DC-SIGN and Toll-like receptor 4 mediate oxidized low-density lipoprotein-induced inflammatory responses in macrophages

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Running Title: DC-SIGN/TLR4 regulates inflammatory response in macrophages

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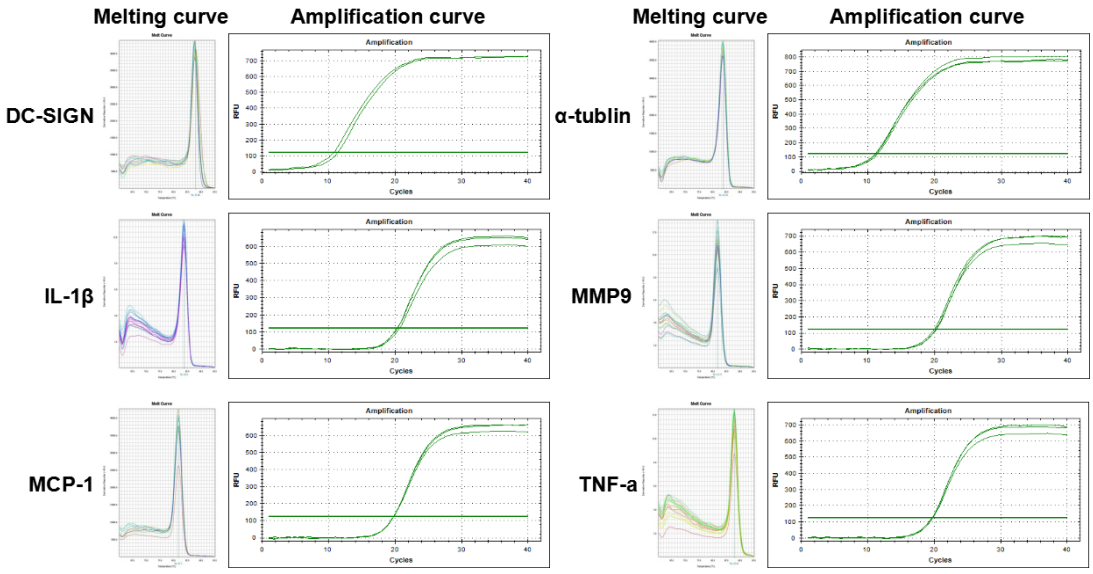
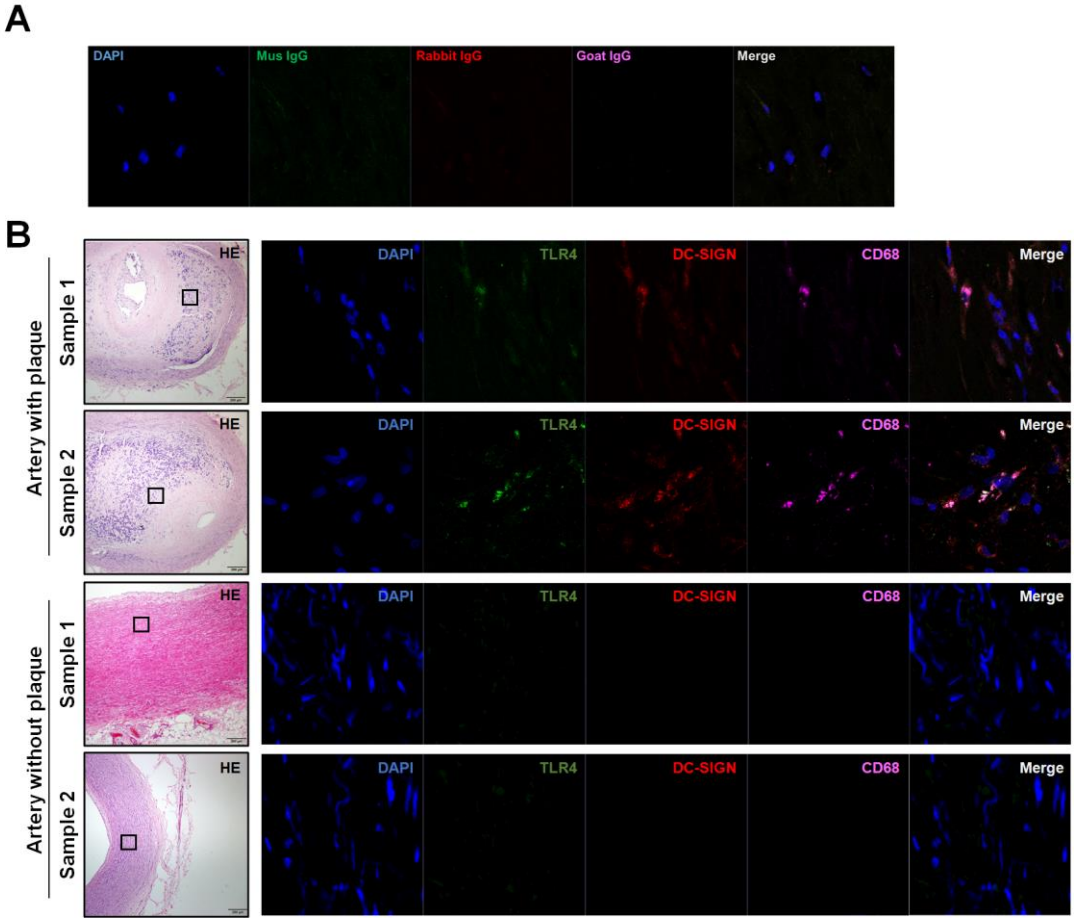
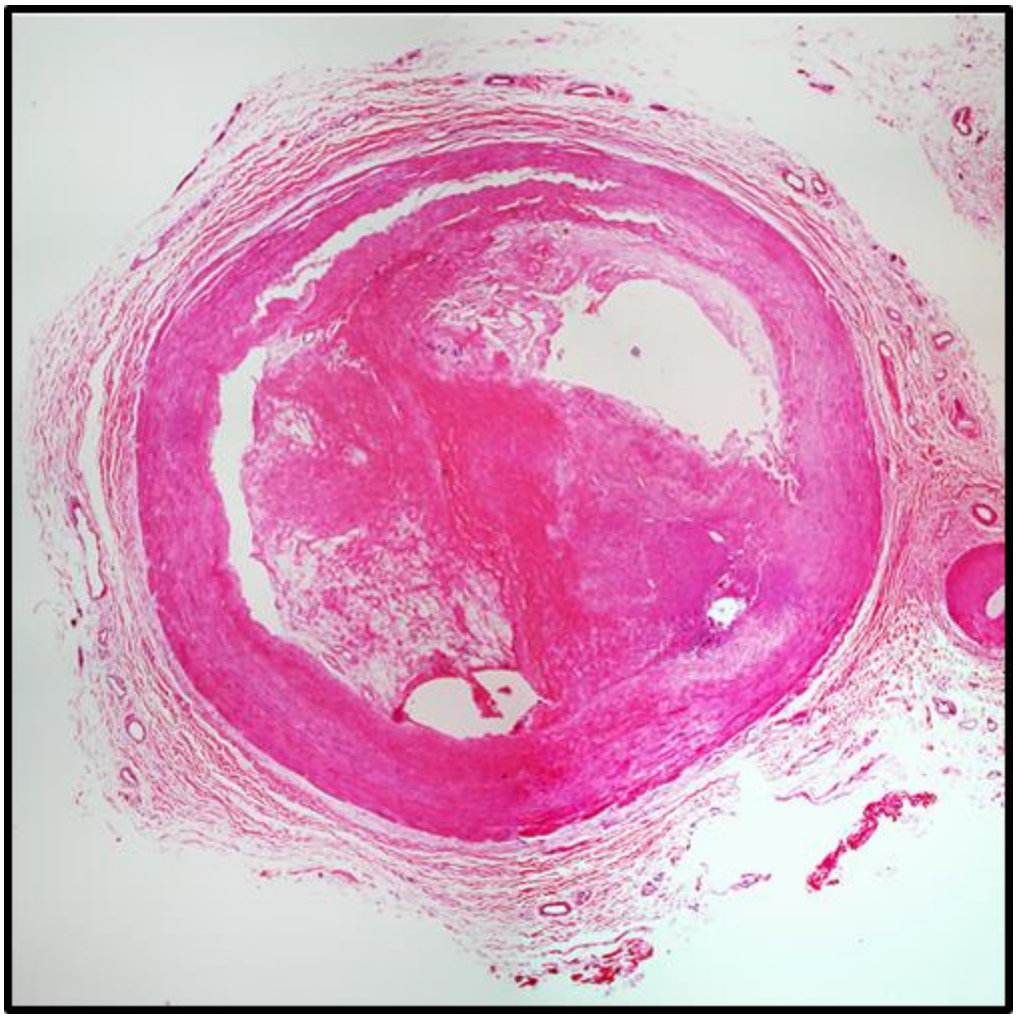
Supplementary DATA



