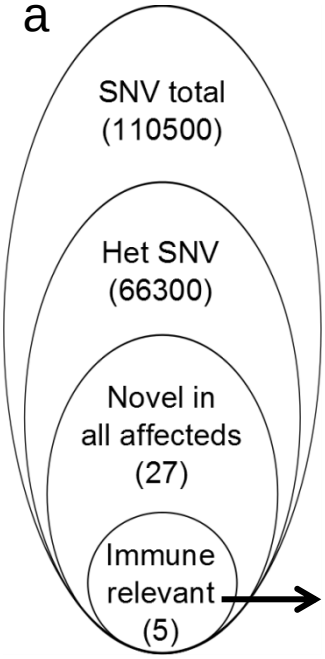
a



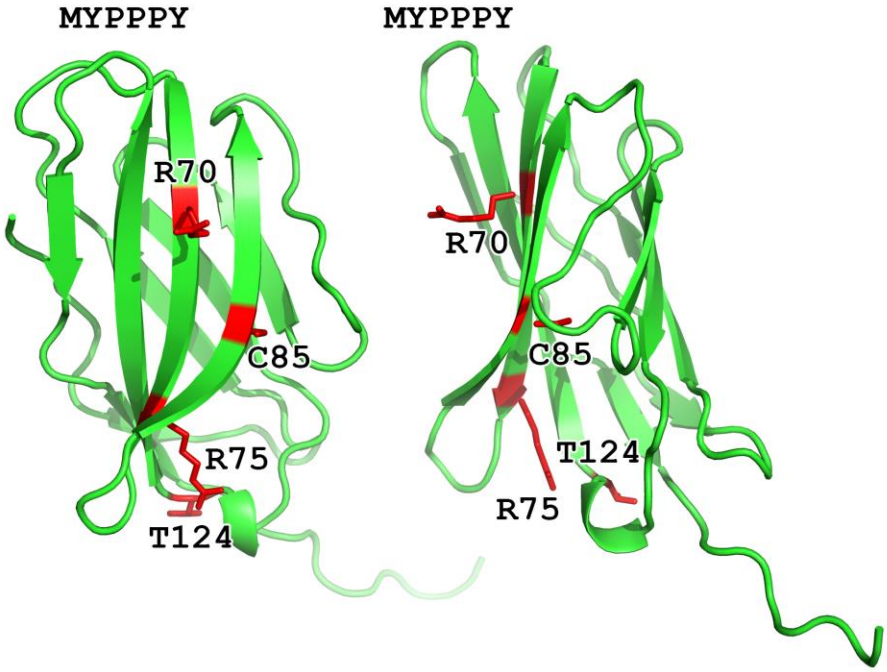
1. CTLA-4 C35*
2. MUC6 T1922L
3. *RNF216* 3‘UTR variant
4. *FGF7* 3‘UTR variants
5. *C1QTNF6* 5‘UTR variant

b

Chr	Gene	Position	Location	Effect	Amino Acid Change	REF	ALT	GENE ID
		152192097			D668C	C	CGG	
1	*HRNR*	152192100	Exon	missense	F669C	A	C	NM_001009931.2
		152192101			G670A	ATC	A	
2	*SATB2*	200136745	3'UTR			A	G	NM_001172509.1
2	*TYW5*	200796424	3'UTR			T	C	NM_001039693.2
2	*CLK1*	201729211	5'UTR			AAG	A	NM_001162407.1
2	*CTLA4*	204732770	Exon	stop gained	C35*	C	A	NM_005214.4
3	*PRR23B*	138739752	5'UTR			G	A	NM_001013650.2
5	*ANKH*	14707376	3'UTR			ACG	A	NM_054027.4
7	*RNF216*	5660673	3'UTR			C	T	NM_207116.2
9	*FOXD4*	118004	Exon	insertion	(-)39WKTR	T	TCCTCGTCTTCCA	NM_207305.4
11	*MUC6*	1017035	Exon	missense	T1922L	A	AAT	NM_005961.2
		1017040				GGT	G	
11	*KRTAP5-5*	1651585	Exon	deletion	SCCQSSCCKPY172S	CCTGCTGCCAGTCCAG	C	NM_001001480.2
						CTGCTGTAAGCCTTA		
11	*MPEG1*	58977317	3'UTR			GTAT	G	NM_001039396.1
14	*TMEM55B*	20926018	3'UTR			A	T	NM_001100814.1
15	*HERC2*	28478668	Exon	missense	G1464D	C	T	NM_004667.5
		49777092				A	G	
		49777099				T	A	
15	*FGF7*	49777131	3'UTR			T	G	NM_002009.3
		49777242				A	AGAAT	
		49777255				A	AT	
17	*USP6*	5031733	Intron			T	C	NM_004505.2
17	*KRTAP9-4*	39406474	3'UTR			C	A	NM_033191.2
22	*C1QTNF6*	37584323	5'UTR			A	AGGAGGGAGAG	NM_182486.1
							AGGAGGGGAT	
22	*GGA1*	38029267	3'UTR			G	A	NM_001172688.1

Supplementary Figure 1: Affected-only analysis of whole exome sequencing data revealed heterozygous nonsense mutation in *CTLA4.* a: Filtering strategy for whole exome sequencing data. On average, 110 500 variants were observed per subject. 60 % of the observed variants were heterozygous. Of all heterozygous variants, 27 non-synonymous variants were found in coding or intronic regions in all four affected family members. Five of those variants were found in genes relevant for the immune system. **b:** Table showing novel variants identified by whole exome sequencing in all four affected family members. Chr: Chromosome; REF: reference sequence; ALT: observed sequence; All genomic positions refer to human genome build GRCh37/hg19.

