

SUPPLEMENTARY DATA

Figure S1: Central localization of GFP-paxillin during invadopodia ring expansion.

GFP-paxillin and cortactin-DsRed double transfected BHK-RSV cells were plated on Lab-Tek chambers for 24 hours at 37°C. GFP-paxillin and cortactin-DsRed localizations were followed by time-lapse video microscopy. Kymography analysis of GFP-paxillin and cortactin-DsRed signals along the white arrow shows that GFP-paxillin always has an internal localization compared to cortactin (y=30µm t=99 min). Scale bar is 10 µm.

Figure S2 Dominant negative effect of YF GFP-paxillin overexpression on cellular phosphopaxillin levels.

BHK-RSV cells transiently transfected with WT GFP-paxillin or Y31F/Y118F GFP-paxillin were plated onto glass coverslips for 24 hours, treated for 330 min with 5 mM of orthovanadate, stained for with anti PY31 paxillin specific antibody (red), and DAPI (blue). Bar is 10 µm.

Figure S3: Overexpression of YF GFP-paxillin alters invadopodia organization.

BHK-RSV cells transiently transfected with WT GFP-paxillin or Y31F/Y118F GFP-paxillin were plated onto glass coverslips for 24 hours and stained for F-actin using phalloidin-TRITC (control). White arrows outline invadopodia rosette in WT GFP-paxillin cells and yellow arrow small invadopodia clusters in YF GFP-paxillin cells. Upon 30 min of treatment with 5 mM orthovanadate (+ Na₃VO₄), the invadopodia rings of YF GFP-paxillin transfected cells (yellow arrow) were much thicker than those of WT GFP-paxillin transfected cells (white arrow) or non-transfected cells. Bar is 10 µm.

Movie 1. 24h-plated BHK-RSV cell transiently transfected with Cortactin-GFP without 5 mM orthovanadate treatment. The size of the invadopodia ring stayed the same along the whole movie. Time-lapse videomicroscopy was performed over 130 min with intervals of 5 min

Movie 2: 24h-plated BHK-RSV cell transiently transfected with Cortactin-GFP with 5 mM orthovanadate treatment. Invadopodia rings became larger, fused and generated an invadopodia belt at the cell periphery. Time-lapse videomicroscopy were acquired over 130 min with intervals of 5 min

Movie 3: BHK-RSV cell were transfected with Cortactin-DsRed and GFP-paxillin- and plated on a Lab-Tek chamber for 24h before movie acquisition. Invadopodia ring expansion was induced by orthovanadate treatment. During invadopodia ring expansion, the core protein cortactin was always localized at the periphery of the ring whereas paxillin was mainly localized at the ring center. Images were acquired over 99 min with intervals of 3 min.

