

Short report

Open Access

## Differential requirement for MEK Partner 1 in DU145 prostate cancer cell migration

Electa R Park<sup>1,5</sup>, Ashok K Pullikuth<sup>2</sup>, Evangeline M Bailey<sup>2</sup>, Donald E Mercante<sup>3</sup> and Andrew D Catling<sup>\*2,4</sup>

Address: <sup>1</sup>Department of Biochemistry and Molecular Biology, LSU Health Sciences Center-New Orleans, LA, USA, <sup>2</sup>Department of Pharmacology, LSU Health Sciences Center-New Orleans, LA, USA, <sup>3</sup>Biostatistics Program, School of Public Health, LSU Health Sciences Center-New Orleans, LA, USA, <sup>4</sup>Stanley S Scott Cancer Center, LSU Health Sciences Center-New Orleans, LA, USA and <sup>5</sup>Laboratory of Integrin Signaling and Tumorigenesis, Van Andel Research Institute, Grand Rapids, MI, USA

Email: Electa R Park - [Electa.Park@vai.org](mailto:Electa.Park@vai.org); Ashok K Pullikuth - [apulli@lsuhsc.edu](mailto:apulli@lsuhsc.edu); Evangeline M Bailey - [emckin@lsuhsc.edu](mailto:emckin@lsuhsc.edu); Donald E Mercante - [dmerca@lsuhsc.edu](mailto:dmerca@lsuhsc.edu); Andrew D Catling\* - [acatli@lsuhsc.edu](mailto:acatli@lsuhsc.edu)

\* Corresponding author

Published: 23 November 2009

Received: 16 July 2009

*Cell Communication and Signaling* 2009, **7**:26 doi:10.1186/1478-811X-7-26

Accepted: 23 November 2009

This article is available from: <http://www.biosignaling.com/content/7/1/26>

© 2009 Park et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### Abstract

ERK signaling regulates focal adhesion disassembly during cell movement, and increased ERK signaling frequently contributes to enhanced motility of human tumor cells. We previously found that the ERK scaffold MEK Partner 1 (MP1) is required for focal adhesion disassembly in fibroblasts. Here we test the hypothesis that MP1-dependent ERK signaling regulates motility of DU145 prostate cancer cells. We find that MP1 is required for motility on fibronectin, but not for motility stimulated by serum or EGF. Surprisingly, MP1 appears not to function through its known binding partners MEK1 or PAK1, suggesting the existence of a novel pathway by which MP1 can regulate motility on fibronectin. MP1 may function by regulating the stability or expression of paxillin, a key regulator of motility.

### Introduction

Prostate cancer is the most commonly diagnosed cancer in men. While *in situ* disease usually responds well to treatment, progression to an androgen-hypersensitive, metastatic state is often fatal [1]. Aberrant signaling through the ERK pathway correlates with progression in some models [2], although oncogenic mutations in Ras or Raf occur infrequently in prostate cancer [3,4]. Rather, prostate cancer commonly displays misregulation of cell-matrix adhesion signaling, including alterations in integrin expression and upregulation of Focal Adhesion Kinase (FAK) and p21-Activated Kinase (PAK) [5,6]. We have previously shown that PAK1 can activate MEK1 independently of Raf in model systems [7] suggesting that this alternative pathway to ERK activation may function in

tumor cells lacking oncogenic mutations in Ras and Raf. Indeed, active PAK1 has been shown to stimulate ERK-dependent proliferation of breast cancer cells [8], and is constitutively active in DU145 prostate cancer cells [9].

MEK Partner 1 (MP1) is a putative scaffolding protein that binds MEK1 and ERK [10]. MP1 also binds to the late endosomal protein p14 [11], and the p14-MP1-MEK1 complex regulates trafficking and degradation of the EGFR [12]. Thus MP1/p14 provides a means of colocalizing the endocytosed EGFR and its downstream effectors during proliferation stimulated by EGF. MP1 and p14 also play important roles in cell morphogenesis and migration. MP1 and/or p14 are required for efficient activation of MEK and ERK, and control focal adhesion and actin

dynamics during adhesion to fibronectin [13]. Since ERK is known to regulate focal adhesion disassembly [14,15], these observations implicate MP1-scaffolded ERK in focal adhesion dynamics. However, MP1 also binds active PAK1 [13], itself a regulator of adhesion disassembly [13,16,17], consistent with alternative mechanisms for its involvement in the regulation of cytoskeletal and adhesion dynamics [13]. Elevated PAK activity is associated with altered growth and migratory properties in a number of tumor types, including prostate cancer [18]. We therefore investigated whether MP1 influences ERK and/or PAK signaling during migration of DU145 prostate cancer cells.

## Materials and methods

### Reagents

EGF (Invitrogen) and UO126 were from Calbiochem. Gridded coverslips were from Bellco; glass-bottomed 35 mm microwell dishes were from MatTek Cultureware. Both were coated with fibronectin as described [19].

### Cell Culture

DU145 were maintained in RPMI-1640 containing 10% fetal bovine serum (FBS, Invitrogen).

### siRNA Assays

Cells ( $4.5 \times 10^5$  cells/100 mm dish plated two days prior to transfection) were transfected with 100 nM control siRNA (AGAGAGUAGUAGAGACAAUCGUCUG), MP1 Stealth siRNA (CAACACAGGACUAAUUGUCAGCCUA) or p14 Stealth siRNA (UUAAGAUGCCGCCACUUGGGU-GAGG) (Invitrogen) using Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. Experiments were performed 72 hours post-transfection.

