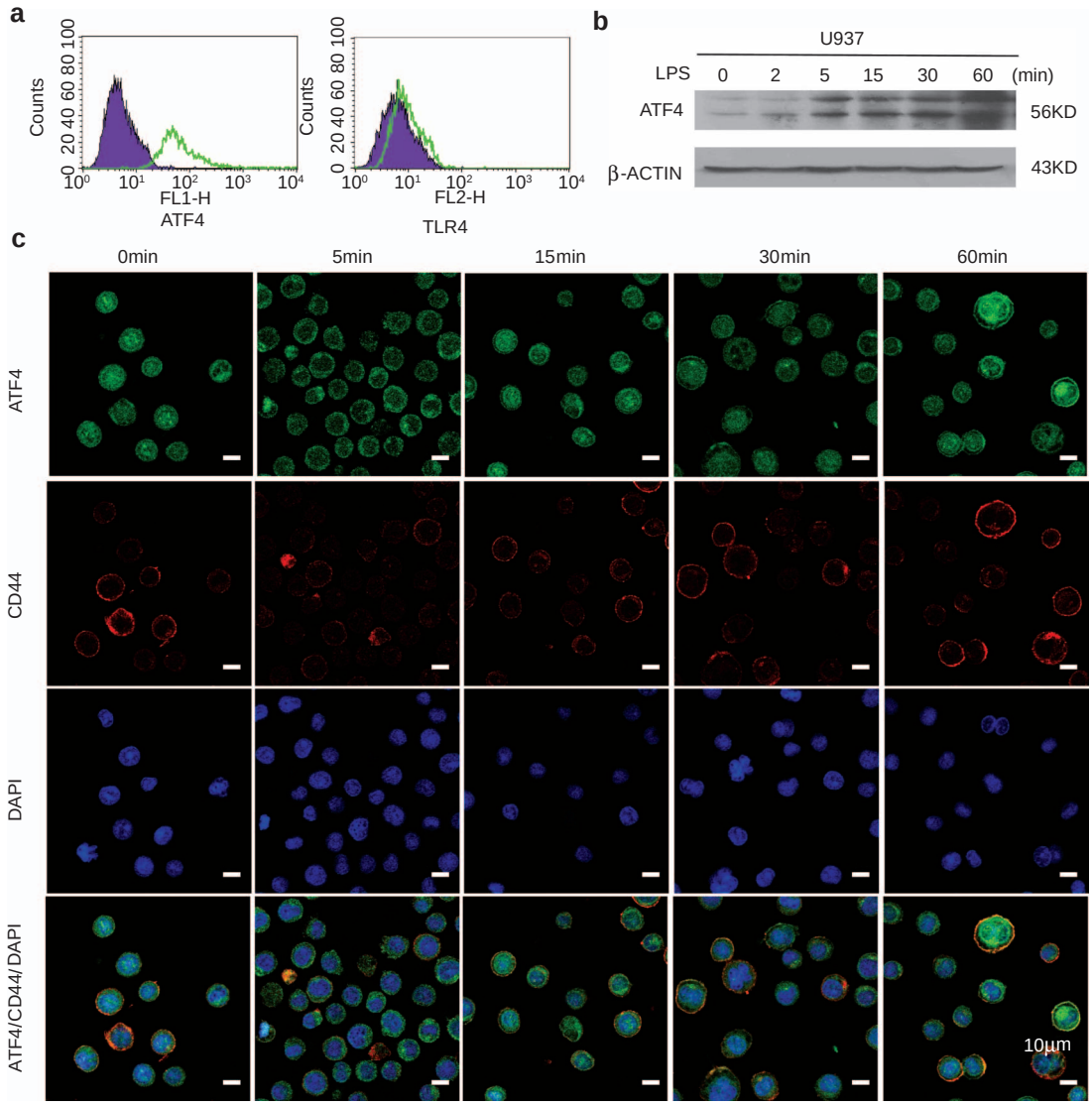
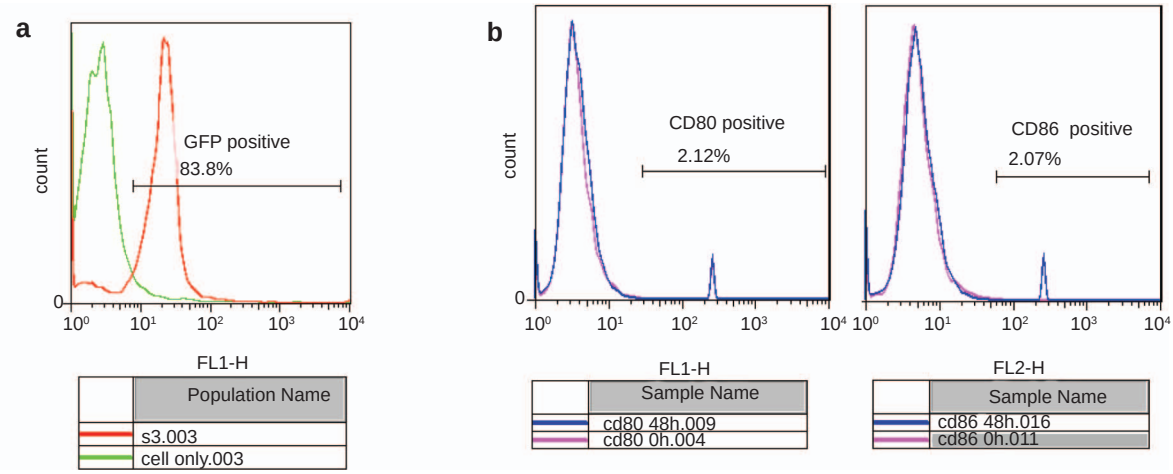
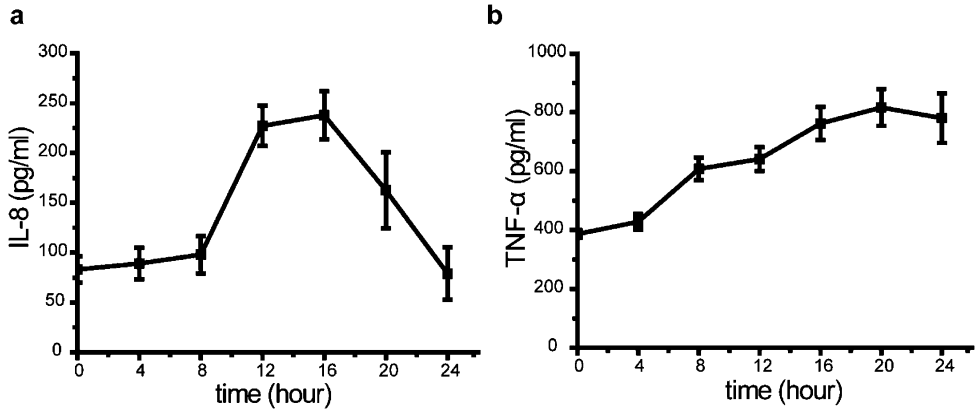


