FIGURE S1

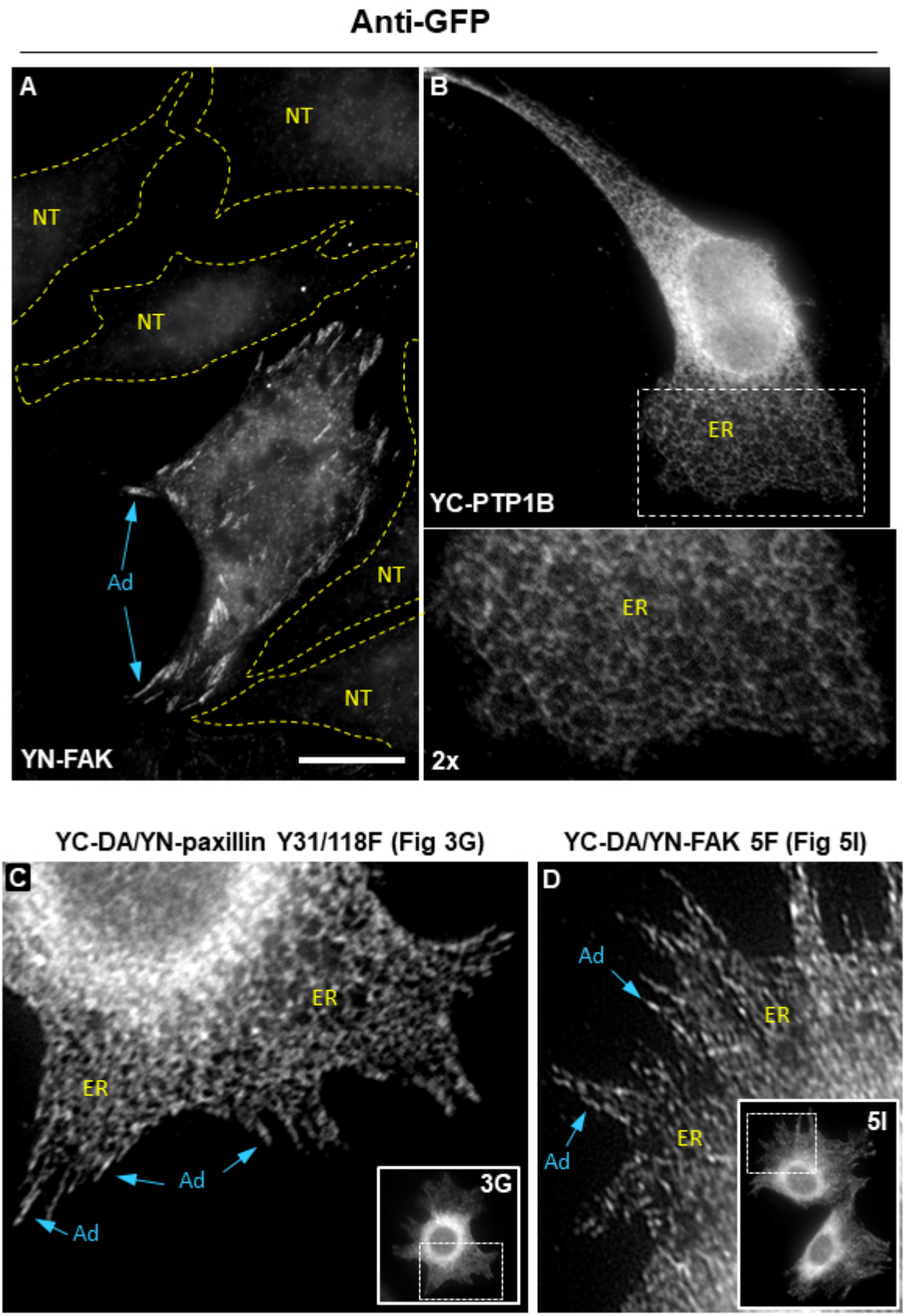

Fig. S1. Recognition of BiFC constructs with anti-GFP.

PTP1B WT cells transfected with YN-FAK (A) or YC-PTP1B (B) and stained with the polyclonal anti-GFP. Note that the signal distribution agrees with the expected localization of each construct, YN-FAK in adhesions (Ad, arrows in A) and YC-PTP1B in the ER (ER label and inset in B). Note the dim and diffuse background signal in non transfected cells (delimited by the yellow dashed lines and labeled NT). In cells co-transfected with BiFC pairs targeted to the ER and adhesion compartments, their co-expression can be recognized by the different signal distribution (C and D). The panels C and D are 4x magnifications of cells shown in Fig. 3G and Fig. 5I (added at bottom) and correspond to the BiFC analysis of PTP1B DA with paxillin Y31/118F (Fig. 3G) and YN-FAK 5F (Fig. 5I), respectively. In these mutants the BiFC signal is negative, but the anti-GFP labeling confirms the co-expression of both BiFC constructs, as revealed by the simultaneous distribution of adhesions (Ad) and ER in panels C and D. Scale bar, 20 µm.

