Appendix
An alternatively transcribed TAZ variant negatively regulates JAK-STAT signaling
Fang et al., 2019

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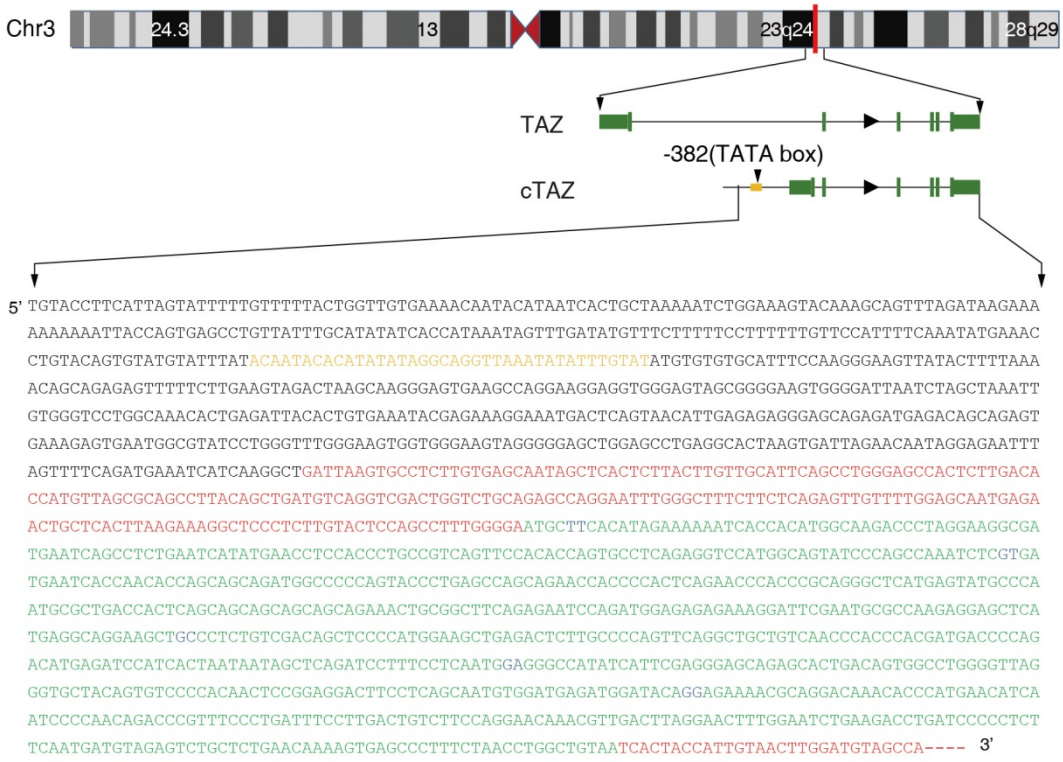
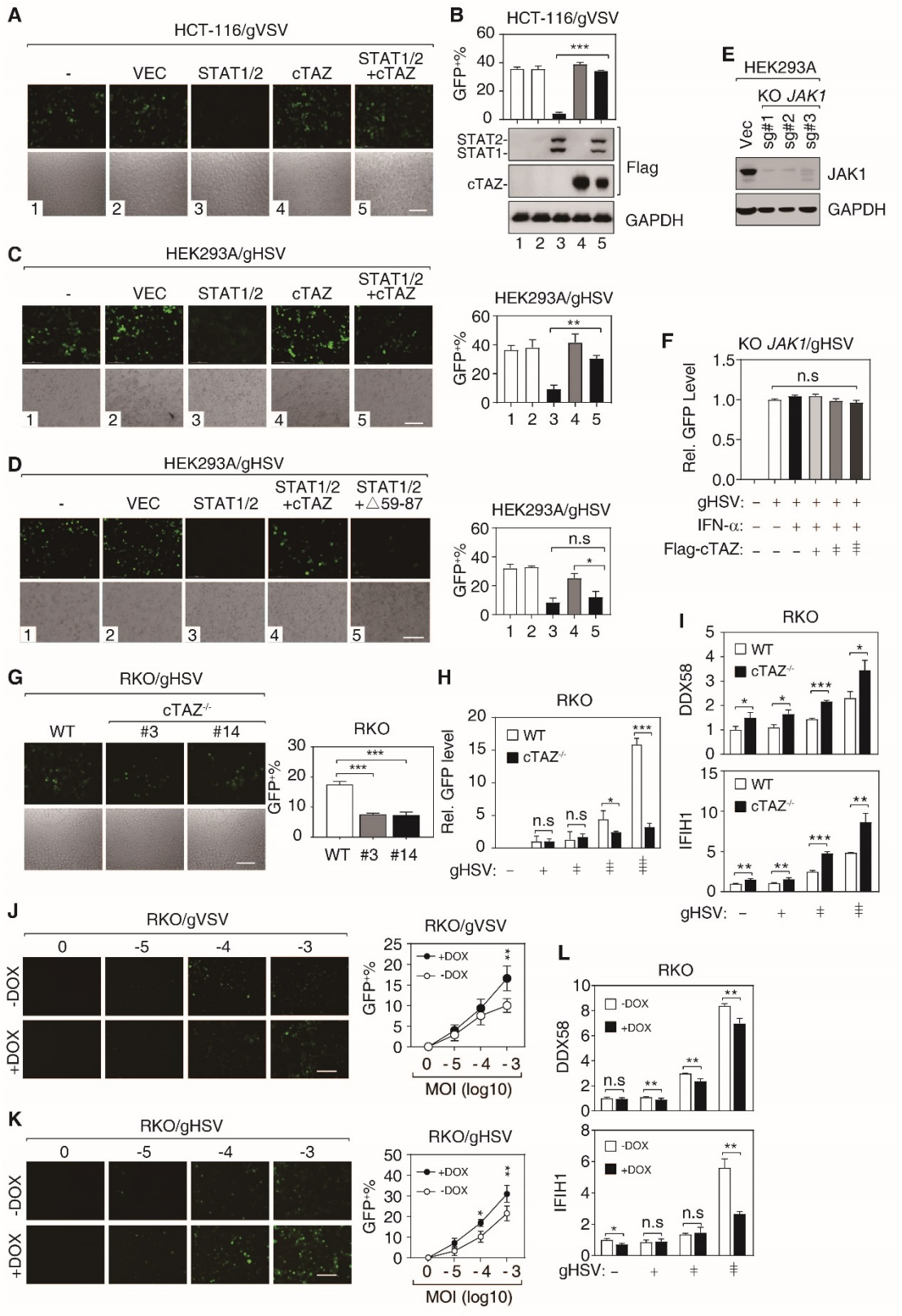


