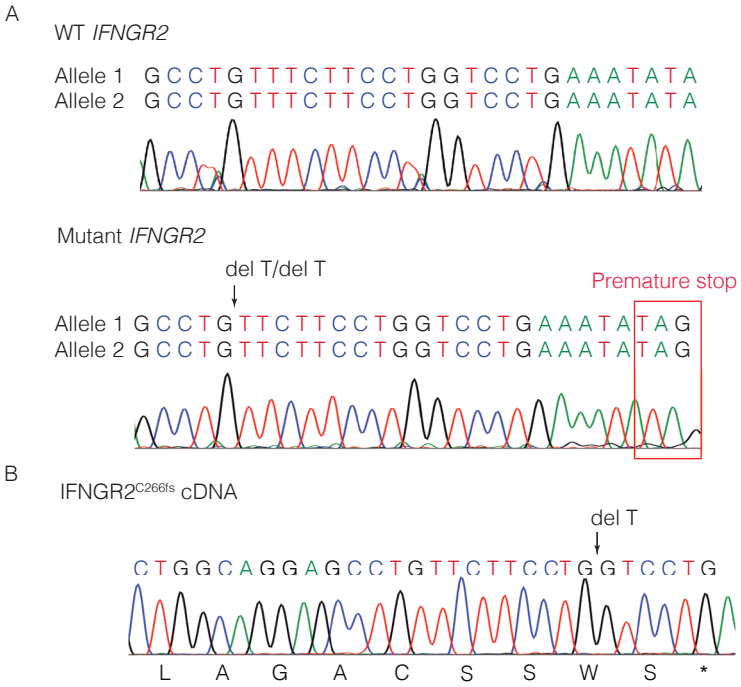
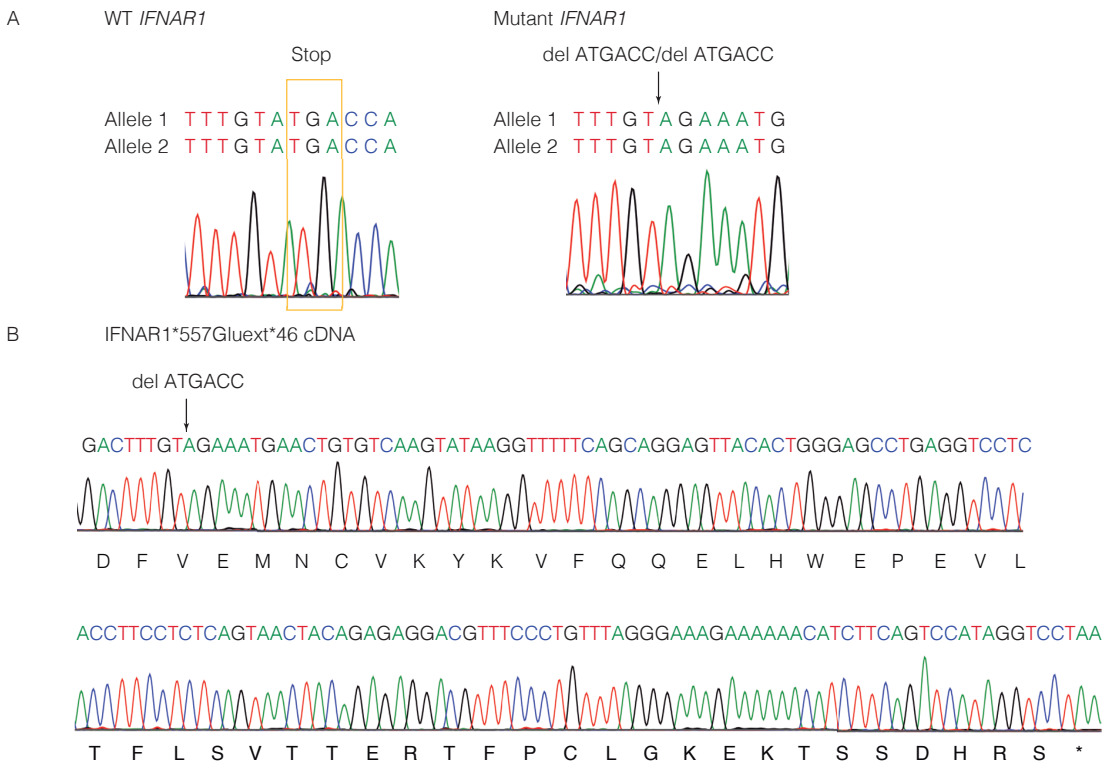
Supplemental material



