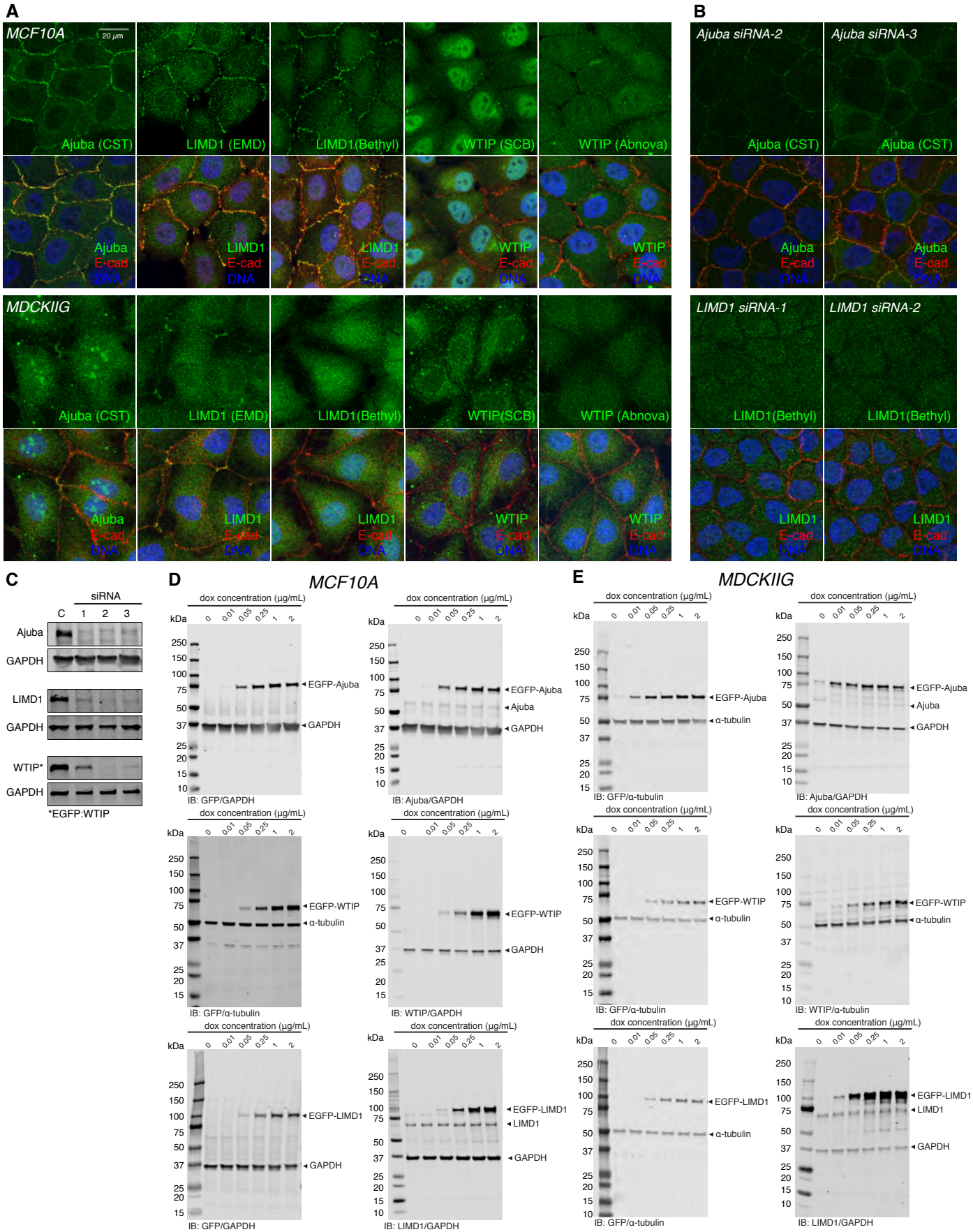
SUPPLEMENTARY FIGURES

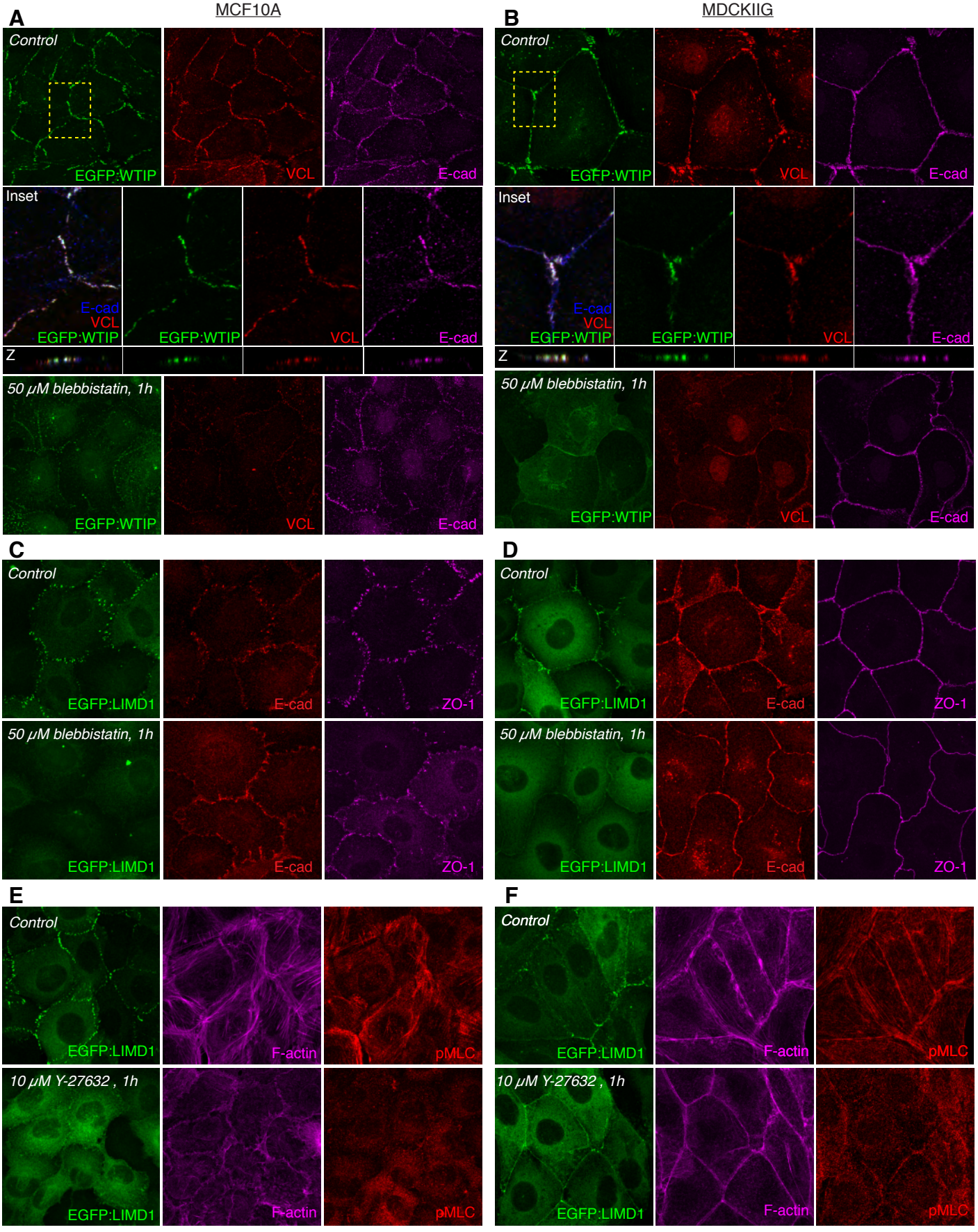
Supplementary Figure S1



Supplementary Figure S1. Evaluation of Ajuba family protein antibodies and Dox-inducible stable cell lines.

(A) MCF10A (top panels) or MDCKIIG (bottom panels) cells were grown at low density and stained with the indicated Ajuba family protein commercially available antibodies (green), E-cad (red) and DNA (Hoechst, blue). **(B)** MCF10A cells transfected with the Ajuba or LIMD1 siRNAs and stained with the indicated antibodies (green), E-cad (red) and DNA (Hoescht, blue) **(C)** Western blot on lysates of MCF10A cells transfected with 3 different siRNA against each of the Ajuba family proteins and blotted using the indicated antisera. Because the WTIP antibody used does not recognize the endogenous protein, MCF10A EGFP:WTIP cells were used to evaluate WTIP siRNAs. **(D)** Western blots on lysates of stable MCF10A cell lines of Ajuba family proteins treated with increasing Dox concentrations and blotted with the indicated antisera. **(E)** Western blots on lysates of stable MDCKIIG cell lines of Ajuba family proteins treated with increasing Dox concentrations and blotted with the indicated antisera. Tubulin or GAPDH was used as a loading control and size markers are at left of each blot.