FIGURE S2

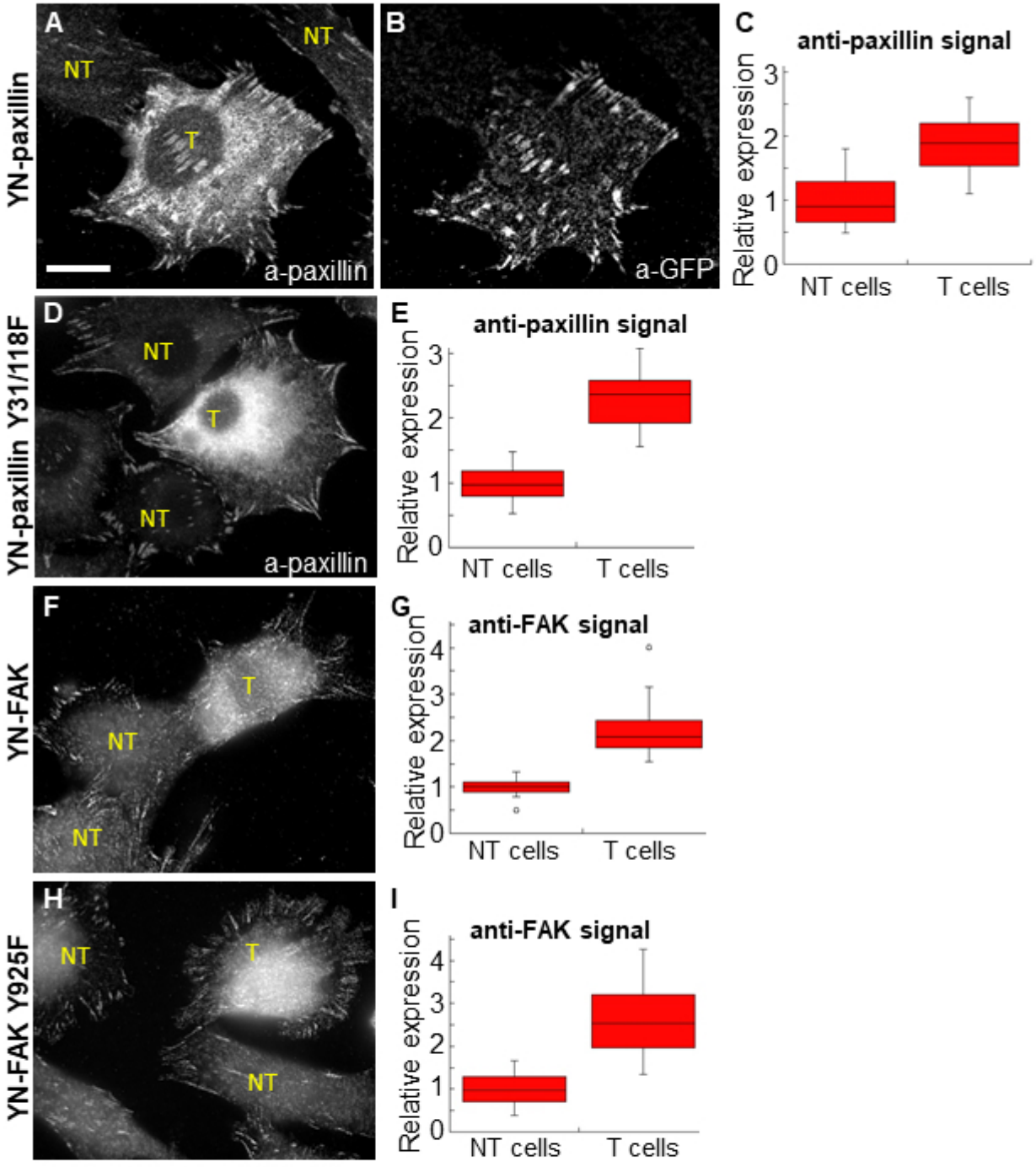

Fig. S2. Distribution and expression levels of BiFC constructs. PTP1B WT cells transfected with YN-paxillin (A-C), YN-paxillin Y31/118F (D,E), YN-FAK (F,G), and YN-FAK Y925F (H,I) were stained with anti-paxillin (A,C-E), anti-GFP (B), and anti-FAK (F-I), and analyzed by wide-field fluorescence microscopy. Transfected (T) and non-transfected (NT) cells were indicated. Box plots show the quantification of mean intensity in non-transfected (endogenous levels) and transfected (endogenous plus exogenous levels) cells. Values were normalized to the mean of NT cells. Boxes enclose 50% of the data with the median value displayed as a line. The top and bottom of the box mark the limits of the lower and upper quartiles. The lines extending from the top and bottom of each box mark the minimum and maximum values in the data set within 1.5*IQD (Inter Quartile Distance) from the lower and upper quartiles, respectively. YN-paxillin, n=27; YN-paxillin Y31/118F, n=26; YN-FAK, n=21; YN-FAK Y925F, n=21. Note that the signal average in transfected cells doubles that in non-transfected cells. Scale bar, 20 μm.

