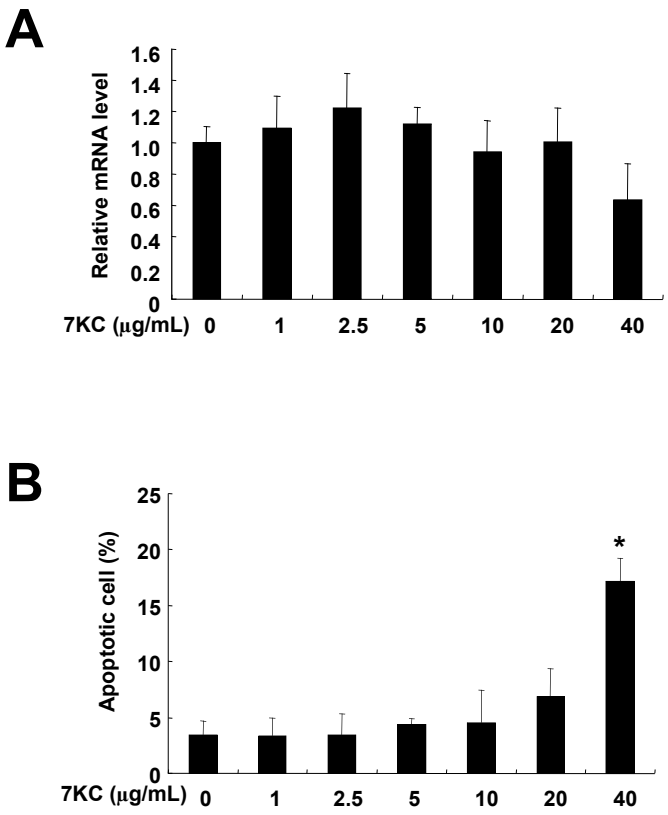
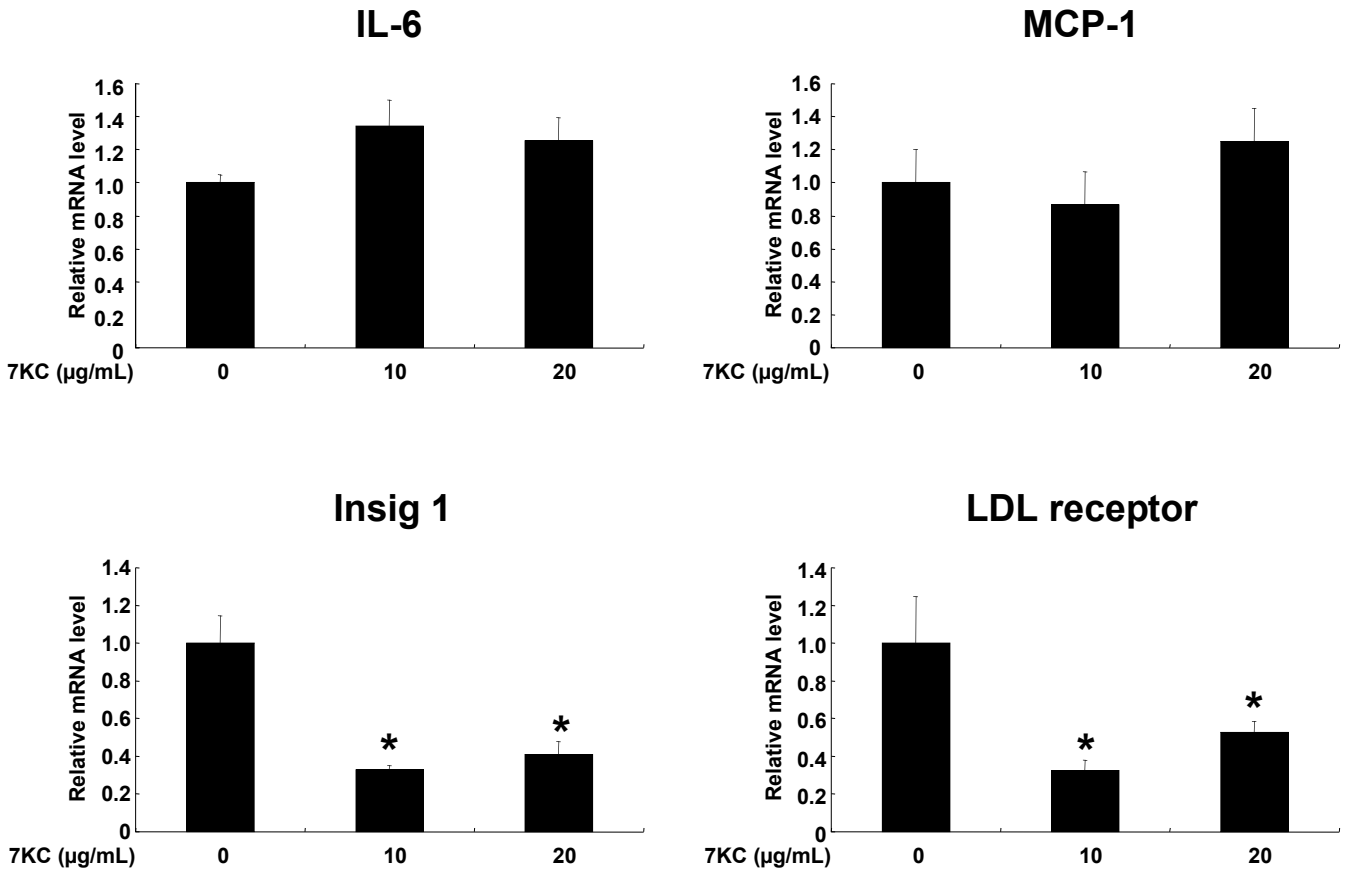
