**SUPPLEMENTAL FIGURES**

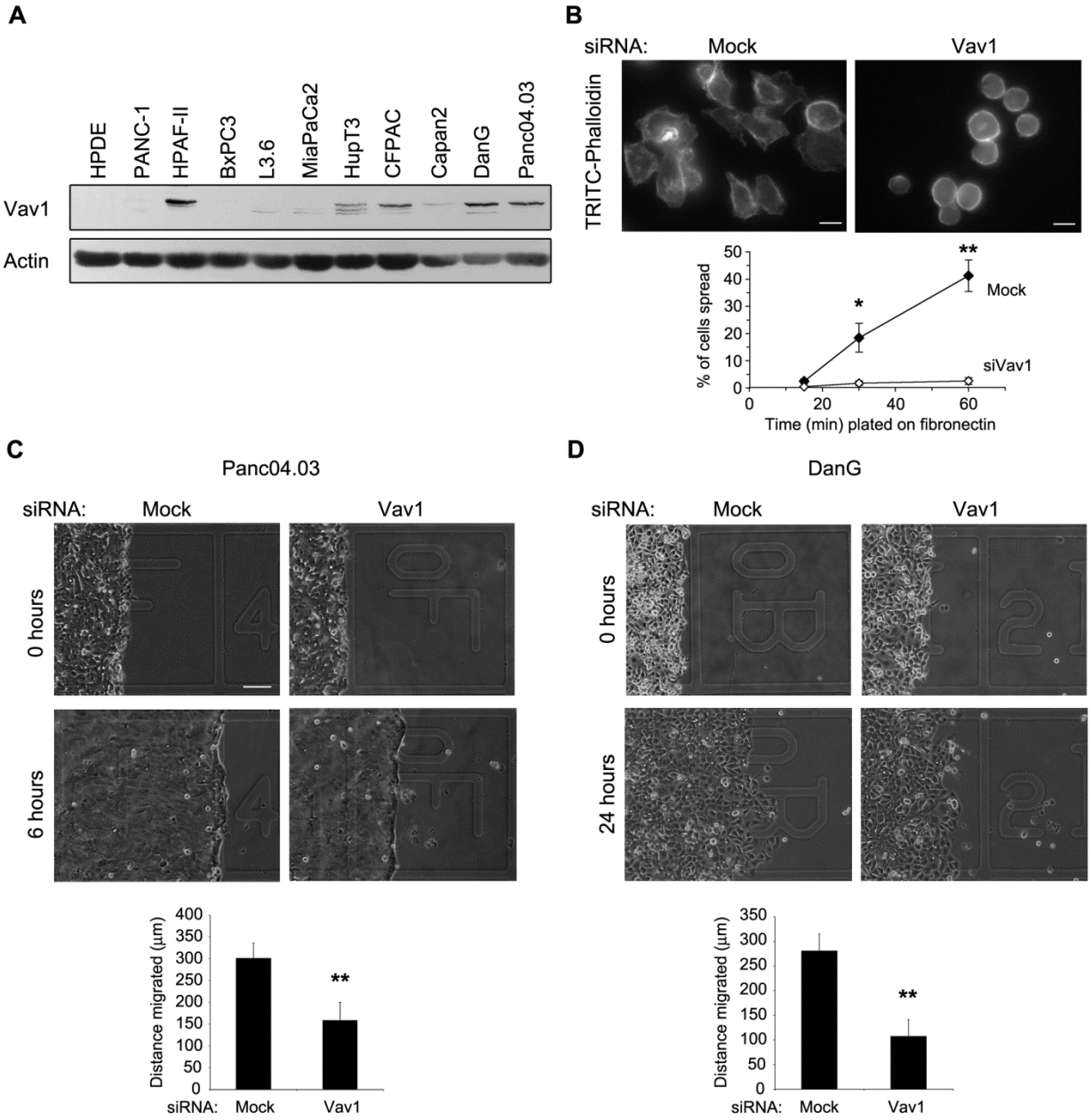


**Figure S1, related to Figure 3. Vav1 enhances the migratory properties of pancreatic cancer cells. (A)** Vav1 is ectopically expressed in pancreatic cancer cell lines. Ten different human pancreatic cancer cell lines were immunoblotted for Vav1 and compared to HPDE control pancreatic ductal epithelial cells. Vav1 is expressed in 80% of the cancer cell lines tested, but not in HPDE control cells. **(B)** Vav1 promotes cell spreading on fibronectin. DanG cells were depleted of Vav1 using siRNA, plated on fibronectin for 15-60 min, and fixed and

stained for actin. The percentage of spread cells was determined at each time point. Cell spreading was essentially blocked within one hour in the Vav1-knockdown cells. Scale bar = 10 μm. Graphed data represent the mean +/- SEM of three independent experiments. **(C)** Vav1 promotes pancreatic cancer cell migration in a wound healing model. Panc04.03 cells were depleted of Vav1 using siRNA, and grown to confluence on gridded coverslips. Cells were wounded by scratching with a pipette tip, and cells were imaged at t=0 hours and t=6 hours post wounding. The distance migrated was determined using iVision software. Scale bar = 100 μm. **(D)** DanG cells were treated as described in (B), but migration distance was measured after 24 hours. Graphed data indicate the mean +/- standard deviation of at least 30 measurements in one experiment, and are representative of three independent experiments. * indicates $p<0.05$; ** indicates $p<0.01$.

