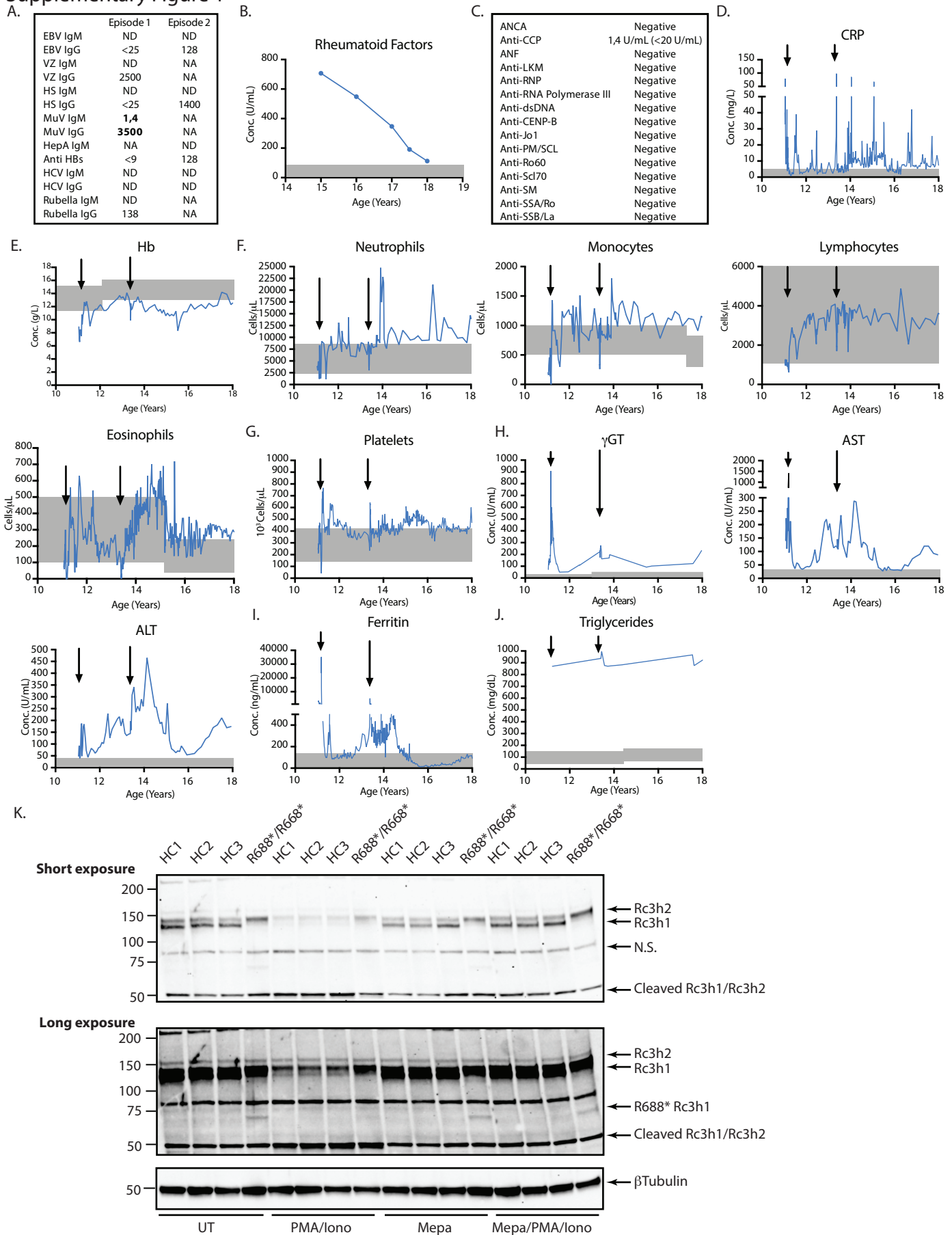


Supplementary Information for:

A human immune dysregulation syndrome
characterized by severe hyperinflammation
with a homozygous nonsense Roquin-1 mutation

Tavernier SJ et al.

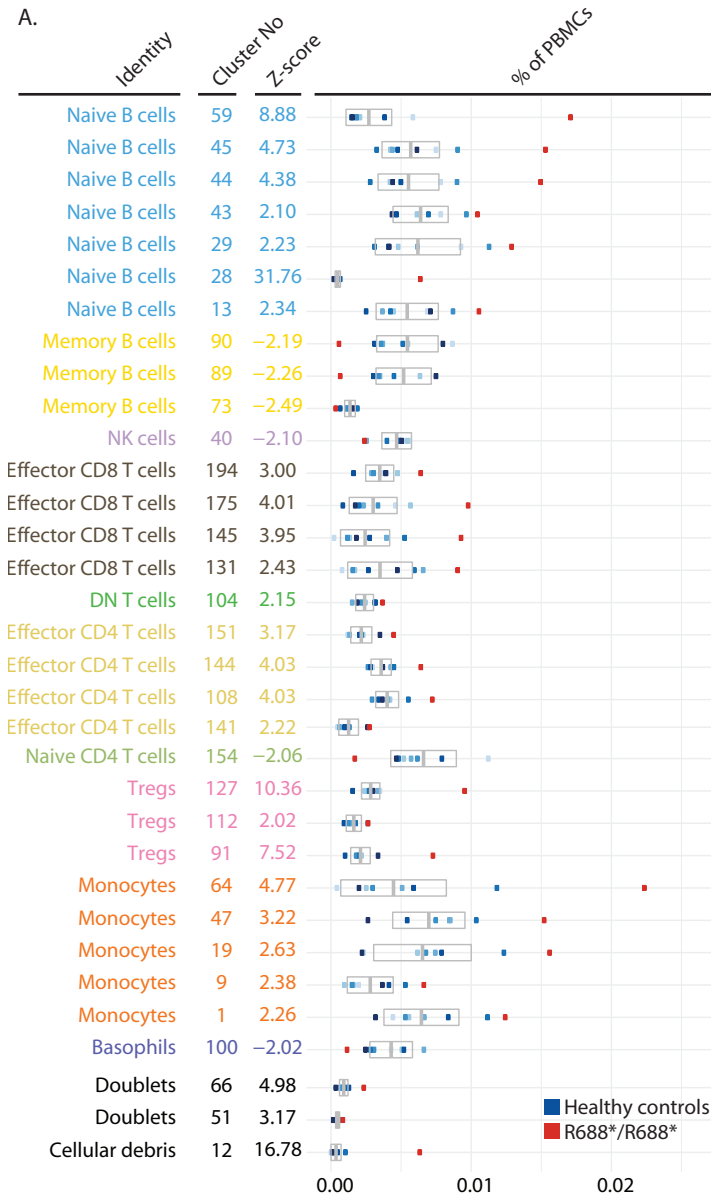
Supplementary Figure 1



Supplementary Fig. 1. (A) Overview of pathogen directed immunoglobulin concentrations measured in the setting of hyperinflammatory episodes. (B) Concentration of rheumatoid factors measured during clinical follow-up. (C) Overview of probed self-antigen directed immunoglobulins. (D-J) Evolution of a number of clinical laboratory parameters. Grey bars depict reference values. CRP: C reactive protein; Hb: hemoglobin; γ GT: gamma glutamyl-transpeptidases; AST: aspartate transaminases; ALT: alanine transaminases. (K) Immunoblot analysis of Roquin-1, its paralog Roquin-2, their cleavage products and the truncated R688* mutant in T cells of 3 healthy controls (HC) and the R688*/R688* proband treated with PMA, ionomycin and/or mepazine. β -Tubulin is used as a loading control. N.S.: nonspecific band. Supp. Fig. 1K is a representative blot for 2 independent experiments.

Supplementary Figure 2

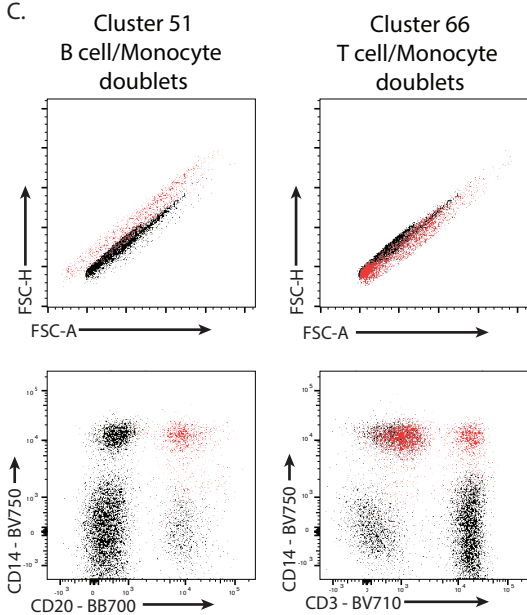
A.



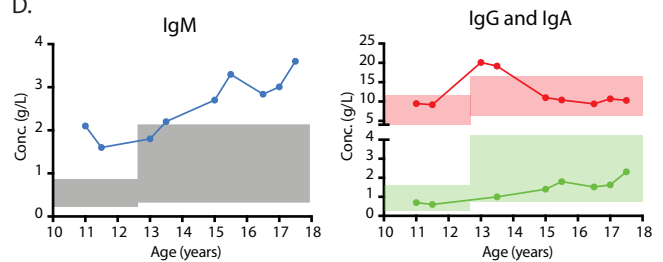
B.

