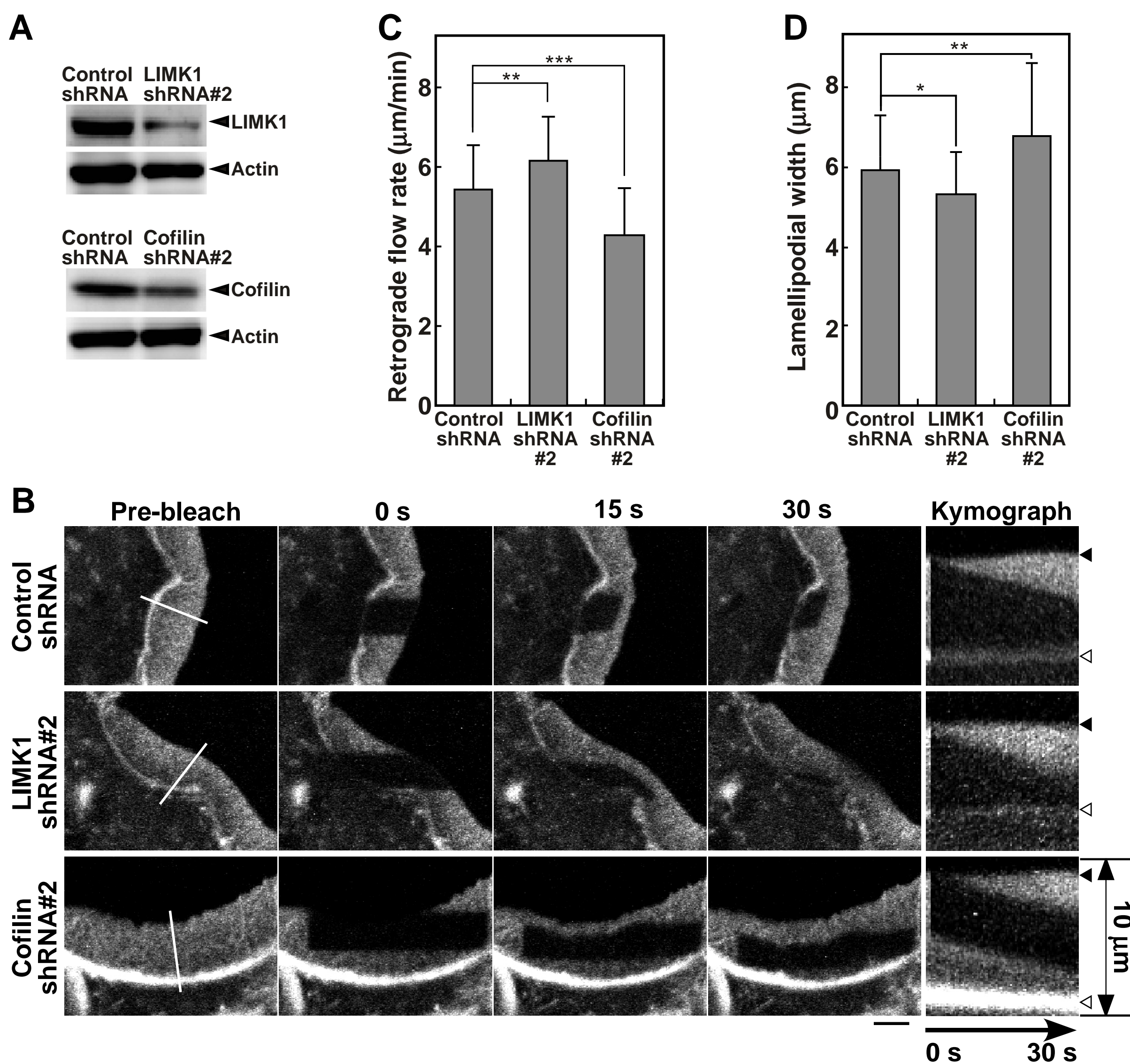

Fig. S1. Effects of another set of LIMK1 and cofilin shRNAs on the rate of actin retrograde flow and the width of lamellipodia in RacV12-expressing N1E-115 cells. A, suppression of LIMK1 or cofilin expression by shRNA plasmids. N1E-115 cells were transfected with control shRNA, LIMK1 shRNA#2 or cofilin shRNA#2. Expression of LIMK1 and cofilin was analyzed as described in Fig. 3A. B, FRAP time-lapse analysis of the effect of LIMK1 or cofilin shRNA#2 on the rate of actin retrograde flow. FRAP time-lapse imaging of YFP-actin and kymograph analysis were carried out, as in Fig. 3B. Scale bar, 5 μm. C, quantification of the rate of actin retrograde flow. D, quantification of the width of lamellipodia. Data are means ± SD of 40 (control shRNA), 42 (LIMK1 shRNA#2) and 70 cells (cofilin shRNA#2) from at least three independent experiments. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Supplemental Fig. S1 Ohashi *et al.*

