Supplementary Information

Tartrate resistant acid phosphatase deficiency causes a bone dysplasia with autoimmunity and a type I interferon expression signature

Authors and affiliations

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SUPPLEMENTARY NOTE

Patient ascertainment.

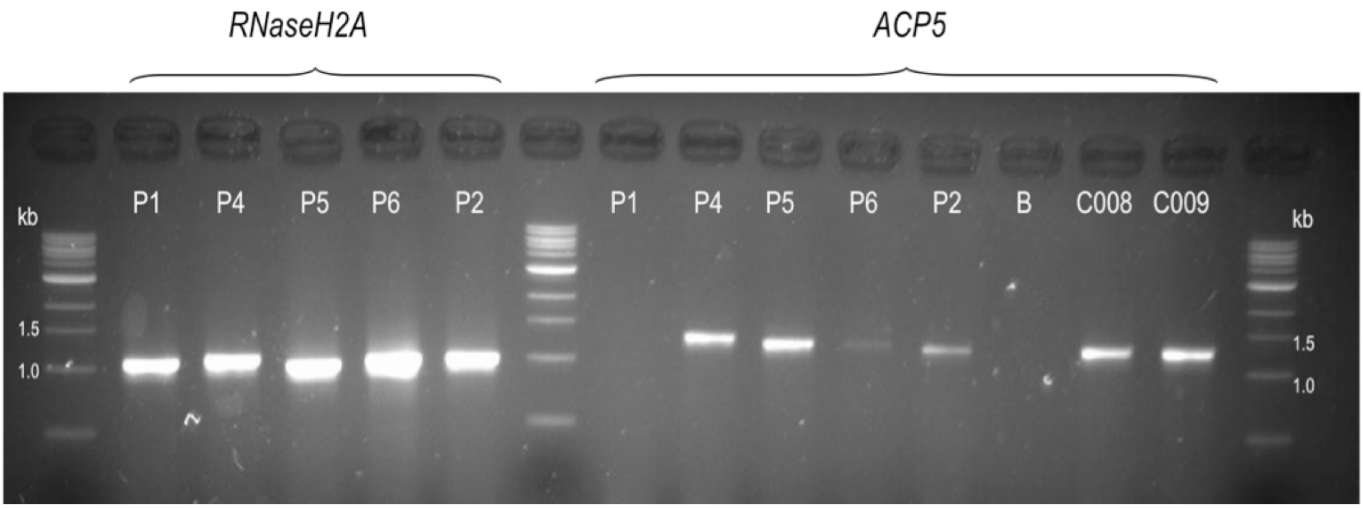
Patients were ascertained based on a clinical suspicion of a diagnosis of SPENCD (see Table 1). Samples were collected for mutation analysis and further investigations as appropriate. Family members and controls samples were recruited accordingly.

Description of patient 5.

Patient 5 was identified with SPENCD at the age of 14 years following a molecular diagnosis in her brother (patient 4). She was under clinical review because of apparently idiopathic short stature, and her only history of immunological disease was of Raynaud's phenomenon. This patient shows a lesser elevation of interferon induced gene transcripts on qPCR, non-elevated titres of ANA and anti-dsDNA antibodies, but a high level of interferon alpha in serum. These data illustrate intrafamilial variability, and possibly suggest that elevated levels of interferon alpha precede the development of serological indices of autoimmunity.

