Supplem Fig S1 Venkatramanan et al

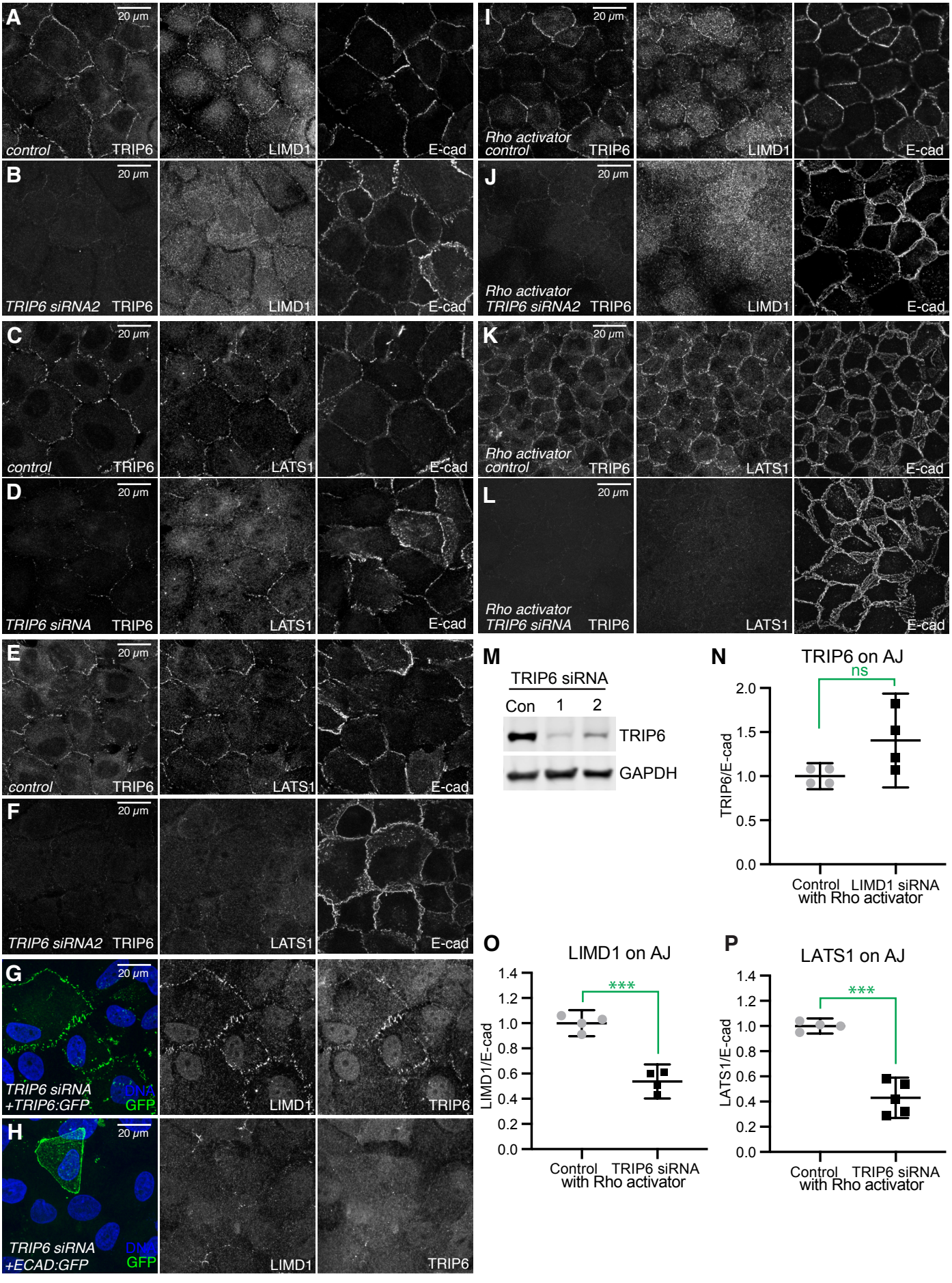

Fig. S1. Validation of TRIP6 siRNAs and their effect on LIMD1 and LATS1 localization
(A-F) MCF10A cells grown at low density and transfected with control or TRIP6 siRNA or siRNA2 as indicated, cultured for 72 hours, then fixed and stained for TRIP6, LIMD1 or LATS1, and E-cad. (G-H) MCF10A cells grown at low density and transfected with TRIP6 siRNA and cultured for 24 hours, then transfected with either TRIP6:GFP (rescue) (G) or ECAD:GFP (control) (H) and fixed, stained for LIMD1 and TRIP6 after a further 48 hours incubation. (I-L) MCF10A cells cultured at high cell density and treated with 1 µg/ml Rho-activator-II for 3 hours before fixation and stained