Supplementary Figures and Tables

Defective kinase activity of IKKα leads to combined immunodeficiency and disruption of immune tolerance in humans.

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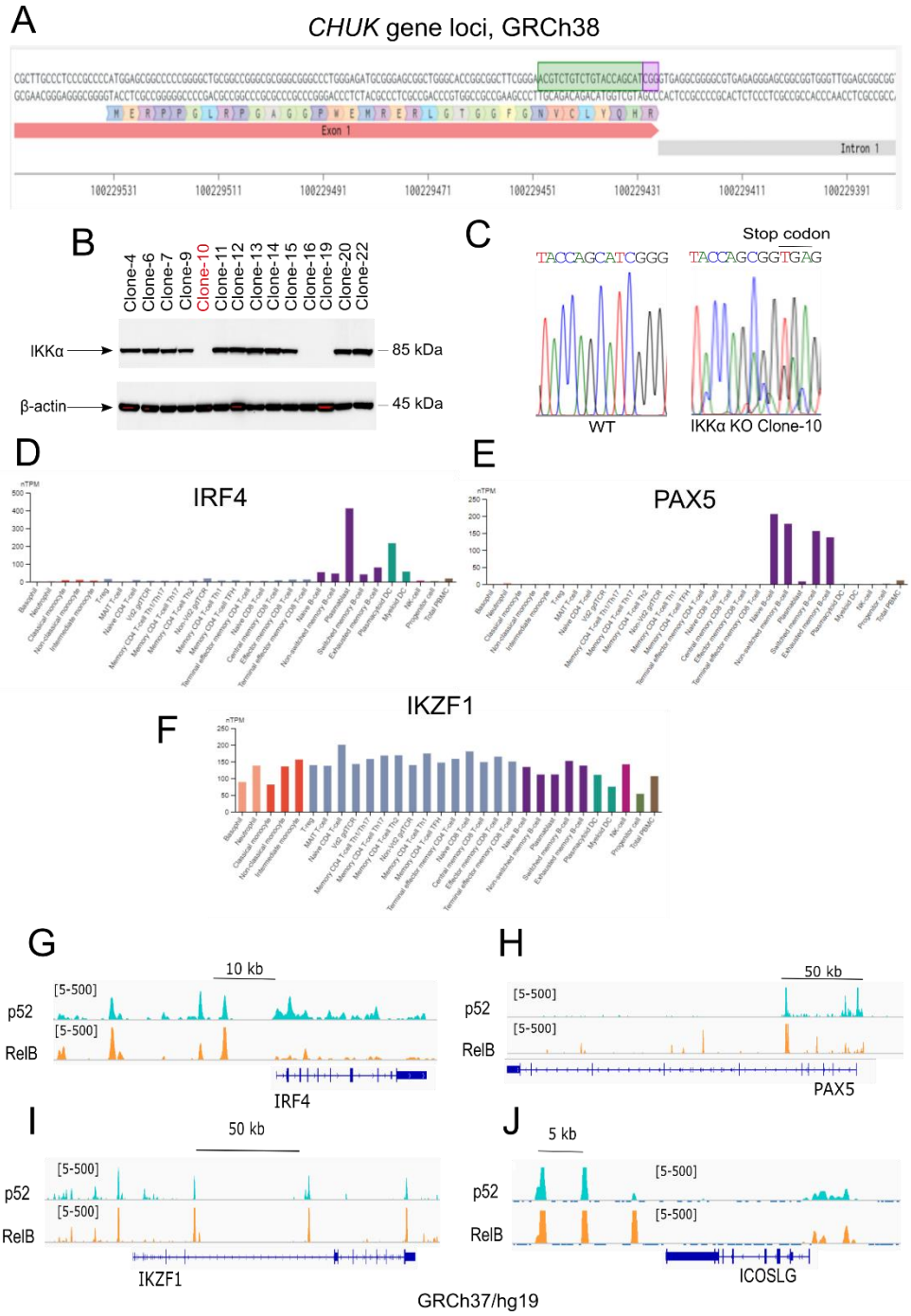

Figure-S1: Editing of *CHUK* gene loci in HEK293T cells and analysis of RelB and p52 binding in the genomic loci of *IRF4, PAX5, IKZF1* and *ICOLG* genes in human B cells.

A) CRISPR/Cas9-mediated *CHUK* deletion strategy in HEK293T cells. A PAM sequence (5'-ACGTCTGTCTGTACCAGCAT-3') in Exon-1 of the *CHUK* gene, coding for IKKα, was selected for targeting. **B)** Western blotting results of IKKα in different clones of genome-edited HEK293T cells. β-actin was used as a loading control. **C)** Sanger sequencing results of genome-edited *CHUK* gene locus in WT HEK293T cells and IKKα-KO HEK293T cells (Clone-10). **D-F)** Expression levels of *IRF4*, *PAX5*, and *IKZF1* were analyzed in 29 blood cell types and total PBMCs from Monaco et al (Ref 58). The transcript expression values (nTPM) are visualized in the figure. **G-J)** p52 and RelB binding in *IRF4, PAX5, IKZF1* and *ICOSLG* gene loci was analysed using the Integrative Genomics Viewer (IGV). ChIP-seq datasets were extracted from a previously published study (GSE55105). Tracks are based on GRCh37/hg19 version of the human genome reference builds.

