Supplemental Material

Results

Fractionation of mouse plasma by high-resolution SEC. APOA1 eluted as a single major peak in fractions 8–16 of plasma (the apparent size of mature, lipidated HDL) when it was isolated from wild type mice (**Fig. 1A, Supplemental Material**). A small shoulder of APOA1, likely lipid-free or poorly lipidated APOA1, eluted later (fractions 17–23). Both cholesterol and phospholipids also eluted as a major peak with a small shoulder (which contained phospholipid but very little cholesterol) that co-eluted with APOA1 (**Fig. 1B and 1C, Supplemental Material)**. In APOE-deficient mice, cholesterol eluted in two peaks (data not shown): a major peak that co-eluted with the major peak of APOA1 and exhibited the apparent size of HDL, and a smaller peak that eluted earlier and was consistent with the expected sizes of LDL and VLDL. Phospholipids eluted in three peaks: a major peak that co-eluted with APOA1 and two smaller peaks with the apparent sizes of LDL/VLDL and lipid-free/poorly lipidated APOA1, respectively.

We next quantified the CEC of fractions isolated by SEC from the five strains of mice (**Flg. 2, Supplemental Material**). The wild type fractions that promoted macrophage CEC eluted as a single major peak of material that co-eluted with APOA1 (**Fig. 2A, Supplemental Material**). In striking contrast, plasma exhibited two major peaks of ABCA1 CEC activity. One co-eluted with APOA1, while the other eluted later and had an apparent MW smaller than that of HDL (**Fig. 2B, Supplemental Material**).

To determine the role of APOA1 in CEC, we fractionated plasma from mice deficient in the protein (**Fig. 2C, Supplemental Material**). Macrophage CEC was reduced by 70% in the peak of material that co-eluted with APOA1. It was also smaller in the small shoulder of the early eluting peak of activity, suggesting that lipid-free APOA1 promotes cholesterol efflux. ABCA1 CEC was also reduced (~50%) in the major peak of material that co-eluted with APOA1. However, there was only a modest reduction of ABCA1 CEC in the late-eluting peak of material. Taken together, these observations indicate that HDL particles containing APOA1 are major mediators of macrophage CEC and ABCA1 CEC but that proteins or other factors not associated with APOA1 are also important contributors.

We next investigated the effects of APOE deficiency and APOA1/APOE deficiency on macrophage CEC and ABCA1 CEC (**Fig. 2E-H, Supplemental Material**). In the APOE-deficient mice, macrophage CEC activity eluted as 3 peaks on SEC (**Fig. 2E, Supplemental Material**): a major peak with the expected size of VLDL/LDL; a smaller peak of activity in the HDL size range; and a small, late-eluting peak. Macrophage CEC was markedly higher in the peak in the VLDL/LDL size range than in the HDL size range, likely reflecting a marked increase in APOB-containing lipoproteins in APOE-deficient mice (37). In contrast, the peak with macrophage CEC activity that co-eluted with APOA1 was ~30% smaller in the APOE-deficient mice than in the wild type mice. These observations confirm that APOB-containing lipoproteins can promote macrophage CEC. They also show that lipoproteins with the apparent size of HDL that contain APOE can promote macrophage CEC.

The pattern of ABCA1 CEC exhibited by plasma from APOE-deficient mice fractionated by SEC differed from that shown by macrophage CEC (**Fig. 2F, Supplemental Material**). Only two peaks of material exhibited ABCA1 CEC: one eluted with the apparent size of HDL and the other eluted later. None of the material with ABCA1 CEC activity eluted with the apparent size of VLDL/LDL. ABCA1 CEC activity was lower in the peak of material with the apparent size of HDL but was not decreased in the late-eluting material. These observations confirm that APOB-containing lipoproteins do not promote ABCA1 CEC and that lipoproteins with the apparent size of HDL that contain APOE can also promote ABCA1 CEC. In contrast, APOE deficiency had no

effect on the ABCA1 CEC activity of the material whose apparent size was smaller than that of mature HDL.