Age at evaluation (yr)	14	17
Immune cell subsets		
T cells		
CD4 T cells (% of T cells)	66,7 (61,0 - 74,2)	69,6 (67,7 - 87,0)
Naïve (% of CD4 ⁺)	54,7 (34,1 - 71,2)	53,0 (54,0-71,0)
CM (% of CD4 ⁺)	32,9 (22,8-54,1)	27,2 (18,7-35,0)
EM (% of CD4 ⁺)	11,0 (5,9 - 19,5)	16,2 (8,7-12,2)
EMRA (% of CD4 ⁺)	1,4 (0,1 - 1,0)	3,6 (0,9-2,1)
CXCR5 ⁺ (% of CD4 ⁺)	9,3 (4,5 - 13,0)	3,2 (2,6-5,4)
CD25 ⁺ CD127 ⁻ (% of CD4 ⁺)	16,5 (4,2 - 7,1)	19,9 (6,5-12,2)
CCR4 ⁻ CXCR3 ⁺ (% of CD4 ⁺)	NA	3,8 (3,5-10,2)
CCR4 ⁺ CCR6 ⁺ (% of CD4 ⁺)	NA	6,4 (0,6-3,5)
IFN γ ⁺ (% of CD45RO ⁺ CD4 ⁺)	25,9 (19,0 - 47,1)	NA
IL-17A ⁺ (% of CD45RO ⁺ CD4 ⁺)	3,3 (0,2 - 2,0)	NA
CD8 T cells (% of T cells)		
Naïve (% of CD8 ⁺)	27,8 (27,0 - 35,5)	30,4 (22,0 - 32,3)
CM (% of CD8 ⁺)	42,6 (29,4 - 73,2)	34,7 (40,0 - 58,1)
EM (% of CD8 ⁺)	10,0 (6,5 - 15,8)	9,8 (6,0 - 8,5)
EMRA (% of CD8 ⁺)	30,4 (10,4 - 45,5)	37,6 (28,8 - 39,9)
CD4 ⁺ CD8 ⁺ (% of CD8 ⁺)	3,7 (0,7-1,4)	11,6 (2,1-5,0)
PD1 ⁺ (% of CD8 ⁺)	NA	11,6 (2,5 - 7,4)
IFN γ ⁺ (% of CD45RO ⁺ CD4 ⁺)	95,0 (41,7 - 90,8)	NA
B cells (% of PBMCs)		
Naïve (% of Bs)	18,3 (4,5-15,4)	13,3 (3,9-7,4)
Transitional (% of Naïve Bs)	91,0 (38,3 - 79,0)	94,4 (51,0 - 71,1)
IgM ⁺ memory (% of Bs)	4,3 (1,0 - 4,4)	2,5 (4,1-20,9)
IgM ⁺ memory (% of Bs)	2,4 (7,3 - 15,9)	1,9 (12,2-19,3)
IgM ⁺ memory (% of Bs)	1,4 (13,2 - 40,6)	1,0 (10,3-20,3)
CD21 ^{low} (% of Bs)	7,34 (0,7-7,21)	NA
NK cells (% of PBMCs)		
CD56 ^{hi} (% of NKs)	9,6 (3,2 - 10,8)	8,4 (4,8 - 12,7)
CD16 ⁺ CD56 ^{hi} (% of NKs)	2,5 (3,3 - 21,6)	1,2 (2,1 - 8,4)
CD16 ⁺ CD56 ^{dim} (% of NKs)	1,8 (0,6 - 3,6)	0,3 (0,5 - 2,0)
CD16 ⁺ CD56 ^{dim} (% of NKs)	94,1 (79,6 - 90,6)	93,3 (85,0 - 93,2)
Monocytes (% of PBMCs)		
CD14 ⁺ (% of Monos)	15,0 (3,8 - 27,3)	29,8 (24,4-27,4)
CD16 ⁺ (% of Monos)	86,3 (91,3 - 98,3)	78,5 (71,5-83,0)
CD16 ⁺ (% of Monos)	13,5 (1,9 - 8,7)	12,1 (4,6-15,5)
DCs (% of PBMCs)		
cDCs (% of DCs)	1,7 (1,3 - 3,4)	0,9 (1,3-2,1)
pDCs (% of DCs)	90,5 (56,3 - 85,8)	56,6 (41,1 - 53,2)
pDCs (% of DCs)	8,0 (13,2 - 41,6)	28,6 (30,9 - 48,6)
Basophils (% of PBMCs)		
	0,5 (0,2 - 1,4)	0,4 (0,5-1,6)
NKTs (% of PBMCs)		
iNKTs (% of NKTs)	0,8 (0,8 - 2,4)	0,8 (2,4 - 5,0)
	2,8 (0,6 - 3,2)	4,1 (5,6-6,7)

C.



D.

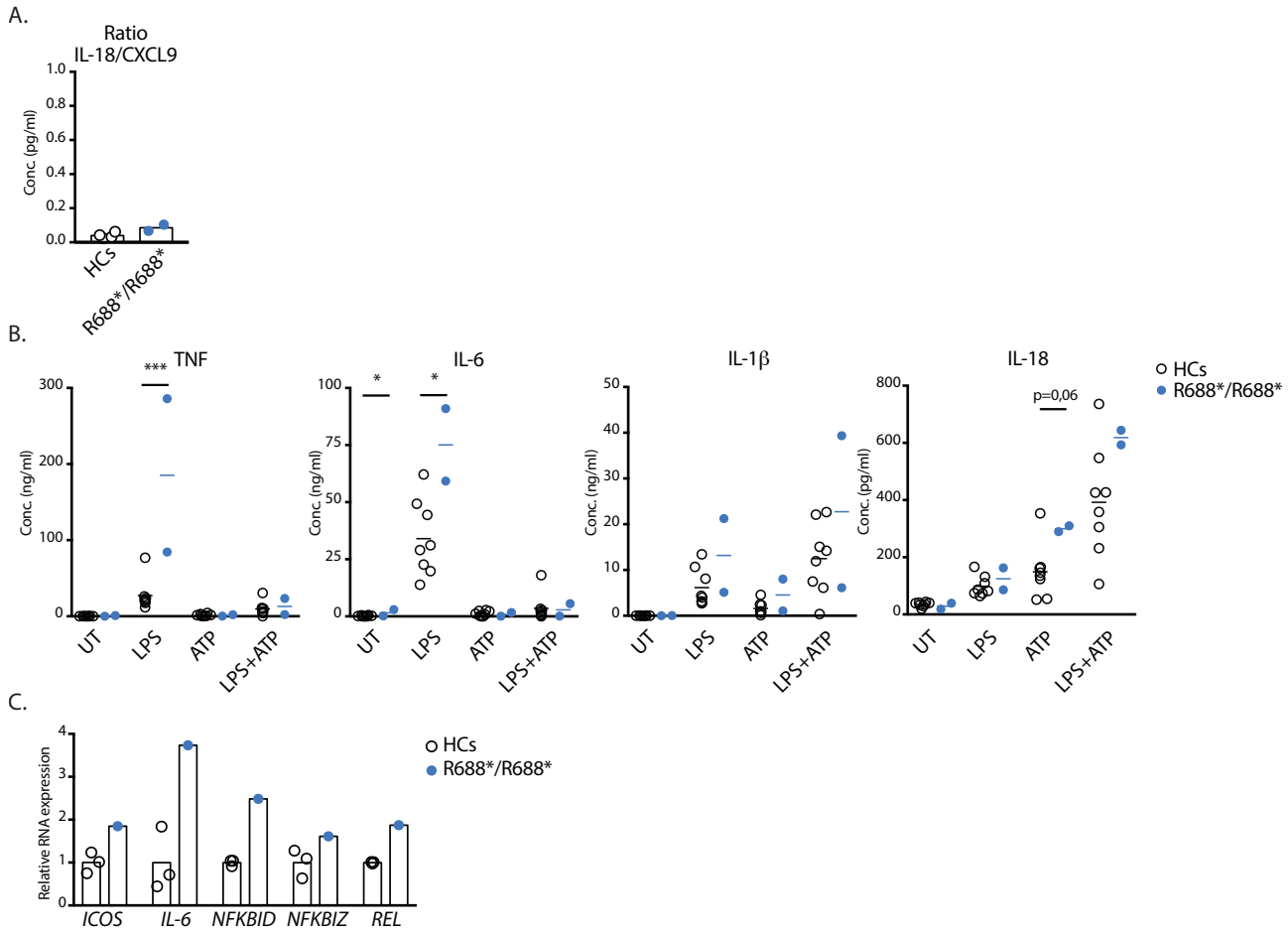


E.

Age (yr)	11	15
IgG	9,5 (4,70-11,9)	10,4 (7,0-16,0)
IgG2	0,56 (0,85-4,10)	0,91 (1,06-6,1)
IgG3	0,301 (0,15-1,49)	0,445 (0,18-1,163)
IgE	<4,4 (0-200)	<4,4 (0-200)