Supplementary Figure 1. Sanger sequencing of IFNGR2 using genomic DNA from the patient's blood and cDNA from the patient's fibroblasts. A. Sanger sequencing of gDNA demonstrates the homozygous *IFNGR2* mutation (798delT) in the patient, which is absent in the control. **B**. Sanger sequencing of cDNA from the proband's fibroblasts demonstrates the premature truncation at residue 270 of the IFNGR2^{C266fs}.



Supplementary Figure 2. Sanger sequencing of IFNAR1 using genomic DNA from the patient's blood and cDNA from the patient's fibroblasts. A. Sanger sequencing chromatograms of gDNA demonstrates the homozygous *IFNAR1* mutation (1671_1821del) in the patient, which is absent in the control. **B.** Sanger sequencing of cDNA from the proband's fibroblasts demonstrates the addition of a novel 46 amino acid sequence to the C-terminus of IFNAR1^{*557Gluext*46}. **C.** Immunoblot of HEK293T cells transfected with either wild-type or mutant IFNGR2, and treated with cyclohexamide (CHX) ± MG132, as indicated. The data shown is representative of three independent experiments.

Supplementary Table 1. Mutations which are homozygous in the patient and not found in the dnSNP, 1000 Genomes, or ExAC databases.

Chromosome	Start	Reference	Patient	Gene
chr1	67242069	-	C	*TCTEX1D1*
chr1	85930490	A	G	*DDAH1*
chr7	76144774	A	T	*UPK3B*
chr11	4592708	-	AG	*C11orf40*
chr12	974308	-	C	*WNK1*
chr12	4736727	T	G	*AKAP3*
chr13	49796269	TC	-	*MLNR*
chr21	34143903	T	G	*GCFC1*
chr21	34727852	ATGACC	-	*IFNAR1*
chr21	34805097	T	-	*IFNGR2*
chr22	38485609	C	T	*BAIAP2L2*
chrX	54785283	A	T	*ITIH6*
chrX	105279192	AA	-	*SERPINA7*

Methods

Genetic analysis

WES was performed on genomic DNA from the patient using the Illumina HiSeq-2000 (Illumina Inc., San Diego, CA), using Agilent SureSelect for library preparation. The average coverage of the exome was 150x. The Burrows-Wheeler Aligner (BWA) was used to map reads to the human reference genome assemby GRCh37[41]. Variants were called using the Genome Analysis Toolkit (GATK)[42], Sam Tools[43, 44] and Picard tools (http://picard.sourceforge.net). The raw reads have been deposited in the Sequence Read Archive, under Biosample accession number PRJNA397405 (SRP114945).

Sanger sequencing

Sanger sequencing was used to validate the mutations in the *IFNGR2* and *IFNAR1* genes identified on the proband by WES and to identify the carrier status of the parents. Two pairs of gene specific nested primers were used to cover the mutation site in each gene: IFNGR2 (F1: 5'-GTGAGAAGAGTGCTGAACTG-3'; F2: 5'-GTGCGTAGGAAGATCATTCT-3'; R1: 5'-ACCATTAAGATGTCTGCGTG-3'; R2: 5'- AGAGATTGTACCATGACACT-3'), IFNAR1 (F1: 5'-

