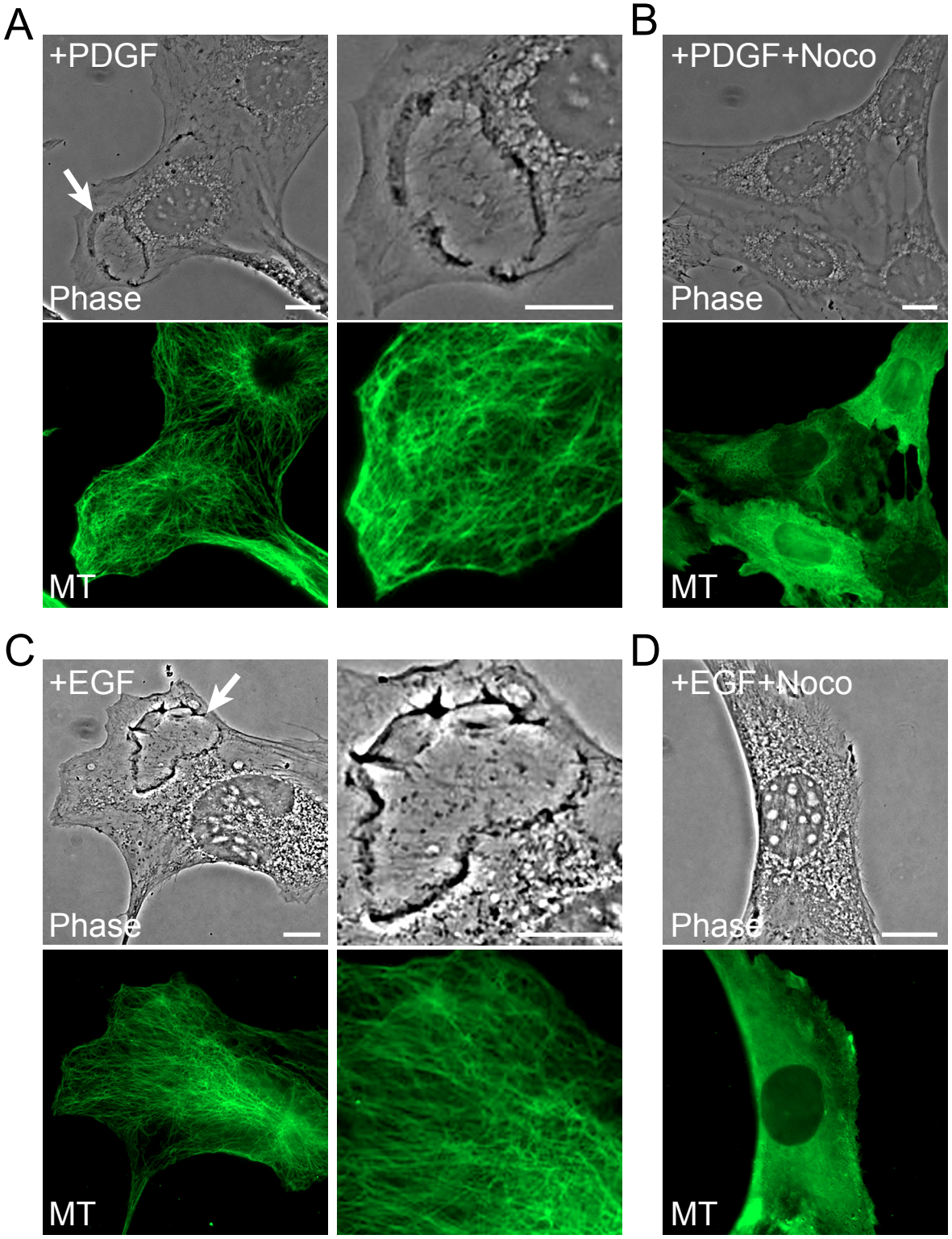


Figure S1. Immunofluorescence imaging of tubulin in MEFs stimu-lated by EGF or PDGF. (**A**) PDGF induced CDR (arrow and enlarged image). Microtubules (MT) underlie CDR. (**B**) Nocodazole blocked PDGF-induced CDR without otherwise altering cell morphology. Tubulin staining shows that nocodazole depolymerized microtubules. (**C**) EGF induced CDR without significantly altering the microtubule network (arrow and enlarged image). (**D**) Nocodazole depolymer-ized microtubules and blocked PDGF-induced CDR. Scale bar = 10 μm