for TRIP6, E-cad and LIMD1 (I, J) or LATS1 (K, L) respectively. (M) Western Blot indicating knockdown efficiency of TRIP6 siRNAs used. Con indicates negative control siRNA. GAPDH is the loading control. (N-P) Quantification of junctional levels of TRIP6 (N), LIMD1 (O) and LATS1 (P) relative to E-cad levels in control and LIMD1 siRNA or TRIP6 siRNA cells cultured at high density and treated with Rho-activator-II. N=4 for N,O, and control in P, N=5 for TRIP6 siRNA in P. Statistical compairisons by unpaired t test are shown, ns indicates not significant, *** indicates $P<0.001$, and error bars indicate 95% confidence interval (ci).

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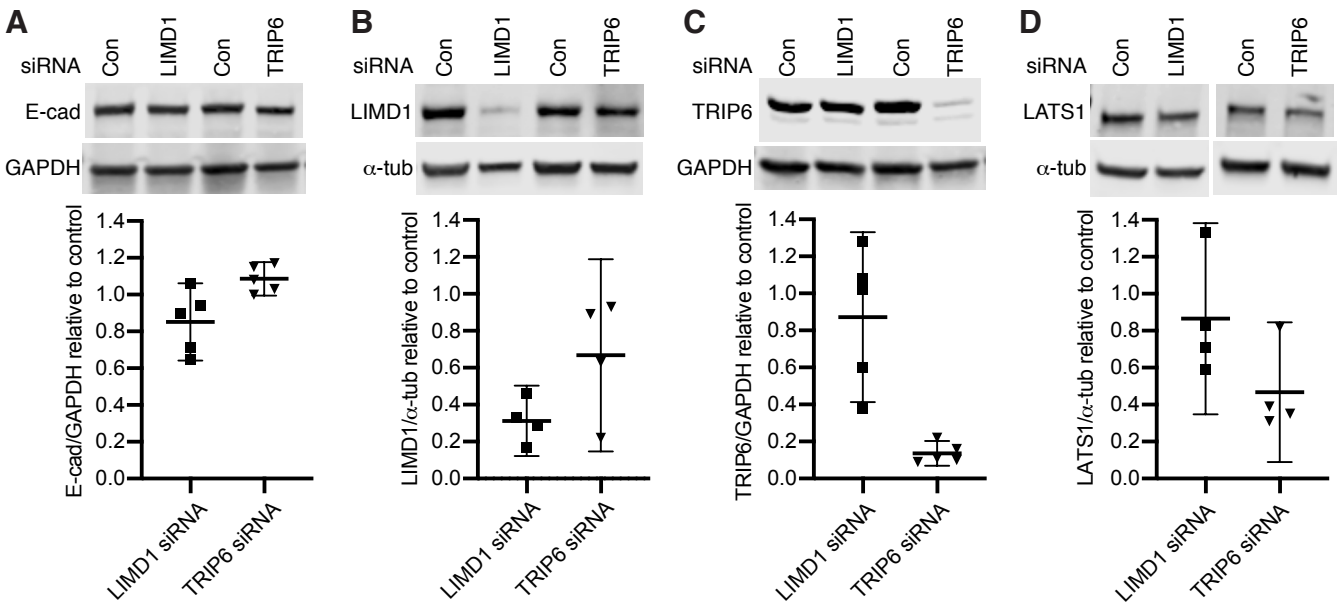