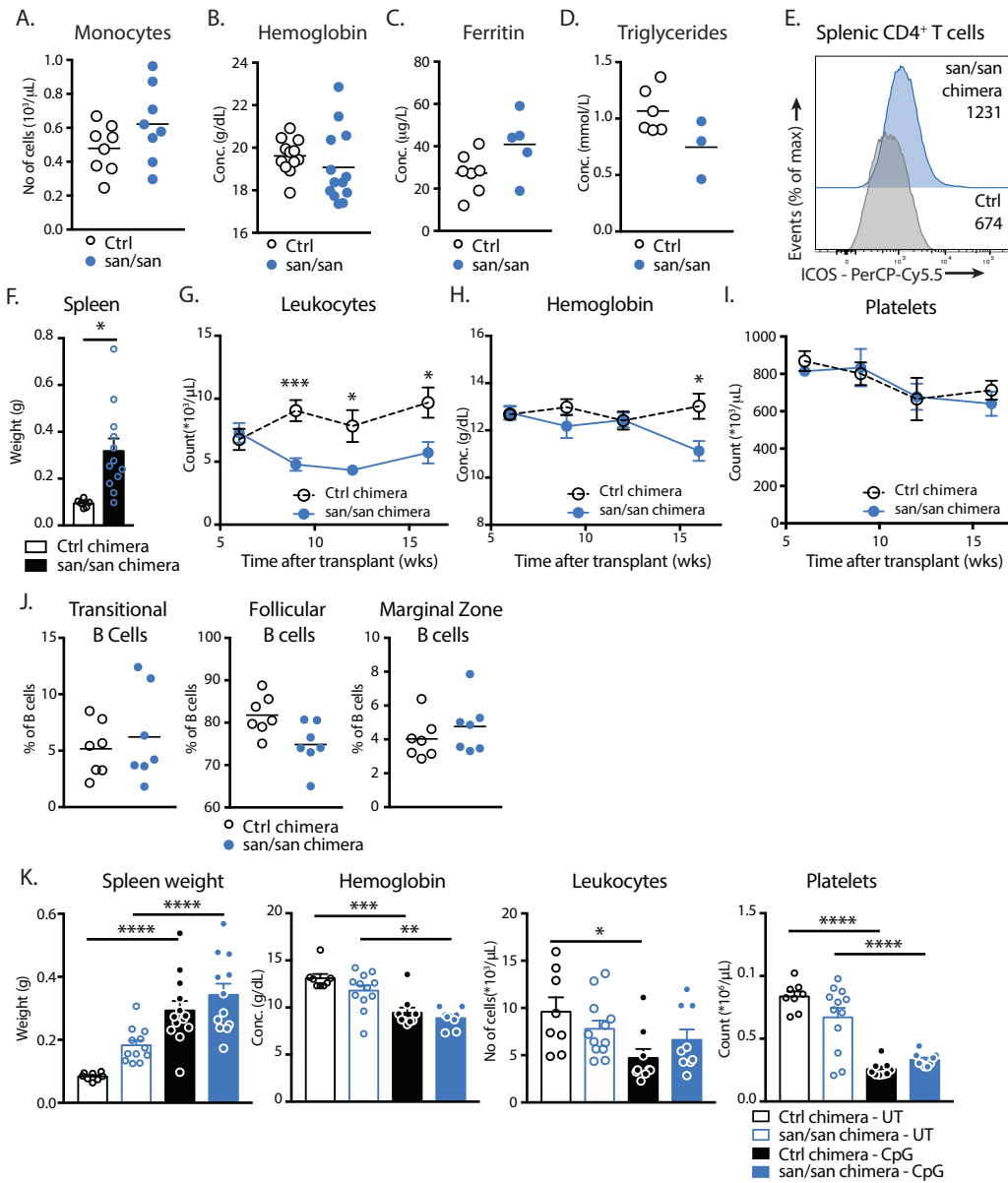
Supplementary Fig. 2. (A) Boxplots and individual datapoints of immune cell clusters with a Z-score higher or lower than 2 or -2 of the R688*/R688* proband and healthy controls (HCs). Color code matches annotated immune cell population. **(B)** Immunological characterization of R688*/R688* proband at the age of 14yrs and 17yrs old. Values between brackets represent the minima and maxima values of a cohort of healthy controls (HCs; n>4). **(C)** 2D scatter plots of cluster 51 and 66 identifying cell doublets on FSC-A by FSC-H and CD14 by CD20 or CD3 by CD20, respectively. Data from specific cluster (red) and viable cells (black) are shown. **(D)** Evolution of serum immunoglobulin (Ig) M (blue), G (red) and A (green) concentration in R688*/R688* proband. Reference values are shown as greyed bars. **(E)** Concentration of IgG subtypes IgG2 and IgG3 and IgE in the proband at the age of 11 and 15 years. Reference values are given between brackets.

Supplementary Figure 3



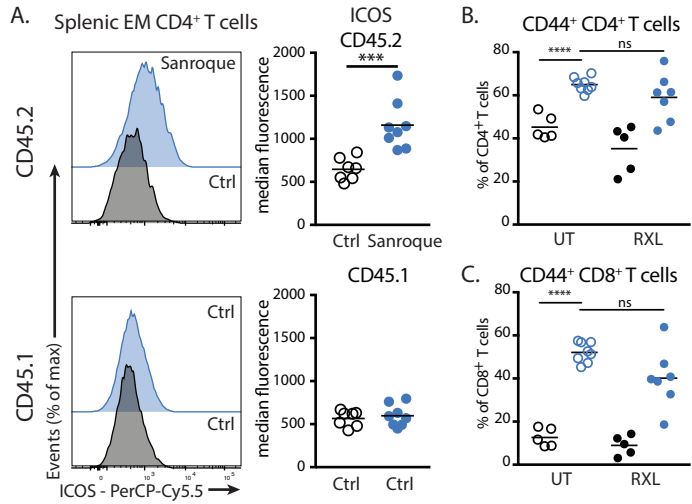
Supplementary Fig. 3. (A) Scatter plot displaying mean ratio of serum concentrations of IL-18 over CXCL9 in R688*/R688* proband (n=2) and healthy controls (HCs; n=4). (B) IL-1 β , IL-18, IL-6 and TNF concentration in the supernatant of monocytes of the R688*/R688* proband (n=2) or healthy controls (HCs; n=8) treated with ATP (5mM) or LPS (100ng/ml). *= $p < 0.05$; ***= $0.001 < p < 0.0001$ (unpaired t-test). Mean concentration is shown. (C) Relative expression of mRNA transcripts of *ICOS*, *IL-6*, *NFKBID*, *NFKBIZ* and *REL* in T cells derived from the R688*/R688* proband or healthy controls (n=3). Data was normalized using the housekeeping genes *HPRT* and *GAPDH*. Mean relative expression is shown. Data is accumulated from 2 different timepoints (Suppl. Fig. 3A), 2 independent experiments (Suppl. Fig. 3B), or is representative of 2 independent experiments (Suppl. Fig. 3C).

Supplementary Figure 4



Supplementary Fig. 4. (A) Mean concentration of blood monocytes in sanroque mice (n=8) and littermate controls (n=8). (B) Mean concentration of hemoglobin in littermate controls (n=12) and sanroque mice (n=13). (C) Mean ferritin concentration in sanroque mice (n=5) and littermate controls (n=7). (D) Mean triglyceride concentration in controls (n=6) and sanroque mice (n=3). (E) Representative histogram of ICOS expression in CD4⁺ T cells chimeras reconstituted with sanroque or control bone marrow cells. (F) Spleen weight in chimeric mice reconstituted with sanroque (n=12) or control bone marrow cells (n=6). * $p < 0.05$ (unpaired t-test). Bar graph show mean and SEM. (G-I) Evolution of leukocytes, hemoglobin and platelets in chimeric mice reconstituted with sanroque (n=16) or control (n=10) bone marrow. * $p < 0.05$; *** $= 0.001 < p < 0.0001$ (unpaired t-test). Mean and SEM are given. (J) B cell maturation in chimeric mice reconstituted with sanroque (n=7) or control bone marrow (n=7). Scatter dot plot with mean. (K) Quantification of spleen weight, hemoglobin, leukocytes and thrombocytes of sanroque ($n^{\text{vehicle}}=12$, $n^{\text{CpG}}=9$) and control ($n^{\text{vehicle}}=8$, $n^{\text{CpG}}=9$) chimeric mice treated with vehicle or CpG. * $p < 0.05$; ** $= p < 0.01$; **** $= p < 0.0001$ (one-way ANOVA with Tukey's correction). Bar graph depict mean and SEM. Data is representative of 1 (Suppl. Fig. 4C, D) or 2 independent experiments (Suppl. Fig. 4E, J) or represents accumulated data of 2 (Suppl. Fig. 4A, B, F, K) or 3 (Suppl. Fig. 4G-I) independent experiments.

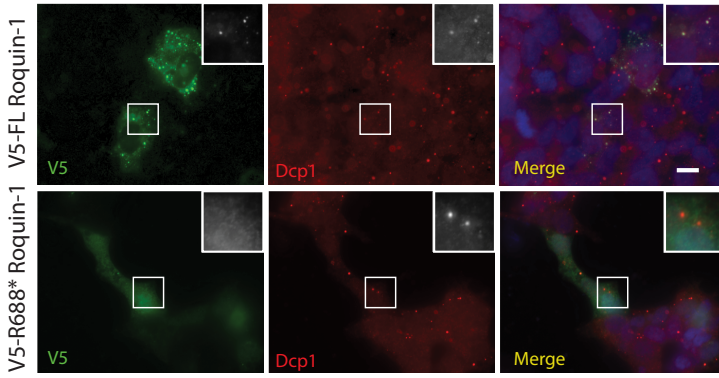
Supplementary Figure 5



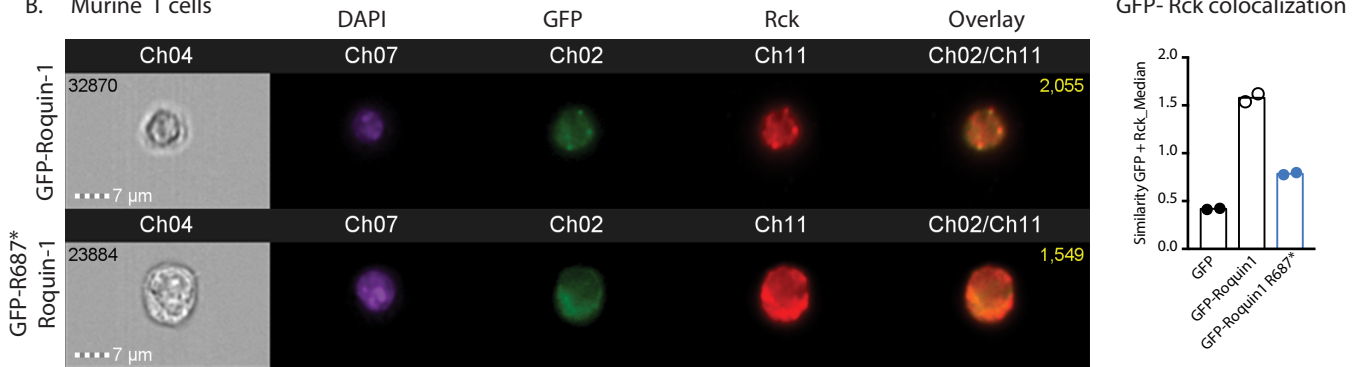
Supplementary Fig. 5. (A) Representative histogram and scatter dot plots of mean ICOS expression in CD4⁺ T cells of mixed bone marrow chimeras reconstituted with sanroque (n=7) or control CD45.2 (n=8) and control CD45.1 mice. ***=0.001<p<0.0001 (unpaired t-test). **(B)** Quantification of CD44⁺ CD4⁺ and CD8⁺ T cells in sanroque (n^{vehicle}=8, n^{RXL}=7) and control (n^{vehicle}=5, n^{RXL}=5) chimeric mice treated with vehicle or RXL. *=p<0.05; **=p<0.01; ****=p<0.0001 (one-way ANOVA with Tukey's correction). Scatter dot plot with mean is plotted.

