Supporting Information

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SI Materials and Methods

Cell Culture. Three different pancreatic ductal adenocarcinoma (PDAC) cell lines were chosen because of their mutation profiles. Bx.Pc3 has wild-type K-Ras, whereas both L3.6pl and Panc-1 have mutant K-Ras. However, L3.6pl represents highly metastatic PDAC and Panc-1 represents a dedifferentiated phenotype. Bx.Pc3, C6, and Panc-1 cell lines were obtained from the American Type Culture Collection and were grown in RPMI medium, Kaign's Modification of Ham's F-12 (F-12K) medium, and Dulbecco's Modified Eagle's Medium (DMEM), respectively. L3.6pl and HPDE were obtained from Craig Logsdon (University of Texas MD Anderson Cancer Center, Houston), and Han14.3 cell line was obtained from Nabeel Bardeesy (Harvard Medical School, Massachusetts General Hospital, Boston). L3.6pl was grown in DMEM; HPDE was grown in keratinocyte medium; Han14.3 was grown in RPMI. All media were supplemented with 10% (vol/vol) FBS, 1% penicillin-streptomycin (pen-strep), and 1% L-glutamine, except keratinocyte medium (supplemented with human recombinant epidermal growth factor 1-53 and bovine pituitary extract) and F-12K medium (supplemented with 2.5% FBS, 15% horse serum, 1% pen-strep, and 1% L-glutamine).

Antibodies. Primary antibodies were purchased from Abcam [plectin (E398P), CD63 (MEM-259), EEF1A2 (EPR1265(B)), CD55 (EPR6689), and RPL28]; R&D Systems [integrin β4 (422325)]; Cell Signaling Technology [Hsp90 (C45G5) and Alix (3A9)]; System Biosciences (Exosome antibody kit containing CD9, CD63, CD81, and Hsp70); and Proteintech (Rab27A and Rab27B). Horseradish peroxidase-conjugated secondary antibodies were purchased from R&D Systems. Primary antibodies (plectin, integrin β4, and Hsp90) were diluted 1:1,000 in 1% milk–Tris-buffered saline Tween 20 (TBST), and secondary antibodies were diluted 1:5,000 in 1% milk-TBST for immunoblotting.

Flow Cytometry. Trypsinized control and plectin-knockdown cells were incubated with biotin conjugated anti-plectin antibody (eBioscience) for 30 min on ice, washed 3 times with PBS, and then with Alexa Fluor 488-conjugated streptavidin (Invitrogen) for 30 min on ice. After antibody incubation and washing, the cells were fixed with 4% paraformaldehyde (PFA) for 15 min at room temperature. Cell nuclei were then stained with DAPI (Invitrogen). Flow cytometry using plectin-targeting peptide (PTP) was performed in a similar manner, except 10 μM PTP was used instead of antibodies. Cell fluorescence data and images were acquired using ImageStreamX Mark II (Amnis), and the data were analyzed using the ImageStream Data Analysis and Exploration Software (IDEAS).

PTP Binding on the Cell Surface. A total of 1×10^4 cells were seeded into wells of a 96-well plate and incubated at 37 °C and 5% CO_2 for 24 h. The cells were washed once with PBS and fixed with 4% PFA for 10 min. The cells were blocked with 1% BSA in PBS for 1 h and incubated with 1 μM fluorescent-coupled PTP in PBS–1% BSA for 2 h. Then, the cells were incubated with HRP-conjugated anti-fluorescein antibodies (Invitrogen) for 30 min. After washing, the cells were incubated with 3,3′,5,5′-tetramethylbenzidine (Sigma) for 10 min, and absorbance was measured at 650 nm.

Immunogold Transmission Electron Microscopy. The cells were washed with PBS, blocked with Aurion blocking solution (Aurion) for 1 h at room temperature and incubated with 5 μg/mL mouse anti-plectin antibody (Abcam, 7A8) or CD63 antibody (Abcam MEM-259) for 1 h at room temperature. The cells were washed with 0.1% BSA-c PBS (Aurion) 6× for 10 min and then incubated with 10-nm gold-conjugated anti-mouse IgG secondary antibody for 1 h at room temperature. The cells were washed with 0.1% BSA-c PBS 6× for 10 min and PBS 2× for 10 min, and fixed with 2.5% glutaraldehyde for 15 min. Cells were embedded and imaged via TEM.

Purification of Exosomes. Exosomes were collected from conditioned media by standard procedures either via ultracentrifugation as previously described (1–3) and also ExoQuick-TC (System Biosciences). For ultracentrifugation, the conditioned media was centrifuged at $300 \times g$ for 5 min to remove dead cells and at $12{,}000 \times g$ for 10 min to eliminate other cellular debris. Exosomes were pelleted by ultracentrifugation at $200{,}000 \times g$ for 16 h using a 45 Ti rotor (Coulter-Beckman). The exosome pellet was resuspended in 300 μL of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) buffer for TEM-negative staining and PBS or lysis buffer for other analyses. For ExoQuick-TC purification of exosomes, 10 mL of conditioned media were centrifuged at $300 \times g$ for 5 min to remove dead cells and at $12{,}000 \times g$ for 10 min to eliminate other cellular debris. The supernatant was then mixed with 2 mL of ExoQuick-TC and incubated at 4 °C overnight. The mixture was centrifuged at $1{,}500 \times g$ for 30 min, supernatants were removed, and centrifuged for additional 5 min to remove all fluid. The exosome pellet was resuspended in 100 μL of buffer.

TEM of Exosomes and Dynamic Light Scattering. A total of 10 μL of exosomes resuspended in Hepes buffer was placed on non–glow-discharged carbon-coated grids for 10 min and then stained with 10 μL of 2% uranyl acetate. After 1 min of incubation, the liquid was removed using filter paper and the grids were allowed to air dry. Digital dark-field images were recorded using a JEOL 1230 transmission electron microscope. For dynamic light scattering, exosomes resuspended in PBS were diluted in deionized water and the size distribution data were collected using Malvern Zetasizer Nano ZS90 (Malvern) and analyzed with Zetasizer Software.

