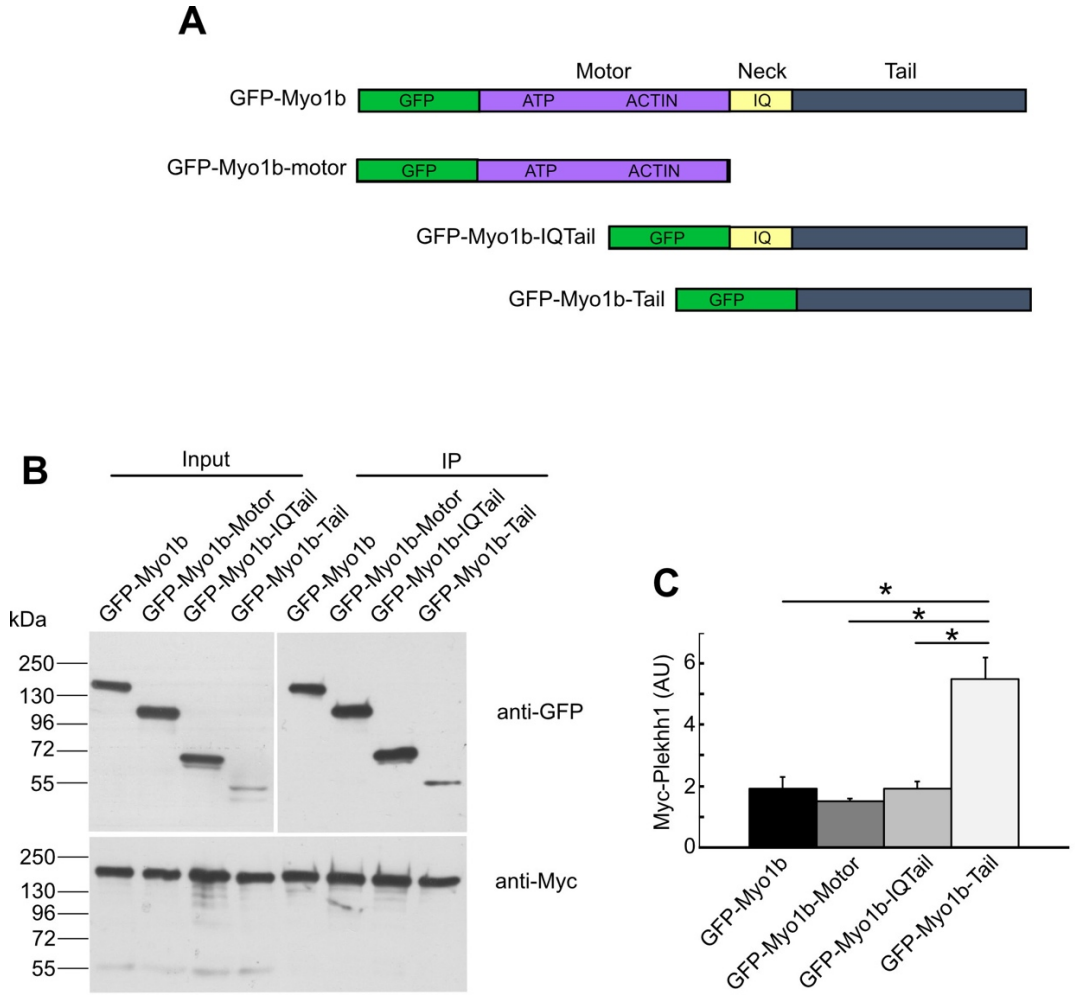
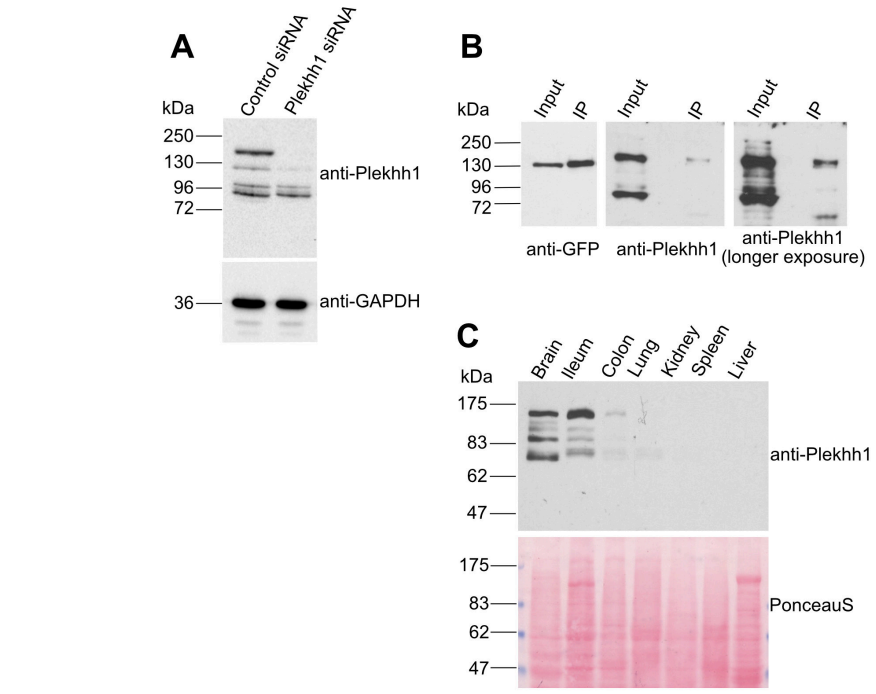
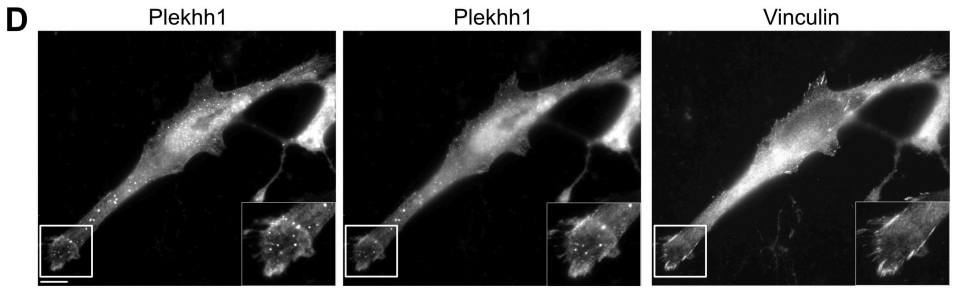


