Expanded View Figures

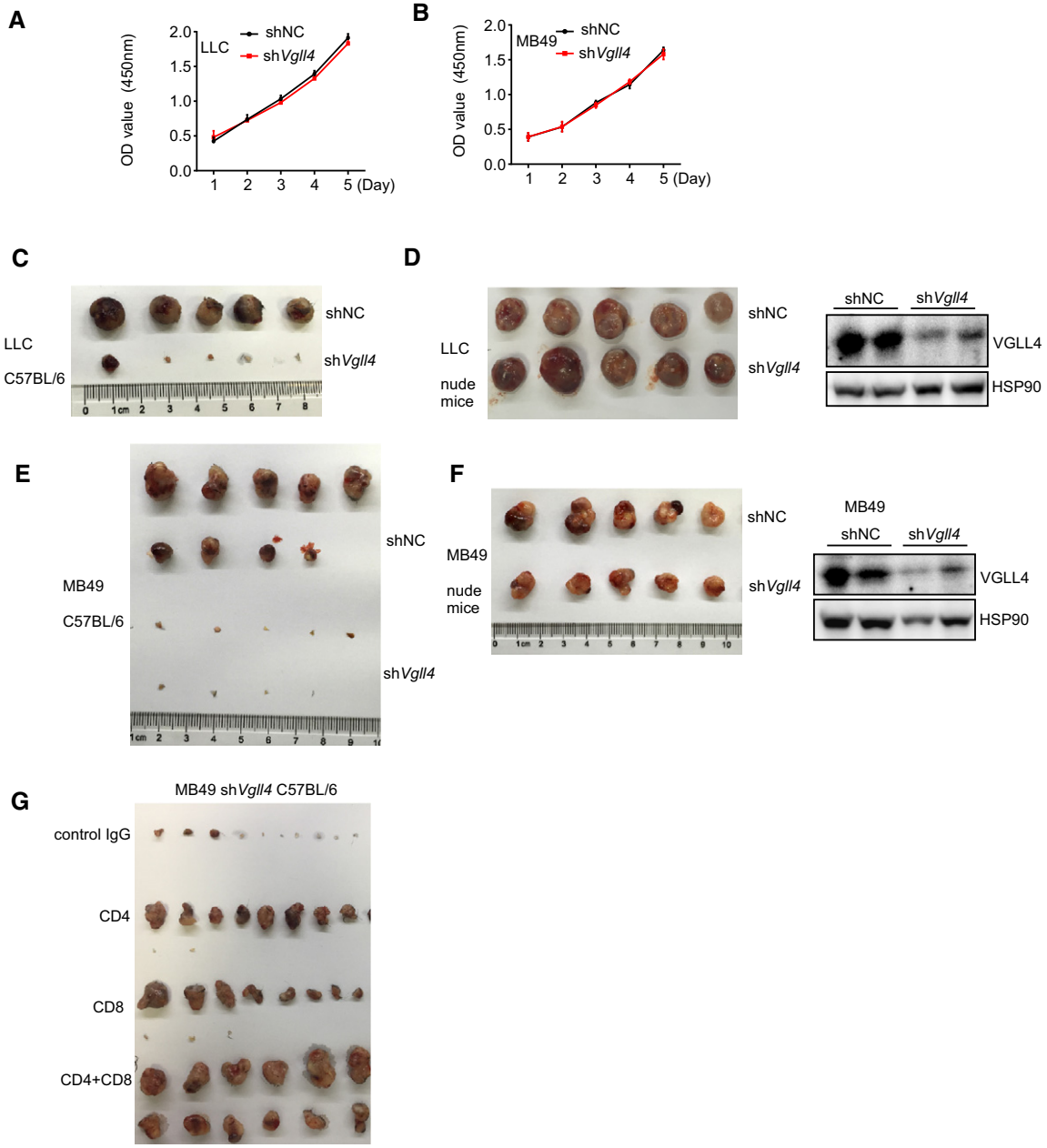

Figure EV1. Loss of Vgll4 in tumor cells does not affect cell proliferation, but inhibits tumor growth in syngeneic mouse model.

