Expanded View Figures

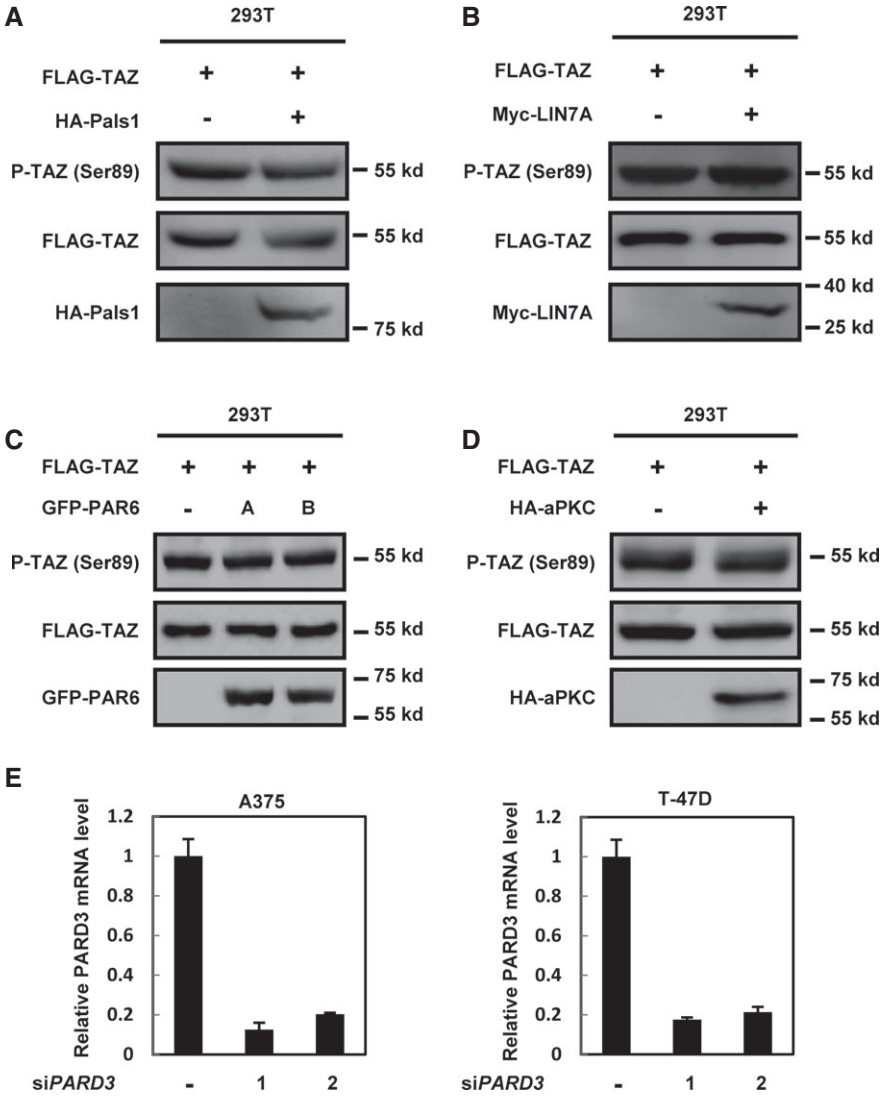

Figure EV1. PARD3 dephosphorylates and inactivates YAP/TAZ.

A, B Overexpression of tight junction proteins Pals1 (A) or LIN7A (B) has no effect on the phosphorylation of TAZ on Ser89. HEK293T cells were transiently transfected with HA-Pals1 and Myc-LIN7A in the presence of TAZ as indicated, and the cells were harvested for Western blot analysis.

C, D The PAR complex components have no effects on TAZ phosphorylation. HA-aPKC and GFP-PAR6A/B were transfected into HEK293T cells, and cell lysates were harvested for Western blot analysis.

E Verification of PARD3 siRNA knockdown efficiency in A375 and T-47D cells. Samples were detected by real-time PCR. The results are average ± SEM of three independent experiments.

