Supplemental Material

Visfatin Promotes Monocyte Adhesion by Upregulating ICAM-1 and VCAM-1 Expression in Endothelial Cells via Activation of p38-PI3K-Akt Signaling and Subsequent ROS Production and IKK/NF-κB Activation

Yu-Ting Lin[a] Luen-Kui Chen[a] Deng-Yuan Jian[a,b] Ting-Chia Hsu[a]
Wei-Chih Huang[a] Tse-Ting Kuan[a] Shao-Yun Wu[a] Ching-Fai Kwok[c,d]
Low-Tone Ho[a,c,d] Chi-Chang Juan[a,d,e]

[a]Institute of Physiology, School of Medicine, National Yang-Ming University, Taipei, Taiwan, [b]Division of Nephrology, Wen-Lin Hemodialysis Unit, Taipei Veterans General Hospital, Taipei, Taiwan, [c]Division of Endocrinology and Metabolism, Department of Internal Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, [d]Department of Medical Research, Taipei Veterans General Hospital, Taipei, Taiwan, [e]Department of Education and Research, Taipei City Hospital, Taipei, Taiwan

Figure S1. Visfatin upregulates ICAM-1 and VCAM-1 expression in endothelial cells through the PI3K/Akt- and p38-dependent pathways. Cells were incubated (A and B) in the absence or presence of 100 ng/ml visfatin for various times (0-24 h) or (C and D) with various concentrations of visfatin (0-100 ng/ml) for 24 h. The expression of ICAM-1 and VCAM-1 were measured. The results are the mean ± SEM for three separate experiments, each in triplicate. $^{*}P < 0.05$ compared with the zero control (A-D) or the untreated control (E-H); $^{\#}P < 0.05$ compared with the visfatin alone group.

