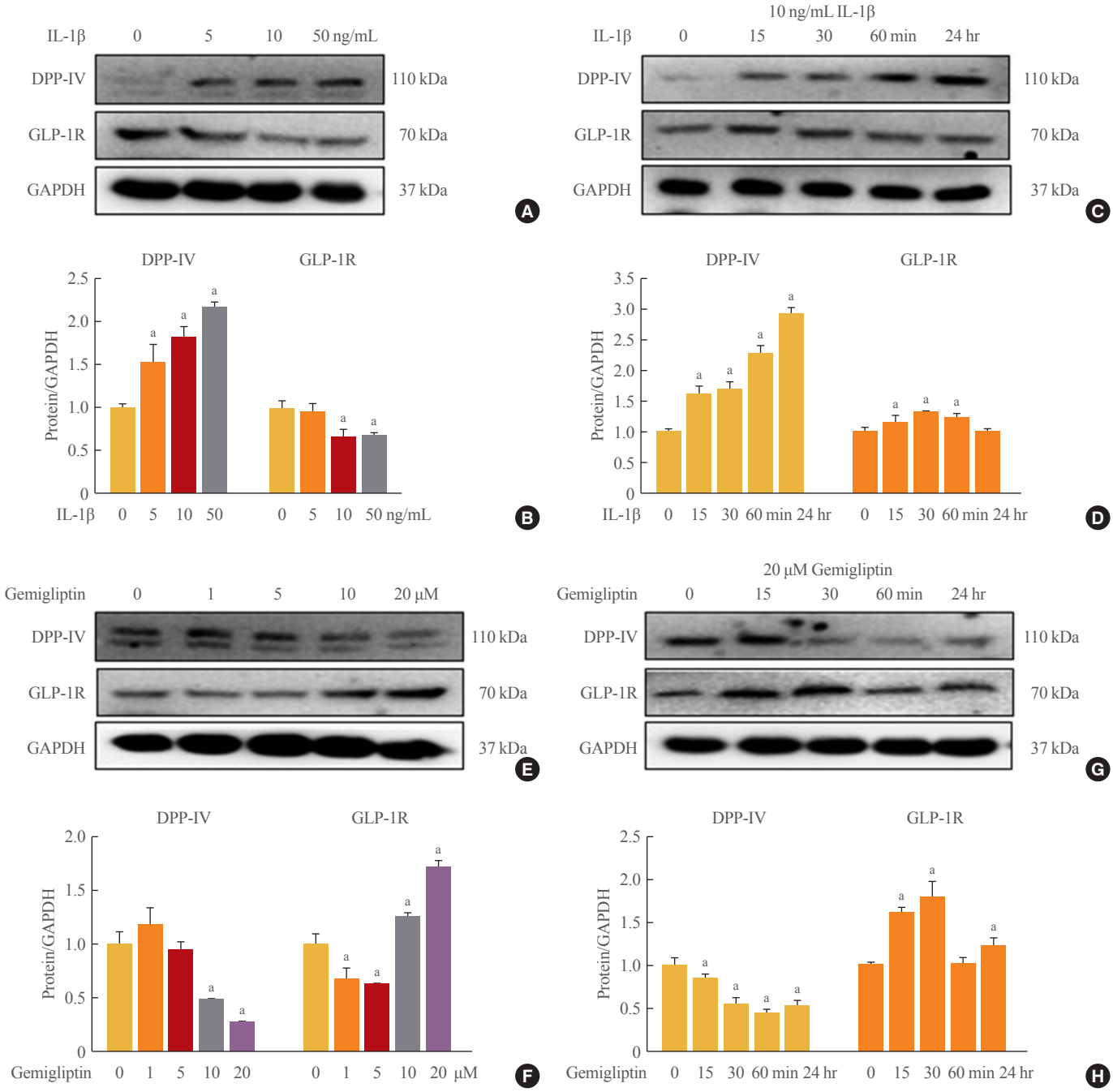
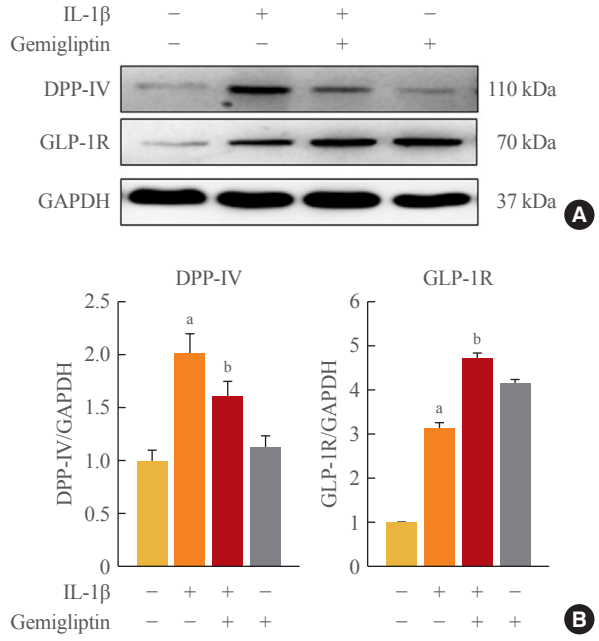
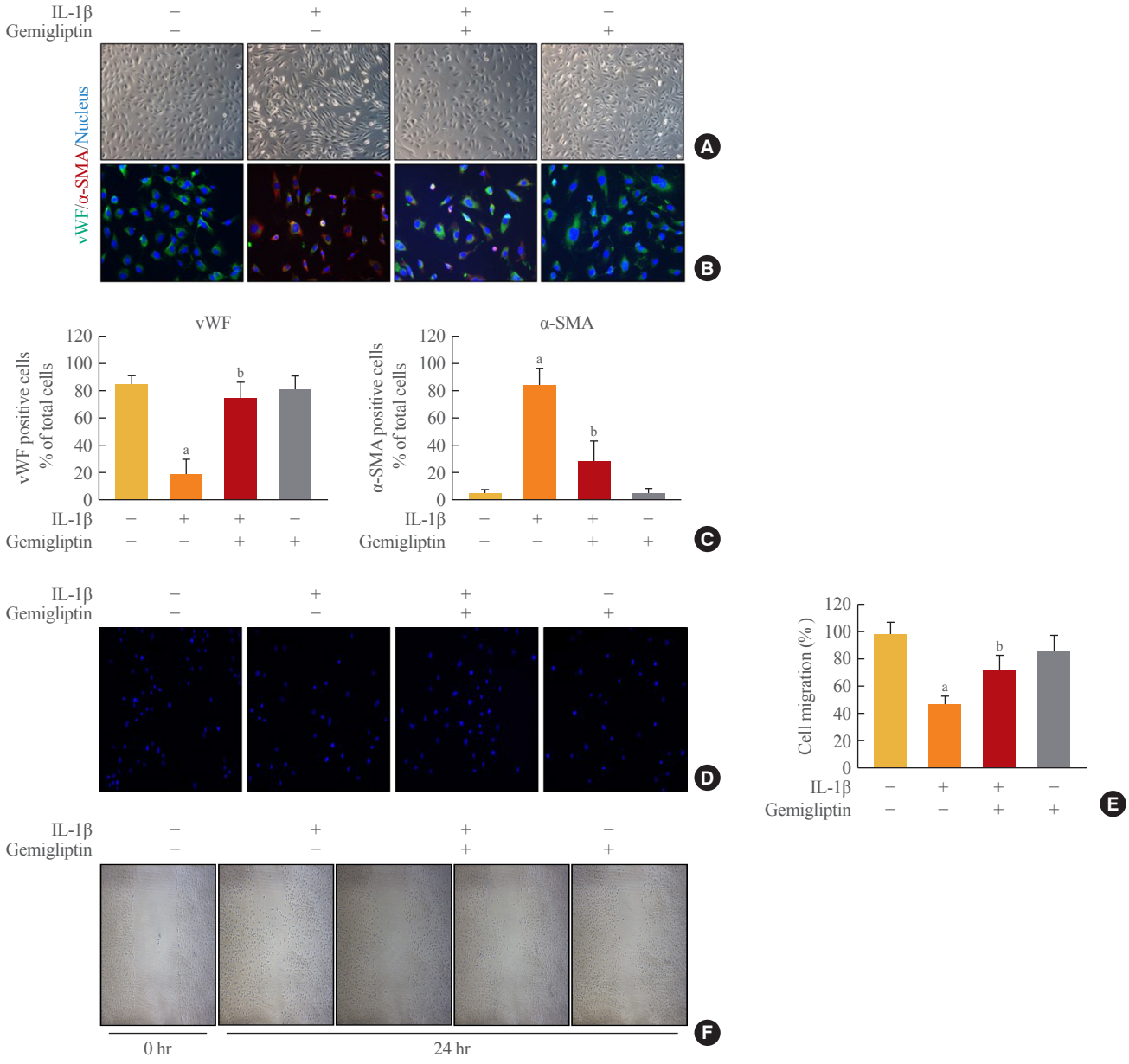


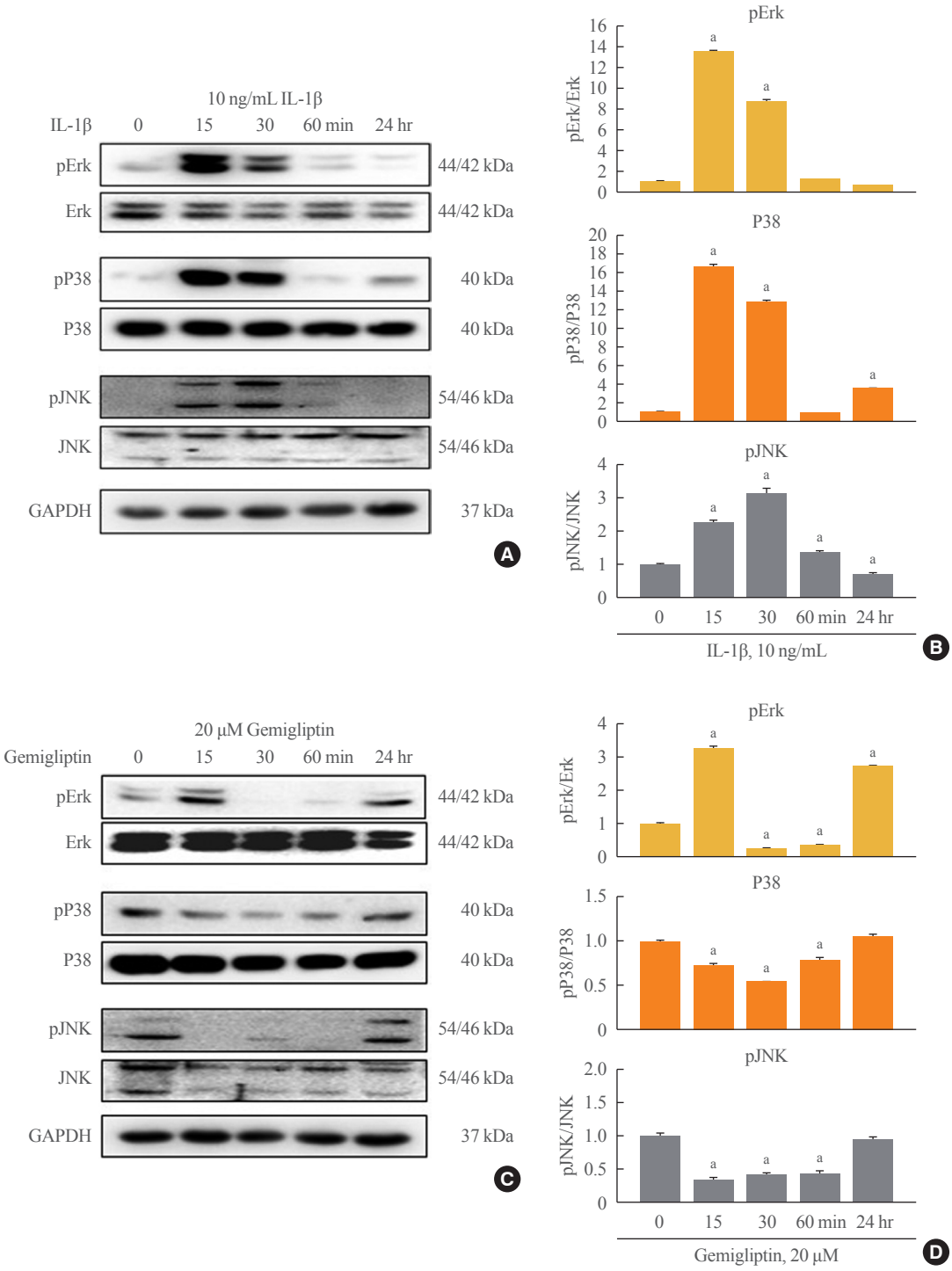
Supplemental Fig. S1. Dose and time-dependent change in the expression of dipeptidyl peptidase IV (DPP-IV) and glucagon-like peptide 1 receptor (GLP-1R) in endothelial cells. (A) Human umbilical vein endothelial cells (HUVECs) were treated interleukin-1β (IL-1β) in the dose-dependent manner (0, 5, 10, 50 ng/mL) for 24 hours and (C) in the time course (0, 15, 30, 60 minutes or 24 hours). Representative Western blot image comparing changes in the expression of the DPP-IV and GLP-1R. (E) Gemigliptin was treated in HUVECs in the dose-dependent manner (0, 1, 5, 10, 20 μM) for 24 hours, and (G) in the time-dependent manner (0, 15, 30, 60 minutes or 24 hours). Representative Western blot image comparing changes in the expression of the DPP-IV and GLP-1R. (B, D, F, H) Quantification of protein expression by densitometry analysis of Western blots. Results were normalized with glyceraldehyde 3-phosphate dehydrogenase (GAPDH; loading control) and expressed as fold-change relative to without IL-1β. Values are mean±standard error of the mean ($n=3$). [a]$P<0.05$, Control.