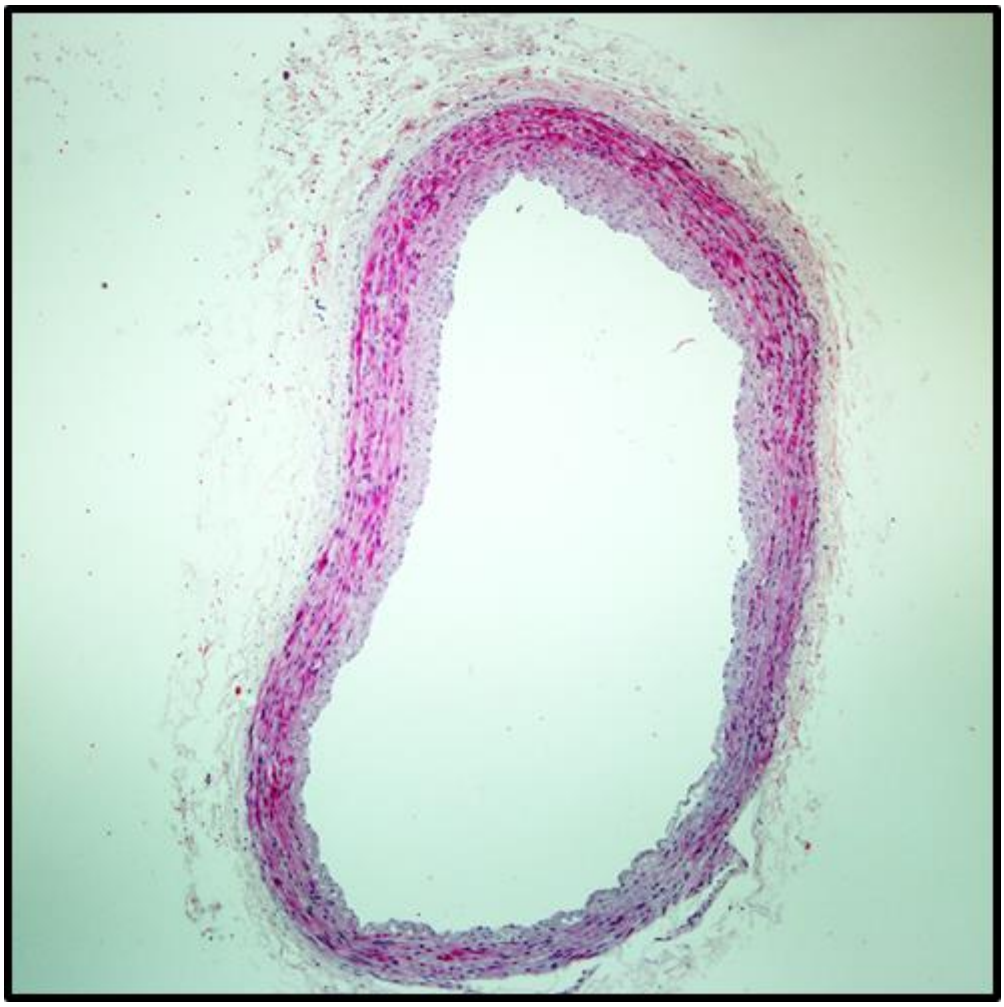
Supplementary Fig.1 The efficiency and specific of primers using for Realtime-PCR.



