Supplementary materials and methods

Measurement of Cell Apoptosis

For Annexin V-PE assay, the Annexin V-PE Apoptosis Detection Kit (BD PharMingen, San Diego, CA) was used. Apoptotic cells were detected by annexin V binding to phospholipid phosphatidylserine (PS), which was translocated from the inner to the outer leaflet of the plasma membrane of apoptotic cells. Approximately 1×10^6 cells were resuspended in 1×binding buffer and incubated with phycoerythrin (PE)-conjugated annexin V and the fluorescent DNA-binding dye 7-AAD in the dark for 15 min at room temperature. The cells were then quantified by FACS analysis.

Supplementary Figures

Figure S1

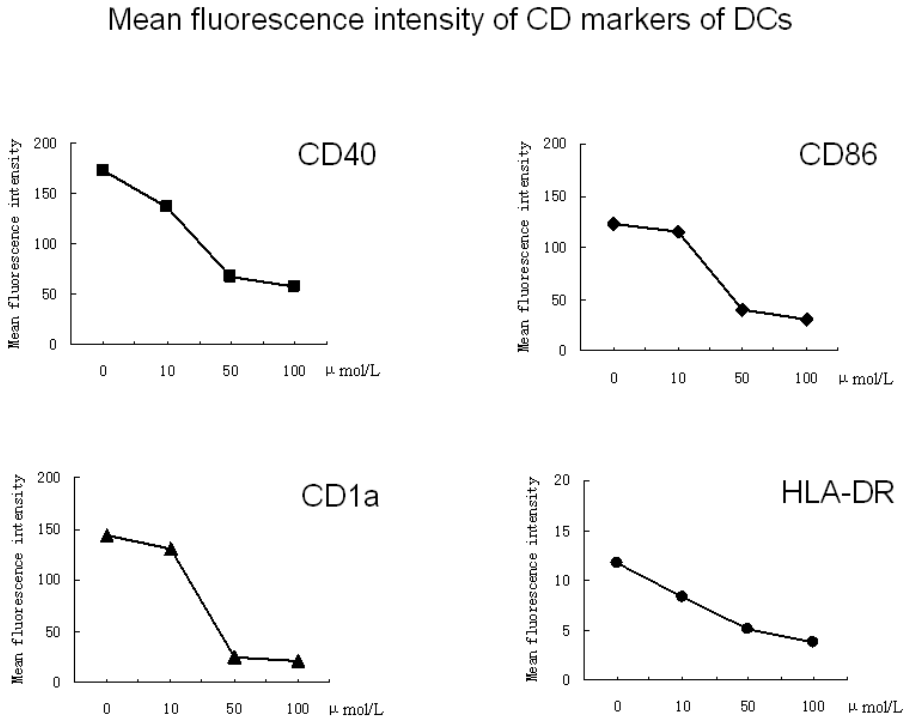


Figure S1 Concentration curves for the effect of Sal B on DCs maturation. Ox-LDL-induced DCs were treated with 0, 10, 50, and 100μM of Sal B, and the phenotypic changes of CD40, CD86, CD1a, and HLA-DR were determined, respectively.