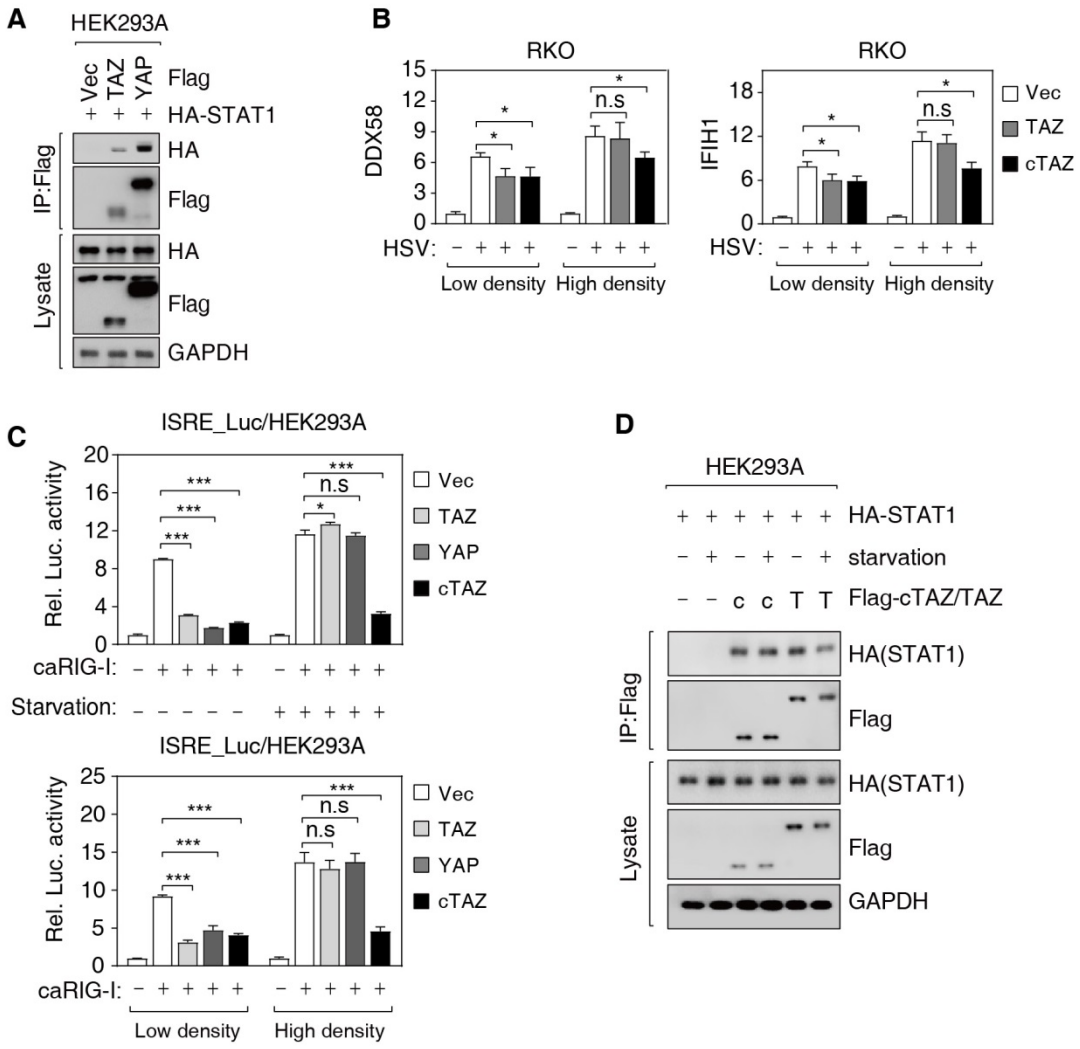
Appendix Fig S1. The promoter, 5'UTR, and exon sequences of cTAZ.
Proscan online tool was used for promoter and TATA box prediction (https://wwwbimas.cit.nih.gov/molbio/proscan/). The sequence in green indicates CDS, red for UTR, black for the predicated promoter, and yellow for the predicated TATA box.



