Expanded View Figures

Figure EV1. TRIP6 knockdown in MCF10A cells activates Hippo signaling, and TRIP6-KO knockout cells are rescued by FLAG-TRIP6 expression.

A Lysates from MCF10A cells infected with lentivirus with a control shRNA (shEGFP) or a mix of two different shRNA against TRIP6 (shTRIP6-1 and shTRIP6-4) were analyzed by Western blotting using the indicated antibodies, and the levels of LATS1 activating phosphorylation were quantified (mean ± SD; $n = 3$; $^{**}P \leq 0.01$, *t*-test).

B MCF10A cells were infected with lentivirus carrying control shRNA (shEGFP), or two different shRNA against TRIP6 (shTRIP6-1, shTRIP6-4) and the levels of TRIP6 and YAP target gene expression were analyzed using RT–qPCR (mean ± SD; $n = 3$; $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, *t*-test).

C MCF10A cells were infected with lentivirus carrying control shRNA (shEGFP), or a mix of two different shRNA against TRIP6 (shTRIP6-1 and shTRIP6-4) and were stained for YAP and TRIP6. Merged image shows YAP (green), TRIP6 (red), and DNA (blue). Quantification of YAP nuclear localization in least 100 cells is shown (mean ± SD; $n = 3$; $^{****}P \leq 0.0001$, Fisher's test). Scale bar = 20 μm.

D YAP, TRIP6, and tubulin levels were measured by Western blotting in MCF10A cells infected with lentivirus carrying control shRNA (shEGFP), or shRNA against TRIP6 (shTRIP6-1) (mean ± SD; $n = 3$; $^{**}P \leq 0.01$, *t*-test).

E LATS1 and LATS2 were depleted from WT and TRIP6-KO 293A cells using siRNA as described (Fig 3B) and were stained for YAP. Merged image shows YAP (green) and DNA (blue). Quantification of YAP nuclear localization in least 100 cells is shown (mean ± SD; $n = 3$; $^{***}P \leq 0.001$, Fisher's test). Scale bar = 20 μm.

F The lysates from (E) were probed by Western blotting with LATS1, LATS2, TRIP6, and tubulin antibodies to test knockdown efficacy.

G Control (WT) and TRIP6-KO HEK293A cells were transfected with 200 ng of control and FLAG-TRIP6 plasmids. (Note that 200 ng of FLAG-TRIP6 plasmid restores approximate wild-type levels (see panel H) of TRIP6 expression). After 48 h of transfection, cells were stained using anti-YAP and TRIP6 antibodies by immunofluorescence. Quantification of YAP nuclear localization is shown (mean ± SD; $n = 3$; $^{****}P \leq 0.0001$, Fisher's test). Scale bar = 20 μm. We compare TRIP6-KO cells to TRIP6-KO cells rescued by FLAG-TRIP6 plasmid (rescue).

H Different amounts (50, 100, 150, 200 ng) of FLAG-TRIP6 plasmid were transfected into HEK293A TRIP6-KO cells and TRIP6 levels in lysates were analyzed by Western blotting using anti-TRIP6 antibodies and compared to those in control HEK293A (WT) cells. 200 ng of FLAG-TRIP6 plasmid (marked with asterisk) was selected to perform the rescue experiment described in panel (G).