Fig. S1. (related to Figure 1): Interaction of Myo1b domains with Plekhh1

A) Schematic representation of Myo1b, its domains and the constructs used in B and C. B) Myc-Plekhh1 pulled down with GFP-Myo1b, GFP-Myo1b-motor, GFP-Myo1b-IQTail or GFP-Myo1b-Tail (IP) from Hek293T cell lysates expressing these constructs (Input) were analyzed by SDS-PAGE and immunoblotted with anti-GFP or anti-Myc antibodies. C) The amount of Myc-Plekhh1 pulled down with GFP-Myo1b, GFP-Myo1b-motor, GFP-Myo1b-IQTail or GFP-Myo1b-Tail was quantified and normalized to the amount of the GFP Myo1b domains immunoprecipitated. Data are shown as an average of 3 experiments. Error bars represent +/- SEM. *P=0.0175 for GFP-Myo1b and GFP-Myo1b-Tail. *P=0.0255 for GFP-Myo1b-motor and GFP-Myo1b-Tail; *P=0.0222 for GFP-Myo1b-IQTail and GFP-Myo1b-Tail.

Figure S2





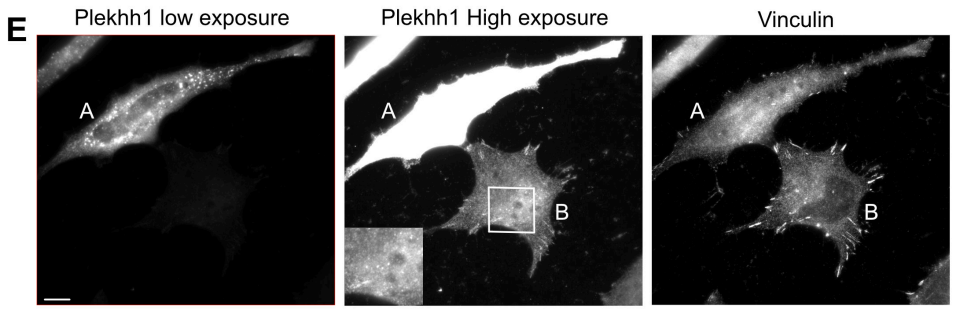


Fig. S2. related to Figure 2: Characterization of Plekhh1 antibodies and tissue expression of this protein

