Supplementary Figure 1

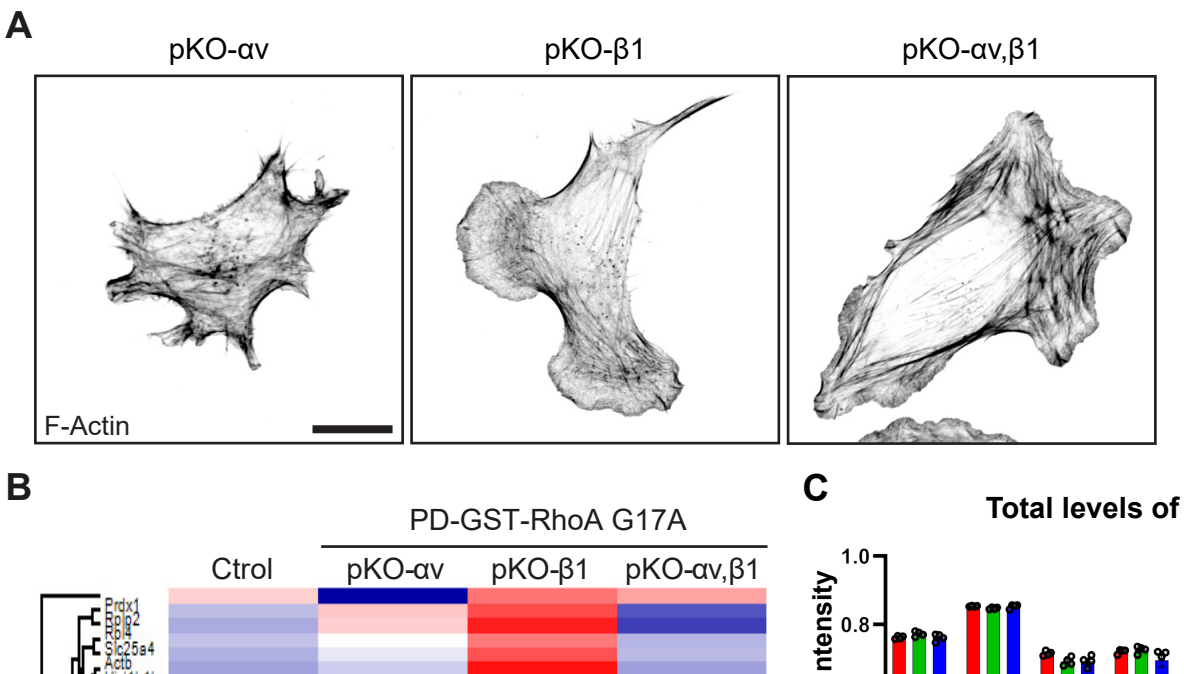
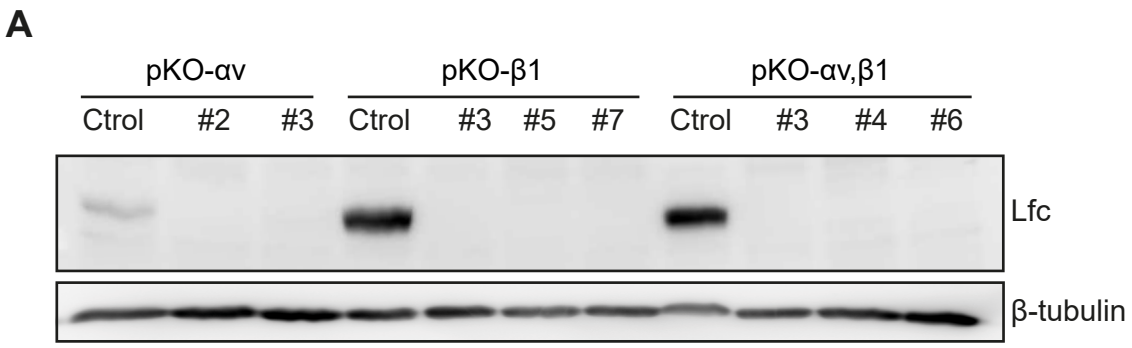
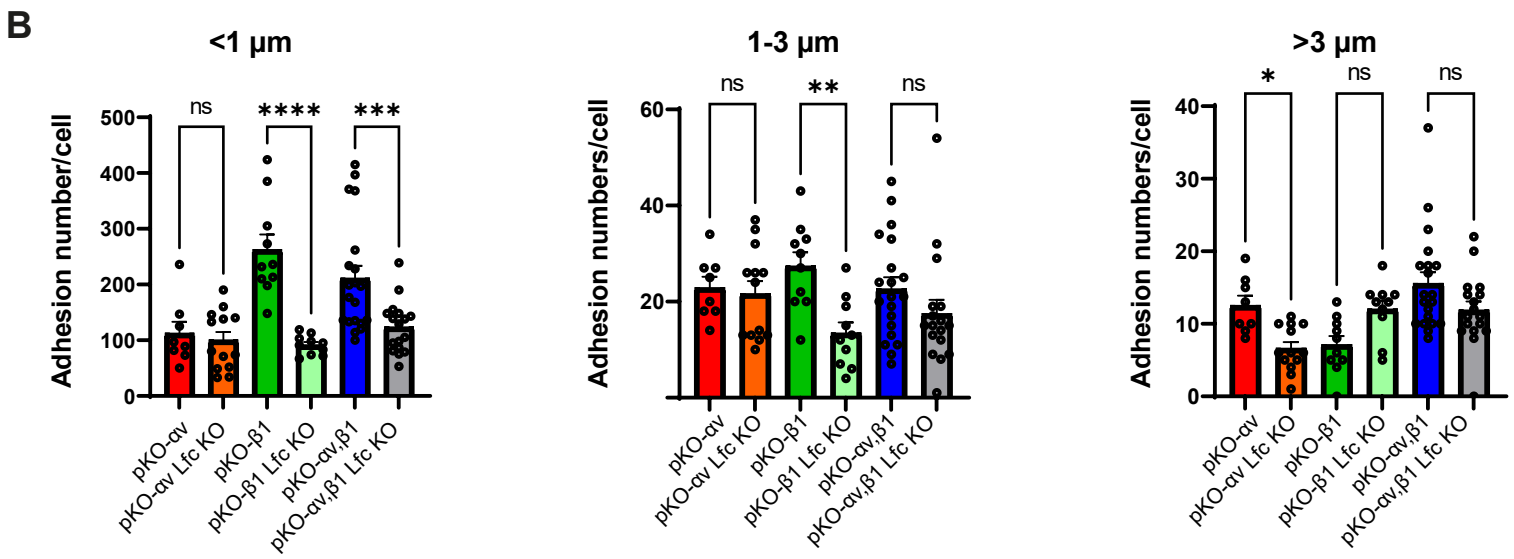


