Supporting Information

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SI Materials and Methods

Antibodies, Plasmids, and Reagents. Mouse anti-myc (9E10) monoclonal antibody, rabbit anti-IRF3 (FL-425) polyclonal antibody and goat anti-VDAC1 (N-18) polyclonal antibody were from Santa Cruz Biotechnology. Mouse anti-Flag monoclonal antibody and mouse anti-HA monoclonal antibody were from Sigma. Rabbit anti-Phospho-IRF-3 (Ser-396) polyclonal antibody, rabbit anti-Phospho-Akt (Ser 473) polyclonal antibody and rabbit anti-VISA polyclonal antibody were from Cell Signaling Technology. Mouse anti-gC1qR monoclonal antibody was from CHEMICON International, Inc. DMEM were purchased from GIBCO. Mitochondrion-Selective Probe MitoTracker Red CMXRos was from Invitrogen. Poly(I:C) was from Amersham Biosciences. ISRE, NF-κB, SV40, and human IFN-β promoter luciferase reporter plasmids and mammalian expression plasmids of human RIG-I, VISA were provided by H. Shu (Peking University, Beijing).

Construction of gC1qR Mutants. The full-length cDNA of human gC1qR was amplified by TaqDNA polymerase using specific primers. The PCR product was then subcloned into the appropriate sites of the expression vector pcDNA6/myc-HisA (Invitrogen). The truncated cDNA of human gC1qR was amplified by TaqDNA polymerase using specific primers. The PCR products were then subcloned into pEASY-T1 vector (Transgen) respectively. The resulting plasmids were digested with BamHI and EcoRI or EcoRI and NotI. The recovered fragments were subcloned into the appropriate sites of the expression vector pcDNA3/Flag (Invitrogen) or pcDNA6/myc-HisA (Invitrogen), respectively

Transfection and Reporter Gene Assays. 293T cells were seeded on 24-well dishes and transfected the next day by standard calcium phosphate precipitation. Within the same experiment, each transfection was performed in duplicate and where necessary, empty control plasmid was added to ensure that each transfection received the same amount of total DNA. The amount of poly(I:C) and expression plasmid was 0.5 μg for each transfection and poly(I:C) adding in the media was up to 20 μg. To normalize transfection efficiency, 0.2 μg of pRL-SV40 plasmid was added to each transfection. Dual specific luciferase reporter assays were performed using Dual-Luciferase Reporter 1000 assay kit (Promega) by following the manufacturer's protocol. Firefly luciferase activities were normalized on the basis of Renilla luciferase expression levels. All reporter gene assays were repeated for at least 3 times. Values are shown as mean $\pm$ SD from 1 representative experiment.

Coimmunoprecipitation and Western Blot Analysis. We seeded 293T cells on 10-cm dishes. The next day, cells were infected 12 h after transfection with different genes (15 μg of each for expression plasmid), with Sendai virus or left uninfected for 12h. Cells were then lysed in lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride). For immunoprecipitation, cell lysate was incubated with 1 μg of the indicated antibody or control IgG and 25 μL of Protein A/G Plus-Agarose (Santa Cruz Biotechnology) at 4 °C for $\approx$4 h. The agarose beads were washed 3 times with 1 mL of lysis buffer after precipitation. The precipitates were analyzed by standard immuno blot with antibody as indicated.

Vesicular Stomatitis Virus (VSV) Plaque Assay. The 293T cells (1×10^5) were transfected with the indicated plasmids for 24 or 36 h before VSV infection. At 1 h after infection, cells were washed with PBS for 3 times and then medium was added. The supernatants were harvested at 12 h after washing. The supernatants were diluted 1:106 and then used to infect confluent BHK21 cells cultured on 24-well plates. At 1 h postinfection, supernatant was removed and 3% methylcellulose was overplayed. At 3 days after infection, overlay was removed and cells were fixed with 4% formaldehyde for 1 h and stained with 0.2% Crystal violet in 20% methanol. Plaques were counted, averaged and multiplied by the dilution factor to determine viral titer as PFU/mL.

