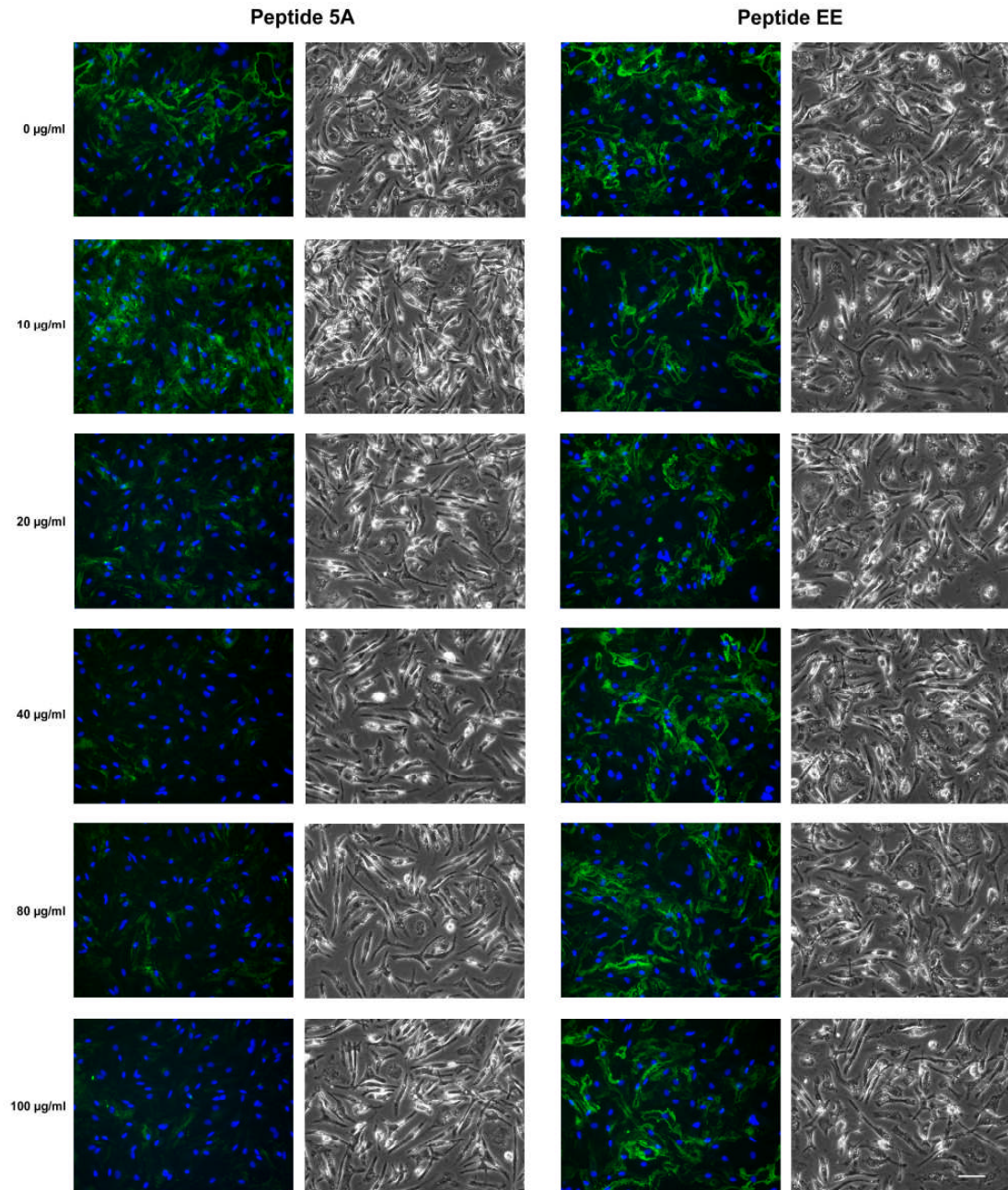
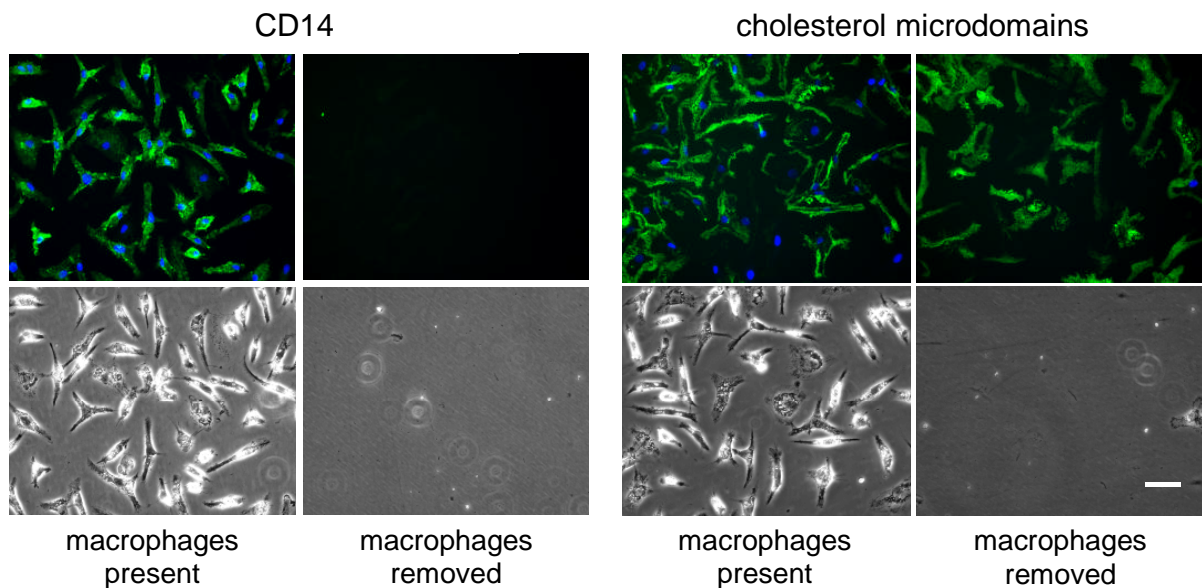


