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Supplementary Information for

Human RIPK1 Deficiency Causes Combined Immunodeficiency and Inflammatory Bowel Diseases

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Supplementary Information Text

Materials and Methods

DNA sequencing

Patients' and family members' peripheral blood was used to isolate genomic DNA using QIAamp DNA Blood Mini Kit (Qiagen) according to manufacturer's instructions. Upon enrichment for exoms using Agilent Exome enrichment or Ampliseq exome panel, whole exome sequencing was conducted on Illumina sequencing platforms. Bioinformatics analysis for detection of rare sequence variants following Mendelian inheritance patterns were performed as described previously (1, 2). PCR amplification of mutated DNA regions was conducted using primers described in the *SI Appendix*, Table S4. Sanger sequencing was performed by Eurofins Genomics (MWG Eurofins) or ABI PRISM Terminator v1.1 Cycle Sequencing Kit (ABI). Sequences were aligned and analyzed by custom alignment software.

Cell culture

Epstein-Barr Virus–transformed lymphoblastoid cell lines (EBV-LCL) from healthy donors and patients as well as RIPK1-deficient Jurkat cells (kindly provided by Dr. B. Seed) and BLaER1 cells were cultured in RPMI-1640 medium supplemented with GlutamAX^{TM} (Thermo Fisher Scientific), 10% v/v fetal bovine serum (FBS), 1% penicillin-streptomycin, 1 mM Sodium Pyruvate, and 10 mM HEPES (all from Gibco, Life Technologies).

HT-29 (ATCC, HTB-38), HCT-116 (ATCC, CCL247), and patient-derived dermal fibroblasts were maintained in DMEM (Thermo Fisher Scientific) supplemented with 1%

L-glutamine, 10% v/v FBS, and 1% penicillin penicillin/streptomycin. All cells were routinely tested for Mycoplasma contamination.

To differentiate BLaER1 cells into monocytes, 80,000 cells per well were cultured in RPMI-1640 medium supplemented with recombinant human IL-3 (10 ng/ml), recombinant human M-CSF (10 ng/ml, both from PeproTech), and β-Estradiol (100 nM, Sigma-Aldrich) for 6 days based on published protocol (3).

Engineering of RIPK1 knockout cell lines using CRISPR/Cas9 genome editing

Two sgRNAs targeting the 5'- and 3'- end of RIPK1 were designed with the online CRISPR Design tool developed by the Feng Zhang's lab. Two targets with low off-target rates were chosen (*SI Appendix*, Table S4). sgRNAs were subcloned into pSpCas9(BB)-2A-GFP vector (PX458, PX459) (Addgene). Plasmids were transfected into HT-29 and HCT-116 cell lines by lipofectamine 2000 (Thermo Fisher Scientific) according to manufacturer's protocols. Single cells were sorted in 96-wells 48 h after transfection. RIPK1 KO in expanded clones was confirmed by Sanger sequencing and Western blot analysis.

Cloning and lentiviral overexpression of RIPK1 mutants

Human wt *RIPK1* was amplified from the verified cDNA sequence clone purchased from Dharmacon GE Healthcare (Accession: BC126254, Clone ID: 8991970) and cloned into the bicistronic pET-IRES-RFP plasmid. PCR-based site-directed mutagenesis has been used to generate patient-specific mutants. For constructing the "all-in-one" Tetracycline-inducible expression system, tetracycline operator and repressor were amplified from

pcDNA4/TO-Flag-H3.3 (a gift from Bing Zhu; Addgene, #47980) and pcDNA6/TR, respectively, and cloned into the pET-IRES-RFP vectors.

Lentiviral packaging plasmids psPAX2 and pMD2.G (kindly provided by Didier Trono, Addgene, #12260 and #12259) were cotransfected with the lentiviral vector encoding RIPK1 into HEK293T cells in 10 cm petri-dishes at 70% confluency. The supernatants containing viral particles were collected every 24 hours for a period of 72 hours. Subsequently, RIPK1-deficient BLaER1, Jurkat, HT-29, and HCT-116 cells were transduced with lentiviral particles as described previously (1). Transduced cells were sorted by RFP mean fluorescence intensity using the BD FACSAria cell sorter (BD Bioscience).

Immunophenotyping of peripheral blood mononuclear cells

Patients' blood samples were washed with PBS (Gibco) and stained with antibodies (*SI Appendix*, Table S5) in BD Brillant stain buffer (BD Biosciences). Except for chemokine receptors that were incubated at 37 °C, surface antigens were incubated at room temperature for 15 min. 1x BD FACS™ Lysing solution (BD Biosciences) was used to remove red blood cells. Sample acquisition was performed on the LSRFortessa™ flow cytometer (BD Biosciences) and data were analyzed using Flowjo V9 (TreeStar).

