Supplementary information

Supplement to:

Severe viral respiratory infections in children with *IFIH1* loss-of-function mutations

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Table S1. Summary metrics of short-read alignment. Related to experimental procedure. PF, pass filter; SD, standard deviation.

	PF unique reads (%)	PF unique reads aligned (%)	Off-bait (%)	Mean bait coverage (%)	At least 2X coverage (%)	At least 30X coverage (%)
Average	0.93	0.96	0.22	69.99	0.99	0.78
SD	0.07	0.04	0.03	15.29	0.01	0.08
Median	0.96	0.97	0.23	68.04	0.99	0.79

Table S2. Summary metrics for variant calling. Related to experimental procedure. Three different variant calling methods were used and the variants in the intersection used for downstream analysis.

Variant caller	GATK	Platypus	SAMtools	Intersection
Total variants (%novel)	287681 (16.74%)	271978 (17.72%)	317820 (18.90%)	242954 (14.14%)
SNV (%novel)	263507 (14.04%)	258548 (16.34%)	296161 (16.63%)	232591 (13.25%)
Indel (%novel)	24174 (46.23%)	13430 (44.34%)	21659 (49.89%)	10363 (34.12%)
FRAME-SHIFT	1644	1333	1415	951
STOP-GAINED	880	942	1082	793
STOP-LOST	89	85	99	76
START-LOST	126	128	144	112
SPLICE-SITE	377	353	446	297
MISSENSE	64057	66559	74945	59144
INFRAME	1854	1102	1811	867
SYNONYMOUS	55698	56964	62381	52261
UTR	23478	16320	19780	14434
LoF (%novel)	2635 (49.44%)	2419 (49.89%)	2710 (46.60%)	1878 (41.64%)
SNP-LoF (%novel)	1116 (30.73%)	1219 (34.12%)	1371 (32.31%)	1023 (30.00%)
Indel-LoF (%novel)	1519 (63.19%)	1200 (65.91%)	1339 (61.23%)	855 (55.55%)

Table S3. Homozygous loss-of-function variants observed in the study population.

Chr, chromosome; MAF, minor allele frequency; NA, not available.

Chr	Position	SNP ID	Gene	Predicted effect	MAF (%) gnomAD	MAF (%) this study
1	26301080	rs375730731	PAFAH2	STOP-GAINED	<0.01	2.08
2	163124596	rs35732034	IFIH1	SPLICE-SITE	0.64	2.08
11	18497187	rs145833201	LDHAL6A	SPLICE-SITE	0.09	0.83
17	79974815	rs143008575	ASPSCR1	SPLICE-SITE	0.09	0.83
X	7889837	NA	PNPLA4	STOP-GAINED	<0.01	0.83
X	127185638	NA	ACTRT1	FRAME-SHIFT	0.18	2.08

