Online Supplement

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LOSS OF THE PROTEIN-TYROSINE PHOSPHATASE DEP-1/PTPRJ DRIVES MENINGIOMA CELL MOTILITY

SUPPLEMENTARY MATERIALS AND METHODS

LOH analysis

DNA extraction from snap-frozen tumor samples and from corresponding peripheral blood samples was done according to standard procedures. Paired genomic DNA samples were amplified by PCR using fluorescently (Cy5) labelled primers. Oligonucleotide sequences were obtained from htpp://www.ncbi.nlm.nih.gov in the UniSTS database. DNA samples of 100 ng were used for PCR according to the instructions of the manufacturer (DyNAzyme DNA polymerase kit, FINNZYMES, Espoo, Finland). Cycling conditions were as follows: 10 min initializing denaturation, followed by 35 cycles of 94 °C for 20 s, 58 °C for 20 s, and 72 °C for 30 s, and a final step at 72 °C for 7 min. PCR products were diluted 1:10 and run on a CEQ8000 genetic analyzer (Beckman Coulter, CA, USA). A difference of 30% or more in the intensity ratio of the two alleles in tumor DNA compared to normal DNA was considered as evidence for LOH.

GST fusion protein expression and purification

GST-tagged catalytic domains of DEP-1 (wildtype or D1205A substrate trapping mutant) were expressed and purified from *E. coli*. Rosetta strains transformed with pGEX6P-1-cat-DEP-1-WT or pGEX6P-1-cat-DEP-1-D1205A plasmids were grown in LB medium

containing 100 µg/ml ampicillin and 30 µg/ml chloramphenicol to OD 0.5. Expression was induced by addition of 0.5 mM IPTG for 5 h at room temperature. Bacteria were pelleted and resuspended in lysis buffer (25 mM Tris pH 7.5, 150 mM NaCl. 1 mM EDTA, 2 mM EGTA, 0.1% beta-mercaptoethanol, supplemented with protease inhibitors) and lysed in a french press. Lysates were cleared by centrifugation (30 min 30000 rpm), filtered (0.45 µm filter pore size) and incubated with GSH-Sepharose (GE Healthcare, Freiburg, Germany) for 1 h at 4°C. Beads were washed four times with wash buffer (25 mM Tris pH 7.5, 150 mM NaCl, 1% Triton X-100, 10 mM beta-mercaptoethanol) and once with wash buffer without Triton. GST fusion proteins were eluted with 20 mM glutathion in wash buffer without Triton. Eluates were concentrated with Amicon Ultra-15 Centrifugal Filter Units (Ultracel-30 membrane; Millipore, Schwalbach, Germany) and further purified by gel filtration using a Superdex 200 column (GE Healthcare, Freiburg, Germany) in 0.1 M HEPES, 150 mM NaCl buffer. Fractions containing the GST fusion proteins were pooled and diluted to a concentration of 0.1 M HEPES, 75 mM NaCl. Proteins were coupled to Affigel 15 agarose (Bio Rad, Munich, Germany) for 4 h at 4°C. Beads were washed five times with 0.1 M HEPES, 150 mM NaCl and stored in wash buffer containing sodium azide.

Substrate trapping

SW480 colon epithelial cells were grown to confluence. Cells were left untreated or treated with 100 µM pervanadate (freshly prepared; 1 mM sodium orthovanadate mixed with 5 mM H_2O_2 and 1 mM HEPES, pH 7.4, final concentration) for 10 min at 37°C, washed with PBS and lysed in NP40 lysis buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 1% NP40, 1 mM EDTA, 2 mM EGTA, 10% Glycerol, 5 mM sodium iodoacetate; supplemented with protease inhibitors); after five minutes, DTT was added to a final concentration of 10 mM. After centrifugation, lysates were precleared by incubation with Sepharose CL 4B (Sigma Aldrich, Taufkirchen, Germany) for 6 h. 100 µg of Affigel 15 agarose-coupled GST- DEP-1

catalytic domains (WT/D1205A) were combined with precleared SW480 cell lysate (100 mg protein) over night, washed four times with buffer containing 50 mM HEPES, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 2 mM EGTA and boiled in 2x SDS sample buffer. Aliquots of the samples were separated by SDS-PAGE and analyzed my mass spectrometry and Western blotting.