Fig. S2. Effect of TRIP6 or LIMD1 knockdown on protein levels

Western blots showing relative amounts of E-cad (A), LIMD1 (B), TRIP6 C), and LATS1 (D) with respect to their loading controls (GAPDH or α-tub) in control and LIMD1 or TRIP6 siRNA treated MCF10A cells, together with quantification of the ratio of protein assayed/loading control levels in siRNA treated cells relative to that in control siRNA treated cells. Values for each replicate are indicated by dots on the scatter plots, error bars show 95% ci.

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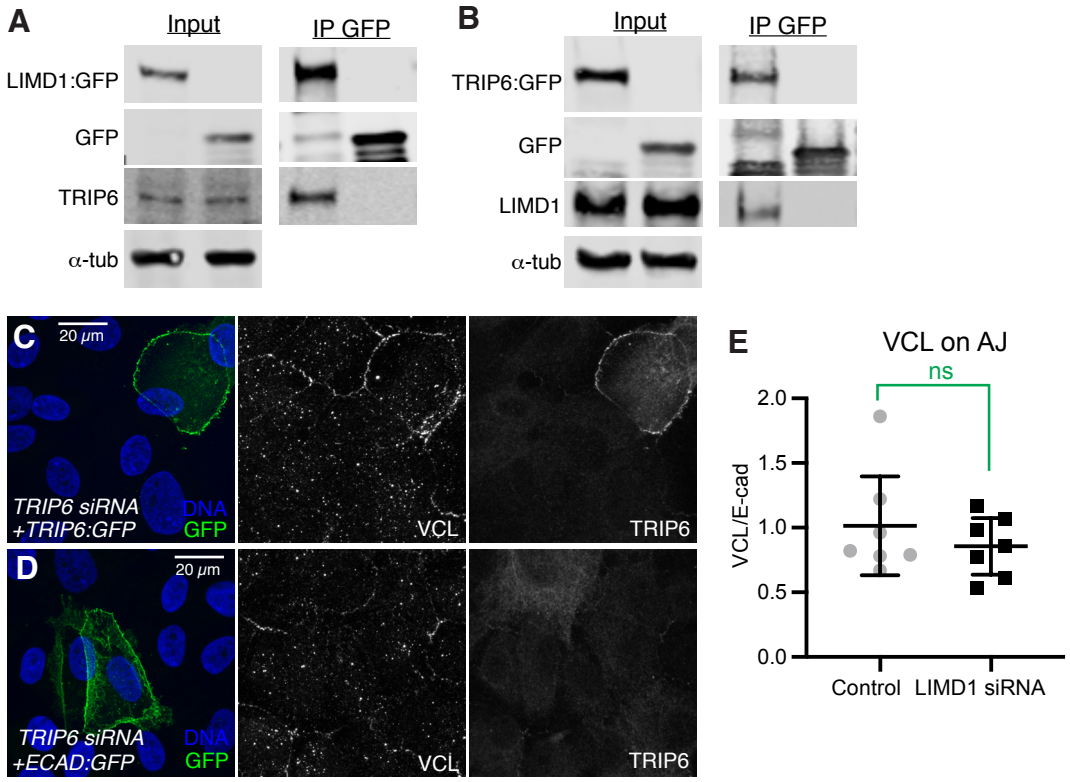

Fig. S3 Association of LIMD1 and TRIP6, and influence of TRIP6 on VCL

(A,B) Show the results of co-immunoprecipitation experiments between TRIP6 and LIMD1. HEK293T cells were transfected with LIMD1:GFP or GFP:V5 (A), or TRIP6:GFP or GFP:V5 (B). Input shows immunoblots on cell lysates, IP GFP shows immunoblots on proteins immunoprecipitated from cell lysates with GFP-Trap magnetic agarose beads. Proteins detected are labelled at left. (C,D) MCF10A cells grown at low density were transfected with TRIP6 siRNA and cultured for 24 hours, then transfected with either TRIP6:GFP (rescue) (C) or ECAD:GFP (control) (D) and fixed, stained for VCL and TRIP6 after a further 48 h incubation. (E) Quantification of junctional levels of Vinculin relative to the E-cad levels in control and LIMD1 siRNA treated cells. Each dot represents results from a confocal image stack containing several cells, N=7 for control and for LIMD1 siRNA. Significance of unpaired t test indicated, ns= not significant, error bars show 95% ci.

