Supplementary Information

ACSS2 promotes systemic fat storage and utilization through selective regulation of genes involved in lipid metabolism

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Figs. S1-S12

Table S1

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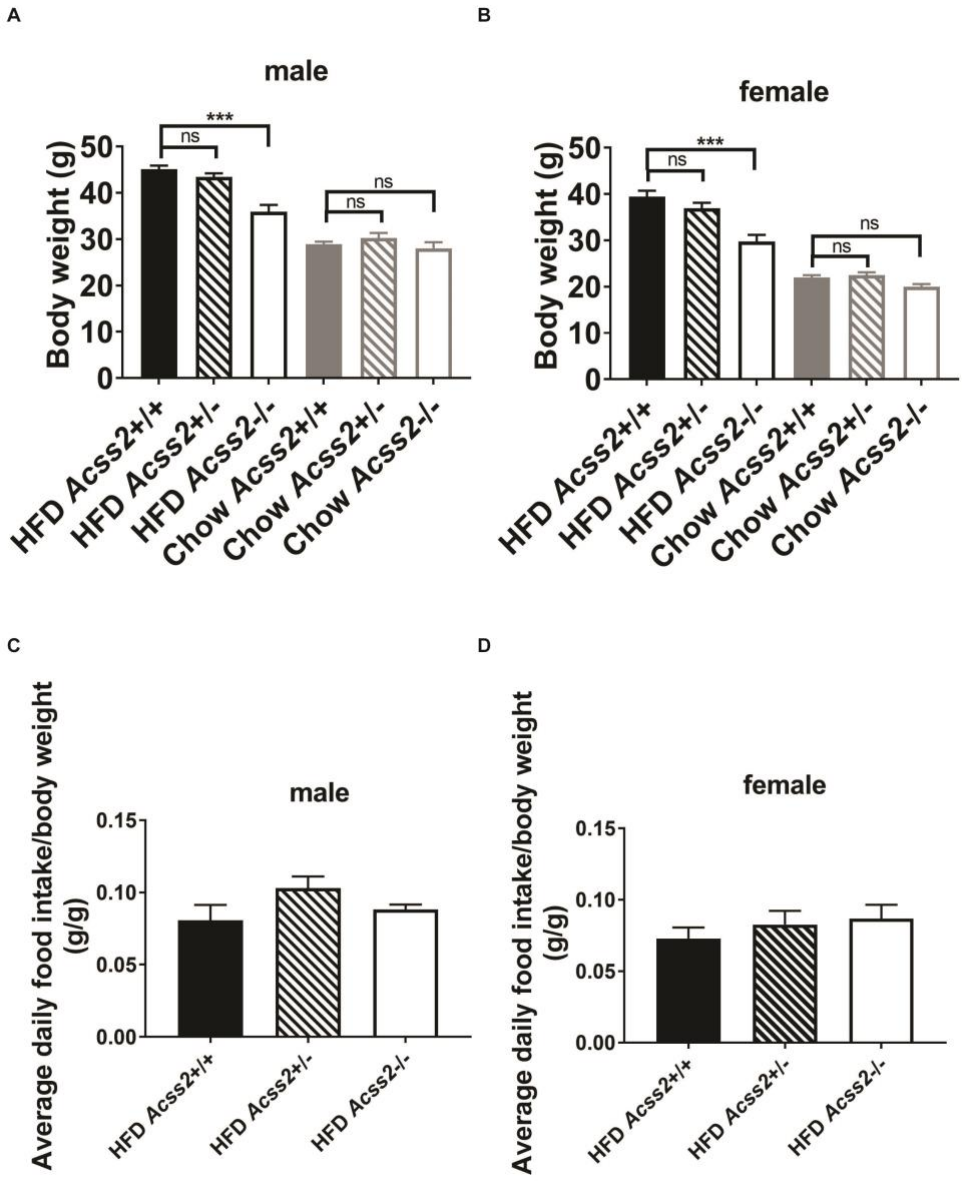

Fig. S1. **(A-B)** Body weights of 20 week-old male (A) or 22 week-old female (B) *Acss2*+/+, *Acss2*+/-, and *Acss2*-/- mice fed chow or HFD starting at 9 weeks as in Fig. 1A, B. **(C-D)** Food intake was measured daily for *Acss2*+/+, *Acss2*+/-, and *Acss2*-/- male (n=6) and female (n=7) mice on HFD. There was no significant difference between genotypes on HFD. Data reflect the mean ± SEM.

