

Supplementary Information

Administration of hydrogen-rich water prevents vascular aging of the aorta in LDL receptor-deficient mice

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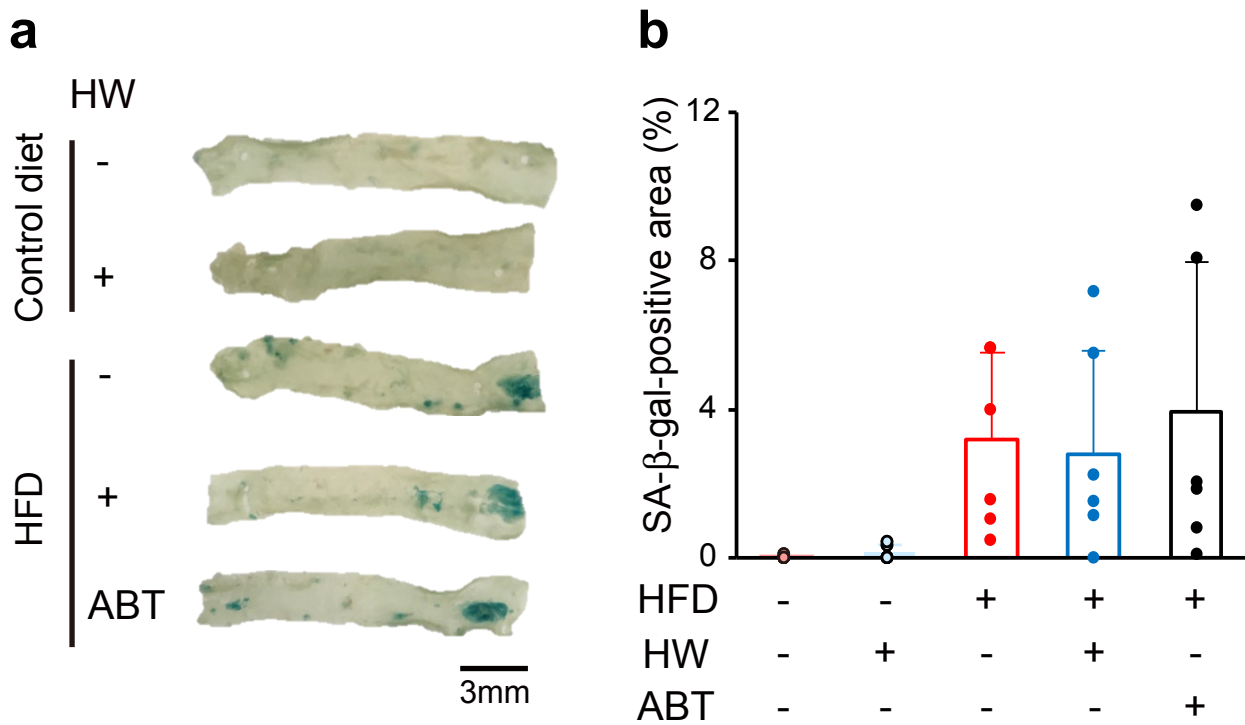
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Supplementary Methods

