Figure S1. Knockdown of p130Cas does not decrease cell spreading area in Ras-transformed cells. (A and B) NIH3T3 cells, iMEFs, and p53^-/- MEFs were infected with a control or Ha-RasV12-expressing retrovirus. (A) Phase-contrast images of cells (Eclipse TE2000-S; Nikon). Bars, 100 µm. (C and D) NIH3T3 cells were infected with Ha-RasV12- and HtrA2/Omi shRNA- or p53R175H-expressing retroviruses (C), together with a control or p130Cas shRNA-expressing retrovirus (D). (B-D) Cell spreading areas were quantified from the F-actin-stained images of Fig. 3 B (B), 3 E (C, right), 4 I (C, left), 5 C (D, left), and 5 H (D, right) using ImageJ software. Data represent the mean ± SD of more than 28 cells; *, P < 0.01. (E and F) NIH3T3 cells (E) and p53^-/- MEFs (F) were infected with a control or Ha-RasV12-expressing retrovirus. (Left) Traction stress maps of cells. Red lines indicate cell outlines. Bars, 20 µm. (Right) Average traction stress magnitude of cells. Data represent the mean ± SD of more than 10 cells. *, P < 0.01.
Figure S2. Oncogenic Ras decreases the F/G-actin ratio in a p53- and HtrA2/Omi-dependent manner. (A) NIH3T3 cells, iMEFs, and p53−/− MEFs were infected with a control or Ha-RasV12-expressing retrovirus. (B) NIH3T3 cells were infected with a Ha-RasV12-expressing retrovirus together with a control or p53R175H-expressing retrovirus. (C) NIH3T3 cells were infected with a Ha-RasV12-expressing retrovirus together with a control or HtrA2/Omi shRNA-expressing retrovirus. (D) NIH3T3 cells were infected with Ha-RasV12- and p53R175H-expressing retroviruses together with a control or p130Cas shRNA-expressing retrovirus. (E) NIH3T3 cells were infected with Ha-RasV12- and HtrA2/Omi shRNA-expressing retroviruses together with a control or p130Cas shRNA-expressing retrovirus. G- and F-actin were separated by ultracentrifugation. (A–E) Supernatants (G-actin) and pellets (F-actin) were analyzed by immunoblotting. The ratio of F/G-actin normalized to the control value is shown. Data represent the mean ± SD; *, P < 0.05.
Figure S3. Caspase-3 is not involved in Ras-induced β-actin cleavage. (A) NIH3T3 cells were treated with 0.5 µg/ml Adriamycin for 18 h or infected with a Ha-RasV12–expressing retrovirus. Caspase-3 cleavage (denoting the active form of caspase-3) or β-actin cleavage was evaluated by immunoblotting. The black arrowhead indicates full-length caspase-3 or β-actin, and white arrowheads indicate cleaved fragments. GAPDH was used as a loading control.

(B) Cells were immunostained with an anti-cytochrome c antibody (red) and the nucleus was stained with DAPI (blue). Bars, 20 µm. Acquired images were analyzed using ImageJ software. Projected images (maximum intensity z-projection of 20 optical sections) are shown.
Figure S4. *Oncogenic Ras does not increase expression of PUMA and BAX*. (A) NIH3T3 cells were infected with a control or Ha-RasV12–expressing retrovirus together with a control or p53R175H-expressing retrovirus. (B) p53−/− MEFs were infected with a control or Ha-RasV12–expressing retrovirus. (C) p53−/− MEFs were infected with a Ha-RasV12–expressing retrovirus together with a control or p53KRKKK-expressing retrovirus. Expression of p53 target genes, PUMA and BAX, were examined by quantitative real-time PCR.
Figure S5. **Bax is localized to mitochondria in Ras-transformed cells.** (A) NIH3T3 cells were transfected with a Mito-DsRed expression vector to visualize mitochondria and cultured for 24 h before a 1-h treatment with 2 µM oligomycin. Images were obtained by three-dimensional structured illumination microscopy. Colors indicate the lengths of mitochondria. Insets show the raw fluorescence images. Bars, 10 µm. (B) NIH3T3 cells were treated with vehicle (ethanol) or oligomycin for 3 h. After subcellular fractionation of the cytosol (Cyto) and mitochondria (Mito), the distribution of HtrA2/Omi was evaluated by immunoblotting. COX IV and α-tubulin were used as mitochondrial and cytosolic markers, respectively. (C) NIH3T3 cells were infected with a control or Ha-RasV12-expressing retrovirus. Cells were transfected with Mito-DsRed and GFP-Bax expression vectors and cultured for 24 h. Images were obtained with a confocal microscope. Bar, 10 µm. (D) NIH3T3 cells were transfected with Mito-DsRed and GFP-Bax expression vectors and cultured for 24 h before a 1-h treatment with oligomycin. Images were obtained with a confocal microscope. Bar, 10 µm. (E) NIH3T3 cells were treated with vehicle, oligomycin for 1 h, or with FCCP (100 µM) for 4 h. Confocal images of cells stained with JC-1. The ratio of red fluorescence (J-aggregate) to green fluorescence (monomer) is correlated with mitochondrial membrane potential.
Video 1. **Live imaging of actin filament dynamics in cell membrane protrusions from a Ras-transformed NIH3T3 cell.** NIH3T3 cells were infected with a Ha-RasV12–expressing retrovirus and transfected with a Lifeact-GFP expression vector to label F-actin. Live imaging of protrusion was performed with an inverted microscope (model IX81; Olympus) equipped with a spinning-disk confocal unit (PerkinElmer), an oil-immersion objective (100x, NA 1.40, PL FL; Olympus), and an EMCCD camera (model C9100-50; Hamamatsu Photonics). Frames were taken every 10 s for 5 min.