Supplementary Figure 6

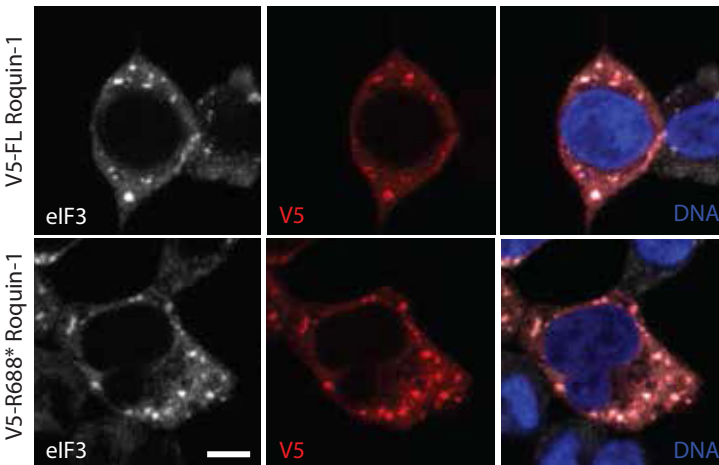
A. HEK293T



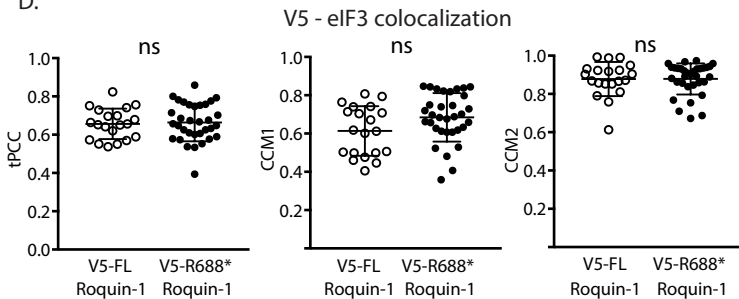
B. Murine T cells



C. HEK293T + Sodium Arsenite

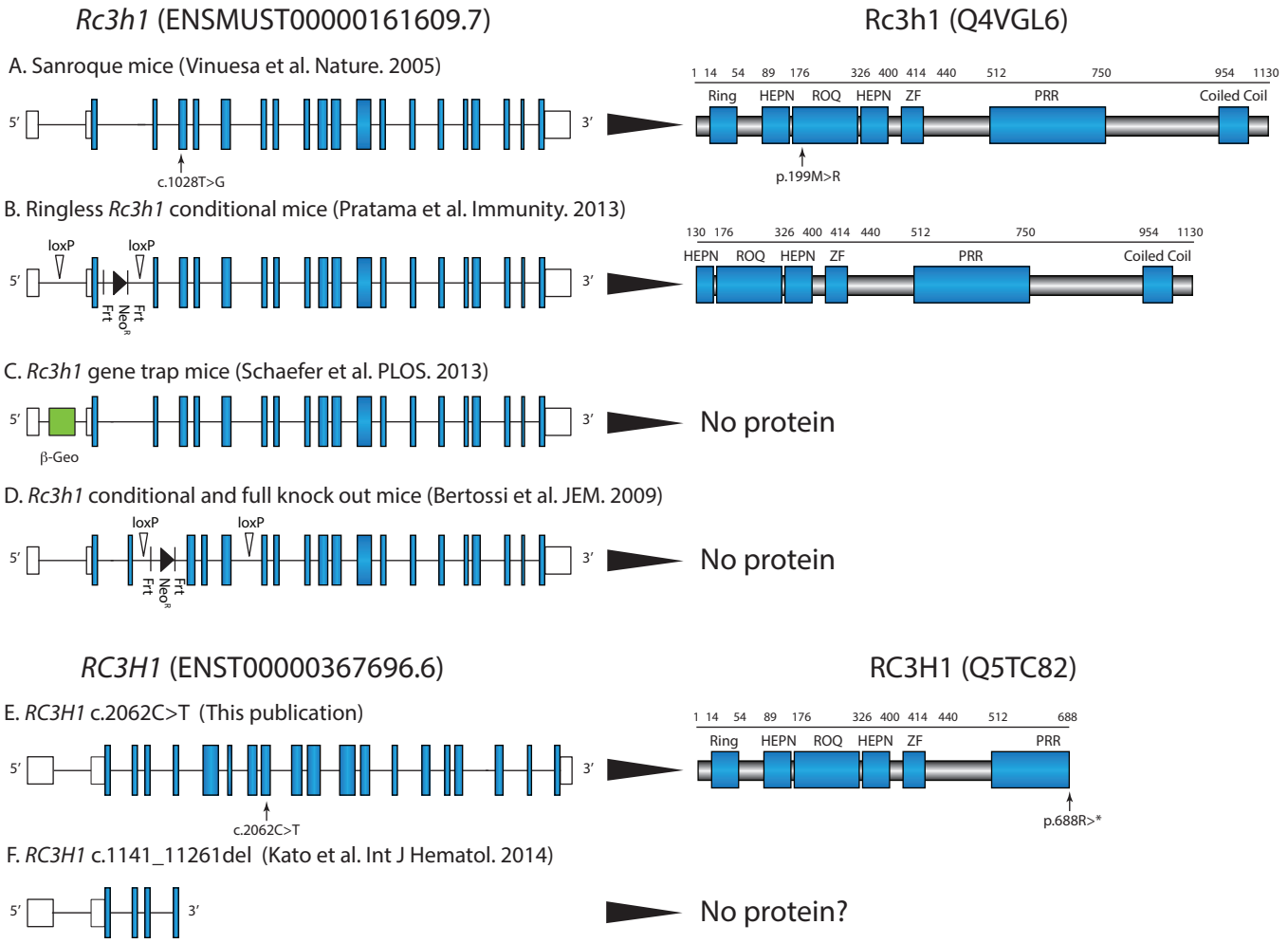


D.



Supplementary Fig. 6. (A) HEK293T cells were transiently transfected with V5 tagged Roquin-1 (V5-FLRoquin-1) or the V5-R688* Roquin-1 variant and subsequently stained with anti-V5 and anti-Dcp1 (P-body marker). Nuclei were revealed using Hoechst. Scale bar = 10 μ M. **(B)** 4-OHT treated murine Rc3h1-2^{fl/fl}; CD4-CreERT2; rtTA CD4⁺ T cells were retrovirally transduced doxycycline inducible constructs encoding GFP fused WT or R687* Roquin-1. After 16h stimulation with doxycycline, cells were stained with anti-Rck (P-body marker) and DAPI. Correlation of GFP and Rck fluorescent signal was calculated using the Similarity Bright Detail Feature. **(C)** HEK293T cells were transiently transfected with V5 tagged Roquin-1 (V5-FLRoquin-1) or the V5-R688* Roquin-1 variant, treated with arsenite and subsequently stained with anti-V5 and anti-eIF3 (Stress granule marker). Nuclei were revealed using Hoechst. Scale bar = 10 μ M. **(D)** Correlation analysis of endogenous eIF3 and Roquin-1 comparing HEK293T cells transfected with V5-FLRoquin-1 and V5-R688* Roquin-1. tPCC: thresholded Pearson correlation coefficient; CCM1/2: Manders coefficient1/2. Mean and standard deviation are plotted. Data shown is representative of 1 (Suppl. Fig. 6A), 2 (Suppl. Fig. 6B) or 3 (Suppl. Fig. 6C, D) independent experiments.