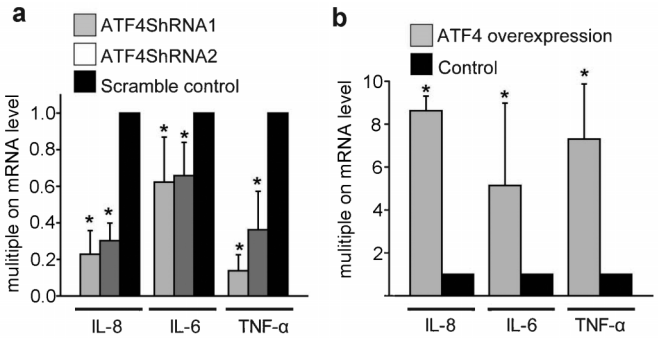
Supplemental Figure 1 TLR4 engagement activates ATF4 in U937 cells. (**a**) Flow cytometry analyzes the expression of ATF4 and TLR4 in U937 cells. (**b**) ATF4 was induced to accumulate after 100 ng/ml LPS stimulation at 0, 2, 5, 15, 30 and 60 min in U937 cells. Whole cell lysate and nuclear extract were analyzed by western blotting with ATF4 antibody. (**c**) Confocal image of ATF4 translocation in U937 cells. Cells were immunostained with ATF4 antibody which was labeled with ALEXA488 (green) and CD44 antibody which was labeled with ALEXA546 (red) and cell nuclei were counterstained with DAPI (blue). Scale bar equals to 10 μm. ATF4, activating transcription factor 4; DAPI, 4′,6-diamidino-2-phenylindole; LPS, lipopolysaccharide; TLR, Toll-like receptor.