Immunoblotting. Cells were grown in 10-cm Petri dishes (BD Falcon) to ∼70% confluence and were lysed in 500 μL of lysis buffer (1% Triton X-100, 1 mM EDTA, and 1 mM protease inhibitor mixture in PBS). Protein concentrations were determined using a bicinchoninic acid (BCA) protein assay kit (Thermo Scientific). Equal amounts (20 μg) of proteins in SDS sample buffer (containing β-mercaptoethanol) were resolved on 5% or 4–15% polyacrylamide gels (Bio-Rad) and transferred onto PVDF membranes for immunoblot analysis.

shRNA Lentiviral Transduction. Lentivirus vector encoding shRNA against human plectin and integrin β4 was obtained from Nabeel Bardeesy (Massachusetts General Hospital and Harvard Medical School, Boston, MA) and Santa Cruz Biotechnology, respectively. shRNA against mouse plectin was also obtained from Santa Cruz Biotechnology. shRNA against plectin isoforms 1a, 1c, and 1f were cloned into pLKO.1 puro by selecting regions of exon 1, which were obtained from The RNAi Consortium (TRC). The target sequences are: 5′-AGGGCCTCTGAGGGCAAGAAA-3′ for plectin-1a, 5′-TCTCTGAAGATGTCTCCAATG-3′ for plectin-1c, and 5′-AGGTGCGCGAGAAGTACAAAG-3′ for plectin-1f. shRNA against Rab27a and Rab27b were designed as described in Ostrowski et al. (4). Cells were infected with integrin β4 shRNA

lentiviral particles according to manufacturer's protocol. Plectin- and Rab27-knockdown cells were created using the same protocol. Briefly, cells were seeded on 12-well plates and grown to 50% confluence. Old media were replaced by media containing polybrene (4 μg/mL). Lentiviral particles were thawed at room temperature and gently mixed before adding to the cells. Three different ratios of virions to cells were used (1:1, 2:1, and 3:2). The infected cells were incubated overnight and media were replaced (without polybrene). Stably transfected cells were selected by puromycin (2 μg/mL for Bx.Pc3, 3 μg/mL for L3.6pl, 4 μg/mL for Panc-1, and 6.5 μg/mL for Han14.3) treatment. To further select stable clones, a single colony was isolated using the trypsin method (5). Down-regulation of each protein was verified via immunoblot.

Sample Preparation for Proteomics Analysis. For each cell line, 150 μg of exosome lysate was diluted with 50 mM ammonium bicarbonate buffer. Samples were then reduced with 10 mM DTT for 1 h at 50 °C, alkylated with 25 mM Iodoacetamide for 30 min at room temperature in dark, and reduced again with 10 mM DTT for 15 min at room temperature. Samples were loaded onto 3 kDa filter (Millipore) to concentrate and to wash twice with 50 mM sodium bicarbonate buffer. After washes, sample volumes were adjusted to 80 μL and digested with trypsin at 1:50 ratio for 18 h at 37 °C. After digestion, samples were collected by reverse spin and the filters were washed twice with 80 μL of 100 mM sodium acetate buffer (pH 5.5). For each cell line, the sample was combined with the washes and the pH was adjusted to pH 5.5 with 10% acetic acid. The parental L3.6pl exosomes were labeled with formaldehyde-H2 (light-label) and the exosomes from L3.6pl transduced with short hairpin RNA against plectin (L3.6pl shPLEC) was labeled with formaldehyde-D2 (heavy-label), respectively. To label each sample, 5 μL of 20% labeling agent was added to the samples, immediately followed by addition of 5 μL of freshly prepared 3 M sodium cyanoborohydride solution. The samples were incubated for 15 min at the room temperature, with vigorous vortex every few minutes. The light- and heavy-labeled samples were combined and purified through C18 purification columns (the Nest Group) following the manufacturer's instructions.

Mass Spectrometric Analysis. An LTQ-Orbitrap Elite mass spectrometer (Thermo Fisher Scientific) coupled with a nano-flow HPLC (Eksigent Technologies) was used in this study; 1.5 μg of sample was injected for the mass spectrometric analysis. The samples were first loaded onto a 1.5-cm trap column (IntegraFrit 100 μm; New Objective) packed with Magic C18AQ resin (5-μm, 200-Å particles; Michrom Bioresources) with buffer A (deionized water with 0.1% formic acid) at a flow rate of 3 μL/minute. The peptide samples were then separated by a 27-cm analytical column (PicoFrit 75 μm; New Objective) packed with Magic C18AQ resin (5-μm, 100-Å particles; Michrom Bioresources) followed by mass spectrometric analysis. A 60-min nonlinear LC gradient was used as follows: 5–7% buffer B (acetonitrile with 0.1% formic acid) versus buffer A over 2 min and then to 35% over 60 min. The flow rate for the peptide separation was 300 nL/min. The mass spectrometric method consisted of 1 MS scan followed by 10 data-dependent MS/MS scans. The MS1 scan was collected in the Orbitrap at a resolution of 120,000 in the profile mode (m/z range of 400–1,800). The dynamic exclusion settings were as follows: repeat count of 1, repeat duration of 15 s, exclusion list size of 500, exclusion duration of 30 s, with an exclusion mass width of 10 ppm. The preview mode for the Fourier Transform Mass Spectrometry master scans function was enabled, and charge state screening was enabled to reject charge states +1 and +4. Monoisotopic precursor selection was also enabled. The collision-induced dissociation fragmentation parameters were as follows: default charge state was 2+; isolation width of 2.0 m/z; normalized collision energy set at 35.0; activation Q was 0.25; and the activation time was 10 ms.

Mass Spectrometric Data Analysis. Raw machine output files of MS/MS spectra were converted to mzXML files and searched with X! Tandem (6) configured with the k-score scoring algorithm (7), against the UniProt human database. The search parameters were therefore as follows: enzyme: trypsin; maximum missed cleavages: 1; static modifications: carboxamidomethylation on cysteine, light dimethyl on N terminus and lysine; dynamic modifications: oxidation on methionine, difference between light and heavy dimethyl labeled on N terminus and lysine; parent monoisotopic mass tolerance: 2.5 Da. Peptide identifications were assigned probability by PeptideProphet (8). Relative quantitation of heavy and light peptide abundance was performed with Xpress (9) version 2.1. Proteins present in sample were inferred using ProteinProphet (8). To minimize interassay variation, the two exosome samples were labeled with different stable isotopes, combined, and analyzed together simultaneously in a single measurement for quantitative comparison.

Cell-Proliferation Assay. The number of viable cells was determined by quantifying ATP presence using the CellTiter-Glo Cell Viability Luminescent Assay according to the manufacturer's protocol (Promega).