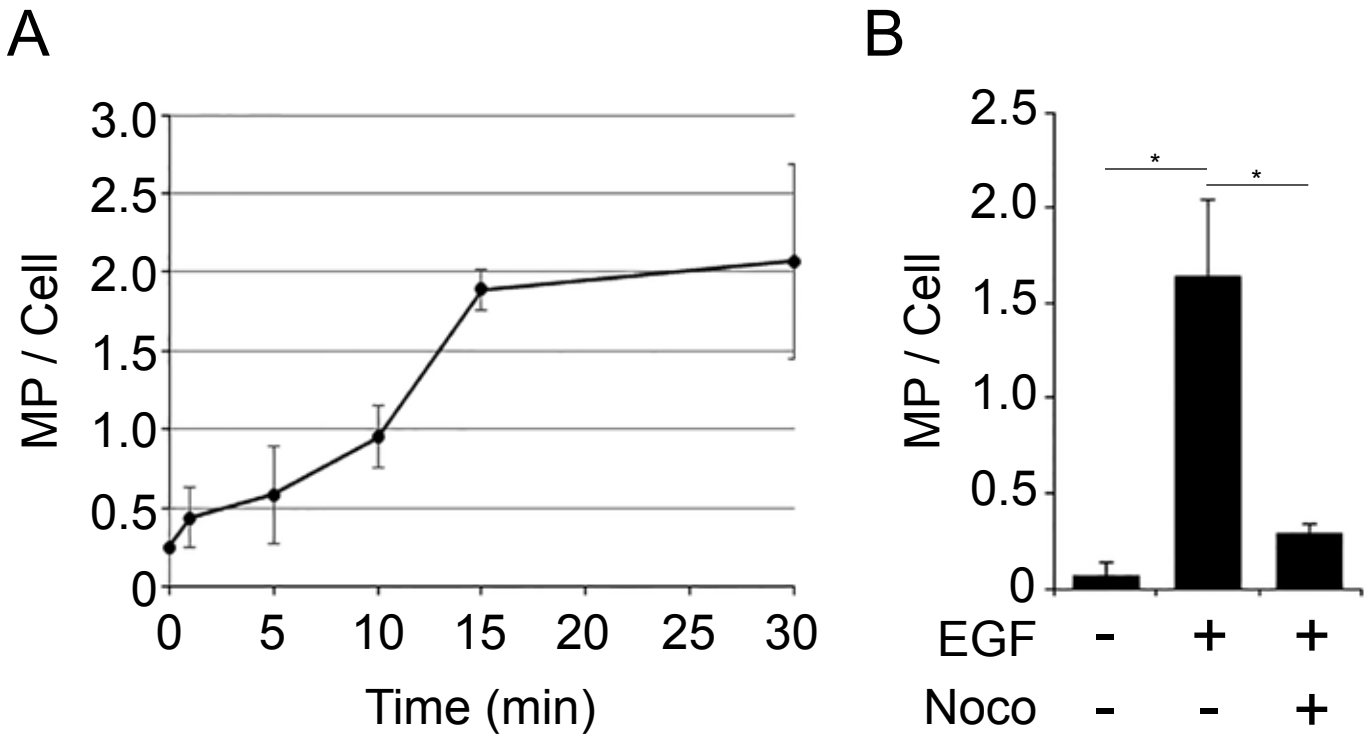


Figure S2. EGF-induced macropinocytosis is inhibited by noco-dazole. (**A**). Time-course of EGF-induced macropinocytosis. MEF were stimulated with EGF in the presence of FDx, then washed, fixed and scored for macropino-somes. The number of macropinosome in EGF-treated MEF increased 15 min after the treatment. (**B**). Nocodazole treatment blocked EGF-induced macropinocytosis. Cells were incubated 15 min with FDx, then washed, fixed and scored for macropi-nosomes. More than 15 cells were observed for each condition. A one-way ANOVA was applied for statistical analysis. *p<0.05.