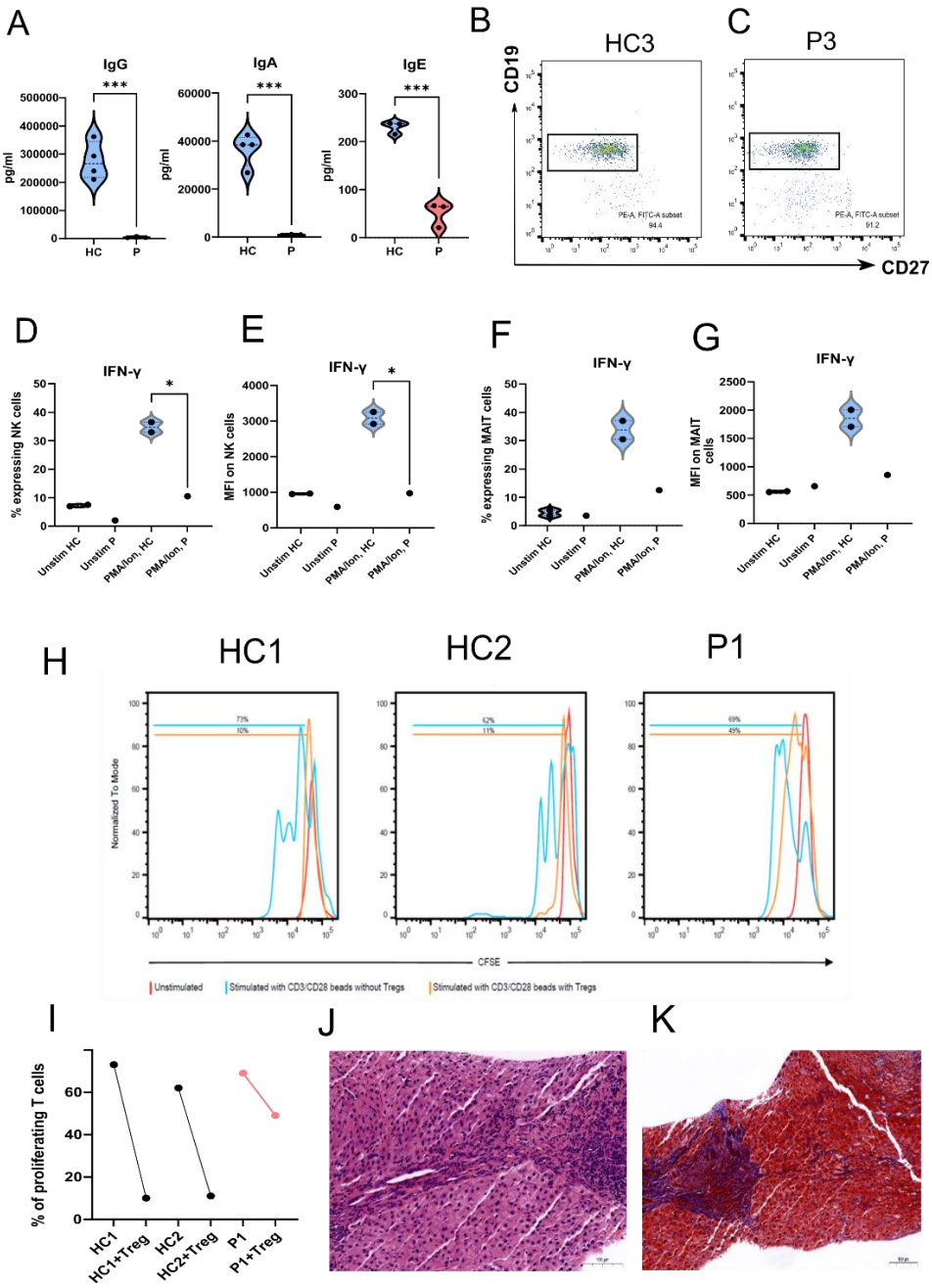

Figure-S2: Analysis of B cell class switching in PBMC culture, IFN-γ expression in NK and MAIT cells, Treg suppression assay and inflammation and fibrosis in liver biopsy sample of patient-1.

A) In vitro class switching experiment using PBMCs of patients (n=3) and healthy controls (n=4) upon in vitro stimulation with recombinant CD40L and IL-4 for 7 days. IgG, IgA and IgE levels in culture supernatant were measured with multiplexed bead-based assay. Two-tailed, non-paired t-test was used for statistical analysis. **B-C)** Flow cytometric analysis of purified naïve B cells from a healthy control and patient sample using CD19 and CD27 markers. **D-G)** Flow cytometric analysis of intracellular IFN-γ expression in NK and MAIT cells with or without stimulation with PMA and ionomycin. Results are from 2 healthy controls and 1 patient. One-way ANOVA was used for statistical analysis. **H)** Treg suppression assay results from 2 healthy controls and patient-1. **I)** Graphical representation of Treg suppression assay in H. **J)** H&E staining (x32) showing portal inflammation and prominent cholestasis around the periportal hepatocytes in liver biopsy sample of P1; scale bar, 100 µm. **K)** Gomori Trichrome staining (x20) showing portal, periportal and perisinusoidal fibrosis in in liver biopsy sample of P1.

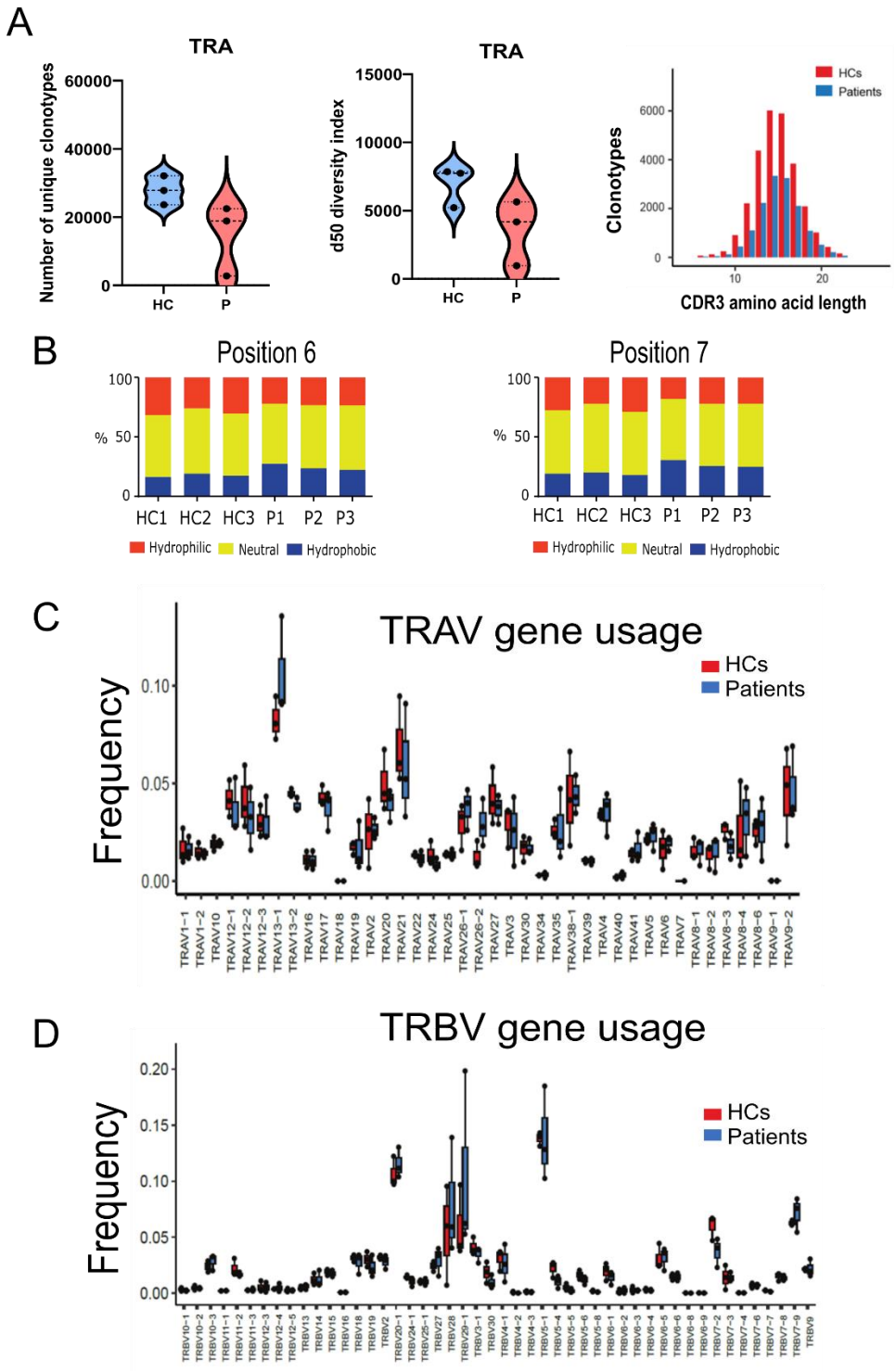

Figure-S3: Gene usage analysis of TCR repertoire in patients and healthy controls.