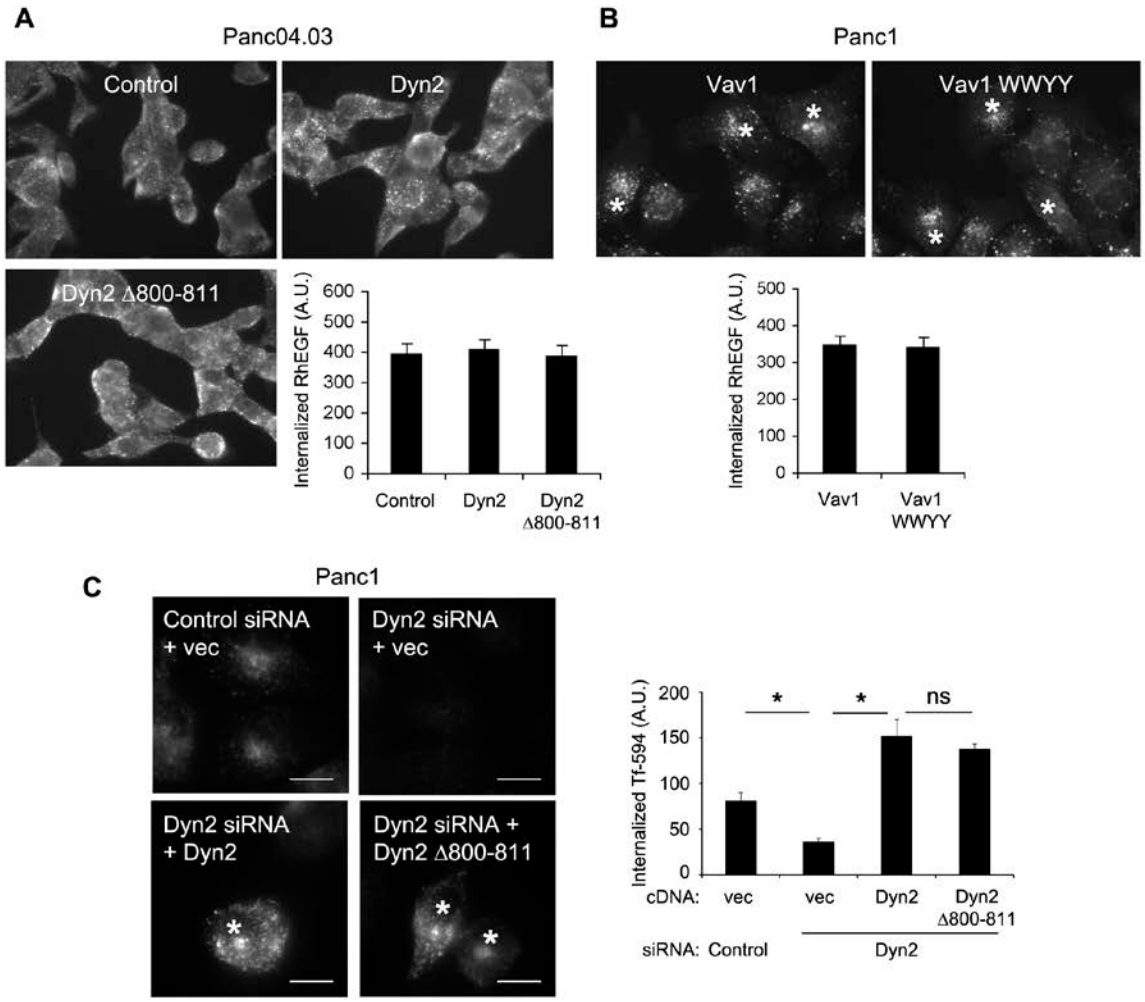


**Figure S2, related to Figure 4. Disruption of the Dyn2/Vav1 interaction does not alter endocytosis. (A)** Panc04.03 cells stably expressing Dyn2, Dyn2 Δ800–811, or a control vector were serum starved and stimulated with Rh-EGF (100 ng/ml) for 10 minutes. Surface Rh-EGF was acid-stripped, and internalized Rh-EGF was quantified. There was no difference in Rh-EGF uptake among the cell lines. **(B)** Panc1 cells were transfected with WT Vav1 or Vav1 WWYY, then serum-starved and stimulated with Rh-EGF for 10 minutes as described in (A). There was no difference in Rh-EGF uptake between cells expressing WT Vav1 or Vav1 WWYY. Asterisks indicate transfected cells. For (A) and (B), graphed data represent the mean +/- standard deviation of at least 60 cells in one experiment, and are representative of 3 independent experiments. **(C)** Dyn2 Δ800-811 is competent for endocytosis. Panc1 cells were transfected with siRNA against Dyn2 or a control siRNA, then transfected to re-express WT Dyn2 or Dyn2 Δ800-811. Cells were treated with transferrin-594 for 20 minutes, then acid stripped and

internalized transferrin was quantified. Asterisks indicate transfected cells. Both WT Dyn2 and Dyn2 Δ800-811 were able to rescue endocytosis defects in the Dyn2 knockdown cells. Graphed data represent the mean +/- standard deviation of at least 30 cells in one experiment, and are representative of 3 independent experiments. * indicates $p<0.05$. Scale bars=10 μm.

