

Supplemental Fig. I. GM-CSF-differentiated macrophages express CD11c. Wild-type bone marrow cells were differentiated into macrophages with (A-C) GM-CSF or (D-F) M-CSF. Macrophages were immunostained for (B and E) CD11c or (C and F) CD68 and counterstained with DAPI. (A and D) Phase-contrast images of GM-CSF- or M-CSF-differentiated macrophages. Scale bar = 50 μ m and applies to all micrographs.

Supplemental Fig. II. Cytokine profile of GM-CSF-differentiated macrophages. Wild-type macrophages were incubated without or with 1 mg/ml LDL for 24 h in the presence of GM-CSF. Macrophages were then incubated 24 h with medium containing GM-CSF but without serum or LDL. Supernatants were harvested and assayed as described in the Methods.

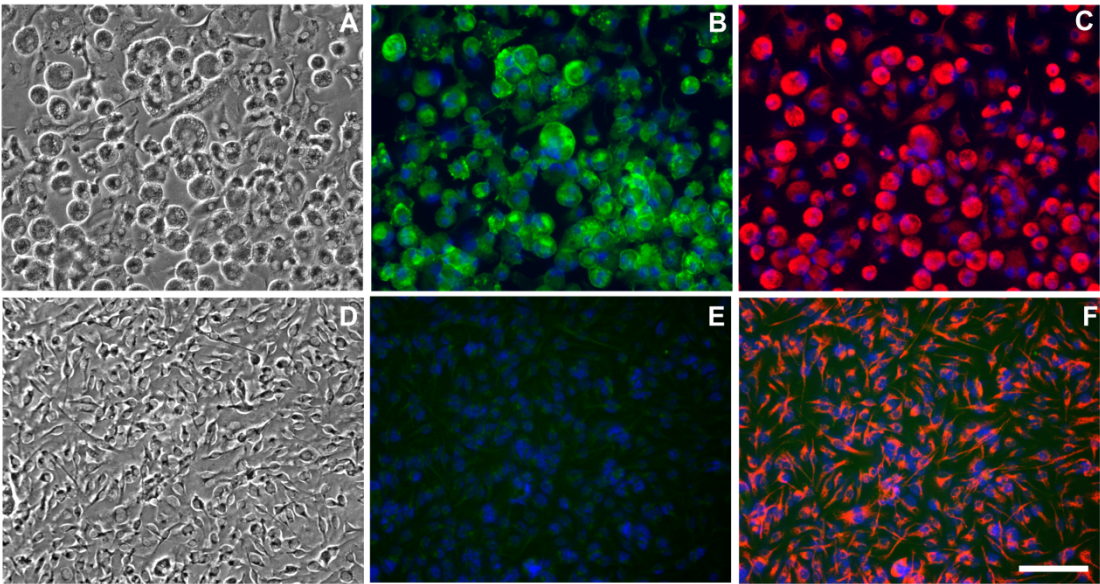
Supplemental Fig. III. Macrophage LDL-derived cholesterol accumulation is constitutive. LDL receptor-null macrophages were incubated with 1 mg/ml LDL for 6 h in the presence or absence of GM-CSF and then cholesterol accumulation was assessed. The basal cholesterol level was 89 nmol/mg cell protein. * = $p < 0.05$.

Supplemental Fig. IV. Macrophage uptake of 125 I-LDL is not affected by excess unlabeled LDL. Wild-type macrophages were incubated 6 h with 25 μ g/ml 125 I-labeled lipoprotein alone or with 500 μ g/ml unlabeled lipoprotein. The incubation media contained GM-CSF but no serum. ns = not statistically different. *** = $p < 0.001$.