Fig. S1. A) Immunostaining of indicated cell types plated on FN for 4 h in serum-free medium. Images show F-actin. Scale bar, 10 µm. B) Pulled-down proteins by GST-RhoA G17A were subject to hierarchical cluster analysis. PDs were performed with cells plated on FN for 45 min in serum-free medium. Heat map shows the Z-score and is representative of three independent experiments. C) Bar graph showing MS intensity of total levels of eight GEFs in the indicated cell types.

Journal of Cell Science • Supplementary information

Supplementary Figure 2





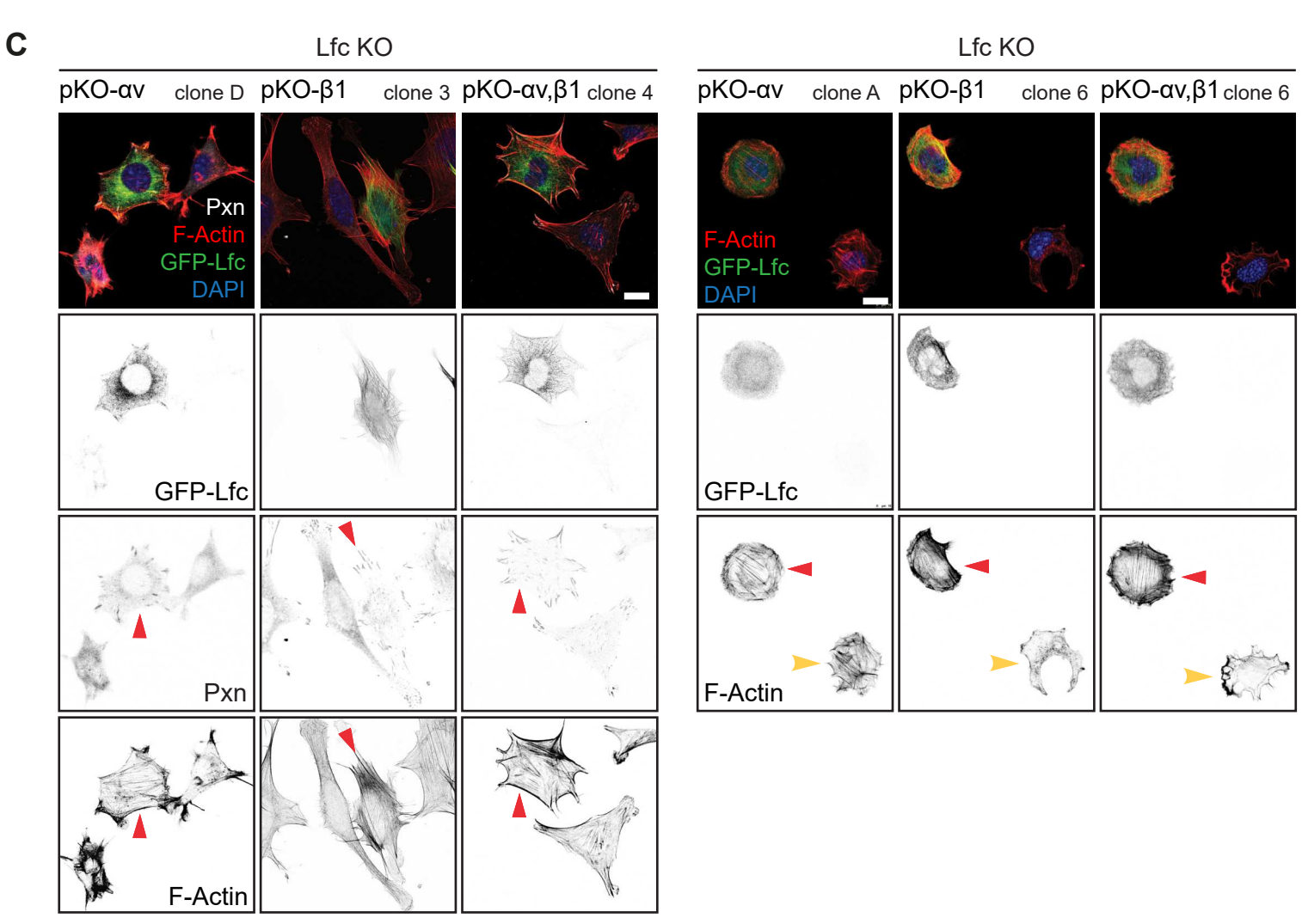


Fig. S2. A) Confirmation of Lfc KO in clones of indicated cells by western blotting. B) Quantification of adhesion number per cell in the indicated conditions. Adhesion size was divided into <1 μm, 1-3 μm and >3 μm. Graphs show the quantification of 8-20 cells per condition. Unpaired t-test between pKO-αv and pKO-αv Lfc KO, pKO-β1 and pKO-β1 Lfc KO, and pKO-αv,β1 and pKO-αv,β1 Lfc KO, *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001. C) Indicated pKO cells overexpressing GFP-Lfc cultured for 45 min on FN-coated glass or on circular FN-coated micropatterns serum-free medium and then visuali ed or stained GFP-Lfc (green), F-actin (red), paxillin (white) and nuclei (DAPI, blue). Scale bar, 10 μm. Red arrows indicate GFP-Lfc expressing cells, and yellow arrows indicate GFP-Lfc non-expressing cells.

