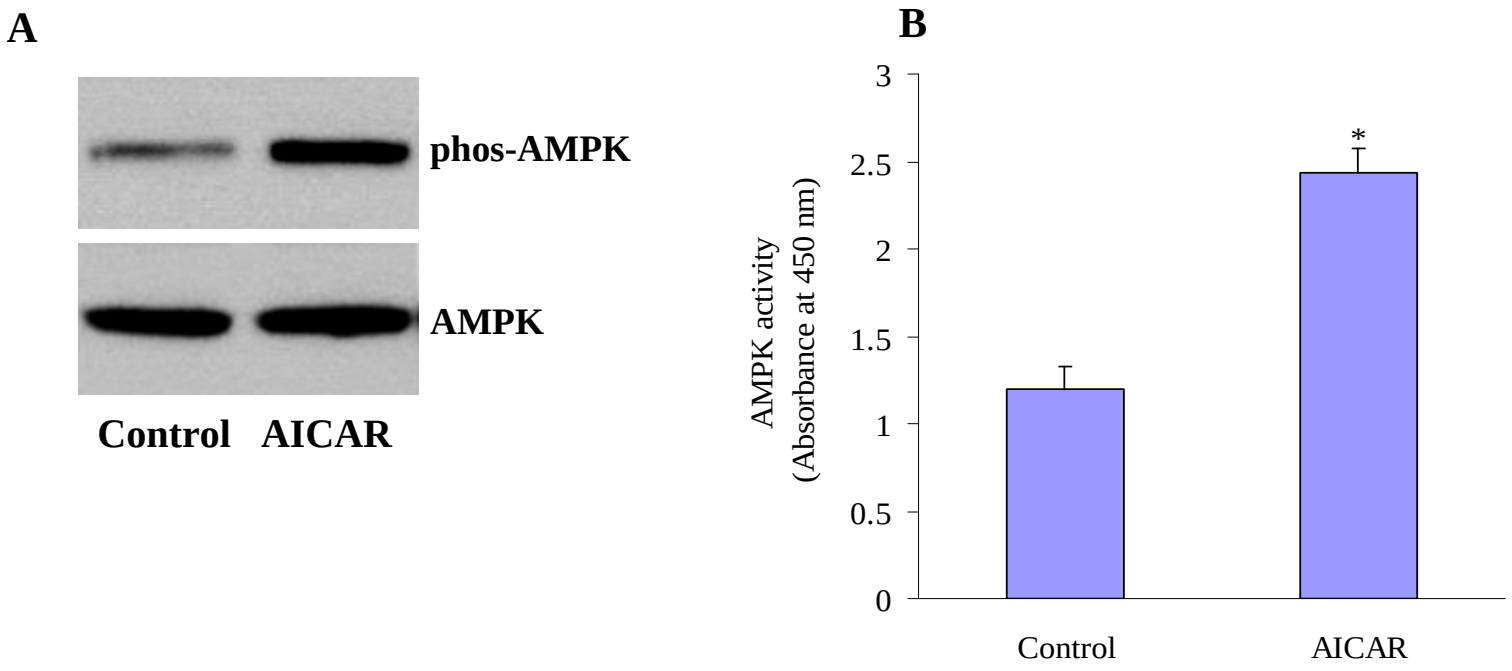
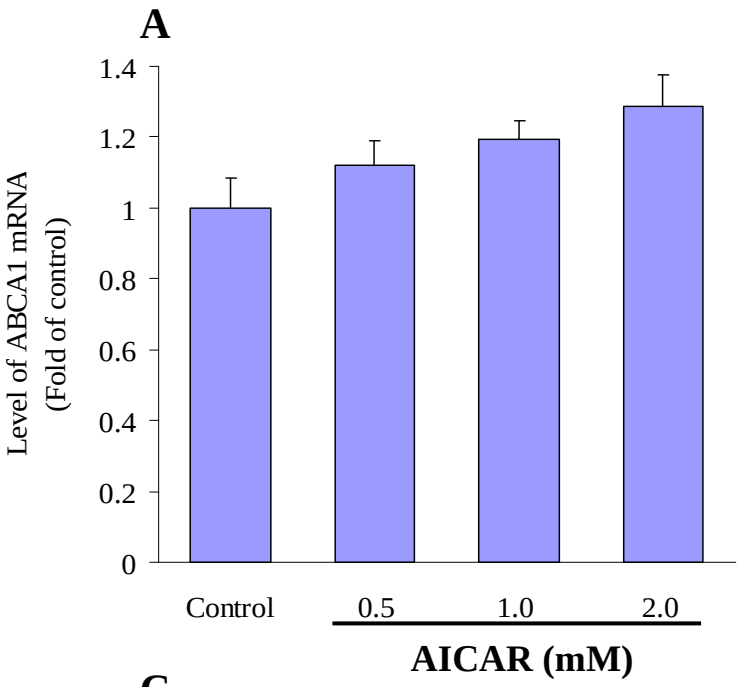
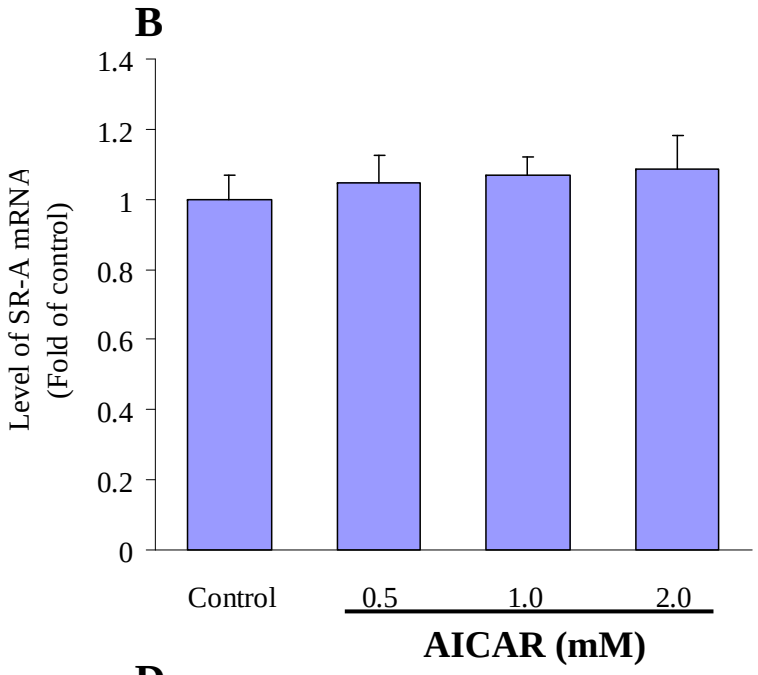
Supplement Fig. 1

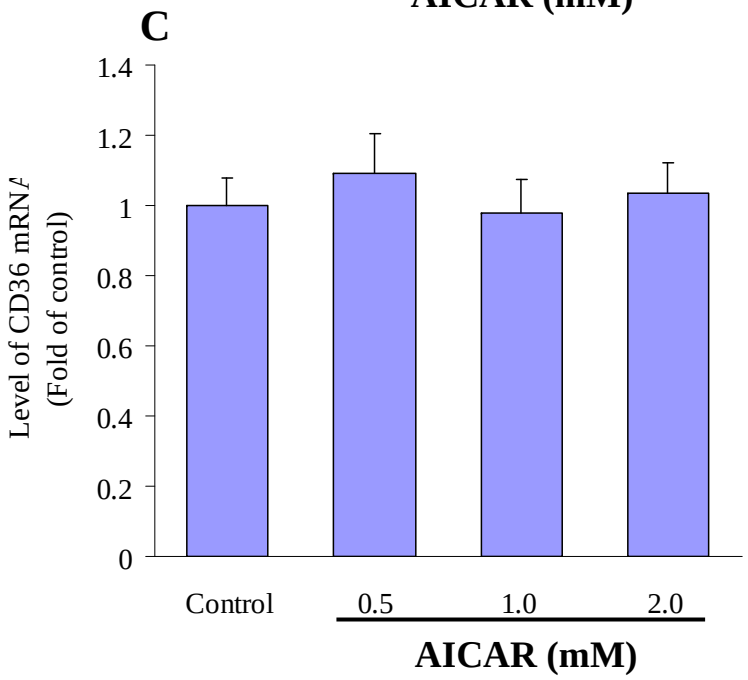


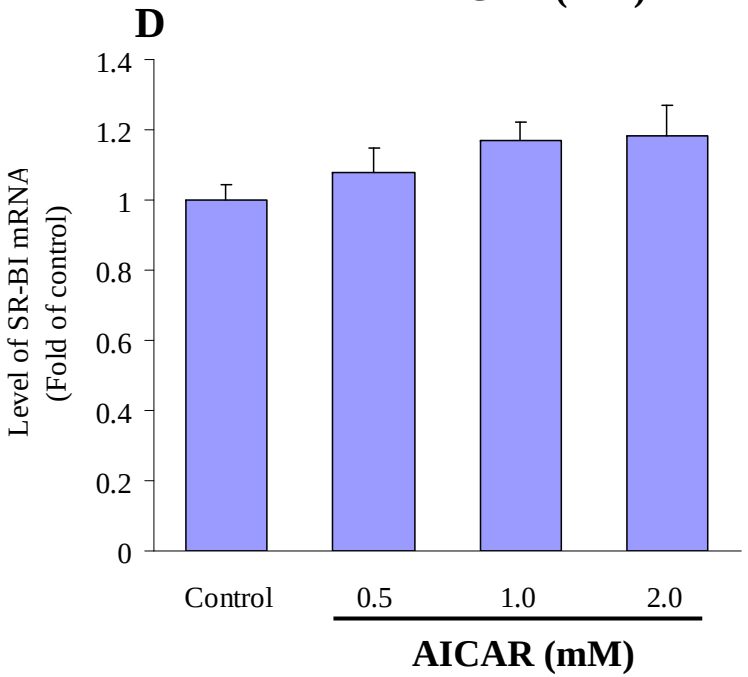
Supplement Fig. 1 Effects of AICAR on AMPK activation in macrophages. J774.1 