A) Number of unique clonotypes, d50 diversity index (number of clonotypes occupying the 50% of repertoires) and CDR3 amino acid length of TRA repertoire of healthy controls (n=3) and patients (n=3). Two-tailed, non-paired t-test was used for statistical analysis. **B)** Distribution of hydrophobic, neutral, and hydrophilic amino acids in positions 6 and 7 in TRB CDR3 region of healthy controls (n=3) and patients (n=3). **C)** TRAV gene usage comparison between patients (n=3) and healthy controls (n=3). Red bars indicate healthy controls and blue bars indicate IKKα^{G167R} patients. **D)** TRBV gene usage comparison between patients (n=3) and healthy controls (n=3). Red bars indicate healthy controls and blue bars indicate IKKα^{G167R} patients.

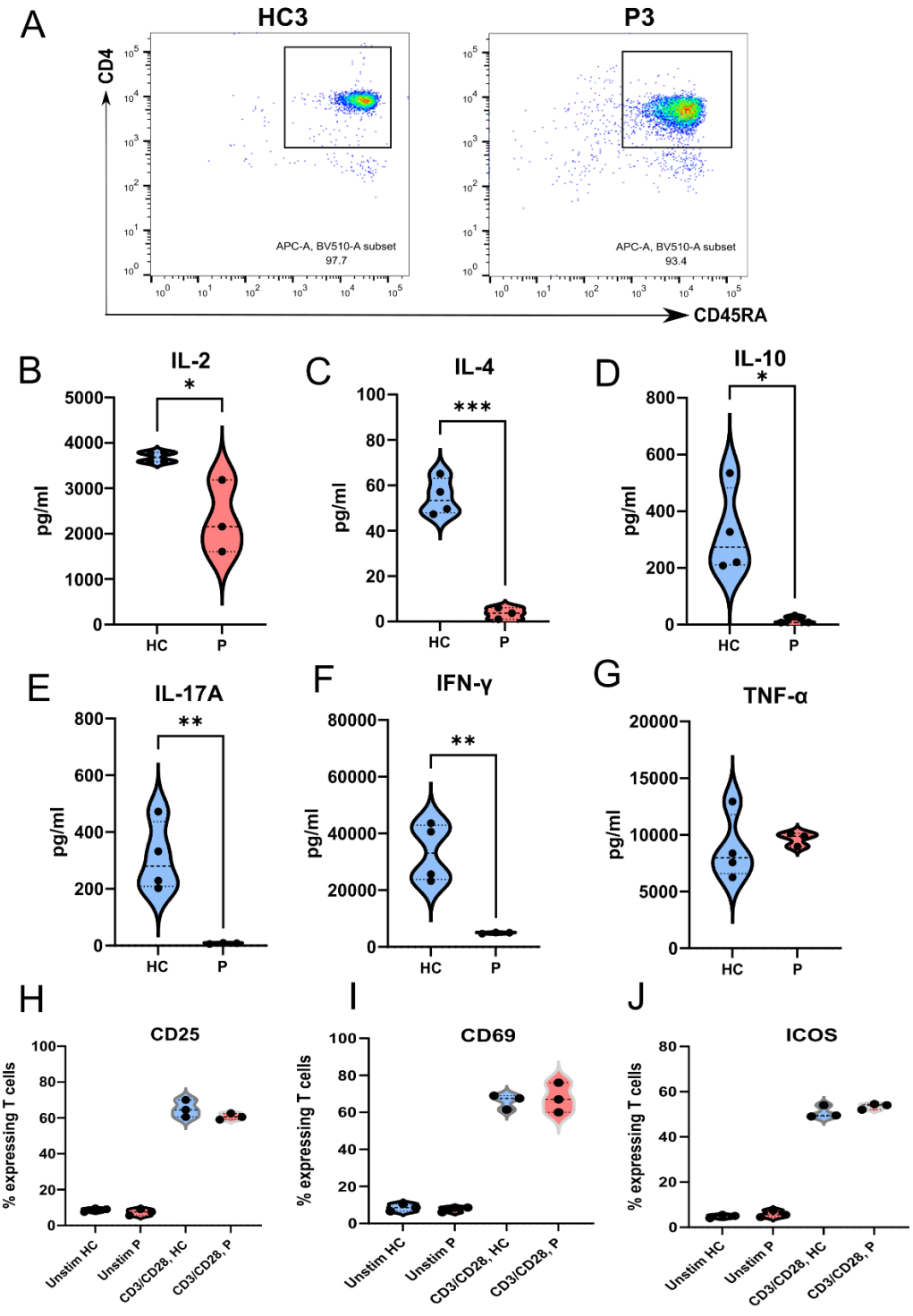

Figure-S4: T cell activation and cytokine release in PBMC culture from patient cells and healthy controls.

A) A representative flow cytometry staining of purified naïve $CD4^+$ $CD45RA^+$ T cells from a healthy control and patient sample. **B-G)** Analysis of the cytokine release from PBMCs 48 hours post stimulation with anti-CD3 and anti-CD28 antibodies (n=4 healthy controls vs n=3 patients). Two-tailed, non-paired t-test was used for statistical analysis. **H-J)** Flow cytometric analysis of the surface expression of CD25, CD69 and ICOS on T cells with or without stimulation of PBMCs with anti-CD3 and anti-CD28 antibodies in cell culture from healthy controls (n=3) and patients (n=3). One-way ANOVA was used for statistical analysis.