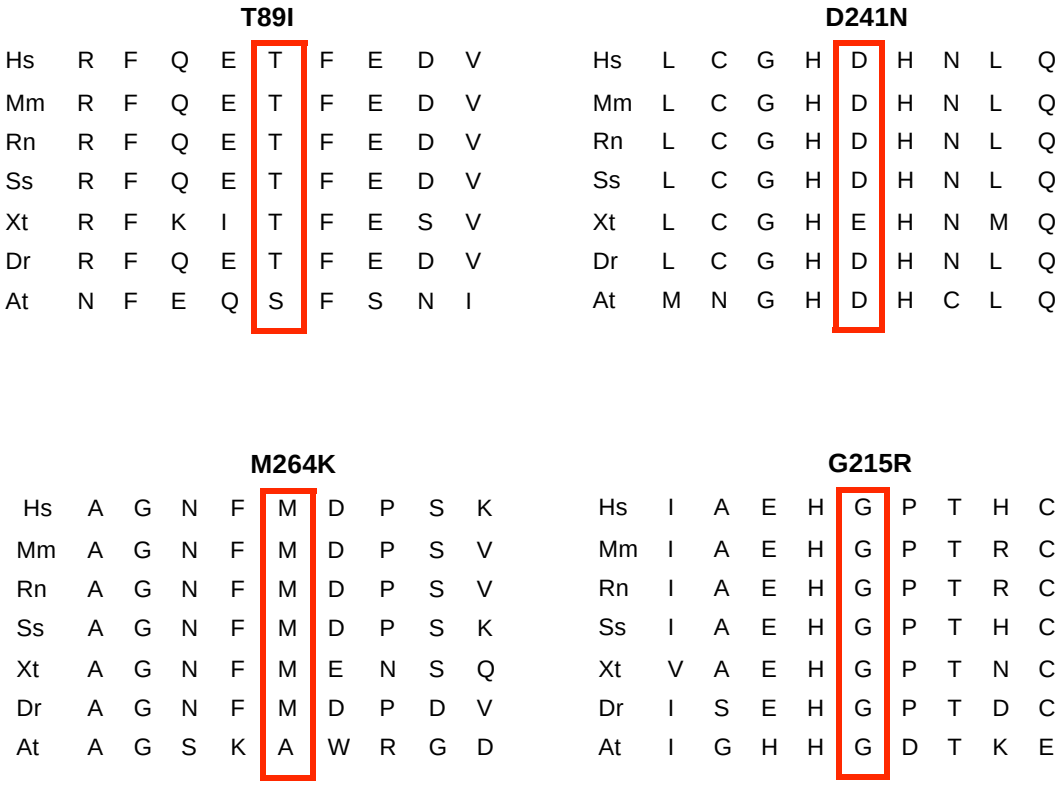
SUPPLEMENTARY FIGURES

Supplementary Figure 1. Reverse transcription PCR (RT-PCR) analysis.



