Expanded View Figures

Figure EV1. Identification of a short TAZ isoform transcribed by an alternative promoter.

A Antibodies targeting C-terminus YAP/TAZ, such as TAZ (CST) and YAP/TAZ, effectively pulled down the smaller protein (asterisk) in Caco2 cells in an IP assay.
B The smaller protein (asterisk) was not pulled down using anti-TAZ (SA) recognizing N-terminus of TAZ.
C cTAZ is expressed in mouse lymph nodes and thymus.
D cTAZ is expressed in human lymph nodes. Protein and mRNA expression of cTAZ in lymph nodes of thyroid cancer patients were analyzed.
E Exogenous YAP/TAZ was not processed to cTAZ. Caco2 cells were transfected with C-terminal HA-tagged TAZ or YAP, and protein expression was determined.
F Information about CRISPR/Cas9 guide RNAs targeting *cTAZ*. UTR and exons were in green.
G The deletion of the alternative exon of *cTAZ* gene. Genomic DNA of *cTAZ* KO cells was subjected to Sanger sequencing.
H No correlation between cTAZ and full-length TAZ expression at mRNA level in human tissues. 492 tissue samples were used in this analysis. Pearson's correlation coefficient R^2 was calculated.
I Low H3K9ac, H3K27ac, H3K4me1, H3K4me2 and H3K4me3 signals around *cTAZ* promoter of MCF-7 cells.

Source data are available online for this figure.

