Tanimura et al., http://www.jcb.org/cgi/content/full/jcb.201503123/DC1

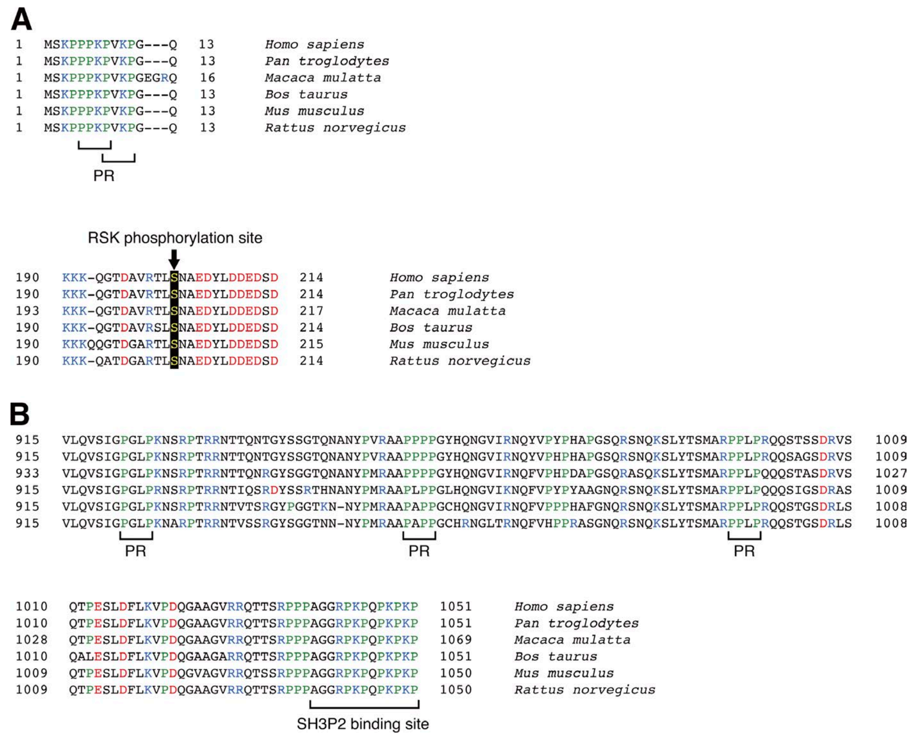

Figure S1. **Amino acid sequences of the N-terminal proline-rich region and C-terminal acidic amino acid cluster of SH3P2 protein as well as the TH2 domain of Myo1E protein are conserved among several mammalian species.** (A) Amino acid sequences of the N-terminal proline-rich region (top) and the C-terminal acidic amino acid cluster (bottom) of mammalian SH3P2 proteins. (B) Amino acid sequences of the TH2 domain of mammalian Myo1E proteins. Acidic amino acids, basic amino acids, and proline are shown in red, blue, and green, respectively. Conserved proline-rich (PR) sequences (PXXP) recognized by SH3 domains are indicated. The RSK phosphorylation site is shown in yellow (A).

