Supporting Information

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SI Materials and Methods

Antibodies. The following antibodies were used for immunocytochemistry and Western blot analysis: anti-myc 4A6 antibody (1:3,000 for Western blotting; Millipore), anti-myc 9E10 antibody (1:800 for immunocytochemistry; Santa Cruz Biotechnology), anti-myosin VIIA antibody (1:200 for immunocytochemistry; Abcam), anti-myosin VIIA antibody (1:1,000 for Western blotting; Santa Cruz Biotechnology), anti-Erk antibody (K23; Santa Cruz Biotechnology), anti-*N*-cadherin antibody (BD Biosciences), and anti-MyRip antibody (Sigma). Anti-myosin IIB (1:5,000 for Western blotting) was kindly provided by R. Adelstein (National Institutes of Health, Bethesda, MD).

Western Blot Analysis. Proteins separated by SDS/PAGE were transferred to a PVDF membrane (Bio-Rad) in a tank apparatus at 70 V for 30 min. The membrane was blocked with 3% nonfat dry milk and 0.05% Tween 20 in Tris-buffered saline for 1 h. For endogenous myosin VIIA detection, the membrane was blocked with 5% normal rabbit serum and 0.05% Tween 20 in Tris-buffered saline for 1 h. The membrane was incubated with primary antibodies, followed by HRP-conjugated secondary antibodies (BioRad). Detection was performed with Super Signal West Pico (Pierce Chemical).

Cell Culture and Transfection. HeLa cells (ATCC) and COS7 cells (ATCC) were cultured with DMEM containing 10% FBS. Human retinal pigmented epithelial (ARPE-19) cells (ATCC) were cultured with DMEM/F12 with 10% FBS and 2 mM L-glutamine (Invitrogen). Both cells were kept at 37 °C with 5% CO_2. Transient transfections were performed with GeneJammer Transfection Reagent (Stratagene) according to the manufacturer's instructions. Plasmid DNA was purified by using Qiagen minior Maxiprep columns. The cells were observed under the confocal microscope at ~14 h after transfection.

Confocal Microscopy. Fluorescence images were viewed with a Leica DM IRB laser-scanning confocal microscope controlled by Leica TCS SP II systems (Leica Microsystems) equipped with a Plan-Apochromat 60× 1.40 N.A. oil-immersion objective (Leica). The images were processed by using Adobe Photoshop software. Time-lapse images were obtained by sequential epifluorescent and phase illumination at 37 °C. Movie files were created with QuickTime (Apple).

Immunofluorescence Staining. Cells cultured on glass coverslips were fixed in a fixation buffer (4% formaldehyde, 2 mM $MgCl_2$, and 1 mM EGTA in PBS) for 20 min at room temperature, washed twice with PBS, and permeabilized with 0.25% Triton X-100 in PBS for 10 min at room temperature. Cells were washed twice with PBS and blocked with 5% BSA in PBS for 1 h at room temperature. The cells were incubated at 4 °C overnight with the indicated primary antibodies and subsequently for 1 h with the fluorescence-labeled secondary antibodies (Alexa Fluor 568– or 647–conjugated antibody; Molecular Probes). For actin staining, Alexa Fluor 568 or 647/phalloidin (Invitrogen) was used.

Subcellular Fractionation. At 24 h after the transfection, the cell pellets were snap-frozen in liquid nitrogen and stored at −80 °C. The cells were resuspended in 60 μL of Solution A [150 mM NaCl, 25 mM Hepes/NaOH (pH 7.4), 1 mM PMSF, 10 μg/mL leupeptin, 2 μg/mL pepstatin A, and 1 μg/mL trypsin inhibitor] and homogenized in a 1.5-mL tube with a pellet pestle (Kimble-Kontes) on ice. After adding 240 μL of Solution A, the samples were centrifuged at 22,000 × *g* for 5 min at 4 °C to remove the nuclear and crude plasma membrane (fraction PM). The supernatant was further centrifuged (600,000 × *g*) to separate the cytoplasm fraction (supernatants, fraction Cy) and the small vesicle-containing fraction (pellets, fraction V).

