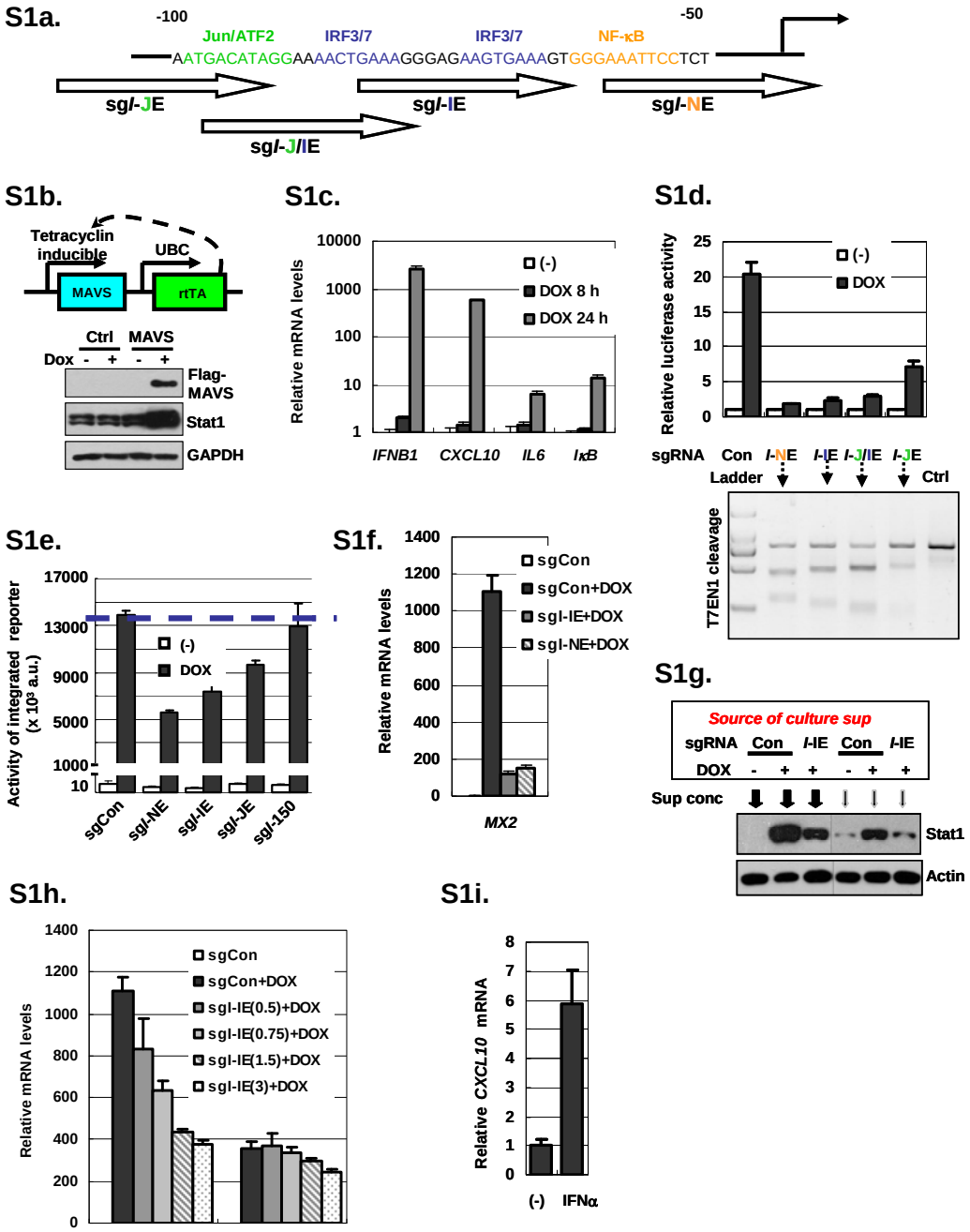
Supplementary Figures:

Supplementary Fig. 1

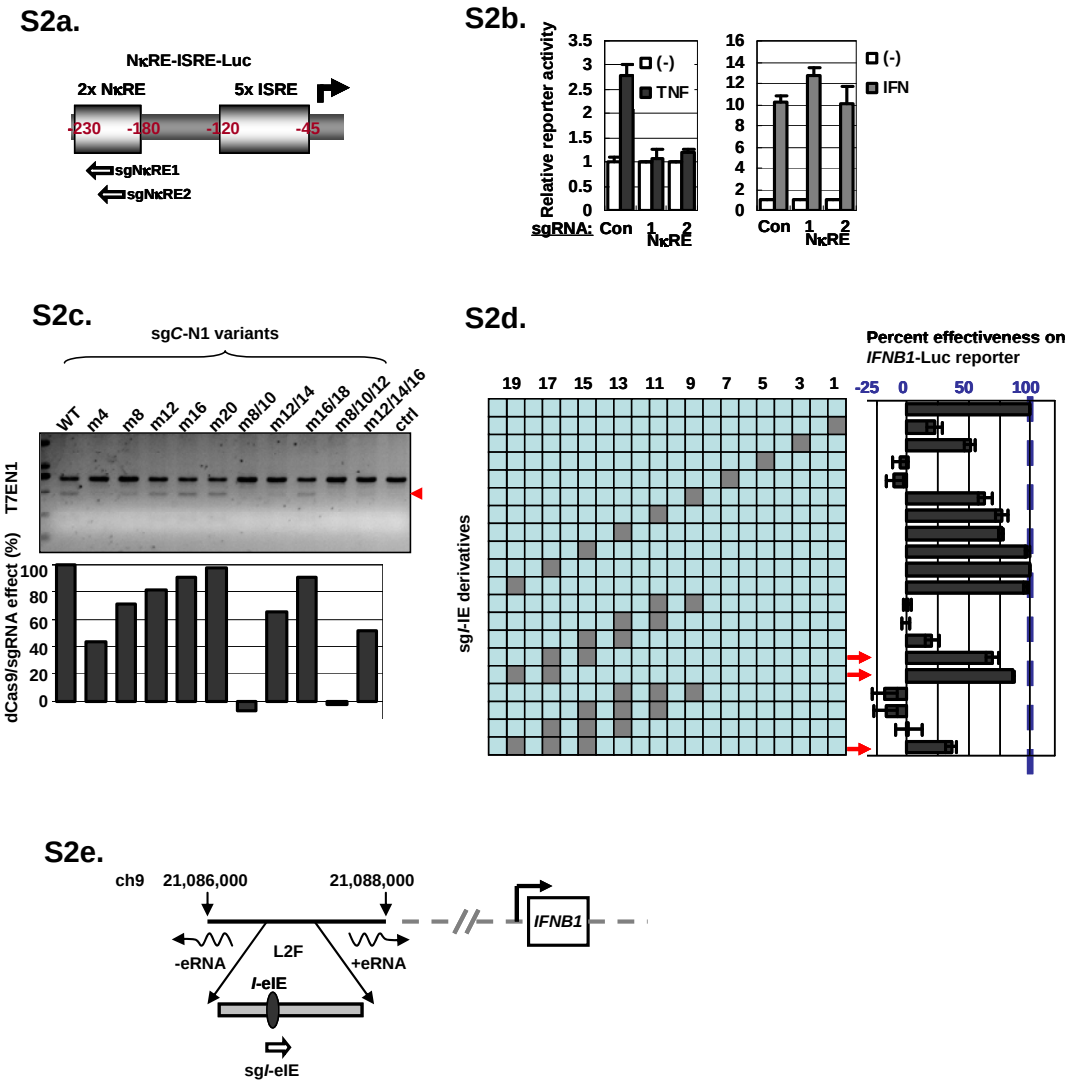


Supplementary Figure 1: Sequence-specific targeting of *IFNB1* *cis*-elements by dCas9/sgRNA. (S1a) Sequences of closely situated virus-responsive elements in human