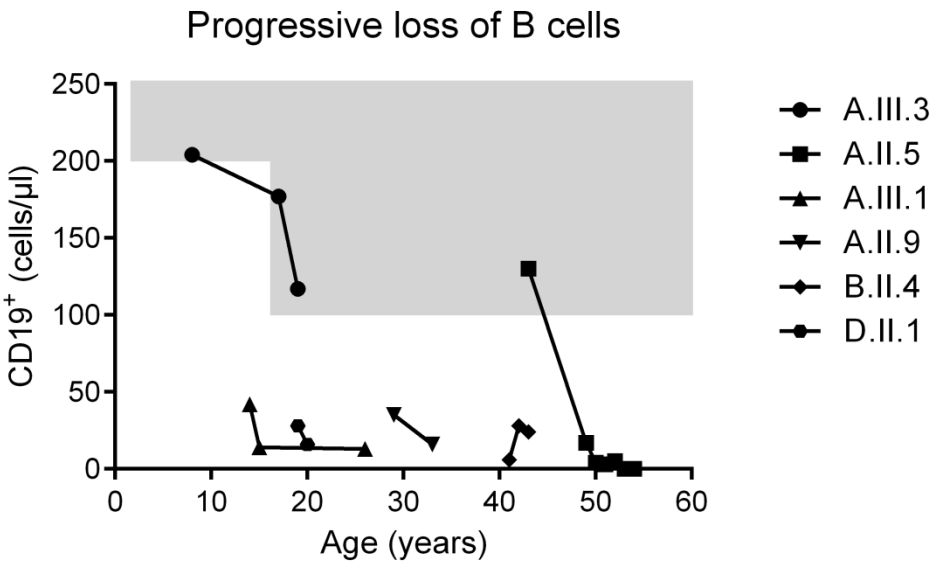
a



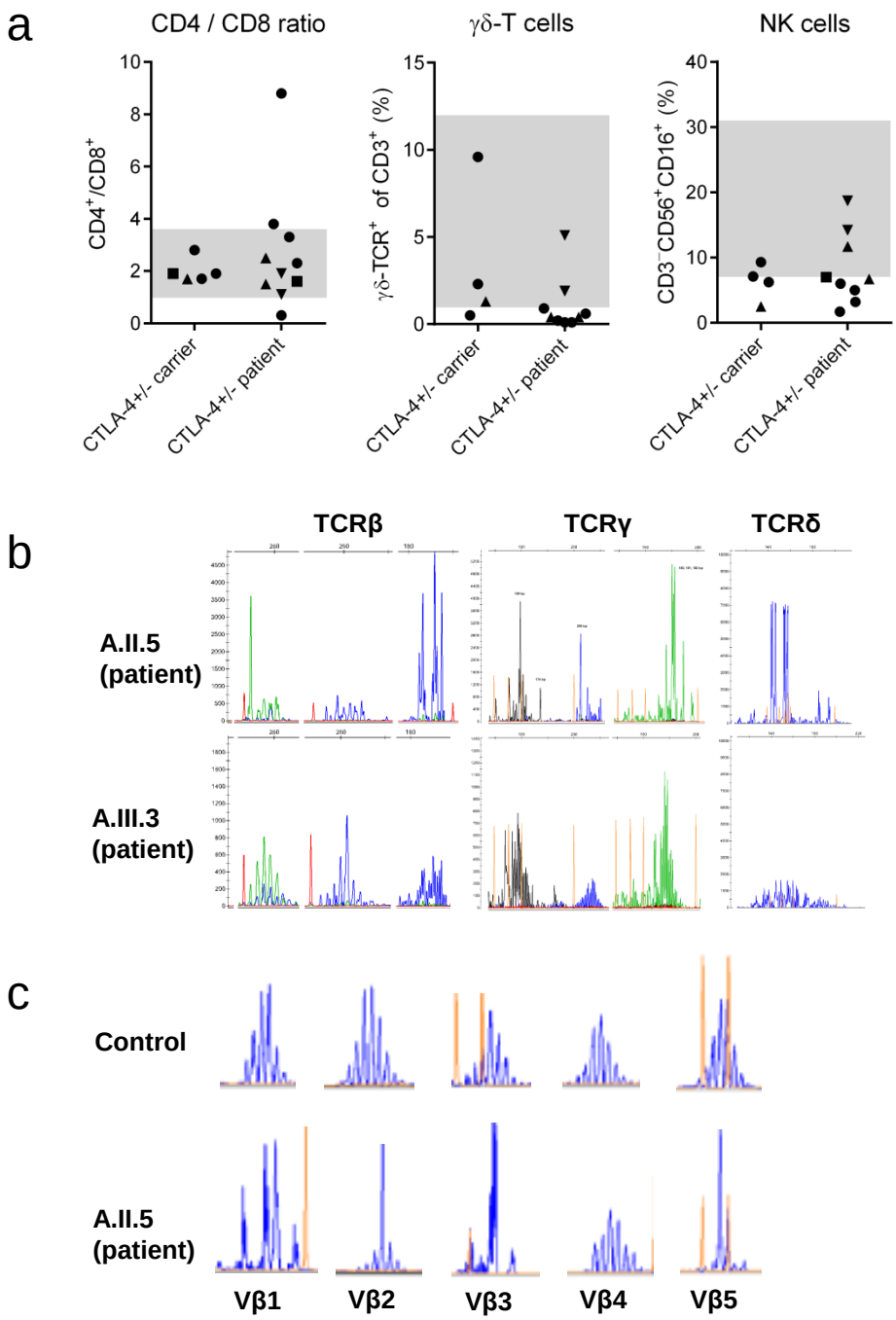
b

Protein Acc.	Gene	Organism	p.70R>W, p.75R>W	p.124T>P
NP_005205.2	*CTLA4*	H.sapiens	65 KATEVRVTVLRQADSQ 80	115 TIQGLRAMDTGLYICKVELM 134
XP_526000.1	CTLA4	P.troglodytes	65 KATEVRVTVLRQADSQ 80	115 TIQGLRAMDTGLYICKVELM 134
NP_001038204.1	CTLA4	M.mulatta	65 KATEVRVTVLRQADSQ 80	115 TIQGLRAMDTGLYICKVELM 134
NP_001003106.1	CTLA4	C.lupus	65 NAAEVRVTVLRQAGSQ 80	115 TIQGLRAMDTGLYICKVELM 134
NP_776722.1	CTLA4	B.taurus	63 KADEVRVTVLREAGSQ 78	113 TIQGLRAMDTGLYVCKVELM 132
NP_033973.2	Ctla4	M.musculus	65 NTDEVRVTVLRQTNDQ 80	115 TIQGLRAVDTGLYLCKVELM 134
NP_113862.1	Ctla4	R.norvegicus	65 NTDEVRVTVLRQTNDQ 80	115 TIQGLRAADTGLYFCKVELM 134
NP_001035180.1	CTLA4	G.gallus	48 NAKEIRVTLLKQTGDK 63	100 TLTGLQANDTGLYVCKMERM 118
XP_002936576.1	ctla4	X.tropicalis	47 KVEEMRFRLLRKMGNQ 62	99 HLSGMQMSDTGMYICKLDIM 117

