Supplemental Figure Legends

Figure E1. IGV plot of large *NFKB1* deletion. IGV plot of patient I:II-1 showing the large *NFKB1* deletion identified by 50% reduction in the number of reads from the whole genome sequencing data mapping to that region.

Figure E2. Numbers of CVID patients per gene in which we identified a likely pathogenic variant fully explaining the patient's phenotype. Assessment of all 390 CVID cases in our cohort identified 31 patients with a monogenic defect in 11 different genes. Variants in *NFKB1* contributed to more than half of all CVID patients with a monogenic diagnosis (16/31, 52%). *NKFB2* and *BTK* were the next most commonly implicated genes, with three explained cases each.

Figure E3. Protein model of high-impact missense variants in and proximal to the ANK domain. Residues observed with missense variants containing a high CADD score (≥20) are highlighted. Most of these are situated on the protein exterior (green) and appear equally in the primary immunodeficiency cohort and non-primary immunodeficiency cohorts (**Figure 2)**. A623G (#19) appears to be located more interior, although mutation of an Alanine to Glycine is a moderate substitution. Equally notable are residues of the Ankyrin repeats which previously have been probed as interaction sites upon NF-κB dimerization. While R614 (#7), K684 (#8), L517 (#13) and R687 (#16) could be considered part of these putative interaction sites, variants at these sites are found in non-primary immunodeficiency patients (**Figure 2**).

Figure E4. Western blot analysis of all tested *NFKB1* variant carriers. Western blot analysis targeting p50, IκBα and GAPDH of *NFKB1* variant carriers. Twelve patients with truncating variants (Arg284*, His513Glnfs*28, c.160-1G>A and Asp451*), one patient with gene deletion (del 103370996-103528207) and three patients with putative protein destabilizing missense variants (Ile281Met, Val98Asp and Ile87Ser) were tested. Relative fluorescence quantification of p50 and GAPDH by Odyssey Infrared Imaging system above and below each western blot. IκBα was not targeted in the Case D western blot.

Figure E5. Serum IgM, IgG and IgA levels in serum of *NFKB1* LOF variant carrier. Each dot represents an *NFKB1* LOF variant carrier and their age. In grey age-dependent reference values.

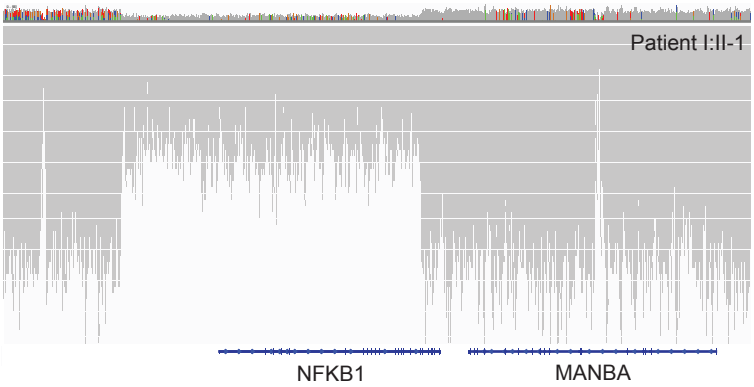
Figure E6. Gating strategy for Figure 5B-E and additional B cell analyses. **(A)** Representative flow cytometry plots of a healthy control, patient A:II-1 (clinically unaffected) and patient A:II-4 (clinically affected). Phenotype of $CD19^+CD20^+$ B lymphocytes. Numbers represent percentages in corresponding quadrants. **(B)** Percentages of $CD27^-IgD^+$ (naïve) or $CD27^-IgD^+CD24^+CD38^+$ (transitional) B cells. (*HD* healthy donor, *NFKB1*$^{+/-}$ individual with *NFKB1* LOF variant.) Only individuals with sufficient B cells could be analyzed. P-values were determined by two-way ANOVA with Bonferroni post-hoc test (naïve B cells) or Student's t-test (transitional B cells), *ns* not significant, **P≤0.01.

Figure E7. Gating strategy Figure 6A and 6B and formation of $CD38^+$ plasmablasts and IgA production. **(A)** Representative flow cytometry plots of a healthy control and patient A:II-4 (clinically affected) after a 6 day culture of CFSE-labeled lymphocytes normalized for B cell number unstimulated, CpG/IL-2 (T cell independent activation) and anti-IgM/anti-CD40/IL-21 (T cell dependent activation). B cells gated on $CD19^+CD20^{-/+}$ and subsequently on $CD27^{++}$ (left) and CFSE and CD38 (right). Numbers represent percentages in corresponding quadrants. **(B)** Plasmablast formation measured by proliferation and CD38 upregulation ($CFSE^-CD38^+$). **(C)** IgA production in the supernatant. (*HD* healthy donor; *CU* clinically unaffected or *CA* clinically affected individuals with LOF variant in *NFKB1*.) Only individuals with sufficient B cells could be analyzed. P-values were determined by two-way ANOVA with Bonferroni post-hoc test, *ns* not significant, **P≤0.01, ***P≤0.001.

Figure E8. Additional lymphocyte numbers in individuals with *NFKB1* LOF variants. Absolute numbers of total lymphocytes ($CD45^+$), $CD4^+$ and $CD8^+$ T cells, NK cells ($CD3^-CD16^+CD56^+$) and invariant natural killer T cells ($CD3^+V\alpha24^+V\beta11^+$). Each dot represents a single individual and their age. In grey age-dependent normal values.

Figure E9. Normal T cell differentiation in individuals with NF-κB1 deficiency. (A) Representative flow cytometry dot plots of a healthy control and patient E:II-1, defining the differentiation of $CD3^+CD4^+$ and $CD3^+CD8^+$ T lymphocytes with CD27 and CD45RA. **(B, C)** Summary of subsets of **(B)** $CD4^+$ T cells and **(C)** $CD8^+$ T cells. (*HD* healthy donor, *NFKB1*$^{+/-}$ individual with *NFKB1* LOF variant.) P-values were determined by two-way ANOVA with Bonferroni post-hoc test, *ns* not significant.

