

# Supplementary Information

## **FAK and paxillin dynamics at focal adhesions in the protrusions of migrating cells**

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### **SUPPLEMENTARY MOVIES S (1 -17) LEGENDS**

**Supplementary Movie S1–3 series.** A live EC transfected with GFP-FAK and mCherry-paxillin. Dual-color images from 0 to 60 min show the dynamics of FAK (green) and paxillin (red). These movies showed that FAK-FAs more than paxillin at cell front. The FAK assemble ahead of paxillin at FAs in the protruding region of cell front.

**Supplementary Movie S1.** Showing FAK in the migrating cell..

**Supplementary Movie S2.** Showing paxillin in the migrating cell.

**Supplementary Movie S3.** Showing the dynamics of FAK (green) and paxillin (red) in the migrating cell.

**Supplementary Movie S4 – 6 series.** At cell front region in a live EC transfected with GFP-FAK and mCherry-paxillin. Dual-color images from 0 to 60 min show the dynamics of FAK (green) and paxillin (red). The three movies correspond to Fig. 1B.

**Supplementary Movie S4.** Showing FAK at cell front.

**Supplementary Movie S5.** Showing paxillin at cell front.

**Supplementary Movie S6.** Showing the dynamics of FAK (green) and paxillin (red) at cell front.

**Supplementary Movie S7.** Showing the dynamics of FAK (green) and paxillin (red) at cell center. The movie corresponds to Fig. 1C.

**Supplementary Movie S8.** Showing the dynamics of FAK (green) and paxillin (red) at cell rear. The movie corresponds to Fig. 1D.

**Supplementary Movie S9.** FAK/Paxillin FI ratio image of an EC determined by computational image analysis, pseudo-color coded a range from cool (low FI ratio) to hot (high FI ratio). The movie corresponds to Fig. 1E.

**Supplementary Movie S10.** An EC transfected with GFP-FAK (green) and mCherry-paxillin (red). Dual-color images show FAK and paxillin dynamics. The movie corresponds to Fig. 2A-left three mages.

**Supplementary Movie S11-13.** Showing the dynamics of FAK and paxillin from 0 to 60 min in lamellipodial protrusion. The three movies correspond to Fig. 2B.

**Supplementary Movie S11.** Showing FAK dynamics at the cell leading edge.

**Supplementary Movie S12.** Showing little paxillin dynamics at the cell leading edge

**Supplementary Movie S13.** Showing the dynamics of FAK (green) at the cell leading edge, but there is little visible paxillin dynamics

**Supplementary Movie S14-15 series.** FAK/Paxillin FI ratio image of an EC determined by image analysis, pseudo-color coded a range from cool (low FI ratio) to hot (high FI ratio). The highest ratio of FAK/Paxillin appears at the cell leading edge.

**Supplementary Movie S14. Showing** FAK/Paxillin FI ratio image in a whole EC. The movie corresponds to Fig. 2A-right.

**Supplementary Movie S15.** Showing FAK/Paxillin FI ratio image at the cell leading edge. The movie corresponds to Fig. 2C.

**Supplementary Movie S16.** An EC transfected with GFP-FAK and RFP-actin. Dual-color images of an EC from 0 to 60 min show the co-localization (yellow) of FAK (green) and actin filaments (red) at the cell leading edge. The movie corresponds to Fig. 5.

**Supplementary Movie S17.** A live EC transfected with mCherry-FAK and GFP-paxillin. Dual-color images from 0 to 60 min show the dynamics of FAK (red) and paxillin (green) in a migrating EC. The movie corresponds to Supplementary Figure S1A.