Supplementary Figure S2



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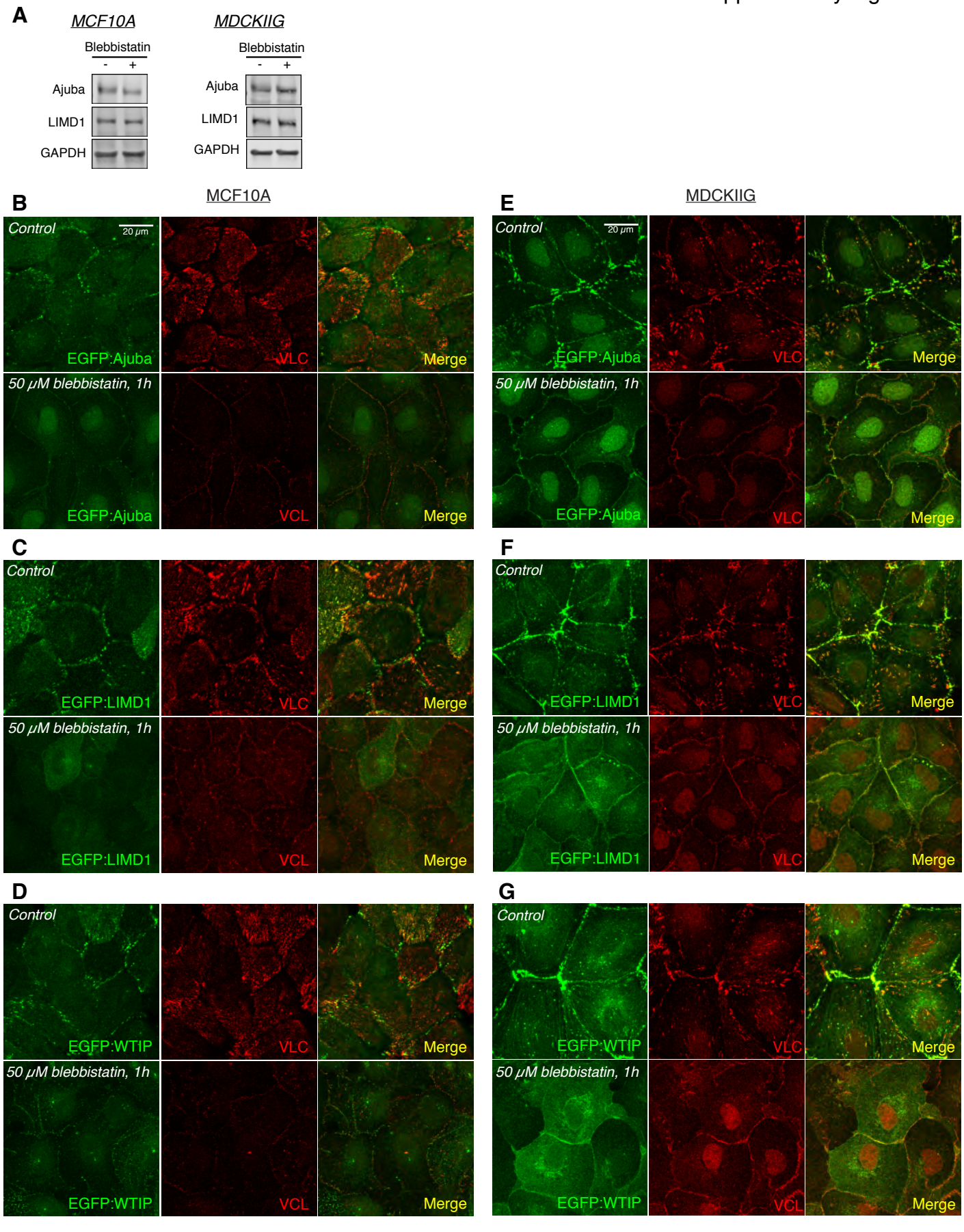
Supplementary Figure S2. Ajuba family protein localization compared to adherens junctions and tight junctions proteins.

(A,B) MCF10A **(A)** or MDCKIIG **(B)** EGFP:WTIP cells plated at low-density (15,000 cells/cm^2) and cultured for 48 hours in total. Transgene expression was induced with Dox (Table 1) for 24 hours before treatments. Cells were treated with DMSO (Control) or 50 µM blebbistatin for 1 hour, fixed in the presence of 0.5% Triton X-100 and then stained with VCL (red) and E-cadherin (E-cad, magenta). Square images are apical slices for MCF10A and Z-projections for MDCKIIG cells, and are representative of at least 3 biological replicates. Insets show higher magnification of the boxed regions (yellow dashes). Vertical confocal slices (Z) are shown at the same magnification as the insets. **(C,D)** MCF10A (C) and MDCKIIG (D) EGFP:LIMD1 cells plated at low-density (15,000 cells/cm^2). Cells were grown for 48 hours in total, induced with Dox for 24 hours before fixation, treated with DMSO (Control) or 50 µM blebbistatin for 1 hour and then stained with E-cadherin (red) and ZO-1 (magenta). Images are Z-projections and are representative of at least 3 biological replicates. **(E,F)** MCF10A (*E*) and MDCKIIG (*F*) EGFP:LIMD1 cells plated at low-density (15,000 cells/cm^2). Cells were grown for 48 hours in total, induced with Dox for 24 hours before fixation, treated with dH_2O (Control) or 10 µM Y-27632 for 1 hour and then stained with phospho-myosin (pMLC, red) and phalloidin (F-actin, magenta). Images are Z-projections and are representative of at least 3 biological replicates.

