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Supplemental Information

Systemic Type I IFN Inflammation in Human ISG15 Deficiency Leads to Necrotizing Skin Lesions

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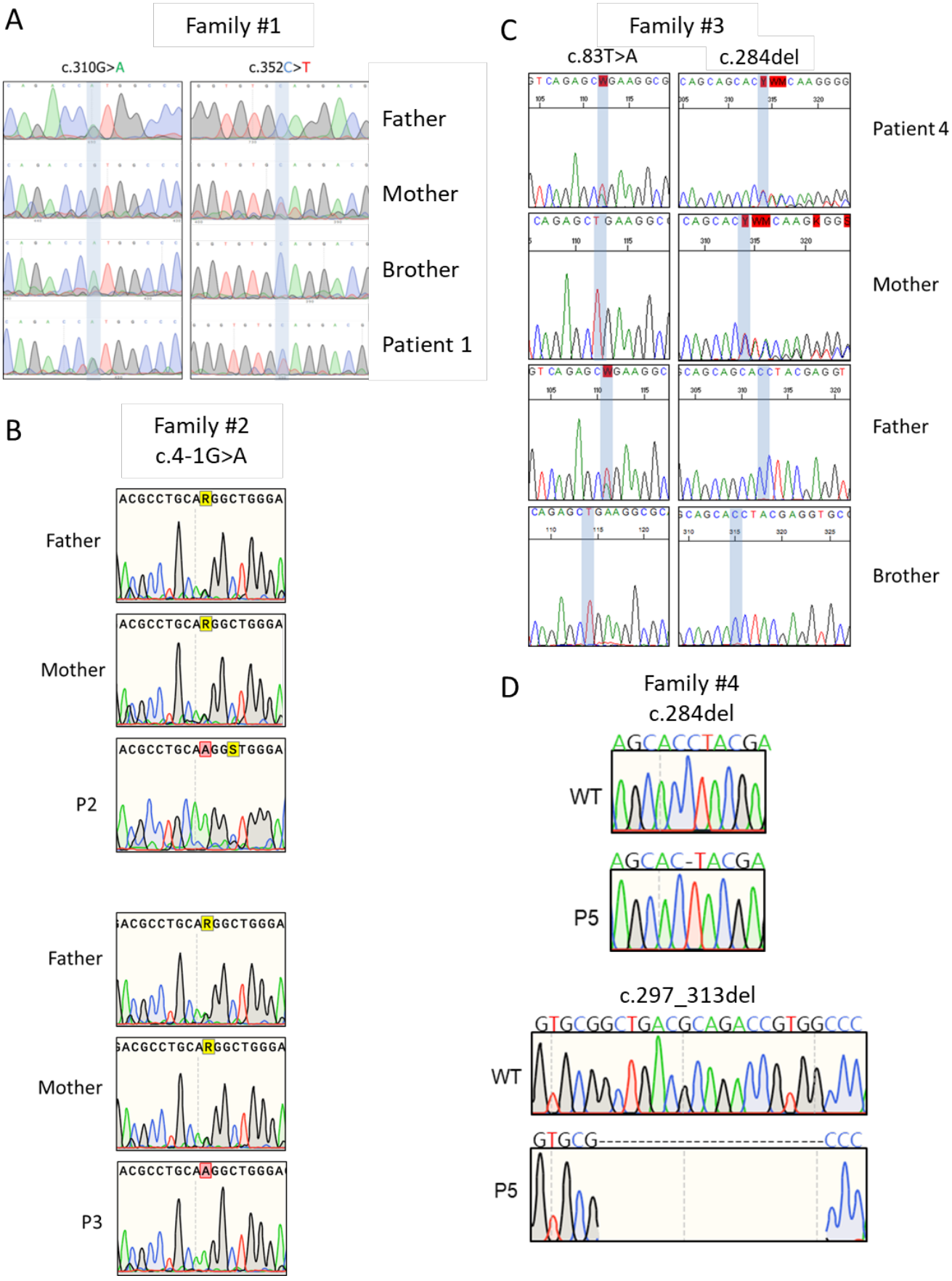

Figure S1, Related to figure 1. Electropherograms showing the *ISG15* variants identified in the different families and confirmed by Sanger sequencing: **A)** Family 1 (c.310G>A, c.352C>T), **B)** Family 2 (c.4-1G>A), **C)** Family 3 (c.83T>A and c.284del), **D)** Family 4 (c.284del and c.297_313del).

