Expanded View Figures

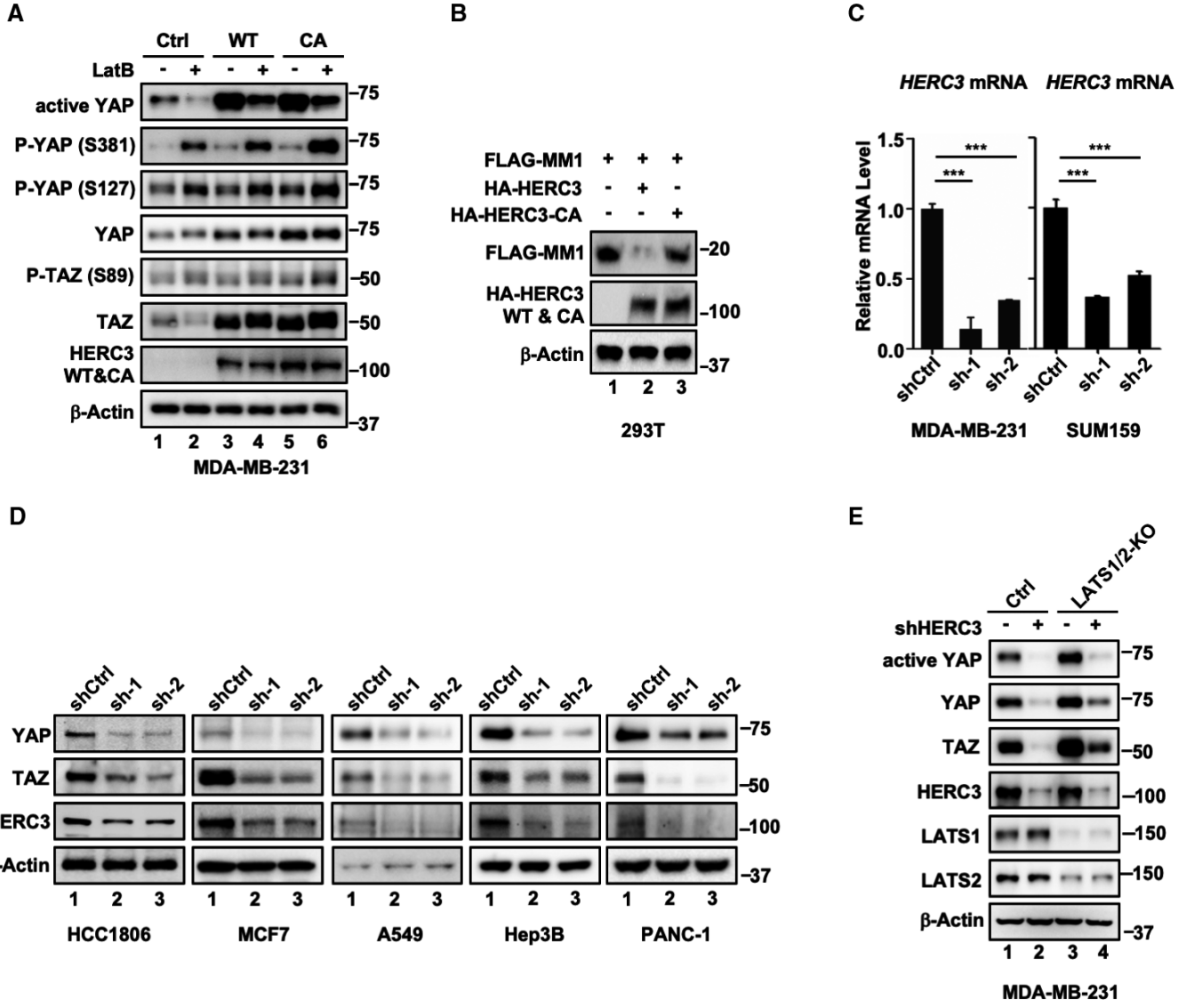

Figure EV1. HERC3 regulates the stability and transcriptional responses of YAP/TAZ.