YFP-EphB2-HCT116 cells transfected with Control or Plekhh1 siRNAs were analyzed by SDS-PAGE and immunoblotted using the home made polyclonal anti-Plekhh1 antibodies and anti-GAPDH antibody for loading control. B) Endogenous Plekhh1 pulled down (IP) with GFP-Myo1b from Hek293T cells expressing GFP-Myo1b (Input) analyzed by SDS-PAGE and immunoblotted with anti-GFP and anti-Plekhh1 antibodies. C) Mouse brain, ileum mucosa, colon, lung, kidney, spleen and liver lysates were analyzed by SDS-PAGE and immunoblot with anti-Plekhh1 antibodies or labelled with Ponceau S for loading control. D-E) Hela cells were transfected with a plasmid encoding untagged Plekhh1, immunolabelled with anti-vinculin and anti-Plekhh1 antibodies and analyzed by epifluorescence microscopy. Left image in D corresponds to a nuclear focal plane and the two right images correspond to a focal plane throughout FA. Note that Plekhh1 is associated with plasma membrane (left image in D), co-distributes with vinculin (two images on the right in D and cell B in E) and forms aggregates in the perinuclear region (left and middle image in D and cell A in E) even at low level of expression (cell B in E). White boxes mark the regions enlarged by1.8 and shown in the inserts. Bars, 10µm

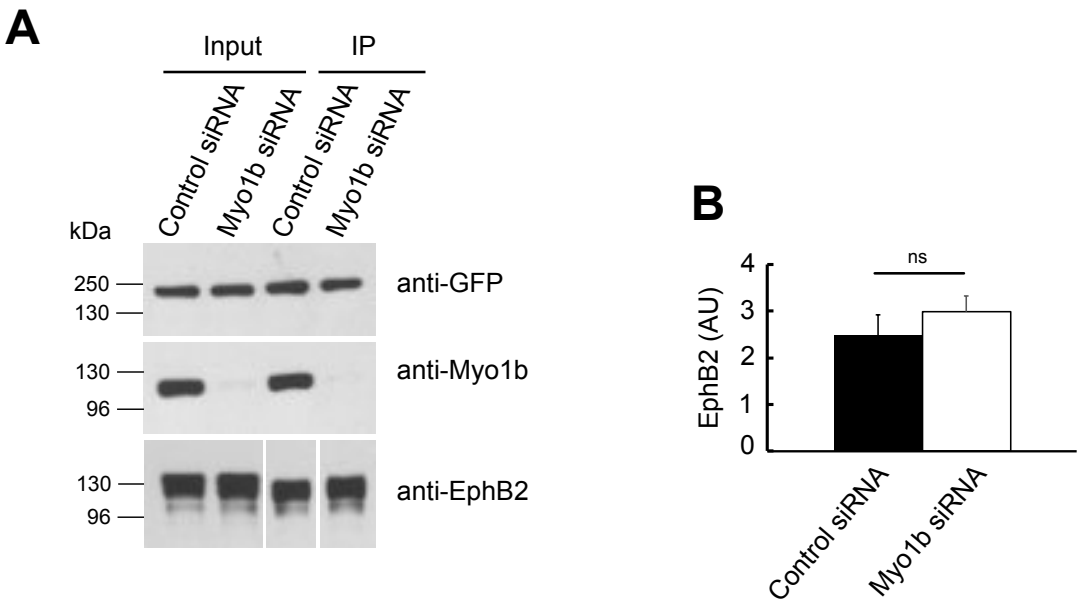


Fig. S3. (related to Figure 4): Interaction of EphB2 with Plekhh1 is independent of Myo1b expression.

Flag-EphB2 pulled down (IP) with GFP-Plekhh1 from Hek293T cell lysates expressing these two recombinant proteins and treated with Control or Myo1b siRNAs (Input) was analyzed by SDS-PAGE and immunoblot with anti-GFP, anti-Myo1b and anti-EphB2 antibodies. The amount of EphB2 pulled down with Plekhh1 was quantified and normalized to the amount of GFP-Plekhh1 immunoprecipitated. Data are shown as an average of 2 experiments. Error bars represent +/- SEM. No significant difference between the two different experimental conditions P=0.4777.