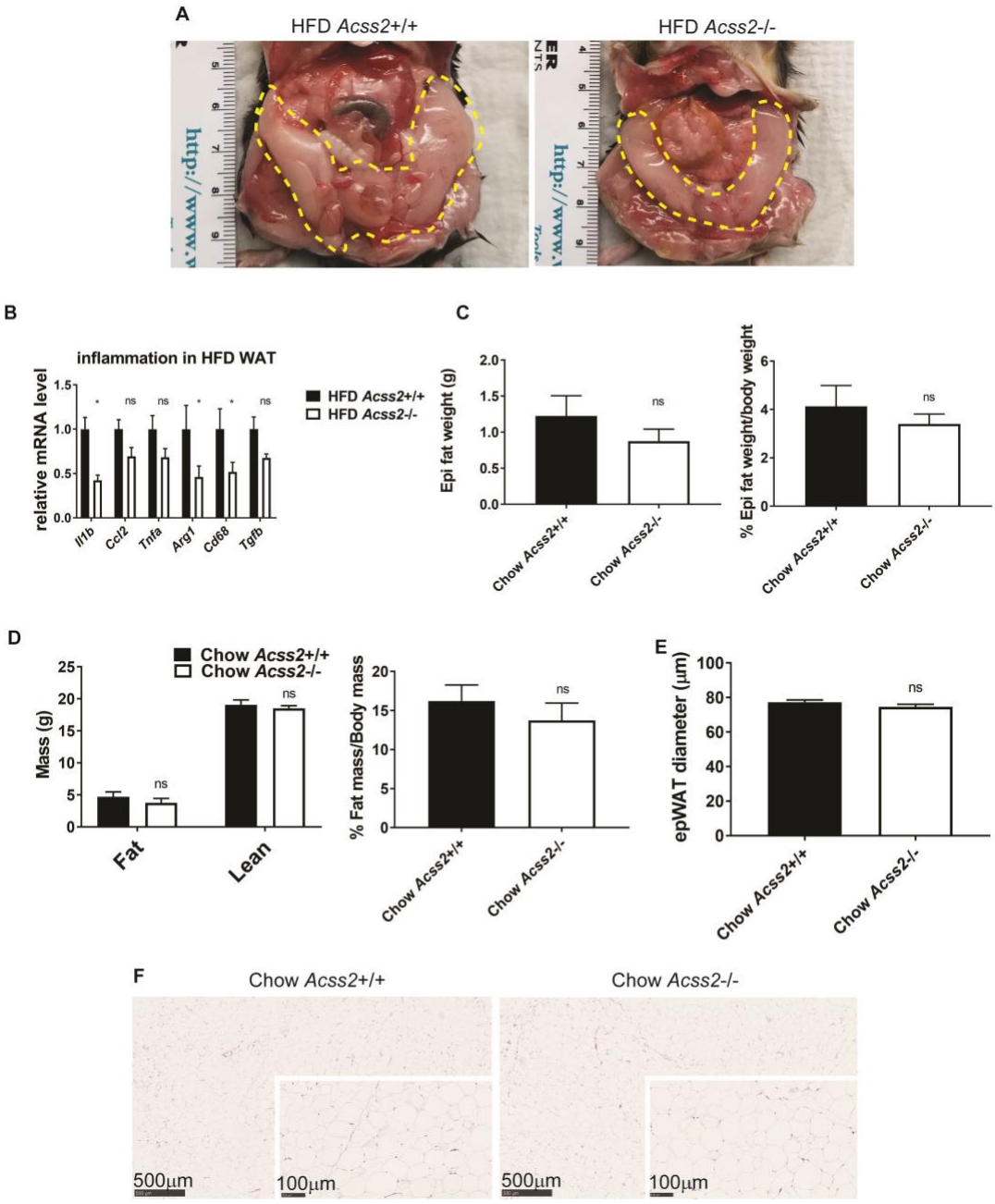

Fig. S2. (A) Representative images of epididymal fat pads of *Acss2*+/+ and *Acss2*-/- male mice fed a HFD for 12 weeks starting at 9 weeks of age. **(B)** mRNA expression of inflammation genes in WAT of male mice on high fat diet (n=7). All data reflect the mean ± SEM. *p<0.05**,** ns: not significant. **(C)** Weight of epi fat was measured for *Acss2*+/+ and *Acss2*-/- male mice (n=5) fed in chow diet, and normalized to body weight. Data reflect the mean ± SEM. ns: not significant. **(D)** Fat composition of *Acss2*+/+ and *Acss2*-/- male mice (n=5) on chow diet. Data reflect the mean ± SEM. ns: not significant. **(E)** Diameters were measured from 60 epWAT cells of male *Acss2*+/+ and *Acss2*-/- mice on chow diet (n=3). Data reflect the mean ± SEM. ns: not significant. **(F)** H&E staining of WAT from male *Acss2*+/+ and *Acss2*-/- mice on chow diet. Arrows denote inflammatory cells. Scale bars, 500 μm and 100 μm.