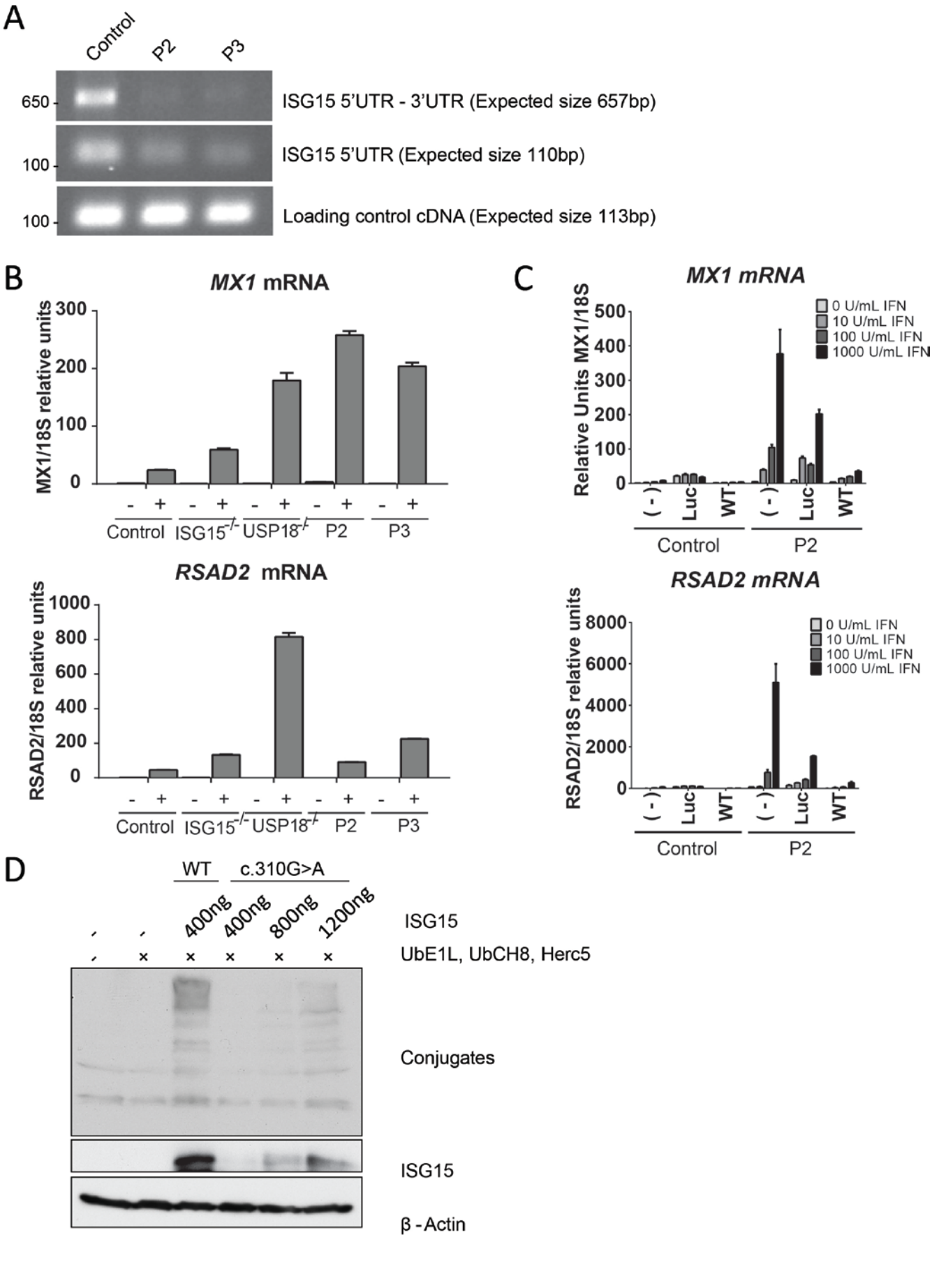
A
Control
P2
P3
650
ISG15 5'UTR - 3'UTR (Expected size 657bp)
100
ISG15 5'UTR (Expected size 110bp)
100
Loading control cDNA (Expected size 113bp)
B
MX1 mRNA
MX1/18S relative units
300
200
100
0
- + - + - + - + - +
Control ISG15-/- USP18-/- P2 P3
RSAD2 mRNA
RSAD2/18S relative units
1000
800
600
400
200
0
- + - + - + - + - +
Control ISG15-/- USP18-/- P2 P3
C
MX1 mRNA
Relative Units MX1/18S
500
400
300
200
100
0
0 U/mL IFN
10 U/mL IFN
100 U/mL IFN
1000 U/mL IFN
(-) Luc WT (-) Luc WT
Control P2
RSAD2 mRNA
RSAD2/18S relative units
8000
6000
4000
2000
0
0 U/mL IFN
10 U/mL IFN
100 U/mL IFN
1000 U/mL IFN
(-) Luc WT (-) Luc WT
Control P2
D
WT
c.310G>A
400ng 400ng 800ng 1200ng
ISG15
UbE1L, UbCH8, Herc5
Conjugates
ISG15
β -Actin

