iScience, Volume *26*

Supplemental information

CircSOBP suppresses the progression of glioma by disrupting glycolysis and promoting the MDA5-mediated immune response

Maolin Mu, Wanxiang Niu, Fang Chu, Qingsheng Dong, Shanshan Hu, and Chaoshi Niu

Supplementary Figure 1

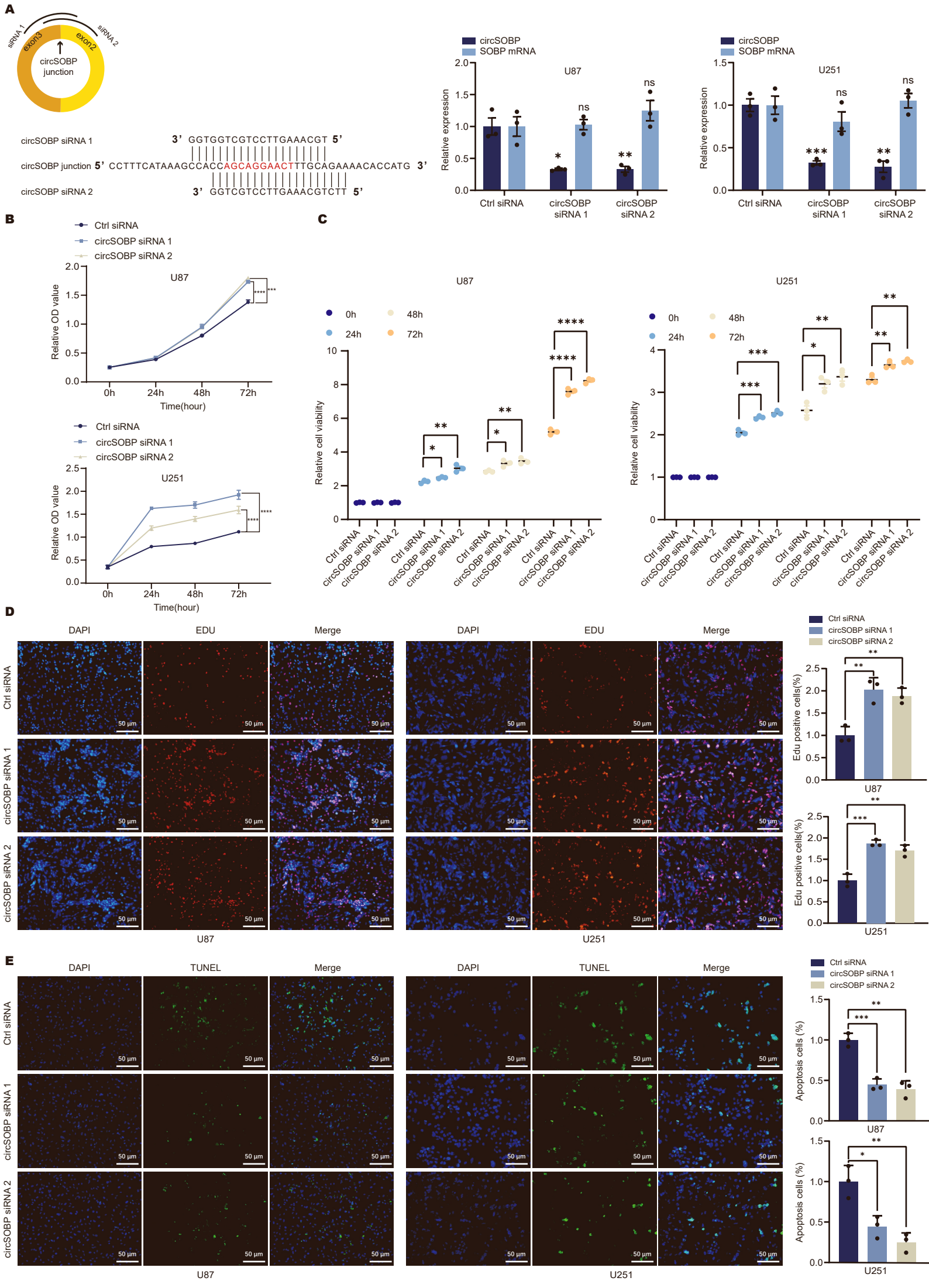


Figure. S1 Knockdown of circSOBP expression promoted the proliferation of glioma cells. Related to Figure 2. (A) The effects of two independent siRNAs on the expression of circSOBP and SOBP mRNA in U87 and U251 cells were assessed by RT-qPCR. siRNA 1 and siRNA 2 are two siRNAs that target the circSOBP back-spliced junction. **(B)** CCK-8 analysis of circSOBP silencing in glioma cells. **(C)** Cell viability was detected at 0 h, 24 h, 48 h, and 72 h after transfection with Ctrl siRNA or CircSOBP siRNAs in U87 and U251 cells. **(D)** Proliferation of U87 and U251 cells was examined by Edu assay. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). Scale bars, 50 μm. **(E)** Apoptosis of U87 and U251 cells was detected by TUNEL assay. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). Scale bars, 50 μm. All statistics of error bars, S.E.M. from three independent experiments. NS, not significant; *P < 0.05; **P < 0.01; ***P < 0.001; ****p < 0.0001 by two-tailed Student's t test. The values of siRNA1-2 were normalized by Ctrl siRNA.

Supplementary Figure 2

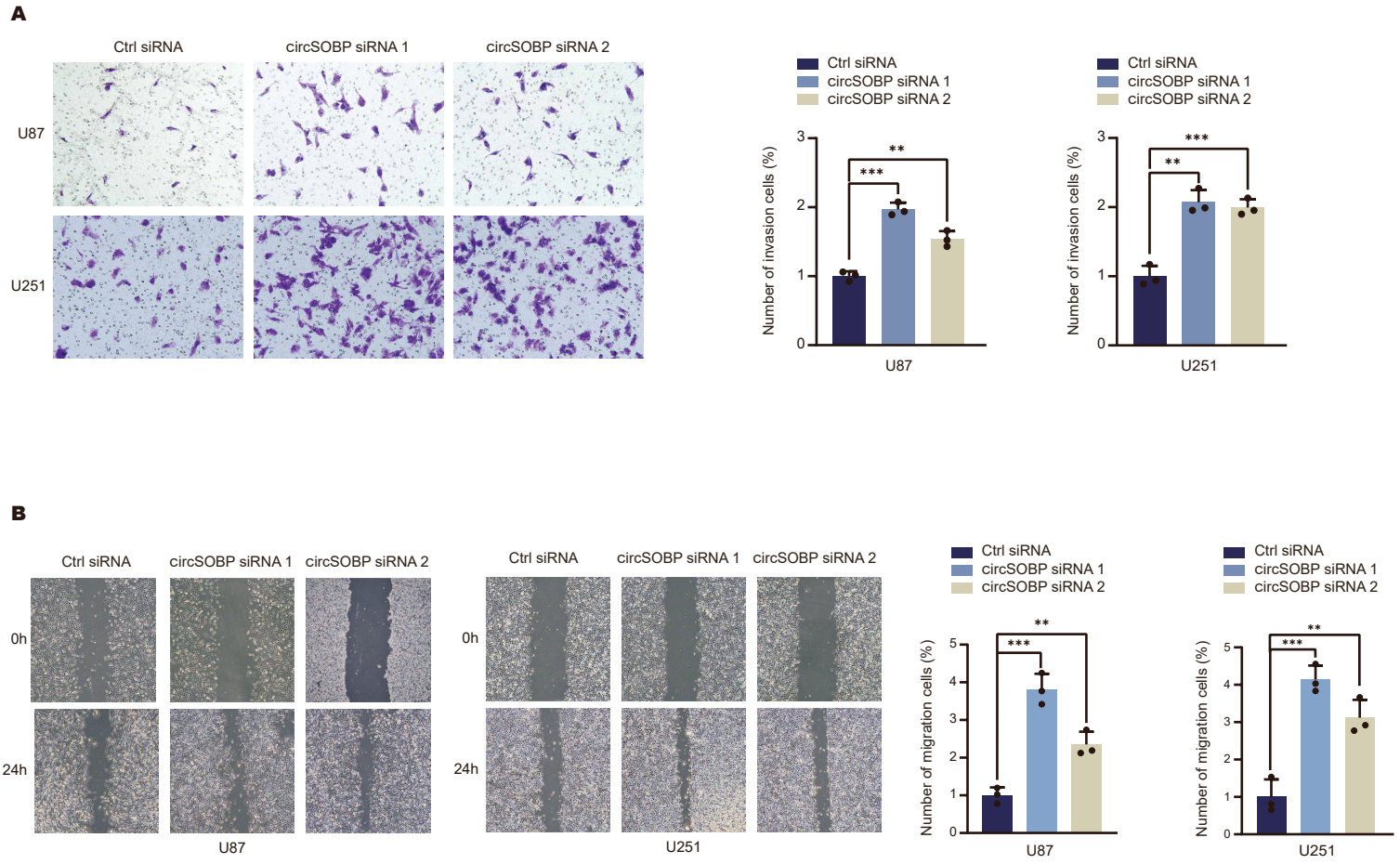
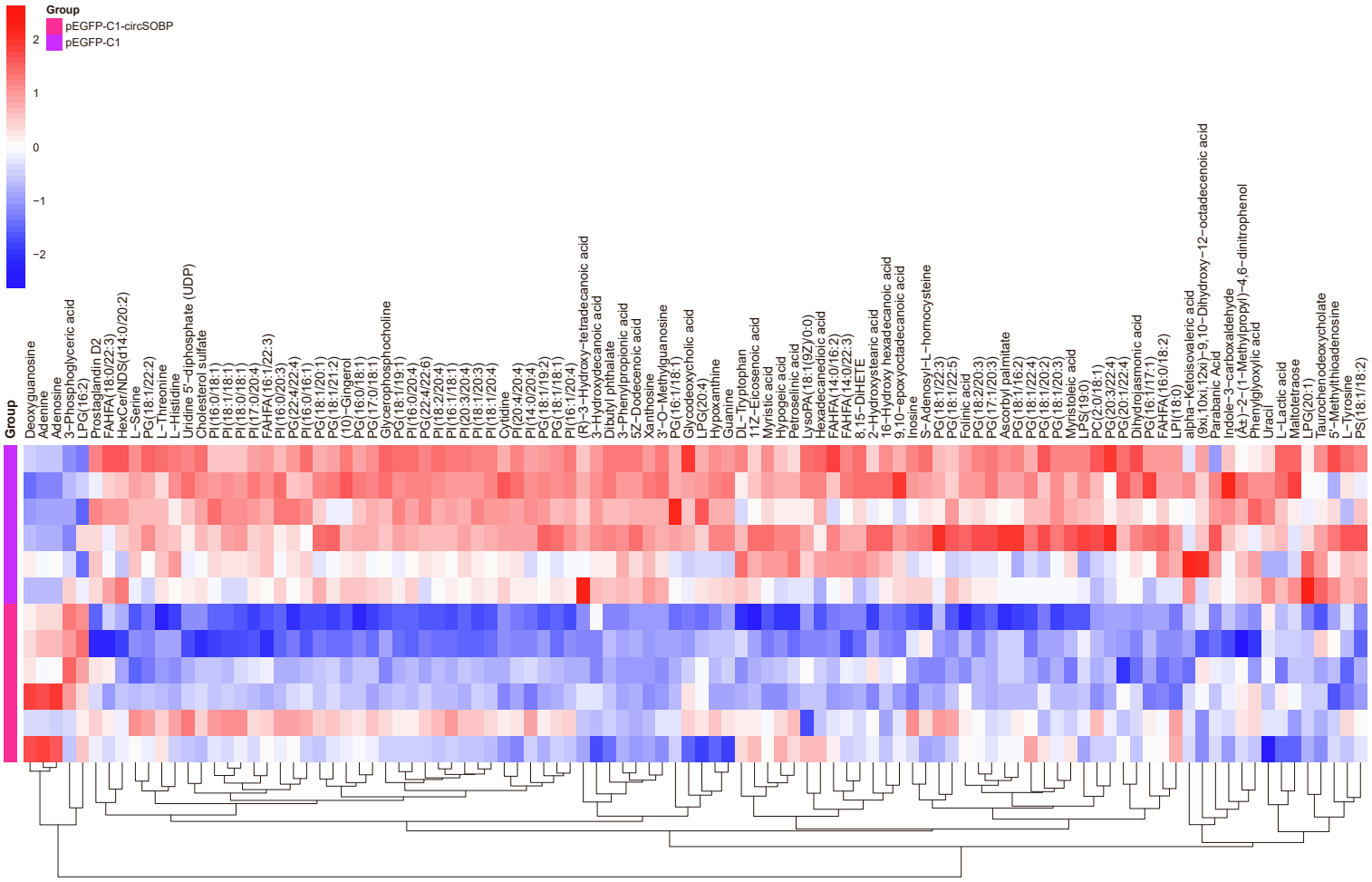


Figure. S2 Knockdown of circSOBP expression promotes the invasive and migratory ability of glioma cells. Related to Figure 2. (A) Transwell assays of glioma cells with silencing of circSOBP. (B) Wound-healing assays of glioma cells with silencing of circSOBP. All statistics of error bars, S.E.M. from three independent experiments. NS, not significant; **P < 0.01; ***P < 0.001 by two-tailed Student's t test. The values of siRNA1-2 were normalized by Ctrl siRNA.

Supplementary Figure 3

A



B

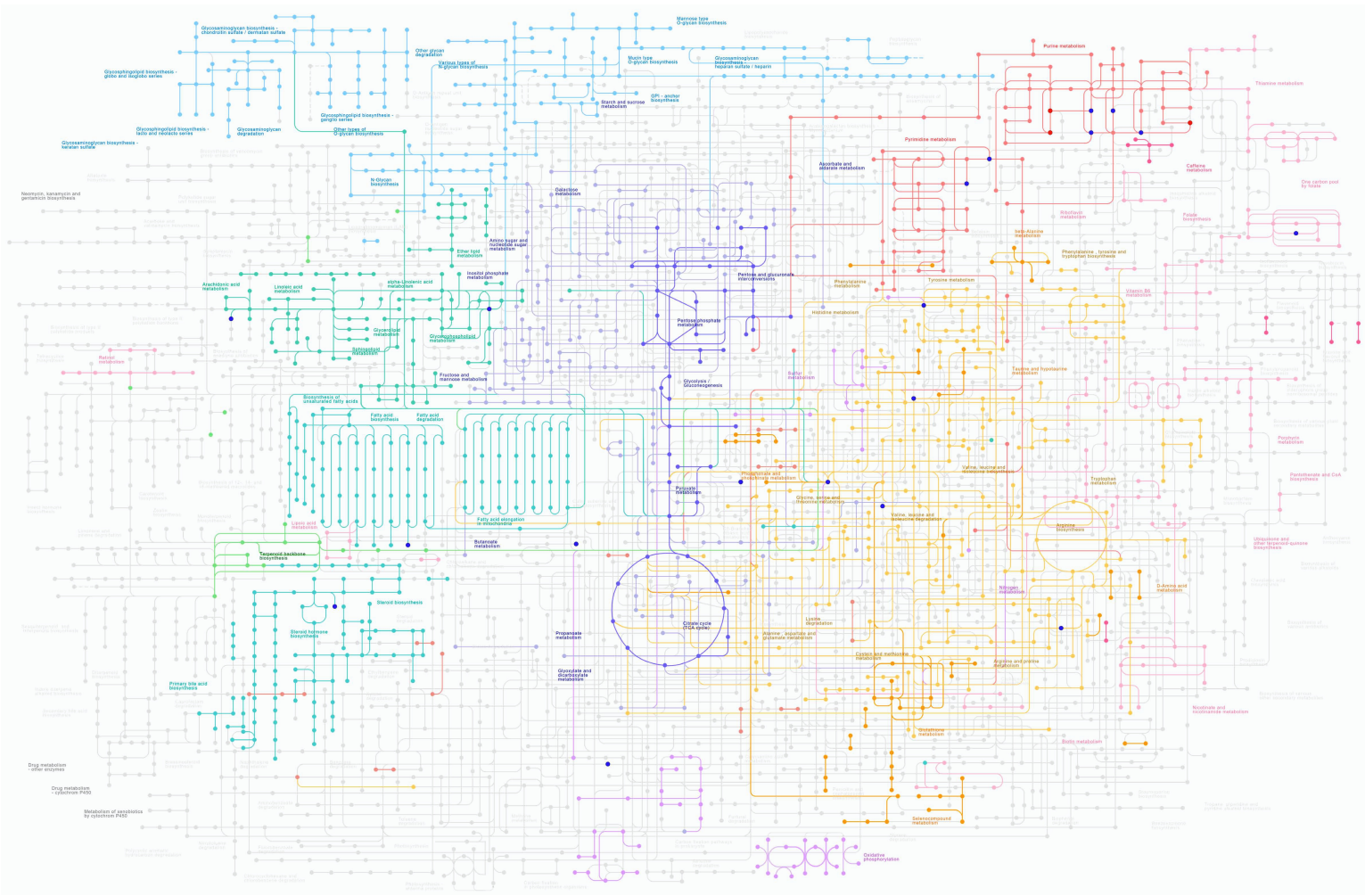


Figure. S3 Comparison of metabolic status of glioma cells with circSOBP overexpression. Related to Figure 3. (A) Heat map of metabolite clusters in the overexpression circSOBP and control groups measured by LC-MS-based metabolomics approach in U87 cells. Horizontal axis represents grouping type; vertical axis represents differential metabolites. **(B)** The differential metabolites of metabolomics were integrated and displayed in a comprehensive metabolic map using KEGG metabolic map. Dots represent compounds; lines indicate the involved metabolic reactions.