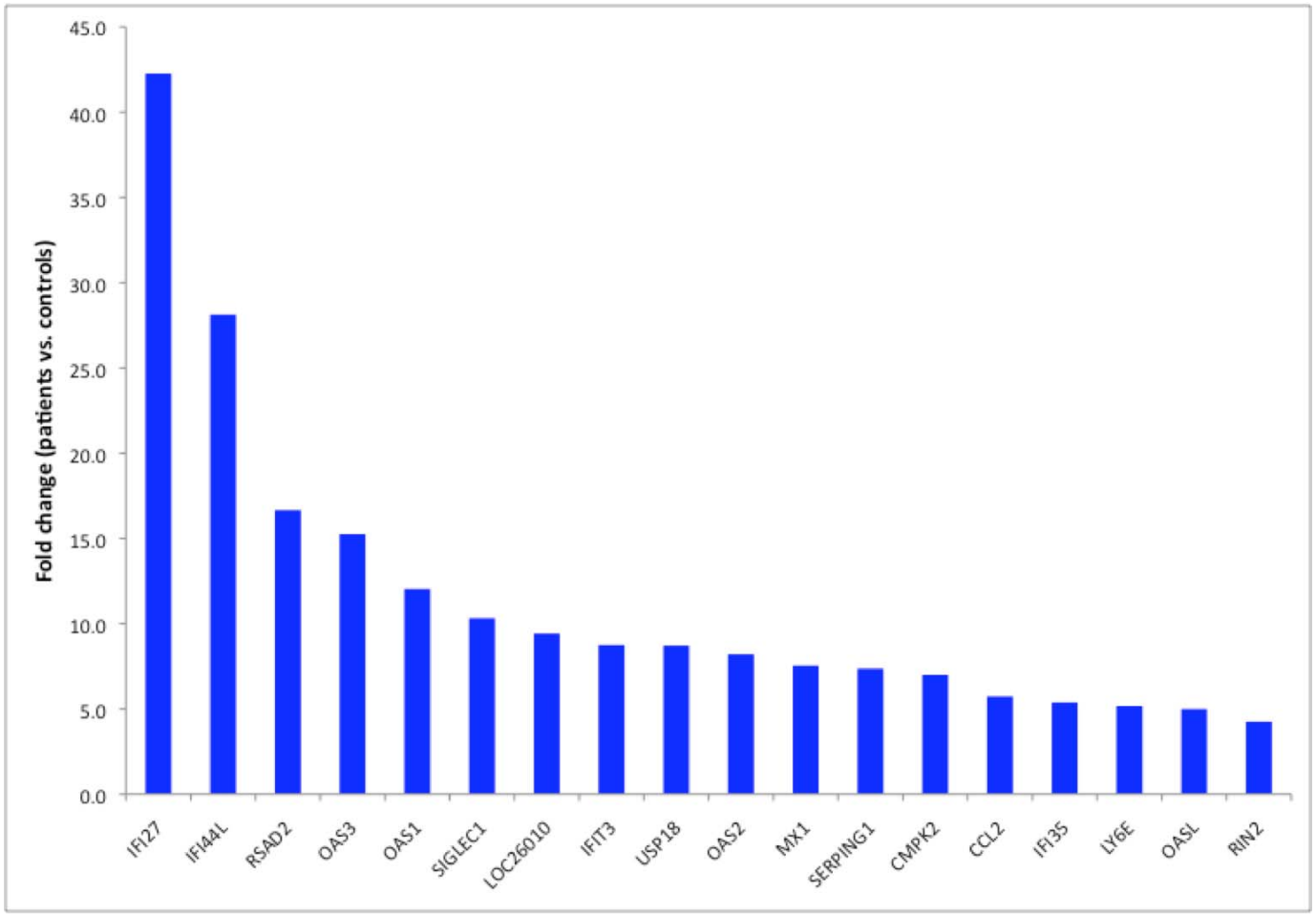
Panel depicts RT-PCR of five patients (P1; homozygous deletion of *ACP5* gene, P4; c.266C>T / T89I, P5; c.266C>T / T89I, P6; c.667C>T / Q223X, P2; c.369C>A / Y123X + c.721 G>A / D241N) and two controls (C008, C009). P represents patient. B is a water blank. RT-PCR was performed using a OneStep RT-PCR kit (Qiagen) with primers specific for *ACP5* cDNA (F: AGGGAGGGAATAAAGGCTCA; and R: TCACATACGTGGGCATCTGT), and *RNaseH2A* as a control gene (F: GCTCCTGCAGTATTAGTTCTTG; and R: TACGTGTGGTTCTCCTTAAACA). *RNaseH2A* PCR product size 1019bp, *ACP5* PCR product size 1256bp. Note complete absence of *ACP5* in P1 compared to normal levels of *RNaseH2A* for the same patient. Note also the reduced levels of *ACP5* in P2 and P6 compared to normal levels of *RNaseH2A*.

Supplementary Figure 2. Sequence alignment of human TRAP with homologues from eukaryotic species.