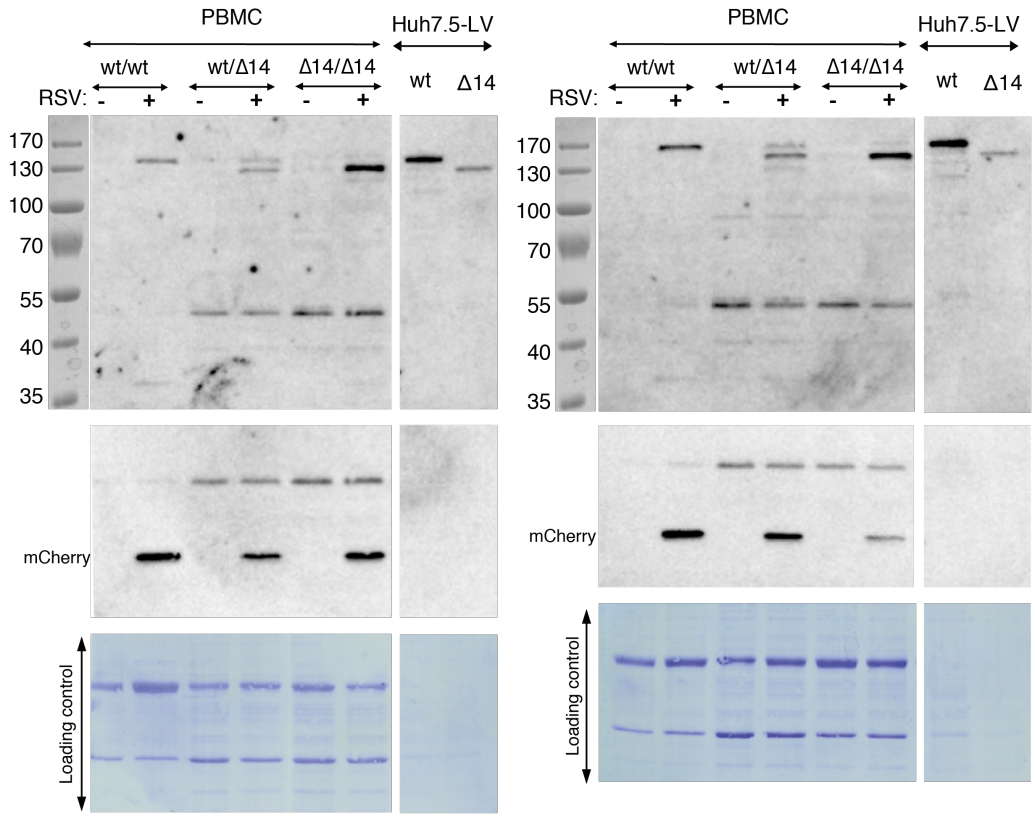

Figure S1. Expression of IFIH1-wt and IFIH1-Δ14 in patients' peripheral blood mononuclear cells. Related to Figure 1.

Expression of IFIH1-wt and IFIH1-Δ14 in peripheral blood mononuclear cells (PBMCs) from an individual homozygous for rs35732034 major allele, from the patient homozygous for rs35732034, and from her heterozygous mother (left) and her heterozygous father (right), as shown by western blot gel with or without RSV infection *in vitro*. Protein expression of both IFIH-wt and IFIH1-Δ14 is boosted by RSV infection, but the increase is particularly dramatic for IFIH1-Δ14. Huh7.5 cells transduced with a lentiviral vector expressing either IFIH1-wt or IFIH1- Δ14 were used as positive controls. Under each gel are shown the expression of mCherry (control for RSV infection) and the

coomassie blue staining (loading control). Molecular weight markers are shown on the left of each gel (kDa).