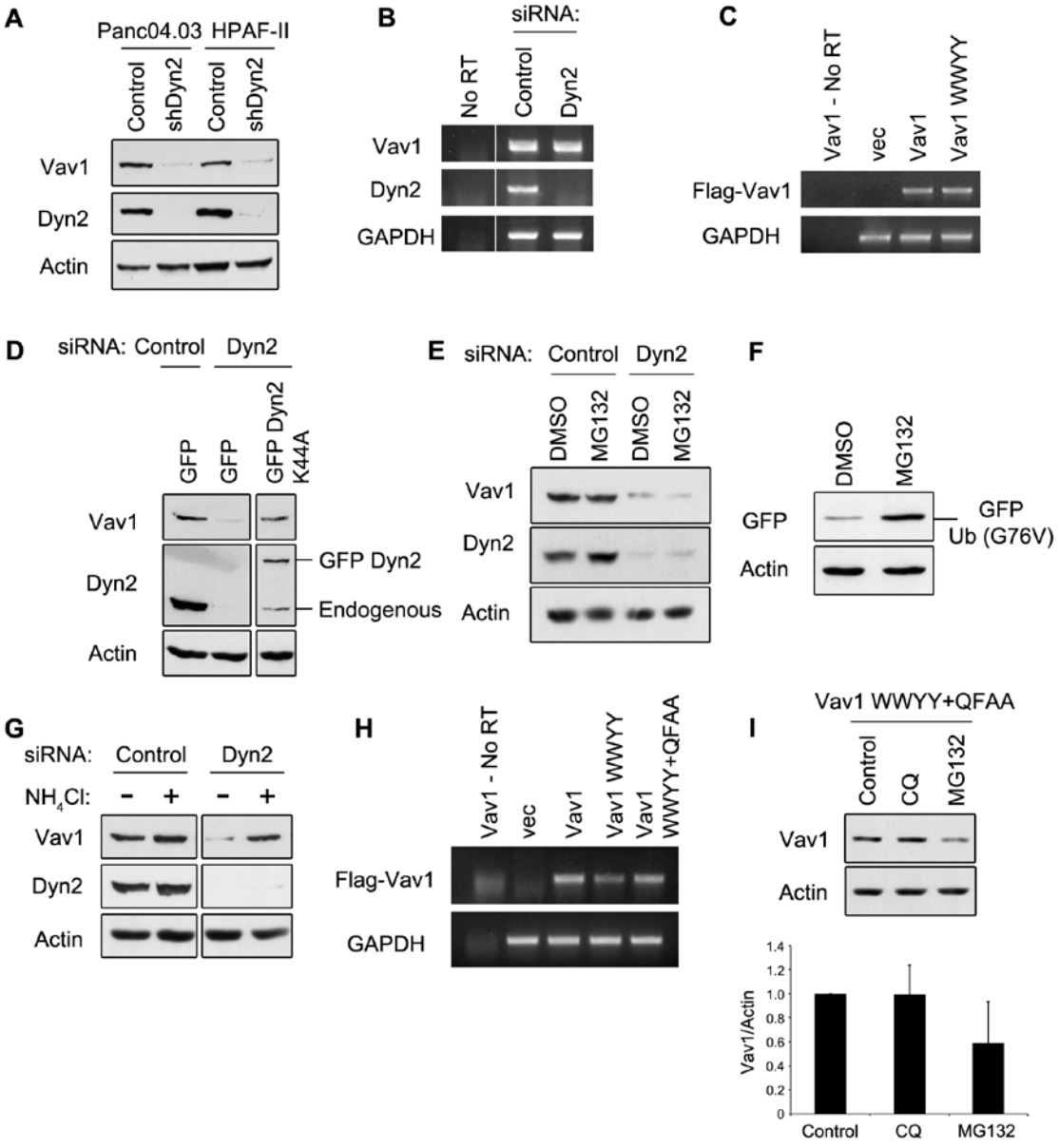


**Figure S3, related to Figure 5. Dyn2 regulates Vav1 protein stability. (A)** shRNA-mediated depletion of Dyn2 decreases Vav1 protein levels in multiple cell lines. Panc04.03 or HPAF-II pancreatic cancer cells were transduced with a lentivirus encoding an shRNA targeting human Dyn2, and lysates were immunoblotted for the indicated proteins. Depletion of Dyn2 reduced Vav1 levels by 80-90% in both cell lines. **(B)** Dyn2 does not regulate Vav1 transcription. DanG cells were transfected with either a control siRNA or siRNA targeting Dyn2. After 72 hours, mRNA was isolated and analyzed by RT-PCR for Vav1, Dyn2, or GAPDH as a loading control. Dyn2 message levels were efficiently reduced by the siRNA, but there was no change in Vav1

