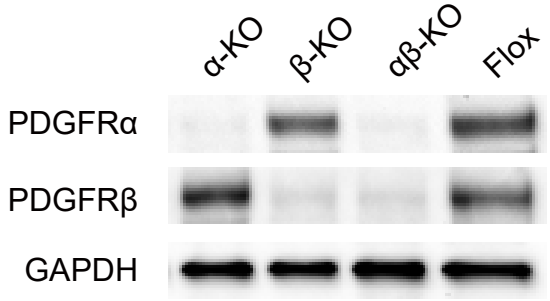
Supplementary Material

Different PDGF Receptor Dimers Drive Distinct Migration Modes of the Mouse Skin Fibroblast

Kohta Yamada[a,b] Takeru Hamashima[a] Yoko Ishii[a] Seiji Yamamoto[a] Noriko Okuno[a] Naofumi Yoshida[a] Moe Yamada[a] Ting Ting Huang[a] Norifumi Shioda[c] Kei Tomihara[b] Toshihiko Fujimori[d] Hisashi Mori[e] Kohji Fukunaga[f] Makoto Noguchi[b] Masakiyo Sasahara[a]

[a]Department of Pathology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, [b]Department of Oral and Maxillofacial Surgery, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, [c]Department of Biofunctional Analysis Laboratory of Molecular Biology, Gifu Pharmaceutical University, Gifu, [d]Division of Embryology, National Institute for Basic Biology, Okazaki, [e]Department of Molecular Neuroscience, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, [f]Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan

