Supplemental Figures for ''Physiological difference in autophagic flux in macrophages from two mouse strains regulates cholesterol ester metabolism''

Peggy Robinet, Brian Ritchey, and Jonathan D. Smith



Supplemental Figure I– AcLDL uptake by macrophages from apoE-deficient AKR and DBA/2 mice. (A) Macrophages from AKR (open bars) or DBA/2 (solid bars) were incubated with DiI-AcLDL and uptake was assessed using flow cytometry. The mean fluorescence intensity±SD of duplicates is shown on the graph. Cells incubated in absence of DiI were used as a control. (B) Macrophages from AKR (open bar) or DBA/2 (solid bar) were incubated with [<sup>3</sup>H]-AcLDL and uptake was assessed by liquid scintillation counting. Results show the mean±SD of duplicates.



**Supplemental Figure II – Bodipy staining of lipid droplets in foam cells from AKR and DBA/2 apoE-deficient aortic root lesion sections.** (A) Lipid droplets in foam cells from aortic root lesions were stained using Bodipy (green) and slides were mounted with Vectashield (Vector Labs) containing DAPI, staining nuclei blue. Images obtained with 63x water immersion lens. (B) The area of the lipid droplets was assessed using Image-Pro plus 7.0 and expressed as percentage of foam cell area (N=4 lesions for AKR and N=3 lesions for DBA/2).



Supplemental Figure III – Mean puncta number per cells in AKR and DBA/2 apo-E deficient macrophages. Cells with  $\geq$ 20 puncta were difficult to count accurately and assigned a value of 20 puncta. Data not normally distributed, lines show median values, not significantly different by non-parametric Mann-Whitney t-test.