When we fractionated plasma from the APOA1/APOE-deficient mice by SEC (**Fig. 2G, Supplemental Material**), we observed a smaller peak of macrophage CEC activity than in the APOE-deficient mice (**Fig. 2E, Supplemental Material**). That peak eluted with the apparent size of VLDL/LDL. The activity in the peak with the apparent size of HDL was markedly lower (90%) than in the wild type mice. The peak also had little ABCA1 CEC activity (**Fig. 2H, Supplemental Material**), suggesting that HDL containing APOA1 and/or APOE promotes efflux mainly by the ABCA1 pathway. Also, the ABCA1 CEC activity of the late-eluting peak was only modestly lower than the activity in the late-eluting peak of the wild type mice.(**Fig. 2H, Supplemental Material**).

In the APOA4-deficient mice, the fractions that promoted macrophage CEC eluted as a single major peak of material that co-eluted with APOA1 (**Fig. 2I, Supplemental Material**) and showed similar activity to the peak from the wild type mice. In contrast, plasma exhibited two major peaks of ABCA1 CEC activity that were moderately reduced compared to those of the wild type mice (**Fig. 2J, Supplemental Material**). Thus, HDL-associated APOA4 does not seem to be a major contributor to macrophage CEC. However, both lipidated and non-lipidated forms of APOA4 can promote cholesterol efflux via ABCA1. Collectively, these data suggest that one or more non-lipidated proteins distinct from APOA1, APOA4, and APOE can promote ABCA1 CEC activity.

Supplemental Figure 1

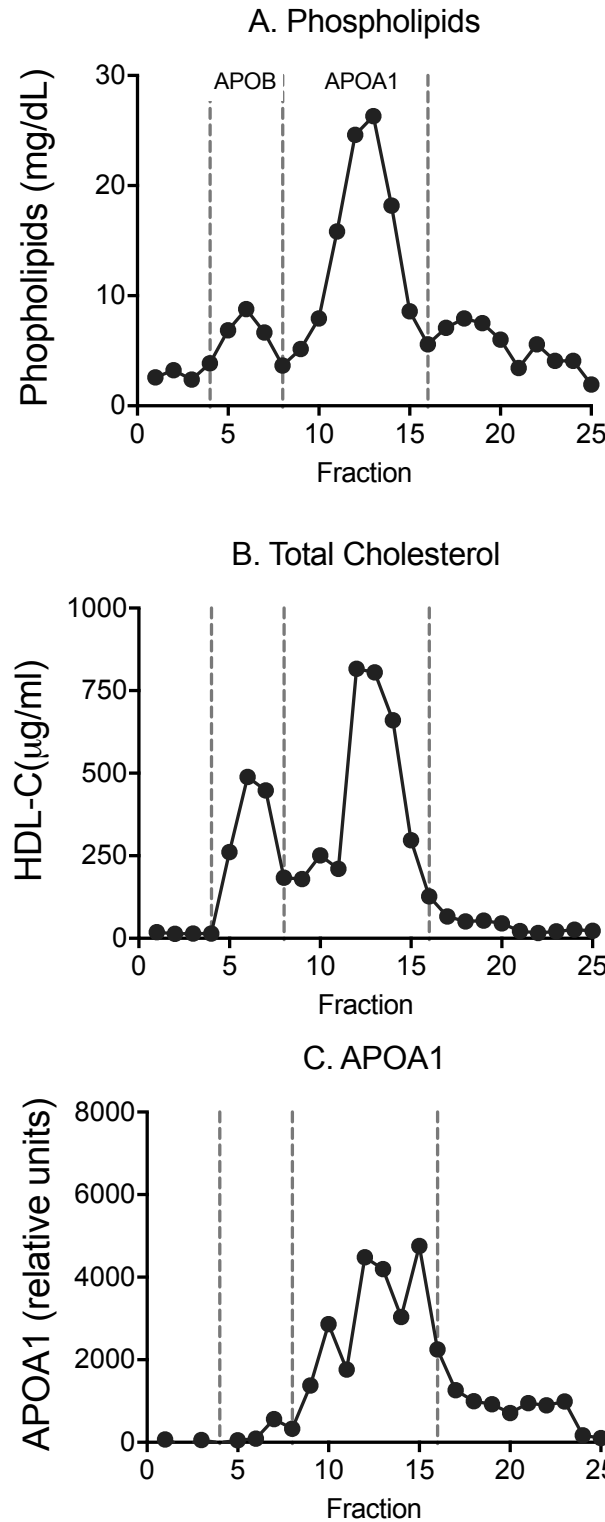


Figure 1. Phospholipid levels, cholesterol levels and APOA1 levels of mouse plasma fractionated by high-resolution size-exclusion chromatography (SEC). Values are averages of duplicate measurements of plasma from 3 different mice.