### Immunoblotting

Cells were typically lysed in RIPA buffer (50 mM Tris, 150 mM NaCl, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 5 mM EDTA, 50 mM NaF) supplemented with 1 µg/mL Leupeptin, 3 mM Benzamidin, 10 nM MicrocystinLR, 100 µM orthovanadate, 5 mM NaPPi, and 1 mM PMSF. Equal amounts of lysate protein were used for immunoblots. Proteins were detected using the following antibodies: MEK1 phospho-S298 [19]; MP1 [13]; MEK1 phospho-S218/S222 (Sigma); phosphoERK1/2 (clone12D4; Upstate) ERK1/2 phospho-T202/Y204 (Cell Signaling); paxillin (Biosource); PAK1 N-20 (Santa Cruz); pS141 PAK1-3 (Invitrogen). p14 antibody #5933 was raised against the peptide sequence Ac-CLKAKAQLVQYLEEPLTQVAAS-OH and affinity purified using standard techniques. Blots were imaged on an LAS-1000 Plus Intelligent Dark Box (Fujifilm) using Image Reader software (Fujifilm); the same software was used for quantification of immunoblot results.

### Adhesion/EGF stimulation assay

Cells were plated on fibronectin essentially as described [13]. For EGF stimulation, cells were washed in serum-free (SF) medium then fed SF medium for 2 hours prior to stimulation.

### Migration assays

Cells were trypsinized and collected in SF medium containing 1 mg/mL soybean trypsin inhibitor (Sigma), pelleted, suspended in SF medium and incubated at 37°C for two hours.  $2 \times 10^6$  cells were allowed to form confluent monolayers on fibronectin-coated grid-etched coverslips for 5-7 hours at 37°C. Cells were treated with 1 µg/mL Mitomycin C (Sigma) and either 25 µM UO126 or DMSO vehicle for an additional 3 hours. The medium was aspirated and the coverslip was scratched 4 times with a 200 µL tip to form a # wound pattern. Loose cells were removed with SF medium; cells were fed fresh SF medium containing 1 µg/mL of Mitomycin C and either 25 µM UO126 or the equivalent volume of DMSO, with or without 1% FBS or 10 ng/mL EGF as indicated. Wounds were photographed in 6 different locations at zero-hour time point, and then incubated at 37°C for 5 hours (transfected cells) or 10 hours (untransfected cells) at which time the same locations were photographed. All photographs were taken with an Olympus IX81 microscope equipped with Slidebook software (Intelligent Imaging Innovations) and the area covered by migrating cells was calculated using ImageJ software (NIH). Parallel cultures were processed for immunoblotting.

### Immunofluorescence

Immunofluorescence was carried out essentially as described [13] using a Leica DMRA2 microscope equipped with Slidebook software. The number and size of vinculin-positive focal adhesions at the wound edge was calculated using Slidebook software.

### Kymography

$2 \times 10^6$  cells were plated on 35 mm FN-coated glass-bottomed Petri dishes in SF medium with 1 µg/mL Mitomycin C and allowed to adhere for 5-7 hours. Confluent monolayers were wounded and imaged starting 10-20 min post wounding and again after 2 hours of migration. All cells were imaged for 5 minutes at 1 frame per second on an Olympus IX81 microscope equipped with Slidebook software. Kymographs were generated using ImageJ software.

### Statistical Analysis

Wound-healing assays were repeated in triplicate and statistical analysis was performed on the pooled results. A general linear model was constructed and linear contrast was used to perform pairwise comparisons of the means for the combined data set. Tukey's Honestly Significant

test was used for post-hoc pairwise comparisons of migration under experimental conditions to that of migration under SF conditions and to each other. ANOVA on pooled data indicated significant differences within the data set.

## Results

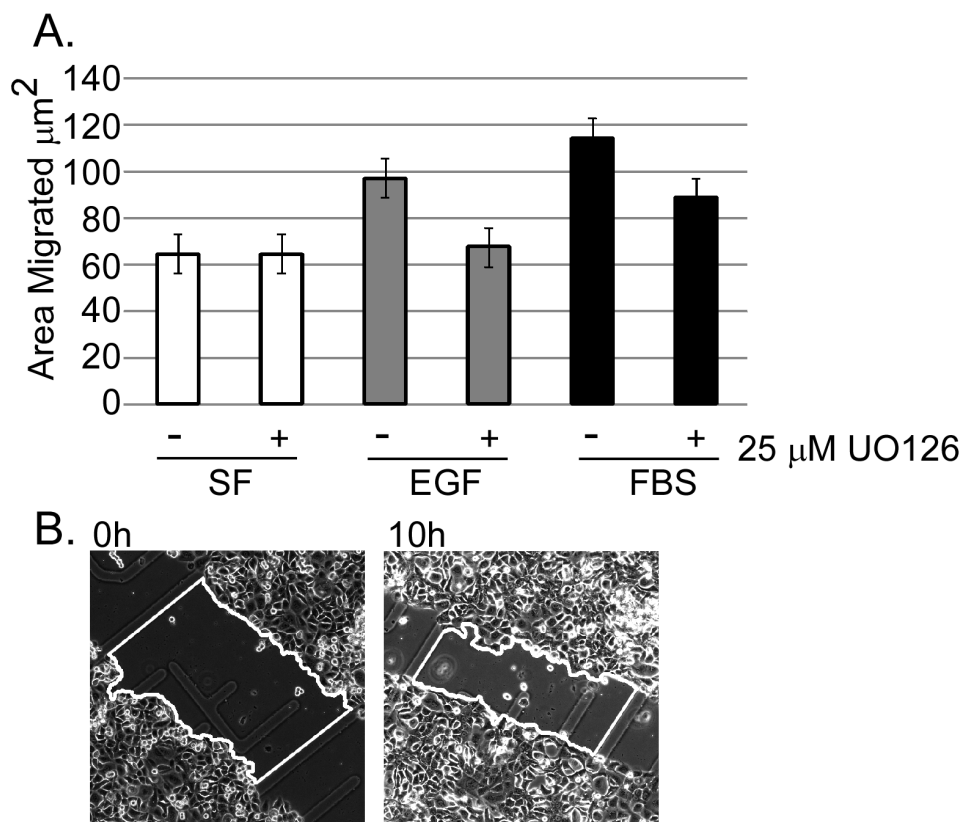
### **ERK activity is required for growth factor-, but not fibronectin-stimulated, DU145 migration**