Figure S2, Related to figure 2. A) Gel electrophoresis of RT-PCR amplicons of *ISG15* in a control, P2 and P3, obtained with primers for: *ISG15* cDNA (from 5'UTR to 3'UTR; expected size = 657 bp), *ISG15* exon 1 (5'UTR; expected size = 110 bp) and a loading control (expected size 113 bp). **B)** hTert-immortalized fibroblasts from a control or P2 were transduced with luciferase or WT ISG15 and sorted. These fibroblasts were treated with the indicated doses of IFN-α2b for 12 h, washed with PBS, and left to rest for 36 h, after which, relative mRNA levels were determined for the genes indicated, in three experiments each, with technical triplicates; data from a representative experiment are shown. Bars represent the mean ± standard deviation (SD). **C)** HEK293T cells were cotransfected with UbE1L, UbCH8 and HerC5 in combination with WT ISG15 or various amounts of ISG15 c.310G>A. Cell lysates were analyzed by western blotting for the indicated antibodies; data from a representative experiment are shown.

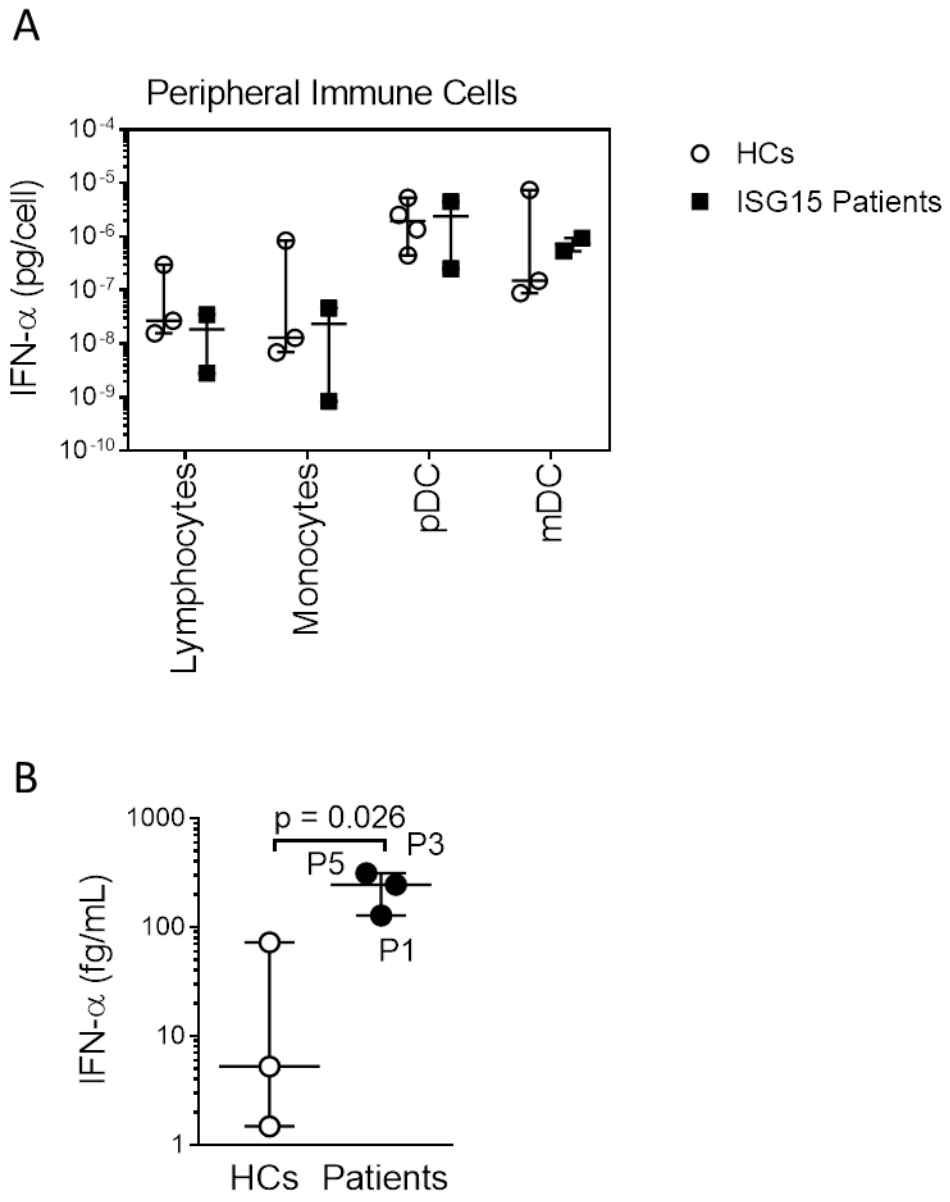

Figure S3, Related to figure 3. A) Detection of cellular interferon production by SiMoA digital ELISA on lysates of the indicated immune cell types. PBMCs from three healthy controls and two samples from P1 were sorted by FACS into lymphocytes ($CD3^+$, $CD19^+$ or $CD56^+$), monocytes ($CD3^-CD19^-CD56^-HLADR^+CD14^+$), myeloid DCs ($CD3^-CD19^-CD56^-HLADR^+CD14^-CD123^-$) or plasmacytoid DCs ($CD3^-CD19^-CD56^-HLADR^-CD14^-CD123^+$) and lysed for quantification. **B)** Single molecular array (SiMoA) digital ELISA quantifying IFNα from plasma of four healthy controls and three patients.