FIGURE S3

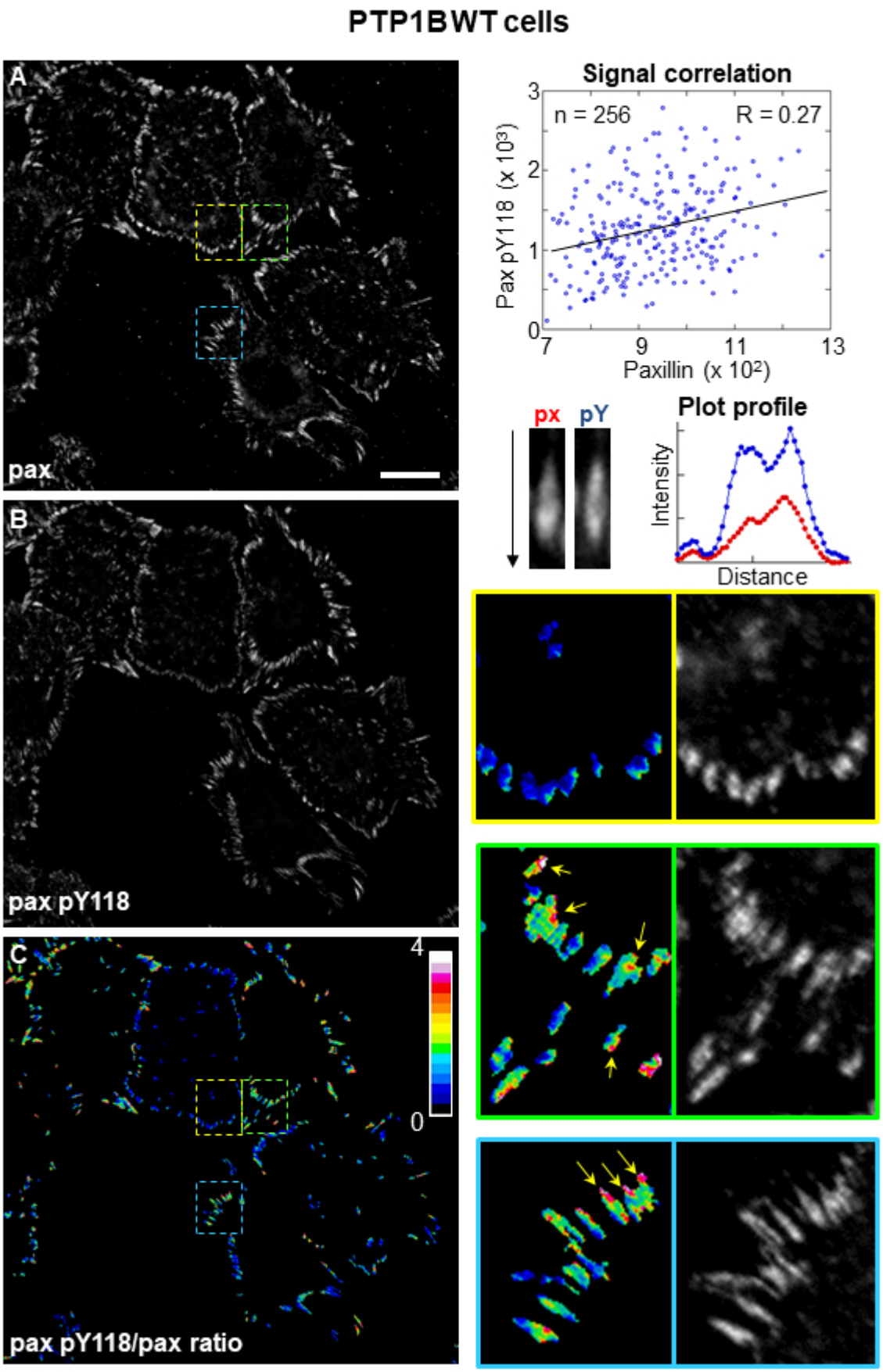

Fig. S3. Phosphorylation level of paxillin in adhesions of PTP1B WT cells. Cells plated on fibronectin were fixed and double stained with a monoclonal anti-total paxillin (pax, A) and a polyclonal anti-phospho-paxillin (pax pY118, B) followed by fluorescent secondary antibodies. Cells were observed by wide-field fluorescence microscopy. Images were processed as indicated in Materials and Methods. The distribution of phosphorylated to total paxillin was assessed by ratio imaging (C). Enlargements of peripheral regions were shown for three different cells (dashed boxes in A,C). Arrows point hotspots of phospho-paxillin within adhesions. The graph displays the average signal of pax pY118 and pax in 256 adhesions of five cells (blue dots) in the field. The Pearson's correlation coefficient R reveals low correlation between both signals. The pax pY118 signal shows high variability (range min/max 115-2792) compared to the pax signal (range min/max 709-1281). The variation of each signal within a single adhesion is shown in the plot profile. The arrow indicates the origin and end of the linescan. Scale bar, 20 µm.

