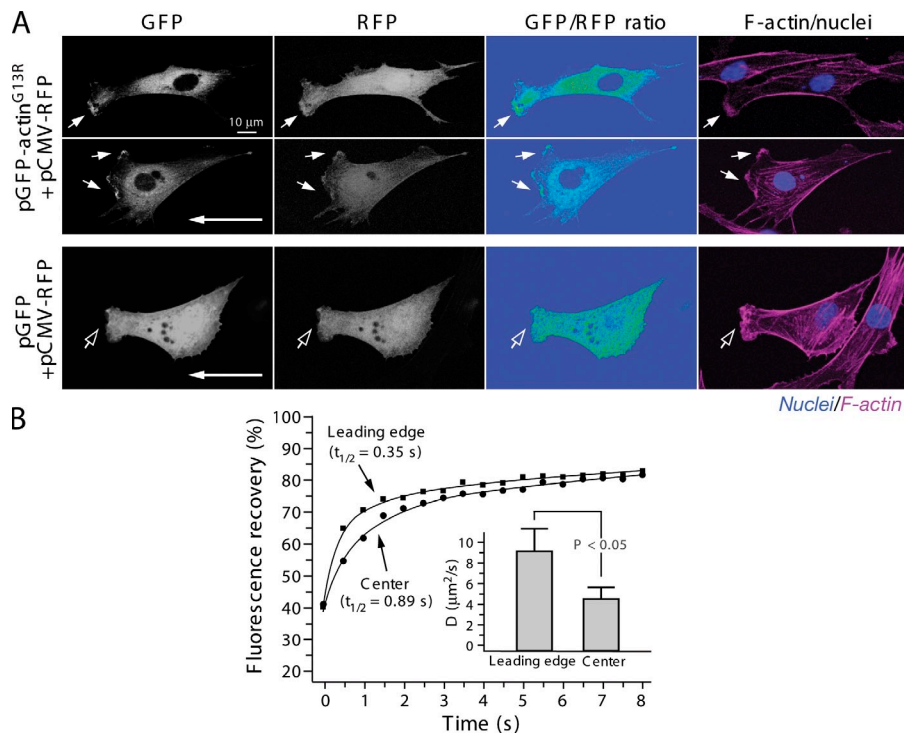Fan et al., http://www.jcb.org/cgi/content/full/jcb.201111088/DC1

Figure S1.   **G-actin localization and dynamics at the leading edge of migrating ECs.** (A) Accumulation of GFP-actin[G13R] at the leading edge. ECs were co-transfected with pCMV-RFP and pGFP-actin[G13R] or pGFP. Migrating cells were fixed and stained with DAPI and Alexa Fluor 633–phalloidin. Images were acquired by confocal scanning. GFP/RFP channel ratios were displayed in pseudocolor using Image-Pro software (Media Cybernetics). The open arrows show absence of preferential GFP/RFP localization at the cell leading edge. The filled arrows show GFP-actin[G13R] accumulation at the cell leading edge. Long arrows indicate the direction of cell migration. (B) Rapid movement of GFP-actin[G13R] at the cell leading edge. ECs were transfected with GFP-actin[G13R]. Migrating cells were subjected to FRAP in a confocal microscope. Representative fluorescence recovery curves at the leading edge and cell center are shown. (inset) Apparent lateral diffusion coefficient D (mean ± SEM; n = 5–8 cells).

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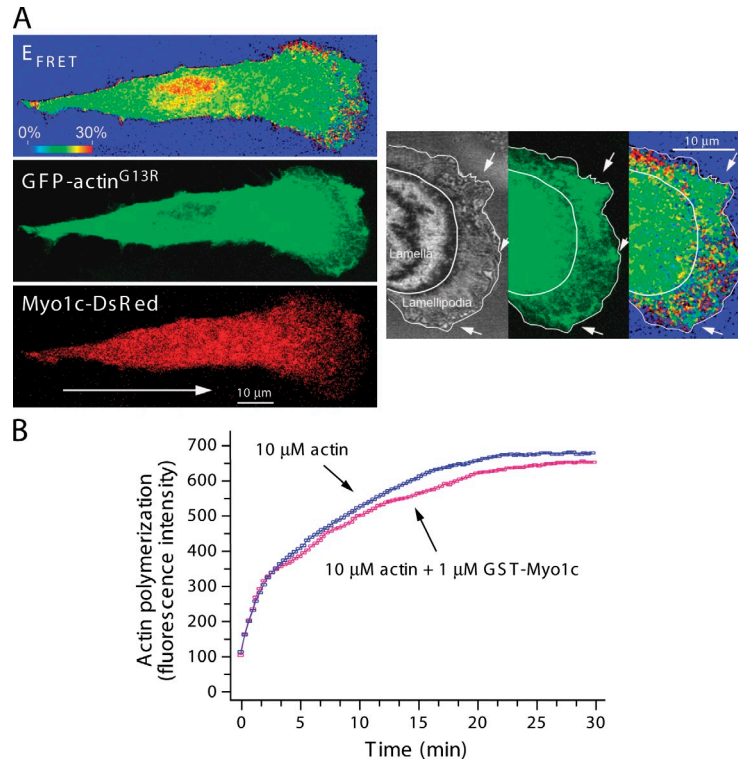Myo1c and G-actin transport in cell migration • Fan et al.   S1