macrophages were incubated with culture medium or AICAR (1.0 mM) for 24 h. (A) Protein samples were immunoblotted with anti-phospho-AMPK (phos-AMPK) and anti-AMPK, respectively. The data are representative of three separate experiments. (B) Two microgram of protein extracts were subjected to AMPK activity assays with SAMS peptide as substrates. The results are means ± SEM from experiments run in triplicate and are representative of at least three independent experiments. $^{*}P<0.05$.

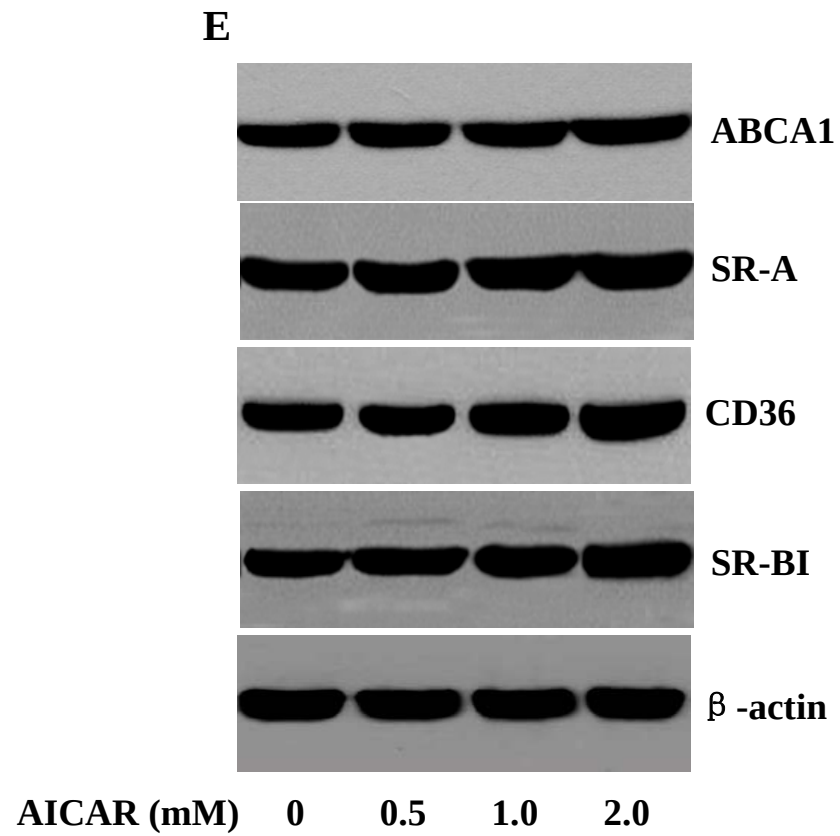
Supplement Fig. 2





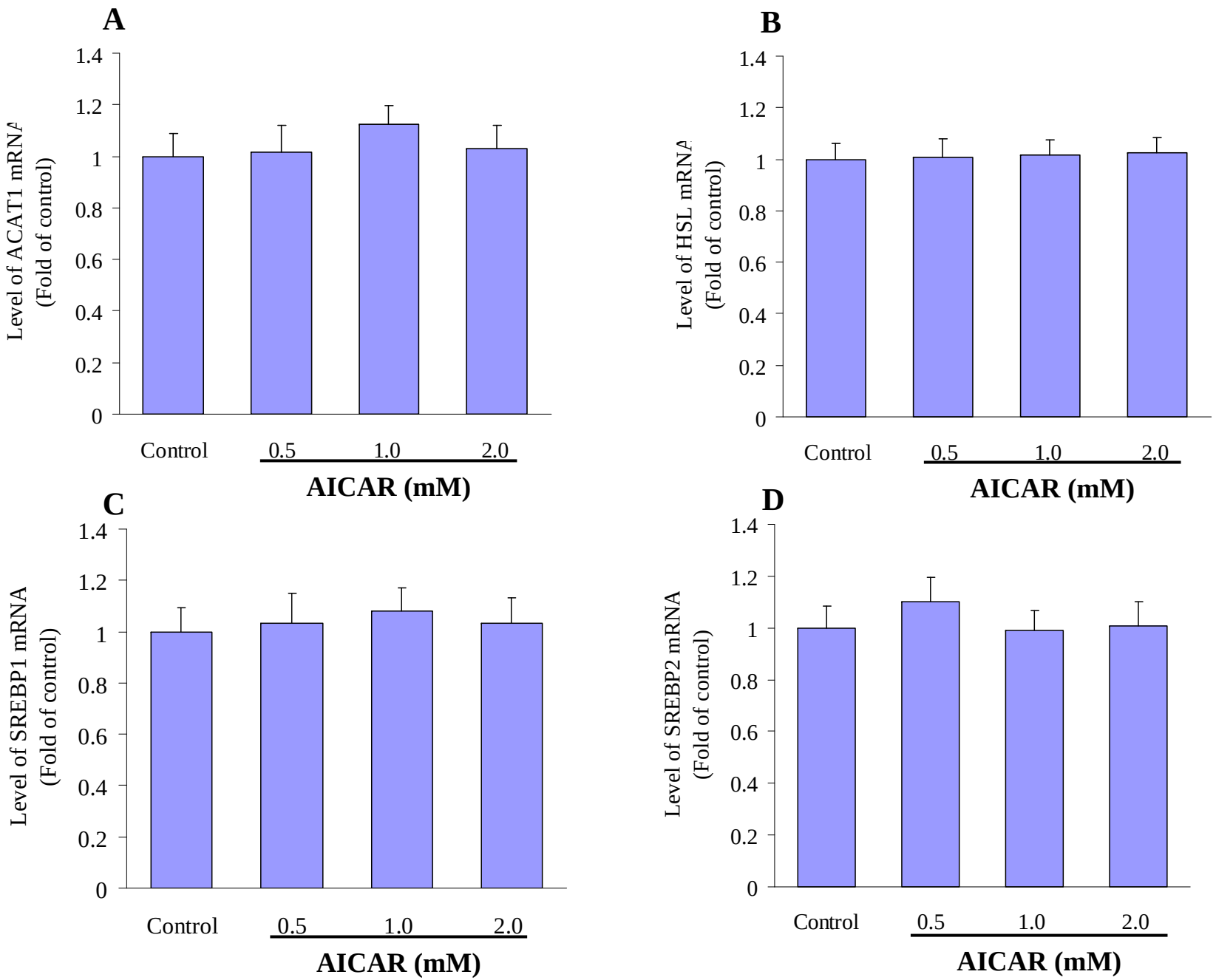