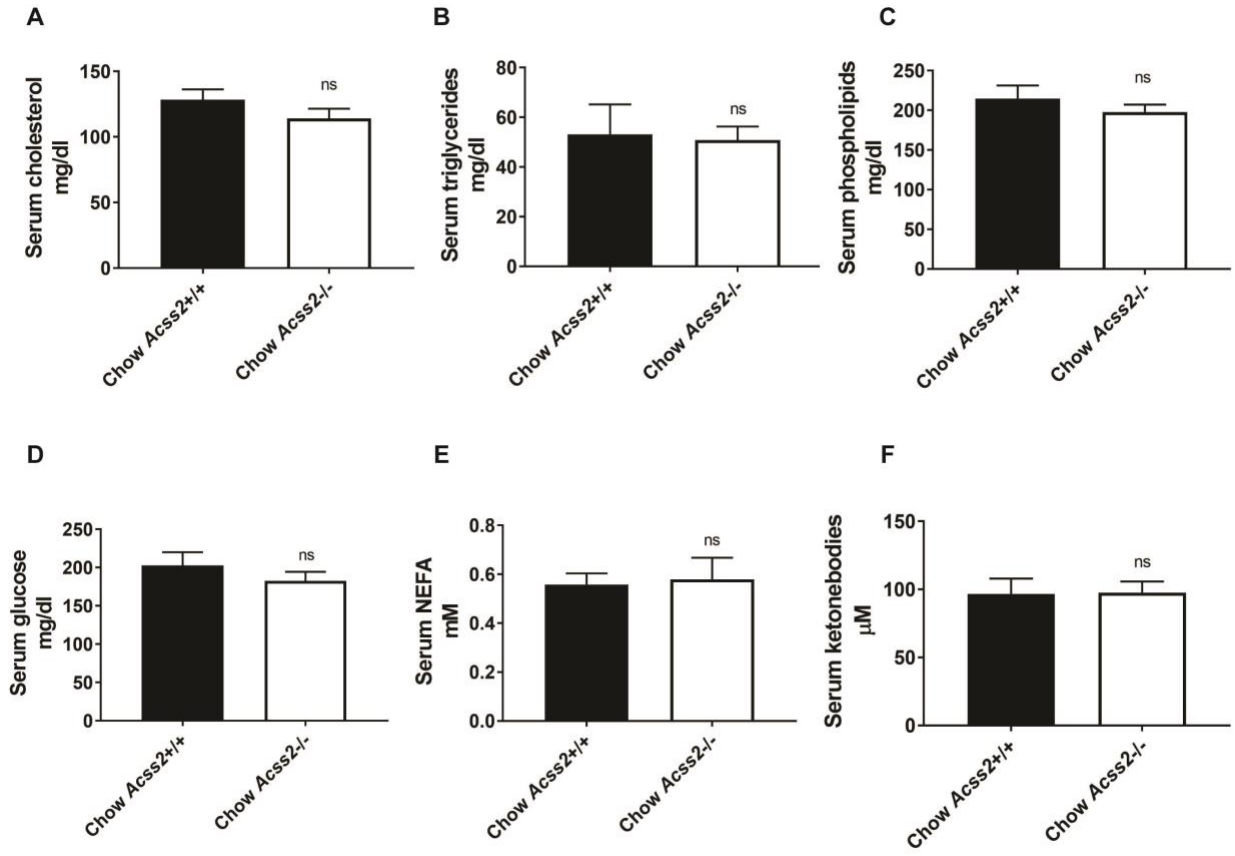

Fig. S3. (A to C) Serum cholesterol (A), triglycerides (B), and phospholipids (C) concentration in *Acss2*+/+ and *Acss2*-/- male mice (n=5) on chow diet. Data reflect the mean ± SEM. ns: not significant. **(D to F)** Serum glucose (D), NEFA (E), and ketone bodies (F) concentration in *Acss2*+/+ and *Acss2*-/- male mice (n=5) on chow diet. Data reflect the mean ± SEM. ns: not significant.

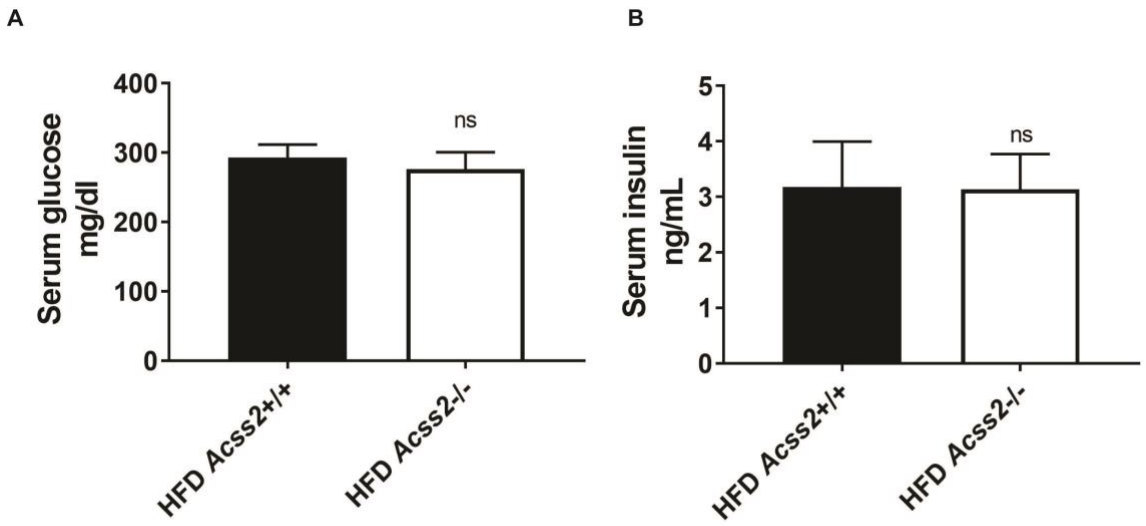

Fig. S4. (A-B) Serum glucose (A) (n=12) and insulin (B) (n=5) concentration in *Acss2*+/+ and *Acss2*-/- male mice fed a HFD for 12 weeks starting at 9 weeks of age. Data reflect the mean ± SEM. ns: not significant.