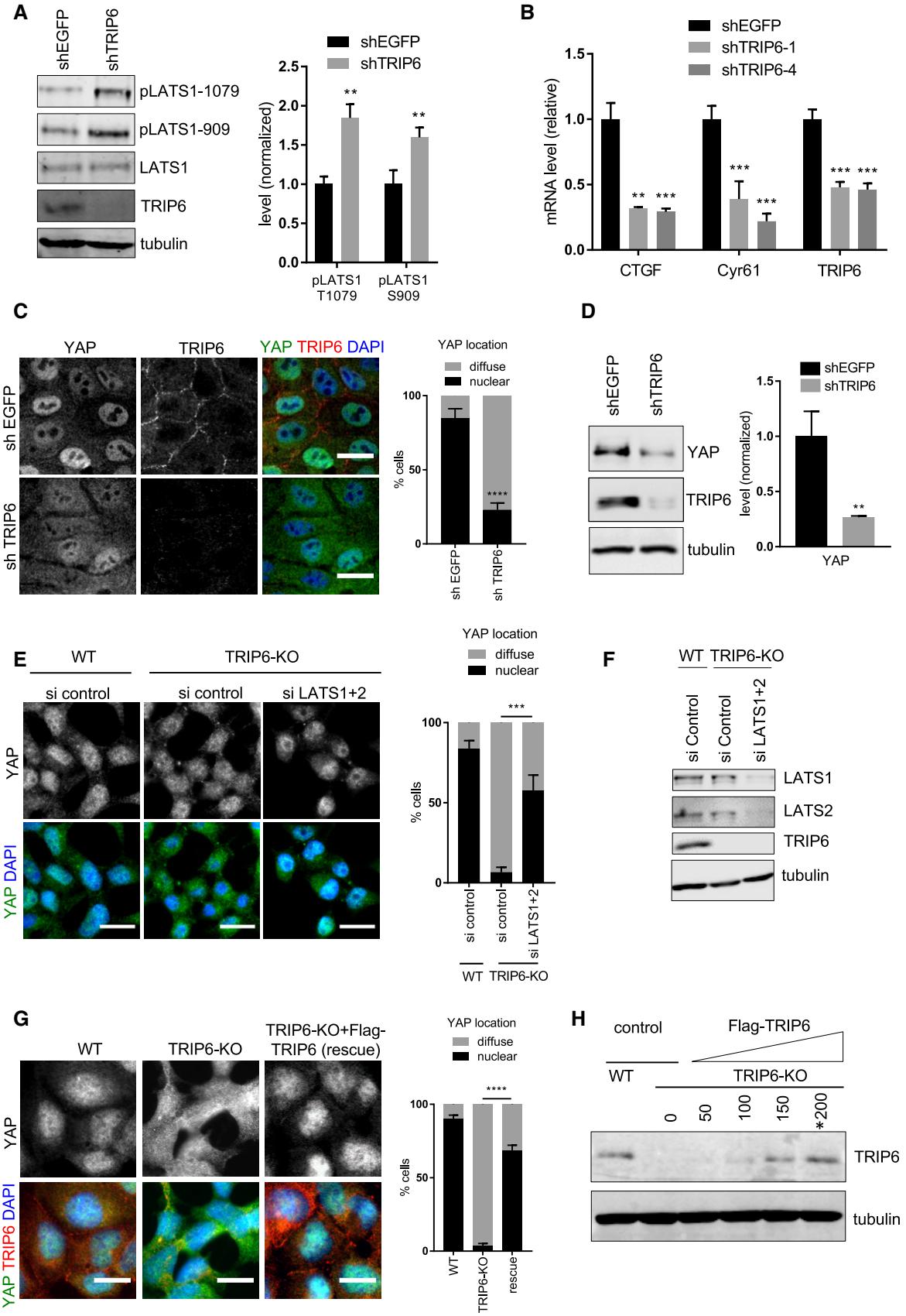

Figure EV1.

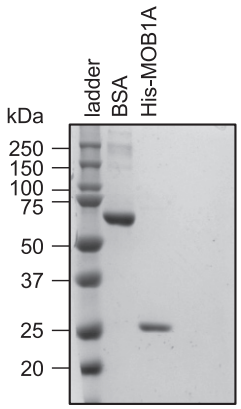

Figure EV2. Levels of BSA and MOB1A.

Coomassie-stained gel showing the amounts of BSA and highest amount of HIS-MOB1A used in Fig 2B.

Figure EV3. Regulation of TRIP6 and LATS1 localization and binding.

A MCF10A cells were stained for TRIP6 and LATS1, and the image was acquired with a confocal microscope. Merged images show LATS1 (green), TRIP6 (red), and DNA (blue). Scale bar = 20 μm. The LATS1-TRIP6 co-localization was confirmed by line scan analysis of the pixel intensity in different fluorescent channels.

B HEK293A cells (WT and TRIP6-KO) were grown, and TRIP6 and LATS1 intracellular localization were determined by immunofluorescence using anti-TRIP6 and anti-LATS1 antibodies. HEK293A (WT) cells were also grown at high density (High density) and treated similarly. Scale bar = 20 μm.