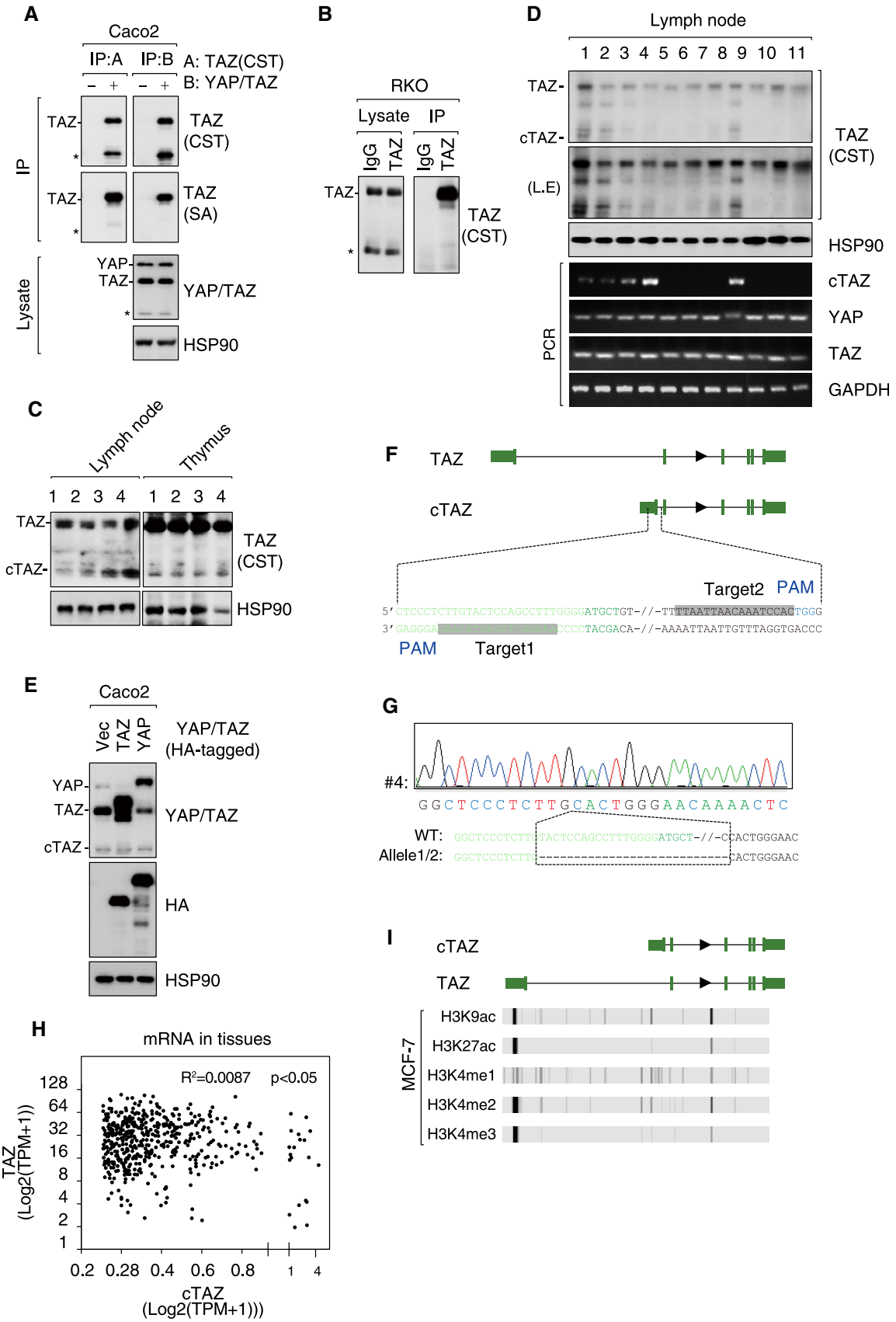


Figure EV1.

Figure EV2. cTAZ was not regulated by Hippo signaling.

A Protein domains and interacting proteins of TAZ. Phosphorylation sites of LATS kinases are shown.

B cTAZ did not interact with LATS2. HEK293A cells were transfected with Flag-tagged cTAZ, TAZ, YAP, WW-mutated TAZ (WW), and WW1/2-mutated YAP (WW1/2), and cell lysates were prepared and used for IP.

C cTAZ was not regulated by serum stimulation. cTAZ- or TAZ-overexpressing MCF-10A cells were stimulated by 10% FBS for 1 h, and protein expression was determined. Ectopic and endogenous TAZ were down-shifted upon serum stimulation, whereas no change for cTAZ was detected.

D cTAZ was not regulated by serum starvation. cTAZ- or TAZ-overexpressing HEK293A cells were serum-starved for 8 h and harvested for IB. The phosphorylation of full-length TAZ was also determined using phos-tag gel. The asterisk indicates a shift of TAZ on regular gel.

E Deletion of LATS kinases stabilized TAZ, but not cTAZ. WT or LATS1/2-double-knockout (dKO) HEK293A cells were transfected with Flag-tagged cTAZ or TAZ. Cells were treated with CHX (50 μg/ml) in a time course (0–4 h), and protein levels were determined. Error bars indicate SD, $n = 3$. ***$P < 0.001$; two-way ANOVA test was used for statistical analysis.

F Serum treatment stabilized TAZ, but not cTAZ. RKO cells cultured in the presence or absence of 10% serum were treated with CHX (50 μg/ml) in a time course (0–6 h), and protein levels were determined. Error bars indicate SD, $n = 3$. ***$P < 0.001$; two-way ANOVA test was used for statistical analysis.

Source data are available online for this figure.