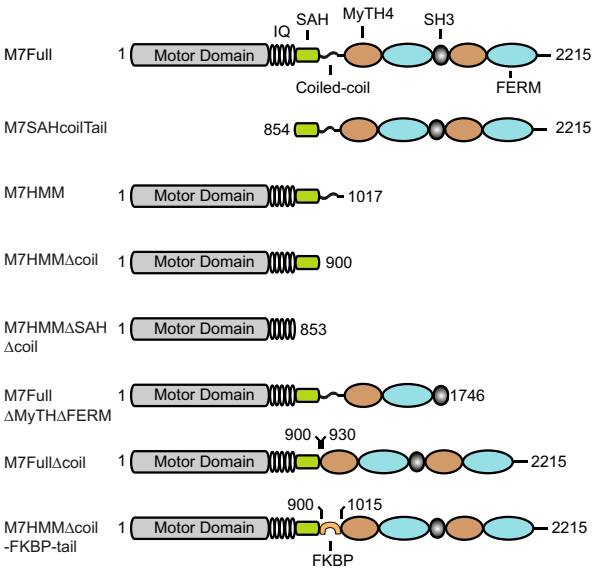


Fig. S1. Schematic diagram of myosin VIIA constructs used in this study. Numbers are amino acid numbers of human myosin VIIA.

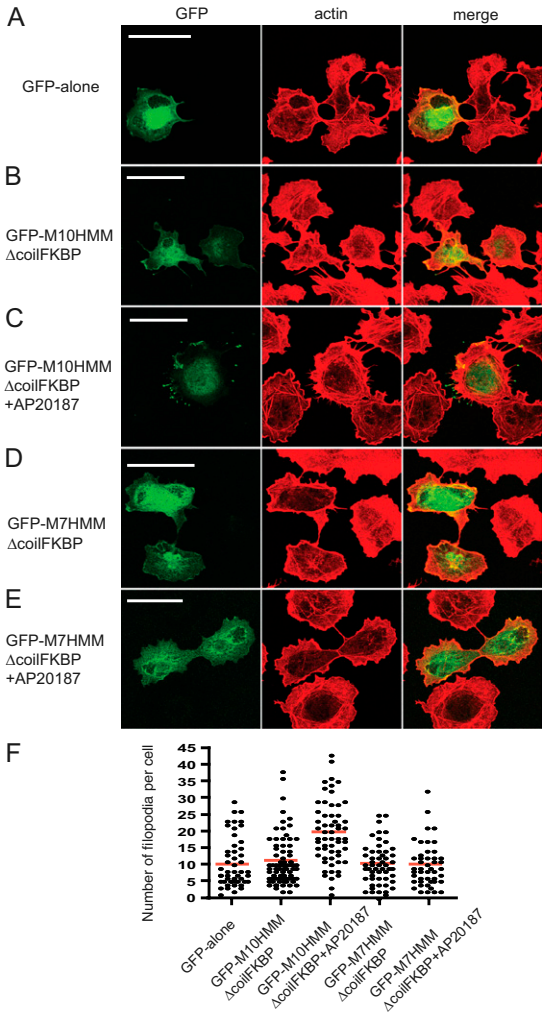


Fig. S2. Dimer formation of myosin VIIA does not induce filopodia/microspikes formation unlike myosin X. COS7 cells were transfected with GFP alone (*A*), M10HMMΔcoil-FKBP (*B* and *C*), or GFP-M7HMMΔcoil-FKBP (*D* and *E*). At 16 h after transfection, 100 nM AP20187 was added to the culture medium (*C* and *E*) and incubated for 30 min. After fixation, the cells were stained with Alexa Fluor 568/phalloidin (red). (Bars = 50 μm.) (*F*) Quantitative analysis of filopodia/microspike induction by various myosin constructs. Dot-plot graph shows the distribution of the numbers of filopodia/microspike per cell, identified by F-actin staining. All peripheral filopodia with the length more than 2 μm were counted for each transfected cells. Red bars show the average filopodial number per cell. The filopodial numbers were 10.0 ± 7.6 (GFP alone, n = 54), 11.6 ± 8.3 (M10HMMΔcoil-FKBP, n = 76), 19.6 ± 9.3 (M10HMMΔcoil-FKBP+AP20187, n = 59), 10.2 ± 6.0 (M7HMMΔcoil-FKBP, n = 54), and 10.0 ± 6.4 (M7HMMΔcoil-FKBP+ AP20187, n = 46). (Bars = 50 μm.)

