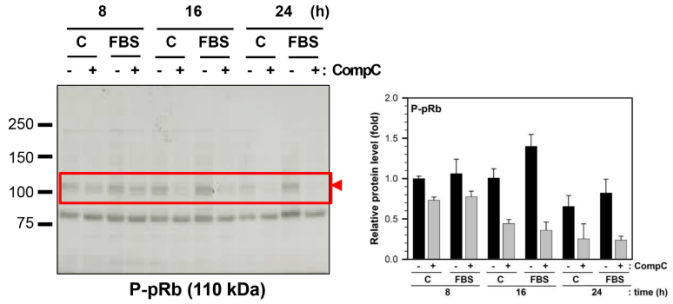
Supplementary Materials: Compound C Inhibits B16-F1 Tumor Growth in a Syngeneic Mouse Model Via the Blockage of Cell Cycle Progression and Angiogenesis

Yun Taek Lee, So Hyun Lim, Boram Lee, Insug Kang and Eui-Ju Yeo

S1-1



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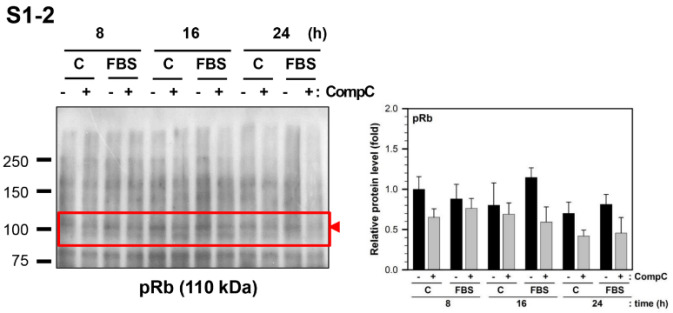

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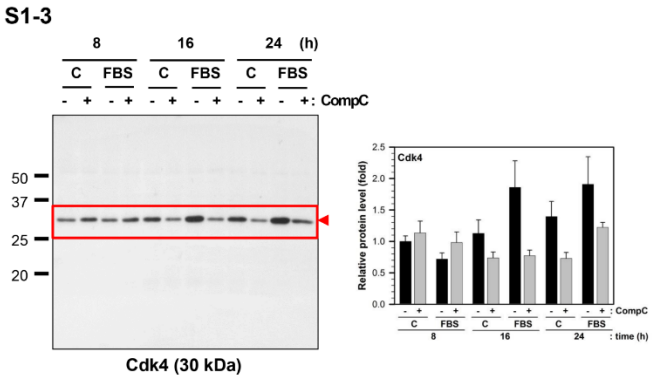

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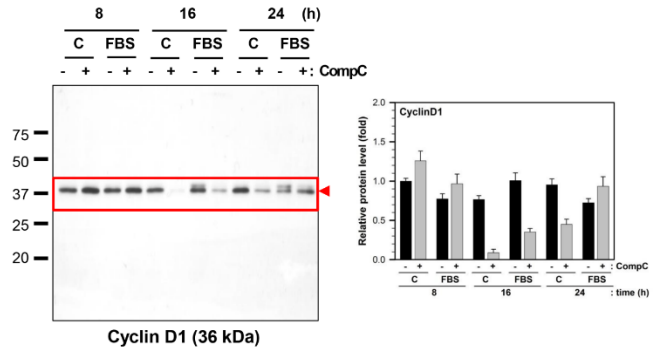

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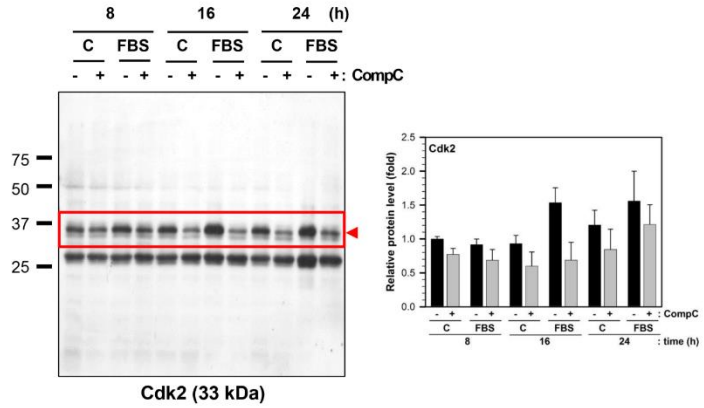
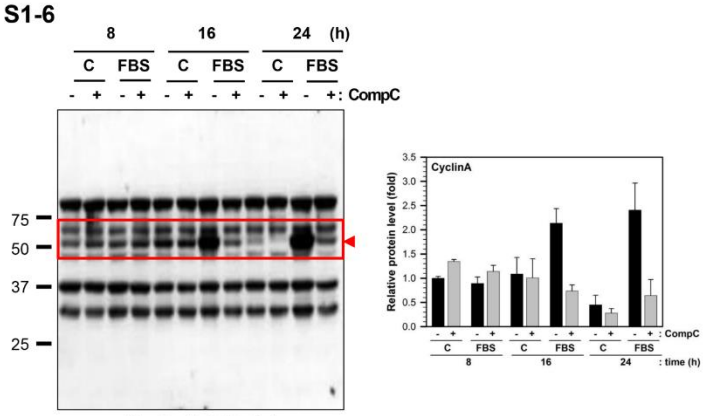


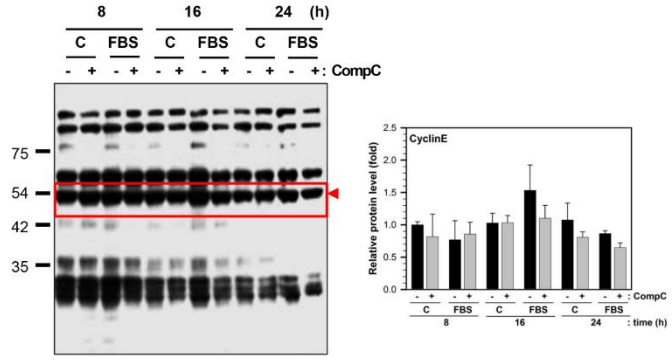
Cdk2 (33 kDa)