Figure E10. T cells of individuals with *NFKB1* variants (*NFKB1*$^{+/-}$) show normal proliferative capacity. 6 day culture of CFSE-labeled lymphocytes unstimulated, anti-CD3/anti-CD28 (T cell receptor stimulation) or IL-15. Percentage of $CD4^+$ or $CD8^+$ T cell specific cell division as measured by CFSE dilution. (*HD* healthy donor, *NFKB1*$^{+/-}$ individual with *NFKB1* LOF variant.) P-values were determined by two-way ANOVA with Bonferroni post-hoc test, *ns* not significant.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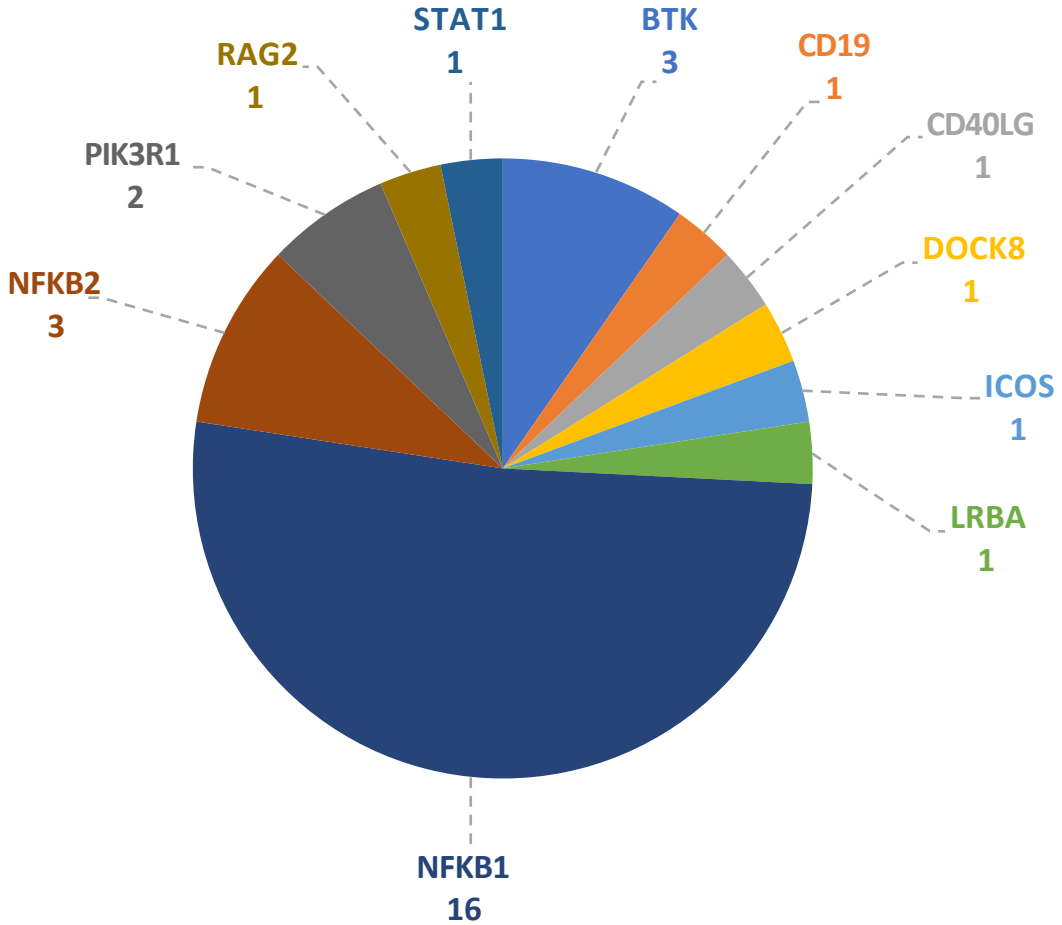
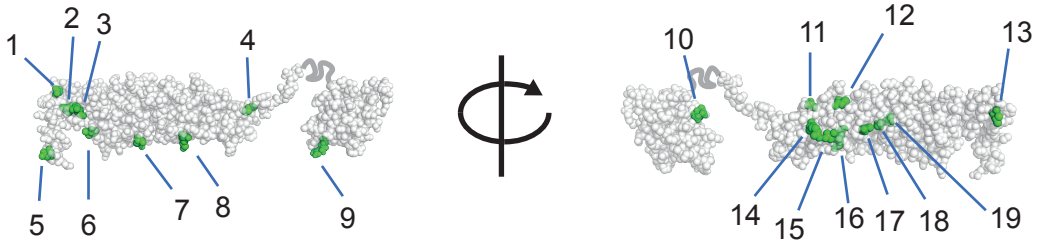
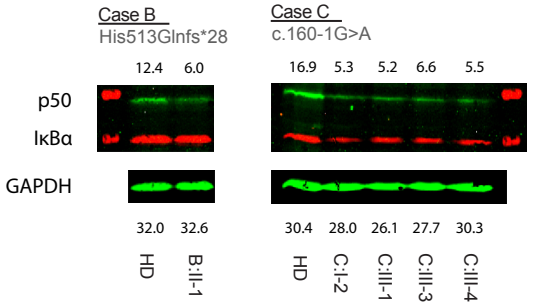
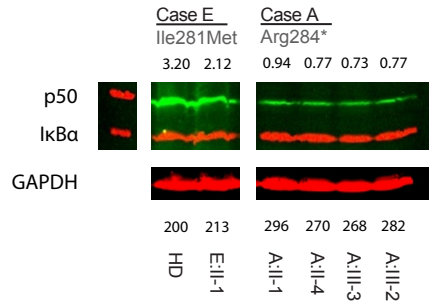
NFKB1
MANBA

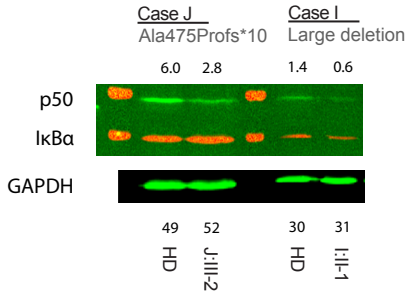

STAT1
1
BTK
3
CD19
1
CD40LG
1
DOCK8
1
ICOS
1
LRBA
1
NFKB1
16
NFKB2
3
PIK3R1
2
RAG2
1

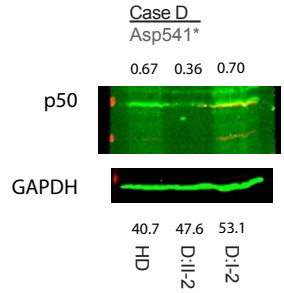


1. D519N
2. V532I
3. R534H
4. P793R
5. N500K
6. V539L
7. R614G
8. K684N
9. R850W
10. V865I
11. A742T
12. C704F
13. L517V
14. R734M
15. D697N
16. R687H
17. S662N
18. A624T
19. A623G


Case B
His513Glnfs*28
Case C
c.160-1G>A
12.4 6.0
16.9 5.3 5.2 6.6 5.5
p50
IκBα
GAPDH
32.0 32.6
30.4 28.0 26.1 27.7 30.3
HD
B:II-1
HD
C:I-2
C:III-1
C:III-3
C:III-4


Case E
Ile281Met
Case A
Arg284*
3.20 2.12
0.94 0.77 0.73 0.77
p50
IκBα
GAPDH
200 213
296 270 268 282
HD
E:II-1
A:II-1
A:II-4
A:III-3
A:III-2