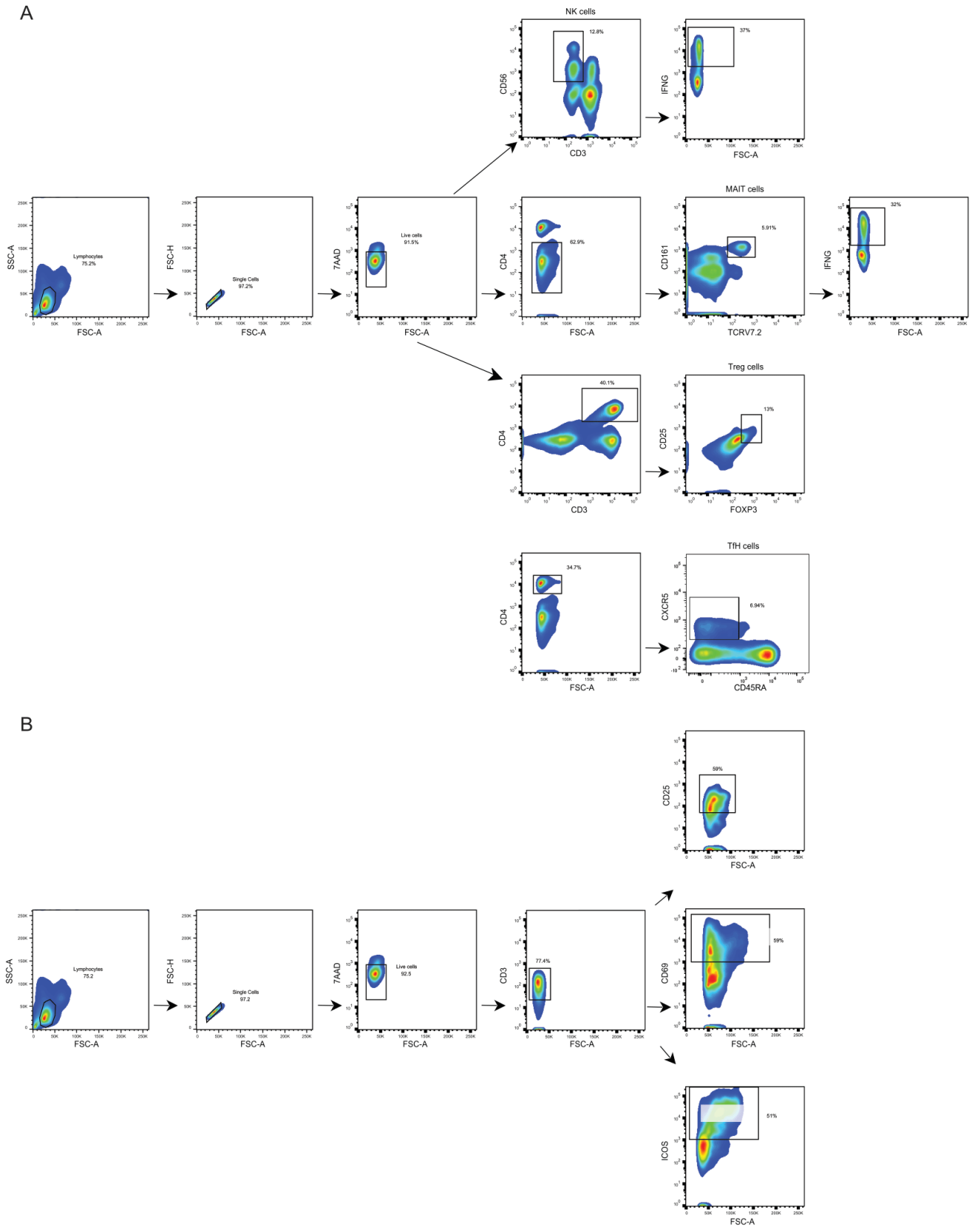

Figure-S5: Gating strategies used for T cell subgroups and NK cells. **A**. Gating strategies for the phenotyping of NK, MAIT, Treg and TfH cells and intracellular staining of IFNG in stimulated NK and MAIT cells. **B**. Gating strategies of the activation markers on stimulated T cells.

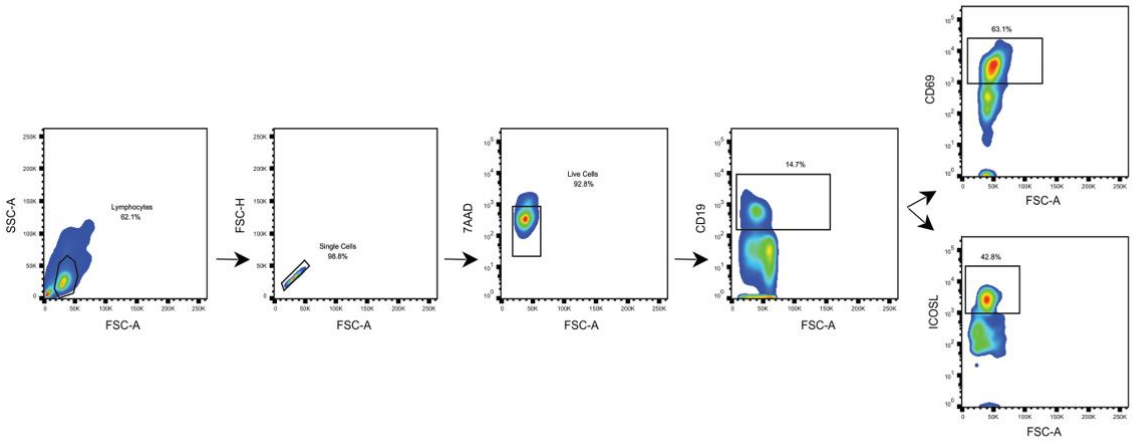

Figure-S6: Gating strategies of the activation markers on stimulated B cells.

Supplementary Table-1: Medical history of patients

Patient-1

The patient has been experiencing frequent lower and upper airway infections since the age of 6 months. Intestinal problems began in the first few months of life. At the age of 2, she was admitted to the child polyclinic due to recurring episodes of diarrhea and vomiting. She also had frequent coughing and expectoration. She experienced fever of unknown origin lasting 3-4 days, which was resolved with intravenous antibiotics. When she was 4 years old, she was diagnosed with bronchitis. She has shown a high susceptibility to infections and has been hospitalized for pneumonia on at least two occasions. For the past 6 months, she has been dealing with persistent oral candidiasis that has not improved. The patient has failure to thrive, as indicated by weight and height measurements at the age of 7 years and 3 months: weight: 15.2 kg (0.12 percentile) and height: 106 cm (0.04 percentile). To address her recurring diarrhea and difficulties with weight gain, she underwent a colonoscopy, which resulted in a preliminary diagnosis of inflammatory bowel disease. This diagnosis was later confirmed by the child gastroenterology service as colitis. She has exhibited poor dental hygiene and has experienced decay in her upper and lower molar teeth. She has been receiving intravenous immunoglobulin (IVIG) and prophylactic trimethoprim/sulfamethoxazole and fluconazole. The patient has presented with icterus, high bilirubin levels, and elevated cholesterol levels. Additionally, hepatosplenomegaly (+5 cm) and hepatic fibrosis were observed. A diagnosis of autoimmune liver disease was made, and a liver biopsy was conducted for further investigation. Inflammatory arthritis was also noted, with synovial thickening in both knees and the left hip joint.

Clinical history of patient-1 after liver transplantation

In August 2023, the patient underwent a liver transplant from her father due to liver failure. At +1 month after the transplant, she developed mild CMV viremia, which was successfully treated with oral valganciclovir. However, 3.5 months after the liver transplantation, she developed cholangitis and required hospitalization at the transplantation centre. Intravenous antibiotic treatment was administered, and there was no significant decline in liver function during this period.