Supplemental Fig. 2 **Macrophage CEC** **ABCA1 CEC**

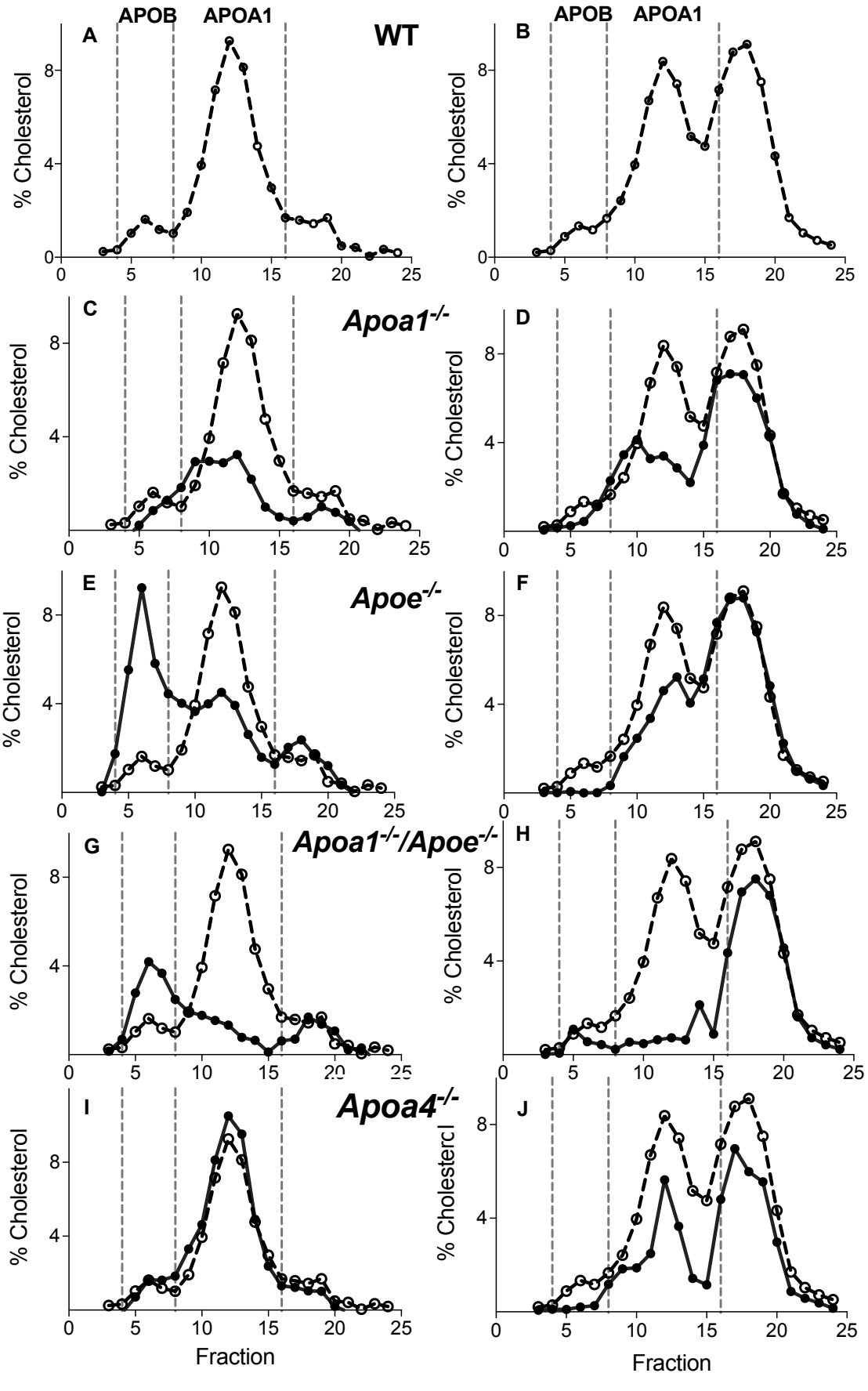


Figure 2. Macrophage CEC and ABCA1 CEC activity of mouse plasma fractionated by high-resolution size exclusion chromatography (SEC). CEC was quantified in fractions isolated from pooled plasma of 5 wild type mice, *Apoa1*$^{-/-}$ mice, *Apoe*$^{-/-}$ mice, *Apoa1*$^{-/-}$;*Apoe*$^{-/-}$ mice, or *Apoa4*$^{-/-}$ mice subjected to SEC. Vertical dotted lines indicate regions of the chromatogram where APOB and APOA1 eluted, as assessed by immunoblotting. Wild type mice (o), apolipoprotein-deficient mice (•).