A, B shVgll4 LLC (A) and MB49 (B) cells grew similar as control cells in regular cell cultures using CCK8 assay. n = 3, mean ± SEM.

C The gross appearance of the primary tumors of control or shVgll4 LLC from C57BL/6 mice.

D The gross appearance of the primary tumors of control or shVgll4 LLC from nude mice. VGLL4 protein expression levels in the tumor lysates from nude mice were analyzed by Western blot and shown in the right panel.

E The gross appearance of the primary tumors of control or shVgll4 MB49 from C57BL/6 mice.

F The gross appearance of the primary tumors of control or shVgll4 MB49 from nude mice. VGLL4 protein expression levels in the tumor lysates from nude mice were analyzed by Western blot and shown in the right panel.

G The gross appearance of the primary shVgll4 MB49 tumors from C57BL/6 mice receiving anti-CD4 alone, anti-CD8 alone, or both anti-CD4 and anti-CD8 blocking antibodies.

Source data are available online for this figure.

Figure EV2. Disruption of VGLL4 expression suppresses PD-L1 expression.

A Cell surface PD-L1 expression is analyzed by flow cytometry in MB49 cells transfected with control or siVgll4 siRNA and stained with PE-conjugated anti-mouse PD-L1 antibody or isotype control antibody.

B Cell surface PD-L1 expression is analyzed by flow cytometry on the IFNγ-stimulated A549 cells transfected with control or siVgll4 siRNA and stained with APC-conjugated anti-human PD-L1 antibody or isotype control antibody.

C Knockdown of VGLL4 by siRNA increases the expression of YAP target genes. The levels of CTGF and CYR61 were determined by qRT–PCR. $n = 3$, mean ± SEM.

D Knockdown of VGLL3 does not affect PD-L1 expression in A549 cells. PD-L1 mRNA levels were analyzed by qRT–PCR in A549 cells transfected with control or siVGLL3 siRNA. $n = 3$, mean ± SEM.

E Depletion of VGLL4 results in reduced PTEN protein levels. A549 or MB 49 cells transfected with control or siVGLL4 siRNA were analyzed with indicated antibodies by Western blot.

F LLC and MB49 tumors respond to PD-L1 blocking antibody treatment. C57BL/6 mice inoculated with LLC ($n = 11$ tumors) or MB49 ($n = 8$ tumors) tumor cells were administrated with anti-mouse PD-L1 antibody every 3 days. Tumor-growth kinetics was measured at the indicated times. mean ± SEM. $^{*}P < 0.05$, $^{***}P < 0.001$, two-tailed Student's *t*-test.

G Expression of PD-L1 in Vgll4-knockdown LLC tumors does not affect YAP target gene expression. Cyr61 mRNA expression levels were analyzed in control or Vgll4-knockdown PD-L1-overexpression LLC tumors by qRT–PCR. $n = 3$ tumors, mean ± SEM, n.s. $P > 0.05$. One-way ANOVA followed by Tukey's test.

H Analysis of proliferation rate of LLC and MB49 cells treated with IFNγ. Control, and Vgll4-knockdown LLC or MB49 cells were treated with IFNγ, and the proliferation kinetics was measured by CCK8 assay. $n = 3$, mean ± SEM.

I Expression of IRF1 in Vgll4-knockdown LLC tumors restored the tumor growth in C57BL/6 mice. Tumor volumes were measured 15 days for nude mice and 21 days for C57BL/6 mice after LLC tumor cell inoculation. $n = 8$ tumors for each group. $^{*}P < 0.05$, $^{***}P < 0.001$, two-tailed Student's *t*-test. The solid line represents the average volume ± SEM.

Source data are available online for this figure.