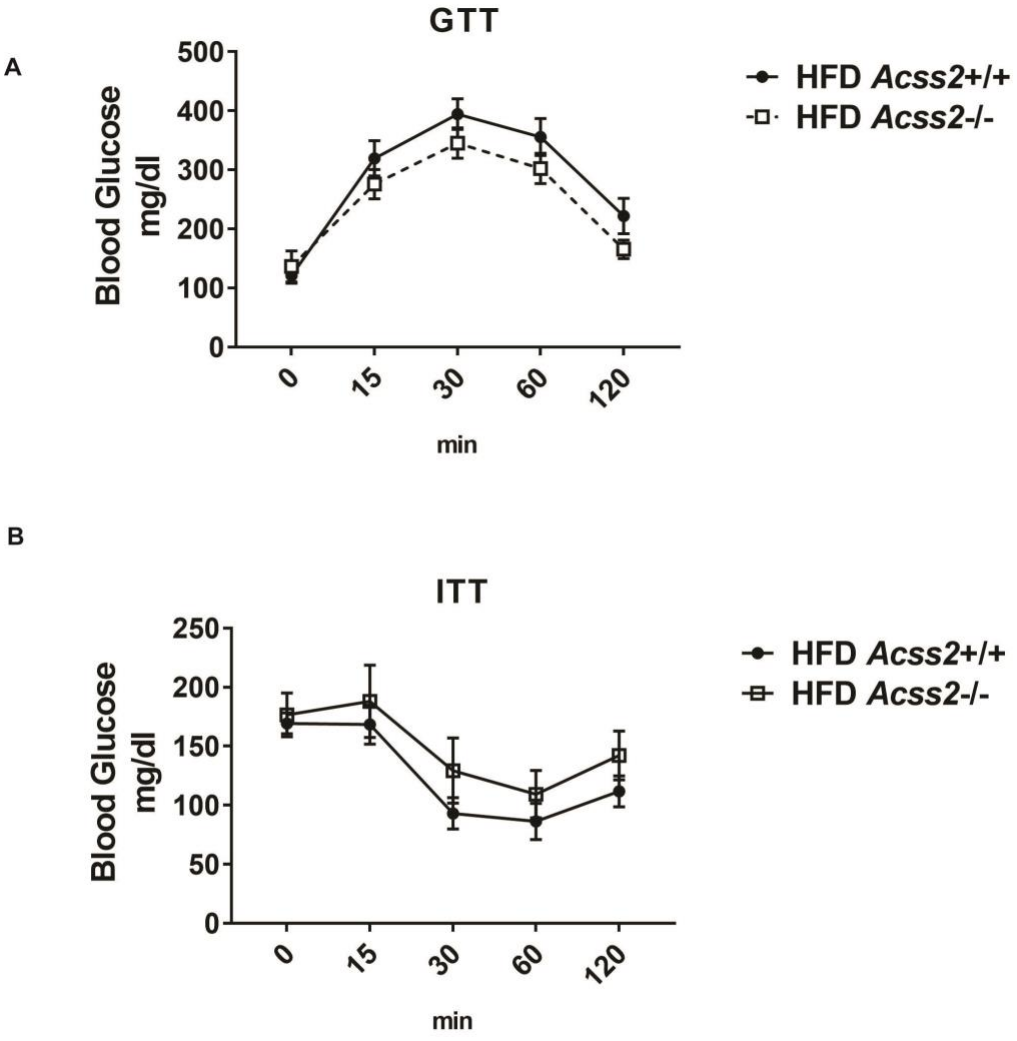

Fig. S5. (A) Blood glucose during glucose tolerance test (GTT) in overnight-fasted *Acss2*+/+ (n=16) and *Acss2*-/- (n=14) male mice fed a HFD for 12 weeks. **(B)** Blood glucose during insulin tolerance test (ITT) in 4 h-fasted *Acss2*+/+ (n=12) and *Acss2*-/- (n=9) male mice fed a HFD for 12 weeks. Data reflect the mean ± SEM.

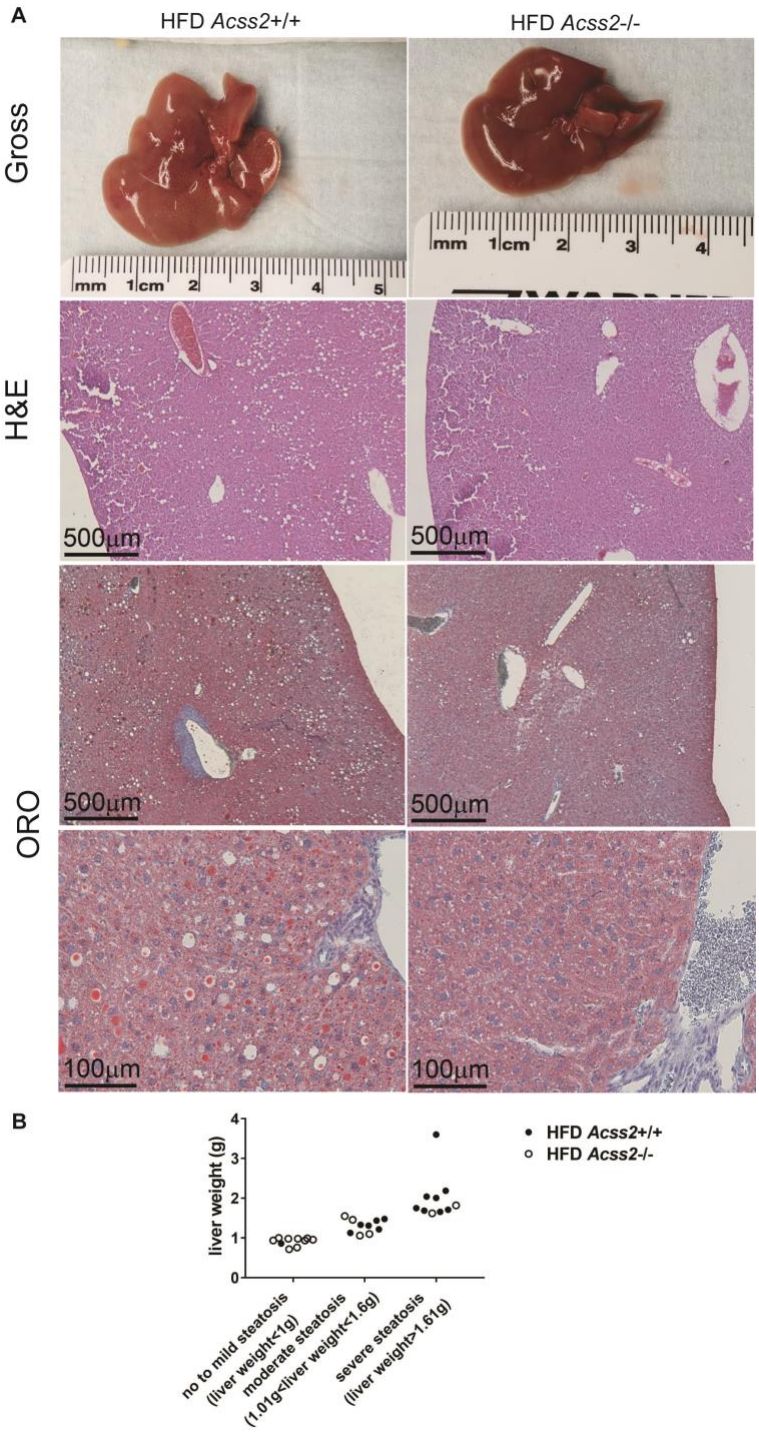

Fig. S6. (A) Gross**,** Haematoxylin and eosin (H&E), and oil red O images of livers from additional representative male mice fed a HFD for 12 weeks. Scale bars, 500 μm and 100 μm. **(B)** Distribution of hepatic steatosis. *Acss2*+/+ and *Acss2*-/- male mice (n=15) fed a HFD for 12 weeks starting at 9 weeks of age were categorized into no to mild steatosis (liver weight<1g), moderate steatosis (1.01g<liver weight<1.6g), and severe steatosis (liver weight>1.61g).