FIGURE S4

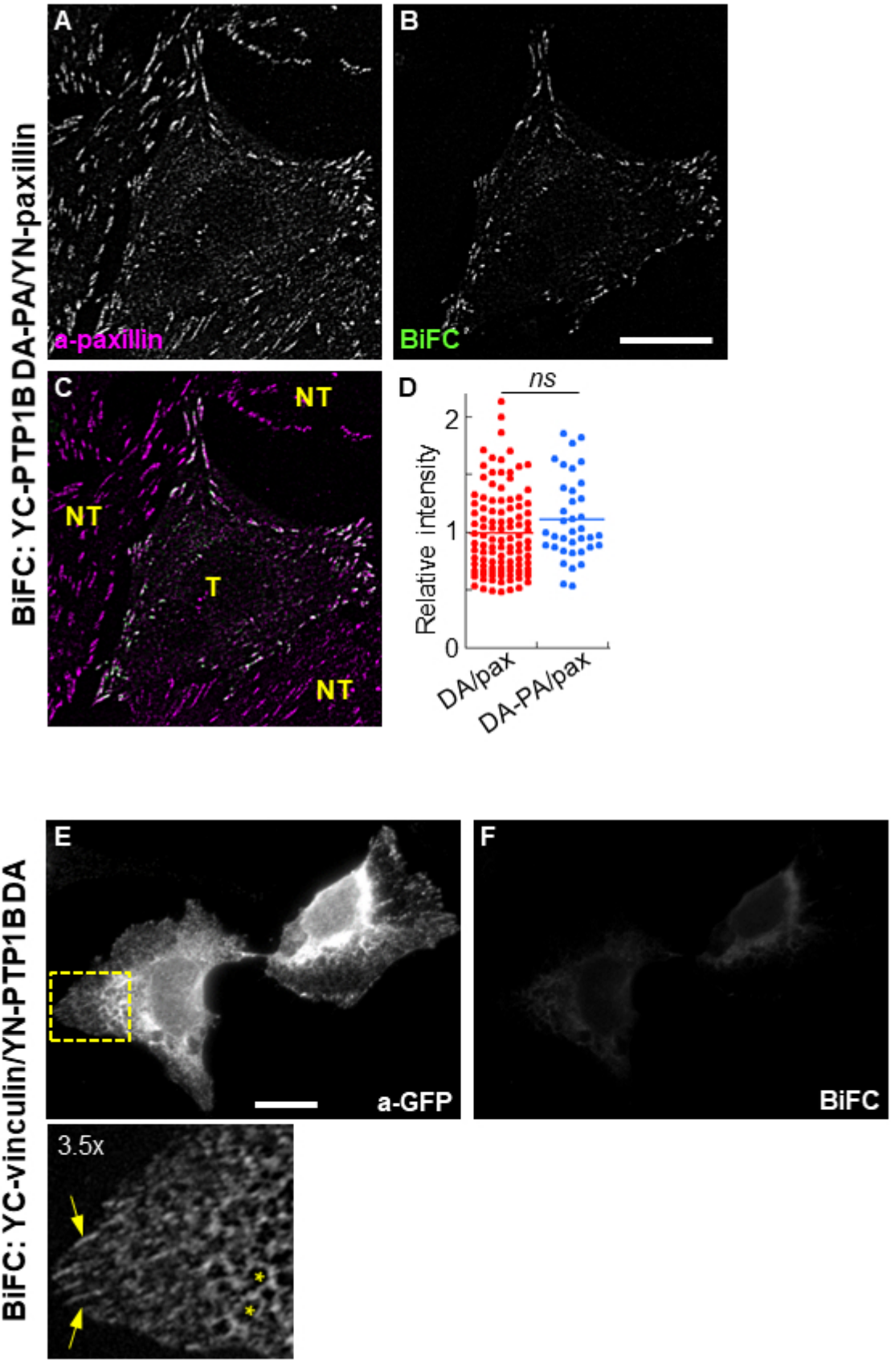

Fig. S4. BiFC analysis of YN-paxillin/YC-PTP1B DA-PA and YC-vinculin/YN-PTP1B DA pairs. PTP1B WT cells were co-transfected with the YN-paxillin/YC-PTP1B DA-PA BiFC pair (A-D) and analyzed by confocal microscopy. Representative images labeled with anti-paxillin (A), displaying BiFC (B), and the merge image (C). T and NT indicate transfected and non-transfected cells, respectively. Quantification of the relative intensity of BiFC to GFP signal (not shown) in peripheral cell-matrix adhesions (D). Ratios were normalized to the mean of DA/pax ratio. DA/pax, n=109; DA-PA/pax, n=37. The mean value is displayed as a line. DA/pax was compared to DA-PA/pax using the Mann-Whitney non-parametric test. *ns* (not significant). PTP1B WT cells were co-transfected with the YC-vinculin/YN-PTP1B DA BiFC pair (E,F) and analyzed by wide field microscopy. Two representative co-transfected cells were stained with anti-GFP and visualized in the red channel (E). They show the combined distribution of both BiFC constructs, YN-PTP1B DA in the ER, and YC-vinculin in peripheral adhesions (yellow asterisks and yellow arrows in the 4.5x magnification, respectively). (F) The same field shown in (E) but visualized in the BiFC channel. Note the lack of BiFC signal. Scale bar, 20 µm.