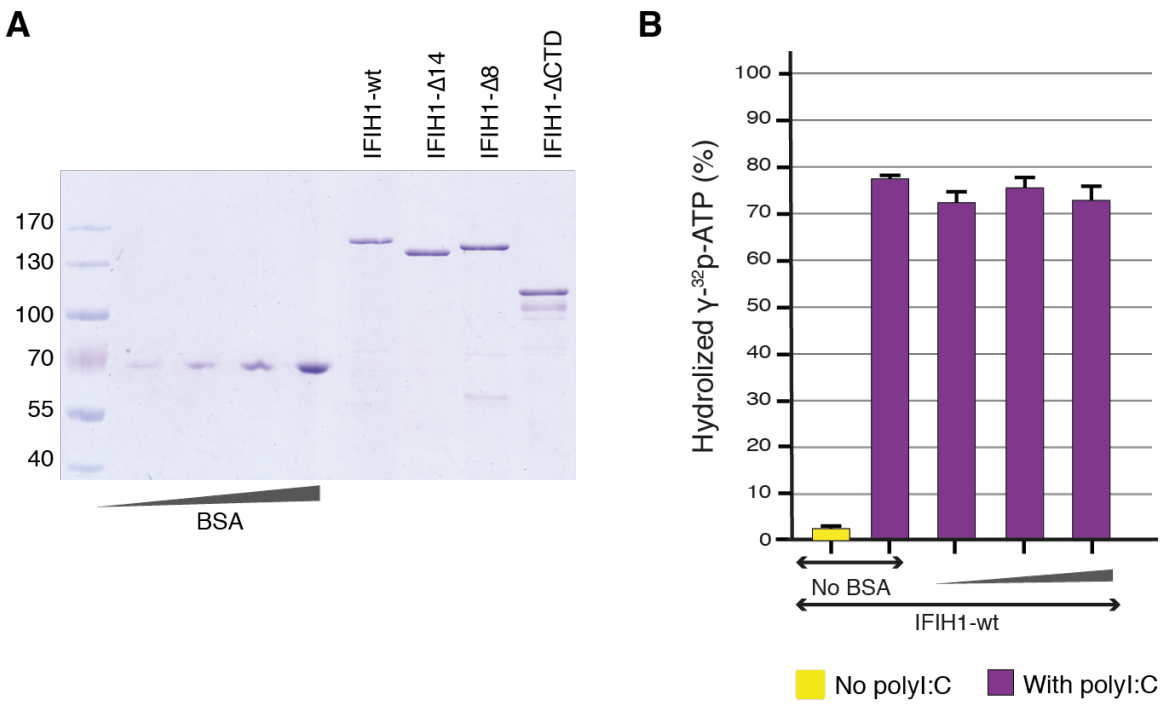

Figure S2. Purification of IFIH1 wild type and mutant proteins. Related to Figure 2. (A) Purified recombinant IFIH1-wt, IFIH1-Δ14, IFIH1-Δ8 and IFIH1-ΔCTD; BSA quantity; 0.1, 0.2, 0.4 and 0.8 µg; (B) BSA does not interfere with ATPase activity of wt protein (300ng wt protein, 150, 300ng or 450ng of BSA, 10ng polyI:C, n=2). Data are represented as mean + SD. BSA, Bovine serum albumin; wt, wild type; polyI:C, polyinosinic:polycytidylic acid.