Figure S2

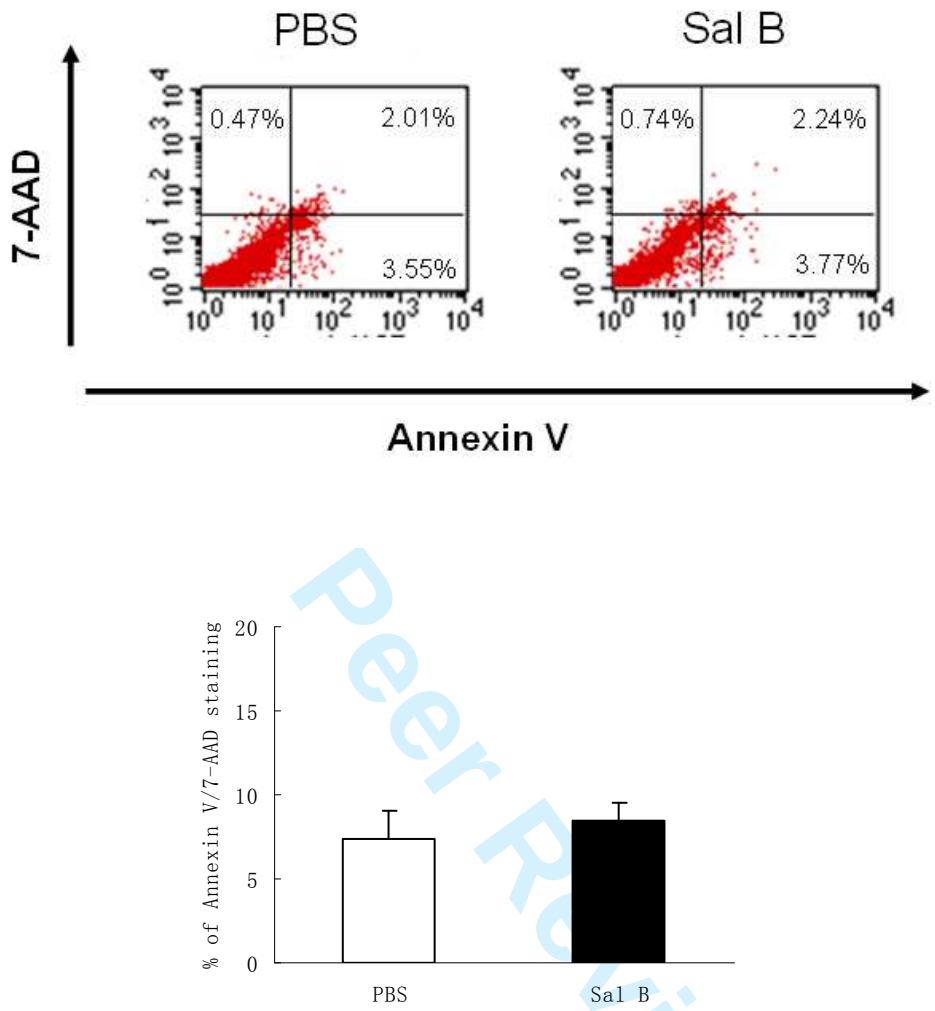


Figure S2 PBMCs were separated and purified with anti-CD14 magnetic beads, then cultured with GM-CSF (100 ng/ml) and IL-4 (40 ng/ml) for 5 days. On day 6, cells were stimulated by Sal B (50μM) and the toxic effects of Sal B were then quantified by FACS analysis after staining with Annexin V and 7-AAD. The results represented the means±SD of three independent experiments.

Figure S3

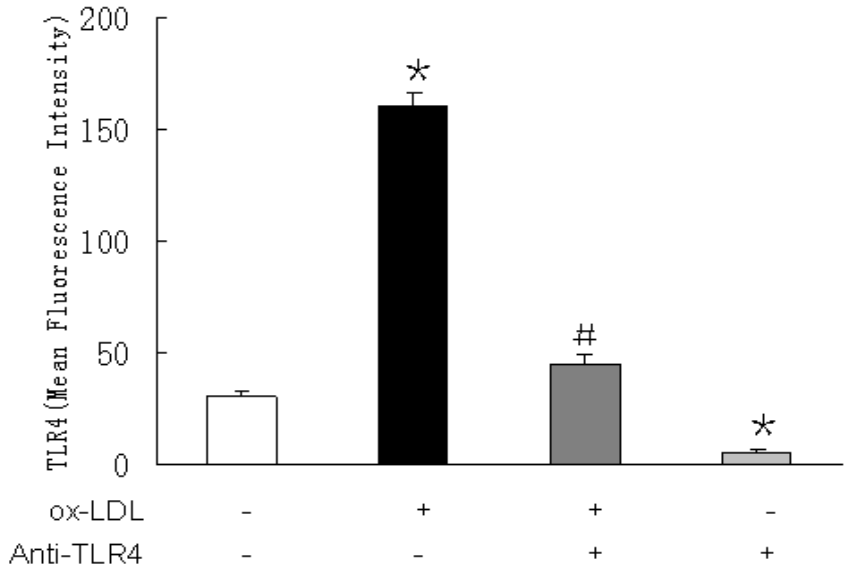


Figure S3 Purified h-monDC was incubated for 5 days in the presence of GM-CSF and IL-4. On day 6, ox-LDL (50μg/ml) was added and the effect of ox-LDL-induced TLR4 expression was determined by FACS after blocking the blocking the membrane TLR4 on matured h-monDC by purified TLR4 neutralizing antibody. Data were presented as means±SD for three times of independent experiments. **P*<0.05 vs. control; # *P*<0.05 vs. ox-LDL.

