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Introduction

Endothelial cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) are up-regulated in early inflammation of atherosclerotic disease⁽¹⁾ which remains a major health problem in western societies and causes 50,000 annual deaths in the United States⁽²⁾. A biomarker and fluorescence imaging system enabling early detection of ICAM activity within heart-aorta prior to formation of pathology-detectable atheromatous lesions will greatly facilitate research in this area. Here we developed an anti-human ICAM-1 single domain antibody (sd-11-4) and tagged it with NIR fluorophore Cy5.5 to detect atherosclerosis in ApoE^{-/-} mouse model using a time-domain *in vivo* small animal fluorescent imaging system⁽³⁾.

Materials and Method

Antibody Labeling

Anti-mouse ICAM-1 antibody (mAb-ICAM-1) (Armenian Hamster IgG1k, BD Biosciences) or llama anti-human sd-11-4 were labeled with the fluorophore Cy5.5 NHS ester (BD Bioscience, Canada) (hereafter mAb-ICAM-Cy5.5 or sd-11-4-Cy5.5).

Animals and Diet

ApoE^{-/-} and C57BL/6 mice (Jackson Laboratories, USA) at 4-8 weeks old were fed with a high-fat diet (HFD) containing 21% fat by weight (Harlan Teklad Inc., USA). Four months later, mice were divided in two groups, one was treated with Lipitor (25mg/kg/day) for one month. Mice were housed in a standard facility with free access to water and food. All the procedures were in accordance with the Canadian Council on Animal Care.

In Vivo Time Domain Optical Imaging

Each month, mice were weighed, shaved, and imaged with the small-animal time domain Optix MX2 pre-clinical imager (ART Advanced Research Technologies Inc., Canada). Scans were performed before (pre-injection scan) and 48 hours after tail vein injection of mAb-ICAM-Cy5.5 or sd-11-4-Cy5.5 (1 ug/g body weight, 100 ul total volume in saline) using a tail vein catheter (SAI Strategic Applications Inc., USA). A 670 nm pulsed laser diode was used to excite Cy5.5. Imaging was done by raster scan with intervals of 1.5 mm. The data were reconstructed as fluorescence intensity (FI), fluorescence lifetime, and fluorescence concentration maps (Conc) with the OptiView software. Fluorescence life time of 1.7-2.0 ns was used to limit non-specific fluorescence.

Ex Vivo Optical Imaging and Immunohistochemistry

At the end of the experiment, mice were deeply anesthetized and perfused with saline. The heart and aorta were excised and imaged in the Optix system, and then frozen on dry ice. Heart-aortas were sectioned with a cryostat and staining with WGA-FITC (Abcam Inc., USA) and Hoechst. Images were acquired using a confocal microscope.

Micro-CT Procedure

Mice were scanned with a micro-CT system (Skyscan 1076, Belgium) at 49 kV and 200 uA using a 0.5 mm aluminum filter. During acquisition, two frames were taken every 0.60° over a 180° step-wise rotation. Nrecon software was used for reconstruction.

