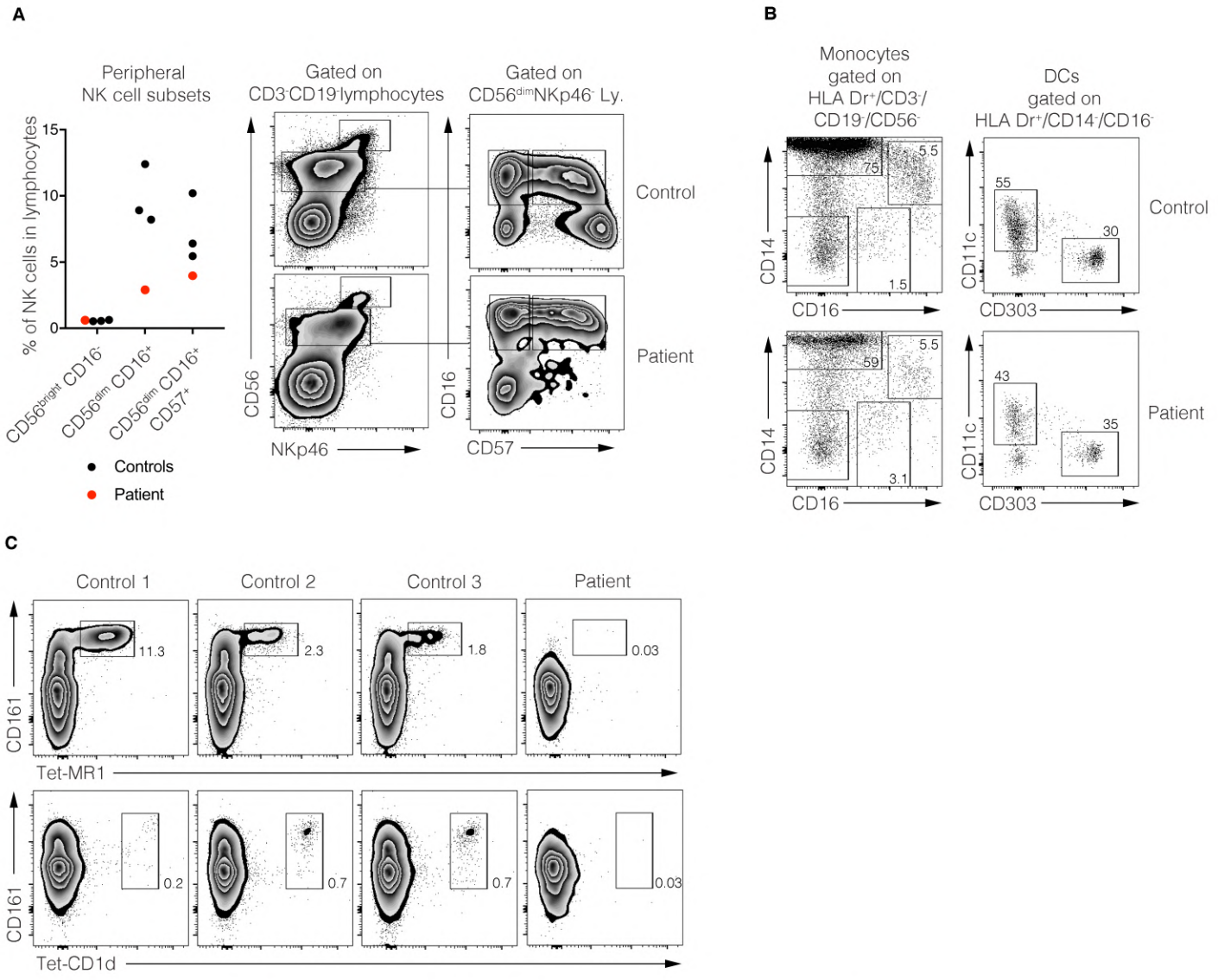
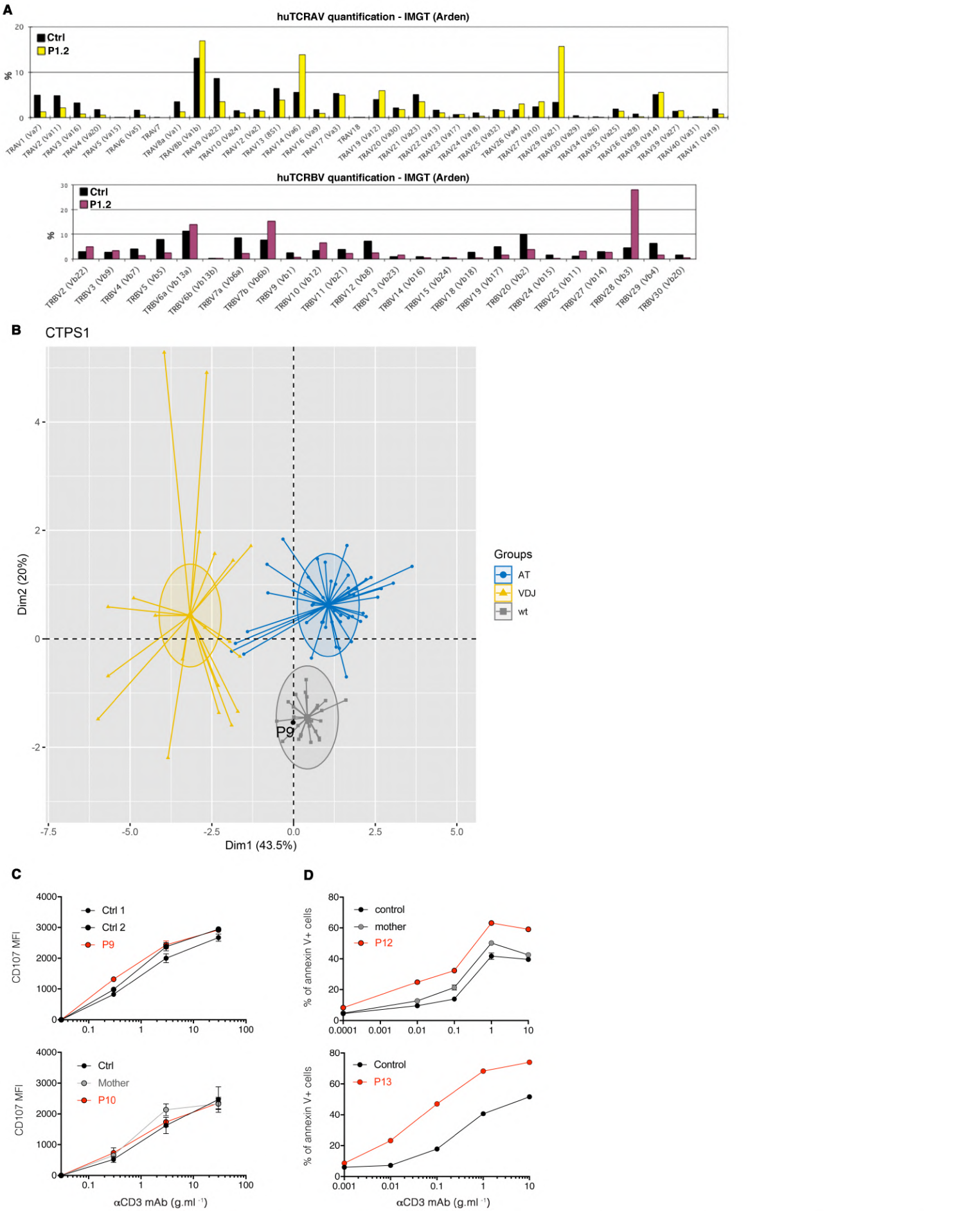
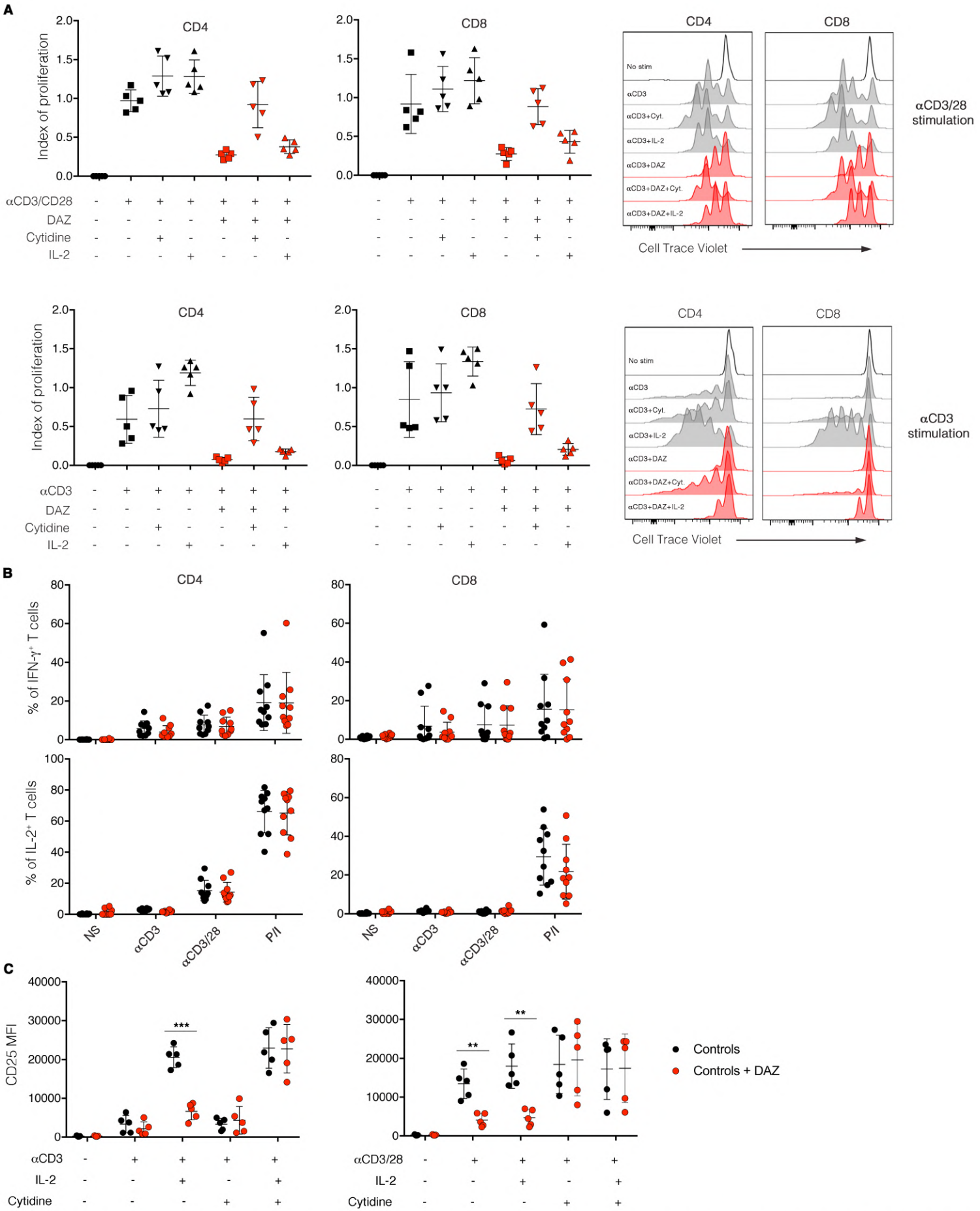


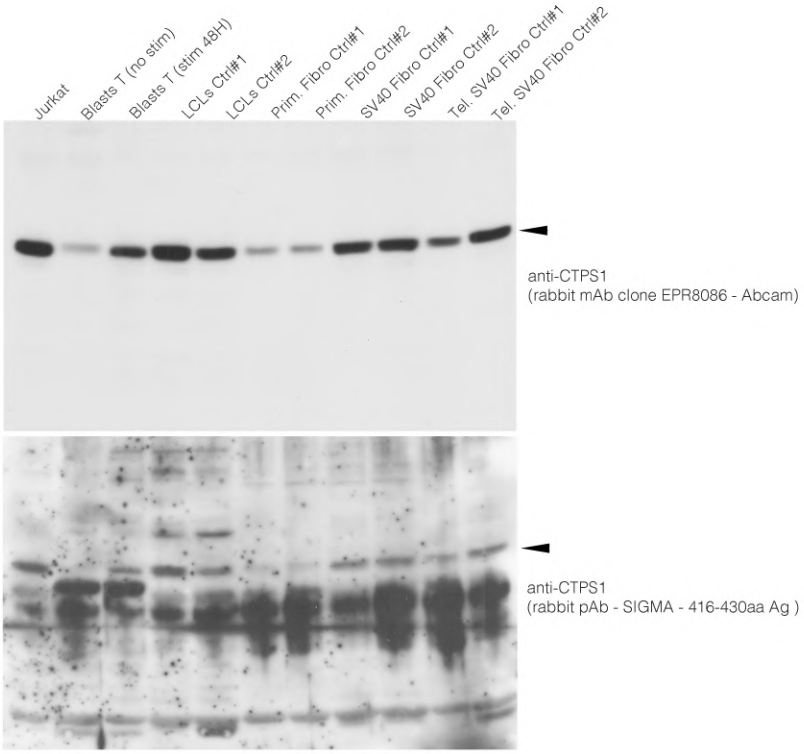
Supplemental Figure 1. Analysis of PBMCs sub-populations in CTPS1-deficient patients. A, % NK cell subsets after gating on CD3-CD19-CD56+NKp46+ and CD57+ lymphocytes in one patient and three controls. **B**, Representative gating strategy of FACS dot plots to identify mDCs, pDCs