IFNB1 promoter are displayed. Jun/ATF2-, IRF3/7- and NFκB-responsive elements were presented in green, blue and orange letters, respectively. The sgRNAs were depicted as block arrows pointing at the NGG PAM sequences. The sgRNAs with coverage of the NFκB-, the IRF-, the Jun/ATF2-responsive elements were respectively referred to as sg*I*-NE, sg*I*-IE and sg*I*-JE. Similarly, a sgRNA that covers parts of both the Jun/ATF2- and the IRF-responsive elements was referred to as sg*I*-J/IE. **(S1b)** MAVS tet-on cells were generated based on a modified lentiviral pTRIPZ vector system that incorporates rtTA and MAVS cDNA in the same construct. The transductants were selected under 1 μg/ml of puromycin and were further maintained as a pool. The control tet-on cells were generated using the original pTRIPZ vector containing RFP. Cells were treated with deoxycyclin (DOX, 2 μg/ml) for 24 h. Total cell lysates were harvested and analyzed on Western blot using indicated antibodies. **(S1c)** MAVS tet-on cells were treated with DOX (2 μg/ml) for 8 or 24 h. Total RNA was harvested and the levels of indicated mRNAs were analyzed by qPCR analysis. **(S1d)** MAVS tet-on cells were co-transfected in triplicates with indicated sgRNAs (see Supplementary Figure S1a for reference), dCas9 and a luciferase reporter containing the 218 bp sequence upstream of first ATG in *IFNB1*. 24 h after transfection, cells were treated with DOX for 24 h and luciferase activities were measured (±STDEV). A T7EN1 assay performed with a separate set of samples was shown below to demonstrate the cleavage activity by each sgRNA. **(S1e)** MAVS tet-on cells were stably introduced with *IFNB1*-Luc (1410-bp) promoter reporter. The cell line was transfected with dCas9 in combination with sg*I*-IE, sg*I*-NE or sg*I*-JE in triplicate wells in a 24-well plate. A sgRNA (sg*I*-150) targeting a more upstream sequence (150 bp to the 5' of the transcriptional start) was used as a negative control. Following the transient

transfection, cells were then treated with 2 μg/ml of DOX for 24 h. After cell lysis, equal amount of protein was subjected to firefly luciferase assay. **(S1f)** Parallel to Fig. 1b, the effect of sg*I*-IE and sg*I*-NE on the relative levels of type I IFN-dependent target *MX2* was examined. **(S1g)** The culture supernatants from MAVS tet-on cells transfected with dCas9/sgCon or dCas9/sg*I*-IE were harvested. The supernatants were used to treat naïve 293T cells for 12 h and protein levels of Stat1 were analyzed. The supernatants were either used at 1:2 (thick arrows) or 1:5 (thin arrows) dilution. **(S1h)** Cells were transfected with increasing dose of sg*I*-IE in combination with dCas9 in 6-well plates as indicated (the amount of sg*I*-IE plasmid in μg unit was denoted). Cells were maintained without drug selection for 24 h and treated with DOX for 24 h. The relative levels of *IFNB1* and *CXCL10* mRNAs were determined. **(S1i)** MAVS tet-on cells were treated for 12 h with 2000 IU/ml of IFNα and the levels of *CXCL10* mRNA were determined by qPCR analysis.

Supplementary Fig. 2



Supplementary Figure 2: Application of dCas9/sgRNA to target both promoter and non-promoter *cis*-elements. (S2a) A schematic display of the NκRE-ISRE-Luc reporter was presented as in Fig. 1a. **(S2b)** Following transfections of dCas9/sgRNA indicated in Fig. S2a, cells were treated with TNFα or IFNα for 12 h. TNFα- and IFNα-inducible reporter activities were presented on the left and right panels, respectively. **(S2c)** Single-, double- or triple-nucleotide mismatched variants of sg*C*-N1 were subjected to T7EN1 assay. The red

arrowhead marks a cleavage product. Additionally, the effects of these sgRNA variants in mediating inhibition of TNFα-induced *CXCL10* expression (taken from the data shown in Fig. 1i) were graphed below the gel picture to show an overall correlation. **(S2d)** Single-, double- or triple-nucleotide mutations were introduced into various positions (marked as grey blocks) in sg*I*-IE targeting the IRF-responsive element of *IFNB1*. The numbers above the blocks indicate the nucleotide position in reference to the NGG on the target DNA. The effects by these sgRNAs were measured after 24 h of DOX treatment. The inhibitory effectiveness by the WT sg*I*-IE (inhibition of DOX-induced relative values *IFNB1*-Luc activity) was set as one hundred percent. Some examples of tolerance were pointed out by red arrows. Error bars represent normalized STDEV arisen from samples of triplicated transfections. **(S2e)** A novel enhancer locus located 20 kb upstream of *IFNB1* gene is displayed. Bi-direction eRNA species were depicted as either '+eRNA' or '-eRNA'. A 550-bp region (L2F) that contains the IRF3-binding element (*I*-eIE) is shown in a zoom-in fashion. The sgRNA targeting this *cis*-element (sg*I*-eIE) was presented as a small block arrow.

Supplementary Information

Supplementary Methods:

Reagents – All chemicals were purchased from Sigma Aldrich if not specifically noted. Recombinant human IFNα (RC036-20ug) and mouse TNFα (RC052-5ug) were from Sangon Biotech. Recombinant IFN-γ was from Pepro Tech (AF-300-02). T7 endonuclease I (T7EN1, M0302L) was from New England Biolabs.