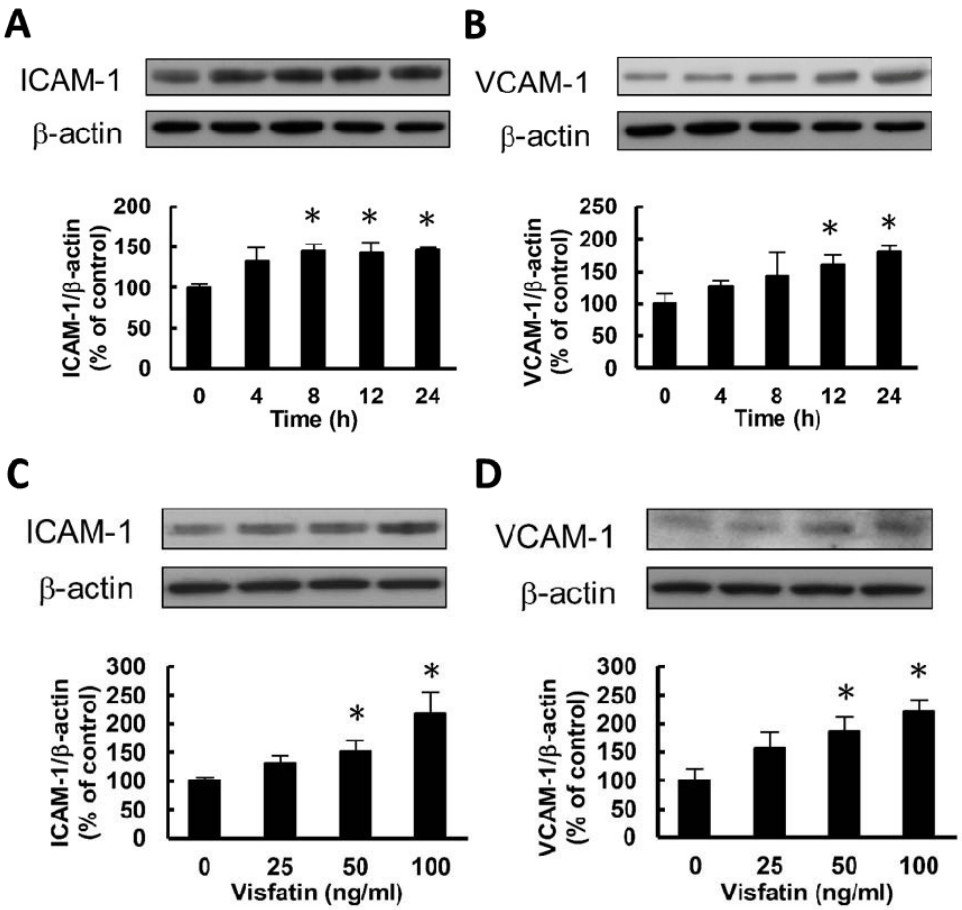


Figure S2. Involvement of p38/PI3K/Akt signaling cascade in visfatin-stimulated ROS production in endothelial cells. Cells were pre-incubated for 1 h in the absence or presence of the ERK1/2 inhibitor PD98059 (PD, 30 μM), PI3K inhibitor LY294002 (LY, 30 μM), p38 MAPK inhibitor SB203580 (SB, 20 μM), or ROS scavenger NAC (5 mM). Cells were then incubated in the absence or presence of visfatin (100 ng/ml) in the continued absence or presence of the inhibitor for a further 30 min. The production of ROS were detected by dihydroethidium (DHE) staining.

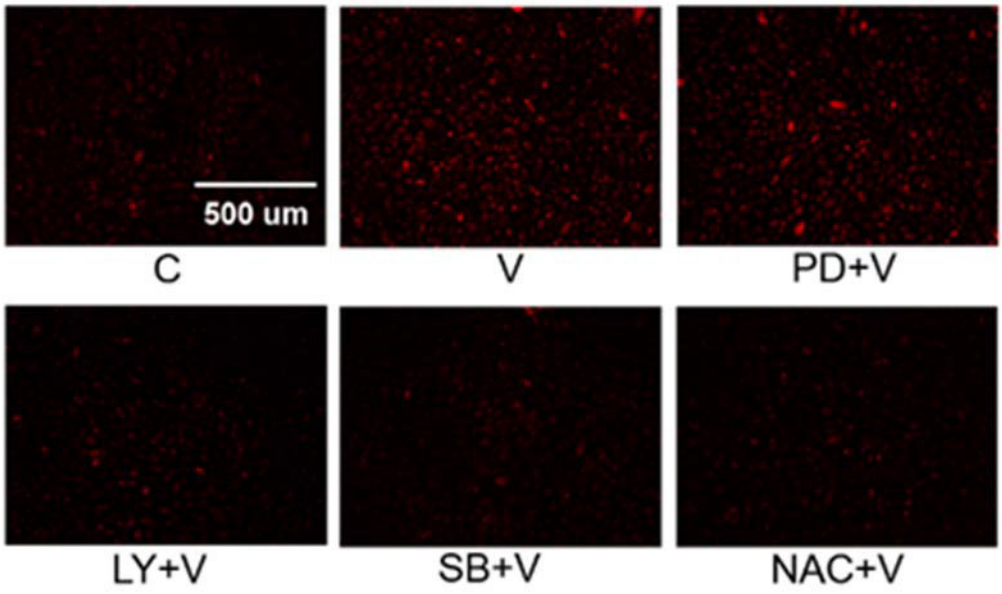


Figure S3. Involvement of NOX in visfatin-stimulated ROS production, and monocyte adhesion in endothelial cells. Cells were pre-incubated for 1 h in the absence or presence of the NADPH oxidase inhibitor DPI (100 nM), followed by incubation in the absence or presence of visfatin (100 ng/ml) in the continued absence or presence of the inhibitor for a further 24 h. (A) The production of ROS and (B) the adhesion of THP-1 monocytes were measured. The results are the mean ± SEM for three separate experiments, each in triplicate. *$P < 0.05$ compared with the untreated control; $^{\#}P < 0.05$ compared with the visfatin alone group.