Supplementary Figure 8

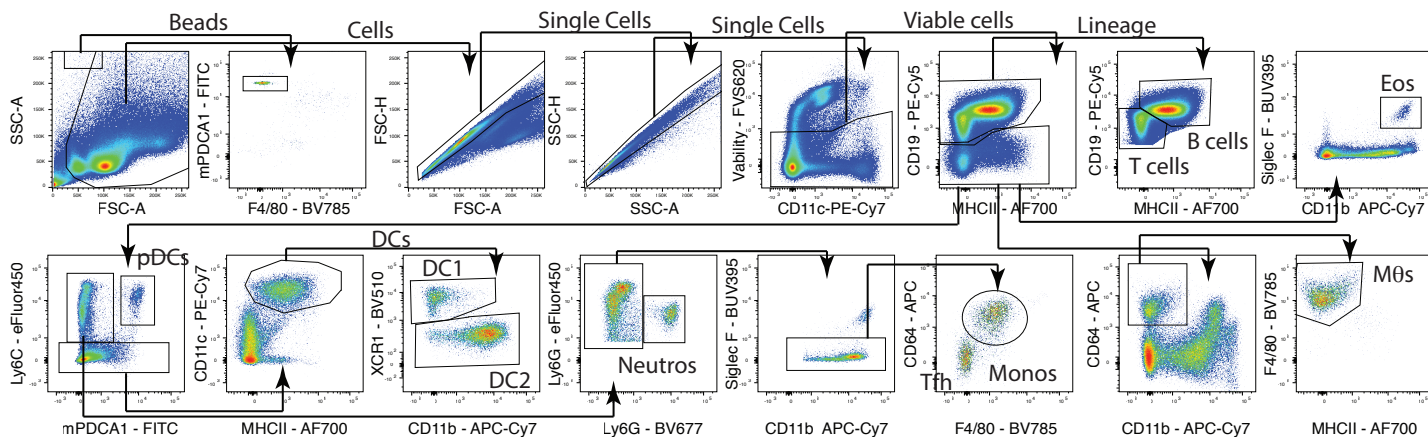


Supplementary Fig. 8. (A-D) Graphical representation of the consequences on transcript and protein level of described murine *Rc3h1* mutants and **(E,F)** reported human *RC3H1* variants.