Plasmids – The lentiviral tet-on shRNAmir expression system pTRIPZ (Open Biosystems) was modified to drive inducible expression of anti-viral signaling adaptor MAVS. Human *MAVS* was first cloned from a Hela cDNA library. *Flag-MAVS* was then used to replace the *RFP-shRNAmir* cassette in pTRIPZ. sgRNAs were designed to mediate a 20-nt base match with the DNA targets and cloned into a U6 promoter-driven expression plasmid (pGL3-U6-puro) as described previously [1]. Human codon-optimized Cas9 expression vector (Addgene No. 44758) was described previously [2] and was used as template to generate the dCas9 (D10A, H840A) plasmid. All promoter reporters used in the present study were constructed based on pGL3-basic vector (Promega). The *IFNB1* promoter reporter used in transient transfection experiments contains a 218-bp sequence upstream of human IFNβ translation-start (proximal promoter), whereas a bigger promoter reporter containing a 1410-bp upstream sequence was used for chromosome integration. In experiments to move the MAVS-responsive *cis*-elements away from the transcriptional start, a series of 15-, 30- or 45-bp spacer were respectively inserted upstream of the TATA-box in the proximal *IFNB1* promoter reporter. The original interferon-responsive reporter (containing five copies of interferon stimulation-responsive element (ISRE) from human *IFIT2* gene) was a generous

gift from Dr. Horvath C (Northwestern University) [3]. The 5xISRE element was first sub-cloned into pGL3-basic to yield ISRE-Luc. To generate a double *cis*-element reporter, duplicated NFκB-responsive element (NκRE) of mouse immunoglobulin kappa chain enhancer [4] was added to the 5' end of ISRE in the ISRE-Luc reporter (NκRE-ISRE-Luc).

Antibodies – Rabbit polyclonal antibody against STAT1 was obtained from Sangon (AB55186). GAPDH antibody was obtained from Santa cruz Biotechnology (SC-32233). Flag antibody (F3165) and mouse serum IgG (I8765) was obtained from Sigma-Aldrich. ChIP antibody against NFκB p65 was purchased from Abcam (ab7970).

Cell culture and transfection – Cell culture reagents were purchased from Invitrogen if not specifically noted. All cells were maintained in DMEM containing 10% FBS and the antibiotics (penicillin/streptomycin) using a 37°C humidified incubator supplied with 5% CO_2. 293T cells were purchased from ATCC and were used to generate the MAVS tet-on cell line. The latter was established using a modified pTRIPZ lentiviral vector system as described above (selected under 1 μg/ml of puromycin). The cell line was determined to be mycoplasma-free using MycoAlert mycoplasma detection kit (Lonza). Transfection of the cells with dCas9/sgRNA and/or various promoter reporter constructs was carried out using Lipofectamine 2000 (Invitrogen). In addition, a stable cell line to monitor the activity of integrated *IFNB1* reporter gene was generated based on the MAVS tet-on cells. Briefly, MAVS tet-on cells were co-transfected in a 5:1 molar ratio with a 1410-bp *IFNB1*-Luc reporter and a pST1374-based plasmid conferring blasticidin resistance. Transfected cells were selected for 10 days in the presence of 10 μg/ml of blasticidin. Clones were isolated, propagated, and tested for DOX-induced luciferase activity.

T7EN1 assay - Sub-confluent (60-70%) 293T cells in 6-well plates were co-transfected with 1.5 μg Cas9 and 2 μg sgRNAs plasmids. The transfected cells were selected for 2 days in the presence of 10 μg/ml of blasticidin and 5 μg/ml puromycin. Afterwards, genomic DNA was harvested from the cells. The regions encompassing the target sites were PCR-amplified using the primers shown in Supplementary Table 1. The amplicons were subjected to the T7EN1 assay as described [5]. Briefly, amplicons were purified (Axygen PCR cleanup kit, AP-PCR-50) and then denatured/re-annealed in NEBuffer 2 (New England Biolabs) using a thermocycler. Re-annealed PCR products were digested with T7EN1 for 30 min and analyzed on 2% agarose gel. The levels of DNA fragments shorter than the expected product sizes reflect the extents of Cas9-mediated cleavage.