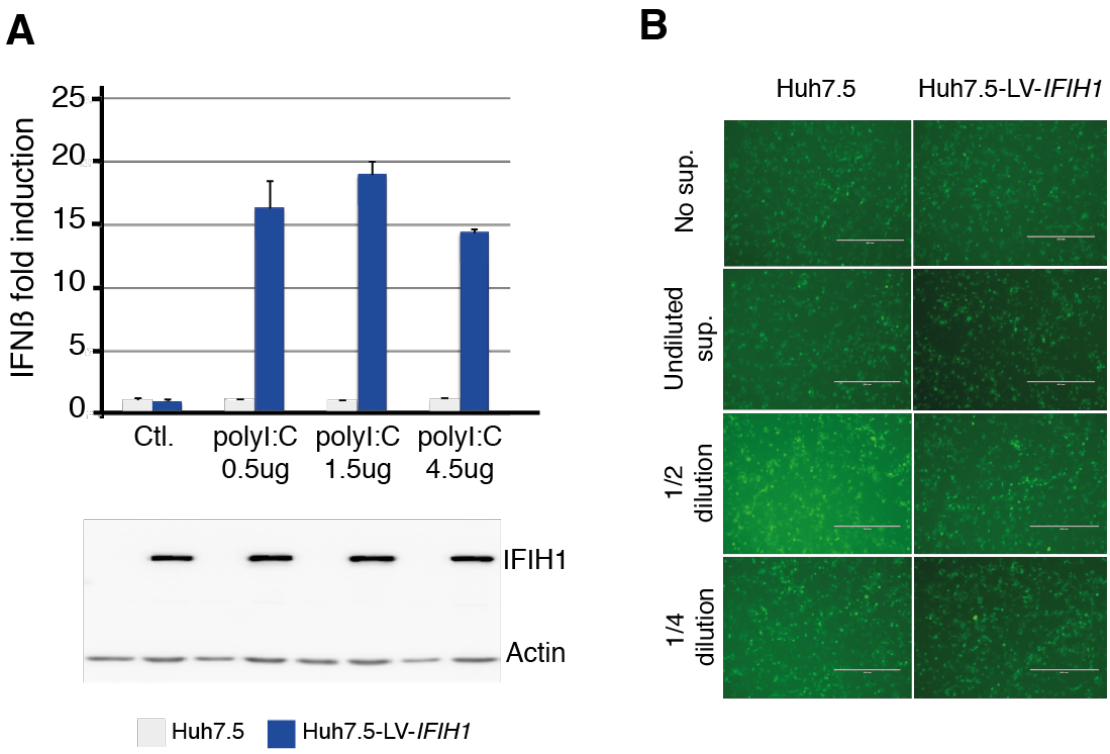

Figure S3. IFIH1 transduction induces IFNβ only in the presence of viral pathogen.

Related to Figure 3.

(A) Huh7.5 cells lack endogenous IFIH1 and do not show any IFNβ induction upon polyI:C stimulation (gray). The cells gain the ability to induce IFNβ after transduction of IFIH1-wt using a lentiviral vector, Huh7.5-LV-*IFIH1* (blue), (n=2); (B) The supernatants of native and IFIH1 transduced Huh7.5 cells were collected and placed on A549 cells for 14 hours; A549 cells were then infected by GFP-tagged vesicular stomatitis virus (VSV-GFP), a rhabdovirus highly sensitive to the antiviral state induced by type 1 interferon. No difference was observed between the two groups, or with the control group (A549 cells without added supernatant) regardless of the supernatant dilution, demonstrating that IFIH1 transduction of huh7.5 cells does not lead to IFNβ induction or to the

establishment of an anti-viral state in the absence of viral stimulation. Data are represented as mean + SD. sup., supernatant.

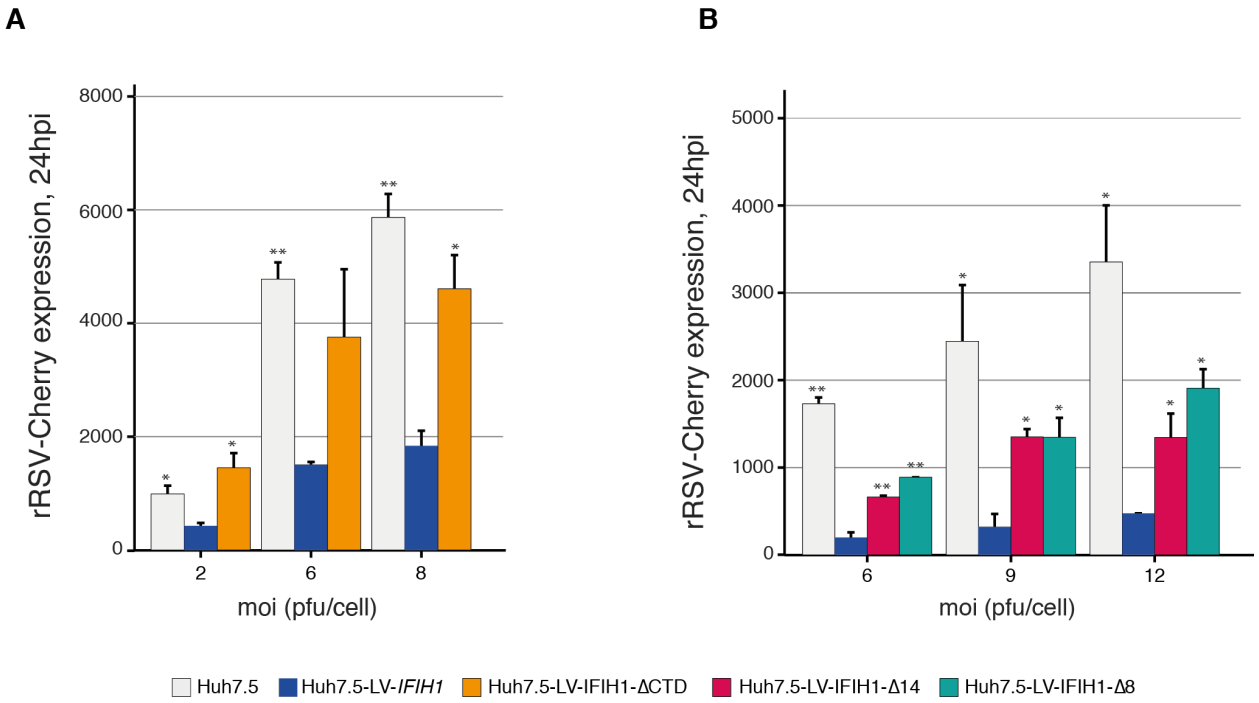

Figure S4. IFIH1 mutant proteins does not reduce RSV replication. Related to Figure 3.