We developed a quantitative wound-healing assay that makes use of gridded coverslips to allow for accurate measurement of migration into a wounded monolayer over time (Fig. 1B; Materials and Methods). To distinguish between wound closure due to cell movement and proliferation, mitosis in these assays was inhibited by the inclusion of 1  $\mu\text{g}/\text{mL}$  mitomycin C; this concentration was determined as the minimal dose needed to prevent DU145 proliferation without inducing cell death under serum-free conditions (data not shown). Additionally, to distinguish between cell spreading and motility, cells were

tracked in real time over the course of the assay (data not shown), and movies were analyzed for directionality and distance migrated. From these assays we were able to determine that DU145 are migrating into the wound in the monolayer over the timecourse of the assay. DU145 cells migrated  $65 \pm 3.5 \mu\text{m}^2$  on fibronectin in serum-free conditions, and pre-treatment with 25  $\mu\text{M}$  UO126 for 3 hr had no effect (Fig. 1A). Migration on fibronectin was increased to  $115 \pm 4.9$  and  $100 \pm 5.3 \mu\text{m}^2$  in the presence of 1% serum or 10 ng/mL EGF, respectively, and these increases were significantly inhibited by UO126 (Fig. 1A). Thus, MEK signaling is required for growth-factor-induced, but not for fibronectin-stimulated, migration of DU145 cells.

### **MPI/P14 is required for migration of DU145 cells on fibronectin**

Since fibronectin-stimulated motility of DU145 cells is insensitive to MEK inhibitor (Fig. 1A), we predicted that



**Figure 1**

**ERK activation is not required for DU145 migration on FN, but is required for migration on FN induced by serum.** A) Results of DU145 wound healing on FN. Shown are averaged results from 4 experiments; error bars represent standard error of the mean (S.E.M.). Migration in response to fibronectin alone was not affected by UO126. Serum and EGF stimulated significant ( $p < 0.0001$ ) increases in migration, that were significantly inhibited ( $p < 0.0001$  for serum,  $p = 0.0004$  for EGF) by UO126 treatment. B) Sample images from wound healing assay; for each condition, six grid squares were imaged at 0 and 10 h post-wounding, and wound area (outlined in white) was measured using ImageJ software (NIH).

MP1 depletion would be similarly without effect on fibronectin-stimulated DU145 migration. However, since MEK activity is required for growth-factor stimulated DU145 motility (Fig. 1A), and MP1 is required for EGF- and serum-stimulated MEK/ERK activation in fibroblasts [13,20], we predicted that MP1 depletion would inhibit growth-factor stimulated motility of DU145 cells. Surprisingly, we found that fibronectin-stimulated motility was inhibited by ~50% following transfection with MP1 siRNA (Fig. 2A, white bars). In contrast, the component of motility stimulated by 1% serum (above and beyond that stimulated by fibronectin alone) was unaffected by transfection of MP1 siRNA, but was substantially inhibited by UO126 (Fig. 2A) as in naïve cultures (Fig. 1A). Consistent with these observations, depletion of MP1/p14 did not substantially inhibit levels of active ERK or active MEK in cells on fibronectin, whether or not they were exposed to growth factor (Fig. 2B), whereas UO126 essentially eliminated ERK activity in both control and MP1 siRNA-transfected cells (Fig. 2B). Note that knock-down of MP1 is difficult to measure accurately due to the presence of closely migrating proteins that cross react with our antibody. However, siRNAs targeting MP1 have been shown to cause a simultaneous reduction in p14 expression, and vice versa [13,21], most probably because the MP1-p14 heterodimer is more stable than the individual subunits. Since our p14 antiserum blots more cleanly, we used p14 levels as a surrogate measure for MP1 knockdown. p14 levels were reduced on average by 82% (Fig 2B; data not shown). Together, these data indicate that MP1/p14 but not MEK signaling is required for fibronectin-stimulated motility of DU145 cells, whereas MEK signaling but not MP1/p14 is required for motility stimulated by serum growth factors. Thus MP1/p14 and MEK play separable roles in DU145 migration.

#### **MP1 depletion decreases membrane activity and peripheral focal adhesions in migrating cells**

We next asked whether membrane protrusion or focal adhesions were altered in DU145 cells lacking MP1/p14. Kymograph analysis allows visualization of the membrane boundary over time; the y-value is determined by the position of the membrane edge, and the x-axis represents time [21,22]. Cells in MP1 siRNA-transfected cultures displayed much less protrusive and ruffling activity at the wound edge relative to control siRNA-transfected cells (Fig. 3A).