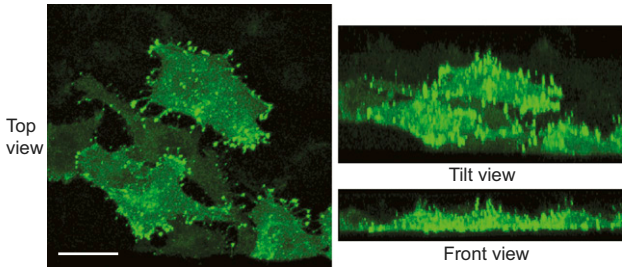


Fig. S3. GFP-M7HMM-FKBP localizes at the tip of both peripheral and dorsal filopodia upon dimer formation. HeLa cells expressing GFP-M7HMM-FKBP were fixed at 30 min after the addition of 100 nM AP20187. The 3D image was reconstructed from confocal optical slices from top to bottom in the cells. (Bars = 10 μm.)

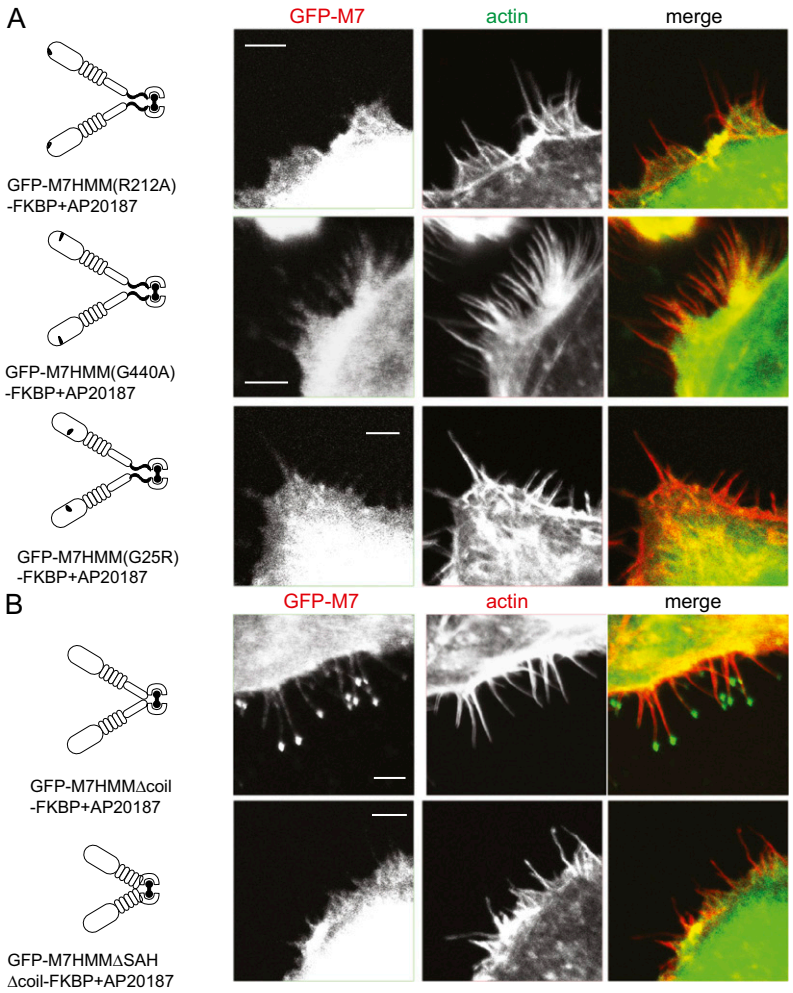


Fig. S4. The motor activity and proper neck length are required for the translocation of myosin VIIA to the tip of filopodia. (*A*) Disruption of the motor activity abolishes the filopodial tip localization of myosin VIIA constructs (GFP-M7HMM-FKBP). HeLa cells were transfected with R212A mutant (*Top*), G440A mutant (*Middle*), or G25A mutant (*Bottom*). (*B*) Elimination of the SAH domain abolishes the translocation of GFP-M7HMM-FKBP to the tip of filopodia. The myosin VIIA HMM construct without SAH domain (GFP-M7HMMΔSAHΔcoil-FKBP) failed to show the tip localization in filopodia in contrast to the construct with SAH domain (GFP-M7HMMΔcoil-FKBP). The cells were fixed at 30 min after the addition of 100 nM AP20187 and stained with Alexa Fluor 568/phalloidin to monitor actin structure. (Bars = 10 μm.)