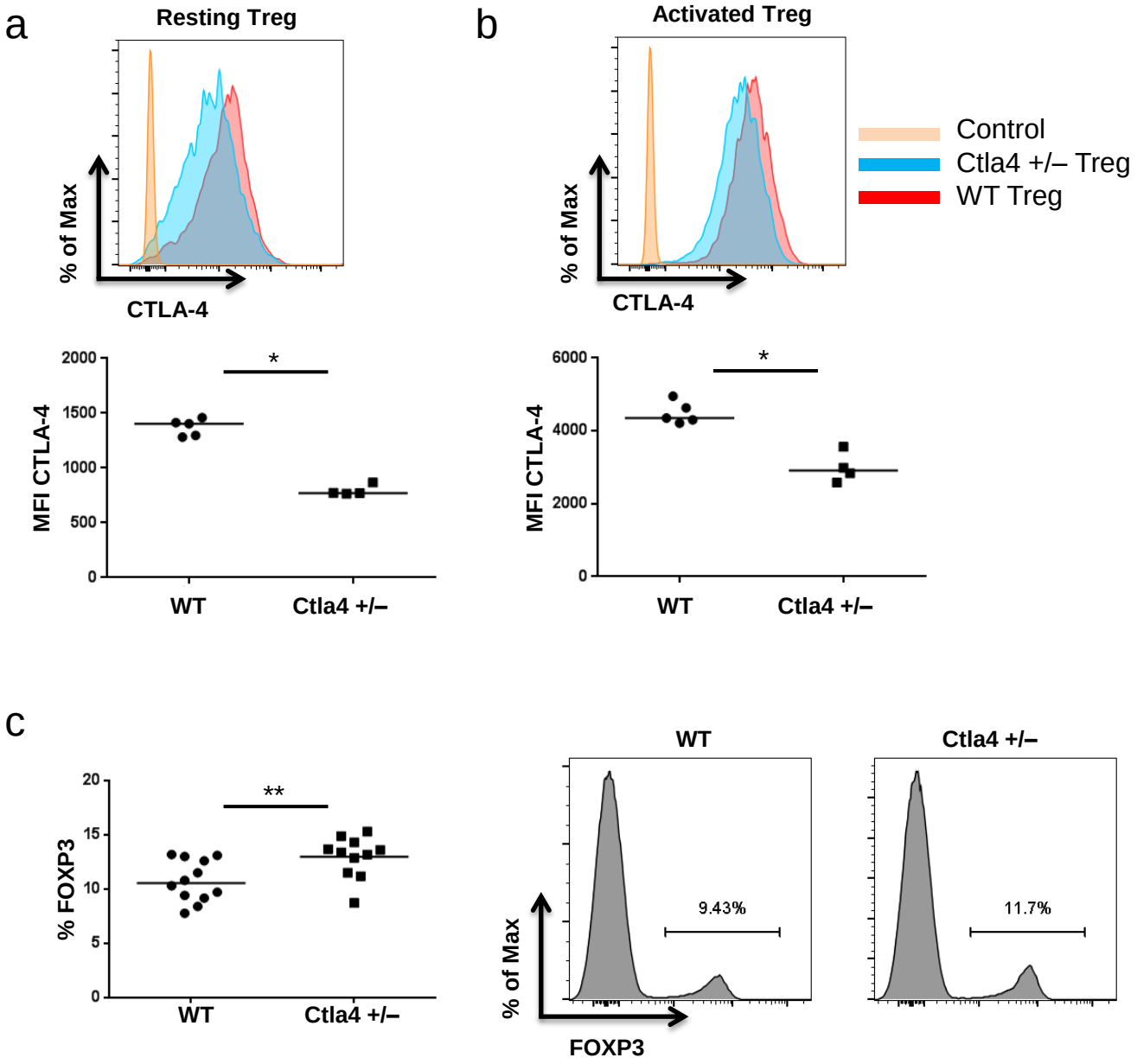
Supplementary Figure 2: ***CTLA4* mutations affect conserved amino acids**. **a:** Structural model of CTLA-4 *(green)* showing ectodomain residues affected by mutations (red). Two orthogonal views of the monomer are shown. The location of the known ^{99}MYPPPY^{104} motif involved in ligand binding is shown. Amino acid numbering is inclusive of signal peptide amino acids. **b:** Protein sequence alignment of CTLA-4 sequence from various vertebrate species. Conserved amino acids affected by mutation are highlighted pink.



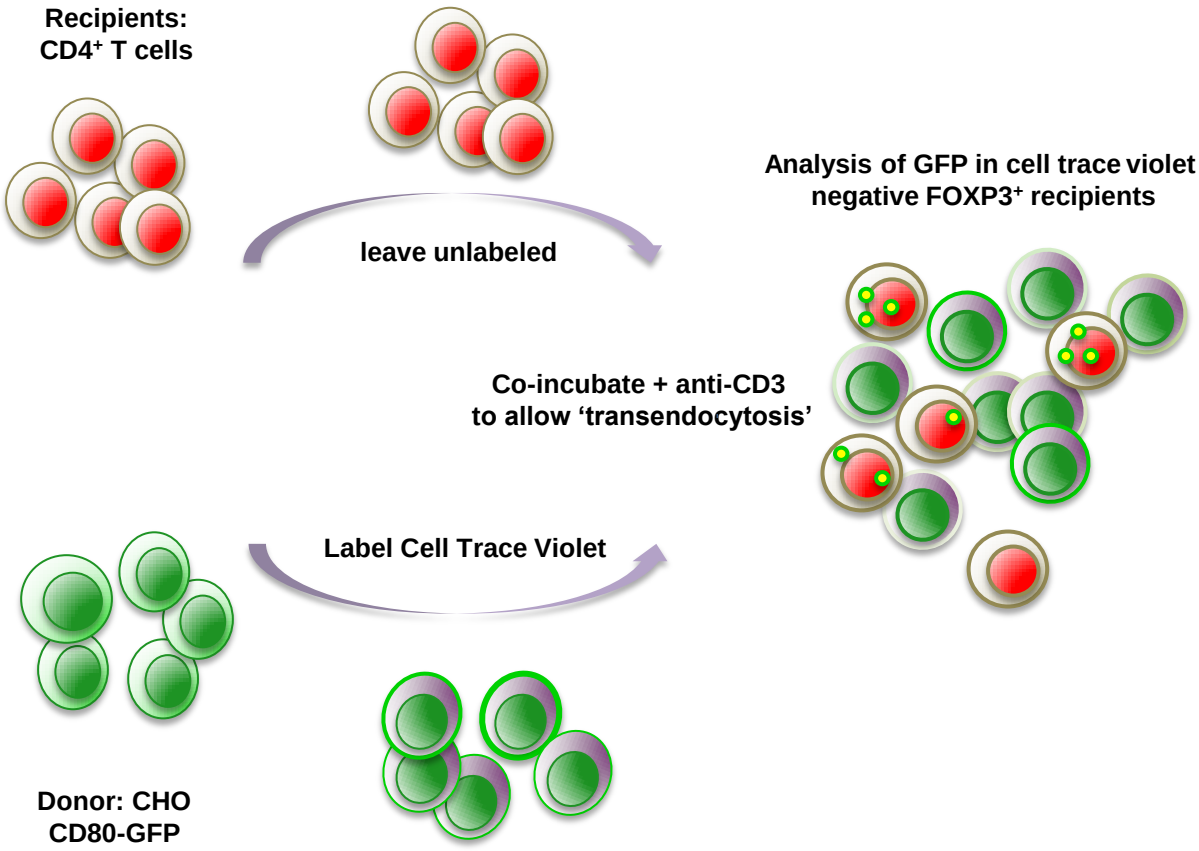
Supplementary Figure 3: Patients lose their B cells over time. Absolute numbers of $CD19^+$ B cells were obtained from differential blood count combined with a lymphocyte panel at different time points for five patients. Gray background indicates normal range.