Two weeks after being discharged from the centre, she experienced fever, diarrhea, and vomiting. Due to her poor overall condition and lack of urine output, she was admitted to the hospital in her hometown. A hemogram revealed pancytopenia (WBC: 930/mm3, absolute neutrophil: 550/mm3, absolute lymphocyte: 310/mm3, Hb: 8g/dl, thrombocyte: 33,000/mm3). She also exhibited signs of renal failure, with elevated levels of creatine (6.8 mg/dl), urea (180 mg/dl), BUN (80 mg/dl), and low levels of potassium (K+: 2.7 mmol/L). Coagulation tests showed impairment (prothrombin time: 18.3 seconds and INR: 1.6), and her CRP was measured at 180 mg/dl.

She received broad-spectrum IV antibiotics, fluid support, and vitamin K. Tacrolimus was discontinued after reaching a level of 16 ng/mL. She was planned to be transferred to the paediatric intensive care unit at our hospital due to the need for haemodialysis. However, during a vomiting episode, she aspirated gastric contents, leading to severe respiratory distress

and requiring intubation. The patient experienced cardiac arrest and was resuscitated through cardiopulmonary resuscitation (CPR).

Upon admission to our paediatric intensive care unit, the patient presented with sepsis, hypothermia, hypotension, diffuse mucosal haemorrhages, and a Glasgow Coma Scale (GCS) score of 3. Blood gas analysis revealed a pH of 6.9, PCO2 of 61 mmHg, PO2 of 29 mmHg, and lactate level of 10 mmol/L. Treatment with meropenem, vancomycin, colistin, amphotericin B, and metronidazole was initiated. Adrenaline, noradrenaline, hydrocortisone, dopamine, and methylene blue supplements were administered. Fresh frozen plasma, vitamin K, tranexamic acid, and platelet transfusions were provided. Haemodialysis was performed once hemodynamic stability was achieved, resulting in significant decreases in creatinine, BUN, and urea levels.

Abdominal tomography revealed perfusion disorder in the transplanted liver parenchyma, dilatation, and diffuse wall contrast in the intrahepatic bile ducts. Hypodense regions consistent with multiple abscesses of varying sizes were observed, primarily located peripherally and near dilated bile ducts. No significant vascular pathology was detected.

Despite receiving all available treatments, including platelets, fresh frozen plasma, vitamin K, tranexamic acid, and factor 7, the bleeding from the mucosa could not be controlled. Unfortunately, the patient succumbed to progressive multiorgan failure and passed away on the third day of hospitalization.

Patient-2

The patient has had frequent upper airway infections and recurrent bronchiolitis since she was 2 months old. She was first hospitalized at the age of 1 due to occasional episodes of diarrhea. In addition to the diarrhea, she experienced recurrent oral and perianal aphthous ulcers and abdominal pain. Due to her sister (referred to as patient 1) being diagnosed with Common Variable Immunodeficiency (CVID), the patient was identified early on for IVIG treatments. She has been receiving IVIG and prophylactic trimethoprim/sulfamethoxazole and fluconazole. Her weight is 13 kg, which falls within the 25th percentile, and her height is 93 cm, which falls between the 25th and 50th percentiles.

Patient-3

The patient has been experiencing frequent episodes of diarrhea and bronchiolitis since birth. She has a high susceptibility to infections and has received treatment with antibiotics. At the age of 5 months, she was admitted to the Children's Hospital due to shortness of breath and was subsequently transferred to the intensive care unit (ICU) after being diagnosed with pneumonia. During her time in the ICU, a CT scan revealed that she had contracted COVID-19. Two months later, her immunoglobulin levels were found to be significantly low, leading to the initiation of IVIG treatment. However, over the past two months, she has continued to experience recurrent infections. There is no history of ear infections or oral candidiasis.

At the age of 3 years and 6 months, her weight is 13.65 kg, which falls within the 25th percentile, and her height is 92 cm, which falls between the 25th and 50th percentiles.

	Patient-1	**Patient-2**	**Patient-3**
Gender	Female	Female	Female
Current age	Deceased at the age of 8.5 years old	Under 9 years old	Under 9 years old
Age at onset of symptoms	6 months	2 months	Since birth
Age at CVID diagnosis	4 years old	1 year old	1 year old
Consanguinity	Yes	Yes	Yes
Clinical features			
Infections			
Microorganisms detected at several infectious episodes	- Infections with rotavirus, SARS-CoV-2 (twice, with a 10-month interval) -Haemophilus influenzae, Bocavirus, and Rhinovirus positive in nasopharyngeal swabs. -Oral and oesophageal candidiasis -Candida positive in stool. -CMV positive in plasma.	-Haemophilus influenzae, and adenovirus positive in nasopharyngeal swabs. -Salmonella gastroenteritis -Oral candidiasis	-SARS-CoV-2
Autoimmunity	Yes	Unknown	Unknown
Secondary lymphoid organs	No tonsils or palpable lymph nodes	No tonsils or palpable lymph nodes	No tonsils or palpable lymph nodes
Hepatosplenomegaly	Yes	No	Yes
Allergies	Not detected by skin prick test	Not detected by skin prick test	Not detected by skin prick test
Hepatic dysfunction	Yes	Yes	Yes
Liver failure	Yes	No	No
Liver transplantation	Yes	No	No
Outcome	Deceased	Alive, receiving IVIG treatment	Alive, receiving IVIG treatment

Supplementary Table-2: Test results of patients

Patient-1

Complete blood count

Test name	Result (14/03/23)	Previous results (31/01/23)	Previous results (29/01/23)	Previous results (28/01/23)	Previous results (6/12/22)	Reference range
Leukocyte (WBC) $x10^9$/L	8.03	6.36	5.84	5.2	11.54	5-13.5
Erythrocyte (RBC) $x10^{12}$/L	***3.38**	3.26	3.12	3.03	3.55	4-5.3
Thrombocyte (PLT) $x10^9$/L	***604**	326	281	284	532	150-450
Haemoglobin (Hb) (g/dL)	***8.7**	**8.3**	**7.9**	**7.8**	**8.5**	11.5-15
Haematocrit (%)	***29.2**	**29**	**27.5**	**26.4**	**31**	34-45
Mean corpuscular volume (MCV) (fL)	86.4	89	88.1	87	87.3	76-91
Mean corpuscular haemoglobin (MCH) (pg/cell)	***25.7**	**25.5**	**25.3**	**25.7**	**23.9**	26-31
Mean corpuscular haemoglobin concentration (MCHC) (g/dL)	***29.8**	**28.6**	**28.7**	**29.5**	**27.4**	33-35.7
Red Cell Distribution Width (RDW) (%)	***19.4**	**20.1**	**19**	**18.6**	**18.9**	11.5-14.5
Neutrophil (%)	***34.8**	40.8	46.2	49	56.8	35-65
Lymphocyte (%)	44.8	42.6	40.6	37.9	33.8	30-55
Monocyte (%)	***13**	7.9	6.5	7.7	4.7	2-9
Eosinophil (%)	***6.2**	7.9	5.8	4.6	4.0	0-6
Basophil (%)	1.2	0.8	0.9	0.8	0.7	0-1.5
Neutrophil count $x10^9$/L	2.79	2.6	2.7	2.55	6.56	1.5-8.0
Lymphocyte count $x10^9$/L	3.6	2.71	2.37	1.97	3.9	1.5-7