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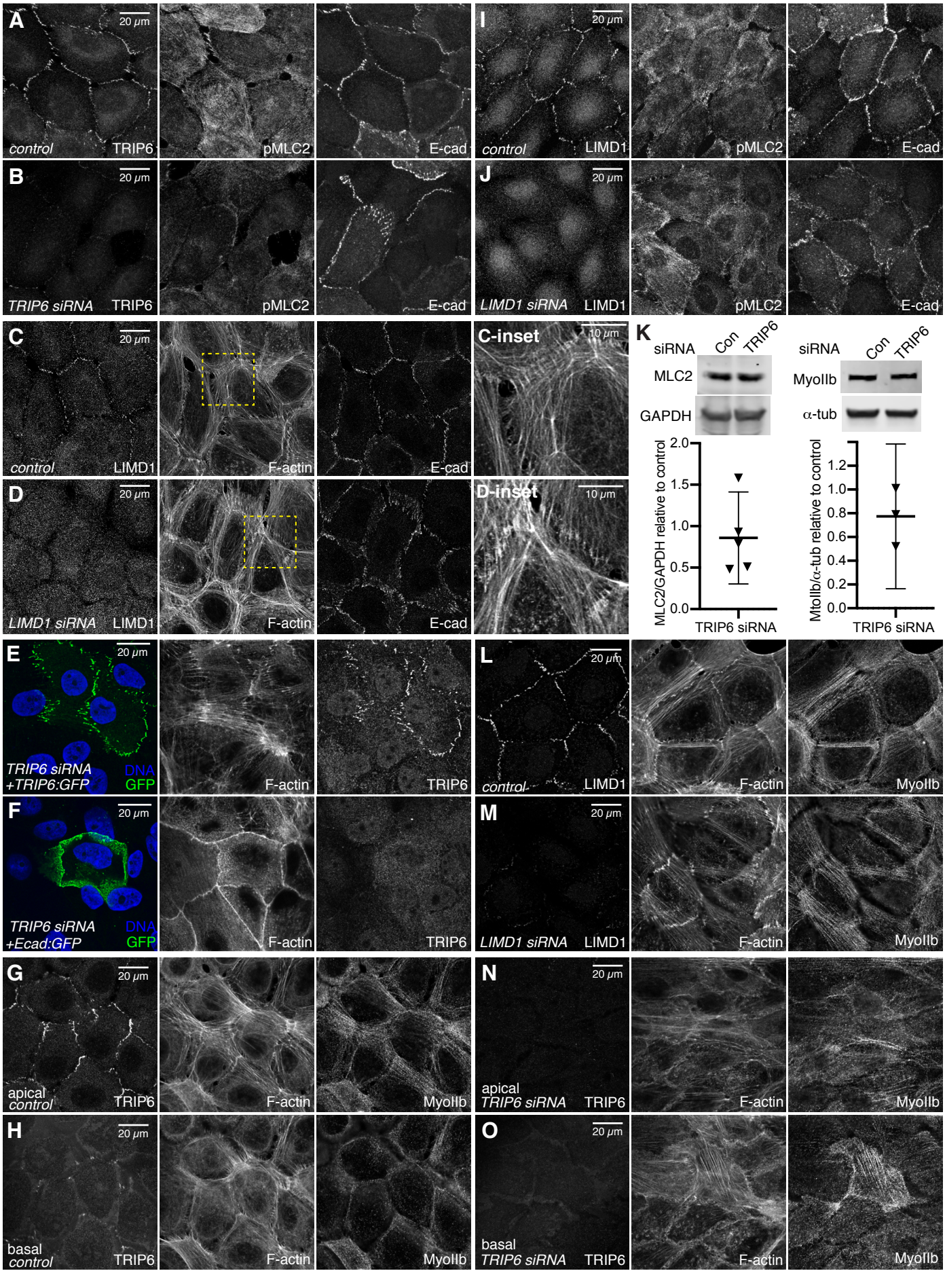