transcript levels. **(C)** WT Vav1 and Vav1 WWYY are transcribed at comparable levels. Panc1 cells were transfected with equal amounts of freshly prepared empty vector (vec), WT Vav1, or Vav1 WWYY. After 48 hours, mRNA was isolated and analyzed by RT-PCR for the transfected Vav1 using a 5' primer specific to the amino-terminal Flag epitope tag. GAPDH was used as a loading control. There was no difference in transcript levels between WT Vav1 and Vav1 WWYY. **(D)** Dyn2 GTPase activity is not required to stabilize Vav1. Panc04.03 cells were depleted of Dyn2 using siRNA, then were transfected to re-express GFP Dyn2 K44A. Lysates were immunoblotted for the indicated proteins. Re-expression of Dyn2 K44A restored Vav1 protein levels in the Dyn2 knockdown cells. **(E)** Inhibition of the proteasome does not block Vav1 degradation. Panc04.03 cells were depleted for Dyn2 using siRNA, then treated with the proteasome inhibitor MG132 (50 μM) or DMSO vehicle control for 6 hours, and lysates were immunoblotted for the indicated proteins. **(F)** Positive control for MG132 treatment. Panc04.03 cells were transfected with GFP-Ubiquitin (G76V), and treated with MG132 as described in (E), and immunoblotted for the indicated proteins. MG132 treatment did increase the protein levels of GFP-Ub (G76V). **(G)** Lysosomal inhibition using ammonium chloride inhibits Vav1 degradation. DanG cells were depleted of Dyn2 using siRNA, then were treated with 15 mM $NH_4Cl$ for 48 h. Lysates were immunoblotted for the indicated proteins. **(H)** Vav1 WWYY and Vav1 WWYY+QFAA are transcribed at comparable levels. Panc1 cells were transfected with equal amounts of the indicated constructs, and were processed for RT-PCR as described in (C). There was no observed difference in transcript levels among the Vav1 constructs. **(I)** Vav1 WWYY+QFAA is insensitive to lysosomal degradation. Panc1 cells were transfected with Vav1 WWY+QFAA and treated with chloroquine (25 μM, 48h) or MG132 (50 μM, 8h). Vav1 levels were normalized to actin and are represented as the mean +/- standard error of five independent experiments.