T cell proliferation and activation

PBMCs isolated from healthy controls, patients, and family members were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE, Thermo Fisher Scientific, 2.5µM) and co-cultured with Biotin-anti-CD3 (Thermo Fisher Scientific) coupled to anti-Biotin

MACSiBeads (Milteni Biotec) with or without soluble anti-CD28 (Thermo Fisher Scientific, 1μg/ml) or with phorbol 12-myristate 13-acetate (PMA, 0.5 ng/ml) and ionomycin (Sigma-Aldrich, 1μM). Activated $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells were analyzed based on CD25 and CD69 expression by BD CANTOII flow cytometer (BD Biosciences) after two days. To analyze T cell proliferative responses CFSE dilution assays were assessed after 4-6 days.

Immunoblotting, ELISA, and cytotoxicity

To analyze the expression of RIPK1 in EBV-LCL and primary fibroblasts, the NF-κB signaling in Jurkat cells upon TNF-α (50 ng/ml, Peprotech) stimulation, as well as IL-1β production and secretion in cell lysates and supernatants of BLaER1 cells upon treatment with LPS (200 ng/ml, 12 hours, Sigma-Aldrich) ± Nigericin (6.5 μM, 2 hours, Sigma-Aldrich), immunoblotting was performed following standard procedures. Cells were lysed in 1x cell lysis buffer (Cell Signaling) and protein concentrations were normalized using Bradford quantification. Equal amounts of cell lysates were then subjected to SDS-PAGE. To detect MLKL oligomerization in HT-29 cells stimulated with TNF-α (100 ng/ml, PeproTech) ± BV6 (100 μM, kindly provided by Genentech) ± Z-VAD-FMK (20 μM, Enzo Life Sciences) the samples were boiled under non-reducing conditions. Proteins were transferred onto polyvinylidene difluoride (PVDF) membranes, followed by blocking in 5% skimmed milk. Primary and HRP-conjugated secondary antibodies are listed in *SI Appendix*, Table S6. Chemiluminescence signals were detected using the ChemiDocTM XRS+ System (Bio-Rad) and analyzed with ImageLabTM software (Bio-Rad).

To quantify IL-1β secretion of BLaER1 cells upon inflammasome activation with LPS (20

ng/ml, 3 hours) ± Nigericin (6.5 μM, 1 hour) ± small molecule inhibitors for NLPR3 (MCC950, 5 μM) and MLKL (NSA, 2 μM, 2 hours before LPS priming), supernatants were collected and measured using the Human IL-1beta/IL-1F2 DuoSet ELISA Kit (DY201, R&D Systems) and a Synergy H1 microplate reader (BioTek Instruments). Cytotoxicity was quantified by lactate dehydrogenase (LDH) release using the CytoTox96® nonradioactive cytotoxicity assay kit (G1780, Promega) according to the manufacturer's instructions.

Electrophoretic mobility shift assay

For electromobility shift assays (EMSA), RIPK1 cDNA sequences were ligated into the pcDNATM6/myc-His A (Invitrogen) expression plasmid to reconstitute RIPK1$^{-/-}$ Jurkat cells with RIPK1 wt and the RIPK1 variant Y426*. Jurkat cells were electroporated using the Amaxa® Cell Line Nucleofector® Kit V (Lonza) using 5.5 μg *RIPK1* plasmid. Cells with stable transcripts were positively selected using RPMI 1640 (1x) + L-glutamine + 25 mM HEPES (Gibco) supplemented with 10% FBS (PAA) and 5 μg/ml Blasticidin (Sigma-Aldrich) as selection medium. Jurkat cells and patient's fibroblasts were stimulated with TNF-α (ImmunoTools; 50 ng/ml) for 30 min. Protein extraction and EMSAs were performed as previously described (4).

Confocal immunofluorescence microscopy

Dermal fibroblasts were seeded onto glass coverslips and fixed with 3.7% paraformaldehyde (Santa Cruz) for 15 minutes at room temperature. Cells were permeabilized by incubation with 0.5% Nonidet P40 (Sigma-Aldrich) at room temperature,

followed by blocking in 3% bovine serum albumin for 20 minutes. RIPK1 was stained by anti-RIPK1 antibody (Cell signaling) for 90 minutes followed by the secondary antibody staining for 45 minutes (*SI Appendix*, Table S7). Actin filaments and nuclei were stained by Alexa Fluor® 594 Phalloidin (Thermo Fisher Scientific) and 4,6 diamidino-2-phenylindole (DAPI; Sigma-Aldrich). Cells were mounted by fluorescent mounting medium (Dako) and visualized using Leica TCS SP5 confocal microscope (Leica). Images were analyzed by Leica LAS AF Lite and ImageJ software.

Dual-luciferase reporter assay

To determine NF-κB activity HCT-116 cells were co-transfected with 300 ng p55-A2-Luc luciferase reporter plasmids containing the NF-κB binding sites and 15 ng pTK-Green Renilla plasmids as an internal control. After 24 hours of incubation, cells were treated with 10 ng/ml TNF-α (Peprotech) for the indicated time points. Cells were then lysed in 1x passive lysis buffer and firefly and renilla luciferase activities were determined according to the protocol of the Dual Luciferase Assay Kit (Biotium) using a Synergy H1 microplate reader (BioTek Instruments).

