# Expanded View Figures

**Figure EV1. TRIM28 is associated with poor survival, increased expression of metastatic gene signatures, and the invasiveness of melanoma cells.**

A Univariate Cox regression analysis of bromodomain gene expression and overall survival in metastatic melanoma. SKCM data (TCGA) were analyzed for bromodomain gene expression in metastases ($n = 367$) and the overall survival of corresponding patients.

B Validation of TRIM28 knockdown using immunoblotting on cell lysates from A375 cells transduced with shLUC, shSCR, shT28-1, or shT28-2 lentiviruses.

C Densitometry (relative to β-actin) of the immunoblot in (B).

D GSEA of gene signatures associated with metastasis of melanoma to distant organs (KAUFFMANN_MELANOMA_RELAPSE_UP or WINNEPENNINCKX_MELANOMA_METASTASIS_UP) in metastases ($n = 367$; *left*) with high (T28hi) or low (T28lo) TRIM28 expression, and in A375 cells (*right*) transduced with two shNTC (shSCR and shLUC) or shT28-1 and shT28-2 ($n = 3$ per construct). RNA-seq data from melanoma metastases were downloaded from The Cancer Genome Atlas (TCGA).

E Eight-week-old female nude mice were injected subcutaneously with $2.5x10^6$ A375 cells transduced with shSCR or shT28-3 lentivirus. Tumor size was measured every 3rd day and tumor size was calculated using the formula $V = (L \times W \times W)/2$ ($n = 10$ mice per group). Results are expressed as mean ± SEM. Statistics were calculated using repeated measures ANOVA.

F Tumor weight analyzed 17 days after subcutaneous injection of cells transduced with shSCR or shT28-3 lentivirus ($n = 10$ mice per group). Results are expressed as mean ± SEM. Statistics were calculated using the two-sided Mann–Whitney *U*-test.

G Eight-week-old female C57BL6/J were injected subcutaneously with $1 \times 10^5$ B16.F10 cells transduced with LKO.1 lentivirus targeting Firefly luciferase (shLuc; $n = 5$) or Trim28 (shT28; $n = 4$). Tumor weight was measured after 14 days, and statistics were calculated using the two-sided Mann–Whitney *U*-test. Results are expressed as mean ± SEM.

H Validation of TRIM28 knockdown in A375 cells using shT28-3 (top panel) and in B16.F10 using an shRNA specific for mouse *Trim28* (bottom panel).

I Reduced expression of the YAP1 target genes *CTGF* and *CYR61* in A375-MA2 cells after TRIM28 knockdown. One-way ANOVA and Dunnett's *post hoc* test were used for statistical testing.

Data information: *P*-values in (E, F, G, and I): $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.
Source data are available online for this figure.