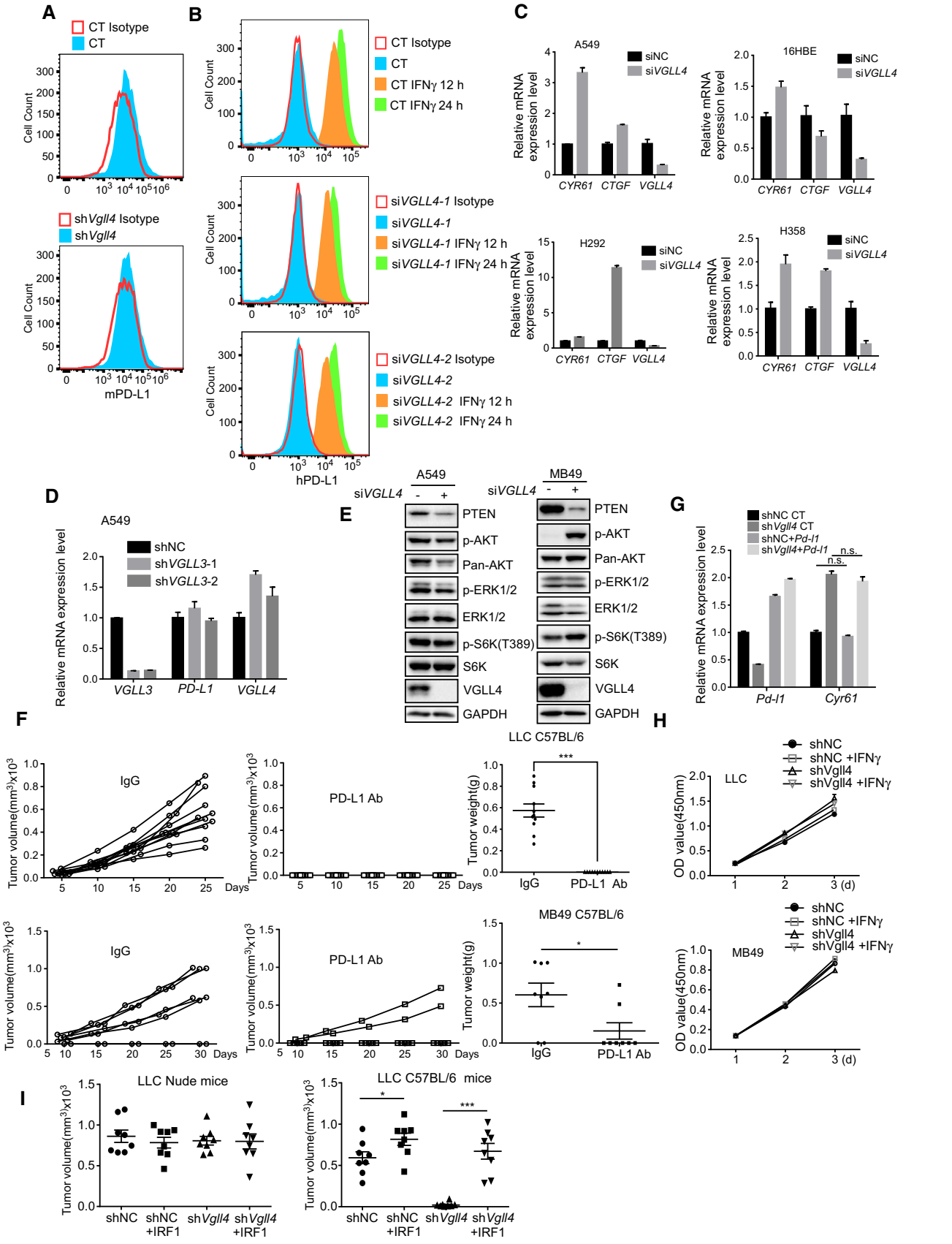

Figure EV2.