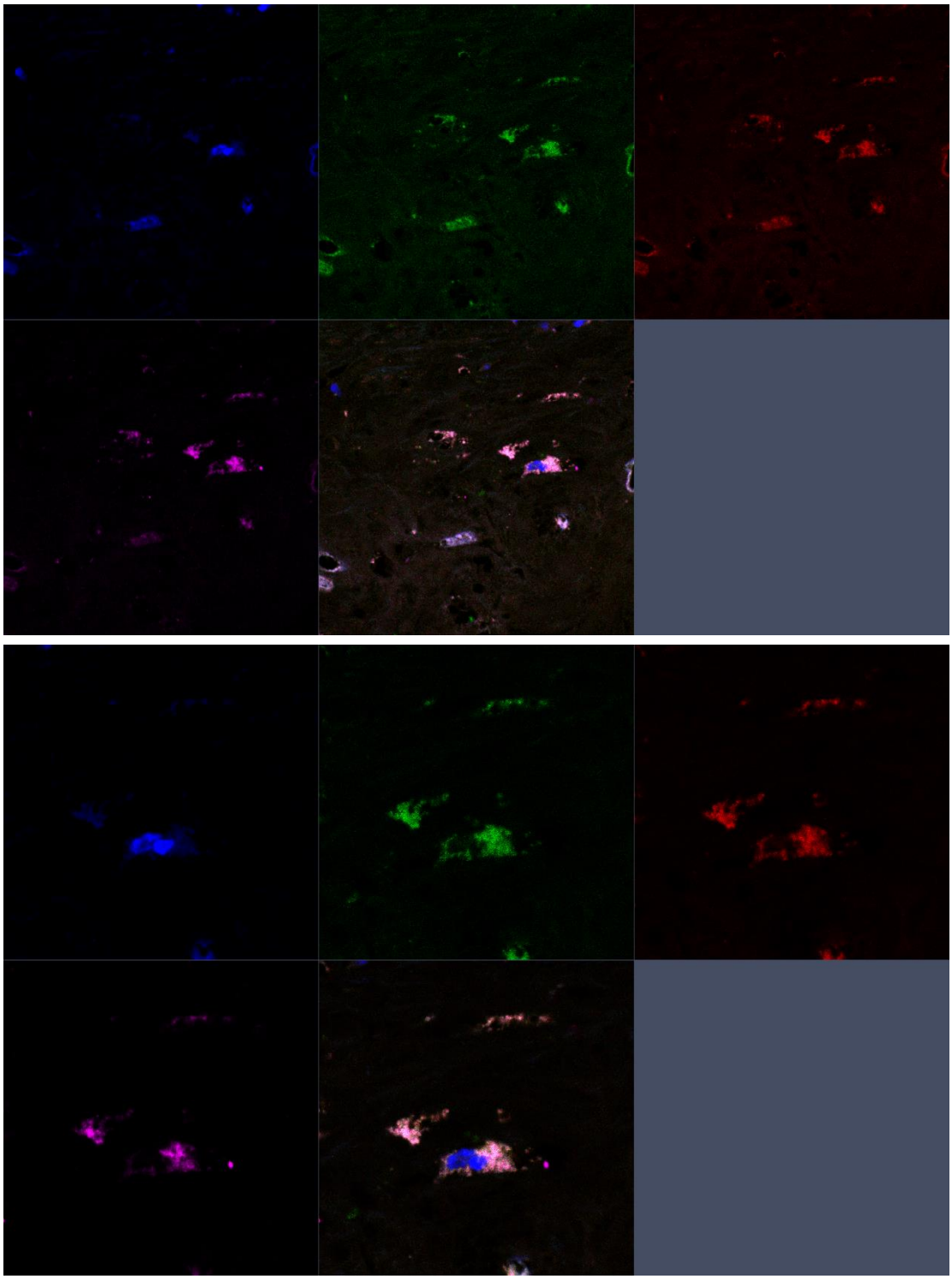
Supplementary Fig.2 The negative staining control of plaques and other two control and atherosclerosis arteries immunofluorescence. (A) DAPI (blue), mouse IgG (green), rabbit IgG (red), goat IgG (pink) respectively. **(B)** Sections were stained with hematoxylin and eosin or immunofluorescence stains for DC-SIGN, TLR4 and CD68.



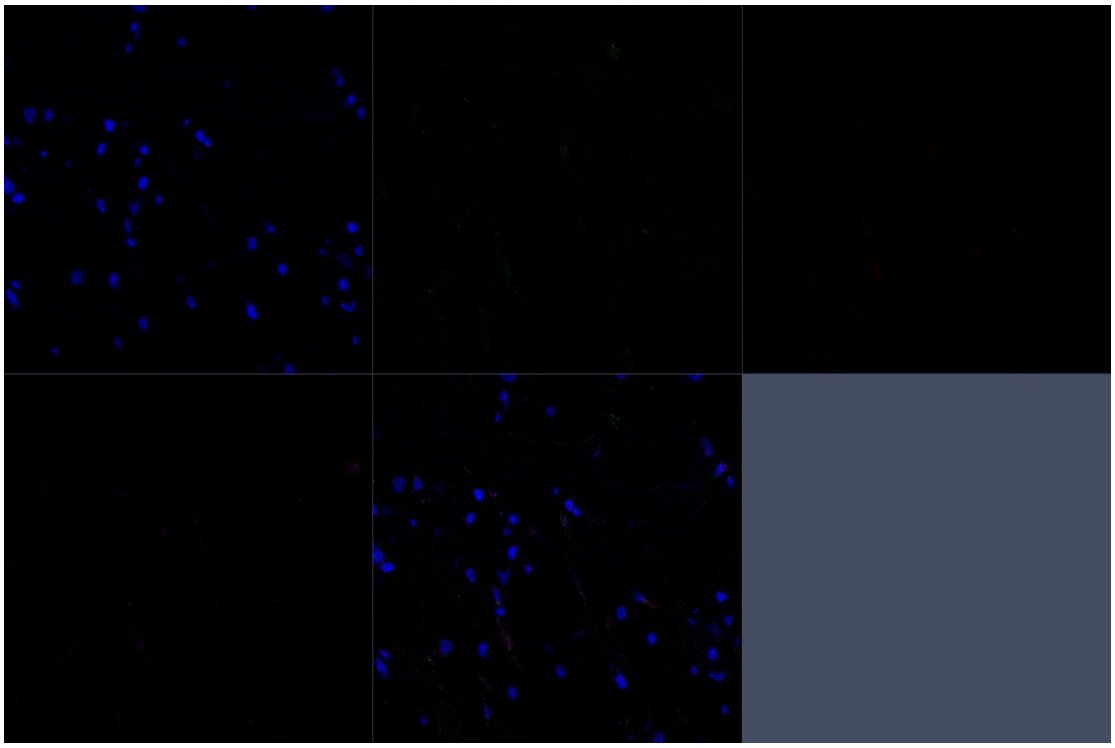
Supplementary Fig.3 Human femoral arteries from patients with angiographic atherosclerotic plaques were assessed with hematoxylin and eosin staining. (The raw data of Figure 1)



Supplementary Fig.4 Human internal thoracic arteries without plaques were assessed with hematoxylin and eosin staining. (The raw data of Figure 1)

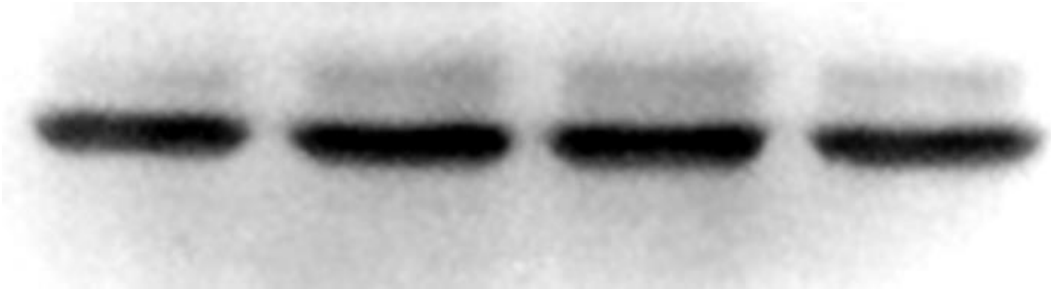


Supplementary Fig.5 Human femoral arteries from patients with angiographic atherosclerotic plaques were assessed by immunofluorescence stains for DC-SIGN, TLR4 and CD68. (The raw data of Figure 1)



Supplementary Fig.6 Internal thoracic arteries without plaques were assessed by immunofluorescence stains for DC-SIGN, TLR4 and CD68. (The raw data of Figure 1)