and different monocyte sub-populations in PBMCs from one control (upper panel) and one patient (lower panel) are depicted. **C**, Representative FACS dot plot analysis of mucosal-associated invariant T cells (MAIT) (upper panel) and invariant natural killer T cells (iNKT) (lower panel) using MR1 and CD1d tetramers respectively and CD161 marker in PBMCs of three controls and one patient. Dot plot graphs were gated on CD3+ T cells.



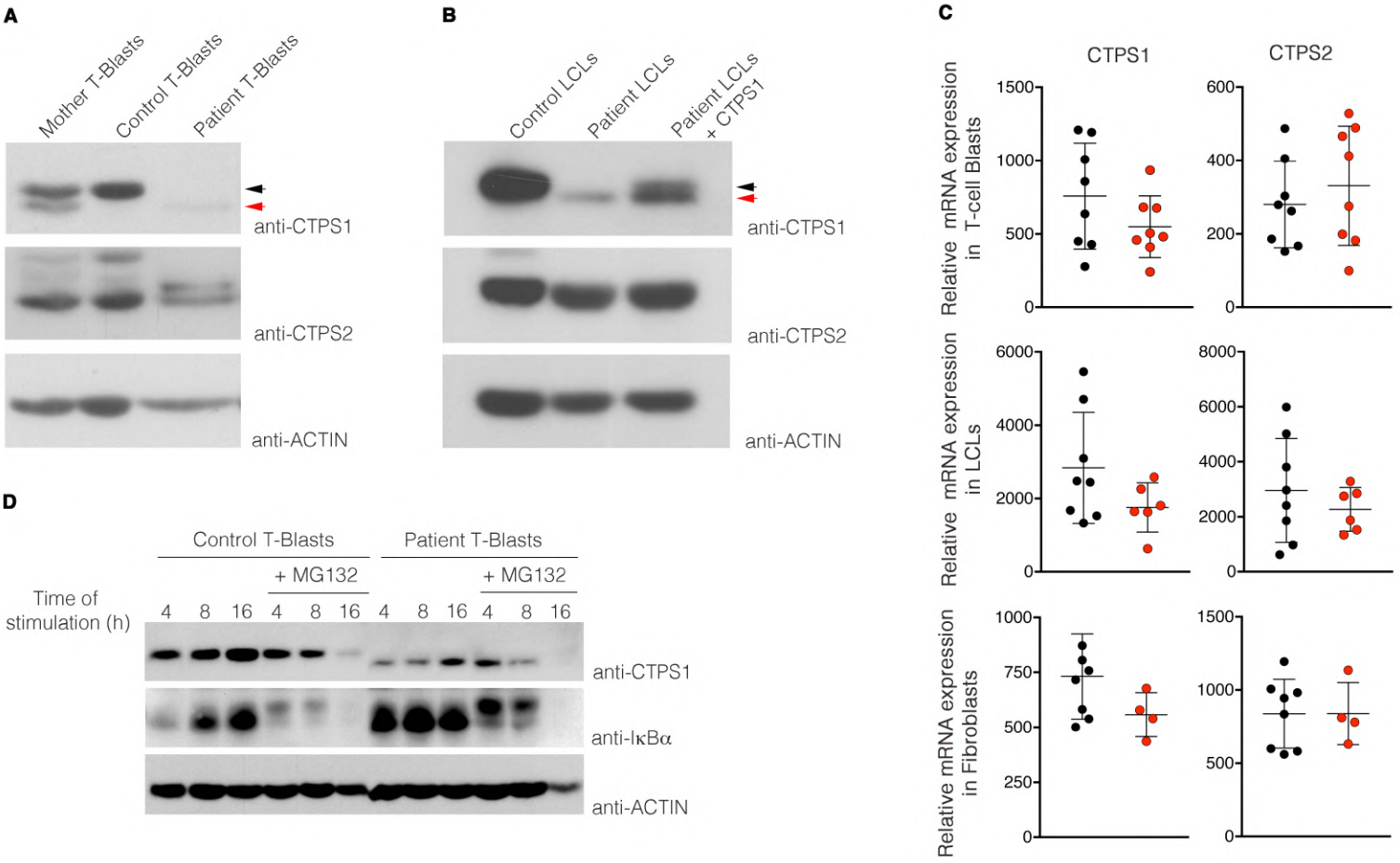
Supplemental Figure 2. Normal T-cell repertoire in CTPS1-deficient patients. A, TCRαβ repertoire analysis by immunoscope of PBMCs from a CTPS1-deficient patient (P1.2). Real-time quantitative PCR of 35 TCRα (TRAV) (upper panel) and 24 TCRβ (TRBV) (lower panel) variable segments, together with TCRα and TCRβ constant segments. The relative usage of each TRAV or TRBV (in percentages) is represented by histograms. The nomenclature used for the TRAV subgroups is from IMGT (ImmuneGeneTics, http://www.imgt.org). **B**, TCRα repertoire was analyzed using PROMIDISα[23]. Unsupervised hierarchical clustering of TCRα transcripts assigned P9 to the group of control individuals, as opposed to the two groups of patients with V(D)J recombination/thymocyte survival defects (AT and VDJ). **C**, Degranulation of CD8+ T-cell blasts based on CD107 expression following anti-CD3 antibody stimulation of control for 3h. Both graphs represent independent experiments. CD107 expression based on MFI calculated from FACS histograms. **D**, Activation induced cell-death of T-cell blasts following anti-CD3 stimulation for 20h. The graph shows apoptotic cells that correspond to Annexin V+ / 7-AAD- (7-AminoActinomycin D) cells. **C-D**, Controls in black, patients in red and mothers of patients in grey.