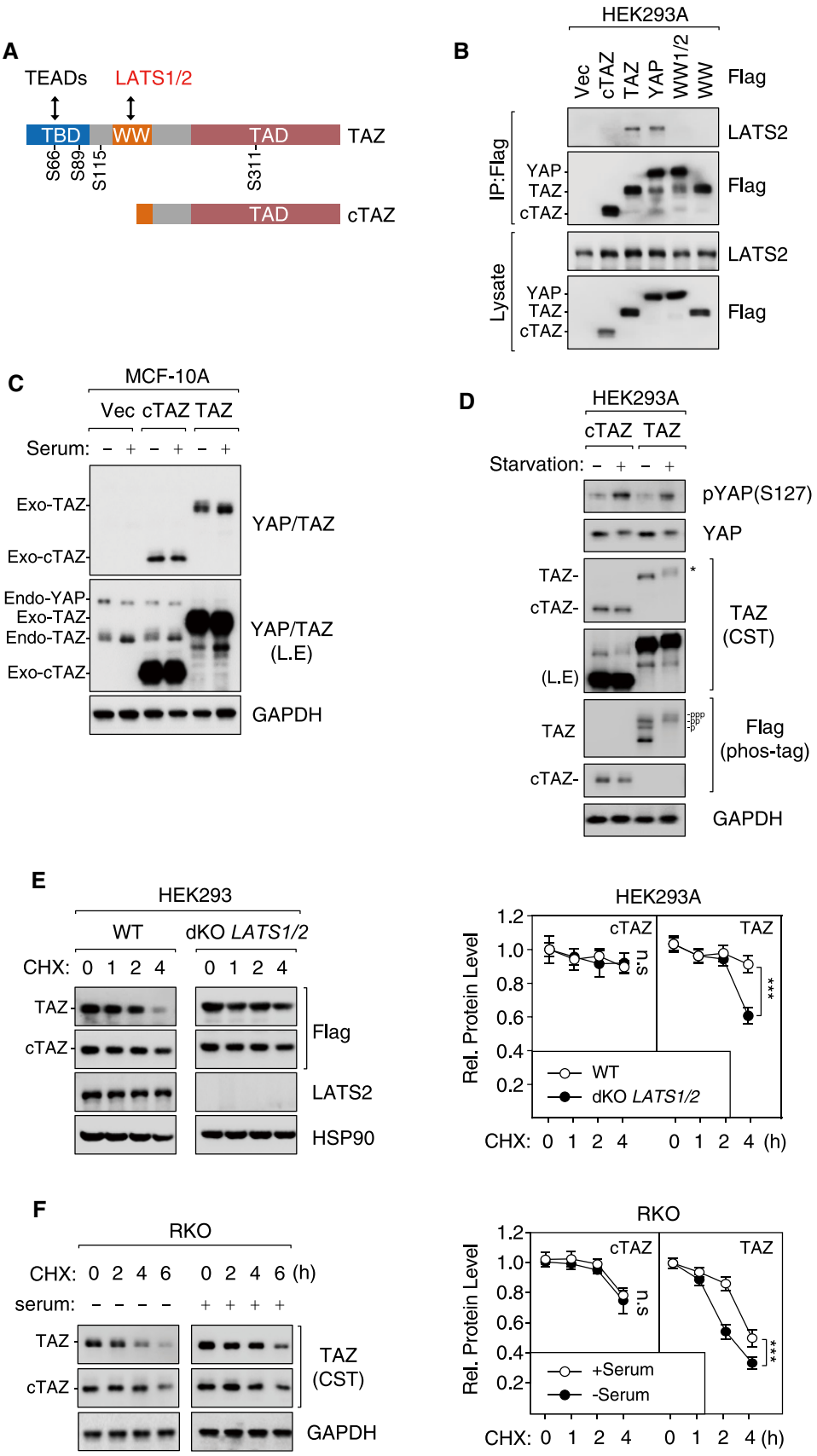


Figure EV2.

Figure EV3. cTAZ had no TEAD-dependent transcriptional activity.

A cTAZ did not interact with TEADs. HEK293A cells were transfected with Flag-tagged cTAZ, TAZ, YAP, WW-mutated TAZ (WW), or WW1/2-mutated YAP (WW1/2), and cells were lysed 24 h later for IP using Flag antibody. The asterisk indicates position of IgG.

B cTAZ could not activate TEAD reporter. HEK293A cells were transfected with 8×GTIIC-luciferase reporter along with cTAZ or TAZ plasmid. Luciferase activity was measured. The expression levels of TAZ, cTAZ, and CYR61 (a TAZ targeting gene) were determined by IB. Error bars indicate SD, $n = 3$. ***$P < 0.001$; Student's *t*-test.