DC-SIGN



α-tubulin

DC-SIGN

α-tubulin

Supplementary Fig.7 The expression level of DC-SIGN was detected by western blot analysis. Human primary macrophages were incubated with oxLDL for increasing time intervals (0, 6, 12 and 24 hours with 50 μg/ml) or increasing doses (0, 12.5, 25 and 50 μg/ml for 6 hours). (The raw data of Figure 2 B and E)

DC-SIGN

α-tubulin

Supplementary Fig.8 The knockdown efficiency of DC-SIGN siRNA was detected by western blot analysis. (The raw data of Figure 3 A)

DC-SIGN

DC-SIGN

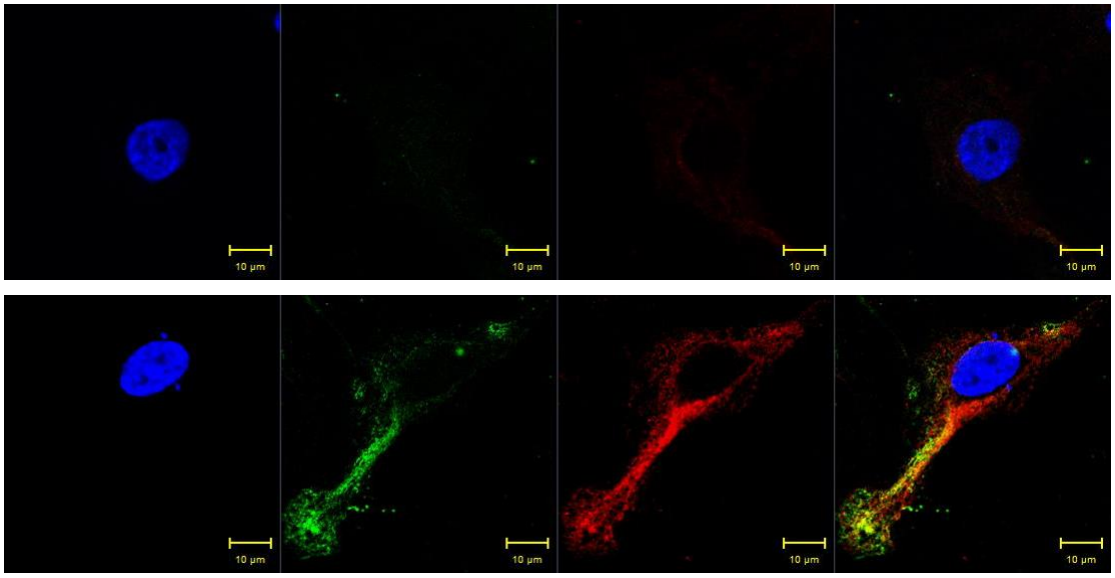
TLR4

TLR4

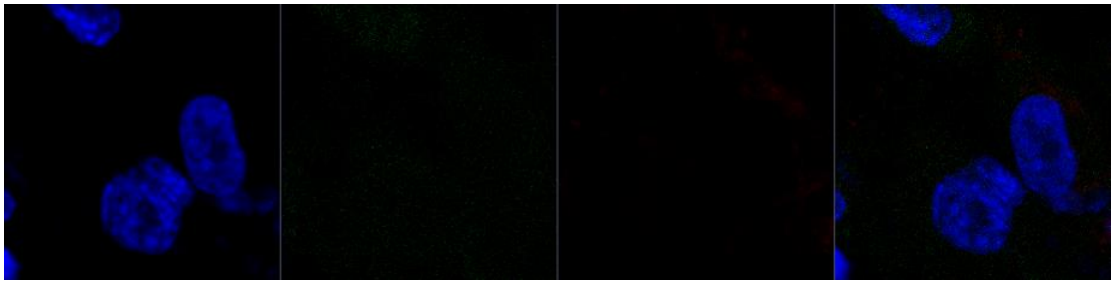
α-tubulin

α-tubulin

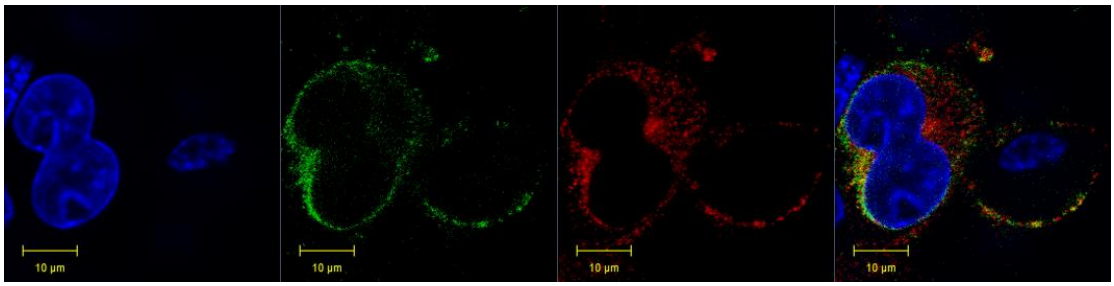
Supplementary Fig.9 The reaction of TLR4 and DC-SIGN had been detected by immunoprecipitated assay. Macrophages were incubated with oxLDL (0, 12.5, 25 and 50 μg/ml) for 6 hours. Cell lysates were immunoprecipitated with a DC-SIGN antibody and probed with an antibody against TLR4. As loading controls, whole cell lysates were probed with antibodies against total TLR4 and α-tubulin. The IgG control had been detected in another Western-blot assay, where the protein level and exposure time were same Western-blot results on the right. (The raw data of Figure 4 A)



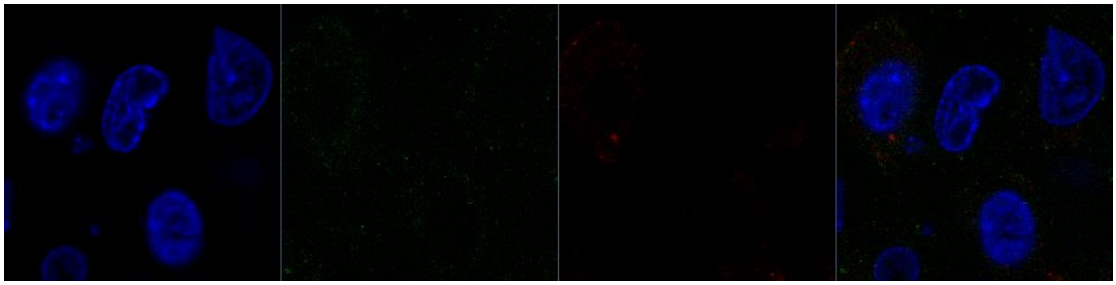
Supplementary Fig.10 The reaction of TLR4 and DC-SIGN had been detected by immunofluorescence assay. Macrophages were stimulated with or without 50 μg/ml oxLDL and stained with DC-SIGN (red) and TLR4 (green). Images were acquired by confocal microscopy (1,200x). Yellow indicates co-localization of the two proteins. (The raw data of Figure 4 B)