FIGURE S5

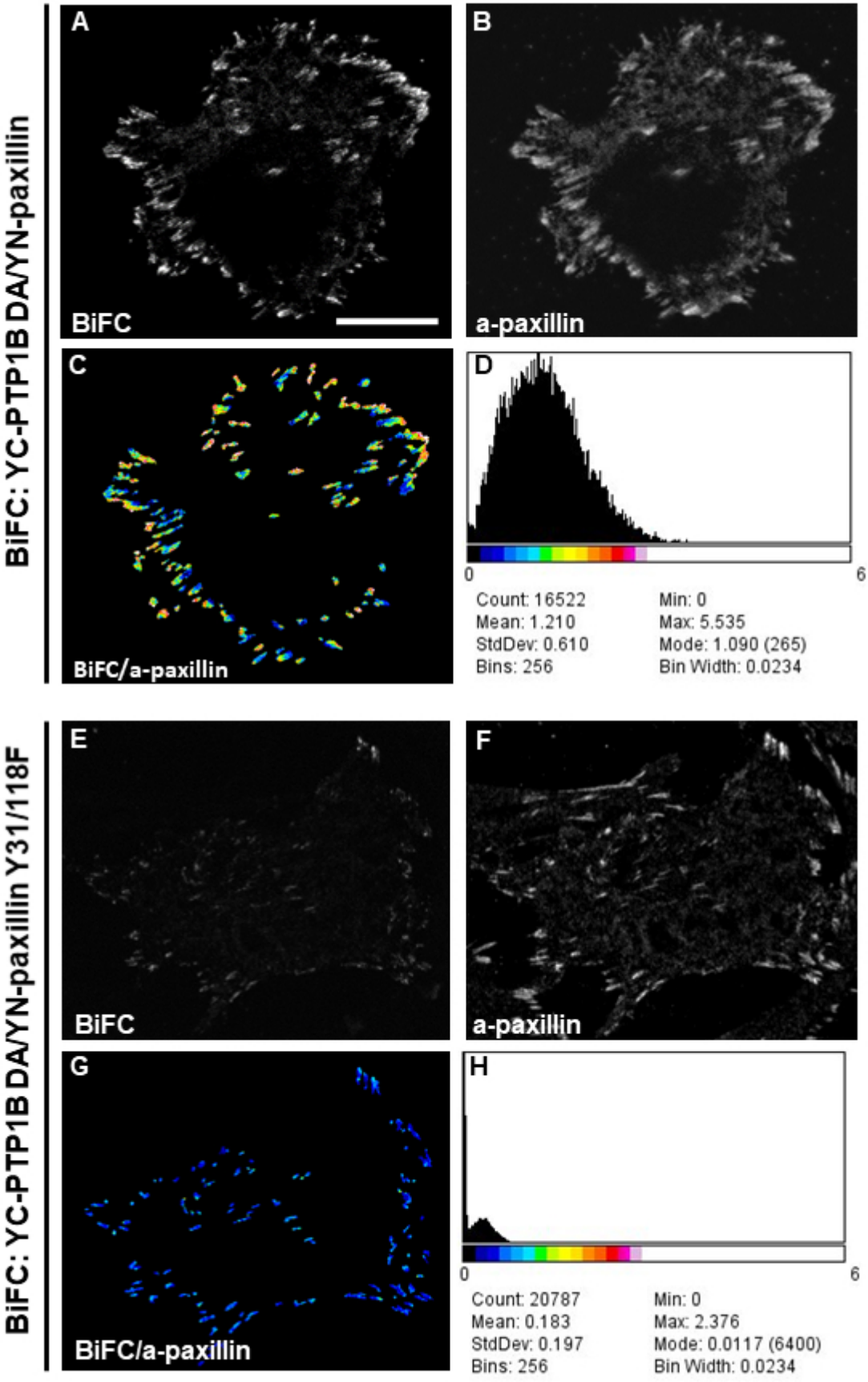

Fig. S5. Analysis of BiFC/anti-paxillin ratio in adhesions. Representative PTP1B WT cells co-expressing YC-PTP1B DA/YN-paxillin (A-C), and YC-PTP1B DA/YN-paxillin Y31/118F (E-G). Cells immunolabeled with anti-paxillin show similar paxillin expression (B,F). However, the positive BiFC signal in cells co-expressing the YC-PTP1B DA/YN-paxillin pair (A) is significantly reduced in cells co-expressing the YC-PTP1B DA/YN-paxillin Y31/118F pair (E). This is better appreciated in the BiFC/anti-paxillin ratios within segmented adhesions (C,G). Ratios are represented by a 16-color palette between a 0-3 range. Histograms representing the frequency distribution of the mean intensity of BiFC/a-paxillin ratios (D, H). The total pixel count as well as the mean, modal, minimum and maximum values are shown. Scale bar, 20 μm.