Supplementary Figure 9

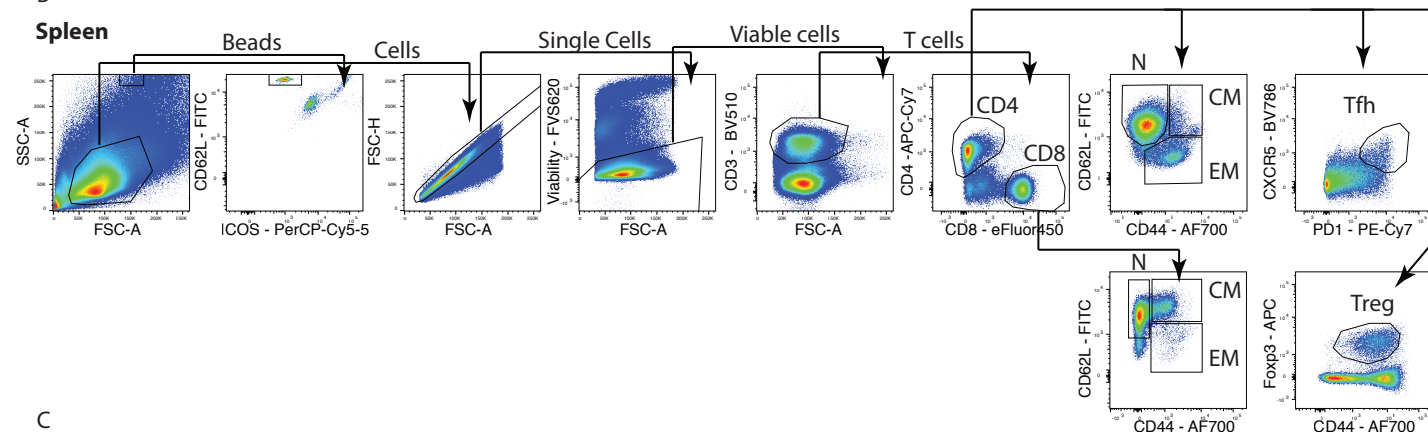
A

Spleen



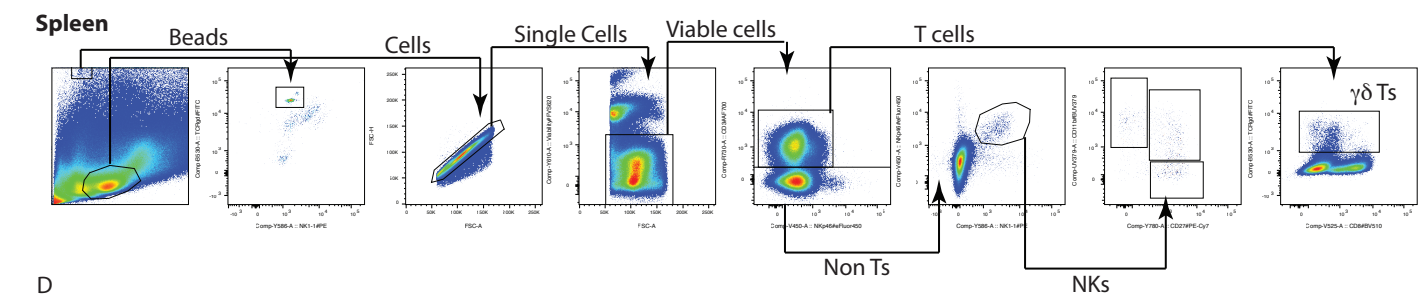
B

Spleen



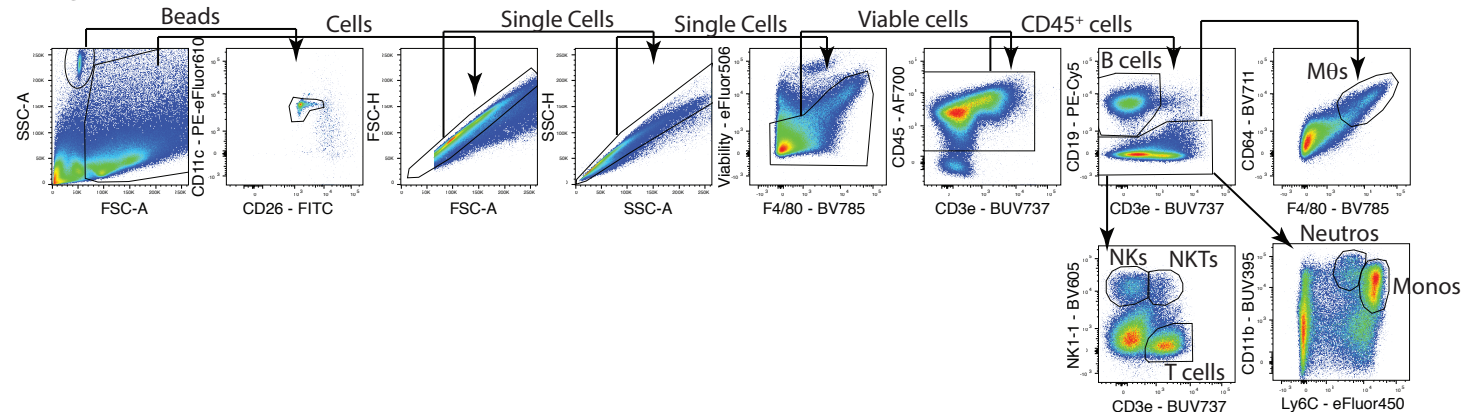
C

Spleen



D

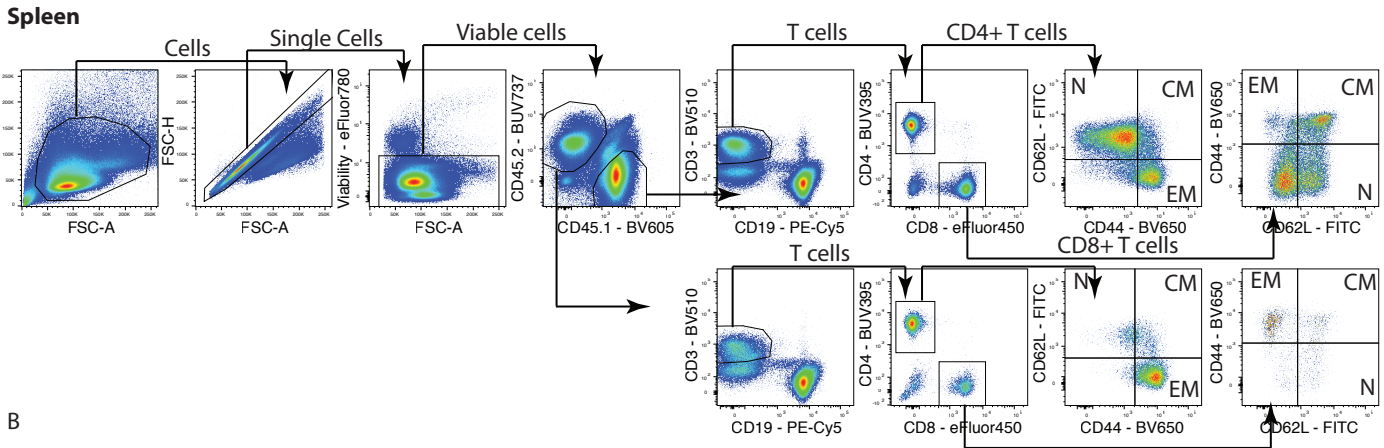
Liver



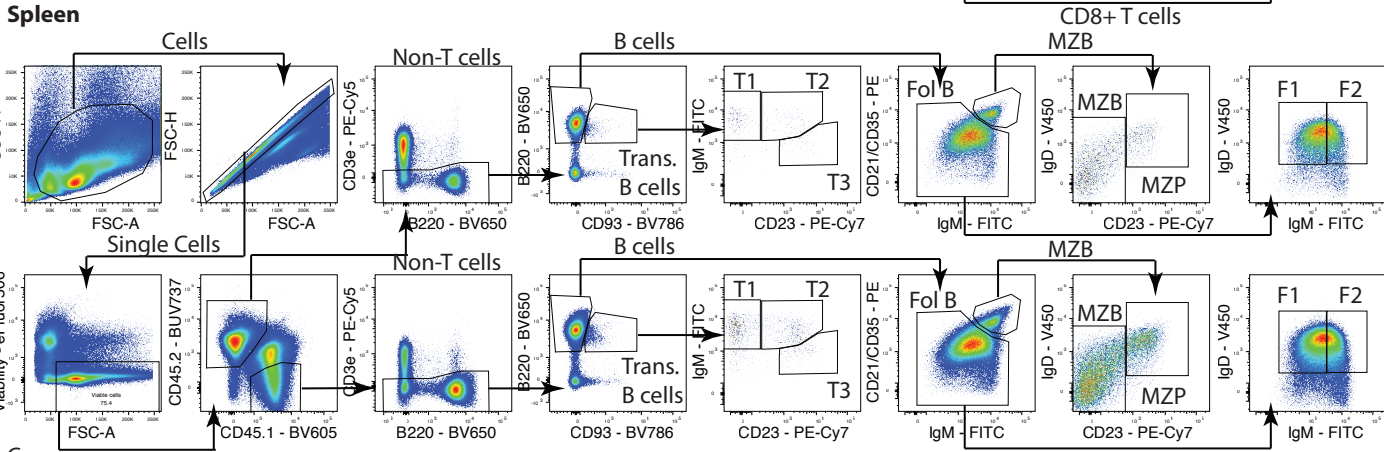
Supplementary Fig. 9. (A-D) Gating strategies of analyzed immune cell populations presented in Figure 4 and Supplementary Figure 4. N: Naive, CM: Central Memory, EM: Effector Memory, Fol: Follicular, MZB: Marginal Zone B cells, MZP: Marginal Zone Plasmablasts, T: Transitional, F: Follicular, EOs: Eosinophils, M0s: Macrophages, Tfh: Follicular T helper cell, Treg: Regulatory T cell, DC: Dendritic Cell.

Supplementary Figure 10

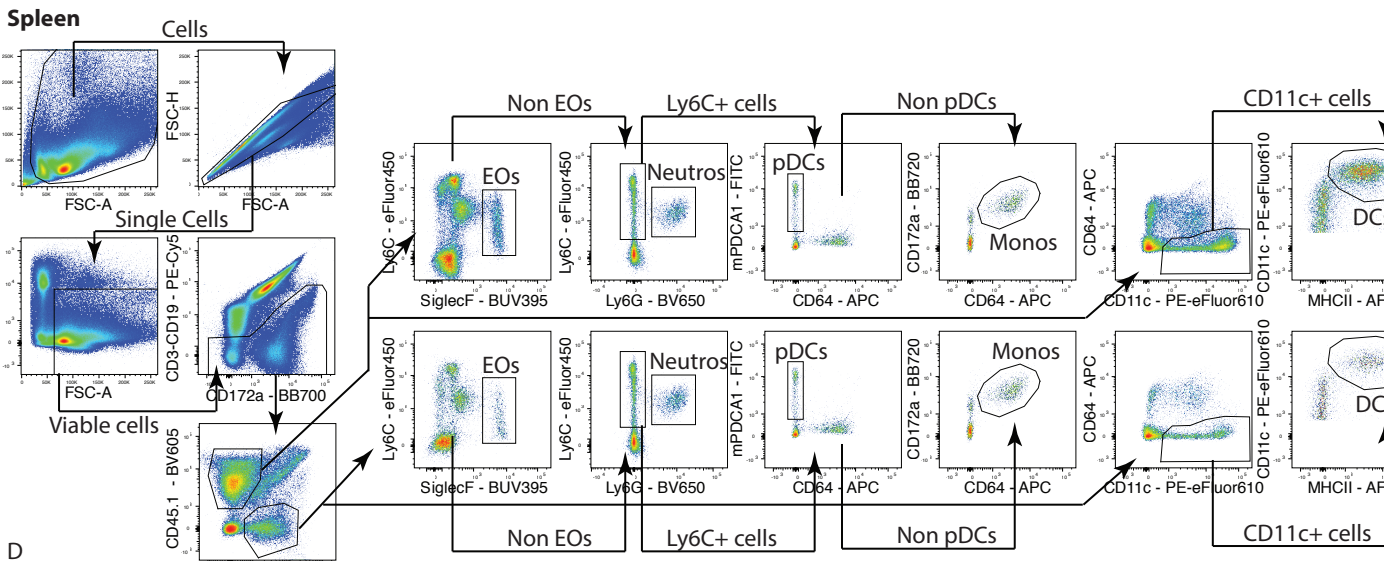
A



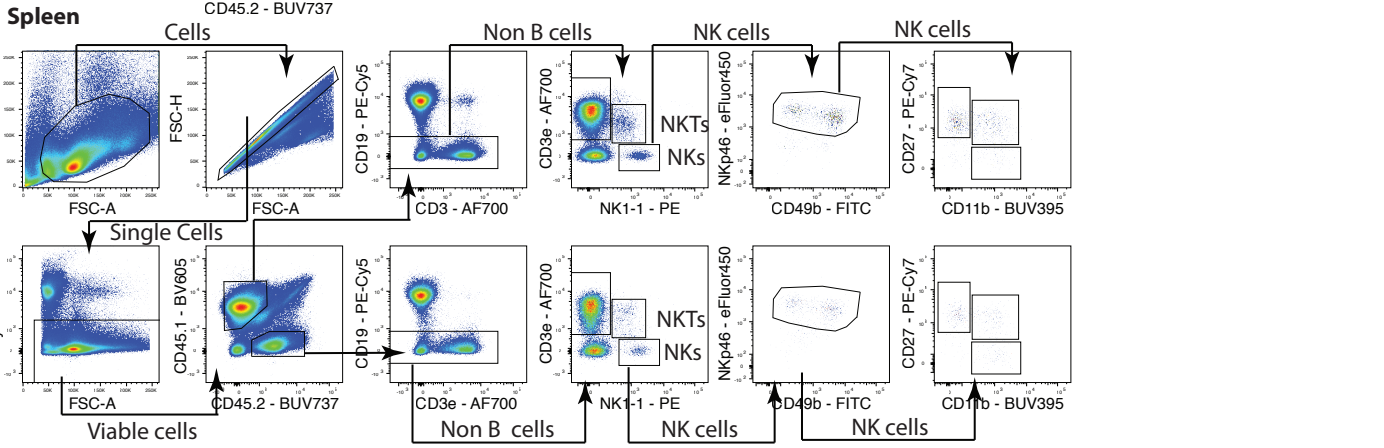
B



C



D



Supplementary Fig. 10. (A-D) Gating strategies pertaining to cell populations presented in Figure 5 and Supplementary Figure 5. N: Naive, CM: Central Memory, EM: Effector Memory, Fol: Follicular, MZB: Marginal Zone B cells, MZP: Marginal Zone Plasmablasts, T: Transitional, F: Follicular, EOs: Eosinophils.

Supplementary Table 1: PID gene list used for filtering WES data (HLH genes in bold)