SUPPLEMENTAL MATERIAL

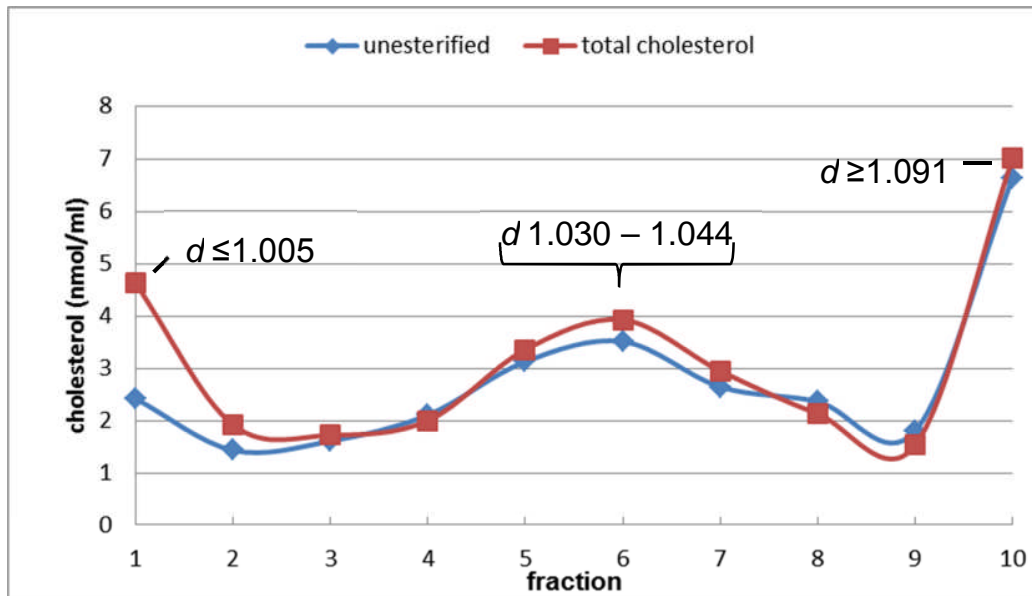
Supplemental Figures



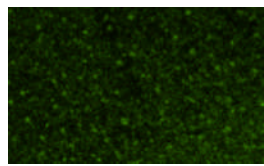
Supplemental Figure I. *Effect of ApoA-I mimetic peptide 5A concentration on mobilization of extracellular cholesterol deposited by human macrophages.* One-week-old human M-CSF differentiated monocyte-derived macrophage cultures were incubated 1 day with 50 µg/ml AcLDL to induce macrophage deposition of extracellular cholesterol microdomains. Following rinsing, macrophage cultures were incubated 1 day with the indicated concentration of either ApoA-I mimetic peptide 5A or control peptide EE without AcLDL. For each peptide, the left-hand column shows cholesterol microdomains visualized by fluorescence microscopy using anti-cholesterol microdomain Mab 58B1 (green), and nuclei imaged with DAPI fluorescence staining (blue). To the right of each fluorescence image is the corresponding phase-contrast microscopic image. Bar = 50 µm and applies to all.