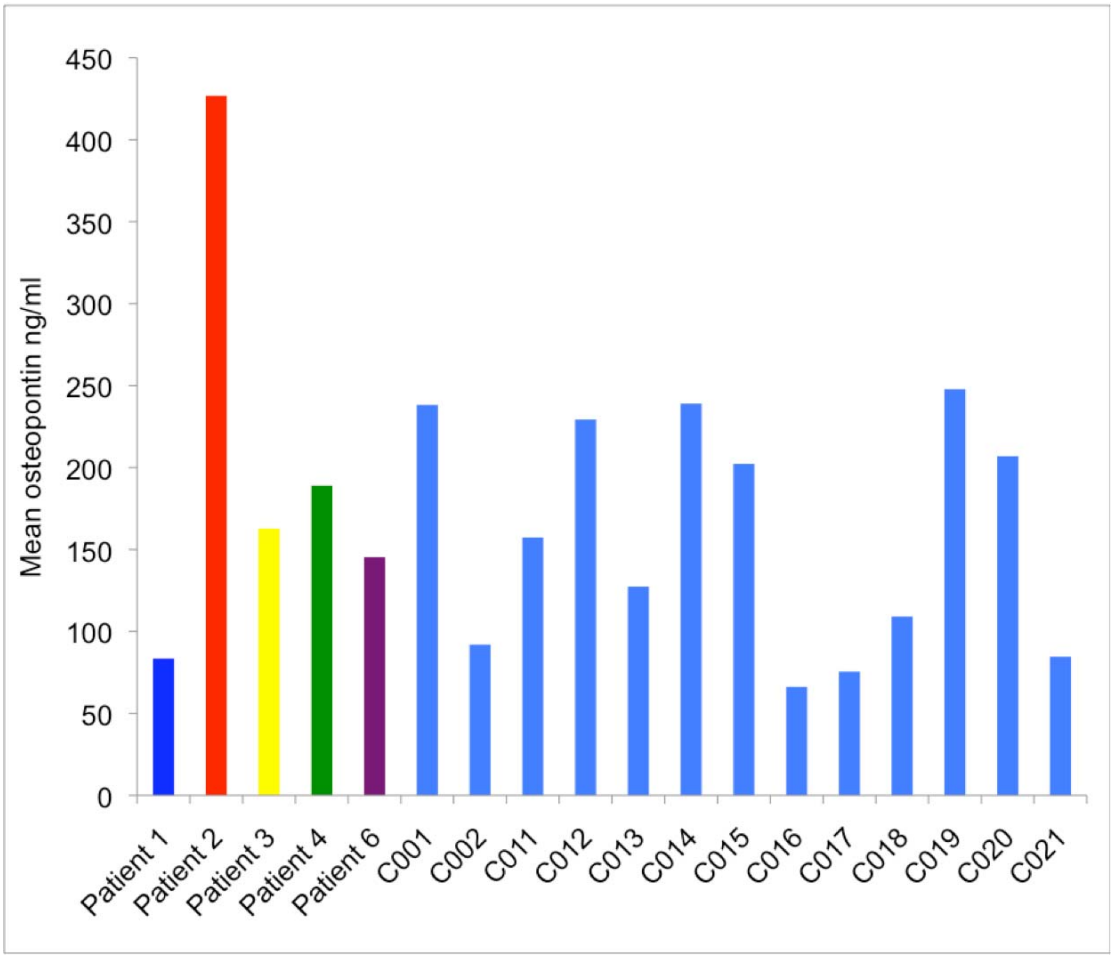
Amino acids altered by *ACP5* missense mutations are boxed in red. Homologues were identified on the NCBI Entrez Protein Database and aligned using CLUSTALW2. Species abbreviations and sequence identifiers are as follows: Hs: *Homo sapiens* (NP_001104505); Mm: *Mus musculus* (NP_031414.1); Rn: *Rattus norvegicus* (NP_062017.1); Ss: *Sus scrofa* (NP_999374.1); Xt: *Xenopus (Silurana) tropicalis (*NP_001008210.1); Dr: *Danio rerio* (NP_999938.1); At: *Arabidopsis thaliana* (NP_198072.1).

Supplementary Figure 3. Microarray analysis in patients with TRAP deficiency.



Whole transcriptome microarray expression analysis was undertaken in three patients, and compared to the data derived from three age-matched control samples. Panel depicts a subset of 18 genes that were four or more fold up-regulated in patients, with a significance level for the comparisons of $p<0.0005$ and a false discovery value of <0.2. Fifteen of these genes are known to be interferon stimulated, characteristic of a type I interferon signature.

Supplementary Figure 4. Plasma osteopontin levels in TRAP deficient patients and controls.



Panel depicts mean levels of osteopontin (ng/ml) in patients and controls. Plasma osteopontin activity was measured with a Quantikine® human osteopontin quantitative sandwich enzyme immunoassay (R&D Sytems). Each sample was measured in duplicate at 2 or 3 dilutions of plasma, and the mean taken of non-conflicting results. Patients and controls were age (Mann-Whitney U test *p*=NS) and sex (chi-squared test *p*=NS) matched. Levels of osteopontin in patients and controls were not significantly different (Mann-Whitney U test *p*=NS). C001 is an unaffected sibling of patients 2 and 3, carrying no *ACP5* mutations.