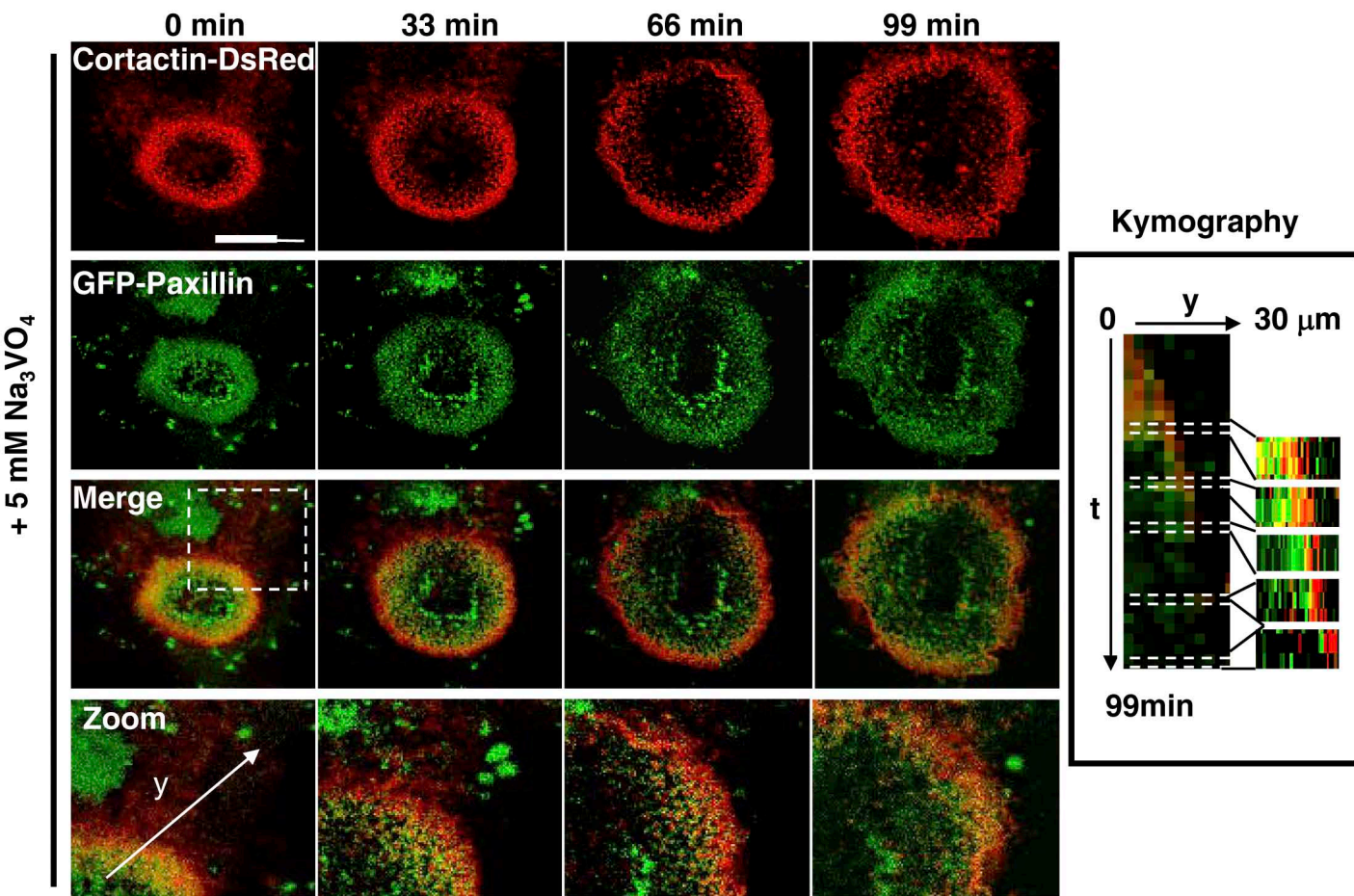
Movie 4A: Transmigration assay step 1: Paxillin-GFP transfected BHH-RSV cell at the top of the HeLa cell monolayer. Movie realized after Z scanning with a confocal microscope Zeiss (LSM 510) and projection in 3D dimensions.

Movie 4B: Transmigration assay step 2: Paxillin-GFP transfected BHH-RSV cell sending a protusion through the HeLa cell monolayer (Time= 50 min). Movie performed after Z scanning with a confocal microscope Zeiss (LSM 510) and projection in 3D dimensions

Movie 4C: Transmigration assay step 3: Paxillin-GFP transfected BHH-RSV cell partially spread under the HeLa cell monolayer (Time= 1h 50 min). The cells form an invadopodia ring

visible from the bottom view. Movie performed after Z scanning with a confocal microscope Zeiss (LSM 510) and projection in 3D dimensions

Movie 4D. Transmigration assay step 4: Paxillin-GFP transfected BHH-RSV cell is spreading efficiently under the HeLa cell monolayer (Time= 1h 50 min) and displays an enlarged invadopodia ring which pulls the transmigrating cell body through the cell monolayer Movie performed after Z scanning with a confocal microscope Zeiss (LSM 510) and projection in 3D dimensions.



