Supplemental Figures:

Figure S1. Characterization of Cy3.5-apoA-I. ***A)***, ABCA1-expressing BHK cells were incubated with 5 μg/ml Cy3.5-apoA-I alone (i) or 5 μg/ml Cy3.5-apoA-I plus 25 μg/ml unlabeled apoA-I as competitor (ii) for 30 min and analyzed by confocal microscopy. Cy3.5-apoA-I was also analyzed in non-induced ABCA1-BHK (iii) and induced Mock-BHK (iv) cells. ***B)***, RAW macrophages uninduced (ii) or induced (i) with 0.3 mM 8Br-cAMP for 20 h to express endogenous ABCA1 were submitted to Cy3.5apoA-I incubation and revealed as in ***A). C)*** Mock- and ABCA1-BHK cells were labeled with 1 μCi/mL ^{3}H-cholesterol for 24 h followed by 20 h mifepristone induction. Cholesterol efflux was performed using 5 μg/ml of either apoA-I or Cy3.5-apoA-I for 6 h. ***D),*** Cy3.5-apoA-I generates nascent HDL particles of sizes comparable to ^{125}I-apoA-I, as analyzed by 2D-gels. After 2h of incubation with either 5μg/mL Cy3.5-apoA-I or ^{125}I-apoA-I, media were concentrated and loaded on 2D gels. Cy3.5-fluorescence was detected using a Typhoon phosphoimager while ^{125}I-radioactivity was directly revealed by autoradiography.

Figure S2. Native and Cy3.5-labeled apoA-I reach the lysosome. ***A),*** ABCA1-expressing BHK cells were co-incubated with 5 μg/ml native apoA-I and 0.1μM LysoTracker Red for 1 h, followed by detection using a primary antibody and an Alexa488-coupled secondary antibody (green). ***B)***, Induced RAW264.7 macrophages were co-incubated with 5 μg/ml Cy3.5-apoA-I and 0.1 μM LysoTracker Green for 5 min, followed by a 1 h chase with LysoTracker Green. ***C)***, Cy3.5-apoA-I colocalizes with lysotracker in BHK cells expressing ABCA1 (BHK-ABCA1), in human skin fibroblasts induced with 22(R)-hydroxycholesterol and 9-*cis*-retinoic acid (HSF), in RAW macrophages induced with 8-Br-cAMP (RAW), and in HepG2 cells (HepG2) after 1h of incubation. The percentage of colocalization was determined as in Figure 2. Please note that mock-transfected BHK cells, uninduced human skin fibroblasts, induced skin fibroblasts from a Tangier patient, and uninduced RAW macrophages failed to bind and internalize significant amount of Cy3.5-apoA-I (not shown).

Figure S3. Sucrose treatment reversibly blocks endocytosis in BHK cells. ***A)*** ABCA1-expressing BHK cells were incubated for 30 min with either 5 μg/ml Cy3.5-apoA-I (*first column*), 10 μg/ml Alexa488-transferrin (*second column*), or 5 mg/ml FITC-Dextran (*third column*) in the absence (*first row*) or presence (*second row*) of 250 mM sucrose. ***B)*** Cells incubated with sucrose for 30 min were washed and incubated for 5, 30 or 120 min in the presence of 5 μg/ml Cy3.5-apoA-I to test for the ability of cells to recover from the treatment. As shown, the endocytosis of Cy3.5-apoA-I resumed after removal of sucrose. ***C)*** ^{3}H-cholesterol efflux was tested for the indicated times after the removal of sucrose from the medium.

Figure S4. **Cytochalasin D does not affect cholesterol efflux in RAW macrophages.** ^{3}H-cholesterol labeled uninduced or induced (+cAMP) RAW cells were pre-incubated in DMEM without or with (+CytD) 6 μM cytochalasin D for 15 min. Cholesterol efflux was performed by incubation with 5 μg/ml of apoA-I with or without 6 μM cytochalasin D for up to 1 h.