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Fig. S4. Effect of TRIP6 or LIMD1 knockdown on actin and myosin

(A-D, I-J, L,M) MCF10A cells cultured at low density transfected with control (A,C,I,L), TRIP6 (B) or LIMD1 siRNA (D,J,M) cultured for 48 hours (LIMD1 siRNA) or 72 h (TRIP6 siRNA), then fixed and stained for TRIP6 or LIMD1, pMLC2, MyoIIb or F-actin and E-cad. Insets in C,D show higher magnification of the boxed regions. (E,F) MCF10A cells grown at low density transfected with TRIP6 siRNA and cultured for 24 hours, then transfected with either pTRIP6:GFP (rescue) (E) or E-cad:GFP (control) (F) and fixed, stained for F-actin and TRIP6 after a further 48 h incubation. (G,H,N,O) MCF10A cells grown at low density transfected with control (G, H) or TRIP6 (N,O) siRNA and stained for F-actin and MyoIIB. Images are projections through 3-7 apical or basal sections as indicated. (K) Western blots showing relative amounts of MLC2 and MyoIIb with respect to their loading controls (GAPDH or α-tub) in control and TRIP6 siRNA treated MCF10A cells, together with quantification of the ratio of protein assayed/loading control levels in siRNA treated cells relative to that in control cells. Values for each replicate are indicated by dots on the scatter plots, error bars show 95% ci.

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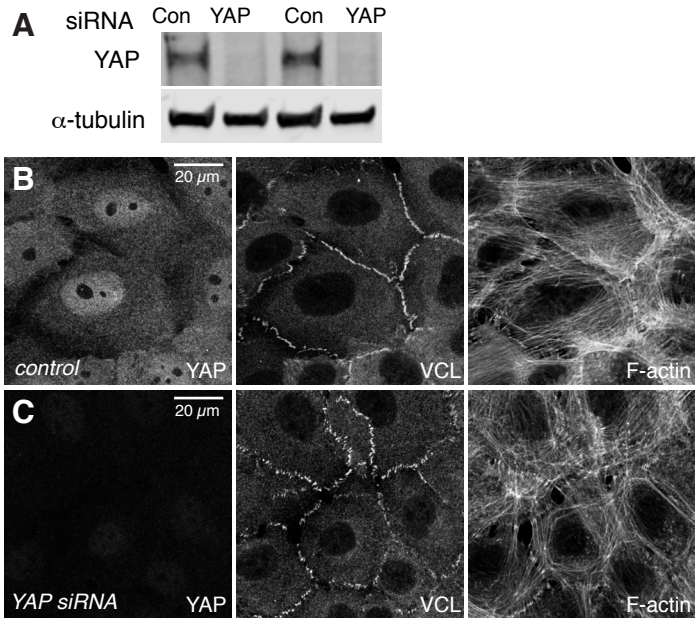

Fig. S5. Effect of YAP knockdown on actin organization

(A) Western blot showing duplicate examples of knockdown efficiency of YAP siRNA used. Con indicates negative control siRNA, α-tubulin is the loading control. (B,C) MCF10A cells cultured at low density were transfected with control (B) or YAP (C) siRNA, incubated for 72 hours and stained for YAP, VCL and F-actin. Images are projections through the entire cell and are representatives of at least 3 biological replicates.