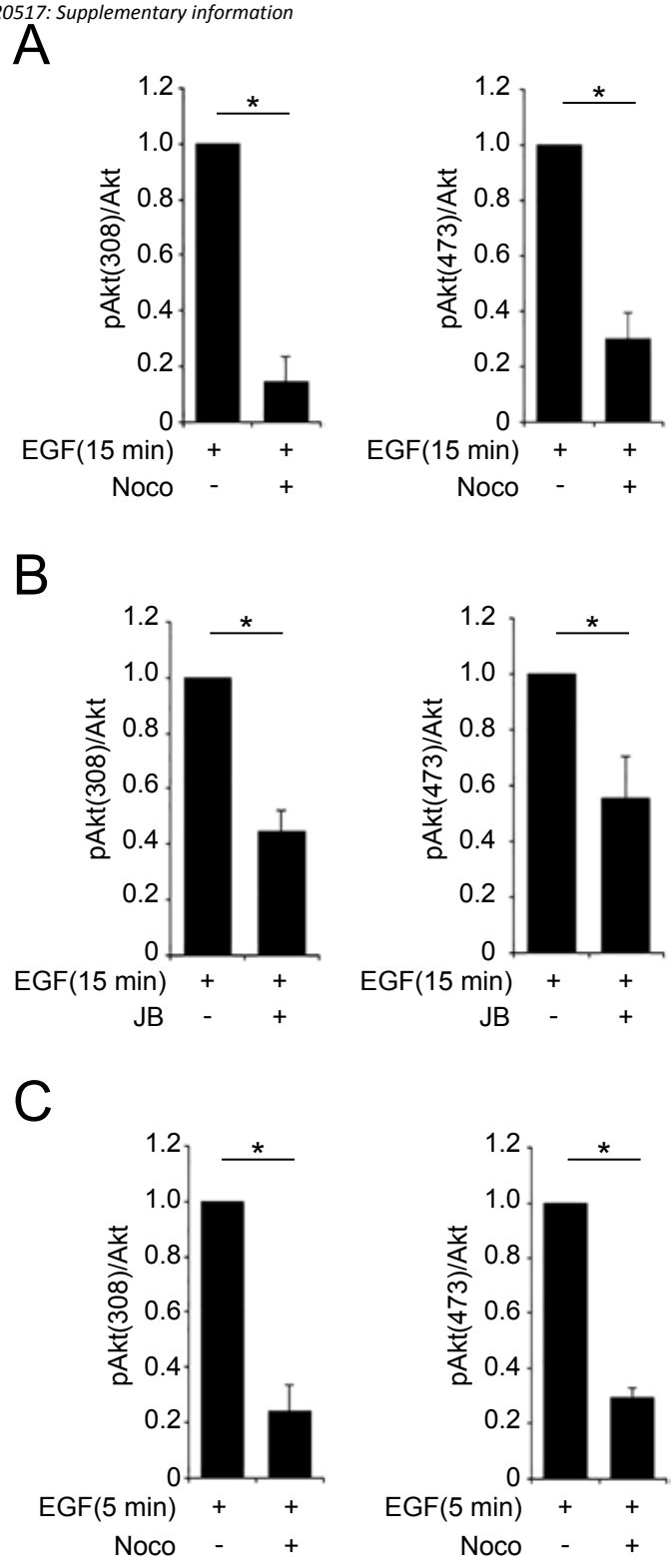


Figure S3. Quantification of western blots confirm that EGF-in-duced pAkt was attenuated by cytoskeletal inhibitors. (**A**) Quantification of western blot results on pAkt(308) and pAkt(473) after EGF stimulation (15 min) with or with-out nocodazole (Noco). (**B**) Quantification of western blot results on pAkt(308) and pAkt(473) after EGF stimulation (15 min) with or without JB. (**C**) Quantification of western blot results on pAkt(308) and pAkt(473) after EGF stimulation (5 min) with or without nocodazole (Noco). Three independent experiments were carried out for the quantification. *p<0.05.