Case J
Ala475Profs*10
Case I
Large deletion
6.0 2.8
1.4 0.6
p50
IκBα
GAPDH
49 52
30 31
HD
J:III-2
HD
I:II-1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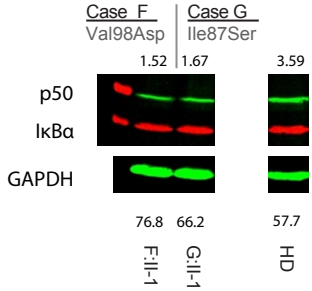
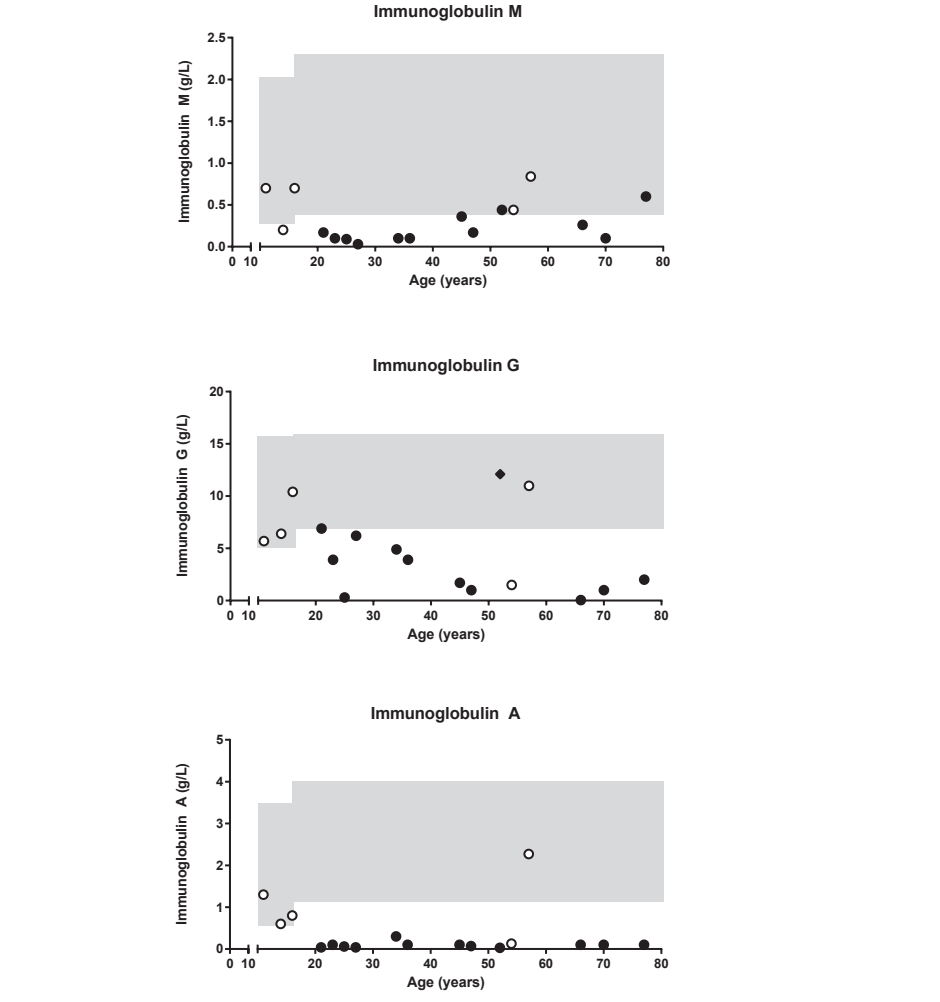
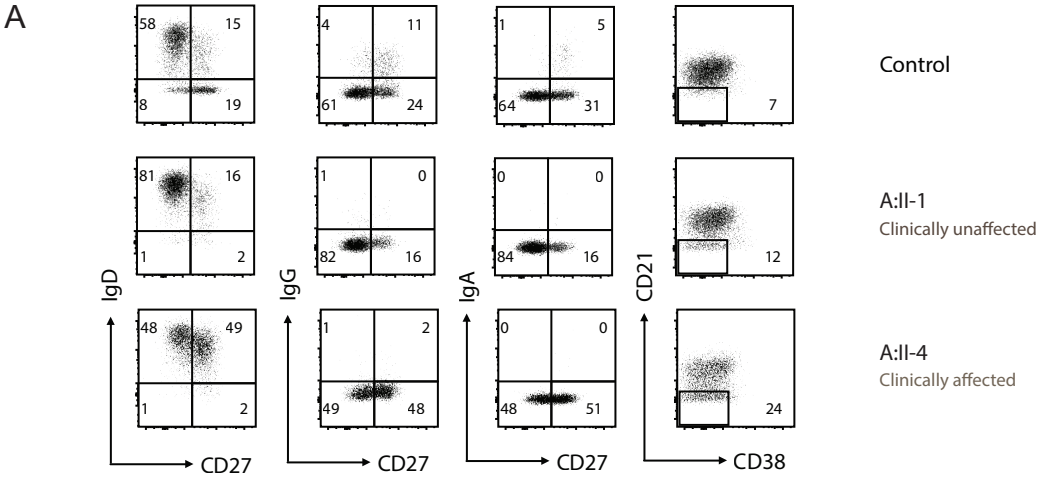
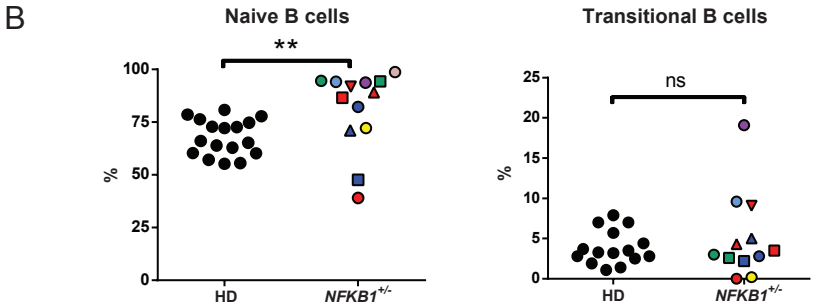
Asp541*
0.67 0.36 0.70
p50
GAPDH
40.7 47.6 53.1
HD
D:II-2
D:I-2


Case F
Val98Asp
Case G
Ile87Ser
1.52 1.67
3.59
p50
IκBα
GAPDH
76.8 66.2
57.7
F:II-1
G:II-1
HD


Immunoglobulin M
Immunoglobulin M (g/L)
2.5
2.0
1.5
1.0
0.5
0.0
0 10 20 30 40 50 60 70 80
Age (years)
Immunoglobulin G
Immunoglobulin G (g/L)
20
15
10
5
0
0 10 20 30 40 50 60 70 80
Age (years)
Immunoglobulin A
Immunoglobulin A (g/L)
5
4
3
2
1
0
0 10 20 30 40 50 60 70 80
Age (years)
Clinically affected
Clinically unaffected
Clinically affected (post IVIG)