Supplemental Fig. S2. Gemigliptin did suppress dipeptidyl peptidase IV (DPP-IV) protein levels in endothelial cells. Human umbilical vein endothelial cells were treated interleukin-1β (IL-1β; 10 ng/mL) in the presence of absence of 20 μM gemigliptin for 24 hours. (A) Representative Western blot image comparing changes in the expression of the DPP-IV and glucagon-like peptide 1 receptor (GLP-1R). (B) Quantification of protein expression by densitometry analysis of Western blots. Results were normalized with glyceraldehyde 3-phosphate dehydrogenase (GAPDH; loading control) and expressed as fold-change relative to without IL-1β. Values are mean±standard error of the mean (n=3). [a]P<0.05, Control vs. IL-1β; [b]P<0.05, IL-1β vs. IL-1β+gemigliptin.



Supplemental Fig. S3. Gemigliptin did inhibited interleukin-1β (IL-1β)-induced endothelial-to-mesenchymal transition (EndMT) and migration in human umbilical vein endothelial cells (HUVECs). HUVECs were treated IL-1β (10 ng/mL) in the presence of absence of 20 μM gemigliptin for 24 hours. (A) Morphological changes in HUVECs induced by IL-1β (10 ng/mL) in the presence of absence of 20 μM gemigliptin were examined using phase contrast microscopy. The original magnification was 100×. (B) Indirect immunofluorescence stain of von Willebrand factor (vWF; green color), α-smooth muscle actin (α-SMA; red color), and nucleus (blue color) were performed in HUVECs cultures in EBM-2 medium with IL-1β (10 ng/mL) in the presence of absence of 20 μM gemigliptin. The original magnification was 200×. (C) Immunofluorescence microscopic results of vWF (endothelial cell marker), α-SMC marker, and 4′,6-diamidino-2-phenylindole (DAPI; nucleus) immunostained in HUVEC cultures. The rate of vWF or α-SMA positively was calculated as a percentage of total cells (DAPI stained cells), as described by the following equation: (number of vWF or α-SMA-positive cells/number of DAPI)×100. (D) Transwell chamber cell migration assays were performed as described in material and methods. Migrated cells in the bottom layer of the transwell chamber were counted in five different areas, (E) and quantification was performed. The original magnification was 100×. Values are mean±standard error of the mean (n=5). (F) Wound healing cell migration assay. EndMT cells at 100% confluence were incubated, scratched linearly, and subsequently stimulated with IL-1β in the presence or absence of gemigliptin. Microphotographs of the wounded area were taken immediately after the scratch was made (0 hour) and 24 hours later to monitor cell migration into the wounded area (the original magnification was 25×). Panels show a representative experiment from six experiments. [a]P<0.05, Control vs. IL-1β; [b]P<0.05, IL-1β vs. IL-1β+gemigliptin.



Supplemental Fig. S4. Time-dependent change in the expression of non-Smad bone morphogenetic protein pathway in endothelial cells. Human umbilical vein endothelial cells (HUVECs) were treated 10 ng/mL interleukin-1β (IL-1β) or 20 μM gemigliptin in time dependent manner (0, 15, 30, 60 minutes, or 24 hours). (A) Representative Western blot image comparing changes in the expression of the extracellular regulated protein kinase (Erk), p38, JNK and the phosphorylation of Erk (pErk), P38 (pP38), JNK (pJNK) in HUVEC after exposure to time dependent of 10 ng/mL IL-1β. (C) Representative Western blot image comparing changes in the expression of the Erk, p38, JNK and the phosphorylation of Erk, p38, JNK in HUVEC after exposure to time dependent of 20 μM gemigliptin. (B, D) Quantification of protein expression by densitometry analysis of Western blots. Values are mean±standard error of the mean (n=3). GAPDH, glyceraldehyde 3-phosphate dehydrogenase. [a]P<0.05, Control vs. IL-1β.