Supplementary Figure 3

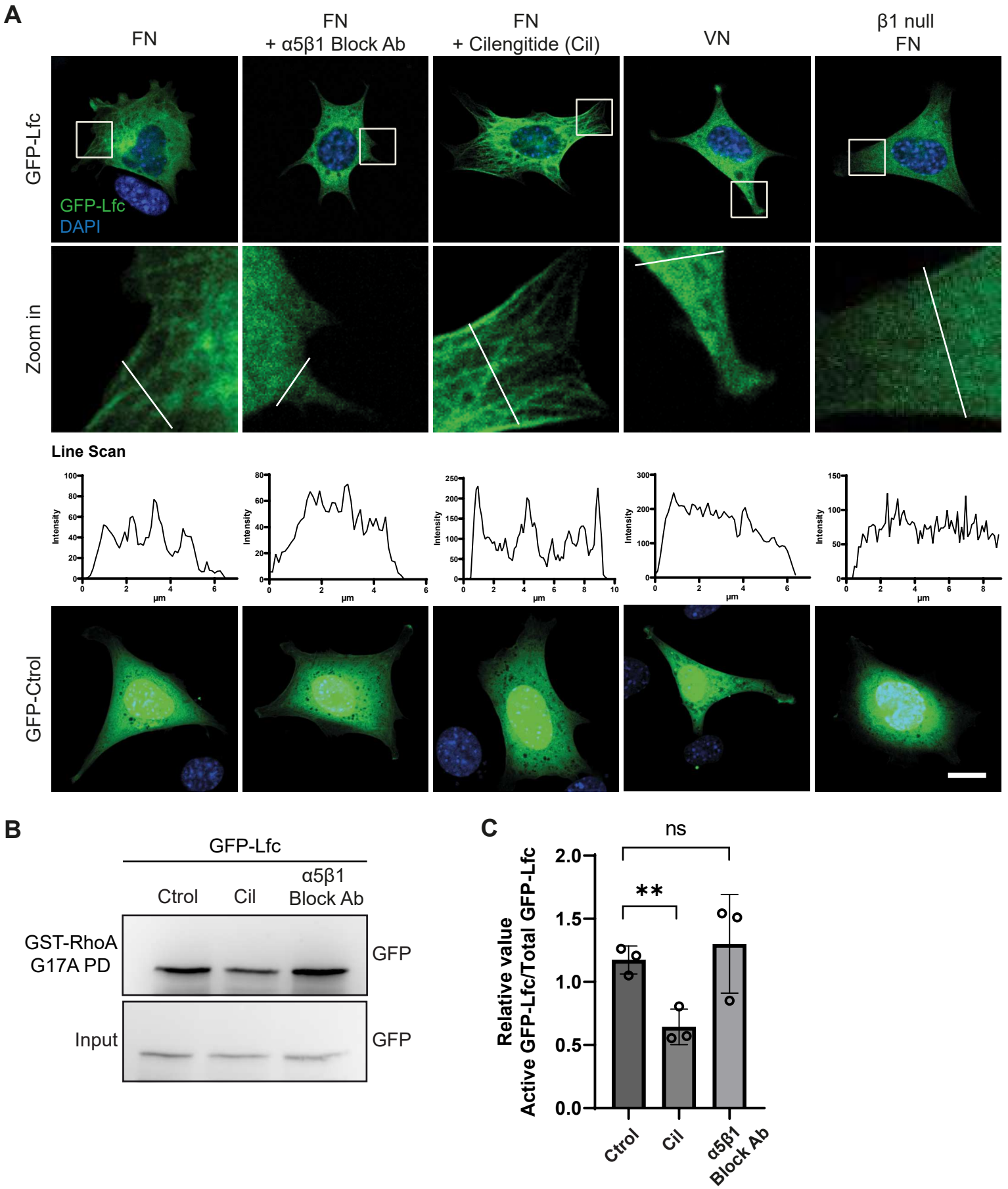


Fig. S3. A) Visualization of GFP-Lfc (green) or GFP alone as a control, in transfected WT mouse fibroblasts seeded on indicated ligand in the presence or absence of indicated compound. β1 integrin-null fibroblasts served as a control. Nuclei were stained with DAPI (blue). Scale bar, 10 μm. The line scan shows the intensity of the GFP-Lfc staining. B) Lfc activity in GFP-Lfc-transfected normal mouse kidney fibroblasts plated on fibronectin for 45 min and treated with either cilengitide (Cil) or α5β1 blocking antibody was shown by western blotting after GST-RhoA G17A PD. C) Graph shows quantification of three independent experiments. Unpaired t-test between Ctrol and Cil, and Ctrol and α5β1 Block Ab, **p<0.01.