Case A
Arg284*
- A:II-1
- A:II-4
- A:III-2

Case B
His513Glnfs*28
- B:II-1

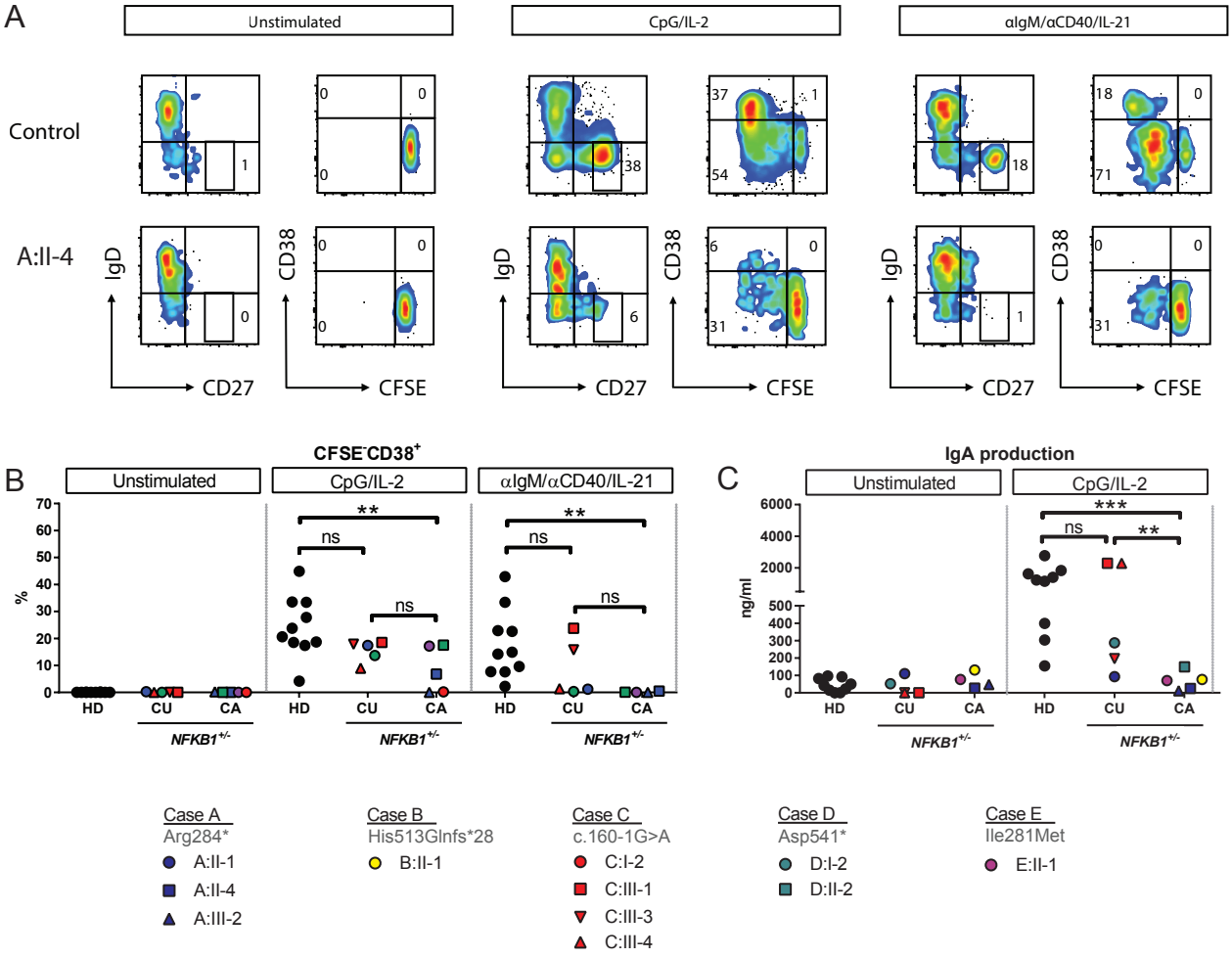
Case C
c.160-1G>A
- C:I-2
- C:III-1
- C:III-3
- C:III-4

