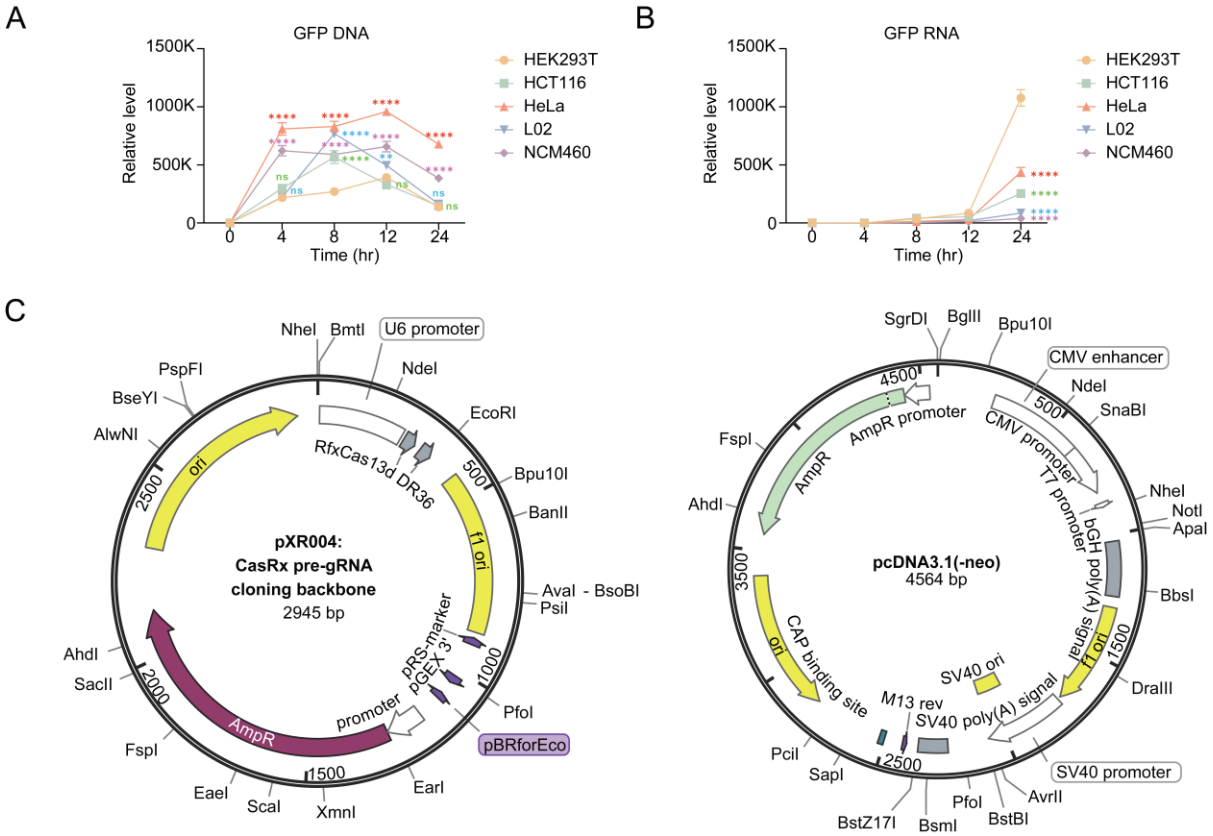


Figure S1. Comparison of plasmid transfection and transgene expression across cell lines.

qPCR analysis of pcDNA3.1-GFP plasmid DNA **(A)** and EGFP mRNA (**B**) levels normalized to those of GAPDH at the indicated time points (≤24 h) in transfected HEK293T, HCT116, HeLa, L02, and NCM460 cells. Statistical P values were calculated using Dunnett's test following ANOVA, with HEK293T cells transfected with pcDNA3.1-GFP serving as the control group. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. ns, not statistically significant. n=3 independent replicates. **(C)** Maps of the pre-gRNA and pcDNA3.1-neo plasmids.

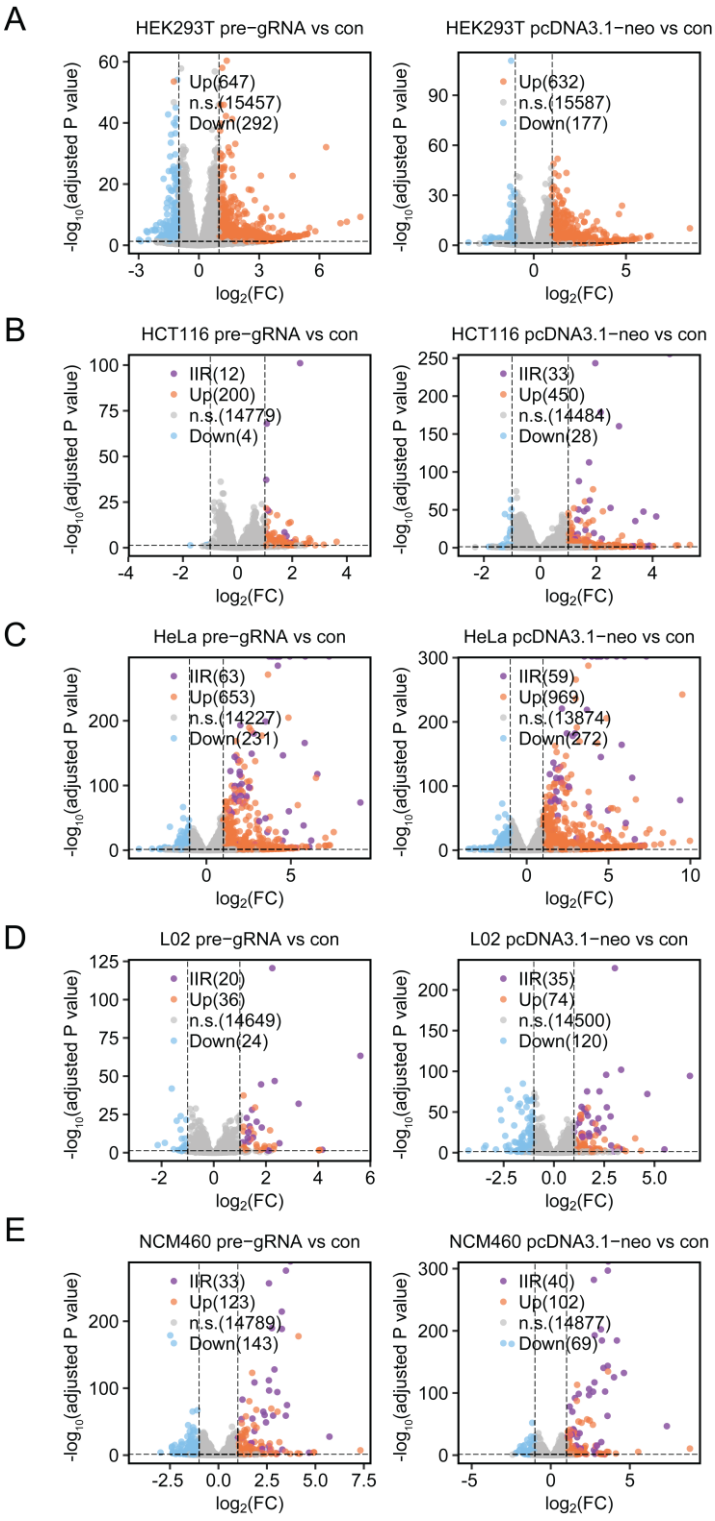


Figure S2. Gene expression varies across cell types after circular plasmid transfection.

Volcano plots of DEGs identified in the HEK293T **(A)**, HCT116 **(B)**, HeLa **(C)**, L02 **(D)**, and NCM460 **(E)** cell lines transfected with the pre-gRNA or pcDNA3.1-neo plasmid compared with the controls. IIR: innate immune response genes related to the RTV BP term, "Up", upregulated (fold change ≥ 2, adjusted p value ≤ 0.05), "n.s. ", not significant, "Down", downregulated (fold change ≤ -2, adjusted p value ≤ 0.05), and "con", control. n=3 independent replicates.