Supplemental Figure 3

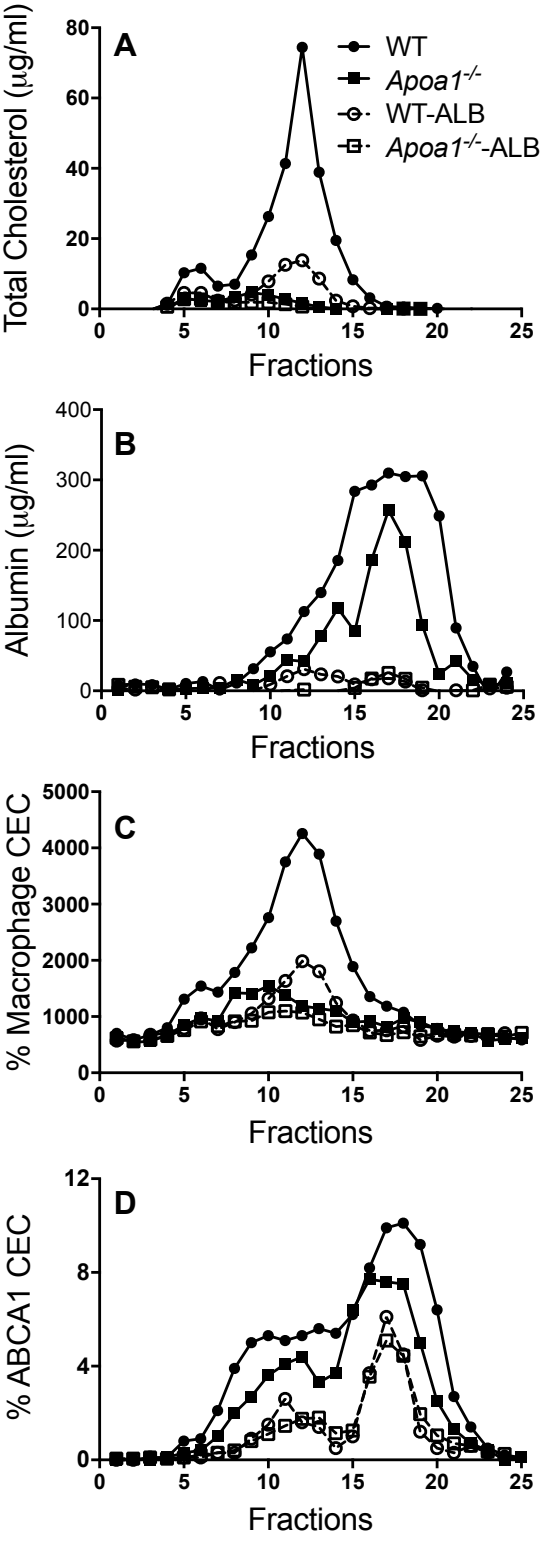


Figure 3. Cholesterol levels, albumin levels, macrophage CEC activity, and ABCA1 CEC activity in albumin-depleted plasma. Plasma from *Apoa1*$^{+/+}$ or *Apoa1*$^{-/-}$ mice was subjected to SEC and each fraction was immuno-depleted of albumin (proteoprep, Sigma, CA). Levels of cholesterol (**A**), albumin (**B**), macrophage CEC (**C**), and ABCA1 CEC (**D**) were quantified in each fraction. Measurements are averages of duplicate determinations from one pooled sample of 3 mice.