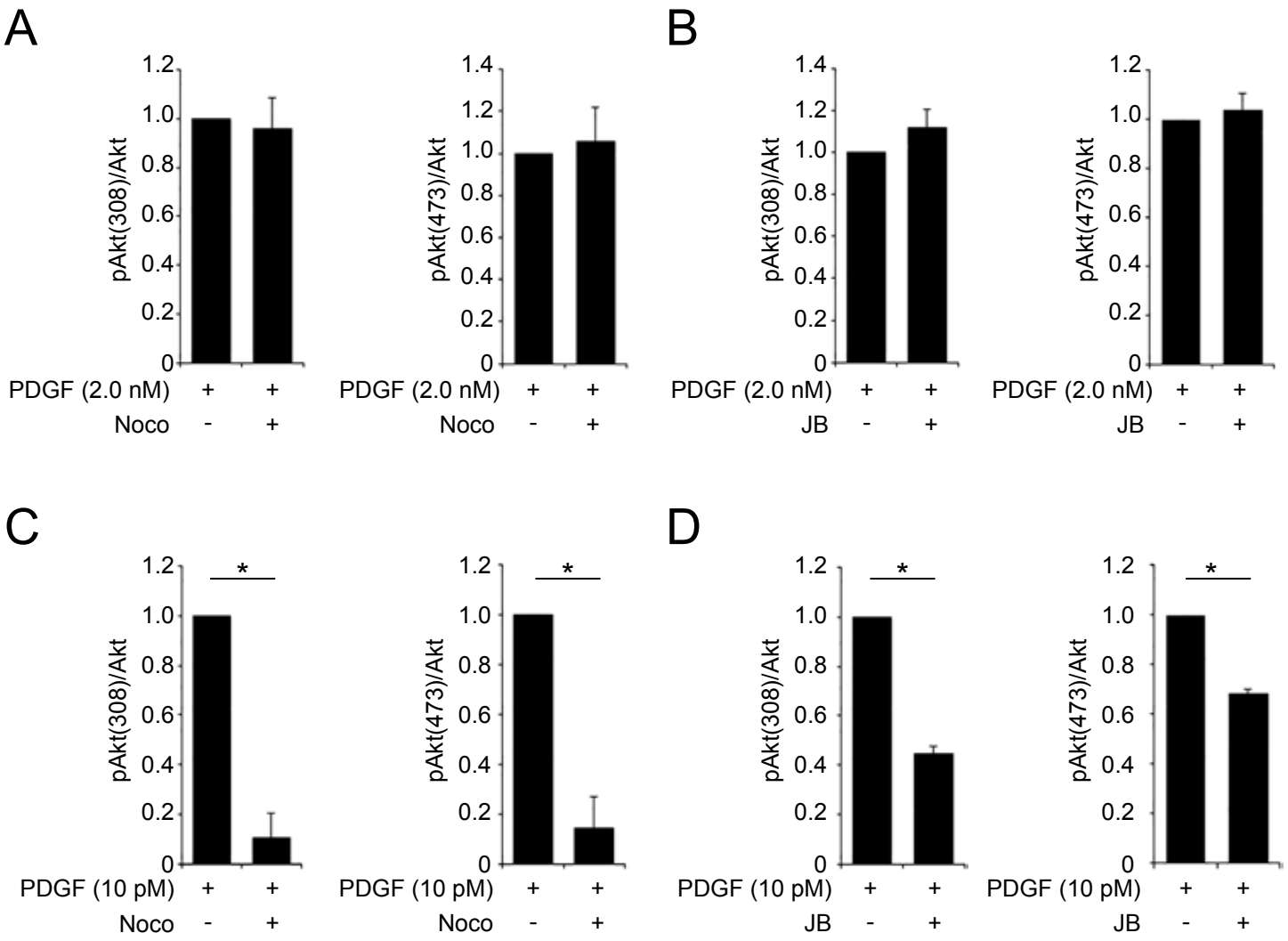


Figure S4. Akt phosphorylation in response to 10 pM PDGF was blocked by cytoskeletal inhibitors. (**A**, **B**) Quantification of western blot results on pAkt(308) and pAkt(473) after PDGF stimulation (2.0 nM) with/without nocodazole (Noco) (A) or J/B (B). (**C**, **D**) Quantification