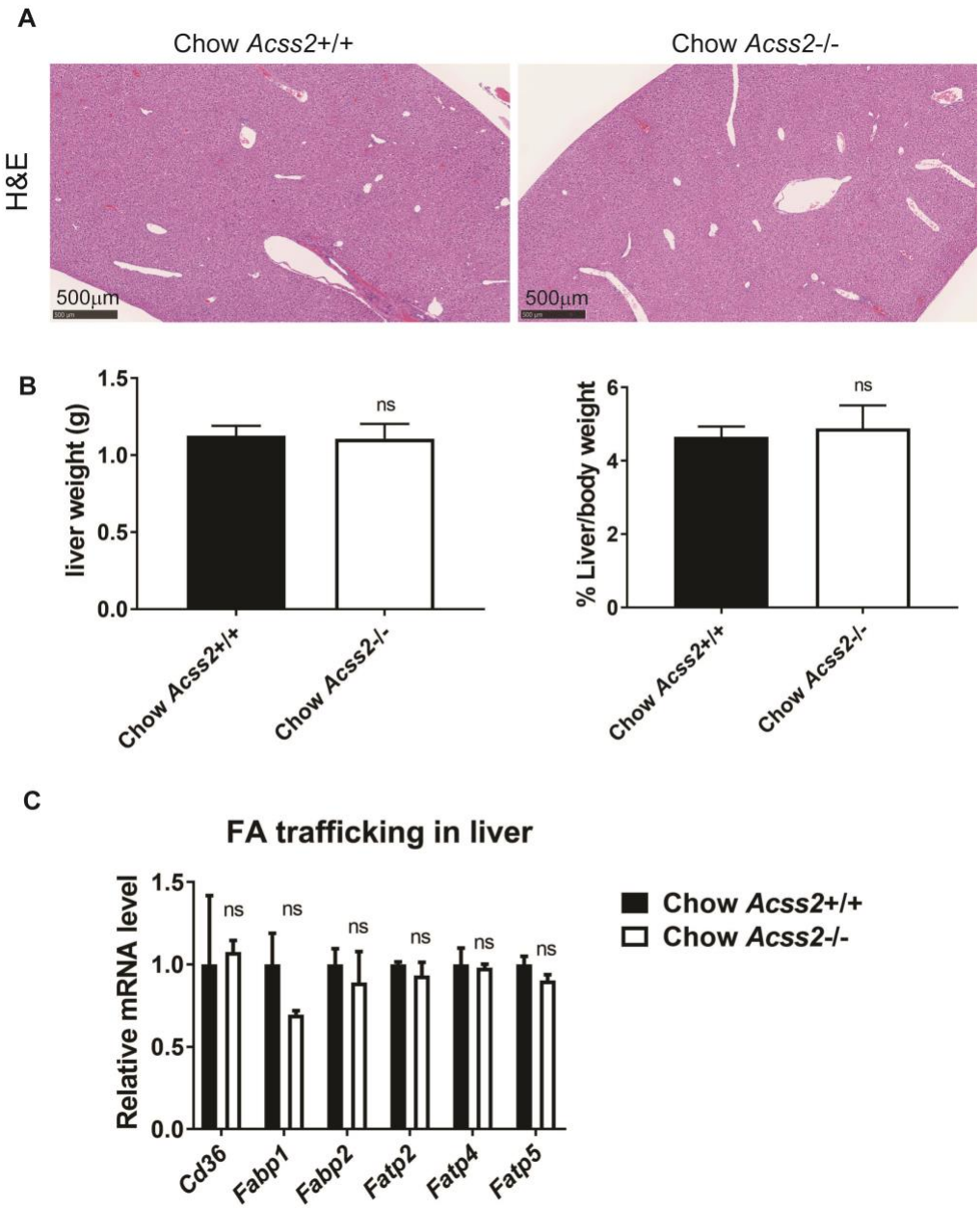

Fig. S7. (A) Haematoxylin and eosin (H&E) images of livers from additional representative male mice on chow diet. Scale bar, 500 µm. **(B)** Liver weights were measured for *Acss2*+/+ and *Acss2*-/- male mice (n=5) fed a chow diet, and normalized to body weight. **(C)** mRNA expression of FA transporters in liver of male mice on chow diet (n=3). All data reflect the mean ± SEM. ns: not significant.