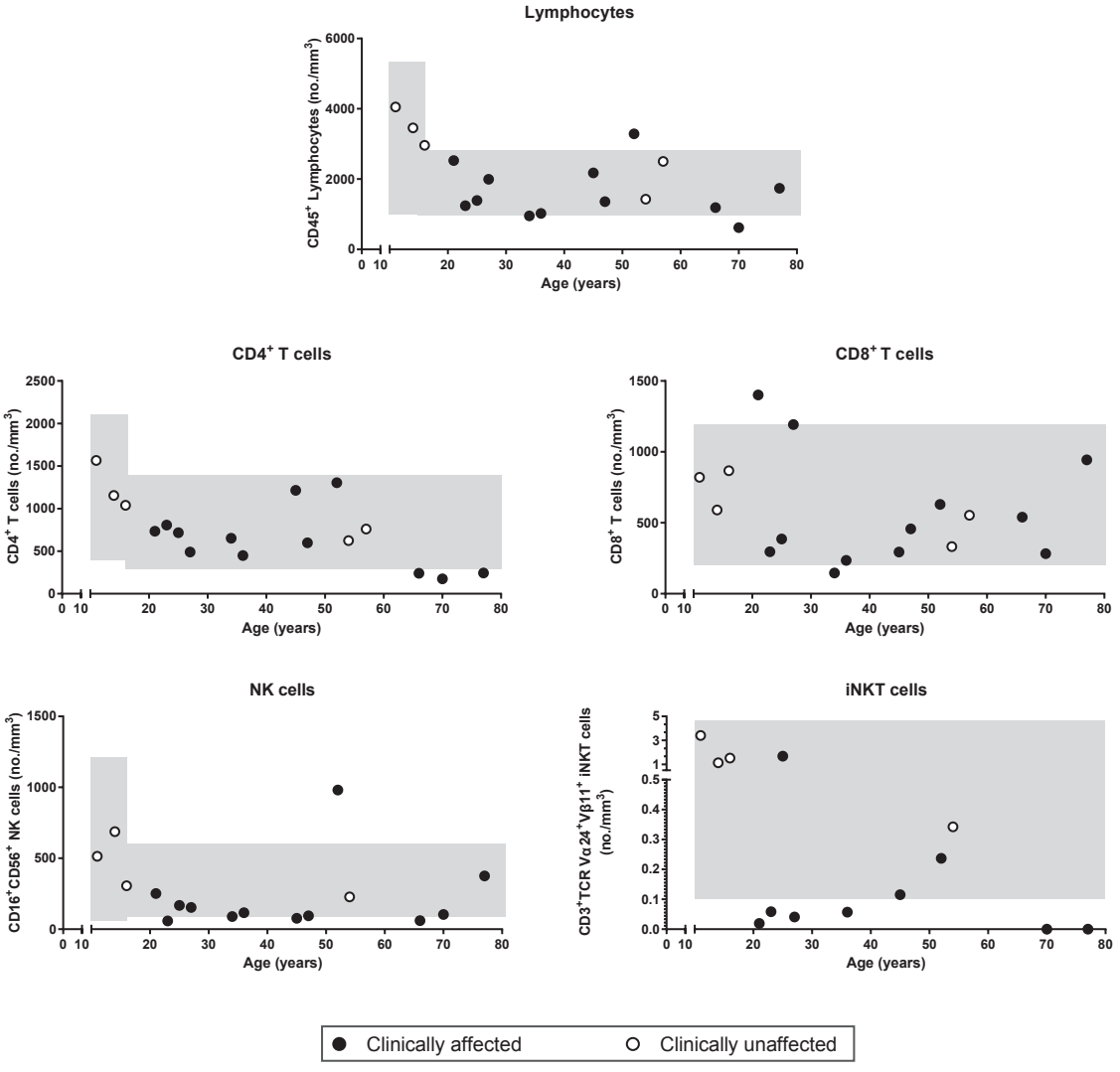
Case D
Asp541*
- D:I-2
- D:II-2

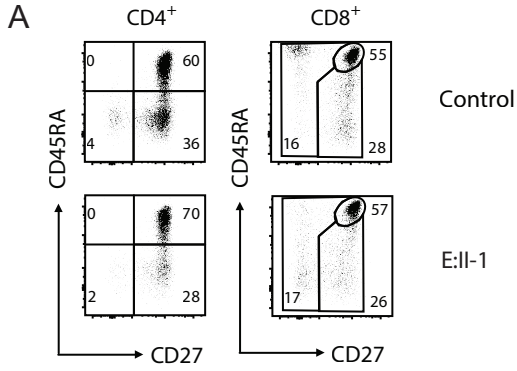
Case E
Ile281Met
- E:II-1

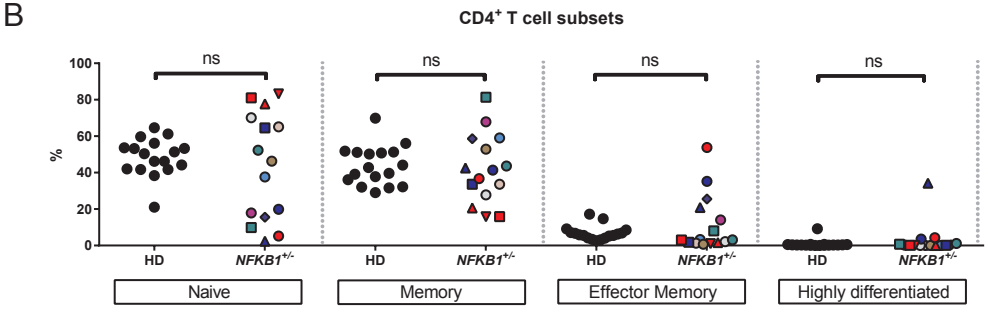
Case I
Large deletion
- I:II-1

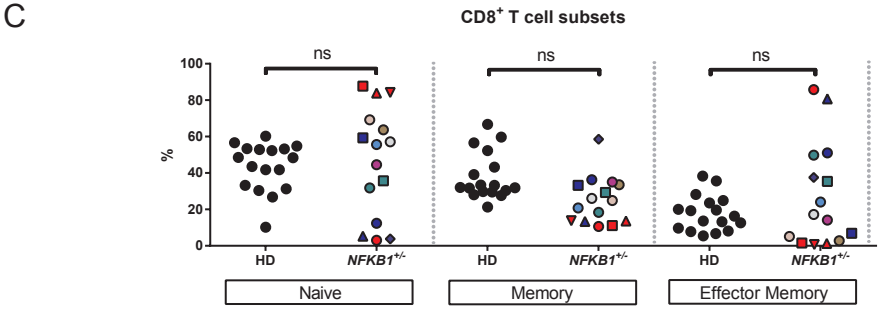
Case J
Ala475Profs*10
- J:III-2


A
Unstimulated
CpG/IL-2
αIgM/αCD40/IL-21
Control
A:Il-4
IgD
CD27
CD38
CFSE
B
CFSE-CD38+
Unstimulated
CpG/IL-2
αIgM/αCD40/IL-21
%
ns
**
HD
CU
CA
NFKB1+/-
C
IgA production
ng/ml
***
Case A
Arg284*
A:II-1
A:II-4
A:III-2
Case B
His513Glnfs*28
B:II-1
Case C
c.160-1G>A
C:I-2
C:III-1
C:III-3
C:III-4
Case D
Asp541*
D:I-2
D:II-2
Case E
Ile281Met
E:II-1


Lymphocytes
CD45+ Lymphocytes (no./mm3)
Age (years)
CD4+ T cells
CD4+ T cells (no./mm3)
Age (years)
CD8+ T cells
CD8+ T cells (no./mm3)
Age (years)
NK cells
CD16+CD56+ NK cells (no./mm3)
Age (years)
iNKT cells
CD3+TCR Vα24+Vβ11+ iNKT cells (no./mm3)
Age (years)
Clinically affected
Clinically unaffected