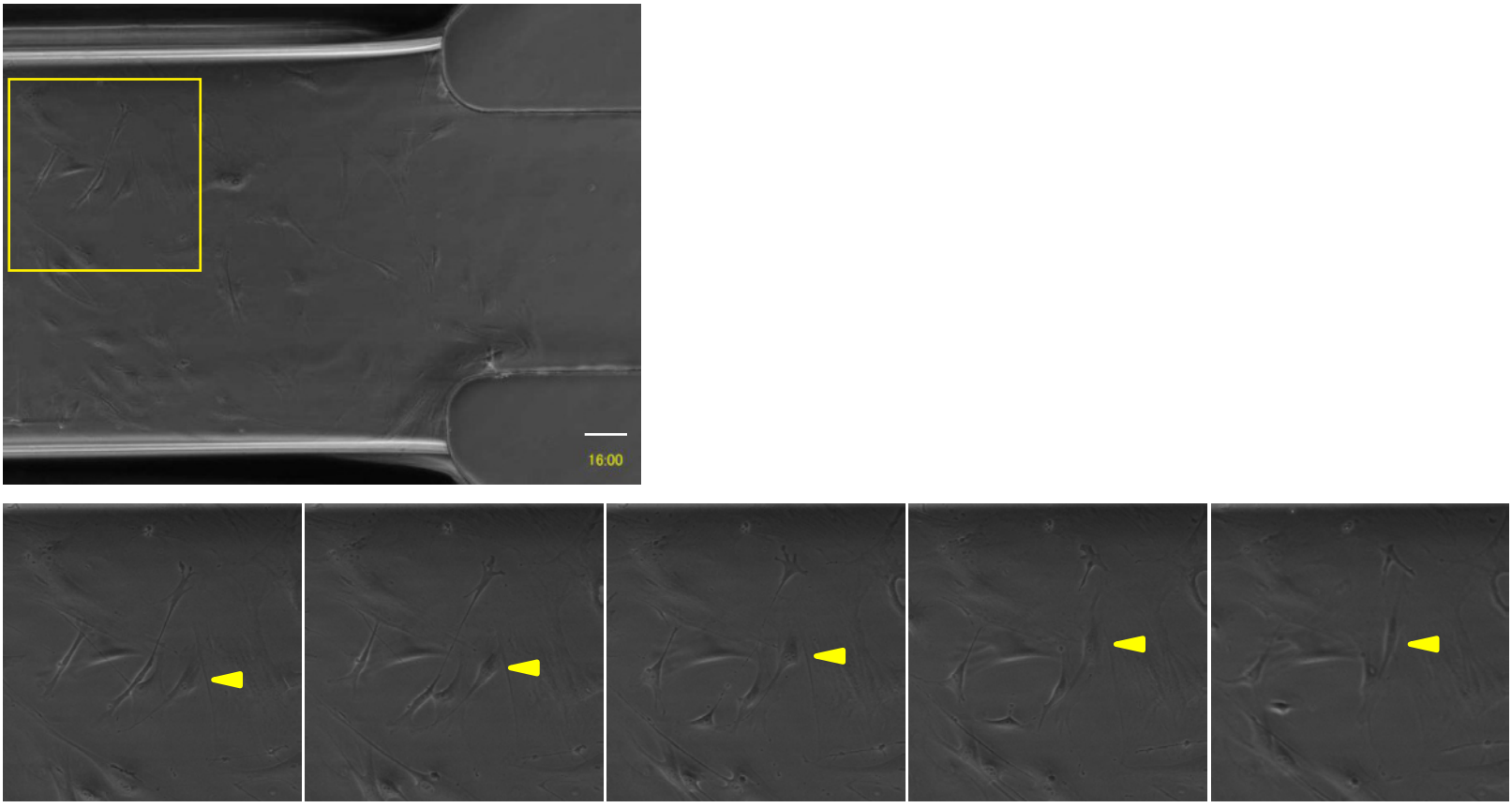
Supplementary Figure 1



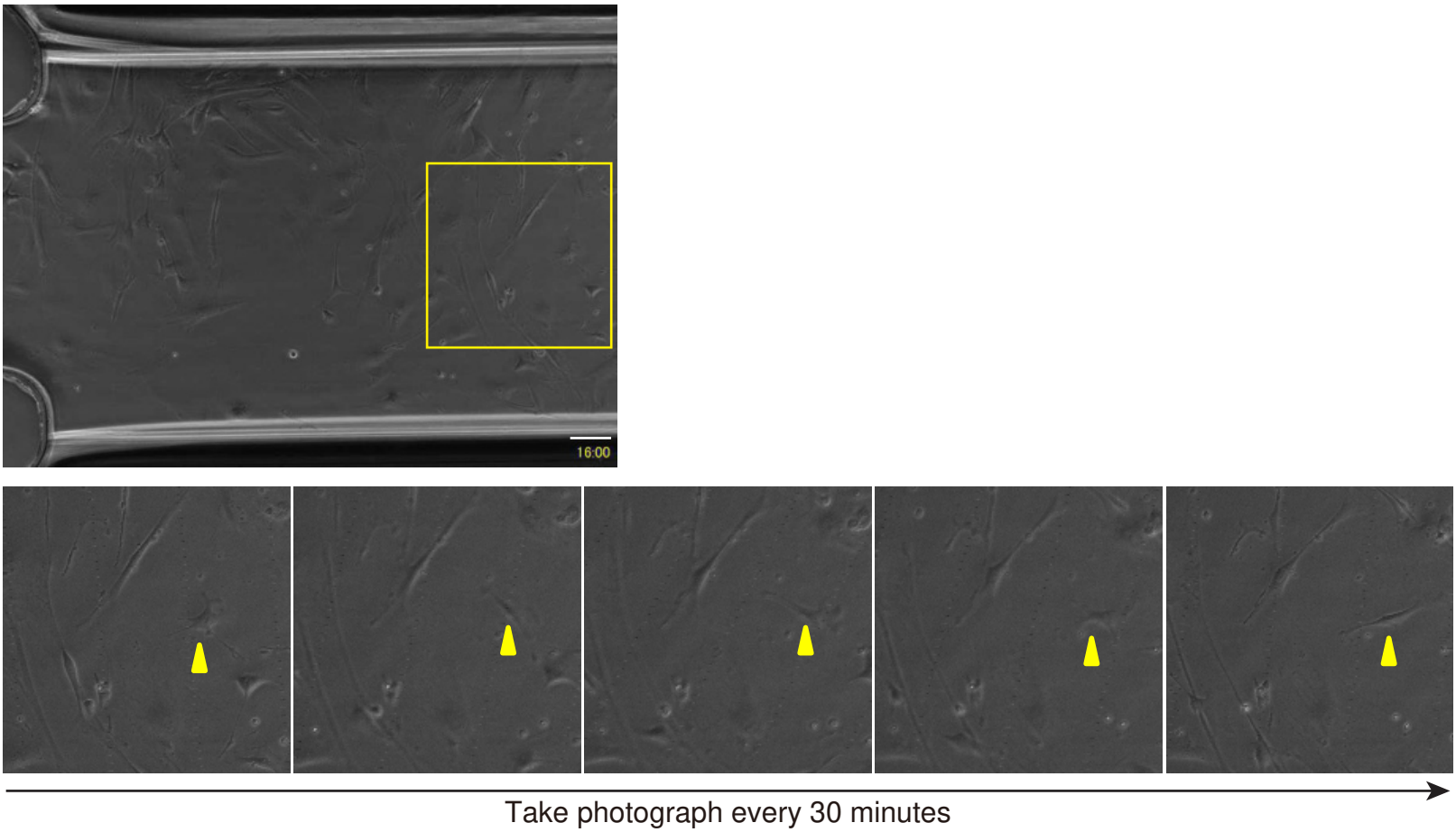
Supplementary Figure 1. Inactivation of different *Pdgfr* genes specifically suppresses expression of the corresponding gene products in fibroblasts. The expression of PDGFR proteins was examined by western blot after tamoxifen-induced *Pdgfr* gene inactivation *in vitro*. In *Pdgfra* gene-inactivated fibroblasts (α-KO), PDGFRα protein was mostly depleted but PDGFRβ protein was preserved. In *Pdgfrb* gene-inactivated fibroblasts (β-KO), PDGFRβ protein was mostly depleted but PDGFRα protein was preserved. In fibroblasts with an inactivation of both *Pdgfra and Pdgfrb* genes (αβ-KO), both PDGFRα and PDGFRβ proteins were depleted. In Flox control fibroblasts (Flox), both PDGFRα and PDGFRβ proteins were expressed. Equal protein loading was confirmed through the use of GAPDH as a housekeeping protein.