Figure S4

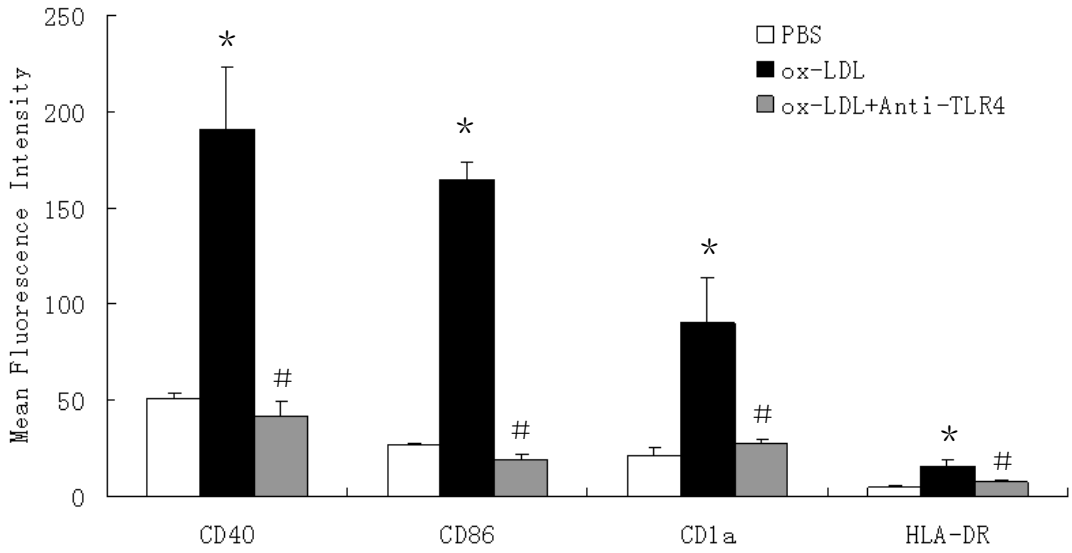
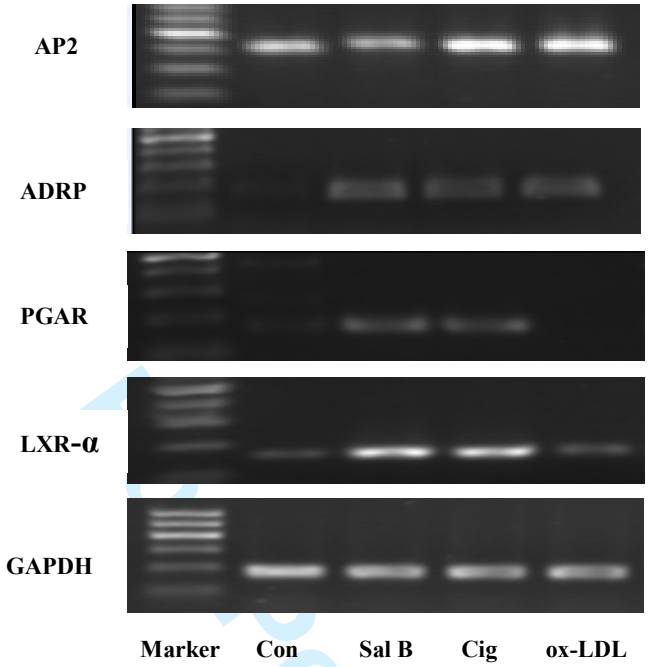


Figure S4 Purified h-monDC was incubated for 5 days in the presence of GM-CSF and IL-4. On day 6, ox-LDL (50μg/ml) was added and the effect of ox-LDL-induced h-monDC phenotype was determined by FACS after blocking the membrane TLR4 on matured h-monDC by purified TLR4 neutralizing antibody. Data were presented as means±SD for three times of independent experiments. *P<0.05 vs. PBS; # P<0.05 vs. ox-LDL.