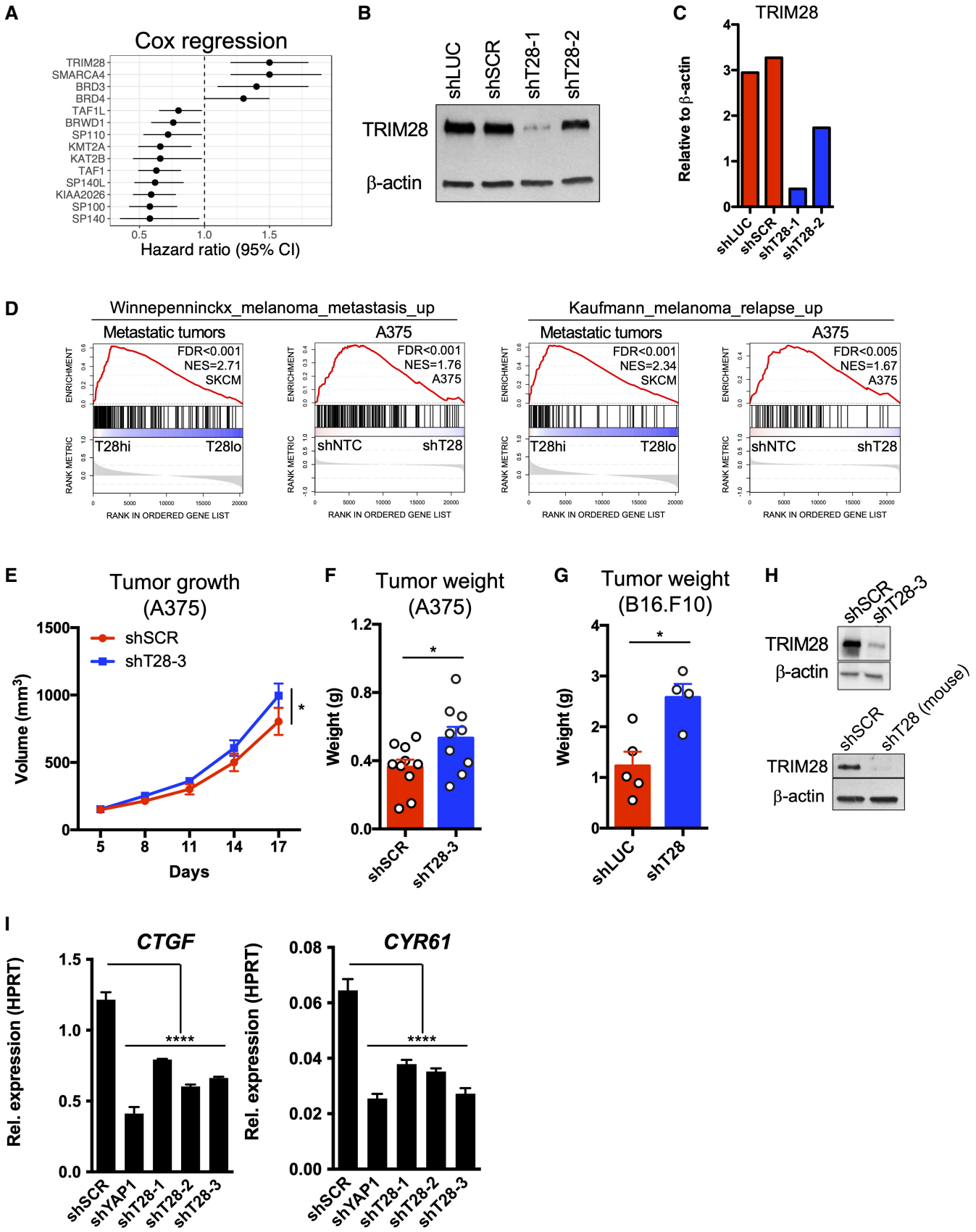


**Figure EV1.**

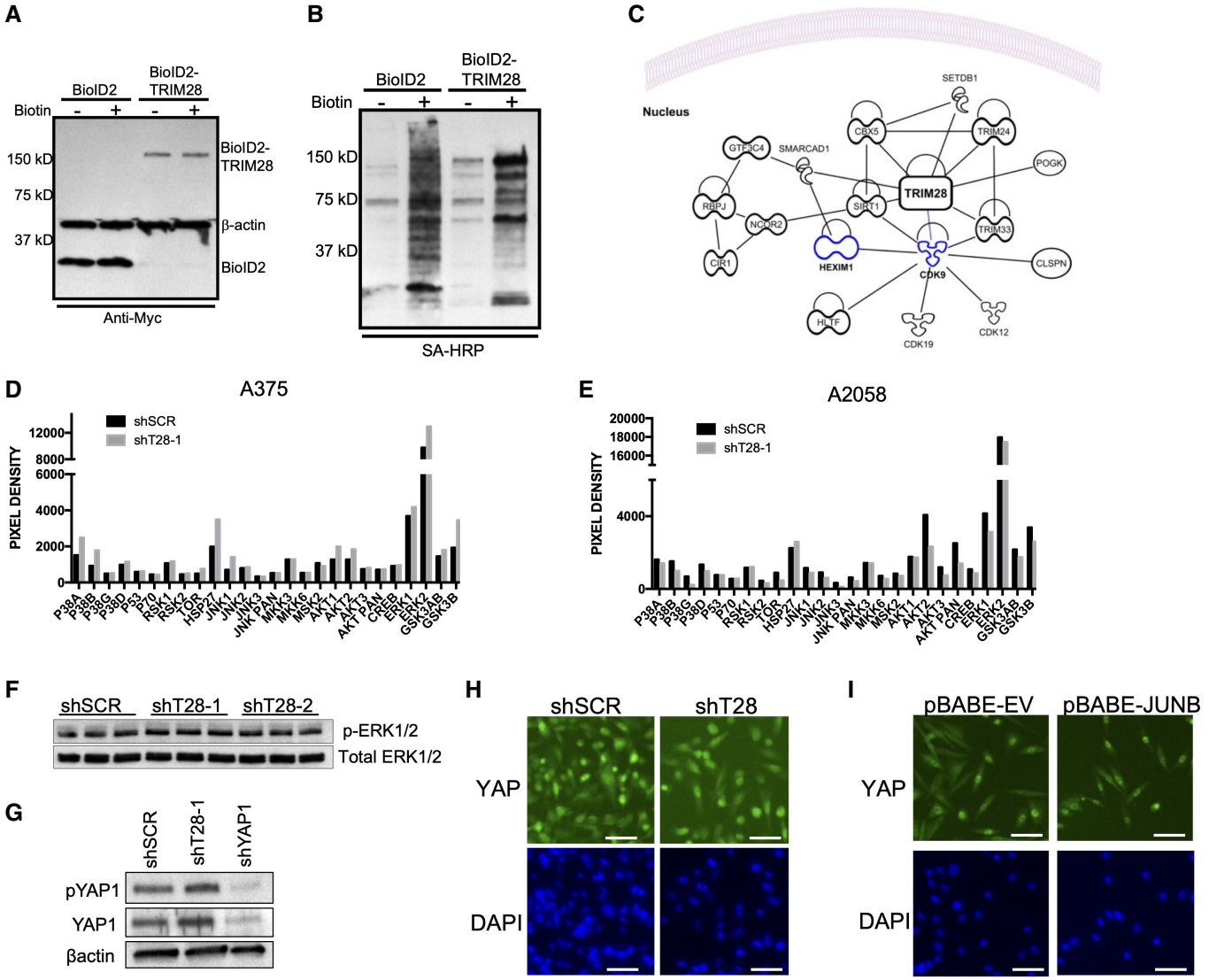


**Figure EV2. TRIM28 interacts with epigenetic regulators and factors regulating transcriptional elongation and does not directly affect ERK or YAP signaling.**

A A375 cells were transduced with pBABE-BioID2-TRIM28 or pBABE-BioID2 followed by selection with 1 μg/ml puromycin. Expression of BioID2 and BioID2-TRIM28 in cell lysates was verified with immunoblotting using an anti-Myc antibody.

B A375 cells were transduced with pBABE-BioID2-TRIM28 or pBABE-BioID2. Transduced cells were then cultured in the presence of 50 μM biotin for 20 h prior to lysis and then bound to Dynabeads MyOne Streptavidin C1 magnetic beads overnight. After the enrichment of biotinylated proteins, they were detected using streptavidin–HRP.

C Protein–protein interaction network based on identified TRIM28 interactors was analyzed by ingenuity pathway analysis (IPA). Displayed interactors are based on experimentally validated direct and indirect interactors. KRAB-ZFN proteins (> 50) are not displayed for increased clarity.

D A375 cells were transduced with shSCR or shT28-1 lentivirus followed by selection in a medium containing 1 μg/ml puromycin cells. Cells were lysed and lysates were applied to Human Phospho-MAPK Antibody Array.