Monocyte count $x10^9/L$	***1.04**	0.5	0.38	0.4	0.54	0.3-1
Eosinophil count $x10^9/L$	0.5	0.5	0.34	0.24	0.46	0-0.7
Basophil count $x10^9/L$	0.1	0.05	0.05	0.04	0.08	0-0.2
Mean platelet volume (MPV) fL	11.5	11.8	12.2	12	11.7	9.2-12.1
Neutrophil to lymphocyte ratio (NLR)	0.76	0.96	1.14	1.29	1.61	

Immunoglobulins

Sample	**Antibody (before IVIG)**	**Result**	**Reference**
Serum	IgA (g/L)	**<0.0667**	0.34-1.81
Serum	IgM (g/L)	**0.03**	0.42-1.81
Serum	IgG (g/L)	**0.192**	5.01-14.59

Sample	**Test name**	**Result**	**Previous results (31/01/23)**	**Previous results (28/01/23)**	**Previous results (27/01/23)**	**Previous results (6/12/22)**	**Reference range**
Serum	Direct bilirubin (mg/dL)	***12.91**	**12.24**	**10.10**	**11.86**	**8.09**	0-0.3
Serum	Total bilirubin (mg/dL)	***13.98**	**12.50**	**12.70**	**12.37**	**8.11**	0.1-1.2
Serum	Indirect bilirubin (mg/dL)	***1.07**	0.26	2.6	0.51	0.02	0-1
Serum	Aspartate aminotransferase (AST) (U/L)	***324**	**225**	**281**	**274**	**244**	0-35
Serum	Alanine aminotransferase (ALT) (U/L)	***160**	**173**	**208**	**212**	**167**	0-35
Serum	Alkaline phosphatase (U/L)	***1861**	**1538**	**1658**	**1578**	**1773**	142-335
Serum	Gamma-glutamyl transferase (GGT) (U/L)	***563**	**386**	**418**	**395**	**587**	6-42
Serum	Lactate dehydrogenase (U/L)	***373**	**323**	**335**	**323**	**425**	120-300
Serum	CRP (mg/L)	***31.9**	**21.8**	**24.4**	**17.5**	**18.3**	0-5

Coagulation tests

Test name	Result	Previous results (31/01/23)	Previous results (28/01/23)	Previous results (27/01/23)	Reference range
Prothrombin time (seconds)	***17.2**	**13.2**	**13.1**	**12.6**	9.4-12.5
INR	***1.53**	**1.15**	**1.14**	1.1	0.83-1.11
Activated partial thromboplastin time (APTT) (seconds)	***43.6**	35.1	35.4	35.9	25.1-36.5
D-dimer (ng/ml)	120	N. A	N. A	112	0-243
Fibrinogen (g/l)	***6.98**	N. A	**6.57**	**6.21**	2-3.93

Lipid profile

Test name	Result	Previous results (31/01/23)	Previous results (08/12/22)	Previous results (07/12/22)	Reference range
Triglyceride (mg/dL)	***501**	**155**	**401**	**426**	0-150
VLDL Cholesterol (mg/dL)	Not calculated	Not calculated	Not calculated	Not calculated	0-30
HDL Cholesterol (mg/dL)	***12**	**17**	**9**	**9**	40-60
Total cholesterol (mg/dL)	**485**	**461**	**687**	**775**	<200 is ideal
LDL cholesterol (mg/dL)	68	413	101	108	<130 is ideal
Non-HDL Cholesterol (mg/dL)	**473**	**444**	**678**	**766**	<130 is ideal

Urine tests

Test name	Result	Unit	Reference range	Previous results (06/12/22)
pH	6.5		5-8	6
Protein	Negative	mg/dL	0-15	Negative
Glucose	Negative	mg/dL	0-100	Negative
Ketone	Negative	mg/dL	0-5	Negative
Bilirubin	***3**	mg/dL	0-1	**3**
Blood	Negative	e/µl	0-10	Negative
Nitrite	Negative			Negative
Urobilinogen	Normal	mg/dL	0-2	Normal
Leukocyte esterase	Negative	Leu/µl	0-15	Negative

Sample	Test name	Result	Unit	Previous results (16/03/23)	Previous results (31/01/23)	Previous results (25/01/23)
Plasma	EBV qPCR	Negative	copy/ml	Negative	Negative	Negative
Plasma	CMV DNA PCR (quantitative)	**1000, positive**	copy/ml	**796, positive**	**200, positive**	Negative

Specific proteins

Sample	Test	Result	Reference
Serum	Alpha-1 antitrypsin (g/L)	***2.42**	1.02-1.57
Serum	Ceruloplasmin (g/L)	***0.864**	0.23-0.48

Patient-2

Complete blood count

Test name	Result	Previous results (14/03/23)	Previous results (22/08/22)	Previous results (26/10/21)	Reference range
Leukocyte (WBC) $x10^9/L$	***20,52**	13.45	13.05	***18.16**	5-14.5
Erythrocyte (RBC) $x10^{12}/L$	4.93	4.78	4.98	4.4	4-5
Thrombocyte (PLT) $x10^9/L$	***458**	***493**	355	***485**	150-450
Haemoglobin (Hb) (g/dL)	***8.7**	***8.3**	***8.6**	***9.4**	11-14
Haematocrit (%)	33.5	***30**	***30.7**	***30.6**	33-41
Mean corpuscular volume (MCV) (fL)	***68**	***62.5**	***61.6**	***69.5**	76-91
Mean corpuscular haemoglobin (MCH) (pg/cell)	***17.6**	***17.4**	***17.3**	***21.4**	24.5-30
Mean corpuscular haemoglobin concentration (MCHC) (g/dL)	***26**	***27.5**	***28**	***30.7**	32.5-35.5
Red Cell Distribution	***20.3**	***19.5**	***19.2**	***20.2**	11.5-14.5

Width (RDW) (%)					
Neutrophil (%)	42.5	29.3	29.1	33.3	30-60
Lymphocyte (%)	48.2	60.7	61.8	54.6	40-70
Monocyte (%)	7.7	8.1	5.9	10.2	2-10
Eosinophil (%)	1.2	1.6	2.9	1.7	0-6
Basophil (%)	0.4	0.3	0.3	0.2	0-1.5
Neutrophil count $x10^9/L$	***8.7**	3.94	3.79	6.06	1.5-8.0
Lymphocyte count $x10^9/L$	***9.9**	***8.16**	***8.07**	***9.91**	2-8
Monocyte count $x10^9/L$	***1.59**	***1.09**	0.77	*1.85	0.3-1
Eosinophil count $x10^9/L$	0.23	0.22	0.38	0.3	0-0.7
Basophil count $x10^9/L$	0.09	0.04	0.04	0.04	0-0.2
Mean platelet volume (MPV) fL	10.2	9.9	9.9	9.9	9.2-12.1
Neutrophil to lymphocyte ratio (NLR)	0.88	0.48	-	-	