To examine focal adhesions in these assays, a monolayer was wounded and fixed after cells had migrated for 5 hrs. Control- and MP1 siRNA-transfected cells display adhesions of similar total number and size distribution (Fig. 3B, C). However, MP1 siRNA-transfected cells have ~50% fewer adhesions at the wound edge (Fig. 3B; defined as adhesions occurring between the cell periphery and the

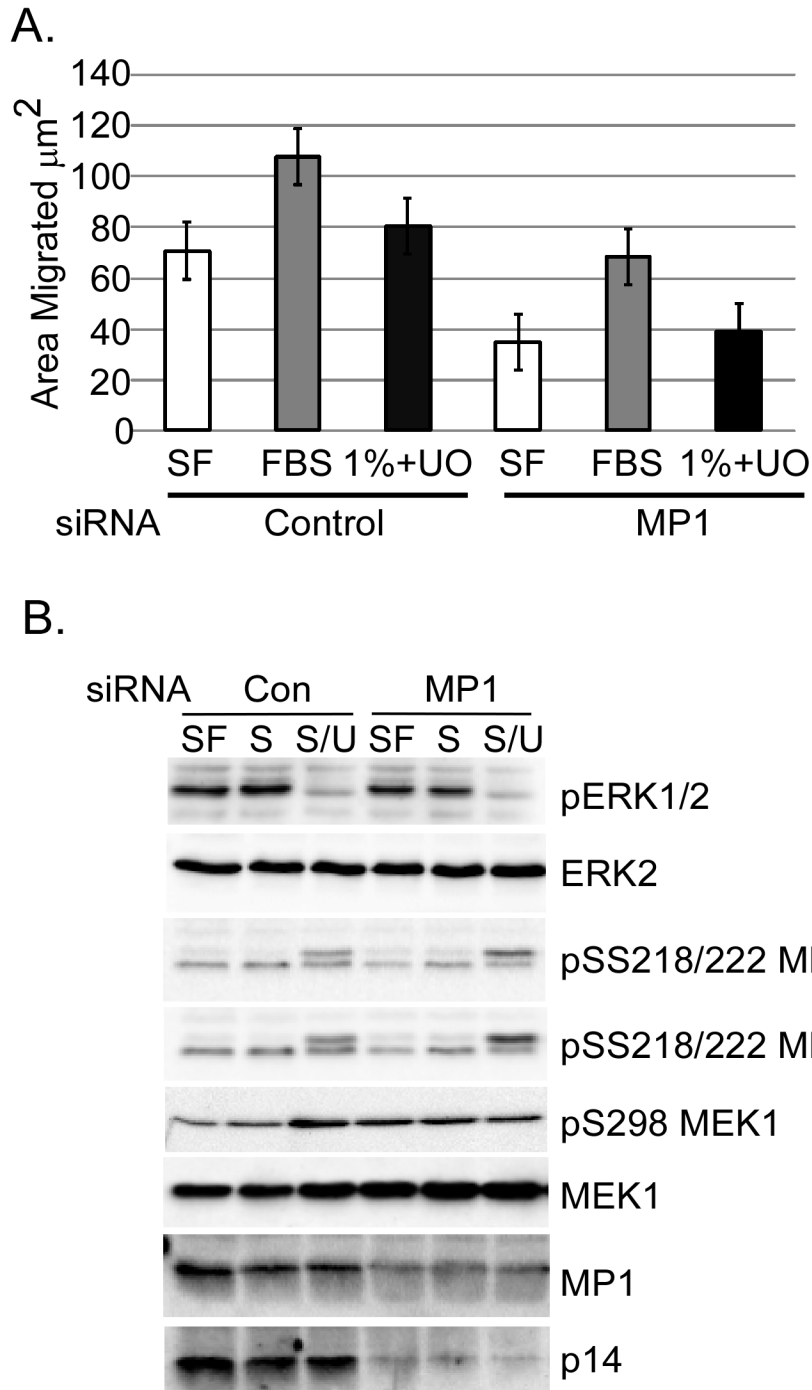
cortical actin ring, not shown; vinculin spots occurring at sites of cell-cell contact were not counted as focal adhesions). This suggests that early nucleation events required for adhesion formation at the wound edge occur at a lower frequency in cells depleted of MP1/p14, or that turnover of this population of adhesions is more pronounced in cells lacking MP1/p14.

#### **Depletion of MP1/p14 modestly inhibits PAK activity**

Membrane protrusion and ruffling are governed in part by localized Rac/PI3-Kinase signaling, while membrane extensions are stabilized by focal complexes that also require PAK activity [16,17]. Focal adhesion maturation and disassembly are complex processes governed by GTPases (including Rac and Rho), kinases (including PAK, FAK, and ERK) and molecular organizing components including paxillin. Since fibronectin-stimulated DU145 motility was insensitive to MEK inhibitor but sensitive to knock-down of MP1/p14, we reasoned that MP1 functions through a MEK activity-independent pathway to regulate membrane and adhesion dynamics. One candidate molecule is PAK1, to which MP1 binds [13]. Group I PAK kinases phosphorylate a number of cellular targets, including MEK1 [7,19,23], paxillin [24], and themselves [25,26]. Depletion of MP1/p14 slightly delays autophosphorylation of PAK1-3 on S141 following adhesion to fibronectin, with phosphorylation recovering around 1 hr and persisting throughout the 5 hr time course. Maximal phosphorylation of MEK1 on S298 is similarly delayed in MP1 siRNA-transfected cells (Fig. 4A). While we cannot exclude that these subtle differences in kinetics of phosphorylation in the population as a whole represent significant quantitative differences at the wound edge, our data suggest that depletion of MP1/p14 has minor effects on the overall activity of the PAKs phosphorylating these substrates.