of western blot results on pAkt(308) and pAkt(473) after PDGF stim-ulation (10 pM) with/without nocodazole (Noco) (C) or J/B (D). Three independent experi-ments were carried out for each panel. *p<0.05.

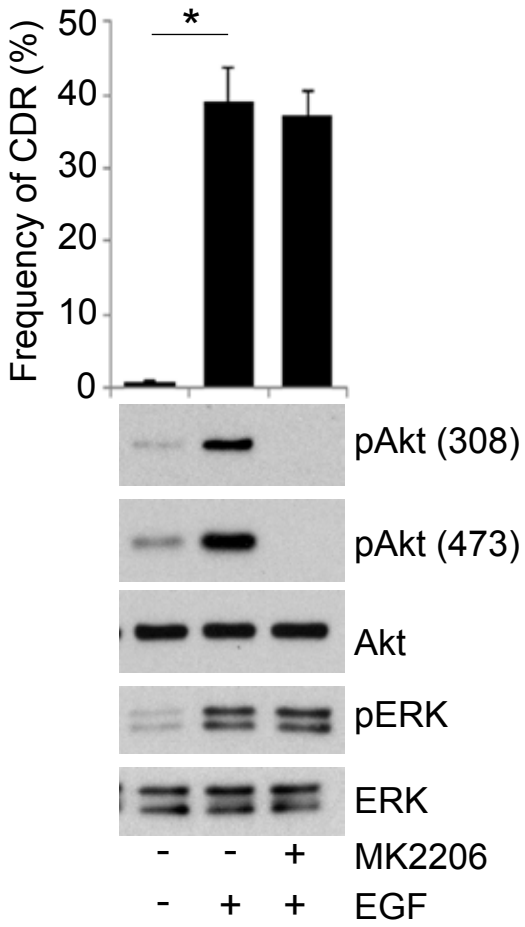
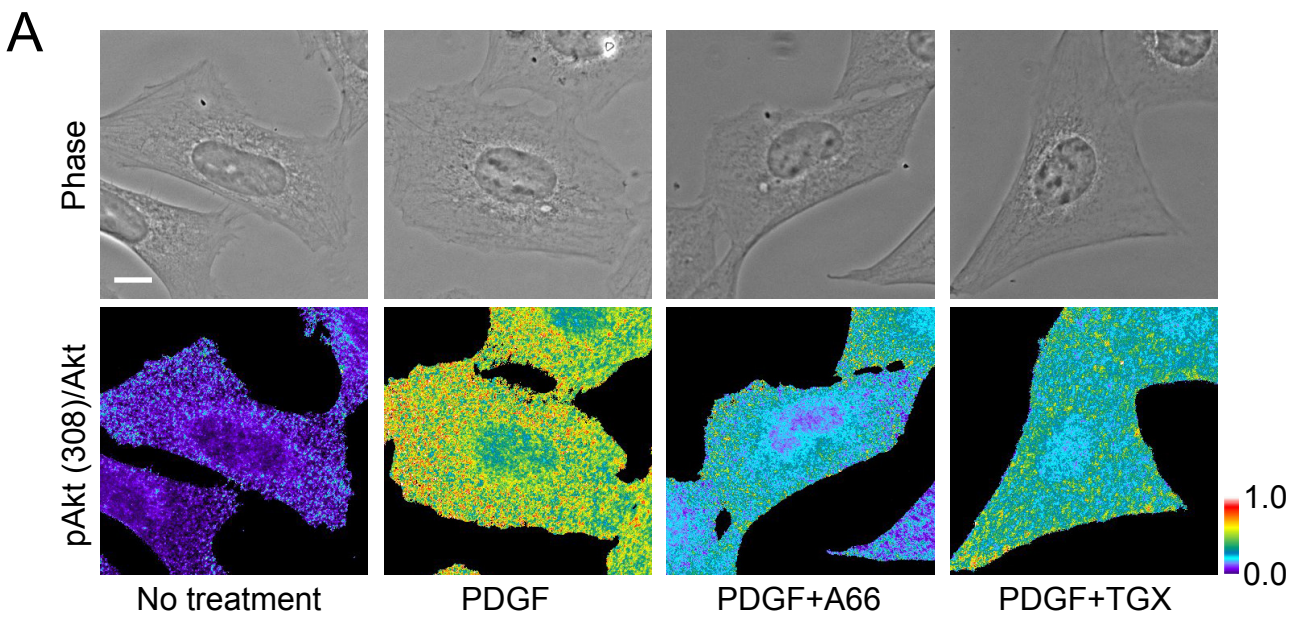
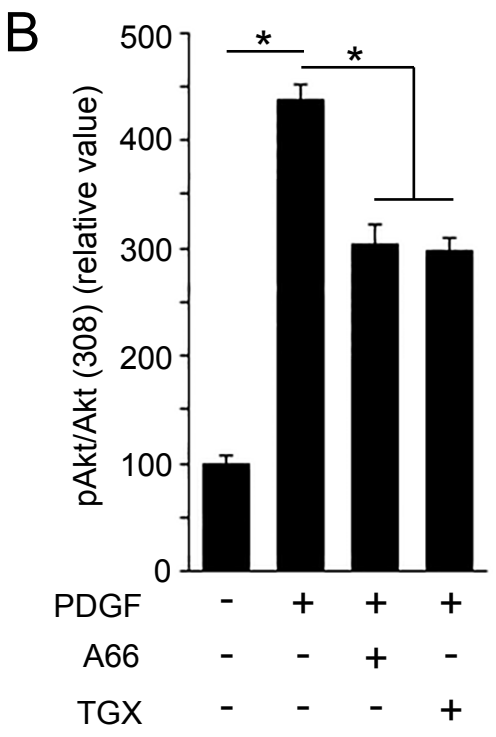