Immunofluorescence Assay. 293T cells were plated at $\approx 1 \times 105$ cells per well onto glass tissue culture chamber slides in a 12-well plate. DNA and poly(I:C) transfections were performed with the standard calcium phosphate precipitation method. At 12 h after transfection, cells were infected with Sendai virus or left uninfected for 3h and were incubated with 150 nM Mito Tracker Red CMXRos (Molecular Probes, Invitrogen) for 30 min at 37 °C. Cells were then washed in PBS (PBS) and incubated with 4% paraformaldehyde for 15 min at room temperature. The fixative was removed by 2 washes with PBS. Cells were incubated in PBS containing 0.2% Triton X-100 (PBST) for 5 min at room temperature and then rinsed twice with PBS. Primary antibodies were diluted in 1% BSA in PBST and incubated with the cells for 1 h at room temperature. To remove excess antibody, the slides were washed 3 times in PBST. FITC-conjugated secondary antibodies were added in the same manner. After washed 3 times, the processed slides were then mounted with nail polish. Imaging of the cells was carried out using LEICA TCS-SP laser scanning confocal microscopy.

Preparation of Cellular and Mitochondrial Proteins. The 293T cells were resuspended with ice-cold RSB (10 mM NaCl, 2.5 mM MgCl2, 10 mM Tris·HCl pH 7.5) after washed with PBS. The cell suspension was homogenized by tight-fitting Dounce homogenizer at 4 °C. Then ice-cold 2.5× MS (525 mM Mannitol, 175 mM Sucrose, 12.5 mM Tris·HCl pH 7.5, 2.5 mM EDTA pH 7.5) was added into the homogenate, followed by 3 times of centrifugation ($1{,}300 \times g$, 5min, 4 °C). The supernatant was centrifugated ($17{,}000 \times g$, 15min, 4 °C). Mitochondria was collected and washed with ice-cold 1× MS for 3 times. For protein extraction, the above mitochondria was lysed with SB (7M Urea, 2M Thiourea, 65 mM DTT, 4% CHAPS), and lysate was stored for assay. Total cellular protein from 293T cells and 293T cells transfected with ectopic genes was extracted by lysing cells with SB after treatment or without treatment. The lysate was resolved on SDS/PAGE followed by immunoblot analysis. Each loading contains 50 μg of protein for SDS/PAGE experiment.

siRNA Information. Control-siRNA: 5′-UUC UCC GAA CGU GUC ACG UTT-3′; gC1qR-siRNA-1: 5′-GGA UGA GGU UGG ACA AGA ATT-3′; gC1qR-siRNA-2: 5′-GAA UGG GAC AGA AGC GAA ATT-3′; gC1qR-siRNA-3: 5′-CAU UUG AUG GUG AGG AGG ATT-3′; gC1qR-siRNA-4: 5′-AGA GUG ACA UCU UCU CUA UTT-3′; gC1qR-siRNA-5: 5′-CGG UCA CUU UCA ACA UUA ATT-3′.