Results

1. Atherosclerosis Detection Using sd-11-4-Cy5.5

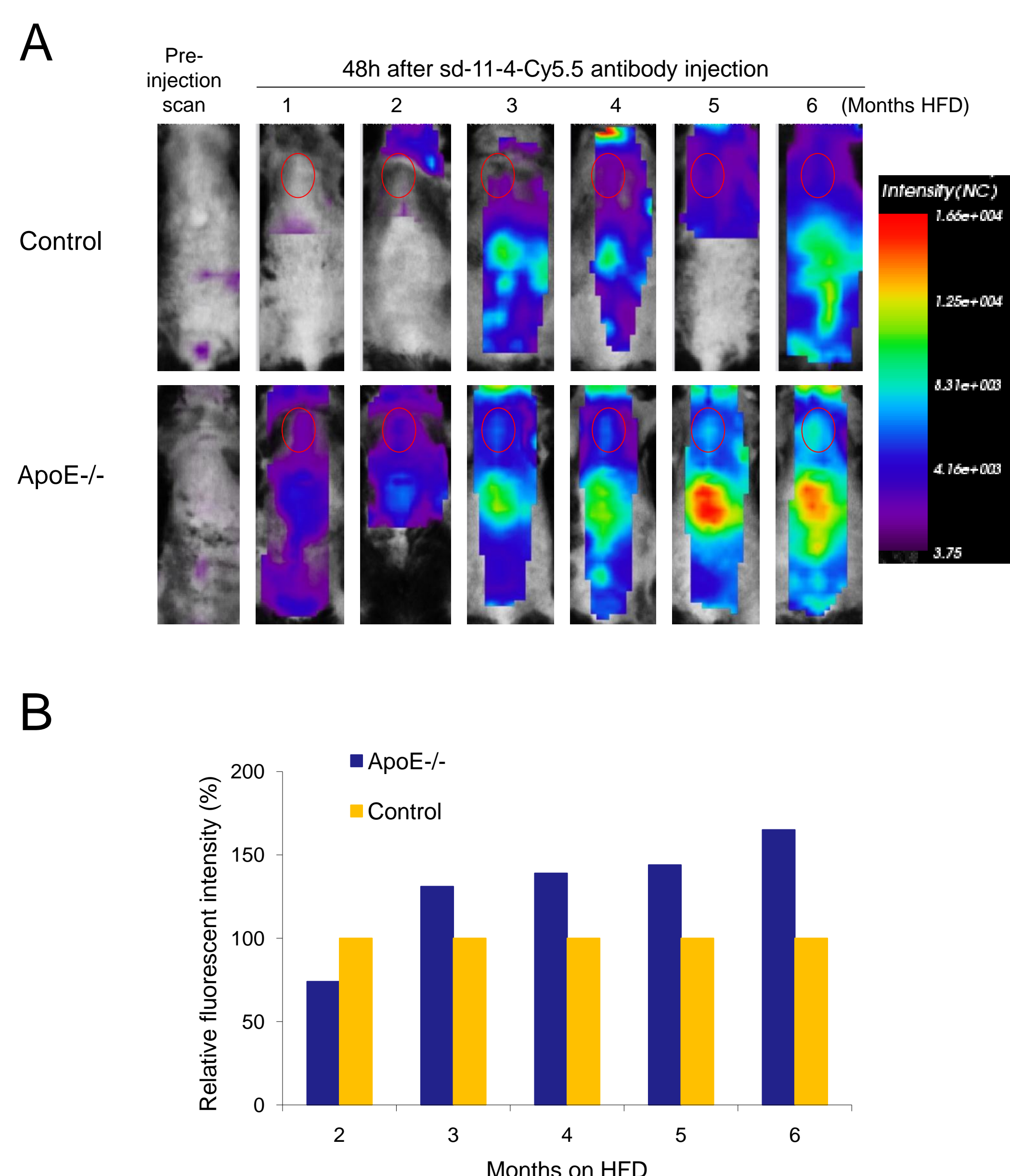


Figure 1. A) Representative fluorescence images of male ApoE^{-/-} and control mice. A strong fluorescent signal on the thorax center (heart-aorta area) was detected in ApoE^{-/-} mice, but not in control mice. **B)** Quantitative analysis of fluorescent signal from ApoE^{-/-} mice (n=9) and control mice (n=5).