E A2058 cells were transduced with shSCR or shT28-1 lentivirus followed by selection in a medium containing 1 μg/ml puromycin cells. Cells were lysed and lysates were applied to Human Phospho-MAPK Antibody Array.

F A375 cells were transduced with shSCR, shT28-1, or shYAP1 lentivirus. After selection, 1 μg/ml puromycin cells were lysed and proteins were separated on 4–20% SDS–PAGE. Proteins were transferred to PVDF membranes and detected with anti-ERK1/2 and anti-phospho-ERK1/2.

G A375 cells were transduced with shSCR or shT28-1 lentivirus. After selection, 1 μg/ml puromycin cells were lysed and proteins were separated on 4–20% SDS–PAGE. Proteins were transferred to PVDF membranes and detected with anti-phospho-YAP1 (pYAP1), anti-YAP1, or anti-β-actin antibodies.

H Intracellular localization of YAP1 after TRIM28 knockdown in A375 cells. Scale bar is 60 μm.

I Intracellular localization of YAP1 after JUNB overexpression in A375 cells. The scale bar is 60 μm.

Source data are available online for this figure.

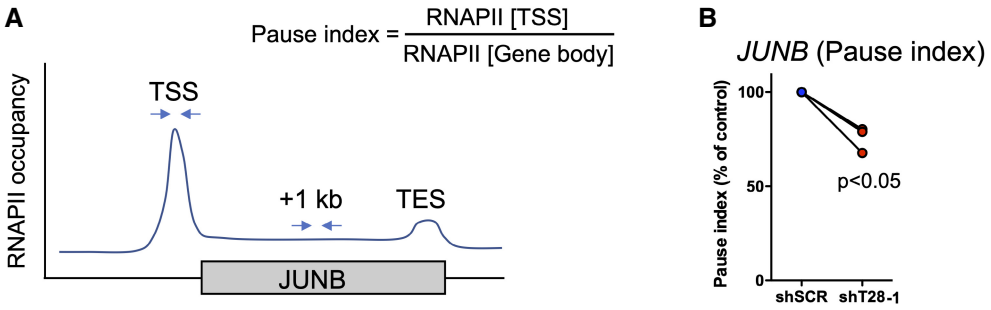


**Figure EV3. ChIP-qPCR validation of reduced RNAPII pausing at the TSS of the JUNB gene.**

A A375 cells were transduced with shSCR or shT28-1 lentivirus followed by selection in 1 μg/ml puromycin. After ChIP against RNAPII, qPCR was performed with primers amplifying precipitated genomic DNA from the TSS and gene body of JUNB (+1 kb).

B Results were normalized to corresponding input samples and three biological replicates are displayed as % pause index compared to shSCR control. The ratio *t*-test was used for statistical testing ($n = 3$).

**Figure EV4. RNA-seq of A375 cells overexpressing JUNB or FOSL1.**

A PCA plot of the different samples in the RNA-seq experiment (EV = empty vector; $n = 4$).

B Volcano plot comparing A375 cells overexpressing JUNB against A375 cells overexpressing FOSL1.

C Volcano plot comparing A375 cells overexpressing FOSL1 against A375 cells transduced with EV.

D Immunoblotting against FOSL1 after overexpression with pBABE-FOSL1 in A375 cells. HSP90 was used as a loading control.

E GSEA analysis of A375 cells overexpressing JUNB compared to cells overexpressing FOSL1 ($n = 4$).

F Validation of JUNB overexpression effect on YAP1 target genes *ANKRD1*, *CTGF*, *CYR61*, as well as *CXCL8* and *CXCL2* in A2058 cells. qRT–PCR was used to determine expression levels and results are expressed as mean ± SEM from three biological replicates ($n = 3$). Two-sided unpaired *t*-tests were used for statistical testing.