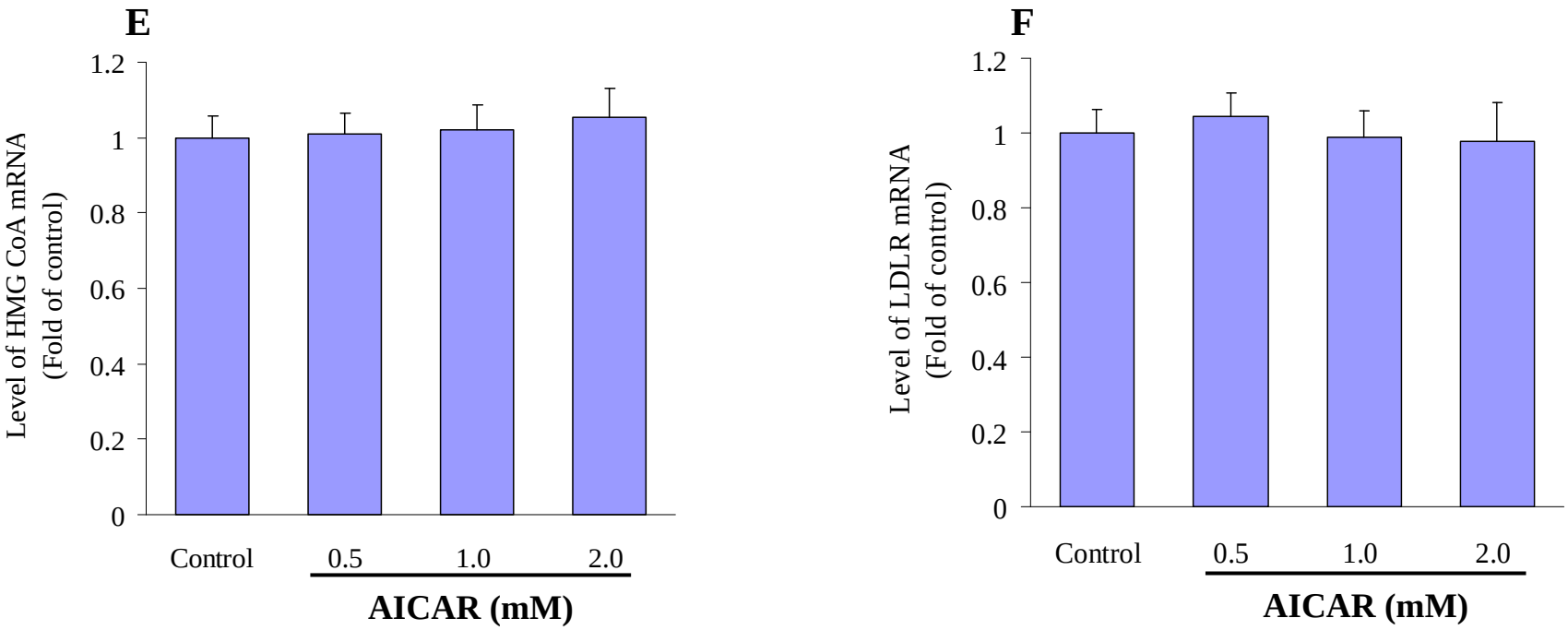




Supplement Fig. 2 Effects of AICAR on the expression of ABCA1, SR-A, CD36 and SR-BI. (A through E), J774.1 macrophages were incubated with AICAR (0.5, 1.0, 2.0 mM) for 24 h, and cell lysates were subjected to real-time quantitative PCR and Western blot to determine the mRNA and protein level of ABCA1, SR-A, CD36 and SR-BI,. (A-D), ABCA1, SR-A, CD36 and SR-BI mRNA levels. The results are expressed as fold of control from at least three independent assays. (E), Relative ABCA1, SR-A, CD36 and SR-BI protein levels. The representative blots from three independent experiments are shown.