A HERC3 and HERC3-CA reverse the reduction in the YAP/TAZ levels induced by LatB. MDA-MB-231 cells stably expressing HERC3 or HERC3-CA were cultured in a medium with or without LatB (1 μg/ml) for 30 min. Cells were harvested for Western blotting analysis with appropriate antibodies as indicated.

B HERC3, but not HERC3-CA, causes MM1 degradation. HEK293T cells were transfected with expression plasmids for HA-HERC3, HA-HERC3-CA, and FLAG-MM1 as indicated. Cells were harvested 24 h post-transfection and subjected to Western blotting analysis with FLAG, HA, and β-actin antibodies.

C HERC3 is efficiently knocked down by shRNA. Total mRNA levels of HERC3 in control or HERC3-deficient MDA-MB-231 cells and SUM159 cells were analyzed by qRT–PCR using primers specific to the indicated target gene. Data are shown as mean ± SEM; $n = 3$ biological replicates. Statistical analysis was performed using two-tailed Student's *t*-test. ***$P < 0.001$.

D Depletion of HERC3 decreases the steady-state levels of YAP/TAZ. HCC1806, MCF7, A549, Hep3B, and PANC-1 cells were stably expressing shCtrl (Control shRNA) and sh-1 and sh-2 (shRNAs against HERC3). Western blotting was carried out to detect levels of YAP, TAZ, HERC3, and β-actin with appropriate antibodies as indicated.

E Depletion of HERC3 decreases the steady-state levels of YAP/TAZ in LATS1/2-KO cells. Western blotting was done as described in panel D. LATS1/2-KO, double knockout of LATS1 and LATS2 in MDA-MB-231 cells stably expressing shCtrl and shHERC3.

Source data are available online for this figure.