Figure S2. **Myo1c interacts with G-actin in lamellipodia of migrating ECs but does not stimulate in vitro actin polymerization.** (A) Spatially restricted Myo1c interaction with G-actin in lamellipodia. ECs were cotransfected with pDsRed-Myo1c and pGFP-actin[G13R]. Migrating cells were subjected to FRET analysis using PFRET software. Arrows indicate lamellipodial domains exhibiting membrane ruffles, GFP-actin[G13R] accumulation, and weak interaction of DsRed-Myo1c with GFP-actin[G13R]. The long arrow indicates the direction of cell migration. (B) Actin (with 10% pyrene-labeled actin) was induced to polymerize by addition of $MgCl_2$ in the presence or absence of GST-Myo1c. Fluorescence intensity was measured with an excitation at 365 nm and an emission at 407 nm. The data shown are from a single representative experiment out of three repeats.

Figure S3. **Role of myosin in G-actin localization and EC migration.** (A) ECs were transfected with pcDNA vector or pcDNA-Myo1c-myc and treated with or without 1 µM jasplakinolide for 1 h. Cells were stained with Alexa Fluor 488–DNase I for visualization of G-actin. Mean fluorescence intensity of G-actin at the cell leading edge was quantified (mean ± SEM; n = 5–8). (B and C) Myosin II inhibitor blebbistatin dismantles stress fibers but does not delocalize G-actin at the cell leading edge. (B) ECs were transfected to express GFP-actin^{G13R} and induced to migrate. Live cells were treated with 0.1% DMSO (vehicle) or 10 µM blebbistatin and imaged by confocal scanning. Arrows indicate G-actin accumulation. (C) ECs were treated with 0.1% DMSO (vehicle) or 10 µM blebbistatin for 30 min and stained with Alexa Fluor 488–phalloidin for visualization of F-actin. (D) Calculation of intracellular Myo1c concentration. (top left) Migrating ECs were stained with anti-Myo1c antibody and visualized with Alexa Fluor 488–IgG and Alexa Fluor 568–phalloidin by confocal scanning. After 3D reconvolution, cell volume was calculated using Volocity software (PerkinElmer). (top right) 3D images of Myo1c were projected to 2D images. (bottom left) Protein in cell lysate from 3 × 10⁴ ECs and 200 ng GST-Myo1c was resolved by SDS-PAGE and immunoblotted with anti-Myo1c antibody. Myo1c in cell lysate was quantified by densitometry, and mean intracellular concentration was determined. (bottom right) Subcellular distribution of Myo1c concentration in migrating ECs is shown as an image conversion of mean concentration of Myo1c using Image-Pro software. Short arrows show the localization of Myo1c at the cell leading edge. Long arrows indicate the direction of cell migration. MM, molecular mass.

A

C

Vehicle   Blebbistatin

F-actin/Nuclei

B

Time (min): 0   15   30

GFP-actin^{G13R}

D

Myo1c   F-actin

Confocal scanning, 3D reconvolution

Myo1c

EC volume = 2.37 ± 0.46 x 10^{-14} m^3

2D projection

(kD)
170 —
130 —
96 —
72 —
56 —
43 —
34 —
26 —

← Myo1c

Myo1c/EC = 5.9 x 10^{-12} g
Myo1c MM = 121.7 kD
[Myo1c]$_i$ = 2.0 ± 0.5 µM

Immunoblot

Myo1c

10 (µM)
5
0

Myo1c concentration