Supplemental Figure 3. IL-2 independent decrease of T cell proliferation in CTPS1 deficient patients. A, Index values of cell proliferation (left panels) in control CD4+ and CD8+ T cells treated with 3-Deazuridine (DAZ) or not. T-cell blasts were stimulated or not with anti-CD3/CD28 beads (upper panels) or anti-CD3 antibody (lower panels) for 48h. Supernatants of untouched and treated T cells were complemented or not with 100U IL-2 (up triangle) or 200µM of cytidine (down triangle). The index values are calculated using FlowJo software from cell trace violet histogram analyses which one representative experiment is shown on the right panels. Data were obtained from two independent experiments. **B**, Frequency of IFN-γ (upper panels) and IL-2+ (lower panels) in CD4 and CD8 T cells of 10 healthy donors treated with 3-deazuridine (DAZ) or not are shown in the dot plot graph. Data are calculated from FACS histogram analysis and normalized on isotype staining. Data were obtained from 2 independent experiments. **C**, Expression of CD25 based MFI calculated from FACS histograms following anti-CD3 antibody or anti-CD3/CD28 beads stimulation of control T-cell blasts treated as in (**A**). Graphs from 2 independent experiments. **A-C**, Each symbol corresponds to an individual healthy donor. Red and black symbols correspond to treatment with DAZ and no treatment, respectively. The horizontal bars represent the median ± SEM. Mann-Whitney tests were used. *, $P < 0.05$; ***, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$



Supplemental Figure 4. Comparison of anti-CTPS1 antibodies in the detection of CTPS1 by immunoblotting. Immunoblots for CTPS1 expression in the Jurkat cell line, control T-cell blasts stimulated or not with anti-CD3/CD28 beads for 48h, two control lymphoblastoid cell lines (LCL) from healthy donors, two primary fibroblast cell lines from healthy donors, two SV40 T-antigen immortalized fibroblast cell lines and two telomerized SV40-transformed fibroblast cell lines. CTPS1 protein expression was detected using a monoclonal rabbit antibody (EPR8086(B)) or a polyclonal rabbit antibody (#SAB111072). Arrows indicate CTPS1.



Supplemental Figure 5. Increased expression of CTPS1Δ18 in the presence of wild-type CTPS1 and analysis of CTPS1 and CTPS2 mRNA expression in cells from CTPS1-deficient patients. A, Immunoblots for CTPS1, CTPS2 and ACTIN protein expression in lysates of activated T cell blasts from the mother of a patient, control or patient T-cell blasts. Black and red arrows indicate CTPS1 and CTPS1Δ18 proteins respectively. **B**, Immunoblots for CTPS1, CTPS2 and ACTIN protein expressions in lysates of LCLs from control, a patient and the LCLs of the patient complemented with WT CTPS1 (left panel). **C**, Dot plot graphs of relative CTPS1 and CTPS2 mRNA expression determined by qRT-PCR of T-cell blasts from eight controls (black circle) and four patients (red circle) (upper panels), and LCLs from 3 patients and 4 controls (middle panels) and fibroblasts from 2 patients and 4 controls (lower panels).The value for each dot is the mean of triplicate values and is normalized on GAPDH gene expression. Data were obtained from four independent experiments. The horizontal bars represent the median ± SEM. **D**, Immunoblots for CTPS1, IκBα and ACTIN protein expression in lysates of control and patient activated T-cell blasts which have been treated with 5μM of MG132 drug during indicated time. Black and red arrows indicate CTPS1 and CTPS1Δ18 proteins respectively.

Supplemental Table 1. Clinical features

Patient	Age at 1st symptoms	Viral infections			Bacterial infections	Extra-hematopoietic manifestations*	Outcome (age in years)	Ref.