Supplemental Figure 4

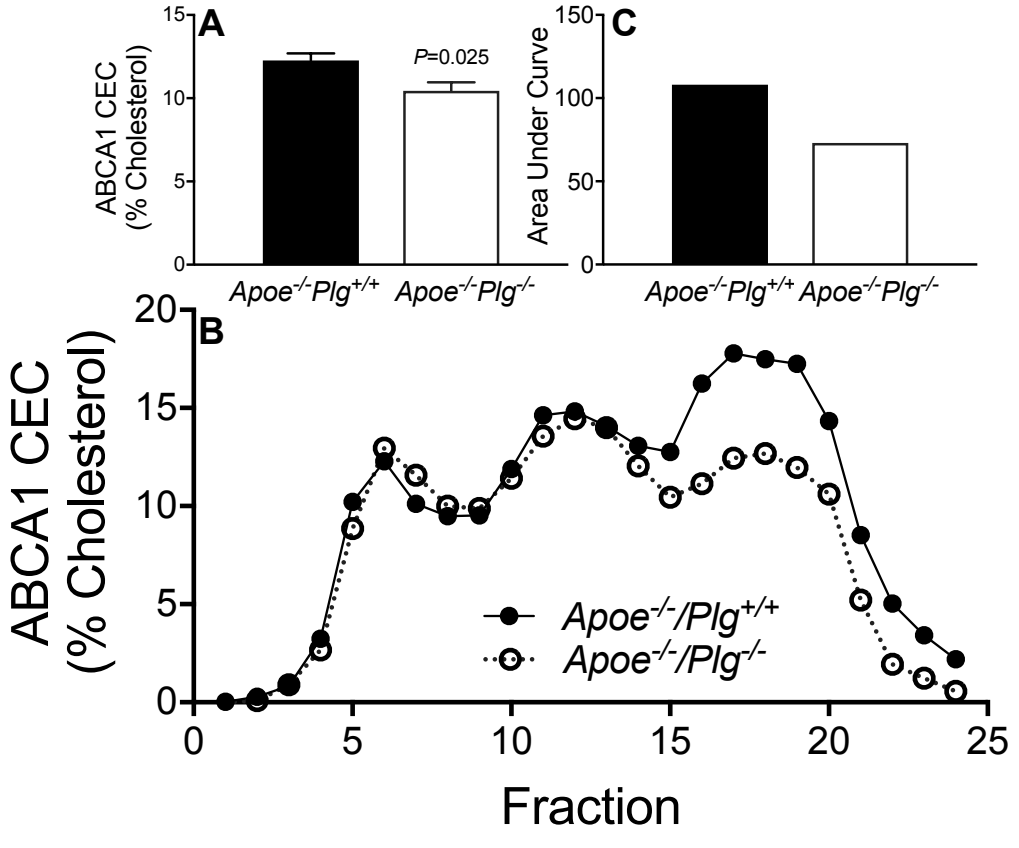
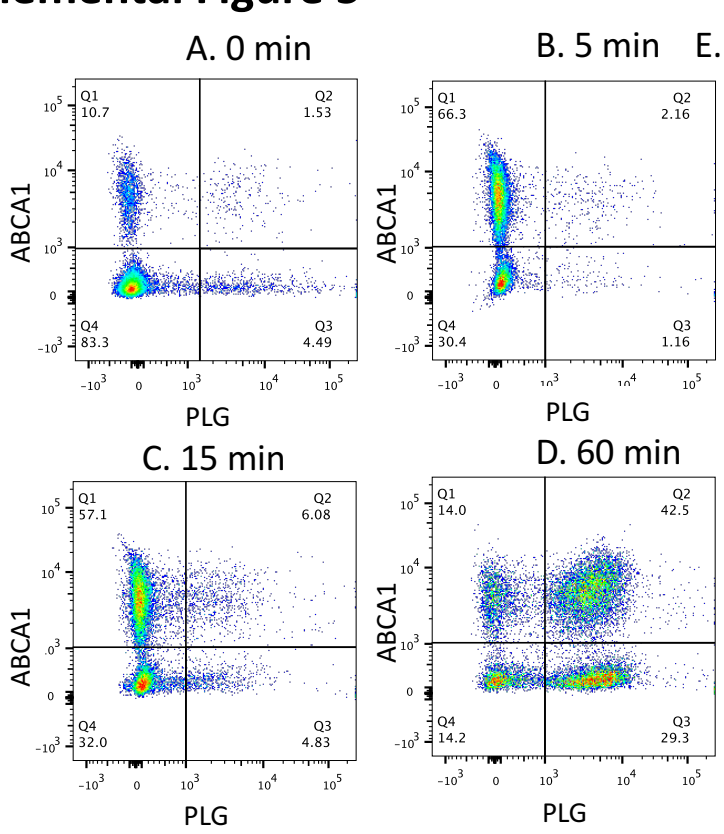