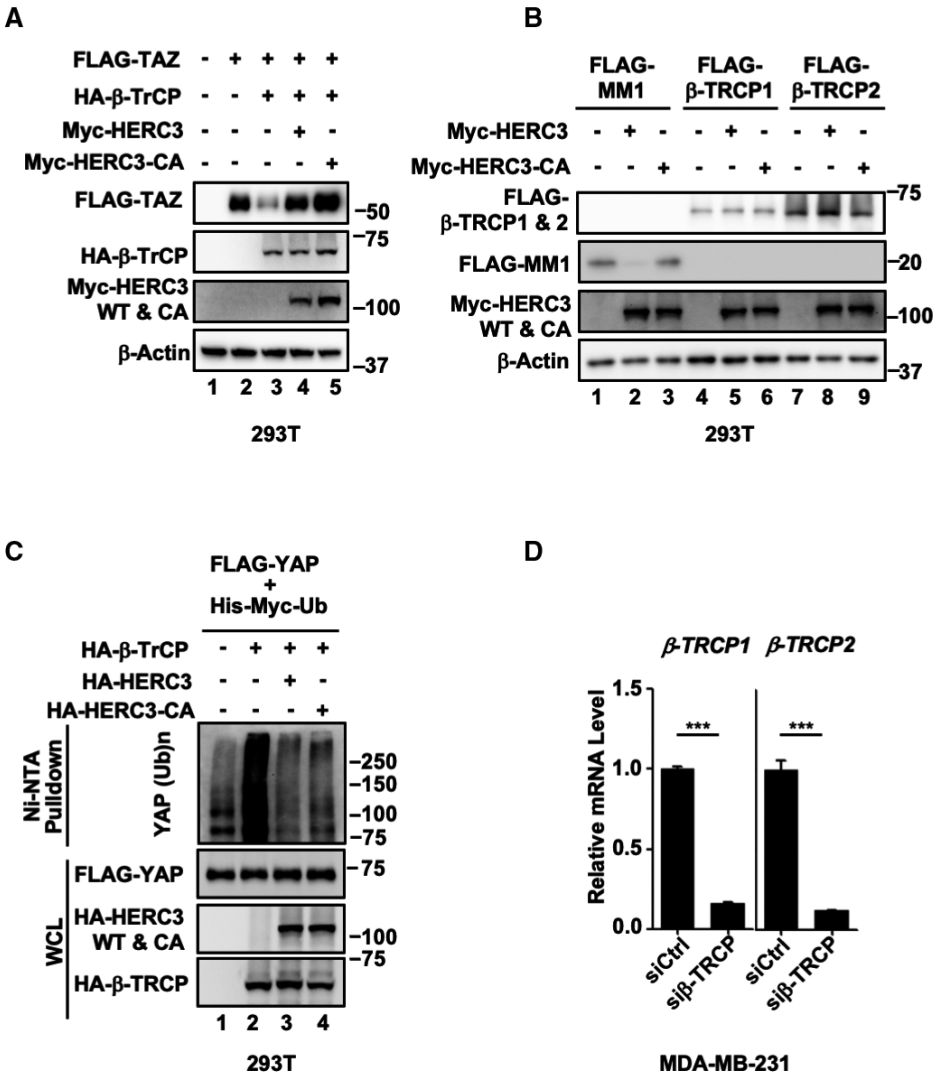

Figure EV2. HERC3 blocks β-TrCP-mediated ubiquitination and proteasomal degradation of YAP/TAZ.

A Western blots showing relevant protein levels in Fig 2B.

B HERC3 does not affect the protein levels of β-TrCP1 and β-TrCP2. HEK293T cells were transfected with expression plasmids for Myc-HERC3, Myc-HERC3-CA, FLAG-MM1, FLAG-β-TrCP1, and FLAG-β-TrCP2 as indicated. Cells were harvested 24 h post-transfection and subjected to Western blotting analysis with FLAG, Myc, and β-actin antibodies.

C HERC3 and HERC3-CA attenuate β-TrCP-mediated ubiquitination of YAP. HEK293T cells were transfected with expression plasmids for His-Myc-ubiquitin and FLAG-YAP, HA-β-TrCP, HA-HERC3, or HA-HERC3-CA as indicated. A total of 24 h after transfection, ubiquitinated proteins were pulled down using Ni-NTA beads and the ubiquitinated YAP proteins were analyzed by using anti-FLAG antibody.

D β-TrCP1 and β-TrCP2 were efficiently knocked down by siRNA. Total mRNA levels of *β-TrCP1* and *β-TrCP2* in MDA-MB-231 cells were analyzed by qRT–PCR using primers specific to the indicated target gene. Data are shown as mean ± SEM; $n = 3$ biological replicates. Statistical analysis was performed using two-tailed Student's *t*-test. ***$P < 0.001$.

Source data are available online for this figure.

Figure EV3. HERC3 promotes tumor cell properties *in vitro*.

A–D Depletion of HERC3 decreases the $ALDH^+$ cell population. The ALDH activity in control and HERC3-deficient cells was analyzed by flow cytometric analysis by using ALDEFLUOR™ kit according to the manufacturer's instructions. (A) MDA-MB-231 cells. (B) Quantitation of $ALDH^+$ cell population in MDA-MB-231 cells. Data are shown as mean ± SEM; $n = 3$ biological replicates. $^*P < 0.05$. (C) SUM159 cells. (D) Quantitation of $ALDH^+$ cell population in SUM159 cells. Data are shown as mean ± SEM; $n = 3$ biological replicates. $^*P < 0.05$.

E, F Depletion of HERC3 reduces the $CD44^+/CD24^-$ cell population. (E) control and HERC3-deficient MDA-MB-231 cells (upper panel) or SUM159 cells (lower panel) were analyzed by flow cytometric analysis with CD24 and CD44 antibodies. (F) Graphical representation of $CD44^+/CD24^-$ cell population. Data are shown as mean ± SEM; $n = 3$ biological replicates. Statistical analysis was performed using two-tailed Student's *t*-test. $^*P < 0.05$. $^{**}P < 0.01$.