Supplementary Figure 4

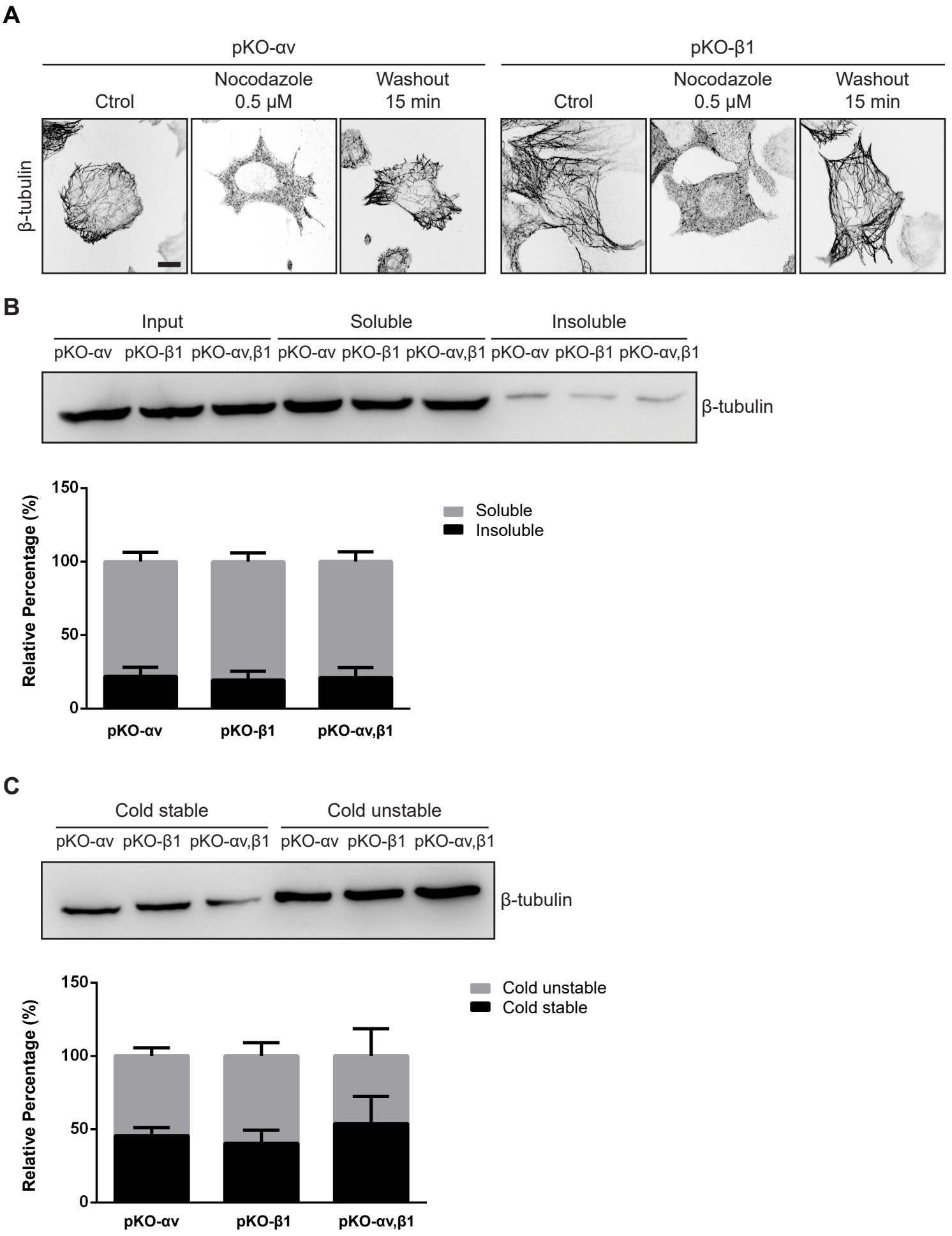


Fig. S4. A) Indicated cell types cultured on FN in serum-free medium and treated either with nocodazole or DMSO (Ctrol) for 2 h followed by 15 min washout and then staining for β-tubulin. Scale bar, 10 µm. B) Microtubule fractionation of indicated cells followed by western blotting. Soluble fraction represents free and insoluble fraction polymerized tubulin. Graph below western blot shows the relative percentage of band densities of each fraction in relation to the sum of densities (density soluble density insoluble 100). uantification of four independent experiments. C) Cold stability assay followed by western blotting. Cold stable indicates tubulin that remained polymeri ed after 30 min incubation on ice. Graph below western blot shows the relative percentage of the band density of each fraction in relation to the sum of the densities (density cold stable density cold unstable 100). uantification of two independent experiments.