Supplementary Figure 2

A α-KO fibroblasts (directional migration)

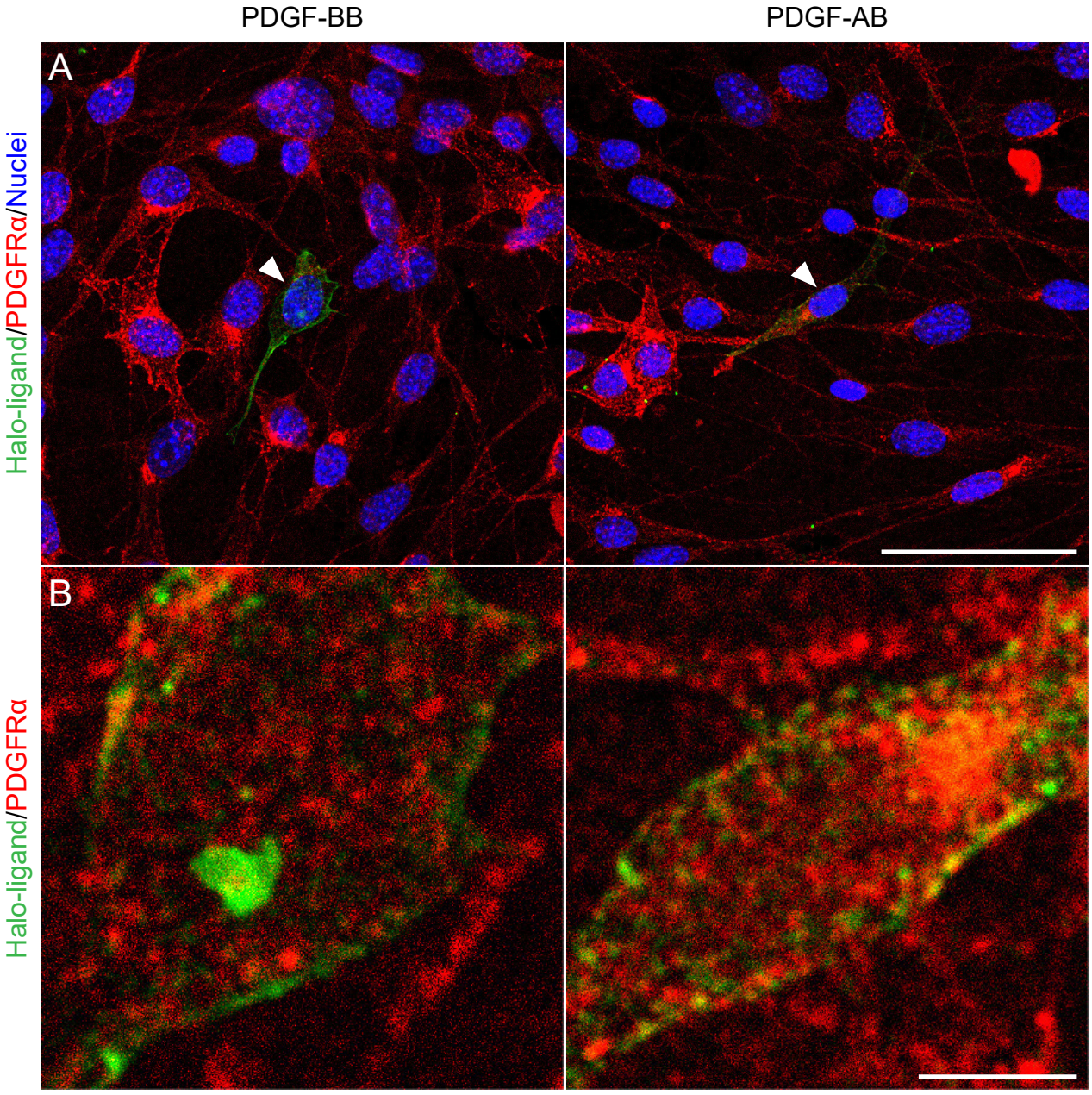


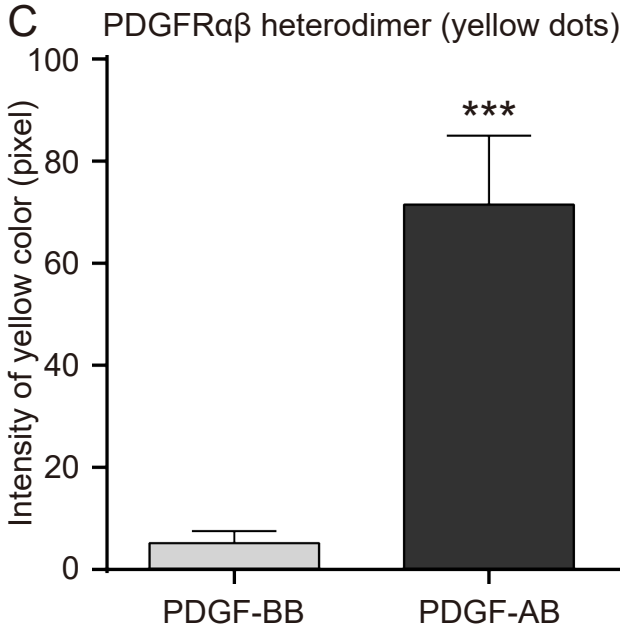
B αβ-KO fibroblasts (random migration)



Supplementary Figure 2. Migration chamber overview and high-magnification time-lapse images of α-KO and αβ-KO fibroblasts. (A, B) The pictures shown here were captured from the videos of different experiments, respectively. (A) The upper image shows an overview of the migration chamber (right part of the chamber) seeded with α-KO fibroblasts. An FBS gradient was generated between 30% (upper border) and 0% (lower border). The lower images show the directional migration of the α-KO fibroblasts focusing on the boxed area in the upper image. The time-lapse images taken at 30 minute intervals from the boxed area were arranged from left to right. Arrowheads highlight the sequential changes of cell shape and location of the same α-KO fibroblast. (B) The upper image shows an overview of the migration chamber (left part of the chamber) seeded with αβ-KO fibroblasts. An FBS gradient was generated between 30% (upper border) and 0% (lower border). The lower images show the random migration of the αβ-KO fibroblasts focusing on the boxed area in the upper image. The time-lapse images taken at 30 minute intervals from the boxed area were arranged from left to right. Arrowheads highlight the sequential changes of cell shape and location of the same αβ-KO fibroblast.