Figure S5. Effects of the Akt inhibitor MK2206 on CDR and signaling by EGF. MK2206 blocked EGF-induced Akt phosphorylation, but not EGF-induced CDR formation or ERK phosphorylation.





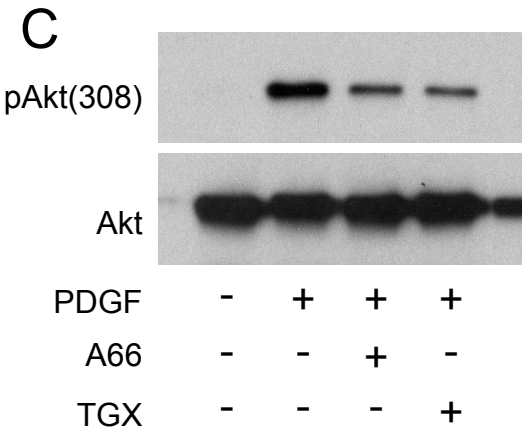


Figure S6. Validation of pAkt/Akt ratio imaging to quantify Akt phos-phorylation in individual cells. (**A**) MEF were treated for 15 min with 2 nM PDGF with or without PI3K inhibitors A66 (p110α-specific) and TGX221 (p110β-specific). After fixation, samples were stained with anti-pAkt(308) and anti-Akt antibodies. Alexa-488-anti-rabbit IgG and Alexa-594-anti-mouse IgG antibodies were applied to detect pAkt(308) and Akt, respectively. Ratio images were calculated by dividing the pAkt(308) fluorescence by Akt fluorescence. Scale bar is 10 μm. Color bar indicates relative values of ratio intensities. (**B**) Quantification of ratio imaging in (A). The ratio value of the PDGF-treated sample was higher than that of no treatment, and was attenuated by A66 or TGX221. More than 10 cells were observed for each condition. *p<0.05. (**C**) For biochemistry, cell lysates were prepared in the same conditions as in (A). Western blot analysis showed that PDGF treatment induced Akt phosphorylation, which was attenuated by A66 and TGX221.