FACS analyses of cherry expressing recombinant RSV in Huh7.5 cells and Huh7.5 cells transduced with a lentiviral vector expressing IFIH1 (Huh7.5-LV-*IFIH*), and compared to (A) Huh7.5-LV-*IFIH1*-ΔCTD transduced cells and (B) Huh7.5-LV-*IFIH1*-Δ14 and Huh7.5-LV-*IFIH1*-Δ8 transduced cells. IFIH1-wt significantly reduced RSV replication at 24 hpi, when compared to either wild type Huh7.5 cells (lacking IFIH1) or Huh7.5 cells transduced with the three mutant vectors, (n=2); *p<0.05, **p<0.01. Data are represented as mean + SD. moi, multiplicity of infection; pfu, plaque-forming unit; hpi, hours post infection.

Supplementary Materials and Methods

Subject recruitment and specimen collection

Between December 2010 and October 2013, we prospectively enrolled previously healthy children suffering from severe lower respiratory tract infection and requiring invasive or non-invasive respiratory support in five specialized Pediatric Intensive Care Units (PICU) from Australia and Switzerland. The study was approved by the respective institutional Human Research Ethics Committees. Written informed consent was obtained from parents or legal guardians.

Children less than 4 years of age that were admitted to PICU due to a severe respiratory infection of proven or presumed viral origin and required respiratory support were eligible. Exclusion criteria were the presence of any significant underlying disease or comorbidity, including prematurity, congenital cardiac disease, chronic lung disease, sickle cell disease, hepatic, renal, or neurologic chronic conditions, solid and hematological malignancies and known primary immunodeficiency. Respiratory support was defined as non-invasive ventilation including high-flow nasal cannulae (HFNC) and continuous or bilevel positive airway pressure (CPAP and BiPAP), or invasive ventilation including conventional and high frequency oscillation ventilation (HFOV).

The following demographic and clinical information was collected: age, gender, weight, ethnicity, type of ventilation, length of ventilation in days, clinical outcome, microbiological diagnostic procedures and results including rapid antigenic test for RSV and influenza, respiratory virus PCR panel, and viral cultures. For each study participant, we obtained a nasopharyngeal aspirate or endotracheal tube aspirate, 1ml EDTA blood in

vacutainer tubes and 2.5ml blood in PAXgene blood RNA tubes. Samples were immediately frozen at -70 degrees Celsius until shipment, and then analyzed in batch.

Screening of respiratory viruses

Viral RNA was extracted from 100ul of nasopharyngeal aspirate using the NucliSens Easymag© (bioMérieux). Respiratory viruses screening was performed using FTD Respiratory pathogens 21 assay (Fast-track Diagnostics) on a Viia7 instrument (Applied biosystems).

Exome sequencing and alignment

Genomic DNA was extracted from whole blood (QIAGEN, 51104). Exome sequencing libraries were prepared with 2 µg to 3 µg of genomic DNA using Agilent SureSelect reagents (Agilent Technologies, 5190-4627, 5190-4631, 5190-6208 and G9611A). Cluster generation was performed using the Illumina TruSeq PE Cluster Kit v3 reagents. The resulting libraries were sequenced as 100-nucleotide, paired-end reads on Illumina HiSeq 2000 or HiSeq 2500 using TruSeq SBS Kit v3 reagents. Sequencing was done at the Lausanne Genomic Technology Facility. Raw sequencing reads were processed using the Illumina Pipeline Software version 1.82. Purified filtered reads were aligned to human reference genome hg19 using Burrow-Wheeler Aligner version 0.6.2 (BWA) (1). PCR duplicates were removed using Picard (http://picard.sourceforge.net/).