Supplementary Table 1. Characteristics of patients and their clinical disease.

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Sex	Female	Female	Male	Male	Female	Male	Male	Female	Female	Female
Country of origin†	France	Austria	Austria	Turkey	Turkey	Pakistan	India	Portugal	Mali	Egypt
Consanguinity (parental relationship)	No	No	No	Yes (first cousins)	Yes (first cousins)	Yes (first cousins)	Yes (uncle-niece)	Yes (first cousins)	Unknown	Yes (first cousins)
Relationship to other patients	None	Twin sister of 3	Twin brother of 2	Brother of 5	Sister of 4	None	None	None	None	None
Birth weight in kg (gestation in weeks)(centile)	2.9 (40) (2^{nd}-25^{th})	2.58 (39) (9^{th})	2.35 (39) (2^{nd})	2.75 (39) (9^{th}-25^{th})	2.66 (40) (2^{nd}-9^{th})	2.87 (39) (25^{th})	2.8 (40) (9^{th})	Unknown	2.2 (40) (0.4^{th})	Unknown
Age at clinical presentation in months (mo)/years (yr)	36 mo	<12 mo	40 mo	22 mo	14 yr	8 mo	2 yr	3 yr	6 yr	4 yr
Features at initial presentation	Seizures	Delayed motor development	Thrombocyto-penia	Spasticity, vasculitic skin rash	Short stature	Short stature	Recurrent infections	Recurrent infections	Nephropathy	Leg pain
Current age in years	27	7	7	11	14	11	8	28	16	11
Skeletal abnormalities										
Last recorded height (standard deviations below the mean)	4	3	1	3	3	5	3	2	6.5	3
Metaphyseal dysplasia	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Platyspondyly	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Nervous system involvement										
Spasticity	No	Yes	No	Yes	No	Yes	No	No	No	Yes
Mild cognitive delay	Yes	Yes	No	No	No	No	No	No	No	No
Intracranial calcification	Yes	No	Not assessed	Yes	Not assessed	Yes	Yes	Not assessed	No	Not assessed
Neuropathy	Yes	No	No	No	No	No	No	No	No	No
Autoimmunity										
Raynaud's phenomenon (RP) or vasculitis	Yes (RP)	No	No	Yes (vasculitis)	Yes (RP)	No	No	No	No	No
Antinuclear antibodies (titer)	Yes (1:1280)	Yes (1:640)	No	Yes (1:640)	No	Yes (>1:320)	Yes (strongly positive on immunoblot)	Yes (1:1280)	Yes (1:1600)	Yes (1:640)
Anti-dsDNA antibodies (titer)	No	Yes (1:320)	No	Yes (100 Farr IU/ml)	No	Yes (1:1280)	Yes (strongly positive on immunoblot)	Yes (>500 Farr IU/ml)	Yes 121 (n<100) ELISA	Yes 33 (n<20) ELISA
Thrombocytopenia	No	Yes	Yes (requiring steroid therapy)	No	No	No	Yes	Yes (requiring splenectomy)	Yes	No
Autoimmune hemolytic anemia	No	No	No	No	No	Yes (requiring therapy with rituximab)	No	No	Yes	No

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Hypocomplementemia (C3 and C4)	No	Yes	No	Yes	Not assessed	No	Not assessed	Not known	Yes	No
Renal involvement	No	Yes (proteinuria; class IV lupus nephritis on biopsy)	No	No	No	No	Yes (proteinuria; class III lupus nephritis on biopsy)	No	Yes (class V lupus nephritis on biopsy)	Yes (microglobulin-emia)
Non-erosive arthropathy (>2 joints)	No	No	No	No	No	No	No	Yes	No	No
Fulfils American College of Rheumatology criteria for diagnosis of lupus	No	Yes	No	No	No	No	Yes	Yes	Yes	No
Treated hypothyroidism	Yes	Yes	No	No	No	No	No	Yes	No	No
Sjögren's syndrome	Yes (chronic sialadenitis with polyclonal B cell and lymphocytic infiltrates – grade IV Chisholm)	No	No	No	No	No	No	No	No	No