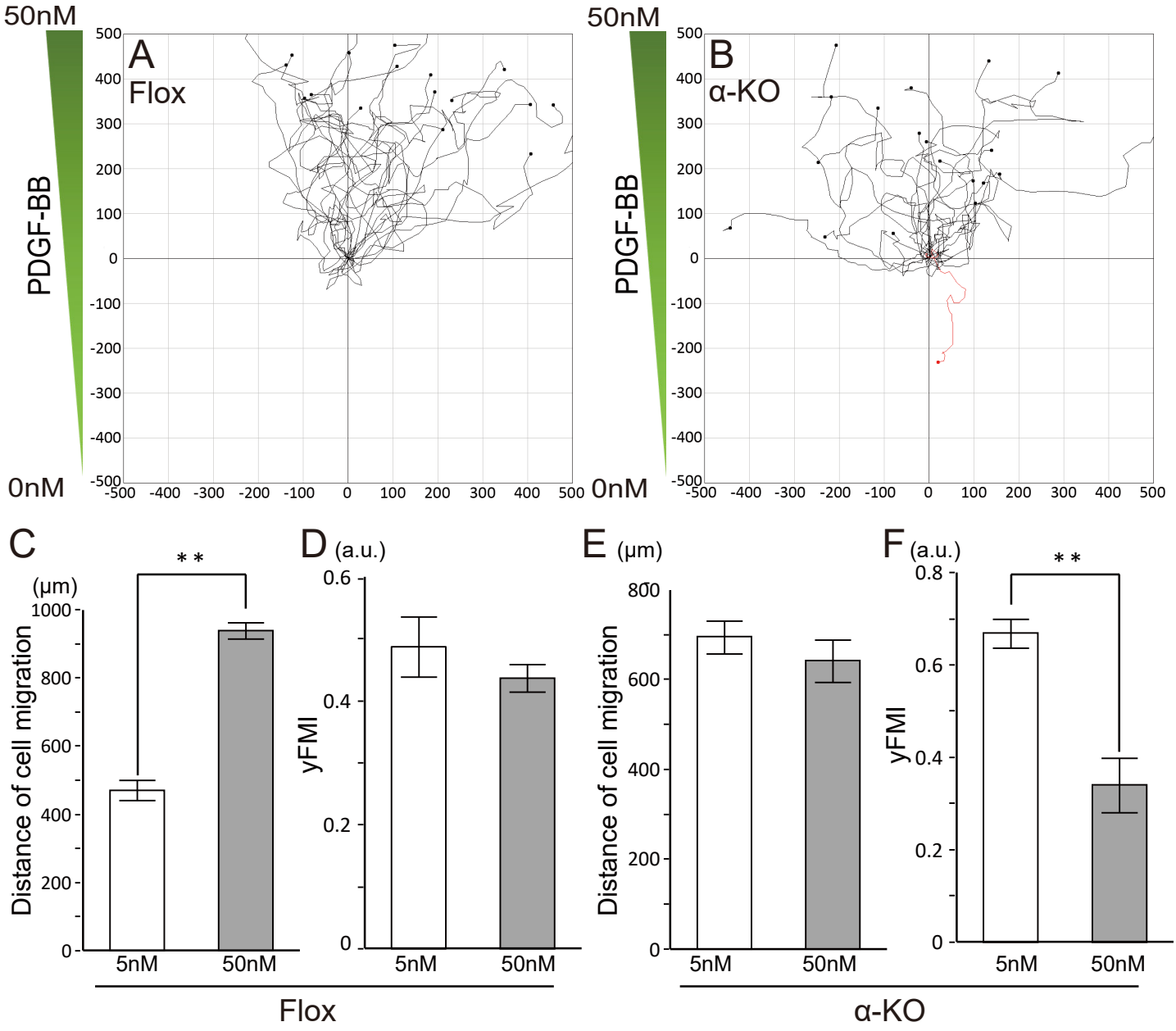
Spplementary Figure 3





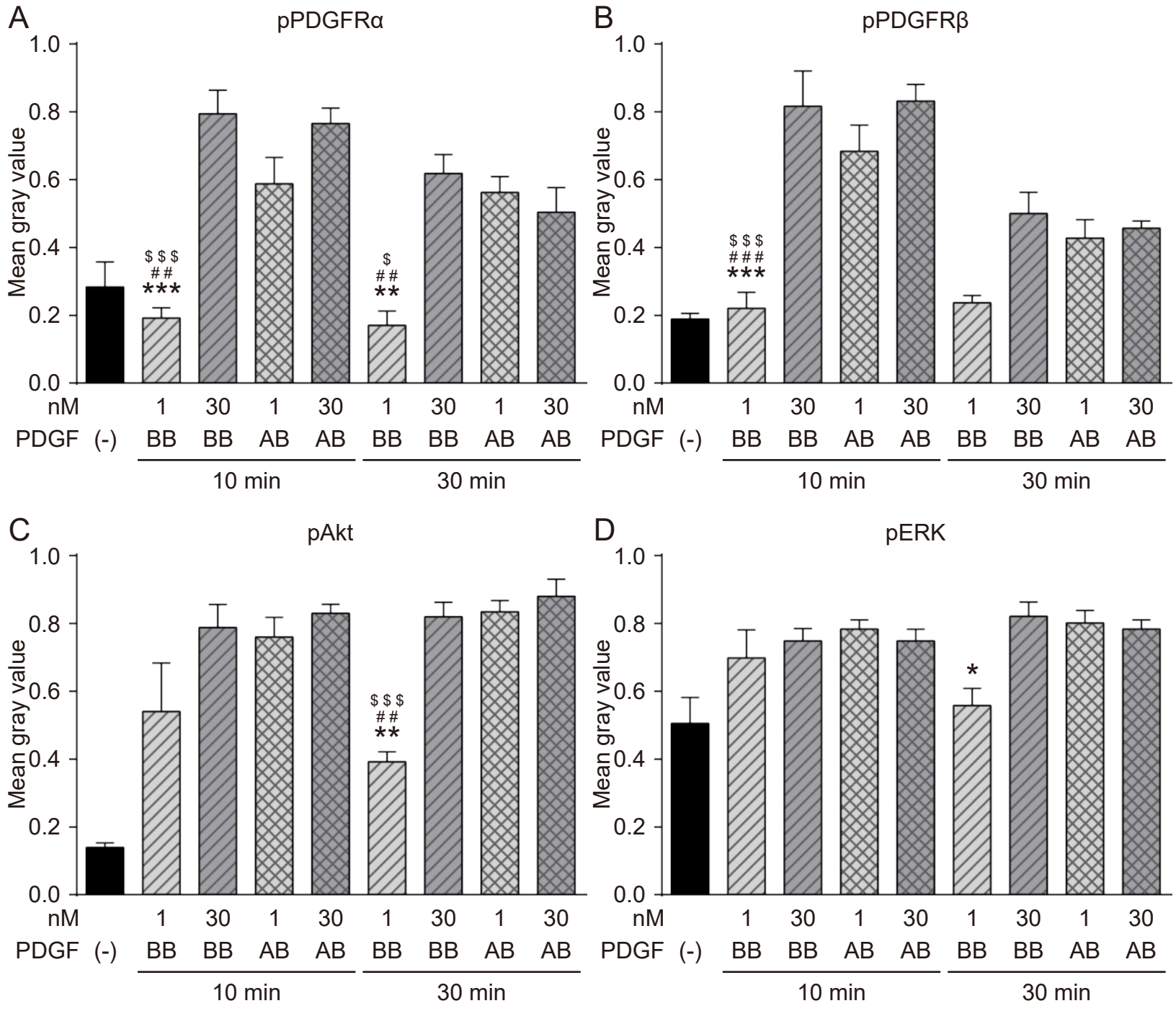
Supplementary Figure 3. Induction of PDGFR heterodimer is less after PDGF-BB stimulation than after PDGF-AB stimulation. To visualize PDGFR heterodimer formation after PDGF simulation, we prepared a gene construct encoding *Pdgfrb-HaloTag* fusion gene. The fusion gene was prepared by inserting *HaloTag* gene (Promega, Madison, WI) between the signal peptide sequence and the mature coding regions of mouse *Pdgfrb* gene using HaloTag fusion construct. The fusion gene was transfected to NIH3T3 fibroblasts by use of Lipofectamine as per the manufacture's protocol (Thermo Fisher, Waltham, MA). After stimulating transfected cells with either of PDGF-BB or PDGF-AB, PDGFRαβ heterodimer formation was visualized by immunofluorescent staining of PDGFRα (red) and by Halo-ligand labelling of fusion protein (green). (A, B) Low magnification views show successfully transfected cells with *Pdgfrb-Halotag* fusion gene (A, arrow heads). At high magnification views (B), association of PDGFRα to PDGFRβ-HaloTag fusion protein is apparently less in PDGF-BB than in PDGF-AB stimulated NIH3T3 cells (B). Nuclei were depicted by Hoechst (blue). Scale bars = 50 μm in A, 5 μm in B. (C) Intensity of the yellow color (PDGFRαβ heterodimer) was significantly lower after PDGF-BB stimulation than after PDGF-AB stimulation. All error bars indicate the mean ± SEM. (*** = $p < 0.001$).