Mass spectrometry

For the identification of interacting proteins by mass spectrometry, material bound to the Affigel 15 agarose-coupled GST- DEP-1 catalytic domains was separated by SDS-PAGE. Protein bands were cut, and subjected to a standard trypsin digestion procedure. Briefly, after reduction by DTT the gel pieces were treated with iodoacetamide. The excess of reagents was removed by three wash cycles with 25mM ammonium bicarbonate followed by 60% aqueous acetonitrile. After drying in a vacuum centrifuge the dehydrated gel pieces were swollen at 0 °C in a solution of 12.5 ng/µl trypsin (Serva, Heidelberg, Germany) in 25 mM ammonium bicarbonate for 30 minutes. The trypsin solution was replaced by 50 µl 25 mM ammonium bicarbonate and digestion was allowed overnight at 37 °C. The supernatant was transferred to fresh Eppendorf tubes and the gel pieces were subsequently extracted by incubation with 25 mM ammonium bicarbonate, 33% acetonitrile in 0.1% trifluoroacetic acid, and 60% aqueous acetonitrile. The supernatants from the extraction steps were combined with the supernatant from the digestion and dried in a vacuum centrifuge. The material was then dissolved in 5 µl of 5% acetonitrile in 0.1% trifluoroacetic acid. For MALDI mass spectrometry a dried droplet preparation was performed using 1 µl of sample and 1 µl of a saturated solution of alpha-cyano-4-hydroxycinnamic acid in 66% acetonitrile/0.1% trifluoroacetic acid. MALDI mass spectra were acquired with an UltraflexII instrument (Bruker, Bremen, Germany) and evaluated by the flexAnalysis and BioTools software packages (Bruker, Bremen, Germany) using the Swissprot database. The samples showing spectra different from background were

further analyzed by ESI mass spectrometry. To this end, the remaining sample from the trypsin digestion was diluted to 20 µl with 1% formic acid and injected into an Ettan nanoLC system (GE Healthcare, Freiburg, Germany) coupled on-line to a LTQ mass spectrometer (Thermo Scientific, Dreieich, Germany). The sample was separated with a Pepmap 75 µm x 15 cm C18 column (Dionex, Idstein, Germany) at a flow rate of 250 nl/min by application of a gradient from 5% to 65% acetonitrile in 0.1% formic acid. The measurements were evaluated by Mascot (Matrix Science, London, UK) using the Swissprot database.