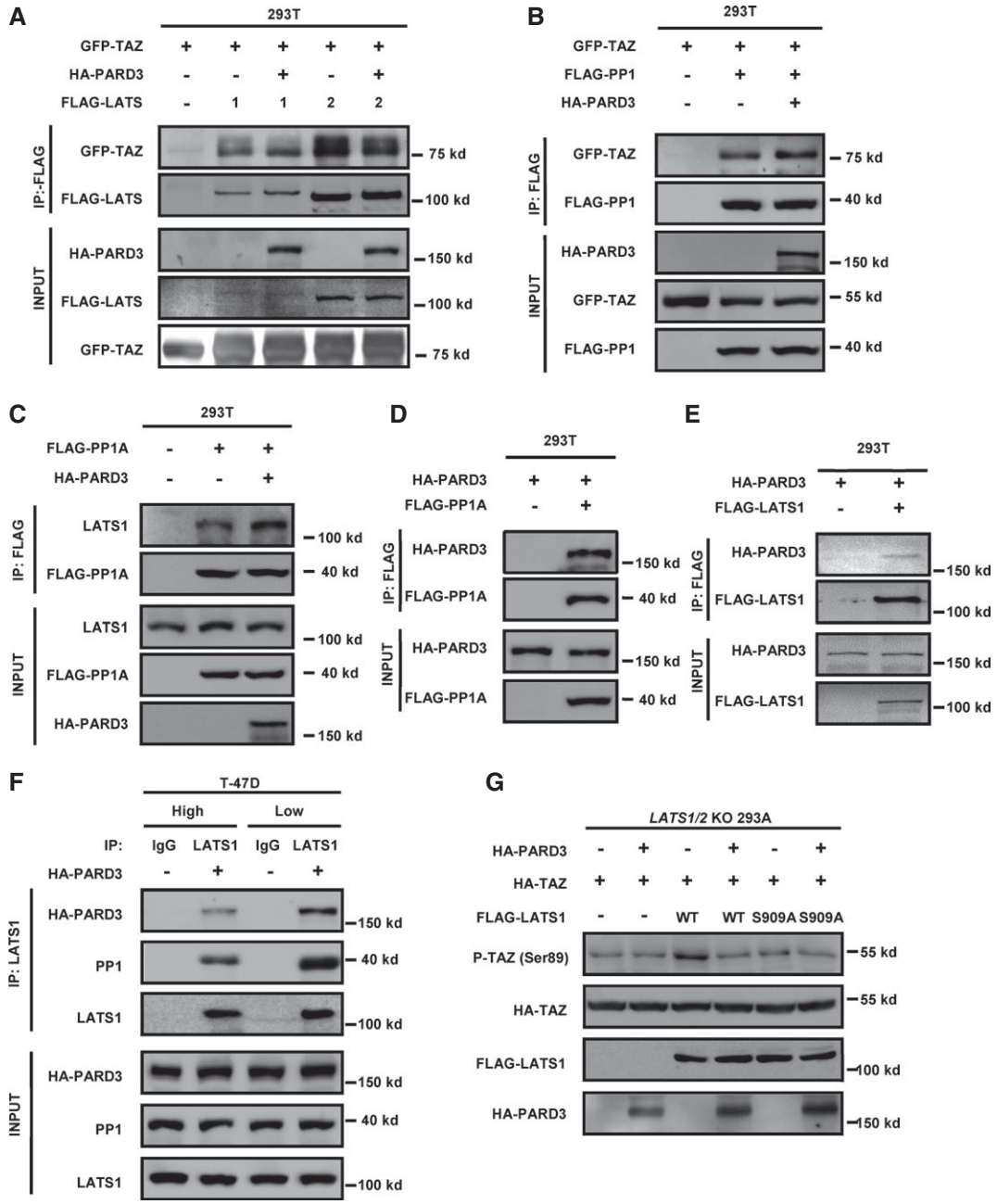

Figure EV2. PARD3 interacts with LATS1 and PP1A.

A PARD3 has no effect on the interaction between TAZ and its kinase LATS1. HEK293T cells were transfected with GFP-tagged TAZ and FLAG-tagged LATS1 in the presence or absence of HA-PARD3, and proteins were immunoprecipitated with FLAG beads for Western blot analysis.