Case A
Arg284*
A:II-1
A:II-4
A:III-2
A:III-3

Case B
His513Glnfs*28
B:II-1

Case C
c.160-1G>A
C:I-2
C:III-1
C:III-3
C:III-4

Case D
Asp541*
D:I-2
D:II-2

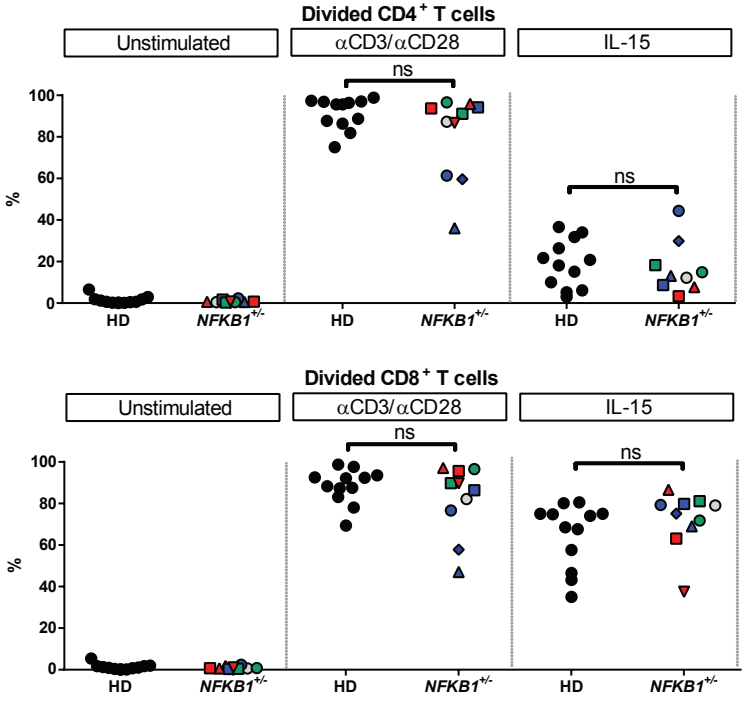
Case E
Ile281Met
E:II-1

Case F
Val98Asp
F:II-1

Case G
Ile87Ser
G:II-1

Case I
Large deletion
I:II-1

Case J
Ala475Profs*10
J:III-2



Case A
Arg284*
A:II-1
A:II-4
A:III-2
A:III-3

Case C
c.160-1G>A
C:I-2
C:III-3
C:III-4

Case D
Asp541*
D:I-2
D:II-2

Case F
Val98Asp
F:II-1

Table E1. Clinical data of individuals carrying *NFKB1* variants

Case ID	Year of birth	Age at PID diagnosis	Clinical diagnosis	Major symptoms at onset	Infections	Autoimmunity / autoinflammation	Malignancy	Survival
Case A								
A:II-1	1961	-	Healthy	-	-	-	-	-
A:II-4	1963	2015	CVID	Sinusitis 2012 Pneumonia 2015	*S. pneumoniae*	-	-	-
A:III-2	1989	1992	XLA	-	Frequent sinopulmonary infections	-	-	-
A:III-3	1995	2005	XLA	Pneumonias 2002	EBV-related splenomegaly 2005 JC-virus 2016	- (splenomegaly)	-	-
Case B								
B:I-1	1939	1967	CVID	Appendicitis-abscess 1965 Bacterial meningitis 1967 Pneumonias 1967	*S. pneumoniae*	AIHA 2005	B-NHL (EBV-negative) 2007	Died 2007: heart attack
B:II-1	1968	2011	CVID	Pneumonias 2010	*S. pneumoniae,* *H. influenzae*	Alopecia areata 1988 Vitiligo 1989 Hypothyroiditis 2003	-	-
Case C								
C:I-2	1938	1990	CVID	Sinusitis Pneumonias ICU respiratory failure (1978) Bronchiectasis	*S. pneumoniae,* *H. influenzae*	Sister and her children have autoimmune disease (MS, IDDM1 & SLE)	Parathyroid adenoma	-
C:II-3	1972	1988	CVID	Pneumonias Bronchiectasis		IDDM1		Died in 2008: during 2nd OLT (1st OLT 2005)
C:II-5	1972	1985	CVID	OMAs Oral ulcers Sore throats	*H. influenzae* *S. pneumoniae* *C. albicans* *A. fumigatus*	-	DLBCL (EBV-neg)	Died in 2011: DLBCL
C:III-1	1999	-	Healthy	-	-	-	-	-
C:III-3	2001	-	Healthy	-	-	-	-	-
C:III-4	2004	-	Healthy	-	-	-	-	-
Case D								
D:I-2	1958	-	Healthy	-	-	Thyroid disease	-	-
D:II-2	1981	1999	CVID	ITP and anemia Hypogammaglobulinemia	Pneumonias Sinusitis	ITP Splenomegaly	-	-

				Asthma	*E. coli*, urinary tract infections	GLILD Periodontitis		
Case E								
E:II-1	1992	1999	ALPS	Bronchiectasis Hypogammaglobulinemia following multiple courses of Rituximab therapy	Chest infections Sinusitis	Autoimmune neutropenia ITP	-	-
Case F								
F:II-1	1946	2000	CVID	Severe pneumonias Bronchiectasis Lung fibrosis Cellulitis R leg	-	Hyperthyroidism	Follicular lymphoma (2005); recurrence (2008)	-
Case G								
G:II-1	1980	2001	CVID	Severe pneumonia Bronchiectasis	COPD lobectomy (2009) *M. avium*	Mild splenomegaly Chronic diarrhea	-	-
Case H								
H:II-1	1973	1997	CVID	ITP and AIHA Splenomegaly	Pneumonia Sinusitis Invasive CMV	Chronic diarrhea with villous atrophy	-	Died in 2008 CMV
Case I								
I:II-1	1991	2009	CVID	Multi-dermatomal shingles (age 12) Recurrent pneumonia Bronchiectasis Sinusitis	*S. pneumoniae* *H. influenzae*	Mild thrombocytopenia	-	-
Case J								
J:III-2	1969	2004	CVID	Recurrent pneumonia Sinusitis, otitis media Recurrent prostatitis	*M. catarrhalis* *H. influenzae* *P. aeruginosa* *S. pneumoniae* 2011 CMV DNA detected in urine	Diabetes (corticosteroid-induced)	-	-
Case K								
K:II-1	1952	1996	CVID	Recurrent pneumonias, otitis and sinusitis Pneumococcal meningitis	*P. aeruginosa* *H. influenzae* *S. pneumonia* *S. marcescens*	AIHA	Peripheral T-cell lymphoma, received CHOP	-
Case L								
L:II-1	1969	1991	CVID	Initially few symptoms – tested for IgG levels when her brother died of bacterial meningitis on		AIHA 1999 (splenectomy)	-	-

				background recurrent respiratory infections and low Ig's. Respiratory infections soon became apparent.				
Case M								
M:II-1	1985	2012	CVID	Respiratory infections, with bronchiectasis and chronic sinusitis. Chronic diarrhea	*P. aeruginosa* (bronchi/sinuses-2012,3,4,5,6), *S. aureus* 2015,6, RSV 2015, atypical myco-bacterium infection 2009, *C. difficile* - year unknown, plantar warts (HPV), ongoing Herpes Zoster, disseminated 2011 (while on azathioprine)	Evans syndrome 2009 (Rituximab) Autoimmune enteropathy 2010 Vitamin B12 deficiency (suspected pernicious anemia)	-	-
Case N								
N:II-1	1959	2015	CVID	Hypogammaglobulinemia. Generalised lymphadenopathy and splenomegaly Pancytopenia	EBV, CMV Neutropenic sepsis (without positive cultures)	Alopecia totalis	Breast cancer	
Case O								
O:II-1	1978	2001	CVID	Frequent bacterial infections Chronic diarrhea	*Giardia lamblia*	-	-	-
Case P								
P:II-1	1961	2004	CVID	AIHA (treated with Rituximab 2012). ITP and autoimmune neutropenia. Recurrent sinus and respiratory tract infections	Chronic Norovirus Rhinovirus *H. influenzae* *S. pneumoniae*	GLILD. Polyarthritis (RA-like: RF/ANA negative)	-	-