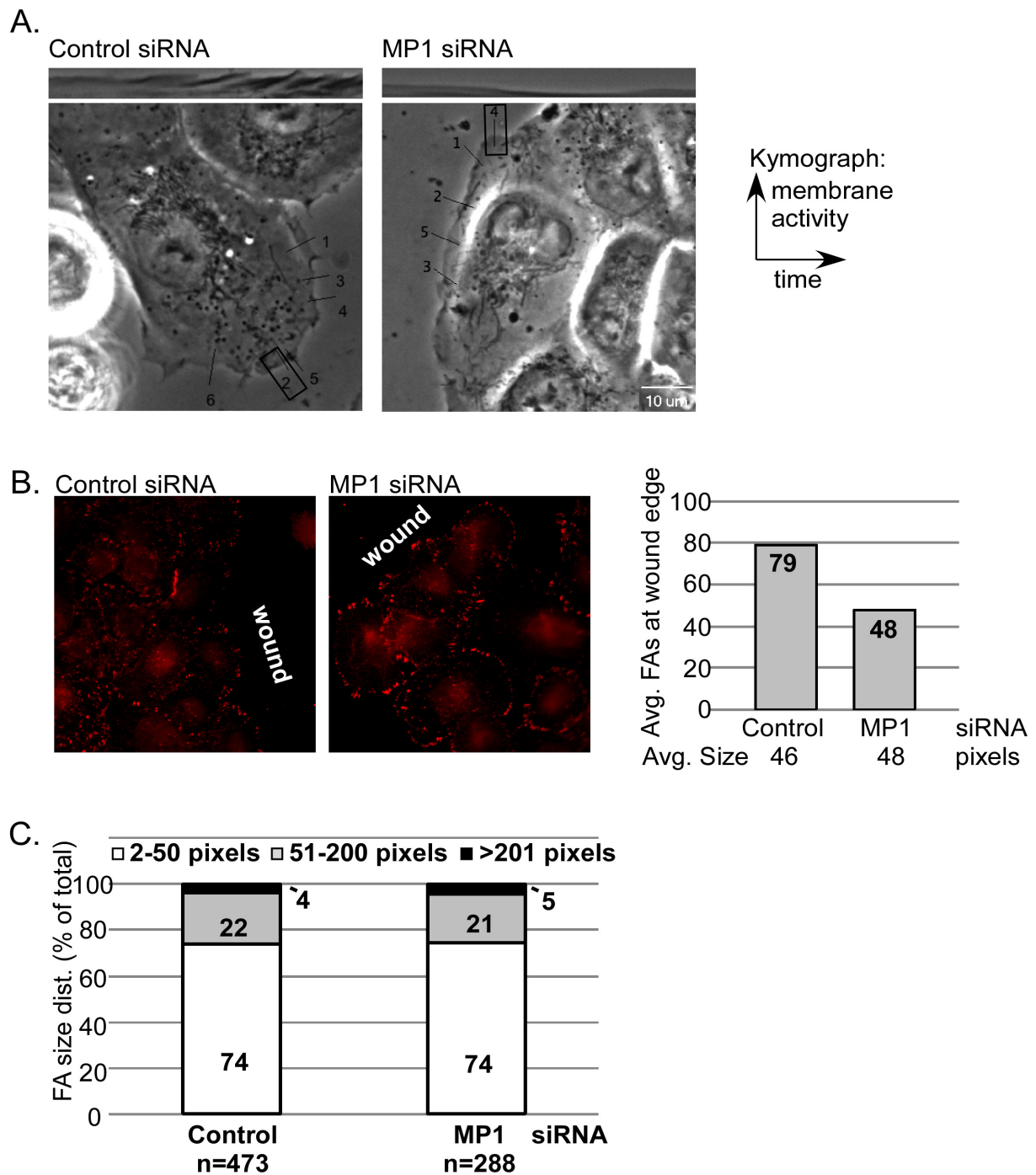
#### **Paxillin is largely absent in DU145 cells depleted of MP1/p14**

To extend these observations, we asked whether PAK phosphorylation of the cytoskeletal linker/adaptor paxillin was regulated by MP1/p14. Paxillin has been shown to play essential roles in focal adhesion dynamics, membrane protrusion and motility [14,27-29]. PAK phosphorylates paxillin on S273, and phosphorylation of this site is required for stabilizing membrane protrusion [24]. Surprisingly, the abundance of paxillin was substantially reduced in cells depleted of MP1/p14 (~60% reduction, Fig. 4B), suggesting that paxillin is poorly expressed or unstable in cells lacking MP1/p14, or that it resides in a compartment not extracted by our lysis buffer. To exclude the latter possibility, we prepared extracts in SDS-PAGE sample buffer which is expected to solubilize all cellular proteins. The abundance of paxillin was similarly reduced (by ~60%) in SDS-PAGE buffer extracts of DU145 cells