B PARD3 does not affect the binding of GFP-TAZ and its phosphatase PP1A. The indicated constructs were transfected into HEK293T cells, and proteins were immunoprecipitated with FLAG beads for Western blot analysis.

C PARD3 enhances the interaction of PP1A with endogenous LATS1. HEK293T cells were transfected with FLAG-tagged PP1A in the presence or absence of HA-PARD3, and proteins were immunoprecipitated with FLAG beads for Western blot analysis.

D, E PARD3 interacts with PP1A and LATS1. The indicated constructs were transfected into HEK293T cells, and proteins were immunoprecipitated with FLAG antibody. Cell lysates were analyzed by Western blot as indicated.

F The interaction of PP1/PARD3/LATS1 is regulated by cell density. HA-PARD3 was transfected into T-47D cells, immunoprecipitation with LATS1 antibody was performed, and the immunoprecipitated samples were used for Western blot analysis as indicated.

G LATS1 S909A mutant is insensitive to PARD3 regulation. WT and S909A were cotransfected into *LATS1/2* KO 293A cells with HA-TAZ. Western blot analysis was performed as indicated.

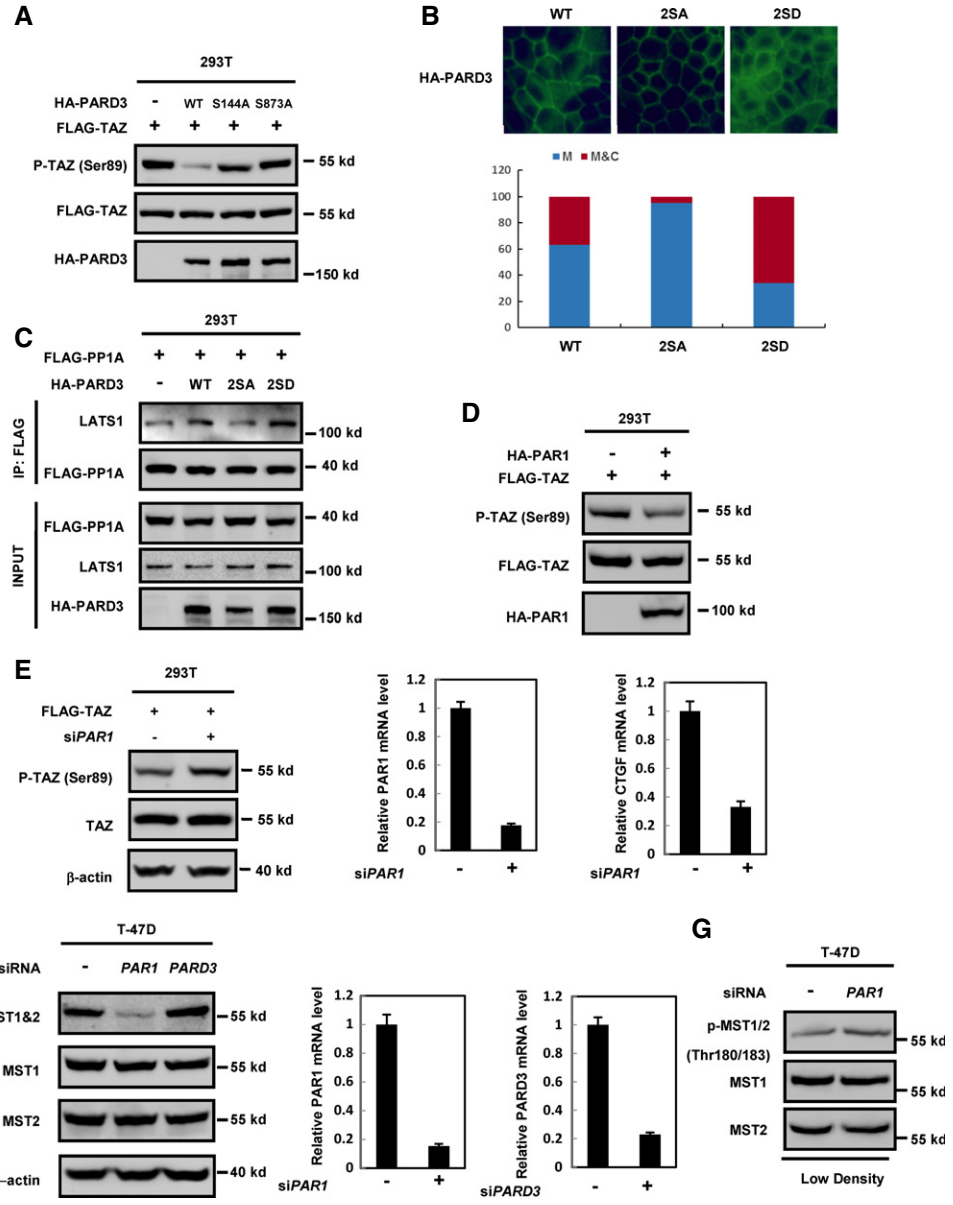

Figure EV3. Phosphorylation of PARD3 at Ser144/Ser873 diminishes TAZ dephosphorylation.

A S144A and S873A of PARD3 diminish phosphorylation of TAZ at Ser89. The indicated constructs were cotransfected with FLAG-TAZ into HEK293T cells. Western blot analysis was performed as indicated.