Supplementary Figure 5

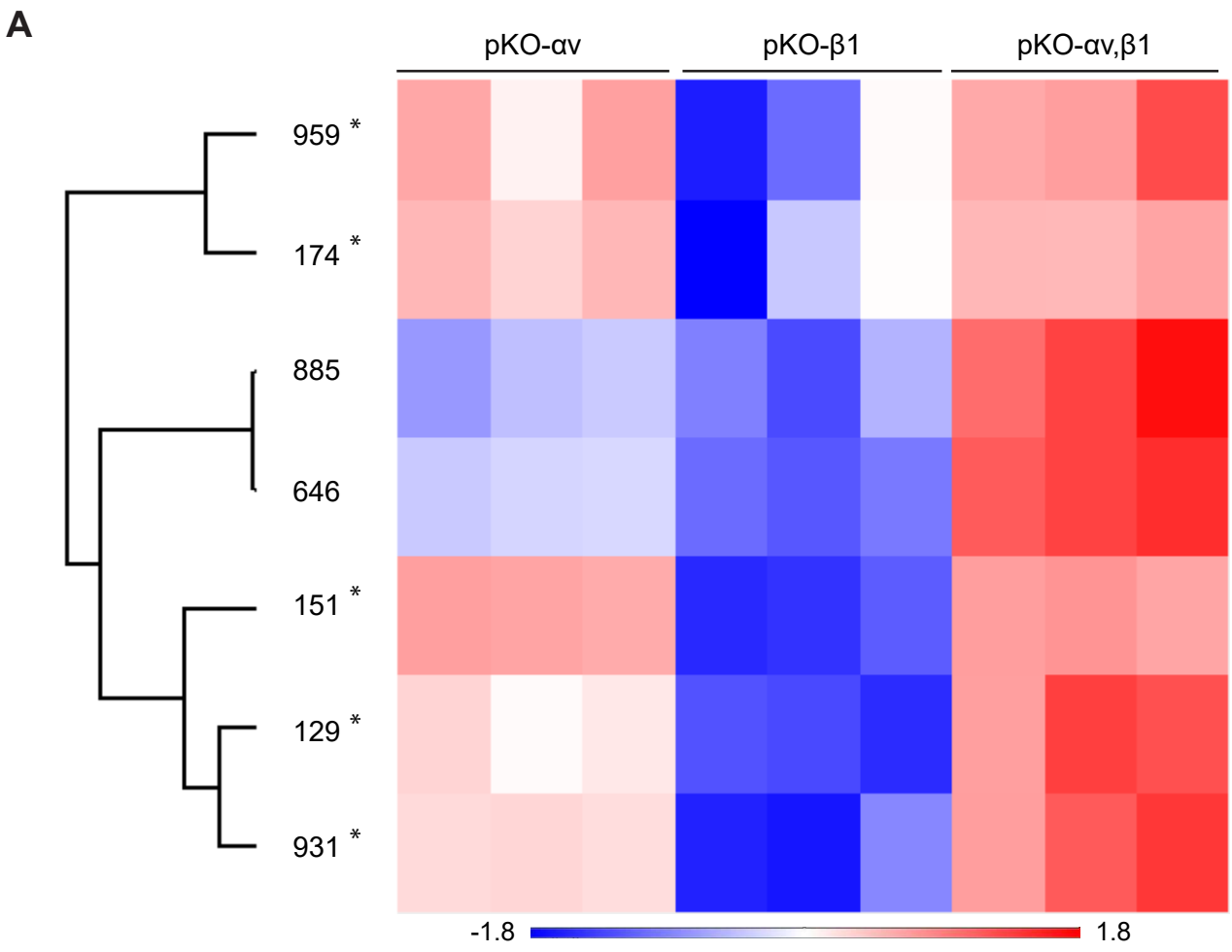
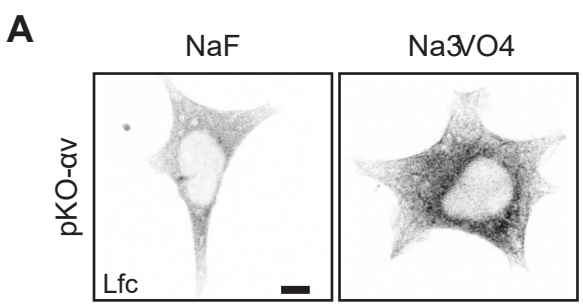
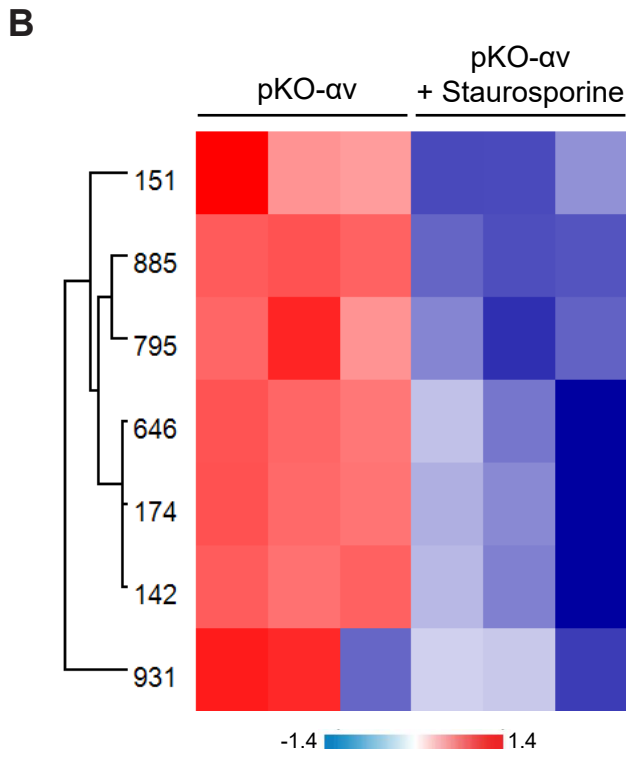
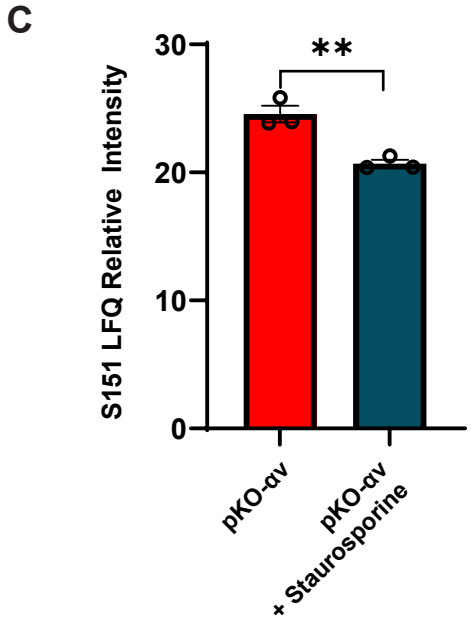


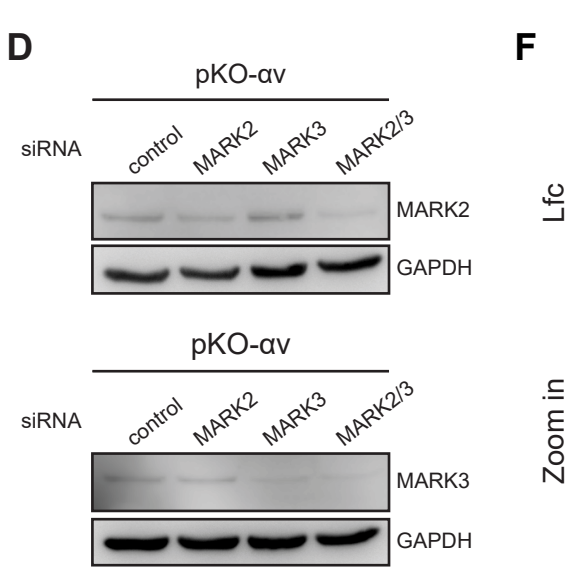
Fig. S5. A) Phospho-enrichment MS analysis of Lfc phosphorylation. Heatmap shows the Z-score of Lfc phosphorylation sites of pKO cells cultured on FN for 45 min in serum-free medium. Phosphorylation sites marked with asterisks are highly phosphorylated in pKO-αv cells when compared to pKO-β1 cells. B) Stably-expressing GFP-Lfc-WT, GFP-Lfc-S151A or GFP-Lfc-S151D in pKO-αv Lfc KO cells showing GFP signals (green). Scale bar, 10 µm. C) Lfc activity of pKO-αv Lfc KO cells overexpressing indicated GFP-Lfc constructs determined by western blotting after GST-RhoA-G17A pull-down. WT, S151A and S151D are highlighted.