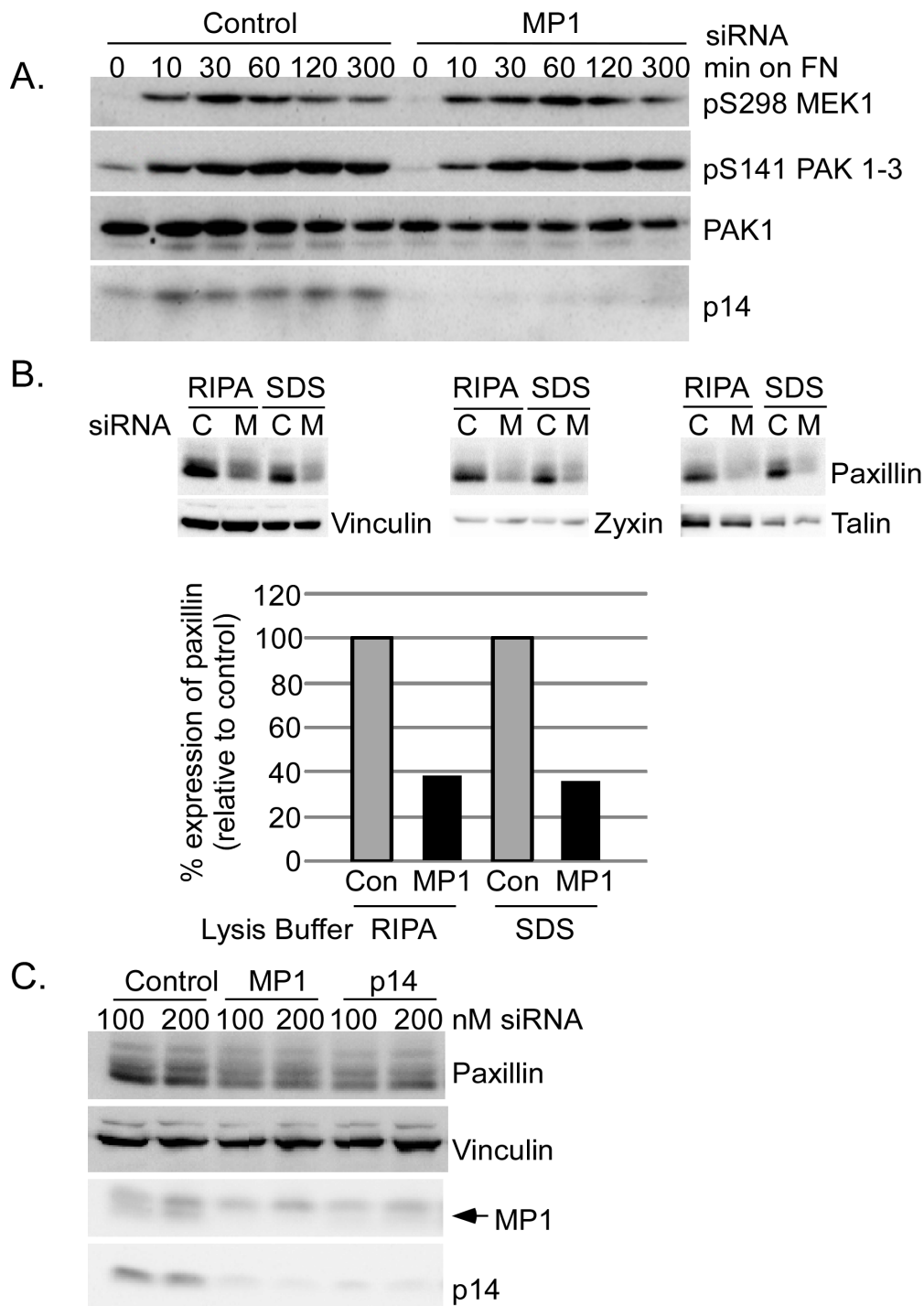


**Figure 2**

**MPI knockdown inhibits migration in DU145 acutely adherent on fibronectin.** A) DU145 cells were transfected with Control (non-targeting) or MPI siRNA. MPI depletion inhibits DU145 migration on FN in serum-free medium (SF) by ~50% relative to control. Migration is induced by 1% fetal bovine serum (1%) in cells transfected with control or MPI siRNA, and the induced component of migration is sensitive to MEK1 inhibition by UO126 (UO). Shown are mean data from three independent experiments; error bars represents S.E.M. B) Representative blot showing MPI knockdown in DU145 cells treated in parallel with cells in wound-healing assay. Two exposures of MEK1 phospho-SS218/222 are shown to emphasize the low level of MEK1 activation in these assays, which nevertheless remains similar between Control and MPI siRNA transfected cells. SF, Serum-Free medium; S, 1% fetal bovine Serum medium; S/U, 1% fetal bovine Serum medium + 25 µM UO126.



**Figure 3**  
**MP1 depletion decreases membrane activity and the number of focal adhesions at the wound edge.** A) DUI45 cells transfected with control or MP1 siRNA were wounded as described. Approximately 2 hours post-wounding, cells were imaged at 1 second intervals for 5 min for kymograph analysis. For each cell, 6-7 kymographs were generated (shown by numbered lines); 5-7 cells per condition were analyzed. Boxes around the number indicate the representative kymograph shown. B) Control and MP1 siRNA transfected cells plated on coverslips and imaged for a wound-healing assay were fixed and stained for vinculin to mark focal adhesions. Average number and size of FA occurring at the wound edge were quantified using ImageJ (chart). C) Size distribution of all focal adhesions in the cells imaged for B. MP1 depleted cells have fewer focal adhesions at the wound edge.



**Figure 4**  
**MPI knockdown modestly delays PAK autophosphorylation in response to FN and decreases paxillin expression.** A) DU145 cells transfected with control or MPI siRNA were plated on FN for the times indicated. Whole cell lysates were analyzed by immunoblot for PAK phosphorylation of MEK1 (pS298 MEK1) and PAK1-3 autophosphorylation on S141. B) Cells transfected with control or MPI siRNA were lysed in RIPA (RIPA) or SDS-PAGE (SDS) loading buffer (see Methods) and expression of focal adhesion proteins was assessed by immunoblot. C, Control siRNA; M, MPI siRNA. Paxillin expression was quantified by densitometry and is expressed as percent of control for each lysis buffer. C) Cells transfected with control, MPI, or p14 siRNA were analyzed for vinculin, paxillin, MPI and p14 expression.

transfected with MP1 siRNA, while expression of other focal adhesion markers vinculin, talin and zyxin was unaffected by knock-down of MP1/p14 (Fig. 4B). Reduction of paxillin levels is unlikely to result from off-target effects of the MP1 siRNA since similar results were obtained with an independent p14 siRNA (Fig. 4C). These data indicate that the MP1 and/or p14 are required to maintain normal paxillin protein levels.