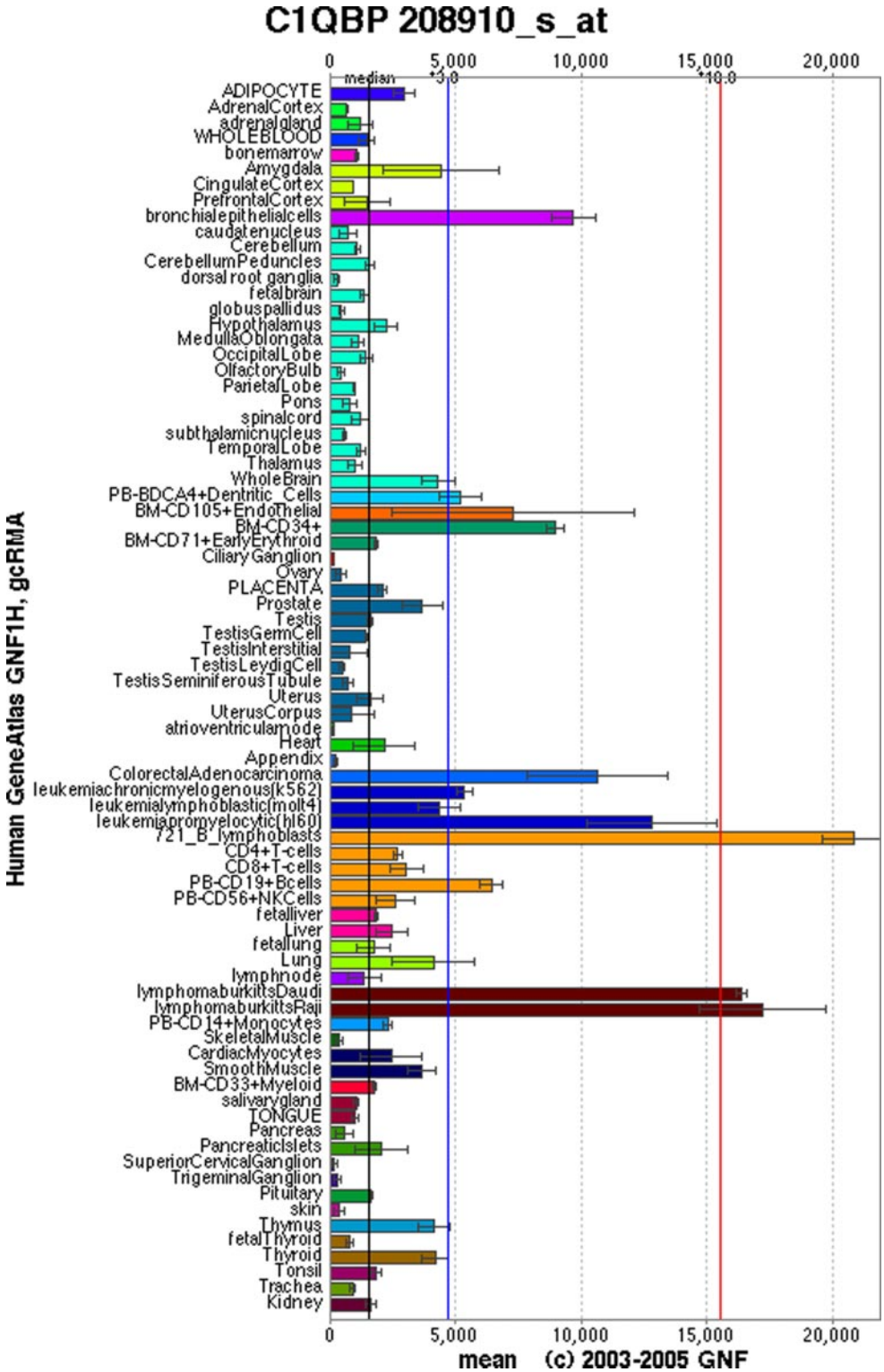

Fig. S1. Human gC1qR mRNA is ubiquitously expressed. Cell and tissue microarray expression data were from the Genomics Institute of the Novartis Research Foundation (GNF) (http://symatlas.gnf.org/SymAtlas), using C1qBP as the keyword identifier. Each bar represents the mean value from duplicate samples.

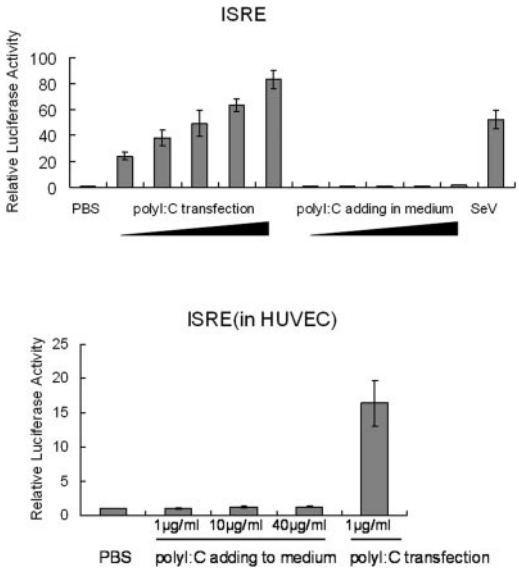

Fig. S2. Poly(I:C) activates only intracellular signaling, not extracellular signaling. 293T cells were transfected with ISRE report and poly(I:C) (range from 0.2 μg to 2.0 μg), or transfected with ISRE reporter only and cultured in medium with poly(I:C) (range from 0.2 μg to 2.0 μg). Reporter assay was perfomed after culture for 18 h.