G TAZ-4SA/YAP-5SA rescue shHERC3-mediated repression of CTGF-Luc reporter activity. HERC3-deficient cells with or without ectopic expression of TAZ-4SA/YAP-5SA and shCtrl MDA-MB-231 cells were transfected with CTGF-Luc and Renilla-Luc. Cells were then harvested and subjected to luciferase assay as described in Fig 1G. Data are shown as mean ± SEM; $n = 3$ biological replicates. Statistical analysis was performed using two-tailed Student's *t*-test. $^{***}P < 0.001$.

H TAZ-4SA/YAP-5SA rescue shHERC3-mediated repression of YAP/TAZ target gene transcription. Total mRNA levels of *CTGF* and *ANKRD1* were analyzed by qRT–PCR using primers specific to the indicated target genes. Data are shown as mean ± SEM; $n = 3$ biological replicates. Statistical analysis was performed using two-tailed Student's *t*-test. $^{***}P < 0.001$.

I TAZ-4SA/YAP-5SA reverse the inhibitory effect of HERC3 depletion on cell proliferation. MDA-MB-231 cells expressing shHERC3 and/or TAZ-4SA/YAP-5SA were analyzed for indicated days by CCK8 assay as described in the Materials and Methods. Data are shown as mean ± SEM; $n = 3$ biological replicates. $^{***}P < 0.001$.