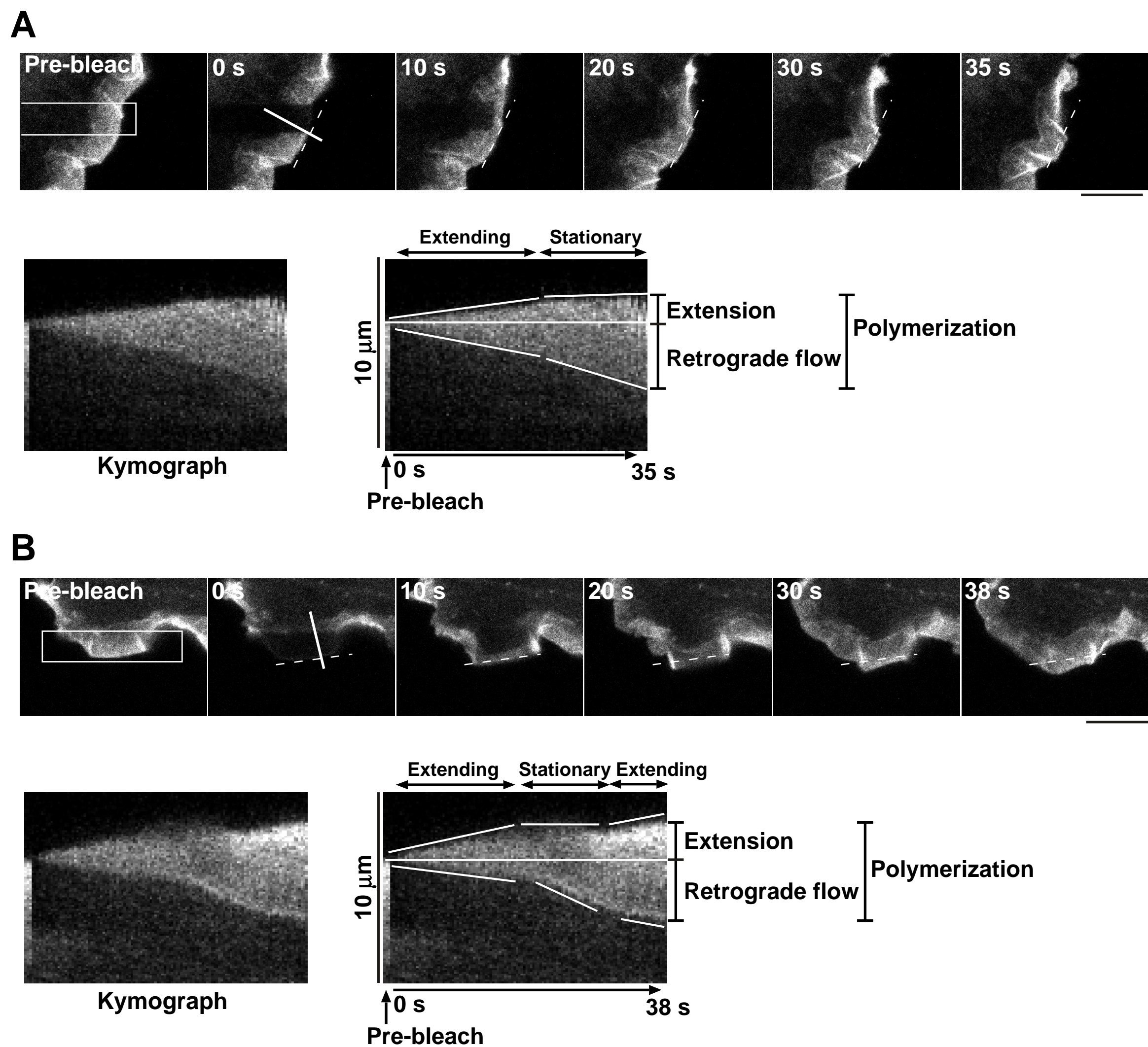

Fig. S2. Two other examples of FRAP time-lapse measurements of the rates of lamellipodium extension, actin retrograde flow and actin polymerization in NRG-stimulated MCF-7 cells. FRAP time-lapse analysis and kymograph analysis were performed as in Fig. 4. Scale bar, 10 µm.