S1-6



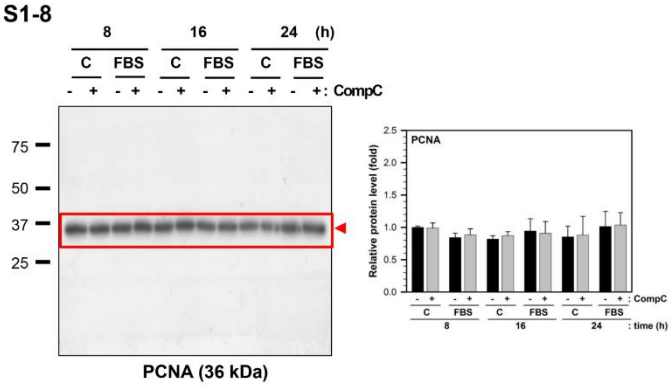
Cyclin A (55 kDa)

S1-7



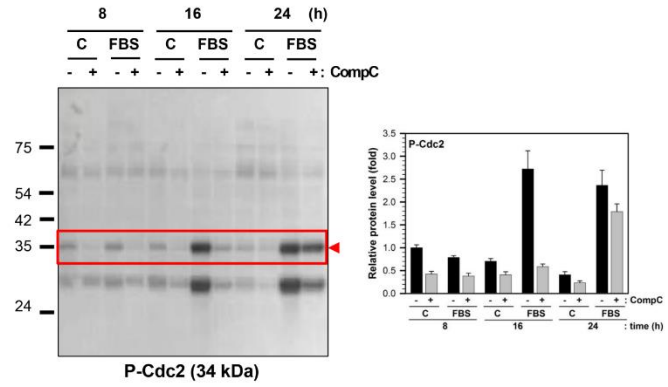
Cyclin E (48 kDa)

S1-8



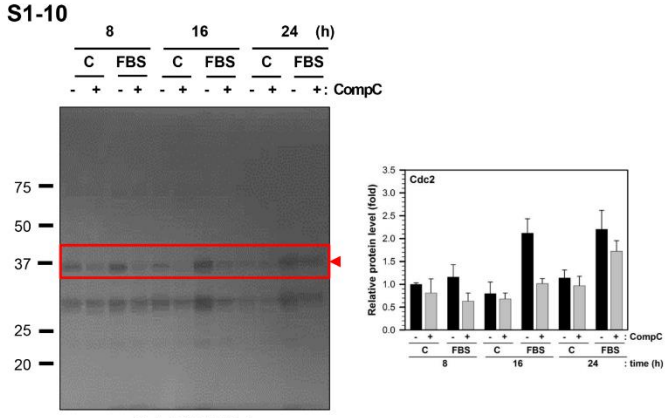
PCNA (36 kDa)

S1-9



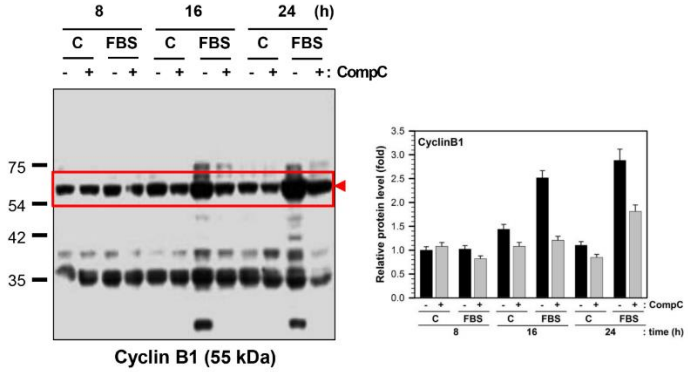
P-Cdc2 (34 kDa)

S1-10



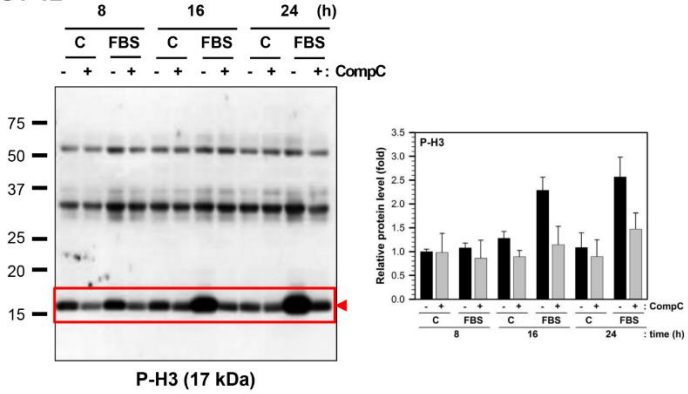
Cdc2 (34 kDa)

S1-11



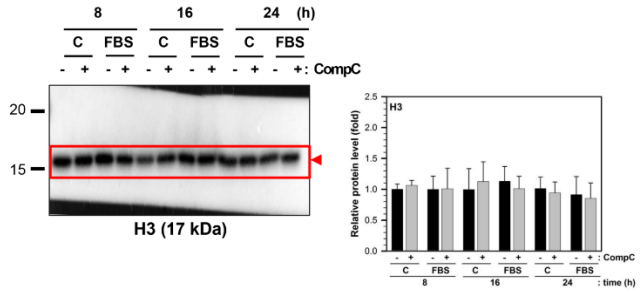
Cyclin B1 (55 kDa)

S1-12



P-H3 (17 kDa)

S1-13



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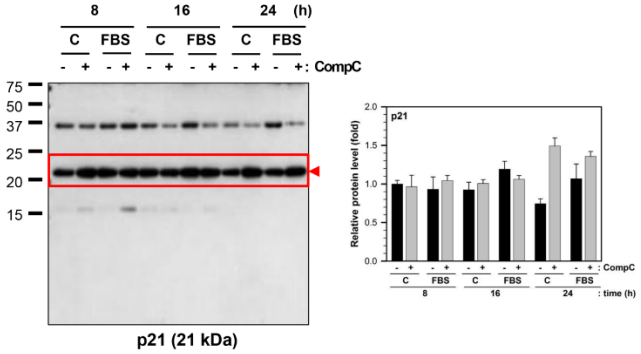