2. Atherosclerosis Detection with mAb-ICAM-Cy5.5

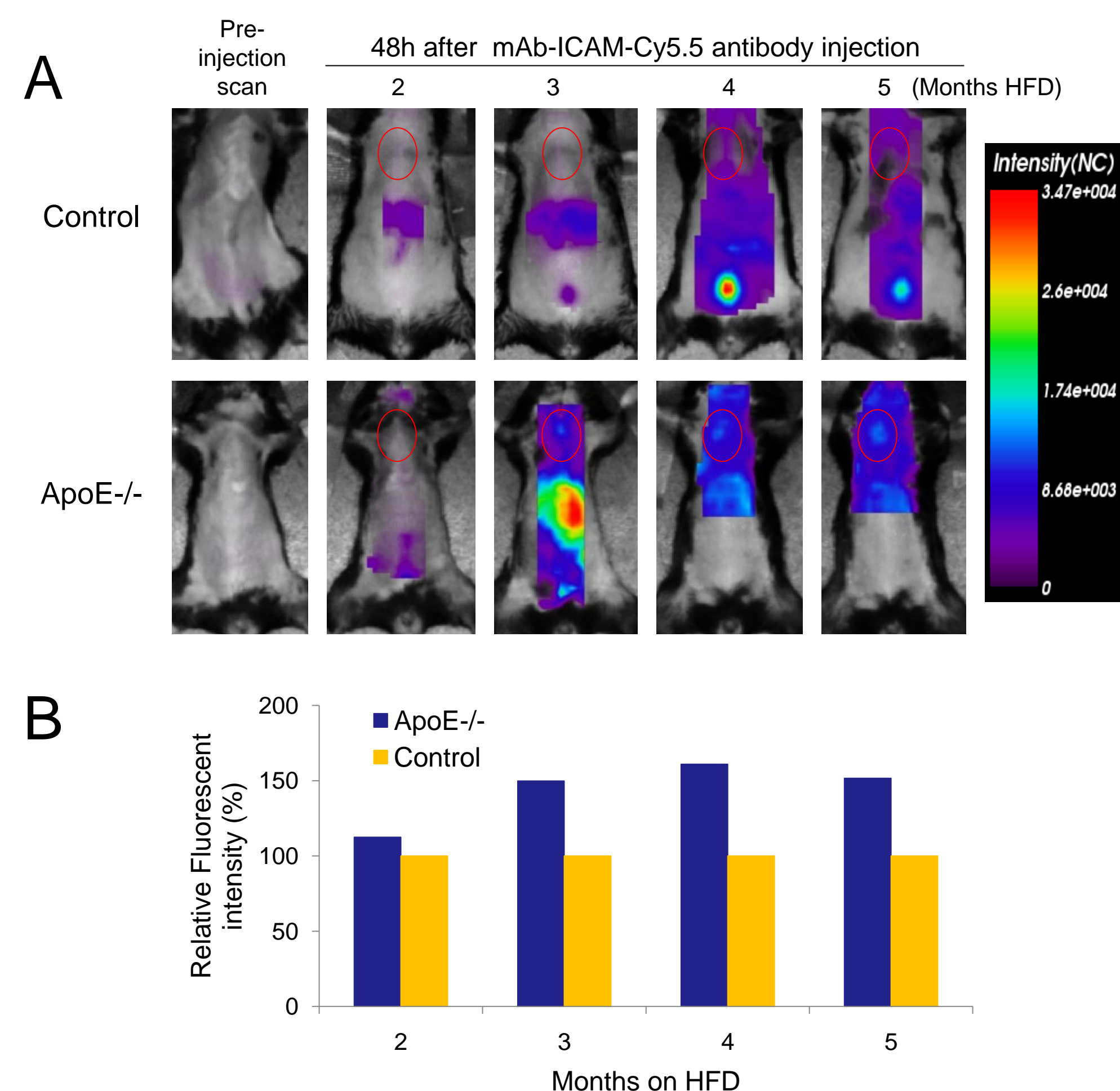


Figure 2. A) Representative fluorescence images of female ApoE^{-/-} and control mice. A strong fluorescent signal on the thorax center (heart-aorta) area was detected in ApoE^{-/-} mice, but not in control mice. **B)** Quantitative analysis of fluorescent signal from ApoE^{-/-} mice (n=8) and control mice (n=5).