Figure 4. Quantification of ABCA1 CEC activity in plasma of *Apoe*$^{-/-}$ (•) and plasminogen deficient *Apoe*$^{-/-}$ mice (o). ABCA1 CEC activity was quantified using [^{3}H]cholesterol-labeled BHK cells with and without induction of ABCA1 expression. (A) Plasma fractionated by high-resolution SEC. Results are the means of triplicate determinations of pooled plasma obtained by combining equal volumes of plasma from 5 different mice (B) Area-under-the-curve for fractions 15–25 of the data in panel A. HDL eluted in fractions 7–14 of this column.

Supplemental Figure 5



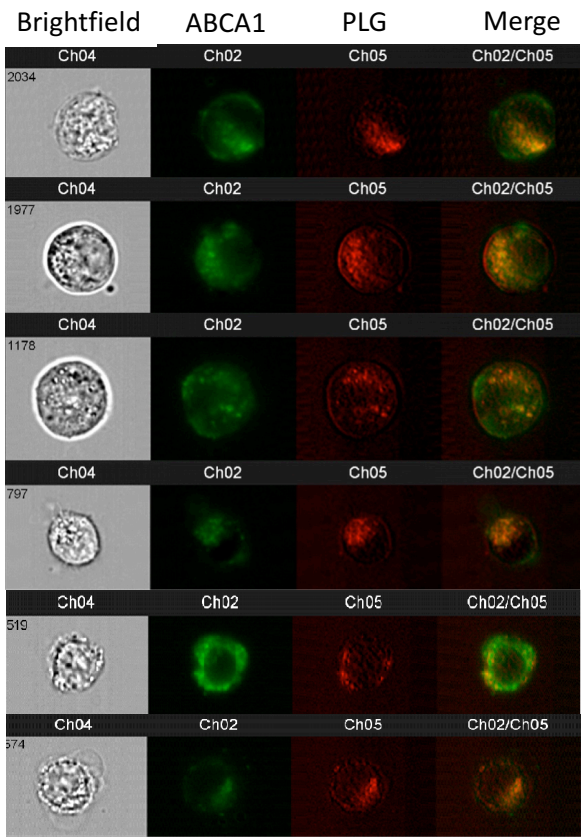
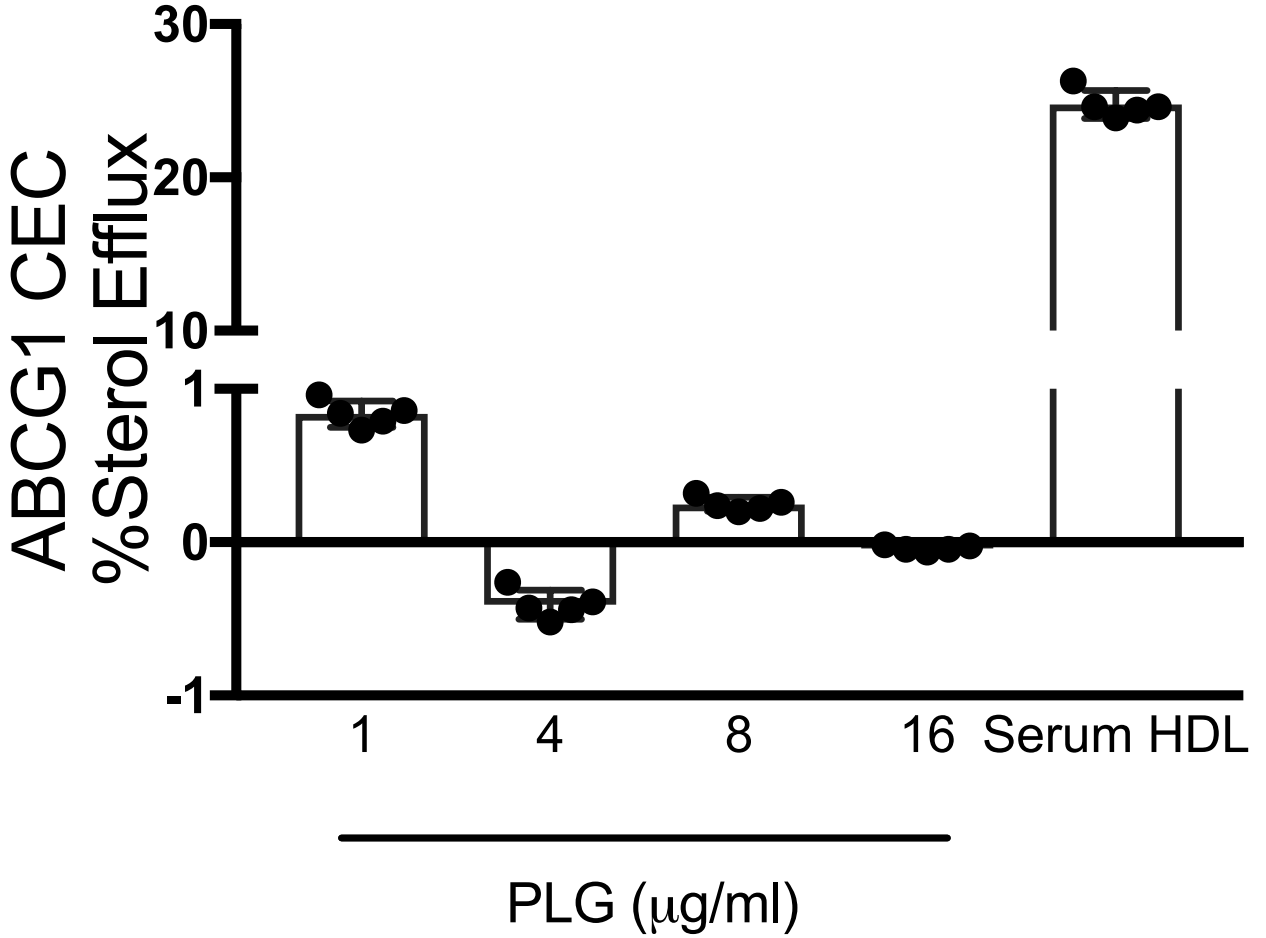


Figure 5. Co-localization of ABCA1 and plasminogen. BHK cells expressing GFP tagged human ABCA1 and not expressing ABCA1 are mixed 1:1 and incubated with 4ug/ml plasminogen for 0(A), 5 (B), 15(C), and 60(D) minutes. Plasminogen primary antibody is detected by APC conjugated secondary antibody. Flow analysis of the cells show and increasing population of cells that are positive for ABCA1 and Plasminogen. The analysis by ImageStream X at 60 minutes (E);green: ABCA1, red: plasminogen, yellow: merge.

Supplemental Figure 6



Supplemental Figure 6. ABCG1-specific CEC (ABCG1 CEC) for plasminogen. ABCG1 CEC of plasminogen and serum HDL were monitored using [^{3}H]cholesterol-labeled baby hamster kidney cells with or without expression of human ABCG1. Each plasminogen concentration is run in duplicate in two independent experiments, serum HDL n=5.