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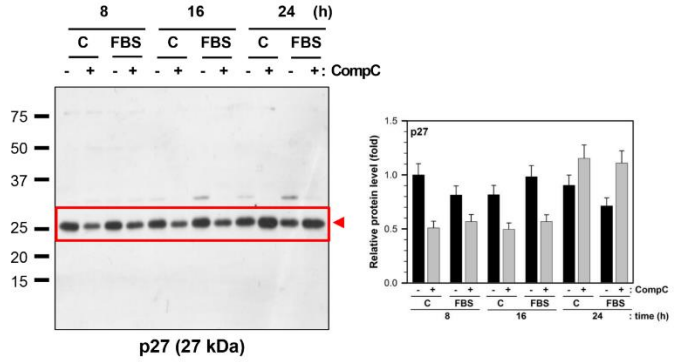

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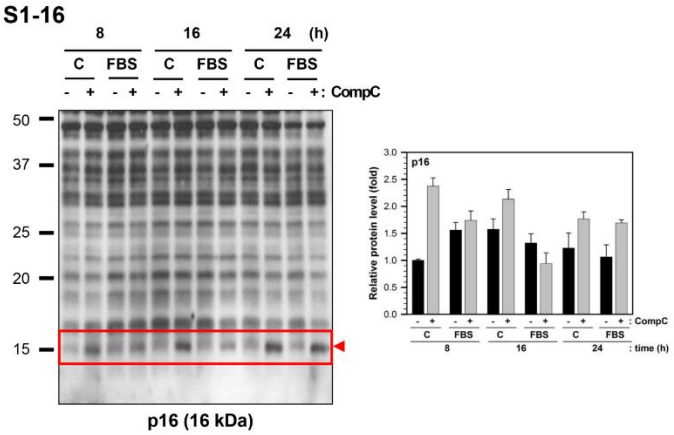


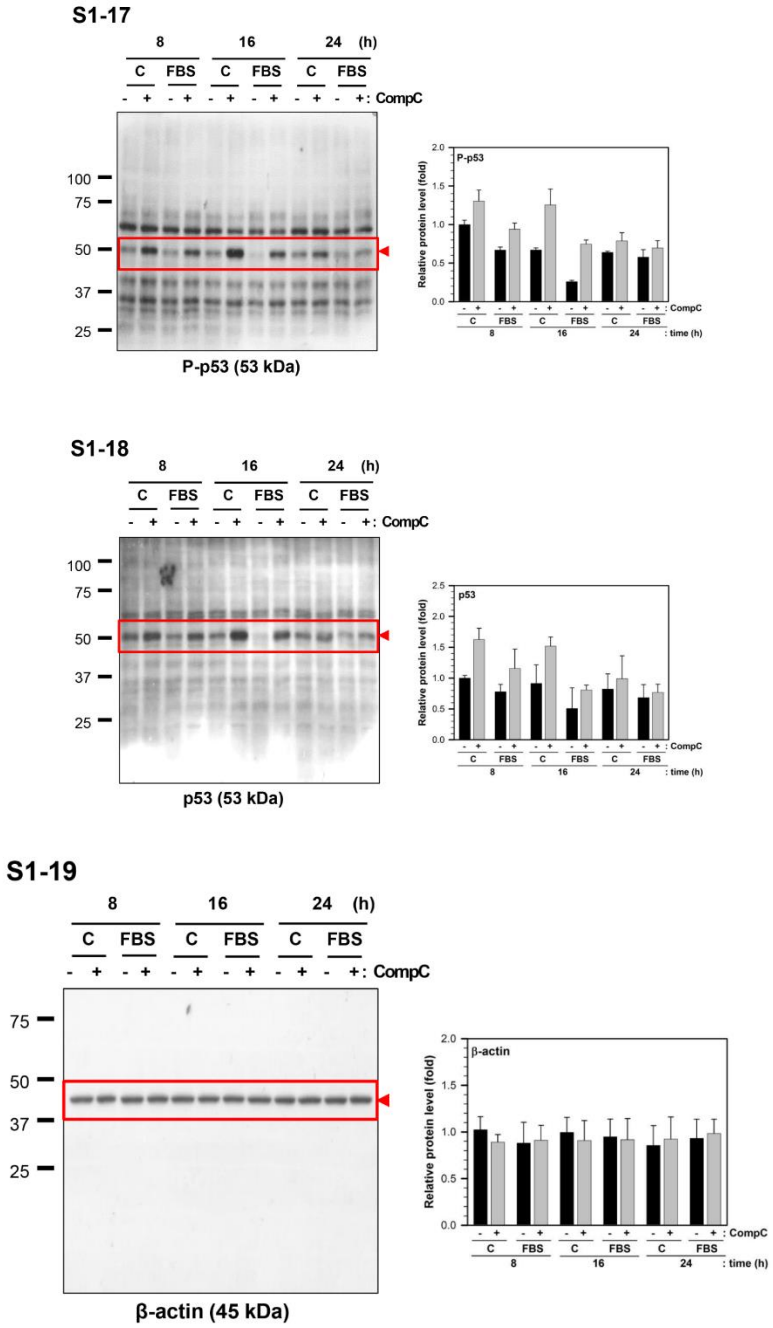
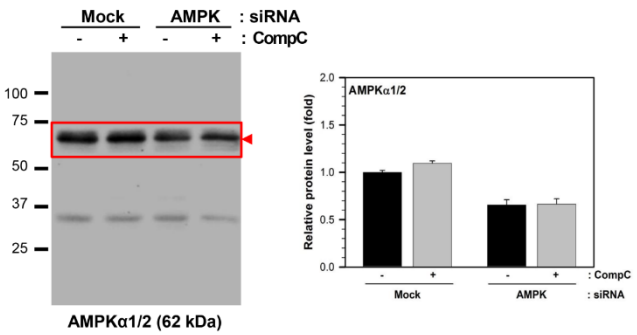
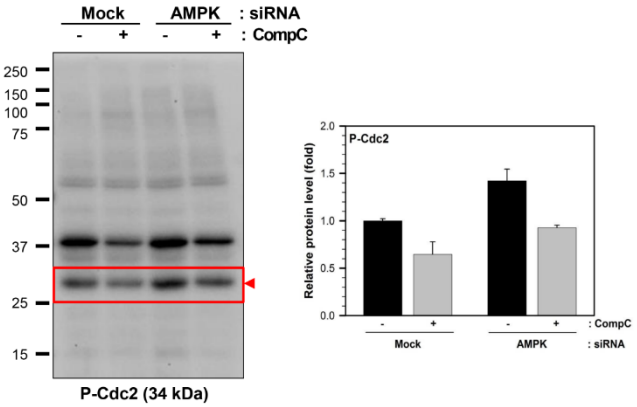


