Results

Normal morphology and unchanged macrophage marker in MORE-PGKO aortas

Aortas from MORE-PGKO mice did not show significant morphological changes compared to LC (Online Figure I A). Inflammation has been considered as an important factor in blood pressure regulation and PPAR-γ is associated with both inflammation and blood pressure regulation (1). We stained aortas with antibodies recognizing macrophage markers. The results showed that F4/80 staining was entirely absent in aortas of both LC and MORE-PGKO mice while the spleen from LC mice served as a positive control with macrophage stained in reddish brown (Online Figure I B). Comparable results were observed using the BM-8 antibody (data not shown) that detects a similar but non-identical macrophage population. Staining with two additional antibodies that detect macrophage, Mac3 and EH-HR3, did not reveal differences between MORE-PGKO and LC aortas either (data not shown). Further, F4/80 protein and mRNA expression levels were not changed in MORE-PGKO aortas compared with LC mice (Online Figure I C~E).

Methods

Histologic analysis

For immunohistochemical staining, sections were incubated at 58°C for one hour, deparaffinized and incubated in antigen retrieval solution (Dako) at 100°C for 14 min. Sections were blocked sequentially with serum, avidin and biotin solutions followed by incubation with primary antibodies against F4/80 (8.33 μg/ml, Bachem), BM-8 (5 μg/ml, Bachem), Mac3 (0.3 μg/ml, BD-Pharmingen), or EH-HR3 (10 μg/ml, Bachem) at 4°C overnight. Staining was visualized using streptavidin-peroxidase and DAB and the sections were counterstained with methyl green/alcian blue. Digital images were obtained using an Olympus BX51 microscope (Olympus America Inc.).

Gene expression analysis

F4/80 gene was assayed using reverse transcription quantitative PCR (RT-QPCR) as described (2). All primer-probe sets were purchased from Applied Biosystems.

Western blot analysis

Total protein was isolated from aortas, subjected to electrophoresis, and transferred to PVDF membranes as described before (3, 4). Membranes were incubated with primary antibodies recognizing F4/80 (Abcam Inc.), and Actin (Santa Cruz Biotechnology Inc.). The membranes were then incubated in secondary antiserums conjugated with horseradish peroxidase and detected as before (4).

Statistics

Mean ± standard error (SE) values were analyzed using Prism (GraphPad Software Inc.). Statistical comparisons between groups were performed by Student's t test. Groups were considered significantly different if P values were ≤ 0.05.

References

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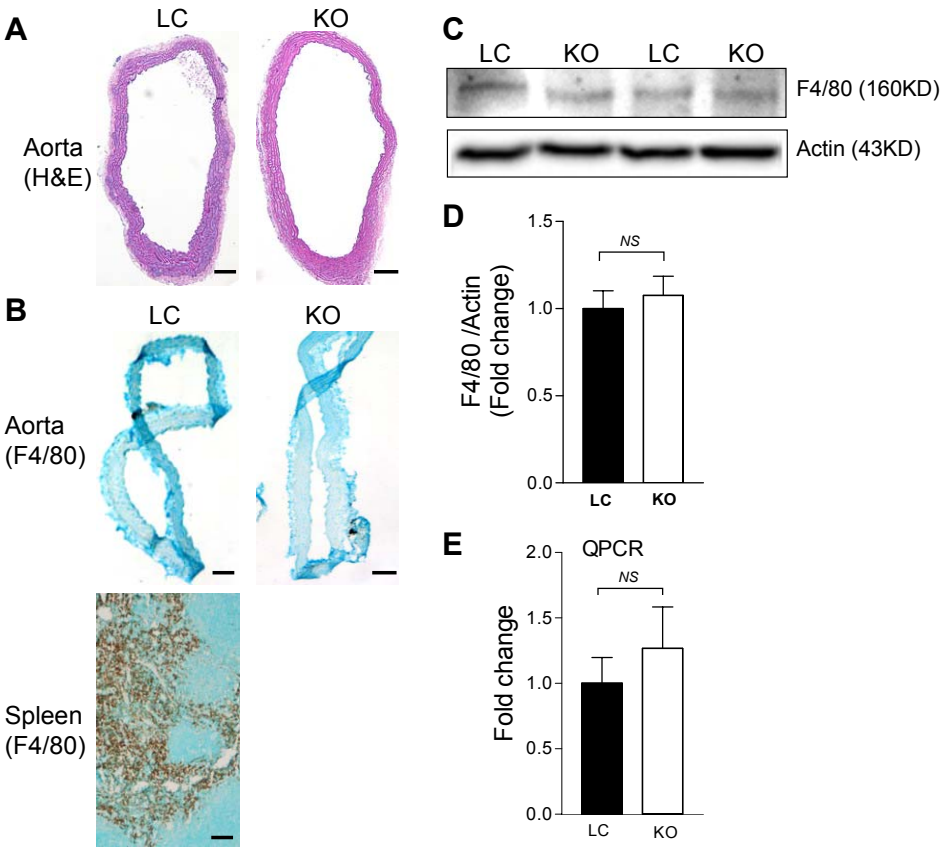
Figure legends

Online Figure I. Normal morphology and unchanged macrophage marker in MORE-PGKO aortas. A. Representative H&E histochemistry of aortas from male LC and MORE-PGKO mice. LC: littermate control; KO: MORE-PGKO. The bars indicate 20 m. **B.** Representative immunohistochemical staining of F4/80 (a macrophage marker) in male mice showing negative results in aortas. Spleen was used as a positive control with reddish brown staining of macrophages. The bars indicate 20 m. **C.** Representative Western blots of F4/80 in aortas from male LC and MORE-PGKO mice. Actin was used as a loading control. **D.** Quantification of F4/80 over Actin. n = 4 for each group. NS: not significant. **E.** RT-QPCR result of F4/80 expression in male LC and MORE-PGKO aortas. β-actin was used as an endogenous control. n = 4 for each group. NS: not significant. The results from female mice were similar for A~E.



Online Figure I