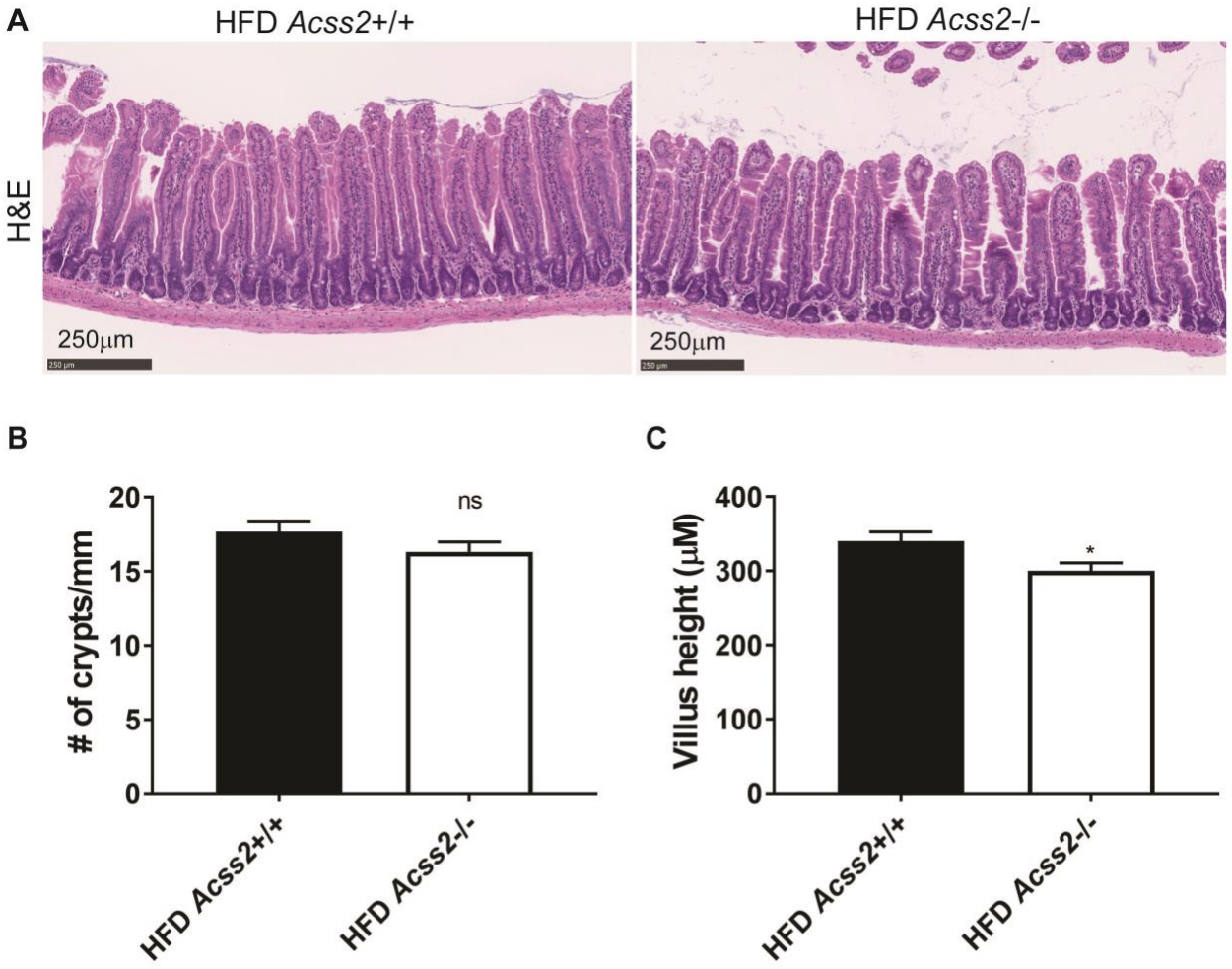

Fig. S8. (A) Haematoxylin and eosin (H&E) images of proximal intestinal samples from *Acss2*+/+ and *Acss2*-/- male mice fed a HFD for 12 weeks starting at 9 weeks of age. Scale bar, 250 µm. **(B-C)** Crypt numbers (B) and villus height (C) of *Acss2*+/+ and *Acss2*-/- male mice fed a HFD for 12 weeks (n=3).

