SUPPROTING INFORMATION FOR THE MANUSCRIPT

SR-B1 uptake of HDL promotes prostate cancer proliferation and tumor progression

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SUPPORTING INFORMATION INVENTORY

EXPERIMENATL PROCEDURES FOR SUPPORTING INFORMATION

Mouse Cohort

12 and 18-week age-matched, male, WT and apoA1-KO mice that were not subjected to subcutaneous prostate cancer cells injections were assessed for plasma total and HDL cholesterol levels, body weight, testes weight, and plasma testosterone levels.

Quantification of mouse serum total and HDL cholesterol

Total and HDL cholesterol from the plasma fraction was then quantified using the Stanbio Cholesterol LiquiColor (Cat. No. 1010) enzymatic-colorimetric assay according to manufacturer's instructions and adapted to a 96-well plate format. In brief, plasma treated with or without the Stanbio precipitating reagent for HDL assay (Cat. No. 0599) was combined with Stanbio liquicolor reagent (1011) for 10 minutes at room temperature, then absorbance at 500 nm was acquired and cholesterol levels calculated versus a standard curve.

Quantification of mouse plasma testosterone

Freshly collected plasma was stored at -80˚C until LC/MS/MS analysis. 60 µl of plasma was spiked with 10 µl of internal standard [25ng/ml, Androstene-3, 17-dione-2, 3, 4-13C3]. Samples were extracted twice with 2 mL of methyl-tert-butyl ether (MTBE, Across). MTBE extracts were dried under nitrogen gas and then reconstituted in 120ul of 50% methanol/water (v/v). The extracted testosterone was quantified using liquid chromatography tandem mass spectrometry (LC/ MS/MS). Briefly, the extracted steroids were injected onto a Shimadzu UHPLC system (Shimadzu Corporation), and separated on a C18 column (Zorbax Eclipse Plus C18 column, 150 mm x 2.1 mm, 3.5 µm, Agilent) using a gradient starting from 20% solvent B [acetonitrile/methanol (90/10, v/v) containing 0.2% formic acid] for 4 min and then to 75% solvent B for 10 min, followed by 95% solvent B for 3 min. Testosterone was quantified on a Qtrap 5500 mass Spectrometer (AB Sciex) using ESI in positive ion mode and multiple reaction monitoring using characteristic parent → daughter ion transitions for the specific molecular species monitored. Data acquisition and processing were performed using MultiQuant software (AB Sciex; version 3.0.1). The peak area ratio of the analyte over the internal standard was used for quantification. Each sample run included a calibration curve with standards for data quantification using the analyte/internal standard peak area ratio.

H&E Staining of tumors

Formalin fixed tumors were paraffin embedded and sectioned. Sections were deparaffinized and stained with hematoxylin and eosin.

Statistical analysis

Cholesterol levels, body weight, and testes weight data are expressed as the mean ± SD. Differences between the values were evaluated by either the Student T-test. Hormone levels were not normally distributed and a non-parametric t-test was performed for the testosterone and dihydrotestoterone levels. An additional parametric Student T-test analysis was performed after removing outliers greater than 2 standard deviations from the mean, normalizing the distribution of the data. Statistics were performed using GraphPad Prism V.8.1.1 software.