C cTAZ could not induce expression of YAP/TAZ target genes. Cells were transfected as in (B). Endogenous *CTGF* and *CYR61* mRNA levels were determined by qPCR. Error bars indicate SD, $n = 3$. ***$P < 0.001$; Student's *t*-test.

D Overexpression of cTAZ could not induce EMT. cTAZ- or TAZ-overexpressing MCF-10A cell lines were established. Expression of TAZ target genes (CYR61 and CTGF) and EMT markers (epithelial marker: E-cadherin; mesenchymal markers: N-cadherin and vimentin) was assessed by IB.

E cTAZ could not induce EMT phenotype. MCF-10A cells (also used in D) expressing cTAZ remained in an organized monolayer, and actin cytoskeleton was similar to that of wild-type cells. On the other hand, full-length TAZ induced cell morphological change and formation of strong actin fibers. Filamentous actin (F-actin) was visualized by FITC-conjugated phalloidin. Scale bar, 100 μm.

F cTAZ could not induce anchorage-independent growth. MCF10A cells (also used in D) were seeded into soft agar; after 3 weeks, colony formation was determined. Error bars indicate SD, $n = 3$. ***$P < 0.001$; Student's *t*-test.

G The expression of TAZ, but not cTAZ, served as a prognostic marker. Lung squamous cell carcinoma (LUSC) mRNA expression data were collected from TCGA database, and the relationship between TAZ (or cTAZ) expression and patient overall survival was analyzed using GEPIA application. The *P*-values are derived by using the Mantel–Cox test.

Source data are available online for this figure.