Supplementary Figure 4



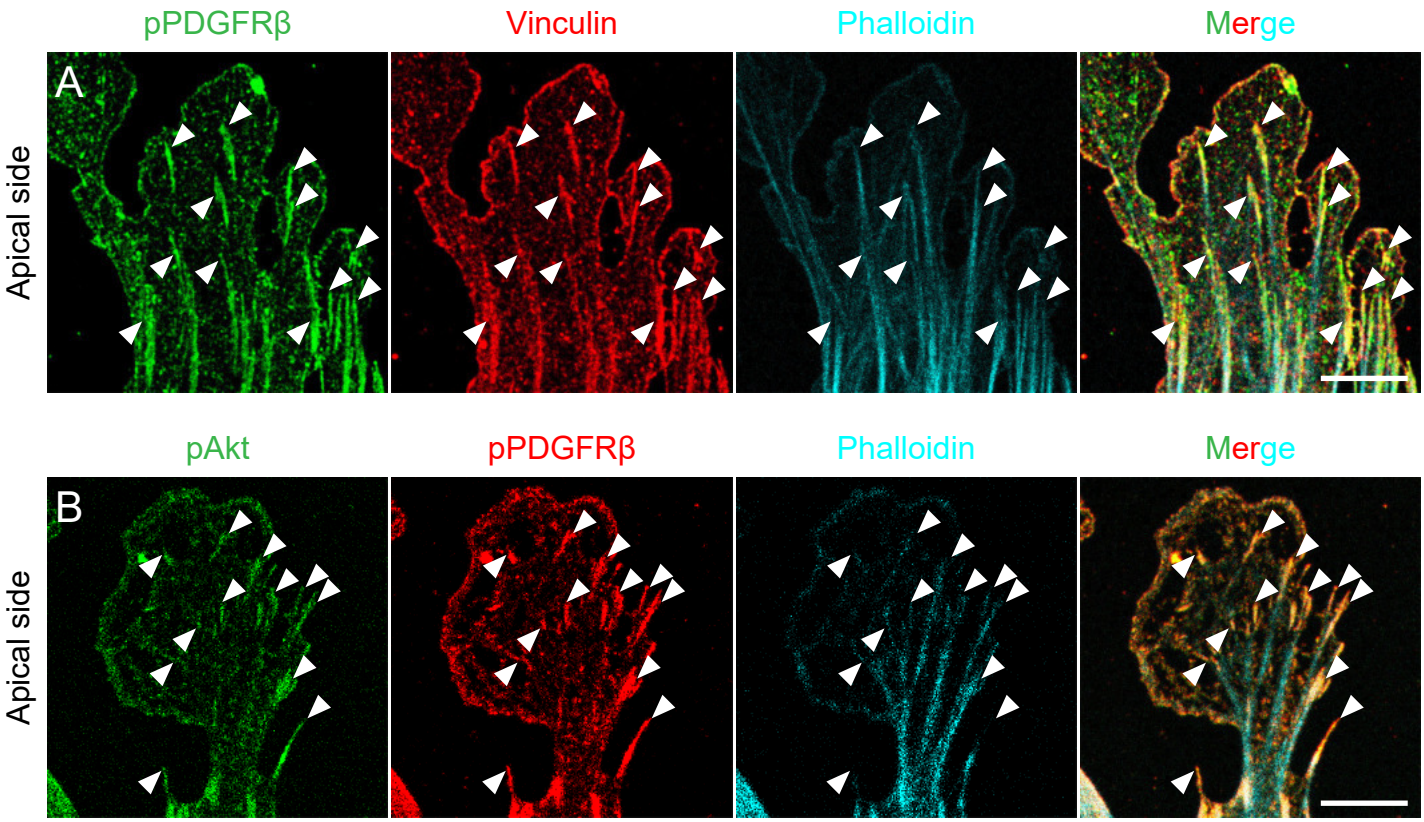
Supplementary Figure 4. A high gradient of PDGF-BB induces directional migration. (A and B) Trajectories of Flox (A) and α-KO (B) fibroblasts in response to a PDGF-BB gradient (50–0 nM). Green triangles indicate the chemoattractant gradient. (C and D) The distances of cell migration (C) and yFMI (D) of Flox fibroblasts stimulated by a high PDGF-BB gradient (50–0 nM) were compared with those obtained in Fig. 2B from cells stimulated with a low PDGF-BB gradient (5–0 nM). (E and F) The distances of cell migration (E) and yFMI (F) of α-KO fibroblasts cultured under a high PDGF-BB gradient (50–0 nM) were compared with those obtained in Fig. 1F from cells stimulated with a low PDGF-BB gradient (5–0 nM). Twenty cells were randomly selected from each experiment for preparing trajectories. Experiments were replicated a minimum of three times, and the representative results are presented. All error bars indicate the mean ± SEM. (** = $p < 0.01$).

