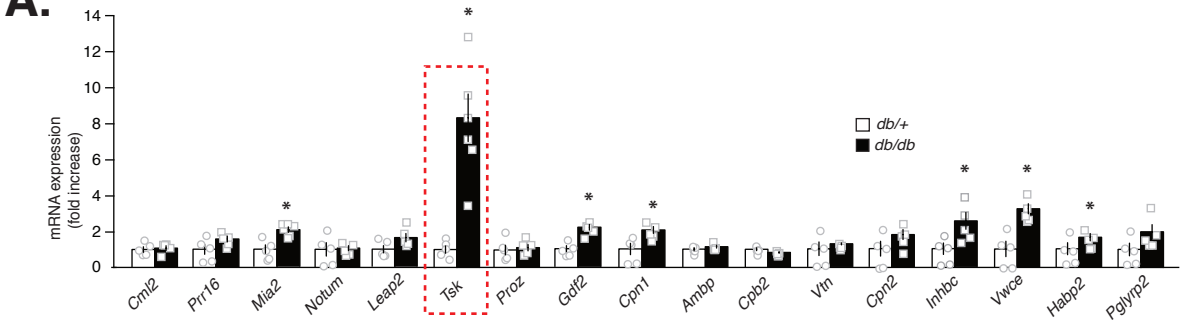
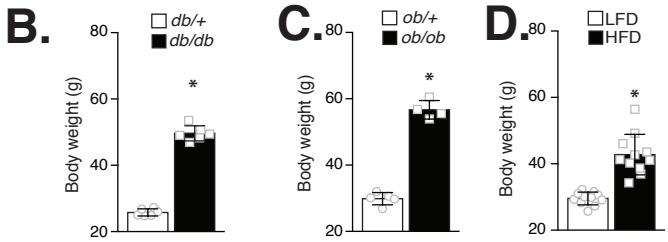
Figure S1 - TSK is a hepatokine induced by obesity.





E

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CHICK (Q65Z9)       1 -MQFLAWFNMLLLLPCFSTTKTCFPGCHCEVESFGLFDSFSLTKVDCSGIGSHIVPVPIPLDT  62
XENOPUS (Q5M7S9)    1 --MAPSWLFLLFIPGMVGSSRSCFPGCQCIVDNFGLFHSFSLTKVDCSGVGPHVVPVSIPLDT  61
DANIO (Q58A48)      1 MASLLCLFFSLLGLAAIGAVKNCHPQCRCEVETFGLFDSFSLTKVDCSRIGPGNTPVPIPLDT  63

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XENOPUS (Q5M7S9)    62 SYLDLSANGIKRINESVLSGPGYTTLINLNLSHNQIVRISFSTFSKLRYLESLDLSHNLLETL 124
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DANIO (Q58A48)      127 SDGCFTGLPLVELDLSENQFKEFNLDLFTTRTQDLPIMVDLSRNLLTSIFRR---TPGHPLYI 186

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CHICK (Q65Z9)       249 FKELPALQVLDLSNNPNLRSLTAEVIFGLNSIQELNLSGTGVSSLPKTVLKYLPSLKSITLRK 311
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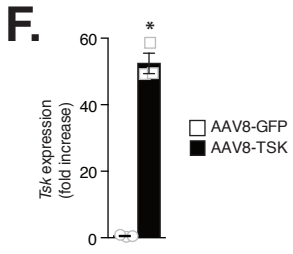


Figure S1. *Tsk* is a hepatokine induced by obesity. (A) Gene expression analysis of all the candidate genes identified in the experimental scheme described in Figure 1A. qPCR was performed in the liver of control and *db/db* mice (n=5-6/group). Data represent the mean ± SEM. Significance was determined by two-tailed, unpaired *t* test. **P* < 0.05 versus controls. **(B to D)** Body weight of **(B)** control and *db/db* mice (n=5-6/group) (10 weeks old), **(C)** control and *ob/ob* mice (n=4-5/group) (12 weeks old) and **(D)** LFD and HFD-fed mice (n=10/group) (11-13 weeks old mice fed a LFD or HFD for 10 weeks). Data represent the mean ± SEM. Significance was determined by two-tailed, unpaired *t* test. **P* < 0.05 versus controls. **(E)** Sequence alignment of TSK protein among species. **(F)** qPCR analysis of *Tsk* expression in the liver of AAV8-GFP and AAV8-TSK mice.