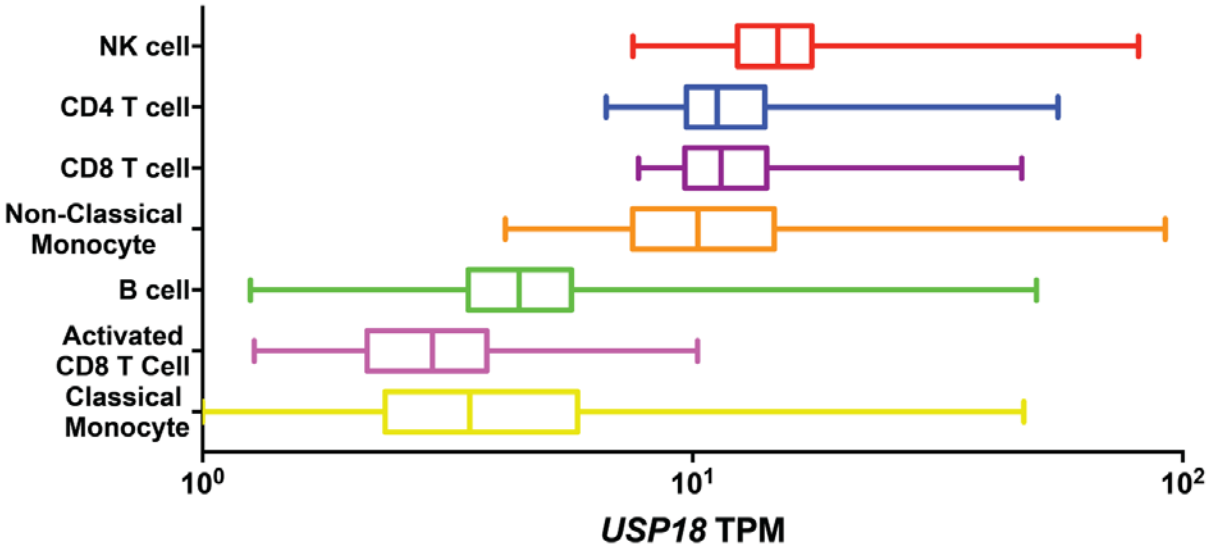

Figure S4, Related to figure 4. USP18 RNA levels in the various immune cell subsets. Mean expression levels of USP18 mRNA in transcripts per million (TPM) within a combined publicly available dataset from The Database of Immune Cell Expression. Boxes indicate interquartile ranges and whiskers indicate maximum and minimum expression across studies.

