Infection occurrence in P1 and P2. CMV, Cytomegalovirus; HIV, Human immunodeficiency virus; Pos., Positive; Neg., Negative.

Lymphocytes		Patient 1 (I-3)		Patient 2 (I-2)					
Age		7y	9y	1y3m	1y9m	2y5m	2y9m	3y	3y8m
CD3^{+} T-cells[3]	10[3] cells/µl	**10.6** (1.0-4.9)	**6.7** (1.0-4.9)	4.4 (1.3-6.5)	3.6 (1.3-6.5)	**6.9** (1.9-3.6)	**4.7** (1.9-3.6)	**6.8** (1.9-3.6)	4.5 (1.9-3.6)
	% of total	**93** (57-81)	**89** (57-81)	**93** (51-77)	**80** (51-77)	**91** (55-79)	**95** (55-79)	**96** (55-79)	**94** (55-79)
Naive (CD45RA^{+}) CD3^{+} T-cells[3]	10[3] cells/µl	8.55 (1.0-6.7)	5.47 (1.0-6.7)	4.32 (1.8-4.2)	4.00 (1.8-4.2)	6.84 (0.8-4.4)	4.95 (0.8-4.4)	6.10 (0.8-4.4)	3.02 (0.8-4.4)
	% of total	75 (61-87)	72 (61-87)	90 (82-94)	87 (82-94)	90 (72-97)	99 (72-97)	86 (72-97)	**63** (72-97)
Memory (CD45RO^{+}) CD3^{+} T-cells[3]	10[3] cells/µl	2.96 (0.5-2.0)	2.20 (0.5-2.0)	0.480 (0.4-3.3)	0.644 (0.4-3.3)	0.760 (0.4-1.6)	1.35 (0.4-1.6)	0.994 (0.4-1.6)	1.48 (0.4-1.6)
	% of total	26 (22-53)	29 (22-53)	10 (9-45)	14 (9-45)	**10** (16-38)	27 (16-38)	**14** (16-38)	31 (16-38)
CD3^{+} HLA-DR^{+} T-cells[2]	10[3] cells/µl	0.684 (0.05-0.7)	0.988 (0.05-0.7)	0.192 (0.1-0.6)	0.506 (0.1-0.7)	**7.140** (0.08-0.4)	0.350 (0.08-0.4)	0.284 (0.08-0.4)	0.336 (0.08-0.4)
	% of total	6 (3-14)	13 (3-14)	4 (2-8)	11 (3-12)	**94** (3-13)	7 (3-13)	4 (3-13)	7 (3-13)
CD3^{+}CD4^{+} T-cells[3]	10[3] cells/µl	**8.6** (0.5-2.7)	**5.0** (0.5-2.7)	3.0 (0.7-4.5)	2.3 (0.7-4.5)	**4.7** (0.6-2.0)	**3.8** (0.6-2.0)	**4.4** (0.6-2.0)	**3.0** (0.6-2.0)
	% of total	**76** (24-47)	**66** (24-47)	**63** (29-55)	52 (29-55)	**63** (26-49)	**76** (26-49)	**63** (26-49)	**63** (26-49)
Naive (CD45RA^{+}) CD4^{+} T-cells[3]	10[3] cells/µl	**6.2** (0.3-2.4)	**3.8** (0.3-2.4)	1.48 (0.5-4.2)	1.93 (0.5-4.2)	4.25 (0.5-6.6)	2.4 (0.5-6.6)	3.69 (0.5-6.6)	ND
	% of total	**55** (17-40)	**50** (17-40)	**55** (19-49)	42 (19-49)	**56** (20-41)	**48** (20-41)	**52** (20-41)	ND
Memory (CD45RO^{+}) CD4^{+} T-cells[3]	10[3] cells/µl	**2.7** (0.2-1.0)	1.2 (0.2-1.0)	0.568 (0.2-1.4)	0.552 (0.2-1.4)	0.76 (0.2-0.8)	**1.3** (0.2-0.8)	0.923 (0.2-0.8)	ND
	% of total	24 (9-23)	16 (9-23)	8 (5-18)	12 (5-18)	10 (8-42)	26 (8-42)	13 (8-42)	ND
CD3^{+}CD8^{+} T-cells[3]	10[3] cells/µl	2.1 (0.3-2.1)	2.0 (0.3-2.1)	1.5 (0.4-3.2)	1.2 (0.4-3.2)	**1.9** (0.3-1.3)	1.00 (0.3-1.3)	**2.6** (0.3-1.3)	1.4 (0.3-1.3)
	% of total	19 (17-37)	27 (17-37)	31 (15-33)	28 (15-33)	25 (9-35)	20 (9-35)	29 (9-35)	29 (9-35)
TCRγδ	10[3] cells/µl	114	152	48	46	76	ND	ND	ND
	% of total	1	2	1	1	1	ND	ND	ND
CD19^{+} B-cells[3]	10[3] cells/µl	0.342 (0.2-2.2)	0.228 (0.2-2.2)	**0.096** (0.5-3.6)	**0.322** (0.5-3.6)	**0.228** (0.3-1.2)	**0.100** (0.3-1.2)	**0.099** (0.3-1.2)	**0.01** (0.3-1.2)
	% of total	**3** (10-27)	**3** (10-27)	**2** (17-41)	**7** (17-41)	**1** (11-31)	**2** (11-31)	**1.4** (11-31)	**0.2** (11-31)
CD20^{+} B-cells[3]	10[3] cells/µl	0.456 (0.2-2.0)	ND	**0.096** (0.5-3.3)	**0.368** (0.5-3.3)	**0.228** (0.3-1.2)	**0.100** (0.3-1.2)	**0.078** (0.3-1.2)	**0.0048** (0.3-1.2)
	% of total	**4** (11-25)	3 (11-25)	**2** (16-41)	**8** (16-41)	**1** (11-29)	**2** (11-29)	**1.1** (11-29)	**0.1** (11-29)
CD3^{-}CD16^{+} CD56^{+} NK-cells[3]	10[3] cells/µl	**0.114** (0.2-0.9)	**0.076** (0.2-0.9)	**0.144** (0.2-1.3)	0.460 (0.2-1.3)	0.304 (0.2-1.2)	**0.075** (0.2-1.2)	0.142 (0.2-1.2)	0.096 (0.2-1.2)
	% of total	**1** (8-28)	**1** (8-28)	**3** (4-15)	10 (4-15)	**4** (5-28)	**1.5** (5-28)	**2** (5-28)	**2** (5-28)