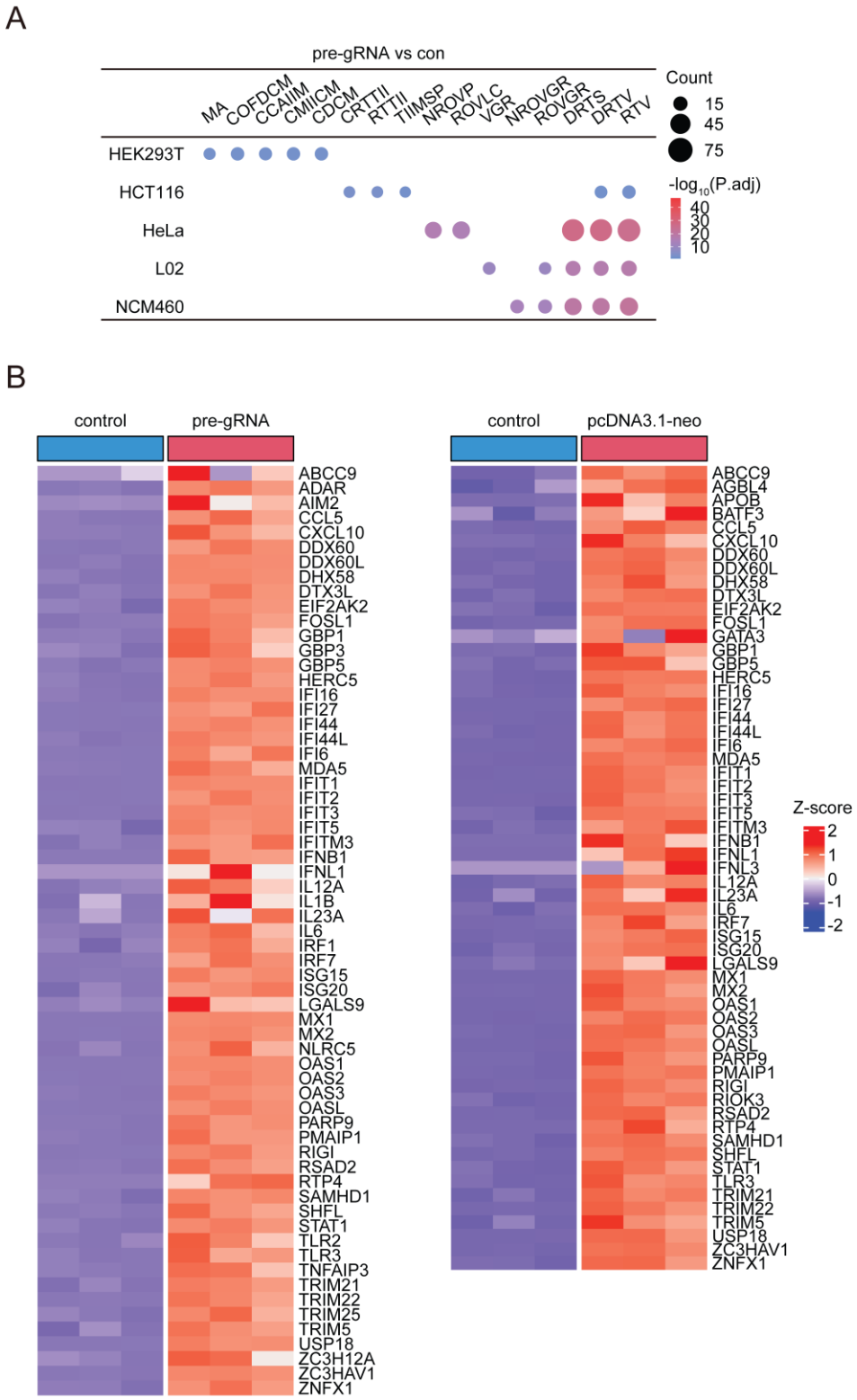


Figure S3. Circular plasmid transfection induces the broad upregulation of IIR genes in HeLa cells.

(A) Dot plots displaying the top five most significantly enriched biological process GO terms among the upregulated DEGs in each cell line following pre-gRNA plasmid transfection versus controls. MA: myofibril assembly; COFDCM: cilium or flagellum-dependent cell motility; CCAIIM: cellular component assembly involved in morphogenesis; CMIICM: cilium movement involved in cell motility; CDCM: cilium-dependent cell motility; CRTTII: cellular response to type I interferon; RTTII: response to type I interferon; TIIMSP: type I

interferon-mediated signaling pathway; NROVP: negative regulation of viral process; ROVLC: regulation of the viral life cycle; VGR: viral genome replication; NROVGR: negative regulation of viral genome replication; ROVGR: regulation of viral genome replication; DRTS: defense response to symbionts; DRTV: defense response to virus; RTV: response to virus. P.adj: adjusted p value. n=3 independent replicates. **(B)** Heatmaps displaying the differential expression of IIR genes in HeLa cells transfected with pre-gRNA (left panel) or pcDNA3.1-neo (right panel) plasmids compared with the controls. n=3 independent replicates.

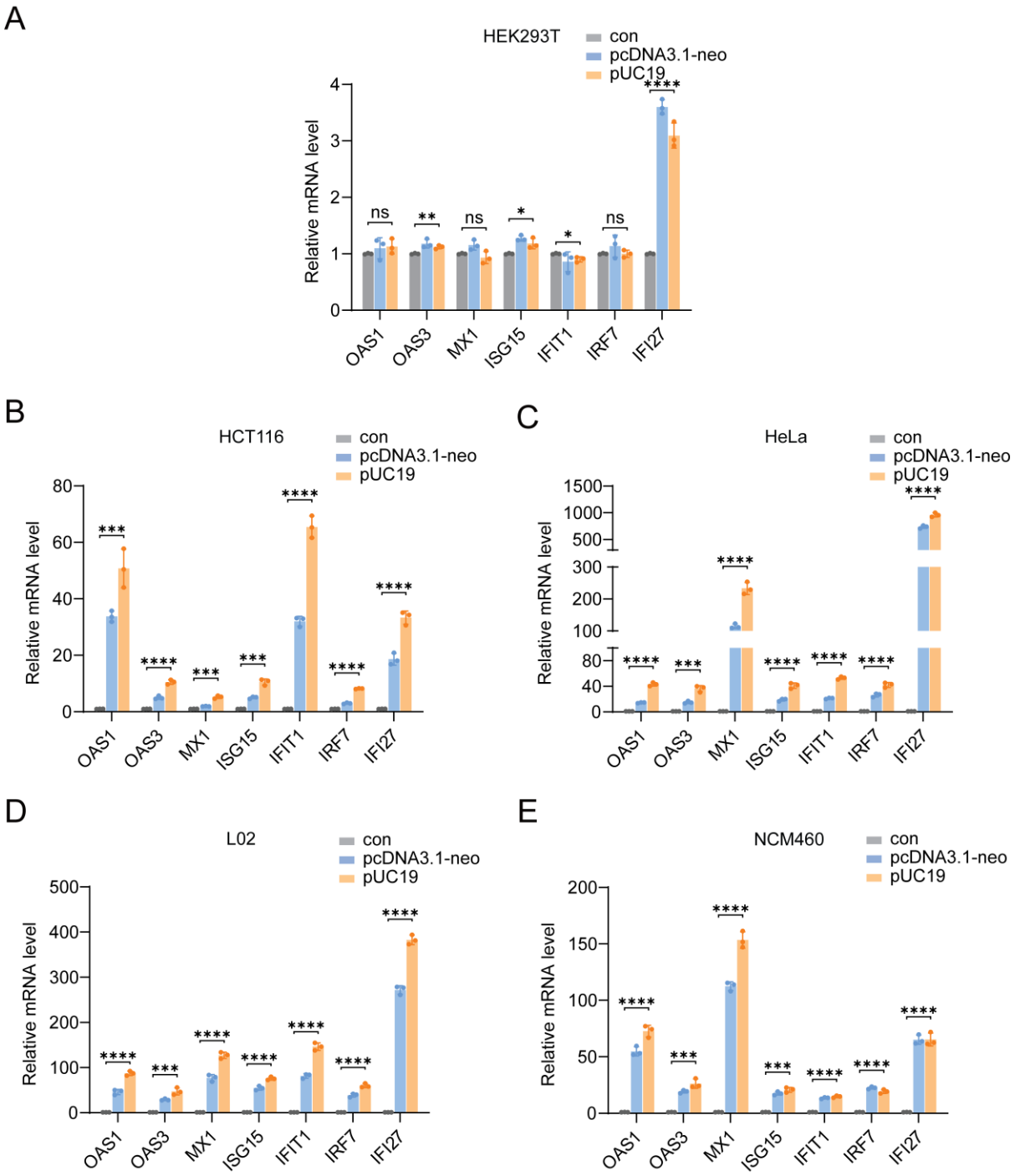


Figure S4. Identification of IIR genes activated by the pUC19 plasmid.

qPCR results at 24 h reveal the relative mRNA expression levels of 7 IIR genes in HEK293T **(A)**, HCT116 **(B)**, HeLa **(C)**, L02 **(D)**, and NCM460 **(E)** cells treated with either transfection reagent, pcDNA3.1-neo, or pUC19 plasmids. Con: control. Statistical comparisons between the pUC19-treated groups and the control groups were performed using two-tailed Student's t tests. *P < 0.05, **P <0.01, ***P < 0.001 and ****P < 0.0001. n=3 independent replicates.

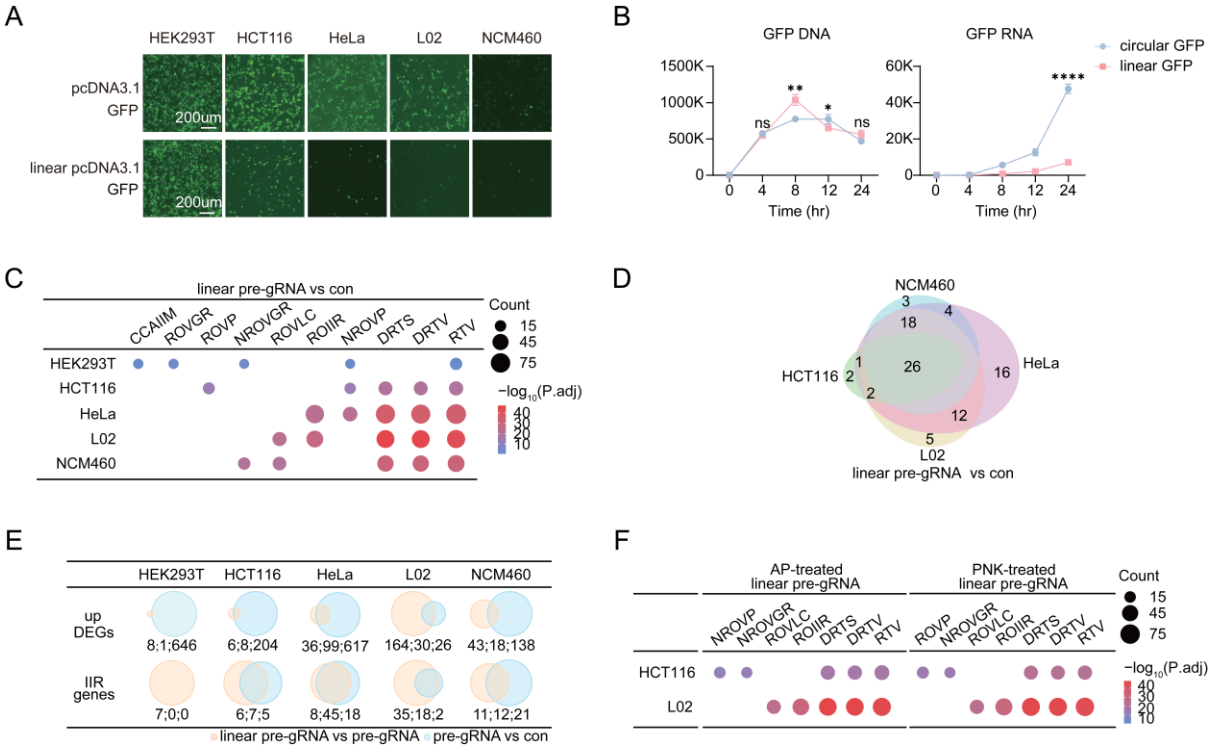


Figure S5. The linear pre-gRNA plasmid significantly activates IIR genes.