FIGURE S6

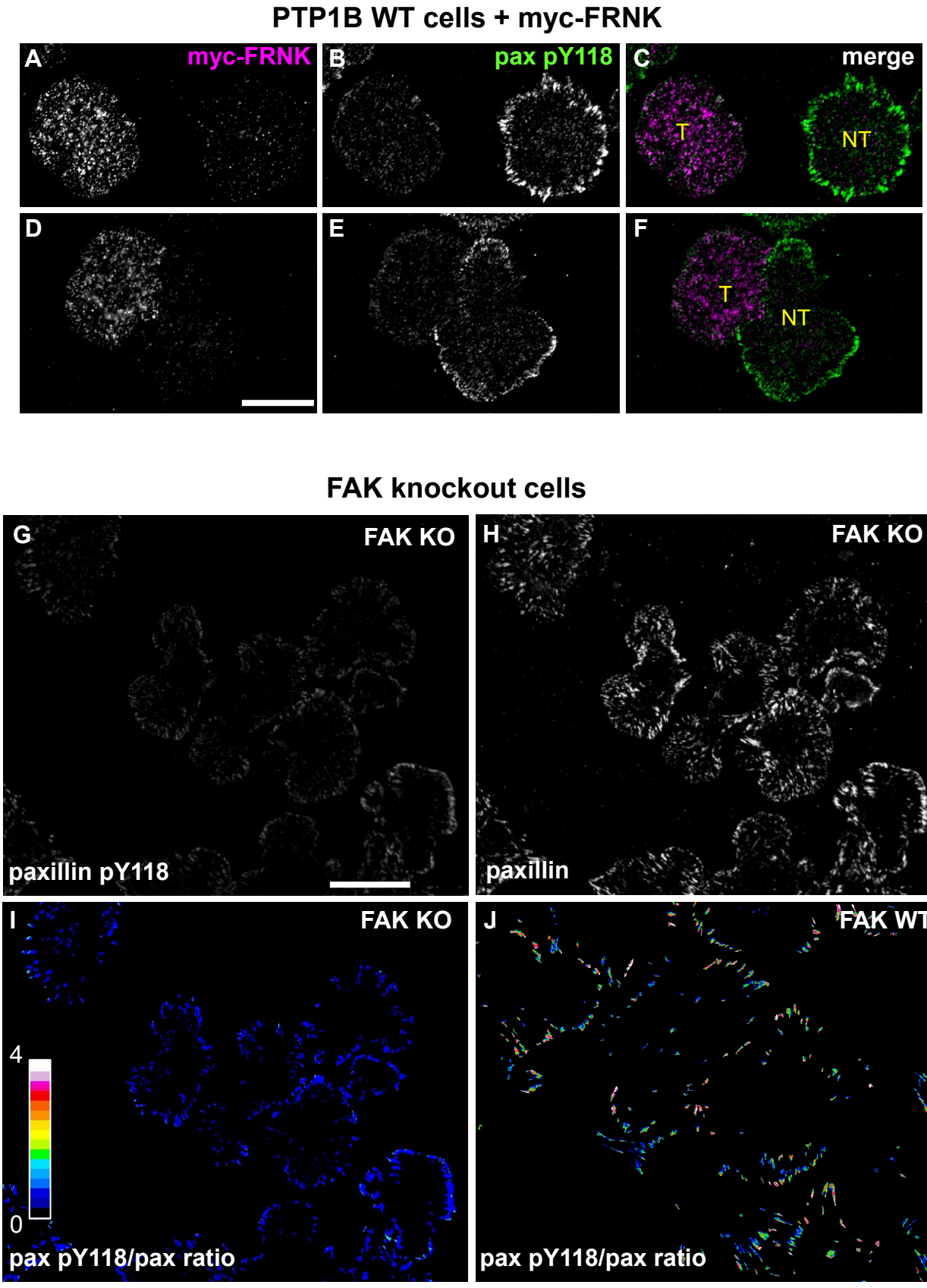

Fig. S6. Phosphorylation level of paxillin in adhesions of cells with impaired FAK function. PTP1B cells transfected with a dominant negative construct of FAK, myc-FRNK (A-F), were plated on fibronectin to form adhesions, and then fixed and double stained with a monoclonal anti-myc (A,D) and a polyclonal anti-phospho-paxillin (pax pY118, B,E) followed by fluorescent secondary antibodies. Cells were observed by wide-field fluorescence microscopy (two representative examples were shown). In merge images (C,F) note that transfected (T) cells show reduced phospho-paxillin signal compared to non-transfected (NT) cells. FAK knockout cells (G-J) co-expressing YN-paxillin and YC-PTP1B DA were plated on fibronectin to form adhesions, and then fixed and double stained with a polyclonal anti-phospho-paxillin (pax pY118, G) and monoclonal anti-paxillin (H), followed by fluorescent secondary antibodies. Cells were observed by wide-field fluorescence microscopy. Note that although paxillin organizes in peripheral adhesions of FAK KO cells, the level of phosphorylation is barely detected. This is better appreciated in BiFC/anti-paxillin ratios within segmented adhesions (I). Images were processed as indicated in Materials and Methods. For comparison, a BiFC/anti-paxillin ratio image from FAK WT cells was shown (J). Scale bar, 20 μm.