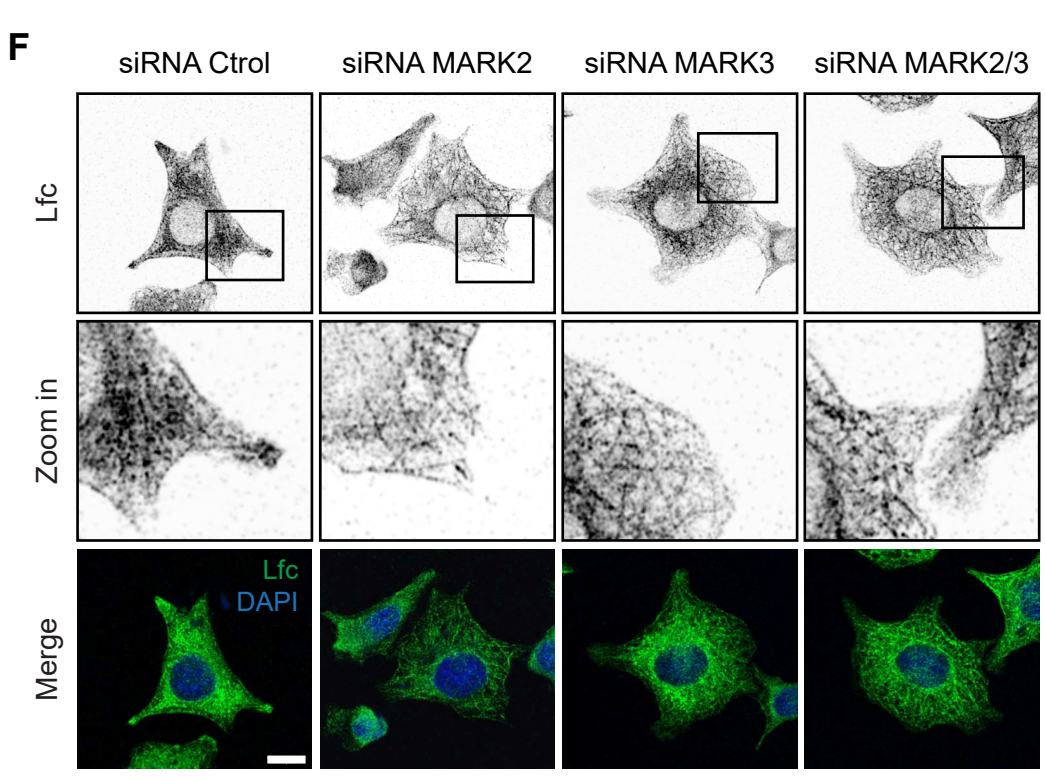
Supplementary Figure 6











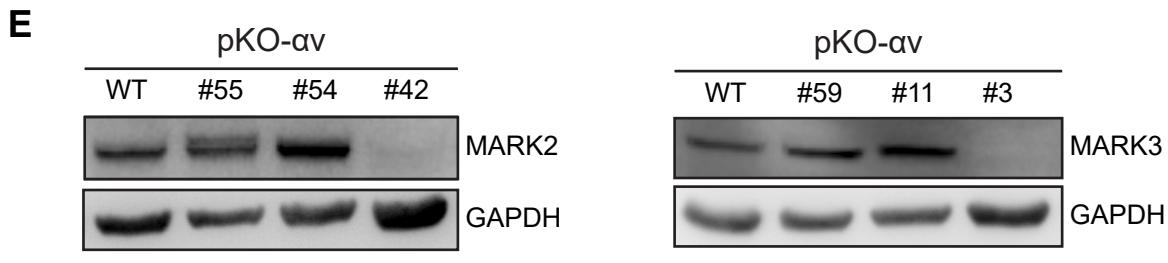
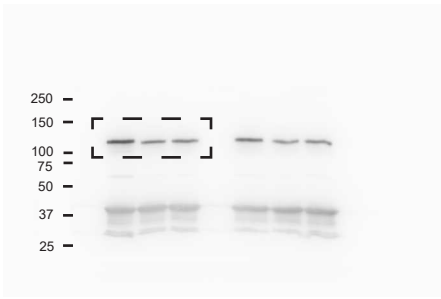


Fig. S6. A) Immunostaining of pKO-αv cells plated on FN in serum-free medium and treated with NaF (1 mM) and Na3VO4 (0,1 mM). Scale bar, 10 µm. B) Phospho-enrichment MS analysis of Lfc phosphorylation in pKO-αv cells with or without 1 nM Staurosporine treatment. Heatmap shows the Z-score of Lfc phosphorylation sites of pKO cells cultured on FN for 45 min in serum-free medium. C) Bar graph showing MS intensity of S151 phospho-site for pKO-αv and pKO-αv with Staurosporine cells from three independent experiments. Unpaired t-test between pKO-αv and pKO-αv with Staurosporine, **p<0.01. D) Confirmation of siRNA-mediated depletion of MARK2 and/or MARK3 by western blotting. Blots are representative examples of two independent experiments. E) Confirmation of Crispr/Cas9-mediated MARK2 and MARK3 KO in pKO-αv cells by western blotting. F) Staining of pKO-αv cells cultured on FN for 45 min in serum-free medium and transfected with indicated siRNA for Lfc (green) and nuclei (DAPI, blue). Scale bar, 10 µm.

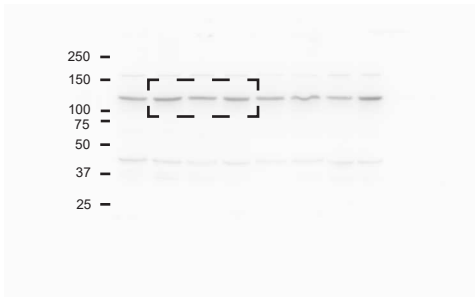
Supplementary Figure 7

Figure 2D

IB: anti GEF-H1/Lfc, RhoA G17A PD



IB: anti GEF-H1/Lfc, RhoA G17A PD input



IB: anti β-tubulin, RhoA G17A PD input

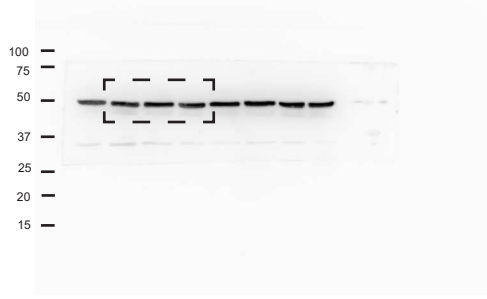
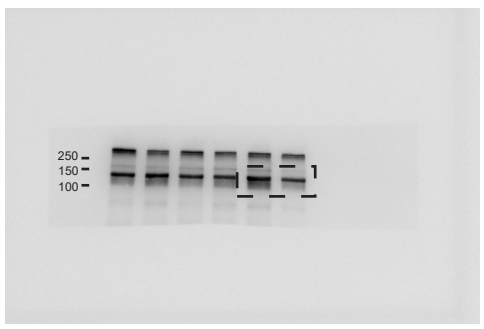