(A) GFP fluorescence in HEK293T, HCT116, HeLa, L02, and NCM460 cells transfected with either circular or linear pcDNA3.1-GFP plasmids. **(B)** qPCR analysis of pcDNA3.1-GFP plasmid DNA and EGFP mRNA levels normalized to those of GAPDH at the indicated time points (≤24 h) in transfected NCM460 cells. Statistical significance was determined using two-tailed Student's t tests. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. ns, not statistically significant. n=3 independent replicates. **(C)** Dot plots displaying the top five most significantly enriched biological process GO terms among the upregulated DEGs in each cell line following linear pre-gRNA plasmid transfection versus controls. CCAIIM: cellular component assembly involved in morphogenesis; ROVGR: regulation of viral genome replication; ROVP: regulation of viral processes; NROVGR: negative regulation of viral genome replication; ROVLC: regulation of the viral life cycle; ROIIR: regulation of the innate immune response; NROVP: negative regulation of viral processes; DRTS: defense response to symbionts; DRTV: defense response to virus; RTV: response to virus. P.adj: adjusted p value. n=3 independent replicates. **(D)** Venn diagram of IIR genes activated by the linear pre-gRNA plasmid compared with the transfection reagent alone across the HCT116, HeLa, L02, and NCM460 cell lines. Con: control. n=3 independent replicates. **(E)** Venn diagrams illustrating the upregulated DEGs and IIR genes between linear pre-gRNA and pre-gRNA plasmid transfections (yellow) and between pre-gRNA plasmid transfections and controls (blue). Con: control. The numerical values corresponding to each Venn diagram region are annotated below and separated by colons. n=3 independent replicates. **(F)** Dot plots displaying the top five enriched GO biological process terms among the upregulated DEGs in HCT116 and L02 cells transfected with linear pre-gRNA plasmids treated with alkaline phosphatase (left panel) or polynucleotide kinase (right panel) compared with the controls. NROVP: negative regulation of the viral process; NROVGR: negative regulation of viral genome replication; ROVLC: regulation of the viral life cycle; ROIIR: regulation of the innate immune response; DRTS: defense response to symbionts; DRTV: defense response to virus; RTV: response to virus. AP: alkaline phosphatase. PNK: polynucleotide kinase. P.adj: adjusted p value. n=3 independent replicates.

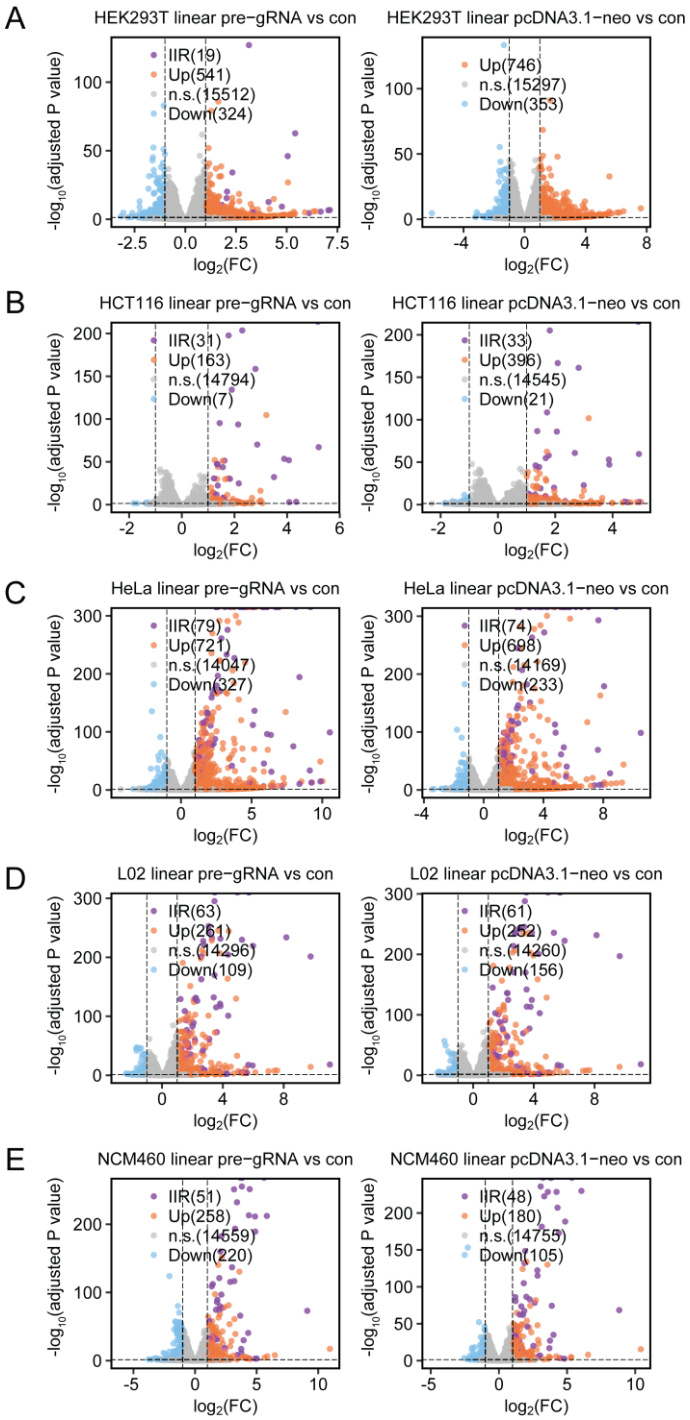


Figure S6. Gene expression varies across cell types after linear plasmid transfection.

Volcano plots of DEGs identified in the HEK293T **(A)**, HCT116 **(B)**, HeLa **(C)**, L02 **(D)**, and NCM460 **(E)** cell lines transfected with linear pre-gRNA or linear pcDNA3.1-neo plasmid compared with the controls. IIR: innate immune response genes related to the RTV BP term, "Up", upregulated (fold change ≥ 2, adjusted p value ≤ 0.05), "n.s.", not significant, "Down", downregulated (fold change ≤ -2, adjusted p value ≤ 0.05), and "con", control. n=3 independent replicates.

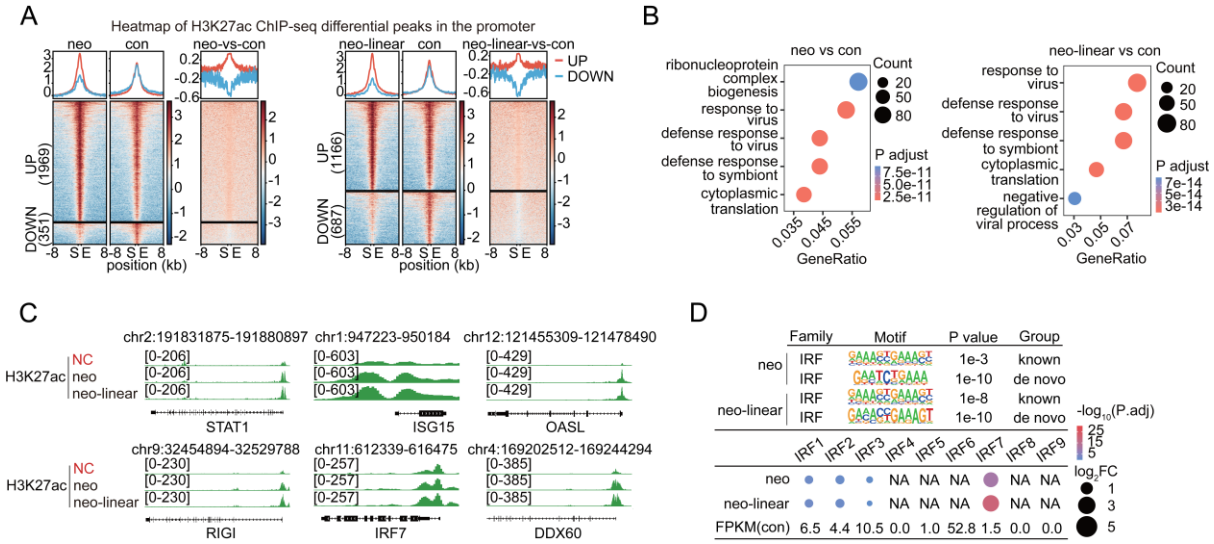


Figure S7. Epigenetic changes in NCM460 cells after transfection with circular and linear pcDNA3.1-neo plasmids.