Figure S1. Quiescent cells were stimulated with 10% FBS for the indicated times (8, 16, and 24 h) in the presence of vehicle (−) or 10 μM CompC (+). Cell lysates were analyzed by western blot analysis using antibodies against total and phosphorylated pRb (P-pRb), Cdks, cyclins, histone H3, Cdk inhibitors, and β-actin as an internal control. The band density of β-actin was used as a normalization control for densitometry analysis of each blot. A representative whole blot and a graph with error bars for western blot analysis of each protein are shown side by side. (supplementary data for Figure 2A,B)

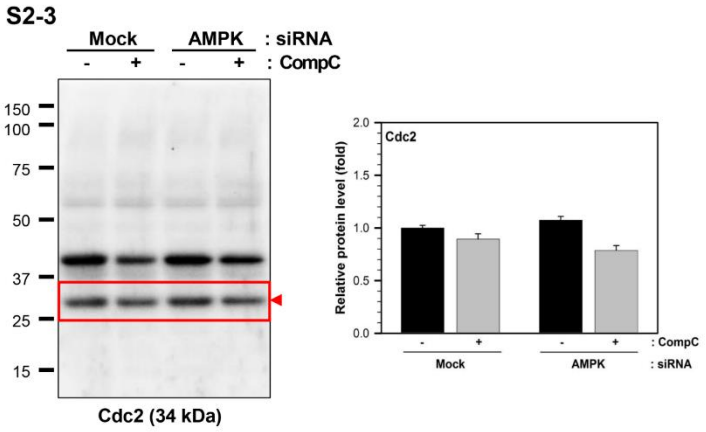
S2-1



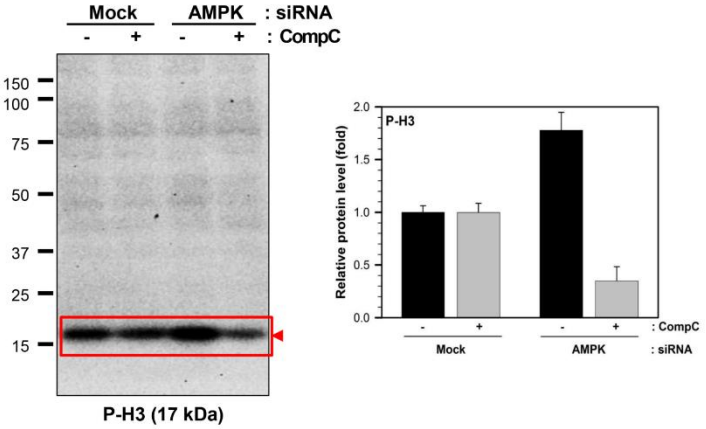
S2-2



S2-3



S2-4



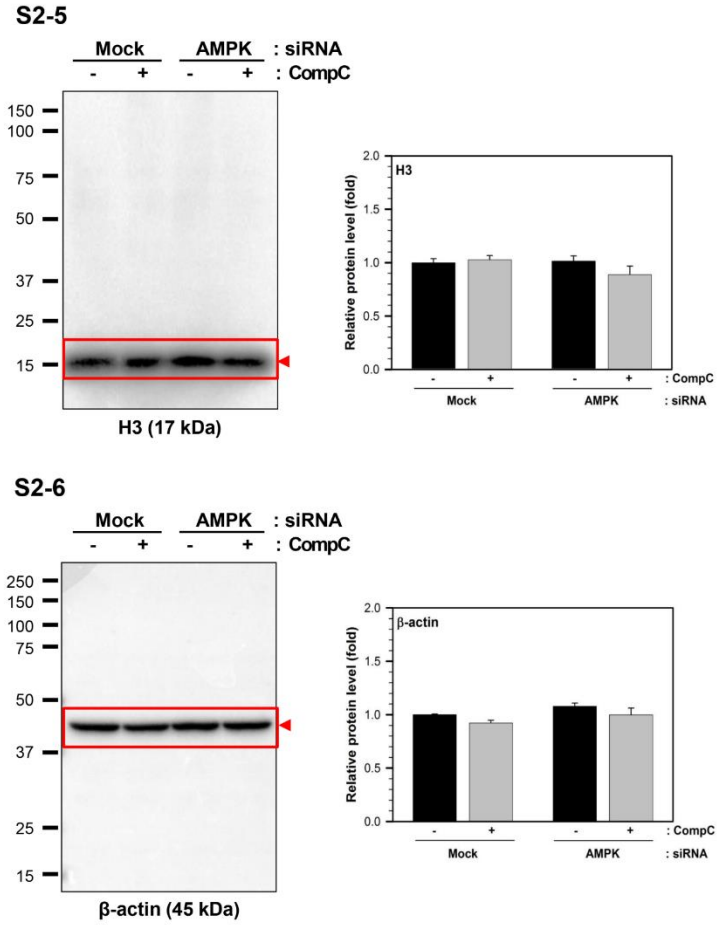
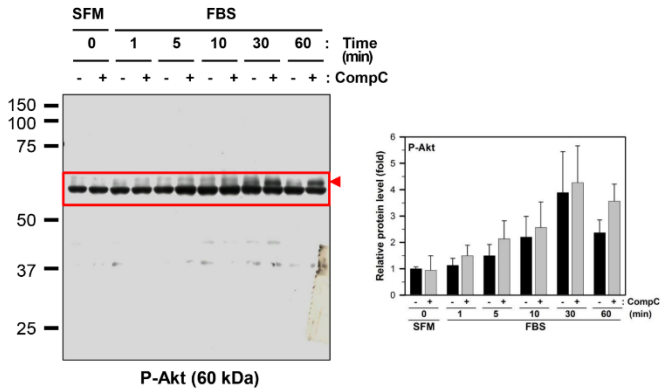