Supplementary Figure 4

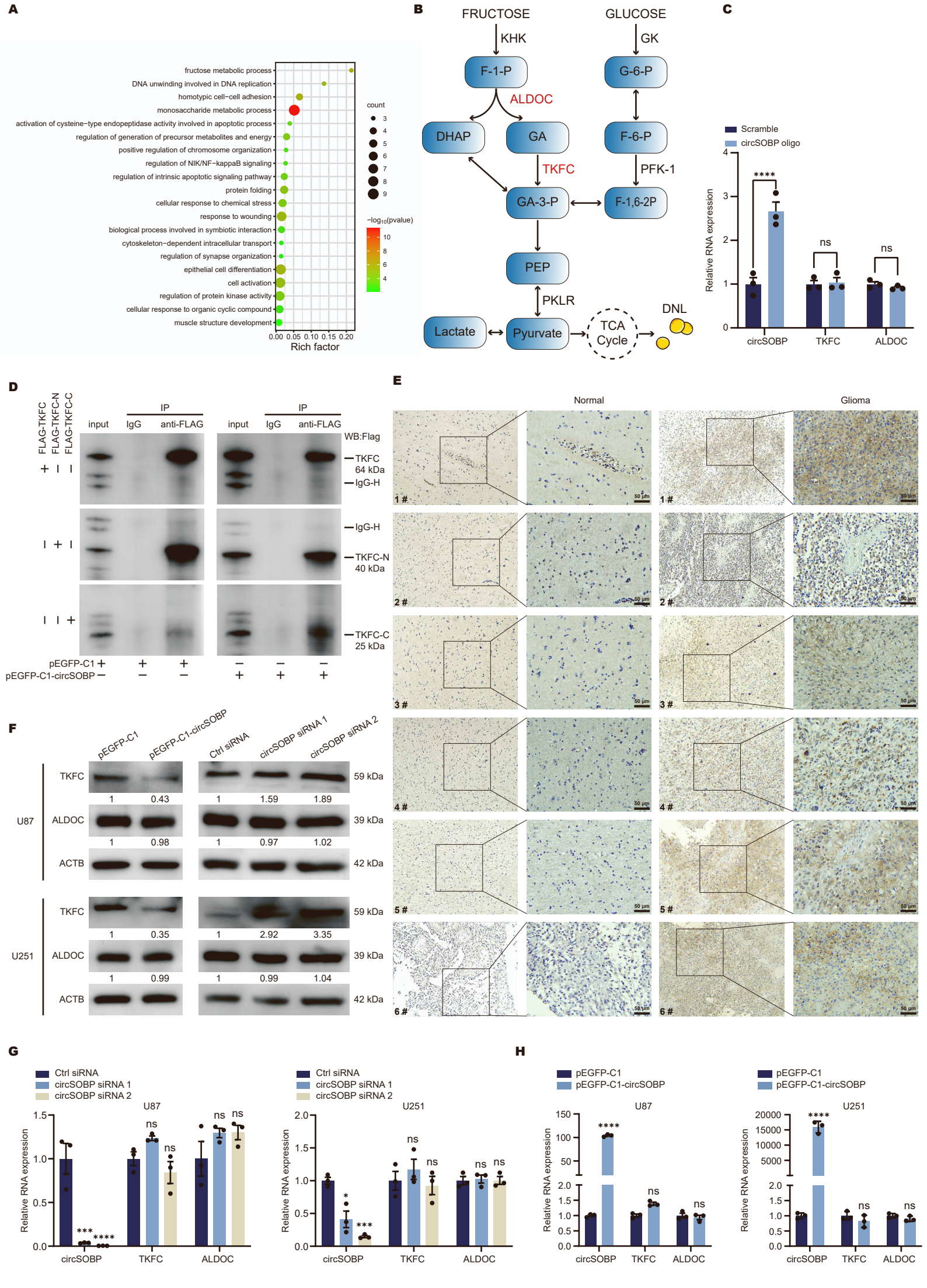


Figure. S4 CircSOBP is primarily associated with the fructose metabolic process and specifically regulates the protein levels of TKFC. Related to Figures 3 and 4. (A) GO enrichment analysis of protein clusters identified by circSOBP pulldown experiment. **(B)** TKFC regulates fructose metabolism. **(C)** RT-qPCR analysis of transcript levels of target proteins in circSOBP pulldown products. **(D)** Western blotting showed the validity of exogenously expressed full-length TKFC and multiple truncated forms of RIP assays. **(E)** IHC staining showed TKFC expression in glioma tissues and normal brain tissues. Scale bars, 50 μm. **(F-H)** Quantitative analysis of target protein expression levels after circSOBP silencing or overexpression. Data in **(F)** suggest protein levels. Data in **(G and H)** suggest RNA levels. All statistics of error bars, S.E.M. from three independent experiments. NS, not significant; *P < 0.05; ***P < 0.001; ****p < 0.0001 by two-tailed Student's t test. Normalized by the value of the control group.

Supplementary Figure 5

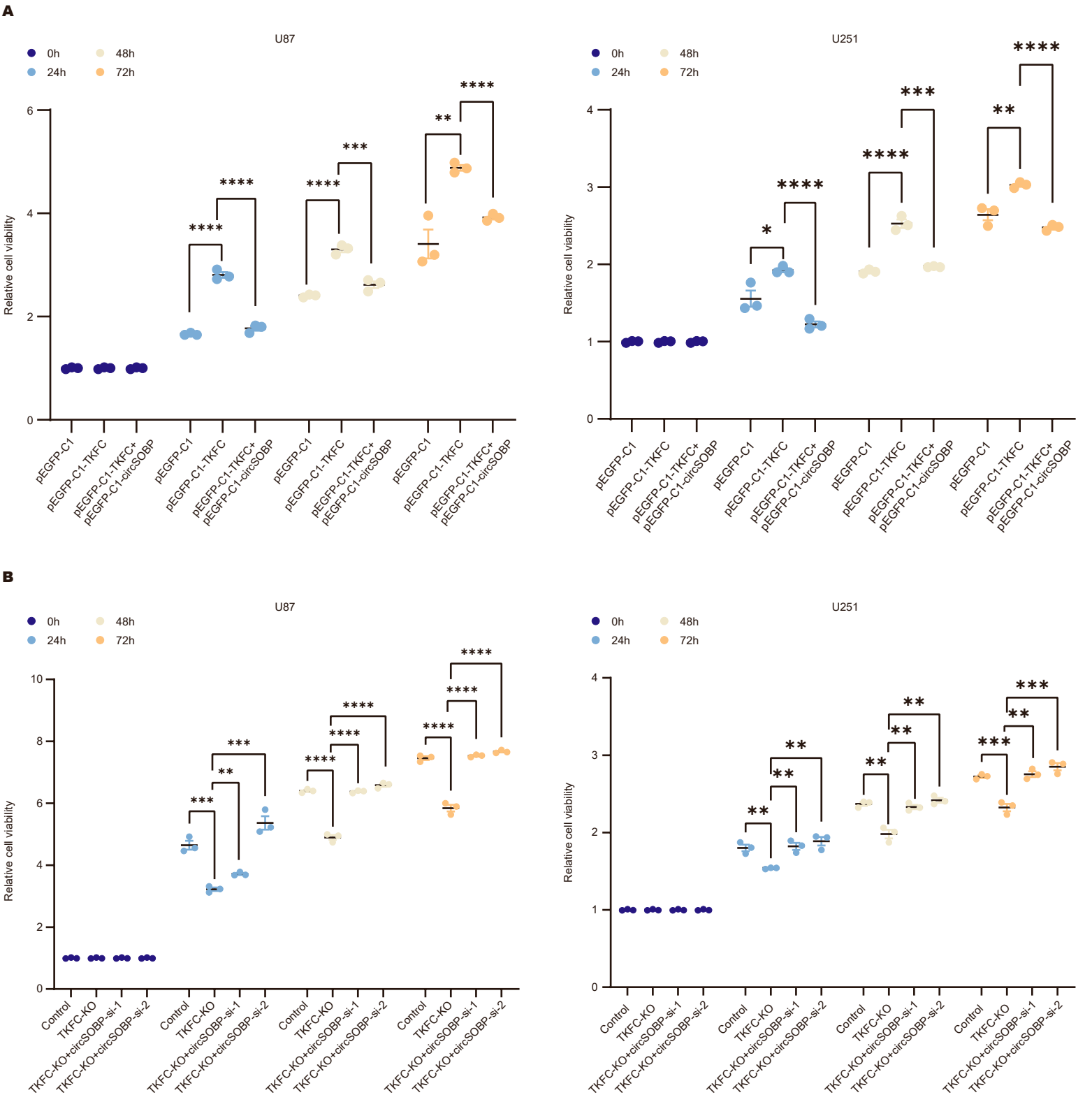


Figure. S5 CircSOBP reverses the promotion effect of TKFC on glioma cell proliferation. Related to Figure 5. (A) Cell viability was detected at 0 h, 24 h, 48 h, and 72 h after transfection with TKFC expression plasmids alone or co-transfection with circSOBP. **(B)** TKFC knockdown alone or co-transfection of circSOBP siRNAs in U87 and U251 cells were analyzed by transwell cell viability assay. All statistics of error bars, S.E.M. from three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****p < 0.0001 by two-tailed Student's t test. Normalized by the value of the control group.

Supplementary Figure 6

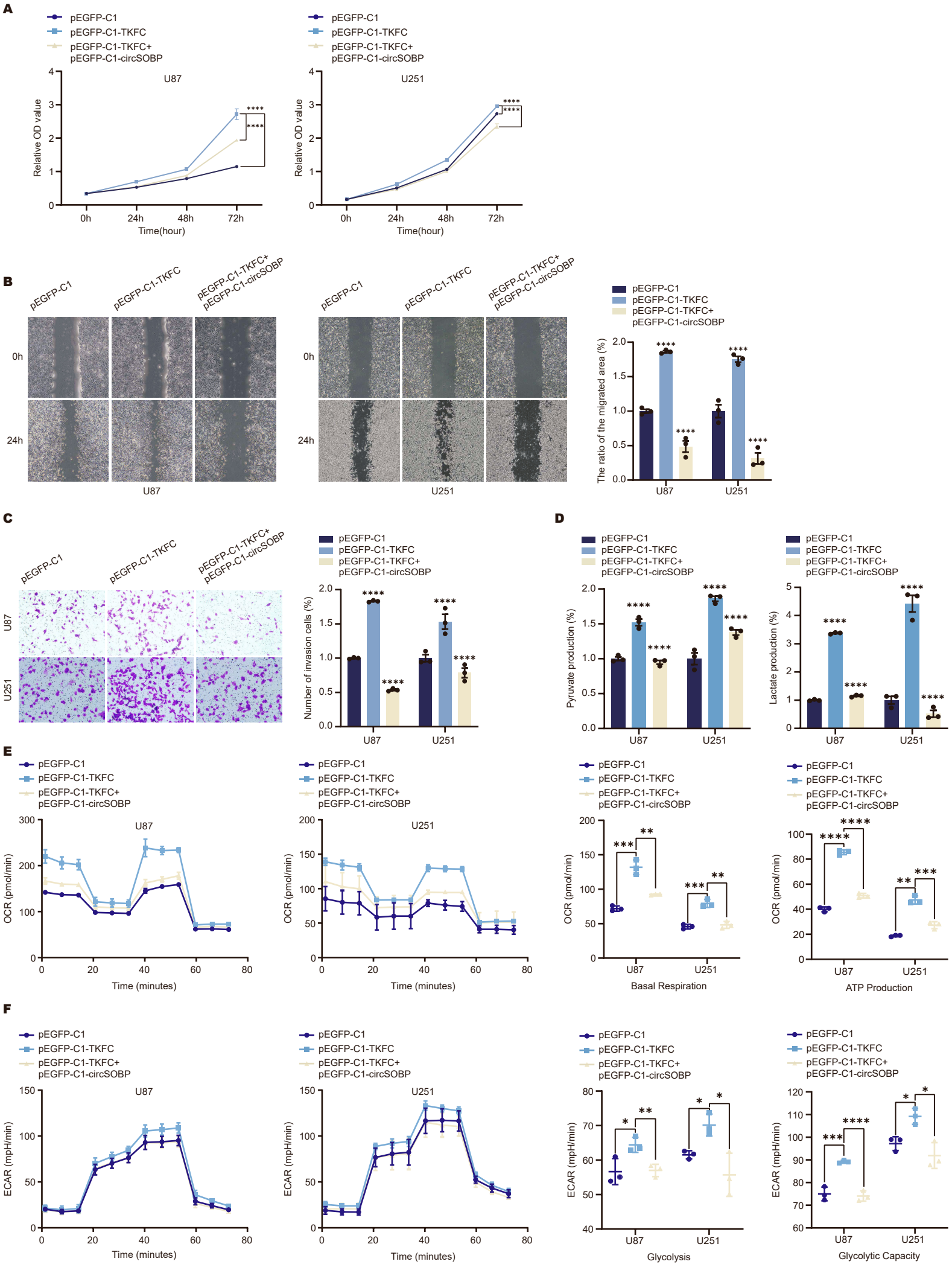


Figure. S6 Ectopic expression of circSOBP reverses the pro-cancer effect of TKFC. Related to Figure 5. (A) CCK-8 assay of TKFC expression plasmids transfected alone or co-transfected with circSOBP in U87 and U251 cells. **(B)** Wound-healing analysis of TKFC expression plasmids transfected alone or co-transfected with circSOBP in U87 and U251 cells. **(C)** Transwell assays in U87 and U251 cells transfected with TKFC expression plasmids alone or co-transfection with circSOBP. **(D)** Pyruvate and lactate contents were measured in U87 and U251 cells treatment by TKFC expression plasmids alone or in co-treatment with circSOBP. **(E)** Oxygen consumption rate (OCR) was used as an indicator of oxidative phosphorylation (OXPHOS) to infer basal respiration and ATP production levels to assay cells with TKFC expression plasmids alone or in co-treatment with circSOBP. **(F)** The extracellular acidification rate (ECAR) was used as an indicator to infer glycolytic flux and glycolytic capacity in cells transfected with TKFC expression plasmid alone or co-transfected with circSOBP. All statistics of error bars, S.E.M. from three independent experiments. NS, not significant; *P < 0.05; **P < 0.01; ***P < 0.001; ****p < 0.0001 by two-tailed Student's t test. Normalized by the value of the control group.