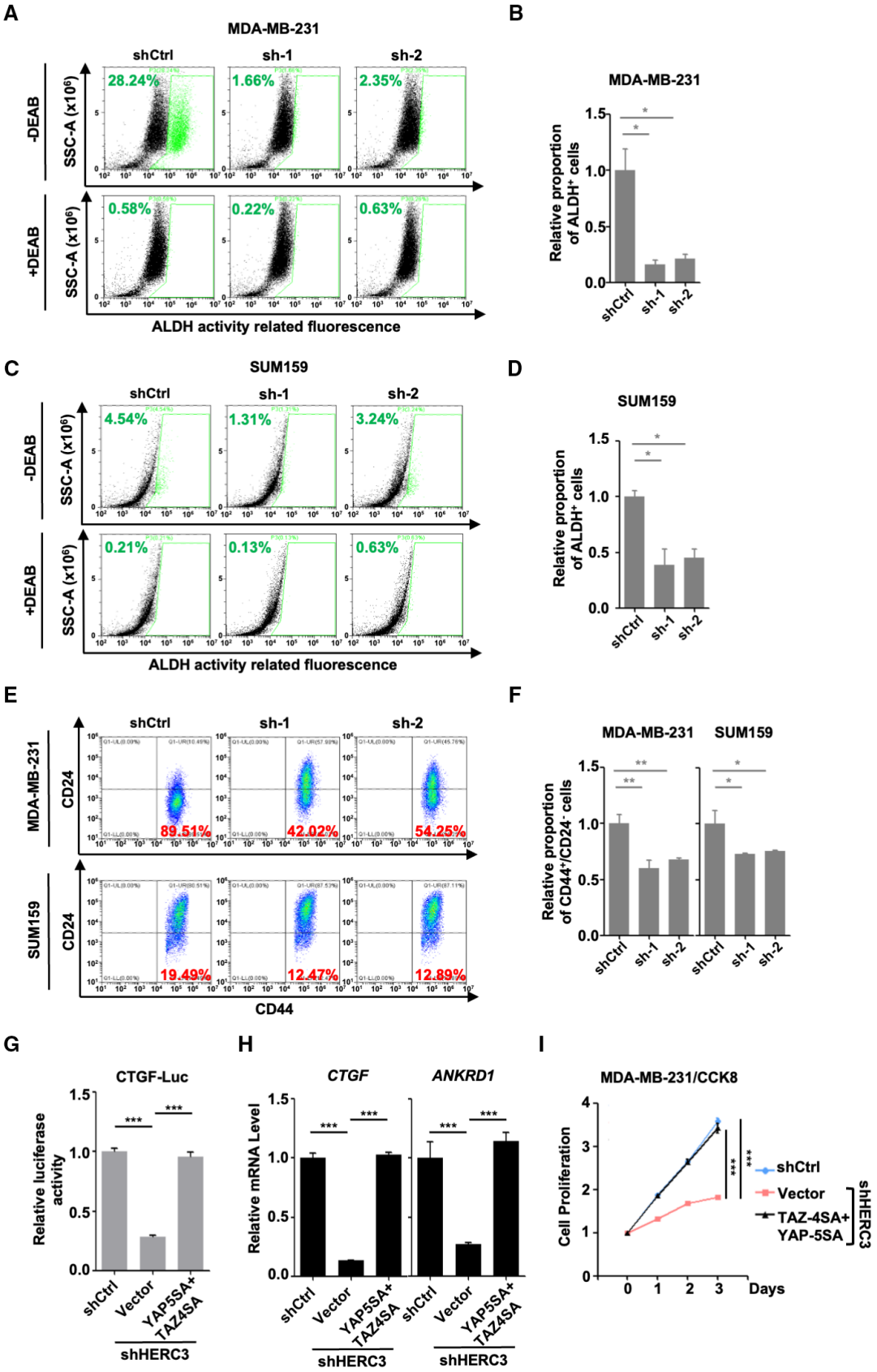

Figure EV3.