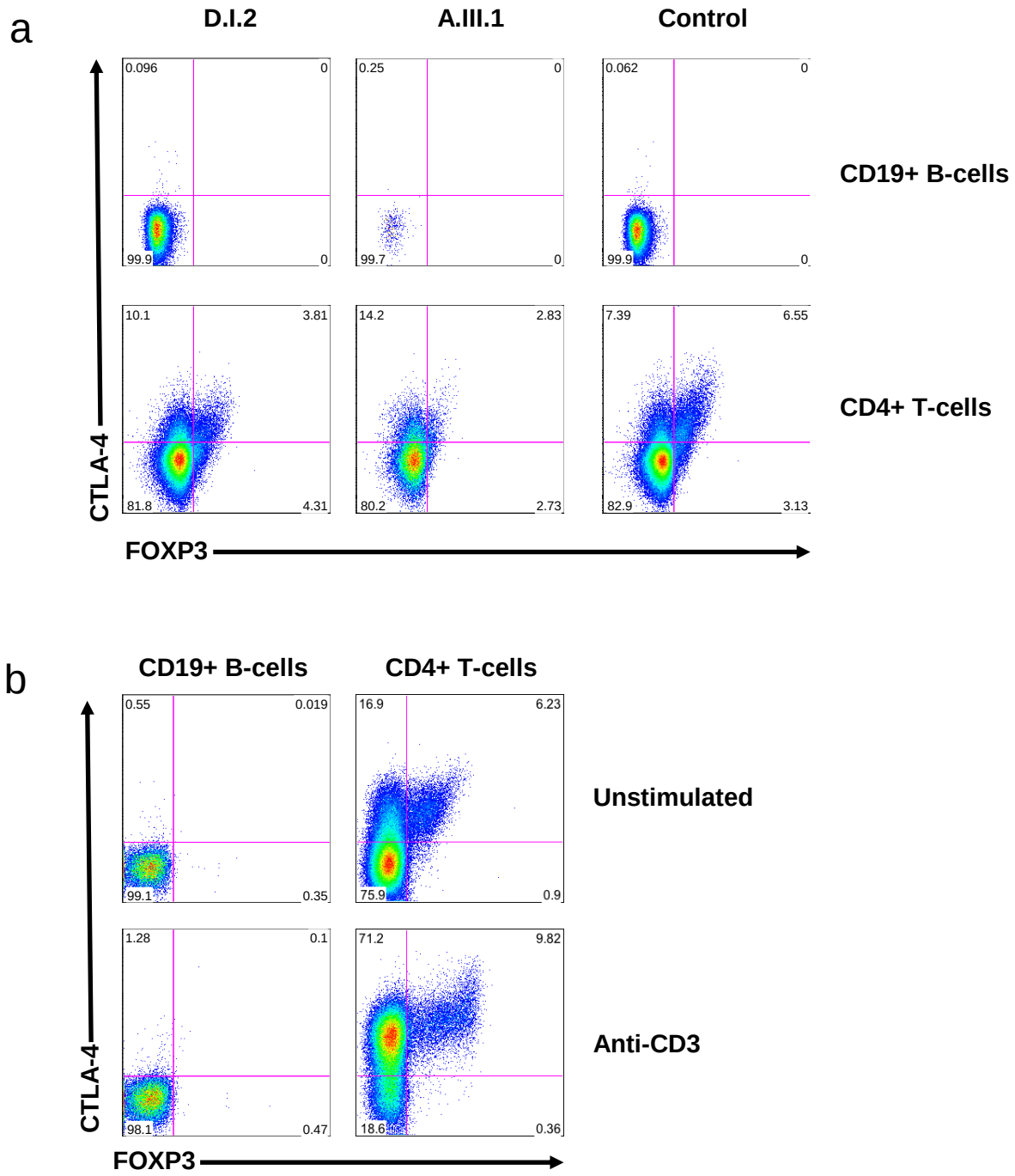
Supplementary Figure 4. Phenotype of peripheral blood lymphocytes. a: Ratio of $CD3^+CD4^+$ to $CD3^+CD8^+$ T cells and percentage of $CD3^+\gamma\delta^-TCR^+$ T cells and $CD3^-CD56^+CD16^+$ NK cells in the peripheral blood of *CTLA4*+/– carriers and patients. Gray background indicates normal range. ● Family A, ▲ Family B, ▼ Family C, ■ Family D. **b:** Peaks of T cell receptor rearrangements represent relative density of PCR products. The TCRβ panel shows Tube A (left) with Vβ–Jβ1.1 to 1.6 (green) and Vβ–Jβ 2.2, 2.6 und 2.7 (blue), Tube B (middle) with Vβ–Jβ 2.1, 2.3, 2.4 and 2.5 and Tube C (right) with Dβ1/2–Jβ rearrangements. The TCRγ panel displays Vγ10 (black), Vγ1–8 (blue) Vγ9 (green) and the TCRδ panel shows TCRδ rearrangements. While TCRγ rearrangements are seen in αβ and γδ T cells, TCRδ rearrangements are specific for γδ T cells. **c:** TCR spectratyping. Expressed T cell repertoire (Vβ1–5 as examples of a total of 24 Vβ families studied) following RT-PCR amplification of cDNA from the blood of a normal control with normal (Gaussian) CDR3 distributions and the patient (A.II.5) showing oligoclonal peaks in Vβ1,2, 3 and 5.



Supplementary Figure 5: Ctla4$^{+/-}$ mice show reduced CTLA-4 protein expression and increased Treg numbers. a–c) CTLA-4 expression levels in spleenocytes of C57BL/6 Ctla4 heterozygous (+/–) or wildtype littermates 14 days after immunization with NP-Ova in Alum. **a**) CTLA-4 expression by resting (CD44^{-}) and **b**) activated Tregs (CD44^{+}). **c**) Percentage Foxp3^{+} within CD4^{+} population. **d**) Percentage B220^{+} among live splenocytes. Lines denote median. P values are derived from Mann-Whitney test. *P≤0.05, **P≤0.01.



Supplementary Figure 6: Transendocytosis Assay Design. T cells (red) are mixed with donor CHO cells expressing CD80-GFP. Donor cells are labeled with Cell Trace violet (purple cytoplasm). Following stimulation, T cells acquire and internalise CD80-GFP from the donor cells – indicated as yellow vesicles. Cell Trace labeled donor cells are gated out in analysis to ensure that only transferred GFP (in recipient cells) is measured.



Supplementary Figure 7: CTLA-4 is not expressed on B-cells. a: Intracellular CTLA-4 and FOXP3 expression levels in $CD3^{+}CD4^{+}$ T cells and $CD3^{-}CD19^{+}$ B cells. **b:** PBMC cultures were stimulated with or without anti-CD3 for 48hrs. Cells were stained as in A.

Table S1: Clinical manifestations associated with *CTLA4* mutations.