C MCF10A cells were grown at low density and until confluency and stained for TRIP6 and FAK. Merged images show FAK (green), TRIP6 (red), and DNA (blue). Scale bar = 20 μm.

D Quantification of LATS1 at cell–cell junctions in MCF10A cells depleted of TRIP6 (see Fig 3A). Intensity of LATS1 was measured at individual cell–cell junctions ($n \geq 48$) (mean ± SD; $n = 3$; ***$P \leq 0.001$, *t*-test).

E Quantification of TRIP6 at cell–cell junctions in MCF10A cells depleted of LATS1 and LATS2 (see Fig 3B). Average intensity of TRIP6 was measured at individual cell–cell junctions ($n \geq 48$) (mean ± SD; $n = 3$; n.s. ≥ 0.05, *t*-test).

F MCF10A cells grown at high density on PDMS membranes and were stretched (or not) at 17% elongation for 2 h; RNA was isolated, and YAP target gene expression was analyzed using RT–qPCR (mean ± SD; $n = 3$; **$P \leq 0.01$, ***$P \leq 0.001$, *t*-test).

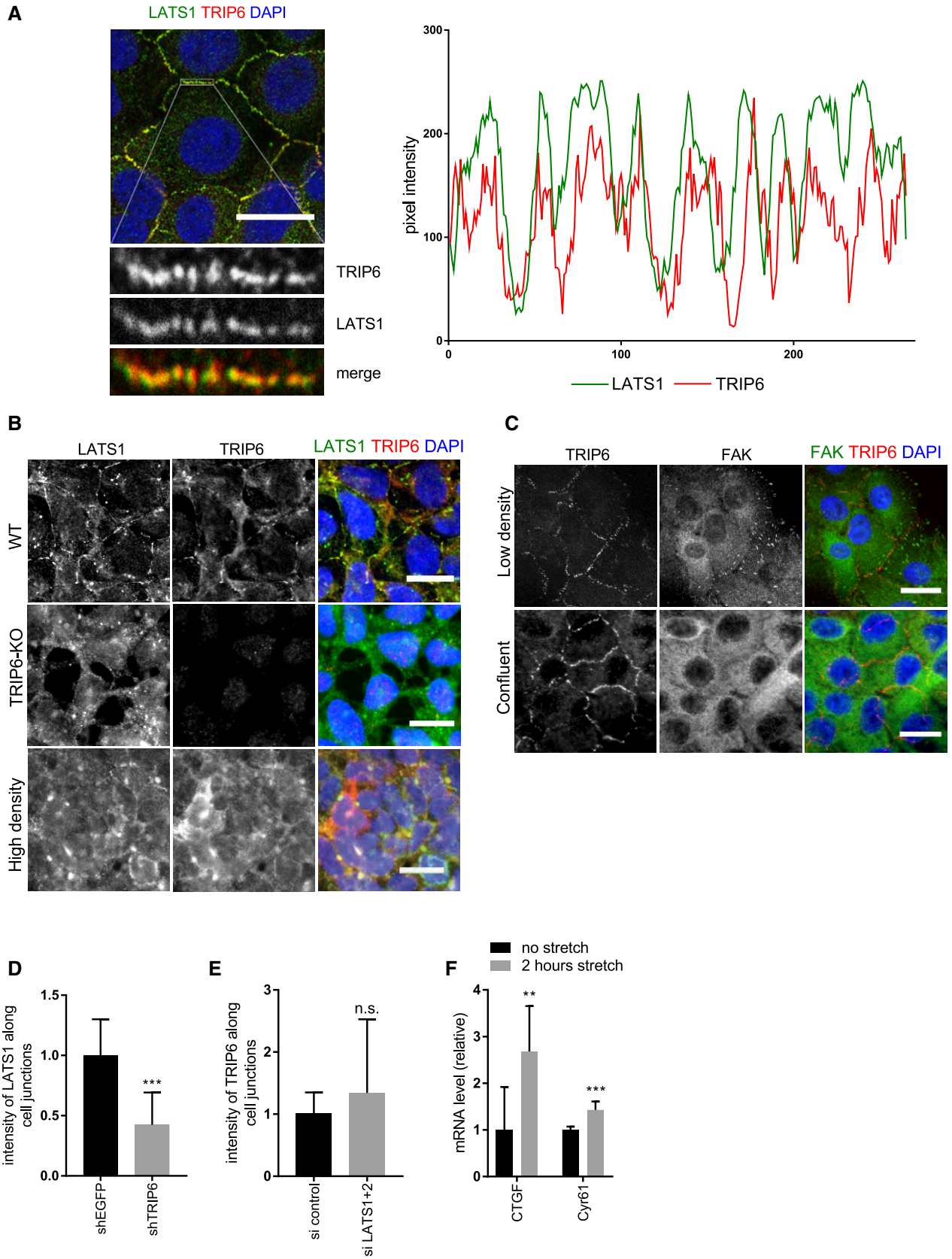

Figure EV3.

Figure EV4. Regulation of TRIP6 and LATS1 localization in HEK293A cells and E-cadherin staining in MCF10A cells after various treatments, TRIP6, LATS1 and 2, and vinculin knockdown.

A HEK293A cells were either not treated (control) or treated separately by serum starvation (no serum), Latrunculin B, Blebbistatin, and EGTA, and then TRIP6 and LATS1 intracellular localization were determined by immunofluorescence using anti-TRIP6 and anti-LATS1 antibodies. Scale bar = 20 μm.

B MCF10A cells were either not treated (control) or treated separately by growth to high density, serum starvation, Latrunculin B, Blebbistatin, or Y27632 treatment, and stained using anti-E-cadherin antibodies by immunofluorescence. Scale bar = 20 μm.

C TRIP6, vinculin, and LATS1 and LATS2 were depleted from MCF10A cells as described (Figs 3B, 5D, and EV1A) and stained using anti-E-cadherin antibodies by immunofluorescence. Scale bar = 20 μm.

D The lysates from (C) were probed by Western blotting for LATS1, TRIP6, and vinculin antibodies to test knockdown efficacy.