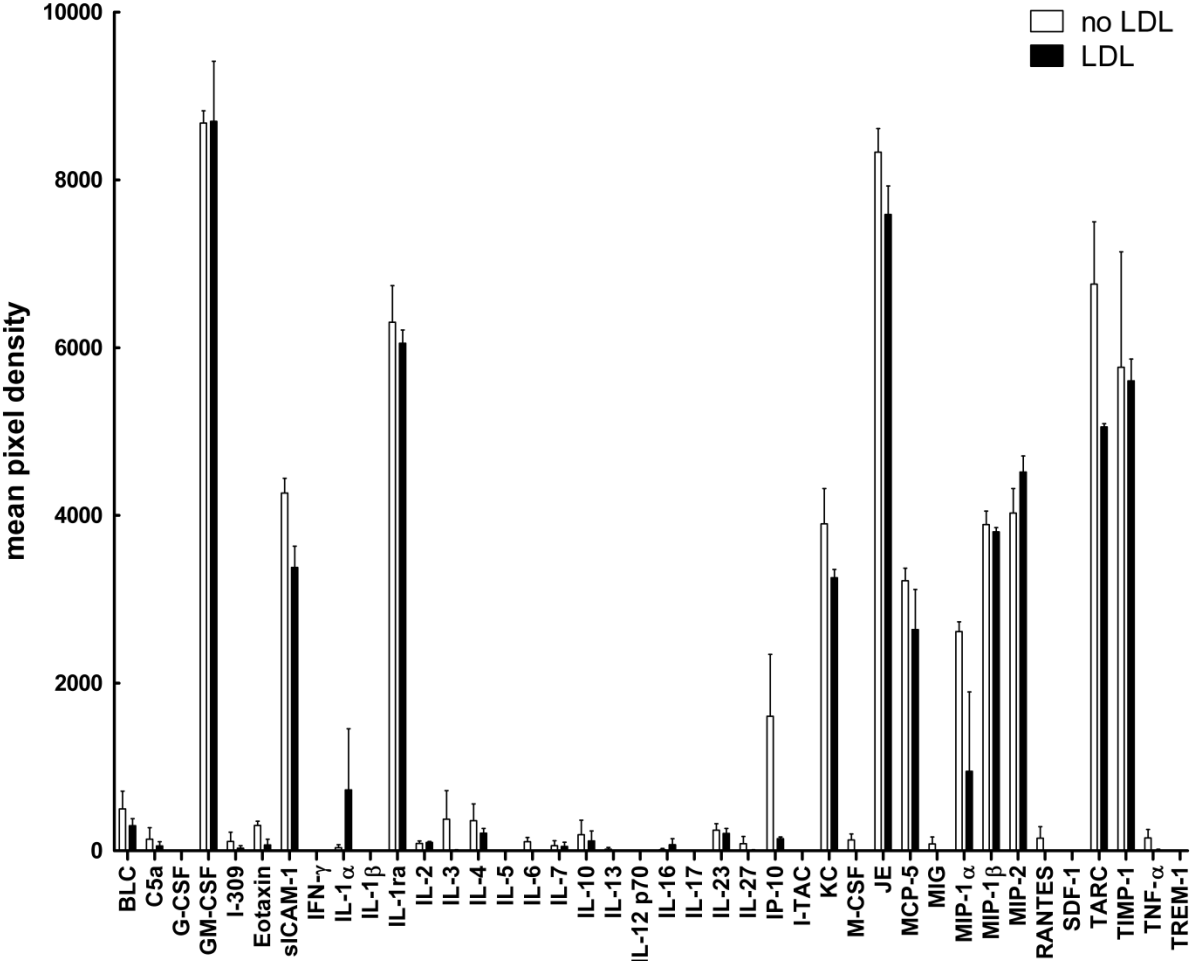
Supplemental Fig. V. Cytochalasin D inhibits LDL-derived cholesterol accumulation but not AcLDL-derived cholesterol accumulation by macrophages. LDL receptor-null macrophages were incubated for 24 h in the presence or absence of 2 μ g/ml cytochalasin D and (A) 1 mg/ml LDL or (B) 25 μ g/ml AcLDL. Cholesterol accumulation was then assessed. The basal cholesterol level was (A) 74 nmol/mg cell protein and (B) 68 nmol/mg cell protein. *** = $p < 0.001$.

Supplemental Fig. VI. Electron microscopy of macrophage uptake of LDL within macropinosomes. LDL receptor-null macrophages were incubated 10 min (A) with 4 mg/ml LDL or (B) without LDL and then LDL was visualized with anti-LDL immunogold labeling. Scale bar = 500 nm and applies to both micrographs.