Note:

H. influenzae strains are non-typeable (= uncapsulated) unless mentioned specifically.

Abbreviations:

AIHA: Autoimmune haemolytic anaemia; B-NHL: B non-Hodgkin lymphoma; CVID: Common variable immunodeficiency; DLBCL: Diffuse Large B cell lymphoma; GLILD: Granulomatous-lymphocytic inflammatory lung disease; ITP: immune thrombocytopenia; OLT: Orthopic liver transplantation; XLA: X-linked agammaglobulinemia

Table E2. Symptoms and organ involvement in individuals carrying *NFKB1* variants

Case ID	Year of birth	Lung	Lymph nodes	Spleen	Liver function	Gastro-intestinal tract	Brain
Case A							
A:II-1	1961	-	-	-	-	-	-
A:II-4	1963	-	-	-	-	-	-
A:III-2	1989	-	-	-	-	-	-
A:III-3	1995	-	Enlarged	Splenectomy for splenomegaly, suspected malignancy	Transaminitis	-	-
Case B							
B:I-1	1939	-	-	Splenomegaly	Hepatomegaly	-	-
B:II-1	1968	-	-	-	-	-	-
Case C							
C:I-2	1938	Bronchiectasis Lung fibrosis	-	-	Transaminitis	-	-
C:II-3	1972	Bronchiectasis	Enlarged (cervical and axillary)	Enlarged	Liver fibrosis (1998) without granulomas, no signs of infection or autoimmunity	-	-
C:II-5	1972	Bronchiectasis	-	-	Liver cirrhosis (1996), suspect of hepatitis C virus infection	Duodenal partial villous blunting, no granuloma and absence of colonic plasmacells	Tremor
C:III-1	1999	-	-	-	-	-	-
C:III-3	2001	-	-	-	-	-	-
C:III-4	2004	-	-	-	-	-	-
Case D							
D:I-2	1958	-	-	-	-	-	-
D:II-2	1981	GLILD	-	Splenomegaly	Normal	-	-
Case E							
E:II-1	1992	Bronchiectasis	Enlarged as a child; biopsies unremarkable	Splenomegaly (previous ITP)	Normal	-	-
Case F							
F:II-1	1946	Bronchiectasis, fibrosis	-	-	-	-	-
Case G							
G:II-1	1980	Bronchiectasis, fibrosis	-	-	-	-	-
Case H							

H:II-1	1973	-	-	Splenomegaly	-	Chronic diarrhea with villous atrophy	CMV retinitis
Case I							
I:II-1	1991	Bronchiectasis	Enlarged (mediastinal)	Splenomegaly	Mild elevation transaminases, gamma-GT	-	-
Case J							
J:III-2	1969	Bronchiectasis (2005) Asthma	-	-	Normal	Intermittent diarrhoea and abdominal pain	-
Case K							
K:II-1	1952	Bronchiectasis (2009)	Previous peripheral T cell lymphoma: CD3+CD8+ (2015)	Splenomegaly (previous AIHA)	-	-	-
Case L							
L:II-1	1969	Chronic left lower lobe collapse, COPD (smoker), no bronchiectasis	-	Splenectomy (AIHA)	Normal	-	-
Case M							
M:II-1	1985	Bronchiectasis. No interstitial disease. Nodule top left lung	-	'Mild' splenomegaly on CT only	Normal	Autoimmune enteropathy (2011)	-
Case N							
N:II-1	1959	NAD	Generalised Lymphadenopathy	Large splenomegaly	Normal	-	-
Case O							
O:II-1	1978	NAD	-	-	Normal	-	-
Case P							
P:II-1	1961	GLILD bronchiectasis	-	Splenomegaly (2012), normalized over time	Nodular regenerative hyperplasia of the liver.	Chronic diarrhea: upon biopsies absence of plasmacells (Norovirus-positive).	-

Abbreviations:

AIHA: Autoimmune haemolytic anaemia; GLILD: Granulomatous-lymphocytic inflammatory lung disease; ITP: immune thrombocytopenia; NAD: No active disease

Table E3. Immunological findings in individuals carrying *NFKB1* variants

Case ID	Year of birth	Absolute lymphocyte count	Abs # CD3+ T cells	Abs # CD3/CD4+ T cells	Abs # CD3/CD8+ T cells	Abs # CD16/56+ NK cells	Abs # CD19+ B cells	IgA level (g/L)	IgG level (g/L) - prior to Ig subst.	IgM level (g/L)	ANA, other autoAbs
Case A											
A:II-1	1961	1.427	0.978 (68.5%)	0.624 (43.7%)	0.332 (23.3%)	0.228 (16.0%)	0.215 (15.0%)	0.13	1.5	0.44	ANA neg
A:II-4	1963	3.288	1.974 (60.0%)	1.305 (39.7%)	0.630 (19.2%)	0.981 (29.9%)	0.323 (9.8%)	0.03	12.1 (post IVIG)	0.44	ANA neg
A:III-2	1989	1.630	1.400 (85.9%)	0.403 (24.7%)	0.941 (57.8%)	0.153 (9.4%)	0.074 (4.5%)	<0.04	6.9	0.17	ANA neg
A:III-3	1995	2.527	2.269 (89.8%)	0.735 (29.1%)	1.401 (55.5%)	0.252 (10.0%)	0 (<0.50%)	<0.04	6.2	<0.03	ANA neg
Case B											
B:I-1	1939	1.189	0.820 (69,0%)	0.240 (20.0%)	0.540 (44.0%)	0.060 (5.0%)	0.290 (25.0%)	<0.1	<0.05	0.26	Coomb's pos
B:II-1	1968	1.936	1.551 (80.1%)	1.257 (64.9%)	0.284 (14.7%)	0.077 (4.0%)	0.291 (15.01%)	0.1	1.7	0.36	Anti-TPO pos
Case C											
C:I-2	1938	1.741	1.316 (75.6%)	0.243 (14.0%)	0.943 (54.2%)	0.375 (21.5%)	0.047 (2.7%)	<0.1	2.0	0.6	-
C:II-3	1972	0.950	0.870 (91.6%)	0.450 (47.4%)	0.410 (43.2%)	0.060 (6.3%)	0.020 (2.1%)	<0.1	1.5	0.20	IDDM1
C:II-5	1972	1.150	0.980 (85.2%)	0.650 (56.5%)	0.331 (28.8%)	0.100 (8.7%)	0.042 (3.7%)	<0.1	1.4	0.20	-
C:III-1	1999	2.959	2.027 (68.5%)	1.038 (35.1%)	0.867 (29.3%)	0.306 (10.3%)	0.597 (20.2%)	0.8	10.4	0.7	-
C:III-3	2001	3.458	1.919 (55.5%)	1.156 (33.4%)	0.591 (17.1%)	0.687 (19.9%)	0.749 (21.7%)	0.6	6.4 (low IgG2)	0.2	-
C:III-4	2004	4.054	2.615 (64.5%)	1.565 (38.6%)	0.821 (20.3%)	0.514 (12.7%)	0.883 (21.8%)	1.3	5.7 (low IgG2 and IgG3)	0.7	-
Case D											
D:I-2	1958	2.5	1.373 (54.9%)	0.761 (30.4%)	0.553 (22.1%)	NA	0.173 (6.9%)	2.3	11.0	0.84	-
D:II-2	1981	1.200	0.679 (55.6%)	0.504 (42.0%)	0.140 (11.7%)	NA	0.027 (2.2%)	<0.3	4.9	<0.1	-
Case E											