Figure 5C

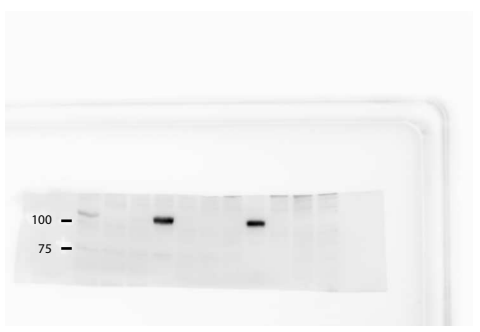
IB: anti GEF-H1/Lfc, RhoA G17A PD



IB: anti GEF-H1/Lfc, RhoA G17A PD input

Supplementary Figure 2A

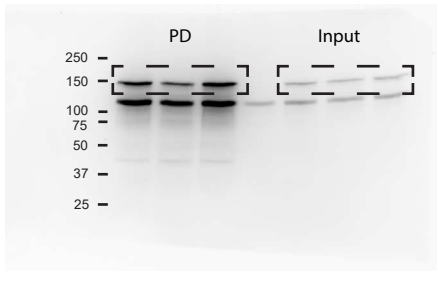
IB: anti GEF-H1/Lfc



IB: anti β-tubulin

Supplementary Figure 3B

IB: anti GEF-H1/Lfc



Supplementary Figure 4B

IB: anti β-tubulin

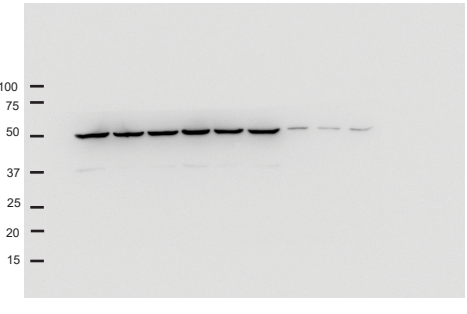
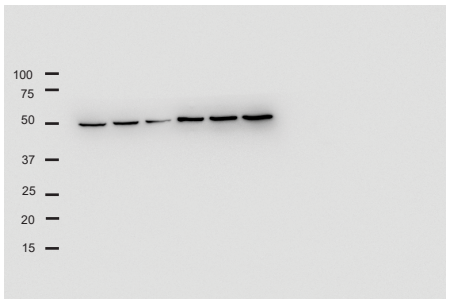


Fig. S7. Uncropped WBs. Dotted rectangles highlight the areas shown in the indicated figures.

Supplementary Figure 8

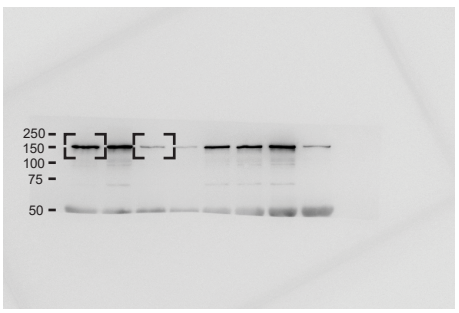
Supplementary Figure 4C

IB: anti β-tubulin

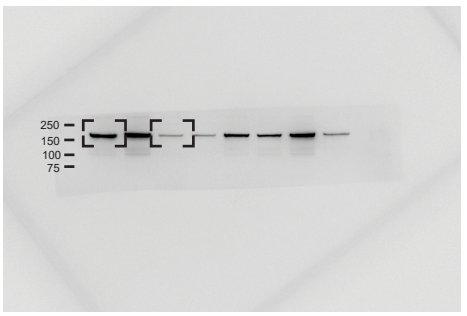


Supplementary Figure 5C

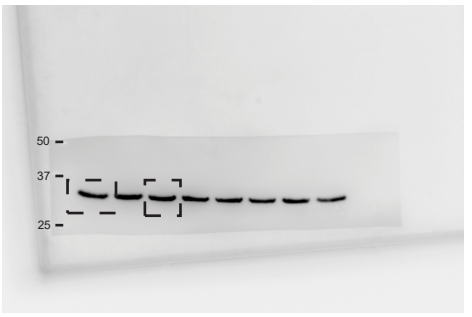
IB: anti GEF-H1/Lfc, RhoA G17A PD, A mutants



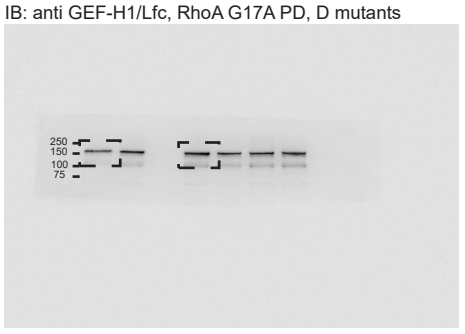
IB: anti GEF-H1/Lfc, RhoA G17A PD input, A mutants



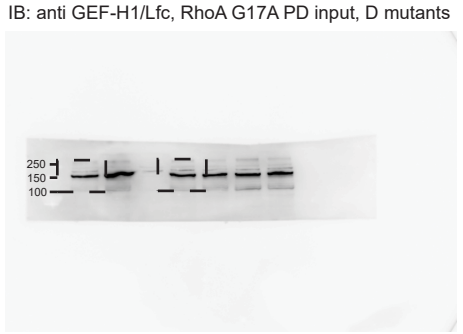
IB: anti GAPDH, RhoA G17A PD input, A mutants



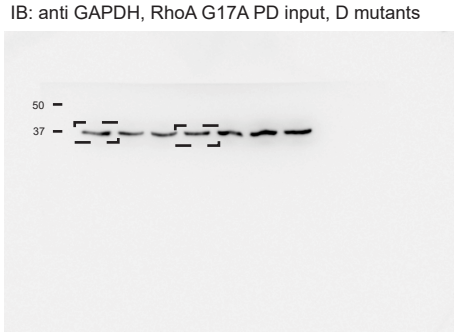
IB: anti GEF-H1/Lfc, RhoA G17A PD, D mutants