SUPPLEMENTARY FIGURES AND TABLES

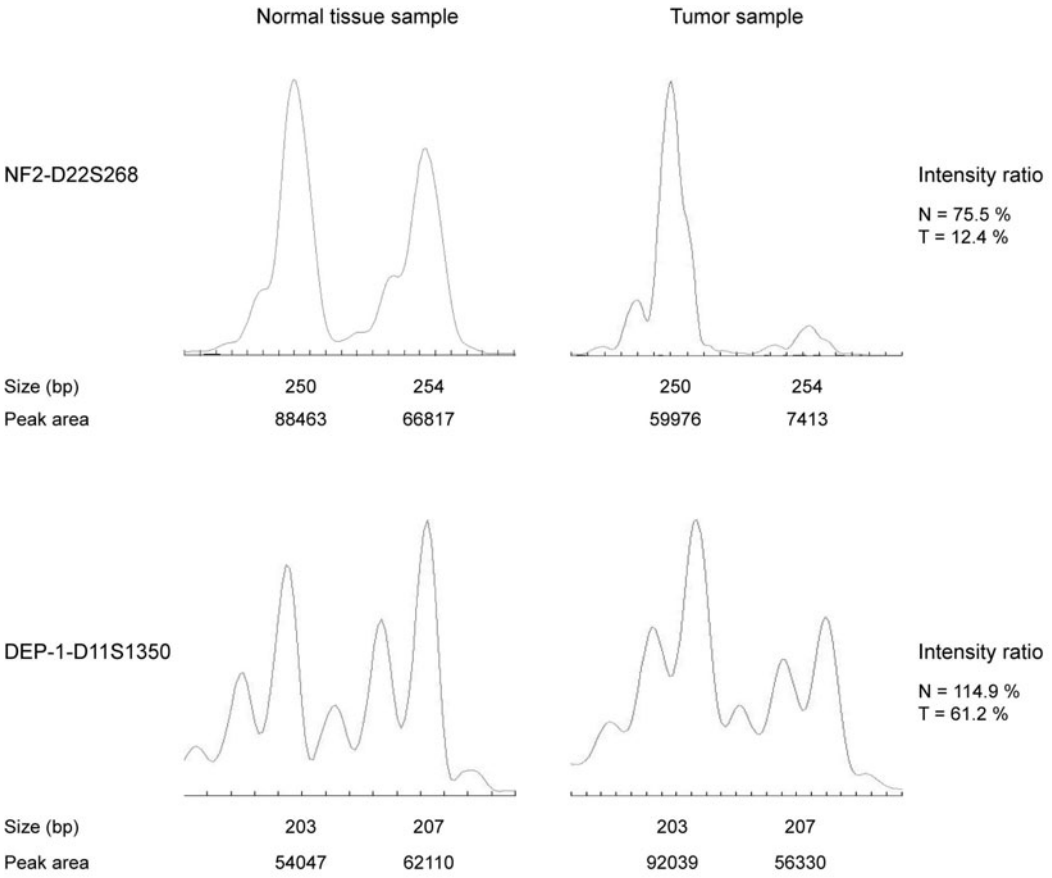

Figure S1. Examples for LOH analysis. Electropherograms of PCR products of two microsatellite markers located in the *NF2* and *DEP-1* region, respectively, are shown. A difference of 30% or more in the intensity ratio of the two alleles in tumor DNA compared to nomal tissue DNA was considered as LOH.

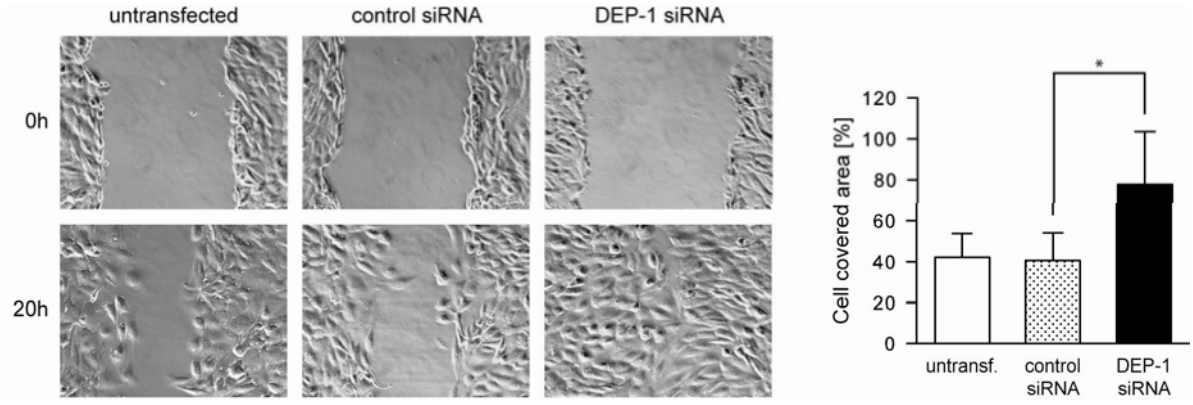

Figure S2. DEP-1 is a negative regulator of meningioma cell migration. Wounding assays with untransfected KT21 cells or cells transiently transfected with DEP-1- targeting or a control siRNA. The assays were performed 72 h after transfection. Images of three positions were taken 0 hours and 20 h after wounding, and the area of the wound covered with cells after 20 h was quantified (mean values ± s.d. of three independent experiments, * $P<0.05$ by *t*-test).

