

# Establishment of rat model with diet-induced coronary atherosclerosis

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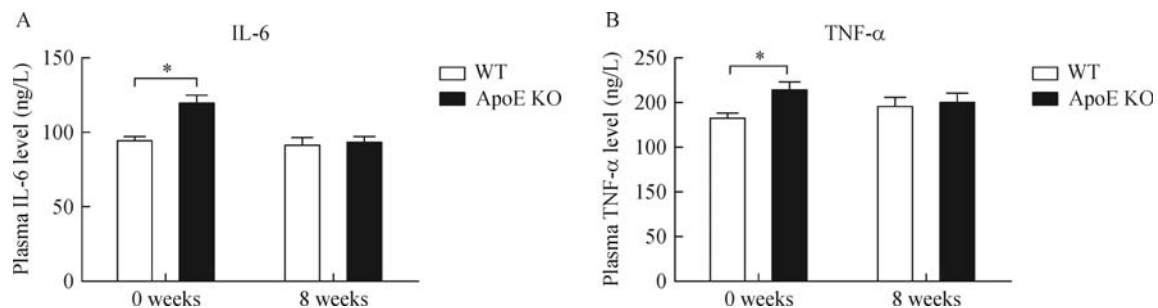
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## Supplementary Data

### 1. Plasma IL-6 and TNF- $\alpha$ levels

Plasma IL-6 and TNF- $\alpha$  levels in WT and ApoE KO rats were measured before and after 8 weeks of Paigen diet using rat Interleukin 6 and rat Tumor necrosis factor  $\alpha$  ELISA Kits (Wuhan Colorful Gene Biotech Co.,LTD, Wuhan, China).

As shown in *Supplementary Fig. 1A* and *1B*, both IL-6 and TNF- $\alpha$  were slightly increased in ApoE KO rats compared with WT littermates before Paigen diet, while did not show any difference after 8 weeks of Paigen diet.

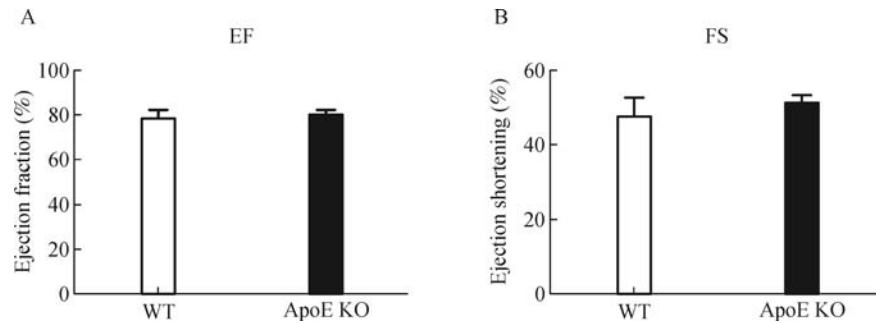


*Supplementary Fig. 1* Plasma IL-6 (A) and TNF- $\alpha$  (B) levels in WT and ApoE KO rats before and after 8 weeks of Paigen diet. n = 6-7, \*  $P < 0.05$ .

### 2. Echocardiography analysis

Echocardiography images were obtained with a high-resolution Vevo 770 imaging system (VisualSonics Inc., Canada). Left ventricle (LV) dimensions and wall thicknesses were determined using parasternal short axis M-mode images at the level of the papillary muscle and averaged from 3 cardiac cycles. Ejection fraction (EF) and fractional shortening (FS) were calculated by the Vevo770 software.

Echocardiography did not show differences on ejection fraction (EF) and fractional shortening (FS) between ApoE KO and WT rats (*Supplementary Fig. 2A* and *2B*) after 10 to 12 weeks Paigen diet feeding, suggesting no significantly changes on cardiac function.