Appendix Fig S2. cTAZ negatively regulated antiviral response.

A. Overexpression of cTAZ repressed the antiviral activity of STAT1/2. HCT-116 cells were transfected with indicated plasmids and infected with gVSV (MOI = 0.01) for 12 hours. GFP signal indicated the infection of gVSV. Scale bar, 100 μm.

B. Quantification of GFP positive cells in S2A. Protein levels were determined by IB.
C. cTAZ overexpression repressed cellular antiviral response. HEK293A cells were transfected with indicated plasmids and infected with gHSV (MOI = 0.01) for 12 hours. (Left) Viral infection was determined by green fluorescence. Scale bar, 100 μm. (Right) Quantification of GFP positive cells.
D. Amino acids 59-87 deleted cTAZ did not affect cellular antiviral response. HEK293A cells were transfected with indicated plasmids. Virus infection and quantification of GFP positive cells were performed as in S2C. Scale bar, 100 μm.
E. JAK1 deletion in HEK293A cells using CRISPR/cas9 technology. Three independent sgRNA were used.
F. cTAZ overexpression did not affect cellular antiviral activity in JAK1 KO cells. Transfection, infection and quantification were performed as in S2C.
G. cTAZ deficiency potentiated cellular antiviral activity. RKO cell lines (WT and two independent *cTAZ*$^{-/-}$ clones) were infected with gHSV (MOI = 0.1) for 12 hours. Scale bar, 100 μm.
H. Deletion of cTAZ enhanced cellular antiviral activity. RKO cell lines (WT and #4 *cTAZ*$^{-/-}$) were infected using gHSV for 12 hours, and fluorescence intensity in lysed cells was measured.
I. cTAZ deficiency promoted ISGs expression. Same cell lines and treatment were used as in S2H. mRNA levels of *DDX58* and *IFIH1* were measured by qPCR.
J and K. cTAZ overexpression enhanced cellular antiviral activity. DOX-inducible cTAZ-overexpressing cell lines were treated with or without DOX (1 μg/ml) for 48 hours, and challenged with gVSV (J) or gHSV (K) for 24 hours, viral infection was determined by GFP intensity. Scale bar, 100 μm.
L. cTAZ overexpression repressed ISGs expression. Same cell lines and treatments were used as in S2J. mRNA levels of *DDX58* and *IFIH1* were measured by qPCR.