		EBV	VZV	Others				
P1.1	1 y	SIM, chronic viraemia	No	CMV, Norovirus, Rotavirus (gut) Parainfluenzae I (RTI)	*H. influenzae* (RTI)	No	HSCT (7 y), died (8 y, gut GVHD)	
P1.2	1 m	SIM	No	Adenovirus, HHV-6, Norovirus (gut)	No	No	HSCT (12 y), died (13y, skin GVHD, PML)	
P2.1	5 y	LPD (CNS)	Severe	No	*H. influenzae* (RTI)	No	HSCT (9 y) a.w. (24 y)	
P2.2	2 y	chronic viraemia	No	No	*S. pneumoniae, H. influenzae* (RTI)	No	HSCT (5 y) a.w. (19 y)	Martin et al, 2014
P3.1	1 y	SIM	Severe	No	*S. pneumoniae* (sepsis, meningitis)	No	died (VZV/SIM) (4 y)	
P3.2	3 m	SIM, chronic viraemia	Yes	HHV6	No	No	HSCT (8 y) a.w. (19 y)	
P4	5 m	LPD (CNS)	No	MV, Adenovirus, Rotavirus (gut)	*N. meningitis B* (meningitis)	No	HSCT (1 y) alive, (7 y, hemiplegic)	
P5	3 m	SIM, LPD	Severe	Norovirus (gut) Parainfluenzae III, Adenovirus, Rhinovirus (RTI)	No	No	died SIM (5 y)	
P6.1	1 y	SIM, chronic viraemia	Yes	Adenovirus, HSV	*Bordetella bronchiseptica*	No	HSCT (4 y) a.w. (14 y)	Kucuk et al, 2016
P6.2	2 y	LPD, chronic viraemia	Yes	Parvovirus B19, HSV	*Pneumococcus*	No	HSCT (7y) a.w. (15 y)	
P7.1	2 m	SIM, chronic viraemia	No	No	RTI, *S. pneumoniae*, meningitis	No	HSCT (7 y) a.w. (8 y)	Trück et al, 2016
P7.2	8 m	No	No	No	Invasive bacterial diseases (otitis...), RTI	No	HSCT (5 y) died (6 y, viral infections)	
P8 §	4 m	No	No	HHV6 (gut)	No	No	HSCT (17 y) a.w. (20 y)	Nademi et al, 2018
P9	3 y	SIM, HLH	Severe	Molluscum	LRTI	No	HSCT (8 y) died (8 y, EBV (CNS))	
P10	2 y	LPD (lung)	Severe	Adenovirus	No	No	HSCT (3 y) a.w. (5 y)	
P11	7 y	No	Severe	Adenovirus, Rhinovirus (RTI)	*H. influenzae* (RTI), *S. pneumoniae*	No	HSCT (12 y) a.w. (14 y)	
P12	1 y	SIM	Yes	Hepatitis C, CMV (meningitis), HPV (warts)	Methicillin-resistant *S. Aureus* (skin), RTI	No	Alive (18 y)	this study
P13	5 m	No	Severe	No	No	No	a. w. (12 y)	
P14	3y	chronic viremia	No	No	recurrent pneumococcal infections: meningitis and septicemia	No	HSCT (3y) a.w.	

y, year ; m, month ; PML, progressive multifocal leukoencephalopathy ; SIM, severe infectious mononucleosis ; CNS, central nervous system; EBV, Epstein-Barr virus; VZV, varicella zona virus; HHV6, human herpes virus 6; HPV, human papilloma virus; LPD, lymphoproliferative disease/lymphoma ; RTI, respiratory tract infection ; CMV, cytomegalovirus ; HSCT, hematopoietic stem cell transplantation ; a.w., alive and well ; n.k. not known ; *not related to infections and immunodeficiency ; § P8 has two siblings who died of fulminant IM and acute VZV before 2 years.

Supplemental Table 2. Immunological data of CTPS1-deficient patients

Patient	**P10**	**P11**	**P12**	**P13**	**P14**
Age (months)	36	24	221	120	37
Cell subsets (10^3 cells.mm^{-3})					
WBC	16.6	9.6	9.3	**3.9** (4.4-9.5)	6.6
Lymphocytes	3.43	3.43	2.33	1.9	1.66
PMN	8.75	3.28	5.67	1.4	4.19
Platelets	509	248	360	266	274
Haemoglobin ($g.L^{-1}$)	109	142	141	129	108
T-cell proliferation					
PHA	low	low	normal	N.D.	N.D.