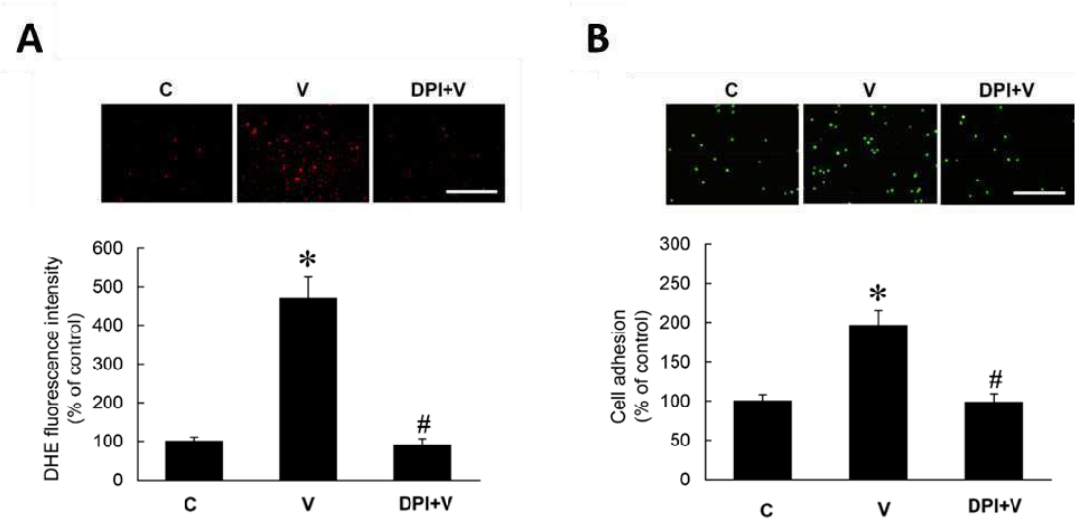


Figure S4. Visfatin promotes NF-κB activation via the p38/PI3K/Akt/ROS signaling cascade. Endothelial cells were pre-incubated for 1 h in the absence or presence of PI3K inhibitor LY294002 (LY, 30 μM), p38 MAPK inhibitor SB203580 (SB, 20 μM), ROS scavenger NAC (5 mM), or IKK inhibitor BAY-11-7082 (BAY, 10 μM). Cells were then incubated in the absence or presence of visfatin (100 ng/ml) in the continued absence or presence of the inhibitor for a further 24 h. NF-κB p65 stain is shown in green and overlaid with a blue image of the DNA-intercalating dye DAPI. The representative images are shown. Scale bar = 50 μm.