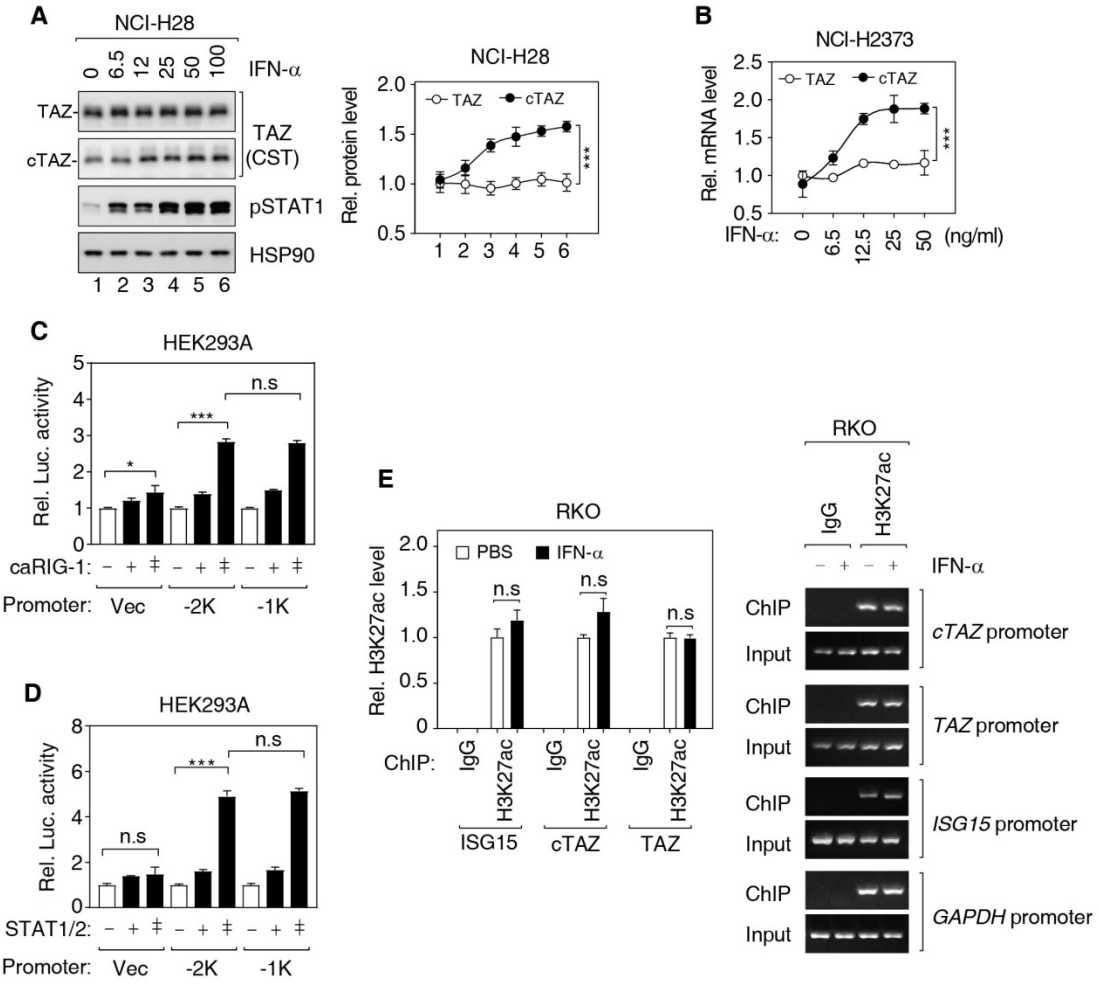
Appendix Fig S3. YAP/TAZ could repress ISGs expression in a Hippo signaling-dependent manner

A. YAP/TAZ interacted with STAT1. HEK293A cells were transfected with indicated plasmids. IP assays were performed using Flag antibody.