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Myositis	Yes (necrosis and inflammatory infiltrates on muscle biopsy and elevated muscle enzymes 10 x normal)	No	No	No	No	No	No	No	No	No
History of recurrent infections	No	No	No	No	No	No	Yes (three episodes of lobar pneumonia; cutaneous herpes simplex, tuberculosis)	Yes (two episodes of lobar pneumonia in childhood)	No	No

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Other	Hypertension; intestinal tract dysmotility; intracranial aneurysm; acute pancreatitis	Libman-Sacks endocarditis; possible cerebral infact	None	None	None	Persistent hypogamma-globulinemia following rituximab	None	Vitiligo	Myelitis. Anticardiolipin syndrome	Rheumatic fever with hypogamma-globulinemia; IGF1 + 2.5SD; BMD + 1.5SD
Described in previous publication (reference number)	Yes (1)	No	No	Yes (1)	No	Yes (2)	Yes (3,4)	Yes (5)	No	No

†As reported by the parents of the patients

Supplementary Table 2. Interferon neutralization assay.

	IFNa2 (Introna) 20iu/ml	IFN beta (Biogen) 200iu/ml	IFN patient 6 6iu/ml	IFN patient 1 16iu/ml	IFN patient 2 12iu/ml
Serum antiIFNa2	>160**	<10	>160	>160	>160
Serum anti beta	<10	>160	<10	<10	10
Serum anti alpha/beta	>160	>160	>160	>160	>160

**Titer as the reciprocal of the dilution which completely inhibits the IFN activity

A neutralization assay was performed on serum from patients 1, 2 and 6, together with an interferon alpha and beta reference. Samples were incubated with serial fold dilutions of anti-interferon alpha, beta and alpha/beta serum. This was then analysed in a standard cytopathic reduction assay and a neutralization titre was determined as a reciprocal of the antibody dilution that suppressed the interferon activity. [6]

Supplementary Table 3. Genes significantly up-regulated fourfold or more in a microarray analysis of TRAP deficient patients versus controls.

Gene name	Gene symbol	Reference sequence	Fold change (patients vs. controls)	*p*-value (patients vs. controls)	*q*-value (patients vs. controls)	References*
Interferon alpha-inducible protein 27	IFI27	NM_001130080	42.3	0.000136	0.115	7,8,9
Interferon-induced protein 44-like	IFI44L	NM_006820	28.1	0.000110	0.100	8,10
Radical S-adenosyl methionine domain containing 2; Viperin	RSAD2	NM_080657	16.7	0.000357	0.169	7,8,10,11
2'-,5'-oligoadenylate synthetase 3, 100kDa	OAS3	NM_006187	15.3	0.000018	0.057	8,9
2'-,5'-oligoadenylate synthetase 1, 40/46kDa	OAS1	NM_016816	12.0	0.000010	0.040	8,9,10
Sialic acid binding Ig-like lectin 1	SIGLEC1	NM_023068	10.3	0.000001	0.012	12
Viral DNA polymerase-transactivated protein 6	LOC26010	NM_001100422	9.4	0.000028	0.074	
Interferon-induced protein with tetratricopeptide repeats 3	IFIT3	NM_001031683	8.8	0.000190	0.123	13,14