Supplementary table 3. Evaluation of lymphocyte subpopulations. Age-matched reference values to absolute and relative cell counts are given in brackets[2,3], specified in leftmost column. Values deviating from the reference ranges are printed in bold.

Chromosome	Position	Position	Position
15	44.38-44.59	rs17583428	rs12594645
15	72.45-73.12	rs2959944	rs2053854
17	43.05-43.38	rs17547180	rs17686001
17	43.83-44.85	rs6503447	rs199515
17	46.58-47.19	rs4793882	rs11653468
17	50.01-51.77	rs203090	rs9899373

Homozygous intervals detected in P1		Homozygous intervals detected in P2	
Chromosome	**Position**	**Chromosome**	**Position**
1	2.4 - 3.81	1	109.82 - 111.63
2	161.61 - 162.67	1	113.09 - 114.81
2	203.47 - 204.48	1	117.55 - 118.87
3	119.89 - 121.63	1	120.05 - 121.29
3	137 - 138.7	1	149.89 - 151.67
3	138.91 - 139.98	2	131.11 - 132.16
3	144.15 - 146.52	4	0.05 - 1.25
3	175.46 - 176.7	5	133.08 - 134.63
3	191.82 - 193.06	5	136.44 - 140.09
3	194.56 - 195.67	5	140.44 - 141.45
4	32.79 - 34.35	5	143.34 - 145.78
4	93.15 - 94.57	5	158.68 - 160.07
4	96.53 - 97.69	7	26.16 - 28.05
4	98.96 - 100.64	8	99.38 - 100.59
4	103.96 - 109.08	8	144.6 - 145.76
4	111.26 - 112.76	9	134.09 - 135.42
4	114.8 - 116.09	9	138.32 - 139.51
4	116.91 - 118.64	10	35.22 - 36.33
4	177.98 - 179.36	10	112.61 - 113.63
4	180.55 - 182.06	10	117.49 - 119.04
5	36.36 - 37.41	12	56.12 - 57.98
5	38.76 - 39.84	15	43.53 - 44.59
5	40.95 - 42.07	15	63.71 - 66.25
5	42.78 - 44.98	15	72.46 - 74.15
5	49.56 - 52.1	15	74.17 - 76.49
5	53.84 - 56.01	15	76.67 - 77.81
5	56.04 - 57.64	15	79.97 - 81.08
5	58.18 - 59.39	15	81.82 - 83.25
5	60.53 - 62.07	15	87.64 - 88.69

Table continued

Chromosome	Position	Chromosome	Position
5	58.18 - 59.39	15	89.72 - 90.89
5	60.53 - 62.07	16	47 - 48.23
5	70.67 - 71.76	16	67.08 - 68.43
5	71.92 - 73.52	17	37.04 - 38.1
6	95.33 - 96.38	17	39.3 - 41.94
6	98.75 - 100.29	17	42.05 - 43.39
6	100.29 - 101.69	17	43.83 - 47.19
6	102.79 - 104.15	17	50.01 - 51.78
7	102.1 - 103.22	17	55.9 - 57.47
7	118.51 - 120.2	17	71.43 - 72.48
11	58.04 - 59.06	23	51.29 - 53.03
11	59.53 - 61.71	23	55.62 - 58.23
11	101.35 - 102.36	23	71.56 - 73.47
13	91.01 - 92.63	23	73.86 - 75.31
13	93.43 - 94.78	23	80.17 - 81.37
14	66.46 - 67.92	23	105.3 - 106.92
15	20.07 - 23.04	23	107.49 - 108.63
15	44.38 - 45.38	23	109.4 - 111.32
15	72.04 - 73.12		
17	43.05 - 44.86		
17	46.59 - 48.29		
17	49.51 - 52.77		
23	53.92 - 54.99		
23	63.19 - 65.38		
23	73.76 - 75.3		
23	107.3 - 108.68		
23	126.32 - 128.1		

Supplementary table 4. Homozygosity mapping of both patients showing homozygous intervals (analysed by PLINK algorithm[4] version 1.07). Upper part: Intersection of homozygous intervals shared by both patients P1 and P2. The interval containing *MAP3K14* is highlighted in dark grey. Lower part: Full list of homozygous intervals in both patients.