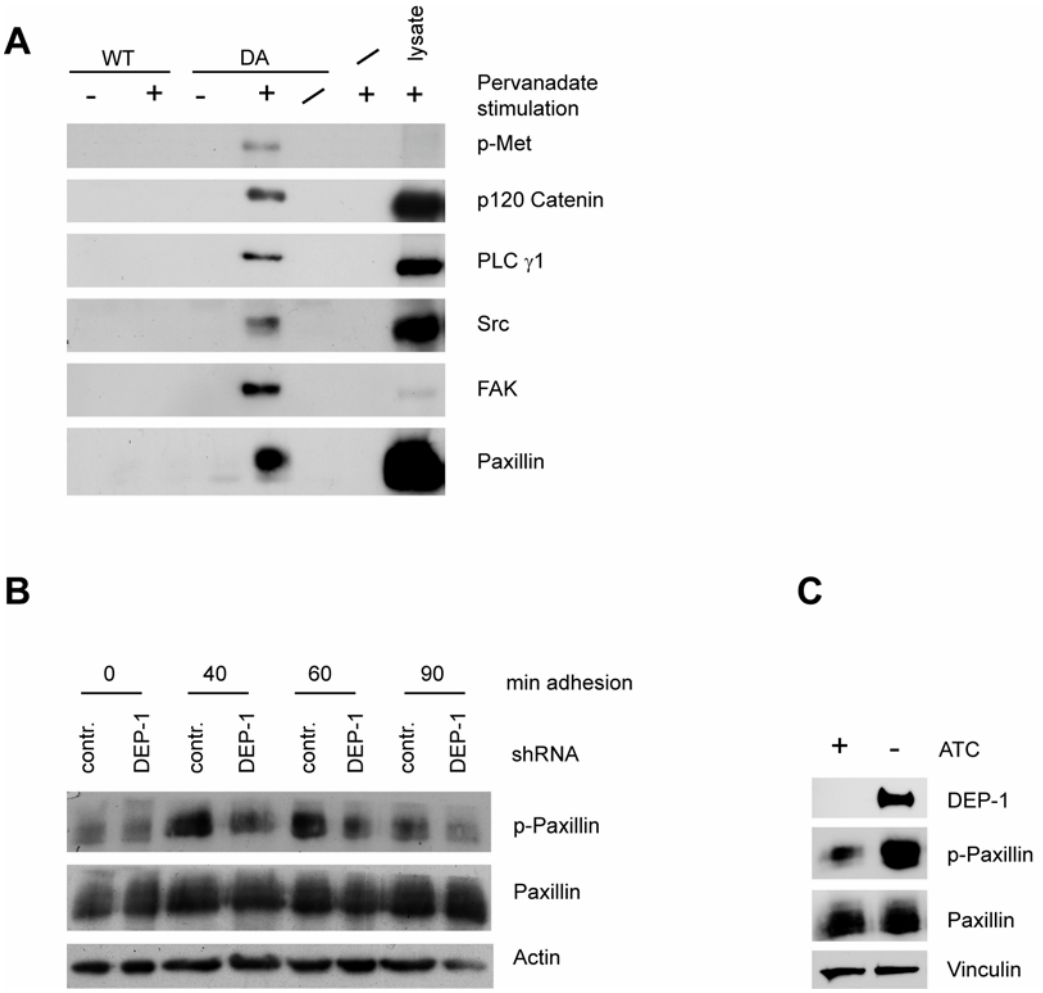

Figure S3. A DEP-1 trapping mutant binds to focal adhesion components, and DEP-1 expression positively correlates with paxillin phosphorylation. **A** GST fusion proteins of the DEP-1 catalytic domain (wildtype (WT) or D1205A trapping mutant (DA)) covalently coupled to agarose beads were incubated with lysates of pervanadate treated or untreated DEP-1 negative SW480 cells. As control, uncoupled beads were incubated with cell lysate (lane 6), and beads coupled to the DEP-1-D1205A catalytic domain were incubated with lysis buffer (lane 5). Trapped proteins eluted from the beads were separated by SDS-PAGE and analysed by immunoblotting. For comparison, SW480 lysate (approximately 1% of the substrate trapping input) was loaded on the gel. **B** HCT116 cells stably transfected with DEP-1-targeting or control shRNA expression constructs were trypsinized, starved in suspension and seeded in fibronectin-coated plates. After the indicated times, adherent cells were lysed

and lysates were analyzed by immunoblotting. **C** SW480 cells stably transfected with an inducible DEP-1 expression construct were grown in the presence (repressed expression) or absence (induced expression) of anhydrotetracycline. Cells were lysed and analysed by immunoblotting.