Analysis of cell death in HT-29 coloncarcinoma cell lines

HT-29 cells were seeded in 48-well plates and cultured in complete DMEM media for 12 h. Cell death was induced by treatment with TNF-α (100 ng/ml, PeproTech) ± BV6 (100 μM, kindly provided by Genentech) ± Z-VAD-FMK (20 μM, Enzo Life Sciences). Treated cells were collected at indicated time points, stained with Annexin V and DAPI (Thermo Fisher Scientific), and subjected to flow cytometry analyses. To screen for cell death responses in

Jurkat cells, Annexin V and DAPI staining or sub-G1 fractions stained with DAPI staining buffer containing 0.25% Triton X100 (Sigma-Aldrich) have been analyzed by flow cytometry.

Statistics

Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software). No method of randomization or blinding was used. No samples were excluded from analysis. Two-tailed unpaired t-test or One-way ANOVA with Dunnett's correction were performed. Graphic data were indicated as mean ± SD. *P* values < 0.05 were considered as statistically significant.

Data availability

The identified *RIPK1* mutations of our patients will be submitted to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) upon publication. Information on the raw whole-exome sequencing data will not be published to protect research participant privacy.

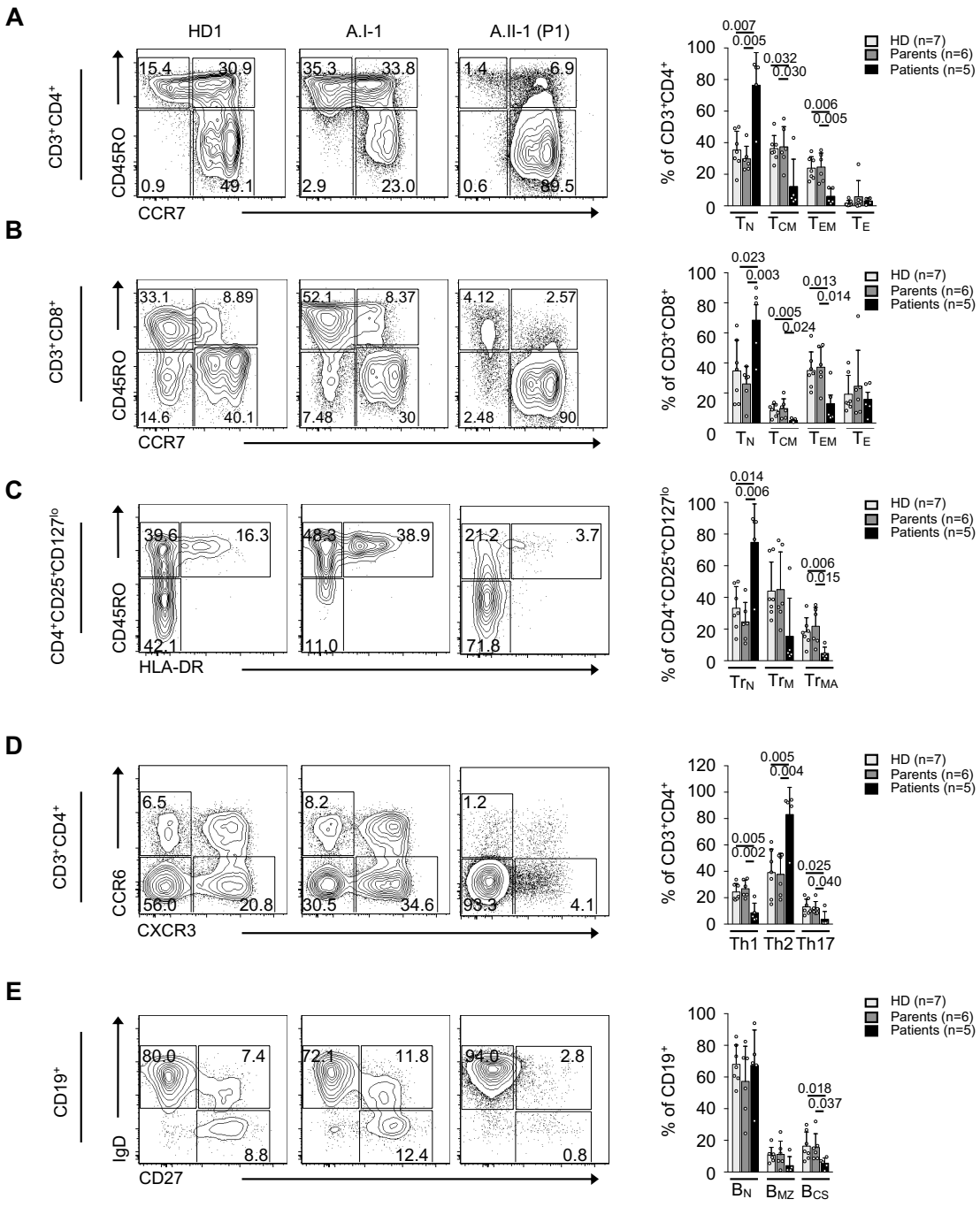

Fig. S1. RIPK1 deficiency is associated with lymphocyte dysfunction.