Supplementary Figure 7

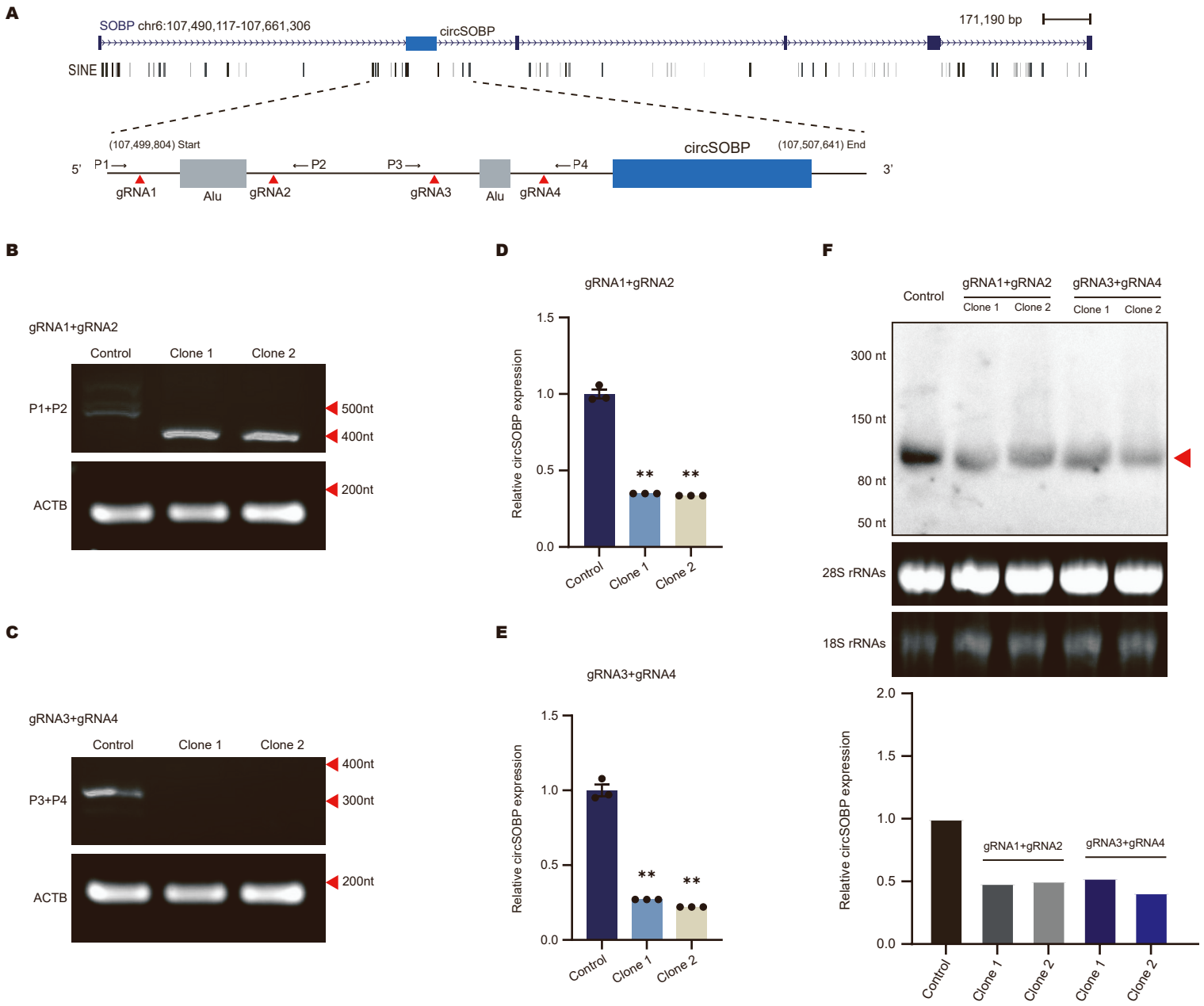


Figure. S7 The formation of circSOBP is dependent on Alu repetitive sequences. Related to Figure 6. (A) The diagram shows that the genomic regions of SOBP exons 2 and 3 contain flanking ALU repeats and long introns. ALU elements and long introns were deleted using CRISPR/Cas9 experiments. Specific primers were used to detect the deletion. **(B and C)** PCR data of CRISPR/Cas9-mediated genomic deletions in 293T cells. Two pairs of gRNAs (gRNA1~gRNA2, gRNA3~gRNA4) were used to mediate the deletion of proximal ALU elements. Two monoclonal clones were picked for each group of ALU element deletion. **(D and E)** RT-qPCR analysis of the effect of each pair of gRNAs that mediated the deletion of ALU elements on circSOBP levels. **(F)** Northern blotting and quantitative analysis of 293T cells with stably absent ALU elements in the proximal segment of circSOBP. Control values were used for normalization. Data in **(D and E)** of error bars, S.E.M. from three independent experiments. NS, not significant; **P < 0.01 by two-tailed Student's t test.

Supplementary Figure 8

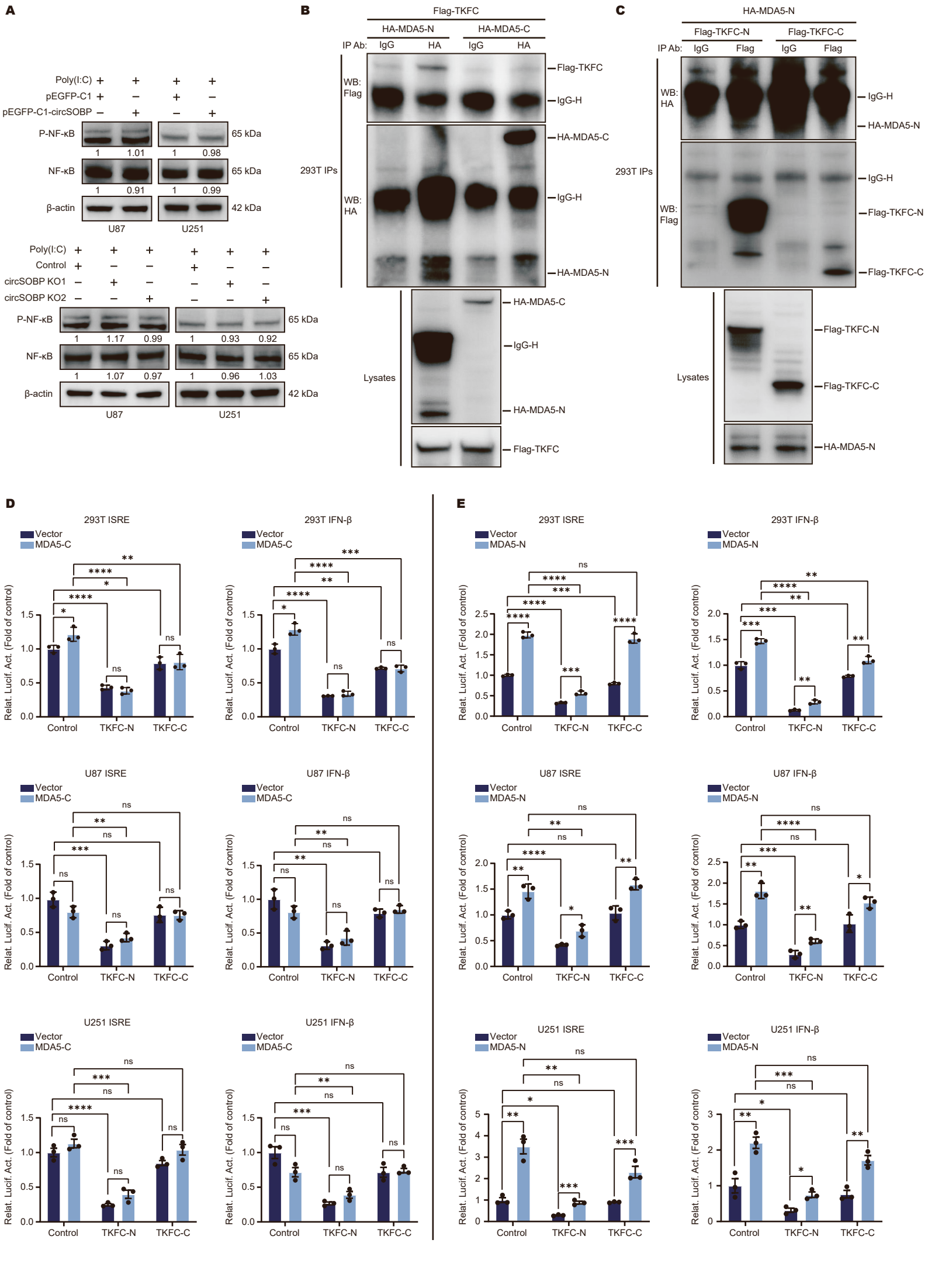


Figure. S8 TKFC bound to MDA5 and inhibited its activation of type I interferon. Related to Figure 6. (A) The effects of circSOBP silencing or overexpression on p-NF-κB and NF-κB protein expression in U87 and U251 cells. **(B)** TKFC interacted primarily with the MDA5-N terminus rather than the MDA5-C terminus. 293T cells were co-transfected with the indicated plasmids and cell lysates were immunoprecipitated with anti-HA antibodies or control rabbit IgG. Immunoprecipitates were analyzed by western blotting with anti-FLAG (top) or anti-HA (middle) antibodies. Western blot analysis of transfected protein expression was performed with anti-HA antibody and anti-FLAG antibody (bottom). **(C)** Based on the results of data **(B)**, it was further verified that TKFC and MDA5 bind mainly through the N-terminal of both. **(D)** MDA5-C was not a downstream target of TKFC to inhibit ISRE and IFN promoters activation in 293T and glioma cells. **(E)** TKFC-N inhibited MDA5-N-mediated activation of the ISRE and IFN promoters in 293T and glioma cells. The RLU values obtained from the renilla luciferase were used for normalization. All statistics of error bars, S.E.M. from three independent experiments. NS, not significant; *P < 0.05; **P < 0.01; ***P < 0.001; ****p < 0.0001 by two-tailed Student's t test. Normalized by the value of the control group.

Supplementary Figure 9

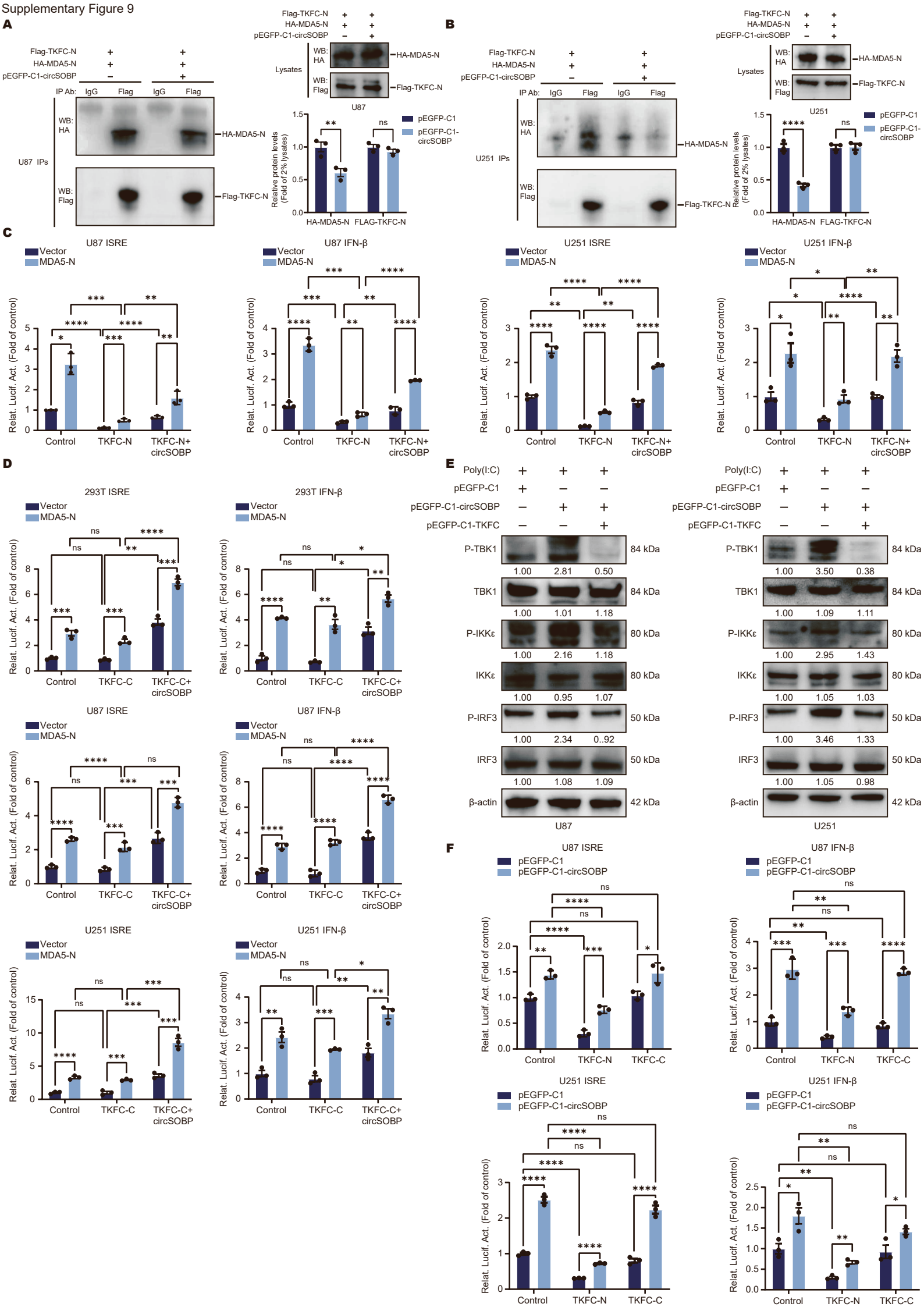


Figure. S9 CircSOBP disrupts TKFC binding to MDA5 and promotes type I interferon transcription. Related to Figure 6. (A-B) CircSOBP disrupted the binding between TKFC-N and MDA5-N. The indicated plasmids were co-transfected in U87 and U251 cells and cell lysates were immunoprecipitated with anti-FLAG antibody or control rabbit IgG. Immunoprecipitations were analyzed by western blotting with anti-HA (top) or anti-FLAG (bottom) antibodies. Western blot results of anti-HA and anti-FLAG antibodies in Lysates were used to do normalized to analyze the expression of transfected proteins. **(C)** Dual luciferase reporter assay showed circSOBP rescued TKFC-N to inhibit MDA5-N-mediated activation of ISRE and IFN promoters in U87 and U251 cells. **(D)** Dual luciferase reporter assay showed circSOBP rescued TKFC-C to inhibit MDA5-N-mediated activation of ISRE and IFN promoters in 293T and glioma cells. **(E)** Expression levels of RIG-I-like receptor signaling pathway proteins in U87 and U251 cells transfected with TKFC expression plasmids alone or co-transfected with circSOBP. **(F)** Effect of circSOBP on TKFC-N and TKFC-C to suppress ISRE and IFN promoter activation in U87 and U251 cells. The RLU values obtained from the renilla luciferase were used for normalization. All statistics of error bars, S.E.M. from three independent experiments. NS, not significant; *P < 0.05; **P < 0.01; ***P < 0.001; ****p < 0.0001 by two-tailed Student's t test. Normalized by the value of the control group.

Supplementary Figure 10

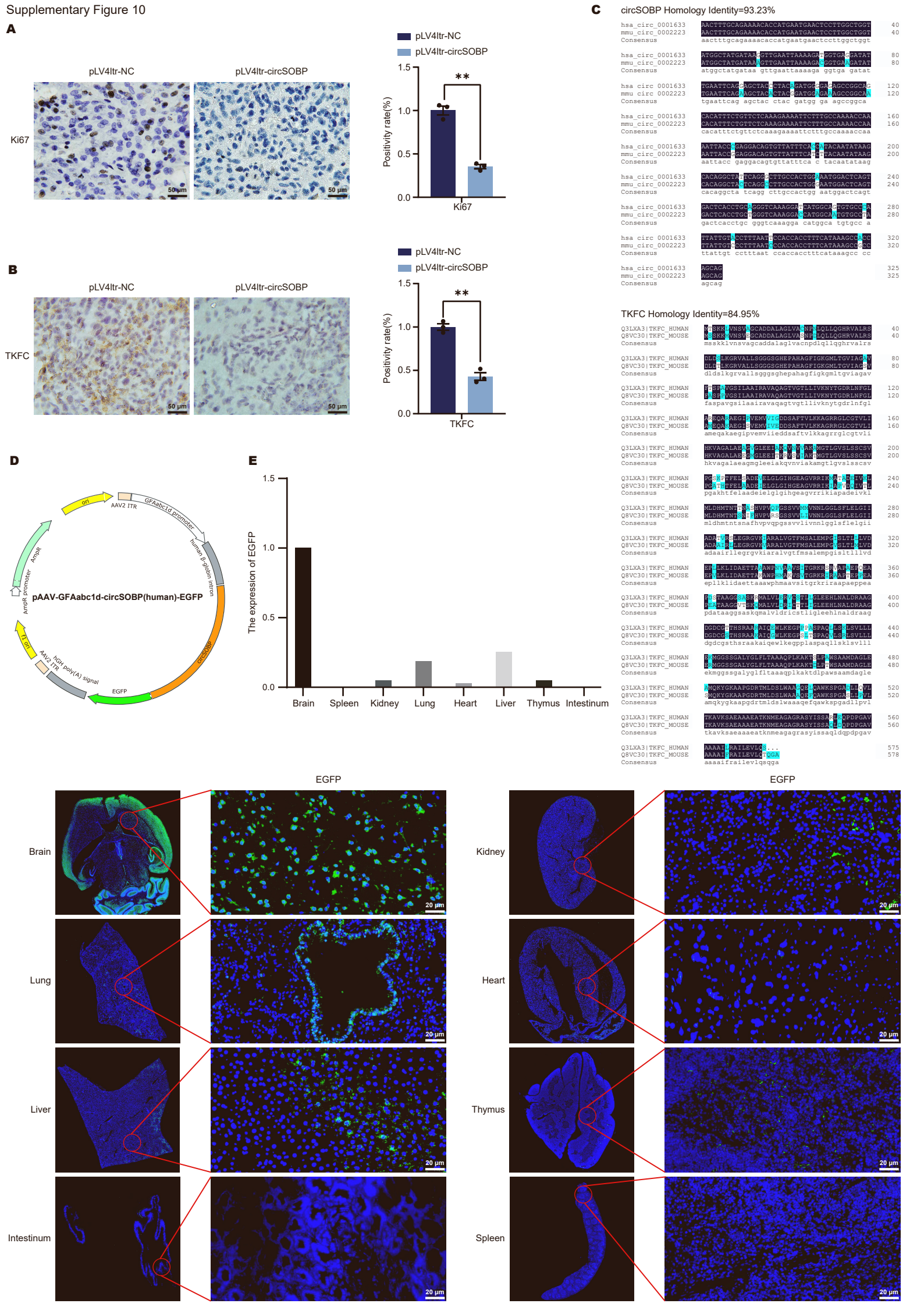


Figure. S10 The effect of circSOBP on glioma *in vivo*. Related to Figure 7 and 8. (A and B) IHC staining showed the expression levels of Ki67 and TKFC in glioma tissues with lentivirus-mediated circSOBP overexpression. Scale bars, 50 μm. **(C)** Homology analysis of circSOBP or TKFC between human and mouse. **(D)** Schematic diagram showing the construction of an adeno-associated viral shuttle plasmid for specific expression of circSOBP (hsa_circ_0001633) in the brain. **(E)** Immunofluorescence (IF) staining was used to analyze the specificity of adeno-associated virus-mediated circSOBP expression in different organs. Scale bars, 20 μm. All statistics of error bars, S.E.M. from three independent experiments. **P < 0.01 by two-tailed Student's t test. Normalized by the value of the control group.