(A) Averaged profiles (top panels) and heatmaps (bottom panels) of H3K27ac ChIP-seq signals within the ±8 kb regions flanking the differential peaks on the promoters identified in NCM460 cells transfected with circular or linear pcDNA3.1-neo plasmids compared with the control. The counts of upregulated and downregulated peaks are displayed on the left side of the heatmaps. Con: control. S: peak start site. E: peak end site. UP: upregulated peaks. DOWN: downregulated peaks. neo: pcDNA3.1-neo, neo-linear: linear pcDNA3.1-neo. n=3 independent replicates. **(B)** GO results for genes with active promoter regions containing upregulated differentially abundant ChIP-seq peaks in NCM460 cells following transfection with pcDNA3.1-neo (left panel) or linear pcDNA3.1-neo (right panel) plasmids compared with the control groups. Con: control. P adjust: adjusted p value. neo: pcDNA3.1-neo, neo-linear: linear pcDNA3.1-neo. n=3 independent replicates. **(C)** H3K27ac ChIP-seq tracks for the STAT1, ISG15, OASL, RIGI, IRF7, and DDX60 genes in NCM460 cells treated with only the transfection reagent or transfected with pcDNA3.1-neo or linear pcDNA3.1-neo plasmids. NC: negative control, neo: pcDNA3.1-neo, neo-linear: linear pcDNA3.1-neo. n=3 independent replicates. **(D)** The most significant Homer motifs (top panel) of the IRF transcription factor family from the known or de novo motif enrichment results, along with dot plots (bottom panels) illustrating the differential expression levels of all genes in the IRF family in NCM460 cells transfected with pcDNA3.1-neo or linear pcDNA3.1-neo plasmids compared with the controls. Only data for genes with increased expression are shown. The average FPKM values of the IRF family genes in the control group are indicated at the bottom. P.adj: adjusted p value. FC: fold change. neo: pcDNA3.1-neo, neo-linear: linear pcDNA3.1-neo. n=3 independent replicates.

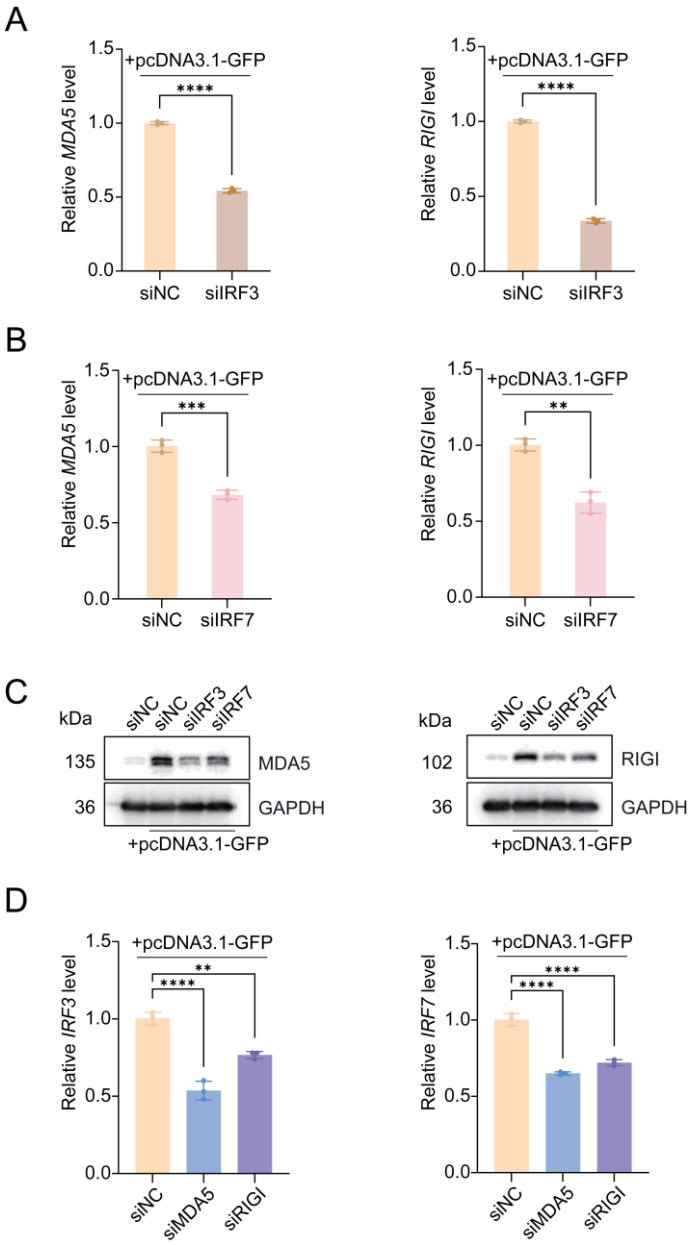


Figure S8. Evidence of mutual regulation between RNA sensors and IRF3/IRF7.

qPCR results at 24 h revealing the relative MDA5 and RIGI expression levels compared with those of the GAPDH gene after knocking down IRF3 **(A)** or IRF7 **(B)** in NCM460 cells transfected with pcDNA3.1-GFP plasmids. Statistical significance was determined using two-tailed Student's t tests. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. n=3 independent replicates. **(C)** Western blot analysis at 24 h showing the MDA5 and RIGI expression levels after knocking down IRF3 or IRF7 in NCM460 cells transfected with pcDNA3.1-GFP plasmids. **(D)** qPCR results at 24 h revealing the relative IRF3 and IRF7 expression levels compared with those of the GAPDH gene after knocking down MDA5 and RIGI in NCM460 cells transfected with pcDNA3.1-GFP plasmids. Statistical P values were calculated using Dunnett's test following ANOVA, with siNC cells transfected with pcDNA3.1-GFP used as the control group. siNC: negative control siRNA. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. n=3 independent replicates.

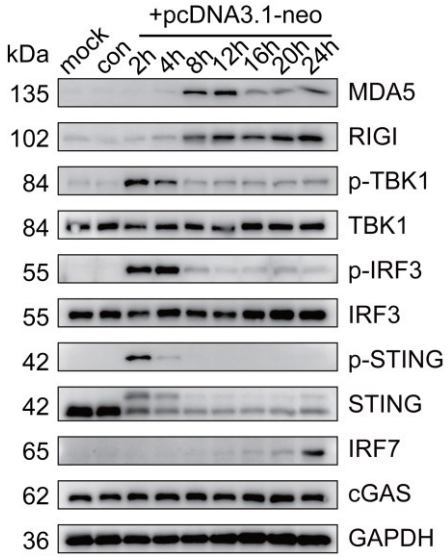
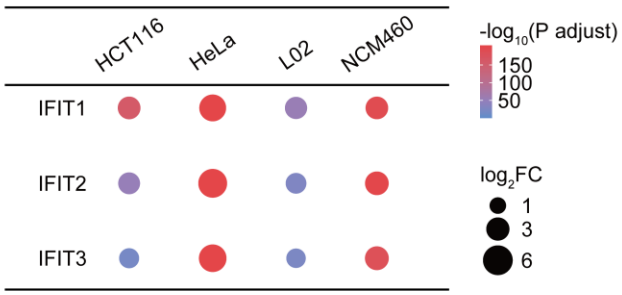


Figure S9. Western blotting of key proteins involved in the DNA- and RNA-sensing pathways.

Western blot assays examining MDA5, RIGI, p-TBK1, TBK1, p-IRF3, IRF3, p-STING, STING, IRF7, cGAS, and GAPDH levels in HeLa cells transfected with the pcDNA3.1-neo plasmid at various time points. Con: control groups treated with only the transfection reagent. mock: wild-type cells.

A



B

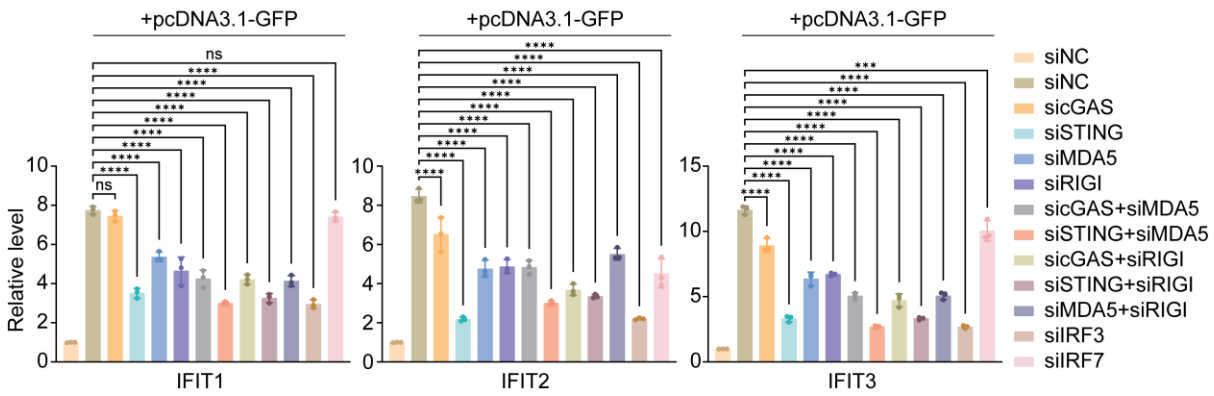


Figure S10. IFIT gene expression levels following the knockdown of key factors in the DNA- and RNA-sensing pathways.

(A) Dot plots showing the differential expression levels of IFIT1, IFIT2, and IFIT3 in HCT116, HeLa, L02, and NCM460 cells following transfection with the pcDNA3.1-neo plasmid. n=3 independent replicates. **(B)** qPCR results at 24 h revealing the IFIT1, IFIT2, and IFIT3 expression levels after the knockdown of cGAS, STING, MDA5, RIGI, IRF3, and IRF7, as well as the double knockdown of factors in the DNA- and RNA-sensing pathways in NCM460 cells transfected with pcDNA3.1-GFP plasmids. Statistical P values were calculated using Dunnett's test following ANOVA, with siNC cells transfected with pcDNA3.1-GFP used as the control group. siNC: negative control siRNA. *P < 0.05, **P <0.01, ***P < 0.001 and ****P < 0.0001. ns, not statistically significant. n=3 independent replicates.