B Immunofluorescence localization of PARD3 WT, 2SD mutant, and 2SA mutant. MDCK cells stably expressing WT, 2SA, or 2SD of PARD3 were stained with HA antibody. The quantification is shown below. 2SA mutant but not the WT and 2SD mutant of PARD3 decreases interaction between PP1A and endogenous LATS1. The indicated constructs were transfected into HEK293T cells, and proteins were immunoprecipitated with FLAG antibody. Cell lysates were analyzed by Western blot.

C Wild type and 2SD but not 2SA mutant of PARD3 increases the interaction between FLAG-PP1A and endogenous LATS1. The indicated constructs were transfected into HEK293T cells, and proteins were immunoprecipitated with FLAG antibody. Cell lysates were analyzed by Western blot as indicated.

D PAR1 induces TAZ dephosphorylation at Ser89. HEK293T cells were transiently transfected with HA-PAR1 and FLAG-TAZ, and the cells were harvested for Western blot analysis.

E Knockdown of *PAR1* increases the phosphorylation of TAZ and inhibits the expression of CTGF. 293T cells were transfected with siRNA targeting PAR1, the phosphorylation of TAZ was detected by Western blot, and the expression of CTGF was detected using real-time PCR.

F Knockdown of *PAR1* but not *PARD3* decreases the phosphorylation of MST1/2 at Thr183/180. T-47D cells were transfected with the indicated siRNAs, and cells were harvested at high density for Western blot analysis. The knockdown efficiencies were determined by real-time PCR on the right.

G Knockdown of *PAR1* increases the phosphorylation of MST1/2 at Thr183/180 slightly. T-47D cells were transfected with the indicated siRNAs, and cells were harvested at low density for Western blot analysis.