3. Fluorescence 3D and Micro-CT Imaging

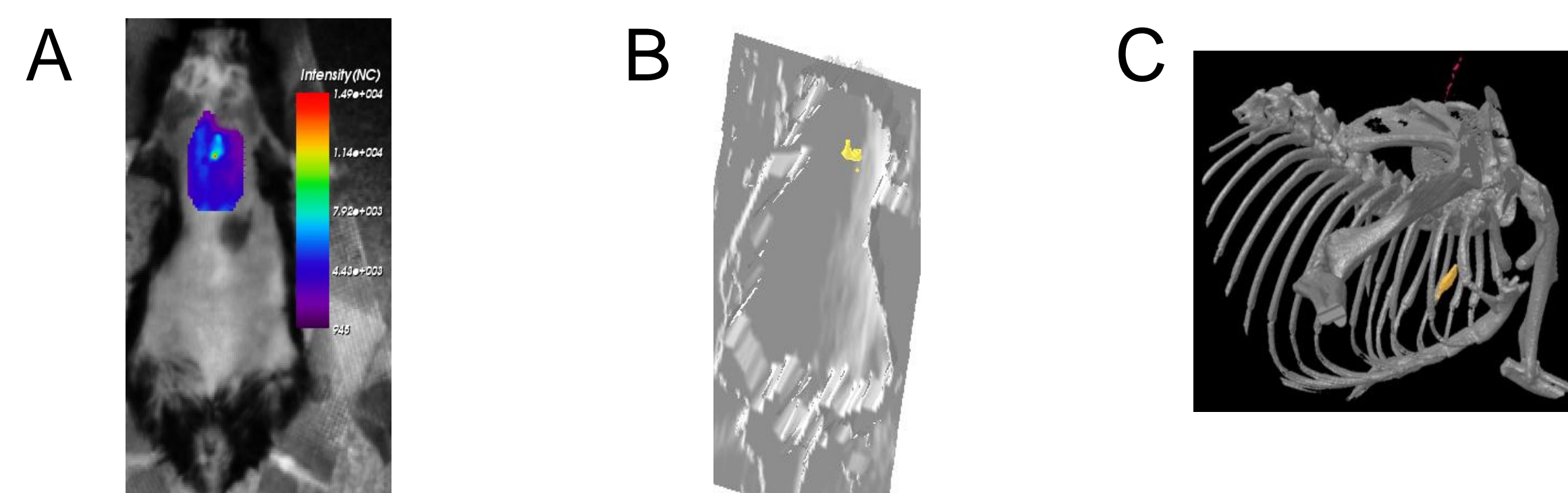


Figure 3. Fluorescence intensity map **(A)** and 3D reconstruction **(B)** of the antibody accumulation the heart-aorta area (orange). **(C)** Micro-CT reconstruction in 3D of the bones and a calcification deposit in the heart-aorta region (orange).