Variant calling and annotation

We used Genome Analysis Toolkit (GATK) (2, 3) version 3.1-1, Platypus (4) version 0.7.9.1 and SAMtools (5) version 0.1.19 to call single nucleotide variants (SNVs) and small insertion and deletions (Indels) from duplicate-marked bam files. With GATK we used HaplotypeCaller for multi-sample variant calling. We followed GATK best practice

to call the variants (https://www.broadinstitute.org/gatk/guide/best-practices?bpm=DNAseq); variants that did not pass GATK filtering were discarded. With Platypus we used callVariants for multi-sample variant calling and variants that did not pass Platypus filtering criteria were discarded. With SAMtools we used mpileup default options for multi-sample variant calling followed by bcftools view to generate the vcf file, variants with quality score below 30 were discarded. Only variants that were present at the intersection of the three variant callers and had a missingness of <5% were kept for downstream analysis.

We used SnpEff (6) version 4.1B to predict the functional impact of variants, and notably to identify putative loss of function variants (LoFs). We defined the following categories of variants as LoFs: stop-gain SNVs, splice site disrupting SNVs, frame-shift indels in the first 95% of the coding region, or deletions removing either the first exon or more than 50% of the coding sequence. We further enriched for LoFs that are more likely to play a functional role by including only LoFs found in genes with a low or medium gene damage index and were affecting all coding transcripts of the gene (7).

RNA sequencing and alignment

Total RNA was extracted from 2.5 ul of whole blood collected in PAXgene tubes using PAXgene blood RNA kit (PreAnalytiX, 762174). Libraries were prepared using the Illumina TruSeq Stranded mRNA (Ribo-Zero Globin) reagents (Illumina, RS-122-2501) using 400ng of total RNA. Cluster generation was performed with the Illumina TruSeq PE Cluster Kit v3 reagents. The resulting stranded libraries were sequenced as 100-nucleotide, paired-end reads on the Illumina HiSeq 2000 using TruSeq SBS Kit v3 reagents. The raw sequencing reads were processed using the Illumina Pipeline Software

version 1.82. Purified filtered reads were aligned to the human reference genome hg19 using STAR (8) version 2.3.0e and the Gencode annotation (9) version 19.

Plasmids

pcDNA3.1(+) containing wild type *IFIH1* was constructed by PCR amplification on pEF-BOS-IFIH1 with sense primer that introduced BamHI site and flag sequence at the N-terminus and with the antisense primer that introduced XhoI site at the C-terminus of IFIH1. The PCR products were digested with BamHI and XhoI and then inserted into pcDNA3.1(+).

pcDNA3.1(+) containing deletion mutant *IFIH1* lacking exon 14 (IFIH1-Δ14) was constructed using a fusion PCR strategy. First, pEF-BOS-IFIH1 was amplified by PCR with sense primer that introduced BamHI site and HA sequence at the N-terminus of IFIH1 and with the antisense primer that inserted the beginning of exon 15 sequence at the end of the exon 13. Second, pEF-BOS-IFIH1 was amplified by PCR with sense primer that introduced the end of exon 13 sequence at the beginning of exon 15 and with the antisense primer that introduced XhoI site at the C-terminus of IFIH1. The resulting PCR products were mixed and amplified by PCR with sense primer that introduced BamHI site and HA sequence at the N-terminus and with the antisense primer that introduced XhoI site at the C-terminus of IFIH1. The PCR products were digested with BamHI and XhoI and then inserted into pcDNA3.1(+).

pcDNA3.1(+) containing deletion mutant *IFIH1* gene lacking exon 8 (IFIH1-Δ8), and pcDNA3.1(+) containing deletion mutant *IFIH1* with stop-gain mutation in exon 10 (IFIH1-ΔCTD), were ordered from life technologies plasmid service. The inserts of the

resulting pcDNA3.1(+) containing IFIH1-wt or mutant plasmids were confirmed by sequencing.