Patients	**A.II.5**	**A.II.8†**	**A.II.9†**	**A.III.1**	**A.III.3**	**B.II.1**	**B.II.2†**	**B.II.4**	**B.III.2†**	**C.II.3†**	**C.II.4**	**D.II.1**	**E.II.3**	**F.II.2†**
Year of birth	**1959**	**1963**	**1965**	**1987**	**1995**	**1958**	**1962**	**1971**	**1984**	**1993**	**2002**	**1993**	**1993**	**1988**
Diarrhea/Enteropathy	yes	yes	yes	yes	yes	yes	yes	yes	-	-	yes	-	yes	yes
GLILD	-	yes	yes	-	yes	nd	nd	yes	yes	yes	-	yes	yes	-
Respiratory infections[#]	yes	yes	yes	-	-	-	-	yes	yes	yes	-	-	yes	yes
Splenomegaly	yes	-	yes	-	yes	nd	nd	-	-	yes	-	yes	yes	-
AI thrombocytopenia	-	-	-	yes	yes	-	-	-	-	yes	-	-	yes	yes
AI hemolytic anemia	-	-	-	-	-	-	-	-	-	yes	-	yes	yes	yes
Lymphadenopathy	-	-	-	-	yes	-	nd	-	-	yes	-	yes	yes	-
Bone marrow infiltration	nd	nd	yes	nd	yes	nd	nd	yes	nd	nd	nd	"reactive changes"	nd	nd
Growth retardation (<3rd percentile)	-	-	-	-	-	-	-	-	-	yes	yes	-	-	-
Kidney disease*	-	-	-	-	-	-	-	-	-	yes	-	yes	-	-
Liver disease*	-	-	-	-	-	-	yes	-	-	yes	-	-	-	-
Psoriasis/other skin disease*	-	-	-	yes	-	yes	yes	-	-	-	-	-	-	-
AI arthritis	yes	-	-	yes	-	-	-	-	-	-	-	-	-	-
AI thyroiditis	yes	-	-	-	Antibodies detectable	-	-	-	-	-	nd	yes	-	-
Brain granulomas	-	-	-	yes	-	-	-	-	-	-	nd	-	-	-
Seizures	-	yes	-	-	-	-	-	-	-	-	-	-	-	-
Solid cancer	-	-	-	-	-	-	-	Stomach	-	-	-	-	-	-
Mutation carriers	**A.I.2**	**A.II.2**	**A.II.3**	**A.II.10**	**A.III.5**	**A.III.6**	**B.I.1†**	**B.II.3**	**D.I.2**					
Year of birth	**1928**	**1955**	**1956**	**1966**	**1992**	**1995**	**1934**	**1964**	**1972**					
Diarrhea/Enteropathy	-	-	yes	3 year episode	-	-	yes	under stress	nd					
Kidney disease*	-	-	-	-	-	-	yes	-	nd					
Liver disease*	-	yes	-	-	-	-	-	-	nd					
Psoriasis/other skin disease*	-	-	yes	-	-	-	yes	yes	nd					
Solid cancer	Colon	-	-	-	-	-	-	-	nd					
Insulin-dependent Diabetes	-	yes	-	-	-	-	-	-	nd					

† deceased; [#] upper and lower tract infections; * details in the supplementary case reports; GLILD: granulomatous lymphocytic interstitial lung disease; AI: autoimmune; nd: not determined

Table S2: Laboratory values of patients and mutation carriers.

Patients	A.II.5	A.II.8	A.II.9	A.III.1	A.III.3	B.II.1	B.II.4	C.II.3	C.II.4	D.II.1	E.II.3	F.II.2
Year of birth	**1959**	**1963**	**1965**	**1987**	**1995**	**1958**	**1971**	**1993**	**2002**	**1993**	**1993**	**1988**
Lymphocyte subsets												
Age at examination	**54**	**30**	**33**	**27**	**18**	**55**	**42**	**17**	**11**	**19**	**19 (15)**	**21 (15)**
Lymphocytes [% of WBC]	13.8↓	nd	62↓	21↓	20.4↓	36↓	13.8↓	9.6↓	24.8↓	13.6↓	21,3↓	30↓
CD3+ [% of PBMC]	93.8↑	79	93↑	95.5↑	80.9	77.8	90.2↑	64.7	70.5	85↑	78.5	93.5↑
αβTCR+ [% of CD3+]	99.7↑	99	99↑	99.5↑	98.9↑	99.3↑	99.0↑	98.3	94	nd	nd	99.8↑
γδTCR+ [% of CD3+]	0.1↓	<1↓	0.2↓	0.1↓	0.6↓	0.4↓	0.4↓	1.94	5.1	nd	nd	<0.2↓
CD4+ [% of CD3+]	19.8↓	66↑	73↑	85.3↑	58.9↑	55.9	55.9	41.4	35.7	62↑	49	91.1↑
CD45RA+ [% of CD4+]	12.3↓	nd	6.4↓	5.4 ↓	21.5↓	18.8↓	37.3	8.1↓	39.4↓	19.8↓	8.2↓	46.9
CD45RO+ [% of CD4+]	78.6↑	nd	nd	92.8↑	75.2↑	71.6↑	58.6↑	90.8↑	58.9↑	nd	87,7↑	59.6↑
CD8+ [% CD3+]	74.1↑	29	19	9.7↓	18.1	22.1	37.1	21.6	30.8	38	27.2	8.6
CD4/CD8 ratio	0,27↓	2.3	3.8↑	8.79↑	3.25	2.53	1.51	1.9	1.1	1.6	1,8	10.6↑
CD3- CD56+ CD16+ [% of PBMC]	6.0↓	nd	1.7↓[1]	3.2↓	5↓	11.7	6.7↓	18.65	14.2	7	5.4↓	1.4↓
CD19+ [% of PBMC]	0.0↓	6↓[2]	2.2↓	0.73↓	10.1	6.5	0.6↓	15.98	15.1	2.39	17.4[3]	11[3]
IgM+ CD27- [% of CD19+]	0.0↓	nd	nd	0.0↓	77.0	39↓	23.3↓	96.48↑	59↓	40↓	92,8↑[3]	nd
IgM+ CD27+ [% of CD19+]	0.0↓	nd	nd	0.0↓	15.5	45.1↑	51.3↑	2.5↓	10	3.9↓	3,6↓[3]	nd
IgM- CD27+ [% of CD19+]	0.0↓	0.0↓	nd	0.0↓	2.35↓	11.8	15.9	0↓	17	4.1↓	0,8↓[3]	nd
CD21 low [% of CD19+]	0.0↓	nd	nd	0.0↓	13.7↑	18.8↑	36.7↑	39↑	17.5↑	3.6	8,5↑[3]	nd
Transitional [% of CD19+]	0.0↓	nd	nd	0.0↓	3.7↑	0.86	0.15↓	nd	nd	1.8	0.0↓[3]	nd
Plasmablast [% of CD19+]	0.0↓	nd	nd	0.0↓	0.08↓	1.02	3.43	nd	nd	7.7↑	0.0↓[3]	nd
Immunoglobulin levels												
Age at examination	**41**	**33**	**33**	**15**	**7**	**55**	**42**	**9**	**11**	**19**	**15**	**18**
IgM [g/l]	<0.17↓	3.25	0.4	0.32↓	0,37↓	0.89	1.39	0.07↓	1.39	0.3↓	1.36	<0.17↓
IgG [g/l]	3.38↓	0.01↓	2.1↓	2.96↓	5.89↓	10.5	6.31↓	0.70↓	9.9	1.3↓	2.11↓	3.36↓
IgA [g/l]	0.26↓	0.11↓	<0.3↓	0.17↓	0.56↓	1.25	0.81	0.07↓	1.17	<0.1↓	0.18↓	0.04↓
IgE [IU/ml]	<17.5	nd	norm	<4	<4↓	40.2	<4.	<2↓	0.92↓	nd	28,7	15
Serology/Virology												
Age at examination	**54**			**27**		**55**	**42**	**7**		**20**	**20 (15)**	**18**
EBV (PCR) [I.E./ml]	(-)			(-)		nd	(+) 2000	(-)		nd	(-)	(-)
CMV (PCR)	(-)			(-)		nd	(-)	(-)		(+)	(+)	(-)
EBV antibodies	IVIG			(+)		(+)	nd	(-)		nd	(-)[4]	nd
CMV antibodies	IVIG			(-)		(-)	nd	(-)		nd	(+)[4]	nd
Diphtheria antibodies	IVIG			(-)		(+)	nd	nd		nd	nd	nd
Tetanus antibodies [IU/ml]	IVIG			(-)		(+)	nd	nd		<0.01	(+)[4]	nd
PPSV antibodies [mg/l]	IVIG			17.98		131.6	15.14	nd		<3.3	nd	nd