APO-BrdU TUNEL Assay. The TUNEL assay was performed according to the manufacturer's protocol (A23210; Invitrogen). Briefly, cells were washed with PBS, then fixed with 1% PFA for 15 min on ice, and then incubated with ice-cold 70% ethanol overnight at −20 °C. The cells were washed with wash buffer twice, incubated with DNA-labeling solution containing reaction buffer, TdT enzyme, and 5-Bromo-2′-Deoxyuridine 5′-Triphosphate for 1 h at 36 °C, washed with rinse buffer twice, and then incubated with antibody solution containing AlexaFluor 488 dye-labeled anti-BrdU antibody. The cells were washed three times with rinse buffer and fluorescence was recorded using FLUOStar Omega microplate reader.

s.c. Tumor Growth and Intratumoral Injection of Exosomes. A total of 500,000 of the control cells were s.c.-injected into the left dorsal flank of male immunodeficient nude mice; 500,000 of the plectin-knockdown or Rab27a-knockdown cells were s.c.-injected into the right dorsal flanks. Tumor sizes were measured with a caliper and the volumes were calculated as $V = ab^2/2$ (a, longest diameter; b, shortest diameter). The same number of exosomes from indicated cell lines were injected into the tumors as described by Bobrie et al. (10). Briefly, exosomes were injected into tumors in 50 μl of PBS at 5 μg per tumor on days 3, 6, 9, 12, 15, and 18. Control tumor was injected with 50 μL of PBS on the same days. All in vivo experiments were performed according to a protocol approved by the University of Virginia Animal Care and Use Committee.

RNA Extraction and Reverse Transcriptase–PCR. Total cell RNA was extracted, and cDNA was synthesized using the FastLane Cell cDNA kit (Qiagen) according to the manufacturer's protocol. Human RNA samples were obtained from Todd Bauer (University of Virginia). PCR amplification of plectin using primers corresponding to individual isoforms. Reactions were repeated in triplicate in three independent experiments. Amplified cDNA fragments were resolved on agarose gel containing ethidium bromide and visualized under UV light (2% agarose gel was used for all plectin isoforms and 1% agarose gel was used for GAPDH). The primer sequences are provided in Table S2.

Migration and Invasion Assay. A transwell-migration assay (BD Biosciences) was used to determine cell migration and invasion. Briefly, the chambers were rehydrated with serum free media at 37 °C and 5% CO_2 for 2 h. After rehydration, 5×10^4 cells were plated in each chamber with serum-free media. The chambers

were placed into the wells containing media with 10% FBS and incubated overnight at 37 °C and 5% CO_2; 8.0-μm polyethylene terephthalate (PET) membrane chambers were used for migration, and matrigel-coated 8.0-μm PET membrane chambers were used for invasion. After overnight incubation, nonmigrating and noninvading cells were removed from the upper membrane surface with a cotton swab. The membranes were washed with PBS, fixed with 4% PFA, and stained with 1% crystal violet in 20% ethanol. To quantify migrating and invading cells, the membranes were imaged at 40× magnification, and the cell numbers were counted in five different fields of view in triplicate (15 measurements total for each group). Percentage of invasion was determined as number of invading cells/number of migrating cells × 100.

Plasmids and Transfections. pGR244 and pGR258, encoding C-terminal fusions of EGFP to full-length plectin-1a and plectin-1f inserts, respectively, were derived from pEGFP-N2 (Clontech) and have previously been described (11). Truncated plectin (PLEC-Trunc), corresponding to exons 1a-9 (containing the actin-binding domain of plectin which also serves as the N-terminal integrin β4 binding region of plectin), was generated by PCR using pGR244 as a template (forward primer: 5′-GCGAATTCACCATGTCTCAG-CACCGGCTCCGTGTG-3′, reverse primer: 5′-GCGGATCCGG-AACTTGCGCTCCTCAAAAGCAGCGG-3′). The PCR product was inserted into pEGFP-N2 using EcoRI and BamHI restriction sites. Plasmids were transfected into cells with Lipofectamine LTX (Invitrogen) according to the manufacturer's protocol. Transfection of the plasmids (pGR244, pGR258, and PLEC-Trunc) was verified via fluorescence microscopy.

Orthotopic Tumor Growth. A total of 750,000 of the control and plectin-knockdown L3.6pl cells were injected into the tail of the pancreas of 50 male immunodeficient mice (5 mice per group per time point); 750,000 of the control and plectin-knockdown Han14.3 cells were injected into the head of the pancreas of 40 male FVB mice (5 mice per group per time point). Pancreas and other organs with metastases were excised, and tumor size, volume, and weight were measured at each time point. Tumor sizes were measured with a caliper and the volumes were calculated as $V = ab^2/2$ (a, longest diameter; b, shortest diameter). Day 0 pancreas weight data were collected from wild-type pancreas without tumor injection.

Confocal Microscopy. A total of 1×10^5 cells were seeded on four-chamber slides (Lab-Tek), transfected with pGR244 and pGR258, and incubated at 37 °C and 5% CO_2 for 24 h. Chamber dividers were removed, and the cells were washed, fixed, and stained with 10 μg/mL wheat germ agglutinin. The slides were then mounted with ProLong Gold antifade reagent with DAPI (Invitrogen). The cells were imaged with Nikon a C1 confocal microscope equipped with a 60× oil immersion objective.

Immunoprecipitation. To detect plectin bound to integrin β4 on the cell surface, cells were incubated with antibodies to plectin for 4 h at 4 °C, washed three times with PBS, lysed, and incubated with Protein G magnetic beads (Invitrogen) for 4 h at 4 °C. Protein G beads were incubated with PBS containing 1% BSA overnight at 4 °C before incubating with lysate to prevent nonspecific binding. The antibody–antigen complex was captured with a magnet and the complex was washed four times with PBS. The complex was resuspended in Laemmelli sample buffer and boiled to disrupt the complex. The supernatant was then analyzed by immunoblotting following SDS/PAGE for integrin β4 binding to plectin. The whole-cell lysates were also evaluated for total plectin expression as a loading control.

Statistical Analysis. Results are presented as means ± SEM. All statistical analyses were performed using a one-way general linear ANOVA, followed by Tukey's test for pairwise comparisons, except for PTP binding on integrin β4-knockdown cells (Fig. 5*B*). For integrin β4-knockdown PTP-binding assay, two-tailed unpaired t test was performed. Significance was asserted at $P < 0.05$.