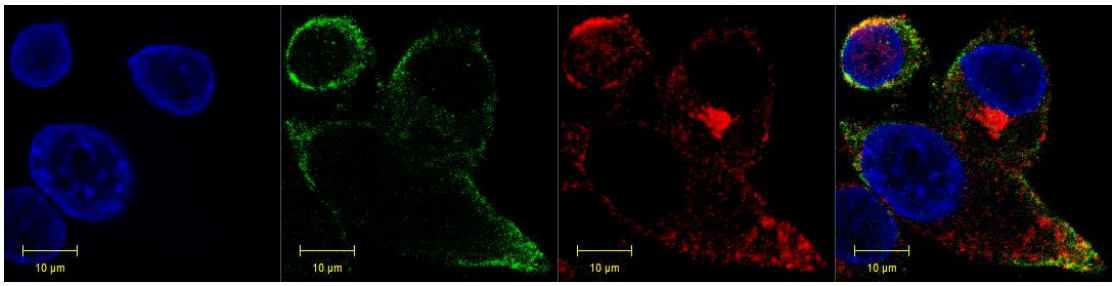
pFLAG-CMV-5.1



pFLAG-CMV-5.1-DC-SIGN



pcDNA3.1 (-)/myc-HisA



pcDNA3.1 (-)/myc-HisA-TLR4

Supplementary Fig. 11 The overexpression efficiency of FLAG-DC-SIGN was measured by immunofluorescence assay. pFLAG-CMV-5.1 or pFLAG-CMV-5.1-DC-SIGN and pcDNA3.1 (-)/myc-HisA or pcDNA3.1 (-)/myc-HisA-TLR4 were transfected into HEK293 cells. The localization patterns of FLAG-DC-SIGN (FLAG: green, DC-SIGN: red) and His-TLR4 (His: green, TLR4: red) were detected by immunofluorescence stains. (The raw data of Figure 4 C and D)

DC-SIGN

α-tubulin

TLR4

α-tubulin

Supplementary Fig.12 The overexpression efficiency of FLAG-DC-SIGN and His-TLR4 were measured by western blot analysis. (The raw data of Figure 4 C and D)

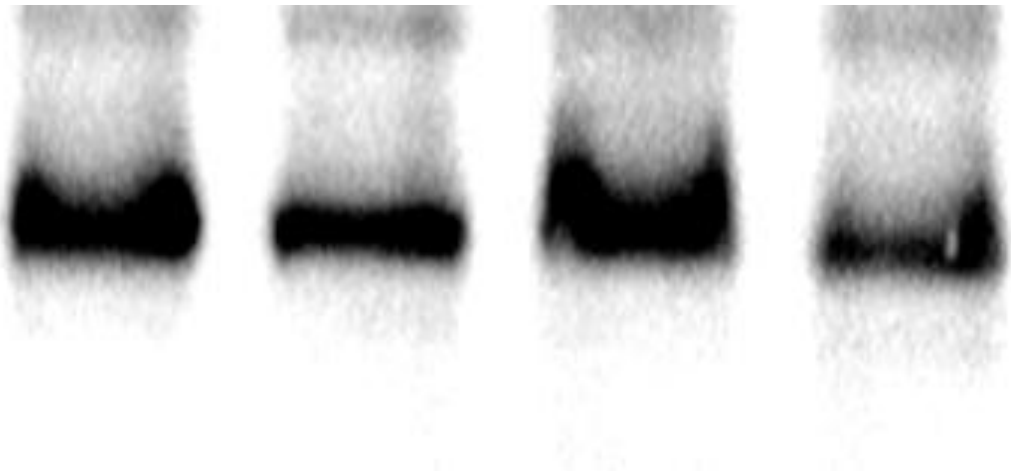
TLR4

His

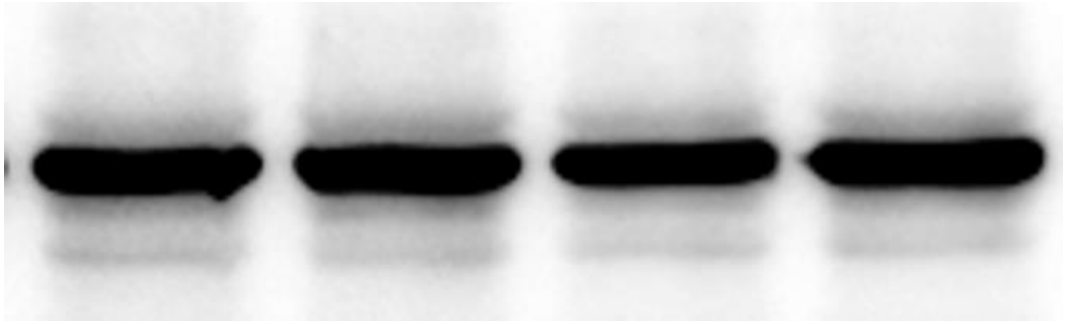
FLAG

Supplementary Fig.13 *In vitro* pull-down of FLAG-DC-SIGN and His-TLR4 fusion proteins. The total cell lysate (10 μg) of FLAG and FLAG-DC-SIGN were absorbed onto anti-FLAG M2 beads and incubated with the whole cell lysate (10 μg) His-TLR4. Elutes were analyzed by SDS-PAGE followed by immunoblotting with anti-

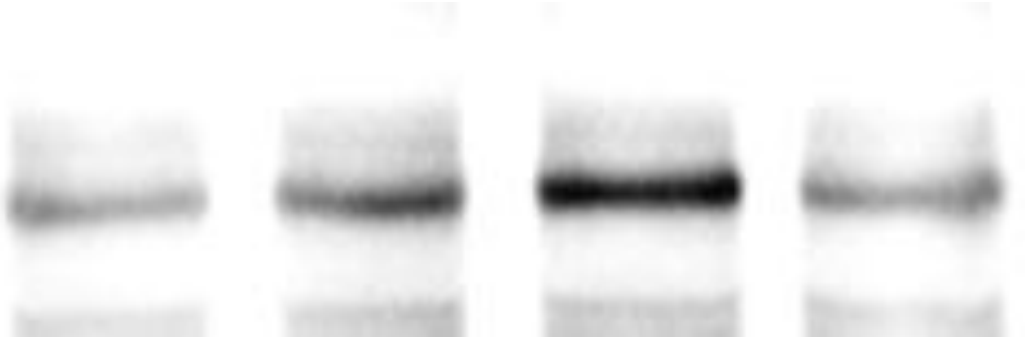
TLR4, anti-His and anti-FLAG. (The raw data of Figure 4 E)