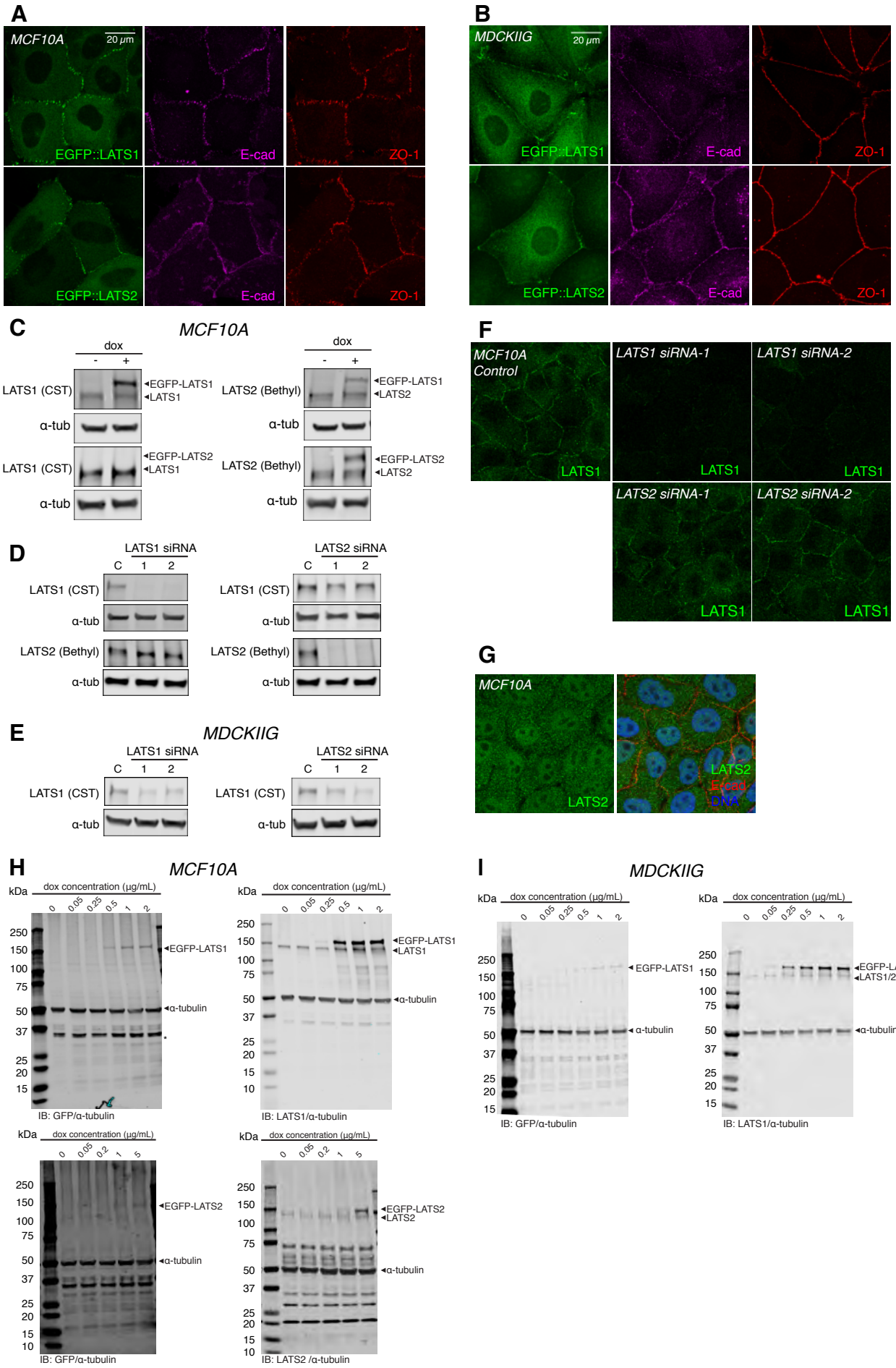
Supplementary Figure S3



Supplementary Figure S3. Ajuba family proteins can localize to focal adhesions.

(A) Western blots on lysates of MCF10A or MDCKIIG cells treated with DMSO (control) or 50 μM blebbistatin for 1 hour, blotted using the indicated antisera. All the blots are from the same experiment and a representative loading control (GAPDH) is shown. **(B-D)** MCF10A or **(E-G)** MDCKIIG cells from the indicated cell lines were plated at low-density (15,000 cells/cm^2) and cultured for 48 hours in total. Transgene expression was induced with Dox (Table 1) for 24 hours before treatments. Cells were treated with DMSO (Control) or 50 μM blebbistatin for 1 hour, fixed in the presence of 0.5% Triton X-100 and then stained with VCL (red) and E-cadherin (E-cad, magenta). Images are basal slices (**B-D**, MCF10A) or Z-projections saturated to show the focal adhesion localization in MDCKIIG cells (**E-G,** MDCKIIG) and are representative of at least 3 biological replicates. Focal adhesion localization is less evident in MCF10A cells.