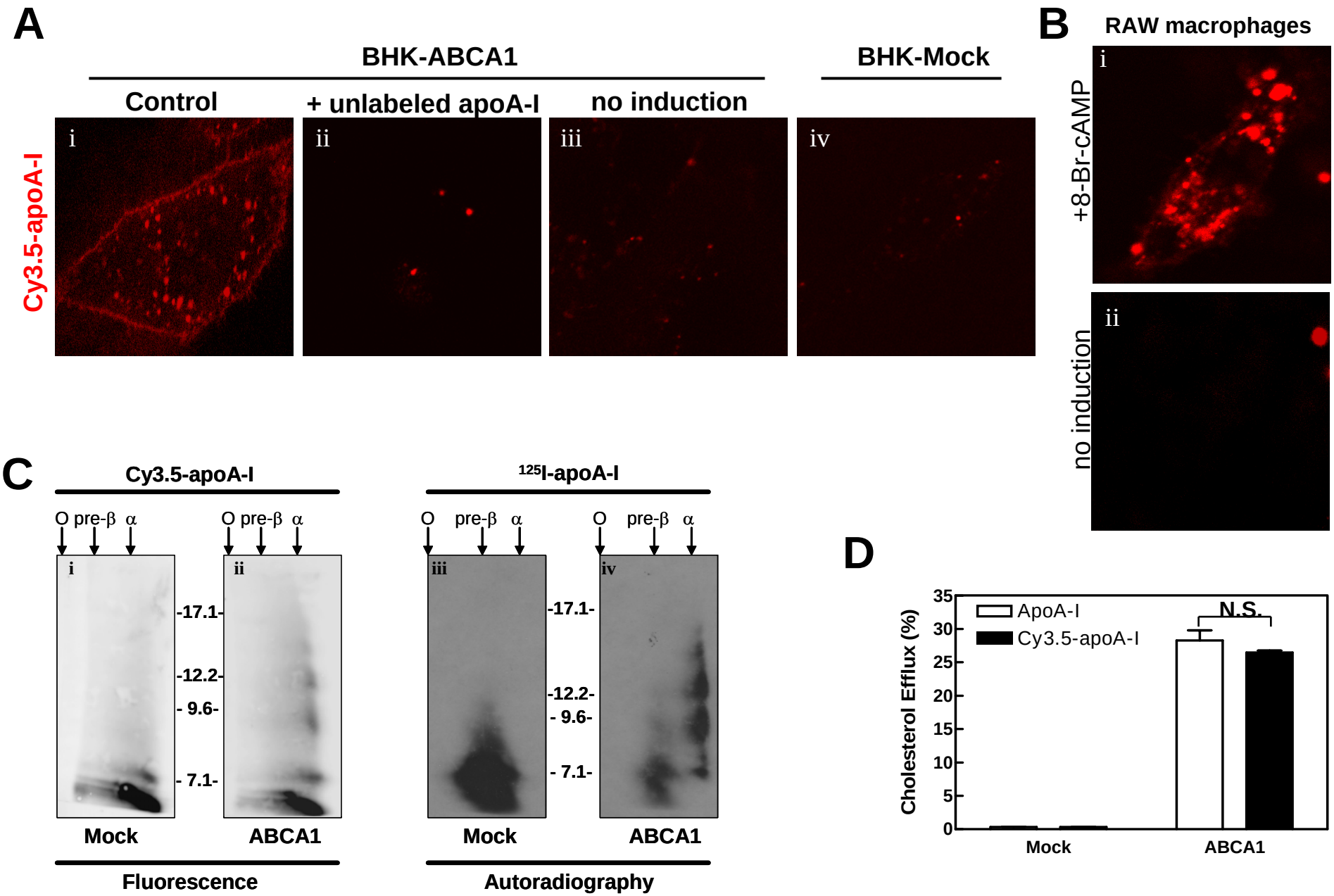


Figure S1, Denis & al.