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WT GFP-Paxillin

YF GFP-Paxillin

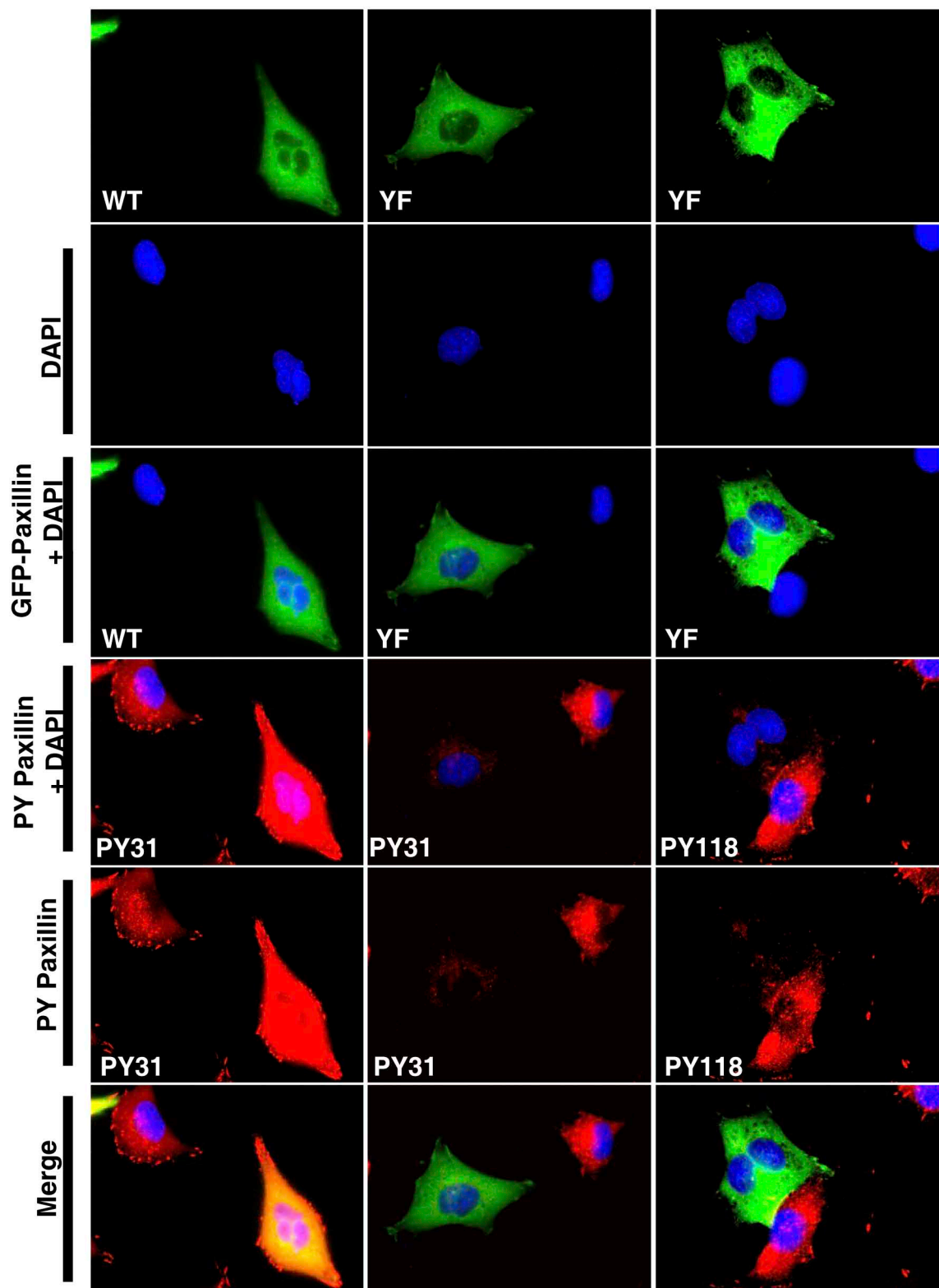
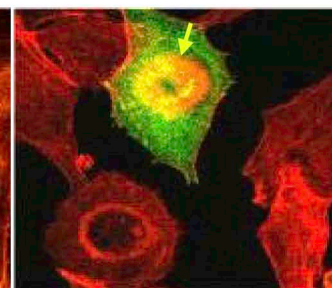
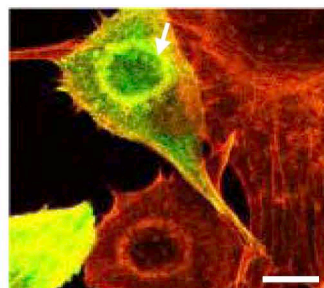
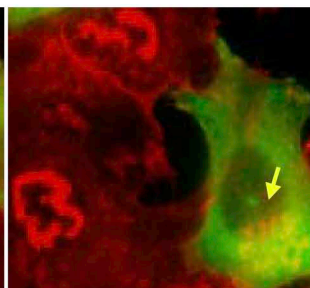
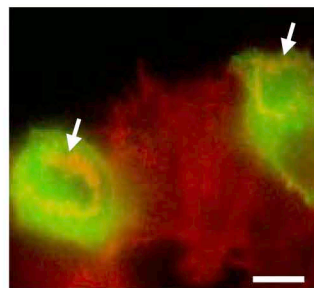
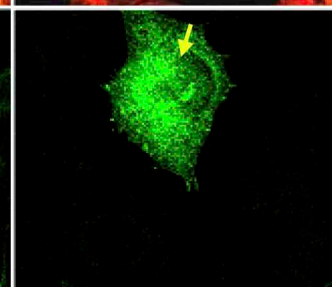
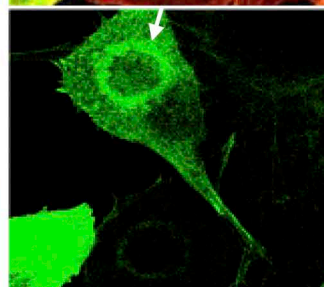
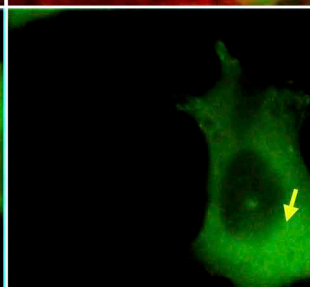
