



Fig. S3. Quantification of different localization of the β_3 -GFP-IPP chimeras with respect to the internal control β_3 -RFP. Example of the procedure for a 3T3/ β_3 -RFP cell co-expressing β_3 -GFP-PINCH. The arithmetic functions of Metamorph are used to build a 16 bit average image and a 16 bit ratio image (obtained by dividing the GFP signal multiplied by 1000 with the RFP signal). Regions to analyze (adhesions positives for RFP and/or GFP signals) are selected in the average image using the threshold function (a) and excluding manually the region corresponding to the highly fluorescent perinuclear area. The selected regions are then applied to the ratio image (b), in which light and dark grey areas correspond respectively to high and low ratio GFP/RFP. Intensity standard deviation (I.S.D.) of the whole adhesion area is read after transferring (with a script) the image within the regions of interest to an image with a black background (c) in which the area surrounding the adhesions could be excluded from the analysis upon thresholding (in light blue) to intensity >1 . (d) The final output is shown after applying the same procedure to a 3T3/ β_3 -RFP cell co-expressing the β_3 -GFP control, in which the uniform ratio GFP/RFP overall the adhesions generates a uniform gray color and low I.S.D. (original GFP and RFP images are shown below). Bar = 20 μm . The maximum possible error of the system (system background noise I.S.D.) was calculated as sum of GFP and RFP background noise I.S.D.s, obtained by analyzing sets of two sequential images in the same channel (respectively GFP or RFP).