B. The inhibitory function of TAZ on ISGs expression was blocked at high cell density. DOX-inducible TAZ- or cTAZ-overexpressing RKO cells were seed at different densities. Following DOX treatment, cells were infected using gHSV (MOI=0.1) for 12 hours. The ISGs expression were determined by qPCR.

C. The inhibitory function of TAZ on ISRE-luciferase activity was blocked at high cell density or under serum starvation. HEK293A cells were transfected with plasmids indicated, plated at different cell densities. For serum deprivation, 12 hours before harvesting cells, culture medium was replaced with serum-free DMEM. ISRE reporter activity were determined by luciferase assays.

D. The interaction between TAZ and STAT1 was down-regulated under serum starvation. HEK293A cells were transfected with indicated plasmids. Cells were serum-starved for 24 hours, and subjected to IP using Flag antibody.



Appendix Fig S4. cTAZ expression was induced by JAK-STAT signaling.

A. IFN-α induced the expression of cTAZ proteins. NCI-H28 cells were treated with IFN-α for 8 hours, and protein expression was determined by IB (Left). Quantification was performed using ImageJ software (Right). Two-way ANOVA was used for statistical analysis, n=3.

B. IFN-α induced the expression of cTAZ mRNA. NCI-H2373 cells were treated with IFN-α for 8 hours, mRNA levels of TAZ and cTAZ were determined by qPCR. Two-way ANOVA was used for statistical analysis, n=3.

C. Overexpression of caRIG-I activated cTAZ promoters. Luciferase reporters for 2K and 1K cTAZ promoters (2000 and 1000 bp upstream of translation starting site respectively) and caRIG-I were transfected into HEK293A cells, luciferase activity was measured 12 hours later.

D. Overexpression of STAT1/2 activated cTAZ promoters. As in S4C, except STAT1/2 was used instead of caRIG-I.

E. IFN-α could not modulate H3K27ac levels of cTAZ promoter. RKO cells were treated with or without IFN-α (50 ng/ml) for 1 hour and subjected to ChIP assays.

Appendix Table S1. Protein expression of cTAZ in different cell lines.

Cell type	cTAZ
293a	×
MCF-7	×
92.1	√
NCI-H28	√
HUVEC	√
RKO	√√
ACHN	√
CaCO2	√√
HCT-15	×
293T	×
MCF-10A	×
NCI-H2373	√
NCI-H2452	√
NCI-H2052	×
LS174T	×
RCC4	√
HCT-116	√
MSTO-211H	×
SW620	×
HeLa	×
MESO-12	×

Appendix Table S2. mRNA expression of cTAZ in human tissues

Tissue	Count mean	Count max	Count median	Positive	Total	Positive %
Adipose_Tissue	3.92	15.19	2.91	213	515	41.36
Adrenal_Gland	3.31	9.74	2.94	21	128	16.41
Bladder	3.41	4.61	2.99	3	9	33.33
Blood	2.67	20.58	1.69	38	444	8.56
Blood_Vessel	3.85	17.20	2.78	220	606	36.3
Bone_Marrow	3.17	6.72	3.07	27	70	38.57
Brain	4.94	98.40	2.67	167	1152	14.5
Breast	4.19	17.02	2.63	73	179	40.78
Cervix_Uteri	2.38	2.62	2.34	5	10	50
Colon	3.32	11.56	2.52	90	308	29.22
Esophagus	4.04	25.15	3.14	214	653	32.77
Fallopian_Tube	3.17	3.70	3.17	2	5	40
Heart	4.21	21.19	3.09	123	377	32.63
Kidney	4.28	7.67	3.59	4	28	14.29
Liver	3.65	8.94	2.80	15	110	13.64
Lung	5.25	77.95	2.90	129	288	44.79
Muscle	4.24	22.36	3.18	40	396	10.1
Nerve	3.68	12.03	2.69	129	278	46.4
Ovary	4.51	20.76	3.47	51	88	57.95
Pancreas	2.80	5.89	2.30	23	167	13.77
Pituitary	3.29	11.56	2.36	34	107	31.78
Prostate	3.33	11.79	2.52	38	100	38
Salivary_Gland	4.68	12.11	3.35	21	55	38.18
Skin	4.98	24.24	3.75	261	812	32.14
Small_Intestine	3.14	10.55	2.37	15	92	16.3
Spleen	2.46	7.86	1.64	18	100	18
Stomach	4.01	10.31	3.26	34	174	19.54
Testis	5.55	42.88	4.16	40	165	24.24
Thyroid	4.29	29.34	2.79	109	279	39.07
Uterus	4.20	7.62	3.67	28	78	35.9
Vagina	4.20	13.01	3.18	33	85	38.82
total				2218	7858	28.23