Figure S2. *Tsk* expression correlates with steatosis in multiple mouse models.

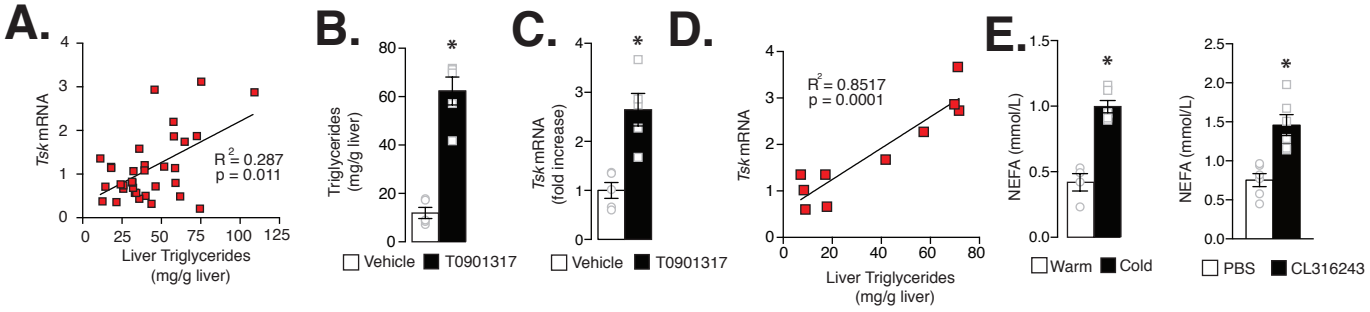


Figure S2. *Tsk* expression correlates with liver steatosis in multiple mouse models. (A) Mice were fed a control or MCD diet for 21 days. Following this period, MCD diet-fed mice were switched back to a control diet for 3, 6 or 12 days. Correlation between *Tsk* transcript levels and hepatic triglyceride content is presented (n=34 mice). **(B)** Hepatic triglyceride content measured in male mice (10 weeks old) injected with either vehicle or T0901317 (n=6 mice/group). Mice were sacrificed 24 hours after the injection. **(C)** qPCR analysis of *Tsk* transcript expression in the liver of male mice injected with vehicle or T090137 (n=6 mice/group). **(D)** Correlation between *Tsk* transcript levels and hepatic triglyceride content in the experiment described in B (n=12). **(E)** Plasma level of NEFA in mice housed at thermoneutrality (30°C) or exposed to cold (10°C) for 6 hours (n=4-6/group) (left part) or in mice injected with saline or CL316243 (0.1mg/kg) (n=6/group) (right panel)**.** In these experiments, male mice (10-12 weeks old) were used. In all the panels, data represent the mean ± SEM. Pearson correlations (two-tailed) were calculated in panels A and D. In panels B, C, and E, significance was determined by two-tailed, unpaired *t* test. $^{*}P < 0.05$ versus control.

Figure S3 - ER stress and inflammatory mediators promote *Tsk* expression and release.