(A-E) Representative immunophenotypical analysis of naïve (T_N), central memory (T_{CM}), effector memory (T_{EM}), and effector (T_E) $CD4^+$ (A) and $CD8^+$ (B) T cells (based on CD45RO and CCR7 expression, $CD127^{lo}CD25^+$ naïve (Tr_N), memory (Tr_M) and activated memory (Tr_{MA}) Treg cells (**C**), $CXCR3^+CCR6^-$ Th1 and $CXCR3^-CCR6^+$ Th17 (**D**), as well

as IgD^+CD27^- naïve (B_N), IgD^+CD27^+ marginal zone (B_{MZ}), and IgD^-CD27^+ switched memory (B_{SM}) B cells (**E**) in PBMC from P1 (left panel). Graphical representations of the immunphenotypical analysis of P1, P5, P6, P7, and P8 showing the mean ± SD of indicated cell populations (right panel). HD, light grey; parents, dark grey; patients, black. Exact *p* values are shown.

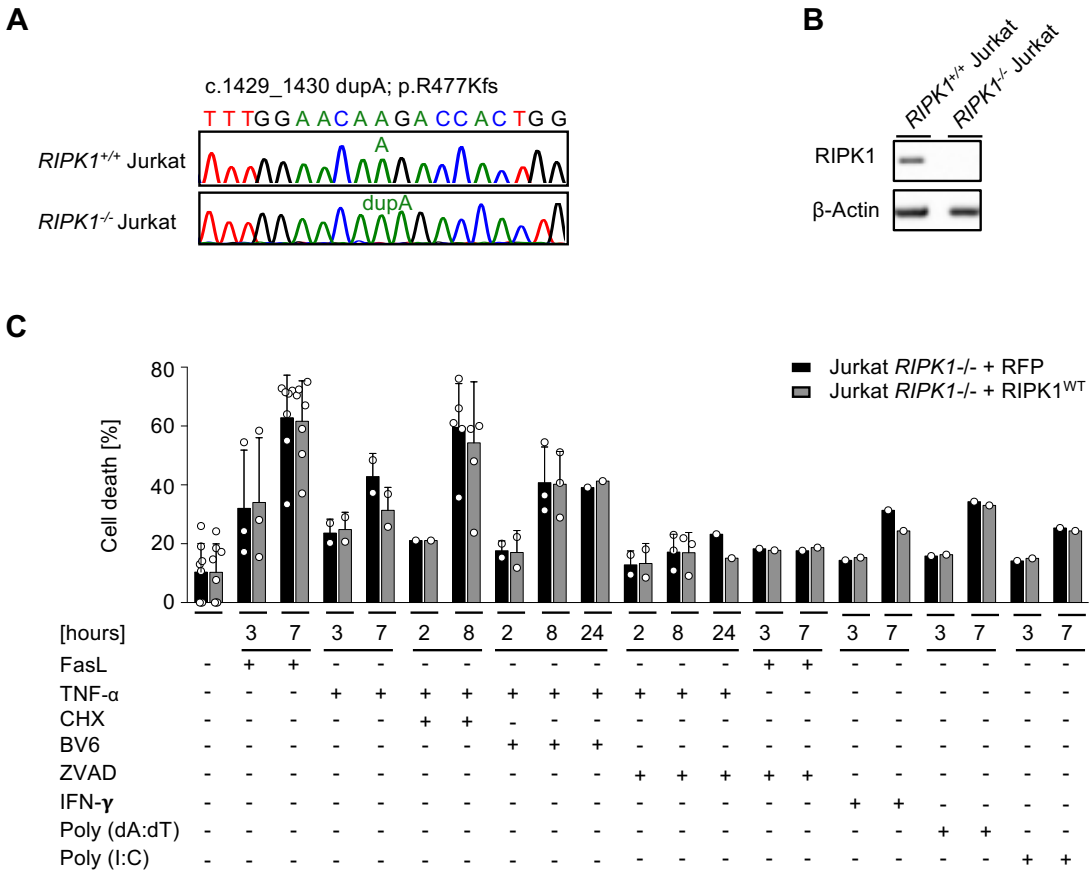

Fig. S2. Assessment of cell death responses in RIPK1-deficient Jurkat cells.

(**A**) DNA Sanger sequencing confirmed a biallelic insertion mutation (c.1429_1430 dupA; p.R477Kfs and (**B**) immunoblotting showed abrogated protein expression of RIPK1 in previously reported RIPK1-deficient Jurkat cells(5). (**C**) Flow cytometry revealed no difference in cell death (Annexin V^+ cells) between RIPK1-deficient Jurkat cells overexpressing either RFP or wt RIPK1 after stimulation with the following agents: Fas Ligand (50 ng/ml), TNF-α (10 ng/ml) ± CHX (250 ng/ml) ± BV6 (100 μM) ± Z-VAD-FMK (20 μM), Fas Ligand (50 ng/ml) ± CHX (250 ng/ml), Interferon γ (100 ng/ml), poly (dA:dT) (10 μg/ml), poly (I:C) (100 μg/ml); Dots indicate the number of experiments.