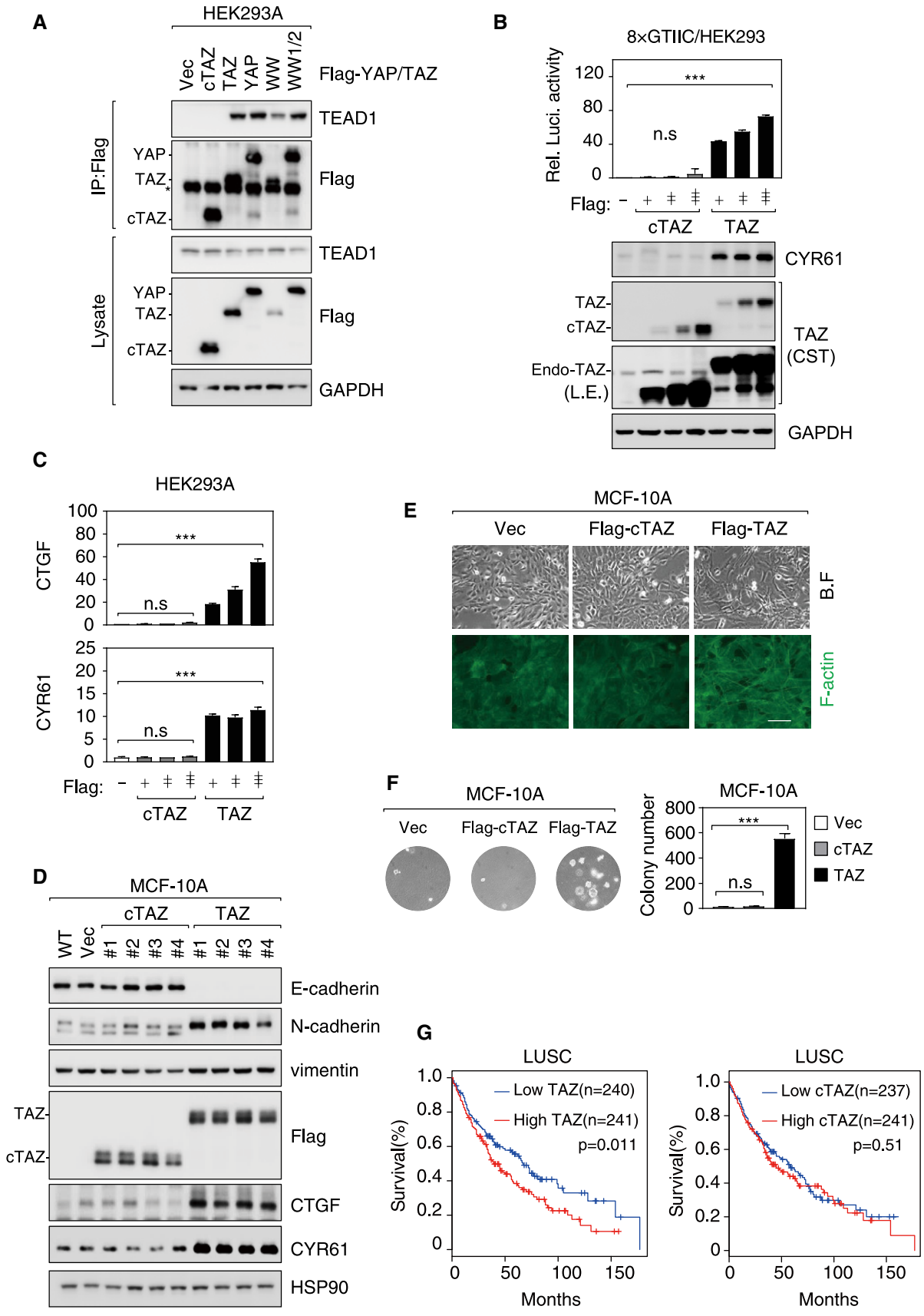


Figure EV3.