Supplementary Table 1 The relationship of circSOBP and clinical characteristics in 94 glioma patients. Related to STAR Methods and Figure 1.

Variable		circSOBP		p-value
		High (n=47)	Low (n=47)	
Sex				1.000
	Male	24	24	
	Female	23	23	
Age(year)				0.804
	≤45	10	11	
	>45	37	36	
WHO grade				0.116
	Low grade (I-II)	12	6	
	High grade (III-IV)	35	41	
Location				0.211
	Frontal	27	18	
	Parietal	2	6	
	Occipital	4	4	
	Temporal	14	19	
Recurrence				0.778
	NO	39	40	
	Yes	8	7	
Histology				0.492
	Pilocytic Astrocytoma	2	1	
	Oligodendroglioma	10	5	
	Astrocytome	2	2	
	Anaplastic Astrocytoma	7	5	
	Glioblastoma	26	34	

Supplementary Table 4 Oligos used in the study. Related to STAR Methods.

Name	Sequence	Application
circSOBP-F	AGCCATACCAGCCAAGGAGT	For circSOBP RT-qPCR
circSOBP-R	ATGGACTCAGTGACTCACCT	
SOBP mRNA-F	AGCAATGGGAGAACTAGACA	For SOBP mRNA RT-qPCR
SOBP mRNA-R	TCCCCAACAGATGATGATCT	
TKFC-F	GAGGTGCTGCTGCTGCC	For TKFC RT-qPCR
TKFC-R	CACCGAGTTCACCAGCTTCT	
ALDOC-F	AGGATAAGGGCATCGTCG	For ALDOC RT-qPCR
ALDOC-R	GCTGACTTTGCCAAGTGG	
circSOBP-gRNA-1	TATCTCGAAAATCTGTCTGC	For crispr / cas9 of circSOBP
circSOBP-gRNA-2	TTGGCTTCTTCATACAATAG	
circSOBP-gRNA-3	AATGGGCTGGCTAGTAACTT	For crispr / cas9 of circSOBP
circSOBP-gRNA-4	CGATAAGAGTTATCTTAAGA	
circSOBP-P1	AGGCAGATATCATAACCTGCC	For circSOBP Alu PCR
circSOBP-P2	AGGGGCAAGATGAAAGAGAGA	
circSOBP-P3	TGCGAGACAGGAATGTTTAAG	For circSOBP Alu PCR
circSOBP-P4	TGCCTATGAACAGGAAGTCAT	
TKFC-gRNA-1	TATCGTGAAGAACTACACTG	For crispr / cas9 of TKFC
TKFC-gRNA-2	CTGAAGAAGGCAGGCCGGCG	
TKFC-gRNA-3	GGCCTAACGTGGCTGCAGTC	For crispr / cas9 of TKFC
TKFC-gRNA-4	TGATTCCACTGCTGCAGGAG	
TKFC-gRNA-5	CGAAGCGGATGGCGCTGGTG	For crispr / cas9 of TKFC
TKFC-gRNA-6	TGGCACCACCCACAGCCGTG	
tag-TKFC-F	ACTATAGGGAGACCCAAGCTTATGACCTCCAAGAA GCTGGTGAACT	For TKFC-FL PCR

tag-TKFC-R	GTCGTCCTTGTAGTCGGATCCGCTCTGCAAGACCTCCAAGATGG	
tag-TKFC(N)-F	ACTATAGGGAGACCCAAGCTTATGACCTCCAAGAAGCTGGTGAACT	For TKFC(N) PCR
tag-TKFC(N)-R	GTCGTCCTTGTAGTCGGATCCCCGTTCCAGCACCAGCG	
tag-TKFC(C)-F	ACTATAGGGAGACCCAAGCTTGCTGCAGTCTCCATTACTGGGC	For TKFC(C) PCR
tag-TKFC(C)-R	GTCGTCCTTGTAGTCGGATCCGCTCTGCAAGACCTCCAAGATGG	
tag-MDA5-F	GCTGGTACCGAGCTCGGATCCATGTCGAATGGGTATTCCACAGACGAG	For MDA5-FL PCR
tag-MDA5-R	TGCTGGATATCTGCAGAATTCATCCTCATCACTAAATAAACAGCATTCTGAATAGTCAAGA	
tag-MDA5(N)-F	GCTGGTACCGAGCTCGGATCCATGTCGAATGGGTATTCCACAGACGAG	For MDA5(N) RT-PCR
tag-MDA5(N)-R	TGCTGGATATCTGCAGAATTCCTGGAGTTCTGGCTCCGGG	
tag-MDA5(C)-F	GCTGGTACCGAGCTCGGATCCCTCAGGCCTTACCAAATGGAAGTTGC	For MDA5(C) RT-PCR
tag-MDA5(C)-R	TGCTGGATATCTGCAGAATTCATCCTCATCACTAAATAAACAGCATTCTGAATAGTCAAGA	
circSOBP antisense-F	AGCCATACCAGCCAAGGAGT	For circSOBP Northern blot
circSOBP antisense-R	TAATACGACTCACTATAGGGAGAATGGACTCAGTGACTCACCT	
circSOBP -fish-oligo-1	TCCACCACCTTTCATAAAGCCACCAGCAGAACTTTGCAGAAAACACCATGAATGAACTCCTTGGCTGG	For circSOBP FISH
circSOBP -fish-oligo-2	TGGCAGTGTGCCCATTATTGTACCTTTAATTCCACCACCTTTCATAAAGCCACCAGCAGAACTTTGCAGAAAACACCATGAATGAACTCCTTGGCTGGTATGGCTATG	
ACTB-F	CCAACACAGTGCTGTCTGG	For ACTB PCR
ACTB-R	GAGTACTTGCGCTCAGGAG	
GAPDH-F	CTTCATTGACCTCAACTACATGG	For GAPDH RT-qPCR
GAPDH-R	CTCGCTCCTGGAAGATGGTGAT	

U1-F	GATACCATGATCACGAAGGTG	For U1 RT-qPCR
U1-R	CTACCACAAATTATGCAGTCG	
si-NC	UUCUCCGAACGUGUCACGU ACGUGACACGUUCGGAGAA	Negative control
si-circSOBP-1	UGCAAAGUUCCUGCUGGUGG CCACCAGCAGGAACUUUGCA	siRNAs of circSOBP
si- circSOBP-2	UUCUGCAAAGUUCCUGCUGG CCAGCAGGAACUUUGCAGAA	
si-TKFC-1	CAGUUGUGAUGAUGGUCAACA UUGACCAUCAUCACAACUGAG	siRNAs of TKFC
si-TKFC-2	GAAAGUUAAUAAACUAUAAUA UUAUAGUUUAUUAACUUUCUU	
Srcamble	TTCTCCGAACGTGTCACGTTCGAACGTGTC	Control probe with 5’biotin labeled
circSOBP-probe	TCTGCAAAGTTCCTGCTGGTGGCTTTATGA	For circSOBP RIP with 5’biotin labeled

Original Data File. Related to STAR Methods.

Figure 1 J

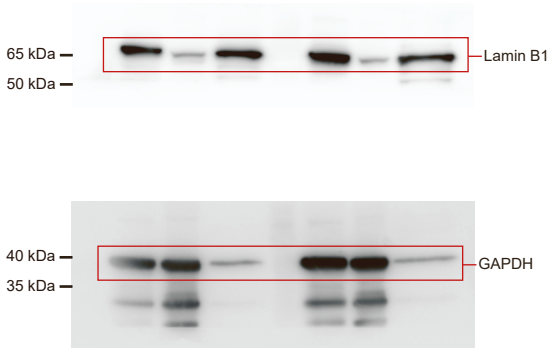


Figure 4 C (U87)

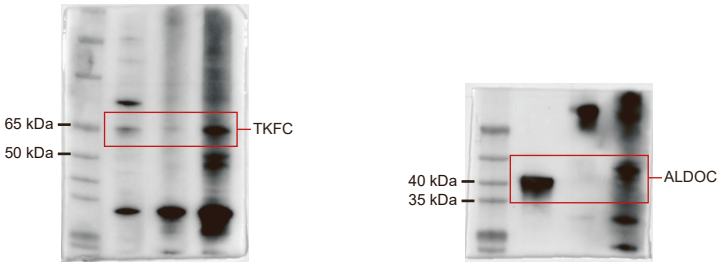


Figure 4 C (U251)

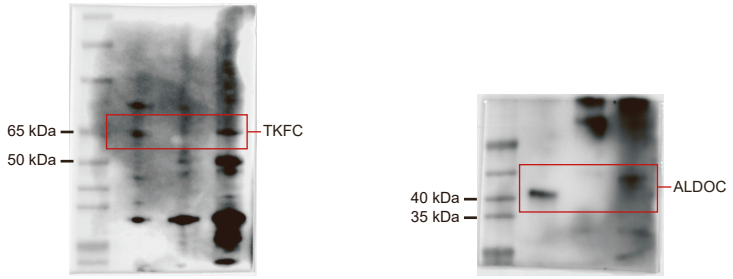


Figure 5 B (U87)

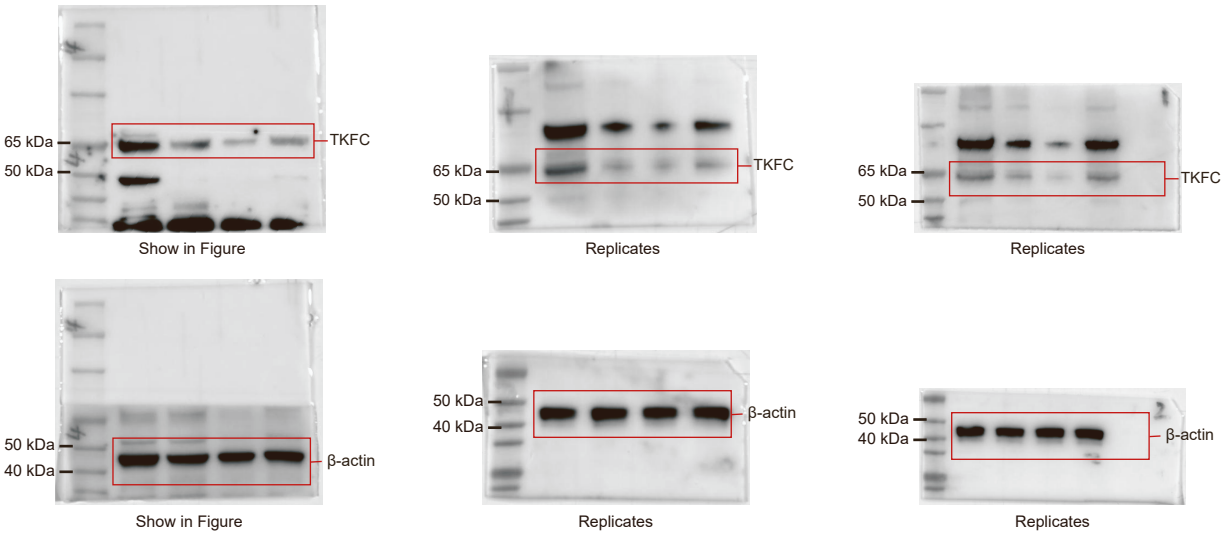


Figure 5 B (U251)

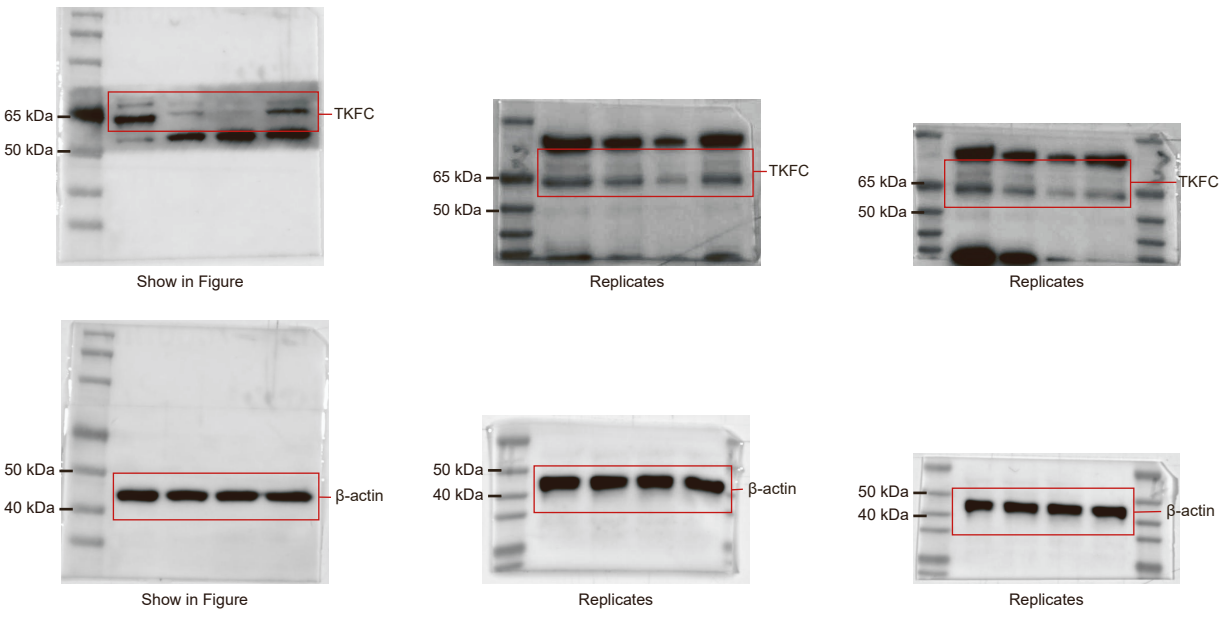
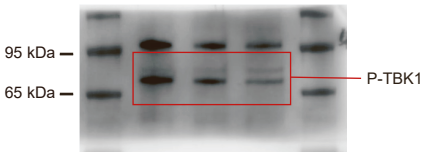
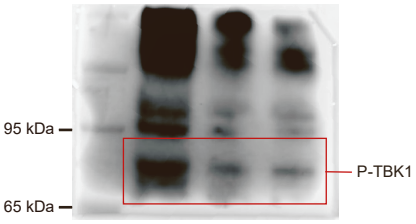


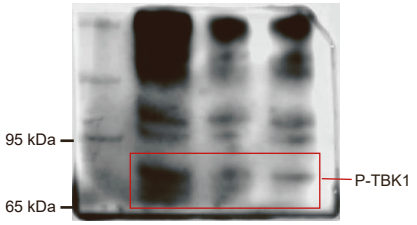
Figure 6 D (U87)