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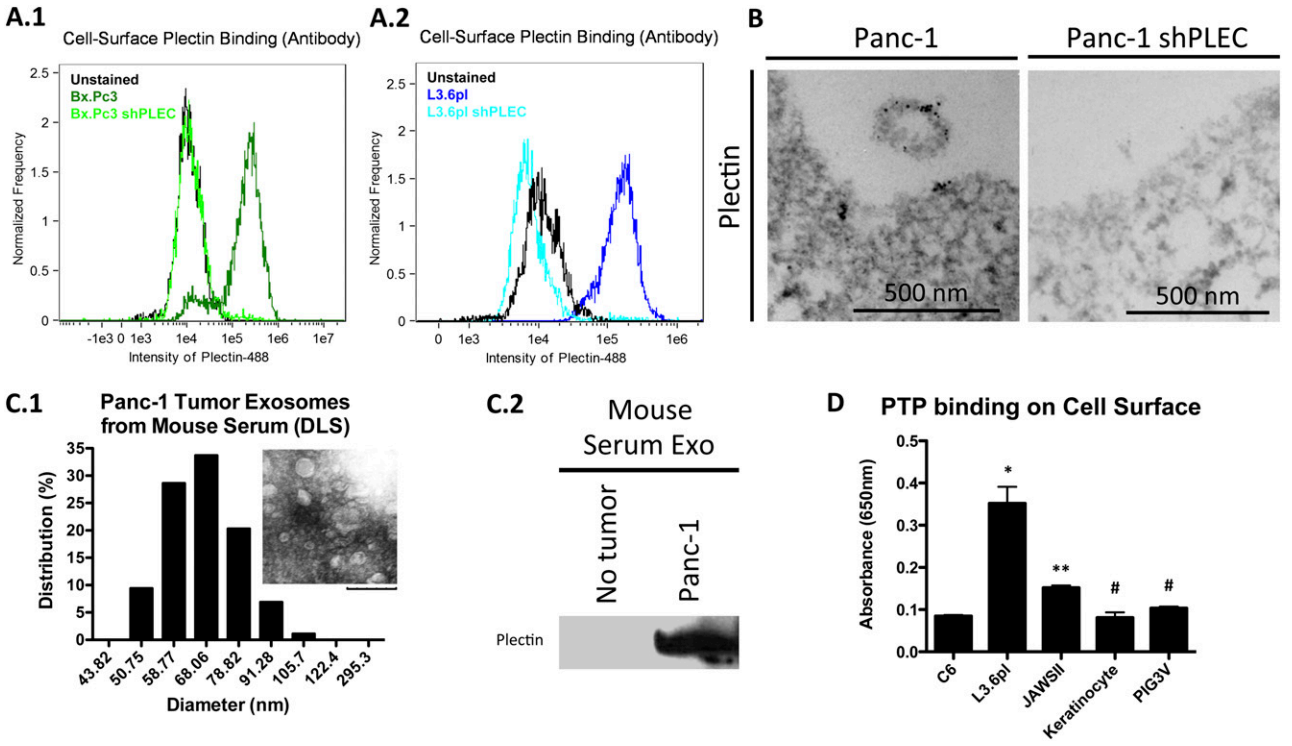

Fig. S1. (*A*) Flow cytometry of unfixed Bx.Pc3 and Bx.Pc3 shPLEC (*A1*), L3.6pl and L3.6pl shPLEC (*A2*). Data from 10,000 cells were collected for all groups. Cells were stained with biotin conjugated plectin antibody and Alexa Fluor 488 Streptavidin, and data were collected using ImageStream^X Mark II. (*B*) Additional TEM images for plectin immunogold staining. (Scale bar: 500 nm.) The images show plectin on the cell membrane and in the exosomes in the control cells but not in the plectin-knockdown cells. (*C1*) Exosomes collected from the serum of a Panc-1 tumor-bearing animal were examined by dynamic light scattering for size distribution and transmission electron microscopy (negative staining) for visualization. (Inset scale bar: 200 nm.) (*C2*) Immunoblot analysis of serum-collected exosomes from immune-deficient mice with and without Panc-1 xenografts. (*D*) PTP binding on the surface of C6, L3.6pl, JAWSII immature dendritic cells, normal human adult keratinocytes, and PIG3V melanocytes. *Significant to C6 ($P < 0.0001$); **significant to both C6 and L3.6pl ($P < 0.0001$); #significant to L3.6pl ($P < 0.0001$).

