Supplemental Material

PAR2 controls cholesterol homeostasis and lipid metabolism in nonalcoholic fatty liver disease

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Table S1. Median (25%, 75%) of laboratory values of NAFLD subjects from Fib 0-2 versus Fib 3-4 cohorts.

Characteristic	Fib 0-2 (n=50)	Fib 3-4 (n=43)	P value[a]
Total Cholesterol, mg/dL	178 (145, 217)	183 (153,234)	0.59
LDL Cholesterol, mg/dL	109 (83, 132)	106 (80, 136)	0.92
HDL Cholesterol, mg/dL	38 (32, 50)	35 (27, 43)	0.026
Triglycerides, mg/dL	152 (112, 230)	168 (105, 236)	0.26
ALT	57 (30,89)	75 (47, 107)	0.35
AST	38 (26, 66)	62 (46, 96)	0.0005

[a]P value from non-parametric Pearson's chi-squared test of median plasma values (Mood's test) from NAFLD/NASH subjects with either Fib 0-2 or Fib 3-4 score in their liver biopsies.

Table S2. Median (25%, 75%) of laboratory values of NAFLD subjects from low hepatic PAR2 versus high hepatic PAR2-expressing cohorts.

Characteristic	Low Hepatic PAR2 Expression (n=42)	High Hepatic PAR2 Expression (n=51)	P value[a]
Total Cholesterol, mg/dL	172 (145, 209)	185 (154,232)	0.26
LDL Cholesterol, mg/dL	95 (79, 131)	116 (87, 144)	0.049
HDL Cholesterol, mg/dL	38 (30, 50)	35 (30, 44)	0.98
Triglycerides, mg/dL	158 (102, 236)	156 (113, 195)	0.75
ALT	59 (33,94)	74 (41, 94)	0.29
AST	43 (26, 76)	50 (38, 80)	0.59
Male (% of total subjects)	40 (17M, 25F)	47 (24M, 27F)	0.54[b]

[a]P value from non-parametric Pearson's chi-squared test of median plasma values (Mood's test) from NAFLD/NASH subjects with either low or high PAR2 expression in their liver biopsies.

[b]P value from Fisher's exact test using number of males (M) versus females (F).