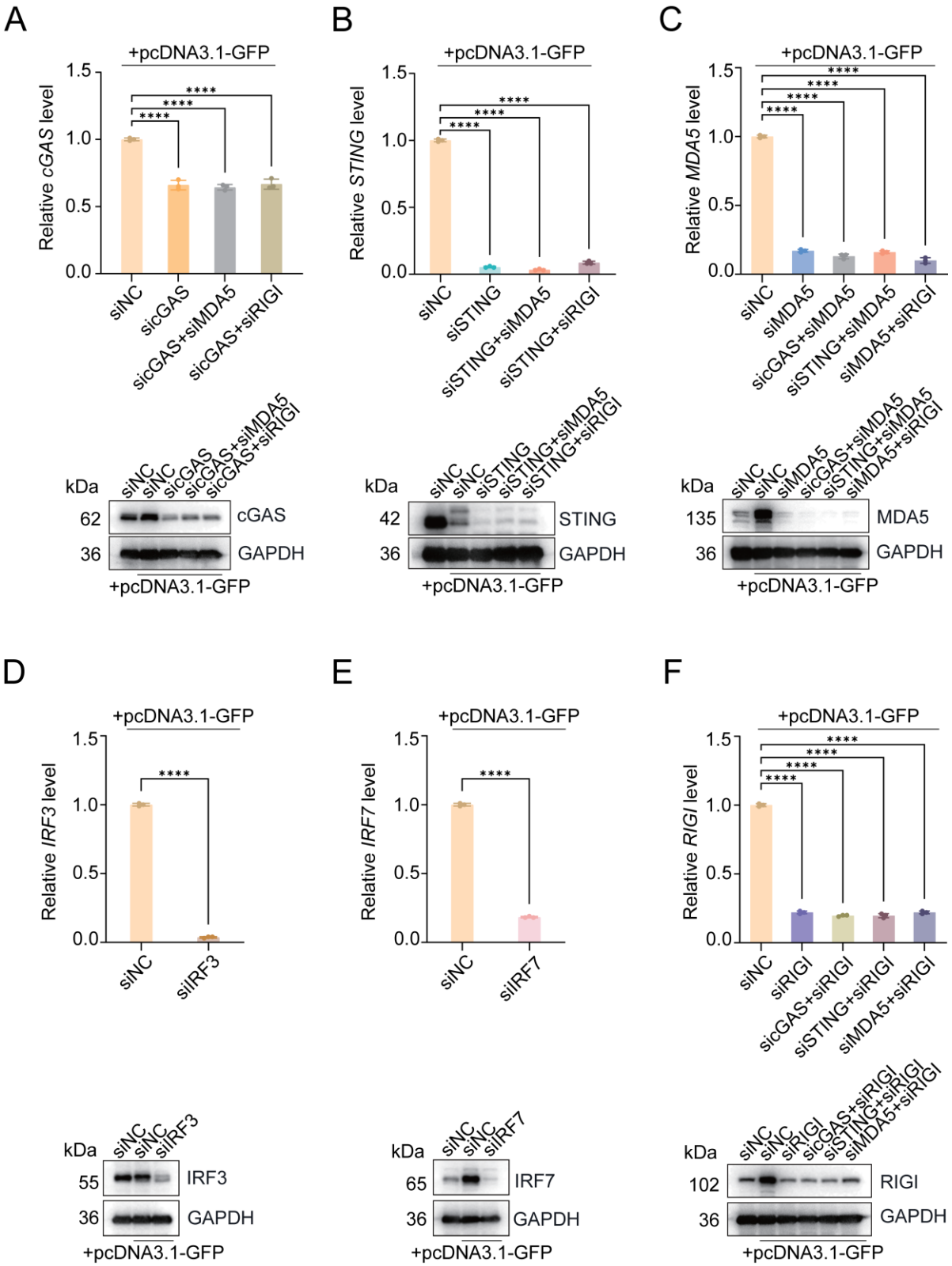


Figure S11. Efficiency of the knockdown of key factors in DNA- and RNA-sensing pathways in NCM460 cells.

qPCR results (upper panel) and Western blot results (lower panel) at 24 h showing the knockdown levels of siRNAs targeting cGAS **(A)**, STING **(B)**, MDA5 **(C)**, IRF3 **(D)**, IRF7 **(E)** and RIGI **(F)** in NCM460 cells transfected with pcDNA3.1-GFP plasmids. Statistical P values were calculated using Dunnett's test after ANOVA

for the data presented in (A), (B), (C), and (F) and via two-tailed Student's t test for the data presented in (D) and (E), with siNC cells transfected with pcDNA3.1-GFP used as the control group. siNC: negative control siRNA. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. n=3 independent replicates.

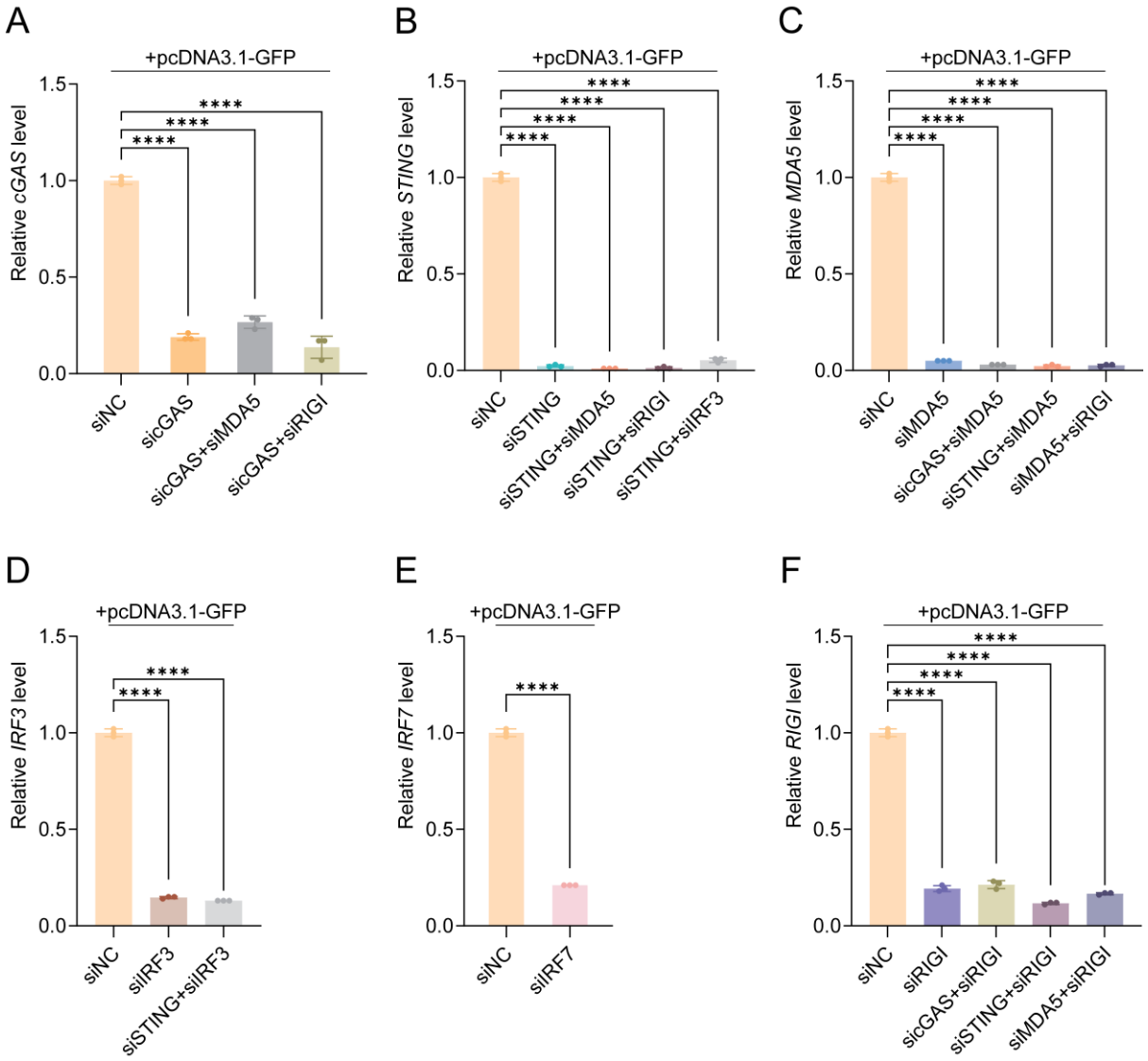


Figure S12. Efficiency of the knockdown of key factors in DNA- and RNA-sensing pathways in L02 cells. qPCR results at 24 h showing the knockdown levels of siRNAs targeting cGAS **(A)**, STING **(B)**, MDA5 **(C)**, IRF3 **(D)**, IRF7 **(E)**, and RIGI **(F)** in L02 cells transfected with pcDNA3.1-GFP plasmids. Statistical P values were calculated using Dunnett's test after ANOVA for the data presented in (A), (B), (C), (D), and (F) and via a two-tailed Student's t test for the data presented in (E), with siNC cells transfected with pcDNA3.1-GFP serving as the control group. siNC: negative control siRNA. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. n=3 independent replicates.