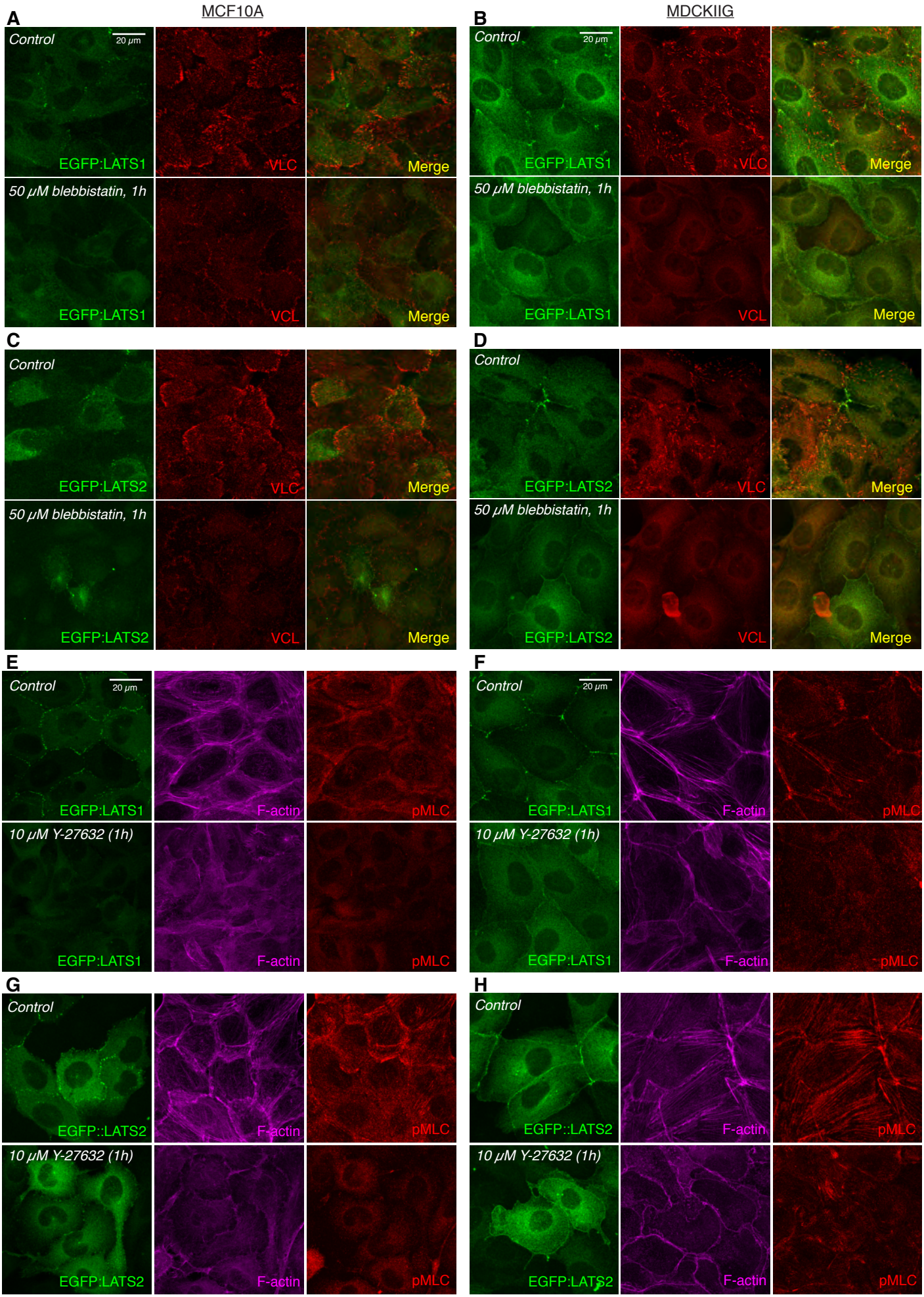
Supplementary Figure S4



Supplementary Figure S4. Localization of Lats proteins, testing of Lats antibodies and Dox-inducible cell lines

(A,B) Cells from the indicated stable cell lines (MCF10A (*a*), MDCKIIG (*b*)) were plated at low-density (15,000 cells/cm^2). Cells were grown for 48 hours in total, induced with Dox for 24 hours before fixation and stained with ZO-1 (red) and E-cadherin (magenta). Images are Z-projections and are representative of at least 3 biological replicates. **(C-E)** Testing of commercially available Lats antibodies. (C) Western blots on lysates of MCF10A EGFP:LATS1 or EGFP:LATS2 cells treated with or without Dox blotted with the indicated antisera. (D) Western blot on lysates of MCF10A cells treated with LATS1 or LATS2 siRNAs and blotted with the indicated antisera. **(E)** Western blots on lysates of MDCKIIG cells treated with LATS1 or LATS2 siRNA and blotted with the indicated antisera. **(F)** MCF10A cells were plated at low-density (15,000 cells/cm^2) and transfected with control, LATS1 and LATS2 siRNAs. Cells were stained with LATS1 antibody (CST). Images are Z-projections. **(G)** MCF10A cells grown at low density and stained with LATS2 antibody (Bethyl, green), E-cadherin (E-cad, red) and DNA (Hoechst, blue) **(H)** Western blots on lysates of stable MCF10A cell lines of EGFP:LATS1 or EGFP:LATS2 treated with increasing Dox concentrations and blotted with the indicated antisera. **(I)** Western blots on lysates of stable MDCKIIG cell lines of EGFP:LATS1 incubated with increasing Dox concentrations and blotted with the indicated antisera. Tubulin was used as a loading control.