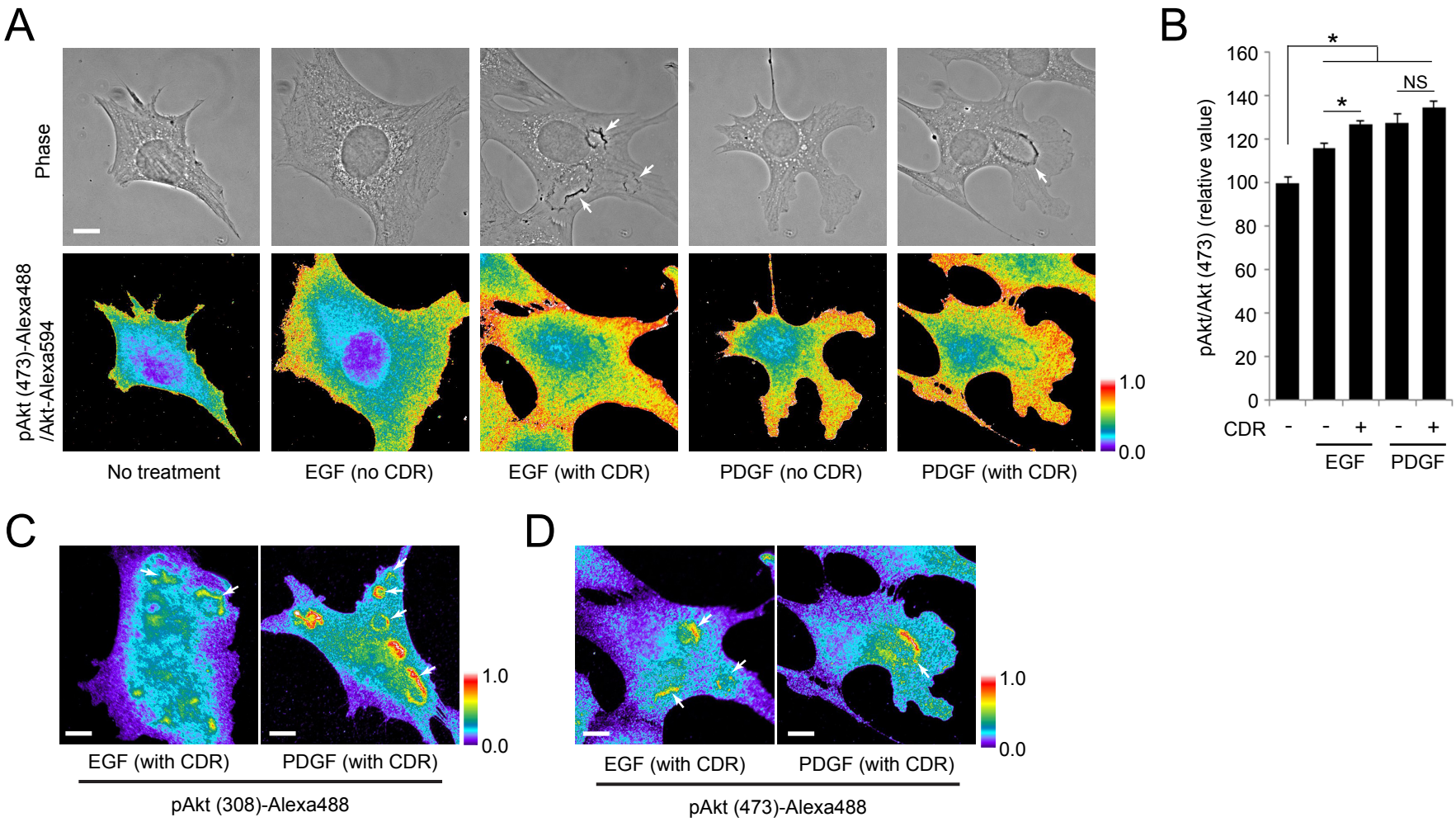


Figure S7. Correlation between the formation of CDR and the magnitude of Akt phosphorylation. (**A**) Phase and ratio (pAkt(473)/Akt) images for MEF in different conditions, as in Figure 4. Scale bar is 10 μm. (**B**) Quantification of ratio imag-ing in (A). The relative ratio values of EGF-stimulated cells showing CDR (EGF, CDR+) were significantly higher than those of EGF-stimulated cells showing no CDR (EGF, CDR-). *p<0.05. More than 10 cells were observed for each assay. *p<0.05. (**C**) pAkt(308) images of MEF showing CDR induced by EGF (left) or PDGF (right). Cells were treated with EGF (16 