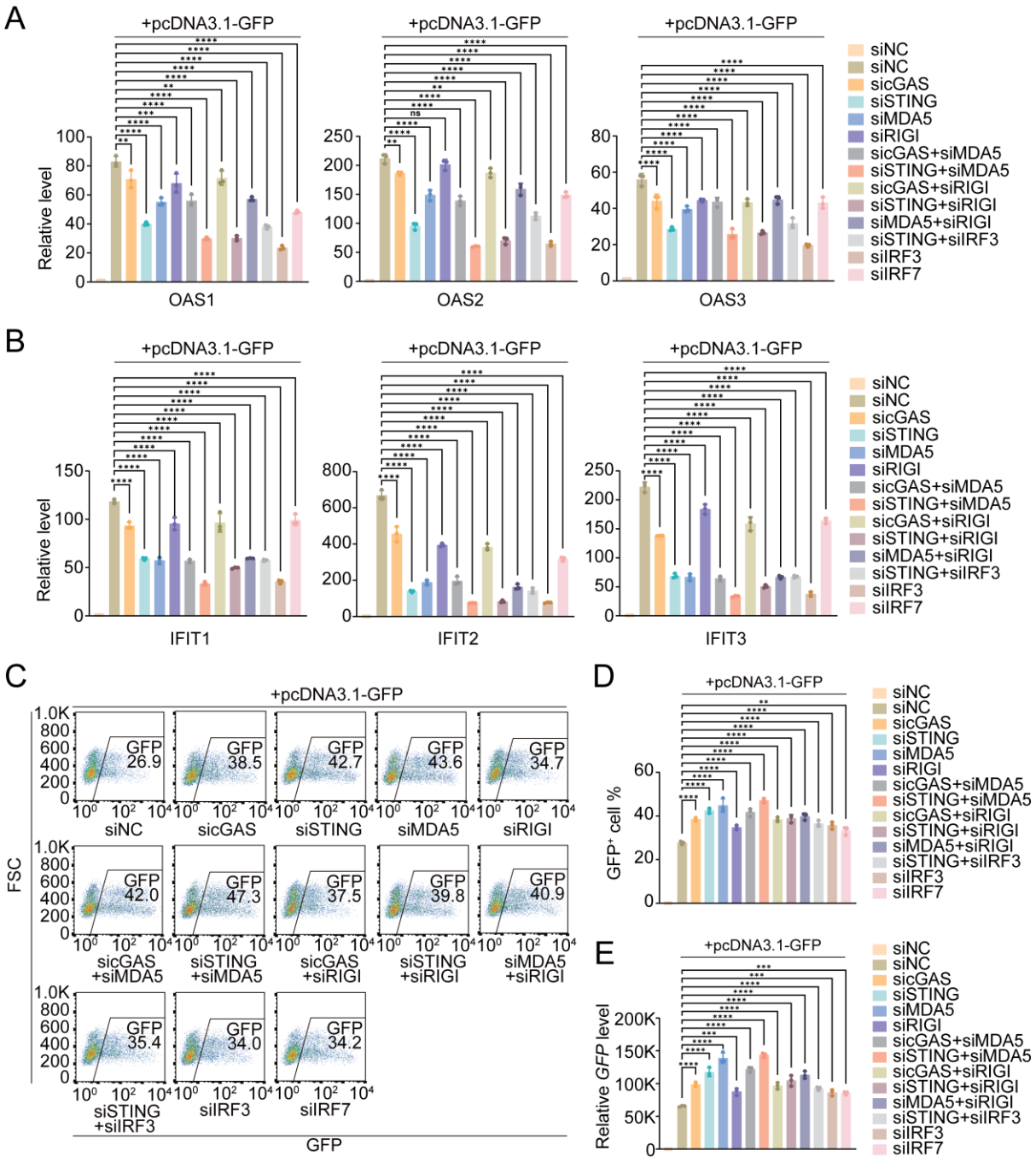


Figure S13. Knockdown of key DNA- and RNA-sensing factors increases the transfection efficiency in L02 cells.

qPCR results at 24 h revealing the relative OAS1/2/3 **(A)** and IFIT1/2/3 **(B)** expression levels compared with those of the GAPDH gene after the knockdown of the cGAS, STING, MDA5, RIGI, IRF3, and IRF7 genes, as well as the double knockdown of factors of the DNA- and RNA-sensing pathways in L02 cells transfected with pcDNA3.1-GFP plasmids. n=3 independent replicates. **(C)** Flow cytometry results of L02 cells transfected with pcDNA3.1-GFP plasmids following the knockdown of cGAS, STING, MDA5, RIGI, IRF3, and IRF7, as well as the double knockdown of factors of the DNA- and RNA-sensing pathways. NC: negative control. FSC: forward scatter. n=3 independent replicates. **(D)** GFP^+ cell ratio obtained from flow cytometry in (C). n=3 independent replicates. **(E)** qPCR results at 24 h revealing the relative GFP expression levels compared with those of the

GAPDH gene after the knockdown of cGAS, STING, MDA5, RIGI, IRF3, and IRF7, as well as the double knockdown of factors of the DNA- and RNA-sensing pathways in L02 cells transfected with pcDNA3.1-GFP plasmids. Statistical P values were calculated using Dunnett's test after ANOVA, with siNC cells transfected with pcDNA3.1-GFP used as the control group. siNC: negative control siRNA. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, ns, not statistically significant. n=3 independent replicates.

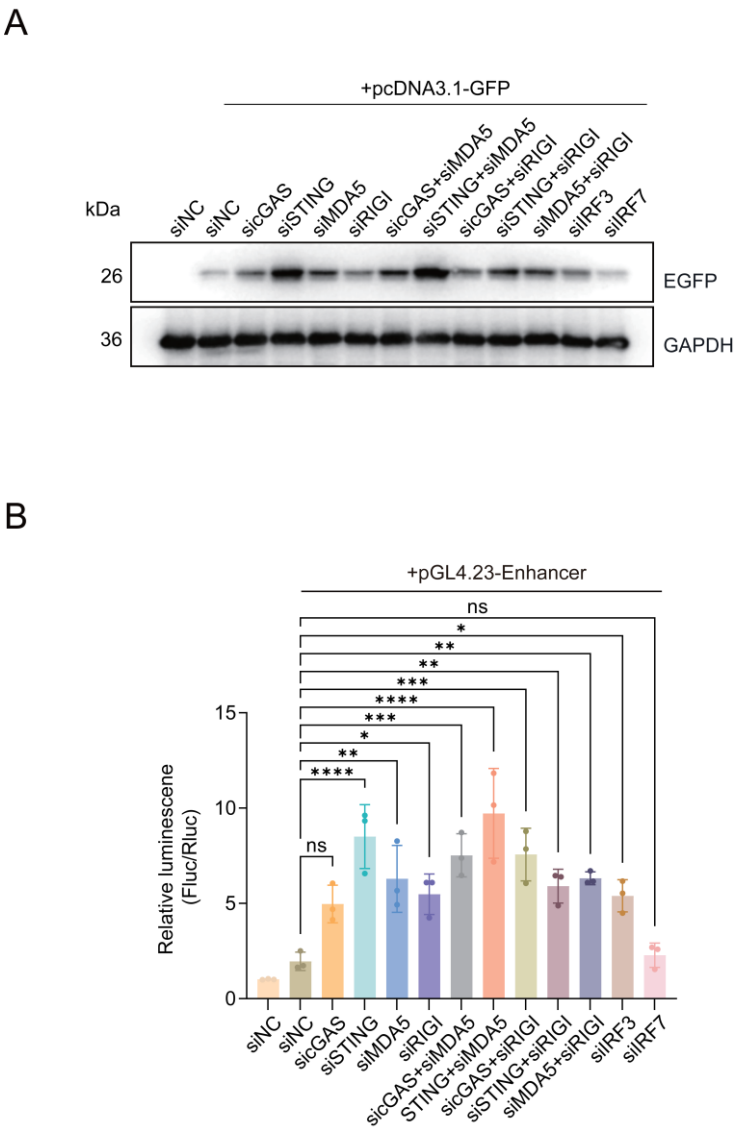


Figure S14. Increased transfection efficiency in NCM460 cells following the knockdown of DNA and RNA sensors.

(A) Western blot analysis at 24 h revealing GFP expression levels in NCM460 cells transfected with pcDNA3.1-GFP plasmids following the knockdown of cGAS, STING, MDA5, RIGI, IRF3, and IRF7, as well as the double knockdown of DNA- and RNA-sensing pathway factors. **(B)** Relative luminescence was analyzed using a dual luciferase reporter assay at 24 hours after the single-gene knockdown of cGAS, STING, MDA5, RIGI, IRF3, and IRF7 or double knockdown of both DNA- and RNA-sensing pathway factors in NCM460 cells transfected with pGL4.23-enhancer plasmids. Statistical significance was determined using Dunnett's test following ANOVA, with siNC cells transfected with pGL4.23-enhancer serving as the control group. siNC: negative control siRNA. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. ns, not statistically significant. Fluc: Firefly luciferase. Rluc: Renilla luciferase. n=3 independent replicates.