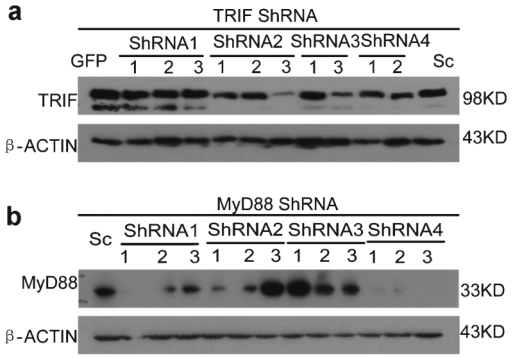
Supplemental Figure 2 The effectiveness of infection in THP-1 cells using lentivirus system. (**a**) Flow cytometry analysis for infection efficiency using lentivirus system (pLv-GFP-bsd plasmid) in THP-1 cells. (**b**) Flow cytometry analysis for the expression of CD86 and CD80 after lentivirus infection in THP-1 cells.



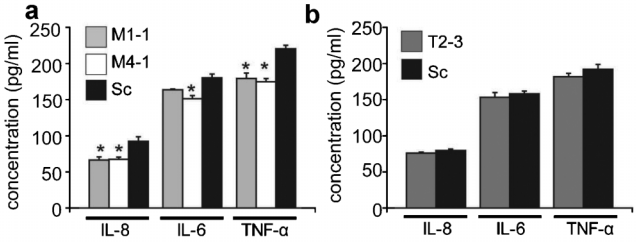
Supplemental Figure 3 A time course ELISA analysis of IL-8 and TNF-α in the supernatant of THP-1 cells that contain overexpressed or knockdown ATF4. Data shown are mean±s.d. of triplicate samples and are representative of three independently performed experiments. ATF4, activating transcription factor 4; TNF, tumor-necrosis factor.



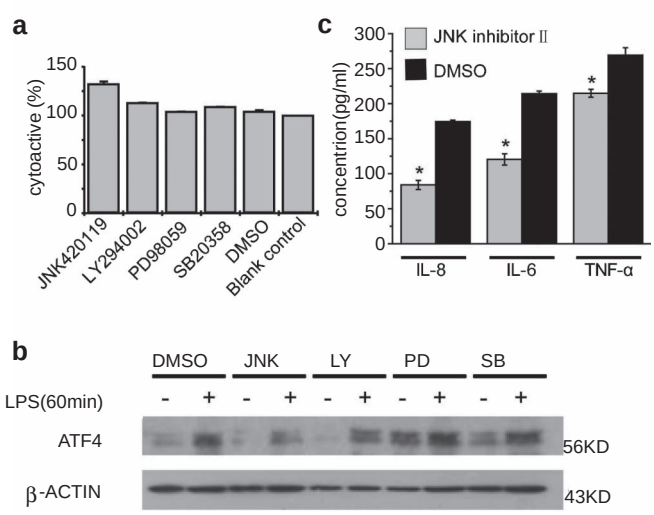
Supplemental Figure 4 Real-time PCR analysis of the amount of IL-6, IL-8 and TNF-α in THP-1 cells which have overexpressed or knockdown ATF4. Data shown are mean±s.d. of triplicate samples and are representative of three independently performed experiments. Asterisks indicate statistically significant differences between the means ($P<0.05$). ATF4, activating transcription factor 4; TNF, tumor-necrosis factor.



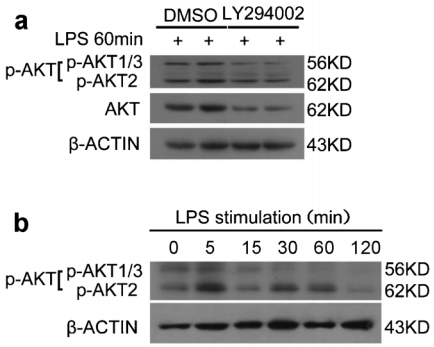
Supplemental Figure 5 MyD88 shRNA and TRIF shRNA screening. MyD88shRNA1~4 and TRIFshRNA1~4 pladmids were transfected into THP-1 cells for 48 h, respectively and the silence efficiency was detected by western blot with corresponding antibodies. The GFP and scramble groups were analyzed as positive controls. TRIF, TIR domain-containing adaptor-inducing interferon β.