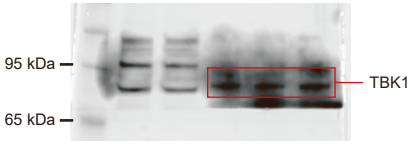
Show in Figure



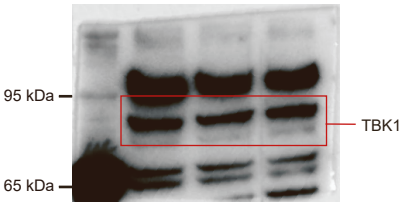
Replicates



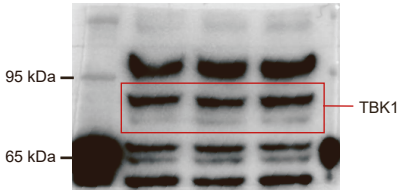
Replicates



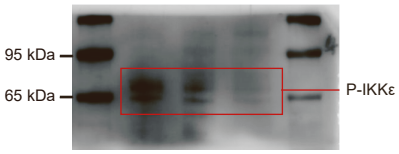
Show in Figure



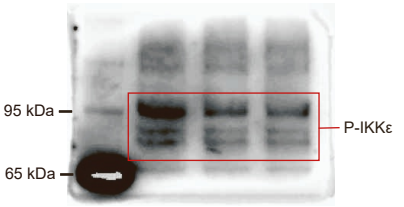
Replicates



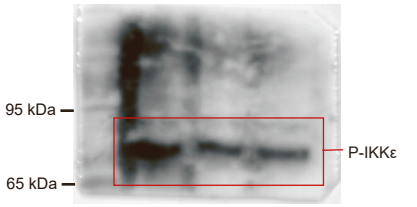
Replicates



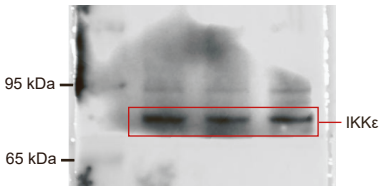
Show in Figure



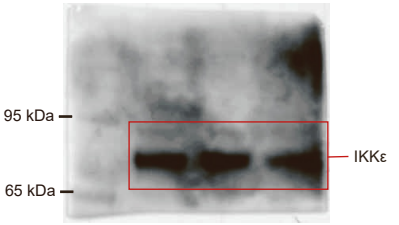
Replicates



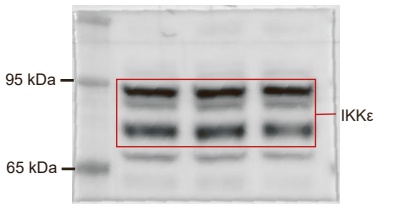
Replicates



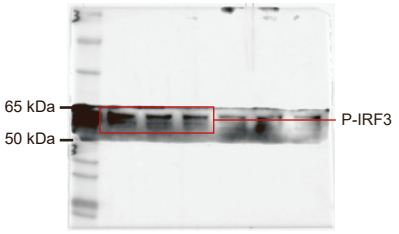
Show in Figure



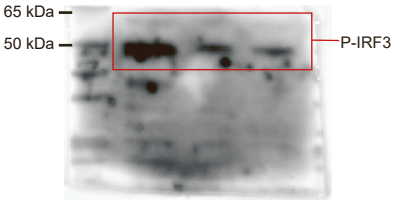
Replicates



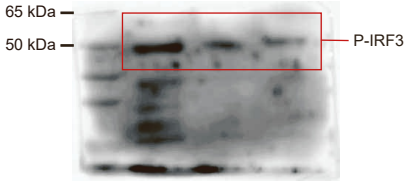
Replicates



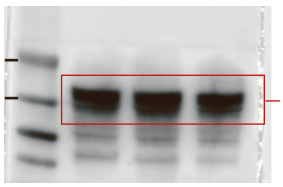
Show in Figure



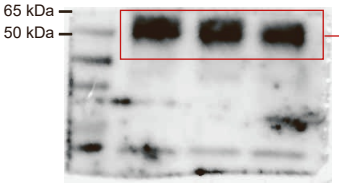
Replicates



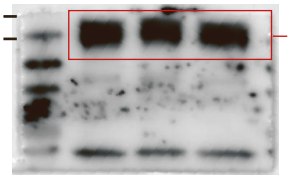
Replicates



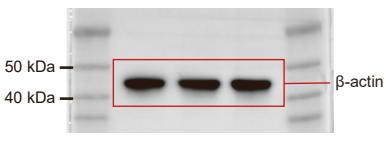
Show in Figure



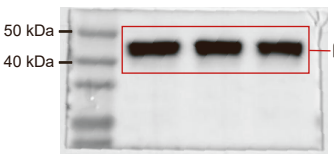
Replicates



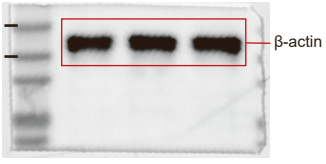
Replicates



Show in Figure

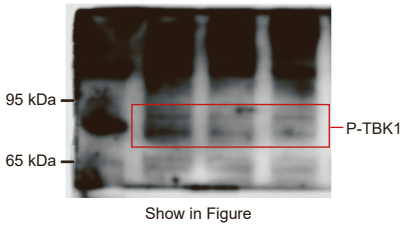


Replicates



Replicates

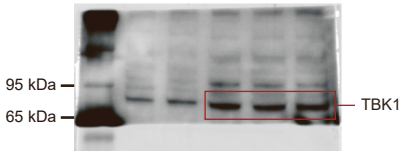
Figure 6 D (U251)



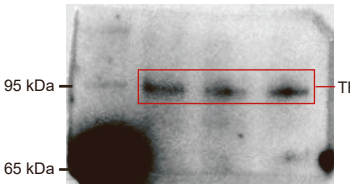
Show in Figure

Replicates

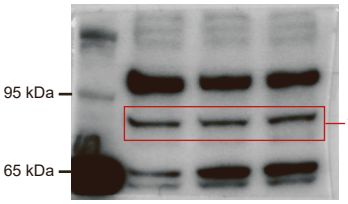
Replicates



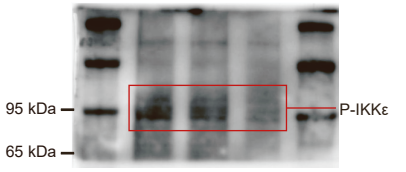
Show in Figure



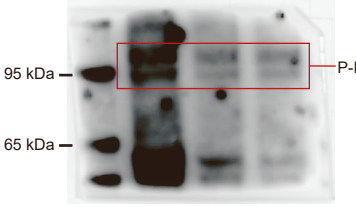
Replicates



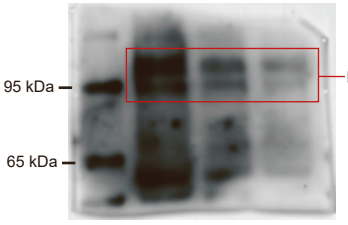
Replicates



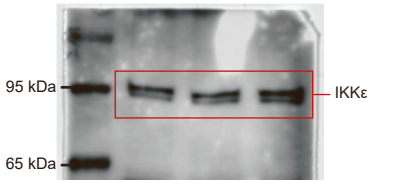
Show in Figure



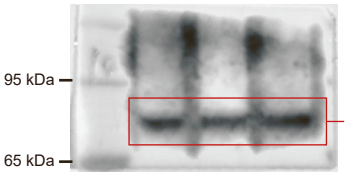
Replicates



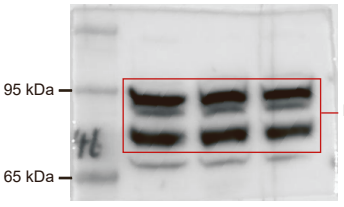
Replicates



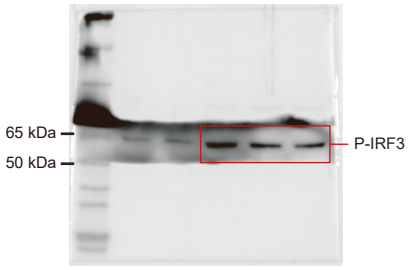
Show in Figure



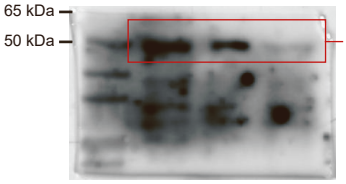
Replicates



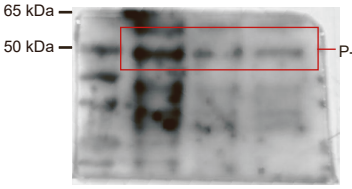
Replicates



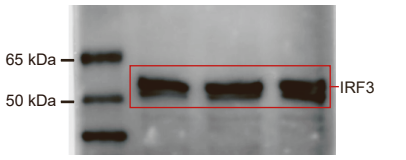
Show in Figure



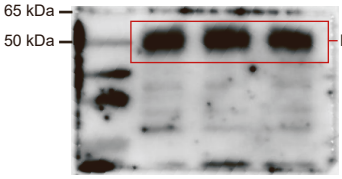
Replicates



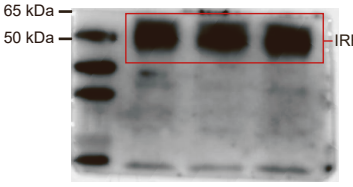
Replicates



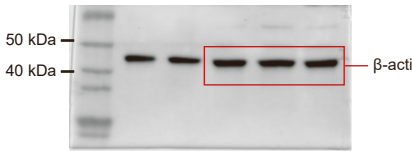
Show in Figure



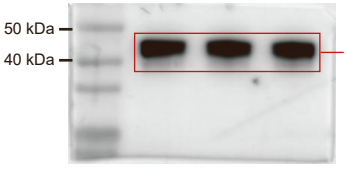
Replicates



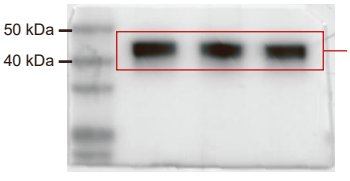
Replicates



Show in Figure



Replicates



Replicates

Figure 6 E (U87)

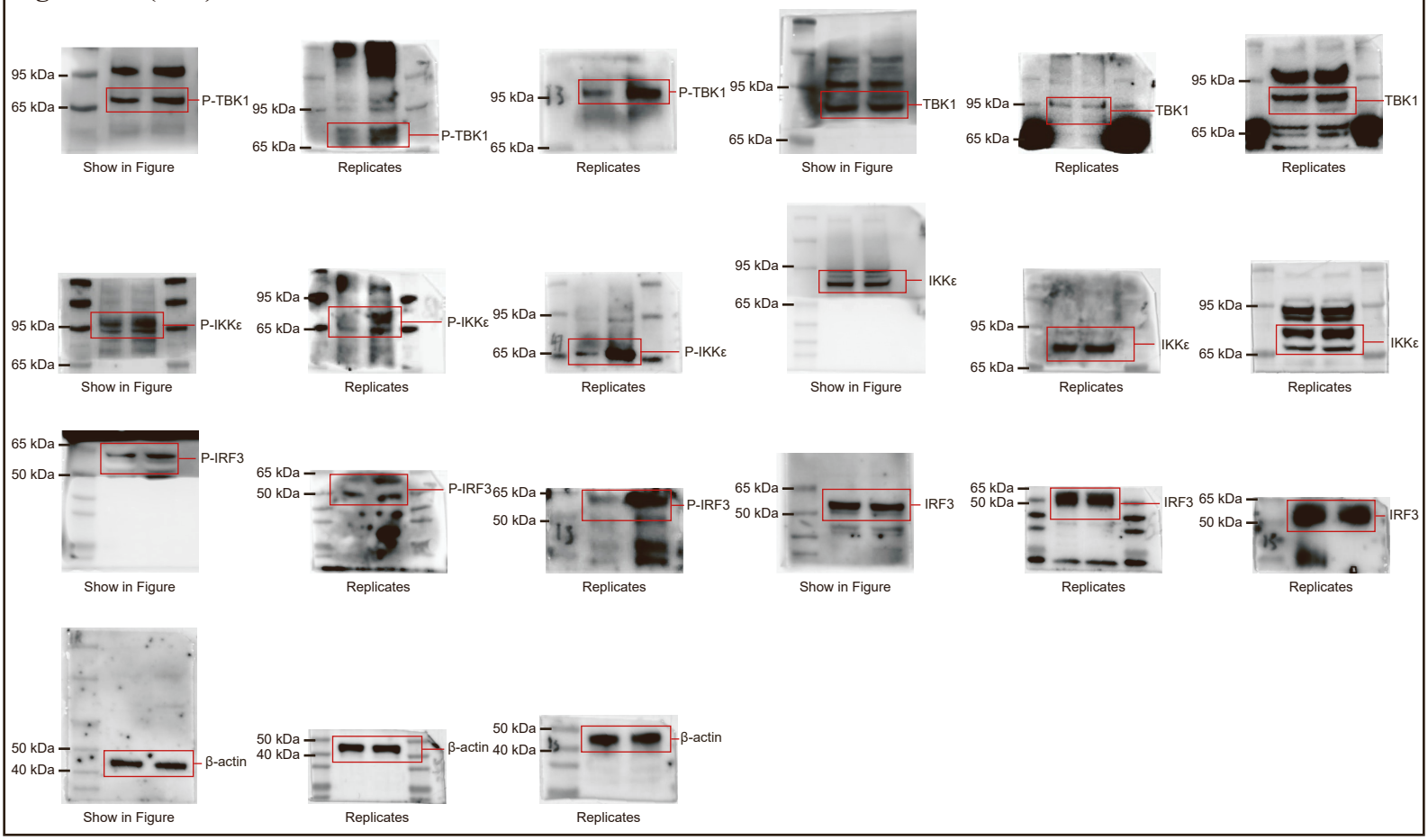


Figure 6 E (U251)