Primary ImmunoDeficiency genes							
PRF1	STIM1	CARD9	MASP1	LRRC32	CDKN1A	IL15RA	CFI
UNC13D	CARD11	IL17RA	MASP2	P2RX7	CREBBP	PTPRC	CFH
STX11	DOCK8	IL17F	COLEC11	PRDM1	EP300	CD3D	CFHR1
STXBP2	TYK2	ACT1	CD46	RELA	ERBB2	CD3E	CFHR2
SH2D1A	PGM3	CLEC7A	CD59	RELB	HDAC3	CD3G	CFHR3
XIAP	CD19	RORC	FCN3	REL	PTPN2	CD247	CFHR4
ITK	RHOH	APOLI	MBL2	SH3KBP1	SP1	CD8A	CFHR5
CD27	IKZF1	RPSA	MEFV	TCF3	KPNA1	ZAP70	THBD
MAGT1	UNC119	STAT3	MVK	TFRC	RET	RAG1	C4B
RAB27A	IKKBK	JAK2	CIA51	TGFB1	MTOR	RAG2	CFI
MYO5A	CD81	ITGB2	NLRP12	TGFB2	MAP3K7	DCLRE1C	CFH
MLPH	TRAC	FUCT1	TNFRSF1A	TGFB3	HDAC1	ADA	CFHR1
LYST	CTPS1	FERMT3	PSTPIP1	TRAF3IP2	AR	AK2	CFHR2
MUNC13-4	BCL10	SLC37A4	NOD2	IL22	NDUFA13	LIG4	CFHR3
CR2	MAP3K14	SLC35C1	LPIN2	IL17A	RPTOR	NHEJ1	CFHR4
MS4A1	DOCK2	RAC2	IL1RN	IRF1	RICTOR	PNP	CFHR5
TNFRSF13B	VAV1	ACTB	IL36RN	CCL8	TP53	PRKDC	THBD
TNFRSF13C	TRNT1	FPR1	SLC29A3	CCL13	EIF4EBP1	LCK	CREB3L3
TWEAK	TAP1	CTSC	CARD14	CLEC4E	CDK4	CORO1A	CREB3L4
BLK	TAP2	CEBPE	SH3BP2	CLEC6A	RPS6KB1	TNFRSF4	CREB5
FCGR2A	TAPBP	SBD5	PSMB8	STAT5a	CDK2	FOXN1	PRKACA
FCGR1	CIITA	G6PC3	NLRC4	IL6	CDK6	ORAI1	PRKACB
CD40LG	RFX5	VPS45	TMEM173	IL6R	MLST8	NOLA3	PRKACG
CD40	RFXAP	HAX1	CECR1	JAK1	TAB1	RTEL1	PRKAR1A
AICDA	RFXANK	ELANE	CNOT3	SOCS3	EIF4E	TERC	PRKAR1B
UNG	WAS	GFI1	ELF4	STAT6	CDKN1B	TERT	PRKAR2A
INO80	WIPF1	JAGN1	MSH5	BCL10	SKP2	TINF2	CXCR5
IGHG1	ATM	LAMTOR2	MSH2	TLR2	PCNA	TCN2	PRKD1
IGKC	MRE11A	TAZ	MLH1	TLR6	CCND2	SLC46A1	AKT1
IL21	NBN	VPS13B	RAD50	WWP1	CCND3	MTHFD1	APCS
IL21R	BLM	C16ORF57	MRE11	PIAS1	TGFB1	SPINK5	BCL6
NFKB1	DNMT3B	CYBB	BOB1	MITF	RORA	SP110	PDCD1
NFKB2	ZBTB24	CYBA	TNFRSF17	ERAP1	IL17RC	TBX1	CD274
LRBA	POLE1	NCF1	TNFRSF1A	GAB2	TRAF6	DGCR6	PDCD1LG2
CTLA4	PMS2	NCF2	TNFRSF18	IL12A	RORC	HRAS	CD28
PRKCD	MCM4	NCF4	TNFSF4	IL6ST	HIF1A	AP3B1	CDX1
PLCG2	RNF168	IKBKG	TNFSF10	GRB2	RORB	BTK	CXCL12
PIK3CD	CHD7	NFKBIA	TNFSF13	PTPN11	IGLC2	IGHM	IL4I1
PIK3R1	SEMA3E	IRAK4	TNFSF13B	JAK1	IRAK1	CD79A	IRF4
AIRE	RMRP	MYD88	VAV2	WWP2	VHL	CD79B	GAB1
ITCH	SMARCAL1	HOIL1	RTP4	SRC	IKKBK	IGLL1	SPRY2
FAS	TREX1	TMC6	NFKBIA	MAP2K6	TGFB2	BLNK	EPS15
TNFSF6	RNASEH2B	TMC8	ICOSLG	CNTF	EGLN1	TCF3	SYK
CASP10	RNASEH2C	STAT2	IFITM1	HCK	IFNGR1	ICOS	EGF
CASP8	RNASEH2A	MCM4	PIK3AP1	FOXO1	HIF1AN	C4B	ELK1
FADD	SAMHD1	UNC93B1	PIK3CA	PIAS3	SIRT1	C2	MAP2K2
NRAS	ADAR1	TLR3	PIK3CB	MAG1	MDM2	C3	SOS2
FOXP3	IFNGR1	TICAM1	PIK3R2	SOS1	TCEB1	C5	JAK3
IL2RA	IFNGR2	TRAF3	PIK3R3	EGFR	TCEB2	C6	IL7RA
STAT5B	IL12RB1	TBK1	CLEC16A	LCP2	CUL2	C7	RAF1
STAT1	IL12B	CXCR4	CD70	SOCS1	INOC1	C8A	
STK4	ISG15	RBCK1	TLR7	PTPN1	MBP	C8G	
TPP2	GATA2	C1QA	TLR9	ESR1	CXCL12	C8B	
IL10RA	IRF8	C1QB	CD180	BCAR1	CD80	C9	
IL10RB	CSF2RA	C1QC	CREB1	IFNAR1	CD86	SERPING1	
IL10	ACP5	C1R	CREB3	CBL	STAT4	CFB	
MALT1	DKC1	C1S	CREB3L1	FYB	NFAT5	CFD	
TTC7A	NHP2	C4A	CREB3L2	GRAP2	GATA3	CFP	
ADAM17	CREB1	CCND1	JUN	MAPK1	CEBPD	MAPK3	
IL2RG	JUND	SHC1	FOS	MAP2K1	NR3C1	ADAM10	

Supplementary Table 2 - Comparison of phenotypes of R688*/R688* patient and published Roquin-1 (and Roquin-2) transgenic mouse strains

	R688*/R688*	Rc3h1^{san/san}	Rc3h1^{9t/9t}	Rc3h1^{-/-}	Rc3h1^{fl/fl} Vav-Cre	Rc3h1^{fl/fl} CD4-Cre	Rc3h1^{fl/fl} CD19-Cre	Rc3h1-2^{fl/fl} CD4-Cre	Rc3h1^{rin/rin}	Rc3h1^{rin/rin} Lck-Cre
	RC3H1 patient	<i>saraoque</i> (M199R)	gene trap	full KO	hematopoietic and endothelial KO	CD4+T cell KO	B cell KO	CD4+T cell KO	Ringless	CD4+ and CD8+ T Ringless
Reference		Vinuesa et al. Nature. 2005; Linterman et al. Immunity. 2009; Lee et al. Immunity. 2012	Schaefer et al. PLoS ONE. 2013; Montufar-Solis et al. Scientific Reports. 2014.	Bertossi et al. J Exp Med. 2011	Bertossi et al. J Exp Med. 2011	Bertossi et al. J Exp Med. 2011	Bertossi et al. J Exp Med. 2011	Vogel et al. Immunity. 2013; Jeltsch et al. Nat. Immunol. 2014.	Pratama et al. Immunity. 2013	Pratama et al. Immunity. 2013; Ramiscal et al. eLife. 2015.
Survival	1 sib dead <i>in utero</i>	Normal	Poor	Perinatal lethal on C57Bl, better survival in CD1 outbred strain	Normal	Normal	Normal	Decreased survival (130 days) due to inflammatory lung pathology	Perinatal lethal (Respiratory distress)	Normal
Mental retardation	+	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dysmorphism	Short stature, Facial dysmorphism, Webbed Neck	Growth defect	Curly tail, caudal spine defect	Developmental pulmonary defect, Caudal spine defect, Growth defect in CD1 outbred background	NA	NA	none	none	Developmental pulmonary defect (thoracic muscle atrophy), Caudal spine defect	none
Immunological phenotype										
Splenomegaly, adenopathy	+	+++	+	+	+	-	+	+++	NA	NA
Anemia	+++ (during hyperinflammatory episodes)	+	NA	NA	NA	NA	NA	NA	NA	NA
Thrombocytopenia	+(during hyperinflammatory episodes)	+(autoimmune)	NA	NA	NA	NA	NA	NA	NA	NA
Monocytosis, increased macrophages	+	+	+	+	+	-	-	+	NA	NA
Neutrophilia	+	NA	NA	-	-	-	-	+	NA	NA
Eosinophilia	-	NA	+	+	++	++	++	-	NA	NA
Increased Tfh (spleen, LN)	-(CXCR5+ CD45RA- CD4+ T)	++	-	-	-	-	-	++	NA	Reduced Tfh (Increased Tfr)
Increased Treg	++	++	++	++	++	-	+	+	NA	-
Increased ICOS on CD4 T cells	+++	++	++	+++	+++	+++	NA	+++	NA	+
Increased OX40 on CD4 cells	++	+	NA	NA	NA	+	NA	++	NA	NA
Increased effector CD4 T cells	-	++	NA	-	++	-	++	++	NA	NA
Increased effector CD8 T cells	+(CD4+ CD8+ T, PD1+ CD27- CD8 T)	+++	++	++	++	++	+	+++	NA	NA
Increased IFNγ+ CD4 T	-	++	NA	NA	NA	NA	NA	++	NA	-
Increased IL-17a+ CD4 T	++	=	NA	NA	NA	NA	NA	+++	NA	-
Increased IFNγ+ CD8 T	+	NA	++	NA	NA	NA	NA	NA	NA	NA
Expanded circulating B cells	+++	-	-	-	Reduced immature and recirculating B cells	-	++	Reduced Splenic B cells	NA	NA
Impaired B cell maturation	+++	NA	-	-	++	-	-	Reduced Follicular and Marginal Zone B cells	NA	NA
Dysgammaglobulinemia	+(IgM, IgG2)	++ (IgG1, IgG2, IgE)	NA	NA	++ (IgG2b, IgG1, IgG3, IgA)	NA	-	-	NA	NA
Systemic auto-immunity										
SLE-like auto-immunity	-	+++	=	NA	-	-	-	-	NA	NA
Auto-antibodies	ANF- RF++	dsDNA ++ RF++	NA	NA	-	-	-	Directed against pancreatic ags, RF-, ANF-	NA	NA
Spontaneous germinal centers in LN, spleen	NA	+++	++	NA	++	-	-	-	NA	NA
Necrotizing hepatitis	++	++	++	NA	-	-	-	-	NA	NA
Inflammatory bowel disease	?(Chronic diarrhea)	++	++	NA	-	-	-	-	NA	NA
Nephritis	-	++ (Glomerulonephritis)	++ (Not specified)	NA	-	-	-	++	NA	NA
Various organ inflammation	-	NA	Lymphocytic pneumonitis	NA	NA	NA	NA	Interstitial pneumonitis, gastritis	NA	NA
Inflammation										
Triglycerides	++	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ferritin	++ (during hyperinflammatory episodes)	NA	NA	NA	NA	NA	NA	NA	NA	NA
Soluble CD25	++ (during hyperinflammatory episodes)	NA	NA	NA	NA	NA	NA	NA	NA	NA
Increased inflammatory serum cytokines	IL-1b, IL-6, IL-10, IL-17a, IL-18, TNFa, IFNγ	IFNγ, IL-6, TNFa	NA	NA	NA	NA	NA	IL-6, IL-10, IL-17a, TNFa, (IFNγ Normal)	NA	IL-21 reduced
Hyperinflammatory episodes	++	Increased lethality in sepsis model	NA	NA	NA	NA	NA	NA	NA	NA

Abbreviations used in the table: NA = not available, ANF = antinuclear factor, RF = rheumatoid factors, SLE = systemic lupus erythematosus.