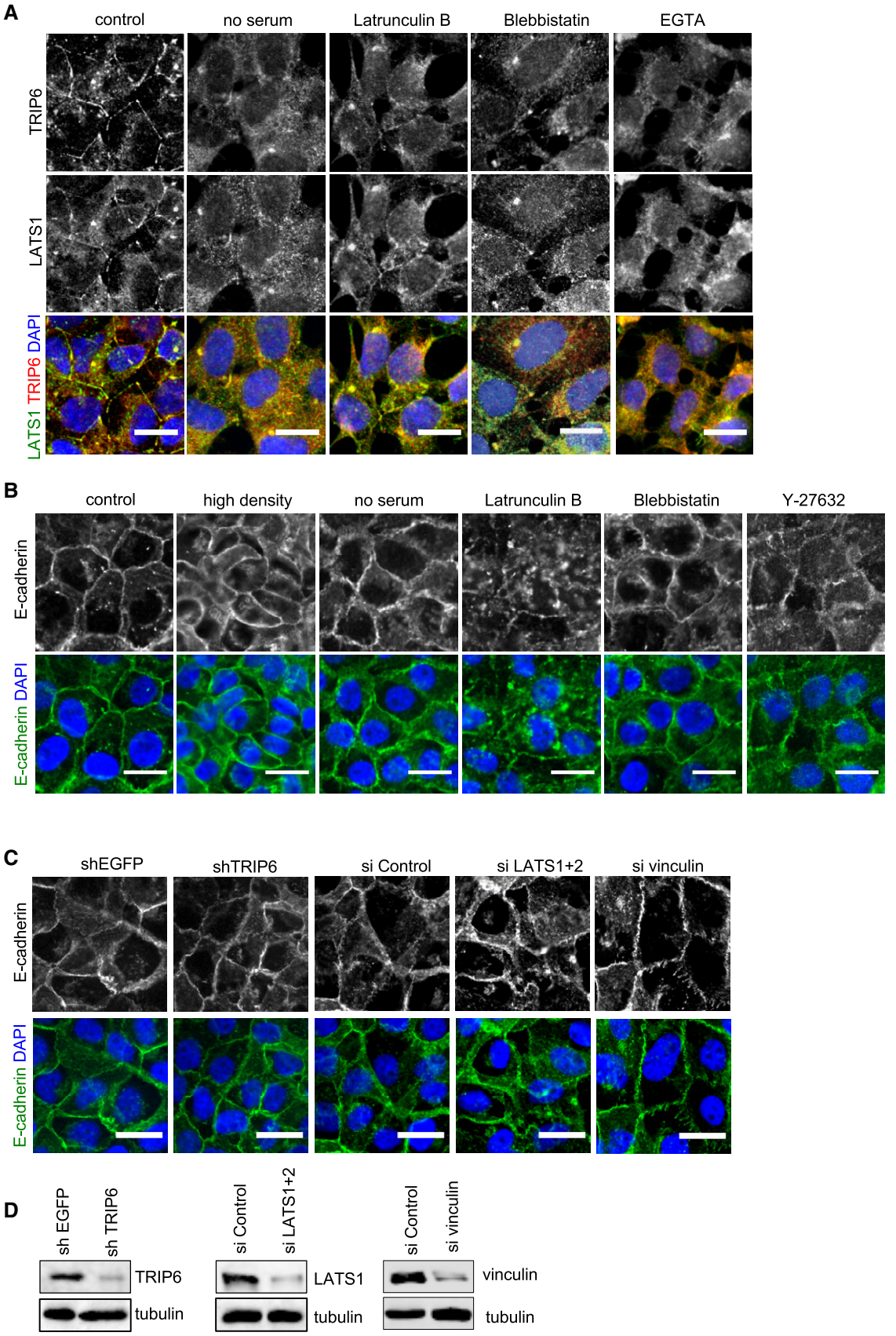

Figure EV4.

Figure EV5. FAK and vinculin co-staining in MCF10A cells, vinculin knockdown efficacy, LATS1 and vinculin co-staining in MCF10A cells, effect of TRIP6 knockdown on vinculin, effect of vinculin knockdown by single siRNAs on YAP activity and localization and rescue of siRNA knockdown are shown.

A MCF10A cells were grown at low density and until confluency and stained using FAK and vinculin antibodies by immunofluorescence. Scale bar = 20 μm.

B Vinculin was knocked down using two different stealth siRNAs in MCF10A cells. MCF10A control cells were treated with control siRNA. Lysates were probed by Western blotting for vinculin and tubulin. The levels of vinculin were determined (mean ± SD; $n = 3$; ***$P \leq 0.001$, *t*-test).

C Vinculin was knocked down as described in (B), and cells were stained using vinculin and LATS1 antibody. Merged images show LATS1 (green), vinculin (red), and DNA (blue). Scale bar = 20 μm.

D TRIP6 was depleted from MCF10A cells as described in Fig EV1A and grown at high density on PDMS membranes and that were stretched (or not) at 17% elongation for 2 h, fixed while under tension, and stained for vinculin. Merged images show vinculin (green) and DNA (blue). Scale bar = 20 μm.

E Vinculin was knocked down by single stealth siRNAs (7662 and 1260) in MCF10A cells and stained using vinculin and YAP antibody. Merged images show YAP (green), vinculin (red) and DNA (blue). Quantification of YAP nuclear localization in least 100 cells is shown (mean ± SD; $n = 3$; **$P \leq 0.01$, ***$P \leq 0.001$, Fisher's test). Scale bar = 20 μm.

F Vinculin was knocked down as described in (E) and the levels of vinculin, and YAP target gene expression was analyzed using RT–qPCR (mean ± SD; $n = 3$; **$P \leq 0.01$, ***$P \leq 0.001$, *t*-test).

G Vinculin was knocked down as described (panel E) in HEK293A cells. Control and vinculin depleted HEK293A cells were transfected with 150 ng of control and EGFP-vinculin (chicken) plasmids. (Note that 150 ng of EGFP-vinculin plasmid restores approximate wild-type levels (see panel H) of vinculin expression). After 48 h of transfection, the levels of vinculin, and YAP target gene expression was analyzed using RT–qPCR. We compare vinculin depleted cells to vinculin depleted cells rescued by EGFP-vinculin (rescue) (mean ± SD; $n = 3$; **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$, *t*-test).