Table S1. Demographic, genetic, and clinical data of RIPK1-deficient patients

Patients	P1	P2	P3	P4	P5	P6	P7	P8
Demographics and Genetics								
Mutation	c.1844T>C,p.I615T	c.1934C>T,p.T645	c.1278C>A,p.Y426*	c.954delG,p.M318fs	c.1934C>T,p.T645M	c.1802G>A,p.C601Y	c.1802G>A,p.C601Y	c.1802G>A,p.C601Y
Consanguinity	not reported	yes	yes	yes	yes	yes	yes	yes
Ethnicity/ Country	Caucasian / Poland	Arab / Kuwait	Caucasian / Germany	Arab / Saudi Arabia	Arab / Israel	North African / Algeria	North African / Algeria	North African / Algeria
Sex	male	female	female	female	female	male	male	female
Age of onset/ diagnosis	6mo/ 3y6mo	1mo/ 3y1mo	1d/ post mortem	1d/ post mortem	1d/ 6y10mo	6mo/ 10y6mo	20d/ 5y5mo	3mo / 3y
Clinical presentation								
Gastrointestinal manifestations								
Colitis	pancolitis	not reported	colitis	colitis	left-sided colitis	left-sided colitis	left-sided colitis	left-sided colitis
Growth failure	+	+	+	+	+	+	+	+
Abdominal pain	+	-	+	+	+	+	+	+
Diarrhea	mucous, bloody	mucous, bloody	bloody	non-bloody	mucous, bloody	non-bloody	non-bloody	non-bloody
Oral lesions	aphthous lesions	-	-	-	ulcers	gingivostomatitis	aphthous lesions	aphthous lesions
Perianal disease	+	+	-	+	+	+	+	+
Others	esophagitis, gastritis with erosions/ulcers, duodenitis with polyps		GI infections (rotavirus/adenovirus)	esophagitis, gastritis		-	-	-
Extraintestinal Manifestations								
Recurrent infections	conjunctivitis, pneumonia, deep-seated infections, episodes of sepsis	otitis media, recurrent URI, LRI, and sinopulmonary infections	septicemia, upper and lower respiratory tract infections	bacteremia, sepsis, skin abscesses, UTI	CMV-associated esophagitis, otitis media, pneumonia, UTI*4	otitis media, omphalitis, pneumonia	otitis media, pneumonia	otitis media
Skin	maculopapular and atopic skin lesions	-	maculopapular exanthema	-	-	-	-	-
Others	hepatosplenomegaly, SJS	not reported	hepatosplenomegaly, liver fibrosis/hepatitis	hypothyroidism, hypocalcemia, hypoalbuminemia	arthralgia, febrile seizures	-	tetany	tetany
Treatment								
Nutrition	AA-based formula (n), TPN (p)	AA-based formula (p)	parenteral (p)	parenteral (p)	TPN (p)	-	-	-
Medication	Ab (p), AZA (p), CS (p), IFX (p)	Ab (p)	CS (g)		5-ASA(n), Ab (p), AZA (p), CS (p), IFX (p),	Ab (p), AF (p)	Ab (p), AF (p)	Ab (p), AF (p), CS (p)
Surgery	ileostomy	rectovaginal fistula		rectoanal fistula	ileostomy, bowel resection	perianal fistula	-	-
Others			HSCT (HLA-identical mother, 1. 12mo, 2. 3y2mo)					
Outcome								
	died (4y, sepsis)		died (19y, cGvHD and pulmonary disease)	died (2y, sepsis)	alive, stable condition	alive	alive	alive

5-ASA, mesalazine; AA, amino acid; Ab, antibiotics; Af, antifungal; AZA, azathioprine; CS, corticosteroids; g, good response; IFX, infliximab; n.r., not reported; LRI: lower respiratory tract infections; n, no response; p, partial response; SJS, Steven's-Johnson syndrome; TPN: total parenteral nutrition; URI: upper respiratory tract infections, UTI: urinary tract infectio

Table S2. Laboratory findings and immune workup of RIPK1-deficient patients.