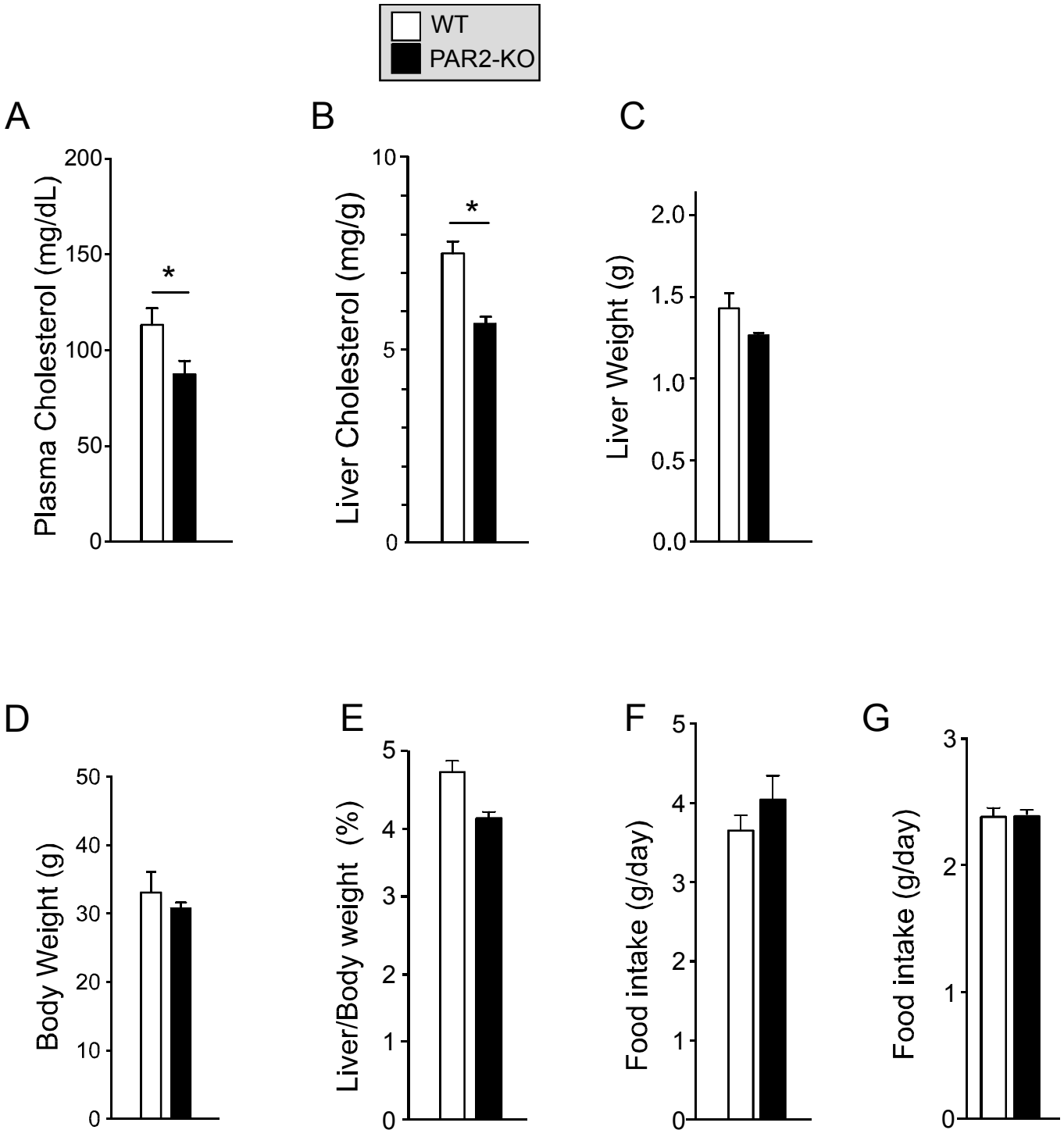

Figure S1. PAR2 deficiency suppresses cholesterol levels in plasma and liver in mice fed a normal diet. Male C57BL/6 wild type (WT-open bars) and PAR2-KO (KO-black bars) mice were fed a normal chow diet (n=5-7) for 16 weeks. (A) At the 16 week end-point, plasma was obtained from non-fasted mice at Z+4 time point and assayed for total cholesterol. (B) Livers from mice in A were harvested and weighed at the 16 week endpoint and assayed for mg cholesterol per g of liver tissue. (C-F) Liver and body weight was measured at the 16 week end point. Mean daily food consumption per mouse was assessed over 16 weeks for (F) normal diet and (G) high-fat diet. *P<0.05 by 2-tailed t-test.

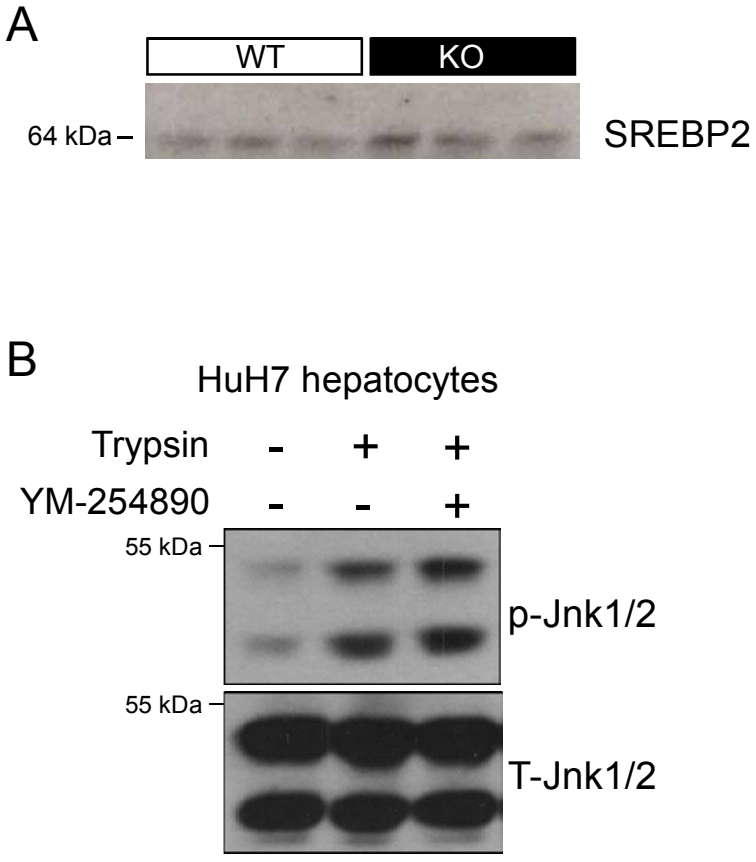

Figure S2. (A) Western blots of liver lysates from WT and PAR2-KO mice fed high fat diet (HFD) for 16 weeks of active 64 kDa proteolytic fragment of SREBP2. (B) The PAR2 agonist, trypsin (10 nM), induces phospho-Jnk1/2 signaling (15 min) in HuH7 hepatocyte lysates which is not affected by the Gq inhibitor, YM-254890 (100 nM), as shown by western blot for phospho (p)-Jnk1/2 and total (T) Jnk1/2.