G Rescue experiment to determine the expression of typical YAP1 and RAS signature genes after siRNA (siNTC or siJUNB) transfection of A375 cells transduced with shSCR or shT28-1. qRT–PCR was used to determine expression levels relative to *UBC*, and the results are expressed as mean ± SEM from three biological replicates ($n = 3$). Two-sided unpaired *t*-tests were used for statistical testing.

H A375 cell lysate was used for immunoprecipitation of endogenous JUNB followed by detection by immunoblotting using anti-YAP1, anti-pan-TEAD, and anti-FOSL1. An isotype IgG antibody was used as a negative immunoprecipitation control.

Data information: *P*-values in (F and G): $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.
Source data are available online for this figure.

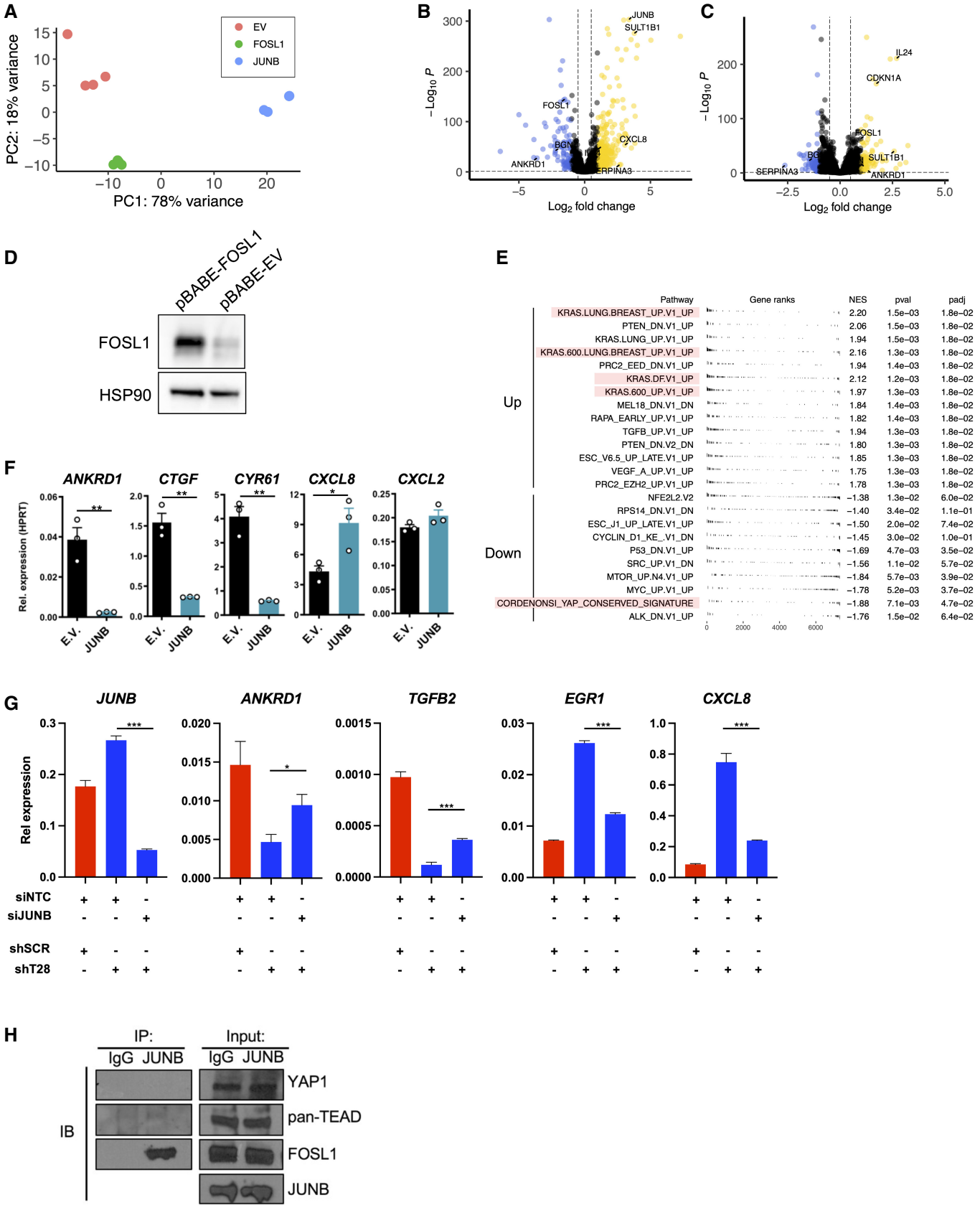


**Figure EV4.**

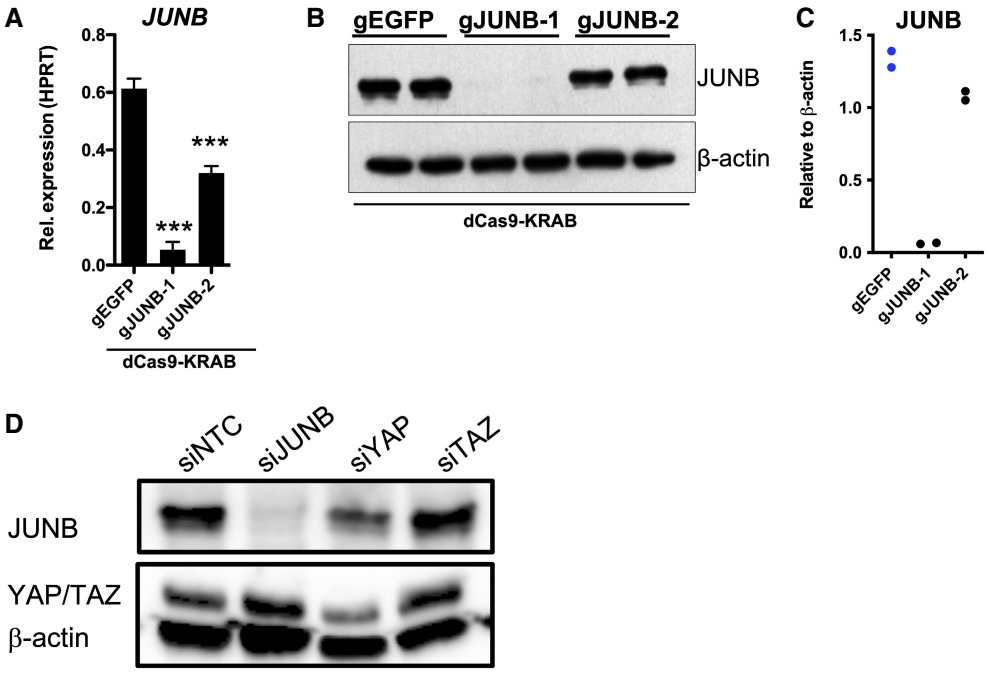


**Figure EV5. Validation of JUNB, YAP, and TAZ expression after CRISPRi and siRNA.**

A Expression of *JUNB* in A375 cells after transduction with dCas9-KRAB lentiviruses expressing gRNA targeting *JUNB* (gJUNB-1 or gJUNB-2) or a control gRNA (gEGFP). qRT–PCR was used to determine *JUNB* expression levels, and the results are expressed as mean ± SEM from three biological replicates ($n = 3$). One-way ANOVA and Dunnett's *post hoc* test were used for statistical testing. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

B Expression of JUNB in A375 cells after transduction with dCas9-KRAB lentiviruses expressing gRNA targeting *JUNB* (gJUNB-1 or gJUNB-2) or a control gRNA (gEGFP). Immunoblotting was used to determine the protein levels of JUNB. β-actin was used as loading control.

C Validation of JUNB knockdown (shown in B) by densitometry. JUNB levels are shown relative to β-actin.

D Validation of siNTC-, siJUNB-, siYAP1-, and siTAZ-mediated knockdown in A375 cells.

Source data are available online for this figure.