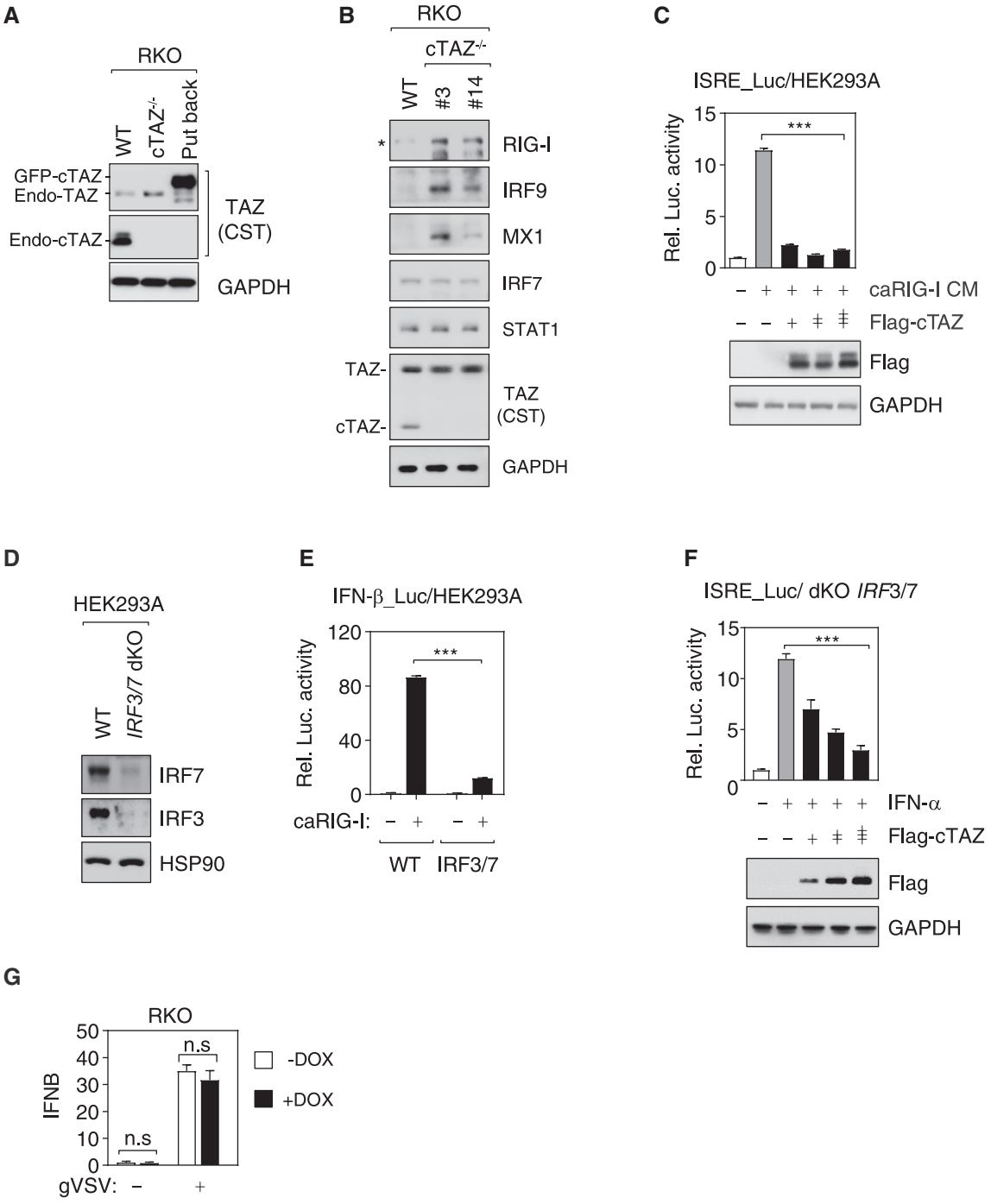

A
RKO
WT
cTAZ-/-
Put back
GFP-cTAZ
Endo-TAZ
Endo-cTAZ
TAZ (CST)
GAPDH
B
RKO
cTAZ-/-
WT
#3
#14
*
RIG-I
IRF9
MX1
IRF7
STAT1
TAZ-
cTAZ-
TAZ (CST)
GAPDH
C
ISRE_Luc/HEK293A
Rel. Luc. activity
15
10
5
0
***
caRIG-I CM
Flag-cTAZ
Flag
GAPDH
D
HEK293A
WT
IRF3/7 dKO
IRF7
IRF3
HSP90
E
IFN-β_Luc/HEK293A
Rel. Luc. activity
120
80
40
0
***
caRIG-I:
WT
IRF3/7
F
ISRE_Luc/ dKO IRF3/7
Rel. Luc. activity
15
10
5
0
***
IFN-α
Flag-cTAZ
Flag
GAPDH
G
RKO
IFNB
50
40
30
20
10
0
n.s
n.s
-DOX
+DOX
gVSV:

Figure EV4.

Figure EV4. cTAZ directly repressed JAK-STAT signaling.

A cTAZ protein levels in cells used for RNA-seq (Fig 2A).

B Depletion of cTAZ induced protein expression of several ISGs. WT RKO cells and cTAZ$^{-/-}$ clones (#3, #14) were used. The asterisk indicates position of RIG-I.

C cTAZ repressed 5×ISRE-luciferase activity. HEK293A cells were transfected with 5×ISRE-luciferase reporter along with indicated plasmids, treated with or without conditional medium (CM) from caRIG-I expressing cells for 12 h, and luciferase activity was measured. Protein levels were determined by IB. Error bars indicate SD, $n = 3$. ***$P < 0.001$; Student's *t*-test.