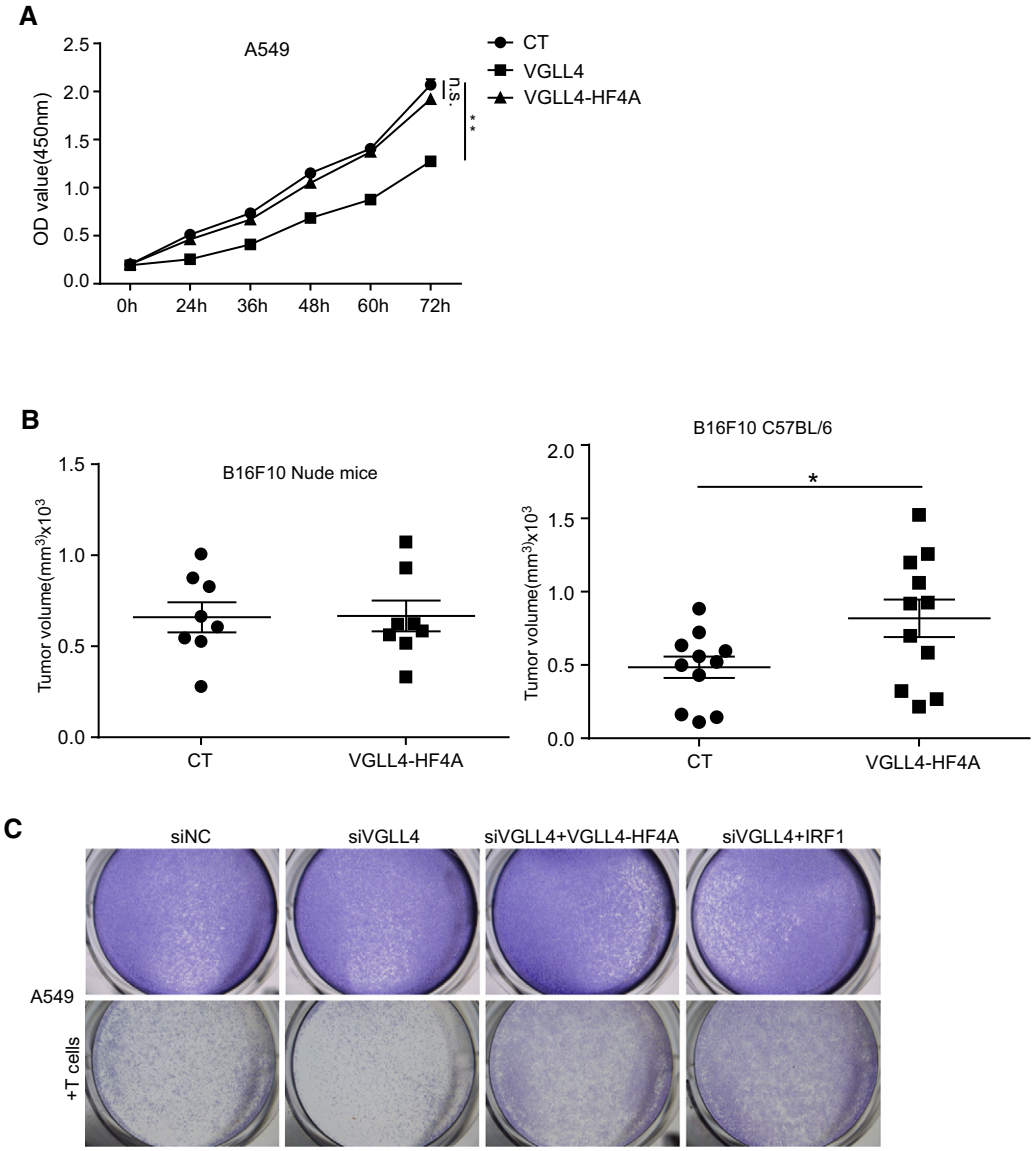

Figure EV3. VGLL4-HF4A rescues the defects of VGLL4-knockdown tumor cells.

A VGLL4 suppresses A549 cell growth *in vitro*. The proliferation of A549 cells with VGLL4 or VGLL4-HF4A expression was detected by CCK8 assay. $n = 3$, mean ± SEM, $^{**}P < 0.01$, one-way ANOVA followed by Dunnett's test.

B Overexpression of VGLL4-HF4A promotes B16F10 tumor growth in C57BL/6 mice. Control or VGLL4-HF4A-overexpressing B16F10 cells were transplanted into nude mice ($n = 8$ tumors each group) or C57BL/6 ($n = 11$ tumors each group) mice, and tumor volumes were measured 21 days after tumor cell inoculation. $^{*}P < 0.05$, two-tailed Student's *t*-test. The solid line represents the average weight ± SEM.