Immunoglobulins

Sample	**Antibody**	**Results (Before IVIG)**	**Reference**
Serum	IgA (g/L)	<0.6	0.34-1.81
Serum	IgM (g/L)	***0.1**	0.42-1.81
Serum	IgG (g/L)	***0.3**	7-16
Serum	IgE (IU/mL)	10.1	0-100

Sample	**Test name**	**Result (15/03/23**	**Previous results (14/03/23)**	**Reference range**
Serum	Direct bilirubin (mg/dL)	**-**	0.06	0-0.3
Serum	Total bilirubin (mg/dL)	*** 0,05**	0.11	0.1-1.2
Serum	Indirect bilirubin (mg/dL)		0.05	0-1

Serum	Aspartate aminotransferase (AST) (U/L)	33	30	0-35
Serum	Alanine aminotransferase (ALT) (U/L)	12	13	0-35
Serum	Alkaline phosphatase (U/L)	182	181	142-335
Serum	Gamma-glutamyl transferase (GGT) (U/L)	7	7	6-42
Serum	Lactate dehydrogenase (U/L)	***361**	***311**	120-300
Serum	CRP (mg/L)	0.7	0.8	0-5

Patient-3

Complete blood count

Test name	**Result**	**Reference range**
Leukocyte (WBC) $x10^9$/L	***13.89**	3.39 – 8.86
Erythrocyte (RBC) $x10^{12}$/L	4.08	4-5
Thrombocyte (PLT) $x10^9$/L	***486**	158 - 374
Haemoglobin (Hb) g/dL	***10.9**	11.1 – 14.7
Haematocrit (%)	***33.8**	36.9 – 49.1
Mean corpuscular volume (MCV) fL	*** 82.8**	87 – 102.2
Mean corpuscular haemoglobin (MCH) pg/cell	26.7	25.6 – 30.8
Mean corpuscular haemoglobin concentration (MCHC) g/dL	32.2	32.2 – 35.5

Red Cell Distribution Width (RDW)	***14.10**	11.2 - 14
Neutrophil %	***28.10**	30-60
Lymphocyte %	63.70	40-70
Monocyte %	6.30	2-10
Eosinophil %	1.4	0-6
Basophil %	0.5	0-1.5
Neutrophil count $x10^9/L$	3.90	1.5-8.0
Lymphocyte count $x10^9/L$	***8.85**	2-8
Monocyte count $x10^9/L$	0.88	0.3-1
Eosinophil count $x10^9/L$	0.19	0-0.7
Basophil count $x10^9/L$	0.07	0-0.2
Mean platelet volume (MPV) fL	***9.1**	9.2-12.1

Immunoglobulins

Sample	**Antibody**	**Result (Before IVIG)**	**Reference**	**Unit**
Serum	IgA	***0.1**	0.19 – 2.20	g/L
Serum	IgM	***0.1**	0.4-1.4	g/L
Serum	IgG	***5.6**	7-16	g/L
Serum	IgE	12.2	0-100	IU/mL

Supplementary Table-3: Possible disease-causing variants identified by WES (minor allele frequency <= 0.01)

Gene	Chr	Variant	Zygosity	Consequence	CADD	PolyPhen	SIFT
				P1 and P2			
CHUK	10	c.499G>A p.Gly167Arg	Hom	Missense	28.5	Possible damaging	Deleterious
TREX1	3	c.341G>A p.Arg114Hi	Het	Missense	18	Possible damaging	Deleterious
CFTR	7	c.3659C>T p.Thr1220Ile	Het	Missense	10	Benign	Tolerated
POLE	12	c.232T>G p.Leu78Val	Het	Missense	16.4	Benign	Tolerated
				P3			
CHUK	10	c.499G>A p.Gly167Arg	Hom	Missense	28.5	Possible damaging	Deleterious
BRCA2	13	c.800G>A p.Gly267Glu	Hom	Missense	13	Possible damaging	Tolerated
KMT2D	12	c.14267A>G p.Lys4756Ar	Het	Missense	22	Benign	Tolerated

Supplementary Table-4: Detailed analysis of IKKα^{G167R} by Missense 3D

Type	Analysis	Criterion
Disulphide breakage	The wild-type residue is not CYS so it cannot form a disulphide bond	The substitution breaks a disulphide bond that was in the wild type. The maximum S-S length for the bond is 3.3 Å
Buried Pro introduced	This substitution does not introduce a proline	The substitution introduces a buried proline.
Clash	This substitution does not trigger clash alert. The local clash score for wild type is 69.50 and the local clash score for mutant is 71.29.	The mutant structure has a MolProbity clash score ≥ 30 and the increase in clash score is > 18 compared to the wild type.
Buried hydrophilic introduced	This substitution does not replace a buried hydrophobic residue with a hydrophilic residue. The wild-type residue GLY is exposed neutral with RSA 20.2% and the mutant residue ARG is buried hydrophilic with RSA 8.0%	The substitution replaces a buried hydrophobic residue with a hydrophilic residue.
Buried charge introduced	This substitution does not trigger buried uncharged residue alert. The wild-type residue GLY is exposed uncharged with RSA 20.2% and the mutant residue ARG is buried charged with RSA 8.0%	The substitution replaces a buried uncharged residue with a charged residue.
Secondary structure altered	This substitution does not alter the secondary structure 'S' (bend)).	A substitution results in a change in the DSSP secondary structure assignment at the variant position.
Buried charge switch	This substitution does not trigger buried charge switch alert. The wild-type residue GLY is exposed uncharged with RSA 20.2% and the mutant residue ARG is buried positive-charged with RSA 8.0%	The substitution switches the charge (+/-) of the buried residue.
Disallowed phi/psi	This substitution does not trigger disallowed phi/psi alert. The phi/psi angles are in favoured region for wild-type residue and in allowed region for mutant residue.	The mutant residue is in outlier region while the wild-type residue is in the favoured or allowed region.
Buried charge replaced	This substitution does not replace a buried charged residue with an uncharged residue. The wild-type residue GLY is exposed uncharged with RSA 20.2% and the mutant residue ARG is buried charged with RSA 8.0%.	The substitution replaces a buried charged residue with an uncharged residue.

Buried Gly replaced	This substitution does not replace a buried GLY residue.	The substitution replaces a buried glycine.
Buried H-bond breakage	This substitution does not result in a complete disruption of all side-chain / side-chain H-bond(s) and/or side-chain / main-chain bond(s) bonds, and the wild-type residue is not buried (RSA 20.2%).	The substitution breaks all side-chain / side-chain H-bond(s) and/or side-chain / main-chain H-bond(s) formed by the wild type which was buried. The maximum H-bond N-O length is 3.9 Å.
Buried salt bridge breakage	The wild-type residue does not form any salt bridge.	The substitution breaks a salt bridge formed by wild-type which was buried. The maximum N-O bond length is 5.0 Å.
Cavity altered	The substitution leads to the contraction of cavity volume by 145.152 Å^3	The substitution leads to an expansion or contraction of the cavity volume of ≥ 70 Å^3. Cavity also refers to a pocket on the surface.
Buried / exposed switch	The wild-type residue GLY is exposed (RSA 20.2%) and the mutant residue ARG is buried (RSA 8.0%)	The substitution results in a change between buried and exposed state of the target variant residue. (RSA < 9% for buried and the difference between RSA has to be at least 5%.)
Cis pro replaced	Wild-type residue is not a cis proline.	A cis proline in the wild type is replaced in the mutant.
Gly in a bend	This substitution replaces glycine originally located in a bend curvature.	The wild-type residue is glycine and is located in a bend curvature (reported 'S' in DSSP).