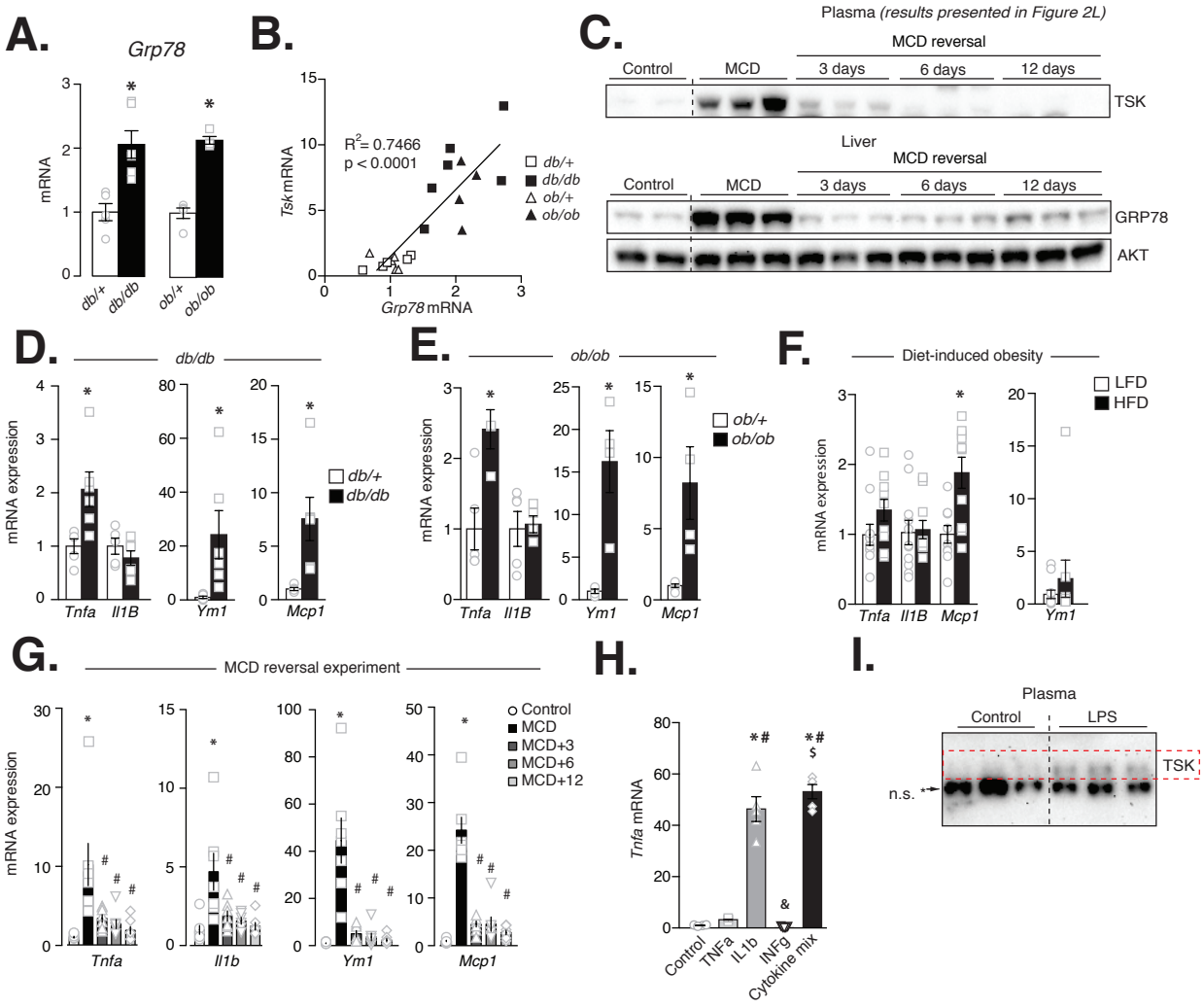
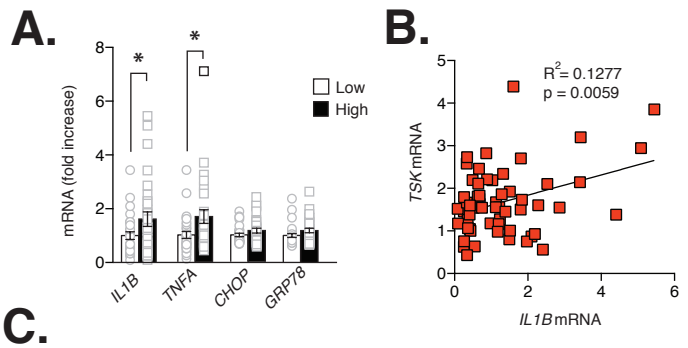