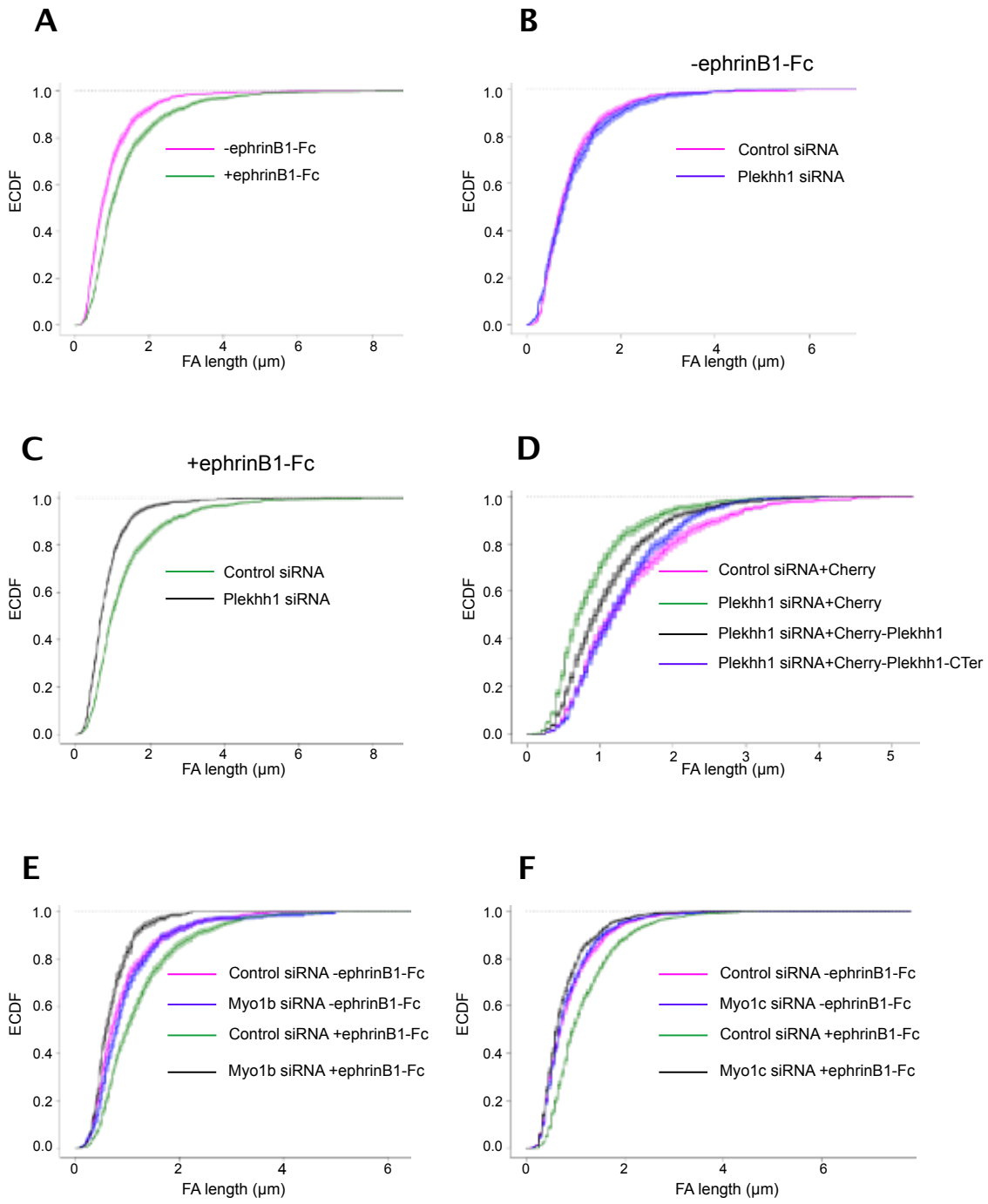


Fig. S4. (related to Figure 7): Distribution of the length of the FA

The distribution of the length of FA has been analyzed for each cell using the Empirical Cumulative Density Functions (ECDFs) (see material and methods). This has been done for all the cells analyzed in Figure 7B, 7C, 7D, 7E, 7G and 7F. A) FA length in cells stimulated or not with ephrinB1-Fc, P= 0.001. B) FA length in non-stimulated cells treated with Control or Plekhh1 siRNAs. The difference is not significative, P= 0.477. C) FA length in stimulated cells treated with Control or Plekhh1 siRNAs, P = 0.001. D) FA length in stimulated cells transfected with Control or Plekhh1 siRNAs and plasmids encoding Cherry, Cherry-Plekhh1 or Cherry-Plekhh1-CTer, P = 0.001 for Control siRNA +Cherry and Plekhh1 siRNA +Cherry, P = 0.001

for Control siRNA +Cherry and Plekhh1 siRNA +Cherry-Plekhh1, P=0.203 for Control siRNA +Cherry and Plekhh1 siRNA +Cherry-Plekhh1-CTer. E) FA length in non-stimulated and stimulated cells transfected with Control or Myo1b siRNA, P=0.001 for Control siRNA-ephrinB1 and Control siRNA+ephrinB1-Fc, P=0.001 for Control siRNA +ephrinB1-Fc and Myo1b siRNA+ephrinB1-Fc F) FA length in non-stimulated and stimulated cells transfected with Control or Myo1c siRNA, P=0.002 for Control siRNA-ephrinB1-Fc and Control siRNA+ephrinB1-Fc, P=0.002 for Control siRNA +ephrinB1-Fc and Myo1c siRNA+ephrinB1-Fc