Supplementary Table-5: Detailed analysis of IKKα^{G167R} by MutPred2

ID	Substitution	MutPred2 score	Remarks	Affected PROSITE and ELM Motifs
IKKA_HUMAN	G167R	0.9	-	ELME000146, ELME000313, PS00007
Molecular mechanisms with P-values <= 0.05			**Probability**	**P-value**
Altered DNA binding			0.47	5.80E-05
Altered Ordered interface			0.31	0.01
Altered Metal binding			0.3	4.40E-03
Altered Transmembrane protein			0.27	5.40E-04
Gain of Helix			0.27	0.03
Loss of Relative solvent accessibility			0.25	0.03
Gain of Acetylation at K162			0.21	0.03
Loss of Catalytic site at D165			0.1	0.04

For more information on the probability and statistical analyses performed by the MutPred2 algorithm, readers are referred to the study by Pejaver, V., Urresti, J., Lugo-Martinez, J. et al. Inferring the molecular and phenotypic impact of amino acid variants with MutPred2. Nat Commun 11, 5918 (2020).

Supplementary Table-6: Detailed analysis of IKKα^{G167R} by CUPSAT

Amino acid	Overall Stability	Torsion	Predicted ΔΔG (kcal/mol)
ALA	Destabilising	Favourable	-0.45
VAL	Destabilising	Unfavourable	-0.82
LEU	Stabilising	Unfavourable	0.34
ILE	Destabilising	Unfavourable	-1.76
MET	Destabilising	Unfavourable	-2.17
PRO	Stabilising	Favourable	0.24
TRP	Destabilising	Unfavourable	-1.56
SER	Stabilising	Favourable	0.23
THR	Destabilising	Unfavourable	-0.88
PHE	Destabilising	Unfavourable	-0.29
GLN	Destabilising	Favourable	-0.68
LYS	Destabilising	Favourable	-1.12
TYR	Destabilising	Unfavourable	-2.02
ASN	Destabilising	Favourable	-1.05
CYS	Destabilising	Unfavourable	-2.12
GLU	Destabilising	Favourable	-0.62
ASP	Destabilising	Favourable	-0.77
ARG	Destabilising	Favourable	-0.83
HIS	Stabilising	Favourable	0.99

Supplementary Table-7: Primer sequences used in this study.

Gene	Assay	Forward primer	Reverse primer
CHUK (IKKα)	PCR	AGTGACGCATTCATTCTCGC	ACGCACTGTCACACTCACTA
CHUK (IKKα)	qRT-PCR	CCTCAAGATGGGGAGACTTC	ACTCATTCTGTTAACCAACTCCA
IRF4	qRT-PCR	TCCGAGAAGGCATCGACAAG	AGGCGTTGTCATGGTGTAGG
PAX5	qRT-PCR	TATCCGACTCCTCGGACCAG	GTTCCACTATCCTCTGGCGG
IKZF1	qRT-PCR	TGGCAGGGCAGAGGGAG	GCATCCATGGTCCTCAGGTT
GAPDH	qRT-PCR	CGACCACTTTGTCAAGCTCA	GAGGGTCTCTCTCTTCCTCT
CCR6	qRT-PCR	AGCACACCACCCAGTGTATG	CGTGGGCCCTCTCTTACTGA
KLRG1	qRT-PCR	TAGCTTTGTGCAGACATGCG	TGCAAAGGGACACTTCTTACAC
GPR15	qRT-PCR	TTGCATTTCAAACCCGGCAG	CCGTCCTCCACAGTCCTAGA
KLRB1	qRT-PCR	CTCTGTCTGCCATGGACCAA	GAACCCTGACAGACATCCCG
GZMK	qRT-PCR	CTCCATCCAGTATGGCGGAC	GTGTGCGCCTAAAACCACAG
NR4A1	qRT-PCR	CATTGTTGCCAAGACCTGCC	ATCTAGCCTCACAGGAGGGG

Supplementary Table-8: Antibodies used in this study.

Antibody name	Cat number	Company	Assay	Conjugate	Dilution
IKKα	A19694	ABclonal	Western blotting (Cell lines)	-	1:1000
IKKα	621401	BioLegend	Western blotting (PBMCs)	-	1:1000
NIK	4994	CST	Western blotting	-	1:1000
NF-κB2 p100/p52	695902	BioLegend	Western blotting	-	1:1000
Phospho-NF-κB2 p100 (Ser866/870)	4810	CST	Western blotting	-	1:1000
FLAG	F9291	Sigma	Western blotting	-	1:3000
β-Actin	4970	CST	Western blotting	-	1:3000
GAPDH	2118	CST	Western blotting		1:3000
α-Tubulin	ab7291	Abcam	Western blotting		1:3000
CD19	363010	BioLegend	Flow cytometry	APC-Cy7	1:100
CD27	356410	BioLegend	Flow cytometry	APC	1:100
IgD	348225	BioLegend	Flow cytometry	BV421	1:100
IgM	314506	BioLegend	Flow cytometry	FITC	1:100
CD38	356606	BioLegend	Flow cytometry	APC	1:100
CD21	354908	BioLegend	Flow cytometry	PerCP	1:100
CD3	317318 317308	BioLegend	Flow cytometry	APC, PE	1:100
CD8	345774	BD	Flow cytometry	PerCP	1:50
CD4	300546	BioLegend	Flow cytometry	BV510	1:100
CD8	344748	BioLegend	Flow cytometry	BV421	1:100
CD56	362508	BioLegend	Flow cytometry	PE	1:100
CD161	339926	BioLegend	Flow cytometry	PB	1:100
TCRV α7.2	351728	BioLegend	Flow cytometry	AF700	1:100
CD25	302604	BioLegend	Flow cytometry	FITC	1:100
FOXP3	320108	BioLegend	Flow cytometry	PE	1:100
CXCR5	356914	BioLegend	Flow cytometry	FITC	1:100
CD31	303106	BioLegend	Flow cytometry	PE	1:100
CD16	360706	BioLegend	Flow cytometry	APC	1:100
CD16	302017	BioLegend	Flow cytometry	APC-Cy7	1:100
ICOSL	309403	BioLegend	Flow cytometry	PE	1:100
CD69	310906	BioLegend	Flow cytometry	PE	1:100
CD45RA	304112	BioLegend	Flow cytometry	APC	1:100
IFN-γ	69007	Proteintech	Flow cytometry	CL488	1:100
ICOS	313508	BioLegend	Flow cytometry	PE	1:100