Amino Acid Mutations			
Amino acid	Overall stability	Torsion	Predicted ΔΔG (kcal/mol)
GLY	Stabilising	Unfavourable	0.65
ALA	Stabilising	Favourable	2.59
VAL	Stabilising	Unfavourable	2.59
LEU	Stabilising	Favourable	3.84
ILE	Stabilising	Unfavourable	3.01
MET	Stabilising	Favourable	3.44
TRP	Stabilising	Unfavourable	1.53
SER	Stabilising	Unfavourable	0.29
THR	Destabilising	Unfavourable	0.42
PHE	Stabilising	Unfavourable	3.94
GLN	Destabilising	Favourable	0.46
LYS	Destabilising	Favourable	4.59
TYR	Stabilising	Unfavourable	1.16
ASN	Destabilising	Unfavourable	0.94
CYS	Stabilising	Unfavourable	1.86
GLU	Destabilising	Favourable	0.56
ASP	Destabilising	Unfavourable	2.08
ARG	Destabilising	Favourable	2.07
HIS	Destabilising	Unfavourable	2.88

Supplementary table 5. NIK^{Pro565Arg} protein stability prediction calculated by the CUPSAT tool. This tool predicts change in the folding stability upon an amino acid point mutation in a protein of known structure[5]. Algorithm was run on http://cupsat.tubs.de/jsp/nmrpredict.jsp. using pdb protein entry 4G3D.PDB (NIK catalytic domain). Proline 565 to Arginine mutation prediction marked in dark grey.

Supplementary Note: Patient Case Reports

Patient 1 (P1) is a 9-year-old girl born to consanguineous healthy parents (**Supplementary Fig. 1a**). The patient had a younger brother who died at the age of 2 years from suspected combined immunodeficiency. Decreased IgG (pre-intravenous immunoglobulin (Ig) substitution treatment) and IgA levels as well as elevated levels of IgM were identified on a single occasion (**Supplementary Table 1**). Cytomegalovirus (CMV) detected by PCR and *Cryptosporidium* present in the stool (**Supplementary Table 2**) were reminiscent of a class switch recombination defect, however no mutations in *CD40*, *CD40L* or *IL21R* were found. Despite supportive treatment including regular intravenous Ig substitution treatment and ganciclovir, she continued to suffer from repeated episodes of viral and bacterial infections as well as from an episode of granulomatous hepatitis and tuberculosis osteomyelitis due to dissemination after Bacillus Calmette Guérin (BCG) vaccination. An allogeneic hematopoietic stem cell transplantation (HSCT) after reduced toxicity conditioning was performed at 9 years of age. She remains clinically well. (A. Ikincioğulları and A. Küpesiz, unpublished).

Patient 2 (P2) is a first-degree cousin of P1 also born to consanguineous healthy parents (**Supplementary Fig. 1a**). She presented with recurrent lower respiratory tract infections, oral and oesophageal candidiasis and severe chronic diarrhea resistant to budesonide-mesalasine (**Supplementary Fig. 1b**). She was tested positive for *Cryptosporidium* on one occasion (**Supplementary Table 2**), later on she also developed cholestasis with deterioration of liver function. Her mother was HLA-identical and used as a donor for an allogeneic HSCT performed without conditioning at the age of 3. As no engraftment was observed after 50 days, a second transplant from the same donor was performed, However, the patient deceased on day 6 following the second HSCT due to rapidly accelerated septic shock and multi-organ failure (A. Ikincioğulları and A. Küpesiz, unpublished).

Supplementary References

1   Center for chronic immunodeficiency, F., Germany. http://www.uniklinik-freiburg.de/cci/live/diagnostik/immunologische/NormwerteCCILaborDiagnostik.pdf. (January 2014).

2   Comans-Bitter, W. M. *et al.* Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. *J Pediatr* **130**, 388-393 (1997).

3   Ikinciogullari, A. *et al.* Peripheral blood lymphocyte subsets in healthy Turkish children. *Turk J Pediatr* **46**, 125-130 (2004).

4   Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**, 559-575, doi:10.1086/519795 (2007).

5   Parthiban, V., Gromiha, M. M. & Schomburg, D. CUPSAT: prediction of protein stability upon point mutations. *Nucleic Acids Res* **34**, W239-242, doi:10.1093/nar/gkl190 (2006).