Methods of electrophoretic mobility shift assay

Nuclear extract was prepared from HUVECs using Nuclear and Cytoplasmic Protein Extraction Kit (keyGEN BioTECH, Nanjing, CN). The probes targeting ICAM-1 or VCAM-1 consensus sequences were biotin-labeled using Light Shift Chemiluminescent EMSA kit (Gzscbio, Guangzhou, CN). Binding assays were performed in 20 µL of reaction mixture system containing 2 µL of HUVECs lysate, 5 mM MgCl₂, 2.5% Glycerol, 0.05% NP-40, 50 ng/µL of poly(dI·dC) and 20 fmol labeled probes for 20 minutes at 37 °C. Reactions were analyzed by electrophoresis on a 6% Native-PAGE at 100 V, and transferred onto Nitrocellulose Membrane (Invitrogen). Forty minutes, after transfer, the membrane was immediately cross-linked by UV-light for 15 seconds and the target bands were detected by chemiluminescence (Tanon, Shanghai, CN).

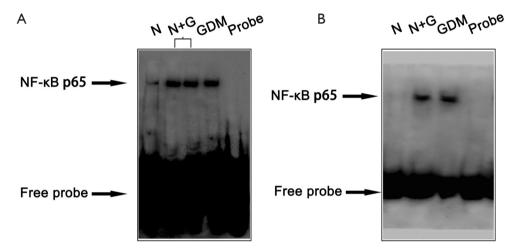


Figure S1 Combination between NF- κ B and the promoter of ICAM-1 or VCAM-1. EMSA was used to detect changes of the combination between NF- κ B and the promoters of ICAM-1 or VCAM-1 under different conditions. (A) the combination between NF- κ B and the promoter of ICAM-1; (B) the combination between NF- κ B and the promoter of VCAM-1. N, normal HUVECs; N + G, normal HUVECs were pretreated with high glucose; GDM, GDM-HUVECs.