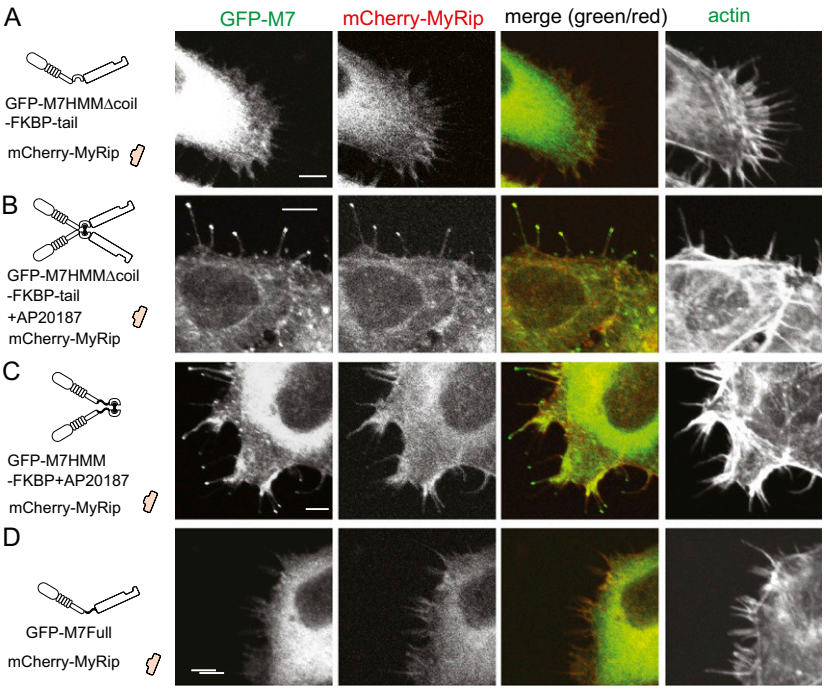


Fig. S5. Myosin VIIA forced dimer transports MyRIP to the tip of filopodia in HeLa cells. (*A*) GFP-M7HMM-FKBP-tail and mCherry-MyRIP showed diffuse localization before the addition of AP20187. (*B*) GFP-M7HMM-FKBP-tail transported mCherry-MyRIP to the tip of filopodia upon addition of AP20187. (*C*) GFP-M7HMM-FKBP without tail domain failed to transport mCherry-MyRIP to filopodial tips. (*D*) GFP-M7Full did not localize at tip of philopodia even when mCherry-MyRIP was coexpressed in HeLa cells.

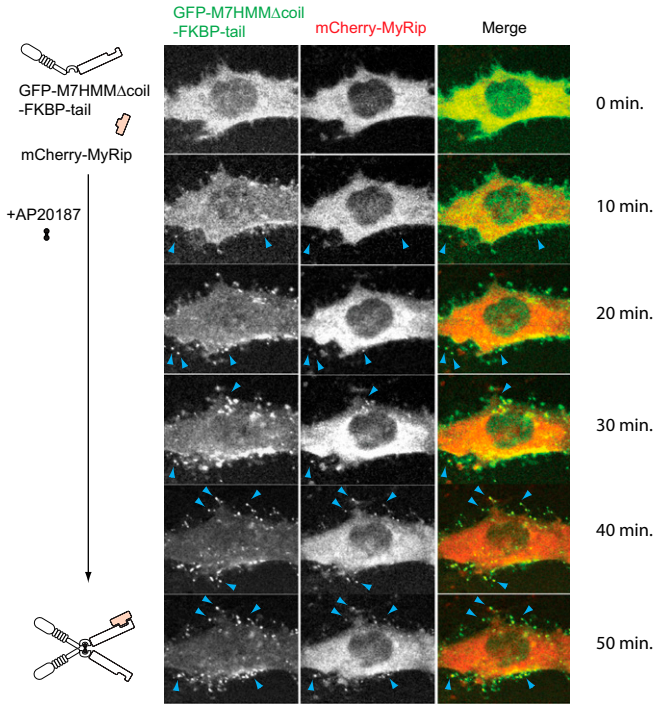


Fig. S6. Time-lapse images show translocation of GFP-M7HMMΔcoil-FKBP-tail and mCherry-MyRip in living cells. The translocation of GFP-M7HMMΔcoil-FKBP-tail and mCherry-MyRip in living HeLa cells was monitored under the confocal epifluorescence microscope after the addition of 100 nM AP20187. Blue arrowheads indicate filopodial tip colocalization.

