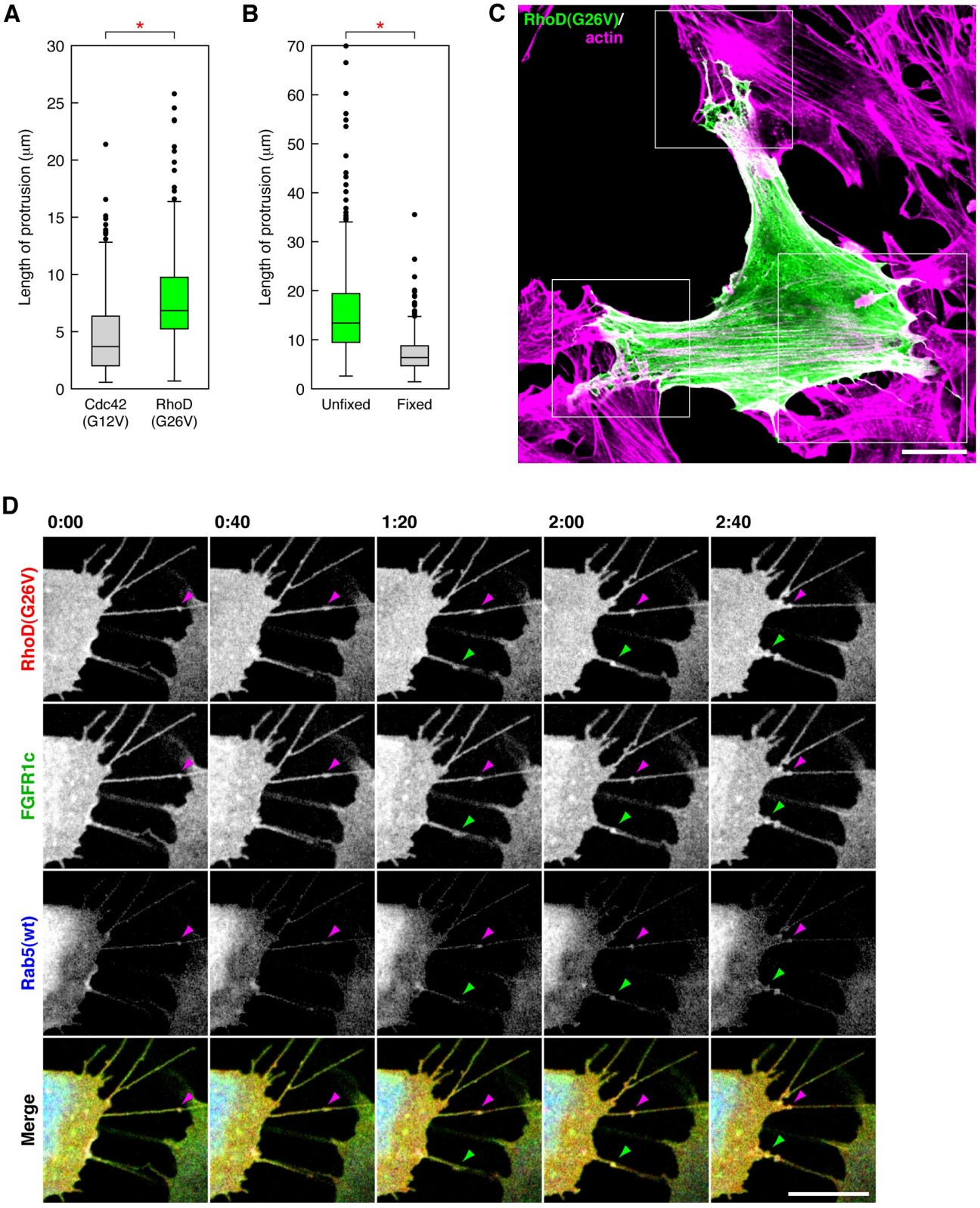
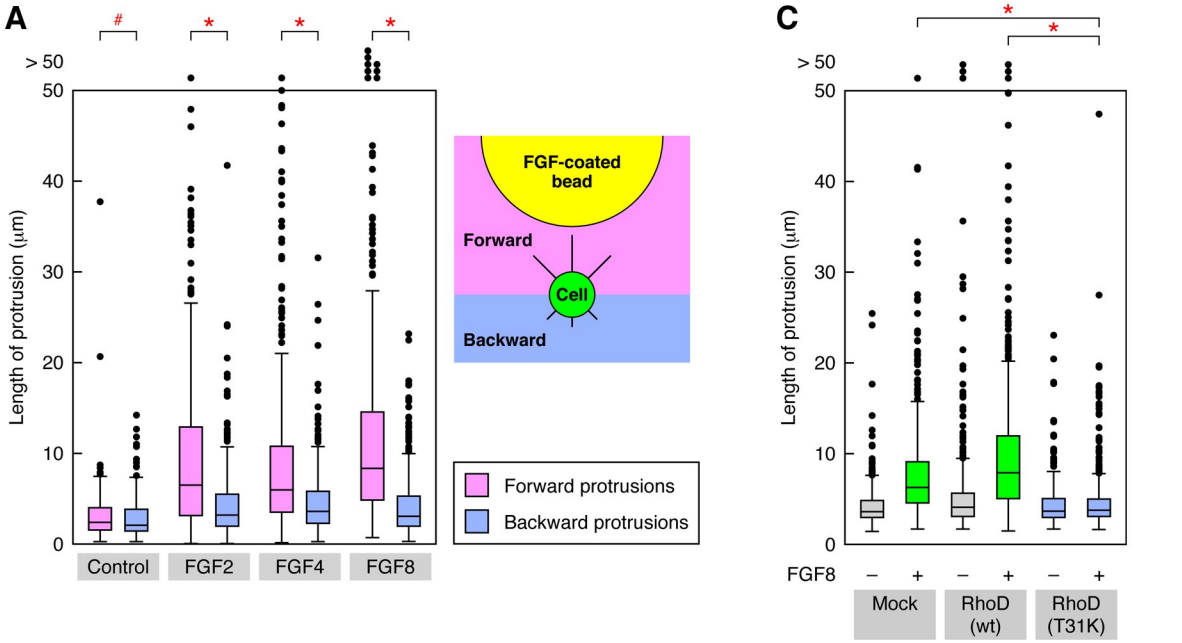
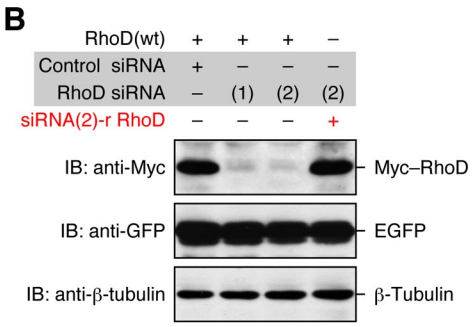
Supplemental Figure S1 K. Koizumi et al.