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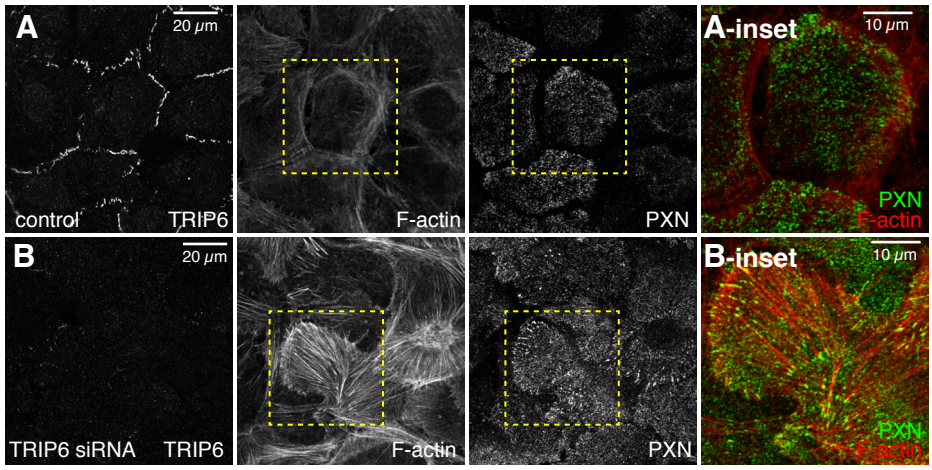