Mutation carriers	**A.I.2**	**A.II.3**	**A.III.5**	**A.III.6**	**B.II.3**	**D.I.2**
Year of birth	**1928**	**1956**	**1992**	**1995**	**1964**	**1972**
Lymphocyte subsets						
Age at examination		**57**	**21**	**18**	**49**	**42**
Lymphocytes [% of WBC]		29.3	nd	nd	25↓	nd
CD3+ [% of PBMC]		81.5	50.9↓	67.6	93.8↑	71.5
αβTCR+ [% of CD3+]		99.1↑	86.9	93.3	98.3↑	nd
γδTCR+ [% of CD3+]		0.5↓	9.58	2.79	1.3	nd
CD4+ [% of CD3+]		59.5↑	54.6	55.2	56.3	65.3↑
CD45RA+ [% of CD4+]		25.3↓	22.0↓	48.3	35.3	24.4↓
CD45RO+ [% of CD4+]		71.0↑	72.3↑	39.8	49.8	nd
CD8+ [% CD3+]		21.3	29.0	32.8	34.1	34
CD4/CD8 ratio		2.79	1.88	1.68	1.65	1.92
CD3- CD56+ CD16+ [% of PBMC]		9.3	6.25	7.11	2.5↓	nd
CD19+ [% of PBMC]		8.6	16.0	11.2	1.9↓	12
IgM+ CD27- [% of CD19+]		75.4	93.7↑	76.6	29.9↓	79
IgM+ CD27+ [% of CD19+]		20.0	4.95↓	18.4	57.5↑	5.82↓
IgM- CD27+ [% of CD19+]		2.99↓	0.32↓	2.43↓	5.43↓	11.3
CD21 low [% of CD19+]		9.46↑	6.18	15.8↑	12.8↑	9.28↑
Transitional [% of CD19+]		3.28	1.37	0.0↓	1.1	0.96
Plasmablast [% of CD19+]		0.09	0.76	0.49↓	0.21↓	0.26↓
Immunoglobulin levels						
Age at examination	**72**	**57**	**21**	**18**	**49**	
IgM [g/l]	0.44↓	1.52	0.42	1.07	0.47	
IgG [g/l]	4.22↓	11.2	8.27	10.7	7.59	
IgA [g/l]	0.25↓	1.45	0.43↓	0.64↓	0.91	
IgE [IU/ml]	<17.5	<4↓	<4↓	21.1	767↑	
Serology/Virology						
Age at examination		**57**	**21**	**18**	**49**	
EBV (PCR) [I.E./ml]		nd	(+) 9500	(+) 500	nd	
CMV (PCR)		nd	(-)	(-)	nd	
EBV antibodies		(+)	(+)	(+)	(+)	
CMV antibodies		(+)	(-)	(-)	(-)	
Diphtheria antibodies		(-)	(-)	(+)	(-)	
Tetanus antibodies [IU/ml]		(-)	(+)	(+)	(+)	
PPSV antibodies [mg/l]		143.83	11.26	131.6	29.07	

From deceased patients B.II.2 and B.III.2, and from mutation carriers A.II.2, A.II.10 and B.I.1, values could not be obtained. [1](CD3-CD16+); [2](CD20+); [3](B cell values from age 15, no B cells detectable later due to Rituximab treatment); [4](Values from age 15 before SCIG); nd: not determined; norm: within the normal range; PPSV: Pneumococcal polysaccharide vaccine

Supplementary notes 1: Case reports

Laboratory values of all individuals studied are displayed in **Table S2**.

Family A

The female index patient in Family A (individual A.II.9 in **Figure 1a**) died at age 37 years following pulmonary insufficiency. She was diagnosed with hypogammaglobulinemia and splenomegaly at age 17. She had recurrent sinusitis and bronchitis. Nephrolithiasis was treated at age 21. She was diagnosed with tuberculosis, involving lungs and esophagus at age 22, followed by multiple recurrent bacterial pneumonias. During the following years her lung function declined substantially; she developed bullous lung disease with pneumatocele formation, pulmonary hypertension and clubbing. An upper right lobectomy at age 36 was followed by a bilateral lung transplant one year later due to pulmonary demise. She gained quality of life for one year before her pulmonary function declined rapidly. She succumbed following pulmonary insufficiency at age 37 years, 15 months after her lung transplant. Laboratory values showed lymphopenia due to very low B- and NK cell counts. At age 33 she had 2.1 g/L immunoglobulin and lacked IgA and IgM (**Supplementary Table 2**).

Her brother (A.II.8) developed grand mal seizures when he was 12 years old. He was diagnosed with tuberculosis at age 24; in the light of our findings here, it is relevant that a possible differential diagnosis considered at that time was sarcoidosis. He suffered from recurrent pneumonia requiring ventilation and ultimately leading to structural lung disease with pulmonary hypertension. In addition, he suffered from wasting enteropathy with watery diarrhea. This led to malnutrition and osteomalazia with recurrent spontaneous fractures of the spine. His laboratory studies showed a relative lymphopenia with decreased CD8 T cell and B cell counts, but normal CD4 T cell and NK cell numbers. IgG and IgM serum levels were borderline low but IgA was decreased. He died at age 34 following his wasting enteropathy and lung disease.

His son (A.III.3) was well until age 15 years, when he developed chronic diarrhea, lymphadenitis (**Figure 2e**) and splenomegaly, and multiple granulomas in his lungs. At age 18, he developed thrombocytopenia (17,000/µl) and his lung condition worsened, requiring systemic immune suppression with corticosteroids. Duodenal and gastric biopsies showed T lymphocytic infiltrates consisting mainly of $CD4^+$ T cells (**Figure 2b**). IgA^+ plasma cells were reduced in the intestinal tissue in line with reduced switched memory B cells in the peripheral blood (**Supplementary Table 2**). Bone marrow histology showed mild focal T cell infiltrates. Furthermore, histological stainings highlighted strongly decreased levels of plasma cells in the bone marrow.