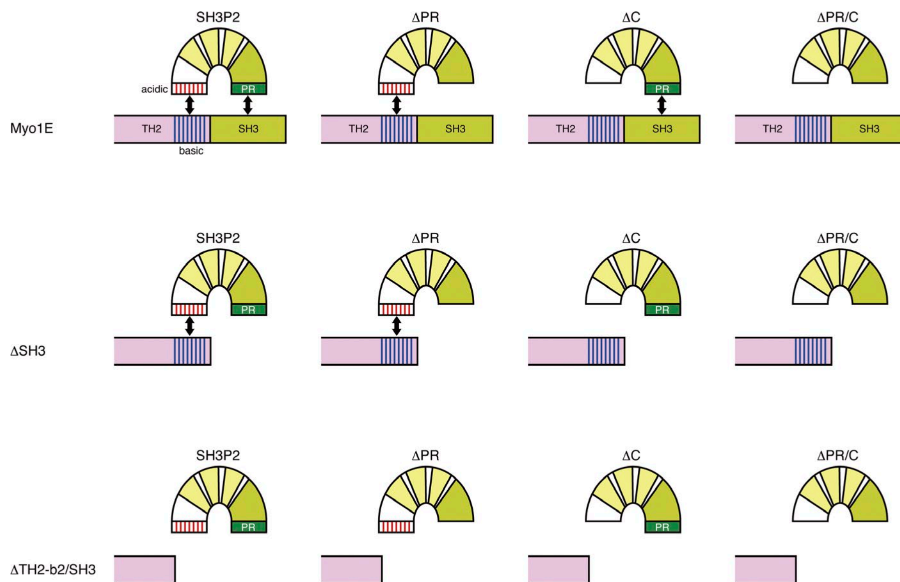

Figure S2. **Putative binding interfaces for the interaction between SH3P2 and Myo1E.** Arrows indicate regions of SH3P2 and Myo1E implicated in the interaction between the two proteins.

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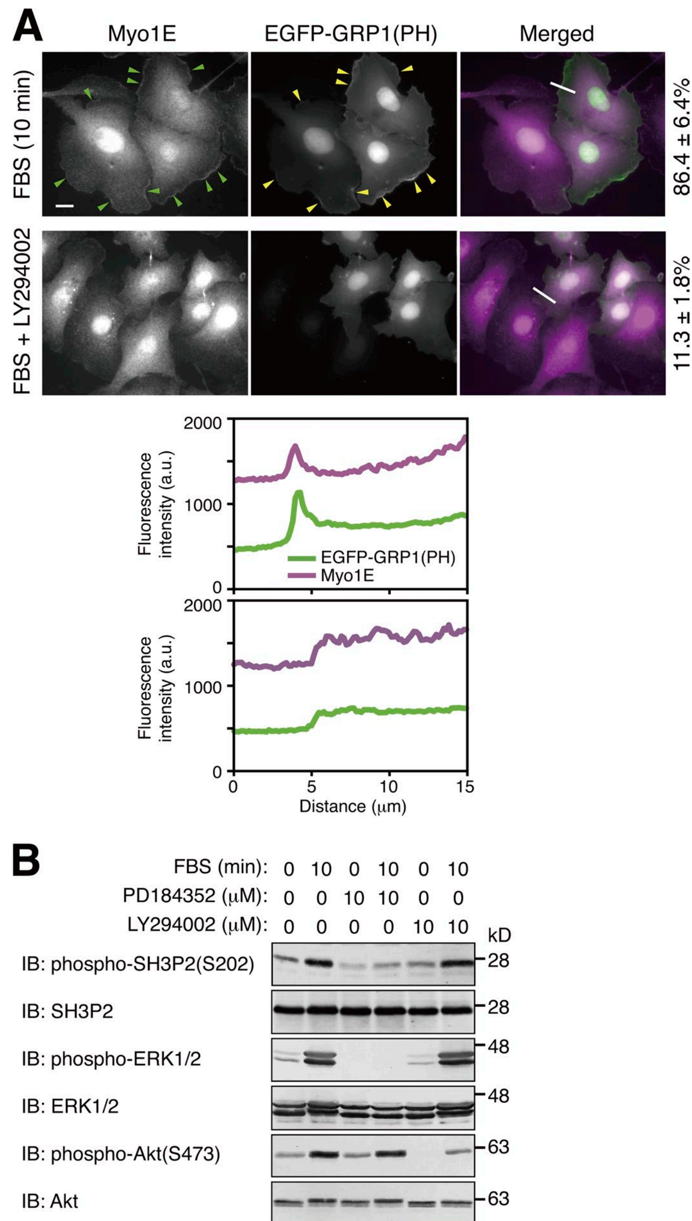