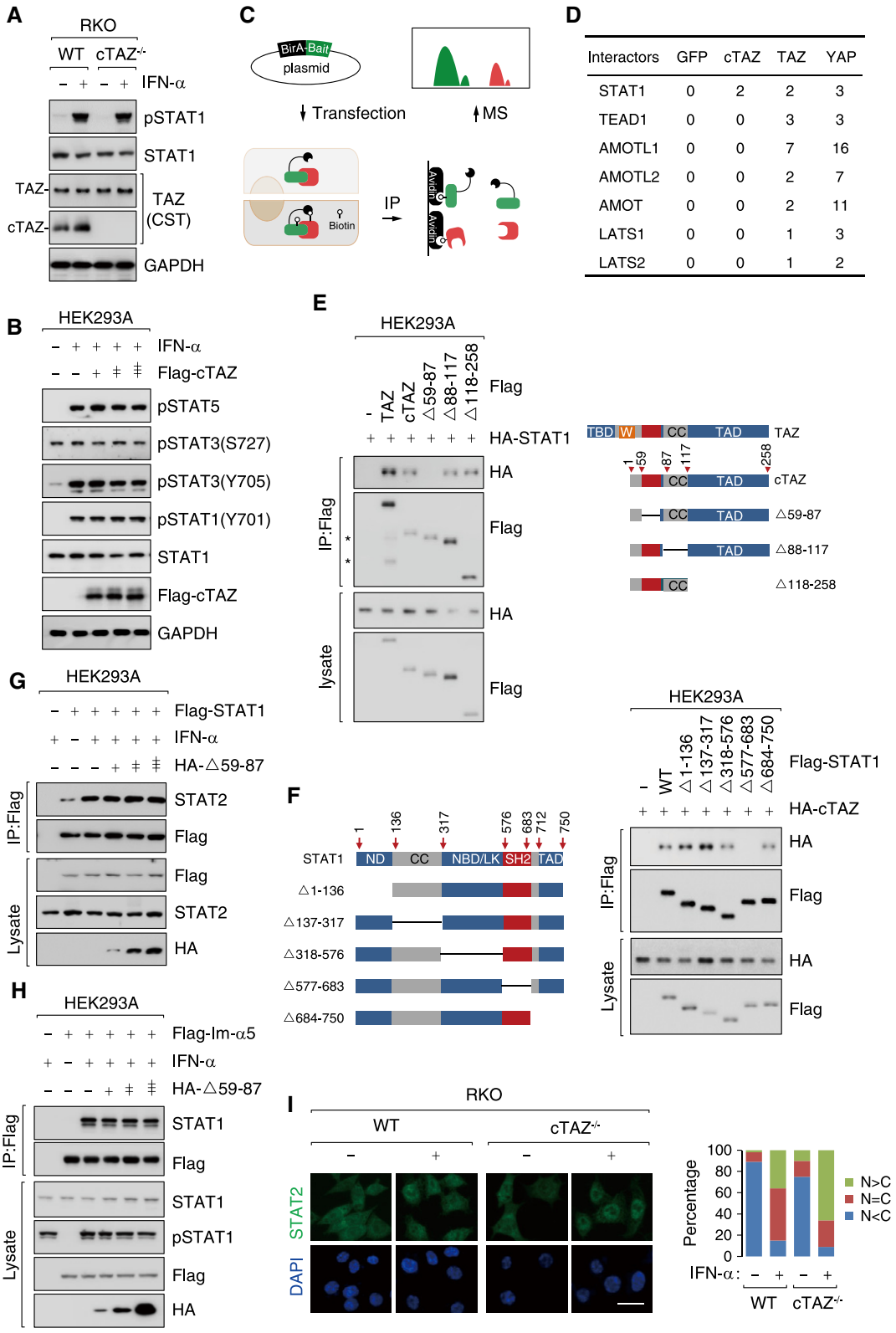
D Knockout (pooled) of *IRF3* and *IRF7* (dKO) using CRISPR/Cas9 technology. The expression of *IRF3*/*7* was assessed by IB.

E Knockout of *IRF3*/*7* significantly downregulated IFN-β-promoter activity induced by caRIG-1. Error bars indicate SD, $n = 3$. ***$P < 0.001$; Student's *t*-test.

F cTAZ repressed 5×ISRE-reporter activity induced by IFN-α in *IRF3*/*7* dKO cells. Error bars indicate SD, $n = 3$. ***$P < 0.001$; one-way ANOVA test was used for statistical analysis.

G cTAZ did not affect *IFNB* mRNA expression. Control or cTAZ-expressing (DOX induced) RKO cells were infected by gVSV for 12 h. *IFNB* expression was determined using qPCR. Error bars indicate SD, $n = 3$. Student's *t*-test.

Source data are available online for this figure.