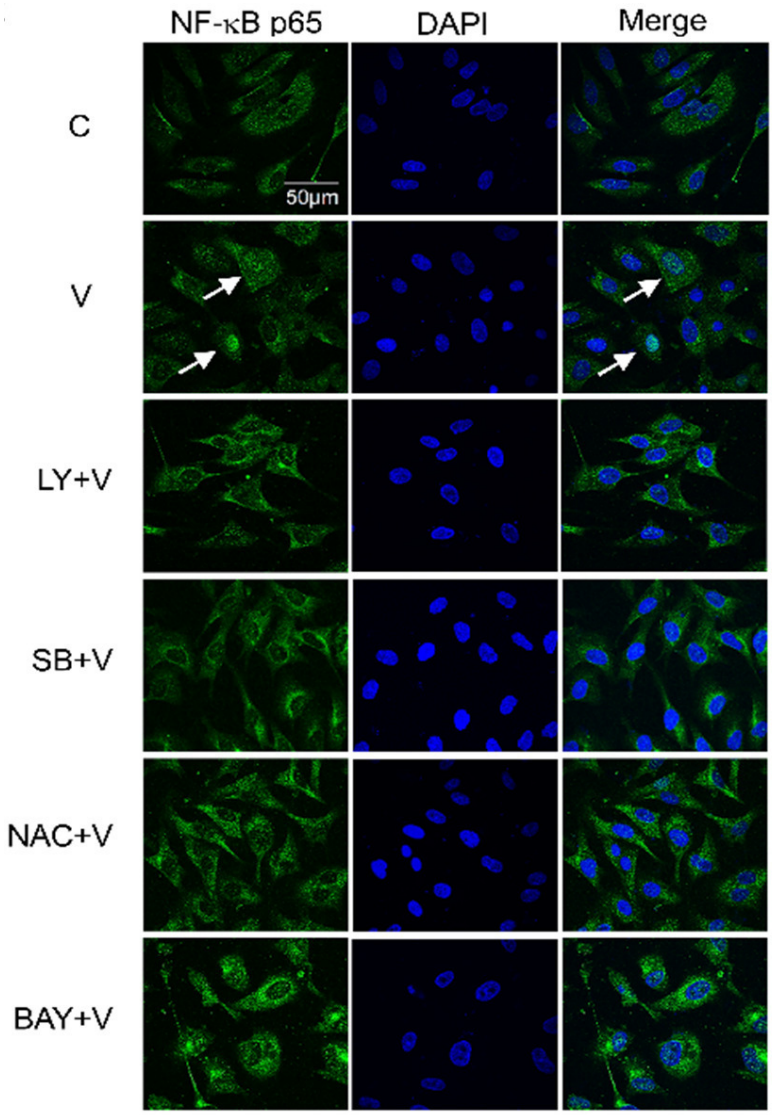
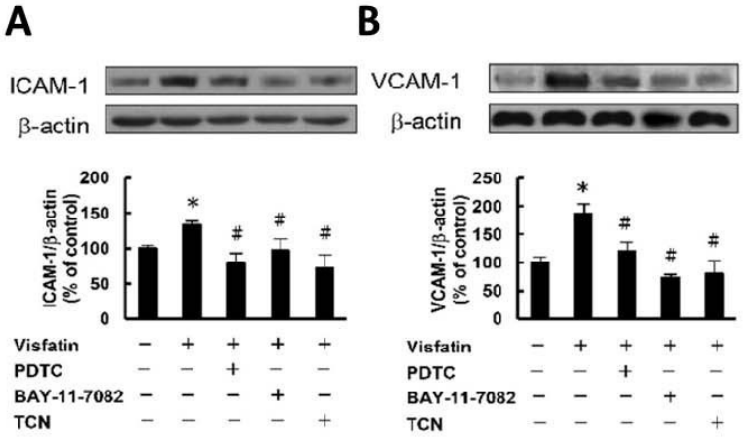
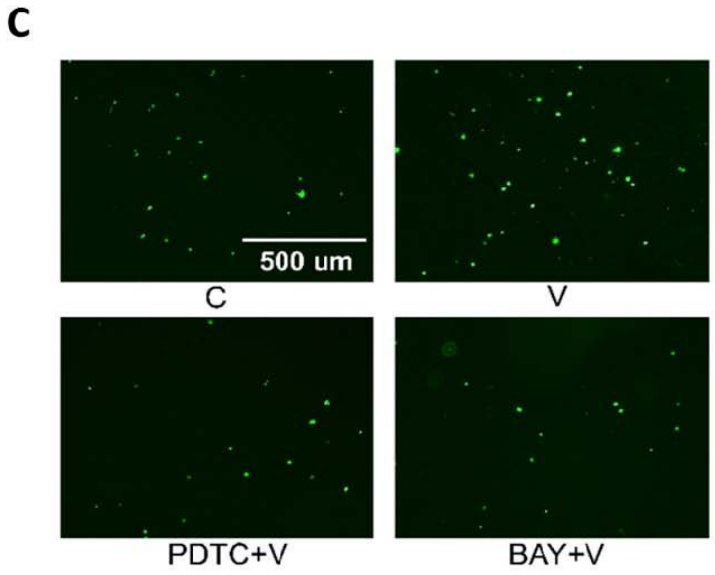