nM) or PDGF (2 nM) for 3 min. Arrows indicate CDR. Scale bar = 10 μm. (**D**) pAkt(473) images of MEF showing CDR induced by EGF (left) or PDGF (right) from (A). Arrows indicate CDR. Scale bar = 10 μm. All color bars indicate relative values of ratio intensities.

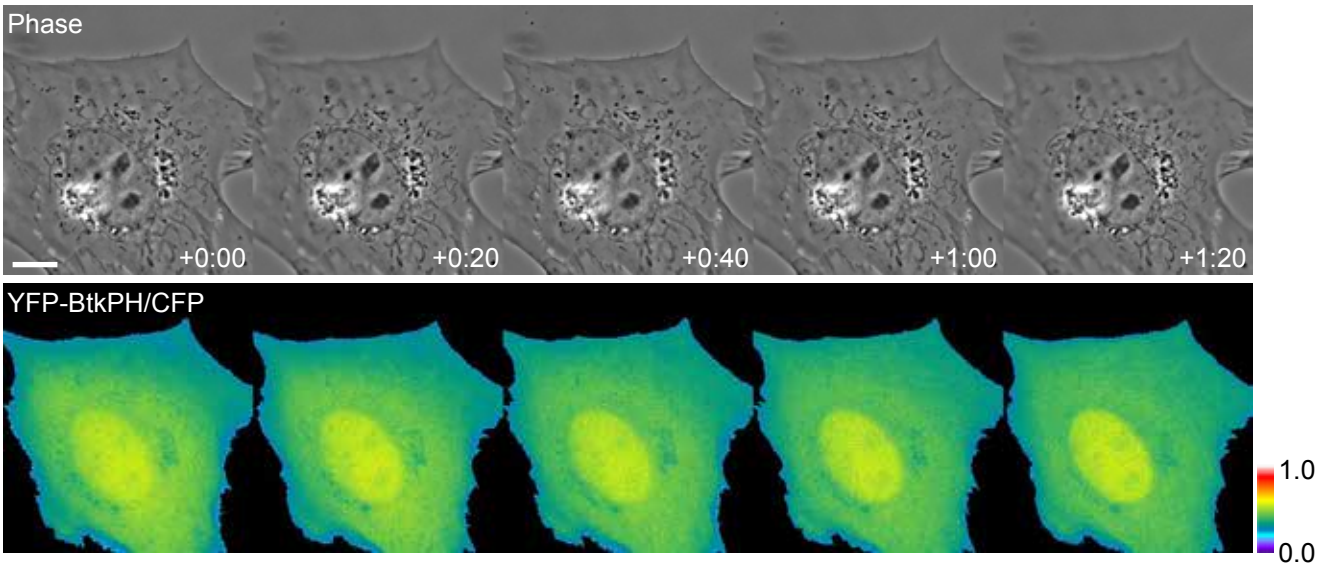


Figure S8. Ratio imaging of unstimulated MEF. Live-cell imag-ing of MEF expressing YFP-Btk-PH, a probe protein for PIP3, and CFP as a volume reference. Time over the observation (no stimulation) is indicated at bottom of phase contrast images (minutes : seconds). Scale bar = 10 μm. Color bar indicates relative value of ratio intensities.