## Discussion

### MP1/p14 regulated signaling events

MP1/p14 is essential for ERK activation during adhesion of fibroblasts to fibronectin, and in this context, depletion of MP1/p14 causes an apparent stabilization of focal adhesions [13]. We therefore predicted that depletion of MP1/p14 would cause inhibition of fibronectin-stimulated ERK activation and stabilization of focal adhesions in DU145 cells, and thus inhibit cell motility. To our surprise, we found that migration of DU145 cells on fibronectin does not require acute MEK activity but does require MP1/p14. Conversely, migration stimulated by growth factors requires acute MEK activity, but does not require MP1/p14. Furthermore, MP1/p14 depletion does not significantly inhibit ERK phosphorylation in response to fibronectin or growth factor stimulation. These data indicate that there are separable MEK activity- and MP1/p14-dependent pathways leading to DU145 motility in response to serum growth factors and fibronectin respectively.

Because Group I PAKs have multiple roles in cell motility [30] and PAK1 binds MP1 [13], we reasoned that MP1 might function through PAK to regulate fibronectin-stimulated DU145 migration. However, depletion of MP1 caused only modest differences in PAK autophosphorylation or PAK phosphorylation of MEK1; phosphorylation of the PAK-dependent substrate cofilin was also unaffected by depletion of MP1 (data not shown). These data suggest that PAK is not a major effector for MP1 in DU145 cell motility.

### MP1/p14 is required for maintenance of paxillin levels

Surprisingly, we found that paxillin was largely absent from DU145 cells depleted of MP1/P14 while expression of other focal adhesion proteins was unaffected. Future experiments will determine whether this loss of paxillin results from decreased mRNA or protein synthesis, or increased turnover of paxillin protein.

### Depletion of MP1/p14 influences membrane dynamics and focal adhesion abundance

We have found that depletion of MP1/p14 results in a loss of paxillin, decreases membrane protrusion, and reduces the number of focal adhesions at the wound edge. Paxillin serves as a signaling nexus at focal adhesions and in the

leading edge of migrating cells. In particular, the GIT1-PIX-PAK complex is stabilized by PAK phosphorylation of paxillin, leading to increased localized Rac activation, membrane protrusion, and migration [24]. This complex localizes to a population of dynamic focal complexes at the leading edge [14,24], suggesting that it may be important for stabilizing membrane protrusion. Alternatively, paxillin may liberate p190A RhoGAP from p120 RasGAP at the leading edge of motile cells [31]. Locally released p190A RhoGAP is free to stimulate hydrolysis of GTP on Rho allowing membrane protrusion. Interestingly, while the loss of paxillin correlates with a failure of DU145 to migrate on fibronectin, it does not preclude serum-stimulated motility.

Our data are the first to indicate that MP1/P14 has important functional roles outside the ERK pathway. These observations suggest that MP1/p14- and paxillin-dependent and -independent pathways for motility may be operational in DU145 cells in a context-specific manner.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

ERP carried out the migration and biochemical assays and drafted the manuscript; AKP provided technical expertise for the microscopy and for quantitative analysis of migration, kymography and focal adhesion size and abundance; EMB performed knock-down experiments investigating paxillin abundance; DEM performed statistical analysis for the migration assays; and ADC was responsible for the overall design and coordination of the study. All authors read and approved the final manuscript.

## Acknowledgements

This work was supported by Department of Defense Prostate Cancer Research Program Predoctoral Traineeship Award W81XWH-05-1-0591 (E.R.P.) and National Institutes of Health RO1 GM068111 (A.D.C.). We thank Dr. Becky Worthylake for her assistance with the imaging experiments and Dr. Wayne Orr (LSUHSC, Shreveport) for helpful discussions.

## References

1. American Cancer Society: **Cancer Facts and Figures 2008**. Atlanta: American Cancer Society; 2008.
2. Bakin RE, Gioeli D, Sikes RA, Bissonette EA, Weber MJ: **Constitutive activation of the Ras/mitogen-activated protein kinase signaling pathway promotes androgen hypersensitivity in LNCaP prostate cancer cells**. *Cancer Res* 2003, **63**:1981-1989.
3. Burger M, Denzinger S, Hammerschmied C, Tannapfel A, Maderstorfer A, Wieland WF, Hartmann A, Stoehr R: **Mitogen-activated protein kinase signaling is activated in prostate tumors but not mediated by B-RAF mutations**. *Eur Urol* 2006, **50**:1102-1109. discussion 1109-1110
4. Cho NY, Choi M, Kim BH, Cho YM, Moon KC, Kang GH: **BRAF and KRAS mutations in prostatic adenocarcinoma**. *Int J Cancer* 2006, **119**:1858-1862.
5. Kumar R, Vadlamudi RK: **Emerging functions of p21-activated kinases in human cancer cells**. *J Cell Physiol* 2002, **193**:133-144.
6. Slack JK, Adams RB, Rovin JD, Bissonette EA, Stoker CE, Parsons JT: **Alterations in the focal adhesion kinase/Src signal transduc-**