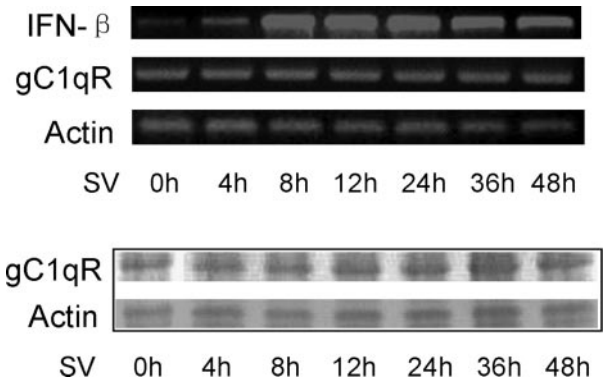

Fig. S3. Human gC1qR in both mRNA and protein level is stable upon viral infection. 293T cells were infected with Sendai virus. Cells were collected at a set of time course to extract total RNA for RT-PCR assay and total protein for Western blot assay.

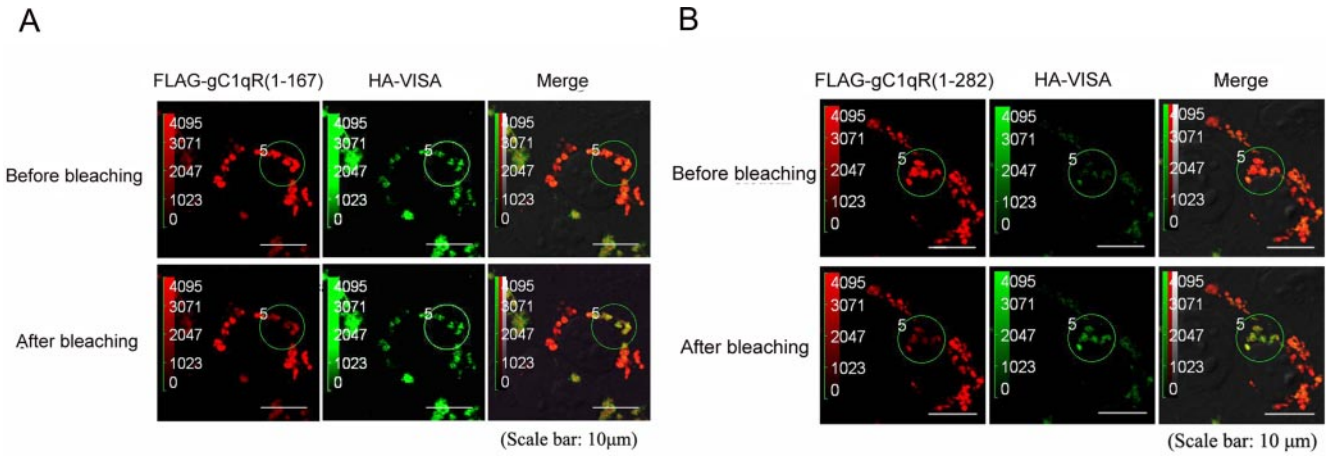

Fig. S4. The image of the interaction of MAVS and gC1qR within the cell by FRET. 293T cells were cotransfected with HA-VISA and FLAG-gC1qR1–167 (*A*) or HA-VISA and FLAG-gC1qR1–282 (*B*). At 12 h after transfection, cells were infected with SV for 3 h. HA-VISA was labeled with FITC by αHA primary antibody and FITC-conjugated secondary antibody. FLAG-gC1qR1–167 and FLAG-gC1qR1–282 were labeled with TRITC by αFLAG primary antibody and TRITC-conjugated secondary antibody. Cells were imaged by Olympus FV1000S laser scanning confocal microscope with a 60× objective lens. Interference filters used in this study were as follows: donor channel (for TRITC) excitation wavelength, 559 nm; donor channel emission wavelength, 578 nm; acceptor channel (for FITC) excitation wavelength, 488 nm; acceptor channel emission wavelength, 519 nm. The control of the whole system and the calculation were performed by the OLYMPUS FLUOVIEW Version 1.7a software (Olympus). The color scale in each picture shows the gray scale of the image, it is positive related with the intensity of the fluorescence. The bleaching time varied from 27 s to 27 s 30 ms, depending on the sample.