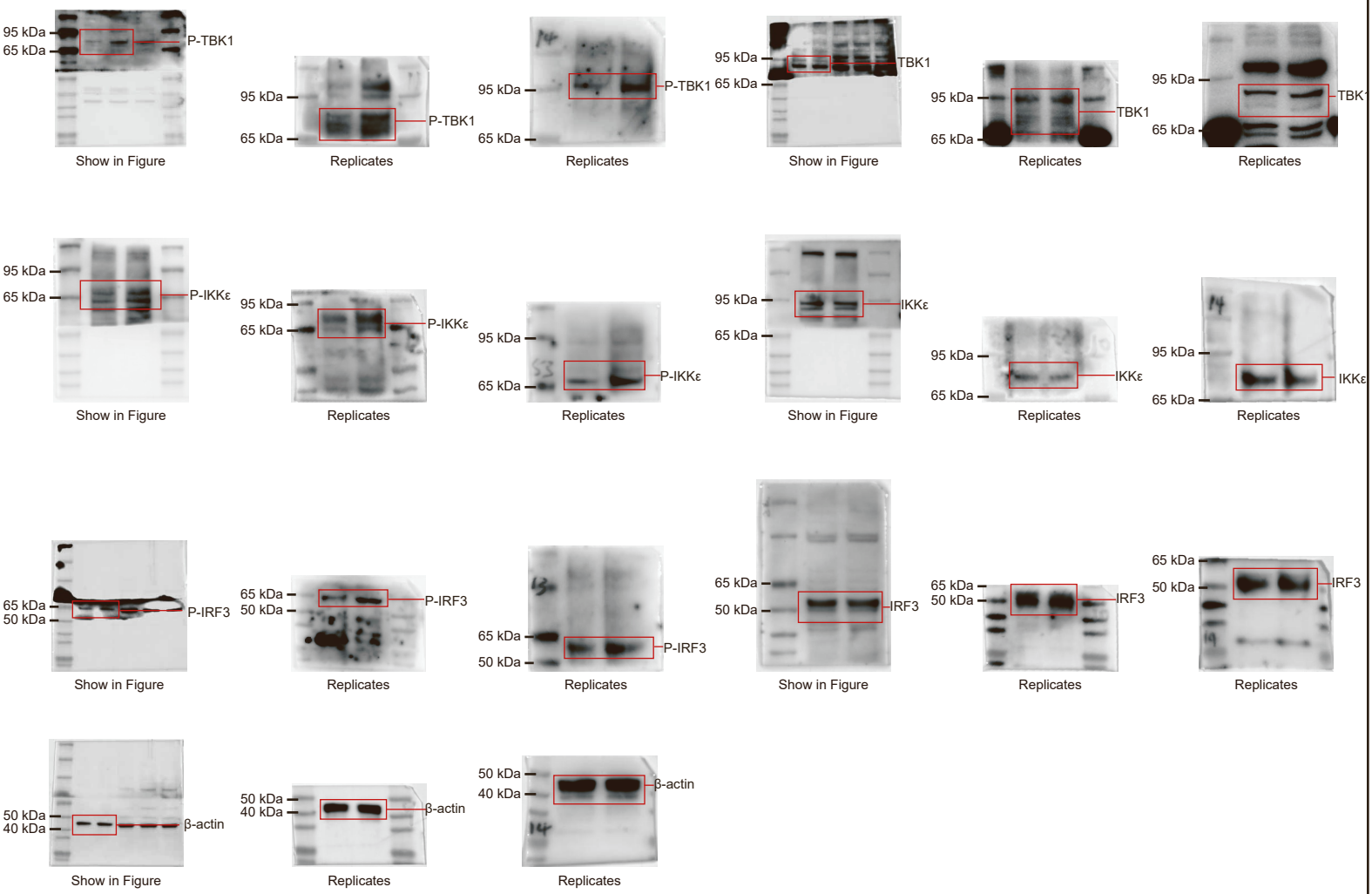


Figure 6 F

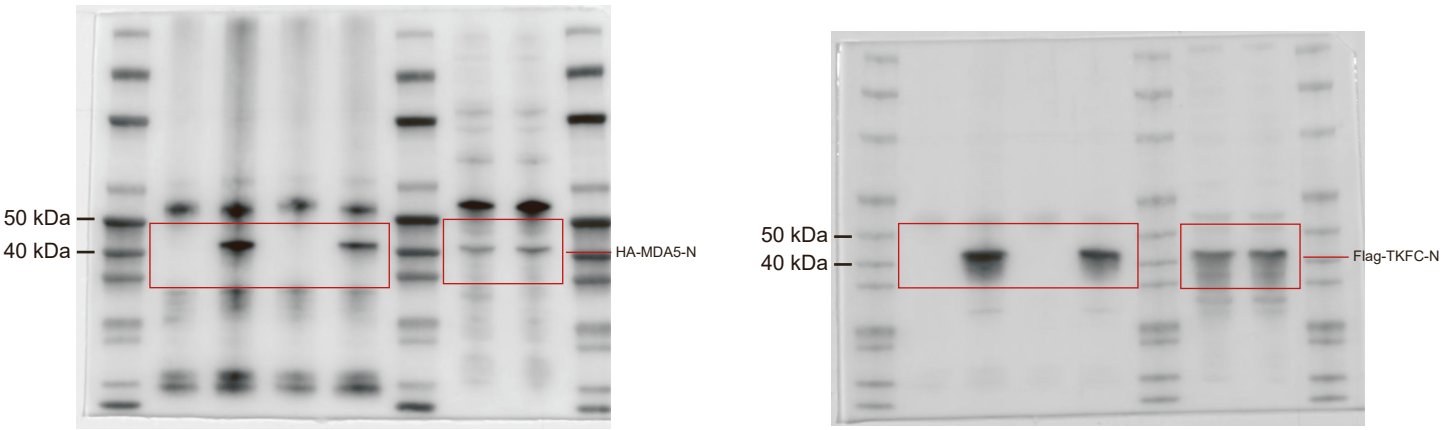


Figure S9 A

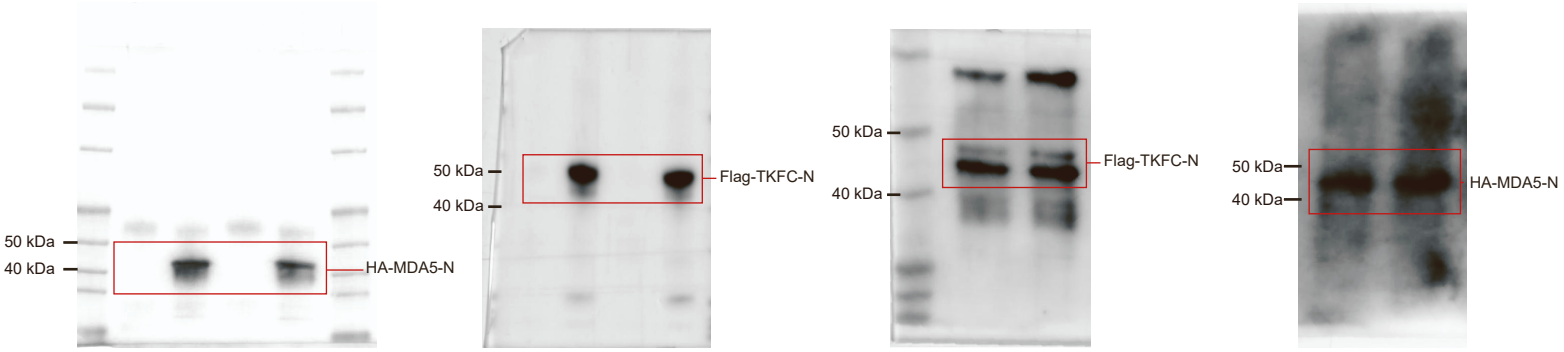


Figure S9 B

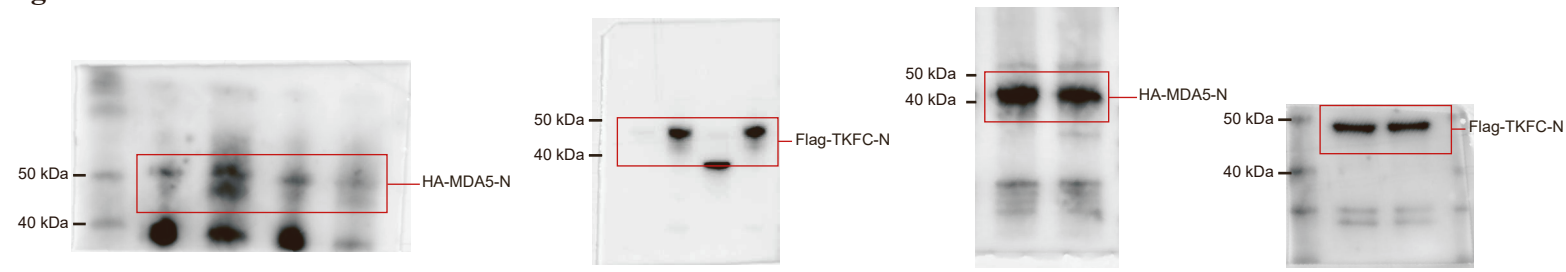


Figure S4 D

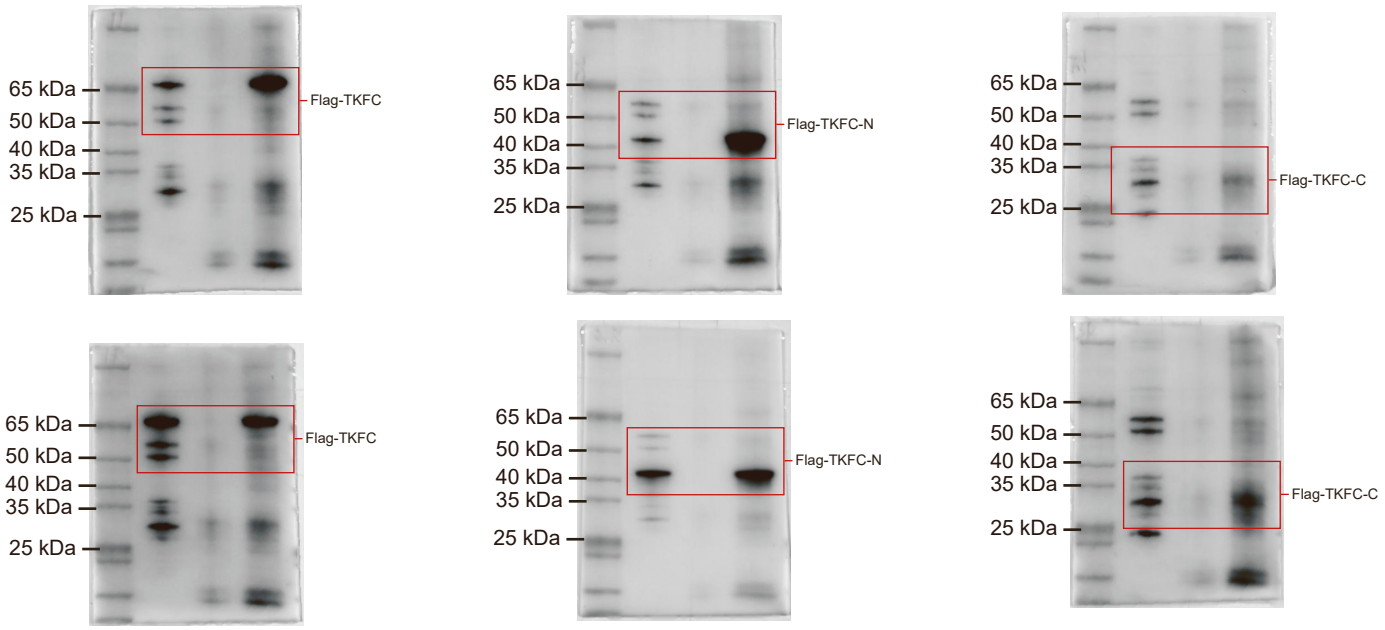


Figure S8 B-C

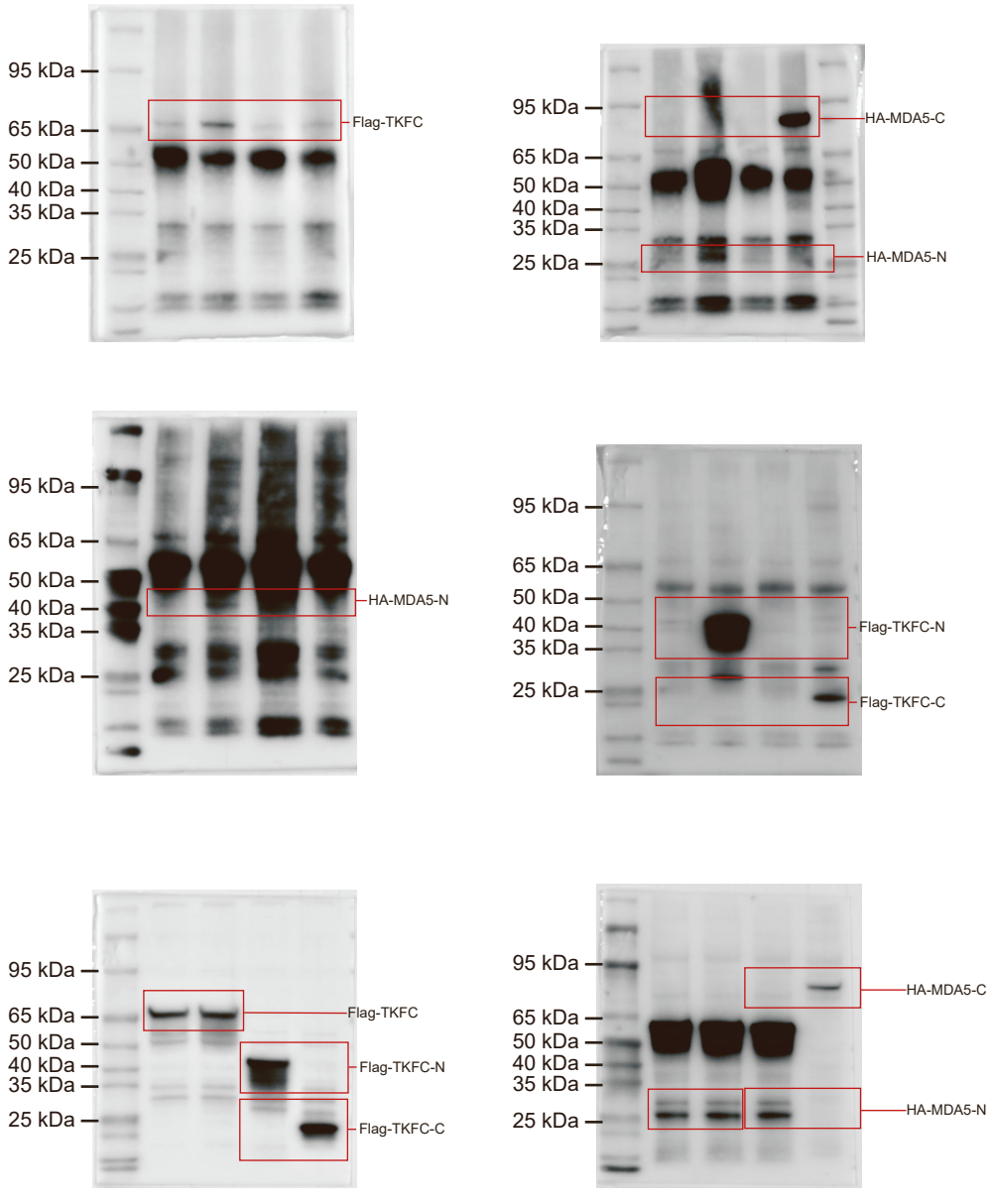
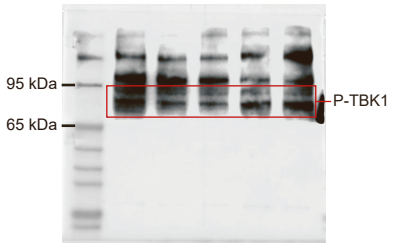
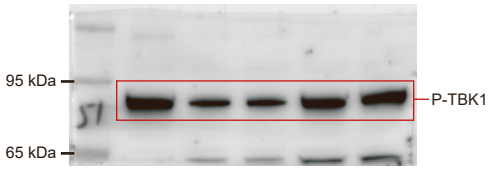


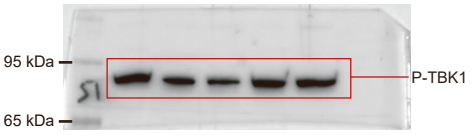
Figure 6 I (U87)