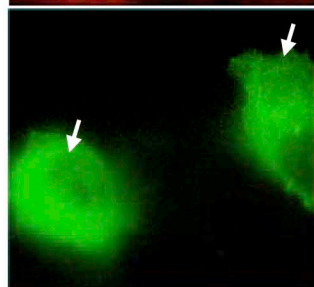
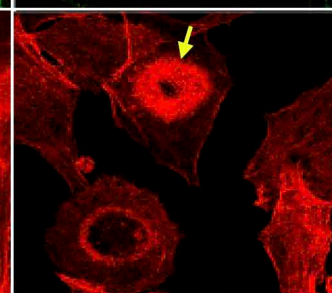
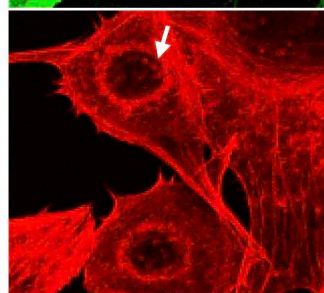
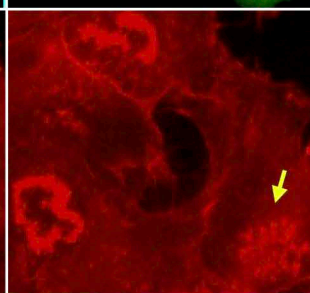
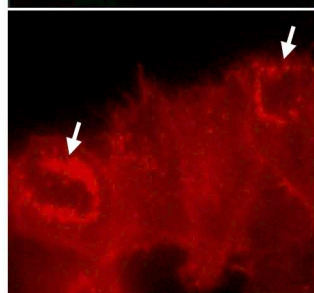


Fig. S1 Badowski et al.

Control**+ 5 mM Na₃VO₄****WT GFP-Paxillin** **YF GFP-Paxillin****WT GFP-Paxillin** **YF GFP-Paxillin****Merge****GFP-Paxillin****Actin****Zoom**