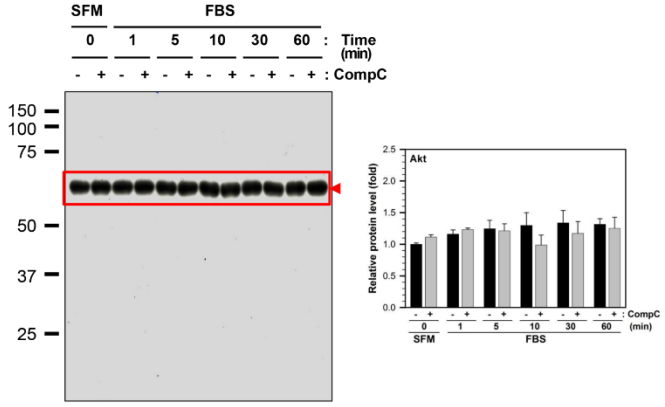
Figure S2. B16-F1 cells were transfected with mock or AMPKα1/2 siRNA for 48 h and then stimulated with 10% FBS in the presence of vehicle (−) or 10 μM CompC (+) for 16 h. Cell lysates were analyzed by western blot analysis using antibodies against AMPKα1/2, total and phosphorylated Cdc2, histone H3, and β-actin. The band density of β-actin was used as a normalization control for densitometry analysis of each blot. A representative whole blot and a graph with error bars for western blot analysis of each protein are shown side by side. (supplementary data for Figure 2C)

S3-1



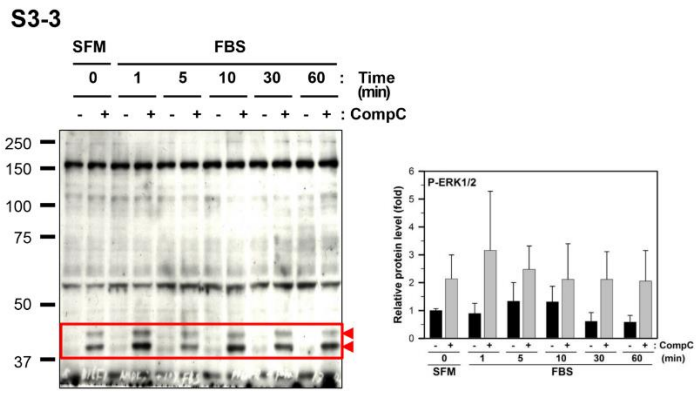
P-Akt (60 kDa)

S3-2



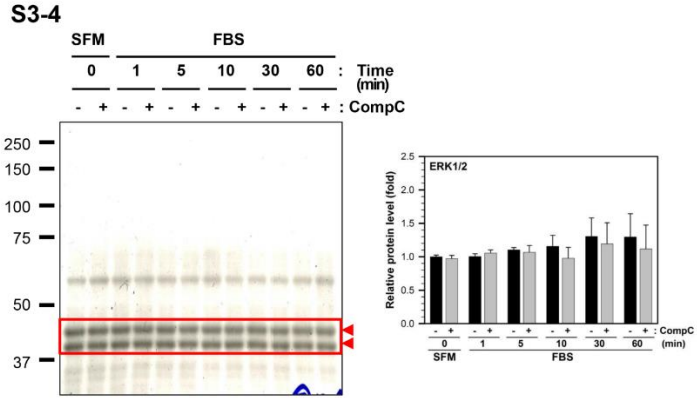
Akt (60 kDa)

S3-3



P-ERK1/2 (42/44 kDa)

S3-4



ERK1/2 (42/44 kDa)

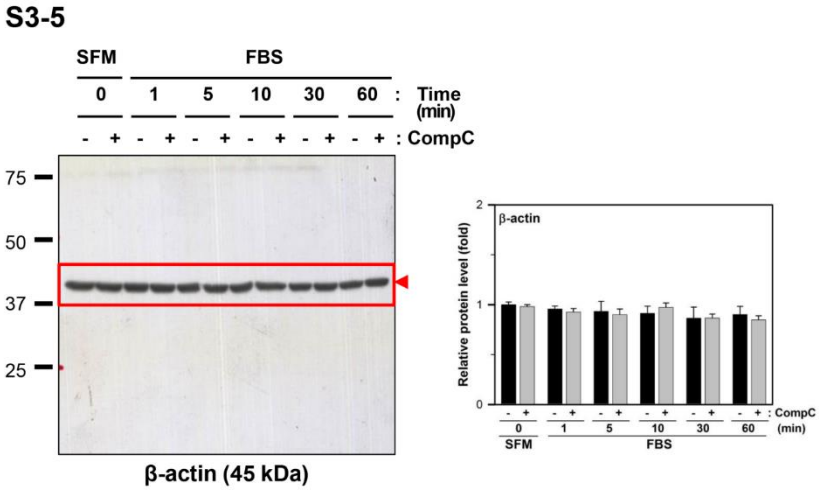
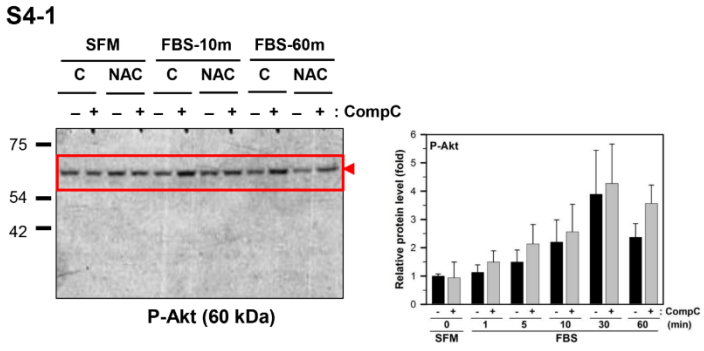
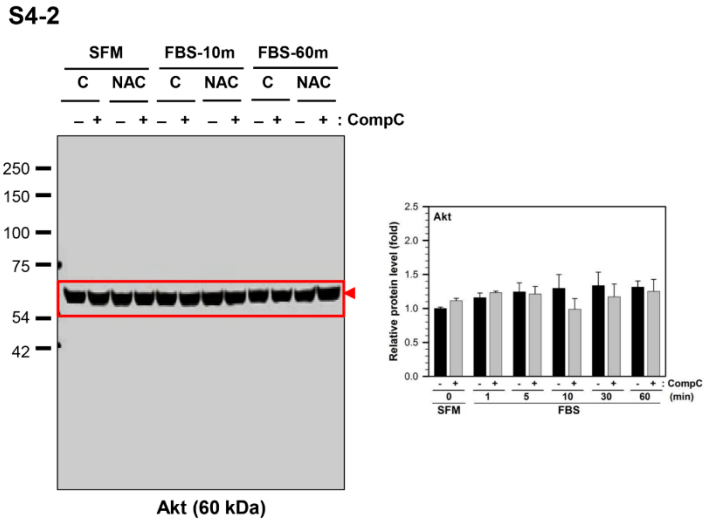