Figure S3. ER stress and inflammatory mediators promote *Tsk* expression and release. (A) qPCR analysis of *Grp78* transcript levels in the liver of control and *db/db* mice (n=5-6/group) and control and *ob/ob* mice (n=4-5/group). **(B)** Correlation between *Tsk* and *Grp78* transcript levels in the experiment described in A (n=20). **(C)** Western blot analyses of plasma TSK levels (top panel) and hepatic GRP78 protein in the MCD reversal study. AKT was used as a loading control. Representative samples are shown. **(D to G)** qPCR analyses of pro-inflammatory gene expression in the liver of **(D)** control and *db/db* mice (n=5-6/group), **(E)** control and *ob/ob* mice (n=4-5/group), **(F)** LFD- and HFD-fed mice (n=10/group) and **(G)** mice included in the MCD reversal study (n=7/group). **(H)** qPCR analysis of *Tnfa* transcript levels in AML12 cells treated with TNFα (5ng/ml), IL1β (20ng/ml), IFNγ (5000U/ml) or a mixture of these cytokines for 8 hours (n=5/condition). **(I)** Western blot analysis of plasma TSK levels in mice injected or not with LPS (2mg/kg). Plasma was collected 12 hours after the injection. In this panel, an arrow point towards a non-specific band (n.s.). In all panels, data represent the mean ± SEM. In panels A, D, E and F, significance was determined by two-tailed, unpaired *t* test. **P* < 0.05 versus controls. Pearson correlations (two-tailed) were calculated in panels B. In panels G and H, One-way ANOVA with multiple comparisons (Tukey's multiple comparison) were performed. In panel G, **P* < 0.05 versus control and # *P* < 0.05 versus MCD diet. In panel H, * denotes significance (*P* < 0.05) versus control, # denotes significance (*P* < 0.05) versus TNFα, & denotes significance (*P* < 0.05) versus IL1β and $ denotes significance (*P* < 0.05) versus IFNγ.

Figure S4. TSK levels are increased in response to steatosis and liver damage in humans.



C.

	Admission		Post-admission		
	n	Number (%) or mean (SD)	n	Number (%) or mean (SD)	p value
Sex (female)	26	18 (69%)	24	16 (67%)	0.96
White blood count (10^9/L)	26	12.3 (5.1)	24	10.0 (6.4)	0.15
Platelet count (10^9/L)	26	53 (36-126)	24	69 (46-114)	0.44
INR	26	4.1 (3.3-5.6)	24	1.9 (1.5-3.2)	0.001
ALT (IU/L)	26	4656 (2119-6921)	24	1925 (909-3010)	0.003
Bilirubin	26	78 (62-116)	24	152 (113-211)	<0.001
Creatinine (mmol/L)	26	180 (110-232)	24	90 (161-127)	<0.001
Lactate (mmol/L)	24	7.1 (4.1-10.8)	24	2.6 (1.8-4.4)	<0.001

Figure S4. TSK levels are increased in response to steatosis and liver damage in humans. (A) mRNA expression levels of pro-inflammatory markers and ER stress markers measured in liver samples isolated from humans with Low or High hepatic triglycerides (n=29/group). Data represent the mean ± SEM. Significance was determined by two-tailed, unpaired *t* test. **P* < 0.05 versus controls. **(B)** Correlation calculated between hepatic *TSK* and *IL1B* transcript levels in the human cohort (n=58). Pearson correlations (two-tailed) was calculated. **(C)** Characteristics of the ALF patients included in the study. Significance for sex was determined by chi squared test. For non-parametric variables (platelet count, INR, ALT, bilirubin, creatinine, lactate) significance was determined using a Wilcoxon rank sum test. For parametric variables (white blood cell count), significance was determined by two-tailed, unpaired *t* test.

Figure S5 - TSK does not affect NAFLD development.