- tion pathway correlate with increased migratory capacity of prostate carcinoma cells. *Oncogene* 2001, **20**:1152-1163.
7. Park ER, Eblen ST, Catling AD: **MEK1 activation by PAK: a novel mechanism.** *Cell Signal* 2007, **19**:1488-1496.
  8. Vadlamudi RK, Adam L, Wang RA, Mandal M, Nguyen D, Sahin A, Chernoff J, Hung MC, Kumar R: **Regulatable expression of p21-activated kinase-1 promotes anchorage-independent growth and abnormal organization of mitotic spindles in human epithelial breast cancer cells.** *J Biol Chem* 2000, **275**:36238-36244.
  9. Horiguchi A, Zheng R, Shen R, Nanus DM: **Inactivation of the NF2 tumor suppressor protein merlin in DUI45 prostate cancer cells.** *Prostate* 2008, **68**:975-984.
  10. Schaeffer HJ, Catling AD, Eblen ST, Collier LS, Krauss A, Weber MJ: **MPI: a MEK binding partner that enhances enzymatic activation of the MAP kinase cascade.** *Science* 1998, **281**:1668-1671.
  11. Wunderlich W, Fialka I, Teis D, Alpi A, Pfeifer A, Parton RG, Lottspeich F, Huber LA: **A novel 14-kilodalton protein interacts with the mitogen-activated protein kinase scaffold mpi on a late endosomal/lysosomal compartment.** *J Cell Biol* 2001, **152**:765-776.
  12. Teis D, Taub N, Kurzbauer R, Hilber D, de Araujo ME, Erlacher M, Offerdinger M, Villunger A, Geley S, Bohn G, Klein C, Hess MW, Huber LA: **p14-MPI-MEK1 signaling regulates endosomal traffic and cellular proliferation during tissue homeostasis.** *J Cell Biol* 2006, **175**:861-868.
  13. Pullikuth A, McKinnon E, Schaeffer HJ, Catling AD: **The MEK1 scaffolding protein MPI regulates cell spreading by integrating PAK1 and Rho signals.** *Mol Cell Biol* 2005, **25**:5119-5133.
  14. Webb DJ, Donais K, Whitmore LA, Thomas SM, Turner CE, Parsons JT, Horwitz AF: **FAK-Src signalling through paxillin, ERK and MLCK regulates adhesion disassembly.** *Nat Cell Biol* 2004, **6**:154-161.
  15. Fincham VJ, James M, Frame MC, Winder SJ: **Active ERK/MAP kinase is targeted to newly forming cell-matrix adhesions by integrin engagement and v-Src.** *Embo J* 2000, **19**:2911-2923.
  16. Manser E, Huang HY, Loo TH, Chen XQ, Dong JM, Leung T, Lim L: **Expression of constitutively active alpha-PAK reveals effects of the kinase on actin and focal complexes.** *Mol Cell Biol* 1997, **17**:1129-1143.
  17. Frost JA, Khokhlatchev A, Stippec S, White MA, Cobb MH: **Differential effects of PAK1-activating mutations reveal activity-dependent and -independent effects on cytoskeletal regulation.** *J Biol Chem* 1998, **273**:28191-28198.
  18. Kumar R, Gururaj AE, Barnes CJ: **p21-activated kinases in cancer.** *Nat Rev Cancer* 2006, **6**:459-471.
  19. Slack-Davis JK, Eblen ST, Zecevic M, Boerner SA, Tarcsafalvi A, Diaz HB, Marshall MS, Weber MJ, Parsons JT, Catling AD: **PAK1 phosphorylation of MEK1 regulates fibronectin-stimulated MAPK activation.** *J Cell Biol* 2003, **162**:281-291.
  20. Teis D, Wunderlich W, Huber LA: **Localization of the MPI-MAPK scaffold complex to endosomes is mediated by p14 and required for signal transduction.** *Dev Cell* 2002, **3**:803-814.
  21. Hinz B, Alt W, Johnen C, Herzog V, Kaiser HW: **Quantifying lamella dynamics of cultured cells by SACED, a new computer-assisted motion analysis.** *Exp Cell Res* 1999, **251**:234-243.
  22. Bear JE, Svitkina TM, Krause M, Schafer DA, Loureiro JJ, Strasser GA, Maly IV, Chaga OY, Cooper JA, Borisov GG, Gertler FB: **Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility.** *Cell* 2002, **109**:509-521.
  23. Frost JA, Steen H, Shapiro P, Lewis T, Ahn N, Shaw PE, Cobb MH: **Cross-cascade activation of ERKs and ternary complex factors by Rho family proteins.** *Embo J* 1997, **16**:6426-6438.
  24. Nayal A, Webb DJ, Brown CM, Schaefer EM, Vicente-Manzanares M, Horwitz AR: **Paxillin phosphorylation at Ser273 localizes a GIT1-PIX-PAK complex and regulates adhesion and protrusion dynamics.** *J Cell Biol* 2006, **173**:587-589.
  25. Bagrodia S, Taylor SJ, Creasy CL, Chernoff J, Cerione RA: **Identification of a mouse p21Cdc42/Rac activated kinase.** *J Biol Chem* 1995, **270**:22731-22737.
  26. Manser E, Leung T, Lim L: **Purification and assay of kinases that interact with Rac/Cdc42.** *Methods Enzymol* 1995, **256**:215-227.
  27. Deakin NO, Turner CE: **Paxillin comes of age.** *J Cell Sci* 2008, **121**:2435-2444.
  28. Efimov A, Schiefermeier N, Grigoriev I, Ohi R, Brown MC, Turner CE, Small JV, Kaverina I: **Paxillin-dependent stimulation of microtubule catastrophes at focal adhesion sites.** *J Cell Sci* 2008, **121**:196-204.
  29. Brown MC, Perrotta JA, Turner CE: **Serine and threonine phosphorylation of the paxillin LIM domains regulates paxillin focal adhesion localization and cell adhesion to fibronectin.** *Mol Biol Cell* 1998, **9**:1803-1816.
  30. Bokoch GM: **Biology of the p21-activated kinases.** *Annu Rev Biochem* 2003, **72**:743-781.
  31. Tsubouchi A, Sakakura J, Yagi R, Mazaki Y, Schaefer E, Yano H, Sabe H: **Localized suppression of RhoA activity by Tyr31/118-phosphorylated paxillin in cell adhesion and migration.** *J Cell Biol* 2002, **159**:673-683.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:  
[http://www.biomedcentral.com/info/publishing\\_adv.asp](http://www.biomedcentral.com/info/publishing_adv.asp)