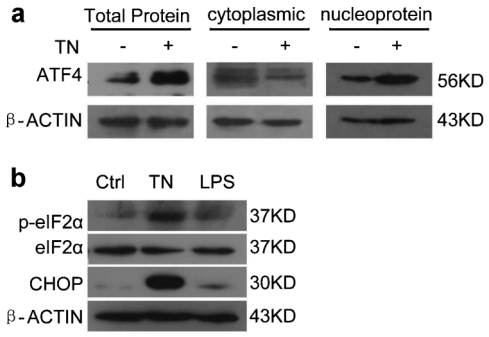
Supplemental Figure 6 MyD88 subpathway affects the secretion of cytokines. ELISA assay for the concentrations of IL-6, IL-8 and TNF-α after MyD88 knockdown (by MyD88 ShRNA 1-1 and 4-1) (**a**) and TRIF knockdown (by TRIF ShRNA2-3) in THP-1 cells (**b**). Data shown are mean±s.d. of triplicate samples and are representative of three independent experiments. Asterisks indicate statistically significant differences between the means ($P<0.05$). TNF, tumor-necrosis factor; TRIF, TIR domain-containing adaptor-inducing interferon β.



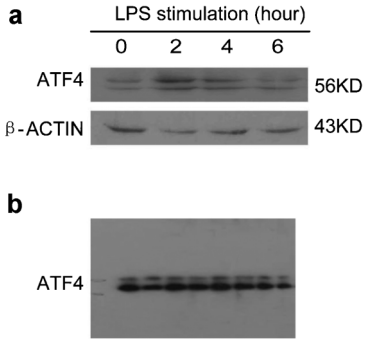
Supplemental Figure 7 The effects of inhibitors of JNK, PI3K, MEK/MAPKK and p38 MAPK on ATF4 activation in THP-1 cells. (**a**) WST-1 analysis of cell viability after the cells were treated by inhibitors. THP-1 cells were cultured in 96-well plate at a density of 4×10^4 cells/well and treated with DMSO, 40 nM JNK inhibitor II, 10 μM LY294002, 20 μM PD98059 and 10 μM SB203580 for 1 h. WST-1 mixture was added to each well and incubated for 2 h. The absorbance of each sample was measured using a microplate reader at wavelength of 450 nm. Data shown are mean±s.d. of triplicate samples and are representative of two independent experiments performed. (**b**) Effects of inhibitors on the ATF4 activation in THP-1 cells. (**c**) Effect of JNK inhibitor II on the secretion of IL-8, IL-6 and TNF-α. The group treated with DMSO was used as a control. Data shown are mean±s.d. of triplicate samples and representative of three independent experiments. Asterisks indicate statistically significant differences between the means ($P<0.05$). ATF4, activating transcription factor 4; DMSO, dimethyl sulfoxide; LPS, lipopolysaccharide; TNF, tumor-necrosis factor.



Supplemental Figure 8 The activation of AKT in the process of LPS stimulation. (**a**) The inhibition of LY294002 on AKT phosphorylation. (**b**) The activation of AKT in the process of LPS stimulation. DMSO, dimethyl sulfoxide; LPS, lipopolysaccharide.



Supplemental Figure 9 Low-dose LPS is not able to induce ER stress. (**a**) Cells were treated with 1 μg/ml tunicamycin (TN) for 12 h and western blot was performed using total protein, cytoplasm protein or nuclear protein. (**b**) The expression of phosphorylated eIF2a and CHOP after the cells were treated with 1 μg/ml tunicamycin or 100 ng/ml LPS for 12 h. ATF4, activating transcription factor 4; DMSO, dimethyl sulfoxide; LPS, lipopolysaccharide.



Supplemental Figure 10 ATF4 level after time-dependent LPS treatment. (**a**) ATF4 level after 2, 4 and 6 h of LPS treatment. (**b**) A full-length blot of ATF4. ATF4, activating transcription factor 4; DMSO, dimethyl sulfoxide; LPS, lipopolysaccharide.