Figure S3. **LY294002 suppresses the serum-induced production of PI(3,4,5)P_3 and localization of Myo1E to lamellipodial tips.** (A) MKN1 cells were transfected for 24 h with a vector for the EGFP-tagged pleckstrin homology (PH) domain of GRP1, which binds to PI(3,4,5)P_3, deprived of serum for 12 h, incubated with or without 10 μM LY294002 for 30 min, and then stimulated with 10% FBS for 10 min. The cells were then fixed and stained with antibodies to Myo1E (magenta), and the fluorescence of EGFP-GRP1(PH) was monitored directly (green). Green and yellow arrowheads indicate colocalization of Myo1E and GRP1(PH) (PI(3,4,5)P_3), respectively, at lamellipodial tips. Bar, 10 μm. The percentages of cells in which Myo1E and GRP1(PH) were colocalized are shown at the right of the merged images as means ± SD for three separate experiments, with $n \geq 25$ cells in each experiment. Fluorescence intensity profiles along the white lines in the merged images are also shown on the right (a.u., arbitrary units). (B) MKN1 cells deprived of serum for 12 h were incubated with or without 10 μM PD184352 or 10 μM LY294002 for 30 min and then stimulated with 10% FBS for 10 min. Cell lysates were then subjected to immunoblot analysis (IB) with antibodies to the indicated proteins. All data are representative of at least three separate experiments.

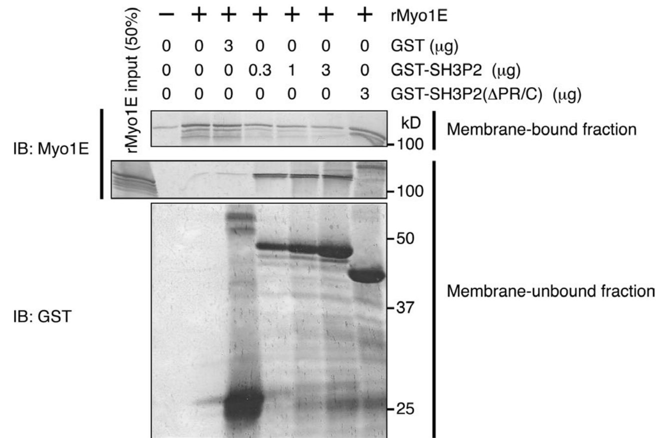

Figure S4. **Membrane association of recombinant Myo1E is suppressed by prior incubation with GST-SH3P2.** MKN1 cells were homogenized in hypotonic lysis buffer with a Dounce homogenizer. The homogenate was centrifuged at 700 *g* for 5 min at 4°C to remove nuclei and intact cells, and the resulting supernatant was centrifuged at 20,000 *g* for 30 min at 4°C to yield a pellet, which was resuspended in hypotonic lysis buffer and used as a semi-intact plasma membrane fraction. A cDNA fragment amplified from wild-type human Myo1E cDNA by PCR with the forward primer 5′-GCCGGATCCATGGGA AGCAAAGGTGTC-3′ (underline indicates the BamHI site) and reverse primer 5′-CGATGCGGCCGCTCGAGTCAGATCTTGGTCACATAG-3′ (underline indicates the NotI site) was digested with BamHI and NotI and cloned into BamHI- and NotI-digested pcDNA3 (Invitrogen). The resulting pcDNA3-Myo1E vector was subjected to transcription and translation in vitro with the use of a TNT T7/T3-coupled reticulocyte lysate system (Promega), and the recombinant Myo1E (rMyo1E) in the reaction mixture was incubated at 4°C first with the indicated GST fusion proteins for 2 h and then in the additional presence of the membrane fraction for 20 min. The reaction mixtures were then centrifuged at 20,000 *g* for 30 min at 4°C to yield the membrane-bound fraction (pellet), and the supernatant was incubated with glutathione-Sepharose beads for 6 h at 4°C, after which the bead-bound proteins were isolated as the membrane-unbound fraction. Both fractions were then subjected to immunoblot analysis (IB) with antibodies to Myo1E or to GST. Data are representative of three separate experiments.