E:II-1	1992	1.046	0.963 (92.1%)	0.709 (67.8%)	0.203 (19.4%)	NA	0.022 (2.1%)	<0.1	3.9	<0.1	-
Case F											
F:II-1	1946	0.619	0.458 (74.0%)	0.174 (28.1%)	0.283 (45.7%)	NA	0	<0.1	<2	<0.1	-
Case G											
G:II-1	1980	1.022	0.684 (66.9%)	0.449 (43.9%)	0.235 (23.0%)	NA	0	<0.1	<3.9	<0.1	-
Case H											
H:II-1	1973	5.458 (splenectomy)	4.857 (89%)	2.129 (39%)	2.620 (48%)	0.262 (4.8%)	0.132 (3%)	0.02	0.1	0.03	Coomb's pos ANA pos
Case I											
I:II-1	1991	1.4	1.102 (78%)	0.717 (51%)	0.349 (25%)	0.166 (12%)	0.122 (7%)	<0.06	0.3	0.09	-
Case J											
J:III-2	1969	1.7	1.336 (78.6%)	0.706 (41.5%)	0.602 (35.4%)	0.019 (1.1%)	0.318 (18.7%)	<0.07	<1.0	0.17	-
Case K											
K:II-1	1952	0.7	0.639 (97%)	0.206 (31%)	0.388 (59%)	0.005 (1%)	0.015 (2%)	0.05	<0.1	<0.1	Coomb's pos
Case L											
L:II-1	1969	1.4	1.129 (77%)	0.687 (47%)	0.405 (28%)	0.249 (17%)	0.080 (5%)	<0.05	10.3 (post SCIG)	<0.05	Coomb's pos
Case M											
M:II-1	1985	1.0	0.902 (90%)	0.621 (62%)	0.223 (22%)	0.055 (5.5%)	0.028 (2.8%)	<0.04	Low pre Ig replacement	0.65	Coomb's pos, ANA neg
Case N											
N:II-1	1959	1.012	0.82 (81%)	0.54 (53%)	0.26 (28%)	0.15 (16%)	0.02 (2%)	<0.07	2.3	0.18	ANA neg
Case O											
O:II-1	1978	1.7	1.39 (82%)	0.97 (57%)	0.40 (24%)	0.06 (3.5%)	0.24 (14%)	0.4	4.6	0.3	ANA neg
Case P											
P:II-1	1961	0.79	0.52 (66%)	0.33 (42%)	0.18 (23%)	0.11 (14%)	0.15 (19%)	<0.1	1.9	0.1	Coomb's pos ANA neg

Abbreviations:

NA: Not Available

Supplemental Methods

Immunophenotyping

Peripheral blood mononuclear cells (PBMCs) were isolated using standard density gradient centrifugation techniques using Lymphoprep (Nycomed, Oslo, Norway). Absolute numbers of lymphocytes, T cells, B cells and NK cells were determined with Multitest six-color reagents (BD Biosciences, San Jose, USA), according to manufacturer's instructions. For the immunophenotyping the PBMCs were resuspended in PBS, containing 0.5% (w/v) BSA and 0.01% sodium azide and incubated with saturating concentrations of fluorescently labeled conjugated monoclonal antibodies. Analysis of cells was performed using a FACSCanto-II flowcytometer and FlowJo software. Patient samples were analyzed simultaneously with PBMCs from healthy controls. The following directly conjugated monoclonal antibodies were used: CD4 PE-Cy7 [348809], CD8 PerCP-Cy5.5 [341050], CD20 PerCP-Cy5.5 [332781], CD21 FITC [561372], CD27 APC [337169], CD38 PE-Cy7 [335825], CD45 APC-Cy7 [348815], IgD PE [555779], and TCRγδ FITC [347903] from BD (San Jose, USA), CD3 Alexa 700 [56-0038-41], CD19 Alexa 700 [56-0199-42] and CD27 APC-eFluor 780 [47-0279-42] from eBioscience (San Diego, USA), CD24 FITC [M1605] and CD27 FITC [M1764] from Sanquin (Amsterdam, the Netherlands), CD45RA (2H4-RD1) PE [6603181], TCRαβ PE [PN A39499], TCR Vα24 FITC [PM IM1589] and TCR Vβ11 PE [PN IM2290] from Beckman Coulter (Brea, USA), IgM FITC [F0317] and IgG FITC [F0158] from Dako (Glostrup, Denmark), IgA FITC [130-093-071] from Miltenyi Biotec (Bergisch Gladbach, Germany).

B and T cell functional assay

To analyze the *ex vivo* activation of T and B cells, PBMCs were resuspended in PBS at a concentration of 5–10×10^6 cells/ml and labeled with 0.5μM CFSE (Molecular Probes) in PBS for 10 minutes at 37°C under constant agitation. Cells were washed and subsequently resuspended in Iscove's Modified Dulbecco's medium (IMDM) supplemented with 10% fetal calf serum (BioWhittaker), antibiotics, and 3.57×10^-

^{4}%(v/v) β-mercaptoethanol (Merck). Labeled PBMCs containing a fixed number of 2×10^{4} (96-well plate) or 10×10^{4} B cells (48-well plate) per well in a flat-bottom plate for 6 days at 37°C and stimulated with saturating amounts of anti-IgM mAb (clone MH15; Sanquin), anti-CD40 mAb (clone 14G7; Sanquin) and 20ng/ml IL-21 (Invitrogen), or 1μg/ml CpG oligodeoxynucleotide 2006 (Invivogen) and 100U/ml IL-2 (R&D Systems), or anti-CD3 (clone 1xE) and anti-CD28 (clone 15E8), or IL-15 (R&D Systems). Proliferation of B and T cells was assessed by measuring CFSE dilution in combination with the same mAbs used for immunophenotyping and analyzed using a FACSCanto-II flowcytometer and FlowJo software.