Figure S5. Visfatin upregulates ICAM-1 and VCAM-1 expression and enhances monocyte–endothelial cell adhesion through the NF-κB-dependent pathway. Endothelial cells were pre-incubated for 1 h in the absence or presence of the NF-κB inhibitor PDTC (10 μM), IKK inhibitor BAY-11-7082 (BAY, 10 μM), or Akt inhibitor triciribine (TCN, 5 μM). Cells were then incubated in the absence or presence of visfatin (100 ng/ml) in the continued absence or presence of the inhibitor for a further 24 h. The expression of (A) ICAM-1 and (B) VCAM-1 and (C and D) the adhesion of THP-1 monocytes were measured. The results are the mean ± SEM for three separate experiments, each in triplicate. $^{*}P < 0.05$ compared with the untreated control; $^{\#}P < 0.05$ compared with the visfatin alone group.


A
ICAM-1
β-actin
ICAM-1/β-actin (% of control)
200
150
100
50
0
*
#
#
#
Visfatin − + + + +
PDTC − − + − −
BAY-11-7082 − − − + −
TCN − − − − +
B
VCAM-1
β-actin
VCAM-1/β-actin (% of control)
250
200
150
100
50
0
*
#
#
#
Visfatin − + + + +
PDTC − − + − −
BAY-11-7082 − − − + −
TCN − − − − +


C
500 um
C
V
PDTC+V
BAY+V

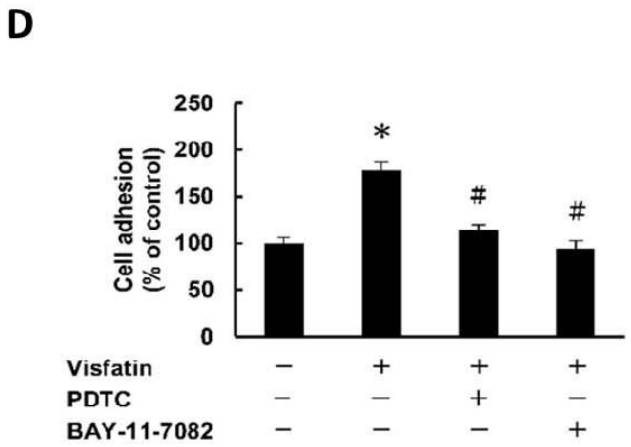

D
Cell adhesion (% of control)
250
200
150
100
50
0
*
#
#
Visfatin − + + +
PDTC − − + −
BAY-11-7082 − − − +

Figure S6. Visfatin upregulates ICAM-1 and VCAM-1 expression and monocyte adhesion in HUVECs through the p38/PI3K/Akt/ROS/IKK/NF-κB pathways. Cells were pre-incubated for 1 h in the absence or presence of the PI3K inhibitor LY294002 (LY; 30 μM), Akt inhibitor TCN (5 μM), p38 MAPK inhibitor SB203580 (SB; 20 μM), NADPH oxidase inhibitor DPI (100 nM), ROS scavenger NAC (5 mM), NF-κB inhibitor PTDC (10 μM), and IKK inhibitor BAY-11-7082 (BAY; 10 μM), followed by incubation in the absence or presence of visfatin (100 ng/ml) in the continued absence or presence of the inhibitor for a further 24 h. The expression of ICAM-1 and VCAM-1 and monocyte adhesion to HUVECs were measured. The results are the mean ± SEM for three separate experiments, each in triplicate. *$P < 0.05$ compared with the untreated control; $^{\#}P < 0.05$ compared with the visfatin alone group.