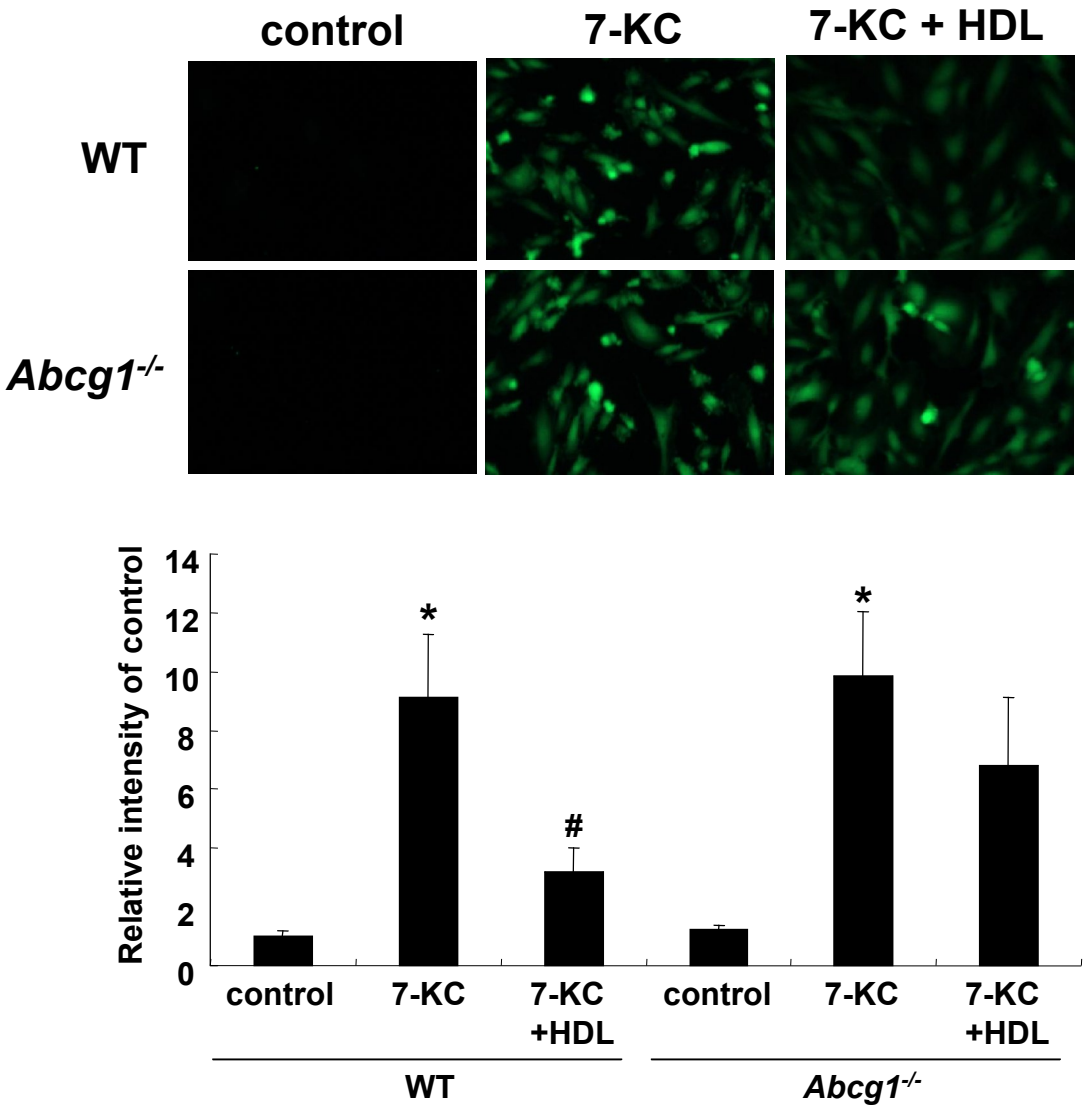
Supplemental Figure 1