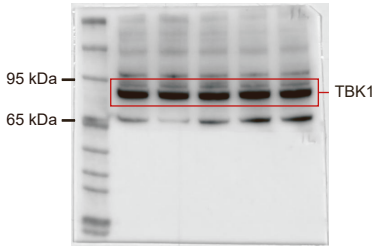
Show in Figure



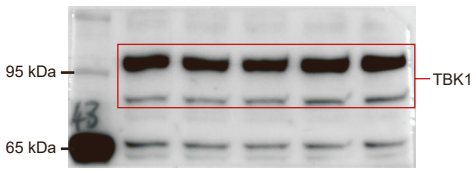
Replicates



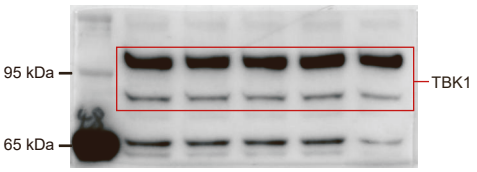
Replicates



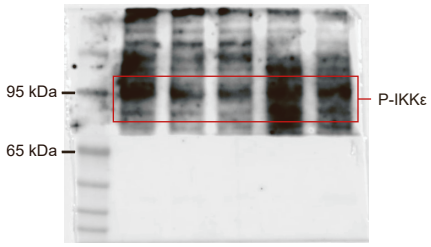
Show in Figure



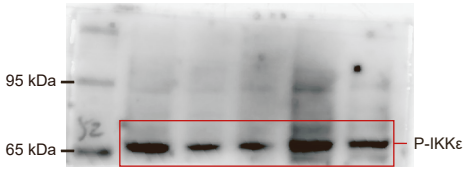
Replicates



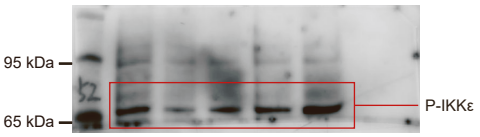
Replicates



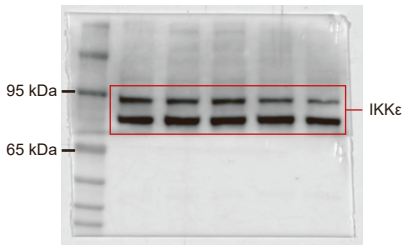
Show in Figure



Replicates



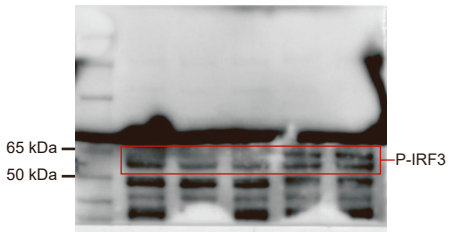
Replicates



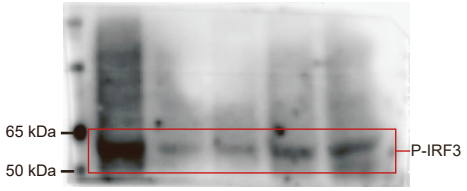
Show in Figure

Replicates

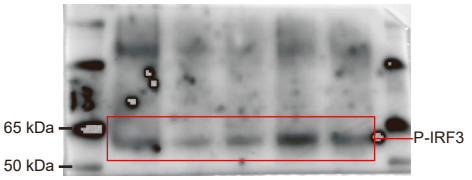
Replicates



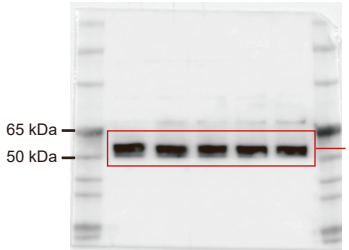
Show in Figure



Replicates

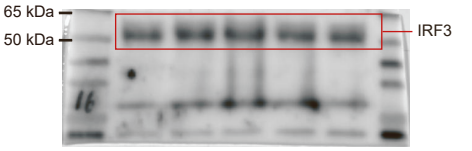


Replicates

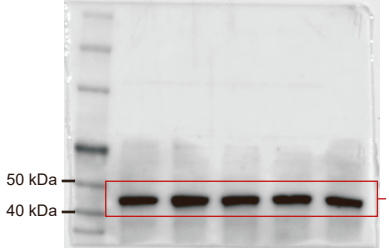


Show in Figure

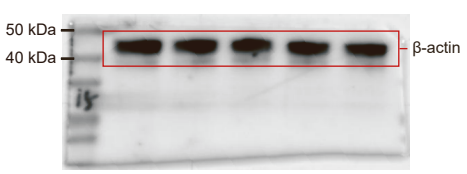
Replicates



Replicates



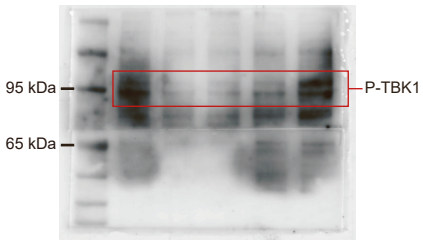
Show in Figure



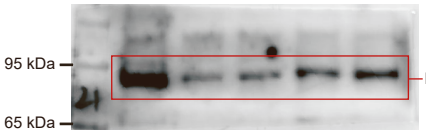
Replicates

Replicates

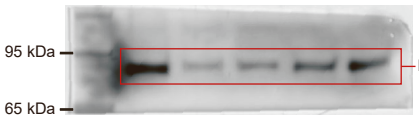
Figure 6 I (U251)



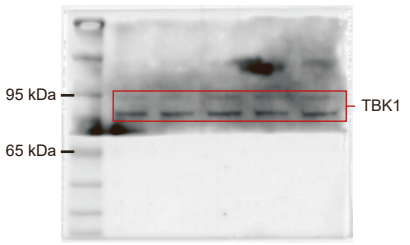
Show in Figure



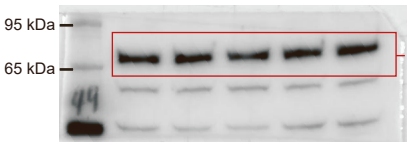
Replicates



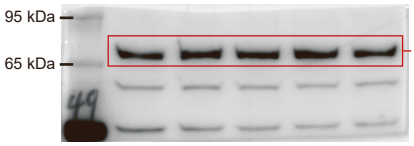
Replicates



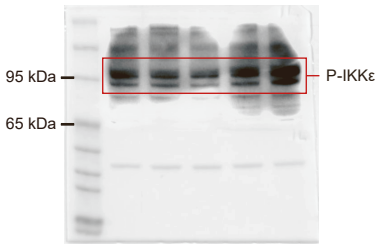
Show in Figure



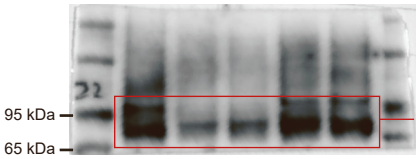
Replicates



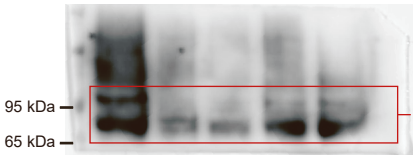
Replicates



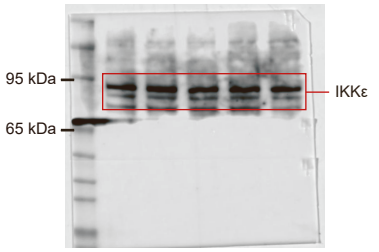
Show in Figure



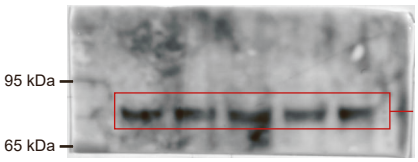
Replicates



Replicates

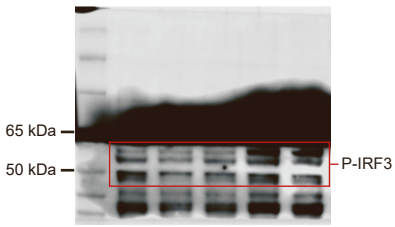


Show in Figure

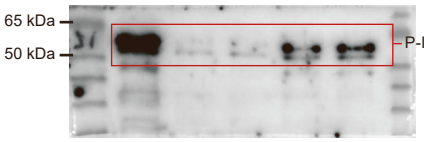


Replicates

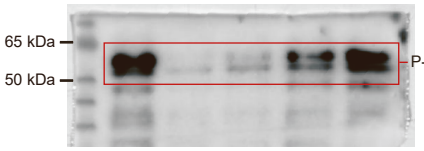
Replicates



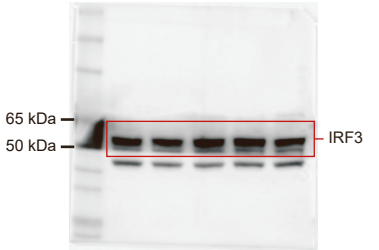
Show in Figure



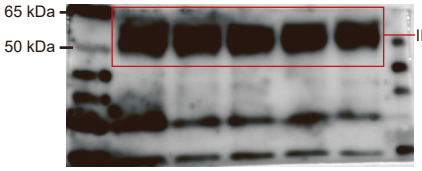
Replicates



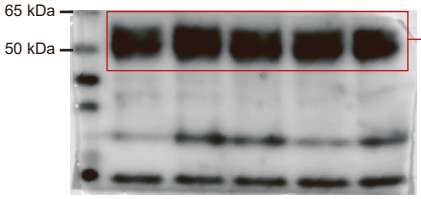
Replicates



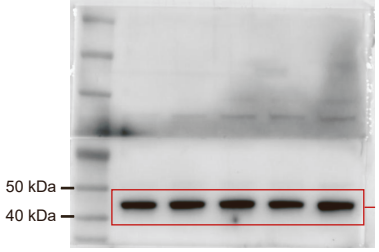
Show in Figure



Replicates

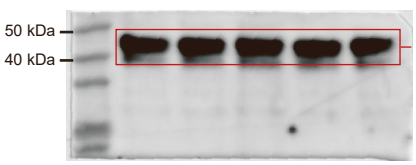


Replicates



Show in Figure

Replicates



Replicates

Figure S4 F (U87)

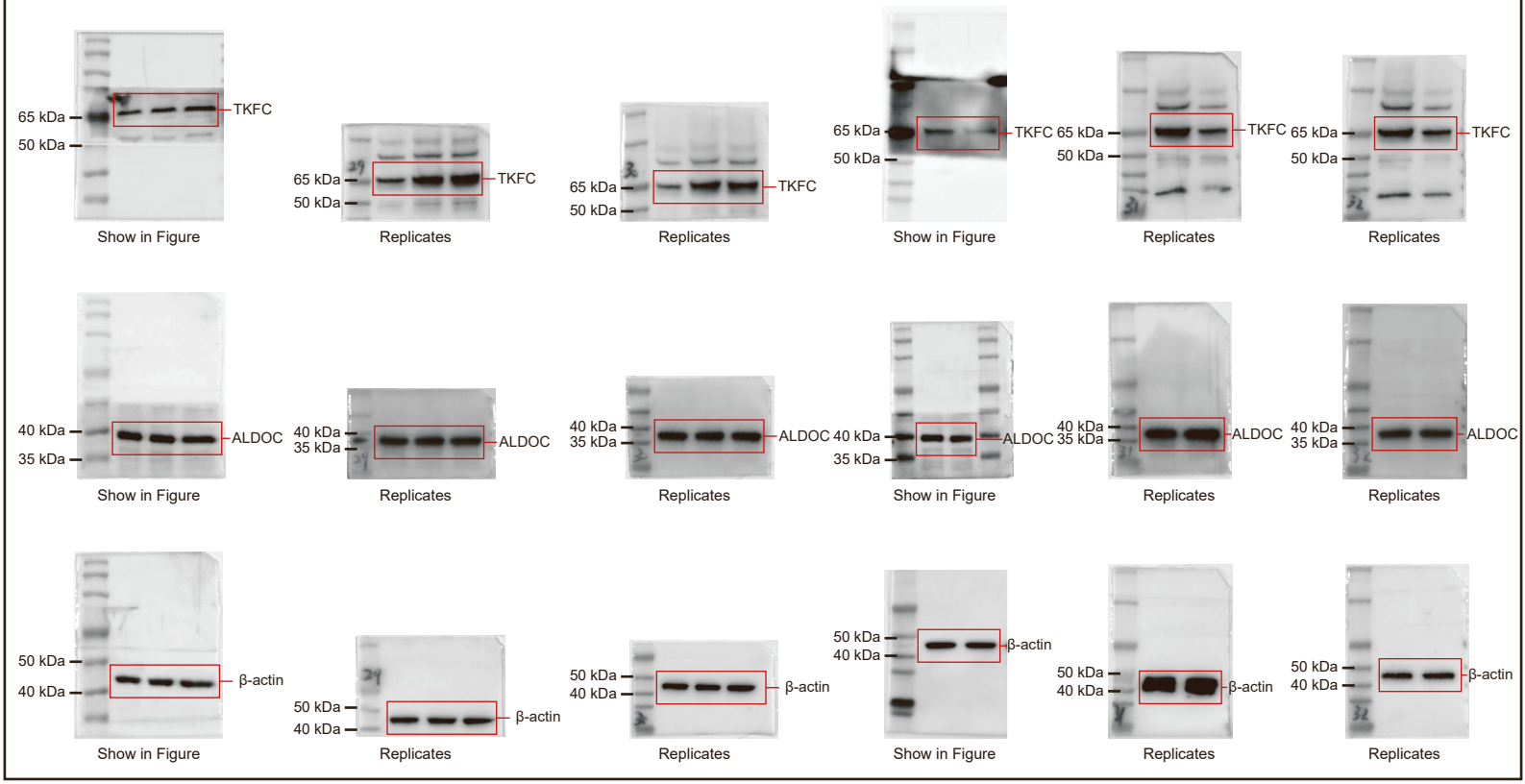


Figure S4 F (U251)

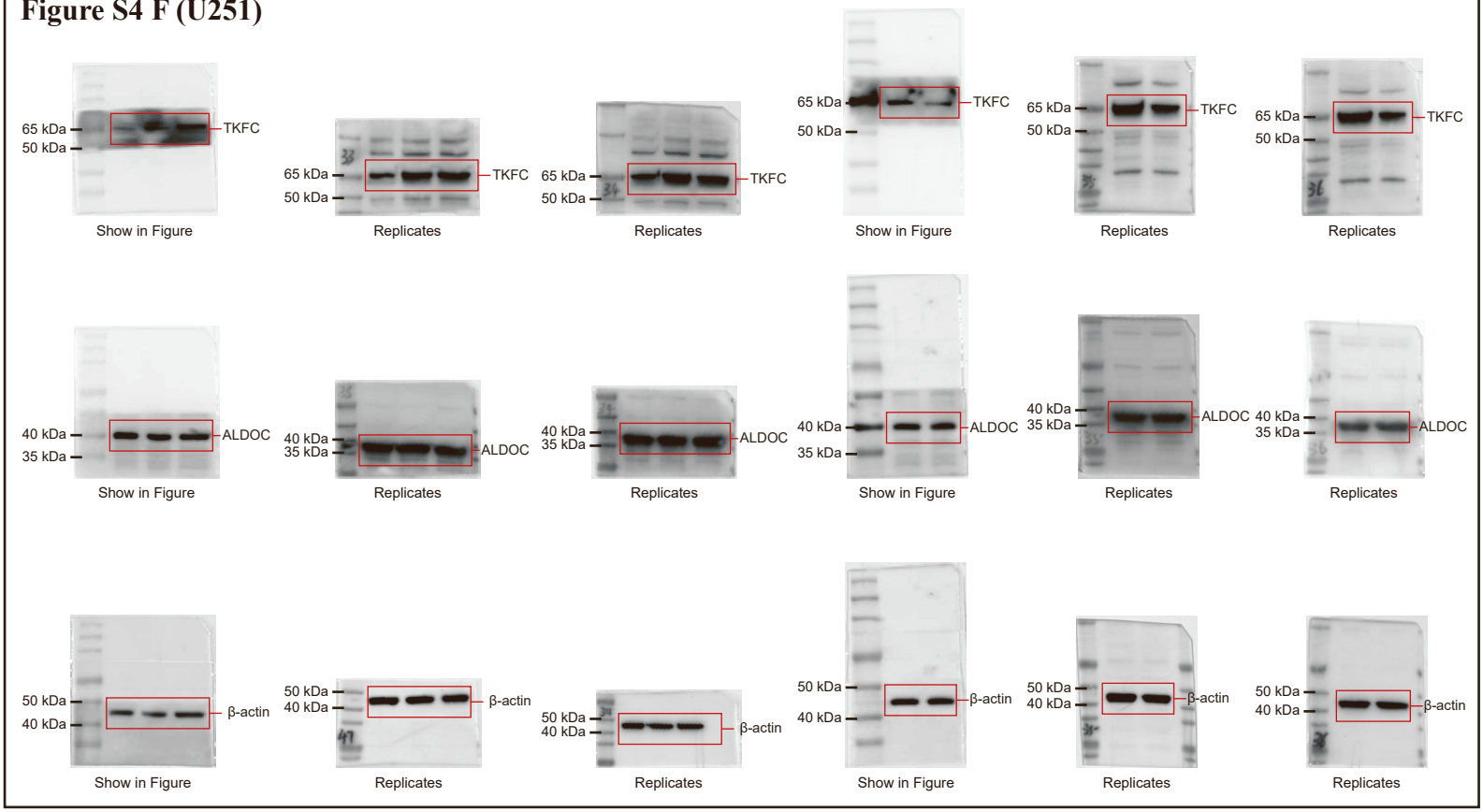


Figure S8 A (U87)

