

**Title:** LJ-1888, a selective antagonist for the A<sub>3</sub> adenosine receptor, ameliorates the development of atherosclerosis and hypercholesterolemia in apolipoprotein E knock-out mice

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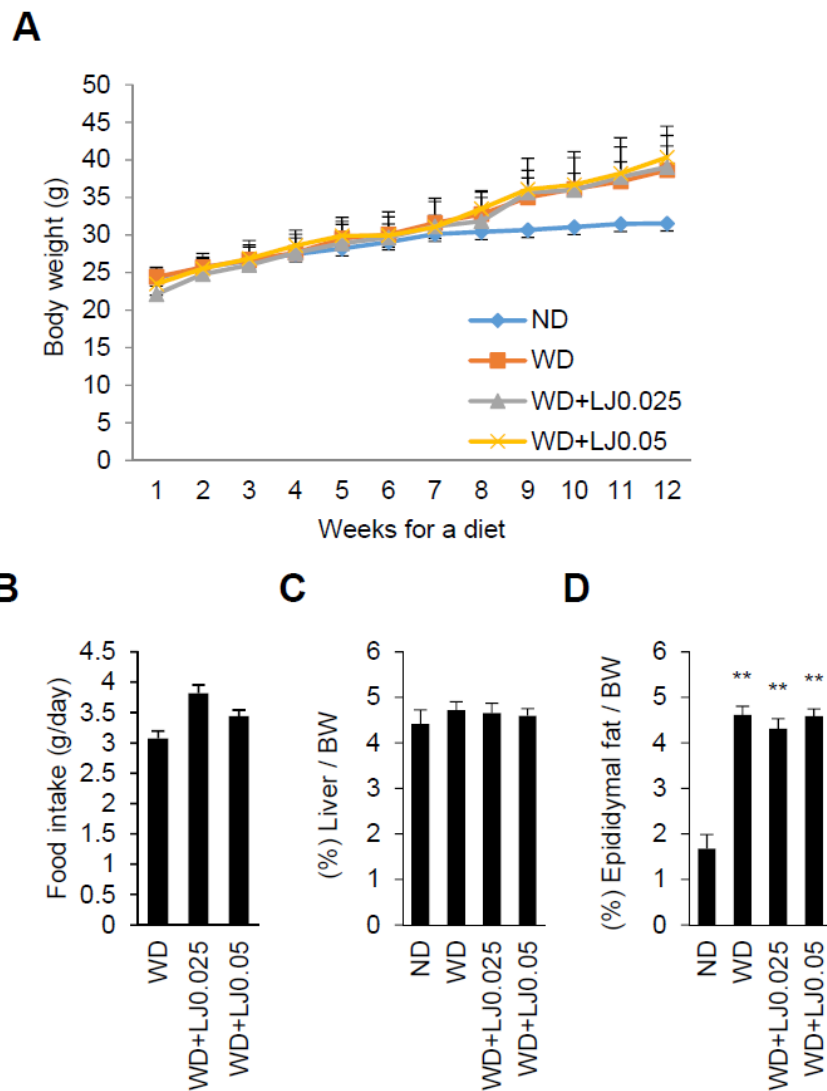
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**Running Title:** LJ-1888 ameliorates atherosclerosis

**Keywords:** LJ-1888, atherosclerosis, hypercholesterolemia, low-density lipoprotein cholesterol (LDL-cho), high-density lipoprotein cholesterol (HDL-cho)

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**Supplementary Fig. 1.** Six week-old apolipoprotein E deficient mice fed normal chow (ND), western diets (WD), and WD with LJ1888 (0.025% and 0.05%). (A) Body weight (g) of indicated mice. (B) Food intake (g/mouse/day). (C) The ratio of liver weight to body weight. (D) The ratio of epididymal fat weight to body weight. , \*\*P < 0.01 versus ND group.



## **SUPPLEMENTARY MATERIALS AND METHODS**

### Plasma lipid analysis

Mice were anesthetized with 0.4 ml of 1.2% Avertin per 25 g body weight, and blood samples were collected from the retro-orbital plexus. Plasma was obtained by centrifugation, and concentrations of total cholesterol, LDL-chol, HDL-chol, TG, glucose, ALT, AST, and ALB were measured using a blood auto-analyzer (Hitachi, Tokyo, Japan).

### Histological analysis

Mice were euthanized with CO<sub>2</sub> gas and perfused with phosphate-buffered saline (PBS) through the left ventricle, and their hearts and aortas were isolated. Hearts were embedded in OCT compound (Sakura, Tokyo, Japan) and frozen at –80°C. Aortas were cut from the proximal ascending region to the bifurcation of the iliac artery; adventitial fats were removed; and each aorta was dissected longitudinally and pinned onto black silicone plates. After fixation with 10% neutralized buffered formaldehyde, the tissue samples were stained with Oil red O for 4 h and washed with PBS. Total aortic areas and lesion areas were measured by Axio Vision (Carl Zeiss, Jena, Germany). Plaque areas on aortic sinuses were measured following cryo-dissection and staining of serial sections with Oil red O, and lesion areas were measured on the resulting images by Axio Vision.

### RNA isolation and real time PCR

Total RNA was isolated using TRIzol reagent (GibcoBRL, Grand Island, NY, USA), suspended in DNase/RNase-free distilled water and stored at –80°C. Complementary DNA was synthesized by the Superscript III First-strand synthesis system (Invitrogen, Carlsbad, CA, USA). Quantitative real-time PCR analysis was performed by 7700 sequence detector (Applied

Biosystems, Foster City, CA, USA) with SYBR Green PCR Master Mix (Applied Biosystems) and specific primer sequences.

Gene	Forward primer	Reverse primer
<i>Ldlr</i>	CTGTGGGCTCCATAGGCTATCT	GCGGTCCAGGGTCATCTTC
<i>Abcg5</i>	TGGCCCTGCTCAGCATCTCTG	GGATTTTTAAAGGAATGGGC
<i>Abcg8</i>	CCGTCGTCAGATTTCCAATGA	GGCTCCGACCCATGAATG
<i>Cyp7a1</i>	CACTCTACACCTTGAGGATGG	GACATATTGTAGCTCCTGATCC
<i>Cyp8b1</i>	GCCCACAGCCTTCAAGTATG	CGACCAGCTTGAAGTCGAAG
<i>Cyp27a1</i>	TTGCTCAGTACTCAGGAGACCA	GCTCTTTGAAAGGTGGTACAGG
<i>Cyp7b1</i>	CAGCTATGTTCTGGGCAATG	TCGGATGATGCTGGAGTATG
<i>LXRa</i>	GCTCTGCTCATTGCCATCAG	CACTTGCTCTGAATGGACGC
<i>LXRb</i>	CGAGCTAGCCATCATCTCGG	AACTGCAAGAACCCTGGCAC
<i>FXR</i>	CGATCGTCATCCTCTCTCCA	ATCAGCATCTCAGCGTGGTG
<i>RXR</i>	TCCTGTTCAACCCTGACTCTAAG	ATGAGCTTGAAGAAGAACAGGTG
<i>Ppara</i>	AGATTCAGAAGAAGAACCGGAAC	CCGATCTCCACAGCAAATTATAG
<i>Pparg</i>	CACAATGCCATCAGGTTTGG	GCTGGTCGATATCACTGGAGATC
<i>Srebf1a</i>	GGCCGAGATGTGCGAACT	TTGTTGATGAGCTGGAGCATGT
<i>Srebf1c</i>	GCTGTTGGCATCCTGCTATC	TAGCTGGAAGTGACGGTGGT