Figure S3. B16-F1 cells were stimulated with 10% FBS for the indicated times in the presence of vehicle or 10 μM CompC. Cell lysates were analyzed by western blot analysis using antibodies against total and phosphorylated Akt (P-Akt) and ERK1/2 (P-ERK1/2), and β-actin. The band density of β-actin was used as a normalization control for densitometry analysis of each blot. A representative whole blot and a graph with error bars for western blot analysis of each protein are shown side by side. (supplementary data for Figure 3A)





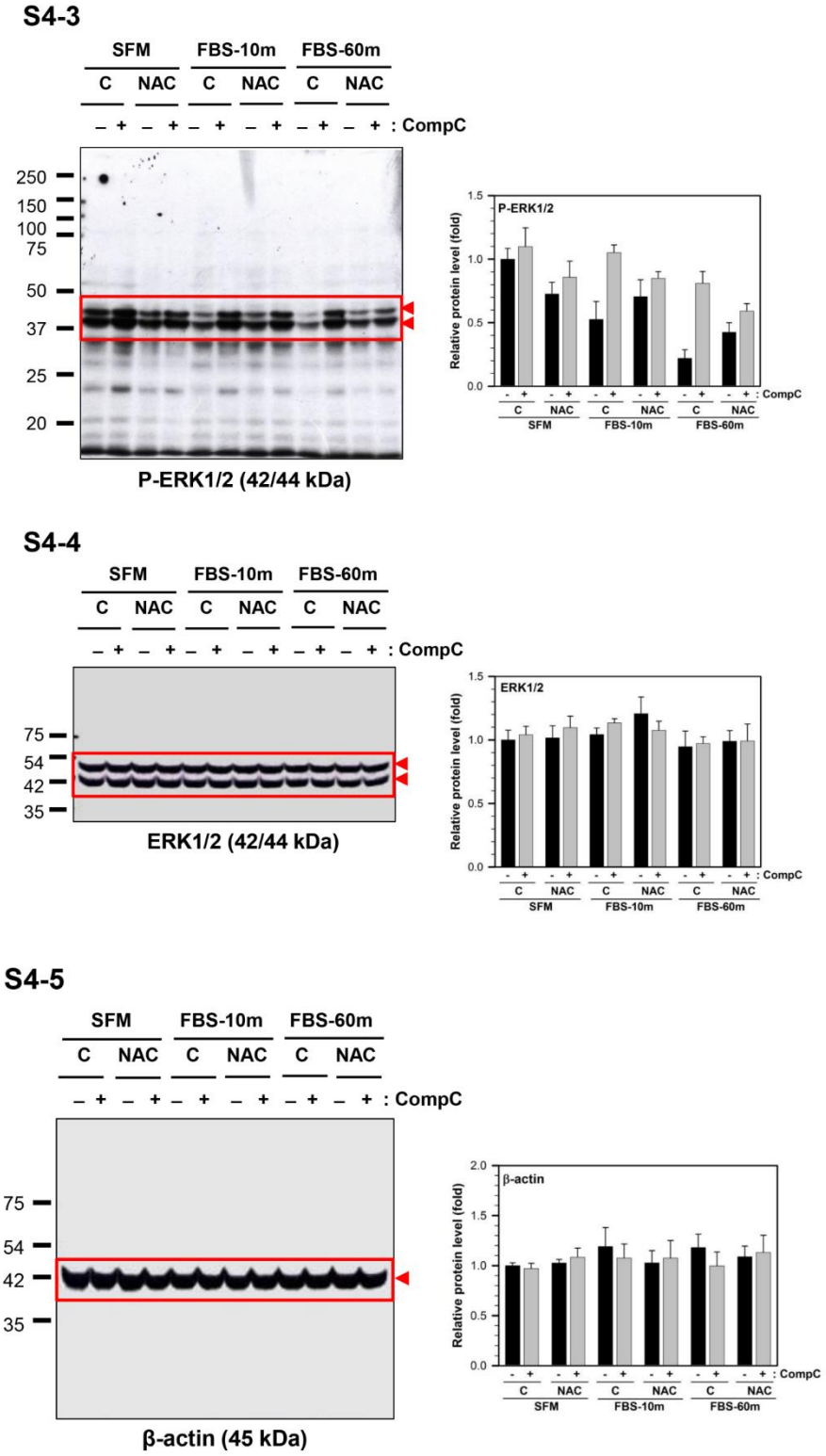
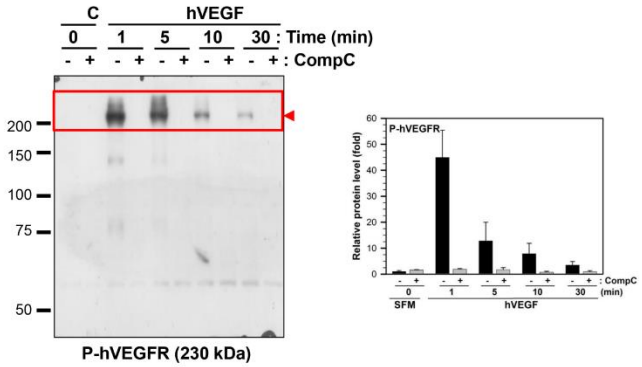