Another brother (A.II.5) of the proband was diagnosed with CVID at age 41, following the detection of an IgG serum level of 3.83 g/L, and an IgA serum level of 0.26 g/L, while IgM was absent. He suffered from chronic sinusitis, recurrent upper respiratory infections, and chronic diarrhea starting at age 42 years. He also had splenomegaly and was diagnosed with autoimmune thyroiditis at age 49. Around the time of the CVID diagnosis, he still had 130 $CD19^+$ B cells/µl. However, during the following 12 years his B cells declined to currently 0 cells/µl (i.e. undetectable). He has been on immunoglobulin replacement for the last 14 years, and never had any interstitial lung disease/granulomatous disease, or thrombocytopenias. Recently, however, he had an episode of arthritis, which responded to doxycycline.

A sister (A.II.3) of the proband suffered from psoriasis and some mild bouts of diarrhea but was not considered to be affected. However, the daughter (A.III.1) developed oligoarthritis at age 10, followed by severe headaches and nausea at the age 15, for which she was evaluated at a tertiary medical referral center. Multiple lesions in the brain were identified (**Figure 2g**) and partly surgically removed, revealing non-caseating granulomas (**Figure 2h**). At the same time hypogammaglobulinemia of 2.96 g/L was diagnosed and intravenous (IV) immunoglobulin replacement therapy was initiated, but soon after discontinued following the patient's wish. Without IgG supplementation, IgG levels remained stable at about 3.4 g/L with no increased rate

of respiratory infections. At age 26 years, she developed bleedings of her gums and shins and was diagnosed with autoimmune thrombocytopenia with platelet counts of 2,000/µl. She was treated with high dose corticosteroids and IV immunoglobulins, but relapsed thrice while reducing this treatment.

Individual A.II.2 is a 58 year old male who lately developed insulin-dependent diabetes mellitus, but was otherwise considered healthy, as was his youngest brother A.II.10 who suffered from diarrhea for three years following an appendectomy, which then resolved. The proband's mother, A.I.2 had reduced serum IgG of 4.2 g/L at the age of 72 years. She developed bowel cancer at age 78 but was otherwise healthy throughout her life. All other family members are considered healthy.

Family B

The index patient in Family B (individual B.II.4 in **Figure 1a**) is a 43 year old female who has suffered from diarrhea since birth. Whereas the diarrhea was mild until age 34, she has suffered from severe watery diarrhea for the last 9 years. This has led to a weight loss of 30 kg from 73 kg to 43 kg during exacerbations of the disease, when major clinical problems included hypokaliemia and malnutrition/wasting. She was diagnosed with *Epstein-Barr-Virus (EBV)*-positive early stage stomach cancer at age 41 years, which was resected *in toto*. Following immune suppression with steroids and cyclosporine A, she now weighs 60 kg, the need for potassium supplementation has ceased, but stools are still loose with a frequency of up to 7 times per day. The histology work-up of gastric and duodenal biopsies showed T lymphocytic infiltrates consisting mainly of $CD4^+$ T cells (**Figure 2a**), some of which formed large aggregates. Eosinophilia, increased number of apoptotic cells, and micro-granuloma were observed, consistent with an autoimmune enteropathy/colitis. Furthermore, we observed a massive infiltration of polyclonal IgA^+ plasma cells in the duodenal mucosa despite very low B cell counts in the peripheral blood (**Supplementary Table 2**). Infection susceptibility was high for the last

decades with recurrent bronchitis over the winter months, but no pneumonia. Laboratory analysis showed a mild leukopenia and mild anemia, but normal thrombocytes. Lymphocyte phenotyping revealed an almost complete loss of peripheral B cells (4 $CD19^+$ cells/µl). Despite this lack, the percentage of switched memory B cells was normal, immunoglobulin G and A serum levels were only borderline decreased, and serum IgM was normal (**Supplementary Table 2**). Bone marrow histology showed severe focal T cell infiltrates consisting mainly of $CD4^+$ T cells (**Figure 2f**) and a reduced granulopoiesis. Plasma cells were only mildly decreased. The analysis of the B cell precursor distribution in the bone marrow revealed a relative expansion of pre-BI cells and a depletion of pre-BII and immature B cells.

Patient B.II.2 developed a chronic gastritis and inflammatory bowel disease at age 15. As the condition worsened, he developed severe wasting disease and finally succumbed at age 23, in 1986. Acute liver failure was noted as the cause of death. He also had psoriasis. Patient B.II.1 is a 56 year old female who suffered from chronic diarrhea since adolescence. She opens her bowels four to ten times a day but is able to maintain her weight. As stool consistency and frequency are worsened by stress, she was labeled as having “irritable bowel syndrome”. At age 44, she was diagnosed with chronic atrophic gastritis requiring replacement with vitamin B 12.

Her daughter, patient B.III.2, suffered from recurrent sinusitis and otitis starting age 7. At age 10, she lost 4 kg of weight (from 37 to 33 kg) and developed dyspnea. By CT imaging, an idiopathic lung fibrosis was detected. Bronchoalveolar lavage showed chronic inflammation and atrophy of the bronchial wall, the biopsy suggested an exogen-allergic alveolitis, a diagnosis which, however, could not be substantiated. Corticosteroid treatment improved her condition, but the lung disease recurred as soon as steroids were reduced. Steroid sparing treatment with cyclosporine A and cyclophosphamide did not improve her lung disease. IgA was significantly reduced (0.07 g/L), but IgG was only slightly reduced (5.33 g/l). Following worsening of her lung

fibrosis despite high-dose corticoid treatments, she received a lung transplant aged 15 yrs. She died one year later due to pulmonary demise.

Another brother (B.II.3) has occasional diarrhea under stress and psoriasis but is otherwise well. The father of the proband (B.I.1) died at age 66 following heart and kidney diseases. The mother of the proband (B.I.2) is well and alive at age 67. She does not carry the mutation.

Family C

Patient C.II.3 (**Figure 1a**) was born to non-consanguineous parents. At the age of 7 years she was admitted to the hospital because of injuries suffered in a traffic accident. No significant health problems had been reported until then, but at physical examination, cachexia (weight and height under the 3rd percentiles) and hepatosplenomegaly were noticed. Imaging revealed an infiltrative lung disease over both lungs and ventral lymph nodes. Since then, she presented with an increased frequency of respiratory infections, pneumonias, and abdominal pain. At age 9 years, she was hospitalized with anemia, thrombocytopenia, and lymphadenopathy. Initially, she was given IV immunoglobulin at high doses and corticosteroids with no lasting response. Splenectomy was performed at age 10. At age 12, her kidney function started to deteriorate, but remained stable until the age of 16 years when she was hospitalized with an H1N1 influenza infection and worsening of her respiratory, kidney, and liver functions. Since then, she had several infections and episodes of septicemia with deterioration of liver function. At age 20 she died of a septic shock. The initial immune investigation at age 7 showed a moderate decrease of IgG, low IgA, and normal IgM levels (**Supplementary Table 2**), while the immunophenotype of peripheral blood lymphocytes was normal. Two years later all immunoglobulin levels were significantly decreased. The ratio of naïve/memory T cells was reversed.