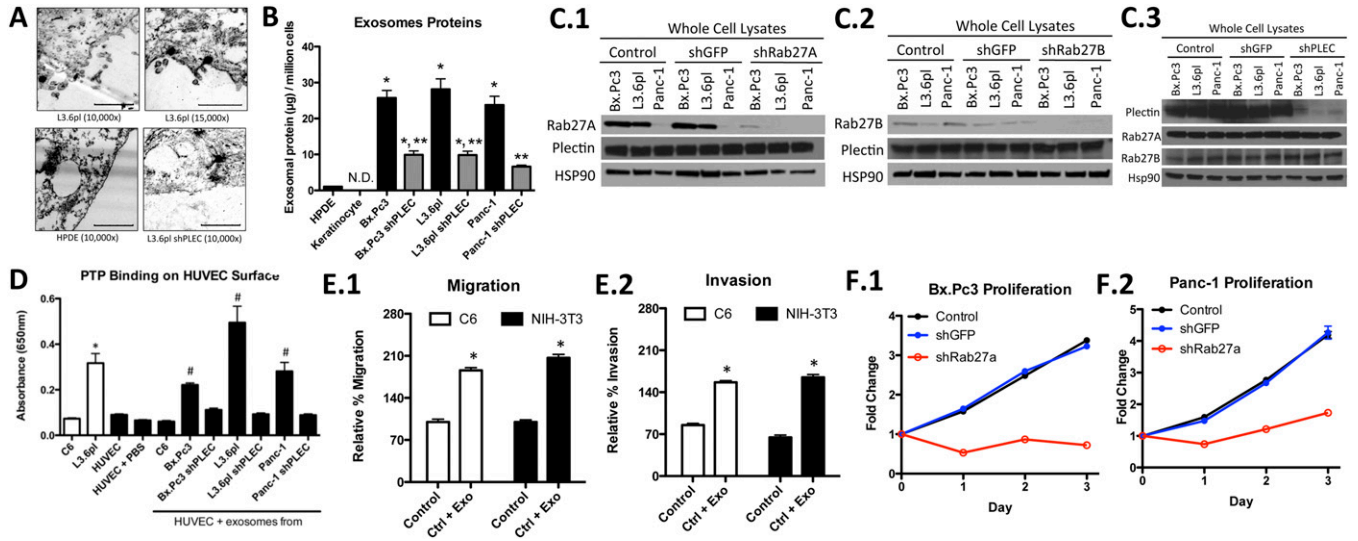

Fig. S2. (*A*) TEM of L3.6pl, HPDE, and L3.6pl shPLEC cells showing cell surface morphology. (Scale bar: 1 μm.) (*B*) NanoSight analysis showing exosome protein content (mean ± SEM). *Significant to HPDE ($P < 0.0001$); **significant to parental controls ($P < 0.0001$). (*C*) Immunoblot analysis showing that Rab27A (*C1*) and Rab27B (*C2*) knockdown does not affect plectin expression levels. (*C3*) Plectin knockdown does not affect Rab27A/B expression levels. (*D*) Cell surface PTP binding on HUVECs with or without exosome treatment. White bars are the controls (C6, negative; L3.6pl, positive). Black bars indicate respective cells treated with plectin-negative or positive exosomes. *Significant to C6 ($P < 0.0001$); #significant to C6, HUVEC, and HUVEC plus PBS ($P < 0.0001$). (*E1* and *E2*) Migration (*E1*) and invasion (*E2*) of C6 and NIH 3T3 cells treated with plectin-positive exosomes. *Significant to control ($P < 0.0001$). (*F*) Rab27A knockdown results in decreased PDAC proliferation. (*F1* and *F2*) Bx.Pc3 (*F1*) and Panc-1 (*F2*). Compared with the controls, Rab27a-knockdown cells grew significantly slower.

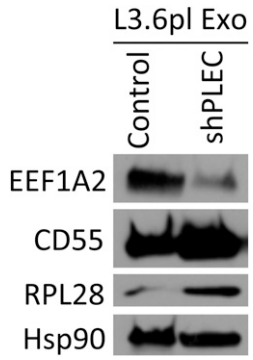

Fig. S3. Immunoblot verification of proteins underexpressed (EEF1A2) and overexpressed (CD55 and RPL28) in plectin-knockdown L3.6pl exosomes from mass spectrometric analysis.