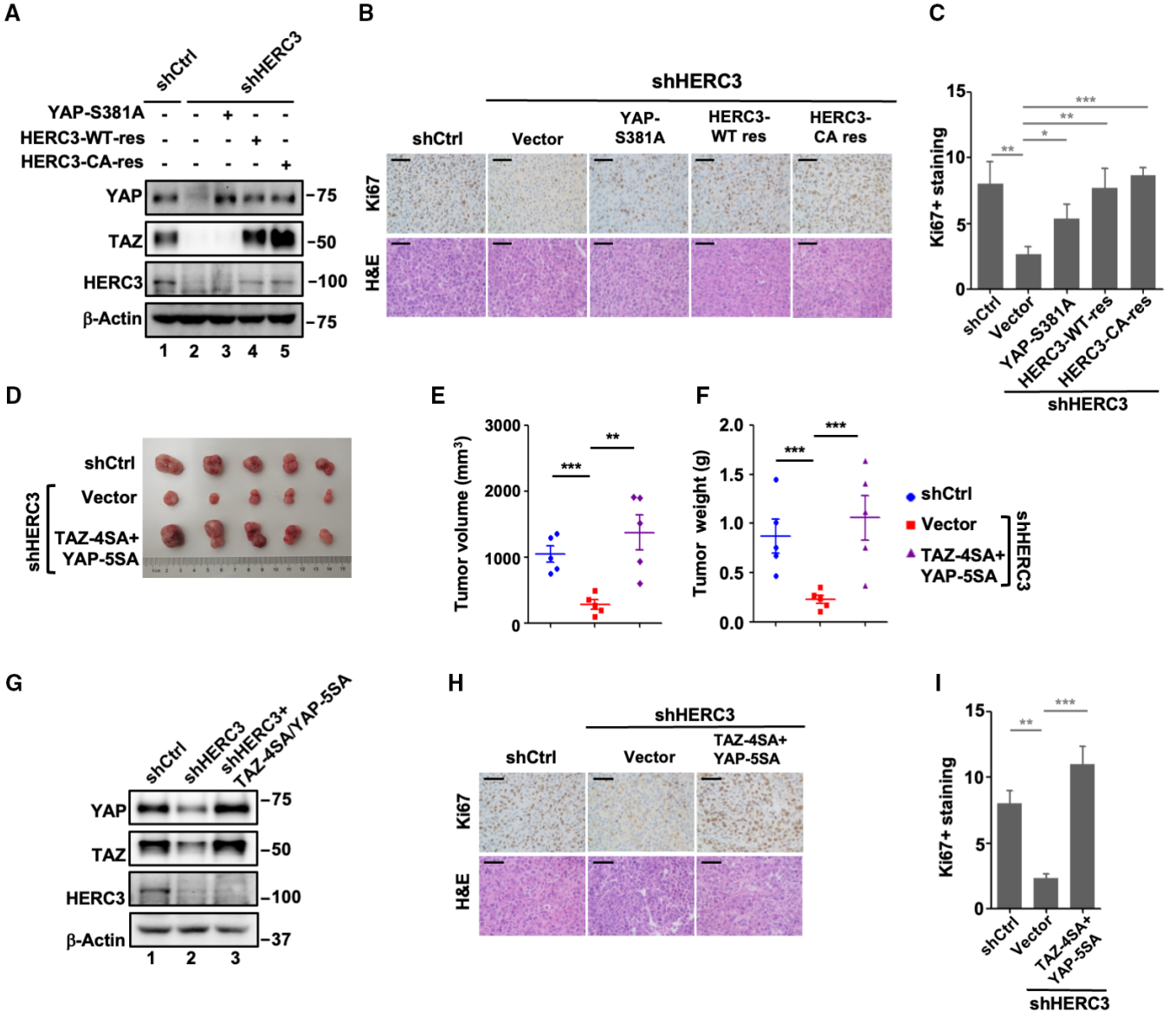

Figure EV4. HERC3 knockdown represses mammary tumorigenesis and metastasis.

A RNAi-resistant HERC3 rescues YAP/TAZ protein levels in HERC3-deficient tumors. Protein levels of YAP/TAZ in tumors (Fig 5L) were analyzed by Western blotting with appropriate antibodies.

B, C RNAi-resistant HERC3 and YAP-S381A rescue cell proliferation in HERC3-deficient xenograft tumors. (B) Representative H&E staining and Ki67 expression from each group's tumor tissues from Fig 5L. Scale bars, 50 μm. (C) Graphical representation of Ki67$^+$ scores from Panel B. Data are represented as the mean ± SEM; $n = 3$ biological replicates. Statistical analysis was performed using two-tailed Student's *t*-test. $^*P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$.

D–F TAZ-4SA/YAP-5SA reverse the inhibitory effect of HERC3 depletion in tumorigenicity. Subcutaneous tumors carrying HERC3 deficiency together with TAZ-4SA/YAP-5SA expression and control were dissected and analyzed at 6 weeks after cell implantation. (D) Tumor morphology. (E) Tumor volume. (F) Tumor weight. Data are shown as mean ± SEM; $n = 5$ biological replicates. Statistical analysis was performed using two-tailed Student's *t*-test. $^{**}P < 0.01$. $^{***}P < 0.001$.

G TAZ-4SA and YAP-5SA are stably expressed at a comparable level to endogenous YAP-TAZ in HERC3-deficient tumors. Protein levels in tumors (Fig EV4D) were analyzed by Western blotting with appropriate antibodies.

H, I TAZ-4SA/YAP-5SA reverse the inhibitory effect of HERC3 depletion on Ki67 expression in xenograft tumors. (H) Representative H&E staining and Ki67 expression from each group's tumor tissues from Fig EV4D. Scale bars, 50 μm. (I) Graphical representation of Ki67$^+$ scores from Panel B. Data are represented as the mean ± SEM. $n = 3$ biological replicates. Statistical analysis was performed using two-tailed Student's *t*-test. $^{**}P < 0.01$. $^{***}P < 0.001$.