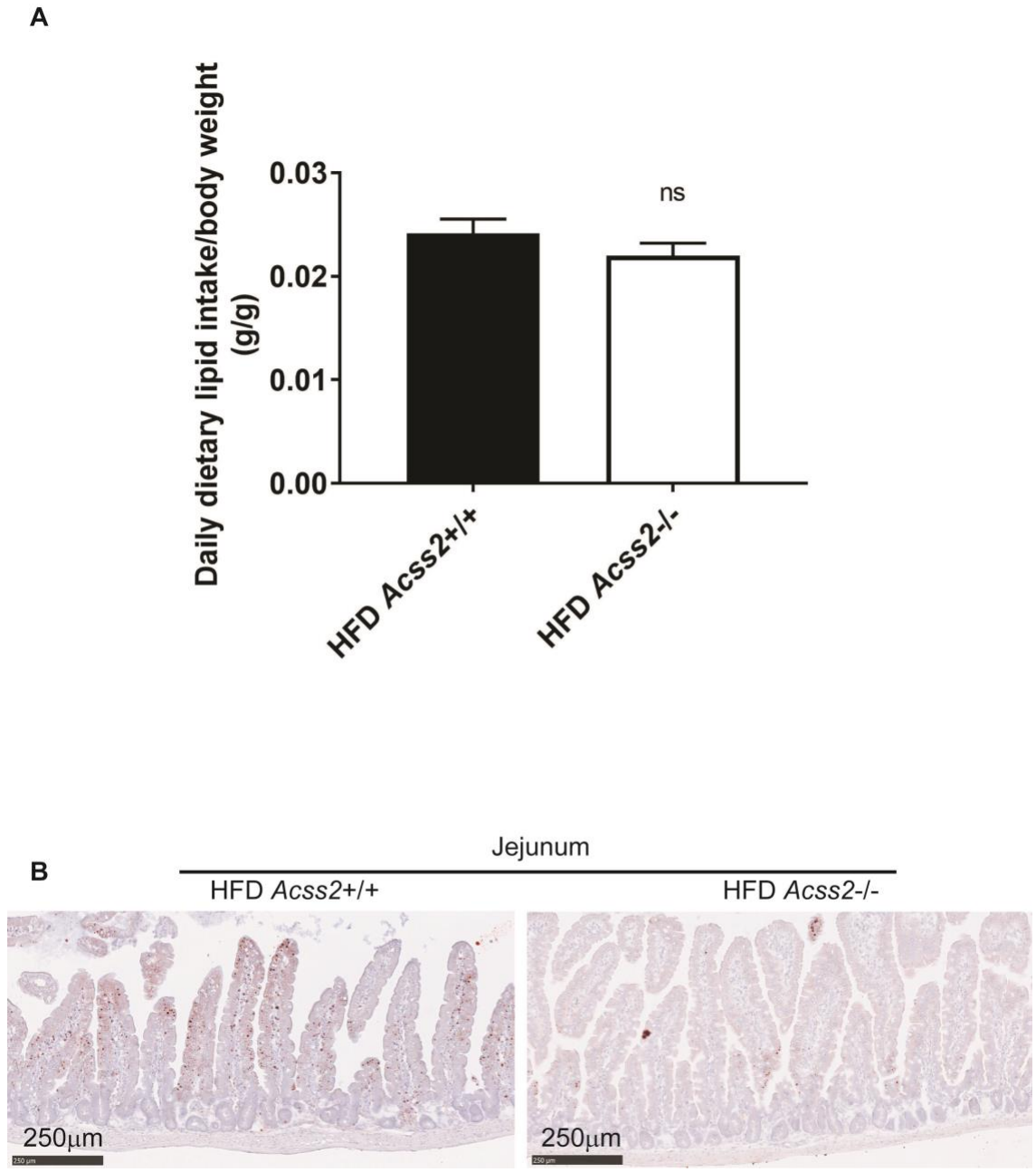

Fig. S9. (A) Daily dietary lipid intake was analyzed in male mice fed a HFD for 12 weeks (n=6), ns: not significant. **(B)** ORO staining of proximal intestines from a representative male mouse of the indicated genotype fed a HFD for 12 weeks. Scale bar, 250 μm.

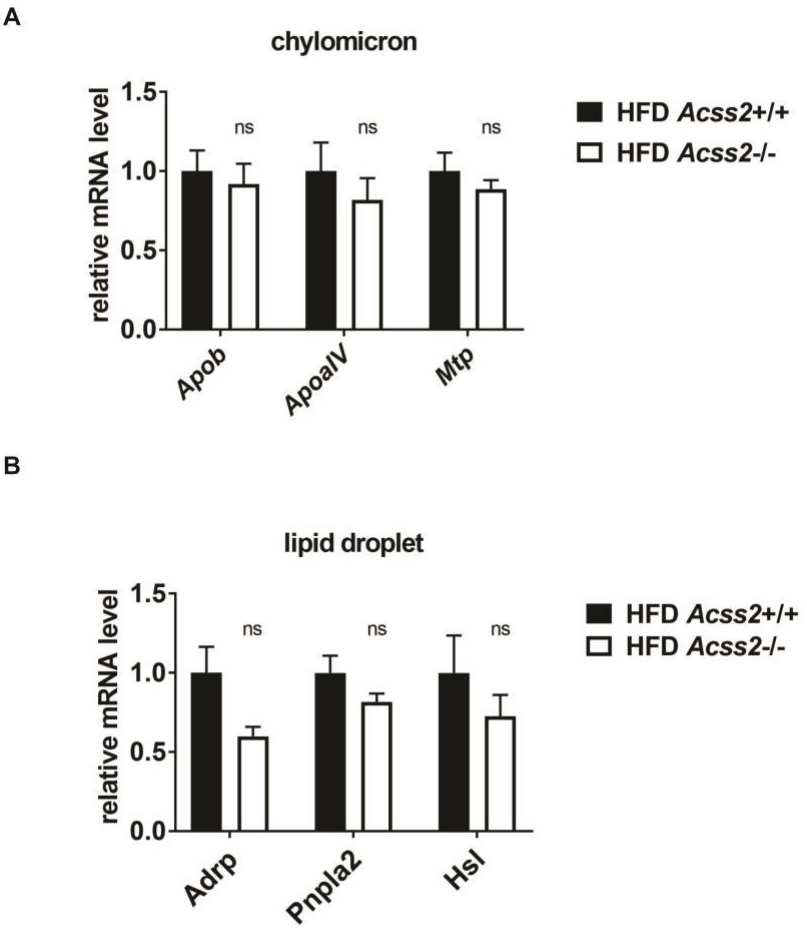

Fig. S10. (A-B) Intestinal mRNA profile of male mice fed a HFD for 12 weeks (n=4). Data reflect the mean ± SEM. ns: not significant.

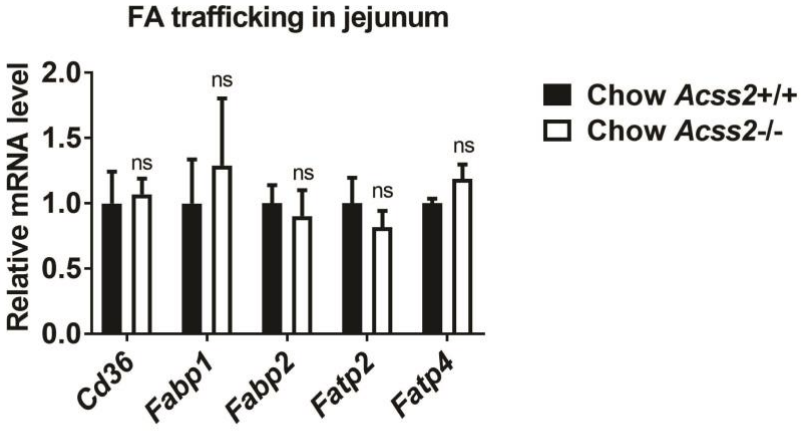

Fig. S11. Intestinal mRNA profile of male mice on chow diet (n=3). Data reflect the mean ± SEM. ns: not significant.