Luciferase activity assay – Sub-confluent (50-60%) MAVS tet-on cells in 24-well plates were co-transfected with 200 ng of the experimental firefly luciferase construct, 1.6 ng of renilla luciferase control and dCas9/sgRNA plasmids (200 ng each). Transfection was performed in triplicates. Treatment with DOX (2 μg/ml), IFNα (2000 units/ml) or TNFα (50 ng/ml) was done 24 h after transfection for the durations indicated in figure legends. Luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega E1910).

RT-qPCR assay – Sub-confluent (60-70%) MAVS tet-on cells in 6-well plates were co-transfected with 1.5 μg dCAS9 (blasticidin-resistance) and 2 μg sgRNA (puromycin-resistance) plasmids. The cells were selected for two days post-transfection in the presence of 10 μg/ml of blasticidin and 5 μg/ml of puromycin. Treatment with DOX (2 μg/ml), IFNα (2000 IU/ml), IFNγ (2000 IU/ml) or TNFα (50 ng/ml) was done for times

indicated in figure legends. Total RNA was extracted using TRIzol reagent (9108) from Takara. Complementary DNA was prepared using HiScript Q RT Super Mix (Vazyme, R123-01). Samples were aliquoted in quadruplicates and quantitative real-time PCR analysis was performed using AceQ qPCR SYBR Green Master Mix (Vazyme, Q141-02). The 18S rRNA was used as internal control for normalization. Average results from quadruplicates were presented (±STDEV). For eRNA analyses, complementary DNA was prepared using a RevertAid cDNA synthesis kit (Thermo scientific, K1621) and random primers. The qPCR primers were shown in Supplementary Table 1.

Chromatin Immuno-precipitation (ChIP) analyses – 293T cells at 50% confluency were transfected in 10-cm plates with plasmids encoding 3xFlag-tagged form of dCas9 and sgRNA (9 μg and 12 μg, respectively). 24 h later, cells in each 10-cm plate were split into a 15-cm plate and selected under blasticidin (10 μg/ml) and puromycin (5 μg/ml) for an additional 24 h. Cells were subsequently treated with or without TNFα (50 ng/ml) for 3 h before subjected to cross-linking (1% formaldehyde) for 10 min at room temperature. 1.375 M of glycine was diluted 1:10 to quench the reactions. After washing, cells were scrapped into a 15 ml tube and the pellets were incubated in 10 ml of cell lysis buffer (5 mM PIPES pH 8.0, 85 mM KCl and 0.5% NP-40) on ice for 10 min. The pellet containing the crude nuclei were further incubated with 1 ml of nuclei lysis buffer (50 mM Tris-Cl pH 8.0, 10 mM EDTA and 1% SDS, supplemented with protease inhibitors) on ice for 10 min. Three hundred μl of nuclear lysates were transfered to 1.5 ml eppendorf tubes and subjected to sonication for four rounds of five 30s/30s ON/OFF cycles using a Bioruptor Standard sonicator (Diagenode, UCD-200). After centrifugation at 13,000 rpm for 15 min at 4°C, the supernatants were transferred to fresh

tubes. The DNA concentrations were measured and samples containing 100 μg of chromatin DNA were aliquoted (for each IP). Each sample was diluted into a final volume of 300 μl and then pre-cleared using protein G-agarose/salmon sperm DNA for 2 h at 4°C. Following pre-clearance, the supernatants were transferred to fresh tubes. 30 μl of aliquots were saved as 'input' DNA. The samples were IPed with antibodies for overnight, which was followed by an additional 2 h with protein G agarose. The beads were then washed for four times with RIPA buffer, four times with the washing buffer (100 mM Tris-HCl pH 7.4, 500 mM LiCl, 1% NP-40 and 1% sodium deoxycholate) and then two times with 1 ml of TE buffer. Each IPed or the 'input' sample was added with 200 μl of TE buffer contain 10 μg of RNase A and incubated at 37°C for 30 minutes. Each sample was then calibrated to required buffer condition, added with 4 μl of proteinase K stock (20 mg/ml) and incubated at 65°C with constant mixing for 6 hours. The supernatant was transferred to a fresh tube and DNA was extracted with phenol/chloroform/isoamyl-alcohol method. The samples were subjected to semi-quantitative PCR analysis using primers corresponding to *CXCL10* proximal promoter (Supplementary Table 1).

1. Shen, B, Zhang, W, Zhang, J et al., (2014) Efficient genome modification by CRISPR-Cas9 nickase with minimal off-target effects. Nat Methods 11: 399-402.
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