Supplementary information, Data S1 Materials and Methods

Constructs, protein expression and purification The full length ISG58 gene was cloned into a pGEX6p-1 vector (Invitrogen) with an N-terminal GST-tag. The plasmid was transformed into *E. coli* strain BL21 <DE3> cells. Cells were cultured in LB medium containing 100 mg/L ampicillin at 37°C, and when the OD_{600} reached 0.6-0.8, they were induced at 16°C with 0.5 mM isopropyl-thio-D-glactosidase (IPTG). The selenomethionyl-derivative protein was derived using the same construct and *E. coli* strain, grown in cultures containing 50 μg/ml SeMet. Cells were harvested after incubating for 20 h by centrifugation at 5000 rpm for 10 min. Cells were then resuspended in a high salt buffer (20 mM HEPES, pH 7.4, 1 M NaCl) and lysed by sonication. The lysate was separated by centrifugation at 16000 rpm for 30min. The supernatant was applied to a GST affinity column (Qiagen, Inc.) followed by intensive washing with the same buffer. The GST tag was removed by prescision digestion on the column at 4°C overnight in a low salt buffer (20 mM HEPES, pH 7.4, 100 mM NaCl). Recombinant ISG58 eluted from the GST column was further purified by anion exchange chromatography using a Q column (GE Healthcare) and gel filtration using a Hiload Superdex-200 column (GE Healthcare) on a FPLC protein purification system.

Crystallization and structure determination Crystallization trials using the hanging drop method were performed. Crystals of a usable size were obtained in reservoir solution containing 100 mM HEPES at pH 6.9, 150 mM sodium formate, 8% PEG

3350. 2.05 Å native diffraction data was collected at the Shanghai Synchrotron Radiation Facility beamline BL17U (SSRF, China, λ = 1.000 Å). Selenomethionine derivative protein was used to obtain heavy atom derivative crystals. Diffraction data was collected at the Diamond Light Source, beamline I04, (Diamond, UK, λ = 0.978 Å). Both sets of data were processed by HKL2000 [4]. The structure was solved using the single-wavelength anomalous dispersion (SAD) method. Heavy atom locations were identified using SHELX [5]. The program Phenix [6] was used to calculate the initial and improved phases. Model building was carried out with ARP/wARP [7], and the structure was refined using Phenix and Coot [8]. In the Ramachandran plot generated using PROCHECK, 97.2% of the amino acids in the final atomic model were in the most favorable region and 2.8% were in the additional allowed region. The atomic coordinates have been deposited in the Protein Data Bank with the accession codes 4J0U. All figures were prepared by Coot and Pymol (http://www.pymol.org).

EMSA assay Model RNAs and DNAs were purchased from Takara Biotechnology Inc. and Sangon Biotech Co. Ltd, respectively. The dsRNAs and dsDNAs were annealed before use by heating and slow cooling. Mutant proteins were purified in a similar manner to wild type proteins as described above. Nucleotides were radio-labeled at their 5’ ends with γ-^{32}P ATP using T4 polynucleotide kinase (NEB). The proteins and nucleotides were mixed in a binding buffer (50 mM HEPES, pH7.4, 100mM NaCl, 2mM $MgCl_2$, 0.1% (v/v) Tween-20) and incubated at 4°C for at least 30 min. The mixture was seperated on a pre-run native 6% acrylamide:bisacrylamide

(29:1) gel in boric acid buffer without EDTA. After electrophoresis at 12V cm^{-1} for 2 h at 4°C, the radioactive signals were detected using photographic films.

VSV-GFP assay The coding sequences of ISG54, ISG56 and ISG58 were amplified by PCR with a C-terminal FLAG tag and inserted into the pReceiver-M02 vector (GeneCopoeia) between the BstBI and XhoI sites. As a negative control, the coding sequence of Renilla luciferase was cloned into the same vector. The K415E/R384E mutant was constructed using site-directed mutagenesis. All the sequences were verified by DNA sequencing.

Recombinant VSV of Indiana serotype was prepared as described previously [9]. The GFP open reading frame was inserted into the pVSV-XN2 plasmid that contains the entire VSV genome, and the expression of GFP can be used to monitor VSV infection. HEK293T cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% penicillin-streptomycin. Vesicular stomatitis virus expressing a GFP reporter (VSV-GFP) is a kind gift from Dr. Glen Barber (University of Miami, Florida). Cells were transfected using polyethylenimine (PEI) and infected with VSV-GFP at an MOI=0.1. Cells were harvested at 8hrs post infection, fixed in 2% PFA, and analyzed on a FACSCalibur flow cytometer (BD Biosciences). Viral infection was calculated as the percent of GFP positive cells multiplied by the mean fluorescence intensity (%GFP*MFI) and was normalized to the negative control which was arbitrarily set as 1.0.

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