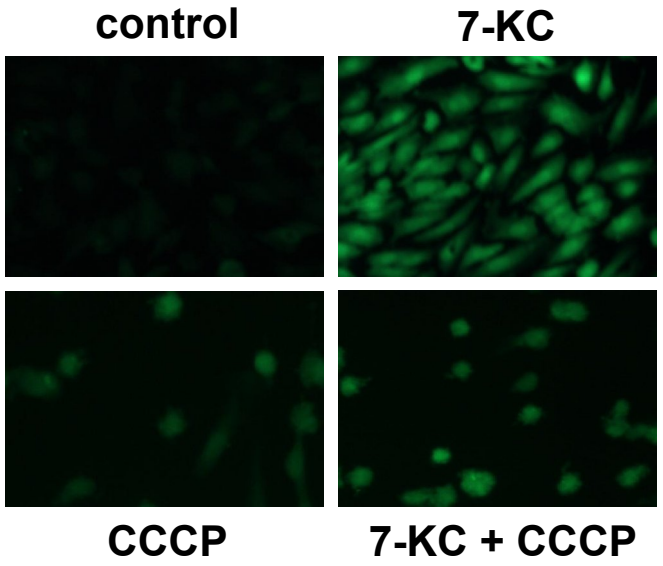
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



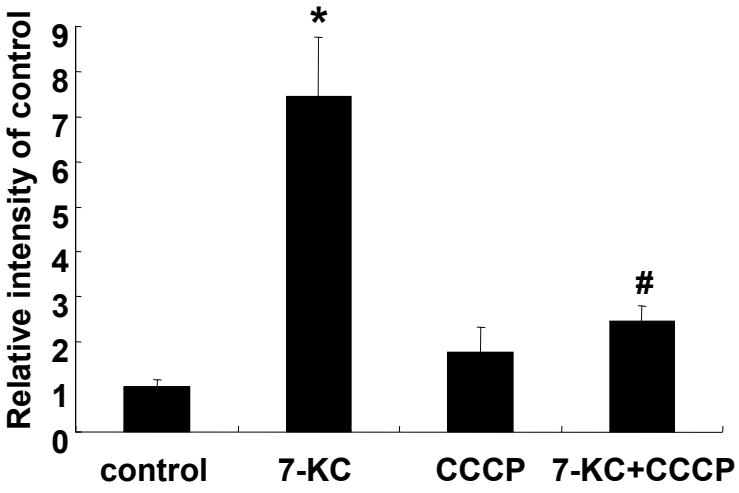


Figure Legends

Supplemental Figure 1

Effects of 7-ketocholesterol (7-KC) on eNOS mRNA levels and apoptosis. HAECs were incubated with 7-KC (1–40 μg/mL) for 16 h. (a) eNOS mRNA levels were determined by quantitative real-time PCR. (**b**) Apoptotic cells were determined by annexin V staining. The results are represented as mean±S.E. $^{*}P<0.05$ vs. control.