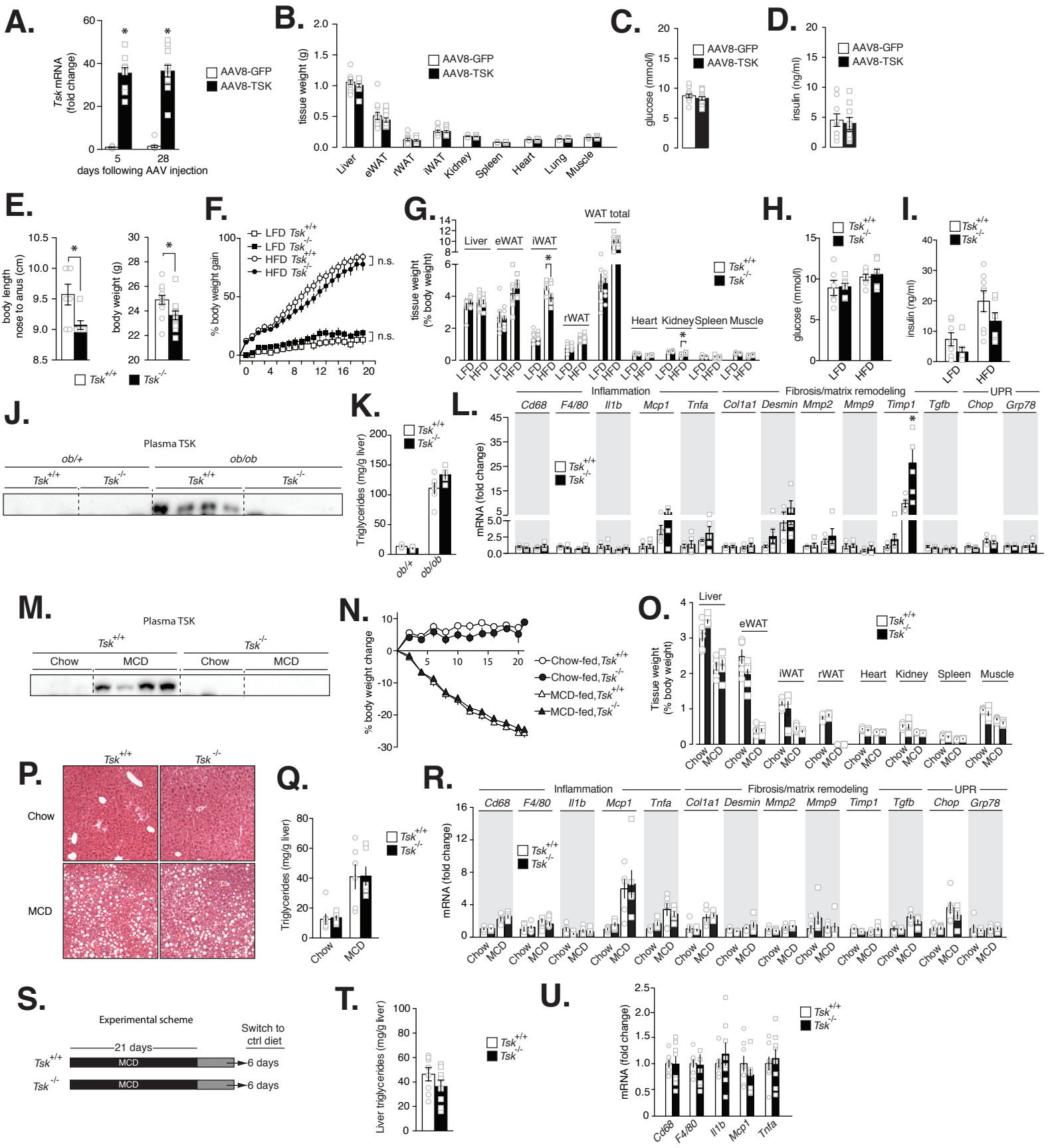


Figure S5. TSK does not affect NAFLD development. (A) Hepatic *Tsk* transcript expression 5 days and 28 days following the injection AAV8-GFP and AAV8-TSK. **(B)** Tissue weight of AAV8-GFP and AAV8-TSK mice (n=12/group). Mice were sacrificed 6 weeks following AAV8 injection. **(C)** Fasting glucose and **(D)** insulin levels measured in plasma of AAV8-GFP and AAV8-TSK mice (n=9-12/group). **(E)** Body length (left) and body weight (right) of wild-type ($Tsk^{+/+}$) and knockout ($Tsk^{-/-}$) (n=7-12/group). **(F)** Body weight gain of wild-type ($Tsk^{+/+}$) and knockout ($Tsk^{-/-}$) (n=6-8/group) fed a LFD or a HFD for 19 weeks **(G)** Tissue weight of wild-type ($Tsk^{+/+}$) and knockout ($Tsk^{-/-}$) mice fed either LFD or HFD diet for 19 weeks (n=6-8/group). Results are presented as % of body weight. **(H)** Fasting glucose and **(I)** insulin levels measured in plasma of wild-type ($Tsk^{+/+}$) and knockout ($Tsk^{-/-}$) mice fed either LFD or HFD diet for 19 weeks (n=6-8/group). **(J)** Western blot analysis of plasma TSK levels in wild-type ($Tsk^{+/+}$) and knockout ($Tsk^{-/-}$) mice bred to the *ob/ob* background (n=4-6/group). Male mice were sacrificed at 16-19 weeks of age. Representative samples are shown. **(K)** Hepatic triglyceride content and **(L)** qPCR analyses of gene expression measured in the liver of mice described in J. **(M)** Western blot analysis of plasma TSK levels in wild-type ($Tsk^{+/+}$) and knockout ($Tsk^{-/-}$) mice fed a MCD diet for 21 days (n=6/group). Representative samples are shown. **(N)** Body weight and **(O)** tissue weight of wild-type ($Tsk^{+/+}$) and knockout ($Tsk^{-/-}$) mice fed MCD diet for 21 days (n=6/group). **(P)** Hematoxylin and eosin stained sections and **(Q)** hepatic triglyceride content of liver samples collected from wild-type ($Tsk^{+/+}$) and knockout ($Tsk^{-/-}$) mice fed MCD diet for 21 days. (**R)** qPCR analysis of various genes in the liver of wild-type ($Tsk^{+/+}$) and knockout ($Tsk^{-/-}$) mice fed MCD diet for 21 days (n=6/group). **(S)** Experimental scheme of the MCD diet reversal experiments. Mice were fed a MCD diet for 21 days before being switched back to a control diet for 6 days (n=8/group). **(T)** Hepatic triglyceride content and **(U)** qPCR analyses of pro-inflammatory gene expression measured in the liver of mice included in the MCD reversal study described in P (n=8/group). In all panels, data are presented as the mean ± SEM. In panels A to E, T and U, significance was determined by two-tailed, unpaired *t* test. $*P < 0.05$ versus controls. In panels F to I, K, L, N, O, Q and R, two-way ANOVA with multiple comparisons was performed between wild-type ($Tsk^{+/+}$) and knockout ($Tsk^{-/-}$) mice for each diet. Only the differences between genotypes within

each diet are presented as significantly different (*$P < 0.05$ versus wild-type ($Tsk^{+/+}$) mice).

Figure S6 - TSKU impact on systemic cholesterol homeostasis.