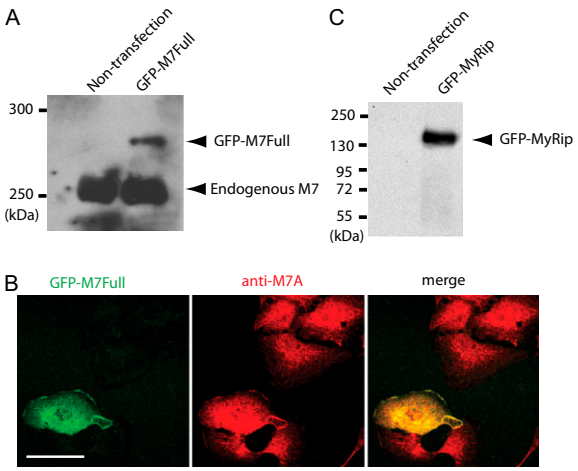
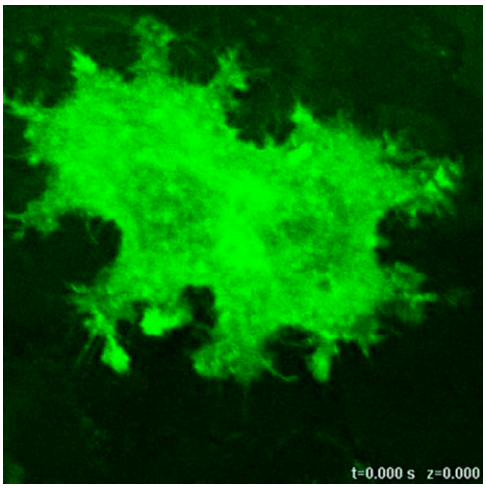


Fig. S7. Expression of endogenous myosin VIIA and MyRip in ARPE-19 cells. (*A*) Western blot analysis of the endogenous and GFP-tagged-M7Full in the cell lysate. At 14 h after transfection of GFP-M7Full, the cells cultured on 10-cm dishes were lysed in 100 μL of lysis buffer [50 mM Hepes (pH 7.5), 150 mM NaCl, 1 mM EDTA, 10 mM $MgCl_2$, 1% Nonidet P-40, 5 mM ATP, and protease inhibitor mixture]. After centrifugation at 22,000 × *g* for 20 min at 4 °C, the supernatants were analyzed by Western blotting using anti-myosin VIIA antibody. As a control, cell lysates were also extracted from nontransfected ARPE-19 cells. The expression level of GFP-M7Full was calculated to be 57.0 ± 12.7% of the endogenous myosin VIIA considering the transfection efficiency (17.5 ± 3.9%). Band density was quantified with ImageJ software. Note that that the apparent low expression level of GFP-M7Full is because of the low transfection efficiency. (*B*) Immunocytochemistry of myosin VIIA shows the colocalization of the GFP signal and anti-M7 signal. At 14 h after transfection of GFP-M7Full, the cells were fixed and stained with anti-myosin VIIA antibody. (Bar = 50 μm.) (*C*) Western blot analysis of endogenous and GFP-tagged-MyRip in the cell lysates. At 14 h after transfection of GFP-MyRip, the cells were precipitated with 5% ice-cold trichloroacetic acid. After washing with PBS buffer, the samples were sonicated with SDS sample buffer and subjected to Western blotting using anti-MyRip antibody. As a control, cell lysates were also extracted from nontransfected ARPE-19 cells.



Movie S1. Dimerizer-induced translocation of GFP-M7HMM-FKBP in a living Hela cell. AP20187 was added to the medium after capturing the first frame (approximately 5 s). Lower right shows time (t) after capturing the image and the Z depth (z) (arbitrary scale) from the bottom of the cell.

Movie S1