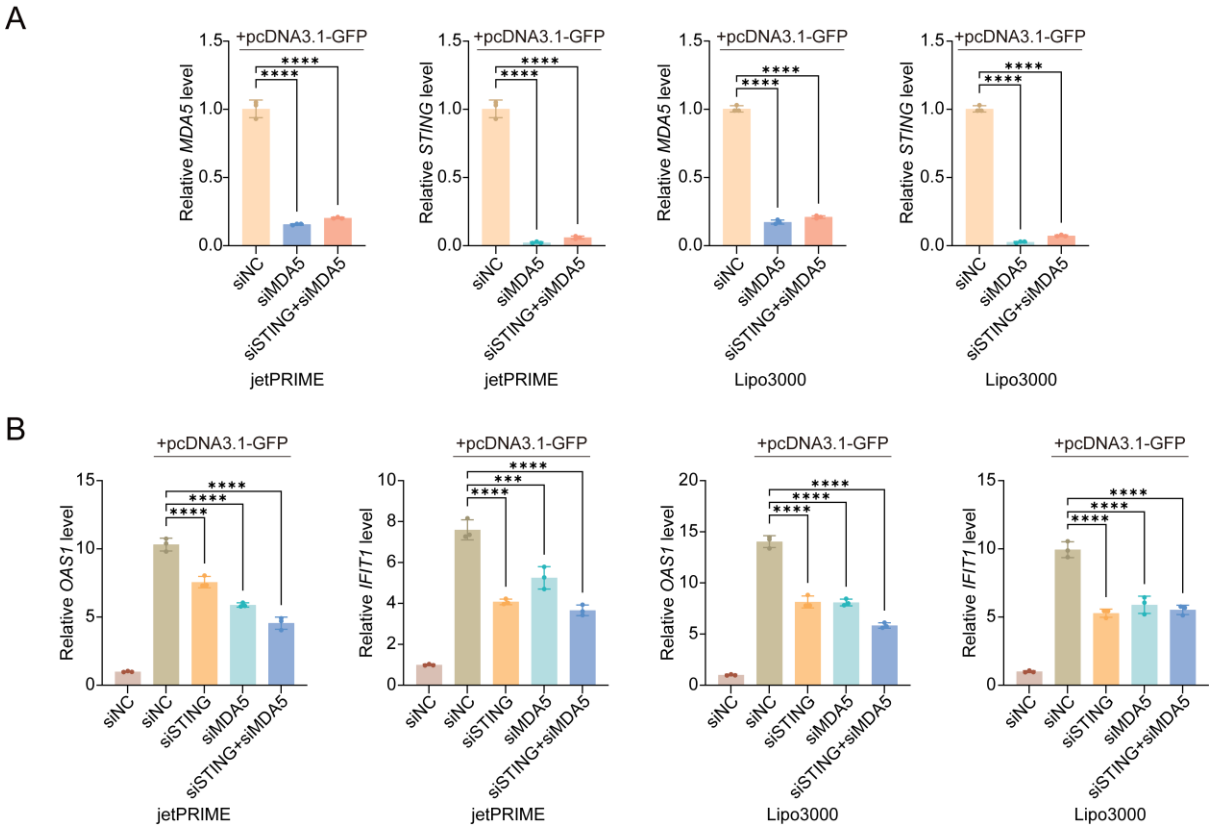


Figure S15. Knocking down STING and MDA5 reduces OAS1 and IFIT1 gene expression independent of the transfection reagent used.

(A) qPCR results at 24 h revealing the knockdown levels of siRNAs targeting MDA5 and STING in NCM460 cells transfected with pcDNA3.1-GFP using jetPRIME or Lipomaster 3000. Statistical significance was determined using Dunnett's test following ANOVA, with siNC cells transfected with pcDNA3.1-GFP used as the control group. siNC: negative control siRNA. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. Lipo3000: Lipomaster 3000; n=3 independent replicates. **(B)** qPCR results at 24 h revealing the relative OAS1 and IFIT1 expression levels compared with those of the GAPDH gene after the knockdown of STING or MDA5 in NCM460 cells transfected with pcDNA3.1-GFP plasmids using jetPRIME or Lipomaster 3000. Statistical significance was determined using Dunnett's test following ANOVA, with siNC cells transfected with pcDNA3.1-GFP used as the control group. siNC: negative control siRNA. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. Lipo3000: Lipomaster 3000; n=3 independent replicates.

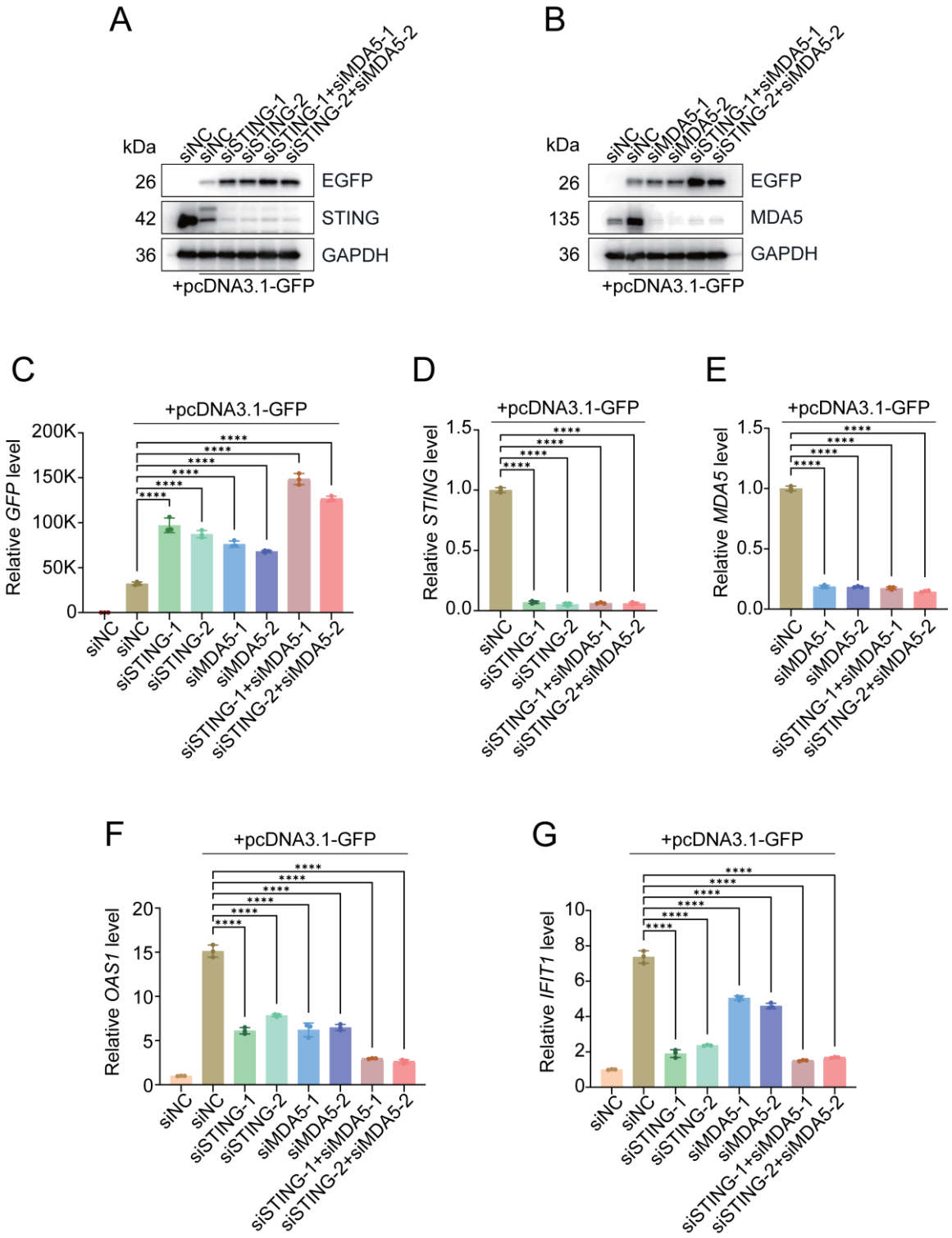


Figure S16. Elimination of the off-target effects of the siRNAs in the STING and MDA5 knockdown experiments.

Western blot analysis at 24 h showing the levels of STING **(A)** and MDA5 **(B)**, as well as the expression levels of EGFP, after STING or MDA5 were knocked down using two siRNAs with different targets in NCM460 cells transfected with pcDNA3.1-GFP plasmids. The siRNAs are designated -1 and -2, representing two different siRNA constructs. qPCR results at 24 h revealing the relative expression levels of GFP **(C)**, STING **(D)**, MDA5 **(E)**, OAS1 **(F)** and IFIT1 **(G)** compared with those of the GAPDH gene after knocking down STING or MDA5

using two siRNAs targeting different sites in NCM460 cells transfected with pcDNA3.1-GFP plasmids. Statistical P values were calculated using Dunnett's test after ANOVA, with siNC cells transfected with pcDNA3.1-GFP used as the control group. siNC: negative control siRNA. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. The siRNAs are designated -1 and -2, representing two different siRNA constructs. n=3 independent replicates.

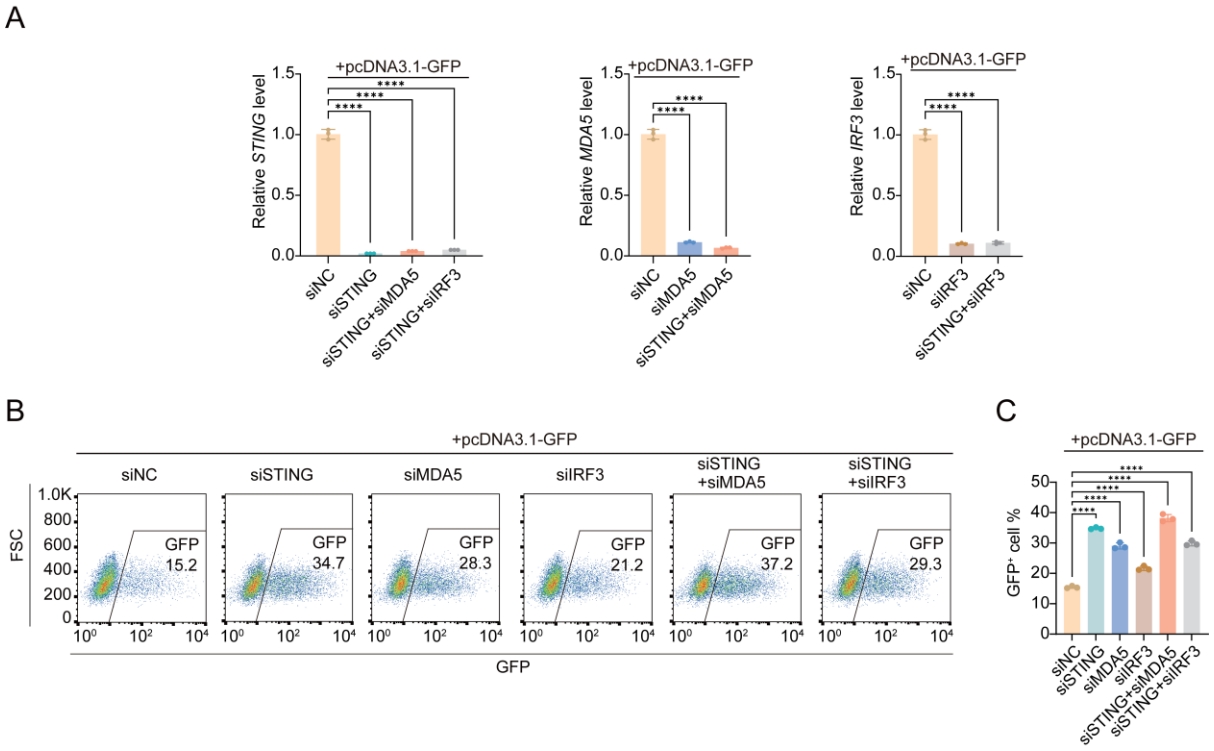


Figure S17. Transfection efficiency was improved with STING/MDA5 knockdown compared with STING/IRF3 knockdown.