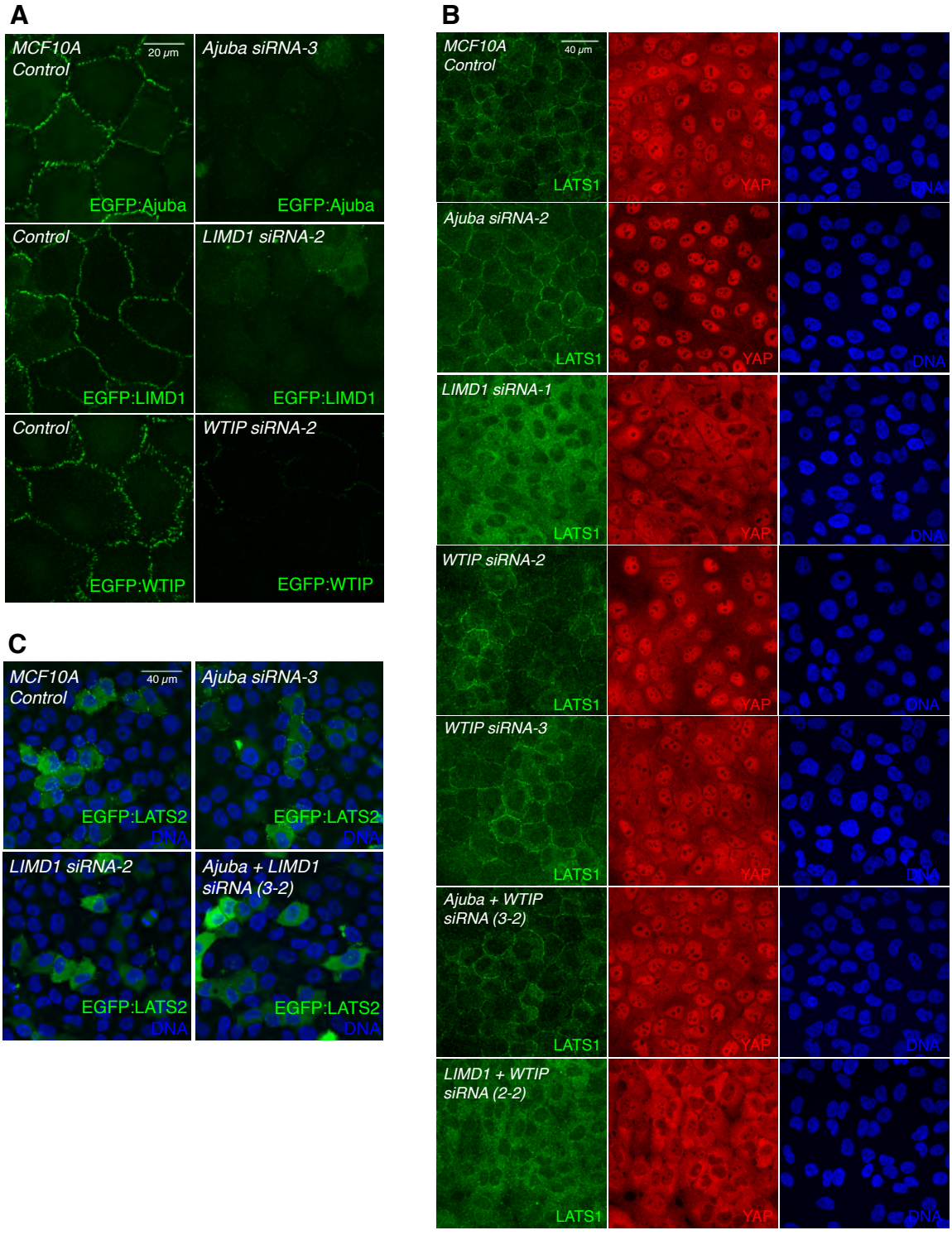
Supplementary Figure S5



Supplementary Figure S5. Lats proteins do not localize to focal adhesions.

A-D) MCF10A **(A,C)** or MDCKIIG **(B,D)** cells from the indicated cell lines were plated at low-density (15,000 cells/cm^2) and cultured for 48 hours in total. Transgene expression was induced with Dox (Table 1) for 24 hours before treatments. Cells were treated with DMSO (Control) or 50 μM blebbistatin for 1 hour, fixed without detergent and then stained with VCL (red) and E-cadherin (E-cad, magenta). Images are basal slices (**A,C**, MCF10A) or Z-projections in MDCKIIG cells (**B,D,** MDCKIIG) and are representative of at least 3 biological replicates**. E-G)** MCF10A **(E,G)** or MDCKIIG **(F,H)** cells from the indicated stable cell lines were plated at low-density (15,000 cells/cm^2) and cultured for 48 hours in total. Transgene expression was induced with Dox (Table 1) for 24 hours before treatments. Cells were treated with dH_2O (Control) or 10 μM Y-27632 for 1 hour, fixed without detergent and then stained with phospho-myosin light chain (S19) antisera (pMLC, red) and phalloidin (F-actin, magenta). Images are Z-projections and are representative of at least 3 biological replicates.