Supplementary Figure 5



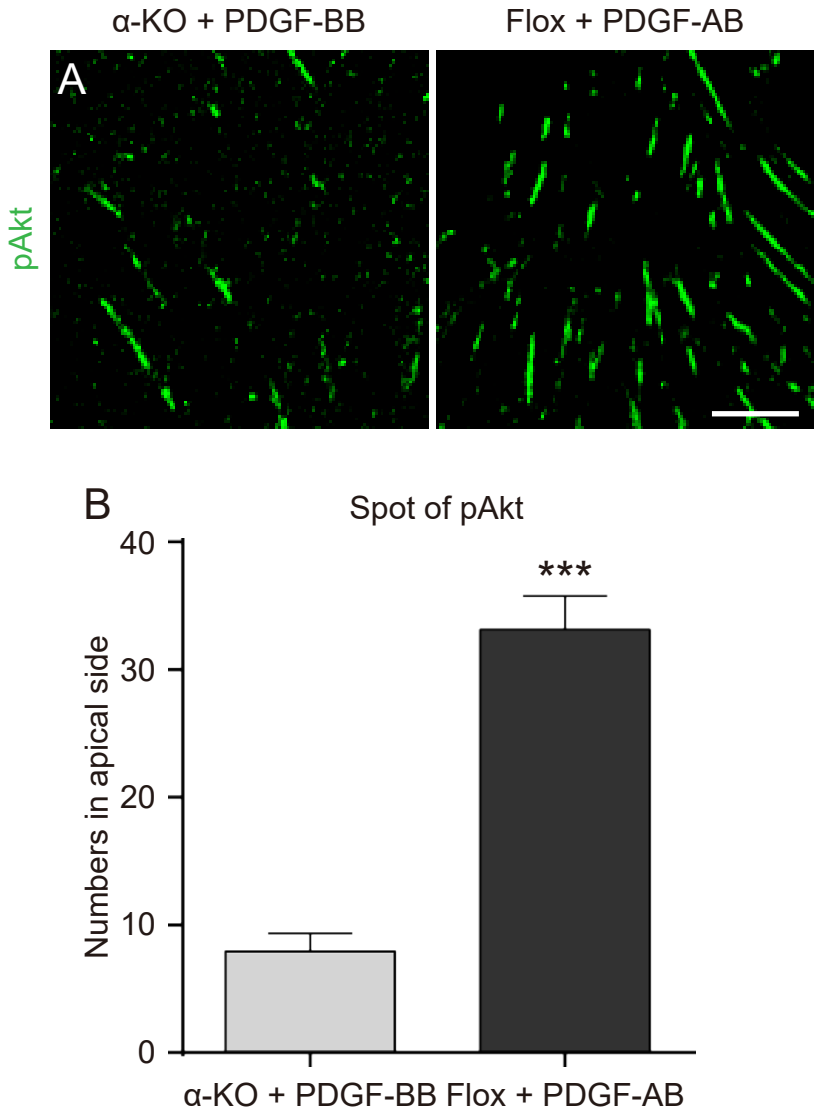
Supplementary Figure 5. PDGFRαβ is more potent activator of Akt and ERK compared with PDGFRββ. (A, B) Western blotting of Figure 3A was repeated three times, and the activation of PDGFRα and PDGFRβ was quantitatively measured. (A) At 10 min of PDGF stimulation, phosphorylated PDGFRα (pPDGFRα) induction after 1nM of PDGF-BB was significantly lower than that after 30 nM of PDGF-BB (∗∗∗, $p<0.001$) and after 1 nM (##, $p<0.01$) or 30 nM of PDGF-AB ($$$, $p<0.001$). At 30 min of PDGF stimulation, pPDGFRα induction after 1nM of PDGF-BB was significantly lower than that after 30 nM of PDGF-BB (∗∗, $p<0.01$) and after 1 nM (##, $p<0.01$) or 30 nM of PDGF-AB ($, $p<0.05$). (B) At 10 min of PDGF stimulation, phosphorylated PDGFRβ (pPDGFRβ) induction after 1nM of PDGF-BB was significantly lower than that after 30 nM of PDGF-BB (∗∗∗, $p<0.001$) and after 1 nM (###, $p<0.001$) or 30 nM of PDGF-AB ($$$, $p<0.001$). At 30 min of PDGF stimulation, pPDGFRβ induction after 1nM of PDGF-BB was tended to be lower than that after 30 nM of PDGF-BB and after 1 nM or 30 nM of PDGF-AB, but the difference was not significant. (C, D) Western blotting of Figure 3B was repeated three times, and the activation of Akt and ERK was quantitatively measured. (C) At 10 min of PDGF stimulation, phosphorylated Akt (pAkt) induction after 1nM of PDGF-BB was tended to be lower than that after 30 nM of PDGF-BB and after 1 nM or 30 nM of PDGF-AB, but the difference was not significant. At 30 min of PDGF stimulation, pAkt induction after 1nM of PDGF-BB was significantly lower than that after 30 nM of PDGF-BB (∗∗, $p<0.01$) and after 1 nM (##, $p<0.01$) or 30 nM of PDGF-AB ($$$, $p<0.001$). (D) At 10 min of PDGF stimulation, pERK induction after 1nM of PDGF-BB was similar to that after 30 nM of PDGF-BB and after 1 nM or 30 nM of PDGF-AB. At 30 min of PDGF stimulation, pERK induction after 1nM of PDGF-BB tended to be lower than that after 30 nM of PDGF-BB and after 1 nM or 30 nM of PDGF-AB, and the difference was significant in the comparison versus 30 nM of PDGF BB stimulation (∗, $p<0.05$).