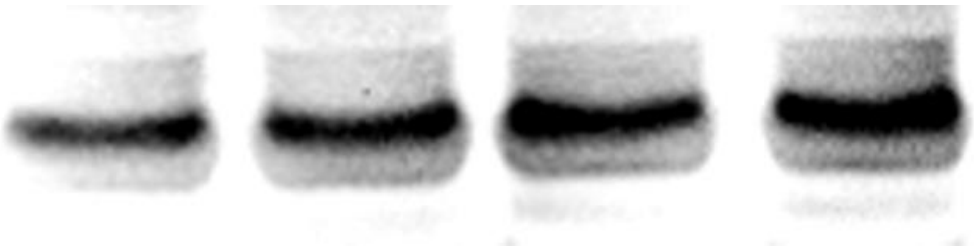
DC-SIGN



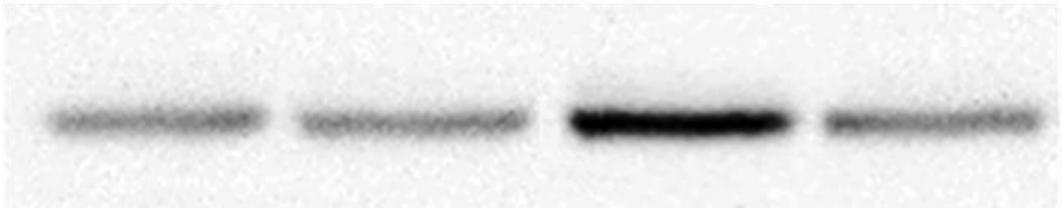
α-tubulin



p-IKKε

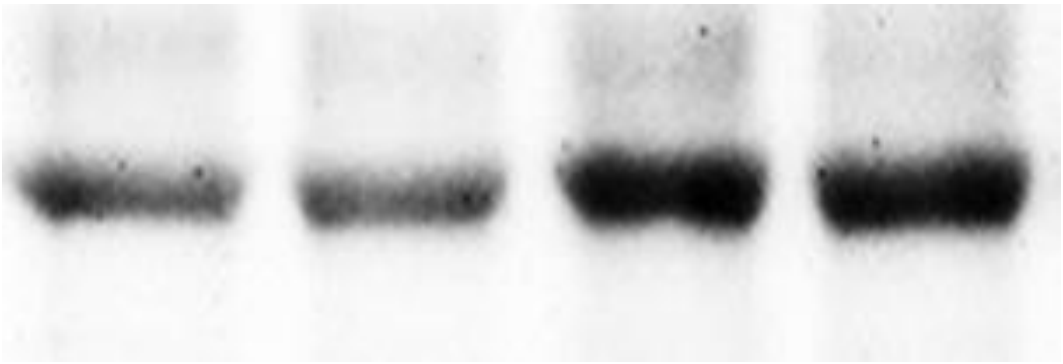


t-IKKε

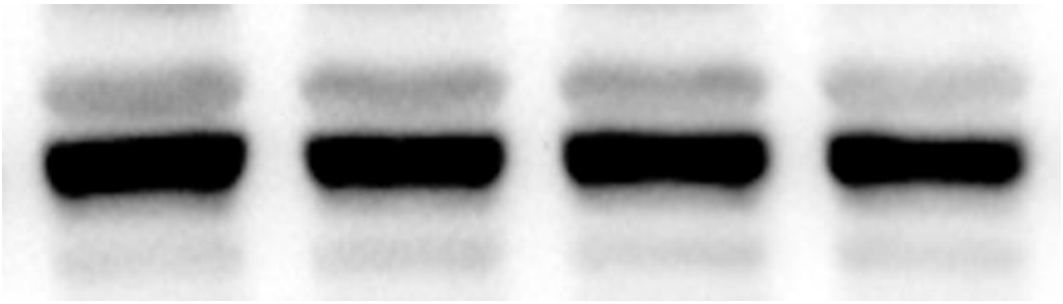


p-P65

t-P65



p-p38



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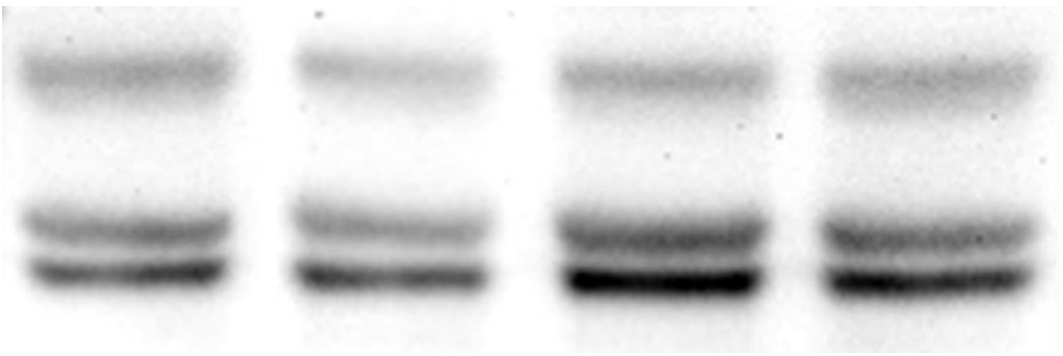
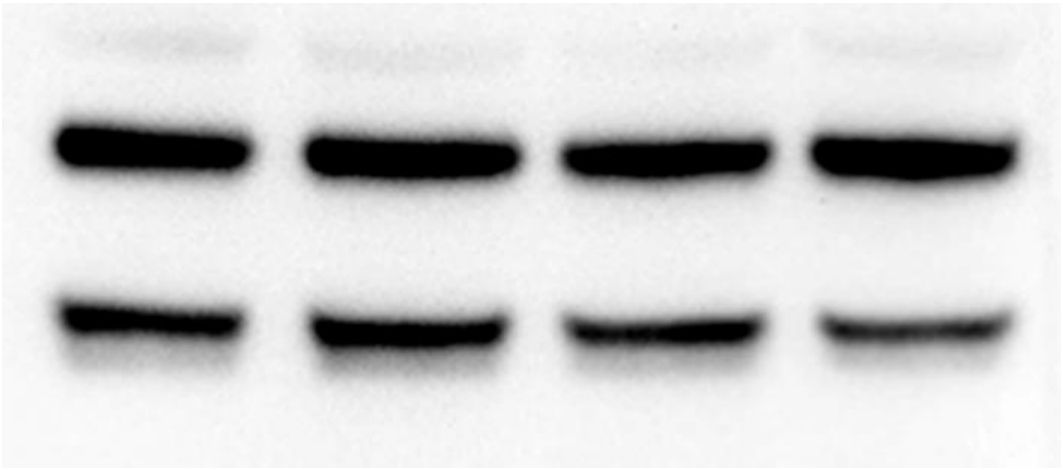
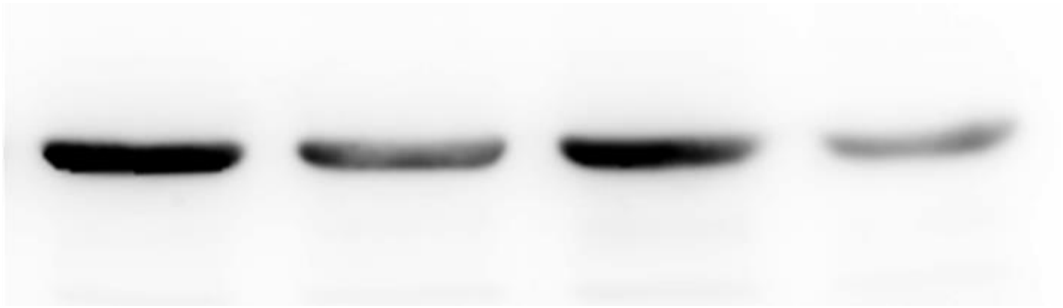