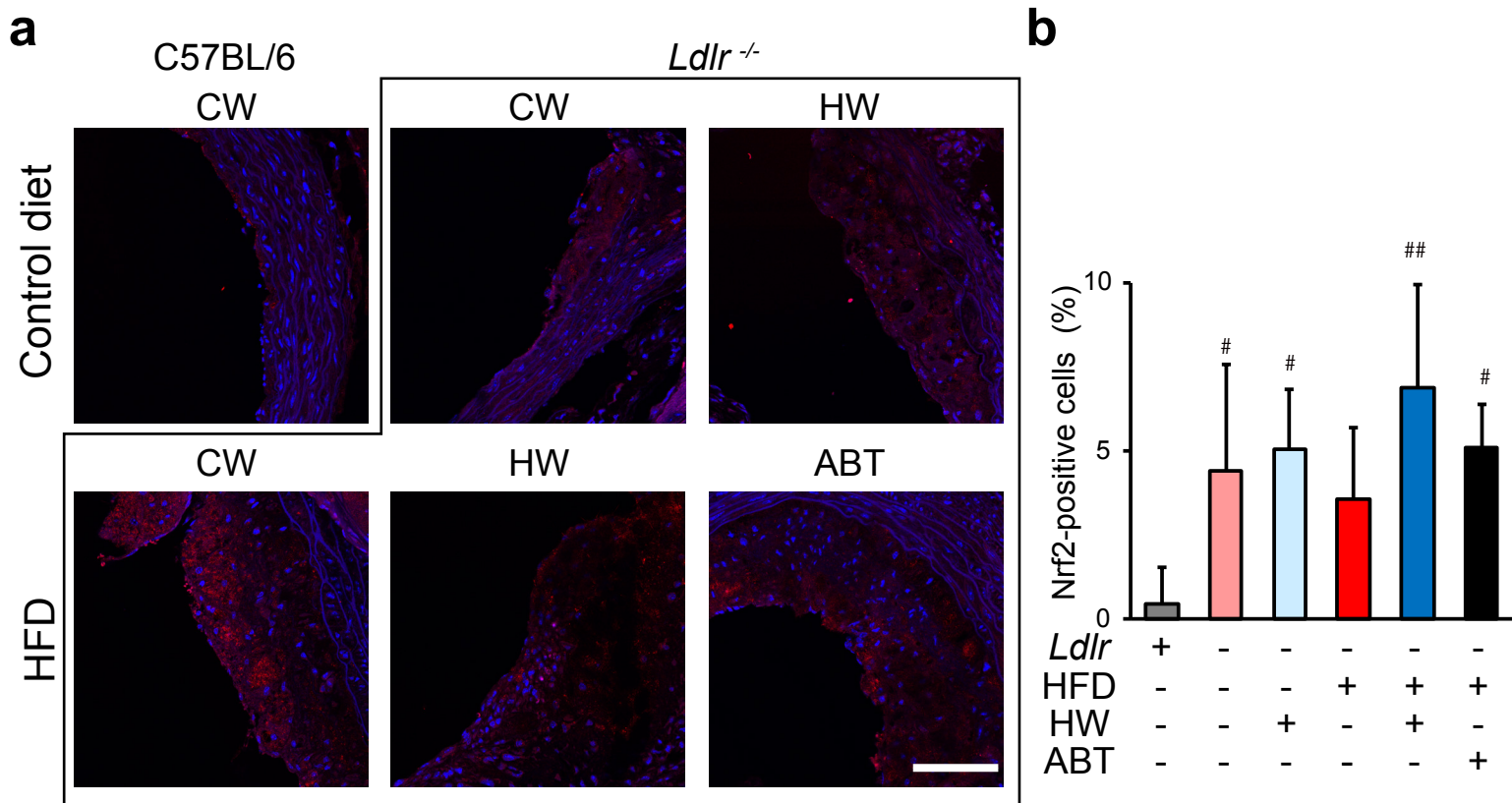
Supplementary Figures 1-4

Supplementary Methods

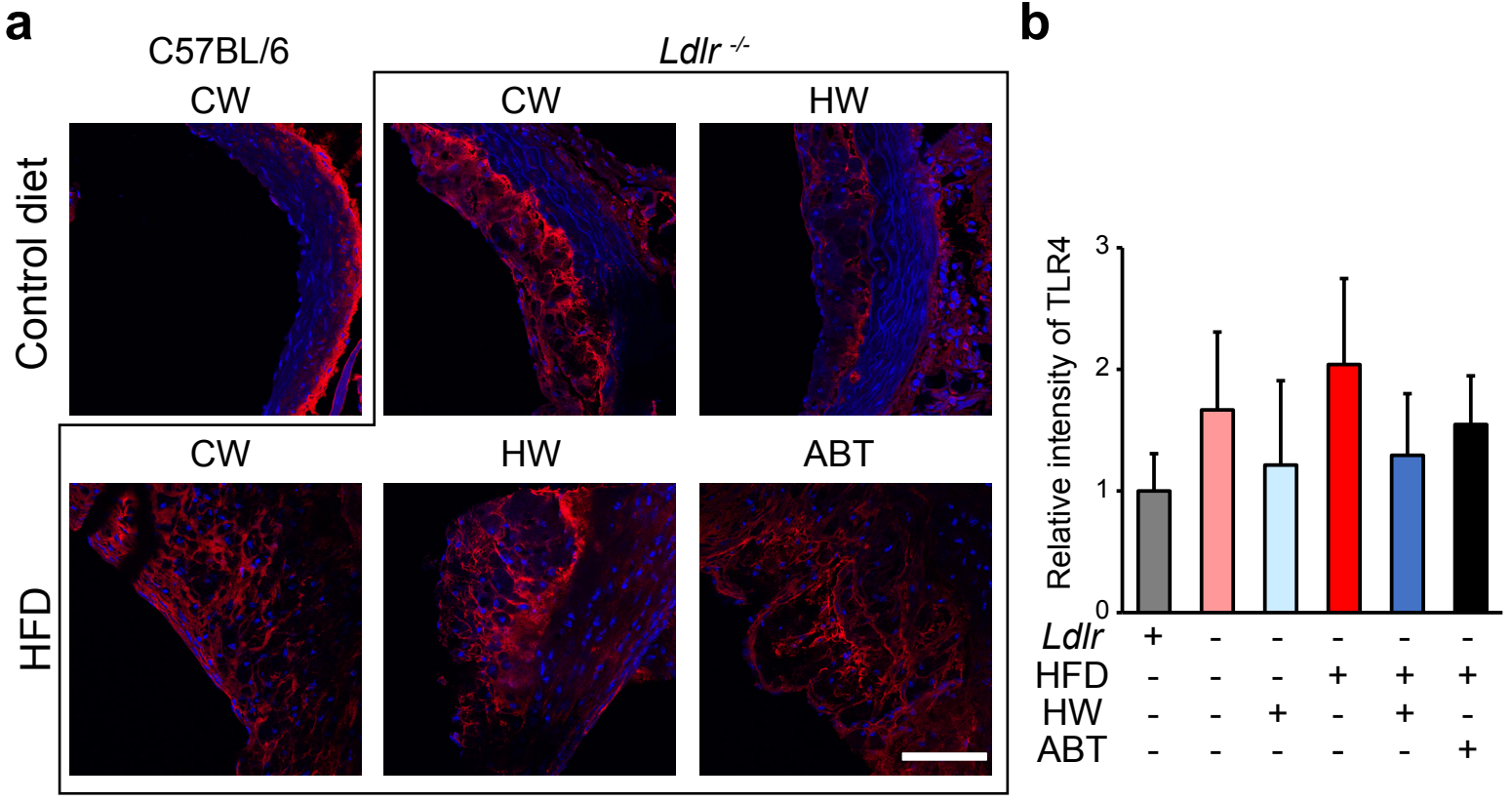
SA- β -Gal assay. SA- β -Gal staining of aortas was performed according to the manufacturer's instructions (Cell Signaling, Danvers, MA, USA). Mouse thoracic aortas were excised and stored in phosphate-buffered saline on ice until fixation. Aortas were fixed with 1 × Fixative Solution for 15 min at room temperature, washed twice with phosphate-buffered saline, and incubated overnight in staining solution at 37 °C.