4. Lipitor Treatment

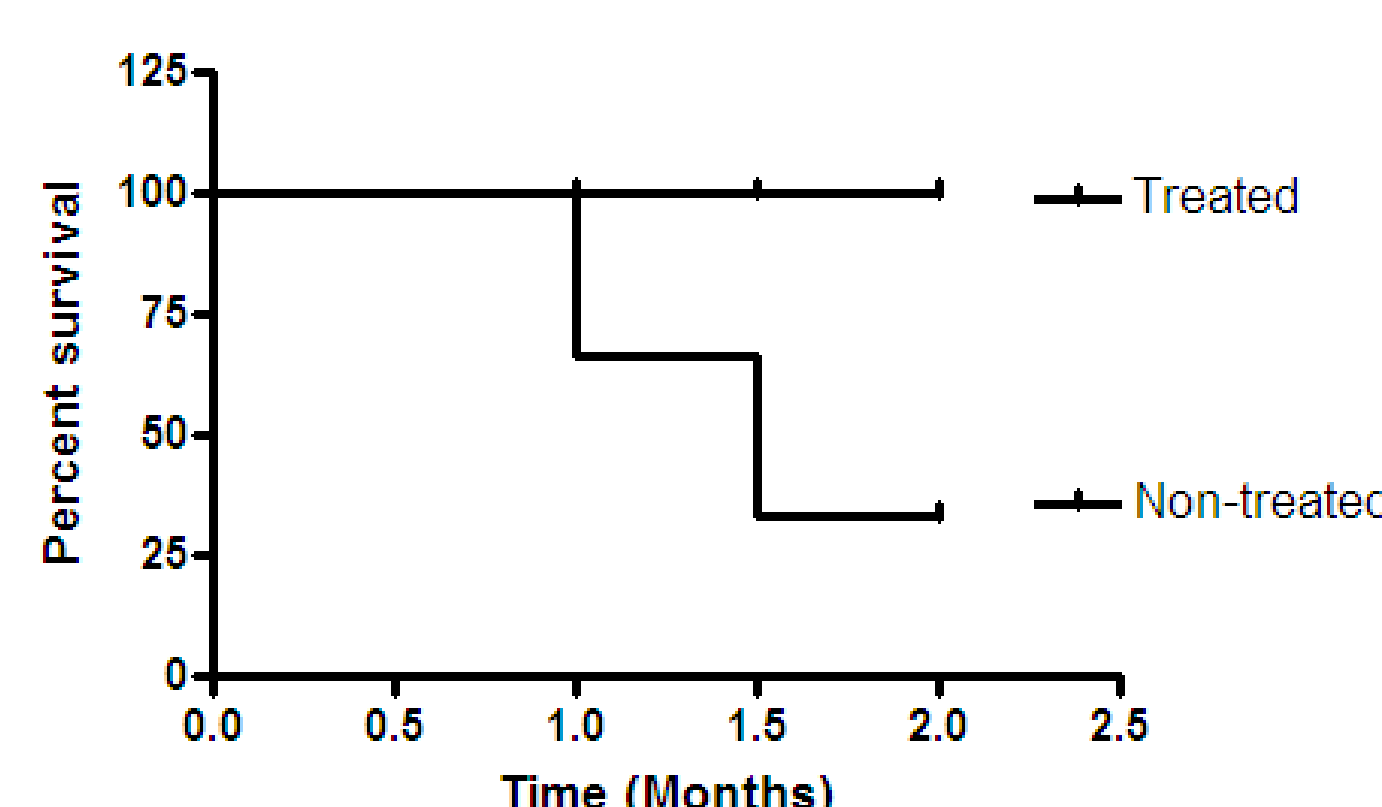


Figure 4. Effect of Lipitor treatment on survival in ApoE^{-/-} mice. Kaplan-Meier analysis revealed a survival benefit in treated ApoE^{-/-} mice. n=3.