p-JNK

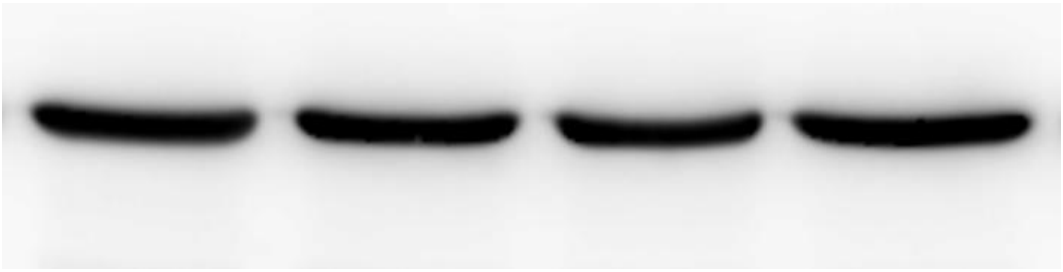


t-JNK

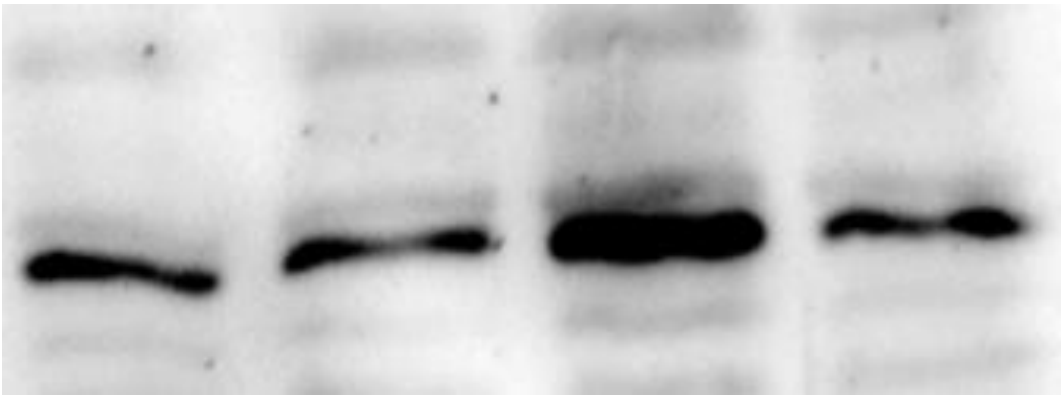
Supplementary Fig.14 DC-SIGN regulated oxLDL-induced signaling pathway. Negative control (NC) or DC-SIGN siRNA was transfected into macrophages treated or not treated with oxLDL (50 μg/ml) for 60 min. Western blot analysis detected the knockdown efficiency of DC-SIGN and the phosphorylation of p38, JNK, IKKε and P65. (The raw data of Figure 5 A)