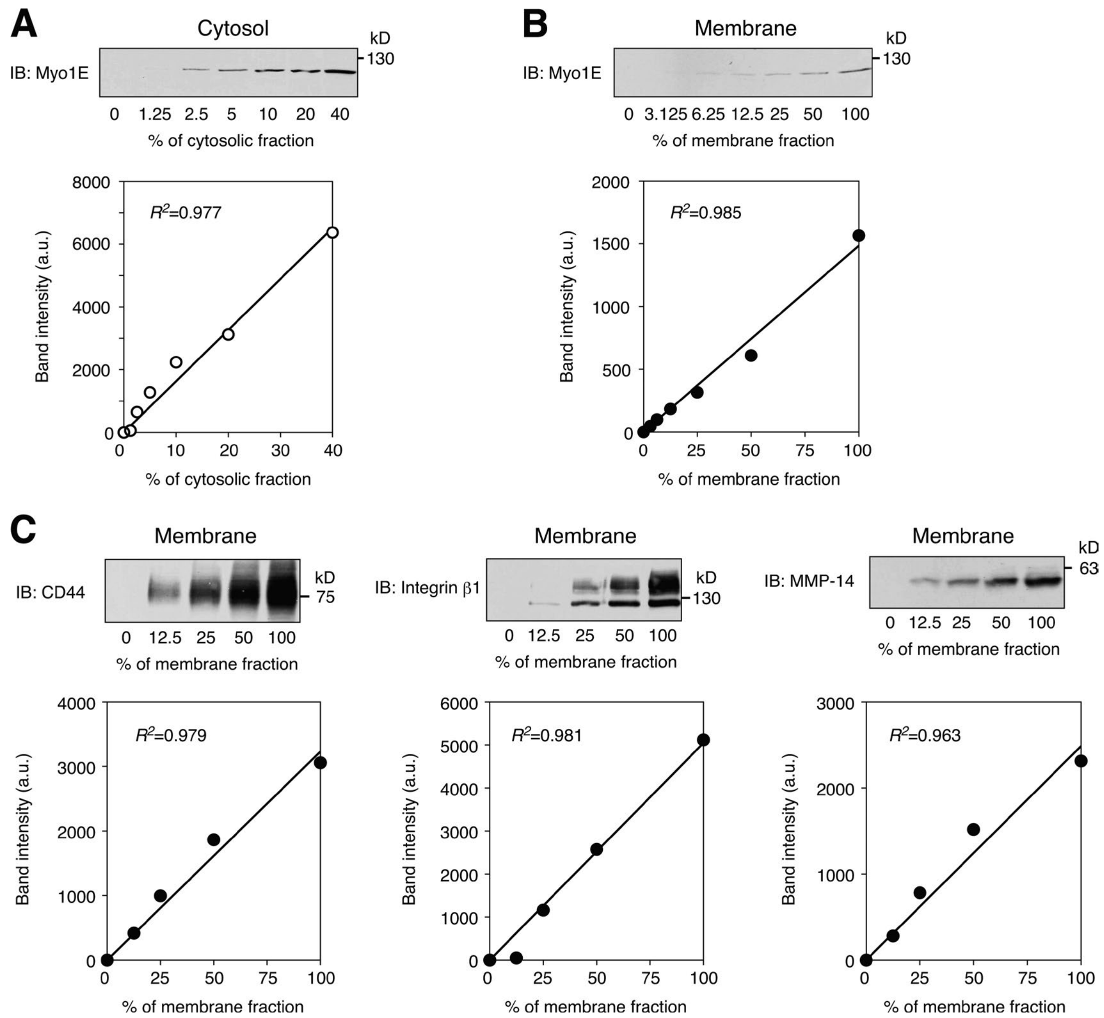

Figure S5. **Quantitative immunoblot analysis of Myo1E in the cytosolic and membrane fractions of MKN1 cells.** MKN1 cells were subjected to subcellular fractionation, and the indicated percentages of the entire cytosolic (A) and membrane (B and C) fractions prepared from cells grown in each well of a 12-well plate were subjected to immunoblot analysis (IB) with antibodies to the indicated proteins. The band intensities of each protein were plotted against the percentage of the subcellular fractions analyzed. The coefficient of determination (R^2) is indicated for each graph. a.u., arbitrary units.