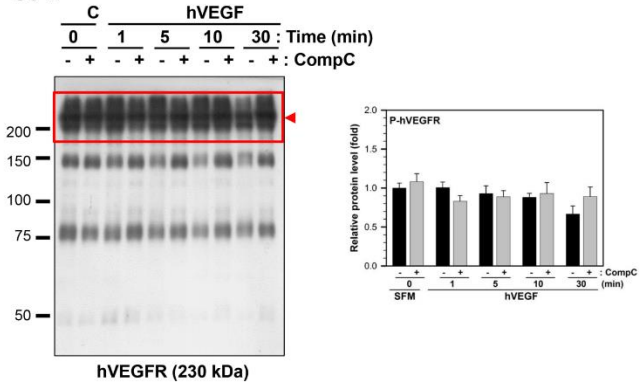
Figure S4. B16-F1 cells were serum-starved by incubation with SFM for 24 h. Cells were pretreated with vehicle alone, 5 mM NAC and/or 10 μM CompC for 1 h. Cells were then stimulated with FBS for 10 and 60 min. Cell lysates were analyzed by western blot analysis using antibodies against total and phosphorylated Akt (P-Akt) and ERK1/2 (P-ERK1/2), and β-actin. The band density of β-actin was used as a normalization control for densitometry analysis of each blot. A representative whole blot and a graph with error bars for western blot analysis of each protein are shown side by side. (supplementary data for Figure 3B)

S5-1



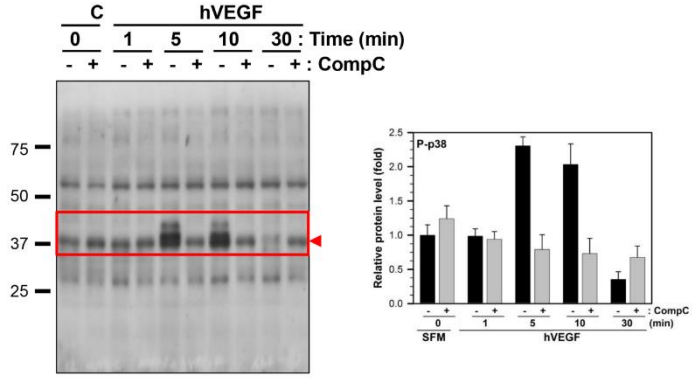
P-hVEGFR (230 kDa)

S5-2



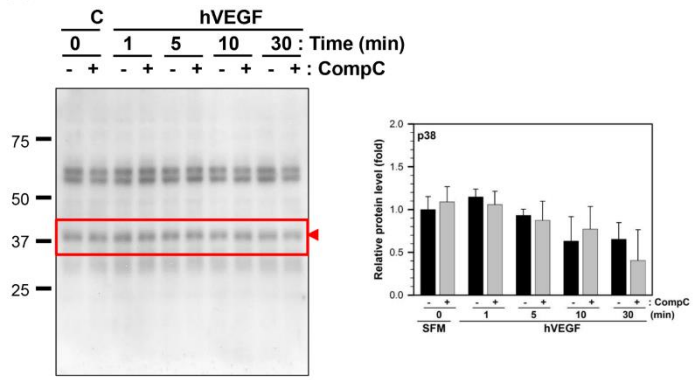
hVEGFR (230 kDa)

S5-3



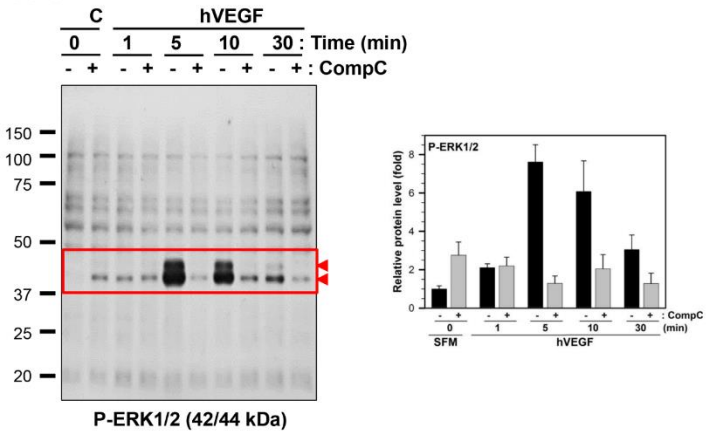
P-p38 (40 kDa)

S5-4



p38 (40 kDa)

S5-5



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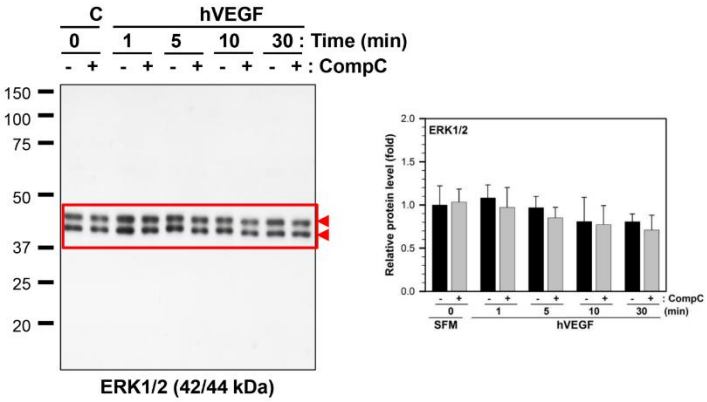

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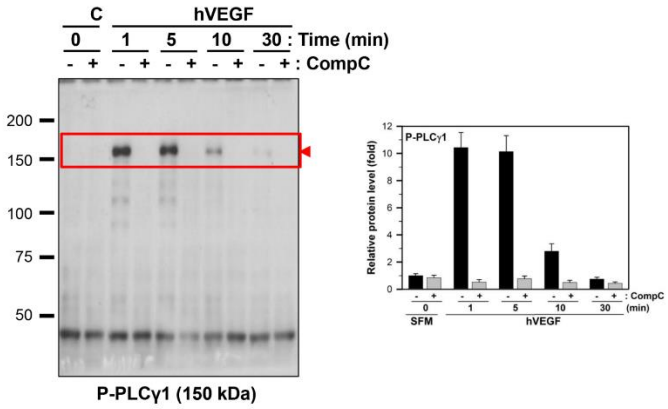