To test IFNβ induction, we used pβ-IFN-fl-lucter containing the firefly luciferase gene driven by the human IFNβ promoter as described previously (10) and pTK-rl-lucter containing the *Renilla* luciferase gene (PROMEGA) driven by the herpes simplex virus TK promoter. pEBS-tom encodes a red fluorescent protein.

Transfection and measure of interferon-β promoter activity

293T cells and Huh7.5 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% Pen/Strep. 100'000 cells were plated into 6-well plates and transfected 24 hrs later with 1.5 μg of pβ-IFN-fl-lucter, 0.5 μg of pTK-rl-lucter and 0.5 μg of pEBS-tom (used as a transfection control), using Gene Juice transfection reagent (NOVAGEN). Additionally, cells were transfected with 1 μg of IFIH1 encoding plasmids. 24 hrs later, Huh7.5 cells (but not 293T cells) were transfected with elicitor RNA using TransMessenger transfection reagent (QIAGEN) according to the manufacturer's instructions. 20 hrs later, cells were harvested and cell lysates were used to measure firefly and *Renilla* luciferase activity (dual-luciferase reporter assay system, Glomax 20/20 luminometer, PROMEGA).

Protein expression in the cell lysates was then checked by Western blotting, using the following primary antibodies: anti-RIG-I (1:1000) (Alexis ALX-210-932), anti-IFIH1 (1:1000) (Alexis ALX-210-935), anti-HA (1:2000) (Enzo Life Sciences ENZ-ABS-118-0500), anti-flag (1:1000) (Sigma F1804-1MG). Immunoblot analyses were developed with the following secondary antibodies: goat anti-mouse and anti-rabbit IgG horseradish peroxidase conjugated whole antibody (1:3000) (Bio-Rad). ImageJ version 1.44p

(http://imagej.nih.gov/ij/) was used for western blot quantifications.

Viruses

Respiratory syncytial virus expressing Green Fluorescent Protein (RSV-GFP, obtained from Mark Peeples (11)) or mCherry (rRSV-mCherry obtained from Jean-François Eléouët (12)) stocks were amplified in A549 (human alveolar adenocarcinoma cell line) cells. RSV-GFP stocks titers were determined using serial dilutions to infect A549 cells. GFP or mCherry expression was measured by flow cytometry on 20'000 cells (*i.e.* 10% of the harvested cells) using BD Accuri C6 Cytometer. Data were analyzed using CFlow Plus software (Accuri, version 1.0.264.15). Fluorescence pictures were acquired using Evos FL epifluorescence microscope. Recombinant vesicular stomatitis virus expressing Green Fluorescent Protein (rVSV-GFP obtained from Jacques Perrault (13)) stocks were amplified in 293T cells and stocks titers were determined using serial dilutions to infect 293T cells. GFP expression was measured by flow cytometry on 20'000 cells (*i.e.* 10% of the harvested cells) using BD Accuri C6 Cytometer. Data were analyzed using CFlow Plus software (Accuri, version 1.0.264.15). Fluorescence pictures were acquired using Evos FL epifluorescence microscope.

Measure of protein stability by pulse chase

Transfected 293T cells were incubated for 30 minutes at 37°C in methionine-free, cysteine-free and FCS-free DMEM. 100 µCi/ml of 35S-methionine + 35S-cysteine labelling mix (HARTMAN Analytic) was added and cells were incubated at 37°C for 30 minutes. The chase (0, 2, 4, and 8 hours) was performed at 37°C in DMEM supplemented with unlabelled methionine and cysteine (10mM). Cell lysates were loaded on 7.5% acrylamide gels, transferred to a PVDF membrane and exposed to autoradiography.

Results were revealed in a phosphorimager (Typhoon, GE Healthcare Life Sciences) and quantified with ImageQuantTL software (GE Healthcare Life Sciences).