Patients	P1	P2	P3	P4	P5	P6	P7	P8
Complete blood counts (10^3 cells/mcl)								
Hemoglobin [g/dL]	10.5 (10.5-13)	12.1 (10.8-12.6)	10.0 (9.0-14.0)	11.0	10.0 (12.0-16.0)	9.9 (12-14,5)	10,8 (12-14,5)	9,2 (12-14)
Platelets	166 (150-350)	426 (234-474)	173 (355-666)	61	831(140-400)	176 (150-400)	215 (150-450)	407 (150-450)
WBCs	5.7 (4-20)	13.16 (7.05-12.08)	4.91 (4.7-14.8)	3.5	20.0 (4-10)	4.5 (4.4-9.5)	5.7 (5.2-11.0)	6.11 (5.2-11.0)
Lymphocytes	0.456 (1.2-13)	10.16 (2.03-5.68)	1.180 (3.2-9.8)	1.96 (3.9-9.0)	6.5 (1.5-4)	1.373 (1.9-3.7)	1.425 (2.3-5.4)	1.41 (2.3-5.4)
Neutrophils	5.016 (1-10)	1.62 (2.34-6.44)	3.2 (1.0-9.0)	0.6	11.4 (2-7.5)	2.97 (2.6-6.3)	3.962 (2.3-6.4)	4.17 (2.3-6.4)
Lymphocyte subsets (10^3 cells/mcl)								
CD3	1.825 (2.8-5.7)	3.67 (1.9-6.2)	0.93 (2.3-6.5)	0.666 (3.1-4.8)	n.d.	1.009 (1.2-2.6)	1.076 (1.4-3.7)	0.909 (1.4-3.7)
CD4	1.308 (1.8-4.4)	2.194 (1.4-4.3)	0.62 (1.5-5.0)	0.470 (2.2-3.3)	n.d.	0.357 (0.65-1.5)	0.591 (0.7-2.2)	0.558 (0.7-2.2)
CD8	0.549 (0.6-1.5)	1.394 (0.5-1.7)	0.25 (0.5-1.6)	0.157 (1.1-1.7)	n.d.	0.552 (0.37-1.1)	0.413 (0.49-1.3)	0.313 (0.49-1.3)
CD4:CD8	2.4 (1.9-4.2)	1.57 (1.3-3.9)	2.48	3.0 (1.5-2.6)	1.0	0.68 (1.5-2.9)	1.4 (1.5 -2.9)	1.78 (1.5-2.9)
CD19	1.271 (0.7-2.8)	3.1 (0.61-2.6)	0.14 (0.6-3.0)	0.710 (1.1-1.9)	n.d.	0.144 (0.27-0.86)	0.228 (0.39-1.4)	0.138 (0.39-1.4)
CD16/CD56	0.09 (0.21-0.8)	0.289 (0.16-1.1)	0.17 (0.1-1.3)	0.57 (0.3-0.7)	n.d.	0.22 (0.1-0.48)	0.121 (0.13-0.72)	0.207 (0.13-0.72)
Immunoglobulins								
IgG [g/L]	4.93 (1.97-6.71)	8.08 (3.0-15.0)	6.46 (2.41-6.13)	6.5 (2.35-4.37)	5.08 (7-16)	8.53 (6.9-11.5)	9,55 (7-11,6)	8.28 (7-11.6)
IgM [g/L]	0.24 (0.21-0.89)	1.42 (0.25-1.15)	3.06 (0.26-0.6)	0.45 (0.34-0.95)	1.16 (0.4-2.3)	0.26 (0.39-0.79)	0,89 (0,4-0,9)	2.39 (0.4-0.9)
IgA [g/L]	0.07 (0.065-0.52)	0.51 (0.16-1.0)	0.43 (0.1-0.46)	1.5 (0.2-0.62)	<0.42 (0.7-4.0)	0.46 (0.68-1.94)	0,91 (0,7-1,6)	0.92 (0.7-1.6)
IgE [IU/mL]	14.57 (0-20)	261 (0-30)	30 (0-170)	22.6 (1-100)	n.d.	n.d.	n.d.	n.d.
Immune work								
T cell proliferation	normal	n.d.	normal	normal	normal	n.d.	n.d.	n.d.
Antibody titers to vaccination	normal	n.d.	normal	TT, DT, HI ↓	normal	n.d.	n.d.	n.d.
NK cell function	not done	n.d.	reduced	n.d.	n.d.	n.d.	n.d.	n.d.
Complement	normal	n.d.	normal	0.170 CEA	elevated c3, c4	n.d.	n.d.	n.d.

n.d., not done.

Table S3. Immunophenotypic characterization of lymphocyte subsets in RIPK1-deficient patients.