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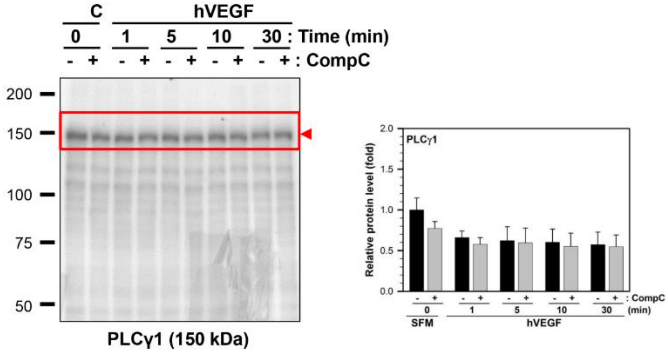

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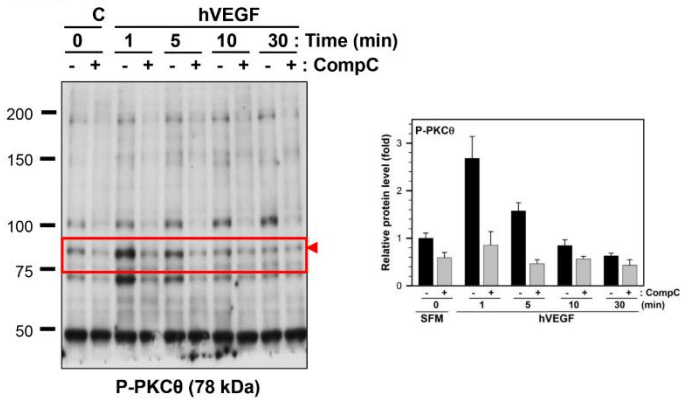

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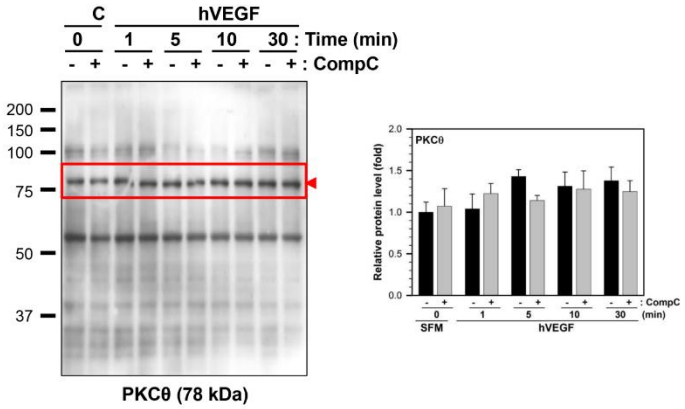

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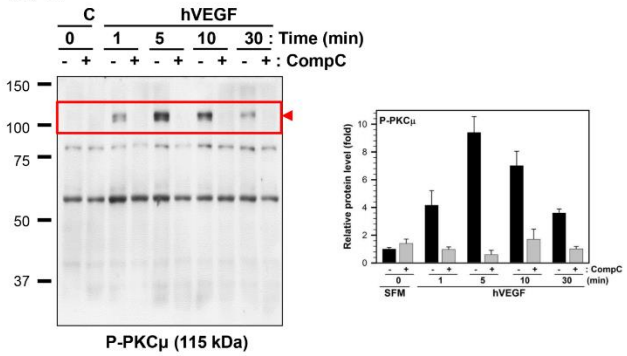

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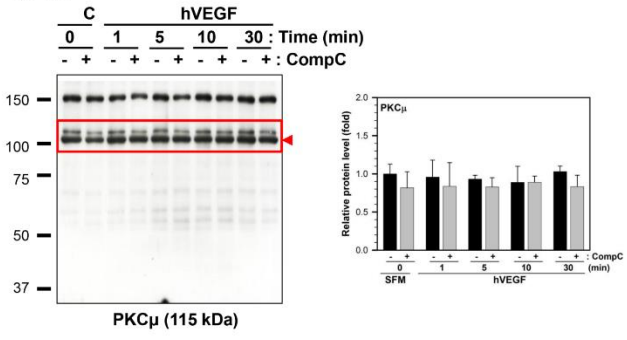

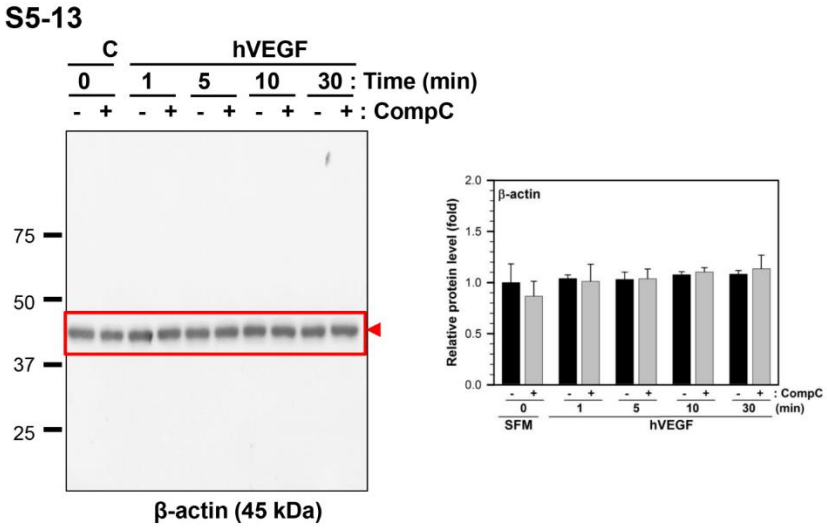

Figure S5. HUVECs were serum-starved by incubation with EBM for 24 h. Cells were treated with EBM containing 50 ng/ml hVEGF for the indicated times in the presence of vehicle (−) or 10 μM CompC (+). Cell lysates were analyzed by western blotting with antibodies against total and phosphorylated hVEGFR and other signaling proteins. The band density of β-actin was used as a normalization control for densitometry analysis of each blot. A representative whole blot and a graph with error bars for western blot analysis of each protein are shown side by side. (supplementary data for Figure 4F)