Recombinant IFIH1 expression

IFIH1 inserts cloned into pcDNA3.1(+) were inserted into pET28-His10Smt3 backbone. pET28-His10Smt3-IFIH1 wt or ΔCTD plasmids were transformed into *E. coli* BL21. Cultures (500 ml) derived from single transformants were grown at 37°C in LB medium containing 50 µg/ml kanamycin to an A_{600} of 0.6. The cultures were adjusted to 0.2 mM IPTG and 2% ethanol and further incubated for 20 hours at 17°C. Cells were harvested by centrifugation and recombinant RIG-I protein was purified from bacteria as previously described (14). Protein concentration was determined using the Bio-Rad dye binding method with BSA as the standard.

Measure of IFIH1 ATPase activity

Increasing amounts of polyI:C were incubated with 200 nM of purified recombinant IFIH1, [(Isqb) γ-^{32}P] ATP (Hartmann Analytic) in a final volume of 15 µl (50 mM Tris acetate pH 6, 5 mM DTT, 1.5 mM $MgCl_2$) for 15 minutes at 37°C. Reactions were then stopped with 1 mM formic acid and 2.5 µl of each reaction were spotted onto TLC PEI Cellulose F plates (MERCK 1.05579.0001) and applied to a migration buffer (LiCl 0.5 M and formic acid 1 N) to separate released $^{32}PO_4$ and non hydrolyzed ATP. $^{32}PO_4$ release was measured in a phosphorimager (Typhoon, GE Healthcare Life Sciences) and quantified with ImageQuantTL software (GE Healthcare Life Sciences). ATPase data were processed as follow: for each sample, the ratio free $^{32}PO_4$/non hydrolyzed ATP was calculated (and reported to the final ATP concentration in the reaction); fold increases were obtained by normalizing the calculated ATPase activity to the ATPase activity of

the protein alone (control without RNA).

Cell culture and transduction with lentiviral vectors

The 293T cells lines were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. All recombinant lentiviruses were produced by transient transfection of 293T cells according to the following protocol. 5 x 10^6 293 cells were plated in 10 cm PD and cotransfected with 15 µg of a plasmid vector (pLV.CMV.IFIH1.IRES-GFP and pLV-U6-empty-PGK-GFP), 10 µg of psPAX2 and 5 µg of pMD2G-VSVG by calcium phosphate precipitation. After 8 hours, medium was changed and recombinant lentiviruses vectors were harvested 24 hours later. Huh7.5 cell lines, platted in 6-well plates, were transduced at an MOI of 2 with the recombinant lentiviruses. Two days after transduction, GFP-expressing cells were sorted by FACS.

Quantitative RT-PCR

Total RNA isolation was performed by using the NucleoSpin RNA kit (Macherey-Nagel) according to the manufacturer's instruction. 200 µg of total RNA from infected cells pellet was used for cDNA synthesis using the Omniscript RT Kit (QIAGEN). Quantitative RT-PCR was performed by using 2 µL of cDNA and 18 µL of Taqman Fast universal PCR master mix (Thermo Fisher Scientific) containing specific primers (20 µM) and probes (5 µM) for human rhinovirus (HRV). 7500 fast Real-Time PCR System (Thermo Fisher Scientific) was used to perform PCR reactions. The ΔΔCT method was used to quantify the mRNA expression levels of endogenous genes. The mRNA expression levels of endogenous genes were normalised to the housekeeping gene 18S rRNA. Viral RNA copy numbers were obtained through the generation of a standard curve obtained with serial dilutions of a plasmid containing HRV cDNA.

Statistical analysis

Independent two-tailed t-tests for all functional tests were performed using R (version 1.65).

Purification of peripheral blood mononuclear cells

Human PBMCs were extracted from heparinised blood using a standard density gradient centrifugation over Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden). Cell viability was enumerated by 0.4% Trypan blue (Gibco, Mulgrave, VIC) exclusion. 10^7 cells were stored until required in liquid nitrogen vapour phase in 1mL freezing media consisting of heat-inactivated FCS (Gibco, Mulgrave, VIC) with 10% DMSO (Sigma, D8418).

Supplementary References

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