ApoA-I uptake vs. cholesterol efflux

Supplemental Figure 1: Live cell confocal images of apoA-I uptake by RAW264.7 macrophages. RAW264.7 cells were incubated for 16 h \pm 0.3 mM cAMP to upregulate ABCA1. Then, 10 µg/mL Alexa Fluor-labeled apoA-I (V93C) was added \pm 0.3 mM cAMP for 2 h. These representative confocal images (magnification of 63x) were taken through the approximate center of the cells. The apoA-I label appears red whereas the CD11b outer membrane marker is green.

Supplemental Figure 2: Effects of endocytosis inhibitors on apolipoprotein-mediated ³Hcholesterol efflux and Alexa Fluor-labeled apoA-I cellular uptake. For cholesterol efflux studies, RAW264.7 macrophages were labeled with ³H-cholesterol and pretreated with 0.3 mM cAMP as described in *Methods*. Then cells were incubated with the appropriate concentration of inhibitor for 1.5 h. Medium containing 10 μ g/ml plasma isolated apoA-I, 0.3 mM cAMP and the appropriate concentration of inhibitor were added to the cells for 1.5 h. The cholesterol efflux and cellular uptake measured by confocal microscopy are shown as a fraction of activity in the absence of any inhibitor. Panel A) Amiloride (in DMSO) was used at 1 mM. Monensin (in DMSO) was present at 25 μ M. Panel B) Cytochalasin D (in DMSO) was added at 1 μ M. For the cholesterol efflux studies, * represents a significant difference compared to the untreated cells determined by an unpaired, two-tailed student's t-test (p<0.05). For the cellular uptake studies, ** represents a significant difference from the untreated cells determined by an unpaired, two-tailed student's t-test (p<0.0005). Error bars represent 1 S.D. of at least triplicate samples from one of two representative experiments.

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Supplemental Figure 1



- cAMP

+ cAMP

Supplemental Figure 2:

A)



B)