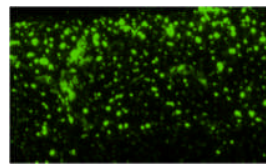
Supplemental Figure II. One-week-old human M-CSF differentiated monocyte-derived macrophage cultures were incubated 2 days with 50 $\mu\text{g/ml}$ AcLDL + 5 μm TO9 to induce macrophage deposition of extracellular cholesterol microdomains. Then, macrophages were removed from one culture and remained in a second culture as labeled. In the upper row, cultures were immunolabeled using either anti-CD14 mouse Mab (left panel-green) or anti-cholesterol microdomain Mab (right panel-green), and nuclei were imaged with DAPI fluorescence staining (blue). In the lower row, macrophages were visualized using phase-contrast microscopy. Upper and lower rows show corresponding sets of microscopic fields. Note that the regions of cholesterol microdomains that surround the attached macrophages and underlie the removed macrophages do not show anti-CD14 immunostaining. Bar = 50 μm and applies to all.



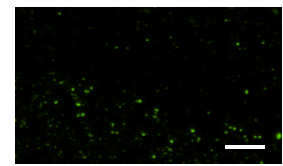
anti-cholesterol
microdomain
Mab58B1
immunostaining



$d \leq 1.005$



$d 1.030 - 1.044$



$d \geq 1.091$

Supplemental Figure III. One-week-old human M-CSF differentiated monocyte-derived macrophage cultures were incubated 2 days with 50 $\mu\text{g/ml}$ AcLDL to induce macrophage deposition of extracellular cholesterol microdomains. Then, macrophages were removed from the culture with EDTA. This was followed by recovery of the cholesterol microdomains from the extracellular matrix using trypsin treatment. The cholesterol microdomain material was subjected to density gradient centrifugation to separate the microdomains from other lipids (e.g., AcLDL) that might have been released from the extracellular matrix. Bar = 12.5 μm and applies to all.

Supplemental Table I

Viability of macrophages

| | % Viability \pm SEM |
|---|-----------------------|
| Human macrophage conditions | |
| AcLDL 1d f/b no addition 1d | 94.0 \pm 0.9 |
| AcLDL 1d f/b ApoA-I (20 μ g/ml) 1d | 92.1 \pm 0.9 |
| AcLDL 1d f/b peptide EE (100 μ g/ml) 1d | 96.3 \pm 0.6 |
| AcLDL 1d f/b peptide 5A (100 μ g/ml) 1d | 94.9 \pm 1.2 |
| AcLDL 1d f/b SPH (125 μ g/ml) 1d | 92.3 \pm 1.7 |
| AcLDL 1d f/b 5A-SPH (100 μ g/ml) 1d | 92.4 \pm 0.2 |
| ABCA1+/+ mouse macrophage conditions | |
| AcLDL+TO9 2d f/b no addition 2d | 97.8 \pm 1.7 |
| AcLDL+TO9 2d f/b ApoA-I (20 μ g/ml) 2d | 98.2 \pm 2.8 |
| AcLDL+TO9 2d f/b peptide EE (100 μ g/ml) 2d | 92.0 \pm 0.3 |
| AcLDL+TO9 2d f/b peptide 5A (100 μ g/ml) 2d | 93.8 \pm 1.1 |
| AcLDL+TO9 2d f/b SPH (125 μ g/ml) 2d | 88.2 \pm 1.6 |
| AcLDL+TO9 2d f/b 5A-SPH (100 μ g/ml) 2d | 94.8 \pm 1.5 |
| ABCA1-/- mouse macrophage conditions | |
| AcLDL+TO9 2d f/b no addition 2d | 92.3 \pm 2.9 |
| AcLDL+TO9 2d f/b ApoA-I (20 μ g/ml) 2d | 97.3 \pm 0.9 |
| AcLDL+TO9 2d f/b peptide EE (100 μ g/ml) 2d | 95.8 \pm 0.6 |
| AcLDL+TO9 2d f/b peptide 5A (100 μ g/ml) 2d | 91.1 \pm 1.5 |
| AcLDL+TO9 2d f/b SPH (125 μ g/ml) 2d | 89.6 \pm 1.7 |
| AcLDL+TO9 2d f/b 5A-SPH (100 μ g/ml) 2d | 91.8 \pm 1.7 |

AcLDL, acetylated low-density lipoprotein; f/b, followed by; ApoA-I, apolipoprotein A-I; SPH, sphingomyelin; 5A-SPH, peptide 5A-sphingomyelin complex; SEM, standard error of the mean.