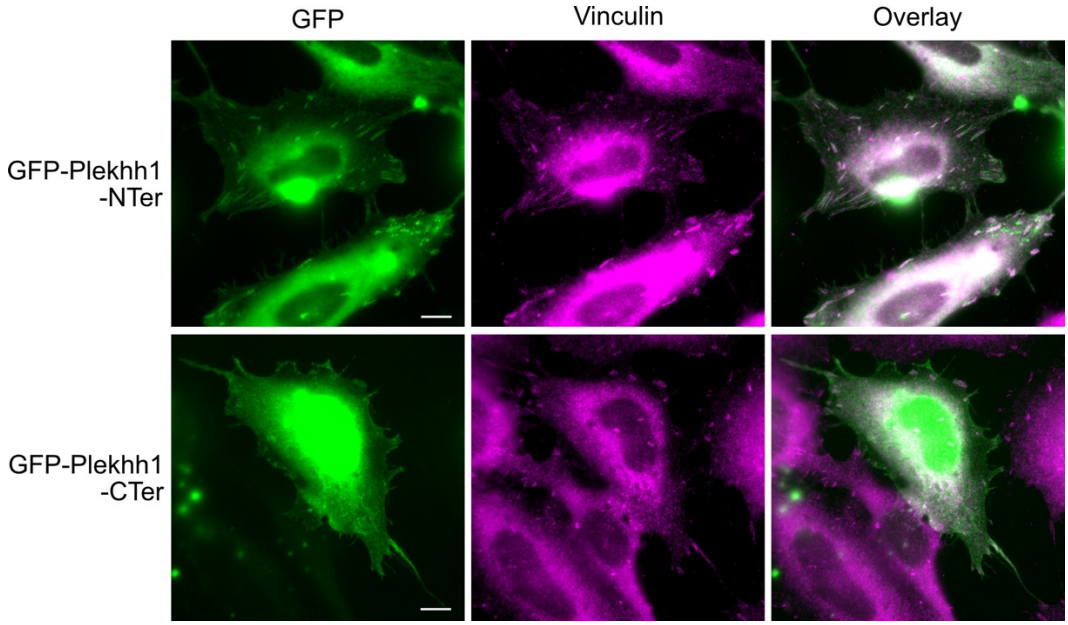
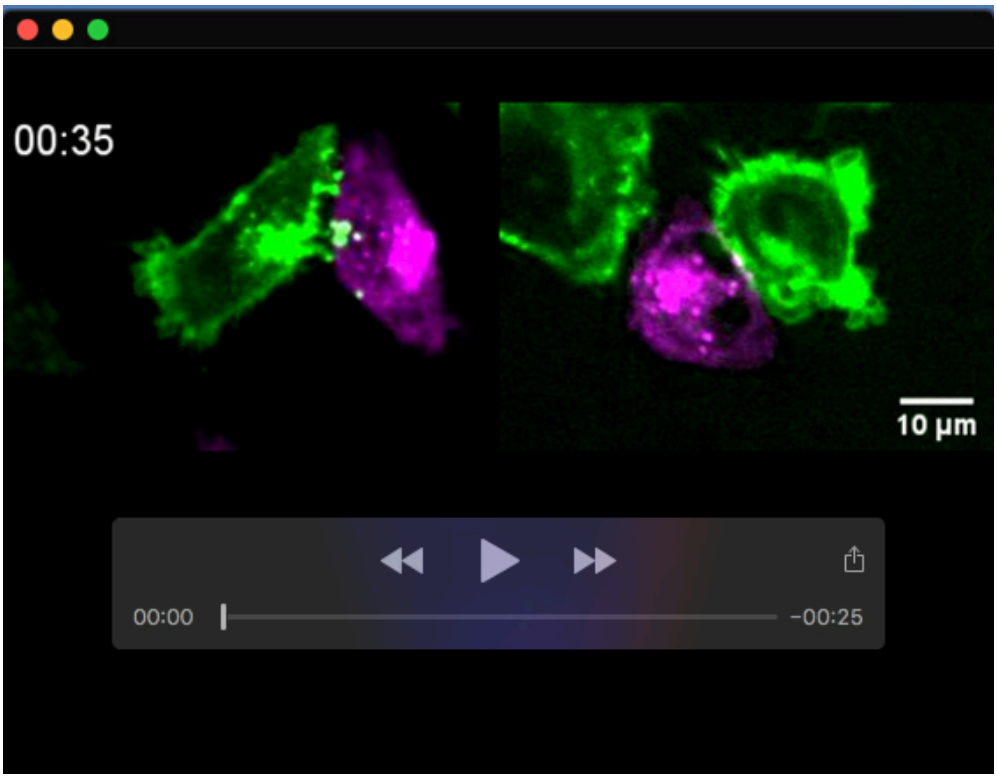
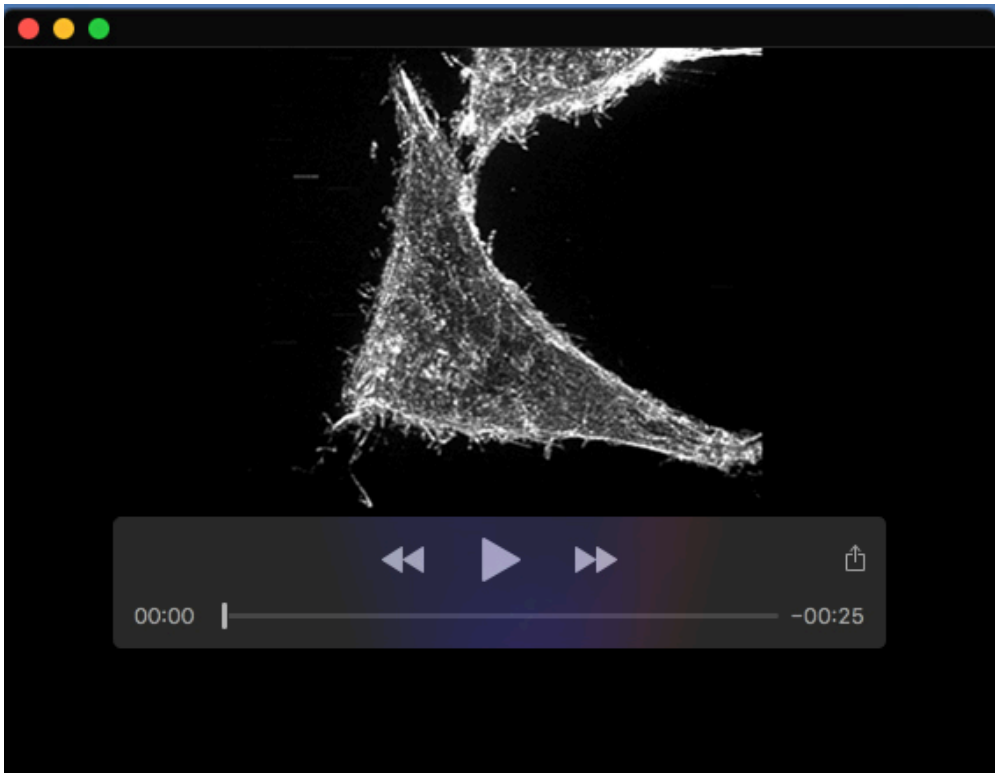


Fig. S5. (related to Figure 7): Plekhh1-NTer is in part associated with FA in contrast to Plekhh1-CTer.