Video 2. **Live imaging of actin filament dynamics in cell membrane protrusions from a Ras-transformed NIH3T3 cell expressing HtrA2/Omi-shRNA.** NIH3T3 cells were infected with a Ha-RasV12–expressing retrovirus together with a HtrA2/Omi shRNA–expressing retrovirus and transfected with a Lifeact-GFP expression vector to label F-actin. Live imaging of protrusion was performed with an inverted microscope (model IX81; Olympus) equipped with a spinning-disk confocal unit (PerkinElmer), an oil-immersion objective (100x, NA 1.40, PL FL; Olympus), and an EMCCD camera (model C9100-50; Hamamatsu Photonics). Frames were taken every 10 s for 5 min.

Video 3. **Live imaging of actin filament dynamics in cell membrane protrusions from a Ras-transformed NIH3T3 cell expressing HtrA2/Omi- and p130Cas-shRNA.** NIH3T3 cells were infected with Ha-RasV12– and HtrA2/Omi shRNA–expressing retroviruses together with a p130Cas shRNA–expressing retrovirus and transfected with a Lifeact-GFP expression vector to label F-actin. Live imaging of protrusion was performed with an inverted microscope (model IX81; Olympus) equipped with a spinning-disk confocal unit (PerkinElmer), an oil-immersion objective (100x, NA 1.40, PL FL; Olympus), and an EMCCD camera (model C9100-50; Hamamatsu Photonics). Frames were taken every 10 s for 5 min.

Video 4. **Live imaging of actin filament dynamics in cell membrane protrusions from a Ras-transformed NIH3T3 cell expressing mutant p53.** NIH3T3 cells were infected with a Ha-RasV12–expressing retrovirus together with a p53 R175H-expressing retrovirus and transfected with a Lifeact-GFP expression vector to label F-actin. Live imaging of protrusion was performed with an inverted microscope (model IX81; Olympus) equipped with a spinning-disk confocal unit (PerkinElmer), an oil-immersion objective (100x, NA 1.40, PL FL; Olympus), and an EMCCD camera (model C9100-50; Hamamatsu Photonics). Frames were taken every 10 s for 5 min.

Video 5. **Live imaging of actin filament dynamics in cell membrane protrusions from a Ras-transformed NIH3T3 cell expressing mutant p53 with p130Cas-shRNA.** NIH3T3 cells were infected with Ha-RasV12– and p53 R175H–expressing retroviruses together with a p130Cas shRNA–expressing retrovirus and transfected with a Lifeact-GFP expression vector to label F-actin. Live imaging of protrusion was performed with an inverted microscope (model IX81; Olympus) equipped with a spinning-disk confocal unit (PerkinElmer), an oil-immersion objective (100x, NA 1.40, PL FL; Olympus), and an EMCCD camera (model C9100-50; Hamamatsu Photonics). Frames were taken every 10 s for 5 min.
Video 6. **Images of F-actin incubated with wild-type HtrA2/Omi.** Alexa Fluor 488–labeled F-actin was incubated with purified wild-type HtrA2/Omi. Images were analyzed by TIRF microscopy using a TIRF microscope (N-STORM; Nikon) equipped with an oil-immersion objective (100×, NA 1.49, CFI Plan Apochromat TIRF; Nikon). Frames were taken every 15 s for 55 min.

Video 7. **Images of F-actin incubated with HtrA2/Omi-S306A.** Alexa Fluor 488–labeled F-actin was incubated with purified HtrA2/Omi-S306A. Images were analyzed by TIRF microscopy using a TIRF microscope (N-STORM; Nikon) equipped with an oil-immersion objective (100×, NA 1.49, CFI Plan Apochromat TIRF; Nikon). Frames were taken every 15 s for 55 min.

Video 8. **Images of F-actin incubated with HtrA2/Omi-S142D.** Alexa Fluor 488–labeled F-actin was incubated with purified HtrA2/Omi-S142D. Images were analyzed by TIRF microscopy using a TIRF microscope (N-STORM; Nikon) equipped with an oil-immersion objective (100×, NA 1.49, CFI Plan Apochromat TIRF; Nikon). Frames were taken every 15 s for 55 min.