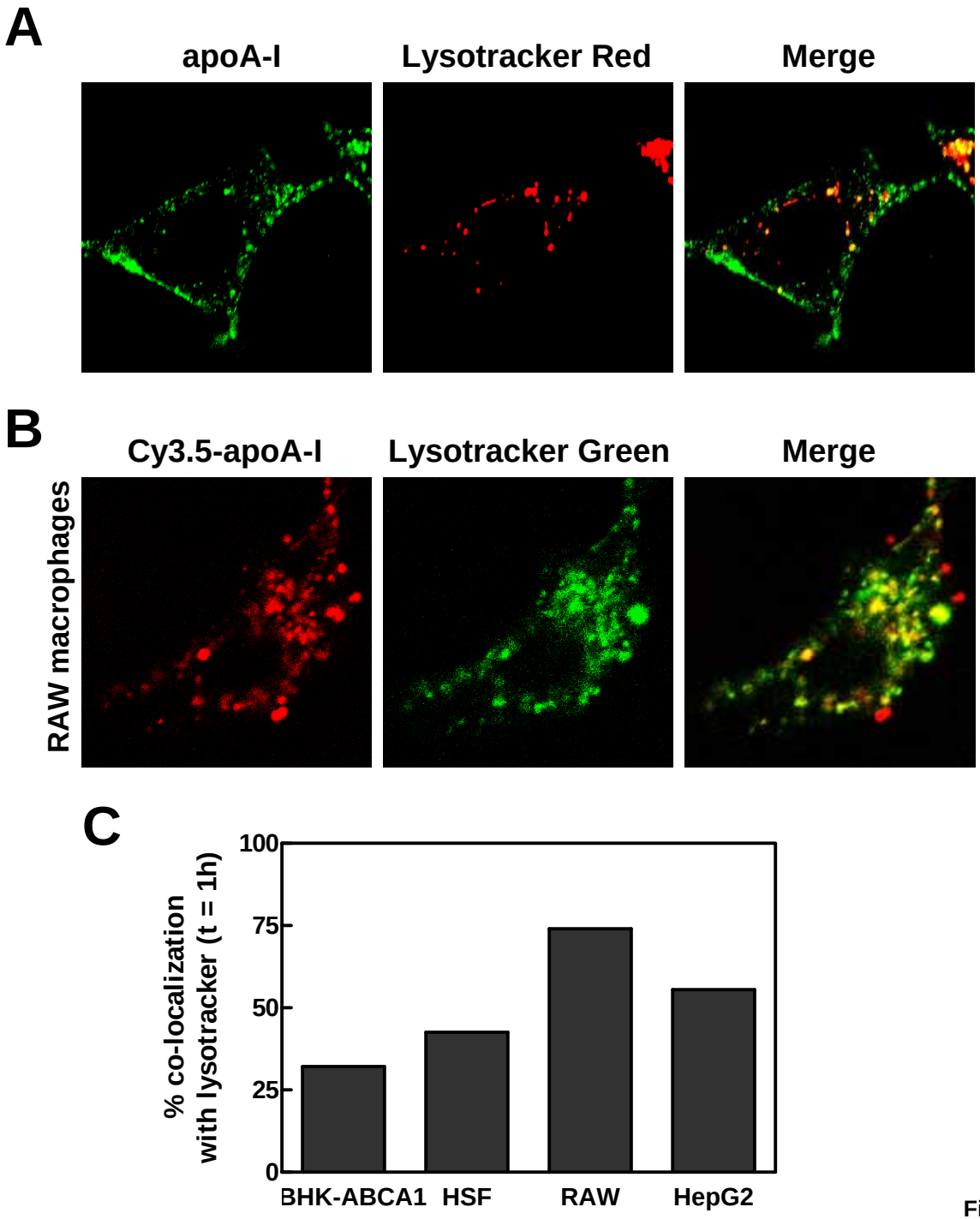
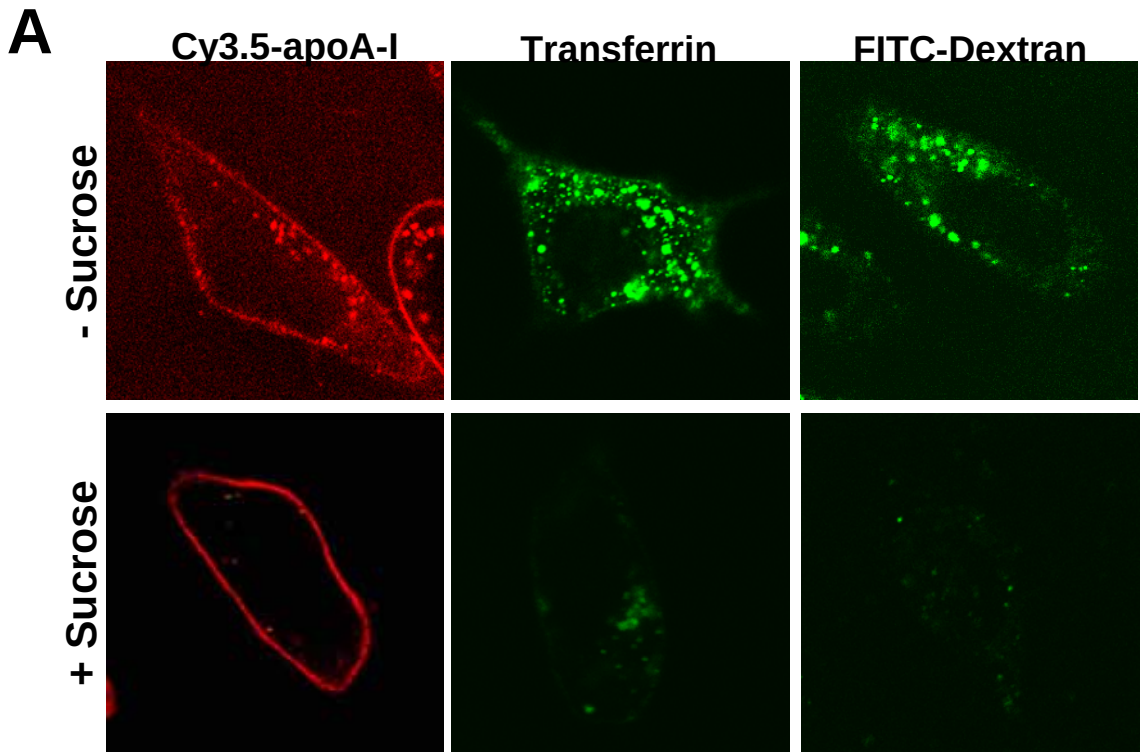
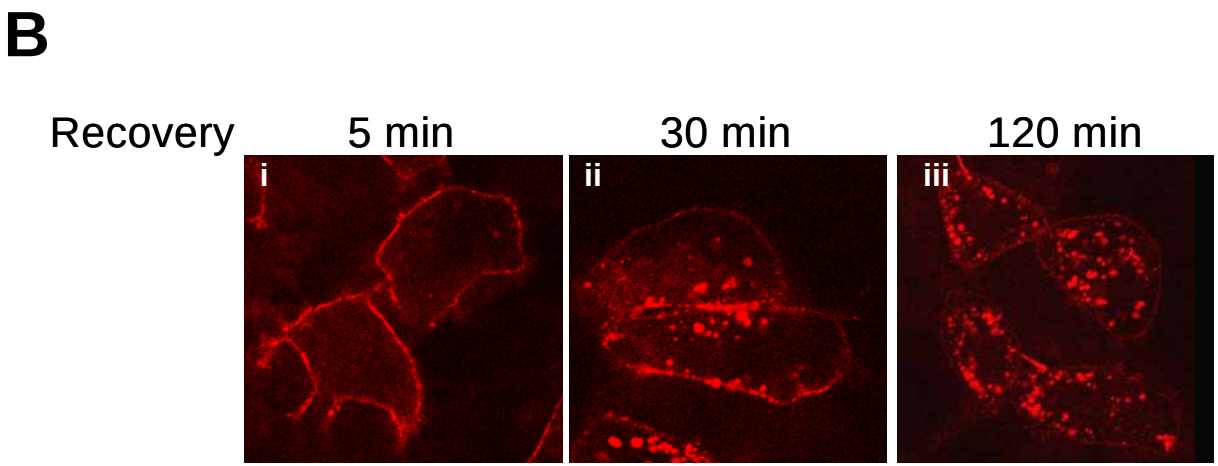