FIGURE S7

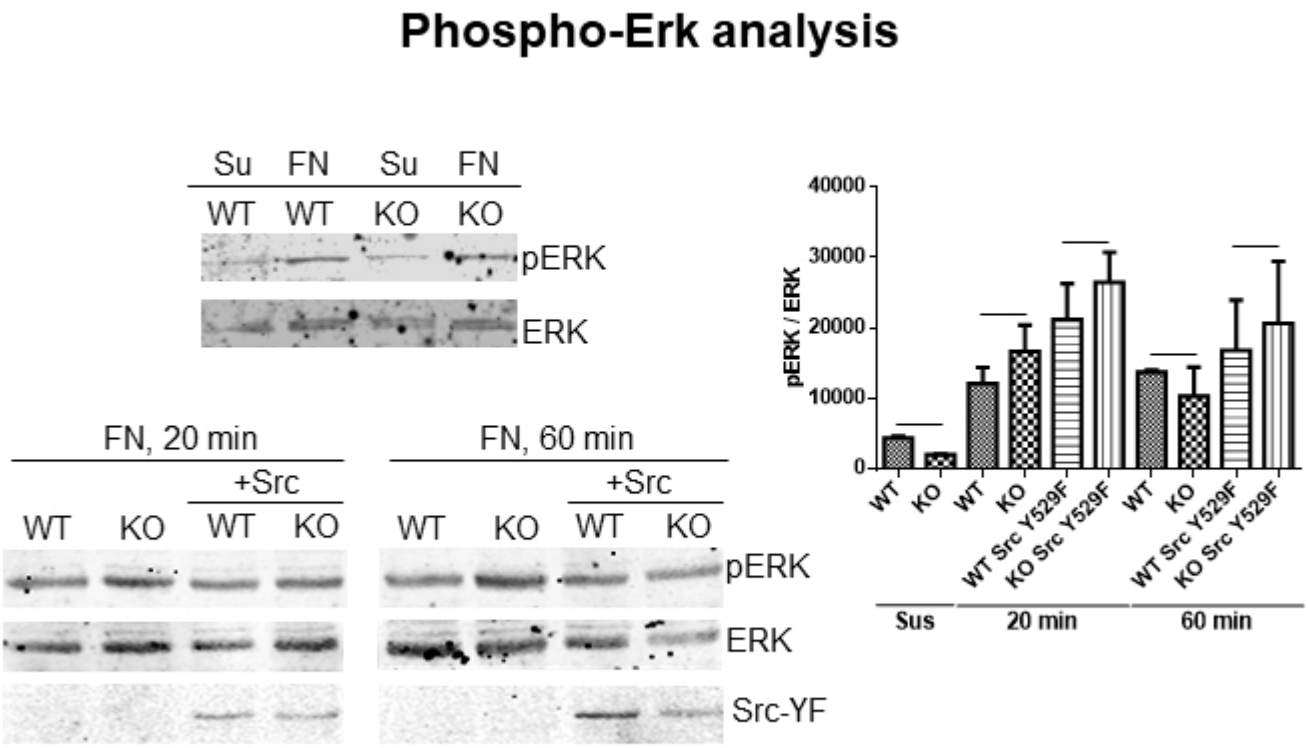

Fig. S7. Assessment of Erk activity. Western blots of serum-starved PTP1B knockout (KO) cells and KO cells reconstituted with wild type PTP1B (WT). The upper panel shows a comparison between cells kept 30 min in suspension and cells plated on fibronectin (10µg/ml) for 20 min. Lower panels show the results of cells non-transfected and transfected with constitutive active Src Y529F-HA plated 20 and 60 min on fibronectin coated dishes. Specific antibodies were used for detection of pERK, ERK, and Src Y529F-HA. Three independent experiments were used for quantification using ImageJ software. Normalized pERK/ERK values, according to LI-COR published protocol, were used for the relative comparison of the different conditions. Bars represent means ± S.E.M. Statistical analysis for normalized pERK/ERK, comparing WT vs KO in each condition, was determined using one-way ANOVA, followed by the Bonferroni´s multiple comparison post hoc test. There was not a significant difference between the means of the indicated pairs.