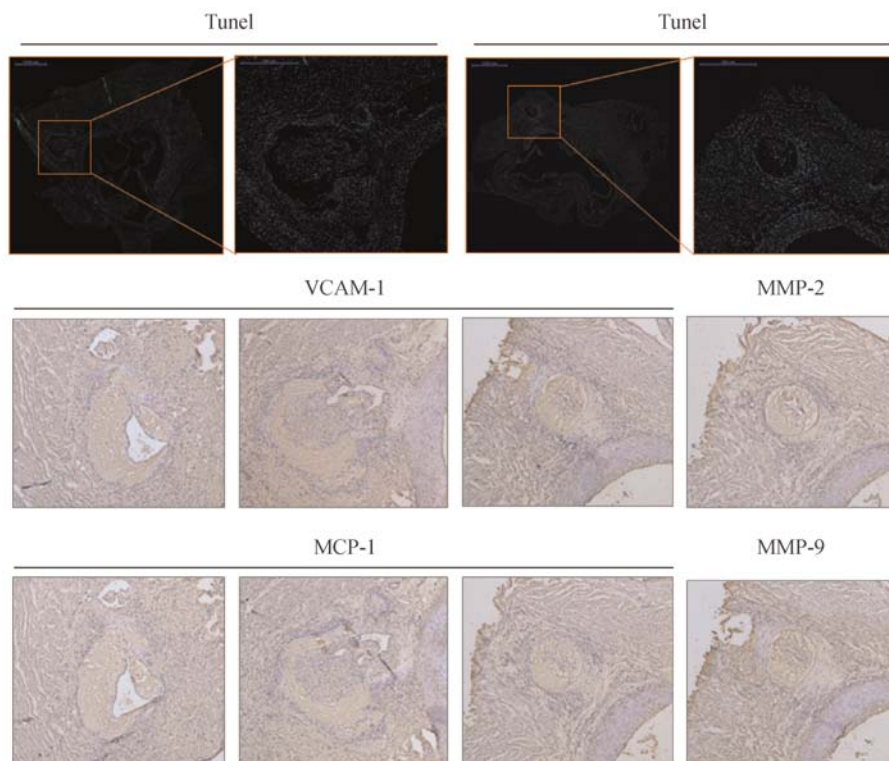


**Supplementary Fig. 2** Left ventricular ejection fraction (A), and short axial shortening fraction (B) were detected by M type echocardiography,  $n = 6-9$ .

### 3. TUNEL, VCAM-1, MCP-1, MMP-2 and MMP-9 immuno-staining in the coronary artery

Apoptosis is an important cell death process, it could accelerate the formation of necrotic core. We measured the apoptosis levels in coronary arterial lesions of ApoE KO rats using TUNEL staining (Roche In Situ Cell Death Detection Kit), there was no obvious apoptosis in the coronary arterial lesions of ApoE KO rats.

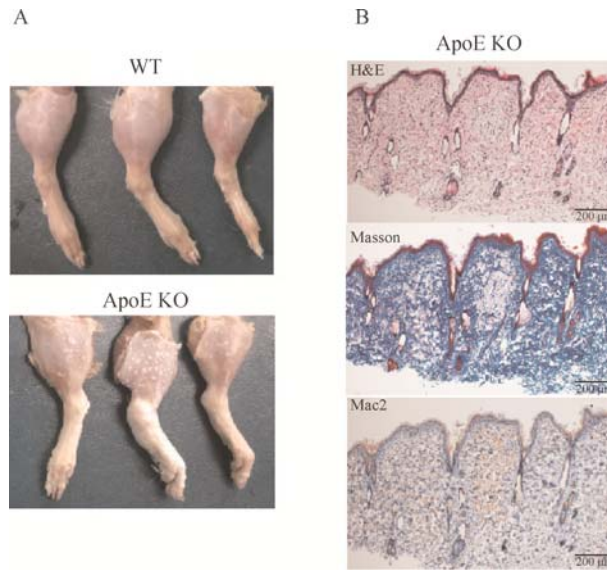
Immuno-detections were performed with VCAM-1, MCP-1, MMP-2 and MMP-9 antibody (Santa Cruz Biotechnology, Dallas, TX) to examine adhesion molecules, monocytes infiltration and matrix metalloproteinase expressions. As show in **Supplementary Fig. 3**, there were no obvious VCAM-1, MCP-1, MMP-2 and MMP-9 expression in the coronary artery lesions of ApoE KO rats.



**Supplementary Fig. 3** TUNEL, VCAM-1, MCP-1, MMP-2 and MMP-9 immuno-staining in the coronary artery of 3 male ApoE KO rats after Paigen diet.

### 4. Skin xanthoma in ApoE KO rats

ApoE KO male rats also exhibited skin xanthoma, which was similar to ApoE deficient patients. The photograph of legs showed obviously skin xanthoma in ApoE KO rats (**Supplementary Fig. 4A**). We also conducted H&E, Masson's Trichrome staining and Mac2 immuno-staining, as shown in **Supplementary Fig. 4B**, skin xanthoma in ApoE KO rats containing collagen fibers and macrophages.



**Supplementary Fig. 4 Skin xanthoma in ApoE KO rats.** Photograph of legs (A), H&E, Masson's Trichrome staining and Mac2 immunostaining (B) of ApoE KO rats after Paigen diet.