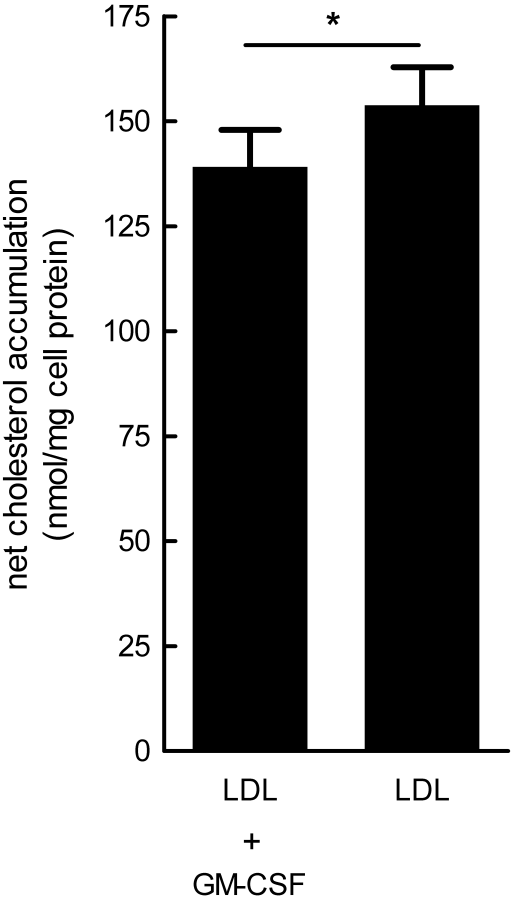
Supplemental Figure I



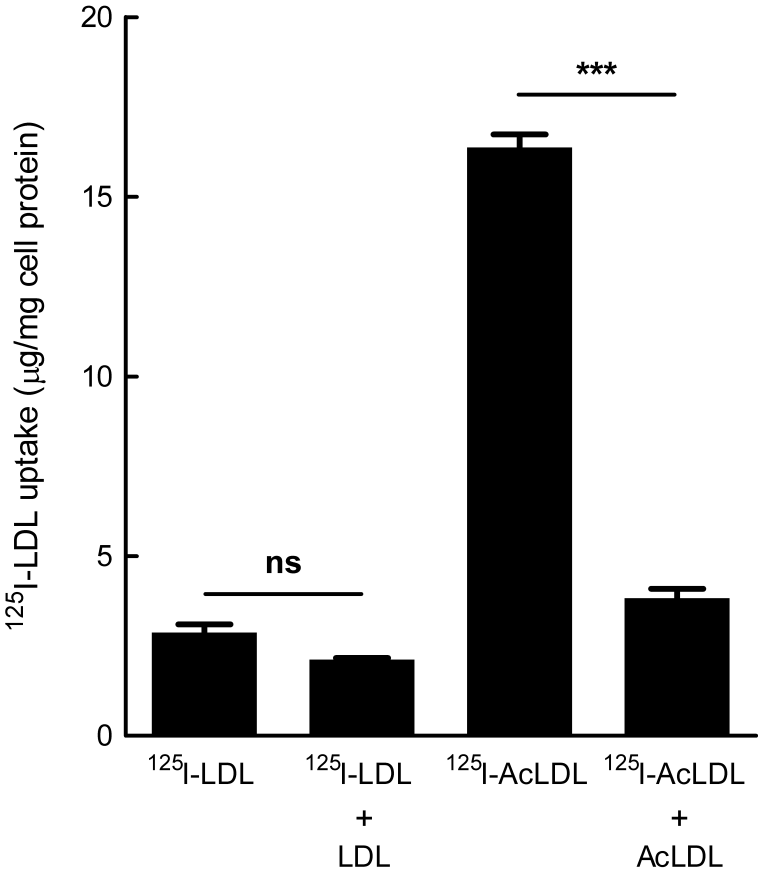
Supplemental Figure II



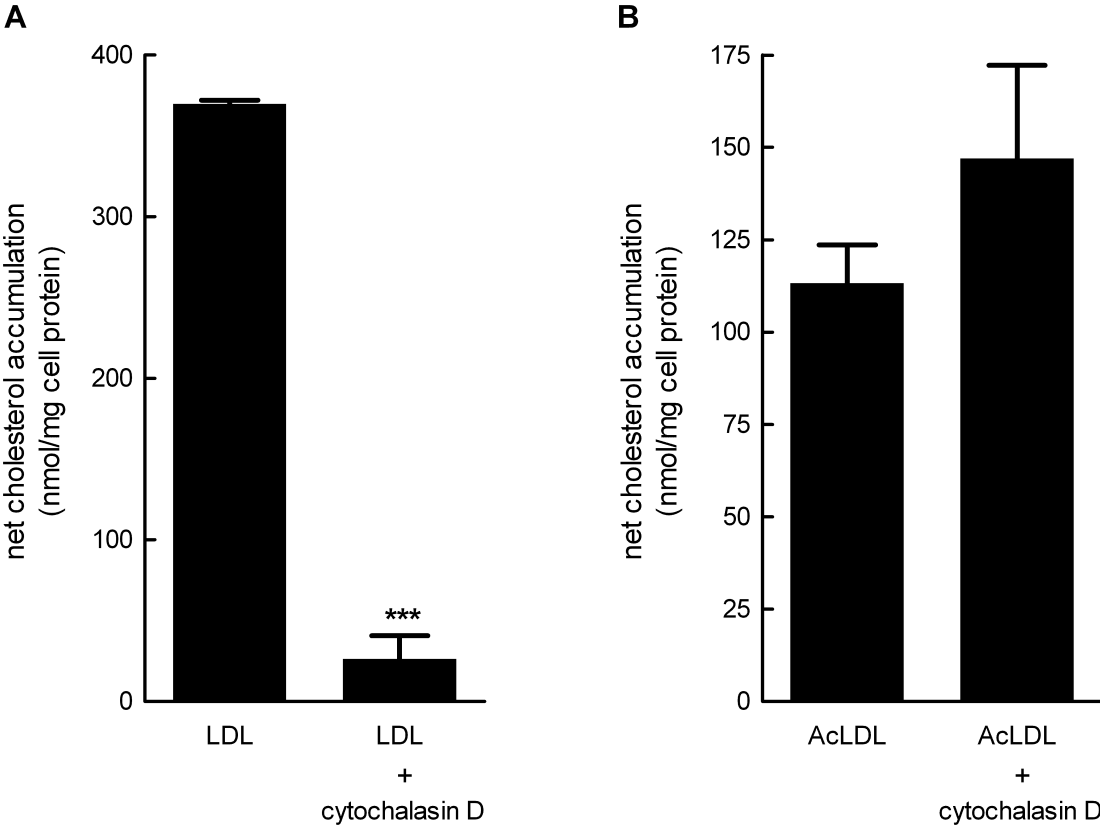
Supplemental Figure III



Supplemental Figure IV



Supplemental Figure V



Supplemental Figure VI