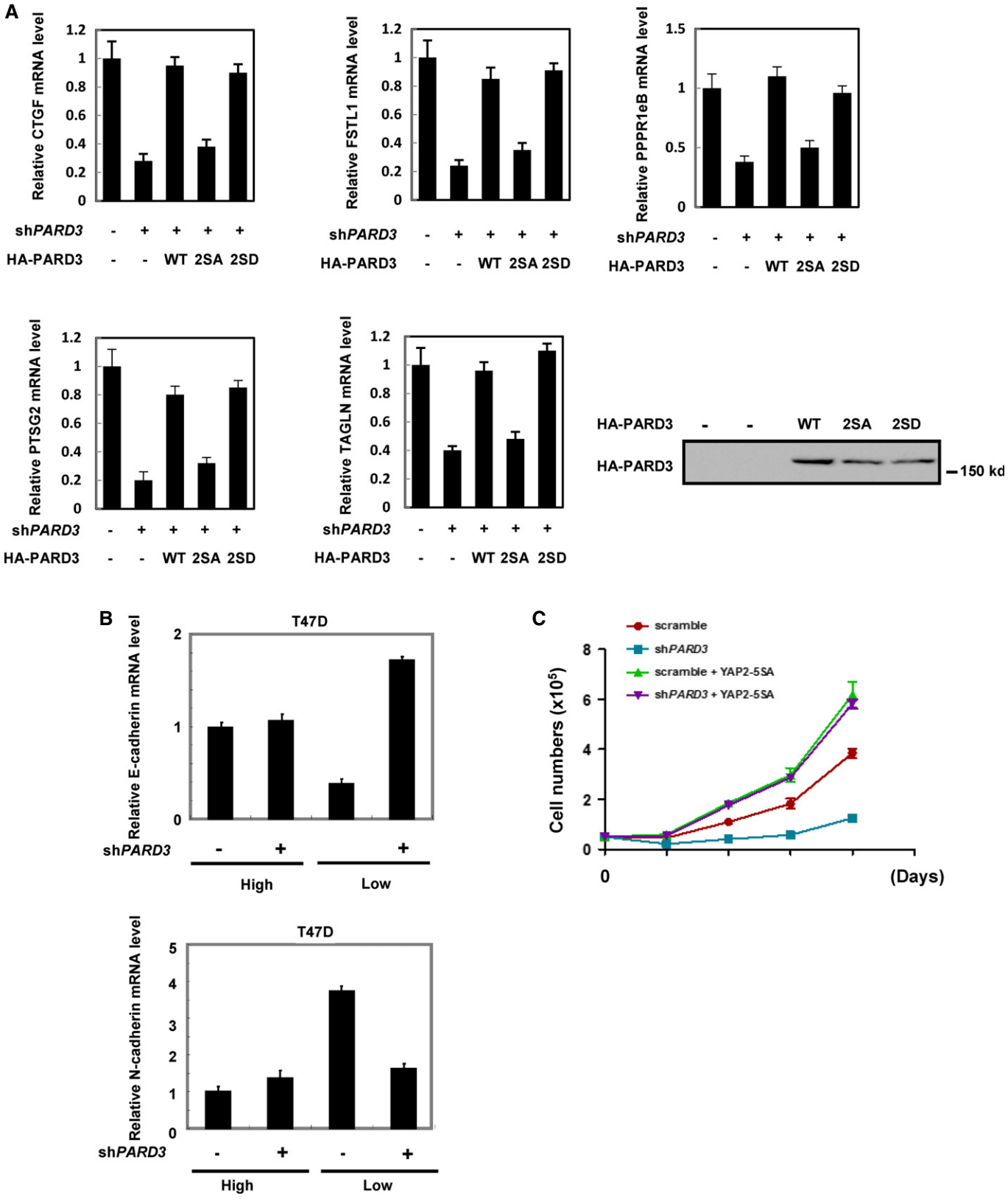

Figure EV4. (continued)