Supplementary Figure 6



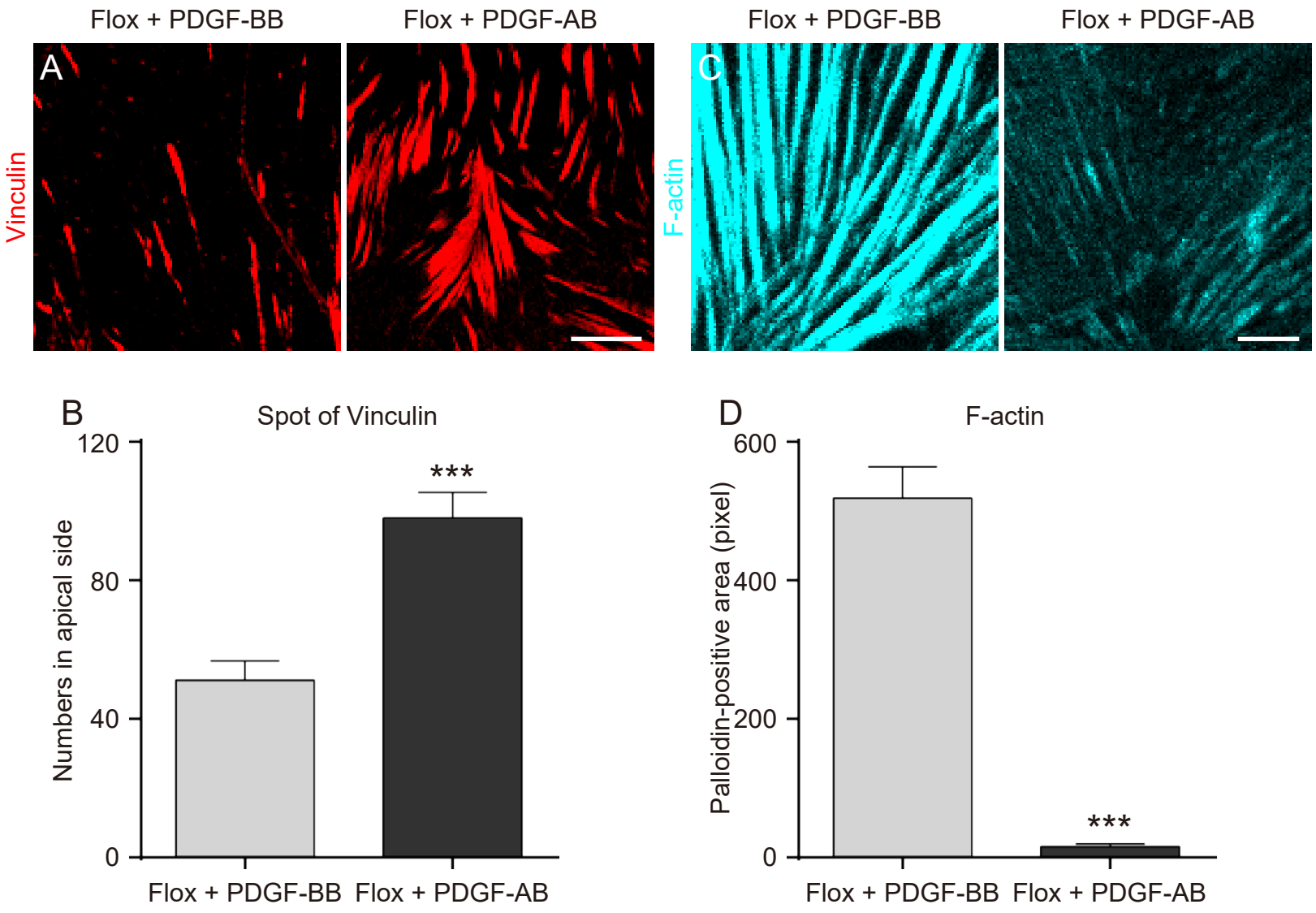
Supplementary Figure 6. Activated PDGFRβ and Akt were localized at vinculin-positive cell adhesion sites that were associating with stress fibers. (A) Immunofluorescence of phosphorylated PDGFRβ (pPDGFRβ, green), Vinculin (red), histochemistry of stress fiber by use of Phalloidin (cyan), and merged pictures of the three in PDGF-BB stimulated α-KO fibroblasts. pPDGFRβ colocalized with the vinculin-positive adhesion points along the stress fiber at the leading edge of α-KO fibroblasts. (B) Immunofluorescence of phosphorylated Akt (pAkt), pPDGFRβ, histochemistry of stress fiber by use of Phalloidin (cyan), and merged pictures of the three images in PDGF-BB stimulated α-KO fibroblasts. pAkt colocalized with pPDGFRβ along the stress fiber at the leading edge of α-KO fibroblasts. Bars = 10 μm

Supplementary Figure 7



Supplementary Figure 7. Spots of activated Akt were fewer in αKO with PDGF-BB stimulation than in Flox fibroblasts with PDGF-AB stimulation. (A) Immunofluorescence of phosphorylated Akt (pAkt) in PDGF-BB stimulated α-KO fibroblasts (α-KO + PDGF-BB) and in PDGF-AB stimulated Flox fibroblasts (Flox + PDGF-AB). Scale bar = 10 μm. (B) Number of pAkt-positive spots in apical side of fibroblasts were more numerous in (Flox + PDGF-AB) than in (αKO + PDGF-BB). ***, $p<0.001$ vs. (αKO + PDGF-BB).

Supplementary Figure 8



Supplementary Figure 8. Spots of vinculin were fewer after PDGF-BB stimulation than after PDGF-AB stimulation in Flox fibroblasts. (A) Immunofluorescence of vinculin in Flox fibroblasts after PDGF-BB stimulation (Flox + PDGF-BB) and after PDGF-AB stimulation (Flox + PDGF-AB). Scale bar = 10 μm. (B) Number of vinculin-positive spots in apical side of fibroblasts were more numerous in (Flox + PDGF-AB) than in (Flox + PDGF-BB). ***, $p<0.001$ vs. (Flox + PDGF-BB). (C) Fluorescence of F-actin using phalloidin-Alexa670 in Flox fibroblasts after PDGF-BB stimulation (Flox + PDGF-BB) and after PDGF-AB stimulation (Flox + PDGF-AB). Scale bar = 10 μm. (D) Intensity of F-actin areas in apical side of fibroblasts were higher in (Flox + PDGF-BB) than in (Flox + PDGF-AB). ***, $p<0.001$ vs. (Flox + PDGF-BB).