Positive: cTAZ (ENST00000472417) transcript count is more than one
Total: the total number of samples involved in this analysis
Positive %: the percentage of samples showing cTAZ expression

https://toil.xenahubs.net/download/gtex_Kallisto_est_counts.gz
https://toil.xenahubs.net/download/GTEX_phenotype.gz

Appendix Table 3. Antibodies used in this study

Antibody	Company	Catalog #	Source	Dilution factor			
				IB	IP	ChIP	IF
pYAP(S127)	CST	13008S	Rabbit	1:1000			
TAZ(CST)	CST	4883S	Rabbit	1:1000	1:500		
YAP/TAZ	CST	8418S	Rabbit	1:1000	1:250		
anti-TAZ(SA)	Sigma	HPA007415	Mouse		1:500		
CTGF	Santa Cruz	sc-14939	Goat	1:500			
CYR61	Santa Cruz	sc-374129	Mouse	1:500			
GAPDH	Santa Cruz	sc-32233	Mouse	1:5000			
Vinculin	GeneTex	GTX113294	Rabbit	1:5000			
HSP90	BD	610418	Mouse	1:5000			
GFP	GeneTex	GTX113617	Rabbit	1:2000			
Flag(M2)	Sigma	F1804-1MG	Mouse	1:2000	1:500		1:250
HA tag	Santa Cruz	sc-7392	Mouse	1:2000			
HA tag	Santa Cruz	sc-805	Rabbit				1:500
E-cadherin	BD	610182	Mouse	1:1000			
N-cadherin	BD	610920	Mouse	1:1000			
Vimentin	BD	550513	Mouse	1:1000			
IRF3	Santa Cruz	sc-33641	Mouse	1:500			
JAK1	Santa Cruz	sc-1677	Mouse	1:500			
IRF7	Santa Cruz	sc-74471	Mouse	1:500			
IRF9	Santa Cruz	sc-365893	Mouse	1:500			
MX1	Santa Cruz	sc-166412	Mouse	1:500			
RIG-I	Santa Cruz	sc-376845	Mouse	1:500			
H3K27ac	Sigma	07-360-S	Rabbit			1:500	
STAT1	BD	610115	Mouse	1:1000	1:500	1:500	1:200
anti-STAT2	BD	610187	Mouse	1:1000			
pSTAT5Y694	CST	4322	Rabbit	1:1000			
pSTAT3Y705	CST	9145	Rabbit	1:1000			
pSTAT3S727	CST	9134	Rabbit	1:1000			
pSTAT1Y701	CST	7649	Rabbit	1:1000			
α-Mouse IgG	MRB	MR-M100	Goat	1:2000			
α-Rabbit IgG	MRB	MR-R100	Goat	1:2000			
AlexFluor 555	Invitogen	A21428	Goat				1:1000
AlexFluor 488	Invitogen	A11001	Goat				1:1000

CST: Cell Signaling Technology;
BD: BD Biosciences;
Santa Cruz: Santa Cruz Biotechnology;
Sigma: Sigma-Aldrich;
MRB: MRBiotech.

