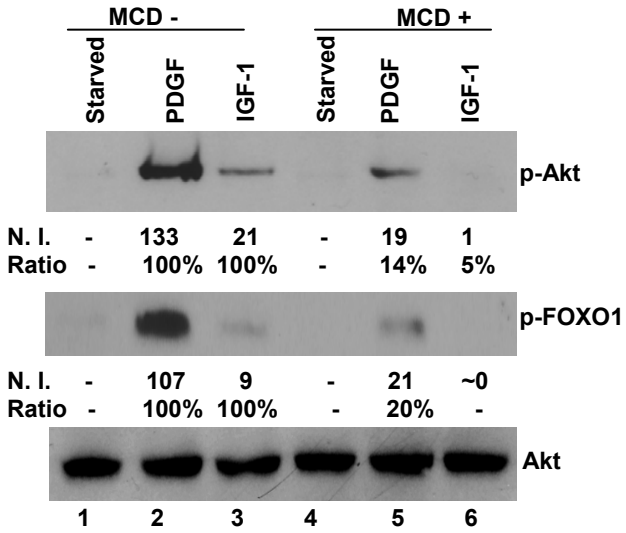
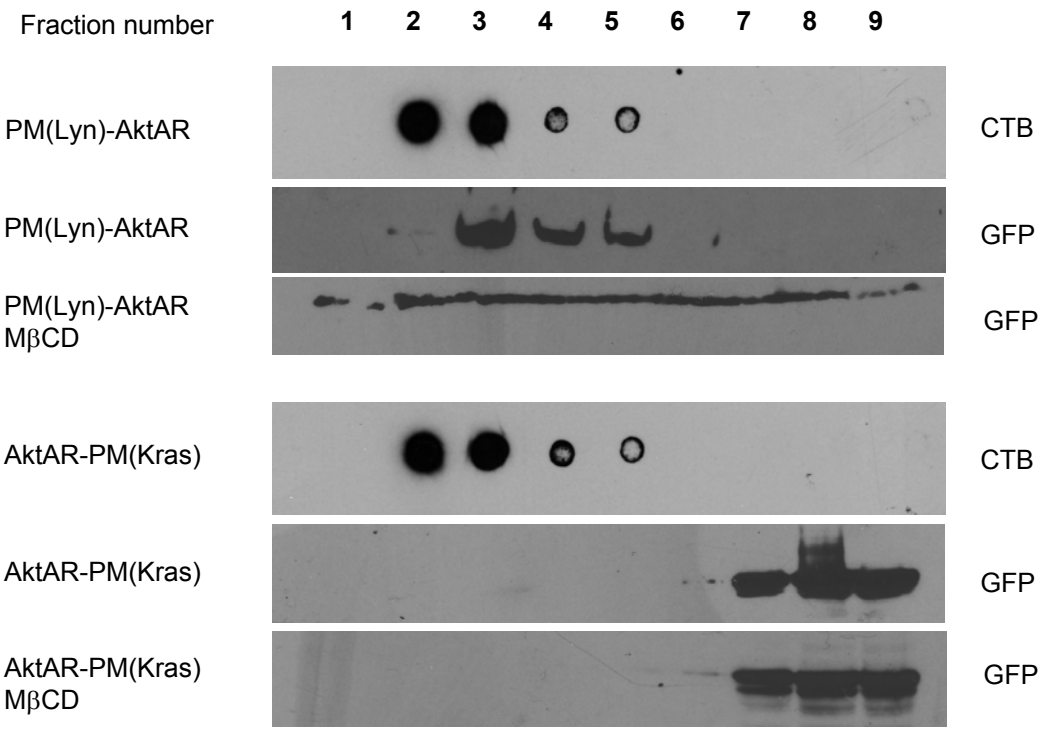
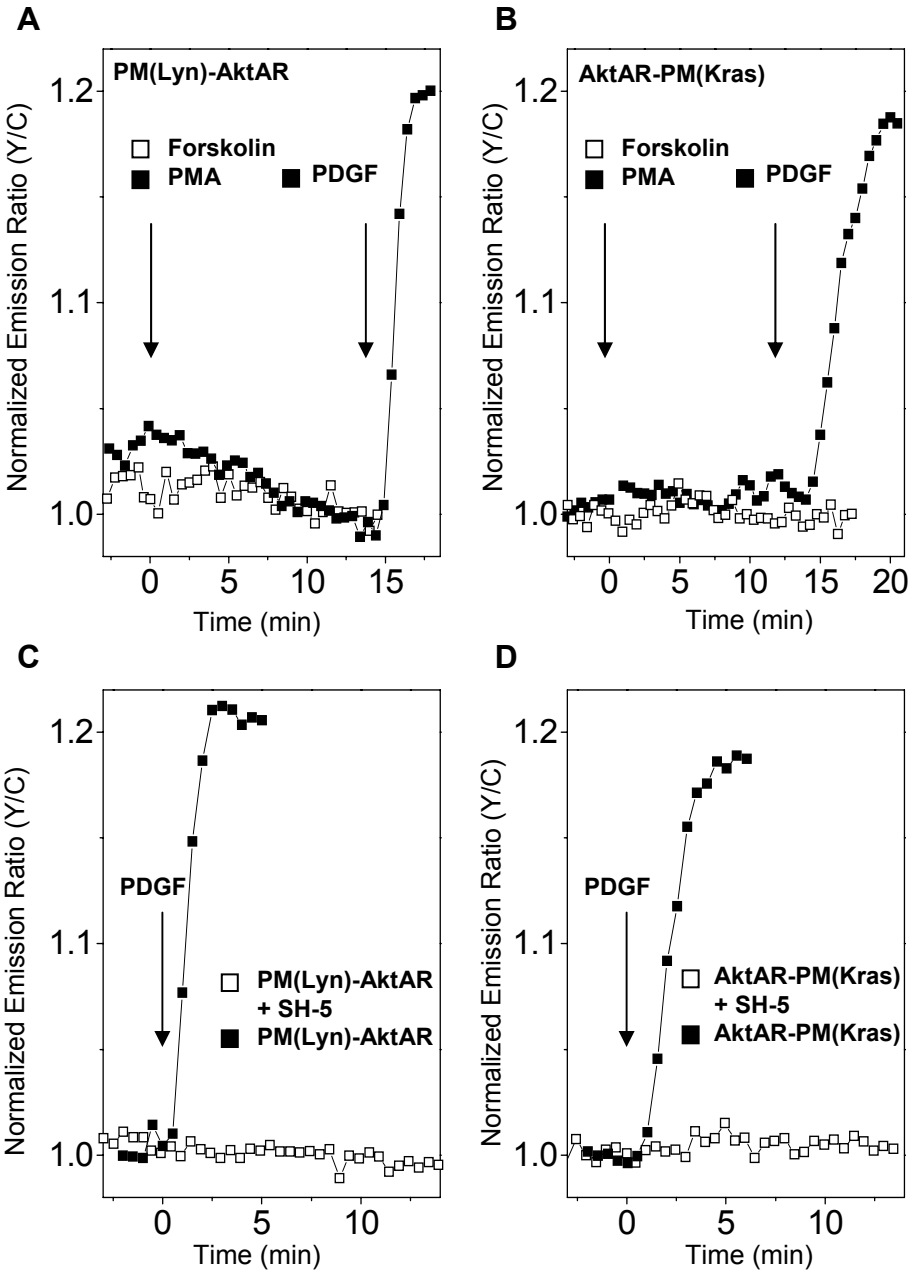