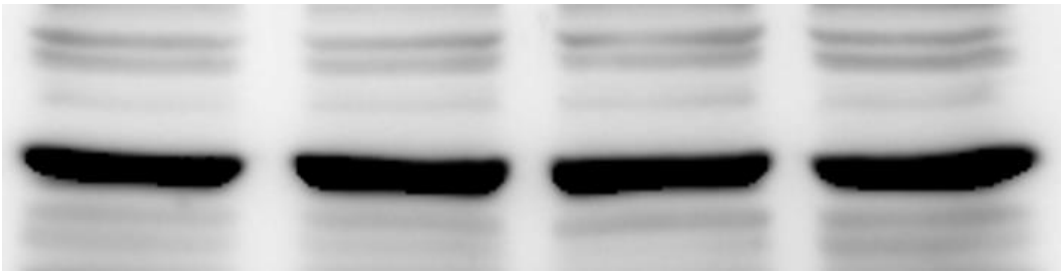
DC-SIGN



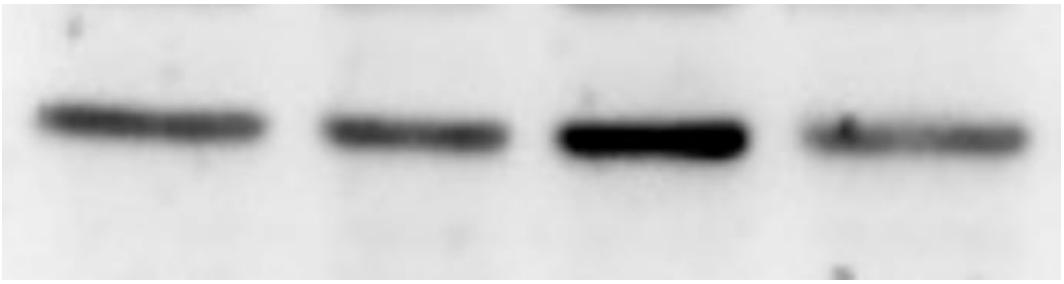
α-tubulin



p-IKKε

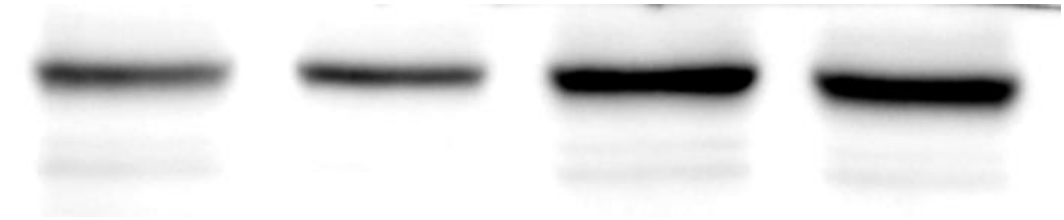


t-IKKε



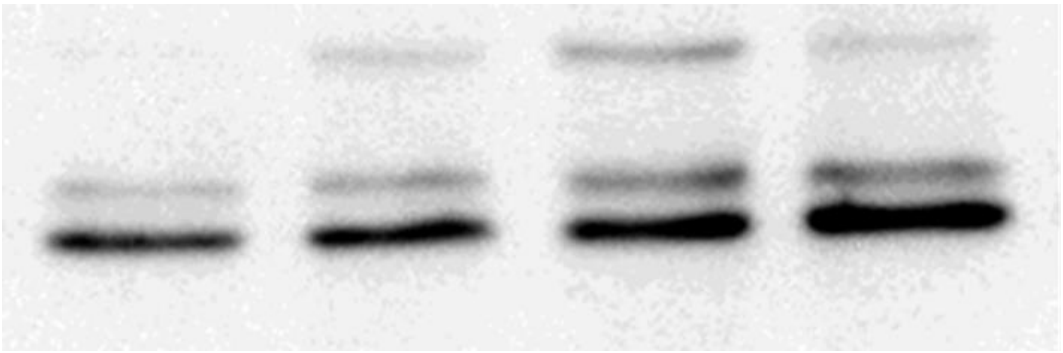
p-P65

t-P65

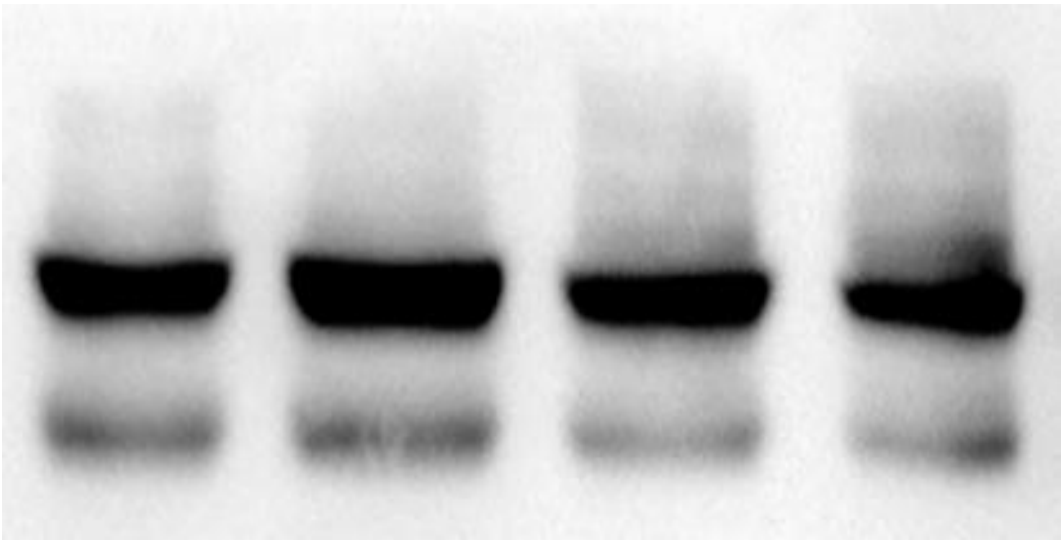


p-p38

t-p38

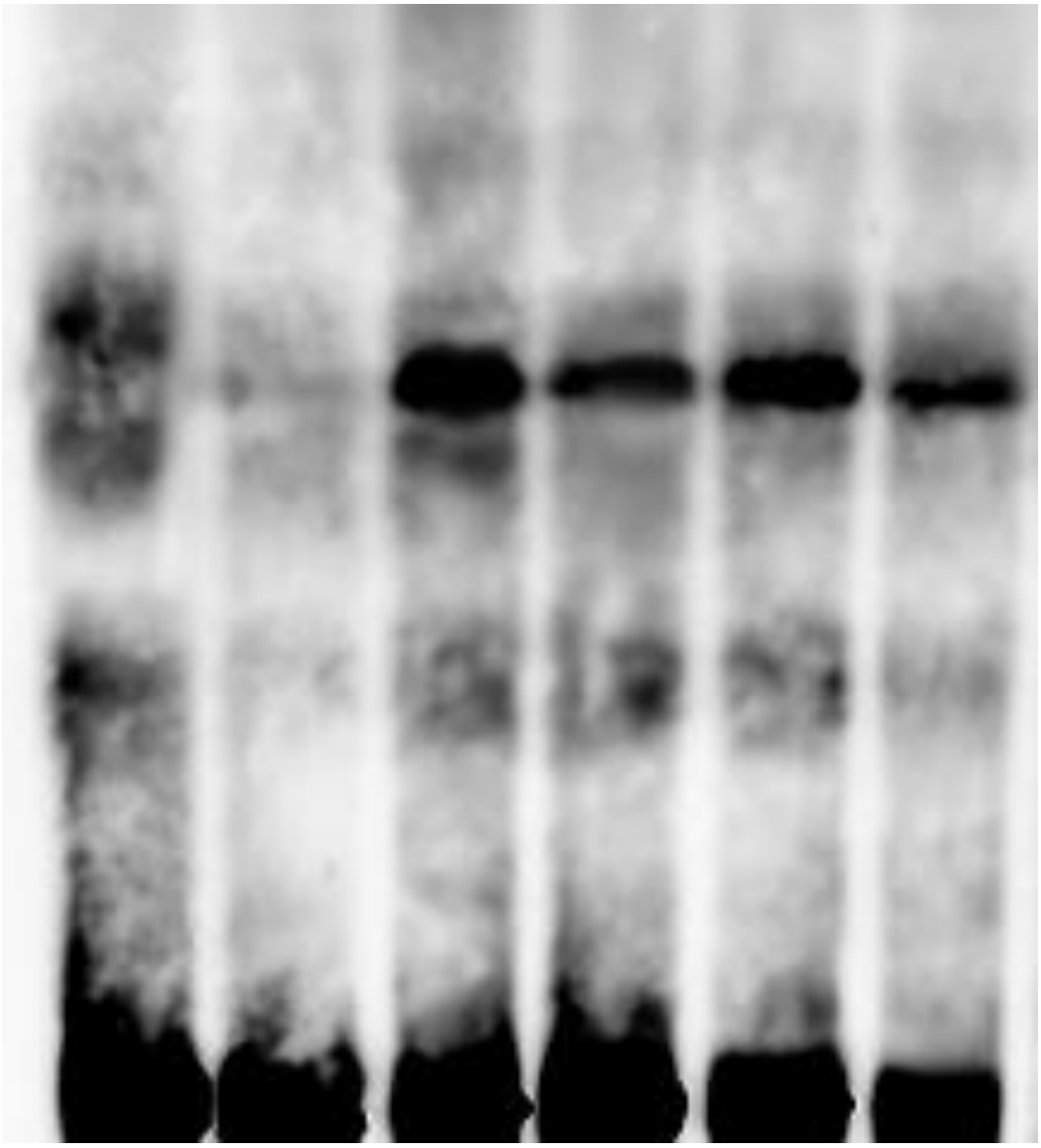


p-JNK



t-JNK

Supplementary Fig.15 DC-SIGN regulated LPS-induced signaling pathway. Negative control (NC) or DC-SIGN siRNA was transfected into macrophages treated or not treated with LPS (62.5 ng/ml) for 60 min. Western blot analysis detected the knockdown efficiency of DC-SIGN and the phosphorylation of p38, JNK, IKKε and P65. (The raw data of Figure 5 B)



Supplementary Fig.16 DC-SIGN regulated P65 activation. Negative control (NC) or DC-SIGN siRNA was transfected into macrophages treated or not treated with oxLDL (50 μg/ml) or LPS (62.5 ng/ml) for 60 min. Nuclear extracts were then prepared and assayed for p65 activation by EMSA. (The raw data of Figure 5 C)