A
RKO
WT cTAZ-/-
IFN-α
pSTAT1
STAT1
TAZ-
cTAZ-
TAZ (CST)
GAPDH
B
HEK293A
IFN-α
Flag-cTAZ
pSTAT5
pSTAT3(S727)
pSTAT3(Y705)
pSTAT1(Y701)
STAT1
Flag-cTAZ
GAPDH
C
BirA-Bait
plasmid
Transfection
MS
Biotin
IP
Avidin
D
Interactors GFP cTAZ TAZ YAP
STAT1 0 2 2 3
TEAD1 0 0 3 3
AMOTL1 0 0 7 16
AMOTL2 0 0 2 7
AMOT 0 0 2 11
LATS1 0 0 1 3
LATS2 0 0 1 2
E
HEK293A
- TAZ cTAZ △59-87 △88-117 △118-258
Flag
HA-STAT1
IP:Flag
HA
Flag
lysate
HA
Flag
TBD W CC TAD TAZ
1 59 87 117 258
CC TAD cTAZ
CC TAD △59-87
TAD △88-117
CC △118-258
F
1 136 317 576 683 712 750
STAT1 ND CC NBD/LK SH2 TAD
△1-136
△137-317
△318-576
△577-683
△684-750
G
HEK293A
Flag-STAT1
IFN-α
HA-△59-87
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STAT2
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Lysate
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HA
H
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Flag-Im-α5
IFN-α
HA-△59-87
IP:Flag
STAT1
Flag
Lysate
STAT1
pSTAT1
Flag
HA
HEK293A
- WT △1-136 △137-317 △318-576 △577-683 △684-750
Flag-STAT1
HA-cTAZ
IP:Flag
HA
Flag
Lysate
HA
Flag
I
RKO
WT cTAZ-/-
STAT2
DAPI
Percentage
100 80 60 40 20 0
N>C
N=C
N<C
IFN-α: - + - +
WT cTAZ-/-

Figure EV5.

Figure EV5. cTAZ attenuated dimerization of STAT1/2.

A Deletion of cTAZ had no effect on the phosphorylation of STAT1 induced by IFN-α. WT and cTAZ$^{-/-}$ RKO cells were treated with IFN-α (50 ng/ml) for 1 h.
B Ectopic cTAZ did not modulate the phosphorylation of STAT proteins induced by IFN-α. HEK293A was transfected with Flag-tagged cTAZ, and treated with or without IFN-α (50 ng/ml) for 1 h.
C The flowchart of BioID assay. BirA*-fused GFP, cTAZ, TAZ, or YAP was stably overexpressed in HEK293A cells and subjected to BioID assays.
D Potential interacting proteins of TAZ and/or cTAZ identified in BioID assay. The values were normalized PSM (peptide–spectrum match).
E The amino acids 59–87 of cTAZ were required for interaction with STAT1. A schematic representation of cTAZ deletion mutants used is shown on the left, CC: coiled-coil domain; TAD: transcriptional activation domain. Transfection and IP assays were performed as in Fig 3A. Asterisks indicate degraded TAZ in lane 2.
F The CC domain of STAT1 was required for interaction with cTAZ. A schematic representation of STAT1 deletion mutants used is shown on the left, ND: N-terminal Domain; DBD/LK: DNA binding domain/linker domain; SH2: SH2 domain; CC: coiled-coil domain; TAD: transcriptional activation domain. Transfection and IP assays were performed as in Fig 3A.
G Amino acids 59–87-deleted cTAZ failed to attenuate STAT1/2 dimerization induced by IFN-α. HEK293A cells were transfected and stimulated by IFN-α (50 ng/ml) for 1 h, and cells lysates were prepared for IP Flag antibody.
H Amino acids 59–87 deleted cTAZ failed to attenuate interaction between STAT1 and importin-α5.
I Deletion of cTAZ promoted nuclear accumulation of STAT2 under IFN-α stimulation. Same cells and treatment were used as in Fig 3E. Scale bar, 50 μm.

Source data are available online for this figure.