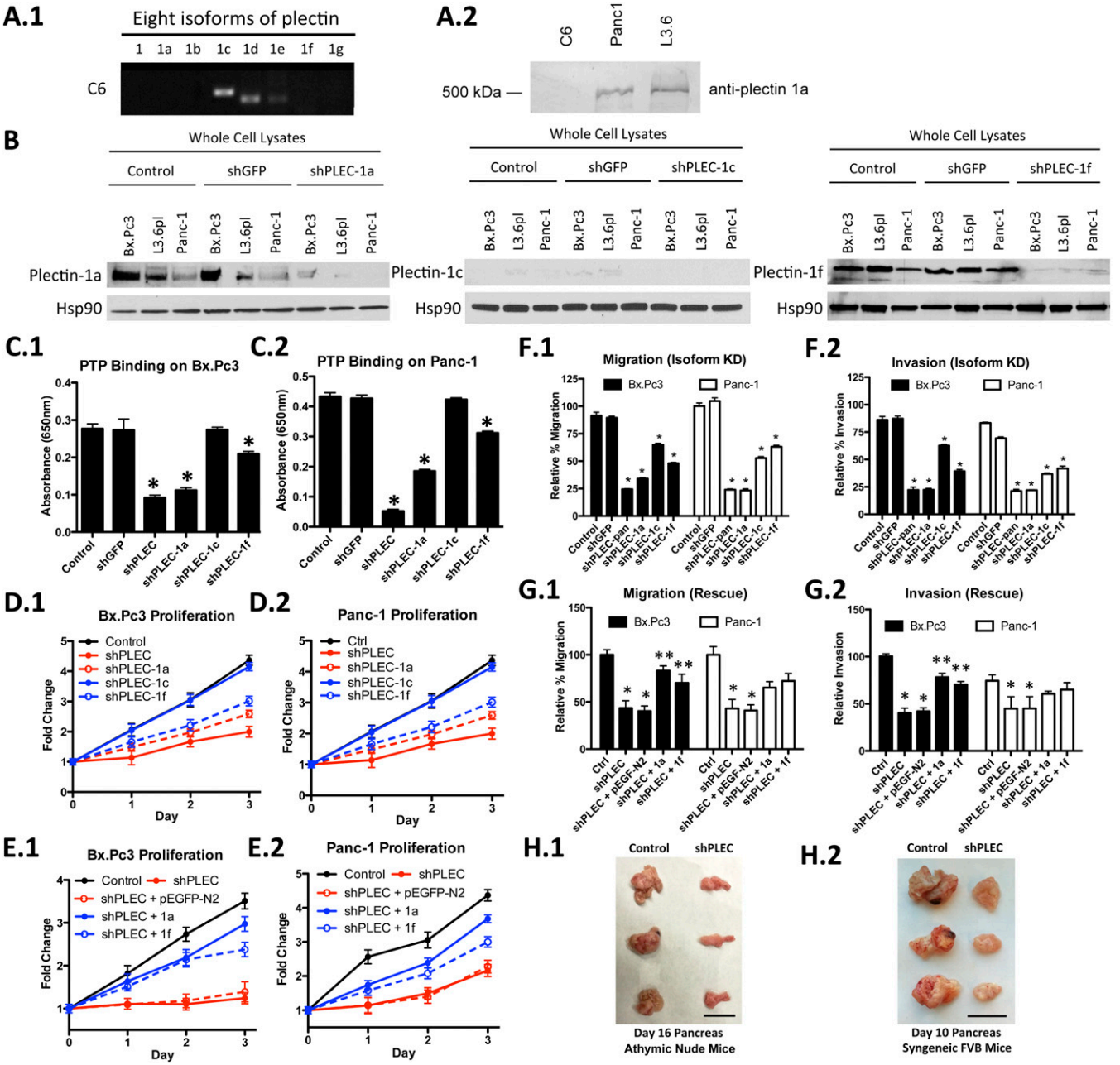

Fig. S4. (*A1*) PCR analysis of plectin isoform expression in C6 cells. C6 cells do not express plectin isoform 1a. (*A2*) Immunoblot analysis using antibodies specific for plectin isoform 1a shows that C6 does not express plectin-1a while Panc-1 and L3.6pl do. (*B*) Knockdown verification of plectin-1a, -1c, and -1f via immunoblotting. (*C*) Cell surface PTP binding on Bx.Pc3 (*C1*) and Panc-1 (*C2*) cell lines with or without plectin isoform knockdown. shRNA knockdown of pan-plectin and specific knockdown of plectin isoforms 1a and 1f resulted in a significant decrease in cell surface PTP binding. *Significant to both control and shGFP ($P < 0.0001$). (*D*) Proliferation of pan–plectin-knockdown and plectin isoform-specific-knockdown Bx.Pc3 (*D1*) and Panc-1 (*D2*) cells lines. (*E*) Proliferation of pan–plectin knockdown and plectin isoform-specific-knockdown Bx.Pc3 (*E1*) and Panc-1 (*E2*) cells lines with and without overexpression of plectin-1a or -1f. (*F*) Isoform-specific knockdown of plectin-1a and -1f resulted in significant reduction of migration (*F1*) and invasion (*F2*) in Bx.Pc3 and Panc-1 cell lines. *Significant to control ($P < 0.0001$). (*G*) Overexpression of plectin-1a and -1f increased migration (*G1*) and invasion (*G2*) in Bx.Pc3. However, transfection of plectin-1a and -1f did not result in significant changes in Panc-1 plectin-knockdown cells. *Significant to control ($P < 0.0001$); **significant to both shPLEC and shPLEC plus pEGFP-N2 ($P < 0.0001$). (*H1*) White light image of pancreas with L3.6pl and L3.6pl shPLEC tumors at day 16. (Scale bar: 20 mm.) (*H2*) White light image of pancreas with Han14.3 and Han14.3 shPLEC tumors at day 10. (Scale bar: 10 mm.)

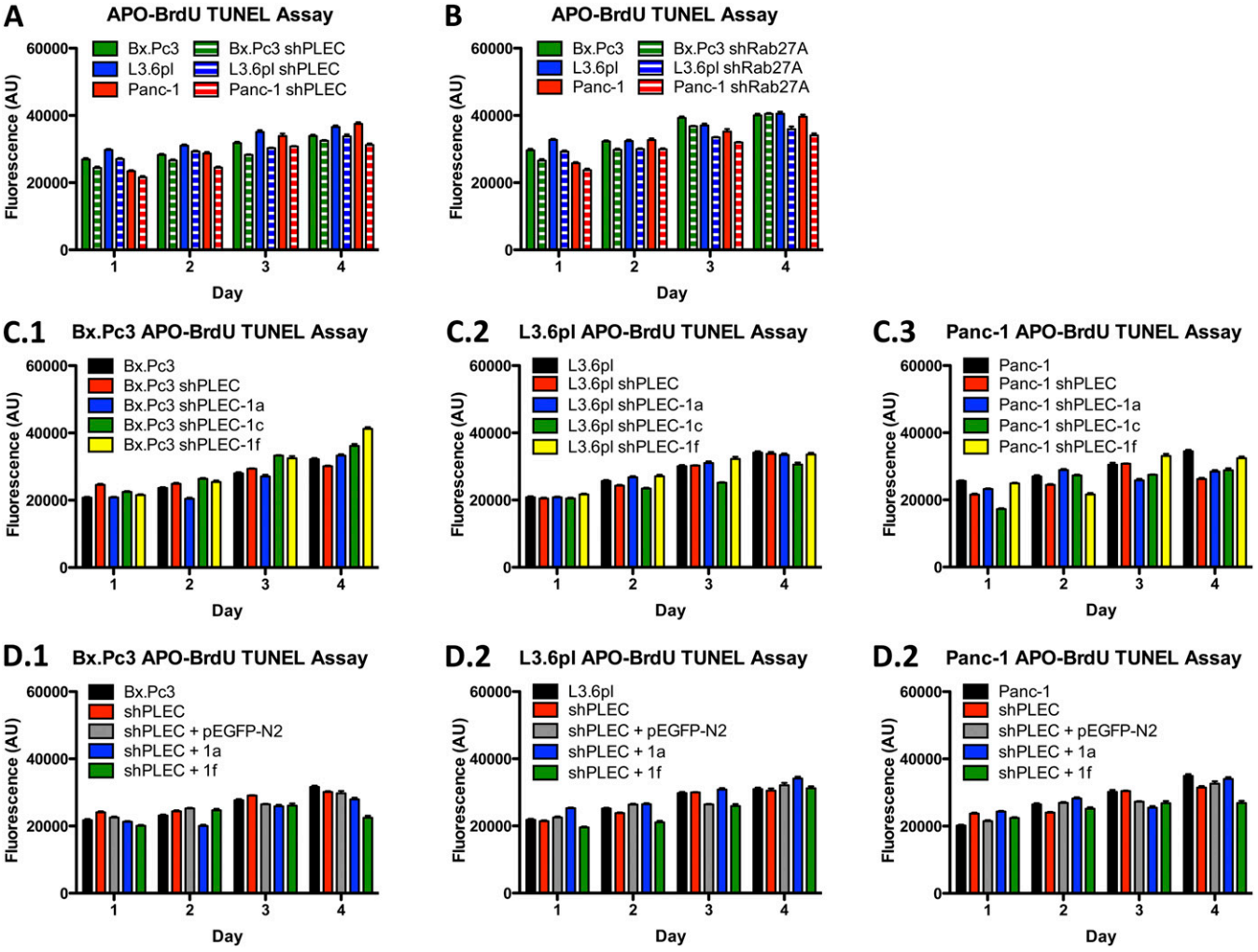

Fig. S5. TUNEL assay accompanying cell viability assay data. (*A*) APO-BrdU TUNEL assay on control and plectin-knockdown PDAC cell lines. (*B*) APO-BrdU TUNEL assay for Fig. 2*F1*: control and Rab27A-knockdown PDAC cell lines. (*C*) APO-BrdU TUNEL assay for Bx.Pc3 (*C1*), L3.6pl (*C2*), and Panc-1 (*C3*) cell lines with isoform specific knockdown. (*D*) APO-BrdU TUNEL assay for plectin-knockdown Bx.Pc3 (*D1*), L3.6pl (*D2*), and Panc-1 (*D3*) cell lines with isoform 1a and 1f rescue.