TAGTATTTCTCTGAACAGCCAT-3'; F2: 5'-TCAACTTCTGAGGAACAAATCG-3'; R1: 5'-TGACTCATCTCATCCAATGC-3'; R2: 5'-GACCTATGATCTGAAGATGT-3').

cDNA sequencing

RNA from skin derived fibroblasts was extracted using the RNeasy Mini Kit (Qiagen) and was reverse-transcribed with the OneStep RT-PCR kit (Qiagen) as per kit protocol. cDNA was sequenced with nested IFNGR2^{C266fs} (F1: 5'-CTTTGACATCGCTGATACCTC-3'; F2: 5'-CTCCAGAGTGTACTGTTTACA-3'; R1: 5'-AAGCGTTTGGAGAACATCTTCT-3'; R2: 5'-GAACATCTTCTTGCTCCTTTT-3') or IFNAR1^{*557next*46} specific primers (F1: 5'-TTCTCTGAACAGCCATTGAAGAA-3'; F2: 5'-GCTACAGTAGAAGAAACTAAT-3'; R1: 5'-TTAGGACCTATGATCTGAAGATGT-3'; R2: 5'-TCTGACTCATCTCATCCAATGC-3').

Cell-culture and stimulation conditions

Fibroblasts were maintained in DMEM supplemented with 10% fetal bovine serum, 50,000 IU penicillin, 50,000µg streptomycin, 10µM HEPES, and 2mM Glutamine. PBMCs were isolated using Ficoll, and were cultured in RPMI medium supplemented with 10% fetal bovine serum, 50,000IU penicillin, 50,000µg streptomycin, 10µM HEPES, and 2mM Glutamine overnight. Cells were stimulated with IFN-α or IFN-γ (R&D systems) 1000 IU/mL for the described time points.

Immunoblotting

Cultured skin fibroblasts were homogenized in PBS that contains 30mM Tris-HCl pH 7.5, 120mM NaCl, 2mM KCl, 1% Triton X-100 and 2mM EDTA supplemented with protease and phosphatase inhibitors (Complete and PhoStop, Roche). Proteins were separated by electrophoresis on 4-15% precast polyacrylamide gels (Bio-Rad) and were transferred to 0.45µm nitrocellulose membrane (Bio-Rad). Membranes were blocked in a 1x solution Tris-Buffered Saline/Tween 20 (TBST) with 5% (w/v) nonfat dry milk for one hour at room temperature and then incubated overnight at 4°C with the specified primary antibody. Primary antibodies used as as follows: pSTAT2-Tyr690 (Cell signaling, 4441), pSTAT1-Tyr701 (Cell signaling, 9167), IFNAR1 (Bethyl, A304-289A), CMV IE1 and IE2 (Millipore, mAb 810R), β-actin (Abcam, ab3280), STAT1 (Cell signaling, 14995), STAT2

(Cell signaling, #4594), GAPDH (Abcam, ab8245). Antigen-antibody complexes were visualized with peroxidase-conjugated secondary antibodies (GE Healthcare) and ECL Western blotting substrate (Pierce). Densitometry of immunoblots was done using the ImageJ analyzer software (1.48v).

Subcellular fractionation

To assess pSTAT1 nuclear translocation after 8 or 24h of stimulation with IFN-α, cells were subjected to subcellular fractionation using a Nuclear Fractionation Kit (Active motif) as per kit protocol. pSTAT1 nuclear translocation was determined by immunoblotting and quantified as previously described. As loading controls for each subcellular fraction, membranes were probed with a polyclonal antibody against Rab5 (Cell signaling, 2143) or PARP (Cell signaling, 9532) for the cytoplasmic and nuclear fraction, respectively.

Gene expression analysis

After 0, 8, 16 or 24h of stimulation with human IFN-α (1000 IU/mL, R&D Systems), RNA was extracted from skin-derived fibroblasts using the RNeasy Mini Kit (Qiagen) and was reverse-transcribed with the iScript cDNA synthesis kit (BioRad). Expression of *IFNGR2* (Hs00194264_m1), the ISGs *IFIT1* (Hs00356631_g1*), IFIT2 (*Hs01922738_s1) and *IRF7 (*Hs01014809_g1) and the housekeeping gene *GUSB* (Hs00939627_m1 Life Technologies, USA) was measured. Results were analyzed using the $2^{-\Delta\Delta CT}$ method.