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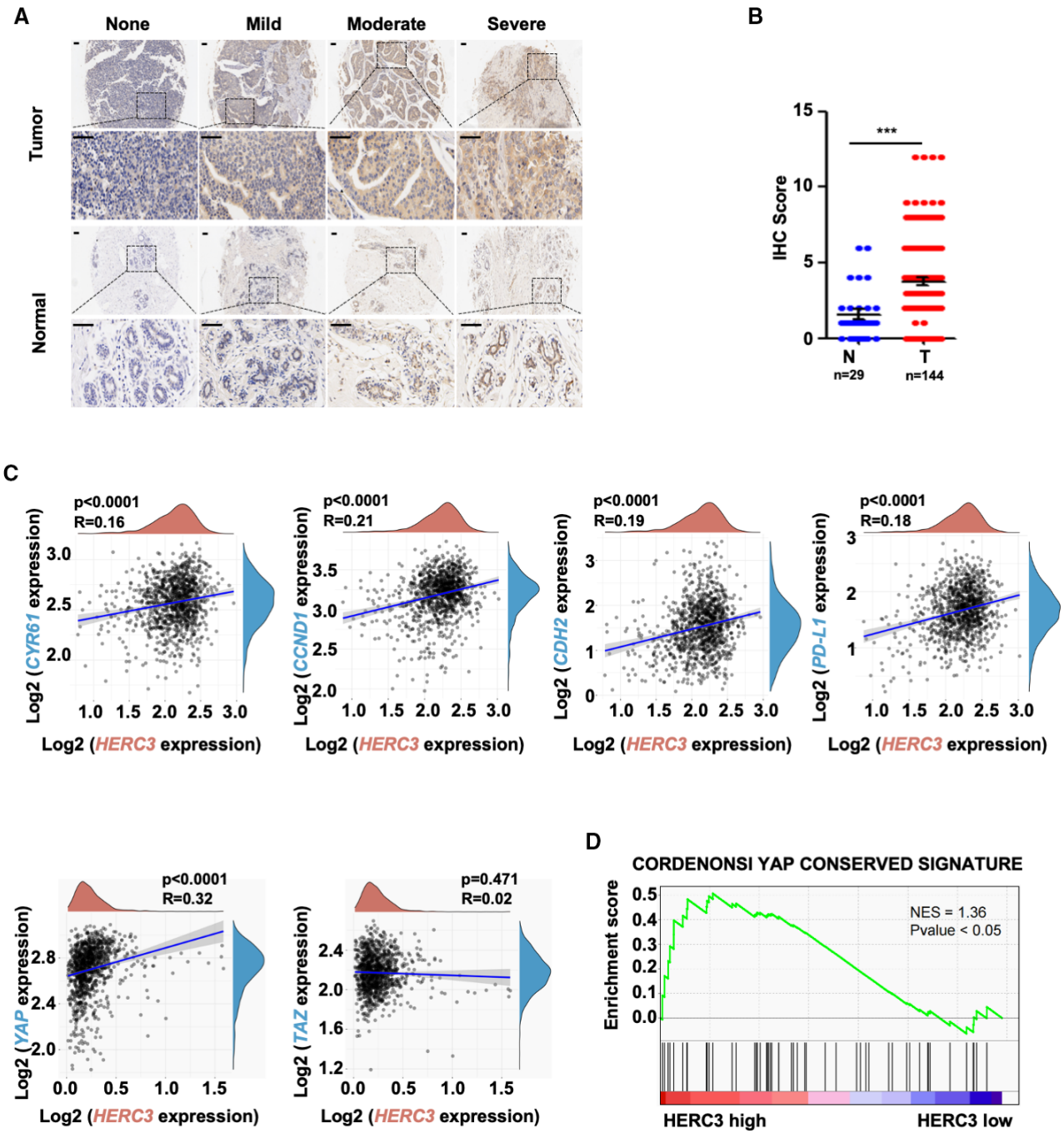

Figure EV5. HERC3 is positively correlated with poor prognosis in breast cancer.

A HERC3 is highly expressed in breast cancer specimens. Representative images of IHC staining of HERC3 in breast tumor samples and adjacent normal tissues of tissue microarrays (Bioaitech). Scale bars, 50 μm.

B Graphical representation of scoring performed on IHC staining in Panel A. Data are represented as the mean ± SEM. $n = 3$ biological replicates. Statistical analysis was performed using two-tailed Student's *t*-test. ***$P < 0.001$.

C Expression level of HERC3 is positively correlated with those of YAP/TAZ target genes (e.g., *CYR61*, *CCND1*, *CDH2*, *and PD-L1*) in breast cancer patients, as analyzed using TCGA database.

D YAP conserved signatures were enriched in breast cancer samples with high HERC3 levels. Transcriptome-wide effects of HERC3 on YAP conserved signature genes in TCGA database were evaluated by GSEA analysis. Red, upregulated genes; blue, downregulated genes. NES = 1.36, *P*-value < 0.05.