OKT3	low	low	low	N.D.	N.D
Serum immunoglobulins ($g.L^{-1}$)					
IgG	16.5 (4.9 – 16.1)	16.7 (6-15)	16.5 (6.9-16.2)	9.2	16.2 (4.9 – 16.1)
IgA	1.45	0.45	3.93 (0.68-3.78)	2.58	0.51
IgM	1.1	0.24	**0.25** (0.45-2.63)	0.87	**0.28** (0.5 – 1.9)
Specific antibodies ($IU.ml^{-1}$)					
Tetanus (> 0.1)	1.58	N.D.	**0.02**	**0.03**	**0.07**
H. influenzae type B (>1)	3.7	1.94	>9.0	>9.0	0.5
S. Pneumoniae (> 0.35)	low	low	N.D.	low	low

WBC, white blood cells. PMN, polymorphonuclear neutrophils. PHA, phytohaemagglutinin. Ig, immunoglobulin. In bold or red correspond to values below or above normal repectively.

Supplemental Table 3 . Immunological features of PBMCs from CTPS1-deficient patients

	P10 (3 years)	P11 (13 years)	P12 (17 years)	P13 (10 years)	P14 (3 years)
T cells					
$CD3^+$ (cells.mm^{-3})	2886	2282	2483	2274	1312
$CD4^+$ (cells.mm^{-3})	1783	845	1376	1423	527
$CD8^+$ (cells.mm^{-3})	923	1330	1175	755	889
CD4/CD8 ratio	2,2	**0.26** (0.9-2.6)	1.6	2	**0.59** (1.3-2.9)
TCRα/β (%)	67	63	52	76	67
TCRγ/δ (%)	5.5	3.8	2.1	2.5	2.2
$CD31^+CD45RA^+$ / $CD4^+$ (recent naïve thymic emigrant) (%)	75	53	51	57	**53** (65-83)
$CD45RO^+$ / $CD4^+$ (memory) (%)	25	45	46	41	**45** (15-35)
$CCR7^+CD45RA^+$ / $CD8^+$ (naïve) (%)	82	59	**31** (49/88)	59	**48** (54-90)
$CCR7^+CD45RA^-$ / $CD8^+$ (central memory) (%)	9.9	24	**48** (10-38)	35	**34** (5-23)
$CCR7^-CD27^-CD45RA^-$ / $CD8^+$ (effector memory) (%)	0.9	5	17	3	6
$CCR7^+CD27^-CD45RA^+$/ $CD8^+$ (exhausted effector memory - EMRA) (%)	0.6	1.9	2.2	0.3	12
$CD127^{low}CD25^+$ / CD4+ (regulatory) (%)	3.9	4.4	5	4.5	5.9
$Vα7^+CD161^+$ / $CD3^+$ (MAIT) (%)	**0.03** (1-8)	**0.01** (1-8)	**0.09** (1-8)	**0.4** (1-8)	**0.3** (1-8)
$Vα24^+Vβ11^+CD161^+$ / $CD3^+$ (NKT) (%)	**0** (>0.02)	**0** (>0.02)	**0** (>0.02)	**0** (>0.02)	**0** (>0.02)
NK cells					
$CD16^+CD56^+$ (cells.mm^{-3})	233	**57** (100-480)	101	**89** (130-720)	159
$CD16^+CD56^+$ (%)	8.2	**1.8** (4-17)	13	4.8	13
B cells					
$CD19^+$ (cells.mm^{-3})	337	342	436	N.D.	239
$CD19^+$ (%)	**12** (14-28)	14	18	10.8	**11.3** (14-28)
$CD21^+CD27^+$ / $CD19^+$ (memory) (%)	**6.4** (11-24)	**5** (13-27)	**14** (16-32)	**1.3** (14-33)	**10.5** (11-24)
IgD^+IgM^+ / $CD19^+CD21^+CD27^+$ (marginal zone) (%)	55	46	42	47	36
IgD^-IgM^- / $CD19^+$ $CD21^+CD27^+$ (switched) (%)	38	49	55	50	62

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