Her sister (C.II.4) was also of small stature and suffered from enteropathy since the age of 9 years. At age 11 she had normal naïve and memory lymphocytes and normal radiosensitivity. She has not been followed up during the last two years.

Family D

A 21 year old female (individual D.II.1 in **Figure 1a**) developed autoimmune hemolytic anemia (AIHA) at the age of 10 years that responded to oral corticosteroids. Subsequently, hypothyroidism and renal impairment were noted and renal biopsy showed a T cell infiltrate. Splenomegaly and generalized lymphadenopathy were noted and a lymph node biopsy was described with “reactive changes”. CVID was diagnosed based on lack of IgA and IgG, which was decreased to 1.3 g/L at age 19. Lung function testing (DLCO -33% predicted) and a chest CT scan suggested granulomatous lymphocytic interstitial lung disease (GLILD). Lung biopsy showed a T and B cell infiltrate and granulomata, consistent with GLILD. She was lymphopenic (**Supplementary Table 2**). Bone marrow histology showed a trilineage haematopoesis with reactive features and no evidence of significant lymphoid infiltrate. High titers of CMV were found in her saliva and urine, but not in blood. She was treated with high dose oral prednisone and at present is treated with mycophenolate mofetil (MMF). Pneumocyctis jerovecii pneumonia developed whilst she was taking high dose prednisolone. Her mother, apparently healthy, carried the same mutation in Exon 2, but she was not clinically evaluated.

Family E

The patient in Family E is a 20 year old female. The clinical history was unremarkable until the age of 12 years, when she developed recurrent AIHA and autoimmune thrombocytopenia (ITP). Chronic abdominal lymphadenopathy and splenomegaly were recognized. From age 14 years onward the patient suffered from recurrent bacterial pneumonias. In addition to pneumonia related changes, CT findings and lung biopsies also revealed features consistent with GLILD (**Figure 2c**). Detailed analysis of the open biopsy showed the development of inducible bronchus-associated lymphoid tissue (iBALT) characterized by focal or densely arranged $CD19^+$ and $CD20^+$ lymphoid follicles and reactive germinal centers. In addition, the iBALT contained T cell infiltrates with predominance of $CD4^+$ cells including a small subset of $Foxp3^+$ Tregs. $CD4^+$ T cells occasionally invaded the bronchial mucosa (**Figure 2d**). However, the majority of

lymphocytes belonged to the B cell compartment and included interfollicular plasma cells. Laboratory findings of E.II.3 included progressive reduction of IgG, IgA and IgM, as well as lack of specific antibodies and gradually evolving lymphopenia. Initial analysis of lymphocyte subsets was normal, but continuous reduction of switched memory B cells and naive T cells was noted over the past 5 years. Class-switched memory B cells also declined over time. Treatment regimens for her multiple autoimmune phenomena included corticotseroids, cyclosporine A, mycophenolate mofetil and rituximab. In addition, subcutaneous IgG replacement therapy was initiated. Nonetheless, lung disease progressed and the patient continued to suffer from frequent relapses of her AIHA and ITP. At age 18, she developed chronic diarrhea. First gastrointestinal biopsies showed only minor irregularities of the small intestinal villous architecture despite the clinical history of chronical diarrhea. The lymphoid tissue of terminal ileum contained hyperplastic lymphoid follicles and plasma cells of all classes with a predominance of IgD^+ and IgA^+ cells. Recently the condition worsened with the development of severe, watery diarrhea. Repeated microbiological work-up could not identify an infectious trigger. Endoscopy revealed mucosal inflammation of the stomach, duodenum and colon. Biopsies clearly showed a progress of the intestinal lesions. Villous blunting was accompanied by reduction of Paneth cells, numerous $CD4^+$ T cells in the tunica propria, mild intraepithelial $CD8^+$ infiltrates, but only rare B and plasma cells. In the colonic biopsies, a moderate increase in apoptotic crypt epithelium was reminiscent of acute graft versus host disease grade 1 and was attributed to progressive autoimmune enteropathy. Treatment with oral budesonide led to a marked clinical improvement.

Family F

The male patient in Family F, born in 1988, died at the age of 23 years. He was diagnosed with AIHA with a pathological Coombs test at the age of 8 years. After splenectomy, the hematological abnormalities resolved temporarily. However, when the disease relapsed, several accessory spleens were found. In 1999, there was a flare of AIHA and ITP, and Evans syndrome

was diagnosed. Surgical removal of 3 of 6 accessory spleens did not induce a remission, and azathioprine and later, mycophenolate mofetil were prescribed. Rituximab (2x 1000 mg in March and in September 2005) induced a lasting remission of the Evans syndrome. Recurrent infections started in 1998, initially with chronic sinusitis. Surgery of the paranasal sinuses was performed in 2000. Subsequently recurrent bronchitis developed, and CT scans revealed bronchiectasis in 2002. A first immunological assessment was performed in 2003, and the serum IgG was found to be reduced (6.4 g/l). The numbers of $CD4^+$ and $CD8^+$ T cells, B and NK cells were normal. Due to continuing recurrent airway infections, a prophylactic antibiotic therapy (trimethoprime/sulfamethoxazole) was started. In light of reduced serum immunoglobulin levels (**Supplementary Table 2**) subcutaneous immunoglobulin substitution (9.6 g/week) was initiated and the infection rate markedly improved. In 2008, the patient complained of diarrhea (up to 20 bowel movements per day), repeatedly with bloody discharge. In several colonoscopies an inflammation of the colon and distal ileum, and rectal ulcerations were diagnosed. Even though biopsies revealed only an unspecific inflammation, Crohn`s disease was suspected. Neither azathioprine, methotrexate nor TNF inhibitors improved the diarrhea. He suffered a substantial weight loss from 110 to 60 kg in 2011. A colectomy was performed, but flares of bloody bowel movements and abdominal pain continued. The patient died at home in 2011. An autopsy was not performed, and the cause of death therefore remained unknown.

Supplementary notes 2: Genetic linkage analysis

We started to perform genetic linkage analysis with microsatellite markers in Family A back in the year 2000. However, no perfect segregating interval could be established under the assumption of a fully penetrant autosomal-dominant trait. Since the family member A.II.3 was healthy but apparently had transmitted the disease to her affected daughter (Figure 1A), we performed an affected-only analysis to allow for a reduced penetrance of the causative mutation.

In the affected-only linkage analysis a variety of regions showed positive scores. The microsatellite marker D2S1384 within 1 Mbp of *CTLA4* achieves the maximum possible LOD score of +1.20 and the 146bp allele is present in all affected individuals and the obligate carrier. However, there are crossovers between D2S1384 and each of the flanking markers D2S1391 and D2S2944.