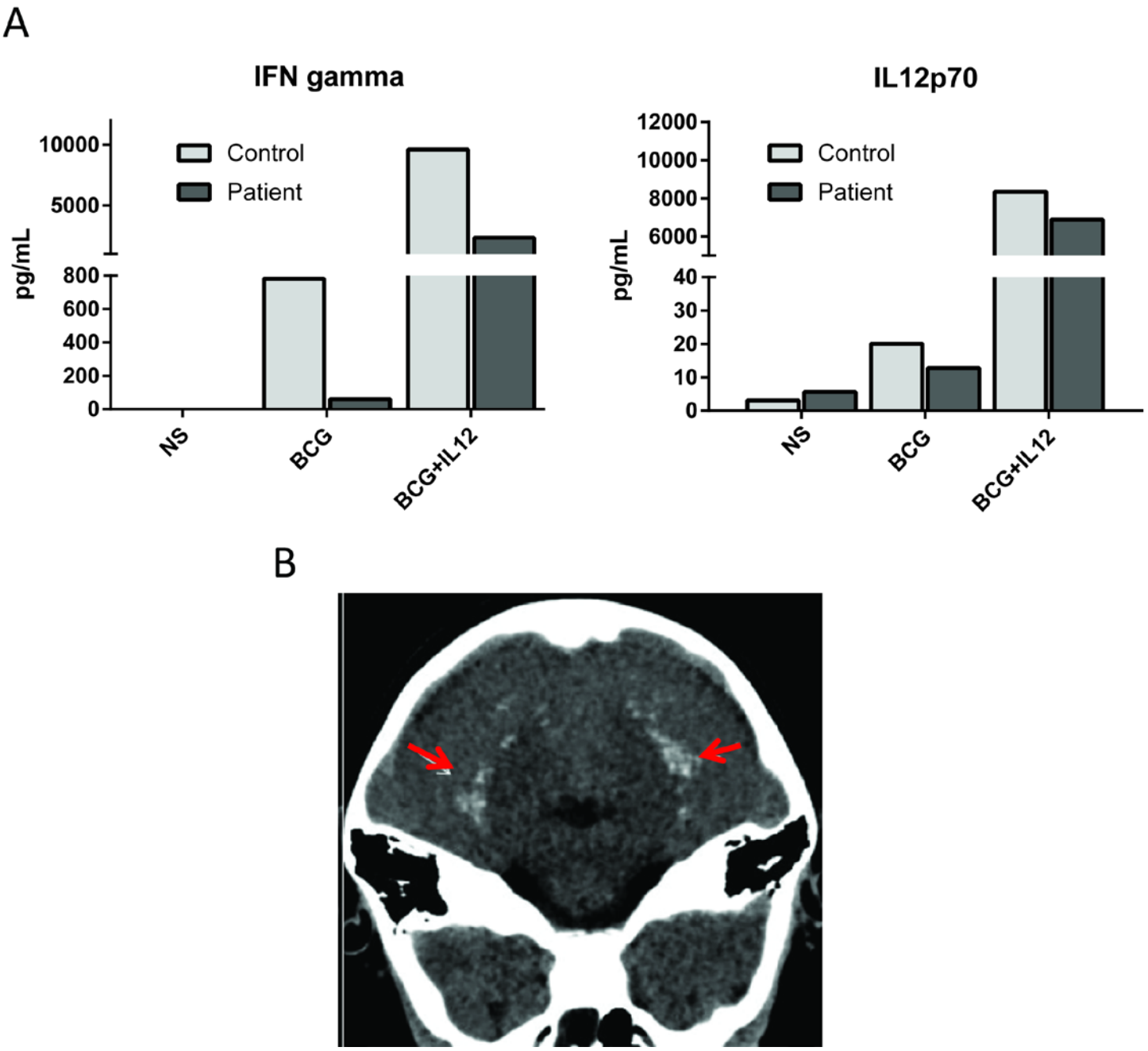

Figure S5, Related to figure 1. A) Cytokine production in the supernatants of whole-blood cells from a control and P1 (patient) left unstimulated or stimulated with BCG alone or BCG plus IL-12. The supernatants were analyzed with a panel of 8 cytokines and the Luminex platform. Levels of IFNγ (left panel) and IL12p70 (right panel) production are shown. **B)** CT scan from P4 showing multiple punctiform lesions of calcium density of cortical-subcortical disposition and following the path of the cerebellar dentate nucleus (arrows).

Previously Reported ISG15-/- Patients (Science 2012 and Nature 2015)											
Demographics				**Genetics**				**Clinical Presentation**			
Case #	**Patient**	**Country**	**Sex**	**Inheritance**	**Mutation**	**Consequence**	**Characterization**	**BCG Vaccination**	**Intracranial Calcificiations**	**Skin Lesion**	**Comments**
1	P1	Turkey	Female	Autosomal recessive	c.379G>T c. 379G>T	p.Glu127* p.Glu127*	Loss-of-expression	Yes	Yes	No/Yes	- Lymphadenopathy after BCG vaccination (MSMD) - Intracranial calcifications - Herpetic lesion on the scapular area