C Expression of VGLL4-HF4A attenuates the T cell-mediated tumor cell killing. Activated T cells and A549 cells were co-cultured in 24-well plates for 4 days and surviving cells stained with crystal violet.

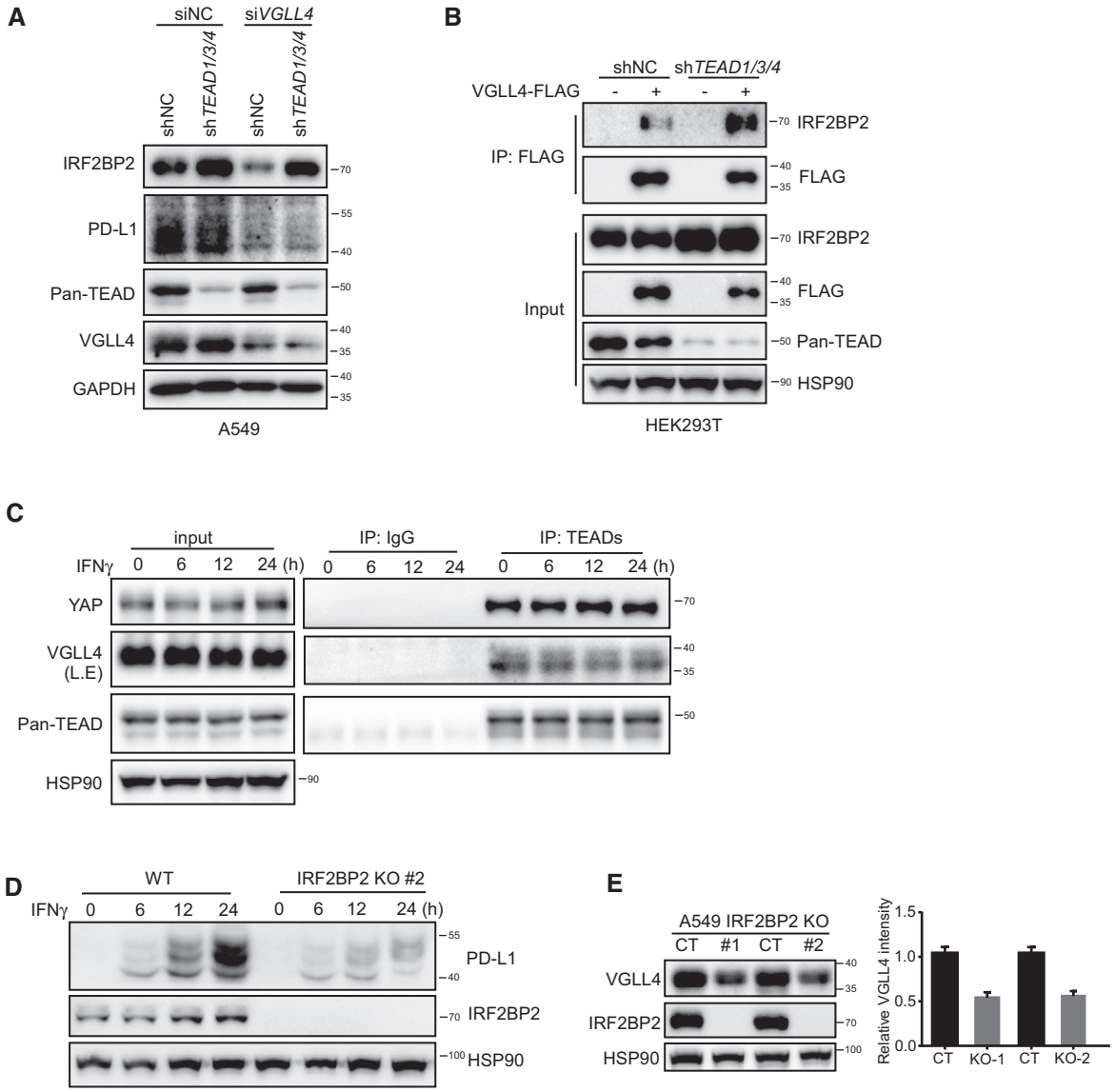

Figure EV4. Knockdown of TEADs increases the association of VGLL4 with IRF2BP2.