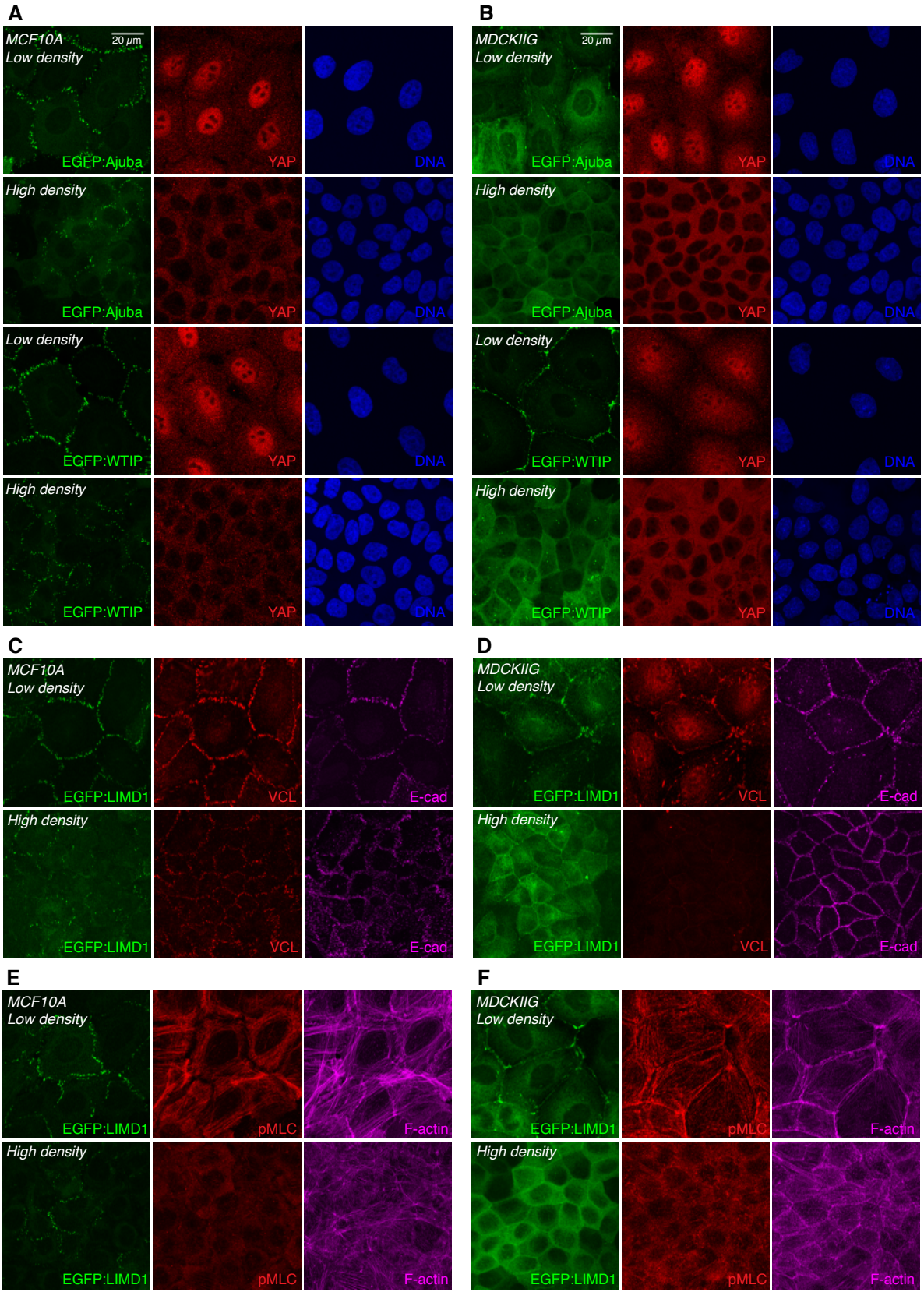
Supplementary Figure S6



Supplementary Figure S6. Efficiency of Ajuba family protein siRNAs and their effects on LATS localization.

(A) Stable MCF10A cell lines of EGFP-tagged Ajuba family proteins were plated at low density, transfected with the indicated siRNA, induced with Dox and then fixed and imaged. **(B)** MCF10A cells at low density transfected with control, Ajuba, LIMD1, WTIP, Ajuba+WTIP and LIMD1+WTIP siRNA stained with LATS1 (green), YAP (red) and DNA (Hoechst, blue). **(C)** MCF10A EGFP:LATS2 cells were transfected with the indicated siRNA and stained with Hoechst (DNA, blue). Images are Z-projections and representative of at least 3 biological replicates.