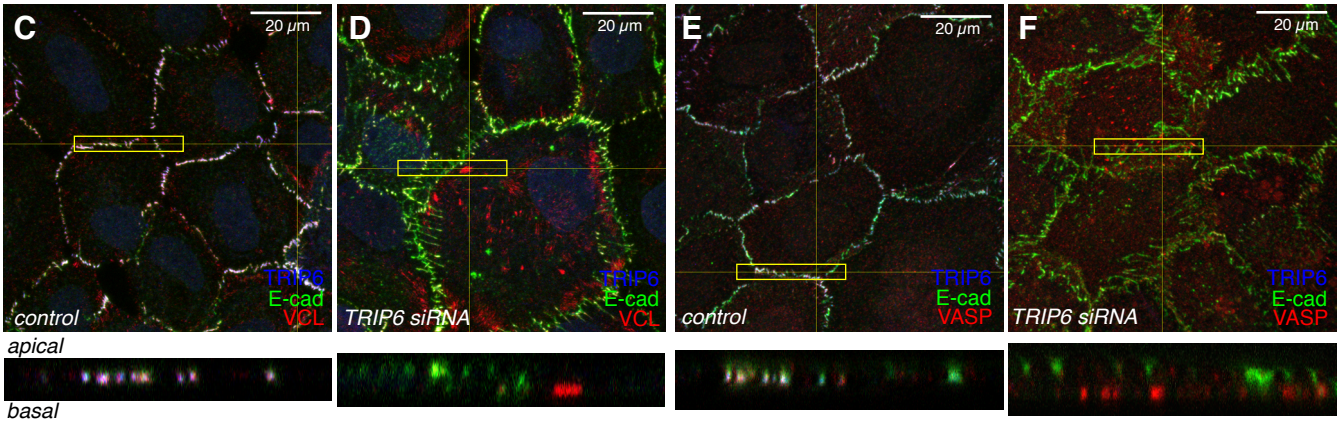

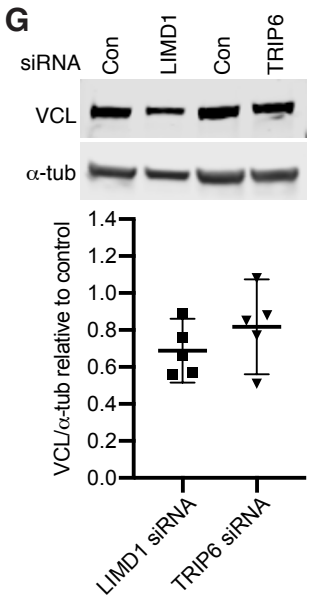

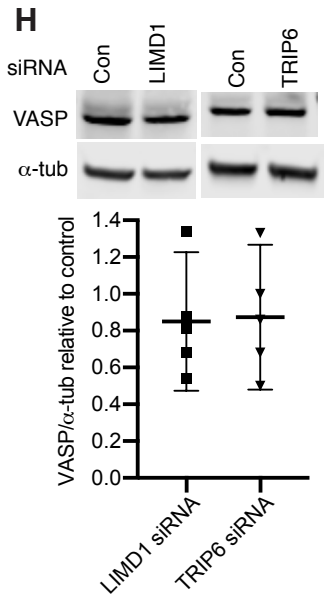

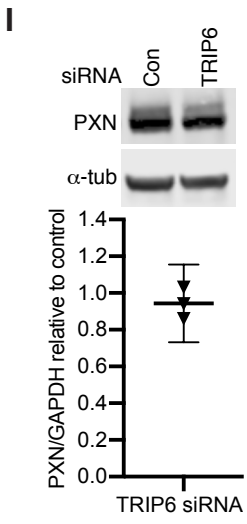

Fig. S6. Effect of TRIP6 knockdown on focal adhesion proteins

(A,B) MCF10A cells cultured at low density transfected with control (A) or TRIP6 (B) siRNA, incubated for 72 hours and stained for TRIP6, PXN and F-actin. Insets show higher magnification of the boxed regions, with PXN in green and F-actin in red. (C-F) MCF10A cells cultured at low density, transfected with control (C, E) or TRIP6 (D, F) siRNA showing the localization of VCL (C,D) or VASP (E,F) (red) relative to E-cad (green) in control and TRIP6 siRNA cells. Projections through the cells are above and XZ slices along the yellow lines within the boxed regions are below, with apical at top. (G-I) Western blots showing relative amount of VCL, VASP, and PXN with respect to the loading control (α-tub) in control and LIMD1 or TRIP6 siRNA treated MCF10A cells, together with quantification of the ratio of protein assayed/loading control levels in siRNA treated cells relative to that in control cells. Values for each replicate are indicated by dots on the scatter plots, error bars show 95% confidence interval.

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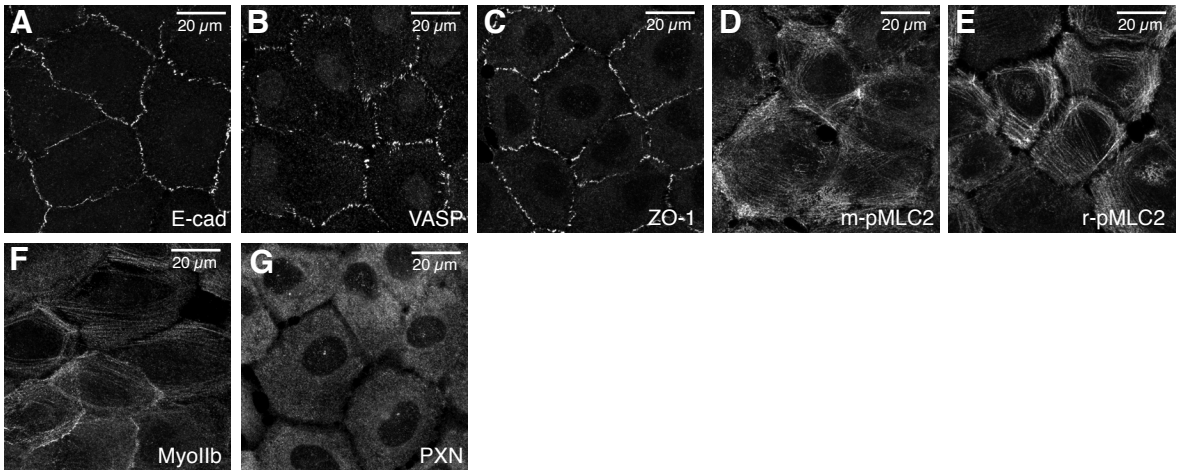

Fig. S7. Additional Antibody Validation

MCF10A cells cultured at low density and singly-labeled for E-cad (A), VASP (B), ZO-1 (C), mouse pMLC2 (D), rabbit pMLC2 (E), Myosin-IIB (F), and PXN (G).