(A) qPCR results at 24 h revealing the knockdown levels of siRNAs targeting STING, MDA5, and IRF3 in NCM460 cells transfected with pcDNA3.1-GFP plasmids. Statistical P values were calculated using Dunnett's test after ANOVA, with siNC cells transfected with pcDNA3.1-GFP used as the control group. siNC: negative control siRNA. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. n=3 independent replicates. **(B)** Flow cytometry revealing the impacts of cGAS, STING, MDA5, and IRF3 single and double (STING/MDA5 and STING/IRF3) knockdown in pcDNA3.1-GFP-transfected NCM460 cells. NC: negative control. FSC: forward scatter. n=3 independent replicates. **(C)** GFP^+ cell ratio from the flow cytometry assay in (B). Statistical P values were calculated using Dunnett's test following ANOVA, with siNC cells transfected with pcDNA3.1-GFP used as the control group. siNC: negative control siRNA. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. n=3 independent replicates.

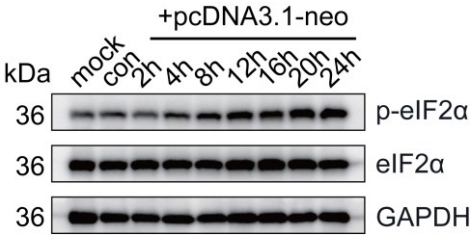


Figure S18. Western blots of eIF2α and p-eIF2α levels.

Western blot assay examining p-eIF2α, eIF2α, and GAPDH levels in HEK293T cells transfected with the pcDNA3.1-neo plasmid at various time points. con: control groups treated with only the transfection reagent. mock: wild-type cells.

Table S1. Common IIR genes identified in low-transfection-efficiency cells transfected with linear DNAs

Genes activated by linear pcDNA3.1-neo plasmids	Genes activated by linear pre-gRNA plasmids
EIF2AK2	EIF2AK2
OAS1	OAS1
RIGI	RIGI
OAS3	OAS3
MDA5	MDA5
STAT1	STAT1
IFIT3	IFIT3
IFIT2	IFIT2
IFI6	IFI6
BST2	BST2
SHFL	SHFL
RSAD2	RSAD2
OASL	OASL
DDX60	DDX60
IFI44L	IFI44
IFI44	PARP9
PARP9	HERC5
NLRC5	NLRC5
MX1	MX1
DTX3L	DTX3L
IFI27	IFI27
LGALS9	LGALS9
USP18	USP18
IRF7	IRF7
IFIT1	IFIT1
ISG15	ISG15
CCL5	

Table S2. Unique upregulated H3K27ac ChIP-seq peaks in HeLa cells transfected with foreign DNA

Upregulated peaks activated by pcDNA3.1-neo plasmids					
chr1:10659789-10660189	chr11:35289431-35289831	chr16:28222626-28223026	chr2:241377773-241378173	chr4:140273062-140273462	chr6:88415753-88416153
chr1:109262660-109263060	chr11:36531515-36531915	chr16:2845516-2845916	chr2:28022397-28022797	chr4:14291538-14291938	chr6:9789838-9790238
chr1:110461729-110462129	chr11:393859-394259	chr16:29973586-29973986	chr2:28519597-28519997	chr4:15010124-15010524	chr7:100781578-100781978
chr1:113277971-113278371	chr11:43977693-43978093	chr16:30671471-30671871	chr2:28631236-28631636	chr4:15044197-15044597	chr7:101426694-101427094
chr1:115297289-115297689	chr11:45920934-45921334	chr16:3071011-3071411	chr2:29120088-29120488	chr4:169237838-169238238	chr7:104738318-104738718
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Note: The notation "chromosome:start_position-end_position" signifies the peak location.

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chr10:91087314-91087714	chr15:58920714-58921114	chr2:172873220-172873620	chr3:41200811-41201211	chr6:2791054-2791454	chrX:118018807-118019207
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chr10:95187454-95187854	chr15:59827343-59827743	chr2:178076806-178077206	chr3:46152963-46153363	chr6:35606561-35606961	chrX:128796776-128797176
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chr11:110586747-110587147	chr15:63664122-63664522	chr2:191878621-191879021	chr3:49383908-49384308	chr6:41888862-41889262	chrX:32471713-32472113
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chr11:122056907-122057307	chr15:67409486-67409886	chr2:193132010-193132410	chr3:5166192-5166592	chr6:43595376-43595776	chrX:45207547-45207947
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chr11:13095322-13095722	chr15:74818135-74818535	chr2:19735916-19736316	chr3:57985708-57986108	chr6:44307281-44307681	chrX:70823159-70823559
chr11:13237251-13237651	chr15:74822931-74823331	chr2:201247497-201247897	chr3:64075700-64076100	chr6:45572582-45572982	chrX:83756841-83757241
chr11:13359403-13359803	chr15:75092169-75092569	chr2:201832549-201832949	chr3:64123961-64124361	chr6:45582337-45582737	chrX:9450136-9450536
chr11:14541564-14541964	chr15:75400590-75400990	chr2:201993272-201993672	chr3:72242721-72243121	chr6:47260143-47260543	chrX:9834369-9834769
chr11:16760049-16760449	chr15:75404887-75405287	chr2:202015598-202015998	chr3:73115632-73116032	chr6:47382497-47382897	

Note: The notation "chromosome:start_position-end_position" signifies the peak location.

Table S3. All primer and siRNA sequences used in this study

Names	Species	Forward primer	Reverse primer
ISG15	human	5'-CGCAGATCACCCAGAAGATCG-3'	5'-TTCGTCGCATTTGTCCACCA-3'
IFI27	human	5'-TGCTCTCACCTCATCAGCAGT-3'	5'-CACAACTCCTCCAATCACAACT-3'
IRF7	human	5'-CCCAGCAGGTAGCATTCCC-3'	5'-GCAGCAGTTCCTCCGTGTAG-3'
MX1	human	5'- AGCGGGATCGTGACCAGAT-3'	5'-TGACCTTGCCTCTCCACTTATC-3'
OAS1	human	5'-TGTCCAAGGTGGTAAAGGGTG-3'	5'-CCGGCGATTTAACTGATCCTG-3'
OAS2	human	5'-CTCAGAAGCTGGGTTGGTTTAT-3'	5'-ACCATCTCGTCGATCAGTGTC-3'
OAS3	human	5'-GAAGGAGTTCGTAGAGAAGGCG-3'	5'-CCCTTGACAGTTTTCAGCACC-3'
OASL	human	5'-CTGATGCAGGAACTGTATAGCAC-3'	5'-CACAGCGTCTAGCACCTCTT-3'
cGAS	human	5'-CACGAAGCCAAGACCTCCG-3'	5'-GTCGCACTTCAGTCTGAGCA-3'
STING	human	5'-CCAGAGCACACTCTCCGGTA-3'	5'-CGCATTTGGGAGGGAGTAGTA-3'
RIGI	human	5'-CTGGACCCTACCTACATCCTG-3'	5'-GGCATCCAAAAAGCCACGG-3'
MDA5	human	5'-TCGAATGGGTATTCCACAGACG-3'	5'-GTGGCGACTGTCCTCTGAA-3'
IRF3	human	5'-AGAGGCTCGTGATGGTCAAG-3'	5'-AGGTCCACAGTATTCTCCAGG-3'
IFIT1	human	5'-GCGCTGGGTATGCGATCTC-3'	5'-CAGCCTGCCTTAGGGGAAG-3'
IFIT2	human	5'-AAGCACCTCAAAGGGCAAAAC-3'	5'-TCGGCCCATGTGATAGTAGAC-3'
IFIT3	human	5'-TCAGAAGTCTAGTCACTTGGGG-3'	5'-ACACCTTCGCCCTTTCATTTC-3'
EGFP	human	5'-GTGACCACCCTGACCTACG-3'	5'-TCAGCTCGATGCGGTTCAC-3'
GAPDH	human	5'-GGAGCGAGATCCCTCCAAAAT-3'	5'-GGCTGTTGTCATACTTCTCATGG-3'

Names	Species	siRNA sequence
IRF3-1	human	GAUCUGAUUACCUUCACGGAA
IRF3-2	human	CCCUUCAUUGUAGAUCUGAUU
IRF7-1	human	GCUGGACGUGACCAUCAUGUA
IRF7-2	human	CCCGAGCUGCACGUUCCUAUA
RIGI-1	human	GGAUUGUUACAGUUCAGAAUU
RIGI-2	human	GCCCUGUUUUUAUACACUUUU
MDA5-1	human	GUAACAUUGUUAUCCGUUAUU
MDA5-2	human	GGUGUAAGAGAGCUACUAAUU
STING-1	human	GGAUUCGAACUUACAAUCAUU
STING-2	human	GGUCAUAUUACAUCGGAUAUU
cGAS-1	human	GGAAGAAAUUAACGACAUUUU
cGAS-2	human	CCAACACUCGUGCAUAUUAUU

Table S4. FPKM values of the cGAS and IFI16 genes in the wild-type HEK293T and HCT116 cell lines

Gene	HEK293T_WT_rep1	HEK293T_WT_rep2	HEK293T_WT_rep3	HCT116_WT_rep1	HCT116_WT_rep2	HCT116_WT_rep3
cGAS	0.019739833	0	0	0	0	0
IFI16	0.030985646	0.015843108	0.039085144	0.016557617	0	0.015505598

Note: "WT" denotes wild type.