Figure S5


AP2
ADRP
PGAR
LXR-α
GAPDH
Marker
Con
Sal B
Cig
ox-LDL

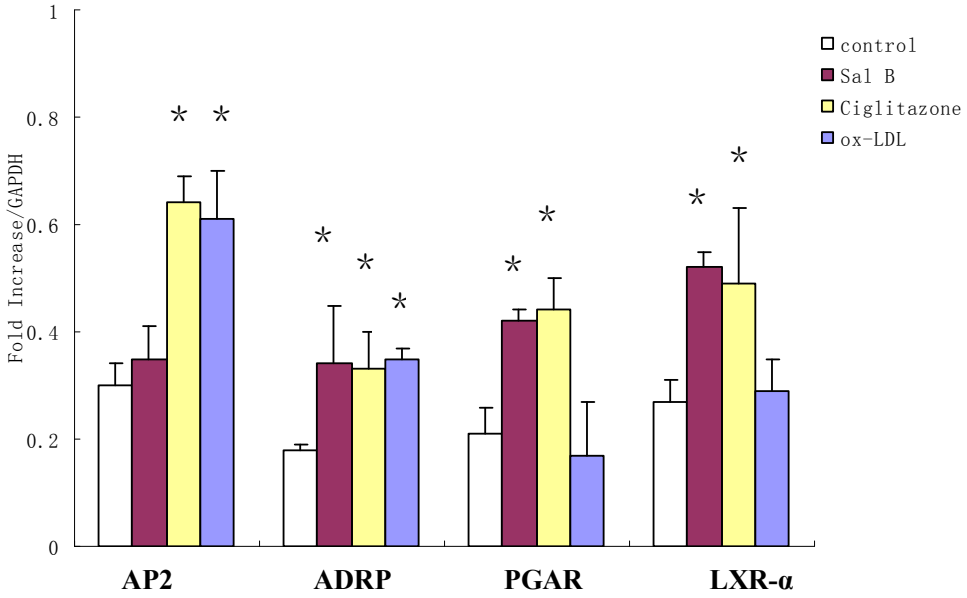

Fold Increase/GAPDH
1
0.8
0.6
0.4
0.2
0
control
Sal B
Ciglitazone
ox-LDL
AP2
ADRP
PGAR
LXR-α

Figure S5 PBMCs derived h-monDC cultured with GM-CSF and IL-4 for 5 days, then stimulated by PBS, Sal B, Ciglitazone and ox-LDL, respectively for 24 h. Then mRNA was extracted and cDNA was reverse-transcribed. The expression of PPAR-γ target genes were analyzed by RT-PCR using specific primers as follows: LXR-α, 5'-acggtgatgcttctggagac-3' and 5'-tagcaatgagcaaggcaaact-3' (NM_001130101.1); AP-2, 5'-gtctccgccatccctattaac-3' and 5'-ggaatgttgtcggttgagaaa-3' (NM_001032280.2); ADRP, 5'-agtggaaaaggagcattggata-3' and 5'-ctgtggtacaccttggatgttg-3' (NM_001122.3); PGAR, 5'-acaagcacctagaccatgaggt-3' and 5'-ctgaattactgtccagcctccat-3' (NM_001039667.1); GAPDH, 5'-agaaggctggggctcatttg-3' and 5'-aggggccatccacagtcttc-3' (NM_002046.3).

(* $P<0.05$, vs control.)

Figure S6

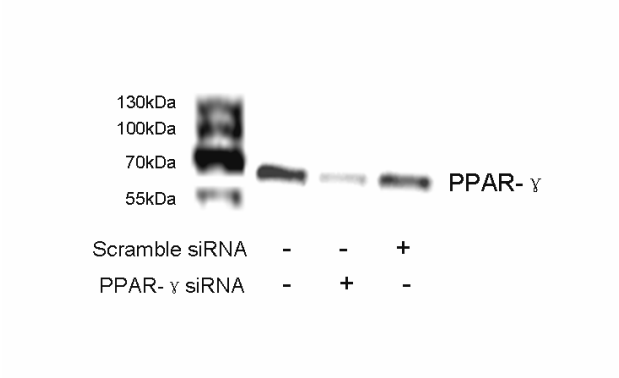


Figure S6 PBMCs derived h-monDC cultured with GM-CSF and IL-4 for 5 days, then cells were transfected with mock conditions (no siRNA), PPAR-γ siRNA, or scramble siRNA for 24 h, respectively.