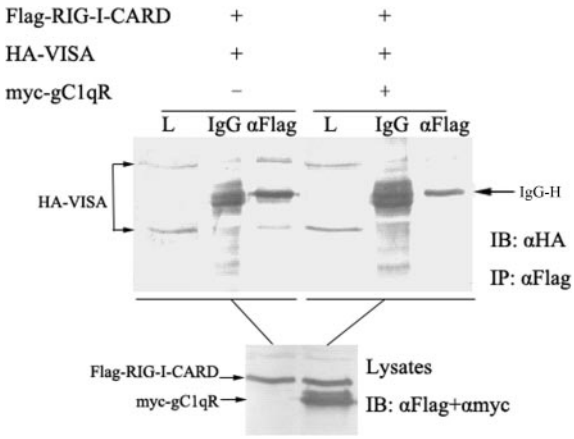

Fig. S5. gC1qR disrupts the interaction between ΔRIG-I and VISA. 293T cells (1×10^6) were transfected with indicated plasmids (8 μg of each). Coimmunoprecipitations were performed with anti-Flag antibody (αFlag) or control mouse IgG (IgG), followed by Western blot analysis assay.

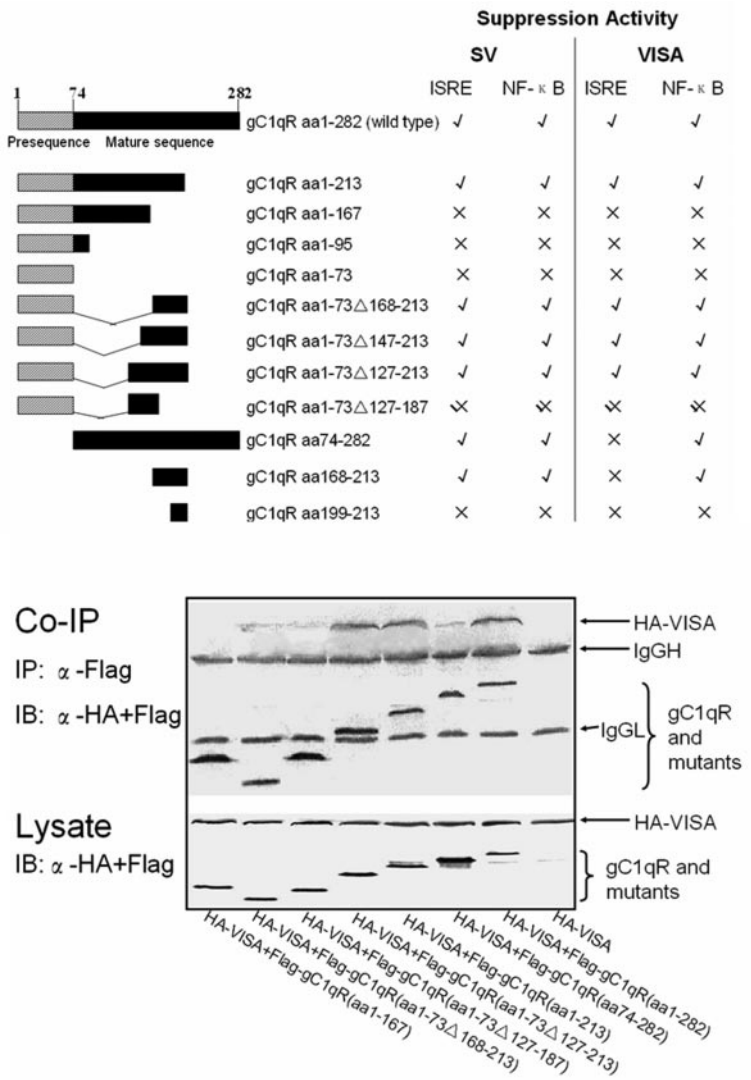

Fig. S6. Mapping of functional region in gC1qR. (*Upper*) Diagram of mutants of gC1qR and the summary of its suppression activity. Different mutants of gC1qR were constructed. The suppression activity of mutants was from the following reporter assay. *Lower*: Interaction of the gC1qR mutants with VISA. 293T cells were cotransfected with different mutants and VISA, respectively. Cellular protein was extracted after transfection and was pulled down with flag antibody. The pull down complex was resolved by immunoblot with HA and flag antibody.