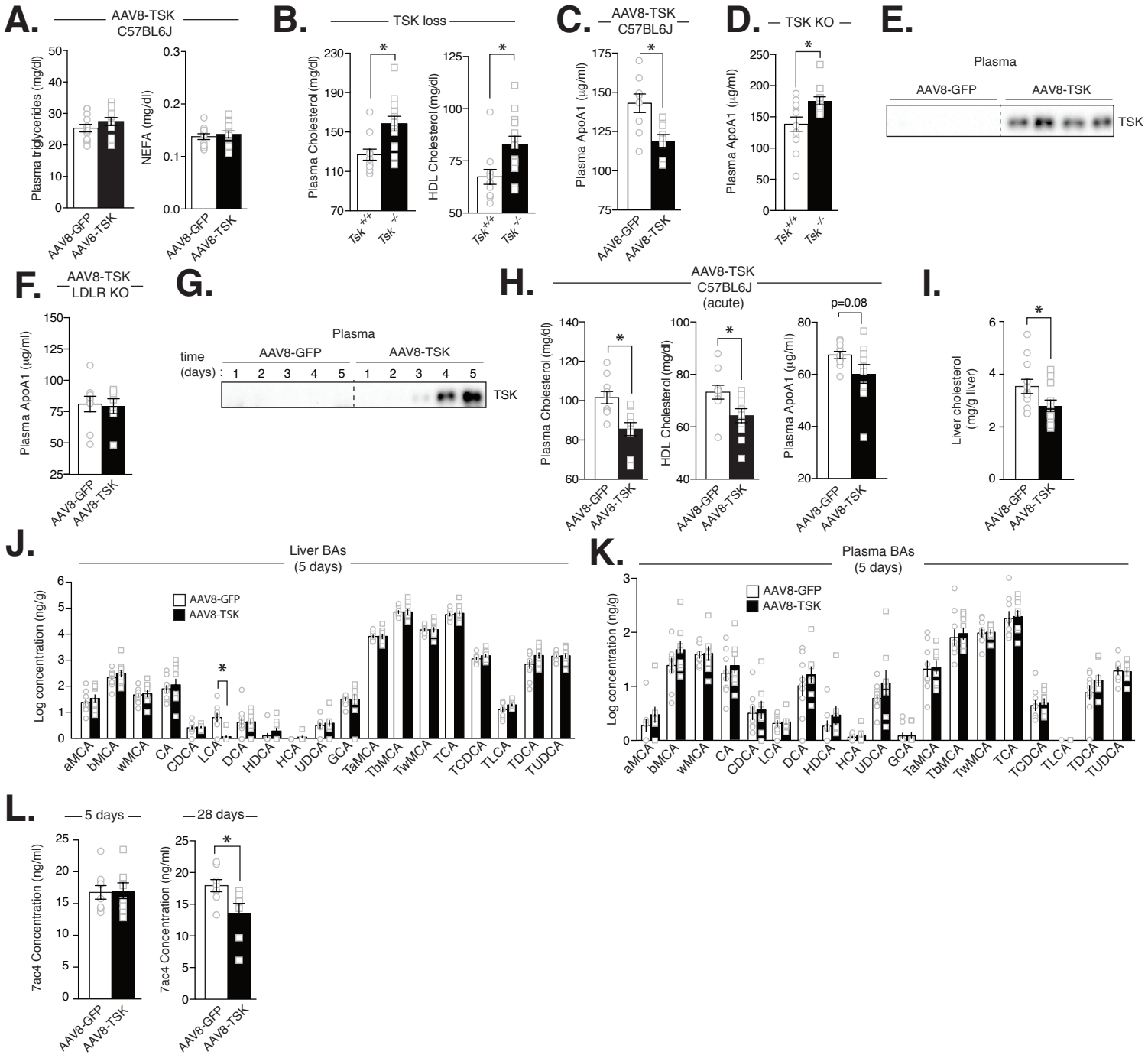


Figure S6. TSK impacts on systemic cholesterol homeostasis. (A) Circulating triglycerides and NEFA levels measured in samples collected from C57BL/6J mice injected with AAV8-GFP or AAV8-TSK (n=12/group). Blood samples were analysed from mice 4 weeks following AAV8 injection. **(B)** Total cholesterol and HDL-cholesterol levels measured in plasma samples collected from wild-type (*Tsk*$^{+/+}$) and knockout (*Tsk*$^{-/-}$) mice (n=11-13/group). **(C-D)** Plasma ApoA1 levels measured in **(C)** C57BL/6J mice injected with AAV8-GFP or AAV8-TSK (n=9/group) and in **(D)** in wild-type (*Tsk*$^{+/+}$) and knockout (*Tsk*$^{-/-}$) mice (n=12/group). **(E)** Western blot analysis and **(F)** ApoA1 levels measured in plasma of LDLR KO mice injected with AAV8-GFP or AAV8-TSK (n=7-9/group). **(G)** Western blot analysis of plasma samples collected from mice injected with AAV8-GFP or AAV8-TSK. Blood was collected 1, 2, 3, 4 and 5 days following AAV8 injection. Representative samples are shown. **(H)** Total cholesterol, HDL-cholesterol and ApoA1 levels measured in plasma samples collected from mice 3 days following the injection with AAV8-GFP or AAV8-TSK (n=10/group). **(I)** Hepatic cholesterol content measured 28 days after the injection of AAV8-GFP or AAV8-TSK. **(J-K)** Bile acid profiling in **(J)** the liver and **(K)** the plasma of mice sacrificed 5 days (n=8-10/group) following the injection with AAV8-GFP or AAV8-TSK. Values are log transformed. **(L)** Circulating levels of 7-alpha-hydroxy-4-cholesten-3-one (7aC4) measured in the plasma of mice sacrificed 5 days or 28 days following the injection with AAV8-GFP or AAV8-TSK (n=8-10/group). In all panels, data are presented as the mean ± SEM. In all panels, excepted panel G, significance was determined by two-tailed, unpaired *t* test. **P* < 0.05 versus control.