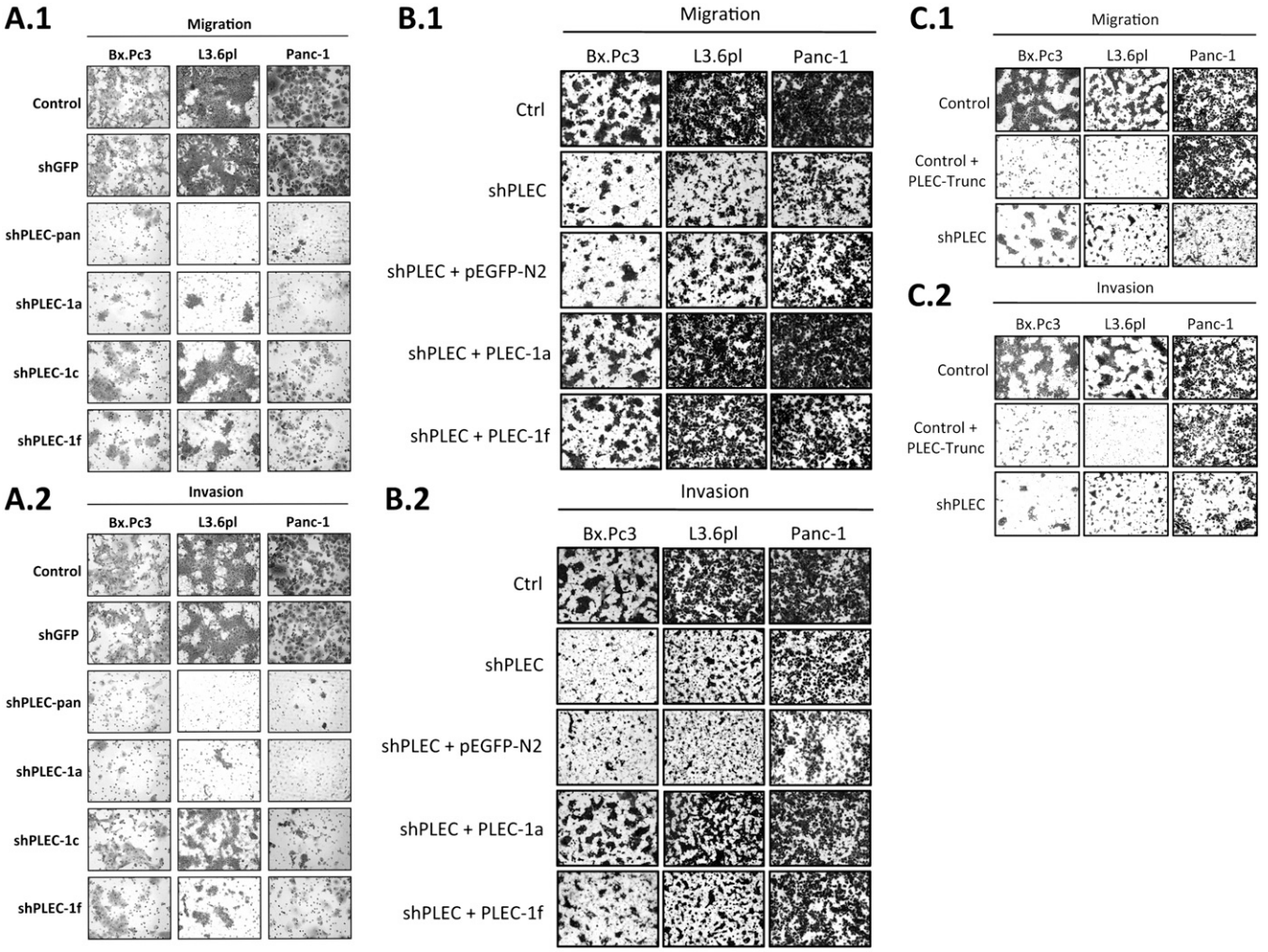

Fig. S6. Representative images of migration and invasion studies. (*A*) Representative images of migratory (*A1*) and invasive (*A2*) cells presented in Fig. 3 *D1* and *D2* (isoform knockdown experiments). (*B*) Representative images of migratory (*B1*) and invasive (*B2*) cells presented in Fig. 3 *D3* and *D4* (rescue experiments). (*C*) Representative images of migratory (*C1*) and invasive (*C2*) cells presented in Fig. 6*E* (PLEC-Trunc experiments in PDAC cells).

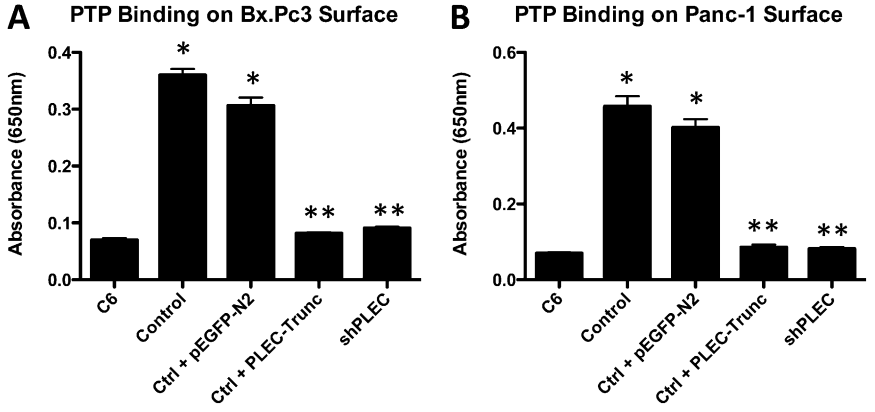

Fig. S7. Cell surface PTP binding of two additional PDAC cell lines expressing PLEC-Trunc. (*A* and *B*) Bx.Pc3 (*A*) and Panc-1 (*B*). When transfected with PLEC-Trunc, plectin expression on the cell surface decreased. *Significant to C6 ($P < 0.0001$); **significant to both control and control plus pEGFP-N2 ($P < 0.0001$).

Table S1. List of proteins from control and plectin-knockdown L3.6pl exosomes with twofold higher or lower difference