FIGURES FOR SUPPORTING INFORMATION

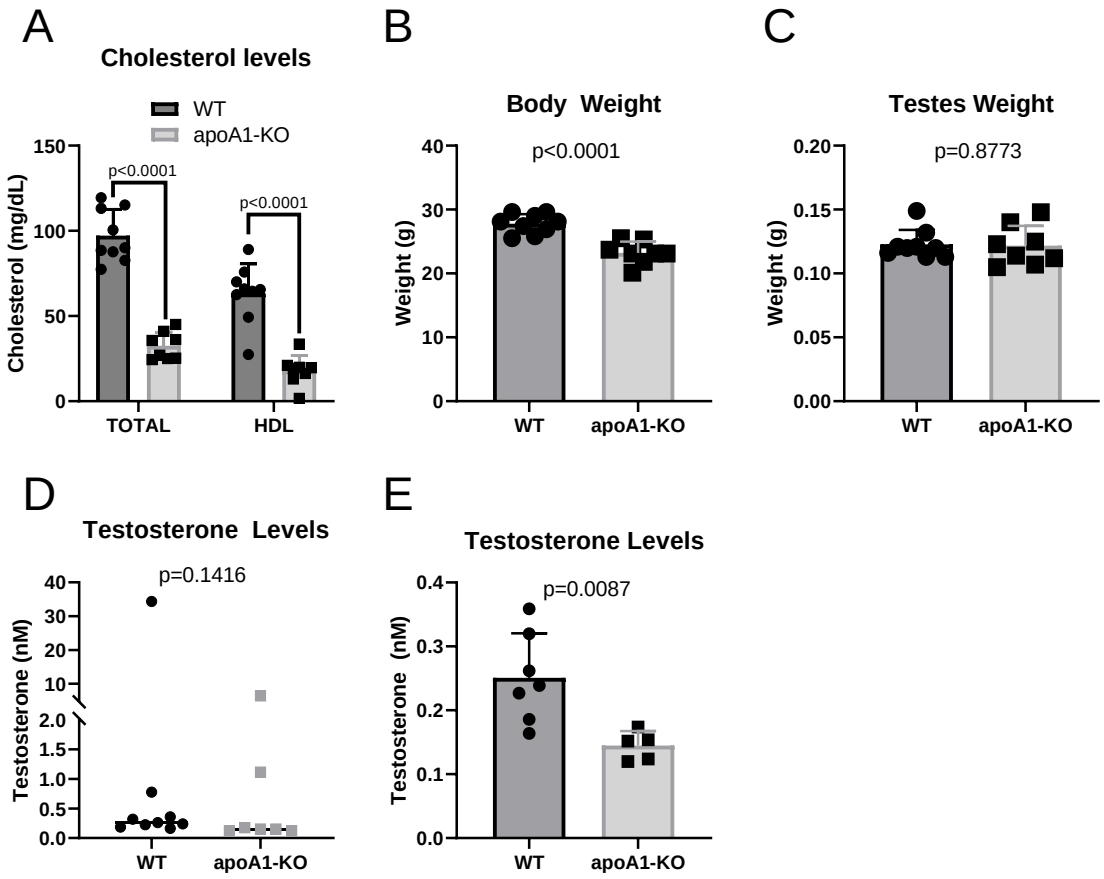

Figure S1. Characterization of WT and apoA1-KO mice. Total- and HDL-cholesterol levels (A), body weight (B), and testes weight (C) in 12 and 18 week old aged matched WT (n=9) and apoA1-KO (n=8) mice (mean ± SD; t-test p-values displayed). D) Serum testosterone levels were not normally distributed (median values; not significant by non-parametric Mann-Whitney t-test). E) Serum testosterone levels after removal of outlier value from each cage were normally distributed (mean ± SD; t-test p=0.0087).

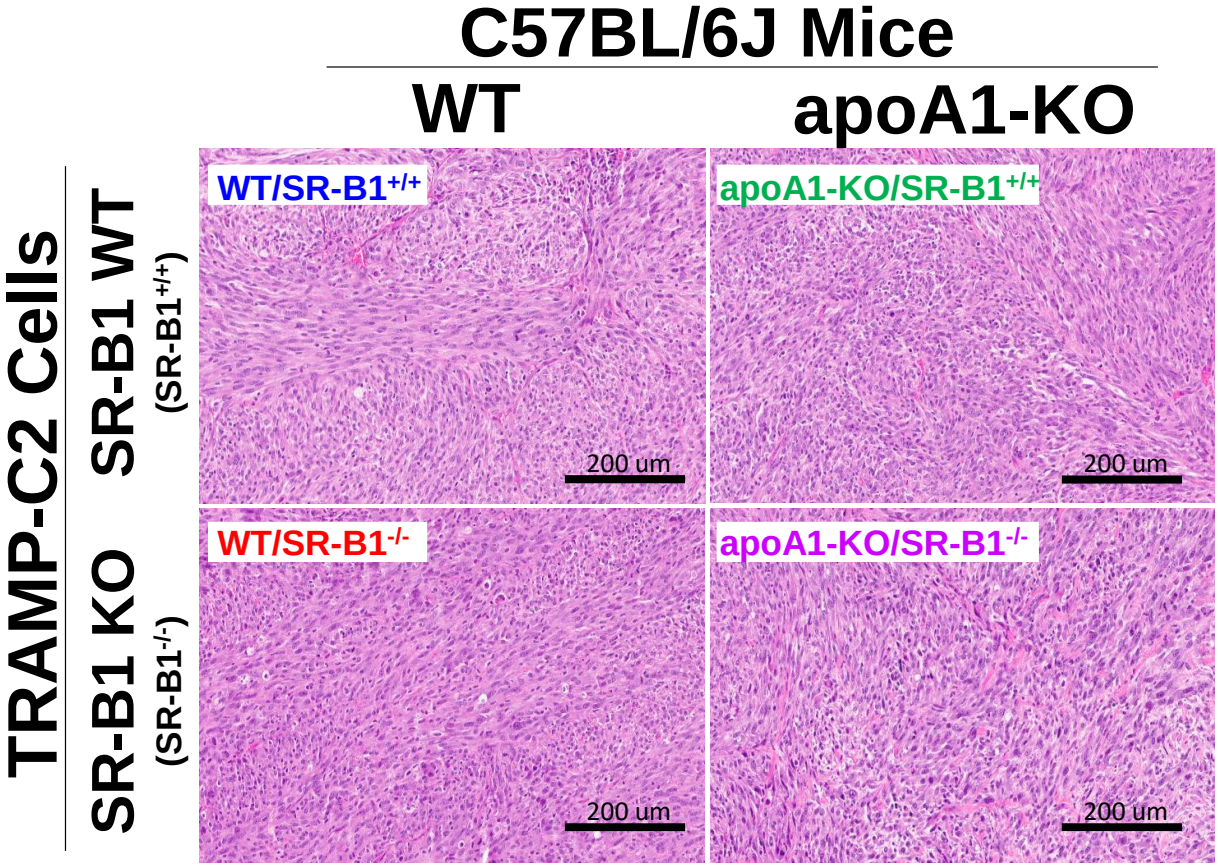

Figure S2. Histology of subcutaneous tumors from study arms. Hematoxylin and Eosin stained tumors from WT/SR-B1$^{+/+}$, WT/SR-B1$^{-/-}$, apoA1-KO/SR-B1+/+, and apoA1-KO/SR-B1$^{-/-}$ at 20x magnification.