Supplementary Table 3 - primer list

<i>RC3H1</i> exon 12	FWD	AAAGGTTAATTGTGGGGAGGA
	REV	GGTCAAACAATGAGAGCCAAT
<i>IL6</i>	FWD	GCTGTGCAGATGAGTACAAA
	REV	GGCTGGCATTGTGGTT
<i>ICOS</i>	FWD	GACCATTCTCATGCCAACTA
	REV	CTATGGGTAACCAGAACTTCA
<i>NFKBID</i>	FWD	ACCTGCGGACCTTTGTCAAC
	REV	CACACGGCTCCTCTTCAACAG
<i>NFKBIZ</i>	FWD	GTGTTCTGGGTAAAGAACTCA
	REV	TGTTCTATGTTTACACCTTGT
<i>REL</i>	FWD	ACCTTCTGACCAGGAAGTTA
	REV	GATCCTGGCACAGTTTCTG
<i>TNF</i>	FWD	CCTCTCTAATCAGCCCTCTG
	REV	GAGGACCTGGGAGTAGATGAG
<i>IFNγ</i>	FWD	TCGGTAACTGACTTGAATGTCCA
	REV	TCGCTTCCCTGTTTTAGCTGC
<i>GAPDH</i>	FWD	TGCACCACCAACTGCTTAGC
	REV	GGCATGGACTGTGGTCATGAG
<i>TBP</i>	FWD	CGGCTGTTTAACTTCGCTTC
	REV	CACACGCCAAGAAACAGTGA
<i>HPRT</i>	FWD	TGACACTGGCAAACAATGCA
	REV	GGTCCTTTTCACCAGCAAGCT
<i>Icos (polyA tail assay)</i>	FWD	gtcacgctgatgtttcttcag
	REV	ttattggctgttcaggagcag

Supplementary Table 4 - Flowcytometry antibodies
Human panels

<i>Epitope</i>	<i>Clone</i>	<i>Fluorochrome</i>	<i>Company</i>	<i>Catalogue Number</i>	<i>Dilution</i>
CD11b	ICRF44	BV480	BD Biosciences	746704	2.5 μ L
CD11c	B-Ly6	BV650	BD Biosciences	563404	0.625 μ L
CD123	32703	BB630-P	BD Biosciences	Custom developed	2.5 μ L
CD127	HIL-7R-M21	BUV737	BD Biosciences	564300	2.5 μ L
CD14	M5E2	BV750	BD Biosciences	746920	1.25 μ L
CD16	3G8	BV570	Biologend	302036	1.25 μ L
CD183	1C6	AF488	BD Biosciences	561730	1.25 μ L
CD185	J252D4	BV421	Biologend	356920	1.25 μ L
CD194	L291H4	PE	Biologend	359412	1.25 μ L
CD196	11A9	BUV496	BD Biosciences	564659	2.5 μ L
CD197	3D12	BUV615-P	BD Biosciences	Custom developed	2.5 μ L
CD20	2H7	BB700	BD Biosciences	745889	0.625 μ L
CD24	ML5	BUV395	BD Biosciences	563818	1.25 μ L
CD25	2A3	BUV563	BD Biosciences	565699	5 μ L
CD27	O323	PE-Cy7	Biologend	302838	1.25 μ L
CD38	HIT2	APC-R700	BD Biosciences	564979	1.25 μ L
CD3e	UCHT1	BV711	Biologend	300464	1.25 μ L
CD4	SK3	BUV805	BD Biosciences	564910	5 μ L
CD45RA	HI100	APC	Biologend	304112	1.25 μ L
CD56	NCAM16-2	BB790-P	BD Biosciences	Custom developed	1.25 μ L
CD8	RPA-T8	BV786	BD Biosciences	563823	0.625 μ L
CRTH2	BM16	APC-Cy7	Biologend	350114	2.5 μ L
FoxP3	PCH101	APC	Thermofisher	17-4776-42	0.5 μ L
HLA-DR	G46-6	BUV661	BD Biosciences	565073	1.25 μ L
ICOS	C398.4A	APC	Thermofisher	17-9949-82	0.25 μ L
IFNg	B27	FITC	BD Biosciences	552887	1 μ L
IgD	IA6-2	BV605	Biologend	348232	1.25 μ L
IL-17A	eBio64DEC17	APC	Thermofisher	17-7179-42	5 μ L
OX40	ACT35	APC	Biologend	350008	1 μ L
PD1	EH12.1	BB660-P	BD Biosciences	Custom developed	2.5 μ L
Streptavidin		PE-Cy5	BD Biosciences	554062	0.625 μ L
TCR gd	B1	BUV395	BD Biosciences	745681	5 μ L
Va24-Ja18	6B12	Biotin	Thermofisher	13-5806-82	5 μ L
Va7.2	3C10	BV605	Biologend	351720	5 μ L
XCR1	S15046E	APC-Fire750	Biologend	372608	2.5 μ L

Mouse panels

<i>Epitope</i>	<i>Clone</i>	<i>Fluorochrome</i>	<i>Company</i>	<i>Catalogue Number</i>	<i>Dilution</i>
anti-goat	Polyclonal	AF647	ThermoFisher	A-21447	1 in 1000
B220	RA3-6B2	BV650	BD Biosciences	563893	1 in 200
CD115	AFS98	PE	ThermoFisher	12-1152-82	1 in 400
CD11b	M1/70	BUV395	BD Biosciences	563553	1 in 200
CD11b	M1/70	APC-eFluor780	ThermoFisher	47-0112-82	1 in 200
CD11c	N418	PE-eFluor610	ThermoFisher	61-0114-82	1 in 500
CD11c	N418	PE-Cy7	ThermoFisher	25-0114-82	1 in 500
CD134	OX-86	PE	Thermofisher	12-1341-82	1 in 100
CD16/32	2.4G2	Unconjugated	In-house	N/A	1 in 400
CD16/32	2.4G2	PE	BD Biosciences	561727	1 in 200
CD161	PK136	PE	Biologend	108753	1 in 300
CD19	eBio1D3	PE-Cy5	ThermoFisher	15-0193-82	1 in 500
CD21/35	7G6	PE	BD Biosciences	552957	1 in 200
CD23	B3B4	PE-Cy7	ThermoFisher	25-0232-82	1 in 200
CD26	H194-112	FITC	BD Biosciences	559652	1 in 100
CD27	LG.7F9	PE-Cy7	ThermoFisher	25-0271-82	1 in 300
CD279	RMP1-30	PE-Cy7	Biologend	109110	1 in 500

Mouse panels - continued

<i>Epitope</i>	<i>Clone</i>	<i>Fluorochrome</i>	<i>Company</i>	<i>Catalogue Number</i>	<i>Dilution</i>
CD3	145-2C11	BUV737	BD Biosciences	612771	1 in 200
CD3	145-2C11	BV510	BD Biosciences	563024	1 in 100
CD3	145-2C11	APC-Cy7	BD Biosciences	557596	1 in 50
CD3	145-2C11	PE-Cy5	ThermoFisher	15-0031-82	1 in 300
CD3	17A2	AF700	ThermoFisher	56-0032-82	1 in 150
CD335	29A1.4	eFluor450	ThermoFisher	48-3351-82	1 in 50
CD38	90	AF700	ThermoFisher	56-0381-82	1 in 200
CD4	GK1,5	APC-Cy7	BD Biosciences	552051	1 in 200
CD44	IM7	red Fluor710	Tonbo Biosciences	TONB80-0441-U100	1 in 200
CD45	30-F11	AF700	ThermoFisher	56-0451-82	1 in 400
CD45.1	A20	BV605	Biolegend	110738	1 in 200
CD45.2	104	BUV737	BD Biosciences	564880	1 in 400
CD62L	MEL-14	FITC	ThermoFisher	11-0621-82	1 in 200
CD64	X54-5/7.1	BV711	Biolegend	139311	1 in 100
CD64	X54-5/7.1	AF647	BD Biosciences	558539	1 in 300
CD8	53-6.7	PE-Cy7	ThermoFisher	25-0081-82	1 in 400
CD8	53-6.7	eFluor450	ThermoFisher	48-0081-82	1 in 400
CD93	AA4.1	Biotin	ThermoFisher	13-5892-82	1 in 50
Clec4F	Polyclonal	Goat	Bio-Techne	AF2784	1 in 100
cRel	1RELAH5	PE	ThermoFisher	12-6111-80	1 in 100
CTLA4	UC10-4F10-11	APC	BD Biosciences	564331	1 in 100
CXCR5	2G8	Biotin	BD Biosciences	551960	1 in 50
F4/80	BM8	BV785	Biolegend	123141	1 in 100
FoxP3	FJK-16s	APC	ThermoFisher	17-5773-82	1 in 300
ICOS	C398.4A	PerCP-Cy5-5	Biolegend	313518	1 in 400
ICOS	C398.4A	APC	ThermoFisher	17-9949-82	1 in 400
IgD	11-26c.2a	V450	BD Biosciences	560869	1 in 200
IgM	II/41	FITC	BD Biosciences	553437	1 in 200
IL-17A	TC11-18H10.1	PE	Biolegend	506904	1 in 200
IL-2	JES6-5H4	APC	ThermoFisher	17-7021-82	1 in 200
Ly6-C	HK1.4	eFluor450	ThermoFisher	48-5932-82	1 in 500
Ly6-G	1A8	BV650	BD Biosciences	740554	1 in 500
MHCII	M5/114,15,2	AF700	ThermoFisher	56-5321-82	1 in 500
mPDCA1	120g8	FITC	In-house	N/A	1 in 200
Rck	Polyclonal	Unconjugated	Bethyl	A300-461A	1 in 100
Ox40	OX-86	PE	ThermoFisher	12-1341-81	1 in 200
Siglec F	E50-2440	BUV395	BD Biosciences	740280	1 in 200
Streptavidin		BV786	BD Biosciences	563858	1 in 200
TCR gd	GL3	FITC	BD Biosciences	561996	1 in 300
Tim-4	54(RMT4-54)	PerCP-eFluor710	ThermoFisher	46-5866-82	1 in 200
TNFalpha	MP6-XT22	PE	ThermoFisher	12-7321-82	1 in 200
XCR1	ZET	BV510	Biolegend	148218	1 in 400