Supplemental Information



segment of the caudal vein quantified

Supplemental Figure 1. Peripheral vasculature of a 19 dpf zebrafish larva fed a HCD supplemented with cholesteryl BODIPY 576/589 C11 for 12 days. The panoramic image is composed of several images stitched together in Photoshop. We routinely observe two areas of vascular lipid accumulation, in the posterior cardinal vein and in the caudal vein, and use the latter for quantitative analysis. Scale, 100 μm.



Supplemental Figure 2. Vascular macrophages accumulate lipid in HCD-fed zebrafish. Images of the dorsal aorta (DA) and the caudal vein (CV) of *mpeg1:EGFP* zebrafish in which macrophages are fluorescent green, fed cholesteryl BODIPY 576/589 C11 (red). Scale, 20 μm.

cholesteryl BODIPY 576/589 C11



NBD-cholesterol



Supplemental Figure 3. Peripheral vasculature of a 20 dpf zebrafish larva fed a HCD supplemented with both cholesteryl BODIPY 576/589 C11 (red) and NBD-cholesterol (green) for 14 days. Scale, $25 \mu m$.



Supplemental Figure 4. Diagram of injection site and imaging area. The zebrafish (7 dpf) vasculature diagram is from [Isogai,S., Horiguchi,M., and Weinstein,B.M. 2001. The Vascular Anatomy of the Developing Zebrafish: An Atlas of Embryonic and Early Larval Development. *Developmental Biology* **230**:278-301]



Supplemental Figure 5. IK17-Alexa488 staining of vascular lesions in the dorsal aorta of adult zebrafish. Frozen cross sections of the dorsal aorta of adult zebrafish fed a HCD for 12 weeks were stained with IK17-Alexa488 in the absence and presence of 10 μ g/ml MDA-LDL, and counter stained with DAPI to visualize nuclei. Scale, 5 μ m. Representative images from 12 sections obtained from 3 different animals.



Supplemental Figure 6. Binding of TT-EGFP to tetanus toxin and to modified LDL. HEK293 cells were transiently transfected with scFv TT-EGFP, and 48 hours after transfection, the supernatants were analyzed for binding to MDA-LDL, OxLDL and tetanus toxin in a microplate assay. Two independent experiments were performed in quadruplicates.



Supplemental Figure 7. IK17 Fab and IK17-EGFP zebrafish homogenate binding to MDA-LDL: Competition assay. The homogenous competition assay was performed as described in Methods. The K_d was calculated according to the Klotz method. (A) Binding of IK17 Fab or IK17-EGFP expressing zebrafish homogenate to MDA-LDL immobilized on the plate was competed with various concentrations of MDA-LDL in solution. Mean±SEM of 3-4 independent experiments. (B) Data of panel A presented in double reverse (Klotz's) coordinates. MDA-LDL concentration is in mol/L, assuming m.w. of 500,000 Da. K_d = slope/intercept of the regression line.



Supplemental Figure 8. Effect of heat shock on vascular lipid accumulation in wild type zebrafish larvae. Wild type (AB) zebrafish larvae were fed a HCD supplemented with 10 μ g/g cholesteryl BODIPY 576/589 C11 for 10 days. One group was subjected to heat shock 2 days prior to imaging and the other was not. Area of lipid deposits (red fluorescence) was normalized to the area of the caudal vein segment. Mean±SEM (9 animals per group). #, p=0.68.



Supplemental Figure 9. Effect of probucol on vascular lipid accumulation and IK17-EGFP binding in HCD-fed transgenic larvae – 10 day time point. Images in Figure 4A in the text show examples of vascular lipid accumulation and IK17-EGFP binding in zebrafish larvae fed HCD \pm probucol for <u>5 days</u>. Images in this Supplemental figure are examples from a <u>10 day</u> feeding time point, with two images for the HCD+probucol group. Scale bar, 25 μ m.



Supplemental Figure 10. Effect of probucol on vascular lipid accumulation in non-transgenic zebrafish. Wild type (AB) zebrafish larvae were fed control diet, HCD, or HCD was supplemented with 0.05% probucol for 12 days. Two days prior to imaging, all diets were supplemented with 10 μ g/g cholesteryl BODIPY 576/589 C11. Area of lipid deposits (red fluorescence) was normalized to the area of the caudal vein segment. 10-12 animals per group.



Supplemental Figure 11. Effect of probucol on IK17-EGFP expression in somites. hsp70:IK17-EGFP zebrafish larvae were fed HCD or HCD supplemented with 0.05% probucol for 10 days. Cumulative intensity of EGFP signal in somites was measured (in arbitrary units). Mean±SEM. 5-10 animals in each group.