A A549 cells were treated with lenti-shTEAD virus or together with siRNA against VGLL4, and cell lysates were analyzed by Western blot with indicated antibodies.

B Knockdown TEADs increase the association of VGLL4 with IRF2BP2. Immunoprecipitation analysis using anti-FLAG antibody was performed in HEK293T cells transduced with lenti-shTEAD virus and followed by transfection with VGLL4-FLAG plasmid.

C The interaction between TEADs with YAP or VGLL4 is not affected in response to IFNγ stimulation. Immunoprecipitation analysis using anti-pan TEAD antibody was performed in A549 cells treated with IFNγ.

D Loss of IRF2BP2 attenuated IFNγ-inducible expression of PD-L1. An independent IRF2BP2 KO A549 clone was treated with IFNγ for the indicated time, and cell lysates were analyzed by Western blot.

E VGLL4 protein levels were reduced in IRF2BP2 KO A549 cells. Immunoblot analysis of control and IRF2BP2 KO A549 cells with indicated antibodies. Quantification of VGLL4 protein levels in control and IRF2BP2 KO A549 cells is shown in the right panel. $n = 3$, mean ± SEM.

Source data are available online for this figure.

Figure EV5. YAP inhibits IFNγ and TNFα-inducible PD-L1 expression.

A miR-130a expression level was analyzed in VGLL4-knockdown A549 cells by qRT–PCR. $n = 3$, mean ± SEM, $^{**}P < 0.01$, two-tailed Student's *t*-test.

B YAP5SA expression increases PD-L1 mRNA levels in A549 cells. A549 cells were transduced with control or YAP-5SA lentivirus and subjected to qRT–PCR analysis. $n = 3$, mean ± SEM, $^{***}P < 0.001$, two-tailed Student's *t*-test.

C WT-YAP inhibits IFNγ-inducible PD-L1 expression. A549 cells were transduced with control or WT-YAP lentivirus and stimulated with IFNγ for the indicated time. PD-L1 mRNA expression was analyzed by qRT–PCR. $n = 3$, mean ± SEM.

D LPA treatment attenuates IFNγ-inducible PD-L1 expression. A549 cells were treated with LPA together with IFNγ and subjected to qPCR analysis. $n = 3$, mean ± SEM, $^{***}P < 0.001$, one-way ANOVA followed by Tukey's test.

E Immunofluorescent staining of YAP (green) counterstained with DAPI for DNA (blue) in A549 cells treated with IFNγ for the indicated time.

F The expression of YAP target genes was analyzed in IFNγ-treated A549 cells for the indicated time. $n = 3$, mean ± SEM.

G YAP-S127 phosphorylation was analyzed in IFNγ-treated A549 cells for the indicated time by Western blot.

H DNA sequence targeted by sgRNA is indicated by the solid line. The seed region is in red. miR-130a KO A549 cells were generated by CRISPR-/Cas9-mediated gene editing, and deleted sequence is shown.

I, J YAP inhibits TNFα-inducible PD-L1 expression. A549 cells were transduced with control, and YAP-5SA or YAPS94A lentivirus and treated with TNFα and subjected to immunoblot (I) and qRT–PCR (J) analysis. $n = 3$, mean ± SEM, $^{***}P < 0.001$, one-way ANOVA followed by Dunnett's test.

K A549 cells were transduced with YAP5SA or YAPS94A lentivirus, treated with TNFα and subjected to qRT–PCR analysis for the expression of YAP target gene CTGF. $n = 3$, mean ± SEM.

L YAP inhibits TNFα target gene expression. A549 cells were transduced with control, YAP-5SA or YAPS94A lentivirus, treated with TNFα and subjected to qRT–PCR analysis. $n = 3$, mean ± SEM.

M Correlation of VGLL4 and YAP expressions was no significant on human non-small-cell lung cancer tissue arrays containing 71 samples (Pearson correlation test; $R = 0.1049$, $P = 0.1049$).