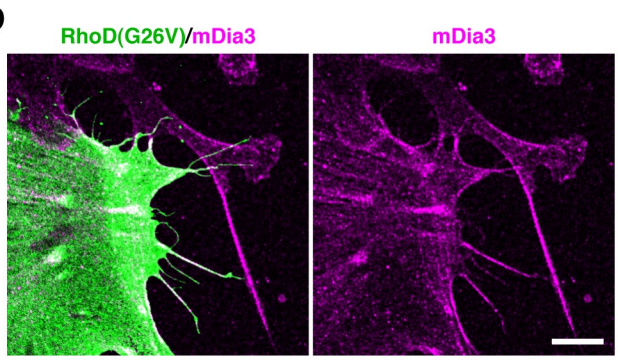


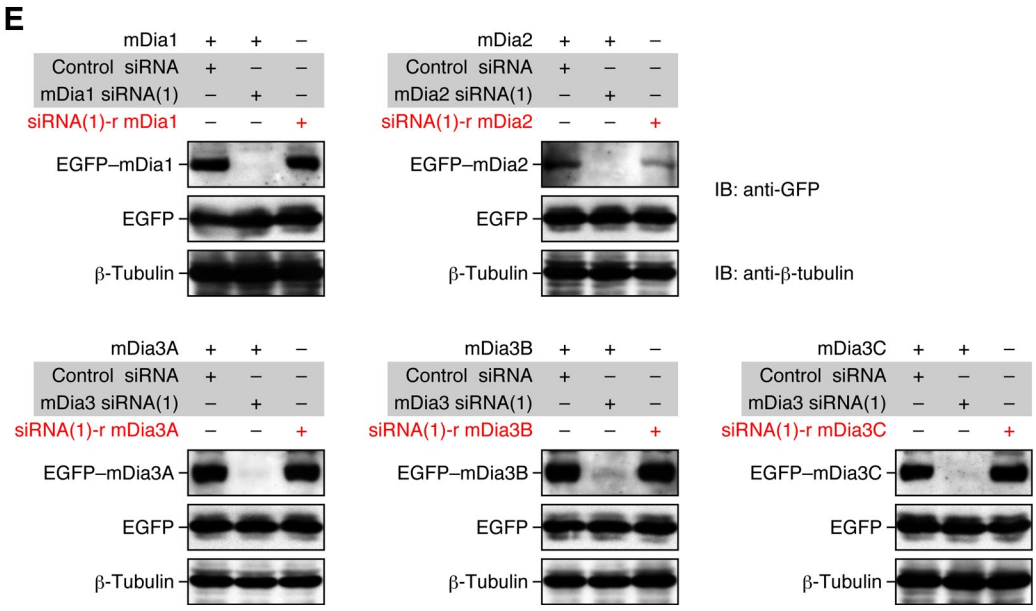
Supplemental Figure S1. Properties of RhoD(G26V)-induced protrusions. (A) The length of protrusions induced by RhoD(G26V) compared with that by Cdc42(G12V). 10T1/2 cells transfected with EGFP–Cdc42(G12V) or EGFP–RhoD(G26V) were fixed with PFA 48 h after transfection. The length is shown as box-and-whisker plots with boxes and whiskers encompassing 75th/25th and 95th/5th percentile, respectively. $n > 200$. * $p < 0.0001$ by *t* test. (B) The length of protrusions induced by RhoD(G26V) in unfixed and PFA-fixed cells 48 h after transfection. $n > 270$. * $p < 0.0001$ by *t* test. (C) Extension of RhoD(G26V)-induced protrusions toward neighboring cells (squared areas). 10T1/2 cells transfected with EGFP–RhoD(G26V) were fixed with PFA. EGFP fluorescence (green) and actin filaments detected with Alexa Fluor 546–phalloidin (magenta) are shown. (D) Time-lapse images of FGFR1c- and Rab5-containing nodules moving through the RhoD(G26V)-induced protrusions. 10T1/2 cells cotransfected with mOrange2–RhoD(G26V) (red), FGFR1c–EGFP (green), and Cerulean–Rab5(wt) (blue) were analyzed by time-lapse imaging at the time indicated. Arrowheads point to FGFR1c- and Rab5-containing nodules moving through protrusions. Scale bars, 10 μm.

Supplemental Figure S2 K. Koizumi et al.









Supplemental Figure S2. Mechanisms of RhoD-mediated protrusion formation. (A) The length of protrusions extending in forward or backward directions to FGF-coated beads in the analysis of Figure 3A. $n > 220$ (control), $n > 320$ (FGF2/4/8). * $p < 0.0001$, # $p > 0.3$ (not significant) by *t* test. (B) Knockdown of exogenously expressed RhoD by siRNAs and RhoD resistant to the siRNA. 10T1/2 cells were first transfected with RhoD Stealth siRNAs and 48 h later with Myc–RhoD(wt) or siRNA(2)-resistant Myc–RhoD together with pEGFP-C1 vector to normalize transfection efficiency. The levels of proteins at 48 h after the second transfection were analyzed by immunoblotting. (C) Interference with the FGF8-induced protrusion formation by the dominant-negative RhoD(T31K). 10T1/2 cells were transfected with EGFP–RhoD(wt) or RhoD(T31K) and serum-starved. They were stimulated with FGF8 for ~60 min, and the protrusion length was analyzed. $n > 400$. * $p < 0.0001$ by *t* test. (D) Localization of endogenous mDia3 in the RhoD(G26V)-induced protrusions. 10T1/2 cells transfected with EGFP–RhoD(G26V) were fixed with PFA, and endogenous mDia3 was detected with the anti-mDia3 pAb. Scale bar, 10 μm. (E) Knockdown of exogenously expressed mDia proteins by each corresponding siRNA and mDia proteins resistant to each siRNA. 10T1/2 cells were first transfected with *mDia* Stealth siRNAs and 48 h later with EGFP–mDia or siRNA(1)-resistant EGFP–mDia together with pEGFP-C1 vector to normalize transfection efficiency. The levels of proteins at 48 h after the second transfection were analyzed by immunoblotting.

MOVIES

Movie 1. RhoD induces two types of thin protrusions. 10T1/2 cells transfected with EGFP–RhoD(G26V) were observed by time-lapse microscopy 48 h after the transfection.

Movie 2. Movement of fluorescent nodules through the RhoD-induced long and immotile protrusions. 10T1/2 cells transfected with EGFP–RhoD(G26V) were observed by time-lapse microscopy 48 h after the transfection.

Movie 3. Movement of FGFR1c- and Rab5-containing nodules through the RhoD-induced protrusions. 10T1/2 cells cotransfected with mOrange2–RhoD(G26V) (red), FGFR1c–EGFP (green), and Cerulean–Rab5(wt) (blue) were analyzed by time-lapse microscopy 48 h after the transfection.