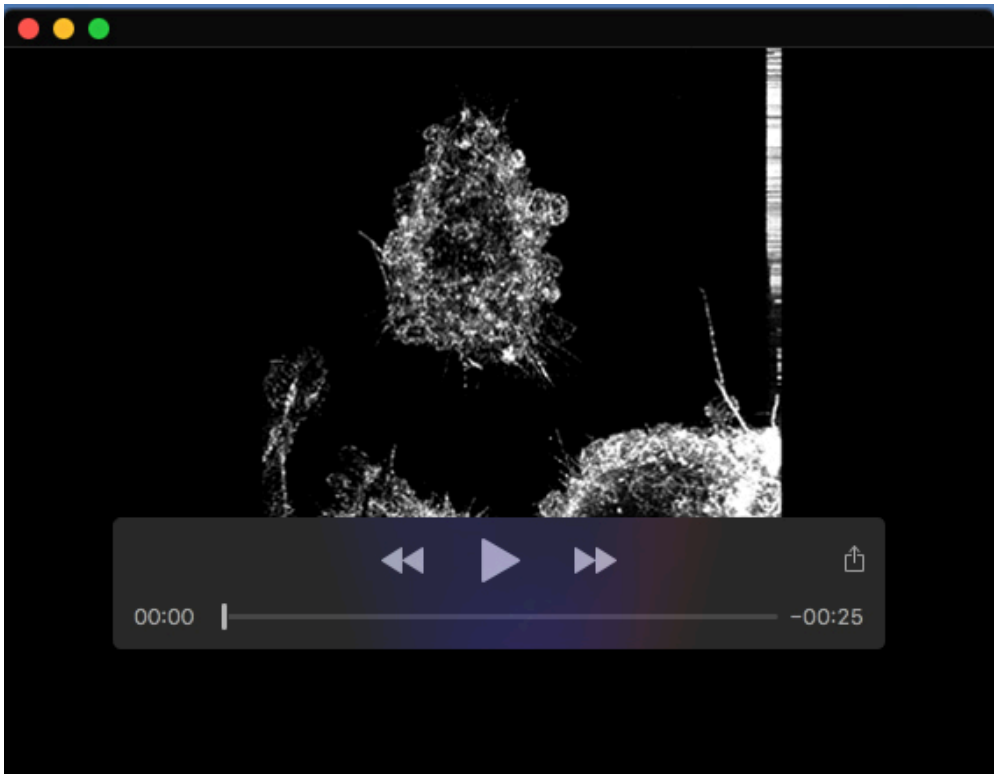
Cellular distribution of GFP-Plekhh1-NTer and GFP-Plekhh1-CTer compared to that of vinculin analyzed by epifluorescence microscopy. Bar, 10µm. Note that GFP-Plekhh1-NTer but not GFP-Plekhh1-CTer co-distributes in part with vinculin (white in overlay).



Movie 1. (related to figure 3) shows YFP-EphB2-HCT116 (green) cell behavior after transfection with Control or Plekhh1 siRNAs and in contact with Cherry-EphrinB1- HCT116 cells (magenta). Images were acquired at 1 frame/5min during 3h. Bar 10µm



Movie 2. (related to figure 5B) shows 3 D reconstruction of the stacks collected with confocal microscopy with Super-resolution module of stimulated YFP-EphB2-HCT116 cells transfected with Control siRNA and labelled with fluorescent phalloidin.



Movie 3. (related to figure 5B) shows 3 D reconstruction of the stacks collected with confocal microscopy with Super-resolution module of stimulated YFP-EphB2-HCT116 cells transfected with Plekhh1 siRNAs and labelled with fluorescent phalloidin. Note the disorganization of the actin filaments underneath the plasma membrane.