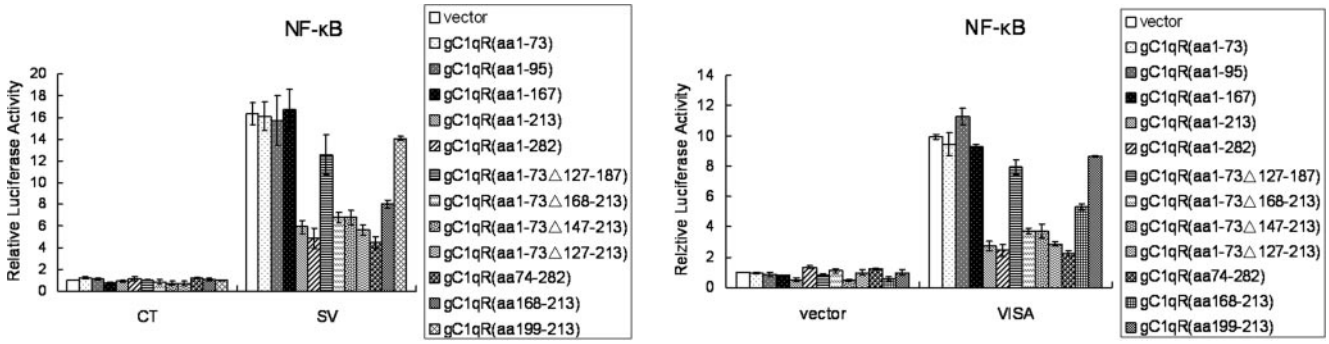

Fig. S7. Influence of NF-κB activation by the gC1qR mutants in cells over expressing VISA or infected with Sendai Virus. NF-κB reporter and gC1qR mutants were cotransfected with VISA, or without VISA into 293T cells, respectively. For cells without ectopic VISA were further treated by virus infection. Reporter assay was then performed after treatment.

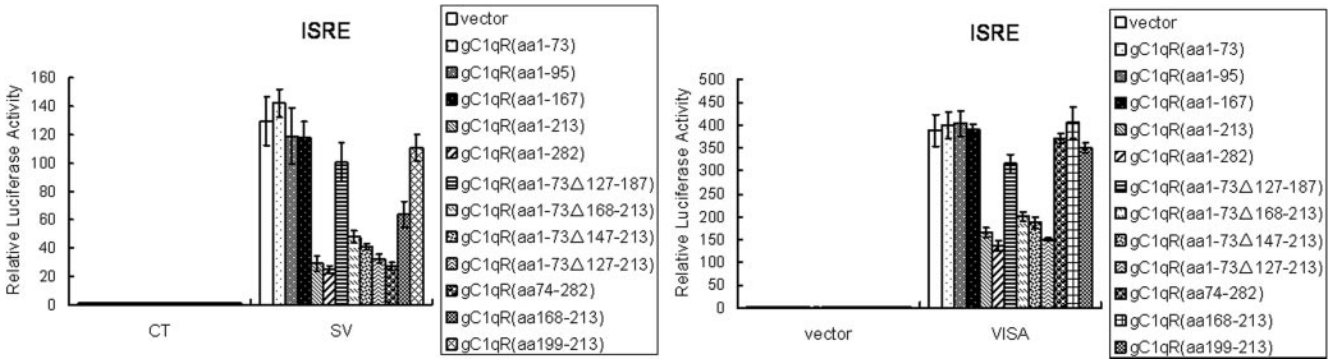

Fig. S8. Influence of ISRE activation by the gC1qR mutants in cells over expressing VISA or infected with Sendai Virus. ISRE reporter and gC1qR mutants were cotransfected with VISA, or without VISA into 293T cells, respectively. For cells without ectopic VISA were further treated by virus infection. Reporter assay was then performed after treatment.

Table1. Primers

Genes	Primer pairs
Human gC1qR	ACAACAGCATCCCACCAA
	CAAAGCCTGCCATGAAAC
Human β-actin	GGGACCTGACTGACTACCTC
	ACTCGTCATACTCCTGCTTG
Human IFN-β	CACTGGCTGGAATGAGACT
	TTTCGGAGGTAACCTGTAAG