Population (%)	HD1	HD2	HD3	A.I-1	A.I-2	A.II-1 (P1)	HD4	HD5	F.I-1	F.I-2	F.II-2 (P6)	F.II-3 (P7)	F.II-4 (P8)	HD6	HD7	E.I-1	E.I-2	E.II-1	E.II-3	E.II-2 (P5)
$CD3^+CD4^+$ $CCR7^+CD45RO^-$	49	30	29	23	45	90	50	39	24	31	88	77	88	16	34	29	27	64	43	41
$CD3^+CD4^+$ $CCR7^+CD45R^+$	31	39	34	34	36	7	26	39	19	31	3	5	4	52	34	49	55	26	44	43
$CD3^+CD4^+$ $CCR7^-CD45RO^+$	15	22	35	35	14	1	19	18	29	32	3	11	3	29	30	20	16	8	12	12
$CD3^+CD4^+$ $CCR7^-CD45RO^-$	1	5	1	3	1	1	3	1	26	4	5	5	4	0	0	0	0	1	0	2
$CD3^+CD8^+$ $CCR7^+CD45RO^-$	40	13	35	5	30	90	65	55	22	32	80	53	83	13	21	31	37	66	64	36
$CD3^+CD8^+$ $CCR7^+CD45R^+$	9	7	12	3	8	3	2	5	3	11	1	1	1	14	7	13	17	4	10	3
$CD3^+CD8^+$ $CCR7^-CD45RO^+$	33	36	41	16	52	4	20	24	51	39	6	17	4	58	35	31	38	15	14	33
$CD3^+CD8^+$ $CCR7^-CD45RO^-$	15	40	8	71	8	3	11	14	22	16	13	27	11	13	34	23	7	14	10	26
$CD4^+CD25^+CD127^{lo}$ $HLA\text{-}DR^-CD45RO^-$	42	12	21	11	35	72	37	22	15	14	62	69	80	14	22	11	14	50	56	32
$CD4^+CD25^+CD127^{lo}$ $HLA\text{-}D^-CD45RO^+$	40	50	55	48	59	21	54	68	79	82	30	28	17	70	70	73	76	42	37	59
$CD4^+CD25^+CD127^{lo}$ $HLA\text{-}DR^+CD45RO^-$	16	35	21	39	0	4	8	8	5	2	4	0	1	13	6	13	7	4	4	4
$CD3^+CD4^+$ $CXCR3^-CCR6^-$	56	39	34	31	53	93	62	47	51	52	94	89	92	15	14	22	19	27	11	47
$CD3^+CD4^+$ $CXCR3^+CCR6^-$	21	30	29	35	22	4	19	18	29	22	4	9	6	34	38	34	19	26	29	21
$CD3^+CD4^+$ $CXCR3^-CCR6^+$	7	14	10	8	10	1	9	10	9	12	1	1	1	20	13	30	22	15	17	14
$CD19^+CD27IgD^+$	80	66	73	72	82	94	51	59	40	24	32	74	69	86	61	53	72	68	81	72
$CD19^+CD27^+IgD^+$	7	12	11	12	7	3	11	9	7	1	1	1	1	6	20	25	15	14	6	11
$CD19^+CD27^+IgD^-$	9	18	12	12	9	1	30	27	32	17	4	7	6	7	13	15	9	10	6	10

Table S4. List of primer sequences.

Primers	Sequence (5' - 3')
sgRNA targets	
sg-Ripk1-T1	ATGACATTCAAGGACATGTCTGG
sg-Ripk1-T2	TCCTTGTCTTTCAAGCGACGAGG
Genotyping KO clones	
gt-RIPK1-KO-T1-F	TTATGAAGGGCGAAGGGAAGGAAC
gt-RIPK1-KO-T1-R	AGGCATTCCTTCCTCCTAGTAGAC
gt-RIPK1-KO-T2-F	TTTCTTTACAGGGTACAGCTCTGCC
gt-RIPK1-KO-T2-R	GTCTTCACCCATCCTCCTGTTTCC
Sanger Sequencing	
seq-RIPK1-DD-F	AGCAATTCAGGAAGCTGGAA
seq-RIPK1-DD-R	CTGAGGCAGCCAACTTTCTG
seq-RIPK1-ex8-F	AATGTTTCATGACACCCATTCTAATG
seq-RIPK1-ex8-R	CCACAATCTATAATAGAGCTGC
seq-RIPK1-P4-F	TGAAATCAGGAAGTGTGAGTCC
seq-RIPK1-P4-R	TGACTGGTGAGCACACTGTT
Cloning	
cl-RIPK1-P1-F	CAGATTGATGAAACTGACCATGACTATGAG
cl-RIPK1-P1-R	GTTTCATCAATCTGAGACTGTGTGAAGCCC
cl-RIPK1-P2/P5-F	AAGGGAGCCATGGTGGGGAAGCTGGCCCAGGCG
cl-RIPK1-P2/P5-R	TTCCCCACCATGGCTCCCTTTATGCCTTCC
cl-RIPK1-P3-R	GCTCGAGTTTATTTTTATTCCTAGGTTAAGGTCTT
cl-RIPK1-P4-F	TGCTGTGCAAAAGGG
cl-RIPK1-P4-R	AGTTGTGAAGAGAATCAGTCTCTTCAACTT
cl-RIPK1-P6-F	GCTCGAGTTTATTTTTATTCCTAGGCTAGATAGT TGGTC
cl-RIPK1-P6-R	GAAAAACTATGCCCGTAAACTGGGCTTCAC
RIPK1_cP_M318IfsTer	TTACGGGCATAGTTTTTCCAGTGCTTTCCC
RIPK1_cP_1F+KPNI	TAAACTATGCGGCCGCCTAGATAGTTGGTCTCAG GCACTGGG
RIPK1_cP_truncXR+NOTI	GGGGTACCGCCACCATGCAACCAGACATGTCCT TGAATG
RIPK1_cP_XR+NOTI	TAAACTATGCGGCCGCTTAAGGTCTTTGCTGTGC AAAAGGGTC
RIPK1_Mutag-F	TAAACTATGCGGCCGCTTAGTTCTGGCTGACGTA AATCAAGC
RIPK1_Mutag-R	AGTTGTGAAGAGAATCAGTCTCTTCAACTT
	AAGTTGAAGAGACTGATTCTCTTCACAACT

Table S5. List of antibodies used for flow cytometry.