IB: anti GEF-H1/Lfc, RhoA G17A PD input, D mutants

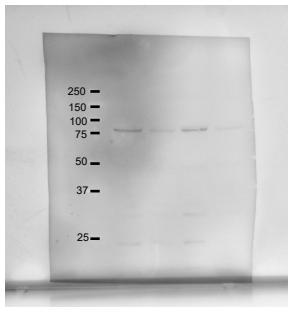


IB: anti GAPDH, RhoA G17A PD input, D mutants

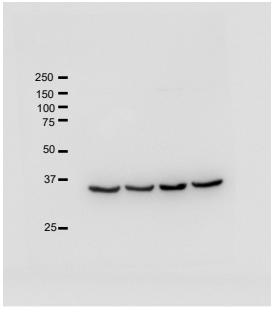


Supplementary Figure 7A

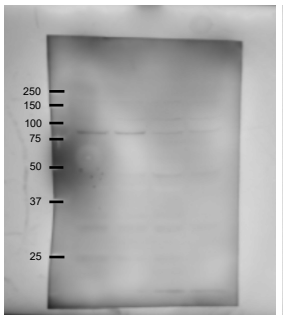
IB: anti MARK2



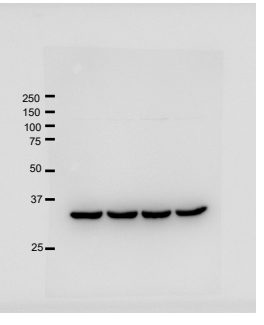
IB: anti GAPDH, MARK2 blot



IB: anti MARK3

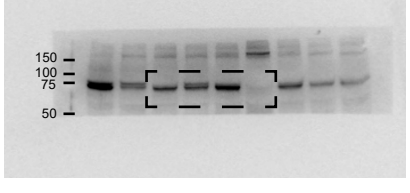


IB: anti GAPDH, MARK3 blot

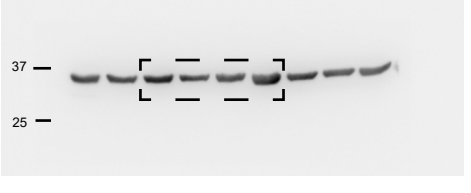


Supplementary Figure 7C

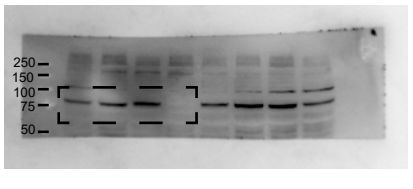
IB: anti MARK 2



IB: anti GAPDH, MARK2 blot



IB: anti MARK 3



IB: anti GAPDH, MARK3 blot

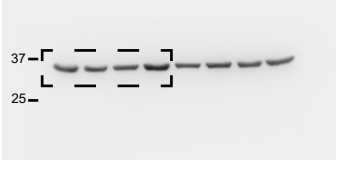
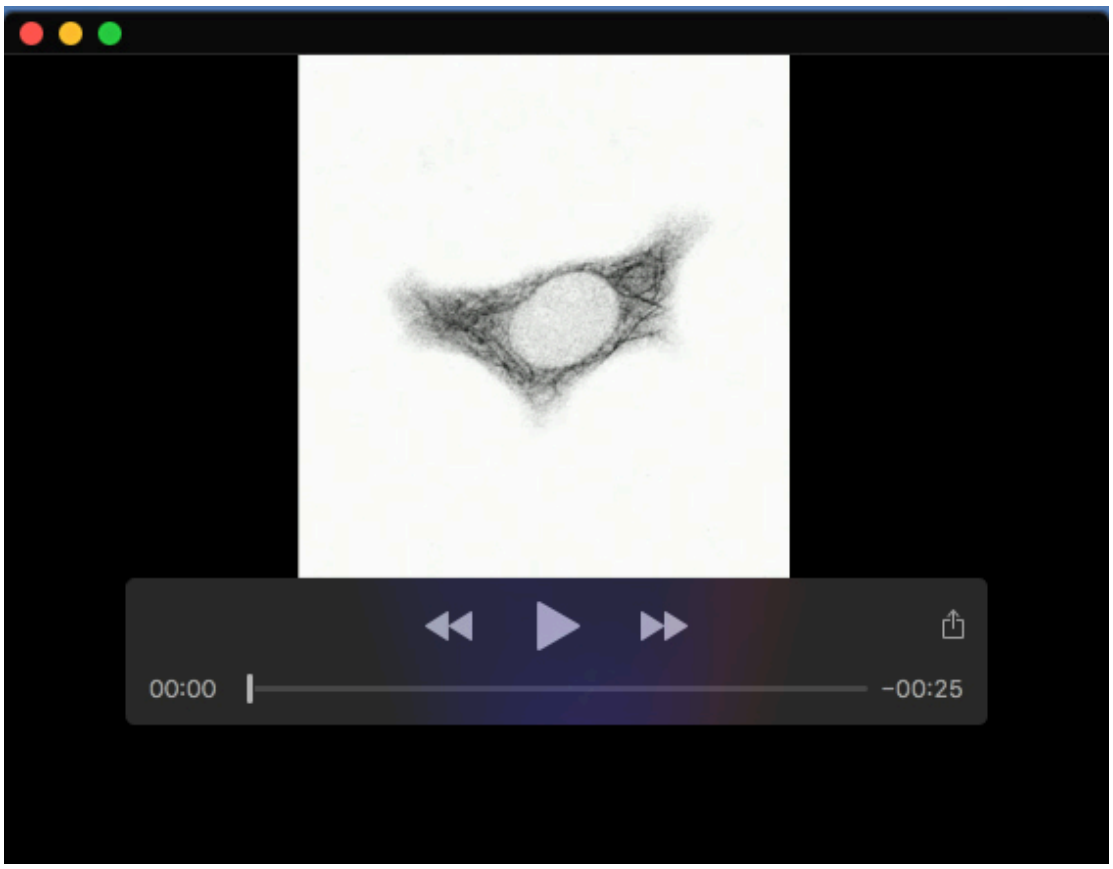


Fig. S8. Uncropped WBs. Dotted rectangles highlight the areas shown in the indicated figures.



Movie 1. Timelapse imaging of pKO-αv cells overexpressing GFP-Lfc on FN-coated glass without serum. Recording started immediately after cells were treated under the microscope with 1nM of Staurosporine. The recording time interval was 10sec/frame.

Journal of Cell Science • Supplementary information