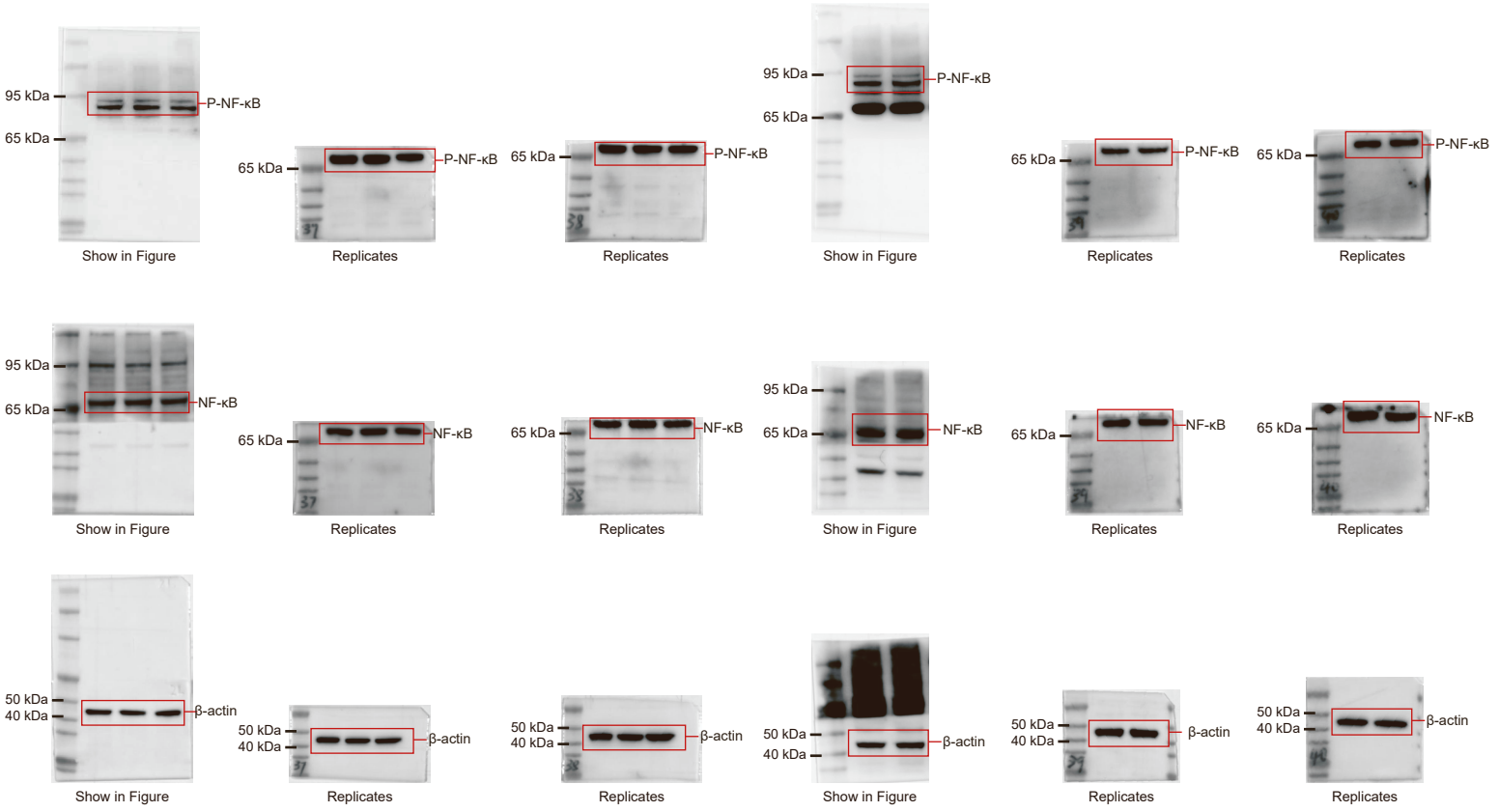


Figure S8 A (U251)

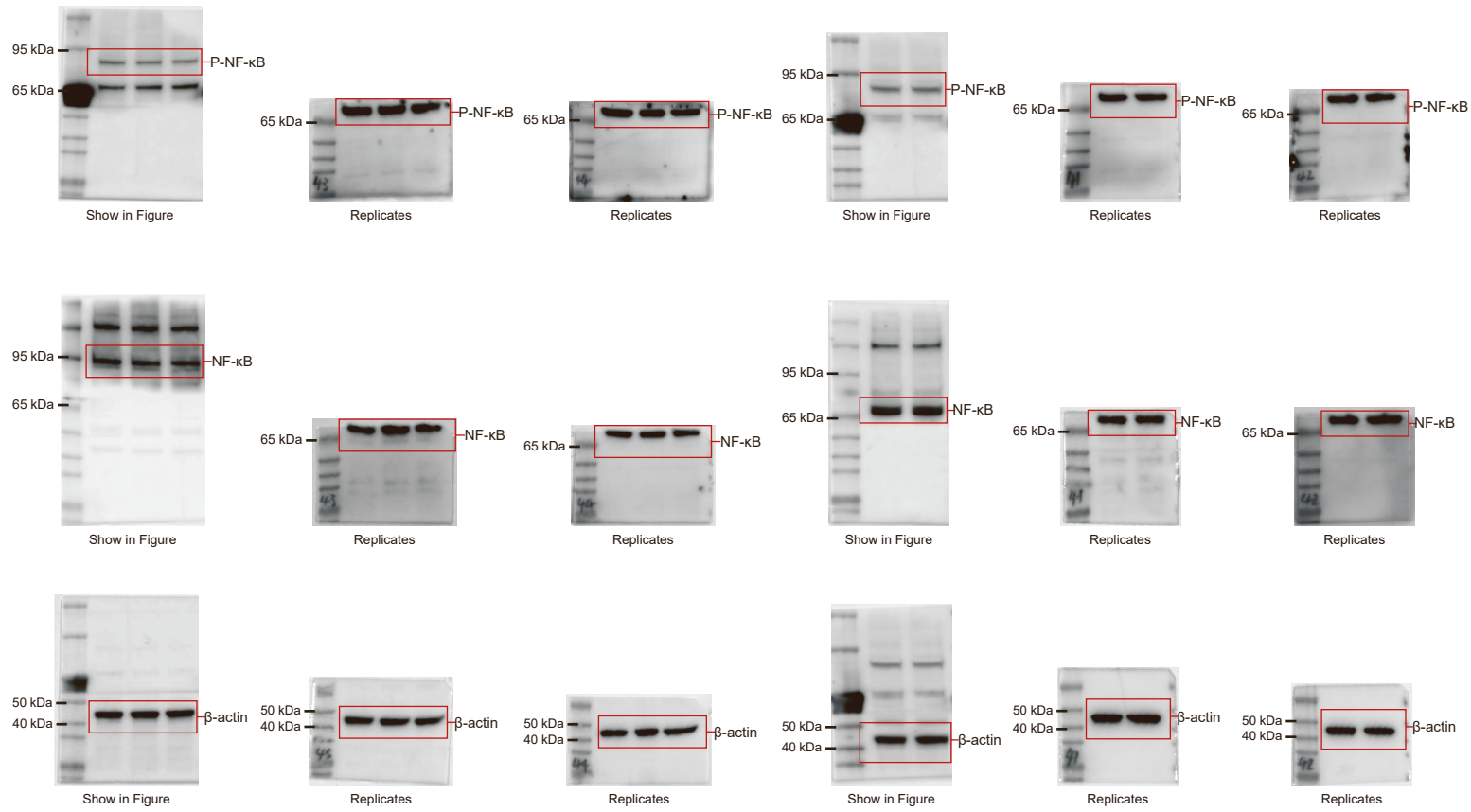
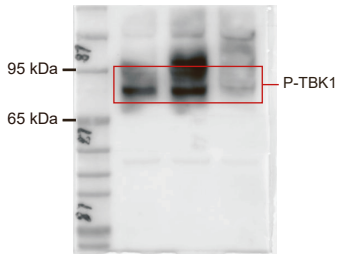
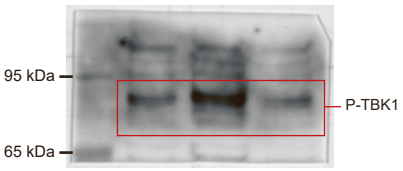


Figure S9 E (U87)

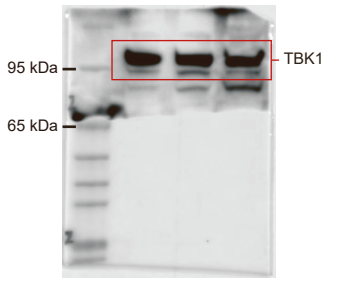


Show in Figure

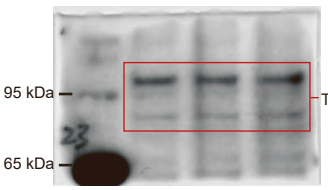
Replicates



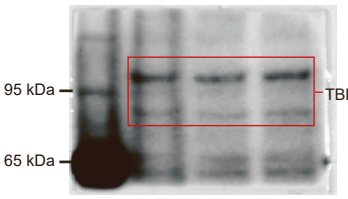
Replicates



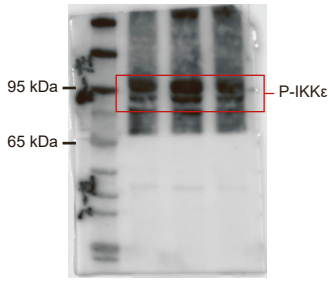
Show in Figure



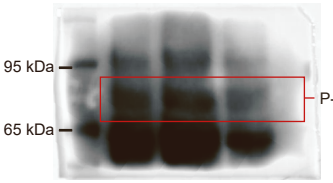
Replicates



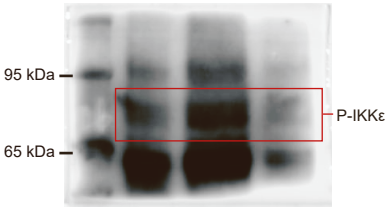
Replicates



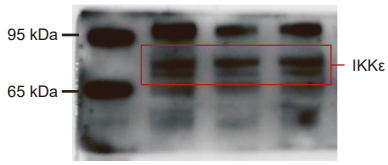
Show in Figure



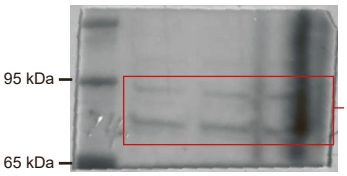
Replicates



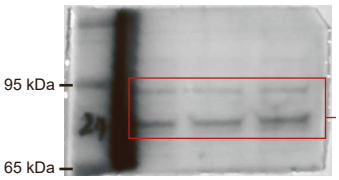
Replicates



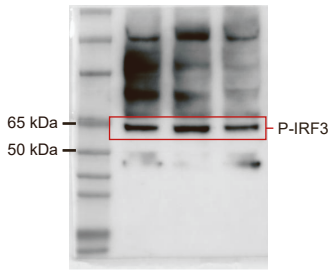
Show in Figure



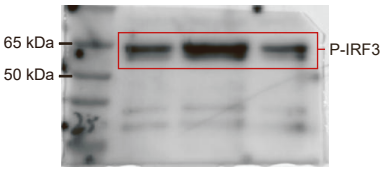
Replicates



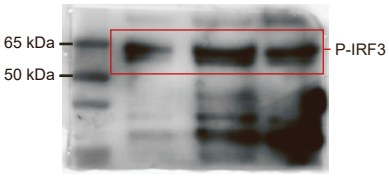
Replicates



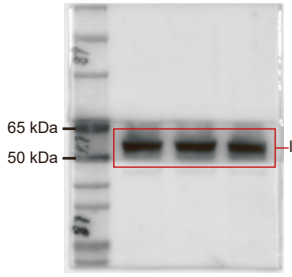
Show in Figure



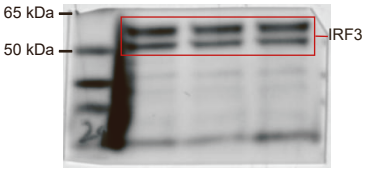
Replicates



Replicates

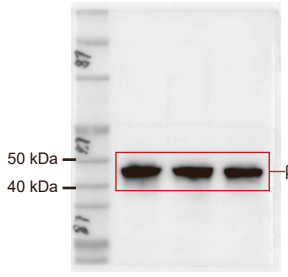


Show in Figure

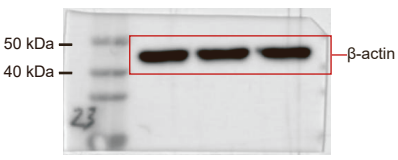


Replicates

Replicates



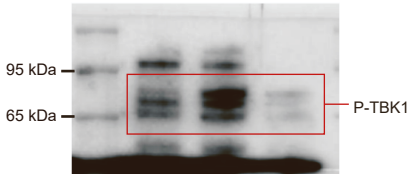
Show in Figure



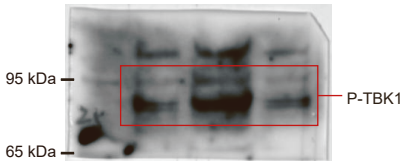
Replicates

Replicates

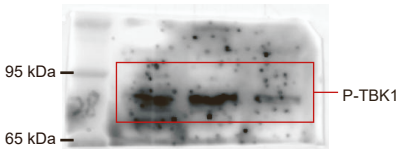
Figure S9 E (U251)



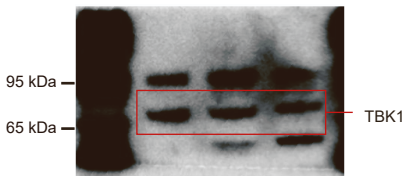
Show in Figure



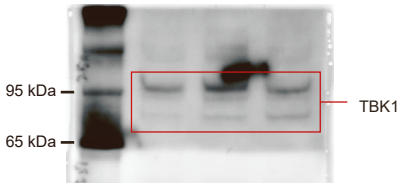
Replicates



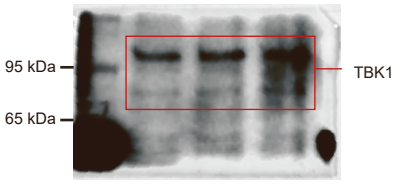
Replicates



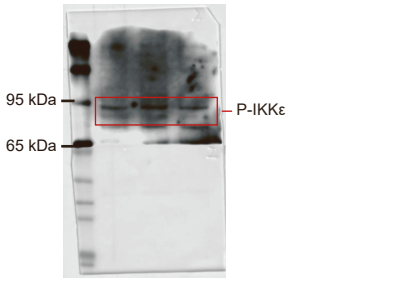
Show in Figure



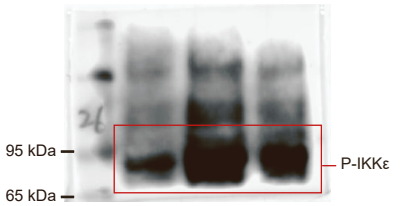
Replicates



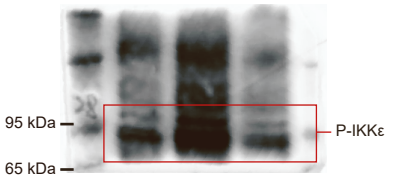
Replicates



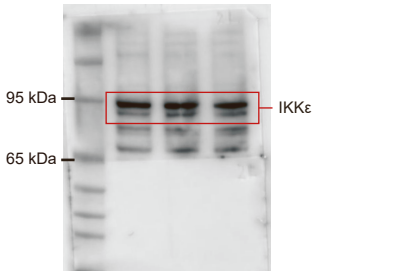
Show in Figure



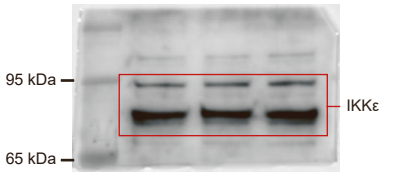
Replicates



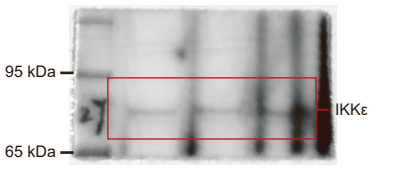
Replicates



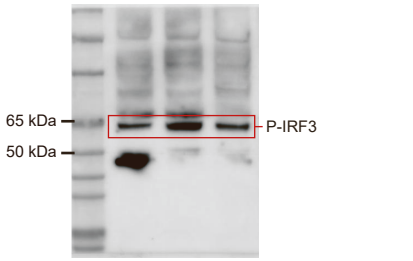
Show in Figure



Replicates

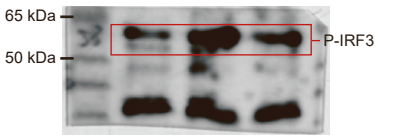


Replicates

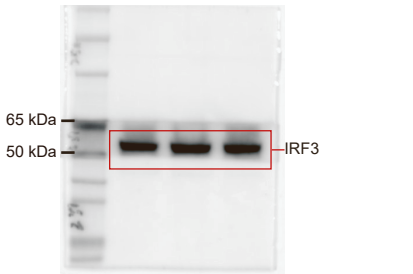


Show in Figure

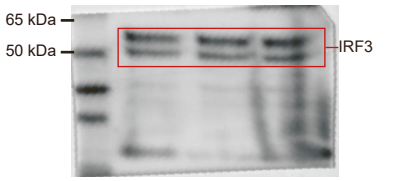
Replicates



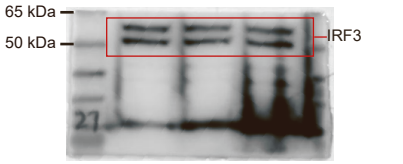
Replicates



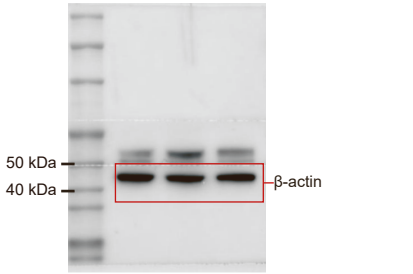
Show in Figure



Replicates

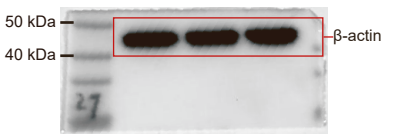


Replicates



Show in Figure

Replicates



Replicates