Figure S2 Denis & al.





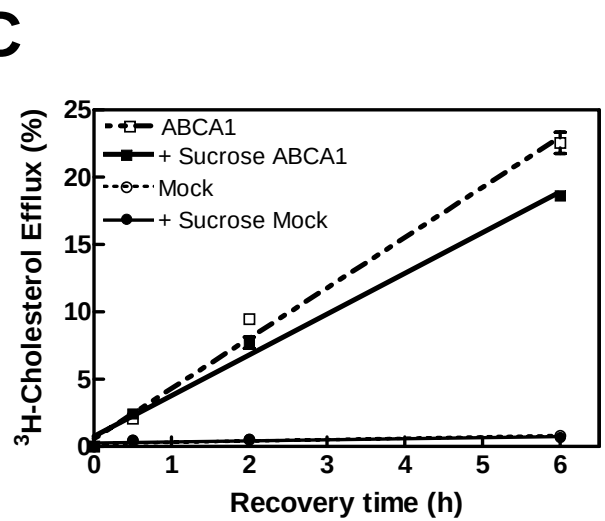


Figure S3, Denis & al.

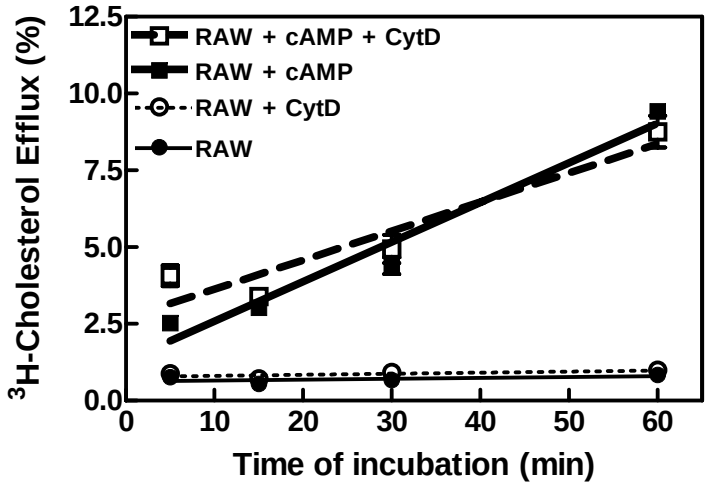


Figure S4, Denis & al.