Gene name	Protein description	L3.6pl/L3.6pl shPLEC
SACM1L	SAC1 suppressor of actin mutations 1-like	20.43
RPL15	Ribosomal protein L15	13.79
PLEC1	Plectin	8.06
EEF1A2	Elongation factor 1-α2	7.99
KIF4A	Chromosome-associated kinesin KIF4A	7.42
SERPINE2	SERPINE2 protein	6.64
VWF	von Willebrand factor precursor	5.71
RPL8	Uncharacterized protein, 60S ribosomal protein L8	4.87
C7	Complement C7 precursor	4.37
DKFZp434N1526	NF-κB inhibitor-interacting Ras-like protein 2	3.83
SERPINF1	Similar to serine (or cysteine) proteinase inhibitor, clade F (α-2 antiplasmin, pigment epithelium-derived factor)	3.80
RAN	GTP-binding nuclear protein Ran	3.52
UBE1	Ubiquitin-activating enzyme E1	3.28
HBG2, HBE1	HBE1 protein, PRO2979, hemoglobin, γA	3.08
ADRM1	Protein ADRM1	3.01
EEF1B2	Elongation factor 1-β	2.97
APP	Amyloid βA4 protein isoform f precursor	2.96
AKR1C2	Aldo-keto reductase family 1, member C2	2.95
HMCN1	Isoform 2 of hemicentin-1 precursor	2.93
SPOCK1	Testican-1 precursor	2.54
CCT3	T-complex protein 1 subunit γ	2.26
KRT10	Keratin 10	2.24
STC1	Stanniocalcin-1 precursor	2.18
RRM1	Ribonucleoside-diphosphate reductase large subunit	2.17
IDH1	IDH1 protein	2.17
RPS20	40S ribosomal protein S20	2.16
EEF1D	Elongation factor 1-δ, eukaryotic translation elongation factor 1δ isoform 1; guanine nucleotide exchange protein	2.11
RPS3A	40S ribosomal protein S3a	2.11
SYNCRIP	Heterogeneous nuclear ribonucleoprotein Q	2.09
HSPA9B	Heat shock 70-kDa protein 9B	2.07
C5	Complement component 5	2.05
PCBP1	Poly(rC)-binding protein 1	2.03
MAT2A	*S*-adenosylmethionine synthetase isoform type-2	2.02
XRCC5	X-ray repair complementing defective repair in Chinese hamster cells 5	2.00
PYCARD	Apoptosis-associated speck-like protein containing a CARD	0.50
RPL28	Ribosomal protein L28	0.47
SLC2A1	Solute carrier family 2, facilitated glucose transporter member 1	0.42
CD55	CD55 molecule	0.41
TUFM	Elongation factor Tu, mitochondrial precursor	0.40
PRDX1	Peroxiredoxin-1	0.37
SERPINC1	Serpin peptidase inhibitor, clade C (antithrombin), member 1	0.34
NAP1L1	Nucleosome assembly protein 1-like 1	0.33
RPL10	60S ribosomal protein L10	0.31
APOE	Apolipoprotein E precursor	0.30
SERPINH1	Serpin H1 precursor	0.29
COL1A1	Collagen, type I, α1	0.27
COL4A2	Collagen α-2(IV) chain precursor	0.18
RPS9	40S ribosomal protein S9	0.16
DMBT1	Isoform 4 of deleted in malignant brain tumors 1 protein precursor	0.08
COL4A1	Collagen type IV α1, Collagen α-1(IV) chain precursor	0.08
YWHAG	14-3-3 protein γ	0.06
COBL	Isoform 6 of protein cordon-bleu	0.04

Table S2. Primer sequences for analyzing expression of plectin isoforms

Oligonucleotide	Forward sequence	Reverse sequence
Human PLEC-1	5′-GTGGTACCCTGCCTGCTG-3′	5′-GAGGTGCTTGTTGACCCACT-3′
Human PLEC-1a	5′-GCCGAAAGAGAACCAGCTC-3′	5′-GAGGTGCTTGTTGACCCACT-3′
Human PLEC-1b	5′-GTTGTGGGTCACGTTGTCAC-3′	5′-TGATGAGGTGCTTGTTGACC-3′
Human PLEC-1c	5′-TGTCTCCAATGGAAGCAGTG-3′	5′-TGATGAGGTGCTTGTTGACC-3′
Human PLEC-1d	5′-CCTGTGACCTCCCACACC-3′	5′-GAGGTGCTTGTTGACCCACT-3′
Human PLEC-1e	5′-TGGACTCTGCCTGGTGGT-3′	5′-GAGGTGCTTGTTGACCCACT-3′
Human PLEC-1f	5′-ACGAGCAGGACTTCATCCAG-3′	5′-GAGGTGCTTGTTGACCCACT-3′
Human PLEC-1g	5′-GAGGGAGGTCTTGCTGGAG-3′	5′-GAGGTGCTTGTTGACCCACT-3′
Rat PLEC-1	5′-GATCGTACCTGCCTCTCTGC-3′	5′-AGACACCACAGGGGTTTCAG-3′
Rat PLEC-1a	5′-GAGTTACTGTCCAGAGCGGC-3′	5′-GAGGTTGTCCTCTGAGCTGG-3′
Rat PLEC-1b	5′-CTGTACATTGTCCCCTGGCT-3′	5′-TGACAACATGACCCACGACT-3′
Rat PLEC-1c	5′-AGTGGCAGTGGCTGAAGGTA-3′	5′-CACAGAACCACCTCCACTCC-3′
Rat PLEC-1d	5′-ACAGGCTTGCTGCCTTAGAG-3′	5′-ACGATCTTCATTGGGGACAG-3′
Rat PLEC-1e	5′-ATTGATCAGCAGAAGCTCCG-3′	5′-ACTTAGGGGCTGAGAACGGT-3′
Rat PLEC-1f	5′-CATGGCCCATCTGCTGAC-3′	5′-ACGGTCTCGTTCATCTTTGT-3′
Rat PLEC-1g	5′-TCTTCACTTCGCAGAGGGAG-3′	5′-TACACAACACAGTTGCCCGT-3′
Mouse PLEC-1	5′-CTTGTGCTTAGAACCGGAGC-3′	5′-ACCTGTAGATTGGTGACGCC-3′
Mouse PLEC-1a	5′-AGCACCTGAGAACTGAGGGA-3′	5′-GAGGTTGTCCTCTGAGCTGG-3′
Mouse PLEC-1b	5′-TCGTGGGTCATGTTGTCACT-3′	5′-ACTGATGTGCCTCTGAGCCT-3′
Mouse PLEC-1c	5′-AAGTGGAGGTGGTTCTGTGG-3′	5′-ACTGATGTGCCTCTGAGCCT-3′
Mouse PLEC-1d	5′-CAGGCTTGCTGCCTCAGA-3′	5′-ACGATCTTCATTGGGGACAG-3′
Mouse PLEC-1e	5′-TGTGCAGTCCTGGACACAGT-3′	5′-TTTGAGGGAGCTGATCTCGT-3′
Mouse PLEC-1f	5′-GTTCTTCTCCCGCCAGCAT-3′	5′-TTGTACTTCTCCCGCACCTC-3′
Mouse PLEC-1g	5′-TCAGCCTTCAACACAAGCAC-3′	5′-CTGCGAAGTGAAGACTCCCT-3′