N Correlation of PD-L1 and YAP expressions was no significant on human non-small-cell lung cancer tissue arrays containing 71 samples (Pearson correlation test; $R = 0.1852$, $P = 0.1220$).

Source data are available online for this figure.

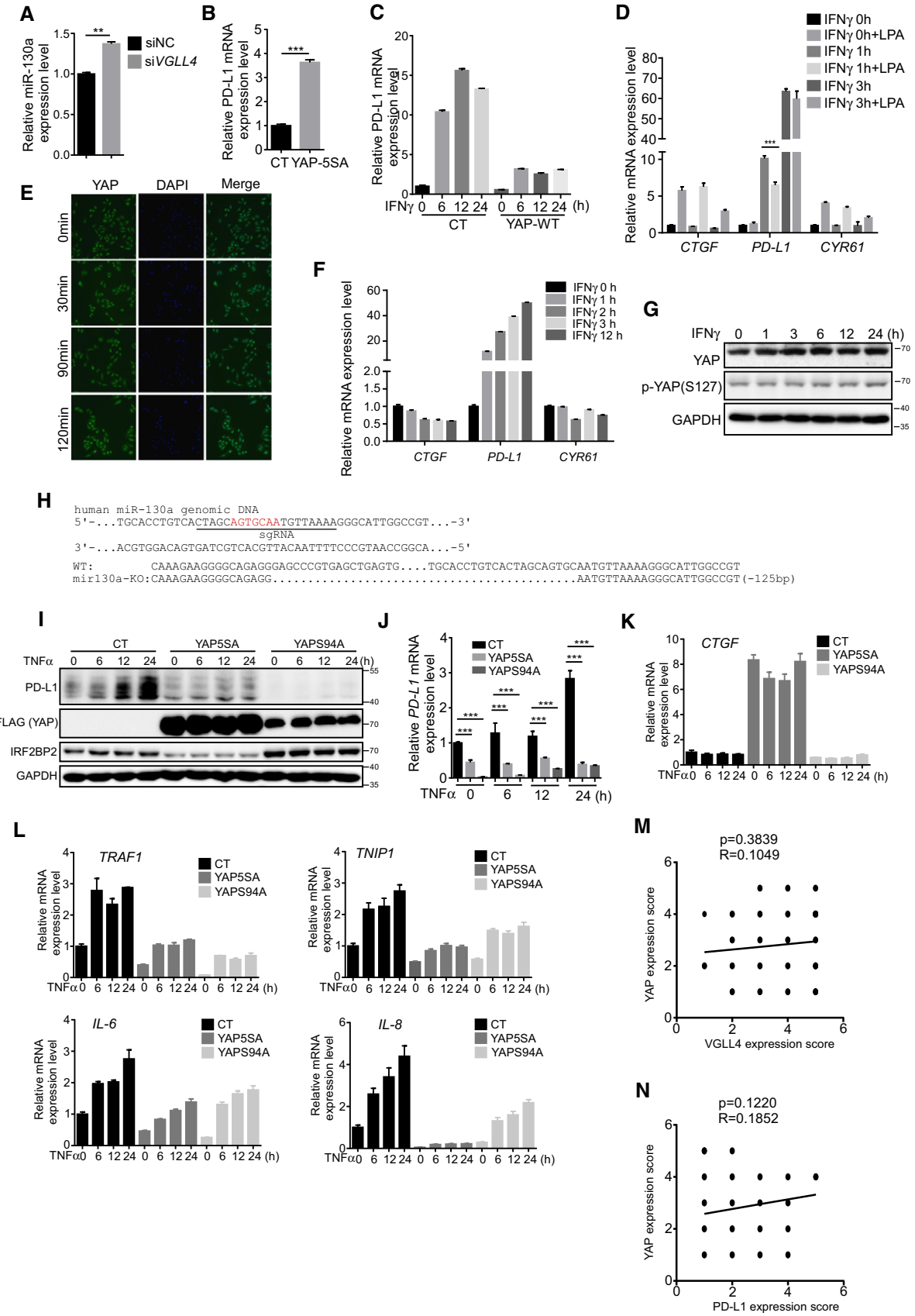

Figure EV5.