Supplemental Figure 2

Effects of 7-ketocholesterol (7-KC) on IL-6, MCP-1, Insig 1 and LDL receptor mRNA levels. HAECs were incubated with 7-KC (10–20 μg/mL) for 16 h. IL-6, MCP-1, Insig 1 and LDL receptor mRNA levels were determined by quantitative real-time PCR. The results are represented as mean±S.E. $^{*}P<0.05$ vs. control.

Supplemental Figure 3

Effects of ABCG1 and HDL on ROS production by 7-ketocholesterol (7-KC) in mouse EC. Mouse ECs were isolated from aortas in WT and *Abcg1*$^{-/-}$ mice. Mouse aortic ECs were incubated with 7-KC (10 μg/mL) in the presence or absence of HDL (100 μg/mL) for 16 h. Intracellular ROS was determined after 30 min of pulse, using CM-H_2DCFDA. The results are represented as mean±S.E. $^{*}P<0.05$ vs. control. $^{\#}P<0.05$ vs. 7-KC alone.

Supplemental Figure 4

Effect of carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) on ROS production by 7-ketocholesterol (7-KC). HAECs were incubated with 7-KC (10 μg/mL) for 16 h. CCCP was added 4 h before intracellular ROS determination. Intracellular ROS was determined after 30 min of pulse, using CM-H_2DCFDA. The results are represented as mean±S.E. $^{*}P<0.05$ vs. control. $^{\#}P<0.05$ vs. 7-KC alone.

Materials and Method

Quantitative real-time PCR analysis

Total RNA was isolated from HAECs using RNeasy Minikit (Qiagen, Valencia, CA). cDNA was synthesized from 1 μg of total RNA with SuperScript reverse transcriptase (Life Technologies, Inc.). Real-time PCR was performed as described previously (61). Expression levels of human eNOS, IL-6, MCP-1, Insig1, LDL receptor and β-actin mRNAs were determined using the specific primers as follows: forward eNOS (5'-GAAGAGGAAGGAGTCCAGTAACACAGAC-3') and reverse eNOS (5'-GGACTTGCTGCTTTGCAGGTTTTC-3'), forward IL-6 (5'-CCCTGAGAAAGGAGACATGTAACA-3') and reverse IL-6 (5'-ACCAGGCAAGTCTCCTCATTGA-3'), forward MCP-1 (5'-GAAGAGGAAGGAGTCCAGTAACACAGAC-3') and reverse MCP-1 (5'-GGACTTGCTGCTTTGCAGGTTTTC-3'), forward Insig1 (5'-GGACGACAGTTAGCTATGGGTGTT-3') and reverse Insig1 (5'-GAGTCATTTGTACAGTCAGCCCGA-3'), forward LDL receptor (5'-CGTGCTTGTCTGTCACCTGCAAAT-3') and reverse LDL receptor (5'-AGAACTGAGGAATGCAGCGGTTGA-3'), and forward β-actin (5'-CCAGGATCAAGGTCGGAAAG-3') and reverse β-actin (5'-GCATCGGACTTGCAGACCA-3'). All mRNA levels were normalized by β-actin mRNA levels.

Apoptosis assay

HAEC were treated with 7-ketocholesterol (7-KC) for 16 h. Apoptosis assay was performed by staining HAEC with Alexa 488-labeled annexin V using Vybrant Apoptosis Assay kit (Invitrogen). The number of Annexin V-positive cells were counted and expressed as a percentage of the total number of cells in at least three separate fields (containing 1,000 cells) from duplicate wells.