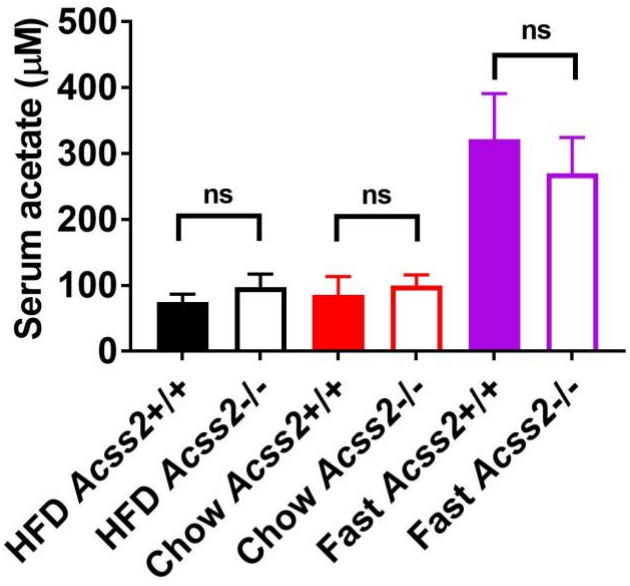

Fig. S12. Serum acetate concentration in *Acss2*+/+ and *Acss2*-/- male mice fed HFD (n=11), chow diet (n=7), and 48 hours fast (n=6). Acetate level was measured using acetate colorimetric assay kit (Biovision, CA). Data reflect the mean ± SEM. ns: not significant.

Table S1. qPCR primers used in this study

gene name	primer sequences
cyclophilin	5’tggagagcaccaagacagaca (F)
	5’tgccggagtcgacaatgat (R)
Cd36	5’ggaactgtgggctcattgc (F)
	5’catgagaatgcctccaaacac (R)
Fabp1	5'ggtgacaactttcaaaggcataaa (F)
	5'tgtcgcccaatgtcatggta (R)
Fabp2	5'ctcggtgtaaactttccctacagtc (F)
	5'tttatttccctcaatggtccag (R)
Fatp2	5'caacacaccgcagaaacca (F)
	5'atttcccagggctttttca (R)
Fatp4	5'acccaaagctgccattgtg (F)
	5'gcatgcggaatccaagtaca (R)
Fatp5	5'gaccactggactcccaaagc (F)
	5'gac agcacgttgctcacttgt (R)
Ppara	5’cgtacggcaatggctttatc (F)
	5’aacggcttcctcaggttctt (R)
Pparg	5’cccaccaacttcggaatca (F)
	5’tgcgagtggtcttccatcac (R)
Srebp1a	5'ggccgagatgtgcgaact (F)
	5'ttgttgatgagctggagcatgt (R)
Srebp1c	5'ggagccatggattgcacatt (F)
	5'ggcccgggaagtcactgt (R)
Rxra	5’tgcccatccctcaggaaa (F)
	5’gcggtccccacagatagc (R)
Lxrb	5’ctcccacccacgcttacac (F)
	5’gccctaacctctctccactca (R)
Acca	5'ggcagctctggaggtgtatg (F)
	5'tccttaagctggcggtgtt (R)
Fas	5’gctgcggaaacttcaggaaat (F)
	5’agagacgtgtcactcctggactt (R)
Scd1	5’ccggagacccccttagatcga (F)
	5’tagcctgtaaaagatttctgcaaacc (R)
Elovl2	5'tcaatgctttcttggacaacatg (F)
	5'ggtaagagtccagcaggaacca (R)
Hcs	5'gccgtgaactgggtcgaa (F)
	5'gcatatatagcaatgtctcctgcaa (R)
Hcr	5'cttgtggaatgccttgtgattg (F)
	5'agccgaagcagcacatgat (R)
Dhcr24	5’aggcagctggagaagtttgtg (F)
	5’cctcgcggttcatatagcaatc (R)
Lss	5'gtggtccgctcttcctcttg (F)

	5'agctggcagagagatgtgtga (R)
Gpat1	5’caacaccatccccgacatc (F)
	5’gtgaccttcgattatgcgatca (R)
Acox1	5’ctgctcagcaggagaaatgg (F)
	5’tgggcgtaggtgccaatta (R)
Acox2	5'gacggtcctgaacgcattt (F)
	5'cattcatggcaataccatgtaagtt (R)
Ehhadh	5'ATGGCTGAGTATCTGAGGCTG (F)
	5'GGTCCAAACTAGCTTTCTGGAG (R)
Dbp	5'cactgtgtgctgttaaaggagtca (F)
	5'cactcctgtggatcgcagaa (R)
Acaa1	5'gcagaagcaggatgactttgc (F)
	5'caatctcagcacggagcat (R)
Scp2	5'CCTTCTGTCGCTTTGAAATCTCC (F)
	5'GCTTCCTTTGCCATATCAGGAT (R)
Acsl5	5'gaaagcctcactcggaagct (F)
	5'ttgtgaataccaacaggaaattca (R)
Mgat2	5'ccttcgcggtccttcagt (F)
	5'gaggcctacgaagatgacgat (R)
Dgat1	5'ggaatatccccgtgcacaa (F)
	5'catttgctgctgccatgtc (R)
Dgat2	5'ccgcaaaggctttgtgaa (F)
	5'ggaataagtgggaaccagatcag (R)
Apob	5’cgtgggctccagcattcta (F)
	5’tcaccagtcatttctgcctttg (R)
apoaIV	5'acagtttcagaagacggatgtca (F)
	5'cgtactagcatccccaagtttg (R)
Mtp	5’cctaccaggcccaacaagac (F)
	5’cgctcaattttgcatgtatcc (R)
Adrp	5'gacctctgcggccatgac (F)
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Pnpla2	5'ccgctggagagtgcagtgt (F)
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Hsl	5'cctcatggctcaactcc (F)
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