Assessment of IFNGR2 turnover

The open reading frame cDNA clone for *INFGR2* (GeneCopoeia) was cloned into the pcDNA6/V5-His A expression vector (ThermoFisher) using the InFusion HD Cloning Kit (Takara Bio USA). The mutant IFNGR2 vector was generated using the following primers for cloning: Forward 5'-GTGGCGGCCGCTCGAGACCATGCGACCGACGCTGC-3' and reverse 5'-ATAGGCTTACCTTCGAAGGACCAGGAAGAACAGGCTCCTGCCAGCAC-3'). HEK293T cells maintained in DMEM supplemented with 10% fetal bovine serum, 50,000 IU penicillin, 50,000µg streptomycin, 10µM HEPES, and 2mM Glutamine were transfected with the wild type or mutant

89 expression vectors using the TransIT-LT1 transfection reagent (Mirus Bio). Two days after
90 transfection, the cells were incubated for 4, 6, or 8 hours in 100μg/mL cyclohexamide
91 (Calbiochem) or 100μg/mL cyclohexamide and 5μM MG-132 (Calbiochem). At the end of the
92 incubations, the cells were harvested and lysed on ice using a solution of 25mM Tris-HCl pH 7.4,
93 150mM NaCl, 1mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, and 1x EDTA-free
94 Protease Inhibitor Cocktail (Roche). After electrophoresis using 4-15% precast polyacrylamide
95 gels (Bio-Rad), the blots were analyzed with an anti-IFNGR2 (AF773, R&D Systems) or a β-actin
96 (Sigma) antibody. Densitometry of immunoblots was done using the ImageJ analyzer software
97 (1.48v).

98 **Fluorescence-activated cell sorting (FACS) analysis**

99 Standard flow cytometric methods were used for staining of cell-surface proteins. Anti-human
100 mAbs to the following molecules with the appropriate isotype-matched controls were used for
101 staining: CD14 (Biolegend, 325606), HLA-DR (Biolegend, 307617) and IFNGR2 (R&D systems,
102 FAB773). IFNGR2 studies were done on PBMCs. For HLA-DR upregulation and STAT1
103 phosphorylation studies, fibroblasts were stimulated with IFN-γ (1000 IU/mL) for the specified time
104 points prior to analysis with flow cytometry. For intracellular staining experiments, cells were
105 permeabilized and fixed using the BD Phosflow Lyse/Fix Buffer and Phosflow Perm Buffer III (BD
106 biosciences) and subsequently stained with an anti-human mAb to pSTAT1 (Cell Signaling,
107 #9174). All flow cytometry data was collected with an LSRFortessa (BD Biosciences, San Jose,
108 Calif) cell analyzer and analyzed with FlowJo software (Tree Star, Ashland, Ore).

109 **Viral infection**

110 The HCMV laboratory strain AD169 was incubated with patient and control fibroblasts at a
111 multiplicity of infection (MOI) of 1 for six hours. Where specified, fibroblasts were pre-treated for
112 17 hours with human IFN-α (1000 IU/mL, R&D Systems).

113 **Statistics.** All data is presented as mean ± S.E.M, and compared using the unpaired Student's *t*
114 test for single comparisons or two-way ANOVA for multiple comparisons, as specified in the figure

legends. Statistical analysis was performed using GraphPad Prism software (version 6.0). For quantification of the % pSTAT1 in the nuclear and cytoplasmic fraction of control and patient fibroblsts, the nuclear and cytoplasmic fractions were first normalized to either PARP or Rab5, respectively, and summed to obtain total pSTAT1. The ratio of pSTAT1 for each fraction relative to the total pSTAT1 content is shown.