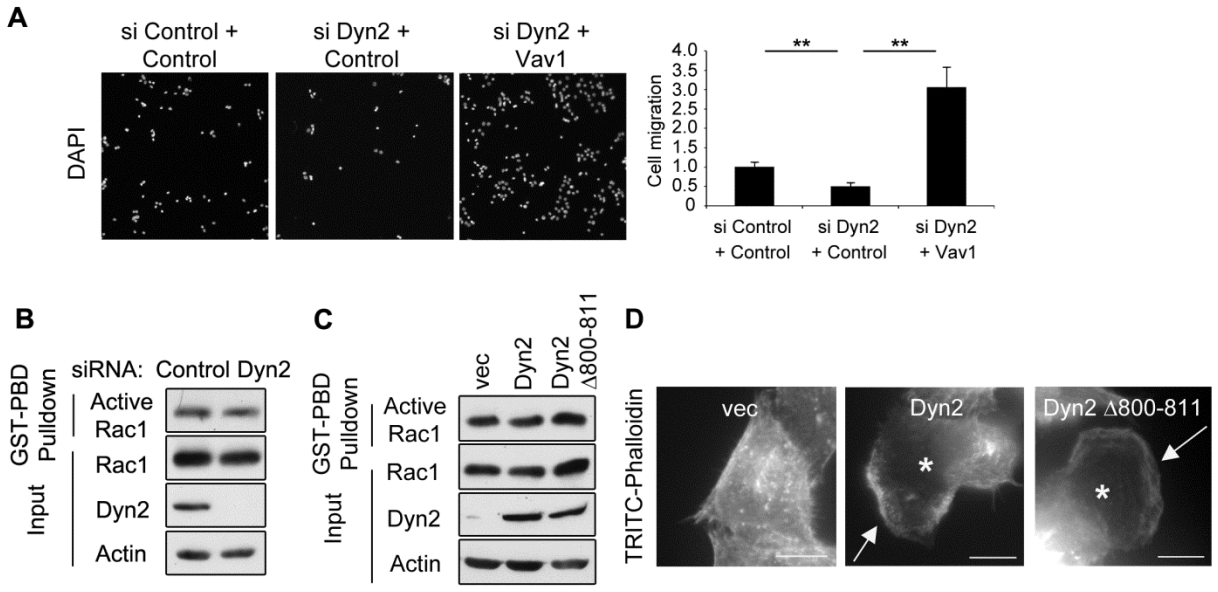


**Figure S4, related to Figure 7. Dyn2 regulates Rac1-mediated migration through modulating Vav1 protein levels. (A)** Re-expression of Vav1 restores defects in cell migration in Dyn2 knockdown cells. Panc04.03 cells were depleted of Dyn2 using siRNA, and then cells were transduced with either a control lentivirus or a virus encoding Vav1 to overcome the loss of Vav1 protein stability. Cells were seeded in transwell migration assays and incubated for 16 hours before being stained with DAPI. The number of cells that migrated through the filter was determined. Three fields from two independent experiments were scored, and the mean +/- standard deviation of the six fields is shown graphically. **(B)** Knockdown of Dyn2 does not impair Rac1 activation in cells that do not express Vav1. Panc1 cells, which do not express Vav1, were transfected with a control siRNA or siRNA against Dyn2, and Rac1 activation was assessed by GST-PBD pulldown. **(C)** Dyn2 Δ800-811 does not affect Rac1 activation in cells that do not express Vav1. Panc1 cells were transfected with empty vector (vec), WT Dyn2, or Dyn2 Δ800-811 and subjected to a GST-PBD pulldown to assess Rac1 activation. **(D)** Dyn2 Δ800-811 does not affect lamellipod formation in cells that do not express Vav1. Panc1 cells were transfected with empty vector (vec), WT Dyn2, or Dyn2 Δ800-811, serum-starved, and stimulated with EGF (50 ng/ml) for 20 min, and were stained for myc-Dyn2 (not shown) or actin. Lamellipodia are indicated by arrows; asterisks indicate transfected cells. Scale bar = 10 μm.

| Vav1 status: | Dyn2 status: | Percent of patients |
|---|---|---|
| Negative | Low | 8.2 |
| Negative | High | 34.7 |
| Positive | Low | 12.2 |
| Positive | High | 44.9 |

**Table S1, related to Figure 2. Dyn2 and Vav1 are co-upregulated in human pancreatic tumors.** Forty-nine pancreatic cancer patients were screened for both Dyn2 and Vav1 expression by immunohistochemistry. Note that 45% of tumors were positive for both Vav1 and Dyn2 overexpression.

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### siRNA and Plasmid Transfections

siRNA transfections were performed using Lipofectamine RNAi Max (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. siRNA targeting human Dyn2, human Vav1, and a nontargeting control siRNA were purchased from Dharmacon (Thermo Fisher Scientific, Lafayette, CO). Sense strand sequences: Vav1 CGUCGAGGUCAAGCACAUU; Dyn2 GACAUGAUCCUGCAGUUCA; Lamp2a: GGCAGGAGUACUUAUUCUAUU (Seki et al., 2012).

Plasmid transfections were performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. GFP-Rac1-V12 was a gift of Dr. Bruce Horasdovsky (Mayo Clinic, Rochester, MN). Human Vav1 (pCDNA3.1 Flag) constructs were described previously (Gomez et al., 2005). Of note, in order to obtain comparable protein levels of WT Vav1 and Vav1 WWYY due to differences in protein stability, at least 10-fold higher amounts of Vav1 WWYY cDNA were transfected compared to WT Vav1 for Rac1 activation assays.

Mutation of QF 762/763 to AA was performed using the primers: forward 5'-CTGGACACCACCTTGGCGGCCCCCTTCAAGGAGCCTGAAAAG-3', reverse 5'-CTTTTCAGGCTCCTTGAAGGGGGCCGCCAAGGTGGTGTCCAG-3'. Myc-tagged rat Dyn2 was generated by amplifying Dyn2 from pCR3.1 (Cao et al., 1998) using the primers: forward 5'- AAGCTTGGCACCATGGGCAACCGCGGGATGGAAGAG-3', reverse 5'-GAATTCCCCCCCAGAACACTGTCCCCTGCAGCCTAGTCGA-3'. The PCR product was digested with HindIII and EcoRI for cloning into the pCDNA3.1 myc-His B vector (Invitrogen, Carlsbad, CA). Rat Dyn2 Δ800−811 was generated in pCDNA3.1 myc-Dyn2 by deleting amino acids 800-811 using the primers: forward 5'-CCTCATTCCTATGCCTGTGGGGCGG<u>CCCGGG</u>CCACAGAATGTGTTTGC-3', reverse 5'-GCAAACACATTCTGTGG<u>CCCGGG</u>CCGCCCCACAGGCATAGGAATGAGG, with the underlined sequence introducing a silent SmaI restriction site. mCherry Vav1 was generated by cloning Vav1 from pCDNA3 into mCherry C3 vector (Clontech, Mountain View, CA).

Panc04.03 cell lines stably expressing Dyn2 or Dyn2 Δ800−811 were generated using the Tsin-PGK lentiviral vector, a gift from Dr. Jan van Deursen (Mayo Clinic, Rochester, MN).

Virus was packaged in 293T cells, and collected 48h after transfection. 48 hours after viral transduction, Panc04.03 cells were selected with 1 μg/ml puromycin for 3 weeks to select for stable expression.