2	P2[1]	Iran	Male	Autosomal recessive	c.336_337insG c.336_337insG	p.Leu114fs p.Leu114fs	Loss-of-expression	Yes	Yes	No	- Ulcers and fistulizing lymphadenopathies after BCG vaccination (MSMD) - Intracranial calcificiations
3	P3[1]	Iran	Male	Autosomal recessive	c.336_337insG c.336_337insG	p.Leu114fs p.Leu114fs	Loss-of-expression	Yes	Yes	No	- Lympadenitis close to the site of BCG vaccination (MSMD) - Intracranial calcificiations
4	P4[2]	China	Female	Autosomal recessive	c.163C>T c.163C>T	p.Gln55* p.Gln55*	Loss-of-expression	No	Yes	No	- Basal ganglia calcifications - Epileptic seizures
5	P5[2]	China	Female	Autosomal recessive	c.163C>T c.163C>T	p.Gln55* p.Gln55*	Loss-of-expression	No	Yes	No	- Basal ganglia calcifications
6	P6[2]	China	Female	Autosomal recessive	c.163C>T c.163C>T	p.Gln55* p.Gln55*	Loss-of-expression	No	Yes	No	- Basal ganglia calcifications

[1, 2] indicates siblings

Table S1, Related to figure 1. Features of previously reported ISG15-deficient patients