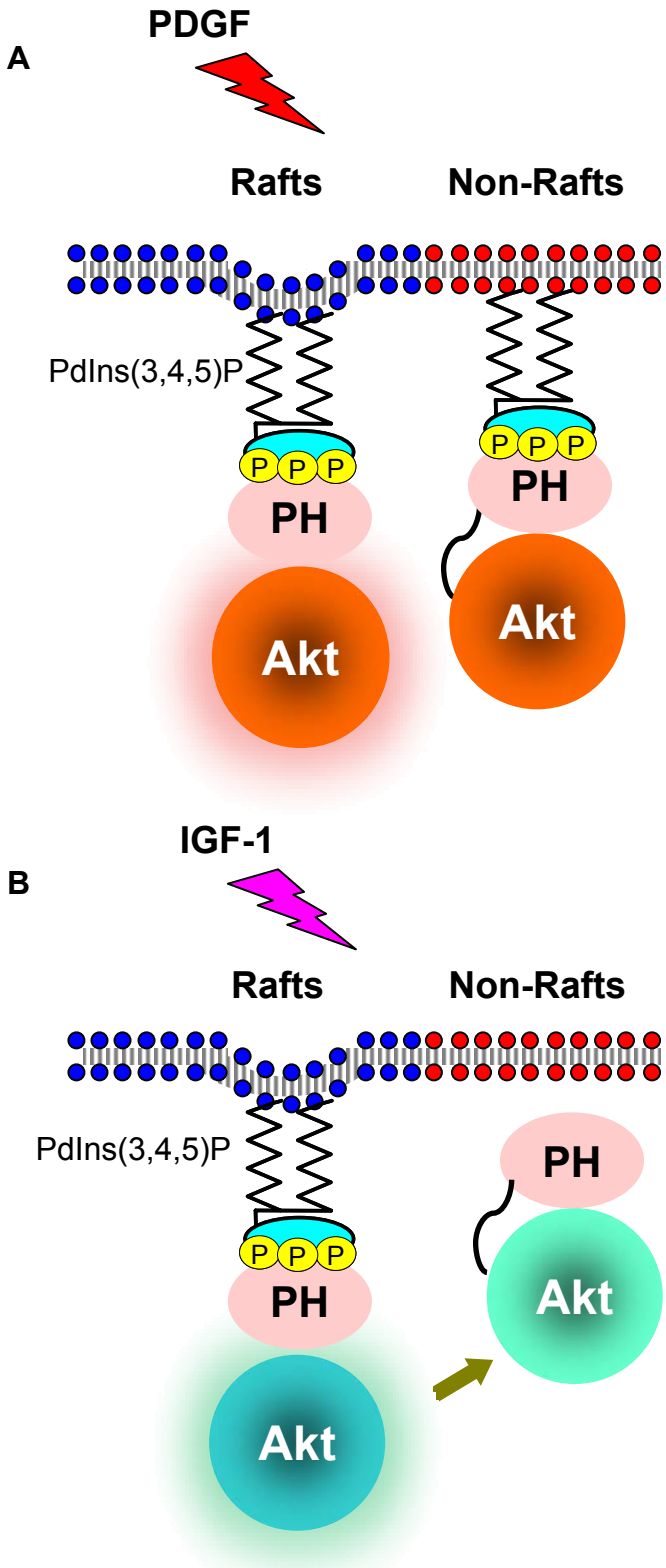
Supplementary Figure 1. Effects of raft disruption on Akt activation. NIH 3T3 cells were serum-starved for 24 h then stimulated with either 50 ng/ml PDGF or 400 ng/ml IGF-1, with or without pre-incubation with 5 mM MβCD for 30 min. Cells were lysed and subjected to western analysis with total Akt, phospho-Akt and phospho-FOXO1 antibodies. The normalized intensity (N. I.) was calculated with UN-SCAN-IT.