Supplemental Fig. S2 Ohashi *et al.*

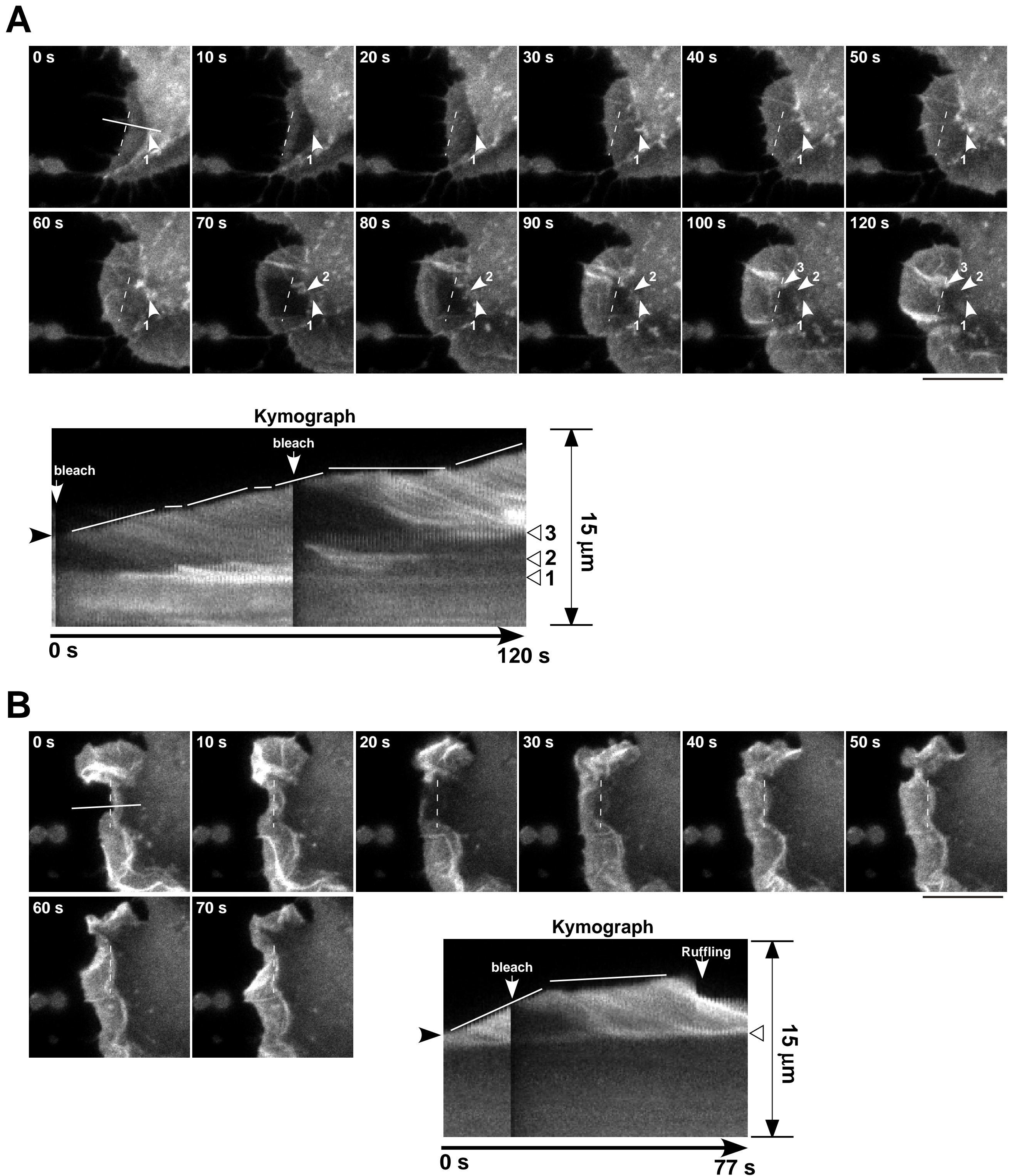

Fig. S3. The longer FRAP time-lapse imaging of NRG-stimulated MCF-7 cells. A, the periodic extension and pausing of lamellipodia. FRAP time-lapse analysis and kymograph analysis were performed as in Fig. 4. Photobleaching was performed at 2 s and 62 s. Fluorescence images were acquired every 1 s for 120 s. Numbers indicate the positions of the stepwise formation of new adhesion structures. Scale bar, 10 µm. See also supplemental movie S6. B, ruffling of lamellipodia. FRAP time-lapse analysis and kymograph analysis were performed as in Fig. 4. Photobleaching was performed at 18 s. Fluorescence images were acquired every 1 s for 77 s. Ruffling was shown to occur at 60 s. Scale bar, 10 µm. See also supplemental movie S7.

Fig. S4. Effects of LIMK1 and cofilin shRNAs on expression of each protein. MCF-7 cells were transfected with control, LIMK1 or cofilin shRNAs and cultured for 48 h. Cell lysates were immunoblotted with anti-LIMK1, anti-cofilin and anti-β-actin antibodies.