**Co-immunoprecipitation, GST-pulldowns, and Western blots**

Reagents were purchased from Sigma Aldrich (St. Louis, MO), unless otherwise indicated. For analysis of protein in cell lysates, cells were lysed in NP-40 lysis buffer (20 mM Tris-HCl (pH 8), 137 mM NaCl, 1% NP-40, 10% glycerol, 2 mM EDTA, 2 mM $Na_3VO_4$, 15 mM NaF, and Complete protease inhibitors (Roche, Indianapolis, IN)), and protein concentration was determined by BCA assay (Thermo Fisher Scientific, Lafayette, CO). 50μg of soluble protein was resolved by SDS-PAGE, transferred to PVDF membrane, and blocked using 5% milk in PBS. Primary antibodies were: anti-Dyn2 (Henley et al., 1998), MC63 anti-Dynamin (Henley and McNiven, 1996), anti-Vav1 (monoclonal, Novus Biologicals, Littleton, CO), anti-Vav1 (polyclonal) (Billadeau et al., 2000), anti-actin (Sigma Aldrich, St. Louis, MO), anti-Rac1 (Millipore, Billerica, MA), anti-Hsc70 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-GFP (Roche, Indianapolis, IN), anti-Lamp2a (Abcam, Cambridge, MA). Secondary antibodies conjugated to horseradish peroxidase were obtained from Biosource (Invitrogen, Carlsbad, CA). Immunoreactive bands were detected using SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific, Lafayette, CO). Bands were quantified using Molecular Analyst Software (Bio-Rad, Hercules, CA), and images were processed using Adobe Photoshop. Adjustments were applied uniformly over the entire image.

For immunoprecipitation of endogenous Vav1 and Dyn2, or Vav1 and Hsc70, DanG cells were lysed in hypotonic lysis buffer (10 mM HEPES pH7.5, 10 mM NaCl, 1 mM $KH_2PO_4$, 5 mM $NaHCO_3$, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM EDTA, 10 mM sodium pyrophosphate, 1 mM $Na_3VO_4$, 1 mM PMSF, and complete protease inhibitors (Roche, Indianapolis, IN)). Lysates were first pre-cleared with Protein G beads (Santa Cruz, Santa Cruz, CA). Over 1 mg total protein was immunoprecipitated with 7.5 μg anti-Vav1 antibody (Novus Biologicals, Littleton, CO) and Protein G beads, or with Protein G beads alone as a control. 50 μg of lysate was loaded as an input control. Samples were resolved by SDS-PAGE and immunoblotted as described above. For Vav1/Hsc70 binding experiments, co-precipitated Hsc70 was normalized to immunoprecipited Vav1, and compared to control cells.

GST fusion proteins were expressed in E. coli BL21 cells and purified using glutathione beads (Amersham-Pharmacia, GE Healthcare, Piscataway, NJ ) according to the manufacturer's protocol. For pulldowns from mammalian cell lysate, cells were lysed in TEN100 lysis buffer (20 mM Tris (pH 7.4), 0.1 mM EDTA, 100 mM NaCl, Complete protease inhibitors (Roche, Indianapolis, IN)), and at least 1 mg lysate was incubated with GST protein-conjugated beads for 1.5 h at 4°C. Beads were washed with NTEN300 buffer (20 mM Tris (pH 7.4), 0.1 mM EDTA, 300 mM NaCl, 0.05% NP-40, Complete protease inhibitors (Roche, Indianapolis, IN)), and proteins were eluted by boiling with 1X SDS-PAGE sample buffer and resolved by SDS-PAGE. 30-50 μg of the lysate was loaded as an input control. Pulldowns using purified protein were performed similarly. His-Dyn2-PRD was purified using $Ni^{2+}$-coated beads (Roche, Indianapolis, IN) according to the manufacturer's protocol, and 3.4 μg of purified protein was incubated with GST-Vav1-SH3 for 1 h in TEN100 buffer, then washed as above with NTEN300.

For protein turnover assays, DanG cells were transfected with siRNAs as described above. 72 h post-transfection, cells were treated with cycloheximide (50 μg/ml, Sigma Aldrich, St. Louis, MO) for 0-6 h, and then were lysed in NP-40 lysis buffer as described above and analyzed by western blot. Vav1 levels were determined by immunoblotting and were normalized to actin at each time point, and compared to t=0. For lysosomal inhibition, cells were treated with chloroquine (25 μM, Sigma Aldrich, St. Louis, MO) or $NH_4Cl$ (15 mM, Sigma Aldrich, St. Louis, MO) 6 hours following transfection, and for 48h prior to lysis. For proteasome inhibition, cells were treated with MG132 (50 μM, Sigma Aldrich, St. Louis, MO) for 6-8 h prior to lysis. As a positive control for proteasome inhibition, cells were transfected with GFP-Ub (G76V) (Addgene plasmid 11941, (Dantuma et al., 2000) and protein levels were assessed in the presence or absence of MG132.