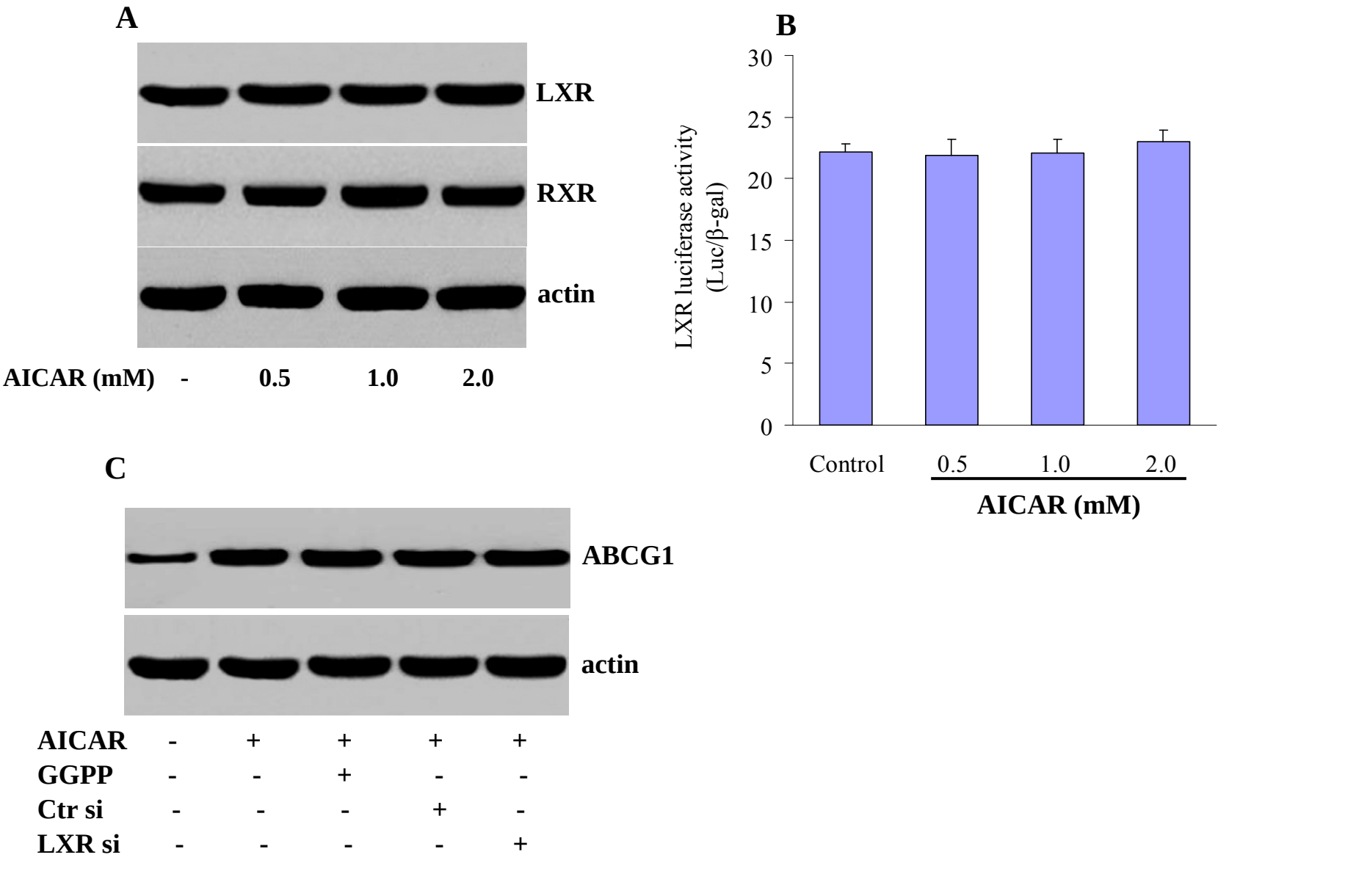
Supplement Fig. 3





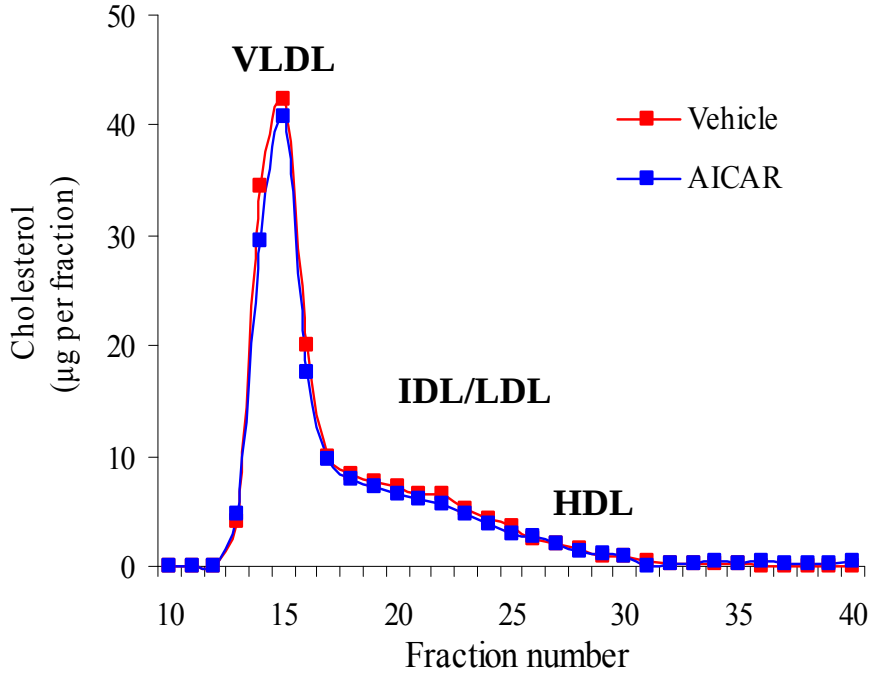
Supplement Fig. 3 AICAR did not affecting the lipid synthesis-related genes expression in macrophages. (A-F) J774.A1 macrophages were incubated with indicated concentration of AICAR (0.5, 1.0, 2.0 mM) for 24 h. Total RNA were extracted and subjected to the real-time quantitative PCR to determine the gene expression levels of (A) ACAT1, (B) HSL, (C) SREBP-1, (D) SREBP-2, (E) HMG CoA and (F) LDLR. The results are expressed as fold changes compared with the control from at least three independent assays.

Supplement Fig. 4



Supplement Fig. 4 AICAR did not alter LXR expression and activation. (A) J774.A1 macrophages were incubated with AICAR (1 mM) for 12 h. Nuclear extracts were isolated and subjected to Western blot to determine the protein level of LXR α , RXR, and actin. (B) Macrophages were transfected with a luciferase reporter plasmid containing LXREs upstream of the thymidine kinase promoter (LXRE-tk-Luc) in the presence of β -galactosidase as a reference plasmid. Twelve hours after transfection, cells were treated with AICAR (0.5, 1.0, 2.0 mM) for 24 h. Luciferase and β -galactosidase activities were then determined in the cell lysates. The values represent the means ± SEM are expressed as luc/ β -gal. (C) Macrophages were pretreated with GGPP (20 μ M) for 2 h, or transfected with LXR specific siRNA. After then, cell were incubated with AICAR (2 mM) for 24 h, cells were lysed for ABCG1 protein assays by Western blot. Beta-actin was used as an internal control.

Supplement Fig. 5



Supplement Fig.5 Size fractionation of plasma lipoproteins. ApoE$^{-/-}$ mice, fed a high fat cholesterol diet, were randomly administered with either AICAR (n = 8, 200 mg · kg^{-1} · d^{-1}) or vehicle (n = 8, 0.9% NaCl) for 12 wks. After then, the plasma were pooled together and fractionated by fast protein liquid chromatography (n = 3 to 4 mice of each group). Data are expressed as the mean ± SEM. VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.