H Vinculin was knocked down as described in Fig 5D in HEK293A cells. Different amounts (50, 150, 250, 350 ng) of EGFP-vinculin plasmid were transfected into the vinculin depleted cells, and vinculin levels in lysates were analyzed by Western blotting using vinculin antibody and compared to those in control HEK293A cells. 150 ng of EGFP-vinculin plasmid (marked with asterisk) was selected to perform the rescue experiment described (panel G).

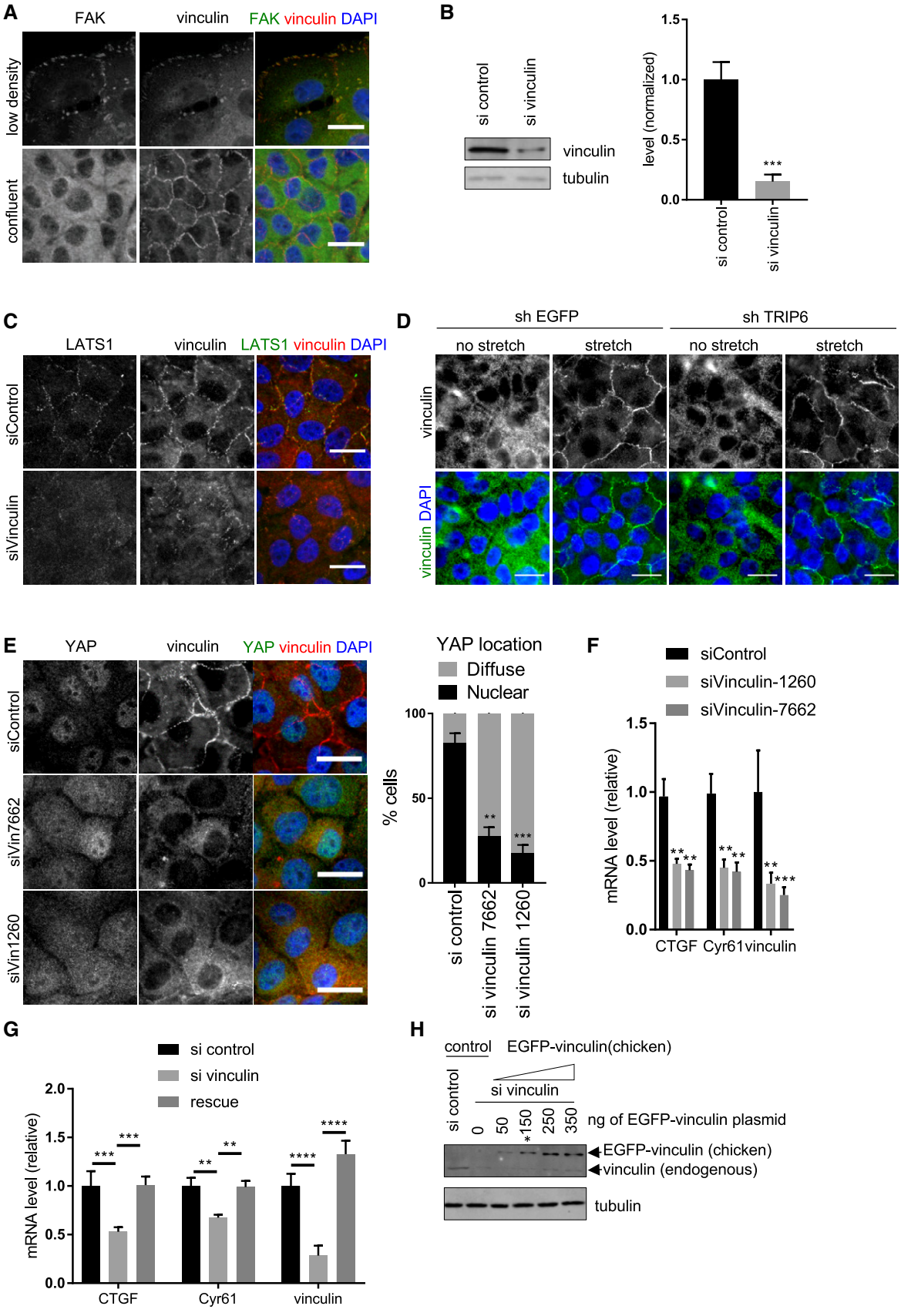

Figure EV5.