**Rac1 Activation Assays**

GST-PAK1-PBD (generously provided by Dr. Vijay Shah, Mayo Clinc, Rochester, MN) was transformed into *E. coli*, lysed in PBD lysis buffer (PBS containing 10 mM DTT, 1% Triton X-100, 10 mM $MgCl_2$, and Complete protease inhibitors (Roche, Indianapolis, IN)), and purified using glutathione beads (Amersham-Pharmacia, GE Healthcare, Piscataway, NJ). For pulldown assays, cells from one 100-mm dish were lysed in Rac1 assay buffer (50 mM Tris (pH 7.4), 500 mM NaCl, 5 mM $MgCl_2$, 0.5% NP-40, 10% glycerol, 1 mM $Na_3VO_4$, and Complete protease

inhibitors (Roche, Indianapolis, IN)), vortexed, and centrifuged at 14,000 rpm for 5 min at 4°C. The supernatant was promptly incubated with 10 μg GST-PAK1-PBD for 10 min at 4°C, then washed once with Rac1 assay buffer, and boiled with sample buffer. 5% of the input was analyzed by western blot for total protein levels. Active Rac1 levels were normalized to actin or total Rac1, and the ratio was then normalized to control siRNA-transfected cells.

**Transwell Migration Assay**

For transwell migration assays, filters with 10 μm or 12 μm pores (PFA12, Neuroprobe, Gaithersburg, MD) were coated with 0.1 mg/ml gelatin, and the bottom well of the blind well chamber (Neuroprobe, Gaithersburg, MD) was filled with medium containing 10% FBS. $1.0x10^5$ cells (Panc04.03 or Panc1) were plated on top of the filter in serum-free medium, and were allowed to migrate for 16 hours. The cells on the top of the filter were removed with a cotton swab, and the cells on the lower face of the filter were fixed and stained with Hoechst 33342 (Cambrex Bioscience) and imaged by fluorescence microscopy using a 10x objective. The number of cells migrated through the filter was determined by DAPI stain, and normalized to control siRNA-transfected cells. Cells were also analyzed in parallel by immunoblotting to confirm protein knockdown and overexpression.

**Lamellipodia Formation Assays**

To measure formation of lamellipodia, Panc1 or HPAF-II cells were serum-starved overnight, then stimulated with EGF (50 ng/ml, Sigma Aldrich, St. Louis, MO) for 20 min. Cells were fixed and stained for the transfected proteins and stained for actin with TRITC-Phalloidin (Sigma Aldrich, St. Louis, MO) or for cortactin (4F11, Millipore, Billerica, MD) as a marker of lamellipodia. Transfected cells were costained for the exogenously expressed proteins (anti-Dyn2 (Henley et al., 1998), anti-myc (Cell Signaling, Danvers, MA), or anti-Vav1 (Novus Biologicals, Littleton, CO)). Secondary antibodies conjugated to AlexaFluor 488 were purchased from Invitrogen (Carlsbad, CA). For colocalization of Dyn2 and Vav1, Panc1 cells were transfected with Vav1, then stained for Vav1 (Alexa Fluor 594) and endogenous Dyn2 (Alexa Fluor 488). For HPAF-II cells stained for actin, gamma changes were applied to images to emphasize actin staining in the lamellipodia versus the very intense actin staining in the cell body. The percent of cells forming lamellipodia was scored, and at least 100 cells were scored

per experiment. For siRNA-mediated knockdown experiments, parallel dishes of cells were lysed and analyzed by western blot to confirm protein knockdown.

**Cell spreading**

DanG cells were transfected with the indicated siRNAs. After 72 hours, the cells were trypsinized, incubated on ice for 20 min, and replated on fibronectin-coated coverslips, which were prepared according to the manufacturer's protocol (Calbiochem, Darmstadt, Germany). The coverslips were incubated at 37°C, then fixed after 15, 30, or 60 minutes and stained using TRITC-Phalloidin. Cells were imaged by fluorescence microscopy and were scored as "spread," "not spread," or as having an intermediate appearance. At the time of plating, half of the cell suspension was lysed and the Vav1 knockdown was confirmed by immunoblotting (data not shown).

**Wound healing assay**

Panc04.03 or DanG cells were transfected with the indicated siRNAs, and grown to confluence on gridded coverslips. 72 hours post transfection, cells were serum-starved overnight. Then, a wound was introduced by scratching the monolayer with a pipette tip. Cells were washed thoroughly and imaged using phase contrast microscopy and a 10x objective. Fresh medium containing 10% FBS was replaced, and the same fields of cells were imaged again after 6 hours (Panc04.03 cells) or 24 hours (DanG cells). The distance migrated was measured using iVision software, and images were adjusted using Adobe Photoshop. Three fields were imaged per condition, and at least 10 distance measurements were taken per field. Following imaging, the cells were lysed and the Vav1 knockdown was confirmed by immunoblotting (data not shown).