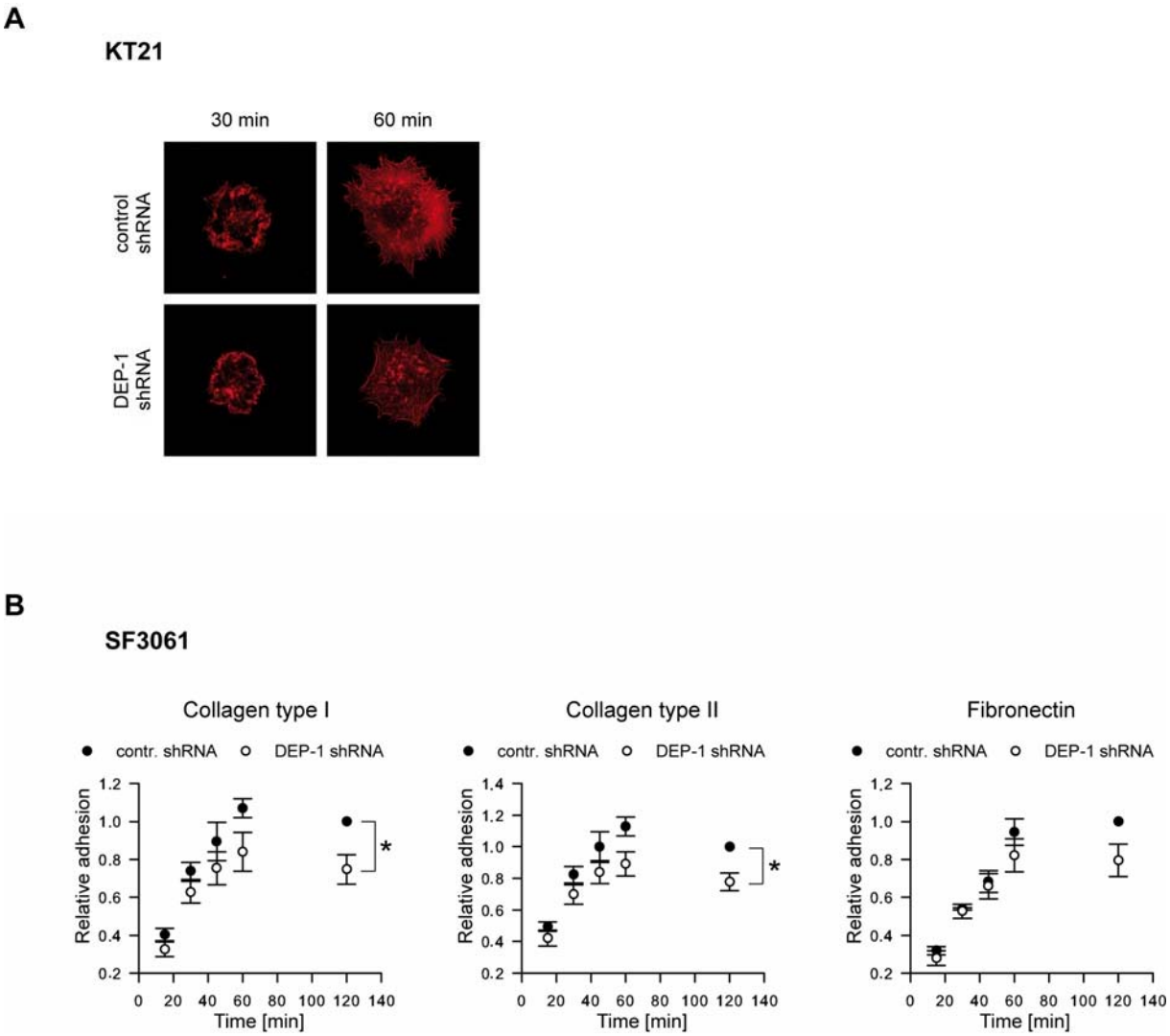

Figure S4. Impaired adhesion of DEP-1 depleted cells. **A** KT21 cells stably transfected with non-targeting shRNA, or with DEP-1-targeting shRNA, as indicated, were seeded on fibronectin-coated cover slips. After indicated times, cells were fixed and filamentous actin was stained with TRITC-phalloidin. A representative example is shown. **B** SF3061 cells

stably transfected with DEP-1-targeting or control shRNA expression constructs were fluorescently labeled with CellTracker™ Green CMFDA and starved for 1 h in suspension before they were seeded in 96-well plates coated with the indicated matrix proteins. At different time-points, non adherent cells were removed, adherent cells were lysed, and fluorescence of the lysates was determined. Measurements were performed in 6 replicates; fluorescence was normalized to the 120 min value of the control shRNA transfected cell line. Graphs display mean ± s.e.m. of four independent experiments; two-way ANOVA and Bonferroni's multiple comparison test was applied to test for significant differences between the two cell lines ($^{*}P<0.05$).

Table S1. LOH of *PTPRJ* and *NF2* in human meningiomas. Results for individual genetic markers.

PTPRJ

Marker	Number of tumors (informative, %)	LOH (%)
D11S4183	21 (81)	-
D11S4117	26 (100)	2 (8)
D11S1784	26 (100)	-
D11S1350	25 (96)	10 (40)

NF2

Marker	Number of tumors (informative, %)	LOH (%)
D22S929	18 (69)	6 (33)
D22S268	18 (69)	12 (67)
D22S421	24 (92)	4 (17)

Table S2. Histological features of tumors formed by KT21 cells in an orthotopic xenotransplantation model

	Tumor formation	Sheeth-like tumor growth	Brain infiltration	Bone infiltration	Tumor necrosis
control shRNA	5/5	5/5	1/5	2/5	0/5
DEP-1 shRNA	5/5	5/5	3/5	3/5	3/5