5. Ex Vivo Imaging and Immunohistochemistry

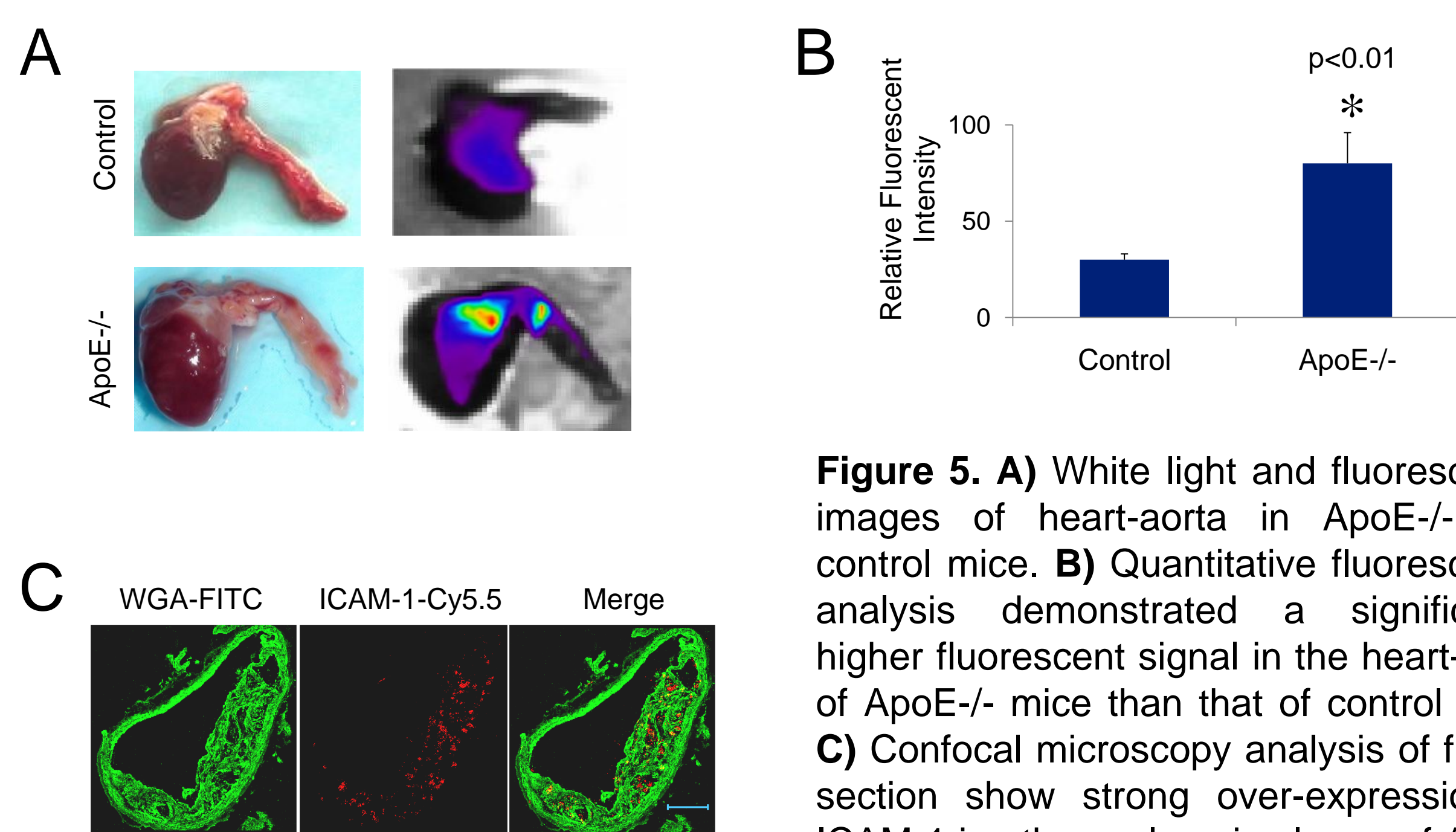


Figure 5. A) White light and fluorescence images of heart-aorta in ApoE^{-/-} and control mice. **B)** Quantitative fluorescence analysis demonstrated a significantly higher fluorescent signal in the heart-aorta of ApoE^{-/-} mice than that of control mice. **C)** Confocal microscopy analysis of frozen section show strong over-expression of ICAM-1 in atherosclerosis plaque of ApoE^{-/-} mice but not in control mice (data not shown). Scale bar : 200 um.

Summary and Conclusions

- Using a new single domain anti-ICAM antibody sd-11-4, we detected *in vivo* atherosclerotic progression in ApoE^{-/-} mice.
- In vivo* fluorescence 3D and micro-CT imaging confirmed atherosclerotic plaques present in heart-aorta position of ApoE^{-/-} mice.
- For the first time, our results revealed sd-11-4 could noninvasively detect atherosclerosis and monitor treatment by prospective optical imaging.

References

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- Ma *et al. Appl. Opt.* 2007. **46**, 1650-1657