**Immunofluorescence**

Fluorescence micrographs were acquired using a Zeiss Axiovert 35 epifluorescence microscope (Carl Zeiss) using a 63x objective, unless otherwise indicated, and a Hamamatsu OrcaII camera (Hamamatsu Photonics, Hamamatsu City, Japan) with iVision software. Images were processed using Adobe Photoshop software (Adobe). Adjustments were applied uniformly to the entire image.

**Dynamin inhibitors**

Dynasore (Calbiochem, Darmstadt, Germany) was used at 80 μM, and MiTMAB (Calbiochem, Darmstadt, Germany) was used at 10 μM for 24 hours, as described previously (Eppinga et al., 2012). Parallel sets of samples were evaluated for Transferrin uptake to confirm that the compounds inhibited endocytosis (data not shown).

**Endocytosis assays**

Panc1 cells were transfected with empty vector, Vav1, or Vav1 WWYY for 48 hours, and serum-starved overnight. Panc04.03 cells stably expressing control vector, Dyn2, or Dyn2 Δ800-811 were plated on coverslips and serum-starved overnight. All cells were then incubated with Rhodamine-conjugated EGF (Rh-EGF, 100 ng/ml, Invitrogen, Carlsbad, CA) for 10 min. Surface Rh-EGF was stripped by incubating with ice-cold 0.5 M NaCl, pH 2.5, for 2 min, 2 min, and 1 min, all on ice. Cells were then fixed using 3% formaldehyde and permeabilized using 0.0001% digitonin for 2 min, and stained for the overexpressed proteins using antibodies against Vav1 (Novus Biologicals, Littleton, CO) or Dyn2 (Henley et al., 1998). Cells were imaged for Rh-EGF using a 40x objective and levels of intracellular fluorescence were quantified in at least 60 cells per experiment.

For transferrin uptake experiments, Panc1 cells were transfected with siRNA against Dyn2, then 24 hours later transfected with empty vector, WT Dyn2, or Dyn2 Δ800-811. Cells were serum-starved for 20 minutes, then incubated with transferrin conjugated to AlexaFluor-594 (Transferrin-594, 10 μg/ml, Invitrogen, Carlsbad, CA) for 20 min. Surface Transferrin-594 was stripped by incubating with DMEM pH3.5 for 1 min at room temperature, then washed thoroughly prior to proceeding with fixation and permeabilization as described above. Cells were stained for myc-Dyn2 (Cell Signaling, Danvers, MA). Cells were imaged for Transferrin-594 using a 63x objective and levels of intracellular fluorescence were quantified in at least 30 cells per experiment using iVision software.

**Lentiviral-based shRNA delivery**

A VSV-G pseudotyped lentivirus (a gift from Dr. Yasuhiro Ikeda, Mayo Clinic) was used to deliver a shRNA vector targeting Dyn2 (Sigma MISSION RNAi library, obtained from the Mayo Clinic Comprehensive Cancer Center RNA Interference Shared Resource). Briefly, media containing viral particles was collected from an HEK-293T packaging cell line, supplemented with 8µg/mL Polybrene (Millipore, Billerica, MA), and applied to target cell populations for a 96 hour period. Protein levels were detected by SDS-PAGE and western blot analysis of whole cell lysates.

**RT-PCR**

mRNA was isolated from cells using RNEasy Plus (Qiagen, Valencia, CA). First-strand synthesis was performed using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. PCR for Vav1, Dyn2, and GAPDH was performed for 20 cycles using the following primers: Vav1: Forward 5'-CCCCCTGGAGCCATTGGACC-3', Reverse 5'-CCGGAAAGCCTTTTTCTCTG; Dyn2: Forward 5'-TCACCACTTACATCCGGG-3', Reverse 5'-TTGTTGGACATGAAGCCC-3'; GAPDH: Forward 5′-CATGACAACTTTGGTATCGTG-3′, Reverse 5′-GTGTCGCTGTTGAAGTCAGA-3′; Flag-Vav1: Forward 5'-ACGATAAGAAGCTTGAGC-3', Reverse 5'-GGAAGGGCATGATCCCC-3'. Primers were designed to generate a product spanning multiple exon boundaries. The 5' primer for Flag-Vav1 included the amino-terminal Flag epitope tag to target only ectopically expressed Vav1. As a negative control, reverse transcriptase was excluded from one sample per experiment. One fifth of the reaction product was resolved on a 1.0% agarose gel, stained with ethidium bromide, and imaged using Kodak 1D 3.5.3 Software.

**Human pancreatic tumor analysis**

Human pancreatic tumor samples were previously analyzed for Dyn2 expression or Vav1 expression by immunohistochemistry (Eppinga et al., 2012; Fernandez-Zapico et al., 2005). 49 patients overlapped between these previously published data sets, and the Dyn2 and Vav1 status were compared among these patients.

**Statistical Analysis**

Microsoft Excel was used for calculations. Unless otherwise indicated, all graphs represent the mean +/- SEM of at least 3 independent experiments. A two-tailed, paired Student's t-test was used to determine statistical significance, and * indicates $p<0.05$; ** indicates $p<0.01$.

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