New ISG15-/- Patients											
Demographics				**Genetics**				**Clinical Presentation**			
Case #	**Patient**	**Country**	**Sex**	**Inheritance**	**Mutation**	**Consequence**	**Characterization**	**BCG Vaccination**	**Intracranial Calcification**	**Skin Lesion**	**Comments**
7	P1	US	Female	Autosomal recessive	c.310G>A c.352C>T	p.Val104Met p.Gln118*	Loss-of-function Loss-of-expression	No	Yes	Yes	- Intracranial calcifications - Skin lesions on the inguinal area
8	P2	Saudi Arabia	Male	Autosomal recessive	c.4-1G>A c.4-1G>A	p.Gly2AlafsTer4[a] p.Gly2AlafsTer4[a]	Loss-of-expression	Yes	Yes	Yes	- Inguinal lymphadenopathies after BCG vaccination (MSMD) - Skin lesions on the neck and inguinal regions, and alopecia at the left occipitoparietal area of the scalp
9	P3	Saudi Arabia	Male	Autosomal recessive	c.4-1G>A c.4-1G>A	Splicing alterations	Loss-of-expression	Yes	Yes	Yes	- Skin lesions in the right axilla
10	P4	Spain	Female	Autosomal recessive	c. 284del c.83T>A	p.Thr95ThrfsTer5 p.Leu28Gln	Loss-of-expression Loss-of-function	No	Yes	Yes	- Cerebellar and basal ganglia calcifications - Skin ulcerations of the leg, groin, vulva and perianal regions
11	P5	Argentina	Female	Autosomal recessive	c. 284del c.297_313del	p.Thr95ThrfsTer5 p.Arg99Argfs*?[b]	Loss-of-expression Loss-of-expression	Yes	Yes	Yes	- BCG disease (MSMD) - Recurrent pneumonia - Basal ganglia calcifications - Skin ulcerations of the leg and vulvar regions.

[a] predominant splice variant

[b] indicates a frameshift with no predictable stop codon

Table S2, Related to figure 1. Features of new ISG15-deficient patients

	ISG5 MSC score	c.310G>A 1:1,014,290		c.352C>T 1:1,014,332		c.4-1G>A 1:1,013,983		c.83T>A 1:1,014,290		c.284del 1:1,014,264	
		Score	MSC-pred	Score	MSC-pred	Score	MSC-pred	Score	MSC-pred	Score	MSC-pred
CADD	24.1	26.9	High	35	High	12.7	Low	25	High	23.2	High
SIFT	0.157	0.00	High	NA		NA		0.00	High	NA	
PolyPhen	0.243	1.00	High	NA		NA		0.999	High	NA	

Table S3, Related to figure 1. *In silico* analysis of the effect of the ISG15 variants.

		CyTOF Imunophenotyping			
		Patient	Healthy controls (n=6)		
			Mean	Min	Max
Granulocytes	Neutrophils	43.95	48.54	25.77	72.19
	Eosinophils	1.65	3.8	1.14	7.25
	Basophils	0.17	0.19	0.07	0.42
CD4 T cells	Total	20.03	15.48	9.27	26.08
	Th1	1.6	3.24	1.18	8.41
	Th2	17.12	7.31	5.44	8.86
	Th17	0.86	1.98	1.17	2.46
	Naïve	15.17	4.88	3.53	6.11
	CM	2.62	4.16	2.62	6.37
	EM	0.9	4.82	1.69	11.4
	EMRA	0.03	0.05	0.01	0.09
CD8 T cells	Total	12.26	8.35	4.73	11.51
	Naïve	0.26	0.06	0.01	0.25
	EMRA	0.03	0.08	0.01	0.33
	EM	1.52	6.12	2.14	9.49
	CM	10.45	2.09	0.96	3.22
NKT		0.28	0.77	0.21	1.76
NK cells	Total	1.03	5.61	0.92	13.27
	CD56high CD16-	0.16	0.42	0.06	1.23
	CD56low CD16-	0.13	2.81	0.37	7.04
	CD56lowCD16+	0.64	1.35	0.08	2.41
B cells		9.19	2.62	1.13	5.12
Monocytes	Classical monocytes	0.84	1.44	0.66	4.2
	Non-classical monocytes	0.25	0.38	0.17	0.7
	Intermediate monocytes	0.12	0.12	0.04	0.21
Dendritic cells	pDCs	0.12	0.12	0.04	0.21
	mDCs (Total)	1.11	1.52	0.49	3.79
	mDC (CD1c+)	0.14	0.11	0.04	0.19
	mDC (CD1c-)	0.96	1.39	0.44	3.63

Table S4, Related to figure 3. Immunophenotyping of the patient