Gene name	Gene symbol	Reference sequence	Fold change (patients vs. controls)	*p*-value (patients vs. controls)	*q*-value (patients vs. controls)	References*
Ubiquitin specific peptidase 18	USP18	NM_017414	8.7	0.000009	0.040	15
2'-,5'-oligoadenylate synthetase 2, 69/71kDa	OAS2	NM_002535	8.2	0.000099	0.099	8,9,10
Myxovirus (influenza virus) resistance 1, interferon-inducible protein	Mx1	NM_001144925	7.5	0.000300	0.146	8,9,10,11
Serpin peptidase inhibitor, clade G	SERPING1	NM_000062	7.4	0.000087	0.099	10
Cytidine monophosphate (UMP-CMP) kinase 2	CMPK2	NM_207315	7.0	0.000087	0.099	
Chemokine (C-C motif) ligand 2	CCL2	NM_002982	5.7	0.000389	0.179	9
Interferon-induced protein 35	IFI35	NM_005533	5.4	0.000076	0.099	9
Lymphocyte antigen 6 complex, locus E	Ly6E	NM_002346	5.2	0.000190	0.123	8
2'-,5'-oligoadenylate synthetase-like	OASL	NM_003733	5.0	0.000092	0.099	16
Ras and Rab interactor 2	RIN2	NM_018993	4.3	0.000047	0.094	

*References provided are to studies demonstrating stimulation by interferon and/or up-regulation in autoimmune phenotypes.

Supplementary Table 4. Assessment of circulating inducers of type I interferon activity in TRAP deficient patient sera compared to patients with idiopathic lupus.[17]

Patient	Serum interferon alpha titer (IU/ml)	Innoculum (ul) to PBMC	Interferon alpha (IU/ml) produced in cell culture
Lupus 1	37	50	60
Lupus 2	35	50	250
Lupus 3	75	25	16
Patient 1	25	50	<2
Patient 2	18	50	<2
Patient 6	50	50	<2

25 / 50 ul aliquots of lupus / SPENCD patient serum were incubated with 475 / 450μl of freshly isolated peripheral blood mononuclear cells (PBMC)($1.5x10^6$/ml) at 37° Centigrade for 18 hours. Supernatants were then removed and type I interferon activity measured as described in the Methods section.

Supplementary Table 5. Interferon gamma levels in TRAP deficient patients.

Patient	Interferon gamma level (pg/ml)
1	N.D.
2	N.D.
3	N.D.
4	N.D.
5	N.D.
6	N.D.

Interferon gamma levels were assayed in six patients using a quantitative sandwich enzyme immunoassay (Quantikine Human IFN-g Immunoassay, R&D Systems), with a sensitivity of 8 pg/ml. All results were below the limit of detectability (N.D.) of the assay.

Supplementary Table 6. Expression of *ACP5* in different human cell types.

Cell type	Fold relative to pDC
PBMC	1
Mo	7
M1	69
DC	81

PBMC = peripheral blood mononuclear cells; Mo = monocytes; M1 = macrophages; DC = dendritic cells; pDC = plasmacytoid dendritic cells.

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood of healthy volunteers by density-gradient centrifugation over Ficoll-Paque (Amersham Biosciences). Plasmacytoid dendritic cells (pDCs) were isolated to >97% purity using the EasySep® Human Plasmacytoid DC Enrichment Kit (StemCell Technologies). Monocytes were isolated from PBMC and 1 x 10^6 were seeded in a 24-well culture plate with 1ml RPMI/10% FBS. RNA was harvested after overnight culture. Macrophages were cultured by seeding 1 x 10^6 monocytes in a 24-well culture plate for 7 days with 100ng/ml GM-CSF. Dendritic cells were cultured by seeding 1 x 10^6 monocytes in a 24-well culture plate for 7 days with 100ng/ml GM-CSF + 20ng/ml IL-4. RNA was extracted using the Qiagen RNeasy kit. qPCR of human *ACP5* was performed using SensiMix SYBR Low-ROX kit master mix (Bioline, London, UK) and compared with *18S.*

Supplementary Table 7. Expression of *ACP5* and *Mx1* following stimulation of human pDCs.

Stimulus	Fold relative to unstimulated pDC	
	ACP5	*Mx1*
Interferon	0.6	63
CpG	1.4	2.6

Plasmacytoid dendritic cell (pDC) cultures (1-2 x 10^5/well in a 96-well round-bottom tissue culture plate, total volume 200µL) were incubated with Universal Type I Interferon (500U/mL, PBL Interferon Source) or Type A CpG ODN 2216 (200nM, InvivoGen) for 4 hours. qPCR of human *ACP5* was performed using SensiMix SYBR Low-ROX kit master mix (Bioline, London, UK) and compared with *18S.*

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