Antibody (anti-)	Clone	Cat. No.	vendor
CCR4-PE-Cy7	L291H4	359410	BioLegend
CCR6-BV786	11A9	563704	BD Biosciences
CCR6-PE-Cy7	11A9	560620	BD Biosciences
CCR7-BV421	150503	562555	BD Biosciences
CD3-APC-H7	SK7	560176	BD Biosciences
CD3-BUV395	SK7	54001	BD Biosciences
CD3-BUV496	UCHT-1	564809	BD Biosciences
CD4-APC	SK3	565994	BD Biosciences
CD4-BB515	RPA-T4	564419	BD Biosciences
CD4-BUV395	RPA-T4	564724	BD Biosciences
CD8-BUV496	RPA-T8	564804	BD Biosciences
CD8-BUV737	SK1	564628	BD Biosciences
CD8-Pacific Blue	RPA-T8	558207	BD Biosciences
CD10-BV421	HI10a	562902	BD Biosciences
CD10-PE	HI10a	340921	BD Biosciences
CD11c-BV421	B-ly6	562561	BD Biosciences
CD11c-BV650	B-ly6	563604	BD Biosciences
CD14-APC-Fire780	M5E2	301853	BioLegend
CD14-BV786	M5E2	563698	BD Biosciences
CD16-APC	3G8	557758	BD Biosciences
CD19-BUV395	SJ25C1	563549	BD Biosciences
CD19-BV737	SJ25C1	564303	BD Biosciences
CD19-PE	HD37	RO80801-2	Dako
CD19-PerCP Cy5.5	hib19	302230	BioLegend
CD20-PE-Cy7	2H7	560735	BD Biosciences
CD21-BUV737	B-Ly4	564437	BD Biosciences
CD21-PE	B-Ly4	555422	BD Biosciences
CD25-PE	M-A251	555432	BD Biosciences
CD27-APC-R700	M-T271	565116	BD Biosciences
CD27-BV786	L128	563327	BD Biosciences
CD28-BB700	L293	745905	BD Biosciences
CD28PerCP-Cy5.5	CD28.2	302922	BioLegend
CD38-APC	HB7	340439	BD Biosciences
CD38-PE-Cy7	p67.6	333946	BD Biosciences
CD38-BV650	HB-7	356620	BioLegend
CD45-BV480	HI30	566115	BD Biosciences
CD45-V500	HI30	560777	BD Biosciences

CD45RA-APC	HB7	550855	BD Biosciences
CD45RA-BUV737	HI100	564442	BD Biosciences
CD45RA-PE	HI100	555489	BD Biosciences
CD45RO-BB515	UCHL1	564529	BD Biosciences
CD45RO-BV786	UCHL1	564290	BD Biosciences
CD56-PE-CF594	NCAM16.2	562289	BD Biosciences
CD57-BB515	NK1	560845	BD Biosciences
CD57-FITC	NK-1	555619	BD Biosciences
CD62L-BV650	DREG-56	563808	BD Biosciences
CD69-APC-Cy7	FN50	310914	BioLegend
CD123-BV421	9N5	562517	BD Biosciences
CD123-BV786	7G3	564196	BD Biosciences
CD127-APC	A019D5	351316	BioLegend
CD127-BUV737	HIL-7R-M21	564300	BD Biosciences
CXCR3-BV421	1C6	562558	BD Biosciences
CXCR3-PE-CF594	1C6/CXCR3	562451	BD Biosciences
HLA-DR BV711	G46-6	563696	BD Biosciences
HLA-DR PE	L243	307606	BioLegend
HLA-DR-PE-Cy7	G46-6	560651	BD Biosciences
IgD-BB515	IA6-2	560651	BD Biosciences
IgM-BV421	g20-127	562618	BD Biosciences

Table S6. List of antibodies used for western blot analysis or EMSA.

Antibody (anti-)	Cat. No.	Vendor
beta-Actin-HRP	SC-47778	Santa Cruz
Caspase-1	BML-SA101-0100	Enzo Life Sciences
IL-1 beta/IL-1F2	AF-201-NA	R&D Systems
MLKL (EPR17514)	AB184718	Abcam
NFκB-p50/p105	Sc-114X	Santa Cruz
NFκB-p65	8242	CST
NLRP3 (D2P5E)	13158	CST
phospho-NFκB-P65 (Ser536)	3033	CST
phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	4370	CST
RIPK1 (D94C12)	3493	CST
Vinculin	V9131	Sigma-Aldrich

Table S7. List of antibodies used for confocal microscopy analysis.

Antibody (anti-)	Cat. No.	Vendor
Goat anti-Rabbit IgG (H+L), Alexa Fluor 488	A27034	Thermo Fisher Scientific
Phalloidin, Alexa Fluor 594	A12381	Thermo Fisher Scientific
RIPK1 (D94C12)	3493	CST

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