### **SUPPLEMENTARY FIGURE S(1-3) LEGEND**

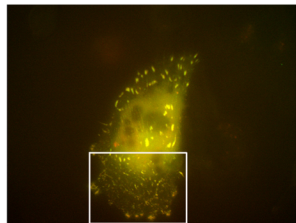
Figure S1. Image analysis of dynamics of FAK and paxillin at FAs in the protrusions of cell front in a living EC transfected with mCherry-FAK and GFP-paxillin. (A) Co-localization of FAK (red) and paxillin (green) yielded a yellow color. Boxed region was selected as regions of interest shown in B. This figure corresponds to Movie S17. (B) Identified FAs were labeled as numbers. The pairs of FAK (left) and paxillin (right) at the same FAs were selected for quantification analysis. (C) The time courses of normalized intensities (y-axis) of FAK (green) and paxillin (red) in four quantified FAs (No. 2, 5, 15 and 16, which are arrowed and labeled in B) are plotted. (D) The values of the time correlation for all 17 FAs in the same cell (including the four FAs in C) are plotted as a function of time shift between FAK and paxillin at FAs. Also plotted is the mean curve for all 17 FAs in this cell. The results of time correlation analysis of this cell (17 individual FAs) yielded a time shift of  $1.70 \pm 0.30$  min (mean  $\pm$  s.e.m.). The value is significantly different from 0 ( $p < 0.01$ ) indicates the time shift at dynamic FAs.

Figure S2. The results of time correlation analysis of independent experiments on twelve migrating cells (201 individual FAs) yielded a time shift of  $2.46 \pm 0.24$  min (mean  $\pm$  s.e.m.), which is significantly different from 0 ( $n=12$ ,  $p < 0.001$ ).

Figure S3. Swapped fluorophores (Green- and mCherry-) between FAK and paxillin did not show the significant difference in the time shift of FAs dynamics. Cells were transfected with GFP-FAK/mCherry-paxillin and mCherry-FAK/GFP-paxillin. These plasmid-transfected cells were tracked and quantified as showed in Fig. 3, 4 and Fig. S1, S2. The results of time correlation analysis of independent experiments on thirteen migrating cells (206 individual FAs) yielded a time shift of  $2.62 \pm 0.27$  min (mean  $\pm$  s.e.m.) (GFP-FAK/mCh-Pax lane). The results of time correlation analysis of independent experiments on twelve migrating cells (201 individual FAs) yielded a time shift of  $2.46 \pm 0.24$  min (mean  $\pm$  s.e.m.) (mCh-FAK/GFP-Pax lane). Compared the results between the two groups, the difference is not significant statistically. The two-tailed  $p$ -value equals 0.67 ( $n_1 = 13$ ,  $n_2 = 12$ ).

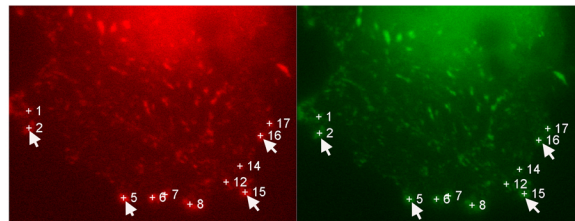
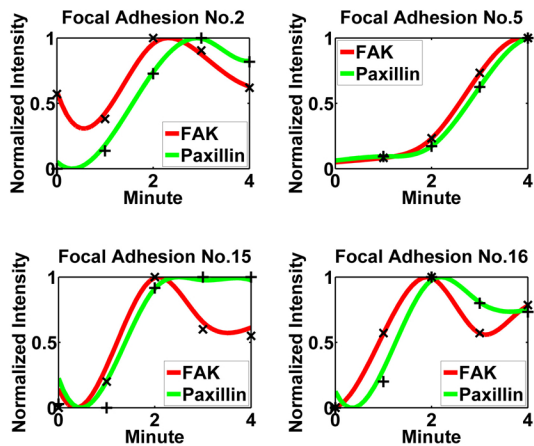
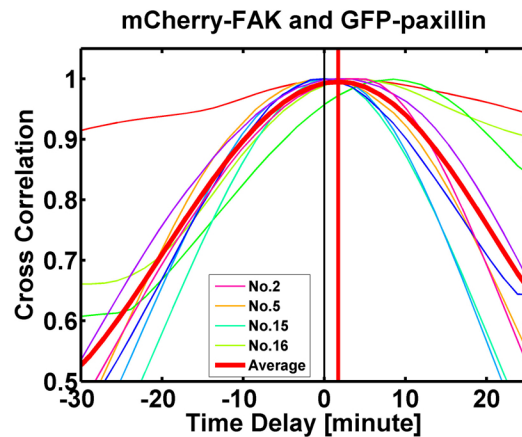
**A**

mCherry-FAK and GFP-Paxillin

**B**

mCherry-FAK

GFP-paxillin

**C****D**

mCherry-FAK and GFP-paxillin