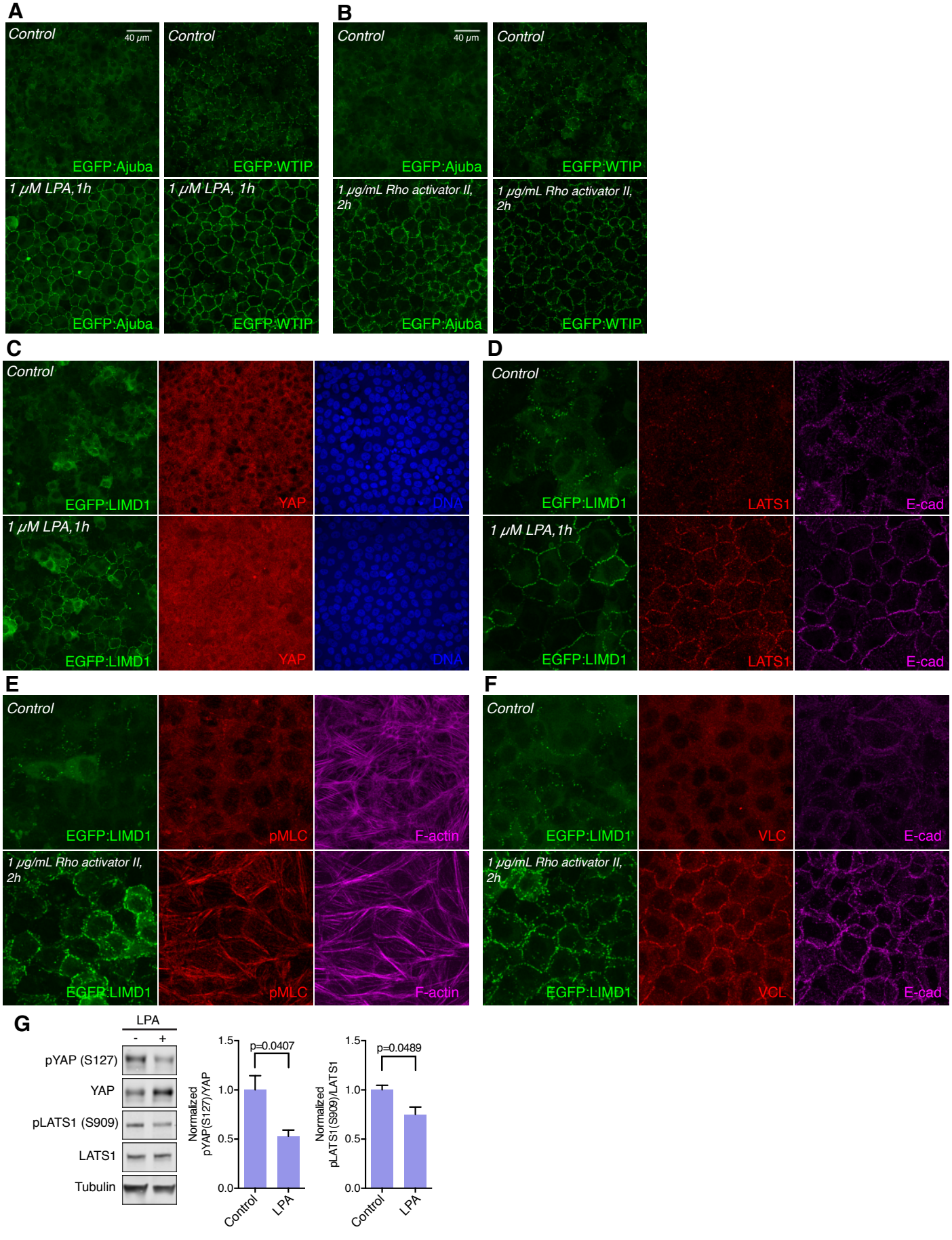
Supplementary Figure S7



Supplementary Figure S7. Regulation of Ajuba family proteins localization by cell density.

(A,B) Cells from the indicated stable cell lines (MCF10A, A) and MDCK, B) were plated at low-density (15,000 cells/cm^2) and high-density (150,000 cells/cm^2) conditions as indicated. Cells were grown for 48 hours in total, induced with Dox for 24 hours before fixation and stained for YAP (red) and DNA (Hoechst, blue). **(C-F)** MCF10A (C,E) or MDCKIIG (D,F) EGFP:LIMD1 cells plated at low and high density as indicated were stained with vinculin (VCL, red) and E-cadherin (E-cad, magenta) (C,D) or with phospho-myosin light chain antibody (pMLC, red) and phalloidin (F-actin, magenta) (E,F). Images are Z-projections and are representative of at least 3 biological replicates.

Supplementary Figure S8



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Supplementary Figure S8. Effects of LPA and Rho activator II on Ajuba family proteins, LATS1, and YAP.

(A,B) Stable MCF10A cell lines of the indicated Ajuba family proteins cells grown at high density and mock-treated with water (control) or treated with 1 μM LPA for 1 hour (A), or Rho activator II for 2h (B). Images are Z-projections and represent at least 3 biological replicates. **(C-F)** MCF10A EGFP-LIMD1 cells were grown at high density, induced with Dox 24 h before treatment and mock-treated with water (control) or treated with 1 μM LPA for 1 hour (C,D) 1μg/mL Rho activator II for 2 h (E,F). After treatment, cells were stained with **(C)** YAP (red) and DNA (Hoechst, blue), **(D)** LATS1 (red) and E-cadherin (magenta), **(E)** with pMLC (red) and phalloidin (F-actin, magenta), or **(F)** vinculin (VCL, red) and E-cadherin (E-cad, magenta). Images are Z-projections and representative of at least 3 biological replicates. **(G)** Western blots on lysates of MCF10A cells mock-treated with water (control) or treated with 1 μM LPA for 1 hour, blotted using the indicated antisera. All the blots are from the same experiment and a representative loading control (Tubulin) is shown. Histograms show quantitation of the pYAP (S127) over YAP ratio and pLATS1 (S909) over LATS1 ratio from 3 biological replicates normalized by to the ratio in the control. Error bars indicate SEM and *p*-values less than 0.05 are shown.