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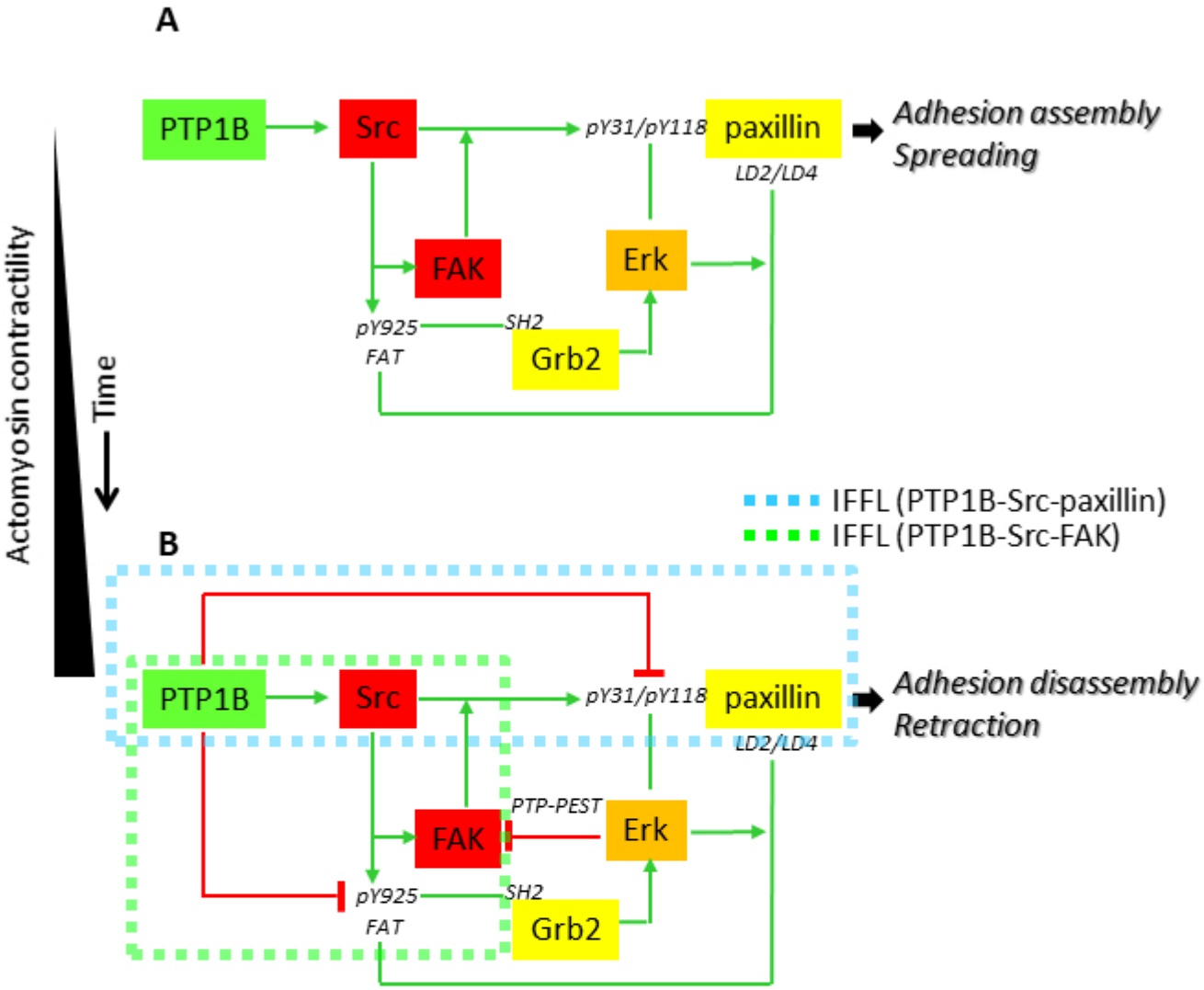

Fig. S8. Model of PTP1B regulation mediated by FAK and paxillin. PTP1B is an upstream activator of Src, which phosphorylates and activates FAK. Both Src and FAK phosphorylate paxillin, leading to adhesion assembly and spreading. In turn, phosphorylation of FAK FAT domain at Y925, recruits Grb2, and promotes Erk activation (A). With a time-delay, PTP1B dephosphorylates FAK and paxillin, leading to adhesion disassembly and lamellar retraction. PTP1B regulation configurates incoherent feedforward loops (IFFLs) that tunes paxillin (dashed blue box) and FAK (dashed green box) outputs depending on their phosphorylation states (B).

Table S1. Conservation of charged amino acids in regions of helices 1, 2 and 4, close to Y925. The *Conservation* column refers to the specific amino acid conservation, while the *Charge conservation* column refers to conservation of the charge type (positive or negative). In bold face, very high conservation values.

Amino acid	Conservation (%)	Charge conservation (%)	
Y925	96		
D922	97	**100**	Helix 1
K923	39	48	
E926	25	38	
R962	96	**98**	
D969	42	63	Helix 2
E970	96	**99**	
K1032	100	**100**	
D1036	100	**100**	Helix 4
D1039	98	**98**	
R1042	99	**100**	