Supplemental Fig. S4 Ohashi *et al.*

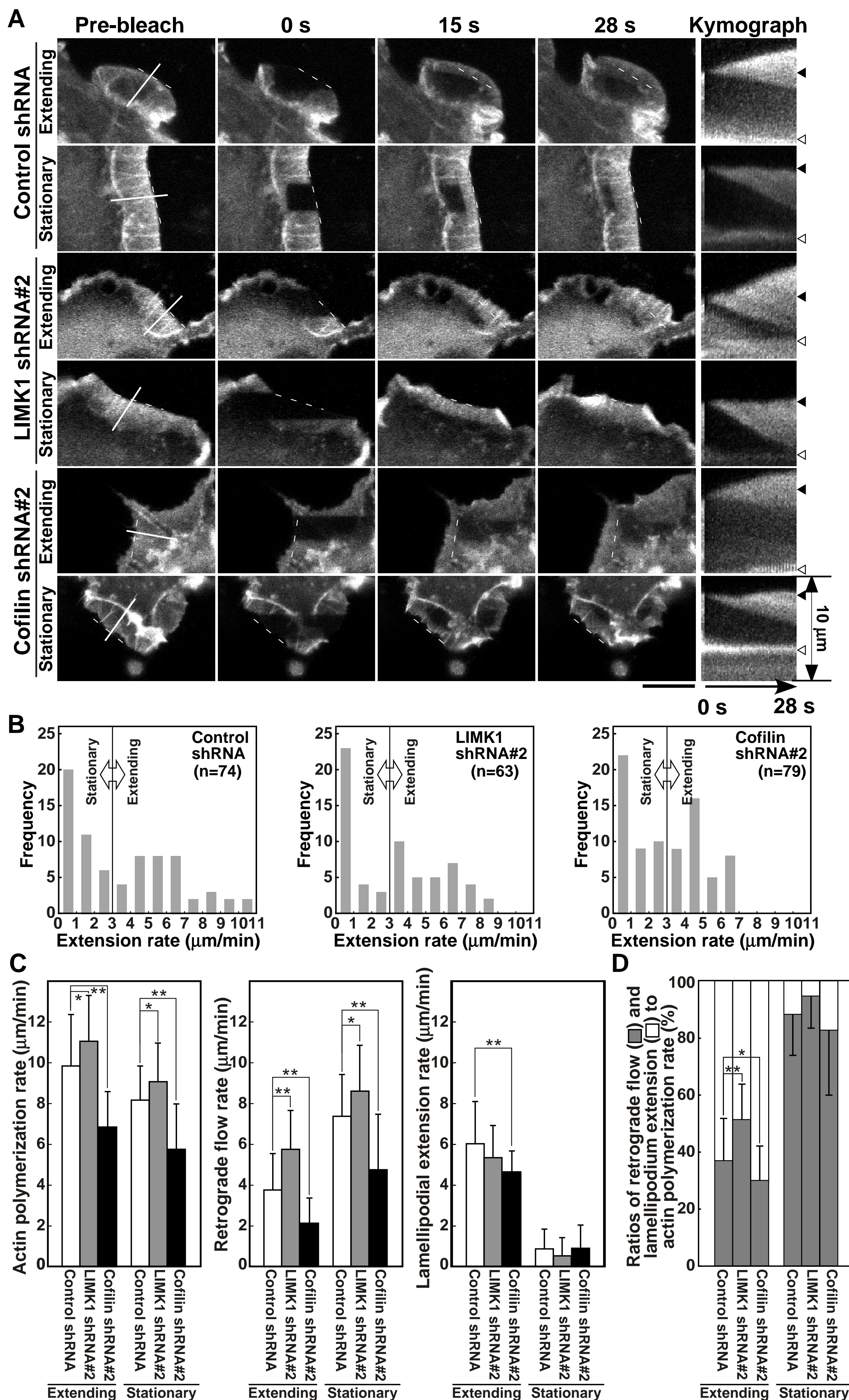

Fig. S5. Effects of another set of LIMK1 and cofilin shRNAs on the rates of actin polymerization, retrograde flow and lamellipodium extension in NRG-stimulated MCF-7 cells. A, FRAP time-lapse analysis. MCF-7 cells were cotransfected with YFP-actin and control shRNA, LIMK1 shRNA#2 or cofilin shRNA#2. Cells were treated with NRG and subjected to FRAP analysis, as in Fig. 6A. Scale bar, 10 μm. B, histograms of cell numbers for lamellipodium extension rates. Lamellipodia were classified into stationary and extension phases, as in Fig. 6B. C, quantitative analysis of the rates of actin polymerization, retrograde flow and lamellipodium extension. Lamellipodia were classified into extension and stationary phases, as in B. Data are means ± SD of 37 (control shRNA), 30 (LIMK1 shRNA#2) and 41 cells (cofilin shRNA#2) for extending lamellipodia, and 37 (control shRNA), 33 (LIMK1 shRNA#2) and 38 cells (cofilin shRNA#2) for stationary lamellipodia. D, ratios of conversion of actin polymerization into actin retrograde flow and lamellipodium extension. Data are calculated from C. *$p < 0.05$; **$p < 0.001$.