Supplementary Figure 2. PM(Lyn)-AktAR and AktAR-PM(Kras) are specifically targeted to lipid rafts and non-raft regions, respectively. HEK 293 T cells overexpressing either PM(Lyn)-AktAR or AktAR-PM(Kras) were subjected to sucrose density gradient fractionation, with or without 10 mM MβCD pre-incubation. Horse-radish peroxidase-conjugated Cholera toxin B subunit was used to determine the lipid raft containing fractions. All the samples were subjected to western analysis with a GFP antibody.



Supplementary Figure 3. Specificity of plasma membrane targeted AktAR. (A) PM(Lyn)-AktAR is insensitive to PKC and PKA activation. NIH3T3 cells were treated with either 50 ng/ml PMA, followed by 50 ng/ml PDGF (n = 3), or 50 μM forskolin (n = 5). (B) AktAR-PM(Kras) is insensitive to PKC and PKA activation. NIH3T3 cells were treated with either 50 ng/ml PMA, followed by 50 ng/ml PDGF (n = 4), or 50 μM forskolin (n = 3). (C) PM(Lyn)-AktAR specifically reports Akt activity. Representative time courses show the response of PM(Lyn)-AktAR in NIH 3T3 cells stimulated with 50 ng/ml PDGF in the absence (n = 4) and presence (n = 3) of 6 μM SH-5. (D) AktAR-PM(Kras) specifically reports Akt activity. Representative time courses show the response of PM(Lyn)-AktAR in NIH 3T3 cells stimulated with 50 ng/ml PDGF in the absence (n = 4) and presence (n = 3) of 6 μM SH-5.



Supplementary Figure 4. A refined model for regulation of Akt activity in different plasma membrane microdomains by PDGF and IGF-1. (A) PDGF stimulates PI(3, 4, 5)P_3 production both in lipid rafts and non-raft regions. The two pools of PI(3, 4, 5)P_3 regulate the activities of raft and non-raft Akt relatively independently. (B) IGF-1 stimulates PI(3, 4, 5)P_3 production mainly in lipid rafts, which leads to activation of Akt. Akt signaling in the non-raft regions is highly dependent on that in the raft regions.

Supplementary Figure 4