Appendix Table 4. Oligos used in this study

Use	Oligos	Sequences
sgRNA	IRF3_sg#1F	CACCGATTACCTTCACGGAAGGAAG
	IRF3_sg#1R	AAACCTTCCTTCCGTGAAGGTAATC
	IRF3_sg#2F	CACCGTCTCCGGACACCAATGGTGG
	IRF3_sg#2R	AAACCCACCATTGGTGTCCGGAGAC
	IRF3_sg#3F	CACCGCAACCCTTCTTTGCGGTTG
	IRF3_sg#3R	AAACCAACCGCAAAGAAGGGTTGC
	IRF7_sg#1F	CACCGCTGAGCGCGTACACCTTGTG
	IRF7_sg#1R	AAACCACAAGGTGTACGCGCTCAGC
	IRF7_sg#2F	CACCGTGCCCCAGCTGGTGACAAGG
	IRF7_sg#2R	AAACCCTTGTCACCAGCTGGGGCAC
	IRF7_sg#3F	CACCGCCGCACGGTGCTGCAGAAGG
	IRF7_sg#3R	AAACCCTTCTGCAGCACCGTGCGGC
	JAK1_sg#1F	CACCGTGTCGGACAGGGAGCCCCTC
	JAK1_sg#1R	AAACGAGGGGCTCCCTGTCCGACAC
	JAK1_sg#2F	CACCGAGGGACATCTTGTCATCAA
	JAK1_sg#2R	AAACTTGATGACAAGATGTCCCTC
	JAK1_sg#3F	CACCGTGAGCTGGCATCAAGGAGAG
	JAK1_sg#3R	AAACCTCTCCTTGATGCCAGCTCAC
	cTAZ-sg#1F	CACCGCCCAAAGGCTGGAGTACAAG
	cTAZ-sg#1R	AAACCTTGTACTCCAGCCTTTGGGC
	cTAZ-sg#2F	CACCGCTTTTAATTAACAAATCCAC
	cTAZ-sg#2R	AAACGTGGATTTGTTAATTAAAAGC
qPCR	DDX58_F	TGGTTTAGGGAGGAAGAGGTG
	DDX58_R	CCCAACTTTCAATGGCTTCAT
	IRF7_F	CGGCTGGAAAACCAACTTCC
	IRF7_R	GGGCTTGGAGTCCAGCATGT
	IFIH1_F	TTCAACCACAGTTCAGCCAA
	IFIH1_R	TGCTCTTGCTGCCACATTCT
	STAT1_F	CCCTTCTGGCTTTGGATTGA
	STAT1_R	GTCAGGTTCGCCTCCGTTCT
	OAS1_F	GGGTGGAGTTCGATGTGCTG
	OAS1_R	AGTGCTTGACTAGGCGGATG
	MX1_R	CAGAGGCAGGAGACAATCAG
	MX1_F	TTCAGGTGGAACACGAGGTT
	IRF9_F	GAAGACTCGCCTGCGCTGTG
	IRF9_R	TGTGCTGTCGCTTTGATGGT
	VSV_1F	ACGGCGTACTTCCAGATGG
	VSV_1R	CTCGGTTCAAGATCCAGGT
	cTAZ_F	AAGGCTCCCTCTTGTACTCC
	cTAZ_R	CATCTGCTGCTGGTGTTGGT

	TAZ_F	GCTGGGAGATGACCTTCACG
	TAZ_R	CTGCTGGCTCAGGGTACTGG
ChIP	TAZ_F	CCCCAAGTCCGTGGTAAACT
	TAZ_ R	TGGGTAAGAGGAGACGGGTG
	GAPDH_ F	GATGCCAGGAGCCAGGAGATG
	GAPDH_ R	TCAGGCAAAGGCCTAGGAGGG
	ISG15_F	CCGCTCACTCTGGGGCATG
	ISG15_R	GCTTCGGCAGGCAGCACCG
	CTAZ_F	ATCTGGAAAGTACAAAGCAGTT
	CTAZ_R	ACCTGCCTATATATGTGTATTGT
shRNA	YAP1_F	CCGGGCCACCAAGCTAGATAAAGAACTC GAGTTCTTTATCTAGCTTGGTGGCTTTTTG
	TAZ_F	CCGGGCGTTCTTGTGACAGATTATACTCG AGTATAATCTGTCACAAGAACGCTTTTTG