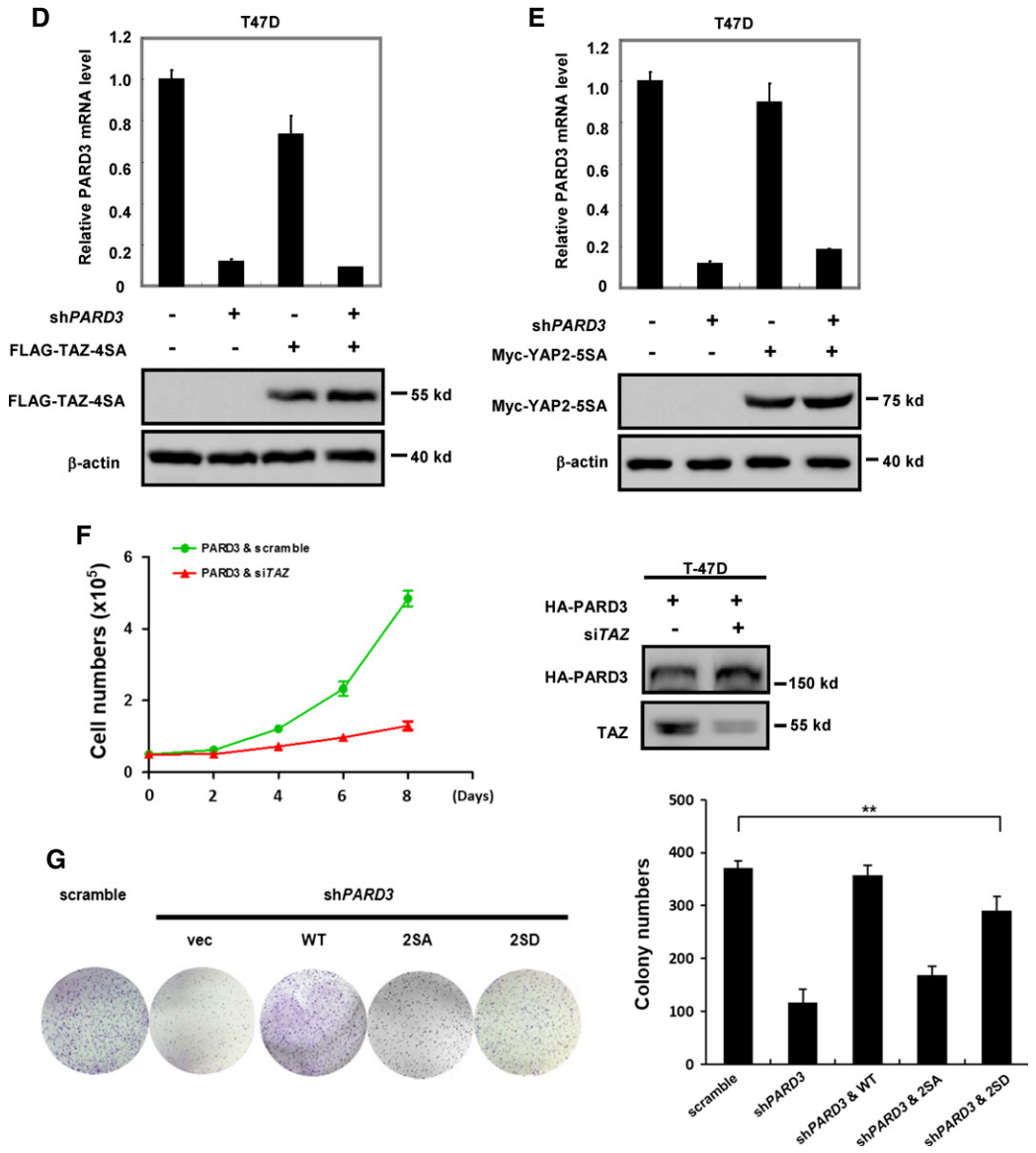

Figure EV4. Knockdown of *PARD3* reduces the function of YAP/TAZ.

A WT and 2SD mutant, but not 2SA mutant, of PARD3 rescue the expression of target genes inhibited by *PARD3* knockdown. T-47D cells with stable knockdown and overexpression of WT and 2SA and 2SD mutants of PARD3 were used as indicated. The expression of target genes was analyzed by real-time PCR. The efficiency of endogenous *PARD3* knockdown is shown above, and the expression of exogenous HA-PARD3 was verified by Western blot. The results are average ± SEM of three independent experiments.

B Knockdown of *PARD3* increases E-cadherin expression and decreases N-cadherin expression at the mRNA level in T-47D cells at low cell density. *PARD3*-knockdown T-47D cells were analyzed by qPCR. The results are average ± SEM of three independent experiments.

C Knockdown of *PARD3* inhibits T-47D cell growth which can be rescued by overexpression of YAP2-5SA. The results are average ± SEM of 3 independent experiments.

D, E Overexpression of TAZ-4SA (D) and YAP2-5SA (E) *PARD3*-knockdown T-47D cell. It was verified by Western blot and real-time PCR.

F Knocking down *TAZ* inhibits the growth of T-47D cells overexpressing PARD3. Growth curves of *TAZ*–knockdown T-47D cells overexpressing PARD3 were determined. Cell lysates were analyzed by Western blot to verify HA-PARD3 and TAZ expression. The results are average ± SEM of 3 independent experiments.

G WT and 2SD mutant, but not 2SA mutant, of PARD3 rescue colony formation inhibited by *PARD3* knockdown. T-47D stable cell lines (5×10^3 cells) were cultured in 6-well plates for 3 weeks. Colonies were visualized by crystal violet staining and counted. The results are average ± SEM of three independent experiments. $^{**}P < 0.01$.