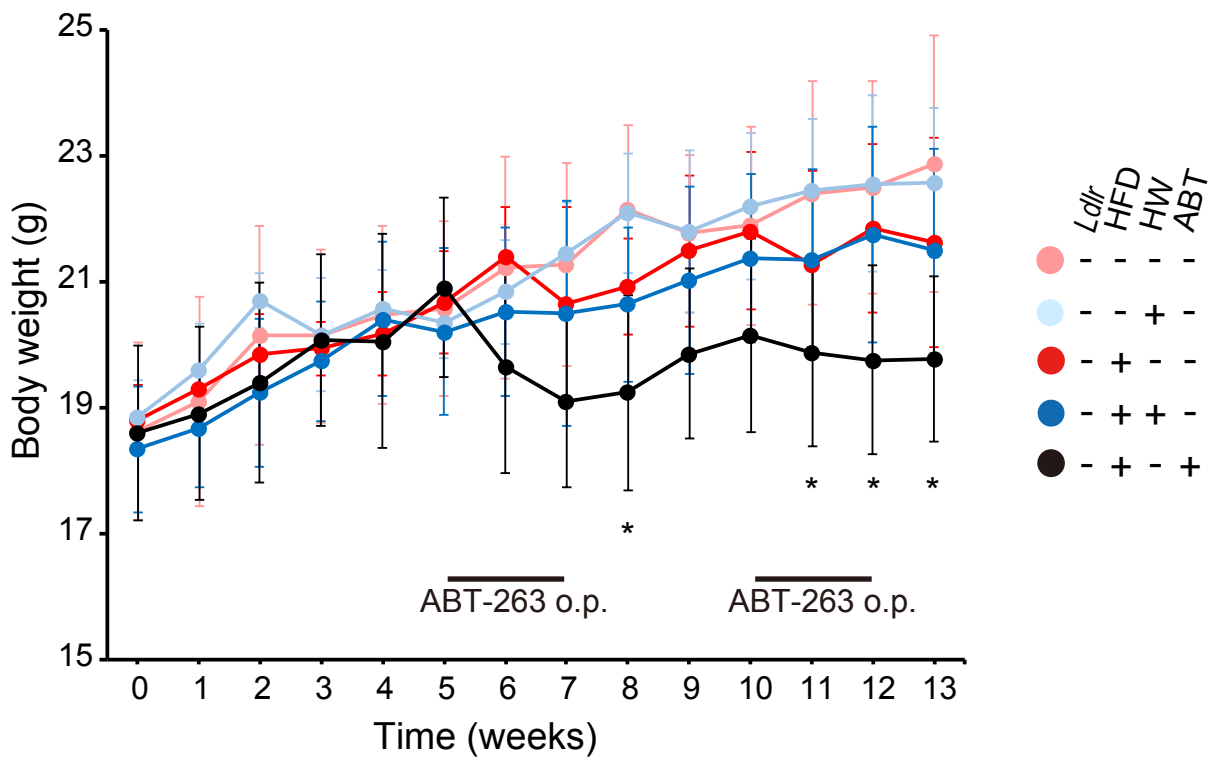
Supplementary Figure 1. Drinking HW did not decrease aortic SA-β-gal staining in *Ldlr*^{-/-} mice. **(a)** The SA-β-gal-positive area in the thoracic aorta was increased in the HFD-fed groups. **(b)** Quantitative analysis of the SA-β-gal-positive area in the aorta. Neither HW nor ABT-263 (ABT) suppressed the increase in the SA-β-gal-positive area. Values are the mean ± SD (n = 6).



Supplementary Figure 2. Drinking HW increased Nrf2 positive cells in the aorta of HFD-fed *Ldlr*^{-/-} mice. (a) Immunohistochemical staining for Nrf2 (red) and Hoechst nuclear staining (blue) in the aortic intima. Nrf2-positive cells were increased in HFD mice given control water (CW). This increase was further enhanced by both HW and ABT-263 (ABT). Scale bar: 100 μ m. (b) Quantitative analysis of Nrf2-positive cells in the aortic intima. #*P* < 0.05 and ##*P* < 0.01 vs. C57BL/6 *Ldlr*^{+/+} control mice.



Supplementary Figure 3. Effect of drinking HW on the expression of TLR4 in the aorta of HFD-fed *Ldlr*^{-/-} mice. **(a)** Immunohistochemical staining for TLR4 (red) and Hoechst nuclear staining (blue) in the aortic intima. Scale bar: 100 μ m. **(b)** Quantitative analysis of TLR4-intensity in the aortic intima.



Supplementary Figure 4. Weight gain in *Ldlr*^{-/-} mice was prevented by ABT-263. Values are the mean \pm SD (n = 6). **P* < 0.05.